Ingestion of Microplastics by Coral

Alyssa Hoekstra from Virginia Commonwealth University

OVERVIEW AND OBJECTIVES:

This QUBES lesson explores the impact of microplastics on coral. Participants will first get a background on both coral and microplastics, and then the lesson will delve more specifically into how microplastics in the ocean affect coral. The study "Patterns, dynamics and consequences of microplastic ingestion by the temperate coral, *Astrangia poculata"* by Rotjan RD, Sharp KH, Gauthier AE, Yelton R, Lopez EMB, Carilli J, Kagan JC, & Urban-Rich J (2019) is the basis for the lesson. The hands-on portion of the lesson will use select data from this study for analyses in R including plots and a paired permutation test. Participants will then be asked to answer questions about the lesson and use critical thinking to apply what they have learned to explore potential future directions and applications. Expected completion time is 1 class period (1.5 hours).

Astrangia poculata coral polyps consume microplastic beads (in blue) preferentially over brine shrimp eggs (yellow); these beads have the potential to be vectors of novel microbes.
PHOTOGRAPH COURTESY ROTJAN LAB

Source: https://www.nationalgeographic.com/environment/article/these-corals-choose-to-eat-plastic-over-food

Objectives and lesson components:

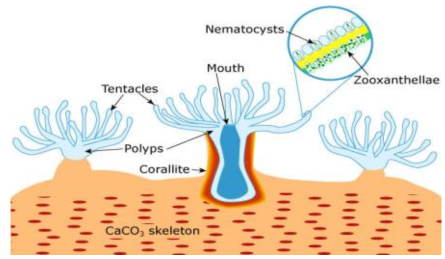
1) **Environmental background:** Learn about coral and microplastics in the environment and how they interact.
2) **Research Review:** Review a study by Rotjan et al. (2019) where coral preferred ingesting microplastics
3) **Statistical Analyses in R:** Use the data collected by Rotjan et. al (2019) to work with data and perform analyses in R
4) **Thinking and connecting the lesson:** Consider what implications these findings might have for future research on microplastic pollution and coral reef threat mitigation

See Appendix I included at the end of the lesson for list of included lesson materials, including supplemental links.

ENVIRONMENTAL BACKGROUND:

CORAL

Coral polyps are soft-bodied animals which may or may not have a hard exoskeleton made of calcium carbonate which are the building blocks for coral reef. In hard coral, polyps are symbiotic with certain kind of single-celled algae (zooxanthellae) which live inside the polyps and give them their color – they also provide organic material for nutrients to host coral. Coral also obtain food by using their tentacles to ingest zooplankton and even small marine creatures, like copepods or eggs. Coral reef provide a marine habitat for fish and their food, act as a shoreline barrier from waves and storms, and are important for the fishing industry and local economies. As many as 60% of the world's coral reef are under immediate local threats, with that number increasing to 75% under threat when considering global threats like climate change. Threats to coral reef such as pollution, climate change, and human activity may cause disease, death, and coral bleaching. Coral bleaching occurs when water temperatures are too warm, and the symbiotic algae are expelled, causing a food source and nutrient loss for the coral. A combination of these can exacerbate the harmful effects to coral. Recently, scientists have been focusing on the effect of microplastic pollution on coral reef.



https://www.usgs.gov/media/images/simplified-coral-anatomy

For more information on coral, see the below link and be sure to check out additional links on the left of the webpage:
https://coral.org/coral-reefs-101/coral-reef-ecology/

MICROPLASTICS

Microplastics are plastics less then 5mm in diameter found in the ocean. They are categorized into two main sources: *primary sources* which are manufactured to be that size – such as microbeads found in cosmetics, and *secondary sources* which start as larger plastics, but are broken down in size from the sun and ocean waves. It is thought that up to 8 million tons of plastic enter the ocean each year; plastics in the ocean is a monumental issue, and as more trash and plastics end up in the ocean, more microplastics are created. The United States banned the use of microbeads in 2015, but there is still the issue of secondary sources and other countries who do not have the same ban. Microplastics pollute the ocean and impact marine habitats. They are also consumed by marine animals, which can have detrimental effects to their health and further up the food web as these animals are consumed further up. Research is continuing into the effects of microplastics on the ecosystem and how humans may even be impacted by consuming microplastics which have made their way into the food web. As microplastics are becoming more pervasive in the ocean, scientists are studying the impact on organisms. We know that coral are under threat from other sources, but as more is understood about microplastics, they are becoming an interest.

https://journals.openedition.org/factsreports/docannexe/image/5290/img-2.jpg

For more information on microplastics, see:
https://www.youtube.com/watch?v=ZHCgA-n5wRw

WHAT IMPACT DO MICROPLASTICS HAVE ON CORAL?

Research is ongoing to study the effect of microplastics on coral. As the effects may depend on the species and type of coral, researchers are studying both temperate and tropical coral, as well as hard and soft coral and how these effects vary between species. Some of the emerging research has shown impacts on the following:

Overall health: Microplastics can reduce coral growth and overall health. Exposure to microplastics can cause coral bleaching and death.

Disease: Microplastics can carry pollution and microbes to coral, whether from ingestion or contact

Impact mechanisms: Cleaning, feeding, and photosynthetic mechanisms of coral can be altered by microplastics.

Barriers to food: Microplastics can act as physical barriers to food, blocking coral from having access to food particles.

Feeding preference: Coral may prefer to ingest microplastics over their typical foods, and after consuming microplastics they are less likely to ingest their typical food

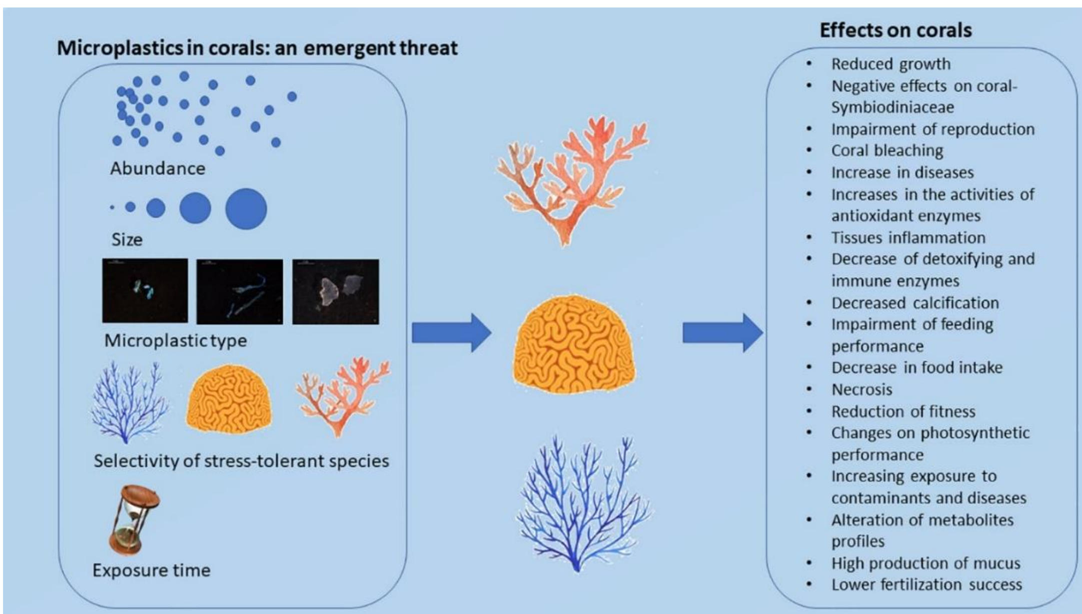


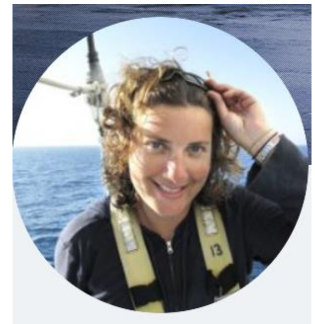
Image from: Oliveira Soares et. al (2020)

For a summary of studies on microplastic threats to coral, see Oliveria Soares et. al (2020) which is linked in Appendix I and also included on the QUBES website as a PDF.

But what does all of this mean? How does this impact overall coral reef health and threats? Scientists are still studying this and how impacts vary across species. Microplastics may just be one piece of the threat puzzle for coral with the exacerbation of outside stressors, like pollution and climate change. As more plastics are used and enter the environment, eventually breaking down into microplastics, these issues and threats facing marine animals like coral will continue to become more prevalent and worrisome.

<u>RESEARCH REVIEW</u>: INTRODUCTION TO RESEARCHER AND DATA

MEET THE PRINCIPAL INVESTIGATOR behind the data for the lesson: **Randi Rotjan, PhD**



Dr. Rotjan is a Research Assistant Professor of Biology at Boston University (BU) and the BU Marine Program. She spends her time out in the field studying marine ecology as well as in the lab, focusing on marine protected areas. She is involved with various marine organizations in both member and leadership roles, including Women Working for Oceans (W2O), The Nature Conservancy – Caribbean, Phoenix Islands Protected Area, Explorers Club, and others.

Watch a video introducing Dr. Rotjan's lab: https://youtu.be/K4fFRDMa1jk

According to her BU lab's website:

> "Research in the Rotjan lab focuses on marine ecology and global change. The main goal is to examine how marine species, communities, and ecosystems respond to the complex multitude of stressors emerging in the contemporary world ocean, and how they will respond to the future ocean change that we expect in the coming decades. In other words, we take a multi-level and systems ecology approach to examining global change. We are also big fans of ocean exploration, which is critical and necessary to set and calibrate ecological baselines. We are a part of a growing global movement to democratize the oceans, making marine science and exploration accessible and available to all. **We apply our science and exploration to conservation. Our simple credo is to help make the world a better place.**"

Other researchers from the study:
Koty Sharp, Roger Williams University
Anna Gauthier, Boston University
Rowan Yelton, Boston University
Eliya Baron Lopez, New England Aquarium
Jessica Carilli, UMass Boston
Jonathan Kagan, Harvard Medical School
Juanita Urban-Rich, UMass Boston

As they were studying coral from the wild, they noticed that almost every coral appeared to have ingested microplastics. Interested in the mechanisms behind this, she and the study team set out to study why this occurring. The QUBES lesson is going to use data from this study to assess ingestion of microplastics of the northern star coral *(Astrangia poculata).*

SCIENTIFIC DATA
Background

Watch this video by Dr. Randi Rotjan on *Astrangia poculata*: https://youtu.be/Chu-rYqJ0Z0

Astrangia poculata, also known as northern star coral, is a temperate coral found off of the waters of the New England coast in the Atlantic Ocean. This coral is being studied to be used as a model for other corals based on their physiology. While other corals are more sensitive to threats, *Astrangia poculata* is able to withstand and adapt better, making them a good model for trying to understand and apply these mechanisms to other more threatened coral. For their study, Rotjan et. al (2019) harvested the coral off of the coast of Rhode Island to first establish if and what kind of microplastics were being ingested by wild coral, and then to perform experiments on harvested coral to better understand ingestion patterns.

Rotjan et. al (2019) Study Overview

Note: you should read the full paper which is included as a PDF on the QUBES website, and also linked in Appendix I.

Study Title: Patterns, dynamics and consequences of microplastic ingestion by the temperate coral, *Astrangia poculata*

Authors: Rotjan R, Sharp K, Gauthier A, Yelton R, Lopez E, Carilli J, Kagan J, Urban-Rich J.

Study objectives:

> "(i) to determine if *A. poculata* ingests microplastics in the wild, (ii) to examine patterns of microplastics ingestion and egestion in the laboratory, and (iii) to identify consequences of microplastic ingestion on subsequent feeding or as a vector for novel microbes."

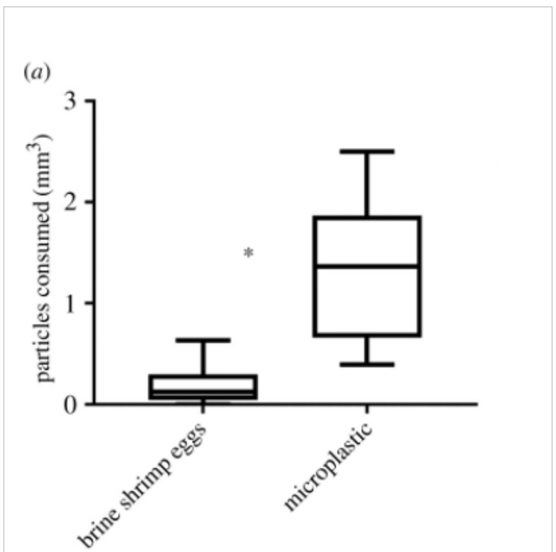
Methods:

> Wild coral was collected off the coast of Rhode Island and dissected in the lab. The microplastics per polyp were counted and classified by shape (fiber, beads, miscellaneous). Harvested coral was brought to the lab. Experiments were run to establish localization of ingested microplastics (top/middle/bottom), feeding behavior of foods following exposure to microplastics or brine shrimp eggs, food preference of microplastics versus brine shrimp eggs, and if microbes such as e. coli could also be ingested with microplastics as a vector

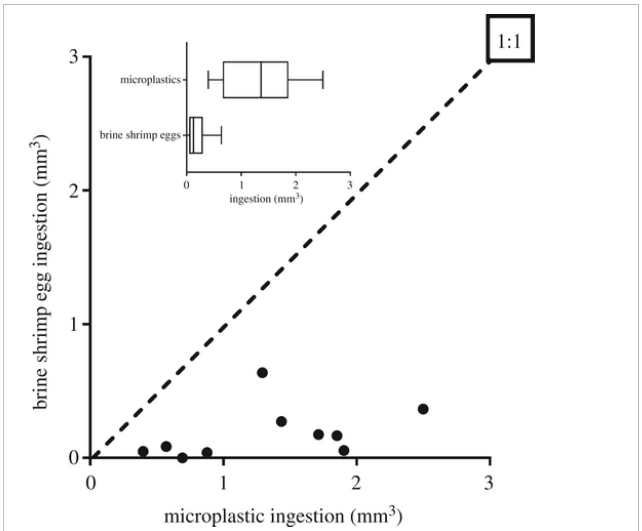
Highlighted Findings:

> Wild Corals: average of 112 microplastics found per polyp, with highest concentration being fiber types
> Harvested Corals: preferred microplastics to brine shrimp eggs; ingested less food after being exposed to microplastics; also ingested microbes and e. coli with the microplastics.

Highlighted Figures from Rotjan et. al (2019) paper: Figures 3 and 5



"Figure 3: Comparison of brine shrimp egg (BSE) consumption versus microplastic bead (MP) consumption. (a) Ingestion of MPs and BSEs in a 50/50 choice assay. (b) Size and shape similarity of BSE and MP beads. Error bars in (a) represent ± 1 s.e.m."



"Figure 5: Comparison of brine shrimp egg (BSE) versus microplastic bead ingestion in *A. poculata* (n = 10 corals). Scatter plot shows paired preference, relative to a dashed line representing no preferential ingestion; any colony (dot) on the dashed line would have ingested an equivalent amount of brine shrimp and microbeads. Box plot shows the means of ingestion preference (inset)."

Data Source for Lesson

Data from the Rotjan et. al (2019) study was obtained from Dryad. The spreadsheet includes the below data on separate sheets which was used for the figures and analyses.

1. Wild coral – number and type of plastics found per polyp and per mass
2. Harvested coral - % of ingested microplastics located in the top/middle/bottom section
3. Harvested coral - Particles consumed per mm3 for brine shrimp eggs and microplastics
4. Harvested coral - Amount (per mm^3) of food type or microplastic ingested after brine shrimp egg or microplastic ingestion
5. Polyp volume

Click here to access the Dryad site with the data.

> Note: The statistical analysis that was run in the paper for preference of microplastic vs brine shrimp egg ingestion were run on polyp-level data (n=325), whereas the data we have only uses the per mm3 amounts for each colony (n=10) as provided in the data spreadsheet. Therefore, we will not be able to run the exact statistical analyses on the polyp-level data, but we will run the same tests used and see if we get similar results for the polyp-level data. The figures/plots we will recreate did use the colony-level data in the paper.

STATISTICAL ANALYSES IN R

For this lesson, we are going to use data behind Figures 3 and 5 of the Rotjan et. al (2019) paper looking at coral ingestion preference of microplastics over brine shrimp eggs. In the study, harvested coral were exposed to the same amount of microplastics and brine shrimp eggs and given the opportunity to feed on them. The amount of each particle ingested was then counted. The authors ran paired permutation tests on polyp-level data (n=325) for their statistical analyses, then plotted the colony-level data (n=10). From the available data, we have access to the colony-level data. We are going to test normality of this data, plot using a boxplot, then perform a paired permutation test and finally use a scatterplot to better visualize ingestion preference of coral.

SCIENTIFIC QUESTION

When given an equal option, do coral preferentially ingest microplastics over brine shrimp eggs?

HYPOTHESIS

Similar to the polyp-level data, the amount of particles/mm3 consumed at the colony-level data will be significantly higher for microplastics consumed than brine shrimp eggs consumed.

DATA ANALYSIS USING R

We will be using R to analyze the data. R will allow us to pull in the data and use code to perform statistical analyses and create plots. Make sure you have R and R studio downloaded and installed on your computer before starting the lesson.

Before analyzing the data, we are going to do an overview of a few important concepts.

> Watch a helpful YouTube video for getting started with R: https://www.youtube.com/watch?v=lVKMsaWju8w.
> The R and R project websites also have "Help" sections.
> Links for R/R Studio downloads:
> https://www.r-project.org/
> https://www.rstudio.com/products/rstudio/#rstudio-desktop

Distribution of data: Normality

Normality of data is the distribution of the data around the mean. When data is normally distributed, half of the values fall to either side of the mean and when plotted will be in a symmetrical bell-curve distribution. If data is normal, we can say it came from a normally distributed population. Tests like the Shapiro-Wilk test (described further below) can be used to test the null hypothesis that the data is normally distributed; a p value less than 0.05 (or wherever the alpha is set) would signify that data is not normally distributed, whereas a p value greater than 0.05 would signify that we cannot reject the null hypothesis and we can assume the data is normally distributed. Whether or not the data is normally distributed will indicate which statistical test should be used.

Parametric vs non-parametric tests: which to use?

Before analyzing data, it is important to select the appropriate statistical test. Choosing an appropriate test will not only depend on the hypothesis being tested and the types of variables, but also how the data within the population is distributed. Tests can broadly be categorized into two categories: Parametric and Non-parametric. The first and main distinction between them is that parametric tests should only be used when data is normally distributed, whereas non-parametric tests can be used even if data does not fit normal distribution.

Parametric tests

- Assumptions to use:
  - Data has a normal distribution
  - There is a linear relationship within the data
  - Data is independent
  - Data from groups have similar variance
- Uses mean as measure of central tendency

Non-parametric tests:

- Data does not need to meet the criteria for a parametric test
- Data does not need to fit normal distribution
- Uses median as measure of central tendency

Common Tests Used

Parametric test	Non-Parametric equivalent
Paired t-test	Wilcoxon Rank sum Test
Unpaired t-test	Mann-Whitney U test
Pearson correlation	Spearman correlation
One way Analysis of variance	Kruskal Wallis Test

https://www.healthknowledge.org.uk/public-health-textbook/research-methods/1b-statistical-methods/parametric-nonparametric-tests

Overview of analyses and plots to be employed for this lesson

Histogram:

A histogram is a visual representation of the frequency and distribution of data.

Shapiro-Wilk test for normality:

A test of normality will tell the researcher if data is normally distributed. When data is not normally distributed, it can skew results. By doing a Shapiro test, one can select an appropriate statistical test for testing the hypothesis based on the distribution of the data, or perform additional steps (like log transforming) to transform the data to a normal distribution.

Boxplot:

A boxplot plots the data in a way that shows five different summaries of the data: the minimum and maximum and represented by points at the top and bottom of the plot, the first and third quartile of the median are represented by the top and bottom of the box, and the median is the middle line. This is a good way to view a summary of the data meaningfully.

Paired Permutation test:

A permutation test can be used when the data is not normally distributed. Permutation tests take the observed data, shuffle it, and recalculates it for a number of times to calculate a distribution of the statistic. The distribution of the permutation statistic is then compared against the original observed statistic see if it is significantly different; you will be able to see how likely the difference is if the data were randomized. In a paired permutation test, the data from two conditions are paired, and we want to see if there is a difference between these paired numbers. There are several different methods for calculating paired permutations.

For our lesson, we are going to use the paired Wilcoxon test, which compares medians and is appropriate for small sample sizes. It is important to note that we are using a paired permutation test for our analyses as we compare the particles consumed per mm3 of brine shrimp eggs vs microplastics across colonies.

> See the following link for a more detailed explanation of the paired Wilcoxon test: https://www.datanovia.com/en/lessons/wilcoxon-test-in-r/#signed-rank-test-on-paired-samples

Scatterplot:

A scatterplot can be used to visualize the relationship between two variables. Points are plotted based on the two variables using the x axis and y axis.

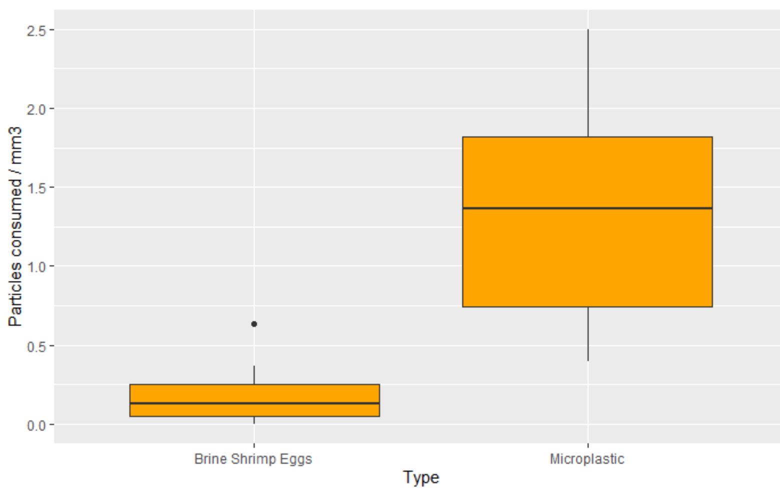
Analyses in R

1) Download the excel sheet titled “Rotjan et al data” from the QUBES website. The data spreadsheet has been formatted and modified to only include the data needed to run the analyses in R. (tip: when downloading the spreadsheet, make sure the file name matches what is in the R markdown script – sometimes extra characters are added at the end after download from QUBES)
2) Download the R Markdown file titled “R Markdown - QUBES Lesson Coral and MPs.rmd” from the QUBES website and load it into R Studio
3) Complete the steps in the R Markdown to run the following
    i) Check normality of data
        a. Histogram
        b. Shapiro-Wilk test for normality
    ii) Boxplot
    iii) Paired Permutation test
        a. Median and interquartile range
        b. Paired boxplot
    iv) Scatterplot

THINKING AND CONNECTING THE LESSON: EXERCISE QUESTIONS

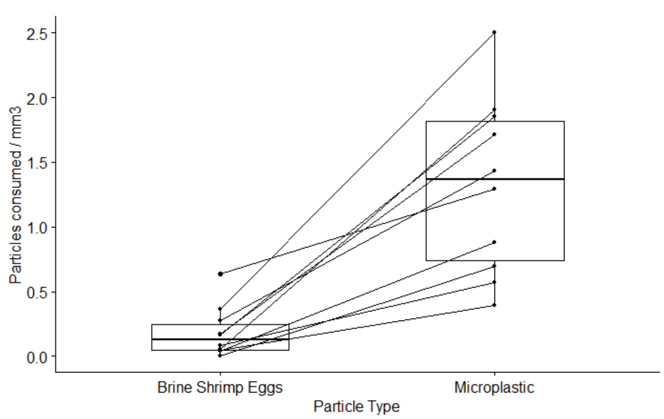
Findings and Questions from R

1) Histogram and normality of data
   - Why is it helpful to first visualize the data as a histogram?
     It allows you to see the distribution of the data. If it follows the bell curve shape, it is likely to be normally distributed.
   - Record the p-values for the normality of the:
     Microplastics – Particles consumed / mm3 p=0.8__________
     Brine Shrimp Eggs – Particles consumed / mm3 p=0.04__________
   - What can you tell about the normality of the data based on this? Did the Shapiro Test match up with what you expected following the histograms?
     Yes – the microplastic was bell curved shaped and the Shapiro test showed the data was normally distributed, whereas the brine shrimp egg data was distributed heavily to one side of the graph and was not able to be determined as normally distributed.
2) Boxplot



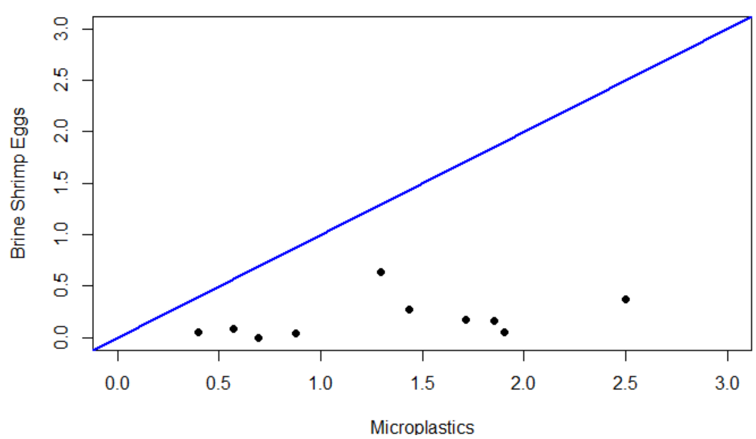
   - From visualizing the data, what can you infer?
     The microplastics seem to have a higher consumption than the brine shrimp eggs. The median for microplastics is at about 1.3, whereas the median for brine shrimp eggs is at about 0.125.

3) Paired Permutation test : Wilcoxon test and related analyses/plots



- Do you think the paired boxplot we created is more helpful than the first boxplot? Why? Potential answer: Because the data is paired, the paired boxplot allows us to see where each of the paired data points falls on the plot. This is important when visualizing the information to see maintain the paired relationship.
- Median values calculated: Brine Shrimp Eggs 0.127 _______Microplastics 1.364 __________
- Interquartile range (IQR) calculated: Brine Shrimp Eggs 0.197 _______ Microplastics 1.079 __________
- Record the effect size you calculated: ________ 0.886 What does this mean? There was a large effect size, showing that the difference between the two groups was not only significant, but strong.
- P value of the two-sided test: 0.00195 _______ What does this tell you about the data? The median number of microplastic particles consumed / mm3 is significantly different than the median number of brine shrimp egg particles consumed / mm3 for the coral.
- P value of the one-sided test: 0.00097 _______ What does this tell you about the data? The median amount of Brine Shrimp Egg particles consumed/mm3 was significantly less than the median amount of Microplastic particles consumed/mm3.

4) Describe the relationship shown in the scatterplot.



The line through the middle signifies a neutral preference, and dots on either side would signify a preference in that direction. Each dot represents one colony, with the dot placed on where the relative preference was based on the amount of brine shrimp eggs consumed vs microplastics. All of the dots fell on the microplastics side, showing a preference for microplastics.

Critical Thinking Questions:

5) Did your findings support the hypothesis? How did your findings compare to what was in the Rotjan et al. (2019) paper? Yes – the hypothesis was supported. Using the colony-level data, we also found that microplastics/mm3 consumption was significantly higher than brine shrimp eggs/mm3

consumption. We were able to recreate the plot from figure 3 and figure 5 using the data from the spreadsheet in R.

6) What do you think are possible implications of the findings of this research?
If coral ingest microplastics over their usual sources of food, it could be detrimental to their health. Microplastics can harbor disease from microbes and make the coral sick, they can make the coral feel full and the coral will then not eat and will not get enough nutrients. Given that the microplastics and brine shrimp eggs were similar in size, the fact that significantly more microplastics were consumed shows that coral prefer them and may actually seek them rather than food.

7) How do you think outside stressors like pollution and climate change are relevant to the issue of microplastics and coral, specifically for ingestion?
As microplastics can carry pollutants and microbes, it is just one way for pollution to make it's way into coral. As coral are also facing these outside stressors from the environment, having the added stress of microplastics impact on their mechanisms is just one more threat to their health, especially if their mechanisms are already misfunctioning due to pollution or climate change.

8) Based on what you learned, what do you think research should focus on next?
(Potential answers - not all-inclusive):
Do tropical coral species also ingest microplastics preferentially over food when given the chance?
Is amount of microplastic ingestion linked to coral bleaching or death rates
Relationship of microplastic ingestion to coral health
How do other environmental factors influence the effects of microplastics? (ie water temperature, ecological richness, water clarity, access to food, etc)

9) What are actions humans can take to help mitigate this problem?
(Potential answers - not all inclusive) Use less plastics and reuse them. Recycle. Focus on cleaning up plastics at shoreline before they become microplastics. Focus efforts on how to clean up plastics that have already reached the ocean. Better attempts to control pollution. Biodegradable plastics and materials. Bans on harmful and unnecessary plastics.

CONCLUSION OF LESSON:

Analyzing the colony-level data can bring us to the same conclusion as the Rotjan et. al (2019) study: when given the option, coral preferentially ingested microplastics over brine shrimp eggs. The ingestion of microplastics by coral has implications for nutrition intake and health. This could lead to lack of proper nutrition in the coral or consumption of microplastics that contain vectors for disease. Microplastic ingestion is an added threat to coral who are already facing multiple threats to their health and survival; for coral who are already sensitive, these threats are then multiplied. The preference for ingestion of microplastics is just one piece of the puzzle, and researchers will continue studying how different species of coral are affected and in what ways. It is also an indication of just how pervasive microplastics are for marine life. As researchers and others work to find solutions to these problems – both coral threats and microplastics – we can all do our part by using less plastic, recycling, and making sure plastic does not end up in the ocean.

APPENDIX I: Included Materials

1. Master lesson: Student and Instructor
2. PowerPoint introduction to QUBES lesson
3. R markdown – "R Markdown - QUBES Lesson Coral and MPs.rmd"
4. Data spreadsheet – "Rotjan et al data"
5. Supplemental Materials
    a. Environmental issue
        i. Coral: https://coral.org/coral-reefs-101/coral-reef-ecology/
        ii. Microplastics in ocean: https://www.youtube.com/watch?v=ZHCgA-n5wRw
        iii. Astrangia poculata: https://youtu.be/Chu-rYqJ0Z0
    b. Research Papers:
        i. Rotjan et. al (2019) http://dx.doi.org/10.1098/rspb.2019.0726
        ii. Oliveria Soares et. al (2020) https://doi.org/10.1016/j.marpolbul.2020.111810
    c. Statistics
        i. Paired Permutation using Wilcoxon test https://www.datanovia.com/en/lessons/wilcoxon-test-in-r/#signed-rank-test-on-paired-samples

Sources:

Oliveira Soares, M., Matos, E., Lucas, C., Rizzo, L., Allcock,L., & Rossi, S. (2020). Microplastics in corals: An emergent threat. Marine Pollution Bulletin *(161, A)* doi: https://doi.org/10.1016/j.marpolbul.2020.111810.

Reichert, J., Arnold, A., Hoogenboom, M., Schubert, P., & Wilke,T. (2019). Impacts of microplastics on growth and health of hermatypic corals are species-specific. *Environmental Pollution, (254, B).* https://doi.org/10.1016/j.envpol.2019.113074.

Rotjan R., Sharp K., Gauthier A., Yelton R., Lopez E., Carilli J., Kagan J., Urban-Rich J. (2019) Patterns, dynamics and consequences of microplastic ingestion by the temperate coral, Astrangia poculata. *Procedures of the Royal Society B. 286*: 20190726. http://dx.doi.org/10.1098/rspb.2019.0726

Rotjan, Randi D. et al. (2019), Data from: Patterns, dynamics and consequences of microplastic ingestion by the temperate coral, Astrangia poculata, Dryad, Dataset, https://doi.org/10.5061/dryad.3qc6328

https://www.fisheries.noaa.gov/feature-story/resilient-new-england-coral-teaching-us-about-future-reefs

https://coral.org/coral-reefs-101/coral-reef-ecology

https://oceanservice.noaa.gov/facts/coral-pollution.html

https://oceanservice.noaa.gov/facts/microplastics.html

https://www.nationalgeographic.com/animals/invertebrates/facts/corals-1

https://www.nationalgeographic.org/encyclopedia/microplastics/

https://www.nationalgeographic.com/environment/article/these-corals-choose-to-eat-plastic-over-food

https://schmidtocean.org/person/randi-rotjan/

https://www.bu.edu/biology/people/profiles/randi-rotjan/

http://sites.bu.edu/rotjanlab/

https://www.wbur.org/earthwhile/2019/06/26/northern-star-coral-boston-university-study-microplastic-pollution

https://www.forbes.com/sites/priyashukla/2019/06/29/some-corals-prefer-to-eat-microplastics/?sh=238c68ad6caf

https://reefbites.com/2018/05/09/microplastics-and-negative-implications-on-coral-reef-health/

https://www.datanovia.com/en/lessons/wilcoxon-test-in-r/#signed-rank-test-on-paired-samples

https://rcompanion.org/handbook/K_01.html

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