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Comparison of swab DNA extraction methods for examining sea star dermal microbiomes

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ABSTRACT: Marine invertebrates are surrounded by and interact with an array of microbes, yet their microbiomes remain largely unexplored. With a seemingly endless choice of nucleic acid extraction kits, there is a need to assess the compatibility across approaches to determine whether microbiome results are comparable across studies employing different extraction methods. In this study, 5 kits were compared for extracting DNA from dermal swabs from 2 sea star species: *Pisaster ochraceus* and *Dermasterias imbricata*. DNA yield varied by kit, as did the ease of PCR amplification. Using 16S rRNA amplicon sequencing, differences in microbial richness and diversity were observed between sea star species, but not among extraction kits. Relative abundances of the most abundant prokaryotic phyla were largely attributed to sea star species rather than kit: the *D. imbricata* microbiome was dominated by *Proteobacteria*, whereas *P. ochraceous* had more even representation of *Proteobacteria*, *Spirochaetota* and *Bacteroidota*. Our results suggest that, despite some differences in ease of amplification, all 5 extraction kits examined here provide comparable and suitable results for characterizing sea star dermal microbiomes.

KEY WORDS: Echinoderm · Amplicon sequencing · 16S rRNA gene

1. INTRODUCTION

Microbiomes, the collection of microorganisms residing on or within an animal, are known to influence the health, physiology, behavior, and ecology of their hosts (Apprill 2017). Recent microbiome research has focused on humans and captive animals, with limited research on wild organisms (Hird 2017). Despite being surrounded by a vast and diverse array of microbes, marine invertebrate microbiomes remain largely unexplored, with most research focused on corals, sponges and shellfish. Existing at this dynamic organism–water interface, dermal microbiomes play particularly important roles in pathogen protection, and there is emerging evidence that some marine diseases result from microbial dysbiosis, or a disruption to an organism's microbiome (Egan & Gardiner 2016). Still, relatively little is known about the role of the host microbiome in the onset and progression of marine diseases, particularly for invertebrates.

Between 2013 and 2015, an outbreak of sea star wasting disease (SSWD) in the Northeast Pacific affected populations of at least 20 asteroid species and had cascading ecological impacts (Hewson et al. 2014, Montecino-Latorre et al. 2016). Although the causative agent and etiology of the disease remains elusive, shifts in microbial community richness and composition are associated with the onset and progression of the disease (Lloyd & Pespeni 2018, Aquino et al. 2021). Whether SSWD is initiated by a causative agent or not, a disruption of natural microbiomes could play a role in the onset and progres-

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sion of disease, or microbiomes could be altered in response to failing host biology as a result of disease.

Swabs are often used to sample dermal microbiomes, and many commercial kits are available for extracting DNA from swabs. The choice of extraction kit has been shown to influence the resulting microbial community profile (e.g. Bjerre et al. 2019, Pearman et al. 2020). Thus, obtaining unified methods to extract and characterize sea star dermal microbiomes is important not only to further investigate SSWD etiology, but also to better understand the ecology and physiology of echinoderms more generally (e.g. Jackson et al. 2018). Previous work with sea star microbiomes has used a range of different extraction kits and methods, complicating the ability to compare between studies (e.g. Jackson et al. 2018, Lloyd & Pespeni 2018, Aquino et al. 2021, Loudon et al. 2023). In the present study, DNA was extracted from dermal swabs obtained from 2 common intertidal sea star species (*Dermasterias imbricata* and *Pisaster ochraceus*) using 5 DNA extraction kits frequently used for medical and environmental studies. Parameters related to extraction success (DNA yield, ease of PCR amplification) were compared, as well as microbial richness, diversity and composition.

2. MATERIALS AND METHODS

2.1. Sample collection and DNA extraction

Dermal swabs were collected from 11 asymptomatic sea stars (n = 8 *Pisaster ochraceus* and n = 3 *Dermasterias imbricata*) in Heriot Bay, British Columbia, Canada (50.1153° N, 125.2205° W). Individuals were rinsed with 0.22 µm filtered seawater and a sterile swab (Puritan Medical Products) was rolled along the aboral surface of each arm for ~10 s, resulting in 5 swabs per individual, 1 swab per arm for each extraction kit. Swabs were placed in cryovials and stored at −70°C at the Hakai Institute's Quadra Island Observatory until extraction. DNA was extracted using 5 kits, with minor modifications made to manufacturer's protocols (Table 1). An extraction negative, consisting of a new swab, was also included, for a total of 12 swabs per kit. DNA was quantified using a Qubit Fluorometer (Invitrogen) and samples were normalized to 2.5 ng µl^{-1} using nuclease free water, or when the concentration was less than 2.5 ng µl^{-1}, undiluted DNA was used for PCR reactions.

2.2. Library preparation and bioinformatic processing

The V4 region of the 16S rRNA gene was targeted for microbial community profiling using the primer pair 515F-Y (Parada et al. 2016) and 806RB (Apprill et al. 2015) with a fusion primer-based approach (Kozich et al. 2013), as detailed in Text S1 in the Supplement at www.int-res.com/articles/suppl/a089p119_supp.pdf). Successful triplicate PCR reactions were pooled, purified using SPRI paramagnetic beads (Beckman Coulter) and quantified using a Quant-iT™ dsDNA Assay Kit. Samples and negatives were pooled in equimolar amounts (7.5 ng DNA per sample) and sequenced using an Illumina MiSeq V3 600-cycle kit. A

Table 1. Modifications made to manufacturer protocols for each extraction kit

Extraction kit	Abbreviation	Modifications	Elution vol. (µl)
Qiagen DNeasy Blood & Tissue	Q.BT	Increased volumes of Buffers ATL and AL (QIAGEN) to 500 µl and volume of 100% ethanol to 250 µl	100
Qiagen DNeasy Blood & Tissue +	Q.BTQ	Added extra step using QIAshredder spin column QIAshredder tubes	100
Qiagen DNeasy PowerSoil Kit[a]	Q.PSO	TissueLyser used for bead beating (5 min at 25 Hz, reorient tubes, 5 min at 25 Hz)	50
Qiagen DNeasy PowerSoil Pro	Q.PSP	TissueLyser used for bead beating (5 min at 25 Hz, reorient tubes, 5 min at 25 Hz)	50
Zymo Research Quick-DNA Fungal/Bacterial Microprep	ZYMO	Added dithiothreitol (final concentration of 10 mM) to the Genomic Lysis Buffer in place of beta-mercaptoethanol Added swab directly into ZR BashingBead™ Lysis Tube TissueLyser used for bead beating (5 min at 25 Hz, reorient tubes, 5 min at 25 Hz)	20

[a]Kit discontinued

series of troubleshooting steps were carried out for samples that did not initially amplify (see Text S1). In addition to DNA yield, amplification success was used as a metric for evaluating kits.

Sequences were quality controlled and denoised using the cutadapt and dada2 plugins in QIIME2 (Bolyen et al. 2019) and classified using the naïve Bayes classifier (Bokulich et al. 2018) trained to the Silva database version 138. Detailed bioinformatic methods are available on GitHub (https://github.com/hakaigenomics/seastar-swab-extmethods) and in Text S1. Potential contaminant amplicon sequence variants (ASVs) were identified and removed using the *decontam* package in R (Davis et al. 2018). Low read count samples (<9000 reads per sample post-decontamination) were also removed, leaving 46 samples containing 2921 ASVs, with a mean read count of 287 362 reads per sample. The 16S rRNA gene sequence data are available in the NCBI Sequence Read Archive under BioProject number PRJNA839850.

2.3. Statistical analyses

All visualizations and statistical tests were conducted in R version 4.2.2 (R Core Team 2022). A significance value of 0.05 was used for all statistical tests. Richness was calculated using the *breakaway* package in R (Willis & Bunge 2015), while alpha diversity (Shannon Index) was calculated using the *DivNet* package (Willis & Martin 2022). Differences in richness and Shannon diversity due to extraction kit and sea star species were determined using repeated measures analysis of variance (ANOVA) models or *t*-tests in the *rstatix* package (Kassambara 2022). When ANOVA and *t*-test assumptions were not met, we used the non-parametric Skillings–Mack and Wilcoxon tests in place of ANOVAs and *t*-tests, respectively (Table S1). Permutational multivariate analysis of variance (PERMANOVA) in the *vegan* R package was used to test for differences in community composition among kits and between species (Oksanen et al. 2022), while principal coordinate analysis (PCoA) ordination plots in the *phyloseq* R package (McMurdie & Holmes 2013) and heat maps in the *ampvis2* R package (Anderson et al. preprint https://doi.org/10.1101/299537) were used to visualize these differences. PERMANOVA and PCoA used Bray-Curtis dissimilarity on ASV abundances normalized to the total reads per sample (i.e. proportions).

3. RESULTS

3.1. DNA extraction and amplification

The 5 extraction kits had different DNA yields, with higher, more variable yields from the Q.PSO and Q.PSP kits, and lower, more consistent yields from the ZYMO and 2 Q.BT kits (range: 6 to 3000 ng; average 401 ng. Fig. 1A). These results align with other studies that also found variable DNA yields among extraction kits (e.g. Pearman et al. 2020). Ease of PCR amplification varied among kits, with the ZYMO kit having the highest success rate (91% amplified without troubleshooting) and the Q.PSO the lowest success rate (55%, Fig. 1B). All samples except one (*Dermasterias imbricata* swab 3 with the PowerSoil Kit) were successfully amplified either in the first PCR or after some degree of troubleshooting, but not all were retained after sequencing due to low read counts.

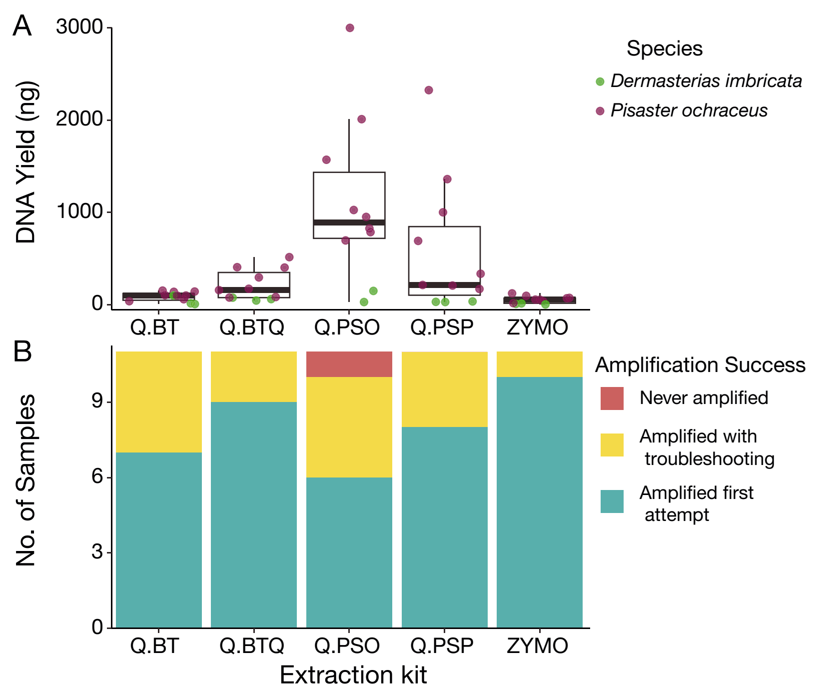

Fig. 1. (A) DNA yields obtained from each extraction kit. Box plots show median value (dark black line), interquartile range (box) and minimum/maximum value (vertical lines). (B) Proportion of samples successfully amplified in the initial PCRs and after troubleshooting for each kit. See Table 1 for definition of kit names

Interestingly, higher DNA yields did not confer greater amplification success. In fact, the kit yielding, on average, the most DNA (Q.PSO) required the greatest degree of troubleshooting.

3.2. Richness and diversity among kits

Estimates of richness ranged from 577 ASVs (Q.PSP), to 861 ASVs (ZYMO), with an average of 724 ± 66 (±SE) ASVs across all kits. There were no significant differences among the 5 extraction kits in terms of ASV richness with both species combined (Table S1, Fig. 2A), nor within *D. imbricata* or *Pisaster ochraceus* (Table S1). However, *P. ochraceus* had significantly higher ASV richness than *D. imbricata*, with an average of 823 (±77 SE) and 477 (±108 SE) ASVs, respectively (Fig. S1A). There were no significant differences in Shannon diversity among the 5 kits with both species combined, nor within *D. imbricata* or *P. ochraceus* (Table S1, Fig. 2B) Similar to richness, *P. ochraceus* had significantly higher microbial diversity than *D. imbricata* Fig. S1B).

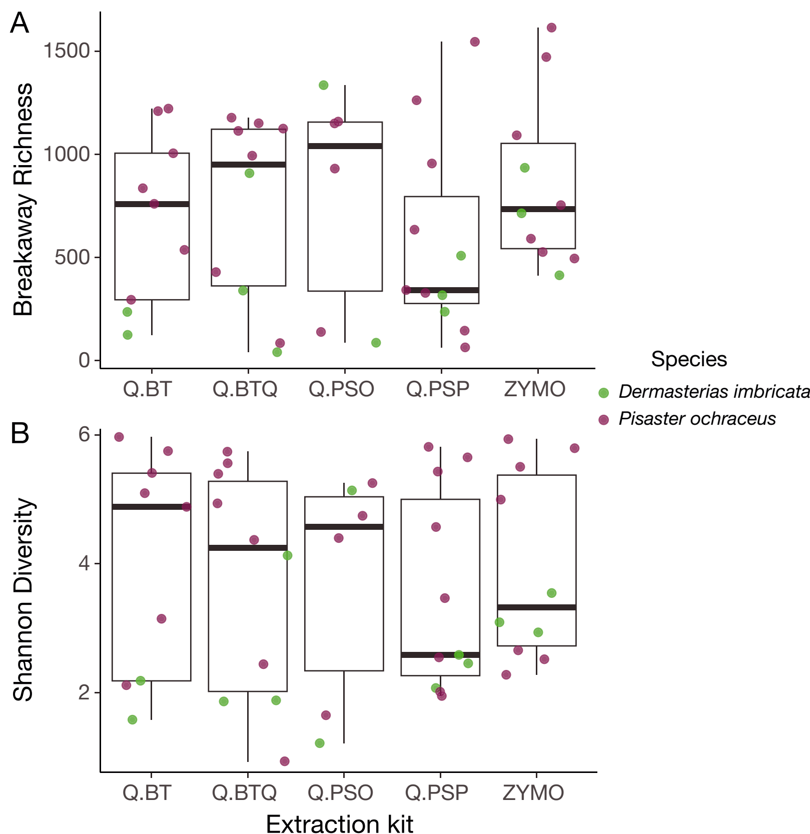

Fig. 2. Median estimates of (A) richness and (B) Shannon diversity for each extraction kit, with sea star species indicated by color. Box plots show median values (dark black line), interquartile range (box) and minimum/maximum values (vertical lines)

3.3. Microbial community composition

Microbiome samples clustered by sea star species rather than extraction kit (Fig. 3A) and there was no obvious grouping by extraction kit within each species. Samples also generally clustered by individual specimen (Fig. 3B). Dispersions were homogeneous among the 5 extraction kits (betadisper, p = 0.96), and a PERMANOVA revealed a significant effect of sea star species (p = 0.001) on beta diversity, but no significant effect of extraction kit (p = 0.85), nor any interaction between extraction kit and species (p = 0.95).

While broad trends in relative abundances of the top 12 phyla were consistent among kits, certain phyla had notably different abundances (Fig. 4). For example, *D. imbricata* swabs from the Q.PSO kit had a greater proportion of *Cyanobacteria* and *Bacteroidota* (Fig. 4A), while *P. ochraceus* from the Q.PSO kit had higher proportions of *Campylobacterota* (Fig. 4B). For the phylum *Bdellovibrionota*, which was found almost exclusively on *D. imbricata*, there was a relatively higher proportion detected in samples extracted by the Q.BT and Q.PSP kits (23.2 and 15.4%, respectively), compared with only 2.7% detected using the Q.PSO kit (Fig. 4A).

Microbiome compositions between the 2 sea star species were also notably different (Fig. 4). While the dermal microbiome of *D. imbricata* was dominated largely by *Proteobacteria* (ranging from 72 to 80% of read counts), the *P. ochraceus* microbiome had more even representation of *Spirochaetota* (average 42.8%), *Proteobacteria* (average 25.1%) and *Bacteroidota* (average 17%). There was a particularly large difference in the relative abundance of *Spirochaetota* between the 2 sea star species, with much greater abundances on *P. ochraceus* (average of 42.8% across all kits) compared with *D. imbricata* (average of 0.3%).

4. DISCUSSION

Five DNA extraction kits were compared in terms of their ability to characterize sea star dermal microbiomes. All kits yielded measurable DNA from

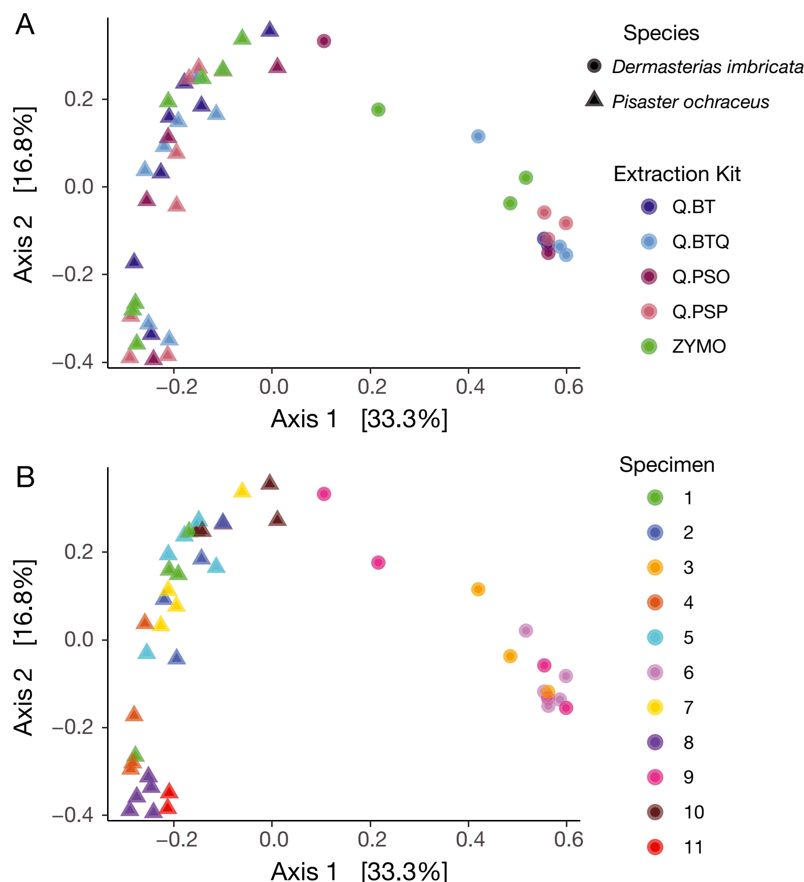

Fig. 3. Principal coordinate analysis (PCoA), based on Bray-Curtis dissimilarity index, showing each swab with (A) extraction kit and (B) specimen, with species indicated by shape

swabs and most samples were successfully amplified, with varying amounts of troubleshooting. No significant differences in richness or diversity were found among the 5 extraction kits for *Pisaster ochraceus* or *Dermasterias imbricata*. Instead, most of the observed variability in richness and diversity was attributed to the 2 sea star species, as well as some inter-specimen variability (Fig. 3). These results suggest that any of the 5 kits tested here are likely appropriate options for detecting ecological variability and patterns in sea star microbiomes. These findings align with previous studies that also found minimal or no effect of kit on microbial richness and diversity (e.g. Evans et al. 2018). However, other studies have detected differences in microbial community profiles based on extraction kit (e.g. Pearman et al. 2020) and thus choice of kit remains an important consideration in microbiome studies and may require optimization for a specific sample type.

While it is not possible to determine the most accurate results in terms of richness, diversity and community composition without the use of mock communities, the 5 kits were evaluated to see if they produced comparable results. The ZYMO kit detected the highest number of ASVs, but did not have the greatest Shannon diversity (Fig. 2). The Q.PSP kit had noticeably lower richness and diversity values compared with the other kits. However, all estimates of richness and diversity had quite high margins of error, due largely to the relatively small sample size and the distinct differences in richness and diversity between the 2 sea star species (Fig. S1). While it is somewhat arbitrary to determine which kit is best for examining dermal microbiomes, selection may depend on the research priorities and factors such as DNA yield, ease of amplification and cost per sample.

Extraction kit did not have a significant effect on beta diversity and dispersion was homogenous among all 5 kits (Fig. 3). In general, all kits revealed similar trends in relative abundances of prokaryotic phyla; however, some taxa had notably variable abundances among kits. For example, there was a wide range in the abundance of phylum *Bdellovibrionota* detected across the 5 kits, from a mean read percentage of 2.7% detected by the Q.PSO kit to 23.2% detected by Q.BT. Further, the Q.PSO kit detected a higher proportion of *Cyanobacteria* and, to a lesser extent, *Bacteroidota* on *D. imbricata* than the other 4 kits (Fig. 4).

While our primary goal was to compare among extraction kits, notable differences in prokaryotic diversity and composition were observed between 2 common intertidal sea star species: *P. ochraceous* and *D. imbricata*. Specifically, *D. imbricata* microbiomes had lower diversity and richness and were dominated by *Proteobacteria*, whereas *P. ochraceous* had more even representation from the top 3 most abundant phyla: *Proteobacteria*, *Spirochaetota* and *Bacteroidota*. While the 2 species had 2287 ASVs in common, there were 619 and 15 ASVs unique to *P. ochraceous* and *D. imbricata*, respectively. Evans et al. (2018) found similar results, with microbial communities clustering by tunicate species, but not extraction kit. These results also align with previous research that found high variability in microbial taxa among different sea star spe-

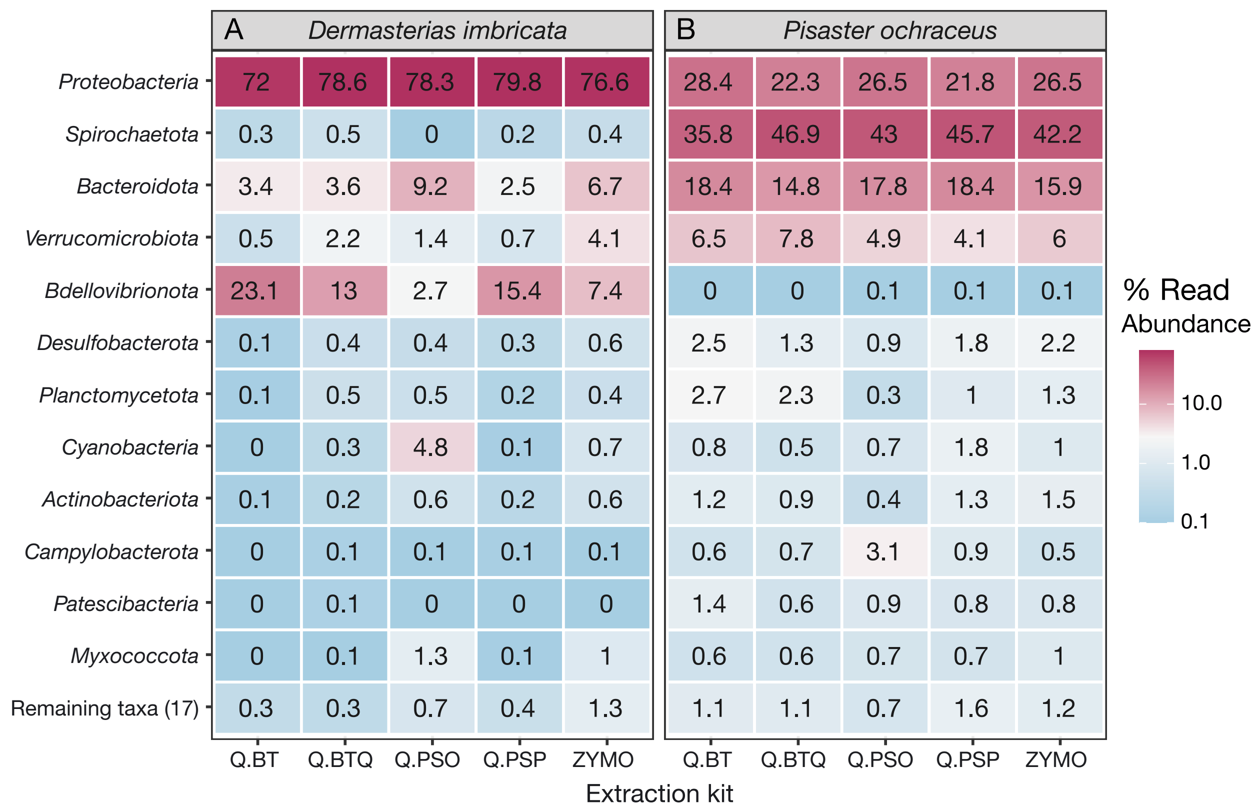

Fig. 4. Percentage read abundances of the top 12 prokaryotic phyla found on swabs from (A) *Dermasterias imbricata* and (B) *Pisaster ochraceous*

cies (Jackson et al. 2018), including the predominance of *Spirochaetota* on *P. ochraceus* (Lloyd & Pespeni 2018, Loudon et al. 2023). Even though phylosymbiosis is increasingly well-documented across animals (Mallott & Amato 2021), it is interesting to note the existence of seemingly different microbial communities on 2 sea star species that vary in their susceptibility to sea star wasting (Montecino-Latorre et al. 2016) — an observation that warrants continued investigation. Reliably characterizing sea star microbiomes is important in the context of marine diseases (e.g. sea star wasting disease) as well as understanding the microbial landscape of echinoderms and marine invertebrates more broadly.

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LITERATURE CITED

Apprill A (2017) Marine animal microbiomes: toward understanding host–microbiome interactions in a changing ocean. Front Mar Sci 4:222

Apprill A, McNally S, Parsons R, Weber L (2015) Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol 75:129–137

Aquino CA, Besemer RM, DeRito CM, Kocian J and others (2021) Evidence that microorganisms at the animal–water interface drive sea star wasting disease. Front Microbiol 11:610009

Bjerre RD, Hugerth LW, Boulund F, Seifert M, Johansen JD, Engstrand L (2019) Effects of sampling strategy and DNA extraction on human skin microbiome investigations. Sci Rep 9:17287

Bokulich NA, Kaehler BD, Rideout JR, Dillon M and others (2018) Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6:90

Bolyen E, Rideout JR, Dillon MR, Bokulich NA and others (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857

Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ (2018) Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome 6:226

Egan S, Gardiner M (2016) Microbial dysbiosis: rethinking disease in marine ecosystems. Front Microbiol 7:991

Evans JS, López-Legentil S, Erwin PM (2018) Comparing two common DNA extraction kits for the characterization

of symbiotic microbial communities from ascidian tissue. Microbes Environ 33(4):435–439

Hewson I, Button JB, Gudenkauf BM, Miner B and others (2014) Densovirus associated with sea-star wasting disease and mass mortality. Proc Natl Acad Sci USA 111: 17278–17283

Hird SM (2017) Evolutionary biology needs wild microbiomes. Front Microbiol 8:725

Jackson EW, Pepe-Ranney C, Debenport SJ, Buckley DH, Hewson I (2018) The microbial landscape of sea stars and the anatomical and interspecies variability of their microbiome. Front Microbiol 9:1829

Kassambara A (2022) rstatix: pipe-friendly framework for basic statistical tests. R package version 0.7.1. https://CRAN.R-project.org/package=rstatix

Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol 79: 5112–5120

Lloyd MM, Pespeni MH (2018) Microbiome shifts with onset and progression of Sea Star Wasting Disease revealed through time course sampling. Sci Rep 8:16476

Loudon AH, Park J, Wegener Parfrey L (2023) Identifying the core microbiome of the sea star *Pisaster ochraceus* in the context of sea star wasting disease. FEMS Microbiol Ecol 99:fiad005

Mallott EK, Amato KR (2021) Host specificity of the gut microbiome. Nat Rev Microbiol 19:639–653

McMurdie PJ, Holmes S (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLOS ONE 8:e61217

Montecino-Latorre D, Eisenlord ME, Turner M, Yoshioka R and others (2016) Devastating transboundary impacts of sea star wasting disease on subtidal asteroids. PLOS ONE 11:e0163190

Oksanen J, Simpson GL, Blanchet F, Kindt R and others (2022). vegan: community ecology package. R package version 2.6-4. https://CRAN.R-project.org/package=vegan

Parada AE, Needham DM, Fuhrman JA (2016) Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol 18: 1403–1414

Pearman JK, Keeley NB, Wood SA, Laroche O and others (2020) Comparing sediment DNA extraction methods for assessing organic enrichment associated with marine aquaculture. PeerJ 8:e10231

R Core Team (2022) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Willis AD, Bunge J (2015) Estimating diversity via frequency ratios. Biometrics 71(4):1042–1049

Willis AD, Martin BD (2022) Estimating diversity in networked ecological communities. Biostatistics 23(1): 207–222

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