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Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA  
SANTA CRUZ

***Dinophysis* and Diarrhetic Shellfish Poisoning Toxin  
at Santa Cruz Municipal Wharf,  
Santa Cruz, California**

A thesis submitted in partial satisfaction  
of the requirements for the degree of

MASTER OF SCIENCE

in

OCEAN SCIENCES

by

**Dana M. Shultz**

March 2018

The dissertation of Dana M. Shultz is  
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Tyrus Miller  
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2018

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## Abstract

### DINOPHYSIS AND DIARRHETIC SHELLFISH POISONING TOXIN AT SANTA CRUZ MUNICIPAL WHARF, SANTA CRUZ, CALIFORNIA

by  
Dana M. Shultz

Diarrhetic Shellfish Poisoning Toxins (DSTs) are produced by the marine dinoflagellate, *Dinophysis*. DSTs can bioaccumulate in shellfish and cause gastrointestinal illness when humans consume high levels of this toxin. Although not regulated in the U.S., recent studies in Washington, Texas, and New York suggest DSTs may be widespread throughout U.S. coastal waters. This study describes a four-year time series (2013-2016) of *Dinophysis* concentration and DST level in California mussels (*Mytilus californianus*) from Santa Cruz Municipal Wharf (SCMW) in Monterey Bay, California. Results show a maximum *Dinophysis* concentration of 9,404 cells/L during this study and suggest *Dinophysis* persists as a member of the background phytoplankton community throughout the year. DSTs in California mussels were found in persistent low levels throughout the course of this study, and exceeded the FDA action level of 160 ng/g 19 out of 192 weeks sampled. *Dinophysis* concentrations alone are a positive but weak predictor of DST level in California mussels, and basic environmental variables (temperature, salinity, and nutrients) do not sufficiently explain variation in *Dinophysis* concentration at SCMW. Overall, this study demonstrates that *Dinophysis* concentrations on the central coast of California, at SCMW, are producing DSTs that accumulate in local shellfish throughout the year, occasionally reaching levels of concern.



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## Chapter 1: Background and Introduction

Phytoplankton form the base of the food chain in the ocean's sunlit surface waters. Coastal areas with high levels of primary productivity from phytoplankton generally boast efficient food webs that support a diversity of life, including fish and marine mammals (Ryther, 1969; Kudela et al., 2005); however, phytoplankton also have the capacity to negatively impact ocean ecosystems (Hallegraeff, 1993). Such events are referred to as harmful algal blooms (HABs). HABs include any phytoplankton event that negatively impacts human health, socioeconomic interests, or aquatic ecosystems (Anderson et al., 2012). HAB causing phytoplankton can produce toxins that accumulate in the food web and contaminate seafood, they can cause blooms resulting in hypoxic conditions that harm marine life and fisheries, and they can hurt the coastal tourism industry by discoloring water at popular destinations (Hallegraeff, 1993; Anderson et al., 2012). The majority of toxin-producing HABs in marine waters are caused by dinoflagellates, and include several well-documented syndromes such as paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), Ciguatera fish poisoning (CFP), and diarrhetic shellfish poisoning (DSP) (Smayda, 1997; Burkholder, 1998; FDA, 2011).

DSP is caused by a suite of lipophilic toxins produced by HAB-causing species within the dinoflagellate genus *Dinophysis*. Diarrhetic Shellfish Poisoning toxins (DSTs) include okadaic acid (OA) and its analogues: dinophysistoxin 1 (DTX-1) and dinophysistoxin 2 (DTX-2) (Reguera et al., 2014). These toxins inhibit protein phosphatases in the cells of mammals, yeast and higher plants (Cohen et al., 1990).

When high levels of DSTs bioaccumulate in seafood and are consumed by humans, hyperphosphorylation of ion channels in the intestines causes nausea, vomiting, diarrhea, abdominal pain, headache, and fever, all of which generally pass within a few days (Cohen et al., 1990; Cordier et al., 2000; FDA, 2011). In addition to causing gastrointestinal illness, okadaic acid is a proven tumor promoter in rodents (Fujiki and Suganuma, 1999). Historically, yessotoxins (YTX) produced by the dinoflagellates *Protoceratium* and *Lingulodinium* were also associated with DSTs, but recent evidence suggests that these toxins should be excluded from the DST group since YTX does not cause the same symptoms as DSTs (Paz et al., 2008). The most common cause of DSP in humans is consumption of contaminated shellfish, especially mussels, but large-scale DSP outbreaks have been associated with consumption of other types of seafood, such as brown crabs (*Cancer pagurus*) (Reguera et al., 2014; Torgensen et al., 2005). The regulatory limit for DSTs in Europe is 160 ng OA equivalents (combined free OA + DTX-1 + DTX-2 and acyl-esters of the same compounds) per g shellfish meat (=160 µg/kg), and the regulatory limit in China and Australia is 200 ng/g. Although not routinely monitored in U.S. coastal waters, the Food and Drug Administration (FDA) recommends that all shellfish products with DSTs measuring above 160 ng/g be removed from the market (FDA, 2011).

*Dinophysis* and DSTs have a worldwide distribution. DSTs are the main HAB threat to aquaculture in Northern Japan, Chile, and Europe (Reguera et al., 2014). DSTs were first isolated, described, and linked to two species of *Dinophysis*,

*D. acuminata* and *D. fortii*, in Northern Japan in the 1980's (Yasumoto et al., 1980; Yasumoto et al., 1985). In China, DSTs historically measured under or slightly above the regulatory limit (200 ng/g) and were not considered a threat to aquaculture until 2011, when more than 200 illnesses were linked to mussels (*Mytilus galloprovincialis*) containing DSTs 40 times the European regulatory limit (Li et al., 2012). DSP events in Europe are widespread and associated with both *D. acuminata* and *D. acuta* (Reguera et al., 2014). In the Galician Rías in Spain, DST remained above the regulatory limit in shellfish from autumn 2005 through March 2006 (Reguera et al., 2014). During an extreme DST event in Northern Portugal, DST in shellfish was measured to be 112 times the regulatory limit, and was linked with local illness (Reguera et al., 2014). Large scale outbreaks of DSP are generally associated with DST levels in shellfish one or more orders of magnitude above the regulatory limit, but illnesses have been linked to DST in shellfish as low as 3 times the regulatory limit (Reguera et al., 2014).

North America has historically been considered a “DSP-free” region with less toxic strains of *Dinophysis* than other areas, but multiple recent studies have recorded high levels of DSTs in shellfish (Reguera et al., 2014). In the summer of 2011, DST in blue mussels from Washington State measured 2-10 times the FDA action level of 160 ng/g and DST in mussels from British Columbia measured 5 times the FDA action level (Taylor et al., 2013; Trainer et al., 2013). The high levels of DST in shellfish led to three illnesses in Washington, marking the first clinical report of DSP in the U.S., and 62 illnesses in British Columbia. In the summer of 2011 in New

York, mussel tissue was found to have DST over 7 times the FDA action level (Hattenrath-Lehman et al., 2013). In Texas, the first shellfish harvesting closure in the U.S. as a result of high DSTs occurred in 2008, when oysters (*Crassostrea virginica*) were found to contain DST 2-3 times the FDA action level (Deeds et al., 2010). These events in Washington, Canada, and Texas strongly suggest that North America can no longer be considered “DSP-free”. More work is needed to describe and understand the toxicity of North American strains of *Dinophysis* and how per cell toxin varies over geographic space and with changing environmental conditions.

The genus *Dinophysis* has over 120 identified species, only twelve of which are considered toxin-producers. Toxic species of *Dinophysis* include: *D. acuminata*, *D. acuta*, *D. caudata*, *D. fortii*, *D. infundibula*, *D. miles*, *D. norvegica*, *D. ovum*, *D. sacculus*, *D. tripos*, *D. mitra* (= *Phalacroma mitra*), and *D. rotundata* (= *Phalacroma rotundatum*); however, the ability for *D. rotundata* to produce toxin has been called into question (Gonzales-Gil et al., 2010; Reguera et al., 2014). Five toxic *Dinophysis* species have been recorded in samples from Santa Cruz Municipal Wharf in the past twenty years (*D. acuminata*, *D. caudata*, *D. fortii*, *D. tripos*, and *D. rotundata*) (Weber, 2000; Sutherland, 2008). A study by G. Carl Scradler in 1981 found three additional toxic species, *D. acuta*, *D. norvegica*, and *D. ovum*, in Monterey Bay phytoplankton samples. Identification of *Dinophysis* to species level is time consuming and some species of *Dinophysis* are difficult to identify based on morphological characteristics alone. For example, *D. acuminata*, *D. ovum*, and *D. sacculus* are generally considered the *D. acuminata* complex, because identification

using light microscopy is unreliable. The most reliable way to identify species of the *Dinophysis acuminata* complex is to sequence their mitochondrial genes (Raho et al., 2008).

Species-specific conceptual models of *Dinophysis* abundance have been developed in other systems, such as the Galician Rías and along the coast of Ireland. In the Galician Rías, early spring blooms of *D. acuminata* have been attributed to anomalous upwelling patterns, suitable ciliate prey populations, and persistent winter populations of *D. acuminata* (Diaz et al., 2013; Velo-Suarez et al., 2014). A large bloom event of a second toxic species in the Galician Rías, *D. acuta*, was attributed to extreme hydroclimatic anomalies (Diaz et al., 2016). On the southern coast of Ireland, high-density thin layers of *D. acuta* accumulate as a result of wind-driven transport and coastal jets (Raine et al., 2010; Farrell et al., 2012). On the Gulf Coast of the U.S., *D. ovum* populations have been linked to favorable temperature and salinity ranges, as well as the availability of ciliate prey (Harred and Campbell, 2014). It is suggested that a model describing *Dinophysis* abundance in Monterey Bay should be species-specific and take into consideration physical, chemical and biological variables.

The following chapter provides baseline information on *Dinophysis* and DST levels in shellfish at Santa Cruz Municipal Wharf, in Monterey Bay, California over a four-year period from 2013-2016.

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## **Chapter 2: *Dinophysis* and Diarrhetic Shellfish Poisoning Toxin Levels at Santa Cruz Municipal Wharf: 2013-2016**

### **1. Introduction**

Harmful algal blooms (HABs) are macroalgal and microalgal events that have the potential to cause ecological and economic harm such as wildlife die offs, human illness, and monetary loss for fishing and tourism industries (Anderson et al., 2012). Over the past several decades, negative economic and ecosystem impacts of HABs have been increasingly observed worldwide (Anderson et al., 2012). The majority of toxin-producing HABs in marine waters are caused by dinoflagellates, and include several well-documented syndromes such as paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), Ciguatera fish poisoning (CFP), and diarrhetic shellfish poisoning (DSP) (Smayda, 1997; Burkholder, 1998; FDA, 2011). The toxin producing marine dinoflagellate, *Dinophysis*, is the primary causative organism of DSP (Reguera et al., 2014).

*Dinophysis* has the ability to produce a suite of lipophilic toxins that bioaccumulate in shellfish and can cause gastrointestinal illness in humans. Diarrhetic Shellfish Poisoning is the illness associated with human ingestion of these toxins, and the associated toxins are collectively referred to as Diarrhetic Shellfish Poisoning Toxins (DST). The symptoms of DSP include: nausea, vomiting, diarrhea, abdominal pain, headache, and fever, all of which generally pass within a few days (FDA, 2011). DSTs were first isolated and described by Yasumoto et al. (1980,

1985) in studies spurred by his own experience with food poisoning after eating cooked shellfish during a DSP event in Japan in the late 1970's (Reguera et al., 2014).

Yasumoto et al. linked two species of *Dinophysis*, *D. fortii* (1980) and *D. acuminata* (1985), to DSTs, marking the first studies to suggest that members of the genus *Dinophysis* produce toxin. DSTs include okadaic acid (OA), dinophysistoxin 1 (DTX-1), and dinophysistoxin 2 (DTX-2), each of which has a slightly different chemical structure, but produce the same toxic effect in consumers (Reguera et al., 2014). *Dinophysis* is the main HAB threat to aquaculture in Northern Japan, Chile, and Europe, but DSTs are not monitored in the U.S. Although not monitored, the U.S. Food and Drug Administration (FDA) recommends that all shellfish products measuring above 160 ng/g DST (combined free OA + DTX-1 + DTX-2 and acyl-esters of the same compounds) be removed from the market (FDA, 2011).

The first clinical report of DSP in the U.S. occurred in June 2011 in Washington State when three people fell ill after consuming recreationally harvested shellfish (Trainer et al., 2013). The first DSP outbreak in British Columbia, Canada also occurred in the summer of 2011 (late July-August), resulting in 62 illnesses and large-scale product recalls (Taylor et al., 2013). In the summer of 2011 in New York, mussel tissue was found to contain DST 7 times the FDA action level of 160 ng/g (Hattenrath-Lehman et al., 2013). Prior to these events in the summer of 2011, the first closure of shellfish harvesting in the U.S. because of DSTs occurred on the Texas Gulf Coast in March-April 2008 (Deeds et al., 2010; Campbell et al., 2010). While there is evidence of DSTs on the east coast of the U.S. dating back to the

1980's (Reguera et al., 2014), the more recent events in Washington and Texas have demonstrated the need to better understand and measure DST occurrence in all U.S. coastal waters.

*Dinophysis* has historically been observed as a member of the phytoplankton community in Monterey Bay and along California's coastline (Jester et al., 2009; Southern California Coastal Ocean Observing System, 2017), yet little is known about the ecology of *Dinophysis* in California's coastal waters. Multiple factors make *Dinophysis* difficult to study. In Monterey Bay, *Dinophysis* represents a small portion of the phytoplankton population, and does not form dense blooms in surface waters like other dinoflagellate genera, such as *Cochlodinium fulvescens*, *Ceratium spp.*, and *Akashiwo sanguinea* (Ryan et al., 2009). There are also no documented occurrences of DSP in humans within California, although given the symptoms, it is possible that mild DSP events have gone unrecognized. As a result, *Dinophysis* has not attracted attention to the same extent as other toxic dinoflagellates that have large bloom events and discolor local waters. In addition, *Dinophysis* was not successfully cultured in a laboratory until 2006, when it was found to be a mixotroph requiring a three member trophic chain. *Dinophysis* can only obtain chloroplasts by first feeding on a ciliate that has already fed on a cryptophyte (Park et al., 2006). This trophic chain continues to make studying *Dinophysis* in culture a challenge.

Previous studies have documented multiple toxic species of *Dinophysis* present in Monterey Bay (Weber, 2000, Sutherland, 2008). In the summer of 1999, *Dinophysis* cells were determined to contain OA and DTX-1 (Weber, 2000). In

2004-2005, California mussel tissue tested for DSTs were positive for both OA and DTX-1 at low levels (Sutherland, 2008). To date, these previous studies have not been published in the peer-reviewed literature, and a baseline of DST level in shellfish of Central California has not been established. This study seeks to inform our understanding of *Dinophysis* and DSTs in Monterey Bay by investigating three main questions. First, what levels of toxin are present in local shellfish over the period from 2013-2016 at Santa Cruz Municipal Wharf (SCMW) and what are the concurrent concentrations of *Dinophysis*? Second, to what degree do genus-level *Dinophysis* measurements relate to DST levels in shellfish? Lastly, what environmental conditions are most associated with local populations of *Dinophysis*?

## **2. Methods**

### **2.1 Sampling site and sample collection**

Data used in this study were collected weekly at SCMW (36.9573°N, 122.0173°W), from 2013-2016. Phytoplankton and seawater data originated from two methods of collection — a depth integrated whole water sample and a vertical net tow sample. For the depth integrated sample, a Niskin bottle was used to collect equal volumes of water at 0, 1.5, and 3 meters depth, which were then mixed together in a plastic container. To collect a net tow sample, a 20 µm mesh phytoplankton net was vertically dragged through 50' of water (dropped to 10', then, pulled to the surface 5 times), following standard methods employed by the California Department of Public Health monitoring program.

## 2.2 *Dinophysis* analyses

Cell counts were conducted by settling 50 mL of depth integrated whole water (preserved with Lugol's iodine solution) in an Utermöhl settling chamber. Counts were done on a Zeiss Axiovert 200 inverted microscope. The entire slide was counted for a majority of samples (N=146), with a detection limit of 20 cells/L. When phytoplankton biomass was unusually high, such as during a bloom, 10 random fields of view were selected for enumeration, resulting in a detection limit of 600 cells/L. This was the case for 54 samples, most of which contained *Dinophysis*. Eleven of these samples had zero *Dinophysis*, but because of the high limit of detection, these eleven samples were removed from all time series plots and time series analyses of genus level *Dinophysis* data. *Dinophysis* cells were identified to species level for samples from 2013-2014. (Raho et al., 2008). The classification *D. acuminata* complex was used to include the species *D. acuminata*, *D. ovum*, and *D. sacculus*, which are difficult to distinguish morphologically using light microscopy (Raho et al., 2008).

*Dinophysis* presence/absence was determined from the net tow sample. A small portion of the sample (~5 mL) was examined each week on the day of collection using a Leica MZ 12.5 dissecting microscope. Relative abundance was determined for each genus present. Relative abundance index (RAI) observations categorized each genera of phytoplankton by the percent it made up of the whole phytoplankton community (Jester et al., 2009). The categories were: absent (0%), rare (<1%), present (1-10%), common (10-50%), and abundant (>50%). For this

study, *Dinophysis* RAI data was binned into categories of absent (0%) or present (>0%).

### **2.3 DST analyses**

California mussels (*Mytilus californianus*) were collected weekly from SCMW as part of the California Department of Public Health Biotoxin Monitoring Program. These mussels were initially collected from the intertidal zone at Davenport Landing Beach, put into mesh bags of approximately 30 mussels per bag and maintained for various durations in a flowing seawater table of sand filtered water (30 µm pore size) at UCSC Long Marine Laboratory. Next, these bags were deployed off a platform at SCMW for at least one week. Each week, one bag of mussels was removed from the wharf and brought into the laboratory for processing. In the laboratory, mussels were shucked and all tissues from 20-30 mussels, except for the white fibrous muscle tissue, was removed, drained with a colander, and homogenized using a Waring Xtreme Hi-Power Blender. A small aliquot of this homogenate (2g) was hydrolyzed according to the protocol outlined in Villar-Gonzalez et al. 2007 and prepared for DST analysis. Extractions were run on an Agilent 6130 quadrupole liquid chromatography-mass spectrometer (LC-MS) with Select Ion Monitoring (SIM) in negative mode using an Agilent Poroshell 120 SB-C18, 2.1x50mm column with matching guard column. A gradient elution started with 95% water with 2 mM ammonium formate and 50 mM formic acid (A) and 5% acetonitrile with 50 mM formic acid (B) for 1 minute, then to 60% at 6 minutes, and 5% A at 8 minutes, held until 11 minutes before returning to initial conditions.

Injection volume was 50  $\mu\text{L}$  and flow rate was 0.85 mL/min. Okadaic acid, DTX-1, and DTX-2 were monitored using masses 803.5 (OA, DTX-2) and 813.5 (DTX-1). Quantification was based on mass and time, with an external standard curve using certified reference material from NRC-Canada. Minimum Detection Limits (MDL) were 0.5, 0.75, and 1.0 ng/mL on-column, equivalent to 5.0, 7.5, and 10.0 ng/g tissue.

#### **2.4 Environmental samples**

The depth integrated whole water sample was used to determine all environmental variables. Water temperature was measured at the time of collection at SCMW using a NIST-traceable digital thermometer. Beginning March 11, 2015, salinity measurements were conducted in the laboratory using an ECOSense EC300A salinometer. Prior to that date, salinity was calculated from formalin preserved samples using an YSI 3100 Conductivity Meter, cross-calibrated with a YSI 6600v2 sonde deployed at SCMW as part of the Central and Northern California Ocean Observing System; a subset of samples were also analyzed using both the formalin-preserved sample and the fresh sample to ensure continuity and intercomparability of the discrete samples. Duplicate chlorophyll samples were filtered onto a  $\sim 0.7 \mu\text{m}$  glass fiber filter (Whatman GF/F), extracted for 24 hours in 90% acetone and read on a Turner 10AU Fluorometer using the non-acidification technique (Welschmeyer, 1994). Ammonium samples were analyzed using the OPA method and read on a TD700 Fluorometer (Holmes et al., 1999). Urea samples were analyzed using the colorimetric method and read on a Varian Cary 50 Bio UV/Visible Spectrophotometer with a 10 cm pathlength cell (Mulvena and Savidge, 1992).



Nitrate+nitrite, phosphate and silicate were analyzed using a Lachat QuikChem 8500 Flow Injection Analyst System and Omnion 3.0 software (Lachat, 2010).

Nitrate+nitrite is referred to as nitrate for the remainder of the analysis.

## **2.5 Imaging Flow Cytobot (IFCB) images**

Images were obtained using an IFCB, an automated imaging flow cytometer. The design and capabilities of the IFCB are provided in detail in Olson and Sosik, 2007 and Sosik and Olson, 2007. These images are from SCMW integrated whole water and net tow samples brought back to the laboratory and run through the IFCB on the benchtop, as well as samples taken at SCMW, where the IFCB samples from a pumped flow through system at approximately 20 minute intervals. IFCB data are provided primarily to illustrate the presence of various *Dinophysis* species; at the time of this analysis, there were insufficient data to attempt more sophisticated analysis (e.g. Campbell et al., 2010).

## **2.6 Statistics**

The relationship between *Dinophysis* and DSTs in mussels was evaluated two ways. First, a Wilcoxon rank sum test was used to determine if the median DST concentration in California mussels when *Dinophysis* was present in the net tow sample was greater than when *Dinophysis* was absent. This non-parametric alternative to the t-test was chosen because toxin distribution is not normally distributed. Second, *Dinophysis* cell concentrations were compared to DST concentrations using logistic regression. Logistic regression was chosen because it allowed toxin data to be binned around a relevant threshold and also allowed the data

to be modeled without transformation. DST in mussel tissue was binned as greater than or less than 100 ng/g. This level was chosen as a way to group toxin into a “low” category and a “higher” category that is approaching the FDA regulatory limit (160 ng/g). A second logistic regression was run for toxin binned by presence/absence in mussel tissue. The logit link function was used to produce the logistic regression output in terms of the predicted probability of mussel tissue containing toxin greater than 100 ng/g, or presence/absence of toxin, for a given *Dinophysis* concentration.

A stepwise multiple linear regression was used to determine which environmental variables are most associated with *Dinophysis* concentrations. This method was chosen as a way to discern if any statistically significant linear relationships exist between *Dinophysis* and easily collected environmental variables that are a part of the weekly SCMW time series (Schulien et al., 2017). Variables used in this model were log transformed ( $\log_{10}(x+1)$ ), excluding temperature and salinity, which did not require transformation. The multiple linear regression was performed in R (R Foundation for Statistical Computing). To determine if the mean temperature when *D. fortii* is present in the water column is statistically higher than when *D. acuminata complex* is present in the water column, a Welch’s two-sample t-test was used.

Data are presented as boxplots in multiple figures. Boxplots were produced in R using the ggplot2 package. The box is centered on the median, with lower and upper hinges corresponding to the first and third quartiles, and whiskers extending

from the hinge to the largest/smallest value 1.5 times the inter-quartile range. Points beyond that range are plotted individually as outliers from a normal distribution.

### **3. Results**

#### **3.1 Time series (2013-2016)**

Time series data for *Dinophysis* concentration at Santa Cruz Municipal Wharf (SCMW) is presented in Fig. 1A. This time series shows a consistent seasonal pattern and moderate interannual variability in peak *Dinophysis* concentration and in the persistence of high concentrations. During this four-year sampling period, the mean *Dinophysis* concentration was 754 cells/L, the median was 80 cells/L and the maximum concentration in a sample was 9,404 cells/L. The maximum concentration was recorded in July 2013. In that same year, high cell concentrations above 5,000 cells/L occurred from March through October, making 2013 the year with highest concentration in a single sample and the year with the longest time period with elevated *Dinophysis* concentrations. *Dinophysis* cell concentrations often exceeded 200 cells/L at SCMW, the concentration threshold above which toxins in shellfish can reach unsafe levels (Yasumoto et al., 1985), but do not approach 20,000 cells/L, the cell count action level adopted in Washington State (Trainer and Hardy, 2015).

Time series data for DST in California mussels is presented in Fig. 1B. Mussel tissue was found to contain okadaic acid (OA), dinophysistoxin 1 (DTX-1) and dinophysistoxin 2 (DTX-2). DST exceeded the FDA guidance level of 160 ng/g during 19 of the 192 weeks sampled during this four-year period, even though cell densities did not reach the cell count action level of 20,000 cells/L used in

Washington. The mean DST concentration was 51.61 ng/g, the median was 15.5 ng/g, and the maximum concentration was 562.9 ng/g (3.5 times the FDA action level of 160 ng/g). DTX-2 dominated the toxin profile found in mussel tissue during this study. OA was consistently found every year at low levels. DTX-1 was only detected during 9 weeks of this study.

### **3.2 Seasonal Trends**

Monthly boxplots of log transformed *Dinophysis* concentrations are presented in Fig. 2A. *Dinophysis* shows a clear seasonal cycle. Concentrations begin to increase in March and peak in the summer months of June and July, with a smaller peak occurring in October. *Dinophysis* concentrations have a median value of zero for December, January and February.

Monthly boxplots of DST concentration in California mussels are presented in Fig. 2B. The seasonal trends of DST in mussel tissue are more variable than *Dinophysis* seasonal trends. Median toxin concentrations begin to rise in March with highest median values in May and June. Toxin trends from June-November and January-February are characterized by extremely high variability. Interestingly, the variability in December is more constrained, with a non-zero median value. Toxin seasonality appears to track *Dinophysis* seasonality from January through May, but the relationship is unclear from June through December.

### **3.3 *Dinophysis* as a predictor of DST concentration in California mussels**

DST concentration in California mussel tissue was significantly greater during weeks when *Dinophysis* was present in a net tow sample (median = 19.99 ng/g, mean

= 62.80 ng/g) than in weeks *Dinophysis* was absent from the new tow sample (median = 0 ng/g, mean = 23.87) (Wilcoxon rank sum test:  $W=2356$ ,  $p=0.00004$ ) (Fig. 3A).

However, the range of toxin concentrations in each distribution is not distinct.

The results of a logistic regression using *Dinophysis* cell concentration as a predictor of toxin level greater than or less than 100 ng/g in mussel tissue is plotted in Fig. 3B. This level (100 ng/g) was chosen as a threshold to mark when toxin in mussel tissue begins to approach unsafe levels. The probability of mussel tissue containing toxin greater than 100 ng/g significantly increases with increasing *Dinophysis* concentration, but there is a high level of uncertainty represented by the wide confidence intervals seen in Fig. 3B (log-odds ratio = 0.00020,  $p = 0.0447$ ).

When no cells are present, there is a 16% probability mussel tissue will contain toxin at a concentration greater than 100 ng/g. When cell counts are high, there are less data to constrain this relationship and variability becomes too high to make an accurate prediction. A logistic regression was also conducted with presence/absence of toxin in mussels as the binary response variable. The results of this second logistic regression reveal higher *Dinophysis* concentrations are more likely to coincide with DST presence in California mussels, but results were not statistically significant (log-odds ratio 0.00017,  $p=0.13$ ), indicating cell concentration data alone are a weak predictor of toxin presence/absence in mussel tissue.

### **3.4 Environmental Context**

A stepwise multiple linear regression (MLR), run forward and backward, was used to begin to explore which environmental conditions are associated with

*Dinophysis* concentrations at SCMW. Relevant environmental variables collected as part of the SCMW time series and known to be associated with phytoplankton ecology are shown in Fig. 4. Ammonium, nitrate, phosphate, silicate, urea, water temperature, salinity and nitrate:phosphate ratio were entered into the model. Results of the stepwise MLR are presented in Table 1. The MLR with the lowest Akaike information criterion (AIC) score contained ammonium, silicate, urea, and salinity as predictor variables of *Dinophysis* concentration. *Dinophysis* abundance increased with decreasing ammonium, decreasing silicate, decreasing urea and increasing salinity. Trends for ammonium and silicate are not significant at  $p < 0.05$  ( $p = 0.07$ ,  $p = 0.08$ , respectively), trends for urea and salinity are significant at  $p < 0.05$  (Table 1). The overall adjusted R-squared for the model is 0.2446 ( $p = 1.279e-11$ ). When the stepwise regression is run, water temperature is the first variable to be removed from the model, followed by the N:P ratio, nitrate, and phosphate.

### **3.5 *Dinophysis* species at SCMW**

The Imaging Flow Cytobot (IFCB) captured images of multiple species of toxigenic *Dinophysis* at Santa Cruz Municipal Wharf between 2015-2017. Images representing the diversity of species seen by the IFCB are presented in Fig. 5 and include: *D. fortii*, *D. tripos*, *D. rotundata*, *D. caudata*, and species in the *D. acuminata* complex. In the two years of available microscopy data that identify *Dinophysis* to species level (2013-2014), these five species seen in the IFCB images, as well as *D. acuta*, *D. norvegica*, and *D. odiosa* were identified (Table 2). *D. acuminata* complex and *D. fortii* were the most abundant species in Lugol's

preserved samples, followed by *D. rotundata*. Fig. 6 shows temperature ranges versus concentration of *D. acuminata* complex, *D. fortii*, and total *Dinophysis* concentration (*Dinophysis* spp.). Visual interpretation of this data suggests *D. acuminata* complex has a broad temperature range, while *D. fortii* might favor a higher temperature range of 15-17°C. A Welch's two-sample t-test was used to determine if the mean temperature when *D. fortii* is present (14.6°C) is statistically higher than the mean when *D. acuminata* complex are present (13.9°C). Results show there is no significant difference ( $t=1.3088$ ,  $p=0.09681$ ) between the two temperature distributions.

#### **4. Discussion**

DST at Santa Cruz Municipal Wharf (SCMW) was found at persistent low levels in mussel tissue throughout this four-year study (2013-2016). In three out of the four years, DST exceeded the FDA action level of 160 ng/g during multiple events. During these three years, peak toxin level ranged from 2-3.5 times the FDA action level. During the 2011 DSP event on the West Coast of the U.S. and Canada that caused human illness, DST in blue mussels from Washington State measured 2-10 times the FDA action level, and toxin in mussels from British Columbia measured 5 times the FDA action level (Trainer et al., 2013, Taylor et al., 2013). At SCMW, DST in shellfish was overall lower, but within the range, of values seen in the 2011 events in Washington and British Columbia; persistent low levels of DST are notable.

*Dinophysis* cell concentrations at SCMW peak during the summer months (May-July), but were found in low background concentrations throughout the year.

Similar to the dynamics observed at SCMW, globally, *Dinophysis* is generally a small fraction of the phytoplankton community, but can contaminate shellfish at concentrations as low as 200 cells/L (Reguera et al., 2014, Yasumoto et al., 1985). *Dinophysis* at SCMW often exceeds 200 cells/L, but concurrent DST does not always reach levels of concern. Monitoring efforts in Washington have adopted a higher cell concentration action level of either 20,000 cells/L, or when relative abundance of *Dinophysis* increases from *present* to *common* (Trainer and Hardy, 2015). The maximum *Dinophysis* concentrations at SCMW 2013-2016 only approached half of that threshold (9,404 cells/L), and *Dinophysis* were only described as common five weeks during this four-year study. During those five weeks, DST concentrations were 21.7, 25.5, 41.39, 150.86, and 334.97 ng/g. A more sensitive cell concentration threshold than currently in place in Washington would be required to capture events of the magnitude observed in this study; however, choosing a threshold of *Dinophysis* concentration as an early warning of DST in shellfish is complicated because the relationship between *Dinophysis* concentration in the water column and toxin level in shellfish is not straightforward.

Our study presents the first published long-term record of paired *Dinophysis* concentration and DST level in California mussels at SCMW. Previous studies and publicly available data for Monterey Bay report *Dinophysis* concentrations similar to those observed in this study, as well as the occasional detection of concentrations higher than captured in our study. This study found a mean *Dinophysis* concentration of 754 cells/L, median of 80 cells/L and maximum concentration of 9,404 cells/L for



weekly data from 2013-2016. At the southern end of Monterey Bay, *Dinophysis* concentration measured weekly at Monterey Wharf from 2013-2016 has a mean of 263.2, median of 30, and maximum of 7,935 cells/L (Southern California Coastal Ocean Observing System, 2017). In 2004, *D. acuminata* was the dominant species at SCMW with a mean concentration of 1,000 cells/L and a maximum of 5,000 cells/L, while *D. fortii* had an average concentration of 1,400 cells/L (Sutherland, 2008). In 2005, *D. fortii* was the dominant species at SCMW with a mean cell concentration of 2,300 cells/L and a maximum of 21,000 cells/L, while *D. acuminata* had a mean concentration of 8,700 cells/L (Sutherland, 2008). In October 2011 at SCMW, *Dinophysis* concentration was 18,900 cells/L and in June 1999, *Dinophysis* concentration was recorded as 20,000 cell/L (Southern California Coastal Ocean Observing System, 2017, Weber, 2000). Together, this data indicates that maximum *Dinophysis* concentrations at SCMW and in Monterey Bay have the potential to approach Washington's action level of 20,000 cells/L, but it is rare.

The relationship between *Dinophysis* concentrations in the water column and DST levels in shellfish tissue is not straightforward. As expected, the distribution of toxin values in mussel tissue when *Dinophysis* is present in the net tow is significantly higher than when it is absent, but these distributions overlap. When *Dinophysis* concentration is zero, logistic regression shows there is still a 16% chance mussel toxin will be over 100 ng/g, a concentration approaching the FDA action level. Ultimately, when *Dinophysis* is present, that does not mean that toxin will be found in the mussels, and when *Dinophysis* is absent, that does not mean mussels will

be free of toxin. The relationship between *Dinophysis* cells and toxin levels in shellfish is known to be complex, and is dependent on multiple confounding factors. Toxin level in shellfish is affected by the percentage of the mussel's diet that is composed of *Dinophysis*, mussel depuration rates (which can vary by season), and the toxicity of cells present (Reguera et al., 2014). With the current state of knowledge, the only way to be sure of the toxin level in mussel tissue is to test it.

Although genus level *Dinophysis* concentrations alone are not a strong predictor of DST level in shellfish at SCMW, understanding *Dinophysis* ecology and environmental drivers of *Dinophysis* abundance is integral to fully understanding and eventually predicting DST concentration in shellfish. In this study, *Dinophysis* concentrations at SCMW were not found to correlate strongly with observed environmental parameters that could inform predictive and conceptual models. Stepwise multiple linear regression showed *Dinophysis* has a negative relationship with nutrients (silicate, urea, ammonium) and a positive relationship with salinity. *Dinophysis*' association with low nutrient levels is consistent with dinoflagellate preference for a stratified water column that develops following upwelling pulses (Smayda and Reynolds, 2001). When diatoms have drawn down surface nutrient concentrations, dinoflagellates such as *Dinophysis* can vertically migrate between deeper waters with ample nutrients and sunlit surface waters; however, this conceptual model for *Dinophysis* is complicated by its dependence on ciliate prey. In addition to preferring a low nutrient environment, a positive relationship with increased salinity suggests that *Dinophysis* concentrations are associated with

upwelling pulses. Overall, the regression model has a fairly low  $R^2$  of 0.2 ( $p < 0.05$ ), indicating that the variables entered into this model (nutrients, temperature, salinity) alone are not enough to predict *Dinophysis* concentrations at SCMW.

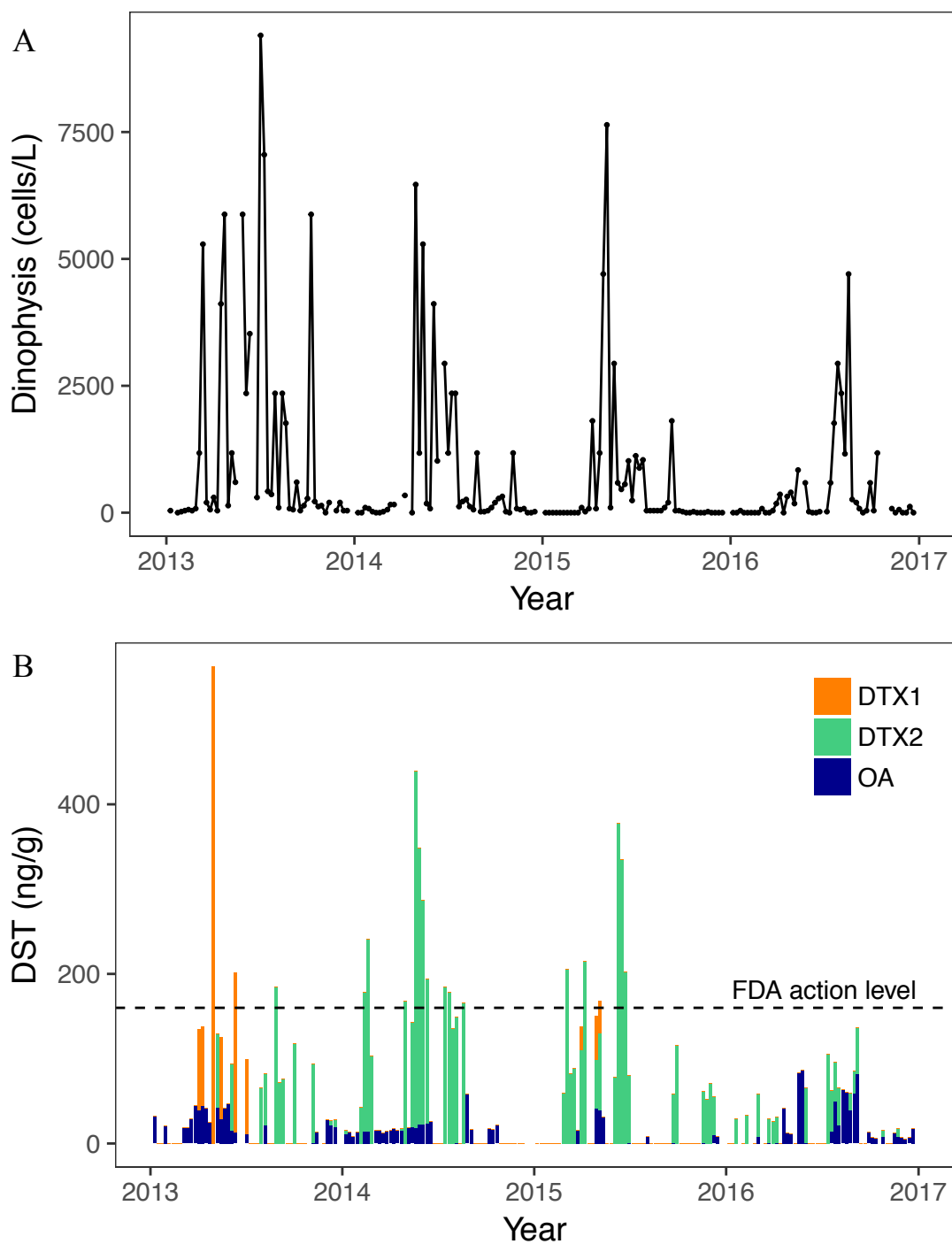
Conceptual models of *Dinophysis* abundance in other systems require knowledge of factors beyond temperature, salinity and nutrients. These species-specific models take into account physical transport via upwelling and coastal jets, stratification in the water column, and predator prey population dynamics between *Dinophysis* and *Mesodinium rubrum* (Farrell et al., 2012; Diaz et al., 2013; Velosuares et al., 2014; Harred and Campbell, 2014). In addition, this analysis at SCMW might not show strong environmental drivers of *Dinophysis* concentration because this genus tolerates a broad range of environmental conditions, as evidenced by its presence throughout the year. It is suggested that consideration of physical (transport, upwelling, stratification, temperature, salinity), chemical (nutrients) and biological (ciliate prey) variables as they relate to specific species of *Dinophysis* would be required to successfully predict *Dinophysis* abundance at SCMW.

In a two-year (2013-2014) weekly study of species composition of *Dinophysis* at SCMW, *D. acuminata* complex and *D. fortii* were found to be the species in highest abundance, and other toxin producers were found in low concentrations (Table 2). *D. acuminata* complex dominated the *Dinophysis* population in 2013, while both *D. acuminata* complex and *D. fortii* dominated in 2014. Visually, Fig. 6 suggests *D. fortii* may prefer a higher temperature range, but there is no significant difference between the temperature distributions for *D. acuminata* complex and *D.*

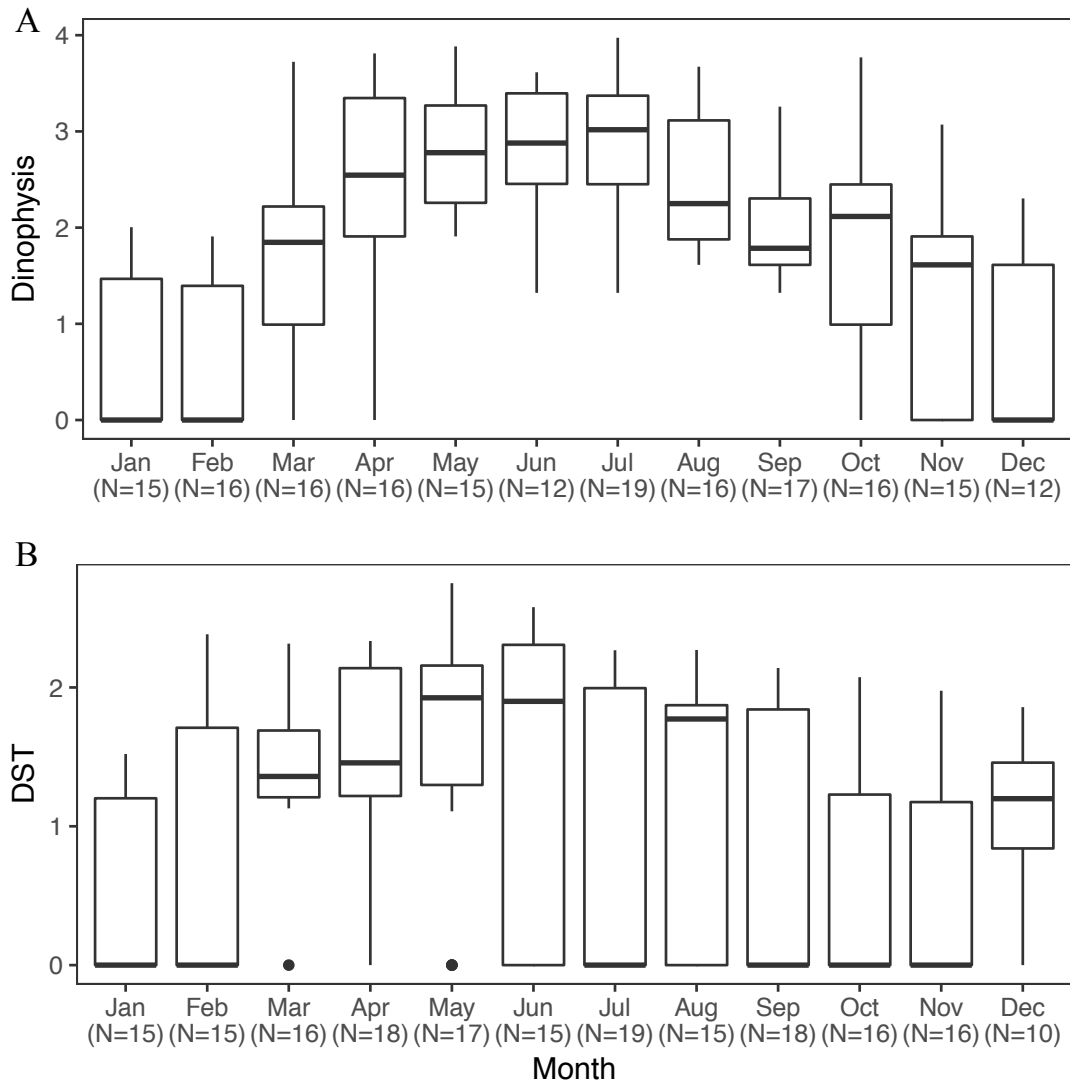
*fortii*. Sutherland (2008) found the average temperature when *D. acuminata* was present to be 15.9, while the average temperature when *D. fortii* was present to be 16.1; however, no test to determine if there was a significant difference was performed. A longer data set or laboratory experiments will be required to confidently describe *Dinophysis* species temperature ranges, and determine if the temperature range for *D. fortii* is significantly different from that of *D. acuminata* complex. It is important to continue to understand *Dinophysis* dynamics at the species level and, if possible, to determine toxin profiles for the most abundant species of *Dinophysis* at SCMW by bringing them into culture in the laboratory.

## **5. Summary**

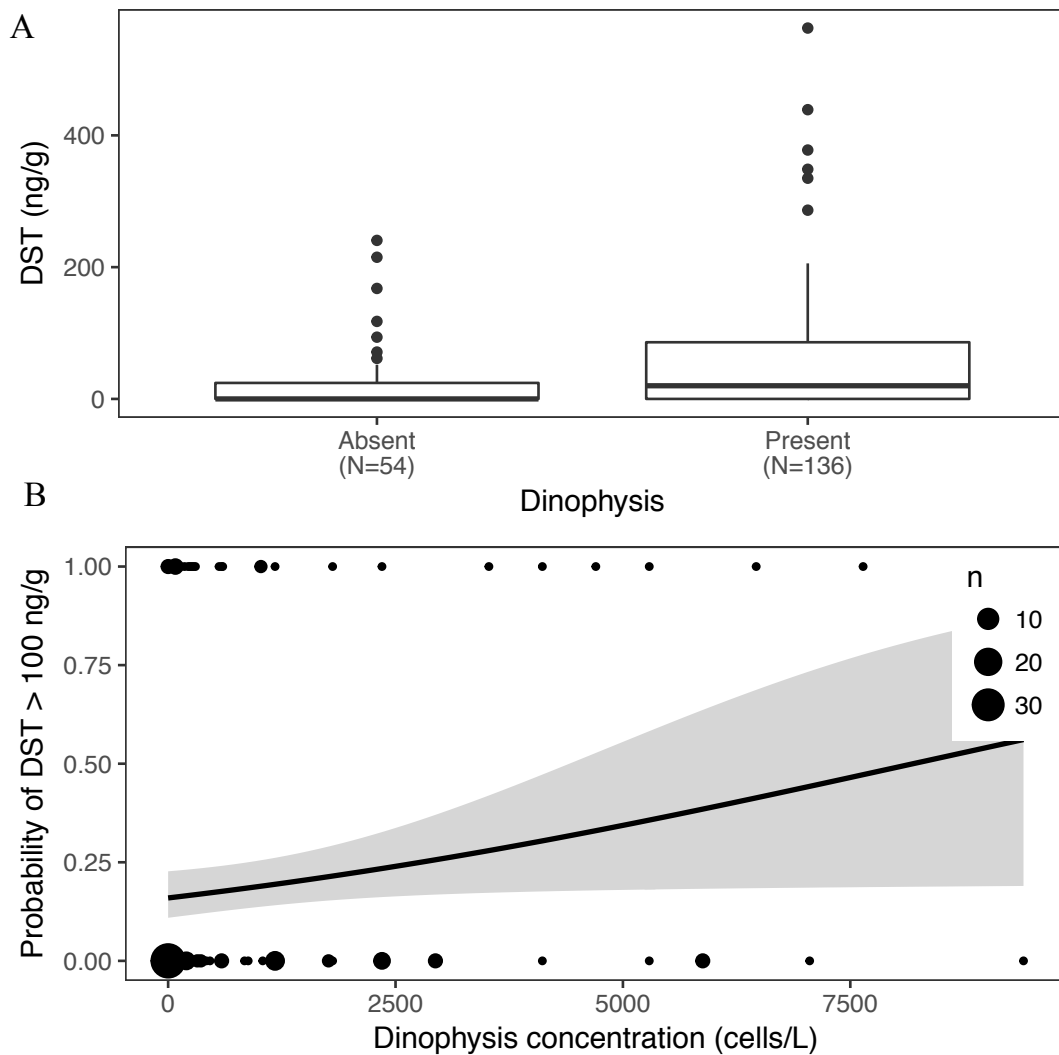
*Dinophysis* is present year-round at SCMW and DST is present in shellfish at persistent low levels throughout the year, with occasional peaks above the FDA action level. Multiple species of toxic *Dinophysis* are found at SCMW, mainly *D. acuminata* complex and *D. fortii*, with highest concentrations found in summer (May-July). *Dinophysis* concentrations at SCMW are not well explained by temperature, salinity, or nutrient data. Future predictive models of *Dinophysis* could benefit from work to understand physical transport of *Dinophysis*, population dynamics of *Mesodinium rubrum*, and potential differences in environmental preference between *Dinophysis* species.



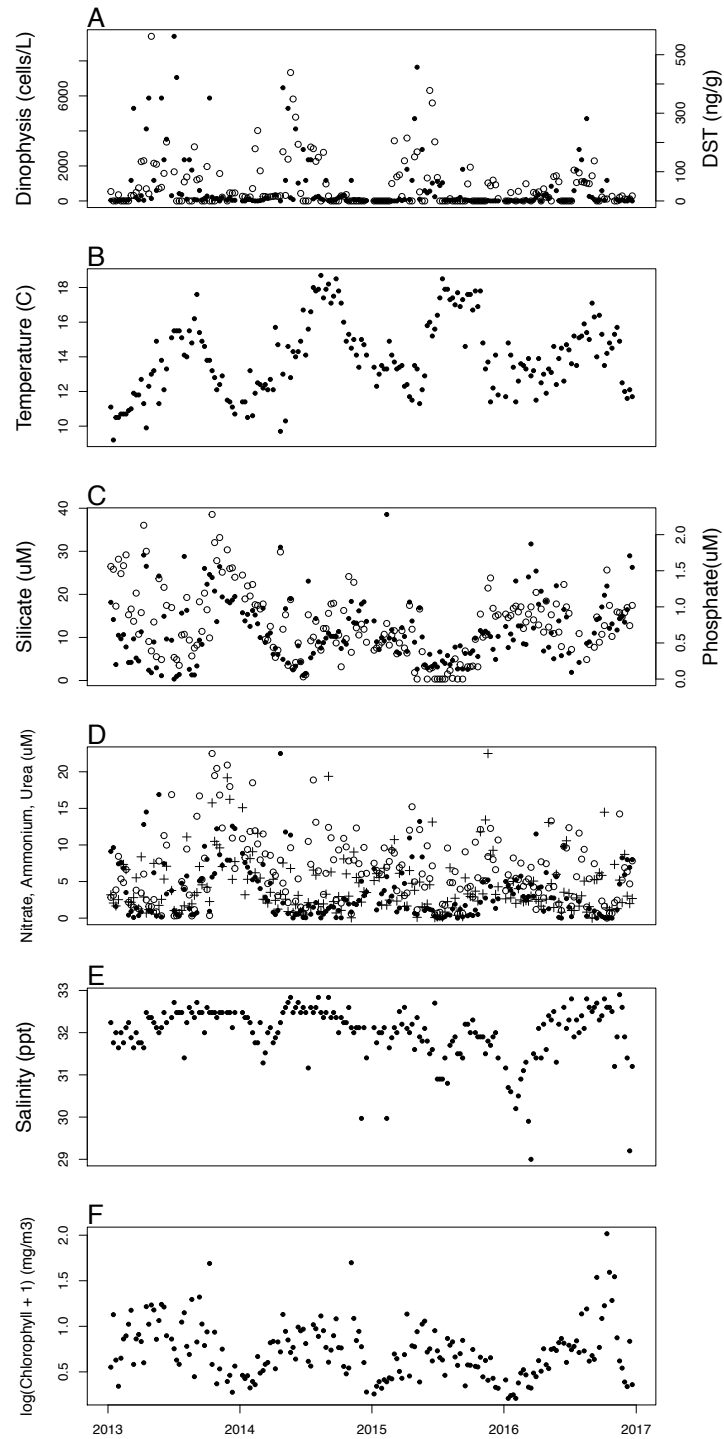
**Fig. 1.** Weekly time series data (2013-2016) at Santa Cruz Municipal Wharf. (A) *Dinophysis* concentrations (cells/L). (B) DST concentration (ng/g) of okadaic acid (OA), dinophysistoxin 1 (DTX-1), and dinophysistoxin 2 (DTX-2). Dashed line at  $y=160$  ng/g toxin, the FDA action level for DST.



**Fig. 2.** Monthly binned seasonal trends for (A)  $\log_{10}(x+1)$  transformed *Dinophysis* (cells/L) and (B)  $\log_{10}(x+1)$  transformed DST (ng/g). The box is centered on the median, with lower and upper hinges corresponding to the first and third quartiles.

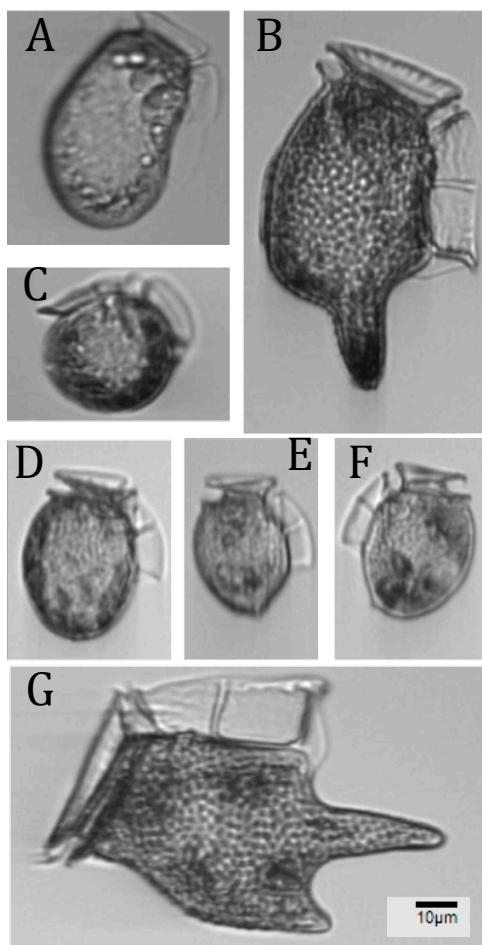


**Fig. 3.** Relationship between *Dinophysis* and DST in California mussel tissue. (A) Boxplot of *Dinophysis* presence and absence in net tow sample versus DST in mussel tissue. The box is centered on the median, with lower and upper hinges corresponding to the first and third quartiles. (B) Fit of predicted probabilities from logistic regression model of mussel tissue toxin greater than 100 ng/g in relation to *Dinophysis* concentration (cells/L). This model has a scatterplot overlay with data from SCMW that went into the logistic regression — samples with toxin greater than 100 ng/g are plotted along  $y=1.00$  and samples of toxin less than 100 ng/g are plotted along  $y=0.00$ , both in relation to *Dinophysis* concentration. Point size relates to the number of samples (N) for a given *Dinophysis* concentration.

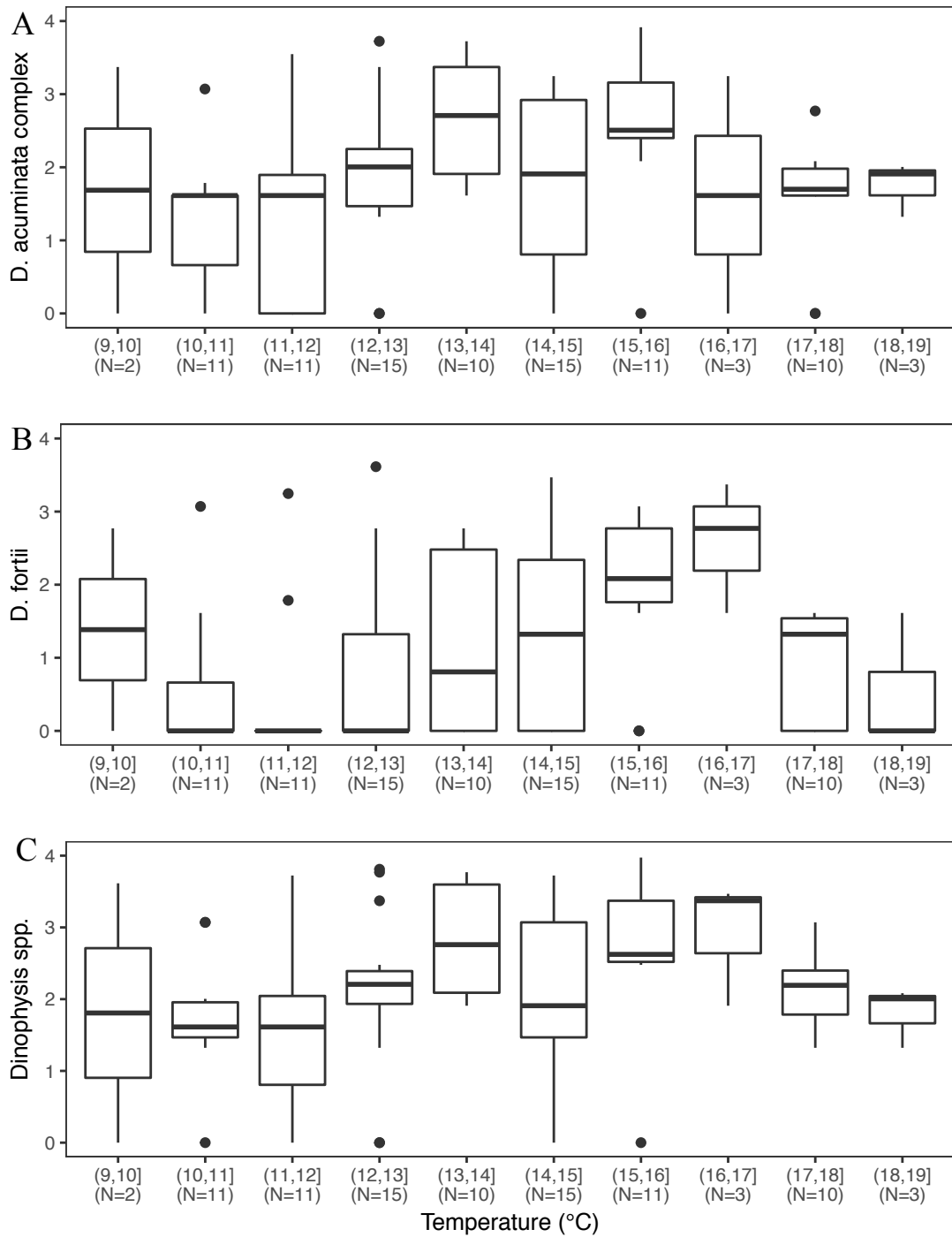


**Fig. 4.** Data from SCMW time series are provided. (A) *Dinophysis* (closed symbols) and DST (open symbols), (B) temperature, (C) silicate (closed symbols) and phosphate (open symbols), (D) nitrate (closed symbols), ammonium (open symbols), and urea (+ symbols), (E) salinity, and (F) chlorophyll *a*.





**Fig. 5.** Images of *Dinophysis* species diversity at SCMW detected by an Imaging Flow Cytobot (IFCB), 2015-2017. (A) *D. fortii*, (B) *D. caudata*, (C) *D. rotundata*, (D-F) *D. acuminata* complex, (G) *D. tripos*



**Fig. 6.**  $\log_{10}(x+1)$  transformed *Dinophysis* concentration data (2013-2014) with one-degree temperature bins for (A) *D. acuminata* complex, (B) *D. fortii*, and (C) *Dinophysis* spp. The box is centered on the median, with lower and upper hinges corresponding to the first and third quartiles.

**Table 1.** Results from a stepwise multiple linear regression (both direction) of environmental variables (ammonium, nitrate, phosphate, silicate, urea, water temperature, salinity and nitrate:phosphate ratio) used to model *Dinophysis* concentration. Model multiple  $R^2 = 0.2446$ ,  $p < 0.05$ .

Variable	Coefficient	p value
Ammonium ( $\mu\text{m}$ )	-0.7667	0.0724
Silicate ( $\mu\text{m}$ )	-0.5509	0.0802
Urea ( $\mu\text{m}$ )	-1.2769	0.0334
Salinity (ppt)	0.5924	0.0000

**Table 2.** *Dinophysis* species identified by microscopy in weekly samples at SCMW over a two year period, 2013-2014.

<i>Dinophysis</i> Species	Proportion of weeks present (N=98)	Mean concentration (cells/L)	Maximum concentration (cells/L)
<i>D. acuminata</i> complex	0.76	625.68	8228.57
<i>D. fortii</i>	0.46	229.71	4114.29
<i>D. rotundata</i>	0.30	75.16	1763.27
<i>D. acuta</i>	0.12	9.67	587.76
<i>D. caudata</i>	0.10	21.46	1175.51
<i>D. tripos</i>	0.05	7.02	587.76
<i>D. norvegica</i>	0.05	1.22	40.00
<i>D. odiosa</i>	0.01	6.00	587.76

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### Chapter 3: Conclusions and Future Directions

This thesis demonstrates that Diarrhetic Shellfish Poisoning Toxins (DSTs) persist in shellfish at Santa Cruz Municipal Wharf (SCMW) throughout most of the year, and occasionally reach concentrations above the FDA action level of 160 ng/g. At SCMW, *Dinophysis* reaches its highest abundance during the summer, but can persist as a member of the background phytoplankton community in low levels throughout the winter months. *Dinophysis* concentration alone is a weak predictor of DST level in shellfish at SCMW and more work is needed to understand and describe the mechanisms driving trends in DST concentrations. Considering *Dinophysis* concentrations along the California coastline are similar to concentrations found at SCMW (Southern California Coastal Ocean Observing System, 2017), this thesis suggests the possibility that DSTs in shellfish might be present in persistent low levels along the length of California's coastline.

Drivers of *Dinophysis* abundance are complex and difficult to describe using simple models that contain few variables, such as NPZ (nutrient, phytoplankton, zooplankton) models (Reguera et al., 2012). In this study, temperature, salinity and nutrient data describe only 20% of the variability in *Dinophysis* abundance at SCMW. In other systems, such as the Galician Rías and the southern coast of Ireland, conceptual models that successfully describe variation in *Dinophysis* abundance require knowledge of physical (transport, upwelling, stratification), chemical (nutrients) and biological (ciliate prey) drivers (Raine et al., 2010; Farrell et al., 2012; Diaz et al., 2013; Harred and Campbell, 2014; Velo-Suarez et al., 2014 Diaz et al., 2016).



In addition to a better understanding of physical, chemical, and biological drivers of *Dinophysis* populations at SCMW, developing species-specific models of *Dinophysis* abundance will be integral to understanding mechanisms that drive *Dinophysis* variability. Modeling *Dinophysis* at the species level is important because different species of *Dinophysis* can have different environmental niches, such as different optimal temperatures for maximum growth rates (Reguera et al., 2012; Gobler et al., 2017). As noted by Gobler et al. (2017), analyzing environmental drivers of *Dinophysis* abundance at the genus level likely masks nuances of species-specific relationships with environmental variables. It is suggested that future work involving *Dinophysis* abundance at SCMW should consider the benefits of collecting *Dinophysis* data identified to species level.

During the four-year study period of this thesis, *Dinophysis* was morphologically identified to species level during two years, 2013-2014 (N=98, weekly samples). The species *D. acuminata* complex, *D. fortii*, *D. rotundata*, *D. caudata*, *D. acuta*, *D. norvegica*, *D. tripos*, and *D. odiosa* were identified in samples from SCMW. The classification of *D. acuminata* complex was used to refer to the species *D. acuminata*, *D. ovum*, and *D. sacculus*, which are difficult to distinguish based on morphology alone. Molecular work to sequence the mitochondrial genes, Cytochrome b (*cob*) and Cytochrome c oxidase 1 (*cox1*), would be required to confidently identify the cells of *D. acuminata* complex to species level (Raho et al., 2008). *D. acuminata* complex and *D. fortii* comprised the majority of the *Dinophysis*

populations both years, followed by *D. rotundata*. This pattern is consistent with previous work at SCMW (Weber, 2000; Sutherland, 2008).

In addition to modeling *Dinophysis* abundance, the ability to predict when shellfish will accumulate high levels of DST is of interest, as it would allow potential monitoring efforts to be concentrated during times when risk of DST in shellfish is anticipated to be highest. To successfully anticipate times of high risk, it is essential to understand the toxicity and toxin profile of *Dinophysis* species present at SCMW. To determine toxicity, *Dinophysis* strains would need to be cultured in the laboratory, and the average toxin present per cell of *Dinophysis* could then be measured. The most straightforward way to determine toxin profile would also require *Dinophysis* to be cultured in the laboratory, where the type, or types, of DSTs (okadaic acid (OA), dinophysistoxin 1 (DTX-1), and dinophysistoxin 2 (DTX-2)) produced by each strain of *Dinophysis* could be unambiguously identified. Culturing *Dinophysis* in the laboratory remains complex, mainly because *Dinophysis* is an obligate heterotroph, and *Dinophysis* was not cultured in this study; however, the two years of *Dinophysis* data identified to species level and corresponding toxin data were used to investigate if the toxin profile of *Dinophysis* species present at SCMW could be determined from two years of weekly monitoring data.

Trends in abundant *Dinophysis* species and DST type between the years 2013 and 2014 were considered to determine if a likely toxin profile could be established for the dominant *Dinophysis* species each year. Results are presented in Fig. 1 and Table 1. Fig. 1 shows the relative percentage of species present (Fig. 1A) and toxin

type (Fig. 1B) in 2013 and 2014. In 2013, when *D. acuminata* complex dominated the *Dinophysis* population (76%), DST type is relatively evenly distributed between OA (24%), DTX-1 (43%), and DTX-2 (33%) in mussel tissue over the course of the year. In 2014, when *D. acuminata* complex and *D. fortii* both composed similar proportions (34% and 45%, respectively) of the *Dinophysis* population, toxin in mussels was dominated by DTX-2 (86%) with low levels of OA (14%) and no DTX-1. Visual analysis of these trends does not suggest a link between one species of *Dinophysis* with a particular type of DST; however, grouping the data by year masks any potential trends that may exist between *Dinophysis* species and DST type on a shorter time scale.

To further investigate a link between *Dinophysis* species and type of DST, linear regressions of log transformed ( $\log_{10}(x+1)$ ) *D. acuminata* complex, *D. fortii*, *D. rotundata*, *Dinophysis spp.* (sum of all species present), and *D. acuminata* complex + *D. fortii* versus each DST type, also log transformed ( $\log_{10}(x+1)$ ), were run (Table 1). *Dinophysis* concentration and DST concentration in California mussel tissue was measured weekly. Linear regressions were run for *Dinophysis* concentrations measured concurrently with toxin data (week of collection), as well as with *Dinophysis* concentrations meant to simulate the cumulative exposure to *Dinophysis* mussels would experience over the past two and three weeks before the toxin in their tissue was measured. This was done by taking the *Dinophysis* concentration averaged over the given week and the prior week (two week average), as well as taking the *Dinophysis* concentration averaged over the given week and the prior two weeks

(three week average). In addition to simulating cumulative exposure, this method was used to produce comparable results to similar analyses conducted by Sutherland (2008). Overall, the linear regressions conducted with the data available show no strong linkage between any species of *Dinophysis* and particular DST type. No significant relationship was found between *Dinophysis* species and OA. A significant relationship with  $R^2 = 0.0829$  was found between DTX-1 and *D. acuminata* complex using a three week average of *D. acuminata* complex concentration. Although significant, the low  $R^2$  value suggests *D. acuminata* complex concentrations do not have a strong relationship to DTX-1. A significant relationship with  $R^2 = 0.1009$  was found between DTX-2 and *D. fortii* using a three week average of *D. fortii* concentrations. Again, although this relationship is significant, the low  $R^2$  value suggests *D. fortii* concentrations do not have a strong relationship with DTX-2.

These results are not consistent with results reported in Sutherland (2008). Using correlations, Sutherland found a strong correlation between OA and *D. fortii* ( $r=0.65$ ,  $p<<0.01$ ) using a three-week average of *Dinophysis* concentrations. Considering only the summer months of 2014, Sutherland found a strong relationship between *D. fortii* + *D. rotundata* and DTX-1 ( $r=0.77$ ,  $p<<0.01$ ) using a two week average of *Dinophysis* concentrations, although no significant correlation was found when considering the whole data set (2004-2005). For total DST (OA + DTX-1) Sutherland found a significant correlation between *D. acuminata* and DST ( $r=0.36$ ,  $p<0.01$ ). It is suggested that culture experiments will be required to unambiguously determine the toxin profile of *Dinophysis* species present at SCMW.

**Figures and Tables:**

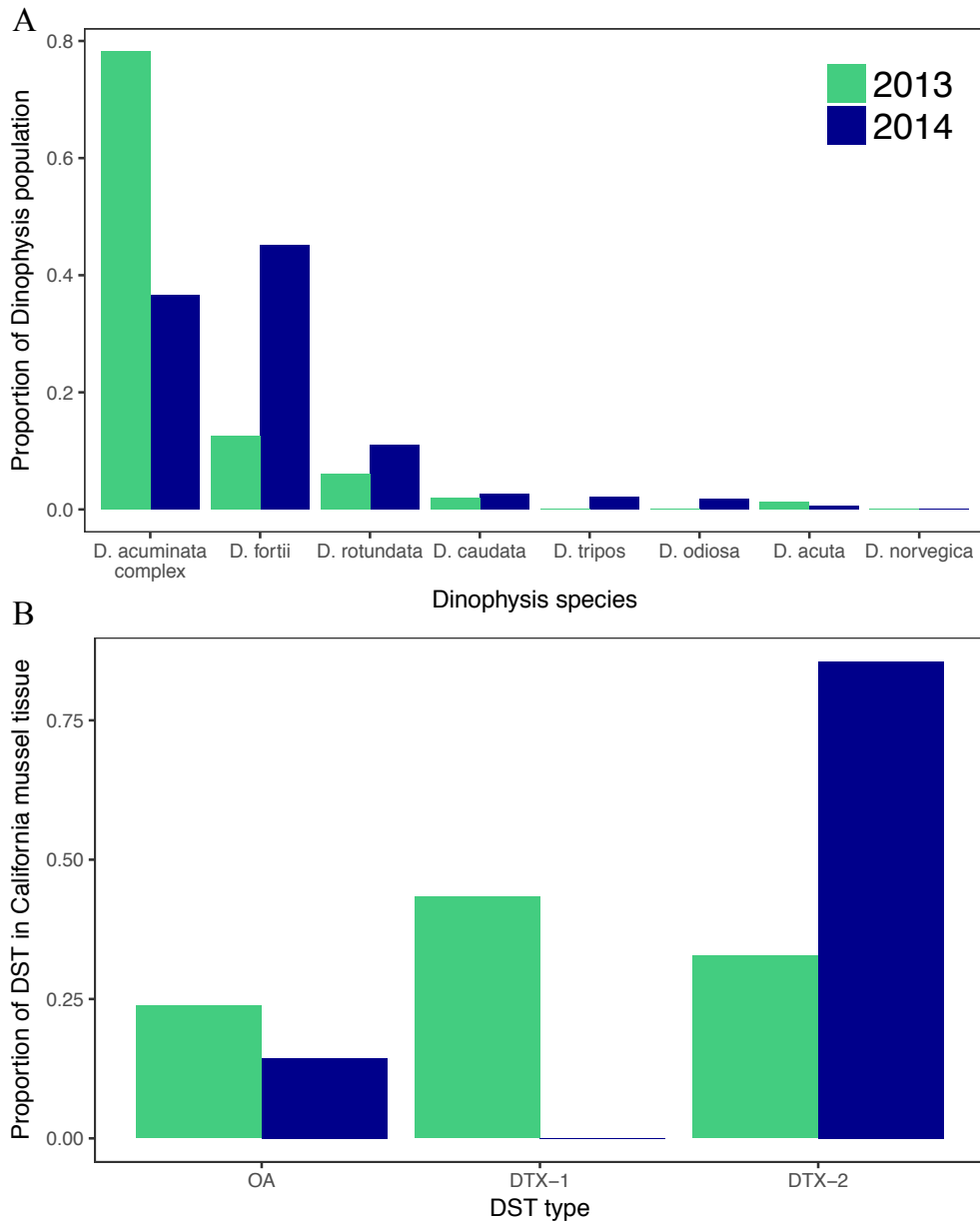


Fig. 1. (A) Proportion of each *Dinophysis* species relative to total *Dinophysis* abundance and (B) proportion of DST type in California mussel tissue relative to total DST concentration in 2013 and 2014 at Santa Cruz Municipal Wharf.

**Table 1.** Linear regression results of log transformed ( $\log_{10}(x+1)$ ) *Dinophysis* concentrations and log transformed ( $\log_{10}(x+1)$ ) DST concentrations in California mussel tissue. Statistically significant results ( $p < 0.05$ ) are in bold. Species listed as *D. acuminata* refers to *D. acuminata* complex, which can include *D. acuminata*, *D. ovum*, and *D. sacculus*.

DST type	<i>Dinophysis</i> species	Week of collection			Two week average			Three week average		
		R <sup>2</sup>	p	slope	R <sup>2</sup>	p	slope	R <sup>2</sup>	p	slope
OA	<i>D. acuminata</i>	0.0234	0.1530	0.0015	0.0000	0.9550	0.0001	0.0004	0.8700	0.0003
	<i>D. fortii</i>	0.0005	0.8290	-0.0005	0.0007	0.8170	0.0007	0.0161	0.2810	0.0039
	<i>D. rotundata</i>	0.0002	0.8890	0.0008	0.0001	0.9380	0.0007	0.0002	0.9130	-0.0013
	<i>Dinophysis spp.</i>	0.0150	0.2530	0.0009	0.0011	0.7700	0.0003	0.0054	0.5360	0.0008
	<i>D. acuminata + D. fortii</i>	0.0135	0.2790	0.0009	0.0002	0.8970	0.0001	0.0041	0.5880	0.0007
DTX-1	<i>D. acuminata</i>	0.0041	0.5510	0.0029	<b>0.0651</b>	<b>0.0216</b>	<b>0.0146</b>	<b>0.0829</b>	<b>0.0128</b>	<b>0.0205</b>
	<i>D. fortii</i>	0.0018	0.6894	-0.0043	0.0004	0.8629	-0.0026	0.0001	0.9450	0.0013
	<i>D. rotundata</i>	0.0080	0.4060	0.0222	0.0007	0.8130	0.0104	0.0000	0.9800	-0.0014
	<i>Dinophysis spp.</i>	0.0017	0.7010	0.0014	0.0346	0.0964	0.0082	<b>0.0606</b>	<b>0.0344</b>	<b>0.0133</b>
	<i>D. acuminata + D. fortii</i>	0.0013	0.7340	0.0013	0.0420	0.0664	0.0097	<b>0.0578</b>	<b>0.0392</b>	<b>0.0141</b>
DTX-2	<i>D. acuminata</i>	0.0033	0.5910	-0.0033	0.0032	0.6180	-0.0040	0.0047	0.5600	-0.0060
	<i>D. fortii</i>	0.0152	0.2502	0.0156	<b>0.0504</b>	<b>0.0439</b>	<b>0.0374</b>	<b>0.1009</b>	<b>0.0058</b>	<b>0.0604</b>
	<i>D. rotundata</i>	0.0020	0.6790	0.0139	0.0165	0.2538	0.0619	0.0169	0.2690	0.0788
	<i>Dinophysis spp.</i>	0.0003	0.8708	0.0007	0.0039	0.5799	0.0034	0.0080	0.4480	0.0059
	<i>D. acuminata + D. fortii</i>	0.0000	0.9830	-0.0001	0.0011	0.7692	0.0020	0.0040	0.5917	0.0046
DST	<i>D. acuminata</i>	0.0002	0.8840	0.0011	0.0150	0.2770	0.0107	0.0187	0.2454	0.0148
	<i>D. fortii</i>	0.0048	0.5190	0.0108	0.0302	0.1210	0.0354	<b>0.0775</b>	<b>0.0163</b>	<b>0.0656</b>
	<i>D. rotundata</i>	0.0093	0.3700	0.0370	0.0152	0.2730	0.0730	0.0103	0.3900	0.0761
	<i>Dinophysis spp.</i>	0.0034	0.5890	0.0030	0.0314	0.1135	0.0118	<b>0.0596</b>	<b>0.0361</b>	<b>0.0200</b>
	<i>D. acuminata + D. fortii</i>	0.0014	0.7240	0.0021	0.0267	0.1450	0.0118	0.0475	0.0622	0.0194

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