

Domoic acid in a marine pelagic food web: Exposure of southern right whales *Eubalaena australis* to domoic acid on the Península Valdés calving ground, Argentina



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ABSTRACT

The gulfs that surround Península Valdés (PV), Golfo Nuevo and Golfo San José in Argentina, are important calving grounds for the southern right whale *Eubalaena australis*. However, high calf mortality events in recent years could be associated with phycotoxin exposure. The present study evaluated the transfer of domoic acid (DA) from *Pseudo-nitzschia* spp., potential producers of DA, to living and dead right whales via zooplanktonic vectors, while the whales are on their calving ground at PV. Phytoplankton and mesozooplankton (primary prey of the right whales at PV and potential grazers of *Pseudo-nitzschia* cells) were collected during the 2015 whale season and analyzed for species composition and abundance. DA was measured in plankton and fecal whale samples (collected during whale seasons 2013, 2014 and 2015) using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The genus *Pseudo-nitzschia* was present in both gulfs with abundances ranging from 4.4×10^2 and 4.56×10^5 cell l^{-1} . *Pseudo-nitzschia australis* had the highest abundance with up to 4.56×10^5 cell l^{-1} . DA in phytoplankton was generally low, with the exception of samples collected during a *P. australis* bloom. No clear correlation was found between DA in phytoplankton and mesozooplankton samples. The predominance of copepods in mesozooplankton samples indicates that they were the primary vector for the transfer of DA from *Pseudo-nitzschia* spp. to higher trophic levels. High levels of DA were detected in four whale fecal samples (ranging from 0.30 to 710 $\mu g g^{-1}$ dry weight of fecal sample or from 0.05 and 113.6 $\mu g g^{-1}$ wet weight assuming a mean water content of 84%). The maximum level of DA detected in fecal samples (710 $\mu g DA g^{-1}$ dry weight of fecal sample) is the highest reported in southern right whales to date. The current findings demonstrate for the first time that southern right whales, *E. australis*, are exposed to DA via copepods as vectors during their calving season in the gulfs of PV.

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1. Introduction

Phycotoxins are secondary metabolites produced by certain species of phytoplanktonic microalgae. These toxic microalgae are consumed and their toxins bioaccumulated in organisms such as

fish, molluscs, krill, copepods and other pelagic invertebrates (Carreto et al., 1986; Shumway, 1990; Turrieff et al., 1995; Bagøien et al., 1996; Teegarden and Cembella, 1996; Hwang and Tsai, 1999; Lincoln et al., 2001; Tester et al., 2001; Bargu et al., 2002; Lefebvre et al., 2002, 2012; Costa et al., 2004) which act as transmission vectors. As a result, adverse effects of phycotoxins have been recorded in top predators such as sea birds (Fritz et al., 1992; Work et al., 1993; Beltran et al., 1997; Gayoso and Fulco, 2006), sea lions (Lefebvre et al., 1999, 2010; Scholin et al., 2000; Buckmaster et al., 2014), whales (Geraci et al., 1989; Fire et al., 2010; Lefebvre et al., 2016), dolphins (De La Riva et al., 2009; Lefebvre et al., 2016) and

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humans (Carreto et al., 1981; Perl et al., 1990; Mons et al., 1998) producing different types of gastrointestinal and neurological damage and even death.

Domoic acid (DA) is a potent water-soluble neurotoxin naturally produced by several species of the diatom genus *Pseudo-nitzschia*. This neurotoxin has been causative of many lethal incidents of marine mammals due to its transfer and accumulation through the trophic web via planktivorous vectors (Gulland, 1999; Lefebvre et al., 1999; Kreuder et al., 2005; Fire et al., 2010; De La Riva et al., 2009). Chronic exposure of sea lions to DA also produces sublethal effects, causing degenerative heart disease, chronic epileptic syndromes and reproductive failures through abortions, death in the uterus and premature parturition (Scholin et al., 2000; Brodie et al., 2006; Goldstein et al., 2009; Zabka et al., 2009). However, little is known about the effects of DA in baleen whales, such as right whales. It has been suggested that concentrations of DA that are not lethal in humans and monkeys, can cause symptoms of intoxication in whales that could lead to their death through chronic exposure in multiple feeding events (Fire et al., 2010).

Studies conducted in the northern hemisphere have demonstrated that North Atlantic right whales (*Eubalaena glacialis*) are exposed to DA mainly through the calanoid copepod *Calanus finmarchicus*, a primary prey in their spring and summer feeding areas along the northeastern US and eastern Canadian coasts (Leandro et al., 2010a; Doucette et al., 2012). Likewise, DA was detected in fecal samples of blue whales (*Balaenoptera musculus*) and humpback whales (*Megaptera novaeangliae*) during a toxic bloom of *P. australis* in Monterey Bay, California (Lefebvre et al., 2002). Similarly, trace levels of DA were detected in tissues (Rowntree et al., 2013) and blood samples (Wilson et al., 2015) collected from dead southern right whales (*Eubalaena australis*) that stranded at Península Valdés (PV). In addition, recent studies have documented the presence of frustule fragments of *Pseudo-nitzschia* spp. potentially producing DA as well as microcrustacean remains, mainly copepodite 5 mandibular gnatobases of *Calanus australis* (a common species in Argentine Sea), in fecal samples of living and dead whales from the Nuevo (GN) and San José gulfs

(GSJ) respectively (D'Agostino et al., 2015, 2016). These findings indicate that southern right whales could be exposed to DA while feeding in the area and copepods could have act as a primary vector of this neurotoxin. However, the transfer of DA from the toxic phytoplankton to southern right whales through zooplankton vectors has not been studied previously.

The gulfs of PV, GSJ which opens to the north and GN which opens to the south (Fig. 1) are important calving grounds for the southern right whale population in the western South Atlantic Ocean. The whales arrive at PV in late austral fall and remain in the area during the winter and spring months where they reproduce and give birth to their calves. In spring, as denser zooplankton patches follow the spring phytoplankton blooms, adults and juveniles begin to filter zooplankton (mainly copepods) by skimming-feeding at the sea surface or by diving to greater depths (Sironi, 2004; Menéndez et al., 2007; Hoffmeyer et al., 2010; D'Agostino et al., 2016). Previous studies confirm that spring phytoplankton blooms in both gulfs are dominated by diatoms with *Pseudo-nitzschia*, in some cases being the most abundant genus (Gayoso, 2001; Sastre et al., 2007; Cadaillón, 2012). Therefore, the feeding of southern right whales in GN and GSJ show a certain degree of temporal overlap with the potentially toxic *Pseudo-nitzschia* blooms occurring in the area during spring.

In the PV calving ground, the population of southern right whales has been increasing at a rate of between 4.57 and 6.2% annually during the last 15 years (Crespo et al., 2014). However, in recent years the population experienced a series of high mortality events with 753 dead whales recorded from 2003 to 2016, 91% being calves (report Southern Right Whale Health Monitoring Program [SRWHMP]; 2003–2016). The effect of phycotoxins on the health of the southern right whale was suggested as a possible explanation for the high mortality events (Rowntree et al., 2013; Wilson et al., 2015). In this region, the transfer of DA from the phytoplankton to zooplankton has been previously documented (Cadaillón, 2012). However, knowledge about the levels of DA to which whales are exposed while at PV area and the potential vectors of this potent neurotoxin is still incipient. In this context,

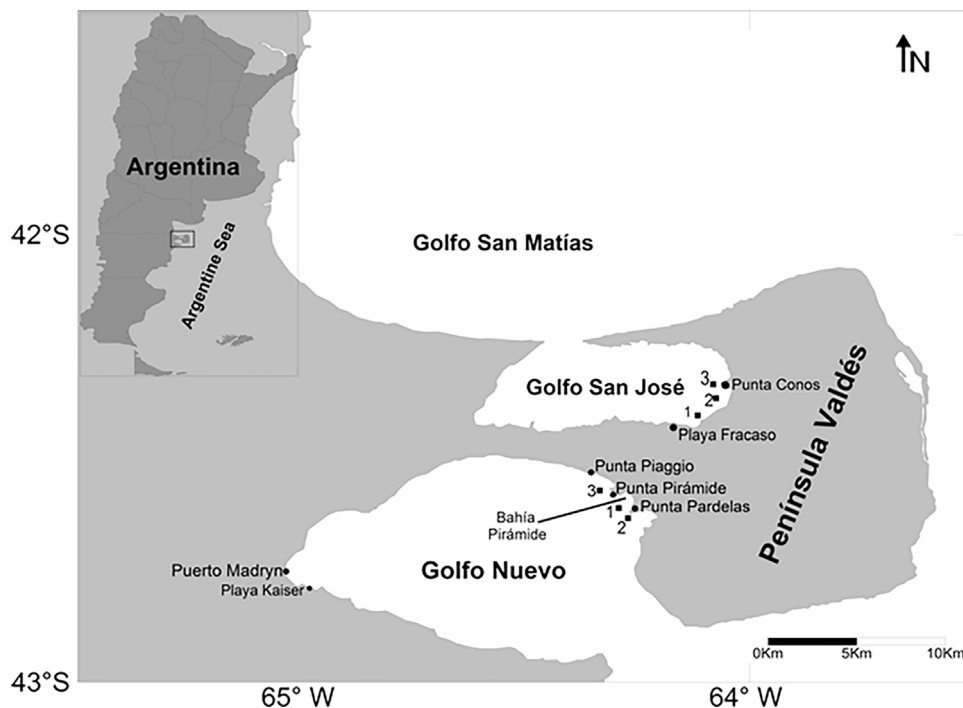


Fig. 1. Study area showing the location of sampling sites in Golfo Nuevo and Golfo San José, Península Valdés, Argentina.

the main objectives of this study were to (1) identify the *Pseudo-nitzschia* spp. that are potentially producers of DA, (2) identify the probable vectors carrying this toxin between trophic levels at PV, and (3) quantify the levels of DA in phyto- and mesozooplankton and fecal samples of live and dead *E. australis* at PV.

2. Materials and methods

2.1. Sample collection

Phyto- and mesozooplankton and water samples were collected at approximately monthly at three stations in both GN and GSJ (Fig. 1) during the 2015 whale season (June–December in GN and July–November in GSJ).

Water samples were collected at 3 m and 10 m depth using a 2.5 l Van Dorn bottle. One liter samples at each depth was mixed and 500 ml were taken for the analysis of chlorophyll *a* (chl *a*) and phaeopigments, and a 250 ml aliquot was fixed with Lugol's solution at a final composition of 0.4 ml 100 ml⁻¹ (Ferrario et al., 1995) for quantitative phytoplankton analyses. Phytoplankton samples for both qualitative and phycotoxin analysis were collected using oblique net tows from 20 m depth to the surface using a 20 µm mesh net while traveling over a 7 min period at a speed of 2 knots. Samples were put in 500 ml plastic bottles. Mesozooplankton samples (mainly southern right whale prey and potential predators of *Pseudo-nitzschia* spp.) for quantitative taxonomic and phycotoxin analysis were collected using 335 µm mesh net equipped with a flow meter (General Oceanics Model 2030R) on mouth of the net. Net tows were performed obliquely from 30 m to surface for 7 min period at a speed of 2 knots and the samples were put in 250 ml plastic flasks. Net tow samples and mesozooplankton samples for taxonomic analysis were fixed with 4% formaldehyde, whereas samples for DA extraction were placed in portable coolers and immediately processed after return to the laboratory (see below). The mesozooplankton samples collected in GN in October could not be used in the analyses of DA, because of an intense *P. australis* bloom in October in the gulf and long chains of *P. australis* found in the respective mesozooplankton samples made the attribution of DA to mesozooplankton impossible.

A total of 14 fecal samples from live and stranded whales were collected in GN from 2013 through the 2014/2015 whale seasons (see Table 6 and Fig. 1 for details). Fecal samples from live whales were collected using a 125 µm mesh net. Samples of stranded individuals were directly collected from the intestine from individuals during necropsy. Both types of fecal samples were placed in plastic flasks in portable coolers and immediately frozen (–20 °C).

2.2. Laboratory procedures

2.2.1. Pigment extraction

For chl *a* determination the seawater samples were filtered through GF/F filters (25 mm and 0.7 µm in pore size) and the filters were stored at –20 °C. Chlorophyll *a* was extracted with 5 ml 90% acetone in darkness over a 24 h period at 4 °C. Extracts were centrifuged at 1,680 ×g for 5 min. Chlorophyll *a* and phaeopigments were quantified using a spectrofluorometer (Shimadzu RF-5301PC) at λEx/λEm: 430/671 nm and concentrations were estimated according to Holm-Hansen et al. (1965) equations. The chl *a* values were corrected for phaeopigments by acidification with HCl (0.1 N).

2.2.2. Composition, abundance and domoic acid extraction of plankton samples

Phytoplankton samples were identified and enumerated in the Laboratorio de Hidrobiología of the Universidad Nacional de la

Patagonia “San Juan Bosco”. Net tow samples for *Pseudo-nitzschia* spp. identification were examined with a phase contrast microscope (Olympus CX31), whereas *Pseudo-nitzschia* cells in bottle samples were enumerated with an inverted microscope (Leica DMIL) according to the procedures described by Utermöhl (1958). Cleaning of *Pseudo-nitzschia* spp. frustules was performed using the method of Hasle and Fryxell (1970). Naphrax mounted slides were examined with an optical microscope equipped with phase contrast and selected samples were examined by SEM (Jeol JSM-6360 LV) for species identification. Phytoplankton abundances were expressed as cells per liter (cells l⁻¹).

Phytoplankton samples for DA extraction were filtered through GF/F filters (25 mm and 0.7 µm in pore size) and frozen (–20 °C) until analysis. Filters were transferred into FastPrep tubes containing 0.9 g of lysing matrix D (Thermo Savant, Illkirch, France) and 0.5 ml methanol were added. The samples were homogenized by reciprocal shaking at maximum speed (6.5 m s⁻¹) for 45 s in a Bio101 FastPrep instrument (Thermo Savant, Illkirch, France). After homogenization, samples were centrifuged (Eppendorf 5415 R, Hamburg, Germany) at 16,100 ×g at 4 °C for 15 min. Supernatants were transferred to spin-filters (0.45 µm pore-size, Millipore Ultrafree, Eschborn, Germany) and centrifuged at 800 ×g at 4 °C for 30 s. The filtrates were transferred to autosampler vials and analyzed by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS).

Mesozooplankton samples for species identification and enumeration were examined under a stereo microscope Nikon SMZ645. Mesozooplanktonic organisms and other potential prey of whales which could possibly consume *Pseudo-nitzschia* spp. were identified to the lowest possible taxonomic level using appropriate literature (Boltovskoy, 1981, 1999; Kirkwood, 1982; Cervellini, 1988; Harris et al., 2000; Young, 2002). According to the abundance of organisms observed *a priori* in the samples, total or aliquot counts were applied. In the latter case, samples were subsampled (1/10) (Boltovskoy, 1981) and all individuals were then identified and counted. Mesozooplankton abundances were expressed as number of individuals per m³ (ind m⁻³).

Mesozooplankton samples for DA extraction were filtered through GF/F filters (47 mm and 0.7 µm in pore size) and frozen (–20 °C) until analysis. Filters were cut in half and transferred into FastPrep tubes containing 0.9 g lysing matrix D (Thermo Savant, Illkirch, France) and 1 ml methanol was added. Samples were homogenized as described above. Filtrates of the same samples were combined and dried in a gentle nitrogen stream and reconstituted with methanol to a final volume of 0.5 ml. Subsequently, the extracts were filtered through centrifugation filters (0.45 µm pore-size, Millipore Ultrafree, Eschborn, Germany) at 16,100 ×g at 4 °C for 5 min at 4 °C. Samples were transferred into an autosampler vial for LC–MS/MS analyses.

2.2.3. Domoic acid extraction of whale fecal samples

Right whale feces were lyophilized (OPERON FDU-8606, Korea) to remove the water and were placed at –20 °C until DA extraction. Fecal samples were thawed at room temperature. Aliquots of all fecal samples (ca. 10 mg) were transferred into FastPrep tubes containing 0.9 g lysing matrix D (Thermo Savant, Illkirch, France) and 1 ml methanol were added. The samples were homogenized by reciprocal shaking at maximum speed (6.5 m s⁻¹) for 45 s in a Bio101 FastPrep instrument (Thermo Savant, Illkirch, France). After homogenization, samples were centrifuged (Eppendorf 5415 R, Hamburg, Germany) at 16,100 ×g at 4 °C for 10 min. The supernatant was transferred to a spin-filter (0.45 µm pore-size; Millipore Ultrafree, Eschborn, Germany) and centrifuged at 16,100 ×g at 4 °C for 5 min. The filtrate was transferred to an autosampler vial; the final volume was adjusted to 1 ml with methanol and stored at 4 °C until use. The residues were re-

extracted once as described above. Accordingly, two extracts were obtained for each fecal sample and they were analyzed by LC–MS–MS/MS separately. One sample (BFA9, see details in Table 6) had extremely high values of DA outside the calibration range. Therefore this sample was divided in three aliquots and each of them was consecutively extracted 20 times ($n=60$) and DA was determined in each individual extract. DA values of the other samples (BFA11, BFA12, BFA13) with high DA levels, which were only extracted twice, were extrapolated by adding the proportion of 3rd-20^{iest} extraction and total extracted DA (1st to 20^{iest} extraction) of sample BFA9.

2.2.4. Domoic acid determination

Mass spectral experiments were performed on an ABI-SCIEX-4000 triple quadrupole-linear ion trap hybrid mass spectrometer coupled to an Agilent model 1100 LC. The LC equipment included a solvent reservoir, in-line degasser (G1379A), binary pump (G1311A), refrigerated autosampler (G1329A/G1330B), and temperature-controlled column oven (G1316A).

The analytical column (50×2.0 mm) was packed with C8 phase. The analytical column (50×2 mm) was packed with $3 \mu\text{m}$ Hypersil BDS 120 Å (Phenomenex, Aschaffenburg, Germany) and maintained at 25°C . The flow rate was 0.3 ml min^{-1} and gradient elution was performed with two eluants, where eluant A was 2 mM ammonium formate and 50 mM formic acid in water and B was 2 mM ammonium formate and 50 mM formic acid in methanol/water (95:5 v/v). The gradient was as follows: 3 min isocratic with 100% A, then linear gradient until 10 min to 50% A, then return to initial condition until 11 min followed by 9 min column equilibration at 100% A. Total run time: 20 min $10 \mu\text{l}$ of sample were injected.

Selected reaction monitoring (SRM) experiments were carried out in positive ion mode by selecting the following transitions for DA (precursor ion > fragment ion): m/z 312 > 266 (quantifier) and m/z 312 > 161 (qualifier). Dwell times of 250 ms for each transition and the following source parameters were used: curtain gas: 10 psi, temperature: 600°C , ion-spray voltage: 5500 V, nebulizer gas: 60 psi and auxiliary gas: 60 psi, interface heater: on, Collision gas (CAD): medium, entrance potential: 10 V, collision energy and collision cell exit potential 30 V and 12 V, respectively. Sample concentrations were calculated from a calibration curve with external standards. A standard solution of DA was purchased from the Certified Reference Material (CRM) Programme of the Institute of Marine Biosciences, National Research Council, Halifax, NS, Canada.

The DA concentrations of plankton samples were expressed as nanograms per net tow (ng NT^{-1}) whereas the levels of DA from fecal samples were expressed as micrograms per gram of dry fecal sample ($\mu\text{g g}^{-1}$). Water content in the fecal samples was estimated by weight loss through 24 h of lyophilisation.

3. Results

3.1. Chlorophyll *a* (chl *a*) and phaeopigments

In GN the highest chl *a* values were recorded on 09-Oct-2015 at all three sampling stations (Table 1) which were associated with *P. australis* blooms (Table 2). In GSJ the highest chl *a* values were generally recorded in spring (Table 1), however no blooms of *Pseudo-nitzschia* spp. were recorded in spring in this gulf (Table 2). Phaeopigment values in both GN and GSJ showed similar patterns to that of Chl *a* (Table 1). In GN the phaeopigment values were highest during the spring bloom of *P. australis* (09-Oct-2015) and in GSJ higher values of this pigment were detected during spring (September, October and November; Table 1).

Table 1

Chlorophyll *a* (chl *a*) and phaeopigment values recorded during the 2015 whale season in Golfo Nuevo (GN) and Golfo San José (GSJ). – = no data.

| Sample date | Sample location | Chlorophyll <i>a</i> [$\mu\text{g l}^{-1}$] | | | Phaeopigments [$\mu\text{g l}^{-1}$] | | |
|-------------|-----------------|--|-------|-------|---|-------|-------|
| | | St. 1 | St. 2 | St. 3 | St. 1 | St. 2 | St. 3 |
| 08-Jun-15 | GN | 0.53 | 0.47 | 0.55 | 0.28 | 0.31 | 0.33 |
| 29-Jul-15 | GN | 0.46 | – | – | 0.26 | – | – |
| 04-Sep-15 | GN | 1.04 | 0.92 | – | 0.47 | 0.48 | – |
| 09-Oct-15 | GN | 4.44 | 6.52 | 4.45 | 0.85 | 1.22 | 1.22 |
| 12-Nov-15 | GN | 0.79 | 0.83 | 0.92 | 0.50 | 0.50 | 0.47 |
| 21-Dec-15 | GN | 0.57 | 1.34 | 0.54 | 0.47 | 0.92 | 0.58 |
| 03-Jul-15 | GSJ | 0.62 | 0.74 | 0.73 | 0.87 | 0.58 | 0.53 |
| 10-Aug-15 | GSJ | 1.58 | 0.85 | 0.74 | 0.65 | 0.48 | 0.45 |
| 24-Sep-15 | GSJ | 3.05 | 2.53 | 0.94 | 1.18 | 0.85 | 0.47 |
| 27-Oct-15 | GSJ | 2.56 | 3.91 | 1.76 | 1.29 | 2.18 | 0.94 |
| 26-Nov-15 | GSJ | 0.83 | 0.98 | 1.55 | 0.87 | 0.88 | 1.43 |

3.2. Plankton and DA analysis

Phytoplankton species determination in net tow and bottle samples showed that the genus *Pseudo-nitzschia* was present in both gulfs during the sampled whale season (Table 2). In GN and GSJ the *Pseudo-nitzschia* species found were: *P. australis*, *P. calliantha*, *P. fraudulenta*, *P. pungens* and *P. pseudodelicatissima* (Table 2). In bottle samples the genus *Pseudo-nitzschia* was observed in densities between 2.26×10^2 and $4.56 \times 10^5 \text{ cells l}^{-1}$ in GN and between 4.40×10^2 and $8.80 \times 10^2 \text{ cells l}^{-1}$ in GSJ (Table 2). The most common *Pseudo-nitzschia* species in both gulfs was *P. australis* (Table 2). In fact, during October there was a bloom of *P. australis* in GN (Table 2) recorded in both net tow (Table 2; St. 1, 2 and 3) and bottle samples (Table 2; St. 1 and 3), which reached a maximum density of $4.56 \times 10^5 \text{ cells l}^{-1}$.

Domoic acid was detected either at low levels or was absent in phytoplankton samples from GN and GSJ, with the exception of those collected during the *P. australis* bloom in GN in October (DA = 5050, 6139, 3248 ng NT^{-1} ; St. 1, 2 and 3 respectively; Table 3). The highest levels of DA in phytoplankton samples from GSJ (112 and 151 ng NT^{-1} ; St. 1 and 2 respectively) coincided with the identification of the species *P. australis* and *P. pungens* in net tow samples of the same stations (Tables 2 and 3). In most cases, a clear correlation was found between the presence of *Pseudo-nitzschia* cells in phytoplankton from both gulfs and the occurrence of DA. However, in some months in GN (November and December) cells of *Pseudo-nitzschia* spp. were identified in net samples even though DA was not detected in phytoplankton samples of the same date (Tables 2 and 3). In contrast, in some cases no diatoms of the genus *Pseudo-nitzschia* were identified in net tow samples, in which DA was detected (September in GN and July, September and November in GSJ; Tables 2 and 3).

Mesozooplankton analyses revealed the presence of copepods (copepodites and adults), cirripeds (larvae), amphipods (adults), decapods (larvae), euphausiids (larvae, juveniles and adults), molluscs (larvae), echinoderms (larvae), appendicularians (larvae) and fishes (larvae) as potential predators of *Pseudo-nitzschia* spp. (Table 4). In all but one the mesozooplankton samples from GSJ (St. 1, 27-Oct-15) and all samples from GN, copepods were numerically dominant within the group of organisms that could act as potential vectors of DA to higher trophic levels including the southern right whales (Table 4).

No clear correlation was found between the detection of DA in phytoplankton samples from both gulfs and the occurrence of this neurotoxin in mesozooplankton samples. In GN, only four samples of mesozooplankton were positive for DA ranging from undetected to 84 ng NT^{-1} (Table 5). While in GSJ, DA was detected in a larger number of samples, although they were in low concentrations, with DA values ranging from undetected to 21 ng NT^{-1} (Table 5).

Table 2
Pseudo-nitzschia species recorded in net tow and bottle samples collected during the 2015 whale season in Golfo Nuevo (GN) and Golfo San José (GSJ). – = no data; * = bloom; P: Present in net tow samples.

| <i>Pseudo-nitzschia</i> spp. quantitative analysis [cells l ⁻¹] | | | | | | |
|---|---------|---------------------|----------------------|-----------------------|-------------------|-------------------------------|
| Golfo Nuevo | | | | | | |
| Sample date | Station | <i>P. australis</i> | <i>P. calliantha</i> | <i>P. fraudulenta</i> | <i>P. pungens</i> | <i>P. pseudodelicatissima</i> |
| 08-Jun-15 | 1 | 0 | 0 | 0 | P | 0 |
| | 2 | 0 | 0 | 0 | P | 0 |
| | 3 | 0 | 226 | 0 | P | 0 |
| 29-Jul-15 | 1 | 0 | 0 | 0 | 0 | 0 |
| | 2 | – | – | – | – | – |
| | 3 | – | – | – | – | – |
| 04-Sep-15 | 1 | 4400 | 0 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 | 0 | 0 |
| | 3 | – | – | – | – | – |
| 09-Oct-15 | 1 | 364980* | 0 | 0 | 0 | 0 |
| | 2 | P* | 0 | 0 | 0 | 0 |
| | 3 | 456225* | 149310 | 0 | 0 | 0 |
| 12-Nov-15 | 1 | P | 0 | P | 0 | 0 |
| | 2 | P | 2520 | P | 0 | 0 |
| | 3 | P | 0 | P | 0 | 0 |
| 21-Dec-15 | 1 | P | 0 | P | 0 | 0 |
| | 2 | 0 | 0 | P | 0 | 0 |
| | 3 | 0 | P | 440 | 0 | 0 |
| Golfo San José | | | | | | |
| 03-Jul-15 | 1 | P | 0 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 | P | 0 |
| | 3 | 0 | 0 | 0 | 0 | 0 |
| 10-Aug-15 | 1 | P | 0 | 0 | P | 0 |
| | 2 | P | 0 | 0 | P | 0 |
| | 3 | P | 0 | 0 | P | 0 |
| 24-Sep-15 | 1 | 0 | 0 | 880 | 880 | 0 |
| | 2 | P | 0 | 0 | 0 | 0 |
| | 3 | P | 0 | 880 | 880 | 0 |
| 27-Oct-15 | 1 | P | 880 | 0 | 0 | 0 |
| | 2 | P | 0 | 0 | 0 | 0 |
| | 3 | P | 880 | 0 | 0 | 0 |
| 26-Nov-15 | 1 | P | 0 | 0 | 0 | P |
| | 2 | 880 | 0 | 440 | 0 | 0 |
| | 3 | P | 0 | 0 | 0 | P |

Table 3

Domoic acid (DA) levels in net tow samples collected during the 2015 whale season in Golfo Nuevo (GN) and Golfo San José (GSJ). – = no data; <LOD = DA levels were below the limit of detection (LOD = 0.64 ng NT⁻¹). Phytoplankton net tow: approximately 21150 l of seawater filtered.

| DA levels in phytoplankton [ng NT ⁻¹] | | | | |
|---|-----------------|---------|------|------|
| Sample date | Sample location | Station | | |
| | | 1 | 2 | 3 |
| 08-Jun-15 | GN | 26 | 194 | 13 |
| 29-Jul-15 | GN | <LOD | – | – |
| 04-Sep-15 | GN | 12 | 22 | – |
| 09-Oct-15 | GN | 5050 | 6139 | 3248 |
| 12-Nov-15 | GN | <LOD | 0 | <LOD |
| 21-Dec-15 | GN | <LOD | 0 | <LOD |
| 03-Jul-15 | GSJ | 46 | 33 | 52 |
| 10-Aug-15 | GSJ | 112 | 151 | 49 |
| 24-Sep-15 | GSJ | 3 | 18 | 14 |
| 27-Oct-15 | GSJ | 8 | 1 | 2 |
| 26-Nov-15 | GSJ | 8 | 88 | 54 |

3.3. Domoic acid in southern right whale fecal samples

Domoic acid was not detected in samples collected from dead whales in 2013 (n = 3) (Table 6). Only one of the samples collected from a living whale in 2014 (n = 7) had detectable levels of DA (BFA9, 710 ± 75 µg g⁻¹ dry weight of fecal sample [Table 6]; 113.6 ± 12 µg g⁻¹ wet weight assuming a mean water content of 84%). The sample was collected on 19-Nov-14 and belonged to a living lactating female (Table 6). All but one of the samples

collected during the whale season 2015 (n = 4) had detectable levels of DA (Table 6). The lowest level from 2015 was collected from a living adult (BFA13) seen skim feeding in Bahía Pirámide (BP, GN, Fig. 1), on 15-Nov-15 (0.30 µg g⁻¹ dry weight of fecal sample [Table 6]; 0.05 µg g⁻¹ wet weight assuming a mean water content of 84%). The highest level of DA in 2015 was recorded also a living adult in BP on 28-Oct-15 (BFA12, 9.10 µg g⁻¹ dry weight of fecal sample [Table 6]; 1.46 µg g⁻¹ wet weight assuming a mean water content of 84%).

4. Discussion

The genus *Pseudo-nitzschia*, was represented in GN and GSJ by *P. fraudulenta*, *P. pungens*, *P. australis*, *P. calliantha* and *P. pseudodelicatissima*, five species that have been previously reported as recurrent components of the phytoplankton community of these gulfs (Gayoso, 2001; Sastre et al., 2001; 2007; Santinelli et al., 2002; Santinelli, 2008; Cadaillón, 2012). The first three species have been associated with toxicity events, both in GN and GSJ as well as in the Argentine Sea (Negri et al., 2004; Sastre et al., 2007; Almandoz et al., 2017) while *P. calliantha* is known as a species potentially producing DA in other regions of the world (Lelong et al., 2012) and *P. pseudodelicatissima* has been reported as non-toxic (Lundholm et al., 2003). In GN, species of the genus *Pseudo-nitzschia* were found from low (2.26 × 10² cells l⁻¹) to bloom (4.56 × 10⁵ cells l⁻¹) abundances. The *Pseudo-nitzschia* bloom occurred during the whale season 2015 showed the highest cell abundance and was caused by *P. australis*. Although the presence of *P. australis* has been previously documented in GN in most cases

Table 4

Abundances (ind m⁻³) of potential predator groups of *Pseudo-nitzschia* spp. within mesozooplankton during the 2015 whale season in Golfo Nuevo and Golfo San José. St=Sampling station, C=copepodites, A=adults, L=larvae, J=juveniles.

| Potential predators of <i>Pseudo-nitzschia</i> spp. | | | | | | | | | | | | | | | | | | |
|---|-----------|----|-----|-----------|----|----|-----------|-----|-----|-----------|-----|-----|-----------|---|----|-----------|---|---|
| Sample collected GN | 08-Jun-15 | | | 24-Jul-15 | | | 04-Sep-15 | | | 09-Oct-15 | | | 12-Nov-15 | | | 21-Dec-15 | | |
| St./ind m ⁻³ | 1 | 2 | 3 | 1 | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Copepods (C-A) | 50 | 6 | 54 | 135 | 75 | 62 | 38 | 327 | 331 | 349 | 720 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Cirripeds (L) | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 124 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Amphipods (A) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Decapods (L) | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Euphausiids (L-J-A) | 0 | 0 | 0 | 0 | 12 | 5 | 20 | 5 | 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Molluscs (L) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Echinoderms (L) | 0 | 0 | 1 | 1 | 12 | 4 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Appendicularians (L) | 0 | 0 | 0 | 0 | 4 | 0 | 2 | 299 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fish (L) | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Sample collected GSJ | 03-Jul-15 | | | 10-Aug-15 | | | 24-Sep-15 | | | 27-Oct-15 | | | 26-Nov-15 | | | | | |
| St./ind m ⁻³ | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | | | |
| Copepods (C-A) | 28 | 70 | 106 | 191 | 21 | 63 | 15 | 9 | 39 | 124 | 187 | 341 | 11 | 4 | 15 | | | |
| Cirripeds (L) | 4 | 2 | 3 | 5 | 0 | 1 | 1 | 0 | 0 | 8 | 3 | 0 | 0 | 0 | 0 | | | |
| Decapods (L) | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | | | |
| Euphausiids (L-J-A) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | | | |
| Molluscs (L) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| Echinoderms (L) | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| Appendicularians (L) | 7 | 0 | 2 | 28 | 0 | 3 | 4 | 0 | 6 | 134 | 7 | 22 | 4 | 2 | 4 | | | |
| Fish (L) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | |

Table 5

Domoic acid (DA) levels in mesozooplankton samples collected during the 2015 whale season in Golfo Nuevo (GN) and Golfo San José (GSJ). – = no data; <LOD=DA levels were below the limit of detection (LOD=0.64 ng NT⁻¹). Mesozooplankton net tow: approximately 84620 l of seawater filtered.

| DA levels in mesozooplankton [ng NT ⁻¹] | | | | |
|---|-----------------|---------|------|------|
| Sample date. | Sample location | Station | | |
| | | 1 | 2 | 3 |
| 08-Jun-15 | GN | 84 | 32 | 18 |
| 29-Jul-15 | GN | 0 | – | – |
| 04-Sep-15 | GN | 0 | – | – |
| 12-Nov-15 | GN | 0 | 0 | 1 |
| 21-Dec-15 | GN | 0 | <LOD | <LOD |
| 03-Jul-15 | GSJ | 7 | 21 | 10 |
| 10-Aug-15 | GSJ | 0 | 2 | 2 |
| 24-Sep-15 | GSJ | 1 | 0 | 0 |
| 27-Oct-15 | GSJ | 5 | 9 | 11 |
| 26-Nov-15 | GSJ | 1 | 18 | 15 |

the cell densities reported for this species were low (Sastre et al., 2001, Cadaillón, 2012) and no bloom of *P. australis* has as yet been reported in either gulfs. In addition, the recorded cell densities of *P. australis* in this study in GN during October 2015 (St 3 = 4.56×10^5 cells l⁻¹) were the highest recorded to date in the entire Argentine Sea (Negri and Inza, 1998; Sastre et al., 2001; Negri et al., 2004; Almandoz et al., 2007; Almandoz et al., 2017). *Pseudo-nitzschia australis* is considered a strong DA producer (12–37 pg cell⁻¹) (Bates, 2000; Kotaki et al., 2000) and for this reason it has been reported to be the most toxic species of the genus *Pseudo-nitzschia* (Trainer et al., 2000). Thus *P. australis* is assumed to be the species primarily responsible for amnesic shellfish poisoning (ASP) problems worldwide (Bates, 2000; Fire et al., 2010) including mortality events of marine mammals (Lefebvre et al., 1999; Scholin et al., 2000; De la Riva et al., 2009; Fire et al., 2010). In Argentine Sea *P. australis* has been suggested to be the major producer of DA (Almandoz et al., 2017). In the quantitative samples of GSJ the *Pseudo-nitzschia* spp. densities were low, with abundances between 4.40×10^2 to 8.80×10^2 cell l⁻¹. Cell abundances observed in this study were much lower than those reported by, Cadaillón et al. (2012) and D'Agostino et al. (2015) for GSJ, where these

Table 6

Domoic acid (DA) levels in fecal samples of southern right whale (*Eubalaena australis*) collected during the 2013, 2014 and 2015 whale seasons in Golfo Nuevo (GN). BP=Bahía Pirámide; – = no data; * = Whale feeding on surface; <LOD=DA levels were below the limit of detection (LOD=0.11 µg DA g⁻¹ fecal sample [dry weight]).

| DA content of fecal samples [µg g ⁻¹] | | | | | |
|---|--------------------|----------------|-------|---------------|----------|
| Fecal ID | Sample location | Date collected | State | Sex/age class | DA |
| BFA1 | GN | 29-Jul-13 | Dead | – | <LOD |
| BFA2 | GN | 06-Oct-13 | Dead | – | <LOD |
| BFA3 | GN | 09-Dec-13 | Dead | – | <LOD |
| BFA4 | BP (GN) | 18-Sep-14 | Live | Adult female | <LOD |
| BFA5 | BP (GN) | 27-Sep-14 | Live | Adult female | <LOD |
| BFA6 | Playa Kaiser (GN) | 05-Oct-14 | Dead | Juvenile male | <LOD |
| BFA7 | BP (GN) | 13-Oct-14 | Live | Adult female | <LOD |
| BFA8 | BP (GN) | 17-Nov-14 | Live | Adult female | <LOD |
| BFA9 | BP (GN) | 19-Nov-14 | Live | Adult female | 710 ± 75 |
| BFA10 | BP (GN) | 22-Nov-14 | Live | Adult female | <LOD |
| BFA11 | Punta Piaggio (GN) | 11-Oct-15 | Live | Juvenile | 1.00 |
| BFA12 | BP (GN) | 28-Oct-15 | Live | Adult | 9.10 |
| BFA13 | BP (GN) | 15-Nov-15 | Live | Adult* | 0.30 |
| BFA14 | BP (GN) | 15-Dec-15 | Live | Adult female | <LOD |

authors have reported that this genus was more numerous and in some cases reached bloom densities (>100,000 cells l⁻¹).

The highest DA level recorded in phytoplankton samples in this study was detected concurrently with the bloom of *P. australis* recorded in GN in October 2015. Although the attribution of toxicity to a species usually involves the proof of the presence of the toxins in cultures or isolated cells (Álvarez et al., 2009), the identification of only *P. australis* in the net tow concentrates in GN from October and the high cell densities recorded in the quantitative analysis (bottle samples) indicate that this species was the main producer of the highest levels of DA recorded in this study. The current results are consistent with those of Almandoz et al. (2017) who found a high association between high cell densities of *P. australis* and the concentration of DA in phytoplankton samples from shelf waters south of PV and GN collected during spring. Although a coincidence between the presence of *Pseudo-*

nitzschia spp. and DA was generally observed in the phytoplankton from GN and GSJ for the rest of the months, an attribution of DA production to one specific *Pseudo-nitzschia* species was difficult to achieve. In addition, DA was also detected in some phytoplankton samples without a detectable presence of *Pseudo-nitzschia* spp. Similar results were reported by Almandoz et al. (2017) and led the authors to suggest that in these cases *Pseudo-nitzschia* cells were in densities below the detection limit of microscopic analysis or that there could be another source of DA, such as other diatom species, early life stages of zooplankton, their eggs or fecal pellets. But in some cases we found the contrary, namely *Pseudo-nitzschia* cells without detectable DA in the respective phytoplankton samples. However, these results seem to support the hypothesis proposed by Almandoz et al. (2017) about low abundances of *Pseudo-nitzschia* spp., because low cell densities of *Pseudo-nitzschia* spp. were recorded in concentrated plankton samples and at the same time this genus was absent or in low abundance in the respective less concentrated quantitative phytoplankton samples.

Domoic acid was first detected in the Argentine Sea in 2000 during a bloom of *P. australis* (Negri et al., 2004). During this event DA was recorded in phytoplankton samples, mussels (*Mytilus edulis*) and anchovies (*Engraulis anchoita*). In addition, in GN DA was detected for the first time in phytoplankton samples in October 2005 and were associated with a spring phytoplankton bloom dominated by *P. pungens* and *P. fraudulenta* (Sastre et al., 2007), the two species that were identified in phytoplankton samples collected during the sampled whale season. However in the present study, low amounts of DA were recorded or DA was absent in phytoplankton samples, in which *P. pungens* and *P. fraudulenta* were identified. Despite the fact that spring blooms of potentially toxic *Pseudo-nitzschia* species are common in both GN and GSJ (Sastre et al., 2007; Cadaillón 2012; D'Agostino et al., 2015), to date no cases of ASP events in wildlife or humans have been documented in PV.

The results presented here indicate that copepods were the main potential vectors for the transfer of DA from *Pseudo-nitzschia* spp. to higher trophic levels including the southern right whales, both in GN and GSJ. Although Cadaillón (2012) reported the transfer of DA from producing microalgae to zooplankton in GN and GSJ, the potential vectors of this toxin from *Pseudo-nitzschia* spp. to higher trophic levels has been uncertain to date. The present findings provide evidence that copepods were dominant among the potential predators of *Pseudo-nitzschia* spp., in the months when the highest levels of DA were recorded in the phytoplankton and mesozooplankton of both gulfs. For example, when the highest levels of DA were detected in phytoplankton in GN in October, copepods were 62.2%, 40.78% and 90.04% (St. 1, 2 and 3, respectively) of the total taxa that could act as potential toxin vectors. Similar results were obtained in GSJ, where copepods were predominant during July and August (July: 70.09%, 96.04%, 95.48% and August: 85.23%, 95.55%, 91.38% at St. 1, 2 and 3, respectively) when the highest amounts of DA were detected in phytoplankton in this gulf. In addition, taking into account that phaeopigments are considered in general indicative of degradation of chl *a* due to grazing by herbivorous zooplankton (Lorenzen, 1967; Helling and Baars, 1985; Head and Harris, 1992), our findings provide compelling evidence that copepods are the main vectors of DA through the pelagic food web of the southern right whale in the PV area. Indeed, maximum phaeopigment levels in GN were detected during the *P. australis* bloom recorded in this gulf in October 2015, where, as mentioned above, copepods dominated the mesozooplankton community and similar phaeopigments-chl *a* ratios were found in GSJ during spring. Furthermore, DA was detected in all phytoplankton samples and in most of mesozooplankton samples which were dominated by copepods among the potential predators of *Pseudo-nitzschia* spp. in GSJ. Thus this

provides conclusive evidence that also in this gulf copepods were the vectors of DA through the food web.

Recent studies have reported that southern right whales are feeding mainly on the predominant copepods during their stay in GN and GSJ (Hoffmeyer et al., 2010; D'Agostino et al., 2016). Thus the evidence that copepods act as a primary vector for DA transfer to the southern right whales demonstrates risk that southern right whales are exposed to when feeding on their calving ground of PV. This evidence is supported by findings recently reported by D'Agostino et al. (2015; 2016), which show the presence of frustule fragments of potentially toxic *Pseudo-nitzschia* spp., as well as remains of microcrustaceans, mainly mandibular gnatobases of copepodites 5 of *C. australis*, in fecal samples of live and dead individuals of southern right whales in GN and GSJ. According to the mentioned authors, a proportion of the *Pseudo-nitzschia* frustule fragments found in the fecal samples of whales probably originated from gut contents of copepods ingested by whales during their stay in this area.

Grazing experiments have demonstrated that copepods (adult and copepodites stages) do not select between toxic and non-toxic *Pseudo-nitzschia* spp. (Leandro et al., 2010b; Shaw et al., 1997; Lincoln et al., 2001; Tester et al., 2001; Harðardóttir et al., 2015). This enables copepods to accumulate large amounts of DA (Tester et al., 2001; Harðardóttir et al., 2015) even after elimination of toxic *Pseudo-nitzschia* (Lincoln et al., 2001; Tester et al., 2001; Maneiro et al., 2005). In addition, copepods have been found to possess a fast DA accumulation rate (< 3 h) (Tester et al., 2001). Recent studies show that DA production increased when cells of *Pseudo-nitzschia* spp. were exposed to grazing copepods (Harðardóttir et al., 2015; Tammilehto et al., 2015). These findings confirm that copepods are efficient vectors for the transfer of DA from producer microalgae to higher trophic levels. The fact that low concentrations of DA in mesozooplankton samples from both gulfs were detected in this study could be due to low cell densities of *Pseudo-nitzschia* spp. and low DA levels found in the phytoplankton samples from both gulfs (except in GN during October) rather to the inability of copepods to act as DA vectors. It should be noted that more numerically important copepod species recorded in the present study (*Paracalanus parvus*, *Ctenocalanus vanus*, *Calanus australis* and *Calanoides carinatus*) have been defined as herbivorous or omnivorous (Boltovskoy, 1981; 1999; Lombard et al., 2010; D'Agostino, 2013; Antacli et al., 2014). These copepod species therefore, can also act as effective DA vectors either by direct consumption of DA producing species or by ingestion of lower trophic level organisms contaminated with this toxin.

This study demonstrates for the first time that living southern right whales *E. australis* are exposed to DA via copepods as vectors on their calving grounds at PV. The analysis of fecal samples from 11 living and three dead southern right whales collected in GN during the calving seasons of 2013 through 2015 provide evidence of consuming prey containing DA. DA was detected in four fecal samples from living whales with values ranging from 0.30 to 710 $\mu\text{g DA g}^{-1}$ dry weight. These levels could be underestimates due to the quantification methodology employed, in which the complete extraction of DA from the fecal samples despite of 20 subsequent extractions performed on the same sample was not achieved. The affected individuals included a lactating female sighted during the 2014 whale season and a juvenile and two adults from the 2015 season whose sexes could not be identified. A previous study of tissues collected from whales that died at PV reported trace levels of DA in only a few of the samples (4 out of 36 whales, Rowntree et al., 2013). More recently, low DA levels were documented in blood samples of a dead male calf and a dead adult female in GN and GSJ, respectively (male calf: 3 ng DA ml^{-1} and adult female: 7 ng DA ml^{-1}) (Wilson et al., 2015). DA levels found in

feces during the present study were the highest reported to date for southern right whales (ranging from 0.30 to 710 $\mu\text{g DA g}^{-1}$ dry weight or from 0.05 to 113.6 $\mu\text{g g}^{-1}$ wet weight assuming a mean water content of 84%). Likewise, the detection of DA in fecal samples from living whales at PV was documented for the first time in this study. It has been reported that a large amount of the orally ingested doses of DA are eliminated through feces (Iverson et al., 1989, 1990; Costa et al., 2010), which is why some authors indicate that the fecal samples could be the best indicators of DA exposure (Fire et al., 2009; Wilson et al., 2015).

Interestingly our results, measured by LC–MS/MS and expressed in wet weight with the exception of BFA13 (0.05 $\mu\text{g DA g}^{-1}$ wet weight assuming a mean water content of 84%), show higher DA values than those reported in feces from northern right whales *E. glacialis* (levels between 0.002 to 0.175 $\mu\text{g DA g}^{-1}$), on their feeding ground in the Great South Cannel and the Bay of Fundy (Leandro et al., 2010a). Given that these levels were found in fecal samples of right whales on their feeding ground, it is expected that these whales experience much higher levels of DA than the southern right whale individuals on their calving grounds. Feeding grounds are considered areas where right whales feed continuously. It has been estimated that a northern right whale on average ingest about 4.61×10^8 copepods d^{-1} on their feeding ground in the Bay of Fundy (Durbin et al., 2002). In contrast, southern right whales migrate from the feeding grounds in South Atlantic and Southern Ocean to PV to reproduce and give birth to their calves and they only feed opportunistically when denser zooplankton patches occur in the area during spring (Hoffmeyer et al., 2010).

Although in the present study no toxin data were available for phytoplankton or mesozooplankton during the 2014 whale season, when the highest levels of DA were found in the fecal sample of BFA9 (710 $\mu\text{g DA g}^{-1}$ dry weight), it can be deduced that this whale was exposed to an intense toxic *Pseudo-nitzschia* bloom. On the other hand, samples BFA11 and BFA12 were collected on 11-Oct-15 and 28-Oct-15 respectively, which indicate that these whales were exposed to *P. australis* bloom recorded in GN on 09-Oct-15. In addition to exposure by feeding on contaminated mesozooplankton, these whales could be directly exposed to DA through the ingestion *P. australis* cells. *Pseudo-nitzschia* spp. is known to form chains whose length may exceed the 335 μm baleen distances of the right whales (Mayo et al., 2001; Leandro et al., 2010a). In fact, the retention of *P. australis* cells during the bloom in GN on 09-Oct-15 in the mesozooplankton net of 335 μm mesh size is clear evidence that a direct exposition of DA of right whales when feeding during a toxic bloom of *Pseudo-nitzschia* spp. is possible. While, sample BFA13 collected on 15-Nov-15 was originated from an adult individual that was feeding at surface. Even though no DA was detected in the phytoplankton sampled at a date close to its collection (12-Nov-15) and the DA levels in the mesozooplankton corresponding to that date were low, the fact that this whale was feeding indicated that it would have been exposed to DA during such an event as demonstrated Leandro et al. (2010a) for northern right whales.

On the other hand, although there were no fecal samples of whales collected in GSJ, previous studies (Sironi, 2004; D'Agostino et al., 2016) as well as our observations have documented that southern right whales feed primarily on copepods in this gulf. Therefore, considering that this study along with the results of Cadaillón (2012), demonstrate the transfer of DA from phytoplankton to mesozooplankton (primarily through copepods in GSJ) it is probable that the whales are also exposed to DA in GSJ through the ingestion of contaminated zooplanktonic organisms, and primarily through copepods.

In this study, the highest level of DA was detected in feces of an adult female that was next to her calf. Rust et al. (2014) reported the presence of DA in the milk of several species of marine

mammals that had been exposed to a *Pseudo-nitzschia* spp. bloom. In fact, has been reported that DA is cleared rapidly in adult mammals (Maucher and Ramsdell 2007; Wittmaack et al., 2015), however the milk of marine mammals may act as a reservoir of DA (Rust et al., 2014). Hence, calves of marine mammals can be exposed to DA through the milk and this exposure may have occurred to the calf of female which defecated sample BFA9. Exposure of calves to DA can lead to developmental abnormalities such as neurological deficits and abnormal behaviors which would affect their survival (Lefebvre et al., 2016). Thus, the transfer of DA from lactating females to their calves through the milk in the PV area would suggest that this neurotoxin is associated with the death of calves in this calving ground. Further research is needed to know if calves are actually exposed to neurotoxins in the PV calving ground and the effects of DA in the first stages of development of southern right whale calves.

5. Conclusions

The findings presented here demonstrate for the first time that the southern right whales are exposed to DA through feeding in their calving ground in the region of PV. This was evidenced by the detection of DA in phytoplankton and mesozooplankton samples from GN and GSJ, and in fecal samples from living southern right whales.

The occurrence of toxic *Pseudo-nitzschia* spp. blooms concurrently with the whale calving season and the detection of high levels of DA in fecal samples of southern right whales, demonstrate that the whales are exposed to natural risks at PV. In addition, finding the highest levels of DA in fecal samples from an adult female with a calf indicates potential risks to calves through the transfer of DA during lactation. Additionally, has been show that in marine mammals the DA cross the placental barrier and accumulate in the amniotic fluid (Goldstein et al., 2009; Hall and Frame, 2010; McHuron et al., 2013) therefore, the calves of southern right whales could be exposed to DA also during gestation.

Undoubtedly, southern right whales are exposed to DA through their diet while on their calving ground of PV as well as in the North Atlantic (Leandro et al., 2010a; Doucette et al., 2012). The health impacts of DA to adults and calves remain unknown. This study highlights the need for understanding the transfer of phycotoxins from mothers to calves. Therefore future studies should continue to analyze DA in both living and dead right whales including the simultaneous sampling of feces from mother-calf pairs. Though difficult, this will allow the documentation of local exposure risks for whales, the levels of toxins that reach individuals as well as a deeper understanding of the transference of phycotoxins to mothers and their calves.

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