Harmful Marine Microalgae in Coastal Waters of Chubut (Patagonia, Argentina)

Alicia V. Sastre, Norma H. Santinelli, Miriam E. Solís, Laura B. Pérez, Soledad Díaz Ovejero, Leilén Gracia Villalobos, Andreana Cadaillón, and Valeria C. D'Agostino

Abstract A Harmful Algal Blooms Regional Monitoring Program has been carried out in Chubut coastal waters (Patagonia, Argentina) since the year 2000. This program surveys an extended shoreline, with bays and gulfs with shellfish natural banks and farms. Paralytic shellfish poison (PSP)-toxin-producing species, *A. tamarense*, have been observed during the study period; in addition, species producing diarrheic shellfish poison (DSP)-toxins, such as *Dinophysis acuminata* and *D. tripos* and *Prorocentrum lima*, and amnesic shellfish poison (ASP)-toxins, as several species of genus *Pseudo-nitzschia*, have been identified. Moreover, the production of the three types of toxins has been proven. Other harmful but nontoxic species have been registered in the area. The aim of this review is to show the temporal and spatial distribution of harmful microalgae species, the environmental factors associated with their occurrence, and their relation to toxic outbreaks during more than 15 years of observations, with special attention focused on the episodes of human intoxications. In addition, we discussed the accumulation and transfer of some phycotoxins through pelagic food webs, from the first trophic levels to large marine mammals, such as whales.

Keywords Harmful marine microalgae · Phycotoxins · Coastal waters · Patagonia Argentina · Southwest Atlantic Ocean

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A. V. Sastre (✉) · N. H. Santinelli · S. D. Ovejero · A. Cadaillón
Instituto de Investigación de Hidrobiología, Facultad de Ciencias Naturales y Ciencias de la Salud, Universidad Nacional de la Patagonia San Juan Bosco, Trelew, Argentina

M. E. Solís · L. G. Villalobos · V. C. D'Agostino
Centro para el Estudio de Sistemas Marinos (CESIMAR), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Puerto Madryn, Argentina
e-mail: miriam@cenpat-conicet.gob.ar; gracia@cenpat-conicet.gob.ar

L. B. Pérez
Laboratorio de Biología, Facultad de Ciencias Naturales y Ciencias de la Salud, Universidad Nacional de la Patagonia San Juan Bosco, Comodoro Rivadavia, Argentina

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1 Introduction

While harmful algal blooms, in a strict sense, are completely natural phenomena that have occurred throughout recorded history, in the past two decades, the impact of such events on public health and economy appears to have increased in frequency, intensity, and geographical distribution (Van Dolah 2000; Hallegraeff 2004). Harmful algal blooms (HABs), commonly called red tides, affect virtually every coastal region of the world. Since the latter term erroneously includes many blooms that discolor the water but cause no harm and also excludes blooms of highly toxic cells that cause problems at low (and essentially invisible) cell concentrations, scientists prefer the term HAB (Anderson et al. 2012).

In the broad sense, UNESCO's Intergovernmental Oceanographic Commission (IOC) coined the term "harmful algal blooms" (HABs) to designate the occurrence of a heterogeneous group of microorganisms that are perceived as harmful. Then, the HAB designation is a societal concept rather than a scientific definition. Blooms are considered to fit the HAB criterion if they cause injury to human health, to socioeconomic interests, or to components of aquatic ecosystems (Reguera 2002; Anderson et al. 2012).

Among the different types of HABs, some may be harmful non-toxigenic. They may cause serious damage to marine wildlife by clogging the fish gills, decreasing oxygen levels in the water column, triggering death by anoxia, etc. Some HABs may injure gill tissues and membranes in fish producing their death by a mechanical effect of their cellular spines and horns (Hasle and Fryxell 1995) or by producing hemolytic substances, as it is the case with some species of Raphydophyceae (Suárez-Isla and Guzmán 1999; Hallegraeff and Hara 2004). On the other hand, some HAB species are toxigenic and produce blooms that cause illness and death of fish, seabirds, mammals, and other marine life, often via toxin transfer through the food web by consumption of filter-feeding organisms that accumulate the toxin-producing species. Human consumers of seafood contaminated by these toxins may also be poisoned, suffering acute toxic symptoms and even fatalities in extreme cases (Anderson et al. 2012). Based on the symptomatology of the intoxication and on the vectors of transmission, different types of poisoning have been defined (Fernández et al. 2002; Wang 2008).

Zooplankton can be an intermediate link in the trophic transfer of phycotoxins to higher consumers, potentially intoxicating marine fish, birds, and mammals (Lefebvre et al. 1999; Scholin et al. 2000). The Valdés Peninsula, declared a World Heritage Site by UNESCO in 2000, and its surrounding gulfs have a rich biodiversity of marine birds and mammals of a great tourist importance; the most representative species is the southern right whale, *Eubalaena australis* UNESCO's Natural Monument, which finds in these waters an important calving ground. Doucette et al. (2006), Leandro et al. (2010), and Fire and Van Dolah (2012), among others, have clearly demonstrated that whales ingest the different toxins that pass through their digestive tract. Thus, harmful algal blooms pose a risk for this protected species as well as for other animals that feed on zooplankton (D'Agostino et al. 2015) and shellfish.

Red tides in the South American Cone, as in other regions of the world, have been recorded as early as the time of the great oceanographic expeditions of the nineteenth century (Reguera 2002). Carreto and Benavides (1993) reported the existence of historical archives in Argentina describing mortalities within the indigenous populations of Ushuaia, caused by shellfish consumption, since 1886. In the Argentine seashore, this phenomenon was first reported in shelf waters off the Valdés Peninsula in 1980, when two fishermen died after eating mussels with high concentrations of paralytic shellfish poison (PSP)-toxins, associated with *Gonyaulax excavata* (Braarud) Balech (Carreto et al. 1981). This species was later transferred to the genus *Alexandrium* Halim under the name *Alexandrium excavatum* (Braarud) Balech et Tangen and synonymized by Balech (1995) as *Alexandrium tamarense* (Lebour) Balech. Molecular studies revealed that *A. fundyense* from eastern USA, *A. catenella* from western USA and western South America, and *A. tamarense* from western South America are all the same species, the so-called *A. tamarense* complex Group I (Lilly et al. 2007), lately renamed *A. fundyense* (John et al. 2014). After strong controversy, the final name adopted for the *A. tamarense* Group I was *A. catenella* (Fraga et al. 2015), priority based on seniority (Prud'homme van Reine and Willem 2017). This study retains the name *A. tamarense* because of the previously published literature for this region and since the confirmation of the presence or absence of alternative ribotypes of this species complex in the eastern South America has not yet been communicated.

Monitoring of PSP-toxins in shellfish in Chubut coastal waters started after the 1980 event. Nevertheless, several toxic outbreaks, some of them including fatal cases, have occurred in different sites of the coast ever since (Vecchio et al. 1986; Esteves et al. 1992; Andrade 2001; Andrade 2002; Santinelli et al. 2002; Baulde 2010; Baulde 2011).

2 The Chubut Province Monitoring Program

Since the year 2000, a Harmful Algae Blooms and Shellfish Toxicity Monitoring Program has been carried out in Chubut coastal waters (Patagonia, Argentina) as part of the Provincial Plan for Prevention and Control of Red Tide. This area covers an extended shoreline of approximately 1600 km, with bays and gulfs with natural shellfish banks and farms. The program includes monitoring of harmful algal blooms and marine environmental conditions, shellfish toxicity control and harvesting closures, educational and training activities, dissemination of information, and detection of intoxicated consumers in public health centers. At the beginning, only the northern coastal zone (North Patagonian gulfs) was controlled, but the southern stations were added several years later. Moreover, amnesic shellfish poison (ASP) and diarrheic shellfish poison (DSP) have been measured since 2005 and 2008,

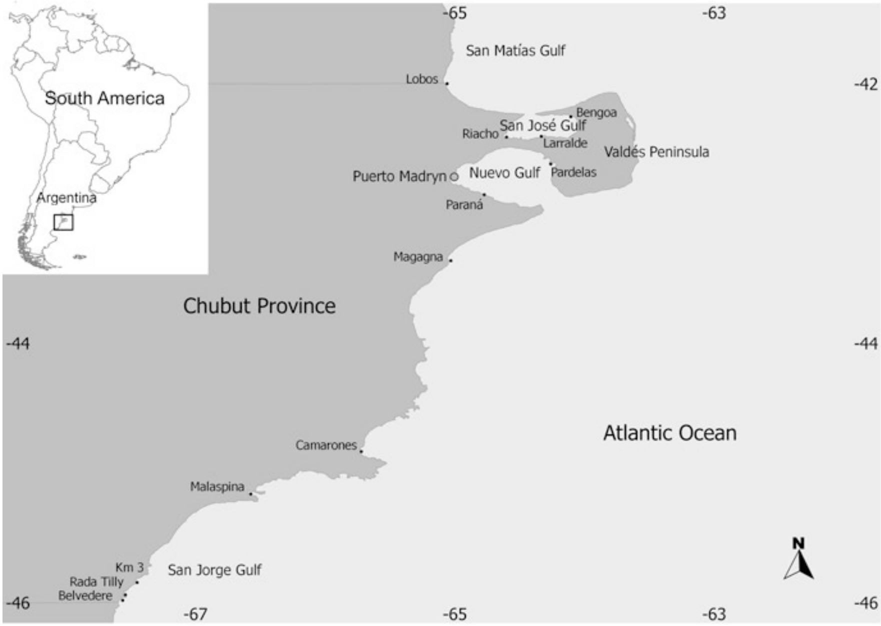


Fig. 1 Study area and location of the sampling stations

respectively. Nowadays, samples are taken monthly or bi-weekly from 12 sites of the Patagonian shoreline.

The study area is located approximately between 42°–46°S and 64°–67°30′W (Fig. 1). We will consider the North Patagonian gulfs of San Matias, San José, Nuevo, and Magagna as part of the northern zone and Camarones and the San Jorge Gulf as the southern zone. Samples were collected from integrated water column; 250 ml subsamples were preserved with Lugol's solution and stored for species identification and enumeration. Afterward, they were counted using the Utermöhl (1958) method with a Leica DMIL phase contrast inverted microscope. In addition, qualitative phytoplankton samples were taken using a 25 μm mesh net through oblique tows on a boat and fixed with formaldehyde at a final concentration of 4%. They were later analyzed at the Instituto de Investigación de Hidrobiología of the Universidad Nacional de la Patagonia.

Living and fixed net samples were observed with an Olympus CX31 phase contrast light microscope. Taxonomic identification of dinoflagellates was carried out following Balech (1995), and diatoms frustules were cleaned following the Hasle and Fryxell (1970) procedure. Scanning electron microscopy observations of the samples were made with a Jeol JSM-6360 LV scanning electron microscopy at the Facultad de Ciencias Naturales y Museo and Universidad Nacional de La Plata and with a Zeiss Supra 40 at the Universidad de Buenos Aires advanced microscopy center.

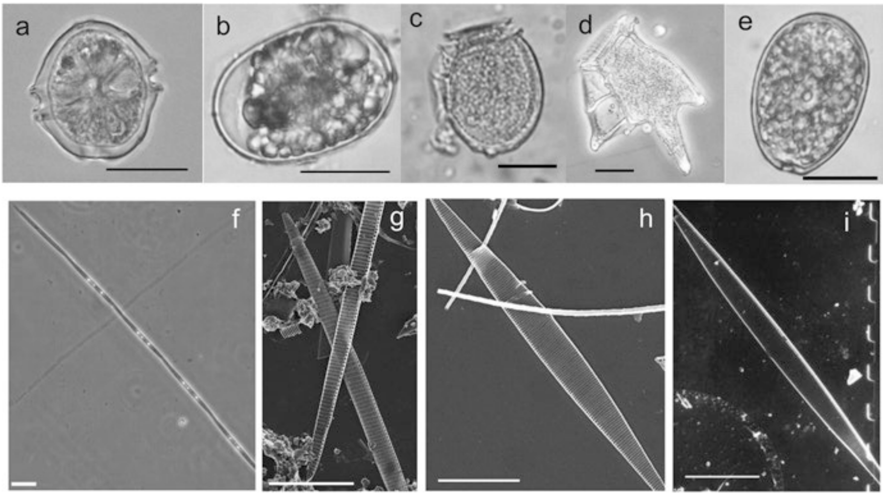


Fig. 2 Main toxin-producing species in Chubut province. (**a**) *Alexandrium tamarense* vegetative cell, (**b**) *A. tamarense* resting cist, (**c**) *Dinophysis acuminata*, (**d**) *D. tripos*, (**e**) *Prorocentrum lima*, (**f**) *Pseudo-nitzschia calliantha*, (**g**) *P. pungens*, (**h**) *P. australis*, (**i**) *P. fraudulenta* (**a–f** LM; **g–i** SEM) (Scale bars = 20 μm)

3 Harmful Toxin-Producing Species

Outbreaks of harmful algal blooms (HAB) have occurred in coastal waters of Chubut province (Patagonia, Argentina) since 1980. Different toxin-producing species associated with these events have been identified (Fig. 2): (i) paralytic shellfish poisoning (PSP), *Alexandrium tamarense*; (ii) diarrheic shellfish poisoning (DSP), *Dinophysis acuminata*, *D. tripos*, *D. caudata*, *D. fortii*, *D. acuta*, *Phalacroma rotundatum*, *Prorocentrum lima*, *P. cordatum*, and *Protoceratium reticulatum*; and (iii) amnesic shellfish poisoning (ASP), *Pseudo-nitzschia australis*, *P. multiseries*, *P. pungens*, *P. fraudulenta*, and *P. calliantha*.

4 Harmful Non-toxigenic Species

Among the harmful non-toxigenic species, the most frequent in the samples were the diatoms *Coscinodiscus wailesii*, *Thalassiosira mala*, *Leptocylindrus minimus*, *Chaetoceros socialis*, *C. concavicornis*, and *Asterionellopsis glacialis*, the dinoflagellates *Prorocentrum micans* and *Lepidodinium* sp., and the silicoflagellates *Dictyocha fibula*, *D. speculum*, and *D. octonaria*. Although they have not caused any proven damage to the fauna or flora in this region, they are responsible for harmful events in other geographic areas (Clément and Lembeye 1993; Hallegraeff 2004; Hasle and Fryxell 1995; Andersen et al. 1995; Hargraves and Maranda 2002; Fryxell and Hasle 2004; Smayda 2006).

5 Environmental Features

The seasonal variability of the hydrographic conditions of both the northern and southern areas is summarized in Fig. 3 and is based on monthly mean temperature, nitrate + nitrite (hereinafter identified as nitrate), phosphate, and silicic acid data collected during the Monitoring Program in Chubut coastal waters. Both areas show the typical seasonal variability of temperate-cold regions. The northern area was characterized by an annual average salinity of 34.06 ± 0.22 (*n* = 818) (not shown in the figure), temperature in the range of 10.7 °C–17.5 °C (Fig. 3a). Nutrient concentrations (Fig. 3b–d) were highest in winter (nitrate ~ 5 μM, phosphate ~ 1.4 μM, and silicic acid ~ 5.7 μM) and lowest from spring to early autumn (nitrate <1 μM or no detected, phosphate <1.1 μM, and silicic acid mainly <2.5 μM). In the southern area, annual average salinity was 33.46 ± 0.41 (*n* = 336) (not shown in the figure), and temperature was between 8.4 °C and 15.5 °C (Fig. 3e). The highest nutrient concentrations (Fig. 3f–h) were also observed in winter, but values were higher than in the northern area (nitrate ~ 10.5 μM, phosphate ~ 1.6 μM, and silicic acid ~ 6.1 μM). The lowest nutrient values were recorded from spring to the end of summer; concentrations of phosphate and silicic acid concentrations were similar to the northern area, but nitrate did not reach undetected levels.

6 Spatial and Temporal Dynamics of Harmful Species

Only the more frequent species in the phytoplankton samples were considered in this analysis. The harmful species showed a large interannual variability in their occurrence and density and differences between sampling sites (Fig. 4). There were years, such as 2001, with great species diversity but others with a strong dominance of a single species, such as 2012 and 2013. *A. tamarense* was an important component within the harmful species in the years 2000, 2004, and 2005 in some stations of the north zone (Magagna, Nuevo, and San José gulfs) and in the 2010 and 2015 in the stations of the south zone (San Jorge Gulf). Among *Pseudo-nitzschia* species, *P. pungens* was well represented until 2010 in the northern area (except 2006 and 2008); *P. australis* until 2006; *P. calliantha* during 2007, 2008, 2009, 2010, and 2015; and *P. fraudulenta* from 2006 onward.

7 Spatial and Temporal Dynamics of *Alexandrium tamarense* and PSP

Alexandrium tamarense was present throughout Chubut province coasts, with maximum cell densities at the end of winter and during spring in the northern zone (Santinelli et al. 2002) and during spring and summer in the south (Pérez et al. 2013)

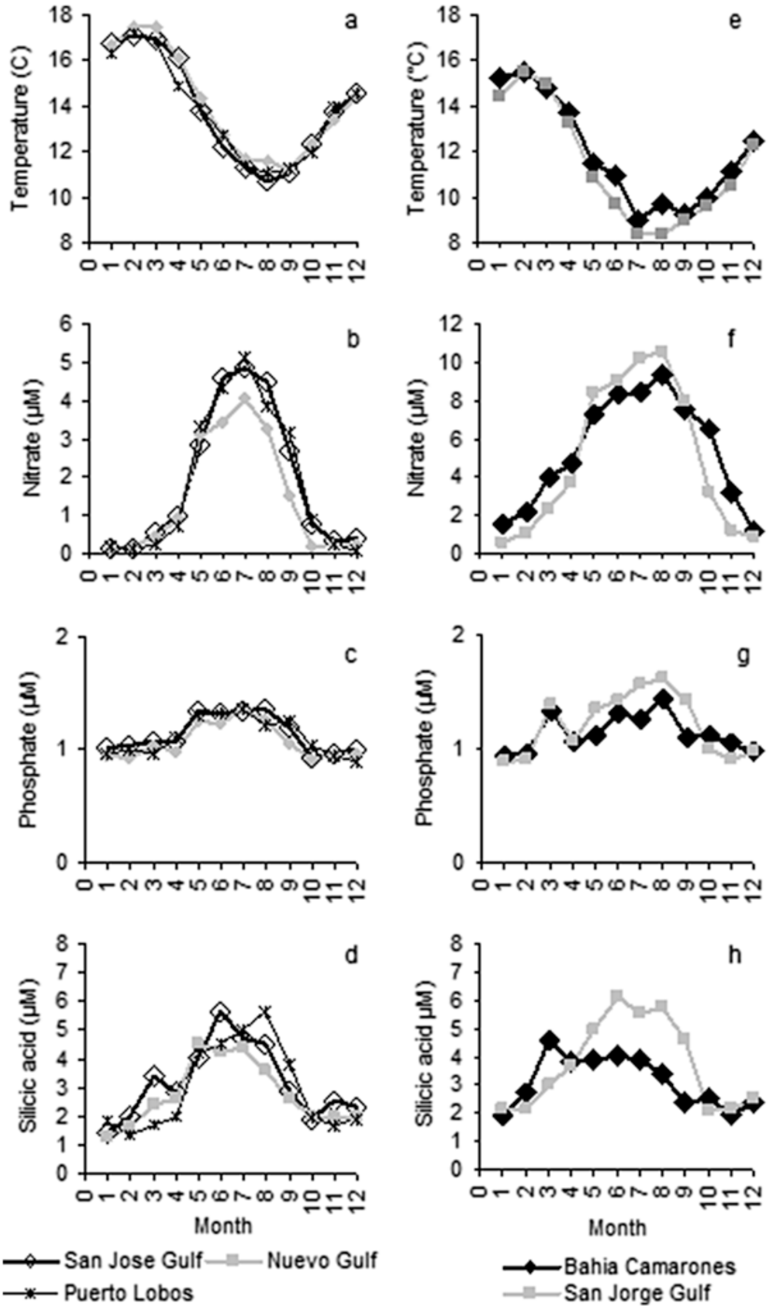


Fig. 3 Monthly mean temperature and seasonal variation of nitrate, phosphate, and silicate in northern (**a–d**) and southern (**e–h**) areas

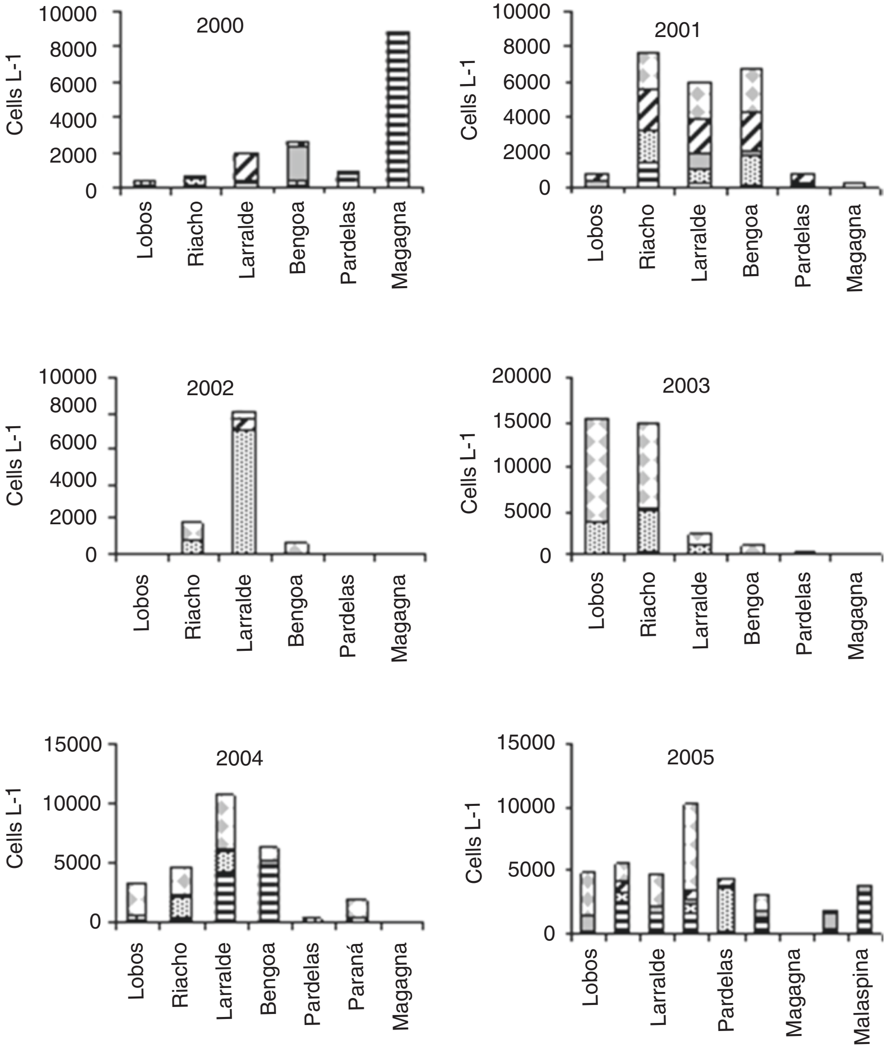


Fig. 4 Spatial and temporal distribution of toxin-producing species.(**a**) Annual average of cell densities between 2000 and 2005, (**b**) Annual average of cell densities between 2006 and 2011, (**c**) Annual average of cell densities between 2012 and 2016

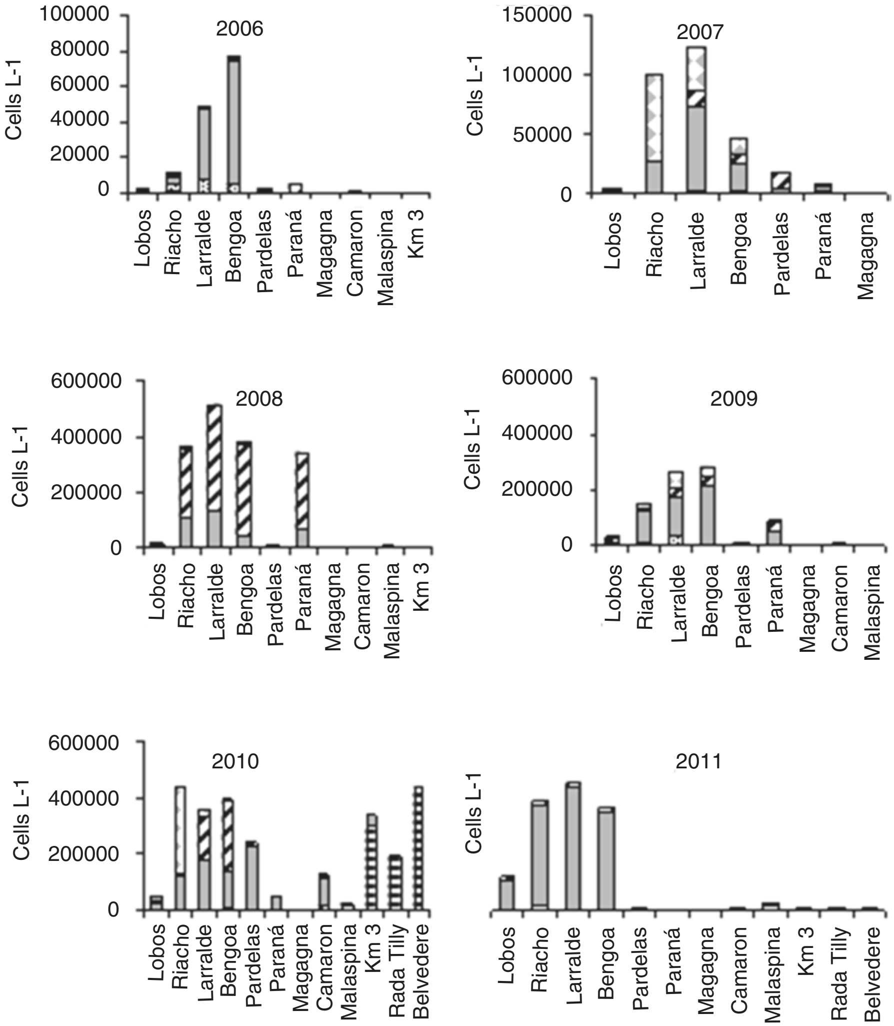
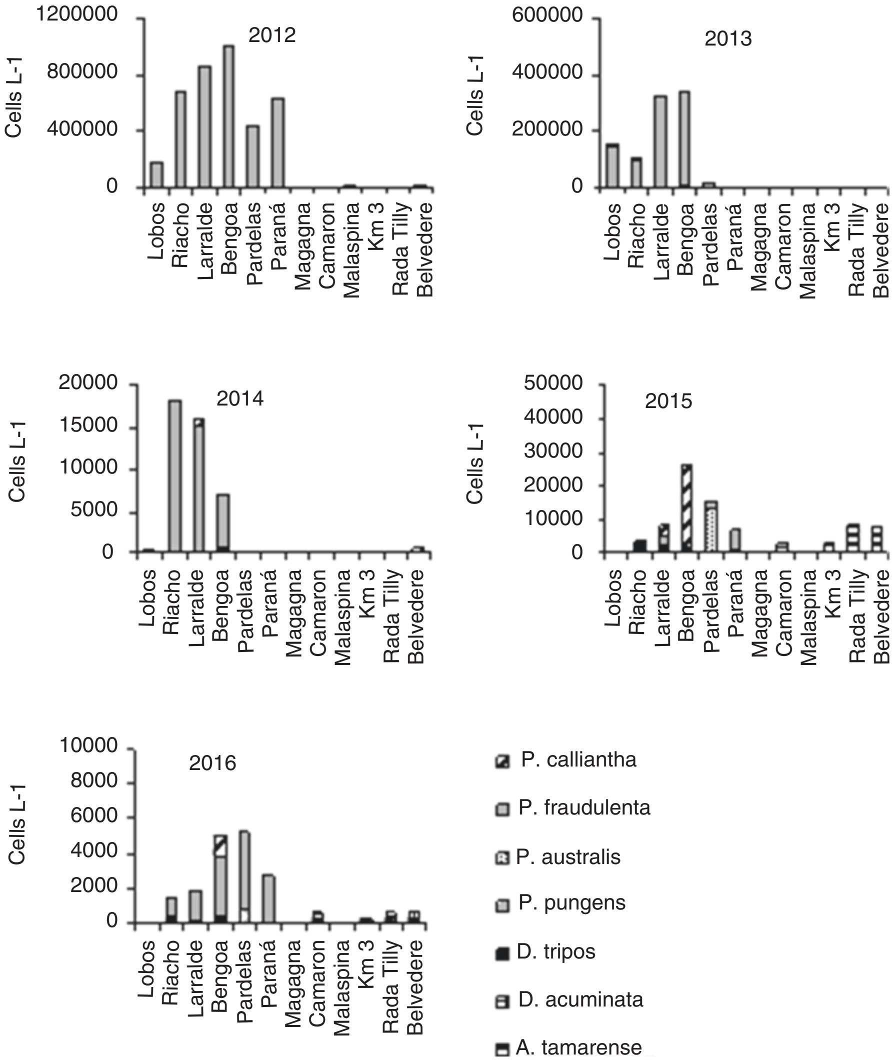


Fig. 4 (continued)



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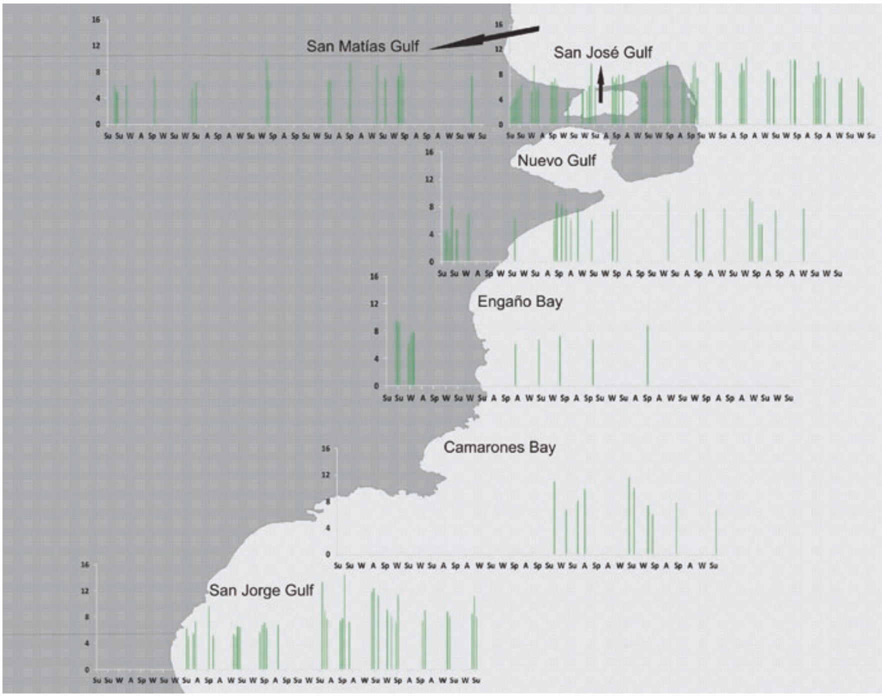

Fig. 5 Seasonal dynamics of *Alexandrium tamarense* density (as ln cell L^{-1}) in Chubut coastal waters between 2000 and 2015

(Fig. 5). This species produced recurrent toxic events during spring and summer associated with a wide interannual variability in toxin concentrations (Andrinolo et al. 1999; Carreto et al. 1996; 1998a, b; Esteves et al. 1992; Reyero et al. 1998; Santinelli et al. 2002).

In the northern zone, cell densities of this species were below 10^4 cells L^{-1} although they led to toxin levels in shellfish, frequently exceeding the regulatory levels established by the World Health Organization (WHO) and by the Argentinian Food Code (AFC) (800 μg STX eq./kg of tissue). The maximum toxin level recorded was 41,114 μg STX eq./kg of tissue in *Aequipecten tehuelchus* (shellfish) in Bengoa station (San José Gulf) in November 2005, following a peak of 6.6×10^3 cells L^{-1} of *A. tamarense* in October. In contrast, high densities of this species were recorded in the Nuevo Gulf in January 1988, (75×10^4 cells L^{-1}), associated with 13,230 μg STX eq./Kg of tissue in *Aulacomya atra* (Esteves et al. 1992) and in December 1993 (2.3×10^6 cells L^{-1}) without toxicity results (Santinelli 2008).

In the southern zone, the highest cell density (7×10^5 cells L^{-1}) and the highest percentage of this dinoflagellate (52%) in relation to the whole phytoplankton community were recorded in January 2010 associated with a toxicity peak of more than 22,000 μg STX eq./kg of shellfish tissue. At this time, five cases of intoxication with a half-hour incubation period and one fatal case occurred in Rada Tilly (Baulde

2010). By late spring (November), the annual maximum of this species (1.9×10^6 cells L^{-1}, 97% of total phytoplankton) was recorded. It should be noted that this has been the record value for all the sampling stations that are part of the monitoring program since 2000. At Belvedere station, the net sample was formed exclusively by *A. tamarense* resting cysts, indicating that the populations were going through an encystment phase. Toxin levels in *Mytilus edulis platensis* reached 88,596 µg STX eq Kg^{-1} of tissue a month later (December).

Blooms occurred in summer with temperatures between 15 and 17 °C and nitrate + nitrite practically depleted. Those from the spring were observed with temperatures between 8 and 10 °C and with undetectable nitrate + nitrite concentrations. One year later (December 2011), a new toxic bloom occurred north of the San Jorge Gulf associated with more than 100,000 µg STX eq Kg^{-1} of shellfish tissue, causing two severe cases of intoxicated people, one of them fatal (Baulde 2011). HPLC analyses of phytoplankton and filter-feeding bivalves, both in the north (Nuevo and San José gulfs) and in the south (San Jorge Gulf), showed that gonyautoxins (GTXs) were the most abundant PSP-toxins. Among these, GTX 1–4 epimers were predominant, and GTX 2 and GTX 3 were present in low concentrations. Small amounts of N-sulfocarbamoyl (C1–C4) and (C1–C2) were detected in mollusk samples but not in phytoplankton and only traces of STX and dc STX in both phytoplankton and shellfish (Reyero et al. 1998; Andrinolo et al. 1999; Sastre et al. 2013).

8 Spatial and Temporal Dynamics of DSP-Toxin-, Pectenotoxin-, and Yessotoxin-Producing Species

Table 1 shows the sites, dates, and environmental conditions in which the highest cell densities of the DSP-toxin-, pectenotoxin-, and yessotoxin-producing species were recorded. The presence of several *Dinophysis* species has been reported since the start of the monitoring program in 2000. *Dinophysis tripos* has also been detected along the coast throughout all the seasons, but peaks of abundance were observed mainly in autumn and winter. It was recorded for the first time in May 2007 with a cell density of 2×10^3 cells L^{-1} in Lobos (San Matías Gulf) and reached a maximum of 26×10^3 cells L^{-1} in Riacho (San José Gulf) in March 2015 (Fig. 6a). *Dinophysis acuminata* was registered along the whole coast (San Matías, San José, Nuevo, and San Jorge gulfs and in Magagna and Camarones) mainly in spring and summer. In November 2007, it reached a maximum density of 5×10^3 cells L^{-1} in the San José Gulf (Larralde) (Fig. 6b). *D. acuminata* is the main agent of chronic and persistent episodes (spring-autumn) of DSP (part of the lipophilic toxin complex), mainly okadaic acid, in bivalves from the Galician coast (Reguera and Pizarro 2008). *Dinophysis fortii* and *D. acuta* were registered in the Nuevo Gulf in January 2006, with a maximum density of 4×10^2 cells L^{-1}. Two species, *Dinophysis caudata* and *Phalacroma rotundatum* (*Dinophysis rotundata*), were

Table 1 Maximum cell density, sampling site and date, and hydrographic data of lipophilic shellfish toxin (LST)-producing species (nutrients in μM)

Species	Cells L^{-1}	Site	Date	T°C	Salinity	NO_3-N	PO_4-P	SiO_2-Si
D. tripos	8400	Bengoa	Aug 2010	11	34.5	2.53	1.20	3.79
D. acuminata	5400	Larralde	Nov 2007	12	34.0	0.03	0.83	0.20
D. caudata	Scarce in net samples	Lobos	Feb 2009	17	34.4	0.16	1.59	0.03
D. fortii	400	Paraná	Jan 2006	17	33.8	0.25	0.78	1.19
D. acuta	440	Paraná	Jan 2006	17	33.8	0.06	0.89	1.53
P. rotundatum	Scarce in net samples	Malaspina	Oct 2005	9	33,7	0,03	0,70	1,07
P. lima	1320	Riacho	May 2004	14	33.7	2	4.17	1.32
P. cordatum	6200	Camarones	Nov 2005	12.5	33.6	0.21	0.95	2.48
P. reticulatum	Scarce in net samples	Pardelas	Oct 2010	12	34.3	0.29	0.73	1.15

not detected in samples for quantitative analysis; they occurred sporadically and were detected in qualitative analysis. *D. caudata* was only found in the San Matias Gulf in summer. These results show that *Dinophysis* species only appeared sporadically in quantitative samples. There are two possible explanations for this finding: on the one hand, the sporadic occurrence in quantitative plankton samples may be due to low cell densities, a common feature among *Dinophysis* spp., which makes it difficult to acquire accurate quantitative information and will often be associated with high counting errors (Reguera et al. 2012). On the other hand, it is known that populations of *Dinophysis* are aggregated in patches or in thin layers of the water column and thus they may escape observation with conventional sampling methods (Escalera et al. 2012).

Other producers of lipophilic toxins present in the study zone were *Prorocentrum lima*, first reported by Santinelli et al. (1995) and related to human intoxications in Puerto Madryn city (Gayoso and Ciocco 2001; Gayoso et al. 2002), *P. cordatum* and *Protoceratium reticulatum*. *P. lima* was most abundant in the northern zone in autumn. *P. cordatum* in the southern coast in summer and *P. reticulatum* was only registered in net samples in the Nuevo Gulf in spring. *D. tripos* could be identified as a pectenotoxin (PTX)-producing species in North Patagonian gulfs and thus most likely responsible for positive DSP mouse bioassays in the region. The PTX-2 production associated with *D. tripos* along the Chubut coast is in accordance with other observations in the Argentine Sea (Fabro et al. 2015) as well as in other regions (Rodríguez et al. 2012). Our study suggests that *D. tripos* blooms associated with the presence of DSP in shellfish are becoming a recurrent phenomenon in the North

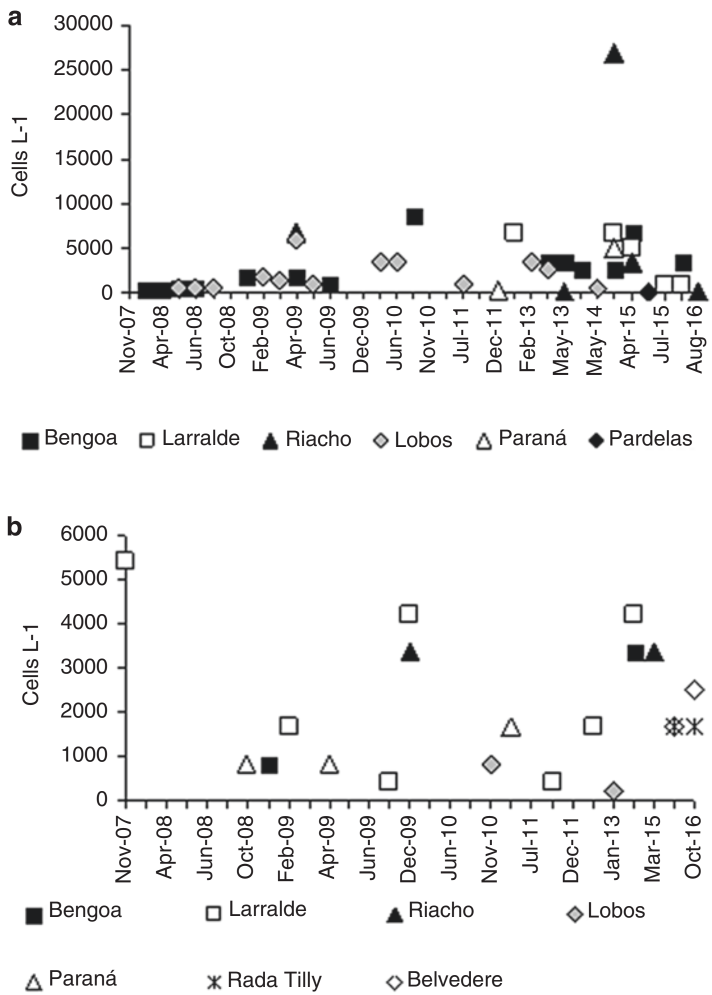

Fig. 6 Cell densities of *Dinophysis tripos* (**a**) and *D. acuminata* (**b**) in different locations of the Chubut coastal area between November 2007 and August/October 2016

Patagonian gulfs (Gracia Villalobos et al. 2015). However, *D. tripos* has never been cited as the causative agent of DSP events when it was the only or the overwhelmingly dominant species of *Dinophysis* in the micro-sized phytoplankton (Reguera et al. 2014). In contrast, *D. acuminata* has been identified as the causative agent of DSP in Southern Brazil (Proença et al. 2007) and, combined with *D. caudata*, in Uruguay (Méndez and Ferrari 2002) and Argentina (Sar et al. 2010, 2012; Sunesen et al. 2014). A coastal species, *D. acuminata*, has a strong negative impact on shellfisheries, because it is an early blooming species with a very long growing season (spring to autumn). This is the most cosmopolitan *Dinophysis* species associated with DSP events (Reguera et al. 2014). Between 2009 and 2016, fishing

Table 2 Maximum cell density, sampling site and date, and hydrographic data of amnesic shellfish toxin (AST)-producing species (nutrients in µM)

Species	Cells L^{-1}	Site	Date	T°C	Salinity	NO_3-N	PO_4-P	SiO_2-Si
P. australis	394,800	Larralde	Nov 2009	12	33.9	0.00	0.78	0.26
P. pungens	2,197,440	Riacho	Nov 2010	12	34.2	0.55	0.86	1.19
P. fraudulenta	4,174,400	Larralde	Dec 2012	13	–	0.00	0.97	0.34
P. calliantha	3,292,800	Bengoa	Dec 2010	13	34.0	0.00	0.96	0.37

authorities had to implement 15 closures for commercial extraction due to DSP-toxins in the Chubut coastal zone.

9 Spatial and Temporal Dynamics of *Pseudo-nitzschia* spp.

P. australis, *P. pungens*, *P. multiseries*, and *P. pseudodelicatissima* have been cited in North Patagonian gulfs (San Matías, San José, and Nuevo Gulfs) (Sastre et al. 1995, 2001). Later, following the taxonomic review by Lundholm et al. (2003), it was verified that according to the ultrastructure of the areolae, the latter species corresponded to *P. calliantha.* Table 2 shows the sites, dates, and environmental conditions in which the highest cell densities of the amnesic toxin-producing species were recorded.

P. fraudulenta has been the most common species in Chubut coastal waters. It has been present in all the sampling stations and showing the highest cell densities. This species, together with *P. calliantha* and *P. pungens*, has several times exceeded densities of 10^6 cells L^{-1}, in the northern gulfs (San Matías, San José, and Nuevo) reaching levels between 2 and 4×10^6 cells L^{-1} in the San José Gulf (Table 2).

The highest densities of *Pseudo-nitzschia* spp. were observed between 2008 and 2012. The ASP-toxin, domoic acid (DA), was detected in continental shelf waters of the Argentine Sea (Southwestern Atlantic Ocean) in July 2000 associated with *P. australis* (Negri et al. 2004). In Chubut coastal waters, this ASP-toxin was measured in phytoplankton concentrates from the Nuevo Gulf (Pardelas station) and Camarones in October 2005, rich in *P. fraudulenta* and *P*. *pungens* (Sastre et al. 2007). Both species co-occurred during the spring bloom at the Pardelas and Camarones stations. Blooms coincided with nutrient decline and increased values of temperature and Secchi disk depth reads. The species *P. australis*, *P. fraudulenta*, *and P. pungens* are worldwide known as DA producers (Lelong et al. 2012).

10 Accumulation and Transport of Some Phycotoxins Through Pelagic Food Webs

Phycotoxins in Chubut coastal waters are concentrated by primary consumers as filter-feeding bivalve mollusks, such as *Mytilus edulis platensis*, *Aulacomya atra*, *Aequipecten tehuelchus*, *Ameghinomya antiqua*, *Ensis macha*, and *Panopea abbreviata*, and by secondary consumers, such as gastropods *Odontocymbiola magellanica*, *Buccinanops cochlidium*, *Buccinanops globulosus*, and *Trophon geversianus*. When PSP-toxin levels in these species exceed the regulatory limits (800 μg Kg^{-1} of shellfish tissue) established by the World Health Organization (WHO), the fishing authority implements closures for commercial extraction. These closures mainly affect the artisanal fisheries and mariculture between September and March.

In addition, Cadaillón (2012) demonstrated the transfer of these phycotoxins (PSP and DA) to zooplankton, the next trophic link in the planktonic food chain, in the study area. This author showed that during spring 2010, ASP-toxins reached a maximum concentration of 42.78 μg DA g $tissue^{-1}$ in zooplankton from Riacho, associated with a sharp increase of *P. pungens* and *P. fraudulenta* populations. In addition, *P. australis* and *P. calliantha* were detected in net samples. Overall, species of the genus *Pseudo-nitzschia* represented 81% of the total phytoplankton community. *A. tamarense* was present in this gulf since the end of winter (September), and STX levels in phytoplankton reached a maximum of 1.866 μg STX eq g $cell^{-1}$ in Bengoa and 3.380 μg STX eq g $tissue^{-1}$ in zooplankton in Larralde (November). In the Nuevo Gulf, the maximum toxin level in zooplankton, 25.75 μg DA g $tissue^{-1}$, was associated with a bloom of *P. fraudulenta*, when this species represented 90% of the total phytoplankton.

Undoubtedly, the presence of toxin-producing phytoplankton species and the possibility of transfer of their toxins to the zooplankton pose a risk to marine organisms, such as fish, birds, and mammals, which are zooplankton consumers belonging to a unique ecosystem of great tourist interest. D'Agostino et al. (2015) found fragments of *Pseudo-nitzschia* spp. frustules in all the southern right whale *Eubalaena australis* fecal samples analyzed. At least four taxa, *P. australis*, *P. fraudulenta*, *P. pungens*, and the *P. pseudodelicatissima* complex, were identified in the feces and water samples analyzed. Micro-sized crustacean remains, mainly copepodite five mandibular gnathobases of *Calanus australis* (a common species in the Argentine Sea), were also found in fecal samples of live and dead whales from the San José Gulf (D'Agostino et al. 2016). These findings indicate that southern right whales may have been exposed to DA, while feeding in this area and copepods could have acted as the main vector of this neurotoxin.

In recent years, there have been an increased number of dead whales found in the Valdés Peninsula area (753 dead whales from 2003 to 2016, Southern Right Whale Health Monitoring Program), and 4 main hypotheses were proposed to explain this phenomenon (Rowntree et al. 2013). One of them, addressed in this study, is the transfer of marine biotoxins along the food web from producing organisms to southern right whales through mesozooplanktonic vectors. Trace levels of STXs and DA were detected in samples of feces, urine, and tissues collected from dead individuals of *E. australis* in the Valdés Peninsula (Uhart et al. 2009; Rowntree et al. 2013). Southern right whale mothers and their calves are exposed to biotoxin-producing algae in the Valdés Peninsula area. High-risk levels of *Pseudo-nitzschia* spp. and *A. tamarense* occurred between 2007 and 2013, the period with the highest number of dead whales (>50 deaths/year) (Wilson et al. 2016). The occurrence of toxic *Pseudo-nitzschia* spp. blooms concurrently with the whale season and the detection of high levels of DA (710 μg DA g^{-1} dry weight) in southern right whale's feces, in some cases higher than those reported during marine mammal mortality events, demonstrate the natural risk to which the whales are exposed during their stay in this area (D'Agostino et al. in press).

11 Conclusions

Distinct environmental conditions in the northern and southern areas of Chubut coastal waters and large differences between seasons have been observed. The composition and abundance of harmful microalgae species showed a large site-specific interannual variability. Some years presented a good diversity of harmful species, whereas others had an almost exclusive dominance of a single species. The PSP-toxin producer *Alexandrium tamarense* was present throughout the entire coast of the Chubut province. In the north, maximal cell densities appeared by the end of winter and during spring; however, it was not necessary to reach high densities to cause toxic events (low biomass toxic HABs). In contrast, cell maxima occurred in spring-summer in the south, and densities exceeding one million cells per liter were necessary to produce a high accumulation of toxins. The similarities between the PSP-toxin profiles in the filter-feeding bivalves and in the co-occurring phytoplankton strongly support the view that the dinoflagellate *A. tamarense* is the source of PSP-toxin contamination in the Valdés Peninsula. GTX1 and STX were the most powerful PSP-toxins in the toxin profile. The predominance of GTX1 may have been decisive in the intoxication episodes registered in the San Jorge Gulf in 2010 and 2011. In addition to human illness, PSP-toxins are cause of major economic losses due to the commercial shellfish harvesting closures.

Among the producers of DSP-toxins and pectenotoxins, high cell densities of *D. tripos* were found mainly in autumn and winter but restricted to the San Matías and San José gulfs, whereas *D. acuminata* peaked in December and was not frequent in all the stations. Closures to commercial shellfish extraction due to DSP-toxins associated with *D. tripos* are becoming a recurrent phenomenon in the North Patagonian gulfs. The other lipophilic toxin-producing species found in the Chubut province coastal zone occurred only sporadically and in low densities.

Although several species of the genus *Pseudo-nitzschia* cited as toxic in other parts of the world occur in high cell densities in Chubut, no human poisoning by

DA has been reported in the region. It has not been confirmed if toxin accumulation has been the probable cause of death in stranded whales. However, it has been demonstrated that whales are exposed to toxins (PSP-toxins and DA) in the Valdés Peninsula since their stay coincides with the toxic phytoplankton bloom season, and it has been confirmed that they feed on contaminated zooplankton in this breeding area.

Chubut coastal waters are an adequate environment for the development of Harmful Algal Blooms and the production of phycotoxins. Therefore, it is necessary to maintain a permanent monitoring to take measures to prevent human poisoning and to understand possible effects on the marine fauna as well as to initiate new lines of research including molecular analysis and the use of satellite images for the early detection of the blooms.

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