2020

Tiny Killers

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TINY KILLERS

MONITORING HARMFUL ALGAE

Sarah Pease
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Grade Level
High School

Subject Area
Biology, Environmental Science, Marine

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Activity Title: Tiny Killers: Monitoring Harmful Algae

Focus: Harmful algae of human health concern in the Chesapeake Bay and the technologies and sampling procedures used to detect them

Grade Level: HS Biology, HS Environmental Science, HS Marine Biology

VA Science Standards:

BIO.1 The student will demonstrate an understanding of scientific and engineering practices by:

a) Asking questions and defining problems

- Ask questions that arise from careful observation of phenomena and/or organisms, from examining models and theories, and/or to seek additional information
- Determine which questions can be investigated within the scope of the school laboratory or field to determine relationships between independent and dependent variables
- Generate hypotheses based on research and scientific principles
- Make hypotheses that specify what happens to a dependent variable when an independent variable is manipulated

b) Planning and carrying out investigations

- Individually and collaboratively plan and conduct observational and experimental investigations
- Plan and conduct investigations or test design solutions in a safe and ethical manner including considerations of environmental, social, and personal effects
- Determine appropriate sample size and techniques
- Select and use appropriate tools and technology to collect, record, analyze, and evaluate data

c) Interpreting, analyzing, and evaluating data

- Construct and interpret data tables showing independent and dependent variables, repeated trials, and means
- Construct, analyze, and interpret graphical displays of data
- Use data in building and revising models, supporting an explanation for phenomena, or testing solutions to problems
- Analyze data using tools, technologies, and/or models to make valid and reliable scientific claims or determine an optimal design solution

d) Constructing and critiquing conclusions and explanations

- Make quantitative and/or qualitative claims regarding the relationship between dependent and independent variables
- Construct and revise explanations based on valid and reliable evidence obtained from a variety of sources including students' own investigations, models, theories, simulations, and peer review
- Apply scientific ideas, principles, and/or evidence to provide an explanation of phenomena and design solutions
- Compare and evaluate competing arguments or design solutions in light of currently accepted explanations and new scientific evidence
- Construct arguments or counterarguments based on data and evidence
- Differentiate between a scientific hypothesis and theory

e) Developing and using models
- Evaluate the merits and limitations of models
- Develop, revise, and/or use models based on evidence to illustrate or predict relationships
- Develop and/or use models to generate data to support explanations, predict phenomena, analyze systems, and/or solve problems

f) Obtaining, evaluating, and communicating information
- Compare, integrate, and evaluate sources of information presented in different media or formats to address a scientific question or solve a problem
- Gather, read, and evaluate scientific and/or technical information from multiple authoritative sources, assessing the evidence and credibility of each source
- Communicate scientific and/or technical information about phenomena in multiple formats

BIO.8 The student will investigate and understand that there are dynamic equilibria within populations, communities, and ecosystems. Key ideas include:
a) Interactions within and among populations include carrying capacities, limiting factors, and growth curves;
b) Nutrients cycle with energy flow through ecosystems;
c) Ecosystems have succession patterns;
d) Natural events and human activities influence local and global ecosystems and may affect the flora and fauna of Virginia

Learning Objectives:
- Students will explain how harmful algae can cause human illness as a result of bioaccumulation and biomagnification
- Students will design a monitoring program using algae cell concentration data
- Students will analyze relationships between harmful algae cell concentrations and harmful algae toxin concentrations in shellfish meat
- Students will compare and contrast different techniques of monitoring harmful algae and shellfish poisoning risk

Total length of time required for the lesson: 1.5 hours

Vocabulary:

Aquaculture – the breeding, rearing, and harvesting of fish, shellfish, algae, and other organisms in all types of water environments (https://oceanservice.noaa.gov/facts/aquaculture.html)

Bioaccumulation – when a chemical or toxin builds up in an organism's body (Fisher, 2012)

Bioindicator – a species, ecological community, or biological process that is typically monitored over time for changes (as in abundance or health) which are used to assess the state of a particular environment (https://www.merriam-webster.com/dictionary/bioindicator)

Biomagnification – the process by which toxins travel from organism to organism in a food web, and higher-level consumers build up more and more toxins at the top of the food web (Fisher, 2012)

Bloom – when there is a rapid increase in the cell concentration of one or a few species of phytoplankton in the water in a particular location; the cell concentration may be so high that the water appears darker in color

Detoxification – the process of a toxin being eliminated from the organism's tissues

Filter-feeder – a method of feeding in which organisms process large volumes of the water they live in to obtain food like phytoplankton (https://www.sciencelearn.org.nz/videos/366-mussels-are-filter-feeders)

Harmful algae – species of phytoplankton known to naturally produce toxins or to form large patches that deplete the oxygen and block the sunlight that other organisms need to live (http://www.coastalwiki.org/wiki/Harmful_algal_bloom)

Harmful algal bloom (HAB) – occur in marine, estuarine, and fresh waters when certain types of phytoplankton bloom, forming sometimes visible patches that may harm the health of the environment, plants, or animals (http://www.coastalwiki.org/wiki/Harmful_algal_bloom)

Imaging FlowCytobot (IFCB) – a robot that can be deployed into the water and programmed to take water samples in which the phytoplankton are photographed, categorized, and counted

Phytoplankton – microscopic, drifting, photosynthetic algae (Fisher, 2012)

Red tide – a commonly used term for a harmful algal bloom, typically referring to a bloom of algae that colors the water red because the algae species that make-up the bloom are reddish in color (https://media.nationalgeographic.org/assets/file/one-ocean-chapter-5.pdf)

Regulatory limit – an established legal limit for toxin concentration in shellfish meat, above which shellfish is considered contaminated and unfit for human consumption (http://www.issc.org/Data/Sites/1/media/2009%20nssp%20guide/section%20vii%20federal%20regulations.pdf)

Satellite imagery – pictures taken from satellites that are used to understand large-scale environmental events (e.g., blooms, fires, hurricanes)

Shellfish – an aquatic invertebrate animal with a shell especially: an edible mollusk or crustacean; e.g., crab, lobster, clam, oyster, etc. (https://www.merriam-webster.com/dictionary/shellfish)

Shellfish poisoning – serious illness that can occur when a person consumes shellfish that have bioaccumulated harmful algae toxins, the most well-known types are paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), diarrhetic shellfish poisoning (DSP) and amnesic shellfish poisoning (ASP) (https://medlineplus.gov/ency/article/002851.htm)

Solid Phase Adsorption Toxin Tracking (SPATT) devices – a tool used to monitor toxins in water, consisting of handmade discs or bags that enclose a special material to which toxins can adsorb (stick) and from which toxins can later be extracted for analysis in a laboratory

Toxin – a chemical substance which damages an organism (https://biologydictionary.net/toxin/)

Background Information:

NOTE: See the procedure, PowerPoint, and worksheets for additional background content needed to conduct this lesson.

Harmful algae are phytoplankton that are considered harmful to the ecosystem and/or to human health. Some harmful algae species produce toxins that can cause human illness. Paralytic Shellfish Poisoning (PSP) and Diarrhetic Shellfish Poisoning (DSP) are two examples of some of the serious human illnesses linked to seasonal harmful algae events in the U.S. These illnesses are caused by eating shellfish or fish that have bioaccumulated harmful algae toxins (Shumway, 1990). Often, these illnesses are associated with filter-feeding shellfish that eat phytoplankton, like bivalves (e.g., clams, mussels, oysters, etc.). Adult oysters can filter up to 50 gallons of water a day (*Oyster Fact Sheet*, 2019)! This means that oysters may bioaccumulate harmful algae toxin in their meat over time, creating the potential for the toxins to be biomagnified up the food web, where they can have a serious effect on human health.

Harmful algal blooms (HABs), sometimes known as red tides, occur when certain species of harmful algae grow so quickly in an area that the color of the water is darkened by the high cell concentrations. HABs tend to occur seasonally, varying by geographic location and by species of harmful algae, and they are often patchy, being moved around by wind and currents. Scientists monitor HABs using satellite imagery, and by sampling water and shellfish samples collected in the field.

Not all harmful algae species produce visible blooms, and this can make monitoring more challenging. One such species, *Dinophysis acuminata*, has been found in the Chesapeake Bay and produces the toxin, okadaic acid. Okadaic acid can bioaccumulate in filter-feeding shellfish meat and cause Diarrhetic Shellfish Poisoning (DSP) in humans, which can lead to gastrointestinal distress, diarrhea, nausea, and vomiting (Landsberg, 2002). In order to monitor harmful algae toxins, one method scientists can employ is to use filter-feeding shellfish, like oysters, as bioindicators by deploying them in waters where harmful algae have been seen, or are expected. These shellfish can then be tested in a laboratory to determine the toxin concentration in their meat and compare results to regulatory limits. In the Chesapeake Bay, no human illnesses related to harmful algae have been reported to-date, but continuing to monitor for harmful algae is essential to protecting human health.

Student Handouts and Other Materials Needed:

Tiny Killers PowerPoint
Harmful Algae Monitoring Activity
Group Activity Sheet
Harmful Algae Monitoring Activity Answer Key

Materials & Supplies, A/V/Tech Support:

For class:

- Highlighters, colored pens or pencils, or markers for students
- Computer and projector for PowerPoint
- Whiteboard and markers or equivalent

For each Bioaccumulation Demonstration you will need:

- 1.5-2 cups granulated sugar/cornmeal/flour
- 15-30 dry small beans/lentils/split peas
- A small sieve or slotted spoon (should catch the dry beans/lentils/split peas)
- Two large mixing bowls or storage tubs
- Two paper plates
- A small Dixie® paper bathroom cup

Procedure:

Before class

Advance preparation of materials – 10 minutes

Before class, print out one copy of "Harmful Algae Monitoring Activity" for each student, one copy of "Group Activity Sheet" for each student group (can be laminated for re-use between classes), and one copy of "Harmful Algae Monitoring Activity Answer Key" for the teacher. Set-up the PowerPoint presentation "Tiny Killers" before class.

Bioaccumulation Demonstration set-up – 5 minutes

Just before class, in one of the large mixing bowls or tubs, mix the sugar (or cornmeal/flour) and beans. (Tip: ideally, you want so few beans that a small scoop of the mixture with the small paper cup only picks up 0-3 beans.) Have the sieve, paper plates, and small paper cup nearby and ready for the Bioaccumulation Demonstration.

Class Part I

Tiny Killers Part I: Introduction – 5 minutes

Present Part I of the Tiny Killers PowerPoint presentation. This section of the PowerPoint includes definitions of phytoplankton, harmful algae, and harmful algal blooms. It also covers harmful algae in the Chesapeake Bay, discussing some examples of harmful algae species, and includes a pronunciation guide for the teacher. The PowerPoint then introduces the first interactive activity.

Bioaccumulation Demonstration & Discussion – 15 minutes

NOTE: With enough materials, this demonstration can be adapted so that pairs or small groups of students can all participate in their own demo.

Describe to the class that the large mixing bowl or tub and its contents represent all of the phytoplankton in the water. The beans represent toxin-producing harmful algae, like *Dinophysis acuminata*, and the sugar (or cornmeal/flour) represents all of the non-harmful algae in the water. Filling the small paper cup represents a scientist taking a water sample to look at the phytoplankton and test for harmful algae toxins. The sieve represents an oyster, or other filter-feeding shellfish, feeding on the phytoplankton in the water.

Have a scientist (student volunteer) take a sample and count the number of beans (toxin-producing harmful algae) they collected by pouring out their sample onto a paper plate. Record on the white board how many toxin-producing harmful algae were in the water sample.

Have an oyster (another student volunteer) feed on the algae for a short period of time (by using the sieve to scoop sugar and beans repeatedly from the bowl or tub, the idea here is that the beans will start to accumulate in the sieve while the sugar/cornmeal/flour passes through), allowing toxin-producing harmful algae to bioaccumulate in the oyster. Record on the white board how many toxin-producing harmful algae were in the oyster.

OPTIONAL: You can re-set and repeat the sampling and oyster feeding if you want to involve more students and collect more data on the white board. This represents replicate sampling.

Ask the students to compare the amount of toxin-producing harmful algae that were reported in the water sample to quantities accumulated in the oyster. Tell the students that to be able to detect toxin with a standard laboratory test, it takes at least 2 toxin-producing harmful algae (beans). Were the scientists able to detect the toxin-producing harmful algae in their water sample/s when it was present? Not always…If the scientist had sampled an oyster, would they be able to detect the harmful algae? Most likely, because the oyster bioaccumulated the harmful algae toxin over time, concentrating the toxin from the water through filter-feeding. Write the definition for bioaccumulation on the board. What could the consequences be for a scientist collecting only water samples? Could they miss a harmful algal bloom?

Have students multiply the shellfish results to mimic how much toxin might be in a meal of 10 oysters. How much toxin-producing harmful algae does the person who eats this meal end up eating? Discuss how the human is exposed to more toxin than the oyster is, this process is called biomagnification. Write the definition of biomagnification on the board. What happens when people eat these oysters? Tell the students that it takes at least 20 toxin-producing harmful algae (beans) to make a person sick from the toxins produced by these algae. If a person eats 10 oysters, how many toxin-producing harmful algae in a single oyster would be enough to raise concerns for human health? (Answer: 20/10=2, so even finding 2 toxin-producing harmful algae in an oyster would be cause for concern.) How many harmful algae in a water sample would be enough to raise concern for human health? Is this amount even detectable? (Even finding one toxin-producing harmful algae in the water sample would be cause for concern!) To protect

human health, would you rather sample water or oysters to monitor these toxin-producing harmful algae and why? Water samples may sometimes miss low cell concentrations of toxin-producing harmful algae…people might get sick. It's often better to directly monitor harmful algae toxin concentrations in oysters or other filter-feeding shellfish.

Regulatory limits are used to determine when to close harvest of oysters or other shellfish so that people don't get sick. Write the definition of regulatory limit on the board. Based on the previous results, what regulatory limit would you set for oysters? Debate whether the limit should be at 1 or 2 toxin-producing harmful algae per oyster: …We know 2 will make people sick; a lower limit of 1 would provide a Margin of Safety, but might needlessly close oyster harvesting areas. Alternatively, you could require a sample of 10 oysters and set a limit for their combined number of toxin-producing harmful algae. Debate the pros and cons of this method, (optional: have different students role-play as oyster growers, health department regulators, or public consumers of oysters).

In the PowerPoint (slide 10), there is a slide to wrap-up and review the main points of this activity.

Class Part II

Tiny Killers Part II: Monitoring to Protect Human Health – 5 minutes

Present Part II of the Tiny Killers PowerPoint presentation. This section of the PowerPoint covers how scientists and regulators protect human health in relation to harmful algae. On slide 12, there is an overview of the types of human shellfish poisonings that are associated with harmful algae. Here there is an optional interactive opportunity for the students, have students break into small groups to quickly look-up some of the symptoms of these specific illnesses and share them with the class. Here are a couple good resources:
https://www.whoi.edu/redtide/page.do?pid=9257 and
http://www.fao.org/3/y5486e/y5486e00.htm#Contents .

The PowerPoint then covers traditional harmful algae monitoring methods, how monitoring is used to close shellfish harvesting areas to protect human health, and the implications of closing shellfish harvest areas. Lastly, this section introduces students to a scientist that researches harmful algae and oysters in the Chesapeake Bay.

Harmful Algae Monitoring Activity – 35-45 minutes

Give each pair or small group of students a copy of the "Group Activity Sheet", and each student their own copy of the "Harmful Algae Monitoring Activity" worksheet to fill out and eventually hand-in. Have each group work together to complete the worksheet. The teacher plays the role of the "lab" in this activity., Pairs/groups of students give one worksheet, with their sampling plan for Year 2, to the teacher and the teacher should use a colored pen to fill in ONLY the lab results that correspond to where and when the students sampled (use the "Harmful Algae Monitoring Activity Answer Key").

As the students consider their lab results, re-emphasize that they don't want to needlessly close shellfish harvesting areas because: it is difficult to open the harvest areas back up; and because watermen lose money while shellfish harvest areas are closed. After some time to record answers to worksheet questions, display PowerPoint slide 19 with the full dataset of toxin concentrations (this is the same data found in the "Harmful Algae Monitoring Activity Answer Key").

NOTE: If the worksheet activity looks like it will take too long to complete in class, have the students do the last set of questions for homework.

OPTIONAL: Have the students graph the cell concentration data and/or the toxin concentration data in Excel.

OPTIONAL: Discuss the activity and any of the worksheet questions as a class, use PowerPoint slide 20 as a backdrop for this discussion.

Class Part III

Tiny Killers Part III: Advancements in Monitoring – 5 minutes

Present Part III of the Tiny Killers PowerPoint presentation. This section of the PowerPoint provides an introduction to some of the technology that is being used to improve harmful algae monitoring in the Chesapeake Bay, and around the world.

Lesson Review and Wrap-Up – 10 minutes

PowerPoint slides 25 and 26 provide a lesson overview of the key take-away points for students, and a series of suggested wrap-up discussion questions to ensure that the students solidify those key take-away points.

After Class

Breakdown and Clean-Up – 5 minutes

The second large bowl or tub can be used to help separate the beans from the sugar/cornmeal/flour, if desired.

Assessment:

Students should be evaluated on their answers during class discussion throughout the lesson, as well as their written worksheet answers. Discussion questions are included within each activity and the PowerPoint.

References:

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Harmful Algae Monitoring Activity **Name**_____

Analysis of Year 1 Sampling Data

Examine the map of the bay and review the site and algae species descriptions, then analyze the Year 1 sampling data through answering the following questions.

How do algae cell concentrations vary between sites? Between species? Between seasons?

Proposal for Year 2 Sampling

Using your analysis of Year 1, create a proposal for where your scientific team will deploy oysters to sample for harmful algae toxins in oyster meat in Year 2. Your team only has enough resources for 9 oyster deployments (for each deployment you must pick one season at one site). Mark an "X" in the table for each of your planned deployments. Think about where you might be likely to find harmful algae toxins.

	Site			
Season	**1**	**2**	**3**	**4**
Winter				
Spring				
Summer				
Fall				

Analysis of Proposal for Year 2 Sampling

Explain what information from Year 1 was important in determining where and when to do your oyster deployments in Year 2.

After you have finalized your team's Year 2 Sampling Plan, collect your harmful algae toxin data from the "lab".

Year 2 Lab Results

Record in the table below the harmful algae toxin data. "ND" means the toxin concentration was too low to be detected ("Non Detect"). All of the toxin data is reported in µg toxin/100 g oyster meat.

	Site							
Season	**1**		**2**		**3**		**4**	
	B toxin	**C toxin**	**B toxin**	**C toxin**	**B toxin**	**C toxin**	**B toxin**	**C toxin**
Winter								
Spring								
Summer								
Fall								

Results and Analysis of Year 2 Data

From your oyster deployments, at which sites and during which seasons, did you detect harmful algae toxins? Does this make sense with what you saw for algae cell concentrations in Year 1? Explain why or why not.

Did you detect any incidences where the oysters exceeded the regulatory limits for seafood? Mark these in your Year 2 Lab Results table.
The regulatory limits are: **80 µg B toxin**/100 g oyster meat, **20 µg C toxin**/100 g oyster meat.

Based on your findings, where are when would you recommend that oyster harvest be closed to protect human health?

Once you fill in all of the lab results, answer the following questions.

Conclusions and Future Work

How successful was your sampling plan at protecting human health, i.e., did you catch all of the incidences when harmful algae toxin in oysters exceeded the regulatory limits? Explain why or why not.

Were there any times where you expected to find harmful algae toxins and didn't? Why might this be?

If you had unlimited resources, how would you design a monitoring plan for these four sites using water samples and/or oyster deployments?

UnHABitable Bay is an estuary that is home to agriculture, aquaculture, and fishing industries. A new monitoring program has collected a monthly water sample from four sites around the Bay to determine the cell concentration in the water for three important algae species. Your team of scientists has been asked to help further develop this monitoring program and help determine where to deploy oysters next year to help monitor any risk of harmful algae-related shellfish poisoning in seafood. You have been given a map of the Bay as well as the first year of data from this new monitoring program to help you make your decisions.

Site Descriptions

All of these sites are used by the public and/or by the aquaculture industry to grow and harvest oysters for use as seafood.

Site 1 is located furthest upriver.
Site 2 is located just offshore from agricultural crop fields.
Site 3 is located in a tributary near industrial chicken coops.
Site 4 is located near a natural oyster reef that is designated as public oyster grounds.

Algae Species Descriptions

Species A is a common, non-harmful algae species. It is a major food source for aquatic filter-feeders.
Species B is a known, toxin-producing harmful algae species. It forms large, visible, red tides when the cell concentration reaches 1000 cells/mL or more. The toxin it produces can bioaccumulate in oysters and can make people sick.
Species C is a known, toxin-producing harmful algae species. It does not produce visible blooms. The toxin it produces can bioaccumulate in oysters and can make people sick.

Year 1 Sampling Data

Algae cell concentrations (in **cells/mL**) are listed for each month and each algae species from all of the water samples collected from the four sites in Year 1.

Site 1

		Algae Species		
Season	**Month**	**A**	**B**	**C**
Winter	Jan	20	0	2
Winter	Feb	22	0	0
Spring	Mar	59	0	0
Spring	Apr	148	3	0
Spring	May	1683	0	1
Summer	Jun	1294	3	0
Summer	Jul	429	6	0
Summer	Aug	32	0	0
Fall	Sep	400	1	1
Fall	Oct	83	0	0
Fall	Nov	34	1	0
Winter	Dec	16	0	0

Site 2

		Algae Species		
Season	**Month**	**A**	**B**	**C**
Winter	Jan	4	2	1
Winter	Feb	33	0	5
Spring	Mar	73	3	0
Spring	Apr	299	0	1
Spring	May	42745	4	0
Summer	Jun	69333	56	0
Summer	Jul	39144	694	1
Summer	Aug	591	1956	3
Fall	Sep	85	8335	8
Fall	Oct	57	39	3
Fall	Nov	3	13	0
Winter	Dec	1	0	0

Site 3

		Algae Species		
Season	**Month**	**A**	**B**	**C**
Winter	Jan	18	4	1
Winter	Feb	25	0	12
Spring	Mar	93	2	7
Spring	Apr	384	7	1
Spring	May	9442	34	0
Summer	Jun	73451	3764	0
Summer	Jul	34462	12776	0
Summer	Aug	2494	745	0
Fall	Sep	62	43	4
Fall	Oct	52	2	1
Fall	Nov	23	0	0
Winter	Dec	22	4	0

Site 4

		Algae Species		
Season	**Month**	**A**	**B**	**C**
Winter	Jan	16	1	0
Winter	Feb	32	2	1
Spring	Mar	85	0	1
Spring	Apr	944	1	3
Spring	May	7593	1	0
Summer	Jun	27899	1	1
Summer	Jul	28484	2	0
Summer	Aug	8384	0	0
Fall	Sep	732	1	0
Fall	Oct	36	0	0
Fall	Nov	14	0	0
Winter	Dec	32	0	0

Harmful Algae Monitoring Activity **ANSWER KEY**

UnHABitable Bay is an estuary that is home to agriculture, aquaculture, and fishing industries. A new monitoring program has collected a monthly water sample from four sites around the Bay to determine the cell concentration in the water for three important algae species. Your team of scientists has been asked to help further develop this monitoring program and help determine where to deploy oysters next year to help monitor any risk of harmful algae-related shellfish poisoning in seafood. You have been given a map of the Bay as well as the first year of data from this new monitoring program to help you make your decisions.

Site Descriptions

All of these sites are used by the public and/or by the aquaculture industry to grow and harvest oysters for use as seafood.

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Algae Species Descriptions

Species A is a common, non-harmful algae species. It is a major food source for aquatic filter-feeders.
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Species C is a known, toxin-producing harmful algae species. It does not produce visible blooms. The toxin it produces can bioaccumulate in shellfish and can make people sick.

Year 1 Sampling Data

Algae cell concentrations (in **cells/mL**) are listed for each month and each algae species from all of the water samples collected from the four sites in Year 1.

Site 1				
		Algae Species		
Season	**Month**	**A**	**B**	**C**
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Spring	Apr	148	3	0
Spring	May	1683	0	1
Summer	Jun	1294	3	0
Summer	Jul	429	6	0
Summer	Aug	32	0	0
Fall	Sep	400	1	1
Fall	Oct	83	0	0
Fall	Nov	34	1	0
Winter	Dec	16	0	0

Site 2				
		Algae Species		
Season	**Month**	**A**	**B**	**C**
Winter	Jan	4	2	1
Winter	Feb	33	0	5
Spring	Mar	73	3	0
Spring	Apr	299	0	1
Spring	May	42745	4	0
Summer	Jun	69333	56	0
Summer	Jul	39144	694	1
Summer	Aug	591	1956	3
Fall	Sep	85	8335	8
Fall	Oct	57	39	3
Fall	Nov	3	13	0
Winter	Dec	1	0	0

Site 3				
		Algae Species		
Season	**Month**	**A**	**B**	**C**
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Spring	May	9442	34	0
Summer	Jun	73451	3764	0
Summer	Jul	34462	12776	0
Summer	Aug	2494	745	0
Fall	Sep	62	43	4
Fall	Oct	52	2	1
Fall	Nov	23	0	0
Winter	Dec	22	4	0

Site 4				
		Algae Species		
Season	**Month**	**A**	**B**	**C**
Winter	Jan	16	1	0
Winter	Feb	32	2	1
Spring	Mar	85	0	1
Spring	Apr	944	1	3
Spring	May	7593	1	0
Summer	Jun	27899	1	1
Summer	Jul	28484	2	0
Summer	Aug	8384	0	0
Fall	Sep	732	1	0
Fall	Oct	36	0	0
Fall	Nov	14	0	0
Winter	Dec	32	0	0

Analysis of Year 1 Sampling Data

Examine the map of the bay and review the site and algae species descriptions, then analyze the Year 1 sampling data through answering the following questions.

How do algae cell concentrations vary between sites? Between species? Between seasons?

Algae cell concentrations are generally lower at Site 1 than the other sites. Site 3 has the highest cell concentrations for all three algae species, with blooms of species A and B. Site 2 also has blooms of species A and B. Cell concentrations are highest for algae species A, and very low for species C. Species A tends to bloom at all of the sites in the late spring, early summer. Species B blooms at sites 2 and 3 in the summer or early fall. Species C doesn't really bloom, but is at its highest cell densities in late winter and early fall.

Proposal for Year 2 Sampling

Using your analysis of Year 1, create a proposal for where your scientific team will deploy oysters to sample for harmful algae toxins in oyster meat in Year 2. Your team only has enough resources for 9 oyster deployments (for each deployment you must pick one season at one site). Mark an "X" in the table for each of your planned deployments. Think about where you might be likely to find harmful algae toxins.

	Site			
Season	**1**	**2**	**3**	**4**
Winter				
Spring				
Summer				
Fall				

Analysis of Proposal for Year 2 Sampling

Explain what information from Year 1 was important in determining where and when to do your oyster deployments in Year 2.

Answers will vary, but students should be thinking about which sites had high cell concentrations of harmful algae (species B & C) at what times of year, and how that might relate to where harmful algae toxin would bioaccumulate in oysters. Hint: Times of year with higher harmful algae cell concentrations often correlate with higher instances of harmful algae toxins in oysters.

After you have finalized your team's Year 2 Sampling Plan, collect your harmful algae toxin data from the "lab".

Year 2 Lab Results

Record in the table below the harmful algae toxin data. "ND" means the toxin concentration was too low to be detected ("Non Detect"). All of the toxin data is reported in µg toxin/100 g oyster meat.

	Site							
Season	**1**		**2**		**3**		**4**	
	B toxin	**C toxin**	**B toxin**	**C toxin**	**B toxin**	**C toxin**	**B toxin**	**C toxin**
Winter	ND	ND	76	6	48	ND	ND	2
Spring	ND	ND	21	ND	50	3	ND	**26**
Summer	25	ND	**122**	19	**684**	ND	8	11
Fall	ND	ND	**1854**	**43**	**97**	2	ND	ND

Results and Analysis of Year 2 Data

From your oyster deployments, at which sites and during which seasons, did you detect harmful algae toxins? Does this make sense with what you saw for algae cell concentrations in Year 1? Explain why or why not.

Answers will vary, students should list when and where they detected harmful algae toxins in their oyster deployments.

For B toxin, toxin concentration correlates somewhat with species B cell concentrations, although cell concentration is not a perfect predictor of toxin concentration (toxin production by the cells may vary by site or by season).

For C toxin, at site 2 in the fall a lot of toxin was produced, but in winter at site 3 (where you might expect to see a lot of toxin), toxin was below detection; this could be again because toxin production may vary through time and space, but it could also be because cell concentrations in Year 2 were lower than in Year 1 at site 3. Cell concentrations will be different from year to year! This also helps to explain why Site 4 had a high toxin event in the spring, either in Year 2 during the spring there was more of species C present at the site, or perhaps the cells that were present at that site produce more toxin than the same species at other sites.

Did you detect any incidences where the oysters exceeded the regulatory limits for seafood? Mark these in your Year 2 Lab Results table.

The regulatory limits are: **80 µg B toxin**/100 g oyster meat, **20 µg C toxin**/100 g oyster meat.

Answers will vary, students should list any incidences that they captured with their sampling plan.

Oysters from sites 2 and 3 exceeded the regulatory limit for B toxin in the summer and fall.

Oysters from site 2 exceeded the regulatory limit for C toxin in the fall, and oysters from site 4 exceeded the regulatory limit for C toxin in the spring.

Based on your findings, where are when would you recommend that oyster harvest be closed to protect human health?

Answers will vary, students should include any sites and seasons where they detected harmful algae toxin concentrations in oysters that exceeded the regulatory limits. This is also a good time to discuss "detoxification" in oysters and other shellfish. Detoxification is the process of toxin being eliminated from the organism's tissues. Rates of detoxification vary with toxin type, shellfish species, and with season (for more info on detoxification and depuration, see [Marine biotoxins, 2004]). In general, filter-feeding shellfish can take some time to eliminate toxins, so often harvest areas remain closed for a while after cell concentrations have declined.

This is when the teacher should reveal the PowerPoint slide with ALL of the lab results. Students can then record these numbers in their table. (Alternatively, students can be given a handout of the full lab results and answer the following questions as homework.)

Once you fill in all of the lab results, answer the following questions.

Conclusions and Future Work

How successful was your sampling plan at protecting human health, i.e., did you catch all of the incidences when harmful algae toxin in oysters exceeded the regulatory limits? Explain why or why not.

Answers will vary. Most likely students will miss some or all of the regulatory exceedances for C toxin because only very low cell concentrations are needed to produce enough toxin to make oysters toxic to humans. Additionally, spring in Year 1 at site 4 showed pretty low cell concentrations of species C, so either more cells were present here in Year 2, or the cells at site 4 produce more toxin than the same

species at the other sites. For B toxin, summer and fall at sites 3 show high toxin, which correlates well with when the blooms of these species occur at this site. At site 2, the oysters might have been high in toxin in the fall either because in Year 2, the bloom occurred later in the year, or because the oysters here detoxify slowly (see previous answer).

Were there any times where you expected to find harmful algae toxins and didn't? Why might this be?
Some students may have expected to find more C toxin at site 3 during the winter. As stated before, it's possible that in Year 2 the cell concentration of species C was lower here at this time of year, or it is also possible that the cells present at this site do not produce as much toxin as species C cells at other sites.

If you had unlimited resources, how would you design a monitoring plan for these four sites using water samples and/or oyster deployments?
Answers will vary. Generally, oyster deployments will provide a more accurate understanding of the risk to human health from consuming oysters than cell concentrations will. Measuring cell concentrations at the same time as toxins might allow us to gain a better understanding of the toxin production of these harmful algae species at different sites throughout the year. If you had to cut out anything, it might not be worthwhile to monitor Site 1 since generally the cell and toxin concentrations are very low. It also might make sense to skip winter monitoring since toxin levels seem to be low at that time of year. In a perfect world, we might want to deploy oysters at all four of the sites year-round to monitor everything. It is worth keeping in mind that the distribution and toxin production of these algae species will likely change as sea surface temperatures rise and species habitat ranges shift due to climate change.