



Investigation Of Bacterial Communities Within the Microbiome of Tiger Sharks (*Galeocerdo cuvier*) in Biscayne Bay, Florida

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Introduction

- Every animal on Earth forms an association with microorganisms¹
- The ocean contains the densest accumulation of microbes on Earth, therefore all marine organisms are exposed to a large and diverse group of microbes. Studies have provided much insight, but little is known about the interaction of microbes in shark health and immune defence and how it varies among different species and in diverse habitats
- There is a large research gap on the physiological status of wild sharks, and specifically how bacterial communities within the microbiome of sharks apply not only to the health of an individual, but the quality of the surrounding marine environment which they inhabit. Most of the current research on the physiological status of sharks comes from captive settings
- The tiger shark is a large (up to 5.5m in length) apex predator found in many oceans throughout the world⁸
- Very few studies have been done on the physiology of these organisms and even fewer have evaluated their immune status; this represents the first study to investigate microbial communities on various tissues of tiger sharks in the wild

Methods

- Sharks captured in South Florida via a circle-hook drumline system and then released once samples were obtained using a bacterial scraper.²
- Two cultures per sample were prepared, and incubated, then subcultured in the lab, bacterial colonies were isolated for gram staining and DNA extraction.
- DNA was extracted via Qiagen QIAmp DNA Mini Kit, and PCR was visualized using 6X TrackIT Cyan/Orange Loading buffer, and UV Transillumination in a Gel Doc system
- Bacterial genus and families were identified via sequencing following PCR and the NCBI BLAST
- Preliminary data analyses were conducted using Microsoft Excel.

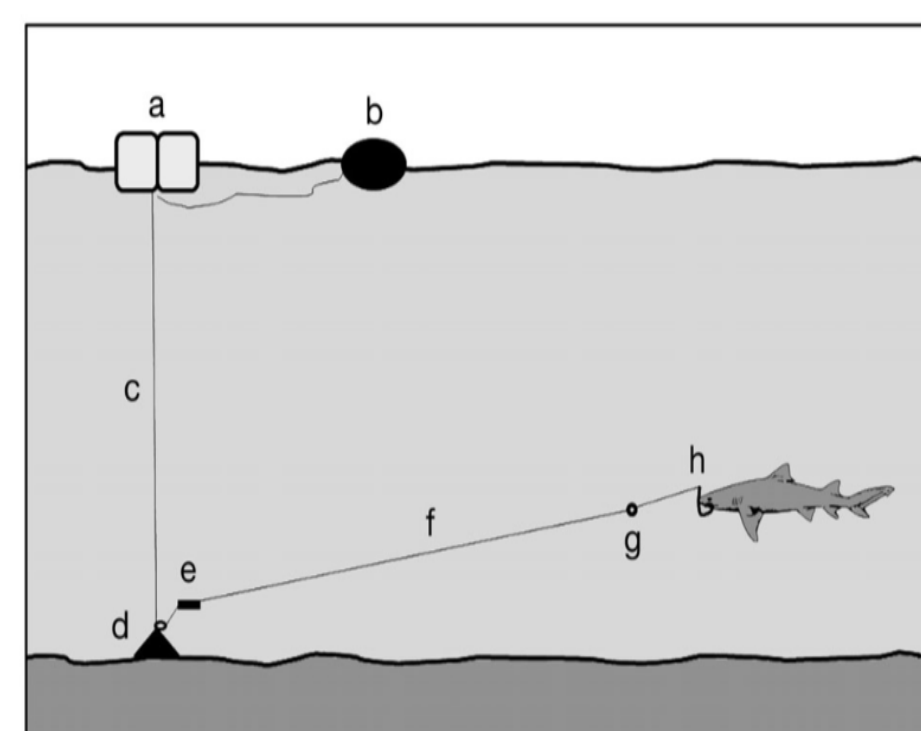


Figure 1. The circle-hook drumline system used to capture sharks.²



Figure 2. Collecting cell scrapings from the inside of the first gill of a juvenile tiger shark (University of Miami Shark Research)

Results

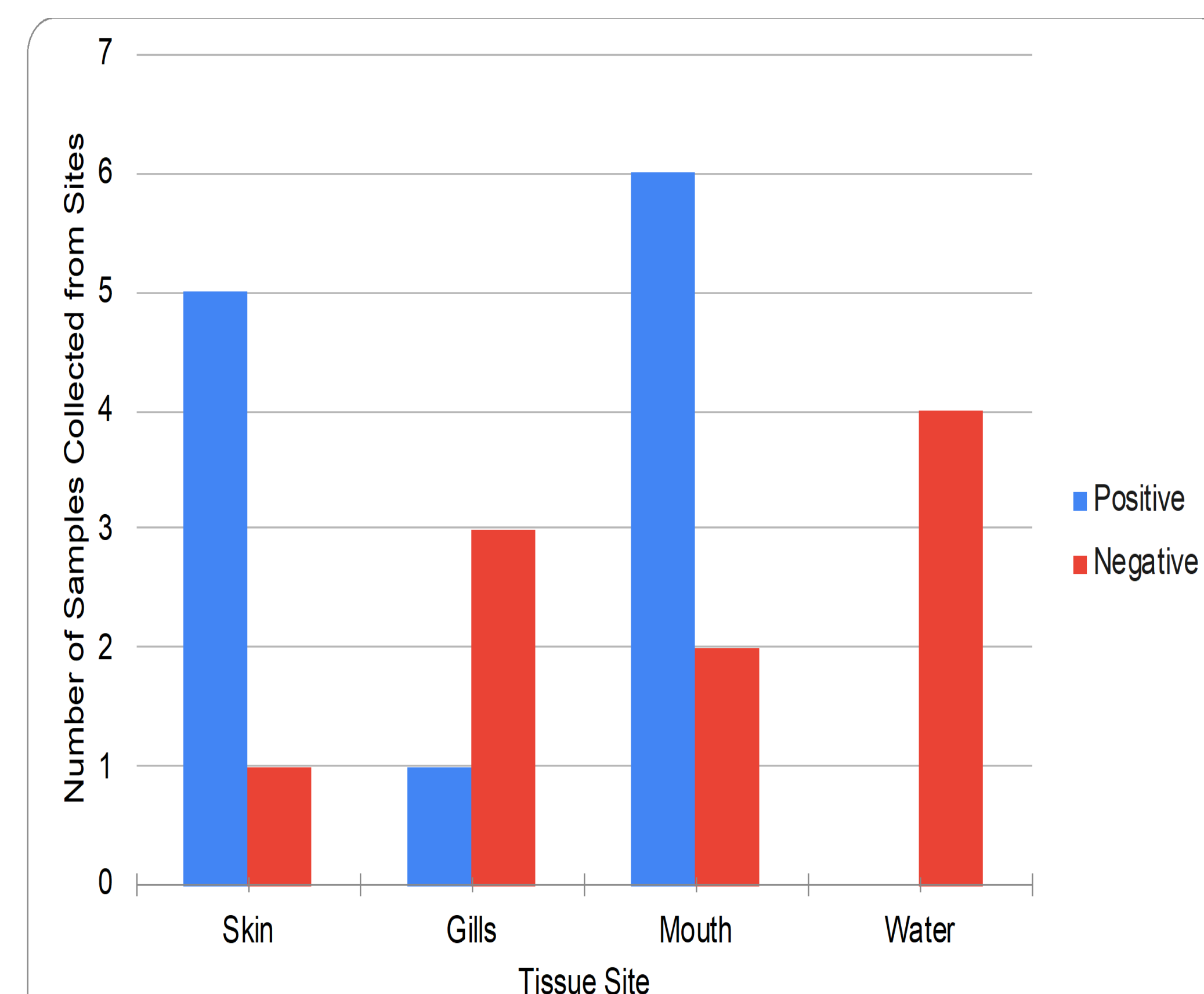
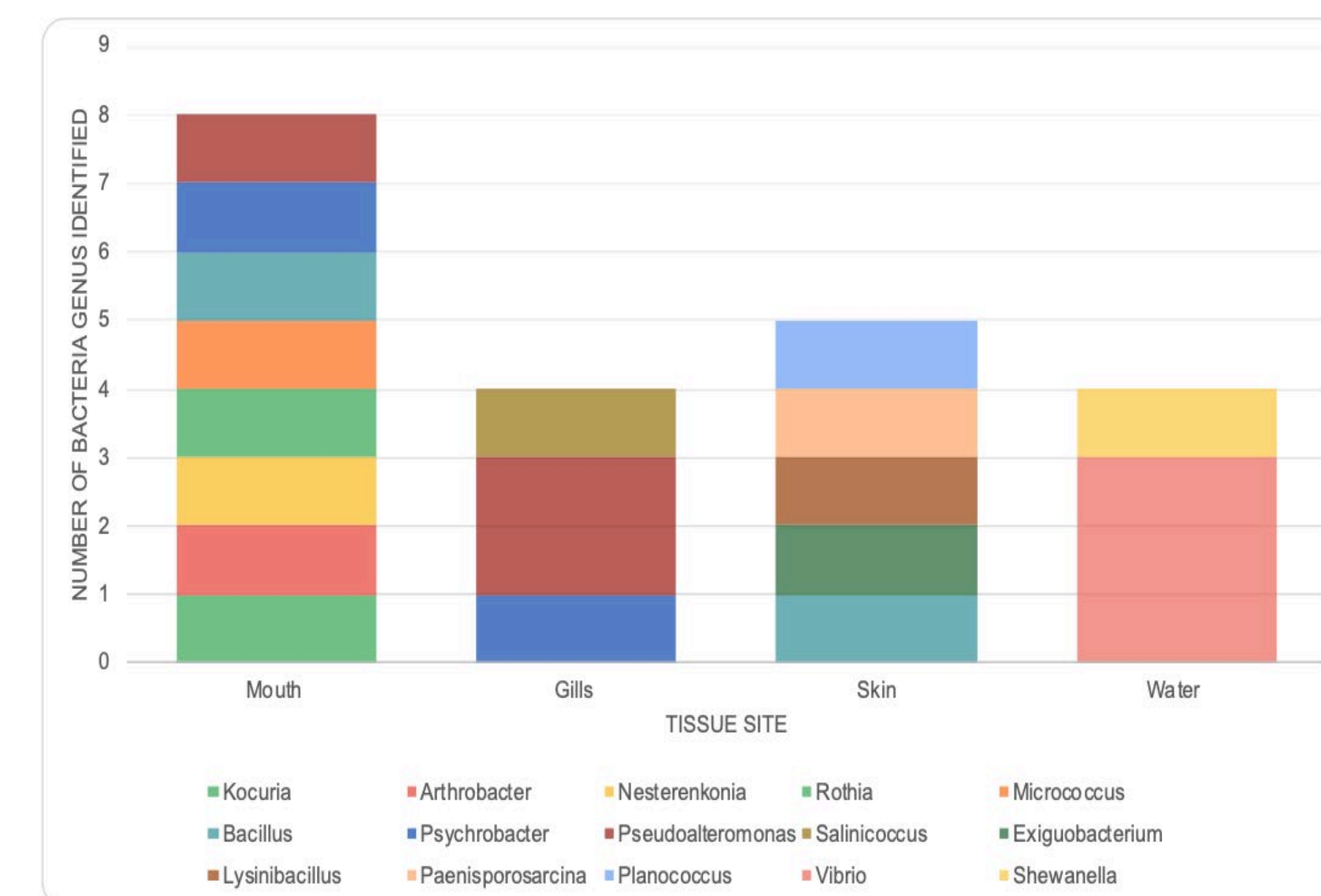


Figure 1. The ratio of gram-negative to gram-positive bacteria found in each of the tissue sites.

Figure 3. Bacterial genus makeup of various tissue sites and water samples of *G. cuvier*. The number of occurrences present in the samples is documented in comparison to the tissue site.



Abstract

Within Biscayne Bay, Florida, a preliminary study was completed on the tiger shark (*G. cuvier*) to create an understanding of the baseline bacterial makeup of the microbiome. Knowing the makeup of a healthy shark's microbiome could lead to helpful insights to environmental and individual organism health. Six tiger sharks were analyzed utilizing both field and laboratory techniques. From three tissue sites and water collection samples, bacterial identification to the genus and family level was done using 16s RNA primers, sequencing and the NCBI BLAST. In addition, samples were subjected to gram staining. Genera identified included Kocuria, Arthrobacter, Nesterenkonia, Rothia, Micrococcus, Bacillus, Psychrobacter, Pseudoalteromonas, Salinicoccus, Exiguobacterium, Lysinibacillus, Paenisporosarcina, Planococcus, Vibrio, and Shewanella. This data can be used to begin to create a baseline for what the "normal" microbiome for tiger sharks is in Biscayne Bay, and future studies could look at the environmental implications for microbiome-host relationships in this species.

References

1. Apprill, 2017
2. Gallagher et al., 2014
3. Holmström et al., 1999
4. Jeong et al.
5. Jørgensen et al.,

6. Marchalonis et al., 1998
7. Pagnanelli et al., 2000
8. Sulikowski et al., 2016

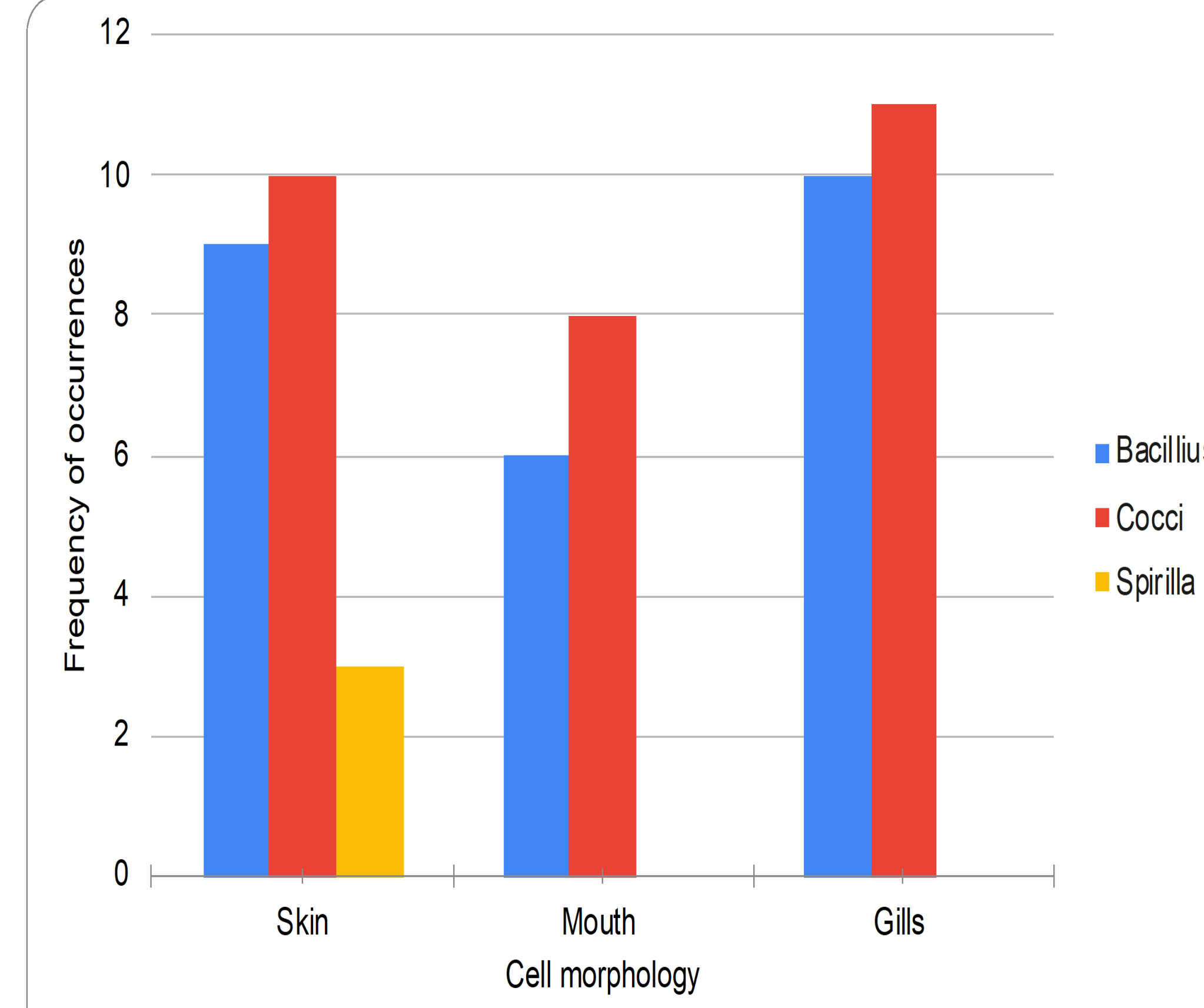
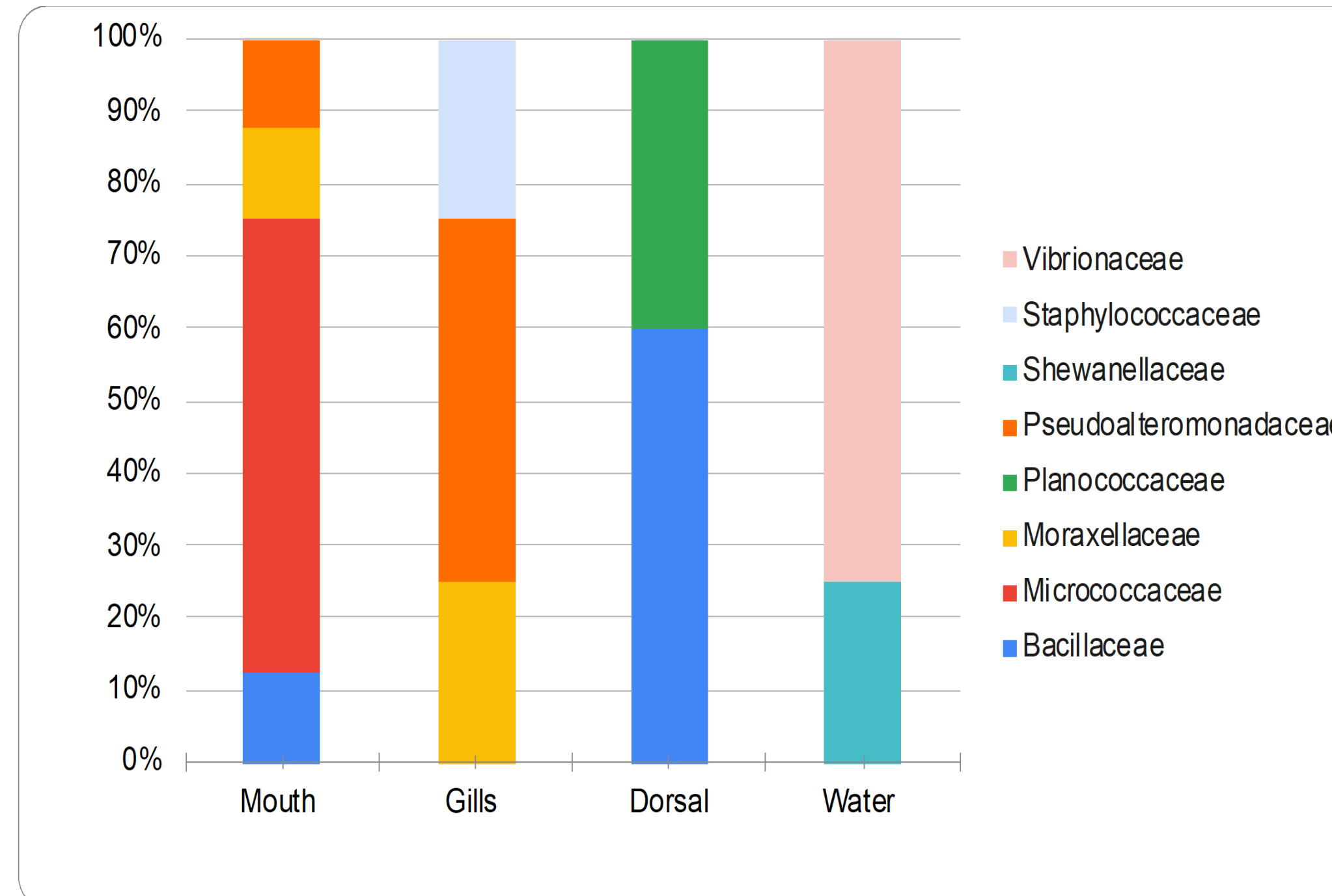


Figure 2. Cell Morphology Occurrences by Tissue Type. Bacillus, cocci and spirilla were quantified by absence or presence on the slides of tissue types skin, mouth, and gills.

Figure 4. Familial make-up of sample results. The percentage of each bacterial family found and identified on the tissue sites.



Sample Site	Family	Bacteria Genus	Gram Stain
Mouth	Micrococcaceae	Kocuria	+
	Micrococcaceae	Arthrobacter	+
	Micrococcaceae	Nesterenkonia	+
	Micrococcaceae	Rothia	+
	Micrococcaceae	Micrococcus	+
	Bacillaceae	Bacillus	+
	Moraxellaceae	Psychrobacter	-
Pseudoalteromonadaceae	Pseudoalteromonas	-	
Gills	Pseudoalteromonadaceae	Pseudoalteromonas	-
	Moraxellaceae	Psychrobacter	-
	Staphylococcaceae	Salinicoccus	+
Skin	Bacillaceae	Lysinibacillus	+
	Planococcaceae	Paenisporosarcina	+
	Planococcaceae	Planococcus	+
	Bacillaceae	Bacillus	+
	Bacillaceae	Exiguobacterium	+
	Pseudoalteromonadaceae	Pseudoalteromonas	-
Water	Vibrionaceae	Vibrio	-
	Shewanellaceae	Shewanella	-

Table 1. Sample sites listed with specific family and genus plus observed gram stain results.

Discussion

- Investigating the relationships that exist in host tissues with bacterial communities may be important to understanding the health of the shark and its environment
- Tiger sharks exhibited both gram positive and gram negative bacteria
- The mouth and skin samples contained the highest diversity of bacterial groups among tissue sites tested
- Found in the mouth and gills, pseudoalteromonas sp. mainly synthesizes biologically active molecules when present. It may provide the host with some protection from other microbes
- Arthrobacter spp. have been shown to absorb heavy metals, behaving like an inorganic adsorbent. This bacterium was found in the mouth of tiger sharks⁷
- Bacillus, which was found in mouth and skin samples, sometimes contains Bacillamide, an algicide that acts against a harmful dinoflagellate. This dinoflagellate is often responsible for many fish deaths during harmful algal blooms, making bacillus an important symbiont for animals living through these events⁴
- Water samples had completely different bacterial families than any of the tissue sites, indicating that there may be limited horizontal transmission from surrounding water

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