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2020

## **Isotopes in the Estuary: Conception and Application of Stable and Radioactive Carbon**

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*Virginia Institute of Marine Science*

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### **Recommended Citation**

Detweiler, D. (2020) Isotopes in the Estuary: Conception and Application of Stable and Radioactive Carbon. VA Sea 2020 Lesson Plans. Virginia Institute of Marine Science, William & Mary. doi: 10.25773/f8rb-6z53

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# ISOTOPES IN THE ESTUARY

## CONCEPTION AND APPLICATION OF STABLE AND RADIOACTIVE CARBON

**Derek Detweiler**

Virginia Institute of Marine Science

**Grade Level**

High School

**Subject Area**

Chemistry, Oceanography,  
and Environmental Science

*The 2019/2020 VA SEA project was made possible through funding from the National Estuarine Research Reserve System Margaret Davidson Fellowship Program which supports graduate students in partnership with research reserves where fieldwork, research, and community engagement come together. VA SEA is currently supported by the Chesapeake Bay National Estuarine Research Reserve, Virginia Sea Grant, and the Virginia Institute of Marine Science Marine Advisory Program.*



**Title:** Isotopes in the Estuary: Conception and Application of Stable and Radioactive Carbon

**Focus:** Scientists use stable and radioactive carbon isotopes to determine the source and age of environmental samples such as plant matter and soils.

**Grade Level:** High School Chemistry, Oceanography, Environmental Science

**VA Science Standards:**

**CH.1** The student will investigate and understand that experiments in which variables are measured, analyzed, and evaluated produce observations and verifiable data. Key concepts include:

1. manipulation of multiple variables, using repeated trials.
2. accurate recording, organization, and analysis of data through repeated trials.
3. mathematical manipulations including graphing, ratio and proportion, construction and defense of a scientific viewpoint.
4. the use of current applications to reinforce chemistry concepts.

**CH.2** The student will investigate and understand that the placement of elements on the periodic table is a function of their atomic structure. The periodic table is a tool used for the investigations of:

1. average atomic mass, mass number, and atomic number.
2. isotopes, half-lives, and radioactive decay.

**CH.3** The student will investigate and understand how conservation of energy and matter is expressed in chemical formulas and balanced equations. Key concepts include:

1. balancing chemical equations.
2. writing chemical formulas.

**Learning Objectives:** At the completion of this lesson, students will be able to:

1. Define and explain the concept of stable and radioactive carbon isotopes.
2. Describe how isotope data is collected and analyzed.
3. Conduct analyses on isotope data by calculating proportions and averages of stable and radioactive carbon signatures.
4. Compare isotope data from different land types within an estuary, including natural forests, wetlands, agriculture, and industry.
5. Assess chemical compounds derived from different land types and their reactions with other compounds.
6. Predict products of two reactants and develop balanced chemical equations based on provided chemical reactions.
7. Communicate scientific results using oral and graphical interpretation.
8. Apply stable and radioactive carbon isotopes in the context of real-world environmental issues and questions.

**Total length of time required for lesson:**

1 hr. 30 min. (or two class periods) with an optional 50 min. supplemental activity.

**Key words, vocabulary:** the following definitions are modified from Google Dictionary.

**Carbon (C)** – belonging to group 14 on the Periodic Table of Elements, it is one of the most abundant elements on Earth, with atomic number 6 and atomic mass 12.011.

**Isotope** – each of two or more forms of the same element that contain equal numbers of protons, but different numbers of neutrons in their nuclei, and hence differ in relative atomic mass but not in chemical properties.

**Carbon-12 ( $^{12}\text{C}$ )** – the most common natural carbon isotope of mass 12, with a nucleus containing six protons and six neutrons. It is the basis for the accepted scale of atomic mass units.

**Carbon-13 ( $^{13}\text{C}$ )** – a natural, stable isotope of carbon with a nucleus containing six protons and seven neutrons. As one of the environmental isotopes, it makes up about 1.1% of all natural carbon on Earth.

**Carbon-14 ( $^{14}\text{C}$ )** – a long-lived, naturally occurring radioactive carbon isotope of mass 14, its nucleus contains six protons and eight neutrons. C-14 is used in carbon dating and as a tracer in biochemistry. As one of the environmental isotopes, it makes up less than 0.1% of all natural carbon on Earth.

**Atomic number** – the number of protons in the nucleus of an atom, which determines the chemical properties of an element and its place on the Periodic Table of Elements.

**Atomic mass** – the mass of an atom of a chemical element expressed in atomic mass units. It is approximately equivalent to the number of protons and neutrons in the atom (the mass number) or to the average number allowing for the relative abundances of different isotopes.

**Radioactive decay** – spontaneous breakdown of an atomic nucleus resulting in the release of energy and matter from the nucleus. Remember that a radioisotope has unstable nuclei that does not have enough binding energy to hold the nucleus together.

**Half-life** – the time taken for the radioactivity of a specified isotope to fall to half its original value.

**Isotope ratio mass spectrometry (irMS)** – measures relative abundance of isotopes in an environmental sample using mass-to-charge ratio of ions.

**Mass-to-charge ratio** – in mass spectroscopy, the mass-to-charge ratio of a cation is equal to the mass of the cation divided by its charge.

**Ratio** – the abundance of one thing compared to another (i.e. a proportion or fraction) denoted by using a colon. For example, 2:1 means 2 to 1, or 2/1.

**Ion** – an atom or molecule with a net electric charge due to the loss or gain of one or more electrons. A negatively charged ion is an anion, while a positively charged ion is a cation.

**Estuary** – a partially enclosed coastal body of brackish water with one or more rivers or streams flowing into it, and with a free connection to the open sea.

**Sea level rise** – an increase in the level of the world's oceans due to the effects of climate change, including increasing temperatures and melting ice sheets.

**Wetland** – land consisting of marshes or swamps; saturated land.

**Nitrate** – a polyatomic ion with the molecular formula  $\text{NO}_3^-$ , a common component of fertilizers in order to maximize crop yield.

**Ammonium** – a polyatomic ion with the chemical formula  $\text{NH}_4^+$ , a common waste product of metabolic processes in animals.

**Carbonate** – a polyatomic ion with the chemical formula  $\text{CO}_3^{2-}$ . In aquatic systems, this compound acts as a pH buffer so systems do not become too acidic.

**Phosphate** – a polyatomic ion with the chemical formula  $\text{PO}_4^{3-}$ . In aquatic systems, this compound is present in minerals and is also used to fuel cellular processes of plants and algae.

**Glucose oxidation** – the transformation of glucose sugar ( $\text{C}_6\text{H}_{12}\text{O}_6$ ) produced by plants and algae to carbon dioxide ( $\text{CO}_2$ ) through respiration.

**Carbon dioxide** – a polyatomic ion with chemical formula  $\text{CO}_2$ . It is a colorless, odorless gas produced by burning fossil fuels. It is absorbed by plants in photosynthesis, but is becoming increasingly important in the context of climate change because it is a greenhouse gas and is responsible for Earth's increasing temperatures.

**Proxy** – a variable that is not in itself directly relevant, but that serves in place of an unobservable or immeasurable variable. For example, stable and radioactive carbon isotopes are proxies for sources of carbon to estuaries.

**Replicate** – the action of copying or reproducing something.

**Background Information:** The following information is simplified from Fry (2006) and Bianchi and Canuel (2011), two collegiate-level chemistry textbooks specializing in isotopes and aquatic environments.

Carbon is one of the most abundant elements on Earth and is found in all living things including bacteria, algae, plants, and animals. Elemental carbon is defined as having an atomic number of 6 and an atomic mass of 12.0107 with 6 protons and 6 neutrons. However, this is only true most of the time. Have you ever wondered why the mass of carbon is not exactly 12.0? This is because variations of the carbon atom, though few, exist naturally in the environment.

A standard atom of carbon described above is more specifically defined as Carbon-12 ( $^{12}\text{C}$ ), which comprises 98.93% of all naturally occurring carbon on Earth. Two variations of carbon, carbon-13 ( $^{13}\text{C}$ ) and carbon-14 ( $^{14}\text{C}$ ), are also present in tiny amounts, and simply differ in their number of neutrons. Carbon-13 has 6 protons, 7 neutrons, and comprises just 1.1% of all carbon on Earth. Carbon-14 has 6 protons, 8 neutrons, and comprises less than 0.1% of all carbon on Earth. Because these variations all contain 6 protons, they are chemically the same. The difference in the number of neutrons is simply due to natural variation caused by billions of years of Earth's environmental history. We call carbon-13 and carbon-14 isotopes of carbon-12.

The technical definition of an isotope is an atom of an element that has a different number of neutrons but retains the same chemical characteristics. In Latin terminology, *iso-* means same, and *-topos* means place. In this context, this means that isotopes have the same location on the Periodic Table of Elements. Though carbon isotopes are the focus of this activity, all elements have various isotopes that comprise their total abundance on Earth. However, there is typically one atomic form that dominates the entire pool by more than 98%. As we will see, there is something hidden in that remaining 2% that scientists can exploit for investigative purposes.

Isotopes can also be characterized as stable or radioactive. Stable isotopes, such as carbon-13, never change or decay in the environment. Radioactive isotopes, such as carbon-14, spontaneously decay and disappear over time. How can these two distinct properties be used in scientific research? The following describes how stable and radioactive carbon isotopes work and how they are used in scientific research.

Scientists can use stable carbon isotopes ( $^{13}\text{C}$ ) to determine the source of materials in a given environment.  $^{12}\text{C}$  dominates in all living things, but biochemical processes such as photosynthesis and respiration in plants also use  $^{13}\text{C}$ . The biochemical pathways for these processes differ between types of organisms, and therefore would differ in the relative amounts of  $^{13}\text{C}$  and  $^{12}\text{C}$  present in tissue. For example, trees, wetland plants, and algae all perform different types of reactions to help them survive in their respective environments. These reactions follow different pathways and utilize different amounts of  $^{12}\text{C}$  versus  $^{13}\text{C}$ . Determining the ratio, or proportion, of these isotopes in an environmental sample therefore provides insight into the source of the material.

Radioactive carbon, or radiocarbon ( $^{14}\text{C}$ ) is formed in the upper atmosphere when nitrogen loses a proton and gains a neutron. On Earth, it is found in trace amounts mostly confined to the atmosphere and surface deposits. The amount of  $^{14}\text{C}$  in the environment is almost constant, but decreases with a predictable decay rate because it is unstable. Decay is measured in terms of half-life, or the time it takes for one half of all unstable nuclei to decay. Carbon-14 has a half-life of 5,730 years, meaning that half of an original parent material will remain after 5,730 years. Determining the ratio of  $^{14}\text{C}$ :  $^{12}\text{C}$  provides

information on the age of material. For instance, a high  $^{14}\text{C} : ^{12}\text{C}$  would indicate younger material such as that derived from plants, since there is still a lot of radiocarbon present. A low  $^{14}\text{C} : ^{12}\text{C}$  would indicate older material such as that derived from industry or agriculture, since there is not much  $^{14}\text{C}$  left.

In practice, stable and radioactive isotopes can be determined from a variety of environmental samples including plant matter, soils, and water and are representative of both living and dead plant matter. While collection and preparation varies, samples can ultimately be analyzed using isotope ratio mass spectrometry (irMS). This analytical technique measures the mass-to-charge ratio of different elemental ions, and the frequency or abundance of each carbon isotope is calculated. The mass-to-charge ratio is

equal to the mass ( $m$ ) of an ion divided by its charge ( $z$ ). The frequency or abundance of each  $m/z$  is indicative of an isotope.

In environmental research, isotopes provide pertinent information about the quality of an environment that might have a mixture of materials from different sources, such as estuaries. Estuaries, like the York, James, and Rappahannock Rivers in Virginia, are partially enclosed coastal water bodies with one or more rivers or streams flowing into them, and with a free connection to the open sea, in this case, the Chesapeake Bay. Estuaries are at the interface between land and sea and experience influences from many surrounding landscapes such as natural forests and wetlands, or more human-perturbed regions such as agricultural or industrial land. By assessing samples from estuarine environments, one can conclude how it is impacted by the surrounding landscape and therefore assess its quality.

Quality of estuaries is thereby impacted by the chemical compounds from different land types. If isotope data suggests that an estuary is impacted by lots of agriculture or industry, scientists might wonder what types of compounds contribute to this impact. For instance, estuaries might receive lots of fertilizer runoff from agricultural land which is usually comprised of nitrate and ammonium, two compounds that optimize the growth of crops. Additionally, factories might release lots of carbon dioxide due to the burning of fossil fuels. Even natural land types influence the types of compounds found in estuaries. For instance, forests release phosphate molecules, which are produced by mineral weathering. Wetlands release carbonate, which buffers aquatic systems from sharp changes in pH. Lastly, the algae that live in the estuary itself produce their own food in the form of simple sugars or glucose. Isotopes allow scientists to draw conclusions about the types of chemical compounds that contribute to complex estuarine water chemistry.

In this activity, students will embark on a journey through an estuary impacted by various land types including natural forests, wetlands, agriculture, and industry. They assess the abundance of each type of carbon isotope associated with each land type. Then, given an unknown sample, determine which land type impacts their estuary “unknown” the most. Through this activity, students will also be introduced to various aspects of the scientific process, including the importance of replication. Replication is the action of copying or reproducing something. In research, it is important to sample an area more than once to assure the accuracy and precision of results. That is, it is important that each replicate is similar in value. Greater similarity indicates greater strength and power in your results.



A supplementary activity, titled “What’s in the Water? Visualizing Chemical Compounds in Estuaries,” provides additional learning about the types of compounds associated with each land type. Here, students are challenged to predict the products of reactants interacting within estuary waters to form new compounds.

**Materials and Supplies:** For each group of 3-4 students, you will need:

1. Estuary display (provided as five 8.5 x 11” sheets in Appendix A, to be assembled)
  - a. Can be assembled on larger poster board (optional)
2. Access to color printer
3. Scotch tape
4. Scissors
5. Three standard-dozen egg cartons (sampling plot)
6. Large bag of rice (soil)
7. ~60 red beads ( $^{12}\text{C}$  isotope)
8. ~115 yellow beads ( $^{13}\text{C}$  isotope)
9. ~60 blue beads ( $^{14}\text{C}$  isotope)
10. Three dimensional model structures for the following chemical compounds. **Note:** Can be built using marshmallow modeling kits or if not available, use the two-dimensional structures in illustrated in Appendix B
  - a. Nitrate ( $\text{NO}_3^-$ )
  - b. Ammonium ( $\text{NH}_4^+$ )
  - c. Carbonate ( $\text{HCO}_3^-$ )
  - d. Phosphate ( $\text{PO}_4^{3-}$ )
  - e. Glucose ( $\text{C}_6\text{H}_{12}\text{O}_6$ )
  - f. Carbon dioxide ( $\text{CO}_2$ )
11. Marshmallow modeling kit (colored pompoms or modeling clay are an additional alternative)
  - a. Pack of toothpicks
  - b. Pack of multi-color miniature marshmallows (Kraft Jet-Puffed FunMallows)
12. 25x30” Post-it Easel Pad chart paper
13. Data sheet (provided in Appendix C)

Round “tokens” cut from colored construction paper can be used as an alternative.

**Teacher Preparation:** For each group of 3-4 students, complete the following prior to lesson:

1. Assemble estuary display by printing each of the provided 8.5 x 11" sheets and either taping them together or gluing on a larger poster board.
2. Remove tops (lids) of standard-dozen egg cartons.
3. Cut bottom portion (the part that holds the eggs) of cartons into fractions of four "plots" or egg holders (2x2) (Fig. 1).
  - a. Note: you will be able to get two 2x2 plots per egg carton, and can combine remaining 1x2 plot from two cartons to conserve resources.

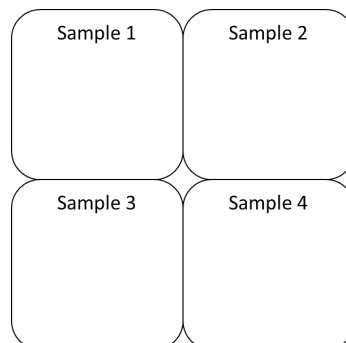


Fig 1: Schematic of sampling plots created from egg cartons.

4. On each estuary display, place newly created plots on each designated site (forest, wetland, agriculture, industry, aquatic and unknown estuarine site).
5. Place beads in each 2x2 plot (representative of samples) according to the following ratios (Fig. 2).
  - a. **Note:** There are five types of "unknown" estuarine environments that can be used in this lesson, each impacted more or less by a certain landscape. Each group will have the same forest, wetland, agriculture, industrial, and and aquatic site. However, each group will be randomly assigned a different unknown to break up the redundancy among groups. Each unknown should match one of the common sites (see procedure).
  - b. **Forest site**
    - i. Sample 1: 5 yellow, 1 red, 2 blue
    - ii. Sample 2: 3 yellow, 1 red, 1 blue
    - iii. Sample 3: 4 yellow, 2 red, 1 blue
    - iv. Sample 4: 5 yellow, 2 red, 1 blue
  - c. **Wetland site**
    - i. Sample 1: 3 yellow, 2 red, 2 blue
    - ii. Sample 2: 4 yellow, 2 red, 4 blue
    - iii. Sample 3: 4 yellow, 3 red, 4 blue
    - iv. Sample 4: 5 yellow, 3 red, 4 blue
  - d. **Agricultural site**
    - i. Sample 1: 3 yellow, 2 red, 1 blue
    - ii. Sample 2: 4 yellow, 2 red, 1 blue
    - iii. Sample 3: 4 yellow, 3 red, 1 blue
    - iv. Sample 4: 5 yellow, 3 red, 2 blue
  - e. **Industrial site**
    - i. Sample 1: 2 yellow, 2 red, 1 blue
    - ii. Sample 2: 2 yellow, 1 red, 0 blue
    - iii. Sample 3: 3 yellow, 2 red, 0 blue
    - iv. Sample 4: 1 yellow, 1 red, 0 blue

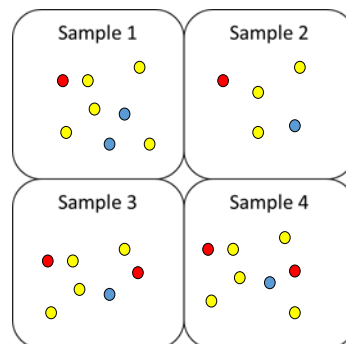


Fig. 2: Example of 2x2 sampling plot for forest site.

- f. **Aquatic site**
  - i. Sample 1: 4 yellow, 2 red, 1 blue
  - ii. Sample 2: 6 yellow, 3 red, 3 blue
  - iii. Sample 3: 2 yellow, 1 red, 0 blue
  - iv. Sample 4: 6 yellow, 3 red, 2 blue
- g. **Unknown estuarine site 1 (forest-influenced)**
  - i. Sample 1: 4 yellow, 1 red, 1 blue
  - ii. Sample 2: 2 yellow, 1 red, 1 blue
  - iii. Sample 3: 5 yellow, 3 red, 2 blue
  - iv. Sample 4: 6 yellow, 2 red, 2 blue
- h. **Unknown estuarine site 2 (wetland-influenced)**
  - i. Sample 1: 3 yellow, 2 red, 3 blue
  - ii. Sample 2: 5 yellow, 3 red, 4 blue
  - iii. Sample 3: 2 yellow, 1 red, 3 blue
  - iv. Sample 4: 4 yellow, 2 red, 4 blue
- i. **Unknown estuarine site 3 (agriculture-influenced)**
  - i. Sample 1: 4 yellow, 1 red, 1 blue
  - ii. Sample 2: 2 yellow, 1 red, 0 blue
  - iii. Sample 3: 3 yellow, 3 red, 1 blue
  - iv. Sample 4: 3 yellow, 2 red, 2 blue
- j. **Unknown estuarine site 4 (industry-influenced)**
  - i. Sample 1: 5 yellow, 5 red, 1 blue
  - ii. Sample 2: 2 yellow, 1 red, 0 blue
  - iii. Sample 3: 4 yellow, 3 red, 0 blue
  - iv. Sample 4: 1 yellow, 1 red, 1 blue
- k. **Unknown estuarine site 5 (no influences – aquatic)**
  - i. Sample 1: 3 yellow, 1 red, 0 blue
  - ii. Sample 2: 5 yellow, 2 red, 2 blue
  - iii. Sample 3: 3 yellow, 2 red, 1 blue
  - iv. Sample 4: 5 yellow, 2 red, 1 blue
6. Fill plots with rice until isotopes are completely covered.
7. **Note:** Steps 1-6 can be simplified by cutting up “tokens” from colored construction paper to match number of beads above, without egg cartons.
  - a. Duplicate as needed and cut colored sheets of paper into respective tokens.
  - b. Four replicate samples are to be placed at each site using the same color ratios as described above.

8. For the supplemental activity, Visualizing Chemical Compounds in the Estuary: use marshmallows and toothpicks to construct three-dimensional models of representative compounds that might be found at each site (Fig 3). As an alternative, illustrations of the structures, provided in Appendix B, can be printed for student reference. If constructing molecules, refer to sheets for correct structure. Be sure to assign each element a color so that students are able to interpret the structure.
- Forest site: phosphate ( $\text{PO}_4$ )
  - Wetland site: carbonate ( $\text{CO}_3$ )
  - Agriculture site: nitrate ( $\text{NO}_3$ ) and ammonium ( $\text{NH}_4$ )
  - Industrial site: carbon dioxide ( $\text{CO}_2$ )
  - Aquatic site: glucose ( $\text{C}_6\text{H}_{12}\text{O}_6$ )
  - Other reactants:
    - Water ( $\text{H}_2\text{O}$ )
    - Hydrogen ion (x2) (H)
    - Oxygen gas ( $\text{O}_2$ )
9. Place each compound at its respective site next to each sampling plot.
10. Provide printed data sheets (Appendix C) for each group.

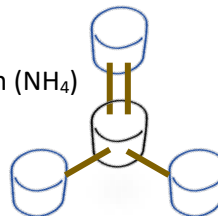


Fig. 3: Example of a carbonate ( $\text{CO}_3$ ) marshmallow molecule. In this example, white marshmallows represent carbon while blue represents oxygen.

## Procedure:

### 1. Introduction (~30 minutes)

- The instructor will introduce stable and radioactive isotopes and their environmental applications using the provided PowerPoint.
- Students will be split into groups of 3-4, each with an assigned display (unknown estuarine site 1, 2, 3, 4, or 5). Depending on class size, some unknowns may have to be assigned more than once.
- The instructor will introduce and explain the activity using the second portion of the provided PowerPoint.

### 2. Activity (~40 minutes)

- Each group will work to collect isotopes from the **known** forest, wetland, agriculture, industry, and aquatic sites (4 replicates per site) and organize them by color.
- Each group will record the number of each isotope for each sample plot according to color and record results on provided assessment sheet. The average of all plots for each site will also be recorded.
- Each group will calculate average ratios of  $^{13}\text{C}$ :  $^{12}\text{C}$  and  $^{14}\text{C}$ :  $^{12}\text{C}$  for each sample plot according to color and record results on provided assessment sheet.
- Repeat steps a-c for the **unknown estuarine site**. At this point, each group should obtain a different result depending on their assigned unknown.
- Each group will plot bar graphs of the average ratios of  $^{13}\text{C}$ :  $^{12}\text{C}$  and  $^{14}\text{C}$ :  $^{12}\text{C}$  on a 25x30" Post-it Easel Pad following the template provided on assessment sheet.
- Based on isotope ratios from the forest, wetland, agriculture, industry, and aquatic sites, students will decide what land type most impacts their estuary (based on result from unknown) and write it on their graph. Students will complete the questions on their assessment sheet.

### 3. Gallery Walk (~5 minutes)

- After completing bar graphs, each group will display their graph around the classroom.
- Students will walk around to observe each graph and the conclusion reached by other groups regarding the land type that most impacts their estuary. Note: the only difference between each graph should be the bar representing the unknown, and the unknown should closely match either the forest, wetland, agriculture, industry, or aquatic site.

### 4. Wrap-up (~15 minutes)

- Students will get back into original groups for a closing discussion led by the instructor.
- Representatives from each group will share results of their unknown with the entire class. The class will discuss the answers to the following questions:
  - Based on your graph, does your prediction about the estuarine site make sense? Why?
  - What factors might contribute to a certain land type having a greater or lesser impact on an estuary as indicated by your isotope analyses. For example, if your estuary exhibited a pattern most similar to agriculture, why might this be so?
  - Explain the pattern observed with  $^{14}\text{C}$ :  $^{12}\text{C}$ . That is, why would certain land types exhibit older material compared to land types with younger material?
  - Describe an environmental issue that you might be able to resolve, or provide information about, by using stable and/or radioactive isotopes.
  - Case Study Question: Sea level rise is a current threat to wetlands due to coastal development and the inability of wetlands to adapt quickly enough. As a result, wetlands can become inundated and disappear over time. They are essentially drowned out. How might isotope signatures shift in response to wetland disappearance.
- The instructor will highlight the importance of stable isotopes and how they can be used to determine the quality of material in estuaries.

### 5. Optional activity for take-home or additional class period.

#### What's in the Water? Visualizing Chemical Compounds in Estuaries

(Note: This activity will work best if students already have experience writing chemical formulas and balancing chemical equations).

#### a. Introduction (~10 minutes)

- Using the provided PowerPoint, the instructor will introduce common chemical compounds and associated chemical reactions for each land type used in the isotope activity.

#### b. Activity (~30 minutes)

- Students will examine the structure of these compounds using the provided marshmallow models or print-outs.
- Students will predict the products of two given reactants and record results on provided assessment sheets.
- If desired, students may also construct the products using marshmallow models.

#### c. Wrap-up (~10 minutes)

- The instructor will review answers using the provided PowerPoint, incorporating previous knowledge of writing chemical formulas and balancing equations.

**Assessment:** Students will be assessed by successful completion of a turned-in data sheet as well as active participation in group discussion and gallery walk. Overall, this activity can contribute to a course's class work and/or participation grade.

### **References**

Bianchi, T.S. and Canuel, E.A. 2011. Chemical biomarkers in aquatic systems. Princeton University Press: Princeton, New Jersey, 396 pp.

Fry, B. 2006. Stable isotope ecology. Springer Scientific: New York, NY, 308 pp.

## **Appendix A: Estuary Display**

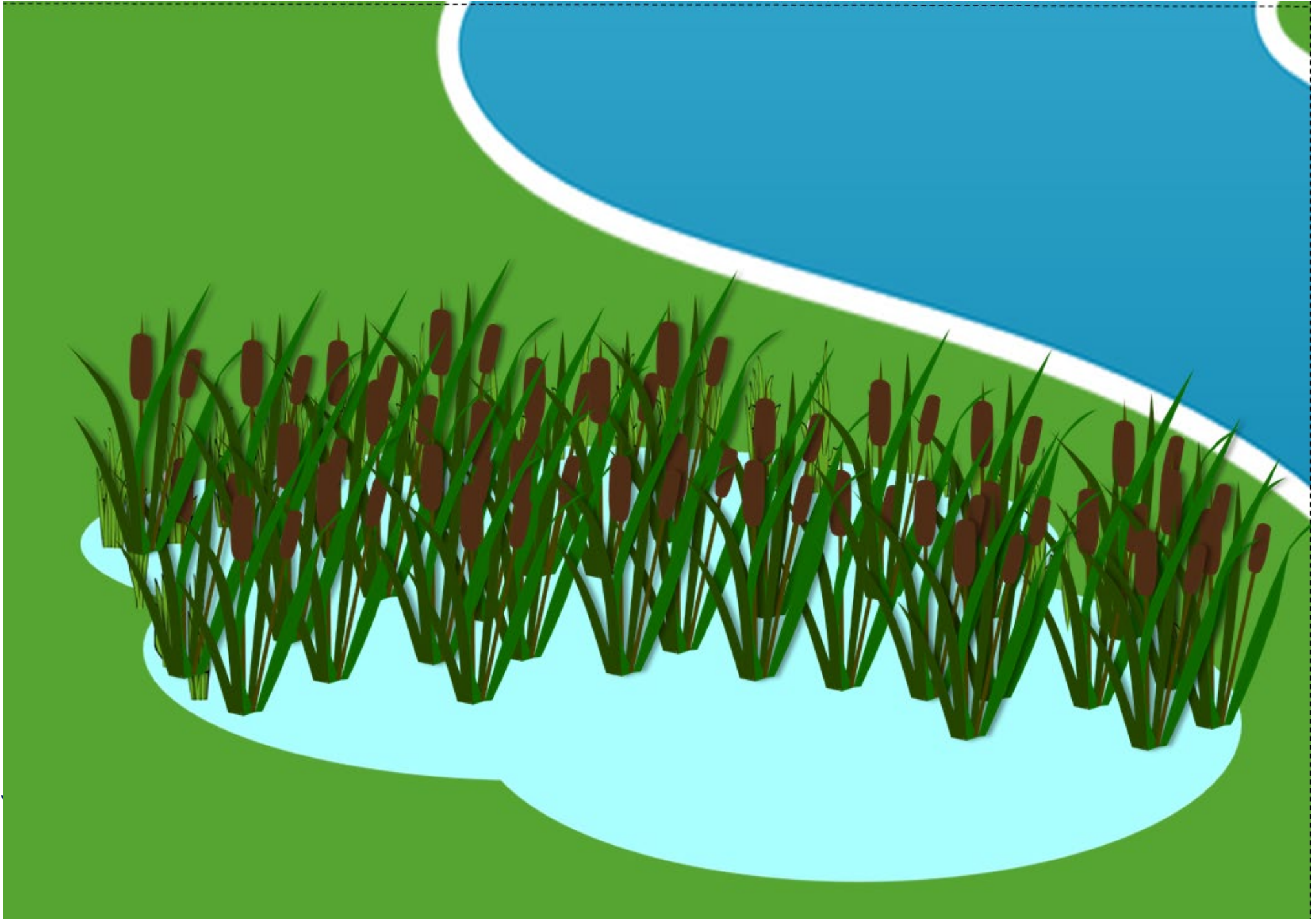
Print the following five 8.5 x 11" sheets  
and assemble to create an estuary landscape display









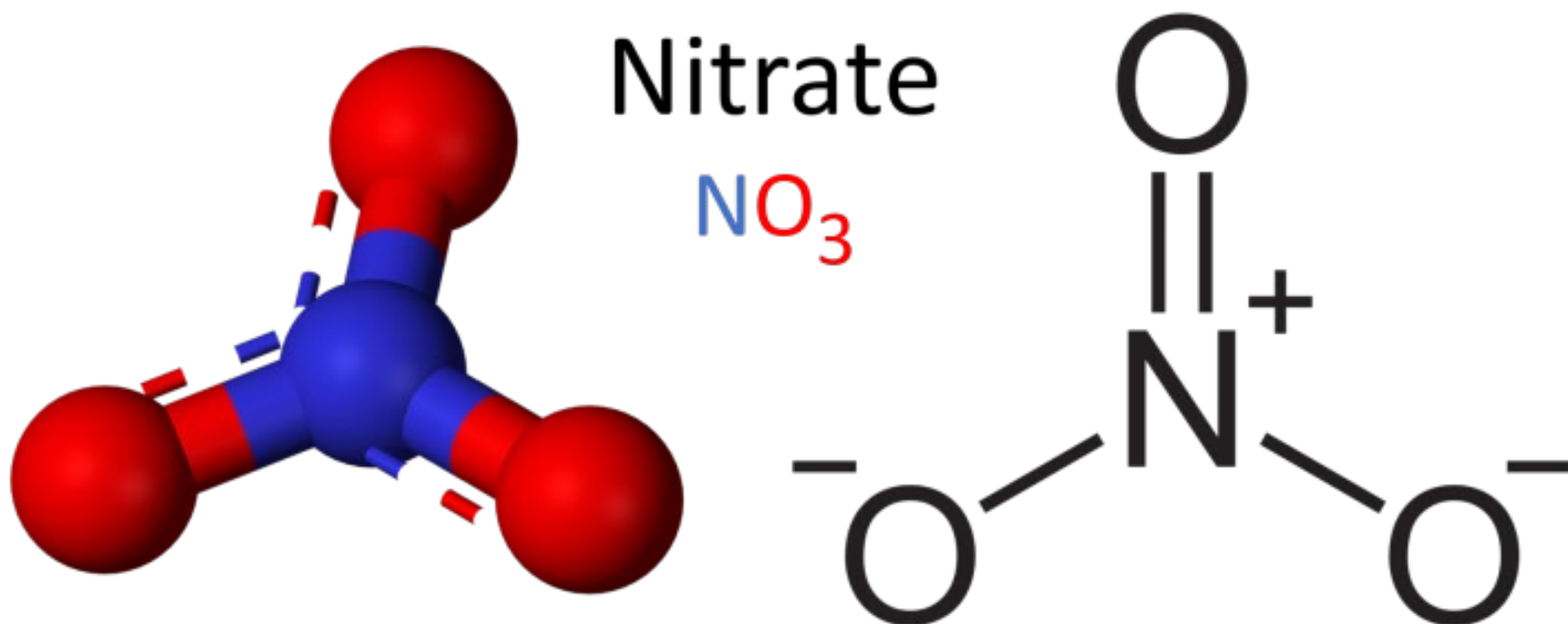




## **Appendix B: Chemical Compound Structures**

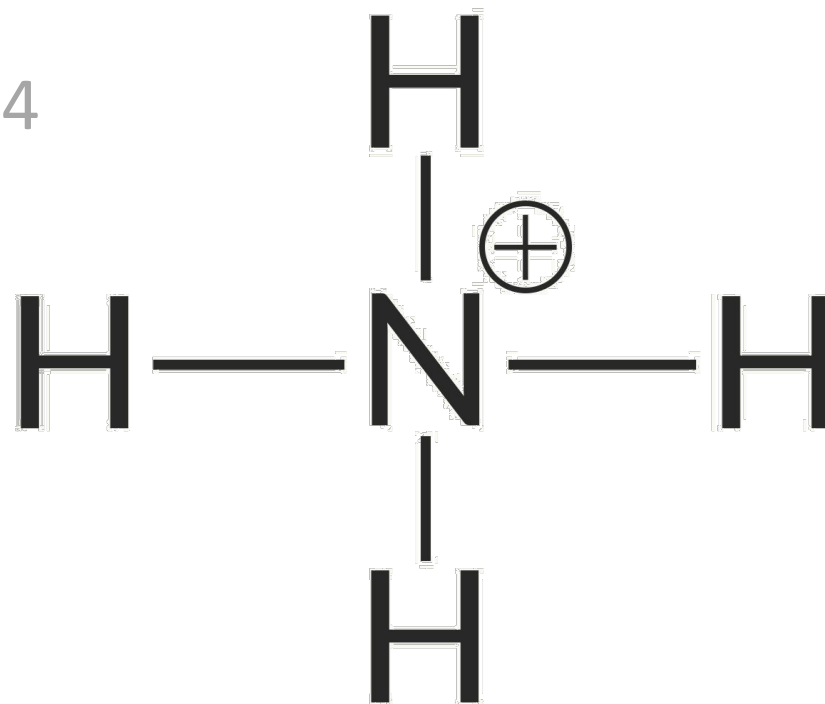
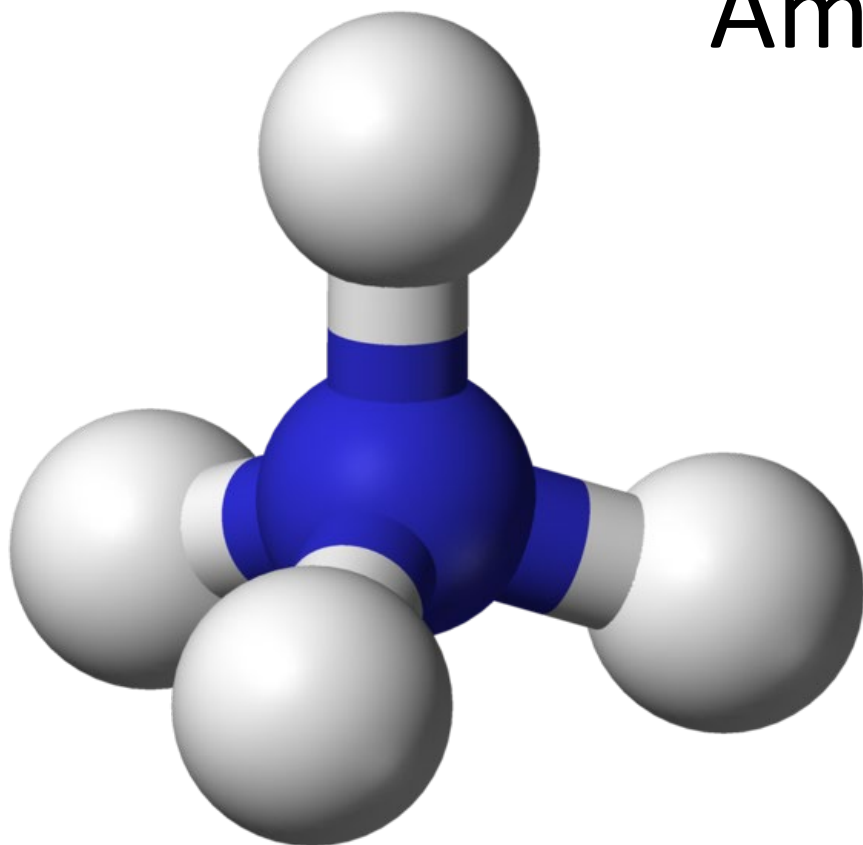
Supplemental Activity – What's in the Water? Visualizing Estuary Compounds

Print the following 6 pages for student reference

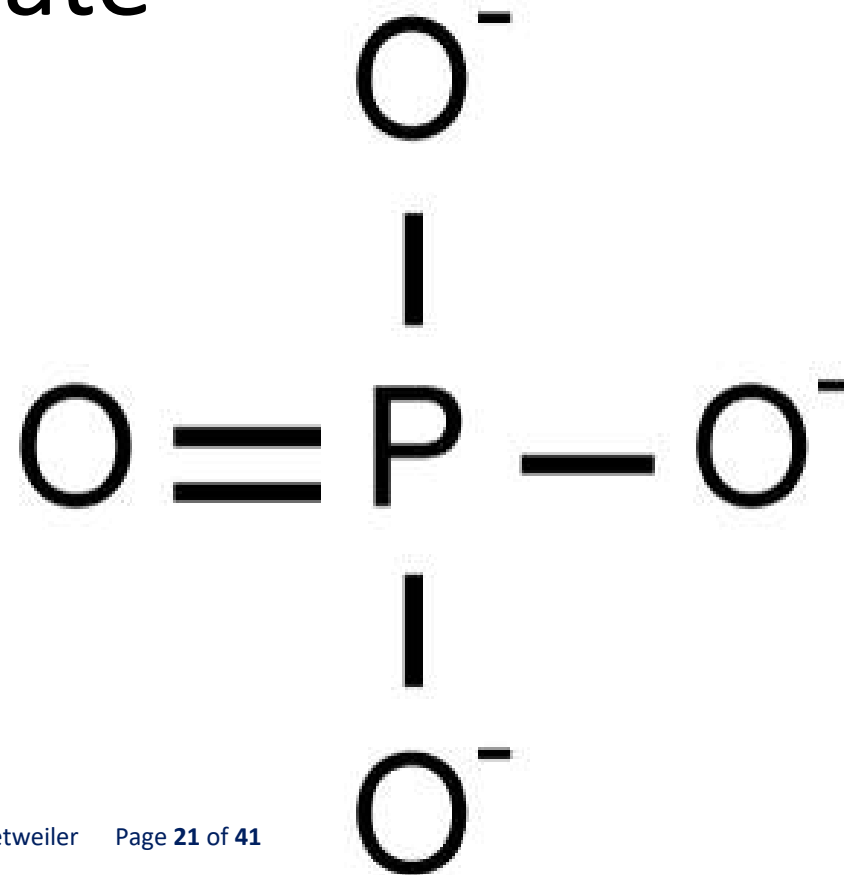
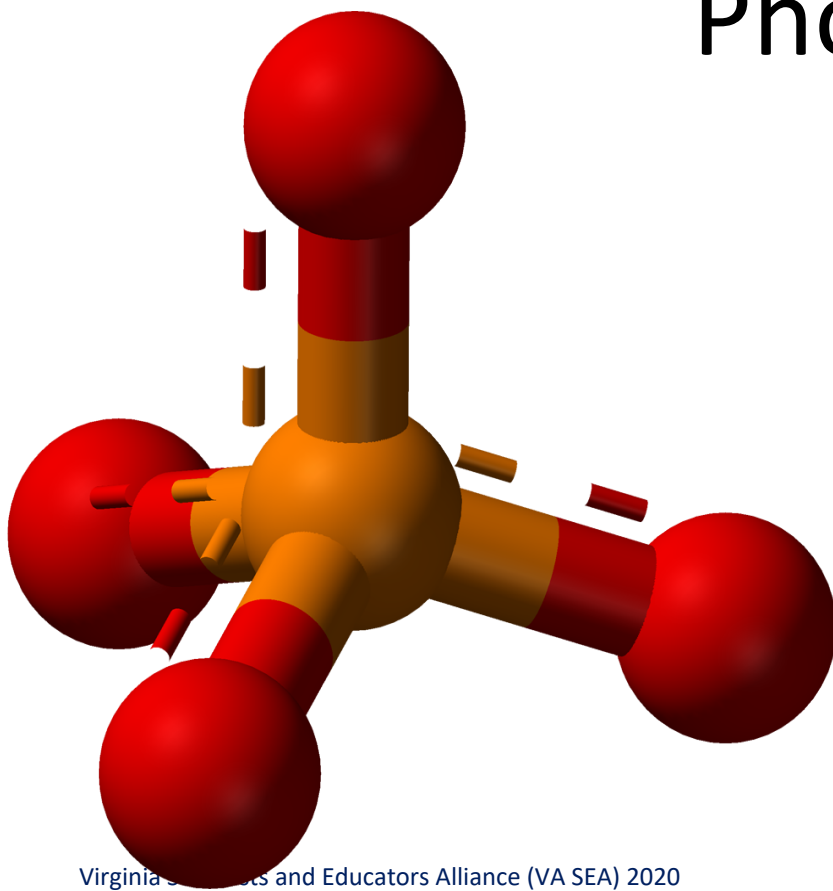




# Ammonium



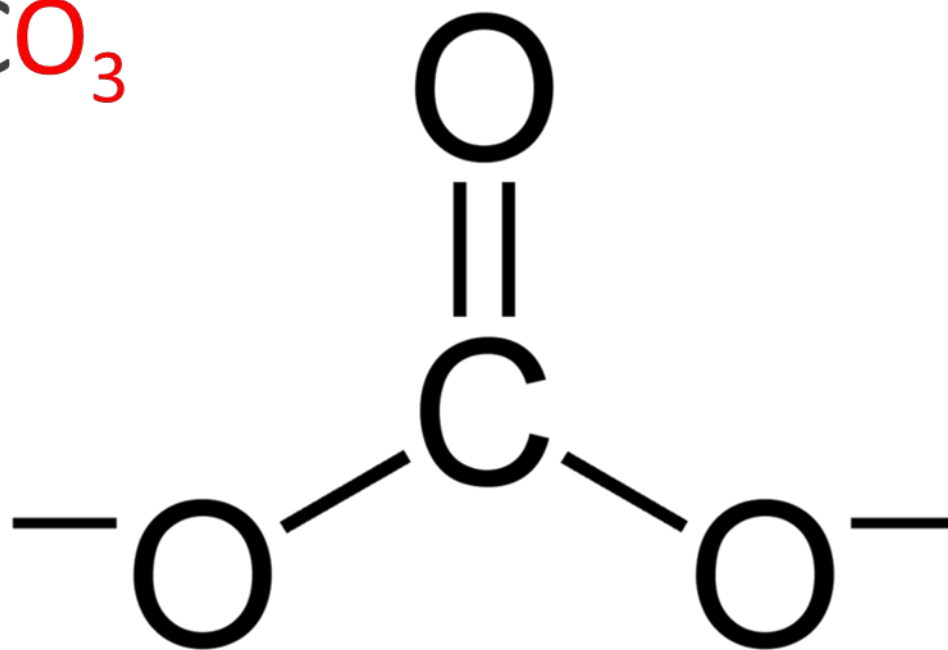
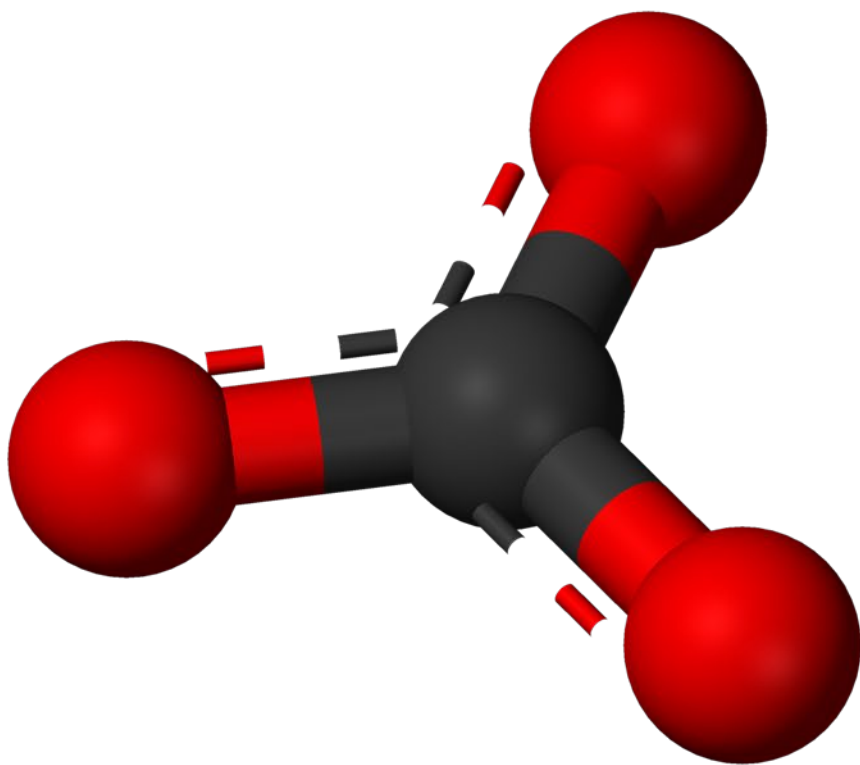
# Phosphate



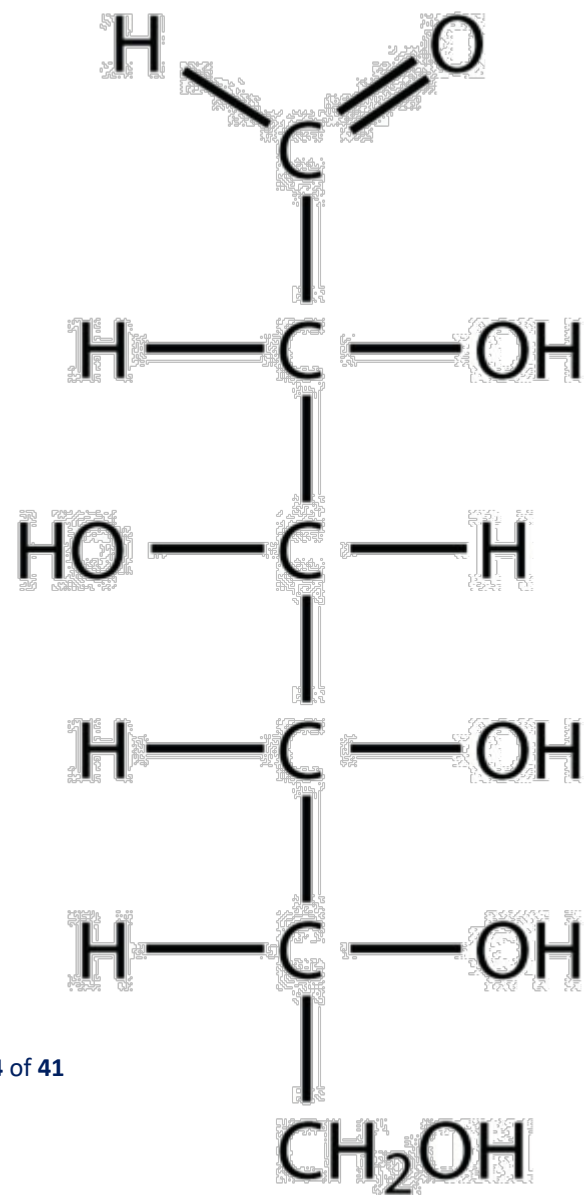
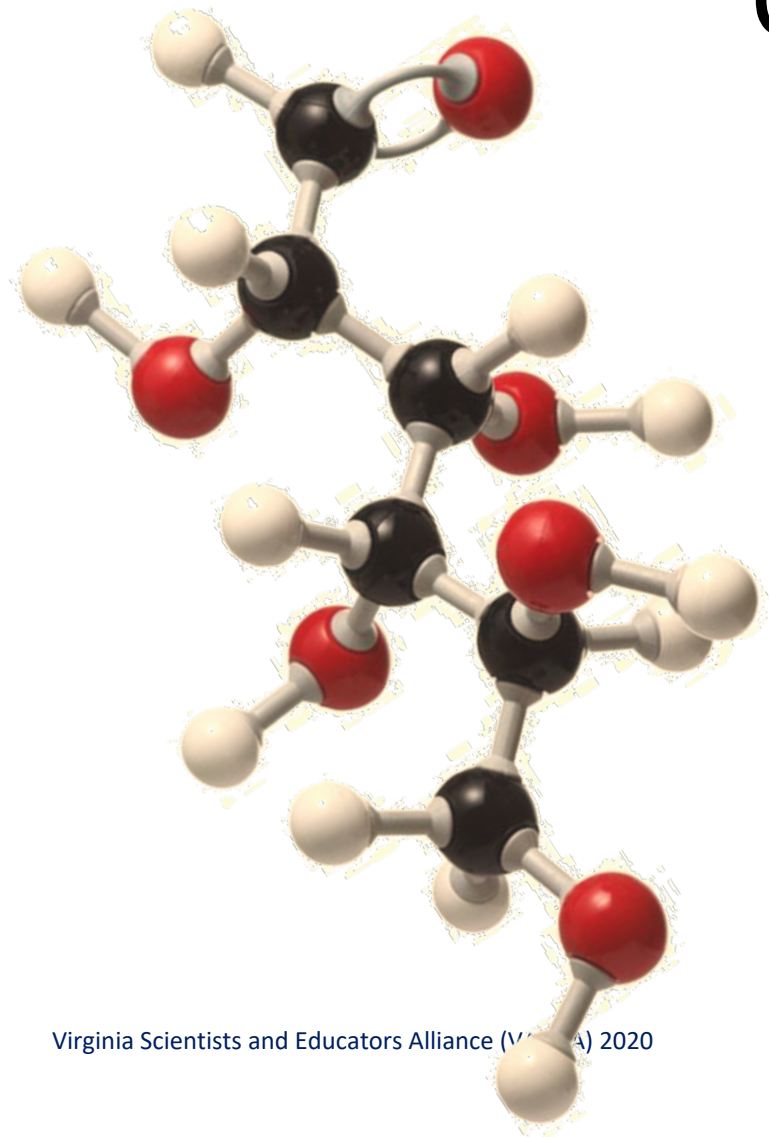




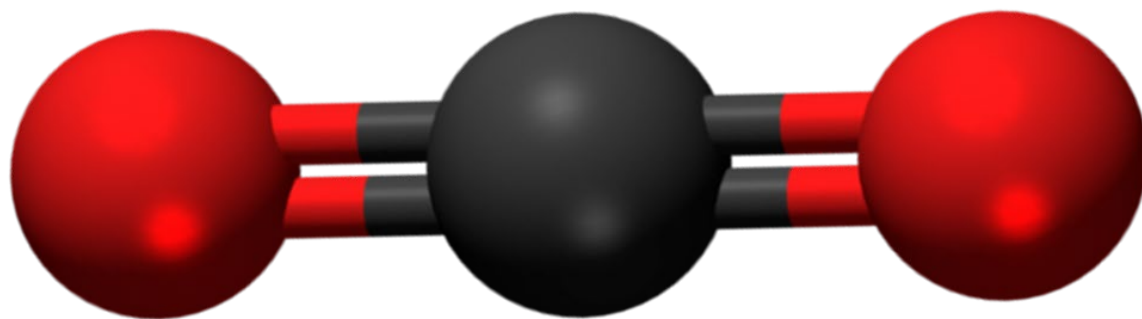
# Carbonate



# Glucose (sugar)



# Carbon Dioxide



## **Appendix C: Data Sheet**

Print one data sheet (4 pages) for each group.

If conducting the supplemental activity,  
print the 5<sup>th</sup> page also.

Name: \_\_\_\_\_

## Isotopes in the Estuary: Conception and Application of Stable and Radioactive Carbon

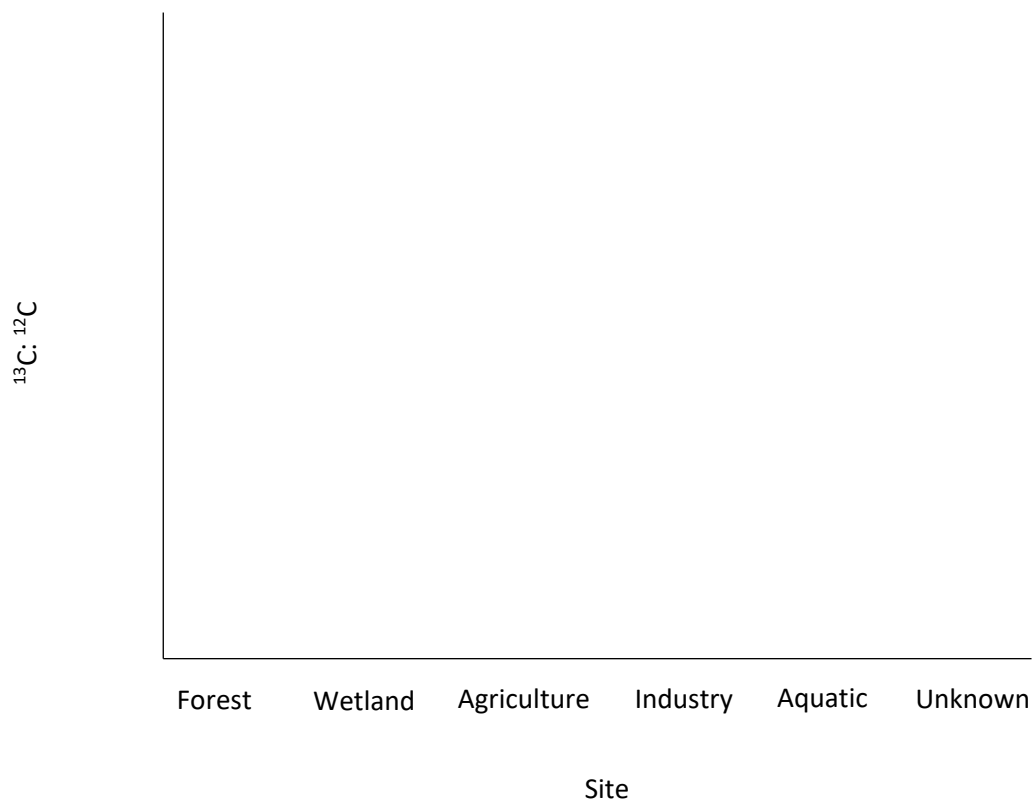
### Data Sheet

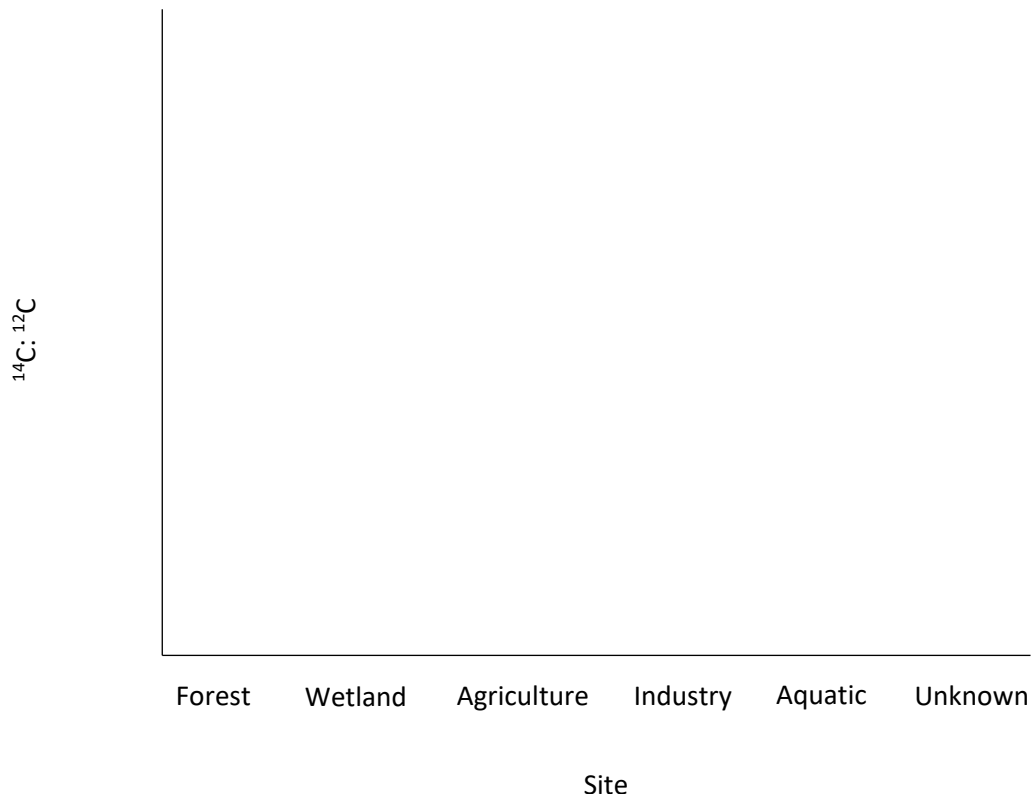
In student groups of 3-4, collect and organize samples from the known forest, wetland, agriculture, industry, and aquatic sites. Each group will also collect and organize samples from an “unknown” estuarine site. Record results here. Be sure to carefully and accurately calculate the average for the number of each isotope and associated ratios of  $^{13}\text{C}: ^{12}\text{C}$  and  $^{14}\text{C}: ^{12}\text{C}$ .

	$^{12}\text{C}$	$^{13}\text{C}$	$^{14}\text{C}$	$^{13}\text{C}: ^{12}\text{C}$	$^{14}\text{C}: ^{12}\text{C}$
<b>Forest</b>					
Sample 1					
Sample 2					
Sample 3					
Sample 4					
Average					
<b>Wetland</b>					
Sample 1					
Sample 2					
Sample 3					
Sample 4					
Average					
<b>Agriculture</b>					
Sample 1					
Sample 2					
Sample 3					
Sample 4					
Average					
<b>Industry</b>					
Sample 1					
Sample 2					
Sample 3					
Sample 4					
Average					
<b>Aquatic</b>					
Sample 1					
Sample 2					
Sample 3					
Sample 4					
Average					
<b>Unknown Estuarine Site</b>					
Sample 1					
Sample 2					
Sample 3					

Sample 4					
Average					

1. Circle your assigned unknown estuarine site: 1 2 3 4 5
2. Create a bar graph for the average isotope ratios at each sample site. For the y-axis, convert your ratio to a whole number (for example, plot 1.5:2.75 as 0.55, because 1.5 divided by 2.75 is equal to 0.55).





3. After analyzing your unknown sample, compare it to the known sites. What land type do you believe most impacted your estuary?  
Circle one: forest wetland agriculture industry no impact (aquatic signature)
4. Based on your graph, does your prediction about the unknown estuarine site make sense? Why?
5. What factors might contribute to a certain land type having a greater or lesser impact on an estuary, as indicated by your isotope analyses. For example, if your estuary exhibited a pattern most similar to agriculture, why might this be so?
6. Explain the pattern observed with  $^{14}\text{C}: ^{12}\text{C}$ . That is, why would certain land types exhibit older material compared to land types with younger material?



7. Describe an environmental issue that you might be able to resolve, or provide information about, by using stable and/or radioactive isotopes.
  
8. Case Study Question: Sea level rise is a current threat to wetlands due to coastal development and the inability fo wetlands to adapt quickly. As a result, wetlands can become inundated and disappear over time. They are essentially drowned out. How might isotope signatures shift in response to wetland disappearance.

Name: \_\_\_\_\_

**Supplemental Activity:**  
**What's in the Water? Visualizing Chemical Compounds in Estuaries**

**Data Sheet**

After reviewing chemical compounds associated with each land type, predict the following products, based on their reaction with other compounds in an estuary.

**Agriculture**



**Wetland**



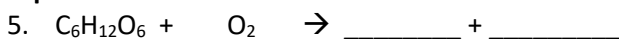
**Forest**



**Industry**



**Aquatic**



## Appendix D: Instructor's KEYS to Data Sheets

### Isotopes in the Estuary: Conception and Application of Stable and Radioactive Carbon

In student groups of 3-4, collect and organize samples from the known forest, wetland, agriculture, industry, and aquatic site. Each group will also collect and organize samples from an "unknown" estuarine site. Record results here. Be sure to carefully and accurately calculate the average for the number of each isotope and associated ratios of  $^{13}\text{C} : ^{12}\text{C}$  and  $^{14}\text{C} : ^{12}\text{C}$ .

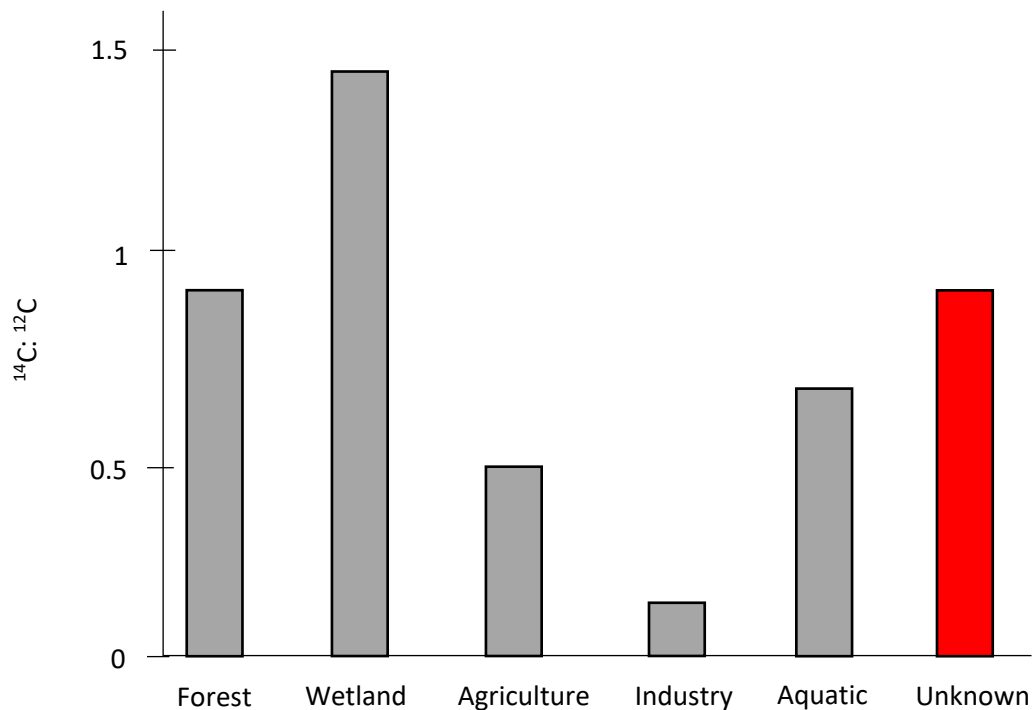
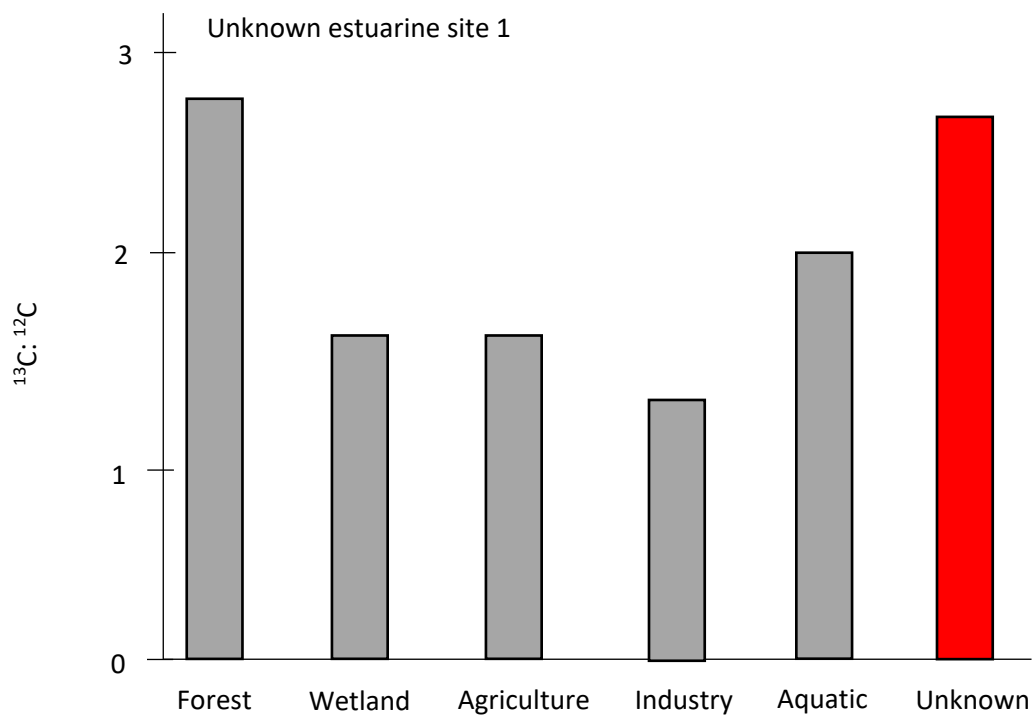
	$^{12}\text{C}$	$^{13}\text{C}$	$^{14}\text{C}$	$^{13}\text{C} : ^{12}\text{C}$	$^{14}\text{C} : ^{12}\text{C}$
<b>Forest</b>					
Sample 1	1	5	2		
Sample 2	1	3	1		
Sample 3	2	4	1		
Sample 4	2	5	1		
Average	1.5	4.25	1.25	4.25 : 1.5	1.25 : 1.5
<b>Wetland</b>					
Sample 1	2	3	2		
Sample 2	2	4	4		
Sample 3	3	4	4		
Sample 4	3	5	4		
Average	2.5	4.0	3.5	4.0 : 2.5	3.5 : 2.5
<b>Agriculture</b>					
Sample 1	2	3	1		
Sample 2	2	4	1		
Sample 3	3	4	1		
Sample 4	3	5	2		
Average	2.5	4.0	1.25	4.0 : 2.5	1.25 : 2.5
<b>Industry</b>					
Sample 1	2	2	1		
Sample 2	1	2	0		
Sample 3	2	3	0		
Sample 4	1	1	0		
Average	1.5	2.0	0.25	2.0 : 1.5	0.25 : 1.5
<b>Aquatic</b>					
Sample 1	2	4	1		
Sample 2	3	6	3		
Sample 3	1	2	0		
Sample 4	3	6	2		
Average	2.25	4.5	1.5	4.5 : 2.25	1.5 : 2.25
<b>Unknown Estuarine Site 1</b>					
Sample 1	1	4	1		
Sample 2	1	2	1		
Sample 3	3	5	2		
Sample 4	2	6	2		

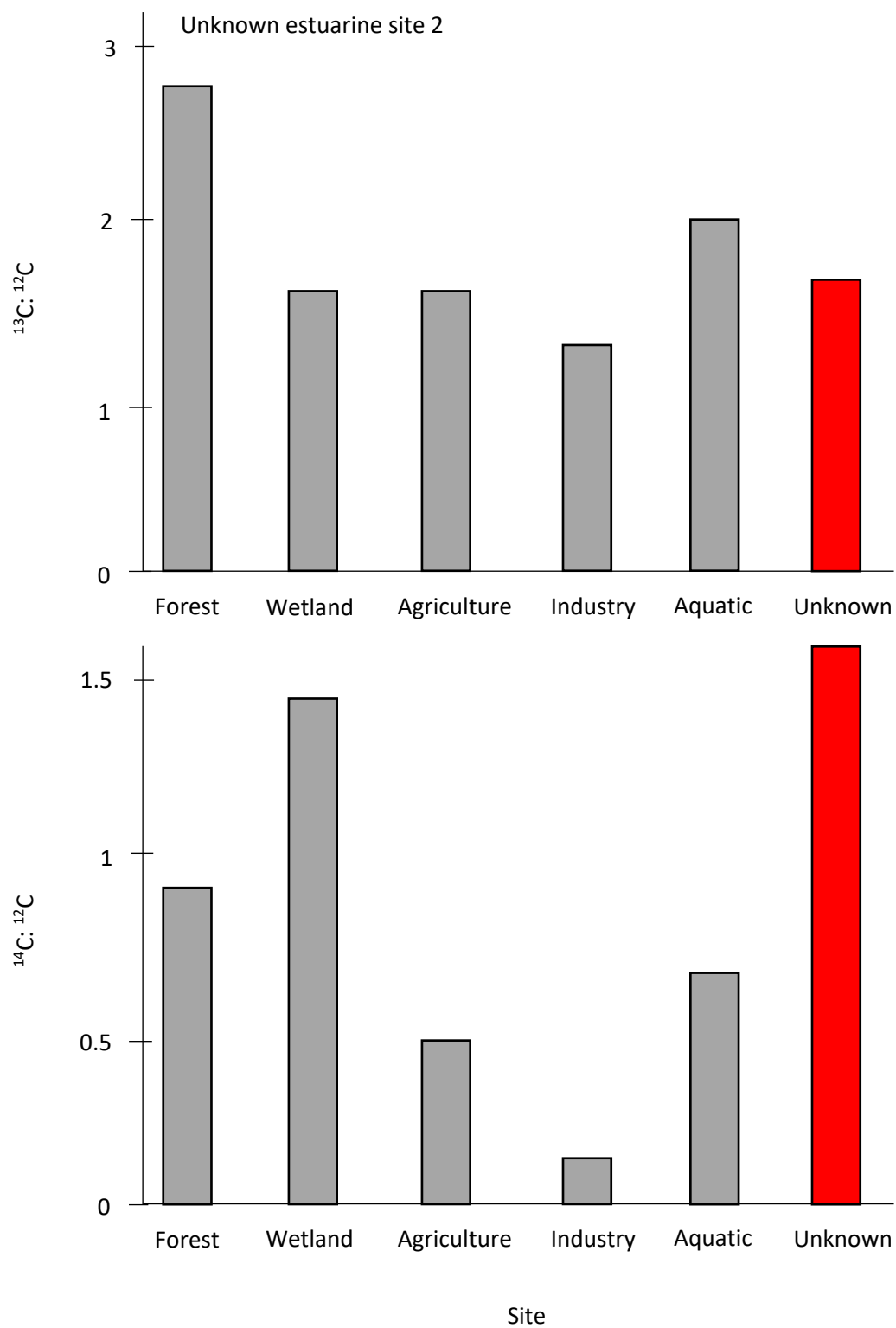
Average	1.75	4.25	1.5	4.25 : 1.75	1.5 : 1.75
<b>Unknown Estuarine Site 2</b>					
Sample 1	2	3	3		
Sample 2	3	5	4		
Sample 3	1	2	3		
Sample 4	2	4	4		
Average	2.0	3.5	3.5	3.5 : 2.0	3.5 : 2.0
<b>Unknown Estuarine Site 3</b>					
Sample 1	1	4	1		
Sample 2	1	2	0		
Sample 3	3	3	1		
Sample 4	2	3	2		
Average	1.75	3.0	1.0	3.0 : 1.75	1.0 : 1.75
<b>Unknown Estuarine Site 4</b>					
Sample 1	5	5	1		
Sample 2	1	2	0		
Sample 3	3	4	0		
Sample 4	1	1	1		
Average	2.5	3.0	0.5	3.0 : 2.5	0.5 : 2.5
<b>Unknown Estuarine Site 5</b>					
Sample 1	1	3	0		
Sample 2	2	5	2		
Sample 3	2	3	1		
Sample 4	2	5	1		
Average	1.75	4.0	1.0	4.0 : 1.75	1.0 : 1.75

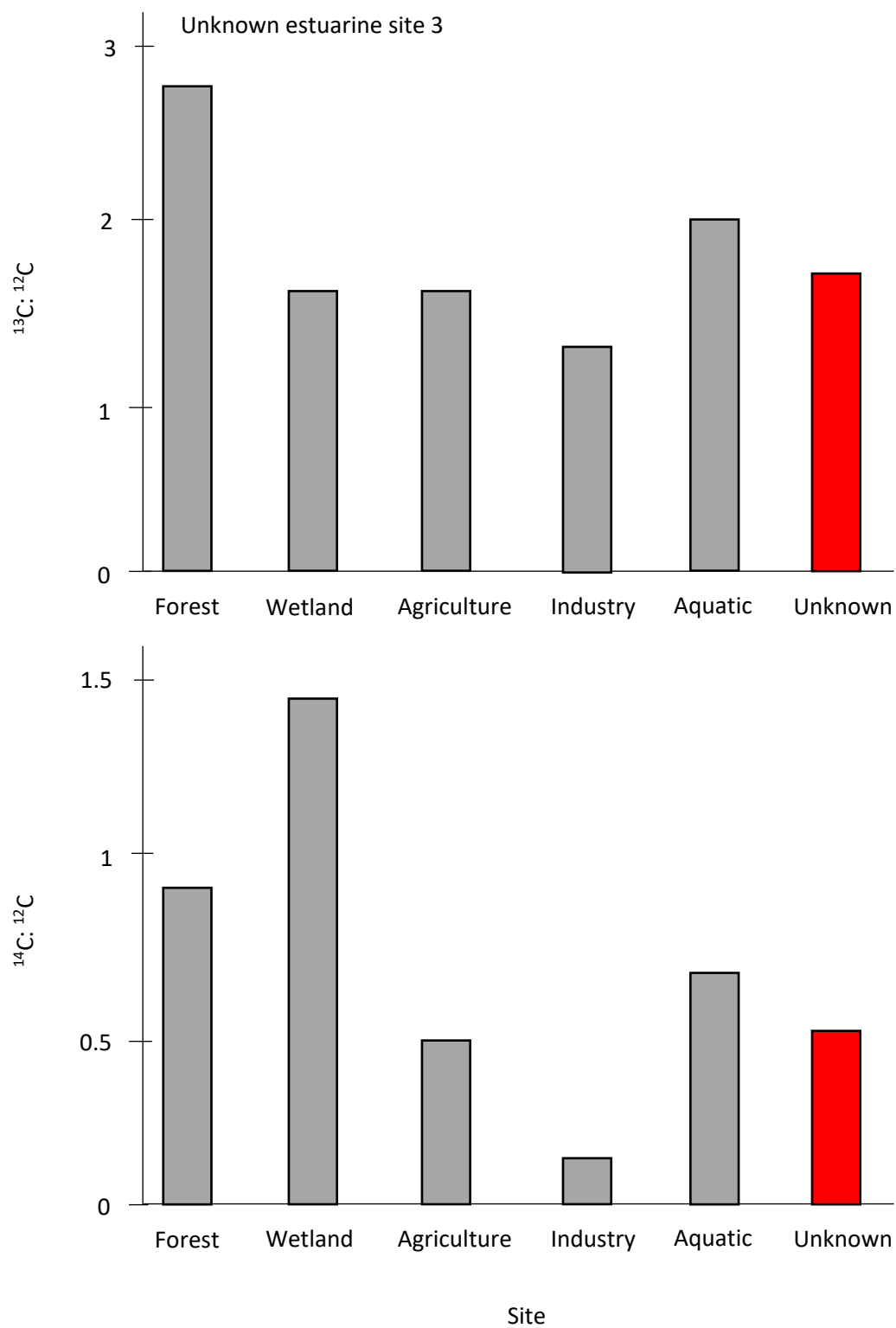
1. Circle your assigned unknown estuarine site: 1 2 3 4 5

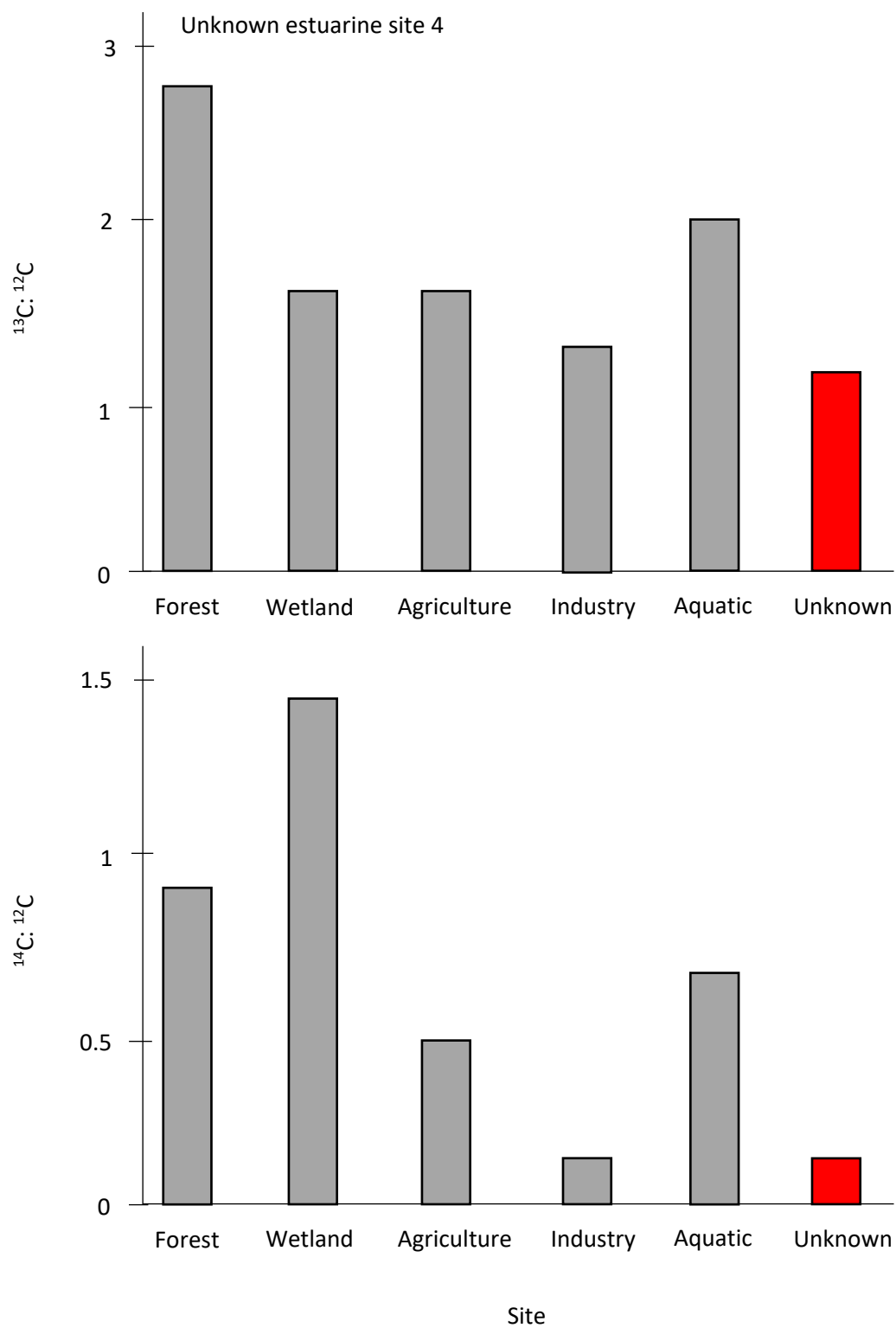
Answer will depend on assigned unknown.

2. Create a bar graph for the average isotope ratios at each sample site. For the y-axis, convert your ratio to a whole number (for example, plot 1.5:2.75 as 0.55, because 1.5 divided by 2.75 is equal to 0.55).

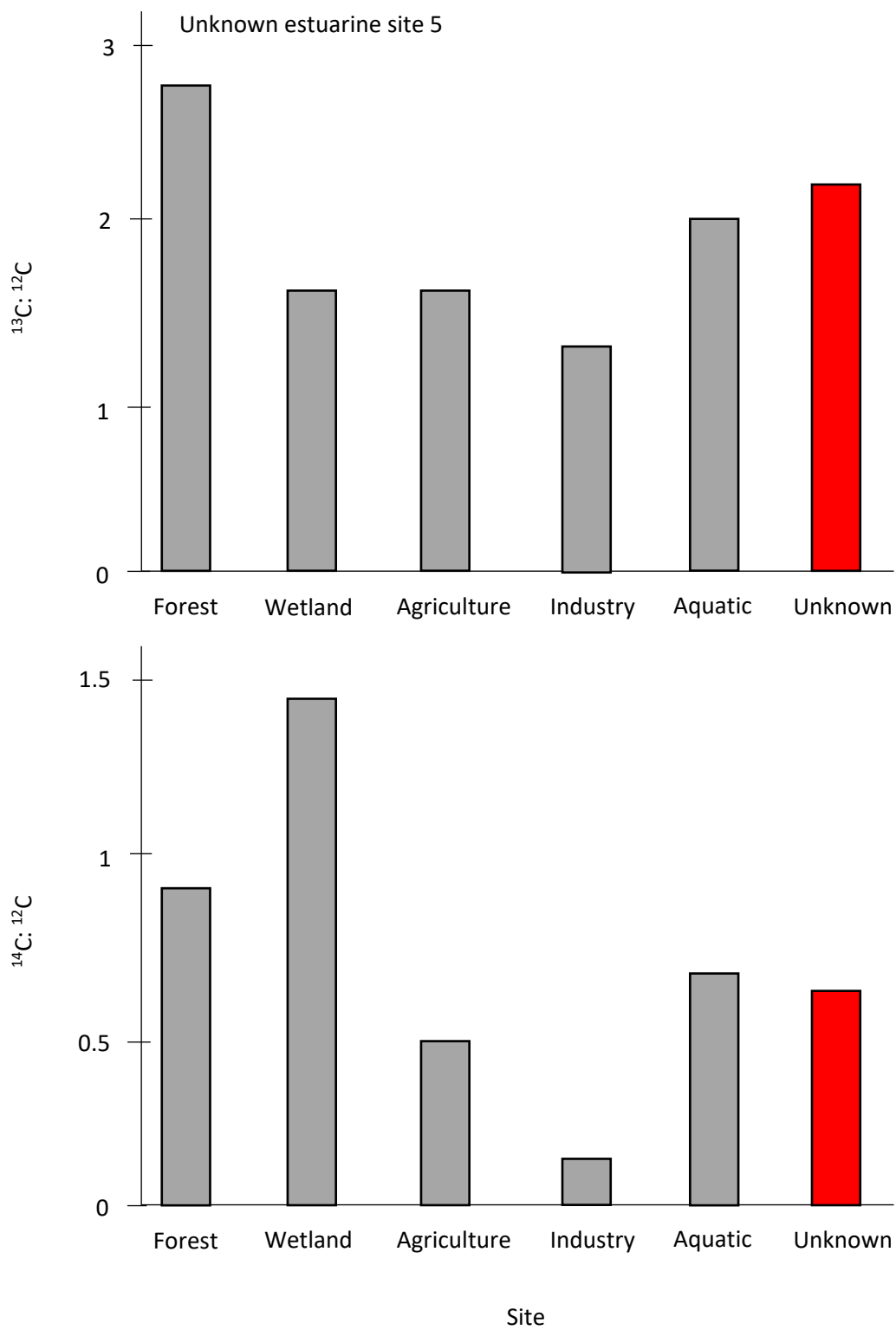












3. After analyzing your unknown sample, compare it to the known sites. What land type do you believe most impacted your estuary?

Circle one: forest wetland agriculture industry no impact (aquatic signature)

Students will reach their conclusion by looking at graphs of their isotope ratios and determining which reference site closely matches their unknown. Unknown site 1 matches the forest site, unknown site 2 matches the wetland site, unknown site 3 matches the agricultural site, unknown site 4 matches the industrial site, and unknown site 5 matches the aquatic site.

4. Based on your graph, does your prediction about the unknown estuarine site make sense? Why?

Student reasoning should include that the ratio of  $^{13}\text{C} : ^{12}\text{C}$  and  $^{14}\text{C} : ^{12}\text{C}$  for the unknown site closely matched that of a reference site and allowed them to narrow down their answer. That is, unknown site 1 matches the forest site, unknown site 2 matches the wetland site, unknown site 3 matches the agricultural site, unknown site 4 matches the industrial site, and unknown site 5 matches the aquatic site.

5. What factors might contribute to a certain land type having a greater or lesser impact on an estuary as indicated by your isotope analyses. For example, if your estuary exhibited a pattern most similar to agriculture, why might this be so?

Students should be able to reach the conclusion that if their unknown estuarine site was impacted by a certain land type, it is because that particular land type is most prominent in the area while the other land types might not be present. For example, if students conclude that their unknown site closely matches agricultural land, their particular estuary might be surrounded by agricultural land (and could experience large amounts of fertilizer or polluted runoff) and outnumber the total area of forest, wetland, or industrial land.

6. Explain the pattern observed with  $^{14}\text{C} : ^{12}\text{C}$ . That is, why would certain land types exhibit older material compared to land types with younger material?

This is a critical thinking question that extends beyond the conclusions made in this activity. Therefore, the instructor may need to provide hints and guidance towards the correct answer.

Certain land types would exhibit older material compared to others depending on how each land type functions and how it is used. For example, industrial sites emit fossil fuels that might find their way into estuaries. Fossil fuels are formed over millions of years, meaning it would have less carbon-14 and therefore a lower  $^{14}\text{C} : ^{12}\text{C}$ . Wetlands also store a lot of material from runoff and build up over time, resulting in a lower  $^{14}\text{C} : ^{12}\text{C}$  as well. On the other hand, forest and agriculture land types would exhibit younger material such as fertilizers, soils, or fresher leaf material that could influence nearby estuaries and therefore have larger  $^{14}\text{C} : ^{12}\text{C}$ .

7. Describe an environmental issue that you might be able to resolve or provide information about by using stable and/or radioactive isotopes.

Answers will vary, but might include how changes in land type might impact estuarine water quality (i.e. removal of forest, construction of some industrial complex), how current land type influences estuarine water quality, and how estuaries differ by location and is reflective of material sources. Other scenarios might involve situations where all of a sudden, there is a large overgrowth of algae, or a major fish mortality event in which isotopes can be used to potentially trace sudden changes in material sources over time.

8. Case Study Question: Sea level rise is a current threat to wetlands due to coastal development and the inability of wetlands to adapt quickly. As a result, wetlands can become inundated and disappear over time. They are essentially drowned out. How might isotope signatures shift in response to wetland disappearance.

Isotope signatures would be expected to shift more towards or potentially dominated by those resembling aquatic environments (if completely inundated) or by forest, agriculture, or industrial environments depending on the land type immediately adjacent to the affected marsh.

## Instructor's KEY to Supplemental Activity Data Sheet

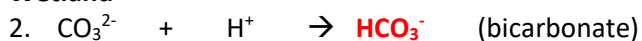
### What's in the Water? Visualizing Chemical Compounds in Estuaries

After reviewing compounds associated with each land type, predict the following products based on their reaction with other compounds in an estuary.

#### Agriculture



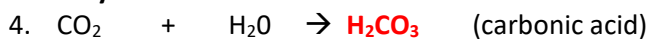
#### Wetland



#### Forest



#### Industry



#### Aquatic

