Identification of phytoplankton species found in digestive tracts of northern anchovies

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Abstract

In 1991, the neurotoxin, domoic acid, contaminated northern anchovies (*Engraulis mordax* Girard) in Monterey Bay, California. Over 100 brown pelicans and Brandt's cormorants died after consuming the contaminated anchovies. The diatom species, *Pseudonitzschia australis* Frenguelli, has been implicated as the domoic acid-producer in this incident. Other *Pseudonitzschia* species have also been shown to produce domoic acid. The stomach contents of anchovies caught in Monterey Bay during the following summer were analyzed to identify the *Pseudonitzschia* species present and the abundance of each species. The *Pseudonitzschia* species present in water samples taken in Monterey Bay at the same time were also obtained. These findings illustrate the risk of possible contamination inherent in the composition of the anchovies' diet and its relation to the plankton composition of the surrounding water. This report also provides a basis for further study on the accumulation of domoic acid by anchovies.

Introduction

In late 1991, the west coast of the United States experienced an outbreak of the neurotoxin, domoic acid. In September of that year, domoic acid apparently caused the death of over 100 brown pelicans (*Pelicanus occidentalis* Ridgway) and Brandt's cormorants (*Phalocrocorax penicillatus* [Brandt]) in Monterey Bay, California, after the birds consumed northern anchovies (*Engraulis mordax* Girard) contaminated with the toxin (Wood & Shapiro 1992; Work et al. 1993). In November of 1991, the health departments in California, Oregon and Washington closed the commercial and recreational shellfish and crab fisheries due to domoic acid contamination (Anonymous 1991; Wood & Shapiro 1992; Todd 1993). Tests found domoic acid in razor clams (*Siliqua patula* Dixon) and dungeness crabs (*Cancer magister* Dana) from both Washington and Oregon waters (Wood & Shapiro 1992).

The only other known mortalities caused by domoic acid occurred in southeastern Canada's Prince Edward Island during the winter of 1987 and 1988. During this event, about 150 people experienced gastrointestinal and neurological dysfunction, and four people died from consuming cultivated blue mussels (*Mytilus edulis* Linnaeus) contaminated with domoic acid (Bird & Wright 1989; Gilgan et al. 1990). In some cases, the victims suffered permanent neurological damage (Addison & Stewart 1989).

Domoic acid is a small, water soluble, heat stable amino acid (Maranda et al. 1990; Villac et al. 1993a) produced by some species of algae (Todd 1990). Domoic acid has many structural similarities to the excitatory neurotransmitters, glutamic and aspartic acids. This molecule also serves as a glutamate agonist with toxic properties to the mammalian and avian nervous system (Bird & Wright 1989; Laycock et al. 1989; Barinaga 1990; Anonymous 1991; Wood & Shapiro 1992). As shown by the two events described above, domoic acid has the capability to accumulate in the tissues of mussels, crabs and anchovies. Roelke et al. (1993) has shown that domoic acid can also concentrate in oysters (*Crassostrea virginica* Gmelin) in lab experiments.

In 1991, when domoic acid appeared in toxic concentrations in Monterey Bay, California, the die-off of pelicans and cormorants served as an early warning that prevented widespread human intoxication (Villac et al. 1993b). The human injuries consisted of only a few cases of mild gastrointestinal problems and one case of memory loss (Villac et al. 1993b). However, the incident in Monterey Bay differed from the Canadian event in many obvious respects. First, domoic acid poisoned birds rather than a mammalian species like humans. Also, in the Monterey Bay event, a different species of diatom, *Pseudonitzschia australis* Frenguelli (=*Nitzschia pseudoseriata* Hasle), rather than *Pseudonitzschia pungens* f. *multiseries* (Hasle) Hasle (=*Nitzschia pungens* f. *multiseries* Hasle) (Hasle 1993), dominated the phytoplankton community during the toxin outbreak (Buck et al. 1992; Fritz et al. 1992; Todd 1993). Both of these diatom species have been confirmed as domoic acid producers (Subba Rao et al. 1988; Bates et al. 1989; Buck et al. 1992; Garrison et al. 1992; Villac 1993b). Finally, mussels were not the vector through which domoic acid passed to the birds. Researchers found northern anchovies containing toxic levels of domoic acid present in the stomachs of the affected waterfowl (Garrison et al. 1992; Todd 1993).

The occurrence of the northern anchovy as a vector for domoic acid is noteworthy not only because the vector was a fish, but also because the toxin was found in the tissues of the fish as well as the gut (Fritz et al. 1992). The northern anchovy is present in the diets of nearly every predatory fish, bird, and mammal species in the California Current (Kucas 1986; Fritz et al. 1992; Work et al. 1993). Any of these animals may be susceptible to the same affliction that killed the cormorants and pelicans in Monterey Bay. However, while there have been instances of both birds and mammals experiencing domoic acid poisoning, no negative effects due to domoic acid intoxification have been observed in fish. Anchovies are also planktophagous throughout all life stages thus increasing the risk of future poisoning events (Kucas 1986).

P. australis and *P. pungens* f. *pungens* are not the only species of diatom genus *Pseudonitzschia* suspected or demonstrated to produce domoic acid. For example, the species *Pseudonitzschia pseudodelicatissima* (Hasle) Hasle (=*Nitzschia delicatula* Skvortzow) has also been implicated as a producer of domoic acid in Eastern Canada (Martin et al. 1990; Haya et al. 1991; Hasle 1993). *Pseudonitzschia delicatissima* (Cleve) Heiden (=*Nitzschia actydrophila* Hasle) (Hasle 1993) has also produced low levels of domoic acid in culture (Smith et al. 1990). Other *Pseudonitzschia* species may produce domoic acid under certain conditions as well. Just the mere occurrence of these organisms does not guarantee that domoic acid will be produced. Bates et al. (1991) found that *P. pungens* f. *multiseries* could produce domoic acid only when cell division ceased and when nitrate and light were present. However, such requirements for production of domoic acid in the other three species have not been established (Fryxell et al. 1990; Martin et al. 1990; Smith et al. 1990; Villac et al. 1993a).

The objective of this research is to identify the species of the genus *Pseudonitzschia* found in the guts of northern anchovies, using fish caught in Monterey Bay in the summer following the toxin event in that area. This report will also compare these findings with the *Pseudonitzschia* composition of net tow samples taken from Monterey Bay at the same time. This information will provide a basis for further research on northern anchovies and their ability to accumulate domoic acid.

Materials and Methods

The anchovies sampled were obtained by the California Department of Health Services[1] on 6 August 1992 in southern Monterey Bay. Stomach content and flesh samples were taken from twenty-four fish. Flesh samples were taken from the dorsal and caudal regions of each fish and frozen for possible future high-performance liquid chromotography analysis for domoic acid. The stomach content samples were taken by removing the stomachs of each fish and gently forcing the contents out into a 20 ml scintillation vial. The wet weight of each sample was then recorded, and a subsample of ten percent of the original wet weight was taken. The remaining sample was then frozen.

In order to analyze the stomach content, each subsample was diluted with 20 ml of sterile filtered seawater. Half of the diluted subsample was then preserved with gluteraldehyde for quantitative analysis. The other half of the diluted subsample was cleaned of organic matter (Hasle and Fryxell 1970) and prepared for qualitative analysis.

The stomach contents of the anchovies were analyzed quantitatively and qualitatitively to determine the abundance of each *Pseudonitzschia* species. For the quantitative analysis, counts of the general shapes (centrics, pennates, etc.) of the cells and cell fragments present in the preserved subsamples were obtained, using an improved Neubauer hemocytometer with a volume of 0.1ml. These counts were repeated three times for each fish. The qualitative analysis involved using the cleaned portion of the subsamples. Using light microscopy, cleaned samples mounted in Hyrax were viewed with an oil immersion 100X objective. Identification of 50 *Pseudonitzschia* and *Nitzschia* cells in each cleaned sample was replicated twice for each fish. The

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identifications were then confirmed using SEM analysis of samples mounted on aluminum stubs and coated with gold-palladium.

The results of the quantitative analysis gave the number of pennate cells per gram wet weight of stomach content for each fish. These data were combined with the results of the qualitative analysis to give the number of cells of each species of *Pseudonitzschia* per gram wet weight for each fish. Information was also obtained as to the occurrence of each species in each of the anchovies.

To estimate the availability of the various phytoplankton species, counts of two samples from net tows in Monterey Bay were also completed. The samples were taken on 6 August 1992 at station Q5, at the southern end of the mouth of Monterey Bay, and also at H3, a station positioned centrally in the bay (Buck et al. 1992). The net tow samples were cleaned of organic matter and mounted in Hyrax. Counts of these samples were obtained using oil immersion 100X objective to identify the species of 50 *Pseudonitzschia* and *Nitzschia* cells. The results of these counts were then used to compare the diatom composition of the anchovy stomach contents to the diatom composition of the water at the time the anchovies were taken.

Results

Pennate diatoms composed 76.7% of the cells in the uncleaned samples of the stomach content. Numerous species of *Rhizoselenia*, another diatom genus, composed a large fraction of the remaining cells in the samples. Some dinoflagellates and centric diatoms were also present. Using the percentage of pennate diatoms per 0.1 ml of sample and the wet weight of the original sample, the number of pennate cells per gram wet weight of each stomach content was obtained. Based on these calculations, the average number of pennate cells found in each fish was 65,465 cells per gram wet weight of sample.

Using light microscopy, 50 pennate cells were identified on two slides for each sample. Of these cells, nine different *Pseudonitzschia* species and a few *Nitzschia* species were identified. Other pennates in the samples were counted simply as unknowns in order to estimate the percentage of pennates occupied by *Pseudonitzschia* species. Six common species of *Pseudonitzschia* were identified. However, *Pseudonitzschia subpacifica* (Hasle) Hasle (=*Nitzschia subpacifica* Hasle) (Hasle 1993), made up the largest portion of the pennates found in each sample. The average abundance of this species was 54,390 cells per gram wet weight of sample. Figure 1 illustrates the abundance of *P. subpacifica* found in each fish.

Five other *Pseudonitzschia* species were also commonly found, although at much lower numbers than *P. subpacifica*. These species were *Pseudonitzschia australis*, *Pseudonitzschia delicatissima*, *Pseudonitzschia pungens*, *Pseudonitzschia pseudodelicatissima*, and *Pseudonitzschia subfraudulenta*. Figure 2 shows the average abundance of all of the *Pseudonitzschia* species over the entire sample of twenty-four fish.

The SEM analysis of the samples confirmed the presence of all of the more common species identified by light microscopy, including *Pseudonitzschia subpacifica*, but with the possible exception of *Pseudonitzschia subfraudulenta* (identification pending). Both forms of *Pseudonitzschia pungens* (*Pseudonitzschia pungens* f. *pungens* and *Pseudonitzschia pungens* f. *multiseries*) were seen. However, the potentially toxic *P. pungens* f. *multiseries* appeared to be the more common of the two forms.

The net tow samples from Monterey Bay revealed a slightly different picture for the composition of the *Pseudonitzschia* species found in the water itself. *Pseudonitzschia subpacifica* dominated the samples, composing 86% of the pennates of the station Q5 sample and 93% of the pennates of the station

Abundance of *Pseudonitzschia subpacifica*

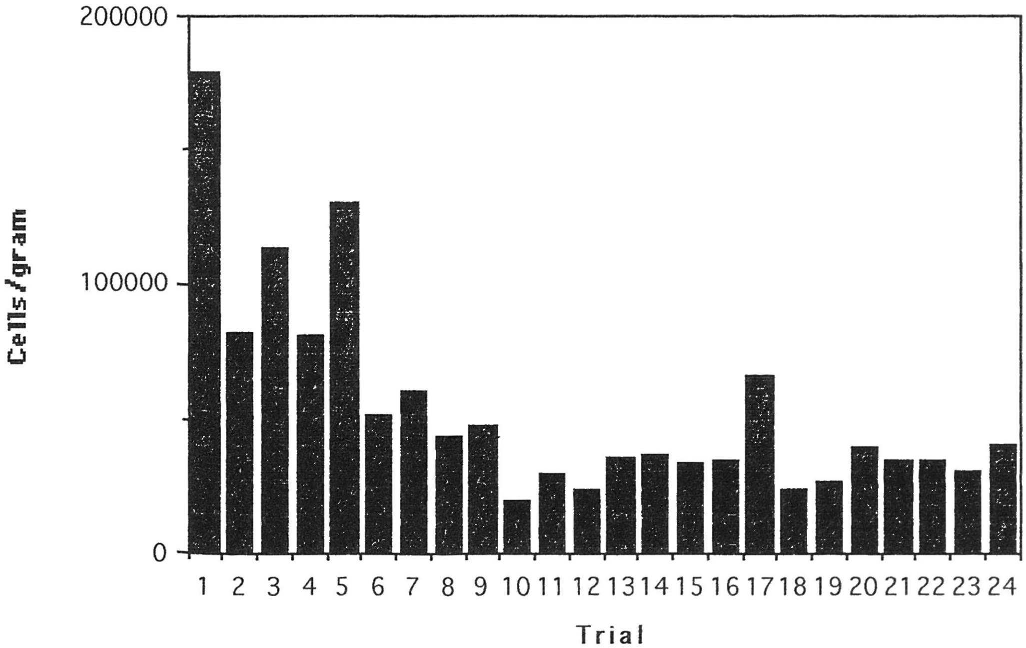

Figure 1- Abundance of *Pseudonitzschia subpacifica* for each fish

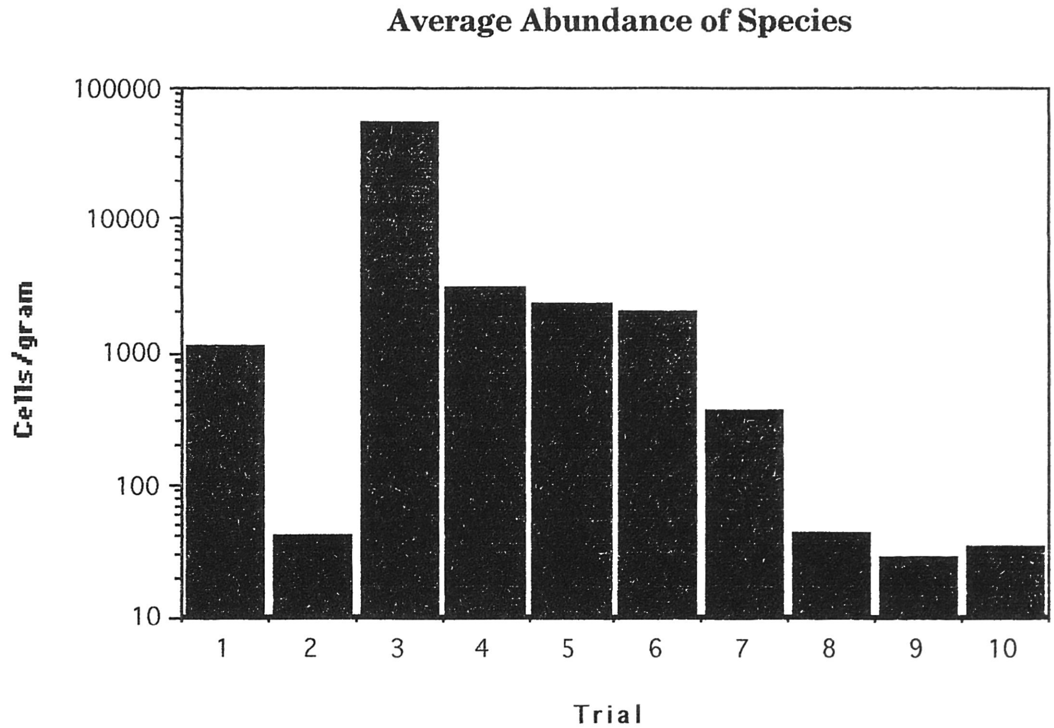

Figure 2- Average abundance of each *Pseudonitzschia* species: species 1- *P. australis*, species 2- *P. fraudulenta*, species 3- *P. subpacifica*, species 4- *P. delicatissima*, species 5- *P. pungens*, species 6- *P. pseudodelicatissima*, species 7- *P. subfraudulenta*, species 8- *P. seriata* f. *obtusa*, species 9- *P. pungiformes*, species 10- *P. seriata*

H3 sample. Other species such as *Pseudonitzschia pseudodelicatissima*, *Pseudonitzschia pungens*, *Pseudonitzschia australis*, *Pseudonitzschia subfraudulenta*, and *Pseudonitzschia delicatissima* made up the remaining fraction of the sample. One *Nitzschia* species, *Nitzschia bicapitata*, was also found in the Q5 sample only. The relative abundance of each species found in the net tow samples is shown in table 1.

Discussion

Northern anchovies are known as filter feeders, and are planktophagous in all life stages (Kucas 1986). Therefore, the possibility of northern anchovies consuming toxic phytoplankton during bloom conditions will vary with the plankton composition of the water. During the domoic acid event in Monterey Bay in late 1991, the stomach contents of anchovies and intoxicated pelicans were found to be dominated by *Pseudonitzschia australis* (Work et al. 1992). *Pseudonitzschia australis*, the diatom species implicated in this toxin event, was also the dominant plankton in the bay waters at that time (Buck et al. 1992; Work et al. 1993). This indicates that the plankton composition of the stomach content of anchovies correlates closely with the plankton composition of the surrounding waters

The results of this study reinforce this idea. *Pseudonitzschia subpacifica* made up most of the plankton found in the anchovy stomach contents and also dominated the net tow samples taken from Monterey Bay on the same day that the anchovies were caught. The other species found in the net tow samples also occurred in similar proportions in the stomach contents of the anchovies. However, the overall number of *Pseudonitzschia* and *Nitzschia* species found in the stomach contents of the anchovies was greater than that found in the net tow samples. This could be due to the fact that the net tow samples came from only two stations in Monterey Bay and did not represent a

Station	% P. subpacifica	% P. pseudodelicatissima	% P. pungens	% P. australis	% P. subfraudulenta	% P. delicatissima
Q5	86	3	4	2	4	1
H3	93	0	3	0	2	2

Table 1- Relative abundance (percentage) of *Pseudonitzschia* species found in net tows from Monterey Bay, CA

composite sample of the northern anchovy habitat. Other reasons might include that some *Pseudonitzschia* species might be more easily filtered by the anchovies than the net.

Northern anchovies occupy and probably feed over large ranges along the west coast of North America. The northern anchovy is abundant throughout the California Current and makes up at least part of the diet of almost every predatory bird, fish, and mammal in the California Current (Kucas 1986). They occur in three distinct subpopulations along the west coast. The anchovies included in this study came from the central subpopulation which ranges from southern California to the northern Baja California peninsula in Mexico (Kucas 1986).

Any higher vertebrate that consumes these fish may be susceptible to domoic acid poisoning. The results of this study found all four of the known diatom, domoic acid-producers in the stomach contents of these anchovies as well as in the water samples. Previous research in this area also indicates that other *Nitzschia* and *Pseudonitzschia* species may be capable of producing domoic acid (Villac et al. 1993; Work et al. 1993). The importance of the northern anchovy to human fisheries and to the diets of so many marine predators increases the danger involved with the contamination of these animals by domoic acid. Also important when considering the risk to humans and other predators of the anchovy is the fact that for the first time, during the Monterey Bay toxin event in 1991, domoic acid was found not only in the digestive tracts of the vector organism (the anchovy), but also the tissues.

This high risk of domoic acid intoxication underscores the need to add domoic acid analysis to routine marine toxin testing. This risk also illustrates the importance of determining what other diatom species might produce domoic acid and how the toxin is produced. Future research should also be done to illustrate how anchovies and other possible domoic acid vectors accumulate domoic acid in their digestive tracts and tissues.

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