Phytoplankton toxins in critical prey species in the Monterey Bay National Marine Sanctuary

A report submitted to the Monterey Bay National Marine Sanctuary
Sanctuary Integrated Monitoring Network (SIMoN)
and
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Overall project summary

Here we propose a study that is a companion project to whichever team is funded to investigate the dynamics of critical prey species in the Monterey Bay National Marine Sanctuary. We believe our project will significantly value-add new data, at modest cost, to the overall research effort, as it leverages the use of samples to be obtained by the main project team and contributes dietary and other information back to that team. In our proposal we describe a phenomenon that is increasingly evident in the sanctuary, namely the frequent presence and often negative effects of the presence of a natural phytoplankton toxin, domoic acid (DA). The toxin contaminates a wide range of organisms including the pelagic prey species listed in this RFP, which then become toxin vectors to their predators, including marine birds and mammal, sometimes resulting in dramatic and much publicized mortality events. Thus our efforts focus on this relatively recently discovered phenomenon of a naturally occurring neurotoxin in the sanctuary and its conveyance throughout the system by prey species that are commonly present throughout much of the California Current upwelling system.

Our proposed project is focused on the analyses of the toxin, DA, in prey species and the analysis of prey gut samples, which typically contain the frustules (shells) of the toxin-producers, species of the diatom genus *Pseudo-nitzschia*. Since the toxin is conveyed by vectors to their predators via gut contents (i.e. delivery of DA to predators is accomplished almost entirely via the gut contents, not body tissues, of the prey), the presence of the toxic cellular material in GI tracts of predators is evidence of their DA contamination (Even apex predators will typically contain diatom debris in their gut or feces when poisoned by DA, if gut contents have not been voided). Thus this study uses both chemical measures of toxin presence (HPLC) and gut content analyses of prey species to show the role of toxin transmission by prey. We will examine two different types of prey samples collected in the Monterey Bay region, the first being "historical" samples, preserved from collections dating to the 1950s, before the toxin and its producers were known, to recently collected samples from 2000 onward, where there are environmental data on cell counts of the toxic species and DA in the water. Historical samples are from the CalCOFI project, while contemporary samples are collected in the sanctuary by colleagues. From these historical samples we will examine the gut contents of thaliaceans (salps, doliolids), which will demonstrate whether the toxic species were present, and krill, which will show whether a key prey taxon, was contaminated by the toxic species. Contemporary samples will be freshly collected specimens obtained by the main team as well as specimens (squid, planktivorous fish) obtained from the commercial fleet in Moss Landing. Contemporary samples will be analyzed in the same manner as the historical samples, but will also be analyzed for DA, to more extensively establish the link between DA and the presence of toxic diatoms in their GI tract. From the main project team and the fishing community, we will analyze prey species for their DA and gut contents, to the extent to which the seagoing efforts provide us with prey species listed in this RFP. Additionally, we will have background water samples of toxic cell abundance and the DA from studies that we are conducting on other funded projects, data that will indicate the pelagic availability of the toxic phytoplankton to the prey.

The Monterey Bay region is the ideal place to study the phenomenon of natural toxins in food webs. First, it is one of the best known coastal ecosystems, due to its long history of marine research activities, which have shown the trophic connections among common pelagic organisms. Secondly, it is a system with the most extensive time series records of DA and toxic phytoplankton presence anywhere. Third, it has a well publicized history of toxic events, which began with marine bird mortalities and, most recently, has been tracked with strandings of sea lions that dramatically exhibit DA-poisoning symptoms. Lastly, there is a possibility of researchers that combine the mix of phytoplankton specialists to experts on prey (and possibly apex predator researchers – if present on the main team) to work together for the first time. We envision the outcome of this project to add significantly to our understanding of the extent to which key pelagic vectors provision apex predators with naturally occurring toxins and to provide an initial view of how long such a phenomenon may have been occurring in the inner waters of the California Current, where toxic phytoplankton have become such a common phenomenon.

Summary of findings for report interval

ORIGINAL GOAL 1

Review available data from the literature on domoic acid (DA) and toxic *Pseudo-nitzschia* in animals from Monterey Bay, both from "regular publications" and from the "gray literature."

Because DA was only recognized on the U.S. west coast in 1991, our efforts related to this review necessarily address the literature from 1991 onward. Three major DA poisoning incidents are recorded in the literature - events in 1991, 1998 and 2000. During the events many brown pelicans, Brandt's cormorants and California sea lions were found dead on the beaches of Monterey Bay, and other, still living specimens, showed neurological symptoms expected of DA intoxication.

Our literature review indicates that the neurotoxin DA in Monterey Bay occurs in digestive tracts or feces of both prey animals commonly consumed by apex predators, in the gut and feces of suspected vectors, and in predators that consumed vectors during the toxic events (Table 1).

We found a wide range of DA concentrations reported in various animals in Monterey Bay, levels we expected to differ depending on the organisms' position in a food chain, and especially whether the organism could directly access the toxic cells or whether it consumed prey containing the toxic cells. DA concentrations in several invertebrate and vertebrate species exceeded the danger levels (i.e. DA concentrations above which human foods are not saleable: 20 µg/g tissue). High DA values ranged from 44 – 2300 ug/g of tissue, with maximum levels in pelagic organisms occurring in partially herbivorous, filter-feeding anchovies (Table 2), a widely consumed prey of many apex predators. Furthermore, benthic filter feeders that can directly collect the toxic cells (e.g., fat innkeeper worm *Urechis caupo*) were expected to have high levels (Tables 1 and 2). As the table indicates, the highest values indeed are found in animals low on the food chain, and the value for the mucus-web feeder *Urechis,* sampled from Elkhorn Slough, is probably the highest level every recorded in an animal, particularly remarkable since it is not simply the level in the gut, but is the "whole body" concentration. (The anchovy value reported by Altwein et al., 1995 was for the gut, not the totally body concentration). Our best explanation of the results is that organisms that directly collect large numbers of cells, like pelagic filter feeding krill and planktivorous fishes (e.g., anchovies and sardines), and the benthic mucus feeder *Urechis,* have the highest levels of DA in their bodies.

TABLE 1. Sources of data on the presence of Domoic Acid (DA) in various organisms obtained in Monterey Bay from 1991 – 2005. Organisms are grouped by trophic level and by year in which they were found to be DA contaminated.

EVENT YEAR	ORGANISM	TROHIC LEVEL	PART ANALYZED	MAX DA µg/ gr tissue	SOURCE
1991	*Doliolum* (doliolid))	filter feeder	whole body	0.6	Haywood 1995
	Oikopleura (larvacean)	"	whole body	9.1	"
	Sagitta spp.(arrow worm)	"	whole body	3.4	"
	Copepods	"	whole body	2.7	"
	Mytilus caliornianus (mussel)	"	whole body	47	Langlois et al. 1993
	Engraulis mordax (anchovy)	"	caudal	4.7	Haywood 1995
	Engraulis mordax (anchovy)	"	viscera	191	Fritz et al. 1992
	Engraulis mordax (anchovy)	"	muscle	38	"
	Engraulis mordax (anchovy)	"	viscera	190	Work et al. 1993
	Engraulis mordax (anchovy)	"	gut	2300	Altwein et al. 1995
	Euphausia (krill)	omnivore	whole body	5.1	Haywood 1995
	Pelagic Polychaetes	predator	whole body	1.3	"
	Pleurobrachia (Sea gooseberry)	"	whole body	1.7	"
	Pelecanus occidentalis (Brown pelicans)	"	stomach	48	Fritz et al. 1992
	Phalacrocorax penicillatus (Brandt`s cormorants)	"	stomach	48	"
	Pelecanus occidentalis (Brown pelicans)	"	stomach	50	Work et al. 1993
	Phalacrocorax penicillatus (Brandt`s cormorants)	"	stomach	50	"
1993	*Dolioletta gegenbauri* (doliolid)	filter feeder	whole body	8.3	Haywood 1995

	Sagitta spp. (arrow worm)	"	whole body	3.3	"
	Calanus pacifica (copepod)	"	whole body	4.2	"
	Pleurobrachia pileus (sea gooseberry)	"	whole body	1.6	"
	Mytilus californianus (mussel)	"	whole body	5.7	"
	Balanus (barnacles)	"	whole body	1	"
	Hydromedusae (jellyfish)	"	whole body	9.5	"
	Brachyuran larvae (crab larvae)	"	whole body	11.6	"
	Euphausia pacifica (krill)	omnivore	whole body	4.9	"
1998	*Engraulis mordax* (anchovy)	filter feeder	gut	71.3	Scholin et al. 2000
	Engraulis mordax (anchovy)	"	viscera	223	Lefebvre et al. 1999
	Engraulis mordax (anchovy)	"	whole body	55	"
	Engraulis mordax (anchovy)	"	body tissue	39	"
	Zalophus californianus (California sea lion)	predator	feces	127	"
	Zalophus californianus (California sea lion)	"	serum	0.2 μg/ml	Scholin et al. 2000
	Zalophus californianus (California sea lion)	"	feces	182 μg/ml	"
	Zalophus californianus (California sea lion)	"	urine	3.72 μg/ml	"
1999	*Emerita analoga* (sand crab)	filter feeder	whole body	13.4	Ferdin et al. 2002
2000	*Engraulis mordax* (anchovy)	filter feeder	viscera	1815	Lefebvre et al. 2002a
	Engraulis mordax (anchovy)	"	viscera	444	Lefebvre et al. 2002b
	Sardinops sagax (Pacific sardine)	"	viscera	588	Lefebvre et al. 2002a
	Atherinopsis californiensis (jacksmelt)	"	viscera	275	Lefebvre et al. 2002b
	Euphausia pacifica (krill)	omnivore	whole body	44	Bargu et al. 2002
	Loligo opalescense (squid)	predator	stomach	0.37	Bargu et al., unpublished
	Scomber japonicus (chub mackerel)	"	viscera	1.4	Lefebvre et al. 2002b
	Citharichthys sordidus (Pacific sanddab)	"	viscera	7.2	"
	Thunnas alalunga (albacore)	"	viscera	4.6	"
	Balaenoptera musculus (Blue whale)	"	feces	207	"
	Megaptera novaeangliae (Humpback whale)	"	feces	10	"
	Loligo opalescens (market squid)	"	stomach	0.37	Bargu et al. *In Press*
2001	*Urechis caupo* (echiuran worm)	filter feeder	whole body	751	Goldberg 2003

	Emerita analoga (sand crab)	"	whole body	278	"
	Dendraster excentricus (sand dollar)	filter/deposit feeder	whole body	13	"
	Callianassa californiensis (ghost shrimp)	deposit feeder	whole body	144	"
	Olivella biplicata (olive snail)	"	whole body	2	"
	Nassarius fossatus (snail)	scavenger	whole body	673	"
	Pagurus samuelis (hermit crab)	"	whole body	55	"
	Citharichthys sordidus (flat fish)	predator	whole body	514	"
	Genyonemus lineatus (White Croaker)	"	viscera	2.8	Fire and Silver 2005
	Leptocottus armatus (Staghorn sculpin)	"	viscera	2.1	"
2004	*Urechis caupo* (echiuran worm)	filter feeder	whole body	1363	Vigilant and Silver 2007
	Cancer productus (red rock crab)	predator	hepatopancreas	372	Cheung, unpublished
2005	*Cancer antennarius* (brown rock crab)	predator	hepatopancreas	264	Cheung, unpublished

TABLE 2: List of organisms, collected from Monterey Bay, with DA concentrations that exceeded the upper limit considered safe for human consumption (i.e. 20 µg DA g-1 for shellfish).

ORGANISM	MAX DA (µg/g tissue)	YEAR	SOURCE
Mytilus californianus (mussel)	47	1991	Langlois et al. 1993
Urechis caupo (echiuran worm)	1363	2004	Vigilant et al. YEAR???
Emerita analoga (sand crab)	278	2001	Goldberg 2003
Engraulis mordax (anchovy)	2300	1991	Altwein et al. 1995
Sardinops sagax (Pacific sardine)	588	2000	Lefebvre et al. 2002a
Atherinopsis californiensis (jacksmelt)	275	2000	Lefebvre et al. 2002b
Euphausia pacifica (krill)	44	2000	Bargu et al. 2002
Callianassa californiensis (ghost shrimp)	144	2001	Goldberg 2003
Nassarius fossatus (snail)	673	2001	Goldberg 2003
Pagurus samuelis (hermit crab)	55	2001	Goldberg 2003
Citharichthys sordidus	514	2001	Goldberg 2003

(flat fish)			
Cancer productus (red rock crab)	372	2004	Cheung,unpublished
Cancer antennarius (brown rock crab)	264	2005	Cheung, unpublished
Pelecanus occidentalis (brown pelican)	50	1991	Work et al. 1993
Phalacrocorax penicillatus (Brandt`s cormorant)	50	1991	Work et al. 1993
Zalophus californianus (California sea lion)	127	1998	Lefebvre et al. 1999
Balaenoptera musculus (blue whale)	207	2000	Lefebvre et al. 2002b

ORIGINAL GOAL 2

Conduct a search, using archived CalCOFI samples, to determine the extent to which toxic *Pseudo-nitzschia* were present in Monterey Bay back into the 1950s.

We had proposed that gut contents of preserved specimens of pelagic filter feeders could provide records of the past occurrence of toxic diatoms. Unfortunately, there is no set of historical samples of phytoplankton for the Monterey Bay region, although there is for zooplankton and fishes. As anticipated, we were able to obtain permission from the curator of the CalCOFI collection at Scripps Institution of Oceanography to remove zooplankton specimens from this remarkable plankton collection and to use them to record phytoplankton in the region that occurred decades ago. We had proposed that herbivorous zooplankton are ideal collectors of *Pseudo-nitzschia* because they provide an integrated sample of the phytoplankton in the surface water column. Salps and doliolids are particularly efficient collectors, since they are non-selective filter feeders of cells >2 microns (Madin 1974), and we have recently found that krill also sample *Pseudo-nitzschia* during toxic events (Bargu et al. 2002). As we proposed in our original request to SIMoN, we removed the gut contents of thaliacean (salp and doliolid) and krill from samples collected on the inner CalCOFI stations of line 67 in Monterey Bay (Figure 1). These samples dated back into the 1950s (Table 3). Gut sample analyses have revealed the presence of toxic *Pseudo-nitzschia* species at least back to 1954, including *P. australis* and *P. multiseries,* the dominant species recorded in toxic events since DA was first recognized in the Monterey Bay area in 1991.

There were additional species of *Pseudo-nitzschia* present in the sample that we have only begun to identify. One of them is *P. turgidula*, known as an oceanic species from the subarctic North Pacific (Marchetti et al. 2006), and hence its presence may indicate offshore water from the main axis of the California Current. Since source waters of the California Current include subarctic waters, the presence of an oceanic

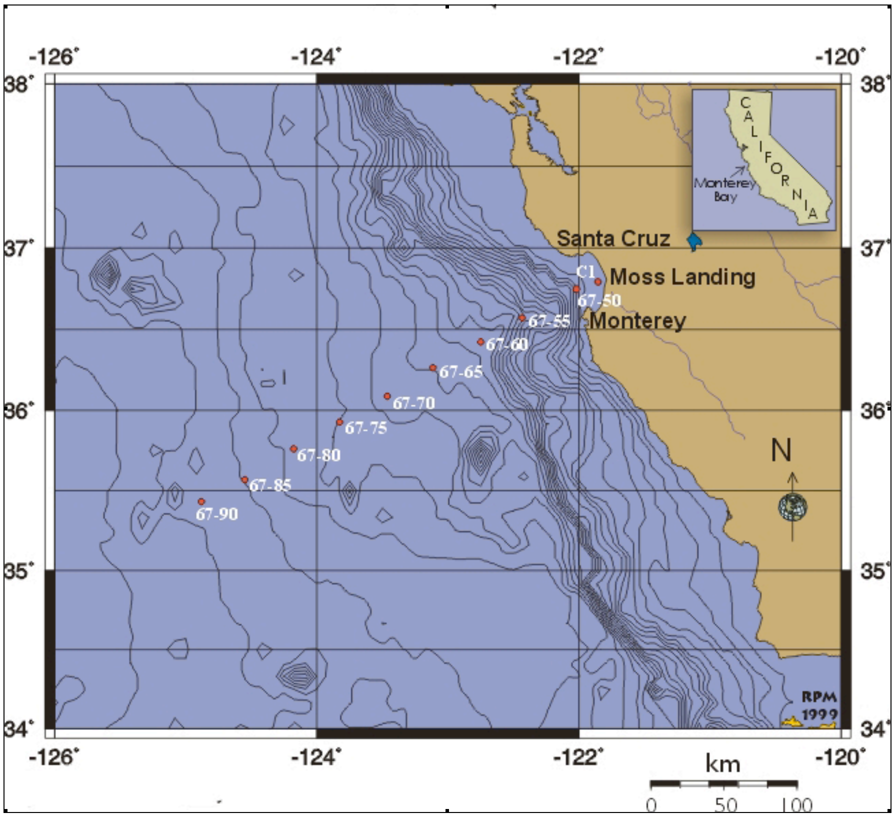


FIGURE 1: Map of CalCOFI line 67 from Monterey Bay, California to XX km offshore. Inner stations on line 67 provided zooplankton samples used in this study (Source: MBARI)

subarctic *Pseudo-nitzschia* species is not unexpected, especially during cold phases of the Pacific Decadal Oscillation (PDO) when subarctic flow is enhanced. As far as we know, no one to date has reported *P. turdigula* in the California Current, but the species is known to produce DA in the subarctic South Pacific (Rhodes et al. 1996) and recently we have obtained preliminary evidence that *P. turdigula* in the northern subarctic Pacific may also produce DA (Bargu and Silver, unpub.). Our analyses of the species composition of other *Pseudo-nitzschia* conspecifics have not yet been completed, due to problems encountered with UCSC's electron microscope.

Our analyses of the historic CalCOFI samples showed that krill specimens from 1954 and 1958 contained mainly centric diatoms and unknown *Pseudo-nitzschia* species. After the large El-Niño event in 1958-1959, *Pseudo-nitzschia* occurred abundantly in salps and doliolids in 1960 and 1961 (Figure 2a). Our results demonstrate that *P.* cf. *turgidula* was the most abundant *Pseudo-nitzschia* species present in those samples (Figure 2b, c). *Pseudo-nitzschia* usually co-occurred with *Chaetoceros*. The abundance of *Pseudo-nitzschia* varied depending on the herbivore examined and the date of the sample.

Our results show that toxic species of *Pseudo-nitzschia* have been present in waters of Monterey Bay, and hence central California, since at least 1958, though only recognized as a toxin-producing genus of diatoms since 1991. Our results here suggest that members of this genus may have been responsible for the bird kill in 1961, an event that inspired

Hitchcock's film, "The Birds," though the causative species of *Pseudo-nitzschia* may be one not currently present in the area (possibly *P. turdigula*). We also show that historical analyses of toxic phytoplankton can utilize preserved specimens of herbivorous zooplankton, thus allowing analyses of links between previous oceanographic conditions and the phytoplankton that occurred during those conditions.

Table 3: Historic CalCOFI samples analyzed for *Pseudo-nitzschia* presence in the guts of planktivores.

#	Expedition	CalCOFI Staline	Lat	Lon	Sample Date	Organism	***Pseudo-nitzschia* presence***
1	C-4910	67-53	36.58	-122.23	10/11/1949	krill+copepod	-
2	C-5408	67-50	36.76	-122.06	8/27/1954	krill	-
3	C-5807	67-50	36.81	-122.08	7/12/1958	krill	+
4	C-5907	67-55	36.68	-122.517	7/20/1959	Doliolid	+
5	C-6010	67-50	36.81	-122.05	9/25/1960	Doliolid	+++
6	C-6010	67-55	36.68	-122.517	9/25/1960	Doliolid	+++
7	C-6107	67-50	36.81	-122.08	7/4/1961	Salp	+++
8	C-6107	67-55	36.68	-122.517	7/4/1961	Salp	+++
9	C-6210	67-50	36.81	-122.08	10/15/1962	Salp	Not analyzed yet
10	C-6507	67-50	36.81	-122.08	7/14/1965	Doliolid	++
11	C-6806	67-50	36.81	-122.08	6/21/1968	Doliolid	++
12	C-7507	67-50	36.8	-122.08	7/16/1975	Doliolid	+
13	C-7905	67-50	36.8	-122.83	5/20/1979	Doliolid	+++
14	C-8005	66.7-50	36.78	-122.05	5/20/1980	Salp	++
15	C-8107	66.7-50	36.78	-122.05	6/30/1981	Doliolid	+
16	C-9904	66.7-50	36.78	-122.06	4/18/1999	Salp	+

- = absent, + = present, ++ = common, +++ = dominant

ORIGINAL GOALS 3 AND 4

Goal 3: Examine specimens of krill and thaliaceans collected by zooplankton net tows during a SIMoN-funded project (headed by B. Marinovic) to determine the extent to which toxic *Pseudo-nitzschia* occur in the gut of thaliaceans and krill when those toxic cells are present in the water.

Benefit: Such data would provide us with insight into the interpretation of the historical CalCOFI samples, as we (Silver and Bargu) had similar and independent measures of the abundance and toxicity of *Pseudo-nitzschia* from the water during the grant period. We expected to compare diatoms from water samples with those in gut contents of krill and thaliaceans.

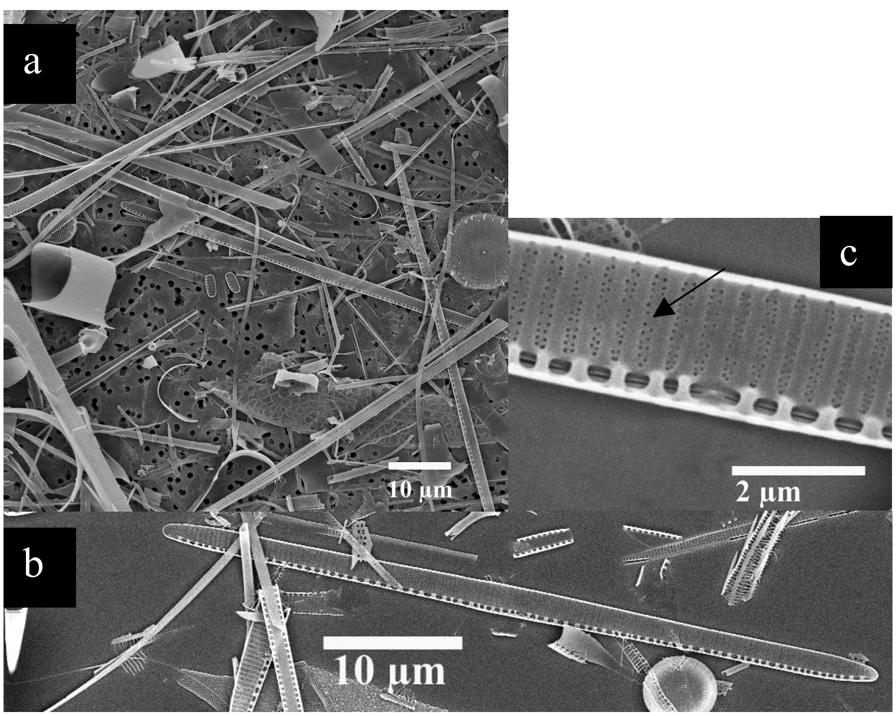
Goal 4: Measure DA in the gut of krill and thaliaceans of samples collected in (3) to determine their DA level.

<u>Benefit</u>: Such data would provide us with information about whether DA was retained in the gut of preserved samples and, if it were, whether we therefore might be able to use historical samples from the gut of these same herbivores to estimate the DA in the water.

Problems Encountered with Goals 3 and 4:

During the study interval - basically between the summer of 2004 and spring of 2007 - the concentration of toxic *Pseudo-nitzschia* in Monterey Bay dropped by approximately an order of magnitude or more at most sites around the bay. In many cases levels of cells and DA were below detection limits. Correspondingly, the levels of DA also dropped by an equivalent amount. Given the reduction in the toxic species and the likelihood we would obtain data of limited usefulness, we focused instead on the CalCOFI –related analyses (Goal 2), in which the SIMoN staff also had expressed particular interest. We also believed this analysis would provide the most novel and useful results.

Figure 2: a. Scanning electron micrograph of thaliaceans (salp) gut content containing *Pseudo-nitzschia* frustules; b. Scanning electron micrograph of *Pseudo-nitzschia* cf. *turgidula* frustule; c. higher magnification view of the frustule from a. An arrow indicates the presence of two rows of large poroids within the striae. A central nodulus is present.



<u>Presentations:</u>

Bargu, S. and M. W. Silver. 2005. Phytoplankton toxins in critical prey species in the Monterey Bay National Marine Sanctuary. Poster. CalCOFI Conference, San Diego, CA-USA

Bargu, S. and M. W. Silver. 2005. An overview of the domoic acid contamination of Monterey Bay food webs. Poster. Third Symposium on Harmful Marine Algae in the U.S. Asilomar, CA – USA.

FUTURE WORK

We hope to extend our SIMoN studies to better understand the historical presence of toxic *Pseudo-nitzschia* in the California Current system, now that we know such studies are feasible using the unique CalCOFI archival zooplankton collection (from 1949 to 2000). We are particularly interested in shifts in species composition in relation to annual and multidecadal climate ocean changes. The following are our questions:

- **Have toxic *Pseudo-nitzschia* been present since the beginning of the last century?**
- **Have there been major species shifts over time?**
- **How far offshore do the toxic *Pseudo-nitzschia* extend, or are they primarily only in the coastal currents?**

We also are exploring the possibility of determining whether DA was produced by some of the other *Pseudo-nitzschia* species that may have been producing toxin, e.g. *P. turdigula*. Using modern ELISA techniques we hope to determine whether recently collected, formalin-fixed specimens of herbivorous zooplankton that contain toxic *Pseudo-nitzschia* retain measurable levels of DA. If so, we may be able to use CalCOFI samples (preserved in formalin) to retrospectively determine toxin presence. Using PCR, we also plan to compare the genetic strains of historical specimens of *Pseudo-nitzschia* with those presently in the region after determining, again, whether formalin-fixed material is suitable for such analyses, as has been reported by some authors. Such future analyses, however, would need major funding, but hopefully the results from this SIMoN project will provide sufficient evidence of the value of such a historical analysis of toxin in a coastal environment that is experiencing changing levels of toxic phytoplankton events.

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