Marine sponges as bioindicators of nitrogen within estuaries on the Oregon coast

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ABSTRACT

As filter feeders, sponges are highly integrated with their environment. Many sponges also host diverse communities of bacteria, including many that are hypothesized to carry out a variety of nitrogen transformations. The presence of these bacteria makes sponges an integral part of the nitrogen cycle in their habitats, and suggests that sponges are an excellent bioindicator of environmental conditions. To test these hypotheses, we collected sponge tissue from two Oregon estuaries, and extracted microbial DNA from these samples. To assess bacterial diversity, we performed Denaturing Gradient Gel Electrophoresis (DGGE) on a fragment of the 16S gene. We also examined nitrogen cycling in sponges by examining the sponge samples for the presence of the *amoA* and *nirS* genes, which encode for enzymes in the nitrification and denitrification pathways, respectively. DGGE results showed diverse bacterial communities, with clear differences between the sites. The results also showed little variation within sites, but were suggestive of seasonal variation. Both functional genes were present in all five species of sponge that we collected. These results suggest that sponges and their associated bacterial communities play a critical role in nitrogen transformations within these bays and that these sponge associated bacterial communities are bioindicators of environmental variation.

BACKGROUND

Figure 1. Map of two collection sites, Netarts Bay and Yaquina Bay.

- Netarts Bay and Yaquina Bay are two estuaries located on the Oregon Coast.
- Netarts is a small community with a population of 748 in 2010
- Its watershed covers 13 square miles
- Newport is a popular tourist destination located on Yaquina Bay with a population of 9989 in 2010
- Its watershed has an area of 253 square miles

QUESTIONS

-What is the abundance of sponge in Netarts Bay and Yaquina Bay, and how do they compare?

-What bacteria are present within the sponge, and what nitrogen transformations are they capable of carrying out?

METHODS AND MATERIALS

Assessment of Sponge Abundance
- 10 m video transects recorded at each site
- Analyzed using CPCe software

Functional Genes
- RT-PCR using *nir*S primers

Assessment of Bacterial Diversity
- Tissue samples collected from each site
- Microbial DNA extracted and amplified using *16*S primers for PCR
- Visualized using denaturing gradient gel electrophoresis (DGGE)

Assessment of Nitrogen Transformation
- Tissue samples collected from each site
- Microbial DNA extracted and amplified using *amo*A and *nir*S primers for PCR
- Visualized using gel electrophoresis

RESULTS

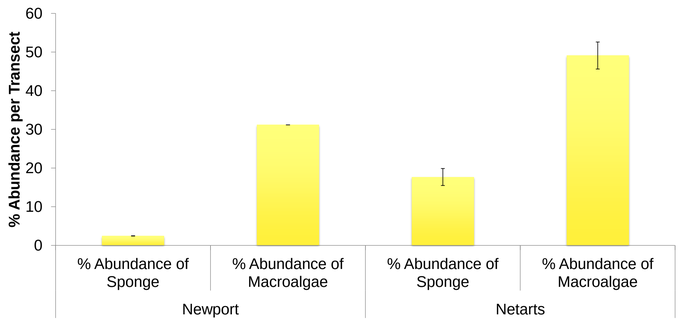


Figure 2. Mean ± standard deviation percent abundance of sponge and macroalgae at both Yaquina and Netarts Bays. ANOVA testing revealed a significant difference between mean sponge percent abundances, as well as between mean macroalgae percent abundances ($p<0.05$)

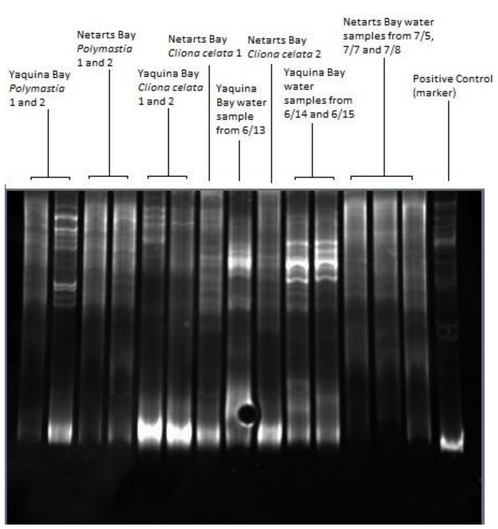


Figure 3. Denaturing gradient gel electrophoresis comparing two species of sponge and water samples from two sites. Each species shows a unique pattern of banding, each representing a different species of bacteria present. Banding patterns also vary within species, between sites.

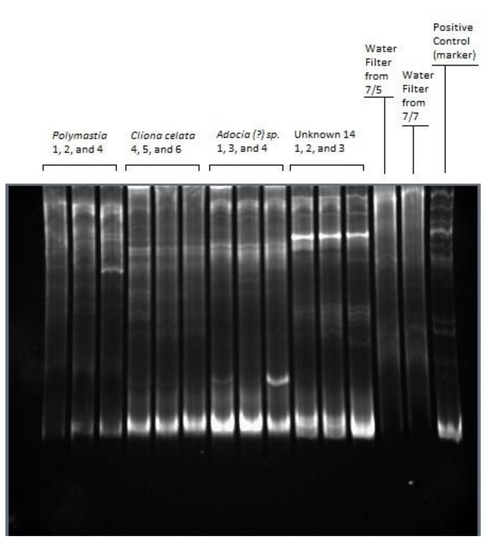


Figure 4. Denaturing gradient gel electrophoresis of four prominent species and water samples at Netarts Bay. Each species shows a unique pattern of banding that remains consistent between individuals.

RESULTS

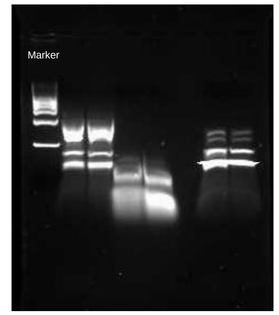
	16S	*amo*A	*nir*S
Cliona celata	+	+	+
Leucilla nuttingi	+	+	+
Polymastia pacifica	+	+	+
Water samples	+	-	-

Table 1. Presence and absence of various genes in sponge species and water samples from Yaquina Bay.

	16S	*amo*A	*nir*S
Adocia(?) sp.	+	+	+
Cliona celata	+	+	+
Polymastia pacifica	+	+	+
Unknown 14	+	+	+
Water samples	+	-	-

Table 2. Presence and absence of various genes in sponge species and water samples from Netarts Bay

Figure 5. Gel electrophoresis of *nir*S RT-PCR . Three different primer sets were used on one individual, each indicating successful amplification of *nir*S mRNA.



DISCUSSION

- Both sponge and macroalgae showed significant differences in percent abundance between sites.
- *16*S DGGE showed unique community composition, with little variation, in each species at each site. Thus, bacterial community compositions differ in response to different environments.
- Functional genes found in all sponge species, but not in water samples, suggesting that nitrogen transformations occur within sponges, not in water.
- *nir*S gene successfully amplified using RT-PCR, indicating use of the gene.

FUTURE DIRECTIONS

- Investigate possibility of seasonal variation of bacterial communities within sites using *16*S DGGE
- Test for activity of *nir*S and *amo*A genes in multiple species using RT-PCR

ACKNOWLEDGEMENTS

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