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***Engraulis anchoita* (Clupeiformes: Engraulidae) eggs and larvae in
the Southeastern Brazilian Bight: new perspectives from a
historical data set (1974-2010)**

Thesis presented to the Oceanographic
Institute of the University of São Paulo and
the School for Marine Science and
Technology of the University of
Massachusetts as part of the requirements
for the title of Doctor of Science, program
of Oceanography, Biological Oceanography
area.

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Universidade de São Paulo
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RESUMO

O principal objetivo dessa tese foi analisar as flutuações de longo-prazo na distribuição e abundância de ovos e larvas de *Engraulias anchoita*, uma espécie de peixe de importância econômica e ecológica, na Plataforma Continental Sudeste do Brasil (PCSE). Nós analisamos amostras e dados abióticos de dezoito cruzeiros oceanográficos realizados durante o fim da primavera e o começo do verão de 1974 a 2010. Dois estoques distintos foram identificados com base no tamanho dos ovos, um predominante e com menor tamanho e outro de maior tamanho ao sul da PCSE. Através de “krigagem” indicativa, foram identificadas áreas de desova ocasional (como ao norte de Florianópolis e a área ao largo da baía de Santos) e áreas em que a desova foi evitada (como em frente à Ilha de São Sebastião e ao Sistema Costeiro Cananéia-Iguape). Usando modelos inflacionados de zeros, os fatores espaciais (diferentes áreas e profundidades amostradas) foram relacionados com a probabilidade de se amostrar falso zero, enquanto os fatores temporais e oceanográficos (diferentes anos e temperatura) foram relacionados com a abundância de ovos e larvas. Apresentamos também uma metodologia mais rápida e mais eficiente para identificar os ovos de *E. anchoita*, comparamos as amostragens realizadas com duas malhagens diferentes e analisamos variações sazonais do tamanho dos ovos capturados. Assim, nossos resultados poderão auxiliar estudos futuros e também no manejo pesqueiro da espécie em questão, ainda não explorada comercialmente na área de estudo.

Palavras-chaves: ictioplâncton, flutuações de longo-prazo, estoques pesqueiros, áreas de desova, modelos inflacionados de zeros.

ABSTRACT

The main objective of this dissertation was to evaluate long-term fluctuations in the distribution and abundance of *Engraulis anchoita* eggs and larvae in the Southeastern Brazilian Bight (SBB). *Engraulis anchoita* is a fish species that is ecologically and economically important. We analyzed samples and abiotic data from eighteen oceanographic cruises conducted during austral late spring and early summer from 1974 to 2010. Two different stocks were detected in the SBB based on egg size, with the predominant stock in the area having smaller eggs than the stock in the region further south. Using indicative kriging, we identified occasional (e.g. Florianópolis - 27 °S and off Santos Bay) and avoided (e.g. off São Sebastião Island and off Cananéia-Iguape Coastal System) spawning sites. Through zero-inflated models, spatial factors (different areas and the local depth) were related to the probability of sampling false zeros and temporal and oceanographic conditions (different years and temperature) with egg and larvae abundance. We also described faster and more accurate methodology to identify *E. anchoita* eggs, and compared the mesh-size efficiency to sample eggs and analyzed how egg size varied seasonally. Our results may support future studies and may assist a future fishery management of *E. anchoita*, a species not yet exploited in the SBB.

Keywords: ichthyoplankton, long-term fluctuations, fish stocks, spawning sites, zero- inflated models (ZI)

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1. GENERAL INTRODUCTION

The family Engraulidae (Clupeiformes: Teleostei), popularly known as anchovies, are small or moderate-sized fishes occurring in all seas from 60°N to 50°S. They typically inhabit coastal marine waters in schools, but some species enter brackish or freshwater (WHITEHEAD et al., 1988). Among the 16 genera and 139 species described (NELSON, 2006) at least nine genera and 26 species are known to occur along the Brazilian coast (WHITEHEAD et al., 1988; MENEZES; FIGUEIREDO, 2003). In the Southeastern Brazilian Bight (SBB), roughly 22°S and 28°S, there are six genera and 12 species, but only the Argentine anchovy (*Engraulis anchoita* Hubbs & Marini 1935) is found offshore from the coast (FIGUEIREDO; MENEZES, 1978). This species exhibits a wide distribution in the western south Atlantic, from Gulf San Jorge (Argentina, 48°S) to Cape São Tomé (Brazil, 20°S) (BAKUN; PARRISH, 1991). Within this wide distribution, three stocks with biological differences are identified: the *patagonic* (48° - 41°S; Argentina), the *bonaerense* (41° - 28°S; northern Argentina, Uruguay and southern Brazil) and the Southeastern Brazilian Bight stock (CARVALHO; CASTELLO, 2013).

In the SBB, the Argentine anchovy together with the Brazilian sardine (*Sardinella brasiliensis* Steindachner, 1879) are the two dominant pelagic fish species in ichthyoplankton samples (KATSURAGAWA et al., 2006). They are important components of the ecosystem, transferring energy and nutrients from the bottom of the food chain to higher predators, such as other fish, marine mammals and seabirds (FREDERIKSEN et al., 2006; CASTELLO, 2007). Cury et al. (2000) described small pelagic fish as mid-trophic-level “wasp-waist” populations, which exert a major control on the dynamics of upper and lower trophic levels.

Beyond the ecological importance, both species are also important fisheries resources. *Sardinella brasiliensis* supports one of the most-important commercial fisheries in Brazil, totaling 74,100 t in 2011 (MPA, 2013), but some stocks are over-exploited (CERGOLE; ROSSI-WONGTSCHOWSKI, 2005; CERGOLE; DIAS-NETO, 2011). On the other hand, *E. anchoita* is economically important to the Argentine and Uruguayan fishery fleets (FAO, 2014), but in southern Brazil anchovy exploitation has only recently started, and in the SBB anchovy populations are in almost-pristine condition, enabling an expansion of exploitation in the near future (MADUREIRA et al., 2009; CARVALHO; CASTELLO, 2013).

Probably due to its fishery importance, most of the pelagic fish studies in the SBB are focused on *S. brasiliensis* (e.g. MATSUURA, 1996; MATSUURA, 1998; GIGLIOTTI et al., 2010; MORAES et al., 2012; DIAS et al., 2014). Regarding *E. anchoita*, most studies available are from the Argentina and Uruguay coasts (e.g. CIECHOMSKI, 1965; 1966; 1973; SANCHEZ; CIECHOMSKI, 1984; EHRlich et al., 2000; HANSEN et al., 2001; CAPITANIO et al., 2005; MARTOS et al., 2005; PÁJARO et al., 2007; PÁJARO et al., 2008; LEONARDUZZI et al., 2010; 2013; PADOVANI et al., 2011; AUAD; MARTOS, 2012; GARCIARENA; BURATTI, 2013; MADIROLAS et al, 2013; MARRARI et al., 2013), fewer from southern Brazil (e.g. WEISS; FEIJÓ DE SOUZA, 1977; LIMA; CASTELLO, 1994; 1995; VASCONCELLOS et al., 1998; VELASCO; CASTELLO, 2005; VAZ et al., 2007; COOKE; MADUREIRA, 2012; TORQUATO; MUELBERT, 2014; COSTA et al., 2016) and even fewer from the SBB (e.g. NAKATANI, 1982; ASANO et al., 1991; MATSUURA et al., 1992; CASTELLO; CASTELLO, 2003; FAVERO et al., 2015a; 2015b).

Contrasting with *S. brasiliensis*, which is restricted to the SBB and spawns only during late-spring and summer (MATSUURA et al., 1992), *E. anchoita* is broadly distributed and spawns all year-round with a peak, in the SBB, during late-spring and beginning of summer (MATSUURA et al., 1992). Anchovies and sardines are oviparous, but the former spawn planktonic eggs that exhibit a particular shape: from almost spherical to strongly elliptical (WATSON; SANDKNOP, 1996). *Engraulis anchoita* eggs usually hatch within 3 days (CIECHOMSKI, 1965), so they are sampled near the spawning area, and in similar environmental conditions to those when spawning occurred (WEBER; McCLATCHIE, 2010).

As small pelagic fish usually exhibit fast growth rates and short lifespans, their stock sizes are characterized by marked fluctuations because of their high dependence on environmentally-driven annual pulses in recruitment (BARANGE et al., 2009; CHECKLEY et al., 2009). The spawning and life cycles of *E. anchoita* and *S. brasiliensis* depend on the intensity of subtropical water penetration on the bottom of the shelf and the formation of retention areas and/or stability of the water column (LIMA; CASTELLO, 1995; MATSUURA; KITAHARA, 1995).

Ichthyoplankton studies began in Brazil during the 1960s, but only after the early 1980s did the research focus turn to understanding the controls exerted by environmental factors on fish eggs and larvae (KATSURAGAWA et al., 2006). Most of

these studies examined the environmental influences on the ichthyoplankton over short to medium temporal scales, especially seasonal (e.g. NONAKA et al., 2000; MUELBERT et al., 2008; GOÇALO et al., 2011; GARBINI et al. 2014; KATSURAGAWA et al., 2014; PORCARO et al., 2014). However, a variety of oceanographic and climatic factors influence distribution and abundance of fish eggs and larvae, not just on seasonal temporal scales, but also on interannual (e.g. FRANCO-GORDO et al., 2008; MUHLING et al., 2013) and decadal ones (e.g. AUTH et al., 2011). The use of ichthyoplankton time-series can greatly improve the ecological monitoring, assessment and management of the oceans (KOSLOW; COUTURE, 2013), but these time-series are often expensive and difficult to maintain, as they usually require systematic sampling programs that require a ship to occur (KOSLOW; WRIGHT, 2016).

Although much attention has been focused recently on multi-decadal time-series of marine organisms (e.g. SÁNCHEZ; CIECHOMSKI, 1995; BRODEUR et al., 1996; MULLIN, 1998; NAGASAWA, 2001; STRATOUDAKIS et al., 2003; HSIEH et al., 2009; AUTH et al., 2011; TURNER et al., 2011), few long time-series data are available, and many that do exist are complicated by changes in methodology made over the years. For example, the California Cooperative Oceanic Fisheries Investigations (CalCOFI) program has sampled zooplankton off the west coast of North America since 1949, but in 1978 the 1m diameter ring net was replaced by a bongo net, with two 0.71m diameter nets (REBSTOCK, 2002) For the Brazilian coast, the Biological Collection "Prof. E. F. Nonato" (ColBIO) of the Oceanographic Institute, University of São Paulo, Brazil, contains archived ichthyoplankton samples from the Southeastern Brazilian Bight since the 1960s, most of which were sampled using bongo nets, but with different mesh sizes. Although these samples do not represent a long-term monitoring program, the ColBIO samples comprise a valuable data set of ichthyoplankton time-series, enabling a large variety of studies, including long-temporal scale evaluations.

Along the Oregon Coast, United States of America, large-scale climate variations, such as the Pacific Decadal Oscillation and El Niño, explained more variation in the concentration and diversity of fish larvae than small-scale factors such as upwelling and Ekman transport (AUTH et al., 2011). Other studies also sought to understand the influence of large-scale environmental factors, such as El Niño/La Niña,

on fish larvae and eggs (e.g. LIMA; CASTELLO, 1994; FLORES-COTO et al., 2008; FUNES-RODRÍGUEZ et al., 2011; BUSBY et al., 2014; MUJICA et al., 2016).

Despite the importance of studies analyzing long-temporal-scale influences on fish, in the Southeastern Brazilian Bight there has not been any study relating long-term environmental factors to the distribution and abundance of the ichthyoplankton. The use of historical data sets can help to fill gaps in the knowledge about fish, increasing the understanding of the distribution and abundance of different species in large temporal scale. Thus we highlighted the relevance of the present study, which analyzed 18 different years (from 1974 to 2010), providing new information about *E. anchoita* early life history.

2. OBJECTIVES

Overall Objective

The main objective of this study is to evaluate long-term fluctuations in the distribution and abundance of *Engraulis anchoita* Hubbs and Marini, 1935 (Clupeiformes: Engraulidae) eggs and larvae in the Southeastern Brazilian Bight, from 1974 to 2010.

Specific Objectives

- Verify locations of the preferred spawning sites of *E. anchoita* in the Southeastern Brazilian Bight, and determine if there was a change in these areas during the studied years (CHAPTER IV);
- Understand how oceanographic factors influence the distribution and abundance of *E. anchoita* eggs and larvae in the Southeastern Brazilian Bight over a long-time-series period (CHAPTER V).

However, to achieve the specific objectives presented, I had to deal with some questions that needed to be answered for a better comprehension of the *E. anchoita* early life history and for the discussion of the main chapters.

- Is there a faster and more accurate way to identify the *E. anchoita* eggs from the total of 1,451 samples?
- The mesh size of the net changed within the studied years, is that a big issue?

- Am I analyzing individuals from different *E. anchoita* stocks?

Those questions were answered in CHAPTERS I, II and III, respectively.

3. MATERIALS AND METHODS

Materials and methods are presented in the following chapters (I-V).

4. RESULTS

Results are presented in the following chapters (I-V).

I. USING NEW TOOLS TO IDENTIFY EGGS OF *ENGRAULIS ANCHOITA* (CLUPEIFORMES, ENGRAULIDAE)

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The family Engraulidae (Clupeiformes: Teleostei), popularly known as anchovies, are small or moderate-sized fishes occurring in all seas from 60°N to 50°S. They typically inhabit coastal marine waters in schools, but some species enter brackish or freshwater habitats (WHITEHEAD et al., 1988). Among the 16 genera and 139 species described (NELSON, 2006) at least nine genera and 26 species are known to occur along the Brazilian coast (WHITEHEAD et al., 1988; MENEZES; FIGUEIREDO, 2003). In southeastern Brazilian waters, there are six genera and 12 species, but only *Engraulis anchoita* Hubbs & Marini 1935 is found offshore from the coast (FIGUEIREDO; MENEZES, 1978).

Engraulis anchoita is important for its role in the marine ecosystem as a secondary consumer near the bottom of the food chain and as essential prey to other fish, marine mammals and seabirds (CASTELLO, 2007). This species is also important to fisheries, in 2011 the capture production in Argentina and Uruguay was about 21,164 tonnes (FAO, 2012). It is estimated that up to 135,000 tonnes of *E. anchoita* could be sustainably exploited along the southern Brazilian coast (MADUREIRA et al., 2009). However, despite its abundance, exploitation of *E. anchoita* has just recently started in southern Brazil, so a better understanding of the species' biology and population dynamics is required (CARVALHO; CASTELLO, 2013).

Anchovies are oviparous, spawning planktonic eggs that vary in shape from almost spherical to strongly elliptical (WATSON; SANDKNOP, 1996). In the Southeastern Brazilian Bight, eggs and larvae of anchovies are very common in ichthyoplankton samples. In some cases, they may account for more than 50% of all ichthyoplankton sampled, with *E. anchoita* as the most abundant species (KATSURAGAWA et al., 2006).

Engraulidae eggs are easy to sort in an ichthyoplankton sample due to their ellipsoid shape, segmented yolk and, usually, absence of oil droplets (CIECHOMSKI, 1965; PHONLOR, 1984a). In the Southeastern Brazilian Bight, there are four egg types of Engraulidae: types A, B, C (*E. anchoita*) and D (NAKATANI, unpublished data).

These groups are distinguished by their morphometric characteristics according to two methods: measurements of the major and minor axes, followed by plotting histograms of frequency distribution (SIMPSON, 1965); and combinations of the two axes in the form of volume and eccentricity of the ellipsoid (PHONLOR, 1984a; KRAUS; BONECKER, 1994). The identification of type C as *E. anchoita* was possible because this is the only species of the family that spawns farthest from the coast, and reproduction studies were conducted in laboratory (CIECHOMSKI, 1965; PHONLOR, 1984a). The egg diameters were measured directly under a binocular microscope or stereomicroscope using micrometers, but this method is more susceptible to errors and is time consuming. Thus, to facilitate the identification of these eggs a method was developed using new and freely available software packages for digital analysis.

The egg samples used in the present study were collected using a bongo net in the Southeastern Brazilian Bight (23°S - 29°S) during the summer of each of the following years: 1975, 1976, 1990, 1991 and 1993 (Fig. I.1). The mesh sizes used in 1975 were 0.505 and 0.333 mm, in 1976 only 0.505 mm and, in the other years (1990, 1991 and 1993), only 0.333 mm. Eggs from 1975 sampled with the 0.505 mm net were used to create a discriminant analysis model, and all the other were used for validation.

All Engraulidae eggs were imaged using a digital camera attached to a stereomicroscope and measured using ImageJ software to automatically obtain measurements of major and minor axes, perimeter, circularity, aspect of the ellipse, and roundness. Volume and eccentricity were calculated following Vanzolini (1977). The analyses were done using the software packages MASS (VENABLES; RIPLEY, 2002), rrcov (TODOROV; FILZMOSER, 2009) and caret (KUHN et al., 2013), from the R software (R CORE TEAM, 2012).

To avoid autocorrelation problems, variables that were highly correlated with other variables (Pearson coefficient higher than 0.95) were excluded. For example, roundness and circularity were highly correlated with each other (0.97), so roundness was excluded. From all the measurements cited above, only volume, eccentricity and circularity were used in the analysis.

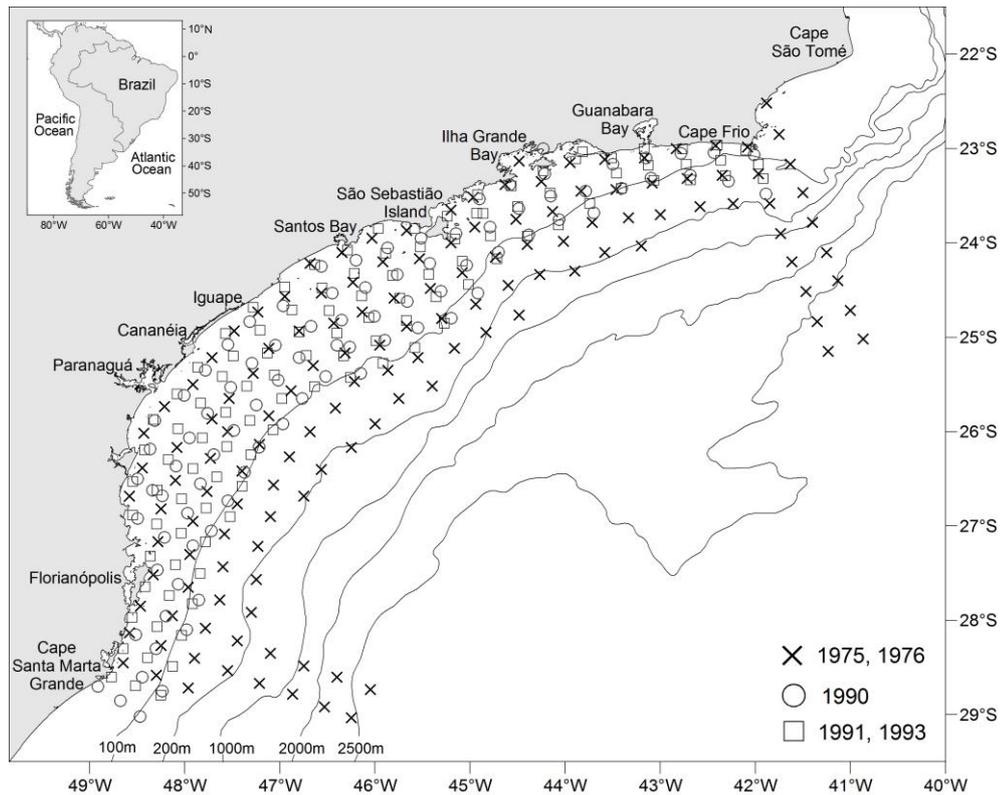


Fig. I.1. Location of the sample stations made in the Southeastern Brazilian Bight during the summer of the following years: 1975, 1976, 1990, 1991 and 1993.

Circles: 1975, 1976; crosses: 1990; squares: 1991, 1993.

Anchovy eggs collected in 1975 with the 0.505 mm mesh-net ($n=1,079$), which were previously separated into four groups based on their morphology by Nakatani (unpublished data) were significantly different from each other and they were discriminated by the measurements used (One Way MANOVA: Wilk's Lambda=0.04, $p<0.01$). To get a model that separates the groups, a Linear Discriminant Analysis (LDA) was done, quantifying the percentage separation achieved by each discriminant function of 86.8%, 13.2% and 0.02% for the first, second and third discriminant functions, respectively. Figure I.2 represents each observation in the space of the first two linear discriminant functions and the measurements that discriminated the groups. It is apparent that groups A and B are the ones with highest volumes, A and *E. anchoita* are more eccentric, and B and D are more circular. It was ensured that the within-group covariance matrices of the explanatory variables were homogeneous to perform LDA.

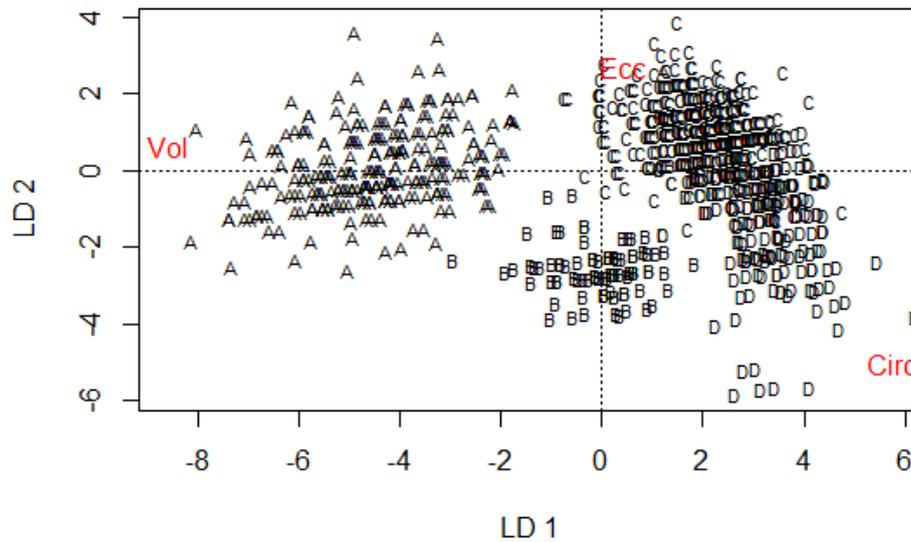


Fig. I. 2. Scatter plot of the different groups (A, B, C, D) of Engraulidae eggs (sampled in 1975) using the first two discriminant functions (LD1 and LD2) and the measurements that discriminated the groups (Vol = volume, Ecc = eccentricity, Circ = circularity, C= *Engraulis anchoita*).

The multivariate observations (each egg) were then classified by Linear Discrimination (LD). The accuracy of the prediction achieved was 92.5%, with groups A, B and *E. anchoita* showing positive and negative predictive value higher than 0.9. Group D had lower accuracy, with positive predictive value of 0.88 and negative predictive value of 0.94, sometimes being mistaken with *E. anchoita*. Of the 209 eggs identified as D by Nakatani (unpublished data), 53 were classified as *E. anchoita* by the model and one as B. On the other hand, of the 504 eggs previously classified as *E. anchoita*, 20 were reclassified as D and two as B (Table I.1).

Table I.1. Contingency table of the counts at each combination of factor levels, total number of Engraulidae eggs classified manually by Nakatani (unpublished data) and by Linear Discrimination prediction, the positive predictive value (PPV) and the negative predictive value (NPV).

	Types of eggs	Reference				Total	PPV	NPV
		A	B	C	D	Prediction		
Prediction	A	273	1	0	0	274	0.99	0.99
	B	0	88	2	1	91	0.97	0.99
	<i>E. anchoita</i>	2	1	482	53	538	0.90	0.96
	D	0	1	20	155	176	0.88	0.94
Total Reference		275	91	504	209	1079		

To obtain a more robust model, 81 eggs that were differently identified in the present study were reclassified by LD, resulting in a model with 100% accuracy and percentage of separation by each discriminant function of 84.7%, 15.3% and 0.03% for the first, second and third discriminant functions respectively.

To validate the new model, 1,266 Engraulidae eggs sampled with the 0.333 mm net from 1975 were reclassified, achieving 90.1% accuracy. Groups A and *E. anchoita* showed again high positive predictive value, of 0.96 and 0.92 respectively, and high negative predictive value, of 0.99 and 0.98. Conversely, groups B and D had high negative predictive value, of 0.98 and 0.91 respectively, but smaller positive predictive value, of 0.61 and 0.87, being mistaken with each other. Of 59 previously identified as B, 19 were reclassified as D and five as A; and of 260 previously identified as D, 72 were *E. anchoita* and 19 were B.

Also, 328 Engraulidae eggs identified previously as A and *E. anchoita* from 1976 were reclassified, and only five *E. anchoita* eggs were identified differently (98.5% accuracy). From 5,146 *E. anchoita* eggs sampled in 1990, 1991 and 1993, only 105 were differently identified (98.0% accuracy).

As the processing of ichthyoplankton samples is very time consuming, the new method can be used to better identify *E. anchoita* and three other types of Engraulidae

eggs from the study area, saving time and being more accurate. Unfortunately, eggs in general are largely undescribed, inclusive of the anchovies ones, so it was not possible to identify the three types of Engraulidae eggs discriminated. In the meantime, the method identifies with high accuracy eggs of *E. anchoita*, one of the most commonly represented species in ichthyoplankton surveys in the studied area, and a potentially important fishery resource for the Brazilian waters. Furthermore, the method presented can serve as a basis for other studies in regions where there are fewer species of Engraulidae.

II. COMPARISON OF TWO BONGO NET MESH SIZE EFFECTS ON THE ESTIMATION OF ABUNDANCE AND SIZE OF ENGRAULIDAE EGGS

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II.1. INTRODUCTION

An historical analysis of the development of quantitative zooplankton collecting systems, from the Hensen net toward four-dimensional biological oceanography, was presented by Wiebe and Benfield (2003). Even with sophisticated sampling systems available to quantify the abundance of planktonic organisms, for example acoustic and optical technologies, less sophisticated net-based sampling devices remain in widespread use. This reliance on simple nets is due to the relatively low expense of the sampling gear, the ease of deployment, and to maintain a standard protocol over a long time series (JOHNSON; FOGARTY, 2013).

Much attention has been focused recently on multi-decadal time-series of marine organisms (e.g. BRODEUR et al., 1996; MULLIN, 1998; NAGASAWA, 2001; STRATOUDAKIS et al., 2003; AUTH et al., 2011; TURNER et al., 2011). Few long time-series data are available, and many that exist are complicated by changes in methodology made over the years. The long-term time-series contain much valuable information if the results obtained with the different methods can be comparable or if calibration factors can be determined for different methods (REBSTOCK, 2002). For example, the California Cooperative Oceanic Fisheries Investigations (CalCOFI) program has sampled zooplankton off the west coast of North America since 1949, but in 1978 the 1m diameter ring net was replaced by a bongo net, with two 0.71m diameter nets on a single frame, so Rebstock (2002) determined whether the estimates of abundance of calanoid copepods collected with the ring and bongo net were comparable. For the Brazilian coast, the Biological Collection "Prof. E. F. Nonato" (ColBIO) of the Oceanographic Institute, University of São Paulo, Brazil, contains archived zooplankton samples from Southeastern Brazilian Bight since the 1960s, but the mesh size used over various years was not the same, what requires studies on the mesh-size effect when sampling different taxonomic groups to allow multi-decadal and multi-annual comparisons.

The ideal sampler for zooplankton is one whose selective characteristics are appropriate, qualitatively and quantitatively, for the problem under study (BARKLEY, 1972). Sampling programs and devices must provide accurate measurements of changes in abundance and levels of precision that permit the detection of variations in key parameters for a proper understanding of the process that influence population dynamics. It is important that the survey design provides adequate spatial and temporal resolution and that the correct sampling gear and mesh size is chosen to minimize sampling bias for the organisms under study (PEPIN; SHEARS, 1997).

A number of studies have documented comparisons of different sampling gears. Pepin and Shears (1997) compared the variability and capture efficiency of bongo nets and Tucker trawls in the collection of ichthyoplankton and other macrozooplankton, concluding that both samplers are efficient at catching a wide range of sizes of larval fish, with bongo nets being more effective for smaller larvae and the Tucker trawl for larger ones. The evaluation of catchability of fish eggs and larval fish with the 0.61 m bongo and the 1 m² Multiple Opening and Closing Net Environmental Sensing System (MOCNESS) was conducted by Johnson and Fogarty (2013), showing that overall catches of fish larvae and eggs were significantly higher for the MOCNESS, which filtered 3.5 times more seawater than the bongo; but bongo nets were more efficient in collecting some larvae taxa in the larger size classes. These authors reinforced the idea that the catchability of sampling gear of larval taxa is species-specific and advanced-stage-specific. In many cases, such as to sample effectively all sizes of leptocephali, two types of nets/trawls will likely be needed (MILLER et al., 2013).

An inter-comparison and evaluation of methods for sampling and determination of zooplankton distribution and biomass was conducted recently including 14 zooplankton sampling instruments: four multiple opening and closing net systems (1- and 10-m² MOCNESS, 1-m² BIONESS, 0.25-m² MultiNet), three high-speed samplers (LHPR, Gulf-V, CPR), one optical plankton counter (OPC), 4 vertically or obliquely hauled nets (WP-2.20- and 60-cm Bongo, 1-m Ring net (CalCOFI)), and two micronekton trawls (MIK ring net, young-fish trawl) (SKJOLDAL et al., 2013). The results of this study showed that different vertical, oblique, and multiple opening/closing net systems produced similar estimates of zooplankton when operated with comparable mesh sized nets, with the mesh size of the net the major influence on the biomass and species composition of the zooplankton community.

Focusing on ichthyoplankton collections, comparisons of different mesh sizes efficiency was documented by some authors (e.g. HOUDE; LOVDAL, 1984; JOHNSON; MORSE, 1994; SOMARAKIS et al., 1998). However, only a few of these studies included fish eggs in the comparison, analyzing the total of eggs sampled (e.g. CHUTE; TURNER, 2001; HERNANDEZ et al, 2011). The studies that compare the mesh size effects on sampling a specific egg family is usually done with Engraulidae, because they are easy to identify due to their ellipsoid shape, segmented yolk and, usually, absence of oil droplets (CIECHOMSKI, 1965; PHONLOR, 1984a). Somerton and Kobayashi (1989) compared the catch of Hawaii anchovy eggs in two plankton nets, one with 0.183 mm mesh size and another with 0.335 mm; MATSUURA and NAKATANI (1980) and SANCHEZ and CIECHOMSKI (1984) compared the catch of *Engraulis anchoita* Hubbs & Marini, 1935 eggs using a bongo net fitted with 0.333 and 0.505 mm mesh size nets, finding contradictory results.

Engraulids are small pelagic fish ecologically important as secondary consumers near the bottom of the food chain and essential prey to other fish, marine mammals and seabirds, at the top of the food chain, linking the bottom with the top (CASTELLO, 2007). Some Engraulidae species are also important fisheries resources (FAO, 2013). Along the southern Brazilian coast it is estimated that up to 135,000 tonnes of *E. anchoita* could be sustainably exploited (MADUREIRA et al., 2009), however its exploitation has just recently started in this area (CARVALHO; CASTELLO, 2013).

Due to the environmental and fisheries importance of the Engraulidae family, the importance of long time-series studies, the lack of information on the catchability of different Engraulidae eggs and the contradictory results of the two studies in the western South Atlantic coast, the main objective of the present study is to compare the bongo net mesh size effects on abundance and size of four types of Engraulidae eggs in the Southeastern Brazilian Bight. The null hypotheses are that the frequency of occurrence, the abundance and the size of the Engraulidae eggs types are the same, regardless of whether the 0.333 mm or the 0.505 mm mesh size net was used.

II.2. MATERIALS AND METHODS

Data collection and preparation

Ichthyoplankton samples were collected at 140 stations from November, 29th to December, 18th 1975; and at 32 stations from March, 8th to 15th 1982. All stations

were located in the Southeastern Brazilian Bight ($23^{\circ}\text{S} - 29^{\circ}\text{S}$) as shown in Figure II.1. The sampling procedure described by Smith and Richardson (1977) was followed, using a bongo net with a 0.61 m diameter mouth opening, equipped with paired cylindrical-conical 0.333 mm and 0.505 mm mesh size nets. To measure filtered water volume a flowmeter was attached at the center of mouth opening of each net. A depressor was applied to the net, which was towed obliquely, from the surface to maximum depth, not exceeding 200 m, and then back to the surface, maintaining a wire angle near 45° . In stations shallower than 60 m the bongo net was lowered twice close to the bottom to increase the volume of water filtered. Towing speed was of about 1.5 - 2 knots. All samples were preserved in 4% buffered formalin in seawater and archived in the Biological Collection "Prof. E. F. Nonato" (ColBIO) of the Oceanographic Institute, University of São Paulo, Brazil.

Engraulidae eggs were sorted and identified by their morphometric characteristics in four groups, type A, B, C (*Engraulis anchoita*) and D, as described by FAVERO et al. (2015a). Major and minor eggs axes were measured automatically through photographs using the program ImageJ. All Engraulidae eggs from each group were measured, except when the number of individuals for a given group exceeded 100. In these instances, 100 eggs were randomly selected for measurement.

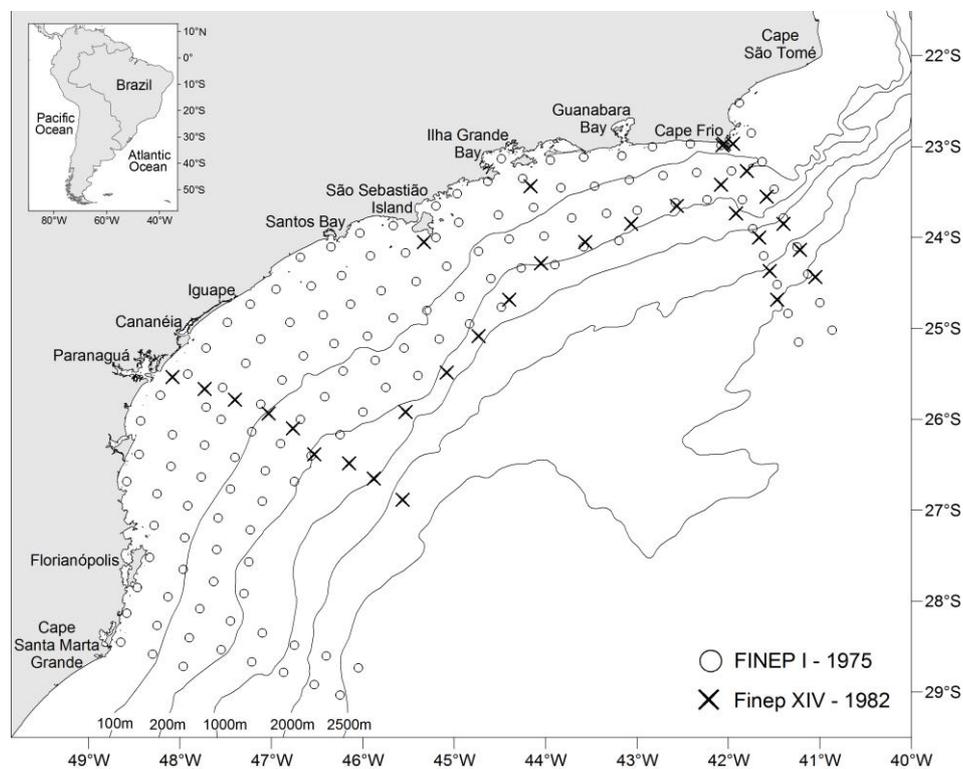


Fig II. 1. Location of the sample stations made in the Southeastern Brazilian Bight during the summer of 1975 and 1982.

Data analyses

Frequency of occurrence (FO) for each egg type in each mesh size net was calculated by $FO = (pi/p)*100$, where pi was the number of stations where the egg type “i” were present, and p was the total number of stations (GUILLE, 1970).

For data descriptions and analyses we standardized the number of Engraulidae eggs by the volume of seawater filtered (eggs/m³).

As our abundance data were not normally distributed and the samples are dependents, to verify an influence of the mesh size on volume of seawater filtered, volume of plankton and abundance of fish eggs, we performed non-parametric Wilcoxon paired-sample test (ZAR, 1999). Although a few studies compare the different mesh-size effect on the plankton catch using consecutive tows (SOMERTON; KOBAYASHI, 1989; CHUTE; TURNER, 2001; TSENG et al., 2011; MAKABE et al., 2012), factors such as tow depth, tow time and net speed, are difficult to control when separate tows are made and compared. As the bongo net was fitted with two different mesh size nets, all factors cited above were the same at each station, enabling a pairwise correlation of the data from the two nets, as done by Matsuura and Nakatani (1980), and Sanchez and Ciechomski (1984). In this case, a paired-sample test will be more powerful than the two-sample test (ZAR, 1999). This analysis was done only in the positive stations, meaning that only at the stations where Engraulidae eggs were collected in at least one mesh size net.

The difference in the egg axes size per mesh was test by the two sample Student’s t-test, with confidence level of 99% ($p<0.01$).

We did all the analysis described using R software (R Core Team, 2012).

II.3. RESULTS

A total of 306,711 fish eggs were collected, with 66,331 (21.63 %) from the Engraulidae family. *Engraulis anchoita* represented 92.89 % ($n=61,615$) of all Engraulidae eggs samples, followed by type D, with 3.26 % ($n=2,160$), type A with 2.95 % ($n=1,960$) and type B with 0.90 % ($n= 596$).

Of 172 oceanographic stations, only at two stations were there no fish eggs. Engraulidae eggs were sampled in 38.95 % of the stations. *Engraulis anchoita* was the Engraulidae with highest FO, of 31.40 %. Again, *E. anchoita* was followed by type D, with 16.28 %, type A with 8.14 % and type B with 7.56% FO.

As Engraulidae eggs were present at only five stations during the year of 1982, all the statistical analysis were calculated with both years together.

For type B eggs, FO was the same in both nets, however in six stations this type occurred only in the 0.505 mm mesh size and at the other six stations, only in the 0.333 mm. For type A, FO was higher in the 0.505 mm mesh net, but in two stations type A eggs occurred only in the 0.333 mm mesh net. For *E. anchoita* and type D eggs, FO was higher in the 0.333 mm mesh net, but they were present in the 0.505 mm mesh net, and were absent in the 0.333 mm mesh net in 10 and 5 stations respectively (Table II.1).

Table II.1. Frequency of occurrence (FO) in the 0.333 mm and the 0.505 mm mesh net; number of stations (N) where the eggs types occurred in just one of the nets.

	<u>FO (%)</u>		<u>N</u>	
	0.333 mm	0.505 mm	0.333 mm	0.505 mm
Eggs total	98.84	98.84		
Engraulidae eggs	27.91	27.91	19	19
Type A	2.91	6.98	2	9
Type B	4.07	4.07	6	6
<i>E. anchoita</i>	25.00	18.60	22	10
Type D	13.37	8.14	14	5

Even with the volume of water filtered being higher in the 0.505 mm mesh net, the median volume of plankton and the median abundance of total fish eggs and of Engraulidae eggs was higher in the 0.333 mm mesh size net. Within Engraulidae, the median abundances of eggs of types A and B estimated for the two mesh sizes were not significantly different, but the median abundance of *E. anchoita* and type D was higher in the 0.333 mm mesh net (Table II.2).

Table II.2. Amplitude and median values of sea water filtered volume (m³), plankton volume (ml/m³) and egg abundance (eggs/m³) collected with 0.333 mm and 0.505 mm mesh nets in the Southeastern Brazilian Bight during 1975 and 1982. P-values (P) of Wilcoxon paired-sample test and number of positive stations (N).

	<u>Amplitude</u>		<u>Median Value</u>		<u>P</u>	<u>N</u>
	0.333	0.505	0.333	0.505		
Filtered volume (m ³)	79.5 - 690.7	85.9 - 690.7	235.1	268.5	<0.01	172
Plankton volume (ml/m ³)	0.02 - 2.67	0.01 - 2.14	0.27	0.18	<0.01	172
Total eggs	0 - 330.7	0 - 128.6	0.97	0.67	<0.01	170
Abundance* (eggs/m ³)						
Total Engraulidae	0 - 54.9	0 - 117.2	0.09	0.01	<0.01	67
Type A	0 - 2.92	0 - 8.21	0	0.04	0.15	14
Type B	0 - 0.76	0 - 2.95	0	0.01	1	13
<i>Engraulis anchoita</i>	0 - 53.75	0 - 114.5	0.24	0	<0.01	54
Type D	0 - 7.11	0 - 2.70	0.03	0	<0.01	28

*Positive stations

The egg abundance differences between the 0.333 and 0.505 mm mesh net varied at each station for each egg type, showing no constant pattern (Fig. II. 2). Type A and type B showed low values of abundance differences between the 0.333 and 0.505

mm mesh net. For *E. anchoita*, there was only one station where the egg abundance sampled with the 0.505 mm mesh net was much higher than the egg abundance sampled with the 0.333 mm mesh, with an abundance difference of 114 eggs/m³; at the other stations, more *E. anchoita* eggs were sampled with the 0.333 mm mesh net, with difference values varying from almost zero to 41.08 eggs/m³. For type D eggs, at most stations the 0.333 mm mesh net sampled more eggs than the 0.505 mm mesh net, and only in a few cases was the 0.505 mm mesh net more efficient than the 0.333 one, but with low difference values, less than 0.08 eggs/m³.

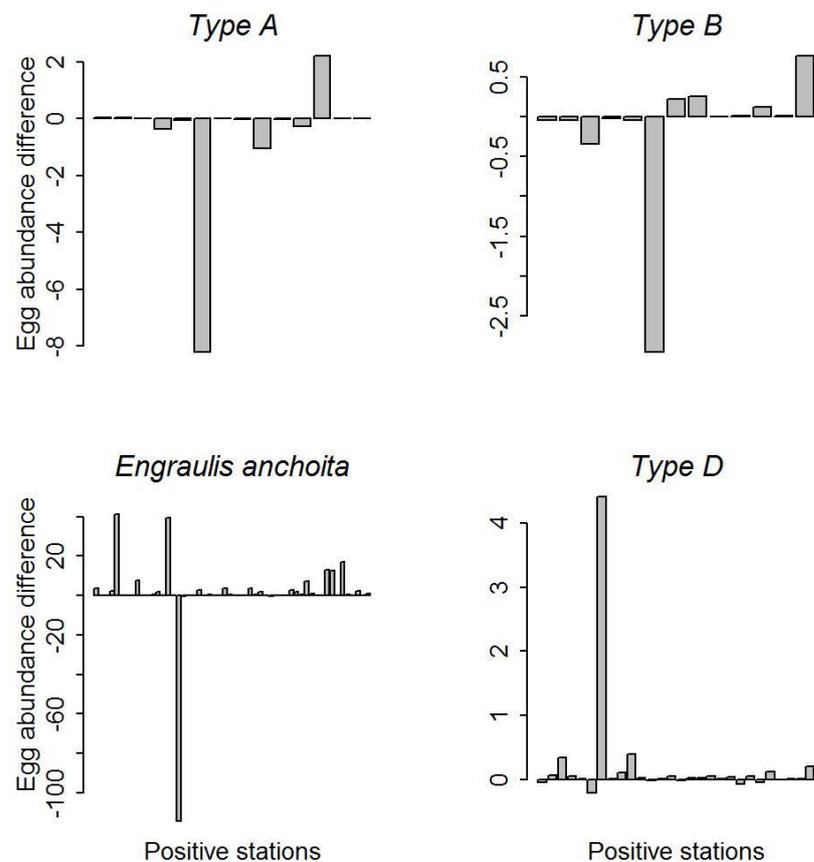


Fig. II. 2. Differences (eggs/m³) between the four types of Engraulidae egg abundances sampled by 0.333 mm and 0.505 mm mesh net in the Southeastern Brazilian Bight, during 1975 and 1982. Positive values mean that the abundance of eggs sampled by 0.333 mm mesh size was higher than that of the 500 mm, and negative values the opposite.

The stations that sampled more *E. anchoita* and type D eggs with the 0.505 mm net were not associated with the stations that presented higher abundance of plankton (Fig. II.3).

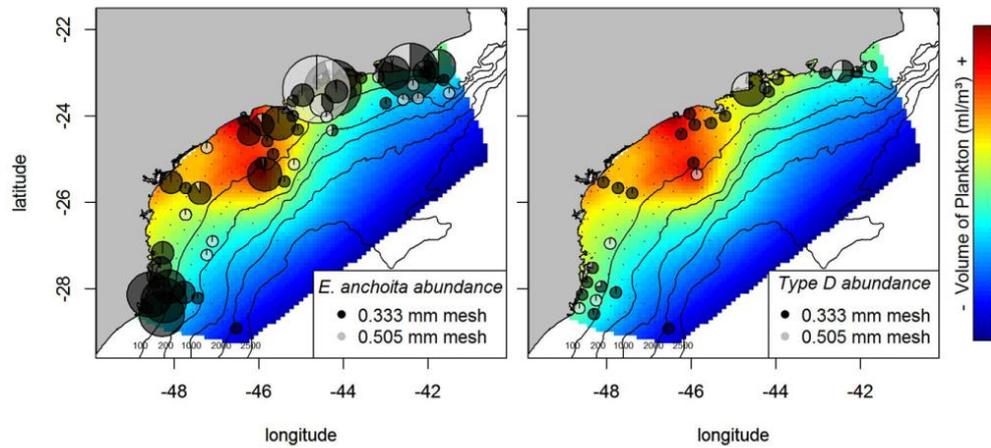


Fig. II.3. Bubble plot of the egg abundance logarithmized (represented by circles) of *E. anchoita* (left) and type D (right), and the proportion of eggs sampled with the 0.333 mm and the 0.505 mm mesh net at each station sampled in the Southeastern Brazilian Bight during 1975 and 1982 and the volume of plankton interpolated, obtained at the same stations during the same years. Small black dots represent the negative stations.

Mean value of the major axis was significantly different between mesh size nets for type A, B and *E. anchoita*; no significant difference was observed for type D. Type A and B eggs also had mean values of minor axis different for both nets, however no significant difference was observed for *E. anchoita* and type D eggs (Table II.3). It is possible to notice in Table II.3 the low size range of the egg axes in the four eggs types identified.

Table II.3. Number of eggs measured (N), size range, mean and standard deviations of the major and minor axes for each Engraulidae egg type sampled in Southeastern Brazilian Bight during 1975 and 1982 using a bongo net fitted with a 0.333 mm and 0.505 mm mesh net. P- values (P) of Student's t-test.

	<u>Major Axis (mm)</u>							<u>Minor Axis (mm)</u>				
	<u>N</u>		<u>Size Range</u>		<u>Mean ± sd</u>		<u>P</u>	<u>Size Range</u>		<u>Mean ± sd</u>		<u>P</u>
	0.333	0.505	0.333	0.505	0.333	0.505		0.333	0.505	0.333	0.505	
Type A	117	274	1.17 - 1.61	1.20 - 1.57	1.39 ± 0.07	1.36 ± 0.06	<0.01	0.62 - 0.82	0.60 - 0.80	0.72 ± 0.05	0.70 ± 0.04	<0.01
Type B	57	91	0.91 - 1.18	0.97 - 1.17	1.10 ± 0.05	1.06 ± 0.04	<0.01	0.63 - 0.78	0.62 - 0.73	0.71 ± 0.04	0.67 ± 0.02	<0.01
<i>E. anchoita</i>	898	559	0.85 - 1.21	0.83 - 1.23	1.03 ± 0.06	1.01 ± 0.07	<0.01	0.45 - 0.63	0.42 - 0.62	0.52 ± 0.03	0.52 ± 0.03	0.42
Type D	194	155	0.76 - 0.99	0.67 - 1.01	0.89 ± 0.05	0.88 ± 0.05	0.08	0.45 - 0.71	0.47 - 0.70	0.56 ± 0.05	0.55 ± 0.04	0.32

No correlations between egg abundance sampled with the 0.333 mm and the 0.505 mm mesh nets were observed for *E. anchoita* and type D eggs, making impossible the establishment of a correction factor (Fig. II.4).

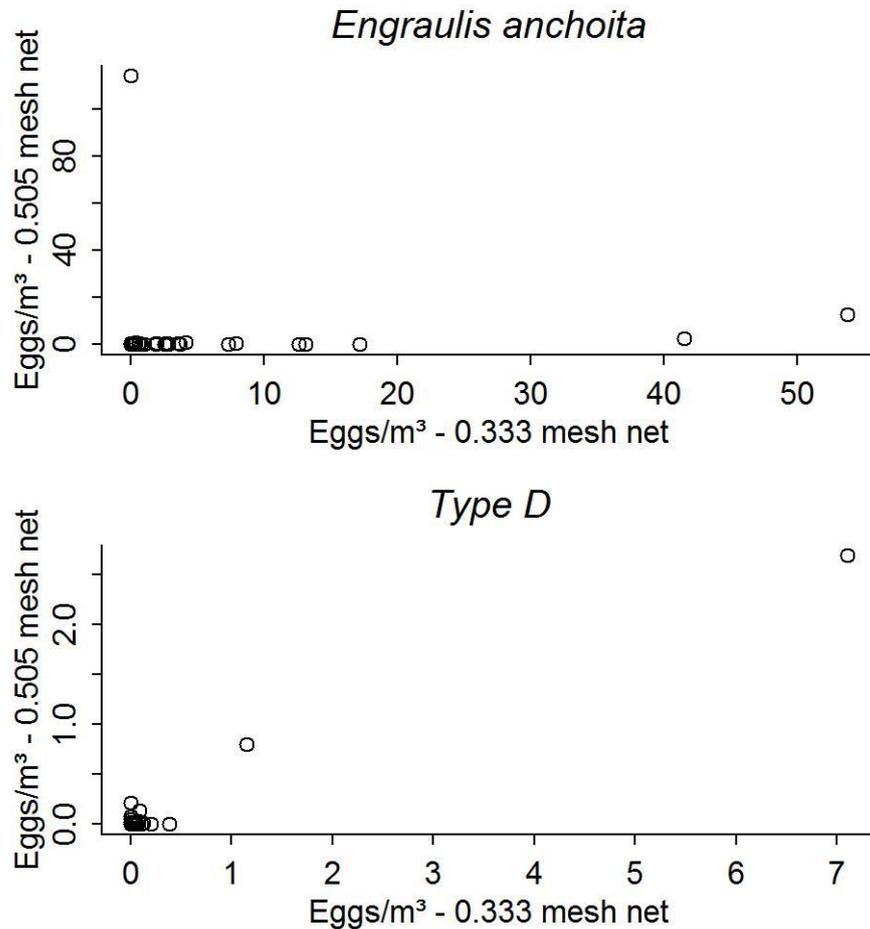


Fig. II.4. Plot of the abundance of eggs sampled with the 0.333 mm mesh net versus the abundance of eggs sampled with the 0.505 mm mesh net for *Engraulis anchoita* and type D eggs.

II.4. DISCUSSION

A bongo net towed at slow speed had been considered to be the best sampling gear for ichthyoplankton surveys (SMITH; RICHARDSON, 1977), but bongo nets do not prevent some problems inherent in the process of sampling plankton, such as clogging, avoidance and extrusion. Clogging is responsible for the reduced ratio between porosity and the filtering area due to the accumulation of particles in the mesh openings (VANNUCCI, 1968). The clogging rate is affected by: composition and density of suspended materials in the water, mesh size, the ratio of filtering area to mouth area and,

the form of the net (SMITH et al., 1968). Avoidance is size-dependent: as larvae grow and develop, they improve their ability to detect net pressure and their swimming ability, evading plankton nets (JOHNSON; FOGARTY, 2013). Extrusion represents the loss of ichthyoplankton through the net meshes by the combined effects of hydraulic pressure, morphology and size of the fish larvae and eggs, net mesh size and stability (VANNUCCI, 1968). Increasing the towing speed may improve the catch efficiency of larger zooplankton, but results in greater loss of small individuals by extrusion. In general, smaller fish eggs and larvae are extremely vulnerable to changes in filtration pressure and extrusion (SMITH; RICHARDSON, 1977). Furthermore, ichthyoplankton patchiness makes sampling of these organisms even more difficult. From all the problems cited, in the present study we can easily eliminate avoidance, as fish eggs cannot swim away from a net.

In a conical net, water flow is evenly distributed throughout its length and, if clogging occurs, it will be distributed evenly over the entire surface. In the combined cylindrical and cone designed net (as in the bongo net used) some of the water is naturally diverted by the cone model and is filtered through the cylinder (CURRIE, 1963). The porous cylindrical part, in front of the filtering cone, probably oscillates in response to eddies shed by the ring, and so cleans itself of accumulated organisms, reducing the rate of clogging (TRANTER; SMITH, 1968).

Fine-mesh nets, with comparable filtered areas, filter initially as well as larger mesh sizes, but clog more rapidly. In relatively clear water of Catalina Island, California, three different mesh size nets maintained high filtering efficiency for 18 minutes, after which the 0.101 mm mesh net clogged to 83 % efficiency and after 30 minutes the 0.201 mm mesh net clogged to 84% efficiency. The 0.333 mm mesh net had about 85% filtration efficiency during one hour of towing (SMITH et al., 1968). Somerton and Kobayashi (1989) studying Hawaii anchovy eggs (*Encrasicholina purpurea*), observed that the catch of the plankton net of a 0.183 mm mesh size was significantly less than in a 0.335 mm one, indicating that the smaller mesh net was likely clogged. Hernandez et al. (2011) found no differences in total fish larvae and egg densities between 0.333 mm and 0.200 mm mesh bongo nets. These authors observed that only two of 22 pairs of samples clogged, mainly because of the low volume of water filtered during their 1–18m oblique tows, of approximately 67 m³. In the present study, since we used conical- cylinder nets of 0.333 and 0.505 mm mesh, larger than the studies discussed above, our tows did not last more than 15 minutes, and the 0.505 mm mesh net did not sample more eggs at the stations with

higher plankton abundance, where the 0.333 mm net would clog, we can also disregard the clogging effect.

A number of studies have documented the effect of mesh size on the retention of larvae, with general conclusion that larger mesh sizes efficiently collect the late ontogenetic stages but underestimate the initial larval stages due to extrusion (e.g. HOUDE; LOVDAL, 1984; LESLIE; TIMMINS, 1989). In contrast, smaller-mesh nets collect smaller size classes of larvae, but are prone to clogging thus reducing their effectiveness in sampling larger size classes (SMITH et al., 1968; TRANTER; SMITH, 1968). In this regard, inappropriate mesh-size nets are one of the most important sources of sampling bias affecting organism size and development stage data (SMITH et al. 1968; COLTON et al. 1980). In our results, the eggs of each Engraulidae type exhibited low variations of the major and minor diameters and the 0.333 mm mesh net sampled type A and B eggs larger than the ones sampled in the 0.505 mm mesh net, the opposite of what was expected. For *E. anchoita* and type D eggs, which were more abundant in samples of the 0.333 mm mesh net, there were no size differences when the two nets were compared. Thus, the different mesh used had no egg size selectivity within each egg type, and our data presented no length-frequency bias.

Another reason why we cannot associate the quantitative difference for *E. anchoita* and type D eggs when comparing both nets with the egg size is because there were minor variations in axes varying from 0.45 to 0.71 mm, smaller than the 0.505 mm mesh aperture and the mesh diagonal. In our study we used nets that had diagonals of approximately 0.45 and 0.71 mm for the 0.333 and 0.505 mm nets, respectively. Smith et al. (1968) summarized the results of Saville (1958) and concluded that the maximum cross-section diameter of an organism must be wider than the mesh diagonal to be fully retained. Some authors considered this “Diagonal rule” too conservative (e.g. LENARZ, 1972; SANCHEZ; CIECHOMSKI, 1984; SOMARAKIS et al., 1998). The engraulid eggs, excluding type D, only had a major axis larger than the 0.71 mm diagonal and all eggs of *E. anchoita* and type D, and some eggs of types A and B had a minor axis smaller than 0.71 mm. Since we cannot confirm whether ellipsoid eggs were retained due to their minor or major axes, we believe that the “Diagonal rule” was not applicable in this study.

Patchiness of planktonic fish eggs and larvae is well described (e.g. MCGURK, 1986; MATSUURA; HEWITT, 1995; BRADBURY et al., 2003; MAYNOU et al., 2006), even for some species of the Engraulidae (HEWITT, 1981). The role of patchiness as a component of the sampling bias was studied by Wiebe and Holland (1968) and Wiebe

(1970; 1971). These studies emphasized the importance of the size analyses, distribution and density of zooplankton patches to understand the sampling bias. The large values of the abundance amplitude in the present study indicate patchiness in the occurrence of the Engraulidae eggs, but no conclusion about the characteristics of these patches can be done with our data. Further studies on the patch size, density and if it is possible for one bongo net to pass through the patch and the other not, or the possibility for one net to pass through a more aggregated part of the patch must be done to confirm if the differences in sampling Engraulidae eggs with different mesh nets is caused by patchiness. The patchiness effect could also explain why eggs of all types were sampled in the 0.505 mm mesh net but were absent in the 0.333 mm mesh net at some stations, and why at one station the 0.505 mm mesh net abundance of *E. anchoita* was much higher than the abundance sampled with the 0.333 mm mesh net, with difference of 114 eggs/m³. If the difference in mesh retention was the only factor involved, this would not happen.

As in our results, in Matsuura and Nakatani (1980) the 0.333 mm mesh net collected more *E. anchoita* eggs than the 0.505 mm mesh net in the Santos area of the southern Brazilian coast in November 1977. However, these authors only sampled five fixed stations and they did not evaluate the abundance difference at each station, or if there was any mesh effect on the egg size. On the other hand, Sanchez and Ciechomski (1984) analyzing *E. anchoita* eggs sampled in the Uruguay and Argentina coasts during the spring of 1981 did not observe any difference in the abundance obtained with the 0.333 and the 0.505 mm mesh net. According to Sanchez and Ciechomski (1984), the difference in the results was caused by the regional variation, as the minor axis of the *E. anchoita* eggs sampled in the Uruguay and Argentina coasts was larger than the ones sampled in the Santos area and also greater than the ones sampled in the present study. The dimension of the minor axis varied between 0.68 and 0.70 mm in the Uruguay and Argentina coasts (CIECHOMSKI, 1973); in the Brazilian southeast coast, the minor axis mean value observed by Matsuura and Nakatani (1980) was of 0.58 mm and, in the present study, of 0.52 mm. It is known that the Southeastern Brazilian Bight, excluding the Santa Marta Cape region, has one unit stock of *E. anchoita* and southern Brazil another one (CARVALHO; CASTELLO, 2013). The southern Brazil stock is the same one named as *bonaerense* off Argentina and Uruguay (CARVALHO; CASTELLO, 2013).

The extent to which clogging, avoidance and extrusion of fish larvae and eggs is a problem depends upon the objective of the study. Ecological studies of ichthyoplankton communities, e.g. dominant species and species composition, will be subject to some bias

from these problems, but this bias may not be serious. However, studies of the distribution and abundance of newly-hatched larvae or the estimate of mortality rates from length frequencies will incorporate serious bias if clogging, avoidance and extrusion losses are overlooked (JOHNSON; MORSE, 1994). As there were no differences in the abundance of Engraulidae eggs of types A and B in the two tested mesh sizes, the results obtained with each net were comparable. On the other hand, our results showed that the 0.333 mm mesh net tends to sample more *E. anchoita* and type D eggs than the 0.505 mm, but as no correlation was observed between these eggs captured in both nets, we could not calculate any correlation factor to facilitate the comparisons. As discussed above, we can discard avoidance, clogging and egg-size mesh-selectivity effect from affecting these results. This way, the difference of the eggs captured in each station seems to occur randomly caused by the egg patchiness or by other factors not analyzed.

In conclusion, the abundance of Engraulidae eggs sampled with bongo net of 0.333 mm and 0.505 mm mesh sizes in the Southeastern Brazilian Bight can be compared if they are types A or B, but if they are *E. anchoita* or type D, it is recommended some caution when comparing these results.

III. SEASONAL AND REGIONAL *ENGRAULIS ANCHOITA* EGG SIZE VARIATION IN THE SOUTHEASTERN BRAZILIAN BIGHT

III.1. INTRODUCTION

Most marine fish eggs are identified by their size, shape and pigment characters. Many newly-fertilized eggs are unpigmented, so the diameter is the most distinguishing feature (BAGENAL, 1971). New tools may be used for automatic identification of fish eggs based on their size and shape, as shown by Favero et al. (2015a). However, fish egg volume varies among different species and among populations of the same species due to temporal, biological, spatial and/or environmental reasons. In addition to fish egg identification, it is important to understand egg-size variation as larger eggs hatch into larger larvae conferring a higher survival potential on the resulting offspring (e.g. BLAXTER; HEMPEL, 1963; BLAXTER; HUNTER, 1982; MARTEINSDOTTIR; ABLE, 1992).

Engraulis anchoita Hubbs & Marini 1935 (Engraulidae: Clupeiformes) is a small pelagic fish, which is important due to its role as a secondary consumer near the bottom of the food chain, and as essential prey for other fishes, marine mammals and seabirds (CASTELLO, 2007). It is economically important to Argentine and Uruguayan fishery fleets (FAO, 2014), and exploitation of *E. anchoita* recently started in southern Brazil (CARVALHO; CASTELLO, 2013). This species is widely distributed in the continental shelf area of the southwest Atlantic, from Vitoria, Brazil (20°S) to Gulf San Jorge, Argentina (48°S) (CASTELLO, 2007). There are three distinct stocks: the *patagonic* (48°-41°S), the *bonaerense* (41°-27°S) and another one from 27° and 20°S (CARVALHO; CASTELLO, 2013). Different methodologies were used to identify three stocks: to distinguish the *patagonic* from the *bonaerense*, Hansen (1994) used growth rates, size-weight relationships and length at first maturation; Castello and Castello (2003) compared the larval growth and length at first feeding from the southeastern and southern Brazil; and Carvalho and Castello (2013) analyzed size and age composition and length-at-age data. Although the parameters used do not inform about genetic discreteness, they can be powerful tools for stock identification purposes (BEGG, 2004).

No study dedicated specifically to *E. anchoita* fish egg size variation in the Southeastern Brazilian Bight has been conducted to date. A few studies on this subject have focused on the south coast of Brazil, and the coasts of Uruguay and Argentina

(CIECHOMSKI, 1973; PHONLOR, 1984a), which cover only the *bonaerense* stock and not the stock most covered in the present study. Thus, the objective of this study was to determine seasonal and regional variability in the size of *E. anchoita* eggs in the Southeastern Brazilian Bight (SBB), considering the factors that may be causing such variation.

III.2. MATERIALS AND METHODS

Study area

The Brazil Current (BC) flows southward along the continental slope of the Southeastern Brazilian Bight (SBB) slope (SILVEIRA et al., 2000) transporting warm and salty Tropical Water (TW) in the upper mixed layer and cold South Atlantic Central Water (SACW) within the pycnocline. An additional water mass exists in the neritic zone, the warm Coastal Water (CW), with lower salinity than the TW (CASTRO FILHO; MIRANDA, 1998). Except for some coastal areas under the influence of waters outflowing from large embayments such as Guanabara Bay in Rio de Janeiro state, oligotrophic conditions prevail in the studied area due to the TW dominance in the upper layers, with oceanic nutrients trapped at the SACW (LOPES et al, 2006).

The intermittent coastal upwelling off Cape Frio is an important process bringing the nutrient-rich SACW into the euphotic zone (VALENTIN et al., 1987). SACW intrusions vary seasonally, advancing toward the coast during spring and summer and then retreating toward the shelf break in autumn and winter (CERDA; CASTRO, 2014).

Another physical process that fertilizes the euphotic zone occurs during the winter, when subantarctic and the Plata River Plume waters are driven northward by southerly winds from the continental shelf of Argentina and Uruguay into most of the inner shelf in the south of Brazil and even the SBB (BRANDINI 1990; STEVENSON et al., 1998; PIOLA et al., 2000; GAETA; BRANDINI, 2006; PIOLA et al., 2008a; PIOLA et al., 2008b).

For the regional variation, the studied area comprised the entire SBB, between Cape São Tomé – Rio de Janeiro state and Cape Santa Marta Grande – Santa Catarina state (roughly 22°S and 28°S). For the seasonal variation, the study was carried in the northern part of the SBB, off Cape São Tomé (22°S) in Rio de Janeiro state to São Sebastião island (24°S), in São Paulo state (Fig. III.1)

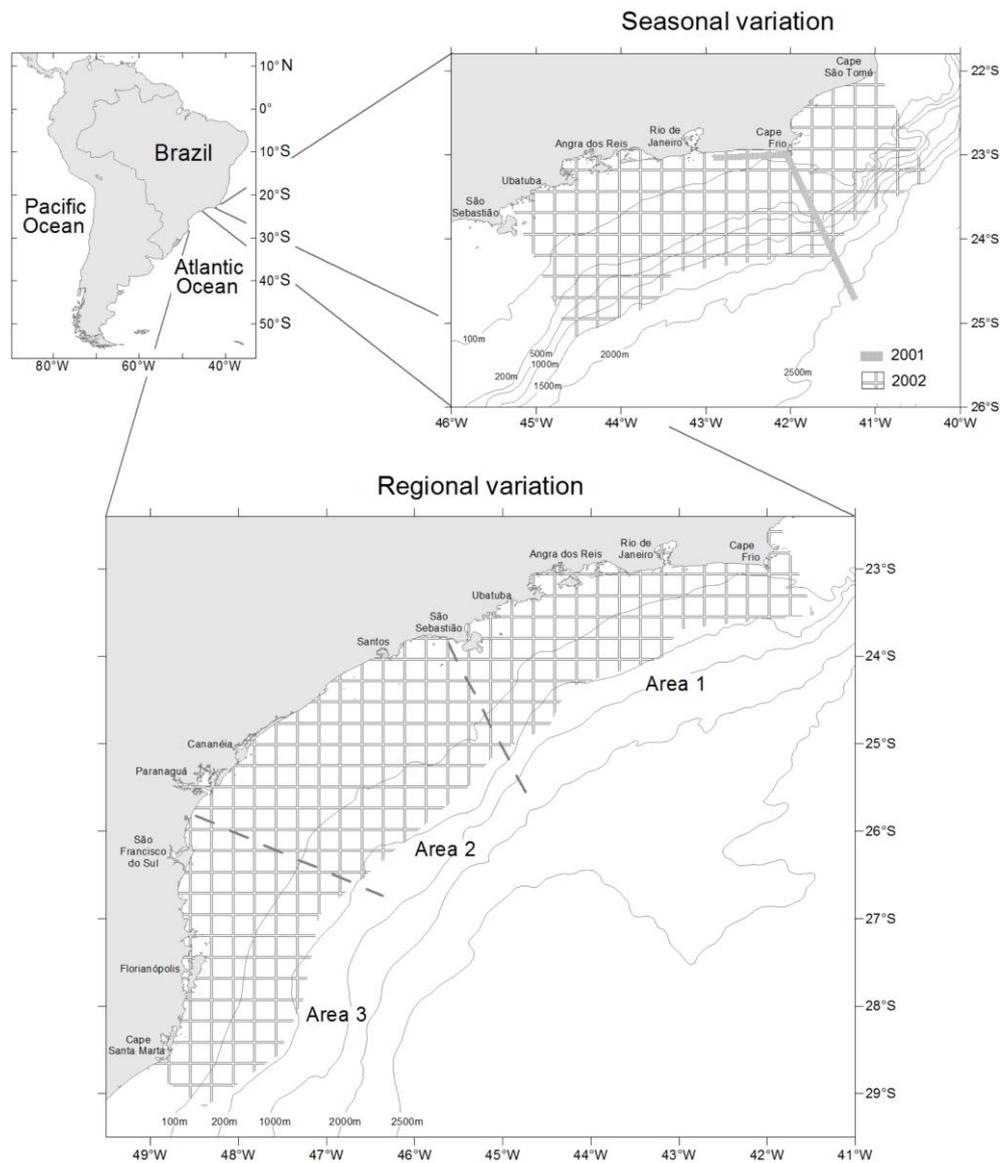


Fig. III.1. Studied area locations.

In situ data

Biotic and abiotic data analyzed for the seasonal study were obtained from four oceanographic cruises that were carried out during summer and winter of 2001 and 2002. During summer and winter of 2001 only one transect was performed at the Cape Frio region, with some oceanographic stations being sampled twice. Summer of 2002 covered 14 transects and winter of 2002 included 13 transects (Table III.1; Fig. III.1).

For the regional study, data were obtained during the summers of five years, as shown in Table III.1. The SBB was divided into three areas: Area 1 from Cape São Tomé to São Sebastião Island, Area 2 from São Sebastião to Paranaguá Bay and Area 3 from Paranaguá Bay to Cape Santa Marta Grande (Fig. III.1). These areas were defined based

on Carvalho and Castello (2013) *E. anchoita* stock identification (Area 3) and on the oceanographic conditions described by Miranda and Katsuragawa (1991) and Mahiques et al. (2004) (Area 1 and 2). We also defined areas based on the spawning map obtained in Chapter IV (Fig. IV.5) so the divisions would not separate any important spawning area.

Table III.1 - Further information about each oceanographic cruise used for this study. N = number of sampling stations.

	Oceanographic Cruise	Date		N
		Initial	End	
Seasonal	Deproas I	2/7/01	2/13/01	19
	Deproas II	7/12/01	7/19/01	17
	Deproas III	1/5/02	1/24/02	72
	Deproas IV	8/3/02	8/21/02	66
Regional	FINEP I	11/29/75	12/18/75	140
	EPM Sardinha	1/10/88	1/30/88	78
	V. Hensen/JOPS	12/28/90	1/11/91	89
	Sardinha I	12/8/91	12/18/91	110
	Sardinha II	1/9/93	1/18/93	108

Temperature and salinity data were obtained from Nansen bottles and reversing thermometers in 1975 and 1988. In the other years, a CTD (conductivity-temperature depth profiler) provided this information.

Ichthyoplankton were sampled as described by Smith and Richardson (1977), using a bongo net with a 0.61 m diameter mouth opening, equipped with paired cylindrical-conical 0.333 mm and 0.505 mm mesh-size nets. To measure the volume of filtered water a flowmeter was attached at the center of mouth opening of each net. A depressor was applied to the net, which was towed obliquely from the surface to maximum depth, not exceeding 200 m. At stations shallower than 60 m the bongo net was lowered twice close to the bottom to increase the volume of water filtered. Towing speed was of about 1.5 - 2 knots. For the present study, we analyzed the samples from the 0.333 mm mesh size net that were preserved aboard in 4% buffered formalin in seawater and archived in the Biological Collection "Prof. E. F. Nonato" (ColBIO) of the Oceanographic Institute, University of São Paulo, Brazil.

Engraulidae eggs were sorted and their major and minor axes were measured through photographs using the program ImageJ. *Engraulis anchoita* eggs were identified

by their morphometric characteristics according to Favero et al. (2015a, Chapter I). Only a sub-sample of about 100 eggs per oceanographic station was measured.

Satellite data

Sea surface temperature (SST) and surface chlorophyll-a concentration (SCC) were obtained from the Moderate Resolution Imaging Spectroradiometer (MODIS) on board the Aqua satellite. These products correspond to level-3 mapped images, on a global scale, with cylindrical equidistant projection, and within a space resolution of 9 km x 9 km, processed by the NASA's Goddard Space Flight Center and distributed at NASA's Ocean Color Web. We have used monthly means to represent the period of the cruises done during summer and winter of 2001 and 2002 (Table III. 1). The idea of using monthly means instead of the exact period of the cruises is supported by including a time-lag between a few processes before a given cruise period (e.g. the time-lag between the availability of nutrients and a primary production for a given area).

Data analyses

Egg abundance was calculated as $N = x.d.V^{-1}$ (TANAKA, 1973), where N is the egg abundance (number of eggs/m² at each sampling station), d is the maximum depth sampled in meters, x is the number of eggs sampled, and V is the volume of water filtered in cubic meters. The mean abundance was calculated for all the sampling stations, not just for the positive ones. The calculation of the frequency of eggs occurrence (FO) was based on Guille (1970).

Analysis of variance (one-way ANOVA) was used to test the differences in the egg major and minor axes, egg volumes, temperature and salinity (at 10 meters depth), when compared per season (winter and summer) and per area in each year. To find means that were significantly different from each other, a Tukey's HSD (honest significant difference) test was performed. TSI (Temperature, Salinity and Ichthyoplankton) diagrams were plotted using temperature and salinity measured at 10 meters and *E. anchoita* presence. We used the 10 meter measurement for the abiotic data because the anchovy eggs mainly occur close to the surface, in the upper 20 m (TANAKA, 1992; SABATÉS, 2008). The analyses described were done using R software (R CORE TEAM, 2015).

III.3. RESULTS

Seasonal variation

Oceanographic conditions

Mean seawater temperature measured at 10 m varied significantly within each period analyzed ($F=8.732$, $df=3$, $p<0.01$). Summer temperatures were higher than winter in both years, but the lowest values were measured during summer (Table III. 2), due to the SACW intrusion that occurred in both years (GOÇALO et al., 2011; ARAÚJO, 2012). Another SACW intrusion indicator was the standard deviation value, which was highest during the summer in both years. Mean salinity at 10 meters also varied significantly within the periods analyzed ($F=15.13$, $df=3$, $p<0.01$), with the summer values being higher than winter values in both years (Table III. 2).

Table III. 2. Mean, standard deviation (sd) and the range of the temperature (°C) and salinity measured at 10 meters depth during summer and winter of 2001 and 2002 in the northern area of the Southeastern Brazilian Bight.

<u>Period</u>	<u>Temperature</u>		<u>Salinity</u>	
	Mean \pm sd	Range	Mean \pm sd	Range
Summer 01	24.65 \pm 3.18	14.70 - 27.65	36.83 \pm 0.42	35.73 - 37.48
Winter 01	22.77 \pm 0.62	22.17 - 24.72	36.04 \pm 0.53	35.14 - 37.14
Summer 02	23.79 \pm 2.86	14.88 - 27.08	36.08 \pm 0.69	34.67 - 37.01
Winter 02	22.32 \pm 1.21	16.50 - 24.44	35.58 \pm 1.04	33.16 - 37.37

SST fields showed larger thermal gradients in the winter due to the differences between the warmer BC and colder coastal waters coming from the south. During the summer of both years this colder coastal water coming from the south was not noticed (Fig.III. 2).

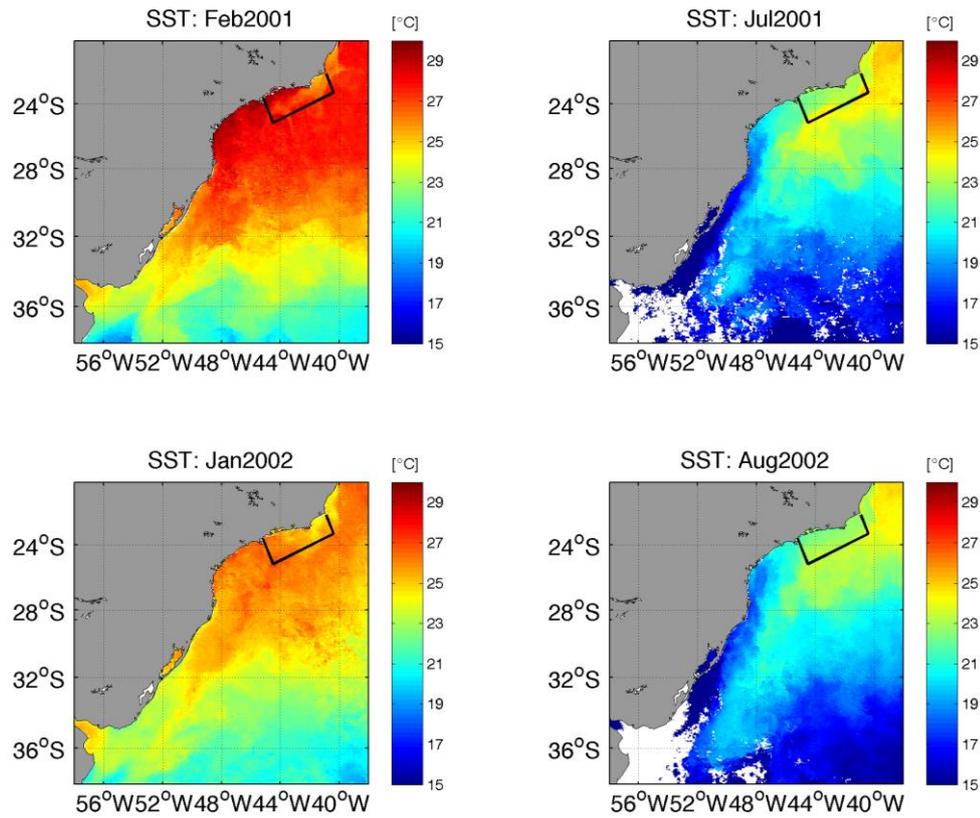


Fig. III. 2. Horizontal distribution of the surface temperature ($^{\circ}\text{C}$) during the summer (left) and winter (right) of 2001 (upper) and 2002 (lower) in the Brazilian, Uruguayan and part of the Argentine coasts. The black line highlights the studied area in 2002.

In terms of the surface chlorophyll-a horizontal distribution, there was a high concentration in the Plata River estuarine and coastal area (around 36°S), but during the winter of both years these high-concentration of chlorophyll-a spread into the southern part of the SBB, reaching around 28°S (Fig. III. 3).

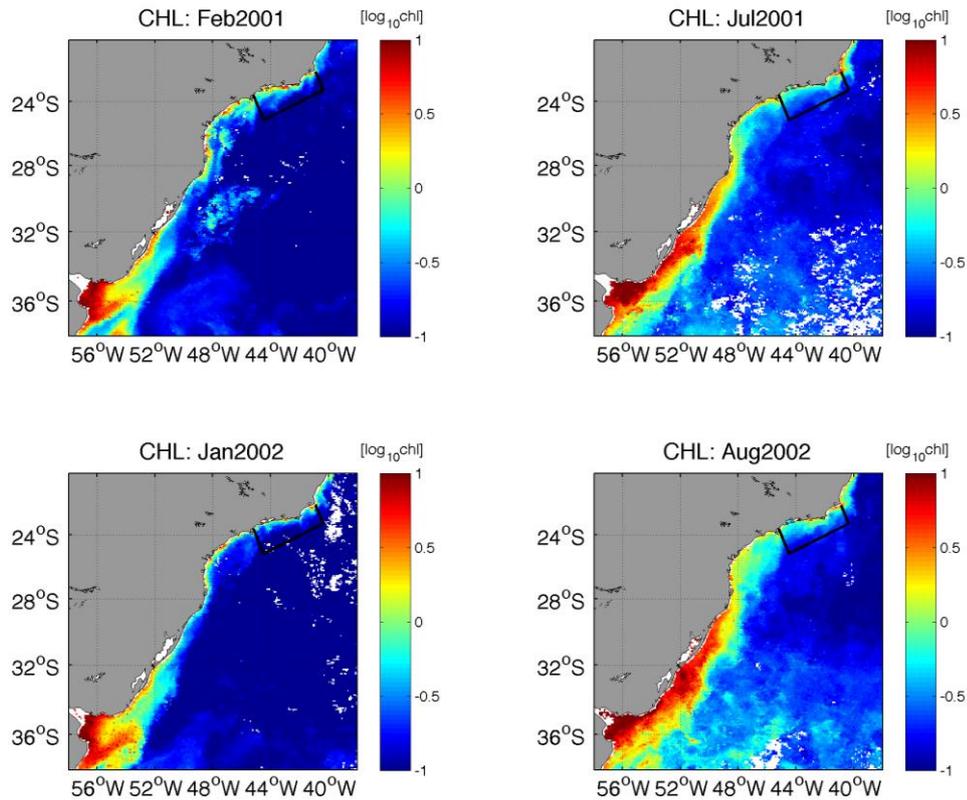


Fig. III. 3. Horizontal distribution of the surface chlorophyll-a concentrations during the summer (left) and winter (right) of 2001 (upper) and 2002 (lower) in the Brazilian, Uruguayan and part of the Argentine coasts. The black line highlights the studied area in 2002.

Further information about water column stratification and water mass intrusions for the same area and period studied, was described by Goçalo et al. (2011) and Namiki (2013).

Engraulis anchoita eggs

A total of 34,996 eggs were sampled, with 10,710 of the Engraulidae family (30.6%). *Engraulis anchoita* represented 98.6% of the Engraulidae eggs sampled (n=10,489) and were more abundant during the summers of both years (Table III. 3).

Table III. 3. Number of *Engraulis anchoita* eggs sampled (N), frequency of occurrence (FO) and mean abundance and standard deviation (eggs/m²) during summer and winter of 2001 and 2002 in the northern area of the Southeastern Brazilian Bight.

Period	N	FO (%)	Abundance (eggs/m ²)	
			Mean	sd
Summer01	99	4.3	1.9	9.3
Winter01	56	27.0	0.7	1.5
Summer02	9904	27.8	32.8	148.8
Winter02	430	18.2	1.4	5.5

During the summer of 2001, on the only transect done, eggs occurred at only one station close to the coast. During the winter of 2001 eggs were sampled at seven stations, all close to the coast (Fig. III. 4 upper).

During the summer of 2002, *E. anchoita* eggs were more abundant at Cape Frio and Cape São Tomé, in the north of the sampled area. On the other hand, during the winter, these eggs were much less abundant, and they had higher occurrence in the southern part of the sampled area (Fig. III. 4 lower).

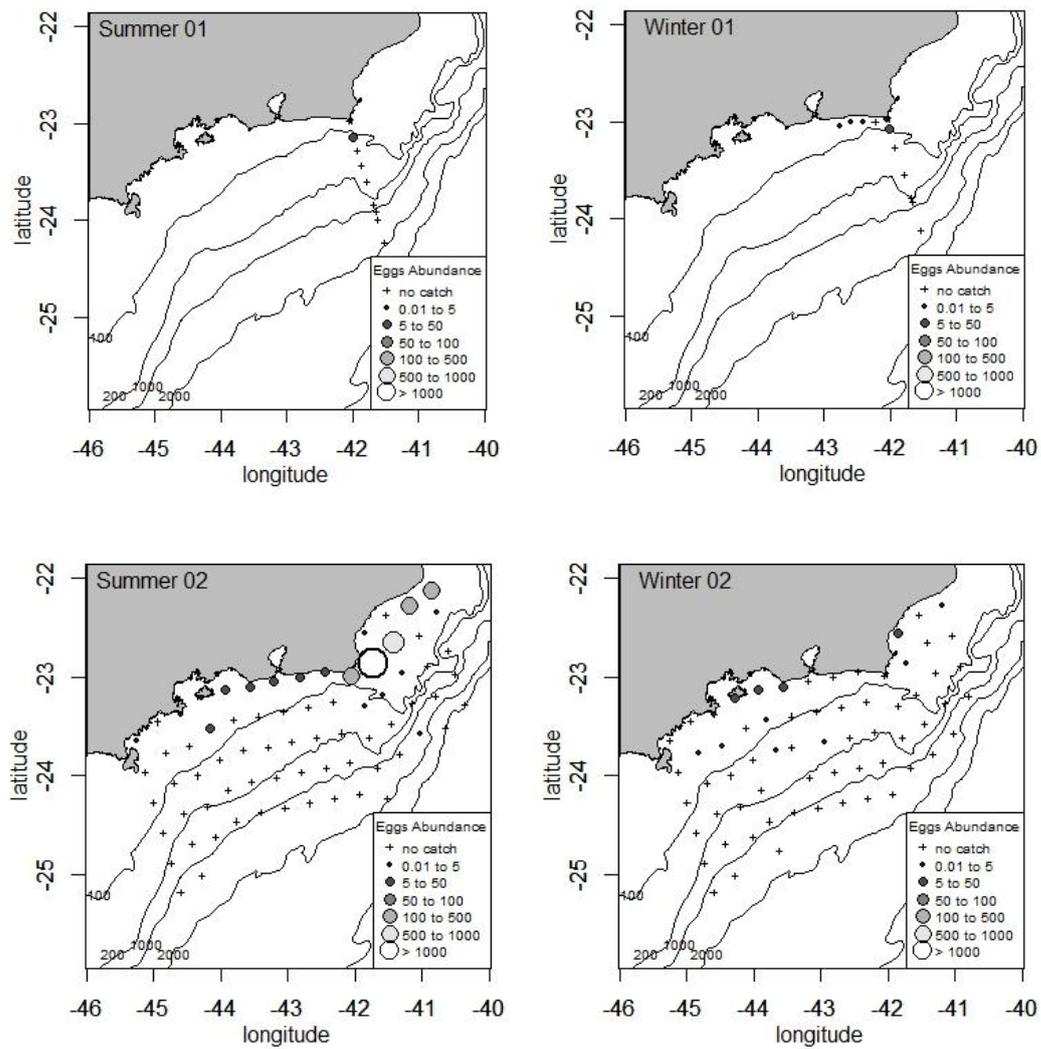


Fig. III. 4. Spatial distribution of *Engraulis anchoita* egg abundance (eggs/m²) sampled during summer and winter of 2001 (upper) and 2002 (lower).

TSI diagrams showed that *E. anchoita* eggs encountered large variations of temperature and salinity, being sampled in a range of temperature and salinity that characterized the three water masses that occurred in the studied area. Low presence of eggs was found in waters saltier than 36.5 (Fig. III. 5).

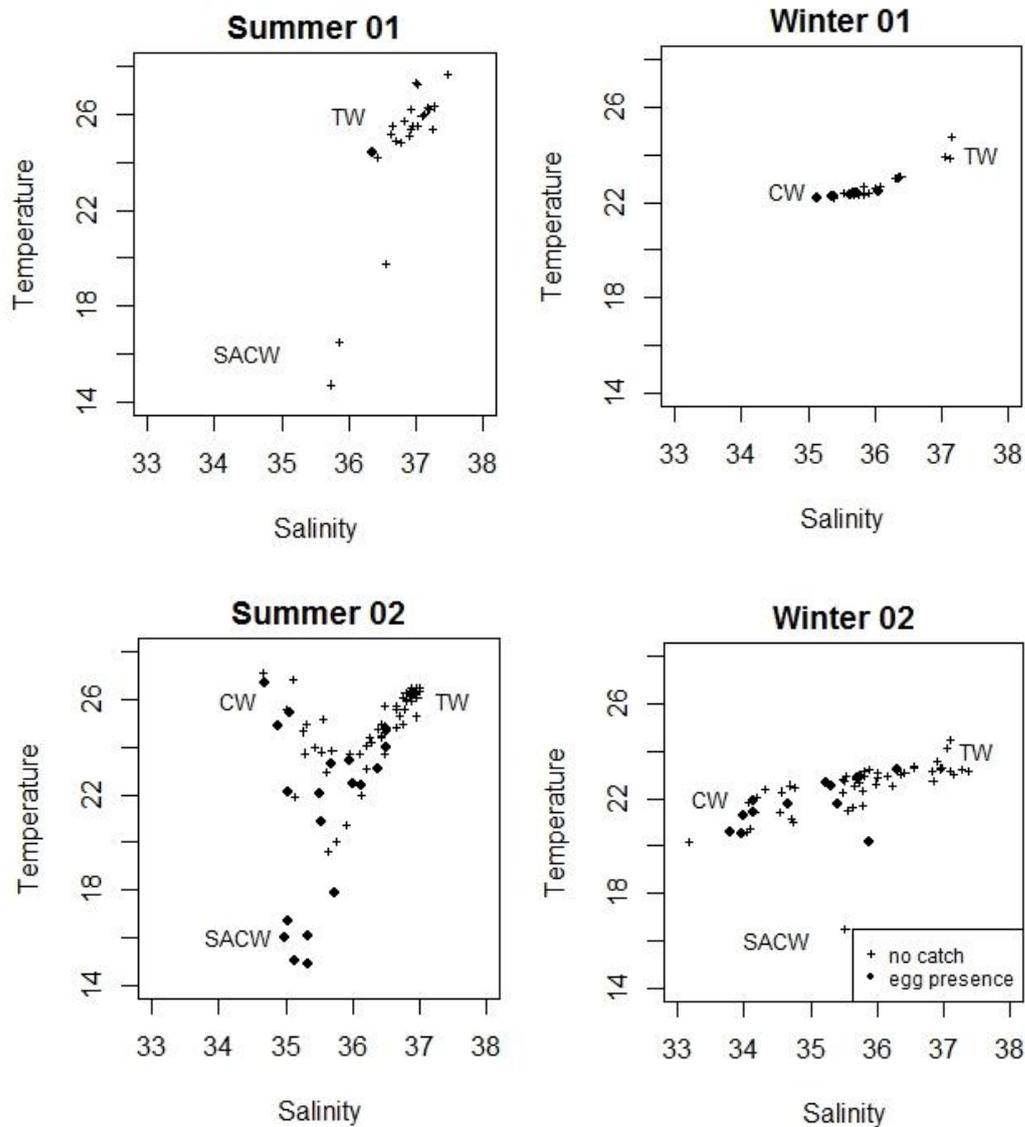


Fig. III. 5. Temperature, salinity and ichthyoplankton (TSI) diagrams for summer and winter of 2001 and 2002. Temperature and salinity at 10 m depth; CW = Coastal Water, TW = Tropical Water, SACW = South Atlantic Coastal Water.

Of the total number of *E. anchoita* eggs that were sampled, 2,794 were measured (Table III. 4). Volume, major axis and minor axis mean values were significantly different within the periods analyzed (Volume: $F=415.4$, $df=3$, $p<0.01$; Major axis: $F=133.2$, $df=3$, $p<0.01$; Minor axis: $F=429.8$, $df=3$, $p<0.01$). *A posteriori* Tukey's tests showed that only the mean values of winter were not significantly different. Eggs were larger in winter than in summer (Table III. 4).

Table III. 4. Mean and standard deviation (sd) values of volume (mm³), major and minor axes (mm) of *Engraulis anchoita* eggs collected during summer and winter of 2001 and 2002 in the northern area of the Southeastern Brazilian Bight. N = number of eggs measured.

Period	<u>Volume</u> (mm ³)		<u>Major axis</u> (mm)		<u>Minor axis</u> (mm)		N
	mean	sd	mean	sd	mean	sd	
Summer01	0.142	0.015	1.031	0.047	0.512	0.026	99
Winter01	0.206	0.018	1.138	0.047	0.587	0.022	55
Summer02	0.177	0.020	1.106	0.053	0.552	0.023	2211
Winter02	0.206	0.021	1.140	0.052	0.587	0.025	429

Regional variation

Oceanographic conditions

Temperature and salinity varied within the three areas analyzed during each year, except in January 1988, when no significant difference in salinity was found. During January of 1988 there was a weak SACW intrusion in Cape Frio (located in Area 1), represented by the lowest standard deviation in temperature calculated for this area; on the other hand, at Cape Santa Marta (located in Area 3) there was a strong SACW intrusion (highest standard deviation for this area). Area 1 was usually the coldest and saltiest area, due to the SACW stronger intrusions in this area. Temperature and salinity values were not significantly different between Areas 2 and 3 (Table III. 5).

Table III. 5: Mean and standard deviation values of temperature (Temp. °C), salinity (Sal.), ANOVA F and p values (*P*) and Tukey *post-hoc* test comparing the three areas during the years studied. N is the number of oceanographic station considered.

		<u>Area</u>			<u>ANOVA</u>		<u>Tukey post- hoc</u>		
		1	2	3	F	P	2x1	3x1	3x2
Dec 75	N	53	44	43					
	Temp.	22.06 ± 2.17	23.76 ± 0.97	23.47 ± 0.87	17.72	<0.01	0	0	0.65
	Sal.	35.99 ± 0.67	35.30 ± 0.76	35.49 ± 0.94	9.76	0	0	0	0.51
Jan 88	N	24	35	19					
	Temp.	24.16 ± 1.37	26.40 ± 1.34	25.40 ± 2.93	10.41	0	0	0.08	0.15
	Sal.	35.41 ± 0.33	35.22 ± 0.60	35.14 ± 0.73	1.27	0.29			
Dec 90	N	25	40	24					
	Temp.	22.71 ± 1.92	24.81 ± 0.56	24.12 ± 2.78	10.44	<0.01	0	0.02	0.31
	Sal.	35.95 ± 0.63	34.84 ± 0.63	34.74 ± 0.86	24.37	<0.01	0	0	0.86
Dec 91	N	33	48	29					
	Temp.	20.34 ± 2.94	24.47 ± 1.91	24.15 ± 1.86	36.11	<0.01	0	0	0.81
	Sal.	36.25 ± 0.70	35.49 ± 0.68	35.54 ± 0.52	24.11	<0.01	0	0	0.06
Jan 93	N	32	47	29					
	Temp.	21.33 ± 3.33	25.13 ± 1.79	24.55 ± 2.82	21.11	<0.01	0	0	0.62
	Sal.	36.01 ± 0.55	35.12 ± 0.59	34.91 ± 0.47	36	<0.01	0	0	0.25

Engraulis anchoita eggs

The horizontal distribution and interannual variability of egg abundance are presented in Chapter V. TSI diagrams showed that *E. anchoita* eggs were sampled in a wide range of temperature and salinity in each area (Fig. III. 6).

A total of 8,809 *E. anchoita* eggs were measured for this study. The number of eggs analyzed by area and year are presented in Table III. 6. Comparing only Areas 1 and 3, it was observed that, in general, eggs in Area 3 were larger than eggs in Area 1, with the exception of January 1988 when the opposite occurred. No size pattern was observed for eggs sampled in Area 2; egg sizes from this area were usually between those of Areas 1 and 3, with no significant differences compared to other areas. During December of 1991 and January of 1993 eggs from Area 2 exhibited the largest sizes (Table III. 6).

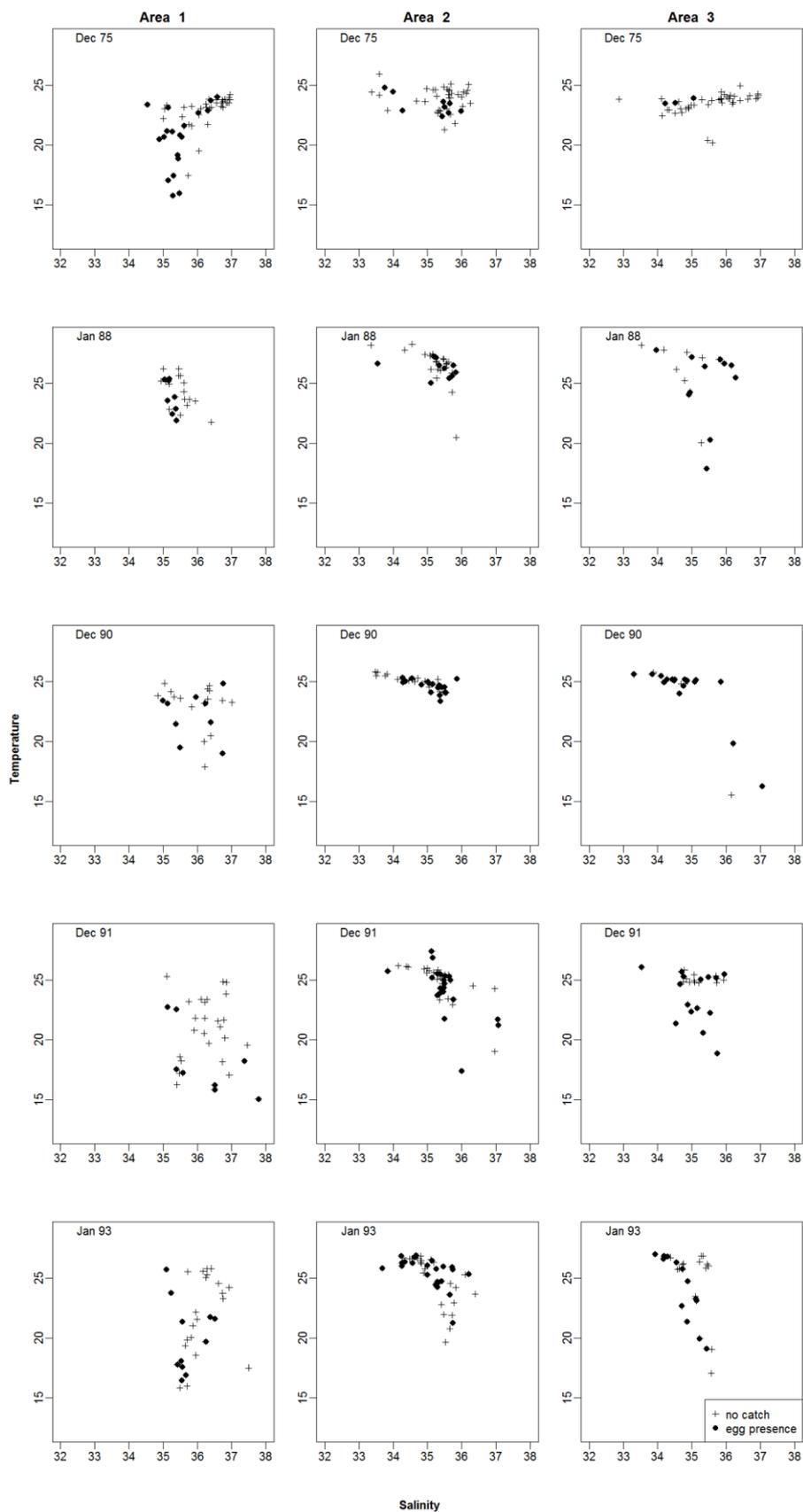


Fig. III. 6. Temperature, salinity and ichthyoplankton (TSI) diagrams for the three areas sampled in five different years.

Table III. 6: Mean and standard deviation values of the major axis (mm), minor axis (mm) and volume (mm³), ANOVA F and p values (*P*) and Tukey *post-hoc* test comparing the three areas during the years studied. N is the number of eggs measured.

		Area			ANOVA		<i>Tukey post-hoc</i>		
		1	2	3	F	<i>P</i>	2x1	3x1	3x2
Dec 75	N	437	139	322					
	Major Axis	1.022 ± 0.06	1.021 ± 0.06	1.037 ± 0.05	7.58	<0.01	0.99	0	0.01
	Minor Axis	0.523 ± 0.03	0.515 ± 0.02	0.533 ± 0.03	26.36	<0.01	0.01	0	0
	Volume	0.147 ± 0.02	0.142 ± 0.02	0.155 ± 0.02	22.62	<0.01	0.06	0	0
Jan 88	N	532	1123	398					
	Major Axis	1.111 ± 0.06	1.066 ± 0.05	1.077 ± 0.05	124.1	<0.01	0	0	0
	Minor Axis	0.548 ± 0.03	0.551 ± 0.03	0.536 ± 0.03	32.57	<0.01	0.19	0	0
	Volume	0.176 ± 0.02	0.171 ± 0.03	0.163 ± 0.03	33.41	<0.01	0	0	0
Dec 90	N	249	400	651					
	Major Axis	1.096 ± 0.06	1.114 ± 0.05	1.121 ± 0.05	18.33	<0.01	0	0	0.09
	Minor Axis	0.553 ± 0.03	0.560 ± 0.03	0.566 ± 0.03	19.13	<0.01	0.01	0	0
	Volume	0.177 ± 0.03	0.184 ± 0.03	0.189 ± 0.02	22.97	<0.01	0	0	0
Dec 91	N	132	544	557					
	Major Axis	1.052 ± 0.05	1.044 ± 0.06	1.043 ± 0.06	1.335	0.26			
	Minor Axis	0.511 ± 0.03	0.534 ± 0.03	0.526 ± 0.03	30.68	<0.01	0	0	0
	Volume	0.145 ± 0.02	0.157 ± 0.03	0.152 ± 0.02	16.77	<0.01	0	0	0
Jan 93	N	391	1583	1351					
	Major Axis	1.066 ± 0.06	1.077 ± 0.05	1.053 ± 0.05	74.4	<0.01	0	0	0
	Minor Axis	0.533 ± 0.03	0.564 ± 0.04	0.545 ± 0.03	223.3	<0.01	0	0	0
	Volume	0.160 ± 0.02	0.184 ± 0.02	0.164 ± 0.02	219.3	<0.01	0	0	0

III.4. DISCUSSION

Fish egg size varies between different species and between different populations of the same species due to temporal, biological, spatial and/or environmental factors. Within the same species, planktonic egg size decreases as the spawning season progresses (BAGENAL, 1971, CHAMBERS; WAIWOOD, 1996). For clupeiforms, this decrease in the egg size may be due to maternal reduction of energy reserves over the spawning season, a switch in the stored energy from reproduction to growth, seasonal changes in the age structure of the spawning populations, changes during oogenesis that are correlated to some environmental conditions (e.g. temperature, photoperiod and food supply) (BLAXTER; HUNTER, 1982; CHAMBERS, 1997) or a co-occurrence of these factors, as suggested by Llanos-Rivera and Castro (2004) for *Engraulis ringens* eggs off the Chilean coast. Ciechowski (1973) reported that size of *E. anchoita* eggs off the Argentinian and

Uruguayan coast decreased 23% throughout the spawning season, probably due to multiple spawning and/or length composition of the spawners correlated with the differences in growth rate in relation to the temperature.

Egg size increases with latitude (LLANOS-RIVERA; CASTRO, 2004). Ciechomski (1973) showed that *E. anchoita* egg-size increased from the north (35 - 36°S) to the south (40 - 41°S), and that this variation could not be correlated with temperature or salinity, as both parameters were similar throughout the sampled area. Temperature and salinity usually exhibits negative correlation with the egg size, as shown for *Engraulis encrasicolus* by Demir (1974) and for *E. anchoita* by Phonlor (1984a). In our results, Area 1, which usually exhibited lowest temperature and highest salinity values, was the area that had the smallest eggs in most of the years analyzed. The exception was in January 1988, when the largest eggs were sampled in that area. During this period, a weak SACW intrusion occurred in Cape Frio (see the horizontal temperature distribution maps from Chapter IV or the sd values in Table III.5) and the values of temperature and salinity were similar within the three areas.

Conversely, in Area 3 highest temperature and lowest salinity values were found, and this area usually had the largest eggs. It is important to highlight that Area 3 encompasses most of the *bonaerense* stock, whereas Area 1 contains only the SBB stock. As the geographic limits of each stock are not well defined, and the eggs from Area 2 exhibited no constant pattern, with sometimes the largest eggs sampled, or with egg sizes exhibiting no significant differences with Area 1 or Area 3, Area 2 will be considered a transition zone, and will not be considered further in this discussion.

Engraulis anchoita individuals from the southeastern Brazil (22°-27°S) exhibited shorter lengths at different ages than those from the southern Brazil (30°-35°S), reflecting poor growth conditions in the former region (CARVALHO; CASTELLO, 2013). The southern region also exhibited a more diverse age composition, with older individuals up to age 4, whereas the southeastern region had a massive presence of 1-year-old anchovies (CARVALHO; CASTELLO, 2013). Relating sizes of females to eggs, two situations can occur: (1) larger females produce larger eggs (e.g. CIECHOMSKI, 1966) or, (2) relative fecundity is higher in larger females (e.g. PÁJARO et al., 1997). Knowing that the individuals from the *bonaerense* stock are larger than the southeastern ones, it is possible to infer that eggs from Area 3 are larger than Area 1 due to differences in the maternal stock.

Anchovies are semi-migratory species that perform trophic and reproductive migrations (CIECHOMSKI, 1973) and no physical barriers occur that would prevent the stocks from moving from one area to another. During the austral winter, adults of *E. anchoita* migrate from Uruguayan and Argentinean waters off the Plata River (35 - 34°S) to Brazilian waters (34- 29°S) where favorable spawning and feeding conditions prevail, returning southward in late spring (LIMA; CASTELLO, 1995).

Even knowing that our studied area is north of the one covered by Lima and Castello (1995), there is a equatorward flow of the colder and fresher Plata Plume Water (PPW) in the inner shelf (PIOLA et al., 2008a; PIOLA et al., 2008b). This flow can carry biota of benthic and planktonic foraminifera, ostracods and micro-bivalves from cold and temperate waters to a tropical bay located at around 23°S (STEVENSON et al., 1998). In addition, the Plata River estuarine front is an important area for the feeding ecology of *E. anchoita*, as the highest stomach fullness values were found at stations close to the surface salinity front (PADOVANI et al., 2011).

The satellite images in Figures III.2 and III.3 probably represent the PPW flow carrying chlorophyll from the south to the north during the winter on the inner shelf. This flow could be guiding the migration of the *E. anchoita* northward, which also happens during the winter. As the eggs obtained during the winter are larger than the ones sampled in summer, one possibility is that larger adults coming from the south would be spawning in or closer to the studied area in winter, but no conclusion can be made with the present data as there have been no studies comparing the length and age structure of *E. anchoita* during different seasons in the SBB, nor have there been any genetic studies of different *E. anchoita* stocks.

Another possibility is that the seasonal variation in egg size may be an adaptive response to the changes in the abiotic conditions encountered by the eggs. An increase in temperature decreases the incubation period (time from fertilization to hatch), and a decrease in the incubation time decreases the egg size (WOOTTON, 1990). Temperature and salinity were higher during the summer than winter in both years and, as expected, the eggs were smaller. Smallest eggs in populations spawning in summer were also observed in the North Atlantic herring (BLAXTER; HEMPEL, 1963).

In conclusion, the regional variations in the egg size of *E. anchoita* were probably caused by the differences in the stocks analyzed, whereas the reasons why there were seasonal variations remain unknown. Two possibilities may occur, one due to difference in the maternal stock and another due to the abiotic variability. As noted above, further

studies are necessary to confirm the hypotheses presented, including comparisons between the size composition of the adults of the different seasons and the different areas, as well as genetic studies. The information that the regional variability may be caused due to different stocks analyzed can be useful to other studies, such as that carried out in Chapter IV, where we characterize the spawning habitat of *E. anchoita* in the SBB relative to environmental conditions. It is important to keep in mind that we are probably dealing with more than one stock, so the preferred spawning conditions can be different for each one.

IV. SPAWNING HABITAT OF *ENGRAULIS ANCHOITA* IN THE SOUTHEASTERN BRAZILIAN BIGHT

IV.1 INTRODUCTION

As small pelagic fish usually have fast-growth rates and short lifespans, their stock sizes are characterized by marked fluctuations because of their high dependence on environmentally-driven annual recruitment pulses (BARANGE et al., 2009; CHECKLEY et al., 2009).

In the Southeastern Brazilian Bight (SBB, roughly 22°S and 28°S), the Argentine anchovy (*Engraulis anchoita*) and the Brazilian sardine (*Sardinella brasiliensis*) are the two dominant pelagic fish species of the ichthyoplankton (KATSURAGAWA et al., 2006). They are important components of the ecosystem, preying on plankton and being a major food source for a variety of fishes, marine birds and mammals. Cury et al. (2000) described small pelagic fish as mid-trophic-level “wasp-waist” populations, which exert a major control on the dynamics of upper and lower trophic levels.

Beyond that, both species are important fisheries resources. *Sardinella brasiliensis* supports one of the most important commercial fisheries in Brazil, totaling 74,100 t in 2011 (MPA, 2013), but some stocks are over-exploited (CERGOLE; ROSSI-WONGTSCHOWSKI, 2005; CERGOLE; DIAS-NETO, 2011). On the other hand, *E. anchoita* is an important commercial fishery in Argentina and Uruguay (21,164 t - FAO, 2013) and its exploitation has only recently started in the southern region of Brazil (CARVALHO; CASTELLO, 2013). In the SBB, the Argentine anchovy is not yet commercially fished.

Due to the fishery importance, most of the pelagic fish studies in the SBB are focused on the *S. brasiliensis* (e.g. MATSUURA, 1996; MATSUURA, 1998; GIGLIOTTI et al., 2010; MORAES et al., 2012; DIAS et al., 2014) and fewer focus on the *E. anchoita* (e. g. ASANO et al., 1991; BAKUN; PARRISH, 1991; MATSUURA; KITAHARA, 1995; CASTELLO; CASTELLO, 2003).

While the Brazilian sardine is confined to the SBB and spawns only during late-spring and summer (MATSUURA, 1990; MATSUURA et al., 1992), the Argentine anchovy occurs from Vitoria, off Brazil (20°S), to Gulf San Jorge, off Argentina (48°S) (CASTELLO, 2007), spawning all year-round with a peak during the late-spring and beginning of summer in the SBB (MATSUURA et al., 1992; Chapter III). This wide range distribution and capacity to survive in different oceanographic conditions makes *E.*

anchoita fish and eggs important components of the pelagic ecosystem in the southwest Atlantic Ocean (MACEDO-SOARES et al., 2014).

The distribution and abundance of small pelagic fish eggs are used to estimate the population size of spawning females and to investigate spawning habitat and its fluctuations (LASKER, 1985; CHECKLEY et al, 2000). Studies on the spawning area are relevant as they provide information that is helpful in many different ways: spawning area distribution can be used to identify boundaries between different populations and/or fisheries stocks (CADRIN et al., 2005); changes in spawning area extension can be used to assess stock health (ZENITANI; YAMADA, 2000; STRATOUDAKIS et al., 2003; GAUGHAN et al., 2004); estimates of spawning area can be used to set cost-effective sampling objectives according to stock state and management needs (MANGEL; SMITH, 1990; SMITH, 1990) and spawning area distribution also can be used as an index of abundance in assessment models (DERISO et al., 1996).

The spatial extent of the spawning area is influenced by biotic and abiotic factors; it affects recruitment and therefore the future structure of the adult population (BELLIER et al., 2007). Hence, there is an interest to obtain the best possible estimates of the distribution and abundance of the fish eggs and, at the same time, characterization of the environment in which they occur (CHECKLEY et al, 2000). That way it is possible to truly describe the spatial extent of spawning areas and their natural variability, in seeking to understand the mechanisms responsible for the interannual variations in small pelagic fish populations (BELLIER et al., 2007).

Thus, the objective of the present study is to characterize the spatio-temporal spawning patterns of *E. anchoita* in the Southeastern Brazilian Bight, in terms of geographic location and some abiotic factors. Throughout the text, the term “spawning habitat” refers to the “realized spawning habitat”, where fish actually spawn in a given year at a given time, as defined by Planque et al. (2007).

IV.2. MATERIALS AND METHODS

Study Area

The studied area comprised the Southeastern Brazilian Bight (SBB), between Cape São Tomé – Rio de Janeiro state and Cape Santa Marta Grande – Santa Catarina state (roughly 22°S and 28°S; Fig. IV.1). Oceanographic and biological descriptions of the

studied area were done by many authors in Rossi-Wongtschowski and Madureira (2006) and recently by Ciotti et al. (2014).

The SBB has a moon crescent-shape with a coastal length of about 1100 km cut by cliffs, small bays and many islands. The isobaths are approximately parallel to the coastline (CASTRO; MIRANDA, 1998). The continental shelf width varies from 230 km at Santos – São Paulo state to 50 km at Cape Frio – Rio de Janeiro state. The average shelf break is about 150 m (MAHIQUES et al., 2010).

Three water masses comprise the neritic zone of the SBB. Transported southwards by the Brazil Current (BC), near the shelf break in the upper 200 m depth layer there is the warm Tropical Water (TW), with temperature higher than 20°C and salinity higher than 36.4. At the lower layer, there is the cool South Atlantic Central Water (SACW) with temperature and salinity below the limits established for TW. Finally, as a result of mixing of the shelf water with waters of continental origin, there is the Coastal Water (CW) characterized by lower salinities (CASTRO; MIRANDA, 1998; SILVEIRA et al., 2000).

No relevant fresh water input influences the SBB as, for example, the influences caused by the Plata River discharge in Uruguay and Argentina (PIOLA, et al., 2005), which enriches even the shelf waters off Santa Catarina state (BRANDINI, 1990). So, except for some coastal areas under the influence of waters outflowing from large embayments such as Paranaguá Bay in Paraná state, and the Cananéia-Iguape Coastal System, south of São Paulo state, oligotrophic conditions prevail in the studied area due to the TW predominance in the upper layers. Oceanic stocks of nutrients are trapped below the thermocline at the SACW (LOPES et al., 2006).

Fertilization of the euphotic zone depends on mesoscale physical processes that are regulated by the seasonal pattern of the wind and the flow dynamics of the BC. During the period analyzed in the present study, the late spring and summer, northeast winds caused Ekman transport of the surface waters offshore, causing onshore bottom intrusions of the SACW. These intrusions may be enhanced by cyclonic eddies and meandering of the BC along the continental slope (CASTRO; MIRANDA, 1998; CAMPOS et al., 2000). Three major coastal upwelling centers are identified in the studied area: Cape São Tomé (22°S), Cape Frio (23°S) and Cape Santa Marta Grande (28°S) (CIOTTI et al., 2014).

As the intrusions events at Cape Frio are usually more intense than in the other two areas, Cape Frio upwelling has been the subject of many studies in physical and biological oceanography (e.g. MAGLIOCCA et al., 1979; CASTELAO; BARTH, 2006).

The upwelling intensification at Cape Frio is related to the local bottom topography, with a strong slope concentrated on the inner shelf, and the 100 m isobath located 7 km from the coast (VALENTIN et al., 1987; RODRIGUES; LORENZETTI, 2001).

Data collection and preparation

All samples used in the present study were previously collected and deposited at the Biological Collection "Prof. E. F. Nonato" - ColBIO (Oceanographic Institute, University of São Paulo).

Ten oceanographic cruises carried out in late-spring and beginning of summer from 1974 to 1993 were analyzed (Table IV.1). These cruises have a wide sampling grid along the SBB and stations from the coast to about the 200 m isobath (Figure IV.1).

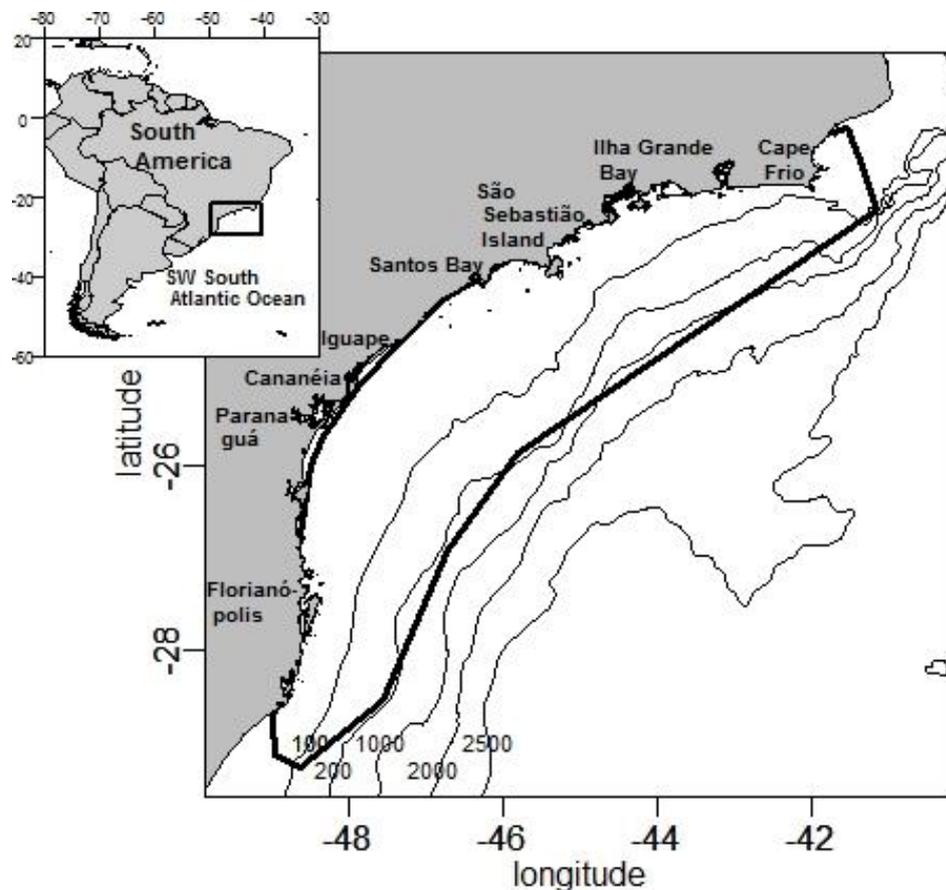


Fig. IV.1. Location of the Southeastern Brazilian Bight in South America (upper figure) and a closer view of the sampled area during the early spring and summer of ten years, between 1974 and 1993 (lower figure).

Plankton samples were collected following Smith and Richardson (1977). A bongo net with a 0.61 m diameter mouth opening, equipped with paired cylindrical-conical

0.333 mm and/or 0.505 mm mesh size nets was used. A flow meter was attached at the center of each mouth opening to measure the filtered water volume. The bongo nets were towed obliquely from the surface to maximum depth, not exceeding 200 m. Samples preserved in 4% seawater buffered formalin from only one cylindrical-conical net were analyzed.

Temperature and salinity data were obtained from Nansen bottles and reversing thermometers until 1988. After this date, a CTD (conductivity-temperature depth profiler) was used.

Table IV.1 - Project name and oceanographic cruise number, month and year of the cruise, number of plankton samples obtained (N) and the bongo mesh-size used in the present study.

Project - Number	Month	Year	N	Mesh-Size (mm)
ICTIO - 1	Nov/Dec	1974	139	0.505
FINEP - 1	Nov/Dec	1975	140	0.505
FINEP - 5	Dec	1976	140	0.505
FINEP - 7	Jan	1978	134	0.505
FINEP - 11	Jan	1980	42	0.505
FINEP - 12	Jan	1981	43	0.505
EPM-Sardinha	Jan	1988	78	0.333
V. Hensen/JOPS	Dec	1990	89	0.333
Sardinha 1	Dec	1991	110	0.333
Sardinha 2	Jan	1993	108	0.333

Under a stereomicroscope with a digital camera attached, Engraulidae eggs were sorted based on their ellipsoid shape, segmented yolk and, usually, absence of oil droplets (CIECHOMSKI, 1965; PHONLOR, 1984), and then imaged. Major and minor axes of the photographed eggs were measured using the program ImageJ. All Engraulidae eggs were measured, except when the number of individuals for a given oceanographic cruise exceeded 100. In these instances, approximately 100 eggs were randomly selected for measurement. *Engraulis anchoita* eggs were identified by discriminant analysis as described by Favero et al. (2015a) (Chapter I).

Data analysis

Kriging Analysis

Geostatistical interpolation technique of ordinary kriging was used to create maps of probability of *E. anchoita* egg occurrence for each year. To predict the response for an unmeasured location, a random function model of spatial correlation was used to calculate a weighted linear combination of the available samples. The most-common geostatistical tool to model the spatial correlation is the semivariogram (or variogram) (CRAWLEY, 2007).

Empirical (experimental) variograms were calculated for each cruise, representing the semivariance between data points as a function of the spatial data between them. Theoretical variograms based on exponential models were adjusted to the empirical variogram by fitting, using the weighted least squares (CRESSIE, 1993). The experimental and theoretical variograms for the cruises studied are presented in Fig. IV.2 based on the fitted parameters in Table IV.2.

As egg abundance distribution was highly skewed toward low values and to limit discrepancies due to differences in the mesh size used (Chapter II - FAVERO et al., 2015b), experimental variograms were calculated using binary-transformed presence-absence data (e.g. BELLIER et al., 2007; GIGLIOTTI et al., 2010). An ordinary kriging analysis performed on a binary-transformed sample population (indicator kriging), allows the estimation of the probability distribution of a variable within a region. No assumptions regarding the distribution of the modelled variable are necessary (MARINONI, 2003).

The mean and the standard deviation of the estimated probability of presence were calculated for every spatial location of the grid for the period sampled, resulting in an average and a variability map. According to Bellier et al. (2007), these maps allow the classification of three categories of spawning area:

- Recurrent spawning sites: areas with high mean and low standard deviation values. Spawning occurs every year in these areas.
- Occasional spawning sites: areas with high standard deviation values. Spawning occurs in some years.
- Unfavorable spawning sites: areas with low mean and low standard deviation. Spawning rarely occurs in these areas.

Numerical methods were implemented using the GeoR library (RIBEIRO; DIGLE, 2001) of the R software (R DEVELOPMENT CORE TEAM, 2015).

Table IV.2. Variogram model parameters for *Engraulis anchoita* eggs in all years analyzed.

Dates	Nugget	Partial Sill	Range
Nov-Dec 1974	0.005	0.156	32.398
Nov-Dec 1975	0.130	0.034	40.000
Dec 1976	0.005	0.114	35.264
Jan 1978	0.002	0.128	37.564
Jan 1980	0.107	0.090	96.000
Jan 1981	0.166	0.192	423.625
Jan 1988	0.150	0.094	45.819
Dec 1990	0.005	0.262	41.782
Dec 1991	0.200	0.068	90.506
Jan 1993	0.220	0.032	39.400

Quotient analysis

Single parameter quotient analysis (SPQ) was used to characterize the spawning habitat of *E. anchoita* relative to surface and subsurface (10 m) temperature, salinity, local depth and distance from the coast (e.g. IBAIBARRIAGA et al, 2007; GIGLIOTTI et al., 2010; MHLONGO et al., 2015). Similar Q values were observed when analyzed separately for each year and each area (as defined in Chapter III), so only the results with all years together were presented.

The quotient value (Q) is the number of eggs found in each environmental variable class expressed as the percentage of the total number of eggs over the study period divided by the percentage of frequency of occurrence of that particular environmental variable class. Assuming that fish select the spawning habitat based on the variable analyzed, quotient values greater than 1 indicate positive selection, whereas values lower than 1 indicate no selection (VAN DER LINGEN et al., 2001).

Confidence intervals of the null hypothesis of even distribution of eggs are computed by bootstrapping procedure in order to test the significance of the quotient values larger or smaller than one. This procedure was repeated 999 times and the 0.025 and 0.975 percentiles of the observed quotient values within each bin were used as the confidence intervals. Preferred values are the ones in which the egg quotient is above the upper confidence interval. Avoided values are those in which the egg quotient is below the lower interval. The tolerance range is the one in which egg quotient values lay between lower and upper confidence limits (BERNAL, et al. 2007). All the analysis was done using R software (R CORE TEAM, 2015).

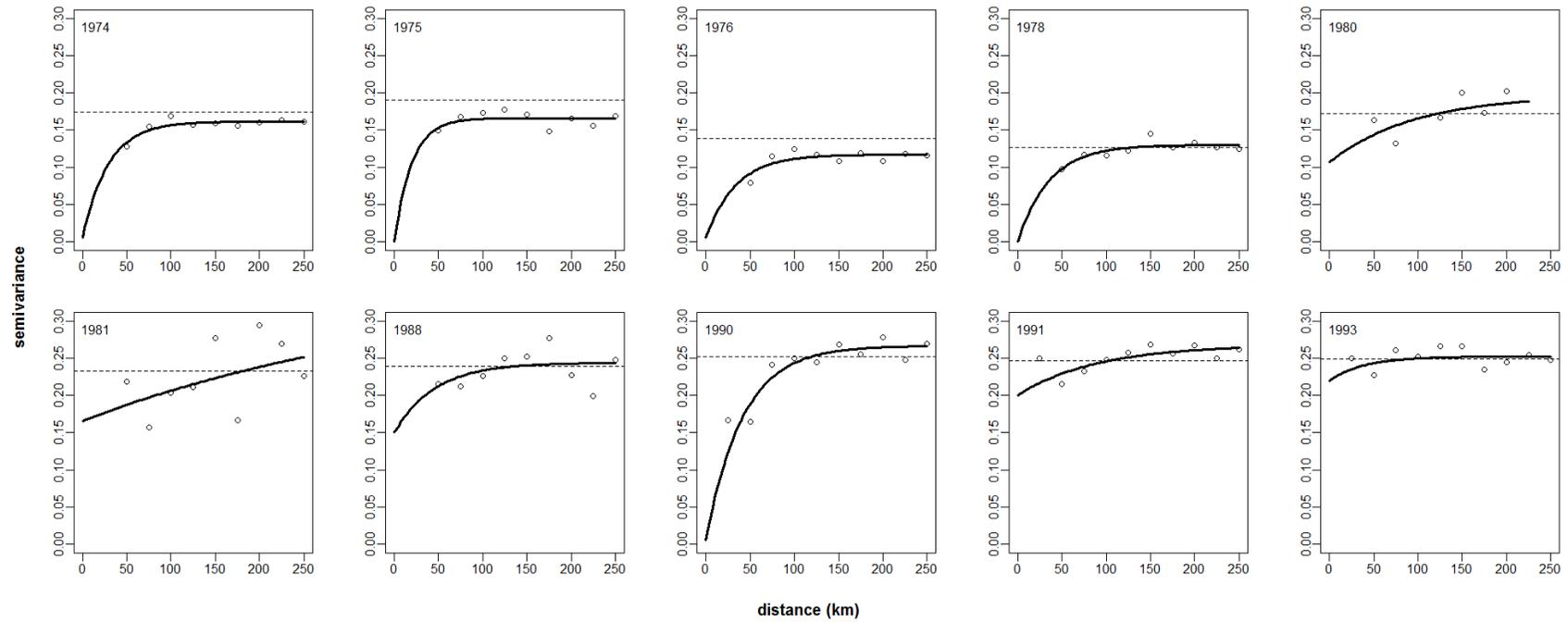


Fig. IV.2. Theoretical fitted variogram adjusted to the experimental variogram for all sampling cruises analyzed. Plain line: theoretical variogram; points: experimental variogram; dotted line: sample variance.

IV.3. RESULTS

Oceanographic conditions

Surface temperature varied from 14.9 to 29.1°C (mean = 24.7 ± 1.9) and at 10 m depth, from 12.7 to 28.3°C (mean = 23.7 ± 2.4). The horizontal distribution of temperature varied each year. Cape Frio upwelling was observed in almost every year, except 1974, 1981, 1988, with some years being stronger than other years (Fig. IV.3).

Surface salinity varied from 28.1 to 38.1 (mean = 35.2 ± 1.1) and at 10 m depth, from 32.7 to 38.2 (mean = 35.5 ± 0.9). Horizontal salinity distribution is shown in Figure IV.4. Salinity increased as the distance from the coast increased. A zone of low salinity values was observed in most years, from 24°S to 27°S, where the shelf is wider. Unlike temperature, salinity measured at the surface and at 10 m depth exhibited similar values, except in 1981 when unusually low values were measured at the surface, and 1991 with high values at 10 m depth.

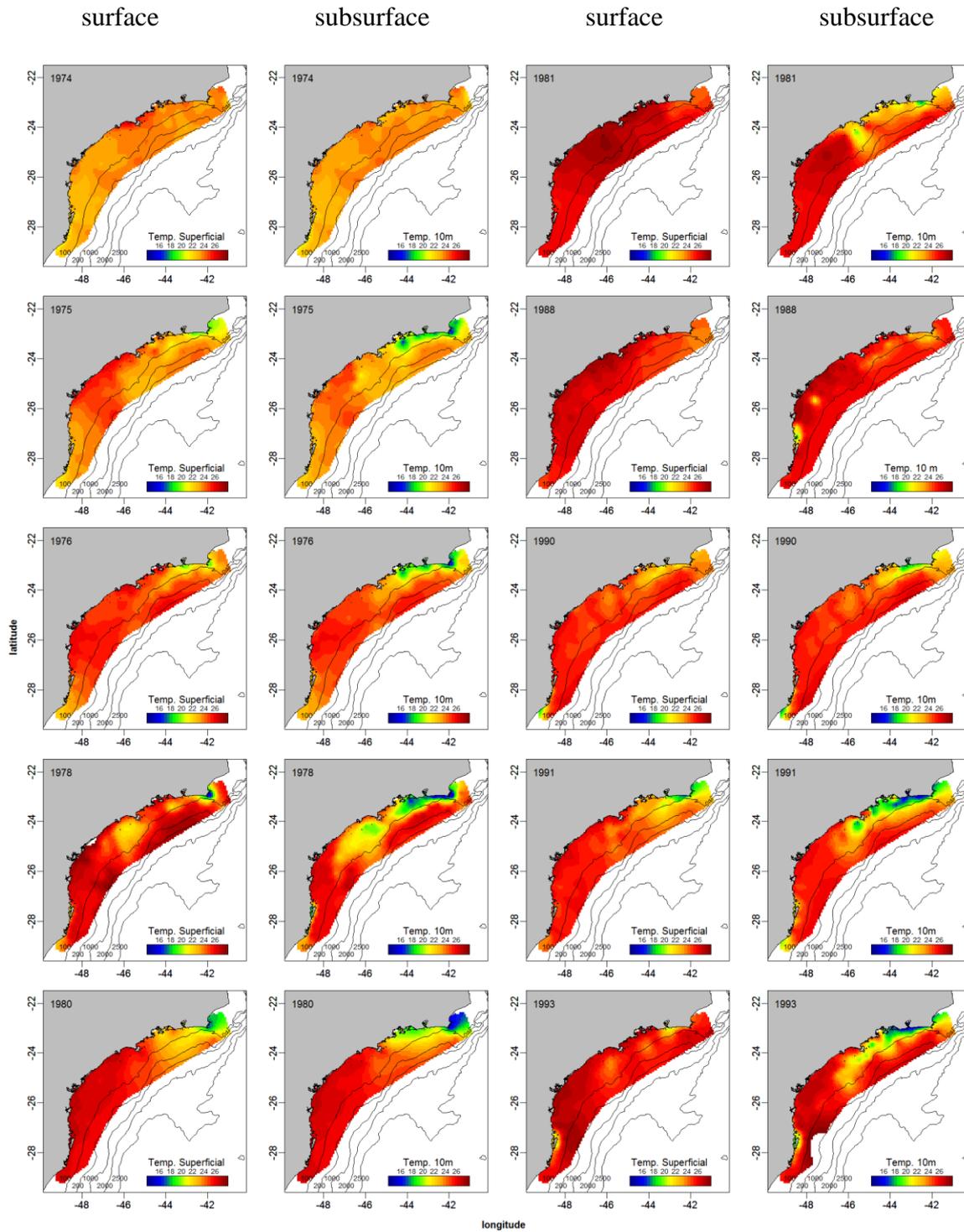


Fig. IV.3. Horizontal distribution of temperature ($^{\circ}\text{C}$) at the surface and at 10 meters depth during the sampled period (1974-1993).

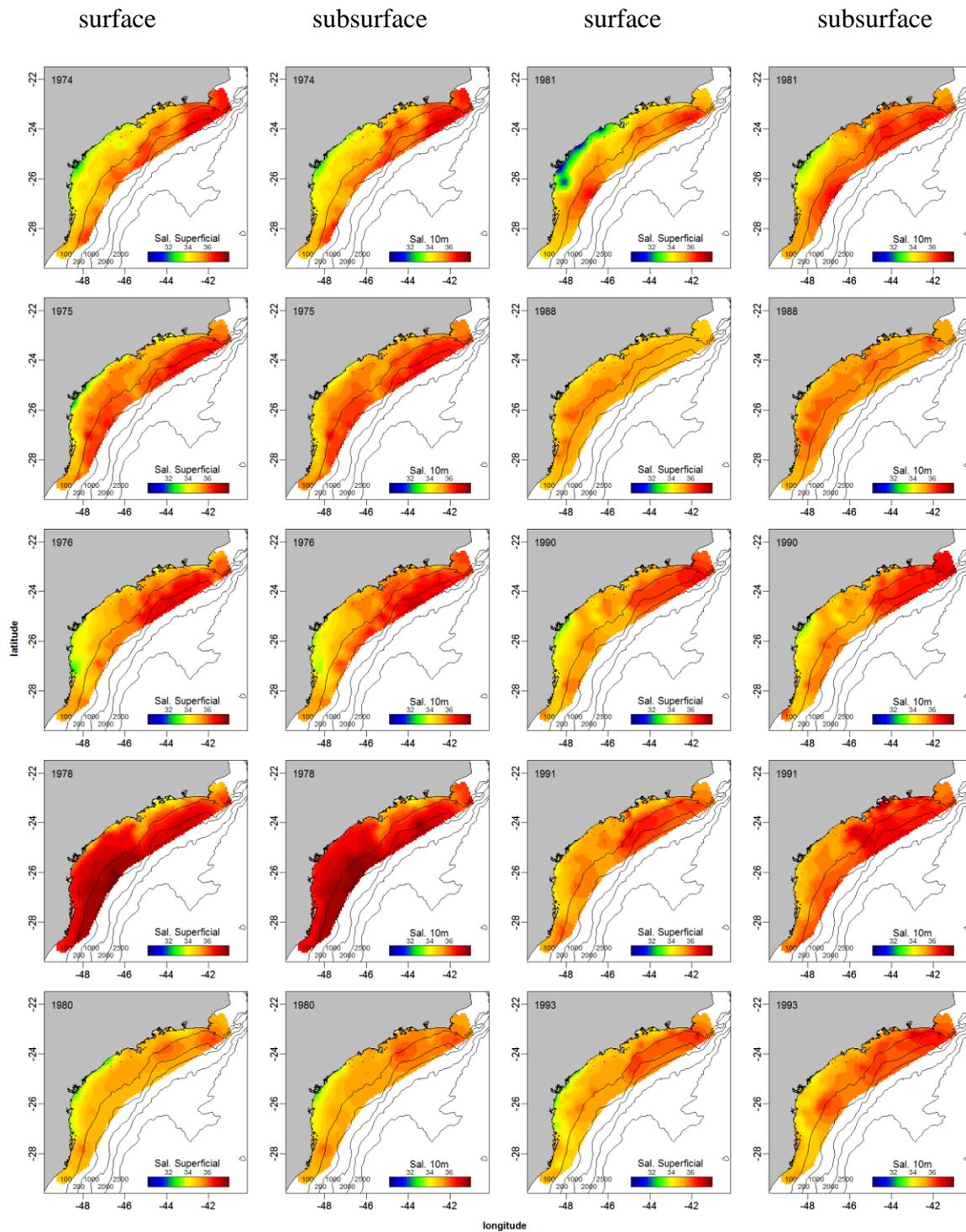


Fig. IV.4. Horizontal distribution of salinity at the surface and at 10 m depth during the sampled period (1974-1993).

Engraulis anchoita spawning area

A well-known occasional spawning site can be identified in the south of the SBB, at about 27°S (Figure IV.5). Other small occasional spawning areas were found in the

central and northern parts of the SBB, between 23°S and 24.5°S, close to the coast. Off the São Sebastião coast, approximately 24.5°S and 44.5°W, there is a well-defined unfavorable spawning site. Another unfavorable spawning site can be found in front of Paranaguá Bay and the Cananéia-Iguape Coastal system, at approximately 25.5°S and 48°W. No recurrent spawning site could be identified, which was also confirmed by the high interannual variability of egg abundance, as observed in Figure IV.6. Egg abundance was usually low (<50 eggs/m²) and high concentrations (>200 eggs/m²) were rare (Fig. IV.6). Table IV.3 summarizes the frequency of occurrence and the abundance of eggs each year.

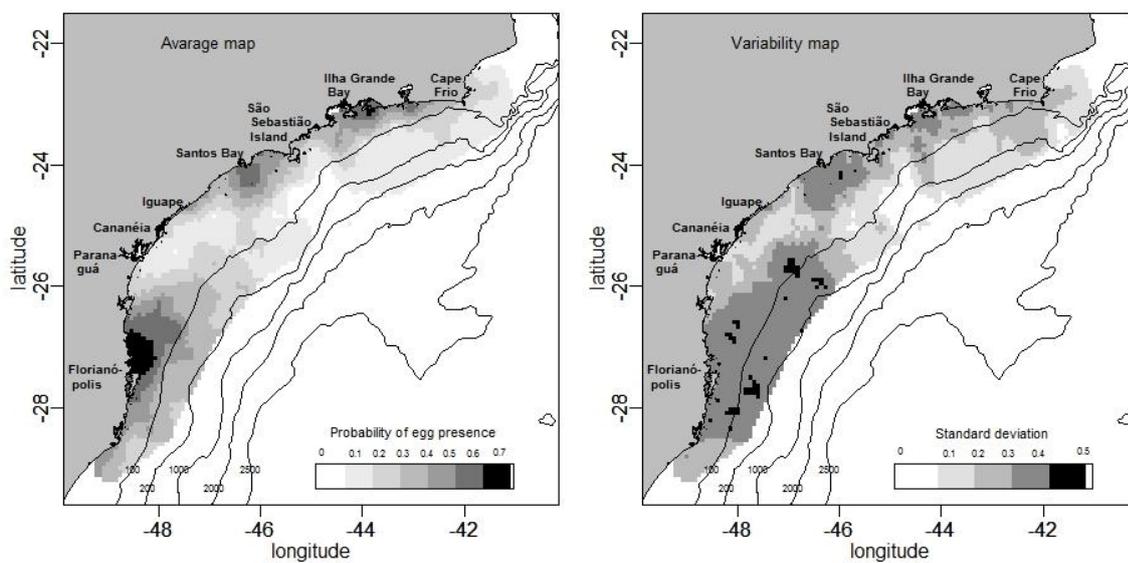


Fig. IV.5. Average (left) and variability (right) maps of *Engraulis anchoita* spawning area for the period of 1974 to 1993.

Table IV.3. Summary table of *Engraulis anchoita* egg samples for all cruises analyzed. Mean, standard deviation (sd) and maximum (Max) abundance of eggs obtained are given in eggs/m².

Dates	Total number of stations	Positive stations	Mean	sd	Max
Nov-Dec 1974	133	29	4.7	28.6	307.9
Nov-Dec 1975	124	31	25.0	231.0	2551.0
Dec 1976	123	20	1.8	9.2	85.0
Jan 1978	123	18	2.5	18.7	183.4
Jan 1980	42	9	21.4	125.1	809.8
Jan 1981	43	15	3.0	10.9	51.7
Jan 1988	78	30	19.7	73.5	506.5
Dec 1990	89	46	16.1	52.3	427.2
Dec 1991	110	47	20.6	68.6	493.6
Jan 1993	108	48	40.1	235.3	2399.7

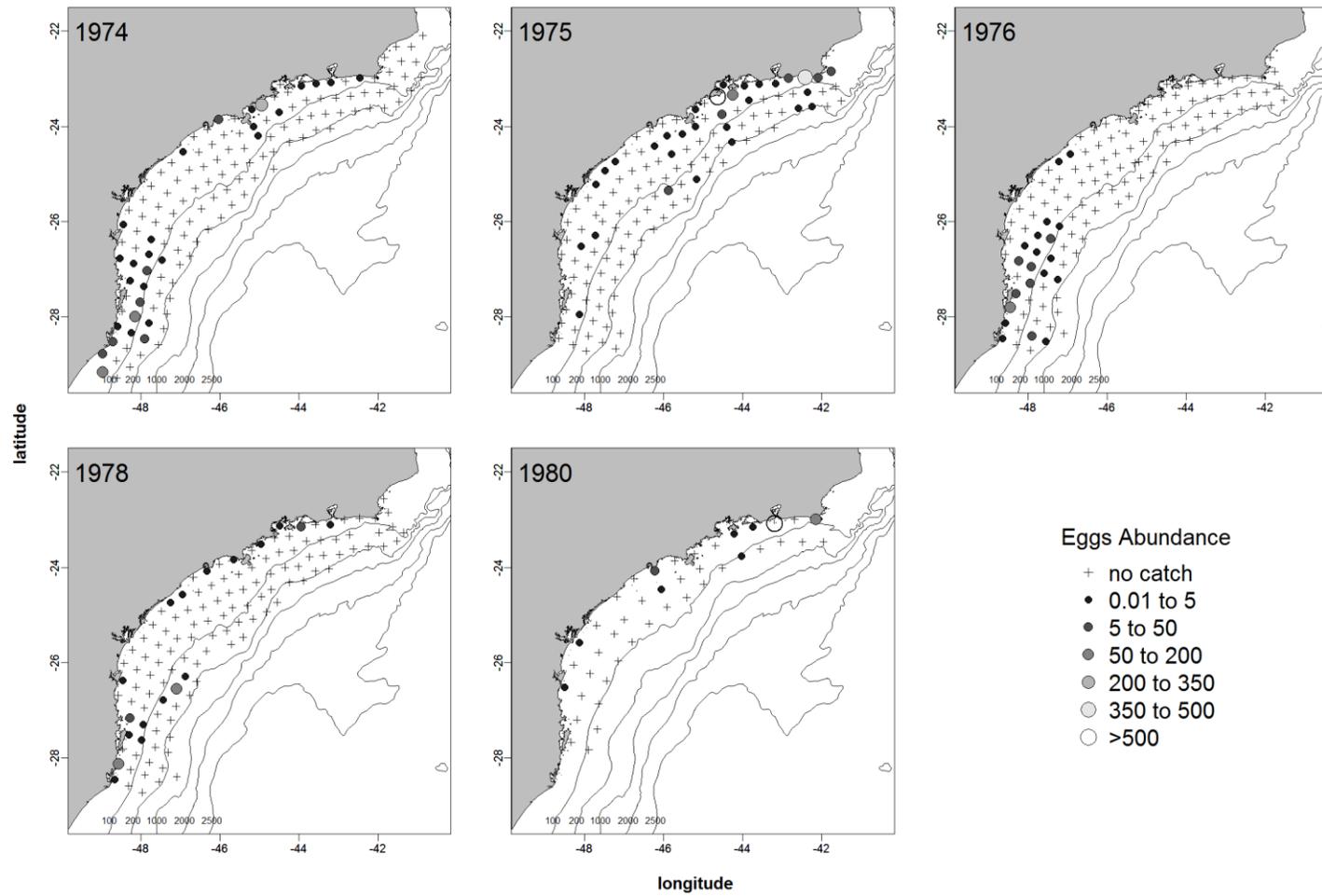


Fig. IV.6. Spatial distribution of *Engraulis anchoita* egg abundance (eggs/m²) sampled in the Southeastern Brazilian Bight during 10 different years.

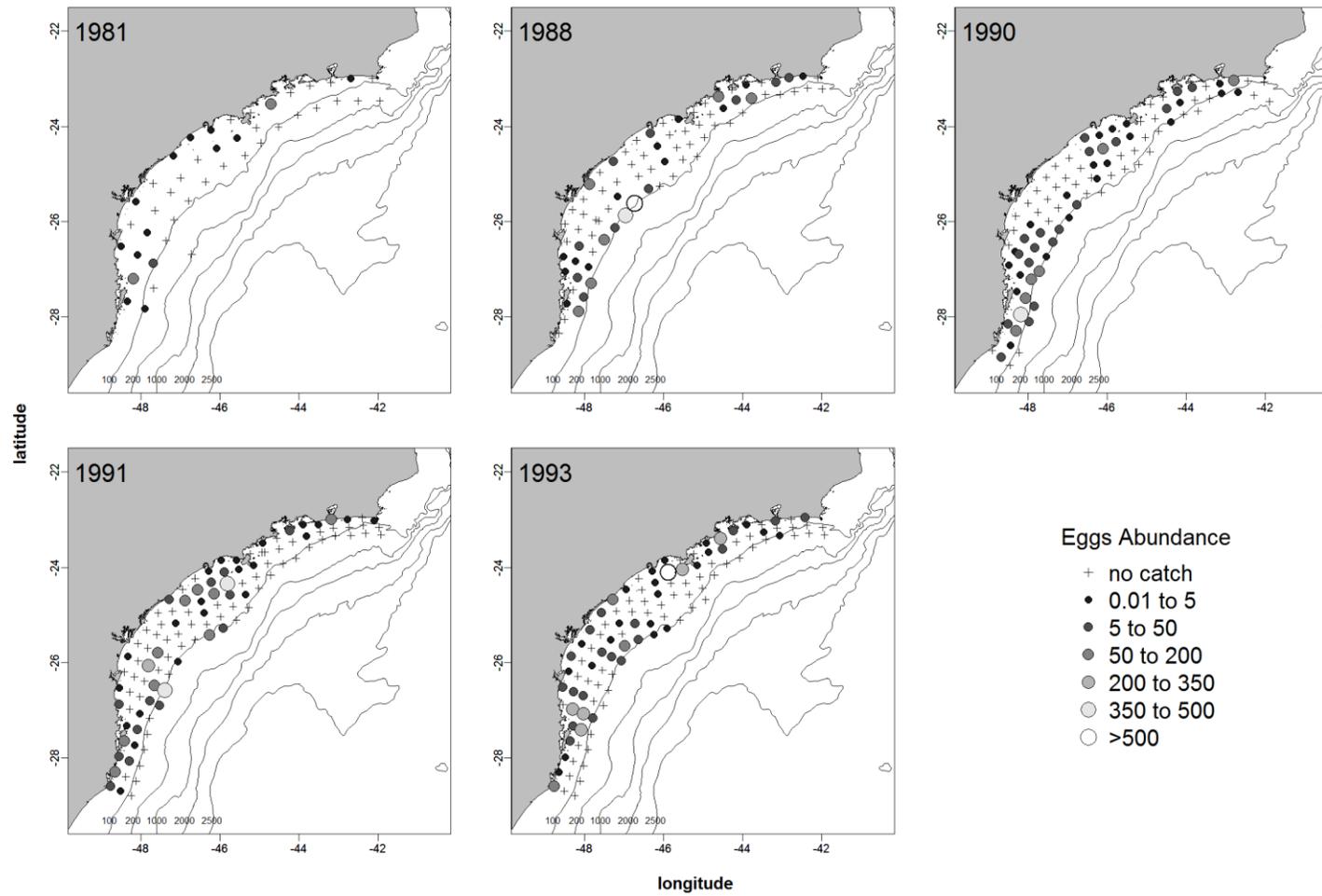


Fig. IV.6 (cont). Spatial distribution of *Engraulis anchoita* egg abundance (eggs/m²) sampled in the Southeastern Brazilian Bight during 10 different years.

Egg abundance sharply decreased toward deeper sites and farther from the coast. Tolerated spawning sites exhibited local depths between approximately 12 to 115 m, with a preference range from 26 to 70 m. Areas with local depths deeper than 115 m were avoided (Fig. IV.7 right). Preferred distance from the coast varied from 3 to 33 km, and distances farther than 55 km were avoided (Fig. IV.7 left).

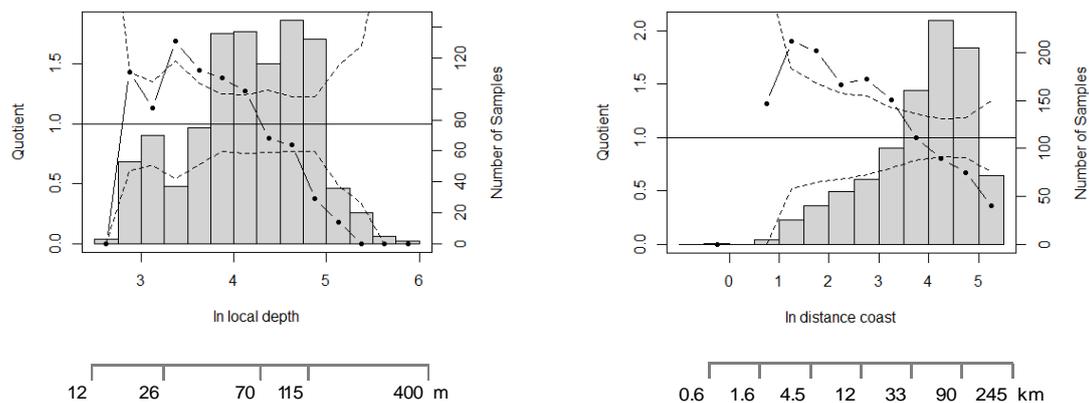


Fig. IV.7. Quotient (Q) curves for the local depth (natural logarithms of the local depth in meters) and distance from the coast (natural logarithms of distance in kilometers) of *Engraulis anchoita* egg occurrence. Histograms represent the number of observations within each bin of the covariate, the continuous black line represents the egg quotient value and the dashed lines represent the upper and lower confidence interval.

TSI diagrams showed that *E. anchoita* eggs were sampled in a wide range of temperature and salinity. Most of the sampling stations with no eggs were within the temperature and salinity range of the Tropical Water (TW) (temperature higher than 20°C and salinity higher than 36.4, according to Castro and Miranda, 1998). Eggs sampled in water colder than 20°C were in the regions influenced by the South Atlantic Central Water (SACW) intrusions in the inner shelf (Fig. IV.8).

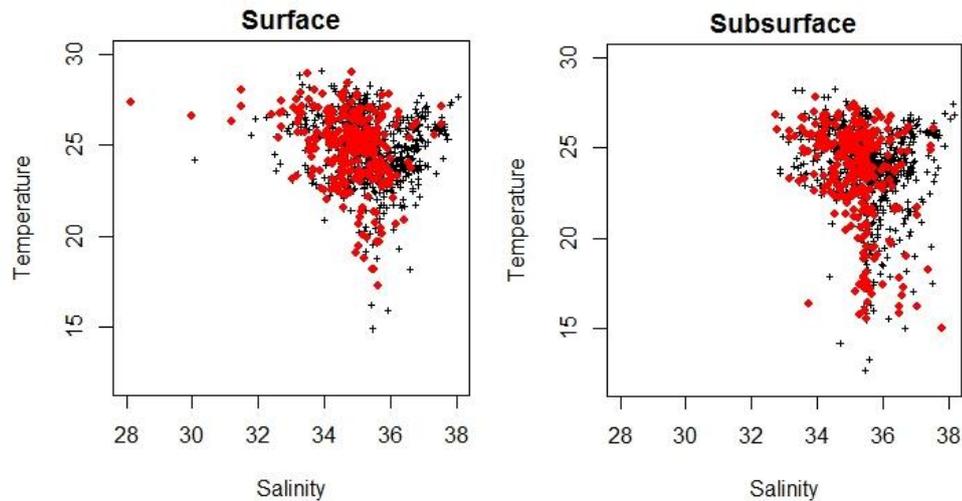


Fig. IV.8. Temperature, salinity and ichthyoplankton (TSI) diagrams for the entire period of study. Temperature ($^{\circ}\text{C}$) and salinity were measured at the surface and at the subsurface (10 m). The red dots represent the stations where *E. anchoita* eggs occurred.

The quotient curve indicated no preferred spawning surface temperature with a tolerance range between 17°C and 29°C . At the subsurface (10 m), tolerance ranged from 15°C to 28°C , with preference from 16°C to 18°C . Surface and subsurface temperatures from 23°C to 24°C were avoided (Fig. IV.9 upper). Surface salinity tolerance ranged from 31 to 36, with preference between 33.5 and 35; any surface salinity values higher than 36 were avoided. At the subsurface, tolerance ranged from 32.5 to 38, with preferred values from 35 to 35.5 and 37 to 37.5; avoided were from 36 to 37 (Fig. IV.9 lower).

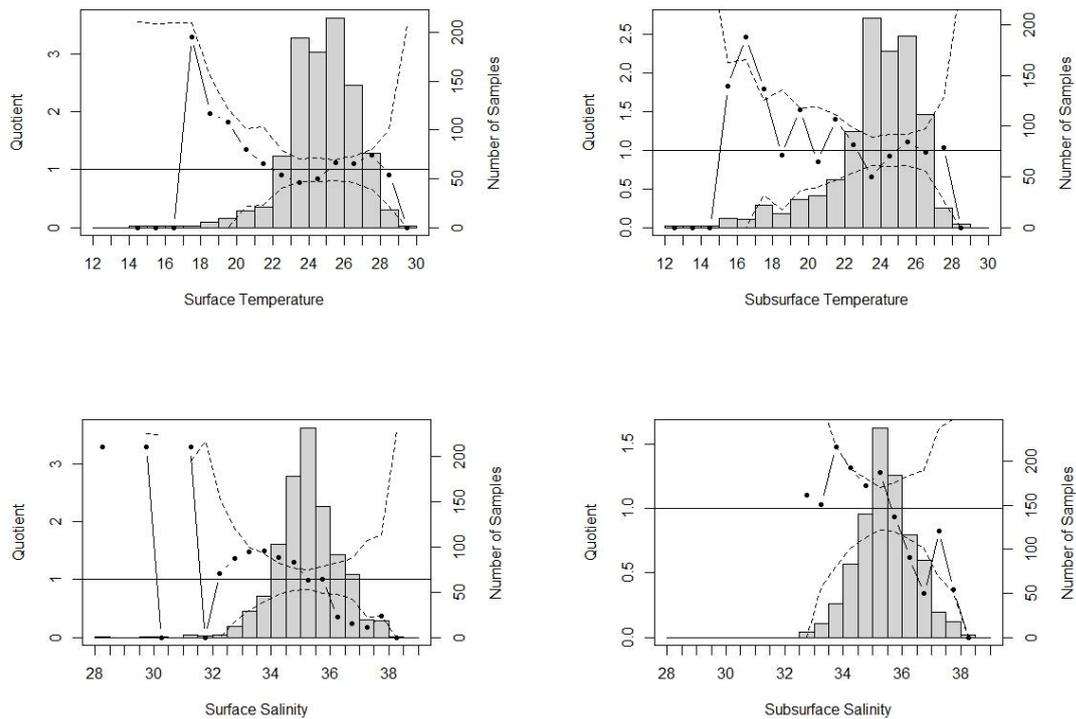


Fig. IV.9. Quotient (Q) curves for the surface and subsurface (10 m) temperature ($^{\circ}\text{C}$) and salinity of *Engraulis anchoita* egg occurrence. Histograms represent the number of observations within each bin of the covariate, the continuous black line represents the egg quotient value and the dashed lines represent the upper and lower confidence interval.

IV.4. DISCUSSION

Although *E. anchoita* eggs were sampled throughout the SBB, a large patch with high probability of egg presence (0.6 - 0.7) was noticed in the southern portion, mainly close to Florianópolis, at around 27°S . According to Gigliotti et al. (2010), the largest *S. brasiliensis* spawning area is also in the southern portion of the SBB, but the highest probability center (>0.75) is located close to Paranaguá Bay (at around 25.5°S), further north of the highest center observed in the present study. In the central portion of the SBB, off Santos Bay, another *E. anchoita* occasional spawning area was found. This area was smaller and with lower presence of eggs (0.5-0.6) than the center off Florianópolis.

A few studies have indicated that the wider continental shelf in the central and southern portions of the SBB provide a more stable environment for spawning

(MATSUURA, 1998; LOPES et al., 2006; MORAES et al., 2012), by providing a thermally-stable environment with low interannual sea surface temperature anomalies (SSTA) (GIGLIOTTI et al., 2010). Further, a quasi-permanent cyclonic cell between São Sebastião and Paranaguá (24°S to 26°S) contributes to larval retention in the area, with highest survival rates of *S. brasiliensis* close to Paranaguá and Santos Bays (DIAS et al., 2014). These same environmental conditions may contribute to the formation of the occasional spawning sites of *E. anchoita* in the southern and central portions of the SBB. Beyond that, a thermal front around Santos observed by Miranda and Katsuragawa (1991) was related with *E. anchoita* shoals occurrence in the area (CASTELLO et al., 1991).

The occasional *E. anchoita* spawning areas found north of São Sebastião Island also exhibited lower probability of egg presence (0.5-0.6) and tended to form small patches along the coast. *Sardinella brasiliensis* also formed small spawning patches to the north, possibly limited by the shelf width that becomes more narrow, and by the proximity of the summer coastal upwelling region of Cape Frio (GIGLIOTTI et al., 2010). In this region, the marked change in direction of the coastline and the shelf break causes strong meandering of the Brazil Current (BC) and eddy formation (CAMPOS et al., 1995). The occurrence of constant northeastern winds during the summer causes coastal wind-driven upwelling, resulting in SACW intrusion from the shelf slope to the coast (CAMPOS et al., 2000). This intrusion brings new nutrients shoreward, increasing their concentrations in the lower euphotic zone, and consequently, leads to an increase in the net production (GAETA; BRANDINI, 2006; LOPES et al., 2006). The increase in zooplankton biomass after the SACW intrusion (LOPES et al., 2006) can promote higher survival rates for the early life stages of pelagic fish (e.g. KATSURAGAWA; MATSUURA, 1992; MATSUURA, 1996).

The intensity of SACW intrusion onto the middle and inner shelf varies from year to year. When the intrusion is weak, there may be an increase in larval mortality and then a decrease in the recruitment (KATSURAGAWA et al., 2006). As an example, we can cite the population of *S. brasiliensis*, which was stable from 1974 to 1987, but then in December 1986 declined when the SACW intrusion was very weak, not reaching the coastal regional and with no vortex in the neritic zone (MATSUURA et al., 1992). This weak SACW intrusion may have led to the sardine recruitment failure in subsequent years, resulting from a decline in the spawning stock biomass (KATSURAGAWA et al., 2006). This was confirmed by the low abundance of *S.*

brasiliensis eggs obtained in January 1988 and in the spawning season of 1991/92 (CASTELLO et al., 1991; MATSUURA et al., 1992; CERGOLE, 1995; MATSUURA, 1998) together with the poor larval nutritional condition in spawning seasons of 1990/91 and 1991/92 (DIAS, 1995). Upwelling also presents a positive effect on the *Engraulis encrasicolus* recruitment in the Bay of Biscay, France (BORJA et al., 1998; 2008; ALLAIN et al., 2001).

In contrast to the pattern described in the previous paragraph, the wind-induced upwelling in Cape Frio tends to transport individuals to unsuitable areas and increase larval mortality, so when mortality rates were lower (January 1980 and 1988), positive SSTA were found, suggesting a weakened upwelling (DIAS et al., 2014). Moreover, in January 1980 unusually high *S. brasiliensis* egg concentrations were found in the northern part of the SBB, coinciding with the period when a high positive SSTA was observed in this area, due to weaker coastal upwelling (DIAS et al., 2014). Indeed, the upwelling effect can induce the confinement of the *S. brasiliensis* spawning zones to the central and southern portions of the SBB (MORAES et al., 2012), not only because of the temperature effect, but also due to the offshore transport associated to the wind-induced upwelling (SOARES et al., 2011).

In our results, as presented in the TSI diagrams (Fig. IV.8) and in the horizontal distribution maps (Fig. IV.6), *E. anchoita* eggs were sampled in the regions influenced by the SACW intrusions on the inner shelf. However the interannual variability of anchovy abundance did not appear to vary due to an influence from the intensity of the SACW intrusion. In the northern SSB, in December 1974, a weak SACW intrusion and low *E. anchoita* egg abundance were observed; in December 1975, a stronger upwelling occurred, with high egg abundance; however, in December 1976, even with a strong SACW intrusion, extremely low egg abundance was found. During these three years, the areas with greatest egg abundances varied in the SBB: higher abundance was found in the south in 1974, followed by higher abundance in the north in 1975, and then eggs were only found in the southern portion in 1976 (Fig. IV.6). According to Lopes et al. (2006), although the nutritional condition of the *E. anchoita* larvae is related to SACW intrusions, anchovy distribution seems to be related to other oceanographic features.

The presence of an estuary is another oceanographic feature that is related to the favorable spawning habitats for anchovies in different regions of the world (e.g. MOTOS et al., 1996; LLORET et al., 2004; BELLIER et al., 2007). Such areas exhibit

the combination of the “ocean triad” of enrichment, concentration and retention (BAKUN, 1996; AGOSTINI; BAKUN, 2002): nutrient enrichment from the freshwater input, concentration in the haline fronts, and retention over the shelf at the edge of river plumes (ALLAIN et al., 2003). In southern Brazil, Uruguay and Argentina, the distribution of *E. anchoita* larvae and eggs is associated with the discharges from the Plata River (Plata Plume Water) and Patos Lagoon (BAKUN; PARRISH, 1991; MATSUURA; KITAHARA, 1995; MACEDO-SOARES et al., 2014). The baroclinic circulation caused by the Plata River and by the Patos Lagoon continental runoff is a mechanism for *E. anchoita* larval retention (VAZ et al., 2007). The importance of haline fronts for spawning and reproduction of different fish species has been widely demonstrated (BAKUN, 1996; 2006; PALOMERA et al., 2007; CARPI et al., 2015).

Nonetheless, in the studied area there is no major freshwater input from large rivers. There are some coastal areas under the influence of waters outflowing from large embayments as the Paranaguá Bay (around 25.5°S) and the Cananéia-Iguape Coastal System (around 25°S). These coastal areas do exhibit lower salinity in most of the years analyzed (Fig. IV.4), but the salinities are within the tolerance limits for *E. anchoita* spawning (Fig. IV.9). Even knowing that these embayments would enrich the coastal area close to them (as noticed in figure III.3 in Chapter III), these areas were classified as unfavorable spawning areas for *E. anchoita* in the present study (probability of egg presence varied from 0.1 to 0.2; Fig. IV.6). The same area is the one that exhibited highest probability of *S. brasiliensis* egg presence (>0.75) (GIGLIOTTI et al., 2010). Although both anchovies and sardines co-occur in the spawning areas, their spawning depths are segregated: *S. brasiliensis* spawns in the warm upper mixed layer, whereas *E. anchoita* spawn in the cool and deep SACW (MATSUURA; KITAHARA, 1995). Even knowing that *E. anchoita* spawn in the cool bottom layer, their eggs and small larvae were found predominantly above the thermocline, co-occurring with the sardine larvae and eggs (MATSUURA et al., 1992; MATSUURA; KITAHARA, 1995). *Engraulis anchoita* was probably not spawning around Paranaguá Bay, possibly due to interspecific competition with *S. brasiliensis*.

Another unfavorable *E. anchoita* spawning area in the SBB is off the São Sebastião Island (Fig. IV.5). When eggs were found in this area, they were located only at the station closest to the island (Fig. IV.6). The same was observed for *S. brasiliensis* eggs by Gigliotti et al. (2010). Islands may influence the oceanic current systems and contribute to ichthyoplankton retention or dispersive mechanisms (LOPES et al., 2006).

Results of a study near São Sebastião Island showed a tendency of fish larvae to concentrate near the southeast of the island (KATSURAGAWA et al., 2006), coinciding with increases in nutrient and plankton concentrations (GIANESELLA-GALVÃO et al., 1997). The local current system flowing close to the coast from southwest to northeast, in the opposite direction from the main flow of the BC, together with the presence of the island, favors larval and food retention in the inner shelf of São Sebastião Island (LOPES et al., 2006).

The area off São Sebastião Island marks the boundary between two main sedimentary zones in the SBB: southward from the island, the deposition processes are associated with the seasonal penetration of waters related to the Plata River runoff and its interaction with the meanders of the BC, and the shelf morphology that favors the establishment of high productivity zones; northward the island, on the inner shelf, organic matter distribution is related mainly to the Cape Frio coastal upwelling, while the middle and outer shelf sediment distributions are a result of a more vigorous meandering of the BC (MAHIQUES et al., 2004). Beyond the oceanographic differences off northern and southern São Sebastião Island (MIRANDA; KATSURAGAWA, 1991), larval fish assemblages also differ when analyzing these two subareas (KATSURAGAWA et al., 2014). It is also important to note that the mineralogy of the surface sediments between Cape Frio (23°S) and the mouth of the Plata River (38°S) is controlled by two oceanographic conditions: the Brazil Current (BC), supplying the shelf with clay minerals from equatorial regions, and the Brazil Coastal Current (BCC), transporting sediments from the Plata River (NAGAI et al., 2014). This sediment distribution shows that area between Itajaí (27°S) and São Sebastião Island (24°S) can be considered as a transitional zone between the dominance of the BC and the BCC (NAGAI et al., 2014).

Mesoscale hydrodynamics features such as coastal upwelling, river plumes and offshore eddies may be related to the *Sardina pilchardus* and *Engraulis encrasicolus* spawning habitat in the Bay of Biscay (France) (BELLIER et al., 2007). These mesoscale hydrodynamic features exhibit large interannual variability (KOUTSIKOPOULOS; LE CANN, 1996; PULLAT et al., 2004; PLANQUE et al., 2007), and as consequence can be linked to the interannual variability of spatio-temporal spawning dynamic of those species (BELLIER et al., 2007). Field surveys show that the spawning patterns of sardine and anchovy off Peru, Chile and Morocco are temporally and spatially variable, suggesting a spawning strategy dominated by

opportunists (BROCHIER et al, 2009). In our results, *E. anchoita* spawning pattern was also highly variable in time and space, and spawning appeared to be related to some mesoscale hydrodynamic features (e.g. SACW intrusions, presence of estuaries and islands, thermal fronts etc.), but further studies are necessary to better understand these relations.

However, it is important to remember that not just abiotic factors influence fish spawning patterns, but also biological ones. Habitat suitability can appear to be density-dependent, meaning that the geographical area occupied by a population expands as population size increases and contracts as it declines (BERTRAND et al., 2004, BARANGE et al., 2009). Positive relationships between spawning area extent and egg abundance have been reported for sardine and anchovy (WATANABE et al, 1996; ZENITANI; YAMADA, 2000; OOZEKI et al., 2007).

Not only intraspecific, but also interspecific egg abundances may be related to spawning patterns. Although further analysis must be done, it was possible to observe the anchovy-sardine egg abundance alternations during the period analyzed. In December 1976, January 1978 and 1981 the mean abundance of eggs was low for *E. anchoita* and high for *S. brasiliensis*, whereas the opposite occurred in January 1980 and 1988 and December 1991 (Fig. IV.10). Fluctuations in population sizes of anchovy and sardines have been found in different oceans (e.g. SCHWARTZLOSE et al., 1999; CHAVEZ et al., 2003; TAKASUKA et al., 2008; BARANGE et al., 2009), but this is the first such report for the SBB. The cause of these fluctuations is unknown but it may be related to variation in spawning habitat (CHECKLEY et al., 2000)

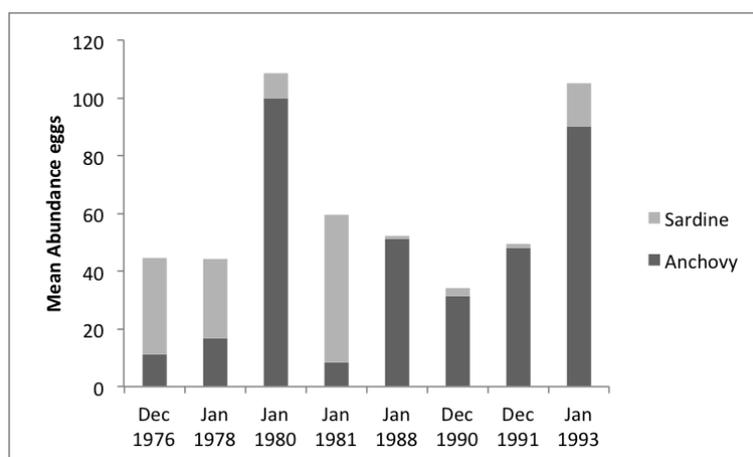


Fig. IV.10. Mean abundance (only positive stations considered) of *Engraulis anchoita* and *Sardinella brasiliensis* eggs during eight years from 1976 to 1993.

Sardinella brasiliensis data were obtained from Gigliotti et al. (2010).

Beyond such alternations, a comparison done between our results and the ones obtained by Gigliotti et al. (2010), showed other differences and similarities in the spawning patterns of anchovies and sardines in the SBB. There was no preferred surface temperature for spawning for both species, whereas the preference for surface salinity was similar for both species. *Sardinella brasiliensis* eggs concentration also decreased toward deeper sites, but preferred spawning sites were closer to the coast than for *E. anchoita*, ranging from 16 to 60 m local depth and tolerating up to 70 m.

Spawning area differences relative to proximity to the coast have been observed in different locations for sardines and anchovies, but without consistent patterns. In the northwestern Mediterranean, *Sardinella aurita* eggs are also normally located closer to the coast than the *E. encrasicolus* ones (PALOMERA; SABATES, 1990; SABATES et al., 2008), thus avoiding competition for food since the early stages of larval development of both species remain at upper levels of the water column (SABATES et al., 2008; COSTALAGO et al., 2015). On the other hand, in the Bay of Biscay, anchovy (*E. encrasicolus*) recurrent spawning sites are located mainly in coastal waters, while sardine (*S. pilchardus*) recurrent sites are less coastal (BELLIER et al., 2007). The same was observed in the southern and central California, with sardine (*Sardinops sagax*) spawning further offshore in more oceanic water, while anchovy (*Engraulis mordax*) spawn inshore, in more-saline upwelled water (CHECKLEY et al., 2000).

Another biological feature that is important to highlight is the presence of different *E. anchoita* stocks in the studied area (CARVALHO; CASTELLO, 2013; Chapter III). Individuals from southeastern Brazil (22°-27°S) exhibited smaller lengths at different ages and a massive presence of 1-year-old anchovies. Meanwhile anchovies from southern Brazil (30°-35°S) exhibited a more diverse age composition, with older individuals up to age 4 (CARVALHO; CASTELLO, 2013). According to Bellier et al. (2007), in the Bay of Biscay coastal and oceanic spawning areas correspond to different components of the *E. encrasicolus* population: the oceanic one is mainly associated with larger individuals, aged 2 years or more (MOTOS et al., 1996); in front of Gironde estuary anchovy size and age may exhibit a more variable distribution (PETITGAS et al., 2003). However, even knowing that further studies are necessary to relate differences in the spawning patterns with different stocks, similar abiotic preferences, shown by the Quotient analysis, were observed for the three areas analyzed in Chapter III, representing two different stocks and a transitional zone.

In summary, this study presented for the first time the spawning habitat of *E. anchoita* based on the best data available in the SBB and geostatistical analysis. Although no spawning areas were classified as recurrent, it was possible to classify some areas as occasional and unfavorable, showing that the spawning habit of *E. anchoita* does not only vary spatially, but also temporally. We discussed some abiotic and biotic factors that could be related to the changes in the spawning habitat of the target species. Shelf width, mesoscale hydrodynamics features and biological interactions all seem to play important roles in defining spawning areas. Future research is necessary to better understand abiotic and biotic factors that influence *E. anchoita* spawning patterns.

V. LONG-TERM FLUCTUATIONS IN THE DISTRIBUTION AND ABUNDANCE OF *ENGRAULIS ANCHOITA* EGGS AND LARVAE IN THE SOUTHEASTERN BRAZILIAN BIGHT

V.1. INTRODUCTION

The small pelagic fish *Engraulis anchoita* Hubbs & Marini, 1935 (Clupeiformes: Engraulidae) exhibits a wide distribution in the western South Atlantic, from Gulf San Jorge (Argentina, 48°S) to Cape São Tomé (Brazil, 20°S) (BAKUN; PARRISH, 1991). Within this wide distribution, three stocks with biological differences are identified: the *patagonic* (48° - 41°S, Argentina), the *bonaerense* (41° - 28°S northern Argentina, Uruguay, and southern Brazil) and the Southeastern Brazilian Bight (SBB) stock (28° - 20°S, Brazil) (CARVALHO; CASTELLO, 2013). All three stocks are ecologically important, transferring energy and nutrients from the bottom of the food chain to higher predators, such as other fish, marine mammals and seabirds (FREDERIKSEN et al., 2006; CASTELLO, 2007). Regarding the fisheries, *E. anchoita* is an important resource in Argentina and Uruguay where the catch was about 21,164 tonnes in 2011 (FAO, 2013). In southern Brazil, the exploitation of the *bonaerense* stock has only recently begun and stocks in the SBB are in almost-pristine condition, but could be exploited in a near future (MADUREIRA et al., 2009; CARVALHO; CASTELLO, 2013).

Probably due to the fisheries difference between stocks, most studies available have focused on *E. anchoita* from Argentina and Uruguay (e.g. CIECHOMSKI, 1965; 1966; 1973; SANCHEZ; CIECHOMSKI, 1984; EHRlich et al., 2000; HANSEN et al., 2001; CAPITANIO et al., 2005; MARTOS et al., 2005; PÁJARO et al., 2007; 2008; LEONARDUZZI et al., 2010; 2013; PADOVANI et al., 2011; AUAD; MARTOS, 2012; GARCIARENA; BURATTI, 2013; MADIROLAS et al., 2013; MARRARI et al., 2013), with fewer studies from southern Brazil (e.g. WEISS; FEIJÓ DE SOUZA, 1977; LIMA; CASTELLO, 1994; 1995; VASCONCELLOS et al., 1998; VELASCO; CASTELLO, 2005; VAZ et al., 2007; COOKE; MADUREIRA, 2012; TORQUATO; MUELBERT, 2014; COSTA et al., 2016) and even fewer from the SBB (Fig. V.1) (e.g. ASANO et al., 1991; MATSUURA et al., 1992; CASTELLO; CASTELLO, 2003; FAVERO et al., 2015a; 2015b).

Beyond the lack of studies, the studied area is oceanographically different from the areas where the *bonaerense* and the *patagonic* stocks occur. In the SBB there are

some coastal areas under the influence of waters outflowing from large embayments such as Paranaguá Bay (around 25.5°S) and the Cananéia-Iguape Coastal System (around 25°S), but with no major freshwater input such as the discharges from the Plata River (Plata Plume Water) located between Argentina and Uruguay and the Patos Lagoon, located in southern Brazil (PIOLA, et al., 2005). It is known that *E. anchoita* eggs and larvae are associated with the freshwater discharges in southern Brazil, Argentina and Uruguay (BAKUN; PARRISH, 1991; MATSUURA; KITAHARA, 1995; MACEDO-SOARES et al., 2014), where baroclinic circulation caused by the Plata River and by the Patos Lagoon runoff is a mechanism for *E. anchoita* larval retention (VAZ et al., 2007).

Another difference between the areas of *E. anchoita* occurrence is that in the SBB nutrient enrichment of the euphotic zone is mainly dependent on mesoscale physical processes that are regulated by the seasonal pattern of the wind and the flow dynamics of the Brazil Current (BC): during the late spring and the summer northeast winds cause Ekman transport of the surface waters offshore, causing onshore bottom intrusions of the South Atlantic Central Water (SACW) (CASTRO; MIRANDA, 1998; CAMPOS et al., 2000). Three major coastal upwelling centers are identified in the SBB: Cape São Tomé (22°S), Cape Frio (23°S) and Cape Santa Marta Grande (28°S), with intrusion events at Cape Frio usually being the most intense (CIOTTI et al., 2014). This upwelling brings new nutrients shoreward, increasing their concentrations in the lower euphotic zone and leading to an increase in the net phytoplankton production (GAETA; BRANDINI, 2006; LOPES et al., 2006). The subsequent increase in zooplankton biomass (LOPES et al., 2006) influences ichthyoplankton survival and distribution (KATSURAGAWA; MATSUURA, 1992; MATSUURA, 1996; KATSURAGAWA et al., 2006; MORAES et al., 2012).

In the southeastern and southern shelf of Brazil, the ichthyoplankton has been studied since the 1960s, but until the early 1980s these studies were mostly related to the identification and description of developmental stages, assessment of fish stocks and spawning biomass, and distribution and abundance of eggs and larvae in a certain area. Since the 1980s, a change in the research focus has occurred, with efforts to understand the control exerted by environmental factors on the early life history of fish (KATSURAGAWA et al., 2006). However, most of these studies examined environmental influences on the ichthyoplankton over short/medium temporal scales,

especially seasonal ones (e.g. GARBINI et al. 2014; KATSURAGAWA et al., 2014; PORCARO et al., 2014).

Along the Oregon Coast, United States of America, large-scale climate variations, such as the Pacific Decadal Oscillation and El Niño, explained more variation in the concentration and diversity of fish larvae than small-scale factors such as upwelling and Ekman transport (AUTH et al., 2011). Other studies sought to understand the influence of large-scale environmental factors, such as El Niño/La Niña, on fish larvae and eggs (e.g. FLORES-COTO et al., 2008; FUNES-RODRÍGUEZ et al., 2011; BUSBY et al., 2014; MUJICA et al., 2016).

Thus, the main objective of the present study is to evaluate long-term fluctuations in the distribution and abundance of *E. anchoita* eggs and larvae in the SBB from 1974 to 2010, and related oceanographic factors.

V.2. MATERIALS AND METHODS

Data collection and preparation

All samples used in the present study were collected in historical oceanographic surveys and stored at the Biological Collection "Prof. E. F. Nonato" - ColBIO (Oceanographic Institute, University of São Paulo).

Oceanographic cruises were selected from the austral late spring and beginning of summer from 1974 to 2010, with the exceptions of two years when sampling occurred in March and May, (Table V.1). Figure V.1 shows the Southeastern Brazilian Bight (SBB) (roughly 22°S and 29°S) and the area concerned for each cruise per decade. Areas were defined based on *E. anchoita* stock identification (CARVALHO; CASTELLO, 2013) (Area 3) and on the oceanographic conditions described by Miranda and Katsuragawa (1991) and Mahiques et al. (2004) (Area 1 and 2). We also defined areas based on the spawning map obtained in Chapter IV (Fig. IV.5) so the divisions would not separate any important spawning areas.

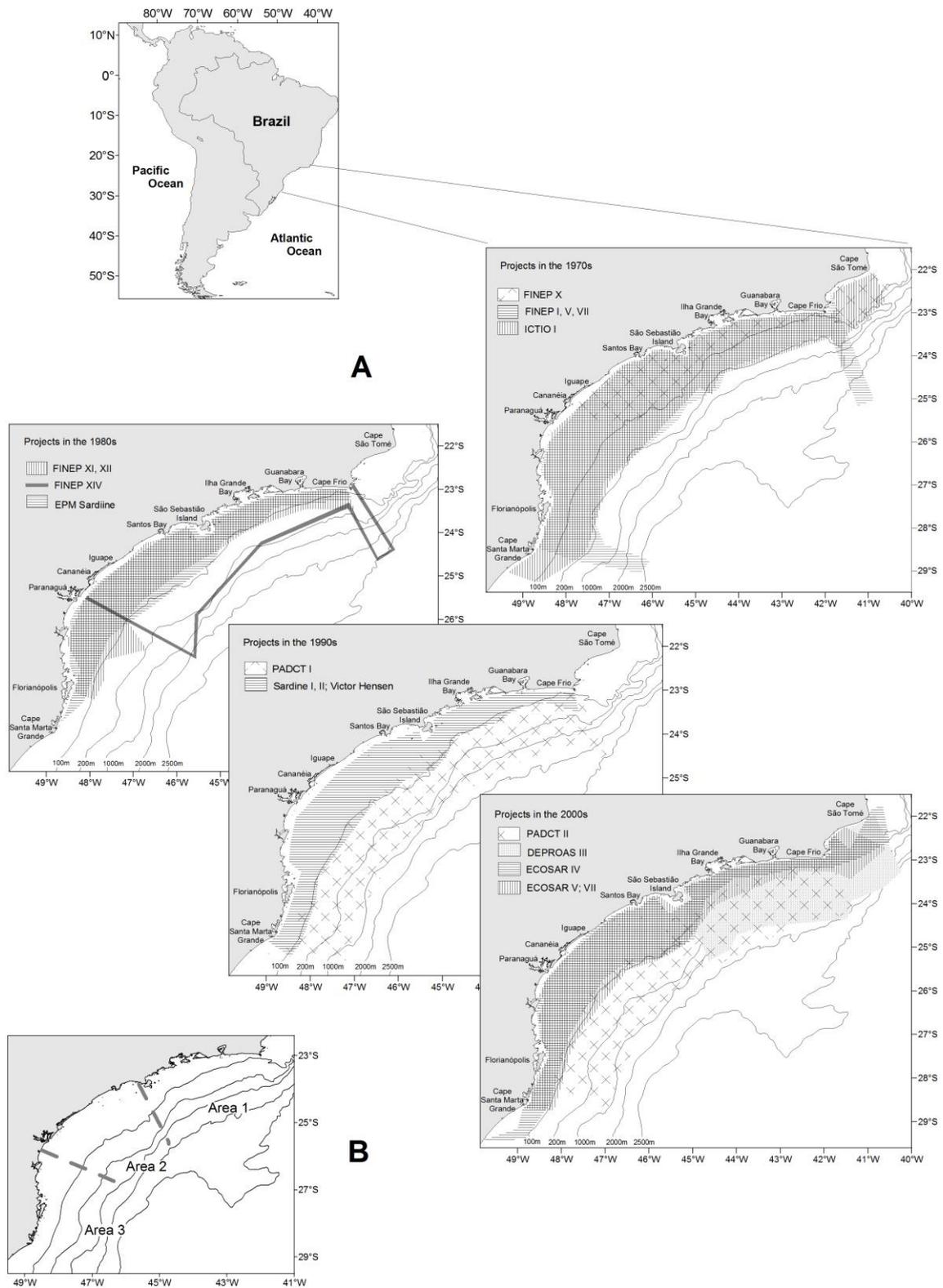


Fig. V.1. Regions sampled during the 18 oceanographic cruises from 1974 to 2010 (A) and the partition in Areas 1, 2 and 3 (B).

Plankton samples were collected, following Smith and Richardson (1977), with a bongo net with a 0.61 m diameter mouth opening, equipped with paired cylindrical-conical 0.333 mm and/or 0.505 mm mesh size nets towed obliquely from the surface to the maximum depth possible, not exceeding 200 m. A flowmeter was attached at the center of each mouth opening to measure the filtered water volume. Samples from only one cylindrical-conical net were analyzed, from which samples were preserved aboard in 4% seawater buffered formalin (Table V.1).

Temperature and salinity data were obtained from Nansen bottles and reversing thermometers until 1988. After this date, a CTD (conductivity-temperature depth profiler) was used. Table V.1 presents further details about each oceanographic cruise.

Table V.1 - Project name and oceanographic cruise number, month and year of the cruise, number of plankton samples obtained, bongo net mesh-size (mm) used in the present study, and El Niño and La Niña intensity of each year. *Information about years of El Niño and La Niña was obtained at: <http://enos.cptec.inpe.br>.

Project-Number	Month	Year	N Samples	Mesh-Size	El Niño*	La Niña*
ICTIO - 1	Nov/Dec	1974	139	0.505		Strong
FINEP - 1	Nov/Dec	1975	140	0.505		Strong
FINEP - 5	Dec	1976	140	0.505	Weak	
FINEP - 7	Jan	1978	134	0.505	Weak	
FINEP - 10	Jan/Feb	1979	83	0.505		
FINEP - 11	Jan	1980	42	0.505	Weak	
FINEP - 12	Jan	1981	43	0.505		
FINEP - 14	Mar	1982	32	0.505		
EPM-Sardinha	Jan	1988	78	0.333	Moderate	
V. Hensen/JOPS	Dec	1990	89	0.333	Strong	
Sardinha 1	Dec	1991	110	0.333	Strong	
Sardinha 2	Jan	1993	108	0.333	Strong	
PADCT - 1	Nov/Dec	1997	47	0.333		
PADCT - 2	May	2001	46	0.333		
DEPROAS - 3	Jan	2002	72	0.333		
ECOSAR - 4	Jan/Feb	2008	46	0.505		Strong
ECOSAR - 5	Nov	2008	60	0.505		
ECOSAR - 7	Mar	2010	55	0.505	Weak	

Under a stereomicroscope with a digital camera attached, Engraulidae eggs were sorted based on their ellipsoid shape, segmented yolk and, usually, absence of oil droplets (CIECHOMSKI, 1965; PHONLOR, 1984a), and then imaged. Major and minor axes of the photographed eggs were measured using the program ImageJ (IJ 1.46r). All Engraulidae eggs were measured, except when the number of individuals for a given oceanographic cruise exceeded 100. In these instances, about 100 eggs were randomly selected for measurement. *Engraulis anchoita* eggs were identified by discriminant analysis as described by Favero et al. (2015a, Chapter I).

Fish larvae available at ColBIO were previously sorted as Engraulidae. *Engraulis anchoita* larvae were identified following Ciechowski (1965), Phonlor

(1984b) and Bonecker et al. (2012). The classification of larvae according to their developmental stage was based on the degree of flexion of the terminal section of the notochord during caudal fin development (RICHARDS, 2006).

Data analysis

Abundance data and frequency of occurrence

Egg and larvae abundance was calculated as $N = x.d.V^{-1}$ (TANAKA, 1973), where N is the egg/larvae abundance (number of eggs or larvae/m² at each sampling station), x is the number of eggs/larvae sampled, d is the maximum depth sampled in meters, and V is the volume of water filtered in cubic meters. The mean abundance of each cruise was calculated for all the sampling stations.

The calculation of the frequency of egg/larvae occurrence (FO) was based on Guille (1970): $FO = (pi/p)*100$, where pi is the number of stations where eggs or larvae were present, and p is the total number of stations.

Modeling *E. anchoita* eggs and larvae abundance

For the modeling we standardized the data using only oceanographic stations up to 200 m and excluding the years when the whole SBB was not sampled (1979, 2002), when the only sampled stations were further away from the coast (1982, 1997, 2001), and when stations were sampled during different months than the other years (beginning of November 2008 and March 2010).

As shown in Figure V.2, many null samples were obtained, indicating that *E. anchoita* eggs and larvae count data might be zero-inflated. Zero-inflated means that the data set contained numerous zeros, and more than expected based on the Poisson or the negative binomial distribution (ZUUR et al., 2009). This excess of zeros may cause overdispersion, so we used zero-inflated models (ZI) to deal with the excessive zeros and overdispersion (ZUUR et al., 2009; OTERO et al., 2016).

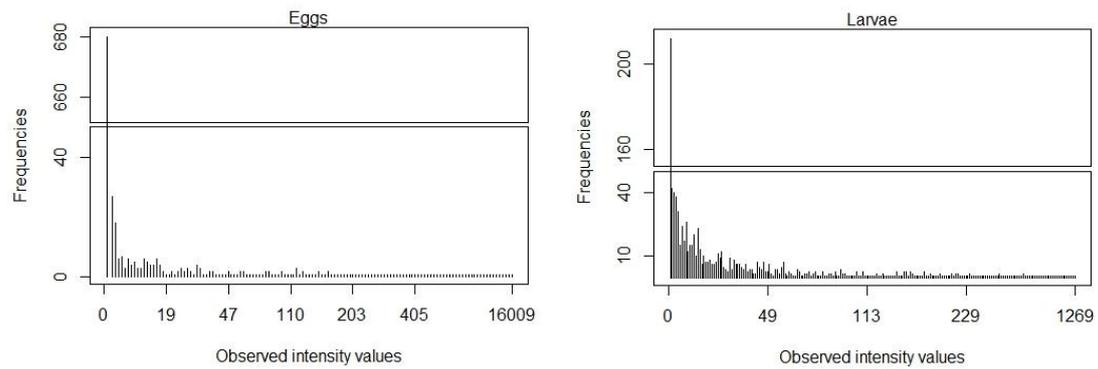


Fig. V.2. Frequency plots for *Engraulis anchoita* eggs (left) and larvae (right) data. The horizontal axis shows the number of eggs or larvae observed at each station, and the vertical axis shows the frequency of observation of each value.

ZI is a mixture model that has two components: the first contains false zeros and the second contains the count data that may produce zeros and values greater than zeros. False zeros are zeros measured due to design, survey and observer errors; they are modeled using a binomial generalized linear model (GLM). The count data were modelled with a Poisson (ZIP) or a negative binomial GLM (ZINB), and under certain covariates, we counted zero eggs or larvae (true zeros) (MARTIN et al., 2005; ZUUR et al., 2009).

Before model fitting, we estimated successive variance inflation factors (VIF) and dropped collinear covariates one at a time until all VIF values were around 3 comparing all the covariates to exclude those that presented collinearity (Table V.2) (ZUUR et al., 2010).

Table V.2. Variance Inflation Factor (VIF) for each explanatory variable considered in the models. Note that the explanatory variable with highest VIF value was dropped in each step (1-4). Temp.Sur. = surface temperature ($^{\circ}\text{C}$); Temp.10m = temperature at 10 meters ($^{\circ}\text{C}$); Sal.Sur. = surface salinity; Sal.10m = salinity at 10 meters; Depth = local depth (m); Distance = distance from the coast (km); Plankton = plankton abundance (mL/m^3).

Covariate	VIF1	VIF2	VIF3	VIF4
Temp.Sur.	3.23	3.07	2.61	1.11
Temp.10 m	3.44	3.41	<u>2.82</u>	
Sal.Sur.	5.06	5.05	1.68	1.67
Sal.10 m	5.17	<u>5.10</u>		
Depth	6.48	2.89	2.80	2.79
Distance	3.62	2.48	2.47	2.42
Plankton	1.08	1.07	1.07	1.02
Latitude	11.87	1.23	1.22	1.15
Longitude	<u>15.73</u>			

From previous knowledge, we hypothesized that the false zeros were caused by: the mesh size used (Chapter II), the area sampled (Chapter III), the distance from the coast and the local depth of each oceanographic station (Chapter IV). The count process was then modeled as a function of surface temperature, surface salinity, distance from the coast, local depth, latitude, areas (as defined in Chapter III) and years sampled. Following Otero et al. (2016), we used the volume of water filtered (in m^3 and \ln -transformed) as an offset in the count part of the ZI models. Model selection was performed excluding non-significant covariates in both parts of the model and models with and without the non-significant covariate were compared using Akaike Information Criterion (AIC), and the one with lowest value was considered the “best” model. We also contrasted the optimal ZI models using Poisson and negative binomial distributions. In the end, we used the residuals of the optimal models for validation.

The data treatment and analyses were performed using R (R CORE TEAM, 2015), and the “MASS 7.3-26” (VENABLES; RIPLEY, 2002) and “pscl 1.04.4” (ZEILEIS et al., 2008) packages.

V.3. RESULTS

Environmental data

Surface temperature obtained during the sample period of each year varied from 14.9°C to 29.1°C (mean = $24.6 \pm 1.9^\circ\text{C}$); at 10 meters depth, the temperature range was from 12.7°C to 28.8°C (mean = $23.6 \pm 2.5^\circ\text{C}$). Horizontal distribution of the surface temperature (the depth considered in the models) is represented in Figure V.3.

Surface salinity varied from 28.1 to 38.5 (mean = 35.4 ± 1.1); at 10 meters depth, the range was from 32.7 to 38.5 (mean = 35.6 ± 0.9). Horizontal distribution of the surface salinity is represented in Figure V.4.

In this section we did not present the horizontal distribution of temperature and salinity at 10 m depth because these variables were excluded in the model, as shown in Table V.2.

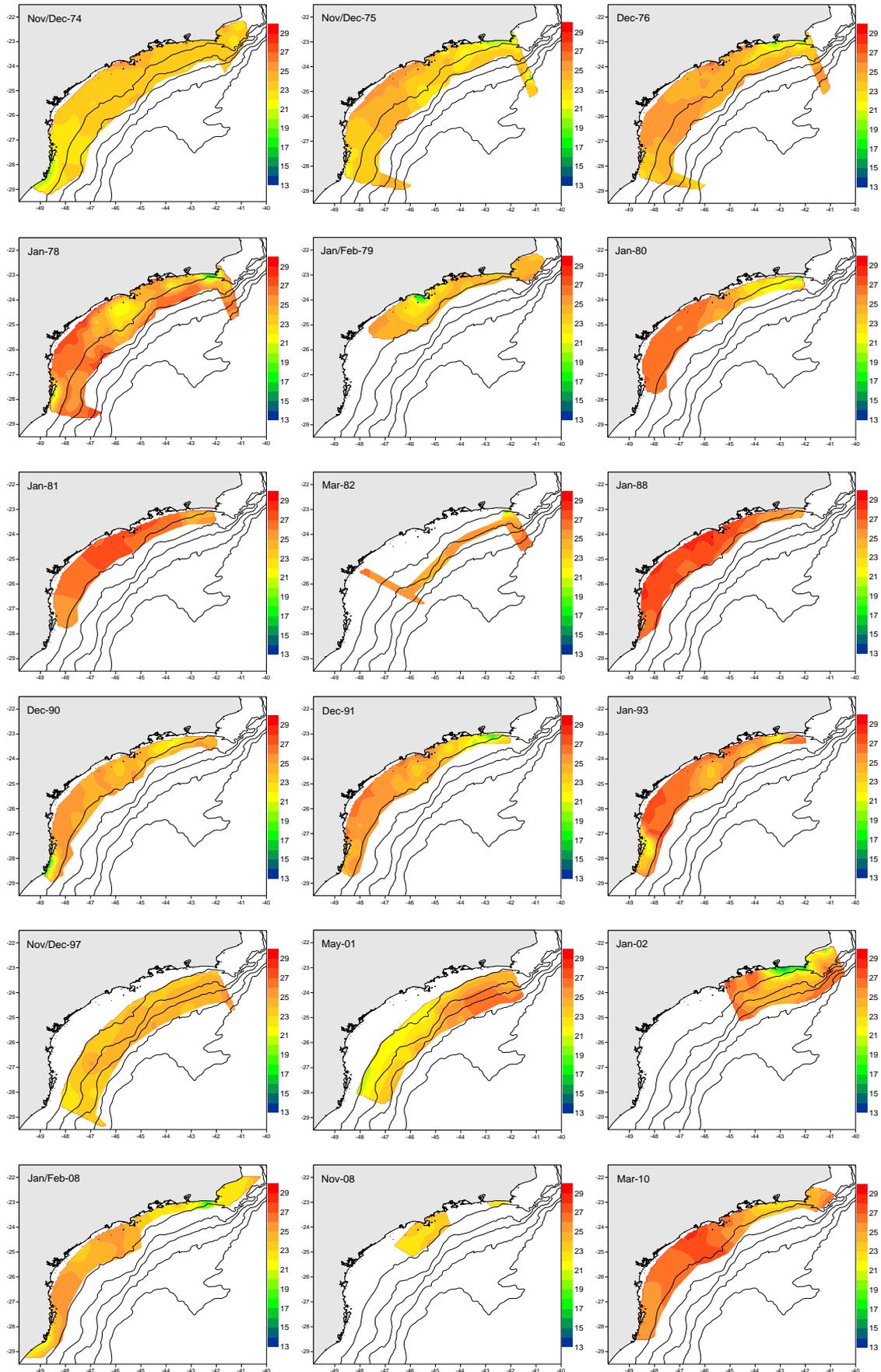


Fig. V.3. Horizontal distribution of salinity obtained at the surface during the sampled period.

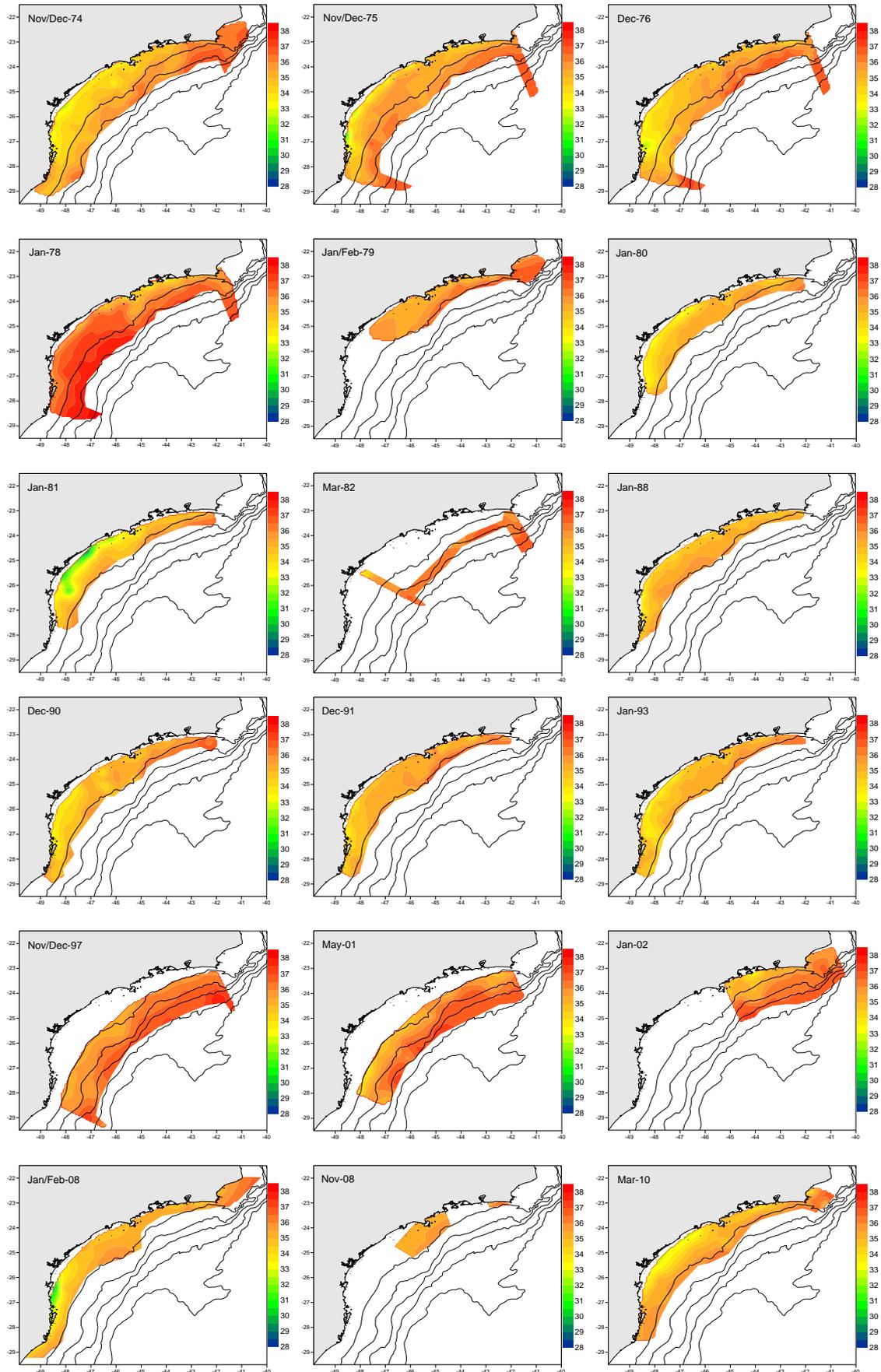


Fig. V.4. Horizontal distribution of salinity obtained at the surface during the sampled period.

Engraulis anchoita data

A total of 102,274 Engraulidae eggs were sampled. *Engraulis anchoita* represented 83.1 % of all engraulid eggs sampled (n = 85,024). Of 1,464 sampling stations, Engraulidae eggs occurred at 29.0% and *E. anchoita* eggs at 24.7 % of them (Table V.3).

Engraulis anchoita larvae totaled 64,403 individuals. Preflexion larvae were the most abundant (n = 39,962), followed by flexion (n = 12,551) and postflexion (n = 11,406). No yolk-sac larvae were obtained and 484 larvae could not be classified by stage of development because they were damaged. Engraulidae larvae occurred at 74.9% of all sampling stations, with *E. anchoita* comprising 69.6% (Table V.3).

Table V.3. Number of stations where *Engraulis anchoita* eggs and larvae were collected (Positive Stations) and their frequency of occurrence (FO) for each year analyzed.

Month-year	Number of stations	<u>Eggs</u>		<u>Larvae</u>	
		Positive Stations	FO (%)	Positive Stations	FO (%)
Nov/Dec-74	139	30	21.6	98	70.5
Nov/Dec-75	140	31	22.1	115	82.1
Dec-76	140	20	14.3	96	68.6
Jan-78	134	18	13.4	86	64.2
Jan/Feb-79	83	19	22.9	58	69.9
Jan-80	42	9	21.4	35	83.3
Jan-81	43	15	34.9	35	81.4
Mar-82	32	2	6.3	18	56.3
Jan-88	78	30	38.5	64	82.1
Dec-90	89	46	42.9	58	65.2
Dec-91	110	47	47.4	82	74.6
Jan-93	108	48	44.4	88	81.5
Nov/Dec-97	47	0	0	18	38.3
May-01	46	1	2.2	12	26.1
Jan-02	72	20	27.8	34	47.2
Jan/Feb-08	46	13	28.3	39	84.8
Nov-08	60	9	15.0	46	76.7
Mar-10	55	3	5.5	37	67.3
Total	1464	361	24.7	1019	69.6

Years that had low *E. anchoita* egg abundance were those sampled in months out of the spawning-peak period, such as November 2008, at stations further away from the coast, such as November/December 1997 or for a combination of both reasons, as in March 1982 and May 2001 (Table V.3 and Figure V.5).

The frequency of occurrence was higher for larvae than eggs in most years (Table V.3). Mean abundance varied considerably, and in some years it was higher for larvae, in others for eggs (Table V.4). However, maximum abundance was commonly higher for eggs than for larvae, with a huge difference value (Table V.4).

Table V.4. Mean, standard deviation (sd) maximum (Max) abundance of eggs (eggs/m²) and larvae (larvae/m²) in each period analyzed.

<u>Month-Year</u>	<u>Eggs</u>		<u>Larvae</u>	
	Mean \pm sd	Max	Mean \pm sd	Max
Nov/Dec-74	4.5 \pm 28.0	307.9	4.4 \pm 9.3	64.5
Nov/Dec-75	22.1 \pm 217.5	2551	18.7 \pm 35.2	220.1
Dec-76	1.6 \pm 8.6	5	8.4 \pm 13.8	99.3
Jan-78	2.3 \pm 17.9	183.4	18.1 \pm 45.2	308.2
Jan/Feb-79	10.3 \pm 62.8	411.4	7.8 \pm 35.5	318.4
Jan-80	21.4 \pm 125.1	809.8	5.9 \pm 9.0	42.5
Jan-81	3.0 \pm 10.9	51.7	14.3 \pm 24.3	112.3
Mar-82	0.3 \pm 1.8	10.3	8.3 \pm 15.7	71.3
Jan-88	19.7 \pm 73.5	506.5	24.9 \pm 68.9	468.7
Dec-90	16.1 \pm 52.3	427.2	4.3 \pm 8.9	53.6
Dec-91	20.6 \pm 68.6	493.6	38.1 \pm 65.0	388.6
Jan-93	40.1 \pm 235.3	2399.7	13.7 \pm 24.9	127.6
Nov/Dec-97	0	0	9.1 \pm 28.5	181.2
May-01	0.2 \pm 1.5	10.1	1.6 \pm 5.9	36.8
Jan-02	32.9 \pm 148.8	1083.5	21.7 \pm 96.7	774.3
Jan/Feb-08	7.2 \pm 36.7	247.3	14.7 \pm 31.3	181
Nov-08	0.1 \pm 0.5	3.3	12.7 \pm 25.8	124.6
Mar-10	2.3 \pm 11.8	64.2	7.1 \pm 15.5	86.1

The spatial distribution of eggs and larvae is shown in Figure V.5. Eggs were usually more aggregated than larvae (fewer stations with higher abundance), while larvae were sampled throughout the studied area, even at stations far away from where eggs were sampled, as in the years 1976 and 1980, and further away from the coast, as in the years 1997 and 2001 (Figure V. 5).

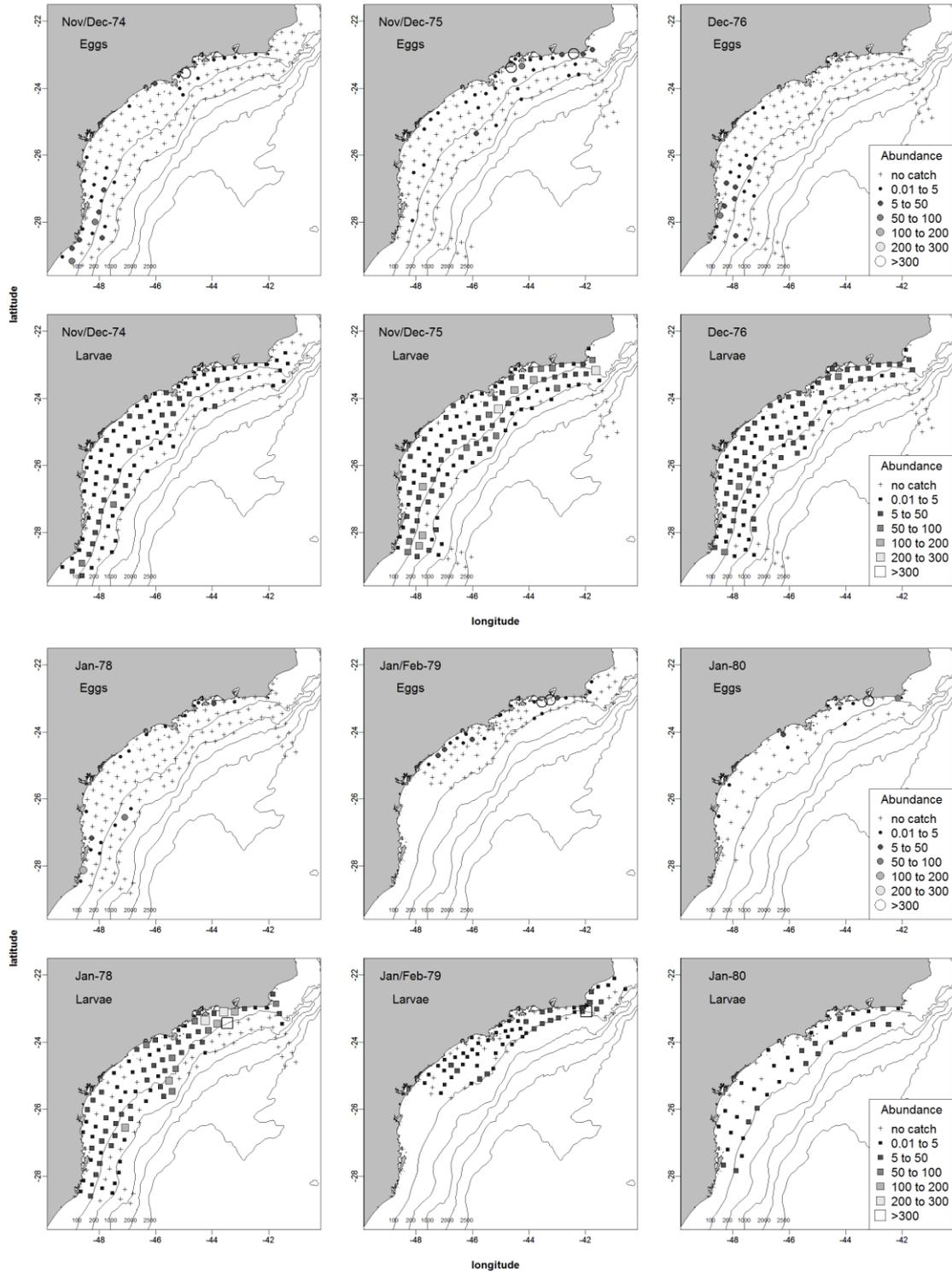


Fig. V. 5. Spatial distribution of *Engraulis anchoita* eggs (upper) and larvae (lower) abundance (in m^2) sampled in the Southeastern Brazilian Bight during 18 different periods.

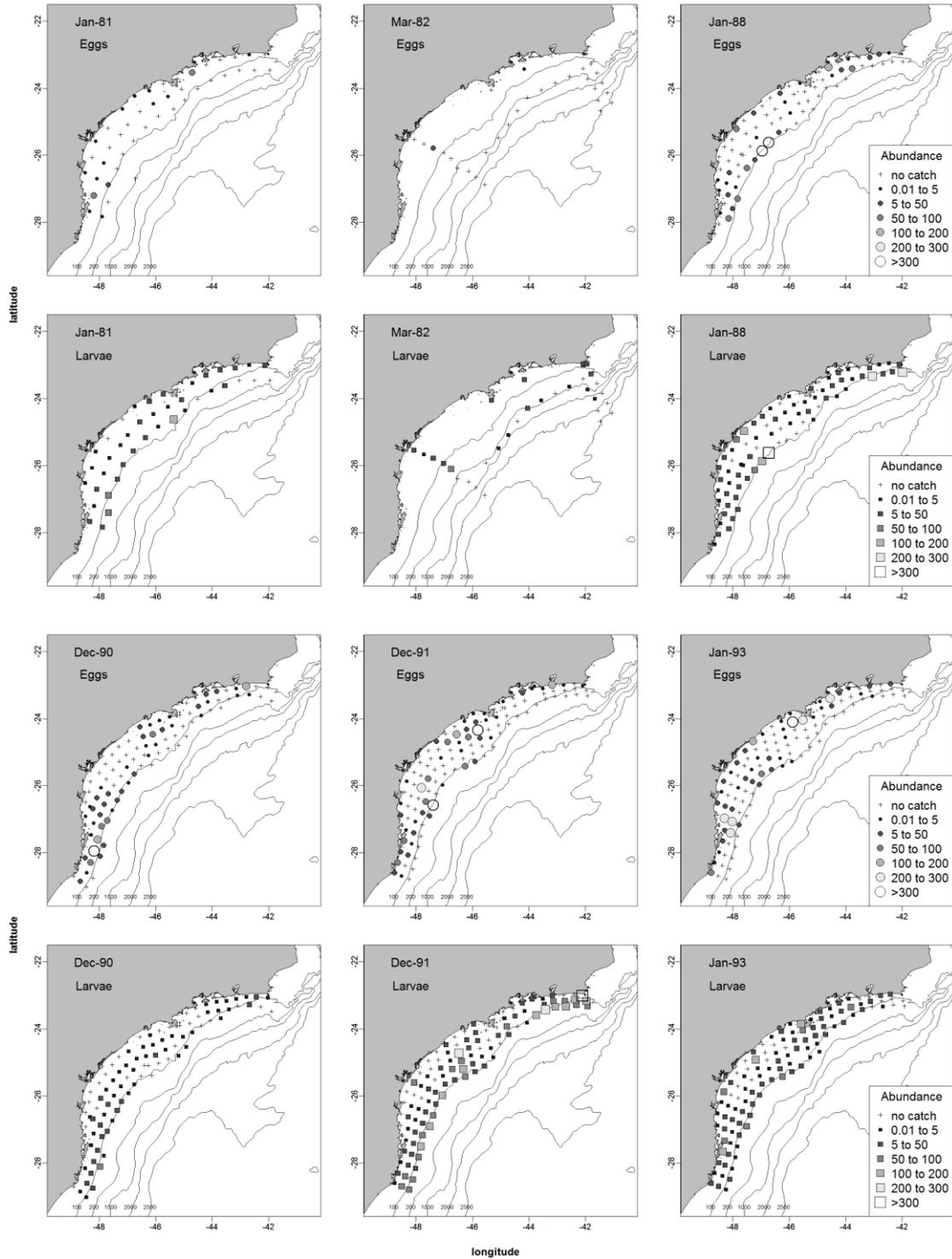


Fig. V. 5. (cont.). Spatial distribution of *Engraulis anchoita* eggs (upper) and larvae (lower) abundance (in m^2) sampled in the Southeastern Brazilian Bight during 18 different periods.

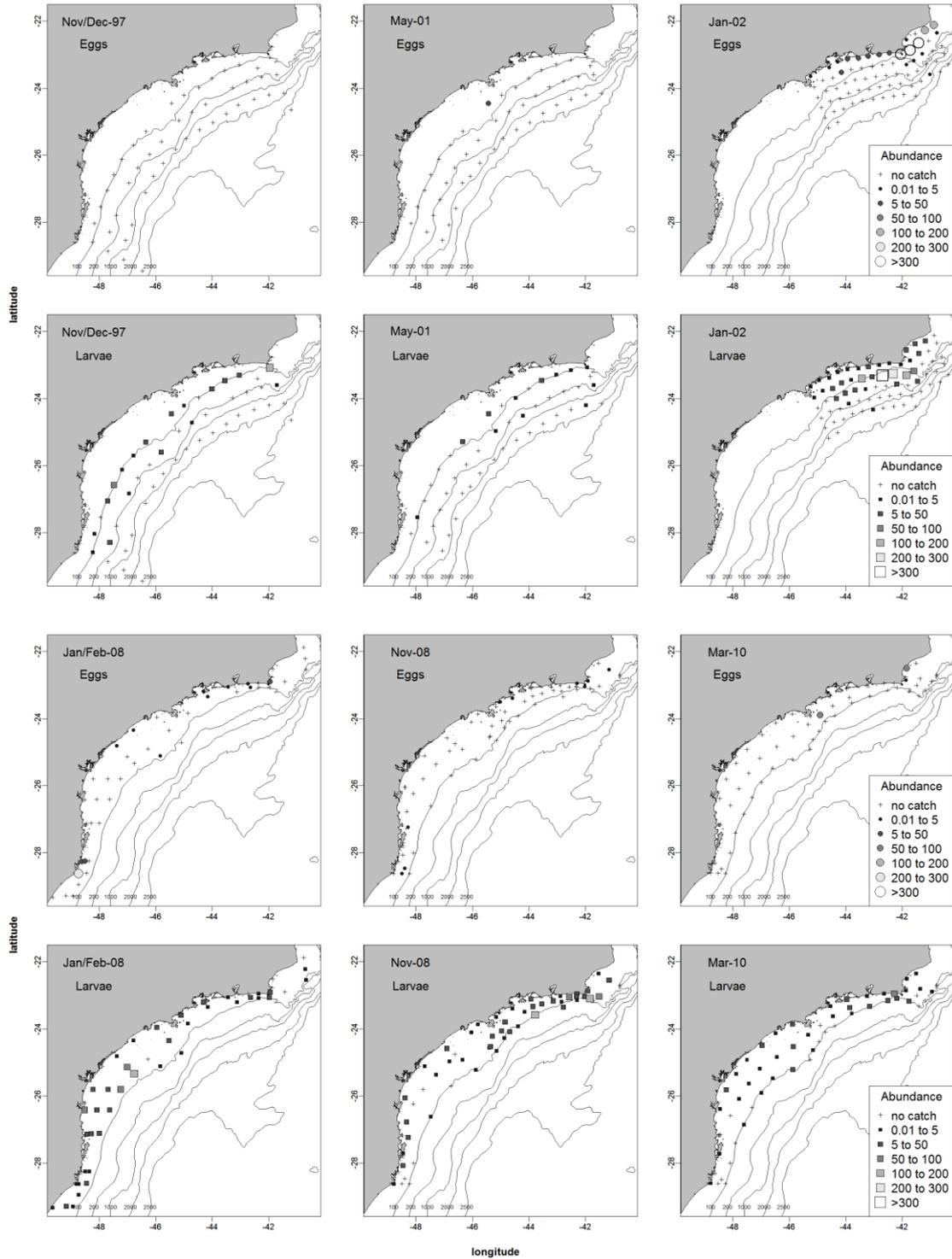


Fig. V. 5. (cont.). Spatial distribution of *Engraulis anchoita* eggs (upper) and larvae (lower) abundance (in m^2) sampled in the Southeastern Brazilian Bight during 18 different periods.

Models relating abiotic and biological data

The results of the final ZINB model fitted to *E. anchoita* egg abundance is presented in Table V.5 and the model selection process in Table V.6. The expected counts of *E. anchoita* eggs decreased as surface temperature and local depth increased (Table V.5). In the years that had significant influence on *E. anchoita* eggs counts, this influence was positive (Table V.6). In those years a 0.333 mm mesh size net was used, and moderate or strong El Niño conditions occurred (Table V.1). Note that the Model 8 had the AIC increased when the non-significant parameter local depth was dropped from final model (Model 7) (Table V.6). In addition, the probability of false zeros was the lowest in Area 3 and higher in Area 2 than Area 1 for both mesh sizes (Fig. V.6). Sampling with the 0.505 mm mesh size increased the probability of false zeros for all areas and it was less probable to catch *E. anchoita* eggs as the local depth increased (Fig. V.6). The final model with a Zero-Inflated Poisson distribution was not more optimal than the ZINB (AIC ZIP = 280376.212) and we could not detect any pattern in the model residuals (Fig. V.7).

Table V.5. Summary of the optimal Zero-Inflated Negative Binomial (ZINB) model fitted to the *E. anchoita* eggs abundance. S.E. = Standard Error; Temp.Sur. = surface temperature (°C); Depth = local depth (m); Mesh 0.505 = 0.505 mm mesh size.

Parameter	Estimate	S.E.	z-values	P-value
<i>Count model coefficients</i>				
Intercept	8.26277	2.695008	3.066	0.00217
Temp.Sur.	-0.372123	0.110103	-3.38	0.000725
Depth	-0.011622	0.006014	-1.933	0.053292
Year 1975	0.336762	0.694043	0.485	0.627522
Year 1976	-0.841905	0.599724	-1.404	0.160372
Year 1978	-0.476433	0.72392	-0.658	0.510455
Year 1980	0.456744	0.800565	0.571	0.56832
Year 1981	0.07341	0.78694	0.093	0.925677
Year 1988	1.880914	0.660089	2.849	0.004379
Year 1990	0.606619	0.556205	1.091	0.275432
Year 1991	1.098819	0.536297	2.049	0.040472
Year 1993	2.45227	0.567984	4.317	1.58e ⁻⁰⁵
Year 2008	-1.005185	0.794611	-1.265	0.205871
<i>Logistic model coefficients</i>				
Intercept	-3.273006	0.624991	-5.237	1.63e ⁻⁰⁷
Depth	0.031516	0.005042	6.251	4.08e ⁻¹⁰
Mesh 0.505	1.437894	0.367584	3.912	9.16e ⁻⁰⁵
Area 2	0.283414	0.345413	0.821	0.412
Area 3	-2.08128	0.513442	-4.054	5.04e ⁻⁰⁵

Table V.6. Comparison between a set of models for *E. anchoita* eggs using the Akaike Information Criterion (AIC). Non-significant covariates with the highest p-value were sequentially dropped from the false zero process and from the count part. TS = surface temperature (°C); SS = surface salinity; Dep.= local depth (m); Dist. = distance from the coast (km); Plank. = plankton abundance (m³); Mesh = mesh size (0.333 and 0.505 mm); Area = 3 different areas sampled; Years = different years sampled. The optimal model is in bold.

Model	False zeros	Count part	AIC	ΔAIC
1	Dep.+Dist.+Mesh+Area	TS+SS+Dist.+Dep.+Plank.+Lat.+Area+Years	4142.80	11.00
2	Dep.+Mesh+Area	TS+SS+Dist.+Dep.+Plank.+Lat.+Area+Years	4140.88	9.08
3	Dep.+Mesh+Area	TS+SS+Dep.+Plank.+Lat.+Area+Years	4138.89	7.09
4	Dep.+Mesh+Area	TS+Dep.+Plank.+Lat.+Area+Years	4136.90	5.10
5	Dep.+Mesh+Area	TS+Dep.+Plank.+Area+Years	4134.96	3.17
6	Dep.+Mesh+Area	TS+Dep.+Plank.+Years	4132.95	1.15
7	Dep.+Mesh+Area	TS+Dep.+Years	4131.80	0.0
8	Dep.+Mesh+Area	TS+Years	4133.65	1.85

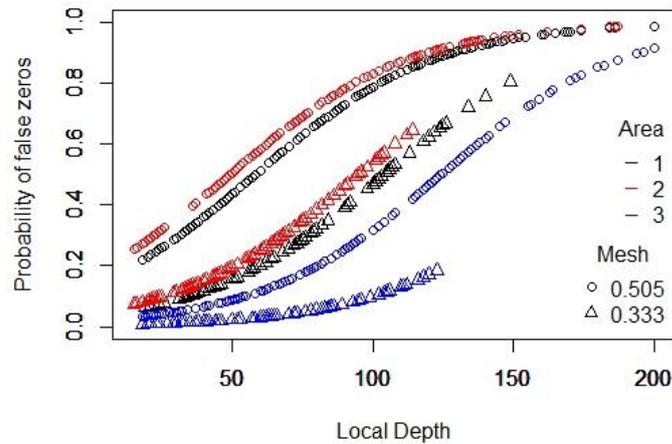


Fig. V.6. Predicted probabilities of false zeros for *E. anchoita* eggs in the different mesh size used and areas sampled as a function of the local depth (m) based on the binomial part of the Zero-Inflated Negative Binomial (ZINB) model.

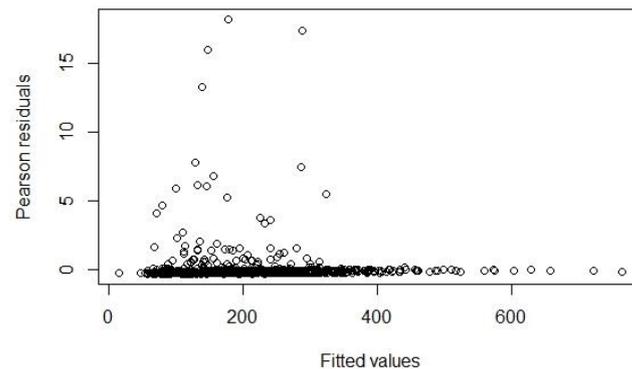


Fig. V.7. Scatterplot of Pearson residuals vs. fitted values for the optimal *E. anchoita* eggs model.

The results of the final ZINB model fitted to *E. anchoita* larvae abundance is presented in Table V.7 and the model selection process in Table V.8. Covariates that explained the larvae abundance and the probability of false zeros were slightly different from the ones observed for eggs. The expected counts of *E. anchoita* larvae decreased as surface temperature and salinity increased. Note that dropping latitude (non-significant) from the final Model 4 slightly increased the AIC value (Model 5), but after that, dropping non-significant covariates caused a decrease in the AIC value (Table V.7). With the exception of the year 1980, all the other years had significant positive

influences on *E. anchoita* larvae counts (Table V.8). The probability of false zeros was highest in Area 1, followed by Areas 2 and 3. After a certain local depth, the probability of false zeros increased as the depth of the sample station increased, irrespective of the area (Fig.V.8). The final model with a Zero Inflated Poisson distribution was not more optimal than the ZINB (AIC ZIP = 87366.607) and we could not detect any pattern in the model residuals (Fig. V.9).

Table V.7. Summary of the optimal Zero-Inflated Negative Binomial (ZINB) model fitted to the *E. anchoita* larvae abundance. S.E. = Standard Error; Temp.Sur. = surface temperature (°C); Sal.Sur. = surface salinity; Depth = local depth (m).

Parameter	Estimate	S.E.	z-values	P-value
<i>Count model coefficients</i>				
Intercept	18.5014	2.93736	6.299	3.00e ⁻¹⁰
Temp.Sur.	-0.14279	0.04079	-3.5	0.000465
Sal.Sur.	-0.51044	0.07162	-7.127	1.03e ⁻¹²
Year 1975	1.67138	0.22173	7.538	4.77e ⁻¹⁴
Year 1976	1.2966	0.22957	5.648	1.62e ⁻⁰⁸
Year 1978	2.42117	0.26064	9.289	< 2e ⁻¹⁶
Year 1980	0.48295	0.30423	1.587	0.112406
Year 1981	1.54079	0.33024	4.666	3.08e ⁻⁰⁶
Year 1988	2.44804	0.29538	8.288	< 2e ⁻¹⁶
Year 1990	0.4884	0.24802	1.969	0.048933
Year 1991	2.58217	0.23529	10.974	< 2e ⁻¹⁶
Year 1993	1.96652	0.24873	7.906	2.65e ⁻¹⁵
Year 2008	1.76852	0.29832	5.928	3.06e ⁻⁰⁹
<i>Logistic model coefficients</i>				
Intercept	-7.305061	1.169268	-6.248	4.17e ⁻¹⁰
Depth	0.051752	0.009023	5.736	9.72e ⁻⁰⁹
Area 2	-1.89996	0.753668	-2.521	0.011704
Area 3	-2.794969	0.798588	-3.5	0.000465

Table V. 8. Comparison between a set of models for *E. anchoita* larvae using the Akaike Information Criterion (AIC). Non-significant covariates with the highest p-value were sequentially dropped from the false zero process and from the count part. TS = surface temperature (°C); SS = surface salinity; Dep.= local depth (m); Dist. = distance from the coast (km); Plank. = plankton abundance (m³); Mesh = mesh size (0.300 and 0.500 mm); Area = 3 different areas sampled; Years = different years sampled. The optimal model is in bold.

Model	False zeros	Count part	AIC	ΔAIC
1	Dep.+Dist.+Mesh+Area	TS+SS+Dist.+Dep.+Plank.+Lat.+Area+Years	8445.40	9.18
2	Dep.+Mesh+Area	TS+SS+Dist.+Dep.+Plank.+Lat.+Area+Years	8440.55	4.33
3	Dep.+Area	TS+SS+Dist.+Dep.+Plank.+Lat.+Area+Years	8438.90	2.68
4	Dep.+Area	TS+SS+Dist.+Dep.+Lat.+Area+Years	8438.24	2.02
5	Dep.+Area	TS+SS+Dist.+Dep.+Area+Years	8438.27	2.05
6	Dep.+Area	TS+SS+Dist.+Dep.+Years	8437.93	1.71
7	Dep.+Area	TS+SS+Dist.+Years	8436.61	0.39
8	Dep.+Area	TS+SS+Years	8436.22	0.0

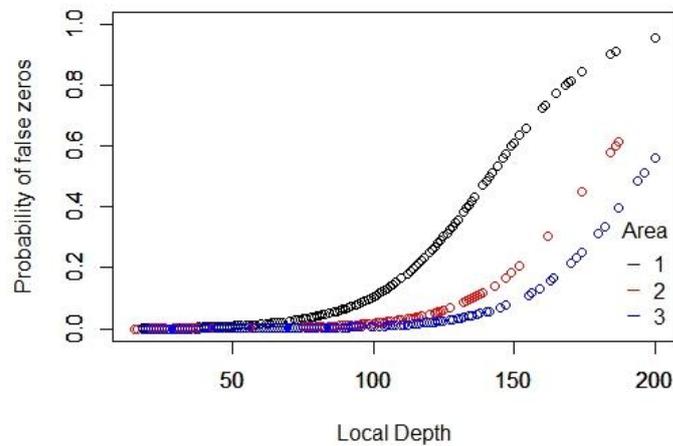


Fig. V.8. Predicted probabilities of false zeros for *E. anchoita* larvae in the areas sampled as a function of the local depth (m) based on the binomial part of the ZINB model.

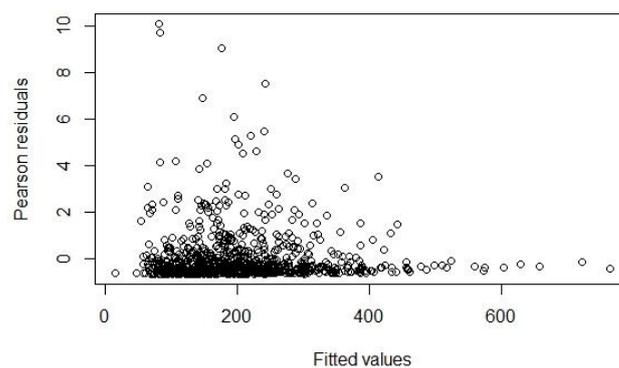


Fig. V.9. Scatterplot of Pearson residuals vs. fitted values for the optimal *E. anchoita* larvae model.

V.4. DISCUSSION

Most ichthyoplankton species exhibit patchy distributions (HEWITT, 1981; MCGURK, 1986; FRANK, 1993; MATSUURA; HEWITT, 1995; BRADBURY et al., 2003; MAYNOU et al., 2006). A combination of biological (e.g. spawning and feeding) with oceanographic factors (e.g. ocean circulation patterns and temperature) drives the distribution and abundance of early life stages of pelagic fishes, contributing to their commonly uneven distributions (BRADBURY et al., 2003). During sampling, such uneven distributions may result in a low number of samples with a high amount of

individuals and a high number of samples with low or zero individuals (METHVEN et al., 2003). Those zeros in the data set, common in fish egg and larvae research (e.g. PENNINGTON, 1983; BORCHERS et al., 1997; FOX et al., 2000; CIANNELLI et al., 2007; KNUTSEN et al., 2007; and the present thesis), can be categorized as false or true zeros. False zeros are those attributed to the absence of the species due to design, survey and observer errors; meanwhile true zeros are those caused by habitat unsuitability or environmental processes (MARTIN et al., 2005; ZUUR et al., 2009).

Data sets with an inflation of zero counts (zero-inflated counts), are harder to categorize within the statistical distribution families that were available and may cause bias in the variance estimates and inferences (WELSH et al., 1996). Not considering the zero inflation and/or overdispersion while modeling may result in incorrect predictions at each site and may cause optimistic conclusions about the significance of the explanatory variables (BARRY; WELSH, 2002; POTTS; ELITH, 2006). Thus, ichthyoplankton were usually modelled considering only the non-zero counts (e.g. CIANNELLI et al., 2007; KNUTSEN et al., 2007) or using a two-step approach (e.g. BORCHERS et al., 1997; FOX et al., 2000). The two-step approach first models only the presence-absence data using the binomial distribution, and then models the abundance considering only the positive stations and using a truncated Poisson or a negative binomial distribution (WELSH et al., 1996). The two-step approach was also used to model cephalopod paralarvae (MORENO et al., 2009; STAAF et al., 2013) that exhibit a data set similar to ichthyoplankton. Models that consider only the positive stations exclude any information carried by the zero counts. Meanwhile, the two-step approach considers the null-samples information, but to make any inference is complicated as there are two sets of environmental influences to deal with, one from the binary and one from the abundance model, usually with different results (BARRY; WELSH, 2002). More appropriate than the approaches cited above is the use of zero-inflated models, which assumes that the abundance data may include zero and non-zero values and also deals with the different types of zeros (MARTIN et al., 2005; MINAMI et al., 2007).

Zero-inflated models have been used in many areas of research (e.g. HALL, 2000; AGARWAL et al., 2002; MAUNDER; PUNT, 2004; MINAMI et al., 2007; OTERO et al., 2016), but, to our knowledge, they have not been used in any ichthyoplankton study to date.

Aggregation or patchiness index curves in pelagic fish species usually exhibit a “U” shape: high initial patchiness of eggs reflecting the adult aggregation behavior followed by a rapid decline as the larvae disperse due to hydrographic factors, and then with an increase of patchiness as the developing fish begin to aggregate in schools (e.g. HEWITT, 1981; HOUDE; LOVDAL, 1985; MATSUURA; HEWITT, 1995). Although we did not calculate these indexes, it is possible to infer that *E. anchoita* eggs are more aggregated than larvae, since the frequency of occurrence of larvae was higher in all periods analyzed, larvae were sampled even in stations far away from where eggs were obtained, and egg maximum abundance was commonly the highest. As consequence, different environmental conditions could influence egg and larvae distribution and abundance, so we modeled the stages separately. This distribution pattern was also observed in *E. anchoita* eggs and larvae for the Uruguayan and Argentinian coasts, where the larvae were more widespread (MARRARI et al., 2013)

Engraulis anchoita egg abundance sharply decreased toward deeper sites, which was also noted in Chapter IV. Katsuragawa et al. (2014) also related greater abundance of *E. anchoita* larvae to shallower stations. Common to both stages, the probability of false zeros increased as the sampling sites became deeper, but for larvae this relationship only occurred deeper than around 75 meters in Area 1 and around 125 meters in Areas 2 and 3 (Fig. V.6 and Fig. V.8). The probability of false zeros was lower in Area 3 than in the other two areas, for both eggs and larvae. Area 3 included the largest patch with a high probability of egg presence as defined in Chapter IV, and as a consequence many larvae were expected.

The difference in the mesh-size probability of false zeros was only significant in the egg binomial part of the ZI model, being higher for the 0.505 mm mesh-size in all areas. This result agrees with Favero et al., 2015b (Chapter II), which showed that FO of *E. anchoita* eggs was higher in the 0.333 mm than the 0.505 mm mesh-size net. It is also important to note in Figure V.6 that until around 50 meters local depth the difference in the probability of false zeros between both mesh sizes was smaller in Area 3 than the other two areas analyzed. This difference may be caused by regional variation. As shown in Chapter III, eggs from Area 3 were usually larger than those from northern areas. According to Matsuura and Nakatani (1980) the 0.333 mm mesh net collected more *E. anchoita* eggs than the 0.505 mm mesh net in the Santos area, in the central part of the SBB; on the other hand, Sanchez and Ciechomski (1984) did not observe any difference in *E. anchoita* egg abundance between both mesh sizes in Uruguay and

Argentina. The locale where the samples came from caused this difference, as the minor and major axes of the *E. anchoita* eggs sampled on the Uruguayan and Argentinian coasts were larger than the ones sampled in the Santos area (SANCHEZ; CIECHOMSKI, 1984).

The non-significant difference of the mesh size probability of false zeros in the larvae binomial part of the ZI model may be related to the fact that we did not obtain any yolk-sac larvae, the initial larval stage that is more prone to extrusion by larger mesh sizes (HOUDE; LOVDAL, 1984; LESLIE; TIMMINS, 1989; JOHNSON; MORSE, 1994; SOMARAKIS, et al., 1998). Matsuura and Nakatani (1980) compared the catches of *E. anchoita* larvae performed with bongo nets fitted with 0.333 and 0.505 mm mesh-sizes, and found that larvae were obtained with both nets in all oceanographic stations, but the finer mesh retained more larvae than the coarse one. However, the authors showed that this difference was only significant when analyzing larvae up to 7 mm standard length.

The *E. anchoita* preference for certain temperature and salinity ranges may change according to the season of the year, latitude and developmental stages, with the early life stages more tolerant than adults (MADUREIRA et al., 2009). This species is widely distributed and is tolerant of a broad range of temperatures and salinities (CIECHOMSKI 1967, MATSUURA; KITAHARA 1995; TORQUATO; MUELBERT, 2014). Brewer (1976), in controlled laboratory experiments, found that *Engraulis mordax* eggs hatch at temperatures between 8.5°C and 28.5°C and larvae tolerated from 7°C to 30°C. However, the same author showed that the number of larvae developing in a normal manner decreased when temperatures were outside of the range from 11.5°C to 27°C, highlighting the importance of temperature in the early life stages. In fact, the present study showed that temperature was a significant covariate in the count part of the model for both, eggs and larvae. These abundances exhibited negative relationships with temperature, in agreement with the results from Macedo-Soares et al. (2014) and Katsuragawa et al. (2014). On the other hand, salinity was a significant covariate only in the larvae count part of the model, also exhibiting a negative relationship, in agreement with Katsuragawa et al. (2014).

Environmental parameters that were not measured in the present study may influence ichthyoplankton abundance and distribution. For example, *E. anchoita* larvae abundance was negatively associated with strong currents near a shelf front (AUAD; MARTOS, 2012) and positively correlated with oxygen stratification, silicate and

phosphate concentration (MACEDO-SOARES et al., 2014). Eggs were positively correlated with abundance of small species of copepods (VIÑAS et al., 2002). Bakun and Parrish (1991) reiterated the importance of a balance between different physical processes that affect water column stability, retention in a favorable habitat and nutrient enrichment that provides appropriate larval food particles in the selection of the spawning habitat.

Some of these environmental conditions that were not evaluated in this study may be masked in the high interannual variation observed in the abundance and distribution of *E. anchoita* eggs and larvae, reinforced with the fact that the different years sampled were a significant covariate for both stages in the count part of the model. *Engraulis anchoita* has a relatively low trophic position in the marine food web (CAPITANIO et al., 2005) and lives only a short period of time (1-4 years) (CARVALHO; CASTELLO, 2013). Those factors make this species very susceptible to environmental variability (BAKUN, 1996), resulting the large interannual fluctuations observed. Those fluctuations in small pelagic fish populations are common in different species worldwide (see LLUCH-BELDA et al. 1992; SCHWARTZLOSE et al. 1999; OOZEKI et al., 2007; CHECKLEY et al., 2009; ASCH; CHECKLEY, 2013).

One oceanic-atmospheric phenomenon that scientists have tried to relate to such small pelagic fish fluctuations is El Niño (e.g. FIEDLER, 1983; KIM, et al., 2005; ASCH, 2015). El Niño is a recurrent, quasi-permanent occurrence of abnormal warming of surface waters in the central equatorial Pacific Ocean. Sometimes, this anomaly can reverse causing the cooling of the surface waters in the same region, a phenomenon named La Niña (DIJKSTRA, 2006). In the Peru–Humboldt system, primary production declines drastically during an El Niño episode, followed by a decline in the zooplankton abundance (BARBER; CHAVEZ, 1983). The Peruvian anchoveta landings do extremely poorly during these episodes (BAKUN et al., 2003).

El Niño is also related to an increase in the precipitation in southeastern South America and as consequence an increase in the river flow in the Plata Basin (ACEITUNO, 1988; ROPELEWSKI; HALPERT, 1989; MECHOSO; IRIBARREN, 1992; PIOLLA, et al. 2005). The higher river discharges in the Plata River estuary and in the Patos lagoon during El Niño are related negatively to recruitment of Whitemouth Croaker *Micropogonias furnieri* (ACHA et al., 2012) and production of Pink Shrimp *Farfantepenaeus paulensis* (MÖLLER Jr. et al., 2009).

In the western South Atlantic, El Niño events are related to negative sea surface temperature (SST) anomalies in the Malvinas Current and positive SST anomalies in the Brazil Current (SEVEROV et al., 2004). According to Macedo-Soares (2015), El Niño induces an increase of Plata River outflow and a decrease of Malvinas current SST, negatively influencing the *E. anchoita* stock from the south of Brazil. Higher Plata River outflow increases offshore transport of *E. anchoita* eggs and larvae far from a suitable area for their development (SANCHÉZ; CIECHOMSKI, 1995) and water cooling can slow larval development, reducing survival rates (PEPIN, 1991).

In the studied area, the occurrence of strong El Niño tends to favor the offshore transport of water and the upwelling of the SACW (PAES; MORAES, 2007). As consequence, the primary production is expected to increase, increasing the *S. brasiliensis* catches one or two years after an intense event. On the other hand, after a “moderate” or “weak” El Niño, or a strong La Niña, the pelagic fishery production would decrease (PAES; MORAES, 2007). Our results showed that years when moderate or strong El Niño occurred, *E. anchoita* egg abundance was positively influenced, but further analyses are necessary to better understand this relationship. As discussed in Chapter IV, it is important to be cautious when relating eggs and larvae distribution and abundance to SACW intrusion. In years when the SACW intrusion is weak, there may be an increase in larval mortality due to a decrease in the zooplankton biomass (KATSURAGAWA et al., 2006). At the same time, the wind-induced upwelling at Cape Frio tends to transport individuals to unsuitable areas and increase larval mortality, so a strong upwelling would also increase the larval mortality rates (DIAS et al., 2014).

We are aware of the imperfection of our data set: samples were not collected every year during the 40-year period, the spatial location of the oceanographic stations varied and two different mesh sizes were used. Beyond that, a wide range of environmental conditions could be considered, as discussed above. Remotely-sensed data could be obtained and used, but they do not cover some earlier years. Nonetheless, we believe that the best long-term *E. anchoita* egg and larvae data from the SBB were analyzed in the present study, using one of best statistical approach available and *in situ* abiotic measurements. Our results provide new information about the early life history of one important pelagic species that, although not yet commercially fished in the studied area, can provide subsidies for the species management in southern of Brazil, Argentina and Uruguay.

5. CONCLUSIONS

The results presented in this doctoral dissertation provide new insights into the early life history of *Engraulis anchoita*, a fish species that is ecologically and economically important. The information provided will not just support *E. anchoita* fishery management, but will also support future studies, presenting faster and more accurate methodology to identify *E. anchoita* eggs (Chapter I), and discussing the mesh-size used to sample them (Chapter II).

Since there are no physical barriers in the ocean, defining stock limits is difficult, particularly for a semi-migratory fish species such as *E. anchoita*. Eggs from the south of the Southeastern Brazilian Bight (SBB) were larger than the ones from the north (Chapter III), showing that eggs from the Santa Marta Cape region probably belong to the *bonaerense* stock, and not to the stock occurring throughout most of the studied area. A seasonal difference between egg sizes was also noticed, with those obtained during the winter being larger than the summer ones (Chapter III). Although it was not possible to conclude from our data what caused this difference, some hypotheses were raised and discussed: 1) larger adults coming from the south would be spawning in or closer to the studied area in winter, guided by the Plata River Water flow; and 2) different egg sizes were an adaptive response to the changes in the abiotic conditions encountered by the eggs.

The high interannual variability in the abundance and distribution of eggs (Chapter IV and V) emphasizes the importance of long-term studies to better understand spawning patterns. Despite not having identified any recurrent spawning sites, a few occasional and unfavorable spawning sites were identified, showing that the spawning habit of *E. anchoita* not only varied spatially, but also temporally. The largest occasional spawning site and with the highest probability of egg presence (0.6 - 0.7) was located around 27°S, close to Florianópolis (Santa Catarina State). On the other hand, a well-marked unfavorable spawning site was located off São Sebastião Island (São Paulo State), with the probability of egg presence between 0 - 0.1. Abiotic and biotic factors that could be related to the changes in the spawning habitat of *E. anchoita* were discussed, with shelf width, mesoscale hydrodynamic features and biological interactions apparently playing important roles in defining spawning sites.

Long-temporal-scale studies are also relevant to better understanding fluctuations in occurrence of ichthyoplankton that are related to environmental conditions. Slightly-different abiotic factors explained egg and larvae abundance and

the probability of sampling false zeros. Common to both stages of development, spatial factors (different areas sampled and the local depth of the oceanographic station) were related to the probability of sampling false zeros, and temporal and oceanographic conditions (different years sampled and surface temperature) were related to egg and larvae abundance. In years that had significant positive influence on *E. anchoita* egg counts, a 0.333 mm-mesh net was used for sampling, and moderate or strong El Niño conditions occurred. Some studies have related El Niño conditions to the abundance fluctuations of pelagic fish, but in our case further investigations are necessary.

The data set used in this dissertation is not a perfect one and we could not explore a wide range of environmental conditions that were probably masked by the high interannual variation observed in the abundance and distribution of *E. anchoita* eggs and larvae. Despite that, using *in situ* abiotic measurements, we analyzed the best long-term *E. anchoita* egg and larvae data from the SBB. Thus, in addition to providing new information about the early life history of one important pelagic species, our results also reinforced the importance of biological collections worldwide, which preserve historical data sets or samples that can be reanalyzed to achieve new perspectives on a species, community or an area.

6. REFERENCES

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