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Assessing biofouling community succession using a metabarcoding approach

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Summary

In this experimental study a metabarcoding approach was used to determine shifts in taxonomical composition of early biofouling communities occurring over 15 days on 184 settlement plates deployed in a New Zealand coastal marina. More than 400 taxa belonging to 7 supergroups, 33 phyla, 73 classes, 132 orders, 195 families and 240 genera were identified using metabarcoding. Non-indigenous organisms were detected in high abundance from the biofilm communities on day one plates, suggesting that for some species their ability to colonize substrates rapidly may play a role in their invasive behavior.

Introduction

Timely detection and accurate identification of marine species is a prerequisite for the accurate assessment of environmental status, sustainable management of marine resources and to prevent or mitigate the spread of invasive or pest species (Lehtiniemi et al. 2015). This task however requires considerable taxonomic expertise, is laborious, and particularly challenging when identifying cryptic species or those at the larval stage. Metabarcoding in combination with high-throughput sequencing allows effective species detection and identification from environmental samples, providing presence/absence or semi-quantitative data on microbial and eukaryotic communities (Wood et al. 2013). Biofouling communities form in marine environments on submerged natural or artificial substrates and are of particular scientific and managerial interests due to their ecological and socio-economical implications. At the early successive stage, biofilms forming on various submerged substrata may host propagules of different taxa (Pochon et al. 2015). Metabarcoding techniques provide a new method for the monitoring and early detection of potential marine pests, particularly in high-risk areas such as ports and marinas.

Materials and Methods

The experiment was conducted in two rounds at Port Lyttelton, New Zealand. Settlement plates (n=184) were deployed in two locations for three time periods (1, 5 and 15 d), after which they were removed, photographed and frozen for subsequent processing (morpho-taxonomy and metabarcoding). Taxonomic identification was performed by visually analyzing the biofilms using a stereo microscope at ×35 magnification. The lowest possible taxonomic level was assigned for each recruit (individual or a colony) detected. From each plate, the biofilm was collected and bulk DNA was extracted using a Power Biofilm® DNA Isolation Kit (MOBIO, USA). The eukaryotic V4 region of the nuclear ribosomal DNA was used as a barcode and was PCR amplified from the biofilm DNA samples with the universal primers Uni18S and Uni18SR (Zhan et al. 2013). Positive amplification products (105) were then sequenced using high-throughput sequencing on a MiSeq instrument (Illumina™). The sequence data was bioinformatically analyzed and taxonomically classified by aligning the sequence reads against the Protist Ribosomal 2 database (Guillou et al. 2013). The results were then compared to those obtained from the morphological analysis by a taxonomist.

Results and discussion

Over the observation period, 441 taxa belonging to 7 supergroups, 33 phyla, 73 classes, 132 orders, 195 families and 240 genera were identified via metabarcoding (Fig. 1). The conventional (morphological) analyses yielded much lower taxonomic resolution and variability (Fig. 2). At the higher taxonomical level (Supergroup or Phylum), the results were more consistent between two methods both in terms of taxonomy and structure (relative abundance).

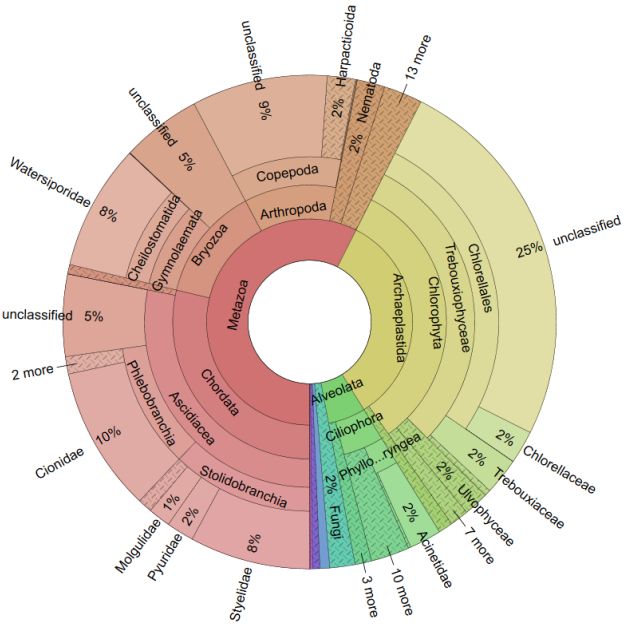

Figure 1. Structure of the biofouling communities identified from the experimental plates using metabarcoding (% of sequence reads).

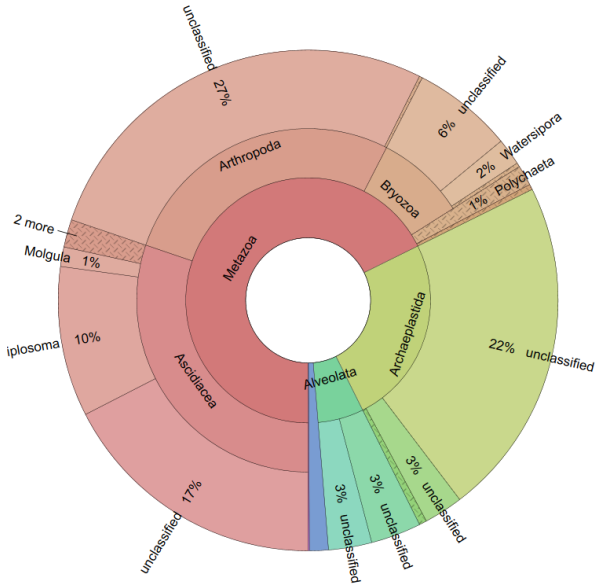

Figure 2. Structure of the biofouling communities identified from the experimental plates using morphology (% of recruit abundance).

Using metabarcoding we identified a shift in the community composition over the observation period from animal-dominated (1-d plates) to plant-dominated (15-d plates). Diverse assemblages (n=395 taxa) were detected after only a single day of deployment. During the study, species adventive in New Zealand were detected (e.g. *Watersipora, Ciona, Molgula* species and Botryllid ascidians), highlighting the potential of this method as an early and effective detection tool.

References

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