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Algal toxins and reverse osmosis desalination operations: Laboratory bench testing and field monitoring of domoic acid, saxitoxin, brevetoxin and okadaic acid

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ABSTRACT

The occurrence and intensity of harmful algal blooms (HABs) have been increasing globally during the past few decades. The impact of these events on seawater desalination facilities has become an important topic in recent years due to enhanced societal interest and reliance on this technology for augmenting world water supplies. A variety of harmful bloom-forming species of microalgae occur in southern California, as well as many other locations throughout the world, and several of these species are known to produce potent neurotoxins. These algal toxins can cause a myriad of human health issues, including death, when ingested via contaminated seafood. This study was designed to investigate the impact that algal toxin presence may have on both the intake and reverse osmosis (RO) desalination process; most importantly, whether or not the naturally occurring algal toxins can pass through the RO membrane and into the desalination product. Bench-scale RO experiments were conducted to explore the potential of extracellular algal toxins contaminating the RO product. Concentrations exceeding maximal values previously reported during natural blooms were used in the laboratory experiments, with treatments comprised of 50 μg/L of domoic acid (DA), 2 μg/L of saxitoxin (STX) and 20 μg/L of brevetoxin (PbTx). None of the algal toxins used in the bench-scale experiments were detectable in the desalinated product water. Monitoring for intracellular and extracellular concentrations of DA, STX, PbTx and okadaic acid (OA) within the intake and desalinated water from a pilot RO desalination plant in El Segundo, CA, was conducted from 2005 to 2009. During the five-year monitoring period, DA and STX were detected sporadically in the intake waters but

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never in the desalinated water. PbTx and OA were not detected in either the intake or desalinated water. The results of this study demonstrate the potential for HAB toxins to be inducted into coastal RO intake facilities, and the ability of typical RO operations to effectively remove these toxins.

1. Introduction

Burgeoning worldwide population has amplified societal interest in developing new and reliable sources of potable water, particularly in naturally arid, highly populated regions. One example of this trend is the semi-arid region of southern California, USA, which has been confronted with shortages of freshwater availability since the early 1900s. Several municipalities and public works agencies have begun to examine seawater desalination as a local source of potable water that can augment existing supplies and address the environmental concerns associated with the current imported water supply.

Seawater desalination faces a number of challenges in order to contribute significantly as an environmentally safe and cost-effective source of potable water in California. One factor complicating the use of desalinated seawater for human consumption is the increasing frequency and impact of nuisance and harmful algal blooms (HABs) in coastal southern California waters (Lewitus et al., 2012). Increases in HAB events have also been documented worldwide, with anthropogenic influences being identified as significant contributors to these increases (Anderson et al., 2002; Glibert et al., 2005; Heisler et al., 2008; Kudela et al., 2008; Paerl and Paul, 2011). HABs can negatively impact coastal seawater desalination facilities through accumulations of high microalgal biomass near intake pipes that increase the solids load to prefiltration processes, increase membrane fouling or biofouling in reverse osmosis (RO) membranes, and increased chemical consumption (Abdul Azis et al., 2000; Ladner et al., 2010; Zhang et al., 2011; Franks et al., url). These concentrated plumes of algal biomass may result in the presence of significant levels of extracellular algal toxins in the intake water and a reduction in dissolved oxygen concentrations as the bloom undergoes decomposition that can make treatment more challenging (Caron et al., 2010). Seawater desalination facilities in the Persian Gulf and the Gulf of Oman have experienced temporary shutdown of their plants during periods of high algal biomass blooms near their intake pipes, until algal biomass sufficiently decreased (Pankrantz, 2008; Nazzal, 2009; Richlen et al., 2010).

While investigations have been conducted to address pretreatment strategies for the removal of high microalgal biomass load prior to introducing the feed water to the RO process (Pearce et al., 2004; Kim and Yoon, 2005; Kwon et al., 2005; Castaing et al., 2010; Desormeaux et al., 2011; Vardon et al., 2011), the fate of microalgal toxins within the desalination process has not been adequately addressed. The wide variety of toxins currently known to be produced in naturally occurring microalgal blooms and the possibility for more than one type of toxin to occur at any given time (Van Dolah, 2000) complicate our understanding of the impact that toxin presence may have on human health via the desalinated product water. In southern California, microalgal species known to produce domoic acid (DA), saxitoxin (STX), brevetoxin (PbTx) and okadaic acid (OA) are frequent, albeit highly variable contributors to the local microalgal community (Caron et al., 2010; Lewitus et al., 2012). The interaction between the potential presence of these toxins and the RO desalination process are an important consideration for existing and future coastal desalination operations in the area. It has been demonstrated in previous work on predicting molecule rejection that molecular weight alone may not determine the rejection capability of a membrane (Verliefde et al., 2006). Rejection capabilities can be impacted by the hydrophobicity of the molecule, surface charge, operating conditions such as the specific flux, and feed water composition. Therefore, it is useful to empirically examine the retention or passage of a molecule of interest.

DA is a neurotoxin produced by species of the diatom genus *Pseudo-nitzschia* in southern California (Caron et al., 2010). It is a hydrosoluble molecule with a molecular mass of 311.14 g/mole. It is generally known that RO membranes are designed to efficiently reject the vast majority of molecules greater than approximately 250 g/mole, making DA an interesting toxin to challenge an RO membrane. DA is seasonally present in the southern California region and therefore has raised concern for successful operation of a desalination plant in the area. Human exposure to DA is typically a consequence of consuming contaminated seafood (generally filter-feeding fish and shellfish). The ensuing condition is referred to as Amnesic Shellfish Poisoning (ASP) and results in symptoms ranging from gastroenteritis (vomiting, diarrhea, abdominal cramps) to confusion, memory loss, disorientation, seizures, coma and/or cranial nerve palsies or death (Perl et al., 1990; Wright et al., 1990).

STX is the parent compound of the group of neurotoxins classified as the Paralytic Shellfish Toxins (PSTs). The known producer of PSTs in southern California is the dinoflagellate *Alexandrium catenella* (Caron et al., 2010; Garneau et al., 2011; Lewitus et al., 2012). STX is the most potent of the more than 30 identified PSTs, and is classified as a chemical weapon in Schedule 1 of the Chemical Weapons Convention (Llewellyn, 2006). The hydrosoluble STX molecule has a molecular mass of 299.3 g/mol, making it closer than DA to the theoretical molecular weight cutoff of an RO membrane. STX is not as pervasive as DA in the southern California area, but recent research has continued to document STX along the California coast (Jester et al., 2009b; Garneau et al., 2011), highlighting it as a concern for successful operation of desalination facilities in the area. Due to the potency of STX, *A. catenella* does not need to be a dominant member of the microalgal community in order to constitute a significant risk to human health (Burkholder et al., 2006). Consumption of a lethal dose can result in death within hours due to muscular paralysis and

respiratory difficulty. Worldwide, over 2000 illnesses each year can be attributed to consumption of seafood contaminated with PSTs, with a 5–10% mortality rate (Hallegraeff, 2003).

PbTxs are a suite of neurotoxins that can be produced by raphidophyte algae found in southern California, specifically *Chattonella marina*, *Heterosigma akashiwo*, and *Fibrocapsa japonica* (Caron et al., 2010; Lewitus et al., 2012). There are 13 derivatives of PbTx that have been identified, the most common to the marine environment are PbTx-2, PbTx-3 and PbTx-9 and of these three, PbTx-2 and PbTx-3 are the most potent (Baden et al., 2005). The molecular masses of the liposoluble PbTxs are large at approximately 899 g/mol. While their size would predict successful rejection by RO membranes, their hydrophobic nature may cause unique solute–membrane interactions, impacting rejection capabilities. Humans that consume seafood containing PbTxs may experience Neurotoxic Shellfish Poisoning (NSP), which causes symptoms of nausea, vomiting, abdominal cramps, paresthesia and respiratory illness and/or failure (Kirkpatrick et al., 2004).

OA is a member of a suite of toxins identified as Diarrhetic Shellfish Toxins (DSTs) which includes dinophysistoxins and pectenotoxins (Caron et al., 2010; Lewitus et al., 2012). DSTs can be produced by members of the dinoflagellate genus *Prorocentrum*, although they are more commonly produced by the genus *Dinophysis*. The two *Dinophysis* species on the west coast of the US known to have the capability of producing OA are *D. acuminata* and *D. fortii* (Yasumoto et al., 1980, 1985; Murata et al., 1982). OA is a liposoluble molecule with a molecular mass of 805 g/mol, much larger than the theoretical molecular weight rejection capabilities of RO membranes but solute–membrane interactions may be negatively impacted by it's hydrophobicity. Ingestion of seafood containing OA and other DSTs leads to Diarrhetic Shellfish Poisoning (DSP) in humans. Symptoms of DSP include abdominal cramps, inflammation of the intestinal tract, and diarrhea (Hallegraeff, 2003).

Microalgae that are capable of producing DA, STX, PbTx and OA are known to be present in southern California waters, although to date only DA and STX have been routinely observed (Anderson et al., 2006; Busse et al., 2006; Mengelt, 2006; Schnetzer et al., 2007; Sekula-Wood et al., 2009; Garneau et al., 2011; Lewitus et al., 2012). The seasonality of these latter toxins throughout the fifteen coastal California counties has been established from an analysis of shellfish tissues by the Marine Biotoxin Monitoring Program (MBMP) of the California Department of Public Health (CDPH) during the period 2002–2007 (Langlois, 2007; Caron et al., 2010). On average, DA concentrations exhibit a strong maximum in spring and minor maximum in fall, while STX concentrations exhibit a maximum in late summer to early fall.

Experimental studies were conducted in the laboratory using a bench-scale RO apparatus to examine if extracellular DA, STX or PbTx at concentrations at or above those observed during substantial blooms of toxin-producing microalgae in the region pose a threat for passage through the RO membrane during standard operations at the RO desalination plant operated by the West Basin Municipal Water District. Operating conditions (i.e. pressure, specific flux and recovery) and feed water composition (i.e. temperature and pH) reflected the conditions experienced at their facility allowing accurate prediction of algal toxin rejection. OA was not included in the bench-scale experiments due to the lack of a commercially available ELISA platform capable of analyzing extracellular OA. In addition, the intake and resulting desalinated water from the pilot desalination plant in El Segundo, CA, was monitored for various microalgal toxins over a five-year period in order to assess the potential for naturally occurring algal toxins to be inducted into seawater desalination operations in southern California. The monitoring program offered insight into the magnitude and duration of toxic HABs in a coastal location during this period.

2. Materials and methods

2.1. Experiments using bench-top reverse osmosis

Experiments designed to directly test the efficacy of RO to remove microalgal toxins common to southern California waters were conducted using a bench-scale RO setup. Monitoring of microalgal toxins in the extracellular phase is not customary and the extracellular concentrations chosen for the bench-scale RO experiments were based upon previously published values for intracellular concentrations, with the assumption that the pretreatment process (micro or ultrafiltration) may efficiently disrupt cells, and in the worst-case scenario release all toxin previously contained within the cell into the extracellular phase (Ladner et al., 2010; Desormeaux et al., 2011). In general, the microalgal toxin concentrations used for the bench-scale RO challenge were derived to exceed typical concentrations of extracellular microalgal toxins observed in US west coast seawater (e.g. ~10 times greater). Concentrations of 50 μg/L extracellular DA (Sigma Aldrich®; St. Louis, MO), 2 μg/L extracellular STX (National Research Council, Institute for Marine Biosciences; Halifax, Canada) and 20 μg/L extracellular PbTx-2 (World Ocean Solutions, LLC; Durham, NC) were used in the laboratory experiments.

The bench-scale RO experiments were undertaken using a SEPA® CF II Membrane Element Cell (Osmonics Inc., Minnetonka, MN) and SCW4 + RO membranes (Hydranautics, Oceanside, CA). This instrument was selected because it could provide a cross-flow environment, not simple dead-end filtration, that more closely represents full-scale RO facilities and was capable of operating under the pressure required for seawater applications. The experimental setup was constructed as a closed system, with water pumped from a reservoir (10 L) containing the algal toxin onto the RO membrane apparatus at a specified pressure, and both permeate and retentate were returned to the reservoir (Fig. 1). The reservoir in the bench-scale RO experiments consisted of 10 L of seawater previously passed through a microfiltration process (20 μm), and 10 L of concentrated seawater collected from the retentate side of the pilot desalination plant in a 20 L polycarbonate carboy. The resulting mixture targeted a conductivity of approximately 85 mS/cm to represent the midpoint of salinity in the feed/concentrate stream for a typical desalination system with 50% recovery.

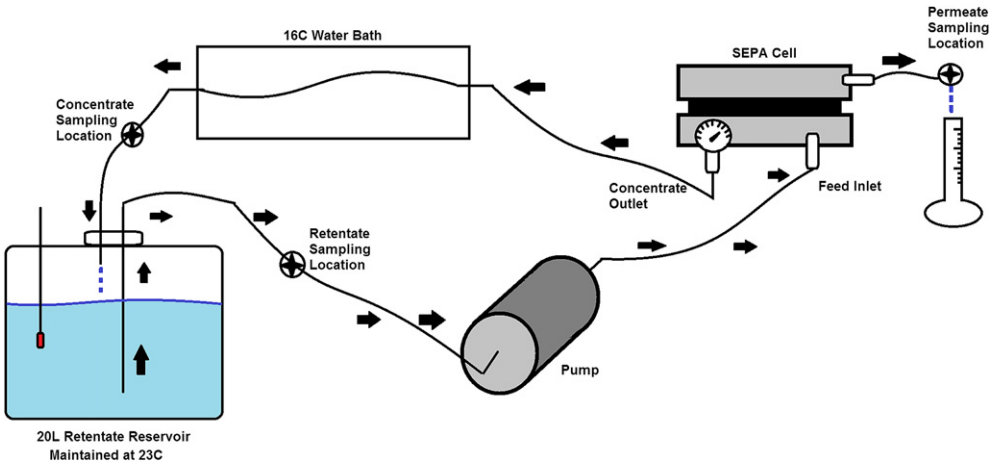

Fig. 1 – Diagram of the bench-scale RO experimental setup. Arrows indicate the direction of water flow through the closed system, and the sampling locations for the retentate, concentrate and permeate are identified.

The closed system approach was used in order to maintain the overall toxin concentration during each experiment. Retentate water was passed through a cooling water bath prior to its return to the reservoir to minimize increases in water temperature, which occurred due to a high pressure pump used in the RO system. Membranes were conditioned at the start of experiments using dechlorinated tap water (tap water treated with 16 μM sodium metabisulfate), followed by rinses with a 0.20 M $MgSO_4$ solution, dechlorinated tap water, a 0.5 M NaCl solution, dechlorinated tap water, a 50/50 mixture of natural, 0.2 μm filtered seawater and the concentrated seawater retentate noted above. The extensive conditioning procedure was designed to establish a steady state operating condition prior to the addition of toxin. The procedure allowed for the confident removal of preservative present on the new membrane and for the membrane to stabilize following the initial compaction experienced after first exposure to high pressure. The conditioned membrane was then challenged with toxin, which was added to the reservoir of the experimental setup. Pressure of the water pumped from the reservoir into the RO unit was operated at a target pressure of 900 psi and adjusted throughout the duration of the experiments in order to maintain a permeate flux of 9 gallons per square foot of membrane per day (GFD) and a cross flow velocity at an average of 0.5 m/sec. Monitoring for conductivity, flux and specific flux (gallons per square foot of membrane per day per unit pressure; SPF) were conducted at regular intervals throughout the duration of each experiment.

Experiments using the bench-scale RO system were performed continuously for 96 h in the DA trial and for 48 h in the PbTx and STX trials. Following the first 96-hour trial with DA, the duration of the bench-scale experiments was shortened to 48 h to allow for an increase in sampling frequency. The reservoir, retentate and permeate were sampled separately in order to trace the fate of the microalgal toxins in the experiments and allow for a mass balance approach to account for all toxins (Fig. 1). For the DA trial, the reservoir, retentate and permeate were sampled initially after addition of toxin, after 1 h, 6 h and every 12 h thereafter. Toxins in the PbTx and STX trials were sampled from the reservoir, retentate and permeate initially, after 1 h, and every 4 h thereafter. All samples were stored at −20 °C until analyses for extracellular toxins using Enzyme Linked ImmunoSorbent Assays (ELISAs). OA was not measured in the laboratory experiments due to the lack of a commercially available ELISA platform capable of analyzing extracellular OA.

The use of a high pressure pump to pass water through the RO membrane resulted in a rise in water temperature, which was addressed by immersing the tubing of the SEPA cell (retentate side) in a 16 °C water bath. A consistent water temperature of 23 °C was maintained in the reservoir, approaching the water temperature typically observed at the pilot desalination plant at El Segundo, CA. A mass balance calculation of the amount of toxin in the reservoir and RO unit system during each experiment was conducted in order to ensure that degradation of microalgal toxins during the bench-scale experiments due to light and temperature fluctuations was negligible.

2.2. *Field sampling*

The intake and resulting desalinated water (permeate) from the pilot desalination plant located in El Segundo, CA, was examined for the presence and concentrations of the algal toxins DA, STX, PbTx and OA to test the efficacy of toxin removal by the RO process. These microalgal toxins were chosen due to their known occurrence in southern California and/or the occurrence of the potential toxin-producing microalgae. Sampling was conducted on a weekly basis during months of full plant operation between 2005 and 2009. The pilot plant operated by the West Basin Municipal Water District (Carson, CA) was co-located at the El Segundo Generating Station (33°55′49.88″N, 118°26′8.31″W; Fig. 2). The intake pipe was located at a depth of approximately 10 m in Santa Monica Bay, CA. The intake water was sampled following passage through a micro-prefiltration system, and the permeate was sampled post-RO-desalination. Samples to

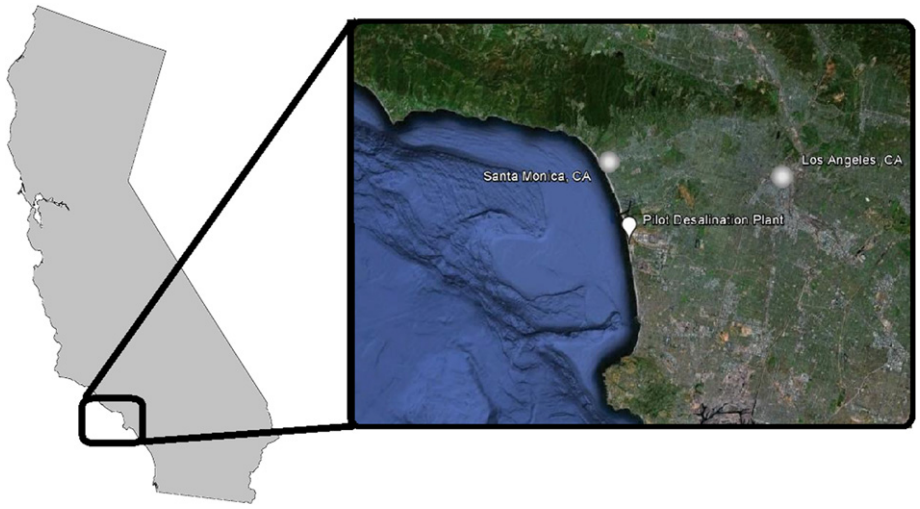

Fig. 2 – Location of the pilot desalination plant operated by the West Basin Municipal Water District in El Segundo, CA, USA, co-located at the El Segundo Generating Station.

be processed for DA, STX and OA were collected in 1 L polycarbonate bottles and stored at 4 °C for no longer than 24 h until processing. Samples to be processed for PbTx were collected in 1 L glass bottles, due to the tendency of PbTx to adsorb to plastic, and stored at 4 °C for no longer than 24 h until processing. Samples for the analysis of intracellular toxins were collected by filtering 200–400 mL of the intake water onto GF/F Whatman filters, which concentrated the cells present in the water and improved the limit of detection for the different algal toxins analyzed. Extracellular algal toxin samples were analyzed on aliquots of seawater collected from the filtrate of the intake water, and from the permeate water of the RO system. All intracellular and extracellular samples were stored at −20 °C until analysis via ELISAs.

2.3. *Analysis of algal toxins*

Concentrations of DA were analyzed using the Amnesic Shellfish Poisoning ELISA from Biosense™ Laboratories (Bergen, Norway) for field samples collected between 2005 and 2008, employing adaptations for intracellular samples and extracellular seawater analyses (Schnetzer et al., 2007). The limit of detection for this method was 0.1 μg/L for extracellular samples and 0.003 μg/L for intracellular samples (the latter value was dependent on the volume of sample filtered). All other DA samples were analyzed using the Mercury Science DA ELISA (Durham, NC). The samples from the permeate of the DA bench-scale experiment were analyzed on both DA ELISA platforms. The limit of detection for the latter method was 0.2 μg/L for extracellular samples and 0.007 μg/L for intracellular samples. Samples for the determination of STX, PbTx and OA were analyzed using ELISA kits available from Abraxis (Warminster, PA). The limit of detection for extracellular STX samples was 0.02 μg/L (intracellular STX was not measured as part of this study). The STX ELISA is capable of detecting STX with 100% efficiency and other PSTs at ≤29%, as reported by the manufacturer, reflecting the ability to identify STX but the low efficiency to detect the other analogs. Data reported here on STX is described in μg/L of STX and not STX equivalents. A detection limit of 0.008 μg/L was achieved for intracellular PbTx and 0.1 μg/L for extracellular PbTx. The PbTx ELISA is reported by the manufacturer to detect PbTx-5 at 127%, deoxy PbTx-2 at 133%, PbTx-2 at 102% and PbTx-3 at 100% efficiency. The limit of detection for intracellular OA samples was 0.008 ng OA/mL. The OA ELISA was capable of detecting OA 100%, as well as DTX 1 and DTX 2 with 50% efficiency, as reported by the manufacturer. Detection of intracellular toxins was more sensitive than the analyses for extracellular toxins because significant volumes of water were filtered for the measurement of intracellular toxins. For intracellular toxin analysis (DA, PbTx and OA) samples were extracted in 3 mL of 10% methanol (Fisher) and sonicated for 60 s. Following sonication, the samples were centrifuged for 10 min at 4000 rpm. Dilutions of the supernatant were prepared with the sample dilution buffer specified by the individual ELISA assays. Extracellular toxin samples (DA, STX, PbTx and OA) were vortexed briefly and diluted with the sample dilution buffer. Intracellular and extracellular samples were handled per manual instructions for each ELISA platform but were optimized prior to sample analysis in order to minimize matrix effects and false positives. Tests for false positives were conducted by analyzing the extraction fluid (10% methanol) and toxin-free seawater diluted to varying degrees and analyzed on each ELISA platform to investigate interference from the solution matrix. The dilution ultimately employed to analyze the laboratory experiments was selected based on these results. Efficacy of the optimization was confirmed using filtered seawater spiked with known concentrations of toxins to investigate the possibility of false negatives. Based on the results of these procedures, intracellular DA concentrations were measured at a minimum dilution of 1:25 on the Biosense ELISA and a minimum 1:10 dilution on the Mercury Science ELISA. Extracellular DA concentrations were measured at a minimum 1:10 dilution on the Biosense ELISA and a minimum 1:2 dilution on the Mercury Science ELISA. Intracellular and extracellular STX

and PbTx as well as extracellular OA samples were measured at a minimum 1:10 dilution. Completed ELISA plates were read at 425 nm on a ThermoMax Microplate reader (Molecular Devices; Sunnyvale, CA) and data compiled by SoftMax Pro software (Molecular Devices; Sunnyvale CA).

3. Results

3.1. *Bench-top RO experiments with DA, STX and PbTx*

The RO process employed in this study resulted in the reduction of DA, STX and PbTx in the permeate of the bench-top setup in all experiments, as evidenced by the lack of detectable concentrations of any of the toxins tested. No DA was detected in the permeate at any time during the experiment, regardless of the DA ELISA used for analysis (Fig. 3A). The DA concentration in the reservoir averaged 53.8 ± 6.9 μg/L throughout the 96-hour experiment, with no perceptible or consistent change over the course of the trial (Fig. 3A). DA concentration in the retentate of the RO setup averaged 52.9 ± 6.4 μg/L over the 96-hour experiment, and no significant trend in DA concentration in the retentate was observed during the experiment (Fig. 3A). The maintenance of a mass balance for DA in the reservoir and retentate signified that the toxin was not significantly degraded during the course of the bench-scale experiment due to light, temperature or microbial processes, and was not appreciably adsorbed or absorbed onto the membrane or other surfaces of the experimental setup. The flux (GFD) and specific flux (SPF) for the DA trial averaged 8.41 ± 0.65 gfd and 0.026 ± 0.001 gfd/psi (average ±1 standard deviation; Fig. 3B), respectively. The applied pressure ranged from 780 to 880 psi in the DA trial in order to maintain a permeate flux near the 9 gfd target. The relative constancy of these values throughout the duration of the 96-hour experiment indicated that membrane performance remained stable during the experiment, with no noticeable fouling that would result in changes in these parameters.

No STX was detected in any of the permeate samples collected for the duration of the 48-hour experiment. The concentration of STX in the reservoir averaged 2.5 ± 0.4 μg/L and the concentration in the retentate averaged 2.7 ± 0.3 μg/L (Fig. 4A). The reservoir water contained 0.4 μg/L extracellular STX prior to the addition of the 2.0 μg/L STX standard, resulting in a higher concentration of STX in the reservoir for the duration of the experiment from the expected value of

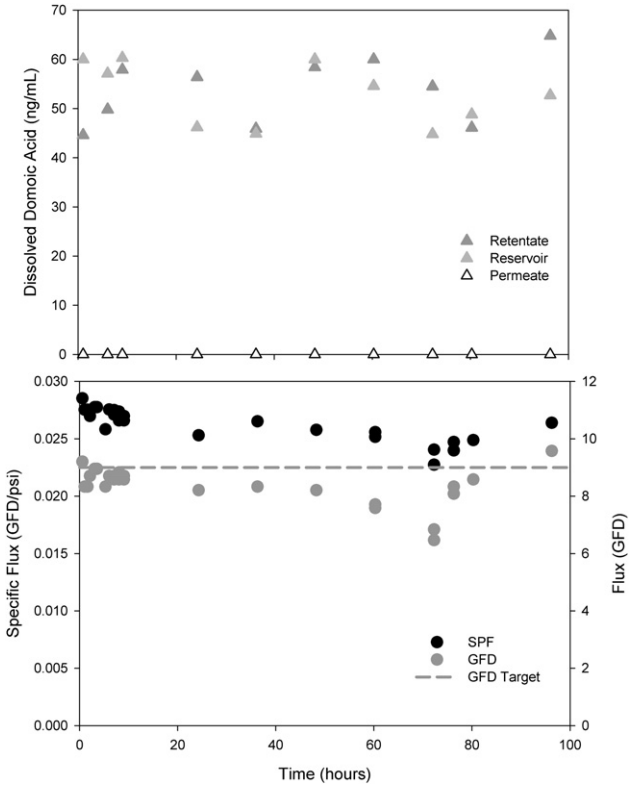

Fig. 3 – Top panel: The change in concentration of extracellular DA over the duration of the bench-scale RO experiment (96 h), as measured in the reservoir (light gray triangles), permeate (open triangles) and retentate (dark gray triangles) streams. The open triangles denote concentrations under the limit of detection of the ELISA method used. Bottom panel: Fouling of the RO membrane over the duration of the experiment, represented by changes in the specific (SPF) and constant flux (GFD).

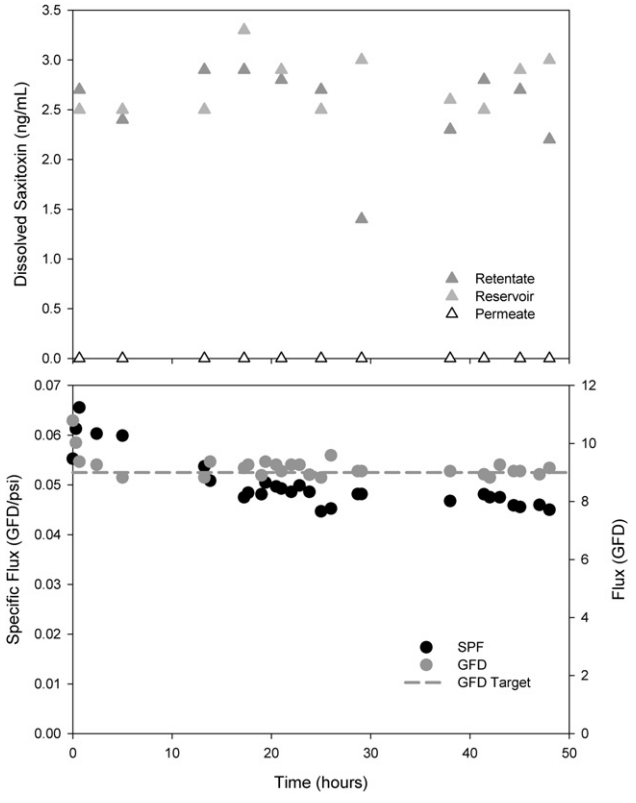

Fig. 4 – Top panel: The change in concentration of extracellular STX over the duration of the bench-scale RO experiment (48 h), as measured in the reservoir (light gray triangles), permeate (open triangles) and retentate (dark gray triangles) streams. The open triangles denote concentrations under the limit of detection of the ELISA method used. Bottom panel: Fouling of the RO membrane over the duration of the experiment, represented by changes in the specific (SPF) and constant flux (GFD).

2.0 μg/L. Toxin levels in the reservoir indicated STX was not significantly degraded due to light, temperature or microbial processes, and was not appreciably adsorbed or absorbed onto the membrane or other surfaces of the experimental setup.

Membrane performance during the STX experiment yielded flux and specific flux values that averaged 9.10 ± 0.20 gfd and 0.033 ± 0.001 gfd/psi, respectively, and indicated no consistent change during the 48-hour experiment (Fig. 4B). The applied pressure in the STX trial ranged from 492 to 787 psi in order to maintain a permeate flux near the 9 gfd target.

No PbTx was detected in any of the permeate samples collected throughout the duration of the experiment, while the reservoir maintained an average concentration of PbTx-2 of 23.0 ± 2.0 μg/L and the retentate maintained an average concentration of 20.5 ± 2.2 μg/L (Fig. 5A). No change in the concentration of PbTx-2 was observed in the reservoir during the experiment, while slight changes appeared to occur in the retentate (approximately 20%). Maintenance of mass balance for PbTx-2 in the combined volume of reservoir and retentate throughout the experiment, indicated PbTx-2 was not significantly degraded due to light, temperature or microbial processes, and was not appreciably adsorbed or absorbed onto the membrane or other surfaces of the experimental setup. Flux and specific flux for the bench-scale RO setup during the PbTx experiment averaged 9.38 ± 0.34 gfd and 0.031 ± 0.002 gfd/psi, respectively, with no marked changes in these parameters over the course of the 48-hour experiment (Fig. 5B). The applied pressure in the PbTx trial ranged from 780 to 880 psi to maintain a permeate flux near the target of 9 gfd.

3.2. *Algal toxins in the intake and desalinated water of a pilot desalination plant*

The intake and desalinated water at the El Segundo pilot desalination plant was monitored for intracellular and extracellular DA during the 2005–2009 study period, for a total 128 samples. Intracellular DA was measured in the intake water most commonly in the spring and early summer months (Fig. 6). The highest intracellular DA concentration of 4.0 μg/L

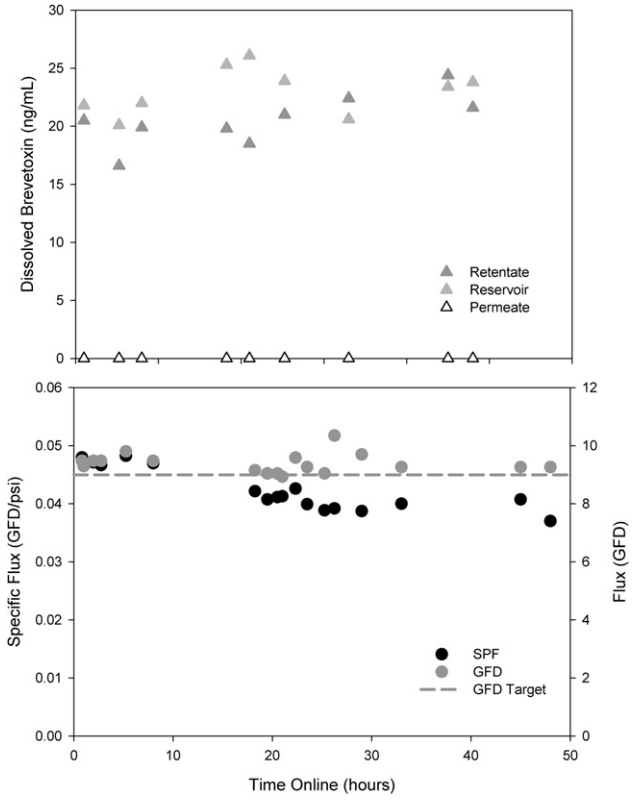

Fig. 5 – Top panel: The change in concentration of extracellular PbTx over the duration of the bench-scale experiment (48 h), as measured in the reservoir (light gray triangles), permeate (open triangles) and retentate (dark gray triangles) streams. The open triangles denote concentrations under the limit of detection of the ELISA method used. Bottom panel: Fouling of the RO membrane over the duration of the experiment, represented by changes in the specific (SPF) and constant flux (GFD).

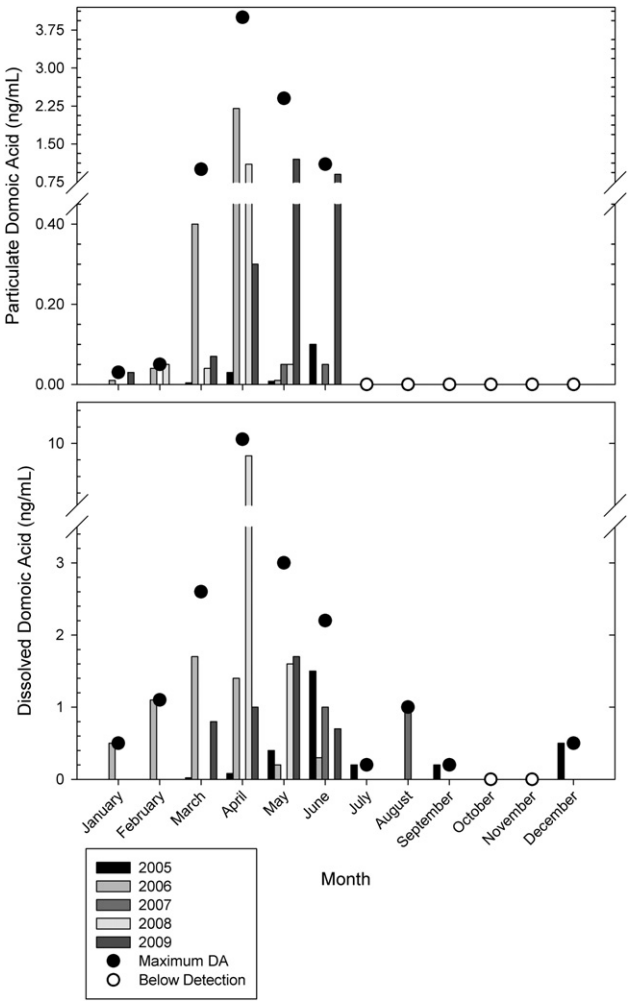

Fig. 6 – The average monthly intracellular (top panel) and extracellular (bottom panel) DA concentration detected in the pre-microfiltrated intake water during the 2005–2009 sampling period; bars are color coded based on sample year. The maximum DA concentration observed for each month, regardless of year, is marked on the graph by a black circle. The open circle denotes concentrations under the limit of detection of the ELISA.

was measured in the intake water during April 2006. Extracellular DA was measured in the intake water most commonly in the spring and early summer months, concomitant with high intracellular DA concentrations, but was also detected infrequently in winter months (Fig. 6). The highest extracellular DA concentration of 10.1 μg/L was detected in the intake water in April 2008. No DA was measured in the desalinated water throughout the extent of the field experiment.

A total of 25 samples of the intake and desalinated waters were monitored for extracellular STX during 2008 and 2009. STX was detected in nearly every month sampled (Fig. 7), with the highest extracellular STX concentration of 0.3 μg/L measured during April 2009. No STX was detected in the desalinated water throughout the experiment.

Monitoring for intracellular and extracellular PbTx in the intake and desalinated water was conducted only in March and April of 2009. PbTx has not been previously demonstrated in the region, although raphidophyte species capable of producing PbTx have been documented in these coastal waters (Caron et al., 2010; Lewitus et al., 2012). Samples were collected multiple times a week, for a total of 12 samples. The 12 samples of the intake and desalinated water yielded no detectable levels of intracellular or extracellular PbTx.

Monitoring of the intake water for intracellular OA occurred during 2008 and 2009. OA has not been previously detected in microalgal samples in southern California, but it has been detected in shellfish samples in central California (Sutherland, 2008). None of the 22 samples analyzed had detectable levels of intracellular OA.

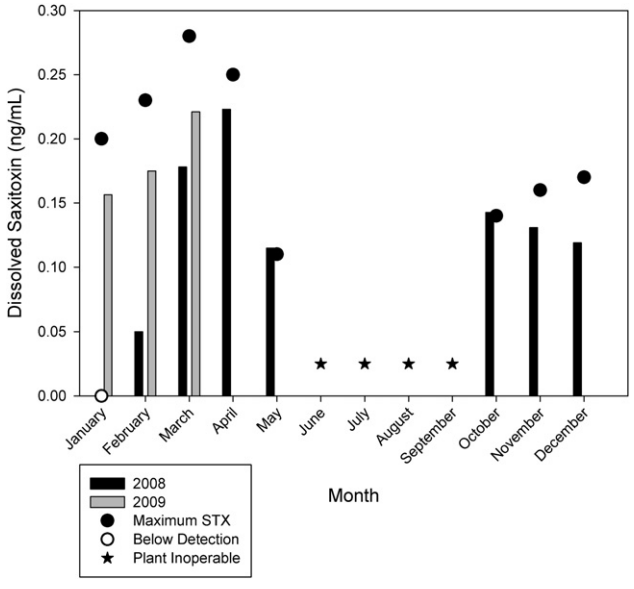

Fig. 7 – The average monthly extracellular STX concentration detected in the pre-microfiltrated intake water during the 2008–2009 sampling period; bars are color coded based on sample year. The maximum extracellular STX concentration observed for each month, regardless of year, is marked on the graph by a black circle. The open circle denotes concentrations under the limit of detection of the ELISA. The star signifies the months samples were not taken due to plant in operation.

4. Discussion

The bench-scale experiments performed in this study were designed to investigate whether algal toxins commonly encountered in coastal ecosystems would be effectively removed from the permeate water produced by commercial RO desalination processes. The membranes, pressures and fluxes employed in our bench-scale apparatus, the toxins and concentrations employed in our laboratory experiments, modeled the full-scale RO desalination plant operated by the West Basin Municipal Water District for the Redondo Beach area in southern California, making the results relevant for other desalination plants planning to operate in the area. The toxin concentrations used in the study were chosen to represent peak challenge concentrations, but were still ecologically relevant concentrations of toxins in coastal waters. The experiments were performed for durations of time that might mimic peak concentrations of these toxins in natural blooms occurring in the region.

Past work in the southern California area (Anderson et al., 2006; Busse et al., 2006; Mengelt, 2006; Schnetzer et al., 2007; Sekula-Wood et al., 2009), analyses of DA concentrations in shellfish from the MBMP data collected by the CDPH (Langlois, 2007; Caron et al., 2010), and monitoring results from the present study have demonstrated that DA is the most prevalent algal toxin in southern California waters. This toxin constitutes a potential threat to human health in many other regions globally (Lelong et al., 2012; Trainer et al., 2012), therefore the results of this study are widely relevant to desalination operations. Our results demonstrated that RO desalination was effective in removing all detectable levels of DA from the RO permeate, even at concentrations typical of an extreme, natural, toxic DA event (up to 50 μg/L of extracellular DA).

The concentration of extracellular DA used to challenge the RO membrane in the bench-scale experiment of this study is comparable to DA concentrations that have been reported in the literature for naturally occurring outbreaks of DA in the southern California region (see Caron et al., 2010 for a review). It is important to note that reports of DA in the literature have frequently focused on concentrations of intracellular toxin rather than extracellular concentrations. The former values are societally relevant because human exposure to DA typically is the result of the consumption of seafood that has become contaminated with DA through the ingestion of toxin-containing cells of *Pseudo-nitzschia*. That trophic connection has led to a strong correlation between DA in planktonic algal organisms and ASP events. However, it is probable that the prefiltration process used to remove the algal biomass load in seawater prior to RO desalination causes morphological damage to microalgal cells, resulting in the release of toxic compounds contained within the cells and may be responsible for a portion of the extracellular DA observed in the intake water from the El Segundo pilot plant over the course of this study.

Detectable quantities of extracellular DA were present in the intake water of the pilot desalination plant during most months of the year during the present study, while intracellular DA was detected the first six months of the year (Fig. 6).

These differences between the detection of extracellular and intracellular DA cannot easily be attributed to methodological differences because our intracellular DA analyses had a lower limit of detection than analyses for extracellular toxin. Another potential driver of the observed discrepancies could be due to prefiltration of the intake water, noted above. Prefiltration of the intake water may cause either the removal or breakage of cells, consequently decreasing the presence of intracellular and increasing the presence of extracellular DA in our samples. Alternatively, active release of DA by *Pseudo-nitzschia* spp. could explain why detectable concentrations of extracellular DA were more prevalent in the samples than intracellular DA. Numerous hypotheses have been explored in hope of explaining the process of production and release of DA by *Pseudo-nitzschia* spp. Laboratory experiments have shown that DA may function as a metal chelator to facilitate the acquisition of iron and copper from the environment (Bates et al., 2000; Rue and Bruland, 2001; Maldonado et al., 2002; Wells et al., 2005), as a deterrent to the grazing activities of zooplankton (Maneiro et al., 2005; Bargu et al., 2006; Olson et al., 2006; Olson and Lessard, 2010) and/or as an allelopathic compound to retard the growth of other algal species competing for nutrients (Subba Rao et al., 1995; Lundholm et al., 2005).

Monitoring the concentrations of intracellular and extracellular DA in the intake water of the pilot desalination plant in El Segundo, CA confirmed the strong spring seasonality of these events in southern California waters previously indicated by analyses of the MBMP shellfish DA data (Langlois, 2007; Caron et al., 2010). Nevertheless, DA was not detected in the permeate water samples from the pilot desalination plant at any time during the five-year monitoring period despite the presence of high concentrations of intracellular DA concentrations (4.0 μg/L in April 2006) and extracellular DA (10.1 μg/L in April 2008). The lack of detectable DA concentrations in the permeate suggest the RO process is effective in removing this dangerous neurotoxin from the desalination product.

Even the smallest concentrations of STX in oceanic waters are reason for human health concern, due to its high toxicity and associated human mortality rates (Hallegraeff, 2003). Our bench-scale RO experiment with STX indicated that RO desalination was effective in reducing the concentration of STX from seawater below detectable levels when the concentration in the reservoir was as high as 2 μg/L. An analysis of PST concentrations in shellfish along the California coast by the MBMP indicates that in recent years, STX has been more prevalent in northern California than in southern California (Langlois, 2007; Caron et al., 2010). Accordingly, most previous studies of this class of toxins on the US west coast have focused primarily on occurrence in central and northern California as well as Oregon and Washington (Jester, 2008; Lefebvre et al., 2008; Jester et al., 2009a). However, our results demonstrate that the extracellular form of STX was present in the El Segundo pilot desalination plant intake water samples throughout much of the year (Fig. 7). A recent study conducted in King Harbor, City of Redondo Beach, CA (Garneau et al., 2011) indicated the sporadic occurrence of intracellular STX and the dinoflagellate, *A. catenella*, a putative producer of this toxin. These findings of the pervasiveness of extracellular STX in southern California highlight the need for a clearer understanding of the distribution and concentration of STX in the region, as well as information on the potential impact of this toxin with respect to desalination operations.

PbTx and OA are not yet recognized as significant contributors to the mixture of microalgal toxins commonly observed in southern Californian coastal waters. OA has been detected in shellfish from Monterey Bay, CA, (Sutherland, 2008) but has not been reported in microalgal samples. PbTx has not been reported in shellfish or microalgal samples in California to date. The concentration of PbTx-2 used in the bench-scale RO experiment was based on the potential production for PbTx-2 demonstrated in laboratory studies of the three raphidophytes that have been observed in southern Californian waters, *H. akashiwo*, *F. japonica* and *C. marina* (C. Tomas, personal comm.). PbTxs are large molecules, and would be expected to be removed effectively by RO. Indeed, the bench-scale RO system employed in this study reduced concentrations of extracellular PbTx-2 below the limit of detection when the concentration in the reservoir was as high as 20 μg/L. PbTxs and OA were not detected in southern California during the monitoring conducted in this study, but known producers of PbTxs from the Raphidophyte class of microalgae and OA producers of the dinoflagellate genus *Dinophysis* have been observed in local waters (Caron et al., 2010).

5. Conclusions

- This study examined the potential impact that the presence of extracellular algal toxins common to coastal waters of southern California may have on the quality of RO permeate by challenging a bench-scale RO unit with high concentrations of DA, STX and PbTx. None of the three toxins tested were detectable in the permeate over the duration of the experiments.
- Field monitoring of intracellular and extracellular algal toxins in the intake waters of a pilot desalination plant located in El Segundo, CA, were conducted over a five year period (2005–2009). DA and STX were present in the water throughout much of the year, with DA present most commonly in the spring and early summer months and STX present in all four seasons.
- Field monitoring of the intake waters was coupled with analysis of algal toxins in the desalination product. No detectable levels of the algal toxins were observed during the five years of monitoring.

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REFERENCES

Abdul Azis, P., Al-Tisan, I., Al-Daili, M., Green, T., Dalvi, A., Javeed, M., 2000. Effects of environment on source water for desalination plants on the eastern coast of Saudi Arabia. Desalination 132, 29–40.

Anderson, C., Brzezinski, M., Washburn, L., Kudela, R., 2006. Circulation and environmental conditions during a toxigenic *Pseudo-nitzschia australis* bloom in the Santa Barbara Channel, California. Marine Ecology Progress Series 327, 119–133.

Anderson, D., Glibert, P., Burkholder, J., 2002. Harmful algal blooms and eutrophication: nutrient sources, composition and consequences. Estuaries 25, 704–726.

Baden, D., Bourdelais, A., Jacocks, H., Michelliza, S., Naar, J., 2005. Natural and derivative brevetoxins: historical background, multiplicity and effects. Environmental Health Perspectives 113, 621–625.

Bargu, S., Lefebvre, K., Silver, M., 2006. Effect of dissolved domoic acid on the grazing rate of *Euphasia pacifica*. Marine Ecology Progress Series 312, 169–175.

Bates, S., Leger, C., Satchwell, M., Boyer, G., 2000. The effects of iron on domoic acid production by *Pseudo-nitzschia multiseries*. In: Hallegraeff, G., Blackburn, S., Bolch, C., Lewis, R. (Eds.), 9th International Conference on Harmful Algal Blooms. Intergovernmental Oceanographic Commission, Hobart, Tasmania, pp. 320–323.

Burkholder, J., Azanza, R., Sako, Y., 2006. The ecology of harmful dinoflagellates. In: Graneli, E., Turner, J. (Eds.), Ecology of Harmful Algae. Springer, Heidelberg, Berlin, pp. 53–66.

Busse, L., Venrick, E., Antrobus, R., Miller, P., Vigilant, V., Silver, M., Mengelt, C., Mydlarz, L., Prezelin, B., 2006. Domoic acid in phytoplankton and fish in San Diego, CA, USA. Harmful Algae 5, 91–101.

Caron, D., Garneau, M.-E., Seubert, E., Howard, M., Darjany, L., Schnetzer, A., Cetinic, I., Filteau, G., Lauri, P., Jones, B., Trussell, S., 2010. Harmful algae and their potential impacts on desalination operations off southern California. Water Research 44, 385–416.

Castaing, J., Masse, A., Pontie, M., Sechet, V., Haure, J., Jaouen, P., 2010. Investigating submerged ultrafiltration (UF) and microfiltration (MF) membranes for seawater pre-treatment dedicated to removal of undesirable micro-algae. Desalination 253, 71–77.

Desormeaux, E., Meyerhofer, P., Luckenbach, H., Kudela, R., 2011. Pilot-testing multiple pretreatment systems for seawater desalination. International Desalination Association Journal, 42–52. First Quarter.

Franks, R., M. Wilf, N. Voutchkov, P. Murkute, Kizer, J. url. A pilot study using seawater reverse osmosis membranes in combination with various pretreatments to meet the challenges of Pacific seawater desalination. Retrieved at: http://www.membranes.com/docs/papers/New%20Folder/A%20PILOT%20STUDY%20USING%20SEAWATER%20REVERSE%20OSMOSIS%20MEMBRANES.pdf. (accessed 24.01.12.).

Garneau, M.-E., Schnetzer, A., Countway, P., Jones, A., Seubert, E., Caron, D., 2011. Examination of the seasonal dynamics of the toxic dinoflagellate *Alexandrium catenella* at Redondo Beach, California, by quantitative PCR. Applied Environmental Microbiology 77, 7669–7680.

Glibert, P., Seitzinger, S., Heil, C., Burkholder, J., Parrow, M., Codispoti, L., Kelly, V., 2005. The role of eutrophication in the global proliferation of harmful algal blooms. Oceanography 18, 198–209.

Hallegraeff, G., 2003. Harmful algal blooms: a global overview. In: Hallegraeff, G., Anderson, D., Cembella, A. (Eds.), Manual on Harmful Marine Microalgae. Monographs on Oceanographic Methodology. UNESCO Publishing, Paris, pp. 25–49.

Heisler, J., Glibert, P., Burkholder, J., Anderson, D., Cochlan, W., Dennison, W., Dortch, Q., Gobler, C., Heil, C., Humphries, E., Lewitus, A., Magniun, R., Marshall, H., Sellner, K., Stockwell, D., Stoecker, D., Suddleson, M., 2008. Eutrophication and harmful algal blooms: a scientific consensus. Harmful Algae 8, 3–13.

Jester, R., 2008. An Investigation into the Prevalence of *Alexandrium* Derived Toxins in Marine Food Webs. University of California, Santa Cruz, Santa Cruz.

Jester, R., Baugh, K., Lefebvre, K., 2009a. Presence of *Alexandrium catenella* and paralytic shellfish toxins in finfish, shellfish and rock crabs in Monterey Bay, California, USA. Marine Biology 156, 493–504.

Jester, R., Lefebvre, K., Langlois, G., Vigilant, V., Baugh, K., Silver, M., 2009b. A shift in the dominant toxin-producing algal species in central California alters phycotoxins in food webs. Harmful Algae 8, 291–298.

Kim, S.-H., Yoon, J.-S., 2005. Optimization of microfiltration for seawater suffering from red tide contamination. Desalination 182, 315–321.

Kirkpatrick, L., Squicciarini, D., Backer, L., Clark, W., Benson, J., Cheng, Y., Johnson, D., Pierce, R., Zaias, J., Bossart, G., Baden, D., 2004. Literature review of Florida red tide: implications for human health effects. Harmful Algae 3, 99–115.

Kudela, R., Lane, J., Cochlan, W., 2008. The potential role of anthropogenically derived nitrogen in the growth of harmful algae in California, USA. Harmful Algae 8, 103–110.

Kwon, B., Park, N., Cho, J., 2005. Effect of algae on fouling and efficiency of UF membranes. Desalination 179, 203–214.

Ladner, D., Vardon, D., Clark, M., 2010. Effects of shear on microfiltration and ultrafiltration fouling by marine bloom-forming algae. Journal of Membrane Science 356, 33–43.

Langlois, G., 2007. Marine Biotoxin Monitoring Program Annual Report. in. California Department of Public Health for California Department of Fish and Game, Sacramento.

Lefebvre, K., Bill, B., Erickson, A., Baugh, K., O'Rourke, L., Costa, P., Nance, S., Trainer, V., 2008. Characterization of intracellular and extracellular saxitoxin levels in both field and cultured *Alexandrium* spp. samples from Sequim Bay, Washington. Marine Drugs 6, 103–116.

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

Lewitus, A., Horner, R., Caron, D., Garcia-Mendoza, E., Hickey, B., Hunter, M., Huppert, D., Kudela, R., Langlois, G., Largier, J., Lessard, E., RaLonde, R., Rensel, J., Strutton, P., Trainer, V., Tweddle, J., 2012. Harmful algal blooms along the North American west coast region: history, trends, causes and impacts. Harmful Algae 19, 133–159.

Llewellyn, L., 2006. Saxitoxin, a toxic marine natural product that targets a multitude of receptors. Natural Product Reports 23, 200–222.

Lundholm, N., Hansen, P., Kotaki, Y., 2005. Lack of allelopathic effects of the domoic acid-producing marine diatom *Pseudo-nitzschia multiseries*. Marine Ecology Progress Series 288, 21–33.

Maldonado, M., Hughes, M., Rue, E., Wells, M., 2002. The effect of Fe and Cu on growth and domoic acid production in *Pseudo-nitzschia australis*. Limnology and Oceanography 47, 515–526.

Maneiro, I., Iglesias, P., Guisande, C., Riveiro, I., Barreiro, A., Zervoudaki, S., Graneli, E., 2005. Fate of domoic acid ingested by the copepod *Arcatia clausi*. Marine Biology 148, 123–130.

Mengelt, C., 2006. Ultravioloet Photoecology, Dark Survival and Seasonal Abundance of *Pseudo-nitzschia australis* and *P. multiseries* in Coastal Waters of Central California. Dissertation. University of California, Santa Barbara.

Murata, M., Shimatani, M., Sugitani, Y., Oshima, Y., Yasumoto, T., 1982. Isolation and structural elucidation of the causative toxin of the diarrhetic shellfish poisoning. Bulletin of the Japanese Society of Scientific Fisheries 48, 549–552.

Nazzal, N., 2009. 'Red Tide' Shuts Desalination Plant. Gulf News, Dubai, UAE.

Olson, M., Lessard, E., 2010. The influence of the *Pseudo-nitzschia* toxin, domoic acid, on microzooplankton grazing and growth: a laboratory and field experiment. Harmful Algae 9, 240–247.

Olson, M., Lessard, E., Wong, C., Bernhardt, M., 2006. Copepod feeding selectivity on microplankton including the toxigenic diatoms *Pseudo-nitzschia* spp., in the coastal Pacific Northwest. Marine Ecology Progress Series 326, 207–220.

Paerl, H., Paul, V., 2011. Climate change: links to global expansion of harmful cyanobacteria. Water Research 46, 1349–1363.

Pankrantz, T., 2008. Red tides Close Desal Plants. Water Desalination Report 44.

Pearce, G., Talo, S., Chida, K., Basha, A., Gulamhusein, A., 2004. Pretreatment options for large scale SWRO plants: case studies of UF trials at Kindasa, Saudi Arabia, and conventional pretreatment in Spain. Desalination 167, 175–189.

Perl, T., Bedard, L., Kosatsky, T., Hockin, J., Todd, E., McNutt, L., Remis, R., 1990. Amnesic shellfish poisoning: a new clinical syndrome due to domoic acid. Canadian Diseases Weekly Report 16, 7–8.

Richlen, M., Morton, S., Jamali, E., Rajan, A., Anderson, D., 2010. The catastrophic 2008–2009 red tide in the Arabian Gulf region, with observations on the identification and phylogeny of the fish-killing dinoflagellate *Cochlodinium polykrikoides*. Harmful Algae 9, 163–172.

Rue, E., Bruland, K., 2001. Domoic acid binds iron and copper: a possible role for the toxin production by the marine diatom *Pseudo-nitzschia*. Marine Chemistry 76, 127–134.

Schnetzer, A., Miller, P., Schaffner, R., Stauffer, B., Jones, B., Weisberg, S., DiGiacomo, P., Berelson, W., Caron, D., 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.

Sekula-Wood, E., Schnetzer, A., Benitez-Nelson, C., Anderson, C., Berelson, W., Brzezinski, M., Burns, J., Caron, D., Cetinic, I., Ferry, J., Fitzpatrick, E., Jones, B., Miller, P., Morton, S., Schaffner, R., Siegel, D., Thunell, R., 2009. Rapid downward transport of the neurotoxin domoic acid in coastal waters. Nature Geoscience 2, 272–275.

Subba Rao, D., Pan, Y., Smith, S., 1995. Allelopathy between *Rhizosolenia alata* (Brightwell) and the toxigenic *Pseudo-nitzschia pungens* f. *multiseris* (Hasle). In: Lassus, P., Arzul, G., Erad, E., Gentien, P., Marcaillou (Eds.), Harmful Marine Algal Blooms. Lavoisier, Intercept Ltd, Paris.

Sutherland, C., 2008. Diarrhetic Shellfish Toxins Linked to Local Dinophysis Populations in the California Coastal Waters of Monterey Bay. MS thesis. University of California, Santa Cruz, Santa Cruz.

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

Van Dolah, F., 2000. Marine algal toxins: origins, health effects, and their increased occurrence. Environmental Health Perspectives 108, 133–141.

Vardon, D., Clark, M., Ladner, D., 2011. The potential of laser scanning cytometry for early warning of algal blooms in desalination plant feedwater. Desalination 277, 193–200.

Verliefde, A., Van Vliet, N., Amy, G., Van der Bruggen, B., van Dijk, J., 2006. A semi-quantitative method for prediction of the rejection of uncharged organic micropollutants with nanofiltration. Water Practice and Technology 1. http://dx.doi.org/10.2166/WPT.2006084.

Wells, M., Trick, C., Cochlan, W., Hughes, M., Trainer, V., 2005. Domoic acid: the synergy of iron, copper and the toxicity of diatoms. Limnology and Oceanography 50, 1908–1917.

Wright, J., Bird, C., de Freitas, A., Hampson, D., McDonald, J., Quilliam, M., 1990. Chemistry, biology and toxicology of domoic acid and its isomers. Canadian Diseases Weekly Report 16, 21–26.

Yasumoto, T., Murata, M., Oshima, Y., Sano, M., Matsumoto, G., Clardy, J., 1985. Diarrhetic shellfish toxins. Tetrahedron 41, 1019–1025.

Yasumoto, T., Oshima, Y., Sugawara, W., Fukuyo, Y., Oguri, H., Igarashi, T., Fujita, N., 1980. Identification of *Dinophysis fortii* as the causative organism of diarrhetic shellfish poisoning. Bulletin of the Japanese Society of Scientific Fisheries 46, 1405–1411.

Zhang, M., Jiang, S., Tanuwidjaja, D., Voutchkov, N., Hoek, E., Cai, B., 2011. Composition and variability of biofouling organisms in seawater reverse osmosis desalination plants. Applied Environmental Microbiology 77, 4390–4398.