**Impact of Introduction of American Bullfrogs on Species Diversity**

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ABSTRACT

This module explores the introduction of the American bullfrog to their ecosystem. Using species diversity calculations and data collection techniques, students explore how the bullfrog affected the species in its community. Students are introduced to calculations like species richness, species evenness, and species diversity indices. Also, this activity provides students an understanding of how this information is gathered in the field and provides them an opportunity to learn how to collect data themselves.

LEARNING OUTCOMES

* Be able to identify the concepts of species richness and biodiversity and compare and contrast them.
* Be able to calculate several indices for species diversity.
* Be able to explain qualitatively the value of a diversity index.
* Be able to explain the formulas (how are they derived) for species diversity.
* Be able to justify why a particular species index would be appropriate to choose for a field project.
* Be able to explain different sampling techniques that could be used for this data collection.

**TEACHING INSTRUCTIONS**

Pre-Class Activity

The pre-class activity introduces students to the issues surrounding the introduction of American bullfrogs to the American Southwest. Students will begin by watching the short video for an overview of the problem and complete the questions to assess their understanding of the video. Additional information about the topic is covered in a blog article. After reading the blog post, students will be able to answer two additional questions. The final question for the pre-class activity will require students to complete research into the control methods of the bullfrog. The answer to the final question will introduce students to the concepts of invasive species control methods as well as the difficulties associated with correcting the impacts of invasive species.

A video outlining ecological sampling methods is included at the end of the pre-class activity. This video introduces students to various sampling techniques and includes pros and cons of the various techniques. If this activity is to be used for a hands-on class activity, a different sampling technique could be assigned to each class group and the students can assess the pros and cons of the technique from their experience.

Part 1: Species Richness and Evenness

The first part of this activity is devoted to the basic measurements of species diversity: species richness and evenness. It begins with general information on what biodiversity is and why it is important. Then, it introduces species richness and evenness using a diagram to explain their calculations. Although this section introduces species evenness, we have decided to approach it using representation of each species compared to the community rather than a particular equation calculation (which is introduced in Part 3). Therefore, students will need to calculate evenness for each species by dividing the number of individuals by the total number of individuals.

To make sure that students understand these measurements, we provide a table of example data using the number of birds of different species in three locations. These are a great opportunity to ensure that your students can properly employ these calculations, understand the differences, and why they are important. I provided a table of the answers in the Answer Key.

The last section of Part 1 has students connect species richness and evenness to the Pre-Class Activity on the American Bullfrog introduction to Arizona.

Part 2: Introduction to Species Diversity - Simpson’s Diversity Index

Simpson's Diversity Index is a measure of diversity. In ecology, it is often used to quantify the biodiversity of a habitat. It takes into account the number of species present, as well as the abundance of each species. Simpson’s Diversity Index measures the probability that two individuals randomly selected from a sample will belong to the different species.The Simpson index ranges from 0 to 1, where 0 represents no diversity (only one species dominating the community) and 1 represents maximum diversity (all species present in equal abundance). A higher Simpson index value indicates higher diversity within the community. It is important to know that there are several different indices that call themselves “Simpson’s Index.” Variations include Inverse/Reciprocal Simpson Index, Simpson’s Index (also called Hunter-Gaston Index in microbiology), and the version we are using in this paper which is often referred to as the Simpon’s Index of Diversity(also called Gini-Simpson Index).

A sample calculation of Simpson’s Index is provided. Students will then calculate the diversity both before and after the introduction of the bullfrogs to an ecosystem and compare their results.

For both Parts 2 and 3, a prior lesson on how to use a spreadsheet or statistical program could help aid in the calculations. The calculations in these parts of were done with Microsoft Excel.

Part 3: Species Diversity - Shannon-Weiner Index

The Shannon-Wiener Diversity Index is widely used due to its ability to incorporate both species richness and evenness in a single metric. It provides a more comprehensive measure of diversity than species richness alone. By quantifying the information content or uncertainty associated with species identification within a community, this index offers a standardized way to compare diversity across different habitats, communities, or time periods.

The Simpson index and the Shannon-Wiener index are both widely used diversity indices in ecology that aim to quantify and compare species diversity within ecological communities. While they serve a similar purpose, there are notable differences in their calculation methods and the information they provide. The Simpson index focuses on the dominance or concentration of species within a community. It considers the probability that two individuals selected randomly from the community belong to the same species. In contrast, the Shannon-Wiener index captures both species richness and evenness within a community. It measures the uncertainty or information content associated with predicting the species identity of an individual chosen at random from the community.

The Shannon-Wiener index, also known as Shannon's entropy, provides a measure of the amount of information required to specify the species identity of a randomly chosen individual from the community. It ranges from 0 to infinity, where 0 represents no diversity (only one species present) and higher values indicate greater diversity. Unlike the Simpson index, the Shannon-Wiener index can increase with both species richness and evenness.

Similar to Part 2, a sample calculation of the Shannon-Wiener Index is provided. Students will then calculate the diversity both before and after the introduction of the bullfrogs to an ecosystem and compare their results.

A review of logarithms could be considered as the natural logarithm is used in Shannon-Wiener Index calculation.

Further topics for discussion could include which diversity index to use for a given ecosystem; or discussions on the construction of each of these metrics which would highlight concepts in probability. In Parts 2 and 3, we used the diversity indices to measure how a particular habitat’s diversity changed over time. These can also be used to compare the diversities of different habitats at the same time.

Part 4: Sampling Techniques

In this activity, we use quadrats to introduce the concept of measuring species richness and species diversity. It is generally accepted that ecosystems that have higher richness and diversity values have higher ecosystem health and stability, and that these ecosystems are more resilient in the face of change (man-made or natural). Having a variety of species can help to provide us not only with food but they also contribute to clean water, breathable air, fertile soils, climate stability, pollution absorption, building materials for our homes, prevention of disease outbreaks, medicinal resources, and more. For example, you can have a marsh that has three frog species in it, but 90% of the frogs belong to just one species. Conversely, a marsh with three species can be more even, with about one third of the frogs belonging to each species; this marsh looks (and acts) much differently. Marshes that are primarily pine don’t have as many deer as a forest that contains a good mixture of oak (with acorns) with pine and maple. Diversity indices can be used to compare different communities, or one community at different points in time, with a higher diversity indicating a healthier community.

The question being explored is What happens to species richness found in an area (the habitat) as the area, or habitat size, increases? This activity will have students go through the scientific method and calculate species richness and using the quadrat method as another way to estimate abundance (or population size). Then, students will calculate species diversity using the Simpson’s diversity index.

As this activity is designed to take students outside to test their hypothesis by counting the number of species (and the number of individuals per species) in 4 randomly selected quadrats (out of a larger area of 16 quadrats). The number of species will then be graphed versus area (or, number of quadrats – one quadrat versus two, three, and all 4 combined). It can be done inside using simulated organisms.

For more information about how to do the quadrat system, please refer to this video: https://youtu.be/wWd3Oo7Mi4E

For students to make a random selection, the quadrats should be numbered across and using letters down the sides. Create one bag/box of letters of the quadrats and another bag/box of numbers. Students should select one letter and one number from the bags and then return them to their appropriate bags, mix, and repeat so that they get 4 combinations (try again if a student gets a duplicate).

Using data obtained from the quadrats it can be extrapolated and used to estimate population sizes for the larger area. Diversity indices can be used to compare one habitat to another of equal size, or to look at one habitat at several points in time. Different areas of the world have different diversity patterns, for example, diversity is usually greater closer to the tropics (zero latitude). With Simpson’s diversity index, diversity runs between zero and one - the larger the number, the more diverse the community. A Simpson’s Diversity Index of 1 indicates complete diversity and a Simpson’s Diversity Index of 0 indicates complete uniformity.

Extensions to the Activity

After completing this activity, students should be able to apply their knowledge to tackling the problem of invasive species. You can divide students into groups and assign/select a locally relevant invasive species. The groups can then devise a solution to address the invasive species and minimize its impact on the environment, as well as further negative impacts from humans. Groups can present their solutions, such as a powerpoint presentation, debate, or oral presentation.

Another extension would be to create a service-learning opportunity where students can research how to address their invasive species and participate in a community event on the topic. Department of Natural Resources, Nature Conservancy and locally active conservation groups are resources that can be utilized.

**PRE-CLASS ACTIVITY**

The introduction of non-native species to new environments can have profound ecological consequences, often disrupting the delicate balance of local ecosystems. The American bullfrog, *Lithobates catesbeiana*, is the largest frog in the United States, weighing up to one pound. It is native to the eastern U.S. but invasive in much of western US and several other continents. The bullfrog has been introduced and spread to many regions around the globe as a food source (for its edible legs) and through aquaculture escape. It is often a dominant species in these new environments, due to a limited number of predators and being a generalist as they will eat anything that fits in their mouths (Adams and Pearl, 2007).

**Part 1:** National Geographic created a video that details the impact of the introduction of the American bullfrog on the desert in the southwest US. It chronicles the voracious appetite of the bullfrog and the repercussions of its introduction on an ecosystem lacking control mechanisms.

**Watch the video on Fearsome Frogs from the National Geographic Society**

<https://education.nationalgeographic.org/resource/fearsome-frogs> and **answer the following questions.**

1. Why were bullfrogs introduced to Arizona?
2. What characteristics made Arizona an ideal habitat for bullfrogs?
3. What types of animals have been found in the stomachs of bullfrogs in Cochise County Arizona?
4. What is one distributing trait of the bullfrog?
5. What predators keep bullfrog populations under control in their native habitat?
6. What species of frog in Arizona has seen dramatic population decline due to the bullfrog?
7. Name one control method used by conservationists in Arizona.

**Part 2:** The Nature Conservancy has a conservation science blog that contains a section (From the Field) detailing the latest research from the field. Another significant concern associated with American bullfrogs is their potential role in disease transmission. Read the blogpost, Recovery: A Plague of Bullfrogs by Ted Williams, which details the impact of all bullfrogs on their environment and methods of controlling these species. While they are generally resistant to many amphibian diseases, bullfrogs can act as carriers for various pathogens, including the chytrid fungus, *Batrachochytrium dendrobatidis*.

**Answer the following questions based on the following blog and your own research,** <https://blog.nature.org/science/2020/01/06/recovery-a-plague-of-bullfrogs/>

1. What is the native range of the American bullfrog?
2. In addition to predatory behaviors, what pathogen can be carried by bullfrogs and what is the pathogen’s impact on the ecosystem?
3. What methods are currently being used to control bullfrog populations in the Western United States?

**Part 3:** Ecological Sampling Techniques

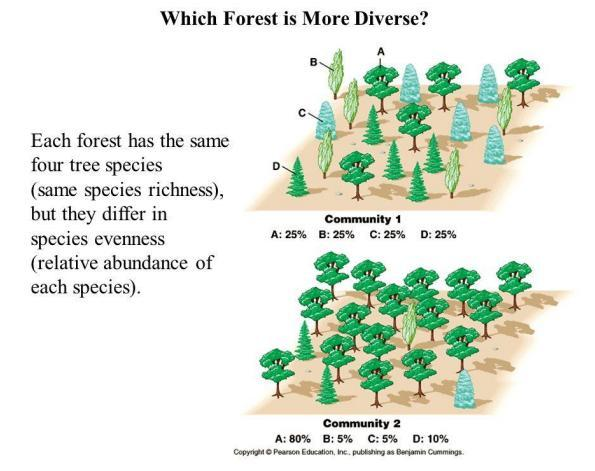
There are a variety of sampling techniques that can be used to estimate population size and ecological impacts. Each method has its pros and cons depending on the environment, species, etc.

Watch the video and answer the questions, <https://www.youtube.com/watch?v=W8jPwBXSqfs>

1. What is the purpose of sampling?
2. Why is sampling conducted?
3. How do you design a sampling strategy?
4. The following sampling techniques were discussed in the video. Explain how to conduct each type of technique and the type of organism that could be targeted.
   1. Quadrants
   2. Line Transects
   3. Belt Transects
   4. Point Transects
   5. Mark and Recapture

**PART 1: SPECIES RICHNESS AND EVENNESS**

Biodiversity describes the variety and variability of all living organisms within a given ecological area. One of the major questions facing ecologists and resource managers is understanding the amount of diversity in a particular area. This is important not only for describing macro ecological trends such as species gradients, but for allowing for the more precise application of limited conservation resources. Additionally, careful analysis of how species are distributed can even help identify some of the process underlying that pattern. Biodiversity can be used to refer to the number of species, their genetic diversity or habitat variety (ecological variations). There are two main components that contribute to biodiversity – species richness and species evenness.

Species richness (S) describes the number of different species present in an area. The more species equals greater richness. To calculate species richness, just count the number of species present. This is also known as *alpha diversity*. Alpha diversity is used to compare the number of species in different communities.

Species evenness (J) describes the relative abundance of the different species in an area. Therefore, similar abundance among species equals more evenness. This can be easily calculated by using the number of individuals of a species by the total number of individuals.

In Figure 1, each forest has the same four tree species; therefore their species richness is the same. However, the representation of each species in the forests is different. In Community 1, all species are equally represented with 4 individuals each; therefore, the species evenness is 25% (4/16). In Community 2, one species dominates the area with two species being very rare; therefore, the species evenness is 80% for one species (16/20) and 5% for another species (1/20).

**Practice Questions**

Using three different urban sites in the table, examine the specific bird data and compare the biodiversity measures.

Table 1. Species counts of birds for three sites.

|  | ***# of individuals counted at site*** | | |
| --- | --- | --- | --- |
| **Species of Bird** | **Site #1** | **Site #2** | **Site #3** |
| Rock Dove (pigeon) | 32 | 44 | 20 |
| Black Capped Chickadee | 4 | 22 | 23 |
| European Starling | 43 | 47 | 25 |
| House Sparrow | 15 | 42 | 25 |
| Song Sparrow | 1 | 6 | 20 |
| House Finch | 23 | 45 | 24 |
| Dark-eyed Junco | 0 | 0 | 20 |
| American Crow | 4 | 4 | 20 |
| Common Grackle | 0 | 5 | 23 |
| American Robin | 0 | 4 | 20 |
| ***Total Number*** | ***122*** | ***219*** | ***220*** |

**Practice Questions**

1. What is the species richness for each site?
2. Do the species seem evenly distributed or is the community dominated by a few species?
3. Calculate species evenness for each site?
4. What do you think is the best indicator of biodiversity, the number of species, the number of individuals, the evenness, or a combination of all three indicators? Why?

**INTRODUCTION OF BULLFROGS**

The introduction of American bullfrogs in the 19th and early 20th centuries to augment frog-leg markets to new environments has had far-reaching ecological consequences. Their predatory behavior, competitive interactions, and potential for disease transmission have disrupted native ecosystems and threatened the survival of many species. Bullfrogs compete with and prey on indigenous wildlife. They devour anything including native frogs, salamanders, bats, fish, crayfish, snakes, turtles, rodents, insectivores, lagomorphs and birds as big as goslings. Bullfrogs also carry and spread the devastating amphibian disease chytrid fungus, to which they’re pretty much immune. Bullfrogs, which can weigh two pounds and produce clutches of 20,000 eggs, dwarf native amphibians in size and fecundity. Where they’ve been introduced, they have few predators. And as global warming destroys habitat of the natives, it expands habitat suitable for bullfrogs.

Understanding the impact of American bullfrogs on their environment is crucial for developing effective management strategies to mitigate their negative effects. By addressing the challenges posed by non-native species like the American bullfrog, conservation efforts can be better focused on preserving and restoring the integrity of native ecosystems.

Table 2. Species counts before and after introduction of bullfrogs.

|  | **# of individuals** | |
| --- | --- | --- |
| **Species** | **Before Bullfrogs** | **After Bullfrogs** |
| Spadefoot toads | 167,295 | 78,290 |
| Oregon Spotted frog | 150,578 | 98,291 |
| Chiricahua Leopard Frog | 126,723 | 25,987 |
| Northern Mexican Gartersnake | 57,239 | 32,093 |
| Sonoran Mud Turtle | 63,829 | 49,983 |
| Barred Tiger Salamander | 75,600 | 42,126 |
| Sonoran Tiger Salamander | 87,864 | 59,209 |
| Desert Hairy Scorpion | 237,974 | 178,923 |
| Bark Scorpion | 487,028 | 328,389 |
| Desert Shrew | 239,772 | 178,273 |
| Mexican Long-tongued Bat | 153,929 | 130,289 |
| Cliff Chipmunk | 274,923 | 230,983 |
| Plains Harvest Mouse | 323,861 | 298,983 |
| Cactus Mouse | 420,392 | 329,298 |
| Western Desert Tarantula | 428,824 | 278,983 |
| Southern House Mosquito | 2,127,982 | 3,582,231 |
| **Total Number** | **5,423,813** | **5,922,331** |

**Questions**

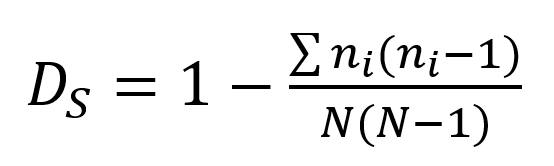
1. Does the species richness change before and after the introduction of bullfrogs?
2. Do the species seem evenly distributed or is the community dominated by a few species?
3. Calculate species evenness for before and after the introduction of bullfrogs?
4. How has the introduction of the bullfrog changed the diversity of the environment? Do you think this will affect the health of the ecosystem?

**PART 2: Introduction to Species Diversity - Simpson’s Diversity Index**

Measuring and quantifying biodiversity is essential for understanding and assessing the health and stability of ecosystems. Simpson's Diversity Index, named after the British statistician Edward H. Simpson, is a widely used tool for quantifying species diversity within a given area or habitat. This index provides valuable insights into the richness and evenness of species present in a community, offering a numerical value that reflects the diversity and distribution patterns of species.

Simpson's Diversity Index (SDI) is a measure that takes into account both species richness (the number of different species) and species evenness (the relative abundance or distribution of each species). It provides a single value that represents the probability that two randomly selected individuals within a community belong to the same species. It is denoted DS and its values range from 0 to 1, where high scores (close to 1) indicate high diversity and low scores (close to 0) indicate low diversity.

The calculation of Simpson's Diversity Index involves summing the squared proportions of each species' abundance within the community. This index ranges from 0 to 1, with 0 representing no diversity (only one species present) and 1 indicating maximum diversity (all species present in equal abundance). The most useful aspect of the SDI is when comparing two sets of data. For example, if one has an SDI of 0.6 and another has an SDI of 0.45, then the set with the SDI of 0.6 is more diverse. The formula for Simpson’s diversity index is:



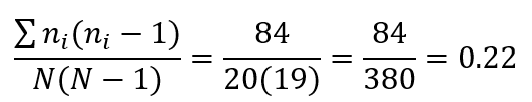
Where DS is the diversity index, n*i* is the number of individuals for each of the *i* different species, and N is the total number of all individuals.

Given the community below, we can calculate the Simpson’s Diversity Index using the formula.

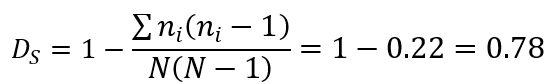
| **Species** | **Common Name** | **# of individuals (ni)** |
| --- | --- | --- |
| 1 | Rabbits | 2 |
| 2 | Foxes | 1 |
| 3 | Grasshoppers | 3 |
| 4 | Beetles | 8 |
| 5 | Squirrels | 1 |
| 6 | Spiders | 5 |
| **TOTAL (N)** |  | **20** |

In performing calculations with Simpson’s Index, it can be helpful to set it up as a table:

| **n*i*** | **n*i-1*** | **n*i (*n*i-1)*** |
| --- | --- | --- |
| 2 | 1 | 2 |
| 1 | 0 | 0 |
| 3 | 2 | 6 |
| 8 | 7 | 56 |
| 1 | 0 | 0 |
| 5 | 4 | 20 |
| **TOTAL = 20** |  | **84** |



Thus the value of Simpson’s Diversity Index for this community is



Since this value is close to 1, it could be said that his community has a relatively moderate to high diversity.

**Questions**

1. Compute the Simpson’s Diversity Index using the data from Table 2 before the introduction of bullfrogs.

2. Compute the Simpson’s Diversity Index using the data from Table 2 after the introduction of bullfrogs.

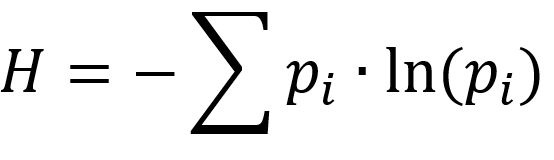
3. Compare the values of Simpson’s index from questions 1 and 2. From this comparison, how has the introduction of bullfrogs impacted the species diversity of this community?

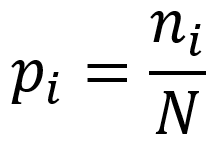
**PART 3: Species Diversity - Shannon-Wiener Index**

In the field of ecology, measuring and quantifying biodiversity is crucial for understanding the complexity and stability of ecosystems. There are many methods that ecologists use to help quantify both richness and evenness. Another widely used tool for assessing and comparing species diversity within a given community is the Shannon-Wiener Diversity Index, named after the American mathematician Claude Shannon and information theorist Warren Weaver. The Shannon-Wiener Diversity Index provides a numerical value that captures both species richness and evenness, offering a comprehensive measure of diversity.

The Shannon-Wiener Diversity Index considers both the number of species present (species richness) and the relative abundance or distribution of each species (species evenness) within a community. It provides a quantitative measure that reflects the uncertainty or information content associated with predicting the species identity of an individual chosen at random from the community. Higher index values indicate greater diversity, while lower values indicate lower diversity within the community.

The calculation of the Shannon-Wiener Diversity Index involves summing the products of the proportion of individuals belonging to each species and the natural logarithm of that proportion, weighted by their respective abundances. The formula for calculating the Shannon-Wiener Diversity Index is as follows:



Where p*i* is the proportion of individuals in the ith species in the data set, that is .

The higher the value of H, the higher the diversity of species in a particular community. The minimum value the Shannon-Wiener diversity index can take is 0 which indicates no diversity. This occurs when only one species is found in that habitat. There is no upper limit to the index. The maximum value for a habitat would equal log(*k*), where *k* is the number of species which occurs when all species have the same number of individuals.

The Shannon-Wiener Diversity Index has various applications in ecological research and conservation. It allows scientists to assess and monitor changes in biodiversity over time, evaluate the effects of disturbances or environmental factors on ecosystems, and compare the diversity of different communities. This index serves as a valuable tool for understanding the health and functioning of ecosystems, informing conservation strategies, and managing natural resources effectively.

The Shannon-Weiner index also can be used to calculate an evenness

1. Compute the Shannon-Weiner Index using the data from Table 2 before the introduction of bullfrogs.

2. Compute the Shannon-Weiner Index using the data from Table 2 after the introduction of bullfrogs.

3. Compare the values of the indices from questions 1 and 2. From this comparison, how has the introduction of bullfrogs impacted the species diversity of this community?

Do you get a similar conclusion as that from the answer to question 3 from Part 2 which used the Simpson Index?

**PART 4: Sampling Techniques**

How do we measure richness and diversity? Biologists obtain data on plant abundance (or slow moving animals, like slugs), richness, and density, by marking off smaller plots, or quadrats, within the habitat. Multiple quadrats are used for replication, and quadrats need to be of equal size, and selected randomly from within the larger habitat. Your number of quadrats should equal at least 10% of your total area. This activity will use a large area divided into quadrats by tape, with different species represented by various materials. Within each quadrat, you will count the number of species, and number of individuals of each species (population size), then calculate population size, species richness, and species diversity for the larger habitat.

Things that can affect species richness and diversity include:

1. Latitude (with greater species diversity at the equator versus the poles).
2. Climate stability (the equator has had a stable climate for longer than temperate / polar areas and so has greater species diversity),
3. Primary productivity (the tropics have a greater productivity versus temperate areas and poles and so has a greater species diversity),
4. Habitat Size (larger habitats support a greater number of species).

Research Question: What happens to species richness found in an area (the habitat) as the area, or habitat size, increases?

**Generate your research hypothesis:**

This will be tested by counting the number of species (and the number of individuals per species) in 5 randomly selected quadrats (out of a larger area of 25 quadrats). The number of species can then be graphed versus area (or, number of quadrats – one quadrat versus two, three, four, and all 5 combined).

**Identify the following variables for the survey:**

Independent Variable:

Dependent Variable:

Controlled Variables:

Procedure: You have an area that is 1 m by 1 m. The area has been divided up into 25 quadrats of 20 cm by 20 cm each.

Quadrats can be made of a variety of materials. PVC pipe and string are a good choice if funds are available and if storage space is available for the constructed quadrats. If budget or storage is a concern an alternative method using string and pencils can be used. Please follow the link to video instructions of this type of quadrat.

1. Sketch the area:

- Include measurements / units

- Include Quadrat Labels – Numbers / Letters

2. Sample only 5 quadrats. But which 5? It is important that you select quadrats randomly – that is, without bias. Maybe you are tired and you don’t want to count so you purposefully select the quadrats with the fewest species. Or, if outside, you select quadrats that are in the shade on a hot sunny day. This is bias.

To make a random selection, the quadrats are numbered across the top and have letters down the sides. Select one letter / one number from the bags your teacher gives you. Write that down. Return the letter / numbers to the bags, mix, and repeat so that you have 5 combinations (try again if you get a duplicate). These are the quadrats you will sample. Circle them on the plan you sketched above.

3. In each of the 5 sample quadrats, count how many different species you surveyed and record this in Table 3.

Table 3: Random Sampling Data for species richness

| Quadrat (Number/Letter) | Species Description | Area of Quadrat (cm2) | Number of species |
| --- | --- | --- | --- |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

4. Calculate species richness for the area by adding up the number of species for all quadrats. Now graph how species richness changes with area (or, number of quadrats). In other words, as we make our area larger, by adding quadrats, does the number of species, inclusive, change? **This is a species-area curve**. More quadrats = bigger area. To do this, you will graph a point for quadrat 1 with its corresponding species richness. The second point is for quadrat 2 but the species richness will be for both quadrat 1 and quadrat 2. And so forth.

Are there the same amount, more, or fewer species as the area gets bigger?

1. Graph your species richness. Make sure to label your axes. And then describe how species richness changes as habitat size (area) increases.

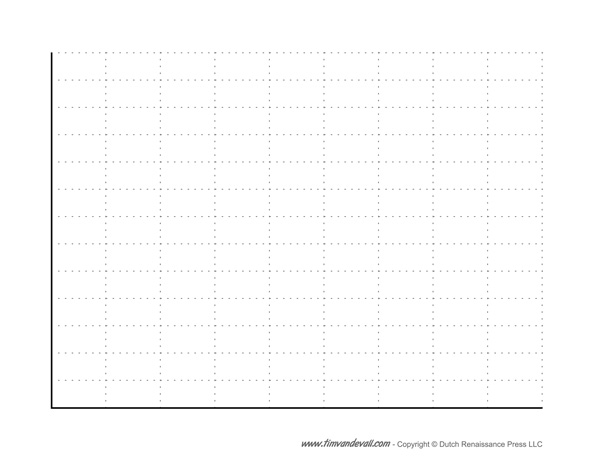
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Figure 1: Species-area relationship curve.

6. Calculate species diversity for the total area based on the number of individuals per species, inclusive, for the 5 quadrats you sampled. Use the Simpson’s Diversity Index (find details in Part 2).

**Species Diversity (Ds) =**

Discussion Questions**:**

1. Why do scientists take a representative sample when estimating the population size of a species?

2. What is a representative sample?

3. Why was it necessary to choose your quadrats randomly (pulling Letter / number combinations from the bag)?

4. What was the dominant “species” within your total area? Does your quadrat sample reflect this? Were there any ‘species’ that you failed to count in your quadrats (but really did exist in the total area?)

5. Predict how the removal of the dominant plant species from the site would affect the population sizes of the other organisms. Explain.

6. In this activity you’ve learned the American bullfrog feeds on all types of organisms, including the Mexican long-tongued bat. This bat is a night pollinator for the Saguaro cactus. When pollinator populations are affected by invasive species there may be an effect on the reproductive success of the plant species the animal pollinates. You have been asked to determine the number of Saguaro seedlings in an area that has been invaded by the American bullfrog. List the steps you would take to calculate an accurate answer in an unbiased manner. Assume the habitat is 2,000 square meters.

7. Population sampling is usually most effective when a species has an even dispersion pattern. It is difficult to determine an accurate estimate when a population has a clumped dispersal pattern. Explain why this would be the case.

8. Based on the bullfrogs you have studied in this activity, could the quadrat sampling method be used to assess a bullfrog population?

1. Why or why not?
2. What sampling method could be used to accurately assess the bullfrog population in a habitat?

**ANSWER KEY**

**Pre-Class Activity**

**Part 1**

1. Why were bullfrogs introduced to Arizona?

The Arizona government introduced bullfrogs to serve as wild game for sportsmen and food for cowboys.

1. What characteristics made Arizona an ideal habitat for bullfrogs?

Few predators, permanent water, plenty to eat

1. What types of animals have been found in the stomachs of bullfrogs in Cochise County Arizona?

Every class of vertebrate found in the county (specific examples include:

1. What is one distributing trait of the bullfrog?

Cannibalism

1. What predators keep bullfrog populations under control in their native habitat?

Gators, snakes, large birds, and humans

1. What species of frog in Arizona have seen dramatic population decline due to the bullfrog?

Leopard frog, mexican garter snake, and sonoran mud turtle

1. Name one control method used by conservationists in Arizona.

Fences and walls

**Part 2**

1. What is the native range of the American bullfrog?  
   Eastern US from the southern part of Maine down to Florida. The bullfrog is also native to the eastern side of Texas and north through Oklahoma, Kansas and southeast Nebraska and Iowa as well as the state of Wisconsin.   
   [American Bullfrog (Lithobates catesbeianus) - Species Profile (usgs.gov)](https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=71)
2. In addition to predatory behaviors, what pathogen can be carried by bullfrogs and what is the pathogen’s impact on the ecosystem?   
   The amphibian disease chytrid fungus, which is devastating for many native amphibians. However, bullfrogs are most immune to the disease.
3. What methods are currently being used to control bullfrog populations in the Western United States?

* Seining and drainage of small ponds and euthanasia of tadpoles.
* Gigs and .22 rifles with copper rounds
* Electro-Frogger Pole

**Part 3**

1. What is the purpose of sampling?

Sampling allows for the collection of a small snapshot of the ecosystem which can best represent the ecosystem and the diversity of the wider community.

1. Why is sampling conducted?

Sampling is conducted so ecosystems can be monitored, managed and conserved. Monitoring allows classification of an ecosystem and allows collection of qualitative and quantitative data.

1. How do you design a sampling strategy?

There are four sampling strategies that could be used: random, systematic, opportunistic and stratified.

* Random - This strategy is used if time is limited and if you are sampling a large uniform area. It involves selection of data from the entire population so that each possible sample is equally likely to occur. A good way to make sure samples are randomly taken is to break an area into a grid and use a random number generator to truly collect a random sample.
* Systematic - A type of random sampling because everything has an equally likely chance of falling into the sampling area, but more structured than random sampling. A fixed periodic interval is agreed upon in advance and then adhered to during sampling. E. g. Every five meters along the line on a transect a quadrant is placed. If a species doesn’t have a uniform distribution, it may be missed. The decision between random and systematic will depend on what kind of area is being sampled.
* Opportunistic - See what is accessible and collect some quick data. It is a non-random technique and can be riddled with bias and may not be representative of the entire community.
* Stratified - Involves proportionally sampling an area. An ecosystem is divided into distinct sections and then it is sampled proportional based on the size of each area.

1. The following sampling techniques were discussed in the video. Explain how to conduct each type of technique and the type of organism that could be targeted.
   1. Quadrants

This is a frame of known size and it can be any shape, but is usually a square. They can be divided into smaller segments. They are placed randomly within an ecosystem and the species within them are recorded. These are good for plants and small, slow animals. Quadrants are not good for highly mobile animals.

* 1. Line Transects

A line is placed through a community and this line acts as a boundary where the

sampling will take place. Once the line is placed, you walk the entire length of the line and record everything observed on the line. Generally line transects are used to sample systematically along an environmental gradient. This technique will capture changes in abundance and distribution along a gradient. It could be used to access an ecosystem as it changes due to altitude or how plants change as you move away from a shoreline. Good for plants and animals that are not highly mobile.

* 1. Belt Transects

Works like a pair of parallel transect lines and everything within the lines is

recorded. These can be used for plants and animals that are not highly mobile.

* 1. Point Transects

Collects data at regular intervals along the transect line and could alternate on either side of the line. These are mainly used to calculate percentage cover and density with plants and animals that are not highly mobile.

* 1. Mark and Recapture

Capture, marking, releasing and then recapturing to determine what tag is present. This mainly focuses on highly mobile animals. The proportion of the marked or tagged animals in the second capture is used to estimate the entire population size.

**PART 1: Species Richness and Evenness**

1. What is the species richness for each site?

Site 1 = 7 species

Site 2 = 9 species

Site 3 = 10 species

1. Do the species seem evenly distributed or is the community dominated by a few species?

Site 1 - there are 3 species with the most number of individuals and several other species that are very rare

Site 2 - half of the species are very common and the other half very rare

Site 3 - the number of individuals of each species seems to be equally represented

1. Calculate species evenness for each site? (I did not round up the numbers)

|  | ***# of individuals counted at site*** | | |
| --- | --- | --- | --- |
| **Species of Bird** | **Site #1 Evenness** | **Site #2 Evenness** | **Site #3 Evenness** |
| Rock Dove (pigeon) | 32/122=26% | 44/219=20% | 20/220=9% |
| Black Capped Chickadee | 4/122=3% | 22/219=10% | 23/220=10% |
| European Starling | 43/122=35% | 47/219=21% | 25/220=11% |
| House Sparrow | 15/122=12% | 42/219=19% | 25/220=11% |
| Song Sparrow | 1/122=0.8% | 6/219=2% | 20/220=9% |
| House Finch | 23/122=18% | 45/219=20% | 24/220=10% |
| Dark-eyed Junco | 0 | 0 | 20/220=9% |
| American Crow | 4/122=3% | 4/219=1.8% | 20/220=9% |
| Common Grackle | 0 | 5/219=2% | 23/220=10% |
| American Robin | 0 | 4/219=1.8% | 20/220=10% |
| ***Total Number*** | ***122*** | ***219*** | ***220*** |

1. What do you think is the best indicator of biodiversity, the number of species, the number of individuals, the evenness, or a combination of all three indicators? Why?

Site 1 has a low species richness and the species that are present are equally represented. Site 2 has a high species richness but is represented mostly by only half the species. Site 3 has a high species richness and is equally represented by those species. Species diversity requires knowledge of both species richness and evenness to get a full picture of how the species are presented.

1. Does the species richness change before and after the introduction of bullfrogs?

No, the species richness stays the same at 16.

1. Do the species seem evenly distributed or is the community dominated by a few species?

Before the introduction of bullfrogs, there were several species with very high population numbers (i.e. Southern House mosquito, Bark scorpion, Western Desert tarantula, and the Cactus mouse), as well as some species with comparatively fewer (Northern Mexican gartersnake and Sonoran mud turtle). After the introduction of bullfrogs, most populations of species decrease, some dramatically. The only species to increase in population numbers is the Southern House mosquito. Therefore, after the introduction of the bullfrog, the ecosystem seems to become more dominated by one species than before.

1. Calculate species evenness for before and after the introduction of bullfrogs?

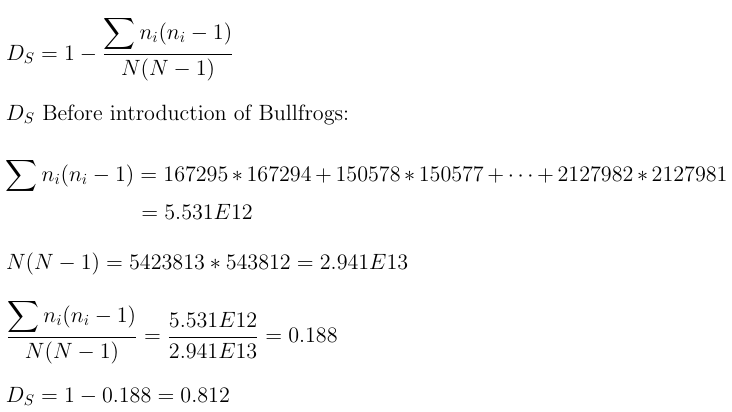
|  | **# of individuals** | |
| --- | --- | --- |
| **Species** | **Before Bullfrogs**  **Evenness** | **After Bullfrogs**  **Evenness** |
| Spadefoot toads | 167,295/5,423,813= 3.08% | 78,290/5,922,331= 1.32% |
| Oregon Spotted frog | 150,578/5,423,813= 2.78% | 98,291/5,922,331= 1.66% |
| Chiricahua Leopard Frog | 126,723/5,423,813= 2.34% | 25,987/5,922,331= 0.44% |
| Northern Mexican Gartersnake | 57,239/5,423,813= 1.06% | 32,093/5,922,331= 0.54% |
| Sonoran Mud Turtle | 63,829/5,423,813= 1.18% | 49,983/5,922,331= 0.84% |
| Barred Tiger Salamander | 75,600/5,423,813= 1.39% | 42,126/5,922,331= 0.71% |
| Sonoran Tiger Salamander | 87,864/5,423,813= 1.62% | 59,209/5,922,331= 0.99% |
| Desert Hairy Scorpion | 237,974/5,423,813= 4.39% | 178,923/5,922,331= 3.02% |
| Bark Scorpion | 487,028/5,423,813= 8.98% | 328,389/5,922,331= 5.54% |
| Desert Shrew | 239,772/5,423,813= 4.42% | 178,273/5,922,331= 3.01% |
| Red Bat | 153,929/5,423,813= 2.84% | 130,289/5,922,331= 2.20% |
| Cliff Chipmunk | 274,923/5,423,813= 5.07% | 230,983/5,922,331= 3.90% |
| Plains Harvest Mouse | 323,861/5,423,813= 5.97% | 298,983/5,922,331= 5.05% |
| Cactus Mouse | 420,392/5,423,813= 7.75% | 329,298/5,922,331= 5.56% |
| Western Desert Tarantula | 428,824/5,423,813= 7.91% | 278,983/5,922,331= 4.71% |
| Southern House Mosquito | 2,127,982/5,423,813= 39.23% | 3,582,231/5,922,331= 60.49% |
| ***Total Number*** | ***5,423,813*** | ***5,922,331*** |

1. How has the introduction of the bullfrog changed the diversity of the environment? Do you think this will affect the health of the ecosystem?

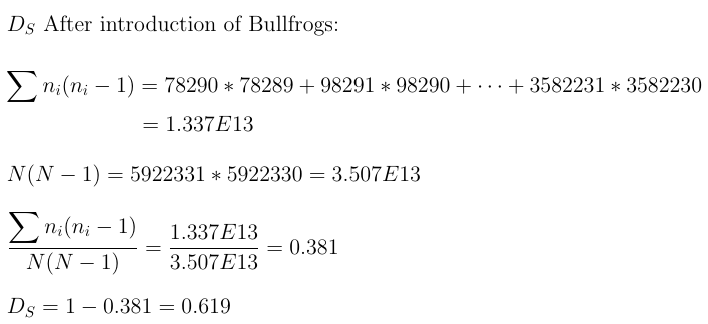
The introduction of bullfrogs caused almost every species to decrease in number. However, the Southern House mosquito increased because its main predators (Oregon Spotted frog, Chiricahau Leopard frog, and Spadefoot toads) decreased due to the competition with the bullfrog.

**PART 2: Introduction to Species Diversity - Simpson’s Diversity Index**

1. Compute the Simpson’s Diversity Index using the data from Table 2 before the introduction of bullfrogs.



Compute the Simpson’s Diversity Index using the data from Table 2 after the introduction of bullfrogs.



1. Compare the values of Simpson’s index from questions 1 and 2. From this comparison, how has the introduction of bullfrogs impacted the species diversity of this community?

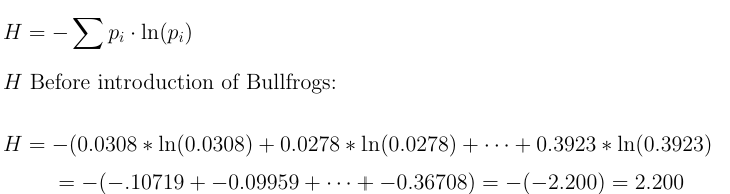
The diversity index was higher before the introduction of the bullfrogs. The community has become less diverse from the inclusion of bullfrogs.

The Simpson’s Index after the introduction of the bullfrogs (0.619) is smaller than the before bullfrogs (0.812) which indicates that the community is less diverse.

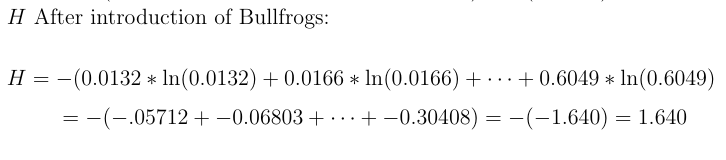
**PART 3: Species Diversity - Shannon-Wiener Index**

1. Compute the Shannon-Weiner Index using the data from Table 2 before the introduction of bullfrogs.

The proportions of each species were computed in Question 7 from Part 1. Using those values converted to a decimal we get the following.



2. Compute the Shannon-Weiner Index using the data from Table 2 after the introduction of bullfrogs.



3. Compare the values of the indices from questions 1 and 2. From this comparison, how has the introduction of bullfrogs impacted the species diversity of this community? Do you get a similar conclusion as that from the answer to question 3 from Part 2 which used the Simpson Index?

The value of the Shannon-Weiner Index decreased from 2.200 to 1.640 after the introduction of the bullfrogs. This indicates that the community diversity has decreased which is the same conclusion that was reached when using the Simpson’s Index.

**Part 4: Sampling Techniques**

1. Why do scientists take a representative sample when estimating the population size of a species?

The most accurate way to determine population size is to simply count all of the individuals within the habitat. However, this method is often not logistically or economically feasible, especially when studying large habitats. Therefore, scientists usually study populations by sampling a representative portion of each habitat and using this data to make inferences about the habitat as a whole.

2. What is a representative sample?

A representative sample is a group or set chosen from a larger statistical population according to specified characteristics that adequately replicates the larger group according to whatever characteristic or quality is under study. A representative sample parallels key variables and characteristics of the larger population under examination. Some examples include sex, age, species, etc.. A larger sample size reduces the likelihood of sampling errors and increases the likelihood that the sample accurately reflects the target population.

3. Why was it necessary to choose your quadrats randomly (pulling Letter / number combinations from the bag)?

It is important that sampling in an area is carried out at random to avoid bias. For example, if one sampled from a school field, but for convenience only placed quadrats next to a path, this might not give a sample that was representative of the whole field. It would be an unrepresentative, or biased, sample.

4. What was the dominant “species” within your total area? Does your quadrat sample reflect this? Were there any ‘species’ that you failed to count in your quadrats (but really did exist in the total area?)

Responses will vary depending on the “species” used and the random assortment of the “species” among quadrats.

5. Predict how the removal of the dominant plant species from the site would affect the population sizes of the other organisms. Explain.

Removing the dominant plant species can have several impacts on other organisms in its environment. These can include increased competition, increased diversity, changes in herbivore populations, shifts in predator-prey dynamics, changes in habitat structure, and invasive species opportunities.

6. In this activity you’ve learned the American bullfrog feeds on all types of organisms, including the Mexican long-tongued bat. This bat is a night pollinator for the Saguaro cactus. You have been asked to determine the number of Saguaro seedlings in an area that has been invaded by the American bullfrog. List the steps you would take to calculate an accurate answer in an unbiased manner. Assume the habitat is 2,000 square meters.

7. Population sampling is usually most effective when a species has an even dispersion pattern. It is difficult to determine an accurate estimate when a population has a clumped dispersal pattern. Explain why this would be the case.

When a species exhibits an even dispersion pattern, individuals are uniformly distributed throughout the habitat. This means that they are spaced relatively evenly apart, which makes it easier to estimate their population size accurately through sampling. In an even dispersion pattern, the probability of encountering individuals is relatively consistent across the entire study area.

On the other hand, a clumped dispersal pattern occurs when individuals of a species are grouped together in clusters or patches within the habitat. This creates areas with high densities of individuals, making it more likely to encounter multiple individuals in those clusters. In contrast, areas with low densities might be missed entirely during sampling efforts. This uneven encounter rate can bias the estimates and lead to underestimation of the overall population size. Estimating the total population based on sampling a few clumps may not be representative of the entire population, especially if the sampled clumps are atypical in size or composition.

8. Based on the bullfrogs you have studied in this activity, could the quadrat sampling method be used to assess a bullfrog population?

1. Why or why not?
2. What sampling method could be used to accurately assess the bullfrog population in a habitat?

Quadrat sampling has proved to be particularly useful in forests when searching

for ground-dwelling amphibians and reptiles (Rodda & Dean-Braley, 2002). For

best results in this methodology of quadrat (or patch) sampling, it will be

important to apply the most appropriate searching technique within each of the

quadrats (e.g., using a rakes over leaf litter).

The mark-recapture method is a widely used technique in ecology to estimate population sizes of animals, especially for species that are difficult to directly count or track. The method involves capturing, marking, and releasing a subset of individuals in the population and then recapturing a new sample later on. By comparing the number of marked individuals in the recaptured sample to the total number of individuals in the second sample, researchers can make statistical inferences about the total population size.

**References**

Adams M.J., and C.A. Pearl. 2007. [Problems and opportunities managing invasive Bullfrogs: is there any hope?](https://doi.org/10.1007/978-1-4020-6029-8_38) In: Gherardi, F. (eds) *Biological invaders in inland waters: Profiles, distribution, and threats.* Invading Nature - Springer Series In Invasion Ecology, vol 2. Springer, Dordrecht.

Rodda G.H. and K. Dean-Bradley. 2002. [Excess density compensation of island herpetofaunal assemblages](https://pubs.er.usgs.gov/publication/1015265). Journal of Biogeography 29: 623-632.