ASSESSING THE HARMFUL MICROALGAE OCCURRENCE AND TEMPORAL VARIATION IN A COASTAL AQUACULTURE AREA, SOUTHERN BRAZIL.

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ABSTRACT

The occurrence of harmful microalgae in aquaculture areas is a potential cause of human health problems and economic and environmental losses. In southern Brazil, coastal aquaculture activities are increasing and the assessment of harmful algal risk is critical. Here, we report the occurrence of six dinoflagellate and two diatom toxic species and other potentially harmful microalgae in a prominent mussel culture area (Armação do Itapocoroy Bight). The highest risk of paralytic and diarrhetic shellfish poisoning was associated with dinoflagellate blooms of *Gymnodinium catenatum* (1.6 104 cells L-1) in autumn, and of *Dinophysis acuminata* complex (>103 cells L-1) from mid-winter through spring. Other potentially toxic microalgae observed were *Pseudo-nitzschia* spp. (diatoms) and *Alexandrium* sp. (dinoflagellate). Stochastic events related to dynamic oceanographic features appear to play an important role in triggering and determining the magnitude of toxic blooms inside the bight. Our results strongly support the need for official monitoring programs in this and other Brazilian coastal aquaculture areas to ensure public safety.

KEY WORDS: microalgae, dinoflagellates, diatoms, cyanobacteria, mussel aquaculture

RESUMO

Ocorrência e variação temporal de microalgas nocivas em área de aqüicultura costeira no sul do Brasil.

A presença de microalgas nocivas em áreas de aqüicultura representa um problema potencial de saúde humana e possíveis perdas econômicas. No sul do Brasil, a atividade de aqüicultura costeira vem crescendo, e o conhecimento do risco causado por microalgas nocivas é essencial. No presente estudo, reportamos a ocorrência de seis espécies de dinoflagelados e duas de diatomáceas tóxicas e outras microalgas potencialmente nocivas em área de aqüicultura na Baía Armação do Itapocoroy, SC. O maior risco de contaminação por venenos com efeito paralisante e diarréico no Homem, esteve associado com florações de *Gymnodinium catenatum* (1.6 104 células L-1) no outono, e de espécies do complexo *Dinophysis acuminata* (>103 células L-1) no período de inverno-primavera. Outras microalgas potencialmente tóxicas observadas foram *Pseudo-nitzschia* spp (diatomácea) e *Alexandrium* sp. (dinoflagelado). Eventos estocásticos relacionados com processos oceanográficos aparentemente desempenham um importante papel na determinação do início e na magnitude das florações tóxicas na Baía de Armação do Itapocoroy. Os resultados indicam a importância da implantação de programa official de monitoramento em áreas de aqüicultura costeira no Brasil, visando garantir a saúde pública dos consumidores dos recursos cultivados.

PALAVRAS CHAVE: microalgas, dinoflagelados, diatomáceas, cianobactérias, moluscos

INTRODUCTION

About 5% of the approximately 5,000 currently described microalgae species are considered to be harmful, as they generate negative economic, environmental or public health impacts (Hallegraeff 2004a). Harmful processes include, but are not limited to, high biomass production, which causes physical, chemical and biological alteration of the marine environment, and production of toxins (Fogg 2002). Toxins produced by microalgae may directly harm organisms, or bioaccumulate in the trophic chain, causing intoxication and, in extreme cases, death of consumers of contaminated mollusks, crustaceans or fish. The main toxins produced by microalgae are the saxitoxins and congeners that cause paralytic shellfish poisoning (PSP), the okadaic acid and congeners that cause diarrhetic shellfish poisoning (DSP) and the domoic acid and derivatives that cause amnesic shellfish poisoning (ASP). Human deaths due to these toxins are estimated to exceed several hundred cases per year (Zingone & Enevoldsen 2000).

The first fully registered DSP outbreak in Brazil occurred recently, in the summer of 2007, covering about 200 km of the southern coast. At least 150 persons were intoxicated and mussel collection and commercialization had to be temporarily suspended (Proença *et al.* 2007). Research on harmful algae in Brazil has significantly increased in the last decade (Odebrecht *et al.* 2002); however, environmental studies focusing on harmful species are scarce. Here, we present the results of a three-years study (January 2000 to December 2002) dedicated to assess the occurrence of harmful algae in a prominent mussel farming area in southern Brazil. In this area, mariculture activities were initiated at the end of the 1980's, currently exceeding 95% of the national production of oysters (*Crassostrea gigas*) and mussels (*Perna perna*). Oyster production has increased from 55 tons produced in 1995 to 2,196 tons in 2003, and mussel production has increased from 190 tons in 1990 to 8,608 tons in 2003 (FAO 2008). The identification of harmful and potentially

harmful species, their toxic profiles, and variability patterns of toxin content per cell, is therefore critical information for the safe management of these aquaculture areas. Sanitation and water quality programs aimed at increasing product quality for both national and international markets are currently being designed in southern Brazil, requiring the knowledge of harmful microalgae risk in coastal aquaculture areas, such as the one provided in this study.

Additionally, we explore environmental conditions that might have contributed to the development of blooms in the study area in order to identify suitable predictors of harmful outbreaks in the region and their proper management. Our bloom concept follows Smayda (1997) in that a species does not have to achieve high population density to be in a bloom state. For example, it may be in bloom at population level of 104 cells L-1 or less, compared to its lower level of 102 cells L-1. In a broader context, our findings contribute to the knowledge of biogeography and events of harmful algae species, which are far hampered by gaps in referenced reports (Zingone *et al.* 2006).

MATERIAL AND METHODS

Study Area

Armação do Itapocoroy Bight (AIB, Fig. 1) is a small coastal area (6.7 km2) with maximum depth of 15m, average depth of 8m, and tidal flushing time of 3.6 days (based on a T 50% tidal prism) (Schettini *et al.* 1999). The AIB's north-facing orientation and the positioning of nearby coastal hills protect it from waves associated with the passage of fronts from the south, providing shelter and favorable conditions for mollusk farming (Schettini *et al.* 1999). The AIB has served as a mussel farming site, mainly for *Perna perna*, since the early 1990's. Measurements of dissolved inorganic nutrients (PO43+ 0.007-4.5 µM; H4SIO4 0.86-50.8 µM; NO3 0.06-5.4 µM; NH4+ 0.75-19.1 µM), and of chlorophyll (0,4-10,1 mg L-1) (Chevarria 1999) indicate that AIB's trophic degree is moderate when compared to the adjacent coastal shelf, and similar to other bays used for mariculture in Santa Catarina state (Proença 2002).

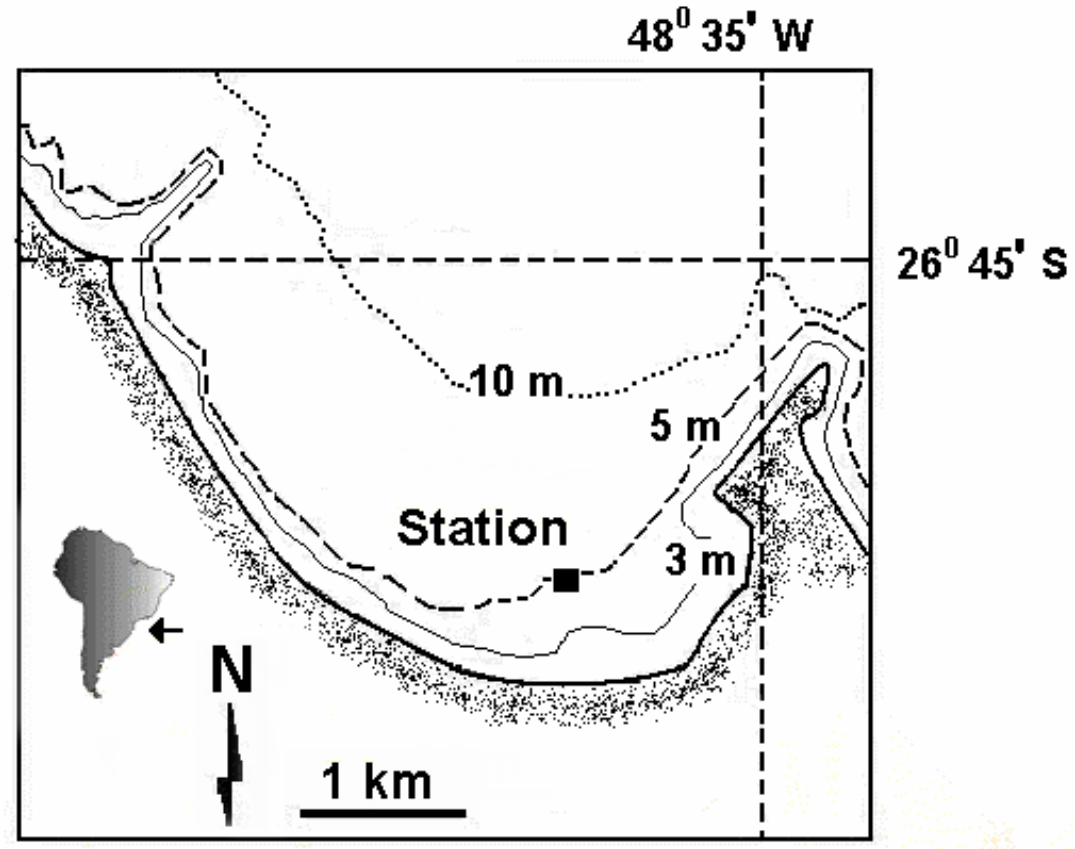

Figure 1. Sampling station located in a mollusk culture area at the bight Armação do Itapocoroy, Santa Catarina, southern Brazil.

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Sampling

Water and plankton samples were collected from a site within the farm area (Fig. 1) at approximately 5 to 14-day intervals, between January 2000 and December 2002, except in February 2002, when no samples were collecte*D.* Vertically integrated water samples were collected from a dinghy using a 2.4 cm-diameter plastic hose adapted with a valve for upper closure (Venrick 1978). The hose was 5 m long, corresponding to the depth of the water column at the collection site. After discharging the content of the hose into a bucket for homogenization, 200 ml aliquots were preserved in amber-color bottles using a 2% Lugol solution (Throndsen 1978) for quantitative analysis of phytoplankton. For the qualitative analysis, samples were concentrated with a conical net (20 µm mesh), stored in plastic bottles, and preserved with formaldehyde 4%. Water temperature (mercury thermometer ±1 °C), salinity (refractometer ±1) and transparency (Secchi disk) were measured at the site.

Analysis

Potentially harmful microalgae were identified to the lowest possible taxon and counted using sedimentation chambers (10 or 50 ml) under a Zeiss Axiovert 135 inverted light microscope, following the Utermöhl method (Hasle 1978). Photographs were taken using a Contax 167 MT camera/ 400 ASA film, or a JVC or Diagnostics Instruments Spot Insight digital camera, either attached to the inverted light microscope or to a Zeiss Axioplan transmitted light microscope. For the taxonomic study of *Pseudo-nitzschia* spp., selected samples from the year 2000 were prepared according to the slow method for cleaning frustules (Christensen 1988) and observed under a Zeiss transmission electron microscope. Cellulose plates of *Alexandrium* spp. were stained with CalcoFluor White (10 mg ml-1 stock solution and 2-10 mg ml-1 working solution of CalcoFluor White 2MR, Fritz & Triemer 1985), and studied under a Zeiss Axioplan epifluorescence microscope equipped with excitement 440 nm and emission 500 - 520 nm filters. Spearman's correlation tests were applied to the abiotic variables.

Table 1. Mean values, standard deviation (parenthesis), minima and maxima of water temperature (°C), salinity and transparency (m; Secchi disk) at Armação do Itapocoroy Bight.

	2000	2001	2002
Temperature	22.5 (4.5) 12.0 (July) 28.5 (March)	22.9 (3.9) 18 (July) 30 (February)	23.6 (3.6) 18.0 (June) 30.0 (January)
Salinity	32.5 (2.8) 21.8 (September) 37.0 (March)	32.0 (2.1) 26.3 (May) 35.0 (June, August, October, November)	31.9 (2.3) 25.1 (August) 35.0 (June)
Transparency	2.7 (0.9) 1.5 - 4.8	3.0 (1.1) 1.4 - 6.0	3.3 (1.6) 1.6 - 7.0

RESULTS AND DISCUSSION

Environmental parameters

Water temperature (T) ranged from 12 to 30 °C (Table 1, Fig. 2). Summer and winter means were 27.3 °C and 18.6 °C, respectively. When comparing the three years, T was significantly colder in 2000 (lowest mean, maximum and minimum T) and warmer in 2002 (highest mean). T variation between any two consecutive samplings was small (average 1.5 °C) except in two occasions, both in 2000: one in February, when T dropped from 28 °C to 20 °C in a week and increased to 27.5 °C in the next week; the other in July, when T dropped from 20 °C to 12 °C in a week and increased to 15.0 °C in the next week. Water salinity (S) varied between 21.8 and 37.0 (average 32.1) with two periods of relatively low values: between July 2000 and March 2001, and between July 2002 and December 2002 (Fig. 2, Table 1). The average rate in S fluctuations was approximately 1.6 per week, with abrupt changes (5 to 10) registered in September 2000, May and October 2001, and August 2002. Water transparency (Tr, Secchi disk) varied between 1.4 and 7 m (mean 3 m;

Fig. 2, Table 1), with no seasonal patterns. Lowest and highest mean annual Tr values were registered in the years 2000 and 2002, respectively. Although the AIB is sheltered from southerly winds and waves, sporadic wind-induced waves from North to East remobilize fine sediments deposited on the bottom of the bight during low energy periods (Schettini *et al.* 1999). Previous studies reported S and Tr values to be inversely correlated in the AIB (Schettini *et al.* 1999; Proença & Schettini 1998). We, however, found no significant statistical correlation among T, S and Tr.

Major shifts in T and S inside the AIB are typically related to dynamical processes in the adjacent shelf, specifically the relative contribution of: (a) warm haline water of tropical origin, (b) cold haline Subtropical Water that upwells onto the shelf (Silva *et al.* 1984), and (c) low salinity water either from the Itajaí-açu river discharge, located approximately 20 km south of the study area (Schettini *et al.* 1999), or from the Brazilian Coastal Current (BCC) originated southward (Lat. 35-32º S) from the mixture of waters of the La Plata River, the Patos Lagoon, and Southwest Atlantic Ocean Subtropical Shelf Water (Piola *et al.* 2005; Souza & Robinson 2004). The BCC moves northward, reaching the study area's latitude (26º S) in the austral winter and spring.

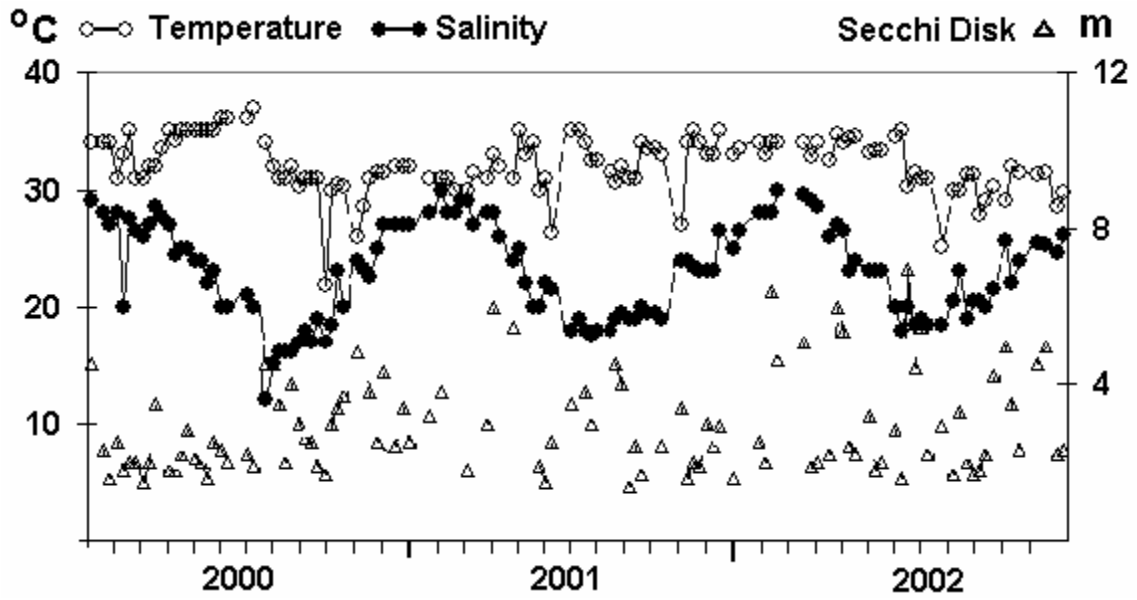

Figure 2. Weekly variation of water temperature (0C) and salinity at the left axis and transparency (m; Secchi disk, right axis) at the bight Armação do Itapocoroy (January 2000 to December 2002).

Microalgae

Traditionally, microalgae species classification is based on general cell and colony morphology and biogeography (Hallegraeff 2004b). However, species definition based solely on these criteria may be impaired due to intraspecific and life cycle morphological variations (Taylor 1987; MacKenzie 1992; Reguera & González-Gil 2001). Analyses of species' genetic composition and cellular ultrastructures are useful in clarifying taxonomy, although in field studies, microalgae are frequently identified through the morphologic characteristics of their morphospecies (Taylor 1993). During our study, we observed a total of six dinoflagellate and two diatom species known to produce toxins according to the IOC Taxonomic Reference List of Toxic Plankton Algae (Moestrup 2004), some of which occurred in abundances considered to be of risk. In addition, we observed five species and genera of dinoflagellates, diatoms and cyanobacteria which have been associated with harmful events elsewhere, or are known to include potentially harmful species (Tab. 2).

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Table 2. Harmful microalgae and their known effects, occurrence frequency, main season and maximum concentration observed in the AIB (2000 - 2002). *Highest contamination risk. SP spring; SU summer; AU autumn; WI winter; ! *Prorocentrum balticum* included (see text).

Species	Harmful Effect	Frequency (%)	Season	Max. cell L^{-1}
Dinoflagellates				
Gymnodinium catenatum Graham	*PSP	3	AU	$1.6\ 10^4$
Dinophysis acuminata Claparède& Lachmann *D. fortii* Pavillard	*DSP	46	WI	$9.8\ 10^3$
D. caudata Saville-Kent	DSP	33	WI; SP	200
D. tripos Gourret	DSP	16	AU; WI; SP	200
P. minimum (Pavillard) Schiller	Toxicity	61	All	! $9.2\ 10^4$
Alexandrium sp. Halim	PSP	41	All	$3.8\ 10^3$
Noctiluca scintillans (Macartney) Kofoid & Swezy	NH_4 accumulation	Sporadic		200
Diatoms				
Pseudo-nitzschia calliantha Lundholm, Moestrup & Hasle *P. pungens* (Grunow ex Cleve) Hasle *P.* cf. *turgidula* (Hustedt) Hasle	*ASP	72	SP; SU	$1.3\ 10^6$
Cyanobacteria				
Trichodesmium erythraeum Ehrenberg ex Gomont *T. thiebautii* Gomont ex Gomont	Discoloration, Toxicity	30	SP; SU; AU	10^4 trichomes L^{-1}

1. Dinoflagellates

1.1. *Gymnodinium*

The potential PSP-toxin producer *Gymnodinium catenatum* was observed in chains up to 14 cells during a two-week period, reaching a relatively high concentration (1.6 104 cells L-1) in March, 2000 (Fig. 3, 4.A), at T 26-28º C and S 31-32. These values correspond to what is described as the species' optimum growth conditions (Band-Schmidt *et al.* 2004). The identification of naked dinoflagellates such as *G. catenatum* may be hindered by the use of preservatives that tend to shrink the cells. However, the preserved cells of *G. catenatum* observed in this study were about 32 mm long and 28 mm wide and therefore significantly larger than cells of Gyrodinium impudicum, the one species that is usually mistaken for *G. catenatum* (Fraga *et al.* 1995). Seasonal blooms of *G. catenatum* are common in Uruguay from late austral summer through the autumn (Méndez & Ferrari 2002), and in autumn in Argentina (Akselman *et al.* 1998). In the AIB, PSP toxins were first detected in mussel extracts from July, 1997 (Proença *et al.* 1999). *G.* cf. catenatum was found in a sample from November, 1998, and final toxicity confirmation came when toxin analysis (bioassays with mice) of *G. catenatum* chains collected in April, 1999, and maintained in culture resulted positive (~ 28 pg STXeq. cell-1) (Proença *et al.* 2001).

Significant environmental changes (T 28º C to 20º C to 26º; S 31 to 35 to 31) took place in the AIB weeks before the *G. catenatum* bloom occurre*D.* These are indicative of the advection of colder and saltier waters, characteristic of upwelled Subtropical Water, STW (Silva *et al.* 1984). The STW is nitrate rich (Niencheski & Fillmann 1997) and this exchange of waters probably triggered the bloom in the bight. The cells that served as inoculum, could have been advected into the AIB or germinated from benthic cysts suspended from sediments. *G. catenatum* produces resting cysts viable in the sediment for many years (Dale 1983). If stimulated by environmental changes, these can germinate in as little as two weeks (Blackburn *et al.* 1989). Preliminary results from an ongoing research on dinoflagellate cysts in the AIB and the adjacent shelf confirm the presence of *Gymnodinium* cysts in the area (Proença, personal communication). The alongshore northward transport of *G. catenatum* associated with the Brazilian Coastal Current was observed in the southern Brazilian shelf in austral autumn (Odebrecht *et al.* 2007). Both, a local origin of vegetative cells and alongshore transport of offshore populations have been claimed as inoculum sources for cases in Tasmania and in Spain, respectively (Hallegraeff & Fraga 1998). In any case, the absence of *G. catenatum* during two consecutive years leads us to suggest that stochastic physical processes are important factors for bloom initiation and success in the AIB.

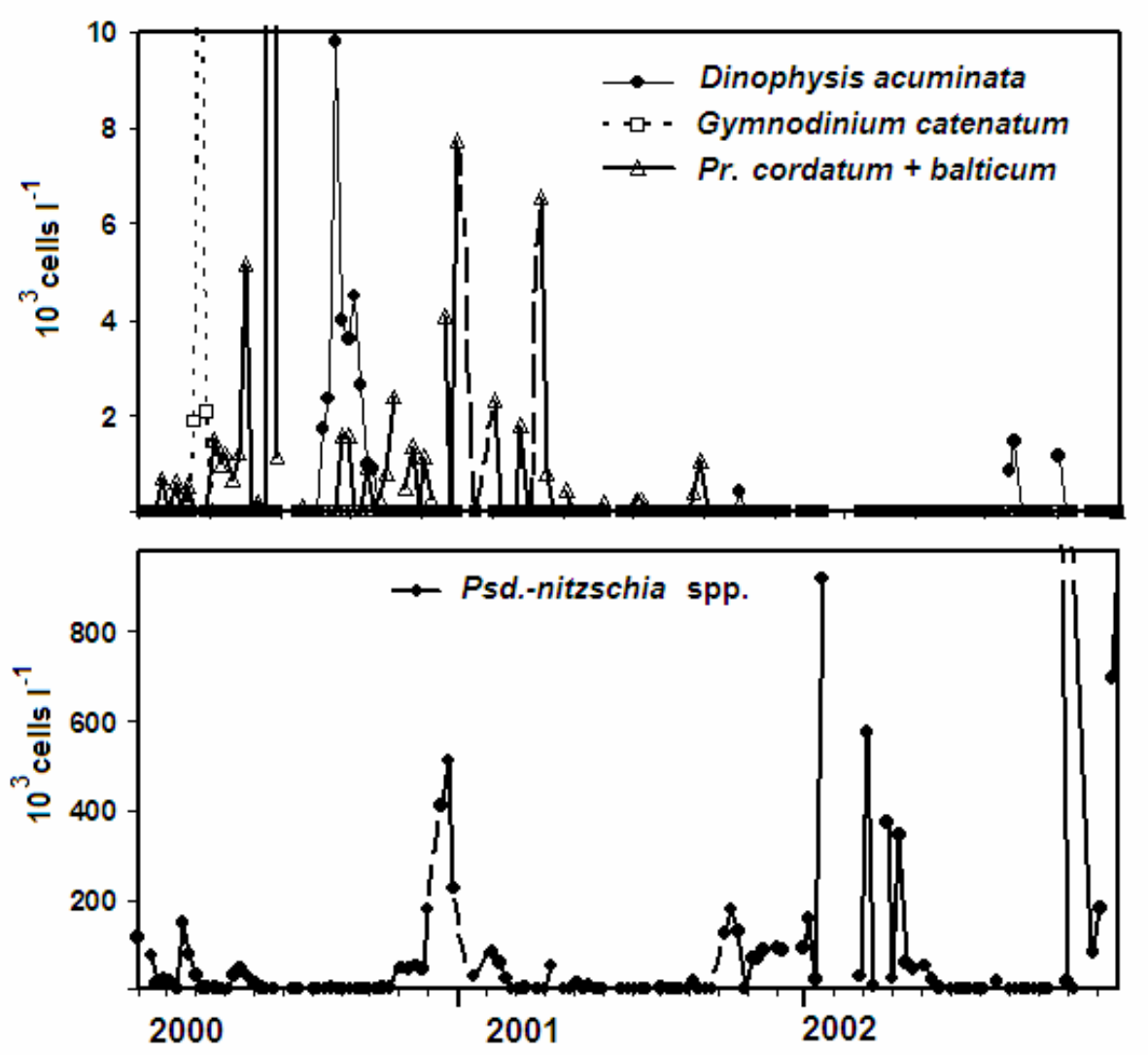

Figure 3. Weekly variation of the dinoflagellates *Gymnodinium catenatum*, *Dinophysis acuminata*, *Prorocentrum minimum* + *balticum* and *Pseudo-nitzschia* spp. (cells l-1) at the bight Armação do Itapocoroy (January 2000 to December 2002).

1.2. *Dinophysis*

Of the potentially harmful identified microalgae, *Dinophysis* presented the highest number of species. High concentration (>103 cells L-1) of the DSP producer *Dinophysis acuminata* complex (i.e. typical *Dinophysis acuminata* morphotype and variations) was observed in the years 2000 and 2002, from mid-winter through spring (Fig. 3, 4). The bloom of *D. acuminata* complex began at the end of July, 2000, a week after a cold water mass (12 0C) had moved into the AIB and it lasted ~50 days. The bloom developed at relatively low T and S values (respectively, 15-17 ºC and 31-32). It reached highest concentration (9.8 103 cells L-1) in three weeks, and died-off by mid-September. Accompanying mice tests for DSP came out positive (test dates: 08/13/00, 08/20/00, 08/27/00, and 09/08/00), and okadaic acid (OA) was detected in phytoplankton and mollusk samples collected in early August (Schmitt & Proença 2000). *D. acuminata* produces OA and other DSP- toxins, and can cause shellfish toxicity at concentrations as low as 2 102 cells L-1 (Lassus *et al.* 1985; Faust & Gulledge 2002). The detection of OA in mollusks from the AIB (Proença *et al.* 1998a,b) and the informal reporting of consumer intoxication indicate a latent problem at the coast of Santa Catarina (Proença *et al.* 1999). Our results seem to reveal a prominent interannual variability in abundance, and seasonal patterns of occurrence of *D. acuminata* complex inside the AIB.

After the winter/spring bloom of 2000, the morpho species was undetectable throughout most of 2001 and 2002, except for occasions in the

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winter/spring of both years in which it occurred in relatively low concentrations (~102 and 103 cells L-1, respectively). These findings are consistent with some studies for the region: Rörig *et al.* (1998a) monitored the AIB from 02/16/96 to 09/01/97 on a weekly basis, detecting *D. acuminata* only once (4.5 102 cells L-1), at the end of the austral winter. Proença (2004) found *D. acuminata* (2.4 103 cells L-1) associated with a bloom of *Myrionecta rubra* and *Prorocentrum micans*, which took place near the outlet of the Itajaí-açu river in winter. However, the most recent and striking DSP outbreak in Brazil, which took place just south of the AIB, happened in summer (January 2007) and *D. cf. acuminata* abundance in the water during the episode reached 5.2 x 104 cells L-1 (Proença *et al.* 2007).

D. acuminata was originally described as widely distributed in cold to temperate waters (Steidinger & Tangen 1996; Faust & Gulledge 2002; Balech 2002), and more abundant in eutrophicated coasts (Taylor *et al.* 2004). Recent studies, however, have found *D. acuminata* also in warm waters. Toxic events caused by *D. acuminata* at the Uruguayan coast, for example, are common in summer and autumn (T 22-25 ºC; S 25-30) (Méndez & Ferrari 2002), and unusual winter blooms in the years 2001 and 2002 (1.1 105 cells L-1) were found to be non-toxic (Méndez & Medina 2004). In addition, various *Dinophysis* spp. are known to achieve modest blooms during coastal upwelling relaxations, such as the observed in the Iberian Peninsula (Reguera *et al.* 1995). Smayda and Reynolds (2001) classified this genus as a transitional life form along the onshore-offshore, mixing-nutrient gradient and adapted to small-scale convective currents. This leads us to suggest that the variations in the magnitude of the seasonal blooms of *D. acuminata* observed in this study are related to dynamic oceanographic features, such as the intrusion of STW into the AIB. However, it is unclear if cells of *Dinophysis acuminata* were transported into the AIB by advection or if local populations grow due to better nutrient conditions associated to the intrusion of the cold, nutrient-rich STW. In addition, it was recently shown that the growth of *D. acuminata* in culture is mixotrophic relying on the autotrophic ciliate *Myrionecta rubra* as food (Park *et al.* 2006). It is likely that similar behavior is observed in the environment and possibly acts as an important growth factor. Other potentially toxic mixotrophic species (*D.* caudata, *D.* fortii and *D.* tripos) and species of unknown toxic profile (*D. diegensis*, *D. operculoide*s, *D. schroederi*, *D. scrobiculata* and *D. cf. bibulbus*) (Table 2, Figs. 4, 5), were observed in low abundance, predominantly in the austral winter and spring.

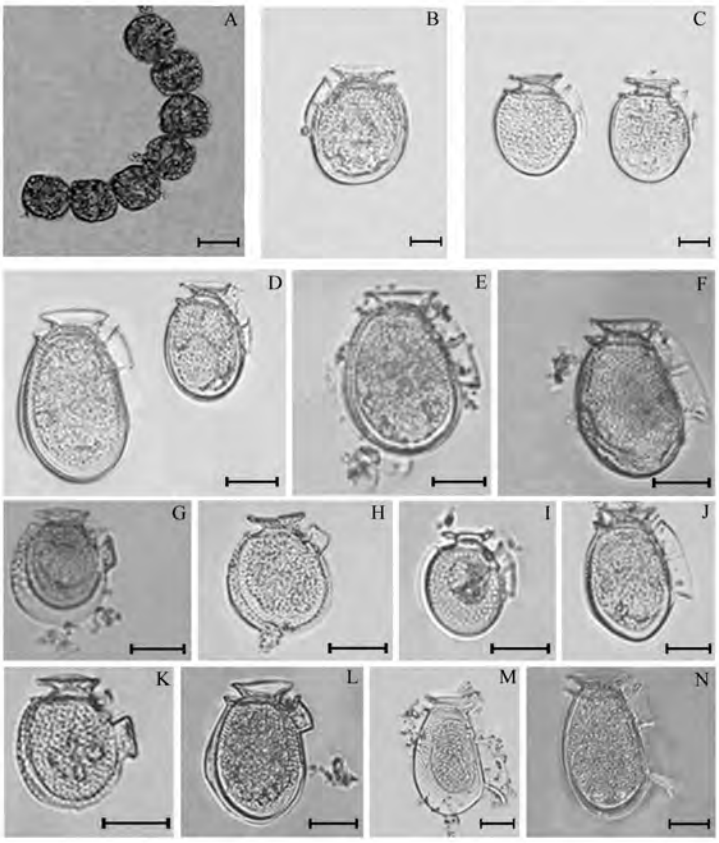

Figure 4. A: *Gymnodinium catenatum*; B, C: *Dinophysis acuminata* complex; D: cells of *D.* cf. schroederi and *D. acuminata*; E, F: *D.* fortii typical cells; G, H, K, L: recently divided dimorphic cells of *D. acuminata* complex; I: *D. acuminata* small cell; J: *Dinophysis* intermediate form; M, N: *D. schroederi*. Scale bar 20 µm.

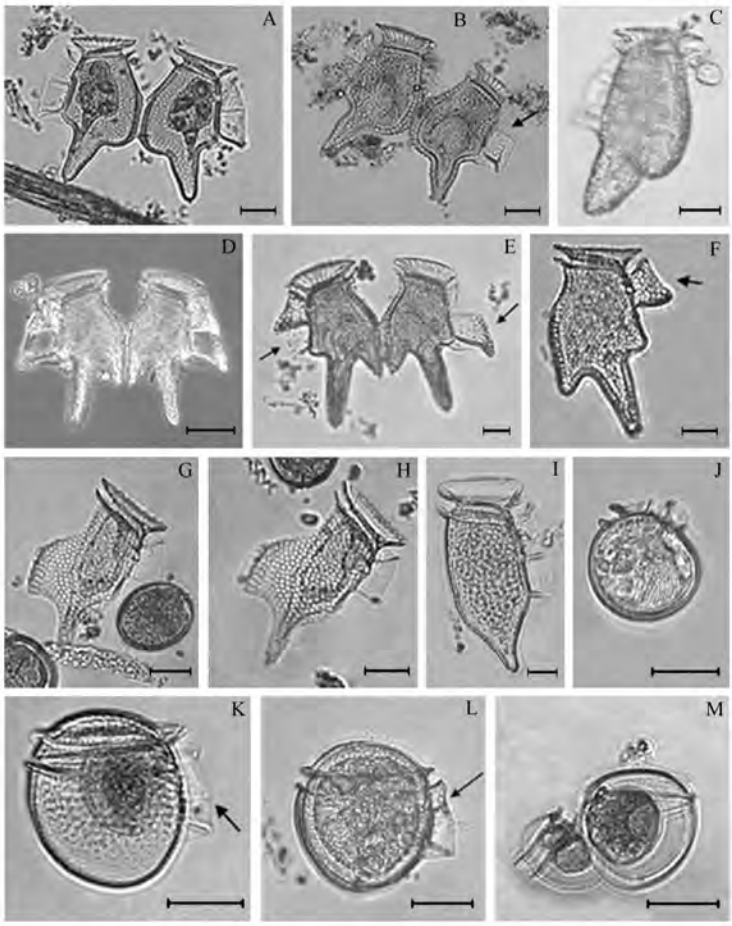

Figure 5. A. *Dinophysis caudata* typical cells in pair; B: recently divided and C: *D. caudata* large form; D: *D. tripos* paired cells and E, F: recently divided cells; G, H: *D. tripos* dimorphic cells showing the *D. diegensis* hypothecal plate; I: *D. diegensis* typical cell; J: *D. cf. bibulbus*; K: *D. scrobiculata*; L: *D. operculoides*; M: probable dimorphic pair. Scale bar 20 µm.

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Balech (1988, 2002) described two varieties of *D. acuminata* for the Southwest Atlantic Ocean and transition forms between them: *D. acuminata* var. *acuminata* (a wider cell with rounded antapex), and *D. acuminata var. lachmannii* (a narrower cell with pointed antapex). In the present study, oval-shaped cells with rounded smooth antapex similar to the description of *D. acuminata* var. *acuminata* were most prevalent (Fig. 4. B, C). The size of the smallest cells (length 35 mm; width 24 mm) was below the size range registered by Faust and Gulledge (2002) and Balech (1988, 2002) for this species (length 38-58 µm, width 30-40 µm; length 39-53 µm, width 33-46 µm, respectively).

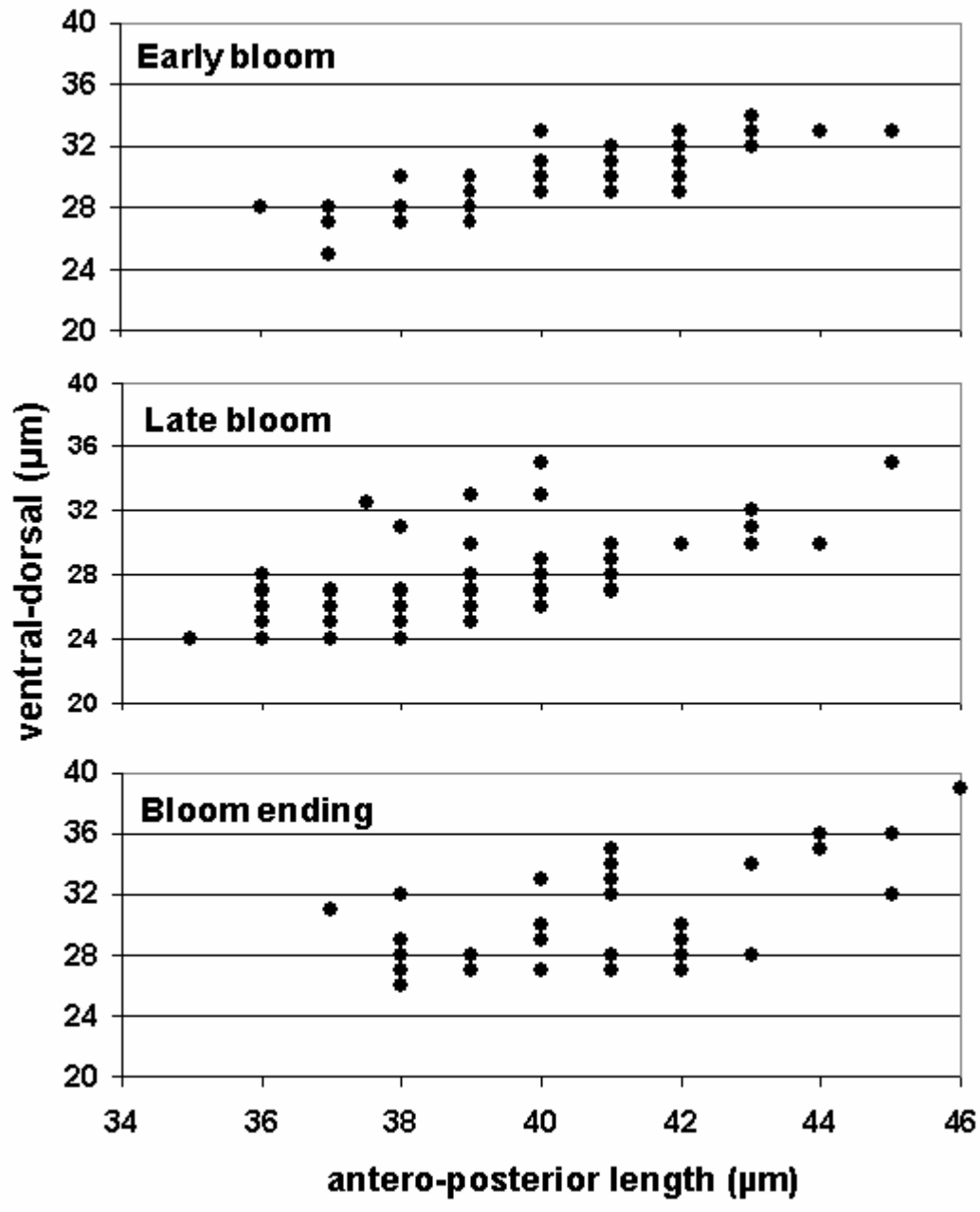

Figure 6. Size variation of *Dinophysis acuminata* complex during the early, late and end of the bloom at the bight Armação do Itapocoroy (August/September 2000).

Phenotypic variations triggered by changes in life cycle and the environment, are common in *Dinophysis* (Taylor 1987). Reguera & González-Gil (2001) suggested that the life cycle of *Dinophysis* spp. is composed of three phases: phase one would be a vegetative phase with two types of division, (1) a longitudinal fission of megacytic cells, resulting in cells of similar size and shape to the mother cell but

incomplete sulcal lists, and (2) a depauperating division, which gives rise to dimorphic cells (one large and one small), united by the megacytic bridge. These small cells would be gametes in phase two, a sexual phase, when couples of dimorphic cells change genetic material and the small cell is engulfed by the larger one, giving origin to a planozygote that later may become an hypnozygote. Phase three would occur through successive non-depauperating vegetative divisions under unsuitable conditions and subsequent growth into intermediate and undersized cells. The noteworthy morphological variability of *D. acuminata* complex observed in our study and the co-occurrence of small cells and cells up to 45% larger (Fig. 6) are logical in light of the polymorphic life cycle described above. Higher numbers of small cells were observed towards the end of the bloom, perhaps due to less favorable environmental conditions as suggested by MacKenzie (1992) for *D.* acuta. It seems plausible to consider these individuals as a continuous size variation of the same morphospecies. Cells formed by one typical *D. acuminata* hypothecal plate and the other hypothecal plate similar in shape but smaller in size would indicate that a depauperating division had occurre*D.* The size of the smaller hypothecal plate of *D. acuminata* complex dimorphic cells (Fig. 4.G, H, K, L) coincided with the size of the small single cells, while the size of the larger hypothecal plate of the dimorphic cells coincided with that of the large single cells. We observed *D. acuminata* complex, *D.* caudata and *D.* tripos with incomplete left sulcal lists, which is characteristic of vegetative cell division (Figs. 4. G, H, K, L; Fig. 5. A, B, E, F), and a swollen cell of *D.* caudata, which could be a megacytic cell (Fig. 5. C). Pairs of *D.* caudata and *D.* tripos joint by the intercalary growth zone (Fig. 5. A, D) resulted from vegetative divisions, while dimorphic cells with one typical hypothecal plate of *D.* diegensis (Fig. 5. I) and the other hypothecal plate of *D.* tripos, lacking the posterior portion of the left sulcal list (Fig. 5. G, H), presumably resulted from depauperating divisions. A dimorphic couple united by their anterior ventral margins (Fig. 5. M) could be exchanging genetic material in the sexual phase.

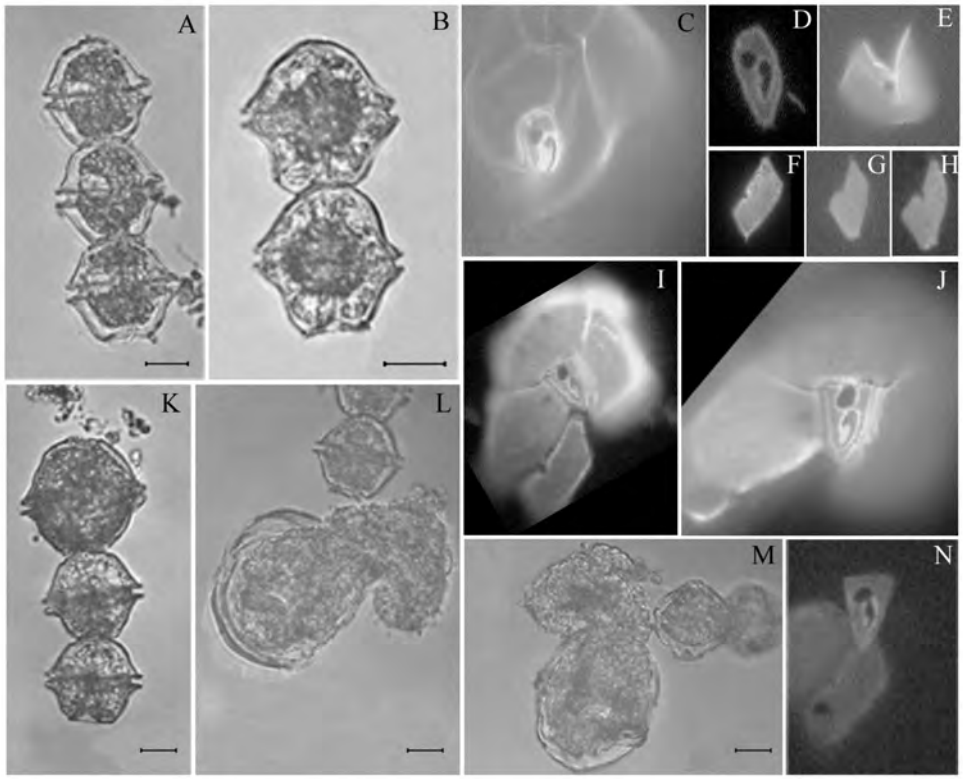

Figure 7. The chain-forming *Alexandrium fraterculus* (A, B); apical pore complex (C, D) posterior sulcal plate (E) and first apical plate (F, G, H); apical pore complex of *Alexandrium* sp. (I, J, N); Chain of *Alexandrium* sp. parasite infected giant cell (K) and emerging vermiform parasite (L, M). Scale bar 20 µm.

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1.3. *Alexandrium*

We found *Alexandrium* spp. in 41% of the samples in every season of the three years, always in low concentration (< 3.8 103 cells L-1). *Alexandrium* comprises 11 toxin-producing species (saxitoxins and congeners); however, many species of this genus are toxic and there is variability in toxicity in some of them, a bloom of any member of this genus should be viewed with concern for possible PSP in local shellfish (Taylor *et al.* 2004). In the AIB, *A. fraterculus* (Fig. 7) was registered in five occasions in 1997 (102 cells L-1, Rörig *et al.*, 1998a), and in 2004 a conspicuous bloom (105 cells L-1) was registered for the first time (Omachi *et al.* 2007). *A. fraterculus* has been found to be non-toxic in the AIB (Proença *et al.* 2001; Omachi *et al.* 2007) as in other places (Steidinger & Tangen 1996; Taylor *et al.* 2004; Landsberg 2002). This is a warm water species mainly found in Brazilian coastal regions, La Plata River outlet, and near the coast of Argentina at 38o S (Balech 1988, 2002).

The observation of giant cells in an *Alexandrium* sp. chain revealed a vermiform parasite infection, typical of the dinoflagellate *Amoebophrya* (Fig. 7. K-M). Similar abnormal cells have been observed in chains of *A. catenella* from the northeast of the United States (Taylor 1968) and of A. affine from coastal waters of Korea (Kim *et al.* 2004). The genus *Amoebophrya* is distributed thoroughly in the Northern Hemisphere with few observations in the Southern Hemisphere (Australia, Park *et al.* 2004; southeastern Brazil, Salomon *et al.* 2006). Three species of *Alexandrium* (*A. tamarense, A. catenella*, and *A. affine*) have been observed infected with *Amoebophrya* (Taylor 1968; Park *et al.* 2004; Salomon *et al.* 2006), but it is possible that other species could also serve as a host. Taxonomic identification of *Alexandrium* species occurring in the AIB merits further study. Balech (2002) registered five *Alexandrium* species in the Southwest Atlantic Ocean*: A. acatenella, A. fraterculus, A. kutnerae, A. tropicale* and *A. tamarense*. Vegetative cells and cysts of *A. tamarense* have been observed at Cassino Beach, about 800 km south of the AIB (Persich & Garcia 2003; Persich *et al.* 2006), without registered recurrence.

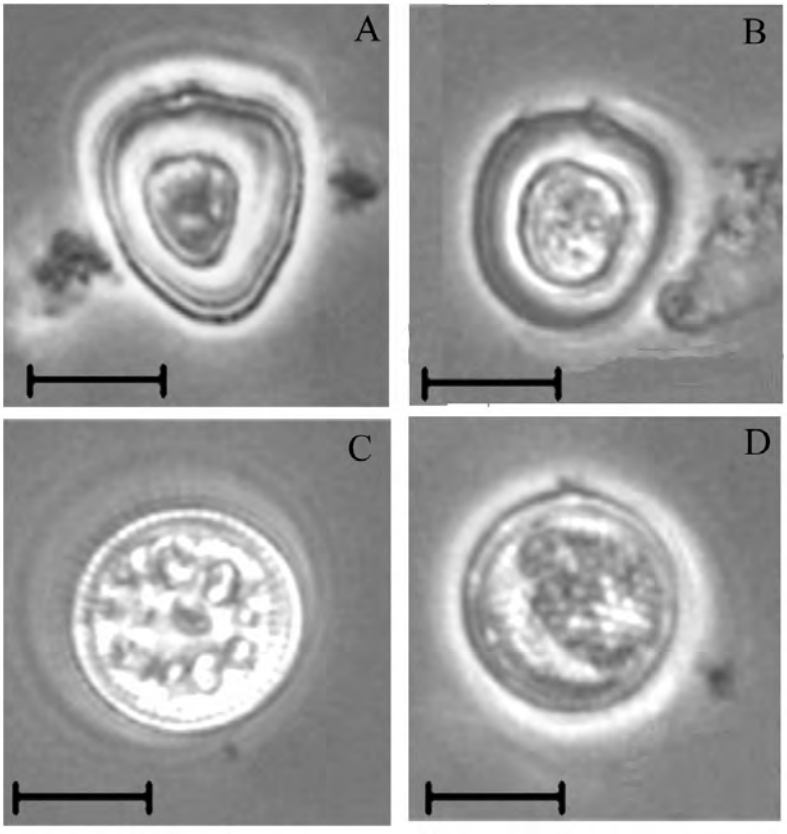

Figure 8. The two small *Prorocentrum* species counted together, A, B: *Prorocentrum* minimum; C, D: *P. balticum*. Scale bar 20 µm.

1.4. *Prorocentrum*

Prorocentrum spp. was found in 91% of the analyzed samples and high abundance of this genus (103-104 cells L-1) occurred between March, 2000 and March, 2001 (Fig. 3). The most abundant species - *P. minimum* + *P. balticum* (Fig. 8) were counted as a group as they may be indistinct during routine countings, due to their tiny size. They co-occurred in 52% of the 71 occasions in which they happened, in wide ranges of T (19-28 ℃), S (29-36) and Tr (1.6-6 m). *P. minimum* is known to produce toxins and presents harmful effects in marine organisms, while *P. balticum* is not known as toxic (Steidinger & Tangen 1996; Faust *et al.* 1999). *P. minimum* had been observed in the AIB reaching 103 cells L-1 in September 1996 (Rörig *et al.* 1998a). Further south in the Patos lagoon estuary, both species are common in autumn and winter, reaching high concentration (106 cells L-1, Abreu *et al.* 1994; Odebrecht *et al.* 1995, Bergesch 2003). Further north in Paranaguá Bay (25030'S, 48030'W), P. minimum occurs from spring to autumn (Mafra *et al.* 2006). The planktonic, cosmopolitan, coastal *Prorocentrum micans* was frequently found (53% of samples) in low concentration (<2 103 cells L-1). *P. micans* is known for extensive water discolorations (Taylor *et al.* 2004; Landsberg 2002). In the non-toxic bloom registered in August 2001 near the outlet of the Itajaí-açu river (Proença 2004), *P. micans* co-occurred with *Myrionecta rubra* and *Dinophysis acuminata*. Other *Prorocentrum* species, *P. scutellum, P. sigmoides* and *P. rostratum* occurred sporadically and in low abundance.

1.5. Other dinoflagellates and silicoflagellates

The dinoflagellates *Ceratium* spp., *Noctiluca scintillans* and the silicoflagellate *Dictyocha fibula*, which are potentially harmful when in high concentration (Landsberg 2002), occurred sporadically in low abundance.

2.2. Diatoms

2.1. ***Pseudo-nitzschia***

We observed *Pseudo-nitzschia*, a potential producer of domoic acid (DA), in 72% of the quantitative samples in all seasons (Table 2). The frequency of occurrence was similar in the three study years (75% in 2000; 65% in 2001; 73% in 2002) but the concentration was higher in the year 2002 (106 cells L-1; Fig. 3) and lowest in the year 2000 (3 - 5 105 cells L-1). Higher abundance of *Pseudo-nitzschia* spp. (>104 cells L-1) was registered from austral spring to autumn (T ≥ 24 ℃, S 27-35, and Tr 1.6 - 5.4 m). Rörig *et al.* (1998a) observed the genus (>105 cells L-1) in the AIB in the autumn and spring of 1996. Our analysis of selected samples from the year 2000 revealed the occurrence of *P. calliantha* in January and December, *P. pungens* in March and May, and *P. cf. turgidula* in March. *P. calliantha* is cosmopolitan with at least two known events of DA production (Lundholm *et al.* 2003). In the study area, detection of DA in a concentrated sample from December 2000 (Proença, unpublished data) coincided with the presence of *P. calliantha* in the water, suggesting a relationship. This species presents a wide distribution in southern Brazil, northward and southward of the AIB (Mafra *et al.* 2006; Moreira 2004). *P. pungens* is also cosmopolitan (Hasle 2002), and a potential DA producer (Rhodes *et al.* 1998; Trainer *et al.* 1998). *P. pungens* has been registered in southern and southeastern Brazil (Odebrecht *et al.* 2001; Villac & Tenenbaum 2001) and in Argentina (Negri & Inza, 1998). The classification of *P. turgidula* as cosmopolitan has been questioned due to its doubtful documentation (Hasle 2002). In the Southwest Atlantic, Negri and Inza (1998) observed *P. turgidula* in high concentration (3.3 106 cells L-1) at low temperature (T 8.9 - 9.7° C). Other potentially noxious diatoms such as *Chaetoceros* spp. and *Coscinodiscus* spp. occurred sporadically in low concentration in the AIB.

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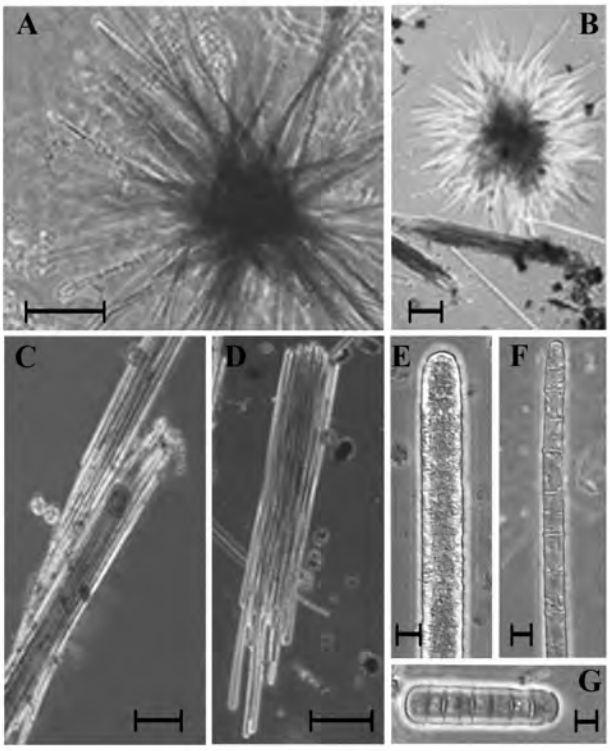

Figure 9. Cyanobacteria "star-like" colonies with radiate trichomes of *Trichodesmium thiebautii* (A, B) and fascicles of *T. erythraeum* (C, D). Single *trichomes* of *T. erythraeum* (E) and *T. thiebautii* (F); G: cf. *Katagnymene pelagica*.

2.3. Cyanobacteria

2.3.1. *Trichodesmium*

The colonial cyanobacterium *Trichodesmium* (Fig. 9) is widely distributed in subtropical and tropical oligotrophic waters (Capone *et al.* 1997; Janson *et al.* 1999) and blooms of some species are harmful to the marine fauna (Landsberg 2002). We found *Trichodesmium trichomes* in 30% of the water samples from the AIB (Table 2), with highest concentration of 2.4 104 *trichomes* L-1 (mostly *T. erythraeum*) in October, 2002. The morphological identification of *Trichodesmium* species is difficult when the *trichomes* are free floating (Janson *et al.*, 1995, 1999) and a close relation with the genus Katagnymene has been proposed (Orcutt *et al.* 2002). We observed colonial morphospecies of *T. erythraeum* and *T. thiebautii* (Fig. 9) predominantly between October and April, but free *trichomes* were found earlier (August, 2002) in net samples for six consecutive months, some resembling Katagnymene pelagicus (Fig. 9. G). Blooms of T. hildebrandtii, *T. erythraeum* and *T. thiebautii* have been registered off the 40 m isobath in the coastal waters of Santa Catarina from austral spring to autumn (Guimarães & Rörig 1997; Rörig *et al.* 1998b). At the continental slope, the Brazil Current transports oligotrophic tropical waters from lower to higher southern latitudes year-round, and meanderings may serve as dispersion mechanism to the adjacent coastal area (Carvalho *et al.* 1998). Previous toxicity bioassays and HPLC analyses of samples from Santa Catarina containing predominantly *T. hildebrandtii* resulted negative for microcystins (Rörig *et al.* 1998b). In Uruguay, non-toxic blooms of *T. erythraeum* are common during the summer (Méndez & Ferrari 2002).

CONCLUSIONS

The occurrence of harmful algae in the AIB poses serious risk of seafood contamination by PSP, DSP and ASP toxins, being produced by *Gymnodinium catenatum*, *Dinophysis acuminata* complex and *Pseudo-nitzschia* spp., respectively. The relationship between fluctuations in the abundance of *Dinophysis acuminata* complex and *Gymnodinium catenatum* and shifts in water temperature and salinity inside of the bight indicate the importance of water advection in triggering their blooms in the AIB, and controlling the magnitude of such events. Although harmful algal blooms are complex ecological phenomena, stochastic physical processes seem to directly influence the probability of DSP and PSP outbreaks in the AIB, and that their prediction would

require the monitoring of ocean currents and meteorological data. The origin of the inocula for the toxic blooms is not clear, and future studies should address this question. When comparing the three years of the study, blooms of dinoflagellates were more frequent and abundant from early 2000 through mid-2001. Contrastingly, the frequency of occurrence of *Pseudo-nitzschia* spp. was approximately constant throughout the study and the risk of ASP outbreaks should be consistently evaluated throughout the year.

The other microalgae identified during this study do not represent an imminent risk to public health or to the mariculture industry, but it would be prudent to also keep them under surveillance.

Our results reinforce the need of public policies for coastal aquaculture management in Brazil. Official programs to (1) evaluate toxins and microalgae, (2) develop handling strategies in case of toxic events, and (3) expand and coordinate research on potentially harmful microalgae, are urgent in order to avoid future health emergencies and economical losses.

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