

WHAT'S IN THE GUT?

INSIGHTS INTO THE GASTROINTESTINAL MICROBIOME IN THE HAWAIIAN GREEN TURTLE

Ronald P. Kittle, III

Co-Authors: KJ McDermid, A Veillet, S Plouviez,

L Muehlstein, GH Balazs

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HAWAIIAN GREEN TURTLE DIET

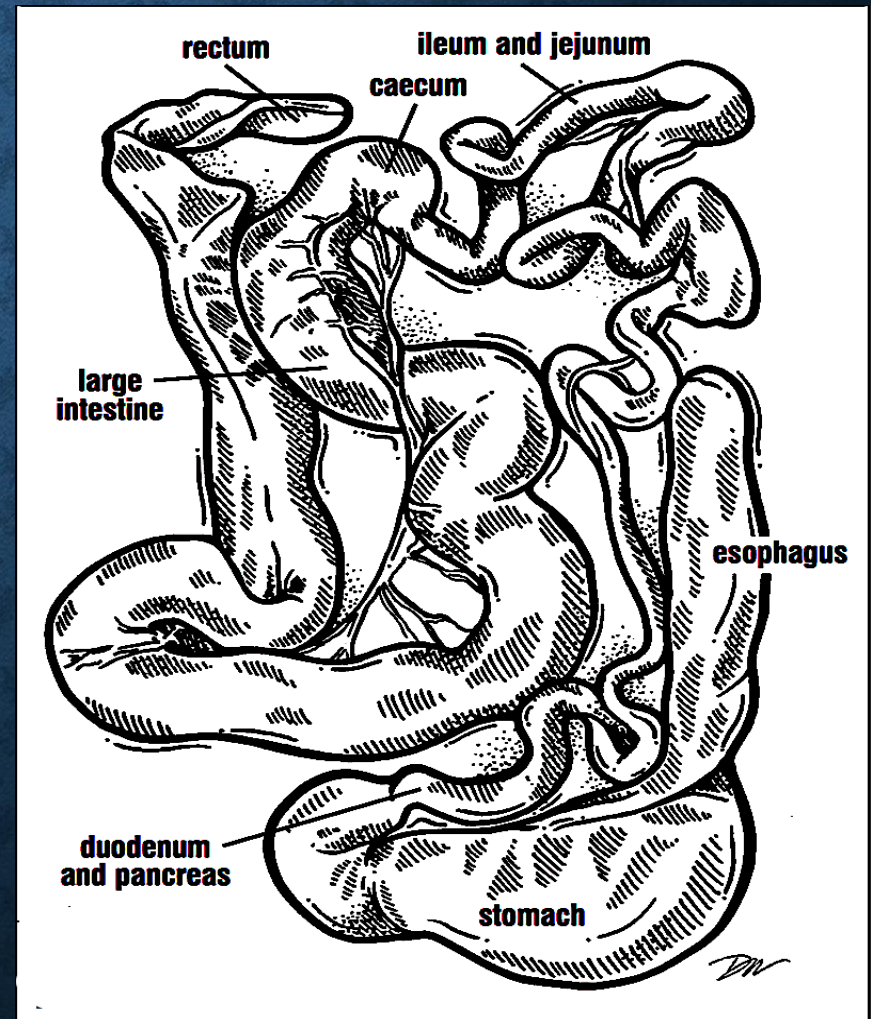
Known To Consume:

- Over 300+ species of macroalgae
- Two seagrasses
- At least one terrestrial shoreline grass
- Fallen leaves of coastal trees.

HINDGUT FERMENTATION

Hindgut Fermenter =
cellulolytic microbes to break
down plant matter in the
caecum and Large Intestine
(ANAEROBIC)

Other Hindgut Fermenters:
Horses, Rabbits, Rhinos, Koalas



IMPORTANCE OF THE MICROBIOME

The microbiota contained within contribute to:

- Provide nutrition and vitamins & energy to host → development
- Stimulation of the immune response
- Help protect against pathogens, neutralize toxins or secondary metabolites from food.
- Regulate gene expression in the host's epithelial tissues.

CHANGES IN MICROBIOME

- Strong links have been documented among ecosystem factors (host habitat, available resources, and diet), microbiome characteristics (richness, diversity, composition, and stability), and host health, fitness, and abundance.
- Change in host diet “can rapidly and dramatically alter composition, relative abundance, and function of gut microbes.”
- Changes in the microbiome may lead to inefficient digestion, lowered immune system, etc.

PREVIOUS MICROBIAL STUDIES OF GREEN TURTLES

- Cloacal & nasal swabs

Cloaca = common cavity for intestinal, urinary, and reproductive tracts

- Cloacal swabs
- Fecal & cloacal swabs of juvenile *C. mydas*
- Pre-rehabilitation and post-rehabilitation

None sampled *in situ* gastrointestinal microflora

MICROBIAL GUT COMMUNITIES

- Dominant microflora in marine herbivores:

Phyla: Bacteroidetes, Firmicutes, Verrucomicrobia

- Microflora in omnivores:

Phyla: Proteobacteria

OBJECTIVE

- The objective of this study was to identify the digestive, gastrointestinal microbiota found in the green turtle (*Chelonia mydas*) in Hawai'i.

PRELIMINARY SAMPLE COLLECTION

Preliminary attempts for cloacal and fecal swabs were attempted

- Sterile Cotton Swab
- Fecal Loop
- Catheter
- Swine & Equine Artificial Insemination Rod
- **No success or contamination from the bladder (due to shared opening) during or immediately after insertion.**
- Minimize contamination → *in situ* directly from the GI tract methods were deemed most appropriate.

METHODS

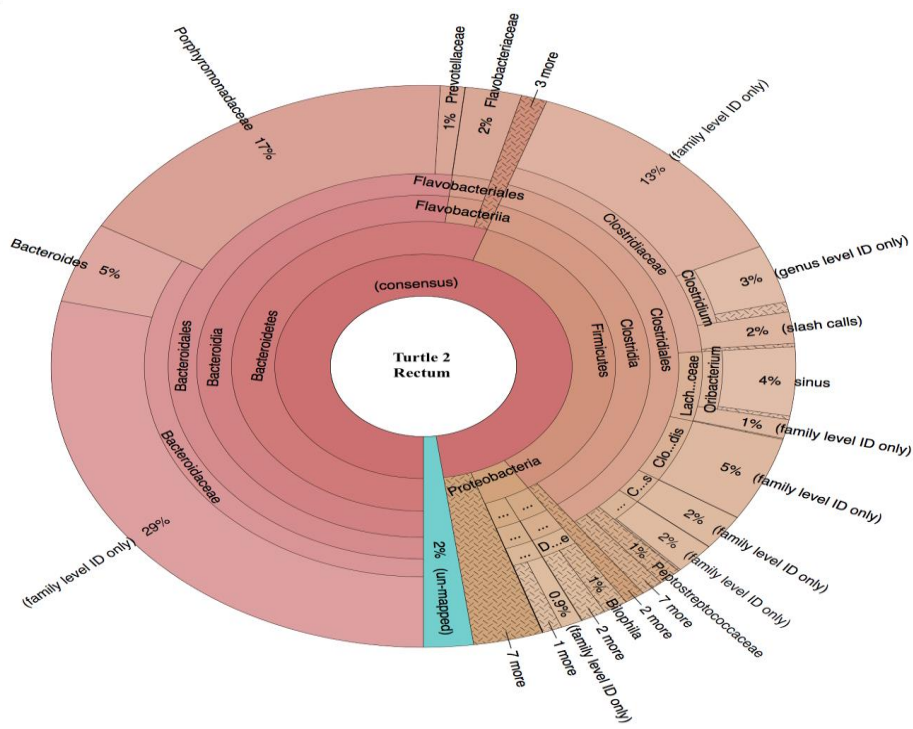
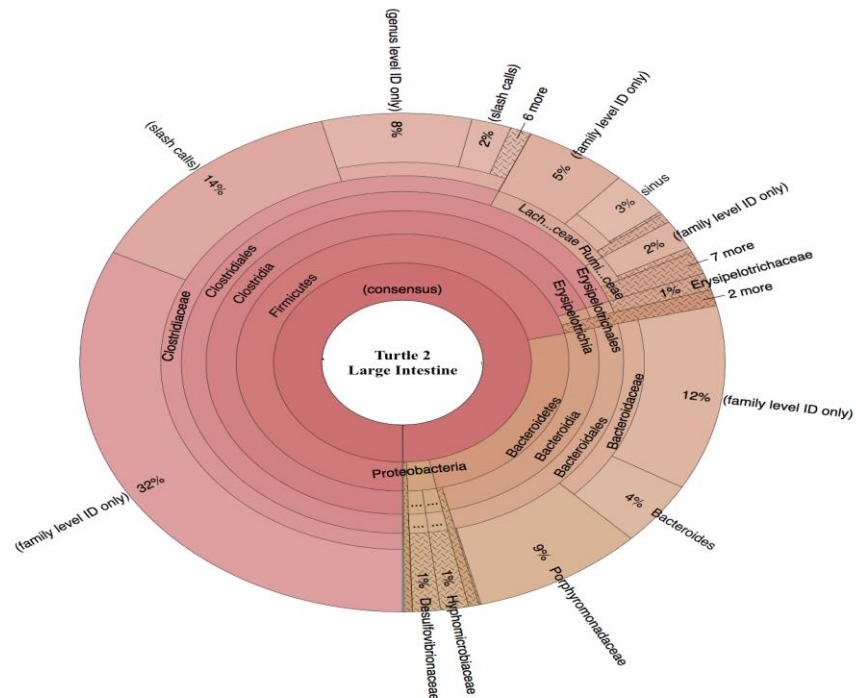
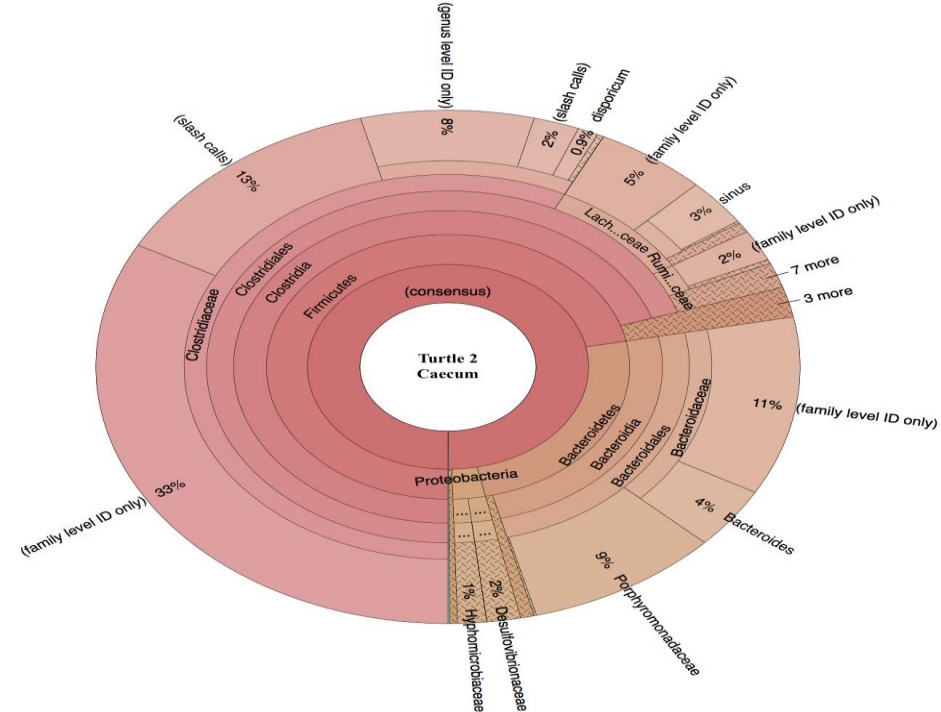
- Eight turtles served as donors for this project, whom were to be euthanized due to mortal injury or terminal illness.
- Fresh gut samples were taken at five locations along the gastrointestinal tract (crop, stomach, small intestine, caecum, and large intestine) from eight turtles for traditional culturing.
- Samples grown aerobically & anaerobically on various selective media

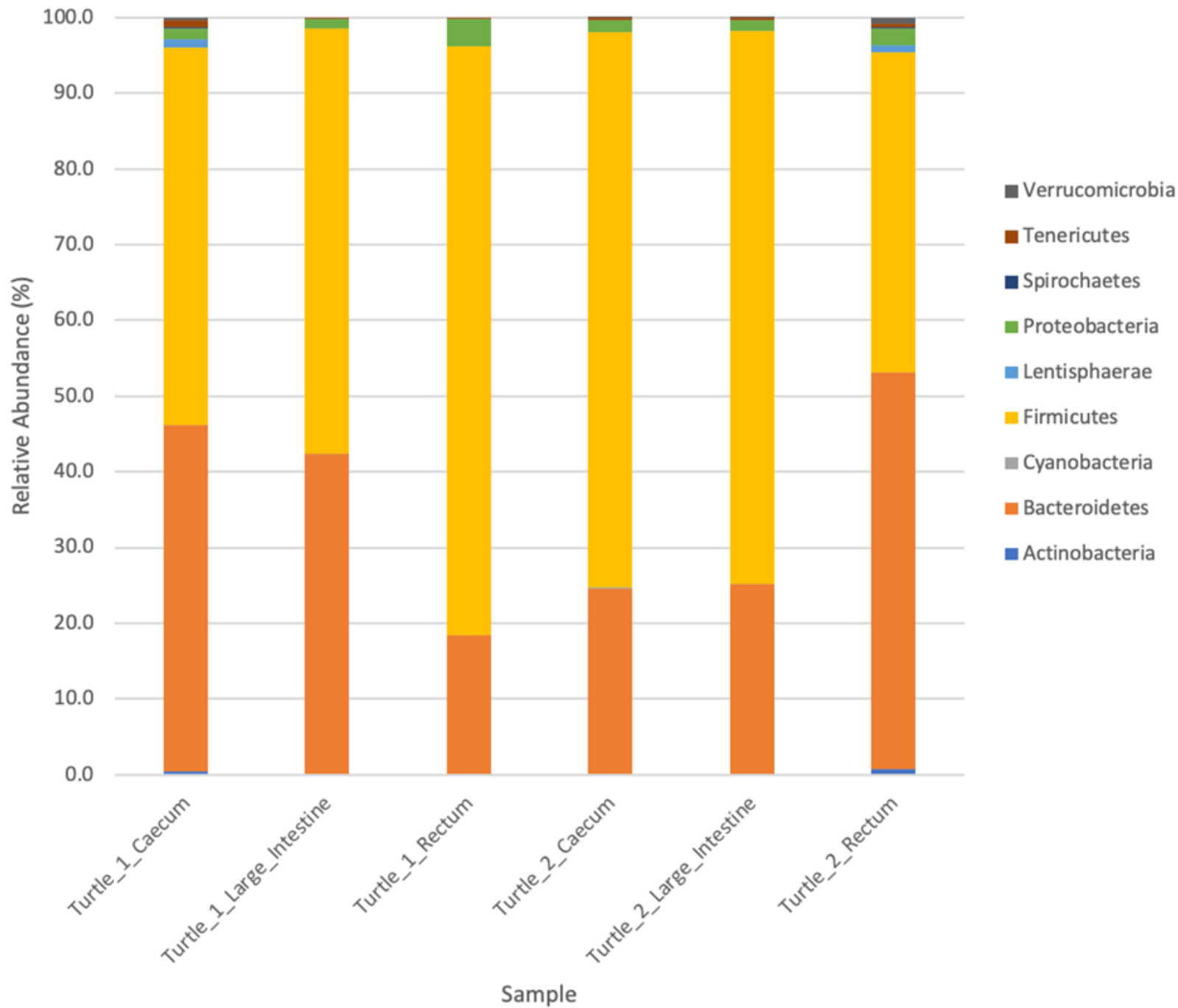
Gastrointestinal Location

Taxa	Crop	Stomach	Small Intestine	Caecum	Large Intestine
<i>Citrobacter freundii</i>	-	+	+	+	-
<i>Escherichia coli</i>	+	+	+	+	+
<i>Klebsiella sp.</i>	+	+	+	-	-
<i>Pantoea agglomerans</i>	+	+	+	+	+
<i>Proteus mirabilis</i>	+	+	+	+	+
<i>Proteus vulgaris</i>	+	+	+	+	+
<i>Pseudomonas sp.</i>	+	+	+	+	+
<i>Salmonella sp.</i>	-	-	+	+	-
<i>Serratia marcescens</i>	+	+	+	+	-
<i>Shigella sp.</i>	+	+	+	+	+
<i>Staphylococcus aureus</i>	-	+	+	+	-
<i>Staphylococcus sp.</i>	-	+	+	+	-
<i>Vibrio sp.</i>	-	+	+	+	-
Total :	8	12	13	12	6

TRANSITION TO 16S rDNA METAGENOMICS

- Much of the diversity is not shown through traditional culturing methods.
- Most identified were facultative aerobes, so not truly the digestive, cellulolytic microbiota that exist in anaerobic conditions..
- Switched to molecular techniques for metagenomics 16S rDNA
 - Two turtles x 3 locations (caecum, large intestine, rectum)
 - ~4.5 million reads sequenced using Ion Torrent PGM
 - Bioinformatics done using dada2 pipeline in R





KEY MICROBIOTA

- *Many species of Clostridium and Ruminococcus* other members of the Phylum Firmicutes in high abundance and are known to produce carbohydrate active enzymes and to degrade cellulose.
- *Clostridium butyricum* produces butyrate and acetate through microbial fermentation of dietary fibers in the lower intestinal tract
- Species of *Bacteroides* produce a variety of enzymes that can degrade laminarin, a polysaccharide of brown algae

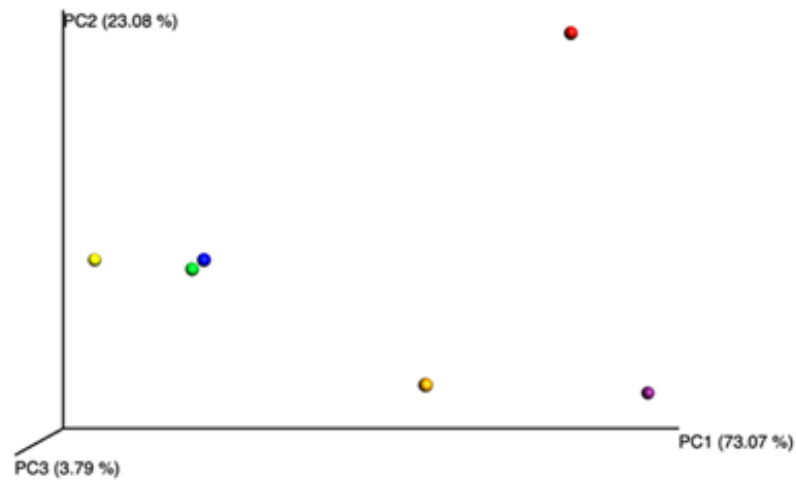
KEY MICROBIOTA

- *Desulfovibrio* (Proteobacteria) in small %, which is important as some red algae are a likely source of sulfate in the form of sulfated algal galactans
- All samples from the green turtle GI tract had dominant microflora consistent with other marine herbivores, i.e. fish, marine iguana, manatee.

	Shannon-Wiener Diversity Index for Bacterial Family (H')		
	Caecum	Large Intestine	Rectum
Turtle 1	2.03 (0.53)	1.76 (0.46)	1.51 (0.39)
Turtle 2	1.55 (0.40)	1.63 (0.43)	2.14 (0.56)

Jaccard's Similarity Index

	T1 Caecum	T1 Intestine	T1 Rectum	T2 Caecum	T2 Intestine	T2 Rectum
T1 Caecum		0.48	0.44	0.54	0.77	0.68
T1 Intestine			0.41	0.62	0.55	0.39
T1 Rectum				0.76	0.56	0.32
T2 Caecum					0.59	0.38
T2 Intestine						0.6
T2 Rectum						

A

● Turtle 1 Caecum

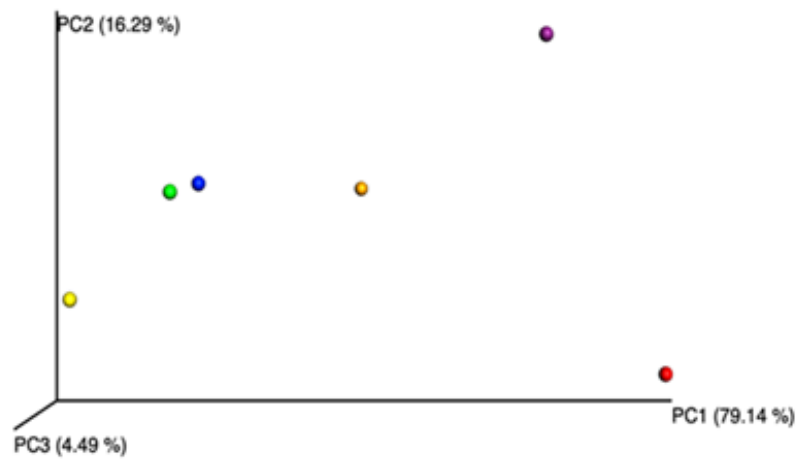
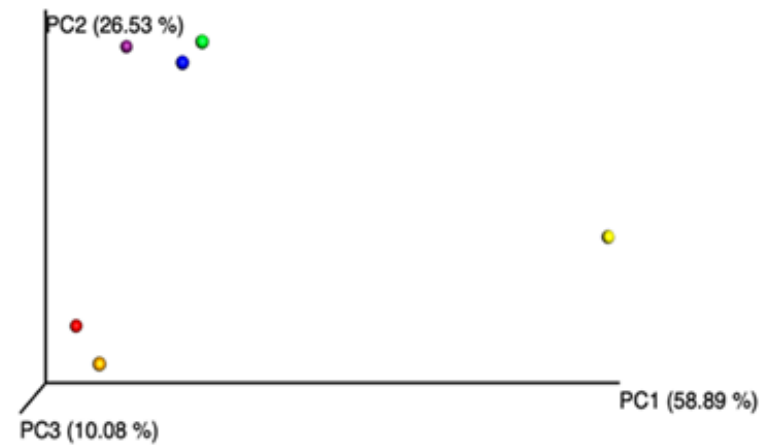
● Turtle 2 Caecum

● Turtle 1 Large Intestine

● Turtle 2 Large Intestine

● Turtle 1 Rectum

● Turtle 2 Rectum

B**C**

FIRMICUTES:BACTEROIDETES

- Ratio used as a proxy of gut health and diseases for various animals.
- Our study (~2.2:1) is consistent with healthy herbivorous hindgut fermenters (horses = 2.0)
- Cloacal swabs of pre-hospitalization green turtles: = 1.77
Cloacal swab samples of post-rehabilitation turtles = 0.56
- Gulf of Mexico juvenile green turtle fecal samples = 2.0
- Gulf of Mexico juvenile green turtle cloacal swabs = 0.47

TAKE HOME MESSAGES

- Studies are needed to compare the microbiomes of green turtles in different habitats, eating different diets, and exposed to different stressors
- The fermentation of carbohydrates to short chain fatty acids “appears to exhibit a high level of redundancy”
- Multiple species in the phyla Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria have the genes that code for carbohydrate metabolism.

TAKE HOME MESSAGES

- Green turtle GI microbiomes might be a potential source of novel microbial genes and enzymes with bioengineering applications in the breakdown of complex plant materials.
- In the future, we may need to “mine the microbiome” of green turtles!
- Accurate and precise enumeration of green turtle microflora may help clarify connections between diet composition and digestive bacteria, as well as provide new tools for assessing the health of green turtles grazing in different locales.

FUTURE STUDIES

- Future studies should compare the GI tract bacteria of more turtles for insight into the healthy, core gastrointestinal microbiome.
- Similar sequencing technologies can be used to analyze diet using more specific primers. Some regions of 16S can amplify algae.