Insects: The *Wolbachia* Story

Sir: I saw a program on the TV where scientists are growing and releasing millions of mosquitoes outside. Why would they do that? A bacterium was mentioned

Image by CDC, Professor Frank Collins

Jessamyn Perlmutter and Robert Unckless

Department of Molecular Biosciences, University of Kansas, USA

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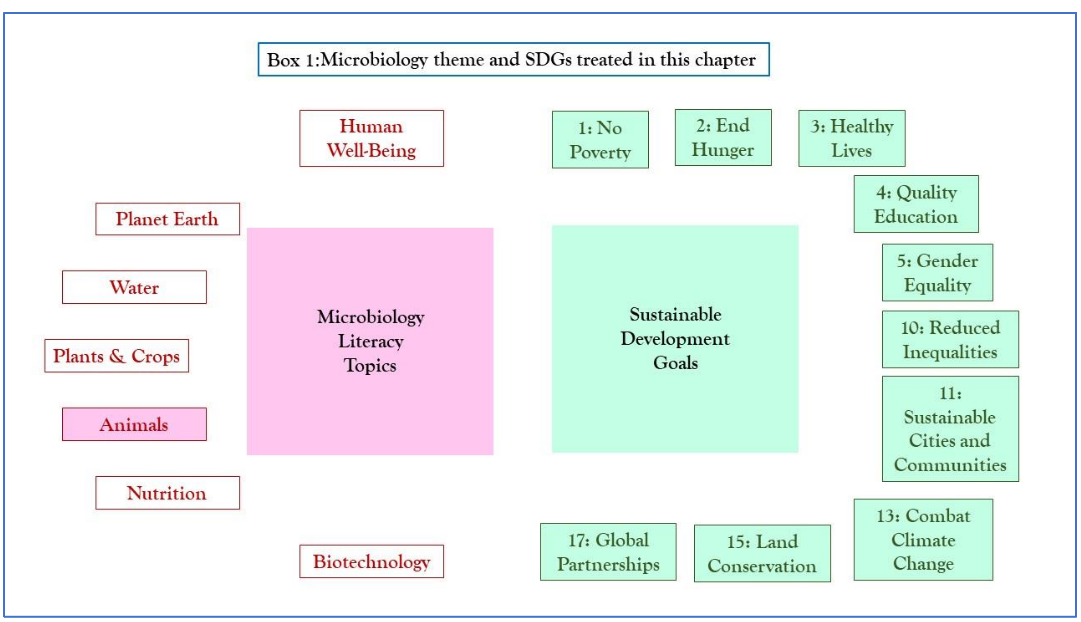
Storyline

Sometimes known as "the world's greatest pandemic", *Wolbachia* bacteria in arthropods are one of the most widespread microbial symbionts on the planet. In fact, they are found in around half of all arthropods, and many nematode species as well. These microorganisms primarily infect the reproductive organs of their hosts, and are vertically inherited from mother to offspring. *Wolbachia* have cunning ways to hijack the reproduction of their hosts to spread themselves in the population, and some strains can even block transmission of viruses that cause diseases in humans, like Zika and dengue. In fact, large efforts are in place around the world to release *Wolbachia*-infected mosquitoes to replace wild populations that have no *Wolbachia*, thereby reducing viral disease transmission to humans. In addition, some pathogenic nematode species need *Wolbachia* to survive, so efforts are underway to develop drugs to kill *Wolbachia*, thus killing the nematodes that cause devastating human diseases. Amazingly, there is now research suggesting a new frontier for *Wolbachia* in agriculture, by protecting crops from viruses spread by insects. Use of these *Wolbachia*-based strategies for arthropod and nematode control contributes to many Sustainable Development Goals (SDGs).

The Microbiology and Societal Context

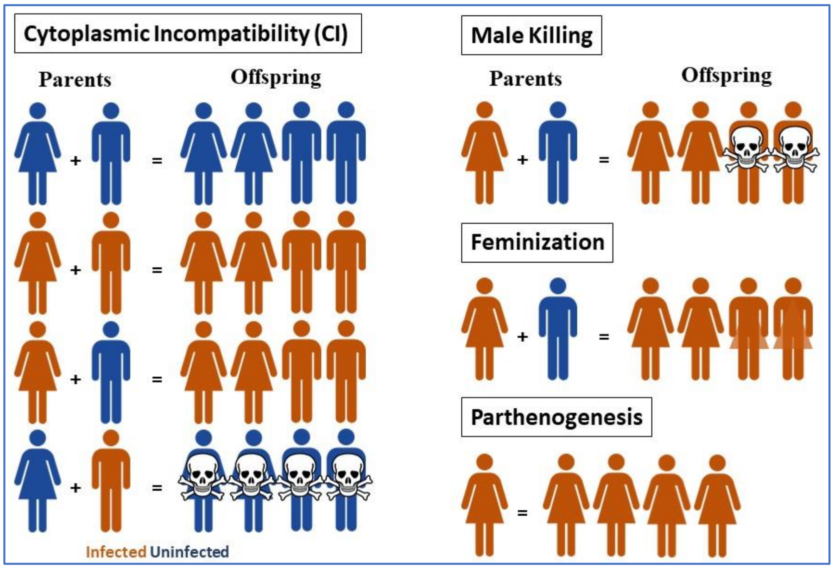
The microbiology: spread of the world's greatest pandemic; arthropod sex manipulation; multipartite and context-dependent host-microbe symbiotic interactions; microbe-induced behavioral changes, reproductive isolation, or extinction; arthropod vector and pest control; river blindness, heartworm, elephantiasis and nematodes; protection of plant crops from infection.
Sustainable Development Goals (SDGs): poverty and equality; hunger and crop health; healthy lives and communities; education and partnerships across the globe; combat effects of climate change



Wolbachia: The microbiology

1. **Wolbachia *are the world's most widespread endosymbionts.*** *Wolbachia* are bacteria that live inside the cells of their hosts (endosymbionts), and are inherited primarily from mother to offspring, a process known as vertical transmission. They are vertically transmitted to offspring inside of eggs, but are not passed through sperm. Since they are spread through the mothers and not through fathers, males are a dead end for the bacteria. Therefore, the fitness of the female host and bacteria go hand in hand; when the female is doing well, so are the bacteria. Fascinatingly, the bacteria will sometimes take things even further and manipulate the reproduction of the host to ensure greater spread in a process called "reproductive parasitism" (see 2). In other cases, the host will become dependent on the microbe, ensuring that all offspring will inherit the bacteria. With a plethora of effective strategies available to *Wolbachia*, these microbes have become incredibly successful at spreading themselves through and across populations. On rare occasions, the *Wolbachia* can even horizontally transmit, meaning they will spread to another host strain or species rather than from parent to offspring, through routes such as eating or preying on. Indeed, it is estimated that these master manipulators can be found in 40-52% of all arthropod species on the planet, and even some filarial nematodes.

2. **Wolbachia *can manipulate arthropod host reproduction.*** Imagine a world where a bacterial pandemic has taken over the human population. This bacterium turns men into women, clones women, or even outright kills just the men. While this is a fictional scenario for humans, it is in fact a reality for the arthropod world. Bacteria like *Wolbachia* will hijack host reproduction to ensure that they are spread to the next generation in a process called reproductive parasitism. There are four main types of parasitism, each of which can be caused by some strains in some hosts. The most common type is cytoplasmic incompatibility (CI). In this method, offspring die when the father has *Wolbachia* but the mother does not. However, if the mother has *Wolbachia*, regardless of the father's status, the offspring live. In a population with individuals that either do or do not have the infection, this gives the infected females an advantage since they can have healthy offspring no matter what, while uninfected females may not. Over time, this could lead to infected individuals outcompeting non-infected individuals in an arthropod population. The second type is male killing, which is exactly what it sounds like. When a mother has a male-killing strain of *Wolbachia*, most or all of her sons will die while her daughters will survive. The third is feminization, where genetically male offspring (containing XY chromosomes, for example) will instead physically develop and reproduce as females. The fourth form is parthenogenesis, which happens when females have female offspring without sperm present; in other words, the females clone themselves and reproduce asexually. One common theme ties all of these manipulations together: they all benefit infected, transmitting females. In each case, the *Wolbachia* are increasing the proportion of infected transmitters in the population, facilitating their own spread.



Box 2. Reproductive parasitism. Adapted from Werren lab.

3. **Wolbachia*-host interactions span a wide spectrum of symbioses*.** While *Wolbachia* are infamous for hijacking reproduction, they also exhibit an array of other symbiotic interactions with other hosts, and exhibit important principles of "multipartite" symbioses, which are relationships between more than 2 organisms. Although many *Wolbachia* are parasites, some are instead considered mutualists, meaning that they have mutually beneficial relationships with their hosts. Mutualism is the case in some arthropods like bedbugs, where the *Wolbachia* provide essential nutrients to the host, and also all *Wolbachia* strains of filarial nematodes. Indeed, the nematodes cannot reproduce without *Wolbachia*, as eggs are either not formed or do not develop without the symbiont there to help. In addition, some of this is context- or strain-dependent. Some strains are capable of parasitism or not, and only in some hosts or environmental contexts. Even parasitic strains are sometimes beneficial in certain ways. Some can help increase the egg-laying of females, for example. Finally, *Wolbachia*-host symbiosis is unique in the animal world since it involves a critical third partner: phages (Figure 1). Phages are viruses that infect bacteria, and *Wolbachia* has a phage called phage WO. This phage has its own DNA, and some of these genes are actually responsible for producing some of the factors that allow *Wolbachia* to hijack reproduction. It is a rare case where a bacterium, a phage, and an animal are all interacting with each other.

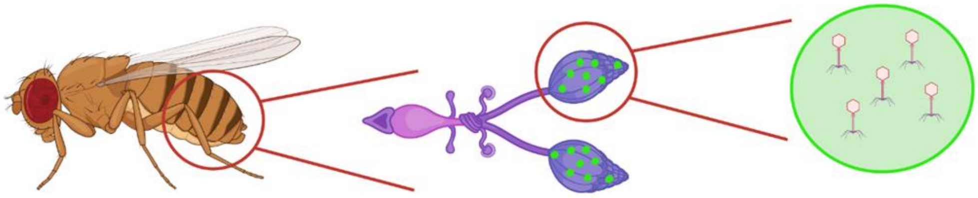


Figure 1. Arthropods, *Wolbachia*, and phages co-exist in a multipartite symbiosis. In this example, a female fruit fly contains *Wolbachia* in her ovaries (female reproductive tract shown, ovaries are dark purple bulbs, *Wolbachia* are green circles). Within the *Wolbachia* are phage WO virus particles (hexagonal shapes with tails). This nested symbiosis of phages in bacteria in an arthropod is similar to Russian Matryoshka dolls that nest inside one another. Image created in Biorender.com.

4. **Wolbachia *shape host behavior and evolution.*** Since *Wolbachia* has such striking impacts on some arthropod populations, the behavior and evolutionary trajectory of these animals can be driven in unique ways by *Wolbachia*. For example, in populations of butterflies with male-killing *Wolbachia*, the population can reach extremes of 95% females or more. Normally, with equal male and female numbers, males will exhibit mate-attracting behaviors and females are picky about preferable male characteristics. This reverses with male-killing, where the scarcity of males forces females to try to attract males and males can be picky about female characteristics. This pickiness is expected to change the proportion of preferable female characteristics in the population over many generations. In other cases, the infection can lead to reproductive isolation and altered mating behaviors. For example, two closely-related fly species *Drosophila recens* and *Drosophila subquinaria* exhibit this pattern. *D. recens* are infected with CI *Wolbachia* and *D. subquinaria* are not. In regions where the populations overlap, if an infected *D. recens* male and an uninfected *D. subquinaria* female have offspring, this would kill their offspring due to CI. Intriguingly, the *D. subquinaria* females have adapted to exhibit a preference for uninfected males, while the *D. recens* females that would not lose their offspring to any male do not exhibit any preferences. In other populations, this preference can compound over long periods of time to ultimately separate populations into two separate species that can no longer interbreed.

5. **Wolbachia *are at the forefront of arthropod control initiatives.*** Some kinds of arthropods are responsible for spreading human disease, and others act as pests. Mosquitoes spread many deadly viruses such as dengue, Zika, chikungunya, and yellow fever viruses. These viruses cause diseases that infect millions every year and kill tens to hundreds of thousands annually. However, a key discovery related to *Wolbachia* may help change that. When an insect is infected with certain strains of *Wolbachia*, the bacteria actually block transmission of the viruses. For example, if a mosquito carries *Wolbachia*, it will have low to no transmission of viruses when biting humans (https://www.youtube.com/watch?v=ut2UxF5gEDI). The abilities of *Wolbachia* to block pathogens and spread itself in a population with reproductive parasitism form the basis of major vector control initiatives around the globe. Several groups such as the World Mosquito Program, Google's parent company Verily, and MosquitoMate have developed facilities, partnerships, and strategies to release millions of *Wolbachia*-infected mosquitoes into the wild to either crash populations or replace them with disease-resistant individuals.

There are two different approaches depending on the desired outcome and circumstances: population suppression or population replacement. Population suppression can happen when millions of infected *males* are released into a wild uninfected population, thus causing offspring death via CI. Over time, fewer and fewer offspring will survive until the population is gone. Note that male mosquitoes do not bite and therefore are not dangerous to humans. This may be helpful when the species is non-native/invasive and unlikely to reestablish itself in that location, or generally in the case of insect pests where eradication is desired. This is sometimes called the "incompatible insect technique (IIT)". The other form is population replacement, which happens when millions of infected *females* are released into a wild uninfected population, where CI takes over to help spread the infection to 100%. You can explore the World Mosquito Program website to view a video on how the initiative works, and how CI helps spread the mosquitoes (https://www.worldmosquitoprogram.org/en/work/wolbachia-method/how-it-works). This strategy in particular is considered highly sustainable since the mosquitoes propagate themselves in the wild, rather than killing the population, and studies show that they maintain their infection and numbers over many years so far. In fact, the incidence of dengue in many trial areas has gone down by as much as 97% at 9 years post release according to the World Mosquito Program.

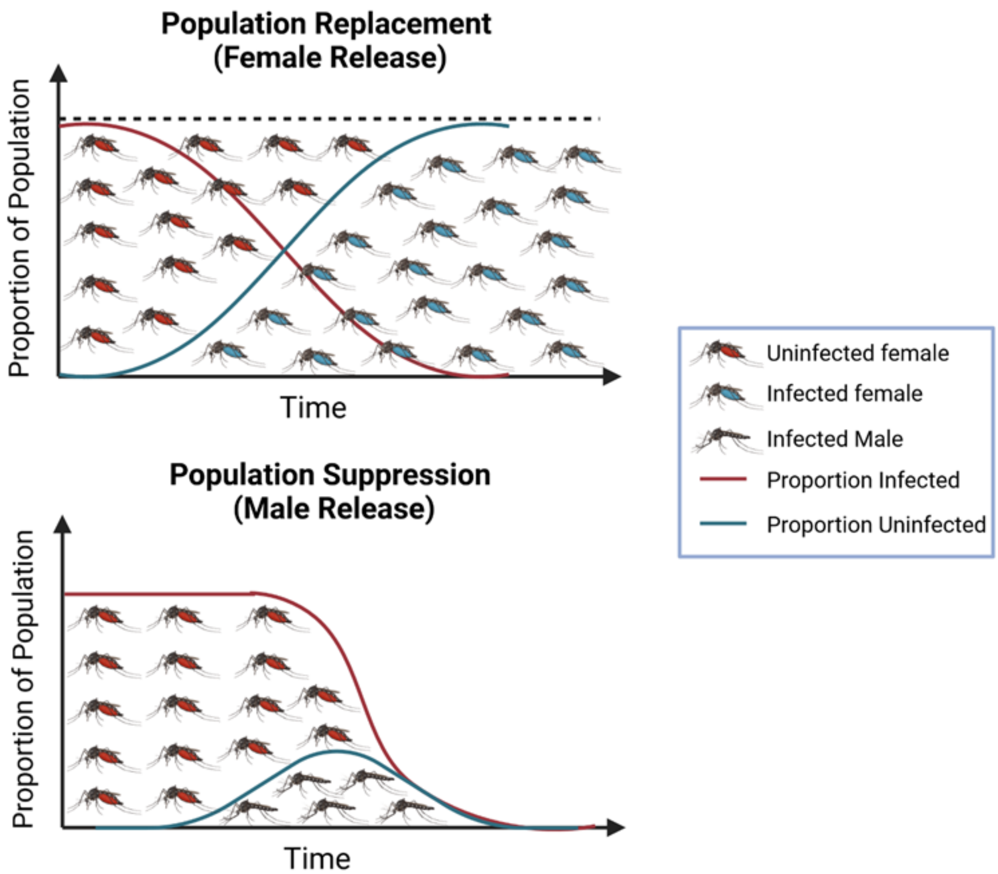


Figure 2. Population replacement and suppression strategies. Population replacement (top) involves large releases of infected females that then outcompete the uninfected wild females. The infected females will have infected male offspring that then induce CI and kill any further offspring they have with uninfected females, reducing the numbers of uninfected individuals in the population over time while infected individuals continue to reproduce. Population suppression (bottom) involves large releases of infected males. The infected males induce CI when they have offspring with wild uninfected females, but do not pass on *Wolbachia* themselves, reducing the entire population size over time. Figure made in Biorender.com, modified from James et al., *Am J Trop Med Hyg*, (2018) *98*, 1-49.

6. **Wolbachia *can help us treat deadly and debilitating filarial diseases.*** Many diseases around the world are caused by filarial nematodes. These parasites spread by blood-feeding insects like black flies and mosquitoes and primarily exist in subtropical parts of the world. These insects breed in bodies of water like rivers, and thus the diseases they spread often occur near sources of water. In the case of river blindness, an infected black fly will spread nematode larvae to humans through a bite. Once inside a human that has been bitten, the nematodes grow into worms in the skin. The adults then reproduce in large numbers and offspring spread through the body. This causes many

symptoms like skin rashes, itching, and inflammation. Importantly the offspring can travel to the eye, where the infection can cause blindness largely through induction of a strong human inflammatory response due to the presence of so many nematodes, including many that die and decompose. Indeed, the disease is called onchocerciasis, aka river blindness, due to it symptoms and proximity to rivers. River blindness affects millions of people around the world, mostly in Africa.

Mosquitoes carry different nematodes that also spread to humans when bitten. These nematodes cause a disease called lymphatic filariasis (aka elephantiasis), that also affects millions around the world. In this disease, the nematodes reproduce in the lymphatic system, where they grow and sometimes die in large numbers. Massive swelling can result from a strong human immune response to both the nematodes and the *Wolbachia* bacteria that dying nematodes shed. This swelling can make limbs look elephant-like, hence the name (Figure 3).

However, once again, *Wolbachia* may be the key to treating these diseases. Since these nematodes have mutualist *Wolbachia* and therefore cannot live without the bacteria, we can treat these diseases with bacteria-targeting drugs. In fact, the antibiotic doxycycline is commonly used to clear the nematodes indirectly by killing the *Wolbachia* they need. Even heartworms in animals can be treated using antibiotics for the same reason. Between *Wolbachia*-targeting antibiotics and other drugs that target the nematodes themselves, such as ivermectin, the treatments available are very effective in curing and preventing these infections. The A-WOL (anti-*Wolbachia*) group is dedicated to developing more *Wolbachia*-targeting medicines to better treat filarial diseases around the globe.

Figure 3. Man in the Philippines with lymphatic filariasis, aka elephantiasis, in his legs.
Photo credit: Centers for Disease Control and Prevention Public Health Image Library #373.

7. ***The future of the* Wolbachia *frontier: Plant and crop protection.*** Just as mosquitoes can carry viruses and spread disease to humans, so can other insects carry pathogens and spread them to plants. In fact, plant pathogens are responsible for large crop losses every year across the globe and contribute to hunger and other food inequalities. One such case is planthoppers that carry Rice ragged stunt virus, which is a destructive pathogen of rice plants (Figure 4). One group recently discovered that *Wolbachia* can block these viruses too, and plans are underway to release *Wolbachia*-infected planthoppers to replace virus-susceptible planthoppers in the wild, and save the rice plants. This is only the beginning, and *Wolbachia*-based plant pathogen blocking may become a major agricultural game changer in the future. This is in addition to potential uses for *Wolbachia* to crash pest populations with CI, such as the invasive medfly (see "Fruit flies" topic).

Figure 4. Rice plants infected with Rice Ragged Stunt Virus (RRSV). Plants develop twisted leaves (left) and an overall "ragged" appearance (right). Photo credits: International Rice Research Institute.

Relevance for Sustainable Development Goals and Grand Challenges

The use of *Wolbachia* in arthropod vector and pest control, and its role in treatment of filarial diseases, relate to largely positive impacts on several SDGs (microbial aspects in italics), including

- **Goal 1. End poverty in all its forms everywhere** (*reduce disease burden and medical costs on the poor, increased educational and work productivity and opportunities*). Medical treatment for arthropod-borne diseases can be expensive, even costing multiple times a family's monthly income. This is a particularly pressing issue for those in poor communities closer to the equator that are hard-hit by mosquito-borne diseases, and poorer individuals who may not have access to clean sanitation. In addition, filarial diseases are common in poorer parts of the world, and using medications to kill *Wolbachia* and the nematodes helps these communities as well. Further, the time spent resting and recovering can reduce work earnings or impede education. Every year, developing economies lose billions of dollars to the cost of tropical diseases including the ones blocked by or treated via *Wolbachia*. The role of *Wolbachia* in blocking these diseases will help reduce financial hardship on individuals and communities across the globe.

- **Goal 2. End hunger, achieve food security and improved nutrition and promote sustainable agriculture** (*increase agricultural productivity, sustainable agriculture*). Recent efforts to extend the use of *Wolbachia* to plants and agriculture have introduced new, safe, and sustainable methods for protection of crops. Many crops are lost to pests or the plant pathogens that pests carry, and elimination of the pests themselves or introduction of *Wolbachia*-infected arthropods that block plant pathogen transmission will help recover crops that would have otherwise been lost. This is a safe, cost-effective, sustainable method (see Goals 11 and 15 for more information).
- **Goal 3. Ensure healthy lives and promote well-being for all ages** (*end neglected tropical diseases and filarial diseases*). The diseases that *Wolbachia*-infected mosquitos block are largely considered neglected tropical diseases, which are diseases that disproportionately impact tropical and poorer regions of the world where few resources are available and research investment is lower. The Zika, dengue, chikungunya, and yellow fever viruses are all carried by mosquitoes. They infect hundreds of millions of people each year and kill hundreds of thousands. The filarial diseases caused by *Wolbachia*-carrying nematodes also have a wide reach. As for filarial diseases, onchocerciasis (river blindness) affects tens of millions of people in dozens of countries mostly in Africa, and lymphatic filariasis (elephantiasis) affects over 100 million people each year across dozens of countries as well (https://www.who.int/tdr/diseases-topics/en/). Treatment of these diseases by *Wolbachia*-targeting drugs (such as the doxycycline antibiotic) or prevention through mosquito releases has the potential to save millions of lives, and significantly improve the quality of life for many millions more.
- **Goal 4. Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all** (*The* Wolbachia *Project*). A group of *Wolbachia* scientists runs The *Wolbachia* Project: Discover the Microbes Within. This is an integrative lab series that brings real scientific research to middle, high school, and college classes through engagement with nature. Students find arthropods, extract their DNA, sequence the DNA, and use computational techniques to analyze *Wolbachia* sequences. The program provides free loaner equipment, materials, and support to classrooms across the US, and also supports classrooms globally. Explore information and materials here: https://www.vanderbilt.edu/wolbachiaproject/.
- **Goal 5. Achieve gender equality and empower all women and girls** (*reduce anomalies in pregnancy, reduce sex-biased disease severity*). Recent research suggests that girls are at higher risk of severe symptoms and death from diseases like dengue and Zika. In addition, pregnant women infected with Zika may give birth to children with various health conditions like microcephaly, which can be a serious disability and the child will require additional care and incur additional medical costs. Further, it is usually women who care for those who are sick, for both mosquito-borne and filarial diseases, costing them time and earning potential. Prevention of the spread and incidence of these diseases with *Wolbachia* will especially

improve the health of girls and women while also reducing financial costs on mothers and female carers.

- **Goal 10. Reduce inequality within and among countries** (*reduce economic burden on poor communities and women*). As covered in Goals 1 and 5, reducing infections with *Wolbachia*-infected mosquitoes and treating filarial diseases will largely help poor communities around the globe, lifting a significant economic and time burden from them. This will, by extension, allow for greater growth and prosperity in these areas.
- **Goal 11. Make cities and human settlements inclusive, safe, resilient and sustainable** (*reduce diseases in urban environments, cost-effective*). Mosquitoes thrive in dense urban environments where humans are available to bite and standing pools of water in gutters, drains, ditches, swimming pools, or other locations are readily available for reproduction (they lay offspring in water). Dense human populations also allow viruses to easily be transmitted to many people. Compared to other disease mitigation efforts, *Wolbachia*-infected mosquito releases are calculated to be cost-saving in most urban locations. This is especially true for the population replacement methods, where the *Wolbachia* will propagate itself among the local mosquito populations and not require further time or monetary investments after the releases beyond monitoring.
- **Goal 13. Take urgent action to combat climate change and its impacts** (*reduce disease impact of climate-induced changes in mosquito behavior and physiology*). Climate change is expected to increase the global risk and incidence of diseases spread by mosquitoes such as dengue. This is due to rising temperatures, which are beneficial to both the mosquitoes themselves, and the viruses they carry. Mosquitoes will have altered reproduction and biting rates at higher temperatures, and could expand their geographical ranges as temperatures rise in currently mosquito-free areas. Increased occurrence of the diseases they carry is therefore an expected outcome of climate change. By 2080, an additional 2 billion people are estimated to be at risk of infection from rising mosquito numbers and geographical ranges. Reducing mosquito populations or replacing them with *Wolbachia*-infected populations will help reduce this impact even if mosquito range, numbers, and behaviors change.
- **Goal 15. Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss** (*reduced pesticides, save crops from diseases*). Currently, agriculture heavily relies on pesticides that can heavily impact the health not only the environment, but animals and insects beyond the target pests, and even humans (see Agrochemicals, Plants and Insects, and Plant Protection topics for more detail). These pesticides can have damaging and cascading effects on the entire ecosystem. *Wolbachia* provides a natural alternative that is relatively well contained. As the so-called "world's greatest pandemic", *Wolbachia* already exist in an estimated half of all arthropod species on planet Earth, so introduction of the mosquitoes does not bring anything to the environment that was not already there, they are simply introduced in larger numbers. With the ability to either eliminate pest populations or replace populations with arthropods containing plant-disease-resistant strains of *Wolbachia*, this provides a cost-effective method to save crops and avoid extra pollutants in the environment. However, there will be relatively small amounts of indirect pollution from the large facilities that produce the mosquitoes, vehicles transporting them, etc.

- **Goal 17. Strengthen the means of implementation and revitalize the global partnership for sustainable development** (*global science, government, NGO, and community participants*). The effort to grow and release mosquitoes around the world has involved a huge public health effort with partnerships from individual to international levels. Mosquito releases are never done without rigorous testing and thorough evaluation, along with adhering to numerous local and international laws, obtaining permits, communications with funders, and knowledge and acceptance from local communities. This involves a strong network across many levels, from citizen involvement in releases to discussions with community leaders, approval processes with governments, partnerships with NGOs, and beyond. Further, the anti-*Wolbachia* (AWOL) team that focuses on filarial diseases also exemplifies global partnerships. They include a diverse range of scientists who research and create new drugs to fight filarial diseases by targeting *Wolbachia* along with numerous academic and industrial partnerships across the globe.

Potential Implications for Decisions

1. Individual

a. Household or business mosquito mitigation measures in release vs non-release areas (Which methods to use? killing them in a release area could reduce the effectiveness of the campaign, other measures like reducing standing water pools around the home may be better)

b. Travel awareness (Vaccines ahead of time when traveling? Travel to areas with endemic nematodes? Availability of treatment drugs in endemic areas?)

2. Community policies

a. Partnership with *Wolbachia* release campaigns (Population suppression or replacement? Approvals from all parties? Consideration of costs, environmental effects? Which partnership organizations or institutions? Community engagement?)

b. Participation in *Wolbachia* Project (Changes to classroom curricula?)

c. Pest control campaigns (Releases of infected pests to block plant diseases? Suppressing pest populations instead?)

d. Local environmental effects (pollution from production, space needed for infrastructure, potential ecological effects)

3. National policies

a. Research funding (increase funding for basic research and development of *Wolbachia*-based tools and treatments? Prioritize funding and infrastructure for mosquito releases, filarial drugs, or pest control?)

b. Arthropod release laws (Allow releases of infected arthropods? Suppressing or population replacement? What requirements should a release permit have? What requirements should there be to monitor? What environmental or ecological concerns are there compared to alternatives? Build infrastructure for rearing locally? Which agencies should be involved? How much financial support should be provided? What timelines are acceptable? Which local experts and leaders should be involved? What level of acceptance by local communities is adequate to permit releases? Use other approaches in tandem? Etc.)

c. Healthcare costs, coverage, and infrastructure

d. Indirect environmental consequences (building of infrastructures for arthropod rearing, pollution from distribution of arthropods via vehicles, etc.)

e. Effects of various approaches on gender/other inequalities and poverty

Pupil Participation

1. Class discussion of the issues associated with arthropod-borne diseases and* Wolbachia*-based strategies to combat disease spread (both mosquito-based and filarial nematodes)

2. Pupil stakeholder awareness

a. *Wolbachia*-based arthropod control has largely positive impacts on SDGs. Which SDG will be the most impacted and why? Which one is most relevant to you and why?

b. What would be some positive and negative consequences of arthropod releases?

c. Would you want releases in your area? What information would you want to know to make a decision?

3. Exercises

a. Model how an arthropod population changes over time using the class as an example. Verbally communicate or give everyone a random card with a sex (male/female, equal numbers) and a *Wolbachia* status (infected/uninfected, equal numbers). This should result in a quarter of the students being uninfected males, a quarter infected males, a quarter uninfected females, and a quarter infected females. Have them pair up with another person in the class with the opposite sex assignment, and ask them to determine if their offspring would survive if the *Wolbachia* were a CI strain. Ask those with dead offspring to sit. For those whose offspring live, have them determine if the offspring would be infected or uninfected. As a class, record the overall infection frequency of the offspring generation from the students' answers (assume 2 offspring per pair), and compare to the starting infection frequency. Either stop the exercise here or carry on for additional generations by giving student new assignments (equal male/female numbers, and *Wolbachia* infection frequency that was calculated for the offspring generation). Ask the class to explain why the infection frequency has changed, and what will happen if they continue each generation.

b. In some cases, population replacement strategies are ideal, while in others, arthropod population suppression is ideal. What are the advantages and disadvantages of each in various scenarios such as invasive pest species vs native mosquito populations? What are the ethical and environmental considerations?

c. What could be done to mitigate the environmental impact of building infrastructure and driving vehicles to release mosquitoes?

d. In areas where *Wolbachia* releases may not be 100% effective, what other sorts of approaches might work well alongside *Wolbachia*-based methods? Consider pests vs disease vectors, and the environmental impacts of additional approaches.

e. What can be done in areas with endemic nematode populations to help treat infected individuals? What kinds of infrastructure and support are necessary? What can be done to prevent infections?

The Evidence Base, Further Reading, and Teaching Aids

General Wolbachia knowledge

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Wolbachia and Heartworm: Why Doxycycline Is Needed in Heartworm Treatment: https://todaysveterinarypractice.com/ahs-heartworm-hotlinewolbachia-heartworm-doxycycline-needed-heartworm-treatment/
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AWOL: https://awol.lstmed.ac.uk/target-diseases

Glossary

Antibiotic: a medicine that inhibits the growth or kills microorganisms, especially bacteria
Anti-*Wolbachia* (A-WOL): a consortium of academic and industrial partners funded by the Gates Foundation to develop new drugs against filarial diseases
Arthropod: an invertebrate animal with an exoskeleton, segmented body, and jointed appendages including insects, spiders, and crustaceans
Asexual: not involving sexual activity, without sex or sexual organs, not involving the fusion of gametes (sperm and egg)
Bacteria: unicellular microorganisms with cell walls, no organelles, and a nucleus
Chikungunya: a viral disease transmitted by the chikungunya virus, causing fever and severe joint pain, among other symptoms
Cytoplasmic Incompatibility (CI): a phenomenon when sperm from a *Wolbachia*-infected father and an egg from an uninfected mother results in inviable offspring
Dengue: a debilitating tropical viral disease transmitted by mosquitoes, causing fever and joint pain
DNA: (deoxyribonucleic acid) a self-replicating material in almost all living organisms that carries genetic information, aka the blueprint for life
Doxycycline: a broad-spectrum antibiotic
Elephantiasis: (aka lymphatic filariasis) a condition where a limb or other body part is grossly enlarged due to infection of lymphatic vessels by filarial nematodes that are spread by mosquitoes
Endosymbiont: symbiosis in which one of the symbiotic organisms lives inside the cells of the other
Evolution: process by which different living organisms develop and diversify from their earlier forms over the history of the planet
Feminization: a phenomenon where genetic male hosts physically develop and reproduce as females due to infection by *Wolbachia*
Filarial: of or relating to any of several parasitic round worms
Fitness: capacity of an organism to survive and transmit its genetic content to reproductive offspring, as compared to competitors
Gene: a unit of DNA that is transferred from parent to offspring, and often produces some product or provides some function to the individual
Heartworm: a filarial parasite transmitted to animals such as dogs that infects the heart
Horizontal Transmission: the spread of an infectious agent from on individual to another, usually through direct contact or contact with bodily fluids or shared air
Host: an animal or plant on or in which another organism lives
Incompatible Insect Technique (IIT): an arthropod pest or vector control technique where lab-reared, *Wolbachia*-infected male insects mate with wild, uninfected insects to induce CI and kill offspring
Inflammation: a localized condition when the body becomes reddened, swollen, hot, or painful in response to injury or infection due to the activity of the immune system
Invasive: plants or animals that spread prolifically and harmfully beyond their natural geographical area
Lymphatic Filariasis: see elephantiasis

Lymphatic System: a network of vessels through which lymph travels (lymph: a fluid with white blood cells)
Male Killing: a phenomenon where certain strains of *Wolbachia* specifically kill male hosts
Microorganism: a microscopic organism, such as a bacterium, virus, fungus, protozoan, or archaean
MosquitoMate: a company producing and releasing *Wolbachia*-infected male mosquitoes, as part of the IIT to control disease
Multipartite Symbiosis: a relationship between 3 or more unlike organisms
Mutualist: symbiosis that is beneficial to both organisms invovled
Neglected Tropical Disease: any of several parasitic, viral, and bacterial diseases causing substantial illness in tropical regions that are historically poor and have not received as much research or funding as other diseases
Nematode: a worm of the phylum *Nematoda*, such as a roundworm
Onchocerciasis: see river blindness
Pandemic: a disease prevalent over a whole country or the world
Parasite/Parasitism: an organism that lives in/on another organism of another species (the host), and benefits by deriving nutrients, usually while harming the host
Parthenogenesis: reproduction from an ovum without fertilization
Phage: short for bacteriophage, a virus that parasitizes a bacterium
Phage WO: the specific phage that infects *Wolbachia*
Population Suppression: an arthropod control technique where the method used results in mass death of the target population
Population Replacement: an arthropod control technique where the method used results in a *Wolbachia*-infected population replacing the previous, uninfected population
Reproductive Isolation: the inability of a species to breed successfully with related species due to geographical, behavioral, physiological, or genetic barriers or differences
Reproductive Parasitism: any of four reproductive manipulations (CI, male killing, feminization, or parthenogenesis) that *Wolbachia* bacteria induce to manipulate arthropod host reproduction
Rice ragged stunt virus: a pathogenic plant virus infecting rice plants that is spread via leafhoppers, causing rice ragged stunt disease where grains develop unfilled and plant density is lost
River blindness: a tropical skin disease caused by nematodes carried on blackflies, where the nematodes can also migrate to the eye and cause blindness
Species: a group of organisms consisting of similar individuals capable of exchanging genes or interbreeding
Strain: a genetic variant or subtype of an organism or species
Subtropical: relating to characteristics of regions bordering or adjacent to the tropics
Sustainable/Sustainability: conserving an ecological balance by avoiding depletion of natural resources
Sustainable Development Goals (SDGs): 17 goals set by the United Nations in 2015 as part of an agenda for sustainable development around the world
Symbiont: an organism living in symbiosis with another, often used to refer to an endosymbiont living with a larger host
Symbiotic/Symbiosis: a relationship between two dissimilar organisms

Tropical: of or relating to the tropical region of the globe, characterized partially by high humidity and temperatures
Vector: an arthropod or other organism that transmits diseases from one animal or plant to another
Vertical Inheritance: transmission of genetic or other material from parent to offspring
Virus: a microscopic infective agent with a nucleic acid molecule and a protein coat, living inside a host
Wolbachia: a genus of intracellular bacteria that infects mainly arthropod species and some filarial nematodes
World Mosquito Program: a non-profit initiative protecting communities from mosquito-borne diseases through releases of *Wolbachia*-infected arthropods around the globe
Yellow Fever: a tropical viral disease affecting the liver and kidneys, causing fever and jaundice, transmitted by mosquitoes
Zika: a tropical viral disease causing fever, rash, joint pain, and fetal anomalies, transmitted by mosquitoes