Assessing biofouling community succession using a metabarcoding approach

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Marine biofouling – issues to ecology and economy

Images: blog.ltc.mq.edu.au; phys.org;
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
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

- 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

<table>
<thead>
<tr>
<th></th>
<th>% seqs reads</th>
<th>seqs P/A</th>
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<tbody>
<tr>
<td>Site</td>
<td>0.3</td>
<td>0.35</td>
</tr>
<tr>
<td>Round</td>
<td>0.02</td>
<td>0.21</td>
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<tr>
<td>Residency time</td>
<td>0.01</td>
<td>0.004</td>
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</table>
Contribution of putative pest species

Watersipora subtorquata

Styela plicata

Ciona savignyj

Corella eumyota

Images: www.msep.org.uk; www.gulfspecimen.org; www.bryozoa.net
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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