Mass fish die-off during a diatom bloom in the Bahía de La Paz, Gulf of California

Mortalidad masiva de peces durante un florecimiento de diatomeas en La Bahía de La Paz, Golfo de California

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López-Cortés D. J., E. J. Núñez-Vázquez, C. J. Band-Schmidt, I. Gárate-Lizárraga, F. E. Hernández-Sandoval, J. J. Bustillos-Guzmán. 2015. Mass fish die-off during a diatom bloom in the Bahía de La Paz, Gulf of California. *Hidrobiológica* 25 (1): 39-48.

ABSTRACT

An unusual fish die-off occurred during a bloom of diatoms from June 10th –July 3rd 2006 in Bahía de La Paz in the Gulf of California. The prevalent organisms were *Pseudo-nitzschia* spp. (2.4×10^6 cells L^{-1}), *Thalassiosira eccentrica* (2.3×10^6 cells L^{-1}) and *Chaetoceros* spp. (9.65 x 10^5 cells L^{-1}). Three toxic species were identified: *P. pseudodelicatissima*-complex, *P. fraudulenta*, and *P. pungens.* Fucoxanthin was the dominant pigment during the bloom, peaking at 9.3 µg L^{-1}. Sea surface temperature dramatically increased from 19.0 °C to 27.0 °C during the bloom, with inorganic nitrogen (1.0 ± 0.6 µM) and $Si(OH)_4$ (15.5 ± 8.0 µM). Low content of domoic acid measured by HPLC-UV from net samples ranged from 24.0 to 52.0 ng per filter and tissue of the chocolate clam *Megapitaria squalida* (0.55 µg g^{-1}) and the white clam *Dosinia ponderosa* (0.06 µg g^{-1}). Domoic acid analysis of dead fish tissues was negative. Fish necropsy indicated that death by asphyxia was probably caused by *Thalassiosira eccentrica* and *Chaetoceros* spp. Alternate mixing processes and instability of the water column, as well as sudden changes of both temperature and nutrients created conditions for proliferation of the diatoms.

Key words: Domoic acid, fish mortality, Gulf of California, *Pseudo-nitzschia, Thalassiosira.*

RESUMEN

Una inusual muerte masiva de peces sucedió durante un florecimiento de diatomeas ocurrido del 10 de junio al 3 de julio en la Bahía de La Paz, Golfo de California. Los organismos dominantes fueron *Pseudo-nitzschia* spp. (2.4×10^6 cels L^{-1}), *Thalassiosira eccentrica* (2.3×10^6 cels L^{-1}) y *Chaetoceros* spp. (9.65 x 10^5 cels L^{-1}). Se identificaron tres especies tóxicas: una pertenece al complejo *P. pseudodelicatissima, P. fraudulenta y P. pungens*. La fucoxantina, con un pico de 9.3 µg L^{-1}, fue el pigmento dominante durante el florecimiento. La temperatura superficial del mar en el transcurso del evento drásticamente se incrementó de 19.0 °C a 27.0 °C, con valores de nitrógeno inorgánico de 1.0 ± 0.6 µM y $Si(OH)_4$ de 15.5 ± 8.0 µM. El contenido de ácido domoico determinado por HPLC-UV fue bajo tanto en muestras de red con un rango de 24.0 a 52.0 ng por filtro como en tejido de almeja chocolata *Megapitaria squalida* (0.55 µg g^{-1}) y almeja blanca *Dosinia ponderosa* (0.06 µg g^{-1}). El análisis de ácido domoico en tejido de peces fue negativo. La necropsia de peces sugiere que la muerte por asfixia probablemente la causó *Thalassiosira eccentrica.* y *Chaetoceros* spp. Procesos de mezcla alternantes y la inestabilidad de la columna de agua, así como cambios bruscos de temperatura y nutrientes, propiciaron condiciones para la proliferación de las diatomeas.

Palabras clave: Ácido domoico, mortalidad de peces, Golfo de California, *Pseudo-nitzschia, Thalassiosira.*

INTRODUCTION

As in other parts of the world, harmful algal blooms in the Gulf of California have increased over the last two decades (Cortés-Altamirano & Licea-Durán, 2004; Gárate-Lizárraga *et al.*, 2009). The primary harmful group dinoflagellates. However, diatoms also develop harmful algal blooms (Licea-Durán *et al.*, 1999). Different species of diatoms like *Thalassiosira* and *Chaetoceros* are noxious, especially for farmed fish, while others like several species of *Pseudo-nitzschia* H. Peragallo are toxigenic (Smayda, 2006; López-Cortés *et al.*, 2006; García-Mendoza *et al.*, 2009).

The genus *Thalassiosira* own to centric diatoms which has one of the greater number of species, with > 100, some of which form mucilaginous colonies (Harris *et al.*, 1995) and can produce harmful algal blooms that generate anoxia. Some species of the genus *Chaetoceros* have long robust setae with spinules that, even in low densities, can produce mechanical damage in fish gills; the fish react producing mucus which leads to asphyxia (Albright *et al.*, 1993). The species of the genus *Pseudo-nitzschia* are limited to marine environments and have wide global distribution (Hasle *et al.*, 1996). Their presence is associated with eddies, upwellings, and post-upwelling conditions (Anderson *et al.*, 2006; Schnetzer *et al.*, 2007).

Presently, 37 species of *Pseudo-nitzschia* have been described, of which 14 produce domoic acid (DA) (Trainer *et al.*, 2012). Seven of these toxigenic species can be found along the west coast of Mexico. These are *P. fraudulenta* (Cleve) Hasle, *P. pseudodelicatissima*-complex, *P. australis* Frenguelli, *P. multiseries* (Hasle) Hasle, *P. delicatissima* (Cleve) Heiden, *P. pungens* (Grunow ex Cleve) Hasle and *P. brasiliana* (Lundholm, Hassle and Fryxell) (Lange *et al.*, 1994; Hernández-Becerril *et al.*, 2007; Gárate-Lizárraga *et al.*, 2007, 2013; Quijano-Scheggia *et al.*, 2011; Table 1). Fish can accumulate DA in their viscera and body tissues without toxic effects (Lefebvre *et al.*, 2012), and they are the principal vectors of transferring DA to higher trophic levels (Trainer et al., 2012; Lefebvre *et al.*, 2012). However, the relation between nutrient concentration in the environment and production of DA remains unclear (Trainer *et al.*, 2009; Lewitus *et al.*, 2012).

Along the west coast of Mexico, DA has been associated with the death of marine mammals and seabirds in several zones (Table 1). Data of the exact environmental conditions associated with these events are scarce, but factors have been found to include wind-driven upwelling and a high ratio of $Si(OH)_4$:N (García-Mendoza *et al.*, 2009). Moreover, the specific conditions that trigger the dominance of *Pseudo-nitzschia* during these blooms, its production of DA, its oceanographic and meteorological conditions are unclear and may be complex (Lewitus *et al.*, 2012). In the southern part of the Bahía de La Paz, a diatom bloom in 2006 occurred during the first 10 days of June, which included different species of *Pseudo-nitzschia* (Gárate-Lizárraga *et al.*, 2007), one species of *Thalassiosira* and *Chaetoceros* spp. (Guluarte-Castro & Bañuelos, 2007; Núñez-Vázquez *et al.*, 2011). The aim of this paper is to describe the hydrographic conditions, related to the diatom bloom dominated by DA producing *Pseudo-nitzschia* spp., which co-occurred with *Thalassiosira* sp., and *Chaetoceros* spp., and to clarify the causes of massive fish death along the southern coast of Bahía de La Paz during summer 2006.

MATERIALS AND METHODS

Study area and sampling sites. Bahía de La Paz is located between 24°06' and 24°47" N, 110°18" and 110°45' W, along the southeastern coast of the Baja California Peninsula, ~180 km north of the mouth of the Gulf of California. In the southern part of the bay, a bloom occurred and subsequently, daily sampling was carried out, from June 20th through July 3rd 2006. Six sites were chosen (Fig. 1): Barco Hundido (BH), El Mogote (EM), Punta Mogote (PM), Marina Palmira (MP), El Caimancito (EC), Punta Prieta (PP), and Balandra (BA).

Sampling, quantification, and identification of phytoplankton. Water samples (n= 19) were collected with Van Dorn bottles at the sea surface. Station EM samples were taken at the surface and 4.5 m. Only at station EC dead fish were collected. One liter of seawater samples from each station were preserved with a Lugol-acetate solution for quantifying (Utermöhl, 1958) and identifying phytoplankton cells. Dominant species were quantified only in samples with high concentrations of chlorophyll a (15.0-23.0 mg m^{-3}) and fucoxanthin (fingerprints of diatoms) (5.8-9.3 mg m^{-3}). Species were identified using an Olympus CH2 light microscope and an inverted Zeiss microscope. Vertical trawls were performed with a 20 µm mesh phytoplankton net at stations EM and PM, where the water was more discolored. An aliquot from these samples was used to identify species with a light microscope (40x) and a scanning electron microscope (see Gárate-Lizárraga *et al.*, 2007). Another part of the net samples was used to analyze DA.

Toxin Analysis. Three concentrated (0.5-1.0 g) phytoplankton samples were collected by net trawling and was used to determine the probable presence of amnesic toxins by mouse bioassay, following the recommendations described by Lawrence *et al.* (1989), based on the mouse bioassay for PSP (Association of Official Analytical Chemists, 1995; Food and Agricultural Organization of the United Nations, 2005). BAL/c strain mice (18–22 g) from the CIBNOR animal laboratory were injected intraperitoneally with 1 mL of each extract. Animals were kept under observation for 48 h, and their clinical signs were recorded.

Bivalves. At station EM, chocolate clam *Megapitaria squalida* (Sowerby, 1835) and the white clam *Dosinia ponderosa* (Gray) 1838 were collected at a depth of 3.0 to 6.0 m for determining concentration of domoic acid for HPLC-UV (Hummert *et al.*, 1997).

Chemical analysis. An aliquot of 30 mL of a sample collected from two net trawls was filtered through a Whatman GF/F glass fiber filter in order to subsequently measure DA concentrations. Filters and bivalves tissue were macerated separately with a methanol/water solution (50:50 v:v) and later quantified by high performance liquid chromatography (HPLC-UV) (model HP 1100, Agilent Technologies, Santa Clara, CA) following the method described by Hummert *et al.* (1997). Identification was achieved by comparing the retention time of the sample with that of the DA standard (National Research Council Canada, Institute for Marine Biosciences, Certified Reference Materials Program, Halifax, NS, Canada). Quantification was performed according to the calibration curve of the standard.

Fish samples. At station EC, photographs and videos of dead or dying fish were taken for identification and clinical signs. Unfortunate only three specimens of dead fish *Mugil cephalus* (Linnaeus, 1758), *Eucinostomu* ssp. and *Pomadasys macracanthus* (Gunther, 1864) were in good enough con-

Table 1 Presence of domoic acid (DA) derived from species of *Pseudo- nitzschia* (P.) diatoms along the Pacific coast of México and associated ecological and socioeconomic impact on higher organisms

Species	Locality/ year	Impact	DA detection (μg g^{-1})	References
Pseudo-nitzschia spp.	Offshore Cabo San Lucas, B.C.S. / 1996	Ecological impact:	Pelicans (37.17)	Ochoa *et al.* (1996), Sierra-Beltrán *et al.* (1997b)
		Pelecanus occidentalis Gmelin, 1789 (150 deaths)	*Scomber japonicus* (Houttuyn , 1872) (142.85)	
P. australis	Northern part of the Gulf of California / 1997	Ecological impact:	3 μg g^{-1} in brain, 3 μg g^{-1} in heart, 6 μg mL^{-1} in blood (*Delphinus delphis*)	SEMARNAT-PROFEPA (1997), Sierra-Beltrán *et al.* (1997a, 1998; 1999), Ochoa *et al.* (1998)
		Gavia immer (Brunnich, 1764) (766 deaths)		
		Balaenoptera. Physalus (Linnaeus, 1758) (4 deaths)		
		Delphinus delphis (Linnaeus, 1758) (55 deaths)		
		Zalophus californianus (Lesson, 1828) (8 deaths)		
P. pseudodelicatissima	Northern part of the Gulf of California/ 2004	Ecological impact:	No data	Sierra-Beltrán *et al.* (2005)
		Sardinops sp. (20 t)		
		Pelecanus occidentales (9 deaths)		
		Delphinus delphis (20 deaths)		
		Zalophus californianus (195 deaths)		
P. fraudulenta, P. pungens, P. pseudodelicatissima-complex	Bahía de La Paz/ 2006	Closed season[2]	*Megapitaria squalida* and *Dosinia ponderosa* (0.06–0.55)	Gárate-Lizárraga *et al.* (2007), Guluarte-Castro & Bañuelos (2007), this study
			Net samples (24–52 ng $filter^{-1}$)	
P. australis	Bahía de Todos Santos B.C./ 2007	No impact	[1]pDA (0.86 μg L^{-1}) (2.73 pg $cell^{-1}$)	García-Mendoza *et al.* (2009)
P. australis	Bahía de Todos Santos B.C/ 2007	No impact	[1]pDA (0.86 μg L^{-1}) anchovy viscera (854 μg g^{-1} viscera)	García-Mendoza *et al.* (2011)
Pseudo-nitzschia sp.	Acapulco, Gro./ 2011	No impact	In seawater (7.6 ng 100 m L^{-1})	Vera-Avila *et al.* (2011)

[1]pDA = particulate domoic acid.
[2]Record mass fish die-off of 106 t caused by *Thalassiosira* sp. and *Chaetoceros* sp.Temporary closed season based on high cell count of *Pseudo-nitzschia* spp.

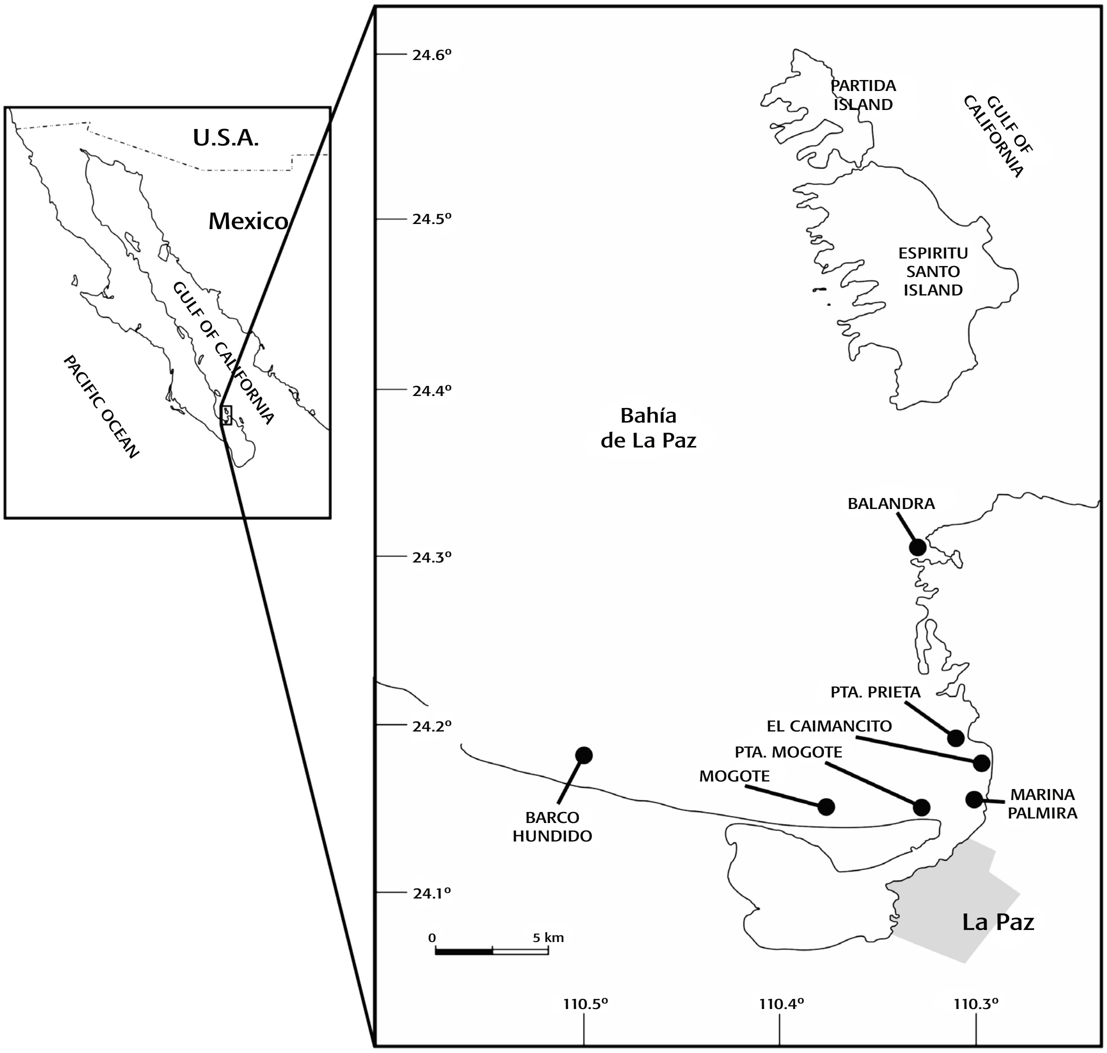


Figure 1. Study area and location of sampling sites (black dots) in the southern part of Bahía de La Paz during a bloom of diatoms (June–July 2006). Barco Hundido (BH), El Mogote (EM), Punta Mogote (PM). Marina Palmira (MP), El Caimancito (EC), Punta Prieta (PP). At EC, dead fish were collected.

servation conditions for necropsy and toxicological analyses by mouse bioassay. Samples were stored at -20 °C before testing and examination At the laboratory, 10 g of viscera and muscle were excised, applying the extraction process for DA described for pellets derived from the phytoplankton tows, except that the viscera and muscle were homogenized at pH 3.0 (Polytron, Kinematica).

Environmental conditions. Temperature (± 0.1 °C) was measured with a bucket thermometer and satellite images of surface sea temperature were downloaded from NOAA CoastWatch program. From the water samples, 500 mL were filtered through a 0.7 µm glass fiber filter (GF/F Whatman, Maidstone, UK) and used to measure NO_2-, NO_3-, NH_4+, PO_4^{3}-, and $Si(OH)_4$ with a continuous flow ion autoanalyzer (Quik Chem Series 8000, Lachat Instruments, Loveland, CO), as described in Strickland and Parsons (1972). Total dissolved inorganic nitrogen (Ntotal) is the sum of the nitrogen compounds (NO_2^-+NO_3-+NH_4^+). The material retained by the GF/F filters was used to identify and quantify photosynthetic pigments by HPLC-UV (model HP 1100, Agilent Technologies, Santa Clara, CA) following the method described by Vidussi *et al.* (1996). Wind data (direction and speed) was obtained from the CIBNOR meteorological station (http://www.cibnor.gob.mx/meteo/ecibmet.html).

RESULTS

Abundance and identification of dominant phytoplankton. The most abundant phytoplankton organism were *Pseudo-nitzschia* spp., *Thalassiosira* sp. and *Chaetoceros* spp. (Figs. 2a-d). The concentration of the dominant species *Pseudo-nitzschia* spp., *Thalassiosira eccentrica* (Ehrenberg) and *Chaetoceros* spp. during the event (from the 1st of June to the 10th of June 2006) at station EM was 0.5 × 10^6 cells L^{-1} and 1.1 × 10^6 cells L^{-1}, 9.65 x 10^5 cells L^{-1}, respectively. During intensive sampling at this site only *Pseudo-nitzschia* and *Thalassiosira eccentrica* were counted. The maximum abundance was on the 21st and 22nd of June 2006. On July 15th of 2006, the concentrations declined for the two species (Table 2). Dominant species of *Pseudo-nitzschia*, in descending order, were *P. fraudulenta*, *P. pungens* (Figs. 2e-f), and the *P. pseudode-*

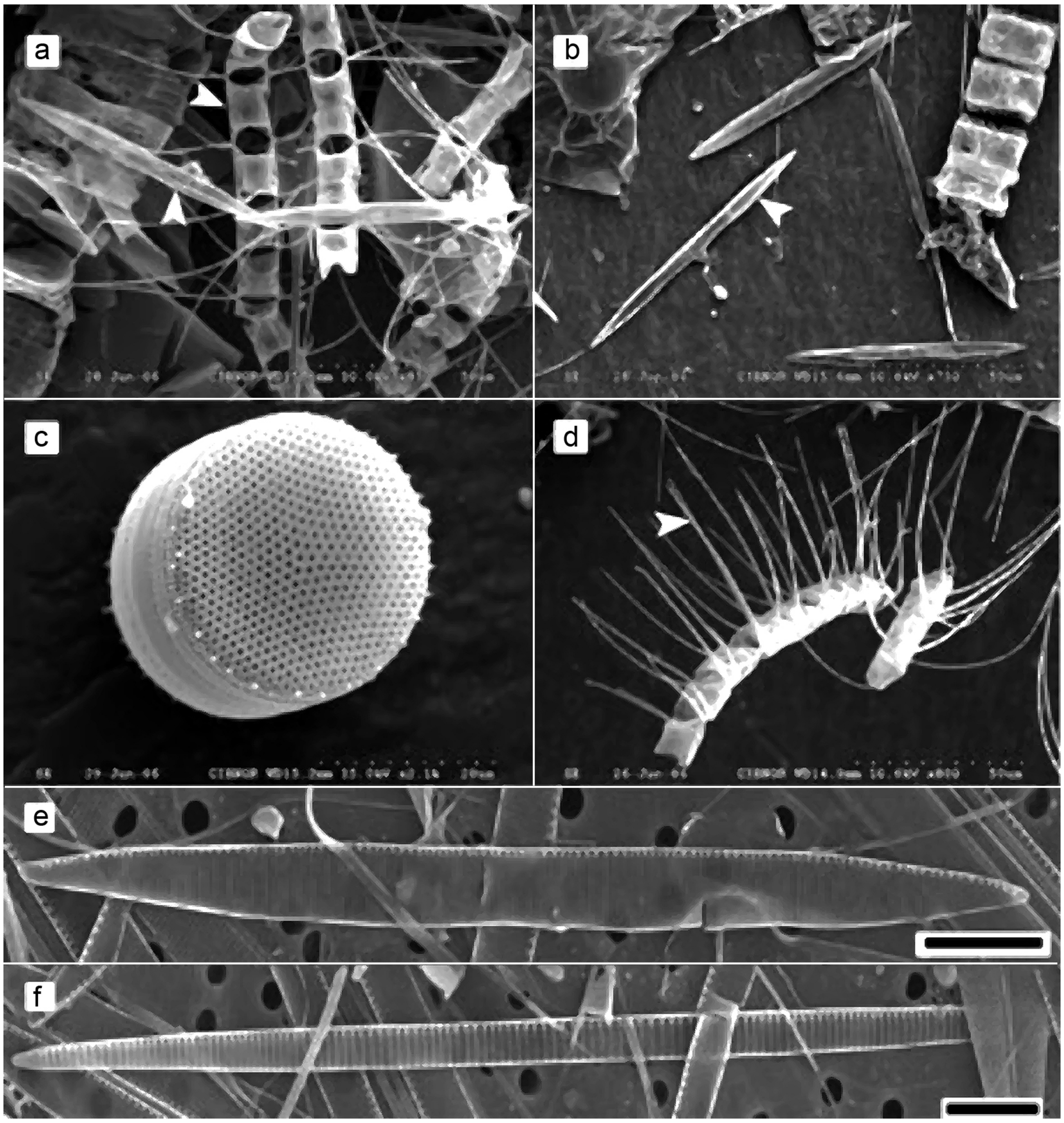


Figure 2a-f. Taxa found during a bloom of diatoms (June–July 2006) in Bahía de La Paz, B.C.S. images taken with SEM. a) *Chaetoceros* sp., *Rhizosolenia* sp., and *Pseudo-nitzschia* sp., b) *Pseudo-nitzschia* sp. Arrowed, c) .*Thalassiosira eccentrica*, d) *Chaetoceros curvisetus*; note the setae (arrowed), e) *Pseudo-nitzschia fraudulenta*, f) *Pseudo-nitzschia pungens* (e, f Images courtesy of Jim Ehrman, Digital Microscopy Facility, Mount Allison University, Sackville, New Brunswick, Canada) and previously published in *Harmful Algae News* 33, 2007). Scale bar = 10 µm.

Table 2. Abundance of cells (Cells L^{-1}) during a diatom bloom in the southern coast of Bahía de La Paz, in June and July 2006

Year 2006	*Pseudo-nitzschia* spp.	*Thalassiosira eccentrica*
	Cells L^{-1}	Cells L^{-1}
1/June	0.5×10^6	1.1×10^6
21/June	2.3646×10^6	1.882×10^6
22/June	2.3×10^6	1.9×10^6
15/July	0.27×10^6	0.038×10^6

licatissima-complex. The chlorophyll a and fucoxanthin maximum concentrations were 15.0 and 9.3 µg L^{-1}, respectively, on June 21st 2006, 23.0 and 6.0 µg L^{-1} on June 22rd 2006, 12.4 and 6.3 µg L–1 on June 23rd 2006, and declined thereafter. Correlation between chlorophyll a and fucoxanthin during this event was positive and significant (r^2 = 0.78, n = 19, α = 0.05).

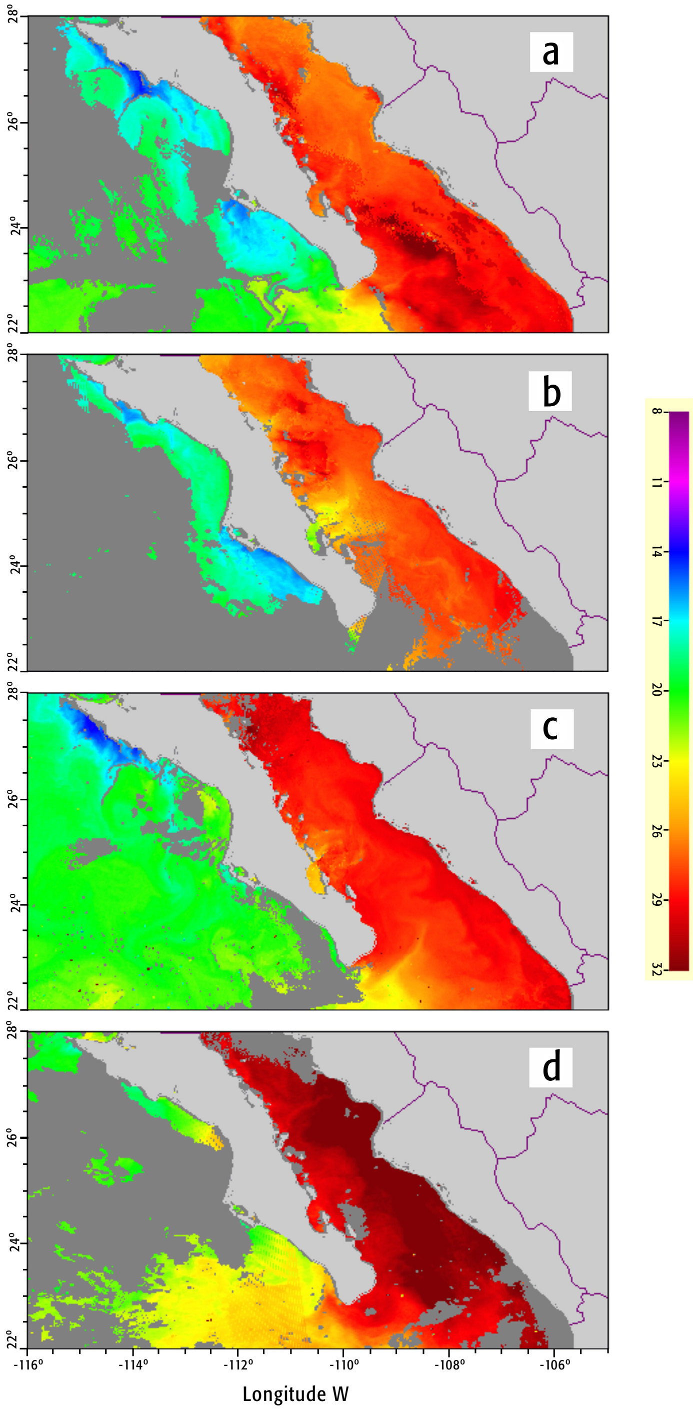
Toxin analysis. Preliminary toxicological analysis by mouse bioassay showed that DA was present in the concentrated net phytoplankton samples. Clinical signs in mice were hypoactivity, difficult breathing, and paralysis of hindquarters. Three mice scratched themselves (ears and shoulders), seven mice had mild to severe shivering, lack of motor coordination and death came within 24 h with the equivalent of >40 µg DA. Symptoms match those described for amnesic shellfish toxins (FAO, 2005). Cyanosis in extremities (mainly in the tail) was also present. Concentrations of DA quantified with HPLC-UV varied from 24.0–52.0 ng $filter^{-1}$ from plankton tows and 0.55 µg g^{-1} of tissue in the chocolate clam *M. squalida* and 0.06 µg g^{-1} of tissue in the white clam *D. ponderosa* (Table 1). These concentrations are below the Mexican health standard NOM 242-SSA1-2009 (DOF, 2010), which stipulates that 20 mg kg^{-1} DA have to be present in tissue before a fishery can be closed.

Fish examinations. Unfortunately by the advanced decomposition state of the fish samples, only three individuals were in good enough conditions to be analyzed. Post-mortem examination of the fish showed congestion and mucus in the gills. The gills had epithelial damage probably caused by *Chaetoceros* spp. setae and by a large accumulation of *Thalassiosira eccentrica*, which caused asphyxia and death. No toxicity (no clinical signs were recorded) was detected in fish viscera and muscle by mouse bioassay.

Environmental conditions. Satellite images of Bahía de La Paz had SSTs of ~27.0 °C on June 1st 2006 (Fig. 3a); seven days later, the temperature declined to 19.0–20.0 °C (Fig. 3b); on June 14th 2006, temperature increased by 5.0 °C to 24.0–25.0 °C (Fig. 3c). On June 28th, temperatures were 28.0–29.0 °C (Fig. 3d). Temperatures recorded at stations in the southern part of the bay during the diatom bloom were 19.0 °C on June 10th. After June 20th, temperature averages were ~around 25.0±1.5 °C and at the end of the month, 27.0–28.0 °C.

A seasonal wind, confined to the area around La Paz, comes from the south, starting in early evening and lasting through midmorning (the "Coromuel"). Daytime winds during the day are typical sea breezes from the northeast. Clearly at the beginning of June, evening winds were relatively high, with a velocity from 3.5 to 5.0 ms^{-1}. The diatom bloom was detected during this period.

Concentrations (in µM) of NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}, and $Si(OH)_4$ ranged from 0.1–0.183, 0.1–2.71, not detectable to 0.5, 0.073–1.44, and 2.94–28.70, respectively. N_{total} fluctuated between 0.70 and 3.31 µM during the sampling period. Between June 20th–23rd, two peaks of 3.31 and 2.69 µM were recorded at station EM (Fig. 4). Figure 4 also shows that N:P ratios were relatively low (0.65 to 6.0).These values are low, compared with the Redfield N:P ratio. Some ratios of $Si(OH)_4$:N (4–28) and $Si(OH)_4$:P (5–56) during the study were higher than the Si:P Redfield ratio (16:16:1; Si:N:P) (Fig. 5).



Figures 3a-d. Satellite images of surface temperatures during a diatom bloom in June–July 2006 in Bahía de La Paz. Surface warm water at ~27.0 °C (June 1, 2006). a) were recorded seven days after; temperature decreased to 19.0 and 20.0 °C, b); on 14 June 2006, an increase of 5.0 °C (24.0–25.0 °C), c) is recorded, end-ing the month on (June 28, 2006) with 28.0–29.0 °C, d). Temperature scale (°C) at right. (Source: National Oceanic and Atmospheric Administration; CoastWatch program)

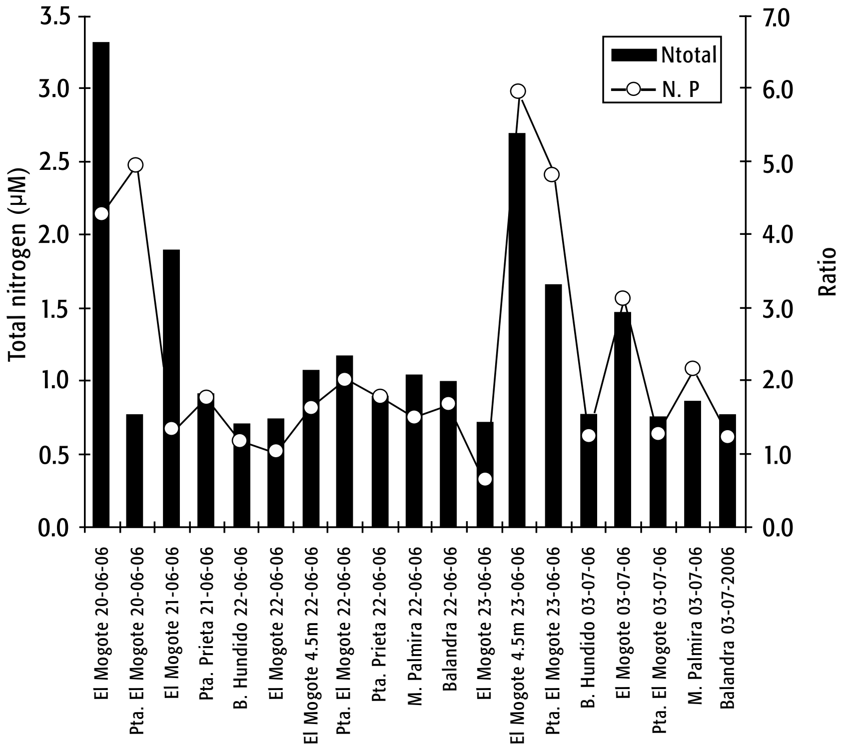


Figure 4. Variation in total nitrogen concentration (μM) and its respective N:P ratio during the diatom bloom (June–July 2006) in Bahía de La Paz. Maxima were recorded at the El Mogote station. The ratios were low (< 6.0), less than the Redfield ratio (16N:1P).

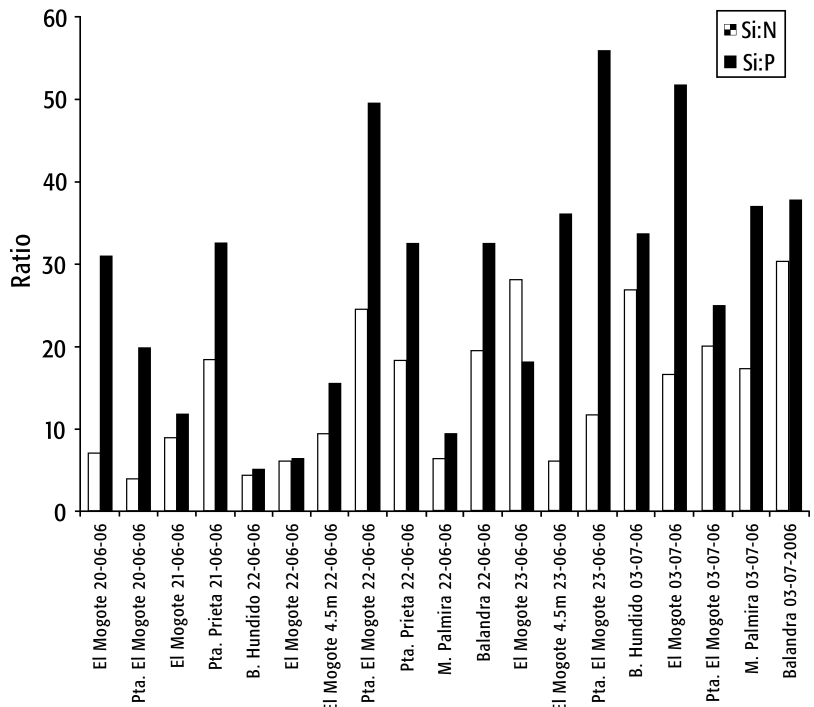


Figure 5. Ratio of Si:N:P during a diatom bloom in Bahía de La Paz, which was higher than the Redfield ratio (16Si:16N:1P). At all stations, there was a domain of silicic acid with big peaks for the El Mogote station.

DISCUSSION

The diatom bloom in Bahía de La Paz was suspected of killing 106 t of fish (*Mugil cephalus*, *Eucinostomus* sp, *Pomadasys macracanthus*), *Scomber japonicum* (Houttuyn, 1872), *Sardinops sagax* (Jenyns, 1842), and *Canthigaster punctatissima* (Günther, 1870) among other species, mostly by *Thalassiosira*, but involving *Pseudo-nitzschia*, with a biomass of 10,800 to 756,800 cells L^{-1} (see also Gárate-Lizárraga *et al.*, 2007; Guluarte-Castro & Bañuelos, 2007). From our observations, fish mortality resulted from obstruction and injuries in gills, caused by *Thalassiosira eccentrica* and *Chaetoceros* sp. respectively. Some species of the genus *Thalassiosira* form noxious blooms and produced mucilage, however *T. eccentrica* did not produce mucilage and there are no records of noxius blooms of this species. Moreoever, as far as the authors are aware this is the first finding of massive proliferation and obstruction of *Thalassiosira eccentrica* in the gills of living wild fish. Observations of gills also showed that long setae of *Chaetoceros curvisetus* Cleve and *Chaetoceros* spp. with spine-like lateral extensions probably worsened injuries; the fish probable response is to produce mucus in the respiratory epithelium, leading to suffocation (Albright *et al.*, 1993; Glibert *et al.*, 2005; Smayda, 2006). Spines, directed toward the seta tip, work as hooks, preventing them from detaching easily, thereby injuring gills tissues.

The high abundance of *Pseudo-nitzschia* genus and detection of DA, suggests that some of the species are the source of DA. Trainer *et al.* (2012) report that some species of this genus are toxic, nevertheless other species produce low levels of toxins (Bates, 1998), such as *P. fraudulenta*, *P. pungens* Hasle, and *P. pseudodelicatissima* (Hasle) Hasle. These species were identified in this work, with the former dominating in abundance. The low production of DA of these species could explain the low levels of DA detected in the phytoplankton net and in the bivalves samples during this event.

Temperatures recorded during this investigation (thermal satellite images) were variable. During this period there were wide temperature changes; *Thalassiosira* and *Pseudo-nitzschia* were most abundant. When abundance declined on June 28th, the thermal range was 27.0–28.0 °C. Aguirre-Gómez *et al.* (1999) reported that sudden changes in temperature can lead to the rapid growth of toxic species in particular, since many of these are capable of enduring variations with the added benefit of not facing competition. During this period of thermal change, we suspect that the conditions for this bloom of diatoms were met, including sufficient nutrients and low competition for nutrients.

Pseudo-nitzschia species tolerate a wide temperature range from 2.0 to 28.0 °C (Hallegraeff,1993), and some species bloom at very narrow temperature ranges, such as *P. australis* that has been found to bloom at 14.0 °C in the northern Baja California Peninsula (García-Mendoza *et al.*, 2009). During this study temperature varied over a wide range (19.0–27.0 °C) when *Pseudo-nitzschia* spp. bloom occurred in Bahía de La Paz.

Pigment signatures of different phytoplankton groups have been a useful tool for identification and detecting algae blooms. We found high concentrations of chlorophyll a and fucoxanthin fingerprint in the diatoms (Jeffrey & Vesk, 1997) and these peaked at the same time at the peak abundance of the three prominent genera. While correlation between chlorophyll a and fucoxanthin was high and significant (r = 0.78).

Wind-driven upwelling events can produce appropriate conditions for proliferation of different species of *Pseudo-nitzschia* (Gárate-Lizárraga *et al.*, 2007; García-Mendoza *et al.*, 2009). When southeastern winds were dominant (> 3.5 ms^{-1}), it was evident that species of *Pseudo-nitzschia*, *Thalassiosira eccentrica*, and *Chaetoceros* spp. proliferated. A similar situation was recorded during a bloom of *Chaetoceros debilis* (Cleve 1894) in June 2003 in the Bahía de La Paz (López-Cortés *et al.*, 2006).

In Bahía de La Paz, among other factors, winds promote mixing of the water column (Obeso-Nieblas *et al.*, 2008), bringing nutrients to the surface. Samples had a range of concentrations of NO_3^- (0.1 to 2.72 µM); PO_4^{3-} (0.073 to 1.44 µM) and Si $(OH)_4$ (2.94 to 28.70 µM). According to Egge and Aksnes (1992), diatoms are dominant when Si $(OH)_4$ is >2.0 µM. Although N:P ratios were low (0.65–6.0), some Si $(OH)_4$:N (4.0–28.0) and Si $(OH)_4$:P (5.0–56.0) ratios were high, compared with Redfield's stoichiometric (16:16:1; Si:N:P), under these conditions, the diatom bloom is likely to have been generated by the higher concentrations of NO_3^- and Si $(OH)_4$, as well as temperature conditions.

Anderson *et al.* (2006) report that, when the $Si(OH_4)$:NO_3^- ratio is higher than the Redfield ratio, particulate DA and cellular DA of phytoplankton were low; also, that the reduction of the $Si(OH_4)$:NO_3^- ratio increased DA content in cells. From their observations, we conclude that the high concentrations of silicic acid in Bahía de La Paz (15.4±9.0 µM) and the high $Si(OH)_4$:DIN ratio was reflected in the low concentrations of DA in our net samples (24-52 ng per filter^{-1}). Although Smayda (2006) concluded that regulation of DA synthesis is complex and affected by many environmental factors.

The main vectors of DA at other trophic levels are filter-feeding organisms, such as bivalves and some fish. Omnivorous and planktivorous fish can accumulate DA in viscera and body tissues (Lefebvre *et al.*, 2002, 2012), even if they do not present toxicological signs during blooms of normally toxic *Pseudo-nitzschia* species. In our mouse bioassays of viscera and body tissue of dead omnivorous fish DA was not detected, probably because ingested phytoplankton was low.

In conclusion, this study found that sudden changes in seawater temperature with high concentrations of nitrogen and silicic acid, probably played a role in triggering blooms of noxious and toxic diatoms. Results suggest that *Thalassiosira eccentrica* and *Chaetoceros* spp. were responsible for the fish die-off. Although cell counts of *Pseudo-nitzschia* were high, toxicity levels were low and not enough to cause accumulation of DA in fish. DA was also low in the tested clams. Future work isolating and cultivating of these phytoplankton will be necessary in order to better quantify toxicity under different environmental conditions.

ACKNOWLEDGEMENTS

We thank Ira Fogel of CIBNOR for English editing. We thank Gerardo R. Hernández García for the figures edition. Ariel A. Cruz Villacorta (CIBNOR), Irena Kaczmarska, and James M. Ehrman of the University of Mount Allison, Sackville, NB, Canada for SEM analysis, Amaury Cordero Tapia, Ivan Murillo (CIBNOR), Alejandra Heredia Tapia at Investigación para la Conservación y el Desarrollo A.C. (INCODE), and Iliana Tejeda (CIBNOR) for technical assistance. Research was funded by Consejo Nacional de Ciencia y Tecnología (CONACYT grant 61226 and SEP grant 20130942) and CIBNOR projects PC 0.11 and PC 0.12. Comments and observations on the manuscript of an anonymous reviewer. Ismael Gárate-Lizárraga and Christine J. Band-Schmidt are fellows of COFAA and EDI of the Instituto Politécnico Nacional. Thanks also to native English speaker editor Miguel Cordoba Matson, who provided an invaluable service in polishing up the English in the final version.

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Recibido: 26 de febrero de 2014.

Aceptado: 25 de abril de 2014.