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Examining the Toxicity, Exposure, and Regulatory Approach to Potential Human Health Risks of the Algal Toxin Domoic Acid

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EXAMINING THE TOXICITY, EXPOSURE, AND REGULATORY APPROACH TO POTENTIAL HUMAN HEALTH RISKS OF THE ALGAL TOXIN DOMOIC ACID

A Dissertation Presented

by

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ABSTRACT

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June 2015

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Domoic acid is a neurotoxin produced by the marine diatom genus *Pseudo-nitzschia* and causes cell death primarily in the area of the brain responsible for long-term memory. The resulting severe illness has been termed amnesic shellfish poisoning. Domoic acid accumulates in shellfish and planktivorous fish that consume *Pseudo-nitzschia*, resulting in exposure to humans through consumption of planktivorous seafood. A regulatory standard in seafood was developed shortly after its discovery in 1987 to protect against acute effects. This regulatory standard has not been revised despite significant recent data in the scientific literature.

This dissertation is divided into four sections: (1) an identification of anthropogenic and natural drivers of nutrient dynamics as well as social dynamics that can contribute to current and future exposure to domoic acid; (2) a review of the weight

of evidence for revisiting the current regulatory standard based on recent low level chronic effects data in the toxicological literature, sensitive subpopulation information and long term seafood consumption data; (3) an analysis of monitoring data on the presence of *Pseudo-nitzschia* in ocean waters and domoic acid in seafood to examine spatial and temporal trends in human exposure; and (4) evaluation of the regulatory framework for natural toxins in seafood with domoic acid as an example.

Nutrient and social dynamics have the potential to drive exposure in humans. Recent toxicological data are not reflected in the current standard as it is based on data for acute toxicity and protects against gross observable neurotoxicity rather than chronic effects. The recent literature has shown that exposure to domoic acid can result in more subtle physical and behavioral brain impacts that have been observed in limited human data as well as extensive data on laboratory animals and marine mammals. Toxicological studies have demonstrated that certain groups such as the young, and the elderly are much more sensitive to domoic acid exposure. This is of particular concern because monitoring data for domoic acid in seafood are limited and may not ensure protection of the public. *Pseudo-nitzschia* is ubiquitous both temporally and spatially. This dissertation concludes that the regulatory approach warrants revisiting.

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LIST OF ACRONYMS

ADI	Acceptable daily intake
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ASP	Amnesic shellfish poisoning
CDC	United States Centers for Disease Control and Prevention
DA	Domoic acid
DIN	Dissolved inorganic nitrogen
DSI	Dissolved silicon
ED50	Effective dose in 50% of a test population
EFSA	European Food Safety Agency
EPA	United States Environmental Protection Agency
FDA	United States Food and Drug Adminisration
FAO	United Nations Food and Agriculture Organization
GI	Gastrointestinal
GluR	Glutamate receptor
iGluR	Ionotropic glutamate receptor
IOC	Intergovermental Oceanic Commission
KA	Kainate acid
LC/MS	Liquid chromatography/mass spectrometry
LD50	Lethal dose in 50% of a test population
LOAEC	Lowest observed adverse effect concentration
LOAEL	Lowest observed adverse effect level

LIST OF ACRONYMS

mGluR	Metabotropic glutamate receptor
MOU	Memorandum of understanding
NMDA	N-Methyl-D-aspartate
NOAEL	No observed adverse effect level
NOAEC	No observed adverse effect concentration
PND	Postnatal day
RfD	Reference dose
TDI	Tolerable daily intake
WHO	World Health Organization

INTRODUCTION

Human population in the coastal zone has increased greatly in recent years. Coastal resource utilization and contamination have intensified due to increases in shipping, aquaculture production, tourism, and residential, commercial, and industrial development. These activities result collectively in higher risks for public health and the global burden of disease[1]. The linkages between ocean and human health are complex and require understanding the relationships in complex chains of cause and effect. Advances in multi-disciplinary scientific research in the coastal zone have allowed for better understanding of the linkages between human activities and public health risks. The increased population in the coastal zone has the dual consequences of both increasing contamination in seafood and also increasing the number of people exposed to this contamination through consumption of seafood.

The U.S. Centers for Disease Control and Prevention estimate that seafood is one of the leading causes of food borne outbreaks of illness in the United States, comprising 15% of all outbreaks with a confirmed source[2]. This is a greater percentage than meat or poultry, which are consumed in much greater quantities. Seafood is one of the most widely marketed, traded, and distributed commodities in the world. Seventy-five percent of the seafood consumed in the United States is imported from other countries[3]. Because seafood is so widely traded, U.S. consumers are exposed to potential seafood contaminants and their associated risks from across the globe.

Monitoring for contaminants is often sporadic and inadequate, even within developed countries. This is particularly problematic because of the large number of source countries. Seafood contaminants can come in a variety of forms. Contaminants can be chemicals (e.g., mercury and persistent organic pollutants), viruses (e.g., hepatitis A, and norovirus), fecal-bacteria (e.g., salmonella and shigella), nonfecal-bacteria (e.g., listeria and vibrio), parasites (e.g., protozoa and nematodes), and biotoxins (e.g., ciguatoxin and domoic acid). Contaminants may be additive or synergistic, and may exacerbate existing medical conditions in people. Contaminants in seafood may be due to uptake from water, sediment, or the food chain, may be introduced during handling or preparation, or be due to inadequate refrigeration. The United States Food and Drug Administration (FDA) regulatory standards are adopted to minimize human health risks from these contaminants while considering the economic costs.

This dissertation evaluates human exposures to the natural toxin domoic acid (DA), a seafood contaminant discovered in 1987. DA is a neurotoxin produced by the marine diatom genus *Pseudo-nitzschia*[4]. DA can accumulate in the edible portion of fish and shellfish that consume *Pseudo-nitzschia*, leading to the potential for risk to human health. This introduction is divided into the following sections, (1) background on the discovery of domoic acid, and (2) presentation of the purpose and organization of the research into four chapters.

Background on the Discovery of Domoic Acid.

Domoic acid was likely historically significant throughout human history but was not identified as a disease vector until 1987. A novel set of disease symptoms was

identified during an epidemiological investigation of disease outbreak by Canadian health authorities in 1987 when blue mussels sourced from mariculture (i.e., marine aquaculture) operations on Prince Edward Island sickened about 156 people and killed three[5, 6] (a fourth developed chronic seizures and succumbed three years later[7]). The acute symptoms of the outbreak were relatable to paralytic shellfish poisoning but had distinct differences. Chief among these acute differences were epileptic seizures and memory loss, the latter of which led to the illness being termed "amnesic shellfish poisoning"[6].

The short term effect of this outbreak was the rapid closing of shellfish beds (at considerable loss of economic productivity) in coastal waters of northeastern North America while the cause was identified. In an extremely rapid and impressive piece of investigative science and policy development, domoic acid was quickly isolated as the source of the disease outbreak, human exposures were estimated, and a regulatory limit was established and enforced[5]. Today that regulatory limit remains unchanged at 20 mg domoic acid/kg seafood[8]. The regulatory limit was established to protect against severe acute effects of a single meal exposure to domoic acid. This limit of 20 mg/kg is not only the Canadian standard, but has been adopted world-wide, including by the United Nations Food and Agriculture Organization/World Health Organization/Intergovernmental Oceanographic Commission. During the nearly thirty years since the development of the standard, there has been growing understanding and concern that chronic exposure to low concentrations of domoic acid could present significant human health challenges.

Domoic acid appears to be unusual because it is an excitotoxin that can cause central nervous system effects at low concentrations[9-13]. The mechanism of

excitotoxicity is binding with glutamate receptors much more strongly than the intended neurotransmitter[14]. By binding with a neuron and failing to release, it causes the neuron to flood with calcium. An influx of water follows the calcium, causing the neuron to swell and burst, causing neuronal cell death.

In addition to being potentially toxic, the diatom genus *Pseudo-nitzschia* is cosmopolitan, with species occurring on the east and west coasts of North America and in fact across the globe[15]. Domoic acid is produced by many species of *Pseudo-nitzschia* and is a neurotoxin of significant concern in the marine environment[16]. The exact function of domoic acid in these diatoms has been a subject of debate, and the environmental factors that lead to its production are still being investigated[17].

Purpose and Structure of the Dissertation.

The issues related to assessing the potential for human risk from domoic acid are complex and cross-disciplinary. *Pseudo-nitzschia* are persistent and present across the globe[15, 18]. Human and anthropogenic nutrient influences on blooms are complex[19, 20]. Due to the severe health effects associated with DA, insufficient protection of human health carries a potentially steep cost ranging from subtle effects to memory and learning to more severe effects such as seizures and death[9, 21]. The economic costs of shellfish bed closures or banning sale of fish above the action level can be substantial. Closures of the Washington State razor clam industry occurred for 13 months in 1991-1992, 13 months in 1998-1999, and 6 months in 2002-2003[22] and the value of that fishery is estimated at more than $20 million annually[23].

Evaluating the potential for human health risks from DA in seafood is a difficult but worthwhile task. The interface of science and environmental policy is a critical

arena. Regulators must weigh economic costs and human health benefits when developing environmental policy and setting a standard. Sufficiently informing the policy process with information on complex scientific issues helps ensure that social benefits and costs are weighed appropriately. Weighing costs of monitoring and enforcement for domoic acid contamination in seafood with human health risks requires an examination of the potential for those risks as its cornerstone. The purpose of this dissertation is to give careful consideration to technical issues regarding toxicity, exposure and current regulation to evaluate the potential for human health risk from domoic acid in seafood.

The central research question is the core that ties together Chapters 1-4. The overarching research question is:

What are social dynamics, toxicity, exposure, and regulatory approach to potential human health risks of the algal toxin domoic acid?

From this core question, the dissertation is structured into chapters, each addressing a separate but related research question pertinent to the potential of human risk from domoic acid.

Chapter 1 The Human Dynamics of Domoic Acid. The research questions for Chapter 1 are:

What are the human dynamics of domoic acid (DA)? Do humans contribute substantially to the levels of DA in seafood and how do social dynamics contribute to an increase in consumption of DA-contaminated seafood?

Both anthropogenic and natural sources of nutrients have the potential to increase levels of *Pseudo-nitzschia* in the environment. Also, domoic acid is produced at different

concentrations by *Pseudo-nitzschia* depending on both the species present and environmental conditions. Chapter 1 examines the current literature on nutrient effects of *Pseudo-nitzschia* bloom dynamics and DA production.

This chapter also addresses the ability of social dynamics to affect human exposure to DA. Fishery production, exportation, and consumption patterns are examined to determine their potential effects on human exposures to DA.

Chapter 2 Toxicity Assessment. The research questions for Chapter 2 are: *What are the long-term effects of exposure to low levels of domoic acid? Is the current toxicological literature sufficient to derive a reference dose that is protective of long–term effects of chronic low-dose exposure? Is there sufficient data to develop a reference dose protective of sensitive subpopulations? Should the reference dose and consumption assumptions in the current action level for seafood be revisited?*

This chapter compiles and synthesizes data documenting chronic effects from low level exposure for humans, laboratory animals, and marine mammals. It also discusses evidence for children and the elderly as sensitive subpopulations and explores similarities and possible relationships between DA exposure and epilepsy and schizophrenia. Human seafood consumption data are also presented. These lines of data are then used to examine the weight of evidence for reevaluating the action level.

Chapter 3 Domoic Acid Exposure. The research question for Chapter 3 is: *What are the spatial and temporal trends in Pseudo-nitzschia cell counts in ocean waters and DA concentrations in seafood and what can we infer about the potential exposures for humans?*

Environmental monitoring data are examined to evaluate the temporal and spatial persistence of *Pseudo-nitzschia* in waters across the globe. *Pseudo-nitzschia* data collected by Plymouth Marine Laboratory Data approximately every two weeks since 1992 are analyzed to evaluate persistence and long term trends as well as the relationships with a number of nutrients. These data are supplemented by a discussion of long term *Pseudo-nitzschia* data sets from the scientific literature. Data from the scientific literature are also analyzed to determine spatial and temporal persistence of DA in seafood across the globe.

Chapter 4 Risk Characterization and Management. The research questions for Chapter 4 are:

Is current knowledge of domoic acid toxicity and exposure to humans sufficiently compelling to reasonably argue that the current standard in seafood be revisited? What lessons can be inferred about the larger regulatory process for natural toxins in seafood?

This chapter utilizes analysis on toxicity and exposure of domoic acid from earlier chapters as a template for evaluating the current FDA regulatory framework for natural toxins in seafood. Attributes of that framework that could warrant revisiting are identified from an examination of current action levels, monitoring programs, communication with the public, and disease surveillance.

CHAPTER ONE

THE HUMAN DYNAMICS OF DOMOIC ACID

Chapter 1 Research Question. What are the human dynamics of domoic acid (DA)? Do humans contribute substantially to the levels of DA in seafood and how do social dynamics contribute to an increase in consumption of DA-contaminated seafood?

Abstract. This paper assesses the question of human social dynamics of domoic acid exposure. Toxicity from DA was first linked to amnesic shellfish poisoning (ASP), an illness identified as an acute response to high concentrations of DA that can cause significant and/or long-term neurological impairments – and some instances, death. The isolation of domoic acid as source of such a challenging symptomology focused early research and regulatory attention on the environmental vectors and epidemiology of ASP. The result of that work led the scientific community to concentrate on bloom dynamics and system attributes that could lead to levels of DA in the environment that could potentially lead to cases of ASP. It influenced the regulatory community to focus regulatory standards to mitigate health risks from ASP.

However, more recent studies in the toxicological literature have demonstrated that exposure to relatively low concentrations of DA can result in significant and permanent effects to the central nervous system (CNS), particularly the brains, of laboratory animals (particularly when exposed to over longer periods of time)

[10, 11, 21, 24]. And, sentinel fauna in the environment have presented symptoms consistent with those studies in areas where chronic exposure to domoic acid was evident[12, 25-28].

These toxicological advances strongly suggest a new focus on developing an understanding of the dynamics of chronic low level concentrations of DA in humans and the environment. This paper therefore frames existing information on bloom dynamics in the context of chronic low level concentrations.

Introduction.

Issues connecting human health and environmental change are, almost by definition complex and uncertain. The study of environmental systems brings with it enormous set challenges. Understanding the source of human disease and challenges to well-being is highly uncertain. Environmental researchers and medical professionals typically maintain a narrow focus and inter-disciplinary connections are not easily made to link environmental change to the impacts on human health.

However, the story of domoic acid (DA) provides an important example of the need to bridge these intellectual divides and to embrace the challenges of this complex system and the potential for its impact of on humans.

The biotoxin is produced by the diatoms of the genus *Pseudo-nitzschia*. However, the majority of information relevant to the study of this organism has not been organized and connected in ways that reflect an understanding of potential interdependencies between bloom dynamics and the various ways in which human systems interact with exposure to DA and, consequently the nature and trends in human health risk. This paper

will assess the larger question of human social dynamics of DA exposure. The effort will tell the story of DA, from the underlying societal drivers, through the potential human health effects, in order to better understand the linkages of this issue within and between environmental systems and human health and well-being. This paper is built around four sections that frame the critical issues influencing the likely increased human exposure to DA.

Each of these sections will develop an integrating theme and address specific questions to better reveal the potential risks of chronic exposure to domoic acid and the relationship of that risk to changes in environmental/social conditions.

Section One: Coastal Social Dynamics and Domoic Acid. This section examines anthropogenic nutrient inputs in coastal areas and their potential to contribute to the condition of common chronic low level concentrations of DA in seafood. An evaluation of coastal system contributions to *Pseudo-nitzschia* population dynamics will allow an understanding of the degree to which humans may contribute to nutrient levels that support and sustain diatom primary production. Specifically, this section will assess the core questions of:

- Do coastal social systems contribute substantively and substantially to the frequency, distribution, intensity and toxicity of coastal DA levels? What are the critical anthropogenic drivers influencing DA dynamics? Is there evidence that these drivers dominate DA dynamics? Are there clear and discernible differences over time and across regions that provide effective insight that could support management action?

Section Two: Human per Capita Seafood Supply. An understanding of the shift towards greater human consumption of planktivorous fish will elucidate the trend

towards greater potential exposure to DA. This section will determine the degree to which:

- There is strong evidence that overfishing of large, high value species has led to a market change to smaller more affordable species. Is it possible that this species switch could elevate the importance of biotransfer toxins such as Domoic Acid? Overfishing of larger predatory species could open markets for planktivorous fish that have a higher potential to contain DA.

Section Three: Global Influences. Globalization of the seafood market can make DA in seafood harder to track and make it more difficult to provide information to the consumer, complicating the exposure picture. The following question will structure this section.

- Seafood consumption is the primary source of DA exposure in humans. And, human consumption patterns of seafood product are changing. Market sourcing for product has become global. Economic globalization and associated seafood consumption patterns may bring more individuals into the global seafood trading market, and may bring new products to market with a potential for DA exposure.

Section Four: Aquaculture. Aquaculture has grown to the point where – when all forms of marine and inland growing are included – nearly half of all of all fish comes from this sector. A significant portion of marine aquaculture (termed "mariculture") species have the potential to contain low levels of DA.

- Continued growth of aquaculture combined with plateauing catches of wild fish will combine to make aquaculture the dominant source of market seafood in the near future. What attributes of aquaculture influence the potential for DA exposure?

The structure, description, analysis, summaries and conclusions of this chapter are designed around these delimitations and questions. While they do not do not constitute a

comprehensive view of the social themes relevant to human well-being and domoic acid, it is argued that they do form a core around which summary conclusions can be structured. They are, we argue, a form of architecture around which a more nuanced understanding of the risk probability of human exposure to domoic acid can be built.

Coastal Social Dynamics and Domoic Acid.

Humans seek contact with the ocean. Humans inhabit coastal areas in high densities and subsequently can have significant impacts on coastal environments. Nutrient levels in aquatic systems have been linked to algal growth and species distribution and the frequency and duration algal blooms – including harmful algal blooms. This section evaluates evidence for whether coastal social systems contribute substantively and substantially to our existing general understanding of coastal DA levels. It identifies the critical anthropogenic drivers influencing DA dynamics. This chapter also examines evidence from the literature and unpublished data about how and under what conditions these social drivers may influence the dynamics of domoic acid production and possible bioavailability to humans. This section examines if there are clear and discernible differences over time and across regions.

***Pseudo-nitzschia* Dynamics.** *Pseudo-nitzschia* is the dominant diatom to produce the domoic acid biotoxin[49]. DA is produced by at least 11 species of diatoms in the genus *Pseudo-nitzschia*, as well as a related species *Nitzschia navis-varingica*[1, 2, 12, 13, 29-31,32]. *P. multiseries* and *P. australis* have been the most abundant DA producers in toxic blooms[13, 32]. These same species appear to be the drivers behind chronic low level concentrations also, although the data connecting them to low levels are, at present, limited. *Pseudo-*

nitzschia pungens and the less frequently recorded *P. fraudulenta, P. multiseries* and *P. australis* appeared to be cosmopolitan[14, 18] (i.e., present across the globe). The diatoms *P. australis* and *P. multiseries*, are widely distributed geographically and temporally and also have strong evidence that they are cosmopolitan. *P. delicatissima* and *P. pseudodelicatissima* also appear to be cosmopolitan in distribution and there are some taxonomic and identification issues[14, 18]. The factors that influence the various environmental conditions favorable for diatom growth depend on naturally variable oceanographic and climatic conditions as well as anthropogenic factors and forcings. For example, large scale oceanic upwelling increases nutrients available for algae, including *Pseudo-nitzschia*, and leads to blooms in regions such as the west coast of North America, Chile, Spain, and Portugal[6].

Geographic regions with large anthropogenic inputs of nutrients and low mixing with open ocean water may also be prime locations for diatom growth due to increased nutrient concentrations. Diatoms can be found in regions with different hydrographic conditions and varying degrees of human influence as diverse as the Bay of Fundy and the Gulf of Mexico[3, 43, 34].

Natural Systems Input. *Pseudo-nitzschia* blooms have been most extensively studied on the west coast of the United States. Ten species of *Pseudo-nitzschia* have been found in Washington State waters. *Pseudo-nitzschia* blooms are a greater problem on the Pacific coast of Washington but the semi-enclosed waters of Puget Sound have also been affected [5,35]. Razor clams have been found to generally contain the highest concentrations of DA in coastal Washington. And, while other species of shellfish have been implicated and area closures have been implemented, razor clams are not present in

Puget Sound[6, 36]. The coastal waters of Washington are not heavily impacted by anthropogenic influences and yet they are most impacted by DA[737]. Puget Sound and the Strait of Juan de Fuca may be affected by eutrophication, where dissolved inorganic nitrogen (DIN) levels are high year-round. *Pseudo-nitzschia* has been present in Puget Sound for decades but DA health related closures in the Sound were not imposed until relatively recently[5, 35, 36].

In California, upwelling contributes to primary production, including diatom growth in most areas. Algal blooms on the California coast begin in spring and are maintained through the summer by upwelling nutrients. Between late summer and fall the concentration of DA-producing diatoms is highest, coinciding with decline of coastal upwelling and nutrient depletion[6, 8, 36, 38]. These blooms are not restricted to nearshore coastal waters. It has been established, for example, that in Monterey, characteristics such as floor topography, water circulation, and coastal upwelling appear to most directly influence algal blooms[9, 39]. There has been an increase in *Pseudo-nitzschia* blooms on the West Coast coinciding with a water temperature shift that has produced cooler water, stronger upwelling, and increased nutrient inputs. This has resulted in greater phytoplankton productivity and a larger northern anchovy population[10, 40]. There is also evidence that a 1998 Monterey DA event may have been triggered by post-El Niño runoff[11, 41]. Thus, in California, it appears that large-scale natural system change is the dominant driver resulting in domoic acid production.

The potential causes of harmful algal blooms have been studied extensively in recent years. And, while algal concentrations cannot be directly predicted from specific oceanographic and environmental conditions such as terrestrial runoff, nutrients, and

temperature[18, 42] research into bloom dynamics can provide important insight into the various anthropogenic factors that contribute to maintaining concentrations of *Pseudo-nitzschia*. This paper focuses on concentrations that may not reach of the level of identified bloom but, nevertheless, may pose both human health concerns and harm to sentinel fauna in the environment[15, 16, 25, 26]. It has been argued that several forms of anthropogenic forcing, including – if not notably nutrient dynamics – influence the production of algal toxins[17, 43].

Anthropogenic input of nutrients in coastal areas. In general, algal growth rates are limited by available nutrients. Nutrient runoff has been proposed as a driver of *Pseudo-nitzschia* bloom dynamics in certain areas of the Pacific coast of North America and in the Gulf of Mexico[6, 36]. It has also been suggested that nutrient inputs from upwelling, combined with wind transport of cells, are important factors in algal concentrations[6].

The complexity of the algal concentrations and the various environmental conditions under which they occur makes it difficult to predict DA concentrations. However, notwithstanding the complexity of natural and human forcing it remains clear that understanding the relationship between anthropogenic nutrient inputs and *Pseudo-nitzschia* blooms is essential for sufficiently clear understanding of the overall risk potential to humans due to domoic acid exposure. Nutrient loading effects depend, minimally, on the ability to determine (1) the quantity of total nutrient inputs (including point and non-point sources), (2) the nutrient supply ratios (relative abundance of nutrient types), and on the (3) chemical form (e.g., organic versus inorganic) of the nutrients. *Pseudo-nitzschia* concentrations typically increase during periods of decreased

upwelling, when there is a transition from a nutrient rich to a nutrient limited environment[535]. Available information on the relationship between specific nutrient attributes and *Pseudo-nitzschia* is summarized below.

Nitrogen. Nitrogen is thought to be the limiting factor in algal growth in many coastal waters. It is also necessary for synthesis of domoic acid and amino acids. Nitrogen limitation is unfavorable for DA production, unlike P or Si limitation[44]. There is evidence for reduced but measurable DA production under N-limited conditions. DA production is increased in laboratory tests using urea (CH_4N_2O) as a nitrogen source, but the growth rate is significantly reduced[25, 45]. For *P. cuspidate*, the nitrate-grown cells are the most toxic[26, 46]. Urea is a form of nitrogen found in fertilizer and animal waste[27,47]. In many coastal systems anthropogenic nitrogen inputs are dominated by run-off of fertilizers and sewage discharge. *Pseudo-nitzschia* increase DA production when using urea (an anthropogenic input) as a nitrogen source[25, 45]. Nitrogen substrates such as urea and ammonium could contribute to *Pseudo-nitzschia* blooms and to the maintenance of seed populations at non-bloom concentrations, particularly during periods of reduced upwelling. For example, elevated ammonium levels from sewage inputs have been suggested as the cause for dense blooms of *Pseudo-nitzschia* in Sequim Bay, WA[5, 35]. There are also recent laboratory studies that have been conducted directly correlating the amount of urea to domoic acid in algal blooms. *Pseudo-nitzschia* is associated with eutrophication and a reduction in the N:Si ratio[1, 28, 31, 48]. And, laboratory studies with *P. cuspidata* and *P. fryxelliana* suggest that reduced N sources from coastal runoff could be important for maintenance of ambient *Pseudo-nitzschia* concentrations, especially during times of low ambient nitrogen concentrations[26].

Phosphorus. Urbanization and agricultural and industrial activities have caused large increases in the influx of both phosphorus and nitrogen in coastal areas[21, 49]. DA production has been triggered by macronutrient limitation of phosphate (PO_4^{3-}) in algae cultures[22, 23, 50, 51]. Depending on the nitrogen/phosphorus ratio (N:P), either can be limiting in aquatic systems. Historically nitrogen was believed to be the limiting factor for algal growth in marine systems while phosphorus was believed to be the limiting factor for algal growth in freshwater systems, although the role of relative water exchange rates and internal biochemical processes that adjust to N:P ratios have increased the complexity of this model[24, 52].

Silicates. The production of domoic acid can also be triggered by macronutrient limitation of silicate ($Si(OH)_4$) in algae cultures[22, 23, 50, 51]. High concentrations of *Pseudo-nitzschia* and DA have been found in waters off the coast of Southern California in the middle of a silicate-depleted cyclonic eddy[29, 53]. A strong correlation has been established between elevated biomass and silicic acid depletion[29, 53], although this relationship is not presented as a uniform finding for all *Pseudo-nitzschia* species. However, the first recorded bloom of *Pseudo-nitzschia*, and the associated outbreak of Amnesic Shellfish Poisoning (ASP) in 1987 was believed to be caused by an unusually dry summer followed by a wet fall, which caused runoff of inorganic silicate, which in turn was believed to sustain a massive bloom of *P. multiseries* for three winter months[30, 54]. Periodic depletion of silicate by the growing diatom cells appears to have stressed the cells and prompted production of DA. The growth of diatoms depends on the presence of dissolved silicon (DSi), and eutrophication can lead to a decrease in DSi. Increased nitrogen and phosphorus loading from anthropogenic activities can also result in

increased diatom production, which in turn can reduce the DSi concentration[21, 49]. There is evidence of a strong synergism between projected future CO_2 levels and silicate-limited growth, which holds the potential of a relationship of future concern over domoic acid potential under anticipated climate change scenarios[31, 55].

Copper. Copper is one of the most widely used metals in the world. The United States is the world's second leading copper producer[19, 56]. Copper is used in a number of commercial and industrial applications including plumbing, building wire, telecommunications, power utilities, in-plant equipment, air conditioning, electrical, business electronics, and industrial valves and fittings. In agriculture, copper compounds are used as fungicides and to prepare copper fungicidal products, algaecides for reservoirs and streams and nutritional supplements in animal feed and fertilizers[19, 56]. Copper compounds are applied as fungicides to foliage, seed, wood, fabric, and leather to protect against blight, downy mildew and rust[56]. The extensive use of copper is likely to lead to an increase in copper concentrations in coastal systems and could be a concern in DA production. For example, monitored levels of domoic acid in Monterey Bay, CA have been argued as being associated with excess copper in runoff from anthropogenic sources[20, 57].

In summary, the relative proportion of nutrients and influences on nutrient dynamics (i.e., silicate and copper), and not simply the total quantity of the nutrient pool, is important because any one nutrient may be limiting for algal growth in a given aquatic condition and/or location[143]. Both chronic and episodic nutrient delivery can promote growth. Management and mitigation of nutrient inputs to the watershed can lead to significant reductions in algal growth, but this relationship and its influence on DA

production is not consistent in every and all situations and there remain significant uncertainties and information gaps[6,8,9,14].

Domoic Acid Production by *Pseudo-nitzschia*. While it is important to understand the conditions that support growth of *Pseudo-nitzschia*, it is also important to understand that concentrations of domoic acid in the environment do not correlate consistently with overall, total *Pseudo-nitzschia* abundance. *Pseudo-nitzschia* will produce different concentrations of DA under different environmental conditions. Domoic acid is believed to play a role in the overall physiology of these diatoms and the amount of the acid produced depends on how much is needed by the cells. The nature of these functions is still under study[58, 59].

The primary species believed to be responsible for DA in seafood are *Pseudo-nitzschia pseudodelicatissima*, *Pseudo-nitzschia cuspidata*, and *Pseudo-nitzschia australis*[5, 35]. In laboratory cultures, cellular production of domoic acid is low during most of the bloom cycle[32, 60]. It appears that as the exponential growth phase decreases and cell division rates decrease as a result of nutrient depletion, DA concentrations have been found to increase[32, 60]. Silicon and phosphorus depletion were found to correlate with increased DA production[1, 22, 23, 31, 50, 51]. A recent mechanistic model designed to predict domoic acid production found that conditions of phosphorus or silicate limitation in conjunction with sufficient light and nitrogen, favors DA production[33, 17].

Bacteria. The presence of certain bacteria may also play an important role in the levels of DA found in blooms. The presence of certain bacterial strains can enhance DA production of *P. multiseries*[34, 61]. Bacteria growing epiphytically on *P. multiseries* may provide metabolic precursors that facilitate diatom production of DA while benefiting

from nutrient release by the diatoms[34, 35, 61, 62]. When the bacterial community of *P. multiseries* was removed with antibiotics, the diatom growth rate increased but it did not produce a significant amount of DA[36, 63]. And, when *P. multiseries* was inoculated with bacteria from the non-toxic *P. delicatissima*, *P. multiseries* did not grow significantly and produced even more DA. *P. delicatissima* did not have its growth affected or produce DA when inoculated with *P. multiseries*. While limited, these data suggest an intriguing potential for a nuanced, potentially significant, relationship between DA and bacteria.

Anthropogenic inputs in coastal areas have the potential to contribute to the condition of both periodically high and chronic low levels concentrations of bioavailable DA in seafood. Coastal systems with large anthropogenic nutrient inputs and coastal upwelling are more likely to provide conditions to support persistent low level concentrations of *Pseudo-nitzschia* populations than areas without both conditions concurrently present in the environment. However, evidence suggests that there is no single environmental condition or anthropogenic input that is exclusively predictive of their presence and persistence. *Pseudo-nitzschia* and associated DA production have been found across large parts of the earth's oceans and have been found to vary greatly both temporally and spatially. As the influence of nutrients and other environmental conditions on *Pseudo-nitzschia* growth and DA production are better defined, the conditions that result in uptake of DA into seafood will be better understood.

Global Production of Fishery Products: Seafood and Domoic Acid.

While anthropogenic nutrient inputs have the potential to increase DA in seafood, social drivers may also influence exposure to DA through changing seafood production and consumption patterns. In order to assess the trends in the potential for human exposure to domoic acid, both a general understanding of the global market for seafood as well as key market attributes holding the greater potential for DA exposure need be reviewed. Seafood consumption is the primary source of DA exposure in humans, and trends in seafood production suggest important changes over the last few decades and hold the potential to change risk potential in humans. Assessment of these changes suggest that exposure to domoic acid may be driven by seafood market forces that are separable from changes to coastal environmental conditions. Simply put, human consumption patterns of seafood product are changing. Indeed, there is evidence that trends in seafood production/consumption patterns infer a gradual shift towards consumption of species that are prone to higher DA concentrations. Analysis of these data allow for better isolation of the market forces most important in determining the risk potential in domoic acid exposure. The remainder of this paper will identify and assess these issues.

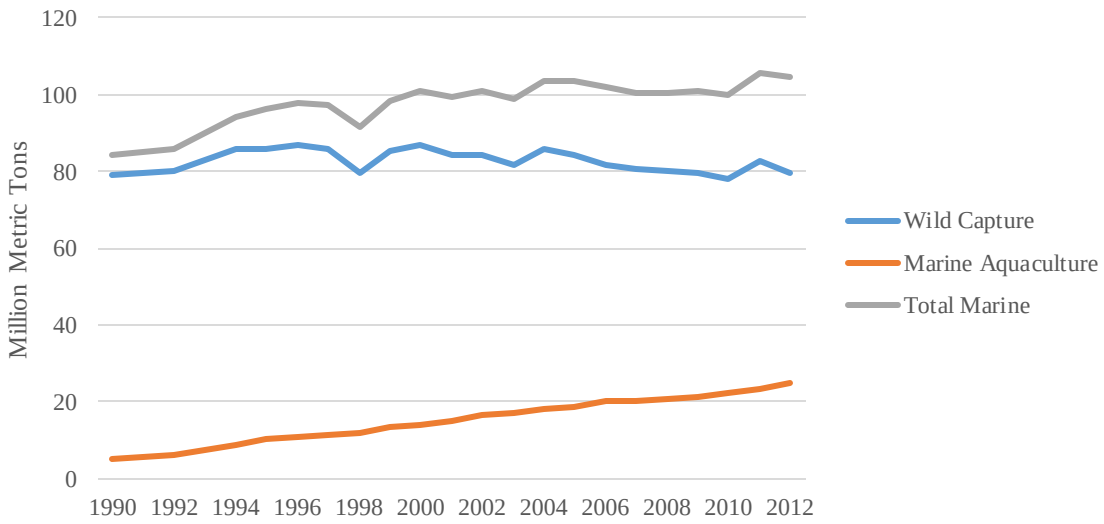
First it will assess generally global seafood market production trends over time and the degree to which changes in production patterns – with a particular emphasis on species composition of that market – can be viewed as altering the risk potential for domoic acid. That will be followed by an assessment of global per capita fish supply and, finally, the assessment will conclude by isolating the role of aquaculture in the market.

Increasing Global Seafood Production. Seafood provides an increasingly important protein supply to feed a hungry and growing world population. Global production of fishery products has grown steadily for the last half century. Fish production has grown at a rate double the general population growth (annual rates of 3.2 and 1.6 percent, respectively) meaning that per capita consumption is increasing with time[3]. Indeed, world per capita consumption has nearly doubled during that period from 9.9 kg in 1960 to 19.2 kg in 2012[3]. The data included are from the broadest definition of "fisheries". That is they include wild capture species from marine sources, wild capture from inland sources, and aquaculture products from marine and inland areas. Each of these classes of fisheries can hold a marginally different potential risk profile as regards domoic acid. The effort here will be to highlight the most notable attributes of each to assess trends in DA risk potential. Since inland production of fish and fish products have not been shown to be associated with domoic acid the key focus of this work will be separate marine production and assessing those trends (where data are separable from inland fisheries).

Seafood Production. Figure 1-1 (and the figures that follow in this section) was constructed using data obtained by querying the United Nations' FishStat database[64] and the Food and Agricultural Organization's (FAO) annual *The State of World Fisheries and Aquaculture* (SOFIA) reports[3, 65-69]. The FAO has collected seafood production data since the 1950s and commodity trading data since the 1970s. At present, these data include reporting from more than 200 state entities and information on 1,967 aquatic species or groups. These data can be used to evaluate trends in fishery, seafood

production and commodity trading and their potential implications for human exposure to DA.

Figure 1-1
Total World Marine Seafood Production

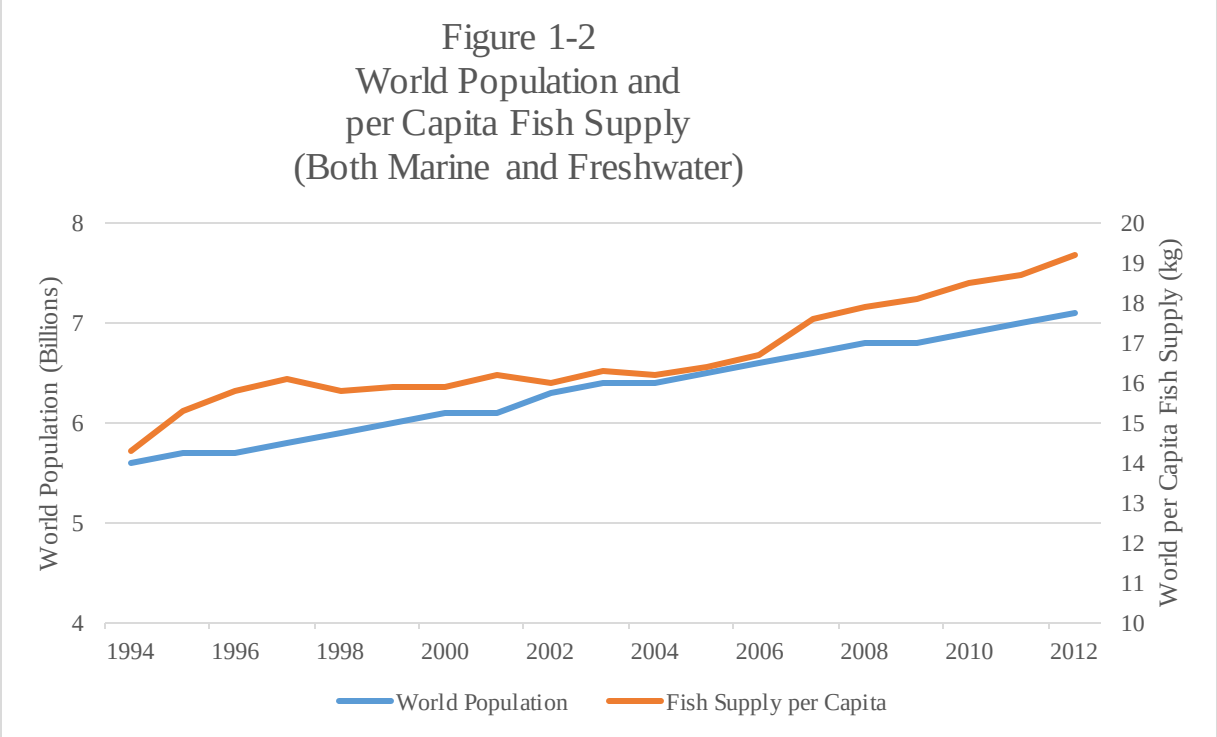


Trends in world marine seafood production are represented in Figure 1-1. These global data best represent the aggregate data needed to understand the possible nature and extent of human exposure to domoic acid. That is, these data exclude the contribution of any inland sources of fishery production.

This figure represents the overall trends in seafood production derived from wild capture and aquaculture sources. It well reveals the fact that global production of wild capture sources since 1990 has remained relatively constant or even declined slightly since the peak in the late 1990s/early 2000s. Wild capture was estimated at 79.3 million metric tons in 1989 and 79.7 m.t. in 2012[70, 3, 69]. Aquaculture was an insignificant

contributor to world marine production in 1990, but has increased dramatically and has accounted for virtually all the growth in world seafood production in the past twenty years[3]. Since aquaculture is an increasingly important source of seafood for the world's population and the processes of its production differ so dramatically from wild capture fishing, it will be the subject of the final analytical section of this paper. Total world marine seafood production has increased from 84 million (metric) tons in 1990 to 104 million tons in 2012 (Figure 1-2).

Human Population and Per Capita Fish Supply. While the world population has risen steadily, the total fish per capita available per person has also increased significantly. The total population of the globe recently edged past seven billion. However, as already noted total fishery production (inland and marine) has grown at a higher unit rate, meaning global fishery supply on a per capita basis is higher today than at any point of recent history. Freshwater and marine data are combined and are not readily separable, but demonstrate pressure on the existing marine biomass and incorporate both steady annual marine wild capture and dramatically increasing annual marine aquaculture.



Per capita marine seafood supply increased from about 14.3 kg/year in 1994 to almost 19.2 kg/year in 2012[3, 69]. Global demand-driven production of seafood is increasing both in total and for the average individual seafood consumer. It should be noted within the context of this paper that these aggregate data include both seafood supply available for humans as food and as other non-food uses such as fishmeal and fish oils. It may be argued that in the case of fish meals and fish oils there could be an indirect risk vector back to humans, albeit at a smaller relative rate give complexity of the number of processing and biotransfer stages between non-food uses and human exposure. Given that these data reveal an increased market supply for seafood as both food caloric and protein source for humans – the continued pace of increased per capita supply suggests unmet market demand. One would expect that there has been a shift in the proportion of

seafood used as human food relative to non-food uses in order to maximize economic rent. And, over the last 30 years that has been the case. In the 1980s direct human consumption of fishery products was about 70 percent of total production. That has increased to about 85 percent of total production today[3].

However, given the focus of the present work, total production while important, needs to be refined by a more detailed understanding of species distribution of global marine seafood production. Some species hold a greater domoic acid risk potential than do others. The reasons for this are both nuanced and straightforward. Chapter Two of this dissertation will examine, in detail, the question of risk exposure for domoic acid in humans. Here, the analysis of seafood species can be built around a limited, but essential, set of toxicological assertion. Notably, (i) it has become increasingly clear that domoic acid can hold a measure of toxicity at low doses, and, (ii) it can be efficiently communicated to humans via the consumption of tissues and organs of low trophic species recently exposed to the toxin and then captured/harvested and then consumed by humans[13, 21, 71-73]. It is, therefore, important to assess any trends in the species structure of global marine seafood in order to assess any changes or trends in domoic acid risk potential.

Global Seafood Production: Trends in Species Composition. Over the past few decades there has a discernable movement toward a global seafood species composition consisting of smaller fish within species and representing lower trophic level species generally. There are several contributing factors. First, global capture fisheries are, at present, almost entirely fished at or beyond their sustainable maximum. The FAO estimates that only about 9% percent of fish stocks can be characterized as being

underfished. Ninety per cent of world stocks thus are characterized as being fished at or beyond their biologically sustainable level (29 per cent) in the most recent data[3]. At this level of fishing pressure more fish representing greater species diversity are brought to market. Since most of the biomass contributing to global seafood markets are represented by smaller species it is rather straightforward to assume that total production is comprised of a larger proportion of smaller species than was the case a few decades ago. Again, the most recent data supports this conclusion[3].

The intense global effort in wild capture harvest is notably evident in fisheries for larger species and for larger individual fish within species. Market forces drive direct effort toward larger fish[74, 75]. Seafood prices for are key to understanding fishing behavior and how effort is targeted. Simply, fish with a higher market value (and, greater potential landed-value profit) hold the greater potential for directly targeted initial effort. Thus, one can infer a consequence of intense overall fishing effort would be a proportionately greater impact on larger species and on larger individuals in within species[74].

With level or increased total effort one would expect that there would be a shift toward smaller fish and lower trophic level species as larger fisher are preferentially caught[76, 77]. The data supports this logic. Indeed, the concept is known as "fishing down marine food webs" in the literature[78]. And, as these larger fish are removed from the system, smaller prey species are more likely to prosper shifting the system balance and economically opening new market opportunities for small fish representing lower trophic level species[79].

Pressure on Top Predator Marine Species: Bluefin Tuna and Swordfish. This concept can be well represented by a general assessment of global fishing effort directed at two particularly high value species; that is, bluefin tuna and swordfish. These fisheries represent well both major attributes of the issue under discussion. They represent larger species of fish and the market for each illustrates the existence of higher demand and profit for larger individuals with species landings.

Figure 1-3
Top Predator Capture
(Bluefin Tuna and Swordfish)

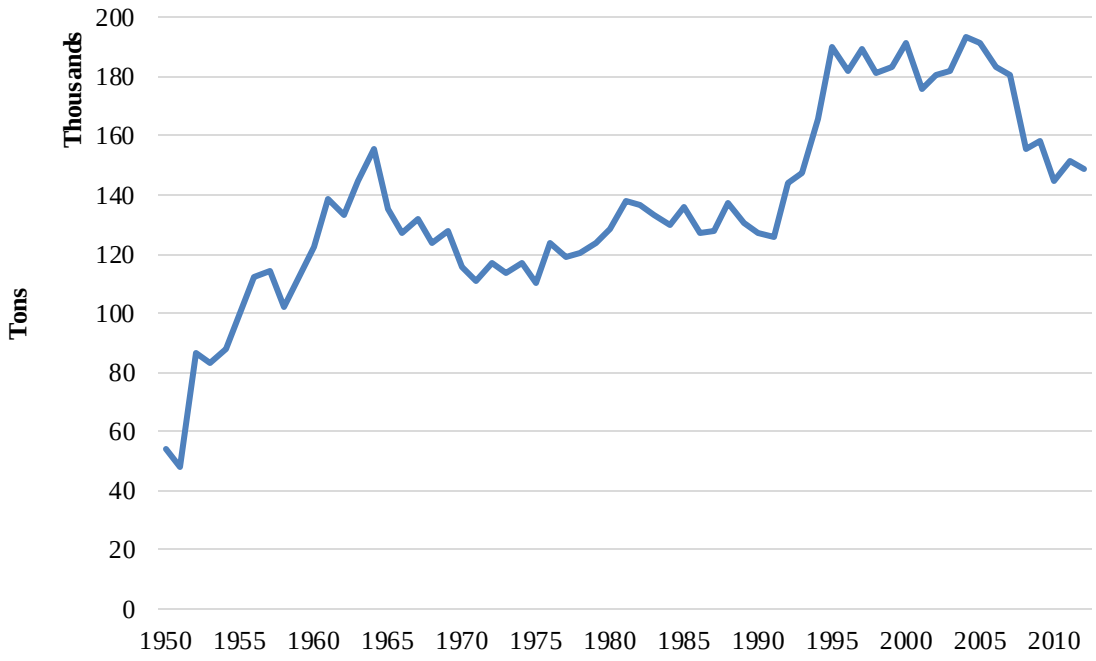


Figure 1-3 depicts the wild capture of Atlantic, Pacific, and Southern Bluefin tuna as well as Swordfish. In 1950, production was less than 60,000 tons[80]. Effort on these stocks has witnessed two large overall increases. The first was in the period immediately after the Second World War and then, again, in the period around the turn of the millennium. Landings peaked about a decade ago at nearly 200,000 tons per year before declining to

about 150,000 tons[80]. The primary economic force driving these landings is overall market preference, and the globalizing of the market (in particular for larger, high value fish) into emerging economy countries where increases in income and wealth have diversified the seafood market by way of increased fishery imports[3, 70]. These conclusions are further supported by the fact that effort directed at them has become globalized as well. Directed effort has extended throughout the global ocean habitats[39] well beyond those in traditional coastal regions[1, 2, 81]. There is also supportive evidence for declines in other large predator stocks. Sharks, tuna, billfish, ground fish, and large reef-associated predators are under substantial pressure across marine habitats[40, 82]. For example, of the 23 tuna worldwide stocks with available data, all are fully exploited, depleted, or recovering[37, 70].

Overfishing of larger predatory species could open markets for planktivorous fish that have the potential to contain DA at higher concentrations than seafood species from higher trophic levels. It also suggests strongly the potential for such a species shift to elevate the relative importance of biotransfer toxins such as domoic acid in calculations of seafoodborne risk potential.

Domoic acid is a water soluble chemical and does not bioaccumulate up through the food web. Rather, the concentration of DA is highest in the planktivorous species at the bottom of the food chain where species feed directly on *Pseudo-nitzschia* diatoms.

Due to increasing demand for seafood and plateauing catches of top predator species, consumption of planktivorous seafood near the bottom of the food chain is likely to increase. One can infer such an increase from Figure 1-4 where total exports for Peru reveals a consistent upward trend. Wild capture production of herring, sardines, and

anchovies was relatively constant from 2002 through 2008, ranging between 18 and 22 million tons[41, 83]. Virtually, all the aggregate data over time supports such a conclusion[3]. However, it is important to note that some of these species are subject to environmental fluctuations (notably, the ENSO cycle in the Pacific) and these influences may affect total production from year to year. And, as total demand for seafood increases, non-food uses of these species may be shifted to food uses. As consumption of lower trophic level species increases, contaminants in these species are, therefore, likely to become more of a concern.

Global Seafood Consumption and Market Demand

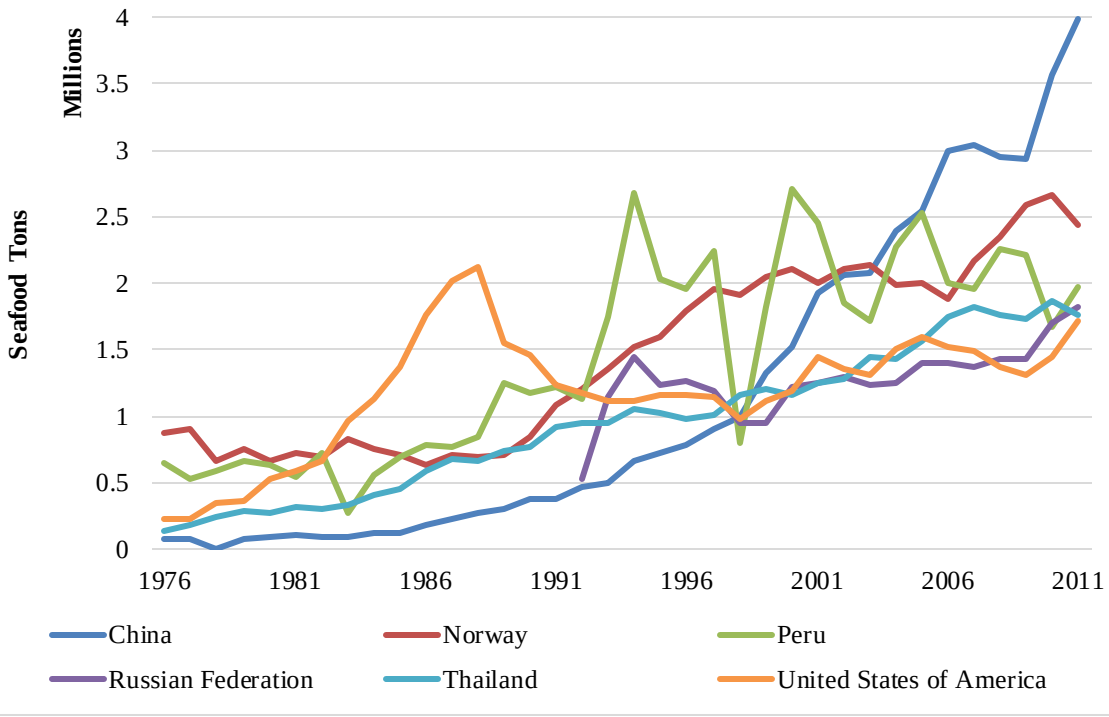
Total global fishery demand is driven by a combination of several factors. These include the increases in human population as previously discussed. Importantly, they also include the influence in global per capita income in many emerging economies (expanding market potential for higher value fisher products); the urbanizing of human population (which can geographically focus import markets by focusing market reach and reducing total commodity transportation costs); and, the associated efficiencies in global transportation networks driven by the broader globalization of commodity trade (which benefits all commodities in trade including seafood).

Seafood consumption is the primary source of DA exposure in humans. And, like overall production functions, human consumption patterns of seafood product are changing[3, 70]. Market sourcing for product has become global. Economic globalization and associated seafood consumption patterns may bring more individuals into the global seafood trading market, may bring new species to market with a potential to human DA

exposure, and may introduce new products (like fish oils) with new and divergent impacts on DA exposure. Seafood is an important commodity that is imported and exported both in the United States and world-wide on a large and broad-ranging scale. Seafood appears to be the most porous international commodity market in terms of trade. More countries trade more seafood to a greater number of other countries than is the case for any other single commodity. Where specified the data presented here on global trade of fish, shellfish, and other organisms includes both marine and freshwater fish. The separation of inland vs. marine has been shown in aggregate in other figures and deriving total direction of trade by species is beyond the need or boundaries of the present analysis.

The United Nations collects marine and freshwater fishery data for 215 countries[37, 3]. The United States is among the top ten countries for fish and fishery product exports and imports (marine and freshwater species), and U.S. imports and exports are substantial and growing[373]. Figure 1-4 depicts the top six exporters of marine seafood in the world and trends in export markets from 1976 to currently available data for 2011.

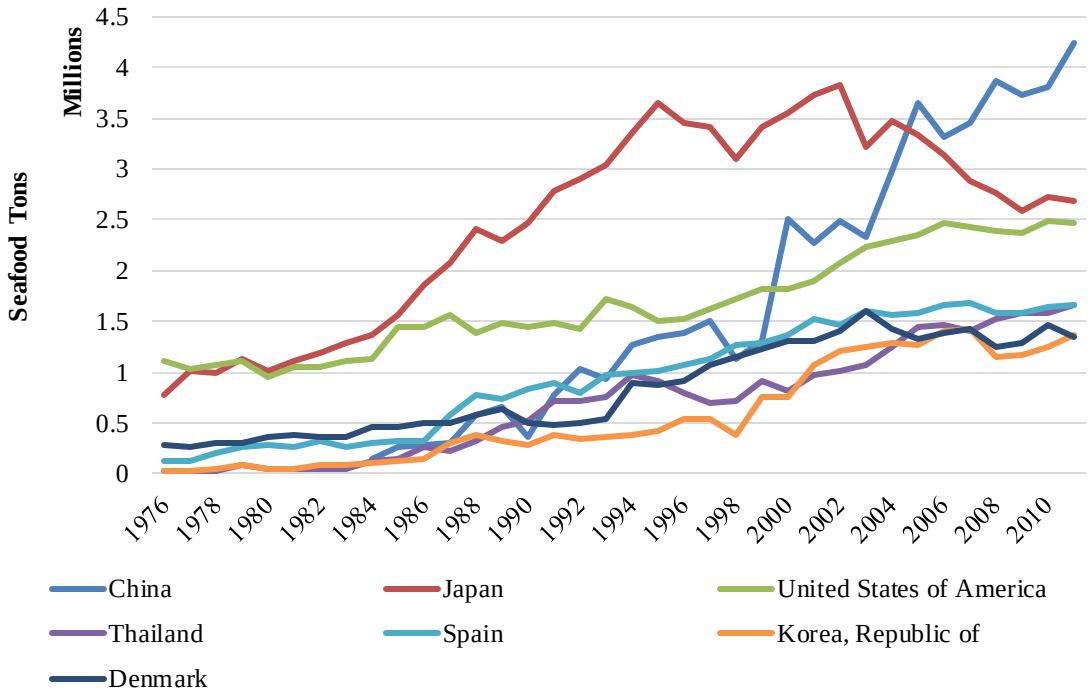
Figure 1-4
Top Seafood Exporting Countries



Chinese production has climbed in recent years to make it the world's largest seafood exporter from 2005 onward[80]. Production by the United States peaked in 1986 (the largest producer at that time). Production by the other top producer nations has increased to varying degrees for all countries during the time period represented in these data. Indeed, data for China, Norway, Thailand and the Russian Federation all reveal strong largely consistent overall growth. The relatively modest relative recent increases for the U.S. (after the production peak of the mid-1980s) represent a heavy reliance on wild capture in fully- and over-fished areas. The fluctuations in production Peru are due to high volume small marine pelagics (i.e., *Anchovita*) which is subject to large production swing due to environmental conditions such as the ESNO cycle[3, 84-87].

The overall view of steady global market growth is similar when viewed through the lens of seafood importing countries (See, Figure 1-5).

Figure 1-5
Top Seafood Importing Countries

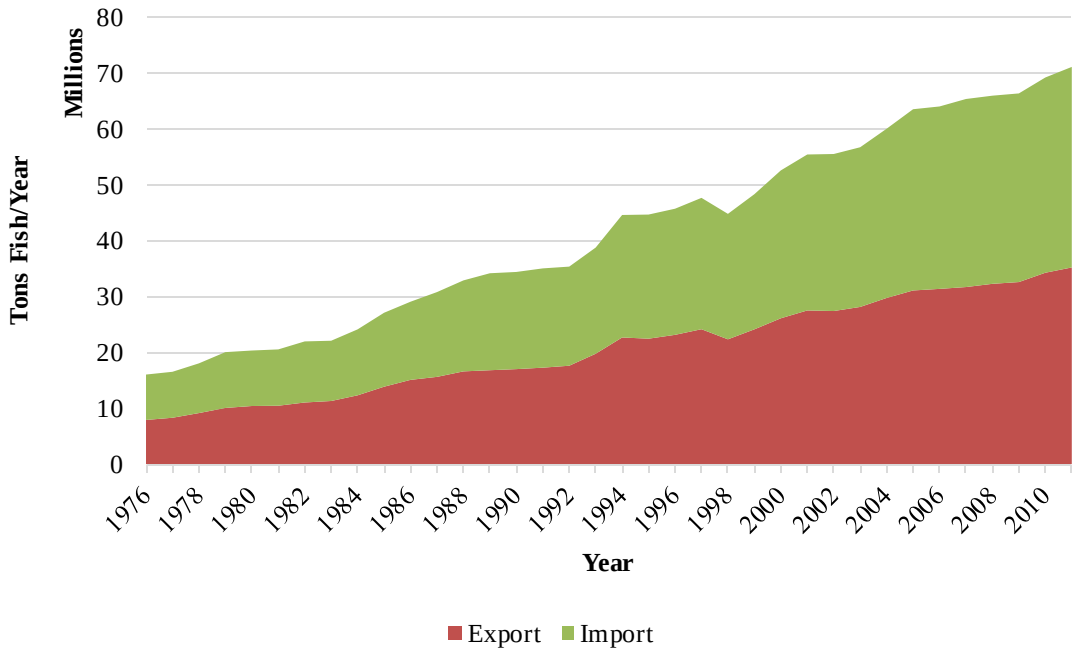


In the figure above, China was by far the greatest importer of seafood (and was the greatest exporter), followed by Japan and the United States[80]. The top seven importing countries depicted all demonstrated substantial growth from 1976 through 2010. Import volumes for the top seven countries were between one and 4.5 million tons in the most recent the year (2010)[80]. The United States ranked second among importing countries at $14 billion of fish in 2008, up from $8.5 billion from 1998 for an annual growth rate of 5.1 percent[37, 70]. The world total imports in 2008 were $102 billion, up from $51 billion in 1998 for a 7.1 percent annual percentage growth rate[41, 83]. Even with a recent marginal

decline in Japanese overall imports, volume is still higher in the latest data than in the earliest year depicted in the figure. Recent reductions in Japanese import volumes represent overall economic challenge during the last several years[41].

Figure 1-6 depicts the view of total global trade in fishery products over the same period. These data reveal the degree to which to which global trade has begun to play a central role in the economics in fisheries.

Figure 1-6
Stacked Chart of World-Wide Seafood Imports and Exports



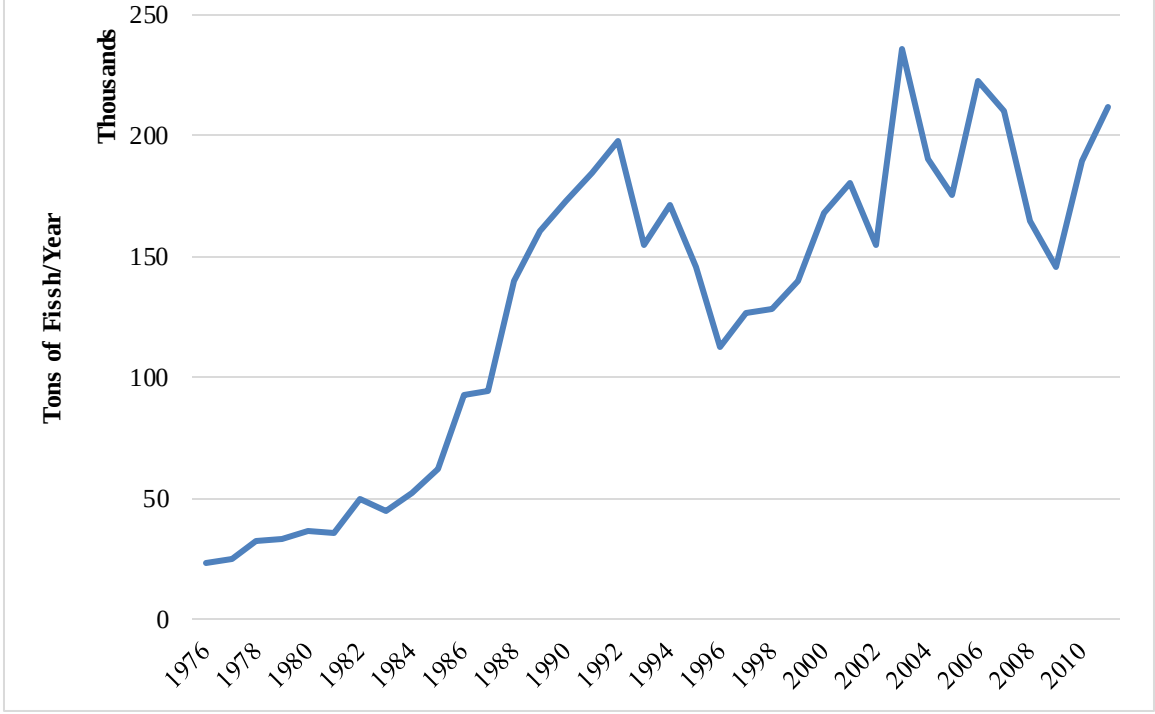
This notable growth in total trade (imports and exports) reveals that more than seventy million tons moved between countries in 2010[80]. The growth of imports and exports means that fish and fish products are moving between countries more than ever before.

This particular attribute of seafood is important in the context of understanding the degree to which domoic acid levels in seafood can be effectively monitored and

regulated. In a market which has 200 state actors trading fishery and seafood products, the complexity of that market makes understanding the exposure of potentially DA contaminated product a significant challenge. Measuring total global trade and sheer number of state actors in that market reveals a challenging policy problem.

Re-exports potentially introduce an even more problematic policy issue in tracking the source of marine fish. Figure 1-7 presents data on re-exports of seafood[37, 80].

Figure 1-7
World Wide Seafood Re-Exports



Re-exporting of seafood occurs when one country imports seafood from another country and in turn exports it to a third country (typically after some form of processing). This adds a further level of complication to product tracking. Even if the country in which the seafood was harvested monitors and manages access to potentially contaminated product

other actors along the supply chain may not. This can introduce high levels of uncertainty on many attributes of the seafood product by the time it reaches its final market sale[88]. While reported re-exporting is still a relatively small part of the global seafood commodities market, it is growing and currently represents a not insubstantial 200,000 – 250,000 tons per year[80].

In short, an individual on one side of the planet may be exposed to contaminants in seafood from the other side of the planet. Monitoring local waters with the implied assumption that seafood harvested will be sold only in regional markets is questionable at best. And, assuming market available product represents product from controlled waters is clearly questionable. This is complicated further by seafood imported from one country to another and then re-exported to a third before it is ultimately consumed[88]. This is a potentially significant problem for a toxin like DA where the source of the toxin (*Pseudo-nitzschia*) is ubiquitous across much of the globe and analysis for DA is spotty or nonexistent (discussed in detail in Chapter 3).

Aquaculture and Domoic Acid: An Essential Attribute of the Question.

In addition to wild capture of seafood, aquaculture is a growing source of seafood and has the potential to result in significant DA exposure. More than 80% of the seafood Americans consume is imported and almost half of those imports are farmed[45, 89]. It has been previously noted how essential farmed product is to the overall ability of the global fishery industry sector to meet changes in demand and to the challenges of feeding a growing global population. Assuming current trends, the United States may need to import as much as 4 million tons of seafood by 2025[46, 90]. Worldwide, aquaculture (both

freshwater and marine) has grown at an average rate of 8.8 percent per year since 1970, far outpacing the growth of capture fisheries (1.2 percent annually) and terrestrial farmed meat production systems (2.8 percent annually) over the same period[46, 90]. As shown in Table 1-1 below, world aquaculture (food fish and aquatic plants) has grown significantly during the past half-century[3]. From a production of below 1 million tons in the early 1950s, production in 2004 was reported to have risen to 59.4 million tons, with a value of US $70.3 billion. This represents an average annual increase of 6.9 percent in quantity and 7.7 percent in value over reported figures for 2002[91]. China is the largest aquaculture producer in the world, growing from 6.5 million tons in 1990, to 21.5 million tons in 2000, and 33 million tons in 2008[37, 70]. While the Chinese numbers are considered rough estimates at best, they do illustrate a dramatic increase in aquaculture to meet fish demand in China. By comparison, U.S. production is much smaller but also growing, with 315,000 tons in 1990, 456,000 tons in 2000, and 500,000 tons in 2008[37, 80, 70].

Table 1-1

World Marine Seafood Production (Million metric tons)

Production	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**
Marine Capture	80.7	79.9	79.6	77.8	82.6	79.7
Marine Aquaculture	20.0	20.5	21.4	22.3	23.3	24.7
Total World Fisheries	100.7	100.4	101.0	100.1	105.9	104.4

With annual wild production topped out at around 80 million tons, much of the change in supply and demand functions are effectively being met by aquacultured product.

Aquaculture is a growing and increasingly important supply of seafood to the world and has provided virtually all the global production growth over the past few decades.

Marine Aquaculture of Molluscs. Freshwater fishes currently dominate aquaculture production, with 28.8 million tons in 2008[83]. The production of freshwater fishes in 2008 was dominated by carp, at 20.4 million tons (71.1 percent)[37, 70]. Molluscs were second at 13.1 million tons, and marine fishes were 1.2 million tons. However it is important to note that aquaculture of some marine shellfish species has increased dramatically. Consumption of shellfish has been a common transfer vector DA risk in the past and any changes in production functions for marine shellfish should be of interest. For instance, abalones, winkles, and conchs have increased more than 100-fold in recent years, from 3,000 tons of aquaculture in 2002 to 359,000 tons in 2008[41, 83]. As shown in Table 1-2, wild capture of several major shellfish groups has remained relatively stagnant or declined, but aquaculture of these groups has significantly increased to meet demand[92]. Production of wild capture oysters has declined from 175,000 tons in 2002 to 127,000 tons in 2008. Wild capture of mussels has also significantly declined during this time period (from 225,000 to 87,000 tons) while scallops and clams remained relatively stagnant. However, all four species of shellfish in Table 1-2 showed gains in aquaculture from 2002 to 2008, demonstrating the increasing importance of aquaculture as demand grows.

Table 1-2

Shellfish Wild Capture and Aquaculture Production (1,000 tons)

Production	**2002**	**2004**	**2006**	**2008**
Oysters:				
Wild Capture	175	150	139	127
Aquaculture	3,884	4,143	4,263	4,164
Mussels:				
Wild Capture	225	189	114	87
Aquaculture	1552	1670	1814	1625
Scallops:				
Wild Capture	750	791	760	764
Aquaculture	1113	1053	1262	1411
Clams:				
Wild Capture	812	856	757	775
Aquaculture	3066	3635	3799	4397

Summary Conclusion.

This chapter provided an examination of the underlying human influences on DA in seafood based on a review of the literature. This chapter linked together (1) anthropogenic sources of nutrients in coastal areas, (2) increasing demand for seafood, resulting in increased consumption of planktivorous species, (3) the globalization of the seafood market, and (4) the growth of marine aquaculture.

Pseudo-nitzschia concentrations and bloom dynamics have been linked to nutrient concentrations in the environment. Temperature-driven mixing of the water column increases available nutrients and leads to seasonal blooms of diatoms. Human inputs of nutrients such as nitrogen and phosphorus have the potential to support higher persistent concentrations of *Pseudo-nitzschia* between bloom events. However, a complete understanding of the conditions that lead to DA production in *Pseudo-nitzschia* is still being developed.

This chapter demonstrated that wild capture of seafood has been relatively stagnant for the past two decades, while demand for seafood and per capita supply world-wide has increased. A focus on species lower on the food chain (i.e., planktivorous species with a greater potential for DA contamination) is likely the only way to significantly increase marine wild capture. This focus could constitute increased capture or diversion of non-food uses to food uses for low trophic level species. The increased globalization of the seafood market exposes individuals to a greater variety of seafood products from a greater number countries than in the past, causing exposure to DA-

contaminated seafood to occur at great distances from its original area of harvest. This makes it difficult to tie together sources of exposure and contamination.

Aquaculture has provided most of the growth of in the seafood market in the last two decades. Since aquaculture occurs in coastal areas where anthropogenic nutrients can lead to persistent *Pseudo-nitzschia* concentrations, uptake of DA into aquacultured planktivorous seafood species is a notable, if significant, concern.

While direct evidence for increased risk exposure is limited at present, the analyses of the various attributes constituting the core of this chapter are strongly suggestive of an increase in risk potential. Trends in coastal social dynamics as well as production and consumption in global seafood are supportive of a concern that exposure to domoic acid is increasing in the global human population.

CHAPTER TWO

TOXICITY ASSESSMENT

Chapter 2 Research Question. What are the long-term effects of exposure to low levels of domoic acid? Is the current toxicological literature sufficient to derive a reference dose that is protective of long–term effects of chronic low-dose exposure? Is there sufficient data to develop a reference dose protective of sensitive subpopulations? Should the reference dose and consumption assumptions in the current action level for seafood be revisited?

Chapter 2 Abstract. Domoic Acid (DA) was unknown prior to the 1987 outbreak of illness on Prince Edward Island, when over 200 people consuming blue mussels were sickened, fourteen exhibited impairment of anterograde memory (impaired memory for events that occurred after exposure), and four patients died. The illness associated with acute exposure in this outbreak was called amnesic shellfish poisoning (ASP). DA binds with receptors in the hippocampus of the brain, which can result in chronic effects such as seizures and effects to long-term memory and spatial navigation. The current regulatory standard for DA is based on observed human effects from estimated ingestion rates of blue mussels during the 1987 outbreak. The data from the 1987 outbreak represent a single exposure to relatively high concentrations of DA during an extreme diatom bloom event. While the development of the regulatory standard was accomplished through impressive and relatively rapid investigative work, the standard

was derived to protect against acute effects and has not been revised despite recent compelling data on subtle and significant effects of chronic exposure.

Recent toxicity studies have elucidated both the mechanism of action and the adverse effects caused by DA. The recent literature has shown that exposure to DA, particularly when the exposure occurs during a critical window of brain development, can result in more subtle physical and behavioral brain impacts at low concentrations. The reference dose also may not adequately protect sensitive subpopulations. Toxicological studies have demonstrated that certain groups such as the young, and the elderly are much more sensitive to DA exposure.

The consumption assumptions used in conjunction with the reference dose to derive an action level may not protect individuals who consume large amounts of seafood regularly. This chapter evaluates the protectiveness of the current action level in seafood in terms of (1) evidence for chronic effects at low doses, (2) protection of sensitive subpopulations, and (3) assumptions about seafood consumption rates. With all these issues taken into account, the weight of evidence indicates a need to revisit reference dose and action level for DA.

Introduction.

The evaluation of a chemical's toxicity characterizes the relationship between chemical dose and the incidence and severity of health effects. It considers factors that influence the dose-response of a chemical including patterns of exposure and age and health variables that could affect susceptibility. Development of a toxicity value typically involves extrapolation of high-dose responses to low-dose responses and from animal

responses to human responses[48, 93]. There are more than five thousand known marine algal species, but only a handful produce chemicals that are known to be toxic to humans or wildlife[49, 94]. DA is a neurotoxin with an extensive body of literature on its toxicity. While there was a recent[50, 21] general review of the toxicology literature, this paper specifically focuses on an evaluation of the toxicological data pertinent to low dose chronic exposure. The regulatory approach for DA has focused only on acute effects from a single exposure, while recent literature has provided evidence for the potential of chronic effects. This chapter evaluates whether there is sufficient toxicological information on chronic low dose effects to develop a toxicity value, called a chronic reference dose (RfD), which is protective of these effects. The current toxicity value is a United Nations Food and Agriculture Organization/World Health Organization/Intergovernmental Ocean Commission (FAO/WHO/IOC) acute provisional reference dose of 0.1 mg DA per kilogram body weight per day (mg/kg/d) that is based on acute effects observed in humans from single high dose exposures during a poisoning incident in 1987[5, 18]. This reference dose was used to derive an action level, which is a concentration in seafood that is considered safe for human consumption.

This chapter reviews chronic toxic effects of DA with an emphasis on the more recently identified subtle effects to brain function, memory, and cognition. This chapter considers (1) the derivations of the current reference dose and action level, (2) the evidence for chronic effects at low doses, (3) the potential for effects to sensitive subpopulations, (4) the protectiveness of seafood consumption assumptions in the action level, and (5) the available data for deriving a chronic action level. The conclusion

discusses whether there is a sufficient weight of evidence that the action level should be revisited.

The first section presents the derivation of the reference dose that forms the basis of the action level in seafood. This section defines the reference dose approach for evaluating toxicity and discusses the toxicological literature for chronic effects of low dose exposure. A reference dose represents a milligram dose of DA per kilogram body weight per day below which adverse effects are not expected to occur[8]. An action level is a regulatory level in food that protects against adverse effects in human. In order to develop an action level in seafood (in milligrams per DA per kilogram of seafood [mg/kg/d]), assumptions are made about the amount of seafood consumed by an individual. This chapter evaluates the toxicological basis of the current action level (i.e., the reference dose) and assumptions about consumption rates.

The second section presents the evidence for chronic effects of DA at low doses. The current reference dose is based on acute human effects but studies have been have been performed in laboratory animals that show more subtle chronic effects. The mechanism of action is discussed on both molecular and systemic levels. Data are presented and trends synthesized for (1) human exposure data, (2) experiments with laboratory animals, and (3) studies on wild marine mammals and birds who received environmental exposures through seafood consumption. This section also discusses the symptoms of long-term domoic acid toxicosis and similarities to chronic brain illnesses.

The third section identifies and evaluates children and the elderly as sensitive subpopulations. This section identifies factors that cause certain groups to be sensitive subpopulations and provides supporting information from the scientific literature.

The fourth section evaluates the ingestion rate assumptions used in the action level. Both the amount per meal and the single meal consumption rate are evaluated. Acute and chronic consumption data are discussed.

The fifth section discusses the available data for deriving a chronic action level. A range of possible chronic action levels is derived and discussed.

Derivation of the Reference Dose and Action Level for Domoic Acid.

The Codex Alimentarius[95] is the international standard-setting body for contaminants in food, and the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures stipulated the Codex Standards as the international standards for food safety. The Codex Committee on Fish and Fishery Products manages risks for seafood safety, while the FAO/WHO/IOC establish RfDs and action levels of chemicals in seafood[95]. In 2004, the Codex Committee requested that FAO/WHO/IOC review scientific data for safe levels of a number of biotoxins in shellfish[8]. The United States Food and Drug Administration (FDA) terms these safe levels "action levels", and the Canadian government refers to them as "maximum residue levels". Action levels are set at an acceptable level a toxin, using scientific information and value judgments. In order to determine the acceptable level of risk, an action level in seafood must combine toxicity and exposure. Therefore, an action level in seafood is comprised of two parts, (1) the toxicity of the chemical and (2) the assumed amount of seafood consumed. This section summarizes the derivation of the current action level for DA.

The first part of the action level development process is the evaluation of toxicity. Toxicity is incorporated through the establishment of a reference dose (RfD). The RfD is defined by the United States Environmental Protection Agency (EPA) as an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of adverse effects[96]. A reference dose is a safe intake of a chemical, usually specified as an acute or chronic RfD, depending on the type of exposure that is being assessed. A chronic RfD can also be referred to as a tolerable daily intake (TDI) or acceptable daily intake (ADI) depending on the nomenclature of the regulatory body that issues it. The derivation of an appropriately protective RfD is critical for DA because some of the effects of DA (such as seizures, permanent memory loss, and death) can be severe.

Process for Deriving a Reference Dose. An RfD is a chemical-specific estimate of oral toxicity that when combined with an estimate of exposure can be used to determine an action level. FDA does not have a well-documented process for deriving an RfD. In contrast, USEPA has a well-documented and transparent approach to deriving an RfD. USEPA maintains and updates RfDs in the Integrated Risk Information System, which is administered by the National Center for Environmental Assessment[97]. USEPA first considers whether the appropriate toxicological data are available. Then, uncertainty factors are applied to the data as needed to account for data lacking in the toxicological literature[97,99].

RfDs are typically derived to protect against chronic non-cancer health effects. RfDs provide a quantitative estimate for health effects of critical concern. The RfD (typically expressed in units of milligrams of chemical per kilogram of body weight per

day, or mg/kg/d) is defined by USEPA as an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without a significant risk of adverse effects during a chronic period of exposure. An RfD can be derived from a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL), with uncertainty factors applied to reflect limitations of the data used[97].

The development of safe toxicity values for use in risk assessment can be traced back to the National Research Council's 1983 "red book" that originally outlined the procedures for risk assessment[98]. Chemicals can cause either cancer or non-cancer effects. There is a separate process for deriving a safe level for carcinogens. Based on a review of the literature, there are no studies evaluating the carcinogenicity of DA[8]. Because no carcinogenicity data are available, the action level for DA is based solely noncancer effects. An RfD is derived using the following equation:

RfD = NOAEL / (UF/MF)

Where:

RfD = Reference Dose

NOAEL = No Observed Adverse Effect Level

UF/MF = Uncertainty Factor or Modifying Factor

Chemicals that cause systemic (noncancer) effects rather than cancer are referred to as "systemic toxicants" because they affect the function of one or more organ systems[99]. Systemic toxicity is assumed to have a threshold dose below which no effects are expected to occur[98]. For systemic effects, there is in theory a range of doses between zero and some finite level that can be tolerated by an individual with essentially no

chance of the toxic effect. This is because the body has homeostatic, compensating, and adaptive mechanisms that must be overwhelmed before a toxic effect occurs[99]. It is important however, that all effects be considered (chronic rather than just acute) and that sensitive individuals (either due to greater exposure or greater susceptibility due to pre-existing conditions) be protected. Although a toxicant's main target site is inhibited at a particular concentration, (based on studies in laboratory animals), there may be other target organs with chronic or secondary acute effects at lower concentrations for which data are not available. It would be impractical, if not impossible, to test all possible endpoints, and therefore the lowest safe dose for all possible significant effects cannot be determined in most cases. Since it is necessary to make regulatory decisions about protecting the public from toxic chemicals, the current risk assessment process with uncertainty factors was developed.

Application of Uncertainty Factors. A reference dose (RfD) is defined as the amount of chemical to which an individual can be exposed on a daily basis over a chronic exposure period without adverse effects[99]. It is based on a no-observed-adverse-effect level (NOAEL) if available. An NOAEL is an experimentally determined dose at which there is no statistically significant effect that would be considered biologically significant[97]. NOAELs may be identified for several toxic endpoints and several NOAELs may be identified from different studies for a particular endpoint. Regulatory values are typically based on the highest NOAEL for the most sensitive endpoint[99]. If an NOAEL cannot be identified for a toxic endpoint due to insufficient experimental data, a lowest-observed-adverse-effect level (LOAEL) is identified and divided by an uncertainty factor to estimate a NOAEL:

NOAEL = LOAEL (experimental dose) / Uncertainty Factor

Once the studies and their endpoints are evaluated, the NOAEL is selected based on an examination of the data. An RfD will then be derived by dividing the appropriate NOAEL by an uncertainty factor:

RfD (human dose) = NOAEL (experimental dose) / Uncertainty Factor(s)

Table 2-1 presents uncertainty factors and modifying factors used by the United States Environmental Protection Agency (USEPA) in deriving an RfD.

Table 2-1

Uncertainty and Modifying Factors Used in Deriving a Reference Dose

Factor Type (EPA Designation)	**Factor Value**	**Purpose**
Uncertainty, intraspecies extrapolation (10H)	10	Account for sensitivity within the human population
Uncertainty, interspecies extrapolation (10A)	10	Account for extrapolation from test animals to humans
Uncertainty, acute to chronic (10S)	10	Account for differences from acute or subchronic experiments to chronic exposure
Uncertainty (10L)	10	Account for differences between LOAEL and NOAEL when no NOAEL is available
Modifying (MF)	1-10	Account for scientific uncertainties of the study and database not explicitly included in the uncertainty factors

Summarized from EPA 1993[99]

Uncertainty factors are usually a value of 10, with each factor representing a specific uncertainty in deriving an RfD. For many chemicals a factor of ten is applied to account for differences in responses between humans and experimental animals (interspecies extrapolation) and a second factor of ten is applied to account for susceptibility of sensitive individuals in the human population (intraspecies sensitivity)[99]. For chemicals with a less complete database (e.g., if only acute or subchronic data are available), a third

factor of ten is applied. EPA sometimes applies an uncertainty factor of three instead of ten and generally does not apply a total uncertainty factor of more than 3,000. Uncertainty factors can modified when case-specific information warrants it based on scientific judgment[100]. A recent paper in the scientific literature concluded that it is difficult to assess whether uncertainty factors overestimate or underestimate the sensitivity differences in human populations and that uncertainty factors continue to be widely utilized by government agencies of many countries[101].

There are a number of concerns with the RfD approach. The focus on a single NOAEL number ignores the shape of the dose-response curve. As scientific knowledge increases, and precursor effects such as enzyme induction become known, it raises questions about what is considered an "adverse effect". Guidelines have not been developed to account for the number of animals used, and some (larger) studies are more reliable than others. Despite these uncertainties, it is the widely used regulatory approach to protect against non-cancer effects due to a lack of a better alternative. The benchmark dose approach has been developed as an alternative to overcome some of these shortcomings. The benchmark dose approach provides a more quantitative alternative to the first step in the dose-response assessment than the current NOAEL/LOAEL process for noncancer health effects, and is similar to that for determining the point of departure proposed for cancer endpoints[96]. It considers the mode of action and whether the effects of concern are likely to be linear or nonlinear at low doses. The next section will examine the toxicological basis of the current RfD for DA and how uncertainty factors were applied to the current RfD.

Current Reference Dose. The RfD for DA was derived immediately after the 1987 outbreak and was never updated. While the deductive work identifying the outbreak and isolating the responsible contaminant was complex and impressive, the RfD dose derived from the 1987 outbreak was relatively simplistic. One hundred and seven people were known to have become ill from consuming DA in blue mussels during the 1987 outbreak. Consumption data were reconstructed for nine of the patients. The concentrations of DA ranged from 31 to 128 mg DA/100 grams of shellfish for these patients. Of these patients, only one of six who consumed between 60 and 110 milligrams of DA showed memory loss and none required hospitalization. All three patients who had consumed between 270 and 290 milligrams of DA suffered neurological symptoms and were hospitalized. One person who consumed 20 mg DA did not become ill. Based on the dose-response relation of this data, it was believed that there was a dose-related increase in severity of symptoms observed in patients consuming between 1 mg/kg and 5 mg/kg, and 1 mg/kg was estimated to be the lowest observed adverse effect level (LOAEL) for acute observable toxicity[8]. No acute effects were observed in one individual estimated to consume 0.33 mg DA/kg body weight. The use of a study on only nine patients (of the more than 150 who were part of the outbreak) introduces significant uncertainty into the RfD.

To account for intra-species susceptibility, and to account for the fact that this was a lowest-observed-effect-level (LOAEL) rather than a no-observed-effect-level (NOAEL), the 1 mg/kg value was divided by a safety factor of 10 to derive an acute RfD of 0.1 mg/kg. Therefore a single factor of uncertainty factor of 10 was used to account for (1) intra-species susceptibility and (2) converting an LOAEL to an NOAEL. EPA

would use a factor of 10 for each of these, for a total factor of 100 (Table 2-1). Data were considered insufficient to derive a chronic RfD[8]. EPA would typically apply an additional factor of 10 to convert from an acute value to a chronic value (Table 2-1). Overall, a chronic RfD derived using the same toxicity data and EPA uncertainty factors would likely be 100 times lower than the current acute value.

FAO/WHO/IOC Action Level. The RfD is used in conjunction with an estimate of consumption to derive an action level that is considered a safe concentration in seafood. The FAO/WHO/IOC adopted the 1987 Canadian action level for the consumption of shellfish. Consumption of other types of seafood were not explicitly considered. The action level by FAO/WHO/IOC was calculated assuming a single meal exposure. The action level incorporated a large meal size to be protective of an acute exposure. An action level in seafood (in units of milligrams of chemical per kilogram of seafood) was obtained through the following equation:

Action Level = (RfD x BW)/CR

Where:

Action Level	=	mg of DA per kg of seafood
RfD	=	Reference Dose (mg/kg)
BW	=	Body Weight (kg)
CR	=	Single Meal Size (kg)

FAO/WHO/IOC (relying on Canada's original calculation after the 1987 outbreak) calculated an action level of 24 mg/kg using an RfD of 0.1 mg/kg, a body weight of 60 kg, and a meal size of 0.25 kg. FAO/WHO/IOC concluded that a meal size of 250 grams would cover the shellfish meal size for 97.5% of shellfish consumers of most countries

for which data was available[8]. This value was then rounded down to 20 mg/kg. FAO/WHO/IOC pointed out that a meal size of 0.3 kg of seafood would yield an action level of 20 mg/kg[8]. The potential concerns with this action level are (1) protection against chronic effects of low doses, (2) protection of sensitive subpopulations, and (3) protection of frequent consumers of seafood.

Chronic Effects at Low Doses

The primary concern with the current action level is that the acute RfD that forms its basis may not protect against the chronic effects of low doses. Scientific understanding of the chronic effects of low dose exposure to DA has improved greatly in the twenty-five years since the current RfD was derived. First, the molecular mechanism of action of DA binding with neurotransmitter receptors that results in excitoxocity and neuronal cell death has been more clearly established. Many areas of the central nervous system vulnerable to these effects have been identified. Loss of neurons has been linked to structural effects in the central nervous system and subsequent functional effects have been determined. This section discusses evidence in the scientific literature for the mechanism of action, as well as evidence for chronic effects at low doses in humans, laboratory animals, and marine mammals. Human data include:

- Chronic effects from acute exposure; and
- An ongoing epidemiological study.

Laboratory animal data include:

- Chronic effects in adult animals;

- Behavioral effects from early life exposures, including both *in utero* and postnatal exposures; and
- Central nervous system structural changes from postnatal exposures;

Marine mammal and bird data include:

- Acute effects; and
- Chronic effects to behavior, the brain, the heart, and reproduction.

Finally, links to DA and the chronic illnesses epilepsy and schizophrenia are discussed.

Mechanism of Action. It is important to understand the mechanism of action before examining the toxicological literature on chronic effects of low dose exposure. Information on the mode of action for DA was extremely limited when the RfD was derived in the aftermath of the 1987 outbreak. A number of published studies have been conducted in the interim that have improved the knowledge on both the molecular mode of action and the organs targeted. These developments are discussed below

Mode of Action. This section provides a brief summary of the current understanding of DA's mode of action. The mode of action for DA is better understood now than during the 1987 outbreak, but research is ongoing. DA is a water soluble tricarboxylic acid with neurotoxic properties. Its potential for toxicity is somewhat mitigated by its toxicokinetics. DA's absorption from the gastrointestinal tract is low, its penetration of the blood-brain barrier is low, and it has a short half-life in the body due to rapid removal by the kidneys[102]. The mechanism of toxicity for DA is excitoxicity by excess activation of the glutamate receptors in neurons, which causes neuron damage and cell death[103].

The two types of glutamate receptors (GluRs) in mammals are ionotropic glutamate receptors (iGluRs) and metatropic glutamate receptors (mGluRs). Domoic acid affects the iGluRs, which form an ion channel that can open or close based on a neurotransmitter. iGluRs include three families of receptors, N-methyl-D-aspartate (NDMA) receptors, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, and kainate acid (KA) receptors. KA receptors include five different types of receptors, including GluR5 and GluR6. DA has a higher affinity for binding with KA receptors than other iGluRs and binds to GluR5 and GluR6 with particularly high affinity. Therefore, much of the toxicological literature has focused on KA receptors.

Toxicity is initiated when DA binds with an iGluR. Glutamate is rapidly removed from iGluRs but DA is not. The five-sided structure of DA makes it less flexible than glutamate, causing it to bind more tightly, resulting in a 30-100 times more powerful effect per molecule than glutamate[89, 104]. iGluRs are ion-gated channels selective to Na^+, K^+, and Ca^{2+}. Stimulation results in cellular influx of extracellular Na^+, Cl^-, and associated water through osmosis. This prolonged binding by DA over-stimulates the neuron, until the neuron swells with water and bursts, killing the neuron[104]. iGluRs are not evenly distributed in the central nervous system, but instead are concentrated in certain areas. This causes neurotoxicity to be particularly significant for these areas, and is reflected in the scientific literature.

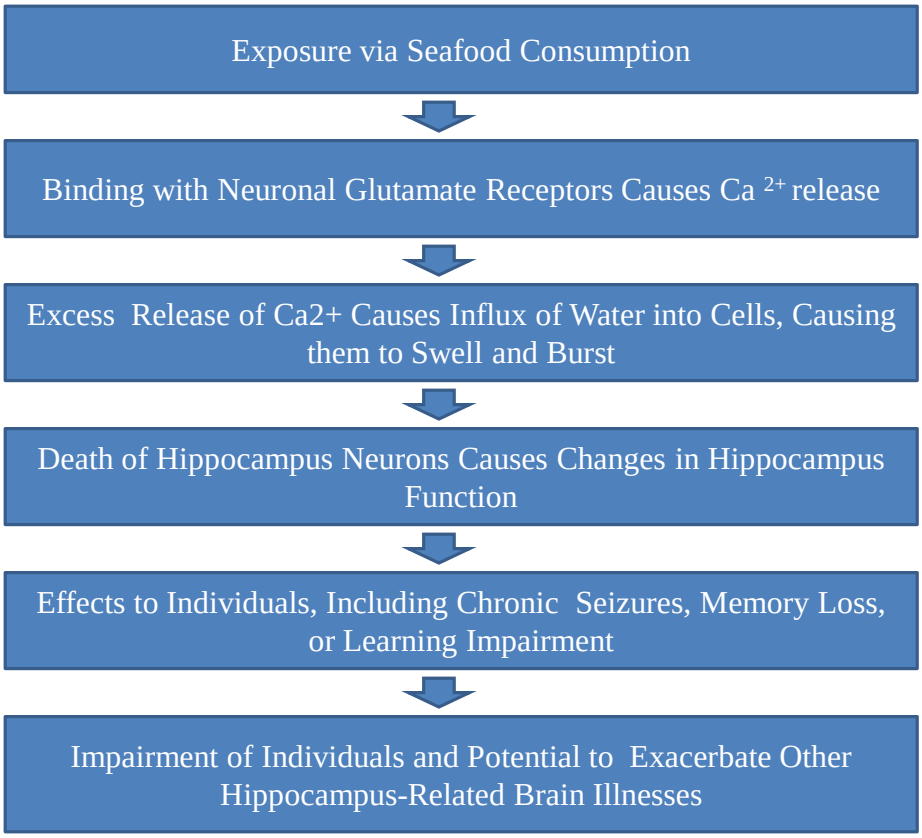
Primary Target Organs. The primary target organs for DA include the hippocampus, thalamus, olfactory bulb, spinal cord, and heart. Neurons with GluR5 and GLuR6 receptors are highly concentrated in the hippocampus, a part of the brain

associated with processing and saving new memories[105]. GluR5 and GLuR6 are present throughout the hippocampus but are most heavily concentrated in the CA 3 region (the middle of the hippocampus), which is where the greatest damage is observed[7]. Other parts of the body that have high numbers of GluR5 and GluR6 are the thalamus, olfactory bulb, heart and spinal cord, and studies have demonstrated effects to these areas[28, 10613]. The literature on the chronic effects of low level exposure to these target organs is detailed later in this chapter.

Adverse Outcome Pathway. An adverse outcome pathway is a conceptual model that depicts existing knowledge about the links between a molecular-level initiating event and adverse outcomes at a higher level of biological organization relevant to risk assessment[107]. An adverse outcome pathway for ecological effects of DA has been published in the scientific literature[14]. A human health adverse outcome pathway is depicted in Figure 2-1:

Figure 2-1

Adverse Outcome Pathway for Domoic Acid



Environmental exposures to DA in seafood can trigger a series of events on a molecular level that cause neuronal cell death, loss of hippocampus function, and result in serious impacts to memory and learning. Figure 2-1 depicts the adverse outcome pathway for the primary target organ, the hippocampus. Adverse outcome pathways could also be developed for other target organs. This figure provides context for understanding the studies in humans, laboratory animals, and marine mammals in the sections that follow.

Human Data for Chronic Effects. The available human data for DA toxicity are currently quite limited. Human data are preferred when developing references doses because it eliminates the need for interspecies extrapolations. Therefore an examination of the human data is a logical first step in examining the toxicological literature on chronic effects of low level exposure. There has been a lone documented outbreak of acute DA poisoning (i.e., amnesic shellfish poisoning) in humans, a 1987 occurrence in Eastern Canada. The available human data are from this outbreak and from initial findings of an epidemiological study being conducted in a Native American tribe in the Northwestern United States. The human data are summarized in Table 2-2:

Table 2-2

Effects in Humans from Environmental Exposures

Test Subject/ Receptor	Exposure Route	Dose Range	Duration	Effects	Concentration (Endpoint)	Reference
107 humans	Mussel ingestion	Not Reported	Single exposure	Ranged from mild symptoms of abdominal cramps and dizziness to seizures, memory loss, and death. Hippocampus neuron death in autopsied fatal cases	Not reported	Perl et al. 1990[108]
9 Humans	Mussel ingestion	1-5 mg/kg (estimated)	Single exposure	Neurological effects	1 mg/kg (LOAEL)	Toyofuku 2006[8]
1 Human	Mussel ingestion	Not reported	Single exposure	Temporal lobe epilepsy	Not reported	Cendes 1995[109]
735 Native Americans	Seafood ingestion	Not Reported	Chronic	Initial findings of lowered mental development indices	Not reported	Grattan 2011[110]

During the 1987 ASP outbreak, 107 people (47 men and 60 women) met the symptomatic definition of amnesic shellfish poisoning and an additional 38 were considered probable cases[21]. Neurological tests were performed on fourteen of the most severe cases and twelve of fourteen exhibited impairment of anterograde memory (impaired memory for events that occurred after exposure). Four patients died of their symptoms within 100 days of exposure and the fifth died of related symptoms three years after exposure. Autopsy of deceased individuals showed brain damage characterized by neuronal necrosis particularly in the hippocampus. Lesions were observed in a number of areas in the brain including the claustrum, secondary olfactory areas, the septal area, and the nucleus accumbens[108]. The claustrum is a thin, irregular, sheet-like neuronal structure hidden beneath the inner surface of the neocortex whose function is largely unknown[111]. The nucleus accumbens is adjacent to the hypothalamus and plays an important role in pleasure, reinforcement learning, fear, aggression, impulsiveness, and addiction[112]. The primary effect of DA is to the hippocampus, a structure important in memory[21] and to the olfactory bulb[13]. The thalamus and subfrontal cortex were damaged in some individuals[21]. These effects were observed with individuals who had appeared to consume larger portions of seafood in the 1987 Canadian outbreak (exact exposures could not be determined for most patients)[21]. The data for this outbreak are important because they represent (1) the only known outbreak of the acute illness amnesic shellfish poisoning and (2) the basis of the current action level.

A single study was found in the literature that performed follow up on the 1987 outbreak and this follow up was for a single patient, an 84-year old individual[7]. During the acute illness, electroencephalograms (measures of brain electrical activity that can

document seizures) initially showed periodic epileptic abnormalities. Eight months after the intoxication, the electroencephalogram was normal. However, one year after the acute exposure, complex partial seizures developed. Electroencephalograms showed epileptic discharges independently over both temporal lobes, with left-sided predominance. Magnetic resonance imaging revealed atrophy of the hippocampus[7]. Although the exposure was acute, the effects to this individual were long-term and permanent. The effects to this individual are strikingly similar to chronic effects in both laboratory animals and marine mammals from coastal systems (discussed in the section below). These chronic effects in marine mammals were recently dubbed "domoic acid epileptic disease" in the literature[13].

Ideally chronic data for a larger human population would be used as the basis of a chronic RfD. A five-year epidemiological study of a Native American tribe in Washington State is currently being conducted but information on the study is limited. The purpose of this five-year ongoing longitudinal cohort study of 625 Native Americans is to determine the incidence and severity of DA-related illness and to identify both exposure and host factors associated with the occurrence of illness, including the effects of repeat low level exposure[113]. Initial data suggests that infants born in years when DA levels in coastal razor clams were above the FDA action level had lower mental development indices than infants born in other years[104]. Members of the tribe consume seafood at high rates, particularly geoducks, a large saltwater clam known to accumulate DA. Concentrations of DA greater than 300 mg/kg in geoducks were found in harvesting areas of several subsistence level Native American Tribes in the Pacific Northwest within the past four years, far in excess of the 20 mg/kg action level[104]. Future results of this

study may provide insights into the effects of chronic low dose exposure in humans and potentially provide the basis of a chronic reference dose.

Laboratory Animal Data for Chronic Effects. Since adequate chronic human data are not currently available, animal data are discussed in this section to evaluate their potential utility in deriving a reference dose for chronic effects of low dose exposure. While studies in laboratory animals have demonstrated long term effects in mammals from early life exposure during a critical brain developmental window, the duration of these exposures has been acute. Laboratory data are summarized for adult, neonatal, and *in utero* rats.

Effects to Adult Laboratory Animals. The evaluation of laboratory data begins with an examination of the studies on effects to adult laboratory mammals and zebrafish. The studies on observed effects in adult animals are summarized below.

Table 2-3

Effects in Adult Animals

Test Species	Exposure Route	Dose Range	Duration	Effects	Concentration (Endpoint)	Reference
Rat (*Rattus norvegicus*)	injection	1 mg/kg	Three exposures spaced one week apart	Behavioral effects, but no neuronal damage	1mg/kg	Schwartz 2014[114]
Rat (*Rattus norvegicus*)	Interperiton eal injection	1-7.5 mg/kg	Single exposure	Behavioral effects	1 mg/kg	Tryphonas et al. 1990a[115]
Cynomolgus monkeys (*M. facsicularus*)	Intravenous injection	4 mg/kg	Single exposure	Behavioral effects and lesions of the hippocampus	4 mg/kg	Tryphonas et al. 1990b[116]
Cynomolgus monkeys (*M. facsicularus*)	Intravenous injection	0.025 0.5 mg/kg	Single exposure	Behavioral effects and excitotoxic lesions of the hippocampus	0.025 mg/kg LOAEL behavioral changes 0.5 mg/kg LOAEL lesions of the hippocampus	Tryphonas et al. 1990b[116]
Rat (*Rattus norvegicus*)	Oral gavage	1-15 mg/kg/d	Daily exposure on days 10-17 of gestation	Mortality, neurotoxicity, accumulation in fetal brains	1 mg/kg/d LOAEL fetal brain accumulation 5 mg/kg/d LOAEL neurotoxicity 15 mg/kg/d LOAEL mortality	Tryphonas et al. 1990a[115]
Zebra fish (*Danio rerio*)	Injection	0.31 mg/kg for six weeks, then 0.18 mg/kg	Once a week for six weeks, then every two weeks for 24 weeks	Neurologic sensitivity	Only one dose tested	Tiedeken et al. 2005[132]

Data are limited for chronic effects to adults from exposure as adults. Many studies have been conducted on effects to adult rats that occur as a result of exposure during a critical neurodevelopmental window in juvenile animals. These studies are discussed in the section on neonatal rats. All but one of the studies conducted in adult animals was via the injection route. A single sub-convulsive injected dose of DA (single 1 mg/kg dose) affected short and long-term the behavior of rats without inducing neuronal damage[114]. Rats are around 20 to 40 times less sensitive than humans to DA administered orally (due to their poor DA gastro-intestinal absorption and faster elimination), which is why most studies are conducted via inter-peritoneal injection[114]. Among the injection studies, LOAELs were 0.025 mg/kg for behavioral changes and 0.05 mg/kg for hippocampus structural changes in monkeys. The limited primate data indicate that monkeys are more sensitive than rodents[129]. Since the available studies on neonatal and *in utero* laboratory animals are in rodents, there is the potential that the rodent data for these groups may in fact underestimate the potential toxicity in humans. For the intraperitoneal route of exposure in rats, there is general agreement that acute effects are observed in adults in the range of 1-4 mg/kg. The only oral study in the literature found somewhat higher LOAELs than interperitoneal injection studies, with an NOAEL for neurotoxicity of 5 mg/kg/d[115]. While the adult data are somewhat limited they do indicate behavioral and hippocampus structural changes at relatively low concentrations, with effects occurring at lower concentrations in monkeys than rats.

Persisting Effects from Early Life Exposure. This section examines the current evidence for persisting effects of early life exposure to DA. It is critical to evaluate data for the developing fetus and neonate because they can often be more sensitive to toxic

chemicals than an adult[117]. This is particularly important for excitotoxic chemicals because the immature synapses of the developing brain make it vulnerable to excitotoxicity[118]. There is a significant body of animal data for potential long-term effects of DA from early life stage exposure. Exposures during a critical developmental window cause long-term structural changes to the brain that manifest themselves in a number of behavioral issues including increased aggression and learning deficits. All currently published studies about early life stage exposure are via the interperitoneal injection route of exposure. These studies are discussed below.

Behavioral Effects from In Utero Exposures. In utero exposures are exposures that occur in the mammalian fetus while inside the mother's womb. In order for these exposures to occur, a chemical must be capable of crossing the placenta. Only two studies were identified that evaluated *in utero* exposures to DA. Table 2-4 summarizes the behavioral effects of *in utero* exposures.

Table 2-4

Behavioral Effects from *In Utero* Exposures

Test Subject/ Receptor	Exposure Route	Dose Range	Duration	Effects	Concentration (endpoint)	Reference
Rat (*Rattus norvegicus*)	Subcutaneous injection in pregnant rats	0.3 – 1.2 mg/kg	Once, prenatal day 13	Behavioral and learning effects	1.2 mg/kg (LOAEL)	Levin et al. 2005[119]
Mice, (C57BL/6)	Interperitoneal injection	1 mg/kg	Injections on either one of three gestational days(gestational day 11.5, 14.5, or17.5) or on all three days	Behavioral effects and severe impairment of learning	1 mg/kg when injected on days 14.5 or 17.5	Tanemura et al 2009[120]

The purpose of these studies was to examine the potential for behavioral effects in juvenile rodents from exposures that occurred while they were *in utero*. Pregnant rodents were exposed to DA via injection that resulted in fetal exposure through the placenta. In both studies, concentrations were selected that did not result in overt toxicity to the mothers.

Significant behavioral effects were observed in both *in utero* studies. Behavioral tests in young 11 weeks after birth indicated severe impairment of learning and memory and anxiety-related disorders and corresponding structural effects in the brains of young rats whose mothers were exposed to 1.2 mg/kg[119]. The rat study[119] investigated a range of doses for a single gestational day, while the mouse study[120] used a single dose, but a range of gestational days. Taken together, these studies indicate that DA is capable of crossing the placenta and causing neurological effects to the fetus that are manifested as behavioral effects later in life. Prenatal exposures to relatively low levels of DA in rats and mice resulted in a spectrum of neurobehavioral issues in adults. Both studies showed severe effects to memory and learning from prenatal exposures, but the study in mice found anxiety-related disorders. The mouse study also complemented behavioral results with findings of alternation in brain structure. These two studies showed collectively that the timing of DA exposure is critical, with progressively greater effects at later gestational days. The studies also demonstrated that even when no maternal toxicity is observed, significant behavioral effects can be observed in adult offspring. This is a significant finding for human risk, indicating that there is the possibility that a pregnant woman could consume DA-contaminated seafood with no apparent ill effects but pass

that DA on to her fetus through the placenta. This could result in the potential for future effects to her child in adulthood.

Behavioral Effects from Post-Natal Exposures. Behavioral effects from post-natal exposures are summarized in Table 2-5.

Table 2-5

Behavioral Effects From Postnatal Exposures

Test Subject/ Receptor	Exposure Route	Dose Range	Duration	Effects	Concentration (Endpoint)	Reference
Rat (*Rattus norvegicus*)	Interperitoneal Injection	Not reported	Single injection, postnatal day 2 or 10	mortality	0.25 mg/kg (LD_{50}) PND2 0.7 mg/kg PND10	Xi et al 1996[121]
				Behavioral effects	0.05 mg/kg	
				Seizures	0.2 mg/kg	
Rat (*Rattus norvegicus*)	Interperitoneal Injection	0.025-1 mg/kg	Twice/day postnatal days 1-2	mortality	0.1 mg/kg (LOAEC)	Levin et al 2006[122]
				Modest hypoactivity in Figure8 maze	0.05 mg/kg (LOAEC)	Levin et al 2006[122]
Rat (*Rattus norvegicus*)	Subcutaneous injection	0.05-0.3 mg/kg	Single dose, postnatal day 0. 5, 8, 14, or 22	"Behavioral effects"	0.08 mg/kg (ED_{50})	Doucette et al 2000[123]
Rat (*Rattus norvegicus*)	Subcutaneous injections	5-20 ug/kg	Daily, postnatal days 8-14	Longer time to eye opening, greater pleasure seeking behavior	20 ug/kg (LOAEC)	Doucette et al 2003[11]
				Seizures when exposed to tests of spatial cognition as adults	5 ug/kg in males (LOAEC) 20 ug/kg in females	Doucette et al. 2004[10]

Table 2-5

Behavioral Effects From Postnatal Exposures

Test Subject/ Receptor	Exposure Route	Dose Range	Duration	Effects	Concentration (Endpoint)	Reference
Rat (*Rattus norvegicus*)	Subcutaneous injections	5-20 ug/kg	Daily, postnatal days 8-14	Mean escape latency by females in water maze as adults	20 ug/kg (LOAEC)	Doucette et al 2007[24]
Rat (*Rattus norvegicus*)	Subcutaneous injections	20 ug/kg	Daily, postnatal days 8-14	Behavioral effects in maze exploration and increased seeking of nicotine as adults	20 ug/kg (LOAEC)	Burt et al. 2008[124]
Rat (*Rattus norvegicus*)	Subcutaneous injections	20 ug/kg	Daily, postnatal days 8-14	Temporal memory dysfunction as adults	20 ug/kg (LOAEC)	Robbins et al. 2013[125]
Rat (*Rattus norvegicus*)	Subcutaneous injections	20 ug/kg	Daily, postnatal days 8-14	Increased startle response at 90 days	20 ug/kg (LOAEC)	Adams et al. 2008[126]
				Decreased water maze performance at 75 days	20 ug/kg (LOAEC)	Adams et al. 2009[127]
				Seizures during exposure to water maze	20 ug/kg (LOAEC)	Perry et al 2009[128]
				Lowered seizure threshold at 160 days	20 ug/kg (LOAEC)	Gill et al. 2010[129]
				Behavioral and molecular indicators of stress	20 ug/kg (LOAEC)	Gill et al. 2012[130]
Rat (*Rattus norvegicus*)	Intraperitoneal injection	1 mg/kg	Single injection on postnatal day 40	Aggressive behavior and seizures during twelve weeks of monitoring	1 mg/kg (LOAEC)	Maucher Fuquay et al 2012[131]

Table 2-5

Behavioral Effects From Postnatal Exposures

Test Subject/ Receptor	Exposure Route	Dose Range	Duration	Effects	Concentration (Endpoint)	Reference
Rat (*Rattus norvegicus*)	Subcutaneous injection	0.10-0.50 mg/kg	Single injection on postnatal day 7	Motor seizures	0.10 mg/kg (LOAEC)	Wang et al. 2000[106]
				Hind limb paralysis	0.33 mg/kg	

All behavioral studies from postnatal exposure were conducted in rats. These studies were conducted to investigate the long-term effects of postnatal exposure to DA during what are believed to be critical time periods of brain development. The brain growth spurt, which lasts until about 2 weeks of age in the rat, is a period of great importance for assessing potential developmental neurotoxins, because the developing nervous system is more sensitive to neurotoxins than the adult nervous system[24]. While doses as high as 0.1 mg/kg resulted in complete mortality in young rats[122], doses as low as 5 ug/kg were found to cause behavioral effects, such differences in eye opening, conditioned place preference and activity levels[11]. Even more significant, rats that are exposed to these low levels early in development, have long lasting developmental effects such as seizures when tested with tasks involving spatial cognition[11] or reduced learning abilities exhibited in water maze experiments (females only[24]). Autopsy results from one of these studies[11] revealed necrosis within the hippocampus and the presence of "mossy fibers" providing physical evidence for the observed behavioral abnormalities. Neonates accumulated high circulating levels of DA and this paralleled their high susceptibility to the toxin[121].

The data in these studies reflected the fact that exposure to DA during the first two weeks of life represents the most sensitive period for neurotoxicity. Neonatal rats are up to 40 times more susceptible to the effects of DA than adults and DA induced reproducible behavioral effects at doses as low as 0.05 mg/kg and induced seizures at doses as low as 0.2 mg/kg via intraperitoneal injection[95, 96, 119, 121]. Levin[122] found that while DA caused significant neurobehavioral toxicity from prenatal exposure, this toxicity was more severe when DA was administered to neonatal rats. The evidence is compelling that the first two weeks of neonatal life is the most sensitive time for rat

exposure to DA. Doucette[123] found a consistent increase in toxicity DA twice as potent in postnatal day 8 rats compared with postnatal day 14 rats. The effective dose for behavioral effects in 50 percent of the test population (ED_{50}) steadily increased through postnatal day 0 (ED_{50} = 0.12 mg/kg), 5 (ED_{50}=0.15 mg/kg), 14 (ED_{50}=0.30), and 22 (ED_{50}=1.06 mg/kg), indicating decreasing toxicity with increased age. Even rats exposed at 22 days postnatal were twice as sensitive compared with studies of adult rats reported in the literature[116]. The studies collectively indicated that the first two weeks of neonatal life are the critical window in behavioral toxicity.

Effects were not limited to neonatal rats, although they appear most sensitive. Older rats could also be induced to show behavioral effects. One study found that among treated forty-day old juvenile rats, ninety-two percent exhibited aggressive behavior and 50 percent exhibited seizures in twelve weeks of monitoring following injections[131]. DA-treated rats showed more aggression over a wider range of time than control rats.

Effects to zebrafish were similar to effects in rats. Fifty percent of embryos treated with 1.2 mg/kg DA displayed convulsions at 2 days post fertilization. Four days post-fertilization, all embryos treated with 4.0 mg/kg DA and higher showed no touch-response reflexes[132].

The studies above collectively indicate significant behavioral effects to memory and learning from postnatal exposure. Behavioral changes in laboratory animals have been accompanied by structural changes in the brain. Numerous chronic behavioral effects have been documented from exposure to low doses of DA. A number of structural effects to the nervous system have also been reported, and these are discussed below.

Structural Changes in Developing Animal Brains. Structural changes resulting from postnatal exposures include effects to the hippocampus, thalamus, olfactory bulb, spinal column, and heart. These studies are summarized in Table 2-6 below:

Table 2-6

Structural Changes From Postnatal Exposures

Test Subject/ Receptor	Exposure Route	Dose Range	Duration	Effects	Concentration (Endpoint)	Reference
Rat (*Rattus norvegicus*)	Interperitoneal Injection	20 ug/kg	Daily, postnatal days 8-14	Mossy fiber sprouting in the hippocampus	20 ug/kg (LOAEC)	Bernard et al. 2007[133]
Rat (*Rattus norvegicus*)	*in vitro*	2 uM exposure to hippocampi of sacrificed 5-6 day old rats	In vitro exposure	Neuronal cell death	2 uM	105 Perez-Gomez et al. 2012[134]
				Abnormal proliferation of cells		105 Perez-Gomez et al. 2012[134]
				Mossy fiber sprouting		106 Perez-Gomez et al. 2014[135]
Rat (*Rattus norvegicus*)	injection	5-20 ug/kg	daily, postnatal days 8-14	Mossy fiber sprouting	20 ug/kg[1]	59 Doucette et al. 2004[10]
				Reduction in hippocampus cell counts		
Rat (*Rattus norvegicus*)	Subcutaneous injections	0.10-0.50 mg/kg, 7-day old rats	Single injection	Spinal cord lesions	0.33 mg/kg LOAEL	Wang et al. 2000[106]

Table 2-6

Structural Changes From Postnatal Exposures

Test Subject/ Receptor	Exposure Route	Dose Range	Duration	Effects	Concentration (Endpoint)	Reference
Rat (*Rattus norvegicus*)	Interperitoneal injections	Single injection, 7-week old rats	1 mg/kg hourly until seizures were induced	Damage to olfactory pathways (animals sacrificed after 7 days)	1 mg/kg	Tiedeken et al. 2013[136, 137]
Rat (*Rattus norvegicus*)	*in vitro*	200 uM	Single exposure	Reduction in ATP-induced elevation in calcium concentrations	200 uM	Nijjar et al. 1999[138]
Rat (*Rattus norvegicus*)	In vitro exposure	0.05-0.25 uM	Single exposure	Marked concentration dependent impairment in activity and integrity of cardiac mitochondria	0.05 uM	Vranyac-Tramoundanas et al. 2008[139]

Table 2-6

Structural Changes From Postnatal Exposures

Test Subject/ Receptor	Exposure Route	Dose Range	Duration	Effects	Concentration (Endpoint)	Reference
Rat (*Rattus norvegicus*)	Intraperitoneal injection	0.25-2.0 mg/kg	Daily, pregnancy days 1-16	Day 22 of pregnancy	0.25 mg/kg NOAEL Decrease in number of live fetuses at term, dose-dependent increase in number of fetuses with visceral or skeletal abnormalities	Khera et al. 1994[140]

[1]It was unclear from the study if structural effects occurred at both 5 and 20 ug/kg

Hippocampus Effects. Three of the studies listed above evaluated the effects of DA to the hippocampus in neonatal exposures[10, 133, 141]. All three studies observed mossy fiber sprouting. Mossy fiber sprouting is a well-established structural effect within the hippocampus found in humans with epilepsy or head trauma[142143]. One of the studies was conducted *in vitro* (in a laboratory vessel), while the remaining two studies were conducted *in vivo*. Both *in vivo* studies exposed rats through daily injections on postnatal days 8-14. These studies were in agreement that the LOAEL for hippocampus effects is 20 mg/kg. DA exposures produced changes indicative of abnormal development and synaptic plasticity of the hippocampus[133]. One study showed that there may be some limited repair to abnormal cell proliferation in some low dose exposure cases, but it could also develop abnormal neural circuits that could be relevant to long term symptoms of disease in other low dose cases[134]. DA exposure resulted in permanent alterations in hippocampal structure and function, including abnormal formation of dentate granule cell axons projecting (i.e., mossy fiber sprouting, which is indicative of epileptic seizure damage)[105, 106, 134, 135]. The magnitude the mossy fiber sprouting was greatest in the mid portion (CA3 region) of the hippocampus. The CA3 region of the hippocampus plays an important role in the encoding of new spatial information within short-term memory [144]. These structural effects to the CA3 region are consistent with spatial memory and learning effects observed in behavioral studies[10, 145].

Olfactory Pathway and Brain Stem Effects. A study was conducted to examine the effects of DA to the olfactory bulb and brain stem[146]. Seven-week old rats were injected hourly until seizures were induced, and then treatment was stopped. Animals

were sacrificed and staining was performed to highlight damage to the olfactory bulb and brain stem. Structural damage in olfactory pathways was associated with levels of DA that induced epileptic seizures[146]. Animals that displayed aggressive behavior had additional neuronal damage to the anterior olfactory cortex[137]. Neuronal damage was also observed in the hippocampus and amygdala (an almond-shaped mass of neurons in the mid-brain believed to be involved in the experiencing of emotions[147]).[146] Most of the literature has identified damage to the hippocampus as the primary cause of long-term epileptic seizures observed after exposure to DA. However, one recent paper has posited that damage to the olfactory bulb and olfactory cortex could be the cause of long term epileptic seizures in exposed animals[13].

Spinal Cord Effects. Spinal cord effects were observed when DA was injected in an immediately adjacent area. Administration of DA in the dorsal neck region resulted in behavioral abnormalities that were due to spinal cord damage (based on observed lesions) rather than damage to the brain[106]. It is unknown if this effect would be significant if injection occurred further from the spinal cord. This study demonstrated that neuron damage from DA is not limited to effects in the brain.

Cardiac Effects. All three types of iGluRs exist in the heart (NMDA, AMPA, and KA receptors) and make the heart a potentially significant location for the effects of DA exposure[21]. In humans AMPA, KA [GluR5, GluR6, and GluR7] and NMDA subtypes of iGluRs showed "differential distribution in the working myocardium, wall of blood vessels, intramural ganglia, and specific components of the conducting system, providing evidence that the molecular targets for excitatory neurotransmission and neurotoxicity" of

DA are present in the human heart[21]. No studies of cardiac effects were conducted with laboratory animals, but cardiac arrhythmias and unstable blood pressure were observed in a number of patients during the 1987 amnesic shellfish poisoning outbreak[108, 148]. In California sea lions, DA-attributed cardiomyopathy varied from mild to severe with a hypothesized mechanism of action is binding with neuroreceptors in the heart[28]. Collectively, this represents compelling evidence for cardiac effects from DA.

Reproductive Effects. A single study was identified on the reproductive effects of DA in laboratory animals[140]. While effects were observed, most were not dose-dependent. An increase in the number of fetuses with visceral or skeletal anomalies was the only effect that was dose-dependent and statistically significant, but was considered an anomaly because effects were not observed in the 1.75 mg/kg dose group[140]. The evidence for reproductive effects is not strong, and additional data are needed to determine if these effects are linked to DA.

In summary, the scientific literature provides substantial evidence that DA causes significant behavioral and structural effects in laboratory animals. When studies in laboratory animals are examined collectively, behavioral effects such as seizures and deficits in memory and spatial learning are linked to damage to the hippocampus. The effects of DA occur primarily in the brain, but can potentially occur in any tissues with significant numbers of iGlurRs. Studies of neonatal rats provide compelling evidence the first two weeks of life represent the most sensitive life stage for DA exposure. The developing brain is particularly sensitive to neuronal damage and low level exposures can result in chronic behavioral effects that manifest themselves later in adulthood. The

biggest shortcoming in laboratory animal data for DA is the lack of a chronic oral exposure study to form the basis of a chronic reference dose.

Typically environmental exposures of chemicals to marine mammals are not considered when evaluating the toxicity of a chemical for humans. However, given the lack of chronic low dose oral exposure studies from the epidemiological literature or from laboratory animal data, combined with the striking effects in marine mammals from chronic exposures, an examination of the marine mammal data is important in illustrating the toxicity of DA. The next section evaluates these exposures.

Effects to Marine Mammals and Birds in the Wild. This section examines the toxicity of DA to marine mammals and birds. DA has been associated with strandings of marine mammals and mortality of seabirds and marine mammals off the California Coast[13]. DA toxicity in birds was first reported in brown pelicans off of Monterey, CA in 1991[149] and in a number of marine mammals along the Central California coast in 1998[27]. Effects to marine mammals have been documented as acute symptoms and as a chronic seizure syndrome. Studies of marine mammals have the advantage of considering actual environmental exposures to DA concentrations in seafood. These environmental exposures are integrated over time, incorporating bloom and non-bloom exposures. DA is persistently present in the bodies of marine mammals on the California coast[150]. These studies would not provide the basis of a reference dose because exposure levels are not quantified, but do provide important information on the effects of chronic exposure to DA in seafood and provide an indication of whether development of a chronic RfD

should be considered. Since these studies were not considered by FAO/WHO/IOC[8], acute and chronic effects are summarized in Table 2-7 below.

Table 2-7

Domoic Acid Effects in Marine Mammals and Birds

Receptor	Effects	Link to DA	Reference
Brown Pelican *Pelecanus occidentalis* and Brandt's Cormorants *Phalacrocorax penicillatus*	Mortality, hemorrhage and necrosis of skeletal muscle.	Viral, bacterial, and chemical hazards ruled out. Stomach contents contained DA, anchovies, and *Pseudo-nitzschia australis*	Work et al. 1993[149]
California seal *Phoca vitulina*	Behavioral effects such as head-rolling, ataxia, seizures and coma. Lesions in the central nervous system and heart.	Classic clinical signs of domoic acid (DA) toxicosis ranging from muscle twitches and ataxia, to seizures and coma. A receptor binding assay was used as a quick screen to identify DA exposure. DA present in 83% of fecal samples	Lefebvre et al. 2010[151]
California sea lion *Zalophus californianus*	Examination of 70 stranded animals showed ataxia and seizures. 69% mortality of stranded animals with clinical signs of DA toxicosis. Post mortem examination revealed cardiac lesions, severe neuronal necrosis in the hippocampus. Acute myofiber necrosis and edema of the heart	Animals exhibited classic signs of DA toxicosis and no signs of infectious disease or other illness. DA detected in serum in 3/7 animals and urine in 7/14 animals tested. *P. australis* bloom of up to 200,000 cells/L occurred during the strandings. Anchovies collected during the	Gulland et al. 2000[27]

Table 2-7

Domoic Acid Effects in Marine Mammals and Birds

Receptor	Effects	Link to DA	Reference
		peak of the bloom had 105 mg DA/kg tissue.	
Pacific harbor seal *Phoca vitulina richardii*	Disorientation, ataxia, and seizures in seals with DA toxicosis. Histopathology revealed hippocampus neuronal necrosis and myocardial necrosis. DA was detected in bodily fluids of both symptom-free animals and animals with DA toxicosis.	Biosense ELISA was used to detect DA in urine, feces, stomach contents, milk, amniotic fluid, fetal meconium, and fetal urine. 65% of urine samples from healthy seals tested positive for DA.	McHuron et al 2013[150]
715 California sea lion *Zalophus californianus*	Tremors, seizures, and mortality. Post mortem analysis showed histopathological changes in the hippocampus.	*Pseudo-nitzschia australis* blooms up to 1.3 x 10^5 cells/L, DA detected in planktivorous fish when 400 sea lions died in Monterey in May-June 1998. DA detected in urine, feces, and serum of animals.	Scholin et al. 2000[41]
Northern fur seal *Callorhinus ursinus*	Ataxia, seizures and coma. Histopathological findings included lesions in the central nervous system and heart, atrophy and extensive loss of granular cells in the hippocampus.	33 stranded seals had clinical or histopathological signs indicative of DA toxicosis. DA detected in 83% of fecal samples	Lefebvre et al. 2010[151]

Table 2-7

Domoic Acid Effects in Marine Mammals and Birds

Receptor	Effects	Link to DA	Reference
California seal *Phoca vitulina*	DA induced increased expression of markers of oxidative stress and glutamine synthetase (GS) redistribution leading to alterations of the glutamine-glutamate- gamma-aminobutyric acid cycle and contributing to the excitotoxicity and seizures	Toxicosis cases were linked to DA based on clinical history, microscopical lesions and DA levels in urine or feces.	Madl et al. 2014[152]
California sea lion *Zalophus californianus*	Clinical symptoms included seizures, ataxia, head weaving, decreased responsiveness to stimuli and scratching behavior. Animals had high hematocrits, and eosinophil counts, and high activities of serum creatine kinase.	265 Californian sea lions diagnosed with DA toxicosis based on clinical signs including seizures, ataxia, head-weaving, decreased responsiveness to stimuli and scratching behavior.	Gulland et al. 2002[12]
164 California sea lions *Zalophus californianus*	551 acute cases were characterized by clinical signs that included ataxia, head weaving, seizures or coma which varied in severity but were continuous during the period of toxicosis, lasting about one week followed by recovery, if treated, or death. They stranded in clusters and had histopathological findings that included hippocampal neuronal necrosis. Twenty-five percent of acute cases developed into chronic cases. 164 cases with chronic neurological disease had symptoms including seizures, lethargy, vomiting, muscular twitching, central blindness and abnormal behavior. Duration of clinical signs from	Criteria used to determine DA exposure in case animals included intermittent seizures (at least 2 weeks apart and/or at least 2 weeks following admission to The Marine Mammal Center), unusual behaviors, stranding individually (not in clusters during blooms of Pseudo-nitzschia algae, like acute DA-exposed animals),	Goldstein et al. 2008[25]

Table 2-7

Domoic Acid Effects in Marine Mammals and Birds

Receptor	Effects	Link to DA	Reference
	initial presentation to death varied from 25 to 1525 days. Seizures were observed in 140 of these cases at intervals varying from hours to weeks, often progressing from simple (not impairing the level of consciousness), partial (focal) to secondary (following a simple seizure) generalized (involving loss of consciousness) seizures. Chronic lesions affected the hippocampal formation and were accompanied by hippocampus atrophy. Cardiac lesions were documented in 102 cases (67 acute, 35 chronic neurological). The cardiomyopathy varied from mild to severe.	and/or hippocampal atrophy evident by MRI.	
California sea lions *Zalophus californianus*	All chronic DA sea lions exhibited significant hippocampus neuron loss (defined as two standard deviations below mean control sea lion values. The study tested whether unilateral neuron loss in chronic DA sea lions was similar to that in human patients with temporal lobe epilepsy. Hippocampal neuron loss is reported to occur in 63–91% of human patients (81%, average). In the present study 79% of sea lions had unilateral hippocampal neuron loss, which is similar to previous reports that used MRI to detect hippocampal atrophy in marine mammals impacted by DA. These findings suggest that unilateral hippocampal neuropathology is common in human patients with temporal lobe epilepsy and in chronic DA sea lions.	Criteria used to determine DA exposure in case animals included intermittent seizures (at least 2 weeks apart and/or at least 2 weeks following admission to The Marine Mammal Center), unusual behaviors, stranding individually (not in clusters during blooms of Pseudo-nitzschia algae, like acute DA-exposed animals),	Buckmaster et al.2014[153]

Table 2-7

Domoic Acid Effects in Marine Mammals and Birds

Receptor	Effects	Link to DA	Reference
		and/or hippocampal atrophy evident by MRI.	
California sea lions *Zalophus californianus*	Eight sea lions that were admitted for neurological effects from DA exposure were examined post mortem. Animals with the domoic acid-associated degenerative nonspecific gross findings, including a globally flaccid heart, multifocal-to-diffuse myocardial pallor, and mild serous pericardial effusion. Distribution of the cardiomyopathy was consistent among animals and had lesion morphology suggestive of an association with the apoptotic pathway. DA-associated degenerative cardiomyopathy affected animals of both sexes, of all age classes after the pup stage, and with either acute neurologic toxicity or chronic neurologic effects. The cardiomyopathy ranged from mild to severe and acute to chronic active	Criteria used to determine DA exposure in case animals included intermittent seizures (at least 2 weeks apart and/or at least 2 weeks following admission to The Marine Mammal Center), unusual behaviors, stranding individually (not in clusters during blooms of Pseudo-nitzschia algae, like acute DA-exposed animals), and/or hippocampal atrophy evident by MRI. Based on lesion morphology and distribution, cardiomyopathy caused by DA was distinguishable from other causes of heart lesions.	Zabka et al. 2009[28]
California sea lions *Zalophus californianus*	Chronic neurologic cases were examined by magnetic resonance imaging and exhibited brain damage including	12 cases diagnosed as acute and 22 diagnosed as chronic DA	Thomas et al. 2010[154]

Table 2-7

Domoic Acid Effects in Marine Mammals and Birds

Receptor	Effects	Link to DA	Reference
	hippocampal and parahippocampal atrophy, temporal horn enlargement, and pathological T2 hyperintensity. Chronic neurologic cases dove shallower for shorter durations, traveled greater distances per day and further from shore, and spent less time hauled-out and more time surface swimming than control animals	cases using criteria from Goldstein et al. 2008.	
California sea lions *Zalophus californianus*	There was increased 3-nitrotyrosine in glutamine synthetase expressing cells and in neurons in animals with DA toxicosis.	Used archived tissue samples of animals classified as having DA toxicosis based on clinical or histopathological findings.	Kirkley et al. 2014[155]
California sea lions *Zalophus californianus*	During 1998-2002, otherwise healthy females with good blubber thickness stranded and exhibited head weaving, ataxia, tremors, and seizures. All 209 animals experienced reproductive failure due to death of the mother (101), spontaneous abortion, or premature birth. Histological analysis of 29 mothers showed severe neuronal destruction of the hippocampus.	Blooms of DA occurred during the strandings of these females	Brodie et al. 2006[156]

Acute Effects in Marine Mammals. These studies were performed to examine the basis of the alarming numbers of marine mammal strandings and deaths observed along the California coast, where the suspected cause was consumption of DA in seafood. Because exposures to marine mammals are environmental, it is difficult to separate acute and chronic effects, although symptoms found in marine mammals following bloom events have been considered acute effects. DA appears to be a significant source of toxicity and illness in California seals. Between 2005 and 2009 nearly half of the seals stranded along the Central California Coast exhibited classical clinical signs of DA toxicity[73].

Short-term effects of DA exposure to marine mammals are strikingly similar to acute toxicity in humans and laboratory animals. Acute symptoms included disorientation, ataxia, head weaving, seizures or coma[154, 151]. These symptoms varied in severity but were continuous during the period of toxicosis[12]. Histopathological findings of acute exposure included hippocampal neuronal necrosis[15, 25, 157]. DA toxicity in marine mammals in Monterey was tied to concentrations in planktivorous fish, including the northern anchovy[41]. These results indicated that shellfish are not always the driver in exposure and toxicity for DA and that planktivorous fish can be a significant source of exposure to higher trophic level species[41]. Collectively, the acute symptoms in the marine mammal studies and their similarities with the 1987 human outbreak[108] indicate that there is the potential for acute effects to humans from consumption of seafood on the west coast. These effects are occurring at subsistence-level consumption rates of planktivorous in marine mammals. Most humans are unlikely to consume planktivorous seafood at

similar rates, but the consumption rate that would not pose a risk to humans is currently unknown.

Long Term Effects in Marine Mammals. These studies were performed to examine the chronic effects observed in marine mammal strandings observed along the California coast, and to examine evidence tying those effects to DA. The authors of a study in California sea lions concluded that exposure to low levels of DA is frequent and measureable even in control animals that did not exhibit overt signs of toxicosis[152]. These studies represent the only available data related to chronic low level exposure to DA in seafood. The effects examined include behavioral effects, brain effects, cardiac effects, and reproductive effects.

Behavioral Effects. Studies were performed to examine the behavioral effects associated exposure to DA in seafood. Examination of 715 sea lions stranded along the California coast with neurological symptoms between 1998 and 2006 confirmed two separate clinical syndromes. The first is acute DA toxicosis that has been documented in humans and laboratory animals[141]. These acute effects of DA to marine mammals were consistent between a number of studies[12, 27, 157]. The second clinical syndrome is a chronic epileptic syndrome characterized by permanent behavioral changes, recurrent seizures and atrophy of the hippocampus[25]. In general, chronic neurological cases were characterized by animals that developed intermittent seizures but were asymptomatic between seizures, exhibited unusual behavior, stranded individually (rather than in groups) and had chronic pathological changes[25, 153, 154]. Pathological changes were

consistent and more extensive than those previously described for acute cases that survived longer in rehabilitation[25].

Brain Effects. A study was conducted to examine similarities between hippocampal neuropathology in the brain of California sea lions that met the criteria for DA toxicosis and human patients with temporal lobe epilepsy[153]. Hippocampi were obtained from control and chronic DA-exposed sea lions. Chronic DA-exposed sea lions had hippocampal neuron loss similar in terms of pattern and extent (but not identical) to those reported previously for human patients with temporal lobe epilepsy[153]. This study provides a strong link between the commonalities of the structural effects of DA exposure and epilepsy.

Cardiac Effects. The hearts of California sea lions that had met the criteria for DA toxicosis were examined for the potential of DA-related effects. Histopathology revealed cardiac effects that ranged from mild to severe[158]. The cardiomyopathy from DA was distinguished from other heart lesions in marine mammals and involves binding of DA with receptors in the heart[28]. The distribution of cardiomyopathy was consistent among animals examined, suggesting a common cause. The authors concluded that degenerative cardiomyopathy in California sea lions represents another syndrome beyond the acute and chronic illnesses associated with exposure to domoic acid and may contribute to morbidity and mortality in marine mammals[28]. These results were supported by other studies that had autopsied marine mammals that had fatal DA toxicosis. Effects observed in these autopsies included lesions of the heart[151, 154] and

edema of the heart[159]. While additional research is needed on the effects to iGluRs receptors of the heart, there is significant evidence for the cardiac effects of DA.

Reproductive Effects. Reproductive effects of DA were examined in California sea lions to determine if DA has contributed to the extensive amount of reproductive failure seen in California sea lions in the last decade[13]. The reproductive failure has been partially associated with DA from *Pseudo-nitzschia* blooms[13]. Given a gestation period of nearly a year and mating each summer, female sea lions spend much of their adult life span pregnant or nursing. Adult females comprise sixty percent of strandings of California sea lions due to DA toxicosis[25] and these animals frequently suffer from reproductive effects including spontaneous abortions and premature births[26]. Reproductive failure as a result of abortion, premature birth, or death of pregnant female sea lions was observed in 209 DA-intoxicated adult females admitted to rehabilitation centers in California in 1998 and 2002[156, 160]. Of these females, 108 died. The other 101 animals survived after aborting or giving birth prematurely, and were released. Tissues from 29 adult animals underwent histological examination. Neuronal atrophy and necrosis in the hippocampus consistent with DA exposure were observed.[161] There have also been recent major population declines of Scottish harbor seals and DA is being investigated as a potential contributor[162]. Collectively these studies provide substantial evidence that DA is making a significant contribution to the decline of marine mammals in some areas.

In summary, marine mammal data provide a critical link in the weight of evidence for revisiting the reference dose and action level. Marine mammal studies have linked

together high cell counts of *Pseudo-nitzschia* with high concentrations of DA in planktivorous fish in the stomachs of marine mammals[27, 41]. This complements studies that have found DA in the bodily fluids of marine mammals exhibiting typical symptoms of DA acute toxicosis[157, 151, 152]. Studies have also documented that many of the acute cases of DA toxicosis progress to a chronic behavioral syndrome in marine mammals[25]. This chronic syndrome is characterized by tremors, seizures, and ataxia (abnormal or uncoordinated movement)[25, 153]. Magnetic resonance imagining or post-mortem examination of the brains of marine mammals with the chronic syndrome revealed severe loss of neurons in the hippocampus[153, 154]. These behavioral symptoms and structural effects in the brains of marine mammals share strong commonalities with effects seen in humans during the 1987 outbreak[7, 108] and in studies with laboratory animals[10, 114-116, 133, 163]. These chronic effects in humans, laboratory animals, and marine mammals share some strong commonalities with the chronic human illnesses epilepsy and schizophrenia. This relationship is examined in the next section.

Commonalities with Epilepsy and Schizophrenia. An important issue for further consideration is the similarities between the chronic effects of DA outlined earlier in this section and those of other chronic brain illnesses. DA primarily affects the hippocampus, a portion of the brain with a primary role in long-term memory and spatial navigation that can be impacted by a number of long-term brain illnesses. While direct data are lacking, DA has the potential to be a contributor to chronic brain illnesses that also affect the hippocampus. Changes in brain function occur throughout life, and some

consequences of early life exposure to chemicals (as was documented for DA earlier in this section) may not manifest themselves until later in life.

Low dose chronic exposures to environmental contaminants may lead to diseases that resemble common illnesses that have other causes, or they may affect function in nonspecific ways that are not diagnosed by doctors as environmental exposures[164]. Many neurological illnesses are diagnosed through identifying a number of symptoms in the patient out of the total range of possible symptoms described for a particular illness. Thus both diagnosis and causes of many illnesses are imprecise and suggest that neurological disorders associated with environmental exposures, including DA exposure, could be potentially misdiagnosed. This section examines commonalities between the effects of DA exposure and temporal lobe epilepsy and schizophrenia. DA has also been suggested as a potential contributor to Huntington's disease and Parkinson's disease but these relationships are not discussed further because significant evidence is lacking[165].

Epilepsy. The effects of DA share their most striking similarity with epilepsy. Epileptic syndromes have diverse primary causes, which may be genetic, developmental, or acquired[166]. Effects to the NMDA family of iGluRs are a recognized cause of epileptic seizures[166] and these receptors are also affected by DA[167]. DA has been used as a model of epileptic seizures[142]. One hypothesis presented (but not tested) in the scientific literature is that dietary exposure to doses of DA that are sub-clinical in pregnant women may be sufficient to damage the fetal hippocampus and initiate epileptogenesis (the gradual process by which a normal brain develops epilepsy)[142]. DA has been shown to

cause epileptogenesis through neonatal exposure, but there is currently no evidence of this effect from *in utero* exposure[10].

Since the initial discovery of amnesic shellfish poisoning (ASP) during the human outbreak in 1987, seizures have been a documented effect of DA exposure[108]. Epileptic seizures occurred in one patient who received long-term care (summarized in Table 2-2)[7]. Postmortem pathology in this patient also indicated severe hippocampus neuronal cell death, and provides evidence supporting the role of excitotoxic injury from DA in development of epilepsy in this individual. The effects in this patient were similar to delayed onset seizures reported in laboratory rats in Table 2-6[10, 11, 168]. When these rats were exposed to new tasks requiring spatial processing as adults, the animals demonstrated a behavioral syndrome that is similar to a stage 2 epileptic seizure[57, 11, 168]. Post-mortem examinations of these rats showed many changes in the hippocampus that are consistent with animal epilepsy including mossy fiber sprouting and a significant loss of neurons[10]. Further support for the similarities between epilepsy and DA exposure comes from Bernard[133] who reported that a series of systemic injections of low dose (no observed acute toxicity) DA during early life development produced physical changes in the hippocampus and behavioral effects that were similar to existing animal models of temporal lobe epilepsy. Rats injected with DA showed subtle changes in cognition and/or emotionality that are characteristic of human temporal lobe epilepsy[124]. Overall, there is strong evidence to suggest that DA could be the cause or contributor to some cases of epilepsy.

Schizophrenia. This section examines evidence for connections between DA exposure and schizophrenia. Schizophrenia is a complex psychiatric illness that affects about one percent of the world's population. It is a debilitating neurological disorder characterized by a range of cognitive and emotional symptoms. Recent studies provide some evidence that many neuropsychiatric disorders are based on neurodevelopmental issues, originating at least in part from structural abnormalities that occurred during critical periods of brain development[169]. Similarly, DA is known to cause brain structural abnormalities when exposures occur during a critical widow in brain development[10, 11]. The effects of DA and schizophrenia have similar symptoms and similar models for how their effects occur.

Low doses of DA during a critical window of brain development results in adult rats with behaviors that mimic a variety of schizophrenia symptoms[170]. These symptoms include psychomotor agitation, altered drug and reward seeking, alterations to working memory, deficits in pre-pulse inhibition (reaction to a weaker stimulus reducing the subsequent reaction to a stronger stimulus) and latent inhibition (delayed development of reaction to a stimulus)[170, 171]. A study examining prepulse inhibition of an acoustic startle response in rats following DA exposure found that the effects in rats are characteristic of human schizophrenia[126]. When these DA-exposed newborn rats reached adulthood, these rats demonstrated evidence of social withdrawal (significantly greater amount of time spent in avoidance behavior and a significantly lesser amount of time spent in social contact) consistent with symptoms of schizophrenia. Low dose exposure of DA to neonatal rats during post-natal days eight through fourteen resulted in an increased

dependence on nicotine in the adult female rats[172]. Altered drug and reward seeking such as nicotine dependence is also considered a symptom of schizophrenia. These studies provide evidence that symptoms of neonatal exposure to DA and schizophrenia share striking commonalities.

The effects of DA and schizophrenia also target the same part of the brain. Numerous studies have focused on neonatal damage of the hippocampus as a model of schizophrenia, because various structural and functional changes in the hippocampus have been consistently implicated in human schizophrenia[173]. DA exposure during a critical developmental window is considered a useful model for advancing the understanding of schizophrenia[125]. Decreased social interaction is a common symptom of schizophrenia and can be readily observed in rats. Low dose exposure to DA in neonatal rats resulted in alterations in glutamate signaling which in turn resulted in social withdrawal[173]. Others report that the time period between the second and third week of life in the rat is a critical period of hyperexcitability within the CA3 subfield of the hippocampus, a limbic region with a marked capacity to generate electrographic seizures[174]. One study concluded that early treatment with DA "may serve as a useful tool to model schizophrenia which in turn may lead to a better understanding of the contribution of glutamate, and in particular, kainate receptors, to the development and/or manifestation of schizophrenia or schizophrenia-like symptoms in the clinical population"[175]. It is important to understand similarities in mechanistic underpinnings for DA effects and schizophrenia and not just the similarities in symptoms and target locations in the brain.

The biological basis for psychotic signs and symptoms in schizophrenia is not well understood[176]. Many abnormalities in several neurotransmitters have been found in the brains of patients with schizophrenia, but much attention has focused on the roles of dopamine and glutamate neurotransmission underlying the disease. Historic research as well as all successful treatments of schizophrenia symptoms, had focused on dopamine receptor blockers[176]. More recently there has been improved understanding of the role of glutamate receptors in schizophrenia, and glutamate agonists have been used successfully for treatment of schizophrenia symptoms[176]. Altered functioning of the glutamate system during critical periods of development is believed to play a role in schizophrenia[126]. Tamminga[177] developed a working hypothesis based on clinical data and theoretical explanations that diminished glutamatergic transmission in the hippocampal glutamate-mediated efferent neuronal pathways and cerebral dysfunction in the hippocampus and its target areas, is the mechanism responsible for schizophrenia.

The primary animal model of schizophrenia, the neonatal ventral hippocampal model, shares key elements with the effects of DA[178]. In this model, excess glutamate in a critical developmental window in a neonatal organism causes neuron death and decreased formation of neural connections. Glutamate plays a critical role in the developing brain, regulating neuronal survival, differentiation, and development of synaptic connections[125]. There is evidence that excessive amounts of glutamate in the developing brain can play a role in the development of schizophrenia[63]. The neonatal ventral hippocampal model of schizophrenia and domoic acid both target iGluRs. The neonatal ventral hippocampal

model involves the triggering of the NMDA subfamily of iGluRs, which can also be targeted by DA[167].

In summary, this review of human, laboratory animal and marine mammal data provided an overview of the chronic effects of low dose exposure to DA and the similarities between DA and chronic brain illnesses. Acute effects in humans and laboratory animals and chronic exposures in marine mammals can cause chronic sub-lethal effects. The behavioral and physiological responses were consistent across humans, laboratory animals, and environmentally-exposed marine mammals. The chronic symptoms of greatest concern include ataxia, tremors and seizures (occurring in humans[7, 108], laboratory animals[106, 179], and marine mammals[27, 151]) and deficits to learning and memory (occurring in humans[110] and laboratory animals[10, 120]). The physiological effects of primary concern include neuron cell death and mossy fiber sprouting in the hippocampus (occurring in humans[7], laboratory animals[115, 116, 124, 145, 180] and marine mammals[153, 154]) neuron death in the olfactory bulb (occurring in laboratory animals[146] and marine mammals[161]) cardiac abnormalities (occurring in humans[108], laboratory animals[139] and marine mammals[25, 28]) and reproductive impacts (occurring in marine mammals[156]). These effects can occur from acute exposure during a critical window in brain development and the effects can be permanent. There is evidence that these effects are occurring in marine mammals from current exposures in seafood.

Human data on the long-term effects of DA is extremely limited. The 1987 outbreak in humans provided information on severe effects of exposure to a single high dose[108]. This single high exposure resulted in chronic epileptic-type seizures and chronic

effects to short term memory in a number of patients[7]. Overall, epidemiological data are lacking but an ongoing study of Native Americans in the Pacific Northwest may provide valuable information on exposure and toxicity[110]. Initial results from this ongoing epidemiology study indicate the potential for more subtle effects to learning.

There is a substantial body of laboratory animal toxicological data on acute and chronic effects of DA exposure. Prenatal exposure studies in laboratory animals documented long-term effects to behavior and learning in offspring at concentrations that did not produce acute effects in their mothers[119, 120]. Rats exposed to low doses during a critical developmental window (postnatal days 8-14) developed long term behavioral changes and physical changes to the brain[10, 11, 24, 128, 181]. These effects included structural and functional changes to the hippocampus, epileptic seizures, memory loss, and behavioral effects[21] that are reflected in laboratory animal and marine mammal data[13, 21, 25]. Both laboratory animal data and environmental exposure to marine mammals indicate that in addition to the short-term syndrome analogous to amnesic shellfish poisoning, there is a long-term syndrome that can include seizures, behavioral changes, and effects to spatial memory and learning[10, 13, 124]. The marine mammal data are particularly compelling because they represent serious effects occurring from actual environmental exposures to DA concentrations found in seafood. While human consumption of seafood is likely considerably lower than consumption by marine mammals, these serious effects to marine mammals raise concerns about the potential for effects to humans, such as subsistence fishers, who could consume high levels of planktivorous fish and shellfish that may contain DA. There are striking similarities between the effects of DA and

chronic brain illnesses. These similarities raise concerns that DA has the potential to exacerbate a number of chronic brain illnesses. When considered as whole, data on effects to humans, laboratory animals, and marine animals provide compelling evidence that chronic effects of low level exposure represent a serious concern, one that may not addressed by an acute domoic acid reference dose.

Domoic acid administered to neonatal rats results in a symptoms, structural abnormalities and mechanisms of action that are observed in epilepsy[133, 142, 167] and schizophrenia[170, 173, 176]. Additional research is needed on whether environmental exposure to DA has the potential to be a cause or a contributing factor in the development of these illnesses.

Protectiveness for Sensitive Subpopulations

A second reason to revisit the reference dose for DA (in addition to concerns about chronic effects of low-level exposure) is the consideration of sensitive subpopulations. An RfD (and subsequent seafood action level) that is developed to protect the general population may not be safe for subpopulations that have greater sensitivity than the general population. This section identifies and discusses two sensitive subpopulations that need consideration when evaluating an RfD for DA. A sensitive subpopulation is defined as any subpopulation that may be at greater risk from exposure to DA than the general population. EPA has not derived an RfD for DA. However, when EPA derives an RfD, sensitive subpopulations are considered when uncertainty factors and modifying factors are applied to a reference dose. As discussed in Section 2, a single factor of 10 was used in the RfD to account for both sensitive

subpopulations and to extrapolate from an LOAEL to an NOAEL. EPA guidance recommends a factor of 10 to account for each of these factors separately. This section focuses on two sensitive subpopulations, children and the elderly. These sensitive subpopulations are discussed below.

Children. Children may be particularly sensitive to DA. There is an increased awareness of the sensitivity of the developing nervous system to toxic injury. There are a number of literature reviews available on early brain development and the predisposition to toxic insult[70, 71] and this is starting to be reflected in government regulations[182, 183]. The EPA has recently revised its risk assessment approach to better account for the sensitivity of children (such as the use of age-dependent adjustment factors for chemicals with a mutagenic mode of action[184]). WHO has recently published guidance on evaluating children's health risks from exposure to environmental chemicals[183]. EPA is required by law to incorporate an additional 10-fold factor in risk assessments for pesticide residue tolerances to take into account the special sensitivities of infants and children as well as incomplete data with respect to toxicity and exposures. Developmental disabilities exact a large toll on children's health in the United States. Developmental disabilities affecting the central nervous system affect large numbers of children and often little is known about the etiology of these conditions[185]. Functional impairment from exposure to toxic chemicals can be difficult to determine and effects of childhood exposure often manifest themselves in adulthood[186]. It is therefore important to consider whether early life exposure to DA has the potential to cause effects to the hippocampus that can be manifested as behavioral and memory issues later in life.

Children are often more sensitive to chemicals because they are still developing, which can affect the interaction of chemicals with their bodies. Several issues that can result in elevated child sensitivity to DA include (1) the state of brain development, (2) the development of the blood-brain barrier, and (3) gastrointestinal absorption and (4) renal clearance of chemicals.

All mammals undergo a significant brain growth spurt after birth[187] and research has shown mammals are particularly sensitive to neurotoxicity during this time[188]. Research has indicated that rats are particularly sensitive to DA during the brain spurt that occurs during the first two weeks of a rat's life[10, 24]. While the brains of rats and human mature at different rates, it is possible to extrapolate from the developmental stage of a rat brain to a human brain. The brain development through postnatal day 14 of the rat equates to the level of brain development for a 49 day-old infant[188, 189]. Therefore, the window of greatest sensitivity for the effects of DA in the developing human brain is expected to be approximately the first two months of life.

The blood-brain barrier is not fully developed for the first 36 months of life, so toxicants such as DA that are slowed by this barrier can affect young children more readily[190]. DA primarily affects the hippocampus and must pass through the blood-brain barrier to cause damage to the brain. The function of the blood-brain barrier is to separate circulating blood and brain extracellular fluid. Endothelial cells along the capillaries restrict passage of large hydrophilic molecules such as DA.

Gastrointestinal absorption is different in children and frequently increased because children need to absorb nutrients more efficiently. A number of factors (gastric

acidity, gastrointestinal motility, enzyme activity, and bacterial flora) increase gastric and intestinal motility in young children[190]. Because gastrointestinal absorption of DA in adults is very low (5-10%), these GI absorption differences in children have the potential to significantly increase uptake of DA[8].

Finally, individuals with a kidney disease that results in impaired renal function will be at greater risk from DA exposure[8]. The kidneys are the only mechanism of elimination once DA is taken up from the gut, as DA is not metabolized in the body[8]. Renal clearance inhibitors in adult rats increased radio-labeled DA concentrations in the brain[191]. Elimination of DA is likely decreased in early childhood because the glomerular filtration rate of the kidneys in newborns is less than 40% of that in adults, and premature infants may have less than 5% of the adult rate[190]. This is likely to lead to significant increases in DA toxicity in young children. Two of the individuals affected in the 1987 Canadian outbreak had reduced kidney function due to renal disease[13].

Prenatal and postnatal sensitivity in children has the potential to be significantly different. In the California sea lion, the long term epileptic disease associated with DA is found most commonly in young animals[25]. Maternal transfer is a significant exposure pathway for DA to the young. Prenatal and postnatal maternal exposure is discussed below.

Prenatal Exposure. Prenatal exposure to DA may be significant. Maternal transfer of DA can lead to effects in offspring which are summarized below. DA readily crosses the placenta, enters brain tissue in prenatal rats, and accumulates in amniotic fluid[192]. Maternal-fetal transfer was found to be 24% between the plasma compartments[131]. One

study suggested that DA in fetal blood is excreted through fetal urine into the amniotic fluid[156]. There it would be diluted and swallowed by the fetus, enter the stomach, and reenter the fetal blood stream[156]. Therefore the fetus and amniotic fluid may act as a reservoir of DA ingested by pregnant females, retained after DA in the mother's body is excreted in maternal urine[156]. There is longer retention of DA in fetal brain than in the mother, indicating the potential for high susceptibility of the fetus to DA[131]. Therefore, the fetal exposure may continue even after DA exposure to the mother ceases.

Postnatal Exposure. DA from maternal plasma readily enters mothers' milk, posing a potential for continual exposure during the lactation and nursing. When mother rats were given a nonlethal dose of DA (1.0 mg/kg) on day 12 of lactation, DA concentrations in milk were 16 times lower than the mother's plasma one hour after exposure injection[193]. However, eight hours after injection, levels in milk were four times the level in the mother's plasma. There was still a quantifiable concentration of DA in milk in the 8-24 hour interval after exposure, whereas DA in the mother's plasma at this time was detectable but not quantifiable. The results suggest that infants could continue to be exposed via milk after DA has been cleared from the mother's plasma. DA was also measurable in the plasma of neonates. While the uptake rate of DA into mother's milk is low, it persists long after maternal exposure ends. This suggests that maternal exposure could result in long term low level exposure for neonates via milk consumption.

The Elderly. In addition to children, the elderly may also be particularly susceptible. In the initial outbreak that led to the discovery of DA toxicity, exposure to

DA was associated with long term neurological deficits mainly in older patients[108]. Eighty percent of the affected individuals in the 1987 outbreak were over 40 and eleven of the thirteen treated in intensive care were over sixty-eight years of age[13]. One study[194] suggested that a "loss of inducible neuroprotective mechanisms may account for increased sensitivity to excitotoxins during aging." Hippocampus neurons from rats 26-29 months old showed a significantly decreased resistance to DA compared to younger rats[194].

Decreased renal function can cause the elderly to be more sensitive to DA. Excretion by the kidneys is the primary mechanism for removal of DA from the body. Renal function may be decreased in the elderly, making it more difficult for the body to remove DA[195]. Renal size and volume decrease with age, accompanied by intra-renal vascular changes and a decrease in the number of glomeruli[196] (clusters of capillaries around kidney tubules responsible for waste removal). The result is a decrease in the excretion rate of DA from the body among the elderly.

In summary, the groups identified above represent sensitive subpopulations for exposure to DA. There is significant evidence that there are subpopulations with documented sensitivity to DA. Children may be at risk due to the sensitivity of their developing brains, an incomplete blood-brain barrier, increased GI absorption, and decreased renal clearance. Neonatal and prenatal mammals lack fully developed kidney function to clear DA from the body efficiently and lack a fully developed blood-brain barrier to limit its entry to target sites in the brain. Laboratory studies indicate particular sensitivity for neonatal rats during the brain "growth spurt". Elderly individuals were

disproportionately represented among the severe cases in the 1987 outbreak and may be particularly sensitive[108]. The uncertainty factor used in the RfD for DA provides less protection than the factor of ten that would typically be used in an RfD derived by EPA and may not be protective of these sensitive subpopulations. The next section considers the protectiveness of consumption assumptions applied in the current action level.

Protectiveness of Consumption Assumptions.

An action level incorporates both toxicity of seafood and the amount of seafood consumed. The RfD represents the toxicity component. The second part of the action level, the assumed consumption rate, is discussed below. The action level incorporates a consumption rate that is reflective of a single meal exposure and may not be protective of chronic consumption of seafood.

Acute Consumption Rate (Single Meal Exposure). Default fish meal sizes are available from a number of agencies, including FAO/WHO/IOC, EFSA, and USEPA. FAO/WHO/IOC used a single meal size of 250 grams of shellfish in deriving the current action level[8]. FAO/WHO/IOC concluded that a meal size of 250 grams would cover 97.5% of shellfish consumers of most countries for which data was available.

The European Food Safety Agency[129] (EFSA) used a single meal size of 400 grams (rather than 250 grams) of shellfish meat, or 0.88 pounds. Neither FAO/WHO/IOC nor EFSA considered meal size data sets for seafood types other than shellfish. EFSA evaluated limited consumption data for the European Union. EFSA believed it was important to use a large meal size for DA due to the severity of its acute toxic effects. The 95th percentile meal size ranged from 70 to 465 g for the four available data sets (two

for France, and one each for Germany and the Netherlands). The largest single meal size identified was 1500 grams and was from the German data set.

EPA assumes a default fish meal size (not shellfish specifically) of 227 grams, which equates to an eight ounce meal[197]. This meal size was taken from the Michigan Anglers Survey[198], where recreational fishers were asked to estimate their typically recreationally caught fish meal size. This is the rate that EPA uses to calculate consumption advisories for water bodies that contain fish with chemical contamination.

The FAO/WHO/IOC, EFSA, and EPA meal sizes represent conservative upper percentile estimates for single meals of shellfish only. These values are appropriate if the purpose is to protect for exposure to a single meal of shellfish. The next section looks at consumption rates over a chronic exposure period that are representative of all types of fish, not just shellfish. Since FDA adopted a regulatory value from Canada when establishing the action level for DA meal size was not separately considered by the agency.

Chronic Consumption Rates of Seafood. Chronic seafood consumption rates are available from a number of sources. Seafood is caught and consumed through commercial, recreational and subsistence fishing. A key consideration in selecting a consumption rate is the population to be protected. Consumption rates can vary greatly from country to country, or within the populace of a given country. Consumption rates are greater among recreational fishers than the general populace and greater still for subsistence fishers. Since the action level contains assumptions about the amount of

seafood consumed, any individuals who consume more seafood than is assumed by the action level will have greater potential for DA exposure.

Selection of a fish consumption rate for use in an action level is a value judgment about what population or subpopulation (and what percentage of individuals within the population or subpopulation) are to be protected by the action level. Chronic consumption rates are typically discussed in term of grams of fish per day averaged over time, rather than in terms of individual meal size. Consumption rates for various groups are presented in Table 2-8:

Table 2-8
Studies Available to Derive a Chronic Seafood Consumption Rate

Receptor	Consumption Rate (g/day)	Reference
Recreational Marine Fishers	5.6 – 24 mean	Moya 2004[199]
Recreational Marine Fishers, various ethnic groups	8 – 116 mean	Moya 2004[199]
Recreational Marine Fishers, various ethnic groups	176, 95th percentile, Asian-Filipinos in San Francisco	Moya 2004[199]
General Population	11.3 – 19 mean	Smiciklas-Wright 2002[200]
Native American Subsistence Fisher	540 mean (not marine data)	Harris 1997[201]

Recreational Marine Fishers. The National Marine Fisheries Service conducts the Marine Recreational Fisheries Statistics Survey[202]. EPA queried these data to develop consumption estimates for a range of geographic locations, ages and ethnicities within the United States[199]. Mean and median consumption rates were not available for all groups.

Mean consumption rates ranged from 5.6 to 24 g/d across regions. Ethnic groups had median consumption rates ranging from 8 to 116 g/d. Ninety-fifth percentile consumption rates ranged as high as 176 g/d (for 70 Asian-Filipino respondents in San Francisco Bay, CA).

General Population. Using data from an EPA consumption survey and a mean meal size of 114 g for all age groups combined, and assuming the consumer eats 3-5 seafood meals per month, exposure would range from 340 to 520 grams/month or 11.3 to 19 g/day for seafood[200]. These data are representative of the general U.S. population rather than regions, age groups, or ethnicities.

Subsistence Fishers. Subsistence fishers are defined as those fishers who rely on non-commercially caught fish and shellfish as a major protein source in their diets. Certain Native American groups may have greater exposure due to consumption patterns that differ from the general population[110]. Typical Native American consumption of seafood can be an order of magnitude greater than the general population[201]. Commercial and recreational fishers and their families, as well as certain ethnicities, may consume seafood at a very high rate[199].

EPA recognizes that for Native American subsistence fishers, eating fish is not simply a dietary choice that can be completely eliminated if contaminant concentrations reach unacceptable levels. Instead, it is an integral component of many Native American lifestyles and cultures[197]. This traditional lifestyle is a "living religion" that includes values about environmental responsibility and community health as taught by elders and tribal religious leaders[201]. Harris and Harper[203] surveyed traditional tribal members in

Oregon with a subsistence lifestyle and determined a consumption rate of 540 g/d that included fresh, dried, and smoked fish. These data were not specific to seafood but instead were for the Columbia River and likely significantly overestimate any subsistence exposures to seafood. The Quinault Indian Nation, which has traditionally consumed subsistence levels of seafood on the Olympic Peninsula in Washington State, recently recommended that the Washington State Department of Ecology use a chronic fish consumption rate of 175 g/d to protect their people[204]. EPA Region 10 has conducted a pilot study of Quinault Indian Nation seafood consumption rates (in only nine individuals) and hopes to perform a full survey in the future[205].

There are significant data on long-term seafood consumption rates for various groups. There are also significant uncertainties in various data sets. Estimates of consumptions rates vary both within and among various fish consumer groups. General population mean consumption rate estimates are lowest at 11.3 to 19 g/d. Recreational fisher mean consumption rates range from 5.6 to 24 g/d, while recreational fisher ethnic group mean consumption estimates range as high as 116 g/d. Upper percentile estimates would range much higher. An estimate of Native American subsistence seafood consumption rates was not identified in the literature. Available data could be used to develop an action level that is protective of long term consumption of seafood, but selection of a specific estimate inherently involves a value judgment.

Deriving an Action Level Protective of Chronic Exposures

The above sections provided strong evidence that the current action level may not be protective of chronic exposure to DA in seafood. The current action level is protective only for single meal exposures and does not consider chronic consumption or the long-term, sub-lethal effects that have been reported in animal studies. Additionally, sensitive subpopulations are not explicitly protected with a separate uncertainty factor. This section will discuss (1) alternatives to the current reference dose, and (2) alternatives to the current action level.

Alternatives to the Current Acute Reference Dose. This section discusses alternatives to the current RfD. There are both alternative acute RfDs that have been proposed and data on chronic effects that could be used to derive a chronic RfD. These are discussed in the sections below.

Alternative Acute Reference Doses from Government Agencies. The FDA and the EFSA have both derived acute RfDs that could be used as alternatives to the current FAO/WHO/IOC value. Table 2-2 summarizes the studies used, uncertainty factors of the current FAO/WHO/IOC RfD, and alternative RfDs proposed by FDA and EFSA[129]. It is important to note that none of these values are specifically protective of chronic exposure.

Table 2-9 Comparison of Currently Available Acute Reference Doses			
Reference Dose (mg/kg/d)	**Study\Critical Effect (mg/kg)**	**Uncertainty Factors**	**Reference**
0.10*	1.0 (LOAEL) in humans (Perl et al. 1990)	Total=10 10 (intraspecies)	FAO/WHO IOC review[8]
0.03	0.9 (LOAEL) in humans (Perl et al. 1990)	Total=30 3 (LOAEL to NOAEL) 10 (intraspecies)	EFSA 2009[206]
0.034	0.5 (LOAEL) in non-human primates converted intravenous dose (Tryphonas et al. 1990)[116]	Total=300 10 (LOAEL to NOAEL) 3 (interspecies) 10 (intraspecies) 5% absorption	Slikker et al. 1998[207]
0.018	0.26 benchmark dose in non-human primates (Tryphonas et al. 1990)[115]	Total=300 10 (LOAEL to NOAEL) 3 (interspecies) 10 (intraspecies) 5% absorption	Slikker et al.1998[207]
*RfD in current use			

The FAO/WHO/IOC RfD represents the basis of the current action level. Application of uncertainty factors involves judgment and different uncertainty factors have been used by different organizations. FAO/WHO/IOC applied a single uncertainty factor to the LOAEL, reducing it by a factor of 10 to account for intraspecies differences (i.e., sensitive individuals within a species) and to convert from an LOAEL to an NOAEL rather than using a factor of ten to account for each separately[51] (i.e., a total uncertainty factor of 100)[8]. During their review of the RfD, FAO/WHO/IOC concluded this was reasonable based on the Canadian outbreak data, since one individual who was estimated to consume 0.33 mg/kg did not become acutely ill. For chronic effects, the

FAO/WHO/IOC concluded that available toxicity data at the time were not sufficient to support the derivation of a chronic RfD.

EFSA performed a recent review of the RfD and derived their own value[206]. Similar to FAO/WHO/IOC, EFSA concluded that there was not a chronic exposure study available to form the basis of a chronic RfD. However, EFSA re-evaluated the data from the 1987 outbreak and determined that the LOAEL for mild signs and symptoms was 0.9 mg/kg/d. EFSA applied an uncertainty factor of 10 to protect sensitive individuals and also applied an uncertainty factor of 3 to convert from an LOAEL to an NOAEL. EFSA's acute RfD, which is more than a factor of three lower than the FAO/WHO/IOC RfD, has not been adopted for use in an action level for seafood.

The FDA's National Center of Toxicological Research developed two RfDs that are three and six times lower respectively than the currently used FAO/WHO/IOC RfD[207]. The FAO/WHO/IOC RfD uses a total uncertainty factor of 10, the EFSA RfD uses a total uncertainty factor of 30, and the two FDA-derived RFDs use a total uncertainty factor of 300. FDA's uncertainty factors for its proposed RfDs were somewhat consistent with the EPA approach[237]. FDA used an interspecies uncertainty factor of 3 for one RfD (0.034 mg/kg in Table 2-9) rather than ten because the study[116] was performed on primates. It has been suggested however that the typical intraspecies uncertainty factor of ten for sensitive subpopulations may not be protective of children[186]. The lowest acute RfD in Table 2-9 (0.018 mg/kg) was derived by FDA based on the benchmark dose approach in a study with rats[115]. FDA has not adopted either of the acute RfDs derived and published by its own scientist, as the basis of an action level,

despite the fact that these RfDs have been available for more than fifteen years. While these lower acute RfDs have been derived, the original RfD is still used as the basis of the current action level.

Data for Deriving a Chronic Reference Dose. This section discusses the available data for deriving a chronic RfD. The relevant chronic toxicological studies in the scientific literature were previously discussed in this chapter. The primary weakness in the literature is the lack of a chronic oral study that could be used as the basis of a chronic RfD. However, the strength of the scientific literature on DA is the numerous studies that have been conducted on behavioral and physiological effects from acute interperitoneal exposures to DA during postnatal days 8-14, a critical window in brain development.

The lack of chronic studies has resulted in FDA, FAO/WHO/IOC and EFSA deriving only acute RfDs. However, humans are not exposed to a single meal of seafood in a lifetime, a year, or even a season. The effects of chronic exposure generally occur at lower concentrations than those associated with acute exposures. The potential for effects from the combined exposure of a number of meals over a given time period means that an acute RfD may not be protective for chronic exposure. For adequate protection, a chronic RfD should be used in conjunction with a chronic consumption rate to derive an action level in seafood. The ongoing epidemiological study in Native Americans in the Northwest[110] may provide a useful basis for a chronic RfD in the future. Any of the currently available acute RfDs could be used to derive a chronic RfD by using an uncertainty factor to account for the acute to chronic conversion.

None of the acute RfDs are based on the numerous toxicological studies for early life stage exposure that resulted in chronic effects in later life. Two of these studies with the lowest LOAELs are summarized in Table 2-10 below.

Table 2-10
Studies Available to Derive a Chronic Reference Dose

Receptor	Duration/Route	NOAEL	LOAEL	Critical Effect	Reference
Rat	Acute/injection	None	0.9 mg/kg injection converted to oral in rats (LOAEL)	Novelty-induced seizure-like syndrome and structural effects to the hippocampus	Doucette et al. 2004[10]
Rat	Acute/injection	None	0.9 mg/kg injection converted to oral in rats (LOAEL)	Hypoactivity in the figure-8 maze	Levin et al. 2006[122]

Available animal data on chronic effects of acute low dose exposure to DA are primarily via the interperitoneal route of exposure, including the two studies summarized in Table 2-10. Although interperitoneal data are not typically used when deriving an RfD, in the absence of suitable oral exposure data, the injected dose could be adjusted to account for the fraction absorbed in the gastrointestinal tract. This approach was used by FDA's National Center for Toxicological Research when deriving an alternative acute RfD for DA in the scientific literature[207]. DA is not well-absorbed from the GI tract but absorption is higher for primates than rats, 4-7% versus 2%[208, 209]. The two injection studies in Table 2-10 were adjusted (1) by the rat absorption rate (2%) to convert it to an

oral dose and then (2) by the ratio of rat to primate oral absorption (2% versus mean of 5.5%) to account for the greater absorption by primates. These adjustments yielded estimated oral LOAELs that could be used as the basis of an RfD protective of chronic effects.

WHO recommends applying an uncertainty factor of 100 to an NOAEL from a chronic study when deriving an acceptable daily intake (analogous to a chronic RfD). The first factor of 10 accounts for animal to human extrapolation and the second factor of 10 accounts for protection of sensitive subpopulations[210]. Recently, WHO published guidance for deriving acute RfDs for the evaluation of pesticide residues in food from agricultural uses[211]. In this most recent guidance, the animal to human factor of 10 was explained as a factor of 2.5 for toxicodynamics and a factor of 4 for toxicokinetics. The factor of 10 for sensitive subpopulations was explained as a factor of 3.2 each for toxicokinetics and toxicodynamics. Toxicokinetics describes the process when a chemical is taken up into the body and is governed by the processes of uptake, distribution, metabolism, and excretion. Toxicodynamics describes the process of the chemical interacting with the body to cause biological effects, including effects at the organ, cellular, and molecular levels. WHO does not have guidance on uncertainty factors for extrapolating from an acute dose to a chronic dose. All of the studies for DA are of an acute duration.

A chronic RfD can be derived if an acute to chronic uncertainty factor is used from another source. EPA does have recommendations for deriving a chronic RfD from acute data. EPA recommends an acute to chronic uncertainty factor of 10[99]. Acute data

with chronic effects were identified in Table 2-10 (both studies had an LOAEL of 0.9 mg/kg/d). If a chronic RfD were derived consistent with EPA's uncertainty factors, then the LOAEL of 0.9 mg/kg would be divided by an uncertainty factor of 1,000 (10 for LOAEL to NOAEL, 10 for interspecies differences, and ten for intraspecies differences)[99]. This would result in a chronic RfD of 0.0009 mg/kg/d.

If the LOAEL values in Table 2-10 were divided by a factor of 100 (10 for interspecies and 10 for intraspecies), then a hypothetical chronic RfD could be as low as 0.0009 mg/kg/d. If the lowest acute RfDs (derived by Slikker of FDA[207]) in Table 2-9 were divided by an uncertainty factor of ten to derive chronic RfDs, the chronic RfDs would be 0.0034 and 0.0018. Therefore potential range of chronic RfDs would be from 0.0009 to 0.0034 mg/kg/d, based on current data and recognizing that the application of uncertainty factors involves professional judgment. The next section discusses how these revised RfDs could be used to estimate a revised action level.

Alternative Action Levels. This section evaluates potential alternatives to the current DA action level of 20 mg/kg in seafood. An action level is a regulatory value in seafood. The previous sections reviewed a range of possible options for a chronic seafood consumption rate and a range of possible options for a chronic RfD. A chronic seafood consumption rate can be used in conjunction with a chronic RfD to calculate a chronic action level.

Recently, due to a reevaluation of the acute RfD and the assumed meal size, EFSA calculated an alternative action level. The Committee on Toxicity of Chemicals in Food Consumer Products and the Environment was tasked by EFSA with considering

whether the current FAO/WHO/IOC action level is protective of public health. They used a revised RfD of 0.03 mg/kg based on an LOAEL of 0.9 mg/kg/d and an uncertainty factor of three to convert from an LOAEL to an NOAEL and a meal size of 0.4 kg[129] to derive an action level of 4.5 mg/kg[206]. This proposed alternative action level is more than four times lower than the FAO/WHO action level of 20 mg/kg, but still only protects for acute effects of single meal exposure. Despite this updated value of 4.5 mg/kg (still an acute action level), FOA/WHO/IOC[8] the EU (Regulation No. 853/2004 of the European Parliament and of the Council of 29 April 2004) and FDA (21 CFR 123.3(d)) continue to use an action level of 20 mg/kg.

The current action level is protective of acute effects and single meal exposures. An action level that incorporated chronic, rather than acute effects, would be calculated:

Action Level = (RfD x BW x AP) / (CR x EP)

Where:

Action Level	=	mg of DA per kg of seafood
RfD	=	Reference Dose (mg/kg/d)
BW	=	Body Weight (kg)
CR	=	Single Meal Size (kg)
EP	=	Exposure Period (days)
AP	=	Averaging Period (days)

This equation is used in conjunction with chronic RfDs and chronic consumption rates in Table 2-11 to calculate a range of possible chronic action levels.

Using data on possible chronic RfDs and long term fish consumption rates, a range of possible chronic action levels can be calculated based on the current data. The low end chronic RfD estimate of 0.0034 mg/kg/d is derived by dividing the 0.034 mg/kg/d NOAEL from Table 2-9 by an acute to chronic uncertainty factor of 10 to convert from an acute RfD to a chronic RfD. The high end chronic RfD estimate of 0.0009 mg/kg/d is derived by dividing an LOAEL of 0.9 mg/kg from Table 2-10 by an uncertainty factor of 1000 (10 for acute to chronic, 10 for interspecies extrapolation, and 10 for sensitive subpopulations). The low end consumption rate is the middle of the mean consumption range reported for recreational fishers from Table 2-8. The high end chronic consumption rate is the upper end of the range of mean values reported for consumption by ethnic marine recreational fishers from Table 2-8. Action levels were calculated using the equation on the previous page. The range of action levels is presented in Table 2-11.

Table 2-11

Range of Possible Chronic Action Levels

Hypothetical Chronic RfD Range (mg/kg/d)	**Consumption Rate Range (kg/d)**	**Hypothetical Chronic Action Level[e] (mg/kg)**
Low toxicity (0.0034)[a]	Low End Consumption (0.015)[c]	14
High Toxicity (0.0009)[b]	High End Consumption (0.116)[d]	0.5

[a]Acute RfD of 0.034 mg/kg from Table 2-9 divided by an acute to chronic uncertainty factor of 10.
[b]LOAEL of 0.9 mg/kg from Table 2-10 divided by an uncertainty factor of 1000 (10 for cute to chronic, 10 for interspecies extrapolation, and 10 for sensitive subpopulations.
[c]Middle of the mean consumption range reported for marine recreational fishers from Table 2-8.
[d]Upper end of the range of mean values reported for consumption by ethnic marine recreational fishers from Table 2-8.
[e]Action levels were calculated using the equation on the previous page.

This range of action levels is not intended to propose a specific change to the current action level, but provide evidence for revisiting the current action level. The current action level is 20 mg/kg, the EFSA action level is 4.5 mg/kg, and the range of hypothetical chronic action levels is 0.5 – 14 mg/kg. This analysis shows the magnitude of difference decrease in converting the current action level to a chronic value could be anywhere from a small fraction to an order of magnitude or more.

A lower action level is likely to be exceeded in a significant number of seafood samples and could result in significant human exposures unless proper monitoring is

conducted. Collating data for 37,032 samples, EFSA estimated that approximately 1% of available European seafood samples exceeded the action level of 20 mg/kg while 3.5% of seafood samples exceeded the EFSA value of 4.5 mg/kg (based on limited available sampling data in Europe)[129]. The current action level of 20 mg/kg is exceeded most frequently in Europe by the United Kingdom (exceeded in 17.1% of shellfish samples analyzed), followed by Ireland (11.3%), France (8.6%), Spain (3.6%), and Portugal (1.2%). A lower action level would be exceeded with even greater frequency.

Summary Conclusion.

The purpose of this chapter was not to actually derive a revised chronic RfD, a chronic seafood consumption rate, or a revised action level protective of chronic exposures. Instead, the purpose of this chapter was to evaluate the weight of evidence for revisiting each of these issues by the appropriate regulatory agencies. The effects of DA share striking similarities to other brain illnesses, most notably epilepsy and schizophrenia. DA has the potential to contribute to the severity of illnesses that impact the hippocampus. There is also the possibility (although no evidence in the current scientific literature), for the symptoms of DA to be mistaken for other illnesses.
The current acute reference dose was developed in the aftermath of the 1987 outbreak in Canada, and has not been updated by FAO/WHO/IOC, although it was reviewed ten years ago[8]. A lower (by more than a factor of three) acute reference dose was recommended recently by a committee of scientists established by EFSA, although this RfD has not been used to create a lower action level. RfDs developed by FDA are three to six times lower than the current FOA/WHO/IOC RfD, and yet FDA has not developed

an action level based on its own scientists' RfD, despite the fact the FDA RfDs have been available for fifteen years.

The available RfDs were developed for acute exposures and a chronic RfD has not yet been established. Given that chronic effects have been demonstrated from acute exposures (in humans, laboratory animals, and marine mammals), a chronic exposure study is a critical need for developing a chronic RfD. In the interim, the RfD should be revisited and consideration should be given to applying an uncertainty factor to an acute study to estimate a chronic reference dose. Consideration should be given to the numerous acute injection studies in neonatal rats that show serious chronic physiological and behavioral effects in later life. Recently DA in seafood has caused striking neurophysiological and behavioral effects when consumed by marine mammals. These effects in marine mammals raise concerns about the level of protection afforded to individuals who consume shellfish and planktivorous fish frequently.

The FAO/WHO/IOC acute reference dose includes an uncertainty factor of 10 to account for sensitive subpopulations. However, it is unclear if this uncertainty factor is truly protective for all identified sensitive subpopulations. Numerous studies in the toxicological literature have identified the developing brain in juveniles as particularly sensitive. Young children lack a fully developed renal system or blood-brain barrier, slowing clearance from the body and allowing it to enter the brain more easily. Fetuses are at risk of greater exposure than the general population. DA passes through the placenta and lingers in fetal brains and amniotic fluid long after maternal concentrations are non-detect. The elderly are also sensitive, as witnessed by the more severe impacts to

older victims in the 1987 outbreak in Canada. The elderly are particularly at risk if they have pre-existing conditions that affect the blood-brain barrier, the hippocampus, or the kidneys. Subsistence fishers, such as certain Native American tribes, recreational or commercial fishers and their families, and some ethnicities, may also be particularly at risk when consuming planktivorous seafood at rates greater than assumed by the current action level. There is not currently any advisory message for DA exposure that has been issued for sensitive subpopulations.

The consumption rate used in the current FAO/WHO/IOC action level is protective of a single meal exposure for most individuals. However, DA exposure through a number of meals over a period of years has the potential for additive effects that cannot be ruled out without a chronic study. When a chronic RfD is developed, the consumption rate should be revised to be commensurate with chronic exposure. An action level based on a chronic RfD and a chronic consumption rate will assure protection of seafood consumers.

CHAPTER THREE

DOMOIC ACID EXPOSURE

Chapter 3 Research Question. What are the spatial and temporal trends in *Pseudo-nitzschia* cell counts in ocean waters and DA concentrations in seafood and what can we infer about the potential exposures for humans?

Chapter 3 Abstract. Current knowledge about human exposure to the algal biotoxin domoic acid (DA) is limited and available data have not been integrated and analyzed. Environmental monitoring data indicate that the diatom *Pseudo-nitzschia*, a cosmopolitan species that is widely distributed across the world, is generally present in low concentrations between blooms. An analysis of available *Pseudo-nitzschia* cell count data is a useful initiating step in determining the potential for human exposure. This chapter makes the range of potential exposures to DA more apparent, establishes a framework for further analysis, and identifies data gaps. Data are sparse and this analysis requires some assumptions and caveats. The first part of this chapter synthesizes available *Pseudo-nitzschia* data and assesses temporal trends and correlations with nutrient concentrations. The second part of the chapter is a literature review of tissue concentrations of DA in various types of fish and shellfish from across the globe. This two-pronged approach examines temporal and spatial evidence for the presence of persistent low-level concentrations of domoic acid in coastal systems.

Introduction

Chapter 1 discussed the potential for human influence on cell counts of *Pseudo-nitzschia* in coastal areas. Chapter 2 discussed recent toxicological data that indicate there are significant chronic effects of low-level domoic acid (DA) exposure. Chapter 3 focuses on the prevalence of DA in the environment and the potential for chronic low-dose exposure in humans. Human exposure to DA is exclusively through the consumption of seafood. Other routes of contact (i.e. inhalation, dermal contact, ingestion of water) are not significant. DA is not present in the water column in significant quantities because of the huge dilution factor of the ocean[212, 213]. The exposure pathway for humans is through consumption of seafood, where planktivorous organisms such as fish and shellfish consume *Pseudo-nitzschia* and accumulate DA in digestive tracts and other tissues. Cell counts of *Pseudo-nitzschia* in coastal waters are therefore an indicator of the potential for DA in seafood.

The focus of DA assessment and regulation has been on presence or absence of *Pseudo-nitzschia* blooms and high concentrations of DA in seafood. Persistent low level cells counts of *Pseudo-nitzschia* in coastal waters and persistent low levels of DA in seafood have been largely ignored in favor of protection against acute outbreak of disease associated with blooms. Environmental monitoring data indicate that the diatom *Pseudo-nitzschia* is a cosmopolitan species that is widely distributed across the world and is typically present at low cell counts between blooms. An analysis of *Pseudo-nitzschia* cell count data is a useful initiating step in determining the potential for human exposure.

The first part of this chapter synthesizes available *Pseudo-nitzschia* data collected from the English Coast by the Plymouth Marine Laboratory in Great Britain and assesses

temporal trends and discusses other *Pseudo-nitzschia* data sets from the published literature. The second part of this chapter collects and summarizes published temporal and spatial data on concentrations of DA in various types of seafood from the scientific literature. Seafood monitoring data are sporadic both temporally and spatially and have not been compiled. Limited monitoring data for DA in seafood are available for the United States (primarily on the West Coast) and across the world (primarily Europe and Asia). Chapter 2 discussed growing evidence for chronic effects of low level exposure and this chapter focuses on the presence of persistent low levels of *Pseudo-nitzschia* in the environment and DA in seafood. This chapter assesses the evidence for (1) *Pseudo-nitzschia* diatoms being widespread and present throughout most of the year, and (2) concentrations of DA in planktivorous seafood being widespread and persistent.

Evidence *for* Pseudo-nitzschia *as a Cosmopolitan Species*

This section examines environmental monitoring data to determine whether the genus *Pseudo-nitzschia* is cosmopolitan (i.e., widely distributed across the world) and is present most of the year at low cell counts between blooms. Diatom populations in the ocean vary greatly both temporally and spatially. The amount of DA in seafood relates to the cell counts of diatoms present in the environment, the DA production by those diatoms, and uptake and persistence in seafood. Most toxic species of *Pseudo-nitzschia* are coastal and therefore readily available for consumption by coastal shellfish and planktivorous fish[146]. Diatoms (including *Pseudo-nitzschia*) tend to occur in high cell counts in upwelling zones where they can remain in the upper part of the water column and receive access to sunlight and nutrients. Worldwide, *Pseudo-nitzschia* diatoms are

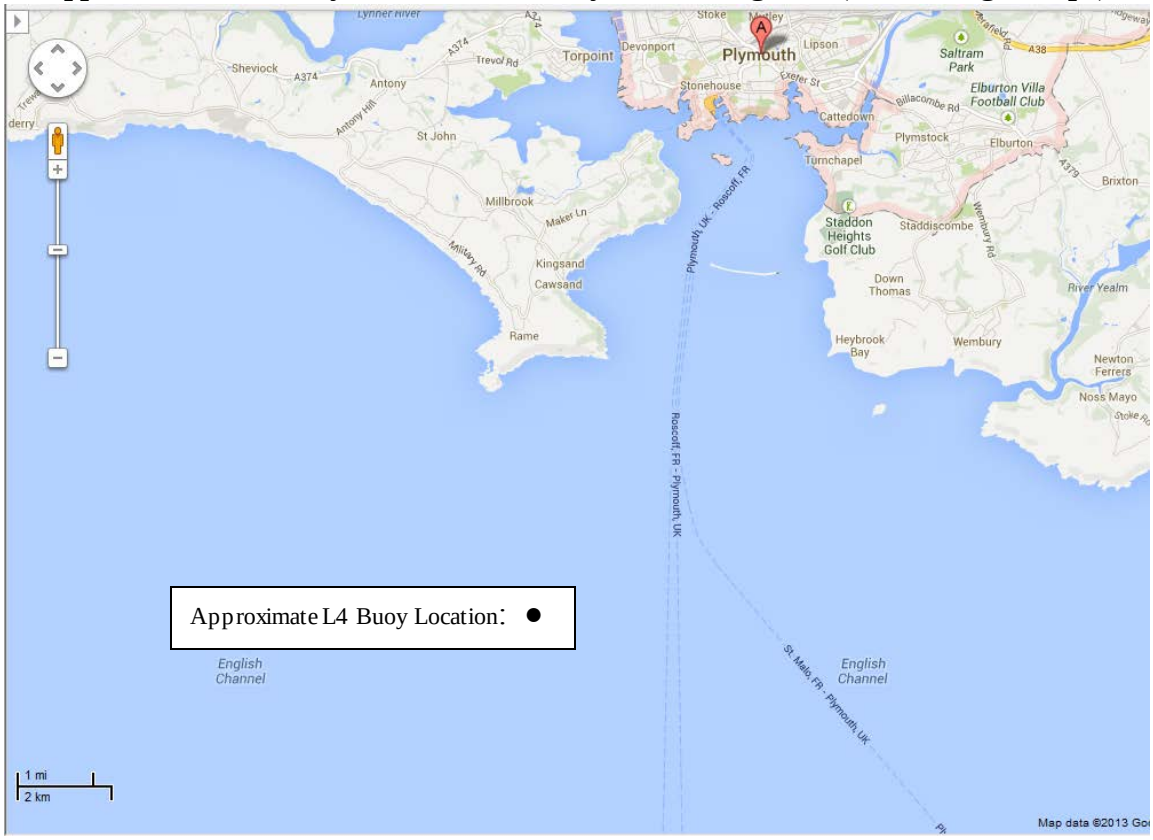
most common on the west coast of continents where these upwelling currents occur[89], but there is also much evidence of *Pseudo-nitzschia* on east coasts, as the first identified outbreak of amnesic shellfish poisoning occurred on the East Coast of Canada. Initial models have been developed to forecast blooms of *Pseudo-nitzschia*[214, 215]. These models can be helpful in determining the need for seafood sampling to protect for acute effects in humans, but models have not been developed to predict concentrations in seafood or to model non-bloom conditions. In coastal areas of low wind and reduced currents, *Pseudo-nitzschia* is able to accumulate in greater cell counts[94].

Materials and Methods for *Pseudo-nitzschia* Data. This section discusses both data sources and analytical methods for examining spatial and temporal trends for *Pseudo-nitzschia*.

Data Sources. Data were collected from two sources, (1) an unpublished data set from the coast of Plymouth England, and (2) data obtained from the published literature.

Plymouth Marine Laboratory Pseudo-nitzschia Data. The Plymouth Marine Laboratory has been collecting *Pseudo-nitzschia* cell count data weekly (weather permitting) off the Coast of Plymouth England since 1992. Plymouth is located on the English Channel approximately 200 miles southwest of London. Data are collected near the L4 buoy located in an area known as the Western Channel Observatory (see Figure 3-1). These data are referred to in the text as the "L4 data".

Figure 3-1
Approximate L4 Buoy Location off of Plymouth, England (from Google maps)



The Western Channel Observatory is an oceanographic time-series and marine biodiversity reference site in the Western English Channel. The buoy is located in about 50 meters of water. The location is typical of temperate coastal waters which are well mixed and contain relatively high nutrient concentrations in the autumn and winter where sea surface temperatures are around 8 Celsius. The salinity of L4 is approximately 34.9±0.40 practical salinity units which is more indicative of marine than estuarine water[216]. The River Tamar is the main source of fresh water in this region. It has a long-term mean flow of 23 cubic meters per second. During spring and summer, temperatures peak at 18 degrees Celsius and weak stratification results in a reduction of nutrient concentrations[217]. Samples are analyzed for phytoplankton and microzooplankton

species abundance and biomass. Paired samples are collected from a depth of 10 meters and preserved with iodine and buffered with formaldehyde. Between 10 and 100 ml of sample, depending on cell density, are settled for more than 48 hours and cells are identified where possible to species level and individuals are counted. Further details of methods used are given in Widdicombe et al.[217]. The L4 sampling location is in a temperate coastal marine region in 50 meters of water[218]. Because it is representative of temperate marine water influenced by coastal nutrient inputs, it is a good surrogate for many coastal waters.

There are 37 known species of *Pseudo-nitzschia* and 14 have been reported to produce DA[219]. Precise determination of each species is extremely difficult and time-consuming and *Pseudo-nitzschia* species are typically lumped into two or three functional groups when reported in the literature. Within the diatoms sampling group at L4, three *Pseudo-nitzschia* functional groups (based on structural similarities in terms of length, width, and shape) are quantified (*Pseudo-nitzschia delicatissima*, *Pseudo-nitzschia pungens*, and *Pseudo-nitzschia seriata*). For much of the analysis of the data in this chapter, the three *Pseudo-nitzschia* species are summed together to obtain a total *Pseudo-nitzschia* count since this chapter's focus is on the presence of persistent low level cell counts of total *Pseudo-nitzschia*. In addition to the weekly phytoplankton counts, weekly L4 data on nitrite, nitrate, ammonia, silicate, and phosphate were also obtained.

Pseudo-nitzschia Data From the Scientific Literature. A literature search was performed to identify other long term monitoring *Pseudo-nitzschia* data sets in the literature. Data sets were identified for Chesapeake Bay, Massachusetts Bay, the Bay of Fundy, and Scotland and are discussed later in this chapter

Statistical Analysis of Data. Analysis of the L4 data used a three-pronged approach consisting of (1) descriptive statistics, (2) visual analysis using figures and (3) non-parametric statistics. These approaches are detailed below. Raw data sets were not available for *Pseudo-nitzschia* studies obtained from the scientific literature and summary statistics, visual analysis and non-parametric statistics therefore could not be performed. Instead the literature data were discussed qualitatively to supplement and provide context to the L4 data.

Descriptive Statistics. Descriptive statistics were used to analyze the data set from the L4 location. L4 data are available for three functional groups *Pseudo-nitzschia delicatissma*, *Pseudo-nitzschia pungens*, and *Pseudo-nitzschia seriata*. The three functional groups were summed to obtain total *Pseudo-nitzschia* cell counts. These data have been collected in the L4 location since 1992. The descriptive statistical approach included evaluation of mean, median, minimum, maximum, standard deviation, and frequency of detection for total *Pseudo-nitzschia* and three functional groups.

Visual Analysis of the Data. A visual approach to data analysis can also be useful when trying to examine trends. Graphs are an effective tool for presenting the pattern of change over time. Total *Pseudo-nitzschia* and functional group cell counts over time at the L4 location were examined to determine if there were any long-term trends. This analysis was performed principally to examine whether or not *Pseudo-nitzschia* is detected more frequently or at higher cell counts in recent data years than it was in past years.

As discussed in Chapter 1, increases in *Pseudo-nitzschia* cell counts have been linked to ambient concentrations of some nutrients in ocean waters. Nutrient

concentrations were plotted along with total *Pseudo-nitzschia* cell counts to attempt to discern relationships between diatom production and nutrients. Weekly nutrient data were collected at the L4 location from the year 2000 onward. Data were available for nitrite, nitrate, ammonia, silicate, and phosphate.

Nutrient data were compared for El Niño (warmer than normal sea surface temperatures), La Niña (cooler than normal), and normal sea temperature years by year-day in order to determine whether sea surface temperature influences nutrient concentrations. El Niño and La Niña are opposite phases of what is known as the *El Niño-Southern Oscillation* (ENSO) cycle. The ENSO cycle is a scientific term that describes the fluctuations in temperature between the ocean and atmosphere in the east-central Equatorial Pacific (approximately between the International Date Line and 120 degrees West)[220]. The ENSO cycle affects the climate not only in the Pacific and tropical areas, but also the North Atlantic and Europe. Studies have shown that ENSO is accompanied by a negative North Atlantic Oscillation index, lower temperatures in northeastern Europe, and greater precipitation which could result in greater runoff and increased inputs of nutrients[85, 221]. The North Atlantic Oscillation Index is a measure of the dominant mode of winter climate variability in the North Atlantic region ranging from central North America and through Europe and into Northern Asia[222]. The index varies from year to year but has tendency to remain in one phase for several years. A positive index indicates an increased pressure difference and results in more and stronger winter storms crossing the Atlantic on a northerly track[222]. This results in warmer and wetter winters for Europe. The North Atlantic Oscillation Index is calculated by projecting 500 millibar height above sea level anomalies of the Northern Hemisphere

onto historic levels from the period 1950-2000. NOAA's Climate Prediction Center provides historical data on sea surface temperatures[220] and categorizes periods as El Niño, La Niña, or normal periods. NOAA characterizes a time period as El Niño if three consecutive months of seas surface temperature are greater than one-half degree Celsius above normal sea surface temperature and a time period as La Niña if three consecutive months are below average sea surface temperatures. Sea surface temperature data are provided in Appendix B. Figures were created that plot *Pseudo-nitzschia* and nutrient concentrations for normal, La Niña, and El Niño years.

Non-Parametric Analysis of the Data. Multivariate time series analysis was used to evaluate the relationship between *Pseudo-nitzschia* and nutrients in the L4 data set. Multivariate analysis is the branch of statistics concerned with analyzing multiple measurements that have been made in a set of data. Multivariate time series analysis evaluates the variance against individual variables and is used to model and explain the interactions among a group of time series variables. Time series are a sequence of data points, in this case weekly nutrient and diatom measurements. Time series analysis accounts for the fact that data points taken over time may have an internal structure (such as autocorrelation, trend or seasonal variation) and examines this structure. Times series analysis looks at the overall pattern of change in an indicator over time.

Statistical tests must have an *a priori* null hypothesis that can never be proven correct but can be rejected with a known risk of doing so incorrectly. This analysis tested the null hypothesis that cell counts of *Pseudo-nitzschia* at L4 are not increasing over time. The probability of rejecting the null hypothesis when it is true (Type I error) was set at $p = 0.05$.

In graphs it is easier to focus on outliers than on subtle changes because gradual changes are hard to detect by eye. Statistical analysis was performed to examine long term trends in *Pseudo-nitzschia* cell counts at L4. The statistical analysis is driven both by the goals of the analysis and by the data set itself. Data were reviewed for:

- Distribution type (normal, lognormal)
- Abrupt Changes (significant changes to the overall patterns)
- Cycles (seasonal, yearly)
- Outliers (data that do not fit the overall pattern of the data set)
- Serial Correlation (correlation of data with itself over successive time periods)

Because of the large number of data points that are below the detection limit (represented by zeros), fitting of the distributions indicated that the data fit a lognormal distribution. This is typical of an environmental data set. Statistics for nonparametric approaches are not as dependent on assumptions about data distribution

Time Series Analysis. Times series analysis was performed on the L4 data set to determine if there was a long-term trend. Time series analyses require that all data are observed, and that there are no gaps with missing data in the time series. Missing data embedded in the series have to be replaced in some way. There are a range of different methods for dealing with missing data. In this case, the missing data were replaced with interpolation from adjacent points. Zeroes in the data must also be replaced for the purpose of log transforming the data. Since the log of zero is undefined, zeroes are replaced with interpolation. The data from 1992 through May 1995 were removed from the analysis because of a several month data gap in the first half of 1995, which might have affected the overall results, as interpolation could not be used to reasonably fill such

a large data gap. Therefore, the data for the period June 1995 through December 2009 were included in the time series analysis.

Exponential smoothing was performed on the L4 data. Exponential smoothing is a weighted moving average model of data. Moving average smoothing (the most common technique) involves averaging of data points across a time period so that nonsystematic components of individual observations are spread across a larger time period and thus "smoothed". A predetermined number of data points (in this analysis either four or twelve) over a specific time period are averaged together, with the new estimated value replacing the observed value. Exponential smoothing assigns exponentially decreasing weights as the observations get further away from the point in time that is being modeled. Smoothing parameters determine the weights assigned to the data points.

One type of smoothing that was performed was the use of a 4253H filter. This transformation consists of several rounds of moving average/median smoothing including:

- A 4 point moving median centered by a moving median of *2*,
- A 5 point moving median,
- A 3 point moving median, and
- A 3 point weighted moving average.

Residuals are computed by subtracting the transformed series from the original series. These steps are then repeated for the residuals and transformed residuals are added to the transformed series.

Pseudo-nitzschia cell counts vary seasonally and therefore autocorrelation analysis was performed for the L4 data. Autocorrelation was performed to look at the similarity in the time lag between data points as a method to find repeating patterns. Partial autocorrelation, where dependence on intermediate elements (i.e., those within the lag) was also performed. Seasonal decomposition was performed to examine seasonal, trend, and irregular components of the time series.

Regression Analysis for Nutrients and Pseudo-nitzschia Levels. Multiple regression was performed on nutrient and *Pseudo-nitzschia* L4 data sets to examine the relationship between independent or predictor variables (nitrate, nitrite, ammonia, phosphate and silicate) and the dependent variable (*Pseudo-nitzschia* cell counts). Partial correlations were calculated to look at the contribution of each independent variable to the prediction of the dependent variable. A distributed lags analysis was also performed for the data. Distributed lags analysis is a technique for examining relationships between variables that involve some delay where a change in one variable causes a delayed change in another. This analysis evaluated whether there is a lagged relationship between nutrient concentrations and a delayed change in *Pseudo-nitzschia* cell counts.

Results and Discussion for the *Pseudo-nitzschia* Data. This section focuses on an analysis of the L4 data collected off the Coast of Plymouth, England by the Plymouth Marine Laboratory and is supplemented by data from other locations in the published literature. Data were analyzed for frequency of measureable cell counts and overall trends in *Pseudo-nitzschia* densities using descriptive statistics, visual approaches, and non-parametric techniques. Collocated nutrient data are analyzed for nutrients that

are covariant with *Pseudo-nitzschia* cell counts. These approaches and their results are detailed in the following sections.

Levels of Concern for *Pseudo-nitzschia* Species. Currently available levels of concern for *Pseudo-nitzschia* provide context for the L4 data. Washington State and Great Britain have developed *Pseudo-nitzschia* action levels in water that would trigger concerns about DA in seafood. While these levels of concern are regarding the potential of acute effects and amnesic shellfish poisoning rather than more subtle effects documented in the recent toxicological literature, they provide a reference point for concern when the L4 data are analyzed.

Washington State performs regular *Pseudo-nitzschia* data collection and has grouped species into three categories according to size and morphological similarities[23]. Each category has its own density of cells that triggers DA testing in seafood. DA testing is triggered when any of the following conditions are met: (1) at least 30 cells per milliliter for *P. australis/heimii/fraudulenta* (short and broad species), (2) at least 1,000 cells per milliliter for *P. pseudodelicatissima/delicatissima* (small and narrow species), or (3) at least 100 cells per milliliter for *P. multiseries/pugens* (long and narrow species). Great Britain does not distinguish between functional groups but instead has a number for total *Pseudo-nitzschia*. The Centre for Environment, Fisheries & Aquaculture Science in Great Britain has set an Action Limit of 150 cells per milliliter for total *Pseudo-nitzschia* species[223]. When this level is exceeded, shellfish samples are tested for DA. The threshold level has since been lowered to 50 cells/ml when it became apparent that DA concentrations of concern could occur at *Pseudo-nitzschia* densities below the original threshold[224]. A cell count of *Pseudo-nitzschia* in water that is protective of chronic low

level exposure to DA in seafood is likely to be significantly lower than these action levels. The cell density data for L4 are discussed below.

Descriptive Statistics. Descriptive statistics are used below to analyze the available data set. L4 data are available for total diatoms, as well as the functional groups *Pseudo-nitzschia delicatissma*, *Pseudo-nitzschia pungens*, and *Pseudo-nitzschia seriata*. Diatom data have been collected in the L4 location since 1992. Data were limited the first year (seven data points in 1992), but were collected almost weekly afterwards. There were 692 measured data points over eighteen years, for an average of 38 data points per year. Summary statistics (mean, median, standard deviation, minimum, and maximum) for the L4 data from 1992-2009 data are presented below.

Table 3-1 Summary of *Pseudo-nitzschia* Observations 1992-2009 at Location L4				
	Pseudo-nitzschia delicatissma	***Pseudo-nitzschia pungens***	***Pseudo-nitzschia seriata***	**Total *Pseudo-nitzschia***
Mean (cells/ml)	31	0.40	2.6	34
Median	0.18	0	0	0.48
Minimum (cells/ml)	0	0	0	0
Maximum (cells/ml)	2850	38	561	2850
Standard Deviation (cells/ml)	186	2.8	23	188
Number of Samples	692	692	692	692
Number of Detects	432	81	235	523
Number of Non-Detects	260	611	457	169
Percent of Samples >0	62.6	11.7	34.0	75.3
Relative Standard Deviation (%)	600	700	885	553

A total of 692 observations represent a fairly robust data set and a larger data set than any that was identified in the scientific literature. *Pseudo-nitzschia delicatissma* dominated the cell counts, with a mean of 31 cells/ml. This represents only a fraction of the total diatoms present (mean of 165 cells/ml). Cell counts of *Pseudo-nitzschia delicatissma* varied more than the other two measured *Pseudo-nitzschia* groups, with a standard deviation of 186 cells/ml (versus 2.8 for *Pseudo-nitzschia pungens* and 23 for *Pseudo-*

nitzschia seriata). Relative standard deviations (the absolute value of the coefficient of variation) were also very high for all categories (553% - 885%) indicating substantial variability in the data.

When the three *Pseudo-nitzschia* species are added together, there are 33 weeks out of a total of 692 weeks that the measured total cell count exceeds the British total *Pseudo-nitzschia* action level of 50 cells/milliliter (roughly 5% of the time). There are 489 observations out of 692 where cell counts are less than the action level but above zero, which demonstrates that *Pseudo-nitzschia* are generally present at low levels most of the year (*Pseudo-nitzschia* is detected in about 75% of all samples). There were 168 observations that were zero (about one quarter of the observations). Recent toxicological literature (summarized in Chapter 2) indicates that DA can cause significant behavioral and learning effects at low doses. Given that the *Pseudo-nitzschia* cell counts are detectable but below levels that would trigger testing of seafood for DA, there is the potential for low-level concentrations of DA in seafood through most of the year.

Visual Approach to the Data. This section uses figures to examine whether total *Pseudo-nitzschia* (sum of the three functional groups) is detected more frequently in recent data years than it was in past years. A similar visual approach to the data is taken to examine long-term trends. This is supplemented by a non-parametric statistical approach to the data. Figure 3-2 below shows total *Pseudo-nitzschia* data for 1992-2009 graphed by year:

Figure 3-2
Total Annual *Pseudo-nitzschia* Concentrations by Year

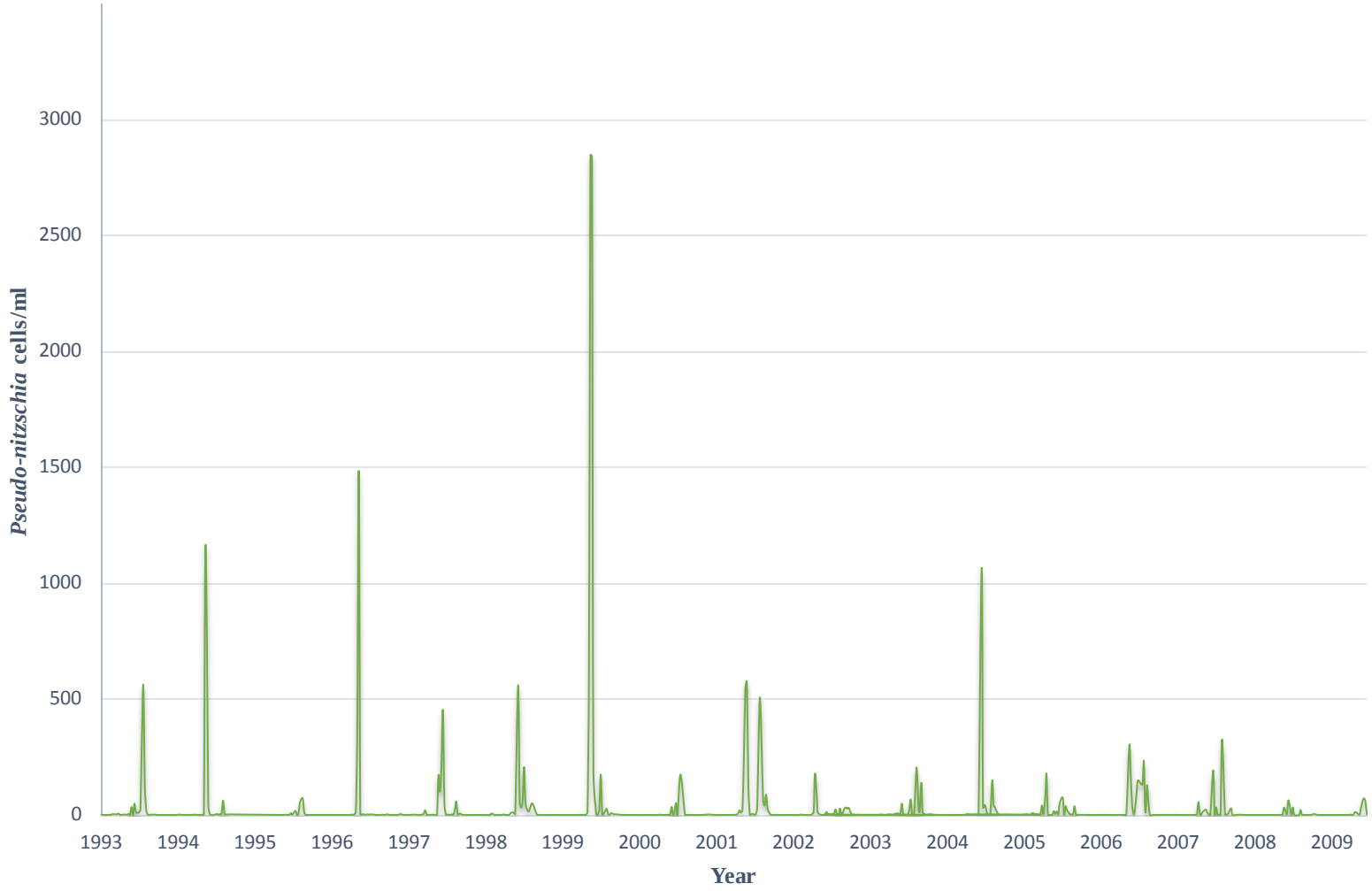


Figure 3-2 illustrates how *Pseudo-nitzschia* cell counts are typically viewed in the scientific literature, where the focus is on high densities during bloom events. There is a repetitive seasonal component to the data while peak heights vary. This figure shows the variable nature of the cell count data and how blooms dominate this graph of the data. The persistence of low level densities is lost in the figure. Between bloom events, cell counts appear to drop to zero, while in actuality *Pseudo-nitzschia* are detected about 75% of the time in the sampling data. Papers in the scientific literature have generally focused on bloom events. There are increasingly moderate peaks leading to a 1999 spike and then a general decline in peaks through 2009. Figure 3-3 puts the data in a log scale scatter plot to better illustrate the range of cell counts:

Figure 3-3
Total Annual *Pseudo-nitzschia* Concentrations by Year, Log Cell Counts

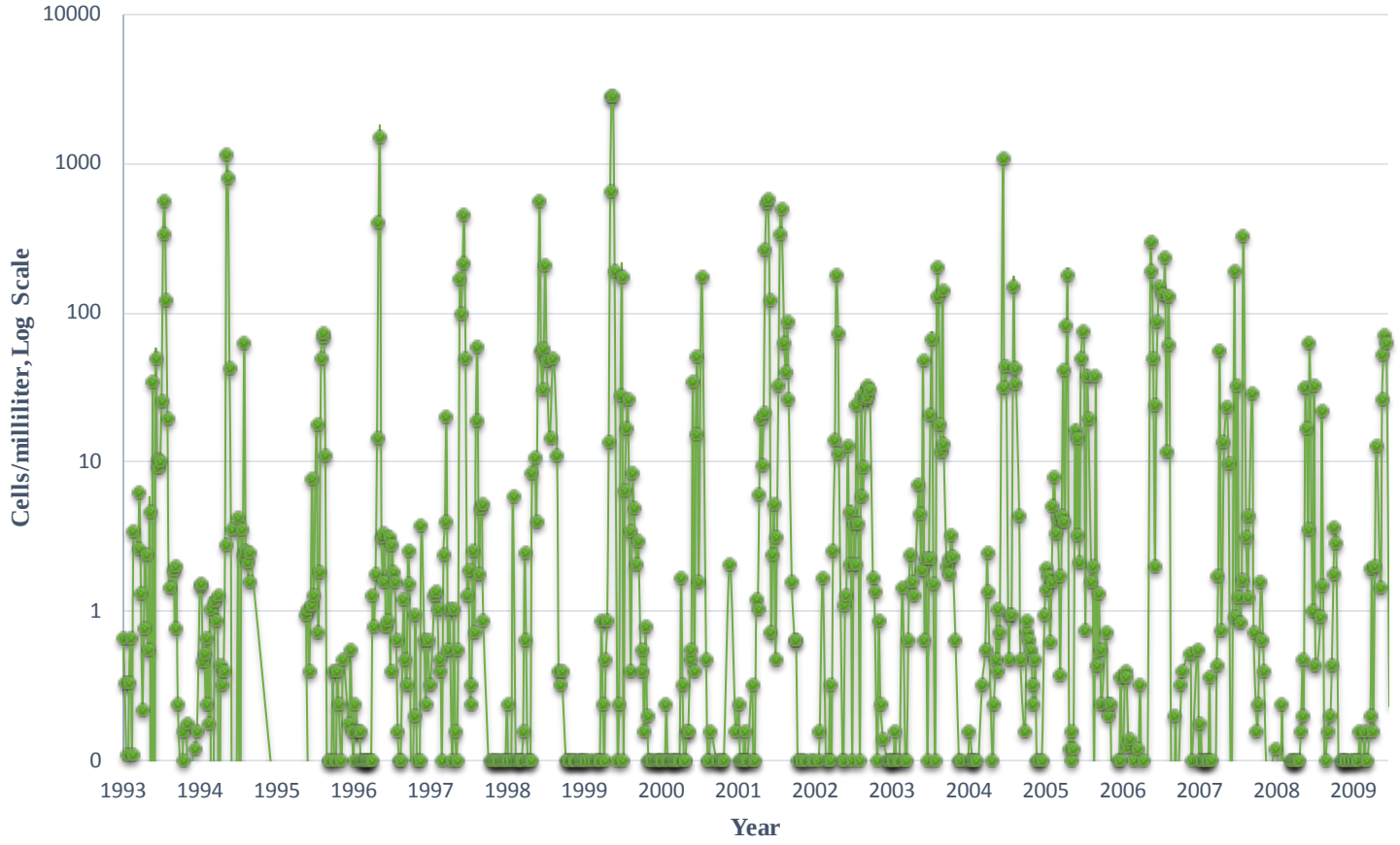


Figure 3-3 shows that persistent lower level cell densities are masked by a focus on the peak bloom events. The data are richer than a bloom focus would elucidate. The previous chapter discussed evidence that chronic low level exposure could be important in terms of toxicity. This chapter provides evidence of consistent presence of low level cell counts.

Figure 3-4 below overlays the weekly cell densities (plotted as calendar day sampled) for each year (i.e., each line representing one year's worth of data), allowing for an easy comparison of the cell count data from year to year.

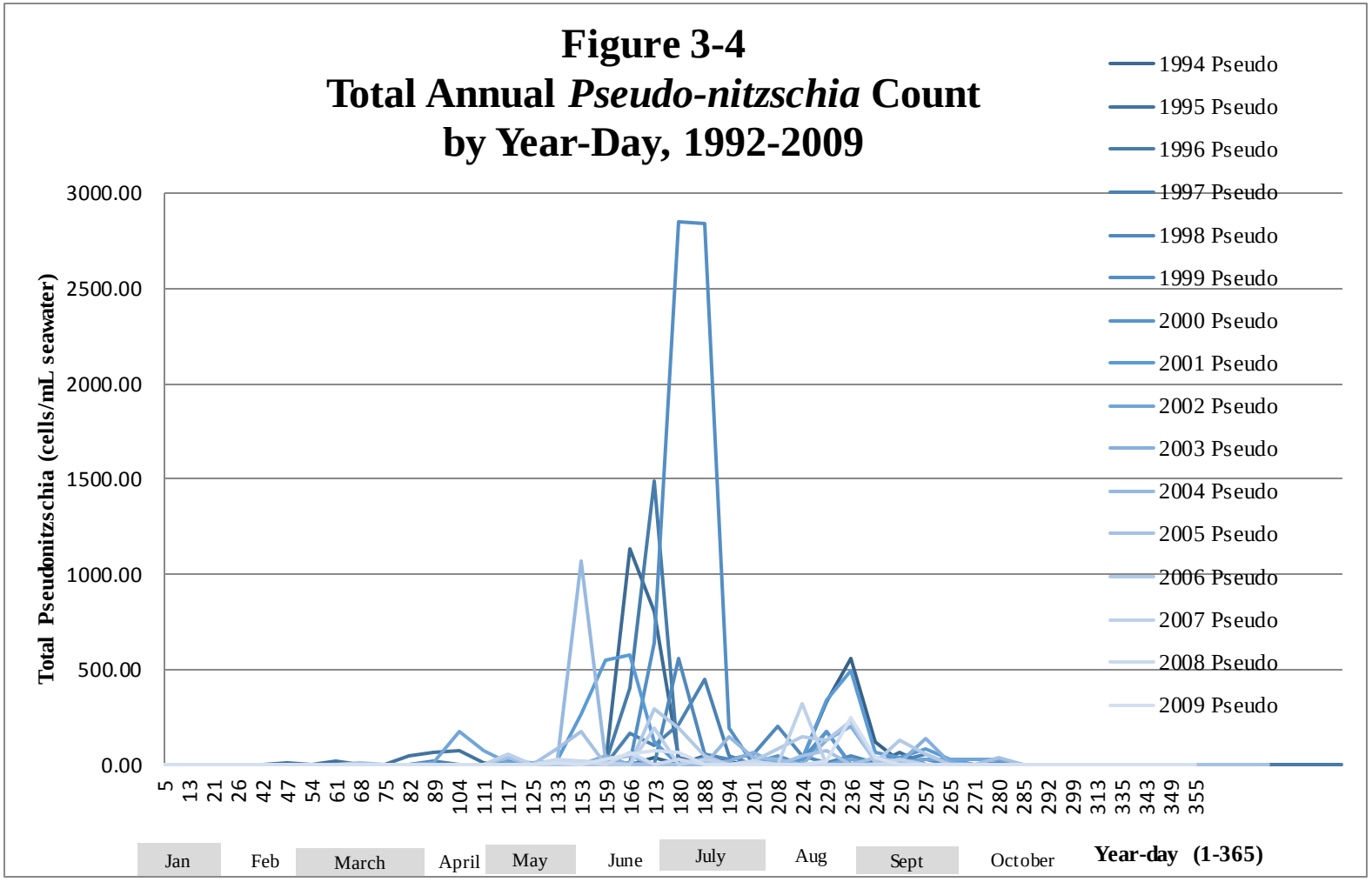


Figure 3-4
Total Annual *Pseudo-nitzschia* Count
by Year-Day, 1992-2009
Total Pseudonitzschia (cells/mL seawater)
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
5 13 21 26 42 47 54 61 68 75 82 89 104 111 117 125 133 153 159 166 173 180 188 194 201 208 224 229 236 244 250 257 265 271 280 285 292 299 313 335 343 349 355
Jan
Feb
March
April
May
June
July
Aug
Sept
October
Year-day (1-365)
1994 Pseudo
1995 Pseudo
1996 Pseudo
1997 Pseudo
1998 Pseudo
1999 Pseudo
2000 Pseudo
2001 Pseudo
2002 Pseudo
2003 Pseudo
2004 Pseudo
2005 Pseudo
2006 Pseudo
2007 Pseudo
2008 Pseudo
2009 Pseudo

This chart is color coded with the earlier years in dark, while progressing through lighter shades for more recent years. The x-axis is depicted in "year-days" where January 1st is day 1 and December 31st is day 365. The data show that there is generally a large bloom in the late spring/early summer (June/July), followed by a smaller bloom in the late summer/early fall (August-September). The exact timing of the blooms varies from year to year. There is also no discernible trend from one year to the next. Most of the higher peaks occur in the earlier years of the sampling period (1993-2000) (also see Figure 3-2). The data show significant variability both month to month and year to year.

One of the primary goals of this chapter is to examine if low levels of *Pseudo-nitzschia* are present throughout most of the year. Persistent low levels of *Pseudo-nitzschia* could result in chronic exposure to DA in seafood for individuals who consume seafood regularly. The current reference dose for DA assumes an acute exposure period and the current action level for seafood assumes a single meal exposure (as discussed in Chapter 2). If *Pseudo-nitzschia* is persistent and present for most of the year, then the protectiveness of the single exposure assumption is called into question. The figures below focus on low density data. The first figure shows *Pseudo-nitzschia* cell count data that are less than 100 mg/L:

Figure 3-5
Weekly Total *Pseudo-nitzschia* Densities
<100 Cells/Milliliter

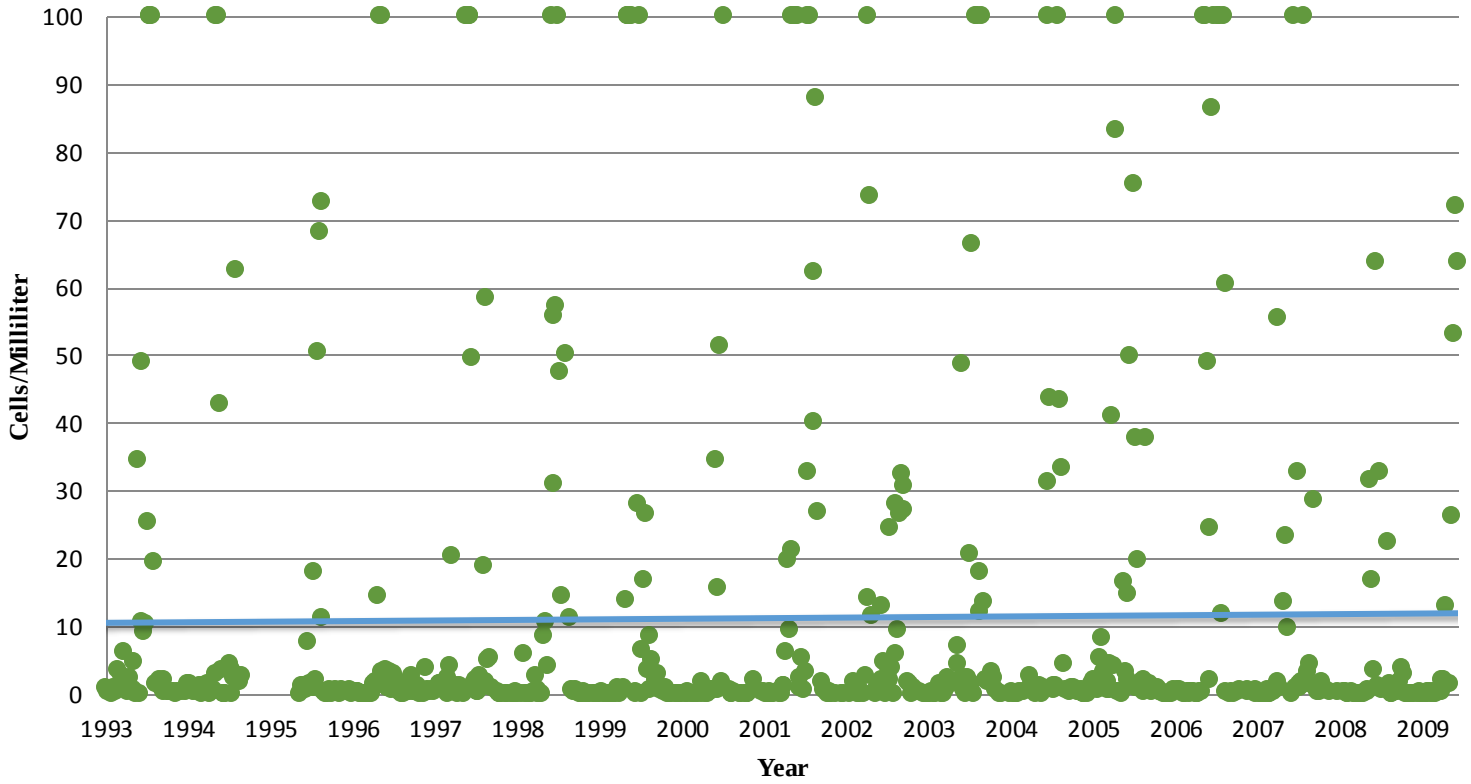
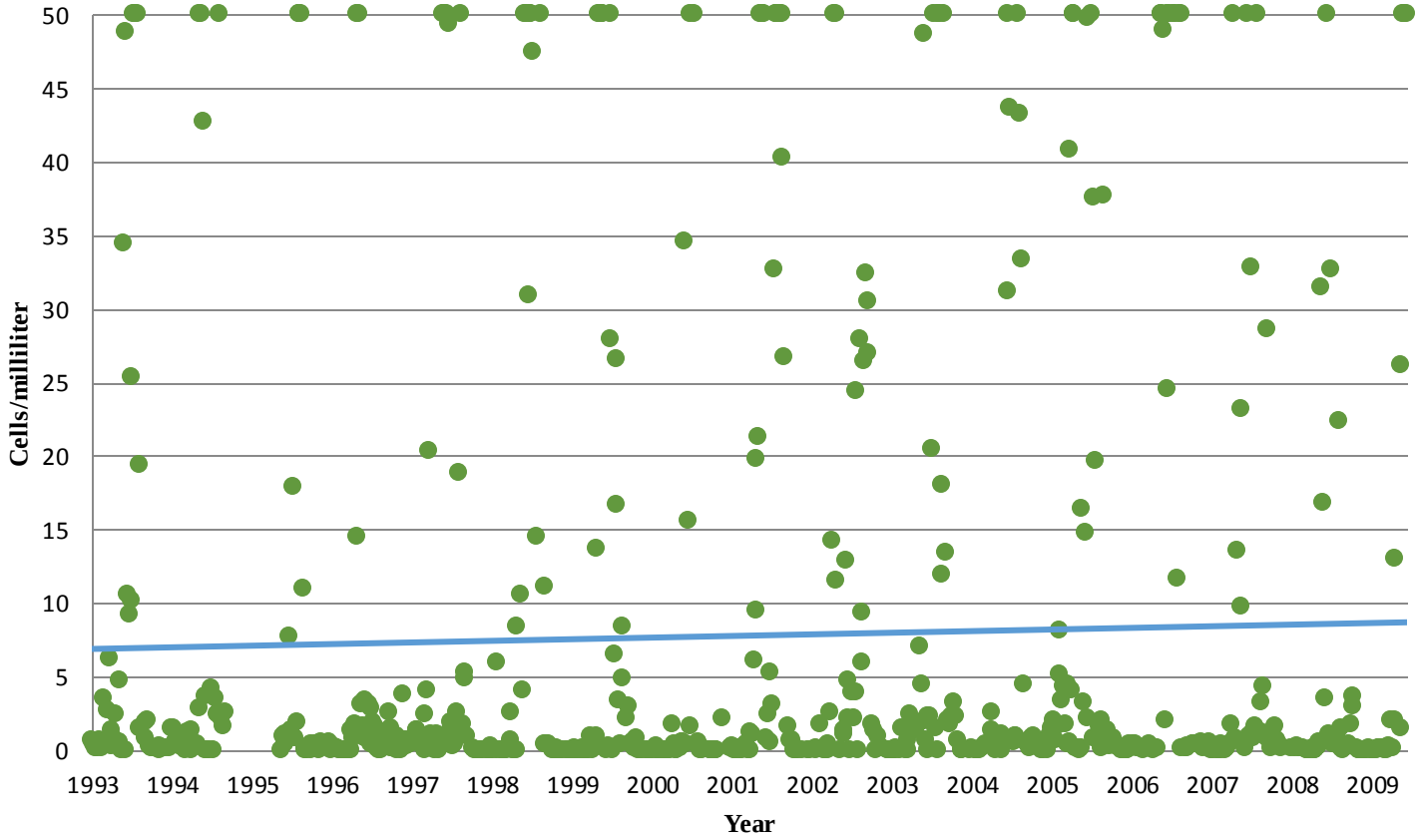


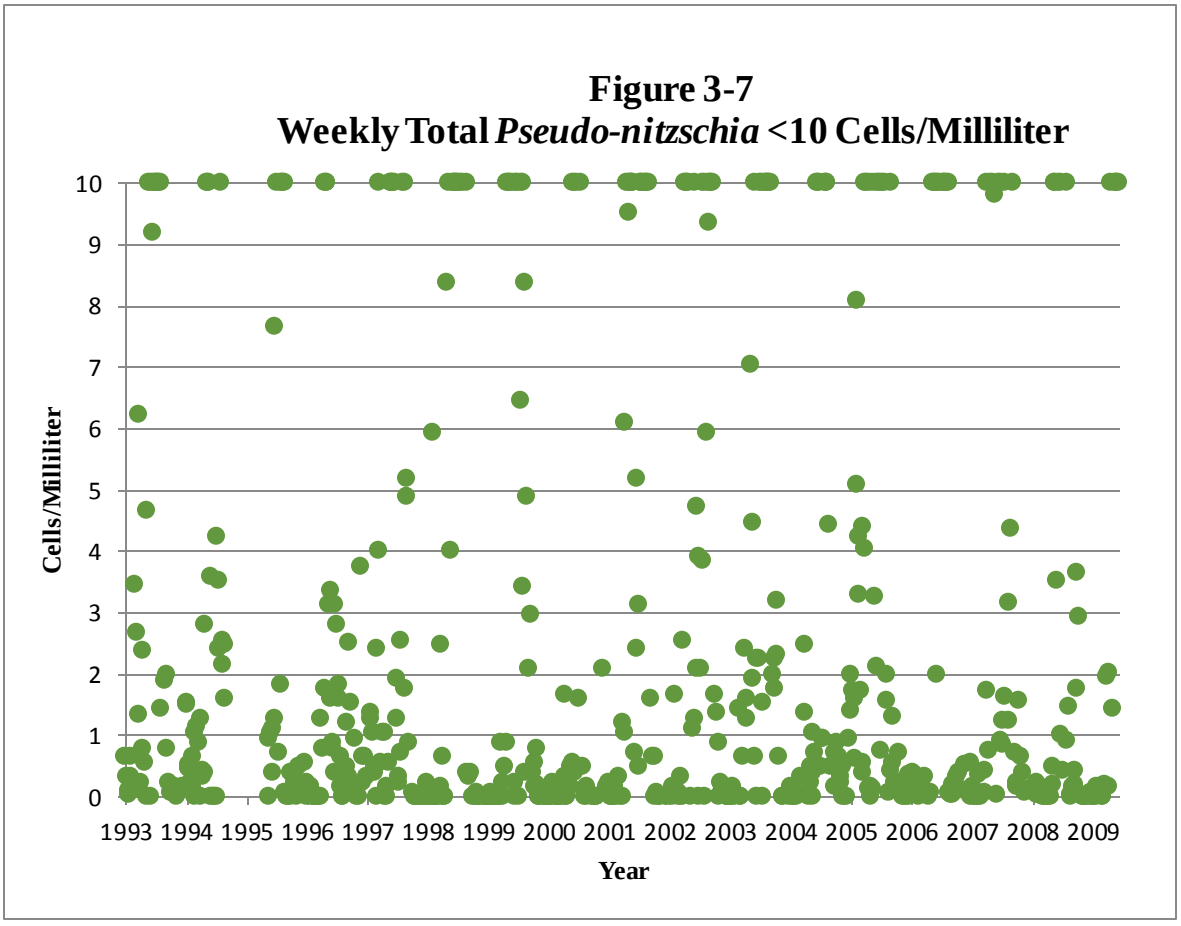
Figure 3-5 shows that cell counts are often detected below 100 cells/ml. There is no discernible trend line over time. Figure 3-6 shifts focus on even lower cell densities, focusing on data less than 50 cells/ml.

Figure 3-6
Weekly Total *Pseudo-nitzschia* Densities <50 Cells/Milliliter



Here too there is no discernible trend as indicated by the straight line. Detections are frequent below 50 cells/ml (the British action level). The British action level may represent a reasonable cut-off for what may constitute a bloom, but demonstrates that lower but measureable cell counts are frequently present and available for production of DA.

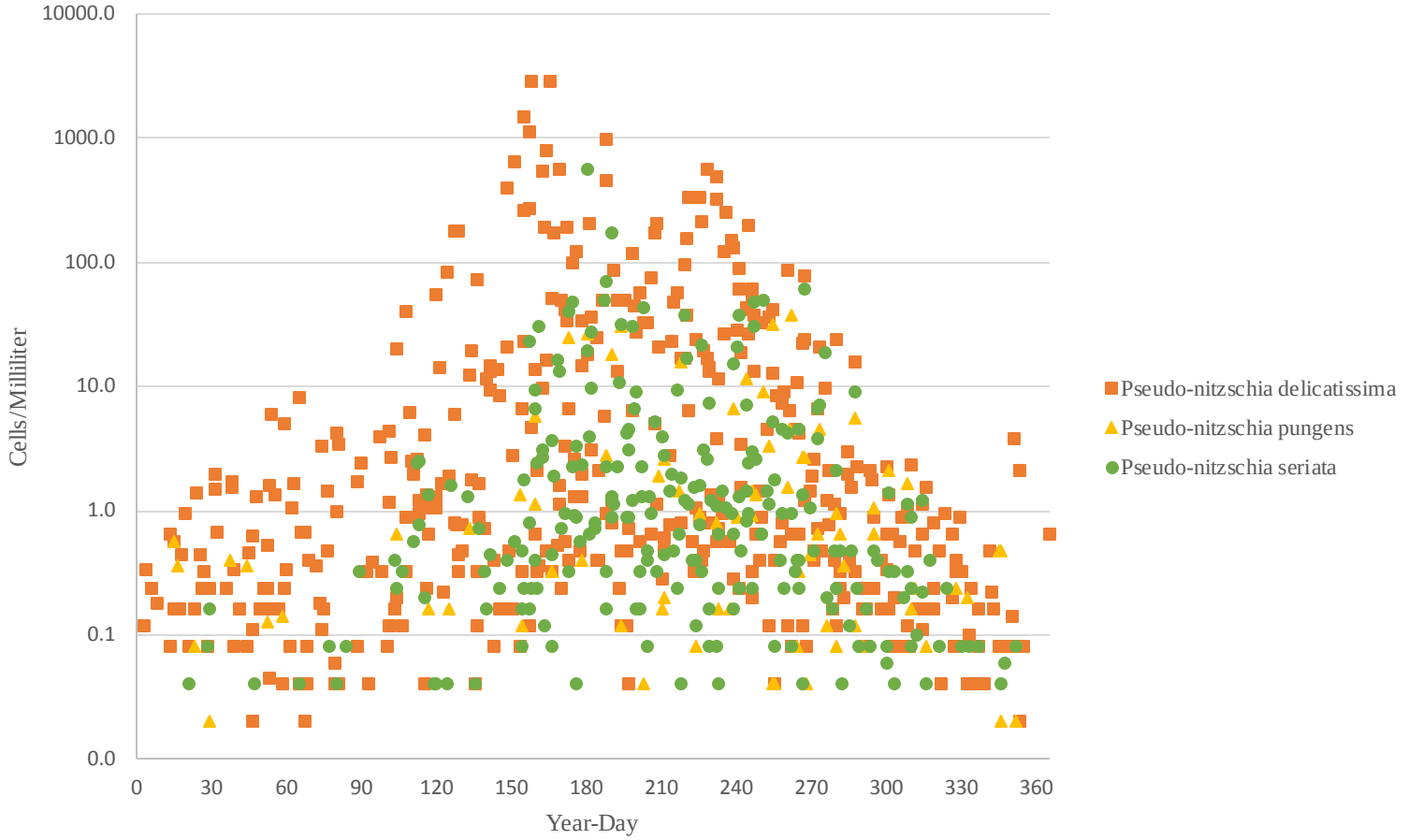
The last figure in this series (Figure 3-7) illustrates cell counts less than 10 cells/ml.



There was also no trend for this figure, and the trend line was left off for ease of viewing low level cell counts. Figure 3-7 illustrates that low cell counts are peristent at the L4 location.

The figures above all represent total *Pseudo-nitzschia* cell counts. Figure 3-8 depicts cell counts by year-day for the three available functional groups of *Pseudo-nitzschia* separately.

Figure 3-8
***Pseudo-nitzschia* Functional Group Cell Counts by Year-Day**



All three functional groups of *Pseudo-nitzschia* depicted produce domoic acid. *Pseudo-nitzschia delicatissima* dominated total Pseudo-nitzschia concentations at L4, with *Pseudo-nitzschia seriata* occasionally producing the top cell counts. *Pseudo-nitzschia pungens* remained at relatively low densities. *Pseudo-nitzschia delicatissima* shows an overall pattern of large blooms in yeardays 120-180 (beginning of May through the end of June) followed by a smaller bloom in yeardays 215-250 (August through mid-September). Blooms of *Pseudo-nitzschia seriata* and *Pseudo-nitzschia pungens* were smaller but their timing was similar.

Figure 3-9 below also presents 1992-2009 data on *Pseudo-nitzschia* functional groups, but presents it year by year, rather than by year-day.

Figure 3-9
***Pseudo-nitzschia* Functional Group Cell Counts By Year**

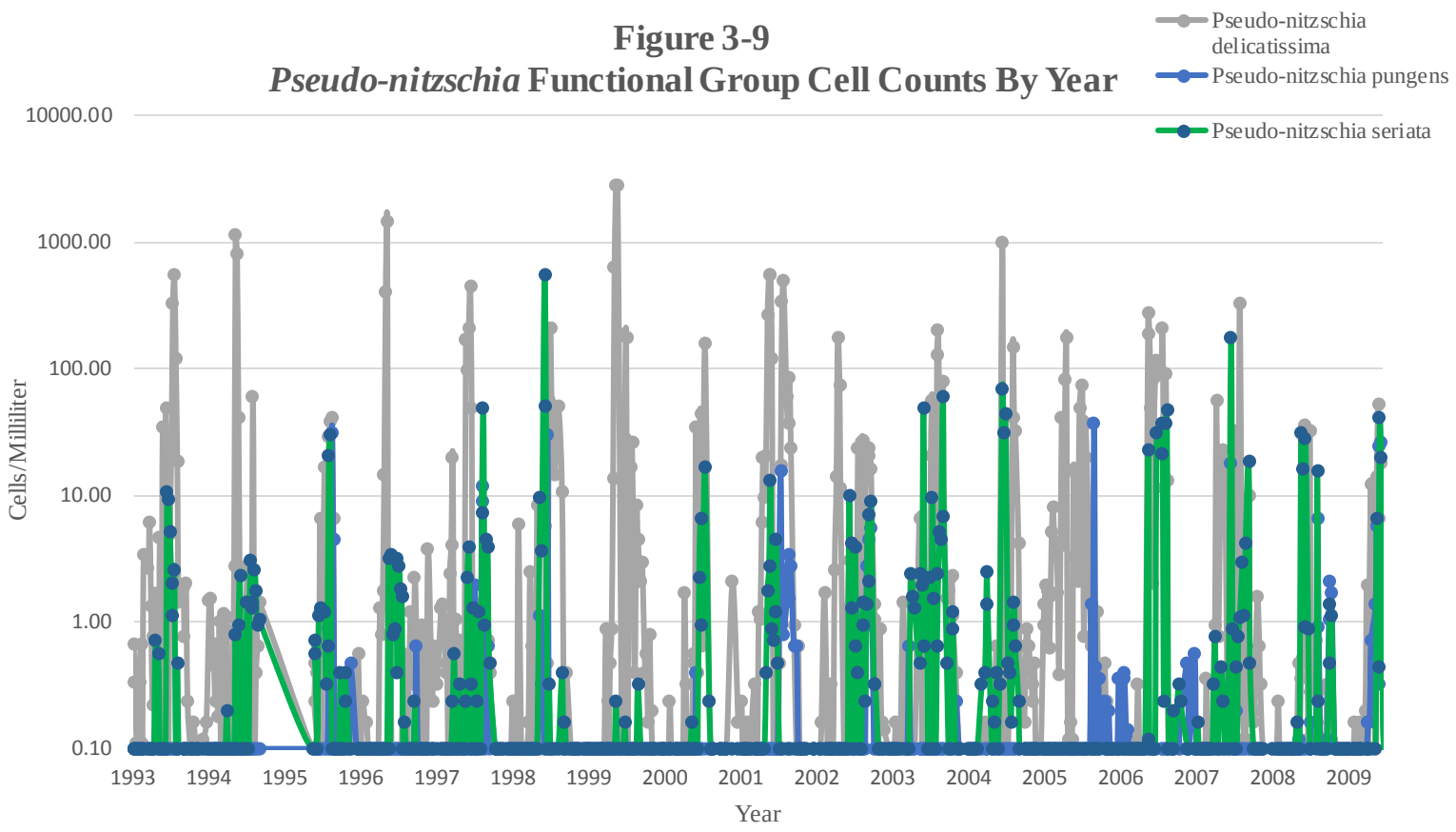
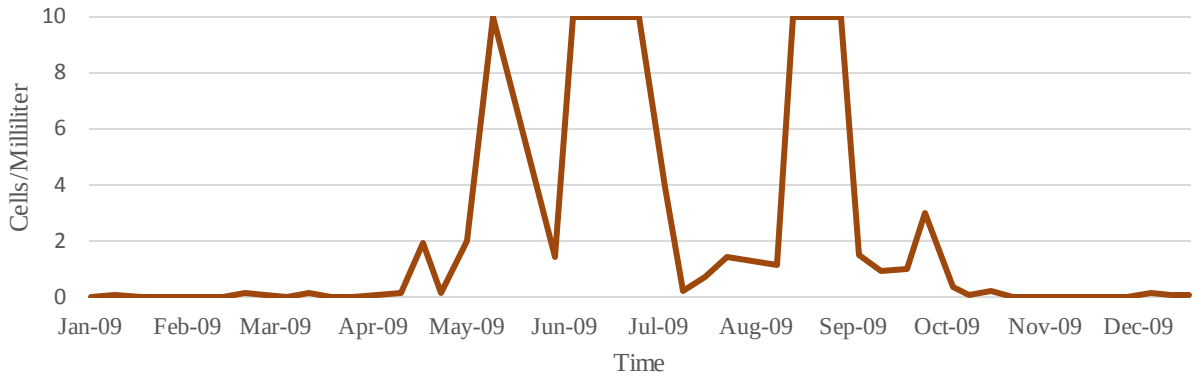


Figure 3-9 shows that *Pseudo-nitzschia delicatissima* is the dominant subgroup in the majority of weeks, but *P. pungens* and *P. seriata* are also significant contributors to total *Pseudo-nitzschia* many weeks. At a given time in a year, any of the three functional groups can dominate the cell counts. Overall there are persistent low cell counts of all three *Pseudo-nitzschia* functional groups.

When considering persistent low level cell counts it can also be illustrative to examine data for a single year. Total *Pseudo-nitzschia* cell counts are presented below for the most recent sampling year in the data set (2009).

Figure 3-10
Total *Pseudo-nitzschia* Cell Counts < 10 Cells/Milliliter
2009 Data



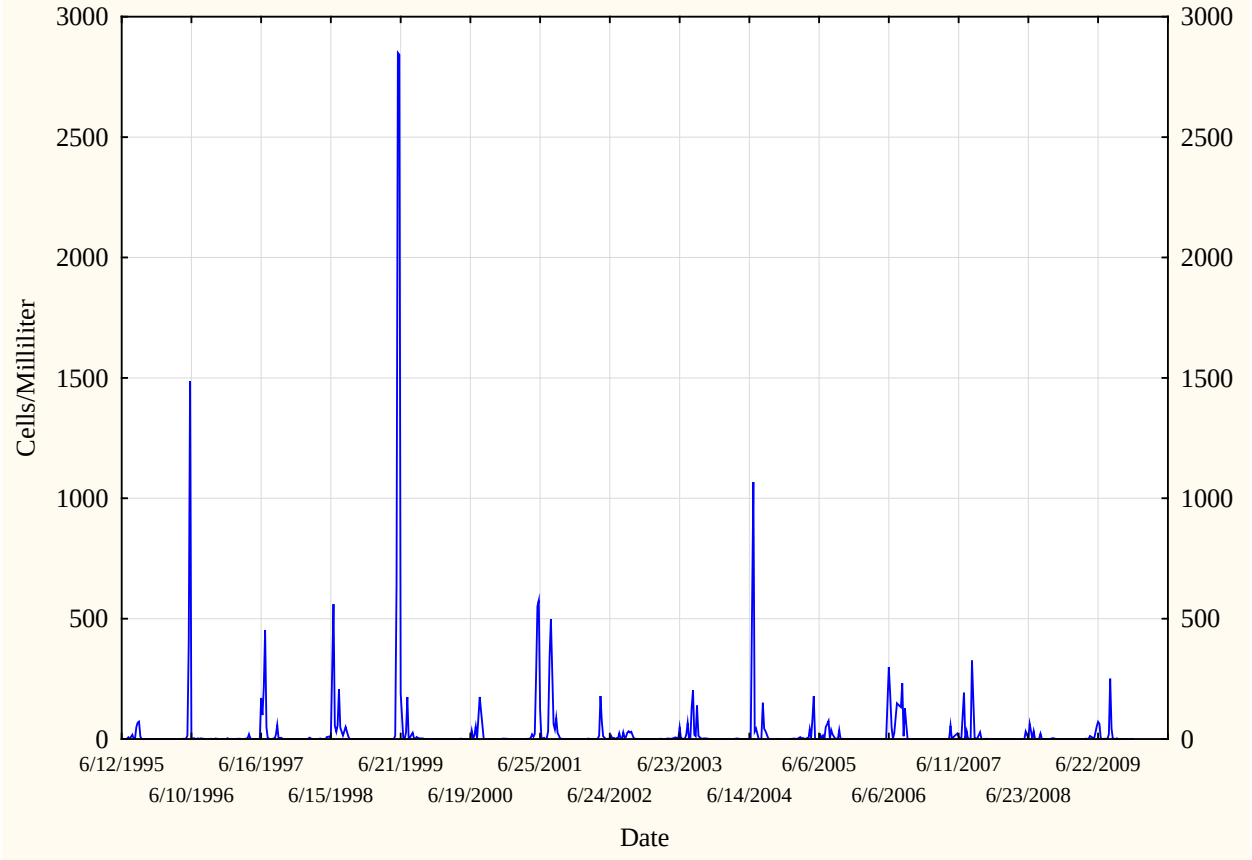
There were forty-four weekly measurements taken in 2009. *Pseudo-nitzschia* was present in detectable densities in 80% of the weekly samples (35 of 44 samples). Eight of these weekly measurements were greater than 10 cells/ml and four of these values exceeded the British Action Limit of 50 cells/ml. Nine of the weekly measurements were recorded as non-detect. Twenty-seven of 44 weekly 2009 measurements were greater than zero, but less than 10 cells/ml. This illustrates that cell counts of *Pseudo-nitzschia*

were present at low levels throughout most of the year, below the action limit, but with the potential to contribute persistent low level concentrations of DA in seafood.

Non-Parametric Approach to Examining Long-Term Trends in the L4 *Pseudo-nitzschia* Data. This section discusses the results of non-parametric statistical analysis to examine the relationship between *Pseudo-nitzschia* cell counts and nutrients.

Time Series Analysis of the Diatom Data 1992-2009. Time series analysis was performed on the data set to determine if there is a long-term trend. Time series analysis assumes that the data consist of a systematic pattern and random noise (error). Times series analysis filters out the noise to make the pattern more evident. The statistical analysis evaluates the data for both seasonality and trend.

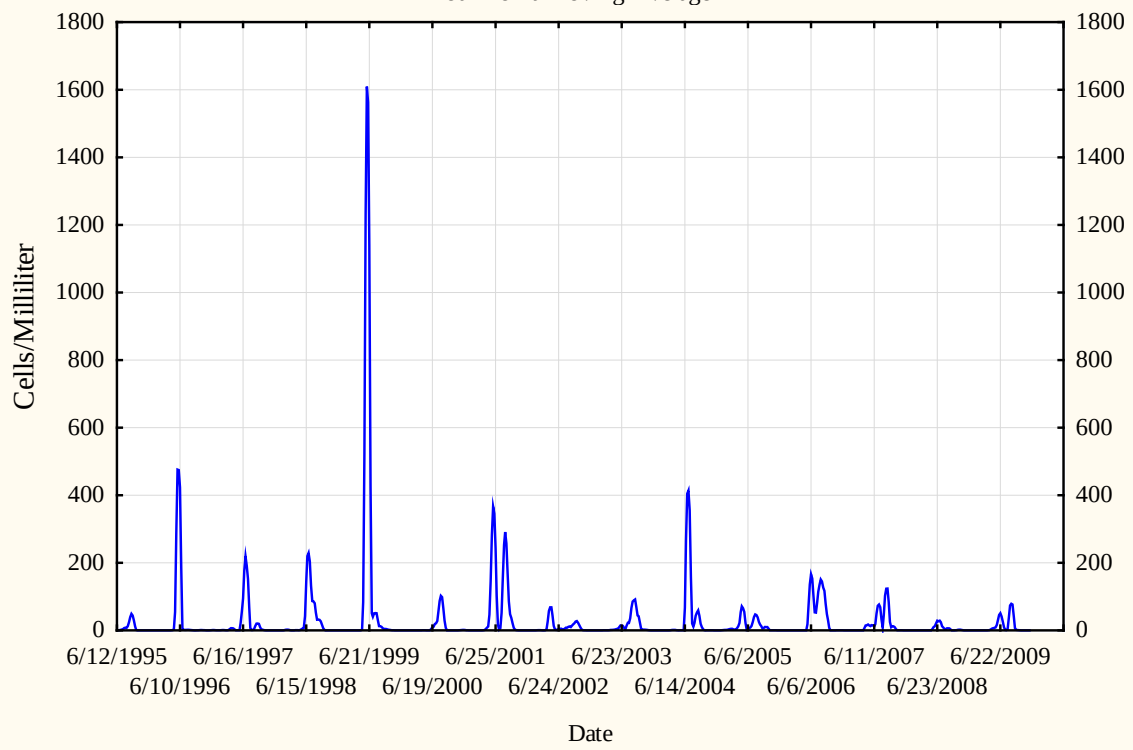
Figure 3-11
Total *Pseudo-nitzschia* by Year



When the total *Pseudo-nitzschia* data are plotted in Figure 3-11, they show steep peaks once a year interspersed with periods of low cell counts.

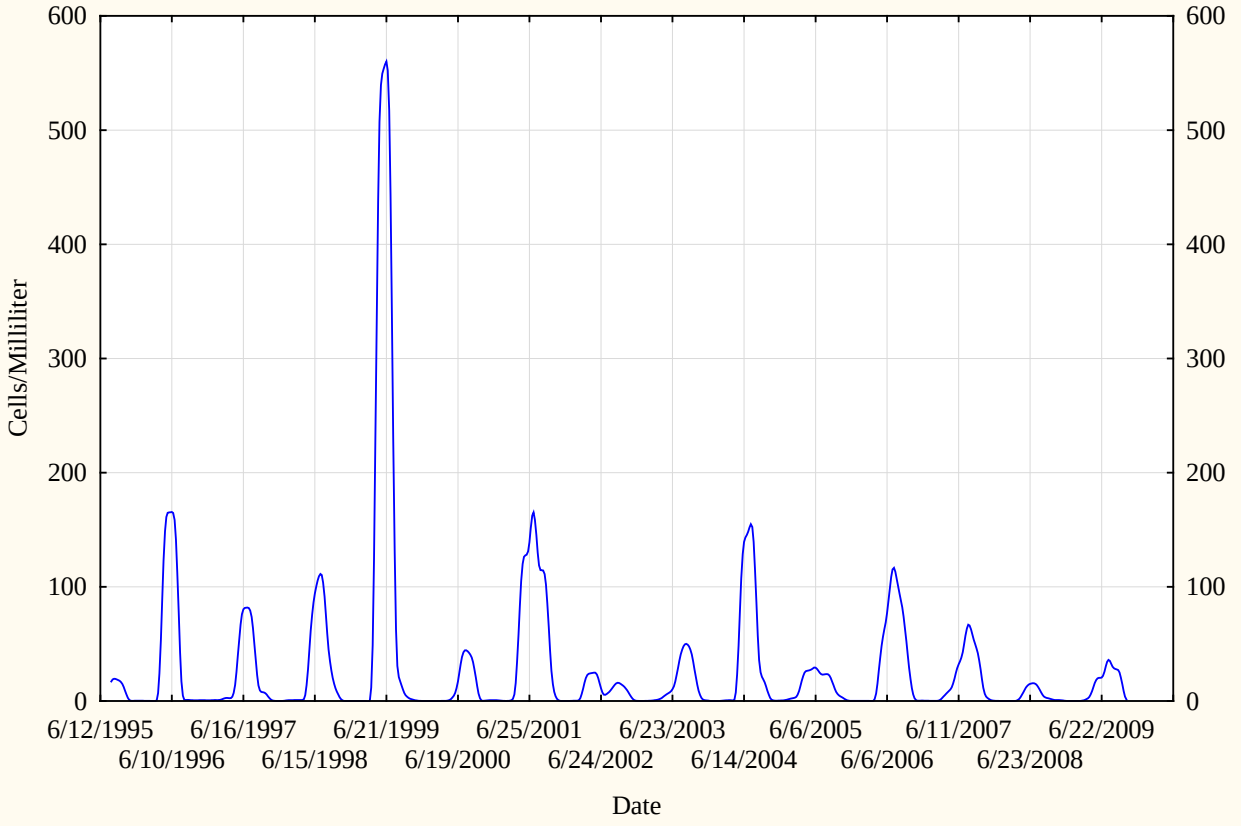
Exponential Smoothing. The time series after exponential smoothing is shown in Figure 3-12:

Figure 3-12
Smoothed Total *Pseudo-nitzschia* Data
Four Point Moving Average



The plot is refined somewhat by averaging four weekly data points over time to represent each week. The data are further refined in Figure 3-13:

Table 3-13
Smoothed Total *Pseudo-nitzschia* Data
Twelve Point Moving Average and T4253H Filter



In this plot, the data were log-transformed and smoothed using a twelve point moving average and a 4253H filter. The smoothed data appear to indicate a declining trend in the peaks, with 1999 cell densities standing out at as significantly higher than any other available years.

Figure 3-14
Total *Pseudo-nitzschia* Data
Smoothed Series and Residuals

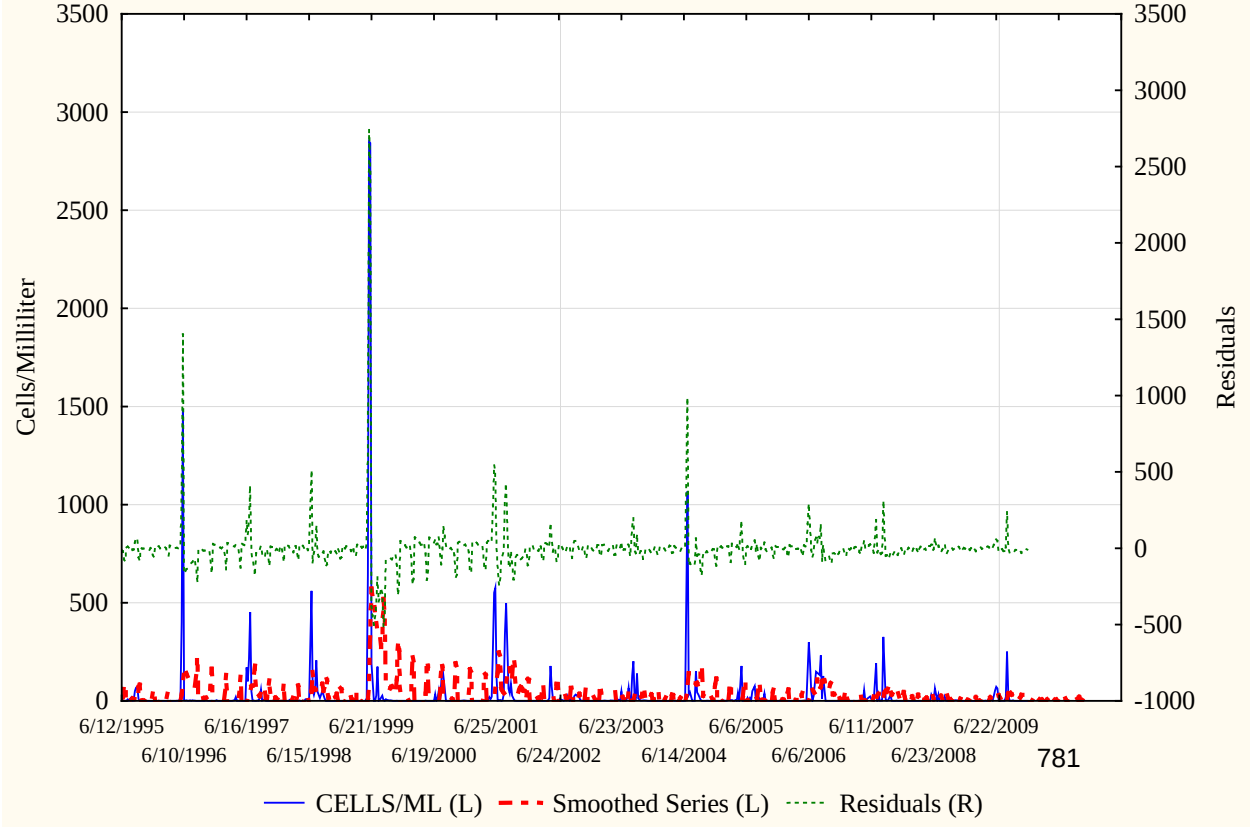
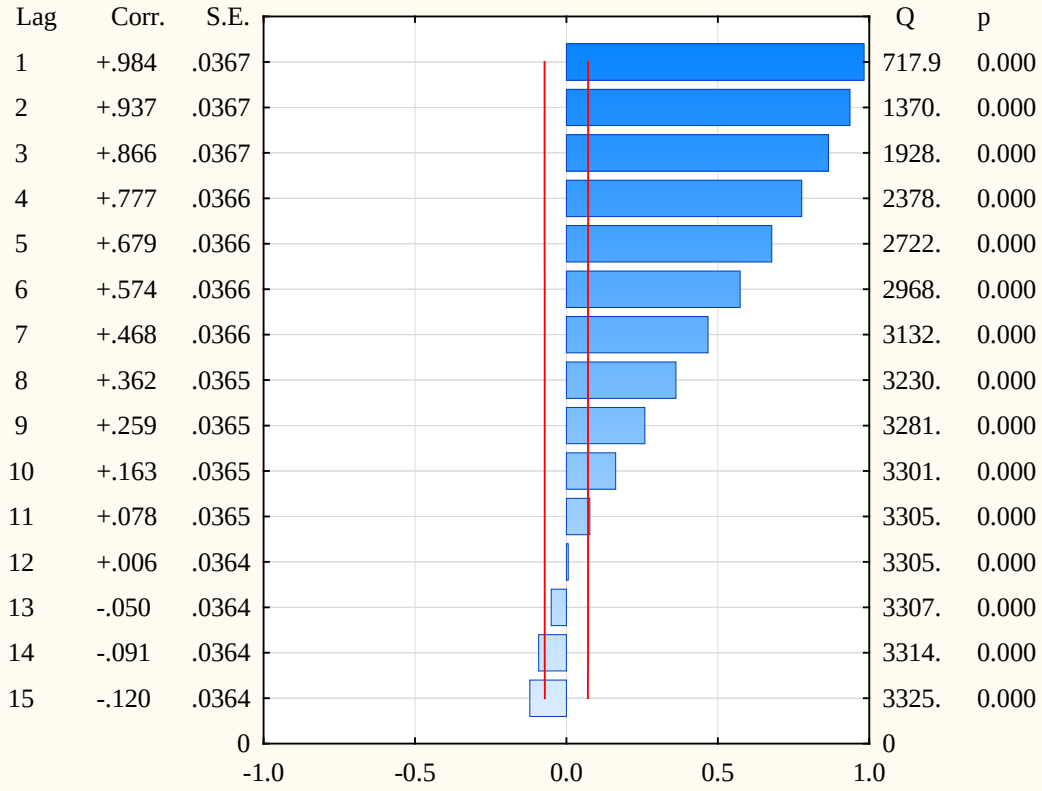


Figure 3-14 shows the original time series (blue solid line), the smoothed series (thick red dashed line), and the residuals (dashed green line). After smoothing, the smoothed series shows no long-term trend. While low cell counts are persistent, there is no upward or downward trend over time. This conclusion does not change with a change in alpha. Figure 3-15 plots the autocorrelation function:

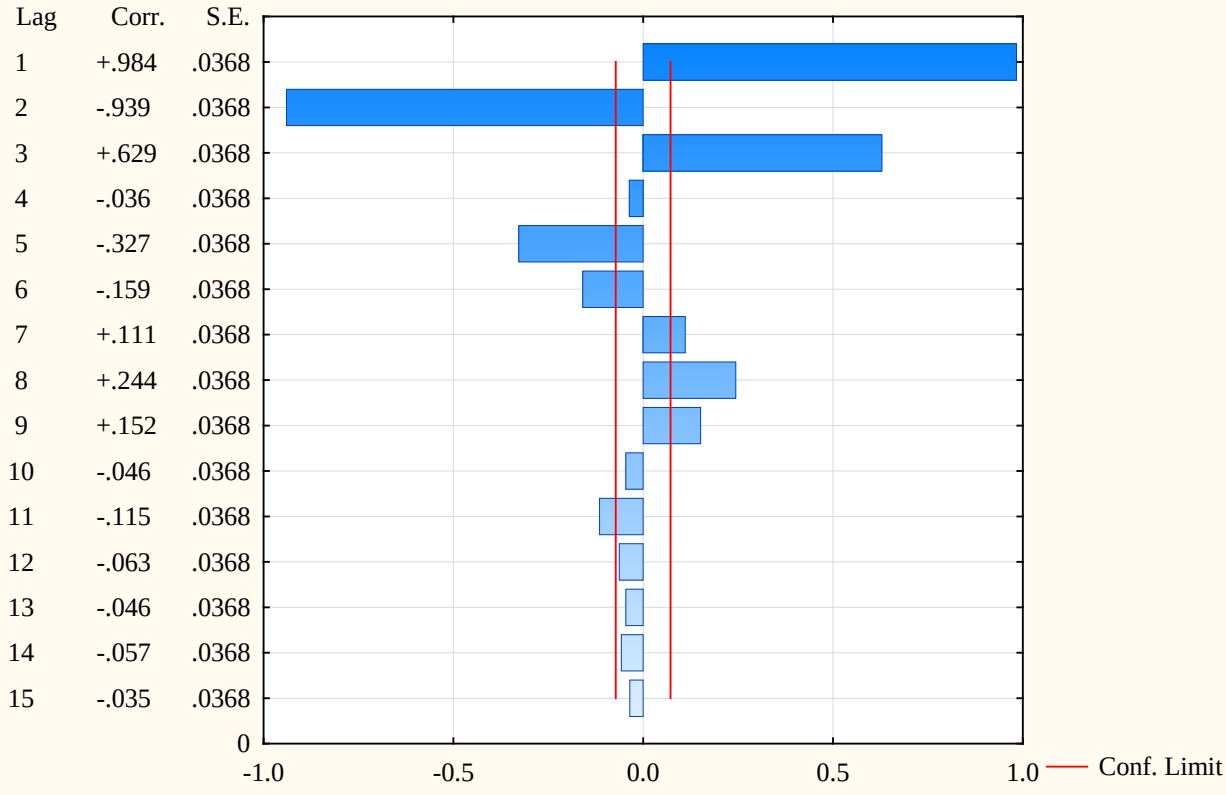
Figure 3-15
Total *Pseudo-nitzschia* Data Autocorrelation Function
Twelve Point Moving Average and T4253H Filter
Lagged by Week



The *Pseudo-nitzschia* data are seasonal data, with highs in cell densities in the warm months and lows in cell counts in the cold months. Autocorrelation is the correlation of a signal with itself. It represents the similarity between data points as a function of the time lag between them and a method of finding repeating patterns. The autocorrelation was high with the preceding and became progressively smaller with each lag (i.e. a slow decay). This indicated a strong seasonal component, where cell counts are most similar to other observations at similar times of the year.

Figure 3-16 plots the partial autocorrelation function:

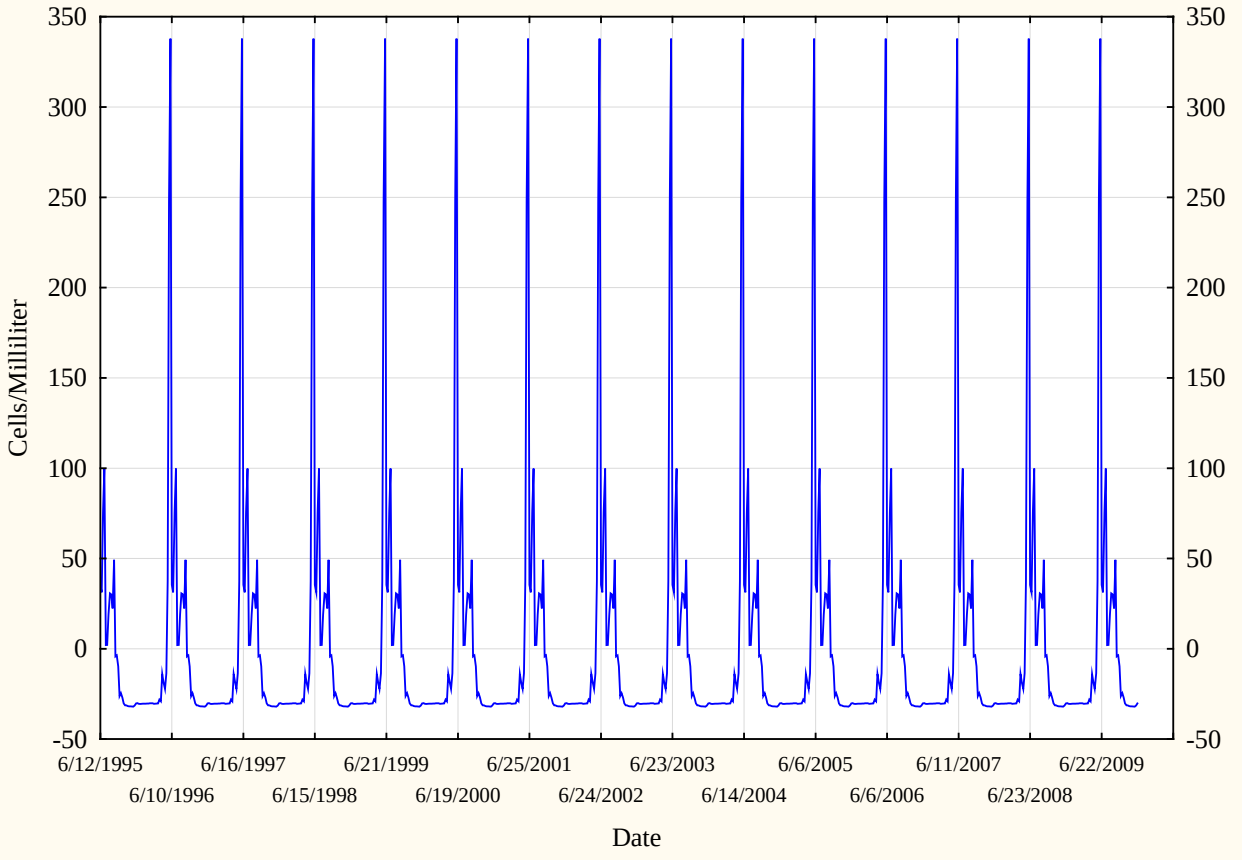
Figure 3-16
Total *Pseudo-nitzschia* Data Partial Autocorrelation Function
Twelve Point Moving Average and T4253H Filter
Lagged by Week



The partial autocorrelation function is an extension of autocorrelation where the dependence on the intermediate elements (i.e., those within the lag) is removed. Above and beyond the very strong partial autocorrelation at lag 1, none of the partial autocorrelations are significant. Each observation is most similar to the previous observation, plus some randomness.

Seasonal factors in the data are plotted in Figure 3-17:

Figure 3-17
Seasonal Factors in the Total *Pseudo-nitzschia* Data



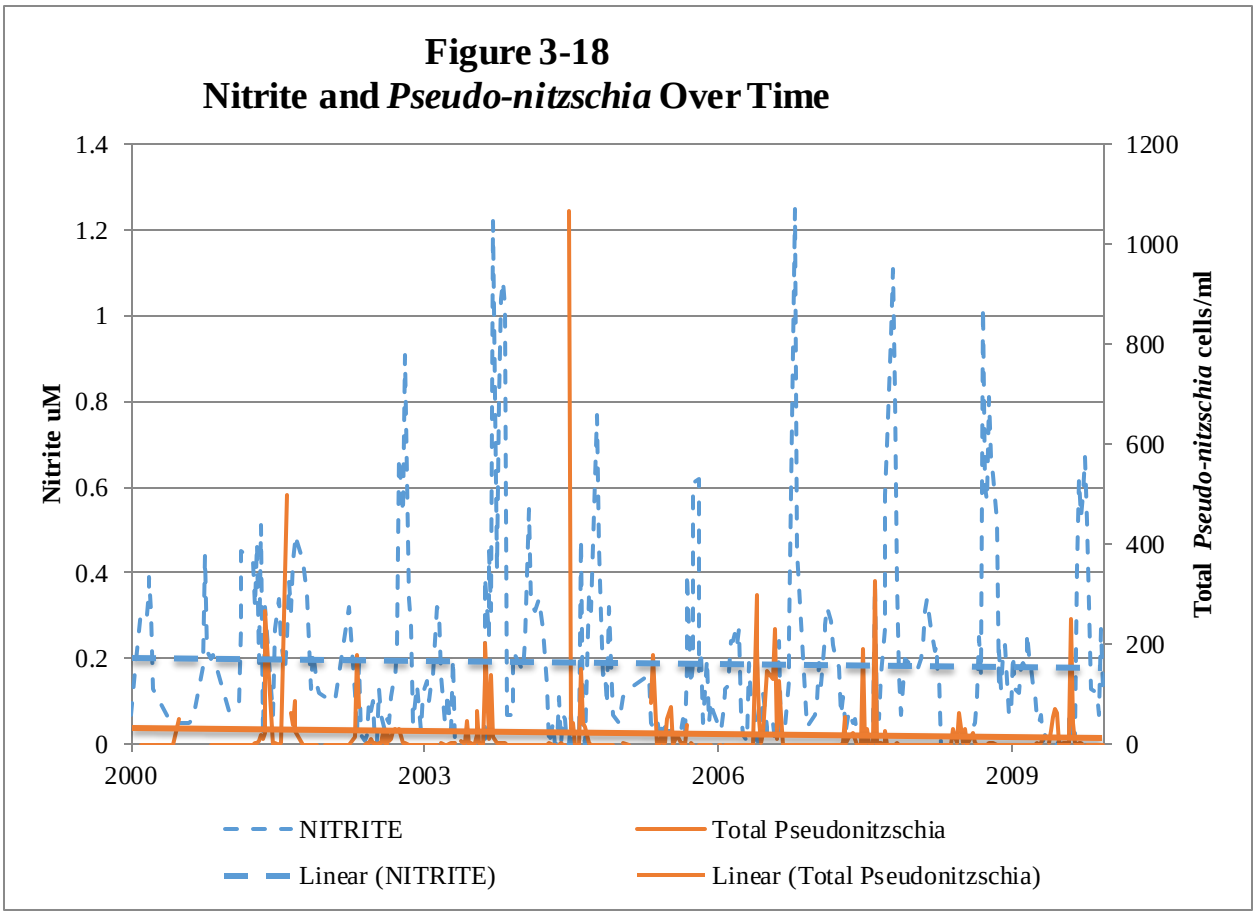
Time series can deconstruct a data set into seasonal, trend and irregular components. As expected, there is a strong seasonal component to the data, as well as significant random noise. There is no long-term trend in the data.

Only the significant statistical findings were presented in the text. This analysis highlighted the important results in the statistical analysis. In summary, the statistical analysis revealed a strong seasonal trend and high correlation to *Pseudo-nitzschia* cell counts in preceding or following weeks. Figure 3-12 appeared to indicate declining peaks over time after smoothing was applied. The data support the conclusion that *Pseudo-nitzschia* is present at relatively low but measureable levels throughout most of

the year. A focus on bloom cell counts has the potential to miss the impact of chronic low level exposure to DA.

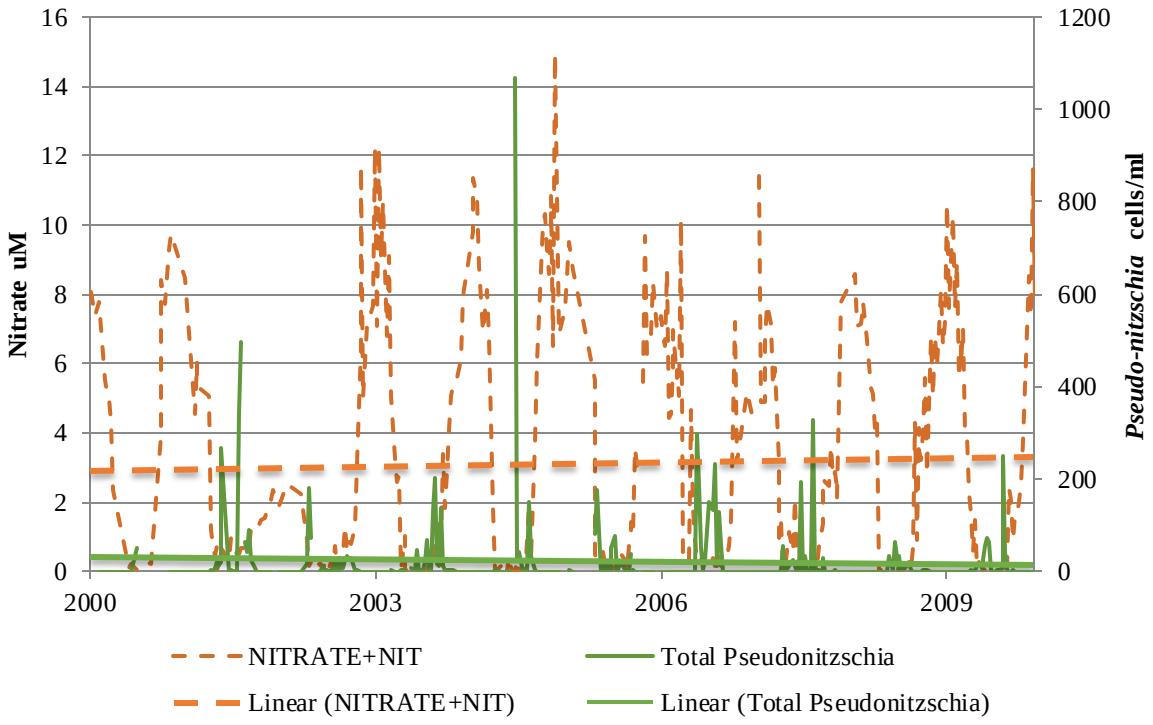
Evaluation of Influence of Nutrients on *Pseudo-nitzschia* Levels. Data from the L4 site were evaluated to determine which nutrients are covariant with *Pseudo-nitzschia* growth by performing paired analysis between each of these nutrients concentrations and total *Pseudo-nitzschia* cell counts. Nutrient concentrations at L4 are high during the well mixed periods (fall and winter) and decrease when the waters become stratified (spring and summer).

Visual Approach to Data. As a first step, the data were plotted to see if there is a visually apparent relation between diatom levels and nutrients.



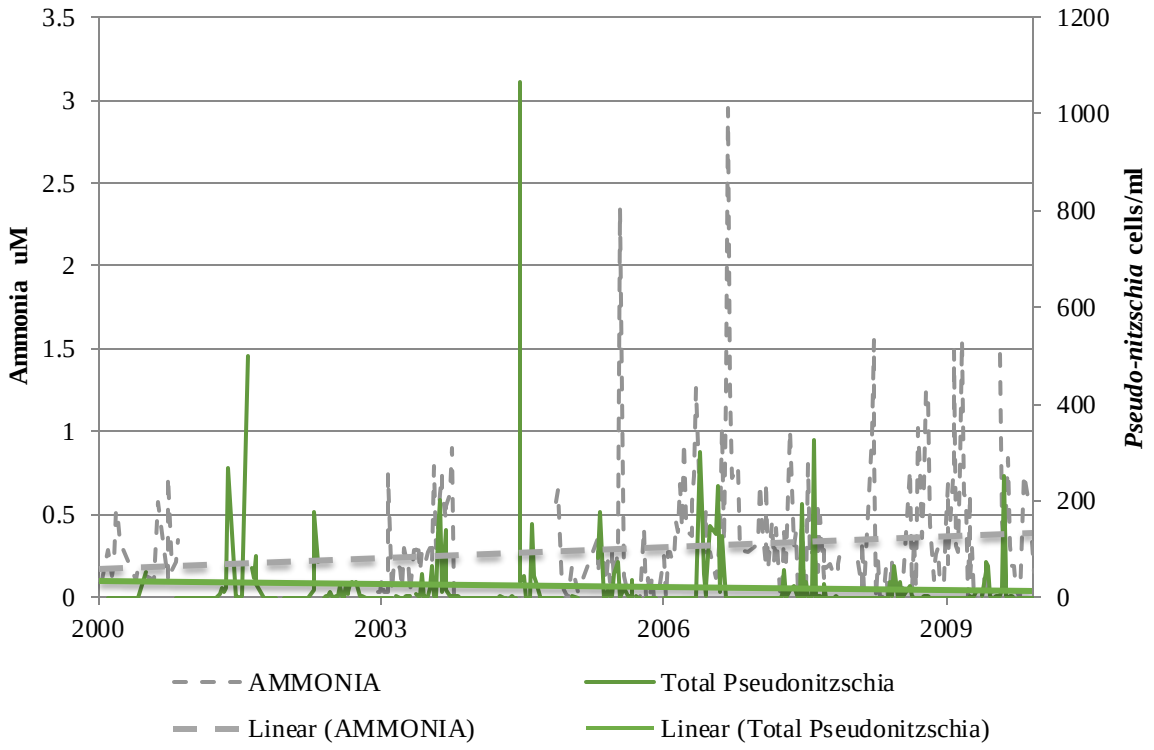
The figure above compares nitrite concentrations and *Pseudo-nitzschia* cell counts from the period 2000 through 2009. There was no clear trend for nitrite or *Pseudo-nitzschia* and and no obvious relationship between them.

Figure 3-19
Nitrate and *Pseudo-nitzschia* Over Time



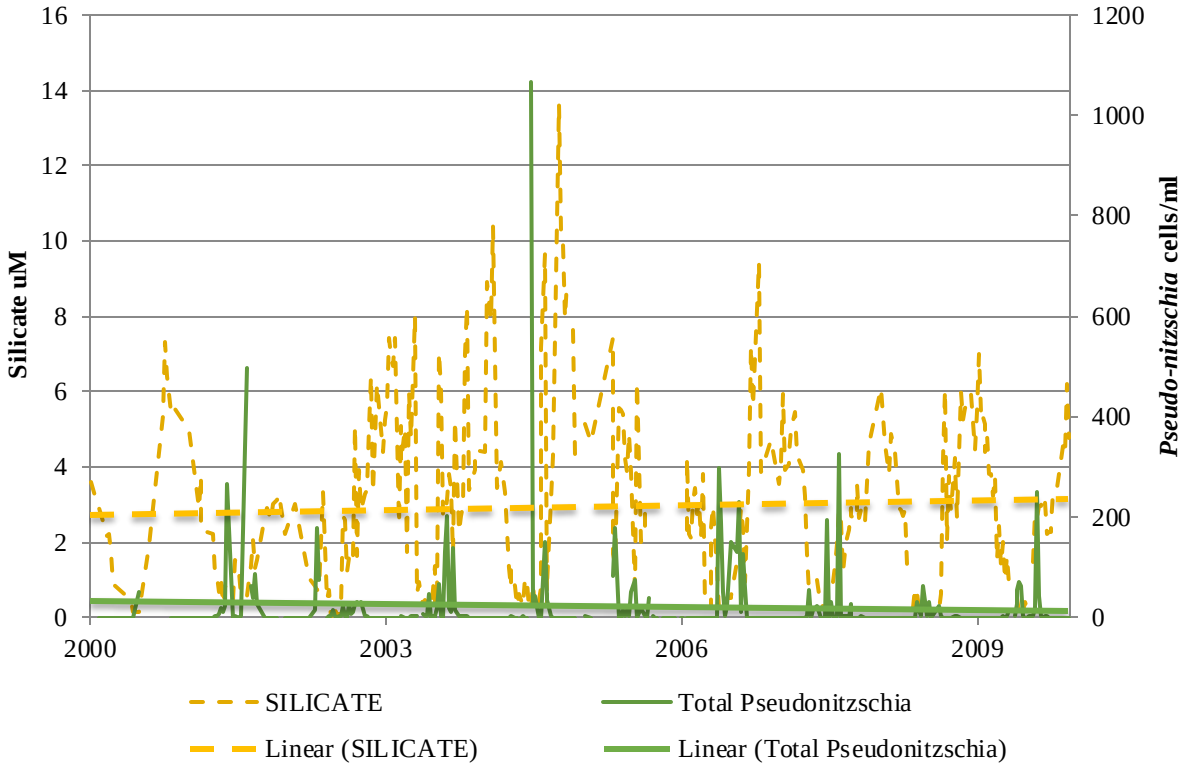
The figure above compares nitrate concentrations and *Pseudo-nitzschia* cell counts from the period 2000 through 2009. There was no clear trend for nitrate (possible slight upward trend) or *Pseudo-nitzschia*. It does appear that nitrate peaks may proceed *Pseudo-nitzshia* peaks.

Figure 3-20
Ammonia and *Pseudo-nitzschia* Over Time

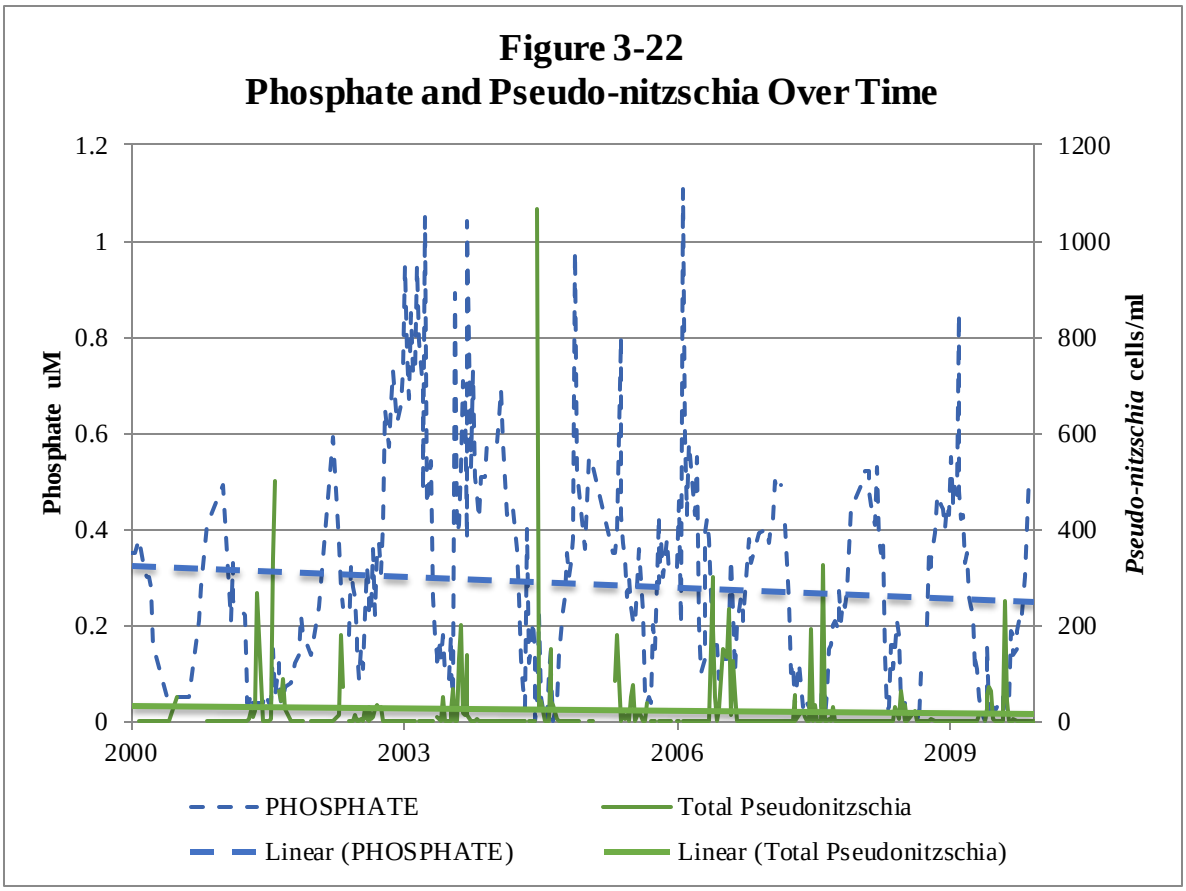


The figure above compares ammonia concentrations and *Pseudo-nitzschia* cell counts from the period 2000 through 2009. There was no clear trend for ammonia (possible slight upward trend) or *Pseudo-nitzschia* and and no obvious relationship between them.

Figure 3-21
Silicate and *Pseudo-nitzschia* Over Time



The figure above compares silicate concentrations and *Pseudo-nitzschia* cell counts from the period 2000 through 2009. There was no clear trend for silicate or *Pseudo-nitzschia*.



The figure above compares phosphate concentrations and *Pseudo-nitzschia* cell counts from the period 2000 through 2009. There was no clear trend for phosphate (possible slight downward trend), although it appears the *Pseudo-nitzschia* peaks are following the phosphate peaks.

Multiple Regression of Pseudo-nitzschia and Nutrients. Multiple regression was performed to determine the whether there is a relationship between *Pseudo-nitzschia* and any of the measured nutrients. The magnitude of the *Beta* coefficients reflects the relative contribution of each independent variable in the prediction of the dependent variable. The dependent variable for this analysis is total *Pseudo-nitzschia* cell count. The independent variables are nitrite, nitrate, ammonia, silicate, and phosphorus. The

null hypothesis is that nitrate, nitrite, phosphorus, or silicate is not correlated with *Pseudo-nitzschia* densities.

The partial correlation represents the contribution of a particular independent variable to the prediction of the dependent variable. The partial correlations were not statistically significant for the *Pseudo-nitzschia* and nutrient data.

Table 3-2

Regression Summary Total *Pseudo-nitzschia* Nutrients

Variable	b*	Std. Err. Of b*	b	Std. Err. Of b	t(233)	p-value
Intercept			45.5	10.4	4.4	0.000018
Nitrite	-0.022	0.070	-8.01	25.3	-0.32	0.75
Nitrate	-0.13	0.099	-3.1	2.4	-1.3	0.19
Ammonia	-0.019	0.065	-4.09	14.1	-0.29	0.77
Silicate	-0.080	0.10	-3.1	3.8	-0.80	0.42

$R=0.22$, $R^2=0.049$, Adjusted $R^2=0.028$

$F(5,23)=2.39$, $p<0.039$, Std. Error of Estimate=82.4, N=239

Table 3-2 contains the standardized regression coefficients (*b**) and the raw regression coefficients (*b*). The magnitude of these *Beta* coefficients allows comparison of the relative contribution of each independent variable in the prediction of the dependent variable. The p values indicate that there is not a statistically significant correlation between *Pseudo-nitzschia* cell counts and any of the measured nutrients.

Table 3-3

Partial and Semi-Partial Correlations Between *Pseudo-nitzschia* and Nutrients

Variable	B* Intercept	Partial Cor.	Semipart Cor.	Tolerance	R^2	t(233)	p-value
Nitrite	-0.022	-0.021	-0.020	0.84	0.16	-0.32	0.75
Nitrate	-0.13	-0.085	-0.083	0.41	0.59	-1.3	0.19
Ammonia	-0.019	-0.019	-0.019	0.96	0.037	-0.29	0.77
Silicate	-0.080	-0.053	-0.051	0.41	0.59	-0.80	0.42
Phosphate	-0.019	-0.013	-0.013	0.47	0.53	-0.20	0.84

Partial and semi-partial correlations and R-square values (Table 3-3) indicated there is not a strong relationship between *Pseudo-nitzschia* and measured nutrients. P-values indicate no statistically significant relationship at a p-value of 0.05.

Distributed Lags Analysis. Distributed lags analysis is a technique for examining the relationships between variables that involve some delay where a change in one variable causes a delayed change in the other variable. This analysis evaluates whether nutrient concentrations are an independent or explanatory variable that affect the dependent variable (*Pseudo-nitzschia* cell count) with some lag. Ammonia was the only nutrient with a statistically significant lag result, and is presented in Table 3-4.

Table 3-4 Lag Analysis for Ammonia				
Lag Unit (Weeks)	**Regression Coefficient**	**Standard Error**	**T(355)**	**P**
0	-25.5	33.9	-0.75	0.45
1	-11.9	36.8	-0.32	0.75
2	68.5	33.9	-2.02	0.044
Lag=2, R=0.16, R2=0.024, N=358				

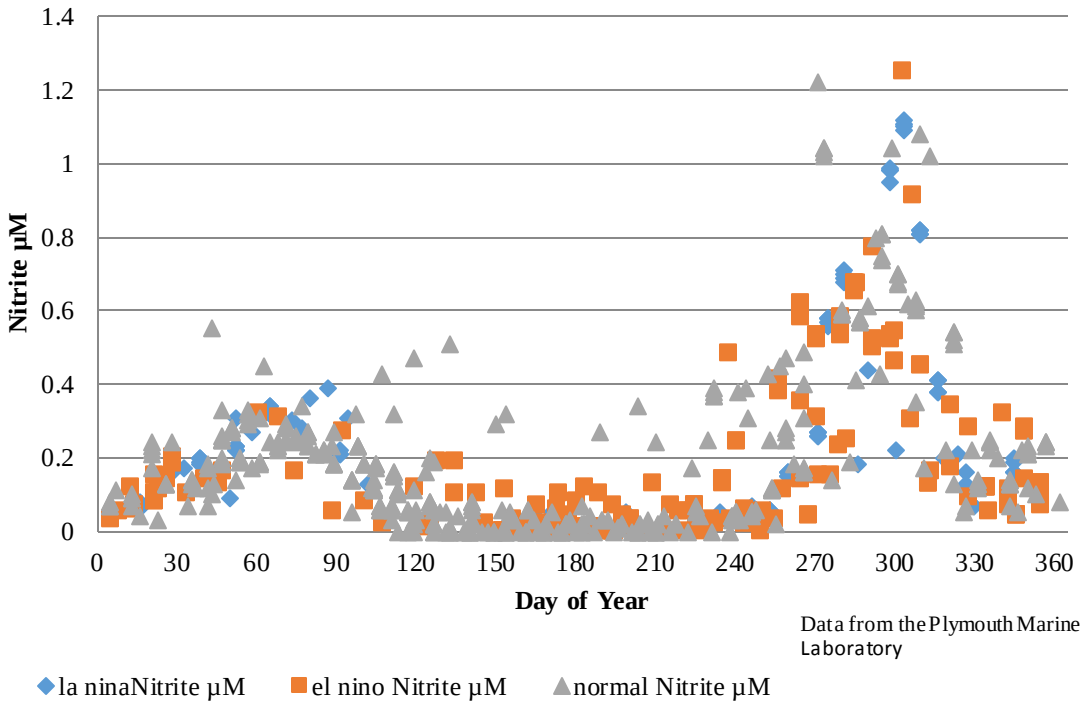
Lags analysis is a useful tool for algae growth because it is reasonable to hypothesize that there will be some lag between an increase in nutrients and a measureable increase in *Pseudo-nitzschia* densities. A two week lag was statistically significant for ammonia at a p value of 0.05. It is unclear if this was a true relationship or an artifact of the data. While the regression analysis for the three combined *Pseudo-nitzschia* functional groups did not show a significant relationship between total *Pseudo-nitzschia* cell counts and nutrient concentrations, there is evidence in the literature that individual functional groups were correlated with nutrients at L4[216]. Downes-Tettmar evaluated seasonal variation in *Pseudo-nitzschia* cell counts and DA in the Western English Channel based on the L4 data. Their study was conducted in the context of blooms, rather than chronic low level cell counts that are being considered in this research and examined functional groups individually. They examined data for a single year (2009) and looked at correlations between three types of *Pseudo-nitzschia* and various environmental factors. The *Pseudo-nitzschia delicatissima* group significantly correlated with hours of lights,

phosphate, salinity, temperature, and rainfall (phosphate and rainfall were negative correlations). *P. pungens/multiseries* group was negatively correlated to all the main nutrients (nitrate, phosphate, silicate, and ammonia), and the *P.seriata* group was negatively correlated to nitrate and positively correlated to temperature.

Effects of ENSO on Nutrient Concentrations. The previous analysis looked at nutrient and *Pseudo-nitzschia* data by year for a visual examination of potential trends or relationships between nutrient concentrations and diatom cell counts.

The figures below present nutrient data for El Niño, La Niña, and normal sea surface temperatures from 2000-2009.

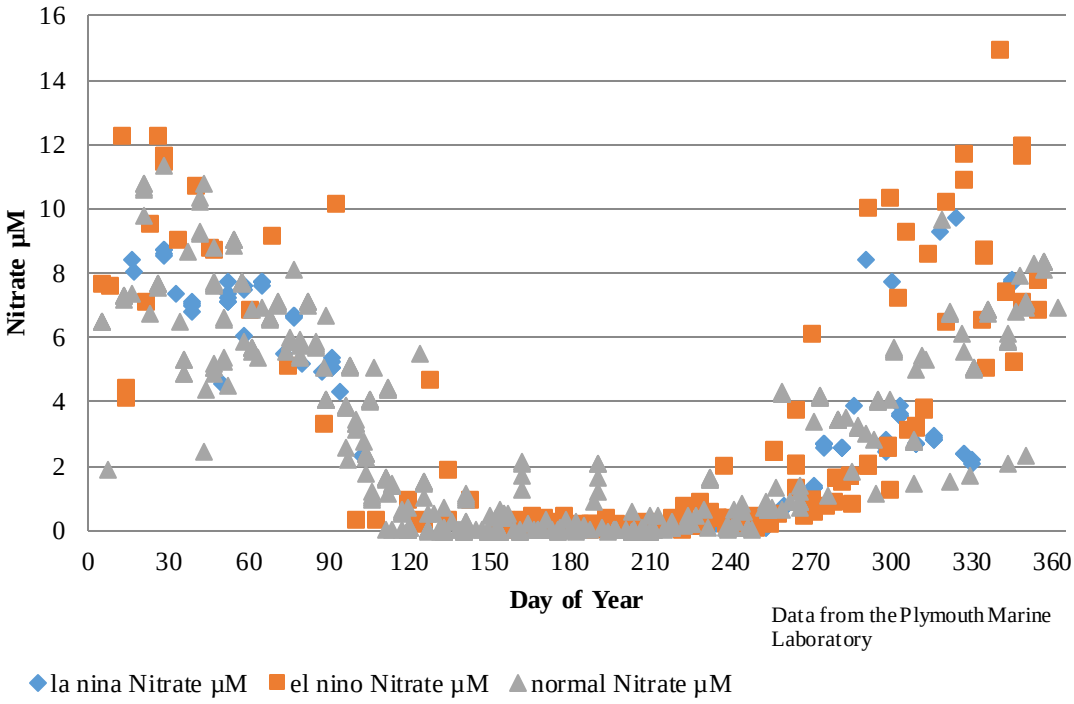
Figure 3-23
Nitrite versus Day of Year 2000-2009



Overall nitrite appears to be under highest under normal conditions. Concentrations under normal and El Niño conditions were similar to each other. The seasonal trends

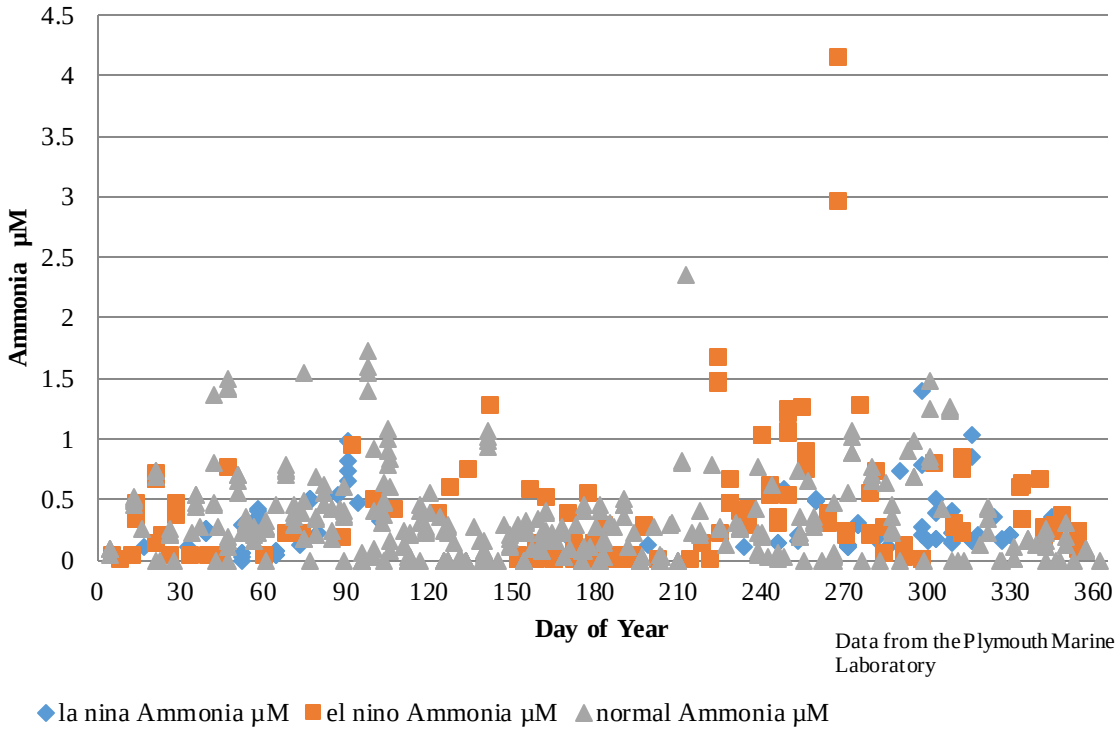
were similar for all three conditions, with a small peak at about day 60 and a much larger peak around day 300.

Figure 3-24
Nitrate versus Day of Year 2000-2009



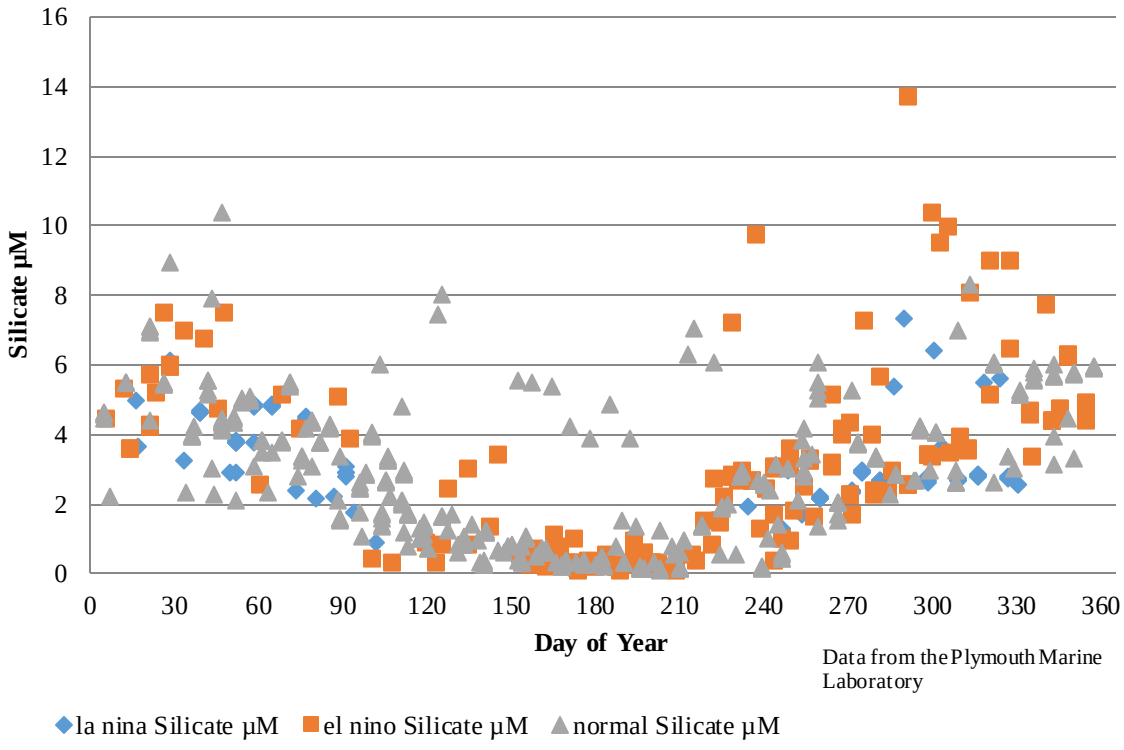
Nitrate appeared generally highest under *El Nino* conditions. All three conditions exhibited highest concentrations early and late in the calendar year with low concentrations in the middle of the summer. The early and late year peaks are similar, whereas the nitrite peaks in the early part of the year were much smaller than the late year peaks.

Figure 3-25
Ammonia versus Day of Year 2000-2009



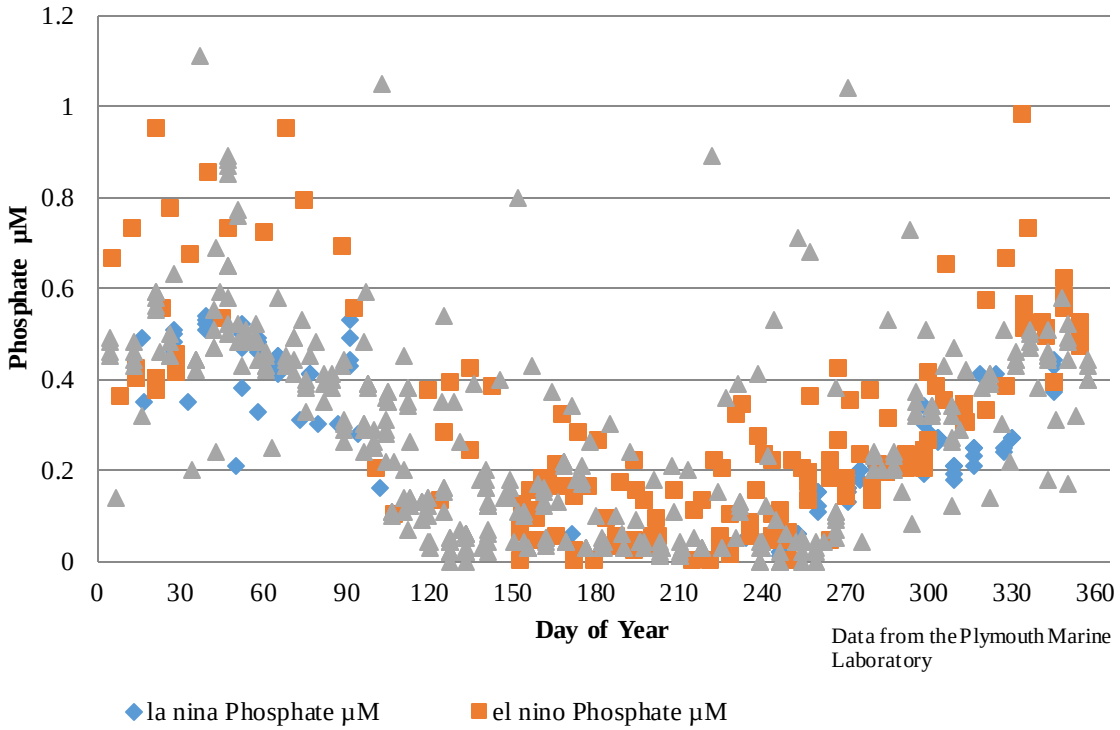
Ammonia concentrations were relatively flat over the course of the calendar year and did not appear to differ significantly under above normal or below normal sea surface temperatures.

Figure 3-26
Silicate versus Day of Year 2000-2009



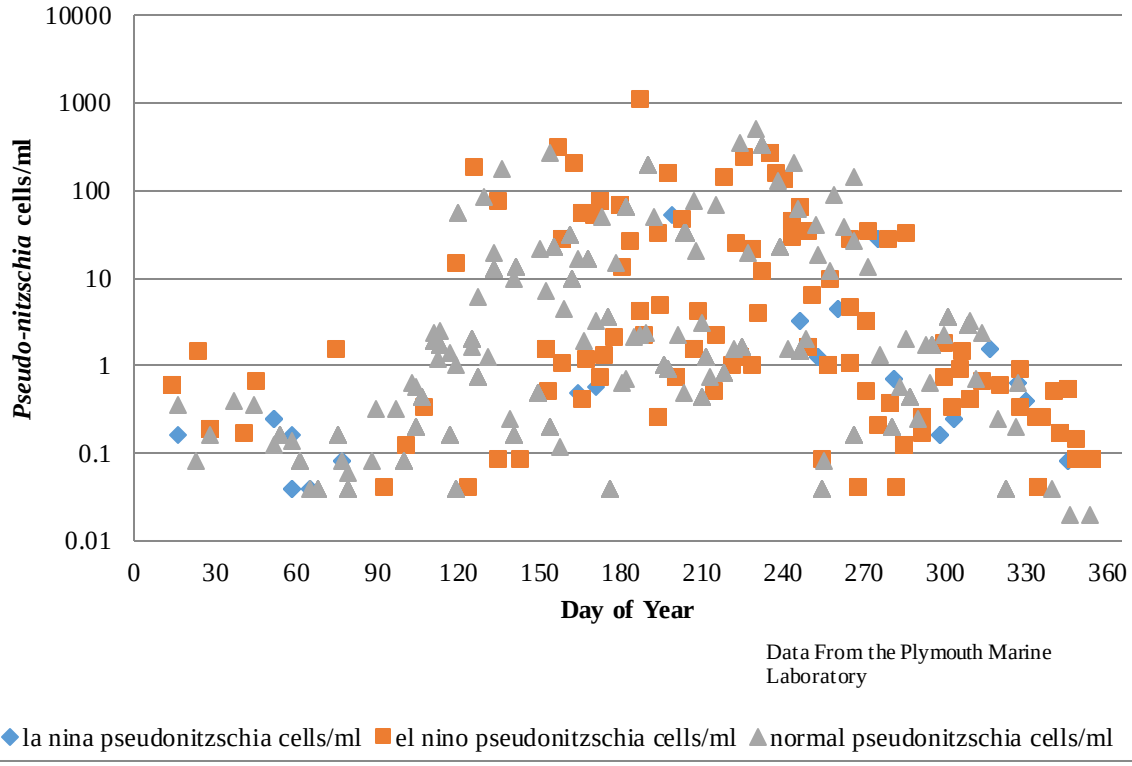
Silicate concentrations showed early and late peaks with lows in the summer. The data did not appear to differ greatly under above normal or below normal sea surface temperatures.

Figure 3-27
Phosphate versus Day of Year 2000-2009



Phosphate concentrations showed early and late peaks with lows in the summer. The data did not appear to differ greatly under above normal or below normal sea surface temperatures.

Figure 3-28
***Pseudo-nitzschia* versus Day of Year 2000-2009**



Finally, *Pseudo-nitzschia* cell counts were plotted by year-day. Previous analysis in this chapter had shown that there is no overall trend from year to year, but this figure looks at the potential for differences in *Pseudo-nitzschia* densities under different sea surface tempearature conditions. *Pseudo-nitzschia* cell counts appeared somewhat lower under La Niña conditions. *Pseudo-nitzschia* showed a reverse trend compared to nitrite, nitrate, silicate, and phosphate, with a peak in the middle of the summer and lows early and late in the year. *Pseudo-nitzschia* was present throughout the calendar year at measureable densities.

Other Long-Term Monitoring Data Sets for *Pseudo-nitzschia*. There are a few long-term *Pseudo-nitzschia* data sets in the published literature, although none provide

weekly data for a time period as long as the L4 data set. The areas monitored include Chesapeake Bay, Massachusetts Bay, the Bay of Fundy, and Scotland. These data sets are briefly reviewed below to provide supporting evidence for widespread geographical occurrence and persistent cell counts of *Pseudo-nitzschia* in the environment. Data on persistent low level cell counts are limited because the published literature has primarily focused on blooms.

Chesapeake Bay. *Pseudo-nitzschia*[225] samples were collected from Chesapeake Bay from 2002 through 2007. *Pseudo-nitzschia* was present year round with abundance highest in the winter and spring. DA was detected in 42% of samples (compared to 75% at L4). Eight stations were sampled 50 times during the five year period (roughly once a month) while seven other stations were sampled between 23 and 46 times. Samples that were found to contain *Pseudo-nitzschia* were also analyzed for DA. DA was detected in 39 of 85 samples that contained *Pseudo-nitzschia* (0.16 to 1.04 picograms DA/ml). Salinity ranged greatly and *Pseudo-nitzschia* was found most often in the areas with highest salinity. There was a statistically significant association between *Pseudo-nitzschia* abundance and both high salinity and low temperature. The author reported that *Pseudo-nitzschia* abundances were similar in data that were collected in Louisiana[225].

A second study was conducted in Chesapeake Bay. Sampling for a number of harmful algae types, including *Pseudo-nitzschia*, was conducted monthly for twenty years (1984-2004) in Chesapeake Bay and in three Virginia Rivers that empty into the Chesapeake[226]. Forty-eight monitoring stations were sampled and *Pseudo-nitzschia* was frequently detected in the Bay. *Pseudo-nitzschia pungens* and *seriata* were two of the most commonly found diatoms over the twenty year sampling period[227]. The paper was

an overview of sampling for all algae species and did not contain quantitative data on *Pseudo-nitzschia*, so detection frequency could not be determined.

Massachusetts Bay. Monitoring for *Pseudo-nitzschia* in Massachusetts Bay began in 1992 by the Massachusetts Water Resources Authority (MWRA) as part of the monitoring program for the new MWRA outfall[228]. The MWRA has consistently detected low to moderate levels of *Pseudo-nitzschia* as part of their monitoring program. *Pseudo-nitzschia* cell counts spiked in 1999 and 2000 and have remained relatively low since then. *Pseudo-nitzschia* densities have decreased dramatically in all monitoring locations (including Cape Cod Bay and Boston Harbor) since the outfall opened. Data were collected on a high frequency near the sewage outfall and on a lower frequency in a larger area including the Massachusetts coast and Cape Cod Bay. Boston Harbor nutrient concentrations decreased dramatically concurrent with the drop in *Pseudo-nitzschia*.

Bay of Fundy. A long-term monitoring program was established at five locations in the Bay of Fundy in 1988. *Pseudo-nitzschia* was observed most of the year with highest cell counts from May to October[229]. Approximately 11 blooms greater than 150 cells/ml were observed between 1988 and 2005. Four stations were sampled between 19 and 33 times per year in the years 1988 through 2005. The paper focused on blooms and did not discuss prevalence of low level cell counts.

Scotland. A single location on the west coast of Scotland was sampled for a three year period[230]. *P. delicatissima* dominated in the spring while *P. seriata* occurred mostly during the summer. Both groups were present in the autumn. Sampling was weekly from April to November and every four weeks for the remaining months. Cells in the *P. delicatissima* occurred in 95% of all samples and in cell counts as high as160 cells/ml (in

April 2002). *P. Seriata* cells occurred in 83% of all samples, with a maximum of 110 cells/ml (in July 2003). Growth was strongly correlated with nitrate, phosphate, and silicate.

None of the published data sets include weekly sampling at a location for the length of time as the L4 data set. However, these data sets do provide further evidence that *Pseudo-nitzschia* is persistent in other geographic areas. While long term data sets are limited, there are a large number of single or short-term sampling events in the published literature. Figure 3-29 presents areas with published sampling results denoted as circles on a world map.

Figure 3-29

Selected Published Occurrences of *Pseudo-nitzschia* Worldwide

This figure illustrates that *Pseudo-nitzschia* is widespread across the globe, present in the coastal waters of all seven continents. The countries represented include Canada[33, 229], the United States[225, 231-233], Mexico[234], Brazil[235, 236], Argentina[237], Chile[18], Iceland[238], Norway[238], Sweden[239], Denmark[240, 241], England[242], Scotland[230], France[243, 244], Portugal[245], Spain[246, 247, 248], Italy[249], Croatia[250], Turkey[251], Morocco[252], Tunisia[253], South Africa[18], India[254], Pakistan[255], Borneo[256], the Philippines[18, 257], Australia[258, 259, 260], New Zealand[261], China[262], Korea[263], Vietnam[29], and Japan[29], as well as the continent of Antarctica[264]. *Pseudo-nitzschia pungens, P. fraudulenta, P. multiseries, P. australis, P. delicatissima* and *P. pseudodelicatissima* are considered cosmopolites (i.e., widely

distributed across the globe) and are documented as the most significant producers of DA[18].

In summary, this section provided evidence for persistent and widespread low level cell counts of *Pseudo-nitzschia*. The L4 data have shown that *Pseudo-nitzschia* is persistent across an 18-year sampling period, where *Pseudo-nitzschia* was present in 75% of samples. Total diatom concentrations have shown a decreasing trend during sampling from 1996-2007[217], but based on the analysis in this chapter, there was no long-term trend in *Pseudo-nitzschia* cell counts at the L4 location.

Nutrient dynamics at the L4 sampling site appear typical of temperate coastal waters[217]. The water column is well-mixed during the winter and fall and this is reflected by higher nutrient concentrations. Weak stratification occurs in spring and summer, which limits the replenishment of nutrients from bottom waters. Nitrite (Figure 3-23), nitrate (Figure 3-24), silicate (Figure 3-26) and phosphate (Figure 3-27) all followed this seasonal pattern (accounting for some year to year variation), with peaks in the winter and fall and lowest concentrations occurring in the summer. Since nutrient concentrations at this location appear seasonal, this indicates that anthropogenic inputs are not a strong influence at the L4 location. While anthropogenic inputs do not dominate nutrient concentrations at the L4 location, they do promote the development and persistence of harmful algal blooms at many locations in the world[43]. The driving force behind nutrient dynamics (i.e., dominance of natural or anthropogenic sources) is site-specific.

Other long term data sets from the scientific literature support the conclusion that low levels of *Pseudo-nitzschia* are persistent over time. In addition to persistence, a

survey of the literature demonstrates that *Pseudo-nitzschia* is widespread across the globe, occurring in coastal waters of all seven continents. The next section focuses on the evidence for the next link in human exposure, persistent low level concentrations of DA in seafood.

Evaluating Domoic Acid Concentrations in Seafood

Exposure assessment is the determination of the intensity, frequency, and duration of actual or hypothetical exposure of humans to an agent that has the potential to pose risk[93]. The previous section evaluated the temporal and geographic persistence of *Pseudo-nitzschia*. The next step in assessing the potential for exposure to humans is examining uptake of DA into seafood. Seafood monitoring data are sporadic both temporally and spatially and have not been compiled. Some seafood data are published, but many are not. While amnesic shellfish poisoning was discovered on the east coast of North America, published monitoring data in this geographic region are very limited. This section examines the spatial and temporal occurrence of DA in seafood based on the published literature to determine if there is evidence to support a conclusion of ubiquitous and persistent low level concentrations of DA in seafood. Since the recent toxicological literature (discussed in Chapter 2) has suggested that low levels of DA may be of significant concern, summarizing the literature data for DA in seafood is an important step in a weight of evidence evaluation of the potential for human exposure and risk. This section discusses (1) the spatial and temporal distribution of available seafood monitoring data for various species, and (2) the potential for exposure in domestic and international food supplies.

Spatial and Temporal Distribution of Seafood Data. In order to understand the potential for human exposure, it is important to examine both the spatial and temporal distributions of DA concentrations globally in seafood. In this section, published data on DA in seafood are compiled and discussed.

Geographical Distribution of DA in Seafood. A number of papers have been published on the concentrations of DA in various types of seafood including shellfish (mussels, oysters, scallops, and razor clams), fish (mackerel, sanddab, combfish, sardines, and anchovies) and other organisms (squid and tunicates). Sampling locations for published studies are presented in Figure 3-30.

Figure 3-30

Published Analysis of Domoic Acid in Seafood

When examining the locations of the published data on DA in seafood it is clear from Figure 3-30 that the data are limited, especially compared to published data on *Pseudo-*

nitzschia. The literature has demonstrated that *Pseudo-nitzschia* is a cosmopolitan species, but this realization has not resulted in widespread sampling for DA in seafood. Published *Pseudo-nitzschia* data were identified for 29 countries (Figure 3-29) but published seafood data are only available for 19 countries (Figure 3-30 and Table 3-5). West coasts tend to have greater upwelling than east coasts of the continents[36]. The west coasts of South America and Africa are of particular concern for their lack of seafood data, since upwelling has the potential to cause relatively high cell counts of *Pseudo-nitzschia* in these areas, resulting in the potential for persistent DA concentrations in seafood (and therefore chronic human exposure).

Another area of significant concern is the east coast of North America. DA was discovered as a human health concern during the amnesic shellfish poisoning in Prince Edward Island in 1987. Despite this fact, the only significant published seafood monitoring data on the East Coast is for the immediate vicinity of the original incident. Further, the only published data for Prince Edward Island are for blue mussels, the shellfish species that caused the initial incident, despite the potential for uptake into other species or in adjacent areas

There are published data for Washington, California, Alabama, and Louisiana. While seafood sampling data are not available for the east coast of the United States, there is evidence that DA is present in the marine food chain in this area. DA was detected in urine and fecal samples recovered from pygmy sperm whales and dwarf sperm whales stranded along the U.S. Atlantic coast from 1997 to 2008. Of the 41 animals analyzed from Virginia, North Carolina, South Carolina and Florida, 24 (59%) tested positive for DA at concentrations of 0.4–1.8 ng/mL in urine and 0.12–13.6 µg DA/in feces as

determined by liquid chromatography–tandem-mass spectrometry[265]. It was unclear whether DA played a role in the strandings, but it is clear that whales are exposed to DA through the food chain in the waters of the southeastern United States. Similar data are available for the waters of the Northeastern United States. As part of an investigation of the potential for DA to contribute to observed reproductive problems in right whales, DA was found in right whale feces as well as krill and copepod samples in the Northeastern United States and Canada[266]. Sixty-nine out of seventy right whale fecal samples collected over 2005 and 2006 tested positive for DA, with detected concentrations ranging from 0.02 – 0.61 µg DA/g fecal matter. These studies indicate that there is continued DA exposure within the food chain of the east coast of the United States. While Figure 3-29 showed that there is extensive evidence in the published literature that *Pseudo-nitzschia* diatoms are widely distributed across the globe, the relative paucity of data on DA in seafood is striking. Based on seafood sampling, DA presents a significant concern for acute effects on the west coast of the United States, which has led regulators in California, Oregon, and Washington to respond with frequent closures of shellfish beds[35, 267, 268]. It is unclear if DA would pose a potential risk for acute concerns in other parts of the United States because it is not regularly evaluated elsewhere in published data, despite the presence of *Pseudo-nitzschia* in most United States marine waters. This point is equally valid for most of the globe where the literature indicates *Pseudo-nitzschia* is widespread but seafood data for DA are extremely limited geographically. Seafood sampling from the scientific literature is summarized in Table 3-5.

Table 3-5
Summary of Published Data on Domoic Acid in Seafood

Geographic location	Species	Reference	Sampling Date	Concentration (mg/kg)
United States (WA)	Razor clams	[269]	1991-1993	<5 - 230
United States (LA)	Menhaden	[270]	2008	ND - 0.31
United States (CA)	Mussels	[271]	2004	5.8
United States (CA)	Chub mackerel	[271]	2004	7.3
United States (CA)	Jack mackerel	[271]	2004	5.5
United States (CA)	Pacific sanddab	[271]	2004	50.1
United States (CA)	Longspine combfish	[271]	2004	9.7
United States (CA)	Mussels	[271]	2003-2004	ND - 2.33
United States (CA)	Shellfish	[272]	2013	0 - 260
United States (CA)	Squid	[273]	2000	<0.5
United States (CA)	Squid	[274] [273]	2009	0.4-0.5
United States (CA)	sand crab	[275]	1999	0.5 - 5
United States (CA)	Shellfish	[272]	2003	ND - <20
United States (CA)	Pacific sanddab	[276]		0.5 - 515
United States (CA)	Anchovies	[277]	1998	223 viscera 39 edible tissue
United States (CA)	Anchovies	[278]	2000	128 - 1815 viscera ND-1.2 body
United States (CA)	Sardines	[278]	2000	169 - 588 viscera 0.2 – 2.2 body

Table 3-5
Summary of Published Data on Domoic Acid in Seafood

Geographic location	Species	Reference	Sampling Date	Concentration (mg/kg)
United States (CA)	Anchovies	[279]	2001	ND – 444 viscera
United States (CA)	Sardines	[279]	2001	ND – 244 viscera
United States (CA)	12 edible fish species	[280]	2001	ND - 2.8 viscera ND edible tissue
United States (CA)	9 species of flatfish	[281]	2004	ND – 53.3 viscera
United States (CA)	Mussels	[32]	2008	ND – 59
United States (CA)	Anchovies	[268]	2011	ND – 155
United States (CA)	Lobster	[268]	2011	ND – 140 viscera
United States (CA)	Crab	[268]	2011	ND – 290 viscera
United States (CA)	Mussels	[268]	2011	ND – 100
United States (CA)	Razor clams	[268]	2011	ND – 97
United States (CA)	Oysters	[268]	2011	ND – 86
United States (WA)	Razor clams	[282]	2002	ND - 295
United States (AL)	Seven fish species	[283]	2011	ND - 0.72
Canada (PEI)	Blue mussels	[108]	1987	5 – 520
Mexico	Tunicate	[284]	2008	8.7 – 15.5 edible tissue
Mexico	Clams	[284]	2008	4.7
Mexico	Mussels	[284]	2008	6.4
Brazil	Mussels	[235]	2008-2009	ND - 98.5

Table 3-5
Summary of Published Data on Domoic Acid in Seafood

Geographic location	Species	Reference	Sampling Date	Concentration (mg/kg)
Argentina	Mussels	[285]	2000	7.7
Argentina	Anchovies	[285]	2000	4.9 edible tissue
Denmark	Mussels	[286]	2004	0.4 - 32
Ireland	Mussels	[287]	1999	0.09
Ireland	Oysters	[287]	1999	0.27 – 0.9
Ireland	Razor clams	[287]	1999	0.09 – 0.66
Ireland	King scallops	[287]	1999	up to 240, 55% of samples over limit of 20 2820 digestive gland
Ireland	King scallops	[288]	2004	ND - 7.3 adductor muscle ND - 296 hepatopancreas
Scotland	King scallops	[289]	2003	up to 63
Scotland	Mussels	[289]	2003	up to 1.3
Scotland	Pacific oysters	[289]	2003	up to 0.3
Scotland	Queen scallops	[289]	2003	up to 0.6
France	Mussels	[243]	1999	ND - 3.2

Table 3-5
Summary of Published Data on Domoic Acid in Seafood

Geographic location	Species	Reference	Sampling Date	Concentration (mg/kg)
France	Mussels	[243]	2000	53
France	Shellfish	[290]	2004	Up to 200
Portugal	Mussels	[291]	2000	54.7-325
Portugal	Crab	[292]	2003	up to 323
Portugal	Shellfish	[291]		ND - 74.2
Portugal	Crab	[292]	2002	ND - 323 edible tissue
Portugal	Sardine	[293]	2002-2003	ND - 128.5 viscera ND edible tissue
Portugal	Octopus	[294]	2003	1 - 166 digestive gland
Portugal	Octopus	[295]	2004	ND - >100
Portugal	Cuttlefish	[295]	2004	ND - 0.7 mantle ND - 242 digestive gland
Greece	Mussels	[296]	2002	ND - 14, 83% <1
Greece	Venus clams	[296]	2003	ND - 5.6, 95% <1
Croatia	Mussels	[297]	2006 – 2008	ND - 6.5

Table 3-5 Summary of Published Data on Domoic Acid in Seafood				
Geographic location	**Species**	**Reference**	**Sampling Date**	**Concentration (mg/kg)**
Morocco	Tuberculate cockles and sweet clams	[298]	2013	ND - 4.9
Morocco	Mussels	[299]	2008-2009	ND – 44
Angola	Bivalves	[300]	2008	ND - 2.5
Australia	oysters mussels clams	[260]	2003-2005	ND - 0.25
Japan	Bivalve	[301]	2006	0.51
Japan	Blue mussels	[302]	2000	0.11 - 1.81
Korea	Surf clam	[303]	2006-2007	1.9 - 4.1
Philippines	Bivalve	[301]	2006	ND – 42
Thailand	Bivalve	[301]	2006	1.8
Vietnam	Bivalve	[301]	2006	ND – 19
Vietnam	Shellfish	[304]	2010	8 – 17

Overall, there were more than 21 species of seafood that had measured levels of DA from across the globe. The largest amount of data for a country is from the United

States, with 33 species from 15 published papers, far more than any other country. Portugal had the second most published data for a country, with 6 species from 4 different published papers. For comparison, the largest amount of data published for a U.S. State is substantially more than this, with data for 12 species for California. Taken as a whole there is a substantial amount of data, with 20 countries represented from across worldwide, but data from any individual country are fairly sparse.

Shellfish. Shellfish and planktivorous fish (such as anchovies and sardines) accumulated the highest levels of DA. Only one study was identified that looked at correlations between the size of a seafood species (king scallops) and the concentration of DA. The size of king scallops was not correlated with the concentration of DA[288], so regulatory limits on shellfish size allowed for collection may not reduce human exposure. While bioaccumulating compounds occur at highest concentrations in the largest members of a species, this same trend does not occur for a non-lipophilic compound like DA [305].

Razor clams have among the highest concentrations of DA in the literature and also retain DA for a greater length of time than other species. Razor clams concentrations are above the regulatory limit of 20 mg/kg more often than any other species in the scientific literature and are heavily tested and regulated on the west coast of the United States[306]. Razor clams had concentrations up to 97 mg/kg in California and 295 mg/kg in Washington State. On a given clamming day up to 60,000 clam diggers have been counted on the 60 miles of beaches in central and southern Washington, indicating that consumption of recreationally caught shellfish is potentially a very significant exposure route[282]. The highest detected concentration from 1991-1999 in

Washington state razor clams was 295 mg/kg in 1998 (n = 445). The standard deviation was very high at a given sampling time and location. The study concluded that a large number of clams must be analyzed to give an accurate picture of the potential for human exposure. For most species of shellfish, DA is found only in the viscera, where it accumulates as shellfish feed on diatoms. Scallops generally have lower concentrations of DA because the adductor muscle, rather than the viscera, is typically consumed. However, DA distributes itself throughout the tissues of the razor clam and can be retained in high concentrations months after a bloom event[282]. Washington State and Oregon have extensive monitoring programs for DA in razor clams. California also monitors for DA in a number of species. Published government monitoring data are not available for other U.S. states.

The maximum DA concentration in mussels from the Prince Edward Island during the 1987 outbreak was 520 mg/kg. Lobsters contained up to 140 mg/kg in their viscera, which may be consumed by individuals who consume lobster hepatopancreas (tomalley). Crab contained up to 323 mg/kg. Measurements of upper water column *Pseudo-nitzschia* and DA abundance suggests that DA is produced throughout the upper 150 meters of the water column and that most of the particulate DA is rapidly lost to the dissolved phase. Comparison of water column DA to particulate DA collected in sediment traps at depths of 150 to 540 meters (uncorrected for sediment trap loss) suggests that about 5% of sea surface DA reaches the seafloor, suggesting that lesser impacts may occur in benthic food webs[172].

Shellfish may be a particularly effective exposure route for humans as most species feed exclusively on phytoplankton (some shellfish are deposit feeders, which can

result in uptake of DA from sediment deposits). Shellfish have only a primitive nervous system and may therefore be relatively immune to the neurotoxic effects allowing them to accumulate concentrations that are potentially toxic to upper trophic level species.

Other Seafood Species. Fish and shellfish preying on planktivorous organisms are exposed to high levels of DA that can accumulate in tissue. This is reflected in Table 3-2. The California sand dab, a predatory flatfish, contained extremely high whole body concentrations of DA with maximum of 515 mg/kg and a mean of 85 mg/kg during bloom events[276]. Concentrations in the sand dab dropped to a maximum of 5.3 mg/kg and a mean of 3.9 mg/kg between bloom events. These whole body concentrations were likely driven by even higher concentrations in the digestive tract. Cuttlefish contained a maximum of 242 mg/kg, while octopus contained a maximum of 166 mg/kg, but edible portions contained less than 1 mg/kg. Concentrations above the acute regulatory limit of 20 mg/kg have been detected in various species in North America, Europe, Africa, and Asia. Published values for seafood are generally in whole body concentrations. It would be useful to have more data for edible tissue (rather than whole body or viscera), which would give a better indication of levels of human exposure.

Seafood Data Outside the Published Literature. There is little published government data for DA in seafood. The California Department of Health Services Marine Biotoxin Monitoring Program monitors for *Pseudo-nitzschia* blooms and for DA in seafood[32]. The data from California's website are not quantified when data are below the regulatory limit of 20 mg/kg. Washington State monitors DA in razor clams at six locations, providing data since 1996 on their website that includes all detected concentration in razor clams[22]. The Oregon Department of Agriculture also monitors DA

in razor clams, but does not make concentration data publically available on their website. Oregon instead provides a map online that shows areas open or closed for razor clams, and releases public announcements of closures[307].

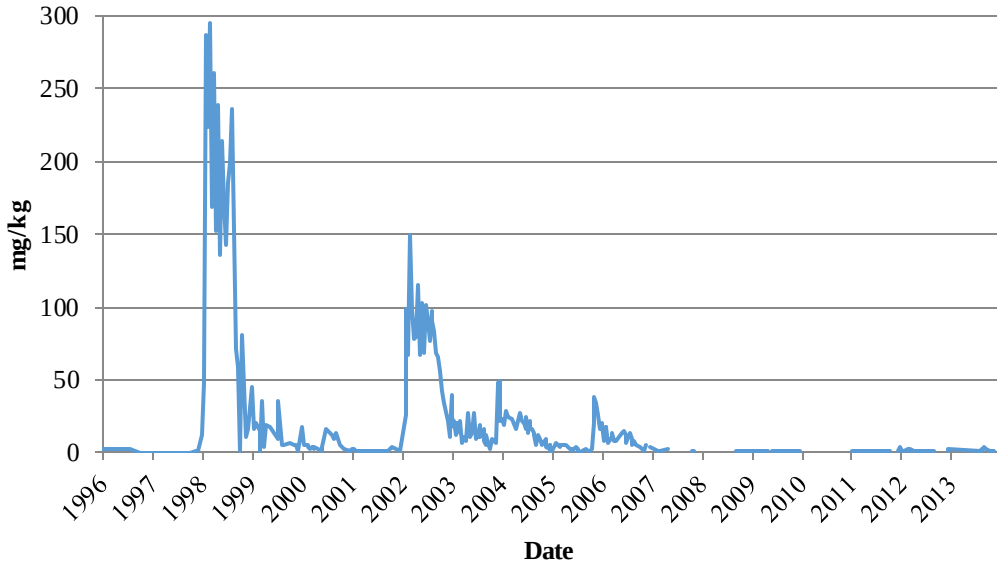
Temporal Distribution of DA in Seafood. Seafood data in the published literature are too spotty temporally to determine if there are any long-term trends at most locations. Most published seafood data represent a snapshot in time for a small area. However, a large study was conducted in Ireland that exhibited significant spatial variability in DA concentrations across 69 sampling locations in Ireland during three sampling events in October 2003, June 2004, and October 2004. The highest DA concentrations were observed near shore and the lowest were observed offshore[288] and could be indicative of other areas and species. These data could indicate that near shore shellfish are of greater concern for DA.

DA was first identified along the California coastline in 1991, which is when the State's monitoring program began. DA has lower acute toxicity than the Paralytic Shellfish Poisoning (PSP) toxin, but has become of greater concern because blooms of *Pseudo-nitzschia* have been of greater frequency and longer duration than most PSP events over the past 20 years[268]. Concentrations of DA above the action level of 20 mg/kg were detected in 52 samples from four California counties in 2012. DA exceeded the action level in at least one location during every month in the period from June through January 2012[268]. DA levels in seafood can change very quickly. On July 9, 2012 mussels and oysters at an aquaculture lease offshore of Santa Barbara were found to contain low levels of DA (7.2 and 4.6 mg/kg, respectively). Within two days the toxin levels increased above the action level, reaching 84 mg/kg and 86 mg/kg by July 16 in

mussels and oysters, respectively. It is challenging to develop an effective monitoring program when seafood concentrations can change quickly in response to *Pseudo-nitzschia* call counts.

The only significant long term publically available data set that quantified all DA concentrations in seafood (i.e., did not report results as "< action level") was for razor clams in Washington State, where data have been collected by the Washington Department of Fish and Wildlife from 1996 through the present at 5 locations[308]. Data for one of those locations (Kalaloch, WA) are presented as an example of temporal changes of DA in seafood in Figure 3-31.

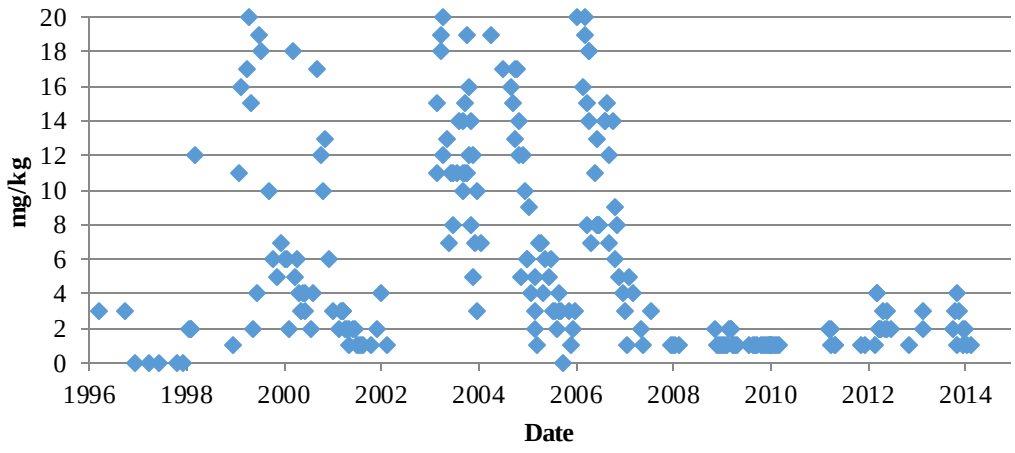
Figure 3-31
Domoic Acid Concentrations in Razor Clams
Kalaloch, WA



Kalaloch, WA generally had the highest concentrations of DA in razor clams among the five locations in Washington State's sampling program. From 1996 through August 2014, 353 samples were collected at this location and 83%, tested positive for DA. Of those 294 positive samples, 71 were greater than the action level of 20 mg/kg. The maximum detected concentration was 295 in November of 1998. The median detected concentration was 7 mg/kg and the mean detected concentration was 25 mg/kg. Some concentrations were dramatically above the 20 mg/kg action level from 1996-2006, but have declined over time however, as seen in Figure 3-31. If only the last ten years of data are included, concentrations are much lower, with a median of 3 mg/kg and a mean of 6 mg/kg.

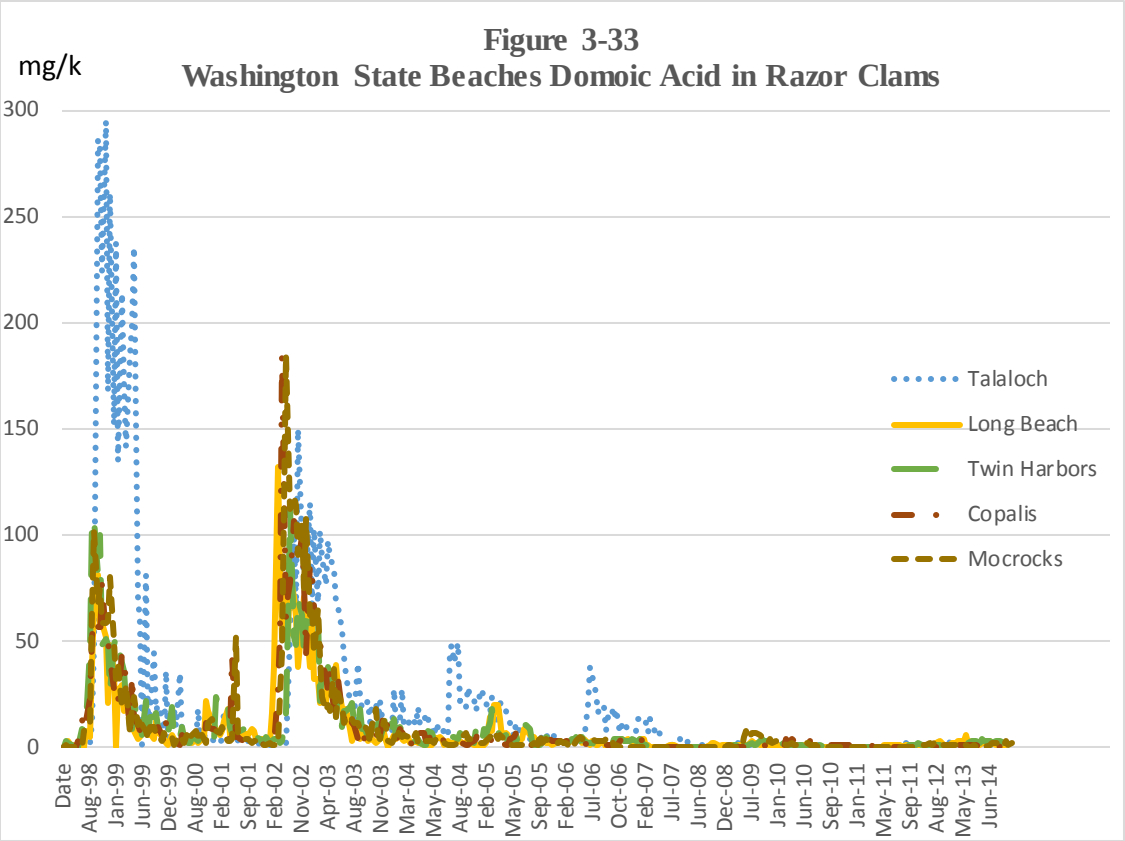
With such dramatic peaks, the persistent lower concentrations of DA are obscured. Figure 3-32 presents only data less than 20 mg/kg, to focus on lower concentrations.

Figure 3-32
Domoic Acid Concentrations in Razor Clams <20 mg/kg
Kalaloch, WA



By removing the higher concentrations, it is easier to see that DA is often present in concentrations that are below the action level of 20 mg/kg, but well above zero. DA concentrations are clearly persistent in this data set at low levels.

The other Washington State locations followed a similar pattern. Figure 3-33 presents data for all five Washington State locations that have been sampled since 1996.



DA concentrations in razor clams for all five locations peaked between 1998 and 2003 and have declined greatly since then. No explanation of the drop off is available in the scientific literature. However, low concentrations of DA have been persistently present in razor clams throughout the sampling period for all five beaches. The action level protects against acute exposures and these data indicate that DA in razor clams is a chronic issue. These data raise the concern that if the current acute action level of 20 mg/kg is not protective of chronic exposures (or sensitive subpopulations), then there is the potential that individuals who consume razor clams regularly on the Washington coast could be at risk.

The data used in preparing Figures 3-31 through 3-33 are presented in Appendix C. While concentrations in razor clams have been low at these Washington beaches in recent years, it is not reflective of the entire Northwest. For instance, the Oregon Department of Agriculture announced on August 29, 2014 that all razor clam fisheries were closed from Florence, OR to the California border (roughly the entire southern half of the Oregon coast) due to high levels of DA[309].

In general, the published datasets on DA in seafood do not contain any comprehensive long-term surveys representing large geographical regions. However, when viewed collectively, the published data do demonstrate that DA in seafood is widely distributed temporally, geographically, and across species with the highest concentrations present in planktivorous species.

Domoic Acid Exposure in the International Food Supply. The section above documented that there is the potential for concentrations of DA in seafood grown or harvested in the United States and other countries and it summarized the wide ranging occurrence of DA in a variety of seafood. This section discusses the implications of this seafood data for international food supplies. As discussed in Chapter 1, seafood is the most widely traded international commodity on the planet. As such, there is the potential for exposure through both domestic and international seafood supplies. Chapter 4 will discuss the regulatory process for DA in seafood consumed in the United States.

DA Stability in Food. DA is relatively stable in seafood, and concentrations do not change significantly with freezing or cooking. When seafood is transported

internationally, it is typically frozen and often transported great distances from one country to another. Freezing Dungeness crab for 90 days at minus 23 degrees Celsius resulted in some redistribution in tissue but did not significantly reduce the concentration of DA[310]. Persistence during freezing indicates that exposure can occur long after seafood has been harvested. Cooking also does not significantly reduce DA concentrations in seafood. Steaming mussels for 10 minutes over boiling water or autoclaving mussel tissue at 121 degrees Celsius (250 degrees Fahrenheit) did not reduce DA tissue concentrations[311, 312]. The stability of DA indicates that it will persistent during any processing that involves heating or freezing.

International Data. There is little published data in the literature for international testing for DA in seafood. Where regular testing programs have been implemented, DA has often been a concern. Shellfish closures for DA have occurred in ten countries, including the United States, Canada, Scotland, Ireland, Spain, Portugal, France, Denmark, New Zealand, and Brazil[267]. These closures represent a subset of countries that could truly be of concern, since the number of countries with *Pseudo-nitzschia* detected in their waters is several times greater, and it does not appear that many countries have significant monitoring efforts.

Commercial shellfish have the potential to cause significant DA exposure in the international food supply. In southern Brazil seven commercial mussel aquaculture farms were sampled in 2008 and 2009 as part of an academic study. During this period, *Pseudo-nitzschia pseudodelicatissima* was detected in cell counts as high as 22,500 cells/ml, far in excess of the action levels for Washington State and Great Britain that

would trigger testing of seafood. The corresponding concentrations of DA measured in the bivalve *Perna perna* surpassed the action level of 20 mg/kg for 13 days, with a maximum value of 98.5 mg/kg. A closure of commercial shellfishing was initiated for one month[235].

Chapter 1 discussed the potential of nutrients to contribute to persistent cell counts of *Pseudo-nitzschia*, thus providing a constant source of DA in seafood. It is common practice in some parts of Asia, such as Vietnam, to add pig feces to aquaculture operations in order to induce algal blooms to feed fish and shellfish[313]. The use of nutrient-rich materials in aquaculture operations has the potential to induce blooms of *Pseudo-nitzschia* and cause uptake of DA into cultured seafood. In 1998, shrimp mortality events at Vietnamese aquaculture farms prompted the collection and analysis of diatoms in water and led to the identification of DA production by *Pseudo-nitzschia*[235] in Vietnamese waters[314]. No testing of DA in shrimp was published in this study. Based on 2009 data, Vietnam produces approximately five percent of the seafood imported into the United States and eight percent of the shrimp consumed in the United States[315]. As aquaculture grows and importing of seafood increases, the potential for DA in seafood due to anthropogenic nutrients may also grow.

Summary Conclusion.

This chapter examined the spatial and temporal trends in environmental densities of the diatom *Pseudo-nitzschia* and the trends of DA in seafood. These data provide a weight of evidence of the potential for exposure of humans to low dose levels of DA in seafood. Persistent low level cell counts of *Pseudo-nitzschia* (which can result in

persistent low level concentrations of DA in seafood) have been ignored as inconsequential. Chapter 1 discussed the potential for human actions to increase exposure to DA. First, anthropogenic sources of nutrients have the potential to support persistent low level densities of *Pseudo-nitzschia* and subsequent concentrations of DA in seafood. Second, as human population and per capita demand for seafood both increase, human social dynamics have the potential to result in higher consumption of lower trophic level seafood. These species are relatively high in DA compared with upper trophic level species. Chapter 2 presented a weight of evidence for potential toxicity of low levels of DA. Chapter 3 supports the conclusion there are persistent low level densities of *Pseudo-nitzschia* in coastal waters and persistent low level concentrations of DA in lower trophic level seafood from those waters.

Statistical analysis on the the L4 data has shown that *Pseudo-nitzschia* is persistent across an 18-year sampling period, where *Pseudo-nitzschia* was present in 75% of samples. Total diatom concentrations have shown a decreasing trend during sampling from 1996-2007[217], but based on the analysis in this chapter, there was no long-term trend in *Pseudo-nitzschia* cell counts at the L4 location.

Nutrient dynamics at the L4 sampling site appear typical of temperate coastal waters[217]. The water column is well-mixed during the winter and fall and this is reflected by higher nutrient concentrations. Weak stratification occurs in spring and summer, which limits the replenishment of nutrients from bottom waters. Nitrite (Figure 3-23), nitrate (Figure 3-24), silicate (Figure 3-26) and phosphate (Figure 3-27) all followed this seasonal pattern (accounting for some year to year variation), with peaks in the winter and fall and lowest concentrations in the summer. Since nutrient concentrations at this

location appear seasonal, this indicates that anthropogenic inputs are not a strong influence at the L4 location. While anthropogenic inputs do not dominate nutrient concentrations at the L4 location, they do promote the development and persistence of harmful algal blooms at many locations in the world[43]. The driving force behind nutrient dynamics (i.e., dominance of natural or anthropogenic sources) is site-specific.

Other long term data sets from the scientific literature support the conclusion that low levels of *Pseudo-nitzschia* are persistent over time. Nonparametric statistics generally did not provide further insight into the L4 *Pseudo-nitzschia* data. Descriptive statistics indicated that *Pseudo-nitzschia* are persistently present at low cell counts throughout most of the year, with the potential for DA production and uptake into seafood through most of the year. Exposures to DA are therefore likely to be chronic rather than acute. Environmental monitoring data from L4 and other locations in the literature indicate that *Pseudo-nitzschia* is globally a cosmopolitan species and is present most of the year at low densities between blooms.

Because of its widespread presence and persistence, *Pseudo-nitzschia* can act as a source of consistent and widespread uptake of DA into seafood. This is supported by the published literature on DA in seafood. Washington State razor clam data indicated persistent low level concentrations of DA in razor clams from 1996 through 2013. The literature on seafood concentrations indicates that levels of DA in seafood are also widespread across much of the planet and temporally persistent in the few areas with published long-term monitoring data. Chapter 4 examines the regulatory process for natural toxins using DA as an example and identifies areas where the regulatory process could be revisited.

CHAPTER FOUR

RISK CHARACTERIZATION AND MANAGEMENT

Chapter 4 Research Question. Is current knowledge of domoic acid toxicity and exposure to humans sufficiently compelling to reasonably argue that the current standard in seafood be revisited? What lessons can be inferred about the larger regulatory process for natural toxins in seafood?

Chapter 4 Abstract. The historical approach to contaminant risk assessment in seafood has largely focused on bioaccumulating chemicals in higher trophic level species. There is likely to be an increase in focus on the associated level of concern about hydrophilic chemicals (such as the neurotoxin domoic acid) in planktivorous fish and shellfish in lower trophic levels as human population and per capita consumption of seafood both increase and humans consume a greater quantity of lower trophic level species. To date, the regulatory focus has been on protection against high acute exposures (i.e., those that would trigger the severe acute effects of amnesic shellfish poisoning).

This chapter utilizes previous analysis on toxicity and exposure to evaluate the protectiveness of the current U.S. Food and Drug Administration (FDA) action level for DA in seafood. The current regulatory framework is discussed and attributes of that framework that could warrant revisiting are identified. These include attributes of action levels, monitoring programs, communication with the public, and disease surveillance.

Other potential regulatory options are discussed for addressing the issue of DA, such as additional toxicity studies, improved analytical techniques, and development of uptake models.

Introduction

This chapter examines the regulatory framework for protecting the public from unsafe levels of DA in seafood and determines what regulatory lessons can be gleaned from information presented in the first three papers. The historical focus of contaminant risk assessment in seafood has been on bioaccumulating chemicals in higher trophic level species. There is likely to be an increasing level of concern about hydrophilic chemicals (such as the neurotoxin domoic acid) in planktivorous fish and shellfish in lower trophic levels as demand for seafood rises and humans consume a greater quantity of lower trophic level species. In the case of domoic acid (DA), the approach has been a focus on protection of acute exposures due to a dearth of chronic toxicity data. *Pseudo-nitzschia* is a cosmopolitan genus but sampling has been limited geographically in scale and scope. Sampling data for DA in edible tissue has been limited in general, but particularly for data below the current 20 mg/kg action level.

Regulation of seafood safety is a complex issue. An examination of regulation of seafood safety by every intergovernmental organization, regional agreement, or country is beyond the scope of this research. While Chapter 2 evaluated chronic toxicity data for DA, and Chapter 3 summarized data world-wide for occurrence of *Pseudo-nitzschia* in ocean waters and for DA in seafood, this chapter focuses on the regulation of seafood safety in the United States as an example. Information from the United Nations Food and

Agriculture Organization/World Health Organization/Intergovernmental Ocean Commission (FAO/WHO/IOC), and the European Food Safety Authority (EFSA) are introduced to supplement the discussion of the United States regulatory approach where appropriate. The current regulatory framework is discussed and attributes of that framework that could require revisiting are identified. This chapter identifies attributes of an effective regulatory program by examining the current approach of using action levels, monitoring programs, communication with the public, and disease surveillance. Other potential regulatory risk management options for addressing the issue of DA, such as additional toxicity studies, improved analytical techniques, and development of uptake models, are discussed.

Current Seafood Regulatory Framework for Natural Toxins in Seafood.

Regulation of seafood safety is a complex process in the United states, with primary food safety protection split among four agencies, (1) the U.S. Department of Agriculture (meat, poultry, and processed egg products), (2) the U.S. Environmental Protection Agency (setting pesticide tolerances in agricultural products), (3) the U.S. Department of Commerce (inspection of seafood), and (4) the U.S. Food and Drug Administration (all other foods including food additives and adulteration). Eleven other agencies have smaller but significant roles in food safety[316].

FDA has responsibility for all toxins in seafood, including natural toxins such as DA. Contaminants can be contained in seafood when harvested, or can be introduced during transport, processing, and packaging. Contaminants can be of natural or man-made origin. This chapter focuses on the regulation of natural toxins present in seafood

during harvesting. DA is used as the primary example to illustrate how natural toxins are regulated.

FDA, from 1938 to the present, has been responsible for assessing and establishing safe levels of contaminants, inspecting, and enforcing safe levels of contaminants in seafood. In the Food, Drug, and Cosmetics Act of 1938, FDA is mandated by Congress to protect the safety of the country's seafood supply. This act charged FDA with setting safe levels of contaminants in seafood in order to protect human health (Federal Law 75-717, 52 United States Statutes at Large 1040). These levels can be tolerances or action levels:

- Tolerances are established through a formal rulemaking process and carry the force of law;
- Action levels are recommended limits that can be quickly established but do not carry the force of law.

State, local, and foreign authorities or private importers are required to ensure seafood meets these levels. These efforts are supplemented by inspection and analysis of a small subset of seafood. More recently, FDA recognized some historical shortcomings in the process and supplemented it through Hazard Analysis and Critical Control Planning (HACCP). The Food Safety Modernization Act was later enacted to further improve on the protection of the nation's food supply. The Centers for Disease Control (CDC) also plays a critical role by collecting information on cases of food-borne illness. CDC's data collection has historically been valuable for determining the extent of disease associated with food-borne illness and the degree to which exposure and risk have been

effectively mitigated. This has not been the case for DA however, because reporting of illness associated with DA is not required by the CDC. While complex and highly diffused, the current system of risk mitigation can be effectively described by a more detailed focus on four significant parts of the system.

The sections below discuss the current role of (1) FDA Tolerances and Action Levels, (2) Hazard Analysis and Critical Control Planning, (3) Centers for Disease Control Disease Surveillance, and (4) the potential impacts of the Food Safety Modernization Act.

Tolerances, Action Levels and Regulatory Limits. Through the Interstate Commerce Clause (United States Constitution Article I Section 8 Clause 3), the federal government has the authority to regulate food products shipped in the United States. The Food, Drug, and Cosmetic Act (FDCA) of 1938 created the Food and Drug and Administration. Section 402(a)(1) of the Food, Drug, and Cosmetic Act covers poisonous and deleterious substances in food. When seafood products transported in interstate commerce are deemed or suspected to contain a contaminant that may pose a risk to human health, the FDA is mandated to take steps to limit the public's exposure. FDA may choose to take a number of steps to protect public health including inspections of shipments, development of an acceptable limit in seafood, and seizure of contaminated seafood. Unavoidable environmental contaminants, including natural toxins such as DA, may be regulated under Section 402(a)(1) when the contaminant "may render injurious" exposure to that food product. The Federal Code of Regulations provides criteria for the establishment of action levels, tolerances, and regulatory limits (this last category is not currently used for natural toxins) for unavoidable contaminants in food for human

consumption (21 CFR 109 and 509). Action levels and tolerances represent limits at or above which FDA can take legal action to remove adulterated products, including shellfish, from the market. Action levels and tolerances are established based on the unavoidability of the poisonous or deleterious substance and do not represent permissible levels of contamination where it is avoidable[317].

Tolerances. Under Section 406 of the Food, Drug, and Cosmetic Act, the agency can establish tolerances for unavoidably added poisonous or deleterious substances. FDA does not consider human health risk exclusively when setting a tolerance. Section 406 requires that the tolerance be established by assessing risk, the feasibility of preventing or reducing the level of the contaminant, and the economic impacts of the removal of large amounts of food from the market [318].

The Food Drug and Cosmetic Act of 1938 describes procedures for establishing tolerances for deleterious substances in food. The FDA publishes a draft tolerance in the Federal Register and a public comment period is established. After the comment period ends, FDA publishes an "order" responding to the comments and establishes a "final" tolerance. Objections can be filed by the public, which must state the grounds for the objection and request a public hearing. If objections raise "material factual issues" then FDA must hold an evidentiary hearing with an administrative law judge[319]. The judge's decision can be appealed to the FDA Commissioner, whose decision can be reviewed by the court of appeals. During this process the tolerance is not enforced. Action levels are established as interim values while tolerances are developed (21 CFR 109 and 509).

FDA has seldom used the tolerance process for regulating contaminants in seafood. There are currently tolerances established for only four contaminants in seafood and none for natural toxins. The established tolerances listed in Table 4-1.

Table 4-1

Tolerances For Seafood

Contaminant	**Level (mg/kg)**	**Food Commodity**	**Reference**
Diquat	0.1	All fish	40 CFR 180.226
Glyphosphate	0.25	All finfish	40 CFR 180.364
	3.0	Shellfish	40 CFR 180.364
PCBs	2.0	All fish	40 CFR 109.30
2,4-D	1.0	All fish	40 CFR 180.142

From the 2009 National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish (published in 2012)[317]

The fact that FDA has only derived four tolerances in seafood suggests that the agency believes the tolerance process is generally not the optimal way to regulate contaminants in seafood. The process for setting tolerances is complex and time consuming. Once established, tolerances are a rigid regulatory tool in situations where research and data are fluid and evolving. FDA has preferred to regulate seafood contaminants through action levels.

Action Levels. FDA has primarily relied on less formal "action levels" that do not require the formal rule making process for tolerances. Action levels are administrative guidelines that define the level of contamination at which the agency may regard food as adulterated [320]. FDA can establish an action level whenever needed, without making the action level "draft" while awaiting a public comment period. The FDA need only publish notice of a new action level in the Federal Register as soon as practicable and make supporting information publicly-available (21 CFR 109.4(2)). The Federal Register notice announces the availability of supporting material for the action level within the Division of Dockets Management. The notice invites public comment on the action level. While FDA is required to seek public comment, FDA is not required to respond to comments formally, as is required in the tolerance setting process. Since FDA need only publish the action level in the Federal Register, and not its derivation or scientific basis, the only way for the public to determine the basis is to obtain information from the docket housed in FDA's Office of Docket Management in Silver Spring, Maryland. Action levels are revoked when a regulation establishing a tolerance for the same substance and use goes into effect[317].

In 1990, FDA redefined the purpose of action levels in response to a circuit court decision that ruled action levels cannot be treated as substantive rules without going through a formal rule-making process. FDA stated that action levels constitute prosecutorial guidance rather than substantive rules, and that action levels do not have the "force of law" of substantive rules, but FDA has the ability to use discretion in their application [55 Fed. Reg. 20,782 (May 21, 1990)]. FDA can take enforcement action (or

recommend court enforcement) regardless of whether or not a particular seafood product exceeds an action level [321]. FDA appears to favor creation of action levels over tolerances because it allows them to establish a regulatory guideline more quickly and allows them greater discretion in terms of enforceability.

Procedures for deriving action levels were outlined in the September 30, 1977 Federal Register. While only four tolerances have been established for contaminants in seafood, twenty action levels have been established for contaminants[317]. These include the pesticides aldrin/dieldrin, carbaryl, chlordane, chlordecone, DDT and its derivatives, endothall and its derivatives, heptachlor/heptachlor epoxide, and mirex, the metals arsenic, cadmium, chromium, lead, nickel, and methylmercury, and the natural toxins saxitoxin, brevetoxin, okadaic acid, DA, ciguatoxin, and azaspiracids[317]. In addition, FDA lists eight chemotherapeutics (used in the aquaculture industry) that are banned from food at any detectable concentration, including seafood (chloramphenicol, clenbuterol, diethylstilbestrol, demetridazole, nitroimidazoles, nitrofurans, fluoroquinilones, and glycopeptides). There are essential differences between action levels and tolerances. Most notably, the proof of evidence remains with the FDA in determination of an action level. If challenged, the FDA must support its determination with evidence. Alternatively, because of the more rigorous and public process of establishing a tolerance, the burden of proof for evidence challenging the tolerance is held by the entity challenging the tolerance determination.

Action Levels for Natural Toxins. FDA has not derived any tolerances for natural toxins in seafood, FDA has established action levels for six natural toxins (toxic substances produced by living organisms), (1) saxitoxin, (2) brevetoxin, (3) okadaic acid,

(4) domoic acid, (5) ciguatoxin, and (6) azaspiracids. FDA action levels for the six natural toxins in seafood are presented in Table 4-2.

Table 4-2
FDA Seafood Action Levels for Natural Toxins

Toxin (Associated Illness)	Concentration (mg/kg)	Seafood Type	Basis
Saxitoxin (paralytic shellfish poisoning)	0.8	All fish	Reconstructed doses in humans that caused acute effects
Brevetoxin (neurotoxic shellfish poisoning)	0.8 mg/kg	Shellfish only	Reconstructed doses in humans that caused acute effects
Okadaic acid (diarrhetic shellfish poisoning)	0.16	Shellfish only	Reconstructed doses in humans that caused acute effects
Domoic acid (amnesic shellfish poisoning)	20 (30 in the viscera of Dungeness crab)	All fish	Reconstructed doses in humans that caused acute effects
Ciguatoxin (ciguatera fish poisoning)	0.00001 P-CTX equivalents for Pacific ciguatoxin and 0.0001 C-CTX-1 for Caribbean ciguatoxin	All fish	Not Available
Azaspiracids (azaspiracid shellfish poisoning)	0.16	All fish	Reconstructed doses in humans that caused acute effects

From the 2009 National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish (published in 2012)[317]

While FDA has procedures for developing action levels, FDA did not develop its own DA action level but instead adopted one that had been developed by Canada[317]. This Canadian standard was subsequently adopted as an international standard by the United

Nations Food and Agriculture Organization, the World Health Organization, and the Intergovernmental Oceanographic Commission (FAO/WHO/IOC)[103].

As seen in Table 4-2, the regulatory approach for natural toxins in seafood has typically relied on the use of acute data from outbreaks to derive action levels. A food-borne illness case cluster of unknown etiology leads to the identification of a natural toxin. Human doses are reconstructed during the outbreak and levels that do not cause gross acute illness are identified. Chronic data are not available and action levels are derived only to be protective of acute exposures and effects[3]. This was the process for DA and this process was paralleled by other natural toxin action levels in Table 4-2. There was insufficient data to derive a chronic action level for any of the natural toxins listed above. Chronic toxicological studies were never performed for natural seafood toxins, so action levels protective of chronic exposure were never developed.

The United Nations and the European Union have established recommended limits on natural toxins in seafood. These levels and their bases are presented in Table 4-3.

Table 4-3 Natural Toxins Seafood Regulatory Levels				
Toxin (Illness)	**US Action Level (mg/kg)**	**Acute Recommended FAO/WHO/IOC Value (mg/kg) (100 or 380 g consumption)**	**Basis of FAO/WHO/IOC Recommended Value[1,3]**	**European Union Health Standard[2,4]**
Saxitoxin (paralytic shellfish poisoning)	0.8	0.11 – 0.42	Based on neurological effects from acute human exposure data. No chronic data available.	0.8
Brevetoxin (Neurotoxic shellfish poisoning)	0.8 mg/kg	NA	Acute value adopted from USFDA. No chronic data available.	NA
Okadaic acid (Diarrhetic shellfish poisoning)	0.16	0.08 – 0.2	Based on GI effects. Acute human and animal data only. No chronic data. Some data to indicate tumor promotion, genotoxicity, and immunotoxicity.	0.16
Domoic acid (amnesic shellfish poisoning)	20 (30 in the viscera of Dungeness crab)	16 – 60	Reconstructed doses in humans that caused acute effects	20
Ciguatoxin (ciguatera fish poisoning)	0.00001 P-CTX equivalents for Pacific ciguatoxin and 0.0001 C-CTX-1 for Caribbean ciguatoxin	NA	NA	NA

Table 4-3 Natural Toxins Seafood Regulatory Levels				
Toxin (Illness)	**US Action Level (mg/kg)**	**Acute Recommended FAO/WHO/IOC Value (mg/kg) (100 or 380 g consumption)**	**Basis of FAO/WHO/IOC Recommended Value[1,3]**	**European Union Health Standard[2,4]**
Azaspiracids (azaspiracid shellfish poisoning)	0.16	0.0063 – 0.024	Acute value based on GI effects. Insufficient data on chronic effects. Initial long-term study showed statistically significant increase in cancer.	0.16
Yessotoxins	NA	8 - 30	Acute animal data No reported effects in humans.	1
Pectenotoxins	NA	20 mg/100g shellfish in some countries.	Acute toxicity data only. No documented Effects to humans. Found in Australia, Italy, Japan, New Zealand, Norway, Portugal, and Spain.	NA
Cyclic Imines	No value but does occur in U.S.	NA	Only LD50 values available. No known effects in humans.	No value but does occur in Europe

[1]From Toyofuku 2006[8]
[2]From Chapter V (2) (c) and (e) of Section VII of Annex III to Regulation (EC) No 853/20045
[3]The FAO/WHO/IOC represents a recommendation by the United Nations, which has no regulatory authority.
[4]The European Union Health Standard represents a regional agreement.

FAO/WHO/IOC does not have regulatory authority and instead of creating enforceable standards for natural toxins in seafood it recommend safe levels. There are three natural toxins (yessotoxins, pectenotoxins, and cyclic amines) being evaluated by the

FAO/WHO/IOC that do not have FDA action levels. These three natural toxins are all produced by cosmopolitan algal species that occur across the world. Yessotoxins are produced by the algae *Protoceratium reticulatum*, and have been detected in algae or seafood in Australia, Canada, Italy, Japan, New Zealand, Norway, and the United Kingdom[8]. Pectenotoxins in molluscs or algae also appear to be cosmopolitan, having been detected in Australia, Italy, Japan, New Zealand, Portugal, and Spain[8]. A provisional value of 20 mg/100 shellfish for pectenotoxins has been adopted in some countries, but not the United States[8]. Cyclic Amines are also ubiquitous, occurring in Canada, Denmark, New Zealand, Norway, Scotland, Tunisia, and the United States, but there is currently no FDA action level[8].

There are currently five natural toxins in fish regulated by the European Union compared to the six regulated by FDA. Four of the regulated toxins are the same in the United States and the EU (saxitoxin, okadaic acid, DA, and azaspiracids), and they have identical guidance levels in the United States and Europe. The United States has an action level for ciguatoxin while the European Union (Commission Regulation No. 854/2004) states that fishery products are not to be placed on the market "containing biotoxins such as ciguatera or other toxins dangerous to human health", inferring that the limit is zero. FDA has an action level for brevetoxin, while the EU does not. The EU has a guidance level for yessotoxins, while the FDA does not. FDA is within the recommended FAO/WHO/IOC range for two toxins (okadaic acid and DA) and above the FAO/WHO/IOC range for two toxins (saxitoxin and azaspiracids).

The DA action level (and other acute action levels discussed above) may work in preventing further outbreak of acute illness, although this is not clear (discussed later in

this chapter). Even when toxicological data in laboratory animals provide evidence that the current guidance levels for DA may not protective, new guidance values were not adopted. FDA and European Union scientists recommended new guidance levels for DA that were never adopted[206]. Because FDA's action level process is not documented and transparent, it is unclear why the proposed revised DA action level was never adopted. There are some inconsistencies in terms of which natural toxins are regulated in the United States and in Europe, and which natural toxins have recommended limits from the United Nations. These inconsistencies do not appear to be related to where the natural toxins actually occur. It is unclear why FDA has not adopted action levels for natural toxins such as yessotoxins, pectenotoxins, and cyclic amines from cosmopolitan species that are likely to occur in the marine waters of the United States when guidance levels are available from FAO/WHO/IOC and EFSA.

Regulatory Limits. Tolerances and action levels are not the only seafood regulatory level options available to FDA. Further complexity was added to the options when FDA developed a process for setting regulatory limits in response to a court ruling. FDA had previously treated action levels and tolerances as functionally equivalent. FDA published a regulation [21 CFR 109.4 (1986)] that stated:

> An action level for an added poisonous or deleterious substance … may be established to define the level of contamination at which food will be deemed adulterated. An action level may prohibit any detectable amount of substance in food.

In this regulation, FDA defined action levels as essentially equivalent to tolerances. However, in 1987 the U.S. Court of Appeals for the District of Columbia Circuit ruled on the case Community Nutrition Institute vs. Young [818 F. 2d 943 (D.C. Cir. 1987)] that

action levels cannot be treated as regulations without the formal comment procedures employed by tolerances. After the Community Nutrition Institute decision, FDA reevaluated its action level policy[323]. The FDA published a new regulation that allows substantive rules, called regulatory limits, to be established by formal notice-and-comment rule making [55 Fed. Reg. 20,782 (May 21, 1990)]. The regulatory limit establishes the level of an unavoidable added poisonous or deleterious substance that renders a food adulterated within the meaning of the Food Drug and Cosmetics Act. A regulatory limit will be established when (1) the substance cannot be avoided by current good manufacturing practices; (2) there is no tolerance established for the substance in the particular food; and (3) there is insufficient information by which a tolerance may be established for the substance, or technology changes that may affect the appropriateness of a tolerance appear reasonably possible [55 Fed. Reg. 20,782 (May 21, 1990)]. The regulatory limit process has not been used for seafood, although it has been used for other food products.

FDA continues to rely on action levels as their regulatory values for natural toxins in seafood. Action levels provide guidance for state, local and foreign entities attempting to comply with FDA regulations and guidance such as Hazard Analysis and Critical Control Plans[321], which are discussed in the next section.

Hazard Analysis and Critical Control Plan (HACCP). FDA has placed much of the responsibility for seafood safety with the producers, processors, and importers of seafood. The risks associated with domestic and imported products led to the creation of the Hazard Analysis Critical Control Points (HACCP) approach to food safety regulation

in 1997[324]. HACCP covers both foreign and domestic production of seafood and provides a systematic process that includes (1) determination of significant hazards, (2) identification of critical control points, and (3) development of control strategies. HACCP is a general risk assessment process that is relies on proper identification of hazards and effective control and is not prescriptive in nature.

Natural toxins in shellfish, are identified and addressed as a hazard in chapter six of the HACCP guidance[325]. FDA identifies a number of seafood types that are of particular concern for DA exposure including mussels, scallops, razor clams, market squid, and anchovies[326]. The natural toxins provisions of HACCP allows state and foreign government agencies, termed shellfish control authorities, to classify waters in which shellfish are found, based on the presence of natural toxins in shellfish meats. Shellfish control authorities can use toxic algal cell counts from monitoring data to classify shellfish harvest areas. As a result of classifications, shellfish harvesting is allowed from some waters and not others, or only at certain times. Shellfish control authorities then regulate shellfish harvests to ensure that harvesting takes place only when and where it has been permitted[325]. HACCP applies to both domestic and imported shellfish.

Domestic Shellfish. Typically for shellfish, state and local authorities identify the natural toxin hazards by monitoring algal cell counts. When algal cell counts reach a certain limit, then tissue samples are collected[306]. Containers of in-shell molluscan shellfish received from a harvester are required to have a tag that discloses the date and place they were harvested (by state and site), type and quantity of shellfish, and information on the harvester. For bulk shipments of shellstock where the shellstock is

not containerized, the shellstock must be accompanied by a bill of lading or similar shipping document that contains the same information[325].

Imported Shellfish. For imported seafood, the burden for ensuring the safety of imported shellfish is placed on importers. U.S. importers must ensure that imported shellfish is harvested and processed in other countries in a manner that is consistent with HACCP. HACCP also provides a process for developing memorandums of understanding (MOUs) with other countries that demonstrate that seafood is harvested and processed in a manner consistent with HACCP. This MOU fulfills the importer's requirement under the seafood HACCP regulation, unless the importer is also a processor. For a country to have an approved MOU, it must provide FDA with:

- Side-by-side comparison of the country's HACCP program with 21 CFR Section 123, the seafood HACCP regulation;
- Side-by-side comparison of the country's sanitation program with FDA's Good Manufacturing Practices regulation, 21 CFR Section 110;
- Side-by-side comparison of the country's low acid canned food and acidified food program with FDA's low acid canned food and acidified food regulations, 21 CFR 108, 113 and 114; and
- A check list of the country's regulatory control system, procedures, etc., to demonstrate the control authority's authority and ability to enforce a HACCP-based control program.

As of March 2014, the only countries that are currently meeting HACCP requirements and have established MOUs with FDA are Canada, Japan, and China[327]. The MOUs may be product and/or processor specific and may not cover all the seafood products from the signatory country.

HACCP relies primarily on self-regulation rather than direct oversight. FDA is not prescriptive in how HACCP plans are developed. Instead, FDA provides a general

framework for states, local authorities and foreign countries to develop their plans. The effectiveness and level of protection of these plans have the potential to vary significantly depending on the state, local authority, or foreign country. FDA has not been successful in reaching MOUs for seafood with other nations, with only three current MOUs. While HACCP attempts to ensure that illnesses are prevented, it does not ensure that diseases are reported when they occur. This issue has been the purview of the Centers for Disease Control.

Centers for Disease Control Reportable Diseases. It is important that steps are taken to ensure that safe levels of contaminants are developed (i.e., tolerances and action levels), and that those levels are met in recreationally and commercially available fish for consumption by the public (i.e., HACCP). However, it is difficult to know whether these efforts have been successful unless information on disease occurrence related to toxins is collected. The Centers for Disease Control (CDC) monitors, identifies, and investigates foodborne diseases. It is also mandated with developing improved epidemiological and laboratory methods through the Public Health Service Act (42 U.S.C. §201). The CDC has developed eight different surveillance networks for foodborne illness, and relies primarily on reporting data from state and local authorities. However, none of these eight surveillance networks collect information on natural toxins in seafood. Certain states have their own mandatory reporting requirements for some illnesses for natural toxins in seafood, but there is no overarching federal requirement.

The CDC, in conjunction with state public health agencies, conducts disease surveillance. The CDC tracks foodborne illnesses related to 31 major pathogens. These 31 pathogens result in 9.4 million domestically-acquired foodborne illnesses per year,

resulting in 55,961 hospitalizations and 1,351 deaths [328]. However, these numbers pale in estimated foodborne illnesses from unspecified causes, with 38.4 million illnesses, 71,878 hospitalizations, and 1,686 deaths annually[329]. The potential contribution of natural toxins, including DA, is of course unproven and unclear.

The incidence of foodborne illness showed a drop immediately after the implementation of 1995 USDA regulations, but has been constant in recent years [330]. Whether the drop was caused by the regulations is unclear. The one exception to this drop in reported foodborne illnesses is cholera, caused by the infectious agent *Vibrio spp*. which can occur in seafood[331]. Coupled with information that harmful algal blooms may be increasing (discussed in Chapter 1), there is cause for concern that the illnesses resulting from natural toxins in seafood, such as amnesic shellfish poisoning, could increase. It is impossible to tell at this point however, since data on illnesses related to seafood toxins is not reportable to CDC[332]. The Food Safety Modernization Act of 2011 gives the Centers for Disease Control and Prevention new responsibilities to enhance federal, state, and local surveillance systems for foodborne illness[333]. It is still unclear what changes may be made to the current system.

State Reporting Requirements. While the CDC does not require reporting of any cases of natural seafood toxin poisoning, states can require reporting from health care providers and local boards of health. During the ten-year period 1978-1987, there were 179 ciguatera outbreaks, with a total of 791 cases reported in those outbreaks[321]. While dated, this effort by the Institute of Medicine remains one of the comprehensive natural toxin data comparatively at the state level. During the same period, there were thirteen

outbreaks of paralytic shellfish poisoning, with 137 cases[321]. Table 4-4 lists states that require reporting of natural seafood toxin poisonings.

Table 4-4
Reportable Illnesses for Natural Toxins in Seafood

State	Amnesic Shellfish Poisoning	Paralytic Shellfish Poisoning	Ciguatera Poisoning	Neurotoxic Shellfish Poisoning	Diarrhetic Shellfish Poisoning
Alabama					
Alaska	X	X	X		
Arizona					
Arkansas					
California	X	X	X		
Colorado					
Connecticut					
Delaware					
Florida		X	X	X	
Georgia					
Hawaii			X		
Idaho					
Illinois					
Indiana					
Iowa					
Kansas					
Kentucky					
Louisiana	X	X	X	X	
Maine		X			
Maryland					
Massachusetts	X	X	X		
Michigan					
Minnesota					
Mississippi					
Missouri					
Montana					
Nebraska					
Nevada					
New Hampshire					
New Jersey		X	X		
New Mexico					
New York					
North Carolina			X		
North Dakota					
Ohio					

Table 4-4 Reportable Illnesses for Natural Toxins in Seafood					
State	**Amnesic Shellfish Poisoning**	**Paralytic Shellfish Poisoning**	**Ciguatera Poisoning**	**Neurotoxic Shellfish Poisoning**	**Diarrhetic Shellfish Poisoning**
Oklahoma					
Oregon	X	X	X		
Pennsylvania					
Rhode Island		X	X		
South Carolina					
South Dakota					
Tennessee					
Texas					
Utah					
Vermont					
Virginia					
Washington	X	X			X
West Virginia					
Wisconsin					
Wyoming					
X = Reporting Requirement This list does not include infectious or parasitic agents					

Despite proximity to the original outbreak of the disease, only one of six New England states (i.e., Massachusetts) collects case information on amnesic shellfish poisoning. In Massachusetts, ciguatera poisoning, paralytic shellfish poisoning, and amnesic shellfish poisoning must be reported by health care providers within 24 hours to the local board of health in the community where it occurred (105 CMR 300.100). The local board of health must in turn pass this information on to the Massachusetts Department of Public Health within 24 of receipt. In Maine, paralytic shellfish poisoning must be reported within 48 hours to the Maine Department of Health and Human Services, but ciguatera poisoning and amnesic shellfish poisoning are not included. Ciguatera and paralytic shellfish poisoning must be reported to the Rhode Island Department of Health on the day they are suspected, but amnesic shellfish poisoning is not included. Connecticut, New

Hampshire, and Vermont do not have any reporting requirements natural seafood toxin illnesses.

There are twelve states that collect case information on poisonings for at least one natural seafood toxin. Amnesic shellfish poisoning is only a reportable disease in six states (Alaska, California, Louisiana, Massachusetts, Oregon and Washington. Paralytic shellfish poisoning and ciguatera poisoning have the greatest number of states collecting case information (ten). Neurotoxic shellfish poisoning cases are collected in two states while diarrhetic shellfish poisoning cases are collected in one state. There are ten coastal states that choose not to collect any data on natural toxin seafood poisoning cases (Connecticut, Delaware, Georgia, Maryland, Mississippi, New Hampshire, New York, South Carolina, Texas, and Virginia). Some additional states have general provisions for reporting clusters of foodborne disease or unusual disease cases. When illness cases are not collected, it is difficult or impossible to evaluate the effectiveness of action levels in protecting public health. Further, if there is no action level for a particular toxin, it is difficult to determine if one may be needed when no illness data are collected for the toxin.

Even if all states were required to report all cases of poisoning by natural seafood toxins, identification of cases of poisoning by can be difficult. For example, when DA was first discovered in Washington State seafood (razor clams) in 1991, it was not considered a natural toxin of concern there. Shellfish samples were collected from Long Beach, WA and extracts were injected into mice to test for saxitoxin, the cause of paralytic shellfish poisoning. The mice began scratching behind their ears, which the technician happened to recognize as a symptom of DA exposure. A telephone survey

was conducted of individuals in the Long Beach area to determine if any individuals had experienced symptoms of DA exposure. Approximately 25 individuals experienced flu-like symptoms that could be attributable to DA exposure, but without further medical follow up, the results were inconclusive (Quick 1992 as cited in Chadsey[306]). This illustrates the difficulty in collecting case information not reportable to CDC for a toxin with a wide range of symptom responses and interpersonal responses to exposure[148]. An inconsistent reporting protocol very likely contributes uncertainty by limiting the overall focus on DA exposure. Limited resources for public health research and reporting are, understandably, directed primarily to more well-established threats to public health.

The more mild symptoms of DA (stomach cramping, vomiting) would be difficult to distinguish from cases of gastroenteritis from other causes. Only more serious cases that resulted in acute effects of immediate seizures and memory loss would likely be identified for reporting. The data collected on shellfish poisoning essentially represents acute data. As discussed in Chapter 2, the effects of DA can cause a short term syndrome (amnesic shellfish poisoning) as well as a chronic syndrome characterized by some combination of seizures, behavioral issues, and effects to learning and spatial memory. In general, reporting systems for foodborne illness are best able to capture the cases of acute illness where those effects can be tied to exposure through a recently consumed seafood meal. The cases of chronic exposure to DA may be difficult to capture, particularly when the effects overlap with other chronic brain illnesses such as epilepsy, schizophrenia, and Alzheimer's disease. Neuro-degenerative, central nervous system effects with loosely defined attributes such as dementia are potentially difficult to classify correctly.

Food Safety Modernization Act 2011. The Food Safety Modernization Act (FSMA) was enacted by Congress in December 2010 (FDA Food Safety Modernization Act (FSMA), P.L. 111-353)[335]. The Act was the greatest expansion of FDA food oversight since the Food, Drug, and Cosmetic Act of 1938. The FSMA mandates that the FDA use science-based food safety standards and introduces a requirement for mandatory traceability of food products[336].

The provisions that most directly affect domestic and imported seafood include:

- Improvement of seafood safety through enhanced inspection and analysis of seafood, identifying high-risk processing facilities, sharing enforcement and compliance information, conducting training and outreach, and improved coordination with other agencies (FSMA, §201);
- Requirements for improved guidance related to post harvest processing of raw oysters (FSMA, §114); and
- Improved inspections of foreign processing facilities by the Secretary of Commerce to evaluate practices and processes in seafood production (FSMA, §306).

There is considerable leeway the Food Safety Modernization Act and uncertainty in how any of these topics will be translated into regulations and guidance. One of the key points of the FSMA is to create a foreign supplier verification program for all foods. Since HACCP already includes a foreign supplier verification program, seafood is exempt from this requirement of the FSMA, which would be redundant. The foreign supplier verification program requirements are more comprehensive and rigorous than HACCP and it is believed that these strengthened requirements will eventually be incorporated into HACCP[337].

In addition, a number seafood safety issues have more recently been considered by Congressional committee but have not acquired the force of law. These include strengthening of coordination among federal programs related to seafood safety, preventing seafood fraud (i.e., mislabeling of species), using third parties to certify the safety of imported seafood, and developing a system to trace domestic and imported seafood production, harvest, processing, importing, and retail[338]. The Food Safety Modernization Act clarifies and expands FDA authority to address seafood safety related to natural toxins such as DA. It is still unclear however, what specific changes may occur in the regulation of natural toxins in seafood.

In summary, ensuring the safety of seafood in the United States relies on international, federal, state, local, and private partnerships. FDA has relied primarily on action levels, with only four tolerances versus 20 action levels in effect for seafood. The reliance on action levels results in standards that have less transparency, scrutiny and public input. Action levels were intended to be placeholders while tolerances were developed, but they have instead become permanent regulatory levels. Action levels are used in conjunction with HACCP to ensure the safety of seafood. The HACCP process provides guidance to the regulatory community. For domestic seafood, FDA provides technical guidance to state and local authorities who make decisions on what toxins to monitor and when to close fishing areas because of natural toxin concerns. For imported seafood, there have been only three memorandums of understanding with other countries regarding the HACCP process. Instead, importers have been tasked with ensuring that seafood from other countries is compliant with the HACCP process. The CDC does not require reporting of illnesses related to natural toxins in seafood, rendering it difficult to

determine (1) if action levels for natural toxins in seafood are effective or (2) if action levels are needed for any additional natural toxins in seafood. This section is used below to guide a discussion of attributes of an effective regulatory system for natural toxins in seafood.

Attributes of an Effective Regulatory System for Natural toxins in Seafood.

This dissertation has argued that recent toxicological studies in laboratory animals and examinations of environmentally-exposed marine mammals indicate that DA has the potential to be toxic at concentrations lower than previously believed. Environmental data indicate that *Pseudo-nitzschia* is both globally widespread and persistently present in coastal waters. Seafood sampling data are scattered and intermittent but indicate persistence of low levels of DA in seafood at locations across the globe. In a globally traded commodity like seafood with limited traceability, the weight of evidence for toxicity and exposure concerns suggests a re-examination of the regulatory approach and this re-examination has been at a minimum recognized and initiated in the Food Safety Modernization Act of 2011. An effective regulatory system is the critical mechanism for the protection of human health from exposure to natural toxins in seafood. The previous sections examined the current regulatory process and this section draws upon that discussion. This section identifies the key regulatory themes of (1) action levels, (2) monitoring, (3) communication, and (4) disease surveillance that collectively maximize the effectiveness of the regulatory system for natural toxins in seafood. DA is used as the primary example to guide discussion of attributes. The core question to be resolved is whether these regulatory issues and associated regulatory responses are sufficiently

adaptable in current management practice to respond to the scientific results on the importance of low level chronic exposure.

Effective Action Levels. Based on the action level analysis from the previous section, there are a number of attributes that contribute to effective action levels. These include (1) public input, (2) transparent and consistent values, (3) appropriately protective values, (4) periodic updating of values, (5) protection of chronic toxicity, (6) protection of sensitive subpopulations, and (6) development of tolerances for natural toxins in seafood. These attributes are discussed below.

Public Input. Tolerances were intended to be the regulatory values for seafood. The process for establishing a tolerance includes a formal public comment process where the FDA is required to consider and respond to public comments. FDA is required to respond to comments on tolerances, similar to other formal rule making processes in the federal government. In contrast, the process for establishing an action level action level is an expedited process that limits timely public input. Action levels were originally intended as interim values that could be established quickly and eventually replaced by tolerances (Code of Federal Regulations [CFR] 21 CFR 109 and 509). Action levels are established and revised according to criteria specified in the Code of Federal Regulations (21 CFR 109 and 509), and are revoked when a regulation establishing a tolerance for the same substance and use becomes effective[317]. The process was created so that FDA could develop safe levels quickly without going through a formal and cumbersome regulatory process that involves formal public comment periods. This would allow FDA to respond quickly to a newly-identified toxic concern in seafood as was the case for DA when the action level was adopted from Canada in a timely manner. The action level for

DA was published in the Federal Register and the FDA had an immediate regulatory tool for an emerging threat to public health. Action levels can then be followed by tolerances with a formal rule making process when the agency deems that sufficient and certain information is available to create more "rigid" regulatory standard. However, tolerances have never been implemented for natural toxins as all action levels are still in effect. In the case of DA, the data in the scientific literature have continued to evolve, which likely has led to FDA continuing with an action level rather than pursuing a tolerance. However, when FDA does not have to respond to comments (as is the case with action levels), there is the potential for insufficient consideration of public input. Because the DA action level has been in effect for 25 years, FDA has not been required to respond to public comments in that time period. This lack of significant public discourse on a regulatory standard is a significant concern. One option for improving public input would be to for FDA to respond to previous public comments if an action level is in effect for a certain length of time, and to periodically solicit new comments and new data from the public.

A Transparent and Consistent Process. FDA outlines the process for creating tolerances and action levels in 21 CFR109 and 509. The regulations provide a general process for the derivation of action levels FDA but do not provide details such as specifying (1) sensitive subpopulations to be protected, (2) uncertainty factors for use in toxicity values, or (3) consumption rates of seafood to be used when deriving tolerances and action levels. FDA has not published a guidance document to document a detailed process for developing tolerances and action levels. In the case of DA, FDA did not develop an action level but instead adopted a value from Canada when DA was

discovered in finfish on the California coast[317]. Action levels for other natural toxins in seafood were also adopted from other sources rather than derived by the FDA[317].

There are few recent examples of FDA deriving an action level for a natural toxin in food. The most recent action level for a natural toxin was for patulin, a mycotoxin produced by the molds *Penicillium*, *Aspergillus*, and *Byssochylamys* in rotting apples. FDA derived an action level for patulin in 2001 in apple juice products[339]. In documenting uncertainty factors applied to the patulin reference dose, FDA did not cite any recent FDA guidance (because it appears no such guidance exists) but instead cited an FDA publication from 1954[340]. Two actions would make action levels and tolerances for natural toxins far more transparent. First, a guidance document for derivation of action levels and tolerances that outlines procedures in detail could be developed by FDA. Second, supporting documentation for individual tolerances and action levels could be placed on FDA's website, where they could be easily accessed by the public.

Appropriately Protective Regulatory Levels. In some cases the FDA process can result in risks in food significantly greater than under EPA. An evaluation of action levels for persistent organic pollutants indicates that consumption of food at maximum levels allowed by FDA would result in exposures that exceed standards set by EPA and ATSDR[341]. For instance, consuming food containing DDT at the FDA action levels would result in adult exposures 90 times the Agency for Toxic Substance and Disease Registry's (ATSDR) Minimal Risk Level (equivalent to a safe chronic exposure level) and 300 times for children[341]. FDA balances risk with health benefits of seafood and the economic costs of removing seafood products from the market[318, 342]. FDA has not been particularly forthcoming with the process for considering these tradeoffs, as action levels

do not require accessible public documentation nor do they require FDA responses to public comments. A recent exposure evaluation of Native American tribes in the Northwest concluded that the mercury action level of 1 mg/kg would need to be reduced to 0.1 mg/kg to afford the Native American tribes the same level of protection as the general public due to their significantly greater seafood consumption rate[343]. It may be appropriate to consider making the levels of protection both consistent among cases and to also make decisions on risk/benefit tradeoffs readily available to the public.

Periodic Updating of Values. EPA and FDA are the primary federal agencies that regulate risks of human exposure to toxic substances. It is therefore instructive to examine how their approaches differ. EPA regulates a list of chemicals many times longer than FDA. EPA has developed the Integrated Risk Information System to organize the process of developing reference doses and to make information on the derivation of values readily available to the public. EPA assigns case managers to chemicals to shepherd them through the development process and to ensure periodic checks on the developments in a chemical's toxicological and epidemiological data in the scientific literature and to ensure that relevant data are incorporated into the regulatory process. FDA does not have an equivalent public process for regularly updating its action levels. The action level for DA appeared to be revisited by FDA in the late 1990s when an FDA scientist published a revised reference dose in a peer-reviewed journal[207]. However, FDA did not adopt this revised (and lower) reference dose. There was no publicly available explanation for why the reference dose was revised but not adopted. As discussed earlier, the action level has been revisited more recently by the World Health Organization[8] and the European Union[344]. FDA could consider developing a

system to regularly re-evaluate their action levels and to make that process open to the public.

Using Chronic Toxicity Data. The action level for DA (and all other natural toxins in seafood) protects only for acute effects of a single meal. The available action levels have been acute because of a perceived lack of data for chronic effects and exposures. However, in the case of DA, chronic effects have resulted from low-level acute exposure when exposure was during a critical window in brain development for juvenile rats. Consideration should be given to using recent animal data that produced chronic effects from acute exposures. Another option is to use an additional uncertainty factor to convert an acute RfD (or action level) to a chronic RfD (or action level). This would result in an appropriately protective action level. Use of these data would protect individuals who regularly consume shellfish or planktivorous fish species that can contain concentrations of DA. Evidence suggests that protection of long-term consumption should be a basic tenet of a regulatory level in seafood.

Protection of Sensitive Subpopulations. In the EPA risk assessment process, sensitive subpopulations are considered. In the reference dose for DA, Canadian officials used a single uncertainty factor of 10 to derive the reference dose and this value was subsequently adopted by FDA. This single uncertainty factor was intended to account for both conversion of a lowest observed adverse effect level (LOAEL) to a no observed adverse effect level (NOAEL) and to account for sensitive subpopulations (i.e., intraspecies sensitivity). This is a narrow margin of safety for sensitive subpopulations considering the increased sensitivity of sensitive subpopulations identified in Chapter 2, including the young, the elderly, individuals with decreased renal capacity, individuals

with blood-brain barrier complications, or individuals with any pre-existing illnesses that affect the hippocampus (e.g., epilepsy, schizophrenia, and Alzheimer's disease). Given recent developments in the scientific literature on the toxicity of DA, as well as its widespread and persistent nature in seafood, evidence argues for revisiting of this regulatory attribute to better ensure the protection afforded sensitive subpopulations by the action level. At a minimum, consideration should be given to developing a consumption advisory that identifies sensitive subpopulations that should be advised to avoid consumption of seafood types that may contain DA. Evidence-based re-evaluations are appropriate for other natural toxins in seafood where new data exist.

Developing Tolerances for Natural Toxins in Seafood. Finally, consideration should be given to using the existing process to develop a tolerance for a natural toxin in seafood if substantial and compelling data exist for a toxin. An action level was intended as a placeholder under FDA regulations while a tolerance was developed. If a tolerance was developed for a natural toxin in seafood, the basis of the action level could be revisited and updated in the more formal rule making process of tolerances.

Effective Monitoring. While FDA does have an action level for DA, there is no specific federal mandate for monitoring of DA in seafood. Effective monitoring includes consideration of appropriate temporal and spatial scales and representative species. Effective monitoring should encompass both imported and domestic sources of seafood.

Temporal and Spatial Scales of Monitoring. Monitoring should consider the appropriate spatial and temporal scales. Frequency of monitoring should be often enough to discover significant temporal changes of DA in seafood. Sampling density and distribution should be sufficient to cover significant spatial changes in DA in seafood.

The details of appropriate monitoring will vary based on the type of seafood, receptors to be protected, and other locale-specific conditions.

Monitoring a Representative Subset of Species. It may not be possible to monitor all commercially harvested species so selection of indicator species of seafood would likely be warranted. An indicator species should highly exposed, commercially important, and widely consumed. Indicator species may serve as continuous monitors of toxins in an area. Indicator species can indicate when blooms (the cause of acute rather than chronic risk) have subsided. Shellfish are useful indicator species because they are sedentary and provide an indication of DA concentrations in a particular area. Fish species are mobile and may integrate potential DA exposures over a larger area. Monitoring species that can maintain their levels of DA for a long time past blooms (i.e., razor clams). Monitoring species should have a wide distribution, residency status (available year-round), and be easily collected.

Effective Domestic Monitoring. FDA provides guidance in HACCP and delegates authority to state and local authorities to make decisions about what monitoring is required. DA was first identified as a problem on the East Coast, but monitoring on the East Coast is very limited. Monitoring is more extensive on the West Coast. After DA was detected in high concentrations in Washington State razor clams in 1991, the entire commercial and recreational fishery was closed for more than a year[306]). Closures of the Washington State razor clam industry occurred for 13 months in 1991-1992, 13 months in 1998-1999, and 6 months in 2002-2003[22]. The value of the razor clam fishery in Washington State is valued at more than $20 million annually[23], so closure of the industry is a substantial economic loss. The Washington State Department of Fish and

Wildlife has developed its own testing program and formed a public-private partnership that includes the U.S. National Oceanic and Atmospheric Administration, the University of Washington, and local communities including the Quinalt Indian Nation. The federal-state-local partnership is called the Olympic Region Harmful Algal bloom (ORHAB) partnership and has developed an extensive monitoring plan that focuses on blooms[23]. The Washington State approach includes collection of *Pseudo-nitzschia* cell counts and particulate DA concentrations in seawater twice a week and DA concentrations in shellfish twice a month at seven locations on the Olympic Peninsula. *Pseudo-nitzschia* species are difficult to identify in samples and require extensive electron microscopy. Washington State classifies cells into three *Pseudo-nitzschia* functional groups (similar to the L4 data discussed in Chapter 3) using light microscopy. A one liter sample of seawater is collected at the same time and location and filtered to capture particulate material on a 0.45 µm filter. Threshold cell counts have been developed for each of the functional groups. If the cell count of *Pseudo-nitzschia* reaches the designated thresholds for any of the three functional groups of *Pseudo-nitzschia*, then a rapid DA toxin test (Jellet Rapid Test, Jellet Rapid Testing Ltd., Nova Scotia, Canada) is performed on the filtered particulate material to give an estimate of particulate DA concentrations, since DA concentrations cannot be reliably predicted by cells counts. Measurement of *Pseudo-nitzschia* functional groups and DA concentrations in seawater give an early warning of the potential for toxic levels in shellfish. Sampling of the Pacific razor clam, *Siliqua patula* (the species that generally has the highest and most persistent concentrations of DA) are monitored twice monthly and immediately after *Pseudo-nitzschia* threshold cell counts are reached[345]. Oregon and California have similar monitoring programs.

Washington State's combination of *Pseudo-nitzschia* cell counts, DA particulate testing, and seafood monitoring has been effective in protecting against acute outbreaks of amnesic shellfish poisoning. The program's effectiveness in preventing effects from chronic low level exposure is an unknown, but the current protocol could be modified for this purpose.

On the East Coast of the United States, monitoring plans have been largely non-existent. In Massachusetts, for instance, the Division of Marine Fisheries (DMF) is responsible for year-round testing of shellfish and shellfish growing areas. Monitoring efforts are more intensive in the spring, summer and, fall. The DMF notifies affected city and town officials of closures. DMF has not considered DA an issue in seafood and therefore does not conduct monitoring. However, if monitoring data are not collected, concentrations in seafood are unknown. As discussed in Chapter 3, monitoring data from the MWRA has indicated the presence of *Pseudo-nitzschia* in Massachusetts coastal waters. The Massachusetts Department of Public Health has not included any data on amnesic shellfish poisoning in their publically released information on reportable foodborne illness[346].

Effective International Monitoring. International monitoring for DA in seafood is an even greater unknown. Only three HACCP memorandums of understanding exist with other countries. Therefore FDA relies primarily on importers to ensure that the monitoring requirements of HACCP are met. Shellfish closures for DA have occurred in ten countries, including the United States, Canada, Scotland, Ireland, Spain, Portugal, France, Denmark, New Zealand, and Brazil[267]. These closures represent a subset of

countries that could truly be of concern, since the number of countries with *Pseudo-nitzschia* detected in their waters is several times this, and could be even greater if more countries performed monitoring.

FDA does not publish analytical data for domestic or imported seafood. The U.S. General Accounting Office was recently tasked with evaluating FDA testing of banned pesticide residues in imported shrimp. The General Accounting Office was critical of the frequency of testing for banned pesticides, stating that when FDA tested for banned pesticides in imported shrimp, they often found them[335]. For example, in 2008 the FDA tested only 34 shrimp samples for residues of nitrofurans (not approved for use in U.S. aquaculture. Six of the samples tested positive. It is unclear how often DA would be detected if it were regularly analyzed for in imported seafood.

As discussed earlier, only three countries (Canada, China, and Japan) have MOUs with FDA for seafood, so the Unites States relies on importers to ensure the safety of most seafood. A significant portion of the seafood imported into the U.S. is not covered by these country-wide HACCP MOUs. Table 4-5 lists countries that import the most seafood into the United States.

Table 4-5

Top Sources of Imported United State Seafood

Country	Percent of Total U.S. Seafood Imports
China	23
Thailand	16
Canada	13
Indonesia	6
Vietnam	5
Ecuador	5
All Other Countries	33

Data from GAO (2011)[315]. Total does not add up to 100% due to rounding.

Only two of the top six seafood importers into the United States have HACCP MOUs. FDA periodically inspects importers to review the adequacy of these "affirmative steps"[327]. Published data for DA in seafood are only available for three of the top six importing countries. China represents almost a quarter of all U.S. seafood imports, but no published data are available for DA in Chinese-harvested seafood. International monitoring for DA remains a significant unknown for imported seafood.

Effective Communication. Effective communication is an attribute that cuts across all aspects of regulatory programs. Action levels, monitoring data and closure decisions are only protective of public health if they are effectively communicated to the public. The target population protected by an action level could be better communicated to state and local authorities and the public. FDA could communicate clearly that the

action level is protective only of acute effects from a single meal exposure, rather than chronic effects resulting from consuming a number of meals over time. FDA could more effectively communicate the level of protection that the current action level provides for sensitive subpopulations.

The effectiveness of monitoring data can vary based on how it is communicated. Most states do not have publically-available information on monitoring data either for *Pseudo-nitzschia* or for DA in seafood. The California Department of Public Health posts monthly seafood biotoxin monitoring reports (including data for DA) on their website, and has these reports available back through 1999[347]. While the posting of these data is potentially very useful, these data could be a more effective communication tool if they were reported differently. California only posts the concentrations if they exceed the current action level. If concentrations are below the action level, they are only listed as "less than 20 mg/kg". This does not give the public any information on how close concentrations in seafood are to exceeding the action level. Individuals who may be a member of a sensitive subpopulation, or may consume multiple meals of shellfish and planktivorous fish, do not have information on what levels of DA they may consume. California and Washington (discussed in Chapter 3) should be commended for making their data publically available in some form. There are data held by many agencies that have not been made public. Finally, areas closed to fishing must be effectively communicated and enforced. Communication efforts must reach both commercial and recreational fishers in order to effectively protect public health.

Disease Surveillance. The effectiveness of a regulatory program cannot be assessed if there is no data collected on illnesses related to natural toxins in seafood. The

decision on collection of case data has been left up to individual states, which has resulted in inconsistencies among states. A federal mandate for collection of data on illnesses related to DA and other natural toxins in seafood would allow for evaluation of the effectiveness of the regulatory program. As discussed in Chapter 2, many of the symptoms of DA toxicity overlap with chronic illnesses of the central nervous system. It therefore would be extremely difficult or even impossible at this time to distinguish illnesses caused by chronic low level exposure to DA. However, a useful first step would be adding the acute illness, amnesic shellfish poisoning, to the reportable disease list. The sudden memory loss associated with high acute exposures appears to be well distinguished from other illnesses (hence its discovery in humans in Prince Edward Island during the only known human outbreak).

Other Potential Regulatory Tools.

There are additional tools that could potentially be used to enhance the current regulatory system. These include (1) improved data on toxicity, (2) modeling of *Pseudo-nitzschia* concentrations, DA production, and DA uptake into seafood, and (3) improved analytical techniques.

Funding of Improved Toxicological and Epidemiological Data. There is a clear need for data on the effects of chronic exposure. An ongoing five-year epidemiological study of a Native American tribe in the Northwest may provide useful data for chronic exposure[104]. Adult laboratory animals have demonstrated chronic effects from acute low level juvenile exposures during a critical window in brain development[11, 24, 119, 122], but a chronic oral exposure study in laboratory animals has not been performed.

A chronic exposure study would provide a firm basis for the development of a chronic action level or tolerance. This dissertation has developed a structured argument, an essential conclusion of which is that there is a clear and compelling need for data to support a chronic reference dose.

Developing an Uptake Model for DA in Seafood. Regulatory agencies in Great Britain and the United States have relied on threshold levels of total *Pseudo-nitzschia* in coastal waters as a trigger for when testing for concentrations of DA in seafood is needed[348]. Human exposure could be more easily reduced if an uptake model could be developed that predicts concentrations in seafood based on the concentration of *Pseudo-nitzschia* present in coastal waters. Models could be used to predict (1) environmental concentrations of *Pseudo-nitzschia* based on physical and chemical parameters, (2) production of DA by *Pseudo-nitzschia*, and (3) uptake of DA into various types of seafood. Current scientific understanding is currently insufficient to accurately predict uptake but efforts are ongoing.

An initial model was created relating a 1.5-year time series of *Pseudo-nitzschia* abundance and domoic acid concentration to physical, chemical, and biological data to predict bloom dynamics of the Santa Barbara Channel in California[349]. The model incorporated satellite ocean color and sea surface temperature data to predict the probability that a remotely sensed phytoplankton bloom contains a significant population of *Pseudo-nitzschia*. A logistic regression model was developed for Monterey Bay by matching *Pseudo-nitzschia* sampling data with parameters such as silicic acid, temperature, nitrate and coastal upwelling[350] and a similar regression model was developed for Lisbon Bay, Portugal[351]. Gaps in current knowledge about the biology of

Pseudo-nitzschia and DA production makes prediction of diatom growth and DA production difficult, especially at a local scale[219]. As *Pseudo-nitzschia* growth models are further refined and uptake models into seafood are developed, modeling has the potential to play an important role in regulation of DA and other natural toxins in seafood.

Improved Analytical Techniques for Exposure. DA has been found in seafood over much of the globe in the last twenty years. Because seafood concentrations change significantly over time in a given location, it is important to develop tools to rapidly detect and quantify concentrations of DA to limit human exposure.

Interdigital Sensor. One promising technology for preventing exposure is the development of an interdigital sensor[352]. The sensor was able to rapidly detect approximately 12.6 mg/kg of domoic acid in seafood, which is below the current action level of 20 mg/kg. This product is not currently commercially available.

ELISA Tests. Two ELISA tests are currently commercially-available for DA[353, 354]. The BS ELISA is a polyclonal anti-body-based test that has been validated for analysis of DA in shellfish tissues[355]. The MeS ELISA is a monoclonal antibody assay developed by the National Oceanic and Atmospheric Administration in conjunction with Mercury Science Inc. and has been validated for analysis for shellfish and dissolved and particulate phytoplankton samples[354]. These tests cannot currently produce identical results to liquid chromatography/mass spectrometry (LC/MS), the gold standard for analytical chemistry. When regression lines were compared, the BS test was higher than LC/MS by a statistically significant amount, while the MeS test was lower than LC/MS by a statistically significant amount. Nonetheless, rapid and inexpensive results are

important in a regulatory framework where decisions to close fisheries must often be made quickly. An initial decision using an ELISA test and later confirmed with LC/MS could represent a useful regulatory protocol.

Development of a Biomarker for Human Exposure. Currently, exposure to DA in humans is diagnosed by examining symptoms that are consistent with acute exposure, and there is no test for exposure. A tool that is able to diagnose repeat low-level exposure has been a critical data gap. A DA-specific antibody response has been identified that is induced by low-level repeat exposure to DA in zebrafish in a laboratory setting[356]. The antibody response and its potential utility as a biomarker for low-level repeat exposure to DA was field-verified by testing with wild naturally-exposed California sea lions[356]. This work could lead to the development of a diagnostic test that could be used to identify low-level repeat exposure in humans or wildlife. A biomarker could be particularly useful since monitoring data are so sparse and chronic DA symptoms are hard to distinguish from other chronic brain illnesses[21].

Summary Conclusion.

This chapter evaluated the regulatory approach to natural toxins in seafood, with DA as an example. The FDA approach for natural toxins has relied on action levels rather than tolerances as guidance values. This reliance on action levels has had the effect of making seafood regulatory values less transparent and less accountable. Action levels lack the public input process of tolerances. On the other hand, action levels do allow FDA a greater deal of flexibility. FDA could change an action level quickly due to new scientific information merely by publishing a revised action level in the Federal Register.

If FDA reviewed the recent scientific literature on the chronic effects of low level exposure and determined a revision to the action level was needed, they would have the ability to make the change quickly. When reliable chronic toxicity data are available to form the basis of a chronic regulatory level, then development of a tolerance for DA (or another natural toxin) in seafood may be warranted.

Identification of attributes of an effective regulatory system represents an important first step in evaluating the regulatory process for natural toxins in seafood. Effective regulatory values for natural toxins in seafood should:

- Incorporate public input;
- Represent transparent values from a consistently-applied process;
- Provide an appropriate level of protection for the public;
- Include periodic updating;
- Incorporate chronic toxicity data and exposure assumptions;
- Provide protection for sensitive subpopulations; and
- Consider development of tolerances for natural toxins when appropriate data are available.

Public input and transparency are critical to ensuring that an agency is accountable for its decisions, and for ensuring that those decisions are made in a rational and consistent manner while considering all relevant information. Protection of chronic exposure is a critical consideration for seafood, since there is a high potential for repeat exposure. The current process for developing acute action levels does not appear to perform strongly in terms of these attributes. Consideration should be given for more protective regulatory values (i.e., based on chronic toxicity data and chronic exposure assumptions).

In order to protect public health, effective regulatory levels in seafood should be accompanied by effective monitoring. Attributes of effective monitoring include:

- Appropriate temporal and spatial scales;

- Representative species;
- Domestic monitoring: and
- Monitoring of imports.

HACCP does not require monitoring for DA and other natural toxins in seafood but instead provides general guidelines for states, local authorities, and importers. HACCP MOUs have only been reached with three countries. Compliance in other countries requires effective implementation of HACCP by importers.

Action levels, monitoring, and closure decisions are only protective of public health if they are effectively communicated to the public. The effectiveness of the regulatory program for natural toxins in seafood can only be assessed if data on cases of illness are both consistently identified and reported.

In addition to assessment of the current regulatory program, it is useful to consider other potential regulatory options including:

- Improved toxicological and epidemiological data;
- Development of an uptake model for DA in seafood; and
- Improved analytical techniques;

Improved data will provide a firm basis for a chronic regulatory level in seafood. Modeling of *Pseudo-nitzschia* populations, DA production, and DA uptake into seafood can aid decisions on the need for monitoring. Improved analytical techniques such as real-time sensors for DA in seafood and biomarkers to determine human exposure to natural toxins such as DA, can further enhance the regulatory process. Periodic reassessment of any regulatory program is a key to its continued improvement.

CONCLUSION

This section synthesizes the information from the previous chapters into an assessment of the issue of DA in seafood and whether the current regulatory approach should be revisited. The first three chapters presented information on DA related to (1) the human social dynamics of DA in seafood, (2) recent toxicity data for DA, and (3) the environmental prevalence of *Pseudo-nitzschia* and human exposure to DA in seafood. Chapter 4 evaluated the FDA regulatory framework for natural toxins. The assessment of DA was used to guide a discussion of the overall regulatory process for natural toxins in seafood.

The Human Dynamics of Domoic Acid in Seafood.

Chapter 1 linked together (1) anthropogenic sources of nutrients in coastal areas, (2) increasing demand for seafood, resulting in increased consumption of planktivorous species, (3) the globalization of the seafood market, and (4) the growth of marine aquaculture.

Pseudo-nitzschia concentrations and bloom dynamics have been linked to nutrient concentrations in the environment. Temperature-driven mixing of the water column increases available nutrients and leads to seasonal blooms of diatoms. Human inputs of nutrients such as nitrogen and phosphorus have the potential to support higher persistent concentrations of *Pseudo-nitzschia* between bloom events. However, a complete

understanding of the conditions that lead to DA production in *Pseudo-nitzschia* is still being developed.

Chapter 1 demonstrated that wild capture of seafood has been relatively stagnant for the past two decades, while demand for seafood and per capita supply world-wide has increased. A focus on species lower on the food chain (i.e., planktivorous species with a greater potential for DA contamination) is likely the only way to significantly increase marine wild capture. This focus could constitute increased capture or diversion of non-food uses to food uses for low trophic level species. The increased globalization of the seafood market exposes individuals to a greater variety of seafood products from a greater number countries than in the past, causing exposure to DA-contaminated seafood to occur at great distances from its original area of harvest. This makes it difficult to tie together sources of exposure and contamination.

Aquaculture has provided most of the growth in the seafood market in the last two decades. Since aquaculture occurs in coastal areas where anthropogenic nutrients can lead to persistent *Pseudo-nitzschia* concentrations, uptake of DA into aquacultured planktivorous seafood species is a notable, if significant, concern.

While direct evidence for increased risk is limited at present, the analyses of the various attributes constituting the core of this chapter are strongly suggestive of an increase in risk potential. Trends in coastal social dynamics as well as production and consumption in global seafood are supportive of a concern that exposure to domoic acid is increasing in the global human population.

Toxicity Data for Domoic Acid.

The purpose of Chapter 2 was to evaluate the weight of evidence for revisiting the DA action level based on concerns about the potential for human health risk from chronic low level exposures to DA in seafood. The effects of DA share striking similarities to other brain illnesses, most notably epilepsy and schizophrenia. DA has the potential to contribute to the severity of illnesses that impact the hippocampus. There is also the possibility (although no evidence in the current scientific literature), for the symptoms of DA to be mistaken for other illnesses.

The current acute reference dose was developed in the aftermath of the 1987 outbreak in Canada, and has not been updated by FAO/WHO/IOC, although it was reviewed ten years ago[18]. A lower (by more than a factor of three) acute reference dose was recommended recently by a committee of scientists established by EFSA, although this RfD has not been used to create a lower action level. RfDs developed by FDA are three to six times lower than the current FOA/WHO/IOC RfD, and yet FDA has not developed an action level based on its own scientists' RfD, despite the fact the FDA RfDs have been available for fifteen years.

The available RfDs were developed for acute exposures and a chronic RfD has not yet been established. Given that chronic effects have been demonstrated from acute exposures (in humans, laboratory animals, and marine mammals), a chronic exposure study is a critical need for developing a chronic RfD. In the interim, the RfD should be revisited and consideration should be given to applying an uncertainty factor to an acute study to estimate a chronic reference dose. Consideration should be given to the numerous acute injection studies in neonatal rats that show serious chronic physiological

and behavioral effects in later life. Recently DA in seafood has caused striking neurophysiological and behavioral effects when consumed by marine mammals. These effects in marine mammals raise concerns about the level of protection afforded to individuals who consume shellfish and planktivorous fish frequently.

The FAO/WHO/IOC acute reference dose includes an uncertainty factor of 10 to account for sensitive subpopulations. However, it is unclear if this uncertainty factor is truly protective for all identified sensitive subpopulations. Numerous studies in the toxicological literature have identified the developing brain in juveniles as particularly sensitive. Young children lack a fully developed renal system or blood-brain barrier, slowing clearance from the body and allowing it to enter the brain more easily. Fetuses are at risk of greater exposure than the general population. DA passes through the placenta and lingers in fetal brains and amniotic fluid long after maternal concentrations are non-detect. The elderly are also sensitive, as witnessed by the more severe impacts to older victims in the 1987 outbreak in Canada. The elderly are particularly at risk if they have pre-existing conditions that affect the blood-brain barrier, the hippocampus, or the kidneys. Subsistence fishers, such as certain Native American tribes, recreational or commercial fishers and their families, and some ethnicities, may also be particularly at risk when consuming planktivorous seafood at rates greater than assumed by the current action level. There is not currently any advisory message for DA exposure that has been issued for sensitive subpopulations.

The consumption rate used in the current FAO/WHO/IOC action level is protective of a single meal exposure for most individuals. However, DA exposure through a number of meals over a period of years has the potential for additive effects that cannot be ruled

out without a chronic study. When a chronic RfD is developed, the consumption rate should be revised to be commensurate with chronic exposure. An action level based on a chronic RfD and a chronic consumption rate will assure protection of seafood consumers.

Human Exposure to Domoic Acid in Seafood.

Chapter 3 examined the spatial and temporal trends in environmental densities of the diatom *Pseudo-nitzschia* and the trends of DA in seafood. These data provide a weight of evidence of the potential for exposure of humans to low dose levels of DA in seafood. Persistent low level cell counts of *Pseudo-nitzschia* (which can result in persistent low level concentrations of DA in seafood) have been ignored as inconsequential. Chapter 1 discussed the potential for human actions to increase exposure to DA. First, anthropogenic sources of nutrients have the potential to support persistent low level densities of *Pseudo-nitzschia* and subsequent concentrations of DA in seafood. Second, as human population and per capita demand for seafood both increase, human social dynamics have the potential to result in higher consumption of lower trophic level seafood. These species are relatively high in DA compared with upper trophic level species. Chapter 2 presented a weight of evidence for potential toxicity of low levels of DA. Chapter 3 supports the conclusion there are persistent low level densities of *Pseudo-nitzschia* in coastal waters and persistent low level concentrations of DA in lower trophic level seafood from those waters.

Statistical analysis on the L4 data has shown that *Pseudo-nitzschia* is persistent across an 18-year sampling period, where *Pseudo-nitzschia* was present in 75% of samples. Total diatom concentrations have shown a decreasing trend during sampling

from 1996-2007[217], but based on the analysis in this chapter, there was no long-term trend in *Pseudo-nitzschia* cell counts at the L4 location.

Nutrient dynamics at the L4 sampling site appear typical of temperate coastal waters[217]. The water column is well-mixed during the winter and fall and this is reflected by higher nutrient concentrations. Weak stratification occurs in spring and summer, which limits the replenishment of nutrients from bottom waters. Nitrite (Figure 3-23), nitrate (Figure 3-24), silicate (Figure 3-26) and phosphate (Figure 3-27) all followed this seasonal pattern (accounting for some year to year variation), with peaks in the winter and fall and lowest concentrations in the summer. Since nutrient concentrations at this location appear seasonal, this indicates that anthropogenic inputs are not a strong influence at the L4 location. While anthropogenic inputs do not dominate nutrient concentrations at the L4 location, they do promote the development and persistence of harmful algal blooms at many locations in the world[43]. The driving force behind nutrient dynamics (i.e., dominance of natural or anthropogenic sources) is site-specific.

Other long term data sets from the scientific literature support the conclusion that low levels of *Pseudo-nitzschia* are persistent over time. Nonparametric statistics generally did not provide further insight into the L4 *Pseudo-nitzschia* data. Descriptive statistics indicated that *Pseudo-nitzschia* are persistently present at low cell counts throughout most of the year, with the potential for DA production and uptake into seafood through most of the year. Exposures to DA are therefore likely to be chronic rather than acute. Environmental monitoring data from L4 and other locations in the literature indicate that *Pseudo-nitzschia* is globally a cosmopolitan species and is present most of the year at low densities between blooms.

Because of its widespread presence and persistence, *Pseudo-nitzschia* can act as a source of consistent and widespread uptake of DA into seafood. This is supported by the published literature on DA in seafood. Washington State razor clam data indicated persistent low level concentrations of DA in razor clams from 1996 through 2013. The literature on seafood concentrations indicates that levels of DA in seafood are also widespread across much of the planet and temporally persistent in the few areas with published long-term monitoring data.

Weight of Evidence for Revisiting the Action Level.

When the weight of evidence for the first three chapters is considered in total, there is significant evidence for a need to revisit the action level for DA in seafood. Human activities can contribute to nutrient concentrations in coastal waters. Human influence on nutrient concentrations can lead to persistent concentrations of *Pseudo-nitzschia* in coastal waters. As population increases in the coastal zone, there is the potential for human impacts to lead to greater increases of DA in seafood. This human influence on the dynamics of DA in seafood can occur across the globe. Drivers of nutrient dynamics, whether primarily natural or anthropogenic, are location-specific.

Seafood is one of the most globally traded commodities. Human activities may lead to increased DA in seafood in one part of the globe and the seafood may be harvested, shipped, and then consumed in another part of the globe. Global trade has many benefits, but global trade in seafood could lead environmental problems in one coastal country causing health effects in another part of the world if effective regulatory monitoring and enforcement are not in place.

Recent toxicological data have demonstrated the potential for subtle but critical chronic effects from consumption of seafood contaminated with low levels of DA. These effects have been observed in thousands of marine mammals from actual environmental exposures to DA in seafood along the California Coast. *Pseudo-nitzschia* is a cosmopolitan diatom genus that is both widespread across the globe and persistent over time. DA has been reported in numerous species of planktivorous fish and shellfish worldwide. When these factors are taken together, there is the potential for risks to humans exposed through seafood consumption that mandates further consideration and an examination of the regulatory process. DA is just one of a number of natural toxins in seafood that are regulated by FDA. DA was used in Chapter 4 to illustrate the current regulatory framework for natural toxins in seafood.

Regulatory Approach for Natural Toxins in Seafood.

The historical focus on contaminants in seafood has been bioaccumulating contaminants in higher trophic level species and this is reflected in FDA's regulatory process. There has been some concern about natural toxins at the lower end of the food chain in shellfish and planktivorous fish also, but regulation of natural toxins has focused on protection of acute effects from single meal exposures rather than chronic exposures.

Chapter 4 evaluated the regulatory approach to natural toxins in seafood, with DA as an example. The FDA approach for natural toxins has relied on action levels rather than tolerances as guidance values. This reliance on action levels has had the effect of making seafood regulatory values less transparent and less accountable. Action levels lack the public input process of tolerances. On the other hand, action levels do allow FDA a greater deal of flexibility. FDA could change an action level quickly due to new

scientific information merely by publishing a revised action level in the Federal Register. If FDA reviewed the recent scientific literature on the chronic effects of low level exposure and determined a revision to the action level was needed, they would have the ability to make the change quickly. When reliable chronic toxicity data are available to form the basis of a chronic regulatory level, then development of a tolerance for DA (or another natural toxin) in seafood may be warranted.

Identification of attributes of an effective regulatory system represents an important first step in evaluating the regulatory process for natural toxins in seafood. Effective regulatory values for natural toxins in seafood should:

- Incorporate public input;
- Represent transparent values from a consistently-applied process;
- Provide an appropriate level of protection for the public;
- Include periodic updating;
- Incorporate chronic toxicity data and exposure assumptions;
- Provide protection for sensitive subpopulations; and
- Consider development of tolerances for natural toxins when appropriate data are available.

Public input and transparency are critical to ensuring that an agency is accountable for its decisions, and for ensuring that those decisions are made in a rational and consistent manner while considering all relevant information. Protection of chronic exposure is a critical consideration for seafood, since there is a high potential for repeat exposure. The current process for developing acute action levels does not appear to perform strongly in terms of these attributes. Consideration should be given for more protective regulatory values (i.e., based on chronic toxicity data and chronic exposure assumptions).

In order to protect public health, effective regulatory levels in seafood should be accompanied by effective monitoring. Attributes of effective monitoring include:

- Appropriate temporal and spatial scales;
- Representative species;
- Domestic monitoring: and
- Monitoring of imports.

HACCP does not require monitoring for DA and other natural toxins in seafood but instead provides general guidelines for states, local authorities, and importers. HACCP Memorandums of Understanding have only been reached with three countries. Compliance in other countries requires effective implementation of HACCP by importers.

Action levels, monitoring, and closure decisions are only protective of public health if they are effectively communicated to the public. The effectiveness of the regulatory program for natural toxins in seafood can only be assessed if data on cases of illness are both consistently identified and reported.

In addition to assessment of the current regulatory program, it is useful to consider other potential regulatory options including:

- Improved toxicological and epidemiological data;
- Development of an uptake model for DA in seafood; and
- Improved analytical techniques;

Improved data will provide a firm basis for a chronic regulatory level in seafood. Modeling of *Pseudo-nitzschia* populations, DA production, and DA uptake into seafood can aid decisions on the need for monitoring. Improved analytical techniques such as real-time sensors for DA in seafood and biomarkers to determine human exposure to natural toxins such as DA, can further enhance the regulatory process.

The purpose of this dissertation was to give careful consideration to technical issues regarding social dynamics, toxicity, exposure and current regulation to evaluate the

potential for human health risk from domoic acid in seafood. The weight of evidence indicates a need for revisiting the current regulatory approach for DA. Further, future developments in the scientific understanding of DA in seafood, including nutrient dynamics, social dynamics, epidemiological data, chronic toxicological studies and exposure data, should be considered in order to make regulatory improvements. Periodic reassessment of any regulatory program is a key to its continued improvement. The regulatory process should be dynamic rather than static and should respond to new knowledge or conditions that cast light on the safety of a contaminant in seafood.

APPENDIX A

PSEUDO-NITZSCHIA DATA FROM THE L4 LOCATION

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
5-Oct-1992	10.43	1.11	0.00	0.00
19-Oct-1992	12.57	2.11	0.00	0.08
26-Oct-1992	7.6	0.33	0.00	0.06
2-Nov-1992	1408.72	0.00	0.00	0.20
9-Nov-1992	5.44	0.11	0.00	0.22
30-Nov-1992	6.96	0.00	0.00	0.00
7-Dec-1992	6.84	0.22	0.00	0.00
4-Jan-1993	8.81	0.33	0.00	0.00
18-Jan-1993	3.37	0.44	0.00	0.00
25-Jan-1993	6.78	0.44	0.00	0.00
1-Feb-1993	5.1	0.67	0.00	0.00
8-Feb-1993	3.37	0.33	0.00	0.00
15-Feb-1993	5.57	0.11	0.00	0.00
22-Feb-1993	6.32	0.04	0.00	0.00
1-Mar-1993	153.95	0.33	0.00	0.00
8-Mar-1993	7.98	0.67	0.00	0.00
15-Mar-1993	4.98	0.11	0.00	0.00
22-Mar-1993	558.49	3.44	0.00	0.00
12-Apr-1993	2167.2	2.67	0.00	0.00
19-Apr-1993	4858.08	6.22	0.00	0.00
26-Apr-1993	1350.76	1.33	0.00	0.00
3-May-1993	11.65	0.22	0.00	0.00
10-May-1993	58.59	0.78	0.00	0.00
17-May-1993	86.08	1.67	0.00	0.72
31-May-1993	35.86	0.00	0.00	0.56
7-Jun-1993	86.71	4.67	0.00	0.00
14-Jun-1993	4.48	0.00	0.00	0.00
21-Jun-1993	272.79	34.44	0.00	0.00
28-Jun-1993	15.45	0.00	0.00	0.00
5-Jul-1993	206.09	48.89	0.00	0.00

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
12-Jul-1993	92.25	0.00	0.00	10.60
19-Jul-1993	418.92	0.00	0.00	9.20
26-Jul-1993	155.86	5.00	0.00	5.20
2-Aug-1993	263.68	23.33	0.00	2.00
9-Aug-1993	1517.5	333.33	0.00	1.12
16-Aug-1993	1194.87	555.56	0.00	2.60
23-Aug-1993	1060.67	122.22	0.00	0.00
30-Aug-1993	71.45	18.89	0.00	0.48
6-Sep-1993	46.11	1.44	0.00	0.00
27-Sep-1993	62.26	1.89	0.00	0.00
4-Oct-1993	40.17	0.78	0.00	0.00
11-Oct-1993	34.72	2.00	0.00	0.00
18-Oct-1993	10.52	0.24	0.00	0.00
1-Nov-1993	13.06	0.08	0.00	0.00
8-Nov-1993	5.34	0.00	0.00	0.10
15-Nov-1993	11.87	0.16	0.00	0.00
29-Nov-1993	7.33	0.10	0.00	0.08
6-Dec-1993	6.2	0.00	0.00	0.00
13-Dec-1993	6.02	0.00	0.00	0.06
3-Jan-1994	8.98	0.12	0.00	0.00
17-Jan-1994	41.35	0.16	0.00	0.00
31-Jan-1994	6.7	1.50	0.00	0.00
7-Feb-1994	31.2	1.52	0.00	0.00
14-Feb-1994	10.17	0.46	0.00	0.00
21-Feb-1994	34.59	0.52	0.00	0.00
28-Feb-1994	16.86	0.24	0.00	0.00
7-Mar-1994	12.56	0.66	0.00	0.00
14-Mar-1994	113.45	0.18	0.00	0.00
21-Mar-1994	116.83	1.00	0.00	0.04
28-Mar-1994	25.6	0.00	0.00	0.00
11-Apr-1994	5.33	1.16	0.00	0.00
18-Apr-1994	19.33	0.88	0.00	0.00
25-Apr-1994	77.81	1.08	0.00	0.20
2-May-1994	334.72	0.00	0.00	0.00
9-May-1994	51.06	0.44	0.00	0.00

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
16-May-1994	55.94	0.32	0.00	0.00
23-May-1994	18.97	0.40	0.00	0.00
30-May-1994	112.08	2.80	0.00	0.00
6-Jun-1994	1664	1136.00	0.00	0.80
13-Jun-1994	1094.12	803.00	0.00	0.00
20-Jun-1994	126.36	41.80	0.00	0.96
27-Jun-1994	436.54	1.28	0.00	2.32
4-Jul-1994	5055.86	0.00	0.00	0.00
11-Jul-1994	272.4	0.00	0.00	0.00
18-Jul-1994	71.49	0.00	0.00	0.00
1-Aug-1994	183.84	2.80	0.00	1.44
8-Aug-1994	217.34	0.00	0.00	0.00
15-Aug-1994	67.72	0.48	0.00	3.04
22-Aug-1994	69.75	0.96	0.00	1.44
29-Aug-1994	1002.41	61.18	0.00	1.28
5-Sep-1994	10.82	0.00	0.00	2.56
12-Sep-1994	12.07	0.40	0.00	1.76
19-Sep-1994	13.93	0.64	0.00	0.96
26-Sep-1994	20.74	1.44	0.00	1.04
12-Jun-1995	25.27	0.00	0.00	0.00
19-Jun-1995	50.92	0.24	0.00	0.72
26-Jun-1995	97.25	0.48	0.00	0.56
3-Jul-1995	65.66	0.40	0.00	0.00
10-Jul-1995	17.56	0.00	0.00	1.12
17-Jul-1995	416	6.46	0.00	1.20
24-Jul-1995	10.73	0.00	0.00	1.28
7-Aug-1995	120.16	16.72	0.00	1.20
14-Aug-1995	12.82	0.40	0.00	0.32
21-Aug-1995	11.34	1.20	0.00	0.64
28-Aug-1995	118.48	28.88	0.88	20.52
4-Sep-1995	146.34	38.00	0.00	30.02
11-Sep-1995	129.91	41.42	31.16	0.00
18-Sep-1995	23.42	6.46	4.56	0.00
25-Sep-1995	9.64	0.08	0.00	0.00
2-Oct-1995	25.74	0.00	0.00	0.00

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
9-Oct-1995	73.56	0.00	0.00	0.00
16-Oct-1995	15.36	0.00	0.00	0.08
23-Oct-1995	19.28	0.00	0.00	0.40
30-Oct-1995	5.94	0.00	0.00	0.00
6-Nov-1995	4.24	0.00	0.00	0.08
13-Nov-1995	6.52	0.00	0.00	0.40
20-Nov-1995	7.94	0.00	0.00	0.24
4-Dec-1995	4.22	0.00	0.00	0.00
11-Dec-1995	13.08	0.00	0.48	0.00
8-Jan-1996	8.66	0.18	0.00	0.00
15-Jan-1996	8.54	0.56	0.00	0.00
23-Jan-1996	29.41	0.16	0.00	0.00
29-Jan-1996	13.58	0.00	0.00	0.00
5-Feb-1996	9.81	0.24	0.00	0.00
19-Feb-1996	42.38	0.16	0.00	0.00
26-Feb-1996	64.62	0.16	0.00	0.00
4-Mar-1996	1.18	0.00	0.00	0.00
11-Mar-1996	9.58	0.00	0.00	0.00
18-Mar-1996	11.55	0.00	0.00	0.00
25-Mar-1996	7.82	0.00	0.00	0.00
1-Apr-1996	6.85	0.00	0.00	0.00
8-Apr-1996	1.98	0.00	0.00	0.00
15-Apr-1996	1.22	0.00	0.00	0.00
22-Apr-1996	2.04	0.00	0.00	0.00
29-Apr-1996	271.42	1.28	0.00	0.00
6-May-1996	118.97	0.80	0.00	0.00
13-May-1996	8.66	1.76	0.00	0.00
20-May-1996	38.53	14.48	0.00	0.00
27-May-1996	445.8	400.90	0.00	0.00
3-Jun-1996	1545.24	1485.80	0.00	0.00
10-Jun-1996	86.8	0.00	0.00	3.12
17-Jun-1996	9.7	1.60	0.00	0.00
24-Jun-1996	46.65	0.00	0.00	3.36
1-Jul-1996	459.89	0.00	0.00	0.80
8-Jul-1996	48.25	0.00	0.00	0.88

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
15-Jul-1996	95.77	0.00	0.00	3.12
22-Jul-1996	123.32	0.00	0.00	0.40
29-Jul-1996	733.22	0.00	0.00	2.80
5-Aug-1996	233.54	0.00	0.00	1.84
12-Aug-1996	74.11	0.00	0.00	1.60
19-Aug-1996	31.74	0.56	0.00	0.08
26-Aug-1996	499.31	0.00	0.00	0.16
2-Sep-1996	280.52	0.00	0.00	0.00
9-Sep-1996	42.72	0.00	0.00	0.00
23-Sep-1996	13.62	1.20	0.00	0.00
30-Sep-1996	15.34	0.48	0.00	0.00
7-Oct-1996	65.26	0.32	0.00	0.00
14-Oct-1996	24.84	2.28	0.00	0.24
21-Oct-1996	9.9	0.88	0.64	0.00
28-Oct-1996	10.84	0.08	0.00	0.00
4-Nov-1996	6.72	0.08	0.00	0.00
11-Nov-1996	9	0.16	0.00	0.04
18-Nov-1996	8.5	0.96	0.00	0.00
25-Nov-1996	7.66	0.00	0.00	0.00
2-Dec-1996	19.31	0.08	0.00	0.00
9-Dec-1996	5.76	0.00	0.00	0.00
16-Dec-1996	19.28	3.76	0.00	0.00
30-Dec-1996	17.37	0.64	0.00	0.00
6-Jan-1997	7.8	0.24	0.00	0.00
13-Jan-1997	6.16	0.64	0.00	0.00
27-Jan-1997	7.56	0.32	0.00	0.00
17-Feb-1997	19.64	1.28	0.00	0.00
24-Feb-1997	5.98	1.36	0.00	0.00
3-Mar-1997	4.76	1.04	0.00	0.00
10-Mar-1997	107.21	0.40	0.00	0.00
17-Mar-1997	50.83	0.48	0.00	0.00
24-Mar-1997	19.04	0.00	0.00	0.00
31-Mar-1997	315.45	2.40	0.00	0.00
7-Apr-1997	40.7	4.00	0.00	0.00
14-Apr-1997	81.5	20.14	0.00	0.24

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
21-Apr-1997	61.74	0.00	0.00	0.56
28-Apr-1997	54.55	1.04	0.00	0.00
12-May-1997	6.66	0.00	0.00	0.00
19-May-1997	32.24	0.72	0.00	0.32
26-May-1997	96.9	0.16	0.00	0.00
2-Jun-1997	142.59	0.00	0.00	0.00
9-Jun-1997	127	0.32	0.00	0.24
16-Jun-1997	524.59	171.00	0.00	0.00
23-Jun-1997	592.19	97.66	0.00	2.24
30-Jun-1997	289.18	209.38	0.00	3.92
7-Jul-1997	703.16	452.20	0.00	0.32
14-Jul-1997	140.56	49.40	0.00	0.00
21-Jul-1997	69.6	0.00	0.00	1.28
28-Jul-1997	150.86	0.00	1.92	0.00
4-Aug-1997	2.96	0.00	0.00	0.24
11-Aug-1997	214.72	0.32	0.00	0.00
18-Aug-1997	660.76	1.36	0.00	1.20
25-Aug-1997	186.39	0.56	0.16	0.00
1-Sep-1997	63.82	0.00	11.68	7.20
8-Sep-1997	201.41	0.00	9.12	49.40
15-Sep-1997	23.12	0.80	0.00	0.96
22-Sep-1997	40.68	0.00	0.32	4.56
29-Sep-1997	25.4	0.72	0.64	3.84
6-Oct-1997	16.98	0.40	0.00	0.48
3-Nov-1997	9.44	0.08	0.00	0.00
10-Nov-1997	6.38	0.00	0.00	0.00
17-Nov-1997	12.82	0.00	0.00	0.00
24-Nov-1997	5.72	0.00	0.00	0.00
8-Dec-1997	6.46	0.00	0.00	0.00
15-Dec-1997	8.01	0.00	0.00	0.00
5-Jan-1998	13.86	0.00	0.00	0.00
12-Jan-1998	5.06	0.00	0.00	0.00
19-Jan-1998	4.98	0.00	0.00	0.00
26-Jan-1998	3.28	0.24	0.00	0.00
2-Feb-1998	3.22	0.00	0.00	0.00

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
16-Feb-1998	24.78	0.00	0.00	0.00
23-Feb-1998	28.36	5.92	0.00	0.00
9-Mar-1998	3.93	0.00	0.00	0.00
16-Mar-1998	6.32	0.00	0.00	0.00
23-Mar-1998	19.78	0.00	0.00	0.00
30-Mar-1998	25.89	0.00	0.00	0.00
6-Apr-1998	8.43	0.00	0.00	0.00
13-Apr-1998	2.14	0.16	0.00	0.00
20-Apr-1998	45.9	2.48	0.00	0.00
27-Apr-1998	321.12	0.64	0.00	0.00
4-May-1998	478.26	0.00	0.00	0.00
11-May-1998	195.82	0.00	0.00	0.00
18-May-1998	305.95	0.00	0.00	0.00
25-May-1998	62.9	8.36	0.00	0.00
8-Jun-1998	2577.99	0.00	1.12	9.50
15-Jun-1998	290.51	0.00	0.32	3.68
29-Jun-1998	1850.13	0.00	0.00	560.50
6-Jul-1998	1024.45	5.70	0.00	50.16
13-Jul-1998	490.22	0.48	30.40	0.00
20-Jul-1998	827.74	57.00	0.00	0.32
27-Jul-1998	699.19	207.10	0.00	0.00
3-Aug-1998	86.22	47.50	0.00	0.00
17-Aug-1998	69.86	14.44	0.00	0.08
3-Sep-1998	193.36	50.16	0.00	0.00
21-Sep-1998	56.04	10.64	0.08	0.40
28-Sep-1998	16.1	0.40	0.00	0.00
5-Oct-1998	19	0.16	0.00	0.16
12-Oct-1998	85.92	0.40	0.00	0.00
2-Nov-1998	8.76	0.00	0.00	0.00
9-Nov-1998	11.24	0.00	0.00	0.00
16-Nov-1998	105.26	0.00	0.00	0.00
23-Nov-1998	203.05	0.08	0.00	0.00
30-Nov-1998	110.25	0.00	0.00	0.00
7-Dec-1998	4.28	0.00	0.00	0.00
14-Dec-1998	3.48	0.00	0.00	0.00

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
4-Jan-1999	4.46	0.00	0.00	0.00
11-Jan-1999	6.04	0.00	0.00	0.00
18-Jan-1999	6.2	0.00	0.00	0.00
25-Jan-1999	6.88	0.00	0.00	0.00
1-Feb-1999	0.86	0.00	0.00	0.00
8-Feb-1999	1.5	0.08	0.00	0.00
15-Feb-1999	1.02	0.02	0.00	0.00
22-Feb-1999	17.52	0.00	0.00	0.00
8-Mar-1999	3.62	0.02	0.00	0.00
15-Mar-1999	107.09	0.00	0.00	0.00
22-Mar-1999	2.72	0.04	0.00	0.00
29-Mar-1999	19.04	0.00	0.00	0.00
5-Apr-1999	30.12	0.00	0.00	0.00
12-Apr-1999	332.36	0.00	0.00	0.00
19-Apr-1999	111.8	0.88	0.00	0.00
26-Apr-1999	141.32	0.24	0.00	0.00
3-May-1999	23.97	0.00	0.00	0.00
10-May-1999	207.42	0.48	0.00	0.00
17-May-1999	271.67	0.88	0.00	0.00
24-May-1999	625.02	13.68	0.00	0.00
31-May-1999	1681.73	642.20	0.00	0.00
7-Jun-1999	3052.44	2850.00	0.00	0.24
14-Jun-1999	2947.5	2842.40	0.00	0.00
21-Jun-1999	258.54	190.00	0.00	0.00
5-Jul-1999	69.38	0.00	0.00	0.00
12-Jul-1999	27.7	0.24	0.00	0.00
19-Jul-1999	265.06	27.74	0.00	0.16
26-Jul-1999	373	174.80	0.00	0.00
2-Aug-1999	41.52	0.00	0.00	0.00
9-Aug-1999	157.2	6.46	0.00	0.00
16-Aug-1999	55.58	16.72	0.00	0.00
23-Aug-1999	58.18	26.60	0.00	0.00
30-Aug-1999	45.58	3.42	0.00	0.00
6-Sep-1999	180.86	0.40	0.00	0.00
13-Sep-1999	129.1	8.36	0.00	0.00

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
20-Sep-1999	82.66	4.56	0.00	0.32
4-Oct-1999	7.98	2.08	0.00	0.00
11-Oct-1999	10.39	2.96	0.00	0.00
25-Oct-1999	8.6	0.40	0.00	0.00
1-Nov-1999	8.66	0.56	0.00	0.00
8-Nov-1999	5.78	0.16	0.00	0.00
15-Nov-1999	5	0.80	0.00	0.00
22-Nov-1999	7.84	0.20	0.00	0.00
29-Nov-1999	7.54	0.00	0.00	0.00
6-Dec-1999	4.61	0.00	0.00	0.00
13-Dec-1999	12.72	0.00	0.00	0.00
3-Jan-2000	20.6	0.00	0.00	0.00
10-Jan-2000	28.43	0.00	0.00	0.00
17-Jan-2000	31.8	0.00	0.00	0.00
24-Jan-2000	13.7	0.00	0.00	0.00
31-Jan-2000	119.33	0.00	0.00	0.00
14-Feb-2000	121.35	0.00	0.00	0.00
21-Feb-2000	73.93	0.24	0.00	0.00
28-Feb-2000	19.51	0.00	0.00	0.00
13-Mar-2000	0.68	0.00	0.00	0.00
20-Mar-2000	1.12	0.00	0.00	0.00
27-Mar-2000	10.76	0.00	0.00	0.00
3-Apr-2000	15.62	0.00	0.00	0.00
10-Apr-2000	40.14	0.00	0.00	0.00
24-Apr-2000	35.06	0.00	0.00	0.00
1-May-2000	28.84	1.68	0.00	0.00
8-May-2000	49.78	0.32	0.00	0.00
15-May-2000	20.84	0.00	0.00	0.00
22-May-2000	150.52	0.00	0.00	0.00
29-May-2000	64.54	0.16	0.00	0.00
5-Jun-2000	76.58	0.00	0.00	0.16
12-Jun-2000	179.89	0.48	0.00	0.00
19-Jun-2000	2333.06	0.56	0.00	0.00
26-Jun-2000	1646.74	34.20	0.40	0.00
3-Jul-2000	12.24	0.40	0.00	0.00

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
10-Jul-2000	91.39	13.30	0.00	2.28
17-Jul-2000	197.8	44.84	0.00	6.56
24-Jul-2000	98.53	0.64	0.00	0.96
7-Aug-2000	335.34	158.08	0.00	16.72
28-Aug-2000	62.58	0.24	0.00	0.24
4-Sep-2000	2.28	0.00	0.00	0.00
11-Sep-2000	5.26	0.00	0.00	0.00
18-Sep-2000	12.08	0.08	0.00	0.08
16-Oct-2000	4.72	0.00	0.00	0.00
23-Oct-2000	2.56	0.00	0.00	0.00
6-Nov-2000	5.42	0.00	0.00	0.00
13-Nov-2000	6.72	0.00	0.00	0.00
20-Nov-2000	5.74	0.00	0.00	0.00
18-Dec-2000	8.58	2.08	0.00	0.00
15-Jan-2001	8.06	0.16	0.00	0.00
29-Jan-2001	2.84	0.24	0.00	0.00
5-Feb-2001	1.54	0.00	0.00	0.00
12-Feb-2001	8.38	0.00	0.00	0.00
19-Feb-2001	3.46	0.00	0.00	0.00
26-Feb-2001	2.74	0.16	0.00	0.00
5-Mar-2001	1.94	0.00	0.00	0.00
12-Mar-2001	3.6	0.00	0.00	0.00
2-Apr-2001	7.02	0.32	0.00	0.00
9-Apr-2001	2.39	0.00	0.00	0.00
16-Apr-2001	75.52	0.00	0.00	0.00
23-Apr-2001	516.3	1.20	0.00	0.00
30-Apr-2001	98.22	1.04	0.00	0.00
7-May-2001	91.92	6.08	0.00	0.00
14-May-2001	150.32	19.76	0.00	0.00
21-May-2001	39.3	9.50	0.00	0.00
28-May-2001	77.16	20.90	0.00	0.40
4-Jun-2001	454.58	264.10	0.00	1.76
11-Jun-2001	863.95	548.72	0.00	2.72
18-Jun-2001	664.41	562.40	0.00	13.30
25-Jun-2001	131.64	121.60	0.00	0.88

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
2-Jul-2001	1681.14	0.00	0.00	0.72
9-Jul-2001	51.65	1.20	0.00	1.20
16-Jul-2001	268.51	0.72	0.00	4.48
23-Jul-2001	59.66	0.00	0.00	0.48
30-Jul-2001	13.2	0.48	2.64	0.00
6-Aug-2001	274.52	17.10	15.58	0.00
13-Aug-2001	947.41	338.20	0.96	0.00
20-Aug-2001	761.68	497.80	0.80	0.00
3-Sep-2001	216.52	60.80	1.44	0.00
10-Sep-2001	138.94	36.86	3.36	0.00
17-Sep-2001	160.12	86.26	1.52	0.00
24-Sep-2001	78.42	23.94	2.72	0.00
8-Oct-2001	9.42	0.96	0.64	0.00
22-Oct-2001	5.68	0.00	0.64	0.00
29-Oct-2001	3.82	0.64	0.00	0.00
5-Nov-2001	77.91	0.00	0.00	0.00
12-Nov-2001	6.36	0.00	0.08	0.00
19-Nov-2001	4.59	0.00	0.00	0.00
26-Nov-2001	40.96	0.00	0.00	0.00
10-Dec-2001	9.16	0.00	0.00	0.00
7-Jan-2002	5.45	0.00	0.00	0.00
14-Jan-2002	25.77	0.00	0.00	0.00
12-Feb-2002	14.3	0.00	0.00	0.00
18-Feb-2002	37.5	0.16	0.00	0.00
4-Mar-2002	23.08	1.68	0.00	0.00
19-Mar-2002	7.1	0.00	0.00	0.00
25-Mar-2002	5.4	0.00	0.00	0.08
2-Apr-2002	1.32	0.00	0.00	0.00
8-Apr-2002	2.56	0.32	0.00	0.00
15-Apr-2002	3.8	0.00	0.00	0.00
22-Apr-2002	170.86	2.56	0.00	0.00
1-May-2002	155.05	14.16	0.00	0.00
7-May-2002	268.21	178.60	0.00	0.00
16-May-2002	82.14	73.34	0.00	0.00
20-May-2002	110.79	11.44	0.00	0.00

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
6-Jun-2002	136.88	0.00	0.00	0.00
11-Jun-2004	121.07	0.00	0.00	0.00
18-Jun-2002	145.1	1.12	0.00	0.00
24-Jun-2002	756.44	1.28	0.00	0.00
1-Jul-2002	296.87	3.04	0.00	9.88
9-Jul-2002	53.2	0.80	0.00	1.28
15-Jul-2002	205.8	0.48	0.00	4.24
22-Jul-2002	38.47	0.00	0.00	0.00
29-Jul-2002	332.98	0.00	0.00	3.92
5-Aug-2002	84.65	0.00	1.44	0.64
12-Aug-2002	59.6	23.94	0.08	0.40
20-Aug-2002	24.41	3.84	0.00	0.00
28-Aug-2002	1.04	0.00	0.00	0.00
2-Sep-2002	247.21	26.98	0.00	0.96
9-Sep-2002	32.97	4.48	0.00	1.44
16-Sep-2002	73.81	9.12	0.00	0.24
23-Sep-2002	82.29	22.42	2.72	1.36
30-Sep-2002	128.34	20.90	4.48	7.04
7-Oct-2002	146.69	23.94	0.96	2.08
14-Oct-2002	68.86	15.96	5.60	8.96
28-Oct-2002	16.82	1.36	0.00	0.32
4-Nov-2002	17	1.04	0.00	0.32
18-Nov-2002	7.28	0.00	0.00	0.00
25-Nov-2002	7.62	0.88	0.00	0.00
3-Dec-2002	5.72	0.16	0.00	0.08
16-Dec-2002	2.06	0.14	0.00	0.00
6-Jan-2003	5.47	0.00	0.00	0.00
13-Jan-2003	13.47	0.00	0.00	0.00
22-Jan-2003	8.49	0.00	0.00	0.00
27-Jan-2003	1.86	0.00	0.00	0.00
3-Feb-2003	11.24	0.00	0.00	0.00
10-Feb-2003	17.92	0.16	0.00	0.00
17-Feb-2003	9.44	0.00	0.00	0.00
3-Mar-2003	48.09	0.00	0.00	0.00
17-Mar-2003	68.69	1.44	0.00	0.00

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
31-Mar-2003	12.94	0.00	0.00	0.00
7-Apr-2003	69.15	0.00	0.00	0.00
14-Apr-2003	210.38	0.00	0.64	0.00
22-Apr-2003	88.7	0.00	0.00	2.40
6-May-2003	9.04	0.00	0.00	1.60
12-May-2003	5.58	0.00	0.00	1.28
3-Jun-2003	20.48	6.56	0.00	0.48
9-Jun-2003	61.43	2.08	0.00	2.40
16-Jun-2003	38.9	0.00	0.00	1.92
23-Jun-2003	64.99	0.00	0.00	48.64
30-Jun-2003	12.2	0.00	0.00	0.64
7-Jul-2003	49.45	0.00	0.00	2.24
14-Jul-2003	24.89	0.00	0.00	0.00
21-Jul-2003	67.84	0.00	0.00	2.24
28-Jul-2003	86.66	20.52	0.00	0.00
4-Aug-2003	1323.5	57.00	0.00	9.50
11-Aug-2003	66.3	0.00	0.00	1.52
20-Aug-2003	1.76	0.00	0.00	0.00
27-Aug-2003	163.2	129.96	0.00	0.64
2-Sep-2003	414.15	199.50	0.00	2.40
11-Sep-2003	132.79	12.92	0.00	5.12
15-Sep-2003	30.34	7.36	0.00	4.56
24-Sep-2003	247.06	78.66	0.00	60.80
29-Sep-2003	114.92	6.72	0.00	6.72
13-Oct-2003	14.62	1.52	0.00	0.48
21-Oct-2003	22.17	1.76	0.00	0.00
27-Oct-2003	30.32	2.24	0.00	0.00
6-Nov-2003	13.43	2.32	0.00	0.88
10-Nov-2003	24.4	1.12	0.00	1.20
24-Nov-2003	10.5	0.40	0.24	0.00
10-Dec-2003	8.28	0.00	0.00	0.00
15-Dec-2003	3.48	0.00	0.00	0.00
21-Jan-2004	5.69	0.00	0.00	0.00
28-Jan-2004	3.04	0.08	0.00	0.08
12-Feb-2004	31.31	0.00	0.00	0.00

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
16-Feb-2004	1.16	0.00	0.00	0.00
23-Feb-2004	0.86	0.00	0.00	0.00
1-Mar-2004	1.59	0.00	0.00	0.00
8-Mar-2004	1.98	0.08	0.00	0.00
29-Mar-2004	6.32	0.00	0.00	0.32
13-Apr-2005	13.38	0.16	0.00	0.40
22-Apr-2004	12.96	0.00	0.00	2.48
26-Apr-2004	3.36	0.00	0.00	1.36
17-May-2004	6.31	0.00	0.00	0.00
24-May-2004	16.38	0.00	0.00	0.24
2-Jun-2004	39.7	0.32	0.00	0.16
7-Jun-2004	115.44	0.64	0.00	0.40
14-Jun-2004	9.38	0.32	0.00	0.08
21-Jun-2004	27.34	0.40	0.00	0.32
6-Jul-2004	1486.97	997.50	0.00	70.30
12-Jul-2004	199.02	0.00	0.00	31.16
21-Jul-2004	215.2	0.00	0.00	43.70
2-Aug-2004	27.81	0.00	0.00	0.48
9-Aug-2004	4.16	0.56	0.00	0.40
16-Aug-2004	3.2	0.80	0.00	0.16
25-Aug-2004	481.91	149.72	0.00	0.96
31-Aug-2004	529.72	41.80	0.00	1.44
6-Sep-2004	95	32.68	0.00	0.64
21-Sep-2004	39.16	4.18	0.00	0.24
27-Sep-2004	24.77	0.48	0.00	0.00
18-Oct-2004	2.24	0.16	0.00	0.00
26-Oct-2004	66.4	0.64	0.00	0.08
1-Nov-2004	12.8	0.88	0.00	0.00
9-Nov-2004	4.66	0.64	0.00	0.00
16-Nov-2004	7.3	0.48	0.00	0.08
23-Nov-2004	6.4	0.32	0.00	0.00
29-Nov-2004	7.02	0.24	0.00	0.00
6-Dec-2004	4.98	0.48	0.00	0.00
13-Dec-2004	5.38	0.00	0.00	0.00
20-Dec-2004	1.38	0.00	0.00	0.00

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
6-Jan-2005	10.52	0.00	0.00	0.00
19-Jan-2005	11.94	0.96	0.00	0.00
24-Jan-2005	11.02	1.40	0.00	0.00
31-Jan-2005	12	1.98	0.00	0.00
7-Feb-2005	7.53	1.74	0.00	0.00
15-Feb-2005	4.13	0.63	0.00	0.00
22-Feb-2005	7.52	1.60	0.00	0.00
28-Feb-2005	17.28	5.10	0.00	0.00
6-Mar-2005	19.43	8.07	0.00	0.00
15-Mar-2005	29.44	3.31	0.00	0.00
21-Mar-2005	76.46	4.25	0.00	0.00
29-Mar-2005	97.7	1.72	0.00	0.00
4-Apr-2005	8.08	0.38	0.00	0.00
11-Apr-2005	20.52	4.40	0.00	0.00
18-Apr-2005	168.2	40.84	0.00	0.00
25-Apr-2005	18.54	4.04	0.00	0.00
4-May-2005	1500.08	83.31	0.00	0.00
9-May-2005	1019.18	178.06	0.00	0.00
16-May-2005	51.89	0.12	0.00	0.00
25-May-2005	75.5	0.16	0.00	0.00
1-Jun-2005	3.08	0.00	0.00	0.00
6-Jun-2005	8.84	0.12	0.00	0.00
13-Jun-2005	48.02	16.34	0.00	0.00
20-Jun-2005	131.31	3.27	0.00	0.00
27-Jun-2005	84.42	14.70	0.00	0.00
4-Jul-2005	50.56	2.12	0.00	0.00
11-Jul-2005	111.79	49.82	0.00	0.00
25-Jul-2005	319.31	75.14	0.00	0.00
1-Aug-2005	139.9	0.76	0.00	0.00
8-Aug-2005	97.72	37.57	0.00	0.00
15-Aug-2005	144.74	19.60	0.00	0.00
30-Aug-2005	2.24	1.56	0.00	0.00
5-Sep-2005	3.64	0.64	1.36	0.00
12-Sep-2005	0.36	0.04	0.04	0.00
19-Sep-2005	101.11	0.08	37.57	0.00

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
26-Sep-2005	39.22	0.00	0.44	0.00
3-Oct-2005	42.6	1.20	0.12	0.00
10-Oct-2005	66.29	0.20	0.36	0.00
17-Oct-2005	4.76	0.16	0.08	0.00
7-Nov-2005	6.32	0.48	0.24	0.00
15-Nov-2005	6.44	0.24	0.00	0.00
22-Nov-2005	5.6	0.20	0.00	0.00
28-Nov-2005	4.04	0.04	0.20	0.00
5-Dec-2005	5.08	0.04	0.00	0.00
12-Dec-2005	4.75	0.00	0.02	0.00
19-Dec-2005	5.46	0.02	0.00	0.00
3-Jan-2006	7.74	0.00	0.00	0.00
9-Jan-2006	8.76	0.00	0.00	0.00
16-Jan-2006	4.38	0.00	0.36	0.00
23-Jan-2006	8.98	0.00	0.08	0.00
6-Feb-2006	2.64	0.00	0.40	0.00
13-Feb-2006	4.24	0.00	0.36	0.00
21-Feb-2006	2.25	0.00	0.13	0.00
27-Feb-2006	2.56	0.00	0.14	0.00
6-Mar-2006	4.02	0.04	0.00	0.00
15-Mar-2006	13.78	0.00	0.00	0.00
20-Mar-2006	5.38	0.06	0.00	0.00
29-Mar-2006	5.88	0.08	0.00	0.00
3-Apr-2006	5.88	0.04	0.00	0.00
11-Apr-2006	69.43	0.12	0.00	0.00
18-Apr-2006	116.12	0.32	0.00	0.00
25-Apr-2006	65.68	0.04	0.00	0.00
4-May-2006	212.17	0.00	0.00	0.04
8-May-2006	36.56	0.00	0.00	0.00
15-May-2006	4.12	0.04	0.00	0.04
23-May-2006	3.56	0.08	0.00	0.00
6-Jun-2006	657.48	276.07	0.00	22.87
12-Jun-2006	279.07	191.12	0.00	0.12
19-Jun-2006	111.03	49.01	0.00	0.00
27-Jun-2006	33.52	2.00	0.00	0.00

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
3-Jul-2006	32.94	24.50	0.00	0.00
10-Jul-2006	325.91	86.58	0.00	0.00
17-Jul-2006	156.43	117.61	0.00	31.04
7-Aug-2006	396.35	94.75	0.00	37.57
14-Aug-2006	529.53	210.73	0.00	21.24
21-Aug-2006	146.08	11.43	0.00	0.24
29-Aug-2006	977.04	89.84	0.00	37.57
4-Sep-2006	154.49	13.07	0.00	47.37
12-Sep-2006	0.48	0.00	0.00	0.08
25-Sep-2006	3.58	0.00	0.04	0.00
3-Oct-2006	12.42	0.00	0.00	0.20
9-Oct-2006	9.06	0.00	0.00	0.04
30-Oct-2006	4	0.00	0.00	0.32
6-Nov-2006	12.92	0.00	0.16	0.24
12-Dec-2006	5.88	0.00	0.48	0.04
18-Dec-2006	2.94	0.00	0.02	0.08
15-Jan-2007	9.06	0.00	0.56	0.00
22-Jan-2007	5.16	0.00	0.00	0.00
29-Jan-2007	3.74	0.00	0.02	0.16
5-Feb-2007	3.72	0.00	0.00	0.00
16-Feb-2007	4.6	0.00	0.00	0.04
20-Feb-2007	6.8	0.00	0.00	0.00
26-Feb-2007	4.12	0.00	0.00	0.00
7-Mar-2007	5.32	0.00	0.00	0.00
13-Mar-2007	2.96	0.36	0.00	0.00
20-Mar-2007	4.36	0.04	0.00	0.00
26-Mar-2007	6.56	0.00	0.00	0.00
10-Apr-2007	1.64	0.08	0.00	0.00
16-Apr-2007	5.16	0.12	0.00	0.32
23-Apr-2007	42.22	0.96	0.00	0.76
30-Apr-2007	104.34	55.54	0.00	0.04
8-May-2007	33.85	0.76	0.00	0.00
21-May-2007	108.6	13.07	0.00	0.44
4-Jun-2007	35.71	22.87	0.00	0.24
11-Jun-2007	39.57	9.80	0.00	0.00

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
25-Jun-2007	442.73	0.00	0.00	0.04
9-Jul-2007	2073.74	0.00	17.97	174.79
16-Jul-2007	1173.61	0.04	0.00	0.88
23-Jul-2007	906.54	32.67	0.00	0.08
30-Jul-2007	231.65	0.60	0.20	0.44
6-Aug-2007	3.32	0.80	0.00	0.04
13-Aug-2007	120	0.88	0.00	0.76
20-Aug-2007	368.54	325.07	0.00	1.08
3-Sep-2007	148.67	0.20	0.00	2.96
10-Sep-2007	9.08	0.12	0.00	1.12
17-Sep-2007	60.63	0.12	0.00	4.24
2-Oct-2007	42.23	9.80	0.00	18.79
8-Oct-2007	7.58	0.24	0.00	0.48
25-Oct-2007	6.64	0.16	0.00	0.00
30-Oct-2007	7.52	0.20	0.00	0.04
12-Nov-2007	8.52	1.56	0.00	0.00
22-Nov-2007	7.16	0.64	0.00	0.00
26-Nov-2007	6.28	0.32	0.00	0.08
11-Dec-2007	7.84	0.08	0.00	0.00
21-Jan-2008	2.32	0.08	0.00	0.04
13-Feb-2008	11.32	0.08	0.00	0.00
20-Feb-2008	6.6	0.24	0.00	0.00
27-Feb-2008	1.32	0.04	0.00	0.00
5-Mar-2008	1.72	0.00	0.00	0.04
17-Mar-2008	1.9	0.00	0.00	0.08
31-Mar-2008	1.32	0.00	0.00	0.00
7-Apr-2008	1.16	0.00	0.00	0.00
14-Apr-2008	1.84	0.00	0.00	0.00
21-Apr-2008	1.12	0.00	0.00	0.00
28-Apr-2008	266.46	0.00	0.00	0.04
6-May-2008	454.47	0.00	0.00	0.00
12-May-2008	428.29	0.00	0.00	0.00
19-May-2008	233.82	0.00	0.00	0.16
28-May-2008	16.48	0.48	0.00	0.00
2-Jun-2008	154.34	0.00	0.12	0.08

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
9-Jun-2008	269.02	0.36	0.00	31.04
16-Jun-2008	233.61	0.52	0.00	16.34
23-Jun-2008	253.03	2.60	0.00	0.92
30-Jun-2008	274.68	35.94	0.00	27.77
14-Jul-2008	389.74	0.12	0.00	0.88
21-Jul-2008	1116.87	32.67	0.04	0.00
28-Jul-2008	6.12	0.28	0.16	0.00
20-Aug-2008	245.66	0.72	0.16	0.04
26-Aug-2008	809.84	0.28	6.53	15.52
2-Sep-2008	368.79	0.32	0.92	0.24
10-Sep-2008	1006.56	0.00	0.04	0.00
15-Sep-2008	24.93	0.00	0.00	0.00
22-Sep-2008	20.11	0.12	0.00	0.04
6-Oct-2008	7.28	0.12	0.08	0.00
13-Oct-2008	5.44	0.32	0.12	0.00
21-Oct-2008	30.26	0.24	1.04	0.48
27-Oct-2008	12.88	0.16	2.08	1.40
3-Nov-2008	13.92	0.12	1.68	1.12
17-Nov-2008	2	0.04	0.00	0.00
26-Nov-2008	3.96	0.00	0.00	0.00
1-Dec-2008	3.12	0.00	0.00	0.00
8-Dec-2008	3.76	0.00	0.00	0.00
15-Dec-2008	3.88	0.00	0.00	0.00
22-Dec-2008	3.36	0.00	0.00	0.00
5-Jan-2009	6.24	0.00	0.00	0.00
13-Jan-2009	4.16	0.08	0.00	0.00
21-Jan-2009	4.48	0.00	0.00	0.00
26-Jan-2009	4.36	0.00	0.00	0.00
11-Feb-2009	2.28	0.00	0.00	0.00
16-Feb-2009	2.04	0.00	0.00	0.00
23-Feb-2009	4.48	0.16	0.00	0.00
2-Mar-2009	4.24	0.08	0.00	0.00
9-Mar-2009	13.4	0.04	0.00	0.00
16-Mar-2009	16.71	0.16	0.00	0.00
23-Mar-2009	1.36	0.00	0.00	0.00

Date	Diatoms	*Pseudo-nitzschia delicatissima*	*Pseudo-nitzschia pungens*	*Pseudo-nitzschia seriata*
	Cells/ML	Cells/ML	Cells/ML	Cells/ML
30-Mar-2009	7.17	0.00	0.00	0.00
14-Apr-2009	70.98	0.20	0.00	0.00
21-Apr-2009	6.84	1.96	0.00	0.00
27-Apr-2009	41.52	0.00	0.16	0.00
5-May-2009	19.4	1.88	0.16	0.00
13-May-2009	244.62	12.25	0.72	0.00
2-Jun-2009	1052.06	0.08	1.36	0.00
8-Jun-2009	101.01	13.89	5.72	6.53
15-Jun-2009	55.11	52.27	0.32	0.44
22-Jun-2009	591.75	6.53	24.50	40.84
29-Jun-2009	354.1	17.97	26.14	19.60
7-Jul-2009	39.65	0.96	2.80	0.16
13-Jul-2009	130.29	0.12	0.12	0.00
20-Jul-2009	1242.68	0.56	0.00	0.16
27-Jul-2009	521	1.12	0.00	0.32
12-Aug-2009	21.62	1.04	0.00	0.12
17-Aug-2009	36.82	13.07	0.00	7.35
24-Aug-2009	288.91	249.93	0.00	1.04
1-Sep-2009	60.2	42.47	0.00	0.84
7-Sep-2009	15.58	0.88	0.00	0.64
14-Sep-2009	39.4	0.56	0.00	0.40
22-Sep-2009	56.14	0.64	0.00	0.40
28-Sep-2009	23.32	2.56	0.00	0.48
7-Oct-2009	97.72	0.12	0.00	0.24
12-Oct-2009	16.7	0.00	0.00	0.12
19-Oct-2009	11.4	0.08	0.00	0.16
26-Oct-2009	3.56	0.00	0.00	0.00
9-Nov-2009	2	0.00	0.00	0.00
1-Dec-2009	4.24	0.04	0.00	0.00
9-Dec-2009	7	0.16	0.00	0.00
15-Dec-2009	4.24	0.08	0.00	0.00
21-Dec-2009	3.08	0.08	0.00	0.00

Date	NITRITE µM	NITRATE µM	AMMONIA µM	SILICATE µM	PHOSPHATE µM
1/17/2000	0.07	8.05	0.1	3.62	0.35
2/2/2000	0.17	7.4	0.11	3.23	0.35
2/21/2000	0.31	7.78	0.29	2.9	0.38
3/13/2000	0.3	5.52	0.13	2.38	0.31
3/20/2000	0.36	5.19	0.22	2.15	0.3
3/27/2000	0.39	4.99	0.53	2.22	0.3
4/3/2000	0.31	4.34	0.47	1.76	0.28
4/11/2000	0.13	2.33	0.33	0.88	0.16
6/12/2000	0.05	0.09	0.1	0.46	0.05
6/19/2000	0.05	0.21	0.18	0.13	0.06
7/17/2000	0.05	0.05	0.13	0.13	0.05
8/21/2000	0.05	0.25	0.1	1.89	0.05
9/4/2000	0.06	0.21	0.58	2.93	0.05
10/12/2000	0.18	3.89	0.1	5.35	0.21
10/16/2000	0.44	8.43	0.73	7.3	0.21
10/26/2000	0.22	7.74	0.16	6.39	0.28
11/13/2000	0.2	9.29	0.21	5.48	0.41
11/19/2000	0.21	9.72	0.35	5.61	0.41
1/16/2001	0.08	8.47		4.96	0.49
2/19/2001	0.09	4.57		2.9	0.21
2/27/2001	0.27	6.05		3.78	0.33
3/4/2001	0.45	5.38		2.32	0.25
4/17/2001	0.43	5.09		2.21	0.22
4/22/2001	0.32	1.18		1.14	0.07
4/29/2001	0.47	0.72		1.12	0.04
5/7/2001	0.19	0.42		0.67	0.04
5/13/2001	0.51	0.71		1.06	0.06
5/20/2001	0.02	0.05		0.37	0.04
5/30/2001	0.29	0.48		0.82	0.04
6/3/2001	0.32	0.7		0.75	0.04
7/1/2001	0.07	0.32		0.46	0.04
7/8/2001	0.27	0.94		1.54	0.06
7/22/2001	0.34	0.63		1.2	0.04
7/29/2001	0.24	0.51		0.57	0.04
8/12/2001	0.17	0.48		0.53	0.15
8/18/2001	0.25	0.68		0.55	0.05
8/29/2001	0.38	0.7		1.02	0.12
9/2/2001	0.31	0.63		1.39	0.09

Date	NITRITE µM	NITRATE µM	AMMONIA µM	SILICATE µM	PHOSPHATE µM
9/9/2001	0.43	0.72		2.06	0.04
9/16/2001	0.47	0.66		1.33	0.04
9/23/2001	0.49	1.24		1.51	0.07
10/21/2001	0.43	1.18		2.68	0.08
11/4/2001	0.35	1.51		2.93	0.12
11/18/2001	0.13	1.53		2.59	0.14
11/25/2001	0.22	1.75		3	0.22
12/9/2001	0.14	2.07		3.13	0.18
12/16/2001	0.12	2.35		3.3	0.17
1/7/2002	0.11	1.89		2.21	0.14
2/12/2002	0.1	2.5		3.03	0.24
4/7/2002	0.32	2.21		1.07	0.59
4/30/2002	0.12	0.93		0.84	0.37
5/6/2002	0.02	0.16		0.74	0.28
5/15/2002	0.19	0.28		0.74	0.24
5/26/2002	0.02			3.33	
6/10/2002	0.01	0.2		0.44	0.18
6/17/2002	0.02	0.17		0.72	0.32
6/23/2002	0.1	0.24		0	0.28
6/30/2002	0.08	0.08		0.15	0.26
7/8/2002	0.1	0.2		0	0.17
7/14/2002	0	0.01		0.21	0.15
7/21/2002	0.01	0.09		0.1	0.09
7/28/2002	0.13	0.25		0.01	0.15
8/4/2002	0.07	0.08		0.32	0.11
8/11/2002	0.05	0.71		2.67	0.22
8/19/2002	0.03	0.14		2.58	0.32
8/27/2002	0.03	0.15		1.23	0.27
9/1/2002	0.06	0.07		1.6	0.22
9/8/2002	0.05	0.26		1.75	0.22
9/15/2002	0.11	0.51		1.58	0.36
9/22/2002	0.14	1.3		5.1	0.22
9/29/2002	0.15	0.55		1.63	0.35
10/6/2002	0.23	0.87		3.9	0.37
10/13/2002	0.67	0.77		2.88	0.31
10/27/2002	0.54	1.25		3.28	0.41
11/3/2002	0.91	3.08		3.4	0.65
11/17/2002	0.34	6.46		5.05	0.57

Date	NITRITE µM	NITRATE µM	AMMONIA µM	SILICATE µM	PHOSPHATE µM
11/24/2002	0.28	11.68		6.41	0.66
12/2/2002	0.05	5.01		3.32	0.73
12/15/2002	0.14	7.08		6.19	0.62
1/6/2003	0.03	7.66	0.03	4.4	0.66
1/13/2003	0.12	12.21	0.03	5.26	0.73
1/22/2003	0.15	7.07	0.12	5.63	0.95
1/27/2003	0.14	12.21	0.03	7.44	0.77
2/3/2003	0.1	9.03	0.03	6.94	0.67
2/10/2003	0.14	10.66	0.03	6.7	0.85
2/17/2003	0.16	8.72	0.75	7.41	0.73
3/2/2003	0.32	6.81	0.03	2.5	0.72
3/10/2003	0.31	9.11	0.21	5.1	0.95
3/16/2003	0.16	5.09	0.2	4.12	0.79
3/30/2003	0.05	3.29	0.18	5.02	0.69
4/6/2003	0.05	2.59	0.06	1.73	0.48
4/13/2003	0.12	2.76	0.3	5.98	1.05
4/21/2003	0.03	0.03	0.24	4.76	0.45
5/5/2003	0.2	1.05	0.24	8.03	0.54
5/11/2003	0	0	0.03	0.61	0.26
5/18/2003	0	0.04	0.15	0.94	0.18
5/27/2003	0	0.05	0.29	0.58	0.14
6/1/2003	0	0.05	0.29	0.35	0.11
6/8/2003	0	0.05	0.34	0.46	0.17
6/15/2003	0	0.05	0.15	0.29	0.13
6/22/2003	0	0.04	0.29	0.29	0.18
6/29/2003	0	0.05	0.27	0.25	0.1
7/6/2003	0	0.04	0.28	0.75	0.1
7/13/2003	0	0	0.23	1.35	0.09
7/20/2003	0	0.04	0.27	0.31	0.18
7/27/2003	0	0	0.3	0.52	0.11
8/3/2003	0	0.06	0.22	7.05	0.05
8/10/2003	0	0.2	0.79	6.06	0.89
8/19/2003	0	0.13	0.3	2.76	0.39
8/26/2003	0	0.13	0.42	2.67	0.41
9/1/2003	0.39	0.85	0.62	3.1	0.53
9/10/2003	0.25	0.91	0.74	3.83	0.71
9/14/2003	0.45	1.37	0.65	3.42	0.68
9/23/2003	0.31	0.74	0.47	1.69	0.38

Date	NITRITE µM	NITRATE µM	AMMONIA µM	SILICATE µM	PHOSPHATE µM
9/28/2003	1.22	3.41	0.55	5.23	1.04
10/12/2003	0.41	1.86	0.63	2.28	0.53
10/20/2003	0.8	2.85	0.9	2.69	0.73
10/26/2003	1.04	4.11	0	2.97	0.51
11/5/2003	1.08	5.04	0	6.99	0.47
11/9/2003	1.02	5.33	0	8.29	0.42
11/23/2003	0.07	5.59	0	3.38	0.51
12/9/2003	0.07	6.13	0	3.9	0.51
12/14/2003	0.22	7.95	0	4.44	0.58
1/21/2004	0.17	9.81	0	4.37	0.58
1/28/2004	0.24	11.36	0	8.9	0.63
2/12/2004	0.55	10.8	0	7.91	0.69
2/16/2004	0.33	8.83	0	10.38	0.58
3/1/2004	0.31	6.91	0	3.44	0.43
3/17/2004	0.34	8.12	0	4.13	0.45
3/29/2004	0.27	6.73	0	3.35	0.44
4/13/2004	0.14	1.81	0	1.34	0.36
4/22/2004	0	0.07	0	0.79	0.26
4/26/2004	0	0.03	0	1.27	0.13
5/10/2004	0.05	0.13	0	0.81	0.07
5/18/2004	0	0.05	0	0.28	0.03
5/24/2004	0	0.04	0	0.64	0.4
6/2/2004	0.11	0.17	0	0.46	0.12
6/7/2004	0	0	0	0.29	0.15
6/14/2004	0.07	0.45	0	1.07	0.21
6/21/2004	0.06	0.08	0	0.94	0.14
6/28/2004	0	0.04	0	0.24	0
7/6/2004	0	0	0	0.21	0.04
7/12/2004	0.07	0.33	0	0.89	0.22
7/21/2004	0	0.03	0	0.22	0.05
8/2/2004	0	0.03	0	0.48	0
8/9/2004	0	0	0	0.78	0
8/16/2004	0	0.85		7.15	0.1
8/25/2004	0.48	1.96		9.65	0.15
8/31/2004	0.04	0.35		0.31	0.1
9/6/2004	0	0.41		0.87	0
9/21/2004	0.35	3.71		3.14	0.04
9/27/2004	0.31	6.07		4.27	0.14

Date	NITRITE µM	NITRATE µM	AMMONIA µM	SILICATE µM	PHOSPHATE µM
10/18/2004	0.77	10.01		13.66	0.23
10/26/2004	0.46	10.32		10.3	0.26
11/1/2004	0.3	9.28		9.93	0.35
11/9/2004	0.16	8.56		7.98	0.3
11/16/2004	0.17	10.19		8.93	0.33
11/23/2004	0.09	10.86		8.91	0.38
11/29/2004	0.12	6.5	0.58		0.98
12/6/2004	0.32	14.93	0.65	7.66	0.52
12/20/2004	0.07	6.85	0.07	4.35	0.48
1/9/2005	0.05	7.55	0		0.36
1/24/2005	0.11	9.5	0.19	5.13	0.55
2/15/2005	0.13	8.73	0.03	4.68	0.53
5/4/2005	0.16	5.53	0.36	7.42	0.35
5/9/2005	0.05	0.53	0.14	1.68	0.35
5/16/2005	0.04	0.29	0.28	1.42	0.39
6/1/2005	0.06	0.4	0.22	5.55	0.8
6/6/2005	0.03	0.55	0.09	5.49	0.43
6/13/2005	0.03	0.21	0.23	5.39	0.37
6/20/2005	0.05	0.39	0.09	4.23	0.34
6/27/2005	0.03	0.36	0.11	3.86	0.26
7/4/2005	0.04	0.18	0.3	4.85	0.3
7/11/2005	0.03	0.12	0.11	3.89	0.24
7/26/2005	0.03	0.11	0.29	0.78	0.21
8/1/2005	0.04	0.49	2.35	6.27	0.2
8/15/2005	0.04	0.5	0.13	2	0.36
8/30/2005	0.03	0.32	0.02	2.36	0.23
9/5/2005	0.06	0.07	0.03	2.99	0.06
9/12/2005	0.02	0.72	0		0.04
9/19/2005	0.18	0.89	0		0.04
9/23/2005	0.4	1.44	0		0.05
10/3/2005	0.14	1.1	0		0.04
10/10/2005	0.19	3.56	0		0.2
10/17/2005	0.61	3.05	0		0.15
11/1/2005	0.62		0.42		0.43
11/7/2005	0.17	5.47	0		0.29
11/15/2005	0.22	9.71	0.12		0.38
11/22/2005	0.05	6.15	0		0.3
12/5/2005	0.2		0.13		0.38

Date	NITRITE µM	NITRATE µM	AMMONIA µM	SILICATE µM	PHOSPHATE µM
12/12/2005	0.05	6.83	0		0.31
12/19/2005	0.1	8.35	0		0.32
12/28/2005	0.08	6.95	0		
1/16/2006	0.04	7.38	0.25		0.32
1/23/2006	0.03	6.78	0		0.46
2/3/2006	0.07	6.51	0.23	2.33	0.2
2/6/2006	0.12	8.72	0.28	4.22	1.11
2/13/2006	0.13	4.41	0.28	2.24	0.59
2/21/2006	0.14	4.55	0.23	2.09	0.43
2/27/2006	0.17	5.87	0.23	3.05	0.44
3/6/2006	0.24	6.98	0.46	3.44	0.58
3/15/2006	0.24	5.59	0.39	2.77	0.53
3/20/2006	0.26	5.8	0.69	3.07	0.48
3/29/2006	0.23	5.06	0.42	2.11	0.43
4/3/2006	0.27	10.12	0.94	3.81	0.55
4/11/2006	0.08	0.3	0.48	0.37	0.2
4/18/2006	0.02	0.28	0.4	0.23	0.1
5/4/2006	0.01	0.32	0.37	0.23	0.13
5/8/2006	0.19	4.64	0.58	2.39	0.39
5/15/2006	0.1	1.84	0.73	2.94	0.42
5/23/2006	0.1	0.91	1.26	1.26	0.38
6/6/2006	0.03	0.27	0.57	0.18	0.15
6/12/2006	0.01	0.27	0.51	0.14	0.18
6/20/2006	0.01	0.35	0.38	0.25	0.16
6/27/2006	0.04	0.43	0.53	0.29	0.16
7/3/2006	0.12	0.19	0.27	0.5	0.09
7/17/2006	0.04	0.16	0.26	0.52	0.13
7/17/2006	0.02	0.1	0.27	0.56	0.13
8/7/2006	0.05	0.38	0.12	1.45	0.13
8/14/2006	0.01	0.16	0.21	2.17	0.2
8/21/2006	0.02	0.54	0.39	2.91	0.34
8/29/2006	0.24	0.38	1.02	2.4	0.23
9/4/2006	0.02	0.15	0.34	0.92	0.1
9/12/2006	0.03	0.2	1.24	2.45	0.2
9/25/2006	0.04	0.42	2.95	3.94	0.26
10/3/2006	0.15	0.76	1.26	7.22	0.23
10/9/2006	0.25	1.48	0.72	5.61	0.21
10/30/2006	1.25	7.19	0.79	9.47	0.38

Date	NITRITE µM	NITRATE µM	AMMONIA µM	SILICATE µM	PHOSPHATE µM
11/6/2006	0.45	3.18	0.29	3.84	0.33
12/12/2006	0.04	5.19	0.28	4.69	0.39
1/15/2007	0.07	4.12	0.32	3.52	0.4
1/22/2007	0.1		0.69	4.14	0.37
1/29/2007	0.19	11.4	0.46	5.92	0.41
2/5/2007	0.14	4.87	0.44	3.94	0.42
2/16/2007	0.25	4.87	0.19	4.07	0.5
2/20/2007	0.28	6.6	0.7	4.33	0.48
2/26/2007	0.32	7.75	0.16	4.96	0.48
3/12/2007	0.29	7.16	0.45	5.46	0.49
3/20/2007	0.23	5.37	0.21	4.35	
3/26/2007	0.21	5.86	0.42	4.2	0.41
4/10/2007	0.18	3.18	0.03	3.91	0.25
4/16/2007	0.07	0.98	0.07	3.22	0.1
4/23/2007	0.11	1.4	0.03	1.66	0.12
4/30/2007	0.06	0.11	0.39	0.7	0.03
5/7/2007	0.02	0.03	0.22		0.04
5/21/2007	0.08	1.13	1.01		0.12
6/4/2007	0.05	0.56	0.31	1.02	0.03
6/11/2007	0.06	2.14	0.39	0.61	0.05
6/18/2007	0.02	0.03	0.03	0.33	0.04
6/25/2007	0.02	0.04	0.08	0.3	0.03
7/2/2007	0.02	0.03	0.03	0.25	0.04
7/9/2007	0.03	1.65	0.5	0.32	0.03
7/16/2007	0.02	0.03	0.03	0.11	0.04
7/23/2007	0.02	0.03	0.03		0.03
7/30/2007	0.02	0.03	0.81	0.93	0.03
8/6/2007	0.02	0.09	0.24	1.39	0.03
8/13/2007	0.05	0.22	0.28	1.94	0.03
8/20/2007	0.37	1.59	0.25	2.87	0.13
8/28/2007	0.05	0.11	0.2	2.55	0.04
9/3/2007	0.07	0.22	0.57	1.23	0.01
9/10/2007	0.06	0.08	0.15	1.73	0.06
9/17/2007	0.16	0.76	0.49	2.13	0.15
9/28/2007	0.27	1.35	0.12	2.33	0.16
10/2/2007	0.58	2.71	0.29	2.89	0.2
10/8/2007	0.7	2.58	0.18	2.67	0.21
10/25/2007	0.95	2.45	0.2	2.61	0.19

Date	NITRITE µM	NITRATE µM	AMMONIA µM	SILICATE µM	PHOSPHATE µM
10/30/2007	1.11	3.6	0.17	3.54	0.27
11/5/2007	0.82	2.7	0.14	2.65	0.21
11/12/2007	0.38	2.86	0.16	2.85	0.25
11/23/2007	0.16	2.4	0.15	2.75	0.24
11/26/2007	0.07	2.13	0.2	2.52	0.27
12/11/2007	0.2	7.8	0.34	4.62	0.44
1/28/2008	0.17	8.6		6.07	0.5
2/8/2008	0.2	7.06	0.22	4.61	0.52
2/21/2008	0.23	7.13	0.06	3.83	0.52
2/27/2008	0.31	7.63	0.35	4.84	0.48
3/5/2008	0.34	7.78	0.05	4.85	0.45
3/17/2008	0.27	6.7	0.5	4.38	0.41
3/31/2008	0.22	5.3	0.98	2.9	0.53
4/7/2008	0.23	5.11	1.55	2.83	0.38
4/14/2008	0.18	4.1	0.9	2.69	0.35
4/21/2008	0.16	4.4	0	2.88	0.38
4/28/2008	0	0.05	0.23	1.41	0.14
5/6/2008	0	0.02	0		0.02
5/12/2008	0	0	0		0.03
5/19/2008	0	0	0.04	0.25	0.16
5/28/2008	0	0	0.18	0.77	0.16
6/2/2008	0	0	0	0.31	0.11
6/9/2008	0	0	0.07	0.69	0.15
6/16/2008	0	0.03	0.23	0.2	0.21
6/23/2008	0	0.02	0		0.18
6/30/2008	0	0.03	0	0.35	0.04
7/14/2008	0.01	0.03	0	0.18	0.04
7/21/2008	0	0.01	0.04	0.12	0.01
7/28/2008	0	0	0	0.18	0.01
8/26/2008	0.04	0.19	0.77	0.18	0
9/2/2008	0.05	0.12	0.05	0.62	0
9/10/2008	0.11	0.7	0.35	4.17	0.02
9/15/2008	0.25	4.29	0.36	6.04	0
9/22/2008	0.16	0.93	0.06	2.05	0.1
9/29/2008	1.02	4.15	1.02	3.75	
10/6/2008	0.6	3.48	0.77	3.38	
10/13/2008	0.57	3.22	0.36	2.84	0.2
10/21/2008	0.81	4.04	0.68	4.24	0.35

Date	NITRITE µM	NITRATE µM	AMMONIA µM	SILICATE µM	PHOSPHATE µM
10/27/2008	0.67	5.6	1.25	4.06	0.32
11/3/2008	0.63	2.82	1.23	2.63	0.34
11/17/2008	0.52	6.76	0.44	5.99	0.39
11/26/2008	0.13	5.08	0.11	5.23	0.46
12/1/2008	0.22	6.79	0.17	5.54	0.47
12/8/2008	0.13	5.94	0.26	5.64	0.45
12/15/2008	0.21	6.98	0.3	5.75	0.44
12/22/2008	0.23	8.14	0.08	5.94	0.4
1/5/2009	0.07	6.49	0.09	4.46	0.45
1/13/2009	0.07	7.19	0.46	5.47	0.43
1/21/2009	0.21	10.59	0.68	7	0.55
1/26/2009	0.13	7.6	0.2	5.46	0.45
1/26/2009	0.13	7.67	0.23	5.44	0.45
1/26/2009	0.13	7.68	0.21	5.42	0.48
1/26/2009	0.13	7.69	0.25	5.5	0.5
2/11/2009	0.12	10.26	0.8	5.16	0.47
2/16/2009	0.18	7.62	1.49	4.38	0.85
2/23/2009	0.19	8.91	0.31	4.9	0.48
3/2/2009	0.18	5.6	0.28	3.8	0.42
3/9/2009	0.22	6.55	0.78	3.79	0.43
3/16/2009	0.26	6	1.55	3.22	0.33
3/23/2009	0.21	7.01	0.62	3.76	0.35
3/30/2009	0.18	4.1	0.6	1.54	0.26
4/6/2009	0.14	3.92	0	2.44	0.24
4/14/2009	0.12	2.31	0.63	1.37	0.22
4/21/2009	0.06	1.65	0.1	2.02	0.11
4/27/2009	0.05	0.56	0.3	0.98	0.13
5/5/2009	0.07	1.51	0	1.65	0.11
5/13/2009	0.01	0.12	0	0.89	0.05
5/21/2009	0.02	0.28	0	1.14	0.02
6/2/2009	0	0.07	0		0
6/8/2009	0	0.06	0.13	0.57	0.04
6/15/2009	0	0.03	0	0.52	0.16
6/22/2009	0	0	0.03	0.16	0.02
6/29/2009	0	0	0.13		
7/7/2009	0.01	0.09	0.03	0.15	0.03
7/13/2009	0	0.05	0.03	0.43	0.02
7/20/2009	0.02	0.04		0.23	0.03

Date	NITRITE µM	NITRATE µM	AMMONIA µM	SILICATE µM	PHOSPHATE µM
7/27/2009					
8/13/2009	0.06	0.14	1.47	1.48	0.05
8/17/2009	0.01	0.08	0.45	2.77	0.01
8/24/2009	0.13	0.38	0.27	2.63	0.05
9/1/2009	0.02	0.04	0.57	2.97	0.05
9/7/2009	0.02	0.04	0.52	2.98	0.04
9/14/2009	0.41	2.39	0.84	3.23	0.19
9/22/2009	0.62	2.05	0.31	3.07	0.18
9/28/2009	0.53	0.9	0.19	2.21	0.14
10/7/2009	0.58	1.6	0.19	2.26	0.15
10/12/2009	0.67	1.67	0.04	2.28	0.19
10/19/2009	0.52	2.03	0.02	2.47	0.21
10/26/2009	0.52	2.54	<0.03	3.36	0.2
11/9/2009	0.13	3.73	0.74	3.45	0.3
12/1/2009	0.12	8.53	0.6	4.56	0.51
12/9/2009	0.07	7.37		4.33	
12/15/2009	0.27	11.62	0.34	6.22	
12/21/2009	0.13	7.76	0.15	4.79	0.5

APPENDIX B

CHANGE IN SEA SURFACE TEMPERATURE DATA (±DEGREE CELSIUS) USED IN THE ENSO ANALYSIS

Year	DJF	JFM	FMA	MAM	AMJ	MJJ
1992	**1.8**	**1.6**	**1.5**	**1.4**	**1.2**	**0.8**
1993	0.3	0.4	0.6	0.7	0.8	0.7
1994	0.2	0.2	0.3	0.4	**0.5**	**0.5**
1995	**1.2**	**0.9**	**0.7**	0.4	0.3	0.2
1996	**-0.7**	**-0.7**	**-0.5**	-0.3	-0.1	-0.1
1997	-0.4	-0.3	0	0.4	**0.8**	**1.3**
1998	**2.3**	**1.9**	**1.5**	**1**	**0.5**	0
1999	**-1.4**	**-1.2**	**-0.9**	**-0.8**	**-0.8**	**-0.8**
2000	**-1.6**	**-1.4**	**-1**	**-0.8**	**-0.6**	**-0.5**
2001	**-0.6**	**-0.5**	-0.4	-0.2	-0.1	0.1
2002	-0.1	0.1	0.2	0.4	**0.7**	**0.8**
2003	**1.2**	**0.9**	**0.5**	0.1	-0.1	0.1
2004	0.4	0.3	0.2	0.2	0.3	**0.5**
2005	**0.7**	**0.5**	0.4	0.4	0.4	0.4
2006	-0.7	-0.6	-0.4	-0.1	0.1	0.2
2007	**0.8**	0.4	0.1	-0.1	-0.1	-0.1
2008	**-1.4**	**-1.4**	**-1.1**	**-0.8**	**-0.6**	-0.4
2009	-0.8	-0.7	-0.5	-0.1	0.2	**0.6**
2010	**1.7**	**1.5**	**1.2**	**0.8**	0.3	

Note: Values are differences in average Celsius temperatures for a three month period compared to historical data.

DJF = December/January/February

JMF = January/FebruaryMarch

FMA = February/March/April

MAM = March/April/May

AMJ = April/May/June

MJJ = May/June/July

Year	JJA	JAS	ASO	SON	OND	NDJ
1992	**0.5**	0.2	0	-0.1	0	0.2
1993	0.4	0.4	0.4	0.4	0.3	0.2
1994	**0.6**	**0.6**	**0.7**	**0.9**	**1.2**	**1.3**
1995	0	-0.2	**-0.5**	**-0.6**	**-0.7**	**-0.7**
1996	0	-0.1	-0.1	-0.2	-0.3	-0.4
1997	**1.7**	**2**	**2.2**	**2.4**	**2.5**	**2.5**
1998	**-0.5**	**-0.8**	**-1**	**-1.1**	**-1.3**	**-1.4**
1999	**-0.9**	**-0.9**	**-1**	**-1.1**	**-1.3**	**-1.6**
2000	-0.4	-0.4	-0.4	**-0.5**	**-0.6**	**-0.7**
2001	0.2	0.2	0.1	0	-0.1	-0.1
2002	**0.9**	**1**	**1.1**	**1.3**	**1.5**	**1.4**
2003	0.4	0.5	0.6	0.5	0.6	0.4
2004	**0.7**	**0.8**	**0.9**	**0.8**	**0.8**	**0.8**
2005	0.4	0.3	0.2	-0.1	-0.4	-0.7
2006	0.3	**0.5**	**0.6**	**0.9**	**1.1**	**1.1**
2007	-0.1	-0.4	**-0.7**	**-1**	**-1.1**	**-1.3**
2008	-0.1	0	0	0	-0.3	-0.6
2009	**0.7**	**0.8**	**0.9**	**1.2**	**1.5**	**1.8**

Note: Values are differences in average Celsius temperatures for a three month period compared to historical data.

JJA = June/July/August

JAS = July/August/September

ASO = August/September/October

SON= September/October/November

OND = October/November/December

NDJ = November/December/January

APPENDIX C

WASHINGTON STATE RAZOR CLAM DOMOIC ACID DATA

Date	Talaloch mg/kg	Long Beach mg/kg	Twin Harbors mg/kg	Copalis mg/kg	Mocrocks mg/kg
Apr-98	0.1	0.1	0.1	3	0.1
Jun-98	0.1	0.1	9	14	0.1
Jul-98	2	0.1	8	8	8
Aug-98	2	8	12	13	10
Aug-98	2	5	47	23	11
Sep-98	12	39	107	52	64
Sep-98	48	66	108	61	102
Oct-98	287	81	81	58	68
Oct-98	282	59	100	51	75
Oct-98	224	59	48	78	59
Nov-98	295	52	51	51	59
Nov-98	168	21	48	50	60
Dec-98	261	48	27	42	80
Dec-98	152	36	48	28	50
Jan-99	238	0.1	52	29	32
Jan-99	135	27	48	21	39
Feb-99	214	30	40	45	19
Feb-99	171	17	36	42	20
Mar-99	142	24	22	31	30
Mar-99	185	16	10	7	7
Apr-99	199	13	9	34	18
Apr-99	236	8	21	15	24
May-99	71	4	13	11	11
Jun-99	59	5	11	8	15
Jun-99	1	7	6	11	11
Jun-99	81	12	22	8	8
Jul-99	11	6	7	6	4
Aug-99	16	7	15	12	10
Sep-99	45	4	15	6	10
Sep-99	17	13	16	9	6
Oct-99	20	8	10	10	6
Oct-99	15	5	6	6	3
Nov-99	2	4	5	2	7

Date	Talaloch mg/kg	Long Beach mg/kg	Twin Harbors mg/kg	Copalis mg/kg	Mocrocks mg/kg
Nov-99	36	2	7	12	4
Dec-99	4	1	6	5	4
Dec-99	19	6	19	2	5
Jan-00	18	4	4	3	1
Mar-00	10	2	3	2	3
Mar-00	35	2	5	1	6
Apr-00	6	3	10	6	4
May-00	5	3	5	5	2
Jun-00	7	3	2	5	8
Jul-00	6	3	2	3	5
Jul-00	6	2	5	2	6
Aug-00	2	4	4	4	8
Sep-00	18	2	3	5	5
Sep-00	5	3	4	6	8
Oct-00	6	4	3	2	4
Oct-00	4	22	5	12	2
Nov-00	3	17	4	10	12
Nov-00	4	13	3	9	14
Nov-00	3	11	3	9	11
Dec-00	4	9	24	8	8
Jan-01	2	6	18	8	7
Feb-01	4	8	20	5	10
Mar-01	17	13	19	7	6
Apr-01	12	18	19	4	3
Apr-01	10	6	10	4	3
May-01	13	9	7	44	4
Jun-01	6	6	5	5	52
Jul-01	3	5	8	5	6
Aug-01	2	6	8	4	6
Sep-01	3	7	9	3	4
Sep-01	3	6	5	2	4
Sep-01	3	6	6	2	4
Oct-01	2	9	5	2	2
Oct-01	2	8	4	1	3
Oct-01	1	4	5	1	3
Nov-01	2	4	4	2	1
Dec-01	2	4	2	2	2

Date	Talaloch mg/kg	Long Beach mg/kg	Twin Harbors mg/kg	Copalis mg/kg	Mocrocks mg/kg
Dec-01	2	4	3	1	1
Dec-01	1	2	5	1	1
Jan-02	1	3	2	1	2
Jan-02	1	16	3	1	1
Feb-02	1	38	4	1	1
Apr-02	1	132	4	27	1
May-02	2	84	2	53	25
Jun-02	4	52	3	185	45
Aug-02	1	80	16	61	185
Sep-02	26	53	60	72	147
Oct-02	52	81	113	78	112
Oct-02	99	71	52	107	118
Oct-02	67	62	48	102	114
Nov-02	150	38	70	87	91
Nov-02	98	59	63	99	107
Dec-02	78	71	44	78	75
Dec-02	80	62	60	44	108
Dec-02	115	38	59	87	63
Jan-03	67	59	61	81	66
Jan-03	103	32	69	66	45
Feb-03	68	32	63	54	65
Feb-03	102	21	22	51	43
Mar-03	90	25	36	39	24
Mar-03	77	21	32	34	17
Apr-03	97	33	38	26	20.03
Apr-03	90	29	29	36	17
May-03	84	30	33	17	37
May-03	68	39	15	15	15
Jun-03	66	29	27	32	26
Jun-03	56	19	10	17	20
Jul-03	43	13	17	13	12
Jul-03	34	18	17	18	14
Aug-03	22	9	19	16	12
Aug-03	11	3	21	12	14
Aug-03	15	6	12.02	11	12
Sep-03	40	13	9	4	12
Sep-03	25	4	18	9	9

Date	Talaloch mg/kg	Long Beach mg/kg	Twin Harbors mg/kg	Copalis mg/kg	Mocrocks mg/kg
Sep-03	18	7	9	9	10
Sep-03	19	7	10	4	11
Sep-03	22	3	5	8	8
Oct-03	12	4	2	10	2
Oct-03	20	4	4	7	11
Oct-03	21	2	7	6	18
Nov-03	13	3	12	6	6
Nov-03	22	4	4	6	4
Nov-03	7	7	11	17	13
Dec-03	11	3	17	15	10
Dec-03	11	0.1	16	7	3
Dec-03	8	4	10	7	2
Jan-04	28	6	14	5	4
Jan-04	11	4	5	3	9
Feb-04	14	9	10	9	6
Feb-04	28	4	8	6	5
Mar-04	10	3	5	10	6
Mar-04	11	5	6	4	5
Mar-04	14	2	5	3	4
Mar-04	15	2	3	1	3
Mar-04	11	3	3	3	7
Apr-04	19	2	4	9	2
Apr-04	11	2	2	7	3
Apr-04	12	1	1	7	2
Apr-04	16	3	1	3	3
May-04	14	1	8	3	3
May-04	8	1	7	7	3
May-04	5	5	3	3	2
May-04	12	4	5	3	4
Jun-04	7	5	4	3	4
Jun-04	3	4	5	4	2
Jun-04	10	1	5	3	2
Jul-04	7	3	5	3	1
Aug-04	47	2	4	4	1
Aug-04	48	2	5	3	1
Aug-04	40	2	5	2	1
Aug-04	49	3	4	2	2

Date	Talaloch mg/kg	Long Beach mg/kg	Twin Harbors mg/kg	Copalis mg/kg	Mocrocks mg/kg
Aug-04	22	3	7	1	4
Sep-04	23	2	6	2	5
Sep-04	19	2	6	1	7
Oct-04	29	4	5	1	4
Oct-04	24	2	3	2	4
Nov-04	23	1	2	3	3
Dec-04	17	1	9	4	2
Dec-04	22	1	3	8	2
Jan-05	28	0.1	3	6	2
Jan-05	22	1	9	5	2
Feb-05	21	2	6	6	3
Feb-05	16	15	12	4	5
Mar-05	24	8	18	3	6
Mar-05	15	20	10	2	8
Mar-05	13	20	8	2	4
Mar-05	22	14	5	5	6
Apr-05	17	5	5	2	5
Apr-05	17	7	6	1	3
Apr-05	14	6	2	1	3
May-05	12	2	4	4	2
May-05	5	1	2	5	
May-05	12	2	6	7	1
Jun-05	10	3	2	4	1
Jun-05	6	4	9	5	1
Jun-05	6	9	11	4	1
Jul-05	9	9	11	3	2
Aug-05	4	6	9	1	1
Aug-05	3	5	3	1	1
Aug-05	5	5	1		2
Aug-05	2	2	1	1	1
Sep-05	1	2	1	2	7
Oct-05	7	4	2	2	2
Oct-05	7	2	5	2	4
Oct-05	4	2	4	2	3
Nov-05	6	2	1	4	3
Dec-05	5	1	2	3	2
Dec-05	6	2	2	3	2

Date	Talaloch mg/kg	Long Beach mg/kg	Twin Harbors mg/kg	Copalis mg/kg	Mocrocks mg/kg
Jan-06	3	2	4	3	2
Jan-06	3	3	4	3	3
Feb-06	2	2	2	2	2
Feb-06	3	2	2	3	2
Feb-06	4	2	2	3	1
Mar-06	3	3	4	3	3
Mar-06	0.1	4	3	2	1
May-06	3	4	5	4	3
May-06	1	4	3	2	5
Jun-06	2	2	5	2	4
Jun-06	3	4	3	2	4
Jul-06	20	2	2	1	4
Jul-06	38	1	2	2	4
Jul-06	34	3	2	1	4
Aug-06	26	1	1	1	3
Aug-06	16	1	1	1	3
Sep-06	20	2	2	0.5	3
Sep-06	19	2	2	1	3
Sep-06	8	0.5	2	1	4
Sep-06	15	1	2	1	3
Oct-06	18	1	1	1	4
Oct-06	14	1	4	1	0.5
Oct-06	18	4	4	0.5	1
Oct-06	7	4	4	3	
Nov-06	11	4	4	4	0.5
Nov-06	13	3	3	4	1
Dec-06	8	4	3	3	1
Dec-06	8	3	3	4	0.5
Dec-06	8	3	4	3	
Dec-06	8	3	3	3	0.5
Jan-07	14	3	3	3	0.5
Feb-07	14	4	4	3	0.5
Feb-07	15	4	1	4	0.5
Feb-07	12	0.5	1	3	0.5
Mar-07	7	1		3	0.5
Apr-07	14	0.1	1	0.5	0.5
Apr-07	9	0.5	0.5	0.5	0.5

Date	Talaloch mg/kg	Long Beach mg/kg	Twin Harbors mg/kg	Copalis mg/kg	Mocrocks mg/kg
Apr-07	6	0.5	0.5		0.5
May-07	8	0.5	0.5	0.5	0.5
May-07	5	0.5	0.5	0.5	
Jun-07	4	0.5	0.5		0.5
Jul-07	3	0.5	0.5	0.5	0.5
Jul-07	0.1	1	0.5	0.5	0.5
Jul-07	1	1	0.5	0.5	0.5
Jul-07	5	1	0.5	0.5	
Aug-07	0.1	0.5	0.5	0.5	
Aug-07	4	0.5	0.5	0.5	0.5
Oct-07	2	0.5	0.5	1	0.5
Nov-07	1	0.5	0.5	0.5	0.5
Jan-08	3	0.5		0.5	0.5
Apr-08	0.1	0.5	0.5	0.5	0.5
May-08	0.1	0.1	0.5	0.5	0.5
Jun-08	1	0.1	0.5	0.5	
Jun-08	0.1	0.1	0.5	0.5	0.5
Jul-08	1	0.1			0.5
Jul-08	1	0.1	0.5		0.5
Jul-08	0.1	0.1	0.5	0.5	
Aug-08	0.1	1		0.5	0.5
Aug-08	1	2		0.5	0.5
Sep-08	0.1	2	1	0.5	0.5
Oct-08	0.1	1	1	0.5	0.5
Oct-08	0.1	0.5	1		0.5
Nov-08	0.1	1	1		1
Dec-08	0.1	1	1		0.5
Jan-09	0.1	1	1	0.5	1
Mar-09	0.1	1	1	0.5	1
Mar-09	0.1	1	1	0.5	0.5
Apr-09	0.1	1	1	0.5	0.5
May-09	2	1		0.5	0.5
May-09	1	1			0.5
Jun-09	1	1	1	0.5	5
Jun-09	1	1		1	8
Jul-09	1	1	1	0.5	4
Jul-09	1	3	2	0.5	7

Date	Talaloch mg/kg	Long Beach mg/kg	Twin Harbors mg/kg	Copalis mg/kg	Mocrocks mg/kg
Aug-09	1	2	1	0.5	7
Aug-09	2	2	1	0.5	7
Sep-09	2	1	3		6
Sep-09	2	1	4		5
Sep-09	1	1	3	1	5
Sep-09	1	1	3	0.5	2
Oct-09	1	1	1	1	4
Oct-09	1	1	4	1	2
Jan-10	1	1	3	0.5	3
Jan-10	0.1	1	2	0.5	2
Feb-10	1	0.1	1	1	1
Feb-10	1	1	2	0.5	2
Mar-10	1	1	2	4	1
Apr-10	1	1	1	2	1
May-10	1	1	1	4	2
May-10	1	1	1	3	1
May-10	1	0.5	1	4	1
Jun-10	1	0.5	1	3	1
Jun-10	1	0.1	1	4	2
Jun-10	1	1		3	2
Jun-10	1	1		1	2
Jun-10	1	1		3	1
Jul-10	1	1		2	1
Jul-10	0.1	1		3	1
Jul-10	1	0.5		2	0.5
Aug-10	1	0.5	1	1	1
Aug-10	1	0.5		1	
Sep-10	0.1	0.5		1	
Sep-10	0.1	0.5		1	
Sep-10	0.1	0.1		1	
Sep-10	0.1	0.5		1	0.5
Oct-10	0.1	0.1		1	0.5
Oct-10	0.1	0.1		1	0.5
Oct-10	0.1	0.1		1	
Nov-10	0.1	0.1		1	
Nov-10	0.1	0.5		1	
Dec-10	0.1	0.5		1	0.5

Date	Talaloch mg/kg	Long Beach mg/kg	Twin Harbors mg/kg	Copalis mg/kg	Mocrocks mg/kg
Jan-11	0.1	0.5		1	0.5
Jan-11	0.1	0.5		1	
Jan-11	0.1	0.5		1	
Jan-11	0.1	0.5		0.5	0.5
Feb-11	0.1	0.5		0.5	
Feb-11	0.1	0.1			
Mar-11	0.1	0.5			
Apr-11	0.1	0.5			
May-11	0.1	0.1		1	
May-11	0.1	0.1		0.5	
May-11	0.1	0.5		1	
May-11	0.1	0.1		0.5	
May-11	0.1	1		0.5	
Jun-11	0.1	1		0.5	
Jun-11	0.1	1		0.5	
Jun-11	0.1	1		0.5	
Jun-11	0.1	1	1	0.5	0.5
Jul-11	0.1	1	1	0.5	0.5
Jul-11	0.1	1	2		0.5
Aug-11	0.1	0.5	1		
Sep-11	2	1	1		
Sep-11	1	1	1		0.5
Sep-11	2	1	1		0.5
Oct-11	1	1	1		0.5
Oct-11	1	1	1		
May-12	1	1	2		
Jun-12	1	1	1		1
Jul-12	0.1	0.1	1	0.5	1
Jul-12	0.1	0.5	1		1
Jul-12	0.1	0.5	1		2
Aug-12	1	1			1
Aug-12	4	1			2
Aug-12	4	2	1		1
Sep-12	2	3	1	0.5	2
Sep-12	2	2		0.5	1
Sep-12	2	1		0.5	1
Oct-12	3	1			1

Date	Talaloch mg/kg	Long Beach mg/kg	Twin Harbors mg/kg	Copalis mg/kg	Mocrocks mg/kg
Oct-12	2	1	1		1
Nov-12	2	2	1	1	1
Nov-12	3	2	2	1	1
Dec-12	2	3	1	1	0.5

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