

PSEUDO-NITZSCHIA BRASILIANA (BACILLARIOPHYCEAE), AN OPPORTUNISTIC DIATOM ON THE COAST OF THE STATE OF RIO DE JANEIRO, BRAZIL

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ABSTRACT

The occurrence of *Pseudo-nitzschia brasiliiana* Lundholm, Hasle & Fryxell, a recently described marine diatom, is reported for two distinct environments on the coast of the state of Rio de Janeiro, Brazil, during the El Niño-ENSO summer of 1998. These occurrences took place almost simultaneously, as a bloom event, which is defined here not only through high cell abundance, but also in terms of high relative contribution to total cell numbers. The blooms occurred at times when each one of these environments was under a specific stress, suggesting an opportunistic behavior for this diatom. In Sepetiba Bay, *P. brasiliiana* was the first species to dominate (2.8×10^6 cells l⁻¹; 85% of the microphytoplankton fraction) after a bloom of the raphidophyte *Chattonella* sp. was subsiding in early February. In Imboassica Lagoon, a oligohaline coastal system ca. 150 km north of Sepetiba Bay, *P. brasiliiana* was the dominant species ($1.3 - 3.6 \times 10^7$ cells l⁻¹; 90% of total phytoplankton) after the lagoon-sea connection was forced opened in mid-February, which allowed the contribution of more saline waters into the lagoon, that probably carried out a seed-stock of *P. brasiliiana* present along the coast during this period.

KEY WORDS: Diatom bloom, Imboassica Lagoon, *Pseudo-nitzschia brasiliiana*, Rio de Janeiro State, Sepetiba Bay

RESUMO

Pseudo-nitzschia brasiliiana (Bacillariophyceae), uma diatomácea oportunista da costa do estado do Rio de Janeiro, Brasil

A ocorrência de *Pseudo-nitzschia brasiliiana* Lundholm, Hasle & Fryxell, uma diatomácea marinha recentemente descrita, é relatada para dois ambientes distintos da costa do estado do Rio de Janeiro, Brasil, durante o evento de El Niño no verão de 1998. As ocorrências aconteceram quase simultaneamente como um evento de floração, definido aqui não apenas pelo alto número de abundância de células, como também pela alta contribuição relativa ao número total de células. As florações ocorreram em momentos em que cada um destes ambientes estava sob estresse, sugerindo um comportamento oportunista para esta diatomácea. Na Baía de Sepetiba, *P. Brasiliiana* foi a primeira espécie a dominar no ambiente (2.8×10^6 células l⁻¹; 85% do microfitoplâncton) após a subsidência de uma floração da rafidofita *Chattonella* sp. no início de fevereiro. Na Lagoa de Imboassica, um sistema costeiro oligohalino ca. 150 km ao norte da Baía de Sepetiba, *P. brasiliiana* foi a espécie dominante ($1.3 - 3.6 \times 10^7$ células l⁻¹; 90% do fitoplâncton total) depois da abertura forçada da conexão entre a lagoa e o mar em meados de fevereiro. Esta abertura permitiu a contribuição de águas mais salinas na lagoa, o que provavelmente introduziu o estoque de células de *P. brasiliiana* que estava presente ao longo da costa durante este período.

PALAVRAS-CHAVE: Floração de diatomáceas, Lagoa de Imboassica, *Pseudo-nitzschia brasiliiana*, Rio de Janeiro, Baía de Sepetiba

ENVIRONMENTAL SETTINGS AND SEQUENCE OF EVENTS

In 1998, the recently described diatom *Pseudo-nitzschia brasiliiana* Lundholm, Hasle & Fryxell (Lundholm *et al.*, 2002) was found to dominate the phytoplankton community at two contrasting sites on the coast of the state of Rio de Janeiro, Brazil: Sepetiba Bay and Imboassica Lagoon (Fig.1). Material collected from Sepetiba Bay on that occasion was used in the study that led to the description of this new species.

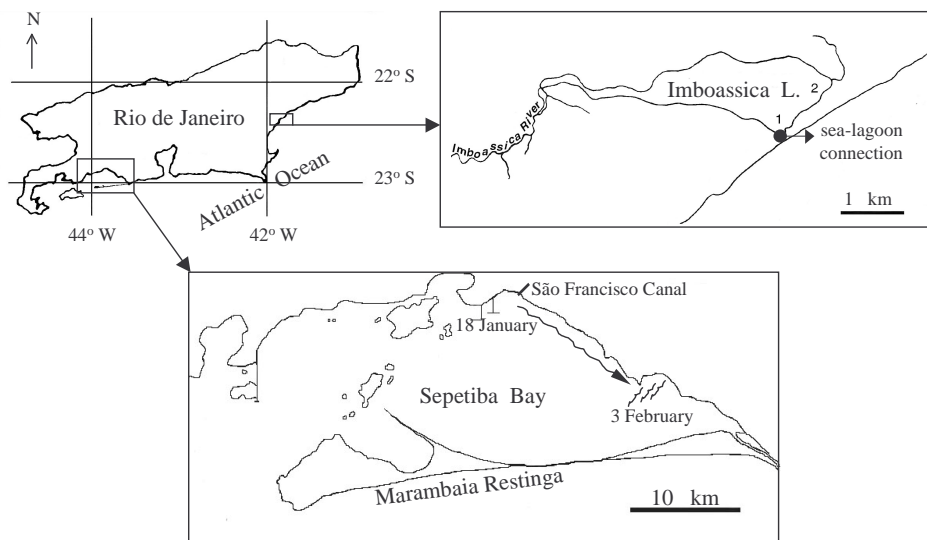


FIGURE 1 – State of Rio de Janeiro and details of Sepetiba Bay (arrow indicates the path of phytoplankton patch from 18 January to 3rd February, when samples were taken) and of Imboassica Lagoon (showing location of sampling stations 1 and 2). In Sepetiba Bay, the estuaries of the rivers Guarda, Guandú and Itaguá are close to the São Francisco canal (and are too small to be pictured on the map at the present scale).

Sepetiba Bay (Fig. 1)

Sepetiba Bay (ca. 305 km²) is separated from the ocean by a sand bar, the Marambaia Restinga. Most of the freshwater input to the bay comes from the eastern side due to the contribution of the rivers Guarda, Guandu and Itaguaí, as well as the São Francisco Canal, whose waters are contaminated by heavy metals and domestic wastes (Amado *et al.* 1999; Feema, 2000). Circulation in the bay is mostly determined by wind and/or tidal-driven currents with a predominant clockwise flow.

On February 1st 1998, newspaper headlines announced a pronounced change of color in surface waters at Sepetiba Bay. It was a bright-green patch of approximately 10 km in length. According to local fishermen, for the previous 15 days, the patch had been travelling from the São Francisco canal towards the interior of the bay. On February 3rd, we were able to sample the patch, which had changed its appearance to long stripes of brownish color. Two days later, the patch was no longer visually detected.

Sampling carried out in the patch and at a site visually outside the patch (Table 1) showed that water temperature was high at both sites, but salinity was markedly lower in the patch. Cell density, estimated from water samples (counting by Utermöhl's settling technique), was high at both sites (over 10⁶ cell l⁻¹), but five times higher in the patch. The patch was characterized by higher chlorophyll *a*, as well as higher total phosphorus and dissolved oxygen, but a lower level of ammonia. The phytoplankton at both sampling sites was dominated (>85% of the nanoplankton) by a raphidophyte (*Chattonella* sp.) which was immediately associated to the bright-green color described by local fishermen and newspaper headlines. The overall shape of the cell, the type of chloroplasts (number, form, and color), and the type and movement of flagella corresponded to those of *Chattonella subsalsa*. Our specimen, however, was smaller than that described for *C. subsalsa* [(14) 20-22µm x 10-13µm, as opposed to 30-50µm x 15-25µm] and the mucocysts of our material were rounded, not panduriformis as in *C. subsalsa*. The cells were extremely sensitive to temperature change, even during short periods of microscope observations; thus, attempts to isolate cells into cultures were not successful. Although fishkill was not observed, as has often been recorded during raphidophyte blooms elsewhere (Hallegraeff & Hara 1995), the media reported cases of skin eruption diagnosed as phytermatoses for those who had direct contact with the water from the patch.

The second most abundant species was *P. brasiliiana*, which comprised 85% of the microphytoplankton in the patch. Since data from the initial period of the phenomenon is not available, we speculate that on February 3rd the *Chattonella* patch had already lost its original characteristics, turning brown in color due to the contribution of this small, opportunistic, pennate diatom. The sampling site outside the patch had 72% of *P. brasiliiana*, but other species were also well represented (*Dactyliosolen fragilissimus*, *Cylindrotheca closterium*), some of them of larger size (*Ceratium hircus* and *Coscinodiscus wailesii*) that can indicate more advanced stages of succession *sensu* Margalef (1980). Even if we assume that a gradual mixing between the patch and the surrounding waters was taking place as the patch was losing its identity and salinity differences were lessened, our data does not allow us to conclude whether *P. brasiliiana* from the patch was seeding the higher abundance found outside or *vice-versa*.

TABLE 1 – Data from Sepetiba Bay taken during the raphidophyte/*P. brasiliiana* bloom on February 1998 and from Imboassica Lagoon taken during the *P. brasiliiana* bloom on March 1998 (refer to Figure 1 for location of stations).

Parameter analysed	Sepetiba Bay		Imboassica Lagoon	
	in the patch	out of the patch	Station 1	Station 2
Phytoplankton (cell l ⁻¹)				
Total	1.0 x 10 ⁷	1.8 x 10 ⁶	1.7 x 10 ⁷	4.7 x 10 ⁷
Microphytoplankton (>20µm)	3.2 x 10 ⁶	3.0 x 10 ⁵	-	-
<i>P. brasiliiana</i>	2.8 x 10 ⁶	2.2 x 10 ⁵	1.3 x 10 ⁷	3.6 x 10 ⁷
Water temperature (°C)	30.0	30.0	27,9	28,8
Salinity (S)	26.88	30.43	20.0	19.2
Dissolved oxygen (ml l ⁻¹)	8.14	5.07	8.32	5.17
Chlorophyll <i>a</i> (µg l ⁻¹)	6.43	1.25	-	-
Ammonia (µM)	0.64	0.81	10,45	30,50
Total phosphorous (µM)	3.10	1.90	1.29	2.40

Imboassica Lagoon (Fig. 1)

Imboassica Lagoon is a small (3.3km²), shallow (mean depth 1.1 m), brackish coastal system located in the northern area of the state of Rio de Janeiro (22°24 'S, 41°42'W) (Panosso & Esteves 2000). This lagoon is separated from the ocean by a sand bar. At times, a lagoon-sea connection is forced opened in order to avoid flooding of the houses that were inappropriately built around the margins of the Lagoon. On each of these occasions, the lagoon-sea connection is kept opened for approximately 15-30 days, after which it is closed by natural sediment deposition. The input of domestic sewage is another anthropogenic disturbance to the lagoon ecosystem.

Phytoplankton communities in Imboassica Lagoon were studied from water samples (counting using Utermöhl's settling technique) for 35 months, fortnightly from November 1996 to May 1998 and monthly from June 1999 to September 2000 (Melo, 2001). Species common to freshwater, brackish and marine systems were found. The waters of the lagoon showed a wide salinity range (0.5 to 35) and its trophic state varied from mesotrophic to hypereutrophic, *sensu* OECD (1982) and Nürnberg (1996). During the studied period, the sandbar was opened three times (16 November 1996, 9 January 1997, and 16 February 1998). Only during the third opening, when Imboassica Lagoon remained connected to the sea for 36 days, a *P. brasiliiana* (Figs 2-4) bloom was observed. There was no change in the color of the water. The sequence of events that took place before, during and after this third opening revealed changes in the phytoplankton assemblages that alternated from a fresh-oligohaline system to more saline conditions, and back to a fresh-oligohaline system (Melo 2001), as detailed below (Fig. 5).

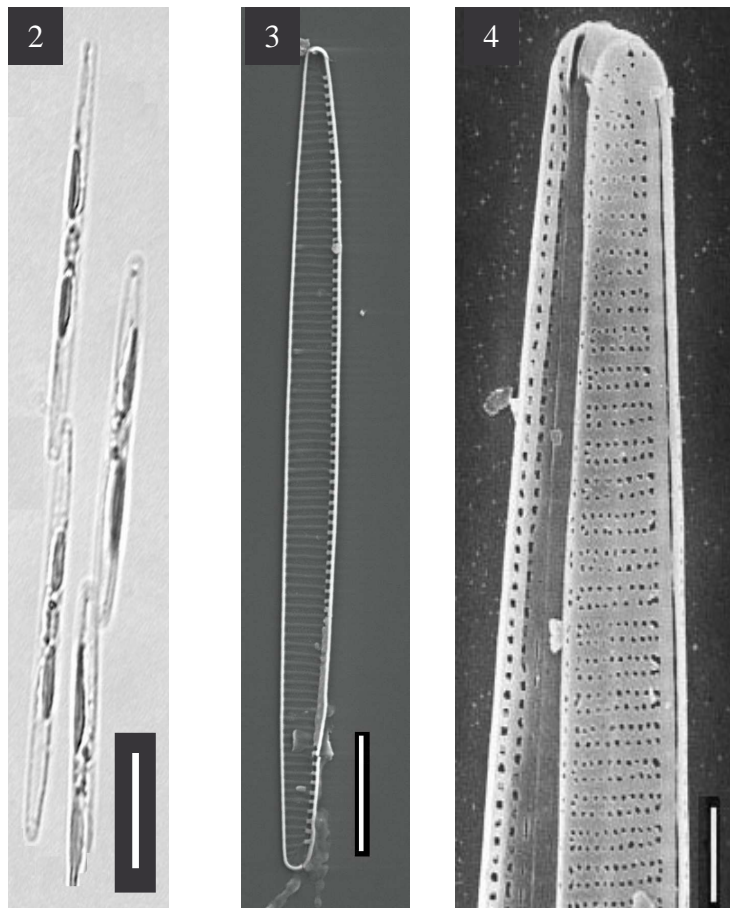


FIGURE 2-4 – *Pseudo-nitzschia brasiliiana* from Imboassica Lagoon. **Fig. 2.** Chain of cells in girdle view (linear with truncate ends). **Fig. 3.** Whole valve (inside view). **Fig. 4.** Detail of bands and valve (outside view). Valve margins are straight along most of the cell length, but taper towards the broadly rounded valve ends. For Sepetiba Bay, apical axis was 12 – 65 µm and the transapical axis was 1.8 – 3.0 µm. For the Imboassica Lagoon, apical axis was 30 – 49 µm and the transapical axis was 2.0 – 3.4 µm. The raphe is eccentric, neither interrupted by a central nodule nor a larger interspace between the two central fibulae. The fibula and interstria correspond in number (20 – 26 in 10 µm) and there are 2 rows of poroids per striae (7 -10 in 1 µm). Scale bars: Fig. 2 = 10µm, Fig. 3 = 5µm, Fig. 4 = 1µm.

Immediately before the third opening of the lagoon-sea connection, the most abundant species, that is, those that contributed with at least 5% of the community, were a cyanobacterium (*Chroococcus microscopicus*), cryptophytes (*Hemiselmis simplex*, *Plagioselmis nanoplanctica*), a chlorophyte (*aff. Elakatothrix* sp.), a raphidophyte (*aff. Chattonella* sp.), and a diatom (*Cyclotella choctawhatcheeana*). During the period of the lagoon-sea connection, *Plagioselmis nanoplanctica*, *Hemiselmis simplex* and *Resultor mikron* were the dominant species (> 50% altogether). *P. brasiliiana* was also observed at 1.2×10^5 cell l^{-1} .

Six days after the closing of the lagoon, *P. brasiliiana* was registered in bloom proportions in two stations (Table 1). The diatom represented, at both sites, ca. 70% of the phytoplankton if the picoplankton fraction (*C. microscopicus*) is considered (Fig. 5bd); otherwise, *P. brasiliiana* comprised 90% of the phytoplankton community (Fig. 5ac). During this *P. brasiliiana* bloom, high values of inorganic nitrogen, especially ammonium, soluble reactive silica, salinity and temperature were registered (Table 1). Fifteen days later, *P. brasiliiana* was no longer observed. *C. microscopicus* became the dominant species (> 60% of cell density), together with cryptophytes (*P. nanoplanctica*, *H. simples*, and *aff. Teleaulax acuta*) and other diatoms (*C. choctawhatcheeana*, *Cocconeis placentula* var. *euglypta*).

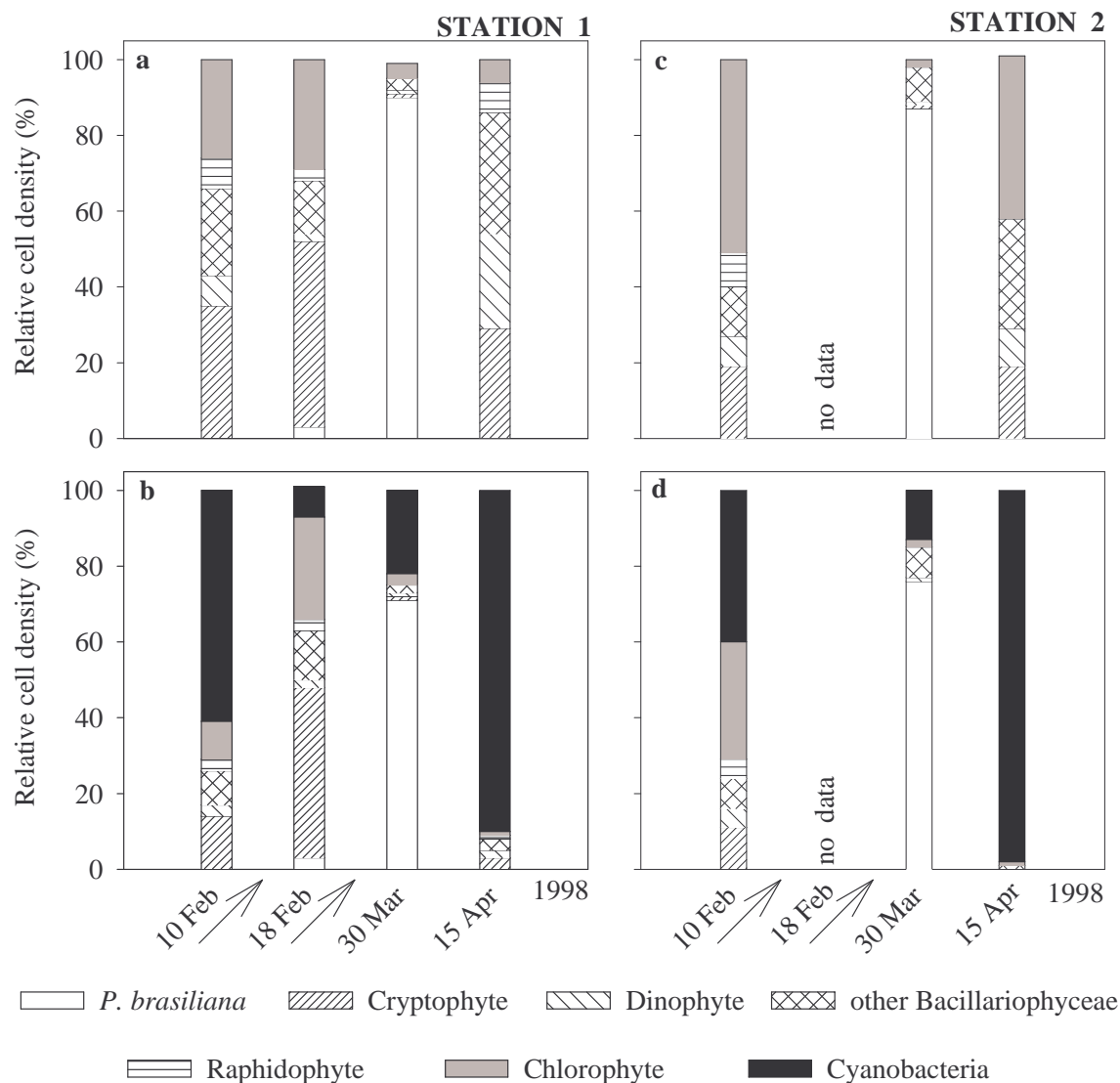


FIGURE 5 – Relative cell density of phytoplankton in Imboassica Lagoon before (10-18 Feb), during (30 Feb) and after (15 Apr) the *Pseudonitzschia brasiliiana* bloom (a and c without Cyanobacteria; b and d with Cyanobacteria). The lagoon-sea connection remained opened between 16 February and 24 March (indicated by an arrow). No data available for Station 2 on 18 February because it was almost dry due to the opening of the lagoon-sea connection.

FINAL REMARKS

Pseudo-nitzschia brasiliiana is a recently described species. It belongs to the *Pseudo-nitzschia americana*-complex, a group of short chain-forming *Pseudo-nitzschia*, some of them often found as epiphytes on other phytoplankton species. The taxonomic revision of this group was long called for (see history of changes on the taxonomic status of *Pseudo-nitzschia americana* in Hasle 1964, Hasle 1993 and Hasle & Syvertsen 1997) and it now includes *P. americana*, *P. brasiliiana* and *P. linea* (Lundholm *et al.* 2002). *Pseudo-nitzschia brasiliiana* has also been reported for other warmer water regions such as Gulf of Panama, Gulf of Mexico, Gulf of California, Vietnam, Indonesia, Thailand, and South Korea (Lundholm *et al.* 2002). As mentioned before, material collected from Sepetiba Bay in February 1998 was used in the study that led to the description of this new species, thus its name: *P. brasiliiana*.

The high abundance and dominance of *P. brasiliiana* in Sepetiba Bay can be interpreted as the initial stages of phytoplankton succession of the microphytoplankton assemblage (*sensu* Margalef 1980) triggered by the impact cause by the raphidophyte bloom. For the event in Imboassica Lagoon, we speculate that salinity played a key role in controlling the increase and decrease of *P. brasiliiana* cell numbers. In this coastal system, average salinity varies from year to year depending on how many times the sea-lagoon connection is opened. During the opening of the sea-lagoon connection, salinity can increase to 36. The genus *Pseudo-nitzschia* is essentially marine (Hasle 1994), although it has been shown in culture that some species can grow in as wide a salinity range as 9 – 45 (Fryxell *et al.* 1990; Jackson *et al.* 1992; Lundholm *et al.* 1997; Villac *et al.* 2004).

We hypothesize that a seed-stock of *P. brasiliiana* was present along the coast of the study areas, at least during part of the 1998 summer period, which provided the inoculum of cells that took advantage of the environmental stress identified for each location. The opportunistic nature of *P. brasiliiana* was indicated by its high abundance and dominance in both environments, which is of concern because 9 species of this genus have shown to produce the neurotoxin domoic acid that may cause Amnesic Shellfish Poisoning (Bates 1998; Fryxell & Villac 1999; Sarno & Dahlmann 2000). *Pseudo-nitzschia brasiliiana* is not (yet!) in the list of toxic species, since one culture isolated from Sepetiba Bay during the episode described in the present paper tested negative for domoic acid production (Lundholm *et al.* 2002).

This first occurrence with an outstanding dominance of *P. brasiliiana* on the coast of Rio de Janeiro raises some interesting questions. Has it been part of the cryptic flora for a long time but was first detected only in 1998? Is it a recent introduction?

There are several possibilities for overlooking the presence of a phytoplankton species: 1) lack of detailed/intensive field sampling; 2) it may be part of the hidden flora and counting procedures at the microscope are below the detection limit; and/or 3) method/expertise is not appropriate for precise identification, which may apply especially in this case, considering that *P. americana* is a recently described taxon and that the identification of the species within the genus *Pseudo-nitzschia* most often requires confirmation with electron microscopy. Nevertheless, one could argue that it could have been detected and misidentified as *P. americana*, a species that can be easily differentiated from the other *Pseudo-nitzschia* species at the light microscope by the rounded cell ends of cleaned valves (as shown in Figs 3-4).

The criteria to establish the status of the new record of a species in a given place as native or introduced can be controversial and highly dependent on the perception/experience of the scientist. Although ballast water is a possible mechanism of transport and introduction of phytoplankton (Hallegraeff & Bolch 1991), as well as mariculture, there are few truly undisputable cases of introduced planktonic organisms (Thresher *et al.* 1999). On the Brazilian coast, the occurrences of the following species have strong arguments in favor of a hypothesis of introduction: *Coscinodiscus waiilesii*, *Alexandrium tamarense* and *Gymnodinium catenatum* (Proença & Fernandes 2004).

In invasion biology, the term cryptogenic is used to refer to a previously unreported species that becomes part of a well-studied biota, often in outstanding cell numbers, but whose origin (native or introduced) is unknown. We propose that this is the status of *P. brasiliiana* on the coast of the State of Rio de Janeiro (and Brazil), as detailed below.

In the case of Sepetiba Bay, intensive shipping activities since the 1970's have created a mechanism for the introduction of species through ballast water discharge (Villac *et al.* 2004). However, as pointed out in Tenenbaum *et al.* (2004), although the phytoplankton of this bay has been intensively studied since the 1970's, routine analysis did not include techniques that could have led to the identification of *Pseudo-nitzschia* at the species level. In short, there is a possible mechanism, but the species could have been overlooked for years.

As for the Imboassica Lagoon, the 35-month intensive survey revealed the presence of 2 undescribed species and 22 new records for fresh and brackish water systems in the state of Rio de Janeiro (Melo 2001). Since 1998, however, *P. brasiliiana* has not been observed in Imboassica Lagoon, which was maintained without a connection with the sea for three consecutive years. In March 2001, however, after a lagoon-sea connection took place, the dominant species were *Chaetoceros* spp. (S. Melo, personal observation). Probably, the seed-stock of *P. brasiliiana* present in the summer of 1998 was no longer in the area.

Pseudo-nitzschia brasiliiana is, therefore, considered to be cryptogenic. On the one hand, its identification is not possible in routine studies (the method most often used is the Uthermölh's settling technique) so we cannot be sure whether it was part of the hidden flora or not. On the other hand, there is a possible vector of introduction (shipping with reception of ballast waters in Sepetiba Bay) and its first occurrence took place as a dominating, opportunistic species. This can be interpreted as an aggressive behavior that some introduced species may have when, outside their original area of distribution, they can grow free from their natural environmental constraints (Carlton 1996). The next step to unravel the true status of *P. brasiliiana* would be the use of molecular biology to trace details of its biogeography, as has been done with other phytoplankton species (Scholin 1998).

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