DNA metabarcoding as a tool for detecting and characterising the spatio-temporal distribution of planktonic larvae in the phylum Echinodermata

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Abstract

Metabarcoding is revolutionising the analysis of biodiversity in marine ecosystems, especially as it provides a means of detecting and identifying cryptic life-stages in field samples. The planktonic larval stage of many species underpins the abundance and distribution of adult populations but is challenging to characterise given the small size of larvae and diffuse distributions in pelagic waters. Yet, planktonic larval dynamics are key to understanding phenomena observed in adult populations, such as the boom-and-bust dynamics exhibited by some echinoderms. Rapid changes in echinoderm population density can have significant effects on local benthic ecosystems, for example, outbreaks of the crown-of-thorns seastar (CoTS) on the Great Barrier Reef (GBR) are responsible for declines in coral cover. Here, we used a DNA metabarcoding approach to investigate the spatio-temporal distribution and diversity of echinoderm larvae on the GBR, including CoTS. Echinoderm larvae were found to exhibit seasonal changes in community composition and richness, consistent with expected fluctuations in larval output based on adult spawning periodicity. Furthermore, this study validates the utility of metabarcoding approaches for the surveillance of CoTS larvae, which could prove useful to future monitoring efforts. Our findings suggest that metabarcoding can be used to better understand the life history of planktonic larvae, and analyses combining environmental (e.g., temperature, nutrients) and oceanographic (e.g., currents) data could deliver valuable information on the factors influencing their spatio-temporal occurrence.

Keywords: echinoderms, metabarcoding, larvae, eDNA, plankton, crown-of-thorns starfish

Introduction

The biodiversity of marine environments is changing at an unprecedented rate due to human-induced habitat disturbances (Beaugrand et al. 2002; Molinos et al. 2015). Ensuring that diversity is preserved or restored is crucial to the functioning and persistence of ecosystems worldwide (Alsterberg et al. 2017). Monitoring marine biota and indices of ecosystem health, such as the presence of certain bioindicator species, is a global research priority (Ceballos et al. 2015; Molinos et al. 2015). For many species, research efforts have focused on quantifying and forecasting the biodiversity of adult populations. However, most marine invertebrates exhibit a pelago-benthic life cycle, whereby adults are primarily benthic, and larvae develop in the water column (Cowen and Sponaugle 2009). Planktonic larval stages can disperse great distances, facilitating the replenishment of, and connectivity among populations. Larval supply, in turn, mediates the establishment and persistence of adult populations (Cowen and Sponaugle 2009; Treml et al. 2015). Despite the importance of the planktonic larval life stage, it is rarely considered in biodiversity monitoring efforts, and little is known of the temporal and spatial stochasticity of planktonic larvae due to the difficulties associated with collecting, detecting and discriminating among species in the field (Uthicke et al. 2015a).

Contemporary genetic tools, such as metabarcoding, can overcome many of the challenges associated with the detection and identification of planktonic larvae (Ko et al. 2013; Uthicke et al. 2015a). Metabarcoding involves the extraction and amplification of DNA within biological samples (e.g., soil and water samples), followed by amplicon sequencing and the taxonomic identification of sequences attributed to each sample. This method not only enables the simultaneous identification of multiple taxa, but it also provides a means of identifying cryptic taxa or life-stages, such as planktonic larvae, that would otherwise be too difficult to detect or identify in the field. It has been used as a rapid and cost-effective tool to quantify diversity in fresh-water and marine environments (Kimmerling et al. 2018; Berry et al. 2019; Glenn et al. 2019). Comparatively fewer studies have applied this approach to plankton diversity, and fewer still in a spatially and temporally structured manner (e.g., Berry et al. 2017).

A distinct ecological characteristic in many marine ecosystems is a phenomenon known as boom-and-bust, that is thought to be influenced by larval dynamics (Uthicke et al. 2009). Boom-and-bust dynamics, describe periods of rapid and exponential growth of benthic adult

populations (Strayer et al. 2017) followed by a sudden population decline (crash), have been attributed in part to the abundance of planktotrophic larval stages (Uthicke et al. 2009). Planktotrophic larvae, those that feed whilst in the water column, are often the product of broadcast spawning, whereby fertilization success increases exponentially rather than linearly with increases in adult densities (Uthicke et al. 2009). This excess supply of larvae can cause rapid increases in adult population sizes especially when there are high larval survival rates (Cruz and Harrison 2017). Additionally, the condition and survival of planktotrophic larvae can be uncoupled from that of their parents (Uthicke et al. 2009; Marshall and Morgan 2011), such that environmental factors influencing planktotrophic larval survival can result in unforeseen changes to adult population densities including 'population explosions' or 'die-offs' (Uthicke et al. 2009). Although boom-bust dynamics are considered natural cycles, there is mounting evidence to suggest that anthropogenic impacts are exacerbating the speed and scale of these natural fluctuations (Uthicke et al. 2009; Matthews, Mellin and Pratchett 2020; Kroon et al. 2021). These accelerated fluctuations in population density may prevent ecosystems from adapting to or compensating for rapidly changing environments (Hoey et al. 2016). Such unpredictable population dynamics therefore pose a challenge for monitoring and management of both at-risk and pest species.

Boom-and-bust phenomena are especially common among echinoderms. Species from the genera *Acanthaster, Diadema*, and *Echinometra* are well-known for extreme cyclical population fluctuations and hold important trophic positions that are pivotal in structuring coral reef communities (Paine 1969; Birkeland 1989; Byrne 2011). For example, density fluctuations of the algal-grazing *Diadema* sea urchins are responsible for the oscillations between coral- and algal-dominated ecosystem states in the Caribbean (Hughes 1994), and transitions between temperate kelp forests and 'urchin-barrens' have also been observed in response to fluctuating population densities of herbivorous sea urchins (Scheibling et al. 1999). Similarly, southern range expansions of sea urchins from Australia and New Zealand into Tasmania are devastating kelp communities (Johnson et al. 2011). The removal of echinoderms from ecosystems can cause 'trophic cascades' and the loss of ecosystem integrity, whilst the rapid proliferation and spread or introduction of a species can cause catastrophic ecosystem 'phase-shifts' (Scheffer et al. 2001). Evidently, changes to echinoderm populations can have significant effects on ecosystem functioning, productivity, and resilience (Paine 1969; Scheibling et al. 1999).

The best-known example of a boom-and-bust species is the corallivorous crown-of-thorns sea star (CoTS) (a species complex consisting of *Acanthaster* cf. *solaris, planci, mauritiensis, benzii* and *elissii;* Uthicke et al. 2009; Byrne et al. 2011; Haszprunar et al. 2017). Population explosions of this species have caused widespread damage, contributing significantly to coral cover loss observed between 1985 and 2012 (De'ath et al. 2012). Outbreaks have been documented periodically since 1962 (Birkeland 1989), with the most recent outbreak underway since 2008-2010 (Westcott et al. 2020). CoTS outbreaks are therefore a critical management issue as decreased coral cover results in the loss of numerous ecosystem services (De'ath et al. 2012). Uncovering the mechanisms that underpin boom-bust cycles is pivotal for an improved understanding of the factors influencing larval supply. Such knowledge will inform the conditions that affect the abundance, diversity, and distribution of adult populations, which is crucial to future management efforts. While other molecular tools such as qPCR demonstrate the utility of DNA-based approaches (Uthicke et al. 2018a, 2019), no study to date has used metabarcoding to study boom-and-bust dynamics.

Here, we use metabarcoding to explore the spatial and temporal dynamics of plankton from the Great Barrier Reef (GBR), Australia. We analysed plankton samples collected from (i) Moore Reef over a 5-year period, and (ii) from 15 reefs across a latitudinal gradient between Fore and Aft Reef and Lizard Island over a 3-year period. To the best of our knowledge, no previous study has used metabarcoding to study echinoderm larvae on the GBR. With this novel application of metabarcoding, we address three main objectives: (1) determine the temporal stochasticity in echinoderm larval occurrence at Moore Reef, (2) document the patterns of occurrence of echinoderm larvae across a latitudinal gradient on the GBR over three years, and (3) determine whether metabarcoding of plankton samples can be used to infer the presence of CoTS larvae at monitored reefs. Given what we know about the boom-and-bust dynamics exhibited by many echinoderms, we expected significant temporal, rather than spatial, stochasticity in the occurrence of echinoderm larvae. This work demonstrates that metabarcoding can be used to gain ecological insights into planktonic larval dynamics of marine invertebrates.

Materials and methods

Collection and processing of plankton samples

Plankton samples were collected as described by Uthicke et al. (2019) as part of ongoing long term monitoring and two sample sets were chosen for this study. The first set of samples were

collected from Moore Reef at regular monthly intervals, spanning a five-year period from 2015-2020 (Fig. 1). A total of 30 samples, consisting of two biological replicates from 15 independent collection dates were chosen. The second set of samples was collected along a latitudinal transect in December of the years 2017, 2018, and 2019. These samples were collected from 15 reefs between Townsville and Lizard Island (Fig. 1). Two samples were collected at each reef in each year, equating to a total of 90 samples.

DNA extraction and PCR amplification

DNA was extracted from the plankton samples at the Australian Institute of Marine Science (AIMS), Townsville, prior to this study as per the protocol outlined in Doyle et al. (2017). The DNA concentration in each sample was quantified using a Nanodrop at AIMS and aliquots were transported to The University of Queensland (UQ) for sample multiplexing and library preparation.

A 313-bp fragment of the COI gene was amplified using the primer set mlCOIintF and jgHCO2198 (forward: 5'-GGWACWGGWTGAACWGTWTAYCCYCC-3'; reverse: 5'-TAIACYTCIGGRTGICCRAARAAYCA-3') designed by Leray et al. (2013) and the Adapterama II multiplexing barcodes described in Glenn et al. (2019). This primer set is commonly used in marine metabarcoding studies and has been shown to amplify DNA from a broad array of marine species, including echinoderms (Leray et al. 2013). The cycling parameters involved 10 minutes at 95°C to activate the polymerase; 35 cycles of denaturation at 95°C for 1 minute, annealing at 48 °C for 30 seconds, elongation at 72°C for 30 seconds; and a final cycle at 72°C for 7 minutes followed by storage at 4°C. PCR amplifications were performed in a total 12.5 µL volume, containing 6.25 µL AmpliTaq Gold 360 Master mix (2 x), 4.25 µL DNA-free water, 0.5 µL forward primer (10 µM), 0.5 µL reverse primer (10 µM), and 1 µL DNA extract (10 ng/µL).

PCR reactions were performed in triplicates to account for amplification biases (Taberlet et al. 2012, Bourlat 2016). The effect of PCR variation was explored in more detail in the Moore Reef dataset, for which triplicates were uniquely tagged allow the differentiation of technical replicates post-sequencing. PCR products were visualised on 2% agarose gels and a clear single band of the expected length indicated successful amplification. 'Failed' amplifications were repeated with five additional cycles to procure more PCR products if possible (Bourlat 2016). Negative controls (no DNA template), positive controls (samples of

known species composition) and blanks were included in each plate. These control samples were used to guide read filtering and reduce noise in the final dataset as recommended by best practice workflows (Taberlet et al. 2012).

Library preparation and sequencing

Individual PCR products were purified using PCR-DX Clean beads following the manufacturer's protocols. Thereafter, products were pooled in equimolar ratios and placed in a limited-cycle PCR to facilitate the ligation of indexed iTru5 and iTru7 primers (Glenn et al. 2019). The second PCR step was performed in triplicates to account for PCR variation in adapter ligation. Each reaction consisted of 25 µL, including 12.5 µL AmpliTaq Gold 360 Master mix (2 x); 5 µL nuclease-free water; 1.25 µL forward primer (10 µM); 1.25 µL reverse primer (10 µM); 5 µL pooled PCR 1 product. Triplicates were pooled and cleaned using PCR-DX Clean beads as above. The final two libraries were pooled in equimolar proportions, to ensure a minimum read depth of 20,000 reads per sample and stored at -20°C until sequenced. Illumina MiSeq 2 x 300-bp paired-end sequencing was performed by the Australian Genome Research Facilityin Melbourne, Australia.

Bioinformatics

The sequencing data was demultiplexed using the python program Mr. Demuxy version v1.2.0 (Glenn et al. 2019). Primer sequences and the reverse complements of the primers were removed from forward and reverse sequences using Trimmomatic version v0.39 (Bolger et al. 2014). FastQC version v0.11.3 was used to confirm the quality of the trimmed reads (Andrews 2010). Initial quality filtering was performed in Trimmomatic using a sliding window of four bases with an average quality of 15 (SLIDINGWINDOW:4:15). A minimum read length equal to the target locus length (313 bp) was set for each primer pair and bases with a quality score below 10 were trimmed (MINLEN:${LENGTH} LEADING:10 TRAILING:10). Paired-end reads were merged using FLASH v1.2.11 with a minimum overlap of 30-bp (Magoč and Salzberg 2011) and only merged reads were used in downstream analyses.

The following filtering steps and taxonomic assignment were performed using VSEARCH version v2.17.1 (Rognes et al. 2016). Quality filtering was performed using the fastx_filter function (fastq_maxee 1). Dereplication and denoising were subsequently performed using the UNOISE3 algorithm (unoise_alpha 2), indel filtering with the fastx_filter function, and

chimera removal using the UCHIME denovo algorithm. Operational Taxonomic Unit (OTU) clustering was performed using swarm version v3.0 with the d=1 (Mahé et al. 2015). Taxonomic identification was performed using SINTAX classification in VSEARCH. The curated MIDORI (Leray et al. *in prep.*) database (release GB241) was used as the reference database from which sequences were identified based on a 97% (sintax_cutoff) similarity threshold (Hebert et al. 2003). Echinoderm sequences were further validated against NCBI GenBank (NCBI Resource Coordinators 2017) sequences on 20 August 2023, and the distribution of identified species was subsequently assessed using the World Register of Marine Species (WoRMS) and the Atlas of Living Australia (Horton et al. 2021, ALA 2023).

Output tables were curated and merged in R version 4.0.2 (R Core Team 2021) and post-VSEARCH processing followed best practice approaches of Alberdi et al. (2018) and Drake et al. (2022). OTU tables were curated following best practices as described in Alberdi et al. (2018) and Drake et al. (2022). Specifically, singletons were discarded to remove low confidence sequences (Alberdi et al. 2018). The removal of "maximum taxon contamination" was performed based on the number of reads present in negative controls (as in Drake et al. 2022). Additionally, in the latitudinal dataset, a sample-based threshold of 0.03% was applied (following Drake et al. 2022), while, in the Moore Reef dataset, a "restrictive additive" approach was used to process PCR/technical replicates (as in Alberdi et al. 2018). While such conservative thresholds may cause the loss of rare taxa it provides certainty of the remaining sequences (Alberdi et al. 2018).

Analyses of larval spatio-temporal variation

The OTU tables derived from the bioinformatic analyses were converted to presence-absence data for analyses and data visualisation. OTUs present in biological replicates were combined in an additive manner, whereby the presence of an OTU in either biological replicate was interpreted as a confirmed presence at a particular time-point/site (as in Burgar et al. 2014; Leray and Knowlton 2015). Analyses involved the total number of OTUs, hereafter referred to as "richness" and the presence/absence composition of OTUs, referred to as "assemblage" in each sample. Abundance was not considered in this study given the biases associated with estimating abundance from read counts (Leray and Knowlton 2015, Bucklin et al. 2016, Deiner et al. 2017; Kimmerling et al. 2018).

All statistical analyses to test for the effect of temporal ("season", "year") and spatial ("site", "longitude", "region") variables on echinoderm (larvae) richness and composition were

completed in R version 4.0.2 (R Core Team 2021) and a significance level of $\alpha < 0.05$ was used for all models. Generalised linear mixed-effects models (GLMMs) were fitted using glmmTMB (Brooks et al. 2017) to analyse the relationship between echinoderm richness in response to the explanatory variables. Generalised linear models (GLM) were also used in cases where the inclusion of random effects was not warranted. All GLMM and GLM models were fitted with a "poisson" distribution given that count data was used as the input for all statistical analyses (Warton 2022). Models with the lowest AIC values were evaluated for overdispersion and heteroscedasticity using DHARMa (Hartig 2019), and models that displayed no significant dispersion or heteroscedasticity were retained (Warton 2022). Redundancy Discriminant Analysis (RDA) was also performed on the Moore Reef dataset, following a Hellinger transformation, to explore the effect of year and season on community composition and species occurrence (Borcard et al. 2011). Plots were also created in R using the packages phyloseq (McMurdie and Holmes 2013) and ggplot2 (Wickham 2009).

Results

Library preparation and sequencing

A total of 18,537,106 paired-end reads were sequenced across all samples, from which approximately 900 unique OTUs were identified (eukaryotes and prokaryotes). We found variation in community composition and read counts in both biological and technical replicates. This variability is exemplified by the variation in read counts between biological replicates (SI: Figs. 1a and 3) and among technical (PCR) replicates (SI: Fig. 1b). Stringent filtering of both datasets was conducted to minimise the effect of amplification/sequencing bias (SI: Figs. 2 and 4). The filtered dataset used in our analyses identified 14 marine eukaryote phyla, including the phylum Echinodermata. Echinoderm OTUs were identified from 23 genera across all five extant classes (Asteroidea, Crinoidea, Echinoidea, Holothuroidea and Ophiuroidea) of the phylum. Echinoderm species detected are all documented as being present on the GBR (SI: Table1).

Analyses of larval spatio-temporal variation

Plankton samples collected at Moore Reef between 2015-2020 were dominated by meroplankton and holoplankton of the Phyla Arthropoda, Chaetognatha and Mollusca (Fig. 2a). Echinoderms comprised between one and eight percent of OTUs identified in plankton samples obtained from Moore Reef (Fig. 2a). Overall eukaryote richness and echinoderm richness followed a similar temporal pattern, with the lowest overall richness for eukaryotes observed in December 2015, June 2016, and January 2017 (Fig. 2a).

Average echinoderm richness was greatest in Autumn (March – May), and lowest in Winter (June – August), with the highest peak observed in January 2018 and the lowest peaks observed in June 2017 and August 2017 (Fig. 2b). Indeed, echinoderm richness was found to be significantly correlated with season and year (SI: Table 2). The samples from Moore Reef were dominated by echinoderms of the class Ophiuroidea (Fig. 2b). RDA analyses indicate that community assemblage is most strongly associated with inter-annual variation (rda, variance = 3.00, F = 2.10, p-value = 0.02), rather than seasonal variation (SI: Table 3). However, the RDA axes explained a small amount of the variation (axis 1: 17.89%, axis 2: 9.67%) in the dataset and should be interpreted with caution.

Samples collected along the latitudinal transect in the years 2017-2019 were dominated by taxa belonging to the phyla Arthropoda, Chordata and Cnidaria (Fig. 4a). Echinoderms

comprised a small proportion of most samples (0-8%) but in a few rare instances they dominated samples e.g., Eddy Reef in 2019 (80%) and Hall-Thompson Reef in 2019 (~50%). Echinoderms were detected in all years, although they were most abundant in 2019 and scarcest in 2017 (Figs. 4b, 5a and 5b). Pairwise "Tukey" comparisons revealed that echinoderm richness was significantly different across the three years studied. However, richness did not differ significantly among the sites sampled, and a clear latitudinal or regional pattern was not detected in this dataset.

Several reads were attributed to the species *Acanthaster* cf. *solaris* (Fig. 3a) and were assigned to eight samples from Moore Reef (Fig. 3a), across the years 2016-2020. Most detections (75%) occurred between the months November-March, however, we also detected CoTS DNA in June 2016 and August 2016 (Fig. 3a). *Acanthaster* cf. *solaris* was detected in 13 samples along the latitudinal transect across the years 2017-2019 (Fig. 5a). Most detections occurred in 2019 (61%) and *Acanthaster* cf. *solaris* were detected in two years at Gibson reef (2017, 2019), Sudbury reef (2018, 2019), and Undine reef (2018, 2019).

Discussion

In this first study of GBR plankton using metabarcoding, we demonstrate that universal CO1 primers can characterise echinoderm larvae present in plankton samples. Here, the primers developed by Leray et al. (2013) exhibited broad taxonomic coverage, recovering 31 genera of the phylum Echinodermata. The results obtained in this study provide empirical evidence for significant seasonal and inter-annual variation in the occurrence of echinoderm larvae. However, we did not find evidence of latitudinal or site-specific differences in echinoderm larval composition among our samples. We also demonstrated that metabarcoding can be used to provide estimates of pest-species occurrence, such as *Acanthaster* cf. *solaris*.

Spatial and temporal variation in echinoderm larval occurrence on the GBR

The first aim of this study was to determine the temporal stochasticity in echinoderm larval occurrence at Moore Reef. Most taxa observed in this study exhibited seasonal fluctuations in occurrence, and the Echinodermata displayed similar patterns to other Phyla such as Arthropoda and Mollusca (Fig. 2a). Peaks in echinoderm larval richness were observed in March and December in this study (Fig. 2b). Correspondingly, echinoderm larvae are usually only present in the zooplankton community following broadcast spawning events, which typically occur in the summer period on the GBR (December – February; McEdward and

318 Miner 2001). Similar seasonal fluctuations in zooplankton richness have also been observed
319 for other taxa such as fish and crustaceans (Stoeckle et al. 2017; Sigsgaard et al. 2017; Berry
320 et al. 2019) and are also shown in this study (Fig. 2a). This is potentially because summer
321 environmental conditions such as temperature and salinity facilitate gamete production
322 (Mercier and Hamel 2010; Uthicke et al. 2015b), while increased phytoplankton availability
323 improves planktotrophic larval survivability (Uthicke et al. 2018b).
324
325 We also found a significant inter-annual difference in echinoderm community composition at
326 Moore Reef (Figs. 2b and 3). *Acanthaster* cf. *solaris* and *Linckia laevigata* were the most
327 frequently detected species (Fig. 3a), which suggests they might be some of the most
328 common species at Moore Reef. Larvae of the genera *Koehleraster* and *Acanthaster* were
329 detected in Winter (June – August) (Fig. 3b) which indicates these groups are potentially less
330 reliant on warmer temperatures as a cue for spawning. However, there is evidence that
331 spawning in echinoderms is complex and correlated with multiple confounding
332 environmental variables (Pearse 1968; Babcock et al. 2011; Bouwmeester et al. 2016;
333 Caballes and Pratchett 2017). There is evidently strong interspecific variation in spawning
334 periodicity among echinoderms which has yet to be thoroughly documented on the GBR
335 (Babcock et al. 1992). Nevertheless, we show that metabarcoding is a valuable tool to capture
336 temporal larval patterns and dynamics.
337
338 The temporal patterns observed in the latitudinal transect dataset corroborated those found in
339 the Moore Reef dataset. A significant inter-annual difference in echinoderm richness was
340 observed across the latitudinal transect samples (Fig. 4b). Richness was significantly greater
341 in 2019 than in 2017 or 2018 which could have resulted from increased larval output, larval
342 survival, or interspecific spawning synchrony. This result indicates that conditions were
343 optimal for spawning and larval survival at the surveyed reefs in 2019; and may explain
344 instances where echinoderms dominated sampled taxa e.g., Eddy Reef and Hall-Thompson
345 Reef (Fig. 4). However, it is also possible that sampling in 2019 coincidently overlapped with
346 a synchronous broadcast spawning event. Based on our results, there is greater variability in
347 the inter-annual occurrence of echinoderm larvae than other plankton (Fig. 4), which is not
348 surprising given evidence of unpredictable spawning behaviour in several echinoderm species
349 (Babcock et al. 2011). Any persistent inter-annual changes in the composition and abundance
350 of echinoderms would only be detected over a longer-term study given that they are long-
351 lived organisms and warrants further investigation (Berry et al. 2019).

The second aim of this study was to document the latitudinal occurrence of echinoderm larvae using the samples collected along a latitudinal transect on the GBR. We did not hypothesise a linear relationship between latitude and echinoderm larval occurrence given the lack of evidence for spatial structuring of adult assemblages in the region studied (ALA 2023). Correspondingly, latitudinal differences in echinoderm larval richness or community composition were not detected in this study, suggesting that the composition and distribution of echinoderm larvae does not differ significantly across the study region. The sites sampled were situated within the North-Central region of the GBR (De'ath et al. 2012) so significant differences in richness and assemblage may only be observed over larger spatial scales. Additionally, currents and constant mixing in the water column are likely to haphazardly disperse planktonic larvae (Trudnowska et al. 2015; Dean et al. 2015). Based on our methods alone, we cannot untangle widespread larval mixing from larval retention, which could lead to similar patterns in larval occurrence if adult populations are similar in taxonomic composition across sites. Scarcely any work has been conducted on the spatial distribution of echinoderm larvae on the GBR to date, so the results obtained herein provide a baseline for future studies. Continual monitoring of larval occurrences could improve projections of adult distributions and identify important larval sources which is pertinent to the management of populations (Doyle and Uthicke 2021).

CoTS detection using a DNA metabarcoding approach

A major aim of this study was to determine whether metabarcoding could be used to detect pest-species such as the crown-of-thorns sea star. We were able to detect *A.* cf. *solaris* larvae at Moore Reef during the summer months (Fig. 3a) which is when CoTS larvae are most often detected in the water column (Uthicke et al. 2019). This result provides further support for previous studies that have suggested this period is the spawning time for CoTS on the GBR (November to January peak; Pearson and Endean 1969; Babcock and Mundy 1992; Caballes and Pratchett 2017; Uthicke et al. 2019; Caballes et al. 2021). We also detected *A.* cf. *solaris* DNA in June 2016 and August 2016 (Fig. 3a) which was not expected. Our assay may have detected fragments of adult CoTS DNA (e.g., environmental DNA fragments from shedding), sperm, or infrequent but possible unseasonal spawning. In the latitudinal transect samples, CoTS larvae were detected most often in 2019 (Fig. 5a), which again indicates a substantial inter-annual variation in larval output, supporting the boom-and-bust characteristics of CoTS. CoTS larvae were also detected twice (over two years) at Gibson

Reef, Sudbury Reef, and Undine Reef, which highlights these reefs as important locations for future studies investigating sources of larval supply as potential targets for control measures (Fig. 5a).

In future, more targeted techniques (e.g., CoTS surveys or ddPCR) could be used to assess whether these reefs are key outbreaking locations or larval source/sink locations. It is worth mentioning however that increased replication and longer-term sampling in metabarcoding studies is essential to adequately capture trends in biodiversity and plankton dynamics (Lacoursière-Roussel et al. 2018; Berry et al. 2019). For example, in our study, more consistent sampling at Moore Reef identified more echinoderm taxa, compared to the latitudinal dataset. Additionally, universal markers are a reasonable choice for most studies, however, custom primers, or a multi-marker approach (Alberdi et al. 2018; Berry et al. 2019) may be required in instances when target taxa are poorly represented by universal primers. For example, primers that target echinoderms specifically, and result in species-specific taxonomic resolution would enable more thorough investigations of echinoderm biodiversity and larval occurrence. Nonetheless, we show that DNA metabarcoding approaches could be invaluable to future management programmes.

Technical variation in DNA metabarcoding studies

In this study, metabarcoding was found to be a useful method for detecting specific species, including pest species such as CoTS. However, like other studies that have quantified replicate variability, we found considerable variance amongst technical and biological replicates (Alberdi et al. 2018; Leray and Knowlton 2015). These findings are further evidence that careful study design that includes ample replication is crucial in metabarcoding studies, particularly if identifying naturally occurring variation is a primary goal. We used stringent filtering of replicates to reveal that, as expected, echinoderm larval richness exhibited significant seasonal stochasticity (Fig. 2b), which is likely to be related to the reproductive seasonality of adults.

Conclusions

Metabarcoding presents a cost-effective and rapid method of gathering information on species occurrence, particularly for cryptic life-stages. Echinoderm taxa identified herein provide a baseline reference for species diversity that can be used to evaluate future species loss, new biological invasions, and changes in community structure. This investigation

contributes to a growing number of studies suggesting that information on the intra and inter-annual variability of larval occurrence can be obtained over large spatial scales using metabarcoding approaches to provide insight into biological parameters such as spawning activity (Lacoursière-Roussel et al. 2018; Berry et al. 2019). Ecologically significant trends can also be identified in response to known environmental gradients which is critical to projecting changes in population density. Likewise, metabarcoding was found to be an efficient method of monitoring CoTS larval occurrence and has the potential to inform pest management efforts on the GBR. Specifically, information on the spatio-temporal occurrence of CoTS larvae can be used to complement and improve models of larval dispersal to identify major source reefs. As sequence databases expand and techniques improve, so too will the capability of metabarcoding to provide robust and comprehensive monitoring of entire ecosystems.

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Contributions

IB, IP, CR and SU conceived and designed the experiments; SU collected the plankton samples; IB, DB and IP designed and performed molecular work. IB and IP analysed the data and IB prepared figures and tables; IB wrote a first draft of the manuscript. All authors contributed to and approved the final version of the manuscript.

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Ethics declarations

Conflict of interest

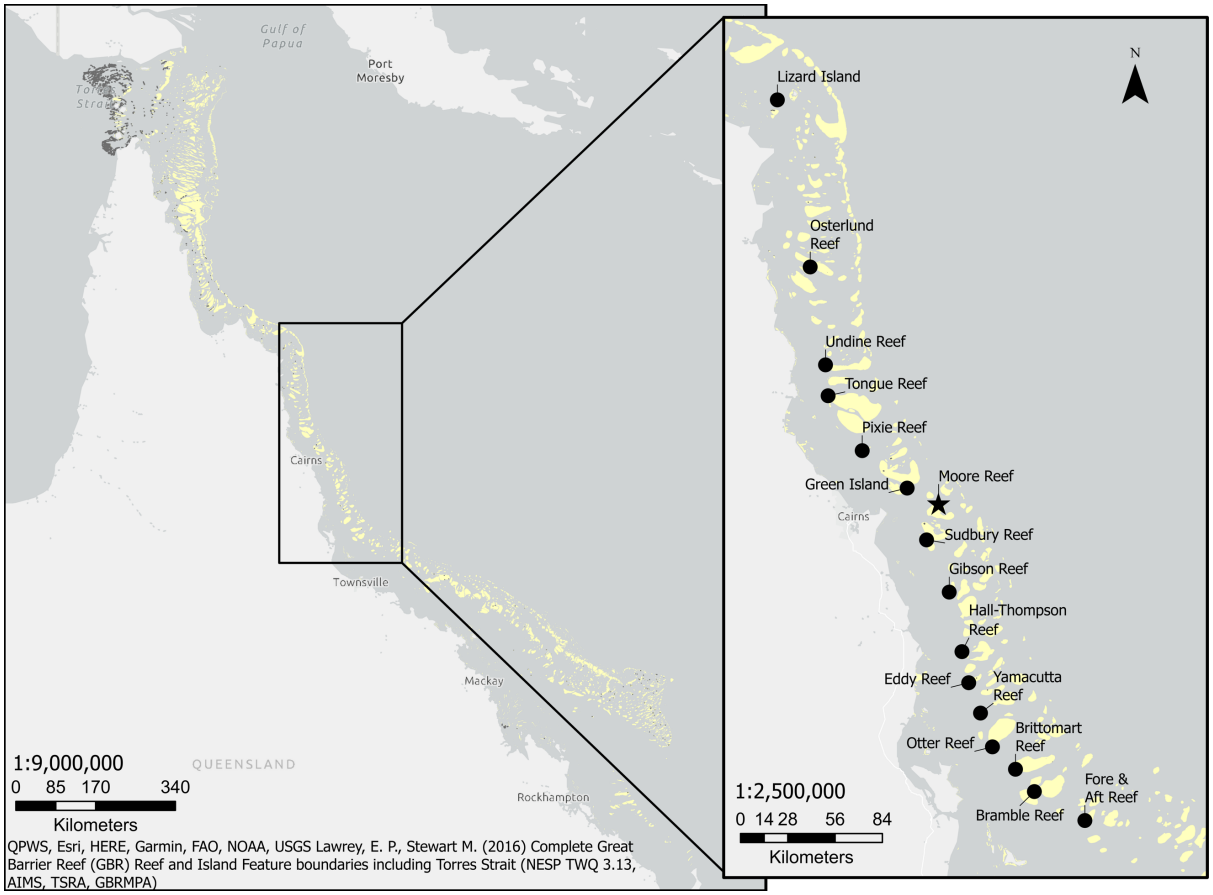
The authors declare no competing interests.

Ethical approval

Collections of plankton samples were approved by the Great Barrier Reef Marine Park Authority (Permit No. G38062.1). Ethical approval for invertebrates used in this study is not required under Australian legislation.

Data availability

Raw sequence reads, intermediate files, and code used to process reads, perform analyses and plot figures will be published in the following UQ eSpace repository: https://doi.org/10.48610/3076584.



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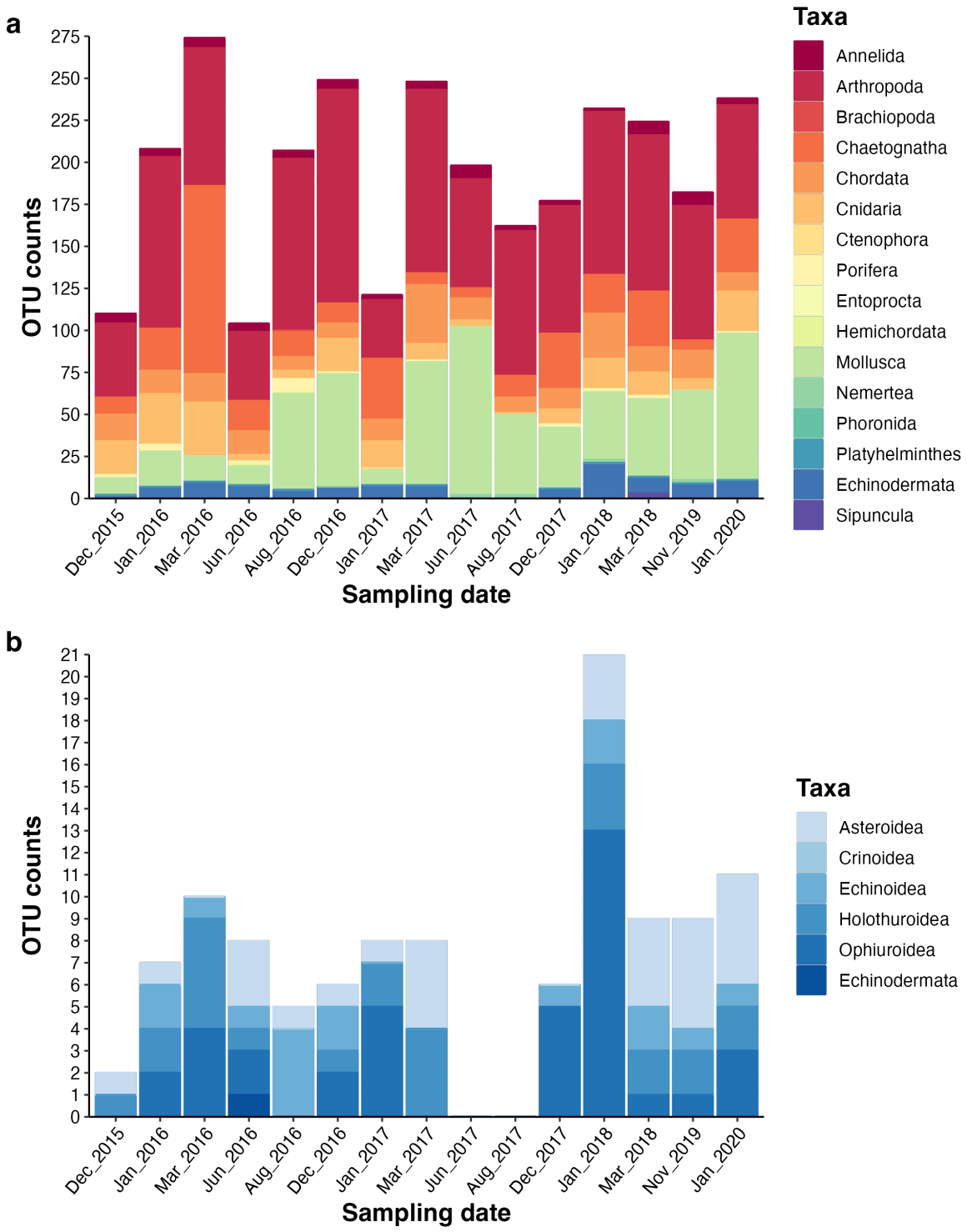
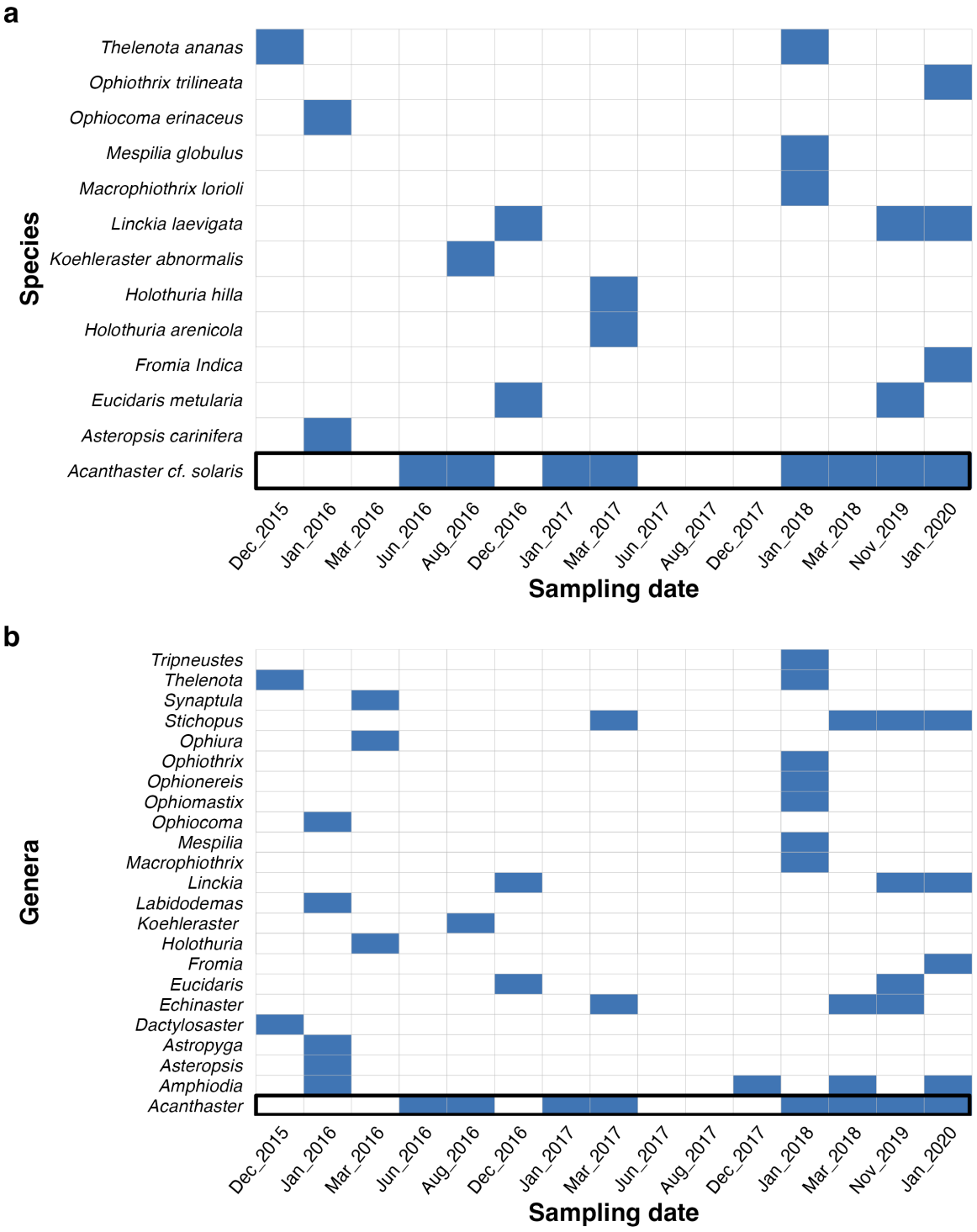

a
OTU counts
Sampling date
Dec_2015
Jan_2016
Mar_2016
Jun_2016
Aug_2016
Dec_2016
Jan_2017
Mar_2017
Jun_2017
Aug_2017
Dec_2017
Jan_2018
Mar_2018
Nov_2019
Jan_2020
Taxa
Annelida
Arthropoda
Brachiopoda
Chaetognatha
Chordata
Cnidaria
Ctenophora
Porifera
Entoprocta
Hemichordata
Mollusca
Nemertea
Phoronida
Platyhelminthes
Echinodermata
Sipuncula
b
OTU counts
Sampling date
Taxa
Asteroidea
Crinoidea
Echinoidea
Holothuroidea
Ophiuroidea
Echinodermata

697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Species
Thelenota ananas
Ophiothrix trilineata
Ophiocoma erinaceus
Mespilia globulus
Macrophiothrix lorioli
Linckia laevigata
Koehleraster abnormalis
Holothuria hilla
Holothuria arenicola
Fromia Indica
Eucidaris metularia
Asteropsis carinifera
Acanthaster cf. solaris
Dec_2015
Jan_2016
Mar_2016
Jun_2016
Aug_2016
Dec_2016
Jan_2017
Mar_2017
Jun_2017
Aug_2017
Dec_2017
Jan_2018
Mar_2018
Nov_2019
Jan_2020
Sampling date
b
Genera
Tripneustes
Thelenota
Synaptula
Stichopus
Ophiura
Ophiothrix
Ophionereis
Ophiomastix
Ophiocoma
Mespilia
Macrophiothrix
Linckia
Labidodemas
Koehleraster
Holothuria
Fromia
Eucidaris
Echinaster
Dactylosaster
Astropyga
Asteropsis
Amphiodia
Acanthaster
Dec_2015
Jan_2016
Mar_2016
Jun_2016
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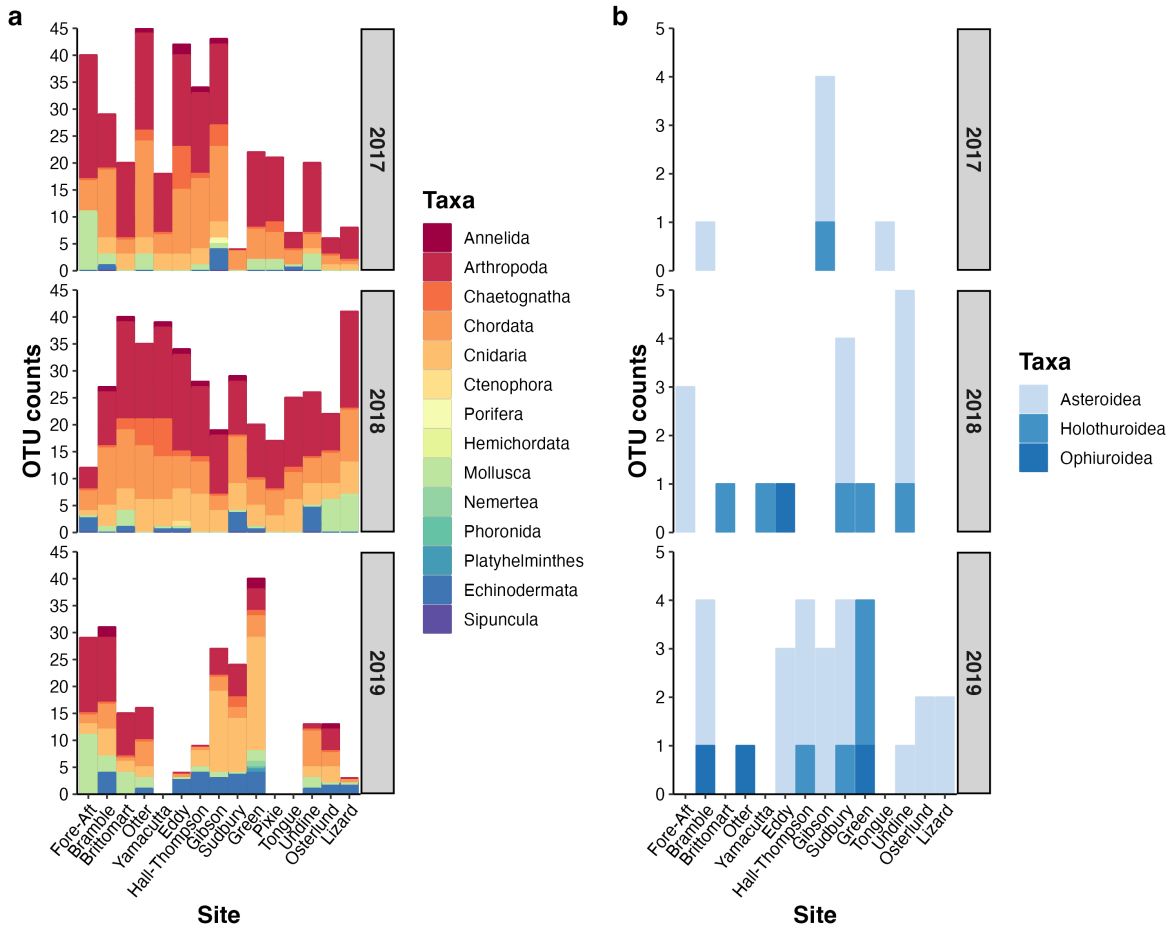
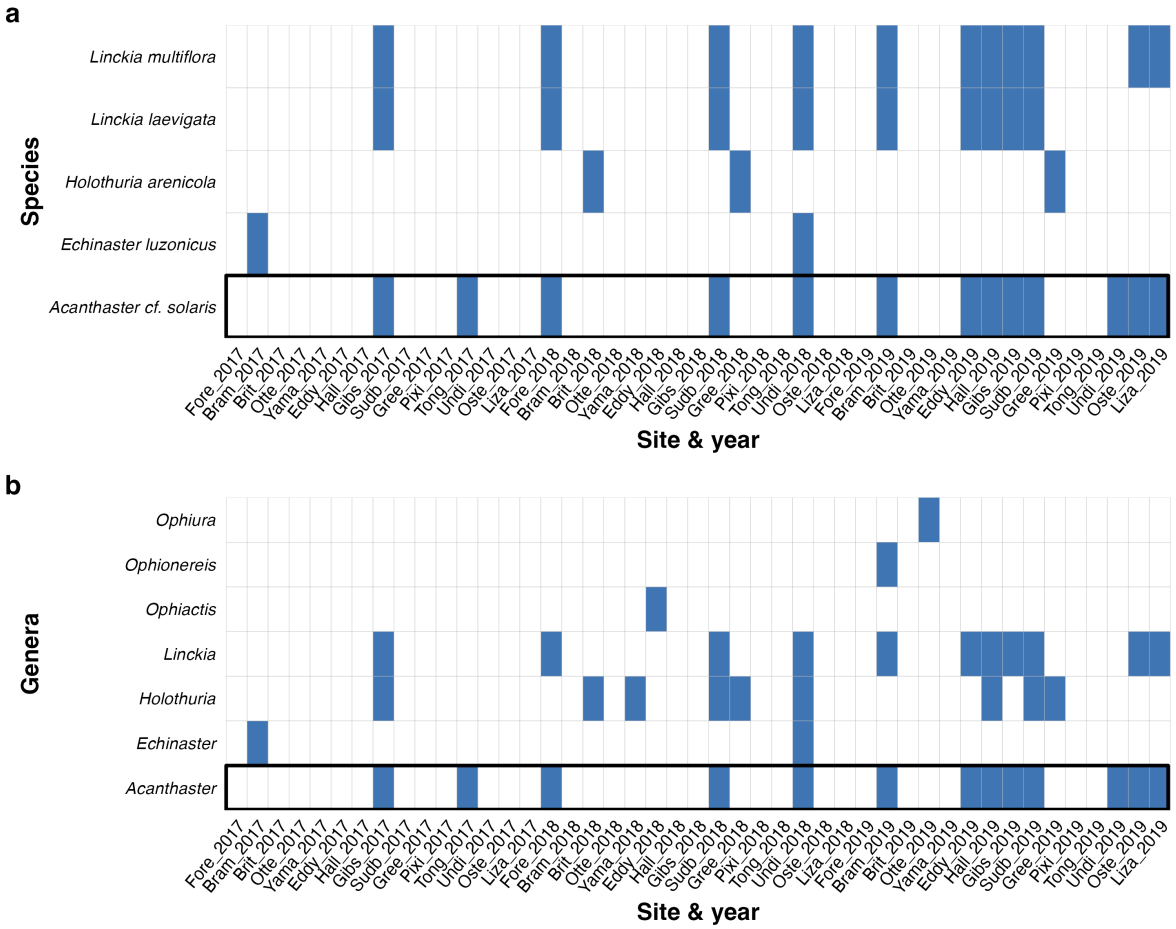
Mar_2018
Nov_2019
Jan_2020
Sampling date

698


a
b
OTU counts
Site
Taxa
Annelida
Arthropoda
Chaetognatha
Chordata
Cnidaria
Ctenophora
Porifera
Hemichordata
Mollusca
Nemertea
Phoronida
Platyhelminthes
Echinodermata
Sipuncula
Asteroidea
Holothuroidea
Ophiuroidea
2017
2018
2019
Fore-Aff
Bramble
Brittomart
Otter
Yamacutta
Eddy
Hall-Thompson
Gibson
Sudbury
Green
Pixie
Tongue
Urdine
Osterlund
Lizard

699


a
Species
Linckia multiflora
Linckia laevigata
Holothuria arenicola
Echinaster luzonicus
Acanthaster cf. solaris
Fore_2017
Bram_2017
Brit_2017
Otte_2017
Yama_2017
Eddy_2017
Hall_2017
Gibs_2017
Sudb_2017
Gree_2017
Pixi_2017
Tong_2017
Undi_2017
Oste_2017
Liza_2017
Fore_2018
Bram_2018
Brit_2018
Otte_2018
Yama_2018
Eddy_2018
Hall_2018
Gibs_2018
Sudb_2018
Gree_2018
Pixi_2018
Tong_2018
Undi_2018
Oste_2018
Liza_2018
Fore_2019
Bram_2019
Brit_2019
Otte_2019
Yama_2019
Eddy_2019
Hall_2019
Gibs_2019
Sudb_2019
Gree_2019
Pixi_2019
Tong_2019
Undi_2019
Oste_2019
Liza_2019
Site & year
b
Genera
Ophiura
Ophionereis
Ophiactis
Linckia
Holothuria
Echinaster
Acanthaster
Site & year

700

Fig. 1 A map indicating the sites from which plankton samples were collected by the Australian Institute of Marine Science (AIMS). Reef names indicate the reef closest to the location from which plankton tows were conducted. Samples were collected from Moore Reef across the years 2015-2020 in January, March, June, August, November, and December. In contrast, samples were collected along a latitudinal gradient (between Fore & Aft Reef and Lizard Island) only in December of the years 2017-2019. Samples from the two datasets were analysed separately in this study. The map was created using data collected by AIMS, TSRA Great Barrier Reef Marine Park Authority (Lawrey and Stewart 2016).

Fig. 2 OTU counts in samples collected at Moore Reef over the years 2015-2020. Counts in PCR replicates were combined in a restrictive additive manner (Alberdi et al. 2018). Counts in biological replicates (two per site) were combined to produce the results shown here. Counts for **a)** the main eukaryote phyla and **b)** Echinoderms are shown. In **b)** the colours on the stacked bars differentiate the five main echinoderm classes. All OTUs not identified to class level are categorised as "Echinodermata".

Fig. 3 Heatmaps showing the presence/absence of OTUs identified as Echinoderms in samples collected from Moore Reef. **a)** OTUs identified to the species level, with *Acanthaster* cf. *solaris* highlighted by a black rectangle. **b)** OTUs identified to genera level, with *Acanthaster* highlighted as a genus of interest.

Fig. 4 OTU counts in samples collected between the years 2017-2019 along a latitudinal gradient on the GBR. Counts in PCR replicates were combined in a restrictive additive manner (Alberdi et al. 2018). Counts in biological replicates (two per site) were combined to produce the results shown here. Counts for **a)** the main eukaryote phyla and **b)** Echinoderms are shown. In **b)** the colours on the stacked bars differentiate the three echinoderm classes detected.

Fig. 5 Heatmaps showing the presence/absence of reads identified as Echinoderms in samples collected along a latitudinal gradient. **a)** OTUs identified to the species level, with *Acanthaster* cf. *solaris* highlighted. **b)** OTUs identified to genera level, with *Acanthaster* highlighted as a genus of interest.