Microbial control of animal behaviour

Miss: I love zombie movies and now I just heard that there are zombie ants – what are these?

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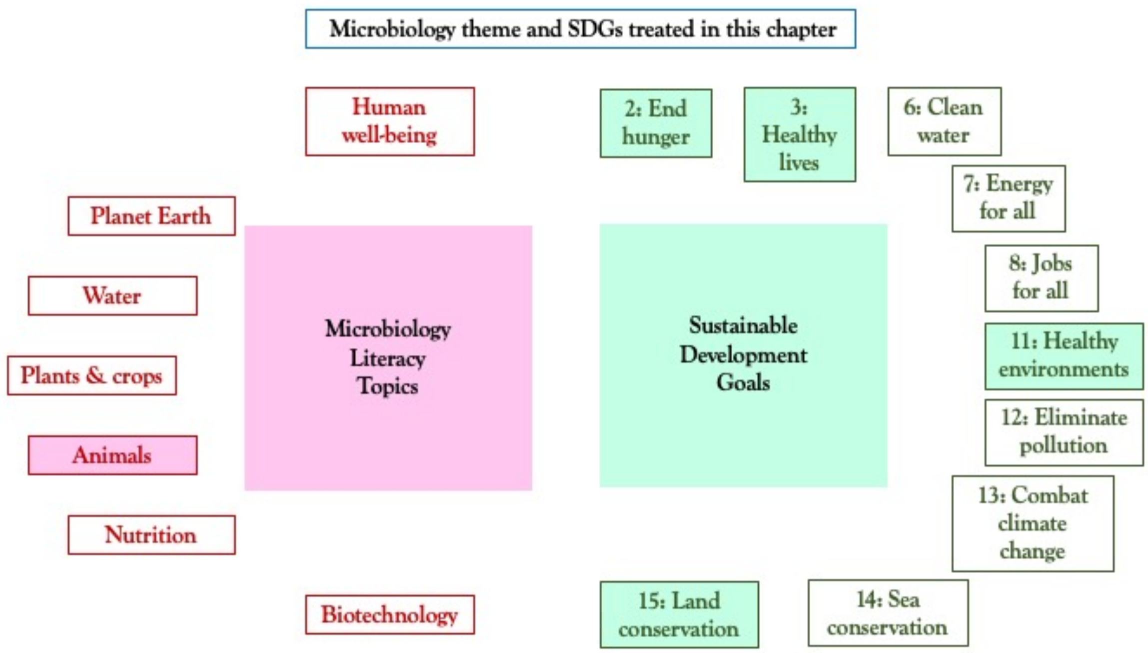
Storyline

Parasites are everywhere! Both larger organisms, such as invertebrates, vertebrates and plants, and microscopically small organisms, such as protozoans, bacteria and fungi, can have a parasitic lifestyle. Some of these parasites, including microbes, can infect animals and control their behaviour. You may think that this is something out of science fiction, and only happens in zombie movies! But, real-life zombie-making parasites most certainly exist! Perhaps, you have heard about zombie ants. These ants are infected with a fungus, that not only "eats" the ants, but also changes their behaviour. As a result, the fungus-controlled ants help the parasite to spread its spores to other ants. Indeed, this is similar to zombies in the movies; they appear to only be motivated to help spread the microbial culprit that infected them and create more zombies! Scientists are incredibly fascinated by the real zombie-making microbes that exist in nature. These parasites are thought to produce interesting new chemicals to interact with their animal hosts, which could lead to the discovery of new medicines. Behaviour-manipulating parasites are also thought of as nature's very own neurologists. Learning how they interact with animal nervous tissue to tweak behaviours will therefore help us understand behaviour and behavioural changes. This information could potentially inform new strategies and therapies to help people with psychiatric and psychological issues. Learning how microbial infections affect animals will also ultimately help us understand their effects on the ecosystems they play a role in. This is especially important if the infected animals are important players in such ecosystems – keystone species or ecosystem engineers, – as is the case with ants. Additionally, the studies into zombie ants and other zombified insects will reveal which neurological pathways are involved in insect behaviour and how those pathways could be interrupted. Such knowledge can be used as ammunition to battle agricultural pests and invasive species.

The Microbiology and Societal Context

The microbiology: the parallels and discrepancies between fictional zombies in pop culture and those found in nature; the impacts of microbial infections on animal behaviour and ecosystem health; microbial production of neuromodulators; microbial take-over of animal biological clocks; future applications in medicine, agriculture and conservation efforts. *Sustainability issues:* human health; global food supply; healthy environments; invasive species and conservation efforts.



Microbial control of animal behaviour: the Microbiology

1. Zombie-making microbes in popular science fiction and in real life. Zombies play a prominent role in pop culture. They are the main subject of video games (e.g., The Last of Us, Zombies Versus Plants), books and movies (e.g., The Girl With All the Gifts, World War Z), and TV series (e.g., The Walking Dead, and iZombie). In these works of science fiction, zombies are often portrayed as "undead" individuals (i.e., living dead) who have been infected by a microbe; often a virus (see the article on the History of Zombies by the History channel and the interview with Joao Araujo, the scientific advisor on the movie The Girl With All the Gifts for a more detailed treatment of this subject). The infection makes individuals forget who they were before and gives them only one goal: attack other humans so the zombie-making microbe can spread. While these popular stories are entirely fictional, they are inspired by real-life microbes that are able to infect animals and hijack their behaviours. For instance, the narrative that infected individuals become "living dead" is completely fictional. In fact, the zombie ants, and other microbe-manipulated animals we find in nature, are very much alive! They are merely called zombies because these infected animals are not in control of their own behaviours anymore; they have been hijacked by the parasite (see the book about Zombie Makers by Rebecca L. Johnson for a more detailed treatment of this subject).

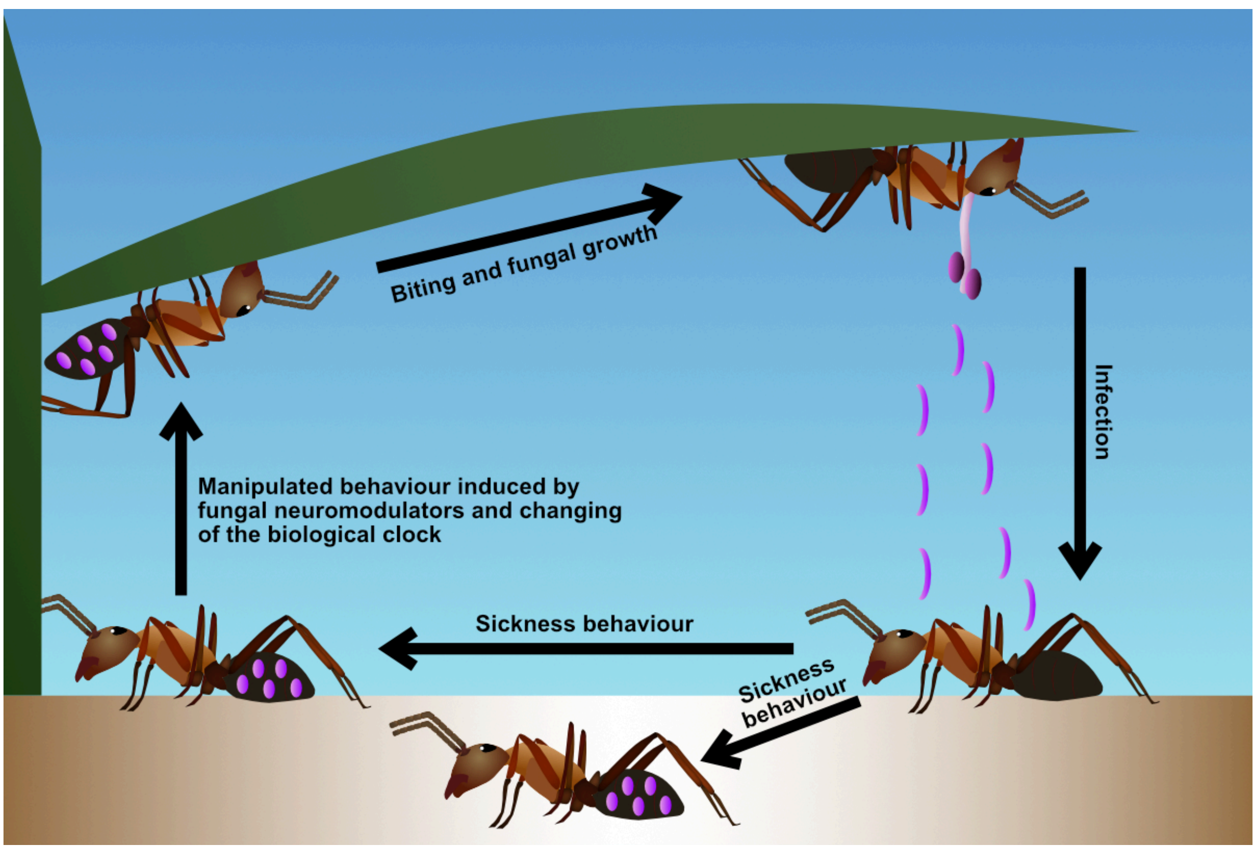
Microbes that manipulate behaviours of their animal hosts can do so as a result of a close co-evolution between the microbe and the animal. For millions of years, there has been an ongoing arms race between the two organisms in which both are evolving to get the upper hand. The microbe evolves to get better at overcoming the animal's immune system and take advantage of its physiological properties to improve its spread to other animal hosts. In turn, the animal evolves abilities to better recognize and fight off the microbial invader and minimize the negative effects on its survival and reproduction (see the links about The Red Queen Hypothesis for a more detailed treatment of this subject). As a result of such a long ongoing battle, certain microbes have evolved the ability to manipulate the behaviours of their animal hosts in a way that the behaviour is fine-tuned to benefit the parasite's survival and transmission. During this time, the microbe "learned" all the ins and outs of its host's brain and how to manipulate it. It has become the ultimate specialist that can pull the strings in the host it co-evolved with, but not in any other animals. Indeed, when we again take the example of the zombie ants, we see that each ant species that gets infected and manipulated has its own specialized species of zombie-making fungus (see the article in WIRED Magazine about The US Having Its Own Species of Ant-Zombifying Fungus for a more detailed treatment of this subject). This makes it highly unlikely that the scenario we see in many fictional zombie stories - a microbe that controls the behaviour of another animal suddenly jumps to humans and causes a zombie apocalypse – could happen in real life. Quite a few years of co-evolution between that microbe and humans would be needed before the microbe could become expert at dealing with and controlling the human brain.

2. General behavioural effects of microbial infections: sickness behaviours. Animal behaviour can also change as an effect of microbial infections that do not involve behavioural control. These types of behaviours are called sickness behaviours. Sickness behaviours are likely the result of the reallocation of energy from baseline physiological processes to processes that deal with the infection, such as immune responses. Additionally, disease (and also therapies) can affect the microbiome, which is thought to have an effect on animal behaviour as well. You might be able to come up with a few sickness behaviours when considering how you felt and behaved when you had a cold or some sort of bacterial infection. You were probably less energetic and chose to sleep rather than do your chores or your homework. You might also have had less of an appetite, which would make eating less of a priority and, in turn, may have upset your mum and dad. Similar to your own experiences, animals, including ants and other insects, behave differently after infection. The daily behaviours of ants are incredibly important for the ant colony to survive. Worker ants within a colony divide the labour: forager ants provide the colony with food, soldier ants protect the colony, and nurse ants take care of the ant queen and her progeny. If a substantial number of ants in one of these behavioural groups gets a microbial infection that affects their behaviour, this will reduce the survival chances of the colony as a whole. Ants are incredibly important players within ecosystems because they are important decomposers (i.e., they use dead organic materials as food) and ecosystem engineers, and they have a large biomass, so are significant members of the food web. Changes in their behavioural ecology resulting from disease could, therefore, have drastic effects for the environments they live in (see the review article about Ants and Their Parasites by C. de Bekker et al., for a more detailed treatment of this subject).

3. Adaptive behavioural effects of microbial manipulators: microbe-controlled behaviours. Spreading from host to host is a very important part of a parasite's life cycle. Any adaptations that make it more likely for a parasite to spread would give it a certain advantage and is, therefore, selected for. One example of such adaptations is microbe-controlled animal behaviour that helps the microbe to spread more easily. This can lead to microbe-induced new behaviours or fine tuning of sickness behaviours, so they benefit the spread of the microbe that is in control. In the zombie ants, for example, carpenter ants that are infected with their specialized species of *Ophiocordyceps* fungus are made to abandon their foraging activities and stray away from the ant colony. This benefits the fungus because this change in behaviour makes it less likely that healthy ants in the colony will attack the infected individual as part of the colony's social immunity. By leading the ant away, the fungus is protecting it to assure survival of the fungus and its production of a fruiting body with infectious spores. When the zombie ant is finally well away from the colony, the fungus induces her to climb nearby plants to a higher position. This will make it easier for the spores to spread and infect more ants! Once the infected ant arrives at a good spot for fungal spread, the fungus makes it bite down with her incredibly strong mandibles, which then stay locked in position to assure that the ant remains in this favourable position. Afterwards, the ant is killed by the fungus, consuming it for the energy it needs to produce the spores needed for spread. The fungus relies heavily on this manipulation to happen. If total control of the ant's behaviour is not achieved, and the ant merely shows sickness behaviours before she dies, the infectious spores needed for the transmission of the fungus are not formed, and the spread to other ants is not achieved (see the article about The Life of a Dead Ant by S. B. Andersen et al., for a more detailed treatment of this subject).

4. Fungi produce neuromodulators to modify the activity of ant brains. A lot of the current research into the microbial control of animal behaviour is focusing on the question of how microbes, which do not have a brain themselves, are able to control animals, which have a brain! One of the strategies used by zombie ant fungi is the production of neuromodulators: molecules that can activate or deactivate neurological processes. *Ophiocordyceps* fungi produce an array of interesting compounds that could have mind-altering effects. Research into these compounds is currently underway, and one type of compound in particular, called alkaloids, could be very interesting. Alkaloids are organic compounds that have pronounced physiological effects in humans and other animals. Caffeine, for instance, is an alkaloid that can be found in tea, coffee or energy drinks. People that consume caffeine generally start to feel more alert, which might cause them to behave more actively or fight the onset of sleep. Which alkaloids the zombie ant fungi exactly produce is currently unknown, and an active part of ongoing research. In addition to alkaloids, other compounds are also produced at the time of behavioural control of the ant. Some of these compounds are completely new with unknown functions. Investigating what they do might lead to new enzyme and drug discoveries that could be developed and tested for medicinal purposes (see the layman's version of an original study that asked How Does a Parasite Create Zombie-Like Behavior? for a more detailed treatment of this subject).

5. Fungi hijack the biological clock of their ant hosts to change the timing of behaviours. Every living organism, including humans, has a biological clock. These biological clocks help the organism and its physiology to effectively anticipate predictable daily changes in the environment, such as light, temperature, the availability of food and the presence of potential predators. Insects, like carpenter ants, also have biological clocks. As a result, the foraging activity of carpenter ants in a colony follows a daily pattern; foraging ants are more active during the night-time compared to the daytime. However, in ants infected with the behaviour-controlling fungus *Ophiocordyceps*, the daily rhythms in foraging activity seem to disappear. Instead, the climbing and biting behaviours that the fungus induces to help spores spread, seem to follow a daily pattern! Eerily, infected ants do not just bite at any given time. They seem to prefer to bite around solar noon. Researchers hypothesize that this is again a way for the fungus to increase its chances of spreading its spores. Biting around a time when fewer healthy ants are around reduces the chance for other ants to interfere by aggressively behaving towards infected individuals. Additionally, fungi need light to produce a fruiting body. So, by making them bite during the daytime, the fungus might be controlling infected ants to seek out a spot with enough light to assure proper fungal development and transmission (see the article about Zombie Ant Fungi Turning Hosts Into Light Seekers by F. S. Andriolli et al., for a more detailed treatment of this subject).



6. The importance of studying fungal parasites and the mechanistic workings of behaviour. With climate changing, fungal infections in plants, animals and humans are on the rise. Despite this, the medical mycology field, which studies and treats fungal infections in humans, is still rather limited. Fungal infections are very difficult to treat without complications and side effects. Because the cells of fungi are fairly similar to those of animals, antifungals often make us sick; they attack both our cells as well as the fungal cells they are meant to kill. A key property of medicines used to treat infectious diseases is *selective toxicity*, i.e. the toxicity of the medicine for the infectious agent – fungus, bacterium, etc. – compared with the toxicity for human cells. Ideally, the medicine should effectively kill the infectious agent but have no effect on us. Selective toxicity is based on differences between us and the pathogen: a medicine with good selective toxicity inhibits an essential activity of the cells of the infectious agent, which is different to the activities of our cells. To identify such differences in fungi, and hence potential targets for new anti-fungals with high selective toxicities, it is incredibly important to study how fungi infect and affect animals. This information can then be used to develop better treatments for fungal infections. Studying fungi that control animal behaviour has another benefit, though. Because of years of co-evolution, these fungi act like nature's own neurologists. Learning how these fungi can so precisely change the behaviour of their animal hosts will also reveal how normal behaviour is regulated and how certain neuromodulating compounds tip the scale towards pathological behaviours. In the long run, such knowledge could again lead to better therapies but, in this case, those related to mental health (see the interview with Neuroparasitologists About the Tiny Critters That Turn Animals Into Zombies for a more detailed treatment of this subject).

Relevance for Sustainable Development Goals and Grand Challenges

The microbial dimension of the study of microbial control of animal behaviour relates to several SDGs, including

- **Goal 2. End hunger, achieve food security and promote sustainable agriculture.** Studies into microbial control of animal behaviour reveal how animal behaviour is regulated and what compounds microbial manipulators use to control that behaviour. This knowledge could be used to battle insect pests that affect and reduce the yields of important food crops. Finding ways to break into something as fundamental as an organism's behaviour is likely to be a more holistic and sustainable approach compared to insecticides against which insects quickly evolve resistance.
- **Goal 3. Improve healthy lives, treat fungal infections more effectively and promote mental well-being.** Studies into microbes, and especially fungi, that infect animals provide us with better insights into fungal infections, how they interact with animals, and how such infections could be treated. Investigation into behavioural control will reveal how behaviour is regulated at the mechanistic and chemical level. This will increase our knowledge with regards to behaviour in general, which will help us better treat mental health issues and behavioural pathologies. Moreover, fungi are known for their ability to produce compounds that have revolutionized medicine and industrial processes in the past (e.g., the discovery of the first antibiotic penicillin, the use of fungal enzymes in the paper and pulp industry). The fungi that control animal behaviour have not been studied in-depth yet. The ongoing investigations could, therefore, lead to the discovery of novel compounds with interesting applications.
- **Goal 11. Sustain healthy environments, improve biodiversity and promote healthy food webs.** Parasites form an incredibly important and abundant aspect of every environment and ecosystem. Animals are generally plagued by a range of parasites, including microbial ones. Negative effects of these parasites on the survival and reproduction of animals could affect food

webs and, therefore, the balance in such ecosystems. Parasites can also act as beneficial players when they promote biodiversity by keeping the growth of certain species populations in check.

- **Goal 15. Conserve and sustainably use land, reduce the effects of invasive species.** Similar to Goal 2, understanding the mechanisms involved in animal control by microbes could help conservation efforts by disrupting essential behaviours in invasive insects that take over the niches of native species and outcompete them. To interfere with the behaviour of invasive species could make them less successful and, therefore, less of a threat to important native species. This could boost species conservation efforts.

Potential Implications for Decisions

Most of the research on microbial control of animal behaviour is still in the fundamental research phase to study the basic biology of this phenomenon and to link the mechanistic workings to behavioural and ecological effects. Depending on the outcome of such studies and the discoveries that are made with regards to novel bioactive compounds, this work could begin to have implications for decision making by various stakeholders in the fields of medicine (i.e., infectious disease and mental health), conservation (i.e., effects on behavioural ecology of keystone species, food webs and biodiversity) and agriculture (i.e., the sustainable control of insect pests).

Pupil participation

1. Class Discussion:
   a. What are your favourite pop culture examples of zombie-making microbes?
   b. Which parts of those examples are purely science fiction and which parts could have been based on real scientific findings? (pupil learning about the subject)
   c. Do you think the fundamental research into behaviour altering microbes to be relevant/important? Why? Or, why not? (pupil stakeholder awareness)

2. Exercises:
   a. Use the internet to find scientific information about other zombie-making parasites, besides zombie ants, to prepare a short, written report or class presentation.
   b. Aspects to be included in the written report or class presentation:
      i. Description and natural history of the zombie-maker of choice
      ii. Find parallels between the scientific discoveries and zombie movies/video games/books. Which parts are based on real science and which parts are not?
      iii. What seems to be the importance of the behavioural manipulation for the parasite?
      iv. What has so far been studied about this zombie-making microbe?
      v. If you were a scientist, what would your next avenue of research be? Formulate as a research question and hypothesis, and predictions of what you think researchers might find.
      vi. What could the societal impacts of your proposed studies and their outcome be?

The evidence base, further reading and teaching aids

1. https://www.history.com/topics/folklore/history-of-zombies#section_5 History of zombies – Origins, pop culture and film; https://www.livescience.com/58096-zombie-science-girl-with-all-the-gifts-movie.html Meet a new kind of zombie, in the film "The girl with all the gifts."
2. R. L. Johnson (2012) Zombie makers: True stories of nature's undead. Available as ebook, audiobook and printed version.
3. https://explorable.com/red-queen-hypothesis Red queen hypothesis - The evolutionary arms race; http://thesciencecafe.com/feature-articles-1/2017/2/20/keeping-up-with-the-red-queen Keeping up with the red queen.
4. WIRED Magazine (2014) Turns out the U.S. has its very own species of ant-zombifying fungus. https://www.wired.com/2014/08/zombie-ant-fungus-in-the-us/
5. C. de Bekker, I. Will, B. Das, R. M. M. Adams (2018) The ants (HymenopteraL Formicidae) and their parasites: effects of parasitic manipulations and host responses on ant behavioral ecology. *Myrmecol. News* 28: 1-24 https://doi.org/10.25849/myrmecol.news_028:001
6. S. B. Andersen, S. Gerritsma, K. M. Yusah, D. Mayntz, N. L. Hywel-Jones, J. Billen *et al.* (2009) The life of a dead ant: The expression of an adaptive extended phenotype. *Am. Nat.* 174: 424-33 https://doi.org/10.1086/603640
7. C. de Bekker for Experiment.com (2016) How does a parasite create zombie-like behavior? https://experiment.com/projects/how-does-a-parasite-create-zombie-like-behavior/results
8. F. S. Andriolli, N. K. Ishikawa, R. V. Isla, T. S. Cabral, C. de Bekker, F. B. Baccaro (2019) Do zombie ant fungi turn their hosts into light seekers? *Behav. Ecol.* 30(3), 609-616 https://doi.org/10.1093/beheco/ary198
9. Medium.com – Love Nature (2016) We asked some neuroparasitologists about the tiny critters that turn animals into zombies. https://medium.com/love-nature/we-asked-some-neuroparasitologists-about-the-tiny-critters-that-turn-animals-into-zombies-89e12c78779a#:~:text=Neuroparasitology%20is%20the%20scientific%20study,hosts%20into%20real%2Dlife%20zombies.

Glossary

Adaptation is the evolutionary process during which a species becomes better suited to its environment through the natural selection for heritable variations that make certain individuals in a population more successful in terms of survival and reproduction than others.
Adaptive. An adaptive trait is a heritable feature that is the result of natural selection and adaptation, which makes an organism better fitted to its environment, maximizing its reproductive success
Antibiotic is a medicine that inhibits the growth of or kills microorganisms
Bacteria are a domain of life consisting of unicellular microorganisms that lack organelles and an organized nucleus.
Biomass is the total mass of organisms in a given area.
Co-evolution is the process of reciprocal evolution which occurs when two or more closely interacting species affect each other's evolution through the process of natural selection.
Decomposers are organisms, such as a bacterium, fungus or invertebrate, that decompose dead organic material so it can be recycled and used as a carbon source by other organisms.
Food webs are the natural interconnection of organic matter and organisms that form the food chains in an ecological community.

Fungi are a kingdom of life consisting of eukaryotic organisms that can range in size from microscopically small, single celled yeasts, to interconnected mushrooms that span an entire forest.
Holistic approaches look at e.g., the organism, situation or problem from a broader instead of from a single perspective by recognizing how the single parts are interconnected to form the whole.
Immune response is the concerted physiological reaction of an organism to the presence of a substance or organism which is not recognized as a constituent of the organism itself.
Invasive species are new to a certain ecosystem and are not normally found there. These species generally cause great economic and environmental harm to the new area they inhabit.
Invertebrates are animals lacking a backbone as part of their body plan.
Mandible. A jaw or crushing organ as part of an organism's mouthparts
Microbiome. All the microbes that inhabit a particular environment, organism or part of an organism, such as the gut of an animal.
Native species are species that normally live and thrive in a certain ecosystem.
Natural history is the scientific study of an organism by ways of observation rather than experimentation.
Niche is the place a species inhabits within a certain environment as characterized by the abiotic factors it favours and the interactions it has with other species in its community.
Parasites are organisms that live in or on other organisms (the host), and take resources at the expense of that organism.
Physiological processes are the ways in which biomolecules, cells, tissues organs and organ systems work together inside a living organism and sustain that organism.
Population is a group of interbreeding individuals of the same species that live within the same area.
Protozoans are single-celled eukaryotes that can be either free-living or parasitic.
Social immunity is the collective action or altruistic behaviour against parasites that benefit the group as a whole rather than the individual.
Solar noon is the time during the day when the sun has reached its highest point in the sky, which is exactly half-way between sunrise and sunset.
Vertebrates are animals that have a backbone as part of their body plan.