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Microbiome composition of *Pisaster ochraceus* sea stars affected by sea star wasting disease

Becky Nesnevich

UVM Honors College Senior Thesis

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Abstract

The largest epidemic of sea star wasting disease is affecting over 20 different species of sea stars in the west coast of the United States. The etiology of sea star wasting disease is currently unknown. Given the important role an organism's bacterial community plays in health and disease, we sought to compare the composition of the microbiome of sick and healthy *Pisaster ochraceus* sea stars. Because tissue types often differ in their microbiomes, we also compared body wall, tube feet, ampullae, stomach, pyloric caeca, gonad, and cross section tissues. To do this, we used 16s amplicon sequencing to classify the bacterial communities and compare across disease state and tissue type. We found some degree of overlap in the microbiome of sick and healthy tissues, with *Tenacibaculum* as the most common genera of higher differential abundance. We also found clustering by tissue type, and stomach and body wall tissue particularly differed from other tissues in their bacterial composition.

Introduction

The relatively recent development of molecular sequencing technologies has allowed biologists to further understand the interdependent relationship between host animals and their bacterial communities, changing our outlook on the role of pathogens in disease. Traditionally, it has been thought that a single pathogen infects a host and causes disease (Lloyd and Pespeni 2018, McFall-Ngai et al. 2013). This is changing in favor of the view that a combination of multiple microorganisms may lead to illness, and that this "polymicrobial" etiology may be more common than previously thought. To name a few examples in humans, acute necrotizing ulcerative gingivitis, lyme disease, and a variety of respiratory diseases are all thought to be polymicrobial (Brogden 2002). Polymicrobial diseases are also common in animals, for example Black Band disease in Corals and bovine respiratory disease (Brogden 2002, Sato 2016). Additionally, certain illnesses could be caused by the disruption of the homeostasis of an organism's microbial community, known as "dysbiosis." This is common in conditions that affect the gut, such as inflammatory bowel disease and Chron's disease, as intestinal bacteria play an important role in digestion (Tamboli et al. 2004, Joossens et al. 2011). This study aims to

understand the role of the microbiome in *Pisaster ochraceous*, the ochre sea star, a species that has been heavily affected by sea star wasting disease (SSWD).

The west coast of North America is currently experiencing the largest epidemic of sea star wasting disease in history. The outbreak of the disease began in the summer of 2013, causing the death of millions of sea stars from Baja Mexico all the way to southern Alaska (Hewson et al 2014). Symptoms of sea star wasting disease begin as white lesions on the body wall tissue. As the disease goes on, sea stars may experience loss of turgor and the progressive disintegration of tissue, which can lead to death in a matter of weeks. About 20 different species of sea stars have been affected, and *P. ochraceous* populations have been particularly decimated. An estimated 75% decrease in the original population of *P. ochraceous* in Washington state has occurred as a result of wasting disease (Eisenlord et al. 2014). *P. ochraceous* plays an important role in Pacific Northwest ecosystems as a keystone species. It preys on the California mussel, *Mytilus californianus*, which tends to outcompete other species in the absence of predators (Pfister 2016). A well-known experiment by Paine (1966) found that when *P. ochraceous* was experimentally removed from its intertidal community, the rich biodiversity that once included various other species like sponges, anemones, barnacles, chitons, and seaweeds significantly decreased.

The cause of sea star wasting disease is currently unknown, but has been associated in one species with a densovirus referred to as sea star-associated densovirus, or SSaDV. A study by Hewson et al. (2014) found that a sample of healthy sunflower stars (*Pycnopodia helianthoides*) became sick with the disease after being inoculated with homogenates prepared from the viral sized fraction taken from already-sick specimens. Stars that were inoculated with heat-killed homogenates remained asymptomatic. However further studies refute this evidence--when other species of sea stars, including *P. ochraceous*, underwent the same experiment, they did not elicit symptoms of wasting disease (Hewson et al 2018). SSaDV has also been identified in museum specimens of sea stars from the west coast, dating as far back as 1942 (Hewson et al. 2014). It is therefore unlikely that this virus alone is causing the current outbreak, as there are likely other factors at play.

Bates et al. (2009) found that that SSWD can also be affected by temperature, season, and locality, suggesting that climate change may play a role in organisms' susceptibility to disease as a result of heat stress and pathogen range expansion. Lab and field experiments at Barkley Sound, British Columbia found that disease prevalence and intensity (mean infection stage) was significantly higher at 14°C than 10°C. Additionally, they found that prevalence and infection stage were significantly higher in early June than in August. June is when the stars begin acclimating to higher summer temperatures, possibly increasing their susceptibility to SSWD. Additionally, symptom progression in *P. ochraceous* specimens slowed significantly at cooler temperatures when compared to summer temperatures, though all specimens eventually died of the wasting disease (Kohl et al 2016). As an emerging marine disease, more research must be done on disease transmission and the sea stars' immune response to SSWD, as well as other environmental factors that could be causing it to develop.

In this study, we sought to understand how the microbiome of *P. ochraceous* sea stars may be affected by sea star wasting disease. Originally, we intended to see if there was a particular tissue type of which the microbiome changes with disease state, because internal organs often differ in their microbial communities. To do this, we classified the microbiomes of sick and healthy *P. ochraceous* sea stars using 16S amplicon sequencing. We took samples of body wall, tube feet, ampullae, stomach, pyloric caeca, gonad, and cross section tissues from both sick and healthy individuals. However, due to having a very small sample size of healthy stars, we were unable to collect a sufficient number of samples of the different tissue types besides the body wall. We therefore did not have enough statistical power to be able to compare all the different tissue types between sick and healthy individuals. However, we were able to compare the microbiomes of sick and healthy body wall tissue, as well as compare different tissue types on a subset of sick individuals. We found significant differences in the microbiomes of sick and healthy stars, namely the higher abundance of taxa from the genus *Tenacibaculum* in sick stars. We also found that there was clustering by tissue type. Stomach tissue was particularly different, with a higher abundance of *Pseudoalteromonas* than the other tissue types.

Methods

Asymptomatic *Pisaster ochraceus* sea stars were collected from Monterey, California (36°36'21.44"N 121°53'23.69"W) on May 4, 2016 or June 8, 2016. Sea stars were kept in separate containers for two weeks and observed until wasting symptoms began. All samples, regardless of showing signs of wasting, were frozen at -80°C after the two week period was up.

67 total samples were taken from 20 different *P. ochraceus* individuals. To start, there were 2 healthy individuals and 4 sick individuals that showed signs of wasting, including lesions, decreased turgor pressure, and the loss of limbs. Sea stars were thawed and dissected in order to collect tissue samples. The various tissue types that were collected included body wall, cross section, tube feet, stomach, pyloric caeca, ampullae, and gonads. The cross section samples were taken directly through one of the rays of the star, with the intention on having all tissue types present in the sample. Once collected, all tissue samples were frozen.

Because there were only 2 healthy individuals at the time of dissection, replicate samples of each tissue type were taken so that there would be an even number of sick and healthy tissue samples (Table S2). Eventually, however, it became clear that one of the healthy individuals was an outlier in the data and was removed from the analysis, thus leaving only one healthy individual to compare tissue types with. Therefore, there was not enough statistical power to be able to compare various tissue types between sick and healthy stars. However, more body wall samples from both sick and healthy stars were added to the experiment, so we were still able to compare the microbiomes of sick and healthy body wall tissue. In total, of the 67 samples, 36 came from sick individuals and 31 were from healthy individuals (Table S1 and S2). 16 of the body wall samples used in the experiment were taken as biopsy punches immediately before freezing. The rest of the 67 Samples were taken after freezing. To ensure that these different body wall tissue collection methods would not interfere with the results, we conducted a principal coordinate analysis between biopsied samples and samples cut from the body wall after freezing (Figure S1).

DNA Extraction

Tissues were thawed and DNA was extracted from each sample according to the protocol for the DNEasy Blood and Tissue Kit by Qiagen (Qiagen, Hilden, Germany). Samples were

defrosted, and a small segment was transferred to a 1.5 ml microcentrifuge tube. DNA was then extracted using a column-based method extraction was done according to the manufacturer's instructions. The quality and concentration of DNA was tested using a NanoDrop 2000 Spectrophotometer (ThermoFisher Scientific).

16S PCR Amplification and Library Preparation

The V3 and V4 region of the 16S bacterial sequences were amplified by polymerase chain reaction. 25 µl PCR reactions contained 2 µl extracted DNA, 12.5 µl 1x MiFi Mix (Bioline), 2 µl each of forward and reverse primers, and 2 µl bovine serum albumin to account for potential inhibitors. The primer sequences are as follows (Illumina adapter overhang nucleotide sequences are underlined): Forward 5'

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and
Reverse 5'

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTATCTAATCC.

A touchdown PCR was run under the following cycling conditions: 95°C for 3 minutes, followed by 30 cycles at 95°C for 30 seconds, 55°C for 30 seconds (decreasing .3°C per cycle), 72°C for 30 seconds, with a final extension at 72°C for 3 minutes. The PCR products were then cleaned with Ampure XP beads and dual-index barcodes were added to the amplicon target. This was done in a 50 µl PCR reaction according to the Illumina 16S metagenomic sequencing library preparation protocol (Illumina, 2013). The PCR products were run on a 2% agarose gel to ensure the sequences amplified sufficiently. The samples were sent to the genomics facility at the Cornell University Institute of Biotechnology to be multiplexed and sequenced in an illumina paired-end miseq run.

Data Processing

The program Qiime was used to match the paired end reads using the command “multiple_join_paired_ends.py” and “multiple_split_libraries_fastq.py” (Caporaso et al. 2010). An OTU (operational taxonomic unit) table based on the similarity of the 16S sequences was built, rarefied to 9,000 reads per sample, using the command pick_open_reference_otus.py (Caporaso et al. 2010). This step also used the RDP classifier and Greengenes Database to do

taxonomic assignment of de novo picked OTUs (McDonald et al. 2012, Wang et al. 2007). Chimeric sequences and low frequency OTUs (OTUs that had fewer than 50 reads in 25% of the samples) were filtered out of the OTU table using the function “filter_otus_from_otu_table.py”. Chimeric OTUs were filtered using VSEARCH (DeSantis et al 2006, Rognes et al. 2016). We then produced a matrix showing the dissimilarity between the different taxa present based on Unifrac distance metric, using the command “core_diversity_analyses.py” (Lozupone and Knight 2005) . Based on the results from the core diversity analysis, it became clear that the only juvenile, individual #24, was an outlier and was removed from any further analysis. We used the command “compare_alpha_diversity.py” and “compare_categories.py” to test for differences in alpha and beta diversity between sick and healthy body wall samples. For the beta diversity analysis, the following methods were used: adonis, PERMANOVA, and MRPP all with the default 999 permutations.

Though the program DESeq2 is commonly used with RNA data, it can be used to test for differential abundance of OTUs with the help of the wrapper “Phyloseq” (McMurdie and Holmes, 2013). DESeq2 was used to assess differential abundance of OTUs between sick and healthy body wall samples. A principal component analysis was done using Phyloseq to compare OTU counts for sick and healthy body wall samples. DESeq2 was also run on a subset of sick samples to test for the effect of tissue type.

Results

Differences in microbiome between sick and healthy individuals

The microbial community differed significantly between samples taken from sick and healthy sea stars for the unweighted data, (adonis, $p=0.015$). However, the weighted data had a higher p value of 0.049, just under the alpha of 0.05. There was no significant difference in alpha diversity ($p=0.053$). In order to visualize how OTU abundance was partitioned among samples we used a principal coordinate analysis. We found some degree of overlap among sick and healthy body wall samples. 63.2% of the variation in the samples was distributed across axes 1 and 2 (Figure 1). We identified 648 OTUs among 24 *P. ochraceous* samples. Of these, 68 OTUs (10%) were relatively higher in sick samples, while 5 OTUs (0.77%) were relatively higher in healthy samples. Among the OTUs that were more significantly more abundant in sick samples,

the most prevalent genus was *Tenacibaculum*, followed by *Reichenbachiella*. The most prevalent taxonomic order in sick body wall samples was Spirochaetales. In healthy body wall samples, the most prevalent taxonomic order was Flavobacteriales (Figure 2).

Differences in tissue types among sick individuals

We used DESeq2 to test for differential abundance between pairs of tissue types in order to compare the number of OTUs present. According to these tests, body wall tissue differed the most from other tissue types, primarily tube feet (5 OTUs up and 1 down in tube feet), ampullae (10 OTUs up and 41 down in ampullae), pyloric caeca (22 OTUs up and 38 down in pyloric caeca), and stomach (36 OTUs up and 4 down in stomach). Stomach tissue also differed from pyloric caeca, ampullae, tube feet, and cross section tissue (Figure 4). Stomach tissue had a higher relative abundance of OTUs from the order Vibrionales than the other tissue types (Figure 5). In particular, the genus *Pseudoalteromonas* from the order Vibrionales was of higher differential abundance in all pairwise comparisons involving stomach tissue.

Cross section tissue had no differentially abundant OTUs except when compared to stomach, which had 25 differentially abundant OTUs (Figure 3). A principle coordinate analysis was performed to visualize differences in OTU abundance among sick tissue types. The PCoA shows clustering by tissue type, and pyloric caeca samples are especially farther away from all the other samples.(Figure 4). Additionally, over 75% of OTUs in pyloric caeca samples were unknown or unidentified. (Figure 5).

Discussion

As sequencing technology advances, we are learning more and more about the complexity of the microbiome and how it relates to disease. As an emerging topic in the world of marine wildlife diseases, there is little existing research on sea star wasting. Because of the important role the microbiome plays in organismal health, we sought to explore the bacterial communities of *P. ochraceous*, an ecologically important species that is heavily affected by the current epidemic. Because of the low sample sizes of healthy tissue types besides body wall, we chose to compare body wall tissue samples between 12 sick and 8 healthy individuals. We also looked at pairwise differences in the microbial communities of body wall, tube feet, ampullae,

stomach, pyloric caeca, gonad, and cross section tissues among a subset of sick samples. This study provides information on the holobiont (the combination of the animal and all the other organisms, living on or in it), and gives us an idea of how the microbiome of *P. ochraceous* changes with sea star wasting disease.

The alpha diversity between sick and healthy body wall samples was not statistically significant ($p = .053$). For the beta-diversity comparisons, our data from the unweighted unifracs matrix yielded a lower p-value ($p = 0.015$) than that of the weighted data ($p = .049$). The difference in these values could be explained by the fact that the unweighted matrix is a qualitative measure, based on whether specific OTUs are present or absent. In contrast, the weighted matrix is a quantitative measure which accounts for differences in relative abundance of observed organisms, so the impact of low-abundant OTUs is diminished (Lozupone et al. 2011, Chao et al. 2005). In our experiment, perhaps there were OTUs of low-abundance that were present in one sample but not the other, disproportionately bringing down the p-value for the unweighted matrix. Additionally, the F-statistic for both the unweighted and weighted matrices were low (1.39 and 3.57, respectively), indicating that the effect of the factor (disease state) may be weak.

In healthy body wall samples, OTUs were most commonly from the order Flavobacteriales (Figure 2). Flavobacteriales is a very large and diverse order with bacteria from many different niches (McBride 2014). *Tenacibaculum*, a genus in the order Flavobacteriales, was also the most prevalent genus within taxa that were of higher differential abundance in sick body wall tissue. Members of the genus *Tenacibaculum* have been known to cause disease in marine organisms, notably tenacibaculosis in fish and spotting disease in sea urchins (Avendaño-Herrera et al. 2006, Masuda et al. 2004). In an earlier experiment done with the same *P. ochraceous* individuals, members of Flavobacteriales including the genera *Tenacibaculum* and *Polaribacteria* were common in sea stars that were in the early stages of wasting disease. *Polaribacteria* may be an opportunistic bacteria that increases in abundance during the later stages of the disease (Lloyd and Pespeni, in review). Looking at the pairwise comparisons of sick tissue types, we had also found that multiple OTUs from the genus *Tenacibaculum* were of higher abundance in body wall tissue than pyloric caeca and ampullae. Perhaps this difference

could be explained by the fact that the sick body wall tissue contained disease lesions, which could potentially contain a higher abundance of pathogenic bacteria.

In the subset of sick samples, stomach tissue differed in microbiome composition from all other tissue types except gonad (figure 3). This was expected given the importance of an organism's microbial community in digestion (Huttenhower et al. 2012). We also found that the pyloric caeca, which is involved in the absorption of nutrients, differed from body wall and stomach tissue. This differed from one study which found no distinction in the microbial community of the pyloric caeca and other tissue types (Jackson 2018).

Stomach tissue from sick individuals had a higher abundance of taxa from the order Vibrionales (Figures 3, 4, and 5). This order includes the ubiquitous genus of marine bacteria, *Pseudoalteromonas*. In our study, *Pseudoalteromonas* was found to be of higher differential abundance in all pairwise comparisons involving stomach tissue, including body wall, tube feet, apullae, and pyloric caeca (figure 3). It was also of higher abundance in sick body wall tissue than healthy tissue. Members of this geographically wide-ranging genus are associated with various marine organisms and possess diverse survival strategies (Holmström and Kjelleberg 1999). Among these strategies are the ability to adapt to extreme-cold conditions (Bosi et al. 2017, Medigue et al. 2005) and the production of biologically-active metabolites, some of which contain antibiotic effects (Holmström and Kjelleberg 1999). Some of these compounds may benefit the host species, as seen in the green algae, *Enteromorpha intestinalis*, and the tunicate, *Cionia intestinalis*, both of which contain *Pseudoalteromonas*, which produces an anti-fouling compound (Holmström et al. 1996). Certain species of *Pseudoalteromonas* are the causative agents of marine diseases, including *P. bacteriolytica* which causes spotting disease in algae (Sawabe et al. 1998). Additionally, other members of *Pseudoalteromonas* have been known to cause cell lysis in marine algae (Kim et al 2009, Lovejoy et al. 1998).

In the case of sea star wasting, *Pseudoalteromonas* is known to be a beneficial genus, having been found in higher abundance in healthy *P. ochraceous* samples (Lloyd and Pespeni 2018). It is possible that the homeostasis of the microbiome is being interrupted, causing the abundance of *Pseudoalteromonas* to decrease with disease. Because we did not have sufficient healthy stomach samples to compare, it is unclear whether our sick stomach samples have a

healthy abundance of *Pseudoalteromonas*. A future study that compares the healthy stomach microbiome to that of stars affected by wasting disease would be necessary to truly see the changes in abundance of this genera.

We found there to be little to no difference in the microbiome of gonad, tube feet, and cross section when compared to the other tissue types with the exception of stomach, which differed from tube feet and cross section tissues (Figure 3). The cross section samples, which were intended to contain a bit of all tissue types, were taken from the rays of the sea stars. The stomach, located in the center of the animal, could not have been included in these samples, thus explaining the microbial differences found between cross section and stomach samples. These results indicate that there may be no specialization of the microbiome of gonad and tube feet tissues.

Sea star wasting disease is a relatively recent phenomenon in the world of wildlife diseases. Because the etiology of the disease is still unknown, a good focal point for research would be the microbiome, given its important role in health and disease. In this study we sought to understand how the bacterial communities of *P. ochraceous* differ between sick and healthy individuals. We also compared the microbiomes of different tissue types among a subset of sick individuals. We found some degree of overlap in the microbial communities of sick and healthy body wall tissue (Figure 1). We also found clustering by tissue type, with stomach and body wall having the highest number of differentially abundant OTUs (Figures 3 and 4). Although this data does not allow us to identify a specific pathogen, this study found some patterns across disease state and tissue types, and can provide a foundation for other microbiome studies. Future studies could focus more on how the microbial community of stomach tissue changes with disease state. More research could also be done on the relationships between specific bacterial taxa (notably *Tenacibaculum*, *Polaribacteria*, and *Pseudoalteromonas*) and the host in order to discern if they are beneficial or harmful.

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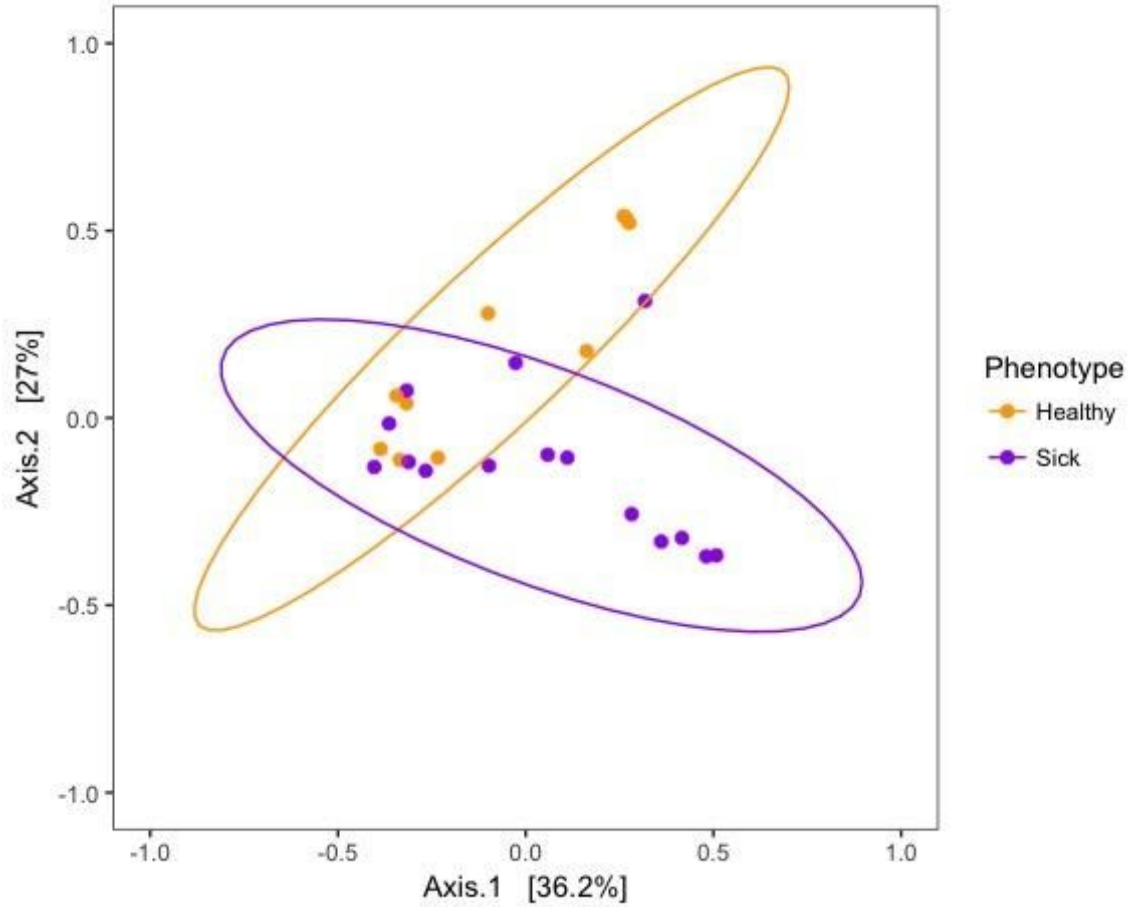


Figure 1. Differences in microbial community between sick and healthy individuals. Principal coordinate analysis plot of microbial communities in samples taken of sick and healthy body wall tissue based on the weighted UniFrac distance matrix. Each point represents one sample. Colors represent the health status of the individual (Orange=Healthy and Purple=Sick).

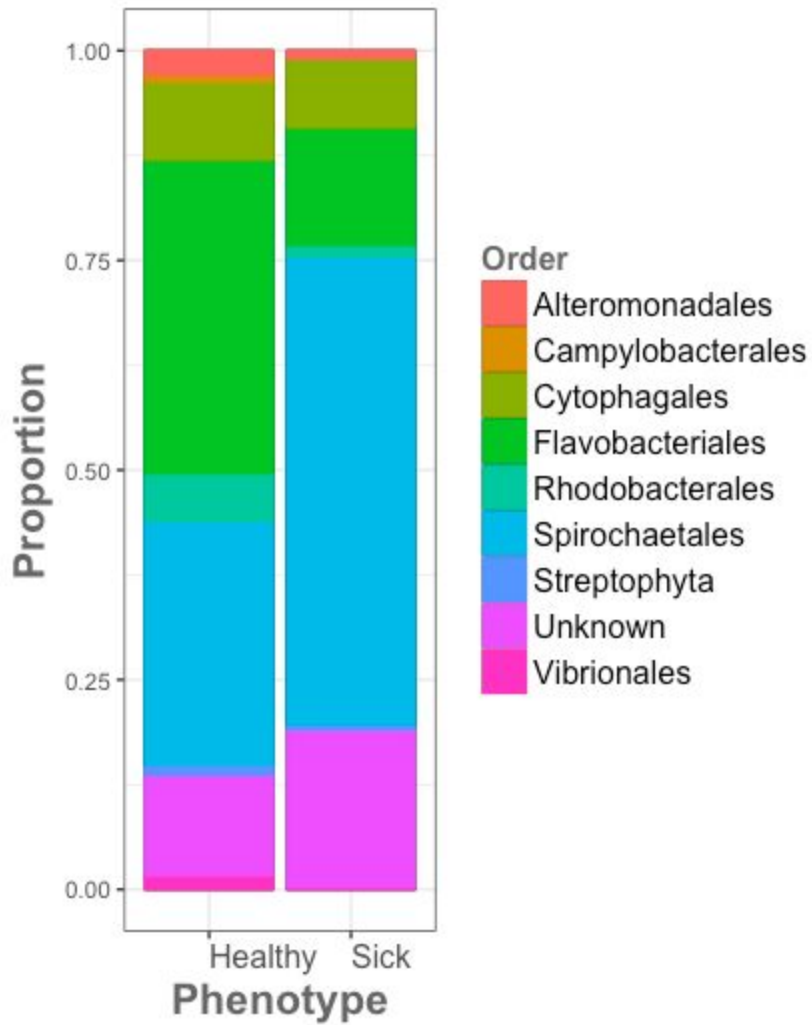


Figure 2. Microbial community differences between sick and healthy *P. ochraceous* samples. Proportions of OTUs classified to the Order level in microbial communities taken from sick and healthy body wall samples.

	Body Wall		Tube Feet		Ampullae		Stomach		Pyloric Caeca		Gonad		Cross Section	
Body Wall			5	1	10	41	36	4	22	38	0	0	0	0
Tube Feet					1	1	14	0	1	0	0	1	0	0
Ampullae							51	0	0	0	0	0	0	0
Stomach									2	26	0	0	1	24
Pyloric Caeca											0	1	0	1
Gonad													0	0
Cross Section														



Figure 3. Microbial community differences between tissue types. DESeq2 results for pairwise comparisons between different tissue types on a subset of sick samples. The numbers refer to the number of differentially expressed OTUs. For each column, the left side (in blue) refers to the number of OTUs that were higher with respect to column label, and the right side (in red) is the number of OTUs that were lower with respect to column label. The chart is colored on a light-dark spectrum: darker colors highlight pairs with more differentially expressed OTUs.

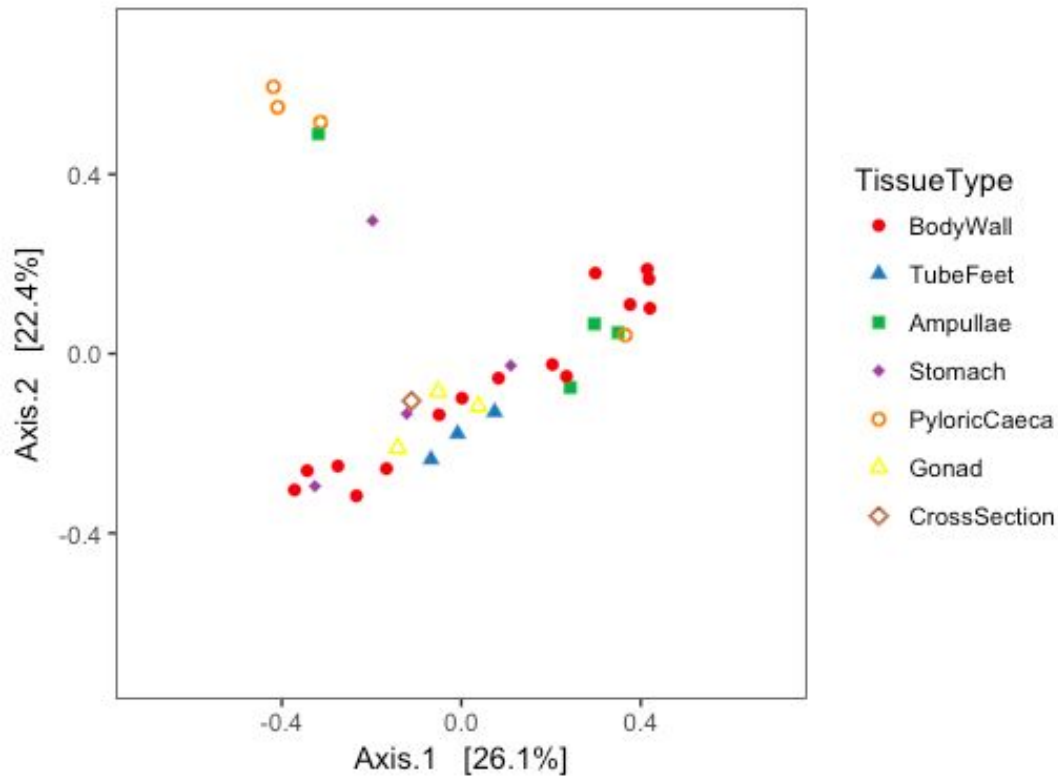


Figure 4. Microbial differences between tissue types. Principal coordinate analysis plot of microbial communities in different tissue types taken from a subset of sick samples. Each point represents one sample, shapes and colors represent the various tissue types (Body wall, tube feet, ampullae, stomach, pyloric caeca, gonad, and cross section). 4 out of 36 samples were removed from this figure for having too few reads after rarefying to 9000 reads per sample. The samples removed were 17-XS, 03-TF, 27-BW, and 10-BW.

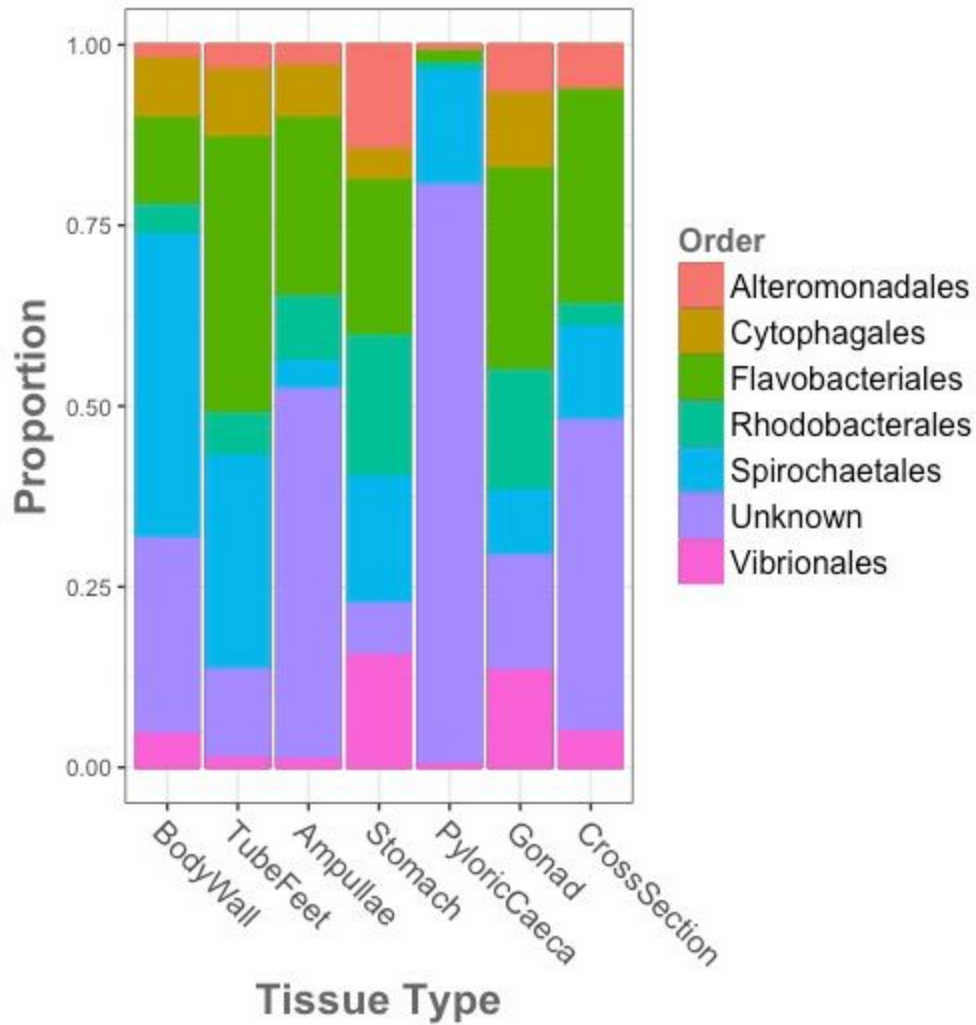


Figure 5. Microbial community differences between *P. ochraceus* tissue types. Proportions of OTUs classified to the Order level in microbial communities taken from different tissue samples on a subset of sick individuals.

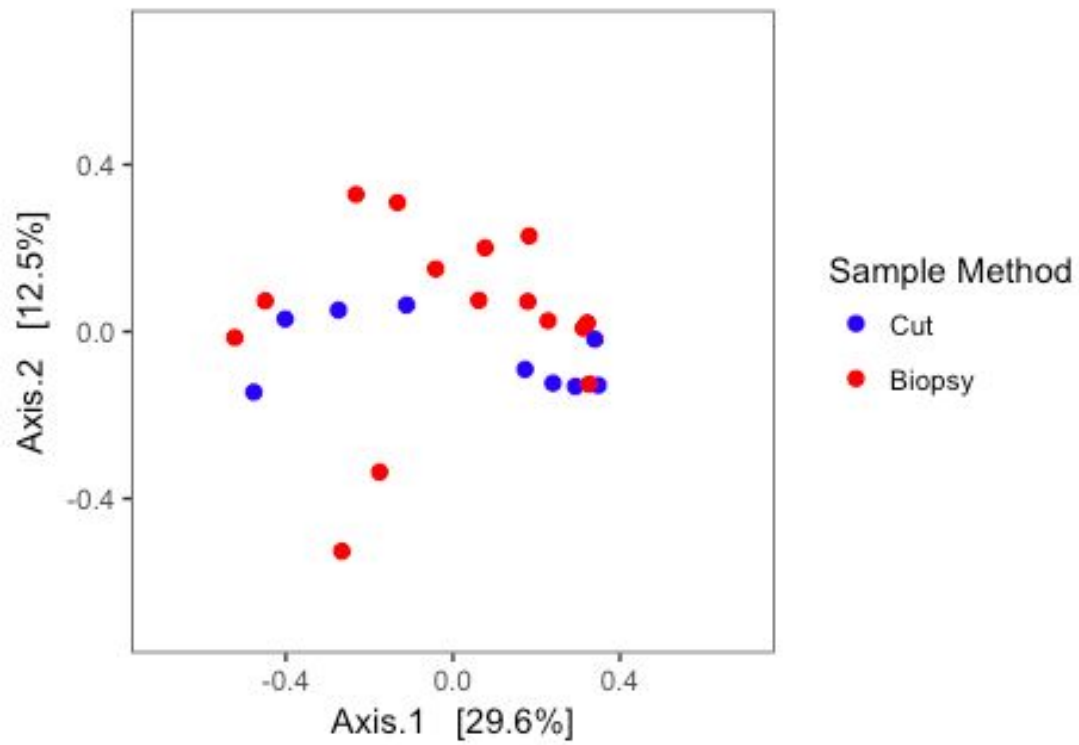


Figure S1. Principal coordinate analysis plot of microbial communities in samples taken as a biopsy punch (red) and cut during original dissection (blue).

Sample ID	Number	TissueType	Health Status	Sample ID	Number	TissueType	Health Status
10-BW	10	BodyWall	Healthy	01-LE	1	BodyWall	Sick
24-TF2	24	TubeFeet	Healthy	01-BW	1	BodyWall	Sick
24-TF	24	TubeFeet	Healthy	01-AB	1	Ampullae	Sick
24-XS	24	CrossSection	Healthy	01-GD	1	Gonad	Sick
24-BW2	24	BodyWall	Healthy	01-DG	1	PyloricCaeca	Sick
24-BW	24	BodyWall	Healthy	01-ST	1	Stomach	Sick
24-DG2	24	PyloricCaeca	Healthy	01-TF	1	TubeFeet	Sick
24-DG	24	PyloricCaeca	Healthy	02-BW	2	BodyWall	Sick
24-ST2	24	Stomach	Healthy	03-LE	3	BodyWall	Sick
24-ST	24	Stomach	Healthy	03-BW	3	BodyWall	Sick
24-BW3	24	BodyWall	Healthy	03-AB	3	Ampullae	Sick
27-TF	27	TubeFeet	Healthy	03-GD	3	Gonad	Sick
27-AB	27	Ampullae	Healthy	03-DG	3	PyloricCaeca	Sick
27-GD2	27	Gonad	Healthy	03-ST	3	Stomach	Sick
27-GD	27	Gonad	Healthy	03-TF	3	TubeFeet	Sick
27-DG2	27	PyloricCaeca	Healthy	05-TF	5	TubeFeet	Sick
27-XS2	27	CrossSection	Healthy	05-LE	5	BodyWall	Sick
27-DG	27	PyloricCaeca	Healthy	05-XS	5	CrossSection	Sick
27-XS	27	CrossSection	Healthy	05-AB	5	Ampullae	Sick
27-ST2	27	Stomach	Healthy	05-GD	5	Gonad	Sick
27-BW2	27	BodyWall	Healthy	05-DG	5	PyloricCaeca	Sick
27-ST	27	Stomach	Healthy	05-ST	5	Stomach	Sick
27-BW	27	BodyWall	Healthy	08-BW	8	BodyWall	Sick
27-TF2	27	TubeFeet	Healthy	09-BW	9	BodyWall	Sick
27-AB2	27	Ampullae	Healthy	15-BW	15	BodyWall	Sick
27-BW3	27	BodyWall	Healthy	17-TF	17	TubeFeet	Sick
31-BW	31	BodyWall	Healthy	17-LE	17	BodyWall	Sick
32-BW	32	BodyWall	Healthy	17-XS	17	CrossSection	Sick
33-BW	33	BodyWall	Healthy	17-BW	17	BodyWall	Sick
34-BW	34	BodyWall	Healthy	17-AB	17	Ampullae	Sick
35-BW	35	BodyWall	Healthy	17-DG	17	PyloricCaeca	Sick
				17-ST	17	Stomach	Sick
				19-BW	19	BodyWall	Sick
				20-BW	20	BodyWall	Sick
				21-BW	21	BodyWall	Sick
				38-BW	38	BodyWall	Sick

Table S1. All samples taken, the individual's identification number, tissue type of the sample, and health status of the individual at the time of freezing. Note: Individual #24 was a juvenile and removed from the analysis.

	Healthy (n=2)	Sick (n=4)	Total
Body Wall	12	15	27
Tube Feet	4	4	8
Ampullae	2	4	6
Stomach	4	4	8
Pyloric Caeca	4	4	8
Gonad	2	3	5
Cross Section	3	2	5
Total	31	36	67

Table S2. Breakdown of samples by disease state and tissue type. Note: n=2 and n=4 refer to all tissue types except body wall. Body wall tissue samples from additional individuals were added in from biopsy punches (see figures S1 and S3).