STATE OF MAINE

DEPARTMENT OF ENVIRONMENTAL PROTECTION

BOARD OF ENVIRONMENTAL PROTECTION

	IN THE MATTER OF
NORDIC AQUAFARMS, INC.	:APPLICATIONS FOR AIR EMISSION,
Belfast and Northport	:SITE LOCATION OF DEVELOPMENT,
Waldo County, Maine	:NATURAL RESOURCES PROTECTION
	ACT, and MAIN POLLUTANT
	DISCHARGE ELIMINATION SYSTEM
	MEPDES)/WASTE DISCHARGE
A-1146-71-A-N	:LICENSE
L-28319-26-A-N	:
L-28319-TG-B-N	:
L-28319-4E-C-N	:
L-28319-L6-D-N	:
L-28319-TW-E-N	:
W-009200-6F-A-N	:
ME0002771	

TESTIMONY/EXHIBIT: NVC/UPSTREAM 4

TESTIMONY OF: **Dr. Kyle Aveni-Deforge**

Date: **December 13, 2019**

Introduction

I am a practicing environmental scientist and work primarily in the marine environment. I have a PhD in Ecology and Evolutionary Biology from the University of South Carolina (Columbia, SC; 2007). That work focused on rocky intertidal ecology and biomechanics including barnacle and mussel disturbance ecology and environmental stress. In my post-doctoral fellowship (University of Hawaii, Hawaii Institute of Marine Biology 2007-2011), I worked on projects that studied biological and ecological responses to environmental fluctuation. We looked at how hydrodynamic environment affect rates of nutrient uptake in submerged sea-grass beds, how algal canopies affect the redox potential of sediments and how short term fluctuations of physical environmental parameters affect biology. Current professional projects are in support of permit work for beach nourishment projects in the Hawaiian Islands. In this capacity, I conduct benthic habitat surveys and water quality analysis to establish baseline environmental conditions. Frequently this work is in support of National Environmental Policy Act (NEPA) requirements, such as Environmental Assessments (EA) or Environmental Impact Statements (EIS), or its parallel state-level program, the Hawaii Environmental Policy Act. I grew up in Belfast and return regularly to visit family.

As an environmental scientist and consultant, I am not an activist. I take a pragmatic approach to coastal development, where good science and best management practices are used to make rational decisions about project scope and project permitting. Fortunately the EA and EIS process within NEPA encourages open and clear communication about environmental consequences of proposed actions. One of my favorite components of this process is the development of alternatives to the desired action, which, when executed in good faith, helps to consider scale and scope of a project in the context of their environmental outcomes.

I typically work in a different Army Corps of Engineers District (Pacific) and different EPA District (9), with perhaps different emphasis on water quality. My expectation that the proposed action would enter the NEPA process through federal regulation of the MPDES/NPDES permitting process via the Coastal Zone Management Act, Essential Fish Habitat Provisions (of the Magnuson-Stevens Act; also see Table 1) or an Army Corp of Engineering dredge permit for pipe placement have not been met. Even so, the preparation of environmental assessments for concentrate aquatic animal production (CAAP) and aquaculture projects is not uncommon for projects like this (USEPA 2006; USEPA 2019).

Table 1: Federal laws applicable to NPDES permits, per EPA NPDES permit writer's manual (USEPA 2010)

Federal law	Year	Federal agency	Legislative citations	Implementing regulations
Endangered Species Act (ESA)	1973	FWS, NMFS	16 U.S.C. 1531 et seq.	50 CFR Part 402
National Environmental Policy Act (NEPA)	1969	CEQ	42 U.S.C. 4321 et seq.	40 CFR Part 6
National Historic Preservation Act (NHPA)	1992	ACHP	16 U.S.C. 470 et seq.	36 CFR Part 800
Coastal Zone Management Act (CZMA)	1972	NOAA	16 U.S.C. 1451 et seq.	15 CFR Part 930
Wild and Scenic Rivers Act	1968	Various	16 U.S.C. 1271 et seq.	36 CFR Part 297
Fish and Wildlife Coordination Act (FWCA)	1934	FWS	16 U.S.C. 661 et seq.	--
Essential Fish Habitat Provisions (EFH)	1996	NOAA	16 U.S.C. 1855(b)(2)	50 CFR Part 600

ABBREVIATIONS

NEPA   National environmental policy act
CEQ   Council on environmental quality
EIS   Environmental Impact Statement
EA   Environmental Assessment
EPA   Environmental Protection Agency
NAF Nordic Aqua Farms
NOAA National Oceanographic and Atmospheric Administration
FFAMP The finfish aquaculture monitoring program implements a tiered monitoring program for facilities that are already in production

TESTIMONY

The proposed project site, like many coastal areas has many intersecting interests, including recreation on the water and at the shoreline (recreational boating in Belfast and Bayside harbors, including sailboats, fishing boats and small craft, like stand-up paddle boards, ocean kayaks and row-boats and water-front parks in Belfast and Bayside), commercial interests (including lobster and shellfish harvest), conservation interests (including eelgrass and habitat areas of particular concern for coastal fisheries) and municipal (including water treatment facility discharges at Belfast and Bayside). Permitting the proposed facility depends on demonstrating that the waste discharged to the nearshore waters of Belfast and Northport is not disruptive to the current uses. To date the data collected to support the application do not appear to be sufficient to demonstrate that there will be no significant impact on the local environment and its stakeholders.

A central component of rationally evaluating potential environmental impacts at the proposed site is the release of nutrients into the water column and their dispersal and dilution thereafter. Three important factors exist for evaluating the discharge into the local environment: local physical oceanographic conditions, local background water quality and waste-water composition. Based on my understanding of the currently available data, these parameters have not been well enough described to make a confident risk assessment for water quality near the project site. Because the proposed project will operate continuously throughout the year and possibly for decades, collecting a thorough data set that describes the background environmental and ecological conditions is important.

The FFAMP proposes a tiered system of guidelines for monitoring aquaculture projects, and NAF has proposed monitoring programs for their facility once it is operational. My testimony asserts that existing knowledge of site water quality and physical oceanography is insufficient to have confidence in our understanding of baseline environmental conditions or how the process waste-water will interact with the environment. Consequently a rational, evidence based decision on the impacts of the proposed action cannot be made. Similarly, the future monitoring program proposed by NAF would not have enough baseline data of the pre-project environment at and near the project site to evaluate environmental impacts.

BACKGROUND WATER QUALITY:

The baseline conditions at the project site were characterized by SONDE casts and water collection on two trips, one in September and the other in October of 2018 (Normandeau 2018). These data are the right type of information to be collecting (temperature, salinity, dissolved oxygen, turbidity, as well as nutrient content). However, these data are not sufficient to describe the receiving water during the proposed action. As NAF will conduct operations throughout the year, the baseline conditions at the proposed outfall should describe a full year's range of variability in water quality parameters. There are many changes that occur in the coastal ocean across seasons, including the strength of stratification, depth of thermocline, available light, available nutrient concentrations and phytoplankton abundance. A suitable monitoring plan would include a minimum of seasonal/quarterly casts and nutrient analysis, accompanied a higher sampling frequency during the period of the year when proposed discharge would likely have its greatest environmental impact[1]. Considering that one

---

[1] The argument for this specific period of time is likely to balance ocean temperature, photosynthetically active radiation and other environmental sources of pollution that could exacerbate the likely impacts of the proposed outfall; site knowledge would be important in making this decision.

possible outcome of nutrient accumulation in the nearshore waters is algal/dinoflagellate bloom, it may be advantageous to begin documenting phytoplankton as a part of baseline monitoring.

PHYSICAL OCEANOGRAPHIC AND METEOROLOGICAL CONDITIONS

The local water currents and residence time in this section of the bay also of high importance to how the proposed discharge is distributed in the water column and advected from the project site. The dispersal models prepared by Ransom were prepared with the best available data and using generally appropriate models. However, without local data for tidal currents, wind forcing and wave structure it is hard to have confidence that these results are representative of the dynamics of real discharge from the proposed site. In fact, there is very little contemporary quantitative data available for local currents and meteorological conditions at or near the project site. A drogue study from the 1990's (Bergund 1995) references work for Normandeau in the mid 1970's (Normandeau 1975; Normandeau 1978). These data sets seem to indicate that there is residual clockwise flow around Islesboro Island, based on the use of Lagrangian drifters and numerical modelling. This is old data, but contradicts the expected plume path, as modeled by NAF.

The numerous islands of Penobscot Bay shield Belfast Harbor and the Little River estuary site from open ocean conditions. The closest buoy that measures oceanographic parameters such as wave period, wave direction,
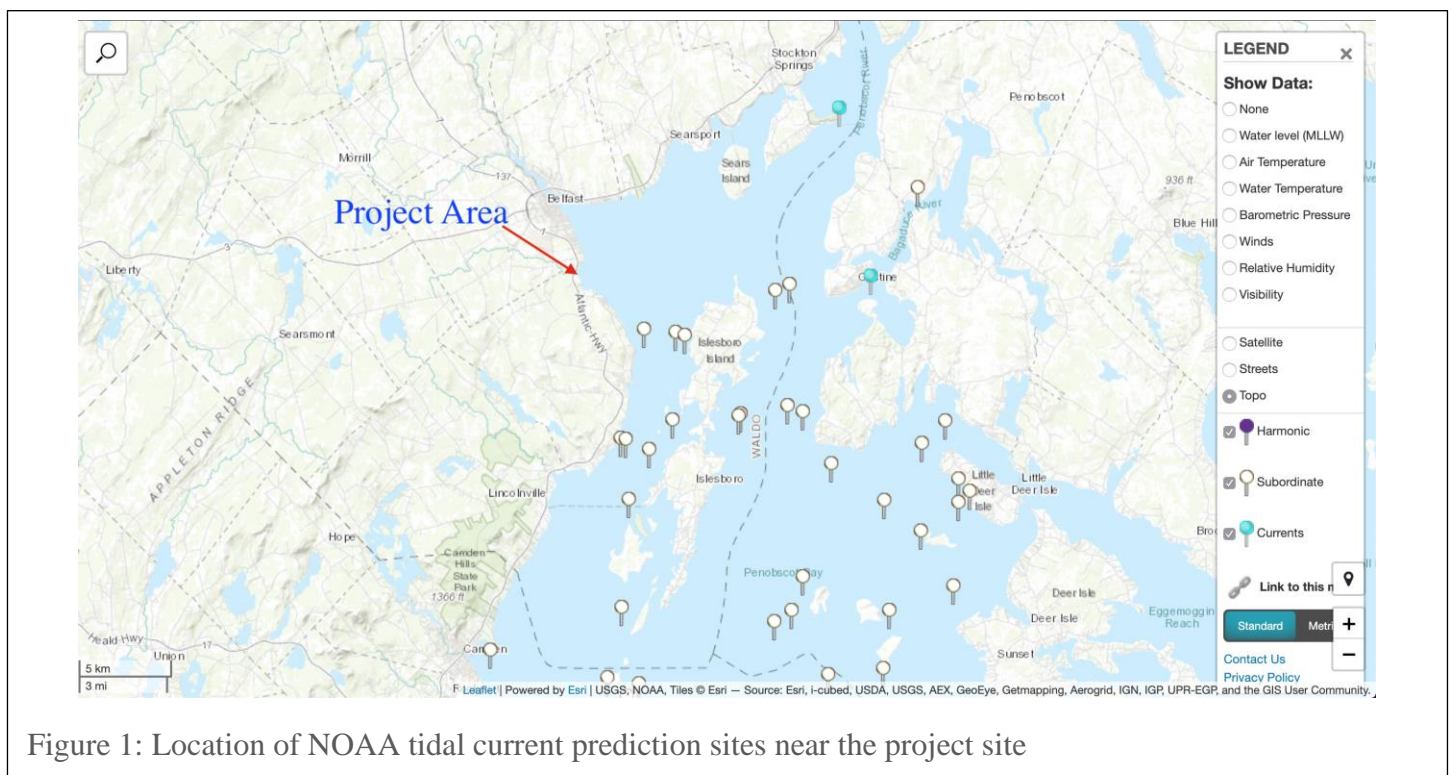

Figure 1: Location of NOAA tidal current prediction sites near the project site

wave, height, wind speed and wind direction is to the south, between Owl's Head and Vanalhaven. With the exception of a pure-south swell, this station is unlikely to provide data that are well correlated physical oceanographic conditions near the project site. Similarly, tidal current data are forecast for a number of stations around Islesboro (Figure 1) and provide bulk flow estimates that are based on tidal change, not measured flow rates. None of them are particularly close to the project site, nor are they anchored by nearby current measurement, or an active tidal monitoring station (the nearest tidal station is NOAA station 8413320 in Bar Harbor).

Given the lack of site-specific information on currents and wave structure, I would expect that seasonal/quarterly current profiles, using an acoustic-doppler current profiler, would be conducted at or near the proposed discharge site. As with background water quality sampling, frequency of measurement would be increased during the period of time when the possible environmental impacts of the outfall would be most likely to occur. These data should be used to parameterize appropriate models to evaluate whether 1.) the plume will reach the sea surface, and if so, under what conditions 2.) the plume will be quickly diluted and 3.) the plume will be carried away from the project site and not be retained near the project site.

PROPOSED NUTRIENT LOAD IN WASTE-WATER

The applicant's discharge calculations are projected as average daily load and average concentration. The timing and fluctuation of the discharge is a potential factor in how the outfall is temporally and spatially distributed in the water column. Average nitrogen in the discharge water is proposed to be more than 45 times the measured background levels. Mean phosphorous discharge is 8 to 16 times the background level. Mean TSS is close to or below background, and mean BOD is about 2 times background levels (Table 2). Given these levels, it is important that the modeling of the plume dispersal is accurate.

Table 2: Proposed discharge and background water quality levels of key pollutants near the discharge site

	Proposed Discharge		Background Levels	
	Daily Total [kg]	Mean Concentration [mg/l]	Min [mg/l]	Max [mg/l]
Total N	673	23	0.17	0.48
P	5.8	0.2	0.012	0.024
BOD	162	2		5.55
TSS	185	6.33	6.9	11

If conditions exist where the plume can reach the surface, the surface is poorly mixed, the plume is cohesive, or local currents to not draw it away as expected by the bulk flow parameters that match the tide unintended and unanticipated environmental consequences may result. The worst case scenario would be a poorly diluted plume that reaches the surface that is then forced onshore by surface winds or tidal currents. No evidence suggests that this is a likely outcome of the proposed outfall.

Another important aspect of nutrient release from the facility is whether it is constant, or pulsatile. While the time-averaged release of nutrients gives a daily mean concentration of nutrient discharge, if the nature of the discharge is not uniform, but sporadic, reflecting process-based activities, such as filter flushing, or periodic maintenance, the instantaneous release of a nutrient may be much higher. A risk analysis of the facility should also include maximum possible discharge levels, the conditions that might trigger un-treated discharge, and the maximum duration of such an accident.

Modeling of the NAF discharge that incorporates actual on-site hydrodynamics with process-relevant discharge scenarios will provide the level of consideration that should allow managers to make reasonable decisions about project related effects on water quality in the receiving water.

RISKS TO LOCAL ENVIRONMENT

The applicant is relying on the present dispersal model to forecast good dilution of the proposed discharge and evaluate environmental risk. Because the dispersal model is not strongly driven by on-site measurements, the

applicant may be underestimating the risk of discharge to the local environment. In fact the only data I have been able to find for the peri-Islesboro currents indicates that net flow, in the 1970's through 1990s, had a residual clockwise flow. The risks associated with underestimating the dilution and dispersal of the outfall could have consequences to a variety of ecosystem functions and services, affecting the stability of local ecosystems as well as how humans can take advantage of the environment.

Nutrient enrichment of a body of water that leads to excessive algal or bacterial growth is called eutrophication Typically, fixed nitrogen is considered to be the limiting factor for primary production in the coastal ocean (e.g. Howarth and Marino, 2006), and increases in nitrogen availability in the water column can promote growth of numerous varieties of phytoplankton and algae. Such growth can lead to general habitat degradation (via reduced water clarity, increased biological oxygen demand leading, in some cases to hypoxia) and harmful algal blooms (e.g. Huisman et al 2005), including red tides and brown tides (see Anderson et al 2008), although tight coupling between red and brown tides and coastal eutrophication is not proven. HABs often result in closure of recreational and commercial fisheries but can also cause closure of beaches and nearshore waters to recreational use. Shellfisheries are documented throughout the soft sediment of the Maine coastline, and are present near the project site (**Figures 2 and 3**).

Eutrophication can also promote the growth of ephemeral filamentous green algae, such as *Ulva spp.,* as well as epiphytes that occupy the leaves of submerged aquatic vegetation, such as eelgrass. Floating mats of green algae, dubbed green tides, have become increasingly common throughout eutrophic coastal waters (e.g. Ye et al. 2011). Blooms of phytoplankton have the short-term effects of reducing water clarity and can rob benthic algae and submerged aquatic vegetation (SAV; such as eelgrass) of light. Coupled with accelerating growth of epiphytes and epibionts, coastal eutrophication can have negative effects on SAV, even leading to loss of beds. There are existing SAV beds of eelgrass near the project site (**Figure 4**).

The proposed action will increase the amount of nutrients in the nearshore. The dilution and removal of those nutrients from the project area is important, yet without local hydrodynamic and meteorological data, accurate predictions of possible plume paths cannot be made.
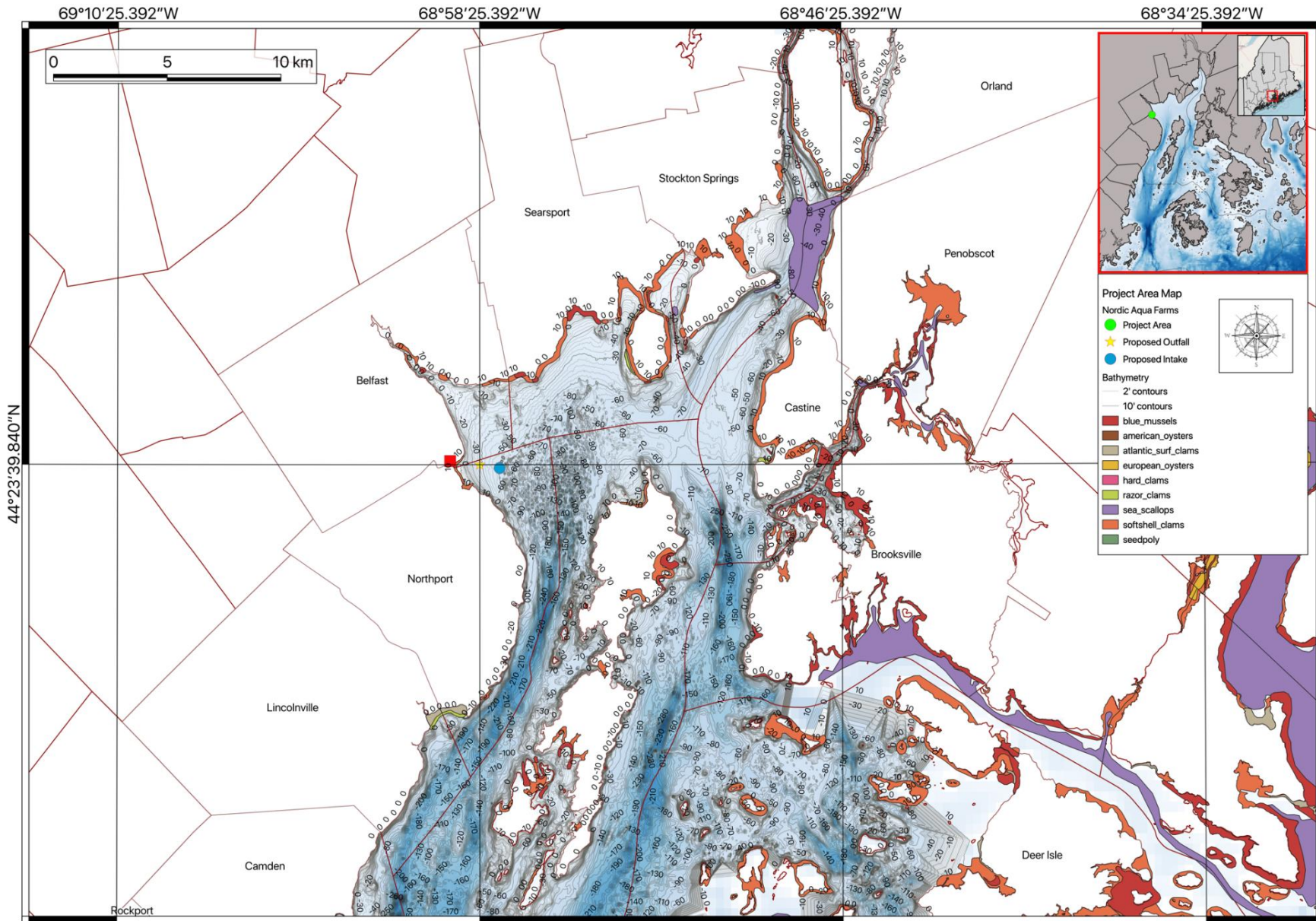
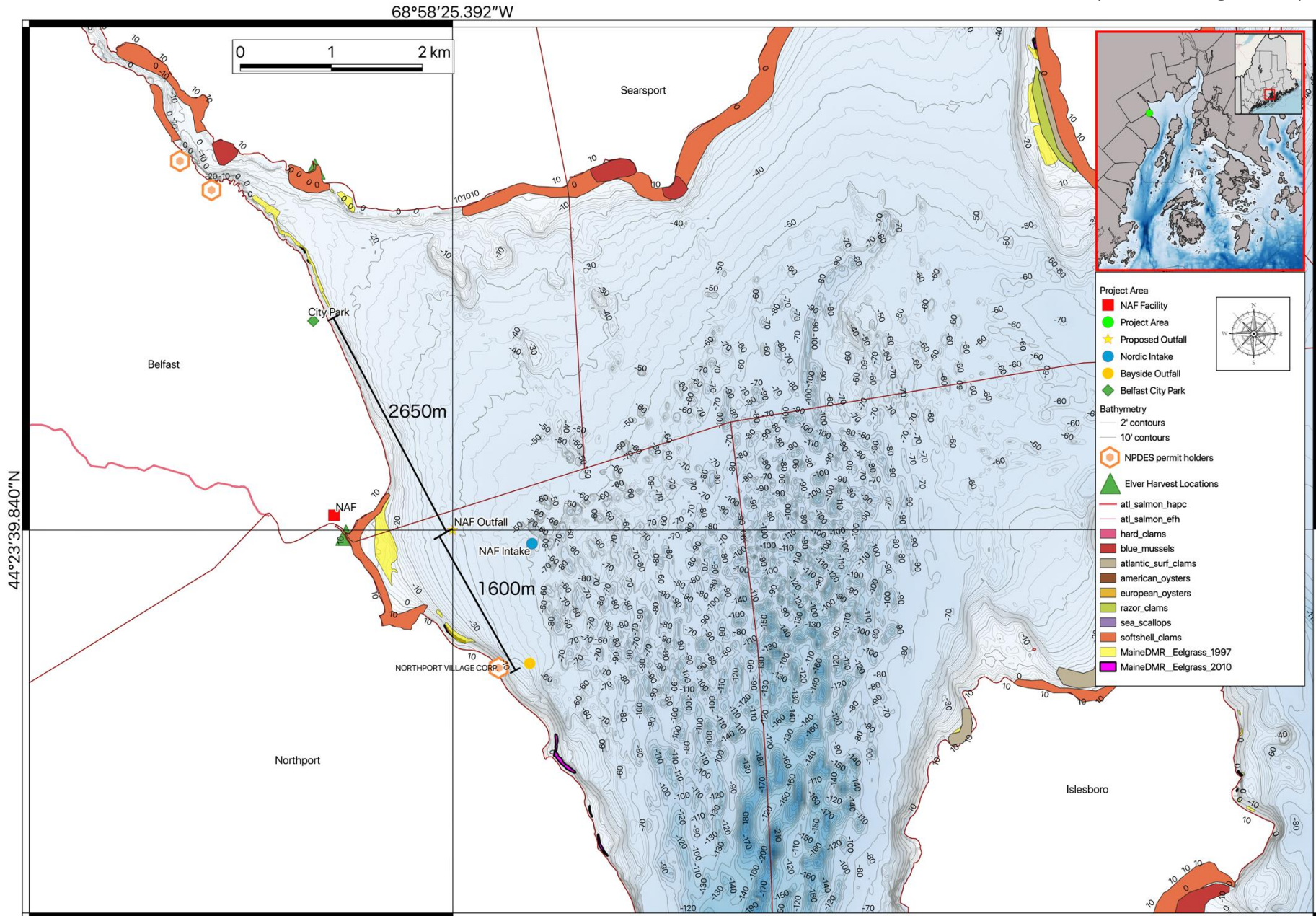**Figure 2:** Distribution of shellfisheries within Penobscot Bay

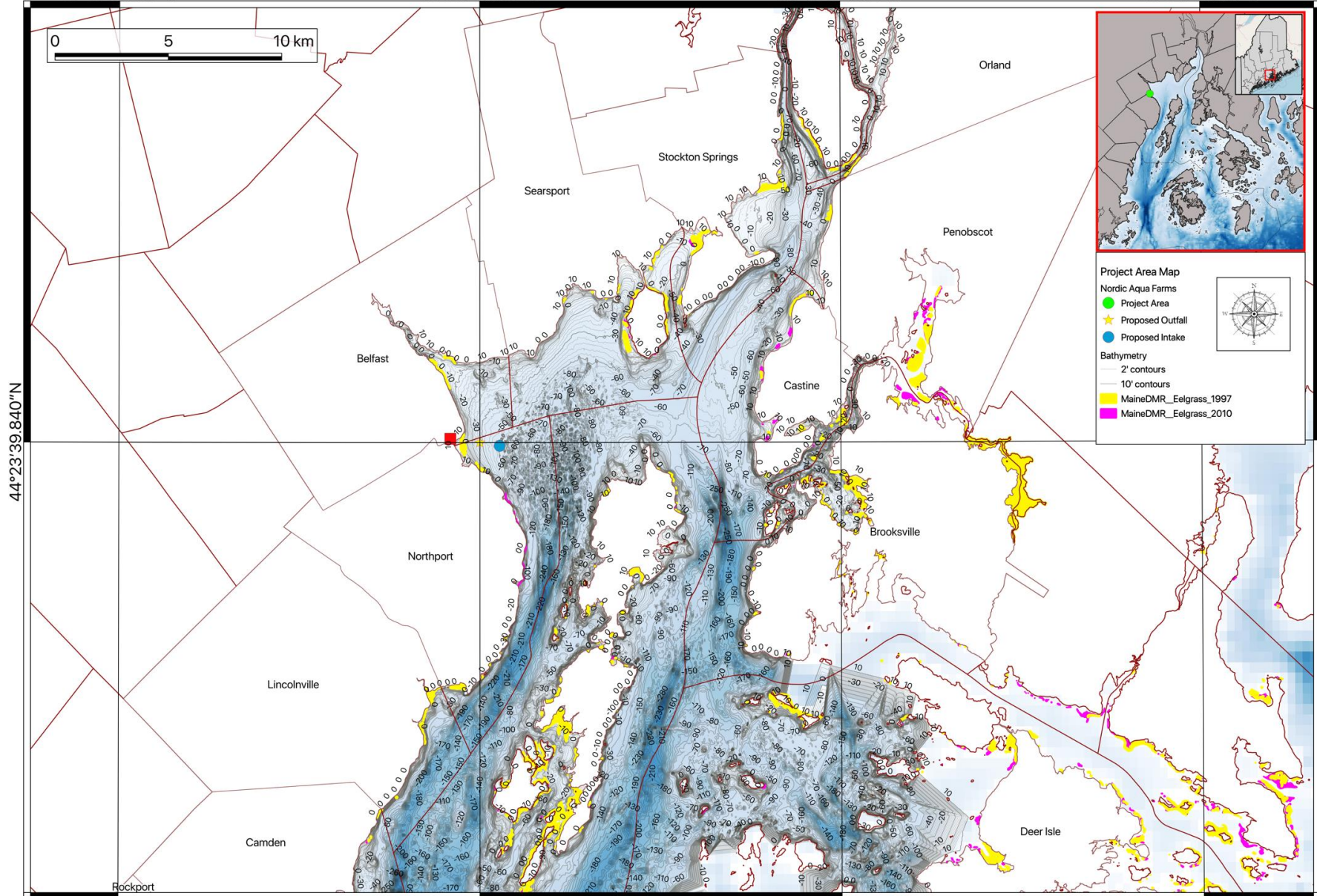**Figure 3:** Distribution of shellfisheries in Penobscot Bay near the project site

Figure 4: Location of eelgrass beds in Penobscot Bay

REFERENCES

Anderson, DM, Burkholder, JM, Cochlan WP, Glibert, PM, Gobler, CJ, Heil, CA, Kudela, R, Parson, ML, Rensel, JEJ, Townsend, DW, Trainer VL, and Vargro GA. 2008. Harmful Algal Blooms And Eutrophication: Examining Linkages From Selected Coastal Regions Of The United States. Harmful Algae. Dec 1; 8(1): 39–53. doi: 10.1016/j.hal.2008.08.017

Bergund, H. (1995) The currents of Penobscot Bay, Maine: Observations and a Numerical Model. Undergraduate Thesis. Department of Geology and Geophysics, Yale University.

Howarth, R. W. and Marino, R. (2006) Nitrogen As The Limiting Nutrient For Eutrophication In Coastal Marine Ecosystems: Evolving Views Over Three Decades, Limnology and Oceanography, 51, 364–376.

Huisman, J., Matthijs, H., Visser, P., Ibelings, B., Mur, L., and Walsby, A.: The Ecophysiology Of The Harmful Cyanobacterium Microcystis, in: Harmful Cyanobacteria, 109–142, 2005.

Normandeau (2018); Normandeau Associates, Inc. Maine Aquaculture Water Quality Summary Belfast Bay Belfast, Maine. Accessed December 5, 2019: Included in Nordic Aquafarms General Application for Waste Discharge License (WDL) / Maine Pollutant Discharge Elimination System (MEPDES) Permit: https://www.cityofbelfast.org/DocumentCenter/View/2291/20181019-mepdes-permit-application-final?bidId=

Normandeau (1975). Normandeau Associates Inc. Environmental Survey Of Upper Penobscot Bay, Maine. Second Quarterly Report for Central Maine Power Company, 385 pp.

Normandeau (1978). Normandeau Associates Inc. An Oil Pollution Prevention Abatement And Management Study for Penobscot Bay, Maine, Vol. II.

USEPA (2010). NPDES Permit Writer's Manual. Chapter 11: NPDES Permit Administration. Accessed December 2019: https://www3.epa.gov/npdes/pubs/pwm_chapt_11.pdf

USEPA (2019). United States Environmental Protection Agency. National Pollutant Discharge Elimination System (Npdes) Permit And Rivers And Harbor Act Section 10 Permit for Kampachi Farms – Velella Epsilon (VE) Offshore Aquaculture Project. DRAFT Environmental Assessment (EA). Accessed December 6, 2019: https://www.epa.gov/sites/production/files/2019-08/documents/velella_environmental_assessment_draft.pdf

USEPA (2006). Compliance Guide for the Concentrated Aquatic Animal Production Point Source Category. Accessed December 5, 2019 https://www.epa.gov/sites/production/files/2015-11/documents/caap-aquaculture_compliance-guide_2006.pdf

Ye NH, Zhang X, Mao Y, Liang C, Xu D, Zou J, Zhuang Z, Wang Q (2011) "Green Tides" Are Overwhelming The Coastline Of Our Blue Planet: Taking The World's Largest Example. Ecol Res 26:477–485

Kyle Aveni-Deforge, PhD
e-mail: kyle@ecologicalmonitoringandanalysis.com
kyle.aveni.deforge@gmail.com

Environmental Scientist

I am a broadly trained scientist, with research and educational experience that links physical, biological and chemical components of an environment to its ecology. I have more than 15 years of experience working at all stages of project development and execution; more than nine years of that experience includes work in the Hawaiian and other Pacific Islands. My recent work has focused on the analysis of coastal erosion, monitoring of water quality characteristics, analysis of marine ecological communities, sampling and analysis of benthic sediments and documentation of environmental compliance for HEPA and NEPA.

Extremely Relevant Professional Experience

Chief Scientist *Ecological Monitoring and Analysis LLC*   2015-*present*

Sand Field Analysis: Managed sand surveys for Royal Kahana Condominiums, Maui County Kahana Bay Regional Sediment Survey, Kahana Bay Environmental Impact Statement and Napili Bay beach restoration projects. Used sub-bottom profiling, jet-probe and coring to quantify available sand and test suitability of grain size distribution, meeting state and federal guidelines for beach restoration. Collaborated with Rising Tide Engineering, Moffatt & Nichol, and Golder.

Benthic Habitat Analysis: Designed benthic habitat evaluation for Royal Kahana Condominiums and Napili Bay restoration projects, participated in development and analysis of Stable Road Beach restoration benthic habitat analysis. Used photographic survey techniques to quantify composition of sensitive benthic communities in support of federal and state permits for beach restoration work.

Water Quality Analysis: Planned and initiated water quality monitoring program to document existing water quality conditions for Royal Kahana Condominiums.

Videographic Coastal Erosion Monitoring and Modeling: Designed and implemented long term photographic/videographic monitoring of nearshore and beach sand volume to build a model that links erosion with observed and forecast physical oceanographic conditions, including long-term sea-level changes, short term sea-level changes (meso-scale eddies), tides and sea-state (wave height, period and direction).

Lead Environmental Scientist *Stable Rd. Beach Restoration Foundation*   2009 – 2015

Lead monitoring and analysis of water quality and benthic habitat in support of permitting and environmental compliance at all stages of the project. This included developing environmental standards for the nearshore project site, offshore sand borrow-site and off-site water quality controls. Worked with other consultants and the Foundation to conduct sand field analysis, dredge monitoring, dredge pipeline routing and stabilization. Coordinated with contractors, oversight agencies (county, state, and federal) and stake holders.

Environmental Scientist   *Oceanit Labs, Honolulu, HI*   2012 - 2014

Worked with Guam/CNMI government to provide expert commentary on technical analyses of biological, ecological, environmental and hydrological consequences of a proposed CVN expansion of Aprah Harbor. Conducted analysis of competing coral growth models for mitigation related planning. Conducted HAPC and EFH analysis for harbor dredge projects. Other work included document review, summary and reporting, proposal writing and habitat equivalency analysis.

Post-Doctoral Fellow   *Hawaii Institute of Marine Biology, Kaneohe, HI*   2007-2010

NSF funded study to evaluate nutrient uptake dynamics in submerged aquatic vegetation in Florida and Hawaii. Used acoustic Doppler velocimetry (ADV/ADCP) to measure hydrodynamic parameters. Studied shallow water light field dynamics on Hawaiian benthic communities. Other activities may have included time series analysis, marine algae ecology, fish pond dynamics, report writing, professional meetings/hobnobbing, peer review, grant writing, public outreach

Education
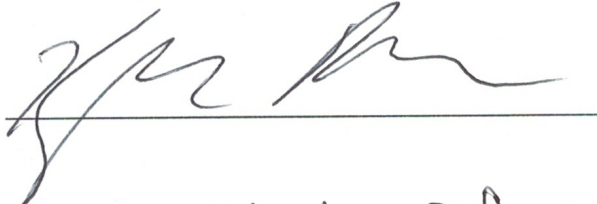
Ph.D. Ecology and Evolutionary Biology University of South Carolina, Columbia, SC   2007

A Biomechanical Investigation of the Advantages to Aggregation in Rocky Intertidal Mussel Beds

Advisor: David S. Wethey

BA Biology Swarthmore College, Swarthmore, PA   1999

I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations.

Date: 12-14

Printed Name: Kyle Aveni-Deforge

Title:

State of North Carolina
County of Durham

Sworn and subscribed before me on this the 14 day
of December , 20 1

Notary Public

Parties Assisting:

Name: Address: Signature:_____

Name: Address: Signature:_____

STATE OF North Carolina

COUNTY OF _Durham_

PERSONALLY APPEARED, _Kyle Avenr-Deforge_, WHO, UNDERSTANDING THE MEANING OF AN OATH, SWORE THAT THE FORGOING TESTIMONY IS TRUE TO THE BEST OF HIS/HER KNOWLEDGE AND BELIEF, THIS 14th DAY OF DECEMBER 2019.

NOTARY PUBLIC _____

MY COMMISSION EXPIRES: 10/15/2024

ANTHONY HOUSE
NOTARY PUBLIC
DURHAM COUNTY, N.C.

Maine Aquaculture
Water Quality Summary
Belfast Bay
Belfast, Maine

Submitted By

Normandeau Associates, Inc.
25 Nashua Road
Bedford, NH 03110-5500
603.472.5191
www.normandeau.com

October 16, 2018

October 16, 2018

Project No. 23631.001

Elizabeth Ransom
Ransom Environmental
Pease International Tradeport
112 Corporate Drive
Portsmouth, NH 03801

Electronically sent via email to elizabeth.ransom@ransomenv.com and drew.fuchs@ransomenv.com

Re: Water Quality Summary
        Belfast Bay
        Belfast, Maine

Dear Ms. Ransom:

Normandeau Associates, Inc. (Normandeau) is pleased to present the results of water quality sampling conducted in Belfast Bay at the proposed Nordic Aquaculture facility intake and discharge locations. Site visits were completed on August 23, 2018 and August 24, 2018 and again on September 7, 2018 consistent with our proposed scopes of work. Water quality data were collected by discrete depth samples submitted for laboratory analysis of multiple parameters as well as in-situ measurements with a YSI water quality data sonde. Samples and measurements were collected from the two proposed intake stations on August 23-24 and from the two proposed discharge stations on September 7. An additional water sample was also collected on September 7 and submitted for laboratory analysis from a location on the Little River below the lower reservoir dam. This report presents our methods for data collection, sampling locations, and results summaries. Original laboratory reports can provided upon request.

Thank you for the opportunity to work with you on this important project, please let me know if you have any questions or wish to discuss this further.

Sincerely,

Normandeau Associates, Inc.

Joel M Detty

Project Manager

Introduction and Methods

Water quality data were collected in Belfast Bay at the proposed Nordic Aquaculture facility intake and discharge locations on August 23-24, 2018 and September 7, 2018. Samples and in-situ measurements were collected from two intake stations and two discharge stations along the proposed submerged intake/discharge pipe route Options 1 and 2A. Samples and in-situ measurements were collected at low tide and high tide at each station and consisted of water column profile measurements using a YSI 6920 water quality data sonde and water sample collection for laboratory analysis. Water samples were collected at discrete depths using a Kemmerer water sampler. Intake location sample were collected August 23-24, 2018 and discharge location samples were collected on September 7, 2018. A single water sample was also collected from the Little River below the lower reservoir dam at low tide on September 7, 2018.

A YSI 6920 multiparameter data sonde was used to record water quality profile readings and was calibrated before and after each sampling event as per manufacturer recommendations. A Kemmerer water sampler was used for collection of water samples and was cleaned with distilled water between each sample as per standard protocol for water quality sampling. As the Kemmerer sampler was unable to collect sufficient sample volume to fill all sample bottles with a single "grab", multiple samples had to be collected from each depth and composited in a clean plastic compositing container. Once the container was full, it was distributed into the individual sample containers which were then preserved and stored as per laboratory instructions. The compositing container was reused for all samples and was cleaned using the same protocol as the Kemmerer water sampler. Nitrile gloves were used during sample collection and were changed after each sample. Laboratory samples were transferred to Alpha Analytical Laboratory in Portsmouth, NH at the end of each sample day within the recommended hold times for all analytes.

Garmin and Trimble GPS units were used to navigate to each station and to mark the location where data collection occurred.

Intake Stations

Two intake stations, Station 1 (intake/discharge pipe Option 1) and Station 2 (intake/discharge pipe Option 2A) were located at the terminus of the proposed pipe routes (See Figure 1). Water quality data was collected from both stations on August 23, 2018 during low tide and on August 24, 2018 at high tide. Before water quality data could be collected, a depth of at least 55 ft. was required at each station. During high tide, a depth of 56 feet was reached at both stations; however, during the low tide samples the observed depth at both stations was approximately 50 ft. Once anchored on station for sampling, a GPS point was recorded to mark the sampling location.

Water Quality Profile Readings

Water quality profile readings were recorded during low tide at Station 2 on August 23, 2018 at 14:36 and at Station 1 at 15:31. The predicted low tide in Belfast Bay on August 23, 2018 was at 15:48. High tide water quality profiles were recorded at Station 2 on August 24, 2018 at 10:00 and at 11:35 at Station 1. The predicted high tide in Belfast Bay on August 24, 2018 was at 10:35. A duplicate reading was also taken at Station 2 at 10:21 as quality control field duplicate. The duplicate reading consisted

of restarting the YSI after the initial profile reading then repeating the standard water quality profile procedure to perform a duplicate measurement. Profile readings were recorded beginning at 0.5 meters below the surface of the water and then repeated every meter down through the profile where the following parameters were recorded: Temperature, Turbidity, pH, Depth, Dissolved oxygen (mg/L and % saturation), Salinity (not recorded on August 23), and Specific Conductance. At both Station 1 and 2 during low tide, YSI readings were recorded to a depth of 15 meters and during high tide to a depth of 17 meters. YSI profile readings for both intake stations are presented in Tables 1 and 2.

Sample Collection

After water quality profiles were complete, water samples were collected for laboratory analysis. Using a Kemmerer water sampler, a total of four samples were collected throughout the water column at each station.   In addition, one field duplicate sample was collected as a quality control. At both stations, the upper samples were collected at a depth of 0.5 meters and the bottom samples were collected approximately 10 feet (3 meters) above the bed surface with two samples collected at equal intervals in between the upper and lower samples. During low tide on August 23, 2018 (predicted low tide at 15:48), samples were collected at 14:59 from Station 2 and at 16:00 from Station 1 at the following depths: 0.5 meters, 4.0 meters, 8.0 meters, and 12 meters. During high tide on August 24, 2018 (predicted high tide at 10:35), samples were collected at 10:59 from Station 2 and at 12:00 from Station 1 at the following depths: 0.5 meters, 4.0 meters, 8.0 meters, and 12 meters. A duplicate sample was collected at Station 2 at 5.0 meters. Samples were analyzed for total suspended solids, nitrogen-ammonia, nitrogen-nitrate/nitrite, total nitrogen, nitrogen-TKN, total phosphorus, chemical oxygen demand and BOD 5-day. Sample collection data and results for both intake stations are presented in Tables 4 and 5.
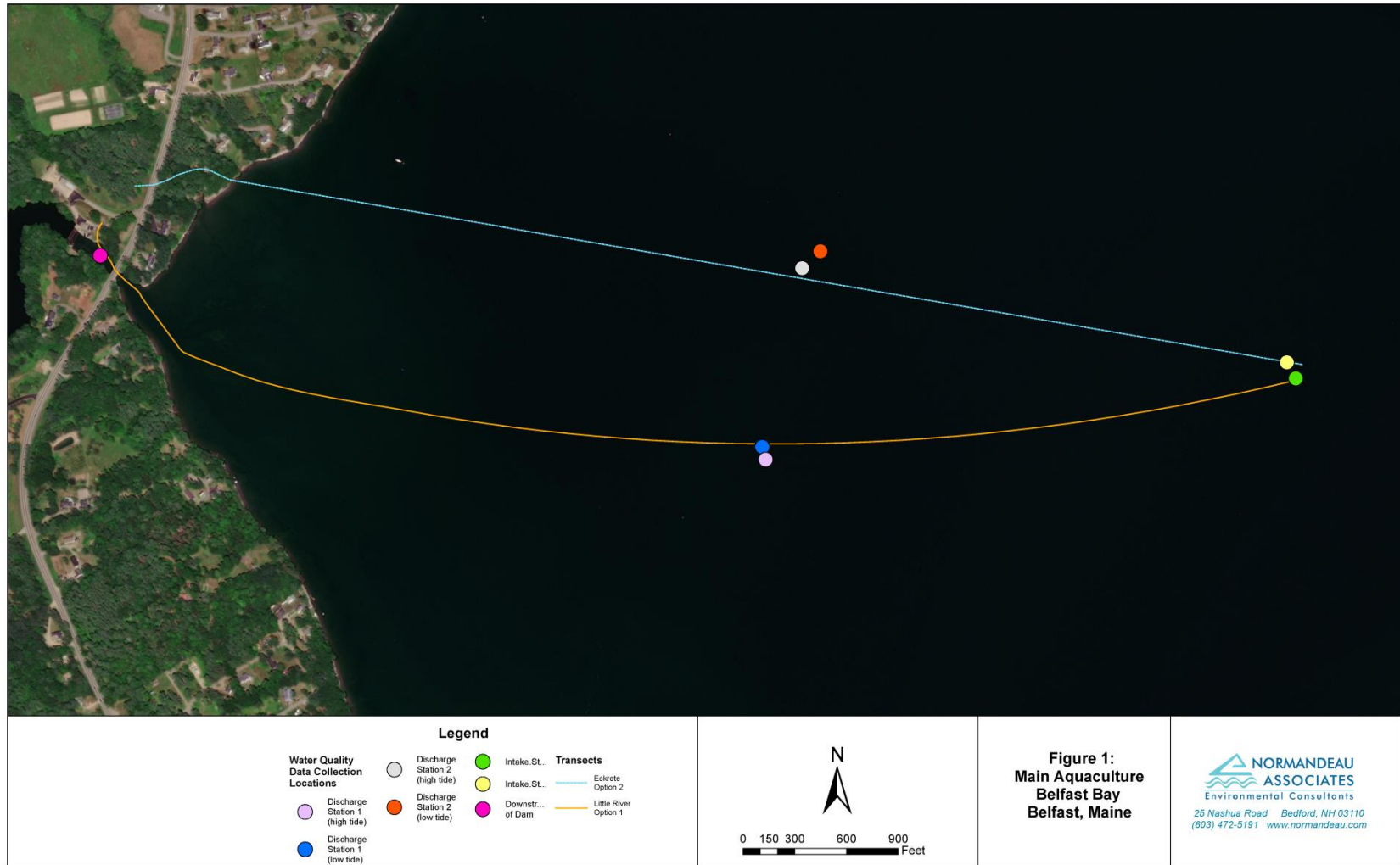
Figure 1. Sampling stations map

Discharge Stations

Two discharge stations, Station 1 (intake/discharge pipe Option 1) and Station 2 (intake/discharge pipe Option 2A) were located along the proposed pipe routes (See Figure 1) closer to shore and in shallower water than the intake stations. Water quality data were collected from both discharge stations on September 7, 2018 during low and high tide conditions. Before water quality data could be collected at high tide, a depth of at least 40 feet was required at each station and 30 feet at each station during low tide. During high tide, a depth of 42 feet was measured at Station 1 and 44 feet at Station 2, while at low tide a depth of 32 feet was measured at Station 1 and 35 feet at Station 2. Once anchored on station, a GPS point was recorded to mark the sampling location. For the discharge stations, 4 GPS points were recorded to mark the sampling locations – i.e. 1 point for each station at high tide and low tide (See Figure 1).

Water Quality Profile Readings

Water quality profile readings were recorded during high tide on September 7, 2018 at Station 1 at 9:00 and at Station 2 at 11:16. The predicted high tide in Belfast Bay on September 7, 2018 was at 9:12. Before water quality profiles were recorded at Station 1, the dissolved oxygen probe was replaced due to an equipment failure and the YSI meter was recalibrated. During low tide on September 7, 2018, YSI profile readings were recorded at Station 1 at 14:39 and at 15:42 at Station 2. The predicted low tide in Belfast Bay on August 23, 2018 was at 15:14. A field duplicate reading was also taken at Station 1 at 14:52. As mentioned previously, this was done by restarting the YSI sonde after the initial profile reading and completing a second duplicate reading following the same standard procedure. Profile readings were recorded beginning at 0.5 meters below the surface of the water and then repeated every meter down through the water column with the following parameters measured and recorded: Temperature, Turbidity, pH, Depth, Dissolved oxygen (mg/L and % saturation), Salinity, Specific Conductance. At Station 1 during high tide, YSI readings were recorded to a bottom depth of 13 meters and during low tide to a bottom depth of 9 meters while at Station 2, readings were recorded to a bottom depth of 14 meters during high tide and 11 meters during low tide. Water quality profile readings for both intake stations are presented in Table 3.

Sample Collection

After water quality profiles were complete, water samples were collected for laboratory analysis. Using a Kemmerer water sampler, a total of four samples were collected throughout the water column at each station.   In addition, one field duplicate sample was collected as a quality control. At both stations, the upper samples were collected at a depth of 0.5 meters and the bottom samples were collected approximately 10 feet (3 meters) from the bed surface with two samples collected at equal intervals in between the upper and lower samples. During high tide on September 7, 2018 (predicted high tide at 9:12), samples were collected at 9:42 from Station 1 and at 11:35 from Station 2 at the following depths: 0.5 meters, 4.0 meters, 7.0 meters, and 10.0 meters. During low tide on September 7, 2018 (predicted low tide at 15:14), samples were collected at 15:08 from Station 1 and at 16:11 from Station 2 at the following depths: 0.5 meters, 3.0 meters, 5.0 meters, and 7.0 meters. A duplicate

sample was collected at Station 1 at 5.0 meters. Samples were analyzed for total suspended solids, nitrogen-ammonia, nitrogen-nitrate/nitrite, total nitrogen, nitrogen-TKN, total phosphorus, chemical oxygen demand and BOD 5-day. Sample collection data and results for both discharge stations are presented in Table 6.

Little River Sample

One sample was collected from the Little River immediately below the lower reservoir dam located off Route 1 during ebb conditions at 13:26 on September 7, 2018 (predicted low tide was at 15:14). The sample was collected approximately 50 feet downstream from the dam in a small channel of running water flowing towards Belfast Bay. As it was an ebbing tide, there did not appear to be any inflow from the bay. The sample was analyzed for nitrogen-nitrate/nitrite, total nitrogen, nitrogen-TKN, and total phosphorus. These results are presented in Table 6.

Table 1. Summary of Water Quality Readings Taken at Intake Locations on August 23, 2018 in Belfast Bay, Belfast, Maine

STATION 1	Temperature (°C)	Specific Conductivity (µmhos/cm)	pH (units)	DO (mg/L)	DO (%)	Turbidity (ntu)
15:31, Low Tide, depth in meters						
0.5	18.96	44,586	8.18	9.02	113.7	0.00
1.0	18.90	44,645	8.17	9.11	116.4	0.00
2.0	18.75	44,696	8.17	9.29	116.8	0.00
3.0	18.41	44,893	8.18	9.61	121.9	0.00
4.0	15.40	47,702	8.07	9.01	108.1	0.00
5.0	15.06	47,765	8.04	8.76	108.9	0.00
6.0	14.65	48,151	8.02	8.76	104.2	0.00
7.0	14.06	48,588	7.98	8.68	97.3	0.00
8.0	12.57	49,040	7.82	7.56	86.5	0.00
9.0	11.69	49,349	7.71	6.02	70.5	0.00
10.0	11.29	49,483	7.71	6.00	67.1	0.00
11.0	11.25	49,517	7.70	6.09	68.1	0.00
12.0	11.29	49,557	7.75	6.01	67.9	0.00
13.0	11.31	49,572	7.77	6.33	71.0	0.00
14.0	11.33	49,583	7.77	6.32	70.8	0.00
15.0	11.35	49,596	7.79	6.17	71.1	0.00

STATION 2	Temperature (°C)	Specific Conductivity (µmhos/cm)	pH (units)	DO (mg/L)	DO (%)	Turbidity (ntu)
14:36, Low Tide, depth in meters						
0.5	18.84	44,544	8.14	9.19	113.5	0.00
1.0	18.84	44,537	8.13	9.23	118.3	0.00
2.0	18.79	44,607	8.13	9.12	120.3	0.00
3.0	18.13	44,800	8.14	8.83	111.6	0.00
4.0	17.90	45,018	8.15	9.77	123.1	0.00
5.0	15.19	47,511	8.01	9.35	109.5	0.00
6.0	14.99	47,834	8.02	8.26	107.9	0.00
7.0	13.75	48,333	7.90	7.58	98.6	0.00
8.0	12.63	48,902	7.8	7.25	84.1	0.00
9.0	11.47	49,362	7.68	6.23	66.4	0.00
10.0	11.50	49,379	7.69	6.16	69.6	0.00
11.0	11.25	49,470	7.72	6.44	72.8	0.00
12.0	11.25	49,500	7.73	6.18	69.0	0.00
13.0	11.29	49,532	7.75	6.22	68.8	0.00
14.0	11.30	49,550	7.77	6.15	69.0	0.00
15.0	11.31	49,555	7.76	6.43	71.6	0.00

Table 2. Summary of Water Quality Readings Taken at Intake Locations on August 24, 2018 in Belfast Bay, Belfast, Maine

STATION 1	Temperature (°C)	Specific Conductivity (µmhos/cm)	pH (units)	DO (mg/L)	DO (%)	Turbidity (ntu)
11:35, High Tide, depth in meters						
0.5	18.73	44,301	8.11	9.42	119.6	0.00
1.0	19.26	44,192	8.10	9.49	119.5	0.00
2.0	18.01	44,511	8.09	9.49	121.3	0.00
3.0	17.70	44,712	8.08	9.23	115.9	0.00
4.0	16.71	45,597	8.00	8.96	107.8	0.00
5.0	15.18	46,999	7.96	8.81	105.6	0.00
6.0	14.02	47,412	7.86	7.89	93.5	0.00
7.0	13.31	48,235	7.86	7.87	91.4	0.00
8.0	13.00	48,385	7.84	7.26	79.8	0.00
9.0	12.87	48,450	7.83	7.25	83.6	0.00
10.0	12.25	48,532	7.77	7.00	79.8	0.00
11.0	11.84	48,649	7.73	6.63	74.6	0.00
12.0	11.48	48,819	7.75	6.51	72.9	0.00
13.0	11.46	48,815	7.76	6.37	71.3	0.00
14.0	11.41	48,835	7.76	6.15	68.8	0.00

15.0	11.41	48,835	7.76	6.34	70.9	0.00
16.0	11.42	48,847	7.77	6.32	69.9	0.00
17.0	11.42	48,848	7.77	6.38	71.4	0.00
STATION 2	**Temperature** (°C)	**Specific Conductivity** (µmhos/cm)	**pH** (units)	**DO** (mg/L)	**DO** (%)	**Turbidity** (ntu)
10:00, High Tide, depth in meters						
0.5	18.26	44,366	8.10	7.64	96.3	0.00
1.0	18.17	44,401	8.13	7.56	95.1	0.00
2.0	18.00	44,440	8.15	7.77	97.7	0.00
3.0	17.85	44,538	8.15	7.86	99.0	0.00
4.0	17.49	45,028	8.13	7.66	95.3	0.00
5.0	15.10	46,832	7.96	7.28	87.1	0.00
6.0	14.76	47,255	7.98	6.72	80.1	0.00
7.0	13.51	47,659	7.88	6.06	70.6	0.00
8.0	12.92	48,748	7.87	6.10	72.0	0.00
9.0	11.91	48,639	7.78	5.64	63.7	0.00
10.0	11.52	48,810	7.77	5.58	62.2	0.00
11.0	11.51	44,818	7.77	5.23	58.6	0.00
12.0	11.47	48,812	7.78	5.41	61.4	0.00
13.0	11.47	48,849	7.79	5.60	62.8	0.00
14.0	11.43	48,855	7.78	5.56	61.3	0.00

15.0	11.43	48,867	7.78	5.49	61.0	0.00
16.0	11.43	48,812	7.79	5.38	60.3	0.00
17.0	11.43	48,880	7.79	5.48	61.4	0.00
10:21, High Tide, depth in meters						
0.5 (duplicate)	18.39	44,393	8.14	7.33	92.7	0.00
1.0 (duplicate)	18.45	44,440	8.14	7.30	92.1	0.00
2.0 (duplicate)	18.02	44,521	8.15	6.76	84.8	0.00
3.0 (duplicate)	17.91	44,590	8.14	7.07	89.1	0.00
4.0 (duplicate)	17.70	44,692	8.14	6.59	82.5	0.00
5.0 (duplicate)	15.67	46,477	8.00	6.83	82.4	0.00
6.0 (duplicate)	14.65	47,149	7.98	6.62	78.6	0.00
7.0 (duplicate)	13.31	47,767	7.87	5.82	67.4	0.00
8.0 (duplicate)	12.64	48,571	7.85	5.35	61.3	0.00
9.0 (duplicate)	12.05	48,605	7.77	5.15	58.2	0.00
10.0 (duplicate)	11.52	48,818	7.77	4.99	56.2	0.00
11.0 (duplicate)	11.48	48,801	7.79	5.27	60.5	0.00
12.0 (duplicate)	11.48	48,821	7.79	5.09	57.3	0.00
13.0 (duplicate)	11.44	48,864	7.78	5.12	57.4	0.00
14.0 (duplicate)	11.42	48,871	7.79	5.09	56.9	0.00
15.0 (duplicate)	11.42	48,881	7.79	5.17	56.2	0.00
16.0 (duplicate)	11.43	48,859	7.79	5.25	58.7	0.00
17.0 (duplicate)	11.43	48,869	7.79	5.17	57.9	0.00

Table 3. Summary of Water Quality Readings Taken at Discharge Locations on September 7, 2018 in Belfast Bay, Belfast, Maine

STATION 1	Temperature (°C)	Specific Conductivity (µmhos/cm)	pH (units)	DO (mg/L)	DO (%)	Turbidity (ntu)
9:00, High Tide, depth in meters						
0.5	18.23	44,242	7.95	8.78	110.1	0.00
1.0	18.15	44,271	7.94	8.67	108.9	0.00
2.0	18.14	44,260	7.93	8.63	108.5	0.00
3.0	18.13	44,280	7.92	8.61	108.3	0.00
4.0	18.13	44,325	7.92	8.59	107.9	0.00
5.0	18.11	44,322	7.92	8.55	107.4	0.00
6.0	17.98	44,409	7.91	8.48	106.7	0.00
7.0	16.02	46,307	7.81	8.34	101.5	0.00
8.0	15.71	46,436	7.78	7.95	96.0	0.00
9.0	15.17	46,779	7.76	7.81	93.6	0.00
10.0	14.60	47,072	7.73	7.65	90.7	0.00
11.0	13.87	47,465	7.64	7.10	82.4	0.00
12.0	13.39	47,701	7.64	6.35	73.4	1.20
13.0	13.05	47,840	7.62	6.03	69.5	2.50

14:39, Low Tide, depth in meters						
0.5	18.75	44,302	7.91	8.71	110.9	0.00
1.0	18.77	44,294	7.91	8.71	110.7	0.00
2.0	18.28	44,327	7.92	8.83	111.3	0.00
3.0	17.91	44,718	7.89	8.75	109.4	0.00
4.0	17.52	45,375	7.88	8.71	108.5	0.00
5.0	16.91	45,892	7.86	8.69	107.0	0.00
6.0	16.50	45,967	7.80	8.10	99.4	0.00
7.0	16.12	46,245	7.78	7.95	96.8	0.00
8.0	15.63	46,527	7.75	7.79	94.1	0.00
9.0	14.56	47,117	7.69	7.53	89.1	0.00
14:52, Low Tide, depth in meters						
0.5 (duplicate)	18.78	44,311	7.96	8.55	108.8	0.00
1.0 (duplicate)	18.74	44,316	7.93	8.61	109.6	0.00
2.0 (duplicate)	18.31	44,350	7.93	8.75	110.4	0.00
3.0 (duplicate)	17.88	44,762	7.90	8.68	108.7	0.00
4.0 (duplicate)	17.39	45,485	7.89	8.68	108.1	0.00
5.0 (duplicate)	17.05	45,844	7.89	8.69	107.5	0.00
6.0 (duplicate)	16.49	46,006	7.81	8.30	100.7	0.00
7.0 (duplicate)	16.04	46,322	7.78	7.93	96.4	0.00
8.0 (duplicate)	15.45	46,681	7.75	7.72	92.7	0.00

9.0 (duplicate)	14.45	47,191	7.68	7.25	85.8	0.00
STATION 2	**Temperature**	**Specific Conductivity**	**pH**	**DO**	**DO**	**Turbidity**
	(°C)	(µmhos/cm)	(units)	(mg/L)	(%)	(ntu)
11:16, High Tide, depth in meters						
0.5	18.72	44,329	7.94	8.64	109.6	0.00
1.0	18.58	44,334	7.94	8.62	109.3	0.00
2.0	18.36	44,327	7.95	8.65	109.2	0.00
3.0	18.28	44,334	7.95	8.65	109.1	0.00
4.0	18.20	44,370	7.95	8.66	109.1	0.00
5.0	18.15	44,435	7.94	8.63	108.4	0.00
6.0	16.97	45,632	7.88	8.42	103.9	0.00
7.0	16.76	45,915	7.88	8.38	103.3	0.00
8.0	16.65	46,046	7.89	8.40	103.4	0.00
9.0	15.93	46,435	7.85	8.35	101.2	0.00
10.0	15.57	46,608	7.85	8.22	99.3	0.00
11.0	14.3	47,312	7.73	7.62	89.1	0.00
12.0	13.61	47,601	7.64	6.71	77.2	0.60
13.0	12.85	48,005	7.61	6.23	71.5	1.80
14.0	12.83	47,817	7.44	5.98	68.5	no data
15:42, Low Tide, depth in meters						

0.5	18.59	44,359	7.93	8.72	110.6	0.00
1.0	18.59	44,361	7.93	8.73	110.7	0.00
2.0	18.57	44,360	7.93	8.73	110.7	0.00
3.0	18.12	44,584	7.93	8.83	111.1	0.00
4.0	17.83	45,061	7.90	8.72	109.3	0.00
5.0	17.60	45,663	7.92	8.76	109.7	0.00
6.0	17.24	45,895	7.91	8.79	109.3	0.00
7.0	15.89	46,047	7.80	8.09	98.1	0.00
8.0	15.35	46,785	7.79	8.02	96.8	0.00
9.0	15.93	46,954	7.71	7.53	89.1	0.00
10.0	13.99	47,407	7.59	6.59	76.0	0.00
11.0	13.60	47,593	7.57	6.32	73.4	1.40

Table 4. Summary of Results of Laboratory Analyses of Water Quality Samples Collected from Intake Locations on August 23, 2018 in Belfast Bay, Belfast, Maine

STATION 1	Solids, Total Suspended (mg/L)	Nitrogen, Ammonia (mg/L)	Nitrogen, Nitrate/Nitrite (mg/L)	Total Nitrogen (mg/L)	Nitrogen, Total Kjeldahl (mg/L)	Phosphorus, Total (mg/L)	Chemical Oxygen Demand (mg/L)	BOD 5-day (mg/L)
16:00, Low Tide								
0.5 meters	10.0	<0.024	<0.033	<0.30	0.195	0.012	1200	<2.0
4.0 meters	14.0	<0.024	<0.033	<0.30	0.225	0.012	640	<2.0
8.0 meters	13.0	<0.024	<0.033	<0.30	0.196	0.009	900	<2.0
12 meters	12.0	<0.024	<0.033	<0.30	0.172	0.014	1200	<2.0
STATION 2	Solids, Total Suspended (mg/L)	Nitrogen, Ammonia (mg/L)	Nitrogen, Nitrate/Nitrite (mg/L)	Total Nitrogen (mg/L)	Nitrogen, Total Kjeldahl (mg/L)	Phosphorus, Total (mg/L)	Chemical Oxtgen Demand (mg/L)	BOD 5-day (mg/L)
14:59, Low Tide								
0.5 meters	11.0	<0.024	<0.033	<0.30	0.221	0.013	1200	<2.0
4.0 meters	45.0	0.031	<0.033	<0.30	0.242	0.012	680	<2.0
8.0 meters	17.0	0.025	<0.033	<0.30	0.273	0.017	1200	<2.0
12 meters	13.0	<0.024	<0.033	<0.30	0.192	0.013	750	<2.0

Table 5. Summary of Results of Laboratory Analyses of Water Quality Samples Collected from Intake Locations on August 24, 2018 in Belfast Bay, Belfast, Maine

STATION 1	Solids, Total Suspended (mg/L)	Nitrogen, Ammonia (mg/L)	Nitrogen, Nitrate/Nitrite (mg/L)	Total Nitrogen (mg/L)	Nitrogen, Total Kjeldahl (mg/L)	Phosphorus, Total (mg/L)	Chemical Oxygen Demand (mg/L)	BOD 5-day (mg/L)
12:00, High Tide (AM)								
0.5 meters	9.6	<0.024	0.090	<0.30	0.185	0.012	790	<2.0
5.0 meters	8.6	<0.024	<0.033	<0.30	0.191	0.012	960	<2.0
9.5 meters	11.0	<0.024	<0.033	<0.30	0.188	0.021	900	<2.0
14.0 meters	11.0	<0.024	0.11	<0.30	0.183	0.019	1300	<2.0
STATION 2	Solids, Total Suspended (mg/L)	Nitrogen, Ammonia (mg/L)	Nitrogen, Nitrate/Nitrite (mg/L)	Total Nitrogen (mg/L)	Nitrogen, Total Kjeldahl (mg/L)	Phosphorus, Total (mg/L)	Chemical Oxtgen Demand (mg/L)	BOD 5-day (mg/L)
10:59, High Tide (AM)								
0.5 meters	10.0	0.039	0.095	<0.30	0.194	0.012	1000	<2.0
5.0 meters	9.2	<0.024	0.10	<0.30	0.235	0.013	810	<2.0
5.0 meters (duplicate)	9.4	<0.024	<0.033	<0.30	0.223	0.013	750	<2.0
9.5 meters	8.5	<0.024	<0.033	<0.30	0.202	0.017	1200	<2.0
14.0 meters	11.0	0.045	0.097	<0.30	0.182	0.024	770	<2.0

Table 6. Summary of Results of Laboratory Analyses of Water Quality Samples Collected from Discharge Locations and Dam on September 7, 2018 in Belfast Bay, Belfast, Maine

STATION 1	Solids, Total Suspended (mg/L)	Nitrogen, Ammonia (mg/L)	Nitrogen, Nitrate/Nitrite (mg/L)	Total Nitrogen (mg/L)	Nitrogen, Total Kjeldahl (mg/L)	Phosphorus, Total (mg/L)	Chemical Oxygen Demand (mg/L)	BOD 5-day (mg/L)
9:42, High Tide (AM)								
0.5 meters	8.5	<0.024	<0.033	0.42	0.418	0.013	1100	<2.0
4.0 meters	8.8	0.024	<0.033	0.78	0.780	0.009	1000	<2.0
7.0 meters	8.6	<0.024	<0.033	0.53	0.531	0.016	1400	<2.0
10 meters	9.0	<0.024	0.046	0.32	0.321	0.015	1100	<2.0
15:08, Low Tide (PM)								
0.5 meters	7.5	<0.024	<0.033	<0.30	0.195	0.015	670	<2.0
3.0 meters	7.8	<0.024	0.034	<0.30	0.238	0.014	860	<2.0
5.0 meters	6.9	<0.024	<0.033	<0.30	0.198	0.012	660	<2.0
5.0 meters (duplicate)	9.5	<0.024	<0.033	<0.30	0.204	0.010	800	<2.0
7.0 meters	10.0	<0.024	<0.033	<0.30	0.142	0.016	750	<2.0
STATION 2	Solids, Total Suspended (mg/L)	Nitrogen, Ammonia (mg/L)	Nitrogen, Nitrate/Nitrite (mg/L)	Total Nitrogen (mg/L)	Nitrogen, Total Kjeldahl (mg/L)	Phosphorus, Total (mg/L)	Chemical Oxtgen Demand (mg/L)	BOD 5-day (mg/L)
11:35, High Tide (AM)								

0.5 meters	7.7	<0.024	<0.033	<0.30	0.259	0.010	1400	<2.0
4.0 meters	9.4	<0.024	0.052	<0.30	0.153	0.014	720	<2.0
7.0 meters	7.4	<0.024	<0.033	<0.30	0.274	0.010	720	<2.0
10 meters	9.4	<0.024	<0.033	0.33	0.333	0.016	800	<2.0
16:11, Low Tide (PM)								
0.5 meters	7.2	<0.024	0.036	<0.30	0.226	0.011	770	<2.0
3.0 meters	7.1	<0.024	<0.033	<0.30	0.247	0.011	690	<2.0
5.0 meters	9.0	<0.024	<0.033	0.48	0.476	0.009	1000	<2.0
7.0 meters	9.3	0.034	<0.033	0.38	0.376	0.014	900	<2.0
BELOW DAM	Solids, Total Suspended (mg/L)	Nitrogen, Ammonia (mg/L)	Nitrogen, Nitrate/Nitrite (mg/L)	Total Nitrogen (mg/L)	Nitrogen, Total Kjeldahl (mg/L)	Phosphorus, Total (mg/L)	Chemical Oxtgen Demand (mg/L)	BOD 5-day (mg/L)
13:26, Ebbing Tide (PM)								
Downstream side of dam	No data collected	No data collected	0.036	0.48	0.480	0.021	No data collected	No data collected

NORDIC AQUAFARMS
SUSTAINABLE AQUACULTURE

Nordic Aquafarms Inc
511 Congress Street
Portland, ME 04101

www.nordicaquafarms.com

October 16, 2018

Mr. Kevin Martin
Commissioner's Office
Maine Department of Environmental Protection
17 State House Station
Augusta, ME 04333-0017

Dear Mr. Martin:

This letter authorizes Attorney Joanna B. Tourangeau of Drummond Woodsum and Elizabeth Ransom of Ransom Consulting to act as agents on behalf of Nordic Aquafarms, Inc. in connection with any applications being filed with the Department of Environmental Protection for Nordic Aquafarms project in Belfast, Maine. These applications include, but are not limited to applications pursuant to Maine statutes implementing the MEPDES Program, the Site Law, and the Natural Resource Protection Act and any other related applications that may be required for this project.

Thank you for your attention to this matter.

Sincerely,

Eric Heim
President

BDN

Todd McLeod | Print Sales Manager

September 24, 2018

AFFIDAVIT OF PUBLICATION

Legal Notices
NOTICE OF INTENT TO FILE
MAINE WASTE DISCHARGE LICENSE/MAINE POLLUTANT
DISCHARGE ELIMINATION SYSTEM PERMIT APPLICATION AND
NOTICE OF PUBLIC INFORMATIONAL MEETING

Please take note that, pursuant to 38 MRSA, Sections 413 and 414-A, **Nordic Aquafarms intends to file a wastewater discharge permit application with the Department of Environmental Protection (DEP)**. This application is for the discharge of up to 7.7 million gallons per day of wastewater from land based aquaculture to Penobscot Bay in Belfast, Maine. **The application will be filed on or about October 19, 2018** and will be available for public inspection at DEP's Augusta office during normal business hours. A copy may also be seen at the municipal offices in Belfast, Maine.

Please take note that, pursuant to Chapter 2 of the Department of Environmental Protection Rules, Nordic Aquafarms intends to hold a **Public Informational Meeting on October 4, 2018 at 6:00 p.m. at the Troy A. Howard Middle School, 173 Lincolnville Ave, Belfast, ME 04915.** The applicant will inform the public of the project and its anticipated environmental impacts, along with information about opportunities for public comments on the project.

A request for public hearing or request that the Board of Environmental Protection assume jurisdiction over this application must be received by the DEP, in writing, no later than 20 days after the application is found acceptable for processing, or 30 days from the date of this notice, whichever is longer. Requests shall state the nature of the issue(s) to be raised. Unless otherwise provided by law, a hearing is discretionary and may be held if the Commissioner or the Board finds significant public interest or there is conflicting technical information.

During the time specified above, persons wishing to receive copies of draft permits and supporting documents, when available, may request them from DEP. Persons receiving a draft permit shall have 30 days in which to submit comments or to request a public hearing on the draft.

Public comment will be accepted until a final administrative action is taken to approve, approve with conditions or deny this application. Written public comments or requests for information may be made to the Division of Water Quality Management, Department of Environmental Protection, State House Station #17, Augusta, ME 043330017. Telephone: (207) 287-3901.

Sept. 21, 2018

This is to certify the advertising

OF: Drummond Woodsum

RE: Notice of Intent to File - Nordic Aquafarms

ON: September 21, 2018

Signed:

Todd McLeod
Print Sales Manager

Then personally appeared the above named Todd McLeod, Print Sales Manager, and acknowledged the foregoing instrument to be his free act and deed in his said capacity and the free act and deed of said corporation.

Before me,

Barbara G. Mower
Notary Public
My commission expires November 9, 2024

bangordailynews.com
P.O. Box 1329 | Bangor, ME 04402-1329 | 207-990-8000 | 800-432-7964

BARBARA G. MOWER
NOTARY PUBLIC
State of Maine
My Commission Expires
November 9, 2024

Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States

Donald M. Anderson,[a,*] JoAnn M. Burkholder,[b] William P. Cochlan,[c] Patricia M. Glibert,[d] Christopher J. Gobler,[e] Cynthia A. Heil,[f] Raphael Kudela,[g] Michael L. Parsons,[h] J. E. Jack Rensel,[i] David W. Townsend,[j] Vera L. Trainer,[k] and Gabriel A. Vargo[l]

[a]Biology Department, MS #32, Woods Hole Oceanographic Institution, Woods Hole MA 02543 USA
[b]Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606 USA
[c]Romberg Tiburon Center for Environmental Studies, San Francisco State University, Tiburon, California 94920 USA
[d]University of Maryland Center for Environmental Science, Horn Point Laboratory, PO Box 775, Cambridge, MD 21613
[e]Stony Brook University, School of Marine and Atmospheric Sciences, Stony Brook, NY 11794-5000
[f]Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, 100 8th Ave. S., St. Petersburg, FL 33701
[g]Ocean Sciences & Institute for Marine Sciences, University of California Santa Cruz, Santa Cruz, CA 95064 USA
[h]Department of Marine and Ecological Sciences, Florida Gulf Coast University, Fort Myers, Fl 33965 USA
[i]Rensel Associates Aquatic Sciences, Arlington WA 98223 USA
[j]School of Marine Sciences, University of Maine, Orono, Maine 04469 USA
[k]NOAA, Northwest Fisheries Science Center, Marine Biotoxins Program, Seattle, Washington, 98112 USA
[l]University of South Florida, College of Marine Science, 140 Seventh Avenue South, St. Petersburg, FL 33701
[*]Corresponding Author: Tel: (508) 289-2321; FAX: (508) 457-2027; E-mail: danderson@whoi.edu

Abstract

Coastal waters of the United States (U.S.) are subject to many of the major harmful algal bloom (HAB) poisoning syndromes and impacts. These include paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP), ciguatera fish poisoning (CFP) and various other HAB phenomena such as fish kills, loss of submerged vegetation, shellfish mortalities, and widespread marine mammal mortalities. Here, the occurrences of selected HABs in a selected set of regions are described in terms of their relationship to eutrophication, illustrating a range of responses. Evidence suggestive of changes in the frequency, extent or magnitude of HABs in these areas is explored in the context of the nutrient sources underlying those blooms, both natural and

anthropogenic. In some regions of the U.S., the linkages between HABs and eutrophication are clear and well documented, whereas in others, information is limited, thereby highlighting important areas for further research.

Keywords: harmful algal blooms, HABs, red tides, eutrophication, nutrients, nitrogen, phosphorus

1. Introduction

Virtually every coastal nation is affected by harmful algal blooms (HABs; Hallegraeff, 1993). It is now widely accepted that there are more toxic algal species, algal toxins, areas affected, fisheries resources impacted, and higher economic losses compared to several decades ago. Reasons for such expansion include natural dispersal of species by currents and storms; dispersal of species through human activities such as ballast water discharge and shellfish seeding; improved detection of HABs and their toxins due to better chemical instrumentation and improved communication among scientists; increased aquaculture operations in coastal waters; and stimulation of HABs as a result of cultural eutrophication (Anderson, 1989; Hallegraeff, 1993; Burkholder, 1998; Glibert et al., 2005a; Glibert and Burkholder, 2006).

In this latter context, there is now consensus on some aspects of the relationship between eutrophication and HABs (Heisler et al., 2008), recognizing that significant questions and challenges remain (Glibert, 2006). Various authors have investigated potential linkages between HABs and eutrophication at the global level (e.g., Smayda, 1989; Burkholder, 1998; Anderson et al., 2002; Glibert and Burkholder, 2006; Glibert et al., 2008). In some instances, the linkage is clear and unequivocal. For example, mandated reductions in pollution inputs to the Inland Sea of Japan in the mid-1970s led to a proportional decrease in the number of red tides and HAB events (Okaichi, 1997). Likewise, increased nutrient loadings to the northwestern Black Sea in the 1970s and 1980s led to an increase and compositional shift in algal blooms (Bodeanu and Ruta, 1998). Subsequently, HABs declined in the 1990s coinciding with a decrease in nutrient loading due to reduced fertilizer usage following the breakup of the former Soviet Union and termination of its agricultural subsidies.

The pathways and mechanisms through which nutrients supplied by human activities can stimulate HABs are often complex and subtle. For example, it is now evident that in addition to the quantity of nutrients supplied through point and non-point sources of pollution, the relative abundance of the major nutrients (e.g., nutrient supply ratios) and the chemical form of those nutrients (e.g., inorganic versus organic) are all important (Smayda, 1989; Anderson et al., 2002; Glibert et al., 2001, 2005a). Furthermore, it is now recognized that nutrient effects cannot be inferred from concentration data alone; in fact, there can be a negative relationship between ambient concentration and biomass due to incorporation of the nutrient into biomass. Nutrient loading or flux rates are the more appropriate measure, but much more difficult to quantify. In addition, the role of mixotrophy, or use of both phototrophic and heterotrophic nutrition, by some harmful algae increasingly has been recognized, and these organisms sometimes have been shown to be indirectly stimulated by nutrient enrichment through consumption of algal prey that are directly stimulated by eutrophication (Burkholder and Glasgow, 1997; Burkholder, 1998; Stoecker, 1999; Lewitus et al., 1999a; Glibert et al., 2005a; Burkholder et al., 2008).

Given that some HABs are stimulated by anthropogenic nutrient inputs, projections of increased nitrogen (N) and phosphorus (P) loadings to coastal waters are worrisome (Seitzinger et al., 2005; Harrison et al., 2005; Burkholder et al., 2006; Howarth, 2008; Bricker et al., 2008). Here we focus on some common HAB events in the U.S., particularly those that cause human illness, but this is not intended to be a comprehensive survey of all blooms, species or regions. For example, some blooms in

the Northeast (e.g., Narragansett Bay; Li and Smayda, 2000), eastern Florida or Florida Bay (e.g., Phlips et al., 1999; Glibert et al., 2004b), the Texas coast (e.g., Laguna Madre; Buskey et al., 2001), and San Francisco Bay (Lehman et al., 2005) are not reviewed herein.

2. Regional HAB phenomena

2.1 PSP in the Gulf of Maine

The most significant HAB problem in the northeastern U.S. is PSP caused by *Alexandrium fundyense*[1]. The affected resources are predominantly shellfish, but higher levels of the food web are also impacted, including lobsters, fish, and marine mammals. Blooms of *A. fundyense* fall into two categories: regional outbreaks occurring in open coastal waters, and "self-seeding" localized blooms in isolated embayments and sounds with little or no input or export of cells to the adjacent coastal waters (Anderson, 1997; Anderson et al., 2005c). A key feature of *A. fundyense* bloom dynamics is a dormant cyst stage that allows this species to overwinter. In the nearshore waters of the Gulf of Maine (GOM), blooms are initiated from two cyst seedbeds, one in the Bay of Fundy, and the other offshore of mid-coast Maine, with delivery of established populations to shore by episodic wind forcings as well as large-scale movements of water masses in the central Gulf (Anderson et al., 2005b). Along this extended transport pathway, cells within the eastern and western segments of the Maine coastal current can enter embayments such as Casco and Massachusetts Bays with downwelling-favorable winds. It is important to recognize, however, that the source populations and associated water masses are delivered from upstream and offshore.

PSP toxicity in the western GOM was sporadic and poorly documented prior to a major bloom in 1972 (Hurst, 1975). PSP toxicity continued at high levels until the early 1990s, decreased for a decade thereafter, and recently has shown signs of increasing again (Anderson et al., 2005a).

2.1.1 Eutrophication linkages An evaluation of potential linkages between northeastern U.S. PSP outbreaks and eutrophication needs to account for the two types of blooms in the region. Riley (1967) challenged the prevailing dogma that coastal nutrients in the GOM were derived primarily from riverine sources. Instead, he suggested that cross-shelf fluxes from the open ocean were important, an inference later validated by Fournier et al. (1977). Nutrients that enter the GOM at depth through the Northeast Channel overwhelm fluxes from the atmosphere or local rivers (Townsend, 1998; Townsend et al., 2006). There has been and continues to be significant inter-annual variability in the magnitude of that nutrient flux into the Gulf, reflecting the relative importance of the source waters and large-scale forcings such as the North Atlantic Oscillation (Townsend et al., 2006).

Coastal currents of the GOM carry populations of *A. fundyense* into embayments such as Casco and Massachusetts Bays where anthropogenic influences may be more significant. Local cyst germination might also provide an inoculum. Poulton et al. (2005), Martorano (1997) and Love et al. (2005) all provided evidence for increasing dissolved inorganic nitrogen (DIN) levels (ranging from 1–10 µM during *Alexandrium* bloom season) along a transect from offshore to inshore within Casco Bay. These data reflect land-based nutrient sources in the Bay (i.e., the city of Portland) and riverine inputs.

Nutrient measurements in the region's rivers and coastal waters are relatively scarce, and suggest that nutrient loads in the Androscoggin and Kennebec (two of the four largest rivers emptying into the GOM) are highly variable (Fig. 1A). Nitrate+nitrite ($NO_3^- + NO_2^-$) levels average about 7.6 µM over an annual cycle, with highest levels (12.7 µM) in the high-runoff months (winter-spring) and the lowest (4.6 µM) during low runoff months (Jun-Oct). These are comparable to or lower than values in other urban regions of the U.S, and once those freshwaters mix with coastal waters, the resulting nutrient

concentrations are diluted (e.g., to ~2.7 µM NO_3^-). Episodic high values of $NO_3^- + NO_2^-$ in excess of 10 µM can occur during bloom season (Fig. 1B), which could be important to *A. fundyense* populations in Casco Bay at that time. The average annual cycle shows no clear changes over the last 7 years, although a longer duration may be needed to detect NO_3^--related changes (e.g., Rothenberger, 2007). Many HABs also use ammonium (NH_4^+) or dissolved organic nitrogen (DON) as their primary N substrate. In Casco Bay, NH_4^+ concentrations are very high, occasionally exceeding 20 µM, and tend to average > 2 µM (Bricker et al., 2007).

Figure 1

Nutrients in Gulf of Maine nearshore waters. *Panel A:* Concentrations of nitrate plus nitrite ($NO_{3-} + NO_{2-}$) and silicate ($Si(OH)_4$) in the Kennebec and Androscoggin Rivers in Maine over the period January 1, 2001 to May 1, 2002 (data from Vorosmarty et al., http://www.gm-wics.sr.unh.edu/). *Panel B:* Concentrations of $NO_{3-} + NO_{2-}$ and $Si(OH)_4$ collected in Casco Bay at the Southern Maine Community College dock, South Portland, Maine (from Townsend et al., http://grampus.umeoce.maine.edu/). *Panel C:* Mixing curves for $NO_{3-} + NO_{2-}$ and $Si(OH)_4$ versus salinity for Gulf of Maine samples collected in a high- (April–May 2000), medium- (June 2000) and low-runoff period (July 2001) (Townsend et al., 2005). Assuming a northern Gulf of Maine coast-wide freshwater source concentration of nutrients similar to the Kennebec-Androscoggin (which was 32 µM $Si(OH)_4$ and 12 µM $NO_{3-} + NO_{2-}$ in April 2000; n=49; http://www.gm-wics.sr.unh.edu/), and coastal seawater concentrations of ca. 8 µM of $Si(OH)_4$ and ca. 8 µM $NO_{3-} + NO_{2-}$ and salinity of 32.5, $Si(OH)_4$ concentrations of 9.5 µM and $NO_{3-} + NO_{2-}$ concentrations of 8 µM at salinity of 30 in April–May are expected (in agreement with those values observed in the mixing curve in Panel C).

Analysis of samples collected from inshore and offshore waters throughout the northern GOM in high-, medium-, and low-runoff periods again suggest that terrestrial sources of $NO_3^- + NO_2^-$ are not significant, except for localized areas of inner Casco Bay as mentioned, based upon the available data. For example, mixing curves of NO_3^- concentrations versus salinity show that there was virtually no low-salinity (terrestrial, freshwater) NO_3^- contribution in July 2001 or June 2000 (Fig. 1C). There was evidence of a terrestrial, freshwater source in these coastal waters in April-May 2000, during and after the high-runoff period but, even then, the NO_3^- sources were primarily from offshore. Data are not available for other N forms offshore.

Considering localized blooms that occur along the New England coast, a separate analysis in each area would be needed to assess whether eutrophication is affecting *A. fundyense* blooms. There is a good likelihood that this is occurring, as these embayments, sounds, and estuaries are often surrounded by housing and other development, often with outdated septic systems. One example, the Nauset Marsh System on Cape Cod, lacks major river input, and NO_3^--contaminated groundwater from septic systems is the largest source of N (Giblin and Gaines, 1990; Howes et al., 2003). Groundwater NO_3^- fluxes are ~5-fold greater than inputs from land runoff, and these two sources combined are comparable to inputs in river-dominated urban areas of the U.S. (Portnoy et al., 1998). In the more highly developed areas of the Nauset system, groundwater NO_3^- concentrations are more than 30-fold higher than in less developed areas. PSP outbreaks within the Nauset Marsh System have increased dramatically over the last several decades. Toxicity occurred in Salt Pond (Eastham) in 8 of 17 years

(48%) from 1975 to 1991, and then in 15 of the last 16 years (94%). PSP is also occurring earlier and lasting longer (H. Lind, pers. comm). The growing nutrient load from NO_3^- contaminated groundwater is a likely reason for the increased frequency of PSP outbreaks within this system.

In summary, PSP outbreaks in the northeastern U.S. take two forms – large regional open-water blooms and localized nearshore outbreaks. For the open-water blooms, the supply of inorganic nutrients from oceanic sources dwarfs inputs from land, so that if there is a eutrophication effect, it is subtle and unquantified. These blooms do impact certain bays and sounds when offshore populations of *A. fundyense* are delivered by winds, and thus there is a potential for local sources of anthropogenic nutrients to enhance bloom magnitude and duration. The strongest linkage to eutrophication is with localized, nearshore PSP outbreaks that recur annually in small salt ponds, embayments, and sounds where flushing rates are low and development pressures can lead to high nutrient inputs from sewage treatment plants, terrestrial runoff, and groundwater sources.

2.2 Brown tides in the Northeast and Mid-Atlantic

Aureococcus anophagefferens is the picoplanktonic pelagophyte that has caused destructive 'brown tide' blooms in northeast and mid-Atlantic U.S. estuaries for two decades (Gobler et al., 2005). The first blooms occurred simultaneously in 1985 in Narragansett Bay, Rhode Island, and Great South Bay and the Peconic Estuary on Long Island, New York (Cosper et al., 1989). Blooms recurred annually in Long Island bays from 1986–88 and sporadically since then, but have not returned to Narragansett Bay. Even during years without blooms, *A. anophagefferens* populations of up to 10^4 mL^{-1} persist (Nuzzi and Waters, 2004). During the 1990s, brown tides expanded south along the U.S. East Coast into bays in New Jersey (Gastrich et al., 2004), Delaware (Popels et al., 2003), Maryland, and Virginia (Trice et al., 2004; Glibert et al., 2007). Low abundance of *A. anophagefferens* has been observed along the entire eastern seaboard of the U.S. from Maine to Florida (Anderson et al., 1993; Popels et al., 2003).

2.2.1 Eutrophication linkages Brown tides are not directly caused by eutrophication from inorganic nutrients, but they may be linked to organic nutrients (below). Other phytoplankton such as diatoms often out-compete *A. anophagefferens* when inorganic nutrient loads are high (Berg et al., 1997; MacIntyre et al., 2004). An examination of spatial and temporal patterns of concentrations of *A. anophagefferens* cells and inorganic nutrients indicates that blooms occurred when inorganic nutrient levels were low (Cosper et al., 1989; LaRoche et al., 1997; Gobler et al., 2002, 2004). Moreover, NO_3^- additions during mesocosm and bottle experiments consistently have yielded reduced *A. anophagefferens* cell densities relative to those of competing algae (e.g., Keller and Rice, 1989; Gobler and Sañudo-Wilhelmy, 2001a). The N-uptake characteristics of *A. anophagefferens* (low K_S and V_{max} for NH_4^+) suggest that this species is well adapted to low nutrient environments (Lomas et al., 1996). The off-shore rerouting of sewage previously discharged directly into western Great South Bay during the early 1980s led to lower levels of dissolved inorganic nitrogen (DIN) there, thus creating a nutrient regime which reduced total annual phytoplankton biomass, but favored dominance by *A. anophagefferens* as blooms began to first develop in the late 1980s (Fig. 2A).

Blooms of *Aureococcus* often occur after 'pre-blooms' of other algae which draw down inorganic nutrients to low levels. Nutrient remineralization processes during and following these pre-blooms can result in enhanced levels of dissolved organic matter (DOM) which can serve as a source of dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) (LaRoche et al., 1997; Gobler and Sañudo-Wilhelmy, 2001b). Cultures (both axenic and non-axenic) and field populations of *A. anophagefferens* have been shown to obtain N from a variety of organic compounds, including urea, amino acids, proteins, chitobiose, and acetamide (Berg et al., 1997, 2002, 2003; Mulholland et al., 2002; Kana et al., 2004). Experimental additions of DOM (glucose, amino acids, DOM from macroalgae) have enhanced the growth and relative abundance of *A. anophagefferens* during field experiments (Gobler and Sañudo-Wilhelmy, 2001a; Kana et al., 2004). Concentrations of DON and DOC often are elevated during bloom initiation, and their drawdown has been associated with bloom development (LaRoche et al., 1997; Gobler et al., 2004).

Brown tides commonly occur in shallow bays where diffusive fluxes from the benthos are important sources of organic nutrients. For example, in the shallow (~1.5 m) lagoonal estuaries of NY, brown tides are persistent events and DON levels are high, due in part to strong benthic-pelagic coupling (Lomas et al., 2004; Gobler et al., 2005). In Maryland's lagoonal bays, both the DON concentrations and the strength of brown tide blooms have increased steadily over the past decade (Trice et al., 2004; Glibert et al., 2007). Since the mid-1990s, mean concentrations of DON in these bays have more than doubled, and the fraction of total chlorophyll composed of brown tide in the summer has increased to an even greater extent (Glibert et al., 2007).

In deeper estuaries where benthic-pelagic coupling is weaker, DON concentrations typically are lower than in shallow lagoons and brown tides occur less frequently. This is the case, for example, in the Peconic Estuary (mean depth > 5 m; Gobler et al., 2005). Both brown tide and DON levels there have decreased since 1996 (Fig. 2B), presumably due to decreased N loading, but DIN levels have remained unchanged. Hence, it is possible that lower DON levels, in conjunction with higher grazing rates on *A. anophagefferens* by the zooplankton community (Deonarine et al., 2006), may be responsible for the absence of brown tides in the Peconic Estuary since the 1990s.

In summary, *A. anophagefferens* thrives in environments where nutrients are dominated by organic rather than inorganic forms. In regions where inorganic nutrient loading is elevated, other phytoplankton with faster growth rates tend to dominate. Thus, brown tides are not directly related to inorganic nutrient loading, but may be indirectly related to eutrophication processes as inorganic nutrients are assimilated and regenerated as organic forms. In some regions, such as the Maryland

Coastal Bays, DON is increasing from agricultural sources, animal operations and organic fertilizers (e.g. Glibert et al., 2007) and direct sources of DON should be considered as contributing to eutrophication in these regions, and therefore to brown tides.

2.3 HABs in the Mid-Atlantic region

In the mid-Atlantic, the frequency of occurrence and, in many cases, the intensity of HABs increasing relative to previous decades. The region has a wide range of high-biomass and/or toxic HABs involving species such as *Prorocentrum minimum*, *A. anophagefferens*, *Microcystis aeruginosa*, *Pfiesteria piscicida*, *P. shumwayae*, *Karlodinium veneficum* (formerly *Karlodinium micrum*, *Gyrodinium galatheanum*), *Heterosigma akashiwo, Chattonella subsalsa* and *Chattonella* cf. *vericulosa*, and *Fibrocapsa japonica* (Marshall et al., 2005a; Burkholder et al., 2005; Moeller et al., 2007; Lewitus et al., 2008). Numerous other potentially toxic or harmful species are also present; for example, six species of *Pseudo-nitzschia* have been found, and of these, 46% contained low levels of domoic acid (Thessen and Stoecker, 2008). Marshall et al. (2005b) identified 34 harmful species for Chesapeake Bay, while Tango et al. (2004) identified 13 in Maryland Coastal Bays. The trend in brown tides in Maryland's Coastal Bays was discussed above, and only a few species and trends are highlighted here.

2.3.1 Eutrophication linkages A recent comprehensive assessment of eutrophication in the nation's waters revealed that most of the estuaries affected by HABs are in the mid-Atlantic, and that this region is the most affected by eutrophication of all coastal regions of the U.S. (Bricker et al., 2007, 2008). With the exception of the Mississippi plume, N loads were found to be highest in the mid-Atlantic. The mid-Atlantic has the nation's two largest estuaries in areal extent (Chesapeake Bay and the Albemarle-Pamlico Estuarine System), which drain watershed areas that are rapidly growing in human and animal populations and changing in land use and N loads. Since 1960, for example, the watershed for Chesapeake Bay has sustained more than a doubling in human population and in use of N- based fertilizers (Kemp et al., 2005; Fig. 3).

Open in a separate window

Figure 3

Temporal changes in land uses, river flow, fertilizer use, and river N concentration in the Chesapeake Bay watershed. From Kemp et al. 2005 with permission of the publisher.

Land use in the region also has changed with a large expansion of confined animal feed operations (CAFOs), primarily swine and poultry (Mallin and Cahoon, 2003; Rothenberger et al., submitted). These have contributed to nutrient loads through surface runoff, leachate into groundwater, and enhanced atmospheric emissions (Burkholder et al., 1997; Mallin, 2000); approximately 80% of emissions of NH_3 from swine operations in the U.S. originate in North Carolina (EPA, 1998; Aneja et al., 2003). Thus, the total N load to the Albemarle-Pamlico water from atmospheric deposition is among the highest for U.S. watersheds (EPA, 2000). CAFOs also have altered the composition of nutrients reaching the estuarine and coastal waters, in particular increasing NH_4^+ and in some cases, urea (Glibert et al., 2005b; Burkholder et al., 2006). Retention ponds also have been shown to serve as

incubation sites for many species (Lewitus et al., 2003; 2008). These major estuaries are also sustaining major loss of wetlands, depletion of shellfish stocks, and global warming, all of which can act synergistically to increase nutrient loads and alter trophic relationships.

An example of frequent high-biomass bloom formers is *P. minimum*. Well-established to be stimulated by eutrophication (Heil et al., 2005; Rothenberger, 2007; Glibert et al., 2008), this species frequently blooms following precipitation events that add significant quantities of nutrients. Blooms are most common in late winter-spring (Albemarle-Pamlico) through early summer (Chesapeake Bay) following major agricultural fertilizer applications (Glibert et al., 2001; Springer et al., 2005; Tango et al., 2005). These blooms now appear to be 10- to 100-fold higher in maximum density in Chesapeake Bay than blooms of the same organism recorded a few decades ago (Tyler and Seliger, 1978).

Blooms of *K. veneficum* are also increasing in frequency in Chesapeake Bay, and have been linked to some fish kills (Deeds et al., 2002; Goshorn et al., 2004). These blooms tend to occur when NO_3^- concentrations are low, and when NH_4^+ or urea concentrations and ambient PO_4^{3-} are elevated. Mixotrophy through consumption of algal prey that are directly stimulated by eutrophication also contributes to *K. veneficum* nutrition and supports faster growth rates (Adolf et al., 2006, 2008).

Other harmful algae that have been linked both directly and indirectly to eutrophication are the potentially toxic, heterotrophic *Pfiesteria* spp. (*Pfiesteria piscicida, P. shumwayae*), which mostly have affected the Albemarle-Pamlico and Chesapeake Bay (Burkholder and Glasgow, 1997; Burkholder et al., 2005; Glibert et al., 2001, 2006a; Marshall et al., 2006; Moeller et al., 2007; Lewitus et al., 2008). Field studies with supporting laboratory experiments have shown that *Pfiesteria* spp. thrive in nutrient over-enriched estuaries where they consume cryptophytes and other microbial prey when preferred fish resources are not available (Burkholder and Glasgow, 1997; Lewitus et al., 1999b; Glasgow et al., 2001; Parrow et al., 2002). They can also act as mixotrophs by retaining kleptochloroplasts from cryptophytes for short periods, apparently used to augment their nutrition (Lewitus et al., 1999b). *Pfiesteria* spp. depend mostly on grazing for their nutrient supplies, but can also take up dissolved nutrients directly (Burkholder and Glasgow, 1997; Lewitus et al., 1999a,b; Burkholder et al., 2001; Glibert et al. 2006a). *Pfiesteria* outbreaks in Chesapeake Bay tributaries also have been associated with high DON and DOC levels (Glibert et al., 2001). For all samples collected from 2000–2001 in Chesapeake Bay (n = 1,614 for water and 156 for sediment), the average urea concentration was an excellent predictor of the percent positive detection of *Pfiesteria* in sediments and in the water column (Glibert et al., 2004a). Laboratory studies have shown that *Pfiesteria* can be stimulated by P (Burkholder and Glasgow, 1997), and that the preference for N form follows a trend of amino acids>urea>NH_4^+>NO_3^- (Glibert et al., 2006a). Urea is now the dominant agricultural N form in the region, and is also a dominant N form in poultry manure (Glibert et al., 2005b). The major effect of eutrophication on *Pfiesteria* spp., nonetheless, appears to be indirectly mediated through abundance of prey that is directly stimulated (Burkholder and Glasgow, 1997; Glibert et al., 2006a).

Large blooms of the toxic cyanobacterium *M. aeruginosa* are common in the summer months in the low-salinity reaches of the estuaries and tributaries of the region (e.g., Jaworski, 1990). In recent years, these blooms have increased as nutrient concentrations have been escalating (Marshall et al., 2005b).

Raphidophytes have caused fish kills and recurring impacts to some mid-Atlantic ecosystems, especially *H. akashiwo*. Based on an analysis of historical phytoplankton monitoring data, these raphidophytes, as well as *Fibrocapsa japonica* and *Chattonella subsalsa,* have increased significantly in abundance over the past 20 years in the Coastal Bays of Maryland (Tango et al., 2004). While inorganic nutrient concentrations decreased from the 1980s to the mid 1990s in the bays where these

species are most common ([Wazniak et al., 2007](#)), significant increases have since been documented in both P and N, especially as NH_4^+ and DON ([Wazniak et al., 2007](#); [Glibert et al., 2007](#)). In laboratory experiments, [Zhang et al. (2006)](#) found that cell yield for *C. subsalsa* and *Heterosigma akashiwo* was higher on NH_4^+ and NO_3^-, respectively, and that *C. subsalsa* did not use urea while *H. akashiwo* readily used this N form. The levels of N used in these experiments were comparable to natural conditions in the highly eutrophic Delaware inland bays.

In summary, the mid-Atlantic region has many HAB species, most of which have increased in frequency, abundance, regional extent and impact over the past few decades as population, agriculture and animal operations, and fertilizer usage have increased. In addition to increased nutrient loads, the estuaries, rivers and embayments of the region are also highly modified by declines in shellfish stocks and wetlands (e.g. [Steel, 1991](#); [Rothschild et al. 1994](#)), leading to multiple stressors which collectively and synergistically lead to habitat change and alterations in trophic structure, including HAB proliferations.

2.4 *Karenia brevis* blooms in the Gulf of Mexico

The most significant HAB species in the Gulf of Mexico is the ichthyotoxic dinoflagellate *Karenia brevis* (formerly *Gymnodinium breve Ptychodiscus brevis*), although other HAB species (e.g., *Pseudo-nitzschia* spp., *Prorocentrum minimum*) are present ([Parsons et al., 2002](#)). Published accounts of widespread west Florida shelf *K. brevis* blooms predate extensive development on that coast ([Lund, 1936](#); [Tester and Steidinger, 1997](#); [Magaña et al., 2003](#)). Although *K. brevis* is the primary species associated with toxic blooms in the Gulf of Mexico, there are now at least five described *Karenia* species known from Gulf waters ([Haywood et al., 2004](#)). Recent blooms are now known to exhibit a sequence of *Karenia* species ([Heil et al., in press](#)) but the toxicity of species other than *K. brevis* is presently under investigation.

2.4.1 Eutrophication linkages
There are two views on the role of nutrients and eutrophication in *Karenia brevis* blooms. [Walsh et al. (2006)](#) examined potential linkages between *K. brevis* outbreaks on the west Florida shelf and the Texas shelf with riverine flux and eutrophication. Increases in nutrient loads to the Caloosahatchee River during the 1970s and 1980s were followed by decreases in the 1990s ([Fig. 4](#)). Overall, [Walsh et al. (2006)](#) did not discern overall differences in the magnitude or frequency of west Florida shelf red tides, or correlations with nutrient data, although the data records were sporadic and the change in total flux relative to earlier periods is unknown. Using a similar approach, [Vargo et al. (2004](#), [2008](#)) evaluated nutrient sources for support of blooms after initiation and transport into coastal waters and estimated that estuarine fluxes of N and P from Tampa Bay, Charlotte Harbor, and the Caloosahatchee River were sufficient to support a major portion of the nutrients needed to sustain moderate *K. brevis* blooms (3×10^5 cells L^{-1}) in localized nearshore areas, but would contribute a low proportion of the nutrients needed to support larger blooms over broader areas.

<u>Figure 4</u>

Available data for total and inorganic N and P concentrations for a 2 square km area at the Caloosahatchee River mouth and the extent of Everglades Agricultural Area (EAA) which is devoted to sugar cane fields for the time frame of 1949 through 2005. Nutrient data has been retrieved from the state of Florida STORET database; available data for the river from a variety of sources are listed on the figure. The change in the areal extent of the EAA has been modified from <u>Brand (2002)</u>.

On the other hand, <u>Brand and Compton (2007)</u> suggested that there has been a significant increase in nutrient inputs to the nearshore waters of west Florida over the past 50 years. Based on their analysis of the State of Florida long-term HAB database, which heavily emphasized bloom periods, they reported that higher nearshore abundance of *K. brevis* during blooms over the 1994–2002 timeframe, compared to the 1954–1963 timeframe, appears to be related to elevated nutrient flux into nearshore coastal waters from urban and agricultural development in the Caloosahatchee River watershed, and eutrophication of Lake Okeechobee, the headwaters for the Caloosahatchee. <u>Turner et al. (2006)</u> used paleo-indicators to assess long-term water quality changes in Charlotte Harbor and found evidence of a 3-fold increase in N loadings between the 1800s and the present, with significant increases in water column NO_3^- concentrations between 1960 and 1980 which have remained essentially constant since the 1980s (see their <u>Fig. 3</u>).

<u>Walsh and Steidinger (2001)</u> and <u>Walsh et al. (2006)</u> hypothesized that N_2 fixation by blooms of the pelagic cyanobacterium *Trichodesmium* spp. and subsequent release of DON, coupled with use of remineralized nutrients from dead fish can lead to *K. brevis* bloom initiation, growth and maintenance on the west Florida and Texas shelves. They estimated that N_2 fixation could supply up to 0.16 µM L^{-1} day^{-1} based on *Trichodesmium* biomass and N excretion rates measured over a 3-yr period (<u>Mulholland et al., 2006</u>). Subsequent decay of dead fish might then be sufficient to supply all of the required N and P for blooms on the order of 10^6 cells L^{-1} or greater. <u>Mulholland et al. (2006)</u> also concluded that observed N_2 fixation and subsequent release of DON by *Trichodesmium* could provide substantial support for a moderate *K. brevis* bloom. A source of P would be required for the Saharan dust-*Trichodesmium*-dead fish hypothesis since P concentrations are extremely low in shelf waters (<u>Vargo and Shanley, 1985</u>; <u>Vargo et al., 2008</u>) and *Trichodesmium* spp. competes with *K. brevis* for available P. Further, based on stable N-isotope measurements of particulate organic matter (POM) collected during 4 years of west Florida shelf red tides, <u>Havens et al. (2004)</u> reported values of $\delta^{15}N_{POM}$ from −2 to 6 ‰, which excludes sewage and upwelled N as a source, but does not exclude commercial fertilizers derived from atmospheric N, which have an isotopic composition close to 0 (e.g., <u>del Amor 2008</u>). However, the low $\delta^{15}N_{POM}$ values indicative of fixed N recorded by <u>Havens et al. (2004)</u> were found only in samples collected in close proximity to a *Trichodesmium* bloom whereas the majority of bloom values (71 in 5 separate blooms) were in the range of 3 to 5 ‰. Thus N from a variety of sources appears to fuel west Florida shelf *K. brevis* blooms.

Mixotrophy may also provide nutrients for *K. brevis* blooms (e.g., <u>Bronk et al., 2004</u>; <u>Jeong et al. 2005a,b</u>; <u>Glibert et al., submitted</u>), as described above for *K. veneficum* and *Pfiesteria* spp. Dissolved organic N (DON) concentrations in west Florida estuaries and coastal waters are about 10-fold higher

than inorganic N concentrations, and while inorganic N:P ratios are low, DON:DOP (dissolved organic P) ratios are consistently higher than the Redfield ratio (Heil et al., 2007). Notably, Hu et al. (2006) found much higher DON flux compared to DIN and DIP. Collectively these data suggest that species capable of using DON, such as *K. brevis*, would be favored over those relying on inorganic N sources (Heil et al., 2007). Concentrations of DON, presumably both terrestrial and marine derived, are sufficient to support blooms $>10^5$ cells L^{-1} but the extent to which this complex N is used by *K. brevis* and other *Karenia* spp. is unknown (Hu et al., 2006; Vargo et al., 2008). Thus, N from various sources appears to fuel west Florida shelf and nearshore *K. brevis* blooms and different nutrient sources may play a role in initiation versus maintenance stages of these blooms.

Hu et al. (2006) hypothesized that "submarine ground water discharge provides the "missing" nutrients that can trigger and support *Karenia* red-tides off west-central Florida". They suggest that increased development and population growth has increased groundwater nutrient levels and this, in turn, contributes to longer and higher biomass west Florida shelf blooms. While Vargo et al. (2008) report elevated near-bottom nutrient concentrations, they generally detected only at the 50 m isobath during years when shelf-break upwelling due to Loop Current meanders occurred.

Elsewhere in the Gulf of Mexico, a link between eutrophication and the frequency and magnitude of *K. brevis* blooms along the Texas coast has been suggested (Buskey et al., 1996; Denton and Contreras, 2004; Biegalski and Villareal, 2005; Walsh et al., 2006). This linkage has been based upon data for nutrient inputs from the Mississippi River (Turner and Rabalais, 1994; Villareal et al., 2001), combined with fish kill and bloom information (Trebatoski, 1988). Walsh et al. (2006), for example, postulated that doubling of the NO_3^- loads in the Mississippi River every decade since the 1950's has led to massive increases in phytoplankton biomass in the river plume. Considering measured water column and near bottom N:P ratios off both Texas and southwest Florida coastal areas where red tides are prevalent, together with modeling results (Walsh et al., 2006), remineralization of this elevated biomass leads to low N:P ratios that favor development of *K. brevis* blooms. Notably, the first documented bloom off Texas occurred in 1955 followed by another in 1976 and 1986, but blooms have occurred almost annually after 1996. Similarly, the increased NO_3^- loading from the Mississippi River coupled with reduced N:Si ratios have been presented as causative factors leading to increased abundance of potentially toxic *Pseudo-nitzschia* spp. in the northern Gulf of Mexico (Parsons et al., 2002).

In summary, *K. brevis* populations may derive nutrients as DON from the cyanobacterial blooms and other aquatic food web sources such as decomposition of fish killed by *K. brevis*, as well as land-based sources. Initiation and development of *K. brevis* in open waters along the west Florida shelf are apparently supported by sources within the aquatic food web, whereas in localized nearshore areas, land-based nutrients may also supply a proportion of the nutrients that sustain the blooms. While it is well known that eutrophication in coastal regions is increasing globally as a result of development and fertilizer use (e.g., Cloern, 2001; Glibert et al., 2005a, 2006b; Howarth, 2008; Bricker et al., 2007), clear evidence to support hypotheses about increased bloom frequency and biomass on the west Florida shelf is still not yet available. Blooms of *K. brevis* along the Texas coast, which are influenced by major nutrient loads from the Mississippi River, have been more clearly linked to stimulation from land-based sources, and additional experimental data will help to clarify the strength of that linkage.

2.5 ASP and PSP in California

California coastal HAB problems are dominated by two organisms: *Alexandrium catenella* which produces saxitoxin (STX), the causative agent of PSP, and several *Pseudo-nitzschia* species whose toxic strains produce domoic acid (DA), the causative agent for Amnesic Shellfish Poisoning (ASP;

alternately called Domoic Acid Poisoning). While other HAB species are present, some of which are linked to nutrient loading (e.g., the dinoflagellate *Lingulodinium polyedrum* and the raphidophyte *H. akashiwo*; Kudela and Cochlan, 2000; Herndon et al., 2003), here we emphasize ASP and PSP syndromes that are regularly monitored by state agencies in California.

Unlike many other ecosystems impacted by HABs, the physical, chemical, and ecological characteristics of the coastal waters of California are largely dominated by upwelling. The boundary along the coast between the upwelled water and the warmer adjacent surface water is usually a front with an associated equatorward jet (Smith, 1992). Consequently, upwelling circulation overrides both the nutrient limitation of stratified waters and the light limitation of well-mixed waters (Hood et al., 1992), and generally nourishes these waters with macronutrients in excess of anthropogenic sources.

2.5.1 Eutrophication Linkages

ASP Potentially toxic *Pseudo-nitzschia* spp. are ubiquitous in California coastal waters, and major toxin events often occur over large spatial and temporal scales (Trainer et al., 2000). Prior to 2000, toxic blooms were considered rare and unusual in southern California (Lange et al., 1994); however, in recent years ASP has become increasingly important in southern regions (e.g. Trainer et al., 2000; Busse et al., 2006; Schnetzer et al., 2007). The apparent synchrony of ASP events in California suggests that there must be large-scale forcings, such as upwelling relaxation, responsible for the otherwise coincidental timing of major DA outbreaks.

Multiple factors have been shown to trigger the production of DA by *Pseudo-nitzschia* (Bates, 1998), but the most thoroughly characterized are macronutrient limitation by either PO_4^{3-} or silicate $(Si(OH)_4)$ in cultures (Pan et al., 1996a,b). *Pseudo-nitzschia* previously has been associated with both eutrophication and a reduction in the ratio of N:Si (c.f. review by Bates et al., 1998; Parsons et al., 2002), and in California there is circumstantial evidence that a massive DA event in Monterey Bay in 1998 was triggered by post-El Niño runoff (Scholin et al., 2000). Recently, Anderson et al. (2006) reported a correspondence between limiting Si concentrations, the ratios of $NO_3^-:Si(OH)_4$ and $PO_4^{3-}:Si(OH)_4$ and the concentrations of *Pseudo-nitzschia* and particulate DA, among other factors.

DA production by *Pseudo-nitzschia* spp. has also been linked to iron (Fe) and copper (Cu) stress. These elements may be indirectly linked to anthropogenic changes, as excess availability of Cu is associated with runoff (Ladizinsky, 2003), and decreasing Fe is associated with modifications to stream and river flow (Johnson et al., 2001). Limitation by Fe directly modulates Si:N ratios in diatoms, as DA may serve as an Fe-acquisition mechanism (Rue and Bruland, 2001; Wells et al., 2005). Recent laboratory and field data also demonstrate that *Pseudo-nitzschia* spp. may increase toxicity when using urea as an N source (Howard et al., 2006), which would come predominantly from anthropogenic inputs (Cochlan et al., 2008). Thus, cultural eutrophication may have the unanticipated consequence of both selecting for *Pseudo-nitzschia* spp. and promoting toxin production (Kudela et al., 2008).

Despite correlative evidence for a connection between ASP and coastal runoff and/or eutrophication, studies are, as yet, lacking to test whether there are direct linkages. In general, blooms of *Pseudo-nitzschia* in California occur during anomalously weak (but not absent) upwelling conditions, typically during a transition from excess to limiting macronutrients (Kudela et al., 2004). However, there is no consistent evidence that *Pseudo-nitzschia* blooms are correlated with runoff events, nor is there direct evidence for trace-metal limitation or stimulation of DA during most blooms (Kudela et al., 2004). An apparent south-to-north trend in bloom events in coastal California waters is consistent with large-scale physical forcing, suggesting that the spatial pattern is indicative of a change in environmental conditions.

PSP PSP toxin events associated with *Alexandrium catenella* occur most years (Price et al., 1991), and large-scale outbreaks appear to exhibit a northward temporal trend (Langlois and Smith, 2001). As with ASP, there are correlative links between PSP occurrences in California and cultural eutrophication, but direct linkages have not yet been established. PSP outbreaks typically initiate on the open coast, and only then move into bays and estuaries (Langlois, 2001). A consistent pattern associated with PSP events is an increase of *A. catenella* in offshore waters, followed by onshore transport during relaxation-favorable winds, with subsequent intoxication of shellfish (Price et al., 1991; Langlois and Smith, 2001). Thus, as with ASP, PSP events appear to be correlated with large-scale oceanographic events, in particular the upwelling-relaxation cycle and the onshore transport of toxic cells.

In summary, both PSP and ASP dynamics in California appear to be dominated by large-scale oceanographic forcings in nutrient dynamics. This does not, however, preclude the possibility that the growth of these algae, their toxicity, and the frequency or duration of toxic events may be exacerbated by anthropogenic nutrient inputs once these populations reach nearshore waters. For example, there is both direct (Cochlan et al., 2008) and indirect (Collos et al., 2004) evidence that some toxic strains of *Alexandrium catenella* and *Pseudo-nitzschia* spp. can use DON for growth, such as urea (presumed to be from anthropogenic sources) that has been measured in appreciable concentrations in California nearshore waters (Cochlan et al., 2008). Although the extent to which such nutrient pulses occur during natural upwelling or runoff is not known for this region, their utilization by HAB species normally found in coastal California suggests that anthropogenic N sources could be potential factors in bloom initiation or maintenance. As with the Gulf of Maine, these localized blooms are significant since they generally occur in populated coastal zones, despite the fact that the total flux from these nutrient sources likely is a minor component in comparison to seasonal upwelling inputs along the open coast (Kudela et al., 2008).

2.6 PSP, ASP, and *Heterosigma akashiwo* in the Pacific Northwest

The dinoflagellate *Alexandrium catenella* is responsible for shellfish harvesting closures in the Pacific Northwest due to PSP toxins. The occurrence of PSP toxins in Washington shellfish was once restricted to the open coast, the Strait of Juan de Fuca and northern Puget Sound. A large bloom originating in the Whidbey Island basin in 1978 spread through large areas of central Puget Sound that were previously unaffected (Nishitani and Chew, 1988; Rensel, 1993) and since then PSP incidents have progressed southward into the remaining inlets of southern Puget Sound (Trainer et al., 2002).

More than ten *Pseudo-nitzschia* spp. are found in the oceanic and inland waterways of Washington. The primary species believed to cause domoic acid DA-related shellfish harvesting closures are *P. pseudodelicatissima*, *P. cuspidata*, and *P. australis* (Trainer et al., 2007). Since 1991, domoic acid has been a recognized problem on the outer Washington coast (e.g., Trainer and Bill, 2004), but over this same period low levels of DA have been measured in Puget Sound shellfish (Trainer et al., 1998). Puget Sound is presumed to be less susceptible to DA closures due to the absence of DA-retaining razor clams in this region.

Fish-killing blooms of the raphidophyte *H. akashiwo* have occurred in Puget Sound and adjacent inland waters of British Columbia, Canada for an unknown period. This alga was observed in the 1960s before marine fish farms were first installed in either region. All evidence from Puget Sound to date indicates that blooms originate in areas remote from the fish farms such as the U.S.-Canada border waters of North Puget Sound and the southern Georgia Strait or in shallow backwaters of central and

northern Puget Sound. The blooms are transported by estuarine circulation (seaward moving brackish river plume) tidal action and winds to net-pen locations in Puget Sound where finfish have been killed (Rensel, 2007).

In Washington inland waterways, there is evidence that both flagellate and non-flagellate HAB species are expanding their scope and magnitude, in some cases possibly due to anthropogenic influences (below). Most of this area is naturally replete with high levels of DIN (10 to 25 µM) due to oceanic upwelling and advection into inland marine waters, but there are seasonally nutrient-sensitive backwaters where HAB development may be related to human-caused perturbations as discussed below.

2.6.1 Eutrophication Linkages Along the open coast, episodic upwelling is the dominant source of nutrients to surface waters, and thus blooms of *A. catenella* and potentially toxic *Pseudo-nitzschia* spp. in those areas are not likely to be sensitive to anthropogenic influence. However, there are two general systems where the linkage between HABs and nutrient pollution in the Pacific Northwest should be examined more closely. Within Puget Sound and the adjacent Strait of Juan de Fuca, inorganic N concentrations are usually high year-round, and are considered saturating for phytoplankton growth except in poorly flushed terminal inlets which make up only ~7% of the area (Mackas and Harrison, 1997). Anthropogenic sources likely have contributed to the elevated NH_4^+ concentrations commonly found in surface waters of the region (W. Cochlan, unpubl. data; Rensel, 2007). Ambient concentrations of reduced N substrates (such as NH_4^+ and urea) are generally about 10-fold lower than NO_3^-, which is primarily of oceanic origin within Puget Sound. In the absence of directed studies to test influences of anthropogenic nutrient enrichment, linking nutrient loading to blooms of *H. akashiwo* remains an elusive possibility in the Pacific Northwest.

With regard to PSP, some anthropogenic stimulation may be occurring since the magnitude, frequency and geographical distribution of shellfish bed closures from PSP toxins have increased over the past 50 years since monitoring began (Trainer et al., 2003). Comparison of maximum yearly PSP toxin values for Puget Sound (averaged for each of the last 5 decades using all shellfish monitoring data) with population estimates of the counties bordering Puget Sound shows a high correlation. The data suggest that increased nutrient loadings to Puget Sound may be contributing to the spatial and temporal increases in PSP in nutrient-sensitive regions that are vertically stratified in the summer months and poorly flushed.

For the occurrence of DA closures, there also may be a linkage to anthropogenic nutrients within Puget Sound. Several species of *Pseudo-nitzschia* are present in Puget Sound and have been for decades (Bill et al., 2006; Horner, 2003), but only since 2003 has their toxicity resulted in shellfish harvesting closures (Bill et al., 2006; Trainer et al., 2007). One possibility is that more potentially toxic strains have entered Puget Sound from offshore initiation sites such as the Juan de Fuca Eddy (Trainer et al., 2002) via occasional summertime reverse flow of surface waters along the south shore of the Strait of Juan de Fuca. It is also possible that environmental factors within the inland waters are stimulating *Pseudo-nitzschia* growth and/or DA production as discussed below.

Aquaculture activities (e.g., fish farms) have been suggested to be stimulatory to the raphidophycean flagellate *H. akashiwo* and eutrophication has been linked to *H. akashiwo* blooms in various locations worldwide (e.g., Wang et al., 2008). In Puget Sound, however, where fish farms are few in number, the evidence is to the contrary. Salmon excrete mostly NH_3 and low levels of urea (Brett and Zala, 1975), but elevated concentrations of reduced N substrates have not been measured downstream of the fish

farms in Puget Sound. NH_4^+ found within the pens is partly oxidized to NO_3^- and diluted within a few meters downstream so that the DIN increase represents only about 5% of the high background flux levels at farm sites (Rensel, 1991).

All commercial fish farms in Puget Sound are, by state aquatic lease requirement, located in non-nutrient sensitive (N-replete) areas (SAIC, 1986) where sunlight, not macronutrients, limits phytoplankton productivity. Certain subareas of Puget Sound are seasonally nutrient-sensitive either by short-term, river-induced vertical stratification during the spring or by summer and fall solar-heating induced vertical stratification of backwaters that allows nutrient stripping of DIN by algae in the surface mixed layer. As *H. akashiwo* cells can migrate to sub-surface, nutrient-rich waters, they might potentially have a competitive advantage over non-motile species. Only some blooms (e.g., Rensel, 1995) occur in such conditions, however, whereas others occur, or at least are transported through, moderately N-depleted mixed layers (Rensel, 2007). The geographic scale of many blooms of *H. akashiwo* (affecting nearly all of Puget Sound in several cases), the brevity of the blooms (a few days to at most a week in duration), and the apparent dependency on warm weather periods and physical transport mechanisms all highlight the importance of naturally occurring physical forcing factors (Rensel, 2007).

Isolates of both *H. akashiwo* and *P. cuspidata* from the Pacific Northwest can grow equally well on NH_4^+ and NO_3^- when supplied as the sole N source (Auro et al., 2006; Herndon and Cochlan, 2007; Auro, 2007), so there is a potential for anthropogenic stimulation of these blooms. For example, several *H. akashiwo* blooms in central Puget Sound have co-occurred precisely with summertime municipal sewage spills into nutrient-sensitive backwater areas in Kitsap County, Washington (Rensel, 2007). Likewise, Trainer et al. (2007) suggested that a toxic bloom of *P. pseudodelicatissima* developed following a pulse of inorganic N (up to 13 μM NO_3^- and 13 μM NH_4^+) after weeks of N-limited conditions in Sequim Bay in 2005. They speculated that a failing septic system was the source of the elevated NH_4^+ concentration in this poorly flushed bay.

In summary, anthropogenic sources of nutrients are considered insignificant for HABs along the outer coast of Washington, but within Puget Sound and the Strait of Juan de Fuca, the potential contribution of pollution-related loading to HAB development remains to be fully understood and quantified. The ability of *H. akashiwo* and *Pseudo-nitzschia* spp. to achieve good growth by using various N sources suggests that reduced N may contribute to their recent success. In these and other cases, the spread of a toxic HAB species into or within new areas such as Puget Sound may be linked to anthropogenic nutrient sources, but as yet directed studies to confirm or refute a link are largely lacking.

2.7 Ciguatera

Ciguatera fish poisoning (CFP) is a circumtropical seafood poisoning caused by the ingestion of marine fish (especially reef fish) that have accumulated toxins produced by species of the benthic dinoflagellate genus, *Gambierdiscus*, especially *G. toxicus* (Yasumoto et al., 1977) and possibly other co-occurring dinoflagellates (e.g., *Ostreopsis llenticularis*; Tosteson et al., 1986).

2.7.1 Eutrophication Linkages The role played by nutrient enrichment in CFP remains unclear because the topic is understudied. Fewer than ten known field-based studies have measured nutrient concentrations concurrently with the abundance of ciguatera dinoflagellates, and only four of these studies were conducted for at least 1 year and reported statistical results (Yasumoto et al., 1980; Carlson and Tindall, 1985; Parsons and Preskitt, 2007). Of these, Carlson and Tindall (1985) reported a significant, positive correlation between the abundance of ciguatera dinoflagellates and nutrient

concentrations. Parsons and Preskitt (2007) did not report a positive correlation in their study of six Hawaiian sites, but *Gambierdiscus* concentrations were highest at the site (Puako) exhibiting the highest amounts of $NO_3^- + NO_2^-$.

Culture-based studies also have been limited. Lechat et al. (1985) reported that growth rates of *G. toxicus* were greater when using higher media enrichments (ES 4%) versus more diluted enrichments (ES 1% and 2%). Durand-Clement (1986) reported that growth of *G. toxicus* increased when the urea concentration was increased from 0 to 1 mM, although this concentration is higher than would be expected in natural habitats. Sperr and Doucette (1996) reported that growth rates did not vary over a range of N:P ratios from 5:1 to 50:1.

The role of nutrients in toxin production is unclear as well. Durant-Clement (1986) reported no differences in toxin production across different media recipes or nutrient concentrations. In contrast, Lechat et al. (1985) indicated that cellular ciguatoxin content increased 10-fold when the metallic salt content (PII-mix) was increased 10-fold. Bomber (1987) did not discern any difference in cellular toxicity for *G. toxicus* grown in regular ES media and low-N ES media. Cellular toxicity also was constant over the range of N:P ratios used by Sperr and Doucette (1996), although toxin levels spiked at an N:P of 30:1 for an unknown reason.

Ciguatera dinoflagellates have two potential sources of nutrients, the water column and benthic substrata. Since they are primarily epiphytic, they can obtain nutrients from macroalgal substatra or co-occurring epiphytes (i.e., the thallisphere; Yasumoto et al., 1980; Withers, 1981). The most li kely role of eutrophication in CFP is through coral reef degradation (Bagnis, 1994). Researchers early on noted that CFP outbreaks often followed disturbances to coral reef environments (e.g., Randall, 1958; Cooper, 1964). The role of nutrient loading in coral reef degradation is complex and some reef sites appear to be more susceptible to nutrient degradation than others (Lapointe, 1999; Parsons et al., 2008). Eutrophication can cause an increase in macroalgae (Lapointe et al., 2004; Smith et al., 2001), providing more habitat for ciguatera dinoflagellates.

In summary, the limited evidence available indicates that any linkages between eutrophication and increased incidents of CFP remain inconclusive. There may be indirect linkages between eutrophication and increased growth of ciguatera dinoflagellates, but further assessment is required to determine the strength of these linkages, and the potential for direct links remains to be examined. Outbreaks of CFP often follow impacts to coral reef ecosystems of which nutrient loading can be a factor, including the expansion of macroalga-based habitat. As ciguatera dinoflagellates are benthic organisms, rigorous assessment of eutrophication linkages will require analysis of not only water-column nutrient loadings, but also of indirect effects mediated through benthic nutrient sources.

3. Conclusions

HAB events in the U.S. are diverse in many ways, spanning a wide range of algal species, poisoning syndromes, and other negative impacts. A common observation is that the initation of large-scale HABs along *open coasts* appears to be unrelated to anthropogenic nutrients, since nutrients supplied by upwelling or advection from offshore water masses (e.g., New England and the Pacific Northwest), or by N_2 fixed from co-occurring blooms (e.g., Gulf of Mexico) can be much larger than terrestrial or atmospheric sources, although data on the diversity of nutrient sources (including organic) are often lacking in these regions. *Localized, nearshore* pollution effects are possible in embayments, estuaries, and sounds, and thus HAB cells delivered to these locations from open waters can be stimulated and sustained by anthropogenic inputs. In regions where HABs originate within estuaries, embayments or nearshore coasts, the role of anthropogenic nutrients is much larger, and in some cases, a dominant

factor in HAB species success. Overall, there is an obvious need for additional research on the relationship between HABs and eutrophication in U.S. estuaries and coastal waters, including many species and regions not covered here. In particular, there is a need for additional information on the fluxes of nutrients (not only concentrations), the role of varying nutrient composition in HAB proliferation, and the interactions between nutrient loads and the complexity of other factors contributing to HABs.

Acknowledgements

Footnotes

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

[1] *Alexandrium tamarense* and *A. fundyense* frequently co-occur and are considered to be varieties of the same species (Anderson et al., 1994). The name *A. fundyense* will be used to refer to both forms.

References

1. Adolf JE, Bachvaroff T, Place AR. Does cryptophyte abundance trigger toxic *Karlodinium veneficum* blooms in eutrophic environments? Harmful Algae. 2008 this issue. [Google Scholar]
2. Adolf JE, Stoecker DK, Harding LW. The balance of autotrophy and heterotrophy during mixotrophic growth of *Karlodinium micrum* (Dinophyceae) J.Plank. Res. 2006;28:737–751. [Google Scholar]
3. Anderson CR, Brzenzinski MA, Washburn L, Kudela R. Circulation and environmental conditions during a toxigenic *Pseudo-nitzschia australis* bloom in the Santa Barbara Channel, California. Mar. Ecol. Prog. Ser. 2006;327:119–133. [Google Scholar]
4. Anderson DM. Toxic algal blooms and red tides: A global perspective. In: Okaichi T, Anderson DM, Nemoto T, editors. Red Tides: Biology, Environmental Science and Toxicology. Elsevier; 1989. pp. 11–16. [Google Scholar]
5. Anderson DM. Bloom dynamics of toxic *Alexandrium* species in the northeastern United States. Limnol. Oceanogr. 1997;42:1009–1022. [Google Scholar]

6. Anderson DM, Glibert PM, Burkholder JM. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries. 2002;25(4b):562–584. [Google Scholar]

7. Anderson DM, Keafer BA, Kulis DM, Waters RM, Nuzzi R. An immunofluorescent survey of the brown tide chrysophyte *Aureococcus anophagefferens* along the northeast coast of the United States. J. Plank. Res. 1993;15:563–580. [Google Scholar]

8. Anderson DM, Keafer BA, McGillicuddy DJ, Mickelson MJ, Keay KE, Libby PS, Manning JP, Mayo CA, Whittaker DK, Hickey JM, He R, Lynch DR, Smith KW. Initial observations of the 2005 *Alexandrium fundyense* bloom in southern New England: General patterns and mechanisms. Deep-Sea Res. II. 2005a;52(19–21):2856–2876. [Google Scholar]

9. Anderson DM, Kulis DM, Doucette GJ, Gallager JC, Balech E. Biogeography of toxic dinoflagellates in the genus *Alexandrium* from the northeast United States and Canada as determined by morphology, bioluminescence, toxin composition, and mating compatibility. Mar. Biol. 1994;120:467–478. [Google Scholar]

10. Anderson DM, Stock CA, Keafer BA, Bronzino Nelson A, Thompson B, McGillicuddy DJ, Keller M, Matrai PA, Martin J. *Alexandrium fundyense* cyst dynamics in the Gulf of Maine. Deep-Sea Res. II. 2005b;52(19–21):2522–2542. [Google Scholar]

11. Anderson DM, Townsend DW, McGillicuddy DJ, Turner JT, editors. The ecology and oceanography of toxic *Alexandrium fundyense* blooms in the Gulf of Maine. Deep-Sea Res. II. 2005c;52(19–21):2365–2876. [Google Scholar]

12. Aneja VP, Nelson DR, Roelle PA, Walker JT. Agricultural ammonia emissions and ammonium concentrations associated with aerosols and precipitation in the southeast United States. J. Geophys. Res. 2003;108(D4):ACD12-1–ACH12-11. [Google Scholar]

13. Auro ME. M.S. Thesis. San Francisco, California, USA: San Francisco State University; 2007. Nitrogen dynamics and toxicity of the pennate diatom *Pseudo-nitzschia cuspidata*: A field and laboratory study; p. 91. [Google Scholar]

14. Auro ME, Cochlan WP, Trainer VL. Nitrogen dynamics of *Pseudo-nitzschia cuspidata* from the U.S. Pacific Northwest. Twelfth International Conference on Harmful Algae; September 2006; Copenhagen, Denmark. 2006. (Abstract) [Google Scholar]

15. Bagnis R. Natural versus anthropogenic disturbances to coral reefs: comparison in epidemiological patterns of ciguatera. Mem. Queensland Mus. 1994;34:455–460. [Google Scholar]

16. Bates SS. Ecophysiology and metabolism of ASP toxin production. In: Anderson DM, Cembella AD, Hallegraeff GM, editors. Physiological ecology of harmful algal blooms. Heidelberg: Springer-Verlag; 1998. pp. 405–426. [Google Scholar]

17. Bates SS, Garrison DL, Horner RA. Bloom dynamics and physiology of domoic-acid-producing *Pseudo-nitzschia* species. In: Anderson DM, Cembella AD, Hallegraeff GM, editors. Physiological ecology of harmful algal blooms. Heidelberg: Springer-Verlag; 1998. pp. 267–292. [Google Scholar]

18. Berg GM, Glibert PM, Lomas MW, Burford M. Organic nitrogen uptake and growth by the Chrysophyte *Aureococcus anophagefferens* during a brown tide event. Mar. Biol. 1997;129:377–387. [Google Scholar]

19. Berg GM, Repeta DJ, LaRoche J. Dissolved organic nitrogen hydrolysis rates in axenic cultures of *Aureococcus anophagefferens* (Pelagophyceae): comparison with heterotrophic bacteria. Appl. Environ. Microbiol. 2002;68:401–404. [PMC free article] [PubMed] [Google Scholar]

20. Berg GM, Repeta DJ, LaRoche J. The role of the picoeukaryote *Aureococcus anophagefferens* in cycling of marine high-molecular weight dissolved organic nitrogen. Limnol. Oceanogr. 2003;48:1825–1830. [Google Scholar]

21. Biegalski SR, Villareal T. Correlations between atmospheric aerosol trace element concentrations and red tide at Port Aransas, Texas, on the Gulf of Mexico. J. Radioanal. Nucl. Chem. 2005;263:997–1005. [Google Scholar]

22. Bill BD, Cox FH, Horner RA, Borchert JA, Trainer VL. The first closure of shellfish harvesting due to domoic acid in Puget Sound, Washington, USA. Afr. J. Mar. Sci. 2006;28(2):437–442. [Google Scholar]

23. Bodeanu N, Ruta G. Development of the planktonic algae in the Romanian Black Sea sector in 1981–1996. In: Reguera B, Blanco J, Fernandez ML, Wyatt T, editors. Harmful Algae. Paris, France: Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO; 1998. pp. 188–191. [Google Scholar]

24. Bomber JW. Ph.D. dissertation. Melbourne, Fl: Department of Oceanography and Ocean Engineering, Florida Institute of Technology; 1987. Ecology, genetic variability, and physiology of the ciguatera-causing dinoflagellate *Gambierdiscus toxicus* Adachi et Fukuyo; p. 147. [Google Scholar]

25. Brand LE. The transport of terrestrial nutrients to south Florida coastal waters. In: Porter JW, Porter KG, editors. The Everglades, Florida Bay and Coral Reefs of the Florida Keys, An Ecosystem Sourcebook. Boca Raton, Fl: CRC Press; 2002. pp. 361–413. [Google Scholar]

26. Brand LE, Compton A. Long-term increase in *Karenia brevis* abundance along the Southwest Florida Coast. Harmful Algae. 2007;6(2):232–252. [PMC free article] [PubMed] [Google Scholar]

27. Brett JR, Zala CA. Daily patterns of nitrogen excretion and oxygen consumption of sockeye salmon (*Oncorhynchus nerka*) under controlled conditions. J. Fish. Res. Board Can. 1975;32:2479–2486. [Google Scholar]

28. Bricker SB, Longstaff B, Dennison W, Jones A, Boicourt K, Wicks C, Woerner J. A decade of change. NOAA Coastal Ocean Program Decision Analysis Series No. 26. Silver Spring, MD: National Centers for Coastal Ocean Service; 2007. Effects of nutrient enrichment in the Nation's estuaries: A decade of change; p. 328. [Google Scholar]

29. Bricker SB, Longstaff B, Dennison W, Jones A, Boicourt K, Wicks C, Woerner J. Effects of nutrient enrichment in the Nation's estuaries: A decade of change. Harmful Algae. 2008 this issue. [Google Scholar]

30. Bronk DA, Sanderson MP, Mulholland MR, Heil CA, O'Neil JM. Organic and inorganic nitrogen uptake kinetics in field populations dominated by *Karenia brevis*. In: Steidinger KA, Landsberg JH, Tomas CR, Vargo GA, editors. Harmful Algae 2002. St. Petersburg, Florida: Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commissionof UNESCO; 2004. pp. 80–82. [Google Scholar]

31. Burkholder JM. Implications of harmful microalgae and heterotrophic dinoflagellates in management of sustainable marine fisheries. Ecol. Appl. 1998;8:S37–S62. [Google Scholar]

32. Burkholder JM, Glasgow HB. *Pfiesteria piscicida* and other *Pfiesteria*-like dinoflagellates: behavior, impacts and environmental controls. Limnol. Oceanogr. 1997;42:1052–1075. [Google Scholar]

33. Burkholder JM, Dickey DA, Kinder C, Reed RE, Mallin MA, Melia G, McIver MR, Cahoon LB, Brownie C, Deamer N, Springer J, Glasgow H, Toms D, Smith J. Comprehensive trend analysis of nutrients and related variables in a large eutrophic estuary: A decadal study of anthropogenic and climatic influences. Limnol. Oceanogr. 2006;51:463–487. [Google Scholar]

34. Burkholder JM, Glasgow HB, Deamer-Melia NJ. Overview and present status of the toxic *Pfiesteria* complex. Phycologia. 2001;40:186–214. [Google Scholar]

35. Burkholder JM, Glibert PM, Skelton HM. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. Harmful Algae. 2008 this issue. [Google Scholar]

36. Burkholder JM, Gordon AS, Moeller PD, Law JM, Coyne KJ, Lewitus AJ, Ramsdell JS, Marshall HG, Deamer NJ, Cary SC, Kempton JW, Morton SL, Rublee PA. Demonstration of toxicity to fish and to mammalian cells by *Pfiesteria* species: Comparison of assay methods and multiple strains. Proc. Natl. Acad. Sci. (U.S.A) 2005;102:3471–3476. [PMC free article] [PubMed] [Google Scholar]

37. Burkholder JM, Mallin MA, Glasgow HB, Jr, Larsen LM, McIver MR, Shank GC, Deamer-Melia N, Briley DS, Springer J, Touchette BW, Hannon EK. Impacts to a coastal river and estuary from rupture of a large swine waste holding lagoon. J. Env. Qual. 1997;26:1451–1466. [Google Scholar]

38. Buskey EJ, Liu H, Collumb C, Bersano JGF. The decline and recovery of a persistent Texas brown tide algal bloom in the Laguna Madre (Texas, USA) Estuaries. 2001;24:337–346. [Google Scholar]

39. Buskey EJ, Stewart J, Peterson J, Collumb C. Tex. Nat. Resour. Conserv. Comm. Austin: Rep. CCBNEP-07; 1996. Current status and historical trends of brown tide and red tide phytoplankton blooms in the Corpus Christi Bay National Estuary Program study area; p. 174. [Google Scholar]

40. Busse LB, Venrick EL, Antrobus R, Miller PE, Vigilant V, Silver MW, Mengelt C, Mydlarz L, Prezelin BB. Domoic acid in phytoplankton and fish in San Diego, CA, USA. Harmful Algae. 2006;5:91–101. [Google Scholar]

41. Carlson RD, Tindall DR. Distribution and periodicity of toxic dinoflagellates in the Virgin Islands. In: Anderson DM, White AW, Baden DG, editors. Toxic Dinoflagellates. New York: Elsevier Science Publishing Co., Inc.; 1985. pp. 171–177. [Google Scholar]

42. Cloern JE. Our evolving conceptual model of the coastal eutrophication problem. Mar. Ecol. Prog. Ser. 2001;210:223–253. [Google Scholar]

43. Cochlan WP, Herndon J, Kudela RM. Inorganic and organic nitrogen uptake by the toxigenic diatom *Pseudo-nitzschia australis* (Bacillariophyceae) Harmful Algae. 2008 this issue. [Google Scholar]

44. Collos Y, Gagne C, Laabir M, Vaquer A, Cecchi P, Souchu P. Nitrogenous nutrition of *Alexandrium catenella* (Dinophyceae) in cultures and in Thau lagoon, southern France. J. Phycol. 2004;40:96–103. [Google Scholar]

45. Cooper MJ. Ciguatera and other marine poisoning in the Gilbert Islands. Pac. Sci. 1964;18:411–440. [Google Scholar]

46. Cosper EM, Dennison W, Milligan A. An examination of environmental factors important to initiating and sustaining "brown tide" blooms. In: Cosper EM, Bricelj VM, Carpenter EJ, editors. Novel phytoplankton blooms: Causes and impacts of recurrnet brown tides and other unusual blooms. Berlin: Springer-Verlag; 1989. pp. 317–340. [Google Scholar]

47. Deeds JR, Terlizzi DE, Adolf JE, Stoecker DK, Place AR. Toxic activity from cultures of *Karlodinium micrum* (=*Gyrodinium galatheanum*) (Dinophyceae) - a dinoflagellate associated with fish mortalities in an estuarine aquaculture facility. Harmful Algae. 2002;1:169–189. [Google Scholar]

48. del Amor FM, Navarro J, Aparicio PM. Isotopic discrimination as a tool for organic farming certification in sweet pepper. J. Env. Qual. 2008;37:182–185. [PubMed] [Google Scholar]

49. Denton W, Contreras C. Water Qual. Tech. Ser. WQTS-2004-01. Austin: Tex. Parks and Wildlife Dept.; 2004. The red tide (*Karenia brevis*) bloom of 2000. [Google Scholar]

50. Deonarine SN, Gobler CJ, Lonsdale DJ, Caron DA. The role of zooplankton the occurrence of harmful brown tide blooms (*Aureococcus anophagefferens*) in US mid-Atlantic estuaries. Aquat. Microb. Ecol. 2006;44:181–195. [Google Scholar]

51. Durand-Clement M. A study of toxin production by *Gambierdiscus toxicus* in culture. Toxicon. 1986;24:1153–1157. [PubMed] [Google Scholar]

52. [EPA] U.S. Environmental Protection Agency. Environmental impacts of animal feeding operations; EPA Office of Water, Standards, and Applied Sciences Division; 1998. [Google Scholar]

53. [EPA] U.S. Environmental Protection Agency. Deposition of air pollutants to the Great Waters. 3rd report to the U.S. Congress, (1) Section A, Chapter 4- water quality issues related to multiple watersheds in the Neuse River Basin. 2000

54. Fournier RO, Marra J, Bohrer R, Van Det M. Plankton dynamics and nutrient enrichment of the Scotian Shelf. J. Fish. Res. Board Can. 1977;34:1004–1018. [Google Scholar]

55. Gastrich MD, Bell JL, Gobler CJ, Anderson OR, Wilhelm SW. Viruses as potential regulators of regional brown tide blooms caused by the alga, *Aureococcus anophagefferens:* a comparison of bloom years 1999–2000 and 2002. Estuaries. 2004;27:112–119. [Google Scholar]

56. Giblin AE, Gaines AG. Nitrogen inputs to a marine embayment: the importance of groundwater. Biogeochem. 1990;10:309–328. [Google Scholar]

57. Glasgow HB, Burkholder JM, Mallin MA, Deamer-Melia NJ, Reed RE. Field ecology of toxic *Pfiesteria* complex species, and a conservative analysis of their role in estuarine fish kills. Env. Health Perspect. 2001;109:715–730. [PMC free article] [PubMed] [Google Scholar]

58. Glibert P, editor. HABs in Eutrophic Systems. Paris and Baltimore: IOC and SCOR; 2006. GEOHAB, Global Ecology and Oceanography of Harmful Algal Blooms Programme; p. 74. [Google Scholar]

59. Glibert PM, Alexander J, Trice TM, Michael B, Magnien RE, Lane L, Oldach D, Bowers H. Chronic urea nitrogen loading: A correlate of *Pfiesteria* spp. in the Chesapeake and Coastal Bays of Maryland, USA. In: Steidinger KA, Landsberg JH, Tomas CR, Vargo GA, editors. Harmful Algae 2002; Proceedings of the Xth International Conference on Harmful Algae; Florida Fish and Wildlife Conservation Commission and Intergovernmental Oceanographic Commission of UNESCO; 2004a. pp. 74–76. [Google Scholar]

60. Glibert PM, Burkholder JM. The complex relationships between increasing fertilization of the earth, coastal eutrophication and proliferation of harmful algal blooms. In: Granéli E, Turner J, editors. Ecology of Harmful Algae. Springer; 2006. pp. 354–354. [Google Scholar]

61. Glibert PM, Burkholder JM, Kana TM, Alexander J, Skelton H, Shilling C. Grazing by *Karenia brevis* on *Synechococcus* enhances its growth rate and may help to sustain blooms. Aquat. Microb. Ecol. submitted. [Google Scholar]

62. Glibert PM, Burkholder JM, Parrow MW, Lewitus AJ, Gustafson D. Rates of direct uptake of nitrogen and nitrogen nutritional preferences by functional types of *Pfiesteria piscicida* and *Pfiesteria shumwayae*. Harmful Algae. 2006a;5:380–394. [Google Scholar]

63. Glibert PM, Harrison J, Heil C, Seitzinger S. Escalating worldwide use of urea – a global change contributing to coastal eutrophication. Biogeochem. 2006b;77:441–463. [Google Scholar]

64. Glibert PM, Heil CA, Hollander D, Revilla M, Hoare A, Alexander J, Murasko S. Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. Mar. Ecol. Prog. Ser. 2004a;280:73–83. [Google Scholar]

65. Glibert PM, Magnien R, Lomas MW, Alexander J, Fan C, Haramoto E, Trice M, Kana TM. Harmful algal blooms in the Chesapeake and Coastal Bays of Maryland, USA: Comparisons of 1997, 1998, and 1999 events. Estuaries. 2001;24:875–883. [Google Scholar]

66. Glibert PM, Mayorga E, Seitzinger S. *Prorocentrum minimum* tracks anthropogenic nitrogen and phosphorus inputs on a global basis: application of spatially explicit nutrient export models. Harmful Algae. 2008 this issue. [Google Scholar]

67. Glibert PM, Seitzinger S, Heil CA, Burkholder JM, Parrow MW, Codispoti LA, Kelly V. The role of eutrophication in the global proliferation of harmful algal blooms. Oceanography. 2005a:198–209. [Google Scholar]

68. Glibert PM, Trice TM, Michael B, Lane L. Urea in the tributaries of the Chesapeake and Coastal Bays of Maryland. Water, Air and Soil Poll. 2005b;160:229–243. [Google Scholar]

69. Glibert PM, Wazniak CE, Hall M, Sturgis B. Seasonal and interannual trends in nitrogen in Maryland's Coastal Bays and relationships with brown tide. Ecol. Appl. 2007;17(5):S79–S87. [Google Scholar]

70. Gobler CJ, Boneillo GE, Debenham C, Caron DA. Nutrient limitation, organic matter cycling, and plankton dynamics during an *Aureococcus anophagefferens* bloom in Great South Bay, NY. Aquat. Microb. Ecol. 2004;35:31–43. [Google Scholar]

71. Gobler CJ, Lonsdale DJ, Boyer GL. A synthesis and review of causes and impact of harmful brown tide blooms caused by the alga, *Aureococcus anophagefferens*. Estuaries. 2005;28:726–749. [Google Scholar]

72. Gobler CJ, Renaghan MJ, Buck NJ. Impacts of nutrients and grazing mortality on the abundance of *Aureococcus anophagefferens* during a New York brown tide bloom. Limnol. Oceanogr. 2002;47:129–141. [Google Scholar]

73. Gobler CJ, Sañudo-Wilhelmy SA. Effects of organic carbon, organic nitrogen, inorganic nutrients, and iron additions on the growth of phytoplankton and bacteria during a brown tide bloom. Mar. Ecol. Prog. Ser. 2001a;209:19–34. [Google Scholar]

74. Gobler CJ, Sañudo-Wilhelmy SA. Temporal variability of groundwater seepage and brown tide blooms in a Long Island embayment. Mar. Ecol. Prog. Ser. 2001b;217:299–309. [Google Scholar]

75. Goshorn D, Deeds J, Tango P, Poukish C, Place AR, McGinty M, Butler W, Luckett C, Magnien R. Occurrence of *Karlodinium micrum* and its association with fish kills in Maryland estuaries. In: Steidinger KA, Landsberg JH, Tomas CR, Vargo GA, editors. Harmful Algae 2002; Proceedings of the Xth International Conference on Harmful Algae; Florida Fish and Wildlife Conservation Commission and Intergovernmental Oceanographic Commission of UNESCO; 2004. pp. 361–363. [Google Scholar]

76. Hallegraeff GM. A review of harmful algal blooms and their apparent global increase. Phycologia. 1993;32:79–99. [Google Scholar]

77. Harrison JH, Caraco NF, Seitzinger SP. Global patterns and sources of dissolved organic matter export to the coastal zone: results from a spatially explicit, global model. Global Biogeochem. Cycles. 2005;19:GBS406. [Google Scholar]

78. Havens JJ, Heil CA, Hollander D, Vargo GA, Ault D, Murasko S, Walsh JJ. Isotopic constraints on nutrient sources supporting the 2001 *Karenia brevis* bloom. In: Steidinger KA, Landsberg JH, Tomas CR, Vargo GA, editors. Harmful Algae 2002; Proceedings of the Xth International Conference on Harmful Algae; Paris. Florida Fish and Wildlife Conservation Commission and Intergovernmental Oceanographic Commission of UNESCO; 2004. pp. 32–34. [Google Scholar]

79. Haywood AJ, Steidinger KA, Truby EW, Bergquist PR, Bergquist PL, Adamson J, MacKenzie L. Comparative morphology and molecular phylogenetic analysis of three new species of the genus *Karenia* (Dinophyceae) from New Zealand. J. Phycol. 2004;40:165–179. [Google Scholar]

80. Heil CA, Glibert PM, Fan C. *Prorocentrum minimum* (Pavillard) Schiller –A review of a harmful algal bloom species of growing worldwide importance. Harmful Algae. 2005;4:449–470. [Google Scholar]

81. Heil CA, Revilla M, Glibert PM, Murasko S. Nutrient quality drives differential phytoplankton community composition on the southwest Florida shelf. Limnol. Oceanogr. 2007;52:1067–1078. [Google Scholar]

82. Heil C, Truby E, Wolny J, Pigg R, Richardson B, Garrett M, Haywood A, Petrik K, Flewelling L, Stone E, Cook S, Scott P, Steidinger K, Landsberg J. The multi-species nature of the 2005 *Karenia* bloom in the eastern Gulf of Mexico. Proceedings 12th International Conference on Harmful Algae; Sept 4–9, 2006; Copenhagen. In press. [Google Scholar]

83. Heisler J, Glibert P, Burkholder J, Anderson D, Cochlan W, Dennison W, Gobler C, Dortch Q, Heil C, Humphries E, Lewitus A, Magnien R, Marshall H, Sellner K, Stockwell D, Stoecker D, Suddleson M. Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae. 2008 this issue. [PMC free article] [PubMed] [Google Scholar]

84. Herndon J, Cochlan WP. Nitrogen utilization by the raphidophyte *Heterosigma akashiwo:* growth and uptake kinetics in laboratory cultures. Harmful Algae. 2007;6:260–270. [Google Scholar]

85. Herndon J, Cochlan WP, Horner R. *Heterosigma akashiwo* blooms in San Francisco Bay. Interagency Ecological Program for the San Francisco Estuary Newsletter. 2003;16:46–48. [Google Scholar]

86. Hood RR, Neuer S, Cowles TJ. Autotrophic production, biomass and species composition at two stations across an upwelling front. Mar. Ecol. Prog. Ser. 1992;83:221–232. [Google Scholar]

87. Horner RA. Identification of some *Pseudo-nitzschia* species from western Washington waters. In: Bates SS, editor. Proceedings of the Eighth Canadian Workshop on Harmful Marine Algae Can. Vol. 2498. Tech. Rep. Fish. Aq. Sci.; 2003. pp. 77–79. [Google Scholar]

88. Howard M, Cochlan WP, Ladizinsky N, Kudela RM. Evaluation of California isolates of *Lingulodinium polyedrum* for the production of yessotoxin. Afr. J. Mar. Sci. 2006;28(2):399–402. [Google Scholar]

89. Howarth RW. Coastal nitrogen pollution: A review of sources and trends globally and regionally. Harmful Algae. 2008 this issue. [Google Scholar]

90. Howes BL, Ramsey JS, Kelley SW, Cote JM. Progress report from the 2001 and 2002 field season: water quality monitoring and hydrodynamic modeling. Massachusetts: Final Report to the Town of Orleans; 2003. Water quality and habitat health of the embayment systems of Orleans, Massachusetts; p. 50. [Google Scholar]

91. Hu C, Muller-Karger FE, Swarzenski PW. Hurricanes, submarine groundwater discharge, and Florida's red tides. Geophys. Res. Lett. 2006;33:L11601. [Google Scholar]

92. Hurst JW., Jr History of paralytic shellfish poisoning on the Maine coast. In: LoCicero VR, editor. Toxic dinoflagellate blooms; Proceedings of the International Conference (lst) Massachusetts Science and Technology Foundation; 1975. pp. 525–528. [Google Scholar]

93. Jaworski N. Retrospective of the water quality issues of the upper Potomac estuary. Aquatic Science. 1990;3:11–40. [Google Scholar]

94. Jeong HJ, Park JY, Nho JH, Park MO, Ha JH, Seong KA, Jeng C, Seong CN, Lee KY, Yih WH. Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*. Aq. Microb. Ecol. 2005a;41:1331-143. [Google Scholar]

95. Jeong HJ, Yoo YD, Park JY, Song JY, Kim ST, Lee SH, Kim KY, Yih WH. Feeding by the phototrophic red-tide dinoflagellates: 5 species newly revealed and 6 species previously known to be mixotrophic. Aq. Microb. Ecol. 2005b;40:133–150. [Google Scholar]

96. Johnson KS, Chavez FP, Elrod VA, Fitzwater SE, Pennington JT, Buck KR, Walz PM. The annual cycle of iron and the biological response in central California coastal waters. Geophysical Research Letters. 2001;28:1247–1250. [Google Scholar]

97. Kana TM, Lomas MW, MacIntyre HL, Cornwell JC, Gobler CJ. Stimulation of the brown tide organism, *Aureococcus anophagefferens*, by selective nutrient additions to *in situ* mesocosms. Harmful Algae. 2004;3:377–388. [Google Scholar]

98. Keller AA, Rice RL. Effects of nutrient enrichment on natural populations of the brown tide phytoplankton *Aureococcus anophagefferens* (Chrysophyceae) J. Phycol. 1989;25:636–646. [Google Scholar]

99. Kemp WM, Boynton WR, Adolf JE, Boesch DF, Boicourt WC, Brush G, Cornwell JC, Fisher TR, Glibert PM, Hagy JD, Harding LW, Houde ED, Kimmel DG, Miller WD, Newell RIE, Roman MR, Smith EM, Stevenson JC. Eutrophication in Chesapeake Bay: Historical trends and ecological interactions. Mar. Ecol. Prog. Ser. 2005;303:1–29. [Google Scholar]

100. Kudela RM, Cochlan WP. The kinetics of nitrogen and carbon uptake and the influence of irradiance for a natural population of *Lingulodinium polyedrum* (Pyrrophyta) off southern California. Aquatic. Microbial Ecol. 2000;21:31–47. [Google Scholar]

101. Kudela RM, Cochlan WP, Roberts A. Spatial and temporal patterns of *Pseudo-nitzschia* spp. in central California related to regional oceanography. Harmful Algal Blooms 2002. In: Steidinger KA, Landsberg JH, Tomas CR, Vargo GA, editors. Harmful Algae 2002; Proceedings of the Xth International Conference on Harmful Algae. Florida Fish and Wildlife Conservation Commission and Intergovernmental Oceanographic Commission of UNESCO; 2004. pp. 347–349. [Google Scholar]

102. Kudela RM, Lane JQ, Cochlan WP. The potential role of anthropogenically derived nitrogen in the growth of harmful algae in California, USA. Harmful Algae. 2008 this issue. [Google Scholar]

103. Ladizinsky N. Master's Thesis. Monterey Bay, CA, USA: California State University; 2003. The influence of dissolved copper on the production of domoic acid by *Pseudo-nitzschia* species in Monterey Bay, California: laboratory experiments and field observations; p. 68. [Google Scholar]

104. Lange CB, Reid FMH, Vernet M. Temporal distribution of the potentially toxic diatom *Pseudo-nitzschia australis* at a coastal site in Southern California. Mar. Ecol. Prog. Ser. 1994;104:309–312. [Google Scholar]

105. Langlois G. Marine biotoxin monitoring in California, 1927–1999. In: RaLonde R, editor. Harmful Algae Blooms on the North American West Coast. Univ. Alaska Sea Grant College Program Report No. AK-SG-01-05. 2001. pp. 31–34. [Google Scholar]

106. Langlois G, Smith P. Phytoplankton. In: Karl HA, Chin JL, Ueber E, Stauffer PH, Hendley JW III, editors. Beyond the Golden Gate—Oceanography, geology, biology and environmental issues in the Gulf of the Farallones. Vol. 1198. U.S. Geological Survey Circular; 2001. pp. 123–132. [Google Scholar]

107. Lapointe BE. Simultaneous top-down and bottom-up forces control macroalgal blooms on coral reefs. Limnol. Oceanogr. 1999;44:1586–1592. [Google Scholar]

108. Lapointe BE, Barile PJ, Yentsch CS, Littler MM, Littler DS, Kakuk B. The relative importance of nutrient enrichment and herbivory on macroalgal communities near Norman's Pond Cay, Exumas Cays, Bahamas: a "natural" enrichment experiment. J. Exp. Mar. Biol. Ecol. 2004;298:275–301. [Google Scholar]

109. LaRoche J, Nuzzi R, Waters R, Wyman K, Falkowski PG, Wallace DWR. Brown tide blooms in Long Island's coastal waters linked to interannual variability in groundwater flow. Glob. Change. Biol. 1997;3:397–410. [Google Scholar]

110. Lechat I, Partenski F, Chungue E. *Gambierdiscus toxicus*: Culture and toxin production. In: Delesalle B, Galzin R, Salvat B, editors. Proceedings of the Fifth International Coral Reef Congress, Tahiti, Antenne Museum-EPHE, Moorea (French Polynesia) 1985. pp. 443–448. [Google Scholar]

111. Lehman PW, Boyer G, Hall C, Waller S, Gehrts K. Distribution and toxicity of a new colonial *Microcystis aeruginosa* bloom in San Francisco Bay estuary, California. Hydrobiol. 2005;541:87–99. [Google Scholar]

112. Lewitus A, Brock LM, Burke MK, DeMattio KAS, Wilde SB. Lagoonal stormwater detention ponds as promoters of harmful algal blooms and eutrophication along the South Carolina coast. Harmful Algae. 2008 this issue. [Google Scholar]

113. Lewitus AJ, Burkholder JM, Glasgow HB, Jr, Glibert PM, Willis BM, Hayes KC. Mixotrophy and nitrogen uptake by *Pfiesteria piscicida* (Dinophyceae) J. Phycol. 1999a;35:1430–1437. [Google Scholar]

114. Lewitus AJ, Glasgow HB, Burkholder JM. Kleptoplastidy in the toxic dinoflagellate, *Pfiesteria piscicida*. J. Phycol. 1999b;35:303–312. [Google Scholar]

115. Lewitus AJ, Schmidt LB, Mason LJ, Kempton JW, Wilde SB, Wolny JL, Williams BJ, Hayes KC, Hymel SN, Keppler CJ, Ringwood AH. Harmful algal blooms in South Carolina residential and golf course ponds. Population Environ. 2003;24:387–413. [Google Scholar]

116. Li Y, Smayda TJ. *Heterosigma akashiwo* (Raphidophyceae): On prediction of the week of bloom initiation and maximum during the initial pulse of its bimodal bloom cycle in Narragansett Bay. Plank. Biol. Ecol. 2000;47:80–84. [Google Scholar]

117. Lomas MW, Glibert PM, Berg GM. Characterization of nitrogen uptake by natural populations of *Aureococcus anophagefferens* (Chrysophyceae) as a function of incubation duration, substrate concentration, light, and temperature. J. Phycol. 1996;32:907–916. [Google Scholar]

118. Lomas MW, Kana TM, MacIntyre HL, Cornwell JC, Nuzzi R, Waters R. Inter-annual variability of *Aureococcus anophagefferens* in Quantuck Bay, Long Island: natural test of the DON hypothesis. Harmful Algae. 2004;3:389–402. [Google Scholar]

119. Love RC, Loder TC, Keafer BA. Nutrient conditions during *Alexandrium fundyense* blooms in the western Gulf of Maine, USA. Deep-Sea Res. II. 2005;52:2450–2466. [Google Scholar]

120. Lund EJ. Annual Report of the Texas Game, Fish, and Oyster Commission (1934–35) Austin: 1936. Some facts relating to the occurrences of dead and dying fish on the Texas coast during June, July, and August 1935; pp. 47–50. [Google Scholar]

121. MacIntyre HL, Lomas MW, Cornwell J, Suggett DJ, Gobler CJ, Koch EW, Kana TM. Mediation of benthic-pelagic coupling by microphytobenthos: an energy- and material-based model for initiation of blooms of *Aureococcus anophagefferens*. Harmful Algae. 2004;3:403–437. [Google Scholar]

122. Mackas DL, Harrison PJ. Nitrogenous nutrient sources and sinks in the Juan de Fuca Strait/Strait of Georgia/Puget Sound estuarine system: assessing the potential for eutrophication. Est. Coastal Shelf Sci. 1997;44:1–21. [Google Scholar]

123. Magaña HA, Contreras C, Villareal TA. A historical assessment of *Karenia brevis* in the western Gulf of Mexico. Harmful Algae. 2003;2:163–171. [Google Scholar]

124. Mallin MA. Impacts of industrial-scale swine and poultry production on rivers and estuaries. Am. Sci. 2000;88:26–37. [Google Scholar]

125. Mallin MA, Cahoon LB. Industrialized animal production – a major source of nutrient and microbial pollution to aquatic ecosystems. Pop. Environ. 2003;24:369–385. [Google Scholar]

126. Marshall HG, Burchardt L, Lacouture R. A review of phytoplankton composition within Chesapeake Bay and its tidal estuaries. J. Plankton Res. 2005a;27:1083–1102. [Google Scholar]

127. Marshall HG, Egerton T, Burchardt L, Cerbin S, Kokocinski M. Long-term monitoring results of harmful algal populations in Chesapeake Bay and its major tributaries in Virginia, U.S.A. Oceanol. Hydrobiol. Studies. 2005b;34:35–41. [Google Scholar]

128. Marshall HG, Hargraves PE, Burkholder JM, Parrow MW, Elbrächter M, Allen EH, Knowlton VM, Rublee PA, Hynes WL, Egerton TA, Remington DL, Wyatt KB, Lewitus AJ, Henrich VC. Taxonomy of *Pfiesteria* (Dinophyceae) Harmful Algae. 2006;5:481–496. [Google Scholar]

129. Martorano CD. MS thesis, Institute for the Study of Earth, Oceans, and Space. Durham, NH: University of New Hampshire; 1997. Nutrient dynamics during blooms of *Alexandrium* spp. in the southwestern Gulf of Maine; p. 132. [Google Scholar]

130. Moeller PDR, Beauchesne KR, Huncik KM, Davis WC, Christopher SJ, Riggs-Gelasco P, Gelasco A. Metal complexes and free radical toxins produced by *Pfiesteria piscicida*. Env. Sci. Technol. 2007;41:1166–1172. [PubMed] [Google Scholar]

131. Mulholland MR, Bernhardt PW, Heil CA, Bronk DA, O'Neil JM. Nitrogen fixation and rate of release of fixed nitrogen by *Trichodesmium* spp. in the Gulf of Mexico. Limnol. Oceanogr. 2006;51:1762–1776. [Google Scholar]

132. Mulholland MR, Gobler CJ, Lee C. Peptide hydrolysis, amino acid oxidation, and nitrogen uptake in communities seasonally dominated by *Aureococcus anophagefferens*. Limnol. Oceanogr. 2002;47:1094–1108. [Google Scholar]

133. Nishitani L, Chew K. PSP toxins in the Pacific coast states: monitoring programs and effects on bivalve industries. J. Shellfish Res. 1988;7:653–669. [Google Scholar]

134. Nuzzi R, Waters RA. Long-term perspective on the dynamics of brown tide blooms in Long Island coastal bays. Harmful Algae. 2004;3:279–293. [Google Scholar]

135. Okaichi T. Red tides in the Seto Inland Sea. In: Okaichi T, Yanagi Y, editors. Sustainable Development in the Seto Inland Sea, Japan - From the Viewpoint of Fisheries. Tokyo, Japan: Terra Scientific Publishing Company; 1997. pp. 251–304. [Google Scholar]

136. Pan Y, Subba Rao DV, Mann KH. Changes in domoic acid production and cellular chemical composition of the toxigenic diatom *Pseudo-nitzschia multiseries* under phosphate limitation. J. Phycol. 1996a;32:371–381. [Google Scholar]

137. Pan Y, Subba Rao DV, Mann KH, Brown RG, Pocklington R. Effects of silicate limitation on production of domoic acid, a neurotoxin, by the diatom *Pseudonitzschia pungens* f. *multiseries* (Hasle). I. Batch culture studies. Mar. Ecol. Prog. Ser. 1996b;131:225–233. [Google Scholar]

138. Parrow MW, Burkholder JM, Deamer NJ, Zhang C. Vegetative and sexual reproduction in *Pfiesteria* spp. (Dinophyceae) cultured with algal prey, and inferences their classification. Harmful Algae. 2002;1:5–33. [Google Scholar]

139. Parsons ML, Dortch Q, Turner RE. Sedimentological evidence of an increase *Pseudo-nitzschia* (Bacillariophyceae) abundance in response to coastal eutrophication. Limnol. Oceanogr. 2002;47:551–558. [Google Scholar]

140. Parsons ML, Preskitt LB. A survey of epiphytic dinoflagellates from the coastal waters of the island of Hawai'i. Harmful Algae. 2007;6:658–669. [Google Scholar]

141. Parsons ML, Walsh WJ, Settlemier CJ, White DJ, Ballauer JM, Ayotte PM, Osada KM, Carman B. A multivariate assessment of the coral ecosystem health of two embayments on the lee of the island of Hawai'i. Mar. Poll. Bull. 2008;56:1138–1149. [PubMed] [Google Scholar]

142. Phlips EJ, Badylak S, Lynch TC. Blooms of the picoplanktonic cyanobacterium *Synechococcus* in Florida Bay, a subtropical inner-shelf lagoon. Limnol. Oceanogr. 1999;44:1166–1175. [Google Scholar]

143. Popels LC, Coyne KJ, Forbes R, Pustizzi F, Gobler CJ, Cary SC, Hutchins DA. The use of quantitative polymerase chain reaction for the detection and enumeration of the harmful alga *Aureococcus anophagefferens* in environmental samples along the United States east coast. Limnol. Oceanogr. Methods. 2003;1:92–102. [Google Scholar]

144. Portnoy JW, Nowicki BL, Roman CT, Urish DW. The discharge of nitrate-contaminated groundwater from developed shoreline to marsh-fringed estuary. Water Resources Res. 1998;34(11):3095–3104. [Google Scholar]

145. Poulton NJ, Keafer BA, Anderson DM. Toxin variability in natural populations of *Alexandrium fundyense* in Casco Bay, Maine – evidence of nitrogen limitation. Deep-Sea Res. II. 2005;52(19–21):2501–2521. [Google Scholar]

146. Price DW, Kizer KW, Hansgen KH. California's paralytic shellfish poisoning prevention program, 1927–1989. J. Shellfish. Res. 1991;10:119–145. [Google Scholar]

147. Randall JE. A review of ciguatera, tropical fish poisoning with a tentative explanation of its cause. Bull. Mar. Sci. Gulf Carib. 1958;8:236–267. [Google Scholar]

148. Rensel JE. Prepared by Parametrix, Battelle Northwest Laboratories and Rensel Associates for the State of Washington Departments of Ecology, Fisheries and Natural Resources. 1991. Phytoplankton and nutrient studies near salmon net-pens at Squaxin Island, Washington. Technical appendices of Programmatic Environmental Impact Statement: Fish culture in floating net-pens; p. 161. appendices. [Google Scholar]

149. Rensel JE. Factors controlling Paralytic Shellfish Poisoning in Puget Sound. J. Shellfish Res. 1993;12(2):371–376. [Google Scholar]

150. Rensel JE. Harmful algal blooms and finfish resources in Puget Sound. In: Robichaud E, editor. Puget Sound Research. Volume 1. Olympia, Washington: Puget Sound Water Quality Authority; 1995. pp. 442–429. [Google Scholar]

151. Rensel JE. Prepared by Rensel Associates Aquatic Sciences for the National Oceanic and Atmospheric Administration Center for Sponsored Coastal Ocean Research (CSCOR) Washington, D.C.: 2007. Fish kills from the harmful alga *Heterosigma akashiwo* in Puget Sound: Recent blooms and review; p. 58. (http://www.whoi.edu/fileserver.do?id=39383&pt=2&p=29109>http://www.whoi.edu/fileserver.do?id=39383&pt=2&p=29109) [Google Scholar]

152. Riley GA. The plankton of estuaries. In: Lauff GH, editor. Estuaries. Vol. 83. Washington, D.C.: Amer. Assoc. Adv. Sci., Publ.; 1967. pp. 316–326. [Google Scholar]

153. Rothschild BJ, Ault JS, Goulletquer P, Heral M. The decline of the Chesapeake Bay oyster population: A century of habitat destruction and overfishing. Mar. Ecol. Progr. Ser. 1994;111(1–2):29–39. [Google Scholar]

154. Rothenberger MB. Ph.D. dissertation. Raleigh, NC: Department of Plant Biology, North Carolina State University; 2007. Long-term Impacts of Changing Land-use Practices on Water Quality and Phytoplankton Assemblages in the Neuse Estuary Ecosystem, North Carolina. [Google Scholar]

155. Rothenberger MB, Burkholder JM, Brownie C. Long-term effects of changing land use practices on surface water quality in a coastal river and lagoonal estuary. Environ. Manag. Submitted. [PubMed] [Google Scholar]

156. Rue E, Bruland K. Domoic acid binds iron and copper: a possible role for the toxin produced by the marine diatom *Pseudo-nitzschia*. Mar. Chem. 2001;76:127–134. [Google Scholar]

157. [SAIC] Science Applications International Corp. Recommended interim guidelines for the management of salmon net-pen culture in Puget Sound. Olympia WA: Washington Dept. Energy 87-5, Ecology No. C-0087110; 1986. p. 48. [Google Scholar]

158. Schnetzer A, Miller PE, Schaffner RA, Stauffer BA, Jones BH, Weisberg SB, DiGiacomo PM, Berlson WM, Caron DA. Blooms of *Pseudo-nitzschia* and domoic acid in the San Pedro Channel and Los Angeles harbor areas of the Southern California Bight, 2003–2004. Harmful Algae. 2007;6:372–387. [Google Scholar]

159. Scholin CA, Gulland F, Doucette GJ, Benson S, Busman M, Chavez FP, Cordaro J, DeLong R, De Vogelaere A, Harvey J, Haulena M, Lefebvre K, Lipscomb T, Van Dolah FM. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. Nature. 2000;403:80–84. [PubMed] [Google Scholar]

160. Seitzinger SP, Harrison JA, Dumont E, Beusen AHW, Bouwman AF. Sources and delivery of carbon, nitrogen and phosphorous to the coastal zone: An overview of global nutrient export from watersheds (NEWS) models and their application. Global Biogeochem. Cycles. 2005;19:GB4S09. [Google Scholar]

161. Smayda TJ. Primary production and the global epidemic of phytoplankton blooms in the sea: a linkage? In: Cosper EM, Carpenter EJ, Bricelj M, editors. Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tide and Other Unusual Blooms. Berlin: Springer-Verlag; 1989. pp. 213–228. [Google Scholar]

162. Smith JE, Smith CM, Hunter CL. An experimental analysis of the effects of herbivory and nutrient enrichment on benthic community dynamics on a Hawaiian reef. Coral Reefs. 2001;19:332–342. [Google Scholar]

163. Smith RL. The physical processes of coastal ocean upwelling systems. In: Summerhayes CP, Emeis K-C, Angel MV, Smith RL, Zeitzschel B, editors. Upwelling in the ocean: Modern processes and ancient records. John Wiley and Sons Ltd.; 1992. pp. 39–64. [Google Scholar]

164. Sperr AE, Doucette GJ. Variation in growth rate and ciguatera toxin production among geographically distinct isolates of *Gambierdiscus toxicus*. In: Yasumoto T, Oshima Y, Fukuyo Y, editors. Harmful and Toxic Algal Blooms. Intergovernmental Oceanographic Commission of UNESCO; 1996. pp. 309–312. [Google Scholar]

165. Springer JJ, Burkholder JM, Glasgow HB, Glibert PM, Reed RE. Use of a real-time monitoring network (RTRM) and shipboard sampling to characterize a dinoflagellate bloom in the Neuse Estuary, North Carolina, U.S.A. Harmful Algae. 2005;4:553–574. [Google Scholar]

166. Steel J, editor. Status and Trends Report of the Albemarle-Pamlico Estuarine Study. Raleigh: NC DEHNR and U.S. EPA National Estuarine Program; 1991. [Google Scholar]

167. Stoecker DK. Mixotrophy among dinoflagellates. J. Eukaryot. Microbiol. 1999;46:397–401. [Google Scholar]

168. Tango P, Butler W, Wazniak C. Assessment of harmful algae bloom species in the Maryland Coastal Bays. In: Wazniak C, Hall M, editors. Maryland's Coastal Bays Ecosystem Health Assessment 2004. Annapolis, MD: Maryland Department of Natural Resources; 2004. http://www.dnr.state.md.us/coastalbays/sob_2004.html. [Google Scholar]

169. Tango PJ, Magnien R, Butler W, Luckett C, Luckenbach M, Lacouture R, Poukish C. Impacts and Potential Effects due to *Prorocentrum minimum* blooms in Chesapeake Bay. Harmful Algae. 2005;4(3):525–531. [Google Scholar]

170. Tester PA, Steidinger KA. *Gymnodinium breve* red tide blooms: Initiation, transport, and consequences of surface circulation. Limnol. Oceanogr. 1997;42:1039–1051. [Google Scholar]

171. Thessen A, Stoecker D. Distribution, abundance, and domoic acid analysis of the toxic diatom genus *Pseudo-nitzschia* from the Chesapeake Bay. Estuaries and Coasts. 2008 [Google Scholar]

172. Tosteson TR, Ballantine DL, Tosteson CG, Bardales AT, Durst HD, Higerd TB. Comparative toxicity of *Gambierdiscus toxicus*, *Ostreopsis* cf. *lenticularis*, and associated microflora. Mar. Fish. Rev. 1986;48:57–59. [Google Scholar]

173. Townsend DW. Sources and cycling of nitrogen in the Gulf of Maine. J. Mar. Sys. 1998;16:283–295. [Google Scholar]

174. Townsend DW, Pettigrew NR, Thomas AC. On the nature of *Alexandrium fundyense* blooms in the Gulf of Maine. Deep-Sea Res. II. 2005;52:2603–2630. [Google Scholar]

175. Townsend DW, Thomas AC, Mayer LM, Thomas M, Quinlan J. Oceanography of the northwest Atlantic continental shelf. In: Robinson AR, Brink KH, editors. The Sea. Vol. 14A. Cambridge MA: Harvard University Press; 2006. pp. 119–168. [Google Scholar]

176. Trainer VL, Adams NG, Bill BD, Anulacion BF, Wekell JC. Concentration and dispersal of a *Pseudo-nitzschia* bloom in Penn Cove, Washington, USA. Nat. Toxins. 1998;6:1–13. [PubMed] [Google Scholar]

177. Trainer VL, Bill BD. Characterization of a domoic acid binding site from Pacific razor clam. Aquat. Toxicol. 2004;69:125–132. [PubMed] [Google Scholar]

178. Trainer VL, Adams NG, Bill BD, Stehr CM, Wekell JC. Domoic acid production near California coastal upwelling zones, June 1998. Limnol. Oceanogr. 2000;45:1818–1833. [Google Scholar]

179. Trainer VL, Cochlan WP, Erickson A, Bill BD, Cox FH, Borchert JA, Lefebvre KA. Recent domoic acid closures of shellfish harvest areas in Washington State inland waterways. Harmful Algae. 2007;6:449–459. [Google Scholar]

180. Trainer VL, Eberhart B-TL, Wekell JC, Adams NA, Hanson L, Cox F, Dowell J. Paralytic shellfish toxins in Puget Sound, Washington State. J.Shellfish Res. 2003;22(1):213–224. [Google Scholar]

181. Trainer VL, Hickey BM, Horner RA. Biological and physical dynamics of domoic acid production off the Washington U.S.A. coast. Limnol. Oceanogr. 2002;47:1438–1446. [Google Scholar]

182. Trebatoski B. Rep. 88-02. Austin, TX: Tex Water Comm.; 1988. Observations on the 1986–1987 Texas red tides *Ptychodiscus brevis*; p. 48. [Google Scholar]

183. Trice TM, Glibert PM, Lea C, Van Heukelem L. HPLC pigment records provide evidence of past blooms of *Aureococcus anophagefferens* in the Coastal Bays of Maryland and Virginia, USA. Harmful Algae. 2004;3:295–304. [Google Scholar]

184. Turner RE, Rabalais NN. Coastal eutrophication near the Mississippi river delta. Nature. 1994;368:619–621. [Google Scholar]

185. Turner RE, Rabalais NN, Fry B, Atilla N, Milan CS, Lee JM, Normandeau C, Oswald TA, Swenson EM, Tomasko DA. Paleo-indicators and water quality change in the Charlotte Harbor estuary (Florida) Limnol. Oceanogr. 2006;51:518–533. [Google Scholar]

186. Tyler MA, Seliger HH. Annual subsurface transport of a red tide dinoflagellate to its bloom area: water circulation patterns and organism distributions in the Chesapeake Bay. Limnol. Oceanogr. 1978;23:227–246. [Google Scholar]

187. Vargo GA, Shanley E. Alkaline phosphatase activity in the red tide dinoflagellate *Ptychodiscus brevis*. PSZN I: Mar. Ecol. 1985;6:251–262. [Google Scholar]

188. Vargo GA, Heil CA, Ault DN, Neely MB, Murasko S, Havens J, Lester KM, Dixon LK, Merkt R, Walsh J, Weisberg R, Steidinger KA. Four *Karenia brevis* blooms: A comparative analysis. In: Steidinger KA, Landsberg JH, Tomas CR, Vargo GA, editors. Harmful Algae 2002; Proceedings of the Xth International Conference on Harmful Algae; Paris. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography and Intergovernmental Oceanographic Commission of UNESCO; 2004. pp. 14–16. [Google Scholar]

189. Vargo GA, Heil CA, Fanning KA, Dixon LK, Neely MB, Lester K, Ault D, Murasko S, Havens J, Walsh J, Bell S. Nutrient availability in support of *Karenia brevis* blooms on the central West Florida Shelf: What keeps *Karenia* blooming? Cont. Shelf Res. 2008;28:73–98. [Google Scholar]

190. Villareal TA, Brainard MA, McEachron LW. *Gymnodinium breve* (Dinophyceae) in the western Gulf of Mexico: Resident versus advected populations as a seed stock for blooms. In: Hallegraeff GM, Blackburn SI, Bolch CJ, Lewis RJ, editors. Harmful Algal Blooms 2000. Paris: Intergovenrmental Oceanographic Commission of UNESCO; 2001. pp. 153–156. [Google Scholar]

191. Walsh JJ, Steidinger KA. Saharan dust and Florida red tides: the cyanophyte connection. J. Geophys. Res. 2001;106:11597–11612. [Google Scholar]

192. Walsh JJ, Jolliff JK, Darrow BP, Lenes JM, Milroy SP, Dieterle DA, Carder KL, Chen FR, Vargo GA, Weisberg RH, Fanning KA, Muller-Karger FE, Steidinger KA, Heil CA, Prospero JS, Lee TN, Kirkpatrick GJ, Whitledge TE, Stockwell DA, Tomas CR, Villareal TA, Jochens AE, Bontempi PS. Red tides in the Gulf of Mexico: where, when, and why? J. Geophys. Res. 2006;111:C11003. [PMC free article] [PubMed] [Google Scholar]

193. Wang S, Tang D, He F, Fukuyo Y, Azanza RV. Occurrences of harmful algal blooms (HABs) associated with ocean environments in the South China Sea. Hydrobiol. 2008;596:79–93. [Google Scholar]

194. Wazniak CE, Hall MR, Carruthers T, Sturgis R, Dennison WC. Linking water quality to living resources in a mid-Atlantic lagoon system USA. Ecol. Appl. 2007;17(5):S64–S78. [Google Scholar]

195. Wells ML, Trick CG, Cochlan WP, Hughes MP, Trainer VL. Domoic acid: the synergy of iron, copper, and the toxicity of diatoms. Limnol. Oceanogr. 2005;50:1908–1917. [Google Scholar]

196. Withers NW. Toxin production, nutrition, and distribution of *Gambierdiscus toxicus* (Hawaiian strain) In: Gomez ED, editor. Fourth International Coral Reef Symposium. Philippines: Manila; 1981. pp. 449–451. [Google Scholar]

197. Yasumoto T, Inoue A, Ochi T, Fujimoto K, Oshima Y, Fukuyo Y, Adachi R, Bagnis R. Environmental studies on a toxic dinoflagellate responsible for ciguatera. Bull. Jpn. Soc. Sci. Fish. 1980;46:1397. [Google Scholar]

198. Yasumoto T, Nakajima I, Bagnis R, Adachi R. Finding of a dinoflagellate as a likely culprit of ciguatera. Bull. Jpn. Soc. Sci. Fish. 1977;43:1021–1026. [Google Scholar]

199. Zhang Y, Fu F-X, Whereat E, Coyne KJ, Hutchins DA. Bottom-up controls on a mixed-species HAB assemblage: A comparison of sympatric *Chattonella subsalsa* and *Heterosigma akashiwo* (Raphidophytes) isolates from the Delaware Inland Bays. Harmful Algae. 2006;5:310–320. [Google Scholar]