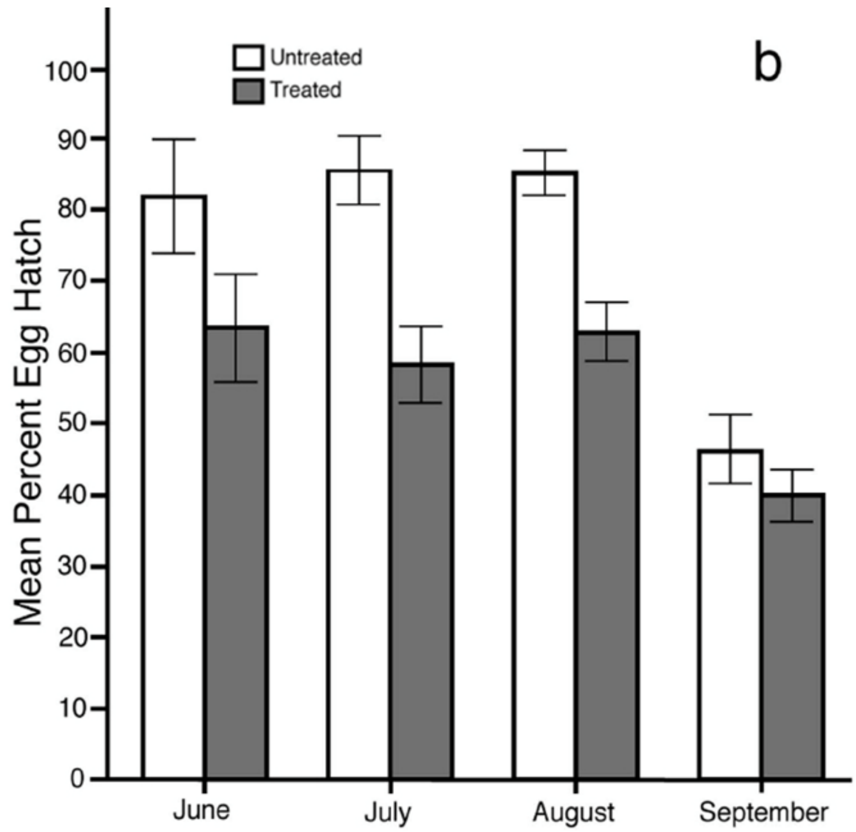
HOW TO USE THIS RESOURCE

Show the following figure and caption to your students. The accompanying Student Handout provides space below the image caption for Observations, Notes, and Questions and space next to the "Background Information" for Big Ideas, Notes, and Questions. The "Interpreting the Graph" and "Discussion Questions" sections provide additional information and suggested questions that you can use to prompt student thinking, increase engagement, or guide a class discussion about the characteristics of the figure and what it shows.



***Caption**: Percentage of* Aedes albopictus *mosquito eggs that hatched at sites where male mosquitoes infected with* Wolbachia *bacteria were released (gray bars) and at sites where the mosquito population was left untreated (white bars). The error bars represent 95% confidence intervals (CI).*

BACKGROUND INFORMATION

Some mosquito-borne pathogens, like the dengue, chikungunya, and Zika viruses, cause diseases for which there are not yet effective treatments or vaccines. Zika virus in particular has spread to more than 80 countries to date, making it a global concern. Zika can cause a debilitating illness of the nervous system called Guillain-Barré syndrome and, when a pregnant woman is infected, severe birth defects including microcephaly. An increasingly common approach to preventing the spread of these diseases is to target mosquito fertility rather than use insecticides, which can have negative environmental effects. One of these techniques involves using the naturally occurring *Wolbachia* bacteria that infect mosquitoes, making them, in some cases, infertile. When male mosquitoes are infected with *Wolbachia*, they must mate with an infected female to produce viable embryos. If they mate with an uninfected female, the early embryo is unable to go through mitosis after fertilization. Consequently, in the field, after mating with a *Wolbachia*-infected male, an uninfected wild female will lay eggs that never hatch. This is called cytoplasmic incompatibility.

In this study, researchers tested whether releasing *Wolbachia*-infected male *Aedes albopictus* (Asian tiger mosquitoes) would suppress populations of this mosquito species in their study area. First, they infected male mosquitoes bred in the lab with a strain of *Wolbachia* known to cause this type of infertility. They worked with homeowners in the neighborhood (a suburban area of Lexington, Kentucky) and the Environmental Protection Agency (EPA) to carefully select the study area where they could release the infected mosquitoes. Each week for 17 weeks, they released 10,000 *Wolbachia*-infected male mosquitoes. The number of released mosquitoes outnumbered the native male population 10 to 1, a ratio expected to allow the released males to outcompete the native males for mating opportunities. The researchers collected adult mosquitoes and eggs at 15 sites where infected mosquitoes were released as well as at 11 sites nearby that were not treated with infected mosquitoes. The hatch rates of the eggs were then observed to assess the impact of the treatment on mosquito fertility.

INTERPRETING THE GRAPH

The figure shows the mean percentage of eggs that successfully hatched during a particular month at the treated sites (gray bars, where infected males were released) and at the untreated sites (white bars, where no infected males were released). For June, July, and August, the error bars, representing the 95% confidence intervals, do not overlap between treated and untreated sites, while for September, the error bars do overlap. The confidence intervals mean that there's a 95% probability that the actual percentage of eggs hatched lies within the range indicated by the bars. A chi-square analysis presented in the publication (not shown) provided evidence that the hatch rates at treated sites were significantly lower than at untreated sites for June, July, and August ($p < 0.0003$, $p < 0.0001$, and $p < 0.0001$, respectively), while the difference in hatch rates for September was not significant. The data show that eggs collected from the treated sites hatched at significantly lower rates than those collected from the untreated sites during June, July, and August, suggesting that the treatment has the potential to suppress mosquito populations by impacting fertility. In September, there was no significant difference in egg hatch rates. In the study, the researchers explain that mosquitoes begin to go through diapause at that time, and therefore the hatch rates of eggs are naturally lower at both the experimental and control sites.

Teacher Tip: Prompt your students to explain the parts of the graph as applicable:

- Graph type: Bar graph
- *X*-axis: Month
- *Y*-axis: Mean percentage of collected mosquito eggs that hatched
- Error bars: 95% confidence intervals

DISCUSSION QUESTIONS

- Describe the differences in the percentages of eggs hatched between treated and untreated sites.
- During which months do you see the greatest impact of the treatment? Why do you think that is?
- Why is it important to include untreated sites in this study?
- Describe what the error bars mean in this figure. How do you interpret the differences between treated and untreated sites based on the error bars?
- What type of statistical analysis would convince you that the decrease in the mean percentage egg hatch in the months of June, July, and August was statistically significant?
- Why do you think the researchers chose to release only male mosquitoes instead of both males and females?
- Why didn't the release of infected males result in zero eggs hatched at the treated site?
- If this site was left untreated the following year, predict what this same graph might look like.

- Based on these results, would you recommend treatment with *Wolbachia* as a way to reduce *A. albopictus* mosquito populations? Why or why not?
- What concerns might scientists or the public have with the release of male mosquitoes treated with a laboratory strain of *Wolbachia*?
- How could this approach to suppressing mosquito populations impact the spread of Zika or other mosquito-borne human pathogens?

SOURCE

Figure 2b from:

Mains, James W., Corey L. Brelsfoard, Robert I. Rose, and Stephen L. Dobson. "Female adult *Aedes albopictus* suppression by *Wolbachia*-infected male mosquitoes." *Scientific Reports* 6, 33846 (2016). https://doi.org/10.1038/srep33846.

CREDITS

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