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Southern right whale (*Eubalaena australis*) calf mortality at Península Valdés, Argentina: Are harmful algal blooms to blame?

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Marine Mammal Science

Southern right whale (*Eubalaena australis*) calf mortality at Península Valdés, Argentina: Are harmful algal blooms to blame?

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ABSTRACT

Península Valdés (PV) in Argentina is an important calving ground for southern right whales (SRWs, *Eubalaena australis*). Since 2005, right whale mortality has increased at PV, with most of the deaths (~90%) being calves <3 mo old. We investigated the potential involvement of harmful algal blooms (HABs) in these deaths by examining data that include: timing of the SRW deaths, biotoxins in samples from dead SRWs, abundances of the diatom, *Pseudo-nitzschia* spp., and the dinoflagellate, *Alexandrium tamarense*, shellfish harvesting closure dates, seasonal availability of whale prey at PV and satellite chlorophyll data. Evidence of the whales' exposure to HAB toxins includes trace levels of paralytic shellfish toxins (PSTs) and domoic acid (DA) in tissues of some dead whales, and fragments of *Pseudo-nitzschia* spp. frustules in whale feces. Additionally, whales are present at PV during both closures of the shellfish industry (due to high levels of PSTs) and periods with high levels of *Pseudo-nitzschia* spp. and *A. tamarense*. There is a positive statistical relationship between monthly *Pseudo-nitzschia* densities (but not *A. tamarense*) and calf deaths in both gulfs of PV.

Key words: *Eubalaena australis*, Península Valdés, calf mortality, *Pseudo-nitzschia* spp., *Alexandrium tamarense*, PSTs, DA, SeaWiFS, MODIS, toxins, harmful algal blooms.

Península Valdés (PV) is an important calving ground for the population of southern right whales (SRWs, *Eubalaena australis*) that live in the western South Atlantic Ocean. PV is located on the northern Patagonian coast of Argentina, south of Golfo San Matías (GSM) and situated between two gulfs, Golfo San José (GSJ) which opens to the north, and Golfo Nuevo (GN) which opens to the south (Fig. 1). SRWs are present at PV from May to December. Most calves are born in August with peak numbers of whales present in August–September (Payne 1986, Crespo *et al.* 2014). Little food is available at PV early in the season (Menéndez *et al.* 2011), and the mothers are primarily fasting while on the calving ground (Thomas and Taber 1984). However, adults and juveniles begin to feed sporadically on spring zooplankton patches later in the season, late September and early October (Payne 1986, Bastida and Rodríguez 2003, Sironi 2004, Hoffmeyer *et al.* 2010). The whales leave PV in October and November (Thomas and Taber 1984, Crespo *et al.* 2014), for their summer feeding grounds.

The SRW mortality rate in the PV region increased suddenly in 2005 (Fig. 2). The average number of deaths went from <6 deaths/year during 1971–2004 to 65 deaths/year during 2005–2014 (Uhart *et al.* 2008*b*, 2009; Rowntree *et al.* 2013, Sironi *et al.* 2014). Between 2005 and 2014, 90% of the deaths were calves <3 mo old. The deaths are anomalous for several reasons: mass deaths are generally rare in baleen whales (Geraci *et al.* 1989, Rowntree *et al.* 2013); the deaths at PV occurred over the entire breeding season and were not grouped into short periods like most unusual mortality events; they were recurrent from year to year; and, they were heavily biased towards newborn calves. A 2010 workshop of the International Whaling Commission (2011) discussed the possible causes for the deaths and proposed the following three most likely causes: (1) a decrease in food abundance, (2) biotoxins produced by harmful algal blooms (HABs), and (3) infectious disease. Kelp gull (*Larus dominicanus*) harassment (feeding on skin and blubber pecked from the whales' backs) in PV was recently added as a fourth potential factor during a second workshop in 2014 (Rowntree *et al.* 1998, Fazio *et al.* 2012, Thomas *et al.* 2013, International Whaling Commission 2015).
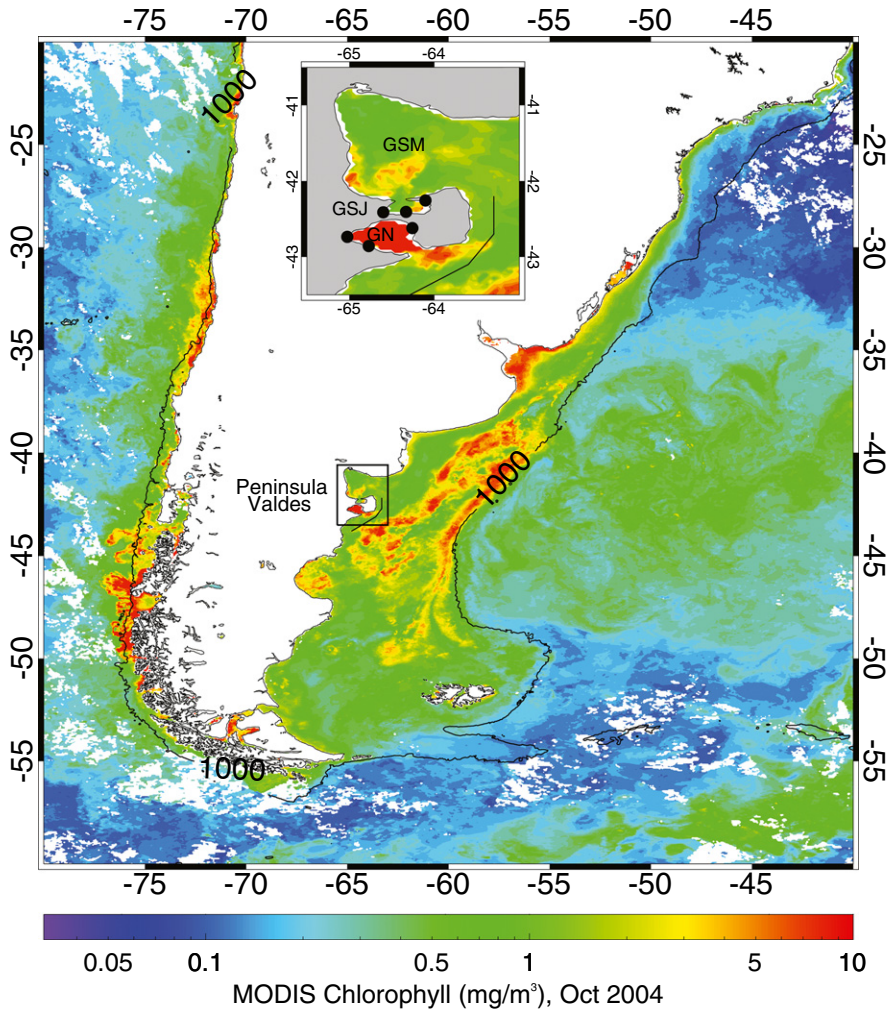
Figure 1. Monthly composite of MODIS chlorophyll from October 2004 for the Patagonia Shelf and shelf-break, Argentina. The 1,000 m isobath is shown in black. The inset shows the PV region south of Golfo San Matías (GSM), with Golfo San José (GSJ) on the north side of the peninsula and Golfo Nuevo (GN) on the south side. Black dots indicate the locations in each gulf where phytoplankton is regularly sampled by the shellfish industry to test for biotoxin producing species. The black line east of Península Valdes (PV) is the general position of the PV tidal front (adapted from Acha *et al.* 2004).

Because biological toxins can result in delayed or remote animal exposure, HABs are obvious potential culprits when investigating unusual marine animal deaths, even in the apparent absence of toxin-producing algae (Flewelling *et al.* 2005). The PV region has a considerable history of HABs. They were first documented there in 1980 (Table 1) when two people died from exposure to paralytic shellfish toxins (PSTs) produced by the dinoflagellate *Alexandrium tamarense* (Carreto *et al.* 1986, Esteves
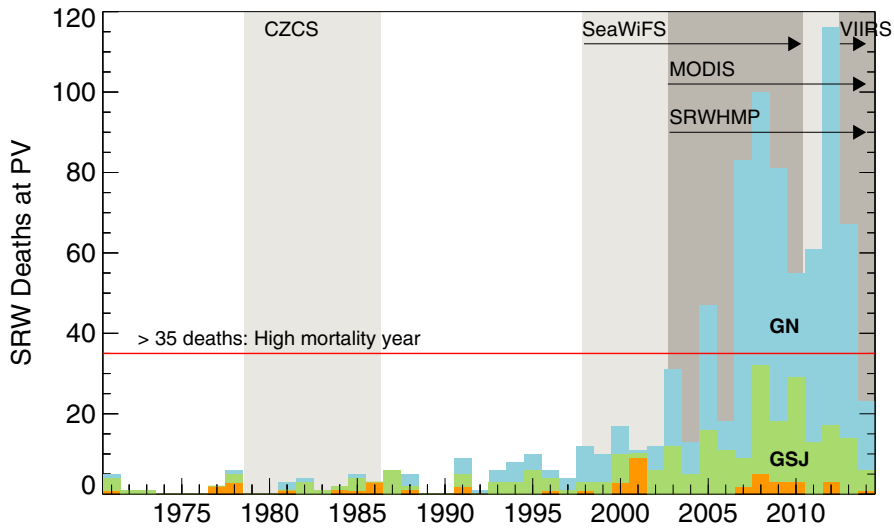
Figure 2. Number of Southern Right Whale (SRW) deaths at Península Valdes (PV) from 1971 to 2014. Deaths are recorded from June to December, when the whales are present at PV. The total number of deaths in Golfo San Jose (GSJ) are shown in green, those in Golfo Nuevo (GN) are shown in blue and those on the Atlantic coast are in orange. Time periods when the satellite ocean color sensors used in this paper were in operation are marked on the plot. Periods with only one sensor in orbit are in light gray, periods with two sensors in orbit are in dark gray. The timing of the establishment of the Southern Right Whale Health Monitoring Program (SRWHMP) is also indicated.

et al. 1992). Two more episodes of human illness and death were attributed to PST exposure in the middle and late 1980s (Table 1). Consequently in 1985 the Ministry of Fisheries of Chubut Province established a shellfish monitoring program in PV, which is one of the main suppliers of bivalve mollusks for human consumption in Argentina (Orensanz *et al.* 1991). Most of the known occurrences of elevated HAB species at PV have not been associated with significant outbreaks of illness or fatalities in humans or other animals (Table 1), despite reaching levels that were deemed high risk or very high risk, according to human risk standards (ANZECC and ARM-CANZ 2000).

The HAB monitoring and research efforts at PV have focused on the dinoflagellate, *A. tamarense* (Table 1), previously *Gonyaulax excavata* (Balech 1971) and *A. excavatum* (Balech and Tangen 1985), which can produce PSTs, including the neurotoxin saxitoxin (STX). Another HAB genus that occurs at PV is the diatom, *Pseudo-nitzschia*, some species of which can produce the neurotoxin DA. Five different potentially toxic *Pseudo-nitzschia* species: *P. pseudodelicatissima*, *P. fraudulenta*, *P. pungens*, *P. australis*, and *P. multiseries*, have been found in the PV region, although some only at low abundances (Andrinoló *et al.* 1999, Sastre *et al.* 2007, Cadaillón 2012). DA analysis was added to the PV monitoring program in 2005, and it was immediately detected in shellfish samples, but at levels below the regulatory limit (Sastre *et al.* 2007). To date there have been no known deaths or episodes of illness in people or animals due to DA exposure in the PV region.

Ocean color satellite data are an important tool for detecting and monitoring algal blooms, including HABs, due to the large spatial scale and the high frequency of

Table 1. List of documented blooms of toxic phytoplankton or mass mortality events at Peninsula Valdes (PV) and on the Argentine Sea. The maximum abundance of phytoplankton is listed where reported, along with an estimated risk level for that abundance (ANZECC and ARMCANZ 2000): VH = very high, H = high, Low = low; GN = Golfo Nuevo, GSJ = Golfo San Jose.

Date	Location	Consequence	Concentration, risk	Reference
		A. tamarense		
November 1980	PV tidal front	2 human fatalities	120,000 cells/L, VH	Carreto et al. 1986
November 1981	PV tidal front		1.8×10^6 cells/L, VH	Carreto et al. 1986
January–February 1985	Engaño Bay	4 human fatalities	Not reported	Vecchio et al. 1986
January 1988	GN	4 human illnesses	750,000 cells/L, VH	Esteves et al. 1992
September 1993	Argentine Sea	massive mackerel die-off	10,000 cells/L, VH	Montoya et al. 1996
October 1993	GSJ		10,000 cells/L, VH	Sastre et al. 2001, Santinelli et al. 2002
November–December 1994	GSJ, GN		10,000 cells/L, VH	Santinelli et al. 2002
September 1995	GN		14,600 cells/L, VH	Gayoso 2001, Gayoso and Fulco 2006
November 1998	GN		4,700 cels/L, H	Gayoso 2001, Gayoso and Fulco 2006
October–December 2000	GN	4,400 dead penguins and other seabirds	21,800 cells/L, VH	Shumway et al. 2003, Gayoso and Fulco 2006
August–September, November 2010	GSJ, GN		3,000 cells/L, H	Cadaillón 2012
		Pseudo-nitzschia		
October 1993	GSJ		345,000 cells/L, H	Sastre et al. 2001, Santinelli et al. 2002
December 1993	GN		6.6×10^6 cells/L, VH	Sastre et al. 2001
October 2005	GN		24,200 cells/L, L	Sastre et al. 2007
November 2010	GSJ, GN		2.5×10^6 cells/L, VH	Cadaillón 2012
		Dinophysis acuminata		
October 1988	GSJ		Not reported	Gil et al. 1989
		Prorocentrum lima		
August 1993	GN		100 cells/L, L	Santinelli et al. 1994
March 1999	GSJ, GN	>40 human illnesses	<100 cells/L, L	Gayoso et al. 2002
		Unknown (but deaths attributed to PST)		
November 2006	GN	>100 dead or dying kelp gulls and royal terns	Unknown	Uhart et al. 2008a

satellite observations (Stumpf *et al.* 2003, Tomlinson *et al.* 2009). Ocean color data primarily measure the concentration of chlorophyll-a in the surface water, generally without the ability to distinguish different types of phytoplankton. However, some HAB species have unique optical properties and specific algorithms have been developed for their detection (Cannizzaro *et al.* 2008). But many genera, such as *Pseudonitzschia* and *Alexandrium,* do not have optically unique properties, and thus cannot be identified by satellite data (Tweddle *et al.* 2010). Potential HABs can be detected by examining chlorophyll anomalies in areas where HAB species dominate the phytoplankton assemblage when in bloom (Stumpf *et al.* 2003). Regardless of which method is used, *in situ* water sampling is needed to identify unequivocally the causative species within a bloom and assess toxicity. Once a HAB has been identified, satellite ocean color data are invaluable for tracking its movement and persistence.

Here we investigate the possibility that HAB biotoxins are the cause or contribute to the SRW deaths. We present the results of analyses of biotoxins in tissue samples collected from dead whales (2004–2010, 2012). The approximate timing of SRW deaths in the PV region (1971–2014) is compared with the abundances of *Pseudonitzschia* spp. and *A. tamarense* (2000–2014) and the timing of shellfish industry closures (2003–2014). Zooplankton data are presented to show the availability of whale prey species in Golfo San Matías (GSM) and PV (1974–1975, 1997–1998 and 2000–2001). Satellite chlorophyll (1997–2014) variability in the region is also examined.

Material and Methods

Whale Deaths

The Ocean Alliance has been recording deaths of SRW at PV since 1971, although effort has increased over time (Rowntree *et al.* 2013), culminating in the development of a formal stranding program in 2003, the Southern Right Whale Health Monitoring Program (SRWHMP). Data presented here are for each gulf (GN and GSJ) between 1971 and 2014. Tissue samples for biotoxin analyses were collected from dead whales in 2004–2010, 2012 by the SRWHMP. Along with tissue sample collection, data on the location, date, age, sex, and length (as a proxy for age) of each dead whale were also recorded. An important caveat is that dates associated with the whale deaths are the dates when the carcasses were discovered, which could be substantially later than the date of death. More details on necropsy procedures can be found in Rowntree *et al.* (2013).

Biotoxins in Dead Whale Tissues

Analyses for the presence of biotoxins were conducted on 118 samples collected from 50 calves and one adult that died at PV. Most samples were analyzed for both PSTs and DA, but a few samples were analyzed only for DA due to small sample size. The analyses were performed by three different laboratories: the National Ocean Service (NOS) National Centers for Coastal Ocean Science in Charleston, South Carolina, U.S.A., the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP) in Argentina, and the Dirección de Salud Ambiental of Chubut Province in Argentina (Table S1). Most of the samples (60%) were collected in 2008. A variety of sample types were tested, predominantly urine, feces, and stomach contents (60%, Table S1).

A detailed description of sample collection, preparation, and toxin analysis methods used by each laboratory is given in Appendix S1.

Abundances of Pseudo-nitzschia *spp. and* Alexandrium tamarense *at PV*

Monthly sampling for *Pseudo-nitzschia* spp. and *Alexandrium tamarense* in GSJ and GN began in 2000 (see locations on Fig. 1). Phytoplankton sampling is conducted by the Ministry of Fisheries, Chubut Province (Argentina). Qualitative and quantitative analyses are made in the Laboratorio de Hidrobiología de la Universidad Nacional de la Patagonia (Hydrobiology Laboratory at the National University of Patagonia) to determine the presence or absence of potentially toxic species, sampling frequency, and the timing of shellfish closures. Phytoplankton samples are collected with a 30 μm mesh plankton net and fixed in 4% formaldehyde (for qualitative analysis) and in Van Dorn bottles and fixed in Lugol solution (for quantitative analysis). Microplankton are quantified with an inverted microscope (Lund *et al.* 1958). Taxonomic identification of *A. tamarense* is carried out following the Balech methodology (Balech 1995). *Pseudo-nitzschia* spp. frustules are cleaned following the method of Hasle and Fryxell (1970) and identified using the scanning electron microscope at La Plata Museum, Facultad de Ciencias Naturales, Buenos Aires. Data presented here are for GN and GSJ from 2000 to 2014.

Shellfish Closures

In response to HAB-related deaths in the early 1980s the Ministry of Fisheries of Chubut Province established a shellfish sampling program in 1985, but data are readily available only starting in 2003. Since then the shellfisheries in both GSJ and GN have been closed annually when levels of PSTs were above the regulatory limit of 80 μg STX eq/100 g. There have been no closures due to DA (the limit for DA is 20 μg/g). Closures are based upon sampling from three sites in GSJ (Riacho, Larralde, and Bengoa) and three in GN (Punta Pardelas, Puerto Madryn, and Playa Paraná) (Fig. 1). Both phytoplankton and shellfish are collected and tested for the presence of PSTs and DA. In winter, when *A. tamarense* and *Pseudo-nitzschia* are absent, shellfish sampling is conducted on a monthly basis. When *A. tamarense* and *Pseudo-nitzschia* begin to appear in plankton samples, shellfish are sampled and tested weekly. If the regulatory toxin limit is exceeded, the shellfishery is closed and sampling is conducted on a monthly basis until *A. tamarense* and *Pseudo-nitzschia* disappear, at which point sampling frequency becomes weekly again. Shellfish closure dates are presented here for GN and GSJ from 2003 to 2014.

Zooplankton (Whale Prey Abundance and Consumption)

To evaluate the temporal availability of whale prey in the PV region, data on mesozooplankton (0.2–20 mm) concentrations were compiled from the published literature for GN, GSJ, and GSM. Data on total mesozooplankton (MSZ) biomass per cubic meter in dry weight (dw) were collected by Ramírez (1996), who sampled GSM seasonally from April 1974 to February 1975. Additional MSZ biomass data for GSM were collected from May 2000 to May 2001 by Hoffmeyer and Pascual (2006) and for GN from December 1997 to September 1998 by Hoffmeyer (this study). MSZ biomass data were derived by multiplying the abundance data with the measured individual biomass values for the copepods *Paracalanus parvus, Ctenocalanus*

vanus, Oithona nana and *Calanus australis* (Fernández Aráoz 1994). Data for the remaining species were estimated using individual biomass values for similarly sized organisms. For example, *O. nana* and *P. parvus* biomass values were used for small MSZ such as cladocerans, invertebrate larvae or other copepods; those of *C. vanus* were used for intermediate sized organisms; and those of *C. australis* were used for other large copepods, fish larvae, and amphipods.

Satellite Data

Ocean color data were analyzed from the Coastal Zone Color Scanner (CZCS), the Sea-viewing Wide Field-of-view Sensor (SeaWiFS) on GeoEye's OrbView-2 satellite, the Moderate Resolution Imaging Spectroradiometer (MODIS) sensor on NASA's Aqua satellite, and the Visible Infrared Imaging Radiometer Suite (VIIRS) on the NOAA/NASA Suomi NPP satellite. Both 8 d and monthly composites were examined. The spatial resolution of the data sets are 0.04° (4 km) for CZCS, 0.1° (9 km) for SeaWiFS, 0.04° (4 km) for MODIS, and for 0.04° (4 km) for VIIRS. The CZCS sensor operated on an intermittent schedule and collected data only 2 h per day on average, with an emphasis on coastal waters of the U.S.A. Monthly composites of sea-surface temperature (SST) from MODIS/Aqua were also used in the multivariate analysis. Monthly composites of CZCS data were obtained from NASA's GSFC's ocean color web (http://oceancolor.gsfc.nasa.gov/). All other satellite data were obtained from the ERDDAP (Environmental Research Division's Data Access Program, http://coastwatch.pfeg.noaa.gov/erddap) server at NOAA's Environmental Research Division (Simons 2011). Data presented are for GN and GSJ from 1997 to 2014. Here a bloom is defined as chlorophyll concentrations >5 mg/m^3, which is more than twice the climatological value of ~2 mg/m^3 for springtime chlorophyll at PV (Williams *et al.* 2013).

Statistical Analyses of Calf Deaths in Relation to Toxin Producers and Other Covariates

The data sets presented here were collected at different intervals by research programs with unrelated aims, so to facilitate statistical analysis of their temporal covariation, values were averaged by months within years (2000–2014), combining all observations within a given month. Calf deaths were treated as the response variable, with log-transformed *Pseudo-nitzschia* and *A. tamarense* cell densities, MODIS chlorophyll and MODIS SST treated as covariates. These variables were scored separately for GN and GSJ, so gulf was treated as a factor. Month (July–December) was treated as a factor in some analyses, and as a scalar covariate in others (7–12), because SST steadily increases and whale densities change through the calving season. Analyses were carried out in R, using the general linear model (glm) function and the quasipoisson family of link functions, to account for the overdispersion of calf deaths per month.

Results

Whale Mortality

Prior to 2005 mortality increased at the same rate that the population was growing (6.8% per year; Cooke *et al.* 2003, Rowntree *et al.* 2013), but in 2005 the mortality

rate increased abruptly (Fig. 2). High mortality years, defined as ≥35 deaths (Rowntree *et al.* 2013), occurred in 2005, 2007–2013. During the 10 yr period from 2005 to 2014, more than three times as many deaths were recorded (649) than in the previous 30 yr (194). Since 2005, 90% of the deaths have been calves <3 mo old (81% of the deaths were calves between 1971 and 2004) (Rowntree *et al.* 2013). The highest mortality year was 2012 when 116 whales died, including 113 calves (97%). More deaths occurred in GN (68%) than in GSJ (27%). However, in 2014 the number of deaths declined to 23.

The greater effort to find dead whales following establishment of the SRWHMP in 2003 does not explain the increase in the mortality rate (Rowntree *et al.* 2013). There is no consistent pattern in the timing of the deaths. Using October 1 to delineate "early" and "late" phases of the calving season, Rowntree *et al.* (2013) found that some years had more deaths early in the season, some years had more late in the season, and some years the deaths were evenly distributed between early and late in the season.

Biotoxins in Whale Tissues

Detectable levels of DA (produced by *Pseudo-nitzschia* spp*.*) were present in only 2 of the 108 samples analyzed (2%, Table 2). DA was found in whole blood samples from an adult female and an unrelated male calf with concentrations of 7 and 3 ng/mL of blood, respectively. The limit of quantification of this method was 1.25 ng/mL of sample. Both whales died in 2005, 22 days apart and in separate gulfs of PV. These deaths coincided with the first record of DA in coastal waters of Chubut province (GN and Bahía Camarones) (Sastre *et al.* 2007). Few blood samples were collected

Table 2. Samples that tested positive for detectable levels of domoic acid (DA) and paralytic shellfish toxins (PSTs) among the 118 tissue samples collected from 51 right whales that died at Península Valdés (PV) in 2003–2010 and 2012 (see Table S1). All positive analyses were performed at the NOAA/NOS laboratory. Liquid chromatography-mass spectrometry (LC-MS) and receptor binding assays (RBA) were used to measure toxin levels. Methods are described in Appendix S1. (A = adult, C = calf, <dl = below the detection limit, GSJ = Golfo San Jose, GN = Golfo Nuevo).

Whale ID	Date collected	Age class	Sex	Length (m)	Sample type	DA (ng/mL or ng/g) LC-MS	PSTs ng STX eq/mL or ng/g RBA	Location at PV
Ea21-05	19 October 2005	A	F	14.21	Blood	7		GSJ
Ea38-05	11 November 2005	C	M	8.48	Blood	3		GN
Ea97-08	2 December 2008	C	F	5.04	Feces	<dl	172	GN
Ea01-09	30 June 2009	C	M	5.5	Liver	<dl	180	GN
Ea01-09	30 June 2009	C	M	5.5	Stomach tissue	<dl	225	GN
Ea01-09	30 June 2009	C	M	5.5	Feces	<dl	800	GN

from other whales for comparison (Table S1). However other sample types such as feces, urine, and gastric content that are typically better indicators of DA exposure all tested negative (limit of detection 2.5 ng/g).

Saxitoxin-like activity (produced by *Alexandrium tamarense*) was detected by receptor binding assay (RBA) (Van Dolah *et al.* 2012) in 4 of the 105 samples analyzed (4%). The four samples were collected from two calves that died in 2008 and 2009, and had STX concentrations in the range of 172–800 ng/g (Table 2). The average detection limit of the RBA was 60 ng STX equivalents per gram of extracted sample or 29 ng STX equivalents per milliliter of urine. LC/MS analyses did not confirm the presence of PSTs as sample matrices caused significant shifts in toxin retention times (compared with reference standards), even with 15-fold sample dilutions.

Abundances of Pseudo-nitzschia *spp. and* Alexandrium tamarense *at PV*

Monthly sampling for *Pseudo-nitzschia* spp. and *A. tamarense* in GSJ and GN from 2000 through 2014 indicates their consistent presence in the region (Fig. 3). Levels of potential risk assessment to humans, as determined by ANZECC and ARMCANZ (2000), are based on cell abundance (cells/L, see Fig. 3). These are classified as potential risks because high cell abundances do not always equate to high levels of toxicity. Cell counts of *Pseudo-nitzschia* in both gulfs were at low risk levels until the end of 2006 when they increased to the high risk category in GSJ. Between 2007 and 2013 the highest cell counts in GSJ were all in the high risk category (Fig. 3). The *Pseudo-nitzschia* cell counts in GN were slightly lower than those in GSJ, but almost all years between 2007 and 2013 had values above the low risk level. *Pseudo-nitzschia* cell counts in both gulfs decreased in 2013 and 2014 with all *Pseudo-nitzschia* values being only of low or moderate risk in 2014.

Only one sample had a high risk level of *A. tamarense* in GN in 2000, but the frequency of high risk and very high risk levels of *A. tamarense* has increased since then (Fig. 3). Between 2007 and 2013 most of the times when *A. tamarense* has been present in either GN or GSJ, it has been at levels of high or very high risk. Values decreased in 2014 when there were no very high risk levels of *A. tamarense* observed in either gulf (Fig. 3). Very high risk levels of both *Pseudo-nitzschia* spp. and *A tamarense* have occured more often in GSJ than in GN.

The abundances of these HAB taxa show a similar pattern, with gradual increases in cell counts from 2000 to 2012, which plateaus at very high risk levels around 2007, and then declines in 2013 and 2014. This pattern is similar to the pattern in the annual whale deaths at PV (red line in Fig. 3) which has high values (>50 deaths/ year) from 2007 to 2013, and a sharp decline to 23 deaths in 2014.

Shellfish Closures

Since at least 2002 the PV shellfish beds have been closed several months every year for harvesting as a result of high levels of PSTs (Fig. 4). The closures usually start in September or October and continue through February or March of the following year. From 2003 to 2012, closure dates began earlier in each consecutive year, indicating that PSTs appeared in shellfish and phytoplankton earlier each year until 2013. In 2003 the closures started in October, but by 2012 they started in August. This trend is statistically significant at the 95% confidence level for the years 2003–2012 ($r^2 = 0.56$ and 0.45 for GSJ and GN respectively, $P < 0.05$ for both, Fig. 4). In 2013 and 2014, however, the closure start dates shifted to later in the year,
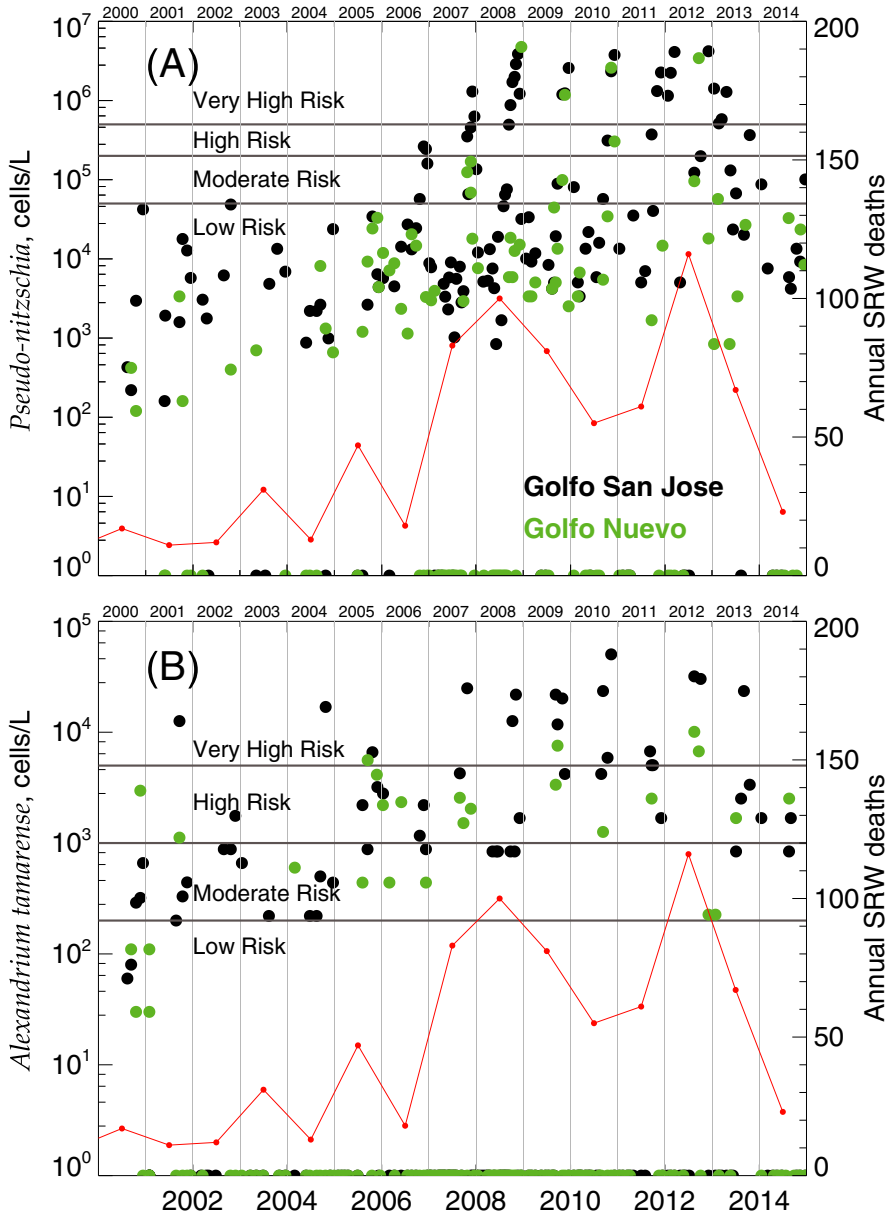
Figure 3. Time series of the maximum abundance of (top) *Pseudo-nitzschia* spp. and (bottom) *A. tamarense* at Penı́nsula Valdes (PV) between 2000 and 2014. Values from Golfo San Jose (GSJ) are black, values for Golfo Nuevo (GN) are green. The data are the maximum value from the three sampling spots in each gulf. Gray lines delineate the different levels of risk of intoxication for human shellfish consumption (ANZECC and ARMCANZ 2000). The annual number of Southern Right Whales (SRW) deaths in Penı́nsula Valdes (PV) is shown as a red line.
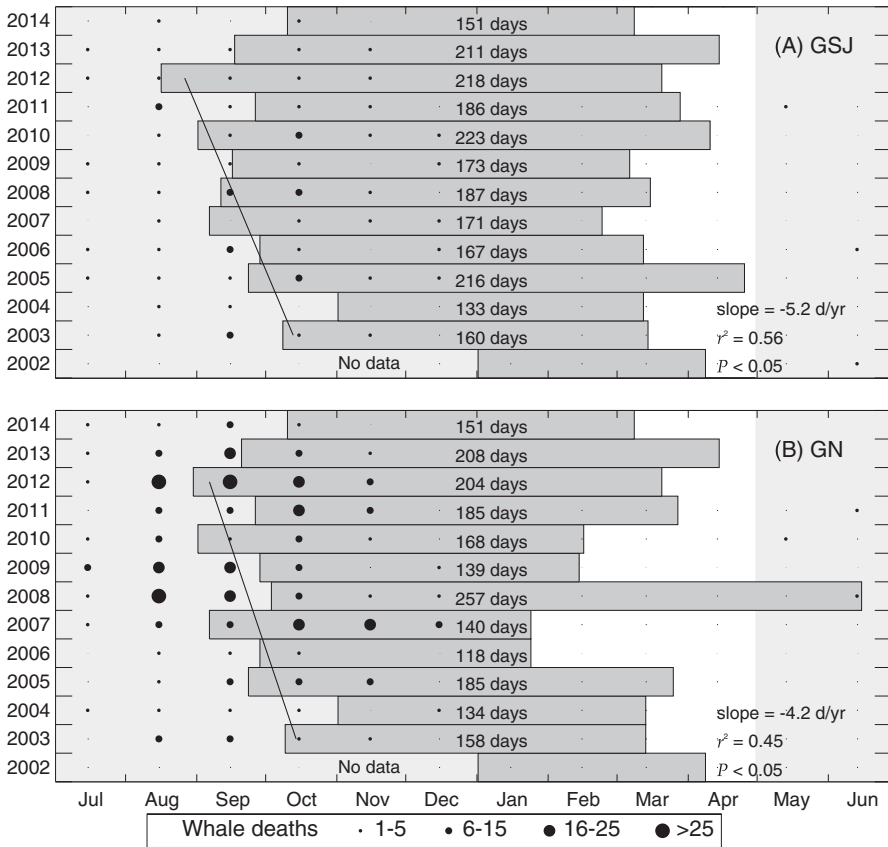
Figure 4. Timing and length (in days) of the shellfish closures (dark gray) from 2002 to 2014 in (A) Golfo San Jose (GSJ) and (B) Golfo Nuevo (GN) due to elevated PST levels. The periods when whales are in the region (May–December) are shown in light gray. Monthly mortality data are shown as black dots, with numbers indicated by dot size as defined above. The slope of the linear regression and the r^2 values are shown for the closing dates. There is no trend for the opening dates ($r^2 = 0.001$ for both GSJ and GN).

September and October, respectively. When these years are included in the analysis the regression is no longer significant (GSJ: $r^2 = 0.17$, $P = 0.17$; GN: $r^2 = 0.16$, $P = 0.19$). No trends were observed in the reopening dates ($r^2 = 0.001$ for both GSJ and GN). The whales can occasionally feed at PV during the period when the shellfisheries are closed. The 2008 closure in GN was particularly long, and lasted until June of 2009, which overlapped with the beginning of the whales' presence at PV. Whale deaths have occurred both before and after closure of the shellfish beds (Fig. 4).

Zooplankton (Whale Prey and Consumption)

MSZ abundance varied seasonally in GSM and GN with annual peaks in spring (October and November), usually following spring phytoplankton blooms, and in autumn (April and May) (Fig. 5). The MSZ spring peak in GSJ occurs in December
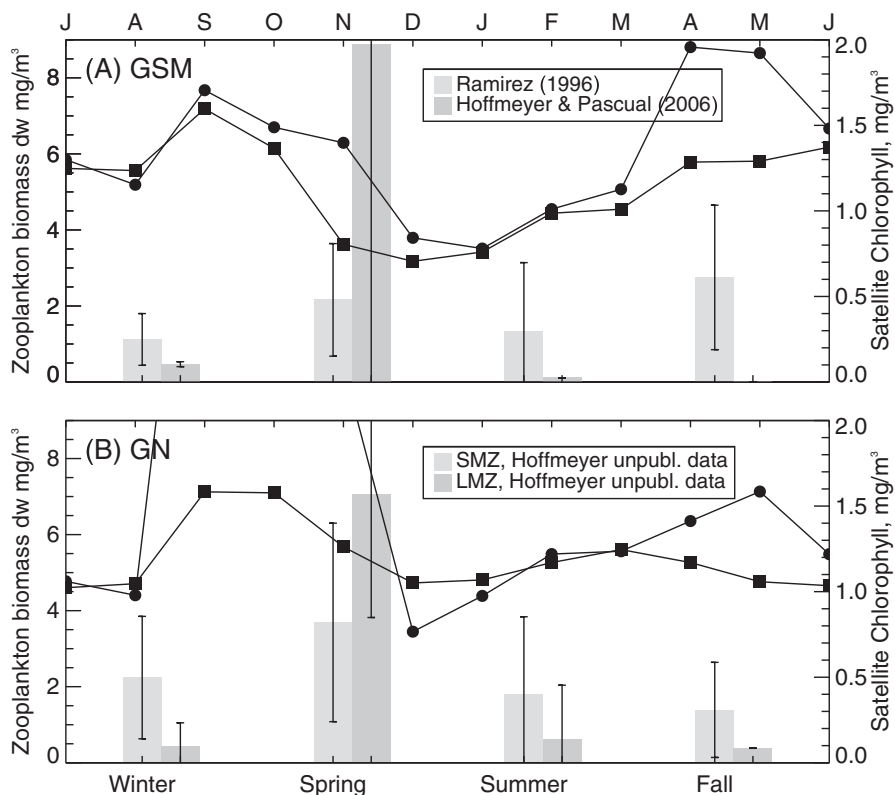
Figure 5. Seasonal changes in zooplankton abundance (bars) in (a) Golfo San Jose (GSJ) and (b) Golfo Nuevo (GN). Data synthesized from Ramírez (1996), Hoffmeyer and Pascual (2006) and unpublished data of MH. The seasonal cycle of phytoplankton is indicated by the monthly averages of satellite chlorophyll data (lines) from SeaWiFS (squares) and MODIS (circles) sensors. In (B) the data are broken down into small mesozooplankton (SMZ) in light gray and large mesozooplankton (LMZ) in dark gray.

(Hernández Moresino *et al.* 2013), which is slightly later than that in GSM and GN. However, in 2010 the MSZ spring bloom in GSJ began in October (MH and CFM, unpublished results), which is consistent with the timing seen in GSM and GN.

Remains of *Calanus* spp. copepods were found in fecal samples collected from both living and dead whales in the PV region (Menéndez *et al.* 2007, D'Agostino 2013). These fecal samples also contained frustules of the diatom *Pseudo-nitzschia*. Those that could be identified to the species level included *P. pungens*, *P. australis*, and the complex *P. pseudodelicatissima* (D'Agostino *et al.*, unpublished results). These species can produce DA, providing evidence that the whales at PV are exposed to potentially toxic diatoms.

Satellite Chlorophyll Data

Spring (September–December) chlorophyll concentrations of ~2 mg/m^3 are typical of the regional PV ecosystem (Williams *et al.* 2013). Satellite data from 1997 to
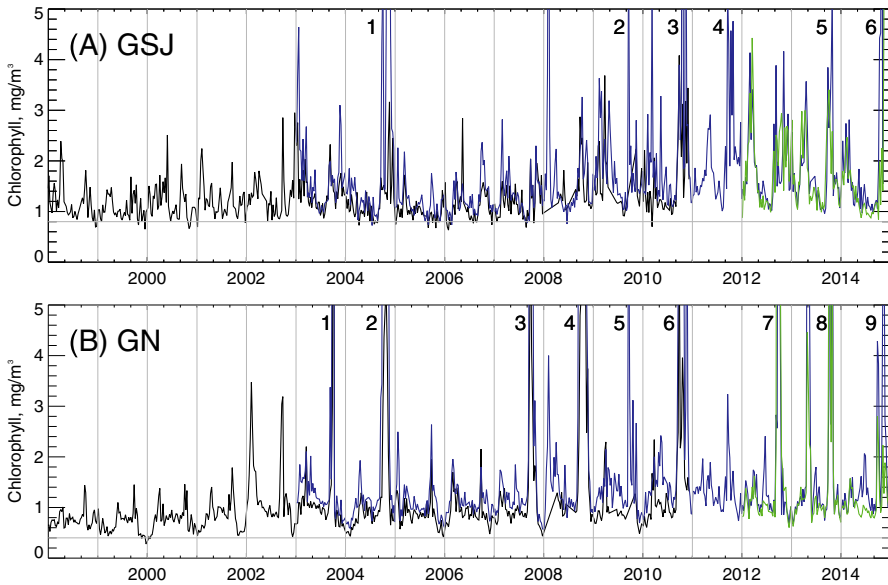
Figure 6. Time series of average chlorophyll densities for Golfo San Jose (GSJ) and Golfo Nuevo (GN) from 1998 to 2014 from SeaWiFS (black line), MODIS (blue line) and VIIRS (green line). Years with spring chlorophyll blooms (>5 mg/m^3) are numbered. Lines are drawn 0.8 mg/m^3 and 0.4 mg/m^3 for GSJ and GN, respectively, which indicate the minimum chlorophyll value at the beginning of the time series.

2002 show chlorophyll levels in GSJ and GN to be generally <2 mg/m^3 (Fig. 6). In 2003 spring chlorophyll values exceeded 5 mg/m^3 in GN, and the following year a bloom (chlorophyll >5 mg/m^3) developed in both GSJ and GN. In most years since 2007 spring blooms have occurred in GSJ and GN. From 2003 to 2014, there have been six blooms in GSJ, and nine in GN. There has also been an increase in the minimum chlorophyll values. At the beginning of the time series the minimum chlorophyll value was ~0.8 mg/m^3 and ~0.4 mg/m^3 in GSJ and GN, respectively. Since 2008 there has been a slight increase in the minimum chlorophyll values observed in both GSJ and GN.

Averages of weekly chlorophyll levels and the number of whale deaths per week from June to December, 2003–2014, are shown in Figure 7 for GSJ and Figure 8 for GN. In GSJ (Fig. 7) the three most significant blooms (chlorophyll >10 mg/m^3) occurred in 2004, 2010 and 2014. Two of these years, 2004 and 2014, were low mortality years, with five and four deaths, respectively in GSJ. The GSJ blooms in 2009, 2011 and 2013 had maximum chlorophyll values just slightly above 5 mg/m^3, which were not sufficient to show up as shaded areas on Figure 7.

GN has had slightly more blooms than GSJ, and they were usually larger in magnitude (Fig. 8). The largest GN blooms occurred in 2004 and 2008, which lasted about two months with maximum chlorophyll values >30 mg/m^3. In 2004, 8 whales died at GN, but in 2008 68 whales died at GN; however, most of 2008 deaths occurred before the development of the chlorophyll bloom. In this case, diatom-dominated blooms with a relatively weak chlorophyll signature, but potentially including *Pseudo-nitzschia* as a component of the phytoplankton assemblage, may have preceded
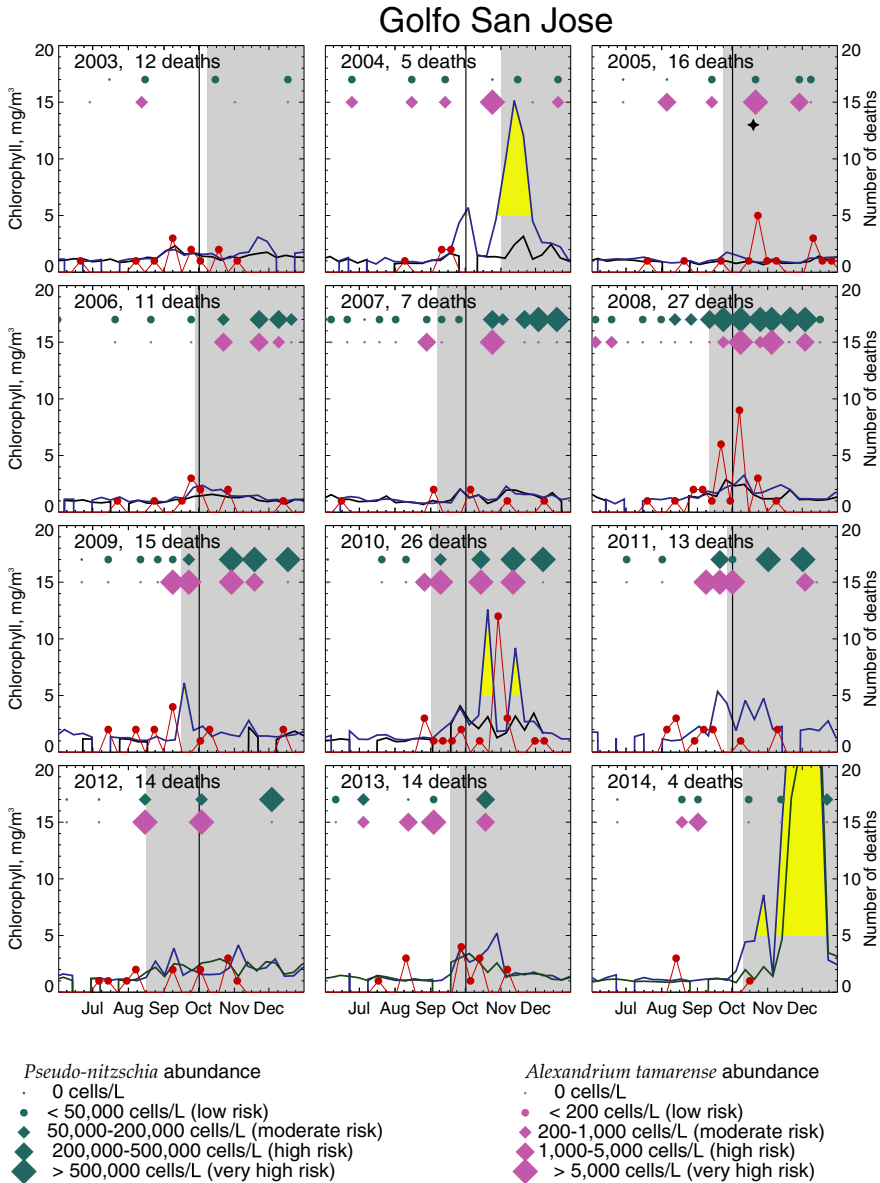
Golfo San Jose

Figure 7. Time series of weekly binned satellite chlorophyll (solid lines of different colors depending on sensor) and number of whale deaths/week (connected red dots) during May–December for Golfo San Jose for the years 2003–2014. SeaWiFS data are shown as a black line, MODIS as a blue line and VIIRS as a green line. Areas shaded in yellow indicate chlorophyll levels above the bloom threshold value (5 mg/m³). Gray indicates periods of shellfish closures. Black lines indicate 1 October and the beginning of the "late" season (see text), when whales begin to feed. The sizes of green and pink symbols indicate the relative abundances of *Pseudo-nitzschia* and *A. tamarense* respectively. Black stars show times when tissue samples from dead whales indicated exposure to biotoxins (from Table 2).
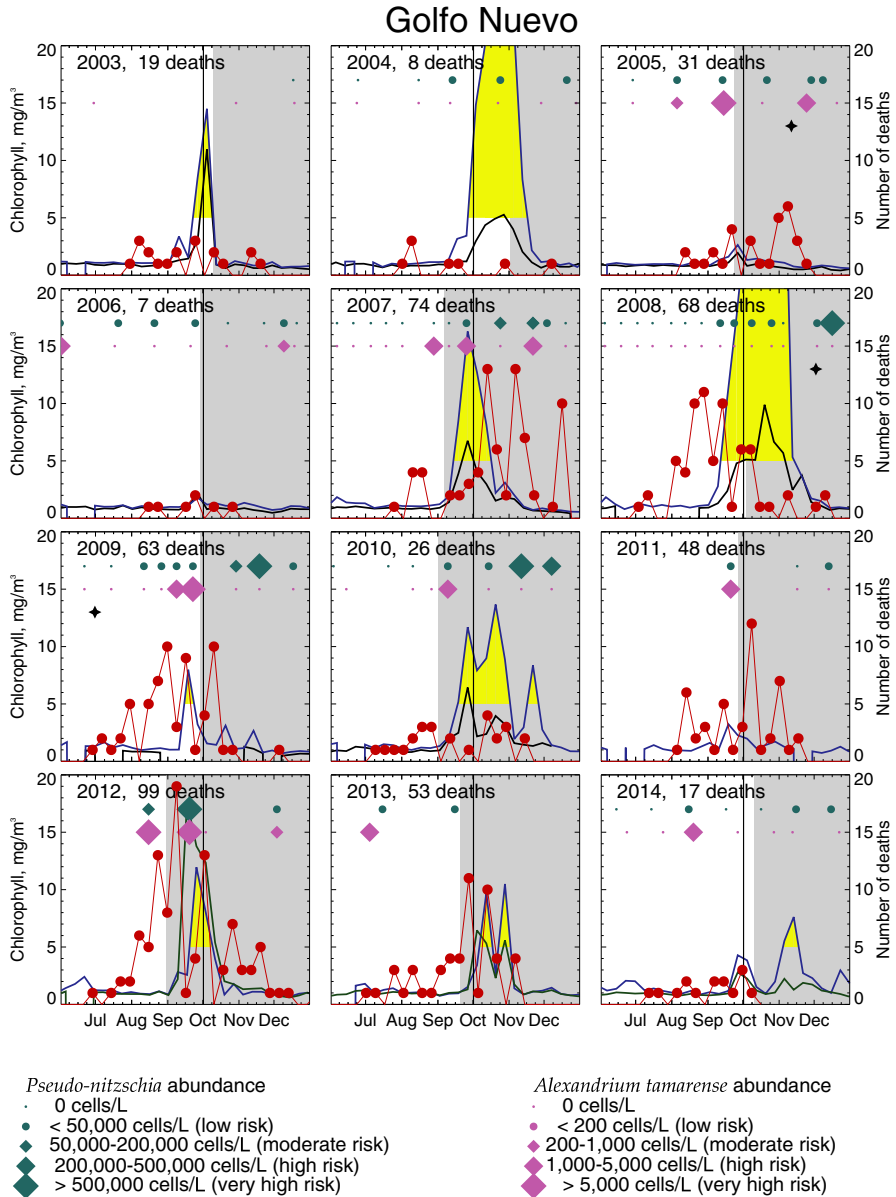
Golfo Nuevo

Figure 8. Same as Figure 7, but for Golfo Nuevo.

the denser chlorophyll bloom detected by satellite. A seasonal succession from diatom- to flagellate- (including dinoflagellates) dominated assemblages is well-documented in coastal waters (Smayda 1980), although the actual drivers of this transition will be specific to the hydrographic characteristics of a given location and their interaction with the phytoplankton community. The blooms in GSJ tend to

develop later in the season, late October or November, whereas the blooms in GN tend to develop late September. Development of the blooms is shown in the animation in Figure S1.

Monthly composites of chlorophyll from the CZCS sensor for PV from August to November between 1979 and 1985 for PV were also examined (Fig. S2). Only 8 of the 28 months had sufficient data coverage of the PV area, none of which indicated the presence of a bloom in either GSJ or GN. However, there was a bloom observed in GSM as well as in the tidal front area (Acha *et al.* 2004; see Fig. 1) in November 1981 at the same time that there was a outbreak of *A. tamarense* in the tidal front area (Carreto *et al.* 1986).

Calf Deaths in Relation to Potentially Toxic Microplankton and Other Variables

Calf deaths vary strongly with seasonality (months and SST) and geography (gulfs), so these were included as nuisance factors in all statistical models to control for their effects. With these controls in place, chlorophyll did not explain significant amounts of variation in calf mortality, and it was therefore dropped from the analyses. *Pseudo-nitzschia* densities consistently emerged as a significant explanatory variable, but *A. tamarense* densities did not, even in the most highly parameterized models. The best model (as judged by reduction of the residual deviance) is one with *Pseudo-nitzschia*, SST (as its gulf-specific difference from the month-specific average), gulf as a factor, and the six months (July–December) as factors (Table 3). The same model with month-number as a covariate is nearly as good (not shown). The positive statistical relationship between monthly *Pseudo-nitzschia* densities and calf deaths is seen in both gulfs (Fig. 9). There is considerable scatter, but on average more calves die in months when nonzero estimates of PN density were above average; the consistency of this pattern implies that it did not arise by chance (Table 3).

In this model, zero-density estimates of *Pseudo-nitzschia* and *A. tamarense* were treated as missing data rather than as true zeros, for two reasons. First, the zeros are discordant with the distributions of the positive (finite) estimates of cell densities, as can be seen clearly in Figure 3; this suggests that the zeros arise from some process

Table 3. Summary of model fit for preferred multivariate analysis of variations in calf mortality and density of biotoxins producing species and chlorophyll. *Pseudo-nitzschia* (PN) density is included, but *A. tamarense* (AT) density is not, nor is chlorophyll, as discussed in the text.

Effect	Estimate	SE	z	P
Intercept	–0.9564	0.7288	–1.31	0.1931
log(1 + PN)	0.2171	0.0427	5.09	2.2e-06***
SST – (average SST)	–0.9822	0.3087	–3.18	0.0021**
as.factor(gulf)GSJ	–1.5466	0.1969	–7.86	1.3e-11***
as.factor(Month)8	1.3281	0.6741	1.97	0.0522*
as.factor(Month)9	1.2191	0.6723	1.81	0.0734*
as.factor(Month)10	0.9670	0.6838	1.41	0.1611
as.factor(Month)11	–0.5347	0.7759	–0.69	0.4926
as.factor(Month)12	–0.7230	0.7507	–0.96	0.3382

Note: The asterisks indicate the significance level according to the *P*-value computed, *** for $P < 0.001$, ** for $P < 0.01$, and * for $P < 0.1$. Dispersion parameter for quasipoisson family taken to be 2.8549. Null deviance: 651.84 on 91 degrees of freedom. Residual deviance: 238.41 on 83 degrees of freedom.
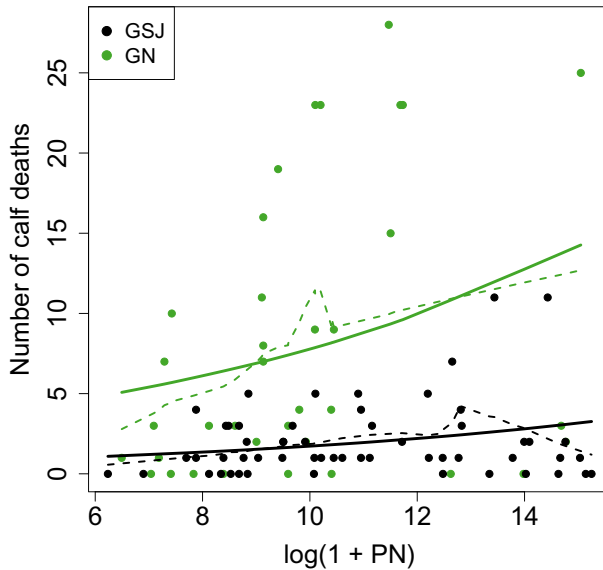
Figure 9. Monthly averaged values of the log of *Pseudo-nitzschia* abundance against the number of calf deaths. Values from Golfo San Jose (GSJ) are black, values for Golfo Nuevo (GN) are green. The dashed lines are smoothed curves through the values predicted by the full model (Table 3) and the solid lines show the predicted values from a model using just *Pseudo-nitzschia.*

different from the one that generates variation among the positive estimates. For example, zeros could result from offshore winds or currents that move *Pseudo-nitzchia* populations away from the shore-based sampling stations. Second, statistical models that include the zeros yield results that are qualitatively consistent with those that do not include them, but the fits are not as good and therefore the significance levels are lower.

DISCUSSION

Are Biotoxins the Cause of the Increased Calf Mortality?

These data indicate that SRW mothers and their calves are being exposed to bio-toxin-producing algae in the PV area. Between 2007 and 2013, high risk levels of *Pseudo-nitzschia* spp. and *A. tamarense*, producers of DA and PSTs, respectively, occurred in both GSJ and GN (Fig. 3). Their increase in abundance at PV has coincided with the increase in whale deaths. For both gulfs there is a positive statistical relationship between calf deaths (Fig. 9) and monthly *Pseudo-nitzschia* densities, but not *A. tamarense* densities. This difference between the two species is interesting because it is the opposite of what drives the closure of the regional shellfishery. While DA, the toxin produced by *Pseudo-nitzschia,* has been detected in the sampling program, it has not been above the regulatory limit. One possible explanation for this apparent paradox relates to the well-documented, highly variable DA content of potentially toxic *Pseudo-nitzschia* species (Lelong *et al.* 2012), such that those cells ingested by shellfish may have contained low DA levels. Moreover, *Pseudo-nitzschia*

blooms can originate offshore as subsurface populations and become entrained into nearshore surface waters via upwelling events (Schnetzer *et al.* 2013) or shoaling of internal tides (Noble *et al.* 2009). Given that cellular toxicity is influenced by numerous environmental factors, and can change depending on fluctuations in population growth status (Trainer *et al.* 2012), whales could be exposed to aggregations of subsurface *Pseudo-nitzschia* cells with a higher DA content than the cells being ingested by shellfish.

Seasonally there is a temporal disconnect between the abundance of potentially toxic phytoplankton species and the whale population, as the peak cell abundances develop at the end of the year (Fig. 7, 8), when most whales have left PV. Another consideration is that the risk levels are those used for human consumption of shellfish (ANZECC and ARMCANZ 2000), and it is not known how relevant they are for SRW calves, which would be indirectly exposed *via* their mother's milk or *in utero* during gestation (Maucher and Ramsdell 2005, Maucher Fuquay *et al.* 2012).

Additional indirect evidence for the exposure of SRWs to biotoxins at PV comes from the shellfishery closure data. From 2003-2012 the timing of the shellfishery closures shifted to earlier in the season, from October in 2003 to August in 2012 (Fig. 4), indicating that the whales were being exposed to potentially toxic phytoplankton earlier in the calving season and for a longer period of time. Between 2012 and 2014 the closure dates have been later, shortening the window of potential biotoxin exposure, and the number of deaths has decreased.

While the whales' dietary targets are not the toxic phytoplankton (*Pseudo-nitzschia* spp. and *A. tamarense*) some phytoplankton (especially long chains of *Pseudo-nitzschia* spp. cells, which can be 100s of micrometers in length) can be filtered directly by the whales when skim-feeding and/or be vectored to whales through their zooplankton prey species (copepods). Adult and juvenile whales feed sporadically on spring MSZ aggregations in late September and early October when the patches are dense enough to be foraged (Payne 1986, Sironi 2004, Hoffmeyer *et al.* 2010). A 5 d feeding event was observed in GN in October 2005, when the whales fed on a dense zooplankton patch composed of calanoid copepods (*Calanoides carinatus* and *Calanus australis*) and juvenile euphausids (Hoffmeyer *et al.* 2010). The most common MSZ species in the region are the copepods, *Ctenocalanus vanus* and *Paracalanus parvus* (Hoffmeyer and Pascual 2006, Menéndez *et al.* 2011). These species are of a size suitable for capture by right whale baleen, which can efficiently retain zooplankton that is 333 μm or larger (Mayo *et al.* 2001).

The presence of *Pseudo-nitzschia* spp. frustules and large quantities of *C. australis* remnants in whales feces collected from live and dead individuals in 2004, 2005, and 2010 (D'Agostino 2013) provides further evidence that whales are likely being exposed to biotoxins at PV through copepods vectors. Gonyautoxins (congeners of STX) and DA were also detected in zooplankton samples of GN and GSJ in 2010 (Cadaillón 2012).

Nonetheless, most of the samples from dead whales had no detectable traces of biotoxins, and when present the toxins were at low levels. For example, the PST values found in fecal samples from PV whales were 172 and 800 ng STX eq/g (Table 2), which was within the range of what is found in fecal samples from apparently healthy feeding North Atlantic right whales (19–1,763 ng STX eq/g; Doucette *et al.* 2012). Water-soluble toxins such as DA and PST are quickly eliminated *via* urine and feces, making detection difficult in exposed animals. Additionally, it may be impossible to detect toxins in calves, particularly if neural damage from toxins occurred *in utero*, months before birth. Female rats excrete DA through feces and

urine but it can cross the placental barrier and is not eliminated from the amniotic fluid, where it can accumulate and continually reexpose the fetus during gestation (Brodie *et al.* 2006, Maucher and Ramsdell 2007, Maucher Fuquay *et al.* 2012). Rodent fetuses are more susceptible to DA than adults, and levels of biotoxins that are nonsymptomatic to adults can cause subsequent developmental effects in exposed fetuses (Brodie *et al.* 2006, Maucher and Ramsdell 2007). Prenatal exposure to DA in rodent fetuses has been shown to have long-lasting neurobehavioral effects (Levin *et al.* 2006, Costa *et al.* 2010).

Many of the whale calves that died were 6 m or longer (74% between 2005 and 2014), suggesting they probably were at least a month old (the average newborn size is 5.5–6.1 m, Best and Rüther 1992). These older calves could have received biotoxins through their mother's milk (*i.e.*, calf 38-05 tested positive for DA and was 8.5 m long, Table 1). Nursing rats injected with DA transfer it to their offspring through their milk (Maucher and Ramsdell 2005, Maucher and Ramsdell 2007). DA has been found in the milk of California sea lions (*Zalophus californiaus*) and harbor porpoises (*Phocoena phocoena*) exposed to *Pseudo-nitzschia* spp. blooms (Rust *et al.* 2014) and has a longer retention time in milk than in blood or feces (Maucher and Ramsdell 2007, Rust *et al.* 2014). There have been no studies examining maternal transfer of PSTs to fetuses or neonates in these species. The dead SRW calf that tested positive for PSTs was quite young, 5.5 m in length and died in June 2009 (Table 1), which is very early in the season (most calves are born in August; Payne 1986). This young calf may have been exposed to PSTs *in utero* since mothers do not usually begin to feed until later in the season and *A. tamarense* was absent from the area at the time of the calf's death (Fig. 7).

It is possible that the early season calf deaths were due to exposure of the fetus to biotoxins when the mother was pregnant. While mating is known to happen at PV, there are only a few instances where multiparous mothers (two or more calves recorded) have been sighted the year before calving at PV, so it is not clear if conception occurs at PV (Payne 1986). It is possible that the females only come briefly to PV to mate, and thus are not likely to be photo-identified during the annual aerial survey, or that conception takes place elsewhere (Payne 1986, Best 1994). If the females become pregnant at PV they are likely exposed to biotoxins while there, but it is also possible that the females could have ingested biotoxins outside of the PV region. Adults, including pregnant females, are known to feed on the Patagonian Shelf and shelf-break and around South Georgia (Tormosov *et al.* 1998, Rowntree *et al.* 2008, Valenzuela *et al.* 2009, Zerbini *et al.* 2015), where prey densities are much higher than at PV (Acha *et al.* 2004) and where toxic HABs were documented in 1980 and 1981 (Table 2; Carreto *et al.* 1986, Esteves *et al.* 1992). However, no data exist on the frequency of HABs in this region.

One period of particularly high whale mortality occurred in GSJ at the end of October 2010 (Fig. 7) when 15 whales died, many of them calves (6 calves, 7 juveniles, and 2 adults). These deaths occurred over a short period of time, and affected all age classes, which is a pattern typically associated with HAB outbreaks, and is unusual at PV. The levels of both *Pseudo-nitzschia* spp. and *A. tamarense* were at "high risk" densities at that time (Fig. 7). DA and PST were also found in both the phytoplankton and zooplankton assemblages in GSJ at this time, and adult whales were observed skim feeding (Cadaillón 2012). While this does not provide conclusive evidence that these biotoxins contributed to the deaths, it adds to the compelling body of evidence that some mothers are likely ingesting biotoxins during their time at PV, which could be passed on to their nursing calves through their milk.

Exposure to multiple toxins could have a synergistic effect, increasing the potency of either or both toxins (Fire and Van Dolah 2012). Concurrent exposure to both DA and okadic acid was associated with a mass mortality of bottlenose dolphins (*Tursiops truncatus*) in Texas (Fire *et al.* 2011). However, North Atlantic right whales in the Bay of Fundy (BOF) have prolonged concurrent exposure to both PST and DA (Leandro *et al.* 2010, Doucette *et al.* 2012) that has not resulted in any unusual mortality event (though calves in the BOF are 6 mo or older). Whether this exposure has effected the overall health and reproductive success of the northern right whale population is not known.

If some of the PV right whale deaths are HAB related, they are unusual in how species- and age-specific they are. In all the years of high whale mortality at PV there have been no reported die-offs of any other marine mammal species, birds or fish, all of which are commonly associated with wildlife mortality attributed to marine HAB toxins (Shumway *et al.* 2003, de la Riva *et al.* 2009).

Are the High Chlorophyll Levels at PV Indicative of HABs?

Since it is not possible to definitively identify phytoplankton species from satellite chlorophyll data, it remains uncertain what species caused the chlorophyll blooms reported here and whether or not they were toxic and classifiable as HABs. However, neither the abundances of *A. tamarense* or *Pseudo-nitzschia* spp. can account for the high levels of chlorophyll measured by satellite. Given a nominal cellular chlorophyll content of 20 pg chl/cell (Anderson *et al.* 1990), an *A. tamarense* abundance of 10^4 cells/L (Fig. 3) would produce 0.2 mg/m^3 chlorophyll. *Pseudo-nitzschia* spp. has a lower cellular chlorophyll content, values range from 0.2 to 1 pg chl/cell (Loureiro *et al.* 2009, Brunet *et al.* 2014), which would only produce 0.2–1 mg/m^3 chlorophyll for the abundances seen at PV.

Chlorophyll blooms have been slightly less prevalent in GSJ than in GN (Fig. 6), but GSJ has had more occurrences of high levels of *Pseudo-nitzschia* spp. and *A. tamarense* (Fig. 3). In 2012 the peak in *Pseudo-nitzschia* spp. and *A. tamarense* in GN was coincident with the chlorophyll bloom, but in 2008 and 2010 the GN peaks in chlorophyll abundance preceded the peaks of *Pseudo-nitzschia* spp. The years with the biggest blooms in GN, 2004 and 2008, had low-risk level abundances of *Pseudo-nitzschia* spp. and *A. tamarense* during the time of the chlorophyll bloom. These observations indicate that the chlorophyll blooms are primarily comprised of other phytoplankton, which is not unusual for many HAB taxa (see Granéli and Turner 2006).

The spatial scales of the two data sets (satellite chlorophyll and phytoplankton abundance) are quite different. The phytoplankton data are the maximum values of samples taken in three different locations in each gulf, all taken very close to shore (see Fig. 1), whereas the chlorophyll data in Figure 7 are averaged across the entire GN. In some periods, notably November 2004, the highest chlorophyll values were in the center of GN (see animation in Fig. S1), and hence coastal samples might not be an adequate representation of what is occurring across the entire gulf. The strong blooms in 2007 and 2008 in GN were coincident with intense green discolorations caused by the dinoflagellate *Lepidodinium* sp., which is not known to be toxic (Sastre *et al.* 2010).

Satellite measurements of chlorophyll are derived for global case-1 waters, meaning waters where chlorophyll is the primary constituent in the water. The algorithm can be much less accurate in coastal waters, when there are other constituents in the water

(sediments, organic matter, *etc.*) or when the regional aerosols significantly deviate from the global aerosol model used for atmospheric corrections. However a comparison of regional algorithms for this area against the standard global algorithm (the one used here) showed the standard algorithm performed best (Dogliotti *et al.* 2009). Comparisons of *in situ* and satellite chlorophyll in the GSM (Williams *et al.* 2010, 2013) and over the Patagonia shelf (Dogliotti *et al.* 2009) have shown that satellite measurements underestimate chlorophyll at concentrations >1 mg/m^3. At chlorophyll >3 mg/m^3, the MODIS algorithm overestimates chlorophyll by ~20% relative to the SeaWiFS algorithm (http://oceancolor.gsfc.nasa.gov/VALIDATION), a bias that is evident in Figure 6.

What Is the Relationship Between the Chlorophyll Blooms at PV and Whale Mortality?

Between 2002 and 2014 there has been a general association between the development of large-magnitude chlorophyll blooms at PV and the increase in whale deaths. However, within years, the timing of major blooms is not correlated with peaks in whale deaths. There were years with strong blooms and little mortality (2004 had 13 deaths in GSJ), and years with no bloom and high mortality (2005 and 2011 had 37 and 61 deaths, respectively, in GSJ). The chlorophyll blooms start to develop in early October or later, which is at the beginning of the "late season" for the whales at PV (Fig. 7), but in 2008, 2009, and 2012 most deaths occurred early in the season, before any bloom developed, and usually before the whales start to feed. Moreover, as discussed above, it does not seem that the increase in the abundance of toxic phytoplankton is responsible for the observed increases in chlorophyll.

Conclusions

(1) SRW mothers and their calves are exposed to biotoxin-producing algae in the PV area. High-risk levels of *Pseudo-nitzschia* spp. and *A. tamarense* occurred between 2007 and 2013, the period with the highest number of deaths (>50 deaths/year). Since at least 2003, the local shellfisheries have been closed in the latter half of the whale calving season because of PST levels above the regulatory action limit. These closures occur at the time when adult whales start to feed on spring zooplankton blooms at PV. Traces of PSTs and DA have been found in samples from a few dead whales, albeit at very low concentrations. Frustules of *Pseudo-nitzschia* spp. have been recorded in fecal samples from whales in the region, indicating there is local direct foraging or trophic transfer of these potentially toxic species. The statistical relationships between month-specific *Pseudo-nitzschia* levels and calf mortality (Table 3), in both GN and GSJ (Fig. 9) suggest that this toxin-producing diatom might be a contributing factor. If the SRW mothers are exposed to DA, it is likely transferred to their calves during nursing, since DA is known to be transferred from mother to young *via* milk in other mammals. Toxic blooms have also occurred on the Patagonian shelf and shelf-break (Carreto *et al.* 1986), where the whales feed after leaving the PV calving ground, thus fetuses could be getting exposed there. More information on HABs in the whales' feeding grounds is essential to understand their role in calf deaths at PV.

(2) Annually, the seasonal development and magnitude of phytoplankton blooms are not correlated with peaks in whale deaths.

(3) Satellite data show that phytoplankton dynamics have changed in the PV region since the 1990s. Spring phytoplankton blooms are a normal seasonal feature;

however, the magnitude of the spring bloom in GN has increased considerably. The first large-magnitude bloom (chlorophyll >5 mg/m^3) occurred in 2004, a year before the first high mortality year. Large-magnitude blooms have been slightly more frequent in GN (nine since 2004), where more (68%) of the whale deaths have occurred, than they are in GSJ (six since 2004).

Literature Cited

Acha, M. E., H. W. Mianzan, R. A. Guerrero, M. Favero and J. Bava. 2004. Marine fronts at the continental shelves of austral South America physical and ecological processes. Journal of Marine Systems 44:83–105.

Anderson, D. M., D. M. Kulis, J. J. Sullivan, S. Hall and C. Lee. 1990. Dynamics and physiology of saxitoxin production by the dinoflagellates *Alexandrium* spp. Marine Biology 104:511–524.

Andrinolo, D., N. Santinelli, S. Otano, V. Sastre and N. Lagos. 1999. Paralytic shellfish toxins in mussels and *Alexandrium tamarense* at Valdes Peninsula, Chubut, Patagonia, Argentina: Kinetics of a natural depuration. Journal of Shellfish Research 18:203–209.

ANZECC and ARMCANZ. 2000. Australian and New Zealand guidelines for fresh and marine water quality. Volume 3. Primary industries: Rationale and background

information. Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Artarmon, Australia.

Balech, E. 1971. Microplancton de la campaña oceanográfica Productividad III [Microplankton of oceanographic campaign Productivity III]. Museo Argentino de Ciencias Naturales "Bernardino Rivadavia." 202 pp.

Balech, E. 1995. The genus *Alexandrium* Halim (Dinoflagellate). Sherkin Island Marine Station, Cork, Ireland.

Balech, E., and K. Tangen. 1985. Morphology and taxonomy of toxic species in the *tamarensis* group (Dinophyceae) *Alexandrium excavatum* (Braarud) comb-nov and *Alexandrium ostenfeldii* (Paulsen) comb-nov. Sarsia 70:333–343.

Bastida, R., and D. Rodríguez. 2003. Mamíferos marinos de Patagonia y Antártida [Marine mammals of Patagonia and Antarctica]. Vázquez Manzini, Buenos Aires, Argentina.

Best, P. B. 1994. Seasonality of reproduction and length of gestation in southern right whales *Eubalaena australis*. Journal of the Zoological Society of London 232:175–189.

Best, P. B., and H. Rüther. 1992. Aerial photogrammetry of southern right whales, *Eubalaena australis*. Journal of Zoology 228:595–614.

Brodie, E. C., F. M. D. Gulland, D. J. Greig, *et al*. 2006. Domoic acid causes reproductive failure in California sea lions (*Zalophus Californianus*). Marine Mammal Science 22:700–707.

Brunet, C., R. Chandrasekaran, L. Barra, V. Giovagnetti, F. Corato and A. V. Ruban. 2014. Spectral radiation dependent photoprotective mechanism in the diatom *Pseudo-nitzschia multistriata*. PLOS ONE 9:e87015.

Cadaillón, A. M. 2012. Floraciones algales nocivas y ficotoxinas en fitoplancton y zooplancton de los Golfos Nuevo y San José [Harmful algae blooms and phycotoxins in phytoplankton and zooplankton of Nuevo and San José Gulfs]. Undergraduate dissertation, Universidad Nacional de la Patagonia San Juan Bosco, Puerto Madryn, Argentina. 168 pp.

Cannizzaro, J. P., K. L. Carder, F. R. Chen, C. A. Heil and G. A. Vargo. 2008. A novel technique for detection of the toxic dinoflagellate, *Karenia brevis*, in the Gulf of Mexico from remotely sensed ocean color data. Continental Shelf Research 28:137–158.

Carreto, J. I., H. R. Benavides, R. M. Negri and P. D. Glorioso. 1986. Toxic red-tide in the Argentine Sea. Phytoplankton distribution and survival of the toxic dinoflagellate *Gonyaulax excavata* in a frontal area. Journal of Plankton Research 8:15–28.

Cooke, J. G., V. Rowntree and R. Payne. 2003. Analysis of interannual variation in reproductive success of South Atlantic right whales (*Eubalaena australis*) from photoidentifications of calving females observed off Península Valdés, Argentina, during 1971–2000. Paper SC/55/O23 presented to the International Whaling Commission Scientific Committee, Berlin, Germany, June 2003.

Costa, L. G., G. Giordano and E. M. Faustman. 2010. Domoic acid as a developmental neurotoxin. Neurotoxicology 31:409–423.

Crespo, E. A., S. N. Pedraza, S. L. Dans, M. A. Coscarella, G. M. Svendsen and M. Degrati. 2014. Number of southern right whales *Eubalaena australis* and population trend in the neighborhood of Península Valdés during the period 1999–2013 by means of aerial and boat surveys. Report of the Scientific Committee SC/65b/BRG07. International Whaling Commission, Cambridge, U.K.

D'Agostino, V. 2013. Caracterización y comparación de restos de organismos planctónicos en material fecal de individuos de Ballena Franca Austral (*Eubalaena australis*), Península Valdés, Chubut, Argentina [Characterization and comparison of planktonic organisms remains in fecal material of individuals of southern right whale (*Eubalaena australis*), Valdes Peninsula, Chubut, Argentina]. Undergraduate dissertation, Universidad Nacional de la Patagonia San Juan Bosco, Puerto Madryn, Argentina. 94 pp.

de la Riva, G. T., C. K. Johnson, F. M. D. Gulland, G. W. Langlois, J. E. Heyning, T. K. Rowles and J. A. K. Mazet. 2009. Association of an unusual marine mammal mortality

event with *Pseudo-nitzschia* spp. blooms along the southern California coastline. Journal of Wildlife Diseases 45:109–121.

Dogliotti, A. I., I. R. Schloss, G. O. Almandoz and D. A. Gagliardini. 2009. Evaluation of SeaWiFS and MODIS chlorophyll-a products in the Argentinean Patagonian Continental Shelf (38°S–55°S). International Journal of Remote Sensing 30:251–273.

Doucette, G. J., C. M. Mikulski, K. L. King, *et al.* 2012. Endangered North Atlantic right whales (*Eubalaena glacialis*) experience repeated, concurrent exposure to multiple environmental neurotoxins produced by marine algae. Environmental Research 112:67–76.

Esteves, J. L., N. Santinelli, V. Sastre, R. Díaz and O. Rivas. 1992. A toxic dinoflagellate bloom and PSP production associated with upwelling in Golfo Nuevo, Patagonia, Argentina. Hydrobiologia 242:115–122.

Fazio, A., M. Bertellotti and C. Villanueva. 2012. Kelp gulls attack southern right whales: A conservation concern? Marine Biology 159:1981–1990.

Fernández Aráoz, N. C. 1994. Estudios sobre la biomasa de Copepoda (Crustacea), con especial énfasis en Calanoida, del Atlántico Sudoccidental [Studies on Copepoda (Crustacea) biomass, with special emphasis on Calanoida, Southwest Atlantic]. Ph.D. thesis, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina. 111 pp.

Fire, S. E., and F. M. Van Dolah. 2012. Marine toxins emergence of harmful algal blooms as health threats to marine wildlife. Pages 374–389 *in* A. A. Aguirre, R. Ostfeld and P. Daszak, eds. New directions in conservation medicine: Applied cases of ecological health. Oxford University Press, New York, NY.

Fire, S. E., Z. H. Wang, M. Byrd, H. R. Whitehead, J. Paternoster and S. L. Morton. 2011. Co-occurrence of multiple classes of harmful algal toxins in bottlenose dolphins (*Tursiops truncatus*) stranding during an unusual mortality event in Texas, USA. Harmful Algae 10:330–336.

Flewelling, L. J., J. P. Naar, J. P. Abbott, *et al*. 2005. Brevetoxicosis: Red tides and marine mammal mortalities. Nature 435:755–756.

Gayoso, A. M. 2001. Observations on *Alexandrium tamarense* (Lebour) Balech and other dinoflagellate populations in Golfo Nuevo, Patagonia (Argentina). Journal of Plankton Research 23:463–468.

Gayoso, A. M., and V. K. Fulco. 2006. Occurrence patterns of *Alexandrium tamarense* (Lebour) Balech populations in the Golfo Nuevo (Patagonia, Argentina), with observations on ventral pore occurrence in natural and cultured cells. Harmful Algae 5:233–241.

Gayoso, A. M., S. Dover, S. Morton, M. Busman, P. Moeller, V. K. Fulco and L. Maranda. 2002. Diarrhetic shellfish poisoning associated with *Prorocentrum lima* (Dinophyceae) in Patagonian gulfs (Argentina). Journal of Shellfish Research 21:461–463.

Geraci, J. R., D. M. Anderson, R. J. Tirnperi, D. I. S. Aubin, G. A. Early, J. H. Prescott and C. A. Mayo. 1989. Humpback whales (*Megapiera novaeangliae*) fatally poisoned by dinoflagellate toxin. Canadian Journal of Fisheries and Aquatic Sciences 46:1895–1898.

Gil, M., V. Sastre, N. Santinelli and J. L. Esteves. 1989. Metal content in seston from San José Gulf, Patagonia, Argentina. Bulletin of Environmental Contamination and Toxicology 43:337–341.

Granéli, E., and J. T. Turner. 2006. Ecology of harmful algae. Springer-Verlag, Berlin, Germany.

Hasle, G. R., and G. A. Fryxell. 1970. Diatoms: Cleaning and mounting for light and electron microscopy. Transactions of the American Microscopical Society 89:469–470.

Hernández Moresino, R. D., L. Villanueva Gomila, R. Di Mauro and P. J. Baron. 2013. Structural differentiation of the mesozooplankton community in two hydrographic domains of a small basin: The frontal system of San Jose Gulf (Patagonia, Argentina) as a study case. Journal of Plankton Research 36:578–584.

Hoffmeyer, M. S., and M. S. Pascual. 2006. Water quality and zooplankton monitoring at the seawater supply circuit of a mollusc hatchery. World Aquaculture 37:31–36.

Hoffmeyer, M. S., M. S. Lindner, A. Carribero, *et al*. 2010. Planktonic food and foraging of *Eubalaena australis*, on Peninsula Valdés (Argentina) nursery ground. Revista de Biología Marina y Oceanografía 45:131–139.

International Whaling Commission. 2011. Report of the southern right whale die-off workshop, 5–18 March 2010, Centro Nacional Patagónico, Puerto Madryn, Argentina. Journal of Cetacean Research and Management 12 (Supplement):367–398.

International Whaling Commission. 2015. Report of the second workshop on mortality of Southern right whales (*Eubalaena australis*) at Península Valdés, Argentina, 5–6 August 2014, Centro Nacional Patagónico, Puerto Madryn, Argentina. Pages 367–398. Document SC/66a/Rep/9.

Leandro, L. F., R. M. Rolland, P. B. Roth, N. Lundholm, Z. Wang and G. J. Doucette. 2010. Exposure of the North Atlantic right whale *Eubalaena glacialis* to the marine algal biotoxin, domoic acid. Marine Ecology Progress Series 398:287–303.

Lelong, A., H. Hégaret, P. Soudant and S. S. Bates. 2012. *Pseudo-nitzschia* (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: Revisiting previous paradigms. Phycologia 51:168–216.

Levin, E. D., W. G. Pang, J. Harrison, P. Williams, A. Petro and J. S. Ramsdell. 2006. Persistent neurobehavioral effects of early postnatal domoic acid exposure in rats. Neurotoxicology and Teratology 28:673–680.

Loureiro, S., C. Jauzein, E. Garces, Y. Collos, J. Camp and D. Vaque. 2009. The significance of organic nutrients in the nutrition of *Pseudo-nitzschia delicatissima* (Bacillariophyceae). Journal of Plankton Research 31:399–410.

Lund, J. W. G., C. Kipling and E. D. L. Cren. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11:143–170.

Maucher, J. M., and J. S. Ramsdell. 2005. Domoic acid transfer to milk: Evaluation of a potential route of neonatal exposure. Environmental Health Perspectives 113:461–464.

Maucher, J. M., and J. S. Ramsdell. 2007. Maternal-fetal transfer of domoic acid in rats at two gestational time points. Environmental Health Perspectives 115:1743–1746.

Maucher Fuquay, J., N. Muha, Z. Wang and J. S. Ramsdell. 2012. Toxicokinetics of domoic acid in the fetal rat. Toxicology 294:36–41.

Mayo, C. A., B. H. Letcher and S. Scott. 2001. Zooplankton filtering efficiency of the baleen of a North Atlantic right whale, *Eubalaena glacialis*. Journal of Cetacean Research and Management (Special Issue 2):225–229.

Menéndez, M. C., A. A. Berasategui, M. S. Lindner, S. L. Diodato, M. D. F. Severini and M. S. Hoffmeyer. 2007. Trophic spectrum of southern right whale *Eubalaena australis* by means of faecal analysis. Biocell 31:347.

Menéndez, M. C., F. Biancalana, A. A. Berasategui, M. D. F. Severini, M. S. Hoffmeyer and J. L. Esteves. 2011. Mesozooplankton composition and spatial distribution, Nuevo Gulf, Patagonia, Argentina. Check List 7:101–107.

Montoya, N., R. Akselman, J. Franco and J. I. Carreto. 1996. Paralytic shellfish toxins and mackerel (*Scomber japonicus*) mortality in the Argentine Sea. Pages 417–420 *in* T. Yasumoto, Y. Oshima and Y. Fukuyo, eds. Harmful and toxic algal blooms. Intergovernmental Oceanographic Commission (IOC) of UNESCO.

Noble, M., B. Jones, P. Hamilton, J. Xu, G. Robertson, L. Rosenfeld and J. Largier. 2009. Cross-shelf transport into nearshore waters due to shoaling internal tides in San Pedro Bay, CA. Continental Shelf Research 29:1768–1785.

Orensanz, J. M., A. M. Parma and O. Iribarne. 1991. Population dynamics and management of natural stocks. Pages 625–714 *in* S. E. Shumway, ed. Scallops: Biology, ecology and aquaculture. Elsevier, Amsterdam, The Netherlands.

Payne, R. 1986. Long term behavioral studies of the southern right whale (*Eubalaena australis*). Report of the International Whaling Commission (Special Issue 10):161–167.

Ramírez, F. C. 1996. Composición, abundancia y variación estacional del zooplankton de red del Golfo San Matías [Composition, abundance and seasonal variation of net zooplankton of San Matías Gulf]. Frente Marítimo 16:157–167.

Rowntree, V. J., P. Mcguinness, K. Marshall, R. Payne, M. Sironi and J. Seger. 1998. Increased harassment of right whales (*Eubalaena australis*) by kelp gulls (*Larus dominicanus*) at Península Valdés, Argentina. Marine Mammal Science 14:99–115.

Rowntree, V. J., L. O. Valenzuela, P. F. Fraguas and J. Seger. 2008. Foraging behaviour of southern right whales (*Eubalaena australis*) inferred from variation of carbon stable isotope ratios in their baleen. Paper SC/60/BRG23 presented to the International Whaling Commission Scientific Commission, Santiago, Chile, 2008.

Rowntree, V. J., M. M. Uhart, M. Sironi, *et al.* 2013. Unexplained recurring high mortality of southern right whale *Eubalaena australis* calves at Península Valdés, Argentina. Marine Ecology Progress Series 493:275–289.

Rust, L., F. Gulland, E. Frame and K. Lefebvre. 2014. Domoic acid in milk of free living California marine mammals indicates lactational exposure occurs. Marine Mammal Science 30:1272–1278.

Santinelli, N., G. Gaille and A. Lettieri. 1994. Harmful algae and PSP toxicity along north Patagonia coast. Harmful Algae News 9:16–17.

Santinelli, N., V. Sastre and J. L. Esteves. 2002. Episodios de Algas Nocivas en la Patagonia Argentina [Harmful algal episodes in Patagonia Argentina]. Pages 197–208 *in* E. Sar, M. Ferrario and B. Reguera, eds. Floraciones Algales Nocivas en el Cono Sur Americano [Harmful algal blooms in the Southern American Cone]. Instituto Español de Oceanografía, Vigo, Spain.

Sastre, V., N. Santinelli, J. L. Esteves and M. E. Ferrario. 2001. Aspectos ecológicos de especies de *Pseudo-nitzschia* en aguas costeras patagónicas [Ecological features of *Pseudo-nitzschia* species in Patagonian coastal waters]. Pages 217–235 *in* K. Alveal and T. Antesana, eds. Sustentabilidad de la biodiversidad [Sustainability of biodiversity]. Universidad de Concepción, Concepción, Chile.

Sastre, V., N. Santinelli, G. Marino, M. Solis, L. Pujato and M. Ferrario. 2007. First detection of domoic acid produced by *Pseudonitzschia* species, Chubut coastal waters, Patagonia, Argentina. Harmful Algae News 34:12–14.

Sastre, V., N. Santinelli and R. Akselman. 2010. Green discolorations in Nuevo Gulf, Chubut Province, Argentina. Harmful Algae News 42:16–17.

Schnetzer, A., B. H. Jones, R. A. Schaffner, *et al.* 2013. Coastal upwelling linked to toxic *Pseudo-nitzschia australis* blooms in Los Angeles coastal waters, 2005–2007. Journal of Plankton Research 35:1080–1092.

Shumway, S. E., S. M. Allen and P. D. Boersma. 2003. Marine birds and harmful algal blooms: Sporadic victims or under-reported events? Harmful Algae 2:1–17.

Simons, R. A. 2011. ERDDAP-The Environmental Research Division's Data Access Program. Available at http://coastwatch.pfeg.noaa.gov/erddap.

Sironi, M. 2004. Behaviour and social development of juvenile southern right whales (*Eubalaena australis*) and interespecific interactions at Península Valdés, Argentina. Ph.D. thesis, University of Wisconsin, Madison, WI. 198 pp.

Sironi, M., V. Rowntree, M. Di Martino, L. Beltramino, V. Rago, M. Franco and M. Uhart. 2014. Updated information for 2012–2013 on southern right whale mortalities at Península Valdés, Argentina. Paper SC/65b/BRG06 presented at the 65th annual meeting of the International Whaling Commission Scientific Committee, Bled, Slovenia, June 2014.

Smayda, T. J. 1980. Phytoplankton species succession. Pages 493–570 *in* I. Miorris, ed. The physiological ecology of phytoplankton. University of California Press, Berkeley, CA.

Stumpf, R. P., M. E. Culver, P. A. Tester, *et al.* 2003. Monitoring *Karenia brevis* blooms in the Gulf of Mexico using satellite ocean color imagery and other data. Harmful Algae 2:147–160.

Thomas, P. O., and S. M. Taber. 1984. Mother-infant interaction and behavioral development in southern right whales, *Eubalaena australis*. Behaviour 88:42–60.

Thomas, P. O., M. M. Uhart and D. McAloose, *et al.* 2013. Workshop on the Southern right whale die-off at Península Valdés, Argentina. Paper SC/65a/BRG15 presented at the 65th annual meeting of the International Whaling Commission Scientific Committee, Jeju Island, Korea, June 2013.

Tomlinson, M. C., T. T. Wynne and R. P. Stumpf. 2009. Evaluation of the use of remote sensing techniques for enhanced detection of the toxic dinoflagellate, *Karenia brevis*. Remote Sensing of the Environment 113:598–609.

Tormosov, D. D., Y. A. Mikhaliev, P. B. Best, V. A. Zaemsky, K. Sekiguchi and R. L. Brownell, Jr. 1998. Soviet catches of southern right whales *Eubalaena australis*, 1951–1971. Biological data and conservation implications. Biological Conservation 86:185–197.

Trainer, V. L., S. S. Bates, N. Lundholm, A. E. Thessen, W. P. Cochlan, N. G. Adams and C. G. Trick. 2012. *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. Harmful Algae 14:271–300.

Tweddle, J. F., P. G. Strutton, D. G. Foley, *et al.* 2010. Relationships among upwelling, phytoplankton blooms, and phycotoxins in coastal Oregon shellfish. Marine Ecology Progress Series 405:131–145.

Uhart, M., M. Montoya and N. Lisnizer, *et al.* 2008*a*. Mortalidad de Gaviota Cocinera (*Larus dominicanus*) por toxina paralizante de los moluscos en Chubut, Argentina [Mortality of kelp gulls (*Larus dominicanus*) by paralytic shellfish poison in Chubut, Argentina]. 12th Reunión Argentina de Ornitología, 5–8 March 2008, San Martín de los Andes, Neuquén, Argentina.

Uhart, M., V. J. Rowntree and N. Mohamed, *et al.* 2008*b*. Strandings of southern right whales (*Eubalaena australis*) at Península Valdés from 2003–2007. Paper SC/60/BRG15 presented to the International Whaling Commission Scientific Committee, Santiago, Chile, June 2008.

Uhart, M., V. J. Rowntree and M. Sironi, *et al.* 2009. Continuing southern right whale mortality events at Península Valdés, Argentina. Paper SC/61/BRG18 presented to the International Whaling Commission Scientific Committee, Madeira, Portugal, June 2009.

Valenzuela, L. O., M. Sironi, V. J. Rowntree and J. Seger. 2009. Isotopic and genetic evidence for culturally inherited site fidelity to feeding grounds in southern right whales (*Eubalaena australis*). Molecular Ecology 18:782–791.

Van Dolah, F. M., S. E. Fire, T. A. Leighfield, C. M. Mikulsk and G. J. Doucette. 2012. Determination of paralytic shellfish toxins in shellfish by receptor binding assay: Collaborative study. Journal of AOAC International 95:795–812.

Vecchio, J. H., O. Gómez, J. A. Orosco, J. C. Tartaglione and G. Gricman. 1986. Intoxicación por veneno paralizante de moluscos (marea roja) [Human intoxication by paralytic shellfish poison (red tide)]. Medicina 46:705–708.

Williams, G. N., N. M. Sapoznik, M. Ocampo-Reinaldo, *et al.* 2010. Comparison of AVHRR and SeaWiFS imagery with fishing activity and in situ data in San Matías Gulf, Argentina. International Journal of Remote Sensing 31:4531–4542.

Williams, G. N., A. I. Dogliotti, P. Zaidman, *et al.* 2013. Assessment of remotely-sensed sea-surface temperature and chlorophyll-a concentration in San Matías Gulf (Patagonia, Argentina). Continental Shelf Research 52:159–171.

Zerbini, A., M. Mendez and H. Rosenbaum, *et al.* 2015. Tracking southern right whales through the southwest Atlantic: New insights into migratory routes and feeding grounds. Paper SC/66a/BRG22 presented at the 66th annual meeting of the International Whaling Commission Scientific Committee, San Diego, CA, May, 2015.

Supporting Information

The following supporting information is available for this article online at http://onlinelibrary.wiley.com/doi/10.1111/mms.12263/suppinfo.

Appendix S1. Methods used for PST and DA sample preparation and analysis.

Table S1. Information about 118 tissue samples analyzed for biotoxins from 51 southern right whales (50 calves, 1 adult) that died at PV from 2003 to 2010 and 2012, broken down by their type, year of collection, age of the whale, whale necropsy condition, where analysed, and the toxin analyzed.

Figure S1. Animation (4013_FigS1.mov) of weekly composites of MODIS chlorophyll for September–November 2003–2014.

Figure S2. Monthly composites of chlorophyll from the CZCS sensor from August–November 1979–1985. There were only 8 of the 28 months with sufficient data coverage of the PV area. None of these indicated the presence of a phytoplankton bloom in either GSJ or GN.

Appendix S1. Methods used for PST and DA sample preparation and analysis.

NOAA/NOS (National Oceanic and Atmospheric Administration, National Ocean Service)

NOAA/NOS analyzed 101 samples from 43 whale calves and 1 adult whale that died between 2004–2010. The samples were preserved frozen and stored in liquid nitrogen or at −80ºC degrees until analysis. Urine samples were centrifuged at 10,000 × g and filtered (0.2 μm) prior to analysis for PSTs. Samples of feces were extracted by combining and homogenizing at a minimum weight of samples when sample volume or mass was limited or a typical weight of 2–10 grams of samples with an equal volume of 0.1N hydrochloric acid. Homogenized samples were boiled for 5 min then volumetrically resuspended. The pH of the cooled mixture was measured and adjusted if necessary to fall between 2.0 and 4.0. These extracts were centrifuged at 3,400 rpm (1,950 × g), then the supernatant was collected and syringe filtered (0.45 μm). Blood card samples (whole blood) were extracted by suspending a filter paper spot containing 100 μL of whole blood in 2 mL of 40% aqueous MeOH. Acidic aqueous tissue extracts and filtered fluid samples were analyzed in a STX receptor binding assay (RBA) to determine the total saxitoxin-like activity of a sample. The receptor binding assay measures competition between radiolabeled saxitoxin and sample or FDA standard (S. Hall, USFDA/CFSAN, Washington, DC) for binding to the voltage-gated sodium channel, the pharmacological target of saxitoxins, to determine the total saxitoxin-like activity of the sample. For DA analysis, samples were extracted by adding four volumes of 50% aqueous methanol, homogenizing, and then probe-sonicating on ice for 2 min. These extracts were centrifuged at 3,400 rpm, and the supernatant collected and syringe filtered (0.45 μm) prior to analysis. Selected samples were cleaned by solid-phase extraction (SPE) using Agilent C18 columns; toxins were eluted using 50% methanol (Wang *et al.* 2012). Samples were analyzed for DA using tandem mass spectrometry coupled with liquid chromatographic separation (LC-MS/MS). This method utilized reversed phase chromatography, using an Agilent 1100 HPLC coupled to an AB-SCIEX API-4000 triple quadruple mass spectrometer in ESI+ mode. Chromatographic separation was performed on a Phenomenex Luna C18(2), 5μm, 150 mm × 2 mm column. Mobile phase consisted of water and acetonitrile in a binary system, with 0.1% formic acid as an additive. Retention time of DA in samples was determined based on the retention time of the certified reference material (Certified Reference Materials Program, National Research Council of Canada, Halifax, Canada). Detection of DA by LC-MS was achieved by the Multiple Reaction Monitoring (MRM) method.

INIDEP (Instituto Nacional de Investigación y Desarrollo Pesquero)

INIDEP analyzed nine samples from two whale calves that died in 2008. All samples were analyzed for DA, and six were also analyzed for STX. All results were negative. Extracts were obtained from each tissue sample for domoic acid (DA) content following the procedures of Wright and Quillian (1995) for DA and AOAC International (2012) for PST. DA concentration was determined by High Performance Liquid Chromatography whit diode array detection (HPLC-DAD) (Wright and Quilliam 1995). The most sensitive method of High Performance Liquid Chromatography whit fluorescence detection (HPLC-FL) was used for the trace determination of DA (Pocklington *et al.* 1990). PST composition was analyzed using the postcolumn derivatization HPLC method (Oshima 1995). Two different columns were used: a YMC-Pack ODS-AQ, 3 μm, 150 mm × 4.6 mm (Waters Corp., Milford, MA) for N-sulfocarbamoyl toxins and an Alltima C8, 3 μm, 150 mm × 4.6 mm (Alltech Associates Inc.,

Deerfield, IL) for the other PSPs. Toxins were identified by comparison of their retention times and fluorescent emission maxima with those of standards, by the disappearance of peaks by eliminating postcolumn oxidation, and by spiking experiments (Onodera *et al.* 1996).

Laboratorio Dirección de Salud Ambiental, Ministerio de Salud, Provincia de Chubut
     The Laboratorio Dirección de Salud Ambiental, Ministerio de Salud lab analyzed eight samples from four whale calves that died in 2012. Biological assays were used to detect PST, and all results were negative. Samples were not tested for DA.

LITERATURE CITED

AOAC International. 2012. Official methods of analysis. Association of Official Analytic Chemists, Arlington, VA.

Onodera, A., Y. Oshima, M. F. Watanabe, C. J. Bolch, S. Blackburn and T. Yasumoto. 1996. Screening of paralytic shellfish toxins in freshwater cyanobacteria and chemical confirmation of the toxins in culture *Anabaena circinalis* Australia. Pages 563–566 *in* T. Yasumoto, Y. Oshima and Y. Fukuyo, eds. Harmful and toxic algal blooms. IOC UNESCO.

Oshima, Y. 1995. Postcolumn derivatization liquid chromatography method for paralytic shellfish toxins. Journal of AOAC International 78:528–532.

Pocklington, R., J. E. Milley, S. S. Bates, C. J. Bird, A. S. W. De Freitas and M. A. Quilliam. 1990. Trace determination of domoic acid in seawater and phytoplankton by high-performance liquid- chromatography of the fluorenylmethoxycarbonyl (FMOC) derivative. International Journal of Environmental Analytical Chemistry 38:351–368.

Wang, Z., J. Maucher-Fuquay, S. Fire, T. Mikulski, B. Haynes, G. Doucette and J. Ramsdell. 2012. Optimization of solid-phase extraction and liquid chromatography–tandem mass spectrometry for the determination of domoic acid in seawater, phytoplankton, and mammalian fluids and tissues. Analytica Chimica Acta 275:71–79.

Wright, J. L. C. and M. A. Quilliam. 1995. Methods for domoic acid, the amnesic shellfish poisons. Pages 113–133 *in* G. M. Hallegraeff, D. M. Anderson and A. D. Cembella, eds. Manual on harmful marine microalgae. UNESCO, Paris, France.

Table S1. Information about 118 tissue samples analyzed for biotoxins from 51 southern right whales (50 calves, 1 adult) that died at PV from 2003 to 2010 and 2012, broken down by their type, year of collection, age of the whale, whale necropsy condition, where analyzed, and the toxin analyzed.

Sample type	Number	Percentage
Feces	29	25%
Urine	21	18%
Intestinal content	20	17%
Stomach tissue	16	14%
Intestinal tissue	13	11%
Gastric	4	3%
Milk	4	3%
Liver	4	3%
Blood	3	3%
Brain	2	2%
Kidney	1	1%
Bone Marrow	1	1%

Sample year	Number	Percentage
2004	1	1%
2005	13	11%
2006	1	1%
2007	8	7%
2008	71	60%
2009	11	9%
2010	5	4%
2012	8	7%

Whale age	Number	Percentage
Calf	117	99%
Adult	1	1%

Whale necropsy condition	Number	Percentage
2	51	43%
3	58	49%
4	9	8%

Analyses location	Number	Percentage
NOAA/NOS	101	85%
INIDEP	9	8%
DSA–Chubut Province	8	7%

Toxin	Number	Percentage
DA	108	92%
PST	105	89%
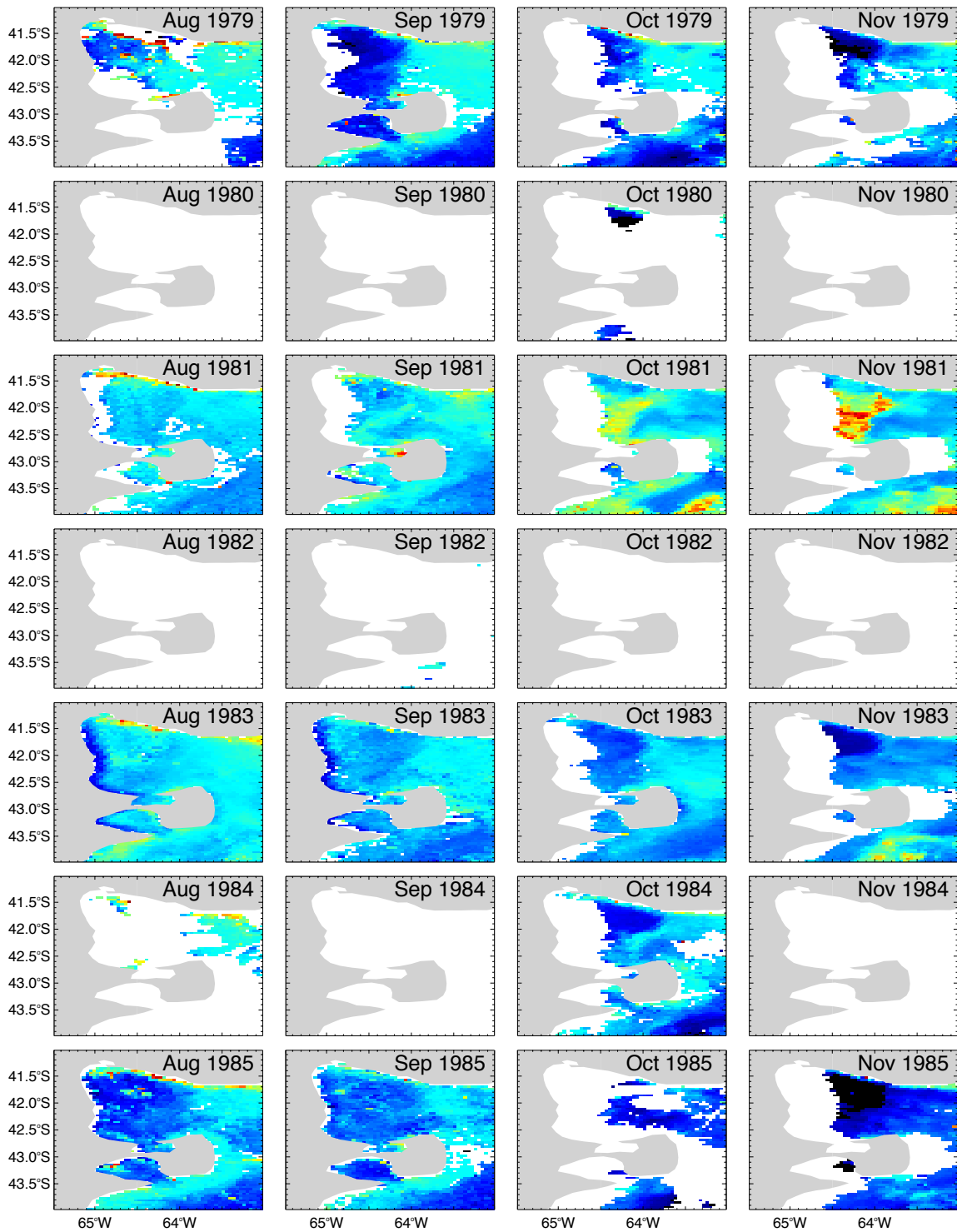
Chlorophyll mg/m³, CZCS data