## Slicing the Pie Activity Packet

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 Pseudonitzschia 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 <br> (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 ?


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



## Exercise 3

| Time |
| :---: | :---: | :---: | :---: | :---: |
| period |

