OVERVIEW

This activity accompanies the BioInteractive *Scientists at Work* video *Genetically Modified Mosquitoes*. In this activity, students are challenged to provide their own questions and ideas for experiments they could conduct to examine the impact of releasing genetically modified (GM) mosquitoes on a local population of wild mosquitoes. Students learn the approaches scientists use to conduct such experiments. They then use math, descriptive statistics, and line and bar graphs to analyze data from treated and untreated (control) areas. Students may optionally use spreadsheets to analyze and graph the data.

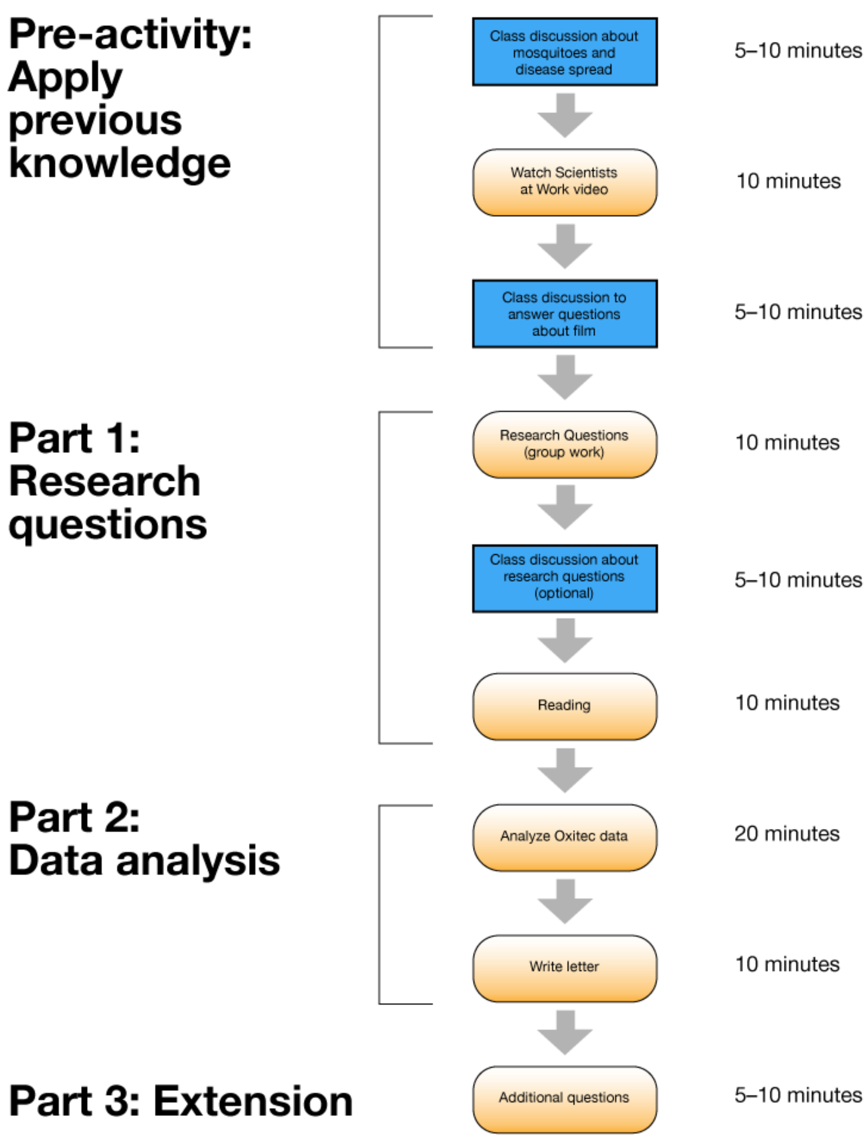

Figure 1. Activity overview. The video in the pre-activity, reading in Part 1, and Part 3 extension may all be assigned as homework.

KEY CONCEPTS

- To explore the impact of GM mosquitoes on wild mosquito populations, scientists use experimental designs that include comparing the estimated density of mosquitoes in treated and untreated areas before and after the release of GM mosquitoes.
- To estimate the population dynamics of an organism that is hard to see and has a large population size, scientists must take numerous samples from the population.
- To exemplify the nature of science or to enhance a discussion on transgenic technology.

STUDENT LEARNING TARGETS

- Describe an experimental design for measuring the impact that releasing GM mosquitoes has on wild mosquitoes; compare this design to one used by scientists.
- Use data to calculate several population statistics.
- Create and analyze graphs to identify patterns.
- Use evidence to form and support claims and conclusions.

CURRICULUM CONNECTIONS

Standards	Curriculum Connections
NGSS (2013)	HS-LS2-2; SEP1, SEP3, SEP5
AP Bio (2015)	2.D.1, 3.A.1, 4.A.5, 4.B.3; SP2, SP3, SP4
IB Bio (2016)	3.5, C.5
AP Env Sci (2013)	II.A, III.A
IB Env Systems and Societies (2017)	2.5
Common Core (2010)	ELA.RST.9–12.7, ELA.WHST.9–12.1 Math.A-REI.3, Math.S-ID.3, Math.S-IC.1, Math.S-IC.3, Math.S-IC.4; MP1, MP2, MP3
Vision and Change (2009)	CC5; DP1, DP2, DP6

KEY TERMS

Aedes aegypti, average density, genetically modified organism (GMO), mating fraction, ovitrap

TIME REQUIREMENTS

Two 50-minute class periods, which includes viewing the video, dependent on how comfortable students are analyzing data and creating graphs. Class time can be shortened if students watch the video at home and complete their graphs for homework.

SUGGESTED AUDIENCE

- High School: Biology (AP/IB)
- College: Introductory Biology

PRIOR KNOWLEDGE

Students should be familiar with:

- the idea that genes code for proteins and that genetic information can be passed to offspring
- what it means for an organism to be genetically modified
- how to compute averages and construct line and bar graphs

MATERIALS

- access to the video *Genetically Modified Mosquitoes*

- one copy of each handout ("Student Handout," "Does Using GM Mosquitoes Work?", and "Data Tables") for each student or group of students
- calculator or access to the "Data Spreadsheet" download and spreadsheet software, such as Microsoft Excel

TEACHING TIPS

- This activity lends itself to students working in groups. Look through the parts required for this lesson and make copies of the handouts accordingly.
- For Step 4 of the "Student Handout," you may refer to the BioInteractive activity "Asking Scientific Questions" to help students develop their research questions.
- For Step 5 of the "Student Handout," students read the "Does Using GM Mosquitoes Work?" document. You may want to review the calculations described in the document with students.
  - The document includes calculations required for computing the ovitrap index (OI), the average density (AD) of *Aedes aegypti*, the relative change in density in an untreated versus treated area, and the fraction of females that are mating with GM males (mating fraction, *M*).
  - You may need to explain that ovitrap (OI) data cannot be used to estimate population size. There are, however, methods for doing that, as explained in the original scientific paper that describes these experiments (Carvalho et al. 2015).
  - When calculating the relative change in mosquito density, you may need to emphasize that the values for average densities in untreated (*U*) and treated (*T*) areas before and after treatment (U_b, T_b, U_a, T_a,) are averages across several months.
- For the calculations in Steps 6–10 of the "Student Handout," decide whether you would like students to use spreadsheet software to analyze the data and to create the graphs described in the activity. Arrange for student access to computers, if necessary.
  - If students use spreadsheets, it will be helpful if they understand basic spreadsheet functions such as adding, writing functions, and computing averages across multiple cells. BioInteractive has tutorials to introduce students to the basics of using spreadsheets in both Microsoft Excel and Google Sheets.
- If you teach a more advanced class, you may want to alert students to the published scientific paper that describes the actual experiments carried out in Brazil (Carvalho et al. 2015).
- The scenario described at the start of the "Student Handout" is based on an actual proposal from the biotechnology company Oxitec to release GM mosquitoes in Florida. To prepare for the potential release, the company submitted an environmental assessment for which thousands of people submitted public comments. In 2016, the Federal Drug Administration (FDA) issued a Finding of No Significant Impact document. In response to request from stakeholders, the Environmental Protection Agency reopened public comment in May of 2018. In November 2018, Oxitec withdrew their proposal with plans of resubmitting.
  - If you discuss this proposal, you may wish to emphasize to students an important aspect of the nature of science: the information gleaned from scientific research may inform discussions of public policy, but making decisions that reflect the values of a community falls outside the purview of science.
- Consider having students visit the website for the Invasive Mosquito Project. It is a citizen science project that provides students, teachers, and anyone interested with the opportunity to collect real data and contribute to a national mosquito species distribution study. The project not only gives individuals an opportunity to explore and collect data, but it also raises awareness of diseases that can be transmitted by mosquitoes and how people can make an effort to protect themselves, communities, and pets from illness.

ANSWER KEY

PRE-ACTIVITY

1. Describe your knowledge of or experience with mosquitoes.
Answers will vary. Many students in the United States are likely to think of mosquitoes as pests that occasionally bite them and cause irritating, itchy welts. However, they may not be aware of the severe health impacts of diseases carried by mosquitoes. You may also want to help students think about the role mosquitoes play in food

webs (for example, as food for birds and bats) and as population control of some nonhuman species by being disease vectors.

2. For what diseases, in humans or other organisms, do mosquitoes act as vectors (carriers)?
Answers include the Zika virus, West Nile virus, Chikungunya virus, dengue, yellow fever, and malaria.
3. Watch the video *Genetically Modified Mosquitoes* and record any questions you have. Briefly discuss the video and your questions with a small group of other students.
Answers will vary.

PART 1: Research Questions

4. Work with your group to develop one or two research questions that would help determine whether releasing GM mosquitoes into the environment is an effective method for reducing wild mosquito populations in your area.

Here students are asked to think ahead and imagine what ideal data would look like. Many, if not most, students will find this thought process to be challenging; it is certainly a challenging process for experienced scientists. Be open to a range of student ideas, but work with them to ensure that the research plan they propose is linked directly to the research question. Many students struggle to design appropriate research experiments, so practice is helpful. If you have previously had students learn about designing controlled experiments, you may want to review those resources at this point. [The BioInteractive activity "Asking Scientific Questions" can be used to help students develop their research questions.]

5. Read through the handout "Does Using GM Mosquitoes Work?" Compare and contrast the question, experimental design, and data described in the reading to your research ideas.

Student answers will vary depending on their research questions and experimental plans. You may want to review the value of including treated and untreated (control) areas.

PART 2: Data Analysis

6. Scientists at the company Oxitec completed an experiment in Brazil similar to the one described in the "Does Using GM Mosquitoes Work?" reading. Some of their data are provided in the "Data Tables" handout. In this same handout, fill in the missing rows for the "Brazilian Data: *Untreated* Area" and "Brazilian Data: *Treated* Area" data tables.
See Tables 1 and 2 for the correct calculations.

Table 1. Answer Key for the "Brazilian Data: *Untreated* Area" table.

	Before GM Mosquito Release				*After* GM Mosquito Release								
	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
L	3	5	6	9	8	9	10	8	5	4	2	1	2
T	10	10	9	10	9	10	10	10	9	9	10	9	9
E	5	13	34	65	85	98	116	112	45	24	7	2	3
OI	30%	50%	67%	90%	89%	90%	100%	80%	56%	44%	20%	11%	22%
AD	0.50	1.30	3.78	6.50	9.44	9.80	11.60	11.20	5.00	2.67	0.70	0.22	0.33

Table 2. Answer Key for the "Brazilian Data: *Treated* Area" table.

	Before GM Mosquito Release				*After* GM Mosquito Release								
	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
L	4	7	7	7	6	3	2	1	1	1	1	1	1
T	10	9	10	8	9	9	10	9	10	9	10	9	10
E	6	30	42	59	31	8	2	1	1	1	1	2	1
OI	40%	78%	70%	75%	67%	33%	20%	11%	10%	11%	10%	11%	10%
AD	0.60	3.33	4.20	6.25	3.44	0.89	0.20	0.11	0.10	0.11	0.10	0.22	0.10

7. On a separate piece of paper, create a line graph by plotting the months on the *x*-axis and AD on the *y*-axis for both untreated and treated areas. Place both untreated and treated data on the same graph.
   See Figure 2 for a sample graph.

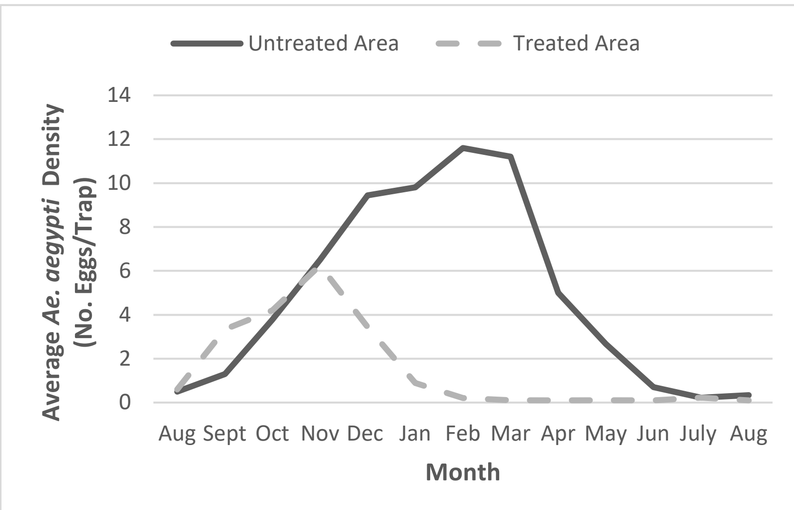

Figure 2. The average *Aedes aegypti* density by month for the untreated and treated areas.

8. Using the information in the *E* and *T* rows of the data tables, calculate the average AD, for the *untreated* and *treated* areas, both *before* and *after* the mosquitoes were released (U_b, T_b, U_a, and T_a). Then use these values to compute the relative change in mosquito density in the untreated versus treated areas.

T_b	U_b	T_a	U_a	relative change $= \frac{\left(\frac{T_a}{U_a}\right)}{\left(\frac{T_b}{U_b}\right)} - 1$
3.70	*3.00*	*0.56*	*5.79*	*-0.92 or -92%*

9. Use the data and evidence you gathered to make a claim about whether the GM mosquito program is effective in Brazil. Make sure to cite specific evidence to support your claim.

The data support the claim that the GM mosquito program seems to be reducing the size of the mosquito population in the treated area. Students may cite multiple sources of evidence, including the higher ovitrap index values in the untreated area versus the treated area from December until June, the higher average density of mosquitoes in the untreated area from December until June, and the higher overall average density of mosquitoes in the untreated area.

10. a. Compute the mating fraction (*M*) for the missing months in the "Mating Fraction Data: Treated Area" table of the "Data Tables" handout.
    See Table 3 for the correct calculations.

Table 3. Answer Key for the "Mating Fraction Data: Treated Area" table.

	Impact of Releases								
	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
F	24	8	1	1	1	1	1	2	1
N	7	1	1	0	0	0	0	0	0
M	77%	89%	50%	100%	100%	100%	100%	100%	100%

b. On a separate piece of paper, create a bar graph by plotting the month on the *x*-axis and the mating fraction on the *y*-axis for the treated area.

See Figure 3 for a sample graph.

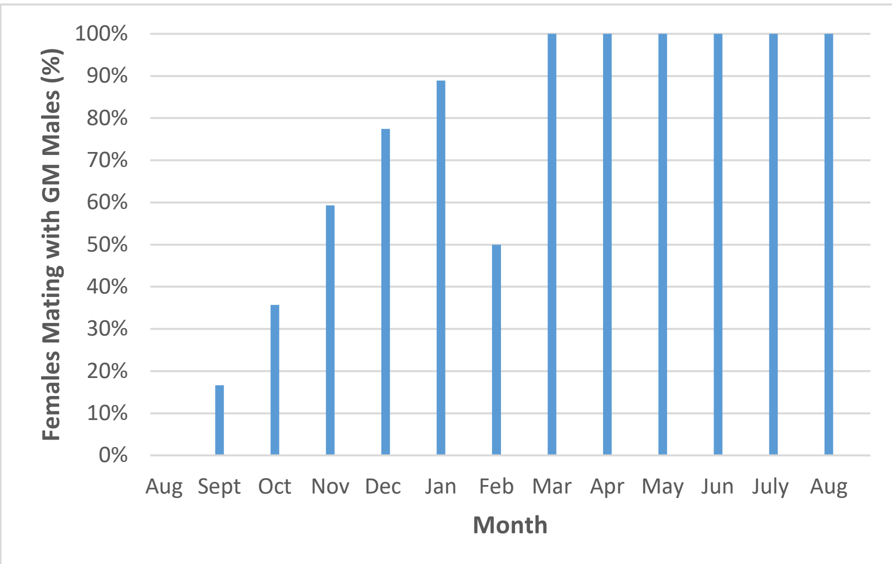

Figure 3. The percentage of females mating with GM males in the treated area.

c. Explain how the mating fraction evidence affects the claim you made in Step 9.

The data on mating fractions provide further evidence to support the claim that the GM mosquito program is causing a major decline in the size of the mosquito population in the treated area. If the decline in the population was due to another variable, the fraction of females mating with GM males would not necessarily increase.

11. Write a short letter to city officials summarizing evidence (based on the Oxitec data) about whether releasing GM mosquitoes may work in your area. Be sure to emphasize the ultimate goal of the research and the GM mosquito technique. Also describe any further questions or concerns you have about the release.

This question provides students with the opportunity to summarize what they have learned in the lesson and provides you with an opportunity to learn what questions students still have that you may want to address in future class sessions. Remind students here that the overall goal of the GM mosquito approach is to reduce and possibly eliminate the Zika virus from places where humans live to improve human health. A good letter to city officials will emphasize this point and how this might be accomplished with the GM mosquito technique.

PART 3: Additional Questions

12. Both the ovitrap index (OI) and the average density (AD) are measures of the population dynamics of *Ae. aegypti*. What ideas do you have for why the researchers use two different measures?

Researchers are often limited by time or funding, so they must make trade-offs. The ovitrap index provides less information than the average density, but researchers can collect OI data from more sites because they do not need to take the time to count all the eggs in each trap. Researchers can thus get a rough idea of trends in population size *from OI data. Average density provides a more accurate description of the population size but is more time-consuming to collect. Researchers may use AD when they have the time or money to count all the eggs.*

13. Why was it valuable for researchers to include an untreated area?

Including a control like the untreated area helps researchers eliminate alternative explanations for patterns they find between the independent and dependent variables. For example, differences in standing water could have influenced mosquito density independently of the GM mosquito program.

14. What additional data do you think the scientists might have or could have collected at each study site on factors that could influence the population dynamics of the mosquitoes?

Student answers will vary, but look for answers that identify environmental factors that reduce population sizes. For example, bats and birds like swallows and nighthawks are important predators of mosquitoes, especially when mosquitoes are most active in the late evening. Bat studies have shown that, while moths may be the most abundant insect in the diets of many bat species, in some cases an individual bat can consume up to 600 mosquitoes in a single night. Knowing about the nightly activities of bats and birds at each study site would help the scientists better understand the effect of the GM mosquitoes on mosquito density.

REFERENCE

Carvalho, Danilo O., Andrew R. McKemey, Luiza Garziera, Renaud Lacroix, Christl A. Donnelly, Luke Alphey, Aldo Malavasi, and Margareth L. Capurro. "Suppression of a Field Population of *Aedes aegypti* in Brazil by Sustained Release of Transgenic Male Mosquitoes." *PLOS Neglected Tropical Diseases* 9, 7 (2015): e0003864. https://doi.org/10.1371/journal.pntd.0003864.

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