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Peruvian fur seals (*Arctocephalus australis* ssp.) and South American sea lions (*Otaria byronia*) in Peru are exposed to the harmful algal toxins domoic acid and okadaic acid

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The apparent global increase of harmful algal blooms (HABs) during the last few decades has negatively impacted aquatic ecosystems and marine mammal health, mainly due to dietary exposure to natural toxins produced by the phytoplankton causing these events (Hallegraeff 1993, Landsberg 2002). Many of the toxins in question are concentrated by planktivorous grazers, and when these grazers are also the primary prey of marine mammals, fatal neurotoxicity and large-scale die-offs can occur (Scholin *et al*. 2000, Flewelling *et al*. 2005, Fire and Van Dolah 2012). Sublethal effects such as reproductive failure and chronic neurological disease have also been identified as severe ecological risks to marine mammals inhabiting productive upwelling zones like the California Current system; mainly as a result of repeated seasonal exposure to the HAB toxins commonly produced there during the breeding season (Brodie *et al*. 2006, Gulland and Hall 2007, Bejarano *et al*. 2008). Much of what is known about HAB impacts on marine mammal health derives from the California Current system, partly due to its unique combination of abundant pinniped populations, frequent HABs, and intense monitoring and study (Reid *et al*. 1958, Sydeman and Allen 1999, Croll *et al*. 2005, Anderson *et al*. 2008). However, little is known about the effects of HAB toxins on marine mammal health in other upwelling zones where these cooccur.

One such region is the Humboldt Current, an upwelling zone along the coast of Peru that is the Southern Hemisphere counterpart to the California Current. The Humboldt Current also

supports abundant populations of marine mammals as well as several genera/species capable of producing HABs (Majluf and Reyes 1989, Trainer *et al*. 2010). Dense accumulations of marine mammal fossils in this area suggest a long history of marine mammal die-offs caused by HAB exposure (Pyenson *et al*. 2014). During an unprecedented mortality event in 2012, over 800 common dolphins (*Delphinus capensis*) were reported dead along the coast of Peru from an unknown cause (MINAM 2012). Though HAB involvement was suspected, analytical results confirming the presence of toxins in dolphin carcasses were inconclusive (IMARPE 2012*a*). While HAB toxins in Peru have been routinely detected or implicated in marine wildlife mortalities since the 1800s, no systematic efforts to investigate the impacts of HAB toxins on marine mammals in this region have yet been carried out (López-Rivera *et al*. 2009, Trainer *et al*. 2010, Reguera *et al*. 2014).

The Peruvian fur seal (unnamed subspecies of *Arctocephalus australis*, hereafter as “FS”) and South American sea lion (*Otaria byronia,* hereafter as “SL”) are two pinniped species that inhabit rookery sites along the Peruvian coast (Committee on Taxonomy 2014). In addition to a long history of anthropogenic health threats, their geographic range also overlaps with that of several toxic HAB species associated with marine mammal health risks in U.S. waters (Majluf and Reyes 1989, Trainer *et al*. 2010, Cárdenas-Alayza 2012). The three major HAB toxins of concern in Peru are (1) domoic acid (DA), a neurotoxin causing mortality, reproductive failure and chronic epilepsy in California sea lions (Scholin *et al*. 2000, Brodie *et al*. 2006, Goldstein *et al*. 2008), (2) saxitoxin (STX), a neurotoxin associated with pinniped, baleen whale and small

cetacean mortalities in the Atlantic ocean (Geraci *et al*. 1989, Hernandez *et al*. 1998, Abbott *et al*. 2009), and (3) okadaic acid (OA), a diarrhetic toxin associated with cetacean mortalities in the Gulf of Mexico (Fire *et al*. 2011). The objective of this study was to investigate the presence of these three HAB toxins in FS and SL at rookery sites in Peru, as well as any cooccurrence of associated HAB species, as a first step toward evaluating potential health risks similar to those observed in well-studied California Current regions.

Our study site is Punta San Juan (PSJ), a 53.8 hectare peninsula on the southern coast of Peru (15°22′S, 75°12′W; Fig. 1). PSJ is the site of the strongest upwelling core of the Humboldt Current system and has an extremely high level of biological productivity (Bakun and Weeks 2008). PSJ is comprised of several rocky beach areas that serve as rookery habitat during the FS and SL breeding seasons (Majluf and Trillmich 1981). Sample collection took place during two field seasons, 11–19 November 2011 and 9–14 November 2012, each occurring during the austral spring breeding season for FS (October–December) and two months prior to the austral summer breeding season for SL (January–February, Majluf and Reyes 1989).

Female FS were restrained at the rookery site using a hoop net and body weight was recorded by suspending the netted animal from a tripod-mounted digital scale. Administration of isoflurane in pure oxygen *via* a facemask (5% for induction, variable 1%–5% maintenance) or intramuscular alfaxalone (2–3 mg/kg; Alfaxan, Jurox, Rutherford, Australia) was used for induction of anesthesia (Adkesson *et al*. 2013). Male FS and SL were anesthetized using a remote drug delivery system (Daninject 3.0 mL darts) and fully reversible combination of butorphanol,

medetomidine, and midazolam, after which they were moved to a stretcher and weighed (Adkesson *et al*. 2012). Animals were restrained for ~30 min after induction of anesthesia, during which time a comprehensive veterinary examination was conducted, recording vital statistics such as heart rate, respiratory rate, blood lactate, and body temperature. Samples for HAB toxin analysis were collected during this period. Body temperature was regulated using a shaded canopy and application of cold seawater as needed, and capture and restraint efforts were designed to minimize disruption to the colony and avoid abandonment of young by lactating dams. Field biologist staff provided monitoring of animals in the days following all procedures. To avoid repeat sampling and facilitate follow-up monitoring, numbered cattle ear tags (All Flex) were attached to the pectoral flipper and ID numbers were shaved in the dorsal pelage.

Urine, stomach contents, and feces were chosen as the target sample types due to their accessibility in live animals, and their tendency to accumulate high concentrations of HAB toxins in exposed marine mammals (Fire and Van Dolah 2012). Urine was obtained by cystocentesis and gastric fluid was obtained *via* nasogastric tube through the oropharynx into the stomach. Feces was obtained using a sterile red-rubber urethral catheter folded into a loop. All samples were stored unpreserved in polypropylene centrifuge tubes at −20°C until extraction for HAB toxins. A total of 64 individuals were sampled, with 49 FS and 15 SL. Animals sampled in 2011 included 31 FS and 7 SL, while animals sampled in 2012 included 18 FS and 8 SL. Seventeen male and 32 female FS were sampled, while all 15 SL sampled were male. All individuals sampled were adults. Due to limited sample volumes, some samples were not tested for all three toxins.

All urine samples were centrifuged at 10,000 × g and filtered (0.45 µm) prior to analysis. For DA, feces and gastric samples were extracted by homogenization in 50% aqueous methanol (4:1, w:v) and probe sonication for 2 min. Extracts were centrifuged at 3,400 × g, and the supernatants were filtered (0.45 µm) and purified using 200 mg C18 solid-phase extraction (SPE) columns, and were stored at −20°C prior to analysis. Extracts were analyzed for DA using liquid chromatography/tandem mass spectrometry (LC-MS/MS) methods outlined in Wang *et al*. (2012). This method utilized reversed phase chromatography, using an Agilent 1100 HPLC coupled to an AB SCIEX API-4000 triple quadrupole mass spectrometer in ESI+ mode. Chromatographic separation was performed on a Phenomenex Luna C18(2), 5µ, 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 observed with a certified DA reference standard (NRC Canada). The detection of DA by MS was achieved by multiple reaction monitoring (MRM) method with TurboIonSpray interface. Four MRM transitions from protonated DA were monitored: m/z 312 → 266, m/z 312 → 248, m/z 312 → 193, and m/z 312 → 161. The limit of detection (DL) of this method was 2.5 ng/g sample, with a signal-to-noise ratio of 3 or higher for the MRM confirmation channel m/z 312 → 161. The limit of quantitation (LOQ) was 5 ng/g with signal to noise ratio of 10 for the MRM quantitation channel m/z 312 → 266.

For OA, feces and gastric samples were extracted by homogenization in 100% methanol (3:1, w:v), followed by centrifugation at 3,000 × g for 5 min. Extracts were filtered (0.45 µm) and stored at −20°C prior to analysis. Samples were

analyzed for the presence of OA and its congeners DTX1 and DTX2, using (LC-MS/MS) methods described in Hattenrath-Lehmann *et al*. (2013). LC separation was performed on an Agilent 1100 series HPLC with a Waters X-Bridge C18 (150 × 3 mm, 5 µm) column, using (1) a mobile phase of water and (2) acetonitrile/water (90:10, V/V), both containing 6.7 mM ammonium hydroxide under gradient elution at a flow rate of 0.4 mL/min (linear gradient from 1 min of 10% B to 90% B at 12 min, held for 3 min, then returned to 10% B at 17 min and held for 4 min). MS detection was in multiple reaction monitoring (MRM) mode using an AB Sciex 4000 QTRAP mass spectrometer. OA and its congeners were analyzed in negative ion mode: OA and DTX2 with MRM transitions of m/z 803.5 → 113.1 and 255.1, and DTX1 with MRM transitions of m/z 817.5 → 113.1 and 255.1. The DL was 0.1 ng/mL for OA , 0.08 ng/mL for DTX2, and 0.13 ng/mL for DTX1 with 5 µL injection of toxins in methanol standards, with signal-to-noise ratios in MRM confirmation channels of 3 or higher. The LOQ was approximately 0.5 ng/g, with a signal to noise ratio of 10 for standards.

For STX, feces and gastric samples were extracted by homogenization in 80% aqueous acetonitrile (4:1, v/v) with 0.1% formic acid, and probe sonication for 2 min. Extracts were centrifuged at 3,400 × g, and the supernatants were filtered (0.45 µm) and stored at −20°C prior to analysis. Samples were analyzed in a STX receptor binding assay (RBA), following procedures outlined in Van Dolah *et al*. (2012). The receptor binding assay measures competition between radiolabeled STX and STX in the sample or in standards (NIST RM 8642) for binding to the voltage-gated sodium channel, the pharmacological target of STX, to determine the total STX-like activity of the sample. The detection limit of this method was 219 ng STX-equivalents/g of

feces or 90 ng STX-equivalents/mL of urine or gastric fluid.

Data for presence and abundance of 20 HAB species/genera[2] endemic to Peru were queried from publicly available data sets curated by the Marine Institute of Peru (IMARPE). These were compiled and used to evaluate the cooccurrence of HABs and toxin exposure in FS and SL, focusing on phytoplankton known to produce the three HAB toxins of interest in this study. The relevant phytoplankton and their corresponding toxins are as follows: (1) *Pseudo-nitzschia* spp. (DA), (2) *Alexandrium* spp. (STX), and (3) *Dinophysis* spp. and *Prorocentrum* spp. (OA). Data for the period 11 January 2011 to 29 December 2012 were selected, using the phytoplankton monitoring site nearest to the study site ("Pisco," 153–206 km from PSJ; Fig. 1). Sampling in Pisco occurred 1–2 times per month at up to nine sampling stations, for a total of 86 sample collections during the study period (IMARPE 2012*b*).

Due to the limited scope of the biotoxin analyses which formed only a portion of the larger health assessment under which they were conducted, only descriptive statistics summarizing toxin results are presented below. The limited sample size and brief duration of this effort prevents in-depth inferences to the general population of FS and SL in the Humboldt Current system. DA was tested in 57 of the 64 animals in this study, and was confirmed present in 26% (n = 15) of these. All DA-positive results were from feces, and concentrations detected ranged from trace levels (above LOD, but below LOQ) to 533 ng/g of sample (ng/g, Table 1). No DA was detected in gastric or urine samples. DA-positive animals were more prevalent during 2011, with 32% (12/37; mean 135 ng/g) of animals testing positive, *vs*. 15% (3/20; 20 ng/g) in 2012.

Comparing between species, 25% (11/44) of FS and 31% (4/13) of SL were positive for DA. Both FS and SL sampled in 2011 had a higher proportion of DA-positive individuals and higher mean DA concentrations compared with their respective 2012 counterparts. Comparing exposure between sexes in FS, 25% (7/28) of males and 28% (8/29) of females were positive for DA. For SL, 31% (4/13) of males were positive for DA.

OA congeners were tested in 18 of the 26 animals sampled in this study (during 2012 only), and OA was confirmed present in 33% (*n*=6) of these. All OA-positive results were from feces, and concentrations detected ranged from 0.5 to 36 ng/g. OA was not detected in gastric or urine samples. Comparing between species, 42% (5/12) of FS and 17% (1/6) of SL were positive for OA. Comparing FS toxin exposure between sexes, 25% (1/4) of males and 50% (4/8) of females were positive for OA. For SL, 17% (1/6) of males were positive for OA. Two individuals (male FS AA1204 and female FS AA1230) tested positive for both DA and OA in each of their feces samples, indicating concurrent exposure to these toxins.

STX was assayed in 48 of the 64 individuals sampled in this study, with STX-like activity detected *via* RBA in 6% (*n* = 3) of these animals. All RBA-positive extracts were from feces, with concentrations ranging from 133 to 818 ng/g (Table 1). STX-like activity was not detected in gastric or urine samples. Comparing between species, 6% (2/35) of FS and 8% (1/13) of SL were RBA-positive. Both of the RBA-positive FS were females, and the RBA-positive SL was male. All RBA-positive extracts were subsequently analyzed by LCMS (data not shown) according to methods described in Fire *et al* (2012), however no STX congeners were detected, and the samples were therefore considered STX-

negative.

Pseudo-nitzschia occurred at elevated levels multiple times during the study period (Fig. 2), with cell abundance ranging from not present to 88,580 cells/L of seawater, approaching the threshold for medium-density blooms (~100,000 cells/L, Anderson *et al*. 2010). An 88,580 cells/L bloom occurred on 21 October 2011, approximately 3 wk prior to the beginning of animal sampling efforts. *Pseudo-nitzschia* was generally absent or at low concentrations (<40 cells/L) during the 3 months prior to animal sampling efforts in 2012. *Dinophysis* and *Prorocentrum* cell abundance ranged from not present to 150,800 cells/L, and although bloom concentrations were reported on several occasions during the study period, they were generally absent or at low concentrations (<420 cells/L) during the 3 mo periods prior to animal sampling efforts. *Alexandrium* was generally absent, with cell concentrations not exceeding 200 cells/L during the study period, and <20 cells/L during the 3 mo periods prior to animal sampling.

Our results confirm DA exposure in both FS and SL from coastal waters known to harbor the DA-producing alga *Pseudo-nitzschia.* Although our brief animal sampling periods provide only a temporal snapshot of DA exposure, it is noteworthy that animals with higher DA concentrations were sampled within 3 wk of the 2011 medium-intensity *Pseudo-nitzschia* bloom, while those with lower DA concentrations correspond to an absence of *Pseudo-nitzschia*. Since *Pseudo-nitzschia* is the only natural source of DA, cell abundance is a reliable indicator of DA exposure in wildlife occupying highly productive upwelling regions (Schnetzer *et al*. 2007). However, since *Pseudo-nitzschia* data from Pisco sampling sites were used as a proxy for cell

abundance at PSJ (a distance of ~155 km), it is not known to what degree the observed *Pseudo-nitzschia* blooms accurately represent local DA exposure in our animals. Nevertheless, *Pseudo-nitzschia* blooms in the U.S. can occur over 1,500 km^2 and last up to 3 mo, making the distance between our proxy site and PSJ somewhat small in comparison (Bates *et al*. 1989, Smith *et al*. 1993). Alternatively, the presence of DA in our 2012 animals may be due to unreported *Pseudo-nitzschia* bloom activity at PSJ, or due to low-level production of DA throughout the year. Future efforts in this region would benefit from frequent phytoplankton monitoring at PSJ before and during animal sampling to more accurately portray the spatial and temporal relationship between the source and fate of DA in this ecosystem, including identifying potential prey vectors for this toxin. Unfortunately, the gastric samples collected in this study were not suitable for taxonomic identification of prey, which could indicate potential phytoplankton grazers that concentrate DA and make it available to FS and SL. However, similar to the northern anchovy (*Engraulis mordax*), a well-known DA vector for California sea lions, the primary prey item of FS and SL is the Peruvian anchovy (*Engraulis ringens*), also a planktivore that grazes on and congregates in regions of high plankton productivity (Muck and Fuentes 1987, Majluf and Reyes 1989). *E. ringens* is found most abundantly within 10 km of the Peruvian coastline, and some of its highest population densities are found in nearshore waters of Pisco and PSJ (Mathisen 1989). Since DA is a water-soluble toxin that is rapidly depurated in mammals, retention of DA in the tissues (and excretion of DA in feces) of FS and SL for longer than a few days is unlikely, suggesting that the source of DA exposure in the PSJ animals are

locally obtained prey items (Perl *et al*. 1990, Truelove *et al*. 1997).

Although many of our animals tested positive for DA, the concentrations were much lower than reported values for live pinnipeds with neurological symptoms of DA intoxication. DA in PSJ animals reached a maximum of 533 ng/g feces, compared with northern fur seals (*Callorhinus ursinus*, up to 18,600 ng/g) and California sea lions (*Zalophus californianus*, 2500-152,000 ng/g) exposed during severe *Pseudo-nitzschia* blooms in the California Current system (Lefebvre *et al*. 1999, 2010; Scholin *et al*. 2000). In addition, clinical signs consistent with DA-related neurotoxicity were not observed in PSJ animals.

Our results also demonstrate OA exposure in PSJ animals inhabiting a region where *Prorocentrum* and *Dinophysis* are endemic, however this exposure occurred when these toxin producers were generally absent prior to animal sampling. It is possible, as with DA, that unreported *Prorocentrum* and *Dinophysis* activity near PSJ or low-level production of OA may be the source of the toxin in these animals. Unlike DA however, OA is a lipophilic toxin that generally has a much longer biological residence time (Matias *et al*. 1999, Svensson 2003), thus any exposure event may conceivably have been weeks or months prior. This, together with our DA results, demonstrate a need for further analysis of FS and SL prey items for both these HAB toxins, in order to determine the vector species and timing of exposure relative to HAB occurrence. Published data for OA in marine mammals are limited, however the concentrations reported here (up to 36 ng/g feces) are comparable to those reported for bottlenose dolphins (*Tursiops truncatus*, up to 10 ng/g feces) sampled in association with *Prorocentrum* and *Dinophysis* blooms

in Texas (Fire *et al*. 2011). The effects of OA exposure in marine mammals are not known, and although chronic exposure has been linked to tumor-promoting activity in rodents, the effects of OA exposure in humans is typically limited to nonlethal gastrointestinal illness (Lloyd *et al*. 2013, Valdiglesias *et al*. 2013).

Although *Alexandrium* is a known member of the phytoplankton community at IMARPE monitoring sites, it was not present in significant abundances or frequency during the course of this study. In addition, STX was not confirmed present in any of the animals sampled at PSJ. Further study over multiple field seasons will be required to more confidently establish whether the presence of this toxin-producing algae represents a concern for marine mammal health in Peru.

As demonstrated through nearly two decades of study in the California Current system, the overlap of HAB toxin distribution and pinniped breeding habitats may result in severe, long-term risk in productive coastal upwelling zones (Bejarano *et al*. 2007, Gulland and Hall 2007). In addition to acute death, population-level impacts from annual, repeated exposure to DA include degenerative cardiomyopathy, abortion and premature births, and chronic epileptic syndrome leading to repeat strandings (Goldstein *et al*. 2008, 2009; Zabka *et al*. 2009). We propose that the Humboldt Current system is a region that deserves at least an initial investigative effort, since it also experiences seasonal increases in HABs that cooccur with pinniped breeding seasons (Majluf and Reyes 1989, Trainer *et al*. 2010). The breeding populations at PSJ represent 36% and 12% of the total Peruvian population of FS and SL, respectively (SC-A, personal observation), thus incorporating HAB toxin analysis

into current PSJ disease surveillance efforts can be used as a model for assessing HAB exposure in pinnipeds throughout the Humboldt Current system (IMARPE 2013, Jankowski *et al*. 2015). Independent of HABs, the impacts of El Niño Southern Oscillation (ENSO) events can be catastrophic on Peruvian FS populations (Arias-Schreiber and Rivas 1998, Stevens and Boness 2003). However, large-scale climatic events like ENSO are also known to exacerbate harmful algal blooms in coastal upwelling systems, due to increased sea surface temperatures and an increased window of opportunity for warm-water HAB species (Moore *et al*. 2010). Future work in PSJ which includes analysis of potential cooccurrence of such HAB species as an additional concern during ENSO events could be beneficial to conservation efforts.

Although the *Pseudo-nitzschia* bloom associated with our 2011 DA-positive animals was only of medium intensity (~100,000 cells/L), such seasonal blooms can have large interannual variability, exceeding 1,000,000 cells/L (Anderson *et al*. 2010). In addition, cellular toxicity levels vary greatly among *Pseudo-nitzschia* species, with a range of over 3 orders of magnitude in DA cell quotas in laboratory cultures (Thessen *et al*. 2009). In the event that a large-scale, high-toxicity *Pseudo-nitzschia* bloom should occur near PSJ during the FS and SL breeding season, a high abundance of DA-contaminated prey may be expected, and potential negative impacts may include morbidity and/or mortality similar to that observed in the California Current system (Bejarano *et al*. 2007). In addition, DA exposure during the breeding and pupping season may carry its own health risks in pinniped prenates due to the critical stage of neurological development occurring in late-term and neonatal mammalian models. Since DA crosses the placenta, penetrates the

fetal brain and is retained and recirculated in the amniotic fluid, DA exposure in pregnant females results in *in utero* toxicity in prenatal animals, resulting in permanent neurological impairment (Ramsdell and Zabka 2008). Further work examining HAB toxin impacts in PSJ pinnipeds would benefit from investigating interannual and sex-class trends in toxin exposure, as such may elucidate potential threats to the recovery of FS and SL in this region.

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Literature Cited

Abbott, G. M., J. H. Landsberg, A. R. Reich, K. A. Steidinger, S. Ketchen and C. Blackmore. 2009. Resource guide for public health response to harmful algal blooms in Florida. Fish and Wildlife Research Institute Technical Report TR-14, Florida Fish and Wildlife Conservation Commission, Tallahassee, FL. viii + 132 pp.

Adkesson, M. J., G. Jankowski and S. Cárdenas-Alayza. 2012. Reversible immobilization of South American fur seals (*Arctocephalus australis*) and South American sea lions (*Otaria flavescens*) using medetomidine, butorphanol, and midazolam. IAAAM 43rd Annual Conference Proceedings, May 2012, Atlanta, GA. Pp. 50–51.

Adkesson, M. J., J. M. Meegan, G. Jankowski, S. Cárdenas-Alayza and J. E. Bailey. 2013. Evaluation of Alfaxalone (Alfaxan) for sedation of South American fur seals (*Arctocephalus australis*). IAAAM 44th Annual Conference Proceedings, April 2013, Sausalito, CA. Pp. 314–315.

Anderson, D. M., J. M. Burkholder, W. P. Cochlan, *et al*. 2008. Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. Harmful Algae 8:39–53.

Anderson, C. R., M. R. P. Sapiano, M. B. K. Prasad, W. Long, P. J. Tango, C. W. Brown and R. Murtugudde. 2010. Predicting potentially toxigenic *Pseudo-nitzschia* blooms in the Chesapeake Bay. Journal of Marine Systems 83:127–140.

Arias-Schreiber, M., and C. Rivas. 1998. Distribución, tamaño y estructura de las poblaciones de lobos marinos

(*Arctocephalus australis* y *Otaria byronia*) en el litoral peruano, en noviembre 1996 y marzo 1997 [Distribution, size, and population structure of the sea lions *Arctocephalus australis* and *Otaria byronia* on the Peruvian coast, during November 1996 and March 1997]. IMARPE. Available at http://biblioimarpe.imarpe.gob.pe:8080/bitstream/handle/123456789/1242/IP%2073.2.pdf?sequence=1.

Bakun, A., and S. J. Weeks. 2008. The marine ecosystem off Peru: What are the secrets of its fishery productivity and what might its future hold? Progress in Oceanography 79:290–299.

Bates, S. S., C. J. Bird, A. S. W. de Freitas, *et al*. 1989. Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada. Canadian Journal of Fisheries and Aquatic Sciences 46:1203–1215.

Bejarano, A. C., F. M. Van Dolah, F. M. Gulland and L. Schwacke. 2007. Exposure assessment of the biotoxin domoic acid in California sea lions: Application of a bioenergetic model. Marine Ecology Progress Series 345:293–304.

Bejarano, A. C., F. M. Van Dolah, F. M. Gulland, T. K. Rowles and L. H. Schwacke. 2008. Production and toxicity of the marine biotoxin domoic acid and its effects on wildlife: A review. Human and Ecological Risk Assessment 14:544–567.

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.

Cárdenas-Alayza, S. 2012. Prey abundance and population dynamics of South American fur seals (*Arctocephalus australis*) in

Peru. M.Sc. thesis, University of British Columbia, Vancouver, BC. 79 pp.

Committee on Taxonomy. 2014. List of marine mammal species and subspecies. Society for Marine Mammalogy. Available at https://www.marinemammalscience.org.

Croll, D. A., B. Marinovic, S. Benson, F. P. Chavez, N. Black, R. Ternullo and B. R. Tershy. 2005. From wind to whales: trophic links in a coastal upwelling system. Marine Ecology Progress Series 289:117–130.

Fire, S. E., and F. M. Van Dolah. 2012. Marine biotoxins: Emergence of harmful algal blooms as health threats to marine wildlife. Pages 374–389 *in* A. A. Aguirre, R. S. 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. 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.

Fire, S. E., J. Pruden, D. Couture, *et al*. 2012. Saxitoxin exposure in an endangered fish: Association of a shortnose sturgeon mortality event with a harmful algal bloom. Marine Ecology Progress Series 460:145–153.

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

Geraci, J. R., D. M. Anderson, R. J. Timperi, D. J. St. Aubin, G. A. Early, J. H. Prescott and C. A. Mayo. 1989. Humpback whales (*Megaptera novaeangliae*) fatally poisoned by dinoflagellate toxin. Canadian Journal of Fisheries and

Aquatic Sciences 46:1895–1898.

Goldstein, T., J. A. K. Mazet, T. S. Zabka, *et al*. 2008. Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (*Zalophus californianus*): An increasing risk to marine mammal health. Proceedings of the Royal Society B: Biological Sciences 275:267–276.

Goldstein, T., T. S. Zabka, R. L. DeLong, *et al*. 2009. The role of domoic acid in abortion and premature parturition of California sea lions (*Zalophus californianus*) on San Miguel Island, California. Journal of Wildlife Diseases 45:91–108.

Gulland, F. M. D., and A. J. Hall. 2007. Is marine mammal health deteriorating? Trends in the global reporting of marine mammal disease. EcoHealth 4:135–150.

Hallegraeff, G. M. 1993. A review of harmful algal blooms and their apparent global increase. Phycologia 32:79–99.

Hattenrath-Lehmann, T. K., M. A. Marcoval, D. L. Berry, S. Fire, Z. Wang, S. L. Morton and C. J. Gobler. 2013. The emergence of *Dinophysis acuminata* blooms and DSP toxins in shellfish in New York waters. Harmful Algae 26:33–44.

Hernandez, M., I. Robinson, A. Aguilar, *et al*. 1998. Did algal toxins cause monk seal mortality? Nature 393:28–29.

IMARPE. 2012*a*. Mortandad de delfines en el litoral de la costa norte, febrero a abril del 2012 [Mortality of dolphins along the northern coast, February to April 2012]. Final report, Instituto del Mar del Perú, Mayo 2012. Available at http://elcomercio.e3.pe/66/doc/0/0/4/7/5/475394.pdf.

IMARPE. 2012*b*. Monitoreo de fitoplancton potencialmente tóxico en Chincha-Pisco [Monitoring of potentially toxic phytoplankton in Chincha-Pisco]. Instituto del Mar del Perú. Available at

http://www4.imarpe.gob.pe/imarpe/index.php?id_seccion=I0108030100000000000000.

IMARPE. 2013. Anuario Científico y Tecnológico IMARPE [IMARPE Annual Scientific and Technological Report]. Volume 13. Instituto del Mar del Perú. Available at http://biblioimarpe.imarpe.gob.pe:8080/bitstream/handle/123456789/2298/Anuario%202013.pdf?sequence=1.

Jankowski, G., M. J. Adkesson, J. T. Saliki, S. Cárdenas-Alayza and P. Majluf. 2015. Survey for infectious disease in the South American fur seal (*Arctocephalus australis*) population at Punta San Juan, Peru. Journal of Zoo and Wildlife Medicine 46:246–254.

Landsberg, J. H. 2002. The effects of harmful algal blooms on aquatic organisms. Reviews in Fisheries Science 10:113–390.

Lefebvre, K. A., C. L. Powell, M. Busman, *et al*. 1999. Detection of domoic acid in northern anchovies and California sea lions associated with an unusual mortality event. Natural Toxins 7:85–92.

Lefebvre, K. A., A. Robertson, E. R. Frame, *et al*. 2010. Clinical signs and histopathology associated with domoic acid poisoning in northern fur seals (*Callorhinus ursinus*) and comparison of toxin detection methods. Harmful Algae 9:374–383.

Lloyd, J. K., J. S. Duchin, J. Borchert, H. F. Quintana and A. Robertson. 2013. Diarrhetic shellfish poisoning, Washington, USA, 2011. Emerging infectious diseases 19:1314–1316.

López-Rivera, A., M. Pinto, A. Insinilla, *et al*. 2009. The occurrence of domoic acid linked to a toxic diatom bloom in a new potential vector: The tunicate *Pyura chilensis*

(piure). Toxicon 54:754–762.

Majluf, P., and J. C. Reyes. 1989. The marine mammals of Peru: A review. Pages 344–363 *in* D. Pauly, P. Muck, J. Mendo and I. Tsukayama, eds. The Peruvian upwelling ecosystem: Dynamics and interactions. ICLARM Conference Proceedings. ICLARM, Manila, Philippines.

Majluf, P., and F. Trillmich. 1981. Distribution and abundance of sea lions (*Otaria byronia*) and fur seals (*Arctocephalus australis*) in Peru. Zeitschrift für Säugetierkunde 46:384–393.

Mathisen, O. A. 1989. Adaptation of the anchoveta (*Engraulis ringens*) to the Peruvian upwelling system. Pages 220–234 *in* D. Pauly, P. Muck, J. Mendo and I. Tsukuyama, eds. The Peruvian upwelling ecosystem: Dynamics and interactions. ICLARM Conference Proceedings. ICLARM, Manila, Philippines.

Matias, W. G., A. Traore and E. E. Creppy. 1999. Variations in the distribution of okadaic acid in organs and biological fluids of mice related to diarrhoeic syndrome. Human & Experimental Toxicology 18:345–350.

MINAM. 2012. Mortandad de aves y mamíferos marinos [Mortality of birds and marine mammals]. Peru Ministerio del Ambiente, Lima, Peru. Available at http://www.imarpe.gob.pe/paita/xtras/inf_mortandad_minam.pdf.

Moore, S. K., N. J. Mantua, B. M. Hickey and V. L. Trainer. 2010. The relative influences of El Niño-Southern Oscillation and Pacific Decadal Oscillation on paralytic shellfish toxin accumulation in northwest Pacific shellfish. Limnology and Oceanography 55:2262–2274.

Muck, P., and H. Fuentes. 1987. Sea lion and fur seal predation

on the Peruvian anchoveta, 1953 to 1982. Pages 234–247 *in* D. Tsukayama and I. Pauly, eds. The Peruvian anchoveta and its upwelling ecosystem: Three decades of change. ICLARM Studies and Reviews 15. Instituto del Mar del Peru (IMARPE), Callao, Peru; Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ), GmbH, Eschbom, Federal Republic of Gemany; and International Center for Living Aquatic Resources Management (ICLARM), Manila, Philippines.

Perl, T. M., L. Bédard, T. Kosatsky, J. C. Hockin, E. C. Todd and R. S. Remis. 1990. An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. New England Journal of Medicine 322:1775–1780.

Pyenson, N. D., C. S. Gutstein, J. F. Parham, *et al*. 2014. Repeated mass strandings of Miocene marine mammals from Atacama Region of Chile point to sudden death at sea. Proceedings of the Royal Society of London B: Biological Sciences 281:20133316.

Ramsdell, J. S., and T. S. Zabka. 2008. In utero domoic acid toxicity: A fetal basis to adult disease in the California sea lion (*Zalophus californianus*). Marine Drugs 6:262–290.

Reguera, B., P. Riobó, F. Rodríguez, *et al*. 2014. Dinophysis toxins: causative organisms, distribution and fate in shellfish. Marine Drugs 12:394–461.

Reid, J. L., G. I. Roden and J. G. Wyllie. 1958. Studies of the California Current system. Reports of California Cooperative Oceanic Fisheries Investigations 6:21–56.

Schnetzer, A., P. E. Miller, R. A. Schaffner, *et al*. 2007. Blooms of *Pseudo-nitzschia* and domoic acid in the San Pedro Channel and Los Angeles harbor areas of the Southern California Bight, 2003–2004. Harmful Algae 6:372–387.

Scholin, C. A., F. Gulland, G. J. Doucette, *et al*. 2000. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. Nature 403:80–84.

Smith, J. C., J. L. McLachlan, P. G. Cormier, K. E. Pauley and N. Bouchard. 1993. Growth and domoic acid production and retention by *Nitzschia pungens* forma *multiseries* at low temperatures. Pages 631–636 *in* T. J. Smayda and Y. Shimizu, eds. Toxic phytoplankton blooms in the sea. Elsevier, Amsterdam, The Netherlands.

Stevens, M. A., and D. J. Boness. 2003. Influences of habitat features and human disturbance on use of breeding sites by a declining population of southern fur seals (*Arctocephalus australis*). Journal of Zoology 260:145–152.

Svensson, S. 2003. Depuration of okadaic acid (diarrhetic shellfish toxin) in mussels, *Mytilus edulis* (Linnaeus), feeding on different quantities of nontoxic algae. Aquaculture 218:277–291.

Sydeman, W. J., and S. G. Allen. 1999. Pinniped population dynamics in central California: Correlations with sea surface temperature and upwelling indices. Marine Mammal Science 15:446–461.

Thessen, A. E., H. A. Bowers and D. K. Stoecker. 2009. Intra- and interspecies differences in growth and toxicity of *Pseudo-nitzschia* while using different nitrogen sources. Harmful Algae 8:792–810.

Trainer, V. L., G. C. Pitcher, B. Reguera and T. J. Smayda. 2010. The distribution and impacts of harmful algal bloom species in eastern boundary upwelling systems. Progress in Oceanography 85:33–52.

Truelove, J., R. Mueller, O. Pulido, L. Martin, S. Fernie and F.

Iverson. 1997. 30-day oral toxicity study of domoic acid in cynomolgus monkeys: Lack of overt toxicity at doses approaching the acute toxic dose. Natural Toxins 5:111–114.

Valdiglesias, V., M. V. Prego-Faraldo, E. Pásaro, J. Méndez and B. Laffon. 2013. Okadaic acid: Mmore than a diarrheic toxin. Marine Drugs 11:4328–4349.

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

Wang, Z., J. Maucher-Fuquay, S. E. Fire, C. M. Mikulski, B. Haynes, G. J. Doucette and J. S. 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 715:71–79.

Zabka, T. S., T. Goldstein, C. Cross, R. W. Mueller, C. Kreuder-Johnson, S. Gill and F. M. D. Gulland. 2009. Characterization of a degenerative cardiomyopathy associated with domoic acid toxicity in California sea lions (*Zalophus californianus*). Veterinary Pathology 46:105–119.

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Figure 1. Location of rookery beaches (shaded gray) and sample collection site (shaded gray), Punta San Juan, Peru. Adapted from Cárdenas-Alayza (2012).

Figure 2. Phytoplankton abundance and temporal distribution in relation to toxin concentrations in PSJ pinnipeds. DA = domoic acid, OA = okadaic acid, STX = saxitoxins.

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[2] *Alexandrium* sp., *Alexandrium peruvianum, Cochlodinium polykrikoides, Dinophysis* sp., *Dinophysis acuminata, Dinophysis caudata, Dinophysis rotundata, Gonyaulax spinifera, Gymnodinium* sp., *Gymnodinium impudicum, Karenia* sp., *Karlodinium* sp., *Karlodinium veneficum, Prorocentrum lima, Prorocentrum minimum, Protoperidinium crassipes, Protoperidinium depressum, Pseudo-nitzschia delicatissima, Pseudo-nitzschia pungens, Pseudo-nitzschia seriata*.

Table 1. Concentrations of domoic acid (DA), okadaic acid (OA), and saxitoxins (STX) detected (ng/g). FS = fur seals, SL = sea lions. <dl = below limit of detection. Date format = mm/dd/yyyy.

				DA			OA			STX		
Animal ID	Species	Date	Sex	Urine	Feces	Gastric	Urine	Feces	Gastric	Urine	Feces	Gastric
AA11-02	FS	11/11/2011	M		<dl							
AA11-03	FS	11/11/2011	M			<dl						<dl
AA11-04	FS	11/11/2011	M		<dl	<dl					<dl	<dl
AA11-05	FS	11/12/2011	M		<dl	<dl					<dl	<dl
AA11-06	FS	11/12/2011	M		<dl	<dl					<dl	<dl
AA11-07	FS	11/12/2011	M		trace						<dl	
AA11-08	FS	11/13/2011	M		trace	<dl						<dl
AA11-09	FS	11/13/2011	M		<dl	<dl						<dl
AA11-11	FS	11/14/2011	M		8	<dl						
AA11-12	FS	11/14/2011	M		<dl	<dl					<dl	<dl
AA11-13	FS	11/15/2011	M		<dl	<dl						<dl
AA11-14	FS	11/15/2011	M		<dl							
AA11-15	FS	11/16/2011	F		<dl						<dl	
AA11-16	FS	11/16/2011	F		<dl	<dl						<dl
AA11-18	FS	11/16/2011	F		<dl	<dl					818	<dl
AA11-19	FS	11/16/2011	F		<dl							
AA11-20	FS	11/16/2011	F		<dl						<dl	
AA11-21	FS	11/17/2011	F		18						<dl	
AA11-22	FS	11/17/2011	F		<dl	<dl					<dl	<dl
AA11-23	FS	11/17/2011	F		<dl	<dl					<dl	<dl
AA11-24	FS	11/17/2011	F		<dl	<dl					<dl	<dl

AA11-25	FS	11/17/2011	F	<dl	<dl					<dl	<dl
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AA11-27	FS	11/18/2011	F	10	<dl					<dl	<dl
AA11-28	FS	11/18/2011	F	<dl	<dl					<dl	<dl
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AA11-31	FS	11/19/2011	F	533							
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OF11-02	SL	11/11/2011	M						<dl		
OF11-03	SL	11/12/2011	M		<dl						<dl
OF11-04	SL	11/12/2011	M	<dl						133	
OF11-05	SL	11/13/2011	M	525						<dl	
OF11-06	SL	11/14/2011	M	215	<dl					<dl	<dl
OF11-07	SL	11/14/2011	M	<dl	<dl					<dl	<dl
OF11-10	SL	11/15/2011	M	10	<dl						<dl
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AA1202	FS	11/10/2012	M		<dl	<dl	<dl				<dl
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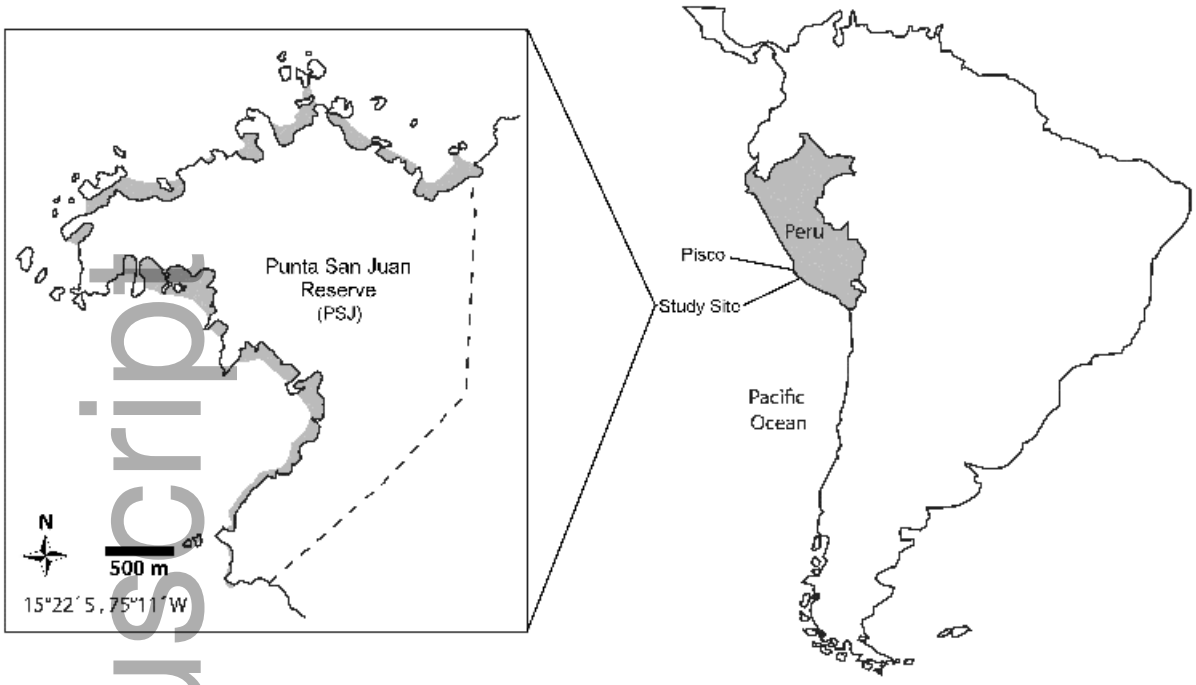
AA1206	FS	11/12/2012	F			36					
AA1207	FS	11/12/2012	F	<dl							
AA1210	FS	11/13/2012	F			<dl					
AA1212	FS	11/13/2012	F	<dl							
AA1214	FS	11/13/2012	F								

AA1218	FS	11/14/2012	F		<dl								
AA1220	FS	11/14/2012	F			<dl		0.8	<dl				<dl
AA1222	FS	11/14/2012	F		<dl			<dl			<dl		
AA1224	FS	11/14/2012	F		<dl	<dl		<dl	<dl		<dl		
AA1226	FS	11/14/2012	F					<dl					
AA1228	FS	11/14/2012	F		<dl			0.5			<dl		<dl
AA1230	FS	11/14/2012	F		19			8			<dl		
AA1232	FS	11/14/2012	F		<dl								
OF1201	SL	11/9/2012	M	<dl		<dl	<dl	<dl		<dl			
OF1202	SL	11/9/2012	M		<dl	<dl		<dl	<dl				<dl
OF1203	SL	11/9/2012	M		11								
OF1204	SL	11/11/2012	M			<dl			<dl				<dl
OF1205	SL	11/11/2012	M			<dl			<dl				<dl
OF1206	SL	11/11/2012	M		<dl						<dl		
OF1207	SL	11/12/2012	M		<dl	<dl		9	<dl		<dl		<dl
OF1208	SL	11/12/2012	M					<dl					



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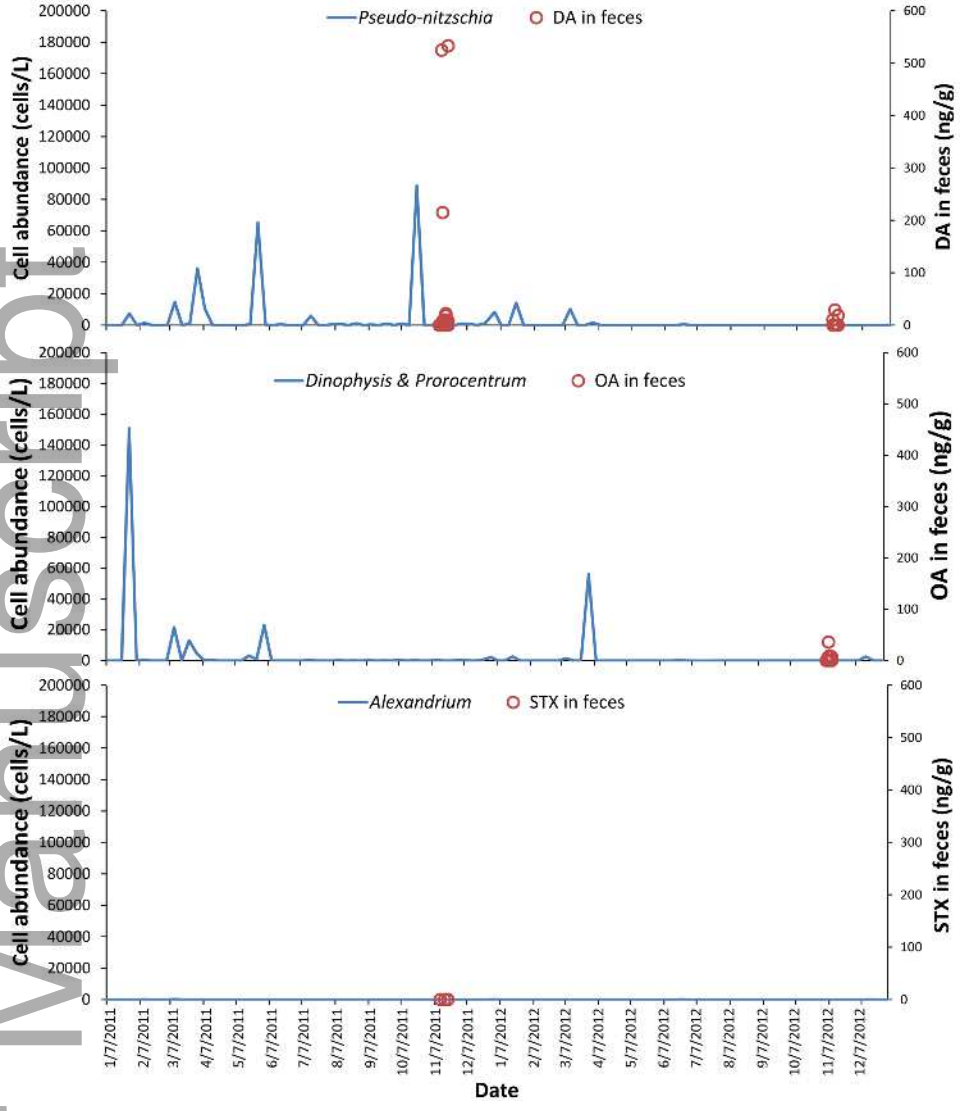

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