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Examination of the Seasonal Dynamics of the Toxic Dinoflagellate *Alexandrium catenella* at Redondo Beach, California, by Quantitative PCR[▽]

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The presence of neurotoxic species within the genus *Alexandrium* along the U.S. coastline has raised concern of potential poisoning through the consumption of contaminated seafood. Paralytic shellfish toxins (PSTs) detected in shellfish provide evidence that these harmful events have increased in frequency and severity along the California coast during the past 25 years, but the timing and location of these occurrences have been highly variable. We conducted a 4-year survey in King Harbor, CA, to investigate the seasonal dynamics of *Alexandrium catenella* and the presence of a particulate saxitoxin (STX), the parent compound of the PSTs. A quantitative PCR (qPCR) assay was developed for quantifying *A. catenella* in environmental microbial assemblages. This approach allowed for the detection of abundances as low as 12 cells liter^{-1}, 2 orders of magnitude below threshold abundances that can impact food webs. *A. catenella* was found repeatedly during the study, particularly in spring, when cells were detected in 38% of the samples (27 to 5,680 cells liter^{-1}). This peak in cell abundances was observed in 2006 and corresponded to a particulate STX concentration of 12 ng liter^{-1}, whereas the maximum STX concentration of 26 ng liter^{-1} occurred in April 2008. Total cell abundances and toxin levels varied strongly throughout each year, but *A. catenella* was less abundant during summer, fall, and winter, when only 2 to 11% of the samples yielded positive qPCR results. The qPCR method developed here provides a useful tool for investigating the ecology of *A. catenella* at subbloom and bloom abundances.

Many estuaries and coastal ecosystems are sites of occasional or recurrent algal blooms formed by species capable of producing noxious or toxic compounds that adversely affect ecosystem structure and function. These harmful algal blooms (HABs) often occur in coastal areas impacted by urbanization or agricultural activity, where their effects can cascade through food webs to negatively impact marine fauna and fishery activities and pose risks to human health (62, 74). A major concern regarding these events is that their frequency and distribution appear to be increasing in coastal environments throughout the world (3, 25, 27–29, 72) including the North American west coast (31, 38, 57, 76).

Paralytic shellfish toxins (PSTs) constitute a suite of harmful neurotoxins commonly produced in marine ecosystems by several species of dinoflagellates within the genus *Alexandrium* (51). Transfer and accumulation of PSTs through marine food webs have been implicated in instances of mass mortality of fish, birds, and marine mammals (12, 24, 55, 59). In humans, PST poisoning manifests itself as paralytic shellfish poisoning (PSP) through the consumption of contaminated seafood. The PSTs are guanidine-based alkaloids, and over 30 analogues have been identified in nature. The parent compound, saxitoxin (STX), is the most potent marine toxin identified at present (66). High concentrations of paralytic shellfish toxins in U.S. waters have been particularly frequent and acute off the northeastern U.S. coast, where most studies of *Alexandrium* bloom dynamics and toxin production have been conducted (5, 15, 17, 49, 52, 77). However, PSTs have also been reported routinely along the entire west coast of the United States (16, 37, 38, 41–47, 56) and in other coastal locations throughout the world (http://www.whoi.edu/redtide/page.do?pid=14899).

The spatial and seasonal dynamics of *Alexandrium* species along the west coast of the United States have been inferred primarily from indirect measurements of PSTs in mussels (i.e., Shellfish Protection and Marine Biotoxin Monitoring Program, California Department of Public Health). Shellfish monitoring activities from 1993 to 2007 in Puget Sound, WA, provide extensive data sets that underscore the high variability in both timing and location of PSP outbreaks. These data indicate that periods of warming and low stream flow appear to favor the accumulation of PSTs in sentinel mussels (56). Analogous information for PSTs along the California coast indicates the potential for measurable levels of toxin in shellfish during any season of the year, but higher average concentrations in shellfish tissue have tended to occur in months with warmer water temperatures (see Fig. 5 in reference 10). Relatively little information relating actual cell abundances of *Alexandrium* species to particulate toxin concentrations in the plankton of these waters is available. Only one study from Monterey Bay, in central California (37), related a range of cell abundances (40 to 3,670 cells liter^{-1}) to particulate toxin concentrations (3.1 to 512 ng STX equivalents [STXeq] liter^{-1}). These particulate toxin concentrations were measured when toxins in sentinel shellfish exceeded the regulatory limit of 80 μg of STXeq per

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100 g of shellfish tissue, as established by the U.S. Food and Drug Administration (37).

Direct measurements of *Alexandrium* abundance in natural water samples and corresponding analyses of STXs are desirable given that a considerable number of experimental studies of *Alexandrium* cultures have indicated that a wide range of physiochemical and biological factors can affect toxin production. Temperature (18), salinity (18, 26, 30, 34), phosphorus and/or nitrogen limitation (26, 30, 39, 60), light intensity (18, 30, 34), and growth phase (2, 20, 26) affect strain- or species-specific physiologies (60) and toxin production by *Alexandrium* species. These observations indicate that a complex suite of environmental parameters could control toxicity in natural ecosystems. Models developed to predict toxic PSP events along the northeastern U.S. coast account for processes such as initial cyst distribution, resuspension of dinoflagellate cysts from coastal sediments, offshore growth conditions (water temperatures and nutrient concentrations), wind forcing and cell transport into coastal areas, rainfall, and river runoff (4, 49). Information linking direct measurements of cell abundances and toxin concentrations in natural samples may provide a mechanism to modify model parameters and ground truth model predictions, thereby improving model output.

Studies leading to fundamental understanding and prediction of PSP events are complicated by, in addition to interpretation difficulties due to variable toxin production, the fact that potent species such as *Alexandrium catenella* can produce quantities of toxin that pose a health risk even at low cell densities (ca. 1,000 cells liter^{-1}) (38). New approaches for accurately and rapidly assessing the presence of these species in water samples are warranted given the difficulties of identifying and enumerating *Alexandrium* species at low natural abundances using traditional microscopy-based techniques.

We developed a quantitative PCR (qPCR) assay specific to *A. catenella* to investigate the temporal dynamics of this toxic alga in King Harbor, Redondo Beach, CA, over a 4-year period (2006 to 2009). The study site is an artificial basin housing several marinas. It experiences intense recreational and commercial activities and has suffered recurrent algal blooms and two massive fish mortality events in 2005 and 2011. Cell abundance information was related to STX concentrations in particulate material in the water column using an enzyme-linked immunosorbent assay (ELISA). Our qPCR approach was based on a molecular beacon targeting the hypervariable D1-D2 region of the large subunit (LSU) rRNA gene (28S LSU rRNA) to specifically detect *A. catenella* strains from the U.S. west coast. Our results revealed that *A. catenella* was a common member of the phytoplankton assemblage in King Harbor throughout the study period but that measurable concentrations of STX were sporadic and unpredictable. Further investigations to elucidate the environmental factors that might promote population growth and toxin production of this noxious alga are facilitated by the development of this new method.

MATERIALS AND METHODS

Study site and sample collection. Weekly samples of surface waters from King Harbor Marina, Redondo Beach, CA (latitude 33.84°N, longitude 118.39°W) (Fig. 1) were collected between 2 May 2006 and 10 December 2009. King Harbor Marina is located in the southern part of Santa Monica Bay. The marina stretches 1 km along the shoreline, with an average depth of 3 to 4 m in the inner basins, and provides 850 boat slips to private vessels. DNA for the development and application of the qPCR method was collected by filtering 50 to 200 ml of seawater onto 25-mm GF/F filters, which were loosely rolled and stored in 2 ml of lysis buffer (100 mM Tris [pH 8], 40 mM EDTA [pH 8], 100 mM NaCl, 1% sodium dodecyl sulfate) at −20°C until processing. The presence of *Alexandrium* cells was determined on duplicate samples using light microscopy and standard settling techniques for 100 ml seawater preserved with acid Lugol's solution (10% final concentration) (78). Whenever *A. catenella* cells were detected, either by visual inspection or by qPCR, we performed STX analyses. Samples that were collected within 2 days prior to and after these sampling dates were included in our toxin analyses.

FIG. 1. Study site in King Harbor in the City of Redondo Beach for the identification and quantification of *A. catenella* cells and STX concentrations. A star indicates our sampling site. (Inset adapted from reference 69.)

Saxitoxin extraction and analysis. Seawater (100 to 200 ml) was collected on GF/F filters and stored frozen at −20°C for later quantification of particulate STX (≥0.7 μm) via an enzyme-linked immunosorbent assay (ELISA; Abraxis LLC, Warminster, PA). This ELISA detects STX with 100% efficiency and the other PSTs with efficiencies of 29% or less. Most commercial assays detect STX because it is the most potent member of the paralytic shellfish toxins, i.e., a dose as small as 1 mg is lethal to humans. Monitoring of STX, the parent compound of the PSTs, can be directly linked to human health risks due to its potency. The particulate fraction of the toxin was analyzed (phytoplankton and other particular material) and does not include extracellular dissolved components that may be released into the surrounding water. STXs were extracted in 3 ml of 10% methanol, sonicated for 15 s, and diluted 1:10. The ELISA can detect an extract concentration as low as 0.015 ng STX ml^{-1}; therefore, the detection limit is 0.002 ng ml^{-1} for a 200-ml sample (i.e., 0.15 ng STX ml^{-1} × 3 ml ÷ 200 ml).

Cultures of *A. catenella* have been shown to contain mainly saxitoxin and neosaxitoxin (neoSTX) (65) but are now known to produce other PSTs. However, the proportions of toxin production that could be found in Redondo Beach are unknown, as no toxin profiles have been published for *A. catenella* of the southern California area. Cultures from Chile were reported to produce toxins in the following proportions: sulfocarbamoylsaxitoxins 1 and 2 (C1/C2), 57 to 76%; gonyautoxins (GTX), 22 to 24%; STX, trace amounts (11, 40). Toxin profiles of 25 different Japanese strains showed the following proportions: C1/C2/C3/C4, 0 to 94%; neosaxitoxin, 0 to 79%; GTX, 0 to 45%; STX, 0 to 13.5% (80). Another study on two different Japanese strains showed that one strain produced GTX-4

TABLE 1. Properties of sequencing primers for the *A. catenella* hypervariable D1-D2 domain, of the molecular beacon, and of the primers for the qPCR analysis targeting this domain

Primer	Sequence[a] (5′→3′)	Length (bp)	T_m[b] (°C)	% GC
D1R	ACCCGCTGAATTTAAGCATA	20	48	40
D2C	CCTTGGTCCGTGTTTCAAGA	20	52	50
UScatMB	[6-FAM]CGCGATCGCTTTGGGCTGTGGGT GTAATGATTCTGATCGCG[BHQ1a~Q]	41	62	56
UScatF	AACAGACTTGATTTGCTTGG	20	51	40
UScatR	CACAGGAGACTTATCATTCATG	22	52	41

[a] Underlined nucleotides represent the stem of the molecular beacon.
[b] T_m was calculated from the equation $T_m = \Delta H/(\Delta S + R \cdot \ln C)$, where ΔH is the enthalpy change (kcal/mol), ΔS is the entropy change (kcal/mol), R is the gas constant (1.987 cal/K · mol), and C is the oligonucleotide concentration. T_m values were calculated using the most accurate nearest neighbor thermodynamic calculation with Santa Lucia values (AlleleID software).

and C4, while the other produced neoSTX and STX (64). This implies the possibility that, during our study, most of the PSTs produced by *A. catenella* were not STX, meaning that while we have detected acute toxicity, we might have underestimated the total toxicity of the samples. Since variations between studies are large, it is virtually impossible to determine exactly the amount of underestimation.

***A. catenella* cultures.** Two *A. catenella* strains were sequenced to provide information for the development of our qPCR approach. One strain (ACRB01) was isolated from King Harbor Marina, City of Redondo Beach, CA (culture establishment and identification by C. Tomas, University of North Carolina Wilmington). A second strain (CCMP1911), cultured from Sequim Bay, WA, was obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (http://ccmp.bigelow.org). Both *A. catenella* cultures were grown in L1 medium and maintained at 18°C and 25 pss (practical salinity scale) under light condition of 100 microeinsteins m^{-2} s^{-1} and a 12-hour/12-hour light-dark cycle. Saxitoxin production by the Redondo Beach strain (ACRB01) was confirmed from analyses of the culture in our laboratory. Toxicity for the CCMP1911 strain was confirmed by the Center for Culture of Marine Phytoplankton.

DNA extraction and sequencing of the D1-D2 region. Extraction of environmental and culture samples (particulate material from 5 to 10 ml of culture) followed previously published standard protocols (13) to minimize sample-to-sample variability associated with traditional DNA extraction procedures that is caused by large amounts of polysaccharides and polyphenols produced by marine algal species (22). Briefly, the frozen GF/F filters kept in lysis buffer were thawed in a 70°C water bath prior to the addition of 200 μl of 0.5-mm zirconia/silica beads (BioSpec Products). Cells were lysed by vortexing (highest setting for 30 to 60 s), followed by heating in a 70°C water bath for 3 to 5 min. Two additional vortexing and heating rounds were conducted to ensure complete cell lysis. The crude lysates were separated from filter debris by forcing the fluid through a 10-ml syringe into a 2-ml DNA loBind tube (022431048; Eppendorf) and stored at −20°C. The crude lysates from the environmental samples were diluted (1:100) prior to use in qPCRs. Aliquots of the culture lysates were further cleaned for cloning and sequencing using phenol-chloroform-isoamyl alcohol (25:24:1), and the DNA was precipitated from the aqueous layer with isopropanol (23). The extracted DNA of the two *A. catenella* strains was quantified using a PicoGreen quantification kit (Invitrogen) and PCRs set up in triplicate (GoTaq polymerase kit; Promega Corporation). The PCR primers used for the amplification of the D1-D2 region (approximately 700 bp) of the large subunit rRNA gene (28S LSU rRNA) were designed by Scholin et al. (71) based on sequence data for *Prorocentrum micans* (48) and targeted the conserved positions 184 to 203 (primer D1R forward) and 816 to 795 (primer D2C reverse) relative to *Saccharomyces cerevisiae* (71). Sequencing primers are given in Table 1. The hypervariable D1-D2 domain has been found to be a useful region of the genome to address the phylogeny and biogeography of *Alexandrium* species (71) and has been widely used (9, 17, 32, 33, 63, 79).

Each PCR mixture contained ~5 to 10 ng of genomic DNA, a 100 nM concentration of each primer, 1× PCR colorless GoTaq Flexi buffer, 3 mM Mg^{2+}, a 200 μM concentration of deoxynucleoside triphosphate (dNTP) mix, 1.5 units of GoTaq DNA polymerase, and 300 ng $liter^{-1}$ of bovine serum albumin. After PCR amplification (heating at 92°C for 3 min, then 35 cycles at 92°C for 1 min, 50°C for 1 min, and 72°C for 1 min as in reference 70) (MyCycler thermal cycler; Bio-Rad Laboratories), amplicons for each strain were purified and concentrated with the DNA Clean & Concentrator-5 kit (Zymo Research Corporation). We used a TOPO TA cloning kit (Invitrogen) according to the manufacturer's protocol. Ligation PCR products were transformed into competent *Escherichia coli* cells by electroporation. Cells were then grown until plasmid DNA was harvested. Plasmids were purified using the UltraClean PCR cleanup DNA purification kit (MO BIO Laboratories) and quantified using PicoGreen quantification. Purified plasmids were then sent out for sequencing (at least 4-fold coverage) at Laragen Inc. (Los Angeles, CA).

Design of quantitative PCR probe and primers. We based our qPCR approach on a previously published quantitative *Taq* nuclease assay (TNA; TaqMan PCR) that was developed for *Alexandrium tamarense* from Japanese waters (33). We converted the original hydrolysis probe into a hybridization probe (i.e., molecular beacon) for increased specificity of the method, and we optimized the primers for our assay (Table 1).

The specific molecular beacon approach for *A. catenella* was designed using the software package AlleleID 6.0 (Premier Biosoft International). Newly obtained D1-D2 sequences for the *A. catenella* strains from Redondo Beach (ACRB01; GenBank accession no. HQ260957) and Sequim Bay (CCMP1911; GenBank accession no. HQ260956) were combined with existing sequences for *A. catenella* available in GenBank (http://www.ncbi.nlm.nih.gov). The latter sequences originated from samples collected off Santa Cruz Wharf, Santa Cruz, CA (strain A3; accession no. AF200667) and from Puget Sound Harbor, WA (accession no. AY056823). We also imported 30 sequences of nontarget species from the GenBank NCBI database (6) into our design program in order to optimize the specificity of the method. Nontarget species included other dinoflagellates (*Ceratium*, *Cochlodinium*, *Dinophysis*, *Prorocentrum*, *Scrippsiella*, *Lingulodinium* spp., etc.) and nondinoflagellate species that are known to commonly co-occur with *A. catenella* (*Pseudo-nitzschia*, *Chattonella* spp., etc.). The specificity of the probe and primer set was evaluated by manual alignment of closely related dinoflagellates using the BioEdit sequence alignment editor, version 7. Specificity was also examined *in silico* on 23 July 2010 using the GenBank nucleotide collection (nr/nt) and the blastn function optimized for highly similar sequences (megablast BLASTN 2.2.24+) (1).

The selected primers UScatF (5-AACAGACTTGATTTGCTTGG-3′) and UScatR (5′-CACAGGAGACTTATCATTCATG-3′) produced a predicted PCR amplicon length of 141 bp. The molecular beacon (UScatMB: CGCGATCGCTTTGGGCTGTGGGTGTAATGATTCTGATCGCG) was labeled with 6-fluorescein amidite (6-FAM) at the 5′ end, and had black hole quencher 1a (BHQ1a~Q) attached to the 3′ end (Table 1). All oligonucleotides were synthesized and purified by high-performance liquid chromatography (HPLC) by Eurofins MWG Operon.

Cell lysates created from an *A. catenella* culture (ACRB01) of known abundance were used to correlate cell numbers to qPCR threshold cycles (C_T) using a standardized protocol. We calibrated our qPCR approach in this manner in order to estimate *A. catenella* cell abundances in natural samples directly from C_T values. Cell abundances provide a much more useful index of the presence of *Alexandrium* in natural samples, although we recognize that variances in copy number within the dinoflagellate (22, 58) could affect this correlation. A calibration curve for this correlation consisted of a 5-point 1:10 serial dilution of a known abundance of actively growing *A. catenella* cells. Cell abundance within the original culture was determined via light microscopy (11,605 cells ml^{-1} ± 1,696 cells ml^{-1}). Each sample was prepared in triplicate from each of the five 1:10 serial dilutions in 1-liter volumes of seawater and seawater filtered through 0.2-μm filters. The filters were processed as described above to produce raw lysates. A serial dilution in natural (unfiltered) seawater was also analyzed to ensure that constituents in natural seawater (e.g., particulate material and nontarget species) would not interfere with the detection of *A. catenella*. The calibration curve spanned overall concentrations from 1.16×10^1 to 1.16×10^5 cells $liter^{-1}$, which corresponded to 5.8×10^{-4} to 0.58 cells per qPCR. A qPCR calibration curve was run in triplicate for each set of standards together with

TABLE 2. Species used in a cross-reactivity PCR to test the specificity of our qPCR assay

Taxonomy	Species	Origin and/or strain	qPCR result
Dinophyceae	*Alexandrium catenella*	Sequim Bay, WA; NWFSC-404	+
	Alexandrium catenella	Redondo Beach, CA	+
	Akashiwo sanguinea	CAS7	−
	Gymnodinium beii	4.19	−
	Lingulodinium polyedrum	Redondo Beach, CA	−
	Prorocentrum gracile	Hermosa Pier, CA; CPG1	−
	Prorocentrum micans	Redondo Beach, CA	−
	Scrippsiella nutricula	7.6	−
	Symbiodinium pulchrorum	NA[a]	−
Bacillariophyta	*Chaetoceros* sp.	California Bight, CA; MO-Cht1	−
	Coscinodiscus sp.	California Bight, CA; MO-Csd1	−
	Ditylum brightwellii	MO-Dtb1	−
	Leptocylindrus danicus	NA	−
	Minutocellus polymorphus	Minuto	−
	Odontella sp.	MO-Odt1	−
	Pseudo-nitzschia australis	Monterey Bay, CA; PN 1	−
	Pseudo-nitzschia delicatissima	San Pedro, CA; SPC 22	−
	Pseudo-nitzschia pungens	San Pedro, CA; SPC 16	−
	Thalassiosira weissflogii	Actin	−
	Thalassiosira rotula	MO-Thr1	−
Bicosoecida	*Cafeteria roenbergensis*	Cflag	−
Chlorophyta	*Chlamydomonas* sp.	I-155	−
	Ostreococcus sp.	MBIC-10636	−
	Pyramimonas cf. *tychotreta*	RS-11	−
Chrysophyceae	*Mallomonas annulata*	I-76	−
	Paraphysomonas bandaiensis	WH1a	−
	Paraphysomonas imperforata	JE4	−
Dictyochophyceae	*Pedinella* sp.	LI1-1	−
	Pteridomonas sp.	NB1	−
Haptophyceae	*Isochrysis galbana*	T-Iso	−
	Phaeocystis globosa	MO-Pg1	−
Pelagophyceae	*Aureococcus anophagefferens*	BT-Darc	−
Raphidophyceae	*Chattonella marina*	Redondo Beach, CA	−
	Fibrocapsa japonica	Redondo Beach, CA	−
	Heterosigma akashiwo	Olisth	−

[a] NA, not applicable.

triplicate sets of unknown samples to facilitate the conversion of C_T values of natural samples to equivalent abundances of *A. catenella*. Controls that did not contain any algae were also included in triplicate in each qPCR.

Optimization of qPCR conditions. Optimization reaction mixtures were prepared with 10 μl of 1:100 crude lysate of an *A. catenella* culture plus 40 μl of reagent master mix (see below) and run on an iCycler iQ real-time PCR detection system (Bio-Rad Laboratories). Initial testing of the thermal protocol was performed with a Mg^{2+} concentration of 4 mM and an annealing temperature of 51°C, as suggested by the AlleleID design software as the optimal theoretical conditions. The annealing temperature was optimized with the gradient feature of the iCycler across a temperature range of 49 to 54°C. The optimal Mg^{2+} concentration was determined by testing reaction mixtures prepared with 4.0 to 7 mM (0.5 incremental unit) Mg^{2+}. The primer set was tested with iQ SYBR green supermix (Bio-Rad) to determine the specificity of amplification based on melting curve analysis.

Environmental samples and standards (cell lysates of an *A. catenella* culture) were diluted 1:100 with sterile Milli-Q water to minimize PCR inhibition due to the presence of lysis buffer and residual cellular contents. The final qPCRs were carried out in 50-μl volumes containing 10 μl of 1:100 crude lysate (~5 to 10 ng of environmental DNA), a 300 nM concentration of each primer, and a 400 nM concentration of fluorogenic probe. Reaction mixtures also contained the following reagents (Promega): 1× PCR colorless GoTaq Flexi buffer, 7 mM Mg^{2+}, 200 μM dNTP mix, and 2.5 units of GoTaq DNA polymerase. All reagents, samples, and standards were prepared on ice prior to thermal cycling. PCRs and a blank control (no DNA added) were set up in triplicate in 96-well PCR plates sealed with flat strip caps (Bio-Rad; 2239441) and centrifuged briefly to remove bubbles. The qPCR thermal cycling conditions were as follows: 1 cycle of heating at 95°C for 3 min and then 40 cycles of 94°C for 15 s (denaturation), 53.2°C for 30 s (annealing), and 72°C for 30 s (extension). Thermal cycling and real-time data collection at the annealing step were performed using an iCycler iQ real-time PCR detection system (Bio-Rad Laboratories).

The specificity of our probe-primer design was tested empirically by analyzing culture lysates of 33 nontarget species from a variety of taxonomic groups (Table 2). These species included a variety of other dinoflagellates, bacillariophytes, chlorophytes, chrysophytes, dictyochophytes, haptophytes, pelagophytes, and raphidophytes, which had been grown to high abundances for the preparation of crude lysates. Prior to use in the cross-reactivity tests, culture lysates were amplified with universal eukaryote-specific PCR primers to ensure the presence of amplifiable DNA.

Statistical analysis. The calibration curves relating qPCR threshold cycle and log cell abundance of *A. catenella* were regressed using the least-squares approximation and the associated statistical tests of Excel software. Nonparametric tests were used since the data failed the normality and homogeneity of variance tests (81). Nonparametric Kruskal-Wallis analyses were used to test differences of the

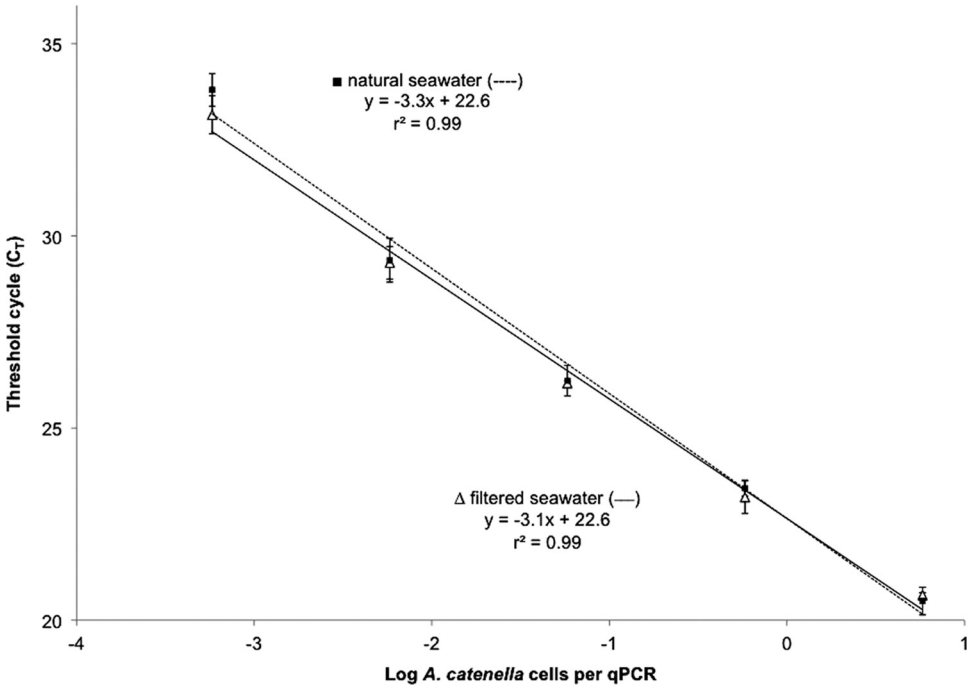

FIG. 2. Calibration curves relating qPCR threshold cycle and cell abundance of *A. catenella* culture, processed as cell lysates. Curves were generated from serial dilutions of *A. catenella* cells spiked into natural (unfiltered) seawater and seawater filtered through a 0.2-μm filter. Cell abundances ranged from 1.16×10^1 to 1.16×10^5 cells liter^{-1}. Error bars represent standard deviations of triplicate lysates.

median values between years. We also tested differences of the median values between seasons, with autumn defined as 21 September to 20 December, winter as 21 December to 20 March, spring as 21 March to 20 June, and summer as 21 June to 20 September. We grouped each variable tested (i.e., STX levels and cell abundances) by season regardless of the sampling year and tested whether each variable was statistically different between seasons.

Nucleotide sequence accession numbers. Sequences of *A. catenella* strains used in this study were deposited in GenBank and assigned accession numbers HQ260957 (668 bp; Redondo Beach strain ACRB01) and HQ260956 (668 bp; Sequim Bay strain CCMP1911).

RESULTS

Calibration, efficiency, and specificity of the qPCR approach. The DNA templates used to calibrate the qPCR approach were crude lysates of unialgal cultures of *A. catenella* for which abundance was determined by microscopy. These calibration curves were employed to directly translate C_T values into cell abundances in natural samples. The range of abundances used in the calibration curves spanned 4 orders of magnitude from 1.16×10^1 to 1.16×10^5 cells liter^{-1} (r^2 = 0.99) (Fig. 2). The annealing temperature chosen for the qPCRs was 53.2°C because it produced the highest relative fluorescence unit (RFU) values at the plateau phase of amplification (>1,600 RFU) and yielded the best reproducibility. In addition, we selected the highest concentration of Mg^{2+} tested (7 mM) because it produced the maximum RFU values and the lowest C_T and had better reproducibility than lower concentrations. Average C_T values decreased from 21.0 to 19.9 with increasing Mg^{2+} concentrations. The postamplification melting curve analysis indicated a single PCR product and confirmed the absence of nonspecific amplicons or primer dimers.

Similar detection efficiencies were achieved from cell culture lysates diluted with natural (unfiltered) or filtered seawater (Fig. 2), indicating that there were no significant inhibitory effects or cross-reactive substances present in natural seawater. The efficiencies of our PCR amplifications (E) were calculated using the equation $E = 100 \times 10^{-1/m} - 1$ (where m is the slope of the calibration curve). An acceptable range for E is generally 90% to 110% for assay validation, which corresponds to calibration curve slopes of −3.6 to −3.1 (36). Thirteen calibration curves were generated to analyze 162 samples in this study. The slopes obtained from these analyses ranged between −3.4 and −3.0, with an average efficiency of 109%. After development of the molecular beacon primer set, additional confirmatory DNA sequencing indicated a 1-nucleotide mismatch between our forward primer (UScatF) and a single 28S rRNA gene clone derived from the Redondo Beach *A. catenella* strain, which was located at the sixth nucleotide position of the primer. This single nucleotide mismatch did not appear to affect the efficiency or usefulness of the molecular beacon assay as originally designed (Fig. 2). These results validated the reproducibility of our qPCR approach.

The specificity of the probe and primer set was examined *in silico* against the GenBank nucleotide collection (1) on 23 July 2010. The BLAST analysis indicated that our molecular beacon hit 47 non-*Alexandrium* sequences, but 83% of these sequences were from terrestrial multicellular eukaryotes (35 sequences) and nonaquatic protists (4 sequences). The remainder, 5 prokaryotic and 3 fungal sequences, were also unlikely to be found in marine samples. Most importantly, neither of the primers matched any of these species, and hence nontarget amplification should have been nonexistent by our qPCR approach. The specificity of our primer/probe design

was also confirmed by negative results for cross-reactivity with culture lysates of 33 nontarget species, which included other dinoflagellates (Table 2).

Sequencing of the D1-D2 region of 28S rRNA gene placed our two *Alexandrium* strains from the U.S. west coast in toxic group I (50), previously known as the North American ribotype (71). This recent phylogenetic grouping, based on D1-D2 domains of LSU rRNA sequence information, indicates that the genus *Alexandrium* includes five taxonomic entities. Groups I and IV are composed of toxic strains from all three morphospecies (*A. catenella*, *A. tamarense*, and *A. fundyense*), with group I dominated by the *A. tamarense* morphotype and group IV by the *A. catenella* morphotype (50). Strains in groups II, III, and V all belong to nontoxic *A. tamarense* (50). BLAST searches revealed that the D1-D2 regions of our two strains corresponded to other *Alexandrium* mophotypes in group I, which now consists of 6 sequences from the morphospecies *A. catenella*, 32 sequences of *A. tamarense*, and 3 sequences of *A. fundyense* from North and South America, the Faroe Islands, Scotland, South Africa, Russia, Japan, and South Korea (50). All of these 41 sequences matched our *A. catenella* D1-D2 sequences for the Redondo Beach isolate and the CCMP1911 specimen through the region employed for designing our qPCR method. Thus, we consider our approach to be specific to the group I *Alexandrium* ribotype and broadly applicable to strains found in many parts of the world.

***A. catenella* abundance and saxitoxin concentration in King Harbor.** *A. catenella* cells were observed in the harbor during every year of the 4-year study, but abundances varied markedly (Fig. 3). Cells were generally detected between February and June but never in July, August, October, December, and January (Fig. 3). Abundances ranged from 26 to 5,680 cells liter^{-1}, and particulate STX concentrations ranged from below the detection limit (lower threshold = 2 ng liter^{-1}) to 26 ng liter^{-1} (Table 3). Maximal cell abundance was detected on 5 May 2006 (Fig. 3). Overall, *A. catenella* was present in 17% of the samples and during all four seasons, but the dinoflagellate was most abundant during spring months, with 38% of the tested samples being positive (range from 27 to 5,680 cells liter^{-1}, median of 244) (Table 3). Overall 18% of the samples collected during the winter months tested positive for *A. catenella*, while only 7% and 2% of the samples from summer and fall months, respectively, were positive. The median value during spring was significantly higher than median values during summer and fall (Kruskal-Wallis, $P < 0.05$, $n = 43$) but did not differ from the winter value (Table 3).

Particulate STX was detected 11% of the time throughout the study period, and these instances were restricted to spring and summer months (Table 3). Concentrations of particulate STX ranged from 3.0 to 26 ng liter^{-1} (Table 3) and were significantly correlated with *A. catenella* cell abundance ($r_s = 0.54$, $P < 0.0001$, $n = 91$). However, maximal STX was observed on 17 April 2008 and did not coincide with the highest *A. catenella* abundance, which was found on 5 May 2006. Moreover, the presence of cells was not necessarily accompanied by measurable toxin concentrations in the particulate fraction of the sample (Fig. 3). Cellular STX content calculated from cell abundances (estimated by qPCR) and particulate STX concentration ranged from 2.0 to 36 fg per cell (median, 16.4 fg per cell; $n = 10$).

The strongest *Alexandrium* year in King Harbor was 2008, when 31% of the qPCR samples ($n = 51$) tested positive for the dinoflagellate between 5 March and 23 June. Cell abundance peaked at 1,920 cells liter^{-1} on 17 April. In 2006, *A. catenella* and particulate STX were present in one-half of the samples ($n = 24$), but sampling did not begin until May, and therefore no conclusion can be reached regarding late winter to early spring 2006. *A. catenella* was less prominent during 2007 and 2009. Cells were detected only five times during 2007 (11% of the samples; $n = 47$), with cell abundances ranging from 83 to 1,440 cells liter^{-1}, and particulate STX was measurable only on 29 June, at 3 ng liter^{-1}. *A. catenella* cells were detected at low abundances during 2009 on 10 and 18 March (36 and 88 cells liter^{-1}, respectively; $n = 40$) (Fig. 3), but STX concentrations were never observed. Kruskal-Wallis analysis showed significant differences between years for *A. catenella* abundances and STX concentrations, but the pairwise comparison procedures (Dunn's method) failed to detect years that were statistically different.

DISCUSSION

Rationale and specificity of the qPCR assay for *A. catenella* in California waters. The molecular beacon-based qPCR method developed and applied in this study was demonstrated to be specific and sensitive for the detection and quantification of *A. catenella* in pure cultures and in mixed natural plankton assemblages. Most *Alexandrium* sequences available to date in GenBank are sequences from the D1-D2 domain of the LSU rRNA and were therefore considered the best possible target for development of our qPCR assay. Furthermore, we chose a molecular beacon approach because of its high specificity compared to SYBR green assays, which do not use an internal probe and report all amplified double-stranded DNA in the sample. This includes nonspecific reaction products or primer dimers, which may generate false-positive signals. Although a recently developed SYBR-based qPCR assay by Dyhrman et al. was found to be an efficient tool to monitor *A. catenella* in Puget Sound (16), the same approach would have likely failed to detect *A. catenella* strains from King Harbor due to mismatches between their forward primer and our D1-D2 sequences, even though the primer was designed to target the North American ribotype. This finding serves as an important reminder that genomic variability in geographically distinct strains of the same species needs to be considered and that probes and primers can be effective only if the taxonomic information on which identifications are based is sound. Taxonomy within the genus *Alexandrium* is currently undergoing revision based on recent phylogenetic analyses (50).

Our *Alexandrium* specimen from King Harbor was identified as *A. catenella* based on morphological characteristics and sequence information for the D1-D2 region of the LSU, which placed the strain within the toxic group I clade defined by Lilly et al. (50). This clade includes not only *A. catenella* but also *A. tamarense* and *A. fundyense* morphotypes. Further BLAST analyses indicated that our qPCR approach would have detected all 41 members within this toxic group I *A. tamarense*/*A. catenella*/*A. fundyense* clade. While *A. catenella* was the only morphotype identified from Redondo Beach samples and is, to date, the only *Alexandrium* species documented on the U.S.

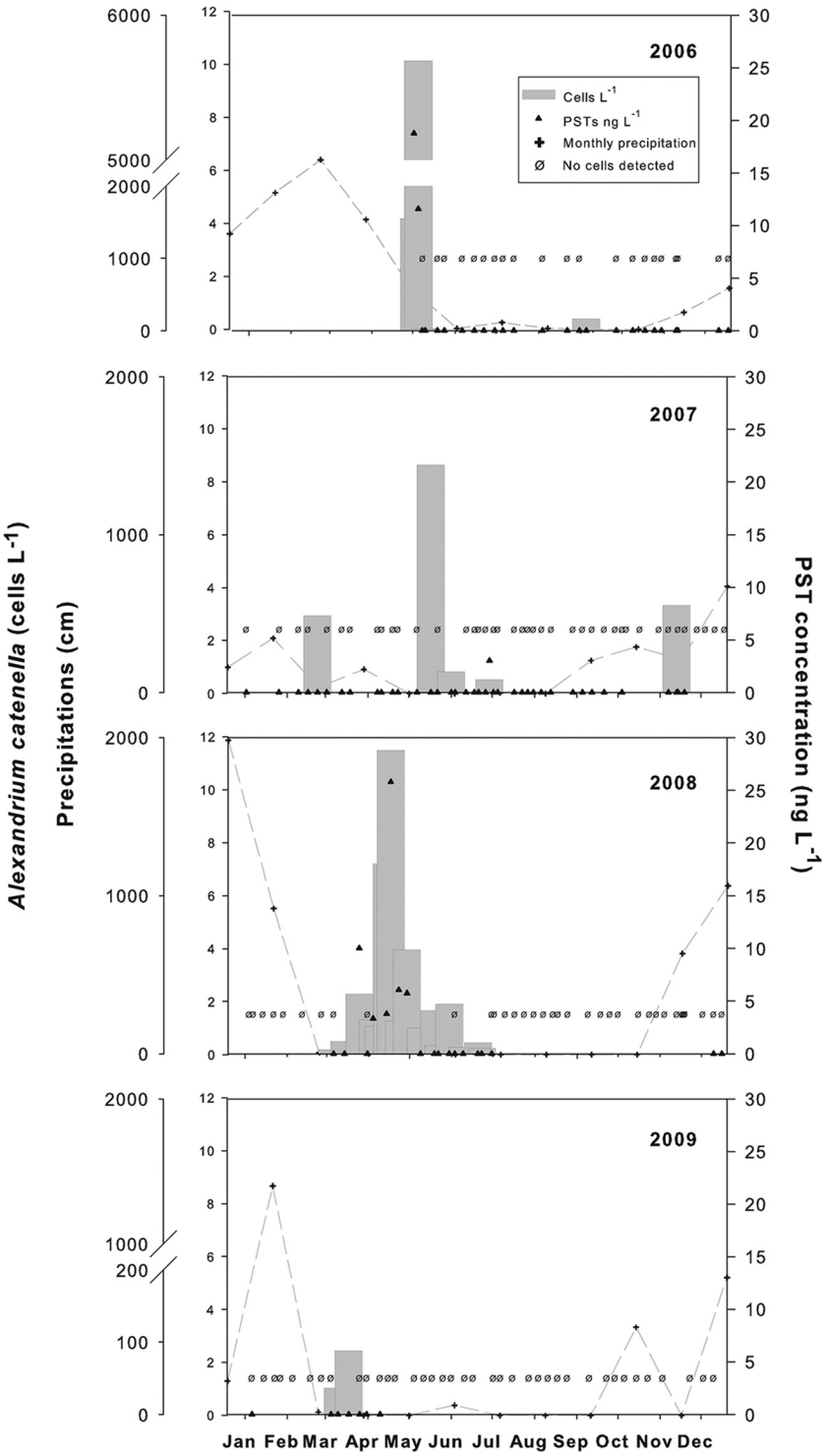

FIG. 3. Abundances of *A. catenella* (bars) from 2 May 2006 to 10 December 2009, particulate STX concentrations (▲) in King Harbor Marina, and monthly precipitations. ∅, cell abundances were below the qPCR detection limit. Note the different scale for cell abundances in 2006. Number of samples, 162.

west coast (10, 16, 37), new insight into species distinctions based on sequence information may lead to reevaluation of geographical distributions. In any case, the detection of all toxic *Alexandrium* species within group I can be revealed by our qPCR approach, which is of ecological relevance for HAB monitoring and early warning of PSP events.

Our qPCR method can significantly improve monitoring programs attempting to provide an early warning of potential

TABLE 3. Seasonal medians and ranges of *A. catenella* cell numbers and particulate STX concentrations measured in surface waters in the King Harbor Marina, City of Redondo Beach

Season	Cells					STX				
	Cell no. (cells liter^{-1})			% positive samples[a]	n[b]	STX concn (ng liter^{-1})			% positive samples	n
	Minimum	Median	Maximum			Minimum	Median	Maximum		
Winter	26	79	488	18	28		BD[d]		0	14
Spring	27	244[c]	5,680	38	47	3.0	6.1	25.8	24	38
Summer	35	83	161	7	43	3.0	3.0	3.0	4	23
Fall	553	533	533	2	44		BD		0	16
Annual	26	171	5,680	17	162	3.0	5.9	26	11	91

[a] Ratio of the number of samples in which cells or STX was detected to the total number of samples analyzed for a given season expressed as a percentage.
[b] n, number of samples.
[c] Significantly different median value (Kruskal-Wallis, $P < 0.05$; performed on all data sets, including zero values).
[d] BD, below detection limit.

PSP events in coastal waters and can be used as a tool for ecological studies of *Alexandrium*. *Alexandrium* rarely is a major component of the marine phytoplankton community along the California coast (≤1,000 cells liter^{-1}) (38, 41) and therefore presents a challenge for detection by traditional microscopical observation. Relative abundance estimates for *A. catenella* for 2006 to 2009 indicate that this species accounted for less than 5% of the total phytoplankton community most of the year and attained 25% under highly favorable environmental conditions but never exceeded 50% (41–44). Absolute cell abundances during our 4-year study in King Harbor determined by our qPCR method never exceeded 6,000 cells liter^{-1}. This maximal value is near the lower detection limit of standard microscopy. For example, microscopy-based counts of coastal water samples typically employ a volume of approximately 25 ml of settled sample, from which ~150 cells per sample or 20 fields of view (×40 magnification) are counted. This method would result in a lower detection limit of approximately 5,000 cells liter^{-1} (19). If every cell in the settled sample was *A. catenella*, this species would be obvious only when it attained the highest abundances observed for this alga in our study of King Harbor. The lower detection limit of 12 cells liter^{-1} afforded by our qPCR assay reduces the detection limit for *Alexandrium* cells by more than 2 orders of magnitude. This lower limit of detection could be further reduced by increasing the volume of sample filtered (100 ml was employed in this study).

Results for amplification of target DNA above the fluorescence threshold in qPCRs (i.e., threshold cycle) were converted to cell abundances of *A. catenella* using an empirical relationship between C_T and cell abundance derived from laboratory cultures of strain ACRB01. The accuracy of this extrapolation is affected by the variability of ribosomal DNA (rDNA) copy number per cell. Factors that impact copy number include physiological status of cells and variance among strains (21). The reported range for nine strains of *A. catenella* was approximately 190,000 to 2,500,000 copies per cell regardless of their geographic origin, although most values were in the range of 200,000 to 500,000 copies per cell (21). The previous authors suggested that, in addition to physiological status, variability in rRNA gene content for *A. catenella* could also be due to the presence of an unstable rDNA pseudogene (as described in reference 79) and/or the presence of extrachromosomal rDNA molecules, but the existence of the latter has not been confirmed. While these potential biases exist, repeated future correlations between cell abundances (culture-based calibration curve) and qPCR results should indicate the degree to which variations in copy number might affect estimates of cell number derived from the qPCR approach and improve the overall accuracy of the method.

Occurrence and seasonal dynamics of *A. catenella* and saxitoxins in King Harbor. *A. catenella* recurred every year in King Harbor Marina during our 4-year study; when it was detected, cell abundances (26 to 5,680 cells liter^{-1}, $n = 162$) generally fell within the lower range of densities previously reported along the coasts of the northeastern United States, central and northern California, and Washington State (Table 4). Cell abundances documented during a 3-year study from Monterey Bay, central California, attained a maximum of 17,387 cells liter^{-1} ($n = 307$), but 82% of the samples had abundances of <1,000 cells liter^{-1} (37). *A. catenella* cells were detected more frequently in Monterey Bay (55% of samples) (37) than in King Harbor (17% of samples). Further north, in Puget Sound, cell abundances ranged from 5 to 812,500 cells liter^{-1} from late April through October 2006 (16). The maximal abundances greatly exceeded the maximal abundance observed during our 4-year survey. Nonetheless, 61% of the cell numbers reported for 41 sites throughout the Puget Sound area during the same period were within the range observed in King Harbor (10 to <10,000 cells liter^{-1}) (see Table 1 of reference 16). Particulate STX concentrations (3.0 to 26 ng liter^{-1}) in King Harbor were also generally lower than concentrations measured from Monterey Bay samples (3.2 to 962 ng liter^{-1}) (37).

Spring was determined to be the most frequent season for the development of *A. catenella* populations and measurable particulate STX concentrations in King Harbor. This trend was substantiated by shellfish monitoring data collected within Los Angeles County between 2003 and 2009, where concentrations from 35 to 306 μg STXeq per 100 g tissue were detected in mussels during springtime only, i.e., between February and May (41–47). This pattern differs somewhat from seasonal trends observed for toxin concentrations in shellfish collected in the other 14 coastal Californian counties from 2002 and 2009 (10). Those data indicated that significant toxin concentrations occurred in shellfish tissues during spring, but maximal concentrations (~1,600 μg STXeq 100 g^{-1} tissue) were usually observed from July to September (10). A trend toward summer-fall toxin maxima for the entire California coastline is also

TABLE 4. Summary of recent reports of *Alexandrium* sp. cell abundances and STX or PST concentrations along the U.S. coasts[a]

Location	Sample period	Depth(s) (m)	*Alexandrium* sp. present	Abundance (cells liter^{-1})	Particulate STX concn (ng STXeq liter^{-1})	STX concn in shellfish (μg STXeq 100 g^{-1})	Reference or source
Penobscot Bay, Gulf of Maine, ME	March–May 1993, 1994, 1998, 2000	Surface	*A. fundyense*	50–200[b]	ND[c]	ND	51
Penobscot Bay to Casco Bay, Gulf of Maine, ME	May–June 1998, 2000, 2001, 2003	1, 5, 10, 20, and 30	*A. fundyense*[d]	<25–2,500	ND	ND	5
Casco Bay, Gulf of Maine, ME	April–June 1998	Surface	*A. fundyense*[d]	0–4,000	0–120[e]	ND	59
Gulf of Maine, ME, NH, and MA	May–July 2005	1, 10, and 20	*A. fundyense*[d]	<10–32,094	ND	BD[f]–4,378	4
Gulf of Maine, ME	May–June 2003	Surface	*A. fundyense*[d]	0–171	ND	ND	15
Massachusetts Bay to Bay of Fundy, Gulf of Maine, ME, NH, and MA	June 2006	Surface	*A. fundyense*	0–10,000	ND	ND	48
Gulf of Maine and Georges Bank, ME	June 2006	Surface	*A. fundyense*	0–22,338	ND	ND	74
Monterey Bay, CA	January 2003–December 2005	Surface	*A. catenella*	0–17,387	BD–962	81–770	37
Puget Sound, WA	April–October 2006	Surface	*A. catenella*	5–812,500	ND	BD–17,000[g]	16
King Harbor, Santa Monica Bay, CA	May 2006–December 2009	Surface	*A. catenella*	0–5,680	0–26	ND	This study

[a] Different methods were used to determine cell abundances and toxin concentrations in the cited studies.
[b] Estimated from Fig. 2.
[c] ND, no data available.
[d] Both *A. tamarense* and *A. fundyense* occur in the Gulf of Maine, but authors considered these to be varieties of the same species; therefore *A. fundyense* was used to refer to both forms.
[e] Original values in pmol STX liter^{-1} were converted using the molecular weight of STX (299.3).
[f] BD, below detection limit. The detection limit is <40 μg STXeq 100 g^{-1} of mussel tissue.
[g] Value is expressed as μg PSTs 100 g^{-1} in the original article.

reflected in shellfish monitoring records from the last 25 years (41) and agrees with 1927 to 1989 observations that show the most significant STXeq levels in mussels between May and October (61). Maximal cell abundances and elevated shellfish toxicity in Monterey Bay were reported for July (37) (Table 4), and the highest STXeq concentrations in shellfish off the Washington and Oregon coasts were observed in summer and fall (14, 74). The late summer-fall period is characterized by a warm and dry climate, weaker seasonal upwelling, and therefore stability in the upper water column and typically lower nutrient levels. Water column stability has been shown to favor *Alexandrium* sp. growth (31), and nutrient limitation has been linked to STX production (26, 30, 39, 60). Accordingly, periods of warming coincided with STX accumulation in sentinel mussels in central and northern California regions (56).

In King Harbor, however, *A. catenella* was seldom observed in summer or fall (only in 7% and 2% of our weekly samples, respectively), and particulate STX was detected once in summer but never in fall. This apparent shift in seasonal occurrence of *Alexandrium*, relative to central and northern California, could be due to earlier seasonal warming of coastal waters in southern California, specific hydrographic features of King Harbor, or both. King Harbor averages approximately 4 meters in depth, and warming and stratification occur relatively early since the site is physically isolated from large-scale mixing events that occur in waters surrounding the embayment. This speculation is consistent with previous observations that *Alexandrium* sp. blooms tend to originate in shallow, near-shore localities and then spread regionally (73).

Substantial interannual variability in the occurrence of *A. catenella* was observed in King Harbor, as has been noted in Puget Sound (monitoring data from 1993 to 2007), where the timing and location of occurrences could not be easily explained by either local or large-scale climate fluctuations (56). Little is known about the factors that drive such significant interannual variability, but precipitation patterns in southern California may be among the factors that could explain these dynamics in King Harbor. This semiarid environment receives an average annual rainfall of only 30 cm, which typically occurs as episodic storm events in late fall and throughout winter (7). Storm water discharges bring more than 95% of the annual runoff from the southern California coastal watersheds into coastal ecosystems (67), implying that these freshwater inputs have a major impact on density stratification and nutrient levels on the coasts during these discrete rainfall events. It is conceivable that any small change in yearly precipitation could result in discernible interannual variations in water stratification and nutrient availability and therefore in *A. catenella* abundances. Variance in precipitation may partly explain why *A. catenella* abundances reached their highest levels in 2006 and 2008, the 2 years of our survey that were characterized by more-than-average monthly rainfalls (National Weather Service data for Los Angeles/Oxnard [http://www.weather.gov/climate; accessed 1 September 2010]).

Our survey indicated that the presence of *A. catenella* in King Harbor Marina was not necessarily associated with detectable STX concentrations. Nearly one-third of our samples in which cells were detected did not contain measurable levels of STX. Similarly, the 3-year survey in Monterey Bay noted that 20% of samples that were positive for *A. catenella* (abundances of <1,000 cells liter^{-1}) had no measurable particulate STX (37). Similar results were obtained in Puget Sound, where almost one-third of the stations revealed no shellfish toxicity despite the presence of *A. catenella* in surface waters (16). The authors of the latter study concluded that the use of absolute cell numbers as an indicator of STXeq levels in shellfish was inappropriate for Puget Sound (16). The use of mussels as sentinel species has been debated (31, 75) because these organisms may depurate toxin in less than 1 h (8). Nonetheless, shellfish toxin profiles should be generally reflective of *Alexan-*

drium abundance in the upper water column (54). Direct measurements of *Alexandrium* spp. in water samples provide information unaffected by factors such as shellfish feeding rate and detoxification kinetics. *Alexandrium* abundance information together with STX levels in the water column, linked to concentration in shellfish, will enhance our capability to predict these toxic events.

Prevalence of PSP events on California coasts. No PSP events from southern California are documented prior to the 1980s (61), and shellfish samples that exceed the regulatory limit were first documented in 2000 (38). In recent years, toxic species of the diatom genus *Pseudo-nitzschia* have posed a more imminent threat and gained wider notoriety. These species produce the neurological toxin domoic acid, which is the cause of amnesic shellfish poisoning (ASP) in humans and which has caused thousands of marine animal mortalities (19, 68). In addition, analyses of algal species composition in King Harbor have revealed the presence of an array of potentially harmful species, including *Prorocentrum* species (*P. micans*, *P. gracile*, and *P. triestinum*) and several raphidophytes (*Chattonella marina*, *Heterosigma akashiwo*, and *Fibrocapsa japonica*) (D. A. Caron et al., unpublished data). The present study demonstrates that *A. catenella* is also a recurrent element of this suite of toxic algae.

The monitoring program of the California Department of Public Health (CDPH) has documented increasing instances of PSP situations along the California coast (41), but these events are still rare compared to the situation in northern coastal locations, especially in Puget Sound (16, 76). Consistent with that finding, *A. catenella* cell abundances in King Harbor exceeded 1,000 cells liter^{-1} only on five occasions, and particulate STXs were detected in four of those situations. Most of the time (81% of samples), *A. catenella* abundances were <1,000 cells liter^{-1}. We were able to detect toxins in only one-third of these low-abundance samples. Low particulate toxin concentrations (3.2 to 14 ng liter^{-1}) were also detected in Monterey Bay samples when no cells were detected, but this situation presumably occurred because cell densities were below the detection level of the method (37), i.e., a filter-based whole-cell hybridization (described in reference 53) using the NA-1 probe (71). These situations indicate the need for a quantification method with a low detection limit such as our qPCR approach to account for concentrations that fall below 1,000 cells liter^{-1}.

During the persistent presence of *A. catenella* in spring 2008, the Marine Biotoxin Monitoring Program (42) detected STXeq three times in mussel samples collected in the vicinity of King Harbor, but these values did not surpass the FDA's regulatory limit of 80 μg per 100 g of tissue. Detectable amounts of STXs were reported off the coast of Palos Verdes, near Portuguese Bend (ca. 15 km south of our sampling location) on 25 March (74 μg STXeq 100 g^{-1}) and on 16 April (41 μg STXeq 100 g^{-1}) (42). Toxins were also detected in mussels from Santa Monica Pier (ca. 20 km north of King Harbor) on 22 April (43 μg STXeq 100 g^{-1}) (42). Over the same period, we measured abundances between 207 and 1,920 cells liter^{-1} and particulate toxin concentrations between 6 and 26 ng liter^{-1} in King Harbor. These observations are in agreement with the danger level defined by Jester et al. (38), which suggests that toxic events occur at cell densities exceeding 1,000 cells liter^{-1}. Although these values were low, toxin transfer from *A. catenella* to shellfish was detected over 40 km of Californian coastline, i.e., from Santa Monica to Portuguese Bend, indicating that our punctual observation of STX production in King Harbor might reflect a broader impact of *A. catenella* on the regional food webs.

Conclusions. A new qPCR approach was developed and employed to generate a 4-year data set of *A. catenella* abundances and STX concentrations in a coastal harbor of southern California (King Harbor, City of Redondo Beach). This study augments existing HAB monitoring programs in California, which generally do not provide quantitative information on *A. catenella* distribution and seasonal dynamics. *A. catenella* was present repeatedly in the harbor, especially during spring. Interannual variability in abundance and toxin concentrations may indicate a potential link between elevated abundances of this dinoflagellate and significant rainstorms during the preceding winter. Given that the ongoing climate change is predicted to increase heavy rainfall events in many regions, including semiarid areas such as the western United States (35), it is conceivable that the occurrence and seasonality *A. catenella* might be affected in the future.

The qPCR method for *A. catenella* developed and applied here allowed comparisons between toxin concentrations in plankton and cell abundances in natural waters and indicated that *A. catenella* is not always accompanied by measurable concentrations of toxins in the particulate plankton. This approach improves our understanding of toxin production by *A. catenella* and of toxin transfer to higher trophic levels. Moreover, the high sensitivity of the qPCR method provides an early-warning tool for monitoring this toxin-producing species. Such tools are critical, especially since regional climate models have predicted longer periods of favorable environmental conditions for *A. catenella* blooms and PST events in the Pacific northwest by the end of the 21st century (57).

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