Scarus spinus, crustose coralline algae and cyanobacteria

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Supplementary Methods

Bite Core Microscopy and CPCe

Percentage surface cover of epilithic biota on the bite cores was analysed in Coral Point Count (CPCe v 4.1, Kohler and Gill, 2006), using a planar photograph of the 22mm diameter bite cores taken at 5x magnification. Within CPCe, a 7 x7 grid was overlaid onto the planar bite core photograph and the biota under each point identified (Nicholson and Clements 2020). Cyanobacterial tufts were coded as separate to algae and represented cyanobacterial filaments > 1 mm in length that were visible at 5 x magnification. Epilithic biota were morphologically identified to species where possible and photographed at magnifications up to 630x using a Leica DMR microscope. In order to quantify the filamentous cyanobacteria and benthic diatoms a standardized microscopic transect sampling method was used (Nicholson and Clements 2020). After the bite cores were photographed for the CPCe and bite mark measurements, a 1cm^2 x 1mm deep scrape was taken from each core surface (Diaz-Pulido and McCook 2002; Bender et al. 2014; Nicholson and Clements 2020). Each scrape was then decalcified for 5 mins with 10% Hydrochloric acid (HCl) (Price and Scott 1992) and centrifuged for 2 min at 2000 rpm. The pellet was spread evenly onto a microscope slide, haphazardly distributed with a dissecting needle and sealed with a 22 x 22 mm coverslip. Ten horizontal transects of the 22mm x 22mm coverslip were made at 630x magnification (10x ocular and 63x objective lens) on a Leica DMR using differential interference contrast microscopy. Along each transect diatoms were counted and each filamentous cyanobacteria encountered was photographed and its length measured in microns (Fig. 2), using either a Leica

DC500 camera or a Jenoptik ProgRes Gryphax camera using Analysis LS or ProgRes Gryphax software. Photographs were used to support taxonomic classification of the filamentous cyanobacteria. CPCe, cyanobacteria lengths and diatom densities values for mid-shelf 2018 bite cores were taken from Nicholson and Clements (2020).

Gene Amplification

16S: Fragments of the V4-V5 variable region of the 16S SSU ribosomal RNA gene (464 bp) were PCR-amplified using the 16S rRNA primer set S-D-Bact-0341-b-S-17 + S-D-Bact-0785-a-A-21: Forward primer (341f): 5′- CCTACGGGNGGCWGCAG-3′, and Reverse Primer (805r): 5' GACTACHVGGGTATCTAATCC-3′ (Herlemann et al. 2011).

18S: Fragments of the V7-V8 hyper variable regions of the 18S ribosomal DNA gene (330-375bp) were PCR-amplified using the 18S rDNA primer set Forward primer (3F): 5′ GYGGTGCATGGCCGTTSKTRGTT 3′, and Reverse Primer (5RC): 5' GTGTGYACAAAGGBCAGGGAC -3′ (Machida and Knowlton 2012).

Both 16S and 18S primer sets were appended with Illumina adapter sequences: Forward overhang 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG and reverse primer: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG (Klindworth et al. 2013).

PCR Methods: The PCR reaction mixture composed of 12.5µl MyTaq™ Red Mix, 1µl (10µM) of each primer, 3µl template DNA, 1µl bovine serum albumin (BSA), 6.5µl Ultrapure molecular biology H2O to make to 25µl. PCR amplifications were carried out in an Eppendorf Mastercycler DNA or a VERITI Thermal Cycler (Applied Biosystems). Amplification conditions for 16S were as follows: an initial denaturation step of 3 mins at 95°C followed by thirty PCR incubation cycles each consisting of: i] denature for 30s at 95°C ii] annealing for 30s at 55°C iii] extension for 30s at 72°C. Final extension for 7 mins 72°C. 18S PCR: initial denaturation 10 min at 95°C followed by: thirty PCR incubation cycles each consisting of: i]

denature for 30s at 95°C ii] annealing for 30s at 57°C iii] extension for 1 min at 72°C. Final extension for 5 min 72°C (Machida and Knowlton 2012). Amplification was verified with gel electrophoresis for 30-40 minutes at 100volts in a TBE SYBRSafe 1.5% agarose gel. PCR products were then purified with AMPure following the manufacturer's instructions, DNA was quantified using an IMPLEN Nanophotometer and Qubit Fluorometer (Invitrogen, Carlsbad, USA)

Bioinformatic Analysis

Illumina MiSeq PE300 sequencing was performed at Auckland Genomics, New Zealand (16S) and Genewiz/Azenta China (18S). Bioinformatic analysis was conducted using QIIME2 2021.4 (Bolyen et al. 2019) within Jupyter notebook (Kluyver et al. 2016), provided by New Zealand eScience Infrastructure (NeSI). DNA sequencing was carried out on one technical replicate per year, which consisted of the pooled scraped surface from the bitecores. Demultiplexed, paired-end sequences were imported into QIIME2 (q2), forward and reverse reads were quality-checked within q2View. Dereplicating, denoising, chimera-removal and trimming were achieved using the DADA2 q2-plugin (Callahan et al. 2016). Poor quality reads were removed based on the quality scores in q2view (16S: p-trim-left-f 20, p-trim-left-r 8, p-trunc-len-f 280, p-trunc-len-r 220. 18S:p-trim-left-f 20, p-trim-left-r 8 ,p-trunc-len-f 280, p-trunc-len-r 230). For taxonomic assignment, reference sequences from the SILVA SSURef NR99 database (Quast et al. 2013) were extracted for the amplicon specific regions: 16S (V4-V5) and 18S (V7-V8), using 'q2-feature-classifier extract-reads' (Bokulich et al. 2018). We reverted to SILVA 132 for 18S as DADA2 is not suitable for classifying Eukaryotes with SILVA 138 (https://benjjneb.github.io/dada2/training). Naïve Bayes classifiers were created and trained using the 'q2-fit-classifier-naïve-bayes'. ASVs were assigned using the 'classify-sklearn' command. Amplicon Sequence Variants (ASVs: Callahan et al. 2017) assigned as

cyanobacteria by SILVA 138 (Quast et al. 2013) using the DADA2 q2-plugin (Callahan et al. 2016) were imported into the 16S database CYDRASIL v.3 (Roush et al. 2021). Multiple alignment was achieved with MAFFT (Katoh and Standley 2013) via 'q2-alignment', and a phylogeny was constructed with FASTTREE2 (Price et al. 2010) with 'q2-phylogeny'. ITOL v.6 (Letunic and Bork 2021) was used to visualize the resulting phylogeny.

Supplemental Methods References

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