WATERCOLORS:

RELATING PROPERTIES OF LIGHT TO ORGANIC MATTER AND ECOSYSTEM PRODUCTION

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Grade Level
High School

Subject area
Biology, Chemistry, Environmental Science

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VIRGINIA INSTITUTE OF MARINE SCIENCE

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Title: Watercolors: Relating Properties of Light to Organic Matter and Ecosystem Production

Focus: Using properties of light (e.g., absorption and fluorescence) to determine the source and composition of dissolved organic matter in estuaries influenced by different land types (e.g., agriculture, industry, forest, wetland) and its subsequent relationship with ecosystem production.

Grade Level: High school biology or environmental science; a portion of this activity is also adaptable to middle school life science or physical science and advanced or AP high school chemistry.

VA Science Standards:

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

a) asking questions and defining problems
   - make hypotheses that specify what happens to a dependent variable when an independent variable is manipulated
   - generate hypotheses based on research and scientific principles

b) planning and carrying out investigations
   - individually and collaboratively plan and conduct observational and experimental investigations

c) interpreting, analyzing, and evaluating data
   - record and present data in an organized format that communicates relationships and quantities in appropriate mathematical or algebraic forms
   - analyze data graphically and use graphs to make predictions
   - consider limitations of data analysis when analyzing and interpreting data

d) constructing and critiquing conclusions and explanations
   - make quantitative and/or qualitative claims based on data
   - apply scientific ideas, principles, and/or evidence to provide an explanation of phenomena or design solutions

e) 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
   - communicate scientific and/or technical information about phenomena and/or a design process in multiple formats

BIO.2. The student will investigate and understand that chemical and biochemical processes are essential for life. Key idea: water chemistry has an influence on life processes.

PS.7. The student will investigate and understand that electromagnetic radiation has characteristics. Key ideas include

a) electromagnetic radiation, including visible light, has wave characteristics and behavior; and
b) regions of the electromagnetic spectrum have specific characteristics and uses.

LS.5. The student will investigate and understand that biotic and abiotic factors affect an ecosystem. Key ideas include

a) matter moves through ecosystems via the carbon, water, and nitrogen cycles;
b) energy flow is represented by food webs and energy pyramids; and
c) relationships exist among producers, consumers, and decomposers.

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

1. Describe how the color of water in various aquatic environments is related to absorbance, fluorescence, and ecosystem production.
2. Define and explain the importance of dissolved organic matter in aquatic environments and its relationship to ecosystem production.
3. Describe how absorbance and fluorescence is used to measure dissolved organic matter.
4. Measure, rank, and compare relative absorbance of dissolved organic matter collected from various sources within an estuary including microbes, agriculture, industry, forests, and wetlands.
5. Interpret how fluorescence data is presented using excitation-emission matrices, contour plots, and the electromagnetic spectrum, with an emphasis on ultraviolet and visible light.
6. Interpret the electromagnetic spectrum, with an emphasis on ultraviolet and visible light.
7. Investigate and determine the source of an unknown sample of dissolved organic matter by generating a contour plot and comparing it to known datasets.

Total length of time required for lesson: 75 min.

Key words, vocabulary: Words are listed in the order they appear in the text and are bolded.

1. **Wetland** – land consisting of marshes and swamps; saturated land.
2. **Estuary** – partially enclosed coastal body of water with one or more rivers or streams flowing into it, and with a free connection to the open sea.
3. **Ecosystem** – a biological community of interacting organisms and their physical environment.
4. **Absorption** – take in or soak up by chemical or physical action, in this case light.
5. **Fluorescence** – radiation emitted by certain substances that have absorbed light.
6. **Ecosystem Production** – the rate at which biomass is gained over time and the total amount of organic matter in an ecosystem available for storage, export, or conversion to carbon dioxide.
7. **Photosynthesis** – process by which plants and phytoplankton use sunlight to make food.
8. **Primary production** – the production of chemical energy and organic compounds by living organisms usually through photosynthesis.
9. **Secondary production** – the generation of heterotrophic (consumer) biomass in an ecosystem by assimilation or transfer of organic matter from primary producers.
10. **Dissolved Organic Matter (DOM)** – a water soluble mixture of dissolved carbon, nitrogen, and phosphorus compounds produced biologically by plants and microbes, geologically by soils and rocks, and artificially by human inputs to aquatic ecosystems.
11. **Microbe** – a microscopic organism such as bacteria or phytoplankton that are often primary producers and form the base of food webs.
12. **Heterotrophic** – acquiring energy through consumption of organic matter derived elsewhere; secondary consumers are heterotrophic and cannot photosynthesize.
13. **Chemical composition** – the identity and relative number of the chemical elements that make up any particular compound; the arrangement, type, and ratio of molecules.
14. **Degradation potential** – the rate at which organic matter breaks down and consumed over time.
15. **Phytoplankton** – microscopic primary producers that inhabit aquatic ecosystems.
16. **Chromophoric dissolved organic matter (CDOM)** – the fraction of dissolved organic matter (DOM) that absorbs light.
17. **Spectrofluorophotometer** – an instrument used to measure chromophoric dissolved organic matter (CDOM) that detects the intensity of light transmitted (absorption) or emitted (fluorescence) at a range of wavelengths that cover the ultraviolet and visible spectrum of light.
18. **Transmit** – passing through a substance, in this case light passing through water.
19. **Emit** – produce and give off something, in this case light.
20. **Wavelength** – the distance between identical points (i.e., adjacent crests) along a wave denoted in nanometers (nm, 10^{-9}).
21. **Ultraviolet light** – light with wavelengths between 100-400nm on the electromagnetic spectrum.
22. **Visible light** – light with wavelengths between 400-700nm on the electromagnetic spectrum; includes colors of the rainbow.
23. **Electromagnetic spectrum** – the range of all types of radiation that travels and spreads out as it goes. Aside from ultraviolet and visible light, radio waves, microwaves, infrared light, x-rays, and gamma rays all make up the electromagnetic spectrum, each with a distinct wavelength range.
24. **Excitation-emission matrix (EEM)** – a three-dimensional scan that results in a contour plot of excitation wavelength versus emission wavelength versus fluorescence intensity.
25. **Excitation** – the energy applied to light at a certain wavelength.
26. **Contour plot** – a type of graph used to plot three-dimensional data (as with excitation-emission matrices). In addition to typical *x* and *y* variables, a *z* variable is plotted as intensity using a color scale and contour lines.

Background Information:

During your lifetime, you may have noticed that not all water looks the same. While the water you cook and brush your teeth with is transparent and clear, the water that flows through rivers, **wetlands, estuaries**, and into the ocean can have distinct coloration. Depending on where you are in the world, the water in these aquatic **ecosystems** can be light blue, dark green, or even brown and black. How and why can water have so many different colors? It mostly has to do with its source and the way it absorbs (takes up) and fluoresces (emits) light from the sun. Furthermore, identifying water sources and measuring **absorption** and **fluorescence** help scientists assess **ecosystem production,** which is essentially the total number of organisms in an ecosystem. For instance, darker-colored water typically means that less sunlight can penetrate the surface to support **photosynthesis** and subsequent **primary and secondary production** leading to low ecosystem production. Lighter-colored water, on the other hand, typically means that more sunlight can penetrate the surface to support photosynthesis and subsequent primary and secondary production leading to high ecosystem production.

Another major component of ecosystem production is **dissolved organic matter (DOM)**. DOM is a mixture of dissolved carbon, nitrogen, and phosphorus compounds that are dissolved in water. DOM is produced biologically by plants and **microbes**, geologically by soils and rocks, and artificially by human inputs to aquatic ecosystems. DOM is important for ecosystem function because it serves as the foundation of aquatic food webs and provides an energy source for **heterotrophic** microbes that acquire their energy by consuming organic matter. However, not all DOM behaves the same. Microbes consume some compounds more easily than others. The ease at which microbes consume compounds depends on **chemical composition** and **degradation potential**, both of which are influenced by the original source of DOM. For instance, “juicy” DOM that is simple in composition and is easily degraded typically comes from aquatic biological sources such as **phytoplankton** and other microbes. This is contrary to DOM that is complex in composition and is not easily degraded. This “stable” DOM typically comes from terrestrial sources such as plants and soils. While phytoplankton, microbes, plants, and soils are natural sources of DOM, human activities also produce DOM that contributes to the total amount of DOM in aquatic ecosystems. The juiciness of DOM from human activities is less certain, but measuring its absorption and fluorescence helps scientists determine its role in aquatic ecosystems.

Because coastal aquatic ecosystems, estuaries in particular, lie at the interface between land and sea, they experience a mixture of DOM from many surrounding landscapes such as natural forests and wetlands, or more human-perturbed areas such as agricultural or industrial land. This presents a challenge to scientists who study estuarine ecosystem production. It can be difficult to determine the relative DOM contribution from each source, but scientists have found a way to exploit how water absorbs and fluoresces light to identify the major contributor of DOM to an estuary in addition to its chemical composition and degradation potential.

DOM absorbs and fluoresces light differently depending on its source and composition. Scientists measure the portion of DOM that absorbs light, collectively referred to as **chromophoric dissolved organic matter (CDOM)**. CDOM is measured with a **spectrofluorophotometer**, an instrument that detects the intensity of light **transmitted** (absorption) and **emitted** (fluorescence) from a sample of water collected from an aquatic environment. CDOM is often measured at a range of **wavelengths** that cover **ultraviolet** and **visible light** on the **electromagnetic spectrum**. These measurements produce **excitation-emission matrices (EEMs)** which are three-dimensional scans that result in **contour plots** of **excitation** wavelength versus emission wavelength versus fluorescence intensity (Figure 1).

Measurements of absorption and subsequent EEMs are then used to quantify overall DOM concentration through absorption intensity as well as source and composition through fluorescence intensity. Though beyond the scope of this activity, DOM source and composition are specifically determined by comparing results to well-established databases, EEMs, and models that are able to assign sources and compositional characteristics to the data in question.

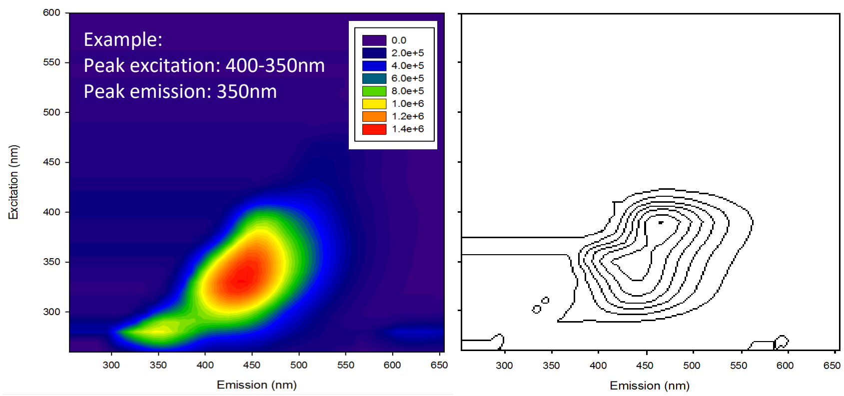

Figure 1: Example of an EEM and contour plot showing excitation and emission wavelengths of CDOM. The intensity of each wavelength is shown by the color scale with warm colors indicating high intensity and cool colors indicating low intensity (data and graphics generated by Derek Detweiler).

The activity presented here will take a qualitative approach to measuring DOM concentration, source, and composition but will still use absorption and fluorescence as guiding principles. Students will characterize DOM from estuaries influenced by natural land uses (forests and wetlands) and human land uses (agriculture and industry) and will ultimately determine the status of ecosystem production within their estuary.

Materials and Supplies:

1. Estuary display (can be found as separate PowerPoint file; four 8.5 x 11'' sheets to be assembled)
    a. Can be assembled on larger poster board (optional)
2. Access to a color printer
3. Scissors
4. Scotch tape or glue
5. Six completely transparent cups or beakers (25-30mL beakers would work best)
6. Tap water
7. Liquid food coloring (pink, yellow, green, blue, and purple or brown or a mixture of many colors)
8. Stirrer (can be anything that stirs cups or beakers)
9. Flashlight (can also use flashlight feature on cell phone)
10. Colored pencils, markers, or crayons (blue, yellow, orange, red)
11. Activity sheets, reference EEMs, and blank contour plots (Appendix A-C)

Teacher Preparation: For groups 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 (can be found as separate PowerPoint file) and either taping them together or gluing on a larger poster board.
2. Fill six transparent cups or beakers (25-30mL beakers would work best) about three-quarters full with tap water.
3. Place one drop of pink food coloring into one beaker, one drop of yellow into a second beaker, two drops of green into a third beaker, two drops of blue into a fourth beaker, and three drops of purple or brown into a fifth beaker. The sixth beaker will contain no food coloring.
4. Stir each beaker so that food coloring is dissolved and cups/beakers are uniform in color.
5. On the display, place the pink beaker on the microbes, the yellow beaker on the factory, the green beaker on the wetland, the blue beaker on agriculture, the brown or purple beaker on the forest, and the colorless beaker somewhere along the open water estuary.
6. Distribute flashlight, colored pencils/markers/crayons, and activity sheets for each group (Appendix A).

Procedure:

1. **Introduction (~30 minutes)**
    a. The instructor will introduce key concepts on relating properties of light to organic matter and ecosystem production using the first portion of the provided PowerPoint.
    b. Students will be split into groups of 3-4 at the assembled estuary displays.
    c. The instructor will introduce and explain the activity using the second portion of the provided PowerPoint.

2. **Activity Part 1: Absorbance (~15 minutes)**
    a. Prior to the start of this activity, lights should be turned off and windows covered to prevent natural light penetration. A small corner or desk light can be turned on so that students can see what they are doing. If windows cannot be covered, an enclosed structure (e.g. cardboard box) can be formed around the cups/beakers described in the next step to block out all but one side of light.
    b. Students will take cups/beakers, representing DOM samples, one at a time from each location (i.e. DOM source) on the display and hold it in front of their face.
    c. Using their free hand, students will hold a flashlight to the bottom of each sample and shine it up and through the cup/beaker. This will act as a qualitative, simplified measure of light absorbance. Alternatively, beakers can be placed on a stable ring stand while the light source is placed underneath.
    d. On the provided activity sheet (Part 1; Appendix A), students will rank each sample from lowest to highest absorbance based on the amount of light that passes through or is absorbed. A brighter color would indicate low absorbance whereas a darker color would indicate high absorbance.
    e. On the provided activity sheet (Part 1; Appendix A), students will answer, and disuss with the instructor, the associated questions to this portion of the activity.
        i. Based on your ranking, which DOM source might contribute to high ecosystem production? What about low ecosystem production? How did you arrive at your answers?

   ii. Based on your ranking, which sample do you expect has the highest concentration of DOM? What about the lowest concentration? How did you arrive at your answers?

3. **Activity Part 2: Fluorescence (~15 minutes)**
   a. Distribute reference EEMs to each group (Appendix B).
   b. After discussing the answers to the questions posed in part one of this activity, students will refer to provided reference EEMs (Appendix B) that are representative of each DOM source.
   c. After a few minutes of orienting themselves with the EEM layout, students will be a given a blank contour plot (Appendix C) representing an unkown sample collected from an estuary dominated by one of the DOM sources on the display. Each group should receive a different unknown. If there are more than five groups, unknown samples can be repeated.
   d. Students will color in their contour plots using the fluorescence intensity scale on the reference EEMs. That is, outside bands should be colored with blue and green whereas inner bands should be colored with yellow and red to ultimately show that red, peak intensities occur at the center of each EEM.
   e. Students will determine the source of their unknown sample by identifying excitation and emission wavelengths at which peak fluorescence is greatest, comparing these wavelengths to those of visible light on the electromagnetic spectrum, and deciding which DOM source it matches from Appendix B.
   f. Students will record their decision on the provided activity sheet (Part 2; Appendix A) and contour plot (Appendix C) and answer the related questions about their unknown sample.
      i. Which wavelength of light had the greatest fluorescence intensity?
      ii. Which color is attributed to this wavelength on the visible light spectrum?
      iii. What is the dominant DOM source in your estuary?

4. **Gallery Walk and Wrap-up (~15 minutes)**
   a. After contour plots are complete and each group decides which DOM source dominates their estuary, plots and associated answers will be displayed around the classroom.
   b. Students will walk around to observe each plot and the conclusion reached by each group.
   c. Students will return to groups for a closing discussion led by the instructor, posing the following question:
      i. Based on the source of your unknown sample and what you observed with other group's conclusions in class, would you expect ecosystem production to be low or high? How did you arrive at your answer? Why or why might not you expect there to be higher rates of photosynthesis or heterotrophic consumption of DOM by microbes?
   d. Students will discuss and record their answers on the activity sheet (Part 2; Appendix A), and representatives from each group will share results of their conclusion with the class while the instructor displays results via the provided PowerPoint.
   e. The instructor will conclude the lesson by highlighting the importance of DOM and its role in ecosystem production.

5. **Extension Assignment**
   a. Assuming the previous components of this lesson plan will take an entire class period, the following questions can be administered as an extension assignment. They are listed on a document separate from the main activity sheet (Appendix F).

i. Based on the source of your unknown sample, sketch what ecosystem production might look like in your estuary. Be sure to depict both primary and secondary consumers as well as heterotrophic microbes that consume DOM.
ii. In the context of ecosystem production, why is it important to determine the chemical composition and degradation potential of DOM derived from human sources?

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

References

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Mannino, A. and Harvey, H.R. 2000. Biochemical composition of particles and dissolved organic matter along an estuarine gradient: sources and implications for DOM reactivity. *Limnology and Oceanography, 45*(4): 775-788.

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Oestreich, W.K., Ganju, N.K., Pohlman, J.W., Suttles, S.E. 2016. Colored dissolved organic matter in shallow estuaries: relationships between carbon sources and light attenuation. *Biogeosciences, 13*: 583-595.

Appendix A: Activity Sheets

Name: ________________________________

Watercolors: Relating Properties of Light to Organic Matter and Ecosystem Production

Part 1: Absorbance

Instructions: Refer to the estuary display in front of you. Each cup or beaker represents DOM samples collected from different sources including wetland, agriculture, forest, microbes, and a factory. There is also an open water sample that was not collected from any source. Your task is to take each sample, hold it in front of your face with a flashlight aimed upwards through the bottom of each sample. If the sample is bright, this means that absorbance is low. If the sample is dark, this means that absorbance is high. This will act as a qualitative, simplified measure of light absorbance.

1. According to the source of each DOM sample, rank the following from lowest (1) (brightest sample) to highest (6) (darkest sample) absorbance based on the amount of light taken up by each sample.

_____ wetland   _____ agriculture   _____ microbes   _____ factory   _____ forest   _____ no source

2. Based on your ranking, which DOM source might contribute to high ecosystem production? What about low ecosystem production? How did you arrive at your answers?

3. Based on your ranking, which sample do you expect has the highest concentration of DOM? What about the lowest concentration? How did you arrive at your answers?

Name: ________________________________

Watercolors: Relating Properties of Light to Organic Matter and Ecosystem Production

Part 2: Fluorescence

Instructions: Now that you have some idea about how DOM from different sources absorbs light, you will assess how these same sources fluoresce light. You will be provided real-life EEMs from various DOM sources that describe their fluorescence intensity at various wavelengths of light. The x-axis represents excitation, or the amount of energy applied to a sample. The y-axis represents emission, or the amount of light given off or produced. Higher light intensity at certain wavelengths of light (in nanometers) are represented by warmer colors (e.g. yellow and red) while lower light intensity are represented by cooler colors (e.g. blue). Depending on the DOM source, intensity will differ according to wavelength along either axis. Take a few minutes to orient yourself to the layout. You will then be given a blank contour plot representing an unknown DOM sample collected from an estuary dominated by one of the DOM sources on the display. Color in your blank contour plot using the provided EEMs as reference for which colors to use for peak intensities. Outside bands should be colored with blue and green whereas inner bands should be colored with yellow and red to ultimately show that red, peak intensities occur at the center of each EEM. After determining the source of your unknown sample by comparing it to peak excitation and emission intensities on the provided EEMs and visible light spectrum, answer the following questions pertaining to your unknown sample. You will need to refer to the visible light spectrum below for question 2.

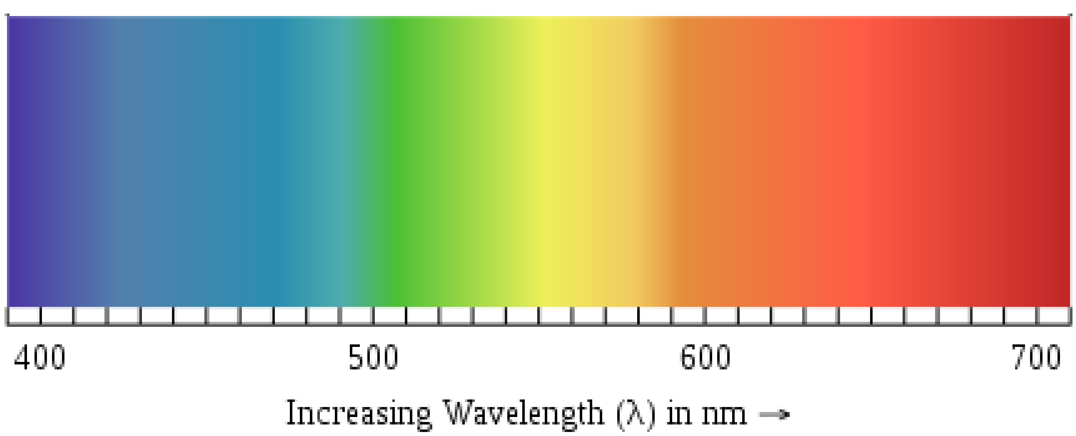


Image source: https://commons.wikimedia.org/wiki/File:EM_spectrum.svg

1. Which wavelength of light had the greatest fluorescence intensity along the emission (y) axis?

   ____

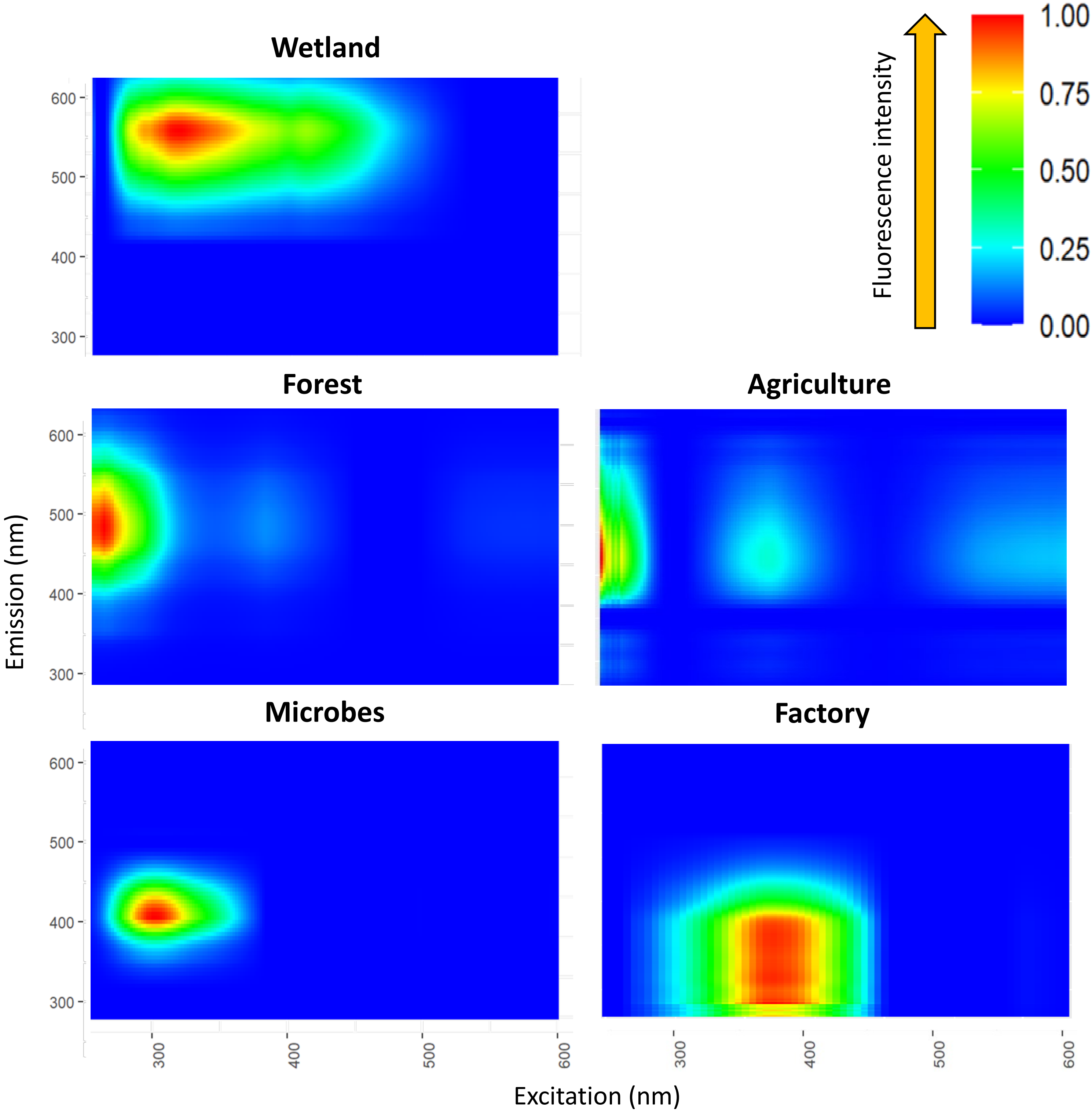
2. Which color is attributed to this wavelength on the visible light spectrum? _____________________

3. What is the dominant DOM source in your estuary? _____________________

4. Based on the source of your unknown sample and what you observed with other group's conclusions in class, would you expect ecosystem production to be low or high? How did you arrive at your answer? Why or why might not you expect there to be higher rates of photosynthesis or heterotrophic consumption of DOM by microbes?

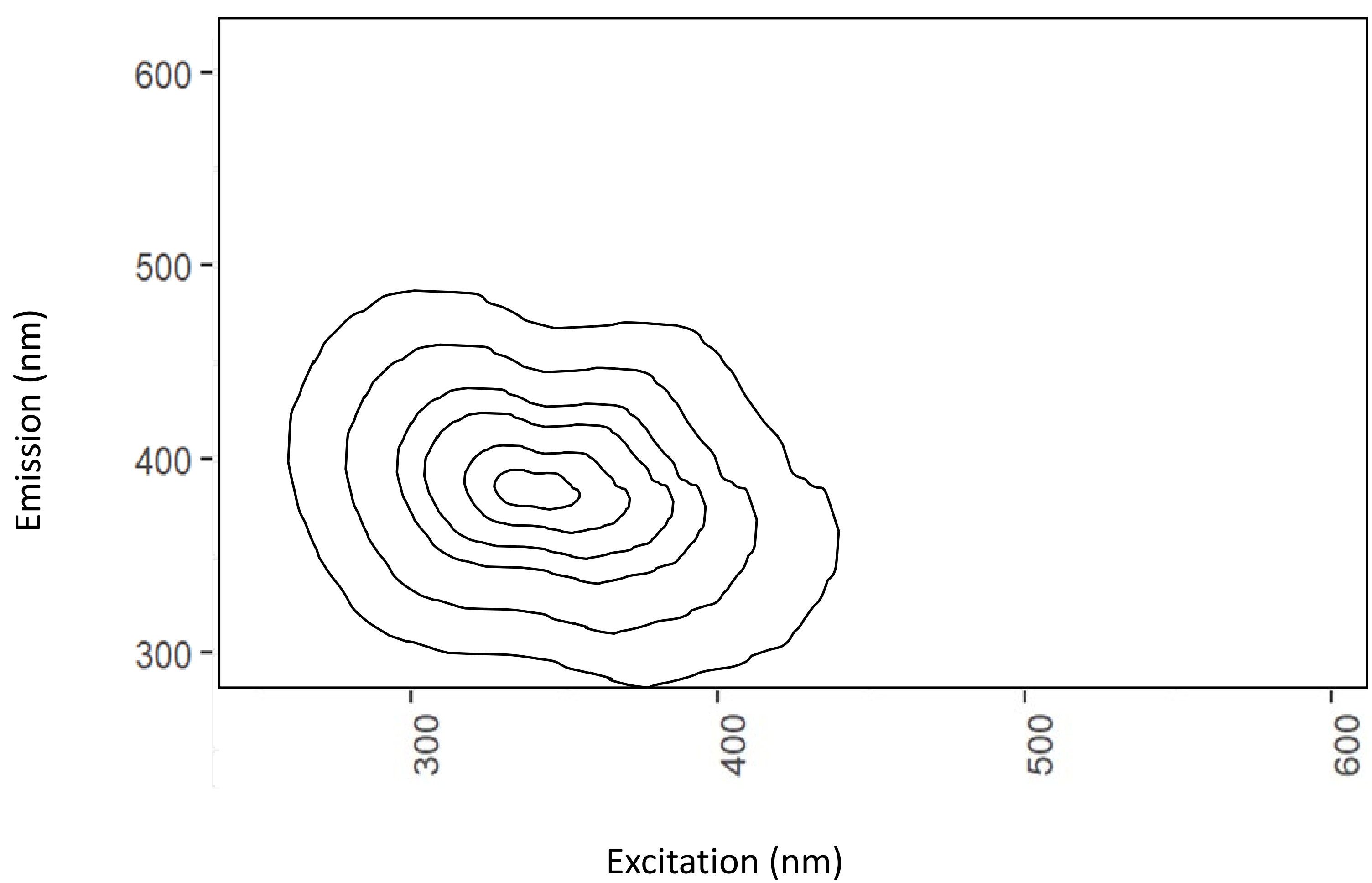
Appendix B: Reference EEMs for Activity Part 2

Exicitation-emission matrices (EEMs) from CDOM measurements of various sources and landscapes

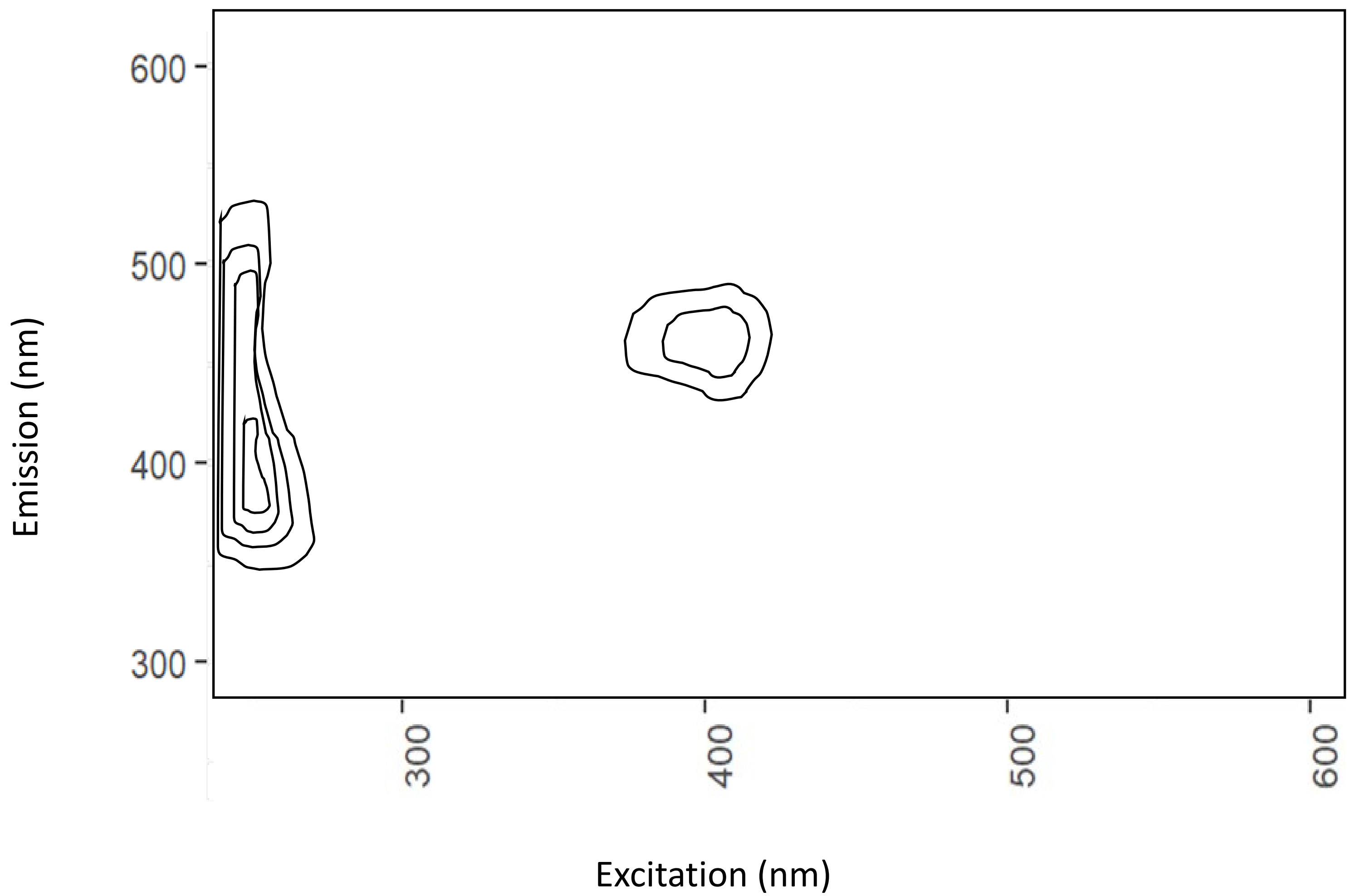


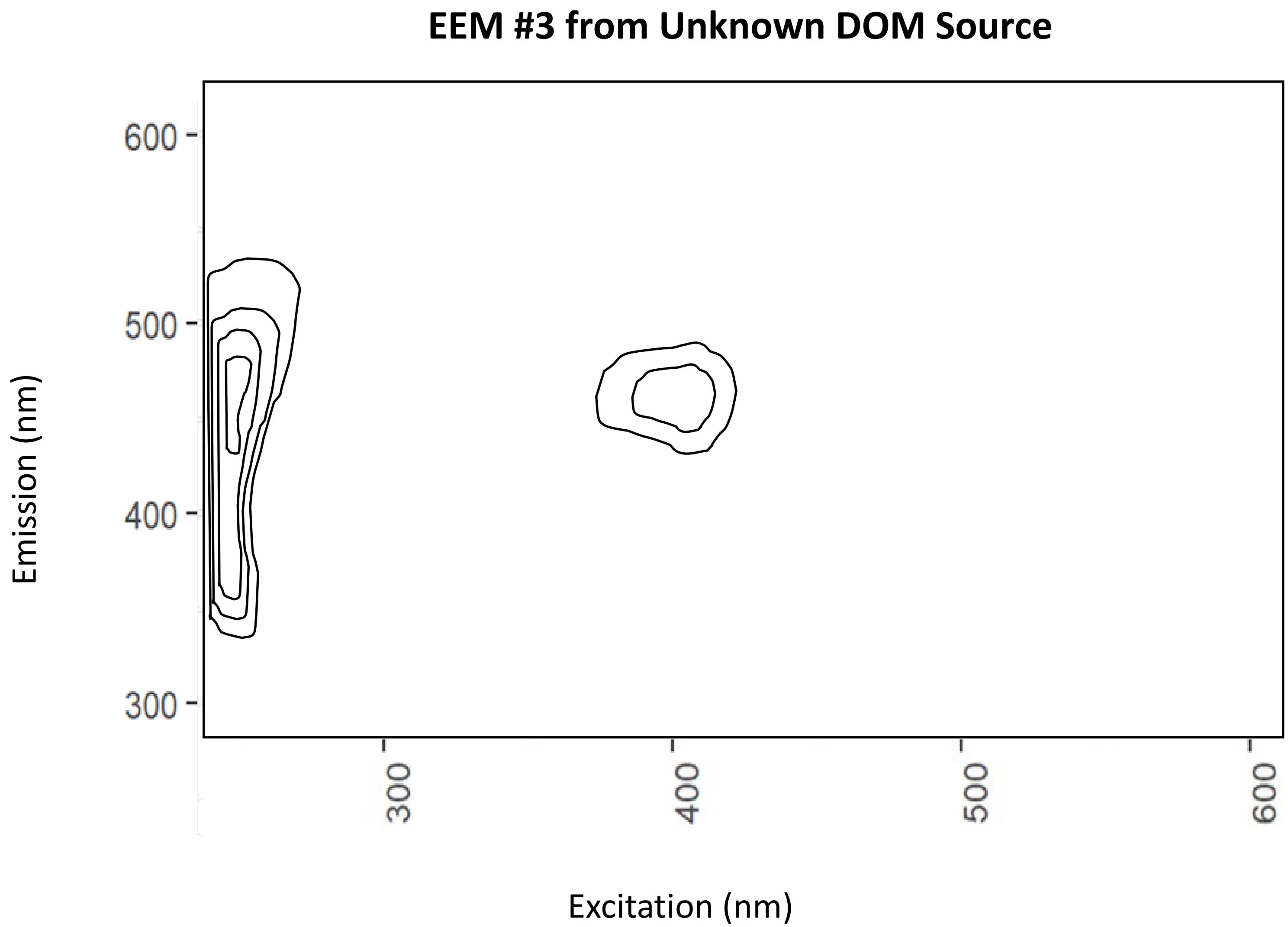
Appendix C: Blank Contour Plots for Activity Part 2

EEM #1 from Unknown DOM Source



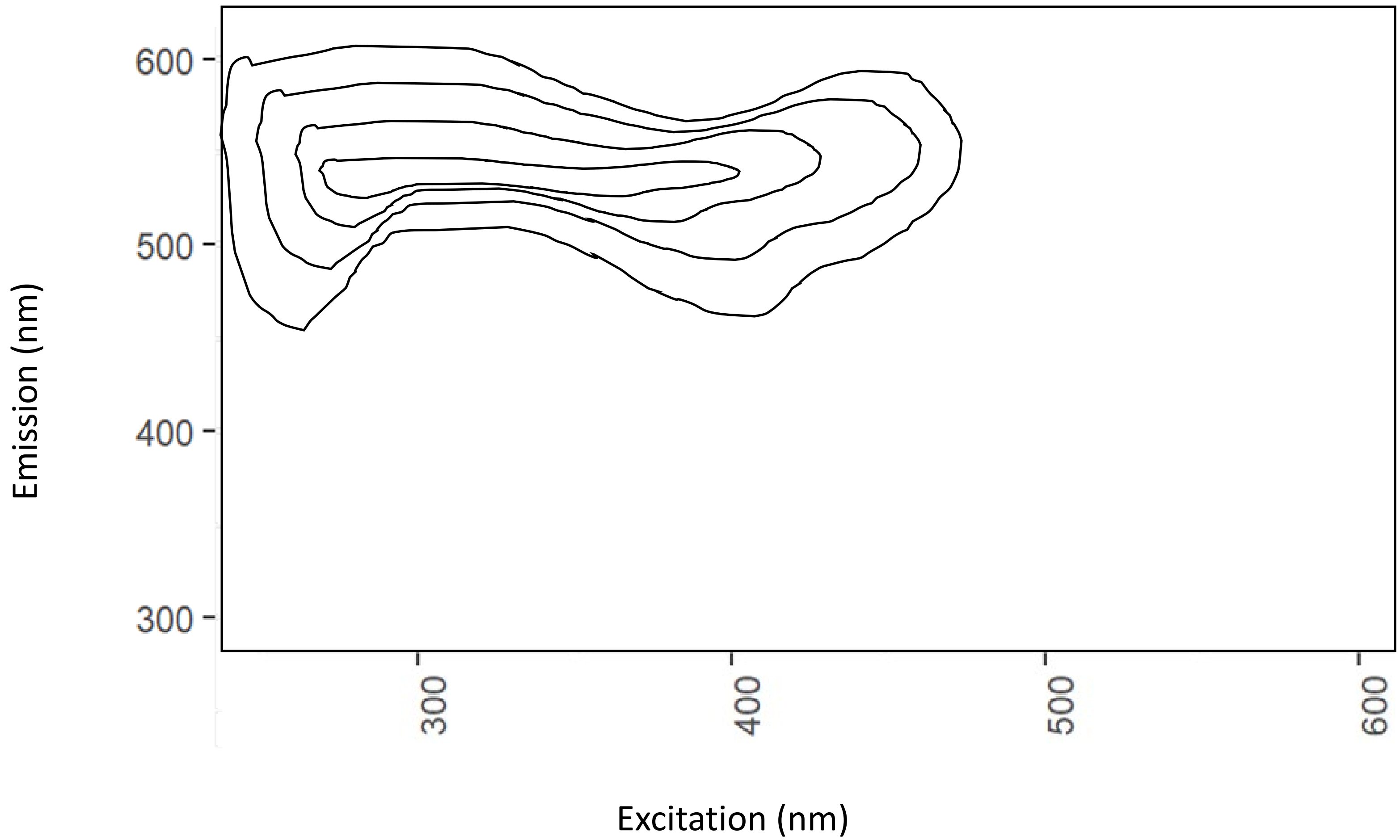
EEM #2 from Unknown DOM Source



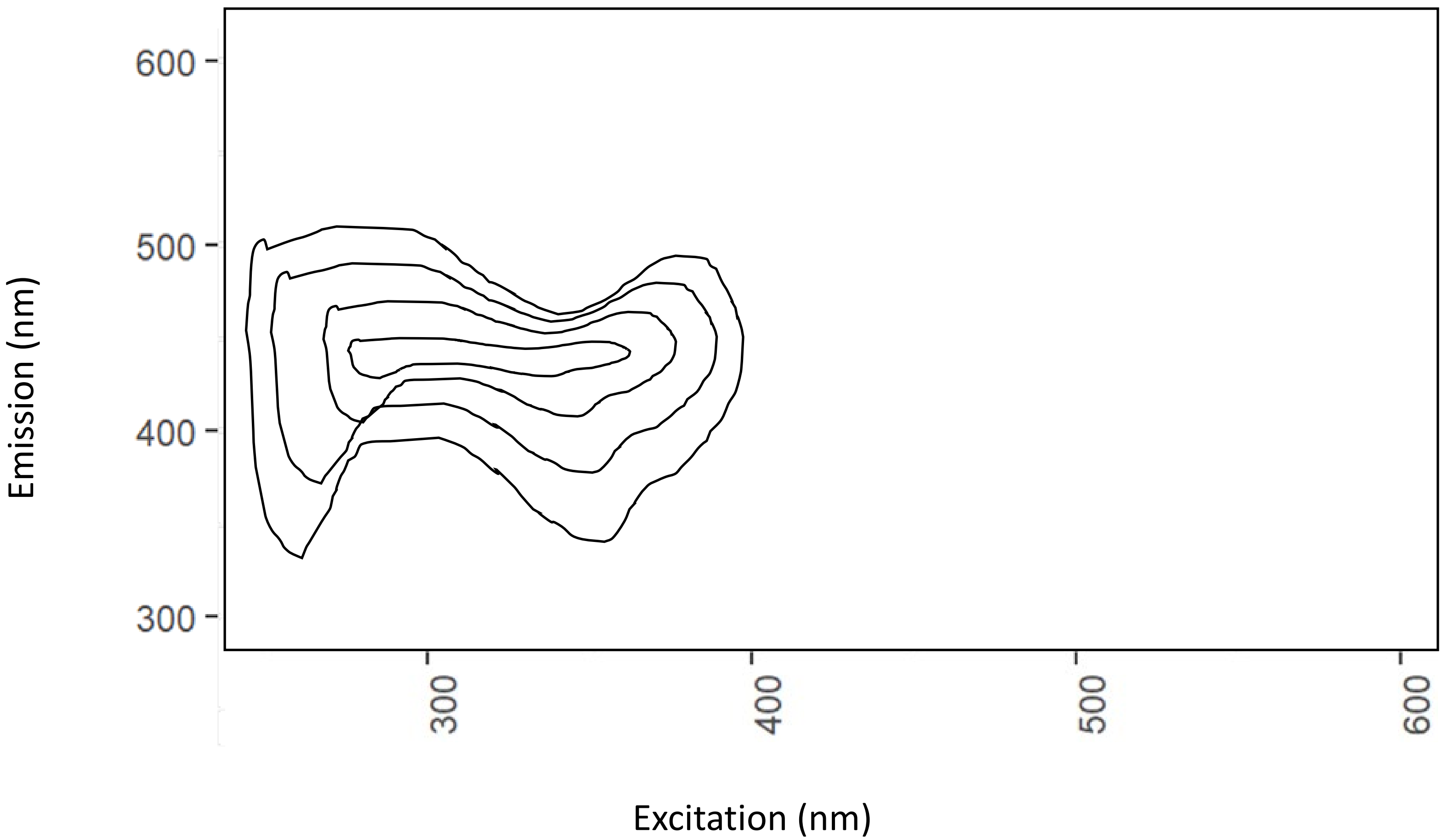


EEM #3 from Unknown DOM Source
600
500
400
300
Emission (nm)
300
400
500
600
Excitation (nm)

EEM #4 from Unknown DOM Source



EEM #5 from Unknown DOM Source



Appendix D: Answer Keys for Activities Part 1 and 2

Name: ANSWER KEY

Watercolors: Relating Properties of Light to Organic Matter and Ecosystem Production

Part 1: Absorbance

Instructions: Refer to the estuary display in front of you. Each cup or beaker represents DOM samples collected from different sources including wetland, agriculture, forest, microbes, and a factory. There is also an open water sample that was not collected from any source. Your task is to take each sample, hold it in front of your face with a flashlight aimed upwards through the bottom of each sample. If the sample is bright, this means that absorbance is low. If the sample is dark, this means that absorbance is high. This will act as a qualitative, simplified measure of light absorbance.

1. According to the source of each DOM sample, rank the following from lowest (1) (brightest sample) to highest (6) (darkest sample) absorbance based on the amount of light taken up by each sample

   4 wetland   5 agriculture   2 microbes   3 factory   6 forest   1 no source

2. Based on your ranking, which DOM source might contribute to high ecosystem production? What about low ecosystem production? How did you arrive at your answers?

   Because microbes exhibited the lowest aborbance out of all DOM sources, one would expect this to contribute to high ecosystem production because more sunlight can penetrate the surface, allowing for higher rates of primary and subsequent secondary production. This is contrary to forest DOM, which exhibited the highest absorbance and would be expected to contribute to low ecosystem production because less sunlight can penetrate the surface, inhibiting primary production. However, instructors should highlight that secondary production can still be high, especially if biomass is attirbuted to heterotrophs.

3. Based on your ranking, which sample do you expect has the highest concentration of DOM? What about the lowest concentration? How did you arrive at your answers?

   Because high absorbance is associated with high concentrations of DOM, the forested sample is expected to have the highest concentration. Therefore, aside from the sample with no DOM source, microbes would be expectd to have the lowest concentration of DOM.

Name: ANSWER KEY

Watercolors: Relating Properties of Light to Organic Matter and Ecosystem Production

Part 2: Fluorescence

Instructions: Now that you have some idea about how DOM from different sources absorbs light, you will assess how these same sources fluoresce light. You will be provided real-life EEMs from various DOM sources that describe their fluorescence intensity at various wavelengths of light. The x-axis represents excitation, or the amount of energy applied to a sample. The y-axis represents emission, or the amount of light given off or produced. Higher light intensity at certain wavelengths of light (in nanometers) are represented by warmer colors (e.g. yellow and red) while lower light intensity are represented by cooler colors (e.g. blue). Depending on the DOM source, intensity will differ according to wavelength along either axis. Take a few minutes to orient yourself to the layout. You will then be given a blank contour plot representing an unknown DOM sample collected from an estuary dominated by one of the DOM sources on the display. Color in your blank contour plot using the provided EEMs as reference for which colors to use for peak intensities. Outside bands should be colored with blue and green whereas inner bands should be colored with yellow and red to ultimately show that red, peak intensities occur at the center of each EEM. After determining the source of your unknown sample by comparing it to peak excitation and emission intensities on the provided EEMs and visible light spectrum, answer the following questions pertaining to your unknown sample. You will need to refer to the visible light spectrum below for question 2.

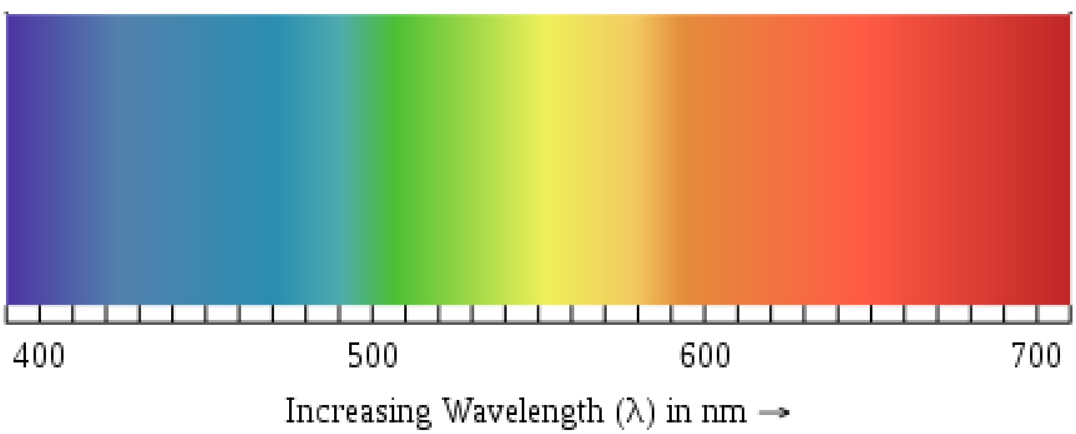


Image source: https://commons.wikimedia.org/wiki/File:EM_spectrum.svg

1. Which wavelength of light had the greatest fluorescence intensity along the emission (y) axis?
   Unknown 1: 400nm
   Unknown 2: 450nm or 475nm or 400-500nm
   Unknown 3: 400nm and 475nm (also accept 450nm)
   Unknown 4: 550nm
   Unknown 5: 450nm

2. Which color is attributed to this wavelength on the visible light spectrum?
   Unknown 1: purple or violet
   Unknown 2: blue or purple and blue
   Unknown 3: purple and blue
   Unknown 4: yellow or yellow-green
   Unknown 5: blue

3. What is the dominant DOM source in your estuary?
   Unknown 1: factory Unknown 2: agriculture Unknown 3: forest Unknown 4: wetland
   Unknown 5: microbes

4. Based on the source of your unknown sample and what you observed with other group's conclusions in class, would you expect ecosystem production to be low or high? Would you expect there to be higher rates of photosynthesis or heterotrophic consumption of DOM by microbes? Explain your answers.

   Microbes: Because absorbance from this source was low, ecosystem production would most likely be high. One would expect there to be high rates of both photosynthesis and heterotrophic consumption of microbes because 1) light would easily penetrate the surface allowing primary production to occur and 2) microbial-derived DOM is considered juicy, so heterotrophs would easily degrade and consume it.

   Factory: Because absorbance from this source was low to moderate, ecosystem production would most likely be moderate to high. There could be high rates of photosynthesis because light can penetrate the surface allowing primary production to occur. Whether or not heterotrophic consumption of DOM will dominate is uncertain since human-derived DOM can be both juicy and stable. However, one might conclude that is primary production is high, secondary production will also be high.

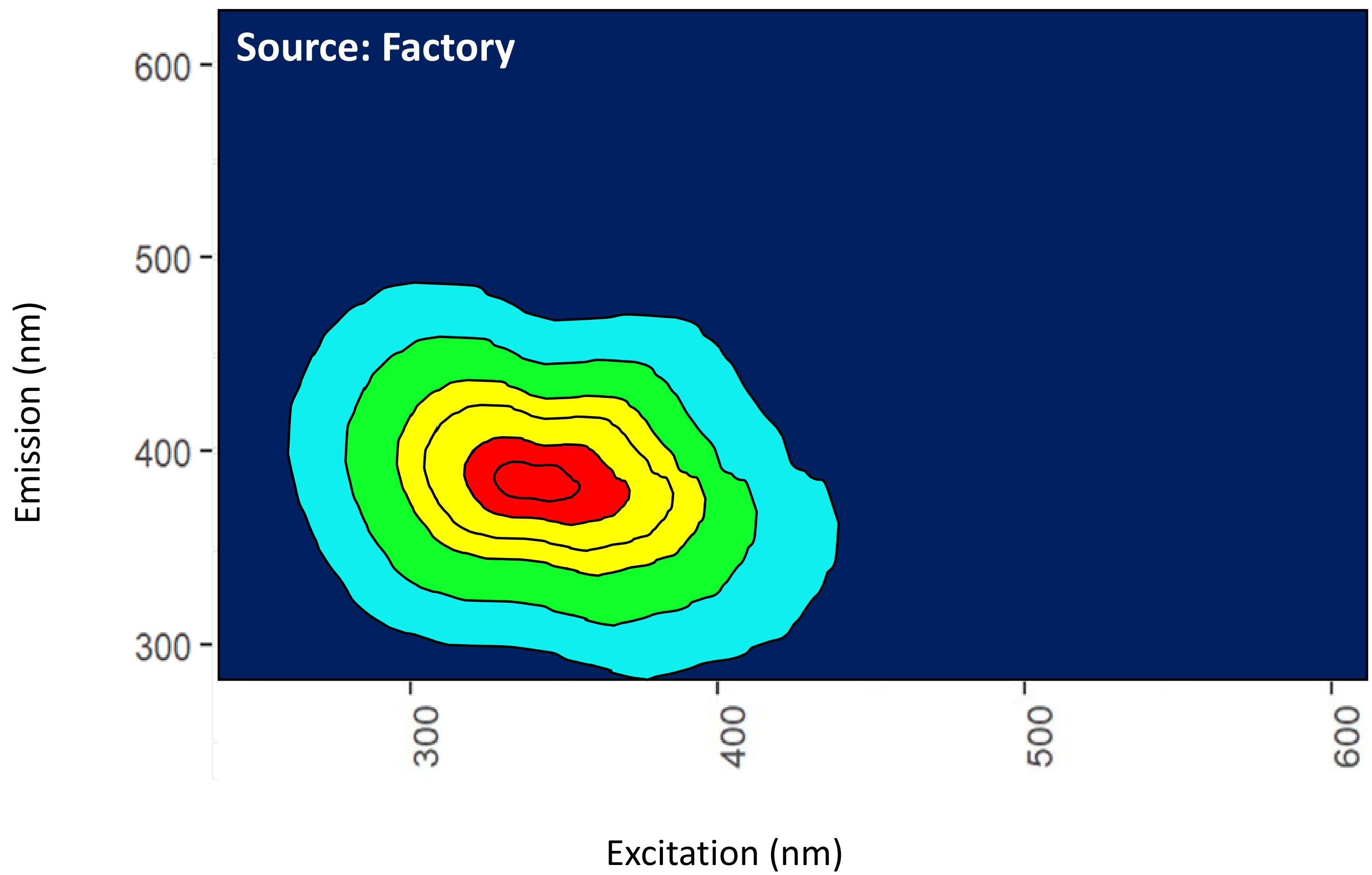
   Wetland: Because absorbance from this source was moderate, ecosystem production would most likely also be relatively moderate. One might expect there to be equal rates of photosynthesis and heterotrophic consumption of microbes since, compared to other unknown samples, it falls in the middle with regards to absorbance.

   Agriculture: Because absorbance from this source was one of the lowest, ecosystem production would most likely be low. One would expect there to be low rates of photosynthesis because light cannot penetrate the surface too far, thereby inhibiting primary production. Whether or not heterotrophic consumption of DOM will dominate is uncertain since human-derived DOM can be both juicy and stable. However, one might conclude that since primary production is low, secondary production might also be low, or, if the DOM is juicy enough, might fuel at least some heterotrophic consumption of DOM.

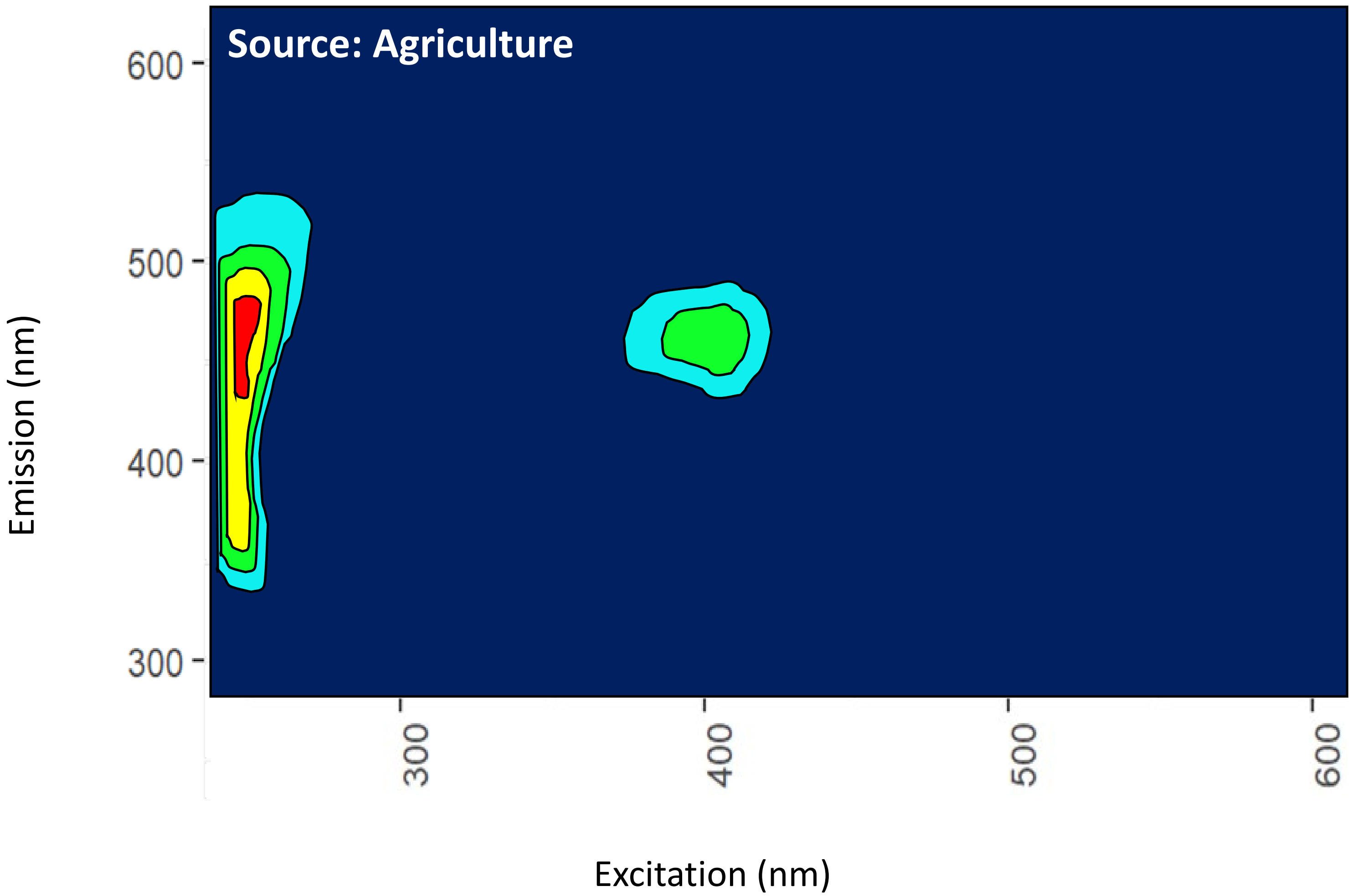
Forest: Because absorbance from this source was very low, ecosystem production would most likely be low. One would expect there to be low rates of both photosynthesis and heterotrophic consumption of microbes because 1) light cannot penetrate the surface allowing primary production to occur and 2) plant or forest-derived DOM is incredibly stable, so heterotrophs would not easily degrade and consume it.

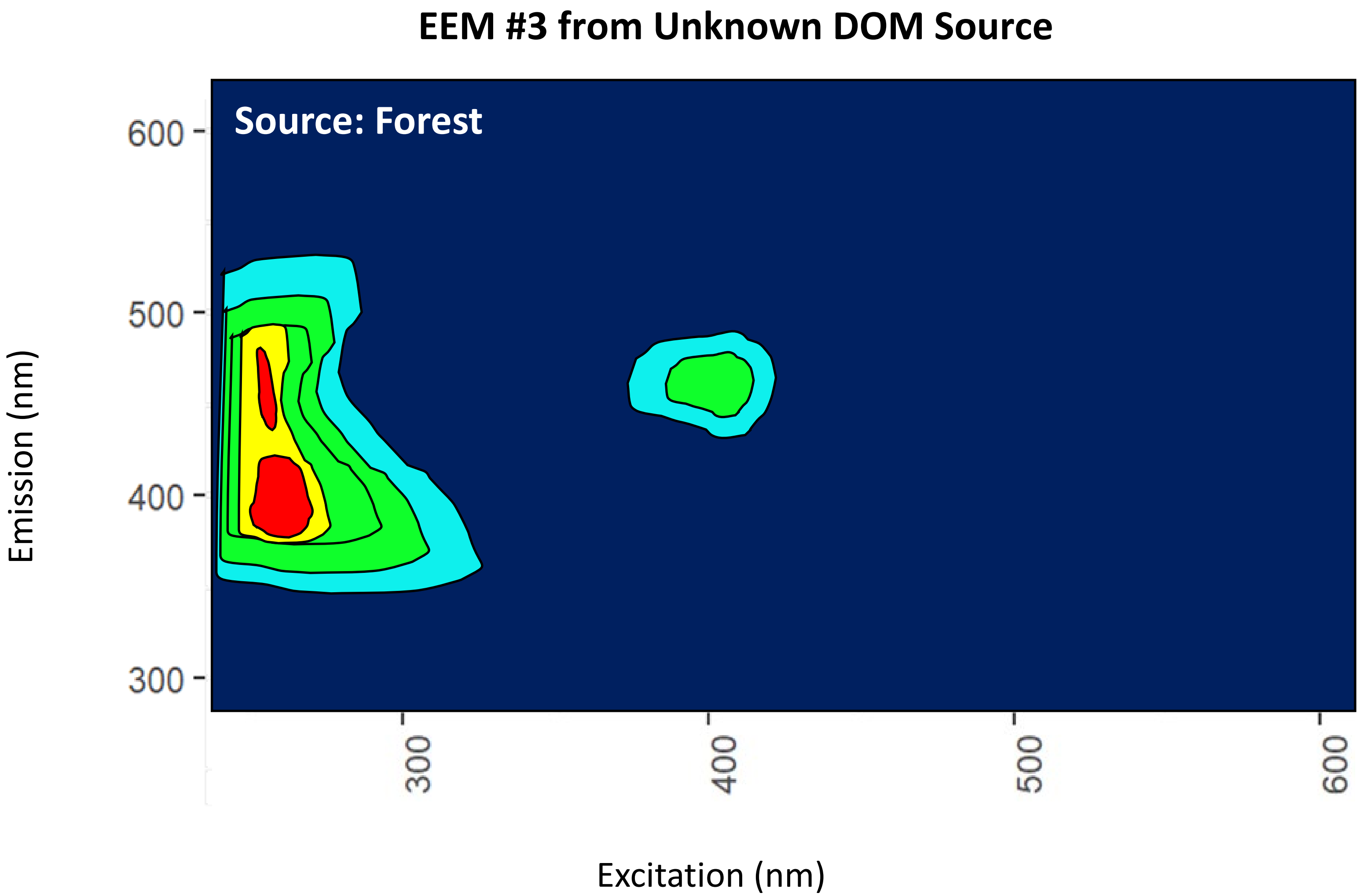
Appendix E: Answer Key for Unknown Sample EEMs in Activity Part 2

EEM #1 from Unknown DOM Source

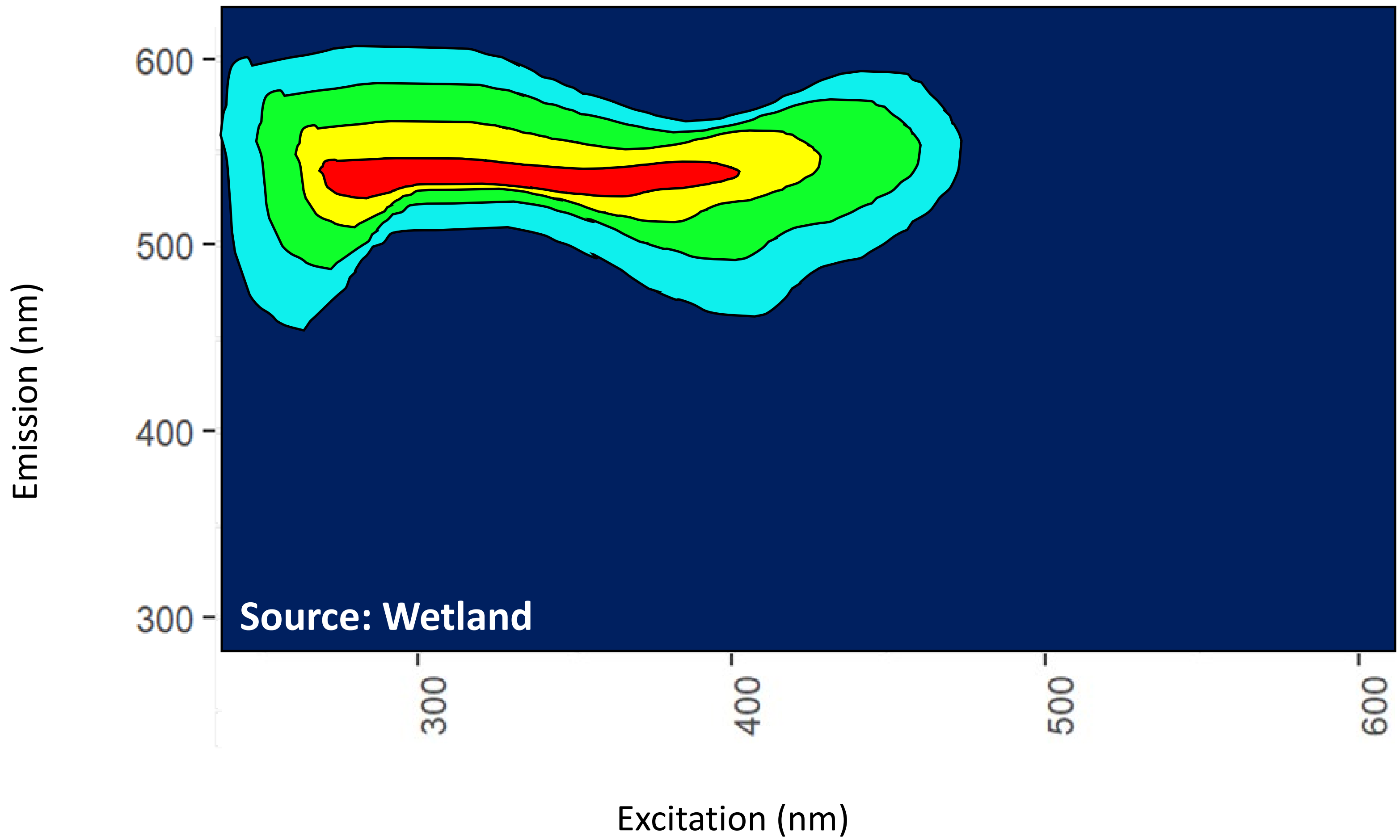


EEM #2 from Unknown DOM Source

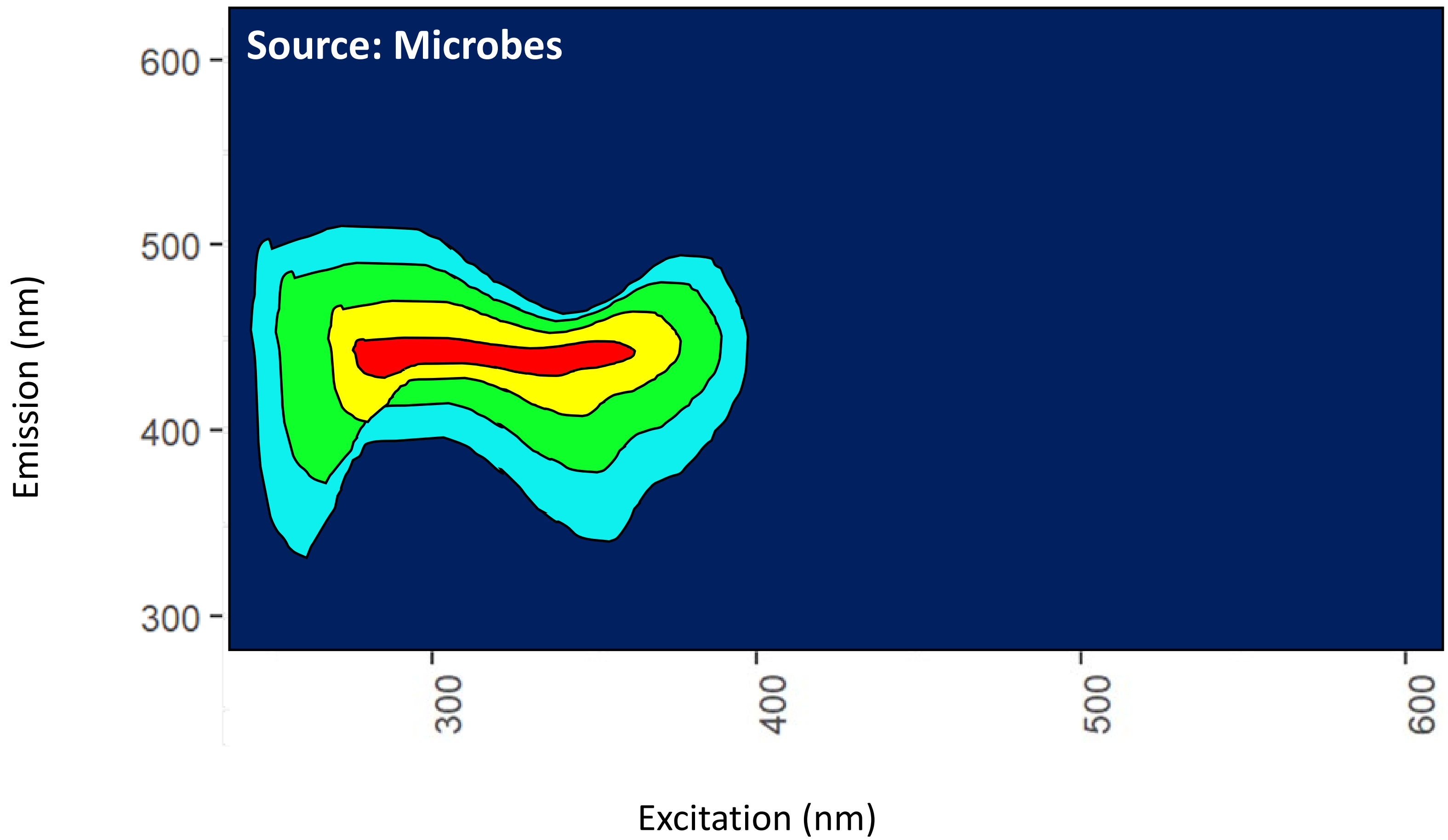



EEM #3 from Unknown DOM Source
Source: Forest
600
500
400
300
Emission (nm)
300
400
500
600
Excitation (nm)

EEM #4 from Unknown DOM Source



EEM #5 from Unknown DOM Source



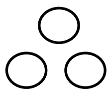
Appendix F: Extension Activity and Answer Sheet

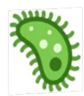
Name: ________________________________

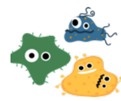
Watercolors: Relating Properties of Light to Organic Matter and Ecosystem Production

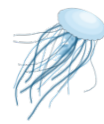
Extension Activity

1. Based on the source of your unknown sample, sketch what ecosystem production might look like in your estuary. Be sure to depict both primary and secondary consumers as well as heterotrophic microbes that consume DOM. Use the following images in your sketch. There may be one or more of each of these groups represented ("primary producer" image from github.com/twitter/twemoji).


DOM


Primary Producer


Heterotrophic Microbe


Secondary Consumer

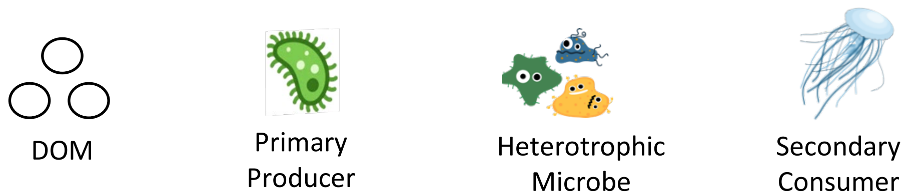
2. In the context of ecosystem production, why is it important to determine the chemical composition and degradation potential of DOM derived from human sources?

Name: ANSWER KEY

Watercolors: Relating Properties of Light to Organic Matter and Ecosystem Production

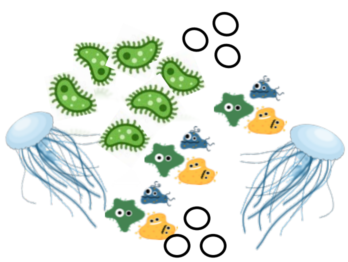
Extension Activity

1. Based on the source of your unknown sample, sketch what ecosystem production might look like in your estuary. Be sure to depict both primary and secondary consumers as well as heterotrophic microbes that consume DOM. Use the following images in your sketch. There may be one or more of each of these groups represented ("primary producer" image from github.com/twitter/twemoji).

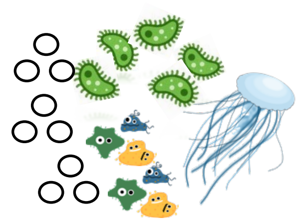


Answers will vary, but examples are provided here. The most important part of this exercise to think about the relative contribution of each to ecosystem production (i.e., number of primary producers versus secondary consumers vs. heterotrophic microbes).

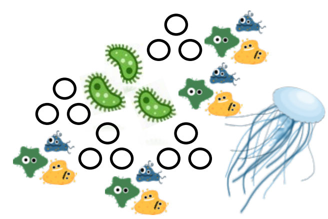
Microbes



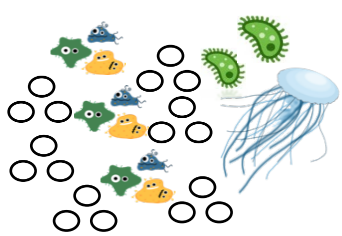
Factory



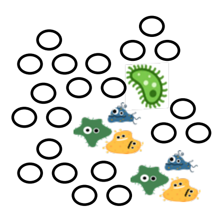
Wetland



Agriculture



Forest



2. In the context of ecosystem production, why is it important to determine the chemical composition and degradation potential of DOM derived from human sources?

It is important to determine the chemical composition and degradation potential of DOM derived from human sources because it would provide necessary insight into ecosystem production and the relative contributions of primary and secondary production to an ecosystem. Additionally, building a factory or constructing a building will create different kinds of DOM and each will result in different environmental impacts. As the coast becomes more developed, areas surrounding estuaries will become less natural (less forests, wetlands, and soils) and more urban (more industrial and commercial). Therefore, it is also important to investigate how “juicy” or stable human-derived DOM is to the heterotrophic microbes that inhabit coastal aquatic ecosystems.