

GREAT AUSTRALIAN BIGHT RESEARCH PROGRAM

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Spatial distribution and diversity in hydrocarbon degrading microbes in the Great Australian Bight I: Functional Gene Distribution

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GREAT AUSTRALIAN BIGHT RESEARCH PROGRAM

The Great Australian Bight Research Program is a collaboration between BP, CSIRO, the South Australian Research and Development Institute (SARDI), the University of Adelaide, and Flinders University. The Program aims to provide a whole-of-system understanding of the environmental, economic and social values of the region: providing an information source for all to use.

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Executive summary

Bacteria are known to efficiently metabolise most components of oil, in particular alkanes and some aromatic compounds. They also efficiently metabolise much of the natural gas that is released during wellhead blowouts. As a consequence, being able to predict the metabolic capacity for oil degradation of bacteria indigenous to the Great Australian Bight will be an important component of the oil spill risk assessment for this area. To address this in part, we studied key genes in three pathways of hydrocarbon degradation: *alkB*, which is involved in alkane degradation, *c23o* which is involved in the degradation of aromatic compounds, and *pmoA*, which metabolises methane. The diversity and relative abundance of these genes was determined in sediment and water samples collected from the Bight. We found numerous copies of each gene in all sediment samples, and of *alkB* and *c23o* in the water samples, suggesting that bacteria in the GAB do have the capacity to degrade oil. Furthermore, the sequences of these genes were unique, suggesting that while these bacteria do have the capacity to degrade hydrocarbons, their rates of response to sources of hydrocarbons, and thus rates of oil degradation, may not be easily predicted based on studies conducted elsewhere.

1 Introduction

Crude oil is a natural product derived from the degradation of organic material over geological time scales (Atlas & Hazen 2011). As a consequence, numerous groups of organisms have developed the ability to metabolize oil (Head et al. 2006, Atlas & Hazen 2011), including bacteria, algae and fungi. Following the recent *Deepwater Horizon* oil spill in the US Gulf of Mexico, approximately one half of the petroleum hydrocarbons released were oxidised by marine microbes (reviewed by Joye 2015). There are several factors that may have contributed to the rapid rates of degradation, including the chemical composition of the spilled oil (which was light and easily degraded) (Atlas & Hazen 2011) the high nutrient levels in the area, the high natural inputs of oil from seeps (Kappell et al. 2014, Yergeau et al. 2015), and the fact that the oil was dispersed, increasing the surface area that is available for oil degradation (Atlas & Hazen 2011, Prince 2015, Kleindienst et al. 2015a, b).

Since crude oil is a complex mixture of different chemicals, the degradation of crude oil is undertaken by a consortium of organisms using different pathways (reviewed in Head et al. 2006). The ability to degrade crude oil is found in diverse, taxonomically unrelated bacteria (Smith et al. 2013). Following the release of crude oil into the environment, the alkanes (linear arrangements of carbon) are degraded most rapidly (Abbasian et al. 2015). Alkane monooxygenase (also referred to as alkane hydroxylase, with the functional marker gene *alkB*) oxidises C₅ to C₁₆ length alkanes (Smith et al. 2013) and is the rate limiting step in this process. Polycyclic aromatic hydrocarbons (PAHs) are also readily degraded, although not as rapidly. The catechol-dioxygenases (including the catechol-2,3-dioxygenase, with the functional marker gene *c23o*; also referred to as *xylE*) are the enzymes responsible for cleaving the aromatic ring and are a conserved step in the metabolism of PAHs (Meyer et al. 1999). This enzyme is thought to control the rate limiting step in the degradation of aromatic compounds (Okuta et al. 1998). Methane can also be released from production wells in large volumes. The enzyme methane mono-oxygenase (with the functional marker *pmoA*) catalyses the first step in the microbial conversion of methane to carbon dioxide under oxic conditions (Inagaki et al. 2004). Following the release of oil into the environment, these processes are thought to occur simultaneously, but to have patterns of relative abundance that proceed in an ecological succession, as depicted below. Indeed, following the *Deepwater Horizon* oil spill, this successional pattern was observed in the waters and sediments of the Gulf of Mexico (Dubinsky et al. 2013, reviewed in Yergeau et al. 2015).

Since the Great Australian Bight (GAB) may have substantial oil and gas reserves that could be developed in the future, we want to better understand the propensity for biodegradation following a potential release of oil into the environment. Consequently, we want to be able to predict the capacity of indigenous organisms in the GAB to degrade hydrocarbons – both as discharged as part of routine operations, and in case of an oil spill. To that end, we characterized the diversity in three genes involved in different pathways in hydrocarbon degradation: *alkB*, involved in the oxidation of alkanes; *c23o*, involved in the “ring breaking” step of the metabolism of aromatic compounds, and *pmoA*, involved in methane metabolism. As our initial forays suggested that the diversity of sequences in the GAB was not well represented by sequences deposited in the National Center for Biotechnology Information (NCBI) databases, we used massively parallel next generation sequencing (Illumina) to capture the diversity of sequences in sediment and water from the GAB. In a companion study, we examine the taxonomic composition of the microbial assemblages found in the sediments of the GAB (Hook et al. 2016).

2 Materials and Methods

2.1 Sample collection

Sediment samples were collected from the surface of cores collected at the stations on transects T2 and T4 shown in Figure 1 below, taken during the *Southern Surveyor* cruise, 3-22 April 2013. A 6-core multicorer from KC (Denmark) was incorporated into an instrumented coring platform that could be controlled from the vessel and allowed reliable collection of sediment samples at depths between 200 and 2000 m (Sherlock et al. 2014). Triplicate sediment cores from each deployment were subsampled for microbial analysis using 30mm diameter minicores. The top 2 cm of each minicore was extruded, placed into a DNA free tube, and frozen until they were transferred to CSIRO laboratories for DNA extraction. Water samples were collected at maximum depth at each Station using a Niskin bottle on the coring platform (located ~ 1m off bottom). Microbial cells were collected by filtration of 2 L seawater through a 0.22 µm pore Sterivex™ GP filter (Millipore®, Massachusetts. Cat. # SVGPL10RC), using a 6 channel peristaltic pump. Pump tubing was rinsed with ~200ml seawater from the appropriate depth prior to cell collection. Pumping continued for 1 min after the sample had cleared the filter to dry. Both ends of the filter were capped, placed in individual snap-lock bags and stored at -80°.

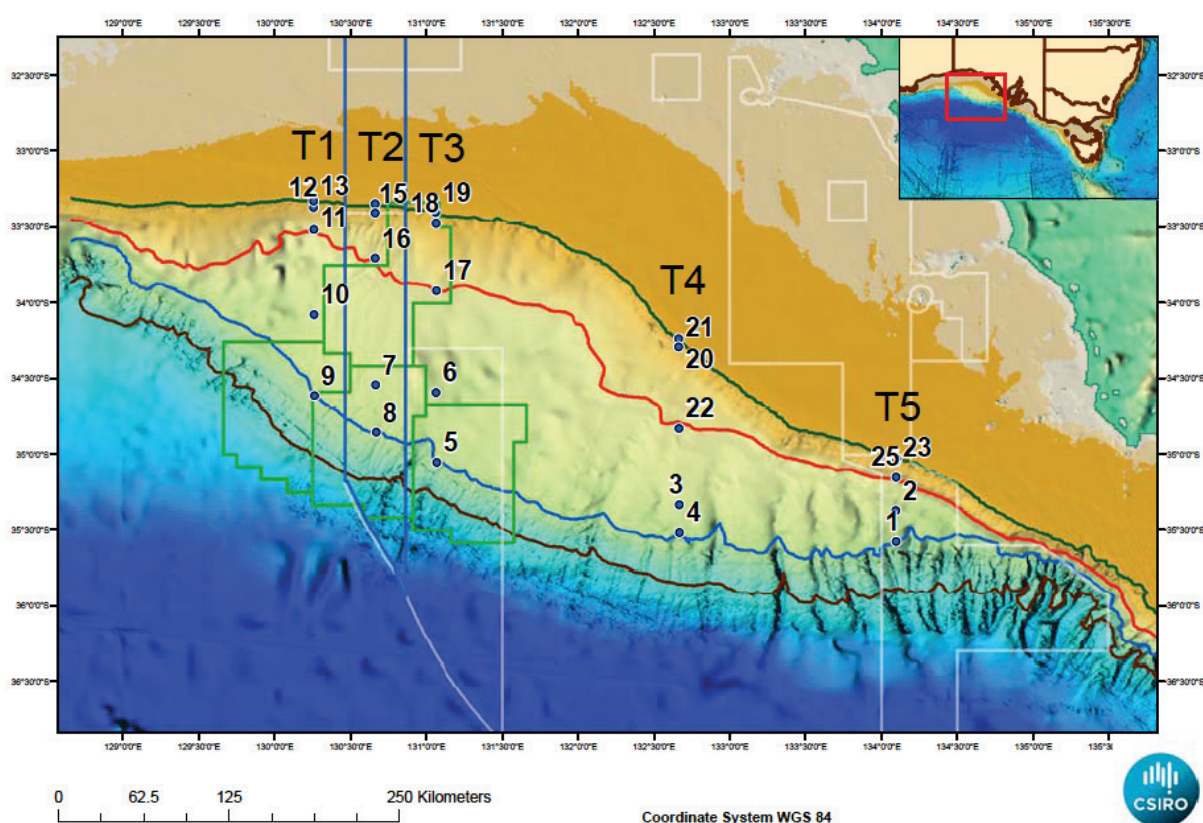


Figure 1. Locations sampled by the *RV Southern Surveyor* for sediments. Green, red, blue and black depth contours are 200, 1000, 2000 & 3000 m respectively, green polygons indicate the BP exploration leases, and the blue polygon the benthic protection zone of the Great Australian Bight Marine Park. T1, T2 etc indicate transect numbers.

2.2 DNA extractions

Sediment

Ten grams of sediment were used for each DNA extraction. DNA was extracted using the PowerMax[®] Soil DNA Isolation Kit (Mo Bio Laboratories Inc, USA), modified as follows: 10 minutes incubation at 70 °C after adding the lysis solution (C1), and extending the incubation times to 30 minutes. Once DNA was eluted, the sample was concentrated to dry pellets in a “speed vac” vacuum concentrator, and then washed in 100% ethanol to remove excess salts.

Water samples

DNA was extracted from sterivex filters following a modified version of the PowerWater[®] Sterivex[™] DNA Isolation Kit (Mo Bio Laboratories Inc, USA) (Appleyard et al. 2011). Filters were removed from -80 °C and brought to room temperature before adding 1875 µL lysis buffer (200 mM sodium phosphate buffer, pH 7.0 containing: 1% CTAB, 2% PVP K30, 0.3 M NaCl and lysozyme at a final concentration of 5 mg mL⁻¹) and 125 µL MT Buffer (MP Biomedicals, LLC, USA) via the inlet valve. Sterivex filters were recapped and attached to a horizontal vortexer (Vortex-Genie 2, Mo Bio Laboratories Inc, USA) and vortexed at speed setting 6 for 1 h. A 3 mL syringe was attached to the inlet of the sterivex and using back pressure from the syringe the contents of the sterivex was removed and split into 2 x 2.0 mL microfuge tubes (approximately 1 mL per tube). 900 µL of Phenol:Chloroform:Isoamyl Alcohol (25:24:1) was added to each tube and mixed by inverting several times. After centrifuging at 13 000 rpm x 10 min, the supernatant was removed and combined in a new 2.0 mL microfuge. Proteinase K was added (20 µL of 20mg/mL stock) followed by incubation at 60 °C for 2 h. 500 µL of Chloroform:Isoamyl Alcohol (CI) (24:1) was added and mixed by inverting several times. After

centrifuging at 13 000 rpm x 10 min, the supernatant was removed to a new 2.0 mL microfuge tube and the CI extraction step was repeated. Following centrifugation the supernatant was removed to a 5.0 mL tube and 3 mL of ST4 Buffer (prewarmed to 65 °C) was added. The barrel of a 20 mL syringe was attached to a filter column that was then attached to a vacuum manifold (Vac-Man® Laboratory Vacuum Manifold, Promega Corp, USA). The contents of the 5.0 mL tube was poured into the syringe barrel and pulled through the filter column using the vacuum. Once the entire volume had been pulled through the column the 20 mL syringe barrel was removed. With the vacuum still flowing the filter was washed with 500 µL ST5 followed by 500 µL ST6 and dried by continuing vacuum flow for a further 2 min after the ST6 was entirely pulled through. The vacuum was turned off and the filter transferred to a new 2.0 mL collection tube and allowed to air dry on the bench for 10 min. To elute DNA, the filter was incubated with 80 µL of 0.1 x TE at 37 °C for 45 min, followed by a final spin at 13 000 rpm x 2 min. The quality and quantity of all DNA was checked using a NanoDrop™ 8000 Spectrophotometer (Thermo Scientific™). DNA was aliquoted into multiple plates, vacuum dried and stored at -20 °C.

2.3 Database Construction

Sequences for *alkB*, *c23o* and *pmoA* were obtained from the NCBI Genbank databases. These sequences were aligned and phylogenetic trees were created using the ARB phylogenetic software package. Multiple probes were designed using principles outlined in previous studies (Stralis-Pavese et al. 2011, Abell et al. 2012, Abell et al. 2014). Upon validation, it became apparent that the diversity in the sequences in the GAB were not captured in the NCBI databases, even though these databases included several previous environmental surveys (Wasmund et al. 2009, Smith et al. 2013). As a consequence, we decided to capture the sequence diversity in water and sediment samples from the GAB via massively parallel sequencing.

2.4 Amplicon Generation and Sequencing

Amplicons for *alkB*, *pmoA* and *c23o* were sequenced using the Illumina MiSeq platform (Illumina, Inc., USA) for all samples, including replicates, from transects 2 and 4 (Figure 1). Illumina overhang adapter sequences were added to gene-specific primer pairs to generate amplicons for next generation sequencing (Table 1). PCR reactions consisted of 2.5 µl 10x ImmoBuffer, 0.5 µl 10 mM dNTP, 1.25 µl 50 mM MgCl₂, 1 µl 10 µM forward primer, 1 µl 10 µM reverse primer, 0.25 µl BSA, 0.2 µl 5U/µl Immolase Polymerase and 10 ng DNA template in a total volume of 25 µl. Cycling parameters were: denaturation at 95 °C x 10 min; 11 cycles of 94 °C x 1 min, 65 °C x 1 min and 72 °C x 30 sec; followed by 24 cycles of 94 °C x 1 min, 60 °C x 1 min and 72 °C x 30 sec; and a final extension at 72 °C x 4 min. Amplicon products were purified using Agencourt AMPure XP (Beckman Coulter, Inc., USA) as per the manufacturers instructions. Second stage PCR incorporating Nextera XT barcodes, purification, library generation and sequencing using the Illumina MiSeq platform (with 300 bp paired reads) were performed according to manufacturers directions.

Table 1. Illumina adapter and primer sequences used to generate amplicons for next generation sequencing.

	Forward sequence 5'-3'		Reverse sequence 5'-3'	Reference
Illumina forward overhang adapter	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[gene specific sequence]	Illumina reverse overhang adapter	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-	
alkB-1F	AAYACNGCNCAYGARCTNGGNCAYAA	alkB-1R	GCRTGRTGRTCNGARTGNCGYTG	Kloos et al. 2006
pmoA189 GC	GGNGACTGGGACTTCTGG	pmoA682	GAASGCNGAGAAGAASGC	Holmes et al. 1995
c230F	AAGAGGCATGGGGGCGCACCGGTTTCATCA	c230R	CCAGCAAACACCTCGTTGCGGTTGCC	Sei et al. 1999

2.5 Bioinformatics

Amplicon sequences were analysed following the bioinformatics workflow established for the Bioplatforms Australia (BPA) funded Biome of Australian Soils (BASE) project (Bisset et al. in revision) (workflow details: <https://ccgapps.com.au/bpa-metadata/base/information>) with the following exceptions: after initial quality control, trimming and merging of read pairs, FASTA format sequences were extracted from FASTQ files and assembled into one file per amplicon. RDP FrameBot (Wang et al 2013) was used to detect frameshift errors in functional gene nucleotide sequences by comparing the DNA sequences to known protein sequences for each gene. FASTA files were submitted separately to the RDP FrameBot pipeline (fungene.cme.msu.edu/FunGenePipeline/framebot/form.spr) resulting in FASTA files containing only frameshift-corrected DNA sequences. These FASTA files were used to generate *de novo* OTUs at 97% similarity using the open reference OTU picking pipeline USEARCH 64 bit v8.0.1517 (Edgar 2010) with the UPARSE algorithm (Edgar 2013) and representative sequences for each OTU. Representative sequences were imported and aligned into the existing ARB databases for each gene as described above.

2.6 Data Analysis and Statistics

Square matrices containing presence and abundance data for all OTUs across all samples were generated using the python command uc2otutab.py provided on the drive5 Bioinformatics and software services site (<http://www.drive5.com/>) to assist in processing OTU data. Rarefaction curves were generated using the rarefaction.single command in MOTHUR (Schloss et al 2009). Preliminary community composition analysis was performed using the Primer-E Multivariate Statistics for Ecologists software package (Version 7; Clarke and Gorley 2015). For community composition analysis, the relative abundance data were fourth root transformed and a Bray-Curtis dissimilarity matrix constructed. Hierarchical agglomerative cluster analysis with the group average method was used to examine groupings in the data, which were then further visualised using principal co-ordinates analysis. Pearson correlations with relevant environmental data collected concordantly were overlaid on the later.

3 Results

3.1 Next-Gen Sequencing results

After QC and removal of chimeras, Illumina sequencing for *alkB* identified 1 417 985 sequences that could be grouped into 626 Operational Taxonomic Units (OTUs – sequences that are 97% similar, the functional equivalent of a species) from both water and sediment samples. Sequencing for *pmoA* produced 1 035 016 sequences from both water and sediment samples that could be grouped into 224 OTUs, and sequencing for *c23o* yielded 2 541 848 sequences from both water and sediment samples that were clustered into 940 OTUs. The rarefaction curves, shown in Figure 2 for *alkB*, demonstrate that we have captured the majority of diversity in the sampled environment with our sequencing efforts.

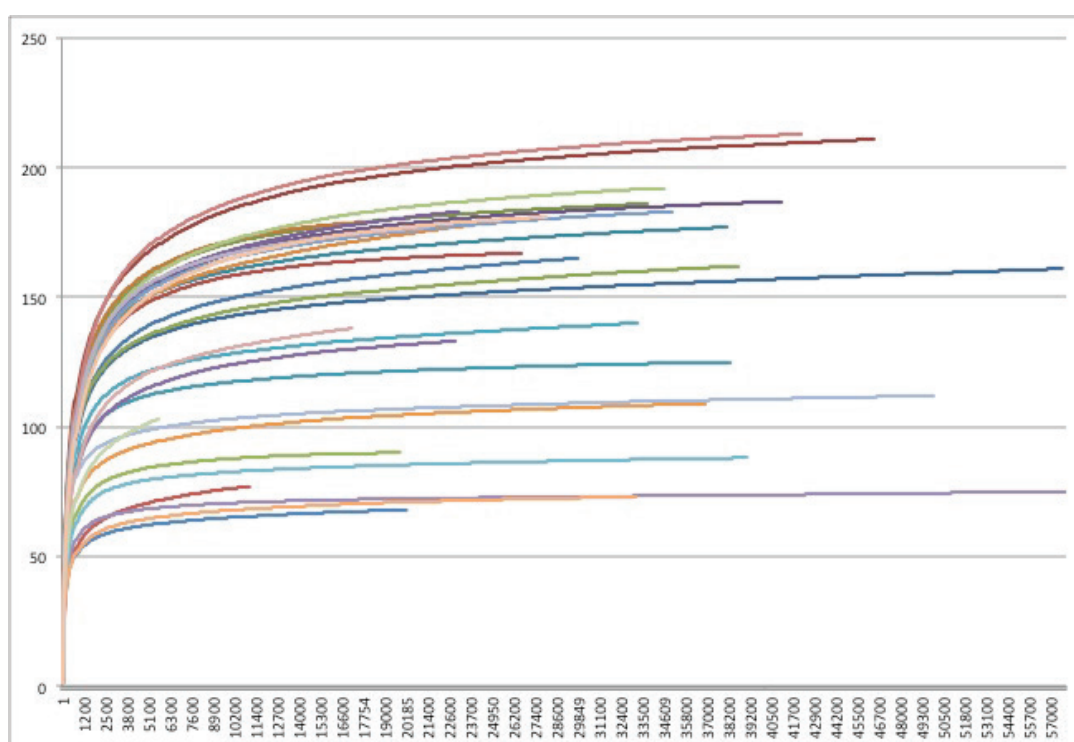


Figure 2. Rarefaction curve for gene sequence diversity for the *alkB* gene. Number of different sequences is shown on the y axis, and the number of sequencing reads are on the x-axis. Each station is shown in a different colour. Although additional new sequences are discovered with the addition of more sequencing depth, the curves have nearly plateaued, demonstrating that our efforts have captured the vast majority of environmental diversity.

3.2 Diversity overview

As depicted above, sediment samples from the Great Australian Bight contained abundant and diverse gene copies of *alkB*, *c23o* and *pmoA*. An overview of this diversity is shown in Figure 3 below.

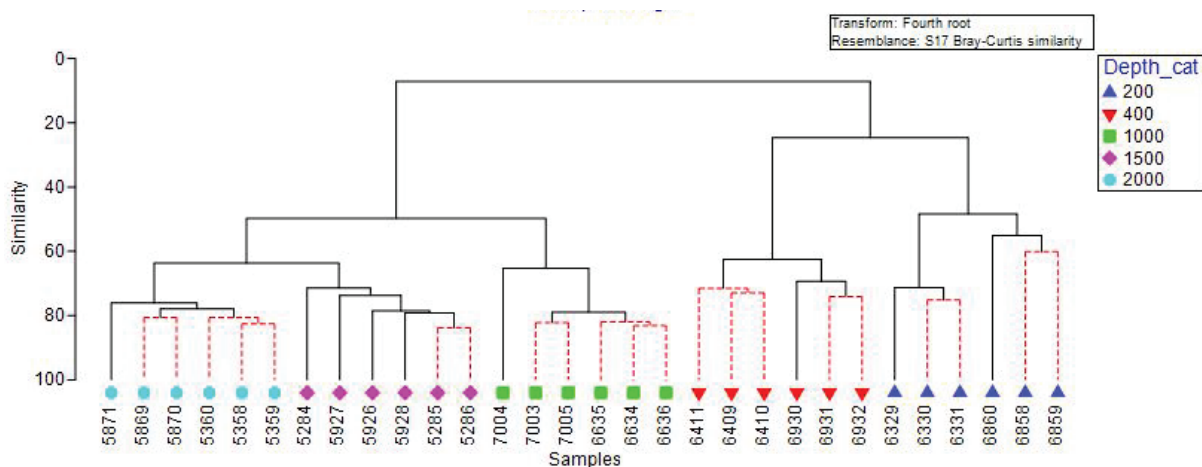


Figure 3. A cladogram showing sequence relatedness amongst sediment samples collected from the GAB. Percent similarity is on the vertical bar. Black lines indicate statistically significant, solid relationships. Red lines indicate relationships with limited statistical support.

Even samples collected from the same station (denoted by sequential numbers) had only 60-80% similarity in composition as determined by these functional genes. Most often, replicate samples formed a distinct cluster, and samples taken at the same depth on the different transects were more closely related than those taken at different depths. There is also a sharp contrast between samples collected in the shallower two depths (200-400 m) relative to the greater depths (1000-2000 m). The environmental factors that are correlated with these differences are shown in Figure 4 below.

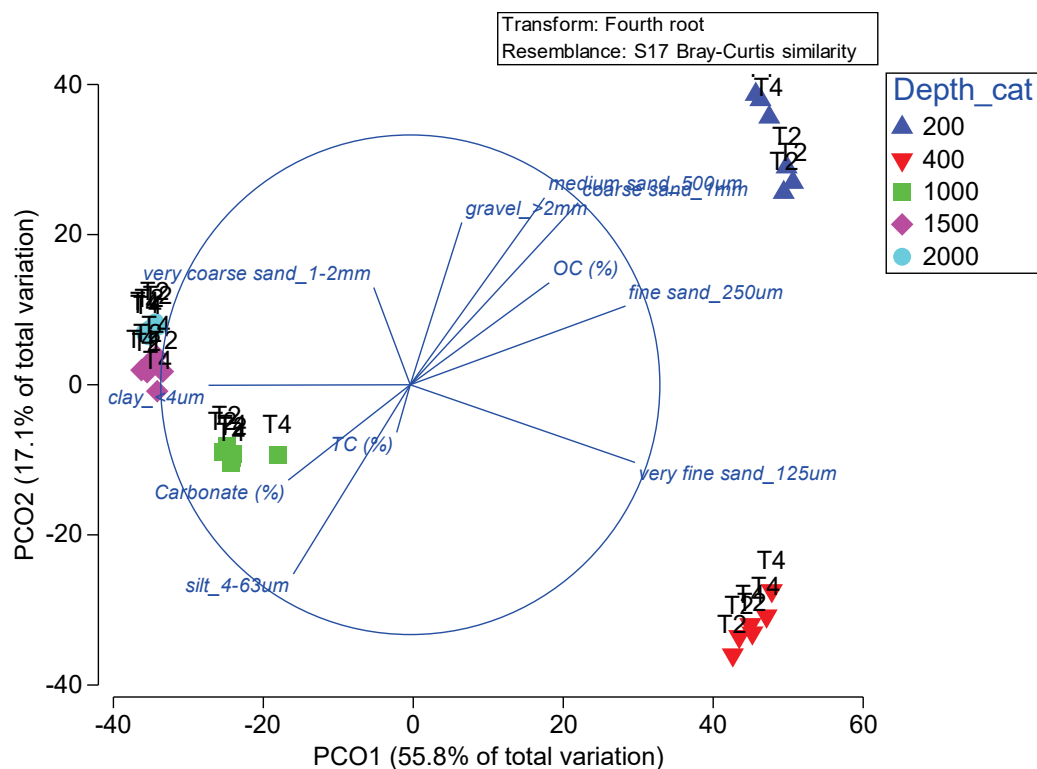


Figure 4. Principal co-ordinates plot showing variables that correlate with differences in diversity for the three selected functional genes, with replicates from each station plotted. For clarity, transects, not sample ID, are shown.

Again, it is apparent that station depth, not transect, is the greatest determinant of sample relatedness, as samples collected at the same depth cluster together, and there is the greatest distance between samples collected at 200-400 meters and those collected at greater depths. Sediment grain size appears to be the primary environmental correlate with this difference. The differences observed in the communities of hydrocarbon degrading bacteria match the differences observed in the sediment grain size at different depths (Table 2 below). The samples collected at 200m and 400m have predominantly sandy sediments, whereas those collected from greater depths have a greater proportion of clay and less sand. As the levels of petroleum hydrocarbons detected in the sediments were consistently very low (Ahmed et al., 2014), we do not think they influence the microbial community composition.

Table 2. Sediment grain size distribution with depth for each of the sampling transects.

Depth	clay_<4um	silt_4-63um	very fine sand_125um	fine sand_250um	medium sand_500um	coarse sand_1mm	very coarse sand_1-2mm	gravel_>2mm
200	0.16	25.84	19.41	20.35	16.67	17.56	2.48	5.25
	0.15	16.64	14.18	23.11	23.42	22.49	9.72	13.93
400	0.75	56.26	24.51	12.98	4.26	1.25	3.50	2.85
	0.64	44.59	23.05	19.08	8.80	3.85	0.38	0.72
1000	27.32	56.37	7.00	5.29	3.74	0.29	1.20	3.40
	36.82	52.72	6.07	2.97	1.40	0.02	6.93	6.58
1500	2.40	61.69	10.70	12.19	11.49	1.52	1.99	1.35
	40.77	40.64	5.55	5.55	6.23	1.26	7.00	4.30
2000	38.45	39.42	6.82	7.28	6.90	1.13	0.73	1.28
	37.61	42.96	5.62	6.09	6.54	1.16	12.56	8.26

The water samples collected are bottom water, and were collected from a Niskin bottle attached to the corer. The water samples had abundant gene copies for *alkB*, less abundant gene copies for *c23o*, and very few copies of *pmoA*. This is not unexpected, as methane oxidisers would be expected to be rare in oxic seawater.

The cladogram showing relatedness of different hydrocarbon degrading bacteria in water samples is shown below in Figure 5. For clarity, only relationships for *alkB* are shown, as these are very similar to the trends found for *c23o*. Again, the samples cluster with depth, with the water samples collected from depths of 1000 m or less forming a distinct clade relative to those collected from 1500 m or 2000 m. However, depth clustering is not as tight as was observed for the sediment samples, and the similarity between samples is lower.

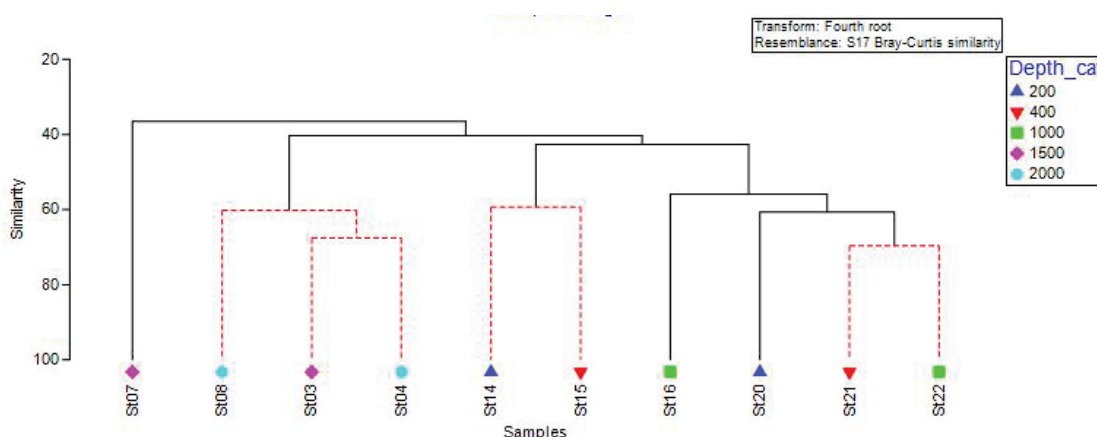


Figure 5. A cladogram showing *alkB* sequence relatedness amongst water samples collected from the GAB. Percent similarity is on the vertical bar. Black lines indicate statistically significant, solid relationships. Red lines indicate relationships with limited statistical support.

Similar trends are apparent if the data are grouped using principal co-ordinates analysis (Figure 6). Again, there is more divergence between sites at the same depth than was observed in the sediment samples, however, there is separation between the two greatest depths and the two shallowest depths, with 1000 m being somewhat in between.

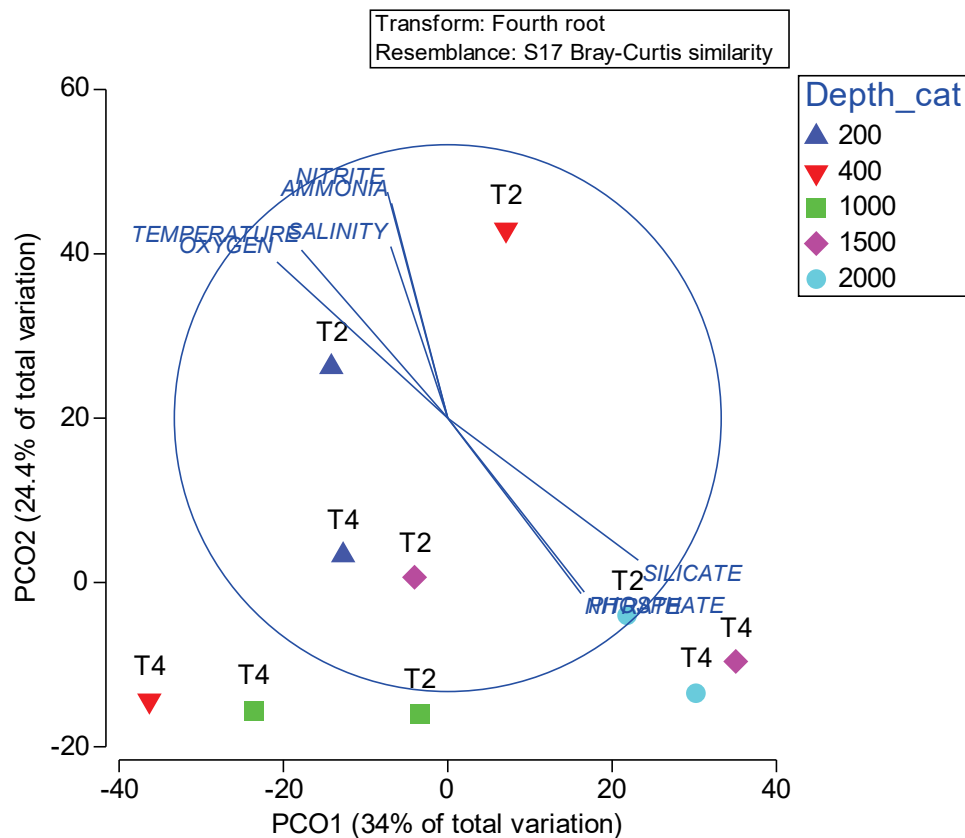


Figure 6. Principal co-ordinate analysis plot showing variables that correlate with differences in *alkB* sequence diversity.

These differences correspond with physico-chemical differences in the water column; dissolved oxygen, temperature, nitrite and ammonia on one hand, and silicate, phosphate and nitrate on the other. As shown in Figure 7, temperature and dissolved oxygen are both higher in the shallow waters, suggesting different water masses.

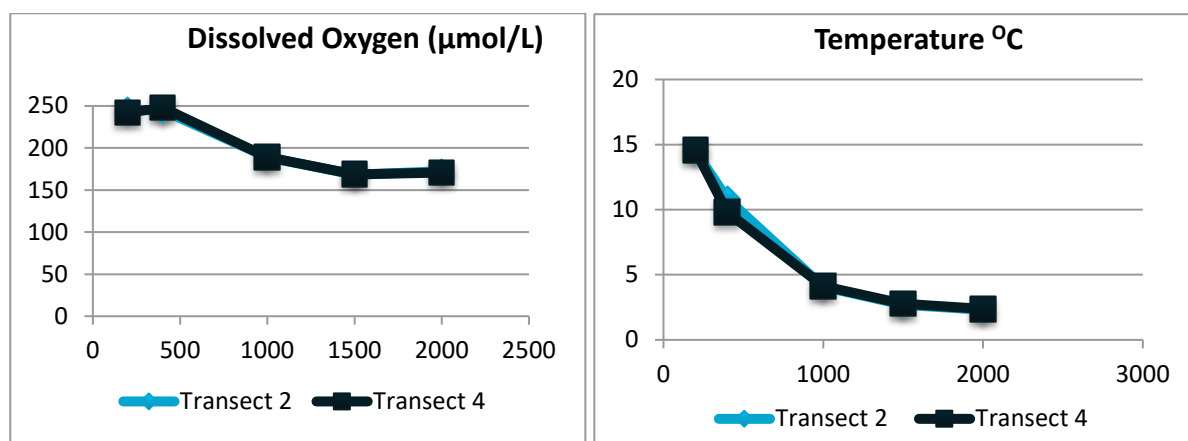


Figure 7. Physical characteristics of the water column for the transects surveyed.

Many of the sequences collected represent novel isoforms of the *alkB* gene that have not been previously described. A cladogram showing the relationships between the sequences identified in this study and those previously available is provided below in Figure 8. The total number of sequences, including the OTUs described in this study (similar sequences grouped together into an OTU at 97% similarity) is shown in black. The number of sequences identified in this project is shown in blue, and the number of sequences identified in the Gulf of Mexico is shown in brown. Clades are grouped at a high (family or above) level of taxonomic classification. It is apparent that the majority of sequences identified in this project are not found in the Gulf of Mexico, and that there are several novel high level clades that have not previously been identified from any environment. The insert shows a high diversity of novel genus level clades within one of the novel family level clades.

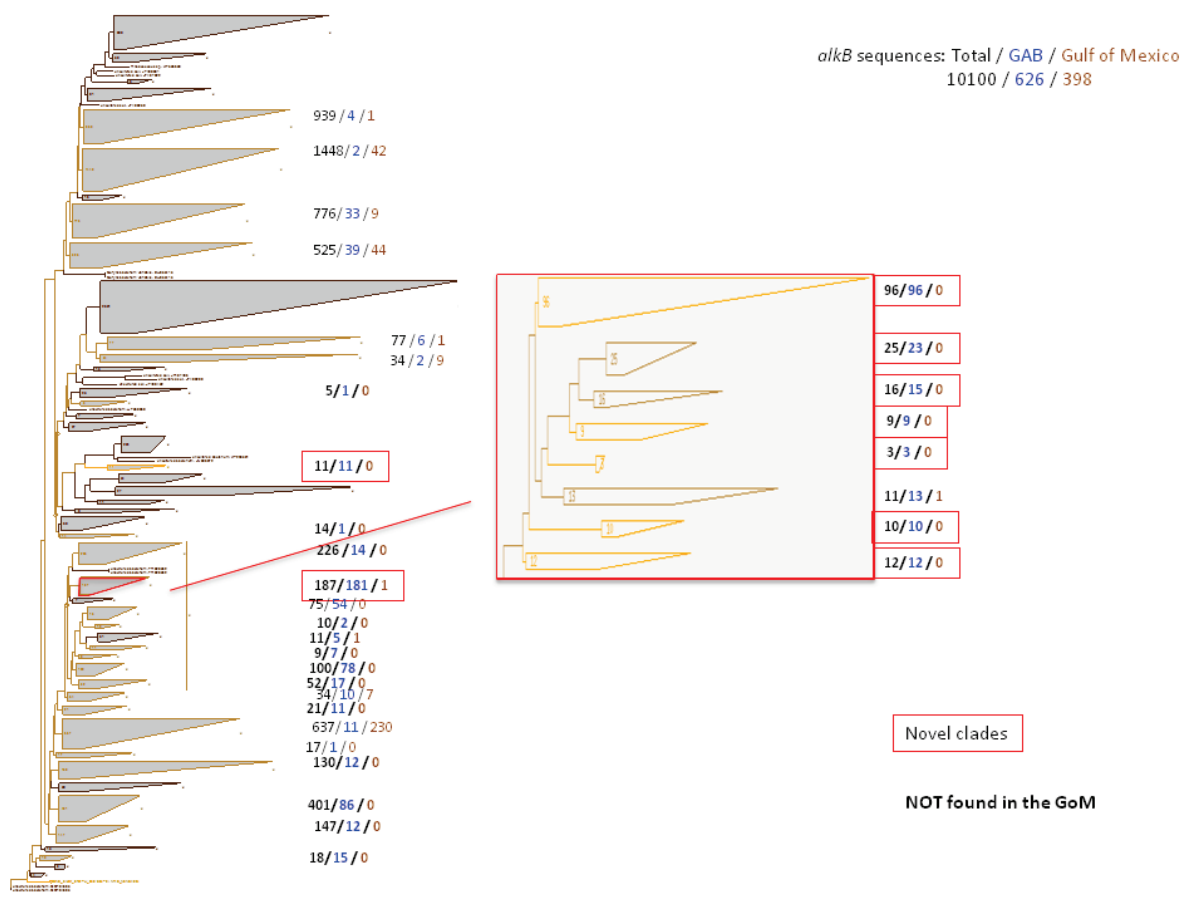


Figure 8. A cladogram, or hierarchical clustering tree based on phylogenetic relationships, based on nucleotide sequences of the *alkB* gene.

Similar results are observed when the phylogenetic relationships based on the nucleotide sequence of *pmoA* are examined (Figure 9). As *pmoA* is a comparatively well studied gene, there are many more sequences in the database. Nonetheless, four novel clades (i.e. clades that were not known at the onset of this work) were identified in this study, and there was low overlap between gene sequences for *pmoA* recorded in the GOM and those measured in this study.

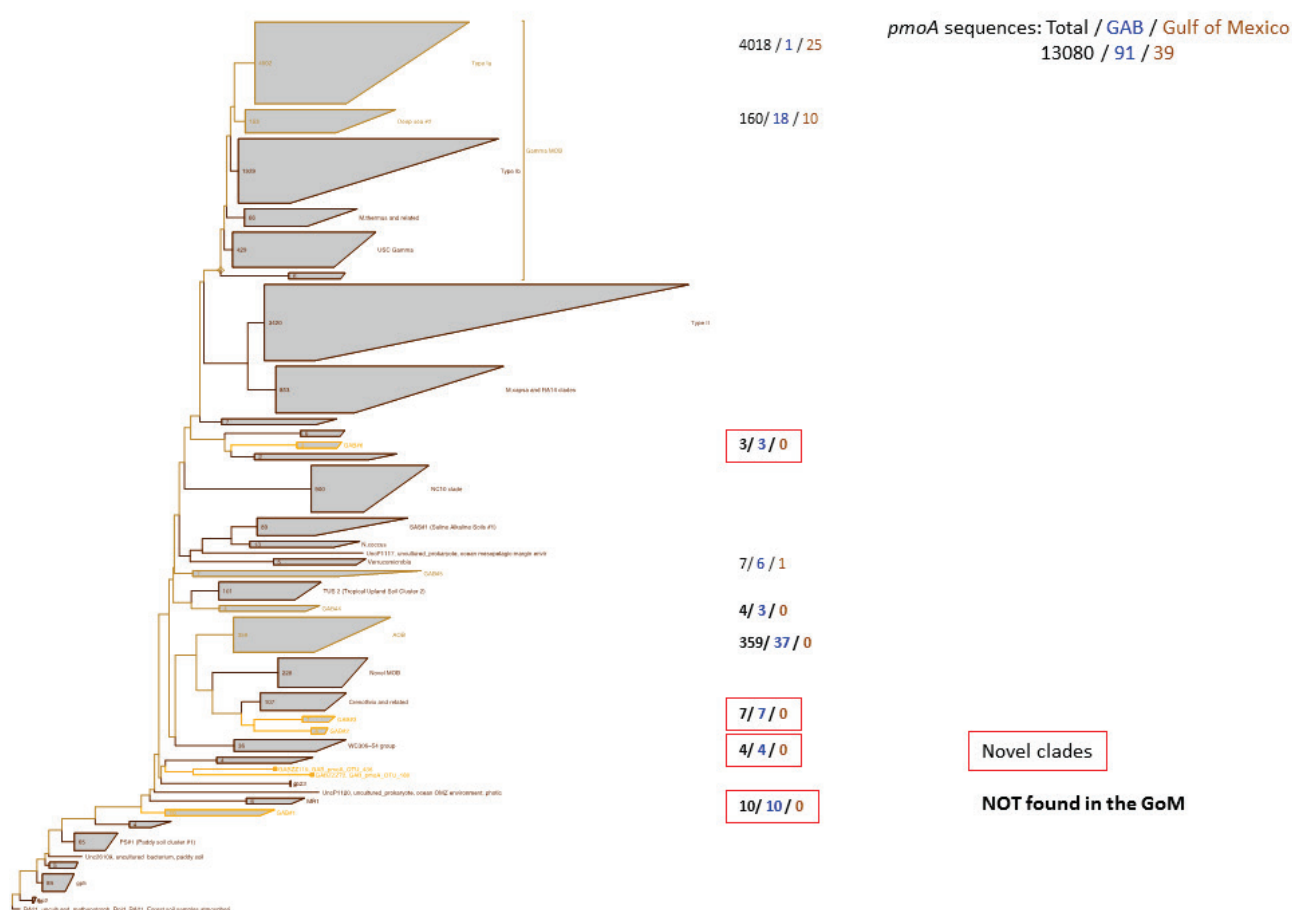


Figure 9. A cladogram based on nucleotide sequences of the *pmoA* gene.

The results were even more striking when the phylogenetic relationships for *c23o* are examined. Of the sequences for the gene currently in our database, one half were identified in the current project. Sixteen novel clades were identified, and none of the gene sequences had homology to those that were identified in the GOM. There are far less published sequences available for *c23o* compared to *alkB* and *pmoA*, as demonstrated by the fact that those collected in this study comprise approximately half of the entire database. That fact, combined with the overall observed novelty of *alkB* and *pmoA* sequences from this study, makes the very high level of observed novelty of *c23o* sequences not all that surprising.

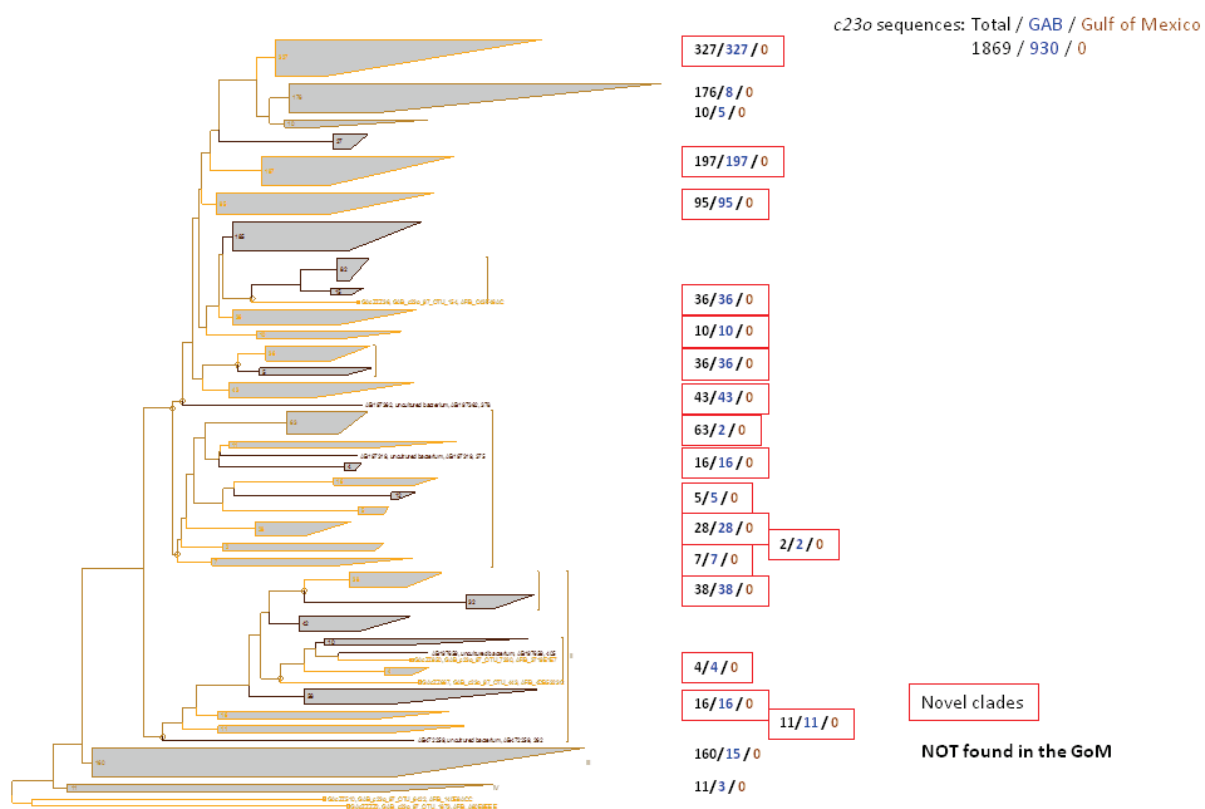


Figure 10. A cladogram based on nucleotide sequences of the *c23o* gene.

4 Discussion

These results indicate that the microbial communities present in the Great Australian Bight have the capacity to metabolise the selected hydrocarbon fractions, even in the absence of an obvious petroleum source. This finding is consistent with the literature on hydrocarbon degrading bacteria. Marine ecosystems normally contain hydrocarbon degrading bacteria (Head et al. 2006, Kostka et al. 2014). Typically, hydrocarbon degrading bacteria make up 1% of the bacterial assemblage in pristine environments, and increase to about 10% in contaminated environments (Atlas 1995).

The gene sequences for all three genes identified in this study are substantially different than what has been seen before. This is consistent with other studies conducted in this region that have identified high degrees of endemism (presentations at the GAB symposium). These differences suggest that while the bacterial assemblages present in the GAB have the capacity to degrade hydrocarbons, they may have different rates of responses to sources of hydrocarbons, and thus degradation may occur at different rates to what has been documented elsewhere. There are also large differences between the GAB and the GOM – which has been well studied due to the recent *Deepwater Horizon* wellhead blowout. The GAB has very low levels of nutrients and no obvious hydrocarbon seeps, whereas the GOM has very high nutrients, due in part to input from the Mississippi River, and abundant natural inputs of hydrocarbons from seeps. Given these differences, it's not surprising that the hydrocarbon degrading microbial communities may have different capacities to degrade petroleum, as evident by the high numbers of novel sequences. These differences also could reflect the novel approach we are taking using a targeted approach to do deep sequencing of functional genes. Most previous studies examining these genes have either used traditional sequencing approaches (which produce only a few hundred sequences) (Wasmund et al. 2009, Smith et al. 2013) or have been conducted using the GeoChip (Lu et al. 2012), although some studies have used metatranscriptomic approaches (Rivers et al. 2013).

Analysis of clone libraries from sediments collected from hydrocarbon seeps in the Timor Sea also indicated a high divergence from known *alkB* sequences (Wasmund et al. 2009). This study analysed fewer depths (approximately 100-400 m), sediment only, and fewer sequences via clone libraries, but nonetheless, had similar trends. They found a high diversity of *alkB* sequences, and a high proportion of highly novel sequences. They found that the diversity of *alkB* sequences decreased with depth. In the GAB, different taxa were more or less abundant at different depths, but our initial analysis does not suggest any decreases in *alkB* diversity with depth. They did not find any correlation between *alkB* diversity and alkane concentration in the sediments, but they did find a higher abundance of *alkB* copy numbers near hydrocarbon seeps, even if the alkane concentrations in sediments were low, suggesting that this gene is a good marker for inputs of hydrocarbons into the environment (Wasmund et al. 2009). By contrast, water samples collected from the northern Gulf of Mexico prior to the *Deepwater Horizon* spill had fewer sequences, but again, a high proportion of novel sequences (Smith et al. 2013). These authors found no obvious trends in *alkB* community composition with depth, but their greatest depths were less than 1000m. Also, they used a clone based approach, meaning they had much less sequencing information to work with (Smith et al. 2013).

Identifying bacteria with the capacity to degrade oil, as well as developing a means of rapidly assessing changes in their abundance, will be an important component of the response and assessment in the event of any oil spills in the area. Previous studies have identified the selected genes as good targets for this sort of an approach. Work conducted following the 2010 *Deepwater Horizon* oil spill, as well as work that has been carried out in contaminated environments elsewhere, has shown an increase in the abundance of these genes. *alkB* and unspecified genes involved in aromatic hydrocarbon degradation were determined to be more abundant in water samples collected from within the hydrocarbon plume than outside it (Lu et al. 2012). Both *alkB* and *c23o* were enriched (though not significantly) in transcriptomes from plume samples (Rivers et al. 2013). Catechol-2,3-dioxygenase was elevated in samples collected from a beach impacted by the *Deepwater Horizon* oil spill relative to an unimpacted beach from the same area (Kappell et al. 2014). A year after the spill, however, no differences in the abundance of alkane monooxygenases or ring cleaving dioxygenases were measured, even though high molecular weight alkanes were more abundant in surface

sediments near the wellhead (Yergeau et al. 2015). Studies conducted after the *Deepwater Horizon* wellhead blowout also identified increased rates of methane oxidation and an increased abundance of methane oxidising bacteria in the months immediately after the wellhead blowout, which is not surprising given the high amounts of methane released after the spill (Crespo-Medina et al. 2014). The rapid increase in methanotrophy was attributed to previously undescribed sequences. These sequences were from a clade with low affinity for methane but high oxidation rates (Crespo-Medina et al. 2014).

These genes have also been shown to be good predictors of bacterial abundances in petroleum contaminated sites that are not associated with a major oil spill. An “overabundance” of *alkB* genes was found to be the best predictor of oil contaminated sites in the Mediterranean, and chronically contaminated sites in the Mediterranean Sea had an increased abundance of catechol-2,3-dioxygenase relative to catechol-1,2-dioxygenase (Bargiela et al. 2015). Increased abundance of *alkB* and *c23o* was measured in soils collected from different oil field contaminated soils in China (Liang et al. 2011).

Although we can measure differences in the structure of these genes, we do not know if these changes would be manifested in differences in how these bacteria respond to petroleum hydrocarbons in the environment, or the rates of microbial oxidation of these hydrocarbons in the GAB as a consequence. This uncertainty is exacerbated by not having a sample near a natural source of oil to compare changes in abundance to the background samples. We would suggest that future studies could perform enrichments of water and sediment collected from the Great Australian Bight to compare both the rates of hydrocarbon oxidation and the microbial response to oil to the rates and response elsewhere in the world.

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