

# GREAT AUSTRALIAN BIGHT RESEARCH PROGRAM

## RESEARCH REPORT SERIES

### Theme 3: Characterisation and assessment of deep-sea benthic biodiversity in the Great Australian Bight

#### THEME REPORT

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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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The authors wish to acknowledge funding support from the Great Australian Bight Research Program (GABRP) - 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.

It is important to also recognise the inclusion of benthic biodiversity data from a parallel program - the Great Australian Bight Deepwater Marine Program (GABDMP), a CSIRO-led research program sponsored by Chevron Australia, with data generated to be made publicly available. These data were provided under a data sharing agreement between the two programs.

A great many staff from CSIRO, SARDI and other participating agencies, as well as the Marine National Facility, helped to acquire biodiversity data during the field surveys and made the variety of land-based contributions that were required to successfully process and document the fauna reported here. Post-processing and analysis have involved a great deal of collaboration by all participating institutions, including the efforts of legal, finance and collections managers. Their collective input has ensured contracting, analysis and handling of material has gone smoothly and efficiently. There are too many individuals to name here, but they are acknowledged in the scientific presentations and papers planned from this project.

Here we would like to make special acknowledgement of other members of the project team (who are co-authors on the project’s two Final Reports): Franzis Althaus, Mark Green, Pamela Brodie, Sharon Hook, Jodie Van de Kamp, Levente Bodrossy and Anthony Chariton (CSIRO), Shirley Sorokin, Maylene Loo and Andrew Oxley (SARDI), and Hugh MacIntosh (Museum Victoria). Finally we acknowledge the valuable contributions made by Stephen Lapidge and Ben Baghurst in their role, at different times, as Research Director for the GABRP.

## EXECUTIVE SUMMARY

Benthic ecology is a foundation element in a system-level study of the marine environment such as that undertaken by the Great Australian Bight Research Program (GABRP). This is because benthic communities are typically highly diverse and influence system productivity, provide structural habitat, alter the physical and chemical condition of the sediment and sediment-water interface, and transfer energy to higher trophic levels.

Our research within the GABRP represents the first detailed and systematic study of benthic biodiversity and benthic ecosystems in the deep-sea of the Great Australian Bight (GAB). Data were generated during two large-scale surveys that, to date, are the deepest (200-3000 m depths) surveys for benthic biodiversity undertaken in Australia's marine jurisdiction.

Research undertaken by the Benthic Biodiversity Theme considered all major components of benthic biota: epifauna (including fishes), infauna, micro- and meiofauna, and the microbial assemblage. A key project output for this previously unsampled region was quality-assured species-level taxonomic data sets for the major elements of fauna. These are foundation data for analyses of faunal composition, diversity, distribution, abundance, assemblage structure and biogeography. Detailed molecular analysis of microbial assemblages provide an initial indication of the extensive biodiversity in this assemblage, and importantly, a preliminary indication of its potential to process any spilled hydrocarbons.

Comprehensive sampling of a large poorly known area such as the deep-sea in the GAB is not feasible. Therefore the use of physical surrogate (covariate) data available at broad scales (remotely sensed oceanographic, hydrographic or acoustic data) are attractive for generating predictive maps of biodiversity and habitat distributions so that spatial extents and changes in them can be used as indicators of ecosystem status. Our project assimilated all relevant covariate data sets and applied two different leading-edge methods of predictive modelling – species distribution (habitat suitability) modelling and gradient forest assemblage mapping – and applied these to the project-generated data sets and to a project-collation of pre-existing historical (museum) data.

The novelty and timing of the work are important because oil and gas exploration may increase significantly in the GAB deep-sea in the next decade. Project data aimed to provide the basis for understanding which indicators and metrics derived from benthic fauna may contribute to an ecological monitoring program to assess the potential future impacts stemming from industry development. This work included a review of current management arrangements and objectives for the GAB. Project data from baseline (unperturbed) sites provides the basis to evaluate indicators and metrics in future comparisons – a reference-site monitoring approach.

The close concordance of patterns between fishes, epifauna, assemblages documented using metabarcoding of the COI and 18S genes, and bacterial and archaeal assemblages as determined using the 16S gene, suggest that there is potential substitutability among these groups for routine ecological monitoring. However, indicators have not yet been identified within any of these faunal groups, nor have the trade-offs between cost and interpretability been examined. This work will need to be part of research into the requirements for ecological monitoring of the GAB deep-sea ecosystem if human activity, especially the development of oil and gas resources, increases.

In terms of benthic ecology, the results reported here transform the GAB from the most poorly known deep-sea region in the Australian marine jurisdiction to the best known.

## INTRODUCTION

### Overview

The research undertaken in the Benthic Biodiversity Theme is an important contribution to the Great Australian Bight Research Program (GABRP) because benthic ecology is a foundation element in any system-level study of the marine environment. Benthic communities are typically composed of a highly diverse fauna that variously influences system productivity, provides structural habitat, alters the physical and chemical condition of the sediment and sediment-water interface, and transfers energy to higher trophic levels. The benthic fauna (Figure 1) is composed of epifauna – animals living on the surface of the seabed, either attached to the substrate itself (e.g. sponges and corals) or living freely upon it (e.g. crabs, echinoderms, molluscs, fishes); infauna – invertebrate animals that exist within or are closely associated with seabed sediments (e.g. small crustaceans and polychaetes); micro- and meiofauna – a highly diverse assemblage of microscopic eukaryotic organisms living on and in sediments, and in the adjacent water column (e.g. Ctenophora and Nematoda); and a microbial assemblage also living on and in sediments and in the water column (e.g. bacteria).

Despite there being good information on the biodiversity of coastal and continental shelf benthic fauna (to about 200 m depth) in the Great Australian Bight (GAB), there was relatively very little known about benthic fauna or ecosystems in deeper waters prior to the establishment of the GABRP (Rogers et al. 2013). This was true of even the larger and more familiar groups of deep-sea epifaunal invertebrates and fishes. It is unsurprising then that the micro- and microbial assemblages present in these habitats were almost completely unknown. Together with infauna, they form part of a key trophic pathway between primary productivity and the epifauna, and to higher order consumers that include a wide range of economically and socially important species. In other, better-studied ocean regions, microbial assemblages include bacteria with the potential to degrade hydrocarbons.

In marine environments where human activities are planned to commence or increase, it is necessary to establish a baseline characterisation in order to assess and understand the extent to which the composition and function of benthic ecosystems may be affected. Oil and gas exploration in the GAB may increase significantly in the next decade, and it seems likely that this expansion will be in water depths greater than 200 m, i.e. depths in which benthic ecosystems were least known.

Any such development will accelerate and expand the need for scientific understanding of the region's marine ecosystems. Whilst it is recognised that the region's coastal and shelf benthic ecosystems have high conservation significance in terms of epifaunal diversity and endemism, these characteristics had not been tested in the deep GAB. There is little information available on most aspects of the ecology of epifauna, including their trophic significance and distributional responses to environmental conditions. Their habitat associations were also unknown because the types and distributions of deep-sea benthic habitats were mostly unclassified and unmapped in the GAB. Benthic infauna are proven indicators of pollution events associated with oil and gas developments, but their potential responses in the deep GAB could not have been evaluated because virtually nothing was known about their diversity, distribution or ecology.





Figure 1. The benthic biota: 'Epifauna' living freely on, or attached to, the seafloor: e.g. fishes, sponges, corals, crustaceans, echinoderms and molluscs (panels 1-9); 'Macrofauna': exist within or closely associated with marine sediments: e.g. amphipods, polychaetes and molluscs (panels 10-12); 'Microfauna': a great variety of small animals: e.g. ciliates & roundworms (panels 13-14); and microbes (panel 15).

Against this background, the Benthic Biodiversity Theme planned two primary areas of study within two separate projects:

- Project 3.1 was a baseline characterisation of the epifauna and infauna that incorporated traditional taxonomic techniques to consistently identify fauna and then used leading edge analytical techniques to identify spatial patterns in assemblage structure and their environmental drivers. This area was considered an essential component of research during the early phase of expanded oil and gas-related activities because of (1) the paucity of existing data, (2) conservation values were untested on the mid- and lower continental slope and continental rise, and (3) the oil and gas lease areas extend across the GAB Commonwealth Marine Reserve. The project both informs the development of ecosystem models within the GABRP and has applications to future management of human activities in the GAB. Its applications are by determining a baseline characterisation of benthic

ecosystems, and helping to identify metrics and indicators that may be relevant to ecological monitoring of the potential future impacts of oil and gas exploration.

- Project 3.2 had a similar focus on characterisation and monitoring, but applied molecular approaches to obtain a broader overview of the fauna present than just the components sampled by the traditional techniques utilised in Project 3.1. Thus, it also incorporated the micro- and meiofauna, and microbes. This project also starts to examine aspects of ecosystem function by quantifying the presence of genes associated with microbial hydrocarbon degradation. It did this by using leading-edge and cost-effective molecular techniques that are innovative and consistent with an Australian national standard. The research is delivered through three sub-projects: 1) a molecular-based program which uses ‘barcoding’ to identify key taxonomic groups of the region; 2) the development of molecular assays for examining the microbial assemblage and the presence of genes associated with microbially-mediated degradation of hydrocarbons; and 3) the development of a rapid and reliable ecogenomic technique which could be used to provide quantifiable data from indicator taxa identified in Project 3.1 and characterised in the barcoding project. Collectively, it is envisaged that these three approaches will provide ecological end-points to reliably and rapidly assess system structure and function.

The strength of the combined approaches of Projects 3.1 and 3.2 is the provision of results and strategies that are familiar and immediately comparable with similar studies in other world oceans, together with results that promise new insights and greater cost-effectiveness for ecological monitoring. Full reports from each project are published separately (3.1 – Williams et al. 2017; 3.2 – Tanner et al. 2017), but outlined below.

## PROJECTS

### 3.1 GAB benthic biodiversity characterisation

#### Objectives

1. Quantify spatial patterns in the physical environment, and composition and abundance of benthic fauna, in BP leases and adjacent continental slope areas of the GAB, to provide baseline metrics that may be relevant to monitoring the potential future impacts of oil and gas exploration on benthic communities.
2. Contribute to determining requirements (including identifying potential indicators and metrics), and identify suitable control regions, for future ecological monitoring in the GAB – especially to detect and quantify ecological impacts from oil and gas exploration on benthic communities of the GAB Marine Park (MP).
3. Contribute data and results to models of ecosystem-level structure and function for the GAB developed in Theme 7.



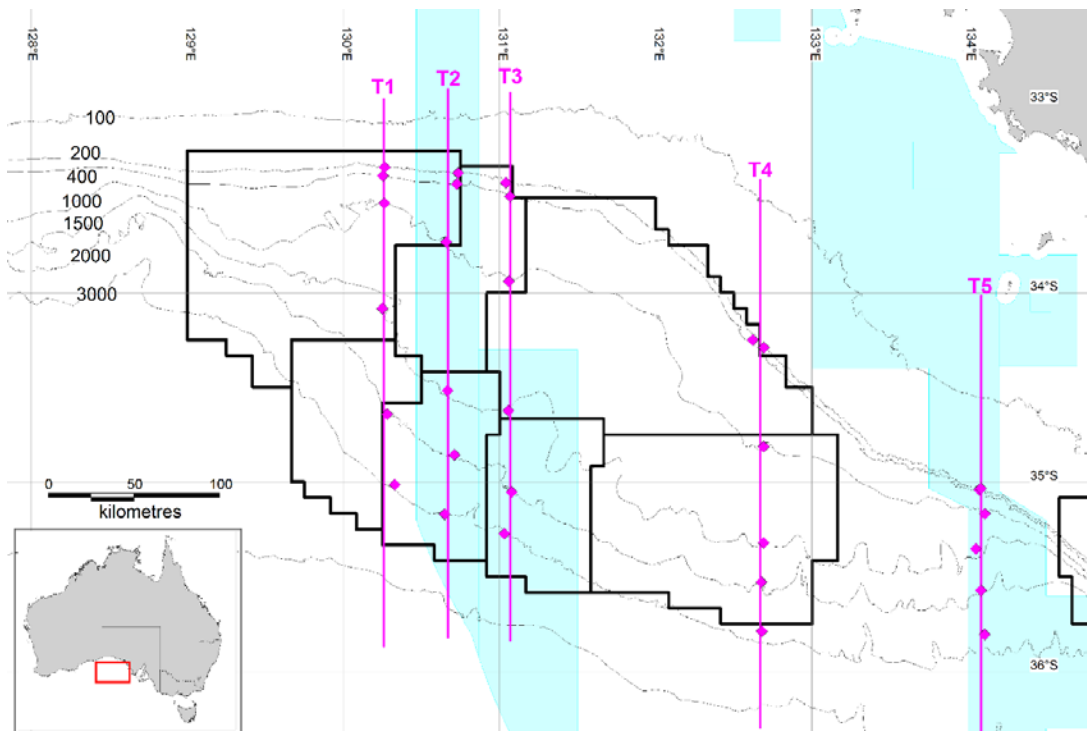
## Key Results and Discussion

### Contribution of the project to the Theme

Project 3.1 characterises the deep-sea epifauna and infauna, the larger elements of the benthos, using conventional taxonomic and analytical techniques (Williams et al. 2017). In contrast, Project 3.2 also incorporates micro- and meiofauna and the microbial assemblage, which requires more specialised ('molecular') techniques for data capture and analysis. Both projects consider benthic fauna in relation to monitoring.

### Data generation

Data for the project were generated during successful large-scale surveys on Marine National Facility vessels RV *Southern Surveyor* (2013) and RV *Investigator* (2015); the latter sampled for both the GABRP and GAB Deepwater Marine Program (GABDMP). Those surveys, to date, are the deepest systematic surveys for benthic biodiversity undertaken in Australia's marine jurisdiction. The GABRP surveys were designed to sample over two gradients along which ecosystem characteristics were expected to vary: depth and east-west (longitude). Sampling was in 6 depth strata – 200 m (shelf break), 400 m (upper continental slope), 1000 and 1500 m (mid- continental slope), 2000 m (lower continental slope), and 3000 m (continental rise) along 5 north-south transects (T1 to T5) at increasing meridians of longitude i.e. a total of 30 sites (Figure 2). Survey collections were used to generate quality assured species-level taxonomic identifications (Operational Taxonomic Units, OTUs) for the major elements of the benthic fauna; these data acted as a foundation for detailed analyses of composition, diversity, distribution, abundance, assemblage structure and biogeography of epifaunal invertebrates and fishes, and infaunal invertebrates. To provide a background for the survey collections, a full suite of environmental co-variate data were generated and compiled to use in the biodiversity analyses. The project also compiled historical benthic biodiversity data from the GAB, and for comparison from southern Australia more broadly, from a comprehensive inventory of museum records.



*Figure 2. Map showing the Benthic Theme study area in the central Great Australian Bight (GAB): sampling sites occur on transects T1 to T5, arranged from west to east, at six depth strata: 200, 400, 1000, 1500, 2000 and 3000 m. Also shown: relevant isobaths (labelled); proclaimed Commonwealth Marine Reserves (shaded light blue); active oil and gas lease blocks (black boundaries).*

## Fishes

A total of 108 species from 49 families was taken by beam trawl in 200-3000 m depths during two surveys in the central GAB in 2015. All samples were on soft sediment habitats, but some were in close proximity to volcanic seamounts and outcropping rocky seabed in submarine canyons. The great majority of species were previously recorded from Australian waters (90%), and from the GAB (75%), but a markedly lower proportion of recorded species occurred at greater depths (1700-3000 m) where there had been virtually no previous sampling: 74% in Australian waters, and 30% in GAB waters. The fauna is dominated by families that typify the deep ocean: rat-tails (Macrouridae), cut-throat eels (Synphobranchidae), morid cods (Moridae), Oreosomatidae (oreo dories), slickheads (Alepocephalidae), cusk eels (Ophidiidae) and halosaurs (Halosauridae). The Macrouridae was most diverse, occurred most frequently, was abundant in 400 m and all deeper strata, and top ranked by biomass and density in area-standardised data. The predominant patterns of biogeographical affinities were strongly modified by depth. Endemic species were most prevalent in shelf break and upper to mid-slope depths (both 52%), and declined with increasing depth (upper to mid-slope species 25%; lower slope/rise 4%), consistent with a general pattern in the Australian ichthyofauna. Fish biomass ( $\text{gm}^{-2}$ ) was significantly related to depth, relatively very low ( $\sim 0.5 \text{ gm}^{-2}$ ) at 200 m depth, highest at 400 m ( $\sim 3.4 \text{ gm}^{-2}$ ), then steadily declining with increasing depth to about  $0.4 \text{ gm}^{-2}$  at 3000 m depth. Depth was the main factor explaining assemblage structure although the lower slope and continental rise (>1500 m depths), that had not previously been sampled in Australian waters, showed relatively little difference to the mid-slope sites (<1500 m depths). There was no clear distinction of sites close to structured habitats, and no longitudinal pattern across a gradient of productivity (higher in the east due to summertime upwelling).

### **Epifaunal invertebrates (megafauna)**

More than 600 species of invertebrate epifauna were collected; ~25% were undescribed and an additional 77 species were new records for Australian waters. The families and genera present were all known to occur in the deep-sea, and many species have been previously recorded in Australia and worldwide; faunal composition was broadly typical for temperate deep-sea regions. The highest diversities (>80 OTUs) were recorded within the Demospongiae, Decapoda, Gastropoda and the combined Echinodermata. Multispecies analyses showed changes in the assemblage structure with depth; sponges and echinoderms dominated the overall biomass and density, with the former being more prominent in shallower depths. The assemblage structure is consistent with a single provincial-scale bioregion in the GAB, with no longitudinal pattern in assemblage, biomass or density distribution. Approximately 70% of species that could be assigned biogeographic data were previously recorded from Australia, with less than half (146 species, 39%) previously known from the GAB. Endemism was low however, with two species, the crab *Choniognathus granulatus* and barnacle *Arcoscalpellum inum* known only from the GAB. Results show a clear eastwards biogeographic affinity, with over twice as many species previously recorded from the southern Pacific (149) than the Indian Ocean (64). This pattern was more dramatic when considering species that occur in only one adjacent region, with 90 species (24%) found in the southern Pacific but not the Indian Ocean, and only 6 species (<2%) from the Indian Ocean but unknown from the Pacific. This suggests that the deep GAB is not an equal mixing zone between western and eastern fauna, but the results are also influenced by the much higher level of collecting in the Pacific vs Indian Ocean.

Our survey of epifauna at baseline (unperturbed) sites provides the basis to evaluate indicators and metrics using a reference-site monitoring approach. A robust (consistent species-level) taxonomic foundation will enable a variety of assemblage-level (composite) metrics (e.g. richness, diversity, distinctness) to be derived, and this is possible across several major taxa including Porifera, Cnidaria, Mollusca, Echinodermata and Crustacea. Species-level data also permit structural and functional changes (including recovery) to be assessed in response to disturbance. Where reference sites should be established can only be determined once the exploration phase of industry development is further advanced because the spatial scales of potential impact are highly activity-specific. However, our data show the high importance of depth to site selection because epifaunal assemblage composition (turnover), diversity, abundance and the proportion of endemic species are all highly correlated with depth.

### **Infaunal invertebrates (macrofauna)**

The total of 200 multi-corer samples for infauna were taken at the 30 depth-stratified stations yielded 1303 individual infauna specimens. These represented at least 258 species, and with some taxa only identified to class or phylum, probably many more. While no patterns in infaunal assemblage structure were found in the analysis of individual samples, the expected depth-related patterns appeared when samples at each site were aggregated. This result suggests that an individual core is too small to provide a representative sample of the assemblage, but that a group of 3 cores is sufficient. Any future studies on infauna in this region should take this into consideration, and preferably use either a single cast of a multi-corer as 1 sample, or utilise a sampler that collects a larger sample. There were several stations at which multiple casts were undertaken. In all cases,

when samples were aggregated, the casts appeared adjacent to each other in the ordination when taken in the same year, but not when taken in different years (2013 vs 2015), indicating substantial temporal variability in the assemblage. This gives added confidence that a single cast provides a good representation of the infaunal assemblage at a site, and that the sampling gear used is not playing a large role in determining what is sampled. Individual samples from a single cast, however, were often separated in the ordination, again indicating that a single core provides an inadequate sample for infaunal analysis. There did not appear to be any unique co-occurring groups of taxa in the sampled region. Taxa accumulation curves suggest that less than 25% of infaunal species present in the region were sampled. This figure increased as taxa were aggregated into higher levels, and reached 100% at the phylum level. Thus, any additional sampling is still likely to produce a high proportion of new species.

### **Predictive models of species and assemblage distributions**

Comprehensive sampling of a large poorly known area such as the deep-sea in the GAB is not realistically feasible. Therefore the use of physical surrogate (covariate) data available at broad scales (remotely sensed oceanographic, hydrographic or acoustic data) is attractive for generating predictive maps of biodiversity and habitat distributions so that spatial extents and changes in them can be used as indicators of ecosystem status. Project 3.1 applied a large suite of environmental covariate data to two different leading-edge methods of predictive modelling: species distribution modelling and gradient forest assemblage mapping. These analyses demonstrate what is presently possible with data available for the GAB, and incorporated both our survey data and historical data. In total, across southern Australia (Sydney to Perth), there were sufficient data to model the distribution of 96 species from 5 phyla that had at least 20 records deeper than 200 m. These models, in aggregate, indicated that the central GAB appears to provide suitable habitat for a relatively high proportion of species, although only in a relatively narrow depth band around the shelf break and upper slope. The assemblage level gradient forests predictions indicated that this same relatively narrow depth band contained 2 distinct assemblages that extended across the whole model domain, while deeper areas were broken up in a longitudinal pattern, although the latter may be an artefact of limited data in deep water. The density, quality and relevance of physical covariate data available for the region is yet to be fully evaluated, and further analysis of the project data will be helpful to fully understand the utility of the data sets currently available for predictive mapping.

### **Ecological monitoring: indicators and metrics**

Project data aimed to provide the basis for understanding how indicators and metrics derived from benthic fauna may contribute to an ecological monitoring program to assess the potential future impacts of oil and gas exploration and/or production on the deep sea ecosystem of the GAB. Project data from baseline (unperturbed) sites provides the basis to evaluate indicators and metrics in future comparisons – a reference-site monitoring approach. We identified several opportunities to develop indicators and metrics by considering the data at both species- and assemblage-level. This assessment was underpinned by the robust (consistent species-level) taxonomic foundation for the collections taken during this and other studies – data were resolved to species-level across several major taxa. Data at the species-level are essential to generate robust metrics for species and many assemblage level indicators, and to subsequently develop knowledge about community structural and functional changes (including recovery) in response to disturbance.

## 3.2 Molecular assessment of benthic biodiversity in the GAB

### Objectives

1. Provide an overview of the biodiversity of sedimentary and pelagic assemblages in the GAB.
2. Develop an effective and rapid molecular monitoring tool to detect any functional changes in the microbial assemblages of the GAB.
3. Assess the presence of hydrocarbon degrading microbes in the GAB, match these to chemical analyses of hydrocarbons in the sediments, and infer the potential of the assemblage to cope with any future increase in hydrocarbon levels.
4. Undertake biodiversity identification using molecular barcoding of selected taxonomic groups of specific interest for future monitoring.

### Key Results and Discussion

#### Contribution of the project to the Theme

Project 3.2 took a broader perspective to characterising the deep-sea fauna, using a suite of leading edge molecular techniques to assess not only the larger species covered in Project 3.1, but also the micro- and meiofauna and microbial assemblages (Tanner et al. 2017). This contrasts with the focus of Project 3.1 on the larger epi- and infaunal elements. Both projects consider benthic fauna in relation to monitoring.

#### Sample collection

Samples for molecular analyses were collected on the surveys described above, either from sediment samples in association with the infaunal collections, or as tissue samples from either epifauna collected in the beam trawls or from infauna.

#### Molecular barcoding

Samples of individual infauna and epifauna from two field surveys (2013 and 2015) in the GAB were DNA barcoded for species identification using the cytochrome c oxidase subunit 1 (COI) region of the mitochondrial genome. The samples that were analysed successfully corresponded to 139 Arthropoda, 106 Echinodermata, 27 Mollusca, 14 Annelida, 11 Sipuncula and 6 Cnidaria species.

#### Microbial assemblages

The diversity and relative abundance of 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, was determined in sediment and water samples collected from the GAB. 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 have the capacity to degrade oil. Furthermore, the sequences of these genes were unique, suggesting that while these bacteria 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. Using the same samples, we analysed the community composition of Bacteria and Archaea. The bacterial assemblage was more diverse than the Archaea, and contained a variety

of taxa related to those that have increased in abundance in response to oil spills in other locations. The sediment and water samples had different microbial communities, and the sediment samples were clustered more tightly, indicating a higher degree of relatedness. The microbial communities in sediment samples collected from the continental shelf (200 and 400 m) differed from those collected from the continental slope (depths greater than 1000 m). Following this, we designed quantitative PCR primers specific to the microbial taxa indigenous to the GAB. Although these bacteria were rare (between  $10^{-5}$  to 0.1% of the total bacterial population), they were present at every station and every sample we analysed. These qPCR assays can be used as both a high throughput screening tool to measure the spatial and temporal duration of impact from routine discharges of petroleum and monitor environmental fate and persistence following an oil spill.

### **Biodiversity identification**

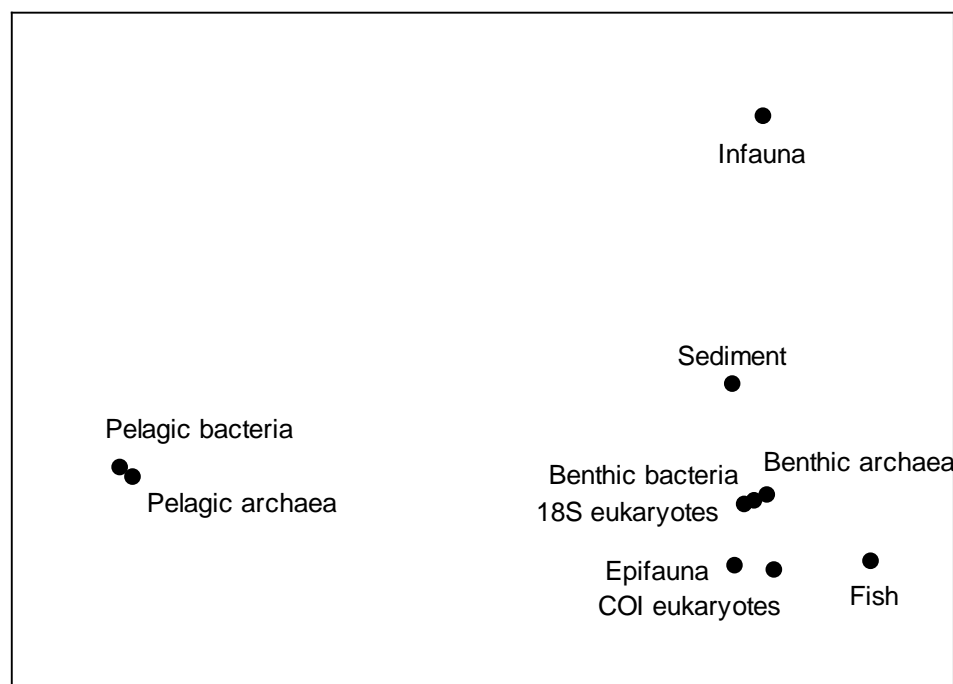
We employed two molecular metabarcoding approaches, using the nuclear 18S rRNA gene and COI, to assess biodiversity in a range of sediment samples from the GAB. For both genes, approximately 1000 OTUs (putative species) were identified, although the majority of these could not be assigned species level taxonomy (86.5% and 95.5%, respectively), either because they are known species that are missing from the barcode databases, or more likely, because they are new species or species not amenable to culture. Only seven of 303 taxa from the GAB that were individually barcoded were identified in the 65 sediment samples that were successfully analysed for the COI gene. Both gene regions show a clustering of assemblage structure with depth, although there were some clear differences in the taxa detected. The results suggest that either gene region would be suitable for rapid environmental monitoring, although substantial work is still required to collect, identify through traditional morphological taxonomy, and individually barcode the species present in the GAB for this approach to be able to tell us what actual species are present and how they vary over space and time.

## **CONTRIBUTION TO THE GABRP**

To explicitly compare the spatial patterns in assemblage structure between the different components of the deep-sea faunal assemblage that we sampled across both projects in the benthic theme, we undertook a 2-stage ordination. This analysis requires matching samples across the different assemblages to be compared, so was undertaken on averages at each station where multiple samples were taken at a station. We also excluded the 3000 m stations, as some analyses are based on 2013 samples only, when the maximum sampling depth was 2000 m. For each assemblage, we created a standard between-station similarity matrix, and then used each element of these matrices as a variable to create a similarity matrix of similarity matrices across assemblages. This second stage similarity matrix was then used in a standard non-metric multidimensional scaling ordination. The results (Figure 3), clearly show the majority of assemblages grouping together in the lower right of the plot, indicating that the spatial patterns within each assemblage are very similar. This grouping encompasses fish, epifauna, the assemblages documented using metabarcoding of both the COI and 18S genes, and bacterial and archaeal assemblages as determined using the 16S gene. The sediments show a somewhat divergent pattern, and the infauna a very divergent pattern. The molecular analyses also examined bacteria and archaea in near-bottom water samples from each station, and while both of these groups showed very similar patterns, they were very different to any of the benthic assemblages.



The close concordance of patterns between most of the components of the benthic community examined suggest that there is potential substitutability for routine ecological monitoring, but that infauna may not provide a good indicator of structure and response alongside other components of the community. In choosing a component to monitor, there would be a trade-off between ease and cost-effectiveness of sampling, versus ability to interpret what any changes mean. In this instance, sampling epifauna and fish requires lengthy trawl deployments and time consuming taxonomy, but it is easier to understand what any changes might mean, and possibly to link them to any known stressors. Molecular approaches, in contrast, require less sampling time, and can be relatively quick in terms of sample processing, but it can also be much more difficult to quantitatively define and interpret any changes and what they mean. This can be partially dealt with in at least two ways. Firstly, a concerted effort to obtain molecular barcodes from a greater range of GAB species will allow more species level assignments to be made when metabarcoding sediment samples, providing a clearer indication of what species are actually present. Secondly, molecular approaches that examine gene function can be employed. We have commenced this work with hydrocarbon degrading functions, so making molecular approaches more applicable for monitoring oil and gas activities, but it will need to be extended to other gene functions to monitor the impacts of other activities.



*Figure 3: Second stage ordination of multiple assemblage and sediment data sets generated by the Benthic Theme, showing the similarities of spatial patterns for each assemblage.*

Research undertaken by the Pelagic Theme was largely structured around the hypothesis that the central GAB (Figure 1, Transects T1-T3) was dominated by year-round down-welling, whereas the eastern GAB (our Transects 4 & 5) experienced summer upwelling, resulting in the central region being dominated by the microbial food-web and the eastern region dominated by the classic food-web during upwelling events. Overall, it appears that this picture is correct. However, we found no

evidence that this difference in pelagic food-webs translated into differences in the benthic assemblages, with no clear longitudinal patterns in any of the assemblages that we studied. This may be due to the fact that despite the food-web difference, there was no apparent difference in long-term pelagic productivity between the east and west. It is also possible that spatial differences only occur on the shelf and near the shelf-break, and that they don't occur in deeper waters that were the focus of our sampling. While no differences in benthic assemblages were found, we did find some differences in sediments from our 2 easternmost 200 m sites compared to the rest of our samples. These sediments were somewhat coarser than the remainder, which is consistent with the upwelling hypothesis which should lead to increased prevalence of bryozoans and other filter feeders. It is possible that more intense effort using gear better suited to sampling bryozoans would show a difference between the east and central GAB, or alternatively that the coarser sediments are being produced on the shelf and then transported off-shelf over time.

We have used data from Theme 1 (Oceanography) to help interpret the patterns in assemblage structure of infauna, epifauna and fish. For all three groups, bottom stress variables derived from the hydrodynamic modelling only explained a very low proportion of the variance in structure. While oceanographic variables were also used in the predictive modelling, the model domain for this work exceeded that of the GAB hydrodynamic model, and thus variables had to be derived from an existing broader-scale model developed by CSIRO.

Data on biomass and abundance of higher level taxonomic groups have been provided to Theme 7 for use in ecosystem modelling.

While we have carried out an extensive and broad benthic research project in the GAB, our characterisation of the assemblages present can still only be described as preliminary. Apart from a few repeat stations for infauna in 2013 and 2015, we only have a single snapshot in time from a relatively low number of widely dispersed samples. Sampling only targeted a single habitat type, only looked at standing stocks and not processes (with the exception of the gene function work on two transects), and did not seek to elucidate the response of any of the assemblages sampled to stressors. Future benthic work in the deep GAB should seek to address some of these gaps by:

- Further analysing the collective benthic data for its potential to define indicators for future ecological monitoring.
- Additional sampling of fauna in additional (structured) benthic habitats.
- Resampling multiple stations at multiple times to examine the extent of temporal variability, and how it relates to spatial variability. Ideally this should include not only between-year comparisons, but also within-year comparison to determine on what time scales the different assemblages are changing.
- Sampling at multiple spatial scales to determine what the characteristic spatial scale of variability is.
- Placing a greater emphasis on barcoding individual species, especially infaunal species, so as to enable a greater suite of species to be identified in metabarcoding. While multiple infaunal specimens were used for barcoding, most did not provide DNA of sufficient quality to be useful, and thus it may be necessary to sort samples on board and freeze them rather than just preserving in DNA quality ethanol.

- To get a better handle on function, a greater range of functional genes should be targeted.
- To start to understand processes and not just standing stocks, studies of benthic pelagic coupling using either benthic landers or on-board incubations of sediment cores should be undertaken. This will allow for example rates of oxygen uptake and nutrient transfer between the sediments and the water column to be measured.
- The population structure and demographic rates of key species should be examined through determination of individual specimen sizes and ages where possible.
- To enhance understanding of how the GAB ecosystem will respond to any potential oil spills, challenge experiments should be carried out on both sediment and water samples incubated on board in the presence of relevant oil. Oxygen uptake and nutrient cycling should be measured in these samples, as well as changes in the microbial assemblages and hydrocarbon levels present.

### Theme 3 outputs

- Project presentations in all four GABRP Symposia (2014-2017), the 2016 International Symposium on Microbial Ecology (Montreal), and the 2017 AMSA Conference Symposium (6 talks).
- Final reports for Projects 3.1 (Williams et al. 2017) and 3.2 (Tanner et al. 2017).
- A media release (September 2016) – SA newspaper and ABC radio (Deep-sea sponges)
- A catalogue of high quality images of benthic epifauna for the research community
- Web-based taxonomic notes on fauna (ScratchPad)
- A set of qPCR assays for use in rapid assessment of the presence of genes associated with microbial hydrocarbon degradation
- Papers published, submitted or in advanced draft for the primary scientific literature:

MacIntosh, H., Althaus, F., Williams, A., Tanner, J., Alderslade, P., Ah Yong, S., Criscione, F., Crowther, A., Farrelly, C., Finn, J., Goudie, L., Gowlett-Holmes, K., Hosie, A., Kuprianova, E., Mah, C., McCallum, A., Merrin, K., Miskelly, A., Mitchell, M., Murray, A., O'Hara, T., O'Loughlin, P.M., Paxton, H., Reid, A.L., Sorokin, S.J., Staples, D., Whitfield, E., Wilson, R.S. (submitted). The deepwater (200-4500 m) invertebrate fauna of the Great Australian Bight: composition, distribution and novelty. Biodiversity Research

O'Hara T.D., Harding C. (2014) A new species of *Sigsbeia* and additional records of ophiuroids from the Great Australian Bight. *Memoirs of Museum Victoria* 72: 131-140

O'Loughlin, P.M., and MacIntosh, H. (2015) New *Prototrochus* species of sea cucumber from Australia (Echinodermata: Holothuroidea: Synaptida: Myriotrochidae). *Zootaxa* 3995, 264-670.

Sasaki, M., and Beheregaray, L. (in prep) DNA barcoding of benthic diversity in the GAB. *Deep Sea Research II*

- Sherlock, M., Marouchos, A., and Williams, A. (2014). An Instrumented Corer Platform for Seabed Sampling and Water Column Characterisation. Proceedings of the OCEANS'14 MTS/IEEE Conference, Taipei. 1-6
- Tanner, J.E., McIntosh, H., Althaus, F. and Williams A. (submitted). Spatial patterns in infaunal and meiofaunal assemblages from the Great Australian Bight continental slope. Deep Sea Research II
- Tanner, J.E., Althaus, F., Sorokin, S.J. and Williams, A. (in prep). Biogeography of the southern Australian continental slope: Do comprehensive surveys accord with museum records?
- Van de Kamp, J., Hook, S.E., Williams, A., Tanner, J.E., and Bodrossy, L. (submitted). First characterisation of deep-sea hydrocarbon degrading microbial communities in the Southern Ocean. ISME Journal.
- van de Kamp J., Hook, S.E., Williams, A., Tanner, J.E. and Bodrossy, L. (in prep) Spatial patterns in microbial diversity based on 16S rRNA sequences in the Great Australian Bight. Environmental Microbiology.
- Williams, A., Althaus, F., Pogonoski, J., Osterhage, D., Gomon, M., Graham, K., Appleyard S.A, Gledhill, D., Bray, D., McMillan, P., Green, M., Doyle, S., Graham, A., Tanner, J.E. and Ross, A. (submitted.) Composition, diversity and biogeographic affinities of the deep-sea (200-3000 m) fish assemblage in the Great Australian Bight, Australia. Deep Sea Research II
- Williams, A., Althaus, F., MacIntosh, H., Loo, M., Gowlett-Holmes, K., Tanner, J.E., Sorokin, S.J. and Green, M. (submitted). Characterising the invertebrate megafaunal assemblages of a deep-sea (200-3000 m) frontier region for oil and gas exploration: the Great Australian Bight, Australia. Deep Sea Research II
- Wiltshire, K.H., Sorokin, S.J., Althaus, F. & Tanner, J.E. (in prep) Assessing environmental suitability of the GAB region for deep sea benthic taxa using species distribution modelling. Deep Sea Research II

### **Theme 3 outcomes**

*Transformed the GAB region from the most poorly known deep-sea region in the Australian marine jurisdiction to the best known.*

*Substantially increased the understanding of how the GAB relates to fauna at similar depths around southern Australia.*

*Improved the ability of stakeholders to understand and monitor potential future impacts on the structure of deep benthic biological communities, including within the GAB Marine Park.*

*Enabled knowledge of benthic biodiversity structure (composition, distribution and standing stock) to be built into ecosystem models for the GAB.*

*An improved ability for stakeholders to implement a monitoring program based on leading-edge and cost-effective techniques that are consistent with an Australian national standard.*

*An understanding of the relative levels of hydrocarbon degraders currently in the GAB that describes the natural baseline before oil and gas exploration commenced.*

*Insights into the capacity of indigenous microbial biota to degrade natural and enhanced levels of hydrocarbons, i.e. 'bioremediation capacity'.*

*A tool to rapidly and cheaply monitor the response of the hydrocarbon degrading community in the event of an accidental release of oil.*

*Improved ability to identify GAB deep-sea fauna through molecular barcoding, and confirmation of the potential for an NGS sequencing-based monitoring technique to provide a rapid and cost-effective approach for monitoring benthic biodiversity in the GAB.*

## CONCLUSION

The compositions, abundances and distributions of deep-sea benthic fauna (microbial, infauna, epifauna and fishes) have been documented in the GAB deep-sea for the first time, using a variety of traditional and novel techniques. With the exception of infauna, all the faunal elements documented showed similar spatial patterns, with depth appearing to play the dominant role in structuring them. There were no east-west spatial patterns detected in any of the assemblages examined, in contrast to the situation in the pelagic. A wide range of other covariates was examined, but all appeared to play a minor role in structuring assemblages in the central GAB. At a broader scale, species distribution models suggest that bottom temperature and oxygen availability play an important role in structuring benthic assemblages across southern Australia. The GAB fauna was more closely related to that off south-eastern Australia than south-western, although both the GAB and southwest regions are still very much under-sampled in comparison to Australia's south-east marine region. In total across the GABRP and the GABDMP, we documented 1,073 species of infauna, epifauna and fishes, of which 275 were new to science.

Genes associated with microbial hydrocarbon degradation are common in both sediment and water samples from the GAB, indicating that microbes in the region have the capacity to degrade hydrocarbons, and may play an important role in processing any spilled oil. However, the sequences found were unique, so their functional responses may not be predictable from studies elsewhere. Bacterial species related to hydrocarbon degraders known from elsewhere were also found, and qPCR assays have been developed that will allow rapid monitoring of the abundance of key bacterial taxa present in the GAB. Metabarcoding of eukaryote genes was also shown to produce similar results in terms of spatial structure in the assemblage as conventional sampling, and may be a useful approach for rapid assessment of faunal changes, although the vast majority of gene sequences were unable to be mapped to specific species.

Benthic biological characterisation will contribute to defining the needs for future ecological monitoring, including to identify key habitats, communities and species, map their distributions, and evaluate methods suited to monitoring change in status.

## REFERENCES

Rogers, P., Ward, T., van Ruth, P., Williams, A., Bruce, B.D., Connell, S.D., Currie, D.R., Davies, C.R., Evans, K., Gillanders, B.M., Goldsworthy, S.D., Griffin, D.A., Hardman-Mountford, N.J., Kloser, R.J., Middleton, J.K., Richardson, A.E., Ross, A., Tanner, J.E., and Young, J. (2013). Physical processes, biodiversity and ecology of the Great Australian Bight region: a literature review. CSIRO, Australia.

Tanner, J.E., Beheregaray, L.B., Bodrossy, L., Hook, S., Oxley, A., Sasaki, M., van de Kamp, J., and Williams, A. (2017). Molecular assessment of biodiversity in the Great Australian Bight. Final Report GABRP Project 3.2. Great Australian Bight Research Program, GABRP Research Report Series Number 26, 150pp.

Williams, A., Tanner, J.E., Althaus, F., Sorokin, S., MacIntosh, H., Green, M., Brodie, P., Loo, M. (2017). Great Australian Bight Benthic Biodiversity Characterisation. Final Report GABRP Project 3.1. Great Australian Bight Research Program, GABRP Research Report Series Number 19, 361pp.





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