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Tiny Drifters Amidst Global Change: Examining Environmental Drivers, Trophic Impacts, and Management Strategies of Estuarine Plankton Communities in the Anthropocene

by

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A dissertation submitted in partial fulfillment of the
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Abstract

Plankton productivity supports estuarine food webs, and has been tied to the success of fisheries, macroinvertebrates, and cultured shellfish yields. Climate change and alterations to nutrient loads are thought to be influencing plankton assemblages, with toxin-producing harmful algal blooms (HABs) on the rise and nutritional quality of plankton declining globally. These shifts in plankton communities may contribute to low biomass yields and toxin-based closures of important fisheries. The objectives of this dissertation are to identify environmental drivers, trophic impacts, and management strategies to understand and respond to changing estuarine plankton communities. To address these objectives, I used a combination of field research in multiple Oregon estuaries to understand environmental drivers of plankton communities, a laboratory-based nutrient addition experiment to investigate the impacts of nutrients on the nutritional quality of plankton, and a social science survey to probe shellfish harvester exposure to algal toxins and their perceptions of risk. I found that discharge shifted plankton communities of the Lower Columbia River Estuary during marine heat waves, favoring the development of two different HABs via two different mechanisms. These HABs included a bloom of *Pseudo-nitzschia* spp. driven by upwelling and subsequent advection into the estuary, and an *in situ* bloom of *Gymnodinium catenatum* fueled by an organic nutrient source and long residence time. I also found that nutrient conditions impact plankton nutritional value, as measured by the biomarker of polyunsaturated fatty acids (PUFAs). This relationship was modulated by seasonality, and high PUFA content emerged through either inorganic or organic nutrient uptake. Finally, I found that combining social and ecological datasets improved assessment of risk of exposure to algal toxins, and that certain demographic groups displayed gaps between their perceived and actual risk of exposure to algal toxins. This research aims to understand plankton regime transitions in toxicity and nutritional value under anthropogenic impact scenarios, and informs management in changing socio-ecological systems. In this dissertation, I identified drought conditions and the summer season as high-risk for estuarine HABs and shellfish harvester exposure to algal toxins. I also found that estuarine plankton can attain high nutritional value via uptake of multiple types of nutrients, providing a high-quality food resource for consumers in these habitats. This information will be useful for adapting future management of estuarine plankton, and has implications for estuarine ecosystems, fisheries, coastal economies, and human health and nutrition.

Dedication

Dedicated to Steve and Joyce.

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Preface

Chapters 2, 3, and 4 are intended for publication in peer-reviewed journals, and therefore may contain some redundant introductory material as they are designed as stand-alone documents. These chapters use “we” to refer to myself and other co-authors who were involved in the development of the work, whereas Chapters 1 and 5 use the first person singular, as I am the sole author of these sections.

Chapter 1: Introduction

Plankton[1] form the base of aquatic food webs, and their productivity has been tied to the success of numerous fisheries (Bacher et al., 1997; Beaugrand et al., 2008; Nedwell, 1999; Ware and Thomson, 2005). Given the pivotal position of plankton in aquatic food webs as primary producers, they are strongly influenced by environmental processes, such as nutrient availability and water quality conditions (McQueen et al., 1986; Menge and Sutherland, 1987). Predominant plankton taxa and their distributions are known to fluctuate with changing climatic conditions over geologic time scales (Falkowski, 2004; Falkowski and Oliver, 2007). Humans have profoundly influenced the environment, particularly over the last several centuries, which has warranted the proposal of a new geological epoch, called the Anthropocene (Crutzen, 2006; Lewis and Maslin, 2015). In this time period, scientists have observed alterations in climate (Edwards and Richardson, 2004; Winder and Sommer, 2012), water quality (Barletta et al., 2019), and nutrient cycling (Duan et al., 2007; Jickells, 1998) that have impacted plankton communities.

Several themes have emerged in studying the response of plankton to a shifting environment due to climate change and other anthropogenic influences. The first theme extensively documents shifts in the abundance and composition of plankton communities due to alterations to nutrient inputs and cycling in estuaries. Cultural eutrophication, most

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[1] Plankton consist of mostly microscopic organisms, and are typically divided into the categories of "phytoplankton" and "zooplankton". Phytoplankton are photosynthetic, while zooplankton are primary consumers. As will be discussed in this dissertation this concept of two categories is evolving, so I simply use the term plankton when not specifically discussing their nutritional strategy in this chapter.

often resulting from excess nutrient inputs into a system (usually nitrogen and phosphorus), is a precursor for algal blooms and low dissolved oxygen, which pose serious threats to the functioning and services of estuarine ecosystems (Bricker et al., 2007). It is often caused by anthropogenic terrestrial sources of nutrients, such as agricultural runoff and sewage. Nutrient enrichment can also occur from groundwater or atmospheric deposition. Shifts in ratios of nitrogen, phosphorus, silicic acid, and iron may also be ecologically relevant, causing shifts in plankton species composition (Jickells, 1998; Turner and Rabalais, 1994). As evidence has emerged that plankton may assimilate both inorganic and organic nutrients using different adaptive strategies, it has become clear that nutrient ratios need to be computed using both nutrient forms (Anderson et al., 2002). Many plankton are primarily photosynthetic, while others utilize organic nutrients via mixotrophy (combination of using inorganic nutrients to support photosynthesis and dissolved or particulate organic nutrient uptake) or heterotrophy (acquisition of both energy and material for growth utilizing organic material) (Heisler et al., 2008). Because different plankton taxa have specific physiological adaptations that allow them to thrive under particular nutrient regimes, anthropogenically-driven fluctuations in nutrient inputs can impact plankton taxonomic composition in exposed areas.

The second theme illuminates a global pattern of increasing observations of harmful algal blooms (HABs). HABs are ecological phenomena that occur when certain plankton taxa accumulate to a harmful degree and cause a host of negative impacts including biotoxin production, hypoxia and dead zones, or physical harmful effects on fish and wildlife from abrasion or secretions (Smayda, 1997). In this dissertation, I largely focus on toxin-producing harmful algal blooms. The recorded number of HABs has increased globally over the last several decades (Hallegraeff, 1993; Van Dolah, 2000). This increase coincides with greater awareness and more frequent observation of these events, but also with rapid environmental changes. Transport of nuisance species through ship ballast water, coastal eutrophication, anomalous weather events like El Niño and marine heat waves, and global climate change are among the proposed causes for this apparent increase (Gobler, 2020; Hallegraeff, 1998; Heisler et al., 2008; Smayda, 2007; Trainer et al., 2020; Van Dolah, 2000).

Lastly, concerns have emerged that nutritional value of plankton is declining globally due to ocean conditions (Hixson and Arts, 2016). Nutritional value can refer to a number of qualities of plankton such as cell size, bulk nutrient content, toxin content, or fatty acid content; the latter two are discussed further in this dissertation. Polyunsaturated fatty acids (PUFAs) are promoters of healthy growth and development for many organisms, and therefore contribute significantly to nutritional quality of a food resource. Boyce et al. (2010) estimated a decline in overall plankton concentration of 1% of the global median per year over the last century. Others have predicted a shift toward smaller plankton with ocean warming (Polovina and Woodworth 2012), and lower plankton N and P content (global average of 1.2% and 6.4% predicted decline over the 21st century: Kwiatkowski et al., 2018). Most relevant to this dissertation, global declines in PUFAs such as EPA and DHA are also expected under ocean warming (2.5 °C) of 8.2% and 27.8%, respectively (Hixson and Arts, 2016). While several of these studies attribute these declines to ocean warming on a global scale (Hixson and Arts, 2016; Paul et al., 2015; Polovina and Woodworth, 2012), others have hypothesized that shifting nutrient regimes are likely to drive changes in nutritional quality at a more localized scale (Hauss et al., 2012; Makareviciute-Fichtner et al., 2021).

Several studies outlined below have suggested that declining plankton nutritional value may limit overall food web quality via the quantity of primary consumers as prey items, as well as the supply of key biomolecules. Dietary PUFAs have been demonstrated to correlate with copepod egg production and hatching success in a number of studies (Jónasdóttir and Kiørboe, 1996; Kleppel and Burkart, 1995; Meyers et al., 2019). Juvenile *Oncorhynchus mykiss* fed on *Daphnia* sp. grown with low PUFA plankton diets had lower growth rates (0.14 ± 0.4 mg fish weight d^{-1}) than those fed with higher quality diets (1.4 ± 0.3 mg fish weight d^{-1}), indicating the importance of high-PUFA plankton to the growth of juvenile fish (Taipale et al., 2018). This result was mirrored in aquaculture studies on other fish species (Harel et al., 2002; Trushenski et al., 2012) and in an invasive freshwater clam (Basen et al., 2011). However, it is known that some marine and estuarine filter feeders discriminate particles based on their perceived quality, demonstrating that high quality plankton (e.g., preferable size, digestibility, nutrient or biomolecule content) may be selectively grazed (Shumway 1985). There is no evidence to suggest that toxic algae are selectively excluded by bivalves, and response is species-specific (Hégaret et al., 2007). Higher PUFA content in the bivalve *Mytilus* sp. has been linked to selective preference for higher quality plankton prey (Zhang et al., 2022). All of this evidence suggests that declines in plankton nutritional quality can impact aquatic food webs, including fisheries that humans rely on for nutrition.

I have chosen to focus on estuarine plankton communities, which represent a nexus between marine and freshwater riverine systems. In river-dominated systems such as those studied in this dissertation, estuaries are typically influenced by oceanographic and riverine processes differentially throughout the year, creating a push and pull of relative ocean vs. riverine influence. Estuarine habitats have increased macro and micronutrient concentrations compared to open ocean areas, differ in salinity, and are often shallower than the critical depth, or the depth at which there is not enough light for photosynthesis to outweigh the physiological costs of respiration (Falkowski and Raven, 2013). Estuaries can receive nutrients both from upwelling processes and from terrestrial runoff (Jickells, 1998). Terrestrial runoff in particular has been shown to provide typically limiting nutrients to coastal plankton communities (Dortch et al., 2001; Rabalais et al., 1996). For example, the Columbia River provides large quantities of silicic acid and iron to plankton communities in the estuary and adjacent coastal ocean, whose growth might otherwise be limited by these micronutrients along the continental shelf (Bruland et al., 2008). Macronutrient runoff (N and P) can dominate inputs in systems that do not receive nutrient subsidies from upwelling, or during non-upwelling seasons (Pinckney, 2001).

Variation in salinity in estuaries can shape the plankton community as well. More ocean-influenced areas of the estuary may be dominated by marine plankton, but the community will shift to brackish and freshwater-dwelling species progressing upriver in an estuary. This can make for overall higher diversity of plankton communities than might be observed in the open ocean. The rate of flushing or retention time in an estuary, which are dependent on discharge and tidal exchange, can dictate the biomass of plankton that accumulates (Cloern et al., 2014). Light limitation may also restrict phytoplankton activity in turbid estuaries (Cloern et al., 2014).

In addition to having unique ecological properties, estuaries also represent an interesting place to study human interactions with aquatic food webs. For millennia, humans primarily interacted with fisheries in the nearshore environment (Erlandson and Rick, 2010). With the advent of fishing technologies, more interaction has taken place in the pelagic environment, though recreational and subsistence fishers still largely interface with the intertidal and nearshore coastal environment (Cooke and Cowx, 2006). The National Oceanic and Atmospheric Administration (NOAA) estimates that 75% of all fish and shellfish harvests rely on estuaries for nursery habitat, and that fish caught in estuaries contribute $4.3 billion to the U.S. economy every year (NOAA Office for Coastal Management, 2021).

Consistent estuarine fishery use makes humans an integral part of food webs in these systems, both influencing and experiencing the impacts of plankton communities on food webs. HABs provide an example of how humans are impacted by changes to estuarine plankton. When HABs occur, shellfish that consume toxin-producing algae through direct or indirect trophic linkages may become contaminated by biotoxins. Human consumption of biotoxin-contaminated shellfish can result in different types of shellfish poisoning that can manifest as mild to severe gastrointestinal illness, neurological symptoms, and sometimes even paralysis or death in severe cases (Berdalet et al., 2016). Prevention of shellfish poisoning requires a multimodal response including ecological monitoring and prediction, fisheries management, effective outreach to shellfish harvesters, and public health mobilization. For my dissertation, I approach the issue of changing estuarine plankton dynamics by using multiple lenses, spanning ecology, natural resource management, and social science.

Each of the following three research chapters are united in their focus on identifying drivers and impacts of changing estuarine plankton communities. In Chapter 2, I use case studies of two marine heat waves to investigate potential drivers of HABs in the Columbia River Estuary. This work places an emphasis on understanding how climate change has and will continue to alter the environmental drivers that shape plankton communities. It also utilizes modeling techniques to make predictions about plankton community shifts that may be indicative of elevated HAB risk. The broader goal of this research is to provide guidance for monitoring efforts as climate change alters estuarine processes via both oceanographic and terrestrial phenomena like marine heat waves and drought.

In Chapter 3, I focus on the interaction of plankton physiology and community ecology in determining the nutritional value of plankton stocks under varied nutrient regimes. This research makes use of fatty acid analysis, a tool that has surged in popularity for use in trophic ecology studies (Budge et al., 2006). This chapter probes how differing nutrient forms (e.g., inorganic nutrients vs. organic nutrients) influence the physiology and community composition of estuarine plankton. It builds on rapidly expanding areas of inquiry including the recognition of widespread mixotrophy among plankton and its impacts to food webs. In this chapter, I ask whether nutritional strategies of plankton communities (i.e., proportion of autotrophy vs. mixotrophy) influence overall community PUFA content. This expands on previous biofuels research to optimize lipid and PUFA production in plankton cultures. Rather than focusing only on the physiological scale as most previous work has done, I evaluate algal community fatty acid profiles, which are the unit of interest when determining available nutrition for estuarine consumers. This represents the scale at which fatty acid content would have ecological and conservation impacts.

In Chapter 4, I address management strategies to cope with HABs, both from a natural resource management perspective and the human dimensions of the issue. This work draws on scientific knowledge of the drivers of HABs to understand HAB distribution in space and time to create an effective and efficient monitoring program in an underserved community. No consistent HAB monitoring program has been implemented for the Coos Bay area coastal community, despite a wealth of recreational and subsistence shellfish harvesting areas. The strength of this applied ecology work lies in the transfer of basic science to address community needs and concerns. Using a socio-ecological lens, this monitoring is paired with an investigation of how shellfish harvesters interface with HABs. Using survey techniques, I gain an understanding of risk of exposure to algal toxins by comparing reported fishery use and consumption rates with observed harmful algae abundance in the estuary. I also investigate how risks posed by HABs and the corresponding management actions and outreach efforts are perceived by shellfish harvesters. Although HAB monitoring and prediction is crucial to preventing negative impacts to shellfish harvesters and coastal economies, successful fishery management and well-received outreach may be equally important in mitigating human impacts of these phenomena. Understanding how communities perceive the issue provides guidance for targeted outreach to increase awareness of HABs and the resources available to shellfish harvesters to aid in making safe harvesting decisions.

This dissertation aims to address multiple issues associated with changing estuarine plankton communities (Fig. 1). The objective of Chapter 2 is to identify environmental drivers of estuarine plankton community shifts during extreme climate events. Chapter 3 aims to understand how plankton community response to environmental change can affect plankton functional traits and nutritional value. The objective of Chapter 4 is to monitor for HABs and gain an understanding of their impacts in a coastal community using a socio-ecological lens. I begin by examining drivers and trophic implications of plankton responses to a changing environment, then shift to work meant to improve societal response to these issues.

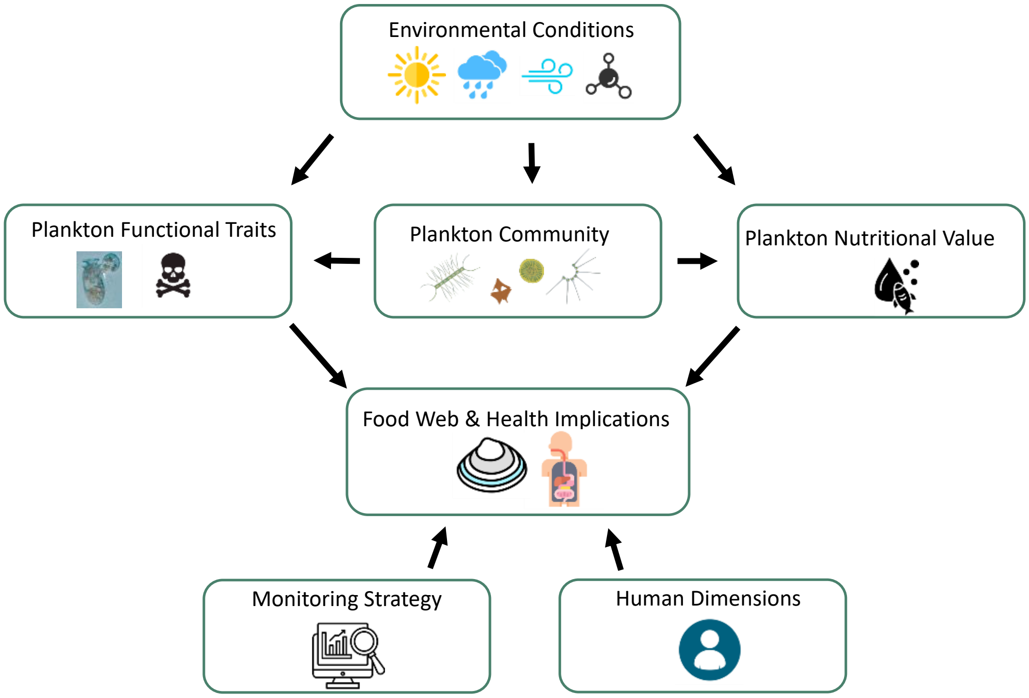

Fig. 1 Conceptual map of this dissertation showing how study objectives relate to each other.

References

Anderson, D. M., Glibert, P. M., and Burkholder, J. M. (2002). Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. *Estuaries*, *25*(4), 704–726. https://doi.org/10.1007/BF02804901

Bacher, C., Duarte, P., Ferreira, J. G., Héral, M., and Raillard, O. (1997). Assessment and comparison of the Marennes-Oléron Bay (France) and Carlingford Lough (Ireland) carrying capacity with ecosystem models. *Aquatic Ecology*, *31*(4), 379–394. https://doi.org/10.1023/A:1009925228308

Barletta, M., Lima, A. R. A., and Costa, M. F. (2019). Distribution, sources and consequences of nutrients, persistent organic pollutants, metals and microplastics in South American estuaries. *Science of The Total Environment*, *651*, 1199–1218. https://doi.org/10.1016/j.scitotenv.2018.09.276

Basen, T., Martin-Creuzburg, D., and Rothhaupt, K.-O. (2011). Role of essential lipids in determining food quality for the invasive freshwater clam Corbicula fluminea. *Journal of the North American Benthological Society*, *30*(3), 653–664. https://doi.org/10.1899/10-087.1

Beaugrand, G., Edwards, M., Brander, K., Luczak, C., and Ibanez, F. (2008). Causes and projections of abrupt climate-driven ecosystem shifts in the North Atlantic. *Ecology Letters*, *11*(11), 1157–1168. https://doi.org/10.1111/j.1461-0248.2008.01218.x

Berdalet, E., Fleming, L. E., Gowen, R., Davidson, K., Hess, P., Backer, L. C., Moore, S. K., Hoagland, P., and Enevoldsen, H. (2016). Marine harmful algal blooms, human health and wellbeing: Challenges and opportunities in the 21st century. *Journal of the Marine Biological Association of the United Kingdom*, *96*(1), 61–91. https://doi.org/10.1017/S0025315415001733

Boyce, D. G., Lewis, M. R., and Worm, B. (2010). Global phytoplankton decline over the past century. *Nature*, *466*(7306), 591–596. https://doi.org/10.1038/nature09268

Bricker, S., Longstaff, B., Dennison, W., Jones, A., Boicourt, K., Wicks, C., and Woerner, J. (2007). *Effects of Nutrient Enrichment In the Nation's Estuaries: A Decade of Change* [NOAA Technical Memorandum]. https://coastalscience.noaa.gov/data_reports/effects-nutrient-enrichment-nations-estuaries-decade-change/

Bruland, K. W., Lohan, M. C., Aguilar-Islas, A. M., Smith, G. J., Sohst, B., and Baptista, A. (2008). Factors influencing the chemistry of the near-field Columbia River plume: Nitrate, silicic acid, dissolved Fe, and dissolved Mn. *Journal of Geophysical Research: Oceans*, *113*(C2). https://doi.org/10.1029/2007JC004702

Budge, S. M., Iverson, S. J., and Koopman, H. N. (2006). Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. *Marine Mammal Science*, *22*(4), 759–801. https://doi.org/10.1111/j.1748-7692.2006.00079.x

Cloern, J. E., Foster, S. Q., and Kleckner, A. E. (2014). Phytoplankton primary production in the world's estuarine-coastal ecosystems. *Biogeosciences*, *11*(9), 2477–2501. https://doi.org/10.5194/bg-11-2477-2014

Cooke, S. J., and Cowx, I. G. (2006). Contrasting recreational and commercial fishing: Searching for common issues to promote unified conservation of fisheries resources and aquatic environments. *Biological Conservation*, *128*(1), 93–108. https://doi.org/10.1016/j.biocon.2005.09.019

Crutzen, P. J. (2006). The “Anthropocene.” In E. Ehlers and T. Krafft (Eds.), *Earth System Science in the Anthropocene* (pp. 13–18). Springer. https://doi.org/10.1007/3-540-26590-2_3

Dortch, Q., Rabalais, N. N., Turner, R. E., and Qureshi, N. A. (2001). Impacts of changing Si/N ratios and phytoplankton species composition. In *Coastal Hypoxia: Consequences for Living Resources and Ecosystems* (pp. 37–48). American Geophysical Union (AGU). https://doi.org/10.1029/CE058p0038

Duan, S., Xu, F., and Wang, L.-J. (2007). Long-term changes in nutrient concentrations of the Changjiang River and principal tributaries. *Biogeochemistry*, *85*(2), 215–234. https://doi.org/10.1007/s10533-007-9130-2

Edwards, M., and Richardson, A. J. (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature*, *430*(7002), 881–884. https://doi.org/10.1038/nature02808

Erlandson, J., and Rick, T. (2010). Archaeology meets marine ecology: The antiquity of maritime cultures and human impacts on marine fisheries and ecosystems. *Annual Review of Marine Science, 2*, 231–251.

Falkowski, P. G. (2004). The evolution of modern eukaryotic phytoplankton. *Science*, *305*(5682), 354–360. https://doi.org/10.1126/science.1095964

Falkowski, P. G., and Oliver, M. J. (2007). Mix and match: How climate selects phytoplankton. *Nature Reviews Microbiology*, *5*(10), 813–819. https://doi.org/10.1038/nrmicro1751

Falkowski, P. G., and Raven, J. A. (2013). Aquatic photosynthesis: Second edition. In *Aquatic photosynthesis*. Princeton University Press. https://doi.org/10.1515/9781400849727

Gobler, C. J. (2020). Climate change and harmful algal blooms: Insights and perspective. *Harmful Algae*, *91*, 101731. https://doi.org/10.1016/j.hal.2019.101731

Hallegraeff, G. M. (1993). A review of harmful algal blooms and their apparent global increase. *Phycologia*, *32*(2), 79–99. https://doi.org/10.2216/i0031-8884-32-2-79.1

Hallegraeff, G. M. (1998). Transport of toxic dinoflagellates via ships ballast water: Bioeconomic risk assessment and efficacy of possible ballast water management strategies. *Marine Ecology Progress Series*, *168*, 297–309. https://doi.org/10.3354/meps168297

Harel, M., Koven, W., Lein, I., Bar, Y., Behrens, P., Stubblefield, J., Zohar, Y., and Place, A. R. (2002). Advanced DHA, EPA and ARA enrichment materials for marine aquaculture using single cell heterotrophs. *Aquaculture*, *213*(1), 347–362. https://doi.org/10.1016/S0044-8486(02)00047-9

Hauss, H., Franz, J. M. S., and Sommer, U. (2012). Changes in N:P stoichiometry influence taxonomic composition and nutritional quality of phytoplankton in the Peruvian upwelling. *Journal of Sea Research*, *73*, 74–85. https://doi.org/10.1016/j.seares.2012.06.010

Hégaret, H., Wikfors, G. H., and Shumway, S. E. (2007). Diverse feeding responses of five species of bivalve mollusc when exposed to three species of harmful algae. *Journal of Shellfish Research*, *26*(2), 549–559. https://doi.org/10.2983/0730-8000(2007)26[549:DFROFS]2.0.CO;2

Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., Dortch, Q., Gobler, C. J., Heil, C. A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H. G., Sellner, K., Stockwell, D. A., Stoecker, D. K., and Suddleson, M. (2008). Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae*, *8*(1), 3–13. https://doi.org/10.1016/j.hal.2008.08.006

Hixson, S. M., and Arts, M. T. (2016). Climate warming is predicted to reduce omega-3, long-chain, polyunsaturated fatty acid production in phytoplankton. *Global Change Biology*, *22*(8), 2744–2755. https://doi.org/10.1111/gcb.13295

Jickells, T. D. (1998). Nutrient biogeochemistry of the coastal zone. *Science*, *281*(5374), 217–222. https://doi.org/10.1126/science.281.5374.217

Jónasdóttir, S. H., and Kiørboe, T. (1996). Copepod recruitment and food composition: Do diatoms affect hatching success? *Marine Biology*, *125*(4), 743–750. https://doi.org/10.1007/BF00349257

Kleppel, G. S., and Burkart, C. A. (1995). Egg production and the nutritional environment of *Acartia tonsa*: The role of food quality in copepod nutrition. *ICES Journal of Marine Science*, *52*(3–4), 297–304. https://doi.org/10.1016/1054-3139(95)80045-X

Kwiatkowski, L., Aumont, O., Bopp, L., and Ciais, P. (2018). The impact of variable phytoplankton stoichiometry on projections of primary production, food quality, and carbon uptake in the global ocean. *Global Biogeochemical Cycles*, *32*(4), 516–528. https://doi.org/10.1002/2017GB005799

Lewis, S. L., and Maslin, M. A. (2015). Defining the Anthropocene. *Nature*, *519*(7542), 171–180. https://doi.org/10.1038/nature14258

Makareviciute-Fichtner, K., Matthiessen, B., Lotze, H. K., and Sommer, U. (2021). Phytoplankton nutritional quality is altered by shifting Si:N ratios and selective grazing. *Journal of Plankton Research*, *43*(3), 325–337. https://doi.org/10.1093/plankt/fbab034

McQueen, D., Post, J., and Mills, E. (1986). Trophic relationships in freshwater pelagic ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences*, *43*(8). https://cdnsciencepub-com.proxy.lib.pdx.edu/doi/abs/10.1139/f86-195

Menge, B. A., and Sutherland, J. P. (1987). Community regulation: Variation in disturbance, competition, and predation in relation to environmental stress and recruitment. *The American Naturalist*, *130*(5), 730–757. JSTOR.

Meyers, M. T., Cochlan, W. P., Carpenter, E. J., and Kimmerer, W. J. (2019). Effect of ocean acidification on the nutritional quality of marine phytoplankton for copepod reproduction. *PLOS ONE*, *14*(5), e0217047. https://doi.org/10.1371/journal.pone.0217047

Nedwell, D. B. (Ed.). (1999). *Estuaries*. Acad. Press, San Diego, CA. pp. 306.

NOAA Office for Coastal Management. (2021). *Research Reserves*. Fast Facts. http://coast.noaa.gov/states/fast-facts/research-reserves.html

Paul, C., Matthiessen, B., and Sommer, U. (2015). Warming, but not enhanced CO2 concentration, quantitatively and qualitatively affects phytoplankton biomass. *Marine Ecology Progress Series*, *528*, 39–51. https://doi.org/10.3354/meps11264

Pinckney, J. L. (2001). The role of nutrient loading and eutrophication in estuarine ecology. *Environmental Health Perspectives*, *109*, 8.

Polovina, J. J., and Woodworth, P. A. (2012). Declines in phytoplankton cell size in the subtropical oceans estimated from satellite remotely-sensed temperature and chlorophyll, 1998–2007. *Deep Sea Research Part II: Topical Studies in Oceanography*, *77–80*, 82–88. https://doi.org/10.1016/j.dsr2.2012.04.006

Rabalais, N. N., Turner, R. E., JustiĆ, D., Dortch, Q., Wiseman, W. J., and Sen Gupta, B. K. (1996). Nutrient changes in the Mississippi River and system responses on the adjacent continental shelf. *Estuaries*, *19*(2), 386–407. https://doi.org/10.2307/1352458

Shumway, S. E. (1985). Particle selection, ingestion, and absorption in filter-feeding bivalves. *Journal of Experimental Marine Biology and Ecology*, *91*, 77–92.

Smayda, T. J. (1997). What is a bloom? A commentary. *Limnology and Oceanography*, *42*(5part2), 1132–1136. https://doi.org/10.4319/lo.1997.42.5_part_2.1132

Smayda, T. J. (2007). Reflections on the ballast water dispersal—Harmful algal bloom paradigm. *Harmful Algae*, *6*(4), 601–622. https://doi.org/10.1016/j.hal.2007.02.003

Taipale, S. J., Kahilainen, K. K., Holtgrieve, G. W., and Peltomaa, E. T. (2018). Simulated eutrophication and browning alters zooplankton nutritional quality and determines juvenile fish growth and survival. *Ecology and Evolution*, *8*(5), 2671–2687. https://doi.org/10.1002/ece3.3832

Trainer, V. L., Kudela, R. M., Hunter, M. V., Adams, N. G., and McCabe, R. M. (2020). Climate extreme seeds a new domoic acid hotspot on the US West Coast. *Frontiers in Climate*, *2*. https://doi.org/10.3389/fclim.2020.571836

Trushenski, J., Schwarz, M., Bergman, A., Rombenso, A., and Delbos, B. (2012). DHA is essential, EPA appears largely expendable, in meeting the n−3 long-chain polyunsaturated fatty acid requirements of juvenile cobia *Rachycentron canadum*. *Aquaculture*, *326–329*, 81–89. https://doi.org/10.1016/j.aquaculture.2011.11.033

Turner, R. E., and Rabalais, N. N. (1994). Coastal eutrophication near the Mississippi river delta. *Nature*, *368*(6472), 619–621. https://doi.org/10.1038/368619a0

Van Dolah F M. (2000). Marine algal toxins: Origins, health effects, and their increased occurrence. *Environmental Health Perspectives*, *108*(suppl 1), 133–141. https://doi.org/10.1289/ehp.00108s1133

Ware, D. M., and Thomson, R. E. (2005). Bottom-Up Ecosystem Trophic Dynamics Determine Fish Production in the Northeast Pacific. *Science*, *308*(5726), 1280–1284. https://doi.org/10.1126/science.1109049

Winder, M., and Sommer, U. (2012). Phytoplankton response to a changing climate. *Hydrobiologia*, *698*(1), 5–16. https://doi.org/10.1007/s10750-012-1149-2

Zhang, X., Li, S., He, J., Wu, Y., Gu, Z., Fan, M., Guo, B., Buttino, I., Liao, Z., and Yan, X. (2022). Microalgal feeding preference of *Mytilus coruscus* and its effects on fatty acid composition and microbes of the digestive gland. *Aquaculture Reports*, *23*, 101024. https://doi.org/10.1016/j.aqrep.2022.101024

Chapter 2: River Discharge Mediates Extent of Phytoplankton and Harmful Algal Bloom Habitat in the Columbia River Estuary (USA) During North Pacific Marine Heat Waves

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Abstract

Marine heat waves (MHW) have been associated with extensive harmful algal blooms (HABs) in the northeast Pacific Ocean, but the degree to which these large-scale oceanographic events are mirrored in nearshore environments has not been well established. We compared phytoplankton assemblages in the Lower Columbia River Estuary (LCRE) during two Pacific MHWs that took place in 2015 and 2019, with observations from 2017, a year with no MHW. These data were paired with environmental data from the summers of 2015 - 2019 to characterize differences in estuarine conditions during MHWs that promote phytoplankton assemblage transitions, and identify HAB-conducive conditions. Bloom densities of HAB taxa, *Pseudo-nitzschia* spp. (4.16×10^6 cells L^{-1}) and *Gymnodinium catenatum* (5.66×10^6 cells L^{-1}), were noted in the estuary during 2015 and 2019, respectively, two years where Pacific MHWs occurred during the summer months. These blooms coincided with estuary temperatures that were 1-2 ℃ above and river discharge volumes 46-48% lower than decadal daily averages. We identified patterns in the densities of several algal taxa associated with MHW-mediated low discharge in the LCRE, such as declines in tychopelagic diatoms and increasing abundance of pelagic marine taxa. We conclude that low river discharge, through extension of saline habitat area and longer residence times, likely contributed to the development of the observed marine HABs in the estuary. MHWs and associated declines in discharge are projected to become more common in the Pacific Northwest with climate change, which may alter late summer phytoplankton assemblages in the LCRE.

Key words: Harmful algal blooms, marine heat waves, estuarine ecology, discharge, *Gymnodinium catenatum*, *Pseudo-nitzschia*

Introduction

Harmful algal blooms (HABs) are occurring more frequently on a global scale, a phenomenon connected to climate change (Hallegraeff 1993, 2010; Van Dolah 2000; Lewitus et al. 2012). HABs often result in fishery closures due to the production of algal toxins, which can accumulate in consumers. Closures aid in protecting public health, but can destabilize commercial and recreational fisheries, create declines in fisheries and tourism revenue, and impact residents' food security, cultural activities, and quality of life (Dyson and Huppert 2010; Poe et al. 2015; Berdalet et al. 2016; Ritzman et al. 2018). The northeast Pacific Ocean experienced a long-lived, persistent MHW, commonly called "The Blob", that began in the winter of 2013, peaked in 2014-2015, and dissipated in 2016 (Bond et al. 2015; Di Lorenzo and Mantua 2016). A shorter-lived MHW (given the name "Blob 2.0") appeared during the summer of 2019 and disappeared after approximately four months (Amaya et al. 2020). Negative ecosystem effects, including a massive marine HAB, associated with The Blob have been extensively documented in the northeast Pacific Ocean (Du et al. 2016; McCabe et al. 2016; Brodeur et al. 2019; Rogers-Bennett and Catton 2019; von Biela et al. 2019; Piatt et al. 2020). Previous temperature anomalies in the Northeast Pacific coastal ocean have resulted in impacts to phytoplankton biomass, productivity, and community composition (Kudela et al. 2006). MHWs can also alter estuarine ecosystems, but HAB studies along the U.S. West Coast have focused on offshore and coastal habitats, leaving gaps in our knowledge of how atmospheric phenomena impact estuaries. Several studies on estuaries outside the Pacific Northwest suggest heat waves negatively affect estuarine water quality (e.g., increased biological oxygen demand, cyanobacteria blooms, low DO, low pH, and microbial pathogen growth) (Wetz and Yoskowitz 2013; Tassone et al. 2022). To our knowledge there is only one published observation of a localized heat wave linked to a HAB within a U.S. west coast estuary (Cloern et al. 2005). Other than this event, the 2015 and 2019 events are the only recorded MHWs associated with estuarine HABs for the region. Though MHW-associated HABs have been observed globally (Roberts et al. 2019; Gao et al. 2021), this field of study is relatively young and events where these phenomena are linked are somewhat sparse (Hobday et al. 2018). Other HABs have been documented in Pacific Northwest estuaries, but were not linked to heat waves and were associated with transport from coastal waters (Lewitus et al. 2012). In the LCRE, algal blooms are relatively rare, with the exception of a recurring late summer *Mesodinium rubrum* bloom (Herfort et al. 2011). In this study, we refer to phytoplankton cell concentrations on the order of 10^4 cells L^{-1} or greater as a bloom. We set this quantitative definition because both *Pseudo-nitzschia australis* and *Gymnodinium catenatum* are known to produce levels of domoic acid and saxitoxins, respectively, that result in consumer contamination at this density (Lefebvre et al. 2002; Costa et al. 2010). This threshold identifies algal densities impactful for fisheries management due to toxin contamination of harvested species.

The LCRE, like the majority of large Pacific Northwest estuaries, is freshwater-dominated (Heady et al. 2014), and therefore thought to be less impacted by oceanographic events like MHWs. Columbia River hydrology is largely influenced by snowpack in low-elevation mountain ranges, with plentiful winter precipitation that falls as rain or snow depending on temperatures, a spring freshet, and dry summers (Tohver et al. 2014). Atmospheric influences such as the position of the deep Aleutian low of the Pacific/North American circulation pattern, El Nino/Southern Oscillation (ENSO) and disruptions of the jet stream, which can contribute to the formation of MHWs, have a large influence on snowpack, and the resulting timing and volume of snowmelt runoff to the LCRE (Cayan 1996; Clark et al. 2001; Mote 2006). The flow regime of the Columbia River is also influenced by a series of storage and run-of-the-river dams (Federal Columbia River Power System 2001). Flow rates to the LCRE may be altered during drought years due to lower snowpack [e.g. 2014-2015 Pacific Northwest "snowpack drought" (Boniface et al. 2016)], warmer temperatures, and exceptionally low summer flows; all characteristics projected under climate change for the region (Hamlet and Lettenmaier 1999). Although the LCRE is river-dominated for much of the year, oceanographic conditions develop during the summer months when river flow is at its lowest (Chawla et al., 2008), creating an inverse relationship between discharge and salinity. Oceanographic inputs to the estuary affect water quality, for example, delivery of upwelling-derived nitrate through tidal exchange (Bruland et al. 2008). The shift from freshwater-dominated to brackish-marine conditions is reflected in the seasonal succession of estuarine plankton (Rollwagen-Bollens et al. 2020). Therefore, the vulnerability of the LCRE to the ecological effects of MHWs could differ significantly depending on the timing of their occurrence.

In this study, we compare phytoplankton assemblages of the LCRE during two MHWs (2015 and 2019) that differed in extent and timing with those in 2017 without

MHW. Our study objectives are to (1) characterize differences in estuarine conditions during MHWs that may promote HABs, and (2) identify phytoplankton assemblage characteristics to aid in identifying transitions to HAB-conducive conditions. This work provides insight into the LCRE's algal community response to two MHWs of differing persistence, and evaluates how hydrologic forcing could play an important role in mediating the impact of MHWs in the LCRE. Particularly during anomalous oceanographic events, understanding the degree and timing of ocean-estuary connectivity is crucial to predicting algal community shifts in estuarine habitat.

Methods

Study area

The LCRE is a river-dominated, drowned river mouth estuary (Heady et al. 2014), with the fourth largest river volume in the United States (Baptista et al. 2015) (Fig. 1). The LCRE food web is largely detritus-driven, and phytoplankton grazing makes up the main living component of food resources for primary consumers (Simenstad et al. 1990). Haertel and Osterberg (1967) described three main groups of plankton in the LCRE: those associated with freshwater, marine plankton that are transported into the estuary from the coast, and plankton indigenous to the estuary that are associated with brackish waters. The study took place at a site in Ilwaco Harbor in Baker Bay, a shallow (<15 m depth) bay just upstream of the river mouth (~5 km) on the northern side of the estuary. We also sampled two nearby sites in the mainstem of the LCRE on the southern side of the estuary during a research cruise aboard the R/V *Oceanus* (Fig. 1a).

Sample collection and analyses

The majority of phytoplankton samples (total *n* = 24) were collected from the surface at Ilwaco Harbor (*n*=18), with additional samples collected in September-October 2015 (*n*=6*)* aboard the R/V *Oceanus,* which provided data on the depth distribution of phytoplankton in the LCRE (surface, mid-depth, and bottom). Surface samples at the Ilwaco site in 2015 and 2017 were collected approximately monthly during the spring and summer of these years, while samples were collected monthly June - July of 2019 and fortnightly August - October of 2019 (Fig. 1b). Whole water samples were collected for algal identification and enumeration using clean glass French square bottles. Samples were preserved in Lugol's Iodine solution (final concentration, 1%). Samples were homogenized and settled in an Utermöhl sedimentation chamber for 24 h and cells were identified and enumerated using a Leica DMIL inverted microscope. Cells were enumerated up to 400 counting units, using 100X, 200X, and 400X magnification, each for several of fields of view per sample in order to adequately capture both smaller cells and rarer, larger taxa. Where replicate samples were available, counts were averaged. Where possible, cells were identified to genus or species, and in some cases complexes or groups that were more practical for identification using light microscopy. For example, species within the genus, *Pseudo-nitzschia,* cannot be identified to species with a light microscope alone; individuals were therefore classified into complexes of *Pseudo-nitzschia* c.f. *australis/fraudulenta*, *P.* c.f. *pungens/multiseries,* or *P.* c.f. *pseudodelicatissima/delicatissima*, which can be differentiated by cell size and shape (e.g., length: width ratio) (Trainer and Suddleson 2005). Phytoplankton abundances are reported as cell concentrations or as a proportion of the total cells counted in the sample. Simpson's Diversity Index (D) was calculated using the '*vegan*' package in R (Simpson 1949).

Collection and analysis of environmental data

Environmental data were collected using continuous sensors at the SATURN-03 endurance station (Fig. 1; www.stccmop.org), including salinity (every 15 s), temperature (every 15 s), chlorophyll (every 3 min), and nitrate (every hour) at three depths (2.4 m, 8 m, and 13 m). Chlorophyll fluorescence was measured using an *in situ* fluorometer (Turner Designs AlgaeWatch in 2015 and a Turner Designs Cyclops in 2019) and calibrated against extracted chlorophyll a (S. Riseman, pers. comm.), and nitrate was measured using an *in situ* ultraviolet spectrophotometer (ISUS Satlantic in 2015, SUNA Seabird Scientific in 2019). Although the SATURN-03 sensor is located across the channel from the water sampling site, we determined that data collected from this sensor suite (i.e., salinity, temperature, nitrate) were highly similar to measurements from grab samples collected at Ilwaco Harbor (data not shown). Discharge data were downloaded from the USGS river gauge at Quincy Washington/Beaver Army Terminal at river mile 53 (river km 85), a point downstream from major tributaries (USGS 2022). Upwelling index data were obtained from the DART Pacific Ocean Coastal Upwelling Index Dataset, courtesy of the National Marine Fisheries Service, Pacific Fisheries Environmental Laboratory (Columbia River DART, Columbia Basin Research, and University of Washington

2021). Upwelling index values were derived from estimated Ekman transport based on mean surface atmospheric pressure fields for every 6 h at the Lincoln City, OR standard location. This index summarizes the direction and velocity of water movement, with positive values representing offshore water movement (upwelling), and negative values representing onshore movement (downwelling). Data for each environmental variable were initially averaged hourly, and were used to calculate day equivalents of elevated temperature and salinity conditions by adding all hours above a certain threshold together and dividing by 24 h (Tables 1, 2). To limit noise from diurnal tidal action, all data for each environmental variable were averaged daily for the generalized additive mixed model analysis. Sensor data were smoothed by calculating 14-d rolling daily averages to capture conditions in the window leading up to each phytoplankton sampling event prior to use in the gradient forest analysis.

Algal toxin analysis

A whole water sample for toxin analysis was collected concurrently with each algal sampling event, filtered onto GF/F filters (400 – 1000 mL per sample, depending on algal density), and frozen at -20^{o}C, pending analysis. Samples in which toxic algal species were present, as determined using light microscopy, were prioritized for toxin analysis of seston (total: *n*=10, October 2015: *n*=6, August-October 2019: *n*=4). Analytes were extracted from filters in deionized water with sonication as in Lefebvre et al. (2008), and four analytical replicates per sample were analyzed. We used ELISA kits (Mercury Science Inc.) and a Molecular Devices Spectra Max M2^{e} plate reader with the necessary toxin standards and controls to assess concentrations of domoic acid (typically reported in ng L^{-1}) and total saxitoxins (typically reported in ppb), the toxins produced by *Pseudo-nitzschia* spp. and *G. catenatum*, respectively. We did not collect shellfish samples during the study to understand whether the algal toxin production translated to accumulation of toxins in shellfish tissues.

Statistical analyses

We used generalized additive mixed models (GAMMs) to characterize the environmental conditions during May through October of 2015 - 2019, the season when HABs most commonly occur, while reducing the variation of environmental variables due to well-documented seasonal changes (Rollwagen-Bollens et al. 2020). We identified periods of statistically significant environmental change in a time series of daily averages of multiple water quality variables using the '*mgcv*' package in R, with Gaussian probability distributions of the variables, and the restricted maximum likelihood (REML) method for smoothness selection (Wood 2017, Simpson 2018). Temporal autocorrelation was explicitly modeled in the GAMM, and model selection was determined by comparing Bayesian Information Criterion (BIC) of models with different error correlation structure and checking for autocorrelation among model residuals.

We used a non-metric multidimensional scaling (NMDS) analysis with the Bray-Curtis dissimilarity index to assess patterns in species composition (Clarke 1993). Species count data were square-root-transformed prior to the NMDS, which was done using the R package '*vegan*' (Oksanen et al. 2011). Vectors of environmental variables were fitted to the NMDS plot using the envfit function in the same package. We used agglomerative hierarchical clustering with average linkage on a Bray-Curtis dissimilarity matrix to find grouping patterns in the phytoplankton assemblage dataset.

We used gradient forest analysis to identify thresholds separating components of phytoplankton assemblages along major environmental gradients. This analysis was performed using the '*extendedForest*' and '*gradientForest*' packages in R (v3.6.2; Ellis et al. 2012). A gradient forest fits a random forest model for each taxon in the phytoplankton assemblage, in which each of 500 trees is fit to a bootstrapped sample and splits are made using a random subset of predictors. Goodness-of-fit R^2 values for each taxon can be distributed in proportion to the importance of each predictor to generate the overall importance of a predictor to phytoplankton species composition. Split density plots show where (on the scale of the predictor) a predictor is splitting trees, and indicate the importance of each predictor based on how much of the variance in the data it explains. Compositional turnover plots show cumulative importance of predictors for each species over gradients of predictor variables. Taxa present in <5% of samples or at very low cell concentrations (< 10 cells ml^{-1}) were excluded from the gradient forest analysis.

Results

Estuarine conditions

Daily average surface water temperatures in the LCRE were elevated by ~1-2 ^{o}C in July-December of 2015 and July-September of 2019, relative to the decadal average at the SATURN-03 site (Fig. 2). For summers included in the study period, temperatures typically increased most rapidly through May and June, peaked in the window of July-September, and began to decrease in September or October (Fig. 2).

Elevated surface temperatures were sustained for a longer period of time during 2015 and 2019 than for any other year during the study period (approximately 49.0 and 47.4 day equivalents >18 ℃; 18.3 and 12.8 day equivalents >20 ℃, respectively) (Table 2). In addition, temperatures did not drop as rapidly during the summer-autumn transition in 2015 as was observed during 2016-2019 (Fig. 3).

Hydrographs for the LCRE varied greatly among the five years. In 2017, a non-MHW year with a strong spring freshet and high discharge throughout the growing season, peak discharge was 111% higher and minimum discharge was 4% lower than the decadal average (Fig. 2). Discharge in 2018 and 2019 also displayed a large freshet pattern, with peak daily average discharges 83% and 92% higher than the decadal average, respectively. The spring freshet was smaller in 2015 and 2016; peak flows were 53% and 5% higher than the decadal average, respectively. For all study years but 2017, minimum discharge was 42 - 76% lower than the decadal average minimum (Fig. S1).

Salinity in the LCRE increased throughout May - October each year, with daily averages <5 PSU typical in May, and >10 PSU typical of late summer. In 2015, however, the smoothed daily average salinity in June was >10 PSU (Fig. 3). The summer of 2015 also had the highest number of day equivalents (7.1 d) of salinity >25 PSU at 2.4 m, compared to a range of 3.8 - 4.4 d for the other years studied (Table 1), showing an extended period of strong marine influence on the surface waters of the LCRE. We observed an extended period of brackish surface water in 2019, which had 72.5 day equivalents of salinity >15 PSU at 2.4 m, compared to a range of 45.5 - 60 d in 2015 – 2018 (Table 1).

Upwelling patterns were similar among years, with the exception of a significant shift toward downwelling in late summer of 2016 (> 0 m^3 s^{-1} 100 m^{-1}), and an earlier peak in upwelling (~120 m^3 s^{-1} 100 m^{-1} in June) and a significant decline in the rate of offshore transport throughout the summer of 2019 (Fig. 3). The highest nitrate concentrations (>20 µM in May 2016-2018) were observed in early summer, with some fortnightly fluctuations. Nitrate concentrations were lower on average in 2015 than other study years, especially earlier in the season (<10 µM in May). No nitrate data were collected in 2019 (Fig. 3). Chlorophyll showed fortnightly variation, with higher overall chlorophyll levels earlier in the summer that decreased as the summer progressed. We observed a small peak in chlorophyll during the 2015 bloom of *Pseudo-nitzschia* spp. and the 2019 *G. catenatum* bloom (z-scores of 1.8 and 2.8, respectively) (Fig. 3).

Algal species composition

We identified 168 species or species complexes and 112 genera, with a mean richness of 25 taxa per sampling date and average sample diversity D of 0.70 (range = 0.17 to 0.91) over the three years for which phytoplankton samples were collected. We identified six phyla (Bacillariophyta, Chlorophyta, Cryptophyta, Cyanophyta, Dinoflagellata, Euglenophyta) and two taxonomically amalgamated groups to capture other less common taxa (small flagellates, and other). Bacillariophyta typically dominated the assemblages, with an average relative abundance of 56%. Among phylum Bacillariophyta, *Navicula* spp. were abundant on all sampling dates, peaking at 69% of total cells in April 2017. *Melosira varians, Skeletonema* spp., and c.f. *Cyclotella/Thalassiosira* spp. were the next most abundant diatoms in spring/early summer. Dinoflagellata had an average relative abundance of 29%, and each of the other taxonomic groups had an average relative abundance of <10% (Fig. 2). A NMDS analysis (stress = 0.12) indicated that phytoplankton samples varied most along the first NMDS axis (Fig. 4) with greater separation of samples among years (i.e., 2015 and 2019 vs. 2017) and seasons (i.e., late summer vs. early summer samples from 2015 and 2019).

Hierarchical cluster analysis indicated that the two algal blooms observed in the study were distinctly different in composition. In 2015, the toxigenic marine diatoms, *Pseudo-nitzschia* spp. (mostly from the *P.* c.f. *australis/fraudulenta* group), dominated the phytoplankton assemblage in early October (average of 56% on 10/1/2015; Fig. 2), in contrast to other sampling dates, which had either undetectable or low (March 2017, August-September 2019) *Pseudo-nitzschia* spp. concentrations. Particulate domoic acid (DA) was measured at relatively low, but detectable, levels during the *Pseudo-nitzschia* spp. bloom (Fig. 6). Average toxin concentrations ranged from 41 – 86 ng L^{-1} from samples taken at the bottom (~13 m), middle (~8 m) and surface of the water column on October 1 and mid water column on October 2, 2015. Although domoic acid was detected at ~8 m, it was not detected at the bottom or surface on October 2. In 2019, the chain-forming marine dinoflagellate, *Gymnodinium catenatum*, peaked at 91% of the total assemblage in mid-August; the bloom persisted through late September. It was not detected in our light microscopy analyses any time outside this bloom period. Once the bloom began to decline (*ca*. 9/12/2019), *G. catenatum* was accompanied by *Euglena* sp. (24%), *Cryptomonas erosa* (16%), and *Mesodinium rubrum* (5%). Overall, species diversity during the peak of the 2019 bloom was very low (D = 0.17) compared to the highest reported diversity from earlier that year (D = 0.80 on 7/10/2019). Saxitoxins were detected during the *G. catenatum* bloom, ranging from 1.14—1.79 ppb, with the highest toxin level detected during the height of the bloom in late August (Fig. 6).

Environmental drivers of phytoplankton assemblage composition

Ordinations with overlaid environmental vectors with points scaled by the relative abundance of phytoplankton taxa (Fig. 5) indicated that densities of the marine planktonic diatoms like *Ditylum brightwellii, Thalassionema nitzschoides, Nitzschia longissima*, and *Pseudo-nitzschia* spp., as well as the planktonic dinoflagellate *G. catenatum* were generally associated with more saline, low-discharge, low-nitrate conditions. Of these, *D. brightwellii*, *T. nitzschoides*, *N. longissima*, and *Pseudo-nitzschia* spp. were also associated with high temperatures and upwelling. On the other hand, the abundance of the freshwater taxa *Ankistrodesmus* sp. and the tychopelagic *Navicula* spp. declined later in the growing season, with higher counts of these taxa observed in samples associated with lower temperatures, higher flows, and low salinity. This is consistent with the gradient forest cumulative density plots (Fig. 7A-E), which indicate a sharp change in cumulative importance of salinity at 13-14 PSU and of discharge at approximately 3,400 $m^3 s^{-1}$ to the abundance of the coastal diatom species, *D.*

brightwellii, which increased under low discharge and high salinity. *Navicula* spp. showed an increase in cumulative importance of discharge at approximately 3,500 $m^3 s^{-1}$ and of salinity at approximately 13 PSU, with its abundance decreasing under low discharge and high salinity. *Ankistrodesmus* sp. showed a sharp change in the importance of discharge at approximately 7,079 $m^3 s^{-1}$, with its abundance decreasing when discharge declined. Temperature, upwelling, and nitrate all contributed to the abundance of c.f. *Cyclotella/Thalassiosira* spp., with increases in cell densities weakly associated with elevated temperature and upwelling index. Cumulative importance of upwelling index to the density of *N. longissima* increased at approximately 50 $m^3 s^{-1}$ 100 m^{-1}, with an increase in abundance during upwelling. *T. nitzschoides* showed an increase in cumulative importance of nitrate at approximately 18 µM, with increasing abundance at lower nitrate concentrations. The gradient forest analysis indicated that the environmental variables of overall greatest importance in defining the phytoplankton assemblage in order were discharge, salinity, temperature, nitrate, and upwelling index (Fig. 7F).

Discussion

Transition to brackish-marine phytoplankton assemblage in MHW years

Every year, shifts in LCRE phytoplankton assemblages accompany the transition from riverine to marine influence in late summer (Rollwagen-Bollens et al, 2020; this study). However, oceanographic conditions accompanying MHWs – temperatures exceeding daily decadal averages by 1-2 °C and anomalously low discharge volumes - were associated with the only blooms of toxigenic species observed in the LCRE during the study period. In a study that temporally overlaps with ours, Rose et al. (2021)

observed spikes in cyanobacterial biomass in late summer of 2017 and 2018 at a site 170 river km upstream of the mouth, though we only observed a muted elevation in cyanobacteria in the summer of 2017 at our downstream site. Although harmful cyanobacteria blooms do occur in parts of the Columbia River, we have not found records of HABs in the lower estuary area of interest for this study with the exception of those discussed herein. *Pseudo-nitzschia* spp. have been observed in the LCRE previously, but did not dominate the assemblage or reach bloom concentrations (Frame and Lessard 2009). Interestingly, the dominant HAB taxon differed between MHWs occurring in 2015 and 2019, with *Pseudo-nitzschia* spp. dominating the former and *G. catenatum* dominating the latter. Both of these taxa occupy marine-brackish habitats; thus, our discussion focuses on drivers of marine and brackish HABs at the LCRE site.

Our results may suggest two different potential mechanisms contributing to bloom development in the LCRE. In 2015, the estuary more closely resembled the coastal ocean in bloom composition; that is, the prolonged period of marine influence in the LCRE resulting from reduced river discharge provided sufficient habitat opportunity to allow offshore *Pseudo-nitzschia* spp. to persist at relatively high densities following tidal exchange. The importance of low discharge in creating appropriate conditions for a bloom is suggested by the timing of the bloom in the LCRE. Offshore, toxic *Pseudo-nitzschia australis* associated with the 2015 North Pacific MHW (National Centers for Coastal and Ocean Science 2015) was prevalent during the summer, while the *P.* c.f. *australis/fraudulenta* complex was not detected in the LCRE at significant concentrations until October 2015 when river flows declined below ~3,400 $m^3 s^{-1}$ and salinity was elevated (>14 PSU). More broadly, the samples collected in October of 2015 revealed higher abundances of marine phytoplankton, including *Pseudo-nitzschia* spp., at deeper depths where salinities were highest in association with the salt wedge (Kärnä and Baptista 2016). Particulate domoic acid (pDA) was detected (<100 ng L^{-1}) when *P.* c.f. *australis/fraudulenta* was present, although toxin levels were less than half the concentration that leads to accumulation in shellfish (ORHAB 2021).

In 2019, *G. catenatum* also bloomed during a period of low discharge (~ 2,800 $m^3 s^{-1}$ - 3,400 $m^3 s^{-1}$ 14-d average) with anomalously elevated temperature relative to the site decadal average. Particulate saxitoxins in water were observed during this bloom, but concentrations were lower than recreational alert levels (10 ppb or $\mu g\ L^{-1}$) for freshwater systems in Oregon, which does not currently have seawater saxitoxin health guidelines (Oregon Department of Agriculture 2021). This bloom did not appear to have been transported into the estuary from a nearshore marine site like the 2015 *Pseudo-nitzschia* spp. bloom. Rather, it is likely to have developed *in situ* nearby the Ilwaco sampling site in Baker Bay, based on its absence in samples concurrently collected from the Columbia River South Jetty, and several other open coast sites in Oregon and Washington (data not shown, M. Rogers pers. comm., 2019). *Pseudo-nitzschia* spp. were present in very low concentrations in the LCRE during the 2019 *G. catenatum* event, but nearby coastal sites had higher abundances (data not shown), indicating a lesser degree of transport into the LCRE than observed during the 2015 bloom event. Given that many dinoflagellates including *G. catenatum* can form resistant cysts to sustain populations through long periods in marginal environments (Blackburn et al. 1989; Hallegraeff et al. 2012), it is possible that cysts transported from an unknown seed area (either offshore or within the estuary) could have seeded the bloom in the LCRE during conditions that favored its proliferation.

We identified clear shifts in phytoplankton assemblages associated with thresholds of discharge and salinity, but the mechanism by which these shifts occur is not clear from this analysis, particularly in explaining the emergence of two different HABs during the transition to marine dominance of the LCRE under low-flow conditions. The observed association of *Pseudo-nitzschia* spp. abundance in the LCRE with upwelling may be due to the effect of wind stress on the movement of the Columbia River plume, which can act as a barrier to onshore transport of marine plankton during downwelling. During upwelling winds, offshore plankton may become entrained within it or subduction may occur (Hickey et al. 2005). The *G. catenatum* bloom did not show the same association with upwelling, and occurred during a significant decreasing trend in upwelling index. Upwelling also supplies inorganic nutrients to surface coastal waters and has a proportionally greater impact on nutrient inputs to the LCRE under low flow conditions (Bruland et al. 2008). Du et al. (2016) documented enhanced *Pseudo-nitzschia* spp. growth in coastal waters during stronger upwelling in 2015, likely due to increased nutrient availability. Nitrate in the LCRE was low in early summer of 2015 compared to other years, which is likely a result of very low discharge during this time, combined with periods of weakened upwelling. During the late summer time frame when the *Pseudo-nitzschia* spp. bloom occurred, nitrate (the preferred N source for *Pseudo-nitzschia* spp. (Cochlan et al. 2008)) increased in the LCRE during a period of stronger upwelling (Du et al. 2016). We did not collect nutrient data beyond nitrate concentrations in the LCRE for 2015-2018 so we rely on previous work in this system to interpret the impact of nutrient conditions during our study period, particularly for 2019.

In contrast, *G. catenatum* is a poor competitor for inorganic nutrients, is not likely to bloom without a source of organic nutrients, and is capable of phagotrophy (Yamamoto et al. 2004; Jeong et al. 2010)). This is consistent with our finding that *G. catenatum* was not associated with inorganic-nutrient-rich marine upwelling, despite being associated with brackish waters in the LCRE. When saline waters extend into the LCRE under low flow conditions, freshwater plankton from upriver may die from osmotic stress when they encounter salinities of 1-5 PSU (Lara-Lara 1990), and contribute to available particulate organic matter (POM) (Small et al. 1990). Long residence time during low flows may slow the flushing of these resources, particularly from lateral bays, creating an optimal food source for a phagotrophic dinoflagellate, which co-occurred with other mixotrophic plankton (e.g., *Mesodinium rubrum*, *Euglena* spp.). Renewing water age in the LCRE is about 20 hours during high discharge conditions, but may exceed 120 hours under low discharge and neap tide conditions in bays with weak circulation, such as our study site in Baker Bay (Kärnä and Baptista 2016). This longer flushing time observed in the late summer may retain POM and provide refuge for plankton (either transported from offshore or grown within the LCRE) that may not establish under faster flowing conditions.

Both HAB taxa observed are also well-adapted to the anomalously warm, saline habitat available in the LCRE during the MHWs that was not present under higher discharge conditions. *Pseudo-nitzschia* spp. are able to grow at temperatures up to 30 ℃ (Zhu et al. 2017). They are rarely found in low salinity waters, and exhibit high mortality rates when exposed to salinity outside 30-35 PSU (Thessen et al. 2005; Ayache et al. 2019). *G. catenatum* can grow at temperatures as high as 29 ℃, though temperate ecotypes grow optimally at temperatures between 12-18 ℃ (Hallegraeff et al. 2012), and will tolerate salinities in the range of 15-40 PSU (Blackburn et al. 1989; Band-Schmidt et al. 2004).

Our analyses suggest that declining river influence during the late summer period of two anomalously warm years (2015 and 2019) was a shared driver in creating a window of habitat availability for two different HABs. Each likely occupied this habitat through a different discharge-related mechanism – an upwelling-fed coastal bloom with tidal advection into an unusually saline LCRE and *in situ* bloom development promoted by long water renewal times and a source of POM in a brackish LCRE.

Characteristics of phytoplankton assemblage transitions

Discharge and salinity were identified as the primary predictors of phytoplankton assemblage composition in the LCRE in the gradient forest analysis. This highlights the importance of the seasonally driven environmental gradient of river dominance vs. ocean influence, and takes a further step in identifying the thresholds at which the resulting phytoplankton transition occurs. The HAB taxa observed during the study period did not exhibit large changes in the cumulative importance of environmental variables in the gradient forest analysis, possibly because they were present at undetectable or low abundance prior to the onset of the HABs. However, several assemblage shifts (e.g., declines in tychopelagic *Navicula* spp. and freshwater taxa, increasing marine pelagic taxa like *Ditylum brightwellii*) may be used to demarcate estuarine niche transitions associated with elevated risk of marine and brackish HABs to help focus monitoring efforts. It should be noted that our species composition dataset is relatively sparse compared to the continuous environmental data from the LCRE. Higher resolution plankton assemblage data may improve environmental threshold estimates of plankton niche transitions, and a larger dataset would allow validation of the gradient forest model. In addition, we only analyzed phytoplankton assemblage data for one year that did not have a MHW, and therefore cannot determine how representative the 2017 community is of typical non-MHW years. In order to better understand how MHWs influence phytoplankton assemblages, more baseline assemblage data are needed to compare anomalous events with normal variability. Although our study captures two HABs during two MHWs, the lack of historical documentation of either MHWs or marine/brackish HABs in the LCRE necessitates continued monitoring to understand the relationship between MHW and HABs in this unique habitat.

Environmental drivers of phytoplankton shifts

Our NMDS analysis with environmental vector overlay indicated that *Navicula* spp. and the freshwater *Ankistrodesmus* sp. were strongly negatively associated with salinity. Although some *Navicula* species in the LCRE are thought to be tolerant of brackish conditions (Simenstad et al. 1984), it is possible that salinity exceeded the preferred range of less salt tolerant species during the extreme low flow conditions experienced in 2015 and 2019. Changes in resuspension of tychopelagic diatoms may also influence the observed shifts in phytoplankton assemblages in our surface water samples. Mixing in the LCRE is governed by complex interactions between tidal transitions, river flow, and density gradient. During the summer low flow period, neap-spring tide transitions in stratification are less disrupted by high flow events, indicating that mixing may be more governed by tidal action. During this time, hydrodynamic models suggest the LCRE is strongly or weakly stratified for most of the tidal cycle, but experiences brief periods (1-2 days) of partial mixing during the transition between the neap-tide and the rest of the tidal month (Jay 1990). Reduced vertical mixing is one possible explanation for the observed decline in tychopelagic taxa during low flow conditions. However, studies on particle movement (Stevens et al. 2017) have focused on the main channel of the LCRE, and flow dynamics are less well understood in the LCRE lateral bays.

This analysis also showed that *Pseudo-nitzschia* spp., *T. nitzschoides*, and *N. longissima,* and *D. brightwellii* (marine pelagic taxa) were positively associated with upwelling and temperature, negatively associated with nitrate, and strongly negatively associated with discharge. *G. catenatum* and *D. brightwellii* (brackish and marine pelagic taxa) were most strongly positively associated with salinity. We observed peaks in chlorophyll in late summer of 2015 and 2019, associated with the growth of these brackish and marine taxa. In contrast, chlorophyll declined significantly throughout the study period during 2017, with very low values in late summer. This may indicate a lack of a transition to marine dominance and the associated phytoplankton assemblage that increased chlorophyll in late summer of MHW years.

As climate change is expected to alter the hydrology of the Columbia River (Hamlet and Lettenmaier 1999), the conditions observed during the described MHW years may become more common in the future. It is likely that low-snowpack, high temperature years will become more frequent, low-flow timeframes may be extended, and flows may be lower during drought conditions, creating potential for a longer, geographically larger window of ocean-influenced LCRE. Currently this LCRE habitat is ephemeral, but has the potential to increase spatially (upriver) and temporally. Our study is limited by the number of years of LCRE phytoplankton monitoring data, making mechanistic explanation of the two different HABs observed during climatically anomalous events challenging.

Despite the increasing occurrence of algal toxin closures for Pacific Northwest beaches, most bays and estuaries remain open to shellfish harvest all year and many are not monitored for HABs to provide early warning of toxin events. Although strong freshwater flows during the winter and spring generally preclude the need for marine and brackish HAB monitoring in river-dominated west coast estuaries, we show that these HABs can develop during anomalously low summer discharge. Sustained monitoring will be essential to understand mechanisms driving estuarine HAB development in a changing climate.

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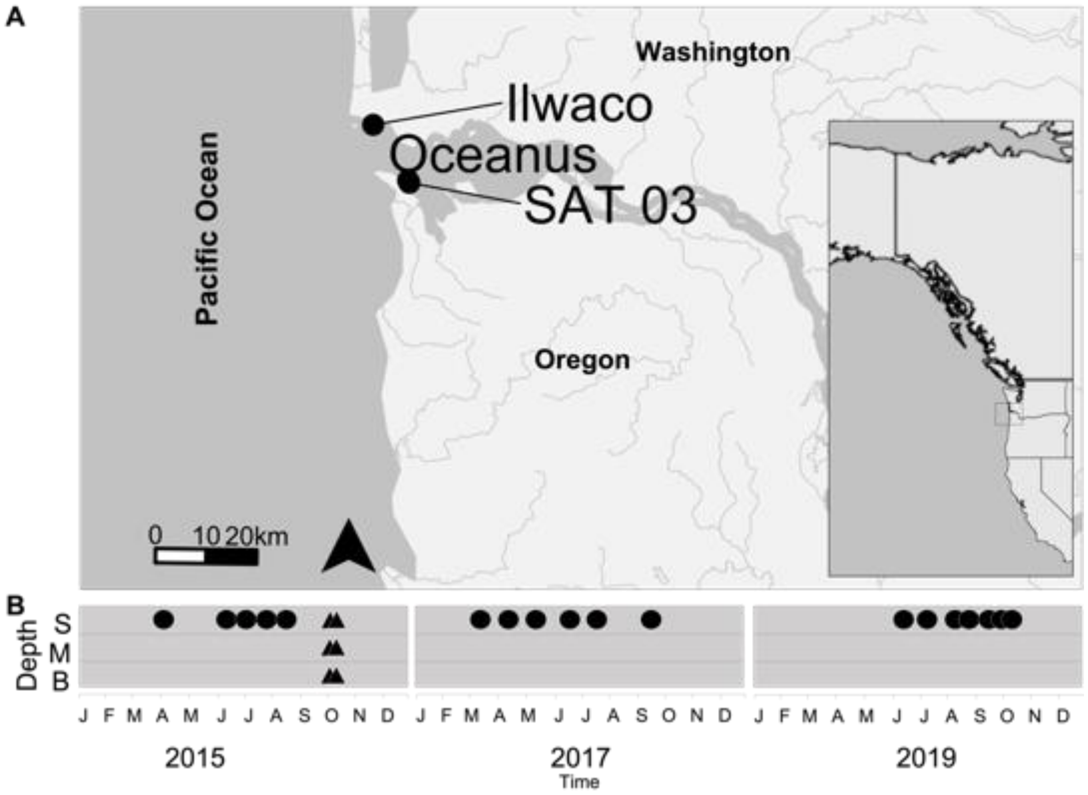

Fig. 1 Map of Lower Columbia River Estuary with regional inset (A) and phytoplankton sampling scheme (B). (A) The Columbia River forms part of the Oregon-Washington border. Phytoplankton and nutrient samples were collected from Ilwaco and Oceanus sites. Environmental data were collected from sensors at Saturn 03 site. Discharge data were collected upstream of the close-up map, and upwelling index was reported for Lincoln City (Oregon Coast). (B) Each point on the timeline represents a phytoplankton sampling event, with the depth of the sample specified on the y-axis (S = surface, M= mid-water column, B = bottom). Circles represent sampling from the Ilwaco site and triangles represent sampling from the R/V Oceanus. Our sampling scheme combined long-term, low frequency, surface samples with short-term, high-frequency, samples of the whole water column. This allowed for characterization of phytoplankton assemblages at multiple temporal and spatial scales.

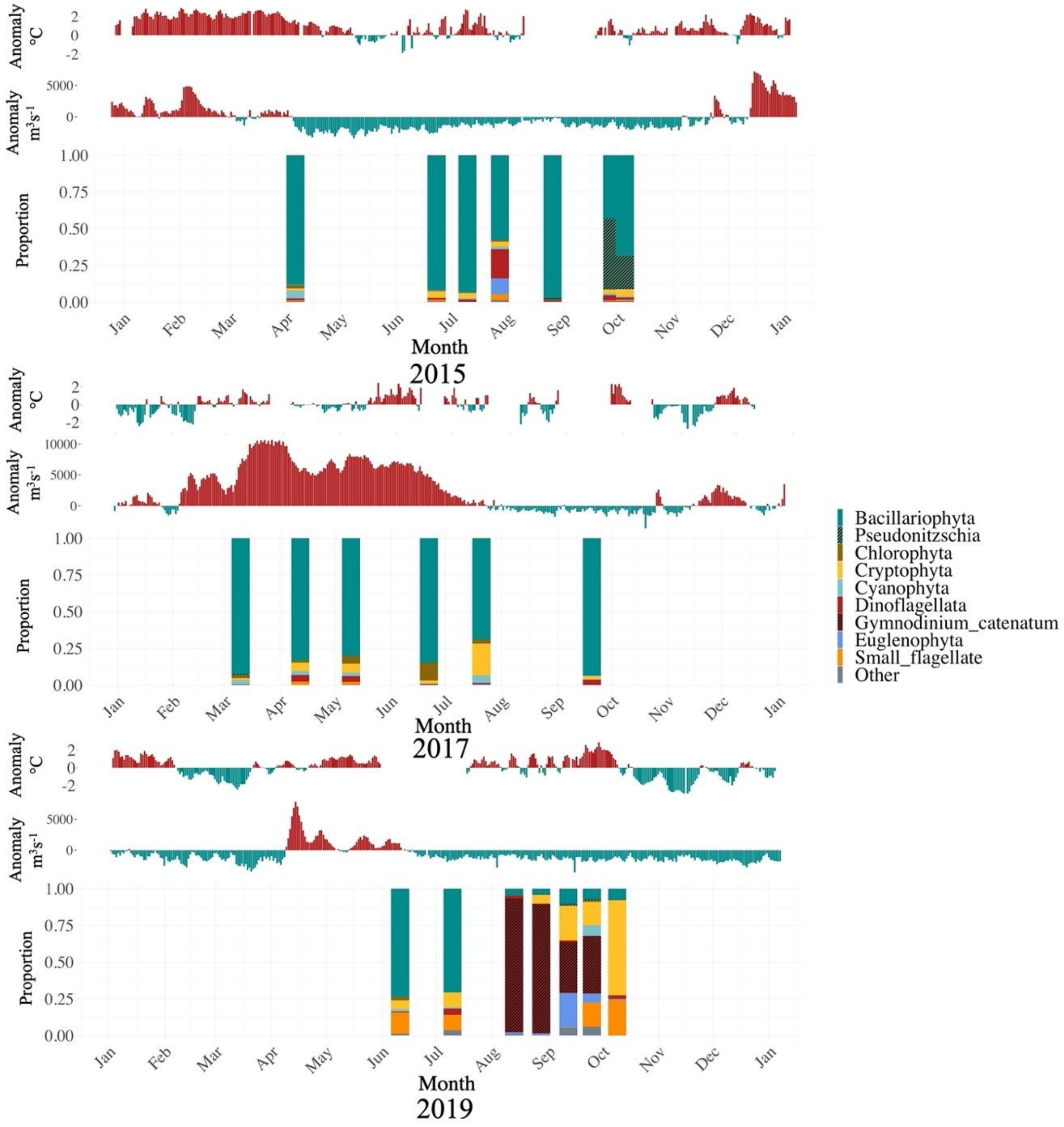

Fig. 2 Bar plots of phytoplankton assemblage composition (bottom) with temperature anomaly in degrees Celsius (top) for 2015, 2017, and 2019. Phytoplankton taxa are divided into phyla (Bacillariophyta Chlorophyta, Cryptophyta, Cyanophyta, Dinoflagellata, Euglenophyta) and agglomerative groups (Small flagellates and Other, which includes a combination of other phyla that were relatively rare). All phytoplankton samples were taken at the surface, except for the October 2015 samples, which are shown as an average of phytoplankton samples from the surface, middle and bottom of the water column. The harmful algal blooms observed during the study period (*Pseudo-nitzschia* spp. and *Gymnodinium catenatum*) are given their own group and color (striped). Temperature anomaly (difference from decadal daily average at Saturn-03 site) is shown

on the same time-scale as phytoplankton bar plots, with warmer-than-average days shown in red, cooler-than-average days shown in blue, and gaps in the time series indicating missing values.

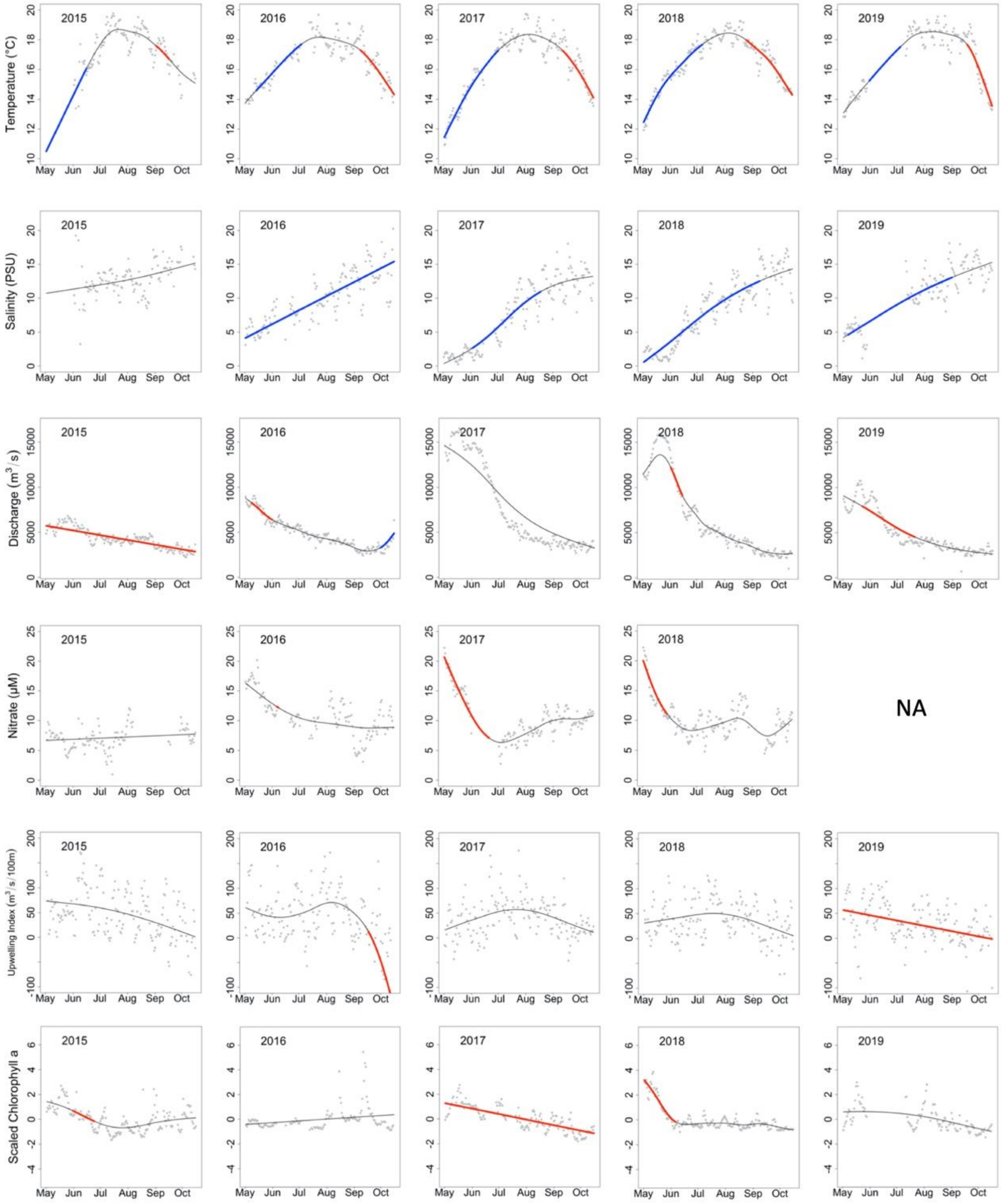

Fig. 3 GAMMs provide a characterization of environmental conditions during the growing season (May-October) of each year from 2015 - 2019, with significantly increasing modeled time frames displayed in blue, and significantly decreasing periods shown in red. Chlorophyll a values for each year were scaled (z-scores) to account for inter-annual differences in sensor calibration. NA: Nitrate data in 2019 was missing.

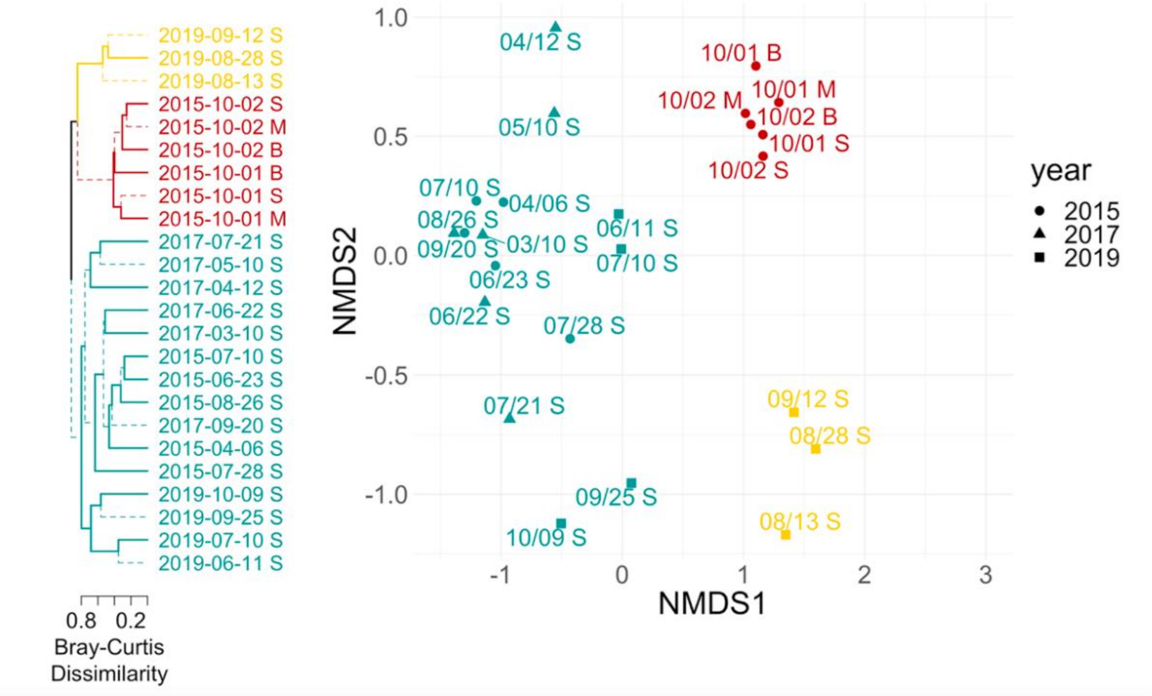

Fig. 4 Two-dimensional ordination using non-metric multidimensional scaling (NMDS) of phytoplankton assemblage data with hierarchical clustering analysis. Each point represents a phytoplankton sampling date and depth (S = surface, M = mid-water column, B = bottom), with more similar communities placed closer to each other. Stress value for the NMDS is 0.12. Shapes depict sampling year (circle = 2015, triangle = 2017, square = 2019). Colors depict cluster identity and dashed lines indicate branches leading to distinct sub-trees.

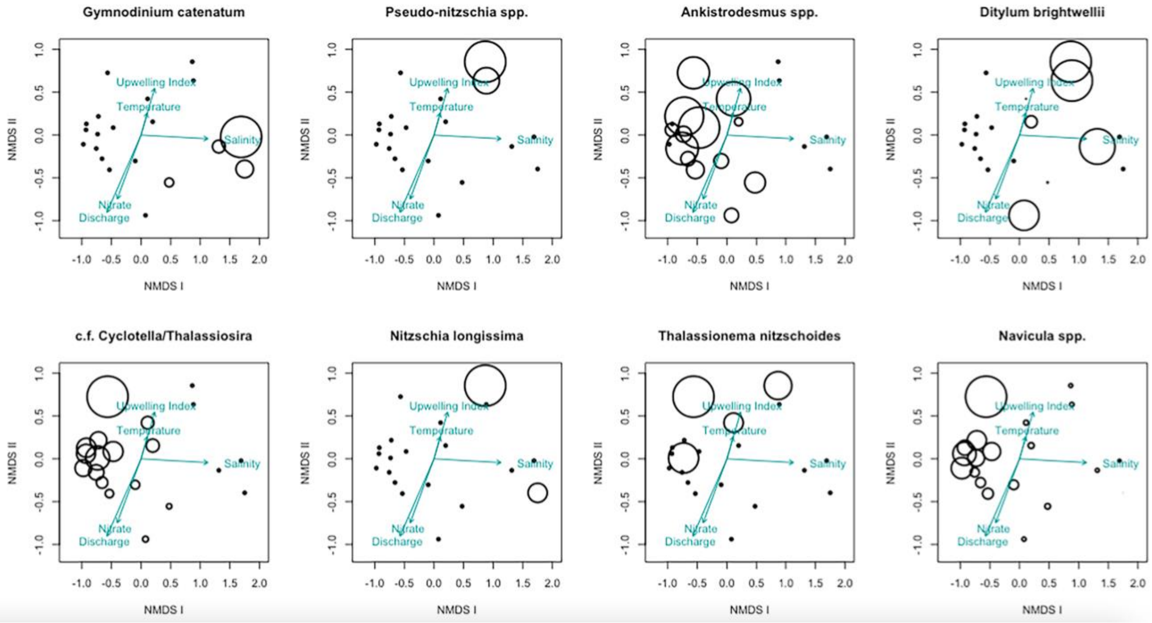

Fig. 5 Non-metric multidimensional scaling plots of phytoplankton assemblage from Ilwaco Harbor samples from 2015, 2017, and 2019, with bubble size proportional to the relative abundance of the selected taxa. Environmental vectors that correspond to the important physical drivers in the gradient forest analysis are overlaid. Phytoplankton taxa were selected either for toxin-production potential (*Pseudo-nitzschia spp.* and *Gymnodinium catenatum*) or because of a notable shift in cumulative importance over the gradient range of environmental predictors in the gradient forest analysis. Where multiple samples were taken (i.e. at different depths) on the same day, phytoplankton abundances were averaged. All measurements of environmental variables corresponding to phytoplankton sampling dates are 14-day moving averages leading up to the date of phytoplankton sampling. Units corresponding to the environmental vectors are: discharge (m^3s^{-1}), temperature (°C), salinity (PSU), nitrate (µM), upwelling index (m^3 s^{-1} 100m offshore transport).

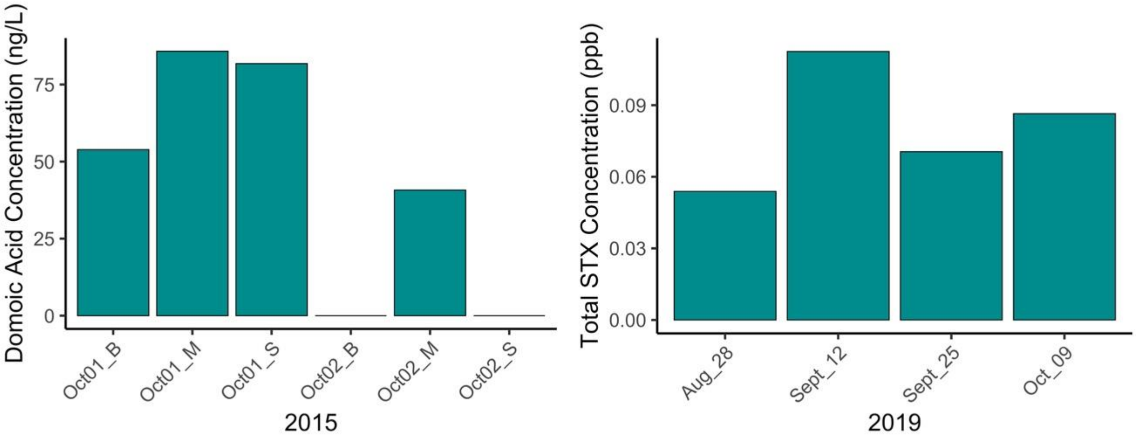

Fig. 6 Concentrations of particulate algal toxins, domoic acid (ng/L) and total saxitoxins (ppb). Domoic acid samples were taken aboard the R/V Oceanus on October 1st and 2nd of 2015 during a bloom of *Pseudo-nitzschia* spp. and total saxitoxin samples were collected from Ilwaco Harbor in August-October of 2019. For each date and depth *n*=1.

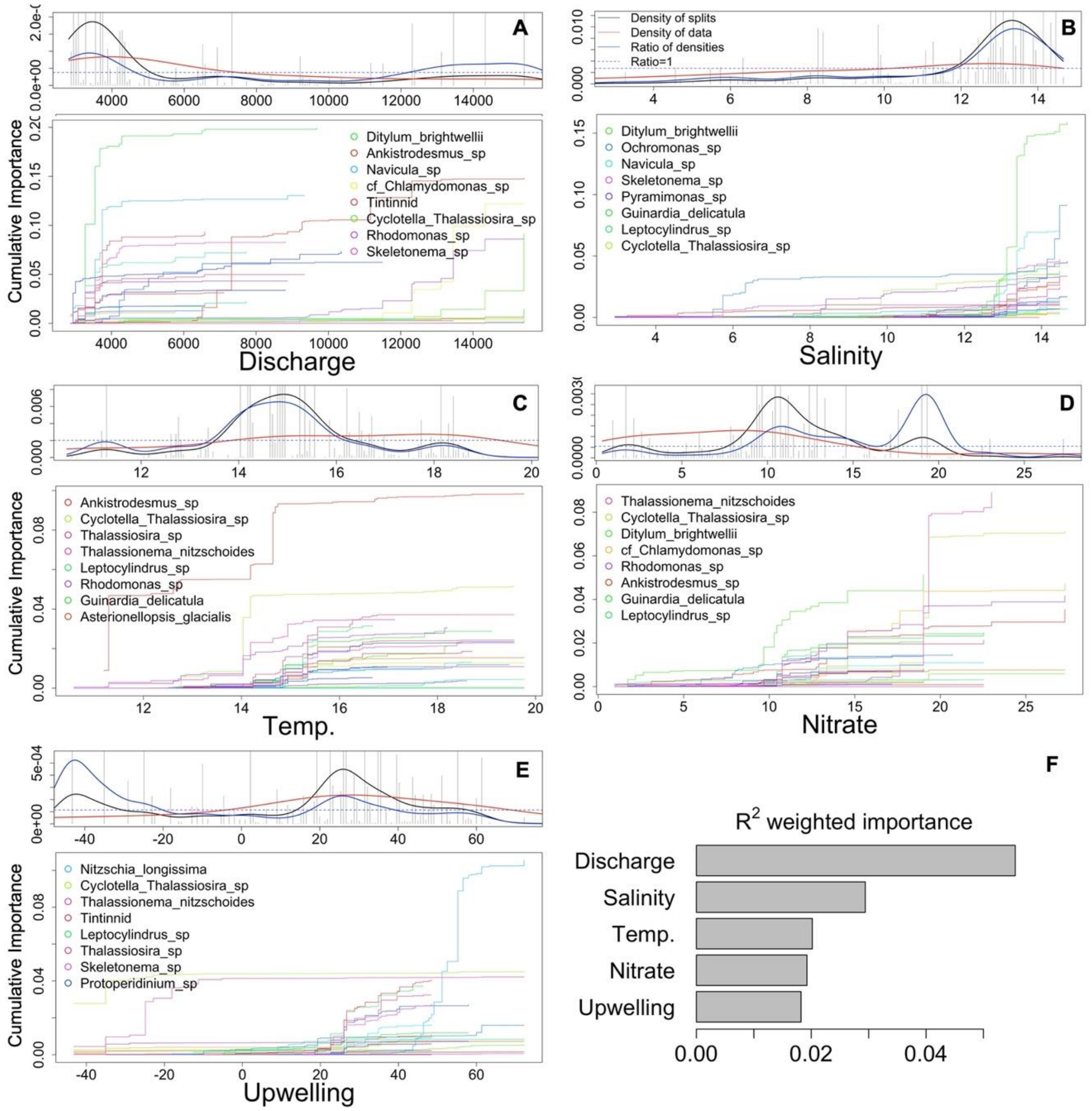

Fig. 7 Gradient forest analysis shown as split density (top, A-E) and cumulative density (bottom, A-E) plots, and overall weighted importance of each predictor (F). (Top panel, A-E) Y axis shows density, x axis shows scale of corresponding predictor variable. Black line shows density of splits from regression trees, red line and gray bars show density of data. Blue line shows the ratio of split density to data density – above dotted blue line indicates that split density is higher than data density. (Bottom panel, A-E) Y axis shows cumulative importance of each variable to the abundance of several phytoplankton taxa ((A) Discharge (m^3s^{-1}), (B) Salinity (PSU), (C) Temperature (°C), (D) Nitrate (µM), (E) Upwelling Index (m^3 s^{-1} 100m offshore transport) to a given species. Different taxa are designated by different colored lines. (F) Overall importance, R^2 for each predictor of the physical drivers.

Table 1 Day equivalents of salinity exceeding 15, 25, and 30 PSU during summer season (June 1st - September 30th) at the Saturn 03 station. Salinity measured at 2.4 m, 8.2 m, and 13.0 m depth. Hourly averages used to calculate day equivalents, with missing hourly salinity measurements imputed using seasonal decomposition-based imputation.

Number of Day Equivalents of Salinity Exceeding 15, 25, 30 PSU (d)					
	2015	2016	2017	2018	2019
>15 PSU					
2.4 m	59.83	53.96	45.50	60.00	72.46
8.2 m	18.29	5.00	11.04	10.79	12.75
13.0 m	156.75	186.88	174.33	215.25	162.67
>25 PSU					
2.4 m	7.13	3.75	4.38	3.88	3.88
8.2 m	17.38	29.21	36.04	42.13	29.33
13.0 m	18.04	47.46	51.13	64.75	55.42
>30 PSU					
2.4 m	0.33	0.00	0.13	0.04	0.21
8.2 m	1.88	0.54	4.96	2.79	0.54
13.0 m	2.42	1.88	15.38	14.71	4.88

Hourly averages of salinity used to calculate number of day equivalents during June-September.

Table 2 Day equivalents of temperature exceeding 15, 18, and 20 °C during summer season (June 1st - September 30th) at the Saturn 03 Station. Temperature measured at 2.4 m, 8.2 m, and 13.0 m depth. Hourly averages used to calculate day equivalents, with missing hourly temperature measurements imputed using seasonal decomposition-based imputation.

Number of Day Equivalents of Temperature Exceeding 15, 18, 20 °C (d)					
	2015	2016	2017	2018	2019
>15 °C					
2.4 m	89.04	76.63	92.08	106.25	73.46
8.2 m	24.46	45.54	43.29	55.08	68.04
13.0 m	15.75	39.75	19.13	35.50	49.63
>18 °C					
2.4 m	48.96	38.29	46.25	45.88	47.38
8.2 m	3.21	8.13	7.54	8.96	14.08
13.0 m	2.50	4.58	2.25	3.88	6.00
>20 °C					
2.4 m	18.29	5.00	11.04	10.79	12.75
8.2 m	0.54	0.04	0.04	1.08	1.00
13.0 m	0.25	0.63	0.25	0.75	0.25

Hourly averages of temperature used to calculate number of day equivalents during June-September.

References

Amaya, Dillon J., Arthur J. Miller, Shang-Ping Xie, and Yu Kosaka. (2020). Physical drivers of the summer 2019 North Pacific marine heatwave. *Nature Communications 11* (1): 1903. https://doi.org/10.1038/s41467-020-15820-w.

Ayache, Nour, Fabienne Hervé, Véronique Martin-Jézéquel, Zouher Amzil, and Amandine M. N. Caruana. (2019). Influence of sudden salinity variation on the physiology and domoic acid production by two strains of *Pseudo-nitzschia* australis. *Journal of Phycology 55* (1): 186–95. https://doi.org/10.1111/jpy.12801.

Band-Schmidt, C. J., L. Morquecho, C. H. Lechuga-Devéze, and D. M. Anderson. (2004). Effects of growth medium, temperature, salinity and seawater source on the growth of *Gymnodinium catenatum* (Dinophyceae) from Bahía Concepción, Gulf of California, Mexico. *Journal of Plankton Research 26* (12): 1459–70. https://doi.org/10.1093/plankt/fbh133.

Baptista, António M., Charles Seaton, Michael P. Wilkin, Sarah F. Riseman, Joseph A. Needoba, David Maier, Paul J. Turner, et al. (2015). Infrastructure for collaborative science and societal applications in the Columbia River Estuary. *Frontiers of Earth Science 9* (4): 659–82. https://doi.org/10.1007/s11707-015-0540-5.

Berdalet, Elisa, Lora E. Fleming, Richard Gowen, Keith Davidson, Philipp Hess, Lorraine C. Backer, Stephanie K. Moore, Porter Hoagland, and Henrik Enevoldsen. (2016). Marine harmful algal blooms, human health and wellbeing: challenges and opportunities in the 21st century. *Journal of the Marine Biological*

Association of the United Kingdom 96 (1): 61–91. https://doi.org/10.1017/S0025315415001733.

Biela, Vr von, Ml Arimitsu, Jf Piatt, B Heflin, Jl Schoen SK Trowbridge, and Cm Clawson. (2019). Extreme reduction in nutritional value of a key forage fish during the Pacific marine heatwave of 2014-2016. *Marine Ecology Progress Series 613* (March): 171–82. https://doi.org/10.3354/meps12891.

Blackburn, Susan I., Gustaaf M. Hallegraeff, and Christopher J. Bolch. (1989). Vegetative reproduction and sexual life cycle of the toxic dinoflagellate *Gymnodinium catenatum* from Tasmania, Australia. *Journal of Phycology 25* (3): 577–90. https://doi.org/10.1111/j.1529-8817.1989.tb00264.x.

Bond, Nicholas A., Meghan F. Cronin, Howard Freeland, and Nathan Mantua. (2015). Causes and impacts of the 2014 warm anomaly in the NE Pacific. *Geophysical Research Letters 42* (9): 3414–20. https://doi.org/10.1002/2015GL063306.

Boniface O. Fosu, S.-Y. Simon Wang, and Jin-Ho Yoon. (2016). The 2014/2015 snowpack drought in Washington State and its climate forcing. *Bulletin of the American Meteorological Society, 97*(12), S19-S24.

Brodeur, Richard D., Toby D. Auth, and Anthony Jason Phillips. (2019). Major shifts in pelagic micronekton and macrozooplankton community structure in an upwelling ecosystem related to an unprecedented marine heatwave. *Frontiers in Marine Science 6*. https://doi.org/10.3389/fmars.2019.00212.

Bruland, K. W., Lohan, M. C., Aguilar-Islas, A. M., Smith, G. J., Sohst, B., and Baptista, A. (2008). Factors influencing the chemistry of the near-field Columbia River plume: Nitrate, silicic acid, dissolved Fe, and dissolved Mn. *Journal of Geophysical Research: Oceans, 113*(C2).

Cayan, Daniel R. (1996). Interannual climate variability and snowpack in the Western United States. *Journal of Climate 9* (5): 928–48. https://doi.org/10.1175/1520-0442(1996)009<0928:ICVASI>2.0.CO;2.

Chawla, Arun, David A. Jay, António M. Baptista, Michael Wilkin, and Charles Seaton. (2008). Seasonal variability and estuary–shelf interactions in circulation dynamics of a river-dominated estuary. *Estuaries and Coasts 31* (2): 269–88. https://doi.org/10.1007/s12237-007-9022-7.

Center for Coastal Margin Observation and Prediction. (2022). Fixed station user interface SATURN-03.2021. http://www.stccmop.org/datamart/observation_network/fixedstation?id=saturn03

Clark, Martyn P., Mark C. Serreze, and Greg J. McCabe. (2001). Historical effects of El Nino and La Nina events on the seasonal evolution of the montane snowpack in the Columbia and Colorado River Basins. *Water Resources Research 37* (3): 741–57. https://doi.org/10.1029/2000WR900305.

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Austral Ecology 18* (1): 117–43. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x.

Cloern, James E., Tara S. Schraga, and Cary Burns Lopez. (2005). Heat wave brings an unprecedented red tide to San Francisco Bay. *Eos, Transactions American Geophysical Union 86* (7): 66–66. https://doi.org/10.1029/2005EO070003.

Cochlan, William P., Julian Herndon, and Raphael M. Kudela. (2008). Inorganic and organic nitrogen uptake by the toxigenic diatom *Pseudo-nitzschia australis* (Bacillariophyceae). *Harmful Algae, HABs and Eutrophication, 8* (1): 111–18. https://doi.org/10.1016/j.hal.2008.08.008.

Columbia River DART, Columbia Basin Research, and University of Washington. (2021). Pacific Ocean Coastal Upwelling Indices. 2021. http://www.cbr.washington.edu/dart/query/upwell_daily.

Costa, Pedro Reis, Maria João Botelho, and Kathi A. Lefebvre. (2010). Characterization of paralytic shellfish toxins in seawater and sardines (*Sardina pilchardus*) during blooms of *Gymnodinium catenatum*. *Hydrobiologia 655* (1): 89–97. https://doi.org/10.1007/s10750-010-0406-5.

Di Lorenzo, Emanuele, and Nathan Mantua. (2016). Multi-year persistence of the 2014/15 North Pacific marine heatwave. *Nature Climate Change 6* (11): 1042–47. https://doi.org/10.1038/nclimate3082.

Du, Xiuning, William Peterson, Jennifer Fisher, Matt Hunter, and Jay Peterson. (2016). Initiation and development of a toxic and persistent *Pseudo-nitzschia* bloom off the Oregon Coast in spring/summer 2015. *PLOS ONE 11* (10): e0163977. https://doi.org/10.1371/journal.pone.0163977.

Dyson, Karen, and Daniel D. Huppert. (2010). Regional economic impacts of razor clam beach closures due to harmful algal blooms (HABs) on the Pacific Coast of Washington. *Harmful Algae 9* (3): 264–71. https://doi.org/10.1016/j.hal.2009.11.003.

Ellis, Nick, Stephen J. Smith, and C. Roland Pitcher. (2012). Gradient Forests: Calculating importance gradients on physical predictors. *Ecology 93* (1): 156–68. https://doi.org/10.1890/11-0252.1.

Federal Columbia River Power System. (2001). The Columbia River System inside story. Bonneville Power Administration, U.S. Bureau of Reclamation, U.S. Army Corps of Engineers. https://www.bpa.gov/p/Generation/Hydro/hydro/columbia_river_inside_story.pdf.

Frame, E. R., and Lessard, E. J. (2009). Does the Columbia River plume influence phytoplankton community structure along the Washington and Oregon coasts? *Journal of Geophysical Research: Oceans, 114*(C2).

Gao, Guang, Xin Zhao, Meijia Jiang, and Lin Gao. (2021). Impacts of marine heatwaves on algal structure and carbon sequestration in conjunction with ocean warming and acidification. *Frontiers in Marine Science 8*. https://doi.org/10.3389/fmars.2021.758651.

Haertel, L., and Osterberg, C. (1967). Ecology of zooplankton, benthos and fishes in the Columbia River estuary. *Ecology, 48*(3), 459-472.

Hallegraeff, G. M. (1993). A review of harmful algal blooms and their apparent global increase. *Phycologia 32* (2): 79–99. https://doi.org/10.2216/i0031-8884-32-2-79.1.

Hallegraeff, Gustaaf M. (2010). Ocean climate change, phytoplankton community responses, and harmful algal blooms: A formidable predictive challenge. *Journal of Phycology 46* (2): 220–35. https://doi.org/10.1111/j.1529-8817.2010.00815.x.

Hallegraeff, G. M., S. I. Blackburn, M. A. Doblin, and C. J. S. Bolch. (2012). Global toxicology, ecophysiology and population relationships of the chainforming PST dinoflagellate *Gymnodinium catenatum*. *Harmful Algae, Harmful Algae--The requirement for species-specific information, 14 (February)*: 130–43. https://doi.org/10.1016/j.hal.2011.10.018.

Hamlet, Alan F., and Dennis P. Lettenmaier. (1999). Effects of climate change on hydrology and water resources in the Columbia River Basin. *JAWRA Journal of the American Water Resources Association 35* (6): 1597–1623. https://doi.org/10.1111/j.1752-1688.1999.tb04240.x.

Heady, W.N., K. O'Connor, J. Kassakian, K. Doiron, C. Endris, D. Hudgens, R. P. Clark, J. and Carter, and M. G. Gleason. (2014). An inventory and classification of U.S. West Coast estuaries. Arlington, VA: The Nature Conservancy. http://www.pacificfishhabitat.org/wp-content/uploads/2017/09/wc_estuaryinventory_final_report_jan15_2015.pdf.

Herfort, Lydie, Tawnya D. Peterson, Victoria Campbell, Sheedra Futrell, and Peter Zuber. (2011). *Myrionecta rubra* (*Mesodinium Rubrum*) bloom initiation in the Columbia River Estuary. *Estuarine, Coastal and Shelf Science 95* (4): 440–46. https://doi.org/10.1016/j.ecss.2011.10.015.

Hickey, B., S. Geier, N. Kachel, and A. MacFadyen. (2005). A bi-directional river plume: The Columbia in summer. *Continental Shelf Research 25* (14): 1631–56. https://doi.org/10.1016/j.csr.2005.04.010.

Hobday, Alistair, Eric Oliver, Alex Sen Gupta, Jessica Benthuysen, Michael Burrows, Markus Donat, Neil Holbrook, Pippa Moore, Mads Thomsen, Thomas Wernberg, Dan Smale. (2018). Categorizing and naming marine heatwaves. *Oceanography 31*, no. 2. https://doi.org/10.5670/oceanog.2018.205.

Jay, D. A., and Smith, J. D. (1990). Residual circulation in shallow estuaries: 1. Highly stratified, narrow estuaries. *Journal of Geophysical Research: Oceans*, *95*(C1), 711-731.

Jeong, Hae Jin, Yeong Du Yoo, Jae Seong Kim, Kyeong Ah Seong, Nam Seon Kang, and Tae Hoon Kim. (2010). Growth, feeding and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. *Ocean Science Journal 45* (2): 65–91. https://doi.org/10.1007/s12601-010-0007-2.

Kärnä, Tuomas, and António M. Baptista. (2016). Water age in the Columbia River Estuary. *Estuarine, Coastal and Shelf Science 183 (December)*: 249–59. https://doi.org/10.1016/j.ecss.2016.09.001.

Kudela, Raphael M., William P. Cochlan, Tawnya D. Peterson, and Charles G. Trick. (2006). Impacts on phytoplankton biomass and productivity in the Pacific Northwest during the warm ocean conditions of 2005. *Geophysical Research Letters 33*, no. 22. https://doi.org/10.1029/2006GL026772.

Lara-Lara, J Ruben. (1990). Primary production in the Columbia River Estuary I. Spatial and temporal variability of properties. *PACIFIC SCIENCE* 44: 21.

Lefebvre, K., M. Silver, S. Coale, and R. Tjeerdema. (2002). Domoic acid in planktivorous fish in relation to toxic *Pseudo-nitzschia* cell densities. *Marine Biology 140* (3): 625–31. https://doi.org/10.1007/s00227-001-0713-5.

Lefebvre, Kathi A., Brian D. Bill, Aleta Erickson, Keri A. Baugh, Lohna O'Rourke, Pedro R. Costa, Shelly Nance, and Vera L. Trainer. (2008). Characterization of intracellular and extracellular saxitoxin levels in both field and cultured *Alexandrium* spp. samples from Sequim Bay, Washington. *Marine Drugs 6* (2): 103–16. https://doi.org/10.3390/md6020103.

Lewitus, Alan J., Rita A. Horner, David A. Caron, Ernesto Garcia-Mendoza, Barbara M. Hickey, Matthew Hunter, Daniel D. Huppert, et al. (2012). Harmful algal blooms along the North American West Coast region: History, trends, causes, and impacts. *Harmful Algae 19 (September)*: 133–59. https://doi.org/10.1016/j.hal.2012.06.009.

McCabe, Ryan M., Barbara M. Hickey, Raphael M. Kudela, Kathi A. Lefebvre, Nicolaus G. Adams, Brian D. Bill, Frances M. D. Gulland, Richard E. Thomson, William P. Cochlan, and Vera L. Trainer. (2016). An unprecedented coastwide toxic algal bloom linked to anomalous ocean conditions. *Geophysical Research Letters 43* (19): 10,366-10,376. https://doi.org/10.1002/2016GL070023.

Mote, Philip W. (2006). Climate-driven variability and trends in mountain snowpack in Western North America. *Journal of Climate 19* (23): 6209–20. https://doi.org/10.1175/JCLI3971.1.

National Centers for and Coastal Ocean Science. (2015). West Coast harmful algal bloom draws attention of Congress. 2015. https://coastalscience.noaa.gov/news/west-coast-harmful-algal-bloom-draws-attention-congress/.

Oksanen, J. (2011). Multivariate analysis of ecological communities in R: vegan tutorial. R package version, 1(7), 1-43.

Oregon Department of Agriculture. (2021). Mussel and clam biotoxin lab results. 8.

ORHAB. (2021). PNW hab bulletin. UW Departments Web Server. https://depts.washington.edu/orhab/pnw-hab-bulletin/.

Piatt, John F., Julia K. Parrish, Heather M. Renner, Sarah K. Schoen, Timothy T. Jones, Mayumi L. Arimitsu, Kathy J. Kuletz, et al. (2020). Extreme mortality and reproductive failure of Common Murres resulting from the Northeast Pacific marine heatwave of 2014-2016. *PLOS ONE 15* (1): e0226087. https://doi.org/10.1371/journal.pone.0226087.

Poe, Melissa R., Phillip S. Levin, Nick Tolimieri, and Karma Norman. (2015). Subsistence fishing in a 21st century capitalist society: From commodity to gift. *Ecological Economics 116 (August)*: 241–50. https://doi.org/10.1016/j.ecolecon.2015.05.003.

Ritzman, Jerilyn, Amy Brodbeck, Sara Brostrom, Scott McGrew, Stacia Dreyer, Terrie Klinger, and Stephanie K. Moore. (2018). Economic and sociocultural impacts of fisheries closures in two fishing-dependent communities following the massive 2015 U.S. West Coast harmful algal bloom. *Harmful Algae 80 (December)*: 35–45. https://doi.org/10.1016/j.hal.2018.09.002.

Roberts, Shane D., Paul D. Van Ruth, Clinton Wilkinson, Stella S. Bastianello, and Matthew S. Bansemer. (2019). Marine heatwave, harmful algae blooms and an extensive fish kill event during 2013 in South Australia. *Frontiers in Marine Science 6*. https://doi.org/10.3389/fmars.2019.00610.

Rogers-Bennett, L., and C. A. Catton. (2019). Marine heat wave and multiple stressors tip bull kelp forest to sea urchin barrens. *Scientific Reports 9* (1): 15050. https://doi.org/10.1038/s41598-019-51114-y.

Rollwagen-Bollens, Gretchen, Stephen Bollens, Eric Dexter, and Jeffery Cordell. (2020). Biotic vs. abiotic forcing on plankton assemblages varies with season and size class in a large temperate estuary. *Journal of Plankton Research 42* (2): 221–37. https://doi.org/10.1093/plankt/fbaa010.

Rose, V, G Rollwagen-Bollens, Sm Bollens, and J Zimmerman. (2021). Seasonal and interannual variation in Lower Columbia River phytoplankton (2005-2018): Environmental variability and a decline in large bloom-forming diatoms. *Aquatic Microbial Ecology 87*: 29–46. https://doi.org/10.3354/ame01967.

Simenstad, Charles, Lawrence Small, and David McIntire. (1990). Consumption processes and food web structure in the Columbia River Estuary. *Progress in Oceanography 25 (1–4)*: 271–97. https://doi.org/10.1016/0079-6611(90)90010-Y.

Simenstad, Charles A, David A Jay, C. David McIntire, Willa Nehlson, Christopher R Sherwood, and Lawrence Small. (1984). The dynamics of the Columbia River estuarine ecosystem volume II. Columbia River Estuary Data Development Program.

Simpson, E. H. (1949). Measurement of Diversity. *Nature 163 (4148)*: 688–688. https://doi.org/10.1038/163688a0.

Simpson, Gavin L. (2018). Modelling palaeoecological time series using generalized additive models. https://doi.org/10.1101/322248.

Small, Lawrence F., C. David McIntire, Keith B. MacDonald, J. Ruben Lara-Lara, Bruce E. Frey, Michael C. Amspoker, and Ted Winfield. (1990). Primary production, plant and detrital biomass, and particle transport in the Columbia River Estuary. *Progress in Oceanography 25* (1): 175–210. https://doi.org/10.1016/0079-6611(90)90007-O.

Stevens, A.W., Gelfenbaum, G., MacMahan, J., Reniers, A.J.H.M., Elias, E.P., Sherwood, C.R., Carlson, E.M. (2017). Oceanographic measurements and hydrodynamic modeling of the mouth of the Columbia River, Oregon and Washington, 2013: U.S. Geological Survey data release, http://dx.doi.org/10.5066/F7NG4NS1.

Tassone, Spencer J., Alice F. Besterman, Cal D. Buelo, Jonathan A. Walter, and Michael L. Pace. (2022). Co-occurrence of aquatic heatwaves with atmospheric heatwaves, low dissolved oxygen, and low pH events in estuarine ecosystems. *Estuaries and Coasts 45* (3): 707–20. https://doi.org/10.1007/s12237-021-01009-x.

Thessen, Anne E., Quay Dortch, Michael L. Parsons, and Wendy Morrison. (2005). Effect of salinity on Pseudo-nitzschia species (Bacillariophyceae) growth and distribution. *Journal of Phycology 41* (1): 21–29. https://doi.org/10.1111/j.1529-8817.2005.04077.x.

Tohver, Ingrid M., Alan F. Hamlet, and Se-Yeun Lee. (2014). Impacts of 21st-century climate change on hydrologic extremes in the Pacific Northwest region of North America. *JAWRA Journal of the American Water Resources Association 50* (6): 1461–76. https://doi.org/10.1111/jawr.12199.

Trainer, Vera L., Marc Suddleson. (2005). Monitoring approaches for early warning of domoic acid events in Washington State. *Oceanography 18* (2): 228-237. https://doi.org/10.5670/oceanog.2005.56.

Van Dolah F M. (2000). Marine algal toxins: Origins, health effects, and their increased occurrence. *Environmental Health Perspectives 108 (suppl 1)*: 133–41. https://doi.org/10.1289/ehp.00108s1133.

United States Geological Survey, National Streamflow Information Program. (2022). USGS 14246900 COLUMBIA RIVER AT PORT WESTWARD, NEAR QUINCY, OR. 2021. https://waterdata.usgs.gov/nwis/uv?site_no=14246900

Wetz, Michael S., and David W. Yoskowitz. (2013). An ‘extreme’ future for estuaries? Effects of extreme climatic events on estuarine water quality and ecology. *Marine Pollution Bulletin 69* (1): 7–18. https://doi.org/10.1016/j.marpolbul.2013.01.020.

Wood, Simon N. (2017). *Generalized Additive Models: An Introduction with R. Second edition.* Chapman and Hall/CRC Texts in Statistical Science. Boca Raton: CRC Press/Taylor and Francis Group.

Yamamoto, T., Oh, S. J., and Kataoka, Y. (2004). Growth and uptake kinetics for nitrate, ammonium and phosphate by the toxic dinoflagellate *Gymnodinium catenatum* isolated from Hiroshima Bay, Japan. *Fisheries Science, 70*(1), 108-115.

Zhu, Zhi, Pingping Qu, Feixue Fu, Nancy Tennenbaum, Avery O. Tatters, and David A. Hutchins. (2017). Understanding the Blob bloom: Warming increases toxicity and abundance of the harmful bloom diatom *Pseudo-nitzschia* in California coastal waters. *Harmful Algae 67 (July)*: 36–43. https://doi.org/10.1016/j.hal.2017.06.004.

Appendix A: Supporting materials for Chapter 2

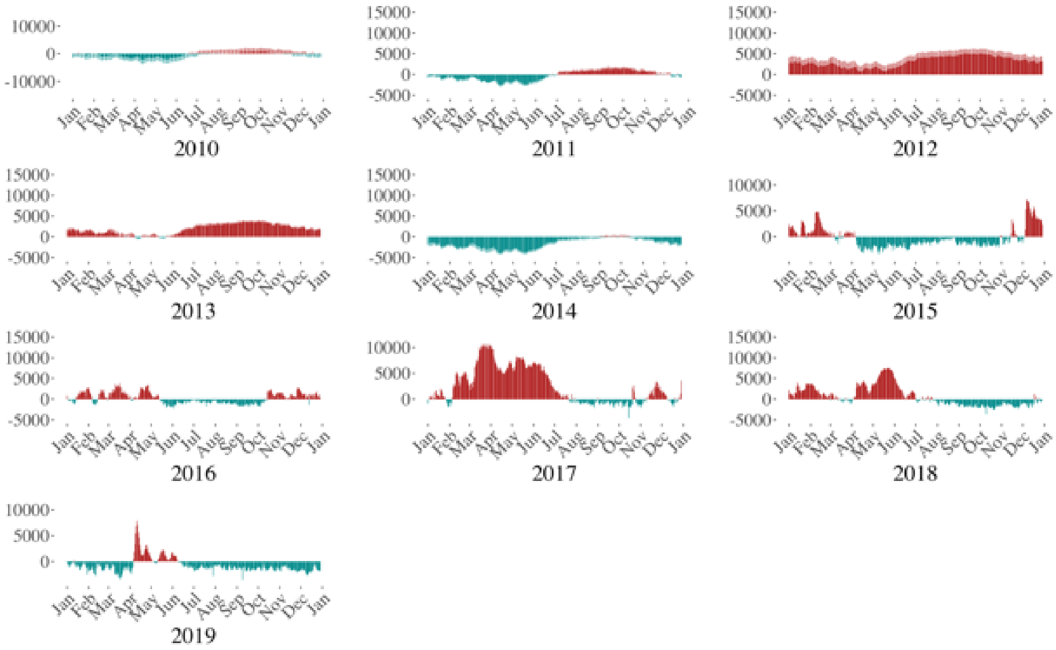

Fig. 8. Daily discharge anomaly for 2010 – 2019 compared to daily decadal average. Discharge data from USGS Quincy Station. Red bars indicate a positive discharge anomaly, blue bars indicate a negative anomaly.

Chapter 3: Effects of Nutrient Availability and Nutritional Strategy on Fatty Acid Profile of Natural Plankton Communities

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Abstract

Plankton are an important part of the foundation of estuarine food webs, providing food resources to consumers, including those of interest for fisheries and aquaculture. These organisms are a critical source of fatty acids, particularly polyunsaturated fatty acids (PUFAs), which are essential for growth and development of many organisms that cannot synthesize these compounds on their own. One aim of this chapter is to investigate how plankton PUFAs are related to the ecologically relevant functional traits of nutritional strategy (i.e., mixotrophy) and toxigenicity. Plankton community composition is strongly influenced by environmental factors including nutrient availability, but it remains unclear how changes in nutrients affect plankton functional traits and PUFA content at the community scale. In this chapter, we investigate the relationship between nutrients, plankton functional traits and PUFAs in an observational study and a laboratory experiment. We first conducted an observational study in 2019 at four sites on the northern Oregon and southern Washington coast to understand the associative relationships between nutrients, plankton functional traits and PUFAs. We then performed a carboy nutrient addition experiment on estuarine plankton samples to test the effects of inorganic nutrient additions (20 μM nitrate) and organic nutrient additions (10 μM urea) compared to controls (2 μM nitrate, 0 μM urea) in June and September of 2021. Plankton were identified via light microscopy and assigned functional traits of mixotrophy and toxigenicity based on literature review. PUFAs were extracted from plankton and measured via gas chromatography-mass spectrometry. We found that field and laboratory responses to nutrients differed in terms of species composition and associated plankton traits, as well as PUFAs. In the summer of 2019, we observed a shift from a non-toxic, autotroph-dominated plankton community to a toxic, mixotroph-dominated bloom of *Gymnodinium catenatum* with elevated community PUFA concentration. In the laboratory experiment, we found the effect of nutrient additions on field-collected plankton communities depended on season (June vs. September). We did not observe significant differences in either mixotrophy or toxicity between nutrient treatments. However, the PUFA docosahexaenoic acid (DHA) was elevated by 56% and 72% in the inorganic and organic N treatments, respectively, compared to the control, and eicosapentaenoic acid (EPA) was 67% and 71% higher in the inorganic and organic treatments than the control in the September round of the experiment. This work is relevant for determining the impacts of anthropogenic nutrient alterations on the nutritional quality of estuarine plankton as a food resource for consumers.

Introduction

Plankton are an essential component of aquatic food webs, and declines in nutritional quality due to toxin production, nutrient content, or fatty acid content, have been shown to disrupt trophic relationships (Jónasdóttir and Kiørboe, 1996; Kleppel and Burkart, 1995; McCabe et al., 2016; Taipale et al., 2018). Seasonal variation strongly influences the composition and abundance of plankton communities and predominant plankton nutritional strategies (i.e., how plankton assimilate nutrients) (Margalef, 1978; Peura et al., 2012). Many phytoplankton taxa are autotrophic (i.e., obligate photosynthesizers). However, the role of mixotrophy (the ability to photosynthesize *and* to be heterotrophic, i.e., to consume other organisms or take up dissolved organic nutrients) has recently been recognized as an important and prevalent adaptive strategy among plankton across habitats (Burkholder et al., 2008; Glibert and Mitra, 2022; Jeong et al., 2021; Millette et al., 2023; Selosse et al., 2017; Stoecker et al., 2017). While autotrophs tend to grow rapidly and outcompete generalist mixotrophs under optimal light and inorganic nutrient conditions, mixotrophy allows plankton to thrive in low light and/or low inorganic nutrient conditions not conducive to solely photosynthetic nutrition (Ward et al., 2011). Mixotrophy is a strategy that is particularly common in estuaries (Stoecker et al., 2017).

Nutrient conditions in estuaries are known to vary seasonally and with proximity to the mouth of the estuary (Lewitus et al., 1998; Partnership for Coastal Watersheds, 2010), with upwelling and freshwater runoff as major drivers of nutrient regimes. Upwelling and precipitation runoff bring inorganic nutrients to the euphotic zone where phytoplankton reside, triggering blooms of autotrophic diatoms in the spring. However, late summer blooms, which are more likely to occur under inorganic nutrient limitation due to relaxation of upwelling currents and lower precipitation, tend to consist of dinoflagellates, a group of plankton that more frequently exhibits mixotrophic behavior (Margalef, 1978; Stoecker, 1999). Understanding the specific environmental drivers of these shifts in taxa and predominant nutritional strategy may aid in predicting plankton response to changing nutrient regimes in estuaries.

Globally, observations of toxigenic plankton are on the rise, yet the factors that influence their abundance remain poorly understood and vary across plankton taxa (Van Dolah, 2000). Some broad patterns across marine and estuarine systems have emerged: (i) toxigenic taxa are often associated with warm, stratified conditions with low inorganic nutrient and high organic nutrient concentrations (Bates et al., 1998; Trainer et al., 2012); (ii) toxigenic taxa are dominated by dinoflagellates that display mixotrophic feeding strategies (Stoecker, 1999; Stoecker et al., 2006), and (iii) production of algal toxins is often linked to inorganic nutrient limitation (Flynn et al., 2018; Stoecker et al., 2006). It should be noted that these broad patterns do not apply to all toxigenic plankton.

The notable prevalence of mixotrophy among harmful dinoflagellate blooms suggests a possible association between nutritional strategy and toxin production, though mechanisms responsible are not well understood. A few studies have speculated about possible ecophysiological mechanisms linking toxin production and mixotrophy, including toxic secretions to catch prey for phagocytosis, allelopathic secretions to enhance competition against autotrophs, and alterations to metabolic pathways (Blossom et al., 2012; Hiltunen et al., 2012; Legrand, 2001; Pomati et al., 2001). For example, Pomati et al. (2001) showed that it is possible to induce algal biotoxin production via altered catabolic pathways when algal cultures are grown on organic nitrogenous substrates for seven days.

In addition to the impacts of nutrients on plankton community composition and toxicity, nutrients may also influence the nutritional value of plankton to consumers by changing cellular biochemical composition, including fatty acid content and particularly polyunsaturated fatty acid (PUFA) content (Bi et al., 2014; Grosse et al., 2019; Harrison et al., 1990). PUFAs are critical for many physiological functions in aquatic organisms, but most consumers cannot synthesize certain PUFAs *de novo* (Parrish et al., 2009). Certain long chain PUFAs such as eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3), and their precursor alpha-linolenic acid (ALA; 18:3n-3), are especially valuable for their important contributions to healthy growth and development of consumers. They are so valuable because most vertebrates have a limited ability to synthesize these compounds and their precursors, which make up the "essential fatty acids" (Hixson and Arts, 2016). Prior research does not show a consistent pattern in the impacts of nutrient limitation on lipid and PUFA content, as there is significant species-specific variation (Reitan et al., 1994). Most studies have altered nutrients in algal cultures, but only two studies investigated PUFA content and nutrient limitation in natural, field-collected plankton communities (Grosse et al., 2017, 2019), which showed a decrease in PUFA content under inorganic N and P nutrient limitations.

Several studies have investigated how nutritional strategy (i.e., autotroph vs. mixotroph) impacts PUFA content. Protozoans, such as mixotrophic or heterotrophic dinoflagellates, are thought to function as "trophic upgraders" of food quality for grazers due to their ability to both synthesize essential PUFA and to concentrate or repackage PUFAs from food sources, whereas autotrophs only contain PUFAs they have synthesized. This suggests that mixotrophs and heterotrophs may differ from autotrophs in terms of PUFA content (Galloway and Winder, 2015; Klein Breteler et al., 1999). Studies of cultured algae found that the degree of mixotrophy influenced fatty acid content. Results were mixed; some studies found that autotrophic cultures produced more PUFAs, but several found that certain monounsaturated or polyunsaturated fatty acids, particularly those associated with storage, were elevated under mixotrophic growth (Calbet et al., 2011; Cerón Garcí et al., 2000; Lari et al., 2019; Rai et al., 2013).

An emerging area of research seeks to understand whether any association between PUFA content and toxin-producing ability exists. Fatty acids and their metabolic pathways have been implicated in toxicity (Alamsjah et al., 2007; Kamaya et al., 2003; Pawlowiez et al., 2014; Wu et al., 2006), suggesting a possible relationship between these processes. For example, enzymes involved in fatty acid synthesis are thought to be structurally similar to enzymes involved in the synthesis of polyketide toxins (Pawlowiez et al., 2014). To our knowledge no research has been conducted to assess associations between toxigenic trait presence and PUFA content in natural systems.

The type and quantity of fatty acids synthesized by a plankton community is influenced by both community composition (phylogenetic relatedness) and environmental factors (Galloway and Winder, 2015; Jónasdóttir, 2019). At the physiological level, environment may influence plankton cellular fatty acid content as a stress response (i.e., switching from structural to storage fatty acids; Grosse et al., 2019). At the plankton community level, PUFA profiles may also be influenced by environmental variation indirectly through changes in community composition. Because certain taxa are known to produce higher levels of certain PUFAs than others, communities with divergent community composition would be less likely to have a similar PUFA profile. For example, dinoflagellates tend to produce slightly more long-chain essential fatty acids (i.e., DHA, EPA, and ALA) than diatoms (Peltomaa et al., 2019). Most other plankton phyla produce lower levels of these PUFAs, with cyanobacteria producing the lowest levels (Galloway and Winder, 2015).

There is a wealth of research on PUFA content changes at the physiological level using lab cultures, but much less work has been done at the community level, which is important for determining the quality of plankton stocks as a food resource for consumers. The aims of this work are to determine how adaptive strategies of plankton are related to community composition shifts, and changes in PUFA content under environmental conditions representative of shifts in nutrient regimes.

In this study we assessed the relationships between nutrient conditions, plankton community composition, functional traits, and fatty acid profiles. Our approach differed from previous work in that it was specifically designed to probe these relationships at the level of the plankton community assemblage, rather than with single cultured species (Bi et al., 2014; Harrison et al., 1990; Reitan et al., 1994). To our knowledge, a quantitative comparison of PUFAs with plankton functional traits has not previously been conducted. This study is also focused on estuarine plankton, which may differ in their PUFA profile response to nutrients from plankton in other habitats (Peltomaa et al., 2019).

We first conducted a field study of estuarine and coastal nutrient conditions, plankton communities, their functional traits and fatty acid profiles to understand how these factors relate to one another in a natural setting. We then conducted a carboy experiment on field-collected plankton communities to test hypotheses developed from field observations. In this experiment, we aimed to test whether inorganic and organic nutrient additions into N-limited estuarine water samples impacted plankton community composition, nutrient uptake rates, abundances of mixotrophic or toxigenic plankton, and production of nutritionally important PUFAs over five days. We expected that the inorganic nutrient addition would promote more rapid nutrient uptake than controls, and the growth of autotrophic, non-toxigenic, high-PUFA plankton. In contrast, we expected the organic nutrient addition to promote growth of mixotrophic, toxigenic plankton that would be lower in key PUFAs than the inorganic nutrient treatment. Using essential PUFAs as a biomarker for nutritional quality, we ask whether mixotrophic, toxic plankton provide an otherwise nutritionally valuable food source, or whether they are lower in PUFA content compared to autotrophic, non-toxic phytoplankton.

Methods

Field sampling and site context

Field sampling of nutrients, phytoplankton, and fatty acid profiles was conducted in 2019. The sites sampled (Table 1) exhibit a gradient of riverine influence, with a mixture of open coast and estuarine habitat. The Columbia South Jetty (CSJ) is an open coastal site that is periodically influenced by the Columbia River plume depending on prevailing winds, though the plume typically extends northward most of the summer (Hickey et al., 2005). Cape Meares (CM) is a sandy beach site with a small freshwater creek input nearby. Ilwaco (IL) is a mesohaline site that is strongly influenced by the Columbia River conditions for most of the year, but ocean influence increases in the summer months as river discharge declines (Chawla et al., 2008). The Willapa (WIL) site is located within a coastal plain estuary that is periodically influenced by the Columbia River plume, has multiple freshwater inputs, and is used for oyster aquaculture operations (Roegner et al., 2002).

All sites were sampled seven times in 2019 (June 10th, July 10th, August 13th and 27th, September 12th and 25th, October 8th). At each sampling event, duplicate whole water grabs were taken at 0.3 m depth for plankton, nutrient analysis, and fatty acid analysis at each site. Plankton were sampled using surface grab samples in glass bottles and preserved with Lugol's solution (final concentration ~1%). Nutrient and fatty acid samples were filtered and processed for storage according to standard protocols (described below). The nutrient concentrations measured included orthophosphate, silicic acid, nitrite, nitrate, ammonium, total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), total nitrogen (TN), and total phosphorus (TP).

Nutrient addition experiment

A controlled nutrient addition experiment was conducted in which samples of both a June and September plankton community were subjected to nitrate or urea addition treatments (Appendix B Fig. 8). Twelve 4-L samples of estuary water were collected in acid-washed carboys from the South Slough Estuary Valino Island site in June and September 2021, with water samples filtered through a 200-µm mesh to exclude large zooplankton, while including microzooplankton (includes mixotrophic or heterotrophic ciliates, dinoflagellates, flagellates, etc.). The Valino Island site represents a mesohaline habitat, which is typically nitrogen-limited during the summer due to the influence of N-limited and P-replete inputs from the coastal ocean; however, this may vary somewhat with relative ocean influence over tidal cycles or seasons, with coastal conditions such as upwelling, and with runoff inputs from precipitation.

Carboys (4 L, n=12 for each round of the experiment) were divided into initial conditions, incubation controls, a nitrate treatment, and a urea treatment. Initial conditions (n=3) were sampled for nutrients, plankton composition, and fatty acids upon returning to the lab. The remaining carboys were all subjected to nutrient treatments and incubated. Controls (n=3) were amended with 2 µM nitrate, nitrate treatment carboys (n=3) were amended with 20 µM nitrate, and urea treatment carboys (n=3) were amended with 10 µM urea and 2 µM nitrate. The 2 µM nitrate added to the controls and urea treatments served as a standard addition. The nutrient amendment concentrations were chosen to be representative of the upper bound of expected natural concentrations of these nutrients in the South Slough Estuary (Schrager et al., 2017).

Carboys were incubated for five days after the nutrient additions in an environmental chamber (12°C) on a 12hr:12hr light:dark cycle at 150 µmol photons m^{-2} s^{-1}. The length of the incubation period was selected to capture the changes that occur in both community composition and physiological responses to understand how the two interact to influence overall PUFA (Marcoval et al., 2008; Meyers et al., 2019). Growth rates of estuarine plankton under conditions where light is not limited, such as the conditions in the environmental chamber, can approach two divisions per day (Alpine and Cloern, 1988), so five days was considered sufficient to observe shifts in the abundance of taxa. Community PUFA content has been shown to change over the course of just 72 hours (Grosse et al., 2019). It was also expected that an incubation of this length would not be long enough for nutrient depletion or starvation of the plankton (Dodds et al., 1993). Urea was chosen as the organic nutrient treatment because of its prevalence in the environment due to expanded use in agricultural fertilizers (Glibert and Burkholder, 2006). Nutrient amendments were only added once at the beginning of the incubation. The pH of the carboys was monitored to indicate if supplemental bicarbonate amendments were needed, but these were not necessary. The experiment was conducted in two rounds (once in June, once in September) to capture the response of plankton communities at different points of seasonal succession to the nutrient amendments.

Whole water samples taken from initial condition carboys and from all incubated carboys at the end of the experiment were used for analysis of inorganic nutrients (nitrate, nitrite, ammonium, orthophosphate, silicic acid, TDN, TDP, TN, TP), and the organic nutrient urea. Plankton community samples (50 mL whole water, preserved with Lugol's, 1%) were collected on day 1, 3, and 5 of incubation without replacement of water during the five-day experiments. To measure the amount of nitrogen uptake, tracer additions of ^{15}N-labeled nitrate and urea were made to subsamples in 200 ml flasks in the same concentrations as the nutrient-amended carboys at the same time as the bulk nutrient additions. After a 6-h incubation during the first day of the experiment, the samples were filtered onto pre-combusted GF/F filters (0.7-µm pore size), and stored at -80°C until analysis. Seston fatty acid samples were collected from the initial conditions carboys and filtered onto a 0.45 µm pore membrane filter and frozen for later fatty acid analysis.

Filtered samples were collected once again at the end of the experiment from each incubated carboy to measure any changes in community fatty acid content.

Laboratory analyses

Nutrients were measured using segmented continuous flow on an Astoria-Pacific AutoAnalyzer to react standard reagents with samples and produce a measurable reaction (Astoria-Pacific International, 2002). Nitrate, nitrite, phosphate, silicic acid, total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP) were measured via spectrophotometric detection; ammonium was measured via fluorometric detection as described by Gordon et al. (1994) and Sakamoto et al. (1990). Total N and P were measured using the Kjeldahl digestion method (Patton and Truitt, 1992). Urea was measured separately using the Environmental Protection Agency’s indophenol blue method (Alam et al., 2016).

Filters collected from the 6-hour isotope tracer incubation subsample were fumigated with hydrochloric acid overnight, dried, and pelletized according to Brandeberry (2020). ^{15}N uptake was measured by isotope-ratio mass spectrometry coupled to an elemental analyzer as described in Morando and Capone, (2018) at the UC Davis Stable Isotope Facility. N uptake was calculated from N atom % using equations as in the protocols of the Joint Global Ocean Flux Study (1994).

Plankton community samples were homogenized, concentrated 1x or 10x, depending on plankton abundance, and settled in a Sedgewick-Rafter counting chamber. Plankton species were identified and counted using a compound microscope and standard identification guides (Bellinger and Sigee, 2015; Hallegraeff et al., 2004; Tomas, 1997). Cells were enumerated up to 400 counting units, using 100X and 200X magnification. Where possible, cells were identified to genus or species, and in some cases complexes or groups that were practical for identification using light microscopy. Plankton were also measured for biovolume calculations to estimate biomass. Plankton abundances are reported as cell concentrations, estimated biomass, or as a proportion of the total cells counted in the sample.

In addition to taxonomic classification, plankton taxa observed in both the experiment and field study were given a binary classification based on their potential to use mixotrophic or heterotrophic strategies (hereafter designated simply as mixotrophy for simplicity) compared to an autotrophic nutritional strategy. Designations of potential for mixotrophy were made using information from a literature review. Where nutritional strategies of taxa had not been definitively classified previously (through direct observations of phagotrophy or organic matter uptake), we classified a taxon as capable of mixotrophy if literature review indicated that growth rate was improved by additions of organic matter either in culture or natural experiments. Plankton were also designated as toxigenic or non-toxigenic, based on literature review of observed taxa. The traits of mixotrophy and toxigenicity may vary at the species or strain level, but our analysis evaluated traits at the genus level due to constraints on identification of certain plankton using light microscopy. Where our taxonomic classifications were known to include species with differing trait designations, we made trait designations based on the best available evidence on commonness of the trait within a phylogenetic clade and in taxa typical of the region. For example, the genus *Alexandrium* was designated as a mixotroph because the majority of species common to the Northeast Pacific are known mixotrophs, even though some species globally are known to lack this ability (Appendix B Table 2).

Fatty acids were extracted three times from GF/F filters using 2:1 chloroform: methanol (by volume) and flushed with N_2 gas (Bligh and Dyer, 1959). To ensure sufficient biomass, technical duplicate samples were combined in the extraction process. A blank was also included with every seven samples, and was used to determine that contamination did not occur during the extraction and transesterification processes. Sulfuric acid (1%) in methanol and n-hexane were used for transesterification of the extracted fatty acid methyl esters (FAMEs). Samples were incubated at 90°C for 90 min, then neutralized with 2% $KHCO_3$, and diluted in n-hexane (Peltomaa et al., 2019). These samples were then vortexed, centrifuged, and the upper phase collected for analysis. Prepared FAMEs were sent to University of Jyväskylä for analysis using a gas chromatograph with mass spectrometer (GC-MS) (Shimadzu Ultra, Kyoto, Japan), using splitless injection, with an injection temperature of 250°C and helium as a carrier gas. Temperatures of the interface and ion source were 250°C and 230°C respectively. We used nonadecanoic acid (19:0) (Nu-Check Prep Inc., Elysian, MN, USA) as an internal standard. Fatty acids were identified by retention times, and concentrations (reported in $\mu g\ L^{-1}$) were calculated from a calibration curve of known standard solutions (GLC standard mixture 566c, Nu-Chek Prep, Elysian, MN, USA).

Statistical analysis of field study data

All statistical analyses were conducted in R version 4.2.2. Descriptive statistics including taxonomic richness and Simpson's Diversity Index (D) were calculated using the 'vegan' package (version 2.6-4; Oksanen et al., 2022) to conduct an initial assessment of phytoplankton in field samples, providing an indication of spatial and temporal variation in composition. Overall fatty acid profiles were initially assessed using non-metric multidimensional scaling (NMDS) to understand variation between sites and over the course of the summer season. R-mode linked to Q mode (RLQ) analysis (Dolédec et al., 1996) was then used to identify co-structures between nutrient conditions and plankton functional traits, as well as between PUFAs and plankton functional traits. This analysis makes use of three different matrices: a site × nutrient conditions matrix or site × fatty acid profile matrix (R), a site × plankton taxa matrix (L), and a taxa × trait matrix (Q). Correspondence analysis (CA) was used to calculate site and taxa weights, which were used in a subsequent principal component analysis (PCA) of R (centered and scaled) and Q matrices. Co-inertia analysis was then performed to find linear combinations of R matrix variables (nutrients and PUFAs) and Q matrix variables (traits) that maximize covariance using the 'ade4' R package (Dray and Dufour, 2007). We used the Ward D2 method of hierarchical clustering of taxa scores from the RLQ analysis to obtain trait syndrome groupings, and the Calinsky-Harabasz criteria to determine the number of groupings (Caliński and Harabasz, 1974). Fourth corner analysis with a false discovery rate p-value adjustment was used to test the significance of relationships between traits and nutrients or PUFAs.

Statistical analysis of experimental data

A two-way analysis of variance (ANOVA) tested for differences between treatments and rounds of the experiment (i.e., June vs. September) for each bulk nutrient type, N uptake rate, PUFAs of interest, and prevalence of mixotrophic and toxigenic plankton as a percentage. When significant interactions were found ($\alpha = 0.05$), one-way ANOVAs stratified by round were conducted. Where the assumption of heterogeneity of variance was not met, we conducted Welch's ANOVAs instead. Tukey's HSD post-hoc was used to test for differences in individual treatments. Variation in phytoplankton community composition was assessed using NMDS, and permutational analysis of variance (PERMANOVA, permutations = 999) was used to test for significant differences between treatment groups and rounds of the experiment using the 'vegan' package in R.

Results

Field observational study

Nutrient conditions overview. Nutrient concentrations varied across field sites and throughout the summer season of 2019 (Fig. 1). TDN and TN tended to be higher earlier in the summer (June – July), and nitrate was highest in mid-summer (August). One exception to this pattern was a strong peak in TN (113 μM on August 27th) at the IL site concurrent with a bloom of *Gymnodinium catenatum*, reflecting the spike in N associated with particulate biomass. The coastal sites tended to have more variability in nitrate, with higher peak values than the estuarine sites, which had more stable nitrate levels throughout the summer. Average silicic acid was highest at the IL site (108.8 µM), followed by the WIL site (42.7 µM), which are both freshwater-influenced.

Phytoplankton composition overview. We identified 80 genera, with a mean richness of 25 taxa per sampling date and average sample diversity (D) = 0.74 (range: 0.17 to 0.91) over the study period in 2019. We identified seven phyla (Bacillariophyta, Chlorophyta, Ciliophora, Cryptophyta, Cyanophyta, Dinoflagellata, Euglenophyta) and two taxonomically amalgamated groups to capture other less common taxa (small flagellates, other). In general, plankton composition at the two coastal sites (CM, CSJ) was very similar, whereas the estuarine sites (IL, WIL) displayed different patterns of community composition. Early in the summer of 2019, chain-forming and/or colonial, autotrophic diatoms, such as *Chaetoceros* spp. (32.3% on June 10), *Thalassiosira* sp. (32% on June 10), and *Asterionellopsis* sp. (8.7% on June 10) dominated the plankton assemblage at all sites except WIL, which was dominated by Cryptophyta (50% on June 10). As the summer progressed, Dinoflagellata, Cryptophyta, and small flagellates increased in relative abundance at all sites. Dinoflagellates became particularly dominant at the IL site during August and September, when a bloom of the mixotrophic, toxigenic *Gymnodinium catenatum* occurred (91% of total assemblage on Aug. 13), which was followed by peaks in other typically mixotrophic taxa including Euglenophyta (24% on Sep. 12), small flagellates (21% on Sep. 25), Cryptophyta (61% on Oct. 8), and Ciliophora (mostly *Mesodinium* sp., and *Strombidium* sp.) (5.4% Oct. 8) **(**Fig. 2).

Fatty acid overview. A total of 63 fatty acids were measured in the field samples. Fatty acid (FA) profiles were significantly different between the sites sampled during the

2019 field season (PERMANOVA: p = 0.006), but did not show significant differences between sampling dates (PERMANOVA: p = 0.33). Early summer samples showed more variability in FA concentrations than samples from later in the summer. Samples from the open coast sites (CSJ, CM) in early summer were characterized by fatty acids typical of marine diatoms, such as EPA. Fatty acid profiles from IL in August (during the *G. catenatum* bloom) clearly clustered away from the rest of the samples, and were characterized by fatty acids typical of flagellates such as *Gymnodinium*, particularly octadecapentaenoic acid (OPA, 18:5n3), and had higher concentrations of EPA, DHA, and ALA than other samples (Fig. 3b, NMDS stress = 0.05). DHA and ALA both peaked in concentration at IL on Aug. 13th, at the height of the *G. catenatum* bloom (Fig. 3a). Although EPA did increase at IL during the *G. catenatum* bloom, it peaked in concentration earlier in the summer at the coastal sites. Peaks of the essential PUFAs (EPA, DHA, ALA) by percent of total fatty acids largely aligned with concentration peaks. One exception is ALA, which made up the greatest proportion of fatty acids at WIL on Aug. 13th (5.6%), despite its higher concentration at IL on the same date.

RLQ analysis

Co-structures between nutrients and plankton traits were identified using RLQ analysis to understand how nutrient concentrations related to the prevalence of the plankton community traits of mixotrophic behavior and toxigenic capability (Fig. 4a), and how the prevalence of these traits relates to the concentration of PUFAs in the whole plankton community (Fig. 4b). PCA analysis separated out samples that were higher in TN, TP, and silicic acid from the those that were higher in other dissolved nutrients, creating a strong gradient along RLQ axis 1 (97.5% of co-inertia). Hierarchical clustering indicated four trait syndromes based on the presence/absence of toxigenic and mixotrophic behavior: non-toxigenic autotrophs, toxigenic autotrophs, non-toxigenic mixotrophs, toxigenic mixotrophs (Appendix B Fig. 10, Calinski-Harabasz value = 786, which was the highest value at four clusters). The RLQ analysis indicated there was a significant global association between nutrients and traits (p = 0.012). Although no nutrient variables were significantly associated with any traits (Appendix B Table 3), trait loadings indicated that mixotrophy was positively associated with TP, TN, and silicic acid, and negatively associated with all other nutrient forms (Fig. 4a). Toxigenicity was most positively associated with TDN, nitrate, nitrite, and ammonium. Concentrations of PUFAs of interest were compared with trait loadings, and revealed that while the global trait-PUFA relationship was significant (p = 0.024) there were no significant associations between specific PUFAs and traits (Appendix B Table 4). DHA, DPA n-3, DPA n-6, and ALA were most positively associated with mixotrophy and negatively associated with toxigenicity (Fig. 4b). DTA and ARA were positively associated with toxicity, and EPA had a weak relationship with toxicity.

Nutrient addition experiment

Nutrient results. Phosphate, silicic acid, nitrite, nitrate, TDP, TDN, and TN tended to be higher in September, whereas ammonium was higher in June, and urea and TP concentrations did not display a significant difference by round of the experiment (Appendix B Table 5). Measurements of nutrient concentrations of initial conditions and final conditions of the carboys confirmed that nutrient additions were successful (Fig. 5, Appendix B Tables 5, 6, 7). The stable isotope incubations indicated that N uptake in the form of either nitrate or urea depending on treatment, was higher in the urea-treated carboys, compared to the controls and nitrate treatments (Appendix B Table 8).

Plankton community. We identified 75 genera, eight phyla (Bacillariophyta, Chlorophyta, Ciliophora, Cryptophyta, Cyanophyta, Dinoflagellata, Euglenophyta, Rotifera), and two taxonomically amalgamated groups to capture other less common taxa (small flagellates, zooplankton) (Appendix B Fig. 11). Mean richness of the experimental carboys was 41 taxa. Bacillariophyta was overall slightly more dominant in June than September (81% compared to 77% of assemblage on average). We also observed that several predominantly mixotrophic phyla (e.g., Euglenophyta, Ciliophora, and Cryptophyta) differed from each other in abundance over the two rounds and throughout the incubation periods. Overall, NMDS (stress = 0.18) paired with PERMANOVA revealed that the initial conditions had significantly different plankton composition from the carboys at the end of the experiment, indicating an effect of incubation ($p < 0.001$) (Appendix B Fig. 13). We also observed a significant difference in composition between the rounds of the experiment, treatments, and days of incubation (PERMANOVA: round $R^2 = 0.21$, $p < 0.001$; treatment $R^2 = 0.12$, $p < 0.001$; day $R^2 = 0.09$, $p < 0.001$) (Fig. 6). The variation along NMDS axis I largely represented seasonal differences in community composition (Fig. 6), while the variation along NMDS axis II largely represented differences between treatments (Appendix B Fig. 12). There were no significant trends in overall plankton biomass, either between treatments, rounds, or days of the experiment (Appendix B Fig. 13).

The average percentage of mixotrophic biomass showed variability in all treatments (initial: 94.1% (SD = 81%), heavily influenced by a few large microzooplankton, control: 41.65% (SD = 59%), nitrate: 19.23% (SD = 12%), urea: 13.00% (SD = 6%)), but did not show statistically significant differences. Percentage of mixotrophic biomass was more similar between the rounds of the experiment (June average: 17.84% (SD = 8%), September average: 31.95% (SD = 36%). Percentage of toxigenic biomass displayed a different pattern of variation, with the highest average proportion of toxigenics in the nitrate treatment (initial: 4.00% (SD = 7%), control: 5.88% (SD = 13%), nitrate: 9.64% (SD = 12%), urea: 2.72% (SD = 3%)) and in the June round (June average: 6.45% (SD = 6%), September average: 3.57% (SD = 4%), though no significant differences were found (Appendix B Table 9).

Fatty acids. There was a significant interaction between round of experiment (i.e., June vs. September) and treatment for the concentrations of each of the fatty acids ($p < 0.05$ for all). The percentage of ALA showed a significant interaction between round and treatment ($p = 0.021$), but % DHA and % EPA did not ($p = 0.056$, $p = 0.726$) (Appendix B Table 10). DHA and ALA tended to be higher in the June round of the experiment than the September round (87% and 175% higher, respectively) (Fig. 7). Upon conducting one-way ANOVAs stratified by round of experiment (Appendix B Table 11), we found that concentrations of EPA and ALA, and percentages of DHA, EPA, and ALA were all significantly different among treatments for both rounds ($p < 0.05$ for all). Concentration of EPA and DHA showed different patterns in each round (Appendix B Tables 12, 13). Concentration of DHA was not significantly different between treatments in the June round ($p = 0.313$), but Tukey's HSD post-hoc indicated that DHA concentration was significantly higher in the nitrate (0.980 $\mu g\ L^{-1}$) and urea (1.083 $\mu g\ L^{-1}$) treatments than the control (0.630 $\mu g\ L^{-1}$) in the September round ($p < 0.050$ for all). In June, concentration of EPA was marginally significantly higher in the initial conditions than the control and urea-treated carboys ($p = 0.054$, $p = 0.038$). In September, concentration of EPA was significantly higher in the nitrate- (2.053 $\mu g\ L^{-1}$) and urea-treated (2.100 $\mu g\ L^{-1}$) than the initial conditions (1.230 $\mu g\ L^{-1}$) or controls (1.443 $\mu g\ L^{-1}$) ($p < 0.050$ for all). Concentration and percentage of ALA were significantly (749% and 194%, respectively) higher in the initial conditions compared to all incubated carboys in both rounds of the experiment ($p < 0.001$ for all). Percent DHA and EPA showed the opposite pattern and were significantly (345% and 119%, respectively) higher in the incubated carboys compared to initial conditions in both rounds ($p < 0.01$ for all).

Discussion

Field study

In the field study, we observed a range of nutrient conditions (Fig. 1), largely associated with seasonal cycles of upwelling, and declines in riverine influence in the more estuarine sites due to lower river discharge in the summer. Dissolved inorganic nutrients (particularly nitrate and total dissolved nitrogen) tended to be higher earlier in the summer at the open coastal sites, likely as a result of upwelling currents which bring nutrient-rich water to the surface of the coastal ocean on the West Coast (Jacox et al., 2018). The early summer season plankton community (Fig. 2), particularly on the open coast, was dominated by diatoms typical of upwelling currents, such as the chain-forming *Chaetoceros* spp., *Thalassiosira* spp., and *Asterionellopsis glacialis.* All of these diatoms are autotrophic, and have a life strategy that allows them to grow and reproduce rapidly under nutrient-replete conditions (Margalef, 1978; Selosse et al. 2017).

The highest values of TN and TP were observed at IL during the *G. catenatum* bloom of August 2019. This may be a reflection of the bloom itself, or of an influx of particulate organic matter (POM) into the lower Columbia River Estuary, a phenomenon which has been known to occur when senescent freshwater plankton encounter higher salinity waters (Lara-Lara, 1990; Small et al., 1990). *G. catenatum* is a mixotroph, capable of both phagotrophy and the uptake of dissolved organic matter (DOM) (Doblin et al., 1999; Jeong et al., 2010; Yamamoto et al., 2004). This influx of POM could have provided an ideal food source to fuel a bloom of this dinoflagellate. The RLQ analysis also indicated that the trait of mixotrophy was positively, but not significantly, associated with TN, TP, and silicic acid. This result was largely driven by the contemporaneous total nutrient peaks and the bloom of *G. catenatum*, which was the peak mixotrophic abundance during the study period. It also reflects the higher abundance of mixotrophs at the lower salinity estuarine sites, which are higher in silicic acid. Mixotrophic cryptophytes made up a high proportion of total plankton at the estuarine WIL site throughout the summer, which may reflect a phenomenon similar to that of the LCRE, with increased organic matter under lower summer flows providing a food resource. IL and particularly WIL are in bays that experience longer residence times compared to open coastal waters (Banas and Hickey, 2005; Kärnä and Baptista, 2016), which may also favor slower-growing mixotrophs. We observed a sharp decline to the lowest calculated concentration of dissolved organic N (TDN – total inorganic nitrogen = 7.83 μM dissolved organic N) during the study period in the timeframe following the height of the *G. catenatum* bloom. Nitrate declined to the lowest observed value (0.5 μM) during this bloom as well. This may be an indication that uptake of both inorganic and organic nutrients was associated with the bloom event. Alternatively, the decline of both dissolved organic and inorganic N could be indicative of niche differentiation, but this seems unlikely given the dominance of *G. catenatum* in the plankton assemblage. These observations from the field aligned with our hypothesis that mixotrophs would tend to be more competitive under low inorganic nutrient availability, because of their metabolic flexibility (Ward et al., 2011).

The development of toxigenic plankton blooms has been associated with high organic nutrient loads, which cannot be utilized by autotrophic taxa, which are less commonly known as toxin producers (Clementson et al., 2004; Hallegraeff et al., 2012; Jeong et al., 2021; Pomati et al., 2001; Ward et al., 2011). We therefore expected to observe a higher proportion of toxigenic taxa under low inorganic nutrient availability, but this expectation only somewhat aligned with our field observations. While dinoflagellates make up a significant portion of toxigenic taxa, plankton that occupy very different niches may also display this trait. During this field study, the two most commonly observed toxigenic taxa were *G. catenatum* and *Pseudo-nitzschia* spp., which were present at different sites, times, and environmental conditions during the field study. *Pseudo-nitzschia* spp. tends to be associated with upwelling and high levels of inorganic nutrients (Du et al., 2016), and this is reflected in the toxigenic autotroph trait syndrome association with dissolved forms of N in the RLQ analysis (Fig. 4a). Our results with regard to toxicity are not clear cut, and perhaps indicate that this trait may be too phylogenetically and functionally widespread to predictably be linked with nutrient levels. We also did not observe any strong associations between any forms of P and either functional trait, likely because the study system is predominantly N-limited (Jickells, 1998).

Nutritionally important PUFAs also displayed a complex pattern of association with inorganic nutrient availability (Fig. 4). We expected PUFA content to be higher for seston samples collected during conditions of high inorganic N availability because these conditions typically favor diatoms. Diatoms grow rapidly and tend to have PUFA content that is among the highest of any of the plankton groups when normalized to carbon content, though it may be comparable or slightly lower than dinoflagellates under some environmental conditions (Galloway and Winder, 2015; Peltomaa et al., 2019). However, concentrations of most PUFAs were positively (not significantly) associated with mixotrophy in the RLQ analysis, potentially indicating support for the idea that mixotrophs can act as trophic upgraders (Klein Breteler et al., 1999; Martin-Creuzburg et al., 2005). Proportionally, EPA was highest at the coastal sites during upwelling (associated with autotrophic diatoms and high inorganic nutrients), while DHA and OPA were highest during the mixotrophic *G. catenatum* bloom (associated with low inorganic nutrients and organic nutrient availability). EPA and DHA are both found in relatively high proportions in marine plankton. EPA is typically found in higher proportions in diatoms, whereas DHA and OPA commonly make up a higher proportion of fatty acids in dinoflagellates (Jónasdóttir, 2019). ALA, a precursor to EPA and DHA, tends to be higher in freshwater ecosystems and was proportionally highest at WIL in August, a possible indication of this site's relative freshwater influence due to its location near the mouth of the Naselle River (Twining et al., 2021). Overall, there was a greater difference in fatty acids between the coastal and estuarine sites than between sampling dates, indicating that riverine vs. marine nutrient influence was associated with differences in PUFA profile. Other studies found a high degree of variability in total PUFA content and specific PUFAs across nutrient conditions (Cashman et al. 2013; Reitan et al., 1994). This is consistent with our field study, as the abundance of individual PUFAs appeared to differ in their response to nutrient conditions and plankton community shifts.

Most PUFAs were less strongly associated with toxicity than with mixotrophy in the RLQ analysis. This lack of strong relationship between PUFAs and toxicity may reflect the disparate phylogeny, and therefore divergent fatty acid profiles, of the two most abundant toxigenic taxa observed during this study, *Pseudo-nitzschia* spp., and *G. catenatum*. The few PUFAs that were associated with the toxigenic autotrophs were ARA (can be elongated and desaturated to EPA and DHA) and DTA (a 2-carbon elongation of ARA) (Twining et al., 2021). ARA tends to be more abundant in diatoms, such as the toxigenic autotroph *Pseudo-nitzschia* spp. It is important to underscore that all relationships observed in the RLQ analysis were not significant and displayed relatively weak associations between functional traits and PUFAs, with variation in the types of PUFAs most abundant in each trait syndrome. These analyses are exploratory in nature and provided direction for our experimental inquiry.

Nutrient experiment

In our nutrient addition experiment, we observed significant differences between the two rounds (June and September of 2021) in nutrient conditions, plankton community, and PUFAs. Dissolved N forms (except ammonium) were higher in the September round of the experiment, due to a precipitation event that increased runoff (Fig. 5). Although the influx of nutrients to the study site via runoff would seemingly promote conditions for autotrophs to thrive, autotrophs were actually somewhat more prevalent in June than in September. Mixotrophs had differing responses to the two rounds, with Euglenophytes and Ciliophora more abundant in June, and Cryptophyta more abundant in September. The South Slough Estuary is very marine influenced in the summer, and the majority of plankton in the estuary is advected from the coastal ocean, which is typically replete with inorganic nutrients from upwelling (Roegner and Shanks, 2001), and may overshadow the effects of nutrients in the immediate vicinity. This pattern may also suggest that seasonal succession (Margalef, 1978) or light availability (Alpine and Cloern, 1988; Cloern, 1999) are more important drivers of predominant nutritional strategy.

The concentration and proportion of DHA, EPA, and ALA tended to be higher in June than in September. This does not entirely reflect the observations from 2019 field study, in which early summer, upwelling-influenced communities had elevated proportions of PUFAs, particularly EPA, but also exceptionally high concentrations of PUFAs during the August 2019 *G. catenatum* bloom. Even though ambient nutrient levels in the estuary were lower in June, the higher PUFA content early in the season may be attributable to PUFA-rich, upwelling-associated plankton advected into the South Slough Estuary (Roegner and Shanks, 2001). It should be noted that the degree of variation in PUFAs in field observations was much greater than that observed during the experiment. This experiment was undertaken with two separate plankton communities from June and September to assess the effect of seasonal succession on the impact of nutrient additions. Given that we observed a significant interaction between concentrations of the key PUFAs by round and treatment type, we can infer that nutrient inputs may affect plankton PUFA profiles differently, depending on the time of year and existing plankton community. In particular, we observed that the nitrate- and urea-treated carboys were higher in DHA and EPA concentration than the controls in September, but showed no significant difference in June (Fig. 7). This may indicate that the September plankton community experienced higher growth rates in response to the nutrient additions, suggesting that the plankton at this time of year were more nutrient limited. It could also suggest that the late season plankton differed taxonomically in a way that increased capacity to synthesize and/or retain these PUFAs or fatty acids in general under nutrient additions. It should be noted that the percentage of these PUFAs was not elevated in the N-treated carboys, providing support for mechanisms such as increased growth or overall fatty acid production. This spike in DHA and EPA upon nutrient input (organic or inorganic) was similar to our field observations in that high PUFA concentrations arose through either inorganic or organic nutrient availability. However, this departed from our field observations, which showed that different nutrient types resulted in different dominant PUFAs (i.e., upwelling-associated, autotroph-dominated, high-EPA community vs. inorganic nutrient-limited, mixotroph-dominated, high-DHA community).

During the incubation period of this experiment, we expected mixotrophs (specialized for low inorganic: organic nutrient conditions: Granéli et al., 1999; Stibor et al., 2003; Stoecker, 1998) to increase in biomass and cell number faster than autotrophic taxa under higher organic nutrient concentrations, as they did in our field observations. However, we observed that there was no significant difference in mixotrophic biomass between treatments. This result reflects the systematic review finding (Granéli et al., 1999) that the prevalence of mixotrophs is highly variable depending on the plankton species present and the environmental conditions encountered by the plankton. While some studies have observed an effect of nutrient treatment on the prevalence of mixotrophy, others have not, suggesting that other factors may be more important in driving mixotroph abundance. The proportion of mixotrophic biomass was even slightly lower in the organic nutrient addition (urea-treated) carboys than the controls and inorganic nutrient addition (nitrate-treated) carboys. Different mixotrophic taxa also displayed different patterns of abundance over the course of the incubation. N uptake was highest in the urea-treated carboys, indicating that mixotrophic activity was occurring, but this did not translate to increased biomass of mixotrophs. This may indicate that these plankton were not growing or reproducing, but were in stasis during the incubation period. Because a majority of plankton in the South Slough Estuary are advected from the coastal ocean (Roegner and Shanks, 2001), it is also possible that the plankton collected for carboy incubations were already stressed by estuarine conditions, and were therefore limited in growth capacity. On the whole, these results indicate that nutrient addition treatments did not have a strong effect on the prevalence of mixotrophs in this experiment.

We also expected to observe more toxigenic taxa in the urea-treated carboys because many toxin-producing taxa are observed in low inorganic to organic nutrient ratio conditions and are also known mixotrophs (Cochlan et al., 2008; Stoecker et al., 2006; Stoecker et al., 2017; Thessen et al., 2009). However, we again saw no significant differences in biomass between the treatments. Though not a significant difference, the highest proportion of toxigenic biomass was observed in the nitrate-treated carboys. This carboy experiment took place in 2021, a year when *Pseudo-nitzschia* spp. was the most dominant toxigenic plankton. *Pseudo-nitzschia* spp. grows well under upwelling conditions (Du et al., 2016), and therefore likely grew well with the addition of nitrate. Our results likely diverged from previous studies on dinoflagellate toxigenic taxa (e.g., Stoecker et al., 2006; Stoecker et al., 2017) because *Pseudo-nitzschia* spp. can grow rapidly on an inorganic N source. This is unlike toxigenic dinoflagellates, which are slower-growing, and may only flourish under inorganic nutrient limitation.

Because we were expecting a shift away from autotrophic diatoms toward more mixotrophic taxa like dinoflagellates, euglenoids, ciliates, and cryptophytes under the organic nutrient treatment, we expected the overall concentration of PUFA to decline. As we did not observe the expected community shifts, we also did not observe a straightforward pattern in PUFAs. Individual PUFAs exhibited different responses to the nutrient treatments. DHA and EPA concentration were significantly elevated in the nitrate- and urea-treated carboys compared to the control in September ($p < 0.050$ for all). There was no significant difference between controls and the N-treated carboys in concentration of ALA or percentage of DHA, EPA, or ALA for either round.

The apparent increase in DHA and EPA concentration in both the nitrate- and urea-treated carboys suggests that production of these PUFAs is bolstered by greater N availability, regardless of its form, potentially through different trophic pathways. Previous studies have found DHA and EPA to be associated with greater N availability (Lau et al., 2021; Strandberg et al., 2022). This result also suggests that individual PUFAs respond differently to changes in nutrient availability, which may be a reflection of plankton taxa responding differently or a direct physiological response (Lang et al., 2011).

The largest difference observed in the essential PUFAs was between the plankton community from the initial conditions in the estuary and the incubated carboy samples, indicating a significant effect of carboy incubation. Proportions of both DHA and EPA were 345% and 119% higher, respectively in the incubated carboys than the initial conditions. EPA concentration showed the opposite pattern in June and was 68% higher in the initial conditions than the incubated carboys, likely reflecting a more diatom-dominated, upwelling-influenced community earlier in the summer. DHA concentration showed no significant difference between initial conditions and incubated carboys in June. Both the concentration and proportion of ALA were significantly higher in the initial conditions compared to all the incubated carboys. These differences between the initial conditions and incubated carboys could have been due to some change in environment caused by the incubation (e.g., light availability, standard addition of nitrate, temperature, mixing), which can all have strong effects on plankton community composition (Winder and Sommer, 2012). Plankton community composition is a primary predictor of community PUFA content (Galloway and Winder, 2015; Strandberg et al., 2015), and appeared to reflect differences in PUFA content in our experiment. It is also reasonable to expect that physiological changes to PUFA content might have occurred within the individual plankton cells as well as plankton community shifts, as previous studies have shown that accounting for taxonomic and environmental variation together explains more variation in community PUFA content (Galloway and Winder, 2015). The increase in DHA and EPA (particularly in September) combined with the decrease in ALA, which can be elongated to the former two PUFAs (Jónasdóttir, 2019; Twining et al., 2021), also suggests the possibility of PUFA bioconversion during the incubation by microzooplankton grazers. PUFA bioconversion is thought to occur to some degree in zooplankton and higher order consumers (Pilecky et al., 2022), and that these essential PUFAs are preferentially retained in grazers (Perhar et al., 2013; Taipale et al., 2011).

There was also a significant difference in overall plankton community composition between initial conditions and the incubated carboys (Fig. 6), suggesting that some aspect of the incubation conditions was an important driver. However, the two rounds of the experiment showed different patterns of community shifts between the initial conditions and the incubated carboys. For example, cell concentrations decreased in the incubated carboys in June, but increased in the incubated carboys in September. In June, Cyanophyta, Euglenophyta, and Dinoflagellata all decreased from the initial conditions, and the diatom *Hemiaulus* sp., which can harbor N-fixing symbionts (Villareal, 1991), was the most abundant taxon. In September, the most notable change in plankton taxa was an increase in small flagellates in the incubated carboys. Overall, there were significant differences between initial and incubated plankton communities, but the pattern of change was not the same for plankton taxa between the two rounds, likely because of seasonal differences in species composition.

While constant for all carboys in the experiment, the amount and intensity of light provided could have been greater than that available to plankton within the estuary. This would have been particularly true for the September round of the experiment, where the incubation provided an additional 30 minutes of light beyond the natural light cycle, likely at higher intensity than would have been observed during this cloudier time of year. This extra light availability would have favored autotrophic growth in the incubated carboys, perhaps overshadowing any changes in the prevalence of this functional trait caused by nutrient amendments, as mixotrophs typically thrive under sufficient irradiance and nutrient limitation (Edwards, 2019; Fischer et al., 2017). Different mixotrophic taxa may also have varied light requirements: some are facultative mixotrophs and require at least some light availability, while some are able to survive periods of time without any light (Wilken et al., 2020).

Insufficient inorganic N limitation could also have caused the lack of distinctive shifts in functional traits between nutrient treatments. It is possible that plankton were not limited by inorganic N for some or all of the incubation in the controls and urea-treated carboys because of background dissolved inorganic N levels (September) or well-provisioned or N-fixing plankton advected from coastal waters (June). If this were the case, the inorganic N source would likely have been taken up first by autotrophs, which can grow faster and outcompete mixotrophs that could have utilized organic N in the urea-treated carboy (Jones, 2000). This combined with the length of the incubation could have allowed for multiple nutritional strategies to take place within the carboys, potentially resulting in the observed weak differences between nutrient treatments. It is also important to note that there is a large degree of variation in nutritional strategy, even within those plankton that are classified as mixotrophs. Particular species of mixotrophs may rely more than others on photosynthesis, dissolved nutrient uptake, or phagotrophy, and this may change over time for a mixotrophic species (Stoecker et al., 2017). Thus, even though we classified a taxon as mixotrophic, it may not have exhibited mixotrophic behavior during the incubation. This variation could have contributed to the results observed in our experiment, but is beyond the scope of this study.

Conclusion

In the 2019 field component of this study we observed a dichotomy of an early-summer, autotroph-dominated community with a distinct fatty acid profile that was higher in EPA, which contrasted with a late-summer, mixotroph-dominated community with exceptionally high concentrations of PUFAs, particularly DHA. These communities corresponded with inorganic nutrient-replete upwelling conditions in the early summer, and a lower inorganic to organic nutrient ratio later in the summer. Based on these observations, we tested the effects of an inorganic and an organic N addition on plankton communities from June and September 2021. In this experiment, we found that nutrient additions impacted communities differently depending on the month of the experiment, and that both inorganic and organic N-treatments displayed higher DHA and EPA than controls in one round of the experiment. There was also a great deal of variation in the response of individual plankton taxa and PUFAs to the nutrient amendments. This illustrates the complexity of processes influencing plankton community composition, functional traits, and fatty acids. Future research could aim to disentangle the effects of light and nutrient limitation on prevalence of mixotrophy in plankton communities. As plankton-derived PUFAs and non-toxic plankton are critical for sustaining aquatic food webs (Hixson and Arts, 2016; Müller-Navarra et al., 2004; Taipale et al., 2016), it is important to understand the factors that influence these characteristics of plankton communities.

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Table 1. Description of study sites.

Site	Code	Coordinates	Year	Location	Description
Cape Meares	CM	45°30’05” N, 123°57’36” W	2019	Surf	Sandy beach, freshwater input
Columbia S. Jetty	CSJ	46°13’40” N, 124°01’14” W	2019	Surf	Sandy beach, plume influence
Ilwaco Harbor	IL	46°18’11” N, 124°02’16” W	2019	Dock	Estuarine, retentive bay
Willapa Bay	WIL	46°30’00” N, 124°01’29” W	2019	Dock	Estuarine, retentive bay
Valino Island	VAL	43°18’57” N, 124°19’16” W	2021	Shore	Estuarine, main channel

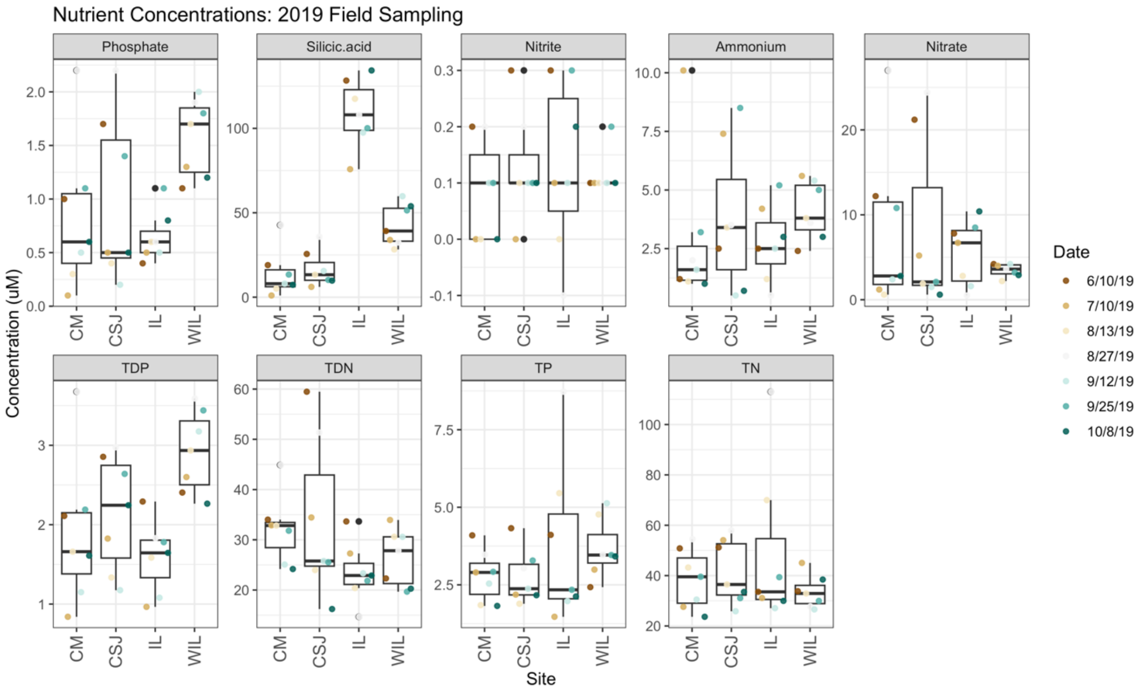

Fig. 1. Boxplots of concentrations (µM) of all nutrient forms from summer 2019 field sampling. Lighter colored points correspond to earlier summer samples, and darker blue points correspond to samples taken later in the summer season. Sites include CM = Cape Meares, CSJ = Columbia River South Jetty, IL = Ilwaco Harbor, WIL = Willapa Bay.

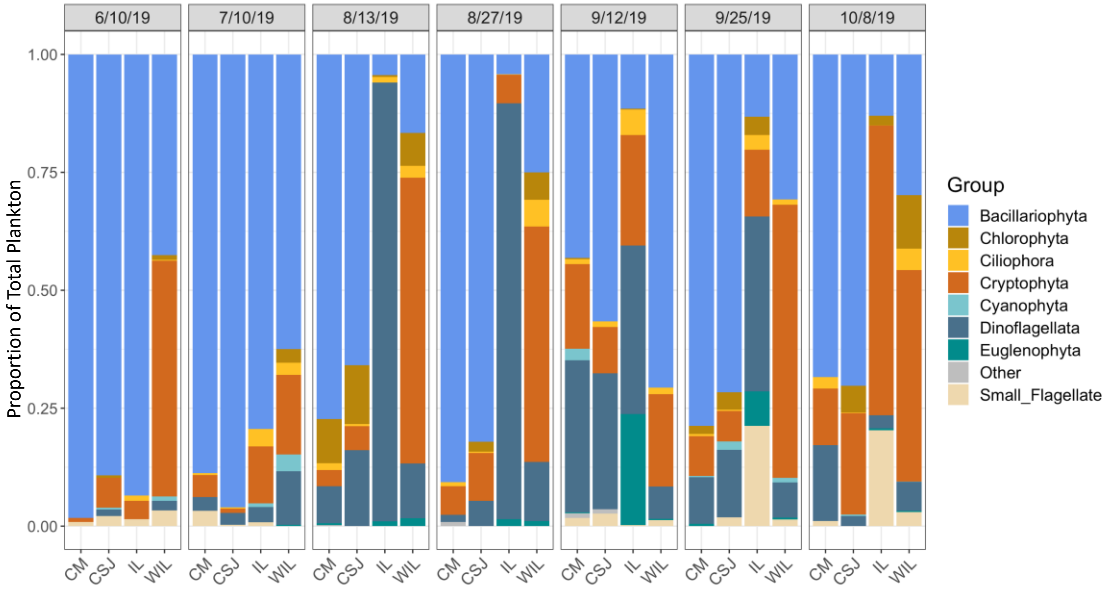

Fig. 2. Barplots of plankton community composition (proportion of total plankton cell concentrations) from field sampling in 2019 summer season at four sites (CM = Cape Meares, CSJ = Columbia River South Jetty, IL = Ilwaco Harbor, WIL = Willapa Bay). Groups are plankton phyla (Bacillariophyta, Chlorophyta, Ciliophora, Cryptophyta, Cyanophyta, Dinoflagellata, Euglenophyta) or taxonomically amalgamated groups of less common taxa (small flagellates, other).

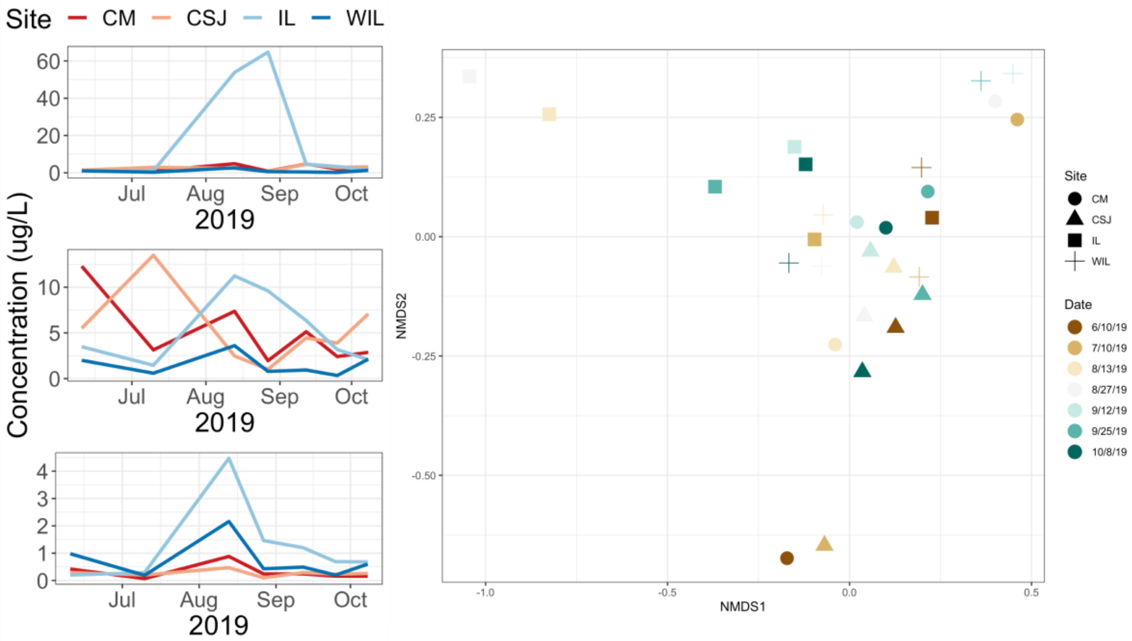

Fig. 3. (a) Plot of concentrations of three key PUFAs: DHA, EPA, and ALA during the 2019 field study. Lines are colored by site. (b) NMDS of whole fatty acid profile (63 total fatty acids as percent of total fatty acids) from field 2019 sampling campaign. Dates represented by different colored points, shapes correspond to sites. Sites include CM = Cape Meares, CSJ = Columbia River South Jetty, IL = Ilwaco Harbor, WIL = Willapa Bay. Stress = 0.05.

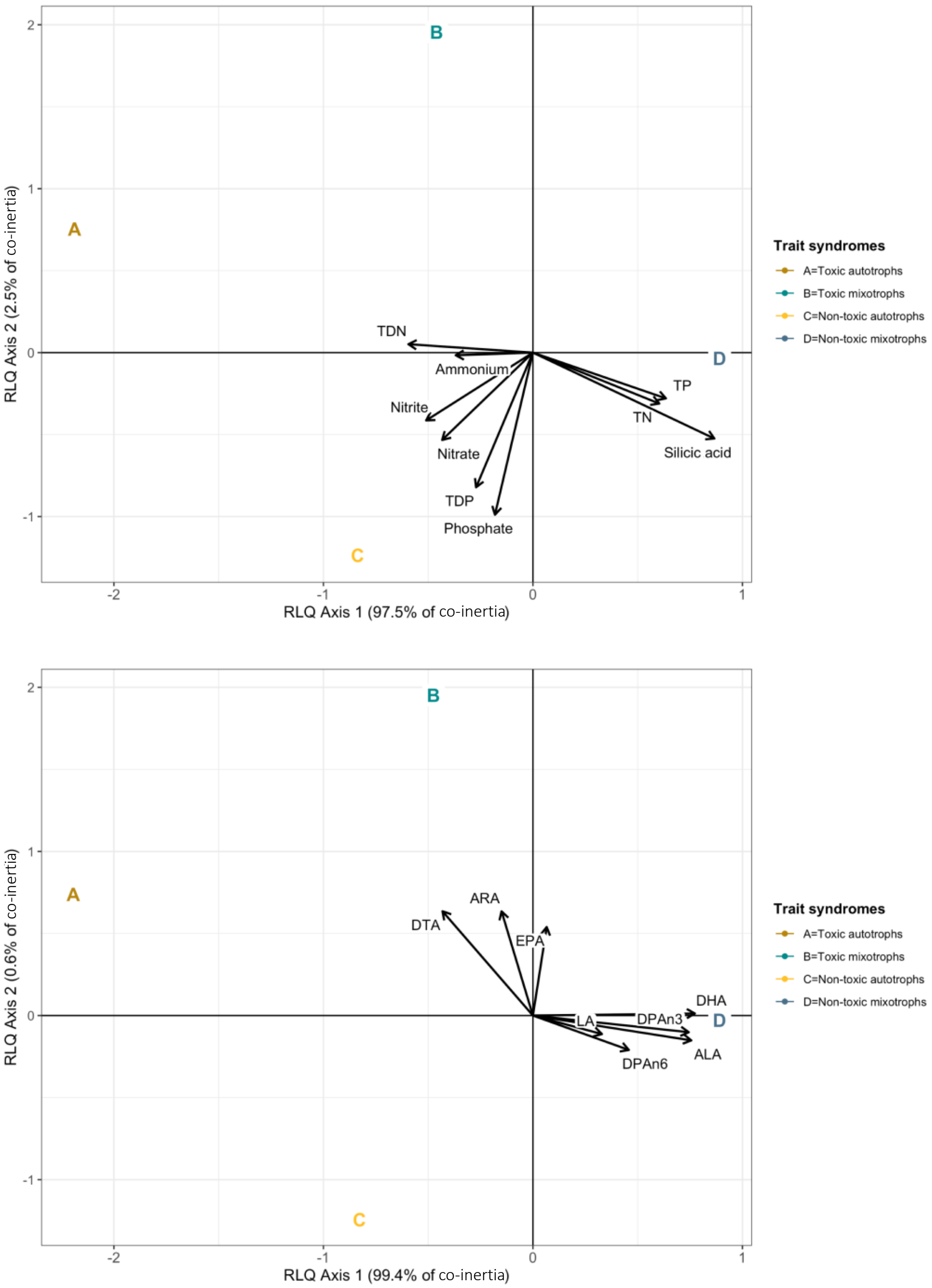

Fig. 4. Biplots showing associations between trait syndrome groupings and (a) nutrient loadings and (b) PUFA loadings. Trait syndromes are indicated by groupings A) toxic autotrophs, B) toxic mixotrophs, C) toxic autotrophs, D) non-toxic mixotrophs. Nutrient

types in (a) include TDN = total dissolved nitrogen, Ammonium, Nitrite, Nitrate, TDP = total dissolved phosphorus, Silicic acid, TN = total nitrogen, TP = total phosphorus. PUFAs in (b) include DTA = docosatetraenoic acid, ARA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, ALA = alpha-linoleic acid, LA = lignoceric acid, and DPA = (omega 3 and 6).

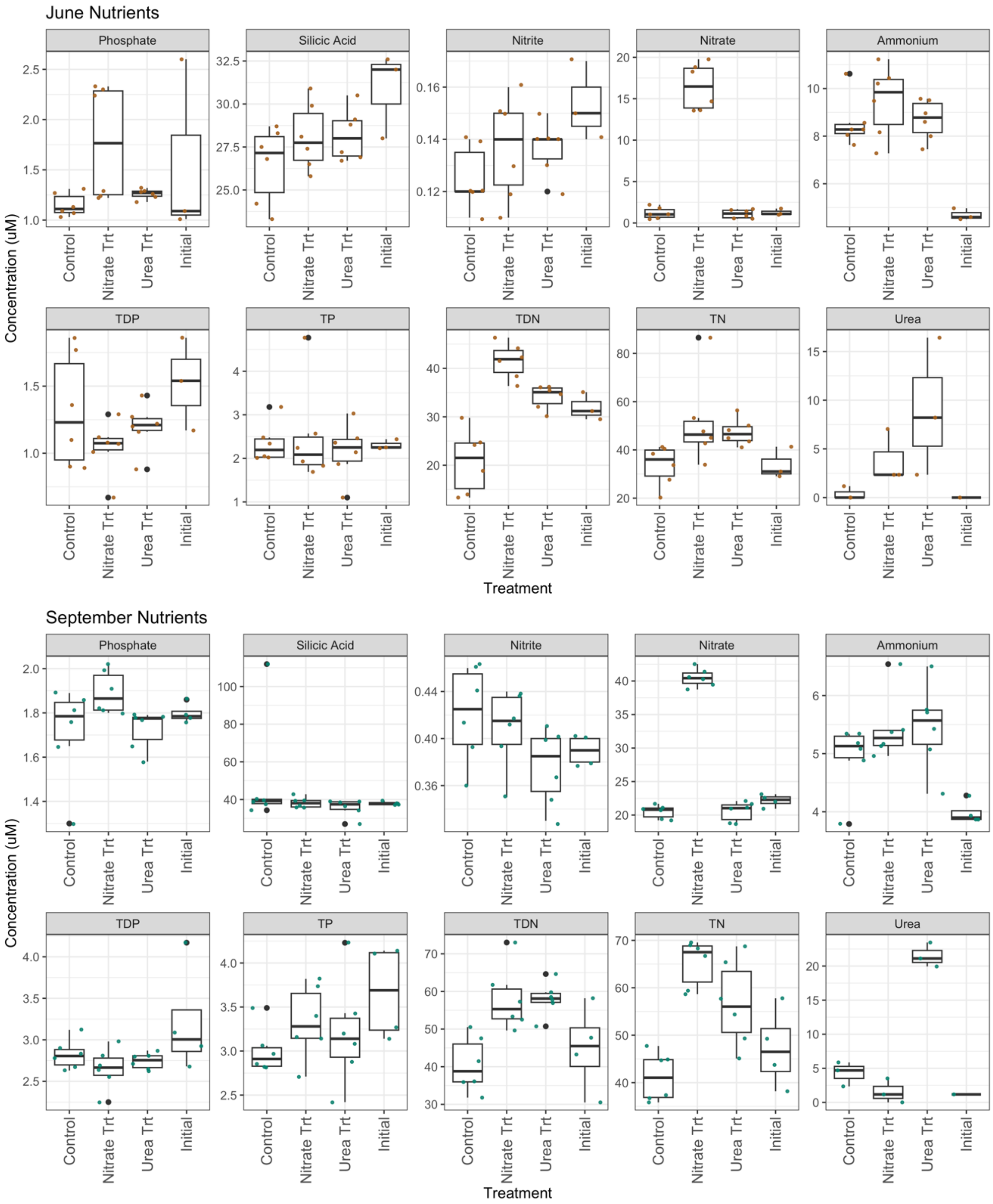

Fig. 5. Boxplots of concentrations (µM) of all nutrient forms from two rounds nutrient addition experiment in June and September 2019 by treatment groups (Control, Nitrate Trt = nitrate-treated carboy, Urea Trt = urea-treated carboy, Initial = initial conditions from South Slough Estuary). Concentrations from June round of experiment shown in brown points, September shown in blue.

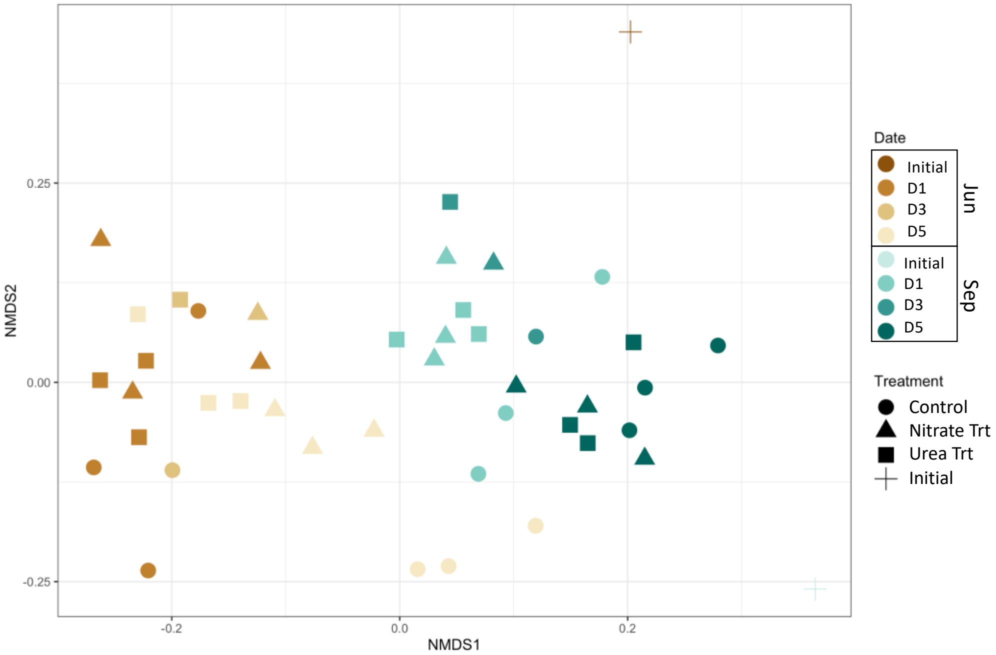

Fig. 6. NMDS of plankton community composition at genus level from experiment. Colors represent different days of the experiment, shapes represent different treatments. Stress = 0.18. Each treatment has three replicates per round, except the initial conditions, which have one sample each for June and September rounds.

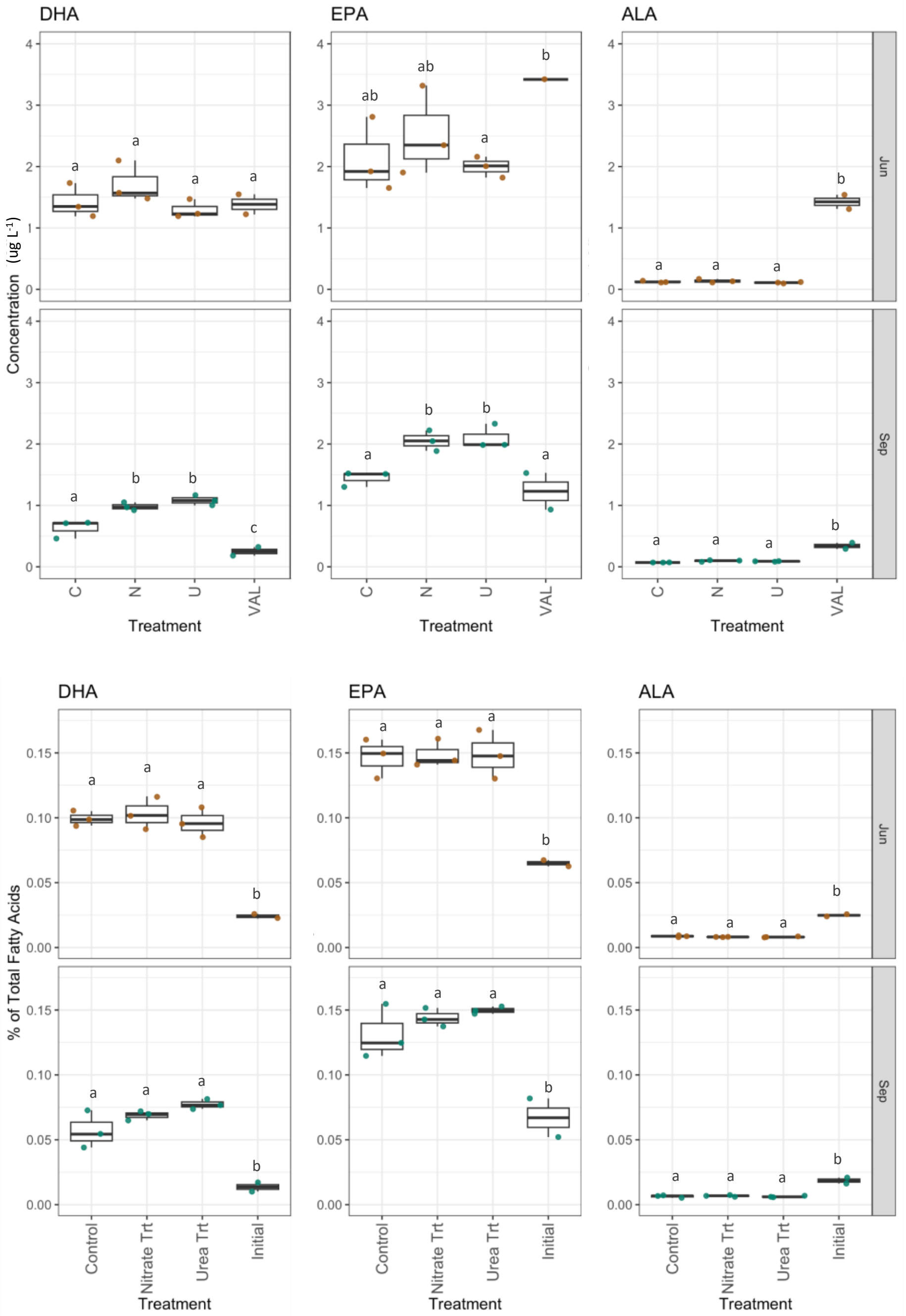

Fig. 7. Boxplots of nutritionally significant PUFAs: DHA, EPA, ALA in Control, Nitrate Trt = nitrate-treated carboys, Urea Trt = urea-treated carboys, and Initial = initial conditions in the South Slough Estuary (Initial). Shown as concentrations in the top two rows and as a percentage of total fatty acids in the bottom two rows. Color of points

represents the round of the experiment. Letters above boxplots represent ANOVA and Tukey’s HSD post-hoc test results.

References

Alam, M., Casareto, B., Yoshimi, S., Sultana, R., and Suzuki, T. (2016). Optimization of dissolved urea measurements in coastal waters with the combination of a single reagent and high temperature. *Journal of Oceanography*, 1–10. https://doi.org/10.1007/s10872-016-0400-2

Alamsjah, M. A., Ishibe, K., Kim, D., Yamaguchi, K., Ishibashi, F., Fujita, Y., and Oda, T. (2007). Selective toxic effects of polyunsaturated fatty acids derived from *Ulva fasciata* on red tide phyotoplankter species. *Bioscience, Biotechnology, and Biochemistry*, *71*(1), 265–268. https://doi.org/10.1271/bbb.60475

Alpine, A., and Cloern, J. (1988). Phytoplankton growth rates in a light-limited environment, San Francisco Bay. *Marine Ecology Progress Series*, *44*, 167–173. https://doi.org/10.3354/meps044167

Astoria-Pacific International. (2002). *Astoria Pacific Instruction Manual and Standard Operating Procedures*.

Banas, N. S., and Hickey, B. M. (2005). Mapping exchange and residence time in a model of Willapa Bay, Washington, a branching, macrotidal estuary. *Journal of Geophysical Research: Oceans*, *110*(C11). https://doi.org/10.1029/2005JC002950

Bellinger, E., and Sigee, D. (2015). *Freshwater Algae Identification, Enumeration and Use as Bioindicators*. Wiley. *Chichester, West Sussex, UK ; Hoboken, NJ*. pp. 304.

Bi, R., Arndt, C., and Sommer, U. (2014). Linking elements to biochemicals: Effects of nutrient supply ratios and growth rates on fatty acid composition of phytoplankton species. *Journal of Phycology, 50*(1), 117–130. https://doi.org/10.1111/jpy.12140

Bligh, E., and Dyer, W. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37(8)*, 911–917.

Blossom, H. E., Daugbjerg, N., and Hansen, P. J. (2012). Toxic mucus traps: A novel mechanism that mediates prey uptake in the mixotrophic dinoflagellate *Alexandrium pseudogonyaulax*. *Harmful Algae, 17*, 40–53. https://doi.org/10.1016/j.hal.2012.02.010

Brandeberry, L. (2020, June 26). *Difficult Combustion Materials: Soils, Sediments, Filters, Wood, Carbonates - Carbon and Nitrogen in Solids*. Stable Isotope Facility. https://stableisotopefacility.ucdavis.edu/difficult-combustion-materials-soils-sediments-filters-wood-carbonates-carbon-and-nitrogen-solids

Burkholder, J. M., Glibert, P. M., and Skelton, H. M. (2008). Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae, 8*(1), 77–93. https://doi.org/10.1016/j.hal.2008.08.010

Calbet, A., Bertos, M., Fuentes-Grünewald, C., Alacid, E., Figueroa, R., Renom, B., and Garcés, E. (2011). Intraspecific variability in *Karlodinium veneficum*: Growth rates, mixotrophy, and lipid composition. *Harmful Algae, 10*(6), 654–667. https://doi.org/10.1016/j.hal.2011.05.001

Caliński, T., and Harabasz, J. (1974). A dendrite method for cluster analysis. *Communications in Statistics*, *3*(1), 1–27. https://doi.org/10.1080/03610927408827101

Cerón Garcí, M. C., Fernández Sevilla, J. M., Acién Fernández, F. G., Molina Grima, E., and García Camacho, F. (2000). Mixotrophic growth of *Phaeodactylum tricornutum* on glycerol: Growth rate and fatty acid profile. *Journal of Applied Phycology*, *12*(3), 239–248. https://doi.org/10.1023/A:1008123000002

Chawla, A., Jay, D. A., Baptista, A. M., Wilkin, M., and Seaton, C. (2008). Seasonal variability and estuary–shelf interactions in circulation dynamics of a river-dominated estuary. *Estuaries and Coasts*, *31*(2), 269–288. https://doi.org/10.1007/s12237-007-9022-7

Clementson, L. A., Parslow, J. S., Turnbull, A. R., and Bonham, P. I. (2004). Properties of light absorption in a highly coloured estuarine system in south-east Australia which is prone to blooms of the toxic dinoflagellate *Gymnodinium catenatum*. *Estuarine, Coastal and Shelf Science*, *60*(1), 101–112. https://doi.org/10.1016/j.ecss.2003.11.022

Cloern, J. E. (1999). The relative importance of light and nutrient limitation of phytoplankton growth: A simple index of coastal ecosystem sensitivity to nutrient enrichment. *Aquatic Ecology*, *33*(1), 3–15. https://doi.org/10.1023/A:1009952125558

Cochlan, William P., Julian Herndon, and Raphael M. Kudela. (2008). Inorganic and organic nitrogen uptake by the toxigenic diatom *Pseudo-nitzschia australis*

(Bacillariophyceae). *Harmful Algae, HABs and Eutrophication, 8* (1): 111–18. https://doi.org/10.1016/j.hal.2008.08.008.

Roegner, C. G., Hickey, B. M., Newton, J. A., Shanks, A. L., and Armstrong, D. A. (2002). Wind-induced plume and bloom intrusions into Willapa Bay, Washington. *Limnology and Oceanography*, *47*(4), 1033–1042. https://doi.org/10.4319/lo.2002.47.4.1033

Doblin, M. A., Blackburn, S. I., and Hallegraeff, G. M. (1999). Growth and biomass stimulation of the toxic dinoflagellate *Gymnodinium catenatum* (Graham) by dissolved organic substances. *Journal of Experimental Marine Biology and Ecology*, *236*(1), 33–47. https://doi.org/10.1016/S0022-0981(98)00193-2

Dodds, W. K., Strauss, E. A., and Lehmann, R. (1993). Nutrient dilution and removal bioassays to estimate phytoplankton response to nutrient control. *Archiv Fur Hydrobiologie. Stuttgart*, *128*(4), 467–481.

Dolédec, S., Chessel, D., Ter Braak, C. J., and Champely, S. (1996). Matching species traits to environmental variables: A new three-table ordination method. *Environmental and Ecological Statistics*, *3*, 143–166.

Dray, S., and Dufour, A.-B. (2007). The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software*, *22*, 1–20. https://doi.org/10.18637/jss.v022.i04

Du, X., Peterson, W., Fisher, J., Hunter, M., and Peterson, J. (2016). Initiation and development of a toxic and persistent *Pseudo-nitzschia* bloom off the Oregon

Coast in spring/summer 2015. *PLOS ONE*, *11*(10), e0163977. https://doi.org/10.1371/journal.pone.0163977

Edwards, K. F. (2019). Mixotrophy in nanoflagellates across environmental gradients in the ocean. *Proceedings of the National Academy of Sciences*, *116*(13), 6211–6220. https://doi.org/10.1073/pnas.1814860116

Fischer, R., Giebel, H.-A., Hillebrand, H., and Ptacnik, R. (2017). Importance of mixotrophic bacterivory can be predicted by light and loss rates. *Oikos*, *126*(5), 713–722. https://doi.org/10.1111/oik.03539

Flynn, K. J., Mitra, A., Glibert, P. M., and Burkholder, J. M. (2018). Mixotrophy in harmful algal blooms: By whom, on whom, when, why, and what next. In P. M. Glibert, E. Berdalet, M. A. Burford, G. C. Pitcher, and M. Zhou (Eds.), *Global Ecology and Oceanography of Harmful Algal Blooms* (pp. 113–132). Springer International Publishing. https://doi.org/10.1007/978-3-319-70069-4_7

Galloway, A. W. E., and Winder, M. (2015). Partitioning the relative importance of phylogeny and environmental conditions on phytoplankton fatty acids. *PLoS One; San Francisco*, *10*(6), e0130053. http://dx.doi.org/10.1371/journal.pone.0130053

Glibert, P. M., and Burkholder, J. M. (2006). The complex relationships between increases in fertilization of the Earth, coastal eutrophication and proliferation of harmful algal blooms. In E. Granéli and J. T. Turner (Eds.), *Ecology of Harmful Algae* (pp. 341–354). Springer. https://doi.org/10.1007/978-3-540-32210-8_26

Glibert, P. M., and Mitra, A. (2022). From webs, loops, shunts, and pumps to microbial multitasking: Evolving concepts of marine microbial ecology, the mixoplankton paradigm, and implications for a future ocean. *Limnology and Oceanography*, *67*(3), 585–597. https://doi.org/10.1002/lno.12018

Gordon, L. I., Jennings, J. C., Ross, A. A., and Krest, J. M. (1994). *A suggested protocol for continuous flow analysis of seawater nutrients (phosphate, nitrate, nitrite, and silicic acid) in the WOCE Hydrographic Program and the Joint Global Ocean Fluxes Study*. (p. 91 (1)). WHP Office Report. https://www.nodc.noaa.gov/archive/arc0001/9900162/2.2/data/0-data/jgofscd/Files/protocols/Chap8.htm

Granéli, E., Carlsson, P., and Legrand, C. (1999). The role of C, N and P in dissolved and particulate organic matter as a nutrient source for phytoplankton growth, including toxic species. *Aquatic Ecology*, *33*(1), 17–27. https://doi.org/10.1023/A:1009925515059

Grosse, J., Breugel, P. van, Brussaard, C. P. D., and Boschker, H. T. S. (2017). A biosynthesis view on nutrient stress in coastal phytoplankton. *Limnology and Oceanography*, *62*(2), 490–506. https://doi.org/10.1002/lno.10439

Grosse, J., Brussaard, C. P. D., and Boschker, H. T. S. (2019). Nutrient limitation driven dynamics of amino acids and fatty acids in coastal phytoplankton. *Limnology and Oceanography*, *64*(1), 302–316. https://doi.org/10.1002/lno.11040

Hallegraeff, G. M., Anderson, D. M., and Cembella, A. D. (2004). *Manual on Harmful Marine Algae*. UNESCO Publishing. Paris, France, pp. 770.

Hallegraeff, G. M., Blackburn, S. I., Doblin, M. A., and Bolch, C. J. S. (2012). Global toxicology, ecophysiology and population relationships of the chain-forming PST dinoflagellate *Gymnodinium catenatum*. *Harmful Algae*, *14*, 130–143. https://doi.org/10.1016/j.hal.2011.10.018

Harrison, P. J., Thompson, P. A., and Calderwood, G. S. (1990). Effects of nutrient and light limitation on the biochemical composition of phytoplankton. *Journal of Applied Phycology*, *2*(1), 45–56. https://doi.org/10.1007/BF02179768

Hickey, B., Geier, S., Kachel, N., and MacFadyen, A. (2005). A bi-directional river plume: The Columbia in summer. *Continental Shelf Research*, *25*(14), 1631–1656. https://doi.org/10.1016/j.csr.2005.04.010

Hiltunen, T., Barreiro, A., and Hairston, N. G. (2012). Mixotrophy and the toxicity of *Ochromonas* in pelagic food webs. *Freshwater Biology*, *57*(11), 2262–2271. https://doi.org/10.1111/fwb.12000

Hixson, S. M., and Arts, M. T. (2016). Climate warming is predicted to reduce omega-3, long-chain, polyunsaturated fatty acid production in phytoplankton. *Global Change Biology*, *22*(8), 2744–2755. https://doi.org/10.1111/gcb.13295

Jacox, M. G., Edwards, C. A., Hazen, E. L., and Bograd, S. J. (2018). Coastal Upwelling Revisited: Ekman, Bakun, and improved upwelling indices for the U.S. West Coast. *Journal of Geophysical Research: Oceans*, *123*(10), 7332–7350. https://doi.org/10.1029/2018JC014187

Jeong, H. J., Kang, H. C., Lim, A. S., Jang, S. H., Lee, K., Lee, S. Y., Ok, J. H., You, J. H., Kim, J. H., Lee, K. H., Park, S. A., Eom, S. H., Yoo, Y. D., and Kim, K. Y. (2021). Feeding diverse prey as an excellent strategy of mixotrophic dinoflagellates for global dominance. *Science Advances*, *7*(2), eabe4214. https://doi.org/10.1126/sciadv.abe4214

Jeong, H. J., Yoo, Y. D., Kim, J. S., Seong, K. A., Kang, N. S., and Kim, T. H. (2010). Growth, feeding and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. *Ocean Science Journal*, *45*(2), 65–91. https://doi.org/10.1007/s12601-010-0007-2

Jickells, T. D. (1998). Nutrient biogeochemistry of the coastal zone. *Science, 281*(5374), 217–222. https://doi.org/10.1126/science.281.5374.217

Joint Global Ocean Flux Study. (1994). *JGOFS PROTOCOLS*. https://www.nodc.noaa.gov/archive/arc0001/9900162/2.2/data/0-data/jgofscd/Files/protocols/toc.html

Jónasdóttir, S. H. (2019). Fatty acid profiles and production in marine phytoplankton. *Marine Drugs*, *17*(3), 151. https://doi.org/10.3390/md17030151

Jónasdóttir, S. H., and Kiørboe, T. (1996). Copepod recruitment and food composition: Do diatoms affect hatching success? *Marine Biology*, *125*(4), 743–750. https://doi.org/10.1007/BF00349257

Jones, R. I. (2000). Mixotrophy in planktonic protists: An overview: Mixotrophy in planktonic protists. *Freshwater Biology*, *45*(2), 219–226. https://doi.org/10.1046/j.1365-2427.2000.00672.x

Kamaya, Y., Kurogi, Y., and Suzuki, K. (2003). Acute toxicity of fatty acids to the freshwater green alga *Selenastrum capricornutum*. *Environmental Toxicology*, *18*(5), 289–294. https://doi.org/10.1002/tox.10127

Kärnä, T., and Baptista, A. M. (2016). Water age in the Columbia River estuary. *Estuarine, Coastal and Shelf Science*, *183*, 249–259. https://doi.org/10.1016/j.ecss.2016.09.001

Klein Breteler, W. C. M., Schogt, N., Baas, M., Schouten, S., and Kraay, G. W. (1999). Trophic upgrading of food quality by protozoans enhancing copepod growth: Role of essential lipids. *Marine Biology*, *135*(1), 191–198. https://doi.org/10.1007/s002270050616

Kleppel, G. S., and Burkart, C. A. (1995). Egg production and the nutritional environment of *Acartia tonsa*: The role of food quality in copepod nutrition. *ICES Journal of Marine Science*, *52*(3–4), 297–304. https://doi.org/10.1016/1054-3139(95)80045-X

Lang, I., Hodac, L., Friedl, T., and Feussner, I. (2011). Fatty acid profiles and their distribution patterns in microalgae: A comprehensive analysis of more than 2000 strains from the SAG culture collection. *BMC Plant Biology*, *11*. https://bmcplantbiol.biomedcentral.com/articles/10.1186/1471-2229-11-124

Lara-Lara, J. R. (1990). Primary production in the Columbia River Estuary I. Spatial and temporal variability of properties. *Pacific Science*, *44*, 21.

Lari, Z., Abrishamchi, P., Ahmadzadeh, H., and Soltani, N. (2019). Differential carbon partitioning and fatty acid composition in mixotrophic and autotrophic cultures of a new marine isolate *Tetraselmis* sp. KY114885. *Journal of Applied Phycology*, *31*(1), 201–210. https://doi.org/10.1007/s10811-018-1549-4

Lau, D. C. P., Jonsson, A., Isles, P. D. F., Creed, I. F., and Bergström, A. (2021). Lowered nutritional quality of plankton caused by global environmental changes. *Global Change Biology*, *27*(23), 6294–6306. https://doi.org/10.1111/gcb.15887

Legrand, C. (2001). Phagotrophy and toxicity variation in the mixotrophic *Prymnesium patelliferum* (Haptophyceae). *Limnology and Oceanography*, *46*(5), 1208–1214. https://doi.org/10.4319/lo.2001.46.5.1208

Lewitus, A. J., Koepfler, E. T., and Morris, J. T. (1998). Seasonal variation in the regulation of phytoplankton by nitrogen and grazing in a salt-marsh estuary. *Limnology and Oceanography*, *43*(4), 636–646. https://doi.org/10.4319/lo.1998.43.4.0636

Marcoval, M. A., Villafañe, V. E., and Helbling, E. W. (2008). Combined effects of solar ultraviolet radiation and nutrients addition on growth, biomass and taxonomic composition of coastal marine phytoplankton communities of Patagonia. *Journal of Photochemistry and Photobiology B: Biology*, *91*(2), 157–166. https://doi.org/10.1016/j.jphotobiol.2008.03.002

Margalef, R. (1978). Life-forms of phytoplankton as survival alternatives in an unstable environment. *Oceanologica Acta*, *1*(4), 493–509.

Martin-Creuzburg, D., Bec, A., and Von Elert, E. (2005). Trophic upgrading of picocyanobacterial carbon by ciliates for nutrition of *Daphnia magna*. *Aquatic Microbial Ecology*, *41*(3), 271–280.

McCabe, R. M., Hickey, B. M., Kudela, R. M., Lefebvre, K. A., Adams, N. G., Bill, B. D., Gulland, F. M. D., Thomson, R. E., Cochlan, W. P., and Trainer, V. L. (2016).

An unprecedented coastwide toxic algal bloom linked to anomalous ocean conditions. *Geophysical Research Letters*, *43*(19), 10,366-10,376. https://doi.org/10.1002/2016GL070023

Meyers, M. T., Cochlan, W. P., Carpenter, E. J., and Kimmerer, W. J. (2019). Effect of ocean acidification on the nutritional quality of marine phytoplankton for copepod reproduction. *PLOS ONE*, *14*(5), e0217047. https://doi.org/10.1371/journal.pone.0217047

Millette, N. C., Gast, R.J., Luo, J. Y., Moeller, H. V., Stamiezskin, K., Andersen, K. H., Brownlee, E. F., Cohen, N. R., Duhamel, S., Dutkiewicz, S., Glibert, P. M., Johnson, M. D., Leles, S. G., Maloney, A. E., Mcmanus, G. B., Poulton, N., Princiotta, S. D., Sanders, R. W., Wilken, S. (2023). Mixoplankton and mixotrophy: future research priorities. *Journal of Plankton Research*, *45*(4), 576–596.

Morando, M., and Capone, D. G. (2018). Direct utilization of organic nitrogen by phytoplankton and its role in nitrogen cycling within the Southern California Bight. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.02118

Müller-Navarra, D. C., Brett, M. T., Park, S., Chandra, S., Ballantyne, A. P., Zorita, E., and Goldman, C. (2004). Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. *Nature*, *427*, 69–72.

Oksanen, J., Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner, H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, and Stier A, Ter Braak C, Weedon J. (2022). *vegan: Community Ecology Package* (R package version 2.6-4) [Computer software]. https://CRAN.R-project.org/package=vegan

Parrish, Christopher C, Kainz, M., Brett, M. T., and Arts, M. T. (2009). *Lipids in aquatic ecosystems*. Springer-Verlag, New York.

Patton, C. J., and Truitt, E. P. (1992). Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory - Determination of total phosphorus by a Kjeldahl digestion method and an automated colorimetric finish that includes dialysis. 46.

Pawlowiez, R., Morey, J. S., Darius, H. T., Chinain, M., and Van Dolah, F. M. (2014). Transcriptome sequencing reveals single domain Type I-like polyketide synthases in the toxic dinoflagellate *Gambierdiscus polynesiensis*. *Harmful Algae*, *36*, 29–37. https://doi.org/10.1016/j.hal.2014.04.013

Peltomaa, E., Hällfors, H., and Taipale, S. J. (2019). Comparison of diatoms and dinoflagellates from different habitats as sources of PUFAs. *Marine Drugs*, *17*(4), 233. https://doi.org/10.3390/md17040233

Perhar, G., Arhonditsis, G. B., and Brett, M. T. (2013). Modelling the role of highly unsaturated fatty acids in planktonic food web processes: Sensitivity analysis and examination of contemporary hypotheses. *Ecological Informatics*, *13*, 77–98. https://doi.org/10.1016/j.ecoinf.2012.10.005

Peura, S., Eiler, A., Hiltunen, M., Nykänen, H., Tiirola, M., and Jones, R. I. (2012). Bacterial and phytoplankton responses to nutrient amendments in a boreal lake differ according to season and to taxonomic resolution. *PLoS ONE*, *7*(6). https://doi.org/10.1371/journal.pone.0038552

Pilecky, M., Kämmer, S. K., Mathieu-Resuge, M., Wassenaar, L. I., Taipale, S. J., Martin-Creuzburg, D., and Kainz, M. J. (2022). Hydrogen isotopes (δ2H) of polyunsaturated fatty acids track bioconversion by zooplankton. *Functional Ecology*, *36*(3), 538–549. https://doi.org/10.1111/1365-2435.13981

Pomati, F., Manarolla, G., Rossi, O., Vigetti, D., and Rossetti, C. (2001). The purine degradation pathway: Possible role in paralytic shellfish toxin metabolism in the cyanobacterium Planktothrix sp. FP1. *Environment International*, *27*(6), 463–470. https://doi.org/10.1016/S0160-4120(01)00101-5

Rai, M. P., Nigam, S., and Sharma, R. (2013). Response of growth and fatty acid compositions of *Chlorella pyrenoidosa* under mixotrophic cultivation with acetate and glycerol for bioenergy application. *Biomass and Bioenergy*, *58*, 251–257. https://doi.org/10.1016/j.biombioe.2013.08.038

Reitan, K. I., Rainuzzo, J. R., and Olsen, Y. (1994). Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. *Journal of Phycology*, *30*(6), 972–979. https://doi.org/10.1111/j.0022-3646.1994.00972.x

Roegner, G. C., and Shanks, A. L. (2001). Import of coastally-derived chlorophyll a to South Slough, Oregon. *Estuaries*, *24*(2), 244–256. https://doi.org/10.2307/1352948

Sakamoto, C. M., Friederich, G. E., and Codispoti, L. A. (1990). *MBARI procedures for automated nutrient analyses using a modified Alpkem Series 300 Rapid Flow Analyzer* (Monograph or Serial Issue 90–2). Monterey Bay Aquarium Research Institute. http://aquaticcommons.org/1971/

Schmitt, J., Larsen, E., Helms, A., Johnson, C., Tanner, B., and Andazola-Ramsey, A. (2010). *Water Quality: Abiotic, Nutrients and Bacteria in South Slough.* Partnership for Coastal Watersheds. http://www.partnershipforcoastalwatersheds.org/water-quality-abiotic-nutrients-and-bacteria-in-south-slough/.

Schrager, H., Bragg, J., Demarzo, A., Yednock, B., Schmitt, J., Helms, A., Tally, J., Rudd, D., Dean, E., Muse, R., Cooper, G., Cornu, C., Gaskill, T., and Wilson, P. (2017). *South Slough National Estuarine Research Reserve Management Plan 2017-2022* (p. 197). South Slough National Estuarine Research Reserve.

Selosse, M.-A., Charpin, M., and Not, F. (2017). Mixotrophy everywhere on land and in water: The grand écart hypothesis. *Ecology Letters*, *20*(2), 246–263. https://doi.org/10.1111/ele.12714

Small, L. F., McIntire, C. D., MacDonald, K. B., Lara-Lara, J. R., Frey, B. E., Amspoker, M. C., and Winfield, T. (1990). Primary production, plant and detrital biomass, and particle transport in the Columbia River Estuary. *Progress in Oceanography*, *25*(1), 175–210. https://doi.org/10.1016/0079-6611(90)90007-O

Stibor, H., and Sommer, Ulrich. (2003). Mixotrophy of a photosynthetic flagellate viewed from an optimal foraging perspective. *Protist*, *154*(1), 91–98.

Stoecker, D. K. (1998). Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. *European Journal of Protistology*, *34*(3), 281–290. https://doi.org/10.1016/S0932-4739(98)80055-2

Stoecker, D. K. (1999). Mixotrophy among dinoflagellates. *The Journal of Eukaryotic Microbiology*, *46*(4), 397–401. https://doi.org/10.1111/j.1550-7408.1999.tb04619.x

Stoecker, D. K., Hansen, P. J., Caron, D. A., and Mitra, A. (2017). Mixotrophy in the marine plankton. *Annual Review of Marine Science*, *9*(1), 311–335. https://doi.org/10.1146/annurev-marine-010816-060617

Stoecker, D., Tillmann, U., and Granéli, E. (2006). Phagotrophy in harmful algae. In E. Granéli and J. T. Turner (Eds.), *Ecology of Harmful Algae* (pp. 177–187). Springer. https://doi.org/10.1007/978-3-540-32210-8_14

Strandberg, U., Hiltunen, M., Syväranta, J., Levi, E. E., Davidson, T. A., Jeppesen, E., and Brett, M. T. (2022). Combined effects of eutrophication and warming on polyunsaturated fatty acids in complex phytoplankton communities: A mesocosm experiment. *Science of The Total Environment*, *843*, 157001. https://doi.org/10.1016/j.scitotenv.2022.157001

Strandberg, U., Taipale, S. J., Hiltunen, M., Galloway, A. W. E., Brett, M. T., and Kankaala, P. (2015). Inferring phytoplankton community composition with a fatty acid mixing model. *Ecosphere*, *6*(1), 1-18.

Taipale, S. J., Kahilainen, K. K., Holtgrieve, G. W., and Peltomaa, E. T. (2018). Simulated eutrophication and browning alters zooplankton nutritional quality and determines juvenile fish growth and survival. *Ecology and Evolution*, *8*(5), 2671–2687. https://doi.org/10.1002/ece3.3832

Taipale, S. J., Kainz, M. J., and Brett, M. T. (2011). Diet-switching experiments show rapid accumulation and preferential retention of highly unsaturated fatty acids in *Daphnia*. *Oikos*, *120*(11), 1674–1682. https://doi.org/10.1111/j.1600-0706.2011.19415.x

Taipale, S. J., Vuorio, K., Strandberg, U., Kahilainen, K. K., Järvinen, M., Hiltunen, M., Peltomaa, E., and Kankaala, P. (2016). Lake eutrophication and brownification downgrade availability and transfer of essential fatty acids for human consumption. *Environment International*, *96*, 156–166.

Thessen, A. E., Bowers, H. A., and Stoecker, D. K. (2009). Intra-and interspecies differences in growth and toxicity of *Pseudo-nitzschia* while using different nitrogen sources. *Harmful algae*, *8*(5), 792-810.

Tomas, C. (1997). *Identifying Marine Phytoplankton*. Elsevier. San Diego, CA, pp. 858.

Twining, C., Bernhardt, J., Derry, A., Hudson, C., Ishikawa, A., Kabeya, N., Kainz, M., Kitano, J., Kowarik, C., Ladd, S., Leal, M., Scharnweber, K., Shipley, J., and Matthews, B. (2021). The evolutionary ecology of fatty-acid variation: Implications for consumer adaptation and diversification. *Ecology Letters*, *24*, 1–23. https://doi.org/10.1111/ele.13771

Van Dolah F M. (2000). Marine algal toxins: Origins, health effects, and their increased occurrence. *Environmental Health Perspectives*, *108*(suppl 1), 133–141. https://doi.org/10.1289/ehp.00108s1133

Villareal, T. A. (1991). Nitrogen-fixation by the cyanobacterial symbiont of the diatom genus *Hemiaulus*. *Marine ecology progress series. Oldendorf*, *76*(2), 201-204.

Ward, B. A., Dutkiewicz, S., Barton, A. D., and Follows, M. J. (2011). Biophysical aspects of resource acquisition and competition in algal mixotrophs. *The American Naturalist, 178*(1), 98–112. https://doi.org/10.1086/660284

Wilken, S., Choi, C. J., and Worden, A. Z. (2020). Contrasting mixotrophic lifestyles reveal different ecological niches in two closely related marine protists. *Journal of Phycology*, *56*(1), 52–67. https://doi.org/10.1111/jpy.12920

Winder, M., and Sommer, U. (2012). Phytoplankton response to a changing climate. *Hydrobiologia*, *698*(1), 5–16. https://doi.org/10.1007/s10750-012-1149-2

Wu, J.-T., Chiang, Y.-R., Huang, W.-Y., and Jane, W.-N. (2006). Cytotoxic effects of free fatty acids on phytoplankton algae and cyanobacteria. *Aquatic Toxicology*, *80*(4), 338–345. https://doi.org/10.1016/j.aquatox.2006.09.011

Yamamoto, T., Oh, S. J., and Kataoka, Y. (2004). Growth and uptake kinetics for nitrate, ammonium and phosphate by the toxic dinoflagellate *Gymnodinium catenatum* isolated from Hiroshima Bay, Japan. *Fisheries Science*, *70*(1), 108–115. https://doi.org/10.1111/j.1444-2906.2003.00778.x

Appendix B: Supporting materials for Chapter 3

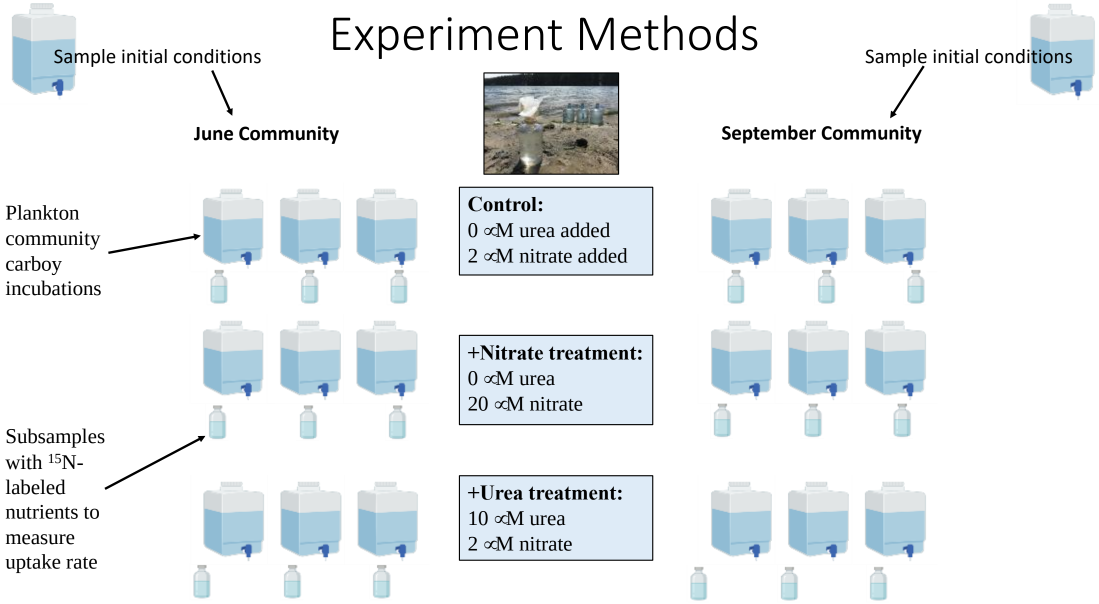

Fig. 8. Diagram of nutrient treatments applied to experimental carboys.

Table 2. List of functional traits by genus.

Genus	Toxigenic	Mixotrophy
Actinastrum		
Alexandrium	x	x
Amphidinium	x	x
Ankistrodesmus		x
Asterionella		
Asterionellopsis		
Asteromphalus		
Athecate dinoflagellate		x
Attheya		
Aulacoseira		
Bacteriastrum		
Centric diatom		
Cerataulina		
Ceratium		x
Chlorella		x
Chaetoceros		
Cochlodinium	x	x
Coelosphaerium	x	
Coscinodiscus		
Cryptomonas		x
Cylindrotheca		x
Cymbella		
Dactyliosolen		
Dictyocha		x
Dinoflagellate		
Dinophysis	x	x
Ditylum		
Eucampia		
Euglena	x	x
Fragilaria		
Glenodinium		x
Gloeocapsa		
Gonyaulax	x	x
Guinardia		
Gymnodinium	x	x
Gyrodinium		x
Hemiaulus		
Heterocapsa		x
Heterosigma	x	x
Isochrysis		x
Karenia	x	x

Leptocylindrus		
Licmophora		
Lingulodinium	x	x
Melosira		
Mesodinium		x
Microcoleus	x	
Microcystis	x	
Navicula		
Nitzschia		
Noctiluca		x
Oscillatoria	x	
Pleurosigma/Gyrosigma		
Prorocentrum	x	x
Protoperidinium	x	x
Pseudo-nitzschia	x	
Pyramimonas		x
Pyrocystis		
Rhizosolenia		
Rhodomonas		x
Scenedesmus		x
Skeletonema		
Sphaerocystis		
Stephanopyxis		
Strombidium		x
Thalassionema		
Thalassiosira		
Thecate dinoflagellate		x
Tintinnid		x
Tropidoneis		
Unknown Cryptophyta		x
Unknown Cyanophyta		
Unknown Chlorophyta		
Unknown flagellate		
Unknown		
Volvox		

References in supplemental file 1 (18KB): trait_matrix_citations.csv

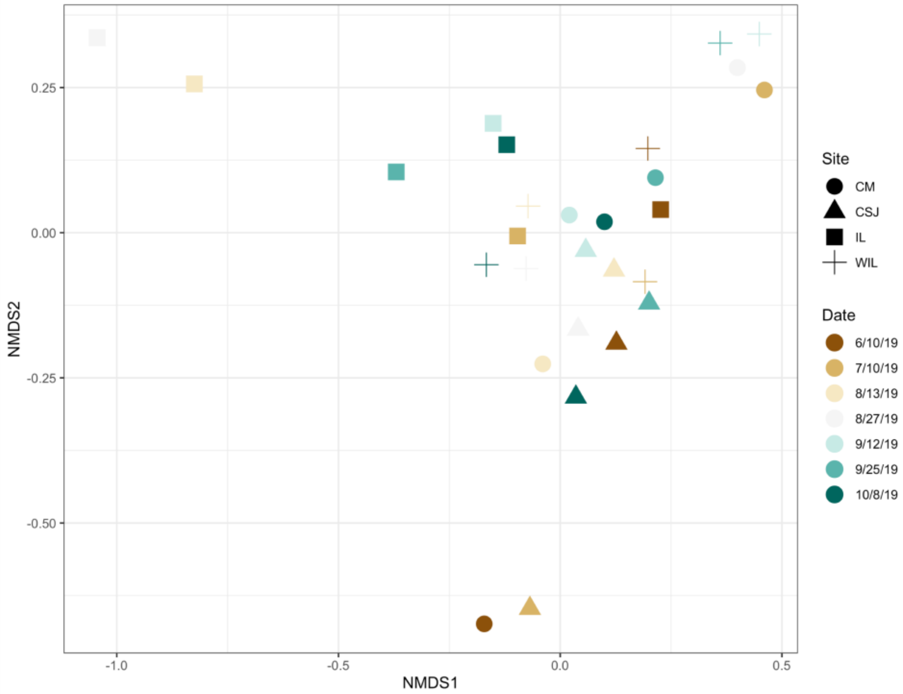

Fig. 9. NMDS of whole fatty acid profile by proportion (%) from field 2019 sampling campaign. Dates represented by different colored points, shapes correspond to sites. Sites include CM = Cape Meares, CSJ = Columbia River South Jetty, IL = Ilwaco Harbor, WIL = Willapa Bay. Stress = 0.07.

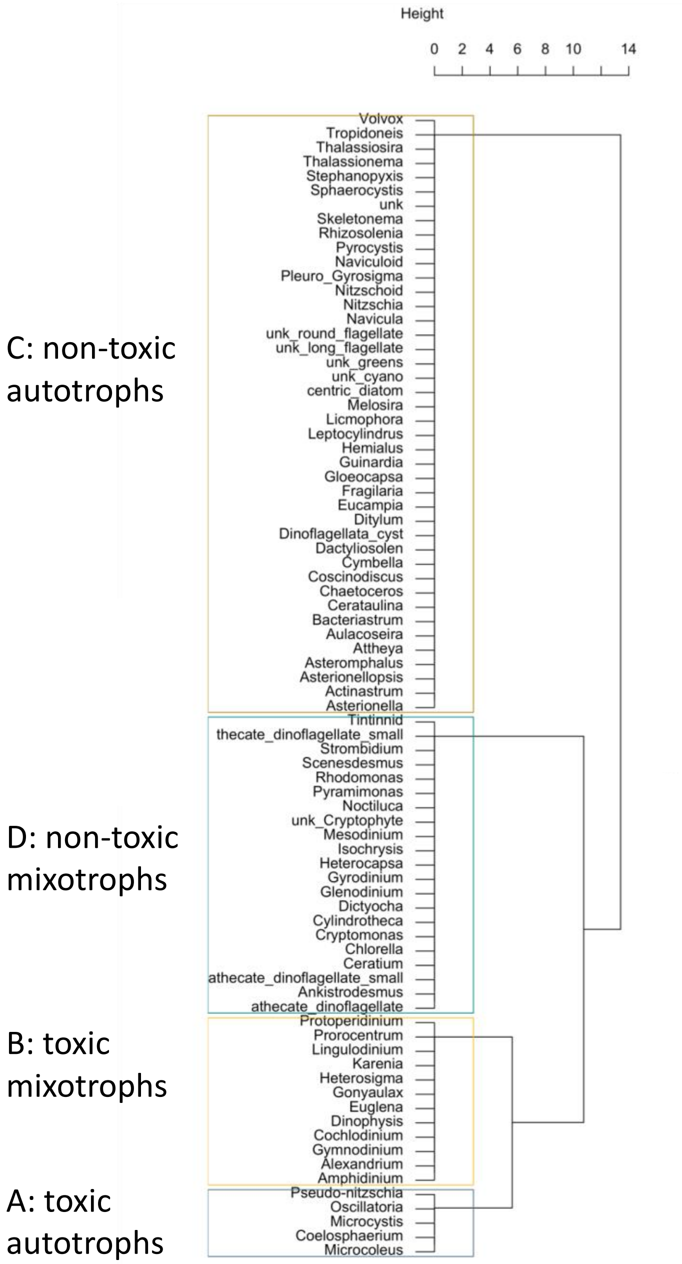

Fig. 10. Dendrogram indicating clustering of plankton taxa into four trait syndromes.

Table 3. Detailed results of fourth-corner analysis for comparison of nutrient forms with plankton traits. TP = total phosphorus, TN = total nitrogen, TDP = total dissolved phosphorus, TDN = total dissolved nitrogen.

Nutrient factor	Trait factor	Obs	Std. Obs	p-value	p-value FDR adjusted
Phosphate	Toxin	-0.017	-0.118	0.919	0.919
Silicic acid	Toxin	-0.323	-1.165	0.255	0.765
Nitrate	Toxin	0.101	0.378	0.666	0.765
Ammonium	Toxin	0.119	0.471	0.623	0.765
TP	Toxin	-0.229	-0.713	0.358	0.765
TN	Toxin	-0.221	-0.688	0.383	0.765
TDN	Toxin	0.198	0.752	0.461	0.765
TDP	Toxin	0.026	0.221	0.855	0.905
Nitrite	Toxin	0.135	0.453	0.630	0.765
Phosphate	Mixotroph	-0.138	-1.259	0.245	0.765
Silicic acid	Mixotroph	0.385	1.344	0.179	0.765
Nitrate	Mixotroph	-0.234	-0.930	0.462	0.765
Ammonium	Mixotroph	-0.176	-0.765	0.680	0.765
TP	Mixotroph	0.288	0.922	0.520	0.765
TN	Mixotroph	0.271	0.876	0.591	0.765
TDN	Mixotroph	-0.280	-1.052	0.366	0.765
TDP	Mixotroph	-0.172	-1.663	0.069	0.765
Nitrite	Mixotroph	-0.265	-0.946	0.481	0.765

Table 4. Detailed results of fourth-corner analysis for comparison of PUFAs with plankton traits.

Fatty acid	Trait factor	Obs	Std. Obs	p-value	p-value FDR adjusted
EPA	Toxin	-0.002	-0.024	0.980	0.980
DHA	Toxin	-0.261	-0.664	0.445	0.663
ARA	Toxin	0.074	0.630	0.557	0.668
ALA	Toxin	-0.262	-0.779	0.377	0.663
LA	Toxin	-0.116	-0.472	0.602	0.722
DPA n-3	Toxin	-0.256	-0.668	0.418	0.663
DPA n-6	Toxin	-0.163	-0.685	0.438	0.663
DTA	Toxin	0.170	0.874	0.407	0.663
EPA	Mixotroph	0.046	0.266	0.847	0.897
DHA	Mixotroph	0.375	0.976	0.451	0.663
ARA	Mixotroph	-0.056	-0.330	0.771	0.833
ALA	Mixotroph	0.363	1.087	0.326	0.663
LA	Mixotroph	0.157	0.690	0.716	0.806
DPA n-3	Mixotroph	0.358	0.960	0.470	0.663
DPA n-6	Mixotroph	0.217	0.919	0.516	0.663
DTA	Mixotroph	-0.193	-0.995	0.395	0.663

Table 5. One-way ANOVA results for concentrations (μM) of nutrients: phosphate, silicic acid, nitrite, nitrate, ammonium, total dissolved phosphorus (TDP), total phosphorus (TP), total dissolved nitrogen (TDN), total nitrogen (TN), and urea. Results stratified by round of experiment (June, September). June phosphate and nitrate were tested with Welch's ANOVA.

Round	Nut. Element	Nut. species	Group var.	Df	F-ratio	p-value
Jun.	N	Nitrite	Treatment	3	2.509	0.094
			Residual	17		
		Nitrate	Treatment	3	50.48	< **0.001**
			Residual	17		
		Ammonium	Treatment	3	13.55	< **0.001**
			Residual	17		
		TDN	Treatment	3	22.81	< **0.001**
			Residual	17		
		TN	Treatment	3	3.344	**0.044**
			Residual	17		
		Urea	Treatment	3	2.248	0.183
			Residual	6		
	P	Phosphate	Treatment	3	2.773	0.134
			Residual	17		
		TDP	Treatment	3	1.962	0.158
			Residual	17		
		TP	Treatment	3	0.208	0.889
			Residual	17		
	Si	Silicic acid	Treatment	3	3.248	**0.048**
			Residual	17		
Sep.	N	Nitrite	Treatment	3	1.982	0.153
			Residual	18		
		Nitrate	Treatment	3	368.8	< **0.001**
			Residual	18		
		Ammonium	Treatment	3	6.313	**0.004**
			Residual	18		
		TDN	Treatment	3	7.392	**0.002**
			Residual	18		
		TN	Treatment	3	13.33	< **0.001**
			Residual	18		
		Urea	Treatment	3	78.70	< **0.001**
			Residual	6		
	P	Phosphate	Treatment	3	2.219	0.121
			Residual	18		
		TDP	Treatment	3	2.732	0.074
			Residual	18		
		TP	Treatment	3	1.705	0.202
			Residual	18		
	Si	Silicic acid	Treatment	3	0.994	0.418
			Residual	18		

Table 6. Tukey's HSD post-hoc tests for nutrient types with significant ANOVA results for difference in nutrient concentrations between treatments for June round.

Silicic Acid	Mean difference	Adjusted p-value
Initial – Control	4.400	**0.029**
Initial – Nitrate Trt	2.767	0.243
Initial – Urea Trt	2.667	0.271
Urea Trt - Control	1.733	0.459
Nitrate Trt – Control	1.633	0.508
Urea Trt – Nitrate Trt	0.100	0.999
Nitrate	Mean difference	Adjusted p-value
Initial – Control	0.088	0.999
Initial – Nitrate Trt	-15.17	< **0.001**
Initial – Urea Trt	0.178	0.999
Urea Trt - Control	-0.090	0.999
Nitrate Trt – Control	15.26	< **0.001**
Urea Trt – Nitrate Trt	-15.35	< **0.001**
Ammonium	Mean difference	Adjusted p-value
Initial – Control	-3.877	< **0.001**
Initial – Nitrate Trt	-4.773	< **0.001**
Initial – Urea Trt	-3.988	< **0.001**
Urea Trt - Control	0.112	0.998
Nitrate Trt – Control	0.897	0.504
Urea Trt – Nitrate Trt	-0.785	0.608
TDN	Mean difference	Adjusted p-value
Initial – Control	11.12	**0.011**
Initial – Nitrate Trt	-9.575	**0.031**
Initial – Urea Trt	-2.182	0.895
Urea Trt - Control	13.30	< **0.001**
Nitrate Trt – Control	20.69	< **0.001**
Urea Trt – Nitrate Trt	-7.393	**0.043**
TN	Mean difference	Adjusted p-value
Initial – Control	0.213	0.999
Initial – Nitrate Trt	-17.69	0.171
Initial – Urea Trt	-13.54	0.372
Urea Trt - Control	13.75	0.203
Nitrate Trt – Control	17.91	0.067
Urea Trt – Nitrate Trt	-4.155	0.923

Table 7. Tukey's HSD post-hoc tests for nutrient types with significant ANOVA results for difference in nutrient concentrations between treatments for September round.

Nitrate	Mean difference	Adjusted p-value
Initial – Control	1.658	0.196
Initial – Nitrate Trt	-18.33	**< 0.001**
Initial – Urea Trt	1.614	0.214
Urea Trt - Control	0.043	0.999
Nitrate Trt – Control	19.99	**< 0.001**
Urea Trt – Nitrate Trt	-19.95	**< 0.001**
Ammonium	Mean difference	Adjusted p-value
Initial – Control	-0.948	0.092
Initial – Nitrate Trt	-1.443	**0.006**
Initial – Urea Trt	-1.476	**0.005**
Urea Trt - Control	0.528	0.423
Nitrate Trt – Control	0.495	0.478
Urea Trt – Nitrate Trt	0.033	0.999
TDN	Mean difference	Adjusted p-value
Initial – Control	4.361	0.828
Initial – Nitrate Trt	-13.00	0.086
Initial – Urea Trt	-13.12	0.082
Urea Trt - Control	17.49	**0.006**
Nitrate Trt – Control	17.36	**0.007**
Urea Trt – Nitrate Trt	0.127	0.999
TN	Mean difference	Adjusted p-value
Initial – Control	6.034	0.553
Initial – Nitrate Trt	-18.03	**0.004**
Initial – Urea Trt	-9.528	0.187
Urea Trt - Control	15.56	**0.006**
Nitrate Trt – Control	24.06	**< 0.001**
Urea Trt – Nitrate Trt	-8.498	0.189
Urea	Mean difference	Adjusted p-value
Initial – Control	-3.128	0.486
Initial – Nitrate Trt	-0.391	0.997
Initial – Urea Trt	-20.33	**< 0.001**
Urea Trt - Control	17.20	**< 0.001**
Nitrate Trt – Control	-2.737	0.330
Urea Trt – Nitrate Trt	19.94	**< 0.001**

Table 8. N uptake rates.

	Mean uptake (nmol L^{-1} h^{-1})	Standard deviation
Control	0.010	0.008
Nitrate Treatment	0.003	0.001
Urea Treatment	1.188	0.424

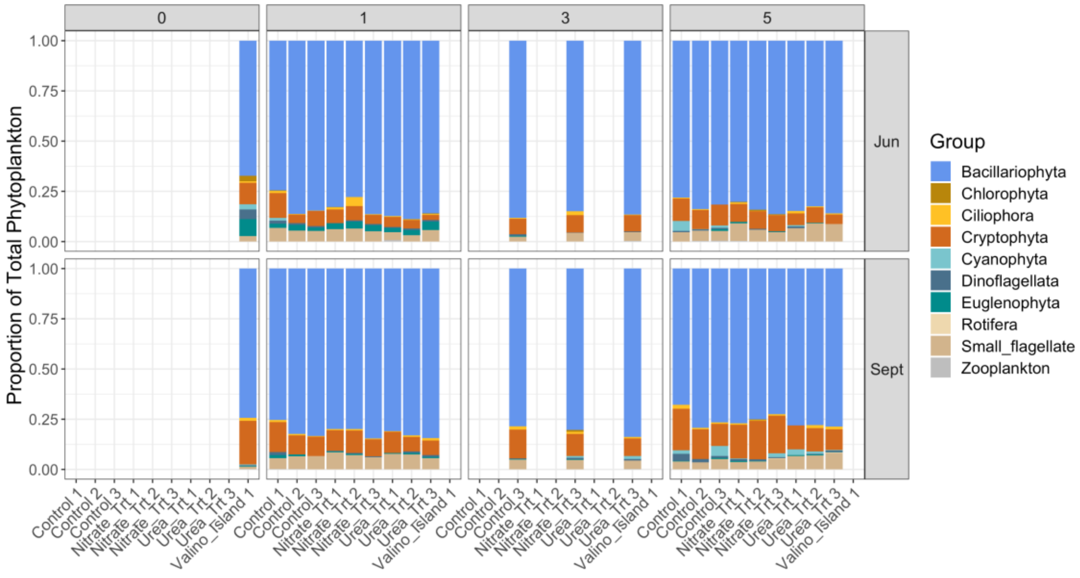

Fig. 11. Barplots of plankton community composition (proportion of total plankton cell concentrations) from nutrient addition experiment, organized by round of experiment (across right side) and day of incubation (across top). Treatment and replicate number are indicated on the x-axis. Groups are plankton phyla (Bacillariophyta, Chlorophyta, Ciliophora, Cryptophyta, Cyanophyta, Dinoflagellata, Euglenophyta, Rotifera) or taxonomically amalgamated groups of less common taxa (small flagellates, zooplankton).

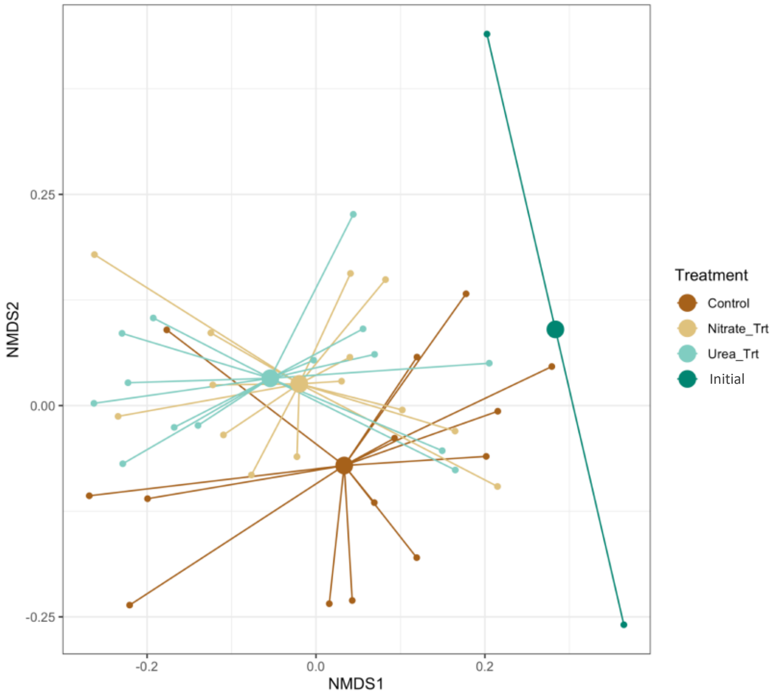

Fig. 12. NMDS of plankton community composition in experiment with spider plots (show group centroid and each sample linked to corresponding centroid in NMDS space) to indicate each treatment of Initial Conditions in the South Slough Estuary, Control, Nitrate Treatment, and Urea Treatment.

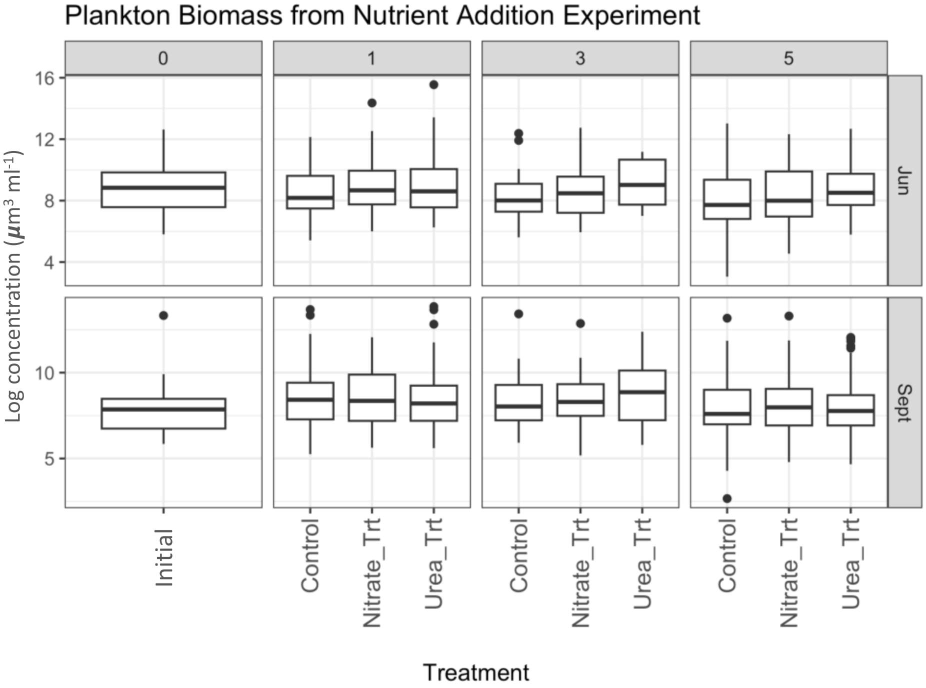

Fig. 13. Boxplots showing plankton biomass by nutrient addition treatment, round of experiment (June or September), and day of the incubation (day 0 = initial conditions in the South Slough Estuary, day 1, 3, and 5 = final day of the incubation).

Table 9. Two-way ANOVA results for biomass of mixotrophic and toxigenic plankton.

Trait	Group var.	Df	F-ratio	p-value
Mixotroph	Treatment	3	0.176	0.911
	Round	1	0.125	0.730
	Treat:Round	3	0.692	0.574
	Residual	12		
Toxigenic	Treatment	3	0.265	0.849
	Round	1	1.011	0.336
	Treat:Round	3	0.021	0.996
	Residual	11		

Table 10. Two-way ANOVA results for concentrations (μg/L) and proportions (%) of PUFAs of interest: DHA, EPA, ALA.

PUFA	Group var.	Df	F-ratio	p-value
[DHA]	Treatment	3	6.396	**0.006**
	Round	1	64.51	**< 0.001**
	Treat:Round	3	4.691	**0.018**
	Residual	14		
[EPA]	Treatment	3	8.227	**0.002**
	Round	1	4.111	0.062
	Treat:Round	3	8.371	**0.002**
	Residual	14		
[ALA]	Treatment	3	268.4	**< 0.001**
	Round	1	117.1	**< 0.001**
	Treat:Round	3	121.2	**< 0.001**
	Residual	14		
% DHA	Treatment	3	56.23	**< 0.001**
	Round	1	51.56	**< 0.001**
	Treat:Round	3	3.198	0.056
	Residual	14		
% EPA	Treatment	3	18.546	**< 0.001**
	Round	1	1.747	0.208
	Treat:Round	3	0.443	0.726
	Residual	14		
% ALA	Treatment	3	183.5	**< 0.001**
	Round	1	32.05	**< 0.001**
	Treat:Round	3	4.510	**0.021**
	Residual	14		

Table 11. One-way ANOVA results of PUFA concentration (μg/L) and PUFA percentage (%) for: DHA, EPA, ALA.

Round	PUFA	Group var.	Df	F-ratio	p-value
Jun.	[DHA]	Treatment	3	1.428	0.313
		Residual	7		
	[EPA]	Treatment	3	4.808	**0.040**
		Residual	7		
	[ALA]	Treatment	3	223.3	**< 0.001**
		Residual	7		
Sep.	[DHA]	Treatment	3	31.37	**< 0.001**
		Residual	7		
	[EPA]	Treatment	3	9.926	**0.006**
		Residual	7		
	[ALA]	Treatment	3	45.52	**< 0.001**
		Residual	7		
Jun.	% DHA	Treatment	3	33.07	**< 0.001**
		Residual	7		
	% EPA	Treatment	3	18.73	**0.001**
		Residual	7		
	% ALA	Treatment	3	383.3	**< 0.001**
		Residual	7		
Sep.	% DHA	Treatment	3	25.27	**< 0.001**
		Residual	7		
	% EPA	Treatment	3	15.74	**0.002**
		Residual	7		
	% ALA	Treatment	3	39.11	**< 0.001**
		Residual	7		

Table 12. Tukey's HSD post-hoc test results for difference between treatments in PUFA concentration (μg/L) and PUFA percentage (%) of total fatty acids for June round.

[DHA]	Mean difference	Adjusted p-value
Initial – Control	-0.038	0.998
Initial – Nitrate Trt	-0.332	0.543
Initial – Urea Trt	-0.088	0.981
Urea Trt - Control	-0.127	0.931
Nitrate Trt – Control	0.293	0.551
Urea Trt – Nitrate Trt	-0.420	0.285

[EPA]	Mean difference	Adjusted p-value
Initial – Control	1.603	0.054
Initial – Nitrate Trt	1.207	0.154
Initial – Urea Trt	1.733	**0.038**
Urea Trt - Control	-0.130	0.990
Nitrate Trt – Control	0.397	0.806
Urea Trt – Nitrate Trt	-0.527	0.649

[ALA]	Mean difference	Adjusted p-value
Initial – Control	1.302	< **0.001**
Initial – Nitrate Trt	1.288	< **0.001**
Initial – Urea Trt	1.315	< **0.001**
Urea Trt - Control	-0.013	0.994
Nitrate Trt – Control	0.013	0.994
Urea Trt – Nitrate Trt	-0.027	0.955

% DHA	Mean difference	Adjusted p-value
Initial – Control	-0.075	< **0.001**
Initial – Nitrate Trt	-0.079	< **0.001**
Initial – Urea Trt	-0.072	< **0.001**
Urea Trt - Control	-0.003	0.979
Nitrate Trt – Control	0.004	0.962
Urea Trt – Nitrate Trt	-0.007	0.824

% EPA	Mean difference	Adjusted p-value
Initial – Control	-0.082	**0.002**
Initial – Nitrate Trt	-0.084	**0.002**
Initial – Urea Trt	-0.084	**0.002**
Urea Trt - Control	0.002	0.999
Nitrate Trt – Control	0.002	0.998
Urea Trt – Nitrate Trt	-2.0×10^{-4}	0.999

% ALA	Mean difference	Adjusted p-value
Initial – Control	0.016	< **0.001**
Initial – Nitrate Trt	0.017	< **0.001**
Initial – Urea Trt	0.017	< **0.001**
Urea Trt - Control	-5.3×10^{-4}	0.732
Nitrate Trt – Control	-5.5×10^{-4}	0.715
Urea Trt – Nitrate Trt	-1.6×10^{-5}	0.999

Table 13. Tukey's HSD post-hoc test results for difference between treatments in PUFA concentration (μg/L) and PUFA percentage (%) of total fatty acids for September round.

[DHA]	Mean difference	Adjusted p-value
Initial – Control	-0.380	**0.021**
Initial – Nitrate Trt	-0.730	< **0.001**
Initial – Urea Trt	-0.833	< **0.001**
Urea Trt - Control	0.453	**0.005**
Nitrate Trt – Control	0.350	**0.018**
Urea Trt – Nitrate Trt	0.103	0.639
[EPA]	Mean difference	Adjusted p-value
Initial – Control	-0.213	0.726
Initial – Nitrate Trt	-0.823	**0.019**
Initial – Urea Trt	-0.870	**0.015**
Urea Trt - Control	0.657	**0.034**
Nitrate Trt – Control	0.610	**0.047**
Urea Trt – Nitrate Trt	0.047	0.994
[ALA]	Mean difference	Adjusted p-value
Initial – Control	0.270	< **0.001**
Initial – Nitrate Trt	0.243	< **0.001**
Initial – Urea Trt	0.253	< **0.001**
Urea Trt - Control	0.017	0.884
Nitrate Trt – Control	0.027	0.667
Urea Trt – Nitrate Trt	-0.010	0.970
% DHA	Mean difference	Adjusted p-value
Initial – Control	-0.043	**0.003**
Initial – Nitrate Trt	-0.055	< **0.001**
Initial – Urea Trt	-0.064	< **0.001**
Urea Trt - Control	0.020	0.080
Nitrate Trt – Control	0.012	0.393
Urea Trt – Nitrate Trt	0.009	0.616
% EPA	Mean difference	Adjusted p-value
Initial – Control	-0.064	**0.007**
Initial – Nitrate Trt	-0.077	**0.003**
Initial – Urea Trt	-0.083	**0.002**
Urea Trt - Control	0.019	0.446
Nitrate Trt – Control	0.013	0.713
Urea Trt – Nitrate Trt	0.006	0.956
% ALA	Mean difference	Adjusted p-value
Initial – Control	0.012	< **0.001**
Initial – Nitrate Trt	0.012	< **0.001**
Initial – Urea Trt	0.012	< **0.001**
Urea Trt - Control	-1.8×10^{-4}	0.999
Nitrate Trt – Control	-3.6×10^{-4}	0.990
Urea Trt – Nitrate Trt	-5.4×10^{-4}	0.965

Appendix β: Supplemental File

Name: trait_matrix_citations.csv

Size: 20KB

Required software: Microsoft Excel

Description: Spreadsheet of plankton traits and citations of literature used to make trait determinations.

Chapter 4: Community-Based, Socio-Ecological Lens Improves Understanding of Harmful Algal Bloom Impacts and Shellfish Harvester Perceptions in Coastal Oregon

The version presented in this dissertation has been approved by all co-authors and the Confederated Tribes of Coos, Lower Umpqua, and Siuslaw Indians Tribal Council.

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Author contributions: TD contributed to the conceptualization of the project, carried out the majority of data collection, analysis, and manuscript writing. SS, JN, AR significantly contributed to the conceptualization of the project and data collection. TP Contributed to project conceptualization and manuscript writing. SC, MNP contributed to the conceptualization of the project and IRB application. MH, DR, SR contributed key background information and feedback on project.

Abstract

Harmful algal blooms (HABs) occur when toxigenic phytoplankton reach high abundance, often producing biotoxins that can enter food chains and contaminate shellfish, making them unsafe for human consumption and necessitating the closure of fisheries to protect human health. Despite decades of development as a management tool, HAB monitoring has rarely been viewed through a socio-ecological lens to understand human dimensions associated with HABs. The goal of this research is to assess shellfish harvester HAB-related health risks and perceptions to target management and outreach. We conducted monthly HAB monitoring (via light microscopy) from October 2020 – August 2022, and monthly toxin testing (via enzyme-linked immunosorbent assay (ELISA)) in 2021 and 2022 in the South Slough and Coos estuaries of southern Oregon. We also conducted in-person and online surveys of local shellfish harvesters from June 2022 - February 2023, collecting demographic information, harvesting practices, and perceptions of HABs. We observed *Pseudo-nitzschia* spp. ($\geq 10^5$ cells L^{-1}) during the summer of 2021, and detected particulate domoic acid at shellfish harvesting sites, typically below alert levels (200 ng L^{-1}). Estimated domoic acid exposures of harvesters were below the recommended daily limit (0.075 mg kg^{-1} body weight), with a pattern of differential exposure related to gender, education, income, distance to harvest, and years of experience harvesting. Survey findings (*n*=140) suggest that intensity of shellfish harvest is highest in summer (80% reported ≥1 summer harvest, 29 - 41% during other seasons). About 64% of shellfish harvesters were concerned about HABs and a majority reported that educational resources, monitoring programs, and fishery closures were

important to their harvesting decisions. Socio-ecological study of this system identifies overlap between toxin risk and human exposure factors to streamline future HAB monitoring. By combining ecological monitoring with information about community fishery use and perceptions, we can more accurately estimate risk of environmental exposures and understand how to best improve management practices.

Introduction

Harmful Algal Blooms (HABs) occur when certain species of microalgae grow rapidly, often creating negative ecological conditions and public health impacts. Coastal and estuarine HABs in the study area of the South Slough National Estuarine Research Reserve and the surrounding area of the Coos estuary tidal basin (southern Oregon coast) have become a concern due to their close proximity to marine waters that are frequently closed to commercial and recreational harvest of shellfish due to elevated concentrations of algal biotoxins. Along the U.S. West Coast, HAB events have caused seabird, fish, and marine mammal mortality events (Fire and Dolah, 2012; Horner et al., 1997; Shumway et al., 2003). Furthermore, HABs in this region can result in accumulation of toxins in shellfish that humans harvest for food, including multiple species of crabs, clams, mussels, and oysters (Moore et al., 2019). In addition to posing a public health concern, the presence of toxins in shellfish can create detrimental impacts to coastal communities when fisheries are closed as a preventive measure (Berdalet et al., 2016; Moore et al., 2019). Recreational fishery closures can create food insecurity for subsistence harvesters, restrict cultural activities for tribal harvesters, impact quality of life for residents, and cause economic losses to coastal areas that benefit from tourism related to shellfish harvesting (Donatuto et al., 2014; Grattan et al., 2016; Holland and Leonard, 2020; Ritzman et al., 2018).

The US Commission on Ocean Policy (2004) has recognized that HABs pose a threat to the environment and coastal communities, and HAB monitoring programs have been developed around the world (Andersen, 1996). With authorization from the Harmful

Algal Bloom and Hypoxia Research and Control Act (HABHRCA), the National Oceanic and Atmospheric Administration (NOAA) prioritized programs to improve research, monitoring, and event response, and is currently developing a nation-wide HAB monitoring network (NCCOS, 2017a, 2017b; NCCOS and US IOOS, 2020). Localized monitoring programs in the Pacific Northwest have also been successful in providing early warning of HABs: Oregon Department of Fish and Wildlife coastal HAB monitoring program, Olympic Region Harmful Algal Bloom Bulletin, and Sound Toxins all contribute to the Pacific Northwest Harmful Algal Bloom Bulletin (https://orhab.uw.edu/pnw-hab-bulletin/) in partnership with the Olympic Natural Resource Center, University of Washington, and the Northwest Association of Networked Ocean Observing Systems. The National Estuarine Research Reserves System has identified HAB monitoring as a management priority, with efforts dedicated to monitoring as a part of the Reserves' System Wide Monitoring Program (SWMP), which employs a network of water quality and meteorological sensors (Dix et al., 2022; Evans et al., 2012; Schrager et al., 2017).

As the science of detecting and predicting HAB events matures, the need for strategies that address a variety of human components alongside the ecological has become apparent. A multi-agency report led by the National Centers for Coastal Ocean Science (NCCOS) (Bauer, 2006), identified human dimensions research as a high priority in the effort to prevent and respond to the impacts of HABs. The Centers for Disease Control also created the Harmful Algal Bloom-Related Illness Surveillance System to record reports of HAB events, fish kills, and associated human illness (Backer et al., 2015). HAB-related human dimensions research has focused on gaining a better understanding of public health (Grattan et al., 2016; Kite-Powell et al., 2008), economic (Dyson and Huppert, 2010; Lim and Kim, 2020), and cultural (Ritzman et al., 2018) impacts of HAB events. Other research (e.g., Gulf Coast region) has emphasized understanding public awareness of HABs and their connection to seafood safety and improving science communication efforts through targeted messaging (Kirkpatrick et al., 2014; Nierenberg et al., 2010). Human-dimensions research on the U.S. West Coast also investigated how people perceive HAB-related risk and management responses to HAB events across multiple communities and cultural contexts (Ekstrom et al., 2020; Ritzman et al., 2018; Roberts et al., 2016). These previous studies on the human dimensions of HABs highlight the importance of analyzing human and natural systems jointly, as each of these components may feed back into the other. However, most long-term monitoring programs (e.g., Andersen 1999; Pacific Northwest HAB Bulletin) do not incorporate human dimensions information directly into monitoring efforts to inform risk assessment.

By combining an ecological HAB monitoring program with a socio-cultural survey of shellfish harvesters at the local level, we illustrate a more complete picture of this complex problem while involving community stakeholders to foster awareness of HABs. Issues associated with HABs emerge from a complex adaptive system (Levin, 1998), in which both ecological and social systems contain individual agents (e.g., plankton, shellfish harvesters) with localized actions (e.g., plankton growth in response to environmental conditions, shellfish harvest based on perception of safety). These local actions may coalesce into patterns at larger scales and create feedbacks, making modeling of these complex processes difficult, but necessary to consider for informed decision-making (Levin et al., 2013). Socio-ecological systems (SES) research uses techniques drawn from social and natural sciences, and can be used to understand systems with complex interactions between behavioral, cultural, political, biological, and physical elements. One of the strengths of this approach is the recognition of the central role that local and Indigenous knowledge plays in SES dynamics (Cote and Nightingale, 2012), particularly where scientific limitations exist (e.g., limited resources, expertise, or spatiotemporal coverage). SES research has been applied broadly since its development (Berkes et al., 2008; Berkes and Folke, 1994), and specifically to the issue of HABs in systems ranging from European marine environments, Chesapeake Bay, to the Olympic Peninsula of Washington State to understand the relationship between HABs and human health, wellbeing, income, scientific literacy, and mitigation response (Crosman et al., 2016; Van Dolah et al., 2013; 2016; West et al., 2021). However, no previous research to our knowledge has integrated time-based algal toxin exposure assessment and risk perceptions with a multi-year HAB monitoring program. This has created a gap in our understanding of how shellfish harvesters' knowledge and perceptions of HABs and their resultant harvesting behavior impact actual exposure to algal toxins, which can vary in time and space.

The primary goal of this research is to assess HAB-related health risks to shellfish harvesters and explore how they perceive HABs in order to more effectively target monitoring, management, and outreach efforts. Understanding how *individuals* with particular demographic risk factors, such as age or gender (Toyofuku, 2006), use the fishery provides crucial information about the risk of exposure to algal toxins and factors that shape risk perception (e.g., how often a person harvests shellfish, length of experience harvesting, shellfish serving size, experience becoming sick from shellfish). Finally, this study provides an opportunity to assess support for potential intervention options and probes participant behaviors regarding the use of HAB monitoring information in decision-making around shellfish harvest.

We hypothesized that exposure to algal biotoxins would vary among recreational shellfish harvesters depending on their level of experience, time of year, proximity to shellfish harvesting sites, and ethnicity. This hypothesis is based on the premise that HABs are more common during the spring and summer seasons (Griffith and Gobler, 2020), and that local coastal residents and Native Americans would harvest shellfish more frequently and therefore have higher chance of toxin exposure (Cisneros-Montemayor et al., 2016; EPA, 2014). We also expected shellfish harvesters to support non-regulatory approaches to HAB management, such as education and monitoring, more than regulatory fishery closures.

Methods

Study area context

Coos County, Oregon, USA has a relatively low coastal population density of 16 people/km^2 (NOAA OCM, 2021b), with the majority (61.5%) of landcover classified as forested and 2.4% classified as developed (as of 2016) (NOAA OCM, 2021a). The county's economy is driven by forest products, tourism, fishing, and agriculture (Bay

Area Chamber of Commerce, 2020). Indigenous people in the Pacific Northwest coastal region have been stewarding and harvesting shellfish since time immemorial (Anderson and Parker, 2009; Confederated Tribes of Coos, Lower Umpqua, and Siuslaw Indians *personal communication,* 2021; Lepofsky et al., 2015; Losey et al., 2004; Mathews and Turner, 2017;). The area of study in the South Slough Estuary and Coos Estuary lies in the homelands of the Hanis and Miluk bands of the Coos Indians; the Confederated Tribes of Coos, Lower Umpqua, and Siuslaw Indians (CTCLUSI) and the Coquille Indian Tribe (CIT) continue to participate in the management of local fisheries in partnership with the state government and other organizations (CTCLUSI, 2021). South Slough National Estuarine Research Reserve (est. 1974) manages 26.5 km^2 spanning the majority of the South Slough and provides scientific and educational resources about the estuary to the public (Schrager et al., 2017).

The South Slough Estuary (2.4 km^2) is a distinct tidal inlet encompassed within the greater Coos Estuary (53.8 km^2) tidal basin (Rumrill, 2006). Both tidal systems are fed by multiple tributaries and join to form a single ocean outlet, creating a complex and fluctuating pattern of mixing between freshwater inputs and high salinity waters from tidal exchange (Hickey and Banas, 2003). Salinity inflow magnitude is largely dependent on the point in the spring-neap tidal cycle, whereas outflow, stratification, and mixing vary seasonally with discharge (Conroy et al., 2020). Most chlorophyll *a* in South Slough is coastally derived and advected into the estuary (Roegner and Shanks, 2001), but phytoplankton abundance and composition vary spatially and seasonally, with greater ocean influence in the lower estuary and during the summer (Hughes, 1997). *Pseudo-*

nitzschia spp., a well-studied toxigenic marine alga in this system, has been observed in blooms that originated in the nearshore coastal environment after upwelling events, were transported into the Coos and South Slough estuaries via tidal action, and rapidly mixed throughout the water column by turbulent flow in estuarine channels (Cziesla, 1999; Ohana-Richardson, 2007; Torres and Shanks, 2018). Continuous state-led HAB monitoring has focused on open-coast sites including regular shellfish tissue testing for algae-derived domoic acid (DA) and saxitoxins and weekly to biweekly phytoplankton sampling along the Oregon Coast (ODFW, 2020; State of Oregon, 2021).

The South Slough and Coos estuaries contain a plethora of habitats for shellfish, and the area has a vibrant recreational shellfishing culture (Ainsworth et al., 2012, 2014; CTCLUSI *personal communication* 2021; Rumrill, 2006). Numerous mudflats support populations of gaper clams (*Tresus capax*), native littleneck clams (*Leukoma staminea*), butter clams (*Saxidomus gigantea*), and cockles (*Clinocardium nutallii*) (SEACOR, 2014). Docks also provide access for shore-based crabbing of Dungeness crab (*Metacarcinus magister*) and red rock crab (*Cancer productus*), which can be found throughout the estuaries and nearshore ocean (Ainsworth et al., 2012, 2014). The nearby intertidal zones support mussels (*Mytilus edulis, M. californianus*), and sandy beaches of the open coast and sand spits are host to Pacific razor clams (*Siliqua patula*). Anyone with a valid Oregon shellfish license can partake in harvesting a myriad of species. ODFW estimated an average of 12,500 clamming trips in the Coos and South Slough estuaries and 14,710 crabbing trips per year are made in the Coos Estuary alone

(Ainsworth et al., 2012, 2014). Most crabbing takes place during slack tides, and mudflats are busiest with clam harvesters during negative tides.

Compared to other Oregon estuaries that are dominated by non-local tourists, Coos Estuary harvesters consist of a higher ratio of local residents (Ainsworth et al., 2014). Many of the region's current residents, including Indigenous people, rely on these fisheries for recreation, cultural practice, economic benefit, food security, and quality of life (CTCLUSI *personal communication* 2021; Donatuto et al., 2014; Lellis-Dibble et al., 2008; Poe et al., 2015).

Algal and environmental sample collection and analysis

Whole surface water 100 mL samples were collected in glass jars via kayak survey at 0.3 m depth every month from four core sites between October 2020 and August 2022 to survey algal taxonomic composition (n= 91) and algal toxins (n= 24) (Fig. 1). These sites geographically correspond with environmental sensors deployed in the estuary for the South Slough National Estuarine Research Reserve SWMP and span degrees of tidal and tributary influence within the South Slough. In April – October 2021 and June – August 2022, sampling was expanded to include phytoplankton (n= 35) and toxins (n= 26) at five other sites based on local knowledge and interest from stakeholder groups (derived from informal discussions over virtual meeting platforms and email and pilot in-person surveys of shellfish harvesters). This expanded sampling included one freshwater site (Tenmile Lake), chosen for its impact on recreational and drinking water impairment. Higher frequency sampling (n= 30) was also performed in September 2021 to capture variation of the estuarine plankton community over a shorter time scale and at different points in the mixed semi-diurnal tidal cycle. In total, 156 phytoplankton samples and 50 toxin samples were analyzed.

Lugol's iodine solution was added to whole water samples (final concentration, 1%) for preservation until analysis using a light microscope. Algal toxin samples were filtered on glass microfiber filters (GF/F, 0.7-µm pore size) for the analysis of toxins associated with particulate matter or in glass vials for later cell lysis by the freeze/thaw method, depending on the toxin of interest. Water quality (temperature, salinity, turbidity, dissolved oxygen) and meteorological data (wind speed, wind direction, total photosynthetically active radiation) data were collected by SWMP sensors (YSI 6600 or YSI 6600 EDS models, Yellow Springs Instrument Company (YSI), Yellow Springs, Ohio) every 15 minutes. Daily precipitation was obtained from the NOAA precipitation gauge in Coquille (~ 15 km SE of SSE) (National Weather Service, 2023).

Laboratory analyses

Whole water algae samples were homogenized and either concentrated (10:1 or 25:1) or 1 ml was sampled directly, depending on particle density, and allowed to settle 15 minutes in a Sedgewick-Rafter counting chamber. Transects were surveyed and cells enumerated up to 400 counting units using a compound microscope on 200X magnification. Plankton were also measured for biovolume calculations to estimate biomass (10 cells measured per taxon, or as many cells as observed in sample for less abundant taxa). Although our sampling methods were designed to target phytoplankton, zooplankton were noted when they appeared during microscopy identifications. Where possible, cells were identified to genus, species, or complexes that were more practical for identification using light microscopy (e.g., genus *Pseudo-nitzschia* was classified into complexes of *Pseudo-nitzschia* c.f. *australis/fraudulenta*, *P*. c.f. *pungens/multiseries*, or *P*. c.f. *pseudodelicatissima/delicatissima*, which can only be differentiated by cell size and shape using light microscopy). Algal abundances are reported as cell concentrations, biovolumes (Sun and Liu, 2003), and as a proportion of the total cells counted or proportion of biovolume to provide multiple ways of assessing the relative importance of toxigenic taxa. Whole water samples for toxin analysis were filtered onto GF/F filters (400 – 1000 mL per sample), and frozen at -20^{o}C, pending analysis. Analytes were extracted from filters in deionized water with sonication as in Lefebvre et al. (2008) or by the freeze/thaw method as recommended by Eurofins Abraxis for microcystins, and two analytical replicates per sample were analyzed. We used ELISA (Eurofins Abraxis) and a Molecular Devices Spectra Max M2^{e} plate reader with the necessary toxin standards and controls to assess concentrations of domoic acid (reported in ng L^{-1}, detection limit = 6.5 ng L^{-1}), total saxitoxins (reported in $\mu g\ L^{-1}$, detection limit = 0.015 $\mu g\ L^{-1}$), and microcystins (reported in $\mu g\ L^{-1}$, detection limit = 0.10 $\mu g\ L^{-1}$).

Algal and environmental monitoring statistical analysis

Regression trees were used to understand the drivers of toxigenic taxa abundance with the R package ‘party’ (v. 1.3, Hothorn et al., 2006). Regression trees, which do not assume a particular form of relationship between predictor and response variables, were conducted with rolling averages of multiple time steps included as possible predictors (15 minutes, 1 hour, 1 day, 7 days, 14 days) to understand the timescale at which environmental variables influenced the abundance of toxigenic taxa. Rolling averages were used to represent conditions influencing the phytoplankton sample while averaging over tidal and shorter-term variation. Non-metric multidimensional scaling (NMDS) with the Bray-Curtis dissimilarity index in package 'vegan' (R 4.2.2) was also used to assess both spatial and temporal variation in community composition over the monthly monitoring period and high-resolution sampling period (Clarke 1993).

Questionnaire sites and protocol

A questionnaire was administered to shellfish harvesters to gain information regarding demographics, harvesting practices, HAB knowledge and perceptions, and HAB management preferences (Appendix C1). Surveys were conducted in two formats: in-person surveys at shellfish harvesting sites to capture the general population of shellfish harvesters, and online surveys specifically recruiting local (CTCLUSI and CIT) tribal members to increase their representation in our study. In-person survey participants were recruited at access points to mudflats and docks known to be popular clamming and shore crabbing locations spanning the South Slough and Coos estuaries. The open nature of these landscapes allowed for attempted recruitment of all harvesters present. This method was chosen given the localized nature of the study and its small pool of potential participants. One harvester from each household was asked to participate in the survey, either by filling in a hard copy of the questionnaire or verbally answering questions asked by the investigators or a trained volunteer. In-person surveys took place within 2 hours of low-tide for clam harvesters or slack tide for crab harvesters during a total of 6 days of negative tides in June and July 2022, and not during a fishery closure due to HABs. Participants were also recruited to fill out an online version of the same questionnaire through monthly advertisements directed at shellfish harvesters in the CTCLUSI newsletter and two advertisements on the Friends of South Slough mailing list. Online surveys took place at will from July 2022 – February 2023, which spanned a period of fishery closures (length of closure varied by shellfish species) due to elevated DA levels. A raffle for gift cards to a local business was offered as incentive to online participants. For both methods of survey, harvesters were required to be over 18 years to participate and were informed that all questions were voluntary (PSU HRRP # 227569-18).

Analysis of questionnaire responses

First, a descriptive analysis of the questionnaire data, summarizing the respondent demographics, shellfish harvesting practices, and knowledge and perceptions of HAB risk was conducted. Questionnaire data were plotted and data analyses were done using R (4.2.2). Respondents were asked if they had previous knowledge of HABs and their connection to seafood safety, to rate their personal risk of consuming algal-contaminated shellfish as “low”, “medium”, “high”, or “I don’t know”, and to rate their concern about HABs on a scale of 1 to 5. Tests for difference in participants’ knowledge and perceptions of HABs based on their experience with shellfish harvesting, HAB-related illness, and demographic factors were performed using Chi square/Fisher’s Exact test of independence, Wilcoxon's signed rank test, or Kruskal Wallis test depending on number of independent variables and sample size. Pairwise Fisher's and Dunn's post hoc tests with multiple test corrections (false discovery rate, Benjamini-Hochberg, respectively) were conducted if omnibus tests found significant differences. These tests were done using R functions 'chisq.test', 'fisher.test', 'wilcox.test', 'kruskal.test', 'pairwise_fisher_test', and 'dunnTest'. Finally, binary logistic regression was used to model differences in concern about HABs (binary-converted Likert scale response data: agree, strongly agree = 1; neutral, disagree, and strongly disagree = 0) between predictors using the R package 'glm' with binomial family logit link function. A number of demographic and shellfish harvesting characteristics (gender, income, education, ethnicity, distance to harvest shellfish, and knowing someone who got sick from eating recreationally harvested shellfish) were included as predictors in the full model, and the best model was chosen using the Akaike Information Criterion (AIC). Coefficients from this model were exponentiated and reported in relation to the 'agree' outcome to present the odds in a more interpretable format.

Exposure assessment

We calculated estimates of DA exposure using average seasonal *Pseudo-nitzschia* spp. abundances, participant harvest frequency, season of harvest, and serving size, combined with information from the literature about *Pseudo-nitzschia* spp. occurrence and shellfish DA contamination. Reports from the literature (Bates et al., 2018; Busse et al., 2006; Du et al., 2016; McCabe et al., 2016; Trainer et al., 2007, 2012; Trainer and

Suddleson, 2005) that included both *Pseudo-nitzschia* spp. abundance and DA concentration in shellfish tissue (mostly from *Siliqua patula,* some data from *Mytilus* spp.*, Venerupis philipinarum*, *Crassostrea gigas, Protothaca staminea*, *Metacarcinus magister*) were used to build one model to estimate shellfish tissue concentration from the natural logarithm of *Pseudo-nitzschia* spp. abundance data ($y = 3.31 \ln(x) – 24.77$, $R^2 = 0.11$). The logarithmic model captured more variation than a linear model, and illustrates the rapid increase in DA observed at the onset of a bloom and the asymptotic nature of DA accumulation in shellfish. For estimates to be included in the model the study was required to have taken place on the U.S. West Coast, and have *Pseudo-nitzschia* spp. and DA concentration in shellfish measurements that were collected within one day of each other during a period of increasing *Pseudo-nitzschia* spp. abundance not following a previous DA event (n = 33). These criteria were meant to ensure that regional *Pseudo-nitzschia* species were used in our estimates, and to include only samples for which shellfish DA concentrations reflected *Pseudo-nitzschia* bloom formation, rather than an accumulation over time or from a previous bloom event. Because seasonal harvest frequency and serving size were collected as discrete ranges in common units to make reporting easier for participants, we converted responses to estimated continuous values and SI unit responses for the purpose of DA exposure calculations (e.g., for harvests per season: 1 harvest/month = 3 harvests/season, >1 harvest/month = 4 harvests/season; or serving size: <3 oz = 85.1 g, 3-8 oz = 155.9 g, >8 oz = 226.8 g). Body weight used in exposure calculation was the national average for reported gender (Fryar et al., 2018). These assumptions made on harvester responses may increase the central tendency of our estimates. For example, if a respondent's true number of harvests is larger than 4 per season or serving size falls outside the conservative estimates we used based on binned responses, we may underestimate true exposure. Therefore, our estimates likely underestimate DA exposure for subsistence-style harvesters.

We calculated an average daily dose (ADD) of DA for the summer season:

$$ADD = Concentration \ x \ Intake \ Rate \ / \ Body \ Weight \ x \ Averaging \ Time \qquad (eq. 1)$$

Or more specifically,

$$ADD = C_{medium} \ x \ IngR \ x \ EF \ x \ ED \ / \ BW \ x \ AT \qquad (eq. 2)$$

where **ADD** = Average daily potential dose of DA (mg kg^{-1} d^{-1}), $\mathbf{C_{medium}}$ = Concentration of domoic acid in shellfish tissue (mg g^{-1}), **IngR** = Ingestion rate (g shellfish d^{-1}), **EF** = Exposure frequency (number of days recreational shellfish eaten per year), **ED** = Exposure duration (0.25 year), **BW** = Body weight (kg), **AT** = Averaging time (91.25 days in 3-month summer season) as in Stuchal et al. (2020). The hazard quotient (HQ) was then calculated to evaluate the potential for health hazards associated with exposure to DA using available health guidelines (Regulatory Action Limit for shellfish, Reference Dose). The HQ was obtained by dividing the exposure dose by the health guideline according to:

$$\mathrm{HQ = (D /RfD)} \qquad \text{(eq. 3)}$$

HQ = Hazard Quotient, **D** = Exposure Dose (mg kg^{-1} d^{-1}), **RfD** = Reference Dose (0.075 mg kg^{-1} bw d^{-1} based on regulatory limit of 20 ppm in shellfish (Kumar et al., 2009)), where

$$D = C_{medium} \ x \ (IngR \ / \ BW) \qquad \text{(eq. 4)}$$

DA exposure and management preference drivers

Tests for difference in calculated exposure to biotoxins among recreational shellfish harvesters were conducted using non-parametric tests (Kruskal Wallis or Wilcoxon's signed rank test, depending on the number of independent variables) based on their years of experience, their proximity to harvest sites, experience with getting sick from eating recreationally harvested shellfish, and demographic factors. Shellfish harvesters' preferences for three different management strategies and the associated demographic and fishery-use drivers of these preferences were examined using binary logistic regression on binary-converted Likert data. Participants were asked to rate their level of agreement with statements such as "[Intervention strategy] is/are important to me to make decisions about harvesting shellfish". They were asked about HAB monitoring programs, publicly accessible educational resources, and fishery closures as potential strategies. Predictors in the full model included gender, income, education, ethnicity, distance to harvest shellfish, and knowing someone who got sick from eating recreationally harvested shellfish.

Results

Phytoplankton community

Over the monitoring period, 136 phytoplankton genera were observed across all sites and sampling times from phyla Bacillariophyta, Charophyta, Ciliophora, Chlorophyta, Cryptophyta, Cyanophyta, Dinoflagellata, Euglenophyta, Ochrophyta, as well as two taxonomically amalgamated groups "small flagellates" and "zooplankton" (Fig. 2). Total estimated sample biovolume ranged from 1.52 x 10^4 μm^3 mL^{-1} to 2.33 x 10^8 μm^3 mL^{-1}, with a median of 2.25 x 10^5 μm^3 mL^{-1}. Estimates of algal biomass tended to be higher in the summer (June, July, August), with some outliers. The most common and abundant toxigenic taxa were *Pseudo-nitzschia* spp., *Microcystis* spp., and *Dolichospermum planctonicum* (Fig. 2). The latter two reached highest concentrations only at the freshwater Tenmile Lake site, though *Microcystis* spp. were occasionally observed in low concentrations in the estuarine sites. *Dolichospermum* spp. reached very high concentrations (>10^7 cells L^{-1}) later in summer both years of the monitoring program. *Pseudo-nitzschia* spp. was present in most estuarine samples collected at low concentrations, but peaked in June and July of 2021 (4.2 x 10^5 cells L^{-1}, recorded on July 27, 2021) throughout the Coos and South Slough estuaries.

Algal toxins

Particulate DA was detected in 83% (33 of the 40) samples for which it was measured, with a maximum of 327 ng L^{-1} and a mean concentration of 31.8 ng L^{-1} (Table 1). Only one sample from the BLM Boat Launch site in the Coos Estuary in June 2021 exceeded the commonly used action limit (200 ng L^{-1}), where shellfish are known to accumulate notable amounts of toxin.

Total saxitoxins were detected in 13% (6 of the 46) samples in which it was measured, with a maximum of 0.198 μg L^{-1} and a mean of 0.005 μg L^{-1}. The highest concentration observed was at the freshwater Tenmile Lake site in July 2022, but this was still below Oregon's drinking water limit of 1 $\mu g\ L^{-1}$ (Farrer et al., 2015) and most of the other detections were low at estuarine sites.

Total microcystins were detected in 79% (11 of the 14) samples tested for this toxin, with a maximum of 3.392 $\mu g\ L^{-1}$ and a mean of 0.443 $\mu g\ L^{-1}$. The highest concentrations were measured in samples from the freshwater site, and three samples were at or above the drinking water limit (1 $\mu g\ L^{-1}$) (Farrer et al., 2015). Only low concentrations were measured in upper estuarine sites.

Questionnaire respondent demographics

A total of 140 shellfish harvesters participated in the survey, with 101 in-person and 39 online respondents. For the in-person questionnaire, the response rate was 91%. Given that the online survey was advertised to CTCLUSI enrolled members and the Friends of South Slough group, only a small percentage (~3%) of those who theoretically received the advertisement responded. However, many of the non-respondents might not have considered themselves shellfish harvesters or had already responded in person, and opted out of the survey for one of these reasons. Reported age ranged from 21 to 86 years old, and the age distribution of respondents skewed older (Fig. 3). More males (n=82) took the survey than females (n=56). Participants with White ethnicity (n=84, 60%) made up the largest proportion of respondents, followed by Native American or Native American and White (n=28, 20%), Asian (n=12, 8.5%), Mixed Race (n=5, 3.5%), Latino (n=3, 2%), Other (n=2, 1%), and Native Hawaiian/Pacific Islander (n=1, 1%). This differs somewhat from Coos County ethnicity data (White = 90.1%, Latino = 7.2%, Mixed Race = 4.6%, Native American = 3%, Asian = 1.3%, African American = 0.7%, Native Hawaiian/Pacific Islander = 0.3%) (United States Census Bureau, 2022). Most respondents' yearly household income was in the $25,000 - $100,000 range (n= 68, 49%), followed by respondents with income above $100,000 (n=34, 24%), respondents with household income less than $25,000 (n=21, 15%), and 12% of respondents chose not to report their income (the highest non-response rate of any demographic question). Median household income for the county is $52,548 (2021 USD) (United States Census Bureau, 2022). Educational attainment ranged from some high school to a graduate degree, with most respondents reporting lower educational attainment than a Bachelor's degree (n = 84), and 39% (n=55) reporting a Bachelor's degree or higher (20% for Coos County). Distance traveled to harvest shellfish ranged from 0 – 4828 km (0 – 3000 miles) with a median of 97 km (60 miles), and 32% reporting a distance of less than 32 km (20 miles) (n=138).

Shellfish harvest, seasonality, consumption

Survey participants reported a wide range of shellfish harvesting and consumption practices in terms of frequency and seasonality of harvest, species targeted, the source of consumed shellfish, and serving size. The number of reported shellfish harvests ranged from 0-166 harvests with a median of 5 harvests in the last year (n = 140). Participants reported the highest harvesting frequency in the summer, as 89% harvested at least once during summer: 57% reported 1 harvest in summer, and an additional 32% reported harvesting shellfish at least once per month during the summer. Frequency of harvests in other seasons was lower, and the percentage reporting at least one harvest per season was 38%, 31%, and 46% for the fall, winter, and spring, respectively (Fig. 4).

While most non-commercial shellfish consumed by participants was obtained from their own harvests (65%), participants also ate shellfish that was given to them as a gift from friends or family (22%), obtained through a trade or barter (6%), or eaten at tribal events (3%) (31% total not self-harvested) (n=136). The category of bay clams (includes gaper, littleneck, butter clams, and cockles) was the most frequently harvested and eaten, followed by crab (includes Dungeness, red rock, and green crab), razor clams, mussels, snails, then other shellfish. Participants consumed non-commercial bay clams from 0 – 44 times in the last year, with a median of 1 time. Two individuals reported consuming recreationally harvested clams 80 or more times in the last year, but these were treated as erroneous outliers based on expert knowledge of subsistence harvesting practices (Russell, *personal communication,* 2023). The number of occurrences consuming non-commercial crab ranged from 0-30, with a median of 2 times consumed in the last year. The typical serving size of non-commercial shellfish tissue ranged from 0 g (3% of participants,), less than 85 g (3oz) (9%), 85 – 227 g (3-8 oz) (47%), to more than 227 g (8 oz) (42%) (n = 139).

Respondent knowledge and perceptions of HAB risk

Of the survey respondents, 81% (n=139) were aware of HABs and their connection to seafood safety prior to taking the survey. Most rated their personal risk of consuming algal-contaminated shellfish as “low” (73%, n= 139). Respondents who rated themselves as “medium” risk (as opposed to “low” or “high”) were significantly younger ($p = 0.01$). We also observed that Native Americans were five times more likely to rate their personal risk as “high” ($p = 0.01$) compared to other ethnicity groups, and people with less than a Bachelor’s degree were twelve times more likely to respond “I don’t know” ($p = 0.012$). Participants were asked to rate their level of agreement with the statement “I am concerned about harmful algal blooms”, and 64% agreed or strongly agreed with this statement (Fig. 5). The best binary logistic regression model for predicting concern about HABs only included gender (pseudo $R^2 = 0.14$, $p=0.044$), and the odds of agreeing with this statement were 0.355 times lower for males than females.

Estimates of harvester DA exposure

Concentration of DA in shellfish was the highest in summer, commensurate with elevated *Pseudo-nitzschia* spp. abundance and increased harvesting effort in this season, suggesting that the summer season carried the highest risk of DA exposure. Estimated acute exposure to DA in the summer ranged from 0 – 0.043 mg kg^{-1} body weight (bw) per meal of shellfish, with a mean of 0.028 mg kg^{-1} bw $meal^{-1}$. Average daily dose for the summer season ranged from 0 – 0.0019 mg kg^{-1} d^{-1} (Table 2). We found that males had 0.006 mg kg^{-1} bw $meal^{-1}$ higher acute DA exposure on average than females ($p = 0.028$). Acute DA exposure was also somewhat lower (difference of averages = 0.004 mg kg^{-1} bw $meal^{-1}$) among participants reporting household income greater than $100,000 than either of the lower income groups and among those reporting a college degree or higher (difference of averages = 0.003 mg kg^{-1} bw $meal^{-1}$) (p = 0.076, p = 0.031 for income and education, respectively). There was also a slight negative correlation between average daily dose and distance to harvest shellfish (r = -0.085; p = 0.18, n = 138) and a positive correlation between total DA exposure and years of experience harvesting shellfish (r = 0.16; p = 0.012, n = 135). We did not observe any significant differences in DA exposure between ethnicity, or any significant correlation between DA exposure and age. It should be noted that only 6% (n=139) of respondents reported ever experiencing illness after eating shellfish harvested recreationally, and the possible type of illness (i.e., response to marine biotoxin, bacterial contamination, shellfish allergies, etc.) is unknown.

Respondent preference for intervention strategies

We observed that all intervention strategies had similarly high levels of support: 90% agreement with fishery closures (n=127), 86% agreement with monitoring programs (n=139), 82% agreement with education resources (n=126). The binary regression for the education intervention statement (pseudo R^2 =0.22) revealed that education (less than Bachelor’s degree, p=0.089) and gender (male, p=0.14) were the most important drivers, indicating that the odds of agreeing with the importance of educational resources were 0.303 times lower for people with educational attainment lower than a Bachelor’s degree, and 2.36 times higher for males than females. For the monitoring intervention, ethnicity (Native American p=0.123, ethnicity White p=0.074), education (less than Bachelor’s degree p=0.099), and gender (male p=0.45) were included in the final model (pseudo R^2 = 0.46). The odds of agreeing with the monitoring intervention statement were 6.9 times higher for Native Americans and 3.8 times higher for Whites compared to other ethnicity groups. The odds of agreeing with this intervention statement were 0.2 times lower for people with less than a Bachelor's degree compared to higher education levels, and 4.1 times higher for males compared to females. For the closure intervention regression, no drivers from the full model were included in the final model.

Discussion

HAB monitoring

To support assessment of biotoxin exposure risk among shellfish harvesters, we investigated spatial and temporal variation in algal community composition, as well as potential environmental drivers in the Coos and South Slough Estuaries. Understanding and communicating these spatiotemporal patterns and causal factors has also been found to be key to increasing shellfish harvesters' trust in HAB management decisions (Ritzman et al., 2018).

Spatiotemporal patterns. Observations of estuarine phytoplankton were consistent with previous work; composition was largely driven by coastal advection into the South Slough and Coos estuaries with some variation along the continuum from coast to upper estuary (Hughes, 1997; Ohana-Richardson, 2007; Roegner and Shanks, 2001; Torres and Shanks, 2018). For example, in the two upper South Slough sites (Elliot and Winchester creeks), mixotrophic taxa (Cryptophytes, Ciliophora, Euglenophytes, Cyanophytes and flagellates) were observed alongside marine plankton. Toxigenic marine taxa were most common in the summer, and plankton community composition varied more between seasons than over the course of a tidal cycle (determined by NMDS, not shown). The highest overall phytoplankton abundances during the monitoring period were observed at low- to mid-estuarine sites, which had a higher proportion of pennate and tychopelagic diatoms. Sites closest to the coast and samples taken during spring and summer tended to have high numbers of centric and chain-forming diatoms particularly in spring and summer, characteristic of upwelling environments (Lassiter et al., 2006). It has previously been noted that to adequately capture HAB taxa in tidally-influenced habitats, both offshore and nearshore monitoring sites should be established in order to keep fishery resources safe (Frolov et al. 2013); this study highlights the importance of this rationale.

Drivers of toxigenic plankton. Using regression tree analysis, we found that the most important and only significant predictor of toxigenic taxa in the estuarine sites was 14-d rolling averaged wind speed (Fig. 7). Wind speed can serve as an indicator of upwelling/downwelling conditions, with stronger prevailing winds more common in the summers when upwelling takes place (Schrager et al., 2017). Upwelling can strongly impact the coastal phytoplankton community composition, most likely on a time scale of days to weeks, and has been documented to increase *Pseudo-nitzschia* spp. abundance (Du et al., 2016; Trainer et al., 2012).

Two additional predictors of toxigenic taxa were related to winds: hourly rolling averaged wind speed, which may be an indicator of wind-driven mixing in the estuary causing resuspension of phytoplankton; and turbidity (14-d rolling average), which can increase following wind mixing. Notably, the more turbid upper South Slough sites typically had plankton communities less influenced by marine organisms, and therefore had lower abundance of *Pseudo-nitzschia* spp. Turbidity can also increase drastically after precipitation events, which can flush out plankton in the estuary (Schrager et al., 2017). There were notable decreases in phytoplankton abundance after precipitation events in the present study. *Pseudo-nitzschia* spp. abundances at a nearby coastal site monitored by ODFW (*personal communication* Hunter, 2023) were comparable (same order of magnitude) to our observations, following the same peak in abundance in the summer of 2021, and were more similar when collected at the same point in the tidal cycle and closely matched in time. However, abundances in the estuary and at Sunset Bay tended to be somewhat higher than at this open coastal site, perhaps due to longer water residence times in the cove of Sunset Bay and slower moving waters of the estuary (Ralston et al., 2015).

The highest densities of *Pseudo-nitzschia* spp. during the monitoring period occurred during the summer of 2021, a year when salinity and water temperature in the lower estuary were higher than the long-term average (over the last 9 to 25 years, depending on the site). The area also experienced longer timeframes of strong northerly winds typical of upwelling during 2021 (Fig. 6). However, salinity was not a significant driver in the regression tree analysis. This is surprising given that HAB taxa are thought to be transported into the estuary from higher salinity coastal waters (Cziesla, 1999; Roegner and Shanks, 2001). This may be due to the limited number of samples collected at low tide, or the fact that plankton transported into the estuary are not growing in response to local conditions, but rather grow in response to the conditions of the coastal ocean. Although elevated temperature has been documented as a potential driver of *Pseudo-nitzschia* spp. blooms (Ohana-Richardson, 2007; Trainer et al., 2020), our regression tree analysis did not implicate temperature as an important driver in the South Slough or Coos estuaries over the range of temperatures observed during the monitoring period.

Toxin production. Unlike abundance, we found that *Pseudo-nitzschia* spp. toxin production tended to be higher at the least saline estuarine site (BLM Boat Launch in the Coos Estuary, Table 1), potentially because of a response due to some aspect of interaction with freshwater at this mesohaline site (i.e., changing nutrient availability such as organic nutrient availability, silicate or phosphorus limitation; high concentrations of trace metals like iron or copper; or higher bacterial concentration) (Lelong et al., 2012; Moreno et al., 2022; Thessen et al., 2005). Other triggers of DA production have been proposed (high salinity, high pH), but are not likely given the low salinity of the site in question (Lelong et al., 2012). A coastal site (e.g., Sunset Bay, Boathouse) likely provides a good indication of incoming marine toxigenic taxa, which were more commonly observed in the estuaries than freshwater or estuarine toxigenic taxa during this monitoring period. However, it may be useful to collect additional toxin samples from a mid to upper estuarine site to check for elevated toxin production by marine taxa (e.g., *Pseudo-nitzschia* spp., *Alexandrium* spp.) in response to freshwater inputs. Although only observed in low abundance, it may also be prudent to periodically sample mid to upper estuary sites to check for estuarine- and freshwater-associated toxin producers (e.g., *Microcystis* spp., *Gymnodinium* spp.).

Risk assessment and risk perception

Toxigenic taxa were most commonly observed during the summer months, presenting the highest risk of HAB-related illness during this time of year. This is compounded by the fact that survey participants reported significantly higher intensity of shellfish harvest during the summer months (Fig. 4). During the monitoring period, *Pseudo-nitzschia* spp. and associated DA presented the greatest algal toxin exposure risk to shellfish harvesters, though DA levels in shellfish remained low enough for fisheries to stay open. Our estimates indicate that the maximum DA exposure level observed among survey participants was slightly more than half of the regulatory limit (hazard quotient = 0.58) (Petroff et al., 2022). This is consistent with the fact that all but one of our DA measurements were below the alert level considered to be the point at which toxins significantly accumulate in shellfish tissues. Other surveys of shellfish consumers have found that a low proportion (5-7%) ingest DA above 0.075 mg kg^{-1} body weight (Andjelkovic et al., 2012; Ferriss et al., 2017), but these may have taken place during periods that spanned higher *Pseudo-nitzschia* spp. activity. Based on long-term fishery closure information, the monitoring period is only somewhat representative of DA exposure for the Coos Bay area, as fishery closures due to elevated DA are not uncommon (State of Oregon, 2021).

There are several facets of uncertainty in our DA exposure estimates at the level of temporal and tidal variation in *Pseudo-nitzschia* spp. abundance, the variation in DA production by *Pseudo-nitzschia* spp., shellfish uptake and depuration rates, as well as uncertainty in the timing of participants' harvests within each season. Furthermore, uptake and depuration rates can vary by shellfish species and during spawning, which typically occurs in spring and summer. Literature values relating the concentration of *Pseudo-nitzschia* spp. cells to concentration of DA in shellfish tissue varied by as much as two orders of magnitude. Given the relatively low R^2 value ($R^2 = 0.11$), the uncertainty of our model is high. The geographically- and species-specific nature of this model also makes comparison with other studies limited, but Trainer et al. (2009) found no significant relationship between *Pseudo-nitzschia* spp. abundance and particulate DA in the Juan de Fuca Eddy. Another study of both natural and cultured *Pseudo-nitzschia* sp. also used logarithmic models to predict DA with R^2 ranging from 0.60 – 0.80, based on predictors which included *Pseudo-nitzschia* spp. cell counts as well as environmental conditions (Blum et al., 2006).

Furthermore, our estimates of DA are likely high, given that *Pseudo-nitzschia* spp. abundances (from which estimates of DA concentration in tissue were made) were measured at high tide when abundances of this marine diatom are typically highest. Tidal action provides a diurnal flushing of *Pseudo-nitzschia* spp. that may reduce its toxic effect on resident estuarine shellfish in comparison to our estimates (Ajani et al., 2013). This coarse estimate could be improved by directly measuring DA in shellfish tissue, especially in conjunction with shellfish harvester surveys. Although we believe the acute exposure to DA was low overall during this study period, it is also important to consider the potential for DA health impacts from chronic, low-level exposure, which are poorly understood (Petroff et al., 2022; Stuchal et al., 2020).

While there was a large degree of uncertainty in estimating shellfish DA from *Pseudo-nitzschia* spp. abundance, we have more certainty regarding behavioral aspects of exposure. Therefore, comparisons of DA exposure among demographic and fishery use groups can provide useful information about relative exposure risks among shellfish harvesters. There are known limitations in accuracy of answers to retrospective survey questions (Pearson and Ross, 1992), though we wouldn't expect this to differ between demographic groups. Male participants showed higher acute DA exposure levels, even though estimates were corrected for an average higher male body weight. We also found that respondents who reported having a Bachelor's degree or higher or reported household income greater than $100,000 had lower DA exposure than lower educational attainment and income groups. This may be an indication that individuals with higher socio-economic status treat shellfish harvest as a sporadic recreational activity, whereas shellfish harvest may be a more common recreational or subsistence activity for lower socio-economic status individuals (Beaumais and Appéré, 2010; Branch et al., 2002). Respondents who lived closer to their shellfish harvesting site or had more years of experience harvesting shellfish tended to have higher DA exposures. This likely reflects factors that influence overall frequency of shellfish harvest. No other demographic groupings appeared to have bearing on DA exposure, despite our expectation that Native American participants would have elevated DA exposure due to higher shellfish consumption rates associated with cultural activities (Grattan et al., 2016).

Our results suggest that there was good alignment in terms of overall perception of risk compared with actual risk, given that most people rated their risk of consuming algal toxin contaminated shellfish as low, and our toxin testing and DA exposure estimates suggest most people were at low risk during the study period. However, there was divergence in perceived risk and relative risk of DA exposure for some demographic groups. Notably, males reported that they were concerned about HABs significantly less than females which is consistent with other research (Roberts et al., 2016), but had higher DA exposure according to our estimates. In contrast, those reporting Native American ethnicity rated their risk of consuming algal toxin contaminated shellfish as “high” more often, but our estimates did not indicate any difference in DA exposure between ethnicity groups. It should be noted that our tests between ethnicity groups are somewhat underpowered because of the unequal sample size between groups. However, this result is similar to previous findings and may reflect greater awareness and concern for environmental contaminants in general (Roberts et al., 2016) or because of tribal outreach about HABs, as well as more cautious shellfish harvesting (i.e., proportionally less harvesting in summer than other ethnicity groups). Overall, 81% of respondents in our study were aware of HABs, which is a lower proportion than those reporting awareness in a study about Florida red tide (Nierenberg et al., 2010), but higher than awareness reported in other studies of awareness of marine HABs (Aditya et al., 2013; Bechard and Lang, 2023). This high level of awareness is surprising, considering that marine HABs in the Pacific Northwest are typically not visible to the naked eye; thus, awareness relies on outreach and education. Similar surveys of stakeholders’ perceptions of HABs in other coastal communities have noted that such an assessment is not likely to apply in all coastal communities. The Coos Bay area has a geographical, historical, and economic context that differs from previously studied communities in the Pacific Northwest (Roberts et al., 2016), and different dominant HAB taxa than other communities assessed for awareness and risk perception nationwide (Kirkpatrick et al., 2014; Nierenberg et al., 2010).

Intervention strategies

Shellfish harvesters overall showed broad support for all HAB intervention strategies asked about in the questionnaire. Despite our expectation that fishery closures would be less supported than the education and monitoring strategies, we found that harvesters most strongly supported the use of fishery closures to prevent HAB-related illness among recreational harvesters over education and monitoring by a small margin. Although there was widespread support for these measures, 36% of harvesters reported that they did not use any information about HABs when deciding whether to harvest shellfish, which could result in harvesting during a HAB event or fishery closure. This indicates that there are likely shellfish harvesters who are aware of HABs and support intervention measures, but do not make use of the available resources. This segment of the population represents an area for improvement in the adoption of HAB monitoring and fishery closure resources. Among those who did use information about HABs, the ODFW website (33%), news (e.g., paper, online, TV) (14%), and word of mouth (13%) were reported as the most commonly used sources. While the state-run website (i.e., ODFW) appears to be the most trusted resource, there is also a significant contribution to dissemination of information happening at the local level, providing support for locally managed monitoring and outreach programs.

These results suggest that local outreach efforts have the potential to increase use of publicly available information about HABs and shellfish harvesting safety. Our findings indicate that the summer season is the optimal time for spreading awareness, since DA exposure was highest in the summer months and is when people are harvesting more frequently. In addition, outreach could aim to target males who had a notable gap in concern about HABs compared to estimated DA exposure. Our results are also suggestive that Native American respondents were more concerned about HABs overall, but were not more vulnerable to DA exposure based on our estimates. This may be an indication of Indigenous knowledge and/or tribal outreach that has been successful, which could potentially serve as a model for local dissemination of information. We also found that 31% of harvesters reported eating non-commercial shellfish that they did not harvest themselves, but received as a gift from friends or family, traded or bartered for, or got from tribal events. Outreach efforts could potentially make use of prosocial behavior (Batson and Powell, 2003) to encourage adoption of HAB information use. In addition to outreach efforts, our harvester behavior data suggests that future HAB monitoring should be prioritized in the summer season. Furthermore, adaptive sampling based on knowledge of community events that result in elevated harvesting activity (e.g., tribal events, local tourism activities) may provide information about HABs when it is most needed.

Conclusion

This study combines monitoring of HABs and their environmental drivers with a risk assessment of shellfish harvester algal toxin exposure using a socio-ecological lens to identify patterns of risk. The Coos Bay area has not previously had a locally-focused monitoring program, and this research provides greater spatial and temporal coverage of HAB monitoring in the area than previous studies. In addition, this assessment of DA exposure at the seasonal scale provides temporally-resolved risk estimates, which may help guide timely management actions. The identification of mismatch between actual and perceived risk of DA exposure among harvesters also provides direction for outreach efforts to target vulnerable demographics. Finally, this study allows discernment of gaps in the utilization of existing informational resources available to harvesters, and provides guidance on effectiveness of management strategies based on harvester preferences.

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Table 1. Algal toxin concentrations by site and date. Toxin concentrations (technical replicate average) are listed as domoic acid (ng L^{-1}) top, saxitoxins (μg L^{-1}) middle, microcystins (μg L^{-1}) bottom. Measurements above alert levels shown in bold, '-' indicates not measured. Concentrations below detection limit listed as 0. Alert levels = 200 ng L^{-1} for domoic acid, 1 μg L^{-1} for saxitoxin and microcystin. Drinking water standard used as fresh and seawater saxitoxin and microcystin guidelines.

Site	5/25/21	6/21/21	7/27/21	8/22/21	9/17/21	6/14/22	7/12/22	8/8/22
Tenmile Lake	-	-	-	-	-	-	-	-
	0	-	0	0	0	0	0.20	-
	0.04	-	**1.00**	**1.04**	**3.39**	0.23	0.32	-
Winch. Creek	40.1	37.1	-	-	-	0	0	-
	0	0	-	-	-	0	0	-
	-	-	-	-	-	-	0.03	0.05
Elliot Creek	6.85	32.6	-	-	-	0	0	-
	0	0	-	-	-	0	0	-
	-	-	-	-	-	-	0.03	0.03
Val. Island	8.80	21.7	-	-	28.9	0	0.42	-
	0	0	-	-	0	0	0	-
	-	-	-	-	-	-	0.03	0
BLM Launch	43.4	**327**	33.8	80.3	21.2	-	-	-
	0	0.03	0	0	0	-	-	-
	-	-	-	-	-	-	-	-
Fossil Point	16.7	30.3	33.6	75.4	18.3	-	-	-
	0	0	0	0	0	-	-	-
	-	-	-	-	-	-	-	-
Charl. Bridge	44.5	26.7	13.6	33.1	11.5	0	0	-
	0	0	0	0	0	0	0	-
	-	-	-	-	-	-	0	0
Boat-house	12.2	31.6	27.9	57.5	26.1	-	-	-
	0	0	0	0	0	-	-	-
	-	-	-	-	-	-	-	-
Sunset Bay	38.5	45.2	28.5	8.75	9.58	-	-	-

	0	0	0	0	0	-	-	-
	-	-	-	-	-	-	-	-

Table 2. Domoic Acid Exposure in Shellfish Harvesters Descriptive Statistics

	Minimum (Non-consumer/consumer)	Maximum	Mean
Average Daily Dose (mg kg^{-1} d^{-1}) (n = 126)	0 / 0.00015	0.0019	0.00058
Exposure Dose (mg kg^{-1} d^{-1}) (n = 139)	0 / 0.014	0.043	0.028
Hazard Quotient (n=139)	0 / 0.19	0.58	0.37

Fig. 1. Map of HAB monitoring sites. Tenmile Lake site is freshwater, all others have some tidal influence. Winchester, Elliot Creek, Valino Island, and Charleston sites correspond to SWMP water quality sensors, meteorological sensor is just upstream from Winchester site at Tom’s Creek station. Coastal sites = Boathouse, Sunset Bay, Coos Estuary sites = BLM Launch, Fossil Point, South Slough Estuary sites = Charleston, Valino Island, Elliot Creek, Winchester.

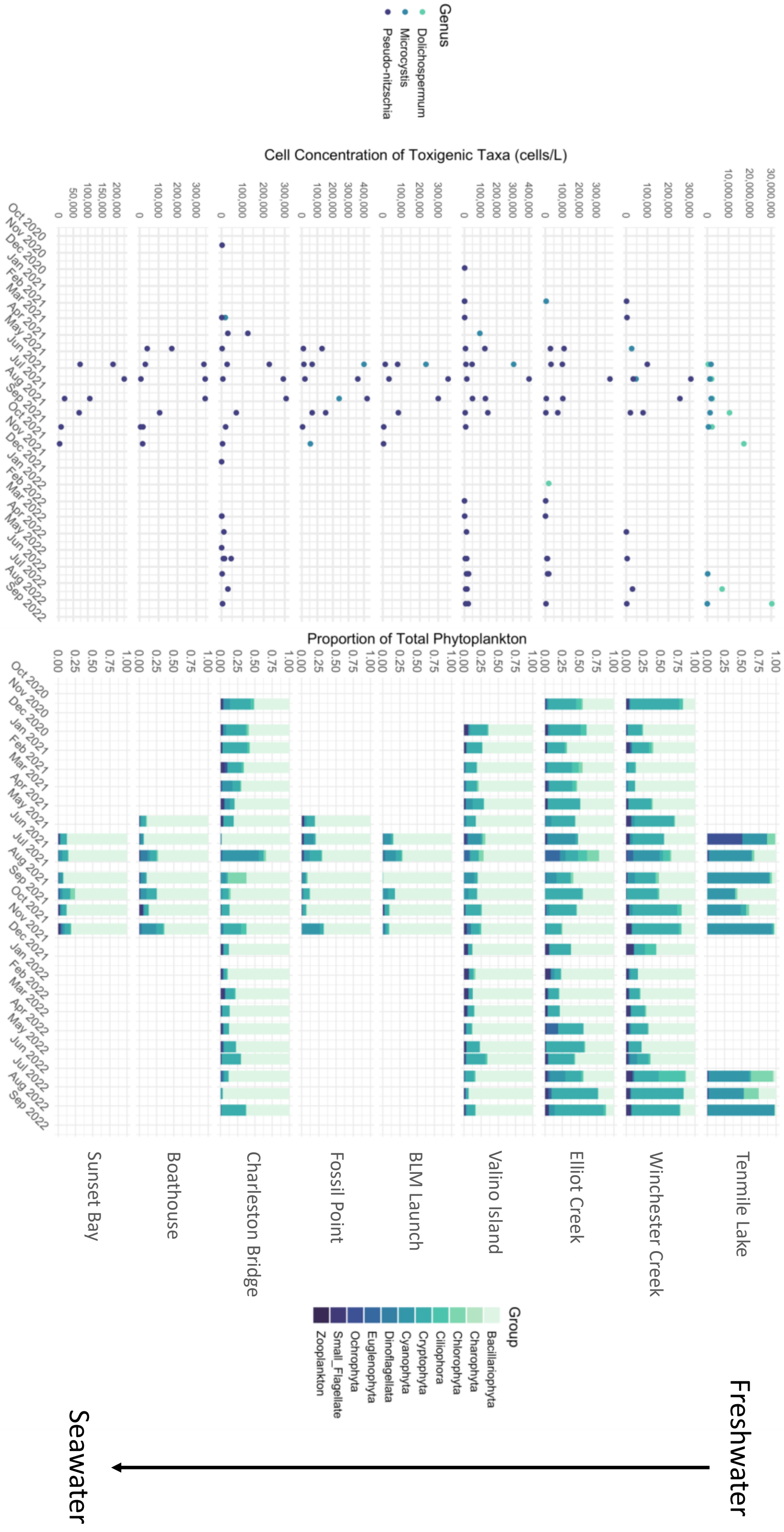

Genus
Dolichospermum
Microcystis
Pseudo-nitzschia
Cell Concentration of Toxigenic Taxa (cells/L)
Proportion of Total Phytoplankton
0.00
0.25
0.50
0.75
1.00
Oct 2020
Nov 2020
Dec 2020
Jan 2021
Feb 2021
Mar 2021
Apr 2021
May 2021
Jun 2021
Jul 2021
Aug 2021
Sep 2021
Oct 2021
Nov 2021
Dec 2021
Jan 2022
Feb 2022
Mar 2022
Apr 2022
May 2022
Jun 2022
Jul 2022
Aug 2022
Sep 2022
Sunset Bay
Boathouse
Charleston Bridge
Fossil Point
BLM Launch
Valino Island
Elliot Creek
Winchester Creek
Tenmile Lake
Group
Bacillariophyta
Charophyta
Chlorophyta
Ciliophora
Cryptophyta
Cyanophyta
Dinoflagellata
Euglenophyta
Ochrophyta
Small_Flagellate
Zooplankton
Freshwater
Seawater

Fig. 2. Phytoplankton monitoring results by site (arranged in order of average salinity from lowest to highest) and date (from October 2020 to August 2022). Cell concentrations (cells L^{-1}) of most dominant toxigenic genera shown in left panel. Relative abundance of cells from each taxonomic group shown as a bar plot in the right panel. Dates with no bar indicate that no sample was taken.

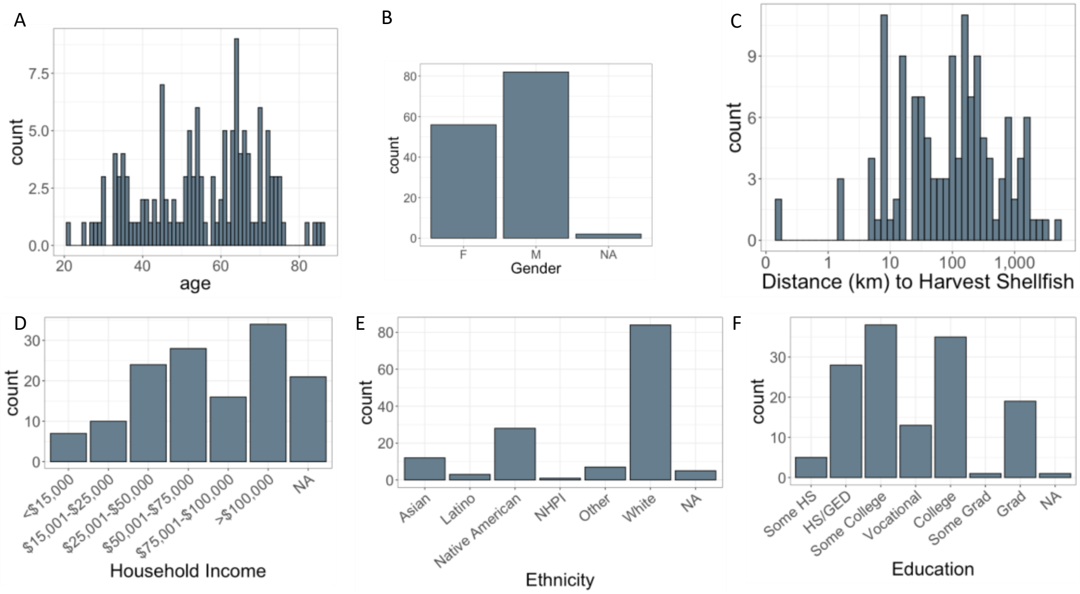


Fig. 3. Count of questionnaire responses from top left to bottom right by: (A) age, (B) gender, (C) distance (in kilometers) to harvest shellfish, (D) household income, (E) ethnicity, (F) education.

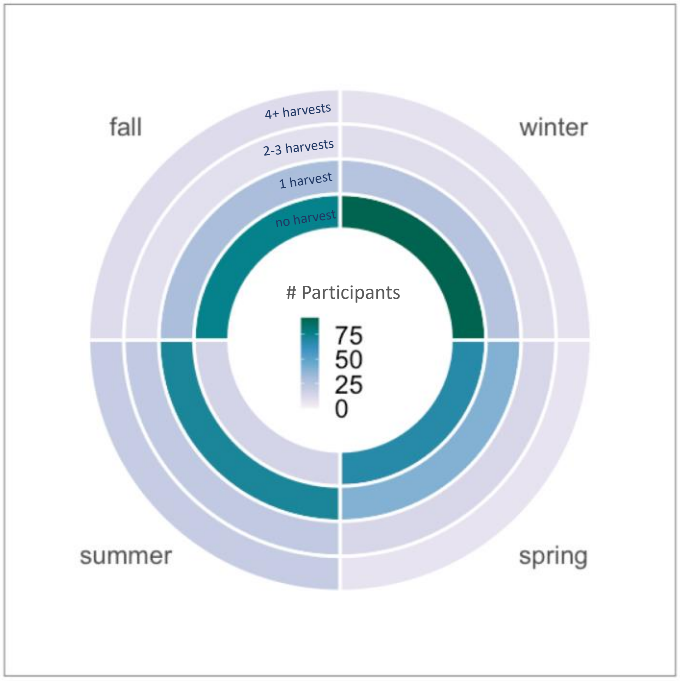


Fig. 4. Circular heat map of intensity of shellfish harvest divided by the four seasons. The innermost ring indicates the response of no shellfish harvest for a given season. Progressing outward, the rings represent 0 harvests per season, 1 harvest per season, 2-3 harvests per season, and 4 or more harvests per season. The color of the field corresponds to the number of participants who responded with a given frequency for each season.

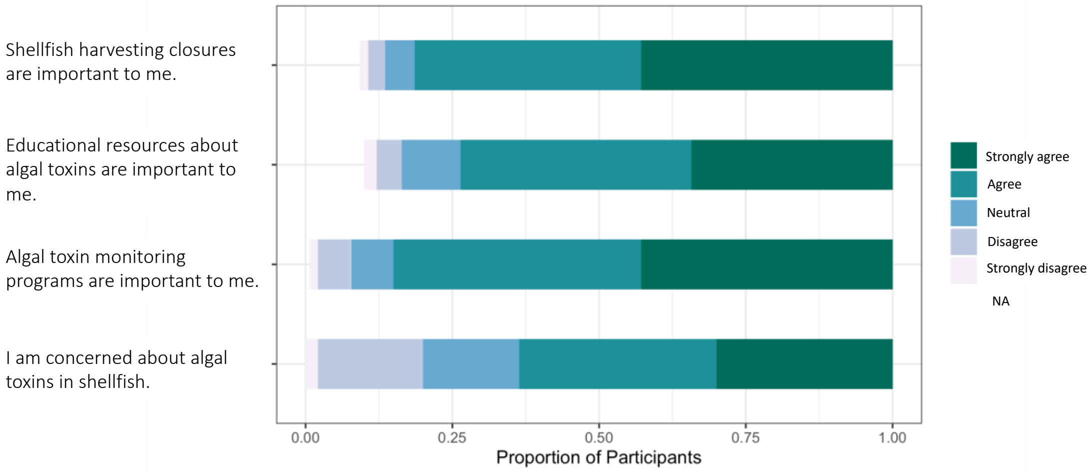


Fig. 5. Proportion of participants responding to each statement on the left, color-coded by their level of agreement with the statement.

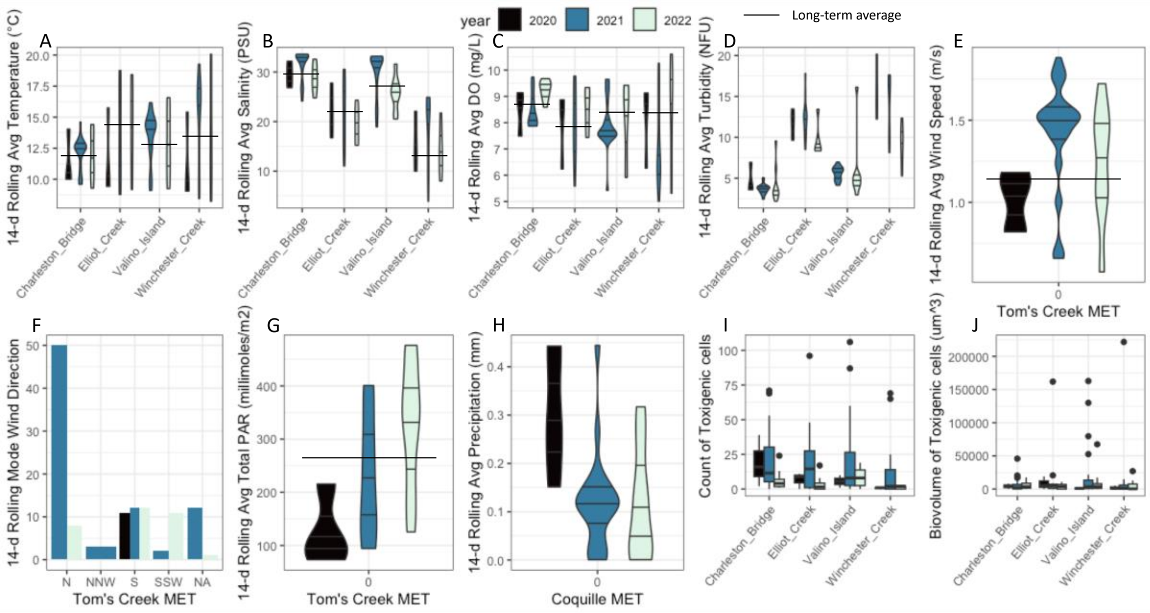


Fig. 6. Violin, boxplot, or bar chart of 14-day rolling averages leading up to the phytoplankton sampling window for each environmental parameter measured from top left to bottom right: A) temperature (°C), B) salinity (PSU), C) DO (mg L^{-1}), D) turbidity (NFU), E) wind speed (m s^{-1}), F) wind direction (rolling mode for categorical variable), G) total PAR (mmoles m^{-2}), H) precipitation (mm). Bottom right two plots indicate I) count and J) biovolume of toxigenic cells (um^3). Colors indicate year, and where available, a black line representing a long-term average for conditions at each site was overlaid. Long-term average is from the last 19 years for Charleston Bridge, 9 years for Elliot Creek, 21 years for Valino Island, 25 years for Winchester Arm, and 5 years for the Tom's Creek Meteorological Station, and is dependent on how long sensors have been deployed as part of the System Wide Monitoring Program.

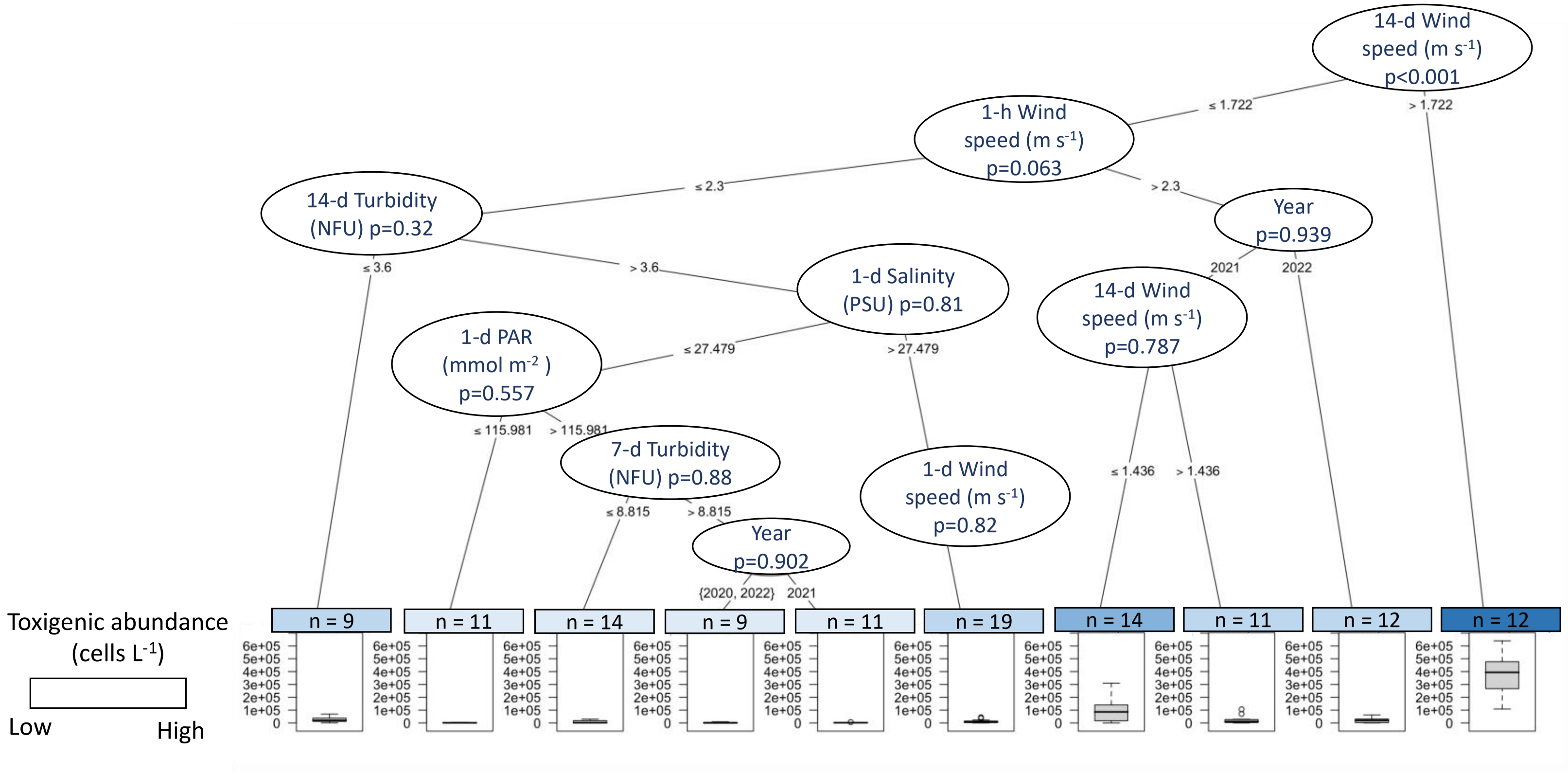

14-d Wind speed (m s^{-1}) p<0.001
≤ 1.722
> 1.722
1-h Wind speed (m s^{-1}) p=0.063
≤ 2.3
> 2.3
14-d Turbidity (NFU) p=0.32
≤ 3.6
> 3.6
Year p=0.939
2021
2022
1-d Salinity (PSU) p=0.81
≤ 27.479
> 27.479
14-d Wind speed (m s^{-1}) p=0.787
≤ 1.436
> 1.436
1-d PAR (mmol m^{-2}) p=0.557
≤ 115.981
> 115.981
7-d Turbidity (NFU) p=0.88
≤ 8.815
> 8.815
1-d Wind speed (m s^{-1}) p=0.82
Year p=0.902
{2020, 2022}
2021
n = 9
n = 11
n = 14
n = 9
n = 11
n = 19
n = 14
n = 11
n = 12
n = 12
6e+05
5e+05
4e+05
3e+05
2e+05
1e+05
0
Toxigenic abundance (cells L^{-1})
Low
High

Fig. 7. Regression tree with concentration of toxigenic taxa as response and all environmental variables (temperature, salinity, turbidity, DO, wind speed, wind direction, total PAR, precipitation) at all time steps available (15-minute RA, 1-hour RA, 1-day RA, 7-day RA, 14-day RA for all variables except precipitation - only 1, 7, and 14-day RA available) were included as predictors. P-values indicate significance of each split. Node color intensity is proportional to the count of toxigenic taxa predicted at that node. Boxplots at nodes indicate toxigenic cell concentration (cells L^{-1}).

References

Aditya, V., Koswara, A., Fitriya, N., Rachman, A., Sidabutar, T., and Thoha, H. (2013). Public awareness on harmful algal bloom (HAB) in Lampung bay. *Mar. Res. Indones*, *38*(2), 71–75.

Ainsworth, J., D'Andrea, A., Vance, M., Groth, S., and Perotti, E. (2014). *Status of Oregon bay clam fisheries, stock assessment, and research.* [Information Reports]. ODFW Marine Resources Program.

Ainsworth, J., Vance, M., Hunter, M., and Schindler, E. (2012). *The Oregon Recreational Dungeness Crab Fishery, 2007-2011.* (Information Reports No. 2012–04). ODFW Marine Resources Program.

Ajani, P., Brett, S., Krogh, M., Scanes, P., Webster, G., and Armand, L. (2013). The risk of harmful algal blooms (HABs) in the oyster-growing estuaries of New South Wales, Australia. *Environmental Monitoring and Assessment*, *185*(6), 5295–5316. https://doi.org/10.1007/s10661-012-2946-9

Andersen, P. (1996). *Design and implementation of some harmful algal monitoring systems*. [IOC technical series]. Intergovernmental Oceanographic Commission.

Anderson, T. L., and Parker, D. P. (2009). Economic development lessons from and for North American Indian economies. *Australian Journal of Agricultural and Resource Economics*, *53*(1), 105–127. https://doi.org/10.1111/j.1467-8489.2007.00426.x

Andjelkovic, M., Vandevijvere, S., Van Klaveren, J., Van Oyen, H., and Van Loco, J. (2012). Exposure to domoic acid through shellfish consumption in Belgium.

Environment International, *49*, 115–119. https://doi.org/10.1016/j.envint.2012.08.007

Backer, L. C., Manassaram-Baptiste, D., LePrell, R., and Bolton, B. (2015). Cyanobacteria and algae blooms: Review of health and environmental data from the harmful algal bloom-related illness surveillance system (HABISS) 2007–2011. *Toxins*, *7*(4), Article 4. https://doi.org/10.3390/toxins7041048

Bates, S. S., Hubbard, K. A., Lundholm, N., Montresor, M., and Leaw, C. P. (2018). *Pseudo-nitzschia*, *Nitzschia*, and domoic acid: New research since 2011. *Harmful Algae*, *79*, 3–43. https://doi.org/10.1016/j.hal.2018.06.001

Batson, C. D., and Powell, A. A. (2003). *Altruism and prosocial behavior.* Springer, British Columbia. pp 463.

Bauer, M. (2006). *Harmful Algal Research and Response: A Human Dimensions Strategy* (p. 58). National Office for Marine Biotoxins and Harmful Algal Blooms, Woods Hole Oceanographic Institution, Woods Hole, MA.

Bay Area Chamber of Commerce. (2020). *Major Industries*. Oregon Chamber of Commerce. https://coosbaynorthbendcharlestonchamber.com/bay-area-info/business-info/major-industries/

Beaumais, O., and Appéré, G. (2010). Recreational shellfish harvesting and health risks: A pseudo-panel approach combining revealed and stated preference data with correction for on-site sampling. *Ecological Economics*, *69*(12), 2315–2322. https://doi.org/10.1016/j.ecolecon.2010.07.012

Bechard, A., and Lang, C. (2023). Seafood consumption during harmful algal blooms: The impact of information regarding safety and health. *Harmful Algae, 123,* 102387. https://doi.org/10.1016/j.hal.2023.102387

Berdalet, E., Fleming, L. E., Gowen, R., Davidson, K., Hess, P., Backer, L. C., Moore, S. K., Hoagland, P., and Enevoldsen, H. (2016). Marine harmful algal blooms, human health and wellbeing: Challenges and opportunities in the 21st century. *Journal of the Marine Biological Association of the United Kingdom*, *96*(1), 61–91. https://doi.org/10.1017/S0025315415001733

Berkes, F., Colding, J., and Folke, C. (2008). *Navigating social-ecological systems: Building resilience for complexity and change*. Cambridge university press, Cambridge, United Kingdom, pp. 388.

Berkes, F., and Folke, C. (1994). Linking social and ecological systems for resilience and sustainability. *Beijer Discussion Paper Series, 52.*

Blum, I., Rao, D. S., Pan, Y., Swaminathan, S., and Adams, N. G. (2006). Development of statistical models for prediction of the neurotoxin domoic acid levels in the pennate diatom *Pseudo-nitzschia pungens f. multiseries* utilizing data from cultures and natural blooms. *Algal cultures, analogues of blooms and applications. Volume 2,* 891-916.

Branch, G. M., May, J., Roberts, B., Russell, E., and Clark, B. M. (2002). Case studies on the socio-economic characteristics and lifestyles of subsistence and informal fishers in South Africa. *South African Journal of Marine Science*, *24*(1), 439–462. https://doi.org/10.2989/025776102784528457

Busse, L. B., Venrick, E. L., Antrobus, R., Miller, P. E., Vigilant, V., Silver, M. W., Mengelt, C., Mydlarz, L., and Prezelin, B. B. (2006). Domoic acid in phytoplankton and fish in San Diego, CA, USA. *Harmful Algae*, *5*(1), 91–101. https://doi.org/10.1016/j.hal.2005.06.005

Cisneros-Montemayor, A. M., Pauly, D., Weatherdon, L. V., and Ota, Y. (2016). A global estimate of seafood consumption by coastal Indigenous Peoples. *PLOS ONE*, *11*(12), e0166681. https://doi.org/10.1371/journal.pone.0166681

Conroy, T., Sutherland, D., and Ralston, D. (2020). Estuarine exchange flow variability in a seasonal, segmented estuary. *Journal of Physical Oceanography*, *50*(3), 595–613.

Cote, M., and Nightingale, A. J. (2012). Resilience thinking meets social theory: Situating social change in socio-ecological systems (SES) research. *Progress in Human Geography*, *36*(4), 475–489. https://doi.org/10.1177/0309132511425708

Crosman, K., Petrou, E., Rudd, M., and Tillotson, M. (2016). The socio-ecological system of razor clams and the Quinault Indian Nation: Modeling the potential impacts of ocean change on a steadfast fishery. *Salish Sea Ecosystem Conference*. https://cedar.wwu.edu/ssec/2016ssec/climate_change_ocean_acidification/10

CTCLUSI. (2021). *History – Confederated Tribes of Coos, Lower Umpqua and Siuslaw Indians*. https://ctclusi.org/history/

Cziesla, C. A. (1999). *The transport and distribution of the toxic diatom Pseudo-nitzschia spp. In the Coos Bay estuary and the adjacent continental shelf* [Thesis, Thesis

(M.S.)--University of Oregon, 1998.]. https://scholarsbank.uoregon.edu/xmlui/handle/1794/9811

Dix, N., H., Ramage, J., and Cianci-Gaskill, S. Dunnigan, J.S. Tanner, K. Cressman, S. McMurray, Y. Rii, R. Guy, R. Jeppesen, E. Smith. (2022). *Recommendations for the National Estuarine Research Reserve System-Wide Monitoring Program regarding the YSI EXO Total Algae sensor.* (p. Technical Report: 35 pp.).

Donatuto, J., Grossman, E. E., Konovsky, J., Grossman, S., and Campbell, L. W. (2014). Indigenous community health and climate change: Integrating biophysical and social science indicators. *Coastal Management*, *42*(4), 355–373. https://doi.org/10.1080/08920753.2014.923140

Du, X., Peterson, W., Fisher, J., Hunter, M., and Peterson, J. (2016). Initiation and development of a toxic and persistent *Pseudo-nitzschia* bloom off the Oregon Coast in spring/summer 2015. *PLOS ONE*, *11*(10), e0163977. https://doi.org/10.1371/journal.pone.0163977

Dyson, K., and Huppert, D. D. (2010). Regional economic impacts of razor clam beach closures due to harmful algal blooms (HABs) on the Pacific coast of Washington. *Harmful Algae*, *9*(3), 264–271. https://doi.org/10.1016/j.hal.2009.11.003

Ekstrom, J. A., Moore, S. K., and Klinger, T. (2020). Examining harmful algal blooms through a disaster risk management lens: A case study of the 2015 U.S. West Coast domoic acid event. *Harmful Algae*, *94*, 101740. https://doi.org/10.1016/j.hal.2020.101740

EPA. (2014). *Estimated Fish Consumption Rates for the U.S. Population and Selected Subpopulations (NHANES 2003-2010). EPA-820-R-14-002.*, Washington, DC: United States Environmental Protection Agency.

Evans, A., Madden, K., and Morehead Palmer, S. (2012). *The Ecology and Sociology of the Mission-Aransas Estuary: An Estuarine and Watershed Profile* [Technical Report]. National Estuarine Research Reserves.

Farrer, D., Counter, M., Hillwig, R., and Cude, C. (2015). Health-based cyanotoxin guideline values allow for cyanotoxin-based monitoring and efficient public health response to cyanobacterial blooms. *Toxins*, *7*(2), 457–477. https://doi.org/10.3390/toxins7020457

Ferriss, B. E., Marcinek, D. J., Ayres, D., Borchert, J., and Lefebvre, K. A. (2017). Acute and chronic dietary exposure to domoic acid in recreational harvesters: A survey of shellfish consumption behavior. *Environment International*, *101*, 70–79. https://doi.org/10.1016/j.envint.2017.01.006

Fire, S. E., and Dolah, F. M. V. (2012). *Marine Biotoxins: Emergence of Harmful Algal Blooms as Health Threats to Marine Wildlife* (p. 553). Publications, Agencies and Staff of the U.S. Department of Commerce.

Frolov, S., Kudela, R. M., and Bellingham, J. G. (2013). Monitoring of harmful algal blooms in the era of diminishing resources: A case study of the U.S. West Coast. *Harmful Algae*, *21–22*, 1–12. https://doi.org/10.1016/j.hal.2012.11.001

Fryar, C., Kruszon-Moran, D., Gu, Q., and Ogden, C. (2018). *Mean Body Weight, Height, Waist Circumference, and Body Mass Index Among Adults: United States, 1999–2000 Through 2015–2016* (National Health Statistics Reports, p. 16).

Grattan, L. M., Holobaugh, S., and Morris, J. G. (2016). Harmful algal blooms and public health. *Harmful Algae, 57*, 2–8. https://doi.org/10.1016/j.hal.2016.05.003

Griffith, A. W., and Gobler, C. J. (2020). Harmful algal blooms: A climate change co-stressor in marine and freshwater ecosystems. *Harmful Algae, 91*, 101590. https://doi.org/10.1016/j.hal.2019.03.008

Holland, D. S., and Leonard, J. (2020). Is a delay a disaster? Economic impacts of the delay of the California Dungeness crab fishery due to a harmful algal bloom. *Harmful Algae, 98*, 101904. https://doi.org/10.1016/j.hal.2020.101904

Horner, R. A., Garrison, D. L., and Plumley, F. G. (1997). Harmful algal blooms and red tide problems on the U.S. west coast. *Limnology and Oceanography*, *42*(5part2), 1076–1088. https://doi.org/10.4319/lo.1997.42.5_part_2.1076

Hothorn, T., Hornik, K., and Zeileis, A. (2006). Unbiased recursive partitioning: A conditional inference framework. *Journal of Computational and Graphical Statistics, 15*(3), 651–674.

Hughes, M. (1997). Temporal and spatial variability of phytoplankton in coastal and estuarine habitats in Coos Bay, Oregon. [Thesis]. University of Oregon.

Kirkpatrick, B., Kohler, K., Byrne, M. M., and Studts, J. (2014). Florida red tide knowledge and risk perception: Is there a need for tailored messaging. *Harmful Algae, 32*, 27–32. https://doi.org/10.1016/j.hal.2013.09.008

Kite-Powell, H. L., Fleming, L. E., Backer, L. C., Faustman, E. M., Hoagland, P., Tsuchiya, A., Younglove, L. R., Wilcox, B. A., and Gast, R. J. (2008). Linking the oceans to public health: Current efforts and future directions. *Environmental Health*, *7*(2), S6. https://doi.org/10.1186/1476-069X-7-S2-S6

Kumar, K. P., Kumar, S. P., and Nair, G. A. (2009). *Risk assessment of the amnesic shellfish poison, domoic acid, on animals and humans*. *Journal of Enivronmental Biology 30*(3), 319-325.

Lassiter, A. M., Wilkerson, F. P., Dugdale, R. C., and Hogue, V. E. (2006). Phytoplankton assemblages in the CoOP-WEST coastal upwelling area. *Deep Sea Research Part II: Topical Studies in Oceanography*, *53*(25), 3063–3077. https://doi.org/10.1016/j.dsr2.2006.07.013

Lellis-Dibble, K. A., McGlynn, K. E., and Bigford, T. E. (2008). *Estuarine Fish and Shellfish Species in U.S. Commercial and Recreational Fisheries: Economic Value as an Incentive to Protect and Restore Estuarine Habitat.* (Memo. NMFSF/SPO-90, 94 p.). U.S. Dep. Commerce, NOAA Tech.

Lelong, A., Hégaret, H., Soudant, P., and Bates, S. S. (2012). *Pseudo-nitzschia* (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: Revisiting previous paradigms. *Phycologia*, *51*(2), 168–216. https://doi.org/10.2216/11-37.1

Lepofsky, D., Smith, N. F., Cardinal, N., Harper, J., Morris, M., White), G. (Elroy, Bouchard, R., Kennedy, D. I. D., Salomon, A. K., Puckett, M., and Rowell, K.

(2015). Ancient shellfish mariculture on the Northwest Coast of North America. *American Antiquity*, *80*(2), 236–259. https://doi.org/10.7183/0002-7316.80.2.236

Levin, S. A. (1998). Ecosystems and the biosphere as complex adaptive systems. *Ecosystems*, *1*, 431-436.

Levin, S., Xepapadeas, T., Crépin, A. S., Norberg, J., De Zeeuw, A., Folke, C., ... and Walker, B. (2013). Social-ecological systems as complex adaptive systems: modeling and policy implications. *Environment and Development Economics*, *18*(2), 111-132.

Lim, W.-A., and Kim, D. H. (2020). *GlobalHAB: Evaluating, Reducing and Mitigating the Cost of Harmful Algal Blooms: A Compendium of Case Studies.* North Pacific Marine Science Organization (pp. 42–54).

Losey, R. J., Yamada, S. B., and Largaespada, L. (2004). Late-Holocene Dungeness crab (Cancer magister) harvest at an Oregon coast estuary. *Journal of Archaeological Science*, *31*(11), 1603–1612. https://doi.org/10.1016/j.jas.2004.04.002

Mathews, D. L., and Turner, N. J. (2017). Chapter 9 - Ocean cultures: Northwest Coast ecosystems and Indigenous management systems. In P. S. Levin and M. R. Poe (Eds.), *Conservation for the Anthropocene Ocean* (pp. 169–206). Academic Press. https://doi.org/10.1016/B978-0-12-805375-1.00009-X

McCabe, R. M., Hickey, B. M., Kudela, R. M., Lefebvre, K. A., Adams, N. G., Bill, B. D., Gulland, F. M. D., Thomson, R. E., Cochlan, W. P., and Trainer, V. L. (2016). An unprecedented coastwide toxic algal bloom linked to anomalous ocean conditions. *Geophysical Research Letters, 43*(19), 10,366-10,376. https://doi.org/10.1002/2016GL070023

Moore, S. K., Cline, M. R., Blair, K., Klinger, T., Varney, A., and Norman, K. (2019). An index of fisheries closures due to harmful algal blooms and a framework for identifying vulnerable fishing communities on the U.S. West Coast. *Marine Policy, 110*, 103543. https://doi.org/10.1016/j.marpol.2019.103543

Moreno, A. R., Anderson, C., Kudela, R. M., Sutula, M., Edwards, C., and Bianchi, D. (2022). Development, calibration, and evaluation of a model of *Pseudo-nitzschia* and domoic acid production for regional ocean modeling studies. *Harmful Algae, 118*, 102296.

National Weather Service. (2023). *NOAA Online Weather Data* [Data set]. https://www.weather.gov/wrh/Climate?wfo=mfr

NCCOS. (2017a). *HABHRCA*. NCCOS Coastal Science Website. https://coastalscience.noaa.gov/research/stressor-impacts-mitigation/habhrca/

NCCOS. (2017b). *PCMHAB*. NCCOS Coastal Science Website. https://coastalscience.noaa.gov/research/stressor-impacts-mitigation/pcmhab/

NCCOS and US IOOS. (2020). *Framework for the National Harmful Algal Bloom Observing Network: A Workshop Report.* National Centers for Coastal Ocean Science.

Nierenberg, K., Byrne, M. M., Fleming, L. E., Stephan, W., Reich, A., Backer, L. C., Tanga, E., Dalpra, D. R., and Kirkpatrick, B. (2010). Florida red tide perception:

Residents versus tourists. *Harmful Algae*, *9*(6), 600–606. https://doi.org/10.1016/j.hal.2010.04.010

NOAA OCM. (2021a). *C-CAP Land Cover Atlas*. Digitial Coast. https://coast-noaa-gov.proxy.lib.pdx.edu/digitalcoast/tools/lca.html

NOAA OCM. (2021b). *Economics and Demographics*. Fast Facts. http://coast.noaa.gov/states/fast-facts/economics-and-demographics.html

Ohana-Richardson, A. (2007). *Population ecology of the diatom genus* Pseudo-nitzschia *within the South Slough, Charleston, Oregon.* [Thesis]. University of Oregon.

Oregon Department of Fish and Wildlife. (2020). *Harmful Algae—Recreational and Commercial Shellfish*. https://www.dfw.state.or.us/mrp/shellfish/razorclams/harmful_algae.asp

Pearson, R. W., and Ross, M. (1992). *Of Retrospective Questions in Surveys'. Questions About Questions: Inquiries into the Cognitive Bases of Surveys 65.* The Russell Sage Foundation.

Petroff, R. L., Williams, C., Li, J.-L., MacDonald, J. W., Bammler, T. K., Richards, T., English, C. N., Baldessari, A., Shum, S., and Jing, J. (2022). Prolonged, low-level exposure to the marine toxin, domoic acid, and measures of neurotoxicity in nonhuman primates. *Environmental Health Perspectives, 130*(9), 097003.

Poe, M. R., Levin, P. S., Tolimieri, N., and Norman, K. (2015). Subsistence fishing in a 21st century capitalist society: From commodity to gift. *Ecological Economics*, *116*, 241–250. https://doi.org/10.1016/j.ecolecon.2015.05.003

Ritzman, J., Brodbeck, A., Brostrom, S., McGrew, S., Dreyer, S., Klinger, T., and Moore, S. K. (2018). Economic and sociocultural impacts of fisheries closures in two fishing-dependent communities following the massive 2015 U.S. West Coast harmful algal bloom. *Harmful Algae*, *80*, 35–45. https://doi.org/10.1016/j.hal.2018.09.002

Roberts, S. M., Grattan, L. M., Toben, A. C., Ausherman, C., Trainer, V. L., Tracy, K., and Morris, J. G. (2016). Perception of risk for domoic acid related health problems: A cross-cultural study. *Harmful Algae*, *57*, 39–44. https://doi.org/10.1016/j.hal.2016.03.007

Roegner, G. C., and Shanks, A. L. (2001). Import of coastally-derived chlorophyll a to South Slough, Oregon. *Estuaries*, *24*(2), 244–256. https://doi.org/10.2307/1352948

Rumrill, S. S. (2006). *The Ecology of the South Slough Estuary: Site Profile of the South Slough National Estuarine Research Reserve. NOAA / Oregon Department of State Lands. 238 pp.* (p. 238). NOAA / Oregon Department of State Lands.

Schrager, H., Bragg, J., Demarzo, A., Yednock, B., Schmitt, J., Helms, A., Tally, J., Rudd, D., Dean, E., Muse, R., Cooper, G., Cornu, C., Gaskill, T., and Wilson, P. (2017). *South Slough National Estuarine Research Reserve Management Plan 2017-2022* (p. 197). South Slough National Estuarine Research Reserve.

SEACOR. (2014). *Recreational Clams in the Coos Estuary*. Oregon Department of Fish and Wildlife.

Shumway, S. E., Allen, S. M., and Dee Boersma, P. (2003). Marine birds and harmful algal blooms: Sporadic victims or under-reported events? *Harmful Algae*, *2*(1), 1–17. https://doi.org/10.1016/S1568-9883(03)00002-7

State of Oregon. (2021). *Shellfish—Recreational Shellfish Biotoxin Closures*. https://www.oregon.gov/oda/programs/FoodSafety/Shellfish/Pages/ShellfishClosures.aspx

Stuchal, L. D., Grattan, L. M., Portier, K. M., Kilmon, K. A., Manahan, L. M., Roberts, S. M., and Morris, J. G. (2020). Dose-response assessment for impaired memory from chronic exposure to domoic acid among Native American consumers of razor clams. *Regulatory Toxicology and Pharmacology*, *117*, 104759. https://doi.org/10.1016/j.yrtph.2020.104759

Sun, J., and Liu, D. (2003). Geometric models for calculating cell biovolume and surface area for phytoplankton. *Journal of Plankton Research*, *25*(11), 1331–1346. https://doi.org/10.1093/plankt/fbg096

Thessen, A. E., Dortch, Q., Parsons, M. L., and Morrison, W. (2005). Effect of salinity on *Pseudo-nitzschia* species (Bacillariophyceae) growth and distribution. *Journal of Phycology*, *41*(1), 21–29. https://doi.org/10.1111/j.1529-8817.2005.04077.x

Torres, N., and Shanks, A. (2018). Pseudo-nitzschia *on the Rising Tide: Phytoplankton Concentrations in the Coos Bay Estuary.* [Poster presentation].

Toyofuku, H. (2006). Joint FAO/WHO/IOC activities to provide scientific advice on marine biotoxins (research report). *Marine Pollution Bulletin*, *52*(12), 1735–1745. https://doi.org/10.1016/j.marpolbul.2006.07.007

Trainer, V. L., Bates, S. S., Lundholm, N., Thessen, A. E., Cochlan, W. P., Adams, N. G., and Trick, C. G. (2012). *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. *Harmful Algae*, *14*, 271–300. https://doi.org/10.1016/j.hal.2011.10.025

Trainer, V. L., Cochlan, W. P., Erickson, A., Bill, B. D., Cox, F. H., Borchert, J. A., and Lefebvre, K. A. (2007). Recent domoic acid closures of shellfish harvest areas in Washington State inland waterways. *Harmful Algae*, *6*(3), 449–459. https://doi.org/10.1016/j.hal.2006.12.001

Trainer, V. L., Hickey, B. M., Lessard, E. J., Cochlan, W. P., Trick, C. G., Wells, M. L., ... and Moore, S. K. (2009). Variability of Pseudo-nitzschia and domoic acid in the Juan de Fuca eddy region and its adjacent shelves. *Limnology and Oceanography*, *54*(1), 289-308.

Trainer, V. L., Moore, S. K., Hallegraeff, G., Kudela, R. M., Clement, A., Mardones, J. I., and Cochlan, W. P. (2020). Pelagic harmful algal blooms and climate change: Lessons from nature's experiments with extremes. *Harmful Algae*, *91*, 101591. https://doi.org/10.1016/j.hal.2019.03.009

Trainer, V., and Suddleson, M. (2005). Monitoring approaches for early warning of domoic acid events in Washington State. *Oceanography*, *18*(2), 228–237. https://doi.org/10.5670/oceanog.2005.56

United States Census Bureau. (2022). *Coos County, OR Quick Facts* [Data set]. https://www.census.gov/quickfacts/cooscountyoregon

U.S. Commission on Ocean Policy: Documents. (2004). https://govinfo.library.unt.edu/oceancommission/documents/full_color_rpt/welcome.html

Van Dolah, E., Paolisso, M., Sellner, K., and Place, A. (2013). Beyond the bloom: Using a socio-ecological systems framework to investigate stakeholder response to harmful algal bloom management in the Chesapeake Bay, USA.

Van Dolah, E. R., Paolisso, M., Sellner, K., and Place, A. (2016). Employing a socio-ecological systems approach to engage harmful algal bloom stakeholders. *Aquatic Ecology*, *50*(3), 577–594. https://doi.org/10.1007/s10452-015-9562-z

West, J. J., Järnberg, L., Berdalet, E., and Cusack, C. (2021). Understanding and managing harmful algal bloom risks in a changing climate: Lessons from the European CoCliME Project. *Frontiers in Climate*, *3*. https://www.frontiersin.org/articles/10.3389/fclim.2021.636723

Appendix C: Supporting materials for Chapter 4

C1. Questionnaire form for shellfish harvester survey.

Portland State University
Shellfish and Harmful Algal Blooms Survey

The information you provide in this survey is anonymous, and will be made public after all survey data has been analyzed. You may end your participation at any time or leave any question blank if you feel uncomfortable or do not wish to proceed with the rest of the survey. You must be 18 years or older to participate. Completing this survey should take 5-10 minutes.

Part I – Demographic and Fishery Use Information

1) What year were you born? Please write in: ___________________

2) Please circle your gender.

Female Male Other

3) What is your ethnic identity? Select all that apply.

- ☐ Alaska Native
- ☐ Asian
- ☐ Black or African American
- ☐ Latino
- ☐ Native American
- ☐ Native Hawaiian and Pacific Islander
- ☐ White/Caucasian
- ☐ Other (write in): _____________

4) If applicable, what is/are your tribal affiliation(s)? Select all that apply.

- ☐ Alsea
- ☐ Chinook
- ☐ Coos (Hanis)
- ☐ Coos (Miluk)
- ☐ Coquille
- ☐ Klamath
- ☐ Lower Umpqua
- ☐ Siuslaw
- ☐ Tillamook
- ☐ Tolowa
- ☐ Other (write in):
- ☐ No tribal affiliation

	8) How often did you **harvest** shellfish in the past year? Please write in your answers below.	9) How often did you **eat** shellfish that were NOT purchased in a store or restaurant (non-commercial shellfish) in the past year?
	Number of harvests in the past year	Number of times eaten in the past year
Razor clams 		
Bay clams (gaper, littleneck, butter clams, cockles) 		
Mussels 		
Crab (Dungeness, red rock, or other)		

		
Snails  		
Other: _______________		
Other: _______________		

5) What is your estimated household income per year from all sources (in US dollars)?

☐ Less than $15,000
☐ $15,001 – $25,000
☐ $25,001 - $50,000
☐ $50,001 - $75,000
☐ $75,001 - $100,000
☐ More than $100,000

6) What is your highest level of education?

☐ Some high school
☐ High school diploma/GED
☐ Some college
☐ Vocational certificate or 2-year degree
☐ Bachelor’s degree
☐ Graduate degree

7) On a typical trip, how far do you have to travel from home to where you can harvest shellfish?

- ☐ ________ miles

10) For each season within the past year, with what frequency did you harvest shellfish to eat? Please mark the approximate number of harvests per season.

	Never	1-2 times a season	About 1 time a month	More than 1 time a month
Winter (Nov. – to Feb.)	☐	☐	☐	☐
Spring (March – May)	☐	☐	☐	☐
Summer (June-Aug.)	☐	☐	☐	☐
Fall (Sept.-Oct.)	☐	☐	☐	☐

11) How have you gotten the non-commercial shellfish you have eaten in the past? Check all that apply.

- ☐ I collected/harvested/caught them myself
- ☐ Given to me by friends or family
- ☐ Purchased, traded, or bartered for them
- ☐ Ate them at tribal events
- ☐ I did not consume non-commercial shellfish

12) What is your approximate serving size when you consume shellfish?

- ☐ Less than 3 oz (less than about 1/3 cup of shellfish meat)
- ☐ 3-8 oz (about 1/3 - 1 cup of shellfish meat)
- ☐ More than 8 oz (more than about 1 cup of shellfish meat)
- ☐ I don’t eat shellfish

13) When you have eaten shellfish, you ate them:

- ☐ With guts
- ☐ Without guts
- ☐ Sometimes with, sometimes without guts
- ☐ I don’t know
- ☐ I don’t eat shellfish

14) How long has it been since you first started harvesting shellfish? _________

15) Have you ever become sick after eating recreationally harvested shellfish?

No Yes Not applicable

16) Do you know anyone personally who has gotten sick after eating recreationally harvested shellfish?

No Yes Not applicable

Part II – Harmful Algal Blooms and Shellfish: Risk Perception and Attitudes

Please read the following statement and answer the questions below.
Harmful algal blooms occur when certain species of microorganism grow rapidly and produce toxins that accumulate in the food chain, and can contaminate shellfish and may cause illness when consumed by humans. Natural resource managers are actively monitoring for algal biotoxin levels in shellfish, and may close fisheries when toxins exceed safe levels. Shellfish are a healthy food source, and can generally be considered safe to eat with proper food handling unless officials have closed the fishery.

17) Were you aware of harmful algal blooms and algal toxin contamination of shellfish prior to this survey?

☐ Yes ☐ No

18) Please indicate your level of agreement with the following statements.

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
I am concerned about algal toxins in shellfish.	☐	☐	☐	☐	☐

19) How would you rate your personal risk of consuming shellfish contaminated with algal toxins?

☐ Low ☐ Medium ☐ High ☐ I don’t know

20a) In the past year, have you used shellfish closure or toxin monitoring information (for example from fish and wildlife, health, or food safety authorities) when deciding whether to harvest shellfish?

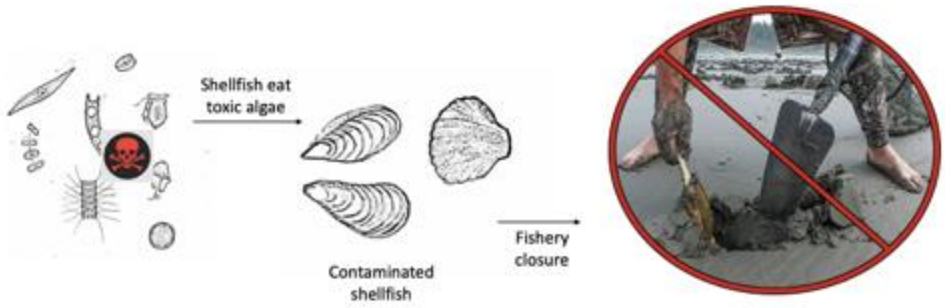
☐ Yes ☐ No

20b) If so, where did you get your information? If not, which would you be most likely to use in the future? Select all that apply.

- ☐ Website
- ☐ Phone hotline
- ☐ Social media posts
- ☐ Signs posted at harvest sites
- ☐ Information with shellfish license purchase
- ☐ Word of mouth
- ☐ Prior knowledge
- ☐ News outlet (paper, online, TV)
- ☐ Other:_______________
- ☐ None of these

21) Please indicate your level of agreement with the following statements.

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
Algal toxin monitoring programs are important for me to make shellfish harvesting decisions.	☐	☐	☐	☐	☐
Publicly accessible educational resources about algal toxins are important for me to make shellfish harvesting decisions.	☐	☐	☐	☐	☐
Shellfish harvesting closure areas when toxins are present are important for me to make shellfish harvesting decisions.	☐	☐	☐	☐	☐



C2. Infographic handout card attached to clipboard for communication about HABs.

Chapter 5: Conclusions

In this dissertation, I sought to understand and develop a management response to the ways in which a rapidly changing world is influencing plankton communities. Human impacts on estuarine ecosystems have increased during the Anthropocene through the effects of climate change, land-use change, and eutrophication, shifting the baseline of knowledge on this topic. This necessitates updating our understanding of where and when HABs occur, how plankton toxicity and nutritional quality impact food webs and fisheries, and how natural resource management can best respond to avoid detrimental impacts to human health, wellbeing, and coastal economies. While each of the previous research chapters delves into a unique aspect of the drivers and impacts of plankton community dynamics, all chapters address related emerging issues due to shifting environmental conditions.

Summary and context of research

In Chapter 2, I determined that Columbia River discharge was a primary driving factor in determining the risk of HABs in the Lower Columbia River Estuary (LCRE) during years with marine heat waves (MHWs). Specifically, when discharge declined below the threshold of ~3,400 $m^3 s^{-1}$, the plankton community became marine-influenced and more prone to marine and brackish HABs. This work builds on a large body of research on the physical and biological processes of the Columbia River plume and the nearby coastal shelf (Baptista et al., 2015; Chawla et al., 2008; Herfort et al., 2016; Hickey et al., 2005; Hickey and Banas, 2003; Kärnä and Baptista, 2016; Miller et al., 2017; Peterson et al., 2017), as well as research on the biological processes of the Columbia upstream from our study site (Rollwagen-Bollens et al., 2020; Rose et al., 2021). I build on this research by contributing a plankton time series to improve understanding of a unique lower estuarine community during extreme climate events. Little work has been done to assess whether the impacts of large-scale MHWs are mirrored in estuarine environments. This research provides evidence that estuarine plankton communities are impacted by MHW via atmospheric impacts on snowpack and subsequent river discharge, as well as transport of MHW-derived marine HABs into estuaries. New research suggests that *Pseudo-nitzschia* spp. blooms may become more common due to seed banks formed during a massive 2015 bloom event (Trainer et al., 2020). I observed this *Pseudo-nitzschia* spp. bloom within the LCRE in October of 2015, several months after its documented establishment in nearby coastal waters (Du et al., 2016), suggesting that the elevated risk of this HAB may extend to estuaries, given conducive conditions. This study also provides the first published account of a toxic *Gymnodinium catenatum* bloom in the estuary. The research presented in this chapter indicates that the LCRE may be seasonally protected from marine HABs, and only at elevated risk during periods of low discharge. Given the critical role of estuarine habitat for fisheries (Lellis-Dibble et al., 2008; NOAA Office for Coastal Management, 2021), it is critical to improve our understanding of how climate change-associated oceanographic events impact estuarine plankton assemblages for effective fishery management in the future.

In Chapter 3, I found that nutrient conditions impacted plankton community composition and the nutritional quality of plankton communities, as the addition of both inorganic and organic nitrogen forms contributed to elevated polyunsaturated fatty acids (PUFAs). Specifically, I observed a mixotroph-dominated HAB of *G. catenatum* with a comparatively PUFA-rich fatty acid profile, and elevated PUFA concentrations with additions of nitrate and urea in experimental conditions. While the plankton functional traits of mixotrophy and toxigenicity were associated with organic nutrients in natural coastal systems, I did not observe a strong relationship between these traits and nitrogen availability in a nutrient addition experiment. This work adds to a rapidly growing area of research, which uses fatty acids as biomarkers of nutritional quality (Brett and Müller-Navarra, 1997; Galloway et al., 2015; Galloway and Winder, 2015; Kainz et al., 2004; Taipale et al., 2016; Twining et al., 2016), and specifically investigates the association of PUFAs with the understudied trophic category of mixotrophs. There has been little previous research to understand how mixotrophy influences the quality of fatty acids synthesized by plankton, or how the ability to produce toxins is associated with PUFAs. I investigated these relationships using natural plankton communities both through observational and experimental study. This differs from most previous research that has been conducted with a single or small number of cultured taxa (except Grosse et al., 2017, 2019), and is a relevant contribution, as fisheries that rely (either directly or indirectly) on plankton stocks for food are impacted by the complex dynamics of plankton communities, rather than single algal taxa. By gaining understanding of the drivers of plankton community functional traits and PUFA profiles, this research may aid in predicting HAB and low nutritional quality events to mitigate impacts to aquaculture and fisheries operations.

In Chapter 4, I identified hotspots of HAB activity in the upper Coos Estuary (CE) and at Tenmile Lake, and found that winds were a major driver of abundance of toxic algae in the Coos Estuary. I found that exposure to the algal toxin domoic acid was at a safe level among shellfish harvesters during the study. I also observed that while harvesters expressed broad support for several HAB management strategies, a significant proportion did not make use of these resources. This research builds on prior socio-ecological research focused on HABs in the Gulf Coast and Olympic Peninsula regions (Kirkpatrick et al., 2014; Nierenberg et al., 2010; Roberts et al., 2016), as well as a body of research on HAB monitoring on the West Coast (Frolov et al., 2013; Lewitus et al., 2012; McCabe et al., 2016; Trainer and Hardy, 2015; Trainer and Suddleson, 2005). This prior research has clearly indicated that HAB monitoring programs and social management strategies to provide information and protect public health must be unique to the place of interest. HAB taxa vary by geographic location, and each coastal community has its own social context which shapes how the public perceives and reacts to HAB events. This research used needs assessment and local and indigenous knowledge to develop a localized HAB monitoring program for the Coos Bay area. This area has historically been underserved in terms of HAB monitoring, with gaps in our understanding of plankton dynamics and prevalence of toxigenic taxa near known shellfish harvesting areas. Another novel aspect of this research is the concurrent collection of ecological and human dimensions datasets which reflexively inform one another. This design allowed for more accurate estimation of algal toxin exposure risk while simultaneously providing information about harvesters' perception of that risk. By assessing gaps between perceived and actual risk, I identified areas to focus future outreach efforts. This information is crucial in tailoring management and outreach for particular demographic groups to target inequities in the risk of exposure to algal toxins and the use of existing resources on shellfish harvesting safety.

Future Research Directions and Recommendations

The research in this dissertation presents several limitations that highlight the need for future work on these topics. While more specific and methodological limitations are addressed in each chapter, several overarching themes emerged for directing future research on the drivers and impacts of estuarine plankton communities. The influence of seasonality was an underlying theme in all chapters, and Chapter 3 probes this concept. I found that nutrient additions impacted plankton nutritional value differently depending on when they were applied, likely because of seasonal differences in community composition, which is an important determinant of PUFA profile. Future research could conduct similar nutrient experiments over a broader range of the year than carried out in this study. This could aid understanding of the time of year added nutrient inputs are likely to have the strongest impact on downstream plankton communities, and thus the nutritional quality of plankton for consumers. In addition, it would be informative to conduct nutrient addition experiments in mesocosms, rather than carboys, to reduce the impact of incubation that I observed (Veldhuis and Timmermans, 2007). Future nutrient addition experiments might also aim to disentangle the interacting effects of light and nutrients on the prevalence of mixotrophy and the abundance of nutritionally important PUFAs.

A significant component of this dissertation involved monitoring plankton communities and identifying drivers of community shifts. In Chapter 2, I examined plankton communities over three years: two with MHWs, one without. Because this study only included one year without a MHW, there was a degree of uncertainty in how well the plankton community of the non-MHW year was representative of a baseline plankton community in the LCRE. More years of plankton monitoring would have made our interpretation of deviation from baseline levels more robust, and future studies of the impacts of climate events on plankton would benefit from long-term monitoring datasets. Similarly, the monitoring program described in Chapter 4 identified winds as a primary predictor of toxic algal abundance in the Coos Estuary over a two-year period. Given the short duration and limited variety of toxic algae present during the study, it is difficult to assess drivers of other types of HABs or whether this driver is consistently important.

This research used biweekly or monthly plankton sampling to integrate over environmental conditions that change at a much faster rate in estuaries. This may obscure more rapid turnover of plankton communities that can occur in estuaries due to dynamic water quality and hydrologic conditions. This is particularly salient in areas of estuaries with low water residence time. The brief assessment of daily plankton community composition in the South Slough Estuary in Chapter 4 revealed that while variation was greater at the monthly scale, there was still notable variation from day to day and at different points in the tidal cycle, which is similar to patterns observed in other estuaries (Cloern, 1991; Muylaert, 2000). The extent to which this fine-scale variation in plankton communities moderates the effects of HABs and nutritional quality on shellfish resources remains poorly understood. For example, it is not clear if tidal action and the ensuing turnover of plankton communities provides a protective influence on the accumulation of algal toxins or low average food quality for estuarine shellfish. Research to answer these questions would aid in determining the temporal scale of plankton monitoring required to detect impacts to estuarine fisheries. Since monitoring programs must balance the desire to capture fine-scale variation with conservation of resources for long term success of the program, research on this topic could help guide monitoring for fishery management.

This work also identified the importance of local HAB monitoring programs. Hyperlocal studies provide several advantages that geographically dispersed studies cannot, including the opportunity for geographically and spatially dense information that is essential for understanding variation between fishing areas and providing nuance in fishery closures. Such nuance could be economically and socially valuable, potentially preventing unnecessary closures or delays of fishing seasons and aquaculture operations. Localized studies also permit the involvement of the community in the research, which can improve monitoring programs through use of local and indigenous knowledge, introduces an aspect of local stewardship of estuarine resources, and promotes awareness of HABs and their impacts to fisheries (Kimura and Kinchy, 2016). The study described in Chapter 4 makes clear that the issue of HABs is not merely an ecological one, but must be considered in the social context of communities impacted by these events. This work suggests that similar localized studies may be of value in other coastal communities, particularly those that are underserved by state-level monitoring programs.

The findings presented in this dissertation illustrate how anthropogenic environmental changes impact estuarine plankton communities, as well as the human dimensions of this issue. These studies depict only a small window of time, and the concept of shifting baselines is a theme of the Anthropocene. Climatic and oceanographic conditions as well as nutrient regimes are continually changing, often beyond the variation that has been historically recorded (Duarte et al., 2009; Little et al., 2017). Continued monitoring will be necessary to help humans best respond to HABs and altered plankton nutritional quality. Future-oriented research and modeling that considers various climate scenarios will also be essential to understand how these changes will affect future estuarine plankton communities, fisheries, and habitats. The problems discussed in this work are complex, and warrant creative, multi-pronged efforts to address them. This dissertation highlights the importance of a transdisciplinary, collaborative approach to address impacts of altered plankton communities amidst global change.

References

Baptista, A. M., Seaton, C., Wilkin, M. P., Riseman, S. F., Needoba, J. A., Maier, D., Turner, P. J., Kärnä, T., Lopez, J. E., Herfort, L., Megler, V. M., McNeil, C., Crump, B. C., Peterson, T. D., Spitz, Y. H., and Simon, H. M. (2015). Infrastructure for collaborative science and societal applications in the Columbia River estuary. *Frontiers of Earth Science*, *9*(4), 659–682. https://doi.org/10.1007/s11707-015-0540-5

Brett, M., and Müller-Navarra, D. (1997). The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshwater Biology*, *38*(3), 483–499. https://doi.org/10.1046/j.1365-2427.1997.00220.x

Chawla, A., Jay, D. A., Baptista, A. M., Wilkin, M., and Seaton, C. (2008). Seasonal variability and estuary–shelf interactions in circulation dynamics of a river-dominated estuary. *Estuaries and Coasts*, *31*(2), 269–288. https://doi.org/10.1007/s12237-007-9022-7

Cloern, J. E. (1991). Tidal stirring and phytoplankton bloom dynamics in an estuary. *Journal of marine research*, *49*(1), 203-221.

Du, X., Peterson, W., Fisher, J., Hunter, M., and Peterson, J. (2016). Initiation and Development of a toxic and persistent *Pseudo-nitzschia* bloom off the Oregon Coast in spring/summer 2015. *PLOS ONE*, *11*(10), e0163977. https://doi.org/10.1371/journal.pone.0163977

Duarte, C. M., Conley, D. J., Carstensen, J., and Sánchez-Camacho, M. (2009). Return to Neverland: Shifting baselines affect eutrophication restoration targets. *Estuaries and Coasts*, *32*(1), 29–36. https://doi.org/10.1007/s12237-008-9111-2

Frolov, S., Kudela, R. M., and Bellingham, J. G. (2013). Monitoring of harmful algal blooms in the era of diminishing resources: A case study of the U.S. West Coast. *Harmful Algae*, *21–22*, 1–12. https://doi.org/10.1016/j.hal.2012.11.001

Galloway, A. W. E., Brett, M. T., Holtgrieve, G. W., Ward, E. J., Ballantyne, A. P., Burns, C. W., Kainz, M. J., Müller-Navarra, D. C., Persson, J., Ravet, J. L., Strandberg, U., Taipale, S. J., and Alhgren, G. (2015). A fatty acid based Bayesian approach for inferring diet in aquatic consumers. *PLOS ONE*, *10*(6), e0129723. https://doi.org/10.1371/journal.pone.0129723

Galloway, A. W. E., and Winder, M. (2015). Partitioning the relative importance of phylogeny and environmental conditions on phytoplankton fatty acids. *PLoS One; San Francisco*, *10*(6), e0130053. http://dx.doi.org/10.1371/journal.pone.0130053

Grosse, J., Breugel, P. van, Brussaard, C. P. D., and Boschker, H. T. S. (2017). A biosynthesis view on nutrient stress in coastal phytoplankton. *Limnology and Oceanography*, *62*(2), 490–506. https://doi.org/10.1002/lno.10439

Grosse, J., Brussaard, C. P. D., and Boschker, H. T. S. (2019). Nutrient limitation driven dynamics of amino acids and fatty acids in coastal phytoplankton. *Limnology and Oceanography*, *64*(1), 302–316. https://doi.org/10.1002/lno.11040

Herfort, L., Seaton, C., Wilkin, M., Roman, B., Preston, C. M., Marin, R., Seitz, K., Smith, M. W., Haynes, V., Scholin, C. A., Baptista, A. M., and Simon, H. M.

(2016). Use of continuous, real-time observations and model simulations to achieve autonomous, adaptive sampling of microbial processes with a robotic sampler. *Limnology and Oceanography: Methods*, *14*(1), 50–67. https://doi.org/10.1002/lom3.10069

Hickey, B., Geier, S., Kachel, N., and MacFadyen, A. (2005). A bi-directional river plume: The Columbia in summer. *Continental Shelf Research*, *25*(14), 1631–1656. https://doi.org/10.1016/j.csr.2005.04.010

Hickey, B. M., and Banas, N. S. (2003). Oceanography of the U.S. Pacific Northwest Coastal Ocean and estuaries with application to coastal ecology. *Estuaries*, *26*(4), 1010–1031. https://doi.org/10.1007/BF02803360

Kainz, M., Arts, M., and Mazumder, A. (2004). Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. *Limnology and Oceanography*. https://aslopubs.onlinelibrary.wiley.com/doi/abs/10.4319/lo.2004.49.5.1784

Kärnä, T., and Baptista, A. M. (2016). Water age in the Columbia River estuary. *Estuarine, Coastal and Shelf Science*, *183*, 249–259. https://doi.org/10.1016/j.ecss.2016.09.001

Kimura, A. H., and Kinchy, A. (2016). Citizen science: Probing the virtues and contexts of participatory research. *Engaging Science, Technology, and Society*, *2*, 331-361.

Kirkpatrick, B., Kohler, K., Byrne, M. M., and Studts, J. (2014). Florida red tide knowledge and risk perception: Is there a need for tailored messaging. *Harmful Algae*, *32*, 27–32. https://doi.org/10.1016/j.hal.2013.09.008

Lellis-Dibble, K. A., McGlynn, K. E., and Bigford, T. E. (2008). *Estuarine Fish and Shellfish Species in U.S. Commercial and Recreational Fisheries: Economic Value as an Incentive to Protect and Restore Estuarine Habitat.* (Memo. NMFSF/SPO-90, 94 p.). U.S. Dep. Commerce, NOAA Tech.

Lewitus, A. J., Horner, R. A., Caron, D. A., Garcia-Mendoza, E., Hickey, B. M., Hunter, M., Huppert, D. D., Kudela, R. M., Langlois, G. W., Largier, J. L., Lessard, E. J., RaLonde, R., Jack Rensel, J. E., Strutton, P. G., Trainer, V. L., and Tweddle, J. F. (2012). Harmful algal blooms along the North American west coast region: History, trends, causes, and impacts. *Harmful Algae*, *19*, 133–159. https://doi.org/10.1016/j.hal.2012.06.009

Little, S., Spencer, K. L., Schuttelaars, H. M., Millward, G. E., and Elliott, M. (2017). Unbounded boundaries and shifting baselines: Estuaries and coastal seas in a rapidly changing world. *Estuarine, Coastal and Shelf Science*, *198*, 311–319. https://doi.org/10.1016/j.ecss.2017.10.010

McCabe, R. M., Hickey, B. M., Kudela, R. M., Lefebvre, K. A., Adams, N. G., Bill, B. D., Gulland, F. M. D., Thomson, R. E., Cochlan, W. P., and Trainer, V. L. (2016). An unprecedented coastwide toxic algal bloom linked to anomalous ocean conditions. *Geophysical Research Letters*, *43*(19), 10,366-10,376. https://doi.org/10.1002/2016GL070023

Millennium Ecosystem Assessment (Program) (Ed.). (2005). *Ecosystems and human well-being: Wetlands and water synthesis: a report of the Millennium Ecosystem Assessment*. World Resources Institute.

Miller, J. A., Peterson, W. T., Copeman, L. A., Du, X., Morgan, C. A., and Litz, M. N. C. (2017). Temporal variation in the biochemical ecology of lower trophic levels in the Northern California Current. *Progress in Oceanography*, *155*, 1–12. https://doi.org/10.1016/j.pocean.2017.05.003

Muylaert, K., Sabbe, K., and Vyverman, W. (2000). Spatial and temporal dynamics of phytoplankton communities in a freshwater tidal estuary (Schelde, Belgium). *Estuarine, Coastal and Shelf Science*, *50*(5), 673-687.

Nierenberg, K., Byrne, M. M., Fleming, L. E., Stephan, W., Reich, A., Backer, L. C., Tanga, E., Dalpra, D. R., and Kirkpatrick, B. (2010). Florida red tide perception: Residents versus tourists. *Harmful Algae*, *9*(6), 600–606. https://doi.org/10.1016/j.hal.2010.04.010

NOAA Office for Coastal Management. (2021). *Research Reserves*. Fast Facts. http://coast.noaa.gov/states/fast-facts/research-reserves.html

Paerl, H. W., Gardner, W. S., Havens, K. E., Joyner, A. R., McCarthy, M. J., Newell, S. E., Qin, B., and Scott, J. T. (2016). Mitigating cyanobacterial harmful algal blooms in aquatic ecosystems impacted by climate change and anthropogenic nutrients. *Harmful Algae*, *54*, 213–222. https://doi.org/10.1016/j.hal.2015.09.009

Peterson, W. T., Fisher, J. L., Strub, P. T., Du, X., Risien, C., Peterson, J., and Shaw, C. T. (2017). The pelagic ecosystem in the Northern California Current off Oregon during the 2014-2016 warm anomalies within the context of the past 20 years: THE PELAGIC ECOSYSTEM DURING 2014-2016. *Journal of Geophysical Research: Oceans*, *122*(9), 7267–7290. https://doi.org/10.1002/2017JC012952

Pinckney, J. L. (2001). The role of nutrient loading and eutrophication in estuarine ecology. *Environmental Health Perspectives*, *109*, 8.

Roberts, S. M., Grattan, L. M., Toben, A. C., Ausherman, C., Trainer, V. L., Tracy, K., and Morris, J. G. (2016). Perception of risk for domoic acid related health problems: A cross-cultural study. *Harmful Algae*, *57*, 39–44. https://doi.org/10.1016/j.hal.2016.03.007

Rollwagen-Bollens, G., Bollens, S., Dexter, E., and Cordell, J. (2020). Biotic vs. Abiotic forcing on plankton assemblages varies with season and size class in a large temperate estuary. *Journal of Plankton Research*, *42*(2), 221–237. https://doi.org/10.1093/plankt/fbaa010

Rose, V., Rollwagen-Bollens, G., Bollens, S., and Zimmerman, J. (2021). Seasonal and interannual variation in lower Columbia River phytoplankton (2005-2018): Environmental variability and a decline in large bloom-forming diatoms. *Aquatic Microbial Ecology*, *87*, 29–46. https://doi.org/10.3354/ame01967

Soga, M., and Gaston, K. J. (2018). Shifting baseline syndrome: Causes, consequences, and implications. *Frontiers in Ecology and the Environment*, *16*(4), 222–230. https://doi.org/10.1002/fee.1794

Statham, P. J. (2012). Nutrients in estuaries—An overview and the potential impacts of climate change. *Science of The Total Environment*, *434*, 213–227. https://doi.org/10.1016/j.scitotenv.2011.09.088

Taipale, S. J., Vuorio, K., Strandberg, U., Kahilainen, K. K., Järvinen, M., Hiltunen, M., Peltomaa, E., and Kankaala, P. (2016). Lake eutrophication and brownification downgrade availability and transfer of essential fatty acids for human consumption—ScienceDirect. *Environment International*, *96*, 156–166.

Trainer, V. L., and Hardy, F. J. (2015). Integrative monitoring of marine and freshwater harmful algae in Washington State for public health protection. *Toxins*, *7*(4), Article 4. https://doi.org/10.3390/toxins7041206

Trainer, V. L., Kudela, R. M., Hunter, M. V., Adams, N. G., and McCabe, R. M. (2020). Climate extreme seeds a new domoic acid hotspot on the US West Coast. *Frontiers in Climate*, *2*. https://doi.org/10.3389/fclim.2020.571836

Trainer, V., and Suddleson, M. (2005). Monitoring approaches for early warning of domoic acid events in Washington State. *Oceanography*, *18*(2), 228–237. https://doi.org/10.5670/oceanog.2005.56

Twining, C. W., Brenna, J. T., Hairston, N. G., and Flecker, A. S. (2016). Highly unsaturated fatty acids in nature: What we know and what we need to learn. *Oikos*, *125*(6), 749–760. https://doi.org/10.1111/oik.02910

Veldhuis, M. J. W., Timmermans, K. R. (2007). Phytoplankton dynamics during an *in situ* iron enrichment experiment (EisenEx) in the Southern Ocean: a comparative study of field and bottle incubation measurements. *Aquatic Microbial Ecology*, *47*(2), 191 – 208. https://doi.org/10.3354/ame047191