Title Page

Is everything everywhere? A hands-on activity to engage undergraduates with key concepts in quantitative microbial biogeography

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Conflict of Interest Notifications Page

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Abstract & Keywords Page

The ubiquity and ease with which microbial cells disperse over space is a key concept in microbiology, especially in microbial ecology. The phenomenon prompted Baas Becking's famous "everything is everywhere" statement that now acts as the null hypothesis in studies that test the dispersal limitation of microbial taxa. Despite covering the content in lectures, exam performance indicated that concepts of dispersal and biogeography challenged undergraduate students in an upper-level Microbial Ecology course. Therefore, we iteratively designed a hands-on classroom activity to supplement the lecture content and reinforce fundamental microbial dispersal and biogeography concepts while also building quantitative reasoning and teamwork skills. In a class period soon after the lecture, the students formed 3-5-person teams to engage in the activity, which included a hands-on dispersal simulation and worksheet to guide discussion. The simulation involved stepwise neutral immigration or emigration and then environmental selection on a random community of microbial taxa represented by craft poms. The students recorded the results at each step as microbial community data. A field guide was provided to identify the taxonomy based on the pom phenotype and a reference to each taxon's preferred environmental niches. The worksheet guided a reflection of student observations during the simulation. It also sharpened quantitative thinking by prompting the students to summarize and visualize their and other teams' microbial community data and then to compare the observed community distributions to the idealized expectation given only selection without dispersal. We found that the activity improved student performance on exam questions and general student satisfaction and comfort with the biogeography concepts. Activity instructions and a list of needed materials are included for instructors to reproduce for their classrooms.

Keywords

1. Experiential learning
2. integrative learning
3. collaborative learning
4. active learning
5. reflective learning

6. Microbiology
7. Ecology
8. Biogeography

Introduction

We designed an active learning exercise for small student teams to explore and reinforce concepts in microbial biogeography and dispersal, which we had identified as challenging for students given past exam performance. We intentionally applied guidelines from experiential, integrative, collaborative, and reflective learning theories to do so. Experiential learning is the process of learning by experience, usually in the form of hands-on application of knowledge, & has been found especially effective in biology education (1, 2, 3, 4). Integrative learning theory aims to encompass multiple learning modalities and the interdisciplinary backgrounds of the students (5). This is crucial to successful collaborative learning, where students work together within small teams and across the larger class community to synthesize and share their knowledge (6). Finally, we asked students to reflect on their experience with this active learning exercise and provide feedback for future implementations. These three approaches are fundamental to the social constructivist learning theory, which posits that students construct meaning based on their previous knowledge and experience (7). In social constructivism, instructor awareness, sensitivity, and adaptation to the student's "come from" is vital to identifying and correcting student misconceptions and misunderstandings (7, 8). Moreover, an instructor can leverage the insights of students from backgrounds different to their own to provide a new perspective or model of the learning topic (7, 8).

The activity presented here is a hands-on, team-based classroom exercise that takes approximately 80 minutes, with natural "pause points" for division into multiple class periods as necessary to accommodate meaningful discussions. The complete instructional materials for the team activity are available within this manuscript and its associated appendices, and are also available on FigShare (https://doi.org/10.6084/m9.figshare.24243421.v1). While the activity was modified on one occasion to be implemented virtually (see Possible Modifications), in our experience, it works best as an in-person experience.

In the course for which the activity was designed, students worked in diverse teams of 4-5, assigned non-randomly using the CATME Teammaker (9). Briefly, the teams were constructed to maximize the diversity of majors represented, minimize out-of-class scheduling conflicts for team projects, and harmonize across student-anticipated efforts. Students worked in their assigned teams on several projects and for in-class activities throughout the semester to encourage collaboration and integration of their understandings of the material. One of the first team activities was a community diversity exercise ("Counting the Uncountable", see doi.org/10.6084/m9.figshare.24258727) that used craft pom microbial communities to ask teams to apply and calculate community diversity metrics and explore taxon abundance concepts in the context of a community (e.g., to identify rare and prevalent taxa). Each different color and size of pom (its phenotype) represented a different microbial taxon. Notably, here we use the term "taxon" to refer to an individual microbial population or species-equivalent, as would be used in today's microbiome research.

The biogeography activity presented here also used the craft pom communities as a model to actively reinforce concepts in microbial dispersal and environmental selection. But first, during a video lecture (flipped classroom), students were introduced to fundamental vocabulary and concepts, including Biogeography, Endemic, Cosmopolitan, Neutral Model, Occupancy, Abundance, Deterministic, and Stochastic. The team activity described here started with a team worksheet that first prompted students to verify their prerequisite biogeography knowledge from the lectures, including the fundamental vocabulary and concepts.

Next, the worksheet provided instructions and data collection forms for a hands-on biodiversity simulation using the now-familiar craft poms (Figure 1). Each team was given an environment card with different parameters to specify its pH and carbon, nitrogen, and water availability. The teams also received a guide that provides the niche preferences of each microbial taxon. Then, teams received a random collection of poms (several dozen), representing a subset of the full possible set of microbes (a.k.a. the metacommunity/regional species pool). Within their teams, the students first modeled how their randomly selected microbes would either die, survive, or thrive over 1 generation given the precise parameters of their team's environment and each taxon's preferred niche. Dead microbes were returned to the instructor, but increases in the thriving populations happened only on paper using simple math. This step represented the environmental selection of fit microbes. Then, the fit community underwent a dispersal event ("Windstorm") that resulted in the emigration of a random subset of their microbes to a neighboring team, and the immigration of a random subset of microbes from a neighboring team into their pom community. This dispersal was performed by mixing or shaking the remaining poms in a container or bag, and then randomly scooping out a small portion

(~25-33%) to physically hand off to a neighboring team. We recognize that population growth is only represented on paper, so the poms undergoing dispersal are not a perfect reflection of the community composition. After the Windstorm-driven dispersal, the environmental selection process was repeated for 2 generations. To practice interpreting and visualizing data, the teams combined their collection of environmentally fit microbial community members with those of other teams to create a biogeographic distribution of microbial taxa across the possible environments (e.g., number of environmental cards/teams).

Finally, the worksheet guided the teams through data analysis and interpretation. First, teams categorized each taxon on an axis ranging from endemic to cosmopolitan, which requires an understanding of these terms and the variable that these terms describe (occupancy). They then used the abundance data to predict where specific taxa would fall on an abundance-occupancy graph and to interpret whether each conformed to the expectation of the neutral model. Finally, teams qualified their confidence in their projections based on their perceived observational effort of the community and taxa (e.g., sampling depth or coverage). They thus were asked to estimate their certainty in a taxon's perceived abundance (Are rare taxa really cosmopolitan/endemic, or just rare? How do we know? What more information is needed, if any, and why?). These follow-up questions were discussed among the whole class to encourage teams to collaborate, to compare their responses, and to elaborate on differing answers to ensure students understand the spectrum of possible correct answers (where applicable) and the key distinctions underlying a correct answer versus an incorrect one. The worksheets were not graded, but the class discussions provided students feedback on their answers, and an instructor-developed worksheet key was provided to students to support exam preparation.

Intended audience and prerequisite student knowledge

This activity is intended for predominately upper-level (third- and fourth-year) undergraduate college students and first-year graduate students in fields related to biology, microbiology, or environmental sciences such as crop and soil sciences, geosciences, environmental engineering, and food safety and toxicology. Before this activity, students were introduced to fundamentals of microbial community ecology, including how to measure diversity, richness, evenness, rarefaction, rarity, and what an operational taxonomic unit (OTU)/taxon could represent. This team activity also assumes that students will have been given a lecture or other instructional materials on the basics of biogeography (our recorded video lessons are available here: doi.org/10.6084/m9.figshare.24243370) such that they can define microbial biogeography, the neutral model, occupancy vs. abundance, stochastic v. deterministic, and endemic vs. cosmopolitan.

Course delivery and learning time

The activity will take approximately 80 minutes to complete after the lecture material. Still, the exact duration depends on the size of the class and the number of teams included (we propose a maximum of 15 teams), with more teams requiring more time for inclusive discussion. However, it has natural break points that enable it to be pursued over multiple class periods and meet the instructor's timing needs. It could be extended with thorough discussion to cover a longer period, for example, a laboratory class period. We have also offered the activity virtually. Please see the Possible Modifications section.

Learning objectives

Upon completion of the associated lecture and this activity, students will be able to:

1. Define the following terms: biogeography, species-area relationships, neutral models, cosmopolitan, endemic
2. Relate spatial observational effort to patterns of microbial diversity
3. Hypothesize how microbial diversity may change across environmental gradients and provide examples
4. Use models of abundance-occupancy to identify taxa that fit the neutral expectation and those that do not
5. Explain how the environmental heterogeneity contributes to patterns of microbial populations and communities over space

This activity complements the associated lecture(s) by giving students an engaging, hands-on application of the biogeography concepts, and the worksheet explicitly connects the activity back to the lecture terms and concepts.

Procedure

Materials

For each *class* of up to 75 students, working in teams of 3-5 students (min 10 teams, max 15 teams), the instructor and students will need the following materials:

1. A mixed pompom pool (composition and taxonomy detailed in the "field guide" Figure 2 & Table 1)
    a. Assorted small (~1 cm) craft pompoms or comparable (~ 10 unique phenotypes) (Amazon, Need 1 unit)
    b. Small (~1 cm) sparkly craft green (or other unique color/size) pompoms or comparable (Amazon, Need 1 unit)
    c. Small (~ 1 cm) sparkly white craft (or other unique color/size) pompoms or comparable (Amazon, Need 1 unit)
    d. Assorted large (~4.5 cm) sparkly pompoms or comparable (~8 unique phenotypes) (Amazon, Need 2 units)
2. 1 large bowl or bag for mixing the pompom pool (this could be done ahead of time, but mixing the pool in front of students reinforces the randomness of their samples).
3. Up to 15 take out containers, cups, or bowls for distributing pompoms to teams (Amazon; Need 1 container per team + some extras)
4. 1 Printed copy of the Activity Environment Cards A-O, with the cards cut out (Appendix 1; Need 1 copy of each card)
5. 22-32 Printed copies of Activity Instructions (Appendix 2; Need 2 copies per team + 2 or more extras)
6. 12-17 Printed copies of Activity Data Sheet (Appendix 3; Need 1 copy per team + 2 or more extras)
7. 22-32 Printed copies of Worksheet (Appendix 4; Need 2 copies per team + 2 or more extras)
8. Classroom or Student-supplied laptops, smartphones, or tablets for entering and sharing class data (at least 1 per team)
9. Up to 30 Pens/pencils (at least 2 per team)

10. 1 pom “field guide” such as the one in Figure 2 can be helpful for students during the simulation. We used an overhead projector to display the field guide to the class.
11. 1 collaborative spreadsheet for data entry (e.g., Google Sheets or OneDrive), such as this one: https://tinyurl.com/368atmaw
    a. These data could also be entered onto a paper form by students and then projected as an overhead to share with the class, but this would take more time because the worksheet would have to be passed around to each team rather than enabling co-working on a digital data form

Of the above materials, each *team* of students will need the following:

- 2 copies of Activity Instructions (Appendix 2)
- 1 Environment Card, randomly assigned to each team
- 1 copy of the Activity Data Sheet (Appendix 3)
- 2 copies of the Biogeography Worksheet (Appendix 4)
- 1 takeout container of mixed pompoms (may be slightly less than full for 15 teams; try to distribute the pompom pool as evenly as possible)
- 1+ computer or laptop for data entry if using digital data form
- 1+ Pen/pencil

Faculty & student instructions

Instructors should provide the prerequisite lecture(s) either in the preceding class session or as a recording assigned before the activity, informing students that the following class session will be an activity. Before the activity day, instructors should obtain the pompoms and takeout containers, then compose the pompom community pool as prescribed in Table 1. Instructors should also prepare the necessary copies of the Activity Instructions (Appendix 1), Activity Data Sheet (Appendix 3), and Worksheet (Appendix 4), as well as print one copy of the Activity Environment Cards (Appendix 2) & cut out the cards. Finally, instructors should prepare a collaborative data form/spreadsheet for teams to combine the data generated during the activity. It should look like an empty copy of the “Original Survey Data” sheet on page 4 of the Activity Instructions. It is recommended that instructors prepare a link to this spreadsheet to make it easier for students to access.

At the start of the class session, instructors should prompt the students to form their teams of 4-5 members or as the instructor decides to organize students. The instructor should introduce the activity and hand out the printed Worksheets (Appendix 4), again 2+ copies per team.

The first step in the activity is for students to take 10 minutes to collaboratively complete the first page of the Worksheet: The Preamble. This page prompts students to define each of the key terms covered in the lecture that will be applied during the activity. Instructors should take 5 minutes to review the definitions of each term to ensure all teams have correct definitions to reference throughout the activity.

Second, the instructor will distribute one copy of the Activity Instructions and the Activity Data Sheet to each team & have students begin reading the instructions. While they read, the instructor will bring out the prepared community pool of pompoms, mix the pool in front of students, evenly divide the pool into the takeout containers, and tuck a random Environment Card into each container. The instructor will then distribute a random container to each team.

Third, the students will work through the activity, with the instructor lightly guiding teams through each step (see Figure 1), especially to coordinate when the "Windstorm" step takes place so that all teams exchange microbes at approximately the same time & not disrupting each other. At the end of the activity, the instructor will provide students with the link to the combined class data spreadsheet & one student from each team will enter the OTU abundance values from their "Finish" column into the appropriate column for their environment. In the meantime, the rest of the team can return their pompoms to the takeout containers and return the containers and Environment Cards to the instructor.

At this point, student teams can begin completing Part 2 of the Worksheet. The instructor should lightly monitor student teams, checking in with teams that seem stuck, quiet, or particularly divided. Depending on the class timing, student progress, and perceived difficulty, the instructor can choose whether to pause teams for class-wide discussion at the end of each of the three pages (for a limited time or for students who are struggling) or allow students to progress through the worksheet & discuss the entire worksheet at the end of the class session.

For the instructor, the clean-up involves recombining the pompoms and Environment Cards from the takeout containers. If the students did write on their Activity Instructions, those can be collected for re-use. Students may retain their completed worksheets unless the instructor wants to grade them. The instructor should later provide students with the Worksheet Answer Key for review and exam preparation.

Tips & Tricks

If there are fewer than fifteen teams, remove environments A, B, and O from the pool because they are redundant with other environments. Environments A, B, and O are on a separate page from environments C – N to easily exclude them during printing.

We recommend printing the Activity Instructions, Data Sheet, and Worksheet as single-sided. This ensures that students are not trying to enter their classroom data on the other side of the same piece of paper as the instructions or questions.

This activity works best when students can co-locate together to discuss. If the classroom has rigid row-style seating, plan to supply an additional copy of the Activity Instructions and Worksheet or provide digital copies of the materials and prompt students with laptops to refer to the digital versions.

We used craft poms, but any collection of small objects could be substituted to assemble the communities and regional species pool, for example: buttons, shells, stickers, etc. We originally tried candies (allergen caution), but found that some students ate some of the community members before the end of the activity, which could impact the teams' resulting species distributions.

Activity timing (80-minute class)

1. (Worksheet) Preamble Review – 10 min; teams work in teams for 5 min review of concepts, 5 min for the instructor to review answers and answer questions.
2. (Activity) Part 1: The Experiment – 15 min; get students started reading the instructions while the instructor mixes and distributes the pompoms.
3. (Worksheet) Part 2: Analysis & Synthesis – Teams should work for 30 min with the instructor circulating between teams to monitor progress and intervene in teams getting stuck on a topic too long.

4. Discussion of Part 2 – 25 min; Instructor leads teams in sharing their answers and sharing their logic.

Suggestions for determining student learning

The students and the instructor can determine student learning through formative and summative assessments. The classroom discussions after the Preamble Review and the Analysis and synthesis parts of the activity enabled students to self-assess their understanding of the concepts and clarify misconceptions at multiple stages. In addition, while the completed worksheets were not graded for correctness, we found it informative to review students' work for accuracy and completeness (Appendix 6). Combined with our notes on the classroom discussions during the activity, this review process informed minor reorganization of the worksheet, suggested areas for improvement in student preparedness for the activity, and provided feedback on our time management. We gave students feedback on their worksheets and a copy of the worksheet key to support their exam preparation. We based this study on several exam questions and used these same questions to assess the impact of the activity on student learning (Appendix 7). The alignment between the Learning Objectives, activity worksheet prompts, and exam questions is provided in Table 2. In our study, these were administered once as a summative assessment in the subsequent exam, but these questions could also be administered before the activity to assess established knowledge.

Sample data

Anonymous data and R code used for visualization and analyses are provided on GitHub (https://github.com/natalie-vandepol/BiogeographyActivity). Two examples of student responses to the questions in Part 2 of the Worksheet are provided in Appendix 6. These two examples highlight some of the variations that can occur in student responses.

Safety issues

There are no safety issues beyond COVID-19 exposure management due to teamwork and material handling associated with this course. A remote teaching modification is provided in the discussion.

Discussion

Field testing

We have used this activity in four semesters of Microbial Ecology (MMG 425) course at Michigan State University, an offering within the Department of Microbiology and Molecular Genetics in the College of Natural Sciences. MMG 425 was an elective for Microbiology and Molecular Genetics majors, a requirement for the MMG majors who opt into the Environmental Microbiology track, and Biosystems Engineering majors. It was also taken as an elective by other majors, including crop and soil sciences, geosciences, environmental engineering, food safety and toxicology, and environmental sciences. It enrolled between 40-65 students and met twice a week for 80 minutes each class period. Most of the undergraduates in MMG 425 were juniors or seniors, though there was an occasional sophomore, and the breakdown was ~ 60-75% MMG majors with ~20-30% engineering majors and ~5-15% other. It was also often taken by first-year graduate students. Most of these graduate students were pursuing Master's degrees in crop and soil sciences or related disciplines, but some were PhD track. Notably, there were no prerequisites for MMG 425 due to the broad and interdisciplinary representation of the enrolled students. This simultaneously offered a challenge and potential for learning enrichment because the students arrived with sometimes very different disciplinary knowledge and cultures.

This activity was created to respond to our initial observation of student underperformance on the biogeography content of the final exam in spring 2018 (SS18) (semesters & class sizes indicated in Table 3). The activity was first introduced in the spring semester of 2019 (SS19) and then continued into the fall semester of 2019 (FS19), fall 2020 (FS20, *virtual offering*), and fall 2021 (FS21). Primary field-testing data were collected in SS19 and fall FS19. Students were informed of the collection of standard classroom data for activity assessment & data gathering was approved by the Michigan State University Institutional Review Board (IRB) (Office of Regulatory Affairs Human Research Protection Program, Study ID STUDY00001727) with exempt determination under 45 CFR 46.104(d) 1.

Instructor Observations

In both SS19 & FS19, we observed student satisfaction with the activity, noting smiles, laughter, and enthusiastic discussion. Some teams divided up responsibilities between members for the simulation and then worked together to answer the discussion questions. It was often evident which team members had watched/attended the lecture before the class session. Still, most students had a laptop to access the prior posted course materials and search the internet for definitions, which enabled students to progress and succeed even without having watched the lecture.

Immediate Student Feedback: Post-Its

In SS19 and FS19, at the end of the activity, we collected Post-It Notes on which students anonymously responded to two prompts:

1. What (about the learning activity) increased your understanding?
2. What (concepts) are you still confused about?

This anonymous Post-It feedback was largely positive. Most students reported an increased understanding of biogeography terms, particularly definitions (Table 4). Most lingering confusion focused on the details of the neutral model and the graphical representation thereof. The activity particularly stressed that endemism/cosmopolitanism strictly described occupancy, which was reflected in the proportion of responses that mentioned both subjects.

Evidence of student learning

Worksheet Responses

The worksheets were noted as complete or incomplete, and personalized feedback was given to build relationships and establish instructor presence. This allowed us to informally assess where students seemed to have struggled (if anything was crossed out) or if a particular team had short or wrong answers or had not completed the worksheet.

Exam Performance

The objective of this activity is to increase student understanding of concepts in microbial biogeography and dispersal. The exam data gathered in testing this activity supported the hypothesis that observing a simulated community over time and applying the course concepts to analyze simulation data would improve student performance on exam questions.

Several exam questions unaffected by the intervention were used to compare students' "baseline performance" between semesters. A large difference in baseline performance would indicate that we should normalize the scores on exam questions related to the intervention. We found that baseline exam performance decreased on the SS19 exam compared to the pre-intervention SS18 exam, while preliminary tests significantly increased performance on the biogeography exam questions. Since we were only interested in determining whether student performance increased, it was deemed unnecessary to inflate the improvement in scores by normalizing the data between the semesters. We did not assess baseline exam performance in FS19.

SS18 was a non-cumulative final exam, whereas SS19 and FS19 were midterm exams. There were several changes necessary for this adjustment, including some recombination of questions and the distribution of question versions between two exam versions: F2-4 became M2a/b/c and F5-7 became M3a/b/c, where F indicates the final exam version, and M indicates the midterm exam version of each question (Appendix S7). The activity described here also inspired two new exam questions (M5 & M6. This meant that, while all 58 students in FS18 answered all the questions, only about half of the students in SS19 and FS19 answered each of questions M2a or M2b, M3a and M3b or M3c, and M5a or M5b (Table 5). Questions M1, M3, M5, & M6 are about the neutral model and the underlying principles of abundance and occupancy. Question M2 is about Endemism and Cosmopolitanism. Question M4 is about how environmental gradients shape microbial diversity.

For questions M1, M2a, M2b, M3a, M3b, and M3c, we saw a statistically significant increase (Wilcoxon rank sum test, $p < 0.08$) in mean percent score in the semesters with the activity (SS19 & FS19) as compared to SS18 (Figure 3). M2c and M4 are the two questions that showed no meaningful changes between the semesters, but we noted that these two questions were those on which students historically did not generally struggle. Only question M5a showed any significant difference in mean score between SS19 and FS19. This indicates that the activity presented here significantly impacted student understanding of microbial biogeography concepts, particularly in areas with the lowest scores.

Possible modifications

We modified this activity during the COVID-19 pandemic in the fall semester of 2020, during which Michigan State University had virtual offerings. The typical in-person lectures were converted to four shorter video lectures, and the team materials (worksheet, environment cards, pom communities) were distributed digitally ahead of the class via Michigan State’s course management platform. The pom communities were provided as digital photographs; the instructor created photos of the random pom communities and then also took photos of those that were dispersed into and out of the community (e.g., three photos per team: the original set, a photo of poms that emigrated, and a photo of poms that immigrated). Notably, the same photo of the poms that immigrated away from one pom community was also used as the photo of the poms that emigrated into another, such that there was a complete “dispersal” inclusive of all teams (these pom community photos are available on FigShare at https://doi.org/10.6084/m9.figshare.24243421.v1). Teams met with the instructor in a synchronous Zoom session where breakout rooms were employed for teamwork (team members were *a priori* assigned to the same room). For discussion, teams were brought together in the main Zoom room. The instructor moved between breakout rooms to address questions. Because of the extra time needed to accommodate the virtual activity took longer than the usual 80 minutes, and the final class discussion occurred at the beginning of the subsequent synchronous period. This modification demonstrates that the activity is flexible to be broken down into smaller sessions or extended according to instruction needs.

Conclusions

In conclusion, the biogeography activity described here is inexpensive and easy to implement. It provides a forum for students to deeply engage with microbial ecology concepts and practice applying them to classroom data in a way that can meaningfully impact student learning and build quantitative reasoning. The active, collaborative, reflective teaching methodologies upon which the activity was founded foster a safe, fun, highly responsive environment where students work with the instructor to explore and learn.

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Figures & Tables

Figure 1 – A flowchart of the Biogeography Activity

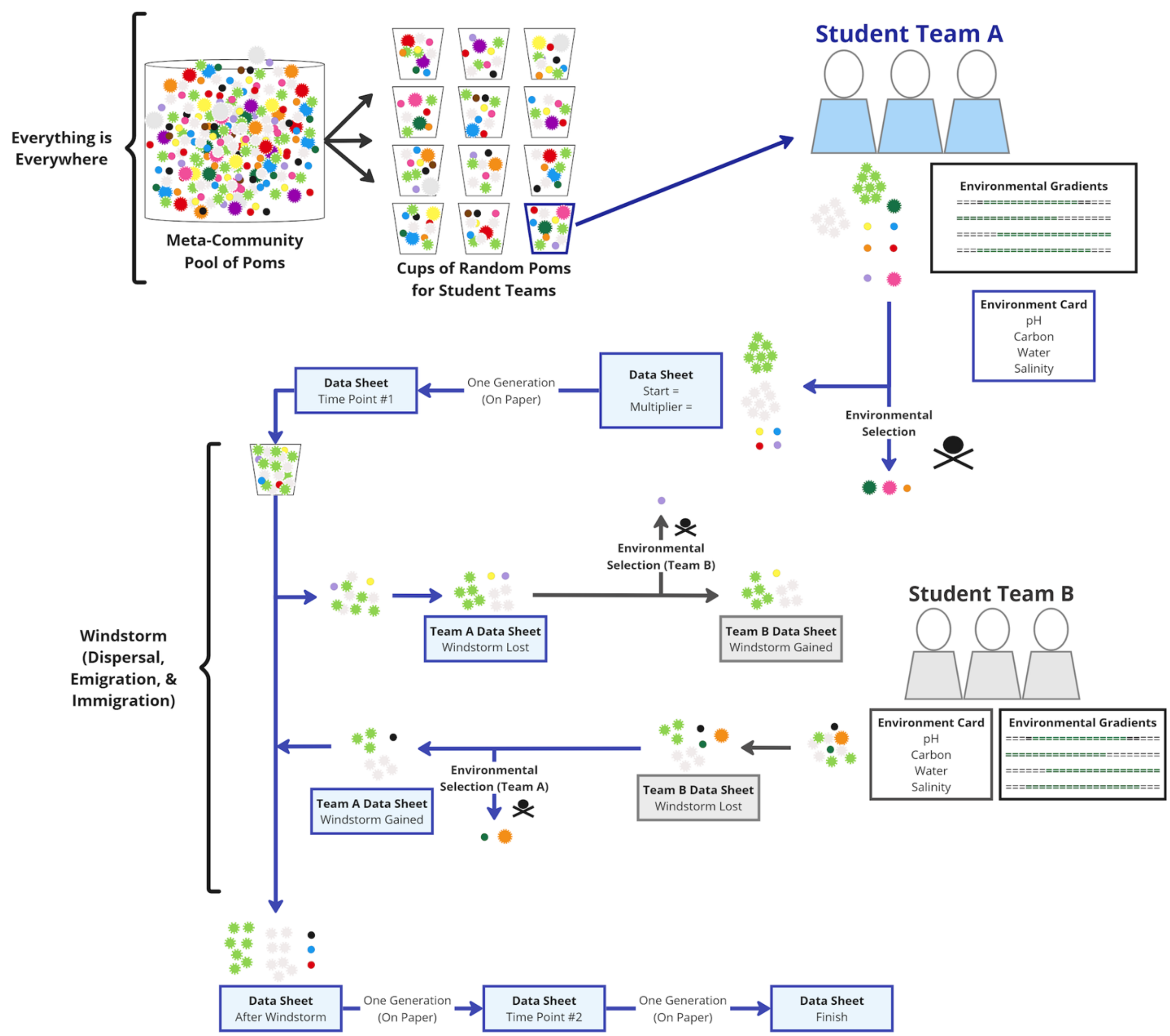


Figure 2 – A "field guide" to the pompoms used in the Biogeography Activity. Numbers and descriptions correspond to the OTU number and description used in the activity sheets.

Figure 3 - Mean Percent score on biogeography exam questions. There is a separate box for each question, labeled by question number. Bars are colored by year, the bar height indicates the mean percent score, and the lines at the top of the bars indicate the standard error. Within each box, the horizontal lines and associated numbers indicate the p-value of Wilcoxon Rank Sum tests between the indicated population means. SS is the spring semester, FS is the fall semester, and the last two digits represent the year of implementation (2018 or 2019).

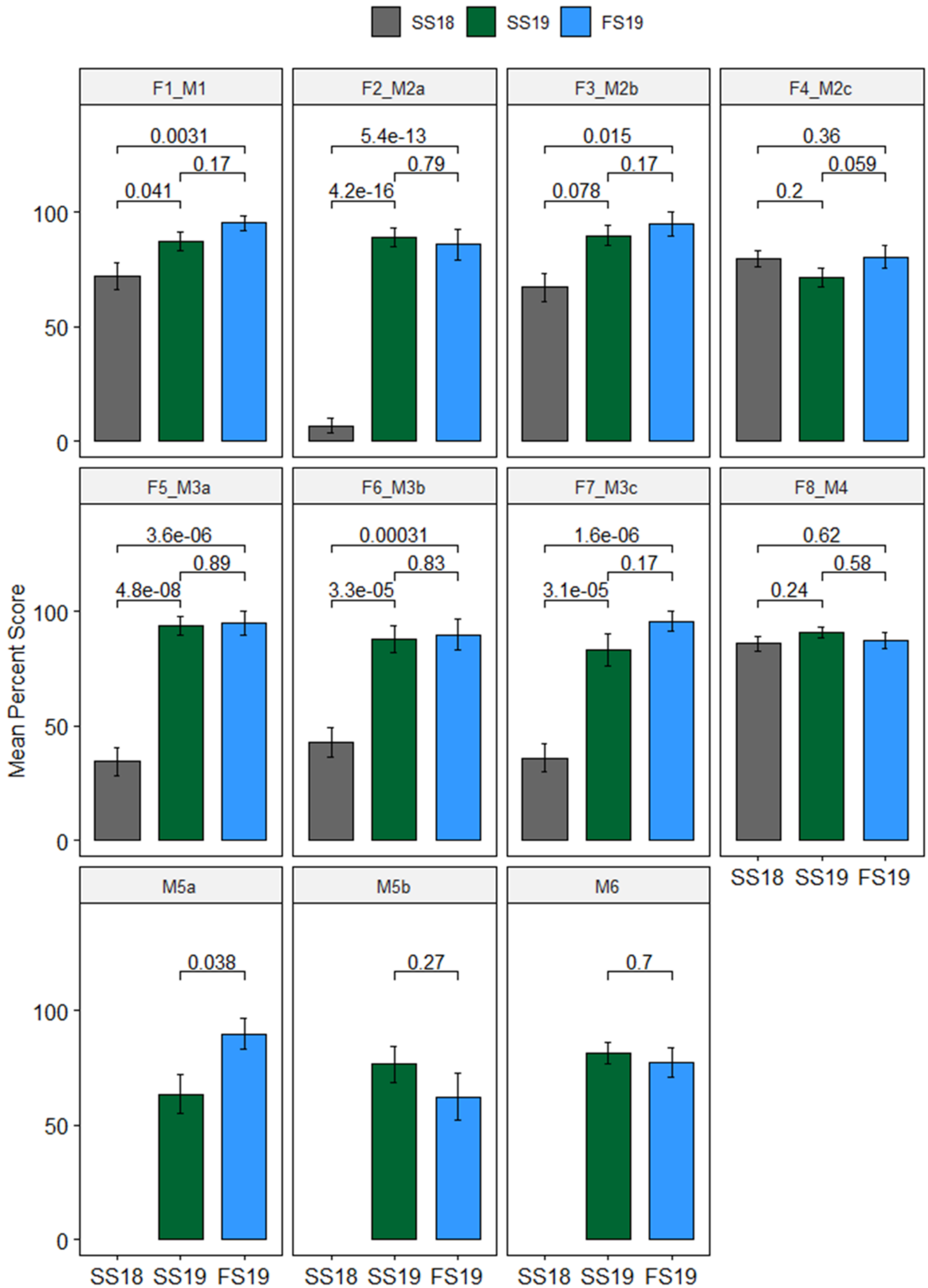

SS18
SS19
FS19
Mean Percent Score
F1_M1
0.0031
0.17
0.041
F2_M2a
5.4e-13
0.79
4.2e-16
F3_M2b
0.015
0.17
0.078
F4_M2c
0.36
0.059
0.2
F5_M3a
3.6e-06
0.89
4.8e-08
F6_M3b
0.00031
0.83
3.3e-05
F7_M3c
1.6e-06
0.17
3.1e-05
F8_M4
0.62
0.58
0.24
M5a
0.038
M5b
0.27
M6
0.7
0
50
100
SS18 SS19 FS19

Table 1 – **The composition of the pom regional species pool for the Biogeography Activity.**

OTU No.	OTU Descriptor	Descriptor	Total in Pompom Pool
1	Small Sparkly Green	Abundant, Persistent (Cosmopolitan)	150
2	Small Sparkly White	Abundant, Patchy	200
3	Small Purple	Competitive exclusion: Med dom. but rare when OTU 10 is present	45
4	Small Black	Medium abundance, patchy, inverse of OTU 1	60
5	Small Green	Medium abundance, gradient increase	35
6	Small Yellow	Medium abundance, gradient decrease	50
7	Small Blue	Medium abundance, persistent	60
8	Small Red	Medium abundance, categorical (A-F, G-O) patchy	50
9	Small Orange	Medium abundance, gradient unimodal	35
10	Small Pink	Abundant in three communities / categorical G-O, otherwise rare (Endemic)	75
11	Small Brown	Rare persistent (Cosmopolitan)	30
12	Small White	Rare patchy correlated with black	30
13	Large Sparkly Orange	Rare persistent	12
14	Large Sparkly Yellow	Rare but present entirely in only one community (Endemic)	17
15	Large Sparkly Green	Rare persistent (Cosmopolitan)	15
16	Large Sparkly Pink	Rare across two communities categorical G-O (Endemic)	15
17	Large Sparkly White	Rare patchy	10
18	Large Sparkly Purple	Rare patchy	7
19	Large Sparkly Blue	Rare singleton	10
20	Large Sparkly Red	Rare singleton	10

Table 2 – Alignment between learning objectives and assessment activities

Learning Objective	**Worksheet Questions**	**Exam Questions**
1. Define the following terms: biogeography, species-area relationships, neutral models, cosmopolitan, endemic	Preamble	F1_M1, F2_M2a, F3_M2b, F4_M2c, M5a, M5b, M6
2. Relate spatial observational effort to patterns of microbial diversity	Part 2, Q7a-7c	F1_M1, F2_M2a, F3_M2b, F4_M2c
3. Hypothesize how microbial diversity may change across environmental gradients and provide examples	Part 2, Q6	F2_M2a, F3_M2b, F4_M2c, F8_M4
4. Use models of abundance-occupancy to identify taxa that fit the neutral expectation from and those that do not	Part 2, Q9a-9c	F5_M3a, F6_M3b, F7_M3c, M5a, M5b, M6
5. Explain how the environmental heterogeneity contributes to patterns of microbial populations and communities over space	Part2, Q5, Q6, Q7c	F2_M2a, F2_M2b, F8_M4

Table 3 – Summary of class sizes and their participation in the student data reported here.

Semester	Students Enrolled	Activity Presented	Data Collected	Students who took the exam
SS18	58	No	Yes	58
SS19	63	Yes	Yes	63
FS19	46	Yes	Yes	44
FS20	49	Yes	No	N/A
FS21	39	Yes	No	N/A

Table 4 - Summary of Student Feedback. After the activity, we asked students for anonymous feedback on Post-It notes. Values indicate the number of responses associated with each main biogeography subject, and "Other" indicates that the response was not specific to any of the categories or was altogether unclear.

Year	Prompt	Endemism/ Cosmopolitanism	Neutral Model & Abundance/ Occupancy	Environmental Selection	Other
SS19	Q1: Increased Understanding	13	23	1	9
	Q2: Still Confused	1	18	3	3
FS19	Q1: Increased Understanding	7	20	1	6
	Q2: Still Confused	0	12	2	10

Table 5 - Number of Students who answered each exam question each semester. Total indicates the number of students who took the exam. The values for each question (columns) and each year (rows) are the number of scores represented in Figure 4 and used in the statistical analyses. Exact prompts for each exam question (e.g., F1_M1) are linked in Appendix 7: Example Exam Questions.

	Total	F1_M1	F2_M2a	F3_M2b	F4_M2c	F5_M3a	F6_M3b	F7_M3c	F8_M4	M5a	M5b	M6
SS18	58	58	58	58	58	58	58	58	57	NA	NA	NA
SS19	63	63	33	30	63	33	33	30	63	33	30	63
FS19	44	43	24	20	44	20	20	23	44	20	24	44

Supplemental Materials

Appendix 1: Activity Environment Cards for use in the activity

Appendix 2: Activity instruction sheet for use in the activity

Appendix 3: Blank Activity data sheet for use in the activity

Appendix 4: Blank Worksheet for use in the activity

Appendix 5: Worksheet answer key

Appendix 6: Sample student worksheets to demonstrate potential student responses

Appendix 7: Exam Questions used in our study

Supplemental Materials

Appendix 1: Activity Environment Cards

ENVIRONMENT C		**ENVIRONMENT G**		**ENVIRONMENT K**	
pH	5	pH	7	pH	9
Water	Scarce	Water	Abundant	Water	Abundant
Salinity	Low	Salinity	Low	Salinity	High
# Carbon Sources	Low	# Carbon Sources	Low	# Carbon Sources	Low

ENVIRONMENT D		**ENVIRONMENT H**		**ENVIRONMENT L**	
pH	5.5	pH	7.5	pH	9.5
Water	Scarce	Water	Abundant	Water	Abundant
Salinity	Low	Salinity	Low	Salinity	High
# Carbon Sources	High	# Carbon Sources	High	# Carbon Sources	High

ENVIRONMENT E		**ENVIRONMENT I**		**ENVIRONMENT M**	
pH	6	pH	8	pH	10
Water	Scarce	Water	Abundant	Water	Abundant
Salinity	Low	Salinity	High	Salinity	High
# Carbon Sources	Low	# Carbon Sources	Low	# Carbon Sources	Low

ENVIRONMENT F		**ENVIRONMENT J**		**ENVIRONMENT N**	
pH	6.5	pH	8.5	pH	10.5
Water	Scarce	Water	Abundant	Water	Abundant
Salinity	Low	Salinity	High	Salinity	High
# Carbon Sources	High	# Carbon Sources	High	# Carbon Sources	High

ENVIRONMENT A	
pH	4
Water	Scarce
Salinity	Low
# Carbon Sources	Low

ENVIRONMENT B	
pH	4.5
Water	Scarce
Salinity	Low
# Carbon Sources	High

ENVIRONMENT O	
pH	11
Water	Abundant
Salinity	High
# Carbon Sources	Low

Appendix 2: Activity Instructions

PART 1: The Experiment

Materials: A Martian rock microbial community (takeout container of pompoms), this worksheet and a pen/pencil. At least one group member should have a laptop to record the final values your group finds.

Instructions: You and your colleagues have characterized the environmental conditions present at several sites that another team previously sampled. They have given you their Original Survey Data regarding the microbial communities present at each site. You expect that environmental conditions are driving community structure over space and have received permission from NASA to test this hypothesis.

You and your colleagues have planted sterile rocks of known chemical/physical composition and can watch in real time as microbes colonize these new, "blank slate" habitats! Each team will independently and carefully evaluate the microbial diversity of one newly colonized rock community at several time points.

- Each collection of pompoms is a Martian rock microbiome that is colonizing the new rock/environment.
- Each piece of pompom is individual from the microbiome community
- Each type of pompom is an operational taxonomic unit (OTU; i.e., or species of microbe).

EXPERIMENTAL PROCEDURE:

1.A. Assemble your new Martian rock community: "Everything is everywhere…"

1. On the Environmental Gradients Table (page 3), mark the **columns** relevant to your environment.
2. Each **row** is an OTU. If there are any "skulls and crossbones" in the relevant columns for that row/taxon, then that taxon will go extinct in your environment. Mark these extinct OTUs with X's in the Start Column of the Community Simulation table (page 4).
3. Get a takeout container of pompoms. Sort them out into OTUs. Determine which OTUs can persist in your environment, and which go extinct (note potential interaction between OTU's 3 & 10).
4. Return the OTUs that go extinct to an instructor. Count the population sizes for each of the surviving OTUs.
5. Record your counts in the "**Start**" column of the Community Simulation table.

1.B. "…but the environment selects."

1. Use the Environment Gradient Table (page 3) to determine the appropriate multiplier for your remaining taxa. For each OTU in your community, look across the 4 columns relevant to your environment. Whichever of these columns has the <u>lowest number</u>, that number is your Multiplier.
2. Record the **Multiplier** for each OTU in the Community Simulation table.
3. For each OTU, calculate the abundance value for Time Point 1.

Time Point #1 = Multiplier * Start

1.C. Windstorm!

1. Put your living OTU pompoms back in your take-out box & shake them up, then (no peeking!) select ~10-15 random pompoms (a small handful). Record these emigrant OTUs in the "**Windstorm Lost**" column.
2. Pass these random pompoms to your neighboring group.
3. Receive immigrant OTUs from a neighboring group.
4. Determine if any of the immigrant OTUs go extinct given your environment (note potential interaction between OTU's 3 & 10). In the "**Windstorm Gained**" column, mark those cells with an X.
5. Record the surviving immigrant OTU counts in the "**Windstorm Gained**" column.
6. For each OTU calculate the abundance value for After Windstorm.

   After Windstorm = Time Point #1 - "Windstorm Lost" + "Windstorm Gained"

1.D. Time Passes

1. For each OTU, calculate the abundance value for Time Point 2.

   Time Point #2 = Multiplier * After Windstorm

1.E. Finish

1. Calculate the Finish abundance.

   Finish = Multiplier * Time Point #2, rounded up to whole integers (e.g., 1.2 → 2).

2. Enter the Finish values in the spreadsheet provided by your instructor.

*************** **Simulation Complete** ***************

Environmental Gradients

OTU No.	OTU Description	pH 4	pH 4.5	pH 5	pH 5.5	pH 6	pH 6.5	pH 7	pH 7.5	pH 8	pH 8.5	pH 9	pH 9.5	pH 10	pH 10.5	pH 11	Salinity Low	Salinity High	Carbon Low	Carbon High	Water Scarce	Water Abundant
1	Small Sparkly Green	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
2	Small Sparkly White	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2
3	Small Purple **	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
4	Small Black	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	☠	2	2	2
5	Small Green	☠	☠	1	1	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2	2	2	2	2	2	2	2
6	Small Yellow	2	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1	☠	☠	☠	☠	2	2	2	2	2	2
7	Small Blue	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
8	Small Red	2	2	2	2	2	2	1	1	1	1	1	☠	☠	☠	☠	2	1	2	2	2	1
9	Small Orange	1.1	1.2	1.3	1.4	1.5	1.6	1.8	2	1.8	1.6	1.5	1.4	1.3	1.2	1.1	2	2	2	2	2	2
10	Small Pink	☠	☠	☠	☠	☠	☠	1	1.5	1.5	2	2	2	2	2	2	☠	2	☠	2	☠	2
11	Small Brown	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	2	2	2	2	2	2
12	Small White	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	☠	2	2	2
13	Large Sparkly Orange	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	☠	2	2	2
14	Large Sparkly Yellow	☠	☠	☠	☠	☠	☠	☠	☠	☠	☠	2	2	2	☠	☠	☠	2	☠	2	☠	2
15	Large Sparkly Green	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	☠	2		2
16	Large Sparkly Pink	☠	☠	☠	☠	☠	☠	2	2	2	2	2	2	2	☠	☠	2	2	☠	2	☠	2
17	Large Sparkly White	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	☠	2	2	2
18	Large Sparkly Purple	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	☠	☠	2	2	2
19	Large Sparkly Blue	☠	☠	☠	☠	☠	☠	1	1	1.5	1.5	2	2	2	2	2	2	2	☠	2	2	2
20	Large Sparkly Red	☠	☠	☠	☠	☠	☠	1	1	1.5	1.5	2	2	2	2	2	2	2	☠	2	2	2

** **CANNOT survive when OTU 10 (Small Pink) is present**

☠ Goes extinct

Original Survey Data (without influence of dispersal)

OTU No.	OTU Descriptor	Community/Environment														
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Small Sparkly Green	33	33	34	33	33	34	33	33	34	33	33	34	33	33	34
2	Small Sparkly White	63	6	60	8	56	0	58	6	61	0	60	6	56	4	56
3	Small Purple	9	10	10	12	11	11	10	12	14	0	16	0	14	0	17
4	Small Black	0	20	0	16	0	16	0	18	0	19	0	15	2	22	0
5	Small Green	0	0	1	2	3	4	5	6	7	7	10	13	14	22	30
6	Small Yellow	20	19	18	15	10	9	8	6	6	5	3	0	0	0	0
7	Small Blue	7	9	7	7	7	10	7	10	7	9	7	6	7	8	6
8	Small Red	10	14	22	20	14	24	0	1	0	1	2	0	0	0	0
9	Small Orange	1	2	5	5	7	10	13	14	10	9	8	7	6	2	0
10	Small Pink	0	0	0	0	0	0	0	0	0	28	0	33	0	25	0
11	Small Brown	2	3	4	2	2	2	3	4	1	2	1	2	3	3	2
12	Small White	0	3	0	3	0	1	0	1	0	3	0	4	0	1	0
13	Large Sparkly Orange	0	1	0	1	0	0	0	2	0	1	0	2	0	1	0
14	Large Sparkly Yellow	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0
15	Large Sparkly Green	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0
16	Large Sparkly Pink	0	0	0	0	0	0	0	2	0	0	0	3	0	0	0
17	Large Sparkly White	0	0	0	1	0	0	0	1	0	1	0	1	0	0	0
18	Large Sparkly Purple	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0
19	Large Sparkly Blue	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
20	Large Sparkly Red	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Appendix 3: Activity Data Sheet

YOUR ENVIRONMENT (A-O): _____

Community Simulation Data Table

OTU No.	OTU Descriptor	Start	Multiplier	Time Point # 1	Wind-storm Lost	Wind-storm Gained	After Wind-storm	Time Point #2	Finish
1	Small Sparkly Green								
2	Small Sparkly White								
3	Small Purple								
4	Small Black								
5	Small Green								
6	Small Yellow								
7	Small Blue								
8	Small Red								
9	Small Orange								
10	Small Pink								
11	Small Brown								
12	Small White								
13	Large Sparkly Orange								
14	Large Sparkly Yellow								
15	Large Sparkly Green								
16	Large Sparkly Pink								
17	Large Sparkly White								
18	Large Sparkly Purple								
19	Large Sparkly Blue								
20	Large Sparkly Red								

Appendix 4: Worksheet

Preamble: Lecture Review

Concisely define the following terms

Biogeography –

Endemic –

Cosmopolitan –

Neutral Model –

Occupancy –

Abundance –

Deterministic –

Stochastic –

Use this space to write clarifications or questions from the biogeography lecture:

PART 2: Analysis & Synthesis

1. List here the environment that your team had and its conditions in pH, Salinity, Carbon, and Water.

2. Generally compare the class simulation values in the shared spreadsheet to those in the **Original Survey Data** spreadsheet on page 4 of the Activity Instructions packet. Are the general trends in abundance similar or different? Briefly explain. *Spend no more than 2 minutes on this question.*

3. Think about the multiplier in this activity
   a. In simple terms, what does the multiplier represent(s)?

   b. Why was the lowest multiplier among all of the environmental conditions selected?

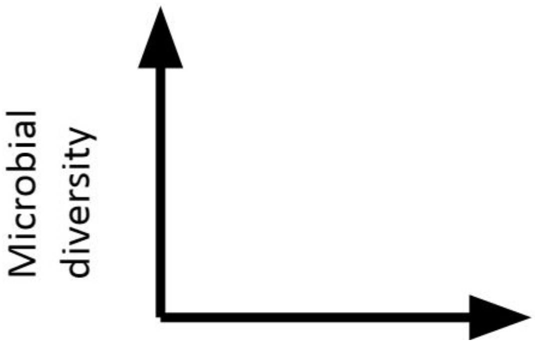
   c. Is the environmental condition a deterministic or stochastic driver of OTU distributions (abundance and occupancy)?

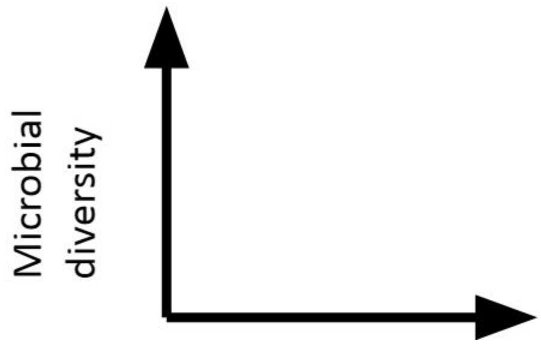
4. Think about the windstorm.
   a. What ecological process does the windstorm simulate?

   b. Is this process a deterministic or stochastic driver of OTU distributions (abundance and occupancy)?

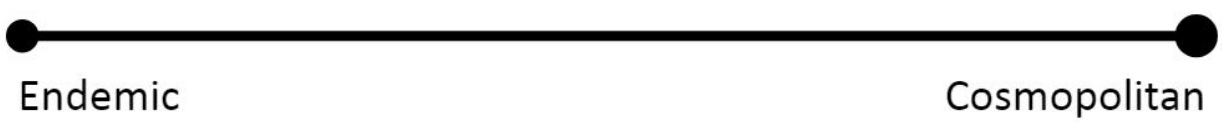
5. Based on the **Environmental Gradients data** and your experience in Part 1, which two environmental factors do you think are most driving the biogeography of these organisms? Briefly explain.

6. Based on your data and what you learned from the lecture, sketch the general relationship you expect to observe between microbial diversity and the environmental factors you selected. Label your axes!





7.a. Looking at the **Original Survey data table**, place the 20 taxa, labeled by their OTU number, on the Endemic-to- Cosmopolitan graph below:



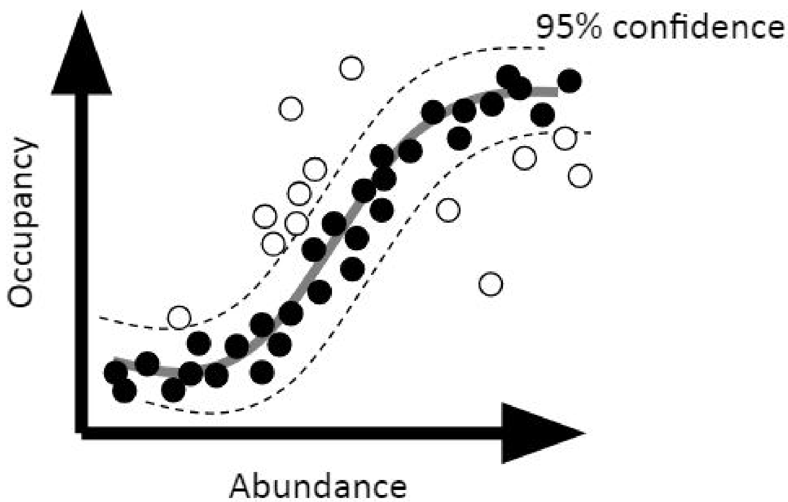
7.b. How confident are you in these placements? Why?

7.c. What additional data would increase your confidence in these placements?

8.a. What biogeographical variable to describe the distribution of taxa is described by terms like endemic and cosmopolitan?

8.b. What biogeographical variable is to describe the distribution of taxa is described by terms like rarity, prevalence, and dominance?

9.a. On the graph below, **1)** circle the axis that describes endemism/cosmopolitanism, **2)** draw lines roughly showing the regions corresponding to endemic and cosmopolitan taxa, and **3)** place OTUs 1, 10 and 11 on the chart.



9.b. In the above graph, what does the solid gray line represent?

9.c. What does it mean when taxa are outside the confidence interval (dashed lines)?

Appendix 5: Worksheet Answer Key

Preamble: Lecture Review:

Concisely define the following terms

- Biogeography - **The study of the spatial distributions of ecological communities and their underlying drivers**
- Endemic - **A population or taxon (e.g., OTU) that is restricted to one environment/set of samples. Endemism can be defined relatively to spatial scale (e.g., endemic to hot springs across the globe, endemic to a specific hot spring in Yellowstone)**
- Cosmopolitan - **A population or taxon (e.g., OTU) that is distributed widely and detected among all/most the samples/environments studied.**
- Neutral Model – **All taxa can be found everywhere, and stochastic factors (e.g., birth, death, immigration, and emigration) drive community structure. Environmental conditions or biotic interactions do not have consequence for distributions in the environment.**
- Occupancy – **Presence/absence of a taxon across all the samples in the dataset. If it is present (detected) in all samples, the occupancy is 1 (100%)**
- Abundance – **The number of individuals of a taxon observed in the community. Dominant taxa have many individuals in the community; rare taxa have few.**
- Deterministic – **The environmental conditions that contribute to a taxon's distribution, including abiotic and biotic factors. Taxa adapt and evolve to thrive in a particular environmental condition (niche) and are selected for or against based on how well the local environment reflects its niche.**
- Stochastic – **The "random"/"drift" factors that contribute to a taxon's distributions, including birth, death, immigration, and emigration.**

<u>PART 2: Analysis & Synthesis</u>

1. List here the environment that your team had and its conditions in pH, Salinity, Carbon, and Water.

   See environment cards.

6. Generally compare the class simulation values in the shared spreadsheet to those in the **Original Survey Data** spreadsheet on page 4 of the Activity Instructions packet. Are the <u>general trends</u> in abundance similar or different? Briefly explain. *Spend no more than 2 minutes on this question.*

   The trends are generally similar, though there are a few OTUs that are not always consistent (e.g., OTU2)

2. Think about the multiplier in this activity.
   a. In simple terms, what does the multiplier represent(s)?

   Cell division given an OTU's relative reproductive fitness.

   b. Why was the lowest multiplier among all of the environmental conditions selected?

   The lowest multiplier is from the environmental condition that is most restrictive to the taxon's growth. It is the limiting resource / strongest environmental filter or constraint for the taxon's success.

   c. Is the environmental condition a deterministic or stochastic driver of OTU distributions (abundance and occupancy)?

   Deterministic

3. Think about the windstorm.
   a. What ecological process does the windstorm simulate?
   Dispersal of individual cells. (Migration is also an acceptable answer, though passive via wind.)

   b. Is this process a deterministic or stochastic driver of OTU distributions (abundance and occupancy)?

   Stochastic

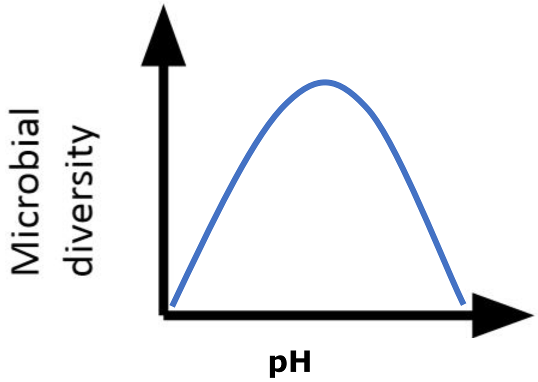
5. Based on the **Environmental Gradients data** and your experience in Part 1, which <u>two</u> environmental factors do you think are most driving the biogeography of these organisms? Briefly explain.

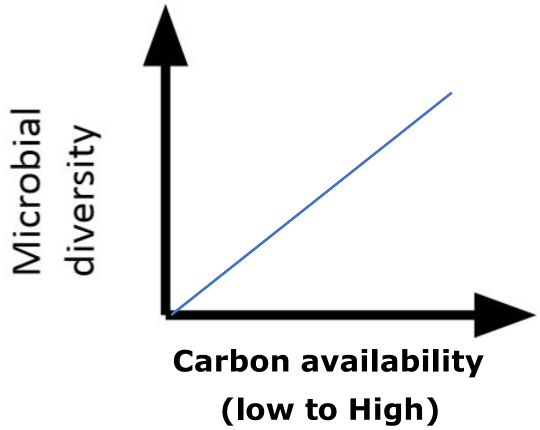
   pH and Carbon.

   pH exhibits a strong environmental gradient that selects for specific OTUs

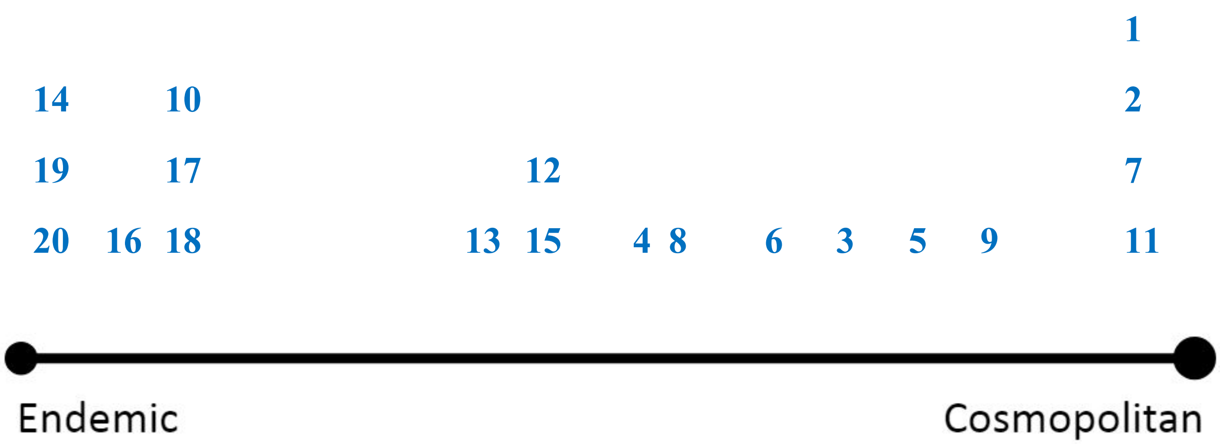
   Carbon availability determines which microbes can survive where, and acts as a strong selective filter (e.g., some OTUs do not survive if carbon availability is low)

6. Based on your data and what you learned from the lecture, sketch the general relationship you expect to observe between microbial diversity and the environmental factors you selected. Label your axes!





7.a. Looking at the **Original Survey data table**, place the 20 taxa, labeled by their OTU number, on the Endemic-to- Cosmopolitan graph below:



7.b. How confident are you in these placements? Why?

Fairly confident in the placement of the abundant taxa (e.g., OTUs 1 - 12 and 14), but not so confident in the less abundant (rare) taxa, since their detection is limited and their placement could only seem endemic.

7.c. What additional data would increase your confidence in these placements?

Additional sampling could clarify whether the less abundant taxa are truly endemic or whether greater observational effort would reveal that they are present in the other samples.

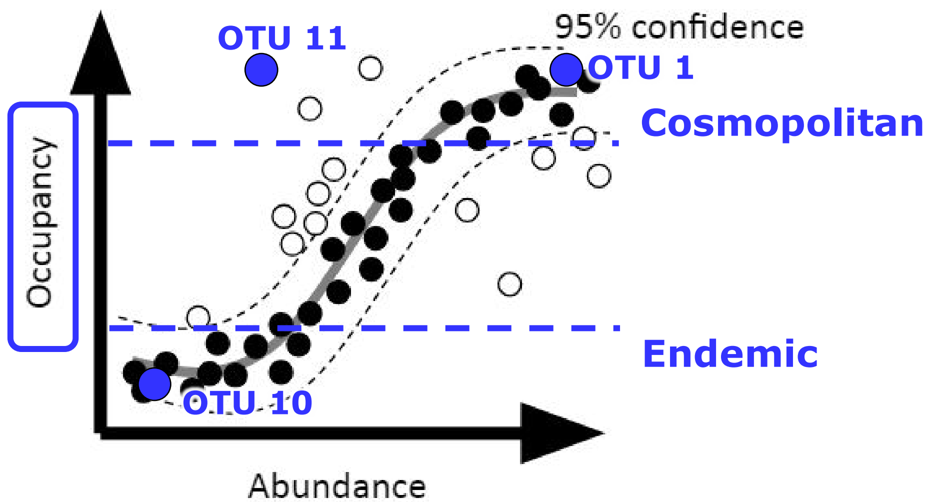
8.a. What biogeographical variable to describe the distribution of taxa is described by terms like endemic and cosmopolitan?

Occupancy

8.b. What biogeographical variable is to describe the distribution of taxa is described by terms like rarity, prevalence, and dominance?

Abundance

9.a. On the graph below, 1) circle the axis that describes endemism/cosmopolitanism, and 2) draw lines roughly showing the regions corresponding to endemic and cosmopolitan taxa and 3) place **OTUs 1, 10 and 11** on the chart.



9.b. In the above graph, what does the solid gray line represent?

The expected distribution of taxa based on a neutral model of abundance and occupancy (e.g., only birth, death, immigration and emigration determine distributions; the environment has no consequence).

9.c. What does it mean when taxa are outside the confidence interval (dashed lines)?

It is statistically very unlikely that those taxa are neutrally distributed because they are outside the 95% confidence interval of the neutral model..

We would expect that some deterministic factor is strongly driving the biogeographic distributions of those taxa (e.g., dispersal limitation, environmental filtering, biotic interactions).

Appendix 6: Sample Data – Student Worksheets

ANSWER WORKSHEET

Team Number: 6
Names of team members present: 4

PART 2: Analysis & Synthesis

1. List here the environment that your team had and its conditions in pH, Salinity, Carbon, and Water.

   - **PH - 4**
   - **salinity - low**
   - **carbon source - low**
   - **water - scarce**

2. Generally compare the class simulation values in the shared spreadsheet to those you found in the previous team activity Counting the Uncountable exercise (**Original Survey Data**). Are the general trends in abundance similar or different? Briefly explain. *Spend no more than 2 minutes on this question.*

   The general trend in abundance is similar between the two spreadsheets. It appears that the small colored OTU's are more cosmopolitan and the Large sparkly colored OTU's tend to be more endemic

3. Think about the multiplier in this activity
   a. In simple terms, what does the multiplier represent(s)?

   The effectiveness of reproduction with the involvement of different environmental factors

   b. Why was lowest multiplier among all of the environmental conditions selected?

   The lowest multiplier is representative of the limiting condition of the environment. Limiting environmental conditions such as PH can decrease the rate of multiplication, so this decrease needs to be taken into consideration for a more accurate OTU population number.

   c. Is the environmental condition a deterministic or stochastic driver of OTU distributions (abundance and occupancy)?

   The environmental conditions would be deterministic.

4. Think about the wind storm.
   a. What ecological process does the wind-storm simulate?

   The wind-storm is an ecological disturbance. This environmental activity is a random dispersal mechanism that impacts the stochastic factors such as immigration, emigration and death. For example a wind-storm could cause erosion of the topsoil and move or destroy the microbial communities that reside there.

   b. Is this process a deterministic or stochastic driver of OTU distributions (abundance and occupancy)?
   The wind storm would be a stochastic driver

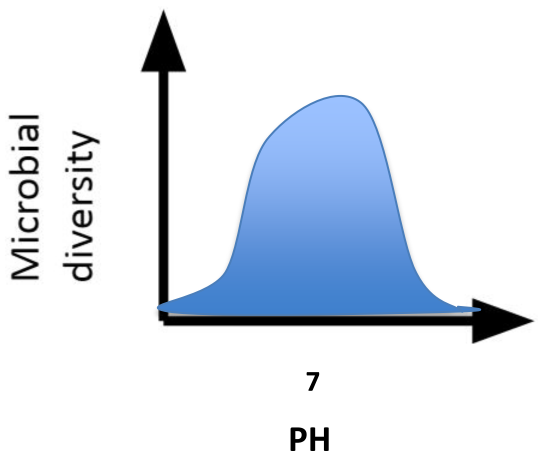
ANSWER WORKSHEET

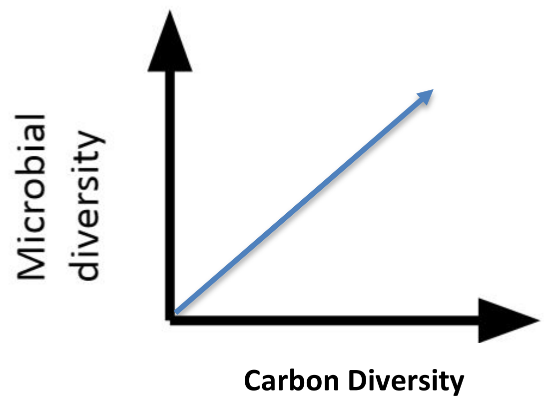
Team Number: 6
Names of team members present: 4

5. Based on the **Environmental Gradients data** and your experience in Part 1, which two environmental factors do you think are most driving the biogeography of these organisms? Briefly explain.

   pH and the availability of carbon are the most important environmental driving factors because they are the most selective.

6. Based on your data and what you learned from the lecture, sketch the general relationship you expect to observe between microbial diversity and the environmental factors you selected. Label your axes!

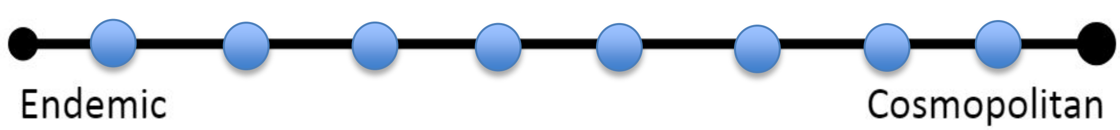




7.a. Looking at the **Original Survey data table**, place the 20 taxa, labeled by their OTU number, on the Endemic-to- Cosmopolitan graph below:

Make Placements from occupancy

14								**9**	
18	**16**	**10**	**8**	**4**		**3**		**7**	
19	**20**	**13**	**12**	**15**	**6**	**2**	**5**	**1**	**11**



ANSWER WORKSHEET Team Number: 6
Names of team members present: 4

7.b. How confident are you in these placements? Why?

We are confident in the cosmopolitan taxa placement but not very confident in the endemic placements because they have not been studied and sampled as thoroughly.

7.c. What additional data would increase your confidence in these placements?

An exhaustive collection of the whole Martian rock microbiome across all environments

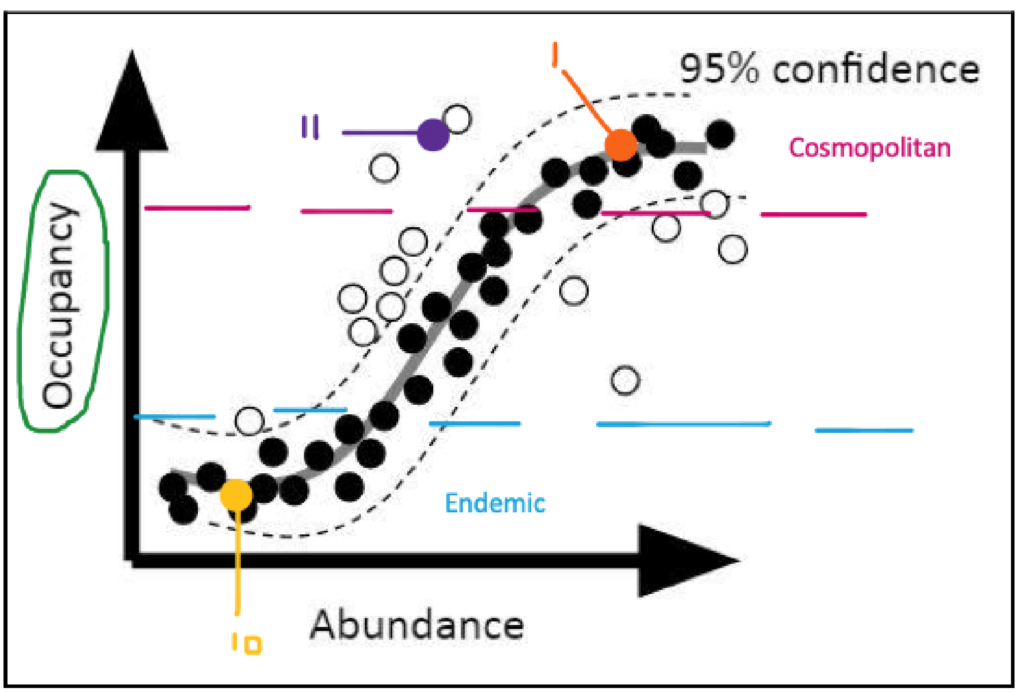
8.a. What biogeographical variable to describe the distribution of taxa is described by terms like endemic and cosmopolitan?

Occupancy

8.b. What biogeographical variable is to describe the distribution of taxa is described by terms like rarity, prevalence, and dominance?

Abundance

9.a. On the graph below, 1) circle the axis that describes endemism/cosmopolitanism, and 2) draw lines roughly showing the regions corresponding to endemic and cosmopolitan taxa and 3) **place OTUs 1, 10 and 11** on the chart



9.b. In the above graph, what does the solid gray line represent?

The solid gray line represents the occupancy/abundance of taxa solely influenced by stochastic factors such as births, deaths, immigration and emigration.

9.c. What does it mean when taxa are outside the confidence interval (dashed lines)?

When taxa are outside the dashed line deterministic factors had an input on their occupancy/abundance of a space.

ANSWER WORKSHEET Team Number: **3**
Names of team members present: **(Redacted)**

PART 2: Analysis & Synthesis

1. List here the environment that your team had and its conditions in pH, Salinity, Carbon, and Water.

The pH is 10. Water is abundant and the salinity is high. The number of carbon sources is low.

2. Generally compare the class simulation values in the shared spreadsheet to those you found in the previous team activity Counting the Uncountable exercise (**Original Survey**). Are the general trends in abundance similar or different? Briefly explain. *Spend no more than 2 minutes on this question.*

I would say that this activity's abundance compared to the previous exercise's abundance is a little different because in this week's exercise, about 50% of each group's OTUs didn't survive. There are no extreme outliers with the number of OTUs per environment, but there does seem to be a few more environments/groups with more OTU's that are at 0 (larger OTUs) because there was a large amount of variance when it came to the environmental conditions in this exercise.

3. Think about the multiplier in this activity
   a. In simple terms, what does the multiplier represent(s)?
   The multiplier represents the environmental conditions that are good for the microbe and allows it to flourish to a certain degree.

   b. Why was the lowest multiplier among all of the environmental conditions selected?

   The lowest multiplier was the limiting environmental condition in the ecosystem.

   c. Is the environmental condition a deterministic or stochastic driver of OTU distributions (abundance and occupancy)?

   Deterministic because the environmental conditions will always result in some OTUs flourishing and others dying out. It is likely that specific environmental conditions will select for specific microbes.

4. Think about the wind storm.
   a. What ecological process does the wind-storm simulate?
   Immigration and emigration

   b. Is this process a deterministic or stochastic driver of OTU distributions (abundance and occupancy)?

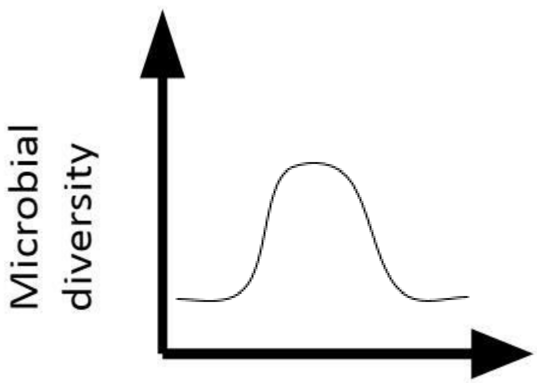
   Stochastic because with each of these ecological processes, you can get a different outcome each time, depending on the environment and the amount of change in the OTUs. It's unlikely that with each round of birth, death, immigration, and emigration, you'll get the same outcome every single time.

ANSWER WORKSHEET Team Number: **3**
Names of team members present: **(Redacted)**

5. Based on the **Environmental Gradients data** and your experience in Part 1, which <u>two</u> environmental factors do you think are most driving the biogeography of these organisms? Briefly explain.

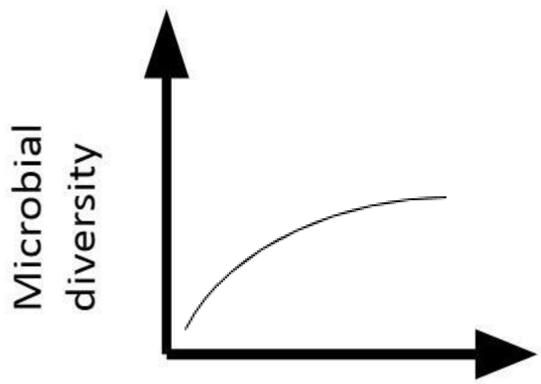
The 2 environmental factors that are driving the biogeography of these organisms that most are the pH and the number of carbon sources. These two factors both contain the largest amount of gradient in each of its factors, so it's likely that at least 50% of the OTUs would be impacted by any change in the factors. In the salinity and water abundance factors, there are only a few OTUs that would suffer from any changes in each of the factors.

6. Based on your data and what you learned from the lecture, sketch the general relationship you expect to observe between microbial diversity and the environmental factors you selected. <u>Label your axes</u>!



Low High

pH

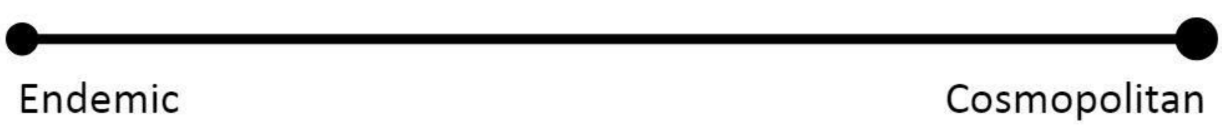


Low High

# of Carbon Sources

7.a. Looking at the **Original Survey data table**, place the 20 taxa, <u>labeled by their OTU number,</u> on the Endemic-to-Cosmopolitan graph below:

22 25 24 23 21 19 18 20 13 16 14 12 11 15 17 8 9 6 7 10 4 3 5 2 1



ANSWER WORKSHEET Team Number: **3**
Names of team members present: **(Redacted)**

7.b. How confident are you in these placements? Why?

I'm pretty confident in where the OTUs are placed. Where the OTUs were placed was based on how many of each of them were present throughout the community. The OTUs that weren't in high abundance were placed near the endemic side. OTUs placed near the cosmopolitan side were the OTUs that had the highest abundance in the community. Although for the taxa that are rarier/more endemic, I am less confident in where they went.

7.c. What additional data would increase your confidence in these placements?

Getting more observations would be helpful.

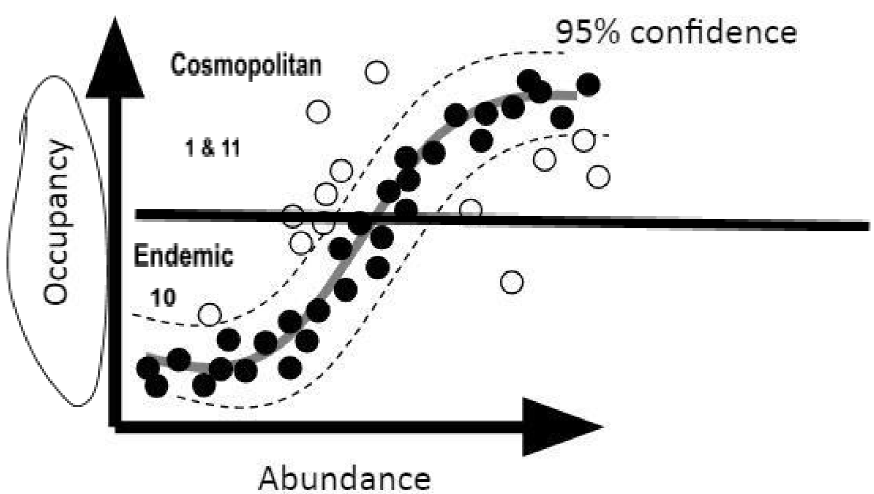
8.a. What biogeographical variable to describe the distribution of taxa is described by terms like endemic and cosmopolitan?

Occupancy is used to describe how many individuals are seen in each sampled community.

8.b. What biogeographical variable is to describe the distribution of taxa described by terms like rarity, prevalence, and dominance?

Abundance is used to describe the number of individuals that make up a taxon/population. Terms such as rarity, prevalence, and dominance are used for a taxa's abundance relative to the other populations' abundances in a community.

9.a. On the graph below, 1) circle the axis that describes endemism/cosmopolitanism, and 2) draw lines roughly showing the regions corresponding to endemic and cosmopolitan taxa and 3) **place OTUs 1, 10 and 11** on the chart.



ANSWER WORKSHEET Team Number: **3**
Names of team members present: **(Redacted)**

9.b. In the above graph, what does the solid gray line represent?

The solid gray line represents the neutral model fit of the relationship between abundance and occupancy based on stochastic factors. It basically summarizes the expectation that taxa with a high abundance (large number of individuals) also have a high occupancy (found in lots of places), and the same with low abundance and occupancy.

9.c. What does it mean when taxa are outside the confidence interval (dashed lines)?

When taxa are outside the 95% CI, it means that they don't fit the neutral model of abundance vs occupancy. More specifically, taxa that are above the model fit are in higher abundance or occupancy than the neutral expectation, while taxa that are below the model fit are in lower abundance or occupancy given the neutral expectation. The taxa that are above the model fit are selected for in the environment, which means that there are deterministic factors that benefit the taxa. The taxa that are below the model fit are selected against in the environment, meaning that there are either local deterministic factors that limit their success or dispersal limitation.

Appendix 7: Exam Questions

F1_M1. Which conveys a neutral expectation of a taxon's abundance and occupancy?

A. A taxon that is very abundant should also be found in few locations.
B. A taxon that is very abundant should also be found in many locations.
C. A taxon that is very rare should also be found in many locations.
D. All of the above
E. None of the above

F2_M2a. In three or fewer sentences, define cosmopolitanism and (**F4_M2c**.) two conditions and/or microbial traits that can lead to cosmopolitan populations.

Cosmopolitanism is when a taxon/population/species is widely distributed in the environment (high occupancy).

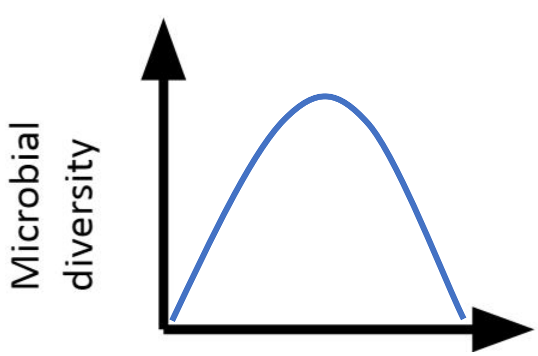
Conditions that promote cosmopolitanism include: 1) high dispersal/low barriers to dispersal; 2) wide niche requirements and high phenotypic plasticity/physiological versatility (related: large genomes)

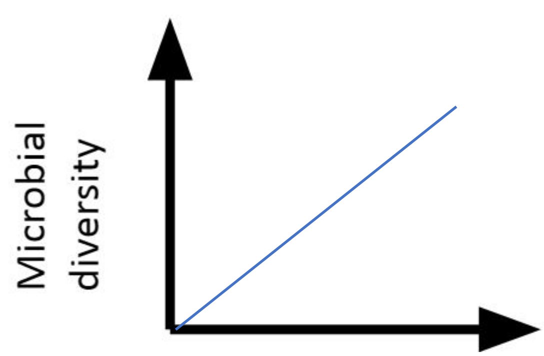
F3_M2b. In three or fewer sentences, define endemism and (**F4_M2c**.) two conditions and/or microbial traits that can lead to endemic populations.

Endemism is when a taxon/population/species has low distribution in the environment (low occupancy) – only found in a few places.

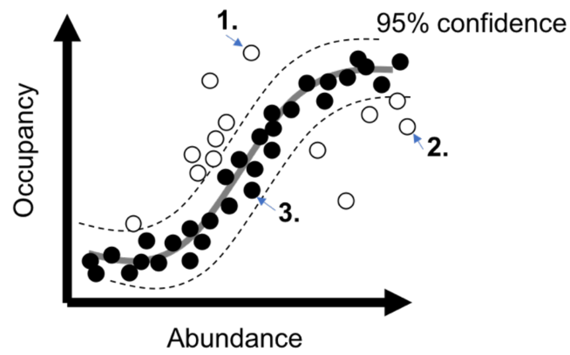
Conditions that promote endemism include: 1) low dispersal; 2) very specific niche requirements (e.g., hot spring) and low phenotypic plasticity physiological inflexibility (related: small genomes)

F8_M4. Based on the lecture content and in-class activity, draw the expectation as to how microbial diversity (y-axis) should change along these environmental gradients (x-axis).





M3, M5, & M6. Consider the abundance-occupancy data below.



F5_M3a (Point #1) & F6_M3b (Point #2). On the chart, which labeled point(s) are driven by selective (rather than neutral) processes?

A. Point #1
B. Point #2
C. Point #3
D. **Point #1 and #2**
E. Points #2 and #3

F7_M3c. On the chart, which labeled point(s) are driven by neutral (rather than selective) processes?

A. Point #1
B. Point #2
C. Point #3
D. Point #1 and #2
E. Points #2 and #3

M5a. Circle the axis that describes the rare-to-prevalent gradient.

x-axis

M5b. Circle the axis that describes the endemic-to-cosmopolitan gradient

y-axis

M6. Provide a one-word answer: What do the individual points on the chart represent?

Populations/OTUs/species.