

## Yessotoxin detected in mussel (*Mytilus californicus*) and phytoplankton samples from the U.S. west coast

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### Abstract

Yessotoxin (YTX) was detected in an algal sample and two mussel samples (0.07–0.10  $\mu\text{g g}^{-1}$ ) collected from Scripps Pier in La Jolla, California during a bloom of *Lingulodinium polyedrum*. Mussel samples collected from Monterey Bay, California also contained measurable YTX (levels up to 0.06  $\mu\text{g g}^{-1}$ ) in samples obtained during a 6-month (weekly) sampling period. *Gonyaulax spinifera* and *L. polyedrum* were identified in background concentrations in Monterey Bay during the time of contamination. An algal sample from Washington coastal waters collected during non-bloom conditions also contained YTX, possibly originating from *Protoceratium reticulatum*.

Three strains of *L. polyedrum* (CCMP1931, CCMP1936, 104A) isolated from southern California coastal waters and one strain of *G. spinifera* (CCMP409) isolated from Maine were tested for YTX production using two methods, competitive ELISA and liquid chromatography–mass spectrometry (LC–MS). The ELISA method detected YTX in the particulate phase in two of three *L. polyedrum* strains. The LC–MS method did not detect YTX in the particulate or dissolved phase of any of the strains.

To our knowledge, this is the first study to test and confirm YTX in environmental samples from California and Washington coastal waters. It is highly likely that *L. polyedrum* was responsible for the YTX contamination in the southern California samples. Future research needs to conclusively determine the biological origin(s) of YTX contamination in central California and Washington coastal waters.

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**Keywords:** *Gonyaulax spinifera*; Harmful algae; *Lingulodinium polyedrum*; *Protoceratium reticulatum*; Yessotoxin

### 1. Introduction

Harmful algal blooms resulting from marine toxins produced by phytoplankton are widespread, and of increasing concern, both nationally and internationally (Anderson et al., 2000; Glibert et al., 2005). Yessotoxin (YTX), a disulfated polyether toxin, was first identified in scallops from Japan, *Patinopecten yessoensis*, for which the toxin class was named (Murata et al., 1987). Since its discovery, close to 90 other analogs have been identified (Satake et al., 1997, 1999; Ciminiello et al., 1998, 2000, 2001; Daiguji et al., 1998; Miles et al., 2004, 2005a, 2005b, 2006; Finch et al., 2005; Paz et al., 2006). There are three known biological origins of YTX, the dinoflagellates, *Protoceratium reticulatum* (Claparède and Lachmann) Buetschli, *Lingulodinium polyedrum* (Stein) Dodge, and *Gonyaulax spinifera*

(Claparède et Lachmann) Diesing. The concentration of yessotoxin in shellfish and laboratory culture studies are summarized in Tables 1 and 2.

The earliest report of potential toxicity for *L. polyedrum* (then called *Gonyaulax polyedra*) from California waters was in 1962 (Schrader and Bliss, 1962). Those authors purified an acid extract from *L. polyedrum* cells and used paper chromatography in conjunction with mouse bioassay to identify a toxin similar to that produced by the dinoflagellate, *Gonyaulax catenella* Whedon and Kofoid (since renamed *Alexandrium catenella* Balech, 1985), which they concluded to be saxitoxin, with an activity of 195 mouse units from  $35 \times 10^6$  cells. Their work has been criticized due to the large amount of saxitoxin used as the standard and possible contamination of the results (Bates et al., 1978). YTX production in *L. polyedrum* has since been detected in both particulate (cellular material) and dissolved (media) phases of *L. polyedrum* cultures (Paz et al., 2004; Armstrong and Kudela, 2006).

*L. polyedrum* blooms frequently in Baja California and southern California coastal waters, and are one cause of the

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Table 1  
Summary of published concentrations of yessotoxin detected in shellfish globally

Shellfish	YTX range ( $\mu\text{g YTX g}^{-1}$ )	Country	Reference
Blue mussels ( <i>Mytilus edulis</i> )	0.5–14.8	Norway	Lee et al. (1988), Ramstad et al. (2001), Aasen et al. (2005)
Blue mussels ( <i>Mytilus galloprovincialis</i> )	0.1–9.0	Italy	Ciminiello et al. (1997), Draisci et al. (1999a)
Greenshell mussels ( <i>Perna canaliculus</i> )	1.6–3.2	New Zealand	Yasumoto and Takizawa (1997), MacKenzie et al. (2001), MacKenzie et al. (2002)
Blue mussels ( <i>Mytilus chilensis</i> )	No quantity published	Chile	Yasumoto and Takizawa (1997)
Blue mussels ( <i>Mytilus edulis</i> )	No quantity published	Japan	Yasumoto and Takizawa (1997)
Blue mussels ( <i>Mytilus edulis</i> )	0.053	Russia	Vershinin et al. (2006)
Scallops ( <i>Patinopecten yessoensis</i> )	No quantity published	Japan	Murata et al. (1987), Yasumoto and Takizawa (1997)
Shellfish (unspecified)	No quantity presented	Ireland	Joe Silke, unpublished data
California sea mussels ( <i>Mytilus californicus</i> )	0–0.10	California	This study

dramatic “red tide” events that occur in these regions (Gregario and Peiper, 2000). There have been anecdotal reports of health problems associated with red tides in southern California caused by *L. polyedrum* (Kudela and Cochlan, 2000), with no attempts before this study to isolate toxins from California isolates of *L. polyedrum* since the early work of Schradie and Bliss (1962).

*G. spinifera* isolated in New Zealand was added to the list of biological producers of YTX (Rhodes et al., 2005) following a shellfish contamination event. Unlike *L. polyedrum*, *G. spinifera* does not typically reach high densities in California, but is frequently present in background concentrations in Monterey Bay, California (M. Silver, unpublished data), and has been reported in bloom concentrations in Tomales Bay, north of San Francisco (G. Langlois, personal communication). The New Zealand isolates have been shown to produce levels of YTX more than 20-fold higher than those of *P. reticulatum* and more than 600-fold higher than *L. polyedrum* for an equivalent cell abundance (Rhodes et al., 2005).

Likely in part due to the large number of YTX-like compounds, there have been conflicting results from studies on the toxicity of YTX and the mode of action is relatively unknown. Yessotoxin has been shown to produce cardiotoxic effects and to be highly toxic to mice when injected intraperitoneally, but not when administered orally (Terao et al., 1990; Ogino et al., 1997; Aune et al., 2002). Other studies suggest the biological targets of YTX to be the lysosomes, the immune system, neuronal tissue and the thymus, with the latter having tumorigenic implications (Franchini et al., 2004a, 2004b; Malagoli et al., 2006; Perez-Gomez et al., 2006). Although there have been no studies directly accessing chronic exposure in low amounts to YTX in humans, this study raises concern about possible human health risks. Due to the potential health implications of YTX ingestion through shellfish, it is a regularly monitored marine toxin in New Zealand, Europe and Japan. In 2002, the European Commission placed yessotoxins in a separate phycotoxin group, and established a regulatory level of  $1 \mu\text{g g}^{-1}$  of YTX equivalents in shellfish meat intended

Table 2  
Summary of published studies quantifying the amount of yessotoxin per cell in culture for all of the known yessotoxin producing species, *Protoceratium reticulatum*, *Lingulodinium polyedrum*, and *Gonyaulax spinifera*

Dinoflagellate	Country	YTX ( $\text{pg cell}^{-1}$ )	Reference
<i>P. reticulatum</i>	Canada	5.0	Stobo et al. (2003)
	Italy	5.83	Boni et al. (2002)
	Norway	18–79	Samdal et al. (2004)
	Japan	0.9–14.0	Satake et al. (1999), Eiki et al. (2005)
	New Zealand	3.0–13.0	Satake et al. (1996, 1999), and MacKenzie et al. (2002), Mitrovic et al. (2005), Rhodes et al. (2005)
	Spain	0–2.6	Paz et al. (2004, 2007)
	United Kingdom	0.3	Stobo et al. (2003)
	United States	0–2.1	Paz et al. (2004, 2007), Cassis (2005)
	<i>L. polyedrum</i>	Ireland	0.3
Italy		1.5	Tubaro et al. (1998), Draisci et al. (1999a)
Norway		0	Ramstad et al. (2001)
Spain		0, 0.3	Riobo et al. (2002), Paz et al. (2004)
United Kingdom		0–0.02	Stobo et al. (2003)
California		0–0.005	This study; Armstrong and Kudela (2006)
<i>G. spinifera</i>	New Zealand	0–200	Rhodes et al. (2005)
	United Kingdom	0	Stobo et al. (2003)
	Maine	0	This study

for human consumption (Directive 2002/225/EC); Japan and New Zealand have similar regulatory language.

Given the worldwide distribution of potentially toxic dinoflagellates, the ubiquity of YTX in other countries, the repeated appearance of this toxin, and the large and frequent blooms of *L. polyedrum* in southern California, we hypothesized that YTX is also likely present in California waters. To address this possibility, we tested mussel samples from Monterey Bay and La Jolla, California, water samples from California, Oregon and Washington, and three strains of *L. polyedrum* isolated from California and one Maine strain of *G. spinifera* for the presence of YTX or related compounds.

## 2. Materials and methods

### 2.1. Mussel samples

Mussel samples, *Mytilus californicus*, were collected weekly at the Santa Cruz Municipal Wharf from March through September 2005 (36°57.48'N, 122°1.02'W) and two samples were collected from Scripps Pier in La Jolla, California (32°52.0'N, 117°15.4'W) during a bloom of *L. polyedrum*, on July 6, 2005 and September 29, 2005. Samples were obtained from mussels suspended in net bags for at least 1 month prior to collection. Immediately upon return to the laboratory, the hepatopancreas was extracted, homogenized and stored at -22 °C. Samples were shipped frozen to the Canadian Food Inspection Agency for YTX analysis using liquid chromatography–mass spectrometry (LC–MS) based on the method of van de Riet et al. (1995). Concurrent net tow and whole-water samples were collected each week from the Santa Cruz Municipal Wharf, immediately fixed in a 4–5% formalin solution and stored at 4 °C for later analysis of phytoplankton species. The fixed whole-water samples were settled in 100 mL aliquots for 24 hours in Utermöhl chambers and analyzed using an Olympus IMT-inverted microscope.

To complement the wharf sampling, additional samples were obtained from monthly transects of Monterey Bay conducted as part of the Center for Integrated Marine Technologies (CIMT) pilot ocean observing system (July 2002 to July 2005) and the Central and Northern California Ocean Observing System (CeNCOOS) (August 2005 to July 2006). Net tow and water samples were collected at 11 sampling stations along seven transect lines within Monterey Bay (data and additional details are available at <http://www.cimt.ucsc.edu>). These samples were fixed in a 4–5% solution of formalin immediately after collection on the ship for later analysis in the laboratory, following the protocols described above.

### 2.2. Water samples

Water was collected from the Scripps Pier in La Jolla, California during a bloom of *L. polyedrum* on June 9, 2005. The water was filtered onto uncombusted glass-fiber filters (Whatman GF/F; nominal pore size 0.7 µm) and stored frozen at -20 °C until analysis for particulate YTX. Additional particulate

YTX samples were collected aboard the R/V *Point Sur* June 27 to July 2, 2004 from the coasts of California, Oregon and Washington. Shipboard water samples were collected using a trace metal clean pump sipper system from near surface (1–5 m) waters; wharf samples were collected using a clean plastic bucket from the surface.

Yessotoxin from the filtered particulate material (hereafter referred to as particulate YTX to distinguish from YTX compounds associated with the dissolved phase, or filtrate) was analyzed using high-pressure liquid chromatography (HPLC) with fluorescence detection (FLD) following the method of Yasumoto and Takizawa, 1997. Yessotoxin standards were obtained from Biosense ELISA kits, since at the time of our experiment, there was no independent, commercially available YTX standard. Samples and standards were mixed with the fluorogenic reagent 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalanyl)ethyl]-1,2,4-triazoline-3,5-dione (DMEQ-TAD) and analyzed on a Hewlett Packard 1090 liquid chromatograph with a Hewlett Packard 1046A fluorescence detector. Draisci et al. (1999b) have positively identified YTX using a combination of liquid chromatography–mass spectrometry with ionspray ionization. The chromatograms from this study were compared to the HPLC data reported previously (Satake et al., 1996, 1997, 1999; Yasumoto and Takizawa, 1997; Draisci et al., 1999a, 1999b) to confirm the identification of YTX. Due to the lack of commercially available standard, we used the limited amount of standard available to analyze the field samples without and with (unspiked and spiked) 25 ng mL<sup>-1</sup> YTX standard added to the particulate samples to confirm that the peaks identified were YTX. As a method control sample, we prepared a particulate sample using the non-YTX producing diatom, *Thalassiosira pseudonana*, and ran this unspiked and spiked with 80 ng mL<sup>-1</sup> YTX standard.

### 2.3. Laboratory cultures

We tested three strains of *L. polyedrum*, CCMP1931, CCMP1936 (from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton), and 104A (received from P. Franks, Scripps Institute of Oceanography; isolated from Scripps Pier in La Jolla, California in 1998) and one strain of *G. spinifera*, CCMP409 (from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton; isolated from West Boothbay Harbor, Maine in 1986) for the presence of YTX. An indirect competitive enzyme linked immunosorbent assay (cELISA) method (Garthwaite et al., 2001; Miles et al., 2002; Briggs et al., 2004; Samdal et al., 2004) and a liquid chromatography–mass spectrometry method (Stobo et al., 2003) were used. Each strain was grown under non-axenic conditions in autoclaved glass flasks with either f/2 or L1 media (Guillard and Ryther, 1962; Guillard, 1975; Guillard and Hargraves, 1993) to approximately 10<sup>3</sup> cells mL<sup>-1</sup>. Culture conditions were standardized at the growth temperatures of 21 °C (±1 °C) and 87 µmol photons m<sup>-2</sup> s<sup>-1</sup> irradiance using Sylvania “grow-lite” spectrally corrected light sources on a 14:10 light:dark cycle.

Preliminary (pre-commercial) YTX ELISA kits were purchased from Biosense Laboratories (Bergen, Norway), which had licensed the method from AgResearch (New Zealand). This method has been compared with directly quantifiable methods such as liquid chromatography–mass spectrometry (Briggs et al., 2004) and has a working range of 50–1100 pg mL<sup>-1</sup> YTX equivalents, with cross-reactivity to YTX, 45-hydroxy-YTX, homoYTX, and 45-hydroxy-homo-YTX (Samdal et al., 2005). For cELISA detection, whole culture material (50 mL, cells plus media) was passed through a sterile 0.2 µm Gelman Laboratory Acrodisc syringe filter, and then through a Waters 500 mg C18 Sep-Pak cartridge to isolate the dissolved phase toxins. The filter was removed, and the dissolved fraction was eluted three times with 5 mL (sequential additions) methanol. The filter was then put inline with a C18 cartridge and the particulate fraction was eluted four times with sequential 5 mL methanol extractions; each elution was collected in separate glass vials. All samples were evaporated to dryness at 65 °C, after which 1 mL of sample buffer (from Biosense) was added to the particulate and dissolved samples, vortexed to dissolve the dried extract and then analyzed following the standard ELISA procedures described by Biosense.

The LC–MS method followed was as described by Stobo et al. (2003) for analyzing particulate, dissolved and whole culture samples. The cultures were filtered onto Whatman GF/F filters and immediately extracted into 25 mL of 100% methanol, then sonicated for 1 min and centrifuged in a Fisher Scientific Marathon 8K centrifuge for 7 min at 4000 rpm. The supernatant (22 mL) was pipetted onto an Oasis-HLB (Waters, 500 mg) SPE cartridge and vacuum filtered (<100 mm Hg). The cartridge was eluted with 10 mL of 100% methanol (Fisher Optima HPLC Grade) into a glass ampoule that was then flame-sealed. Dissolved samples (filtrate from the particulate fraction) were vacuum filtered through an Oasis-HLB (Waters, 500 mg) SPE cartridge after 1.3 mL of 100% methanol was added to 25 mL filtrate. The toxin was eluted and flame-sealed in an ampoule as for the particulate fraction. Total toxin was determined from 25 mL culture aliquots (cells plus media) sonicated for 2 min, centrifuged for 5 min at 4000 RPM and then processed as described above for the dissolved samples. The flame-sealed ampoules were submitted to the NRC Institute for Marine Biosciences (Halifax, NS, Canada) for analysis by LC–MS using electrospray ionization and selected reaction monitoring.

### 3. Results

#### 3.1. Mussel samples

The two mussel samples and the water sample from La Jolla, CA were collected during a bloom of *L. polyedrum* that persisted from June through September, 2005. *L. polyedrum* was observed in water samples as far north as Santa Barbara (G. Langlois, California Department of Public Health, Biotoxin Monitoring Program, personal communication). The highest YTX value recorded for this study (0.10 µg g<sup>-1</sup>) was in the mussel sample collected on July 6, 2005 (Fig. 1). The YTX

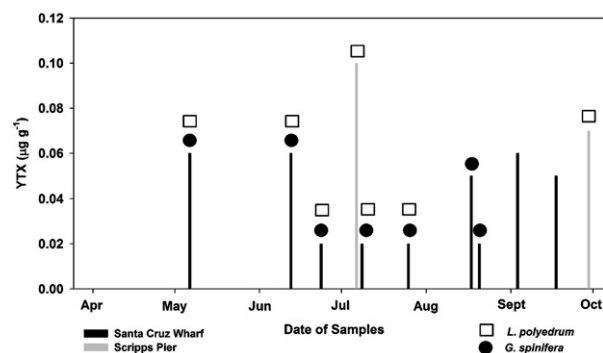


Fig. 1. YTX detected in mussel samples collected weekly at the Santa Cruz Wharf from March through September 2005 (solid black bar) and two samples collected from Scripps Pier, La Jolla, CA during a bloom of *L. polyedrum* (solid gray bars). All samples reported in µg YTX g<sup>-1</sup> shellfish meat. The YTX-producing species identified in whole-water samples during the same month are indicated by (●) for *G. spinifera* and (□) for *L. polyedrum*.

concentration from the second mussel sample collected at this location at a later date (September 29, 2005) during the same *L. polyedrum* bloom was 0.07 µg g<sup>-1</sup>.

YTX was also detected (<0.05–0.06 µg g<sup>-1</sup>) in the samples collected in Monterey Bay (Fig. 1). Several of the fixed whole-water samples (collected concurrently with the mussels) were analyzed for potential YTX-producing species. On May 7, 2005, 0.06 µg g<sup>-1</sup> of YTX was measured from a mussel sample and there were 30 cells L<sup>-1</sup> of *G. spinifera* identified in the associated fixed water sample. Fixed whole-water samples from the May monthly transects in Monterey Bay contained *L. polyedrum* in background concentrations (<5% of the total net plankton assemblage) at 3 of 11 stations. Mussel samples collected in June also exhibited measurable YTX, with a maximum value of 0.06 µg g<sup>-1</sup> YTX on June 13, 2005. The corresponding fixed whole-water sample had 10 cells L<sup>-1</sup> of *G. spinifera*, while 2 out of the 11 Monterey Bay transect stations contained background concentrations of *L. polyedrum*. The July mussel samples exhibited the lowest concentration of YTX; therefore, the fixed whole-water samples were not examined for potential toxin-producing cells. However, the monthly transect samples identified *L. polyedrum* at one station and *G. spinifera* at 4 of 11 stations, all in background concentrations. There was 0.05 µg g<sup>-1</sup> of YTX detected in the mussel sample from August 18, 2005, with 50 cells L<sup>-1</sup> of *G. spinifera* in the corresponding fixed whole-water sample. *G. spinifera* was also identified at 1 of 11 stations in the monthly transect samples. Yessotoxin was detected in the mussel samples from the beginning of September, but potential YTX-producing species were not found. These results are summarized in Fig. 1.

#### 3.2. Water samples

Chromatogram results are shown for the field sample collected from Scripps Pier in La Jolla, California on June 9, 2005 during a bloom of *L. polyedrum* (Fig. 2, solid black line) and for the same sample with 25 ng mL<sup>-1</sup> YTX standard added (Fig. 2, dashed black line) analyzed by HPLC with fluorescence

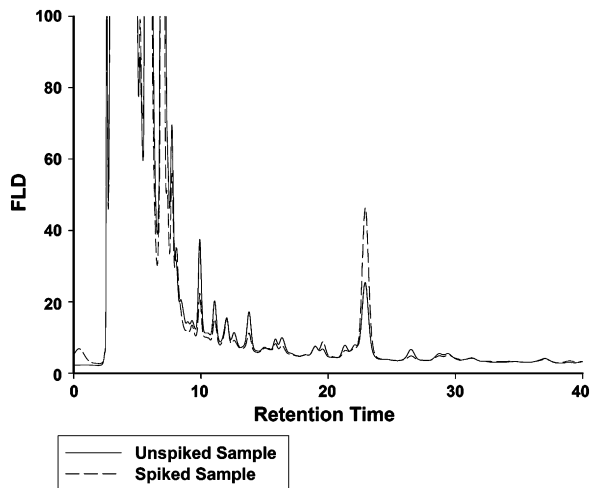


Fig. 2. A field Sample collected from Scripps Pier during a bloom of *L. polyedrum* in June 2005 analyzed using HPLC run unspiked (solid black line) and spiked with  $25 \text{ ng mL}^{-1}$  YTX standard (dashed black line). The 22-min YTX peak (confirmed by separate standard-only chromatograms, not shown) doubled in the spiked sample compared to that in the non-spiked sample.

detection. The 22-min YTX peak was confirmed by separate standard-only samples (data not shown). The putative YTX peak doubled in the spiked sample compared to the non-spiked sample, confirming the presence of YTX. However, due to the lack of a commercially available standard at the time of this experiment, the precise concentration of YTX in the sample cannot reliably be calculated.

Several phytoplankton samples were collected during June and July 2004 in the coastal waters of California, Oregon and Washington. YTX was detected in only one sample collected near Grays Harbor, Washington ( $46^{\circ}51.70'N$ ,  $124^{\circ}17.97'W$ ). This sample was analyzed by HPLC with and without a  $25 \text{ ng mL}^{-1}$  YTX standard spike. The spiked sample (22-min peak) increased by 10% compared to the unspiked sample.

### 3.3. Laboratory cultures

YTX was detected using the cELISA method, in the particulate phase (in the cells) of *L. polyedrum* cultures:  $1.0\text{--}5.0 \text{ fg cell}^{-1}$  for strain 104A and  $0.8 \text{ fg cell}^{-1}$  for strain CCMP1931. YTX was not detected in the particulate phase of strain CCMP1936. YTX was also detected in the dissolved phase (in the media) of *L. polyedrum* strains 104A and CCMP1931 ( $16 \text{ pg mL}^{-1}$  and  $8 \text{ pg mL}^{-1}$ , respectively). The *G. spinifera* isolate (CCMP409) was not analyzed with the cELISA method for the presence of YTX. Despite the application of high sensitivity LC–MS methods, the presence of YTX, 1-homoYTX or 45-hydroxy-YTX could not be confirmed in any of the particulate, dissolved or whole-culture samples of *L. polyedrum* (CCMP1931, CCMP1936 and 104A) or the *G. spinifera* isolate (CCMP409).

## 4. Discussion

YTX is present in central and southern California coastal waters as evidenced by the mussel and water samples collected

in 2005 during both bloom conditions (Scripps Pier samples) and non-bloom conditions (Monterey Bay samples). Based on the co-occurrence of YTX in mussel and water samples during the bloom of *L. polyedrum*, we conclude that *L. polyedrum* field populations in southern California are likely producing low concentrations of YTX and were probably the source of YTX detected in the field samples for this region.

The biological origin of YTX in mussels is not as apparent in Monterey Bay. *G. spinifera* and *L. polyedrum* were in background concentrations when YTX was measured in the mussel samples. These results suggest that there are potentially two YTX-producing species responsible for YTX contamination in mussels from Monterey Bay. YTX has been reported to have a half-life of 24 days in Norwegian blue mussels (*Mytilus edulis*) (Aasen et al., 2005) and 49 days in Greenshell mussels (*Perna canaliculus*) from New Zealand (MacKenzie et al., 2002). Interestingly, Aasen et al. (2005) reported that YTX was present in mussels during the entire sampling year of their study, not just during bloom conditions. They suggested previous contamination and/or the presence of *P. reticulatum* below the detection limit of  $20 \text{ cells L}^{-1}$  as the source of YTX in the mussels during non-bloom periods. These studies illustrate the complexity in determining the biological origin(s) of YTX in the contaminated Monterey Bay mussel samples. It is feasible that the potential YTX-producing species identified in Monterey Bay in low, but detectable, background concentrations could account for the low YTX detected in the mussel samples but future research is needed to identify specific YTX-producers in this region.

There was one sample collected from Washington that contained YTX. A bloom of *P. reticulatum* was reported in this area in August (ca. 1 month after collection of the positive YTX sample), with the bloom peaking on September 2, 2004 (Alan Sarich, Washington Department of Fish and Wildlife). It is possible that this sample was collected during pre-bloom conditions when *P. reticulatum* was in background concentrations (<5% of the total net plankton assemblage) since strains isolated from British Columbia are toxic (Cassis, 2005). The positive result from this study suggests that YTX could potentially be present along much of the U.S. west coast.

Laboratory analyses of algal cultures provide conflicting results as to the toxicity of cultured *L. polyedrum* strains from southern California. The cultures tested exhibit very low or undetectable levels of YTX depending on the method used, with the ELISA method indicating very low YTX production in the particulate phase (in the cells) of the *L. polyedrum* strains (104A and CCMP1931, but not CCMP1936), but with no detectable toxin by LC–MS. The detection of YTX in the dissolved phase (in the media) is most likely due to matrix effects from salt (Ingunn Samdal, personal communication). However, it is highly likely that YTX is present in the dissolved phase from toxin-producing cultures, given that previous investigators have detected YTX in the particulate and in the dissolved phases of other strains of *L. polyedrum* (Paz et al., 2004). The results from the ELISA and LC–MS methods appear to be conflicting. However, Samdal et al. (2005) compared the ELISA to the LC–MS method for yessotoxins in

Norwegian blue mussels and concluded that the ELISA response was 3–13 times greater than that of the LC–MS. Aasen et al. (2005) also compared these two methods using Norwegian mussels and reported the ELISA to be 3–9 times more sensitive than the LC–MS method. They attributed this higher sensitivity to antibodies in the ELISA binding to other YTX analogs whereas the LC–MS only quantifies those YTX congeners that have standards associated with them. Since there have been over 90 YTX analogs identified to date (Miles et al., 2005b), it is possible that the ELISA method used in this analysis was detecting many YTX analogs, whereas the LC–MS was only evaluating YTX, 1-homoYTX and 45-hydroxy-YTX. Additional research is required to determine if the ELISA signal is due to a multitude of YTX analogs (Aasen et al., 2005; Samdal et al., 2005) or due to the presence of closely related, unidentified compounds. Previous published studies have also reported conflicting results on toxicity. Initial studies using several strains of *P. reticulatum* indicated toxin production (Paz et al., 2004) and subsequent studies indicated the same strains to be non-toxic (Paz et al., 2007, B. Paz, personal communication). Future research is needed to improve the detection methods associated with YTX, particularly the development of a sensitive method that would provide consistent results and would be relatively easy to employ in biotoxin monitoring programs. This is essential in order to accurately identify the biological origin(s) of YTX in regions where toxin has been detected in shellfish, such as in Monterey Bay, California.

The levels of YTX measured in this study, during both bloom and non-bloom conditions, are 10 times lower than the EU regulatory limit of  $1 \mu\text{g g}^{-1}$  of YTX equivalents in shellfish meat. These initial results suggest that YTX probably does not pose a significant health risk, if these toxin levels are indicative of the typical concentrations found in coastal California. However, future studies are needed to determine the temporal and spatial variations in seasonal bloom events and resulting toxicity to determine any potential human or wildlife health impacts. Additionally, isolation of potential YTX-producing dinoflagellates and subsequent toxicity determination are required to conclusively identify all of the biological origin(s) of YTX on the U.S. west coast.

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