



Of biofilms and beehives: An analogy-based instructional tool to introduce biofilms in school and undergraduate curriculum

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ABSTRACT

The concept of biofilms and biofilm-based research is largely absent or minimally described in school and undergraduate life science curriculum. While it is well-established that microbes, such as bacteria and fungi, most often exist in multicellular biofilm communities, descriptions in standard biology textbooks continue to focus on the single-celled form of microbial life. We have developed an analogy-based instructional tool to introduce and explain biofilms to school and undergraduate students. The module employs an analogy with beehives, given that biofilms and beehives are both 'superorganism' states, to explain key biofilm features such as development and structure, chemical communication, division of labor and emergent properties. We delivered this analogy-based learning tool to a cohort of 49 students, including middle-to-high school and undergraduate students, and based on participant feedback and learnings, present a formal evaluation of the instructional tool. Further, we outline prerequisites and learning approaches that can enable the delivery of this module in classroom and virtual learning settings, including suggestions for pre-lesson reading, student-centred interactive activities, and specific learning objectives. Taken together, this instructional analogy holds potential to serve as an educational tool to introduce biofilms in school and undergraduate curricula in a relatable and comprehensible manner.

1. Introduction

Typically studied as single-celled organisms, microbes are known to form large multicellular communities known as biofilms [1–3]. Biofilms are highly-organized, structured, three-dimensional aggregates of microbial cells (bacteria or fungi), embedded in a self-produced extracellular matrix [4]. Ubiquitous in the environment, biofilms can be found in lakes, rivers, soil, rocks and wetlands, where they play key roles in recycling of organic matter and nutrients [5]. These effects of biofilms are being increasingly harnessed towards environmental applications such as wastewater treatment and bioremediation [6]. Biofilms are also part of the normal microbial flora of the human body, particularly in the gut, dental and skin microbiome [7–9]. On the other hand, biofilms also have serious environmental and clinical consequences [5]; they are implicated in a range of human infections [10–14] and environmental effects such as water pollution and industrial fouling [15,16]. While biofilms are actively-studied in research laboratories, they are largely absent or

minimally described in school and undergraduate biology curricula [1, 2]. For example, the high school textbook Life on Earth [17] discusses microbial communities in the context of biodiversity, but does not discuss the biofilm mode of microbial life. On the other hand, the widely-used undergraduate biology textbook Concepts of Biology [18] includes a very brief overview of biofilms, with no insights into the processes involved in biofilm structure, formation and function. Given this, an instructional tool that introduces and discusses biofilms in a relatable and comprehensible manner could serve as a valuable addition to school and undergraduate biology curricula.

Analogies have been explored as tools in science education [19–23], and the use of analogies is based on deconstructing systems with respect to their parts, the relation between the parts and the agreement across the themes [20,23–30]. Given this, analogical teaching is inherently suited for building new concepts, and holds value in introducing new concepts in an engaging and relatable manner [23,31,32]. To develop an analogy-based instructional tool for biofilms, we use an analogy with

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beehives, given that biofilms and beehives are both collective organismal states, also known as ‘superorganisms’ [33,34]. Beehives are macroscopic, visible to the naked eye, well-known entities, and provide a relatively familiar analog for microscopic, multicellular microbial biofilms. Using an analogy with beehives, this instructional tool introduces and explains key biofilm features such as development and structure, chemical communication, division of labor, and emergent properties. Since a large segment of research on biofilms has focused on bacteria, we have largely drawn examples from bacterial biofilms.

1.1. Intended audience

This analogy based instructional tool is intended for middle-to-high school and undergraduate students, as part of the biology or life science curriculum. The content draws from fields such as biology, microbiology, chemistry and biochemistry, and can be adapted (as per suggestions provided) for different student levels.

1.2. Learning time

The entire analogy-based lesson, including delivery of the content by an instructor and student activities requires 90 min. The curriculum tool includes instructor guidelines for delivery, suggested student activities, slides for delivery, and sample pre-session and post-session feedback forms.

1.3. Prerequisite student knowledge and pre-session reading material

Middle and high school students, and undergraduates, would be expected to have had a science or biology course. No prior laboratory or field experience is required. To bring students on the same level, the analogy includes a set of pre-session reading materials, which include relevant chapters in biology text books (recommended chapters include basics of biology, biological communities, chemistry of life, cellular life, DNA and genes, diversity of life and bacterial life), as well as a list of key words, across the concepts of biofilms and beehives (Suppl Material). These can be provided to the students a week prior to the session.

1.4. Learning objectives

The learning objectives for this instructional tool are as follows:

1. At the end of the lesson, students will be able to recognize the concept of biofilms as bacterial communities, and contrast it from single-celled microbial life.
2. On completion of the lesson, students will be able to identify the importance of studying biofilms, from both a health and environmental perspective.
3. From the section on development and structure, students will be able to identify and recognize the typical structure of biofilms, the five main stages in biofilm formation, and events influencing these stages.
4. After reading the section on chemical communication, students will be able to recognize the phenomenon of quorum sensing, and the roles of different autoinducer molecules.
5. On completion of the section on division of labor, students will be able to recapitulate the example of division of labor in *B. subtilis* biofilms that contributes to the formation of the biofilm extracellular matrix.
6. Based on their understanding of emergent properties, students will be able to identify the definition of emergent properties, and recognize why antibiotic tolerance is a feature of multicellular biofilms (as opposed to single cells).
7. After the section on limitations of the analogy, students will be able to contrast the key areas in which biofilms are different from beehives by listing at least one key difference.

8. Students will be able to apply their understanding of the analogy to develop at least one idea of their own related to a new idea of investigation on biofilms. It is important to note that here ‘new’ represents what is not stated or explained in the analogy, and not new for the field *per se*, given that students may not have a comprehensive overview of the current status of biofilm research.

2. Procedure

2.1. Materials

Delivery of the analogy-based instructional tool will require a classroom setting (if in-person) or a virtual platform. If in person, the instructor will require equipment for projection of slides (overhead projector, computer). Students will require writing tools and sheets of paper for notes.

2.2. Student instructions

Prior to the lesson, students would be expected to read the suggested pre-session reading materials such as select textbook chapters, as well as familiarize themselves with the meanings of the key words provided to them.

2.3. Faculty instructions for delivery of the module at school and undergraduate level

Prior to the lesson, the faculty instructor will need to share the suggested pre-session reading materials and key words with the students. The instructor will also need to read and understand the modules of the analogy (Table 1), and download and familiarize themselves with the slide deck (Suppl Material) provided for delivery of the analogy. The module includes references to original scientific literature which may be used by the instructor to further clarify concepts. A detailed guideline for the delivery of the analogy, including time to be allocated for each section of the module and additional learning activities, is provided in the Supplementary Material.

For middle-to-high school students, the faculty instructor could begin the session with an overview of microbial life, including different types of microbes and presence of microbes in the environment and the human body. At this time, the instructor could reiterate the beneficial functions of microbes in the environment and human body, while also discussing the deleterious role of microbes in human infections.

In addition to the points above, for undergraduates, the faculty instructor could begin the session with a discussion of the role of microbial communities in the environment and human health, including beneficial and deleterious effects. At this time, the instructor could bring forth the major challenge posed by antibiotic resistance, and how overuse and misuse of antibiotics fuels this concern. This could be tied in to the inherent tolerance of biofilms to antibiotics, when subsequently discussed in the module.

At any stage in the module, if the content poses challenges to understanding, the instructor can revisit the key words with the students to clarify the concept before moving forward.

2.4. Modules of the analogy-based instructional tool

2.4.1. Module 1: Development and structure of beehives and biofilms

The Western or European honeybee (*Apis mellifera*) lives in well-ordered colonies or beehives, consisting of a large queen bee, thousands of female worker bees (which lack a completely developed reproductive system), and a few drone males [35–37]. Worker bees make the 3D honeycomb structures of hives from beeswax secreted from their abdominal glands [38]. At the start of building the colony, scout bees search for suitable locations to occupy, known as ‘scouting behaviour’, based on criteria such as position and volume of the nest

Table 1

Summary of key shared and non-shared features related to the analogy on biofilms and beehives.

Features	Beehives	Biofilms
Development and Structure	Self-produced beeswax Structured solid (at room temperature) beeswax Defined 3D hexagonal structure Honeycomb has mechanical and protective role	Self-produced EPS matrix Slimy, hydrated, dynamic matrix Variable structure, shape, and 3D architecture EPS matrix has mechanical, protective and transport roles
Chemical Communication	'Scouting behaviour' followed by mass recruitment Self-produced pheromones Different types of pheromones based on bee species and function Controls behaviours such as colony development, reproduction, brood rearing Mainly for intraspecies communication, rare pheromones for interspecies communication	Initial reversible attachment followed by irreversible phase Self-produced autoinducers Different types of autoinducers based on microbial species Controls behaviours such as biofilm formation, virulence, bioluminescence Autoinducers for intraspecies, interspecies and interkingdom communication
Division of Labor	Presence of different subpopulations of a single species Subpopulations have committed lifestyle and function	Presence of different subpopulations consisting of single and mixed species Role and function subpopulations may vary depending on genetic and environmental changes
Emergent Properties	Emergent properties result from an active response (shivering in bees leading to thermoregulation of the hive)	Emergent properties can result from an active response (enzymes causing antibiotic degradation) or from passive structures (physical matrix barrier impeding diffusion of antibiotics)

cavity, light, moisture, and temperature [39–41]. Scout bees communicate the presence of an optimum location via a characteristic 'waggle dance' [42], and if several bees come back with the same information, the waggle dance spreads to other bees. When one site is being visited by a sufficiently large number of scouts [43], the recruitment dance results in a swarm, where the queen bee and scouts proceed to establish a colony at the chosen site [44] (Fig. 1A).

In the early stages of biofilm formation, free-floating bacteria (or small bacterial aggregates) attach to a biotic or abiotic surface, via weak chemical forces [45]. This initial attachment of free-floating bacteria is influenced by factors such as temperature, surface properties and nutrients [45]. At this early stage, bacteria interact with the surface in a transient manner, with each reversible contact priming the bacteria for the next stage of irreversible attachment [45]. In the presence of high bacterial densities, these transiently attached bacterial cells secrete extracellular polymeric substance (EPS) [46,47]. EPS consists of a mixture of water, polysaccharides, proteins, and extracellular DNA. The EPS matrix helps bacteria adhere to surfaces and provides mechanical strength to biofilms [45,48]. During further maturation, the biofilm extends from the surface to develop multiple layers of bacterial microcolonies, thereby building a 3D structure. The EPS matrix is a dynamic

substrate that influences the transfer of nutrients and metabolites, resulting in chemical gradients in the biofilm structure [49–51]. Finally, a mature biofilm can disperse, either as clumps of cells or single cells, to seed new surfaces (Fig. 1B).

2.4.2. Module 2: Chemical communication in beehives and biofilms

Chemical communication in beehives occurs through pheromones, secreted from exocrine glands [52]. Honeybee pheromones are a mixture of volatile and non-volatile chemical substances that are transmitted by direct contact. Specific pheromones are released by queen, worker, drone and brood bees, ensuring a broad range of functions [52]. The queen signal is a complex mixture of several chemicals, the main component being Queen Mandibular Pheromone (QMP). QMP is responsible for worker activities, drone attraction and queen rearing. Worker bees produce Nasonov gland pheromones to drive the returning forager bees back to the hive, mark hive entrance, locate food resources and rear future queens [53]. Other important pheromones include alarm pheromones, drone pheromones, which are almost exclusively linked to mating functions, and brood pheromones, that regulate colony development and formation [52] (Fig. 2A).

Similar to the chemical communication in bees, bacteria

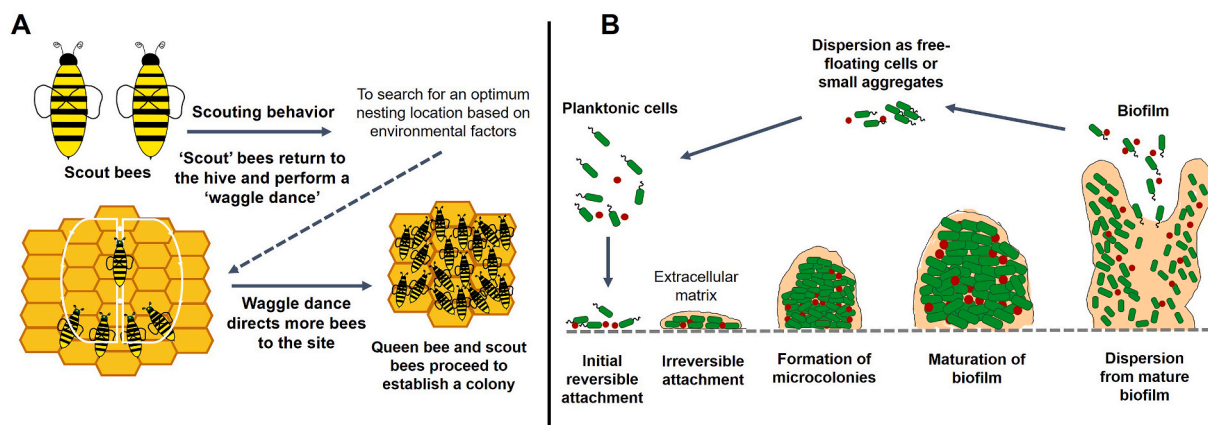


Fig. 1. Development and structure of beehives and biofilms, illustrating shared and non-shared features between the two entities. (A) In *Apis mellifera*, a few scout bees search for an optimum nesting location, the presence and location of which is communicated to the hive using a 'waggle dance', among other characteristic movements. These movements direct more bees, including the Queen bee, to the site, which proceed to establish a new colony. **(B)** In bacterial biofilms, a few free-floating cells or aggregates proceed to initiate biofilm formation following stages of initial reversible and then irreversible attachment, to biotic or abiotic surfaces. Following this, attached bacteria grow to form microcolonies and three-dimensional structures, constituting a mature biofilm. Bacterial cells disperse, as small aggregates or single cells, and can proceed to establish biofilm formation at other sites. Illustrated here is a typical mixed-species biofilm with two different bacterial species shown in green (bacilli) and red (cocci). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

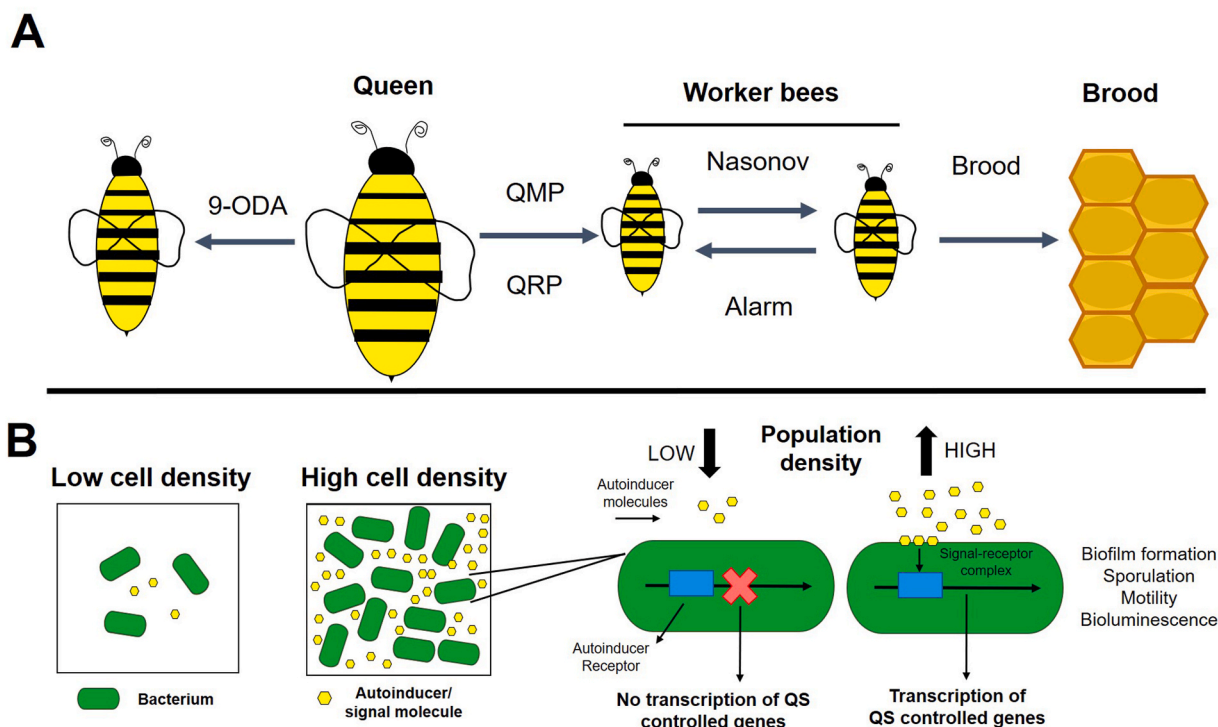


Fig. 2. Chemical communication in beehives and biofilms, illustrating shared and non-shared features between the two entities. (A) In honeybees (such as *Apis mellifera*), chemical communication occurs through specific pheromones, volatile and non-volatile chemical substances transmitted by direct contact. Released by queen, worker, drone and brood bees, pheromones are responsible for a range of functions such as colony development and formation, location of food resources, drone attraction and queen and brood rearing. (B) Similar to the chemical communication in bees, bacteria communicate via signalling molecules (such as autoinducers), in a density-dependent process. At high bacterial cell densities, the signal concentration reaches a certain threshold, following which autoinducer molecules bind to bacterial cell surface receptors, triggering population-wide changes in gene expression. These changes regulate various bacterial group functions such as biofilm formation, motility, sporulation, and virulence. Abbreviations: 9-ODA = 9-oxodecenoic acid, QMP = Queen Mandibular Pheromone, QRP = Queen Retinue Pheromone, QS = Quorum Sensing.

communicate via signalling molecules [54–56]. This phenomenon, known as quorum sensing (QS), depends on bacterial cell density and is mediated by small, diffusible extracellular signal molecules or autoinducers [57]. As the local density of the bacteria increases, the extracellular concentration of autoinducers also increases (Fig. 2B). Autoinducer molecules bind to specific bacterial cell receptors,

triggering changes in gene expression across the bacterial population [58]. These changes in gene expression regulate the various stages of biofilm formation [54,55,59,60]. There are three major classes of autoinducer molecules, N-acylated homoserine lactones (AHLs) or autoinducer-1 (AI-1) primarily found in Gram-negative bacteria, oligopeptides found in Gram-positive bacteria, and autoinducer-2 (AI-2),

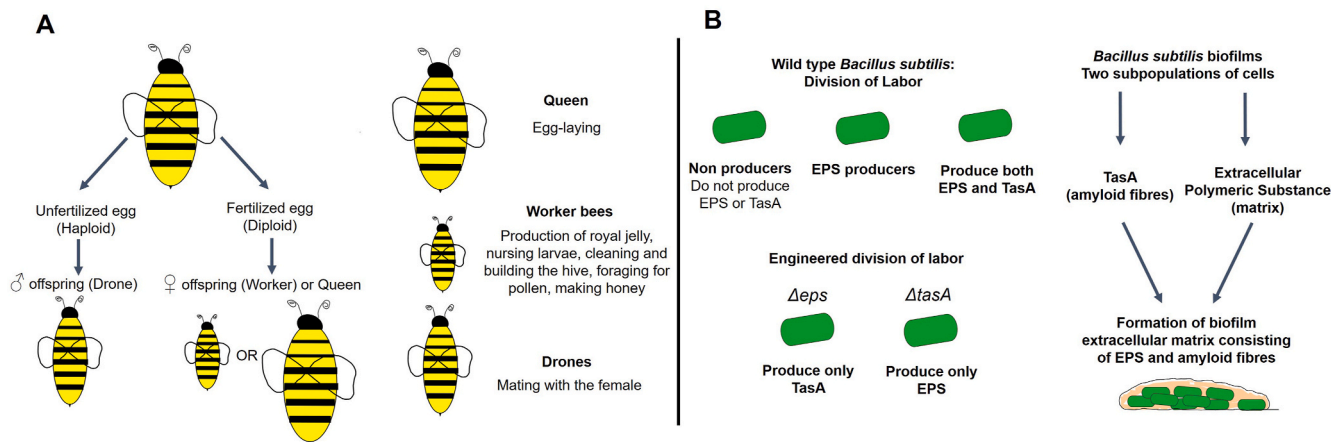


Fig. 3. Division of labor in beehives and biofilms, illustrating shared and non-shared features between the two entities. (A) Honeybees exhibit a unique haplodiploid mode of sex-determination in which the unfertilized haploid eggs produce males, while worker and queen females hatch from the fertilized diploid eggs. In honeybees (such as *Apis mellifera*), these different subpopulations of bees perform specialized functions. Typically, there is only one queen bee per colony, who is fertile and lays eggs in the hive. Drone bees are the sole males of the colony, and their main task is to fertilize a receptive queen. Worker bees do the majority of the work for the colony, with further division of labor within them. (B) A form of division of labor, based on differential gene expression in the bacterial population, is also observed in biofilms formed by the bacterium *Bacillus subtilis*, where division of tasks between subpopulations contributes to the formation of the biofilm matrix.

which is present in both Gram-positive and Gram-negative bacteria, and enables communication across bacterial species [58,61,62].

2.4.3. Module 3: Division of labor within beehives and biofilms

Honeybees exhibit a unique haplodiploid mode of sex-determination in which the unfertilized haploid eggs produce males, while worker and queen females hatch from the fertilized diploid eggs [63–65]. Different subpopulations of bees exist within the hive, with each subpopulation performing specialized functions to maintain the hive integrity (Fig. 3A). Typically, there is only one queen bee per colony, who is fertile and lays eggs in the hive. Drone bees are the sole males of the colony, and their main task is to fertilize a receptive queen. Worker bees do the majority of the work for the colony, and there is further division of labor within them [33,35,66]. Activities divided among worker bees include nursing the developing larvae and the queen, cleaning and building the hive, foraging for pollen, storing honey and nectar in the hive, and protecting the hive from predators.

A form of division of labor, based on differences in gene expression in the population, is also observed in bacterial biofilms [67–71]. For example, in *Bacillus subtilis* biofilms, there is division of tasks between groups of bacterial populations that contributes to the formation of the biofilm matrix [69]. There are two main constituents of the matrix: namely an extracellular polymeric substance (EPS) and a protein TasA (amyloid fibres), which are produced by different *B. subtilis* subgroups. Cells within the biofilm segregate into groups that either produce both components, produce EPS only or produce neither (Fig. 3B). When mutants Δeps (producing only the TasA protein) and $\Delta tasA$ (producing only EPS) were mixed in a culture, they complemented each other by sharing EPS and TasA, to make a biofilm similar to the wild-type (with no mutations). However, these mutants, when studied individually, were deficient for biofilm formation.

2.4.4. Module 4: Emergent properties in beehives and biofilms

As ‘superorganism’ states, beehives and biofilms exhibit collective or emergent properties that are not displayed by individual organisms [72]. In honeybee colonies, a well-known emergent behaviour is thermoregulation [73], that relates to the ability of the honeybee colony to survive as a whole. At low temperatures, bees tend to move closer together and share body heat. Since the centre has more heat, and younger bees cannot shiver, they move inwards. Adult bees shiver to produce heat and move to the middle and outer layers. This heat warms the whole hive (Fig. 4A). As the heat in the centre increases leading to a situation of excess heat, the young bees move to create channels of air exchange, allowing heat from inner regions to flow out towards the older bees. This combined effect enables the hive as a whole to stay warm, a critical factor for survival of the colony.

An important emergent property of bacterial biofilms, that differs from free-floating cells, is increased tolerance to antimicrobials. This results from specific properties of bacteria in the biofilm, as well as the biofilm matrix itself [2]. The EPS matrix reduces diffusion of antimicrobial agents into the inner parts of the biofilm [2]. In the bacterium *Pseudomonas aeruginosa*, components of EPS such as polysaccharide and extracellular DNA, form interactions with antibiotics and impede their penetration through the matrix [74]. Bacteria in biofilms also adopt properties of slow growth and dormancy, with reduce their susceptibility to antibiotics, such as penicillin, that act on actively-growing cells [75]. One group of slow-growing cells in biofilms are persister cells, that exhibit high-level tolerance to antimicrobials [76,77], but can revert to a growing state and repopulate the biofilm once treatment is stopped (Fig. 4B).

2.5. Suggestions for determining student learning

Student learning was assessed using pre-session and post-session feedback forms via Google forms. The forms used a combination of

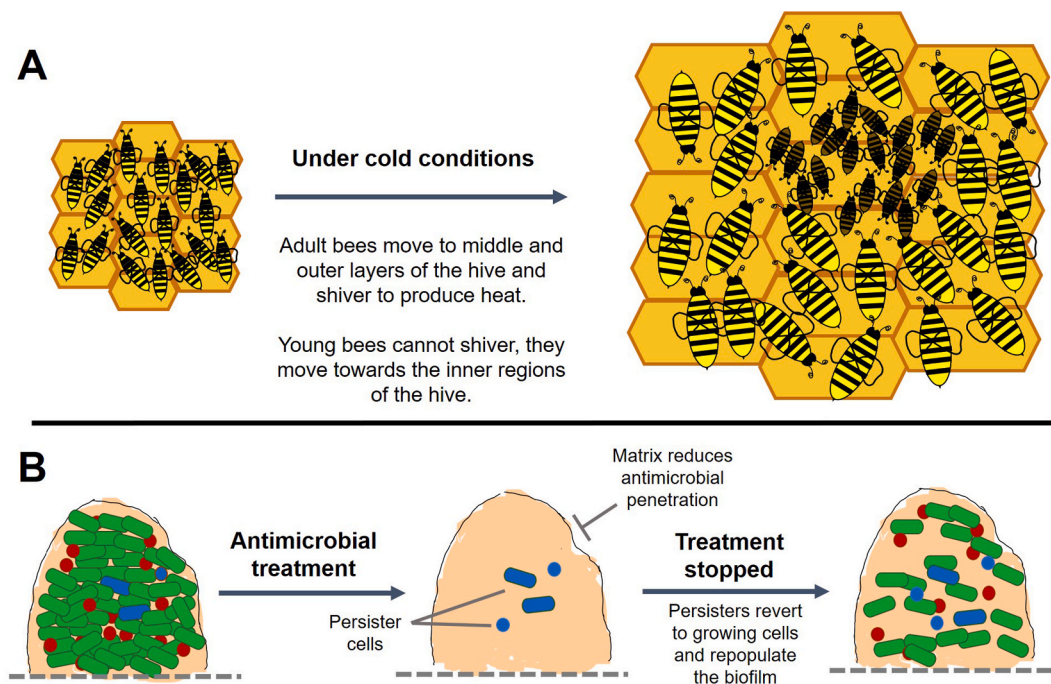


Fig. 4. Emergent features in beehives and biofilms, illustrating shared and non-shared features between the two entities. (A) In honeybee colonies, a well-known emergent behaviour is thermoregulation, which relates to the ability of the honeybee colony to survive as a whole. At low temperatures, bees tend to move closer together and share body heat. Since the centre has more heat, and younger bees cannot shiver, they move inwards. Adult bees shiver to produce heat and move to the middle and outer layers. **(B)** In biofilms, an important emergent property is the increased tolerance to antimicrobial treatments. This results from various factors such as physical and chemical factors in the EPS that reduce the diffusion of antimicrobial agents into the inner parts of the biofilm, as well as the metabolic heterogeneity of the biofilm state that contributes to the formation of slow-growing or dormant cells. These persister cells can tolerate high concentrations of antibiotics, and repopulate the biofilm once antimicrobial treatments are stopped.

multiple choice and free response questions. Pre-session feedback included information on participant demographics, prior science and biology courses, use of pre-session reading materials and familiarity with broad concepts of the analogy. Post-session feedback assessed learning of the content delivered in the modules, particularly with respect to the learning objectives. Students were provided time to fill these forms before and after the session. Both feedback forms are available in the Supplementary Material.

2.6. Sample data

In the pre-session form, students provided data related to demographics, educational level, previous science or biology courses, familiarity with biofilms and beehives and use of pre-session reading materials (Figs. 5–8). In the post-session feedback, student learning data was collected in response to specific content-based questions, anecdotal feedback in response to open-ended questions, and new ideas and hypotheses generated from the modules (Figs. 9–12 and Tables 2–5).

2.7. Safety issues

There are no safety issues associated with the delivery and adoption of this lesson.

3. Discussion

3.1. Field testing

The analogy-based instructional tool was delivered on two separate occasions (session time 90 min) via a virtual format (webinar) to school students (24 students, including middle-to-high school students) and undergraduates (25 students) across India. School students were from different schools across the country, and represented a range of grades. The undergraduates were in year 3 of a 5-year integrated Masters' course in Biotechnology, and had completed basic courses in biology and microbiology prior to this session. Starting in year 2 of the course, undergraduates had the option to select elective courses in biology. Registered participants were provided with instructions and pre-session reading materials via email one week before the session. Explicit written participant consent, or parental consent (in the case of participants

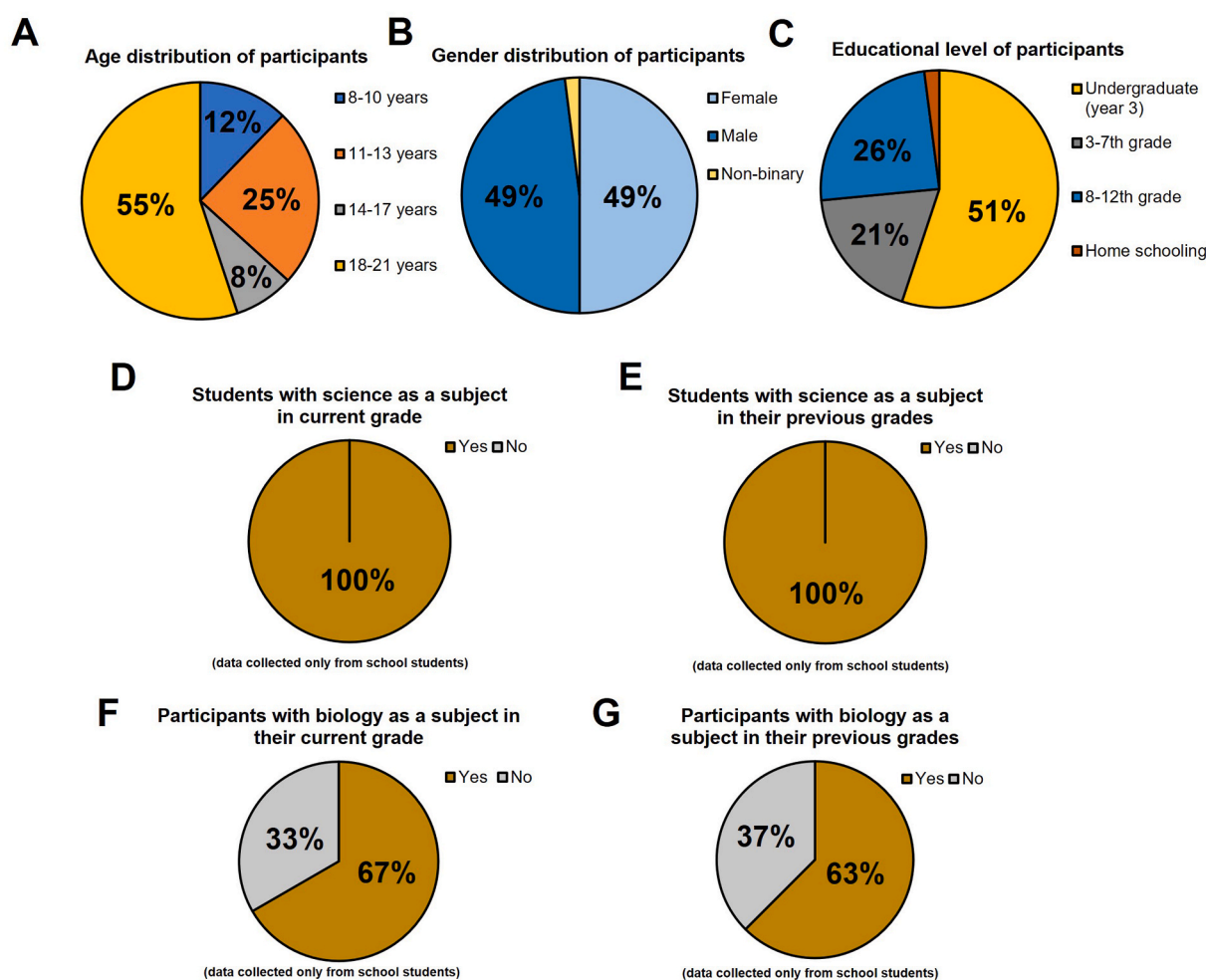


Fig. 5. Distribution of school and undergraduate participants in the session. (A) Based on age, gender and educational level. (A and B) The analogy-based session was delivered to a total of 49 students (middle and high school students and undergraduates) across India, including a range of ages and both genders. (C) Undergraduate students were in the third year of an integrated biotechnology course, whereas school students ranged across middle-to-high school grades, and were studying in different school systems such as private, public, and home schooling. (D and E) All school students ($n = 24$) reported that they had science as a subject in their current grade as well as in previous grades. (F and G) Among the school students, 67% had biology ($n = 16/24$) as a subject in their current grade and 63% had biology ($n = 15/24$) as a subject in previous grades as well. Data for biology and science education were collected only from school students. Percentages are calculated based on total number of school and undergraduate respondents, except when data was not collected for school students ($n=49$ for 5A, B, C and $n=24$, for 5D, E, F, G).

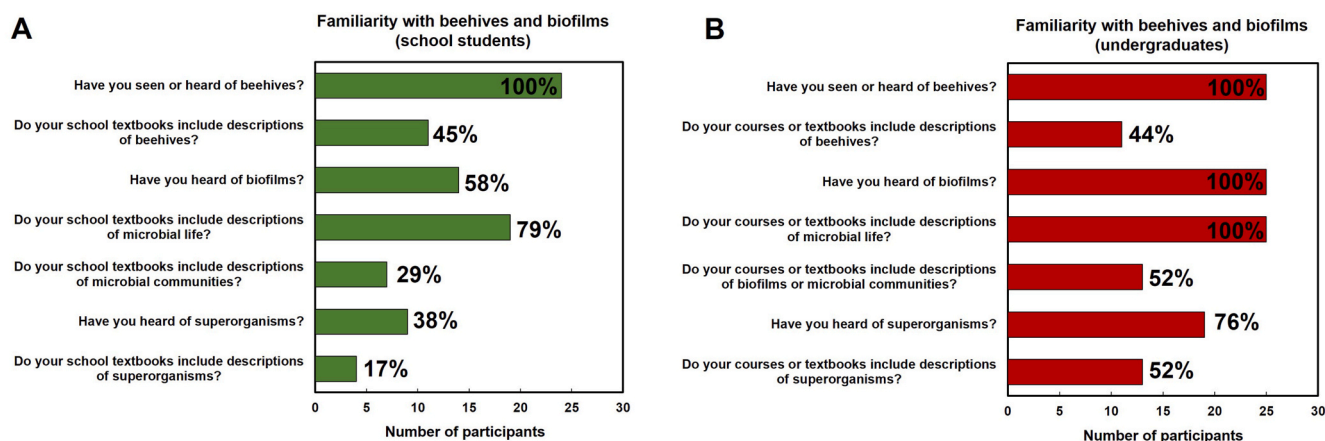


Fig. 6. Prior familiarity of school and undergraduate participants with the key concepts in this analogy-based lesson. (A) Among middle-to-high school students ($n = 24$), familiarity with key concepts such as beehives, biofilms, microbial life and superorganisms varied, and biofilms were notably less represented in school courses and textbooks. (B) On the other hand, while undergraduates ($n = 25$) reported a higher familiarity with the key concepts, including biofilms and superorganisms, feedback revealed that representation of biofilms in undergraduate course material is also limited. Percentages are calculated based on school and undergraduate respondents separately ($n=24$ for school students and $n=25$ for undergraduates). Asterisk (*) indicates the correct response to the question.

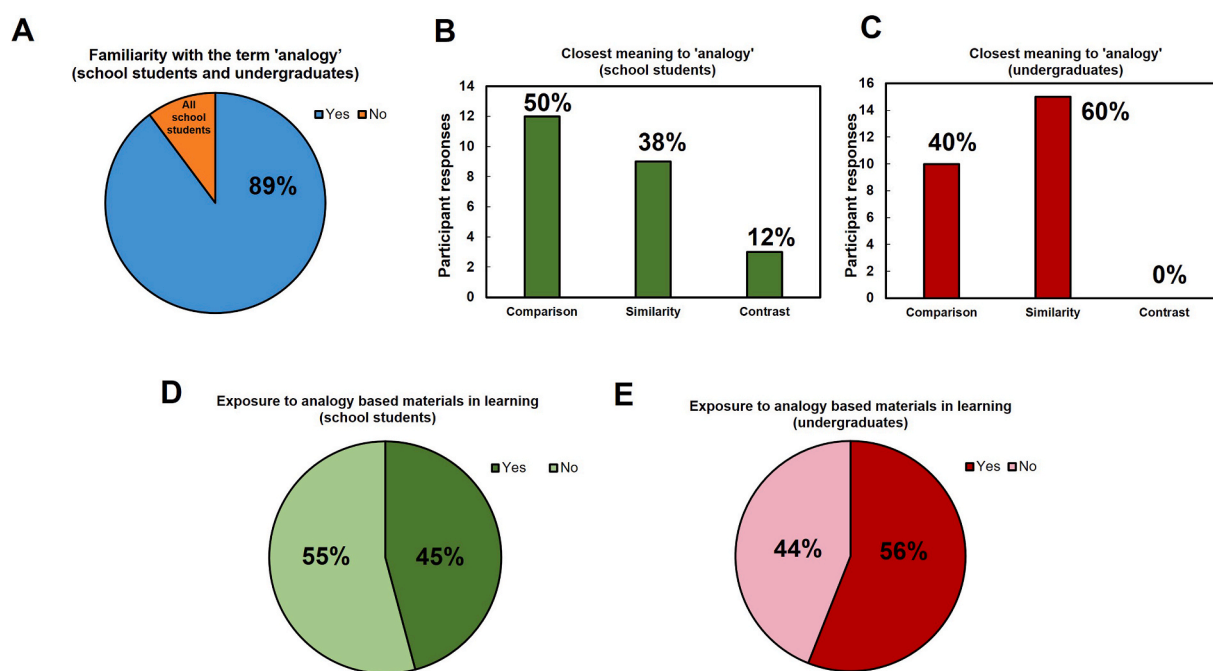


Fig. 7. Prior familiarity of school and undergraduate participants with analogies, and usage of analogies as learning tools. (A) Of all the participants, 89% ($n = 44/49$) reported familiarity with the term analogy. Of the 5 students who were unfamiliar with the term, all were school students. (B) Among middle-to-high school students, 50% ($n = 12/24$) answered that the closest meaning to analogy is 'comparison', whereas 38% ($n = 9/24$) chose 'similarity'. (C) Among the undergraduates, 60% of the respondents ($n = 15/25$) that the closest meaning to analogy is 'similarity', as opposed to 40% ($n = 10/25$) who chose 'comparison'. (D) Based on responses, 45% of school students ($n = 11/24$) had been exposed to some form of analogy based learning. (E) On the other hand, 56% of undergraduates ($n = 14/25$) reported that they had been exposed to some form of analogy based learning. Except in Fig. 7A, percentages are calculated based on school and undergraduate respondents separately ($n=24$ for school students and $n=25$ for undergraduates).

under 18 years of age), was obtained prior to the collection of feedback.

3.2. Evidence of school students and undergraduate learning based on pre-session and post-session feedback

Pre-session feedback (Figs. 5–8) was collected from 24 school students and 25 undergraduates (49 in total). Based on pre-session feedback from 49 school and undergraduate students, the participants in the analogy-based session covered a range of age groups ($n = 6/49$ for 8–10 years, $n = 12/49$ for 11–13 years, $n = 4/49$ for 14–17 years and $n = 27/$

49 for 18–21 years) (Fig. 5A). Participant genders were equally distributed among female ($n = 24/49$) and male groups ($n = 24/49$), with 1 participant self-identifying as non-binary (Fig. 5B). In concordance with age distribution, participants also covered a range of educational levels ($n = 25/49$ undergraduates in year 3, $n = 10/49$ for 3rd–7th grade, and $n = 13/49$ for 8th–12th grade) (Fig. 5C), with 1 participant home schooling in a flexible system. Feedback related to previous science and biology education was collected only from school students, given that it was a pre-requisite for undergraduates in an integrated biotechnology course. For science as a school subject, 100%

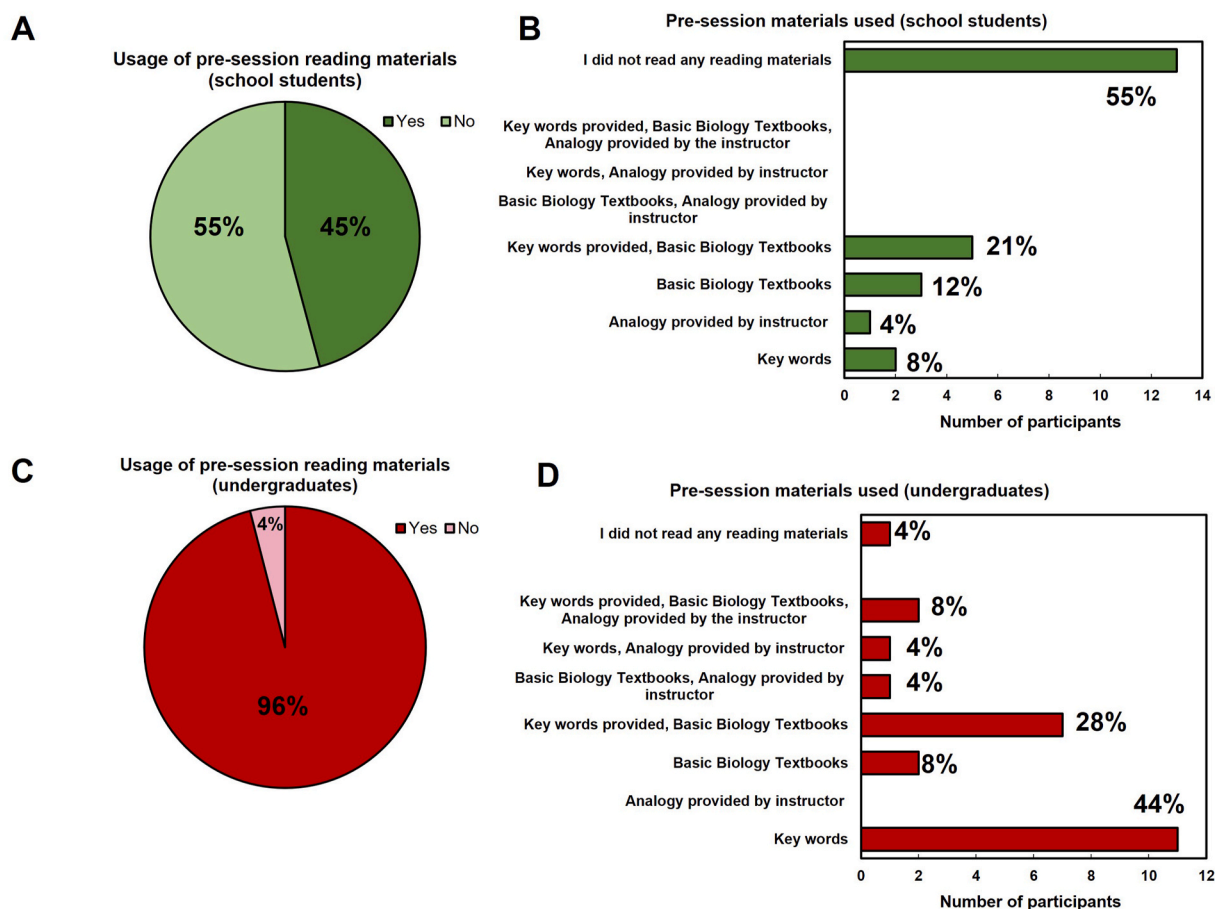


Fig. 8. Participant usage of pre-session reading materials across school and undergraduate students. Prior to the delivery of analogy-based session, participants were provided a set of pre-session reading materials which included key words, relevant concepts in basic biology textbooks and the draft of the analogy. (A) Among the school students, 45% ($n = 11/24$) reported usage of the pre-session materials. (B) The school students reported the highest usage of the key words and basic biology textbook concepts (21%, $n = 5/24$), among the recommended materials. (C) Among the undergraduate students, 96% ($n = 24/25$) reported usage of the pre-session materials. (D) The undergraduates reported the highest usage of the key words (44%, $n = 11/25$), followed by key words and basic biology textbook concepts (28%, $n = 7/25$), among the recommended materials. Percentages are calculated based on school and undergraduate respondents separately ($n=24$ for school students and $n=25$ for undergraduates). Asterisk (*) indicates the correct response to the question.

school students ($n = 24/24$) reported that they had science as a school subject in their current grade, as well as previous grades (Fig. 5D and E). Of the school students, 67% ($n = 16/24$) reported that they had biology as a subject in their current grade, and 63% ($n = 15/24$) responded that they had biology courses in previous grades (Fig. 5F and G).

Based on previous knowledge, 100% of school and undergraduate students ($n = 49/49$) responded that they were familiar with beehives, though less than half of them had descriptions of beehives in their school textbooks (45% ($n = 11/24$) for school students and 44% ($n = 11/25$) for undergraduates) (Fig. 6A and B). This underscores the fact that beehives are familiar entities, and therefore suited to serving as the familiar concept in a learning tool. On the other hand, while 100% of undergraduates ($n = 25/25$) reported prior familiarity with biofilms, only 58% of school students ($n = 14/24$) had heard of the same (Fig. 6A and B). Along similar lines, 100% of undergraduates ($n = 25/25$) reported that their course materials and textbooks included descriptions of microbial life, however, only 52% ($n = 13/25$) reported specific descriptions of biofilms. Among the school students, 79% ($n = 19/24$) reported descriptions of microbial life in course textbooks, and only 29% ($n = 7/24$) stated that this material focused on microbial communities (Fig. 6A and B). Given that students belonged to a wide range of educational levels and schooling systems, this indicates that there is a clear need to include descriptions and discussions related to microbial communities such as biofilms in curricular material both at school and undergraduate level.

Among school students and undergraduates, 89% ($n = 44/49$) responded that they were familiar with the term 'analogy' (Fig. 7A). All of the 5 students who reported being unfamiliar with the term were school students. Further, 50% of school students ($n = 12/24$) selected 'comparison' as the closest meaning to 'analogy', followed by 38% ($n = 9/24$) who selected 'similarity' (Fig. 7B). Among the undergraduates, 40% ($n = 10/25$) selected 'comparison' and 60% ($n = 15/25$) selected 'similarity' as the closest meaning to 'analogy' (Fig. 7C). That the majority of undergraduates selected 'similarity' was unexpected, but could possibly be explained by the fact that analogies often focus on the similar aspects between the two entities, with less attention given to dissimilar features. This is an aspect we have specifically addressed with the section on 'Limitations of this analogy and possible misconception' detailed in the 'Guidelines of delivery' in the Suppl. Material. Further, when examined separately, 45% of school students ($n = 11/24$) and 56% ($n = 14/25$) of undergraduates reported some exposure to analogy based learning tools (Fig. 7D and E). When examined together, 51% ($n = 25/49$) of school students and undergraduates reported exposure to analogy-based learning tools. Taken together, pre-session feedback indicates that beehives are familiar concepts, there is a need to include biofilms in school and undergraduate curriculum, and an analogy-based tool comparing two superorganism states could serve as a unique mode of instruction.

Based on feedback related to pre-session reading materials, 45% of school students ($n = 11/24$) reported the usage of the materials

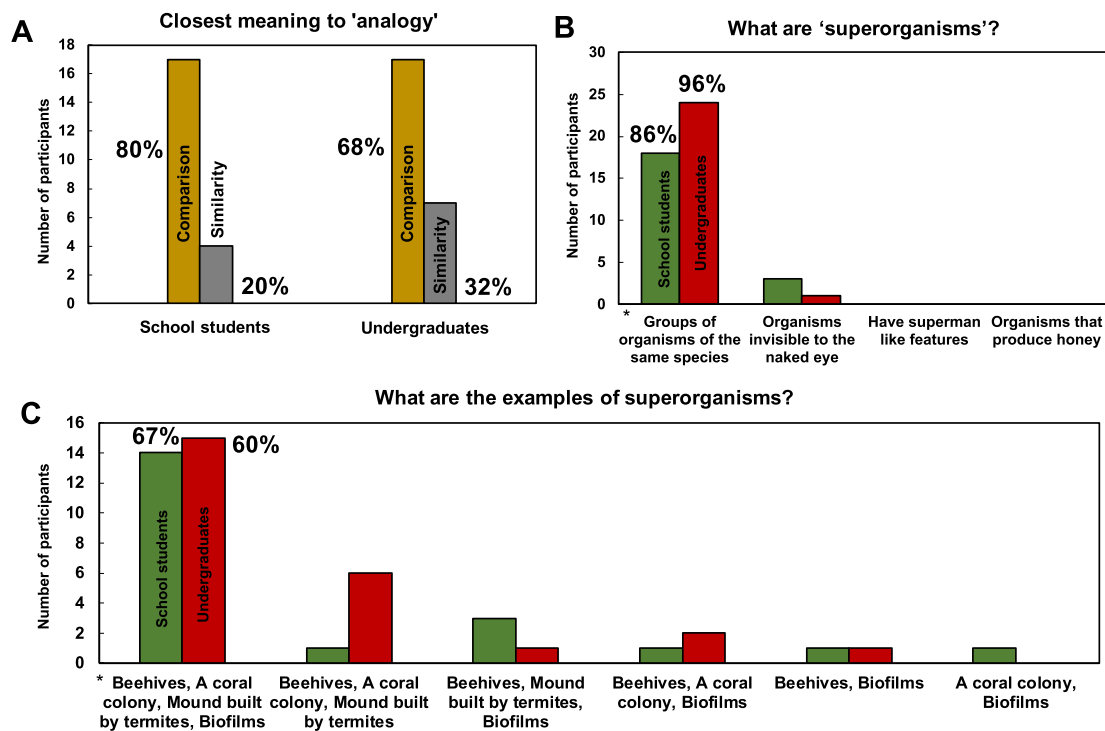


Fig. 9. Post-session feedback from school and undergraduate participants of important concepts such as analogies and superorganisms. (A) Following the delivery of the analogy, 80% of school students ($n = 17/21$) and 68% of undergraduates ($n = 17/25$) reported the closest meaning of analogy as 'comparison'. This is notably higher than the response to the same question prior to the session (Fig. 7B and C), where 50% ($n = 12/24$) and 40% ($n = 10/25$) had answered 'comparison' respectively. (B) Post-session feedback revealed that 86% of school students ($n = 18/21$) and 96% of undergraduates ($n = 24/25$) correctly identified superorganisms. (C) Based on feedback, 67% of school students ($n = 14/21$) and 60% of undergraduates ($n = 15/25$) could identify all the given examples of superorganisms. Percentages are calculated based on school and undergraduate respondents separately ($n=21$ for school students and $n=25$ for undergraduates). Asterisk (*) indicates the correct response to the question.

recommended to them, of which the majority (21%, $n = 5/24$) used the provided key words and suggested reading from basic biology textbooks (Fig. 8A and B). On the other hand, 96% of undergraduates ($n = 24/25$) reported the use of pre-session reading materials, where 44% ($n = 11/25$) used the provided key words and 28% ($n = 7/25$) used key words and basic biology textbooks (Fig. 8A and B).

Post-session feedback (Figs. 9–12) was obtained from 46 respondents, as compared with 49 respondents in the pre-session feedback. It is unclear as to why 3 participants (school students) did not respond or may have left the session prior to completion.

Based on feedback after the session, 80% of school students ($n = 17/21$) and 68% of undergraduates ($n = 17/25$) reported the closest meaning of the term 'analogy' as comparison (Fig. 9A). This is in contrast to feedback obtained prior to the session (Fig. 7B), and indicates a better understanding of analogies as comparative tools. In the pre-session feedback related to superorganisms, only 38% of school students ($n = 9/24$) had heard of superorganisms, and 17% ($n = 4/24$) stated that their textbooks had descriptions of the same (Fig. 6A and B). For undergraduates, 76% ($n = 19/25$) had heard of superorganisms, and 52% ($n = 13/25$) responded that their course material or textbooks had descriptions of superorganisms (Fig. 6A and B). After the session, 86% of school students ($n = 18/21$) and 96% of undergraduates ($n = 24/25$) could identify the correct definition of superorganisms, and 67% ($n = 14/21$) and 60% ($n = 15/25$) of school students and undergraduates respectively were able to identify all the four examples of superorganisms provided (Fig. 9B and C).

Based on post-session feedback, 100% of school students and undergraduates ($n = 46/46$) correctly identified biofilms as bacterial communities (Fig. 10A), and 100% of undergraduates ($n = 25/25$) answered the importance of studying biofilms as to understand their roles in infection and the environment, and to fight antibiotic resistance

(Fig. 10B). However, 62% of school students ($n = 13/21$) included the additional option of 'to learn about beehives' in their response (Fig. 10B). While this was not the response we expected, it indicates that while analogies typically use a familiar entity to better understand an unfamiliar entity, the comparative aspects can be used to foster bidirectional understanding of the entities under discussion. In response to the question related to differentiating biofilms from single-celled bacterial forms, 40% of undergraduates ($n = 10/25$) identified all the relevant features of biofilms, namely increased tolerance to antibiotics, groups of microbes, difficulty in biofilm removal, and that single cells are less commonly observed as compared to biofilms, correctly (Fig. 10C). On the other hand, 56% of undergraduates ($n = 14/25$) identified all features except that single-celled microbes are less commonly observed as compared with biofilms (Fig. 10C). For school students, 33% ($n = 7/21$) identified two important biofilm features namely, tolerance to antibiotics and groups of microbes, and 28% ($n = 6/21$) identified groups of microbes alone as an important feature of biofilms. It is evident from the distribution of responses from school students that while the current form of the analogy provided an overall understanding of biofilms, underscoring that biofilms are more commonly observed as compared with the single form of microbial life would be important. This is notably relevant given that standard biology illustrations continue to depict microbial life as typical single celled forms.

Based on the segment on development and structure of biofilms, 100% of undergraduates ($n = 25/25$) and 57% of school students ($n = 12/21$) identified 'attachment' as the typical first step in biofilm formation (Fig. 11A). The other answers from school students included 29% respondents ($n = 6/21$) who selected 'formation of microcolonies', and 14% who selected 'dispersion' ($n = 3/21$). More than 90% of school ($n = 19/21$) and undergraduate participants ($n = 23/25$) identified the

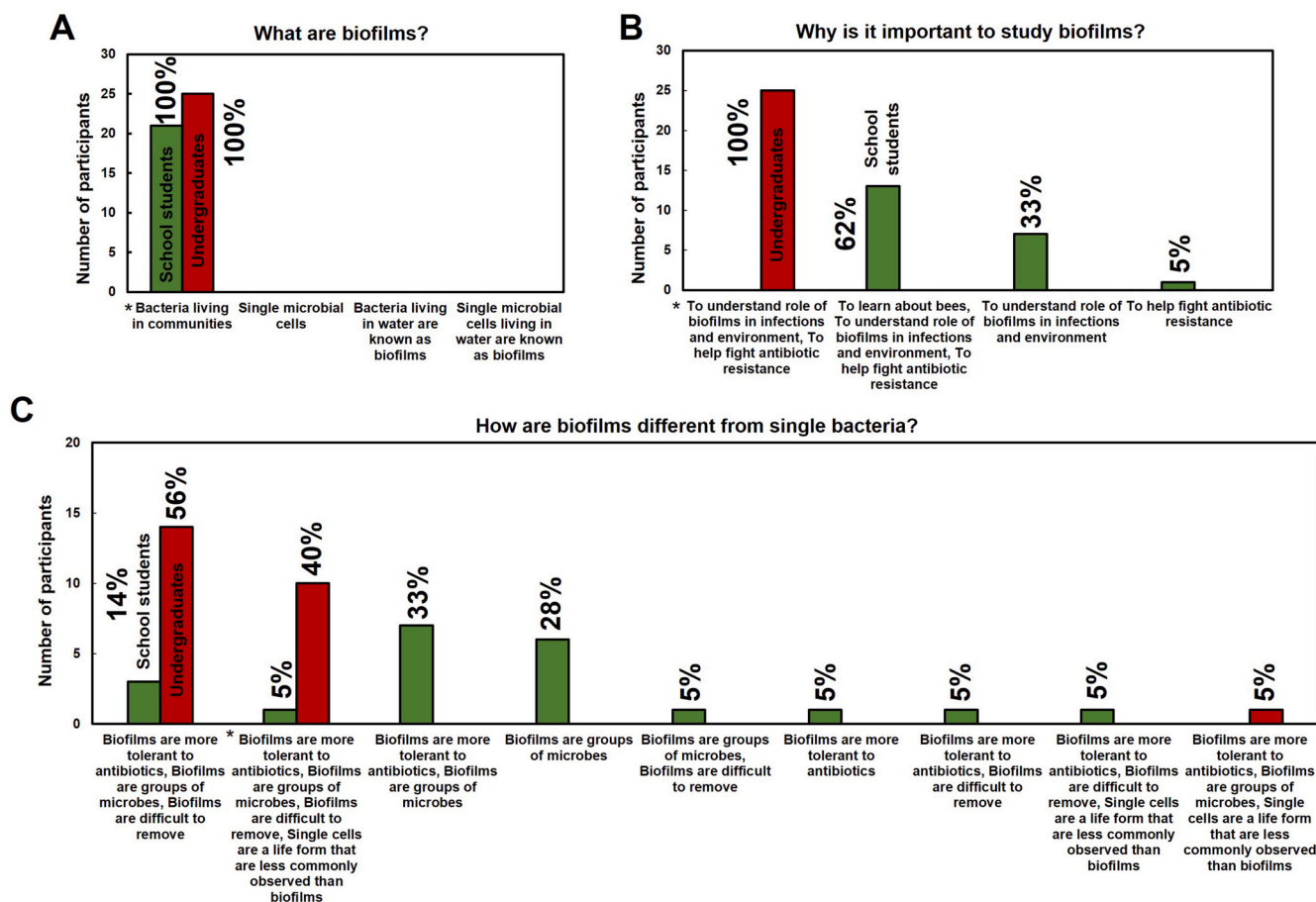


Fig. 10. Post-session feedback of the participants on the overall concept of biofilms. (A) Following the delivery of the analogy, 100% of school students and undergraduates ($n = 46/46$) correctly identified biofilms as bacteria 'living in communities.' (B) Based on feedback, 100% of undergraduates ($n = 25/25$) could identify the importance of studying biofilms. However, 62% of school students ($n = 13/21$), while identifying the correct reasons to study biofilms, also included the option 'to learn about beehives'. (C) Post-session feedback revealed that 96% of undergraduates ($n = 24/25$) correctly identified the differences between biofilms and single bacteria ($n = 14/25$ identified 3 out of 4 differences). However, the responses from the school students were distributed across the options. Percentages are calculated based on school and undergraduate respondents separately ($n=21$ for school students and $n=25$ for undergraduates). Asterisk (*) indicates the correct response to the question.

role of surface properties and cell-to-cell adhesion as important for biofilm formation; with 62% ($n = 13/21$) and 56% ($n = 14/25$) respectively, identifying both features (Fig. 11B). Further, 91% of school students ($n = 19/21$) and 100% of undergraduates ($n = 25/25$) correctly selected the description of EPS in biofilm matrix (Fig. 11C). In the segment on chemical communication, 100% of undergraduates ($n = 25/25$) correctly identified quorum sensing as the term for bacterial cell density dependent communication. On the other hand, the responses from school students varied, with only 48% identifying 'quorum sensing' ($n = 10/21$), followed by 24% selecting 'quorum sensing' ($n = 5/21$) (Fig. 11D). In the question on chemical communication, 92% of undergraduates ($n = 23/25$) and 52% of school students ($n = 11/21$) identified 'autoinducers' as the small molecules (Fig. 11E). This was followed by 'pheromones' as second most common response (from 38% of school students, $n = 8/21$), underscoring the importance of highlighting that pheromones are beehive communication molecules. The segment on division of labor in biofilms was largely focused on examples from *B. subtilis* biofilms, with examples drawn from contemporary scientific literature. Based on feedback, 96% of undergraduates ($n = 24/25$) and 76% of school students ($n = 16/21$) identified the two major matrix components resulting from division of labor in *B. subtilis* biofilms (Fig. 11F). However, only 52% of undergraduates ($n = 13/25$) and 33% of school students ($n = 7/21$) and were able to parse out the fact that absence of either one of these matrix components would result in absence of biofilm formation, with the remaining answering incorrectly

that lack of one component would lead to thicker or normal biofilms respectively (Fig. 11G). This is possibly due to the more complex nature of concepts in this segment of the analogy, and subsequent deliveries could focus on clarifying these aspects, particularly for the school students. Finally, 100% of undergraduates ($n = 25/25$) and 62% of school students ($n = 13/21$) correctly identified emergent properties those arising from groups of populations (Fig. 11H). It is important to note that in the pre-session feedback 76% of undergraduates ($n = 19/25$) and 38% of school students ($n = 9/24$) had reported being familiar with the term superorganisms (Fig. 6A and B).

Based on select anecdotal feedback, several participants were successfully able to recapitulate similarities between biofilms and beehives (Table 2). Importantly, based on responses, participants were able to highlight differences between biofilms and beehives (Table 3). Several important differences stated by the participants (Table 3) indicate that the differences between the two entities was appreciated. Select anecdotal feedback on new ideas that could be explored indicate that the participants were able to leverage comparisons between the two entities to develop novel ideas and lines of investigation for biofilms, not explicitly described in the session (Table 4).

Post-session feedback on the fun, engaging and informative components of the analogy-based instructional session, indicated that the majority (85% and above) of the school ($n = 20/21$ for fun, $n = 19/21$ for engaging, and $n = 20/21$ for informative) and undergraduate ($n = 24/25$ for fun, $n = 23/25$ for engaging, and $n = 22/25$ for informative)

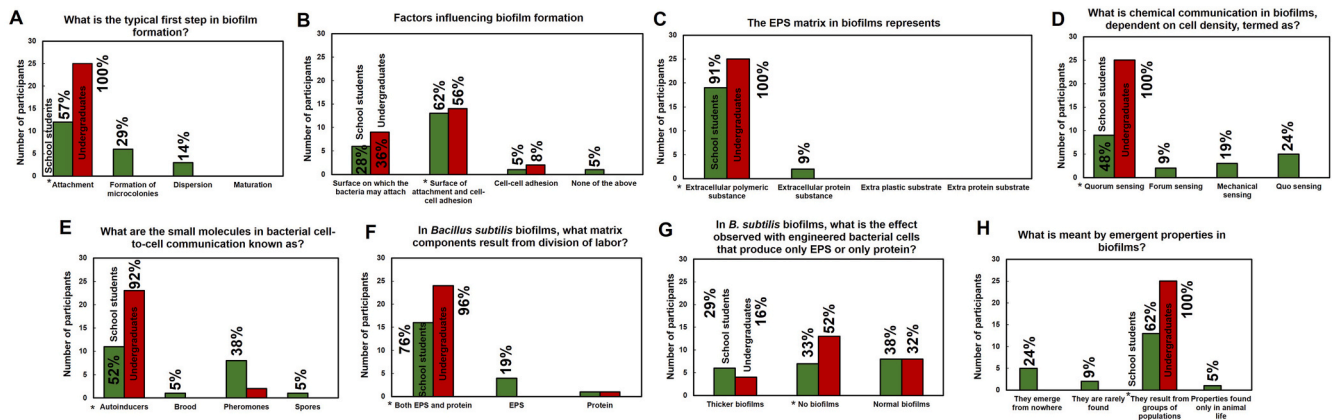


Fig. 11. Post-session feedback of the participants on the specific biofilm features covered in this analogy-based lesson. (A) Based on feedback, 100% of undergraduates ($n = 25/25$) and 57% of school students ($n = 12/21$) correctly identified ‘attachment’ as the first step in biofilm formation. (B) For the question on factors influencing biofilm formation, 62% of school students ($n = 13/21$) and 56% of undergraduates ($n = 14/25$) were able to identify both factors, surface of attachment and cell-to-cell adhesion. (C) The majority of school students (91%, $n = 19/21$) and undergraduates (100%, $n = 25/25$) identified the full form of EPS as extracellular polymeric substance. (D) The majority of undergraduates (100%, $n = 25/25$) identified quorum sensing as the term for chemical communication in biofilms. On the other hand, this was correctly identified by only 48% of school students ($n = 10/21$). (E) Based on feedback, 92% of undergraduates ($n = 23/25$) and 52% of school students ($n = 11/21$) correctly identified autoinducers as the small molecules in bacterial communication, 38% of school students ($n = 8/21$) responded with pheromones. (F) The majority of undergraduates (96%, $n = 24/25$), and 76% of school students ($n = 16/21$) identified EPS and protein as the matrix components in *B. subtilis* biofilms. (G) For the question on the effects observed with engineered *B. subtilis* cells that produce only EPS or only protein, 52% of undergraduates ($n = 13/25$) and 33% of school students ($n = 7/21$) responded with the correct answer ‘no biofilms’. (H) Based on feedback, 100% of undergraduates ($n = 25/25$) and 62% of school students ($n = 13/21$) correctly identified emergent properties as properties that emerge from groups of populations. Percentages are calculated based on school and undergraduate respondents separately ($n=21$ for school students and $n=25$ for undergraduates). Asterisk (*) indicates the correct response to the question.

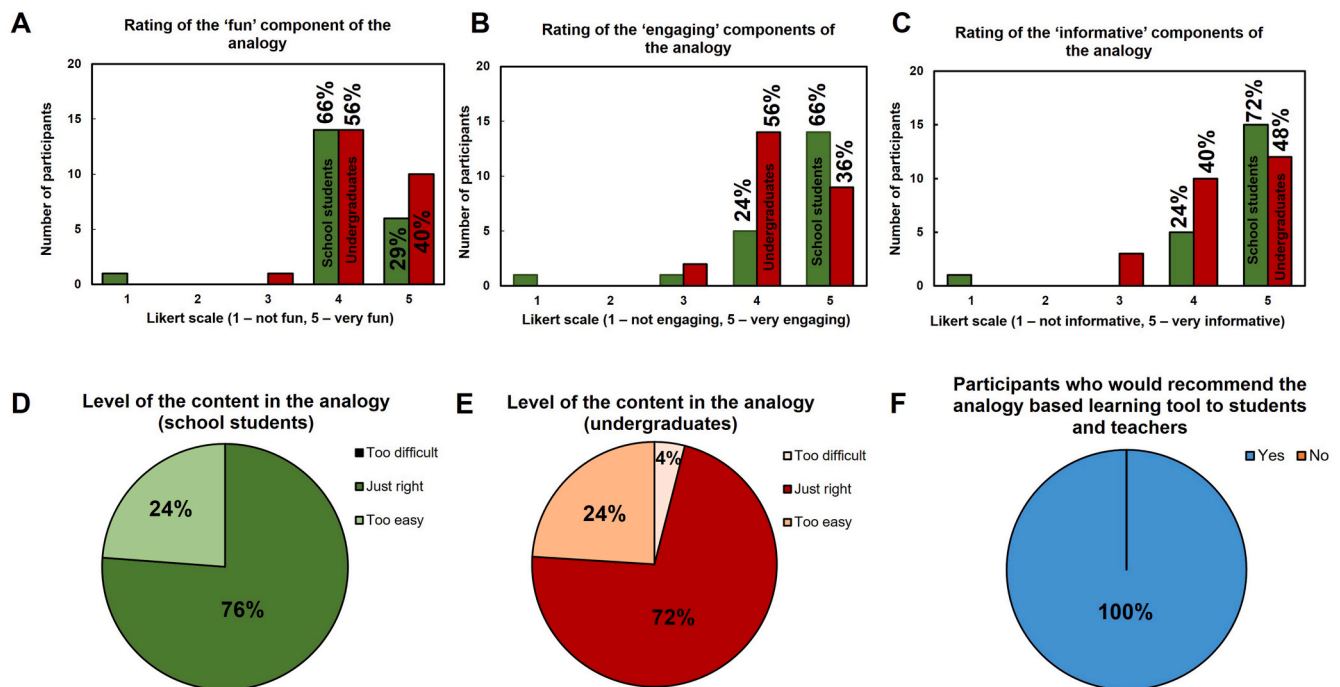


Fig. 12. Post-session feedback of the participants regarding the overall learning experience of the session. (A) Based on feedback, 95% of school students ($n = 20/21$) and 96% of undergraduates ($n = 24/25$) reported the ‘fun’ components of the session to be 4 or 5. (B) With respect to the ‘engaging’ components of the analogy, 90% of school students ($n = 19/21$) and 92% of undergraduates ($n = 23/25$) rated the session as 4 or 5. (C) With respect to the ‘informative’ components of the analogy, 96% of school students ($n = 20/21$) and 88% of undergraduates ($n = 22/25$) rated the session as 4 or 5. For A-C, a Likert scale of rating was used with a rating of 1 as least and a rating of 5 as highest. (D) Majority of the school participants (76%, $n = 16/21$) reported the level of the content as ‘just right’ and 24% ($n = 5/21$) said it was ‘too easy’. (E) For the undergraduates, 72% ($n = 18/25$) reported the level of the content as ‘just right’, and 24% ($n = 6/25$) said it was ‘too easy.’ One undergraduate responded that the session was ‘too difficult’. (F) All participants (school and undergraduate students, $n = 46/46$) responded that they would recommend the analogy-based lesson to students and teachers. Except for Fig. 12F, percentages are calculated based on school and undergraduate respondents separately ($n=21$ for school students and $n=25$ for undergraduates).

Table 2

Select anecdotal feedback in response to the question ‘List one way in which beehives and biofilms are similar’.

Respondent	Feedback
13 years, 8th grade	“They both house multiple members of a species”
13 years, 8th grade	“They both are collections of certain organisms”
14 years, 8th grade	“They both are groups of some living thing that have some special characteristics in common if they are in a group”
20 years, undergraduate	“Biofilms and beehives are both collections of individual organisms that live together in a structured manner with division of labour and emergent properties”
20 years, undergraduate	“Both form 3D structure and show division of labor within colonies”

Table 3

Select anecdotal feedback in response to the question ‘List one way in which beehives and biofilms are different’.

Respondent	Feedback
15 years, 9th grade	“Biofilms can be polymicrobial while beehives consist of bees of the same species”
19 years, undergraduate	“The foraging bees leave and return to the beehive, for collection of nectar and pollen, hence food is not synthesised in the beehive. Whereas, in biofilms, the dispersed bacteria never return to the biofilm and nutrition is synthesised within the colony”
19 years, undergraduate	“Mode of reproduction”
20 years, undergraduate	“Beehive has a central figure as the queen bee. Biofilm has no central coordinating element”

Table 4

Select anecdotal feedback in response to the question ‘Based on this analogy what new ideas could be explored in biofilms?’, indicating that the analogy can lead to the development of new ideas and hypotheses.

Respondent	Feedback
14 years, 8th grade	“I would like to test if there is any ‘leader’ in biofilms, as we observe in beehives”
15 years, 9th grade	“Is there a hierarchy or control that determines which cells attain persister phenotype?”
19 years, undergraduate	“In case a subpopulation in a biofilm gets destroyed, will the remaining members differentiate and acquire properties of the lost group?”
20 years, undergraduate	“Does the distribution of nutrients throughout a biofilm have an effect on what function a group of cell performs?”
19 years, undergraduate	“I would like to test if the persister cells act as a key component in biofilms as the queen bee in beehives”

Table 5

Select anecdotal feedback related to the engaging and informative aspects of the analogy-based session.

Respondent	Feedback
13 years, 7th grade	“The interactive session, the way we imagined different things and how we learnt in a fun way”
15 years, 9th grade	“We ended up learning about two things at the same time. It’s easy to correlate between the two topics because we have better understanding of one of the two topics. We could think about a wider range of aspects and come up with better questions”
20 years, undergraduate	“It used a phenomenon that we are familiar with and could therefore easily relate to. The information was engaging and well placed to ensure that our attention didn’t waver too much. The method of explaining was very simple and to the point”
20 years, undergraduate	“It was more engaging as I was trying to find similarities and difference between two subjects that were compared. It made the understanding of biofilms much easier and interesting”

participants rated these features as 4 or 5 on a scale of 1–5, with 1 being the lowest and 5 being the highest value (Fig. 12A–C, Table 5). This indicates that in addition to the scientific content, the students could relate positively with the learning experience. With respect to the level of the scientific content in the analogy, 76% of school students (n = 16/21) rated the content as ‘just right’ and 24% (n = 5/21) responded that it was ‘too easy’ (Fig. 12D). For the undergraduates, 72% (n = 18/25) rated the content as ‘just right’, with 24% (n = 6/25) responding as ‘too easy’ (Fig. 12E). While this indicates, it was appropriate for both educational groups, depending on the educational level and prior knowledge of the participants, the content of the analogy can be scaled up for undergraduates based on suggestions provided (Suppl Material). It is important to note that 1 undergraduate student rated the content as ‘too difficult’. This student also rated the fun, engaging and informative components of the analogy as 3 out of 5. Finally, 100% of post-session respondents (n = 46/46) responded that they would recommend the analogy-based learning tool to students and teachers for implementation in the curriculum (Fig. 12F).

3.3. Possible modifications of the instructional tool for school students and undergraduates

Based on pre-session and post-session feedback, it is evident that the lesson was informative and engaging for middle-to-high school students, but modifications to the analogy would help reinforce the scientific content at school level. This could be achieved using two approaches: prior to session and during the session. Prior to the session, the modified delivery of the analogy could include an instructor-guided overview of the pre-session reading materials, with a focus on the recommended key words and basic biology concepts in textbooks. During the session, challenging concepts and examples in the modules such as division of labor and emergent properties could be emphasized using student-led Q and A, as well as group enactment of the concepts with assigned roles in the form of name badges or placards. Further, advanced scientific terms such as ‘quorum sensing’ and ‘autoinducers’ could be highlighted using repetition and writing.

Based on feedback, the module was well-suited for an undergraduate curriculum, however the lesson could be modified to include ‘critical thinking points’ to work on in groups, based on application of the concepts covered in the module to open-ended questions. Examples of this include how the ecological effects of natural biofilms or artificial biofilm communities could be harnessed for wastewater management and bioremediation. Further, these open-ended discussions could also focus on understanding the possible advantages of features such as division of labor and emergence in biofilm communities. Further, the session could be modified to enable hands on activities, such as using internet resources or chemistry textbooks to illustrate chemical structures of the relevant molecules, with special emphasis on the functional groups.

Finally, to pace the instructional lesson, particularly for classes with students of various learning levels and abilities, the instructional tool could also be delivered over two sessions of 1 h each with discussion time included between the four module segments.

4. Conclusions

Based on virtual (webinar-based) delivery and feedback, this analogy-based instructional tool is an effective and engaging approach to introduce the concept of biofilms to middle-to-high school and undergraduate students. Analysis of content-related feedback from participants indicates that the learning of the participants aligned with the learning objectives of the module. With the suggestions provided, the instructional tool can easily be adapted to in-person delivery with additional group activities, as well as tailor-made to student knowledge levels.

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Data availability statement

Data generated in this study, including anonymized feedback data, will be made available by the authors' upon request.

CRedit authorship contribution statement

Snehal Kadam: developed the analogy and, Writing – original draft, delivered the analogy-based instructional tool, Formal analysis, the feedback data, and prepared the feedback data related figures. **Ankita Chattopadhyay:** developed the analogy and, Writing – original draft, prepared the analogy-related figures. **Karishma S. Kaushik:** developed the analogy and, Writing – original draft, prepared the analogy-related figures, worked on the revisions for the manuscript, delivered the analogy-based instructional tool, Formal analysis, the feedback data, and prepared the feedback data related figures.

Declaration of competing interest

The authors declare that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biofilm.2021.100066>.

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