Lesson 4:

PPCPs in the Environment

INTRODUCTION

Pharmaceuticals affect the human body, but when these chemicals accumulate in lakes and rivers, aquatic wildlife are impacted as well. As chemicals amass in the environment, wildlife inadvertently consumes them and the effects of pharmaceuticals increase as they move up the food chain. People can make better choices that are not only healthy for them, but will also help prevent pharmaceuticals from ending up in the environment.

STUDENT OBECTIVES

1. Identify and describe the effects of pharmaceuticals that have shown to have adverse effects on aquatic wildlife.

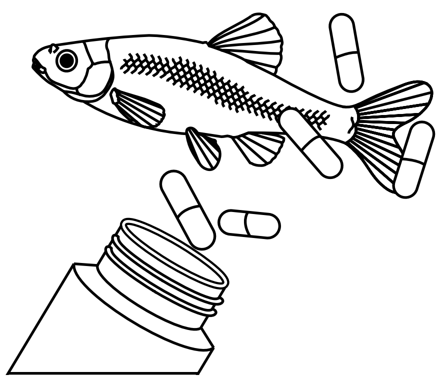
2. Use mathematical representations (graph) to support and revise explanations based on evidence about factors (estrogen) affecting biodiversity (lake populations) and populations (fathead minnows) in ecosystems of different scales (lab, natural water bodies).

3. Evaluate the claims, evidence, and reasoning in the statement that complex interactions in ecosystems maintain relatively consistent numbers and types of organisms in stable conditions, but changing conditions may result in a new ecosystem.

4. Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity.

DAILY ASSESSMENT

Students should be able to accurately graph the given information from A Real Life Small Fish Story Worksheet and be able to describe how the oestrone changed the physiology and survival of male fathead minnows. They should be able to distinguish controlled lab settings with uncontrolled natural environments and discuss possible complications of conducting experiments like this.



STATE AND NATIONAL STANDARDS

COMMON CORE	NAAEE GUIDELINES	NGSS
Math: MP.2 MP.4	3.1 3.1.A 3.1.B 3.1.C 3.1.D	HS-ETS1-2 HS-ETS1-4

9-12 Grade

45 Minutes

Subjects:
Biochemistry,
Environmental science

Setting:
Home and Classroom

Pre-Homework:
Transfer information from the PPCP Worksheet **[Lesson 1]** to the PPCPs in the Environment worksheet.

Complete the unintended use column using the resources given as well as student research.

DSRP vocabulary

Materials:

- ❑ Graphing tools (electronic or hand)
- ❑ See materials section of Gummy Fish demonstration/lab (for flipped classroom.)

DSRP Vocabulary:

- ▹ Bioaccumulation
- ▹ Biomagnification
- ▹ Endocrine disruptors

The Take-Away

Chemical changes to aquatic ecosystems can drastically affect the population of some species and may affect those who depend on those species.

*All instructions that begin with an * are found on the Illinois-Indiana Sea Grant Resource or YouTube page.*

Conventional Classroom Procedure:

1. *Watch video on bioaccumulation vs. biomagnification (bioamplification) (1:22)
2. *Watch Endocrine Disruptors by Dr. Karen Kidd of the University of New Brunswick (28 minutes)
3. Break into lab groups and conduct either the Real Life Small Fish Story and/or the Gummy Fish demonstration/experiment.
4. Discuss the results, conclusions, and solutions in class or assign as a narrative for homework.

Flipped Classroom Procedure:

1. The **night before class**, have students watch/review.fill-in:
    a. *Watch video on bioaccumulation vs. biomagnification (bioamplification) (1:22)
    b. *Watch Endocrine Disruptors by Dr. Karen Kidd of the University of New Brunswick (28 minutes)
    c. *Read *As Pharmaceutical Use Soars, Drugs Taint Water and Wildlife* and *Two-headed salamander isn't radioactive, but is weird.*
2. In class:
    a. Discuss materials from the night before assignment.
    b. Graphing exercise: *A Real Life Small Fish Story*
    c. PPCPs and Gummy Fish lab
    d. Discuss results and create conclusions as well as solutions to the issue.

Resources:

<u>*Related Curriculum on Pollution:*</u>

*University of Illinois. (2011). Pollution solution. *Fresh and Salt*. pp. 136 – 162. (Curriculum)

University of Illinois. (2011). Where Do All the Toxins Go? (Internal View). COSEE's Greatest of the Great Lakes – A Medley of Model Lessons*. (IISG-07-02). pp. G111-130.

<u>*Research articles:*</u>

Daughton, C.G. (2 Dec 2006). *Environmental Life Cycle of Pharmaceuticals*, illustration, USEPA, NERL, Las Vegas, NV.

Daughton, C. G. (2012, 12). Comment on "Life Cycle Comparison of Environmental Emissions from Three Disposal Options for Unused Pharmaceuticals." *Environmental Science & Technology, 46*(15), 8519-8520. doi: 10.1021/es301975v.

Drury, B., Scott, J., Rosi-Marshall, E. J., & Kelly, J. J. (2013, 12). Triclosan Exposure Increases Triclosan Resistance and Influences Taxonomic Composition of Benthic Bacterial Communities. *Environmental Science & Technology*, 130725155410004. doi: 10.1021/es401919k.

Errata: Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change? (2000, 12). *Environmental Health Perspectives, 108*, 598. doi: 10.2307/3454551.

He, Y., Chen, W., Zheng, X., Wang, X., & Huang, X. (2013, 12). Fate and removal of typical pharmaceuticals and personal care products by three different treatment processes. *Science of The Total Environment, 447*, 248-254. doi: 10.1016/j.scitotenv.2013.01.009.

Huerta-Fontela, M., Galceran, M. T., & Ventura, F. (2011, 12). Occurrence and removal of pharmaceuticals and hormones through drinking water treatment. *Water Research, 45*(3), 1432-1442. doi: 10.1016/j.watres.2010.10.036.

Li, X., Zheng, W., & Kelly, W. R. (2013, 12). Occurrence and removal of pharmaceutical and hormone contaminants in rural wastewater treatment lagoons. *Science of The Total Environment, 445-446*, 22-28. doi: 10.1016/j.scitotenv.2012.12.035.

Lubliner, B, Redding, M., & Ragsdale, D. (January 2010). *Pharmaceuticals and Personal Care Products in Municipal Wastewater and Their Removal by Nutrient Treatment Technologies*. (State of Washington Publication No. 10-03-004).

Swan, G. E., Cuthbert, R., Quevedo, M., Green, R. E., Pain, D. J., Bartels, P., Wolter, K. (2006, 12). Toxicity of diclofenac to Gyps vultures. *Biology Letters, 2*(2), 279-282. doi: 10.1098/rsbl.2005.0425.

Thorpe, K., Benstead, R., Hutchinson, T., Cummings, R., & Tyler, C. (2003, 12). Reproductive effects of exposure to oestrone in the fathead minnow. *Fish Physiology and Biochemistry, 28*(1-4), 451-452. doi: 10.1023/B:FISH.0000030627.76841.ed

Zuccato, E. (2007, 12). Pharmaceuticals as Environmental Pollutants. *Drug Safety, 30*(10), 919-990. doi: 10.2165/00002018-200730100-00037

<u>Lectures on pharmaceuticals impact on aquatic environments:</u>

*Barber, L. (July 29, 2014) What are the Effects of Pharmaceuticals in Aquatic Ecosystems (51:38)

*Bennett, B. (July 29, 2014) Overview: How do Pharmaceuticals Enter the Environment? (13:10)

Extensions:

- As a demonstration for a public awareness event, students can replicate the gummy fish activity as a way to show others how chemicals can affect aquatic wildlife and the implications of our actions.
- Have students brainstorm on ways for wastewater treatment facilities to improve their filtration to address chemicals associated with PPCPs.
- Using information on states and facilities that have started treating wastewater for PPCPs, have students create projects on proactive systems, keeping associated costs and feasibility in consideration. Use the He, Huerta, Lubliner, and Li reports as a source. (Resources)

Name ___________________________________ Block/Period_________________

Pharmaceuticals and Personal Care Products in the Environment Worksheet

Name of PPCP	**Targeted Use**	**Unintended Use**
e.g. triclosan	Antibacterial agent found in soaps and other PCPs	Stops luminescence in some bacteria; toxic to algae

Summary of issue:

Examples of PPCPs Intended and Unintended Effects. (Teacher copy)

Nontarget species	**Compound**	**Intended use**	**Nontarget species effects**	**Source**
Fathead minnow	Oestrone (steroidal estrogen)	Replacement therapy in women and in vet med, increase animal growth rate	Male minnow mortality at 307ng/L and 781ng/L. Reduced egg spawn. Female sex characteristics in male spawn.	Thorpe, et.al.
Algae and bacteria	Tricolsan	Antibacterial found in soap, toothpaste, and other PCPs.	Stops luminescence in some bacteria; toxic to algae	Drury, et. al.
Daphnia	Acetaminophen	Analgesic/ anti-inflammatory	Immobilization	Daughton
	Verapamil	Cardiac drug		
	Propranolol	Beta-blocker		
	Diazepam	Psychiatric drug/ muscle relaxant		
Fish	17α-Ethinyl estradiol	Oral contraception	Female sex characterization in male fish	
Mussels	Fluoxetine (e.g. Prozac)	Antidepressant	Elicits significant spawning in males at 10^{-7} M and females at 10^{-6}M	
	Fluvoxamine (e.g. Luvox)		Elicits significant spawning in males at 10^{-9} M and females at 10^{-7}M	
Asian vultures	Diclofenac	Domestic livestock anti-inflammatory drug	Kidney failure (death)	Swan, et.al.

Real Life Small Fish Story (Teacher Information and Instructions)

As a relatively easy way to demonstrate the effects pharmaceuticals have on aquatic ecosystems, a small study was chosen so that students could plot and analyze the data. Overall, the effects of different pharmaceuticals are as varied as the pharmaceuticals themselves. References to other research are listed in the resources. If you have a preserved minnow specimen, it would be helpful as a visual aid to students.

Introduction: The fathead minnows are a popular baitfish and forage fish. According to the U.S. Geological Survey, its use has led to widespread introduction in waterways throughout the United States. A study by Thorpe, et.al tested the effects of a natural steroidal estrogen performed on fat-head minnows in a laboratory setting. Many other studies have witnessed that when estrogens are present in aquatic ecosystems they can affect male fish by changing their sex characteristics so that they develop into hermaphrodites and are able to produce eggs (Daughton, 1999).

Description: The U.S. Geological Survey describes fathead minnows as relatively small (75 to 102 mm) and having a body that is slightly laterally compressed and a head that is slightly flattened dorsally. The fish can reach maturity by age 2. Normal spawning begins when the water temperature is between 15-30^{o}C (59-86^{o}F) and generally peaks in natural waterways in July in the northern United States.

Pharmaceutical – Estrogen: According to Thorpe, et. al, the natural steroid, oestrone, is naturally present in wastewater treatment effluent in concentration between 10 and 20 ng/L in surface water. Estrogens can be found in many forms. Women produce it naturally. Cattle farmers use estrogen in combination with testosterone and progesterone to promote growth in cattle.

Thorpe, Benstead, et. al tested different strengths of the steroid to see how these levels impact physiology and population levels. An adaptation of that study (data approximated) has been created below. The results (in general) are approximately the same as the Thorpe study.

Six sets of six pairs of sexually mature fathead minnows were held in tanks and received clean, 25^{o}C water for 21 days (-21 to 0 days) with 16 hours of light and 8 hours of darkness. Scientists did this to test for compatibility between the pairs. One pair was kept as the control and the other five were given treatments of oestrone (scant, 34, 98, 307, and 781 ng/L) by adding it to flowing water. The following results were recorded (Table 1). Table 2 reveals the mortality results of the experiment. Observations can be made about that information as well.

Students should be able to graph the data given and make observations about the effects of oestrone on the fish. Their graphs should look like Graph 1.

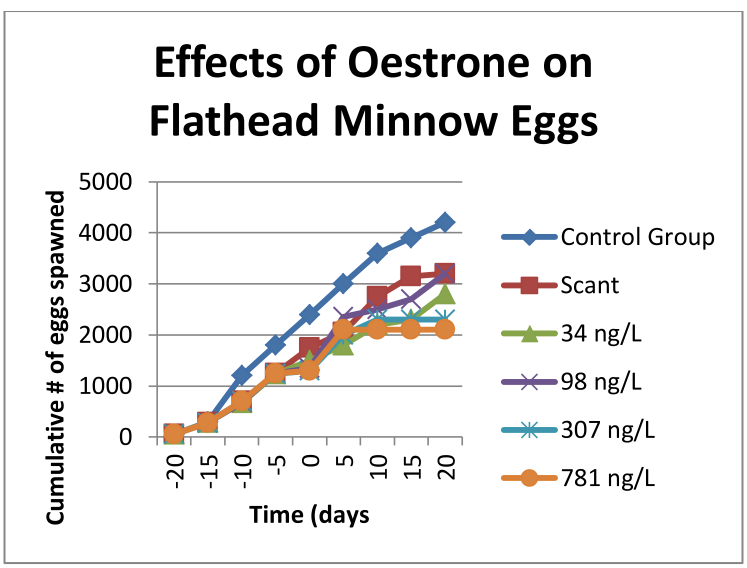
Table 1

Cumulative # of eggs spawned	Treatment amounts →	Control group	Scant oestrone	34 ng/L	98 ng/L	307 ng/L	781 ng/L
Time (days) ᵛ							
-20		50	50	50	50	50	50
-15		300	280	280	275	275	275
-10		1200	700	673	680	700	700
-5		1800	1250	1235	1245	1233	1250
0		2400	1750	1500	1350	1300	1300
5		3000	2050	1800	2350	2000	2100
10		3600	2750	2200	2500	2300	2100
15		3900	3150	2300	2700	2300	2100
20		4200	3200	2800	3200	2300	2100

Table 2

Concentration of oestrone	Male mortality after 20 days
Control	n=0
Scant	n=0
34 ng/L	n=0
98 ng/L	n=0
307 ng/L	n=2
781 ng/L	n=3

Graph 1



A Real Life Small Fish Story (Student Worksheet)

There are many studies on the effects of hormones on aquatic life. You have already viewed some research on this and have a general background.

For this activity, you are going to plot information on exposure of a natural steroidal estrogen, oestrone on fathead minnows.

Table 1 is from an experiment on fathead minnows conducted in a lab. Six sets of six pairs (36 minnows, 18 male, 18 female) of fathead minnows were held in controlled tanks. For the first 21 days, all of the fish were treated the same (25^{o}C of clean water, 16 hours of daylight and 8 hours of darkness). At day 0, oestrone was added to the experimental groups at the levels indicated. The temperature and photoperiod were maintained.

Instructions: Graph the information using tools available to you (e.g. Excel). Properly label the x and y axis. The graph should have the independent value on the x-axis. Properly name this graph at the top of this page. Table 2 will be addressed in the question section.

Table 1

Cumulative # of eggs spawned	Treatment amounts →	Control group	Scant oestrone	34 ng/L	98 ng/L	307 ng/L	781 ng/L
Time (days) ˅							
-20		50	50	50	50	50	50
-15		300	280	280	275	275	275
-10		1200	700	673	680	700	700
-5		1800	1250	1235	1245	1233	1250
0		2400	1750	1500	1350	1300	1300
5		3000	2050	1800	2350	2000	2100
10		3600	2750	2200	2500	2300	2100
15		3900	3150	2300	2700	2300	2100
20		4200	3200	2800	3200	2300	2100

Table 2

Concentration of oestrone	Male mortality after 20 days
Control	n=0
Scant	n=0
34 ng/L	n=0
98 ng/L	n=0
307 ng/L	n=2
781 ng/L	n=3

1. Describe the physiology of fathead minnows. List your source.
2. Describe in a narrative what happened in this experiment.
3. Why do you think the scientists used 36 fish?
4. What are general observations you can make about the graph?
5. What can you say about the control group?
6. At what level (ng/L) do you start to see the oestrone affect the spawn rate?
7. At what level (ng/L) do you see significant decline in spawn rate?
8. How do the increase levels of oestrone affect the spawning rate of the fathead minnows?
9. What are some possible explanations for this?
10. What do the results of *Table 2* say about the levels of oestrone for fathead minnows?
11. Do the effects on the minnows affect higher level aquatic wildlife? Defend your answer.
12. This lab was done in a controlled setting. Describe other factors that may affect the outcome of an experiment like this in different settings and scales of water.
13. What are some possible solutions to this issue (environmentally, engineering, personal change)?

PPCPs and Gummy Fish

A demonstration (or lab for flipped classrooms) can be conducted to show the effects of PPCPs on aquatic wildlife. Some of the results will seem obvious to your students, but by creating an "unknown mixture" of chemicals, you can demonstrate how detecting PPCPs isn't always easy because effluents of wastewater treatment plants do not release just one chemical, it's a mixture of many that may interact with each other.

Materials needed (for class lab, multiply by number of groups):

Note: The gummy fish must be as permeable as gummy bears. If you cannot find fish, you can substitute gummy bears, worms, or gumdrops or create fish out of molds and gelatin.

- 1 green (or red) gummy fish (female)
- 5 clear gummy fish (male)
- Food coloring (green, red, blue, yellow)
- Clean water
- Solutions of 1X green, 2X green, and 3X green (or red if using red fish)
- Solution of "Wastewater Effluent" (water with red, blue, green, and yellow food coloring to make brown)
- 5 clear cups
- Paper towel
- Plastic spoons

Instructions:

1. Before the demonstration, make:
    a. 1 – 1 drop green food coloring/25ml water solution
    b. 1 – 2 drop green food coloring/25ml water solution
    c. 1- 3 drop green food coloring/25ml water solution
    d. 1 – 2 drop each (red, green, and blue)/25ml water solution (wastewater effluent)

2. Put the wastewater effluent solution out of sight until the first part of the demonstration is complete.

3. Explain to the students that the green solution is estrogen. Pour 25 ml of each green solution into 3 clear cups and clean water into the fourth cup. Leave the fifth cup empty for now.
4. Set the green (female) gummy fish aside and place 1 white (male) gummy fish in each solution (clean, 1x green, 2x green, and 3x green).
5. Wait five minutes. During that time have students predict what will happen to the male fish in each solution.
6. After five minutes “fish” ☺ the males from the solutions and lay them next to each other in order of concentration. Have students make observations about the results.
    a. What can you say about color of each male fish?
    b. How does this compare to the female fish?
    c. Since this was estrogen, what can you say about the males’ sex characteristics (based on previous information).
7. Now bring out the “wastewater effluent” and add it to the fifth cup. Explain that wastewater effluent isn’t a simple one chemical output, but a mixture of many chemicals that the wastewater treatment plants are not designed to filter out. One of those chemicals is atrazine as discussed in the article Pesticide Atrazine Can Turn Male Frogs into Females.
8. Pour the solution into the fifth cup and place the last gummy fish in and let it sit for five minutes. While waiting, discuss the possible results of this mixture on aquatic wildlife.
9. Retrieve the male fish. Have students make observations.
10. Ask students what proactive environmentally thoughtful actions can people, industry, and doctors do to help minimize the problems associated with pharmaceuticals and personal care products? Use http://web.extension.illinois.edu/unusedmeds/reducewaste/index.cfm as a resource.