

Assessing biofouling community succession using a metabarcoding approach

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Marine biofouling – issues to ecology and economy



But first....



- **Biofilms** - 'slime' layers are a complex mixture of adsorbed organic matter, marine bacteria, benthic diatoms and other algae, protozoa and fungi, and are precursors for colonization by larger fouling

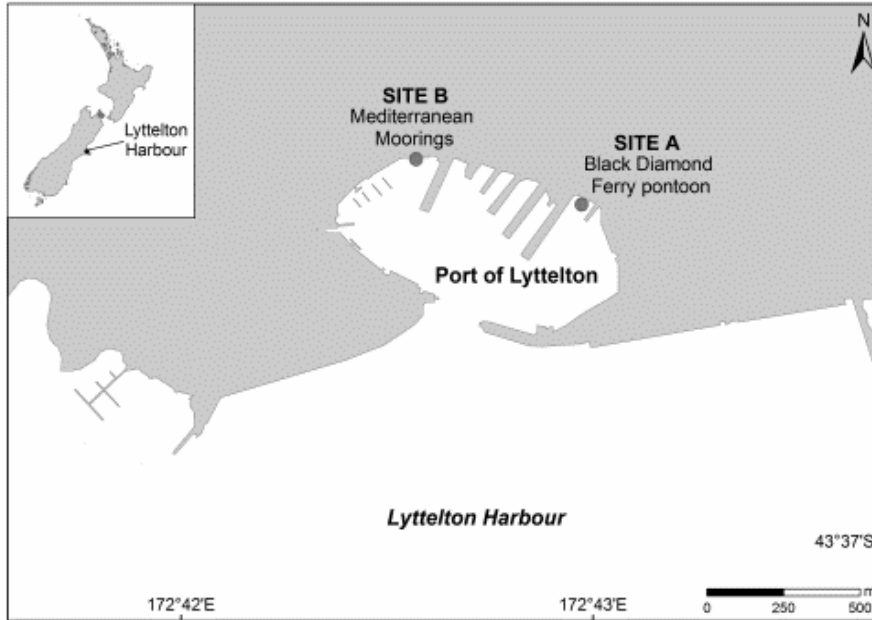
Field experiment on early biofilm communities – Port Lyttelton, New Zealand



Christo



Study site and experiment set-up



- 184 settlement plates
- 2 locations:
Site A, Site B
- 2 experimental Rounds:
2-17 January; 11-26 February
- 3 residency periods:
1 Day, 5 Days, 15 Days

- Stratified design:
116, 40, 28 samples
- 2 analysis methods:
 - NGS metabarcoding (V4 region of 18S rRNA)
 - Morphological ID

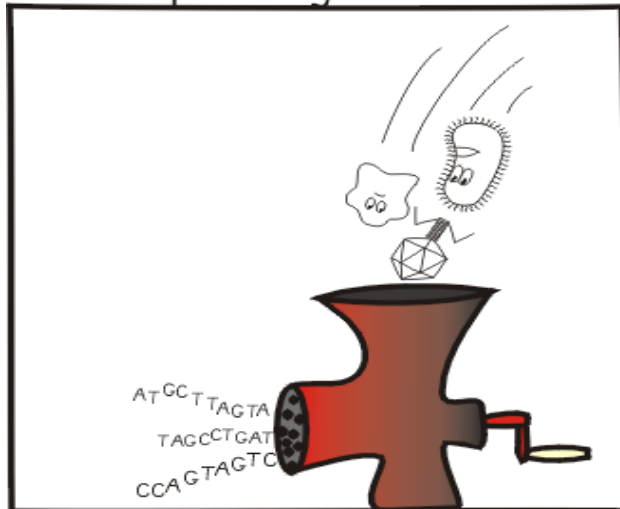


NGS metabarcoding in a nutshell



MiSeq Illumina™ Technology

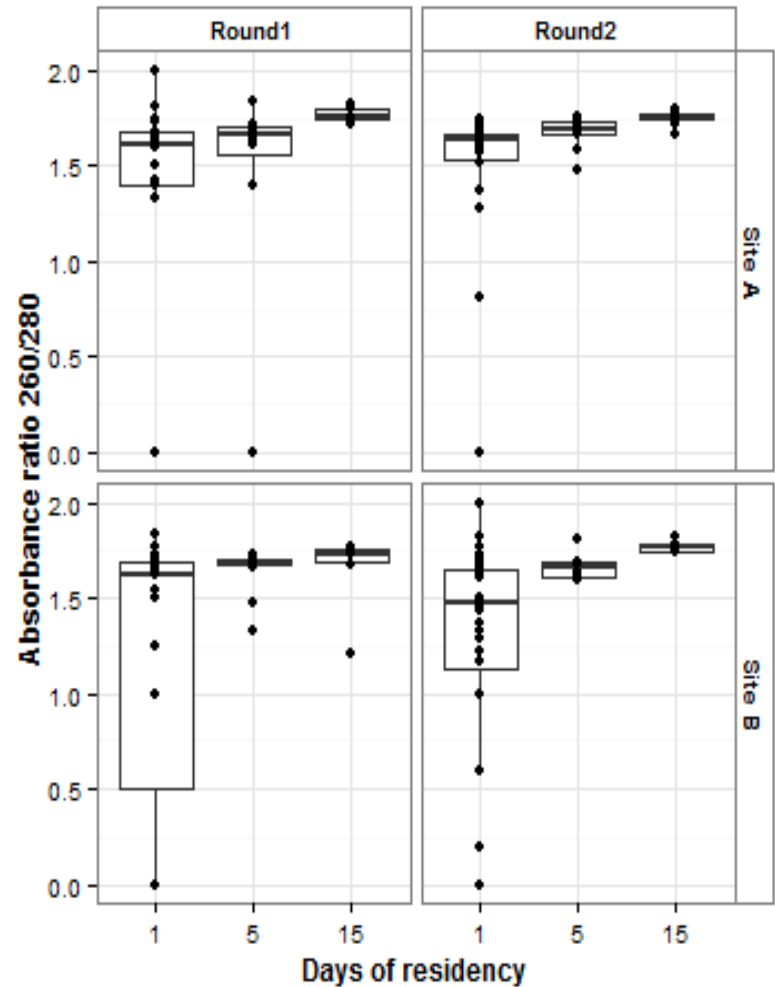
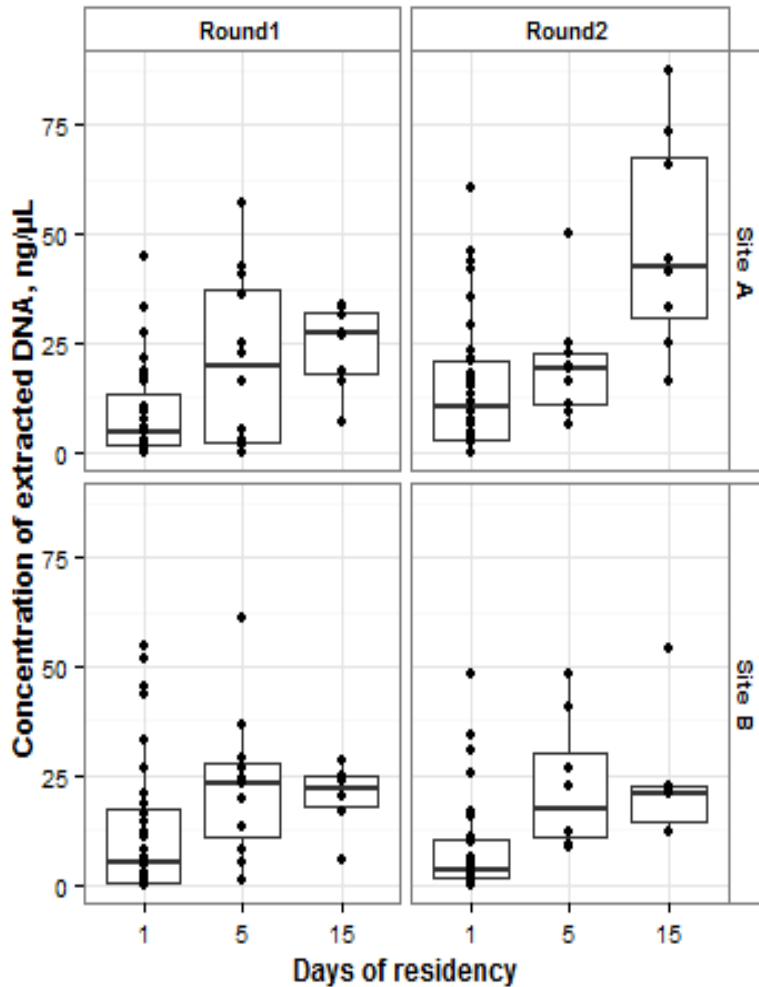
Mass sequencing



by Viktor S. Poór

- High-throughput
- Parallelize sequencing
- Millions of reads at once
- Low-cost sequencing
- Multiplex capability
- Multiple species detection (Metabarcoding)

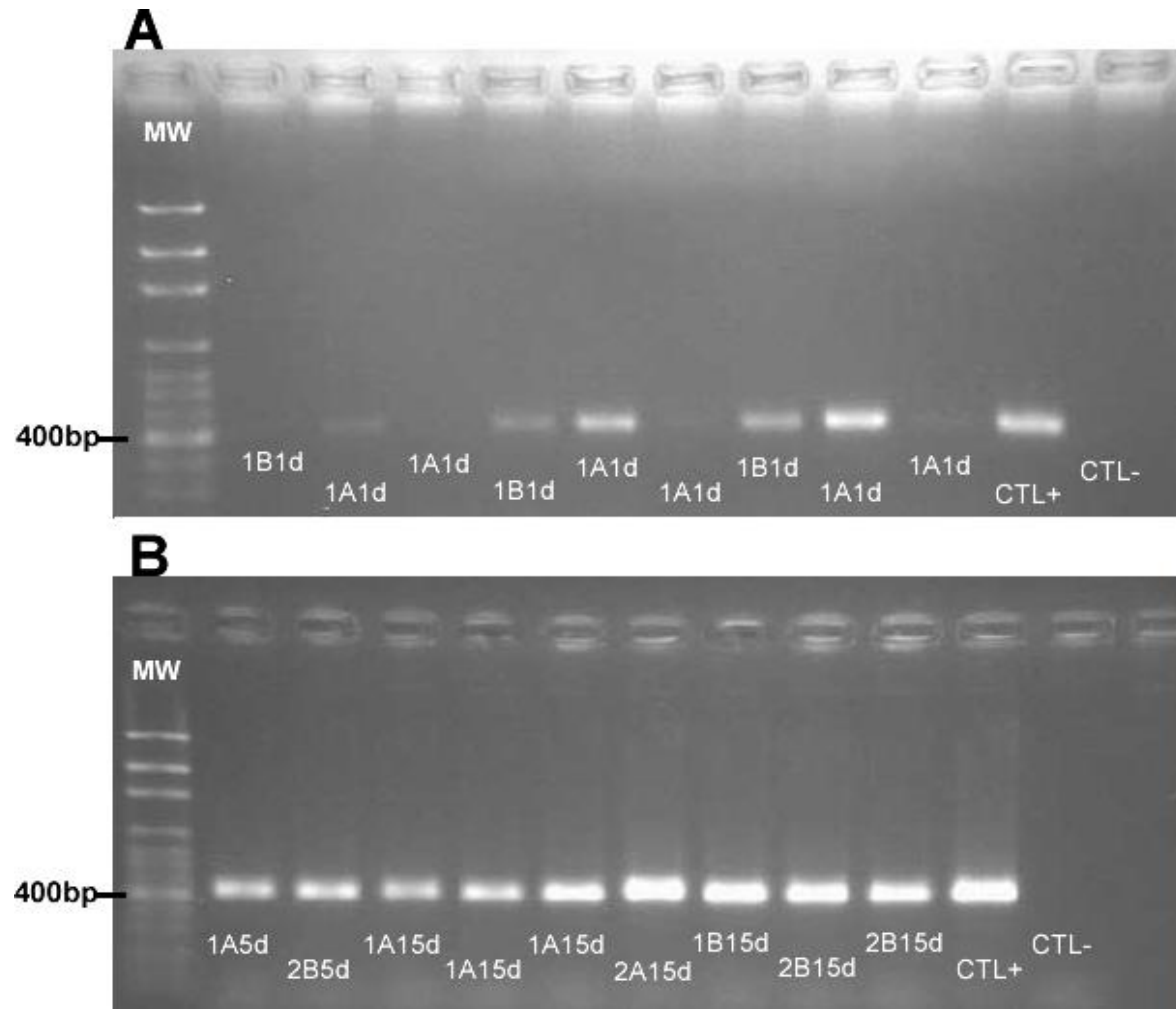
eDNA extractions: quantity and quality issues



PCR amplification: success rate vs. residency

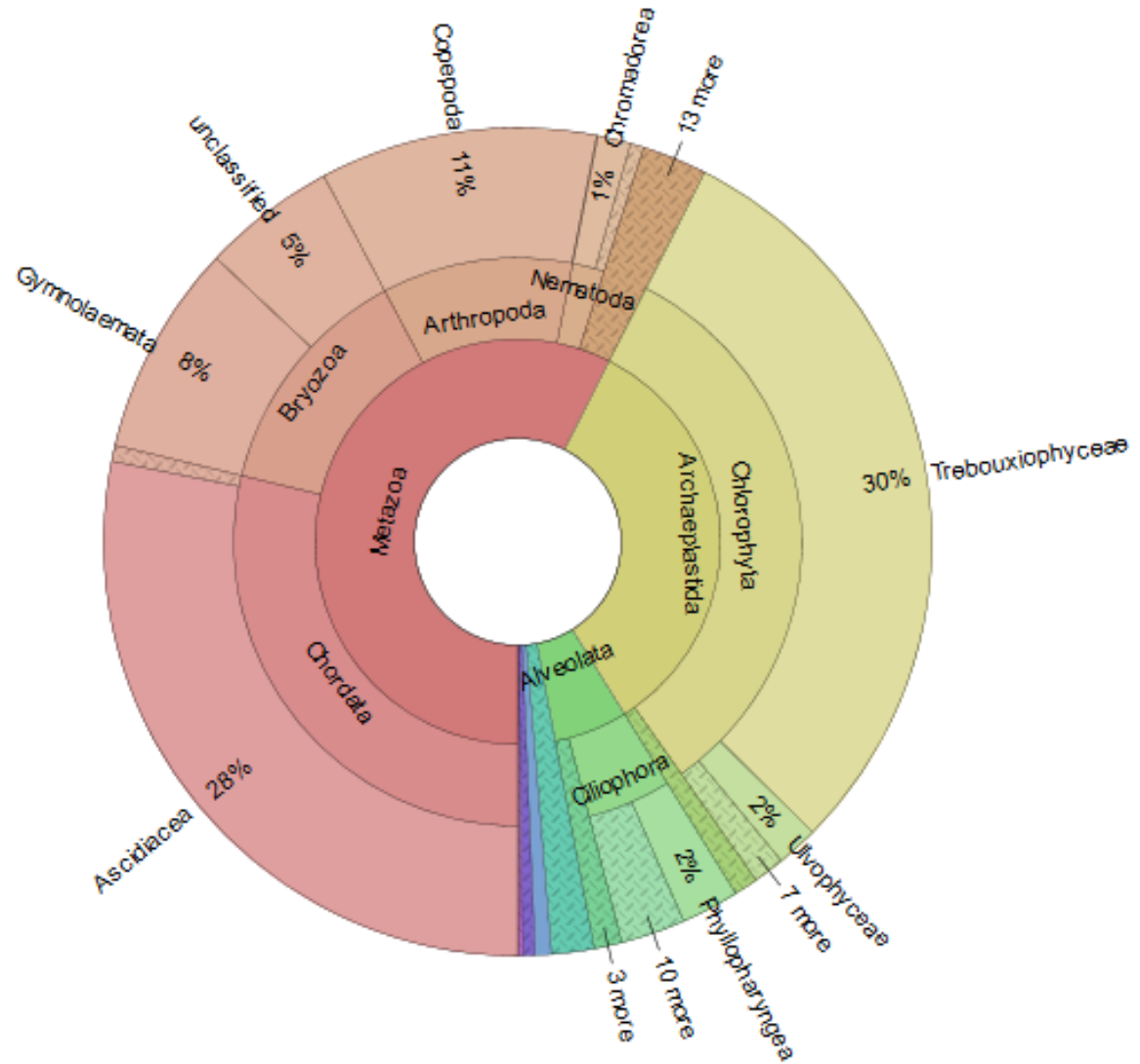
V4 region of 18S rRNA gene was successfully amplified from 105 samples

- 34% for Day 1
- 90% for Day 5
- 96% for Day 15



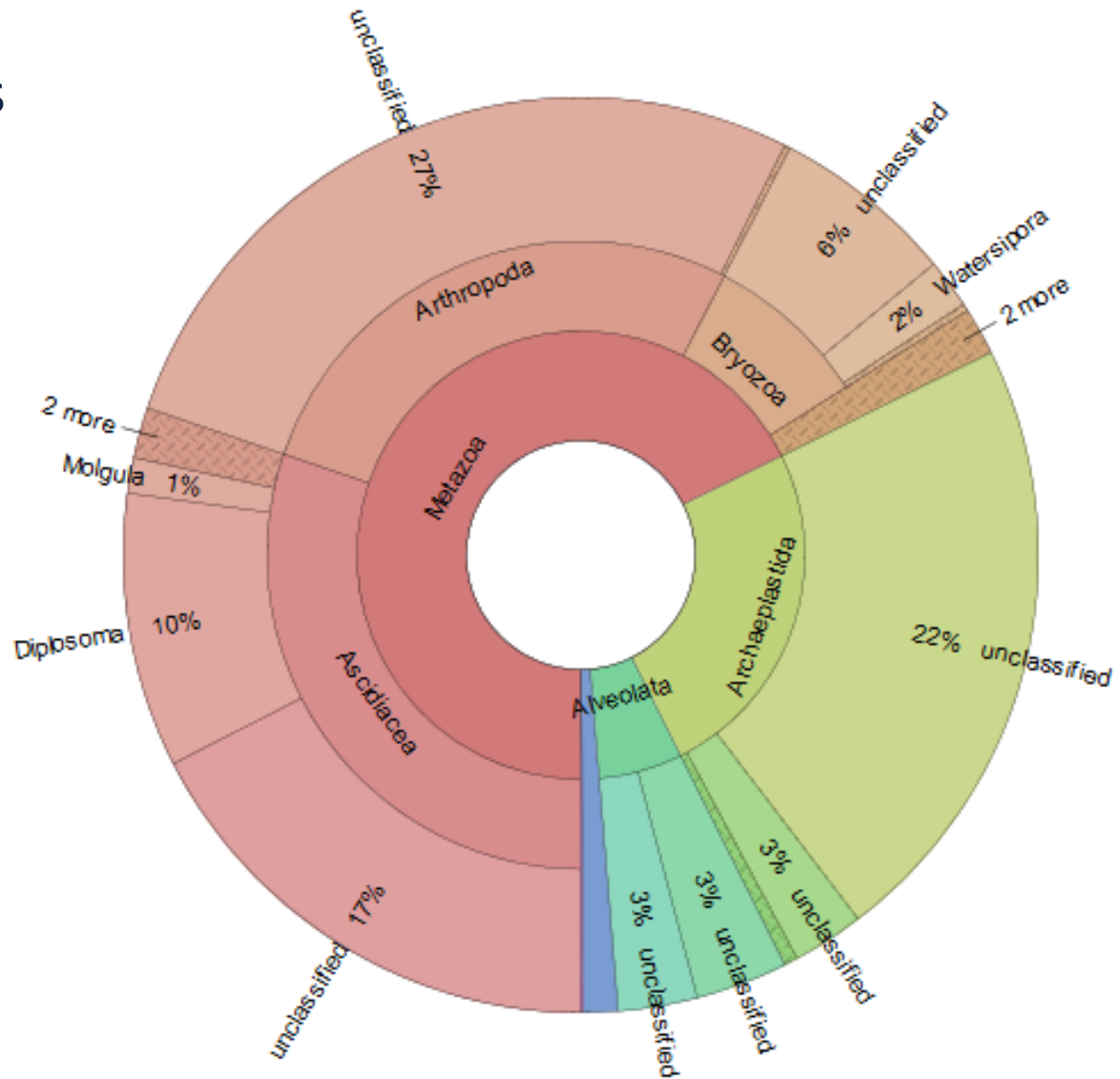
Biodiversity assessed with NGS

- 7 supergroups
- 33 phyla
- 73 classes
- 132 orders
- 195 families
- 240 genera
- 182 species

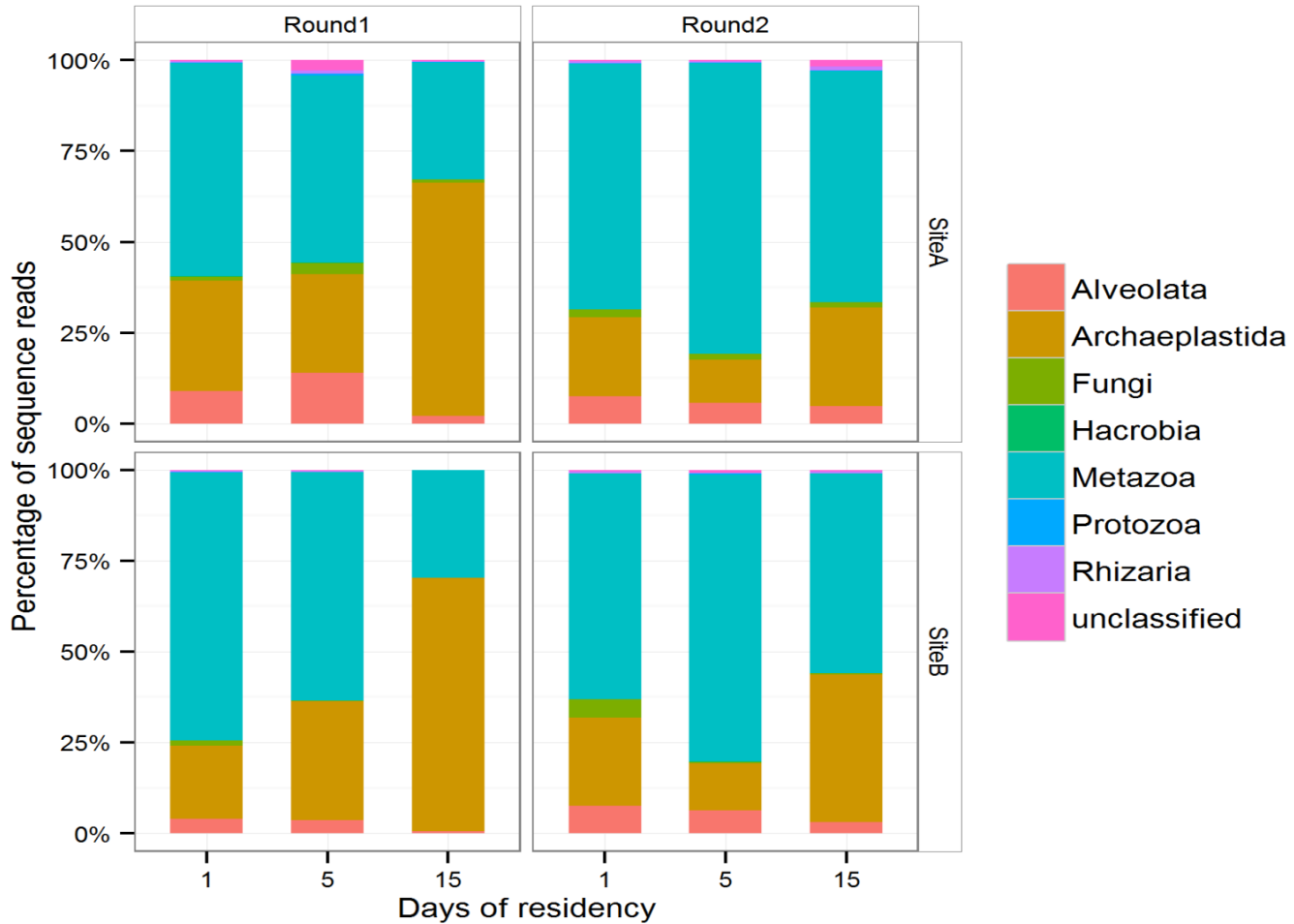


Morphologically assessed biodiversity

- 4 supergroups
- 11 phyla
- 11 classes
- 7 orders
- 7 families
- 6 genera

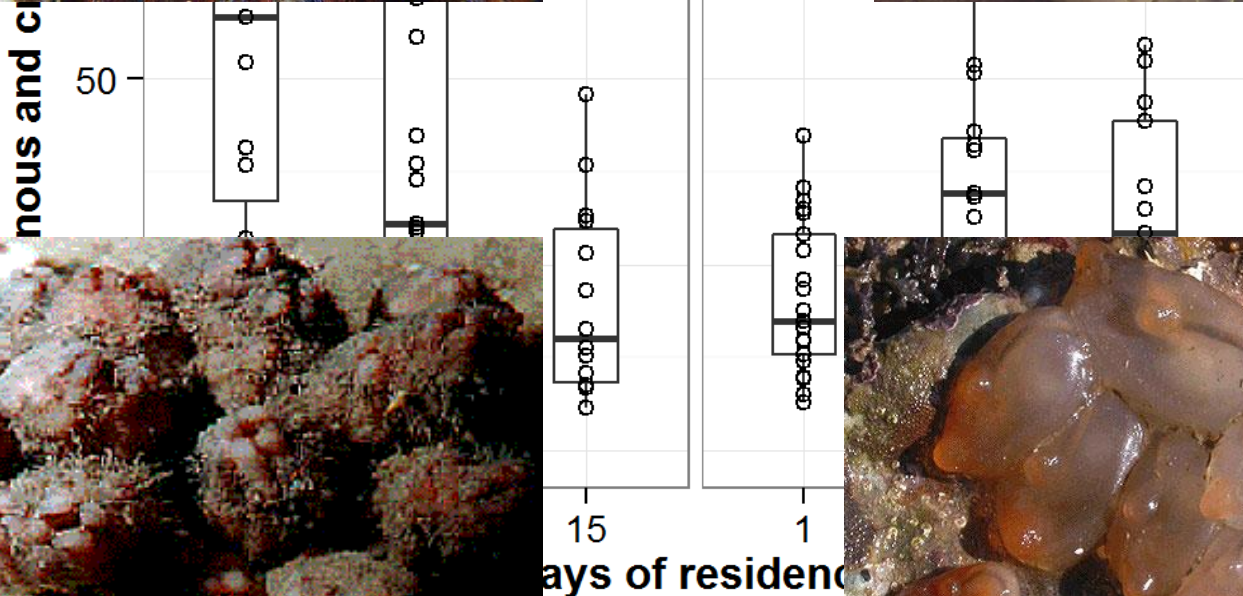
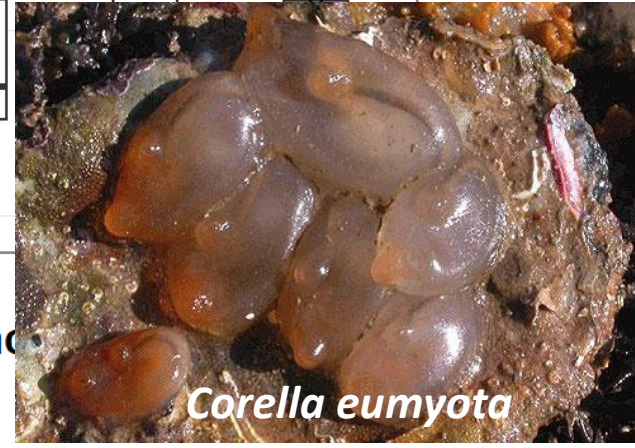
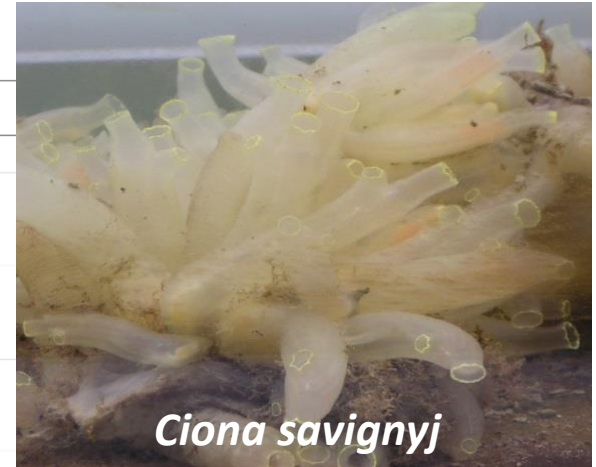


Effect of treatments (NGS data)



| PERMANOVA, P-values | % seqs reads | seqs P/A |
|---------------------|--------------|----------|
| Site | 0,3 | 0,35 |
| Round | 0,02 | 0,21 |
| Residency time | 0,01 | 0,004 |

Contribution of putative pest species



Take home messages

- NGS metabarcoding provided high-resolution taxonomic information, enabling early detection of marine pests
- Additional surveillance of biofouling is recommended for the long-stay vessels and those arriving from the high-risk or invasion outbreak areas
- For efficient NIS detection, we suggest short-term exposure (1-5 days) of sampling substrata in high-risk areas
- However reasonable replication is highly recommended for the short residency sampling



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