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Coastal urbanization influences human pathogens and microdebris contamination in seafood

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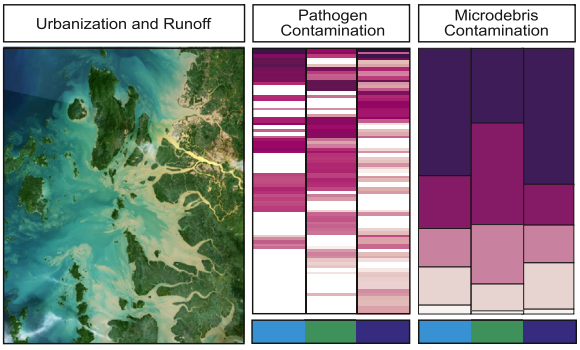
HIGHLIGHTS

- Coastal urbanization increases contamination in seafood.
- We assessed contaminants using next-generation sequencing and infrared spectroscopy.
- 5459 potential human bacterial pathogens belonging to 87 species were quantified.
- 78 different human-derived microdebris materials were identified.

GRAPHICAL ABSTRACT



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ABSTRACT

Seafood is one of the leading imported products implicated in foodborne outbreaks worldwide. Coastal marine environments are being increasingly subjected to reduced water quality from urbanization and leading to contamination of important fishery species. Given the importance of seafood exchanged as a global protein source, it is imperative to maintain seafood safety worldwide. To illustrate the potential health risks associated with urbanization in a coastal environment, we use next-generation high-throughput amplicon sequencing of the 16S ribosomal RNA gene combined with infrared spectroscopy to characterize and quantify a vast range of potential human bacterial pathogens and microdebris contaminants in seawater, sediment and an important oyster fishery along the Mergui Archipelago in Myanmar. Through the quantification of >1.25 million high-quality bacterial operational taxonomic unit (OTU) reads, we detected 5459 potential human bacterial pathogens belonging to 87 species that are commonly associated with gut microbiota and an indication of terrestrial runoff of human and agricultural waste. Oyster tissues contained 51% of all sequenced bacterial pathogens that are considered to be both detrimental and of emerging concern to human health. Using infrared spectroscopy, we examined a total of 1225 individual microdebris particles, from which we detected 78 different types of contaminant materials. The predominant microdebris contaminants recovered from oyster tissues included polymers (48%), followed

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by non-native minerals (20%), oils (14%) and milk supplement powders (14%). Emerging technologies provide novel insights into the impacts of coastal development on food security and risks to human and environmental health.

1. Introduction

Seafood continues to be one of the most-traded food commodities worldwide, yielding over 140 million tonnes available for human consumption and exceeding the combined trade value of sugar, maize, coffee, rice, and cocoa (Asche et al., 2015; FAO, 2016). The global supply of seafood for human consumption has exceeded population growth, increasing at an average annual rate of 3.2% between 1961 and 2013 (FAO, 2016). Given the importance of seafood exchanged as a global protein source, it is imperative to maintain seafood safety worldwide.

Rapid coastal development from urbanization has led to a global sanitization crisis, where 1.9 billion people lack treated wastewater and approximately 4.2 billion people use sanitation facilities and services that are not considered safely managed in ways that eliminate harmful contaminants (UNICEF and WHO, 2019). Wastewater carries numerous contaminants including persistent organic pollutants, nutrients, oils, radionuclides, heavy metals, pathogens, sediments, and debris that can affect the marine environment and associated organisms (Wear and Thurber, 2015). The quality of seafood following harvest is closely related to environmental conditions and microbial constituents of the water it originated from (Feldhusen, 2000). With more than half of seafood exports by value originating in developing countries (Asche et al., 2015), wastewater pollution due to coastal urbanization has the potential to impact food safety and security through contamination.

Seafood is one of the leading imported products implicated in foodborne outbreaks worldwide. For example, 542 seafood-related outbreaks and <5000 illnesses occurred from 2004 to 2013 in the U.S. alone (Center for Science in the Public Interest, 2015). Of these outbreaks, the majority were associated with bacteria (76%), followed by viruses (21%), and parasites (3%) (Iwamoto et al., 2010). Current monitoring protocols for human bacterial pathogens in seafood is not comprehensive, but rather targeted to specific infectious disease agents including *Escherichia coli*, *Listeria monocytogens, Clostridium botulinum, Staphylococcus aureus, Salmonella typhimurium*, *Vibrio vulnificus*, *Vibrio cholerae*, and *Vibrio parahaemolyticus* (DePaola et al., 2010; Elbashir et al., 2018; Yan et al., 2010). Few studies have examined a comprehensive suite of potential human microbial pathogens in current monitoring schemes. The recent development of high-throughput amplicon sequencing of the 16S ribosomal RNA gene allows for rapid identification of numerous human bacterial pathogens from seafood in environments suspected to be contaminated (Ibekwe et al., 2013; Lamb et al., 2017).

Microdebris contamination in seafood is an emerging risk to public health globally (Chan et al., 2019, Kroon et al., 2018a, Lusher et al., 2013, Rochman et al., 2015). Microdebris consist of any persistent, manufactured or processed solid material disposed of or abandoned in the marine and coastal environment (Sweet et al., 2019). In particular, microplastics detected in seafood species have become a growing concern to public health, with cases including fishes (Lusher et al., 2013),

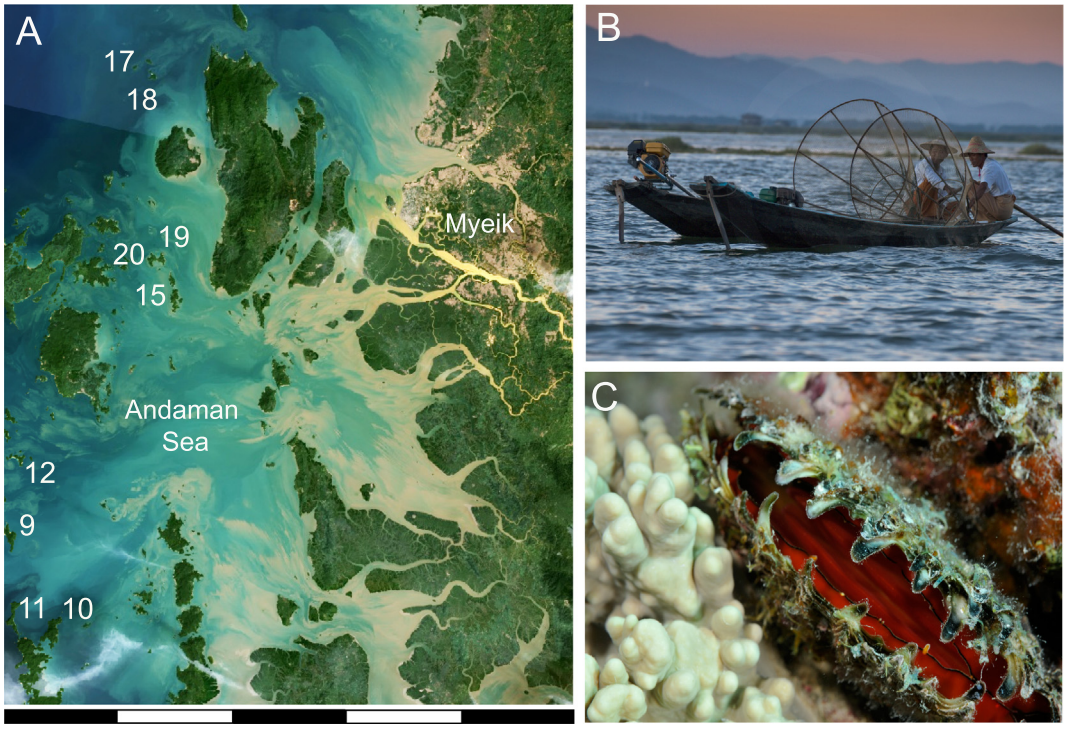

Fig. 1. Study sites spanning the Mergui Archipelago, Myanmar. (A) True color Landsat 8 mosaic image of the Tanintharyi River catchment located near the major city of Myeik in February 2017. Scale bar represents 90 km. The regional image is constructed using the Precipitation Estimation from Remotely Sensed Information using Artificial Neural Networks (PERSIANN) historic rainfall dataset. Turbid water scenes were identified by finding relatively cloud-free images in the days following large precipitation events. (B) Artisanal fishing is the main livelihood in the region, composed of an estimated 10,000 vessels. (C) Common pearl oyster (*Pinctada margaritifera*) an important coral reef fishery in the region, providing food and income. Images courtesy of Samuel Weber, Michelangelo Pignani, and Charles Stirling (Alamy). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

bivalves (Mathalon and Hill, 2014), and crustaceans (Murray and Cowie, 2011; Watts et al., 2014). Toxic harmful additives and compounds found in seawater can accumulate in plastic (Browne et al., 2013; Nobre et al., 2015), and subsequently enter the ocean and marine food webs (Avio et al., 2015; Bakir et al., 2012; Devriese et al., 2017). However, determining the health risks associated with the ingestion of marine microdebris is complicated by the diversity of materials in the environment (Kroon et al., 2018a). Non-plastic microdebris can be misidentified and incorrectly reported as plastic without proper analytical methods (Lusher et al., 2013; Neves et al., 2015), while many microdebris contaminants originally identified as plastics have been later quantified as anthropogenically derived, non-plastic materials (Kroon et al., 2018a; Li et al., 2016). Therefore, it is vital to accurately identify and report the entire suite of microdebris contaminants in order to improve the resolution of health risks associated with consuming contaminated seafood.

Emerging analytical technologies and techniques provide new opportunities to comprehensively identify human-derived microscale contaminants associated with coastal urbanization. Here, we combine next-generation sequencing with infrared spectroscopy that enable the extensive quantification of human-derived bacterial pathogens and microdebris contaminants in seawater, sediment and an important coral reef oyster fishery species along a rapidly developing region of the Mergui Archipelago in Myanmar.

2. Material and methods

2.1. Study location and sampling sites

This study was conducted in the Mergui Archipelago, a 3.4 million hectare area located along the western coast of the Tanintharyi Region of the Andaman Sea in Myanmar (Novak et al., 2009, Fig. 1). The archipelago consists of over 800 islands composed of small rock outcrops to large forested islands surrounded by fringing reefs. Although most islands are unpopulated, over the past 30 years, this region has become increasingly threatened by rapidly growing populations and development (Wongthong and True, 2015). The Mergui Archipelago is situated adjacent to Myeik, a city with a population of over 250,000 people. In this study, we assessed the influence of coastal urbanization on human pathogens and microparticle contamination in seafood using nine coral reefs islands spanning an 86 km gradient of the Mergui Archipelago (Fig. 1). There are currently no data available for waterborne contaminants in this region.

Artisanal fishing is the main livelihood in the Mergui Archipelago with an estimated 10,000 inshore vessels (Ramasamy, 2018). The common pearl oyster, *Pinctada margaritifera*, is an important coral reef fishery in the Mergui Archipelago and a known delicacy in areas of the world (Ramasamy, 2018). The oysters are widely cultured for the production of black pearls and their shell is used extensively in the ornament and button industry (Kimani and Mavuti, 2002). Filter feeding bivalves are continually exposed to ambient environmental conditions, therefore frequently used as bioindicators of contaminant accumulation in monitoring programs (Schwacke et al., 2013). For example, oysters have been shown to accrue numerous chemical contaminants, including toxic metals (Gao and Wang, 2014), arsenic (Hsiung and Huang, 2006) and persistent organic pollutants (Luna-Acosta et al., 2015). Here, we use the common pearl oyster as a bioindicator of microscale contamination due to its widely valued importance as a marine resource.

2.2. Human pathogen contamination

2.2.1. Collection and processing

Three replicate samples of seawater, sediment and oyster gill tissue were collected at each of the nine sites for analysis of human bacterial pathogens. We used gill tissue from oysters as this can be an entry point for pathogens into the body and the disturbance of the gill microbiome has shown to facilitate the establishment and growth of pathogens on the gills and may thus increase the susceptibility of organisms to disease (Llewellyn et al., 2014; Hess et al., 2015). Replicate 2 L seawater samples were collected from surface waters above each site and filtered through 0.22 μm polyethersulfone sterivex cartridges (EMD Millipore, Billerica, MA, USA) using sterile 50 mL polypropylene syringes. Excess seawater was forced from filters and subsequently filled with 1.0 mL of DNA Shield (Zymo Research, Irvine, CA, USA). Cartridges were capped after collection to prevent contamination. Both oyster and sediment samples were collected from three, 20-m haphazardly placed transects along the reef contour at a depth of 4 m ($N = 3$ samples per site, 1 sample from each transect). Oyster tissue samples weighing approximately 500 mg were removed from the gill of each individual using sterile dissection blades and forceps and placed in 1.5 mL of DNA Shield (Zymo Research, Irvine, CA, USA). Sediment samples weighing approximately 2 g were collected from surface sediments using 2.0 mL microcentrifuge tube (Corning, NY, USA) and placed in 1.5 mL of DNA Shield (Zymo Research, Irvine, CA, USA) following the removal of excess seawater.

2.2.2. 16S rRNA gene amplicon data processing

Samples were shipped to Zymo Research (Irvine, CA, USA) for DNA extraction using ZymoBIOMICS®-96 MagBead DNA Kit (Zymo Research, Irvine, CA) and 16S rRNA gene amplicon library preparation with the Quick-16S™ Primer Set V3-V4, a proprietary version of the 341F/785R primer set (see detailed methods in Supplemental Methods M.1). Libraries were 2 × 300 bp paired-end sequenced on an Illumina MiSeq platform. Sequence data was obtained from Zymo Research and processed using the UNOISE2 pipeline (Edgar, 2016) as implemented in the USEARCH package (version 9.2; https://www.drive5.com/usearch/) (Edgar, 2010). The raw forward (R1) and reverse (R2) sequence. fastq files of the 81 samples contained a total of 5,256,442 reads (ranging between 13,857 and 66,583 reads per sample). R1 and R2 paired reads were merged using -fastq_mergepairs. Primer sequences were trimmed using -fastx_truncate and reads were quality filtered with the -fastq_filter script, generating a filtered .fasta file containing 2,136,092 reads with an average length of 418 bp. Unique sequences were identified using the –fastx_uniques script followed by denoising of the sequence dataset with the UNOISE2 algorithm, obtaining 210,156 denoised sequences or 'zero-radius OTUs' (zOTU). The -usearch_global script was then used to generate an OTU (Operational Taxonomic Unit) table at the 100% similarity level. The taxonomy was assigned to each OTU (Operational Taxonomic Unit) based on the All-Species Living Tree Project (LTP) database extracted from the SILVA Small Subunit rRNA reference database (June 2018 release v132) using the -sintax algorithm with the confidence cut-off set at 0.8. The OTU table was converted to the HDF5 biom format and the taxonomic assignment metadata was added. Due to low sequencing depth (<105 reads), five oyster gill tissue samples were excluded from the OTU table (one sample each from sites 10, 12, 15, 19 and 20). No sites were excluded from the analysis. The final OTU table contained 1,252,170 reads belonging to 149,566 OTUs, with an average of 16,476 reads per sample (min 6700; max 33,049). The OTU table, sample metadata and representative sequences of each OTU are available on the Dryad Digital Repository, doi.org/10.7280/d13t1w. Raw sequence data files were deposited in the National Center for Biotechnology Information Sequence Read Archive under BioProject accession number PRJNA626079.

2.2.3. Data analyses

A comprehensive list of potentially pathogenic species of bacteria that lead to human illness were compiled from current online databases (see Supplemental Table A.1 for list and references) and corresponding OTUs were subset from the OTU table. The composition of the bacterial human pathogen community was calculated as the proportion of the

number of reads for each OTU normalized to the total numbers of reads for all OTUs identified as bacterial human pathogens. For compositional data analysis of the communities of human pathogens, we used recommendations and frameworks proposed by Aitchison (1982) and Gloor et al. (2017) accordingly: (1) human pathogens included in our analysis were identified in at least four samples regardless sample type (~5% prevalence overall). A Bayesian-multiplicative replacement was used to impute multinomial proportions under a Dirichlet prior distribution for zeros that appeared in the data set (function: *cmultRepl()* in package: *zCompositions*) (Palarea-Albaladejo and Martin-Fernandez, 2015). The resulting proportions were then transformed using a centered log-ratio transformation (function: *clr()* in package: *compositions*) (van den Boogart et al., 2019). (2) A distance matrix in Euclidean space (function: *vegdist()*; package: *vegan*) (Oksanen et al., 2019) was calculated on the transformed data to obtain an Aitchison distance matrix. The Aitchison distance matrix was then used to assess differences in bacterial human pathogen community among sample types using a permutational multivariate analysis of variance (perMANOVA; function: *adonis()* in package: *vegan*; (Oksanen et al., 2019). Site was included as a factor in the perMANOVA to account for study design and a principal coordinate analysis (PCoA; function: *cmdscale()* in package: *stats*) was performed to visualize the outcomes (R Team, 2019). A multivariate homogeneity of groups dispersion test (function: *betadisper()* in package: *vegan*) was used to determine if the results of the perMANOVA were solely driven by differences in group composition or by differences in group variances, followed by pairwise comparisons of all sample types using a Tukey's Honest Significant Differences test (function: *TukeyHSD()* in package: *stats*). To determine the dissimilarity between sample types, we calculated the Aitchison distance between the centroids of the different sample types (function: *dist_between_centroids()* in package: *usedist*) (Bittinger, 2019). Using the distances between centroids, we determined which sample types are least similar (greatest distance) as well as which sample type is intermediate between the other two sample types. We calculated the overlap in pathogen OTU identity to assess the potential for oysters to contain unique or overlapping pathogen OTU identity with both sediment and water samples.

A heatmap of the mean percent of total pathogens to assess conformity of composition among sample types for individual pathogenic species. Heatmaps were generated using the *ggplot2* package (Wickham, 2016) and the heatmap function in the *stats* package with dendrogram clustering following a Euclidean distance and complete hierarchical clustering (R Team, 2019). All analyses were performed using R v.3.6.3. Script and data are available on the Dryad Digital Repository, doi.org/10.7280/d13t1w.

2.3. Microdebris contamination

2.3.1. Collection

We collected three replicate samples of seawater, sediment and oyster tissue at a subset of four sites located at the most northern extent (sites 17 and 18) and most southern extent (sites 10 and 11) of the 86 km gradient spanning the Mergui Archipelago in this study (Fig. 1). Whole oyster tissue from three replicate oysters were removed from their shells using sterile dissection blades and forceps and placed in 40% formalin for preservation. Microdebris in sub-surface water samples were collected adjacent to reef sites using three replicate 5-minute tows from a plankton net (cod-end mesh size of 100 μm with radius net opening of 15 cm and radius net mesh size of 100 μm). The collected material was rinsed with filtered seawater into 50 mL polypropylene jars and preserved with 70% ethanol (Kroon et al., 2018a). Three replicate sediment samples were collected using a 25 cm^2 quadrat. Sediment was collected using a clean stainless-steel spoon from the top 3 cm of substrate surrounding each reef site and oven-dried for 1 week at 60 °C.

2.3.2. Laboratory

Separation of microdebris was achieved using a density separation technique to separate the lower density particles and fibers from the higher density planktonic material (Thompson et al., 2004; Hidalgo-Ruz et al., 2012; Hall et al., 2015). Plankton samples were poured into clean 250 mL glass graduated cylinders. A hypersaline solution (1.2 mg cm^{-3} NaCl) was added to each cylinder and stirred with a glass stirring rod for 30 s and allowed to rest for 1 h. The supernatant was transferred (via pouring) onto a 0.4 μm polycarbonate filter paper (47 mm) and vacuum filtered. The density separation and filtration steps were repeated three times per sample. Filters were oven dried for 24 h at 40 °C.

Dry sediments were sieved into the size fractions of <0.5 mm. Density separation was conducted using a hypersaline solution (1.2 mg cm^{-3} NaCl) in 500 mL glass graduated cylinders. Each cylinder was shaken for 30 s and allowed to rest for 1 h. The supernatant was transferred (via pouring) onto 0.4 μm polycarbonate filter paper (47 mm) and vacuum filtered. The density separation and filtration steps were repeated three times per sample. Multiple filters were used per replicate if required. Filters were oven dried for 24 h at 40 °C.

Oysters were placed in individual Teflon vessels and digested in 10% potassium hydroxide (KOH) at 40° for ~24–36 h (Tanaka and Takada, 2016; Zhao et al., 2016). Samples were filtered through a 64 μm stainless steel mesh sieve and the remnants were collected and stored in Milli-Q water until vacuum filtration through 0.4 μm polycarbonate filters. Filters were oven dried for 24 h at 40 °C.

2.3.3. Microscopy

Microdebris was identified and quantified using an adapted workflow developed by Kroon et al. (2018b). Each filter was visually inspected under a stereomicroscope (Leica M165C, 0.73×–12.0× magnification) to identify and separate potential microdebris contaminants. Suspected microdebris (both particles and fibers) were removed from each filter using metal needle nose forceps or a hooked microneedle and placed into the middle of concave glass slides and immediately covered with a flat glass cover slide to prevent contamination.

2.3.4. Spectroscopy and chemical assignment

Individual items were analyzed using attenuated total reflectance (ATR) Fourier-transform infrared spectroscopy (Perkin Elmer Spectrum 400 FT-IR Imaging System, USA) in transmission mode (64 scans, 4 cm^{-1} resolution, wavenumber range = 4000–600 cm^{-1}, atmospheric vapor compensation and CO_2/H_2O suppression). Background scans were conducted every tenth sample (Kroon et al., 2018b). Output spectra were compared to the Royal Melbourne Institute of Technology University Organics and Minerals FT-IR spectra database and the Hummel Polymer and Additives FT-IR Spectra Library. Each individual item obtained a percent match (best hit) between the sample and the top reference spectrum was obtained for each individual item, and particles or fibres with a chemical match <70% were excluded from further analysis (adapted from Kroon et al., 2018a, 2018b).

Based on chemical type and search reference spectrum description, each individual item was classified into the following three categories: (1) synthetic pollutants (including polymers, oils and other), (2) semi-synthetic pollutants (including milk supplements, oils and polymers), and (3) naturally occurring pollutants (including non-native minerals and other).

2.3.5. Sample contamination prevention

Steps to prevent contamination were taken during sample collection, processing and analyses (Woodall et al., 2015). All surfaces and tools were regularly cleaned with 70% ethanol, including in-between individual samples. All glassware, hand tools, Teflon vessels, filtration equipment and filter petri dishes were rinsed three times with Milli-Q water and openings were covered with aluminium foil prior to and during use. Air exposure of such equipment was kept to a minimum while

work was being conducted. The diamond cell component of the ATR was cleaned with methanol and lint-free tissue between each sample.

3. Results

3.1. Human pathogen contamination

Through the quantification of 1,252,170 high-quality bacterial operational taxonomic unit (OTU) reads, we detected 5459 human bacterial pathogen OTUs reads belonging to 87 species. A total of 67 species of potential human pathogens were detected in seawater samples (2027 reads, $N = 27$) and 60 species of potential human pathogens from sediment samples (2930 reads, $N = 27$, Fig. 2A). Half the number of bacterial human pathogen species found in the Mergui Archipelago accumulated in oyster gill tissues (502 reads, 44 different species, $N = 22$, Fig. 2A). Three human pathogenic species (*Paraburkholdia insula*, *Selenomonas noxia*, and *Eggerthella lenta*) were detected in oyster gill tissues (6.8% of human pathogens detected), but were not found elsewhere in the environmental samples (Fig. 2B). Potential human pathogens recovered from oyster gill tissues included nine species of bacteria associated with human and agricultural waste (20% of pathogenic species in oysters), including *Bacillus coagulans*, *Bacteroides caccae*, *Bacteroides ovatus*, *Bacteroides uniformis*, *Bacteroides vulgatus*, *Clostridium perfringens*, *Collinsella aerofaciens*, *Coxiella burnetii*, and *Finegoldia magna*. A full list of the human pathogens and their mean abundance found along the Mergui Archipelago can be found in Supplemental Table A.2.

Human bacterial pathogens (determined by species richness and abundance) found in oyster gill tissues are of intermediate composition between seawater and sediment samples (Fig. 2B). Further identification of human pathogens in all sample types showed that oyster gill tissues only contain three unique species and that 93% of detected species within oyster samples were also detected in sediment or seawater samples, allowing for oysters to be representative of seawater and sediment samples for human pathogen species richness. The composition of human pathogen communities showed significant differences among sites (perMANOVA: df = 8, F = 1.96, $R^2 = 0.15$, $p < 0.001$) and sample types (perMANOVA: df = 2, F = 10.81, $R^2 = 0.21$, $p < 0.001$). Subsequent analysis of group variances showed that there are significant differences in the dispersion between oyster gill tissues and seawater as well as seawater and sediment but not oysters and sediment (seawater and sediment: difference = −1.79, $p < 0.001$; seawater and oyster: difference = −1.65, $p < 0.001$; sediment and oyster: difference = 0.14, $p = 0.88$). For the PCoA, 19.2% and 9.0% of the variation in the composition of sample were explained by the principal component one and two respectively. Comparisons of the distance between centroids between sample sites showed that seawater and sediment were most dissimilar (distance between centroids = 7.2), oyster tissue and seawater had the least dissimilarity (distance between centroids = 5.3), and sediment and oyster tissue were intermediate (distance between centroids = 5.4). Therefore, oyster tissue human pathogen communities are of intermediate composition between seawater and sediment samples (Fig. 2A).

3.2. Microdebris contamination

We examined and analyzed a total of 1225 microdebris contaminants in samples of seawater, sediment and oyster tissues, from which 646 individual microdebris contaminants (52.7%) were identified above the >70% chemical match threshold. From the individual microdebris contaminants, we quantified 78 different types of material (Table 1). We found 199 individual microdebris contaminants in oyster tissue ($N = 12$ samples), 351 microdebris contaminants from seawater ($N = 12$ samples) and 96 microdebris contaminants from sediment ($N = 12$ samples) (Fig. 3A). We quantified 47 different microdebris contaminants in oyster tissue of which 34 (72.3%) were also found in the environmental samples (seawater and sediment) (Fig. 3A). We quantified 65 different microdebris contaminants in the environment samples, where almost half (47.7%, 31 different materials) were not found within oyster tissues (Fig. 3A). Overall, oyster tissue contained 30.8% of all individual microdebris contaminants and 60.2% of all contaminants analyzed from samples in the marine environment of the Mergui Archipelago in Myanmar.

The predominant microdebris contaminants recovered from oyster tissues (mean ± SE, $N = 12$ oyster samples) included polymers (48.3% ± 7.2%), followed by non-native minerals (19.7% ± 4.7%), oils (14.3% ± 2.9%) and milk supplement powders (14.4% ± 3.9%) (Fig. 3B). The complete list of microdebris recovered from the oyster tissues can be found in Table 1. Similar categorical patterns in microdebris were quantified in seawater and sediment (Fig. 3B).

4. Discussion

Using next-generation high-throughput amplicon sequencing in combination with infrared spectroscopy, we detected a comprehensive range of microscale constituents of wastewater characterized 87 different species of potential human bacterial pathogens and 646 human-derived microdebris contaminants from 78 different materials from seawater, sediment and an important oyster fishery along the Mergui Archipelago in Myanmar. The potential contaminants found in this study indicate that the Mergui Archipelago in Myanmar has wastewater pollution that can affect downstream food sources spanning over 80 km. Wastewater treatment in Southeast Asia is estimated at 14% (Evans et al., 2012) compared to 100% in North America or 99% in the United Kingdom (Baum et al., 2013). According to the World Health Organization (2019), diarrheal diseases caused by food poisoning and contaminated water resulted in 1.38 million deaths globally of which nearly half a million were children under the age of five (11,900 deaths across Myanmar in 2016).

4.1. Potential impacts of seafood contamination by human bacterial pathogens

Given that the vast majority of all environmental bacteria cannot be grown in the laboratory using standard protocols, where <1% are estimated to be cultivable (Vaz-Moreira et al., 2011), these next-generation techniques provide a culture-independent and explorative alternative to characterize the presence of bacteria that have been associated with risks to human health. Overall, this study presents a comprehensive characterization of 44 different species of potential human bacterial pathogens that can accumulate in seafood using amplicon sequencing of the 16S ribosomal RNA gene. We find that bacterial pathogens vary spatially within the marine environment. For instance, association with sediment enhances survival and persistence. Human bacterial pathogens have been shown to accumulate within sediments or on suspended particles in seawater and subsequently deposited, creating reservoirs of bacteria in the sediment that differ from the water column (Malham et al., 2014). Our results show that benthic coral reef shellfish shared 93% (41 out of 44) of detected human pathogens with sediment or seawater samples, therefore the presence of pathogens in oyster gills is likely to indicate broader environmental contamination.

The predominant bacterial pathogens detected in oyster gill tissues are common to gut microbiota and are frequently detected in water polluted by human and agricultural waste, particularly *Clostridium perfringens* (Li et al., 2015; Labbe and Juneja, 2017), *Collinsella aerofaciens* (Kageyama et al., 2000), *Corynebacterium tuberculostearicum* (Hinić et al., 2012) and *Coxiella burnetii* (Brooke et al., 2013). Seafood contamination by any of these potentially pathogenic bacteria could be detrimental to human health. For example, *C. perfringens* is the third most common cause of foodborne illness, resulting in nearly 4 million illnesses in low mortality countries (WHO, 2015) and economic losses exceeding $613 million per year in the U.S. alone (Scharff, 2018). Moreover,

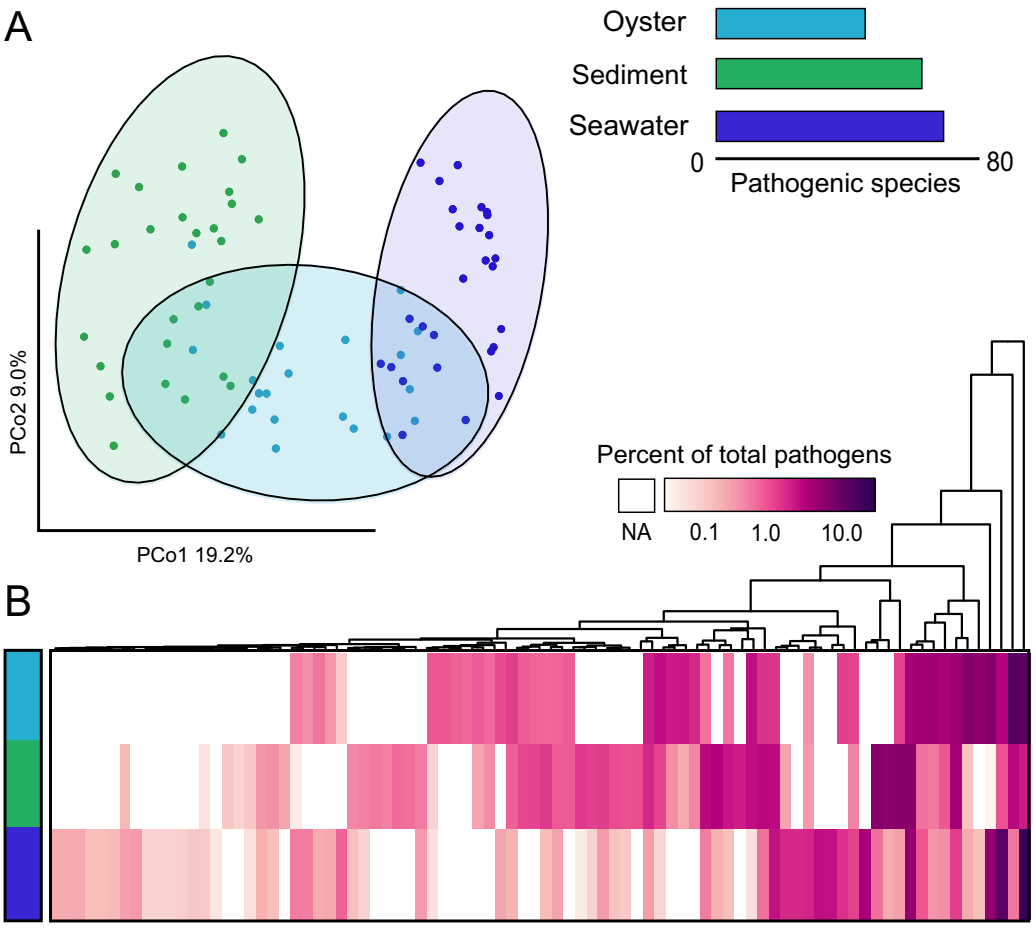

Fig. 2. (A) Spatial representation of the percent composition of human pathogen bacterial assemblages using a principal coordinate (PCo) analysis. The first two axes represent 19.2% and 9.0% of the variation in the composition respectively. Each point represents an individual sample. Bacterial species communities originated from seawater denoted in dark blue, sediment denoted in green and oyster gills denoted in light blue. Ellipses display 95% confidence intervals around the centroid for each sample type. (B) The heatmap illustrates the mean percent composition of bacterial pathogens (columns) of the total pathogenic bacteria quantified in oyster, seawater, and sediment samples (each row). Dendrograms represent hierarchical clustering based on similarities among species composition in samples using Euclidean distance. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

C. tuberculostearicum is a growing concern, as it is multidrug-resistant (Brooke et al., 2013). Recent predictions indicate that heightened drug resistance will result in over 300 million deaths annually and a cost of $100 trillion dollars to the global economy by 2050 (Review on Antimicrobial Resistance, 2016). Many infections from pathogenic bacteria require long-term and ongoing treatment, such as *C. aerofaciens*, which has been linked to inflammatory bowel syndromes such as Crohn's disease and ulcerative colitis (Swidsinski et al., 2002).

While this study did not quantify the level of the potential pathogens detected to determine their level of risk to human health when consuming the oysters, their presence is an indicator that the lack of wastewater treatment within the urbanizing Myanmar coastline is likely a significant source of contamination from a multitude of human pathogens. The extensive diversity of human pathogens that have been shown to accumulate in oysters also suggests that monitoring efforts should be expanded to assess the full risk of seafood consumption. Therefore, these results demonstrate the utility of emerging next-generation technologies. Since sequencing reads are aligned to open-access databases that are continually updated as new bacterial pathogens are archived, there is long-term value in collecting these data in order to monitor change in water quality associated with coastal urbanization.

4.2. Potential implications of microdebris contamination in oysters

The majority of the microdebris contaminants detected in marine sediment and seawater along the urbanized gradient of the Mergui Archipelago of coastal Myanmar were also quantified from oyster tissues, suggesting microdebris pollutants entering in the marine environment are similarly represented in important seafood species (see also Van Cauwenberghe and Janssen, 2014; Bour et al., 2018; Su et al., 2018). Bivalves provide greater sensitivity for detection of contamination because their high rates of filtration can result in the accumulation of particles at greater concentrations than in the ambient water with little metabolic transformation, making their tissues representative of environmental conditions (Schwacke et al., 2013) and ideal sentinels for assessing health risks of poor water quality, including pathogen and microdebris contaminants (Aguirre-Rubí et al., 2018; Jeamsripong et al., 2018; Ruiz-Fernández et al., 2018; Ward et al., 2019).

The predominance of polymer-based microdebris could have implications for both the environment and human health. For instance, harmful additives in plastic can leach into the water and into organisms that ingest plastic (Setälä et al., 2016). Moreover, hydrophobic pollutants in the environment accumulate within plastic particles (Avio et al., 2015; Setälä et al., 2016) and may carry toxins, such as persistent organic pollutants (POPs). The majority of common synthetic polymers can easily absorb POPs like dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs), subsequently entering the ocean and marine food webs (Avio et al., 2015; Bakir et al., 2012; Devriese et al., 2017; Mato et al., 2001; Rios et al., 2007). Several POPs that reach the ocean are additives manufactured into the polymer itself, such as bisphenol A (BPA). There is also significant evidence that suggests microplastic ingestion can disrupt physiological processes in some

Table 1
Chemical type assignment of marine microdebris in oyster gill tissues. A total of 199 marine microdebris items were detected in the gills of oysters collected around four reef sites in the Mergui Archipelago, Myanmar ($N = 12$ sites). Assignment was based on the chemical type as determined by ATR-FTIR. Items were classified as synthetic, semi-synthetic, or naturally-derived according to Kroon et al. (2018a). Brand names removed.

Classification	Particle type	Common use or product	Count
Synthetic pollutants			
Polymers	Poly(butylene-*co*-acrylonitrile)	Polymer	1
	Nylon	Synthetic fabric	3
	Poly(eptm)-g-poly(amide-6)	Nitrile	12
	Poly(ethylene-*co*-methylacrylate)	Plastic	2
	Polyester fabric	Fabric	3
	Polyethylene	Plastic	22
	Polypropylene	Plastic	26
	Teflon	Non-stick coatings	2
	Poly(1-methyl-4-octadecyloxycarbonyl-2-e-butenylene)	Polymer	1
	Poly(1-methylnonadecamethylene)	Polymer	1
	Poly(decamethylenemesaconamide)	Polymer	3
	Poly(S-ethyl-L-cysteine)	Polymer	1
	Poly[bis(stearoyloxy)hexa-2,3,4-triene-2,5-diyl]	Polymer	8
Oils	Alkyd long oil	Surface protectant	1
Other	1-Ethoxysilatrane (migugen)	Agricultural chemical	1
	Calcium stearate	Soap, food additive, manufacturing	4
Semi-synthetic pollutants			
Milk supplements	Full Cream Milk Powder (Brand 1)	Milk supplement	9
	Toddler Vanilla Flavoured Milk Supplement (Brand 2)	Milk supplement	13
	Skim Milk Powder (Brand 3)	Milk supplement	6
Oils	Glisseal grease	Lubricant, vacuum grease	1
	Kerosene	Fuel	15
	Pail-boiled linseed oil	Paint	9
	Stand oil	Paint	9
	Tung oil	Surface protectant	1
Polymers	Cellulose acetate	Biodegradable plastic	1
Naturally occurring pollutants			
Non-native minerals	Talc	Manufacturing, cosmetics	4
	Aluminium silicate	Cosmetics and manufacturing Manufacturing	4
	Bentonite	Cosmetics, agriculture	4
	Chalk	Manufacturing	7
	Calcium carbonate	Food additive	1
	Smectite	Manufacturing	10
	Magnesium stearate	Manufacturing, cosmetics	2
	French ultramarine pigment	Cosmetics and manufacturing Paint pigment	11
Other	Saponin	Cosmetics, pharmaceuticals	1
Total particles			**199**

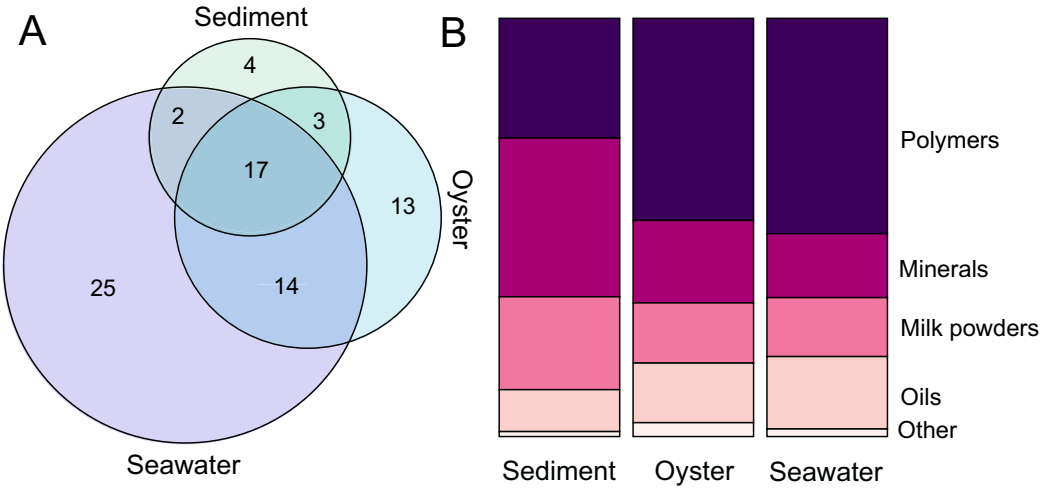

Fig. 3. (A) Diversity of microdebris contaminants shared by seawater, sediment and oyster tissue samples determined by attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectroscopy, and (B) proportions of broadly classified microdebris contaminants quantified from seawater, sediment and oyster tissues.

organisms through absorption of toxins (Watts et al., 2014; Lu et al., 2016). When marine organisms ingest polymer microparticles, POPs are able to absorb into the tissues of the organism (Avio et al., 2015) and possibly transferred to people through food. Therefore, the uptake of microplastics in the marine environment could have far-reaching consequences for human consumption of seafood. Despite limited evidence of bioaccumulation of microplastics in higher organisms, other data that suggest trophic transfer of microplastics can occur in conjunction with direct ingestion (Au et al., 2017). However, the implications of microplastic contamination for food safety and associated public health risks has yet to be fully established. The risk assessment of microplastics in seafood is still in its infancy with little data on the effects to human health (Rainieri and Barranco, 2019).

Over half of the microdebris contaminants detected by FTIR in oyster tissues were composed of non-polymer materials. Although the trophic effects of many of the chemicals remains unknown, it is concerning to find the types of microdebris that can accumulate in marine organisms that haven't been accounted for that may be of particular concern to human health. Several microdebris contaminants found in the oyster tissue can be harmful to human health if ingested, such as kerosene, saponin, and talc (Tshiamo, 2009; Weng et al., 2009; Maiyoh et al., 2015; Chan et al., 2019). However, we did not measure the concentration which would elucidate whether this was a harmful dose. Overall, there are numerous microdebris contaminants found in both the environment and seafood even in underdeveloped locations of the Mergui Archipelago that are potentially harmful when consumed, suggesting that further investigations into the extent of this problem is required at larger scales with quantification.

The large proportion of milk supplement particles found in this study is of particular interest. The prominence of milk supplement in the marine environment demonstrates its extensive usage. Myanmar has recently undergone major political and economic changes, including the opening up of the nation to new corporations that market or sell breastmilk substitutes (Media Monitoring of Breastmilk Substitutes, 2016). Regulation, and enforcement of existing regulations, of such companies has been relatively slow to follow, leading to violations of the code that was adopted by the World Health Organization (WHO). According to the United Nations Children's Fund, Division of Data Research and Policy (UNICEF) (2019), less than one-quarter of all children under 6-months of age in Myanmar are exclusively fed breast milk. Previous work has found that the high use of supplemental milk in Myanmar is linked with anaemia of mothers and has led to increased malnutrition among children (Zhao et al., 2016). Encouragement of breastfeeding is a major global public health initiative, as there are many well-documented medical and neuro-developmental advantages to breastfeeding over the use of milk formulas (Victora et al., 2016; American Public Health Association, 2017).

The prominence of milk supplement we detected suggests a direct fecal-oral link between human waste and potential food products, with potential for transmission of disease or contaminants through seafood consumption. Although the bacterial pathogens detected are also well-known enteric diseases of humans and are also likely tied to wastewater from nearby communities, the presence of milk supplement particles provides additional supporting evidence. Not only do the oysters reflect contamination of the environment, but cultural and socioeconomic aspects of their location. Three separate brands of milk formula were quantified in this study, which may suggest a common ingredient that lends the question to the nutritional quality of supplemental milk. Further work may need to explore milk supplement as a potential persistent organic pollutant.

4.3. Conclusion

The employment of rapid high-throughput 16S rRNA gene sequencing in conjunction with FTIR spectroscopy allowed us to describe a vast array of microcontaminants that are often overlooked in risk assessments of seafood worldwide. While this study provides a detailed characterization of 87 different species of human bacterial pathogens and 78 different microdebris contaminant materials that are found in the marine environment and an important seafood species, little is known about the full extent of risk to public health for many of the contaminants detected. This rapidly developing region in Myanmar is clearly affected by effluent from anthropogenic sources resulting from the lack of wastewater infrastructure. Therefore, the impacts of wastewater contamination on food security and risks to human and marine environmental health will benefit from using emerging technologies to target local infrastructure interventions and monitoring.

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Author contributions

Raechel A Littman: Data curation, original draft preparation, reviewing and editing; Evan Fiorenza: statistical analyses, data visualization, draft preparation, reviewing and editing; Amelia Wenger: study conceptualization and experimental design, sample collection and processing, draft preparation, reviewing and editing; Kathryn Berry: study conceptualization, sample collection and processing, draft preparation, reviewing and editing; Jeroen van de Water: bioinformatics, draft preparation, reviewing and editing; Lily Nguyen: data analysis, draft preparation, reviewing and editing; C. Drew Harvell: study conceptualization, funding acquisition and administration, draft preparation, reviewing and editing; Daniel Parker: draft preparation, reviewing and editing; Soe Tint Aung: field logistical design, sample collection and processing, reviewing and editing; Douglas Rader: study conceptualization, funding acquisition and administration, reviewing and editing; Joleah Lamb: study conceptualization and experimental design, funding acquisition and administration, data analyses and figure preparation, original draft preparation, reviewing and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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