WHO IS LIVING ON THE INTERTIDAL SNAIL *NUCELLA OSTRINA*?

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INTRODUCTION

- The microbiome plays an important role in maintaining the health and fitness of marine invertebrates by facilitating immune defence, aiding in digestion, and enabling adaptation to environmental conditions[1].
- Some bacteria found on the body surface of marine invertebrates have beneficial properties, others can be harmful pathogens, and some may have no functional benefit to the animal at all[2].
- Several studies have examined microbial diversity and activity in marine invertebrates, most have focussed on the gut microbiome, and all studies have focussed on adult invertebrates[3].
- No study to date has investigated the outer microbiome of early juveniles, which is known to be the most vulnerable phase of the life cycle of marine invertebrates[4].
- In addition, a majority of microbial studies have focussed on invertebrates reared in aquaculture facilities; far fewer studies have examined the microbial community associated with wild invertebrates in natural habitats.

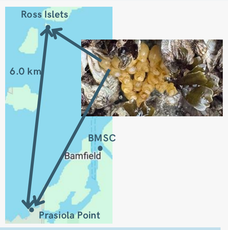
RESEARCH OBJECTIVES

1. Determine an effective methodology for the extraction and amplification of bacterial DNA from both the juvenile and adult stages of *N. ostrina*.
2. Examine the associated microbiome change between the juvenile and adult stages of an *N. ostrina*?
3. Examine the alpha diversity across juveniles and adults *N. ostrina*.
4. Determine how the associated microbiome varies spatially among animals living in different local habitats.

METHODS

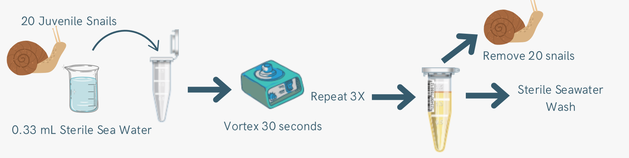
Animal Collection

- Egg capsules from *Nucella ostrina* were collected from the Ross islets and Prasiola Point in Bamfield, BC, and brought back to Bamfield Marine Science Center (BMSC) for DNA extraction.

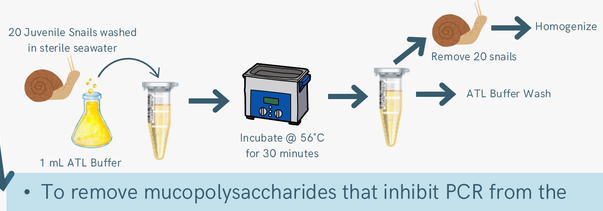


Method Development: DNA Extraction

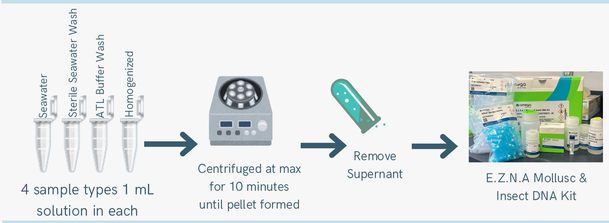
- Sterile Seawater Wash used to remove microbiome from the exterior of the juvenile snails.



- The same snails were then placed in ATL buffer to confirm the complete removal of the exterior microbiome in the previous step.



- To remove mucopolysaccharides that inhibit PCR from the samples, a combination of centrifugation at high speeds and a specific mollusc DNA extraction kit was used.



DNA Isolation

- Polymerase Chain Reaction (PCR) was used to amplify the 16S rRNA sequence, and gel electrophoresis confirmed the presence of the bacterial gene.
- Samples were cleaned and the 16S rRNA gene was barcoded for sequencing

DNA Sequencing

- Amplicons were sequenced at the TRUgen sequencing facility on an Ion Genestudio S5 platform.

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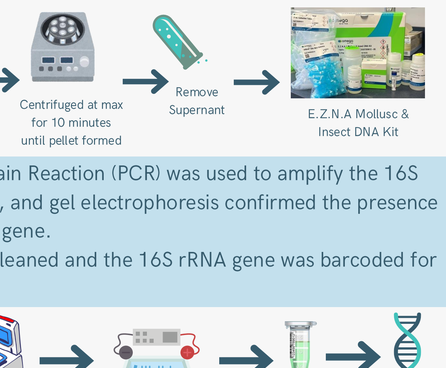

RESULTS

1. Method Development

Juveniles

- Homogenized and ATL Buffer Wash samples were significantly different from Sterile Seawater ($p = 0.001$, $R^2 = 0.38$) (Fig. 1)
- Seawater was significantly different from Sterile Seawater Wash sample ($p = 0.001$, $R^2 = 0.72$) (Fig. 1)

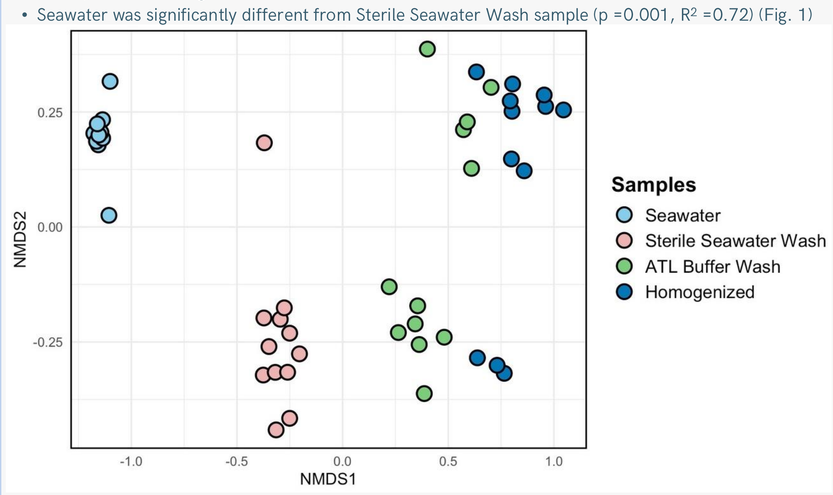

Figure 1. NMDS plot of the community composition of seawater, sterile seawater wash, ATL buffer wash, and homogenized samples of ATL-washed juveniles from the Ross Islets (n = 48).

Adults

- Homogenized samples were significantly different from Sterile Seawater Wash ($p = 0.001$, $R^2 = 0.54$) (Fig. 2)
- ATL Buffer Wash samples were significantly different from Sterile Seawater Wash ($p = 0.001$, $R^2 = 0.70$) (Fig. 2)
- Seawater was significantly different from Sterile Seawater Wash sample ($p = 0.001$, $R^2 = 0.72$) (Fig. 2)

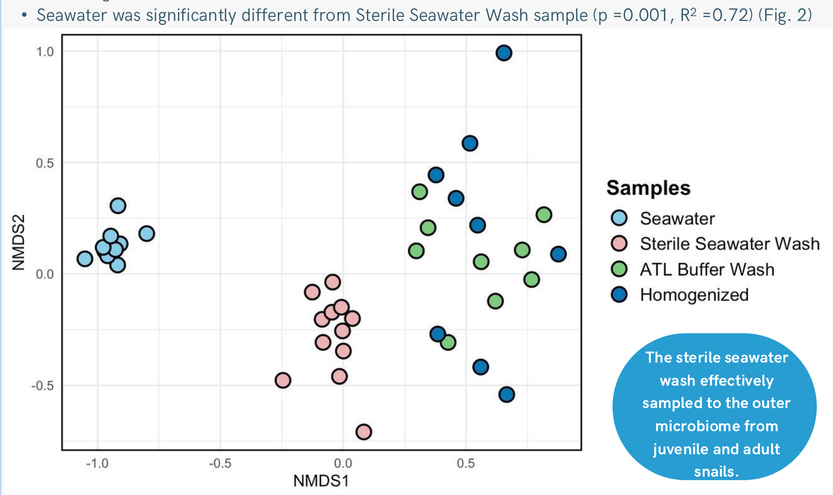

Figure 2. NMDS plot of the community composition of seawater, sterile seawater wash, ATL buffer wash, and homogenized samples of ATL-washed adults from the Ross Islets (n = 38).

2. Life Stages

- **Outer microbial communities differed significantly between the two life stages ($p = 0.001$, $R^2 = 0.44$) (Fig. 3A), with 43.6% of the difference being due to 10 species (Fig. 3B).**

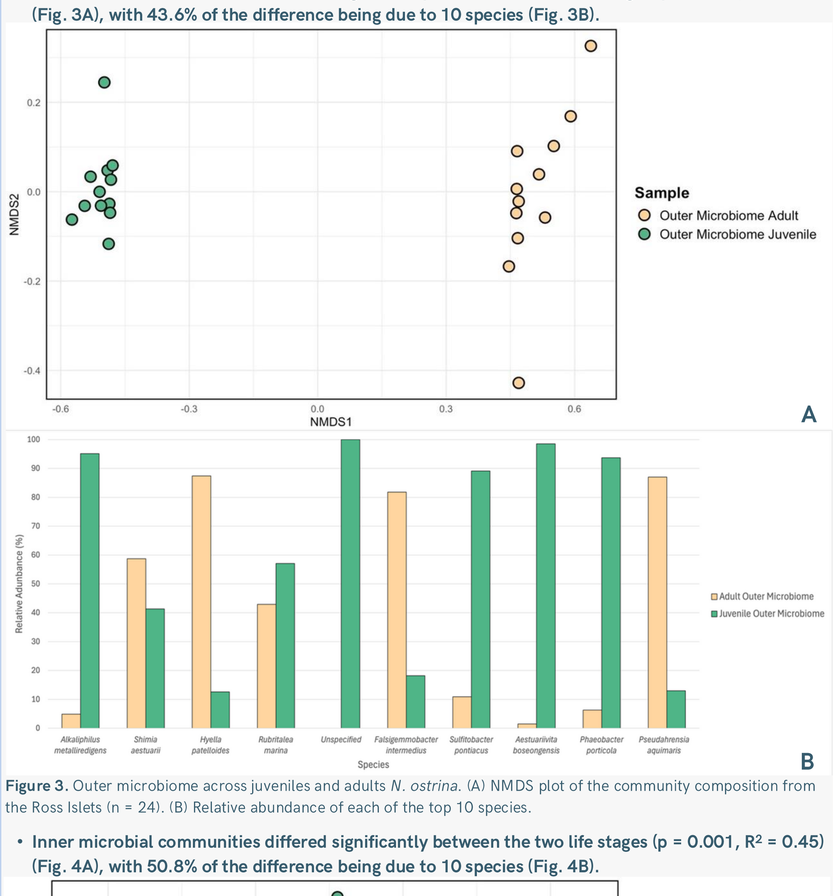

Figure 3. Outer microbiome across juveniles and adults *N. ostrina*. (A) NMDS plot of the community composition from the Ross Islets (n = 24). (B) Relative abundance of each of the top 10 species.

- **Inner microbial communities differed significantly between the two life stages ($p = 0.001$, $R^2 = 0.45$) (Fig. 4A), with 50.8% of the difference being due to 10 species (Fig. 4B).**

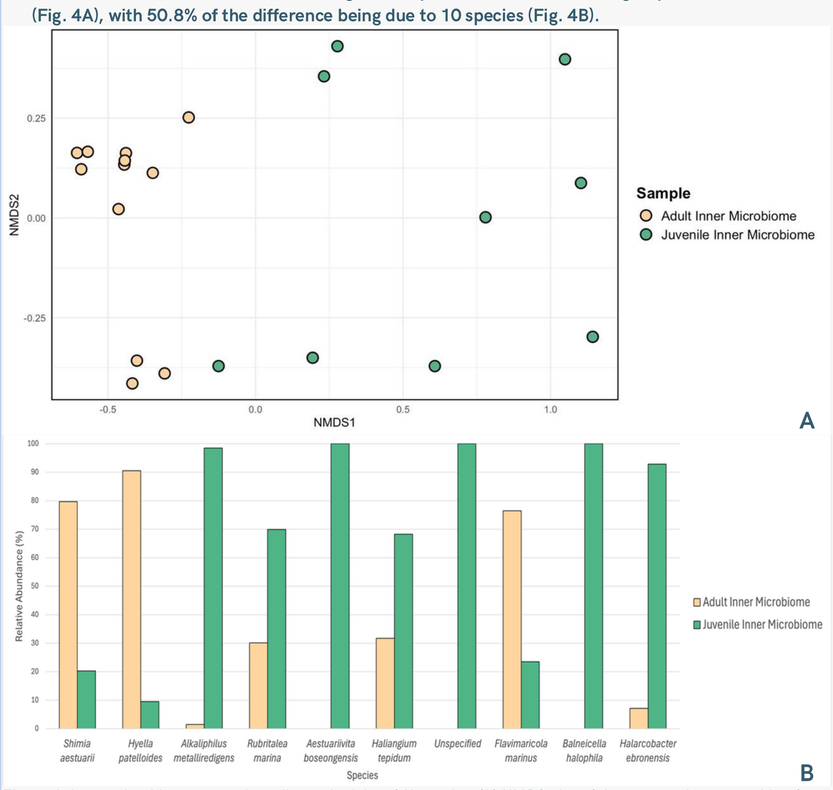

Figure 4. Inner microbiome across juveniles and adults of *N. ostrina*. (A) NMDS plot of the community composition from the Ross Islets (n = 21). (B) Relative abundance of each of the top 10 species.

3. Alpha-Diversity

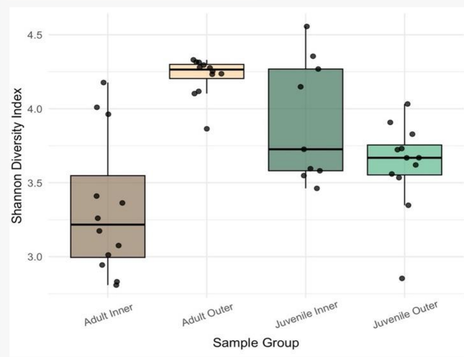

Figure 7. Alpha diversity of the microbial communities associated with *N. ostrina* (n = 44).

- Adult Outer samples exhibited significantly higher diversity than Adult Inner ($p = 4.2e\text{-}05$) and Juvenile Outer ($p = 1.8e\text{-}05$).
- Juvenile Inner samples had significantly greater diversity than Adult Inner ($p = 0.011$).
- Other group comparisons did not reach statistical significance.

4. Spatial Variation

- **Microbial communities differed significantly between the two spatial locations for juveniles ($p = 0.001$, $R^2 = 0.61$) (Fig. 5A), with 38.0% of the difference being due to10 species (Fig. 5B)**

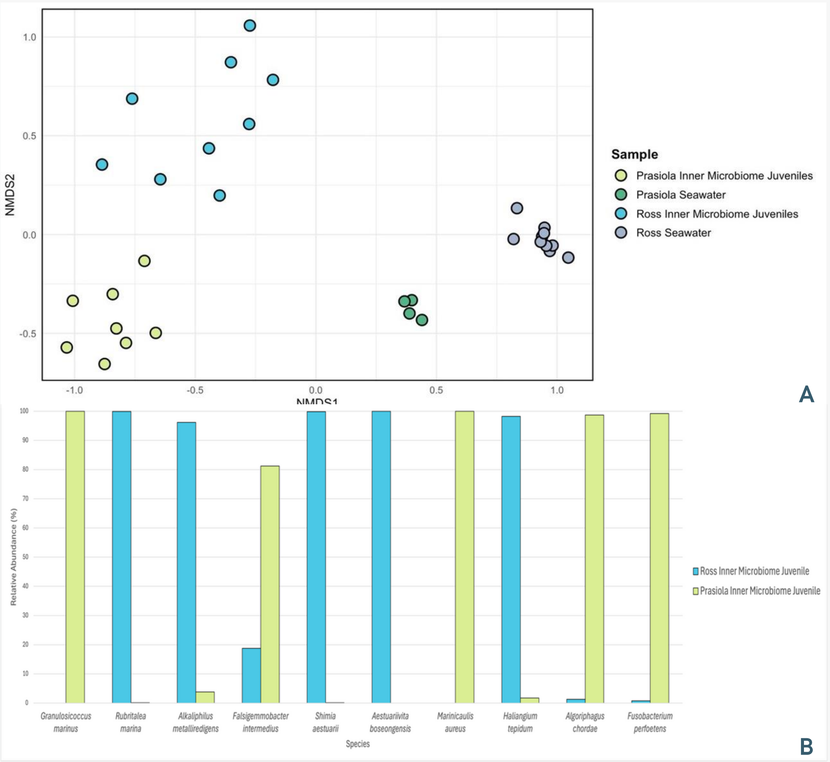

Figure 5. Microbial community on juvenile *N.ostrina* from Prasiola Point and Ross Islets. (A) NMDS plot of the community composition different locations (n = 28). (B) Relative abundance of each of the top 10 species.

- **Microbial communities differed significantly between the two spatial locations for adults ($p = 0.001$, $R^2 = 0.72$) (Fig. 6A), with 52.4% of the differences being due to 10 species (Fig. 6B).**

Figure 6. Microbial community on adult N. ostrina from Ross Islets and Prasiola Point. (A) NMDS plot of the community composition from different locations (n = 29). (B) Relative abundance of each of the top 10 species.

DISCUSSION

The significant difference between the microbiomes associated with wild juvenile and adult *N. ostrina* suggests microbial communities undergo substantial changes with host development. Juveniles are colonized by transient, environmental taxa that may play a protective or opportunistic role during early life stages.

These findings emphasize that microbial acquisition in early life stages is shaped both by environmental influence and host life stage. Spatial variation significantly alters the associated microbiome of both juveniles and adults, indicating that a diverse range of bacteria can colonize. This study underscores the potential of microbiome profiling as a tool to characterize associations between marine invertebrates and the microbial community, and to determine the factors influencing these associations.

FUTURE DIRECTIONS

Future studies should examine the influence of increased temperature associated with climate change on the microbiome of wild early juvenile marine invertebrates to better understand its influence on survival during this vulnerable life stage, and to explore the functional roles of the associated bacterial taxa.

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