Slicing the Pie: Interpreting harmful algal blooms one pie chart at a time

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Abstract

The Earth's oceans are home to a diverse array of life, from large marine mammals to microscopic organisms. Among the most important are the marine phytoplankton, which comprise the basis of marine food webs, and also produce a large percentage of the Earth's oxygen through photosynthesis. Although the vast majority of phytoplankton are essential to ocean health, several dozen species produce potent toxins, and can form what are called Harmful Algal Blooms (HABs). This activity focuses on the importance of HABs, as well as the types of data scientists collect to understand blooms. In the classroom exercises, students will calculate the proportional abundance (% contribution) of five HAB species present in water samples, and use these data to create pie graphs to depict species composition. Students will then compare these results with levels of HAB toxins in water samples collected over the same time period. Thought questions challenge students to develop hypotheses regarding how changes in the HAB community may relate to observed trends in toxin concentrations. This activity was successfully taught to visually impaired students who were able to complete the pie charts and answer the thought questions.

Keywords

Harmful Algal Bloom (HAB), phytoplankton, diatoms, visual impairment, accommodations/adaptations

Introduction

The Earth's oceans are home to a vast and diverse array of life, from large marine mammals, fish and shellfish that are important food sources for the world's population, to microscopic organisms that form the base of marine food webs. These microscopic organisms include plant-like marine algae that are called phytoplankton, named after the Greek words *phyton* ("plant") and *planktos* ("drifter" or "wanderer"). Phytoplankton are members of a large and diverse group of single-celled organisms often referred to as "protists." Like terrestrial plants, they use water (H_2O) and carbon dioxide (CO_2) in the presence of sunlight to create energy (glucose) and oxygen (O_2) via the process of photosynthesis, and are frequently found in or near surface waters of the ocean and down to depths as far as the sunlight penetrates. In addition to serving as an important food source that sustains other animals in the ocean, phytoplankton photosynthesize to produce half or more of the world's oxygen, thereby creating the very air that we breathe. Most phytoplankton are tiny in size and cannot be seen without the aid of a microscope. Despite their small size, they can form massive aggregations (also referred to as blooms) that are sometimes large enough to visualize from space by earth-observing satellite systems.

Most phytoplankton species are both beneficial and necessary to sustaining life in the ocean and on earth; however, a small number of species can have harmful impacts to wildlife, ecosystems, and human populations. Some cause harm by producing potent toxins, which can accumulate in marine organisms that are filter feeders, such as shellfish (oysters, clams, mussels, and scallops), as well as other invertebrates and fish (reviewed by Backer and McGillicuddy 2006; Anderson et al. 2012). This often occurs when conditions are favorable for phytoplankton growth, leading to the formation of high algal densities, also known as a "harmful algal bloom" or HAB. Other phytoplankton species cause harm due to indirect effects of biomass accumulation (for example, by reducing availability of light to bottom communities) or by the physical features of the cells themselves (such as spines that lodge in fish gill tissue). During HAB events, shellfish consume large numbers of phytoplankton through filter feeding, and can accumulate toxins in their body tissues. These toxin-containing shellfish can be dangerous to both humans and wildlife consumers. Blooms of these species sometimes prompt closures of shellfish harvests to protect human health, which can span entire coastlines and cause economic losses to shellfish farmers, fisherman, and seafood suppliers. HABs are also widespread in freshwaters such as lakes, ponds, rivers, and these types of HABs impact all 50 U.S. states. Here, the toxin-producing species are cyanobacteria, and their toxins most frequently harm wildlife and domestic animals (pets and livestock), but can impact human health as well.

Many coastal regions of the U.S. are subject to blooms of multiple HAB species that can co-occur (Anderson et al. 2021). This presents challenges to managers responsible for using critical data to open or close harvests, thus ensuring seafood safety. In the New England region, certain toxin-producing diatoms in the genus *Pseudo-nitzschia* (Fig. 1) have recently emerged as a threat to seafood safety. The first ever shellfish harvesting closure in the region associated with *Pseudo-nitzschia* blooms occurred in 2016, when a massive and unprecedented outbreak caused the closure of shellfish beds along the New England coast, triggering concerns of a region-wide public health emergency and recalls of shellfish (blue mussels, quahogs, clams and European oysters) that were harvested just before the closure (see review by Bates et al. 2018). Blooms of toxic *Pseudo-nitzschia* recurred in 2017, prompting shellfish closures as well as a recall of blue mussels harvested from certain coastal locations in eastern Maine.

The diatom genus *Pseudo-nitzschia* consists of more than 50 species, but only some are considered harmful. These harmful species produce a toxin called domoic acid. In areas with elevated concentrations of toxin-producing species, domoic acid can accumulate in shellfish and fish (Fig. 2) and sicken human and wildlife consumers. Eating contaminated seafood causes the human poisoning syndrome known as "amnesic shellfish poisoning" because memory loss is one of the symptoms. Other symptoms include stomach upset, headaches, confusion, weakness, and seizures. There is no known antidote or cure, and while recovery is possible, some of the effects of domoic acid in humans can be long lasting. Wildlife also experience the effects of domoic acid poisoning, and *Pseudo-nitzschia* blooms have been the cause of abnormal behavior, illness, and mortality in marine mammals (seals, sea lions, dolphins) and seabirds (Bejarano et al. 2008).

Like many phytoplankton species, *Pseudo-nitzschia* diatoms undergo alternating phases of sexual and asexual reproduction (Fig. 3). In growing populations, these diatoms can create long chains or colonies of overlapping cells that allow them to move through the water column (Fig. 1). The life cycle of some diatoms includes a resting stage that facilitates survival during unfavorable conditions, but this has not been documented for *Pseudo-nitzschia* (Amato et al. 2005; Montresor et al. 2013).

Distinguishing toxic from non-toxic *Pseudo-nitzschia* species can be challenging, as visualization of certain key internal cellular structures generally requires advanced microscopic techniques (e.g., Kaczmarska et al. 2005; Fernandes et al. 2014). An alternative approach is to use molecular biology techniques referred to as DNA "fingerprinting" to characterize the *Pseudo-nitzschia* species present in a water sample based on length variation in certain gene regions (e.g., Hubbard et al. 2014). Rather than identifying cells in a sample using a microscope, DNA is extracted and a particular gene region is targeted for analysis, and used to characterize the species composition and proportional abundance of each species in a field sample. By looking at the composition of the phytoplankton communities in a water sample, it can be possible to determine the presence and abundance of toxic species, which provides indication of higher risk of toxicity in seafood.

In this activity for middle school classrooms (Grades 6-8), students will learn about several grade-appropriate scientific concepts through the background information and data exercises focused on HABs, including the importance of phytoplankton to ocean health and life on earth, the life cycle characteristics of phytoplankton species that include both sexual and asexual reproduction, and the relationship between ecosystem health and human health. Through the data exercises, students will learn how scientists can use DNA approaches to study HAB species and analyze "real" data collected by scientists over multiple months and years of sampling to assess changes in HAB communities over time. Students will carry out grade-appropriate calculations of proportional abundance and use these data to complete pie charts depicting changes in HAB species and toxin levels over seasons and across years. The last exercise is designed to illustrate the first reported occurrence of the highly toxic species *P. australis* to New England, so data from both 2014 (pre-occurrence) and 2017 (post-occurrence) will be compared (Clark et al. 2019; Hubbard et al. 2017). Working in groups, students will plot results, and interpret species dynamics in relation to toxin concentrations measured in 2017.

The thought questions are designed to prompt students to reflect on the importance of regular and repeated sampling in scientific studies to adequately observe phytoplankton and HAB dynamics, and their impacts, over varied temporal scales. Students will also be challenged to develop their own hypotheses about which species may be harmful based on the trends they observe over time in relation to toxicity. This activity is timely given the shellfish harvesting closures recently enacted by several New England states due to concerns regarding domoic acid in shellfish (Bates et al. 2018). These events coincided with the appearance of the highly toxic species *P. australis* to New England waters (Clark et al. 2019; Hubbard et al. 2017), which has bloomed annually in the region ever since. Although this activity is based on data from the Northeast US, it has relevance to students throughout the country because freshwater HABs have been documented in all 50 states, and are a growing problem in many regions. The thought questions are designed to encourage students to think about the role of phytoplankton in food webs, which organisms might rely on this food source, and how humans and wildlife may be affected. Figure 2 provides an overview of how HAB toxins enter and are transferred in marine food webs, and could be presented to prompt students to offer possible explanations.

Key Words

Amnesic shellfish poisoning – Harmful Algal Boom-associated human poisoning syndrome caused by consumption of the marine biotoxin domoic acid, causing permanent short-term memory loss, brain damage, and occasionally death in severe cases.

Bp, or base pairs – Unit of double-stranded DNA, defined as two complementary nitrogenous molecular bases, connected by hydrogen bonds.

Diatom – Widespread group of phytoplankton with cell walls composed of silica.

DNA (deoxyribonucleic acid) – Chemical name of the molecule that provides the hereditary material in all living organisms.

DNA fingerprinting – Laboratory technique that allows scientists to identify a particular individual, species, or group based on their respective DNA profiles.

Domoic acid – Neurotoxin produced by certain diatom species in the genus *Pseudo-nitzschia* that causes the HAB poisoning syndrome known as amnesic shellfish poisoning.

Genus - taxonomic rank used in the classification of organisms that is a higher level grouping above species.

Harmful Algal Bloom, or HAB – Rapid growth of a particular algal species, leading to toxic or harmful effects on people, shellfish, fish, marine mammals, and birds.

Phytoplankton – Passively drifting photosynthetic organisms such as diatoms.

Protist – Diverse taxonomic grouping of eukaryotic organisms that are primarily unicellular and sometimes colonial, and include protozoans, most algae, and some fungi-like organisms (slime molds).

RFU, relative fluorescence units – Measurement used in molecular analysis which employs fluorescence detection in the analysis of DNA fragments; samples containing higher quantities of DNA will have higher corresponding RFU values.

<u>Activity Introduction</u>

This activity is focused on harmful algal blooms (HABs), and in particular toxin-producing diatoms in the genus *Pseudo-nitzschia* that can cause illness in humans and wildlife. Students learn how DNA approaches are used to study HAB species, and plot *Pseudo-nitzschia* species composition data collected over multiple years. The thought questions will challenge students to develop hypotheses based on their interpretation of temporal patterns of species dynamics and toxicity (domoic acid concentrations), and to think about the sampling strategies used to investigate community dynamics.

The classroom activity, teacher answer key, and supplemental graphics with enlarged pie charts are provided in the Supplemental Information. This activity includes some modifications for the visually impaired. For example, sketches are provided with thick black lines with high visual contrast that can be seen by students who have some vision, and/or can be printed out on a Pictures in a Flash (PIAF) machine, which elevates black ink when printed on a special paper, enabling students to discern the outline by touch (e.g., Figs. 4 & 5). Some of these students colored in the pie charts (Fig. 6), while others were provided pre-cut slices of paper with various textures (e.g., smooth, rough, dotted) (Fig. 7). Enlarged versions of the pie charts are provided in the Supplemental Information.

Standards

This activity was designed for 6^{th}-8^{th} graders (approximately 11-13 years old). Middle-school standards are provided below, although this activity can also be completed by high school students with enhancement of the activity by asking for students to research HABs in their region (see Modifications and Cross-curricular Expansion section, below).

Next Generation Science Standards (NGSS Lead States 2013)

MS-LS1-6 From Molecules to Organisms: Structures and Processes

Construct a scientific explanation based on evidence for the role of photosynthesis in the cycling of matter and flow of energy into and out of organisms.

MS-LS2 Ecosystems: Interactions, Energy, and Dynamics

Performance Expectations: MS-LS2-4. Construct an argument supported by empirical evidence that changes to physical or biological components of an ecosystem affect populations.

Science and Engineering Practices: MS-LS2-1: Interdependent relationships in ecosystems.

Crosscutting Concepts: MS-LS2.5: Science addresses questions about the natural and material World.

MS-LS2-1 Ecosystems: Interactions, Energy, and Dynamics

Analyze and interpret data to provide evidence for the effects of resource availability on organisms and populations of organisms in an ecosystem.

Grade: Middle School (6-8)

MS-LS2-3 Ecosystems: Interactions, Energy, and Dynamics

Develop a model to describe the cycling of matter and flow of energy among living and nonliving parts of an ecosystem.

Grade: Middle School (6-8)

MS-LS2-4 Ecosystems: Interactions, Energy, and Dynamics

Construct an argument supported by empirical evidence that changes to physical or biological components of an ecosystem affect populations.
Grade: Middle School (6-8)

MS-LS4-1; HS-PS1-2: Patterns: Observed patterns in nature guide organization and classification and prompt questions about relationships and causes underlying them.

MS-ESS3-3 Earth and Human Activity: Apply scientific principles to design a method for monitoring and minimizing a human impact on the environment.

Mathematics through the National Council of Teachers of Mathematics (http://www.nctm.org/Standards-and-Positions/Principles-and-Standards/Principles,-Standards,-and-Expectations/)

Grades 6-8

Understand numbers, ways of representing numbers, relationships among numbers, and number systems

Develop meaning for integers and represent and compare quantities with them.

Understand measurable attributes of objects and the units, systems, and processes of measurement

Understand both metric and customary systems of measurement;

Solve problems involving scale factors, using ratio and proportion;

Formulate questions that can be addressed with data and collect, organize, and display relevant data to answer them

Formulate questions, design studies, and collect data about a characteristic shared by two populations or different characteristics within one population;

Select, create, and use appropriate graphical representations of data, including histograms, box plots, and scatterplots;

Select and use appropriate statistical methods to analyze data;

Find, use, and interpret measures of center and spread, including mean and interquartile range;

Discuss and understand the correspondence between data sets and their graphical representations, especially histograms, stem-and-leaf plots, box plots, and scatterplots.

Develop and evaluate inferences and predictions that are based on data

Use observations about differences between two or more samples to make conjectures about the populations from which the samples were taken;

Make conjectures about possible relationships between two characteristics of a sample on the basis of scatterplots of the data and approximate lines of fit;

Ocean Literacy Principles (National Marine Educators Association 2013)

Principle 5: The ocean supports a great diversity of life and ecosystems

5F. Ocean ecosystems are defined by environmental factors and the community of organisms living there. Ocean life is not evenly distributed through time or space due to differences in abiotic factors such as oxygen, salinity, temperature, pH, light, nutrients, pressure, substrate, and circulation. A few regions of the ocean support the most abundant life on Earth, while most of the ocean does not support much life.

Principle 6. The ocean and humans are inextricably interconnected.

6D. Humans affect the ocean in a variety of ways. Laws, regulations, and resource management affect what is taken out and put into the ocean. Human development and activity lead to pollution (point source, nonpoint source, and noise pollution), changes to ocean chemistry (ocean acidification), and physical modifications (changes to beaches, shores, and rivers). In addition, humans have removed most of the large vertebrates from the ocean.

6G. Everyone is responsible for caring for the ocean. The ocean sustains life on Earth and humans must live in ways that sustain the ocean. Individual and collective actions are needed to effectively manage ocean resources for all.

Climate Literacy Principle

Principle Seven: Climate change will have consequences for the Earth system and human lives.

Materials list

Activity Packet, one per student (See Supplemental Information to download)

Teacher answer key (See Supplemental Information to download)

Printouts of Figures 1-3 to show students

Colored pencils or crayons

Optional: Plush toys or 3D printed models of phytoplankton. The code for producing 3D models, including *Pseudo-nitzschia diatoms*, is publicly available here: https://sites.google.com/site/drjeffreywkrause/diatom-models).

Optional: models of marine mammals impacted by HABs (e.g., seals, dolphins, seabirds)

Optional: bivalve shells (e.g., mussels, clams, scallops)

Supplemental material for visually impaired students:

Enlarged pie charts (See Supplemental Information to download)

Raised print PIAF handouts

Braille writer and paper

Textured paper for creating pie charts

Note that the 3D printed models and raised print figures were designed to accommodate visually impaired students, but were also appreciated by sighted students and teachers so were included in the materials listed above

Safety

Students will be handling paper and pencils and any models the teacher has available. Some objects might be sharp, smelly (bivalve shells), or heavy.

Time Requirement

The activity can be completed in 50 minutes, although it can be longer if a more detailed introduction is provided and/or if some of the thought questions are discussed as a group.

Activity

The teacher will distribute the Activity Packet (See Supplemental Information to download) and writing utensils to the students who will then be assessing the dynamics of *Pseudo-nitzschia* species in New England during 2014 and 2017. Some species in this group produce a potent neurotoxin called domoic acid. This toxin, measured in the metric system (micrograms per liter of seawater), is of concern for communities as it causes a human poisoning syndrome known as amnesic shellfish poisoning, and can also sicken or kill wildlife, including marine mammals (e.g., seals, dolphins, whales) and seabirds.

In the activity, students will identify which species were present from DNA fingerprinting data generated from the water samples collected from July to October of each year. They will calculate the proportional abundance of each of the five species (Fig. 4), depict those data as pie graphs to assess species composition (Fig. 5-7), and compare results over time, along with data on domoic acid concentrations collected over the same time period in 2017 (Fig. 8). The thought questions challenge students to identify temporal changes in community structure and to develop hypotheses regarding which species may be toxin producers, based on comparisons with domoic acid levels obtained at the time of sampling (Fig. 9).

The teacher may choose to break students into groups of 2-4 and ask each group to identify species present at each sampling date based on the “trace” files for a given sample, and the table depicting DNA fragment size (Table 1 in the Activity Packet provided in Supplemental Information). More than one species may be present. Students will calculate the proportional abundance (percent contribution) of each species based on the y axis of trace file figures depicted on page 3 of the Activity Packet, and fill in the worksheet sheet on page 5. If working in groups, perhaps each student could complete two time periods. Students then color in the corresponding pie graphs associated with each sampling time period according to the proportional abundance data recorded in the worksheet for both 2014 and 2017 (Activity packet pages 4 and 5).

Upon completion, the teacher can project the graph for the entire 2017 time series (Activity Packet, page 6), and students can either cut out and tape their completed pie chart on the line graph over the corresponding date, or simply recolor the pie chart provided on the projection. Domoic acid concentrations are presented on the y-axis of this figure, and students can use that information along with the species present in their pie charts to develop hypotheses regarding which species might be toxin producers. The last exercise is designed to illustrate the appearance of the highly toxic species *P. australis* in this region, so data from both 2014 (pre-introduction) and 2017 (post-introduction) will be compared (Activity packet page 7). The thought questions are designed to reinforce concepts regarding the importance of regular and repeated sampling over time to adequately observe HAB dynamics and their impacts over temporal scales. Students will be asked to reflect upon the temporal changes they observe in these datasets (within and across seasons and years) as well as hypothesize which species may be significant toxin producers.

Modifications and Cross-curricular Expansion

This activity could be expanded to include group discussion of additional thought questions, which can also be provided to students who finish the activity early. These questions include:

- Name some photosynthesizers / primary producers in your area. Describe a food web starting with these organisms and ends with humans. What's the shortest web (fewest links) you can think of? Try thinking of a terrestrial web as well as a marine web. If time permits, sketch your web.

- What types of species might be affected by HABs in freshwater systems such as lakes, rivers, and ponds ?
- There are regulations in place to reduce the likelihood of being exposed to unsafe food products. What have been some recent news stories about a contaminated food supply? What was the source of the contamination and how did the government respond to the exposure?
- Have you ever had food poisoning? In retrospect, was there anything you could have done to prevent your exposure?

High school students may also enjoy completing the activity and reviewing the mathematics involved. The assessment questions could be made more challenging by asking these students to investigate recent HAB events in their regions and writing about potential impacts to recreational activities and food safety. Suggestions for further mathematical calculations appropriate for higher grade levels are provided below.

For additional activities related to food webs and the role of photosynthesizers, see the Resources section (below).

Mathematics

Further mathematical calculations could be completed by students who finish the activity early or for higher grade levels. Students could be asked to provide the range of percent composition values per species for each month within and/or across the two years. There would be two values per month per species if only assessing one year, and four values if assessing both years together. Students could also obtain the mean value per month by species to see if the basic species composition trends are similar to the finer scale approach outlined in the Activity Packet.

Reflections

We tested this out both in-person and virtually in 8th grade classrooms with up to 20 students, including at a school for visually impaired students. For the sighted classrooms, there was one teacher present in person and one author via video. For the visually impaired classes, there were 4-5 students with one teacher, one aide, and one author via video. Teachers appreciated the introduction in the beginning as it is relevant to the lesson on protists, as well as the information about the scientific classification of organisms and binomial nomenclature, and the life cycle depiction of asexual and sexual reproduction of a phytoplankton species. In our pre-discussion, students in both groups knew that phytoplankton were an important part of the food web, and that they generate oxygen. We were impressed that a student knew that sea birds could be impacted by contaminated shellfish, as the role of birds in estuarine food webs is probably underrepresented.

During classroom testing of this activity, students enjoyed coloring the pies so much that they preferred to recolor them on the classroom whiteboard for the group discussion instead of cutting out and taping their completed pies. Plus, the teacher preferred not to distribute scissors and tape. After testing the activity, we modified it so that the materials do not have to be printed in color, as we added the words necessary to depict which color should be used for each species. We were mindful to use dark/saturated colors that would be more accessible to the visually impaired.

Students better recognized and interpreted the temporal patterns in community composition by completing the pie charts rather than viewing the data presented in tables. There were plenty of comments from both groups about the value of completing the graphs and interpreting the data. This reinforces the importance of doing/creating the work and not just being handed finished materials. They asked insightful questions about the role of El Niño and the influence of temperature on the results. Some students knew that increased temperatures are sometimes associated with algal blooms. Students asked about questions about sampling strategies employed in field sampling, which led to good discussions about the need for repeated sampling and replicates. Students asked questions about which animals are impacted by *Pseudo-nitzschia* blooms, and provided insightful reflections about the relevance of such science ("people could die") and how toxic algal species could increase their geographic range ("currents"). A student knew that fertilizers from agriculture operations have been associated with algal blooms and eutrophication. We were asked about the acidity of domoic acid, and that led to an interesting discussion about the word "acid," and its chemical composition. Interestingly, the student who was allergic to seafood had some of the most insightful comments and reflections about the relevance of the material presented.

Resources

Additional K-12 activities about food webs and plants in marine/estuarine environments

Aultman, T. and M.C. Curran. 2008. Grass shrimp: Small size but big role in food web. Current: The Journal of Marine Education 24(3):29-33.

Curran, M.C. and T. Fogleman. 2007. Unraveling the mystery of the marsh: Training students to be salt marsh scientists. Current: The Journal of Marine Education 23(2):25-30.

Fogleman, T. and M.C. Curran. 2006. Save our salt marshes! Using educational brochures to increase student awareness of salt marsh ecology. Current: The Journal of Marine Education 22(3):23-25.

Fogleman, T. and M.C. Curran. 2007. Making and measuring a model of a salt marsh. NSTA: Science Scope 31(4):36-41.

Fogleman, T. and M.C. Curran. 2008. How accurate are student-collected data? NSTA: The Science Teacher 75(4):30-35.

Other activities suitable for the visually impaired

Curran, Mary C., Amy S. Bower, and Heather H. Furey. 2017. Detangling spaghetti: Tracking deep ocean currents in the Gulf of Mexico. *Science Activities*. http://dx.doi.org/10.1080/00368121.2017.1322031

Curran, Mary C., Andree L. Ramsey, and Amy S. Bower. 2021. Learning about ocean currents one track at a time. *Science Activities.* DOI: 10.1080/00368121.2021.1885333

Curran, Mary C. and Mindy L. Richlen. 2019. Harmful Algal Blooms (HABs): Track them like a scientist. *Science Activities*. DOI: 10.1080/00368121.2019.1691968.

Curran, Mary C. and Alison Robertson. 2020. Chemistry made easy: Teaching students about the link between marine chemistry and coral reef biodiversity. *Current: The Journal of Marine Education* 34(2):1-11. DOI: https://doi.org/10.5334/cjme.39

Curran, Mary C., Laela S. Sayigh, and Kathleen Patterson. 2019. Eavesdropping on marine mammal conversations: An activity suitable for the visually impaired. *Current: The Journal of Marine Education* 33(2):33–42.

Sukkestad, Kathryn and Mary C. Curran. 2012. Noodling for mollusks. *NSTA: The Science Teacher* 79(8): 38–42.

Thompson, Coral A., Sue C. Ebanks, and Mary C. Curran. 2016. Shrimp Socktail: The shrimp you feel instead of peel. *Current: The Journal of Marine Education* 30(1): 35–47.

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Declaration of interest statement

The authors declare they have no actual or potential competing financial interests.

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Figure Captions

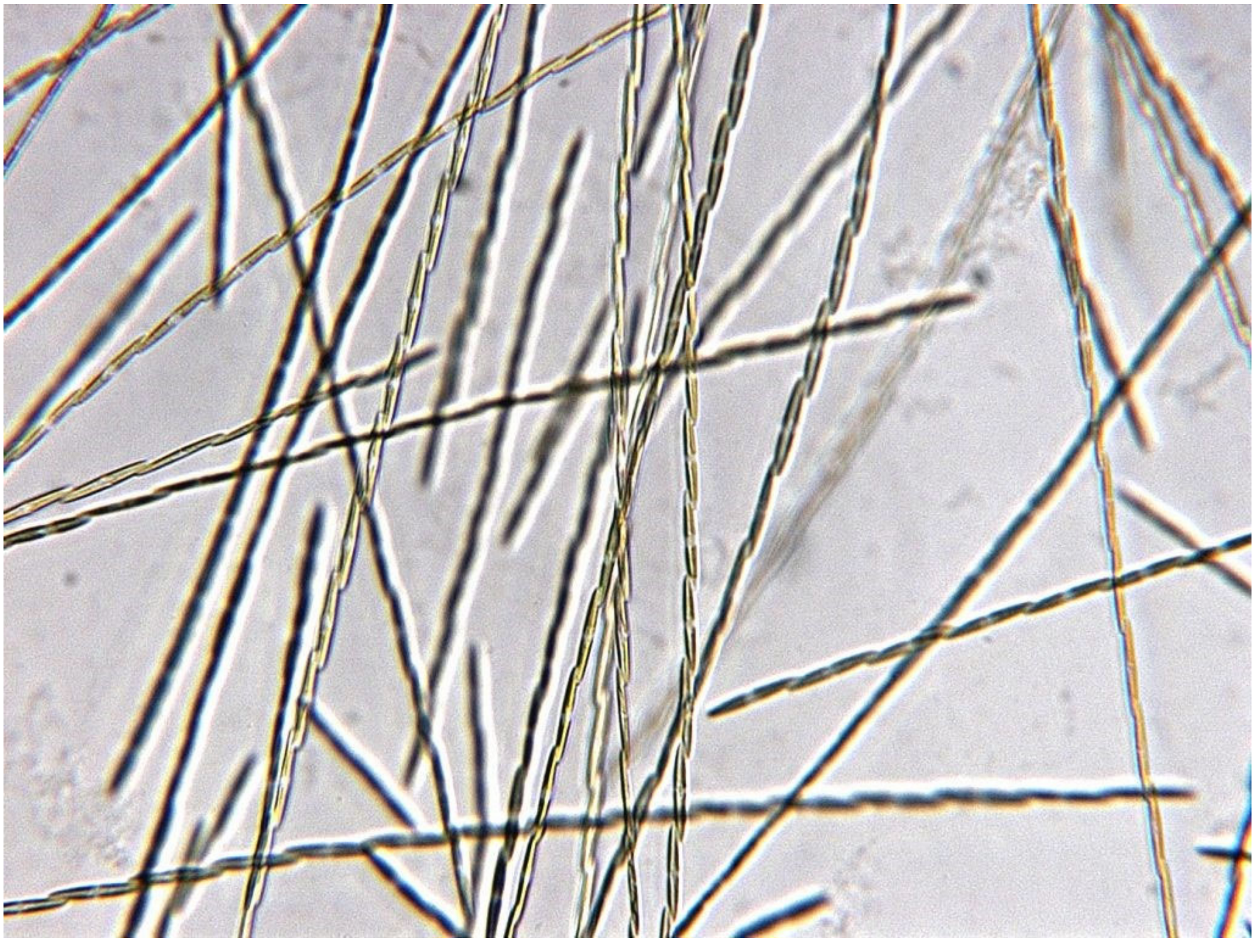


Figure 1. Chains of *Pseudo-nitzschia* sp. isolated from the Gulf of Maine (Photo by XX).

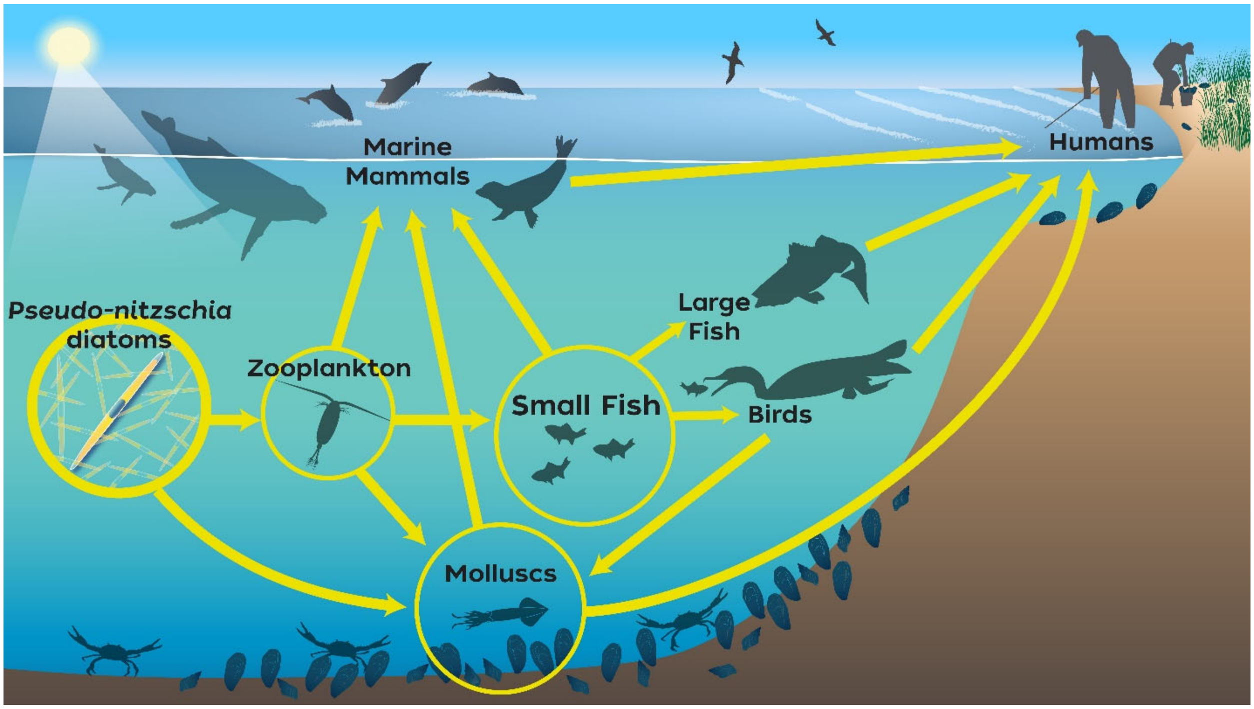


Figure 2. Domoic acid produced by certain *Pseudo-nitzschia* species can accumulate in the marine food web and sicken human and wildlife consumers when these diatoms are eaten by zooplankton, fish, and shellfish that are, in turn, eaten by humans, marine mammals, and seabirds. Illustration created by Natalie Renier, WHOI Graphic Services.

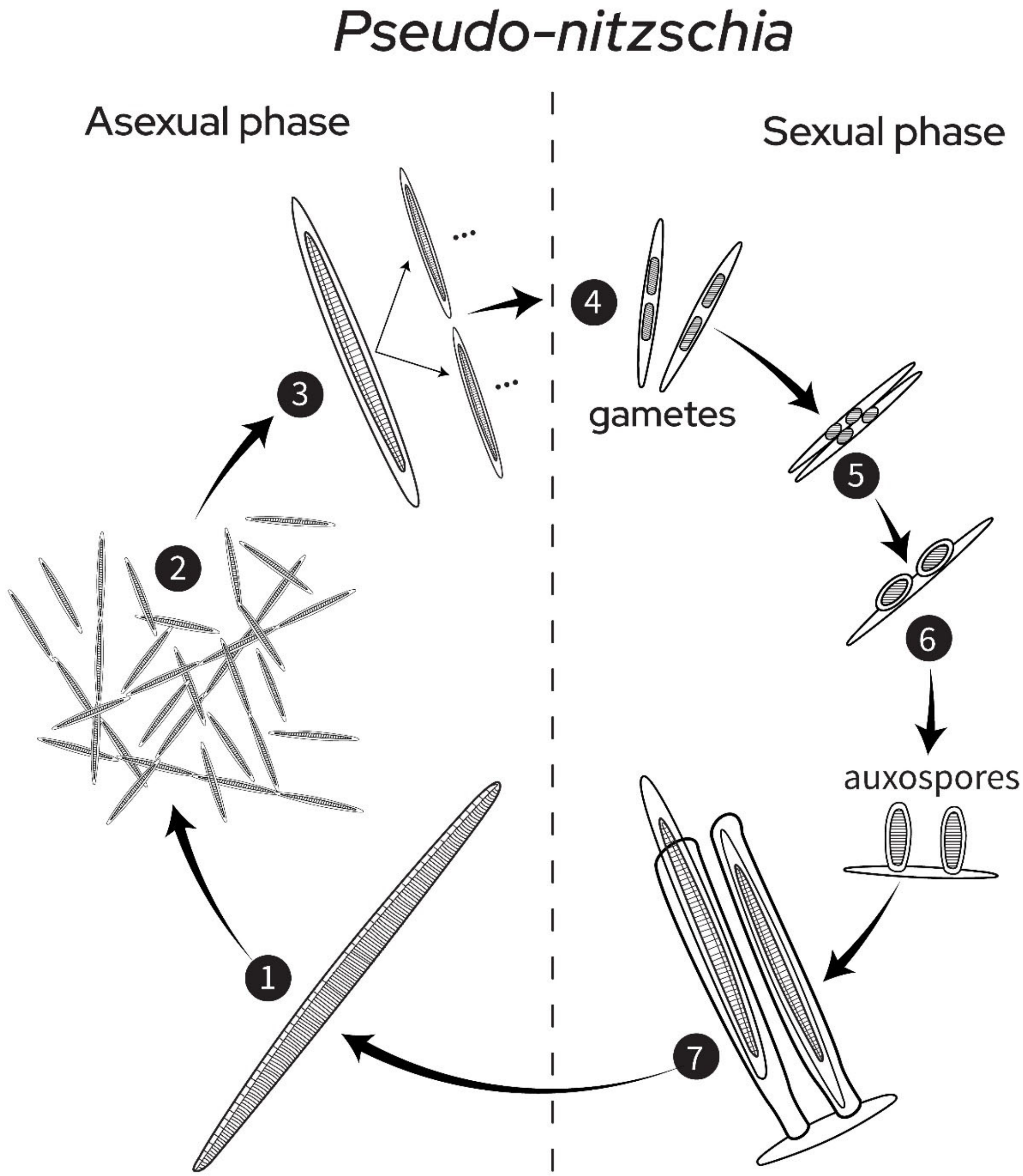
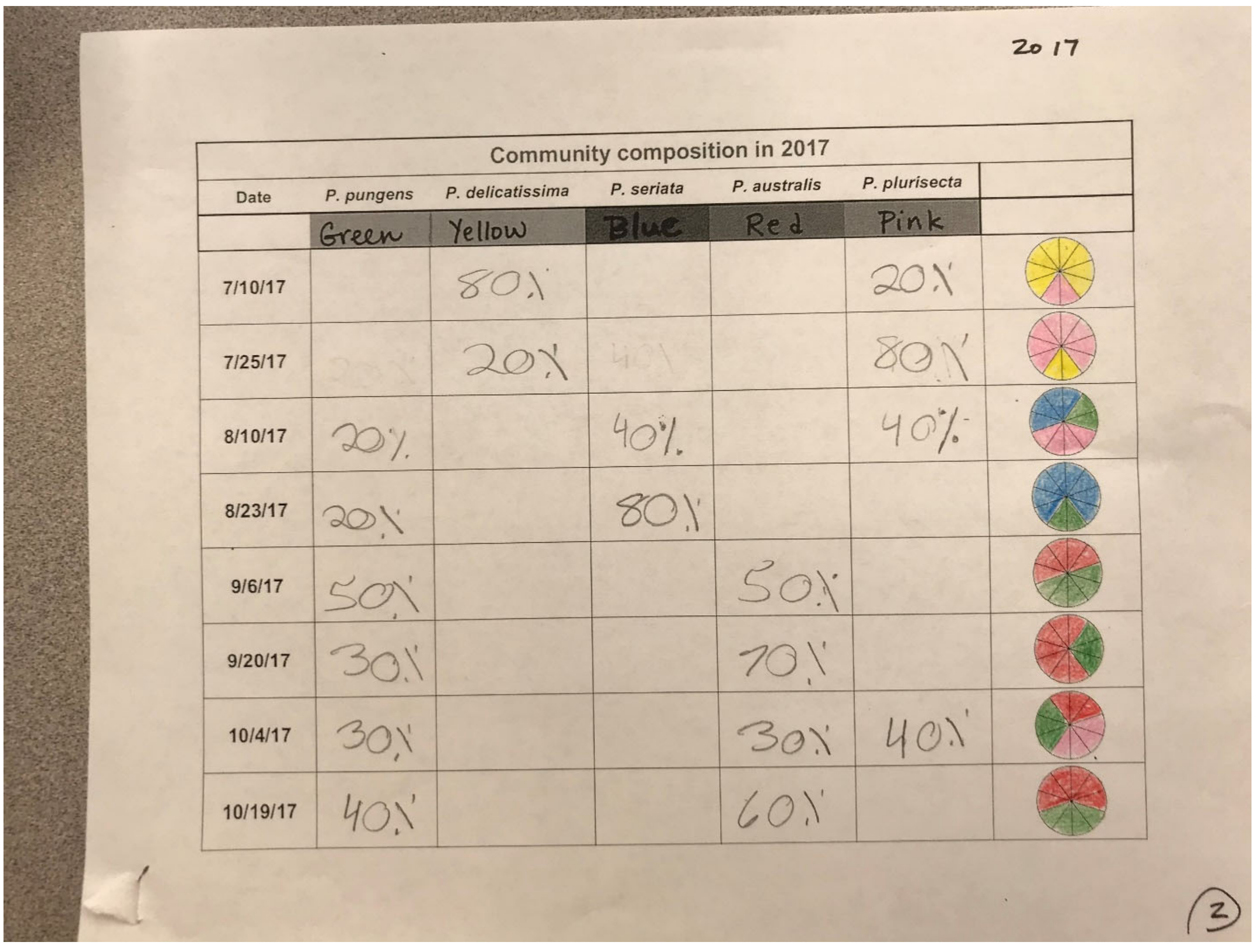


Figure 3. Pennate diatoms such as *Pseudo-nitzschia* are protected by a silica cell wall, called a frustule, which is composed of two nested halves. (2) During asexual cell division *Pseudo-nitzschia* can form long chains of cells, which fragment as they grow; (3) With each division the overlapping halves separate, and each secretes a smaller lower half. Over time, this results in size reduction of the population; (4) Cell size cannot decrease indefinitely, so once the diatom reaches about half of its original size it must reproduce sexually. Two parent cells align and each

one forms two gametes. (5) One parent produces active gametes and the other produces passive gametes. Active gametes migrate to meet the passive gamete and fuse. (6) The fused gametes from a structure called the auxospore. These auxospores elongate and protect the developing *Pseudo-nitzschia* cell until it is fully grown. (7) Developed cells exit the auxospore. Graphic and text adapted from Chepurnov et al. (2005), Davidovich and Bates (1998), D'Alelio et al. (2009), and Sarno et al. (2010). Illustration created by Natalie Renier, WHOI Graphic Services.



2017

Community composition in 2017						
Date	*P. pungens*	*P. delicatissima*	*P. seriata*	*P. australis*	*P. plurisecta*	
	Green	Yellow	Blue	Red	Pink	
7/10/17		80%			20%	
7/25/17		20%			80%	
8/10/17	20%		40%		40%	
8/23/17	20%		80%			
9/6/17	50%			50%		
9/20/17	30%			70%		
10/4/17	30%			30%	40%	
10/19/17	40%			60%		

2

Figure 4. Completed percent species composition table for 2017. The pie charts in the last column of this table can be cut out and taped on the corresponding timepoint on the line graph depicted in Figure 8 (also see Activity Packet; Supplemental Information), or students can recolor the pie charts provided on the figure (which many enjoyed doing).

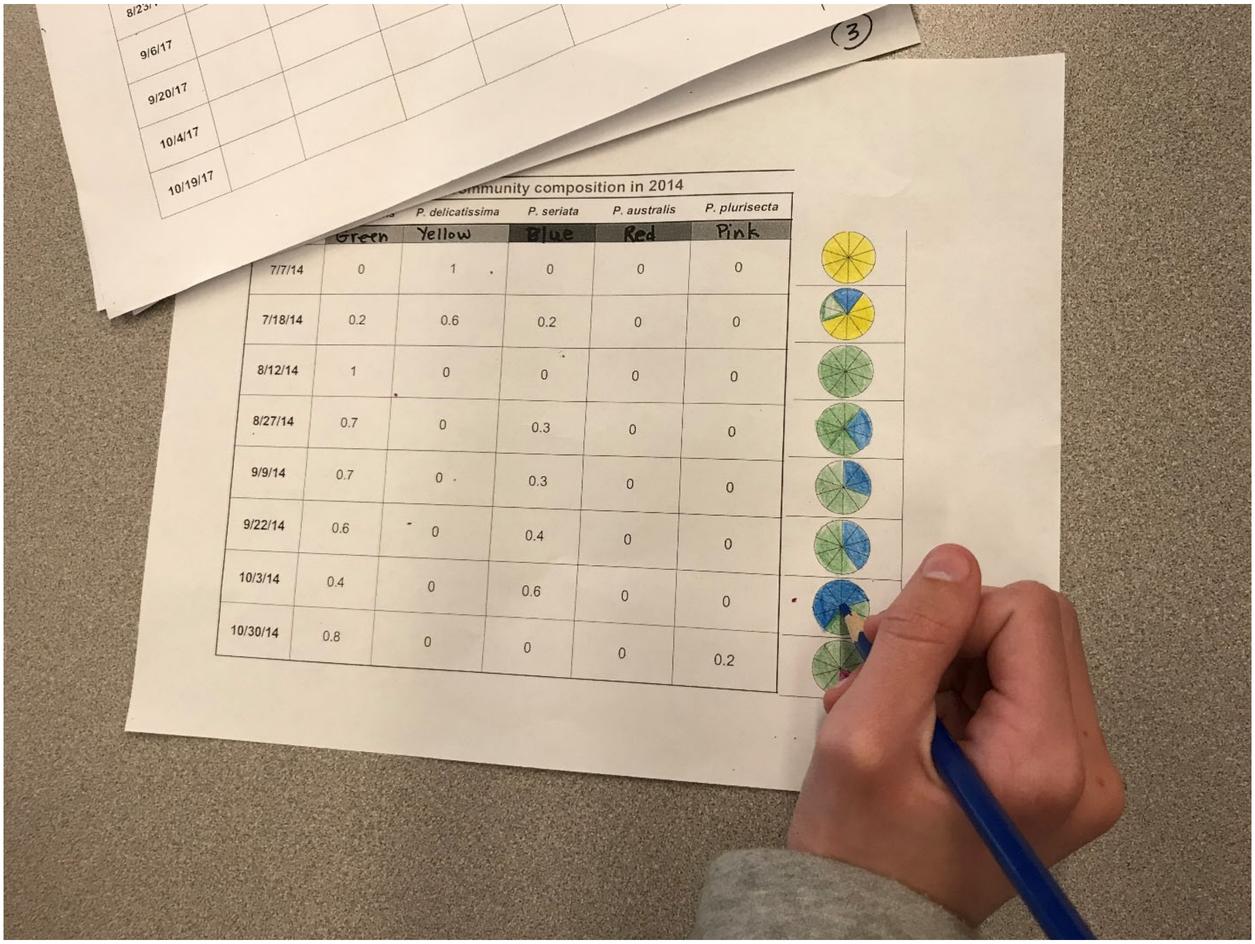


Figure 5. Student completes proportional abundance table and colors in the pie graphs depicting *Pseudo-nitzschia* species composition for each sampling timepoint in 2014.

Figure 6. Student colors in the slices of the pie graph to depict the proportional abundance (percent contribution) of each *Pseudo-nitzschia* species.

573

Figure 7. Student places pre-cut pieces into a pie graph depicting proportional abundance (percent contribution) of HAB species. Materials can have different textures to aid the visually impaired.

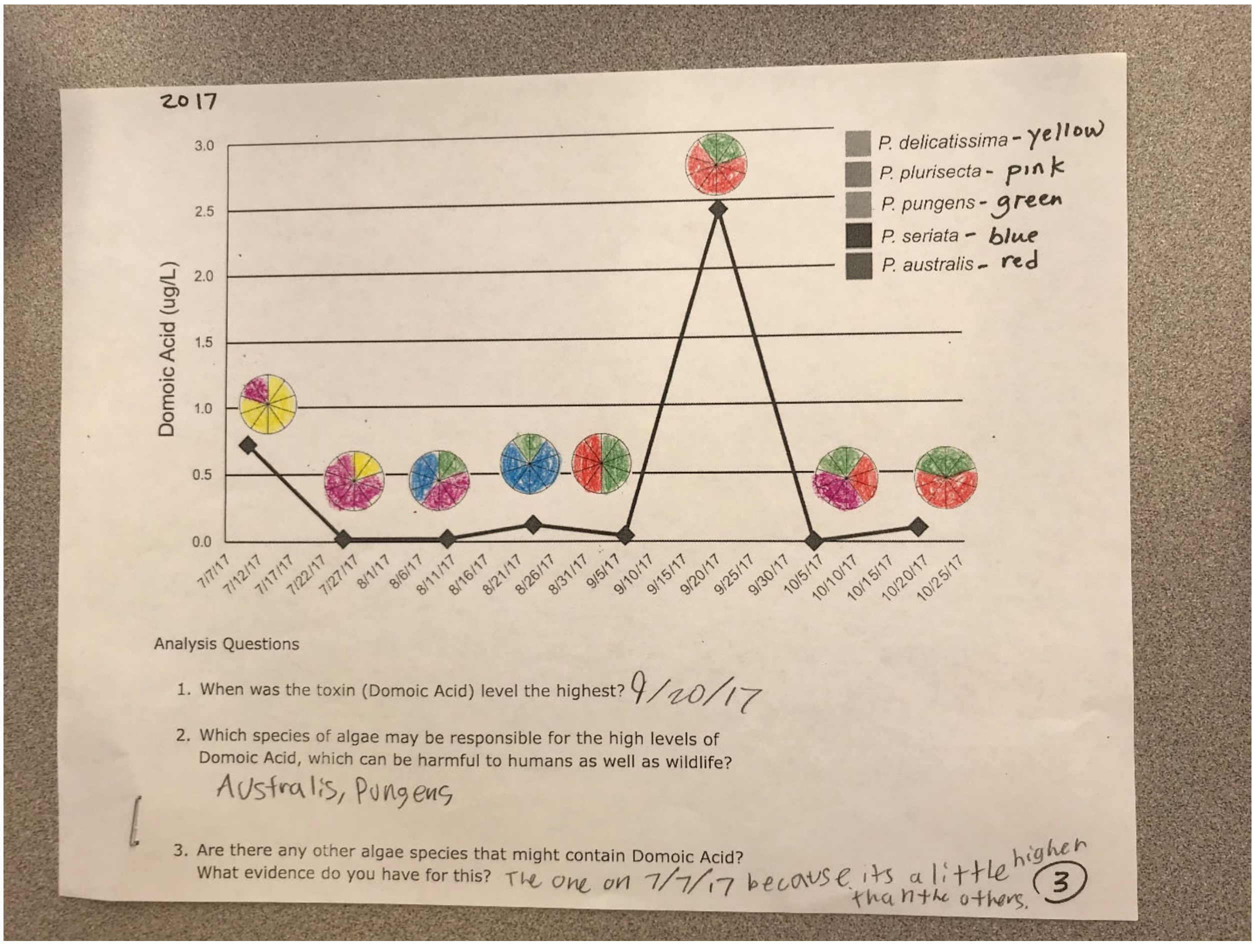


Figure 8. Student superimposes pie charts depicting *Pseudo-nitzschia* community composition on the Exercise Figure 2 (provided in the Activity Packet; see Supplemental Information), which also shows domoic acid concentrations over time in 2017. This figure could be projected onto a white board for the group to complete.

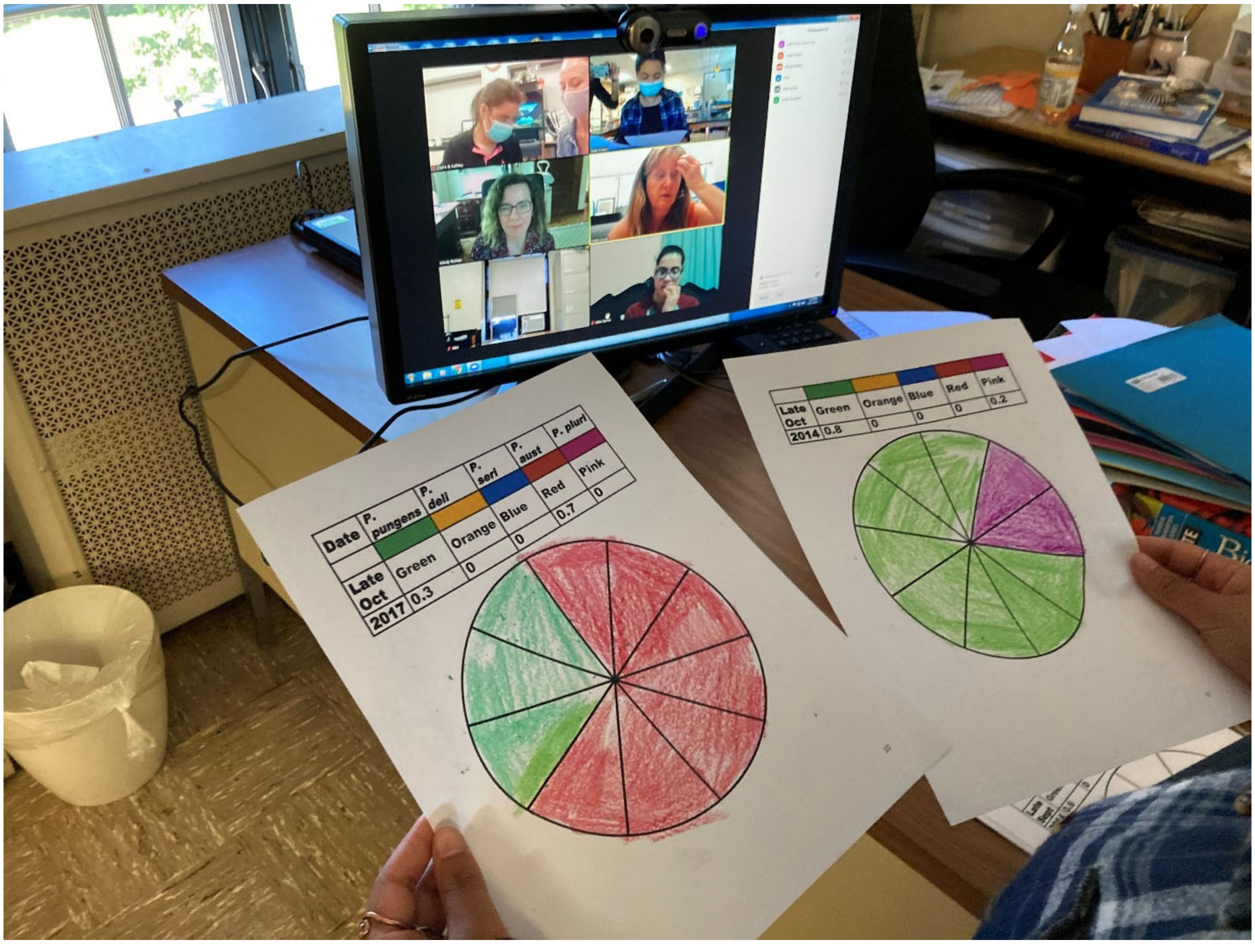


Figure 9. Students on Zoom and in person discuss the proportional abundance (percent contribution) of HAB species within and across seasons and years.

Slicing the Pie Activity Packet **Your Name/s:___________________________**

Harmful Algal Blooms (HABs) are a concern in many coastal ocean regions as well as rivers and lakes. This activity is based on research conducted in the Northeast Atlantic during 2014 and 2017 on a HAB group called *Pseudo-nitzschia*. Some species in this group produce a potent neurotoxin called domoic acid. You will be identifying which species were present using data from DNA collected from water samples collected semi-monthly from July to October of each year. You will then be calculating the proportional abundance (percent contribution) of each of the five species, representing those data as pie graphs, and then comparing those results over time. The thought questions are designed to reinforce your ability to identify changes in the HAB community over time and to hypothesize which species are toxic (based on comparisons with Domoic Acid levels measured at the time of sampling). This toxin is of concern for human health as it causes an illness known as amnesic shellfish poisoning.

The teacher may break you up into groups of 2-4. There are 8 dates for which you must calculate the percent (%) contribution of each species. Each student should could complete at least two dates. A larger print version suitable for the visually impaired is provided the Supplemental Information.

1. Use the information in the Table 1 (below) to identify the species found in the "trace" files for your assigned date/s on page 3 of this Activity Packet. There can be multiple species for any given date. Once you identify the species present in each sample, calculate the proportional abundance (percent contribution) of each species and add it to the Exercise 1 sheet on page 5. An example calculation is shown below. Color in the pie graphs for 2014 (page 4, data provided) and 2017 (page 5).

   Example calculation: The DNA fragment size (base pairs) is oriented on the x axis and the proportional abundance on the y axis. If there is only one peak on the trace file, then there was only one species detected. If, for example, the peak was at 200 base pairs on the x axis, then using the table below, you can determine that this peak represents the species *Pseudo-nitzschia seriata*. You would then use the peak height to determine the proportional abundance of this species (ranging between 0 and 1). In this example, the peak height is 1. If you multiply by 100 to obtain proportional abundance expressed as percent total, then there is a 100% occurrence of that species. Fill in your assigned dates from 2017 and then color in the corresponding pie using the assigned color system. Don't forget to color in the pie graphs for 2014 using the data provided (page 4).

Table 1. DNA fragment size (expressed in base pairs) for each *Pseudo-nitzschia* species

Species	DNA Fragment Size (base pairs)
Pseudo-nitzschia pungens	142
Pseudo-nitzschia australis	150
Pseudo-nitzschia delicatissima	168
Pseudo-nitzschia seriata	200
Pseudo-nitzschia plurisecta	226

2. After you finish your pies, cut them out, put the date on the back, and tape them onto the graph below that depicts domoic acid (in micrograms per liter (ug/L; Exercise 2; page 6). If preferred, or to avoid the need for scissors and tape, you can also recolor the pies on page 5. When was the toxin (domoic acid) level the highest?

3. **The teacher will project the completed graph with all the pies provided (Exercise 2; Answer Key page 6).** Which species may be responsible for the high levels of domoic acid, which can be harmful to humans as well as wildlife? You can abbreviate the first word (the genus) by writing "P." Note that you should underline a genus and species to denote its Latin origin when writing by hand:

4. Are there any other species that might contain domoic acid? Which ones? What evidence do you have for this?

Now let's take a closer look at any similarities over time. We refer to these as temporal changes. Using Exercise 3, complete the pies for 2014 on page 7 using the data in the provided table on page 4. Once those are completed, you will be comparing them to the pies you created for 2017 and answering the following questions. Enlarged blank pie charts suitable for the visually impaired are provided in the Supplemental Information.

1. What similarities or differences do you see within the same month (early vs. late) but within the same year?

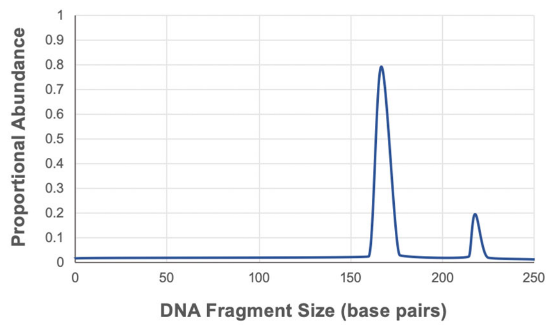
2. What similarities or differences do you see across seasons (summer vs. fall) both within and between years?

3. What similarities or differences do you see between years?

4. What changes in the natural environment could be responsible for these differences (seasonal and annual)?

5. Why do you think *P. australis* is present in the region in 2017 but not 2014?

7/10/17



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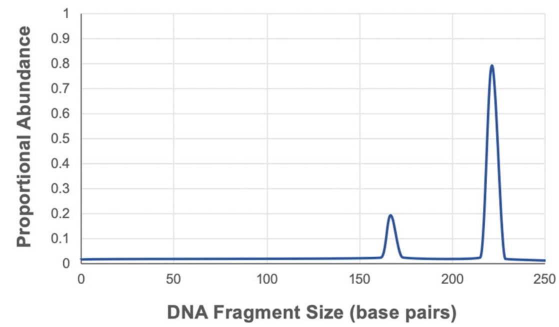
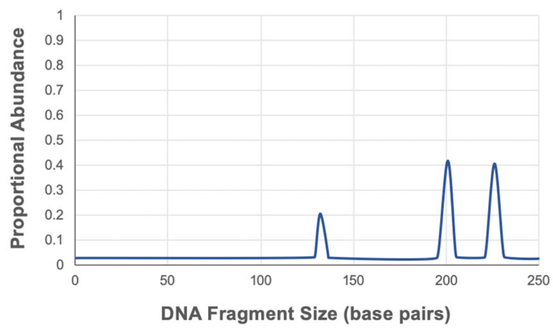


8/10/17



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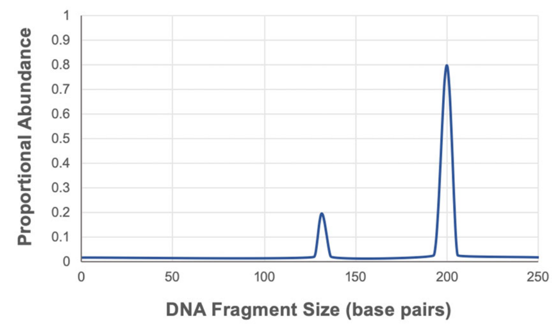
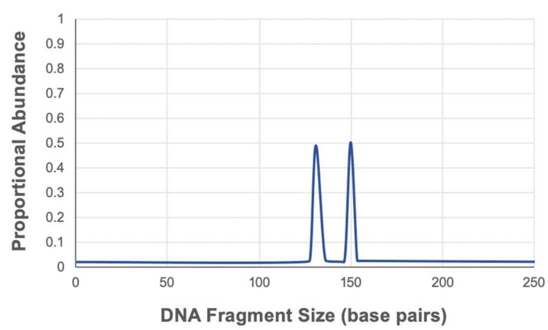
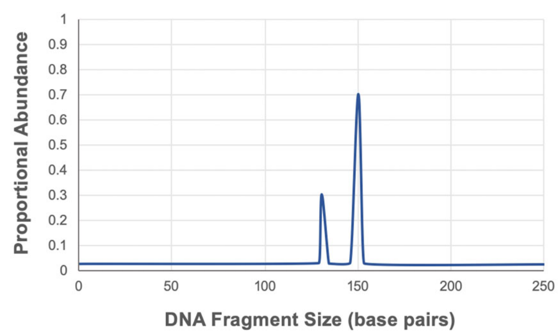


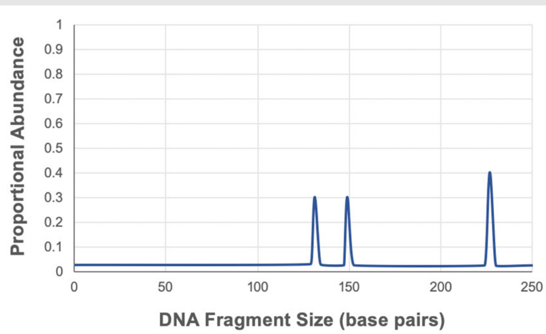
9/6/17



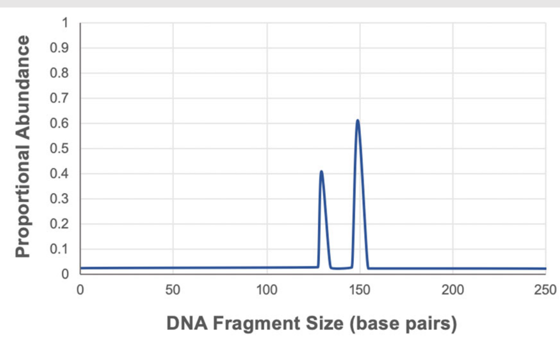
9/20/17



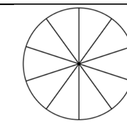
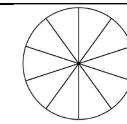
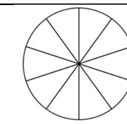
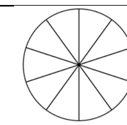
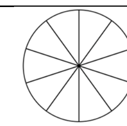
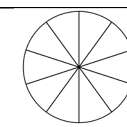
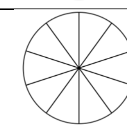
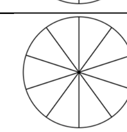
10/4/17

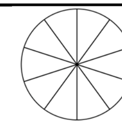
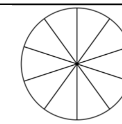
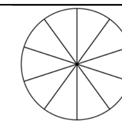
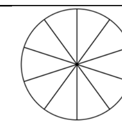
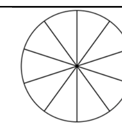
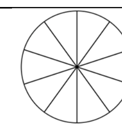
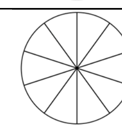
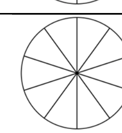


10/19/17

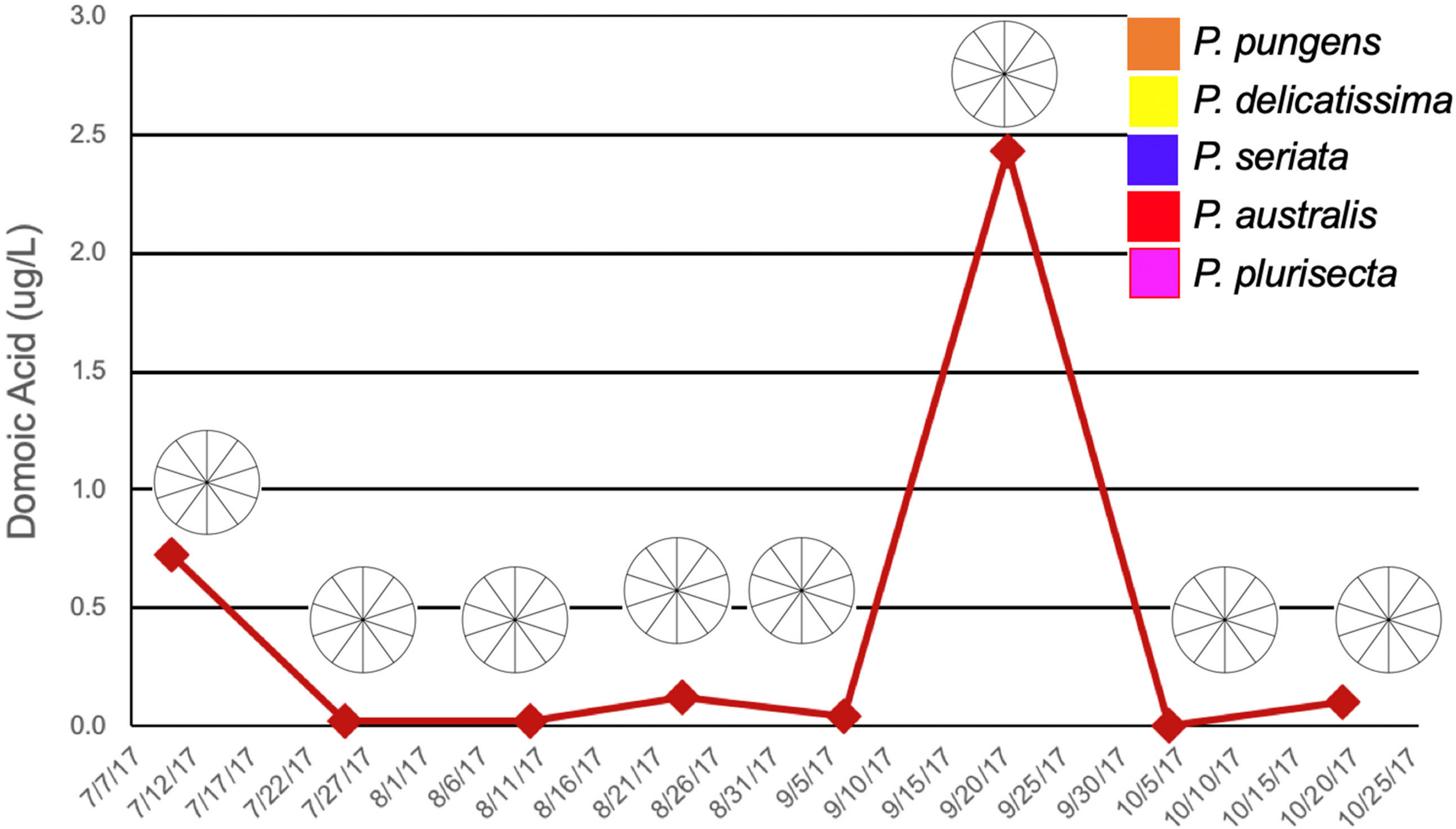


Exercise 1

Community composition in 2014						
Date	***P. pungens***	***P. delicatissima***	***P. seriata***	***P. australis***	***P. plurisecta***	
	Orange	**Yellow**	**Blue**	**Red**	**Pink**	
7/7/14	0	1	0	0	0	
7/18/14	0.2	0.6	0.2	0	0	
8/12/14	1	0	0	0	0	
8/27/14	0.7	0	0.3	0	0	
9/9/14	0.7	0	0.3	0	0	
9/22/14	0.6	0	0.4	0	0	
10/3/14	0.4	0	0.6	0	0	
10/30/14	0.8	0	0	0	0.2	

Community composition in 2017						
Date	***P. pungens***	***P. delicatissima***	***P. seriata***	***P. australis***	***P. plurisecta***	
	Orange	**Yellow**	**Blue**	**Red**	**Pink**	
7/10/17						
7/25/17						
8/10/17						
8/23/17						
9/6/17						
9/20/17						
10/4/17						
10/19/17						

Exercise 2


Domoic Acid (ug/L)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
7/7/17
7/12/17
7/17/17
7/22/17
7/27/17
8/1/17
8/6/17
8/11/17
8/16/17
8/21/17
8/26/17
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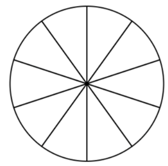
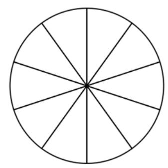
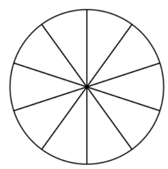
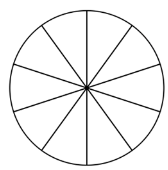
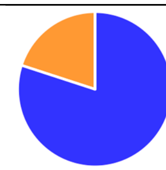
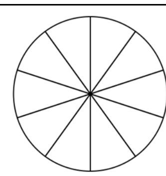
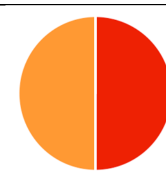
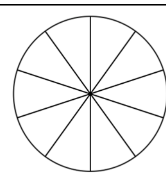
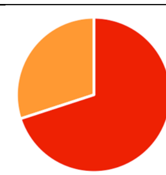
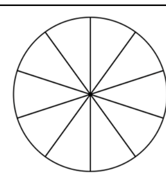
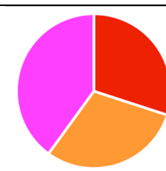
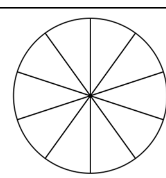
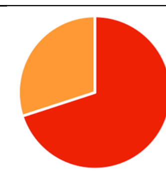
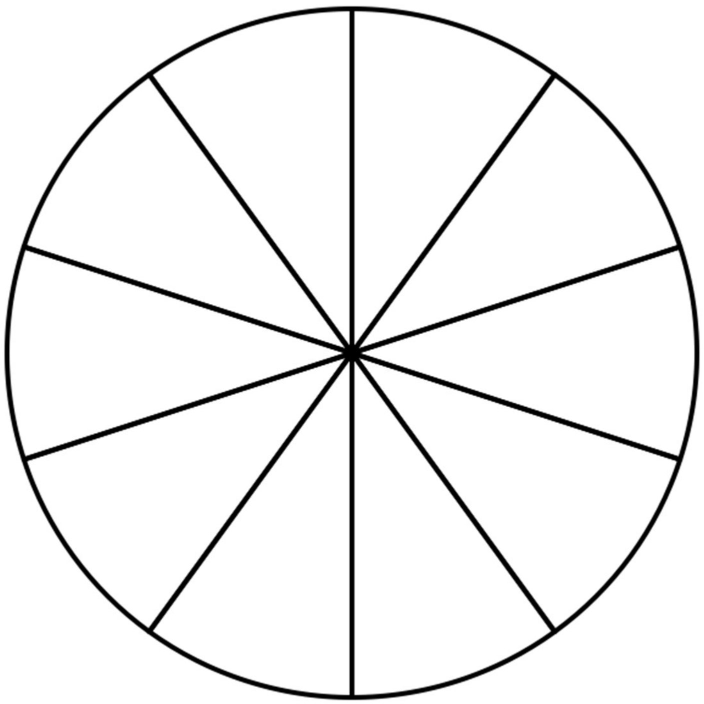
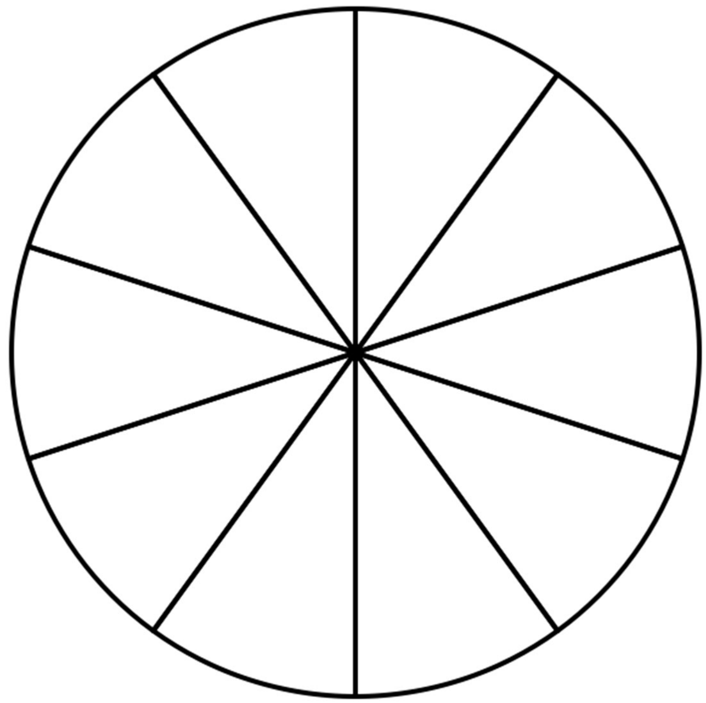
10/15/17
10/20/17
10/25/17
P. pungens
P. delicatissima
P. seriata
P. australis
P. plurisecta

Exercise 3

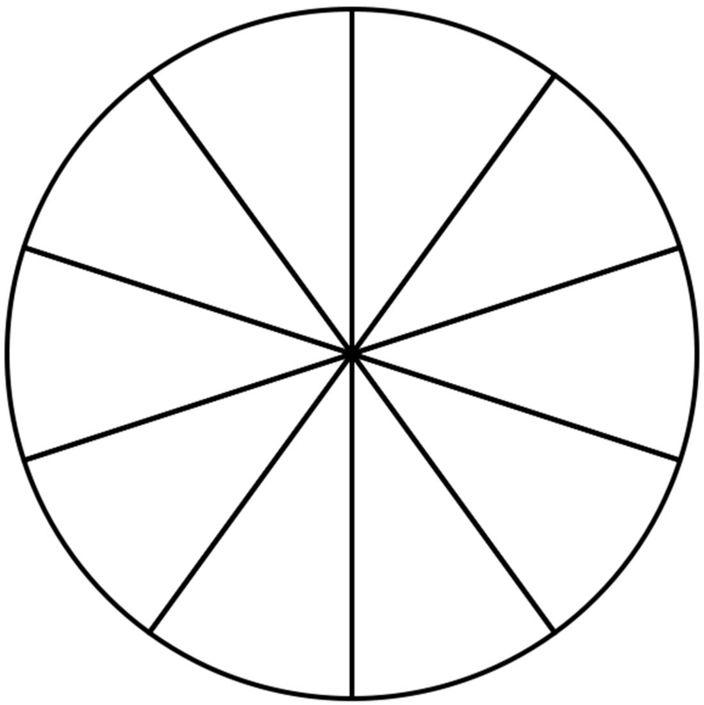
Time period	2014		2017	
Early July	7/7/14		7/10/17	
Late July	7/18/14		7/25/17	
Early Aug	8/12/14		8/10/17	
Late Aug	8/27/14		8/23/17	
Early Sept	9/9/14		9/6/17	
Late Sept	9/22/14		9/20/17	
Early Oct	10/3/14		10/4/17	
Late Oct	10/30/14		10/19/17	

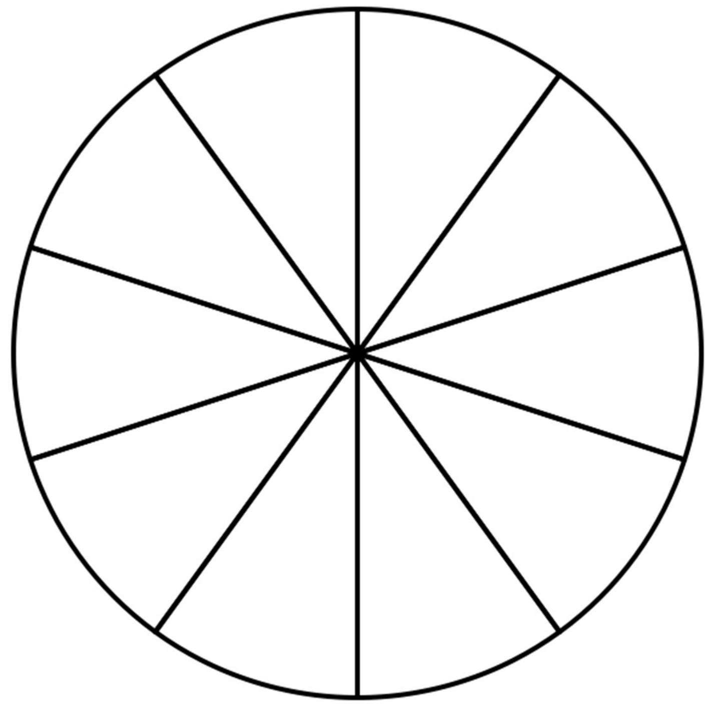
Date	*P. pungens*	*P. deli*	*P. seri*	*P. aust*	*P. pluri*
	Orange	Yellow	Blue	Red	Pink
Early July	Green	Orange	Blue	Red	Pink
2014	0	1	0	0	0
2017	0	0.8	0	0	0.2



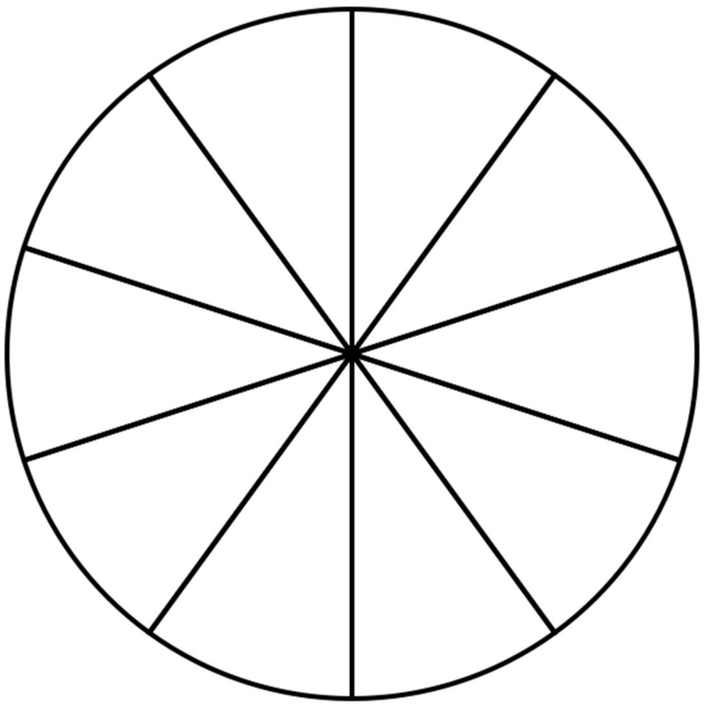


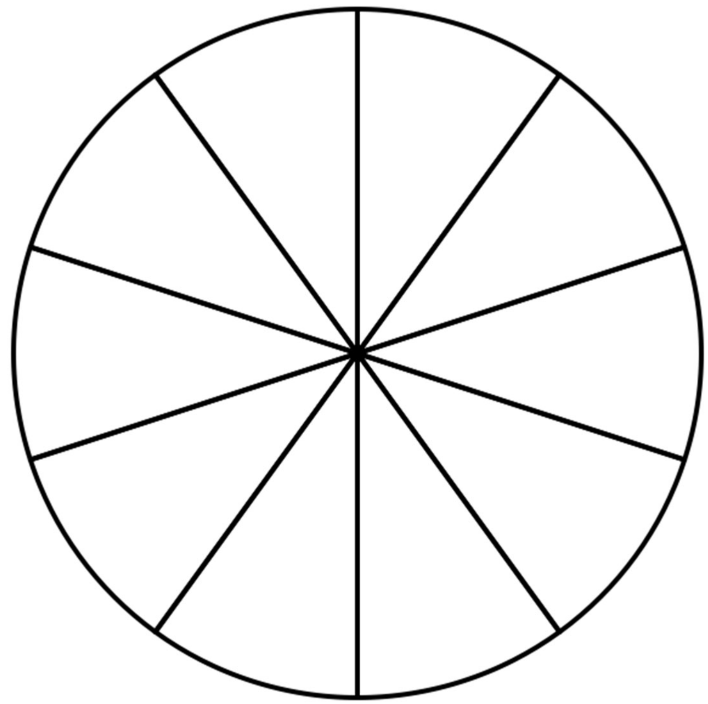
Date	*P. pungens*	*P. deli*	*P. seri*	*P. aust*	*P. pluri*
	Orange	Yellow	Blue	Red	Pink
Late Aug	Green	Orange	Blue	Red	Pink
2014	0.7	0	0.3	0	0
2017	0.2	0	0.8	0	0



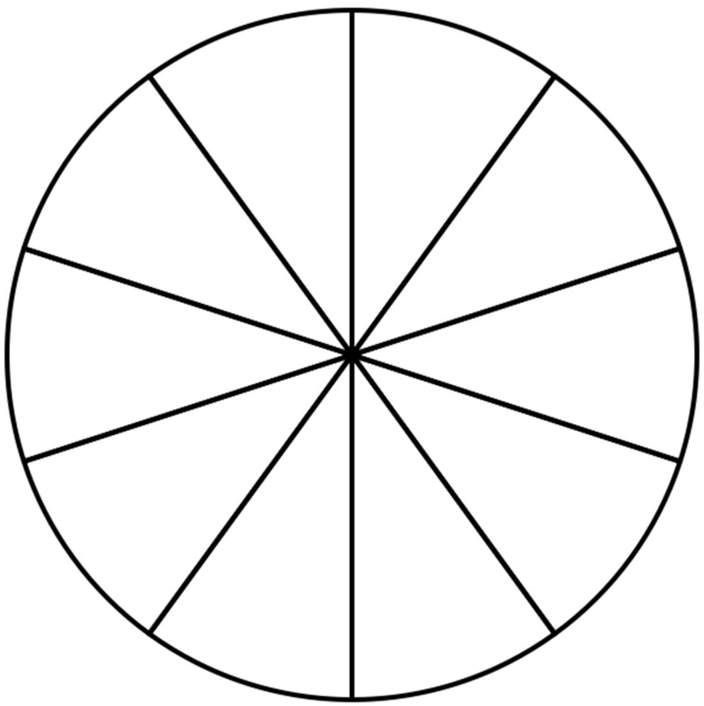


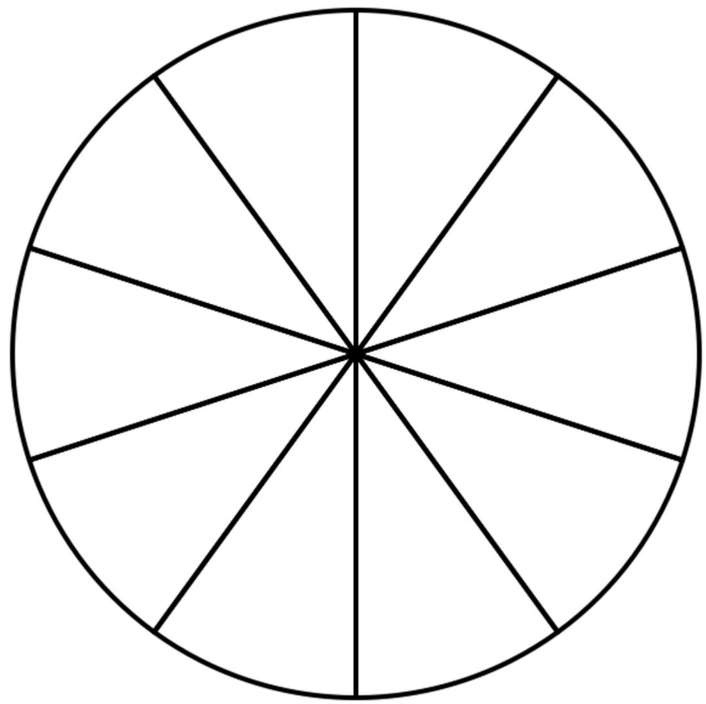
Date	***P. pungens***	***P. deli***	***P. seri***	***P. aust***	***P. pluri***
	Orange	Yellow	Blue	Red	Pink
Early Sep	**Green**	**Orange**	**Blue**	**Red**	**Pink**
2014	**0.7**	**0**	**0.3**	**0**	**0**
2017	**0.5**	**0**	**0**	**0.5**	**0**



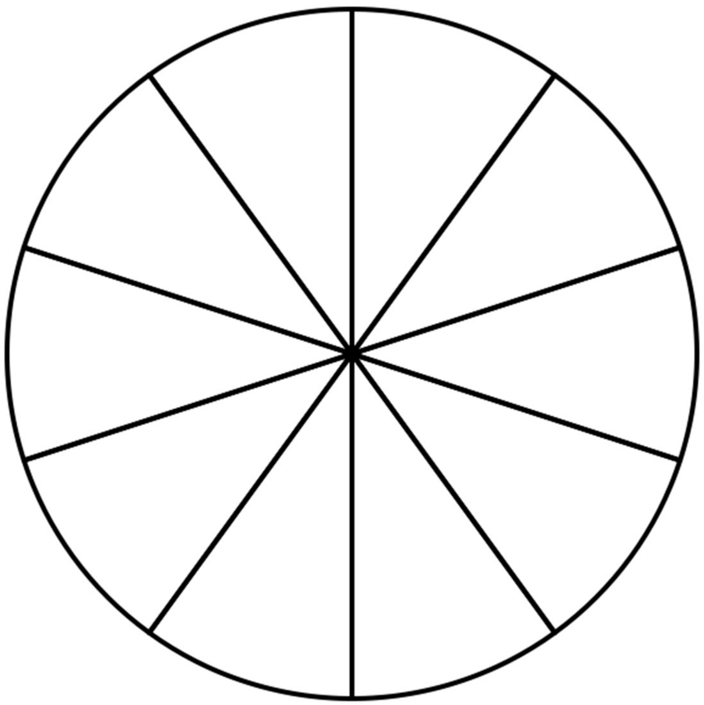


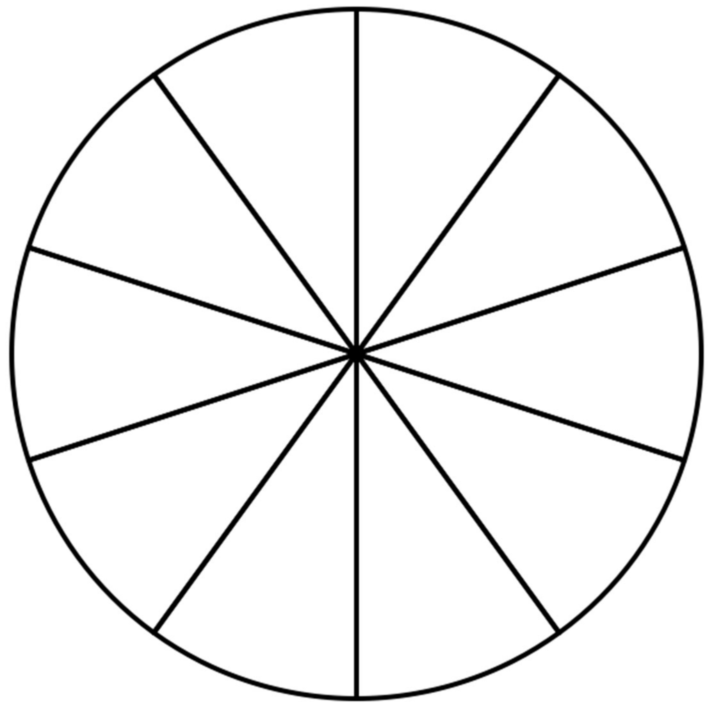
Date	*P. pungens*	*P. deli*	*P. seri*	*P. aust*	*P. pluri*
	Orange	Yellow	Blue	Red	Pink
Late Sept	Green	Orange	Blue	Red	Pink
2014	0.6	0	0.4	0	0
2017	0.3	0	0	0.7	0





Date	*P. pungens*	*P. deli*	*P. seri*	*P. aust*	*P. pluri*
	Orange	Yellow	Blue	Red	Pink
Late Oct	Green	Orange	Blue	Red	Pink
2014	0.8	0	0	0	0.2
2017	0.3	0	0	0.7	0





Assessment Questions

As just discussed, Harmful Algal Blooms (HABs) are a concern in various ocean regions. This activity is based on results from research conducted in the Northeast Atlantic during 2014 and 2017 on a HAB group called *Pseudo-nitzschia*. Some species in this group produce a potent neurotoxin called Domoic Acid. You will be figuring out what species were present by using the DNA extracted from *Pseudo-nitzschia* diatoms present in water samples. You will then be calculating the percent abundance of six species in each sample, representing those data as pie graphs, and then comparing those results between years (2014 vs. 2017) and from month to month. The thought questions are designed to reinforce your ability to see patterns over time and to speculate about which species are toxic (based on comparisons with Domoic Acid levels measured at the time of sampling in 2017). This toxin is of concern for human health as it causes an illness known as amnesic shellfish poisoning, and also sickens wildlife.

The teacher will break you up into groups. There are 8 dates for which you must calculate the percent (%) contribution of each species. Perhaps each student could complete two dates.

1. Use the information in the following table to identify the species found in the "trace" files for your assigned date/s on page 3. There can be multiple species for any given date. Once you identify the species present in each sample, then you will calculate the proportional abundance (percent contribution) of each species and add it to the Exercise 1 sheet on page 4 and color in the pies.

   Example calculation: The DNA fragment size (base pairs) is oriented on the x axis and the proportional abundance on the y axis. If there is only one peak on the trace file, then there was only one species detected. If, for example, the peak was at 200 base pairs on the x axis, then using the table below, you can determine that this peak represents the species *Pseudo-nitzschia seriata*. You would then use the peak height to determine the proportional abundance of this species (ranging between 0 and 1). In this example, the peak height is 1. If you multiply by 100 to obtain proportional abundance expressed as percent total, then there is a 100% occurrence of that species. Fill in your assigned dates from 2017 and then color in the corresponding pie using the assigned color system.

Species	**DNA Fragment Size (base pair)**
Pseudo-nitzschia pungens	142
Pseudo-nitzschia australis	150
Pseudo-nitzschia seriata	200
Pseudo-nitzschia delicatissima	168
Pseudo-nitzschia seriata	200
Pseudo-nitzschia plurisecta	226

2. After you finish your pies, cut them out, put the date on the back, and tape them onto the graph below (Exercise 2; page 5). When was the toxin (Domoic Acid) level the highest?
   ___September 2017___

3. **The teacher will project the completed graph with all the pies provided (Answer Key page 9).** Which species may be responsible for the high levels of Domoic Acid, which can be harmful to humans as well as wildlife? You can abbreviate the first word (the genus) by writing "P." Note that you should underline a genus and species to denote its Latin origin when writing by hand:
   P. australis

4. Are there any other species that might contain Domoic Acid? Which ones? What evidence do you have for this?
   Yes, *P. seri* because the Domoic Acid levels were slightly elevated when they were present.

Now let's take a closer look at any similarities over time. We refer to these as temporal changes. Using Exercise 3, complete the pies for 2014 on page 7 using the data in the provided table on page 6. Once those are completed, you will be comparing them to the pies you created for 2017 and answering the following questions:

1. What similarities or differences do you see within the same month (early vs. late) but within the same year?
   These responses vary. Sometimes the exact same species are present, but in different proportions. Sometimes there are more species for the 2014 date than for 2017 (or vice versa).

2. What similarities or differences do you see across seasons (summer vs. fall) both within and between years?
   These responses vary. There were species that tended to be found early in the year (summer), like *P. deli*. (in both years). *P. pungens* tended to be found later in the year (late summer and fall) in both years. It was often the dominant species when present in 2014 (late summer through fall). *P. aust* was only found in late summer and fall of 2017. *P. pluri* was found only once (fall) in 2014 but then mostly in spring in 2017, with the exception of early October. In both years, there were usually only two species present. These were usually *P. pungens* and *P. seri* in 2014, but *P. pungens* and *P. aust* in 2017.

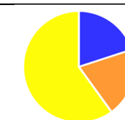
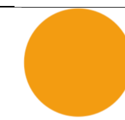
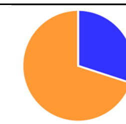
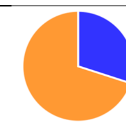
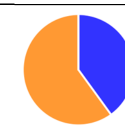
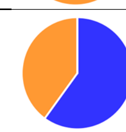
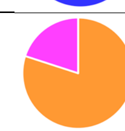
3. What similarities or differences do you see between years?
   These responses vary. There were species that tended to be found early in the year, like *P. deli*. (in both years). *P. pungens* tended to be found later in the year (in both years). It was often the dominant species when present in 2014. It was never the dominant species in 2017, although it was tied with *P. aust* at 50% in early September 2017. *P. aust* was only found in 2017. *P. pluri* was found once once (late October) in 2014 and mostly in early 2017, with the exception of early October. In both years, there were usually only two species present. These were usually *P. pungens* and *P. seri* in 2014, but *P. pungens* and *P. aust* in 2017. There were always at least two species in 2017. The only times there was only one species present was in 2014 (summer).

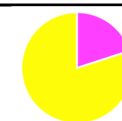
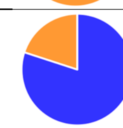
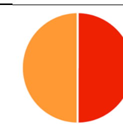
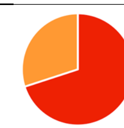
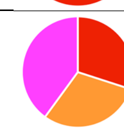
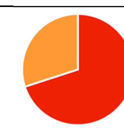
4. What changes in the natural environment could be responsible for these differences (seasonal and annual)?
   There could be changes in the physical environment such as with temperature. There could also be biological factors that differed like the numbers and types of predators or competitors. Diatoms need light to photosynthesize and that could vary seasonally and annually, as could temperature.

5. Why do you think *P. australis* is present in the region in 2017 but not 2014?

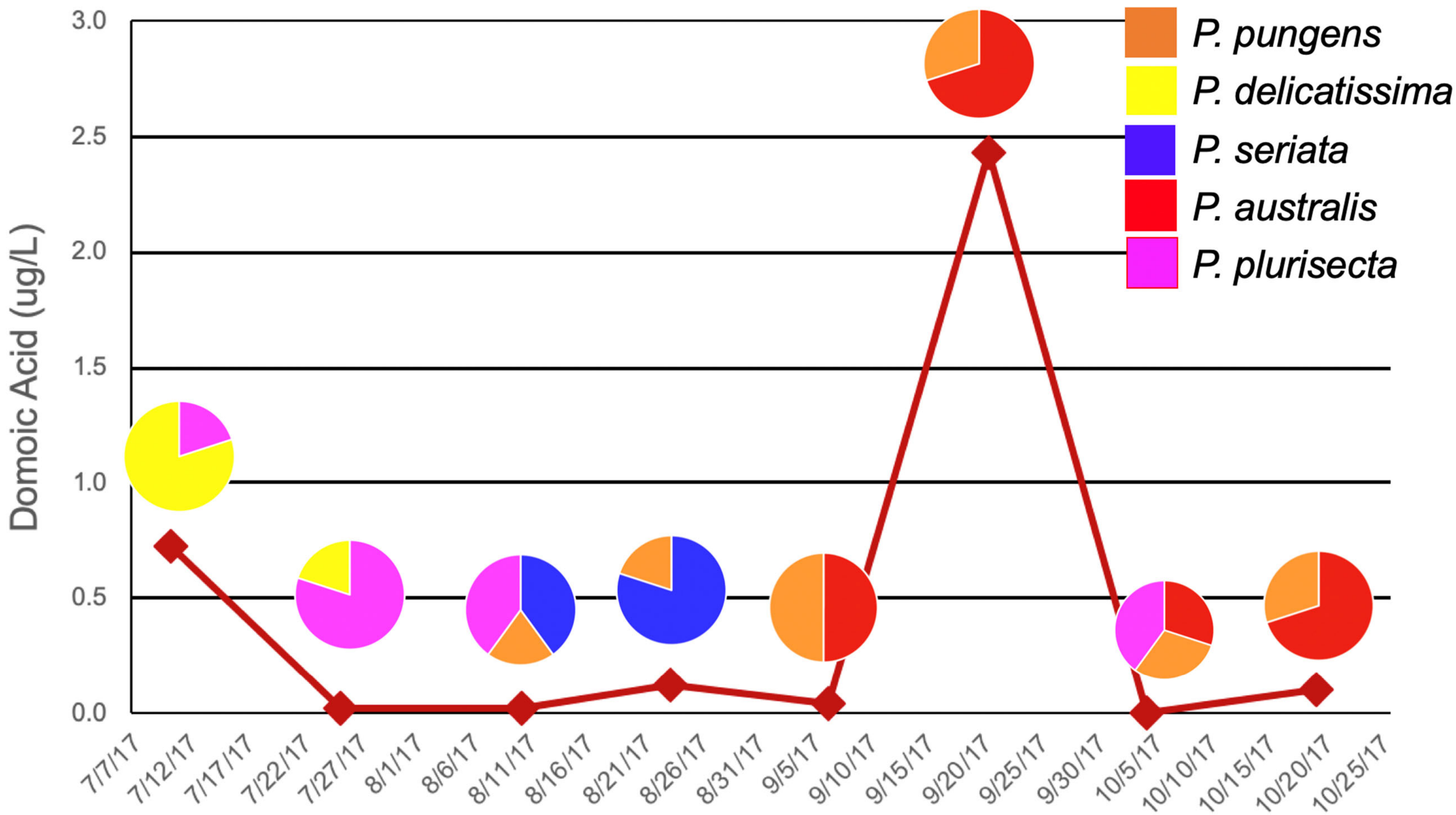
The ultimate source of *P. australis* is not known; however, scientists hypothesize that this species originated from the Scotian Shelf, and was transported to the region by ocean currents.

Exercise 1 – Answer Key

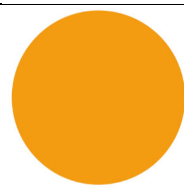
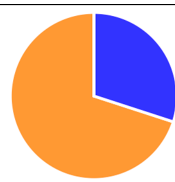
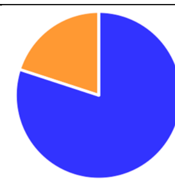
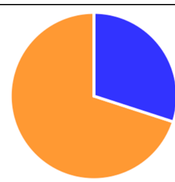
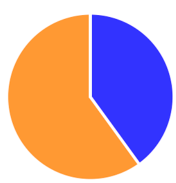
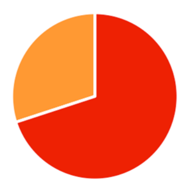
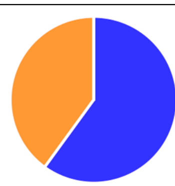
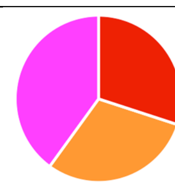
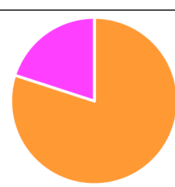
Community composition in 2014						
Date	***P. pungens***	***P. delicatissima***	***P. seriata***	***P. australis***	***P. plurisecta***	
	Orange	**Yellow**	**Blue**	**Red**	**Pink**	
7/7/14	0	1	0	0	0	
7/18/14	0.2	0.6	0.2	0	0	
8/12/14	1	0	0	0	0	
8/27/14	0.7	0	0.3	0	0	
9/9/14	0.7	0	0.3	0	0	
9/22/14	0.6	0	0.4	0	0	
10/3/14	0.4	0	0.6	0	0	
10/30/14	0.8	0	0	0	0.2	

Community composition in 2017						
Date	***P. pungens***	***P. delicatissima***	***P. seriata***	***P. australis***	***P. plurisecta***	
	Orange	**Yellow**	**Blue**	**Red**	**Pink**	
7/10/17	0	0.8	0	0	0.2	
7/25/17	0	0.2	0	0	0.8	
8/10/17	0.2	0	0.4	0	0.4	
8/23/17	0.2	0	0.8	0	0	
9/6/17	0.5	0	0	0.5	0	
9/20/17	0.3	0	0	0.7	0	
10/4/17	0.3	0	0	0.3	4	
10/19/17	0.3	0	0	0.7	0	

Exercise 2 – Answer Key



Exercise 3 - Answer key

Time period	2014		2017	
Early July	7/7/14		7/10/17	
Late July	7/18/14		7/25/17	
Early Aug	8/12/14		8/10/17	
Late Aug	8/27/14		8/23/17	
Early Sept	9/9/14		9/6/17	
Late Sept	9/22/14		9/20/17	
Early Oct	10/3/14		10/4/17	
Late Oct	10/30/14		10/19/17