FPE Seawall Ecological Assessment 2019

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Synopsis: **A report describing the results of ecological surveys carried out at and adjacent to the FPE seawall in 2019.**

REVISION/CHECKING HISTORY

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Executive Summary

Background

This report describes the characteristics of habitats and benthic flora and fauna communities on the Port of Brisbane Future Port Expansion (FPE) seawall and adjacent seabed habitats. This study provides the third ecological assessment of the seawall since completion in 2005.

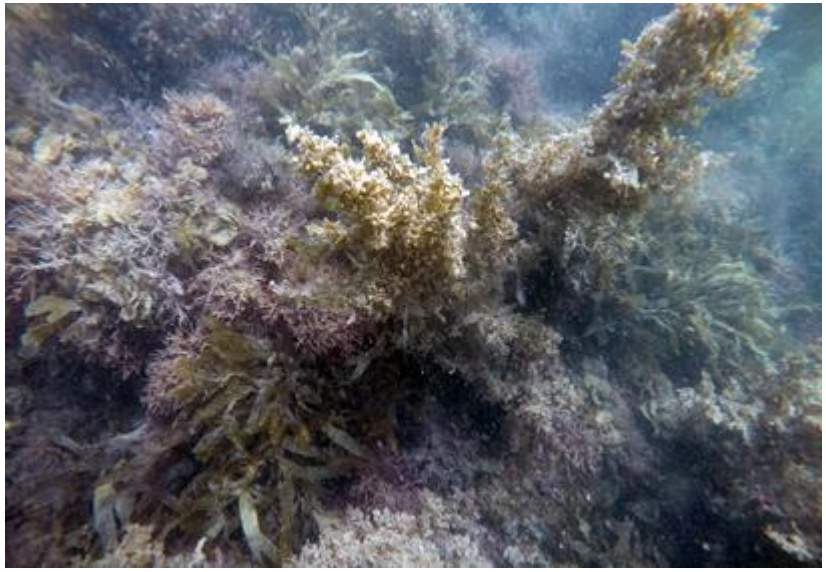
The study involved five key elements: (i) a SCUBA based assessment of seawall flora and fauna communities; (ii) a broad-scale acoustic survey of surrounding seabed habitats; (iii) a description of fish communities using Baited Remote Underwater Videos (BRUVs); (iv) a description of the seabed sediments adjacent to the seawall; and (v) a pilot study of fish assemblages using environmental DNA.

FPE seawall flora and fauna communities

The FPE seawall extends a total length of 4.6 kilometres. The FPE seawall links with other seawalls on the lower Brisbane River to provide the largest length of contiguous intertidal and subtidal hard substrate habitat within western Moreton Bay.

Consistent with previous surveys, the present study found that the FPE seawall supported diverse and abundant benthic flora and fauna assemblages. Shallow areas (<2 m LAT) on the eastern and northern section of the seawall supported a dense macroalgae canopy numerically dominated by brown alga *Sargassum*. The numerical dominance of *Sargassum* in shallow waters is consistent with patterns observed on natural reef systems within Moreton Bay.

Sargassum has a high light requirement and was sparse to absent in deeper and/or more turbid waters, consistent with previous surveys. On the northern and western side of the FPE seawall, deeper areas were numerically dominated by other algae species or were bare. The base of the seawall is subject to sediment deposition which likely limits the benthic assemblages.

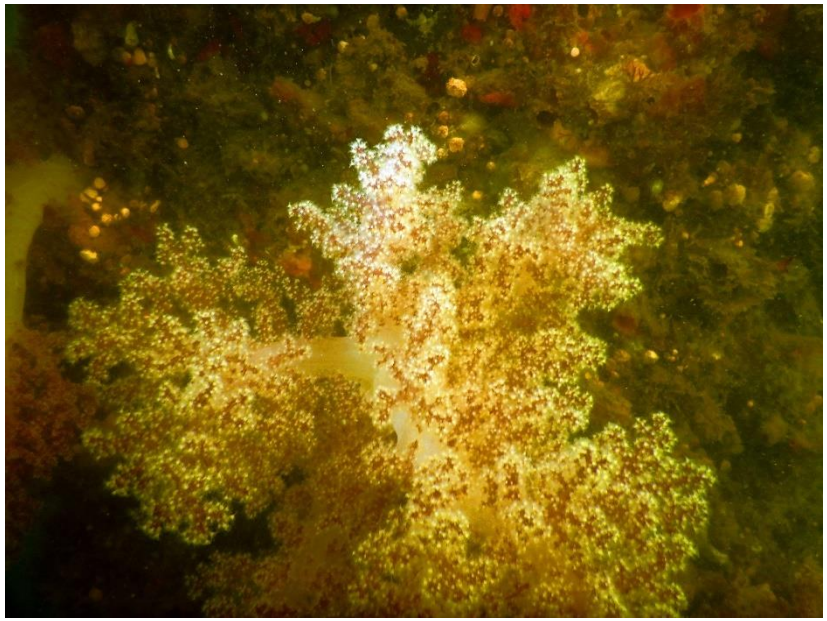


Macroalgae dominated assemblage on FPE rock wall - 2019

The present study represents the first targeted assessment of sub-canopy assemblages on the FPE seawall. A low-density assemblage of attached (sessile) fauna was present under the algae canopy, consisting mainly of filter-feeding organisms (sponges, soft corals, hydrozoans, hard corals, lace corals etc.). Consistent with previous surveys, hard corals were not abundant and comprised of species that tolerate low light conditions. Periodic low salinity and light conditions during flood events, together with shading by macroalgae, limits the development of abundant hard coral assemblages.

Qualitative assessments were undertaken of benthic assemblages on pylons and rock walls along the Brisbane River side of FPE seawall.

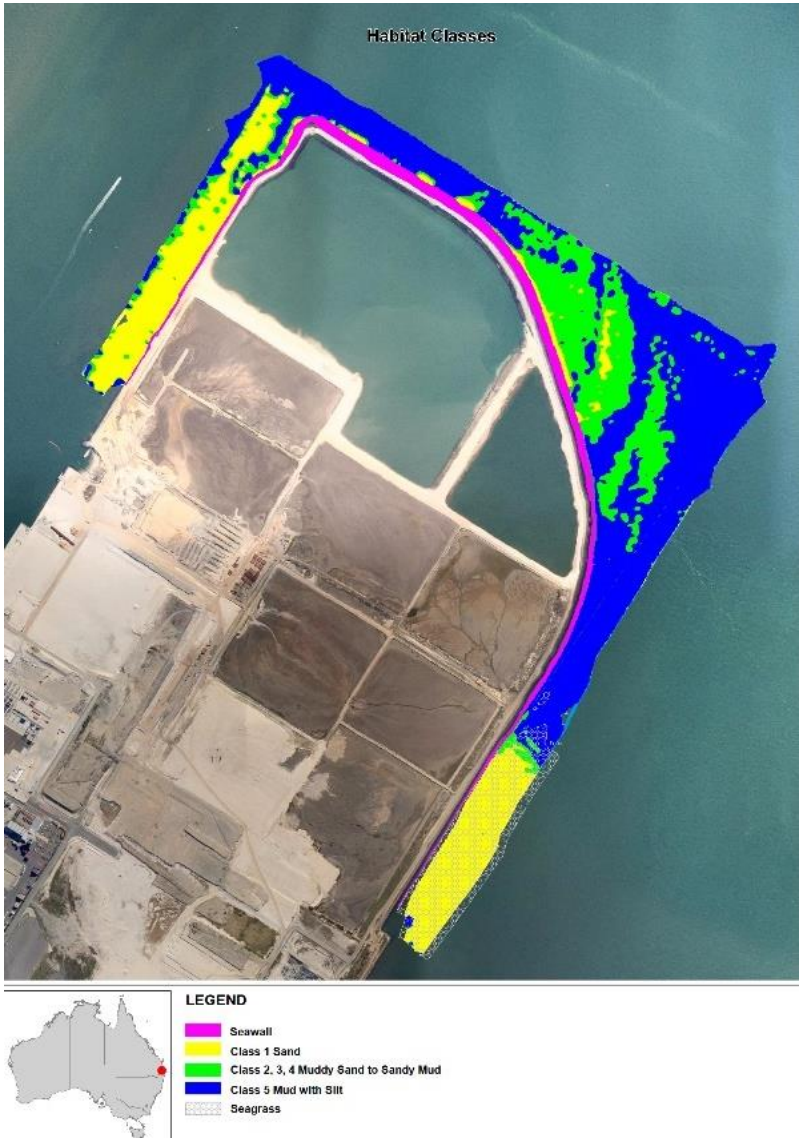
Like the western and northern sides, macroalgae numerically dominated in shallow areas. Pylons supported particularly rich and abundant epifauna assemblages comprised of soft corals, barnacles, oysters, bryozoans, colonial ascidians, solitary ascidians several species of sponges and stinging hydroids.



***Nephthya* soft coral colony growing on pylons**

Soft Sediment Habitats

The nature of benthic habitats directly adjacent to (within 100 m) of the seawall were assessed through acoustic-and video-based surveys. The acoustic system utilised in the 2019 survey provided far greater resolution than that of the 2013 survey, and used different classification techniques, therefore results are not directly comparable.



Seabed classes around the FPE Seawall

Executive Summary

Surface seabed habitats surrounding the seawall were comprised almost entirely of unconsolidated soft sediments. Sediments were primarily comprised of four classes: (i) fine to medium sands; (ii) muddy sand to sandy mud; (iii) mud with silt; (iv) rock wall.

Spatial differences in sediment composition across the study area are likely due to localised differences in hydrodynamic patterns. Several large sediment ridges east of the seawall were arranged in an approximately north-south direction suggesting that sediment transport is occurring from an east to east-south-east weather pattern. Sediments on the crest of the shallowest sediment wave appear to have more sand content, with muddier sediments present in troughs between the sand ridges.

The soft sediment seabeds are largely bare substrate, occasionally containing organic debris and/or surface periphyton layer. However, patches of sparse seagrass (*Zostera muelleri, Halodule uninervis*) and macroalgae were occasionally observed over sandy substrates.

Fisheries and Biodiversity Values

Consistent with the 2013 survey, the results of the present study demonstrated that the FPE seawall represents a high value fish habitat. BRUV surveys identified eight fish species of direct fisheries importance recorded. Yellowfin bream (*Acanthopagrus australis*) was the most abundant species in the 2013 and 2019 surveys. Other common species were the silver biddy (*Gerres subfasciatus*) and mackerel (*Scomberomorus* sp.). Consistent with 2013 survey, painted crayfish (*Panulirus ornatus*) were also observed on diver surveys.

eDNA metabarcoding of water samples was undertaken to test for the presence of marine reef fish, sharks, and dugongs. A total of four classes, 16 orders, 21 families and at least 23 species were detected in a total of eight litres of water. Bony fish were the most species rich group with at least 18 species detected. Three species of elasmobranchs (sharks, rays) were also detected. Dugongs were not detected in this study. Green turtle DNA was detected in one sample. Green turtles are frequent visitors to the area and were observed feeding on the abundant algae and fauna growing on the seawall.

Turtle resting on the FPE seawall

These results support the predictions of the FPE Seawall EIS that seawall habitats provide locally important fisheries habitat values. The FPE seawall also supports the biodiversity values of Waterloo Bay through the provision of habitat (e.g. feeding, shelter, breeding areas etc.) for a multitude of reef-associated algae, invertebrates and fish species.

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1 Introduction

1.1 Background

The Port of Brisbane Pty Ltd (PBPL) has its main port infrastructure at Fisherman Islands, situated at the mouth of the Brisbane River. To meet growing demand for port land, the Future Port Expansion (FPE) project was initiated, resulting in the creation of a 4.6 km perimeter seawall and the associated reclamation of ~230 ha of subtidal seabed. FPE seawall construction commenced in 2002 and was completed in August 2005, and the area within the reclamation is progressively being in-filled with dredged material.

An Impact Assessment Study (IAS) (WBM 2000a) and supplementary IAS (WBM 2000b) for the expansion of the Fisherman Islands port facilities were approved in June 2001. The IAS predicted that the seawall would eventually be colonised by reef associated flora and fauna, and that the seawall was likely to provide both an artificial reef habitat and a fish aggregation site. The seaward side of the seawall now represents an important boat-based recreational fishing area. Investigations into the spatial characteristics and potential environmental values of the seawall communities and nearby seabed habitats were commissioned by the PBPL in 2009 and 2014 (BMT WBM 2009; 2014). These investigations showed that reef associated flora and fauna had colonised the seawall area and that it hosted a variety of commercially important species and providing locally important ecosystem functions (BMT WBM 2009; 2014).

The IAS also identified that the FPE seawall operation had the potential to modify tidal current dynamics within a localised area at Fisherman Islands, and the direction of freshwater flows from the Brisbane River. These changes could alter localised sediment movement and the extent and distribution of seagrasses within the Fisherman Islands area. In conjunction with monitoring of communities colonising the seawall itself PBPL also commissioned monitoring of the effects of the seawall on adjacent marine environments. As part of this monitoring program the nature of the marine sediments and seabed habitats adjacent to the FPE seawall were also categorised by BMT WBM (2009; 2014).

The present study is a continuation of the monitoring program commenced by PBPL in 2014 and involves three main components:

(1) Characterisation of habitat and benthic communities on the FPE seawall.

(2) Fish surveys to quantify fish community assemblages in and around the FPE seawall.

(3) Seabed survey of areas adjacent to the seawall to determine seabed habitats and physical properties of sediments within port limits surrounding the seawall.

In addition, a pilot study was carried out to trial the application of environmental DNA metabarcoding to detect species of interest (fish and other marine vertebrate fauna).

This study reports on the development of both rocky reef and soft-sediment communities of the FPE seawall and the surrounding area and provides an improved understanding of the ongoing changes; both adverse impacts and ecological improvements of the FPE seawall. This information will assist PBPL management with future port planning (i.e. understanding impacts and values of seawall

habitats), and to identify information gaps that require further assessments and/or monitoring activities.

This report is also accompanied by additional deliverables (e.g. digital video imagery and photographs) that can be used by the PBPL management for interpretative and educational activities.

1.2 Study Aims and Objectives

The primary aim of this investigation was to assess the spatial characteristics and environmental values of seawall communities and nearby seabed habitats and to compare these values to previous monitoring results in order to document temporal changes to these communities. The specific objectives of this study were to:

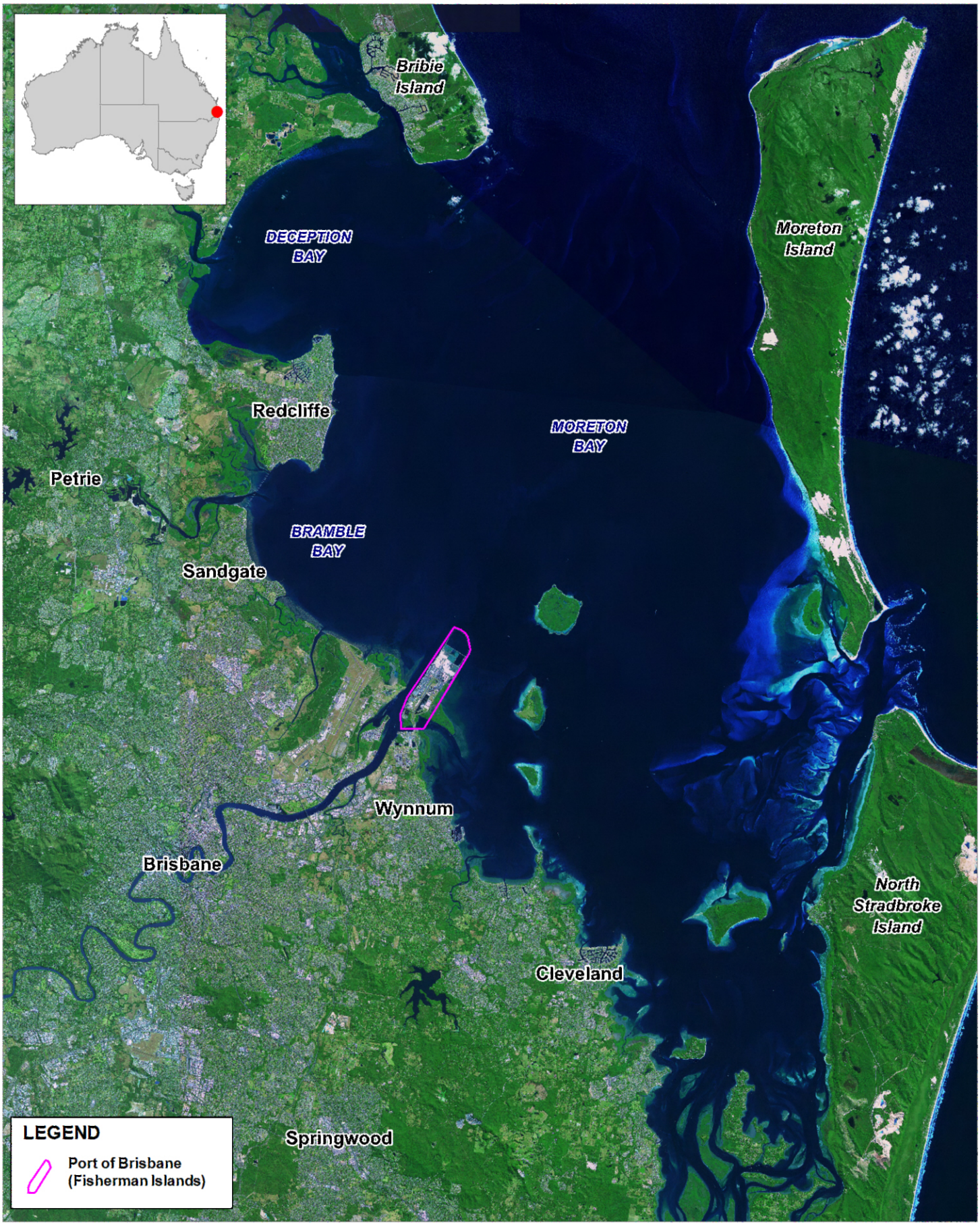
- Quantify spatial and temporal patterns in benthic flora and fauna community structure on the FPE seawall;
- Describe the composition and relative abundance of fish around the FPE seawall;
- Describe spatial patterns and physical characteristics of the soft-sediment habitats surrounding the FPE seawall, and based on comparison to historical data, determine any gross changes in sediment properties over time.

1.3 Study Area Context

The Port of Brisbane is located at Fisherman Islands (27º 22' 57" S, 153º 10' 10" E), which is situated at the mouth of the Brisbane River on the western foreshore of Moreton Bay, Queensland (Figure 1-1). The port facilities at the river mouth (hereafter 'the study area') have been established on land reclaimed over a shallow sub-tidal river delta containing a series of low-lying mangrove islands, collectively called the Fisherman Islands. The area was reserved for harbour purposes in the 1940's. Reclamation commenced in the late 1960's and has been ongoing since that time.

The Future Port Expansion (FPE) reclamation area contains an outer perimeter rock wall (FPE seawall) that is under progressive filling. The FPE seawall extends along the current port quay line into Moreton Bay to the northeast for approximately 1.8 km, before sweeping in a flat-sided horseshoe shape to the south and joining back to the port some 1,400 m south of the start point.

Construction of the present-day port facilities over intertidal and subtidal areas has resulted in extensive changes to the environmental attributes of the Fisherman Islands area. However, significant areas of mangrove, saltmarsh and seagrass ecosystems have also been retained and form part of the Fisherman Islands wetland complex on the southern side of the Port of Brisbane. Situated to the south and east of the FPE seawall lays Moreton Bay Marine Park. The area of the Marine Park adjacent the port is thought to contain one of the largest semi-contiguous seagrass beds in western Moreton Bay. A Ramsar listed wetland is situated to the south of the Port facilities, comprising intertidal portions of the Fisherman Islands wetland complex. The seagrass and mudflats of this Ramsar area are recognised for their importance to dugong, marine turtle and migratory and resident shorebird populations.



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Locality Plan

Figure: 1-1

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BMT WBM endeavours to ensure that the information provided in this map is correct at the time of publication. BMT WBM does not warrant, guarantee or make representations regarding the currency and accuracy of information contained in this map.

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2 Methods

2.1 Seawall Benthic Community Assessment

Benthic habitat characteristics of seawall flora and fauna communities and environmental values were assessed at sites previously surveyed by BMT WBM (2009, 2014) to describe communities and compare changes through time.

2.1.1 Survey Sites and Timing

Field surveys were undertaken over 27 – 29 May 2019. Winds in the month leading up to the sampling period were moderate (15 km/h) and lower than average rain had fallen in the catchment within the one month period leading up to sampling (34.9 mm for 2019 vs 98.4 mm 25 year average) (Figure 2-1). Water clarity was therefore suitable for visual surveys (>2 m visibility).

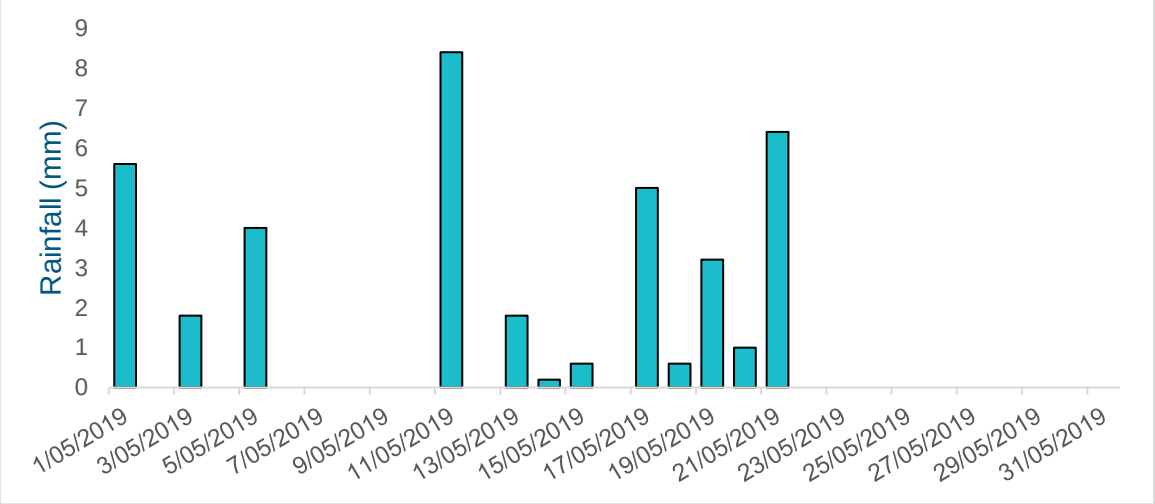

Figure 2-1 Rainfall in May 2019 (Source: Bureau of Meteorology 2019 BoM weather station 040842 Brisbane Aero)

Five quantitative survey sites were surveyed along the FPE seawall (refer Figure 2-2 and Table 2-1). In addition, three qualitative dive sites were surveyed along the north western side of the FPE seawall (see Figure 2-2). Survey data from these sites has not been included in any statistical analysis in this report but summary information and video from these sites has been supplied to PBPL as reference material and for interpretive and educational activities.

Table 2-1 FPE survey sites

Site number	Location	Site type
Site 1	North west wall (Brisbane River)	Quantitative survey site
Site 2	North east wall (north)	Quantitative survey site
Site 3	North east wall (south)	Quantitative survey site
Site 4	South east wall (north)	Quantitative survey site
Site 5	South east wall (south)	Quantitative survey site
Site 6	General Purpose Wharf	Observation site
Site 7	Fisherman Islands No. 4	Observation site
Site 8	Fisherman Islands No. 11	Observation site

The five quantitative sites were divided into three depth strata (relative to LAT): 1.0 m; 2.5 m; and 4.0 m. A submersible depth gauge was used to measure water the depths at each transect, and the time was noted. Tidal prediction data, estimated at 10-minute intervals for the Brisbane Bar (Queensland Maritime Safety), was then used to accurately standardise the depths of each transects relative to the LAT.

One 25m long randomly placed transect was positioned parallel to the depth contour in each depth stratum. A diver (on SCUBA) used paired high-definition cameras with underwater housing and dual 1800 lumen lighting to record the benthic substrate along the transect. Imagery was collected from 20-30 cm above the seafloor, providing a 0.5-1 m wide swath of imagery. One camera collected still imagery every two seconds while the other was used to collect macro and sub canopy imagery. This approach allowed for objective selection of still imagery because stills were collected randomly. The macro images were used to aid in identification. When required, specimens were collected to confirm identifications.

2.1.2 Benthic Cover Analysis

CoralNet was used to quantify benthic cover by projecting a random selection of points over each photo and identifying visually distinct taxa or substrate classes (bare, macroalgae, oysters, barnacles etc.) over each point. CoralNet uses image recognition technology to automatically analyse each image. The program is also a repository and a resource for benthic images analysis. The site deploys deep neural networks which allow fully and semi-automated annotation of images. It also serves as a repository and collaboration platform. Twenty points were identified on each photo on a selection of seven randomly selected photos per transect, giving a total of 140 point identifications per transect. Data were then collated based on the strata and site to calculate percentage cover.

Patterns in community attributes were summarised using simple descriptive statistics (mean, standard error, % cover of different taxa groups), which were plotted and tabulated.

Title:

Location of FPE seawall ecological survey sites

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Patterns in assemblage structure at different sites and depth strata were also analysed using a range of multivariate statistical procedures. For all multivariate analyses, raw data were initially fourth-root transformed and a similarity matrix was generated using the Bray-Curtis measure of similarity. Based on this similarity matrix, the following tests were performed:

- Non-metric multi-dimensional scaling (n-MDS); performed on the similarity matrix to graphically present the similarity of samples based on 2-d configurations (Clarke 1993). Hierarchical cluster analysis was then performed on the similarity matrix using the average linkage method, and groupings were superimposed on MDS plots to check the adequacy and agreement between the two techniques and determine the group membership of samples.
- Two-way crossed ANOSIM; used to determine differences in assemblages among sites and depths.

2.1.3 3D Mosaic Generation

Three-dimensional mapping of the seawall and surrounding seagrass was attempted as a monitoring and stakeholder engagement tool at site 5. Poor visibility associated with a dense school of mysid shrimp, and shallow depths caused the imagery to be unreadable by the 3D model generating software. The imagery collection was attempted more successfully at site 2. The soft sediments adjacent to the seawall, and base of the seawall that was free of long stranded algae species such as *Sargassum* was able to be captured in a 3D model. The wave movement even on calm days at the FPE seawall generates enough movement in the dense macro algae cover to not allow the stitching of imagery.

Notwithstanding this, 400-500 georeferenced photos were taken at site 2 using a wide-angle still camera interfaced with a surface RTK GPS. Photos and positions were built into 3D models using proprietary techniques involving:

- Batch processing of photos to remove poorly focused images.
- Colour correction to bring up red tones and reduce green and blue levels.
- Removal of lens vignetting to homogenise lighting across the field of view.
- Photogrammetry using tie points at precisely known locations.
- Generation of 3D models and orthomosaics from aligned imagery.

2.2 Baited Remote Underwater Video Assessment

Baited remote underwater video (BRUV) assessments were conducted at each of the five quantitative dive sites (Figure 2-2). At each site, the BRUV was lowered to the seafloor within 2 m of the toe of the wall. BRUVs were baited with pilchards as these have repeatedly been the most consistent baits for attracting a wide range of species (Wraith *et at.* 2007). Imagery was collected with a single high-definition submersible video camera.

The first 30 minutes of each recording were analysed, starting from the time that the BRUV reached the seafloor. The time to first (identifiable) appearance (t1st) was recorded for each species, as well as the maximum number of individuals of each species observed within a single frame over the

duration of the recording (max N), max N was then summed to give the total max N for each location. These metrics and total richness were calculated and presented.

2.3 Soft Sediment Habitat Assessment

This component aimed to assess the nature of seabed habitats directly adjacent to (within 100 m) of the FPE seawall. This included the following:

- Soft sediment habitat mapping – using interrogation of 200 kHz single beam echo sounder returns and mapping of 455 kHz side-scan sonar
- Underwater video of soft sediment areas
- Physical qualitative characterisation of sediments.

2.3.1 Soft Sediment Habitat Mapping

2.3.1.1 Acoustic Survey

Approximately 42 km of acoustic lines were surveyed throughout the full extent of the study area, covering the same extent as that surveyed in 2009 and 2014. The number of acoustic lines and distance between lines were chosen to cover and represent as much of the study area as possible over the survey period conducted on May 30th, 2019. A vessel equipped with an RTK GPS was used to determine height above AHD (GDA 2020) for the duration of for the acoustic survey. Vertical error (root mean square error) less than 2 cm was used in the tidal reduction.

Acoustic sounding was conducted using a 200 kHz single beam and 455 kHz CHIRP Lowrance echo sounder and stern transducer. Echo return intensity, depth picks, and sidescan imagery were recorded to a HDS9 unit.

2.3.1.2 Acoustic Data Processing

Side-scan imagery was imported to SonarWiz 7 to perform bottom tracking, nadir removal, and slant-range correction. Time-variable gain (TVG) and automatic gain control (AGC) corrections were adjusted to generate corrected imagery and settings applied to all data. Final mosaic products were exported as geo-tiffs for use in MapInfo 15. Raster imagery of the wall extent was digitised and added to acoustic hardness generated from downscan (single-beam) data.

Single-beam echo return intensity (db), position, and depth under keel were retrieved from Lowrance SL3 files. RTK GPS positioning of height (AHD AusGeiod2020) was used to reduce tides. Measured offsets (under-keel depth, antenna height) and water depth and geoid height were used to convert height above MSL (AHD) to lowest astronomical tide (LAT) using tidal planes described by Maritime Safety Queensland. Seafloor depths relative to LAT were determined as points and interpolated using Triangulation spatial interpolation in Vertical Mapper. Grid cells of 2 m were generated for the soundings. Comparisons between LAT between 2014 and 2019 were made by subtracting grids from the respective years in Vertical Mapper.

Acoustic returns from single-beam echo sounders are composed of a first return directly from the sea floor and a second return, covering twice the distance of the first return. The second return travels to the water surface, is reflected back to the sea floor then back to the transducer. Signal amplitude

of the first return is typically more useful for understanding complexity (roughness) of the seafloor, while the amplitude of the second return is better for defining acoustic impedance or hardness (Chivers *et al.* 1990, Kloser *et al.* 2001; Penrose *et al.* 2005). Analysis of the second return is not appropriate in very shallow waters.

Seafloor hardness was determined by mapping the intensity of the peak backscatter intensity (dB) from the 200 kHz first echo return. The second return hardness was also investigated but was a poorer descriptor of the patterns observed during ground-truthing than peak backscatter intensity; the latter measure was mapped as points and interpolated using MapInfo 15 and Vertical Mapper. Decibel ranges for mud and sand habitats were established based on ground-truthing data. Three transitional habitats between mud and sand were initially established as fine-scale ranges between these two substrates, then transitional sand/mud substrates were combined into one class for the sake of simplicity.

2.3.1.3 Broad Scale Sediment Assessment and Habitat Validation

The acoustic based methodology produced a map of different acoustic substrate classes, which were investigated using other methods. Video assessments were used to ground-truth the mapping results and identify the seabed classes revealed by acoustic analyses. Video surveys were undertaken, as near as possible, along the same transect/grid lines as the acoustic survey. Sixteen drop-camera video transects along the acoustic survey transects were re-sampled, ensuring that major acoustic substrate classes were sampled and validated through this process. This was based on the classes determined by the 200 kHz echo backscatter intensity, where video methods provided a surface view of the substrate.

2.3.2 Benthic Grabs for Sediment

Samples were collected and photographed on May 30th 2019 from 10 of the 16 sites and. Sediment was collected via the use of a stainless steel van Veen grab with a surface gape of 0.028 m^2. Subject to the density and stiffness of the sediment, grabs were able to penetrate and collect surface material to a sediment depth of 0.12 m. Only whole grab samples (i.e. those in which the sampler jaws remained closed following the sample capture) were retained.

On collection, samples were placed onto a tray, photographed, and the following details were recorded:

- Sediment colour
- Field texture (i.e. fine sand, coarse sand, silts, shell fragments etc.)
- Estimation of dominant grain size and composition
- Sediment odour
- Presence of organic material or any foreign objects
- Presence of any marine flora and fauna.

Title:

Acoustic, Drop Camera, and Sediment Sampling Sites

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2.4 e-DNA

2.4.1 Sample Collection and Handling

Water samples were collected by BMT staff on 30th May 2019 at the Port of Brisbane Seawall. Four replicates were taken at two sites (sites 2 and 5) totalling eight water samples. No water control was collected.

Water samples consisting of 1 L were collected and filtered using 0.45 µm mixed cellulose esters with a peristaltic Sentino pump to capture eDNA present in the water. All filtering was carried out by BMT staff. Water membranes were frozen prior to shipping. All samples were shipped frozen to eDNA frontiers and on arrival, samples were stored at -20°C.

2.4.2 Laboratory Methods

2.4.2.1 eDNA Extraction and Analysis

DNA was extracted from half a filter paper using a Qiagen DNeasy blood and tissue kit, following the eDNA frontiers lab's SOPs and detailed in Koziol et al. (2018), Stat et al. (2017), Stat et al. (2018). Each water sample was assigned an individual barcode tag and amplified by PCR using three assays: (i) a proprietary mtDNA 16S assay termed '16S NEST' (ii) a mtDNA COI assay termed 'COI elasmo' (Ward et al. 2005, Fields et al. 2015) and (iii) a 16S mammal assay (Taylor 1996). A library was generated and sequenced using the Illumina MiSeq® platform. Laboratory extraction controls were included to test for contamination.

2.4.2.2 Bioinformatics and Taxonomic Assignments

Bioinformatic tools were used to analyse raw sequence data. Results were demultiplexed and trimmed using ObiTools and quality filtered with Usearch v11 for sequencing errors (maxee=1) and minimum length (COI minlength=100, 16S NEST minlength=50, 16S Mammal minlength=90). Sequences were then dereplicated and unique sequences were transformed into zero radius operational taxonomic units (ZOTUs) to provide sensitive taxonomic resolution (Usearch v11) (Edgar 2018). ZOTUs, in contrast to OTUs are a more exact sequence variant, clustering at 99% to improve taxonomic resolution. Generated ZOTUs were queried against the nucleotide database NCBI (Genbank) and assigned to the species level. Taxonomic assignments were based on an in-house Python script which does further filtering of Blast results (evalue <= 1e-5, %identity >= 94 and qCov >= 100), combines it with ZOTU table results and produces a table containing the taxonomic information available from Blast taxonomy database (accessed August 2019).

It is important to note that barcodes recovered are converted to the lowest possible taxon based on similarities and differences to a DNA database (NCBI's Genbank). This database, and the taxonomic framework that underpins it may contain errors. Accordingly, the DNA taxon identifications should be interpreted as the best available assignment based on currently available information and that errors at species level are possible.

3 Results

3.1 Seawall Benthic Communities

Benthic biota classes recorded on the FPE seawall in May 2019 were as follows (Table 3-1):

- Eight Phaeophyta (brown) algae taxa;
- Seven Rhodophyta (red) algae taxa;
- Two Chlorophyta (green) algae taxa;
- Four Porifera (sponge) taxa;
- Two groups each of Octocorallia (soft corals); and Crustacea (barnacles and crayfish)
- One group each from Scleractinia (hard corals); Chordata (ascidians); Hydrozoa (hydroids); Annelida (polychaete worms); Mollusca (gastropods); and Arthropoda (crustaceans).

Other fauna noted by divers and towed camera, but not included in the transect analysis, included:

- Two groups of Echinodermata (sea urchins and sea cucumbers);
- One group of Annelida (Christmas tree worms);
- One group of Orectolobidae (spotted wobbegong shark);
- One group of Crustacea (mysid shrimps);
- One group of Mollusca (nudibranchs);
- One group of Reptilia (green turtle).

3.1.1 Marine Flora

Macroalgae numerically dominated the benthos at all sites and depths (Table 3-1; and Figure 3-2). Phaeophyta (brown algae), including *Sargassum* sp., *Dictyopteris australis* and various species of *Lobophora*, *Padina* and *Zonaria* were the most widespread and abundant groups. Also common were the red algae *Asparagopsis taxiformis* and *Hypnea* sp. and the green alga *Bryopsis indica.*

Site 1 was numerically dominated by brown algae and red algae which together comprised almost 70% of the total benthic cover at the site. The numerically dominant species represented 14% (*Sargassum* sp.) and 13% (*Hypnea* red algae) to total percentage cover. The remaining benthic substrate was comprised of bare substrate and a sparse benthic fauna cover (oysters, barnacles).

Sites 2 and 3 on the more exposed north-eastern side of the wall had the highest brown and red algae cover. *Dictyopteris australis* was the most species at site 2 (40% cover) and *Hypnea* was the most abundant species at site 3 (25% cover). Bare substrate made up the third highest percentage cover numerically at both sites. Similar algal dominated assemblages were seen among depths.

Sites 4 and 5 were numerically dominated by *Hypnea sp.* and *Dictyopteris australis*. Turfing algae and bare substrate dominated deep water transects at site 4, most likely due to low light availability at this depth. *Sargassum* sp., *Dictyopteris australis* and *Caulerpa* were abundant at site 5.

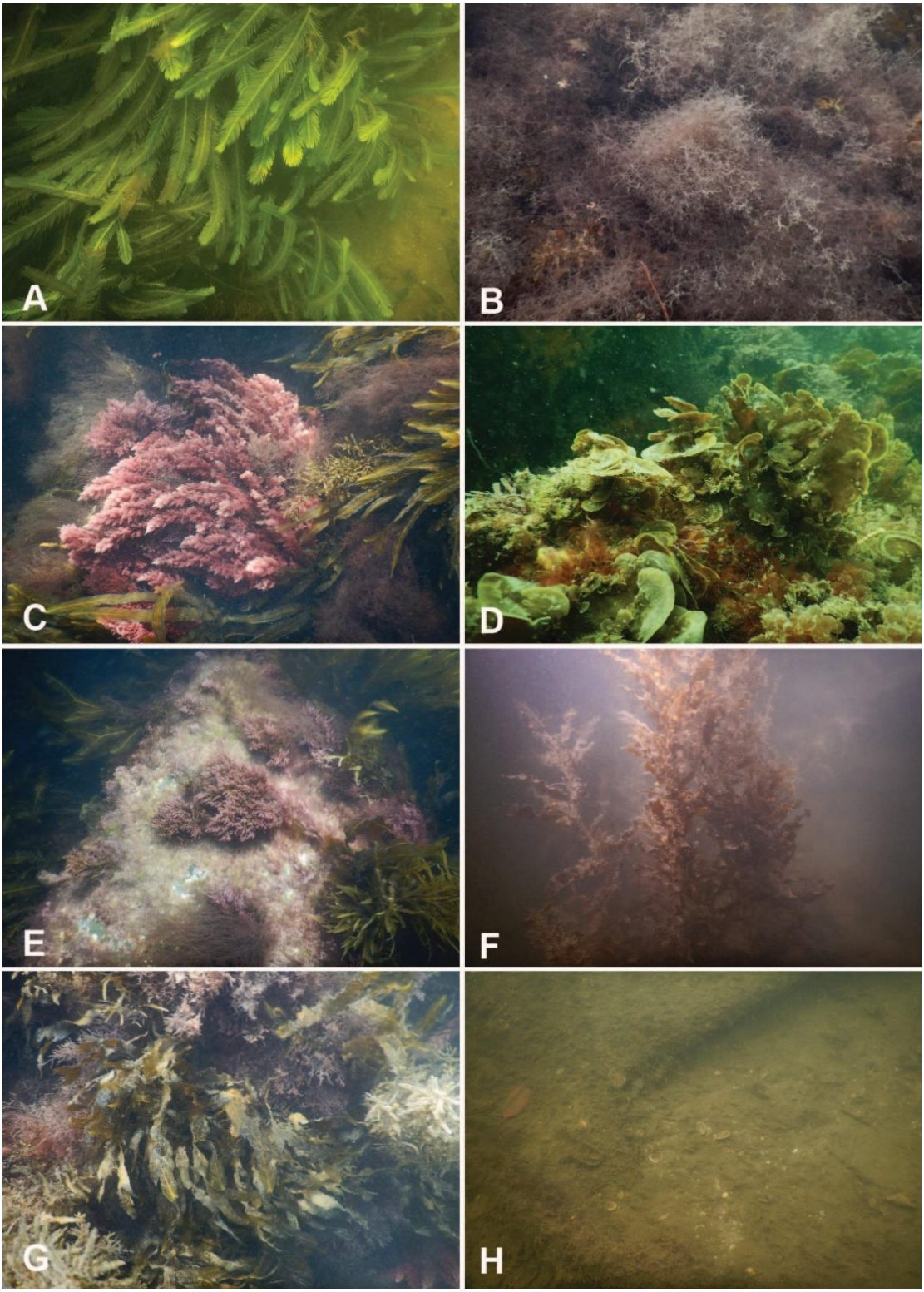


Figure 3-1 Examples of macroalgal communities: *Caulerpa taxifolia* (A); *Hypnea* (B); *Asparagopsis taxiformis* (C); *Zonaria diesingiana* (D); *Pterocladia* (E); *Sargassum flavicans* (F); *Dictyopteris* (G); turfing algae (H).

Table 3-1 Average percentage cover of each benthic group – May 2019

Taxa	Species Name	Site 1	Site 2			Site 3			Site 4		Site 5	
		0.5-1.0	0.5-1.0	1.0-2.0	>2.0	0.5-1.0	1.0-2.0	>2.0	0.5-1.0	1.0-2.0	0.5-1.0	1.0-2.0
Annelida	Sabellidae Fan Worm	0.00	1.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mollusca	*Tristaniopsis alba* (white lace nudibranch)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Oyster	11.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chordata	Colonial Ascidian 1	0.00	0.71	0.00	0.00	0.00	0.00	0.00	2.14	0.00	0.71	0.00
	Solitary Ascidian	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Crustacea	*Panulirus versicolor* (painted crayfish)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Barnacle	3.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Other Crustacean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.00
Echinodermata	*Temnopleurus alexandri* (Alexander's sea urchin)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Holothurian 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Porifera	Orange Sponge	4.29	3.57	0.00	0.00	0.00	0.00	1.43	0.00	0.00	0.71	0.00
	Black Sponge	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	White Sponge	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Yellow Sponge	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Scleractinia	Family Faviidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.43	0.00	0.00	1.43
Hydrozoa	Order Leptothecata (stinging hydroids)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.71
Alcyonacea	Family Clavulariidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.14	0.00	0.00	0.00
Octocorallia	Dendronephthya sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Family Alcyoniidae	2.14	0.00	0.00	0.00	0.00	0.00	0.00	2.86	0.00	0.00	0.00
Total Fauna		21.43	5.71	0.00	0.00	0.00	0.00	1.43	8.57	0.00	1.43	7.14
Brown algae	c.f. *Dictyopteris australis*	9.29	11.43	48.57	49.29	25.00	21.43	15.00	5.00	38.57	16.43	22.86
	Hincksia sordida	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.86	0.00	0.00	0.00
	Lobophora spp.	0.00	2.14	0.00	0.00	0.00	0.00	0.71	1.43	0.00	1.43	0.00
	Padina spp.	5.71	1.43	0.00	0.00	7.14	0.00	0.00	0.71	0.00	0.00	2.86
	Zonaria spp.	0.00	8.57	1.43	1.43	21.43	0.00	12.86	27.86	0.00	14.29	1.43

Results

Taxa	Species Name	Site 1	Site 2			Site 3			Site 4		Site 5	
		0.5-1.0	0.5-1.0	1.0-2.0	>2.0	0.5-1.0	1.0-2.0	>2.0	0.5-1.0	1.0-2.0	0.5-1.0	1.0-2.0
	Sargassum spp.	22.86	5.71	20.71	25.71	10.00	37.14	20.71	2.86	7.14	3.57	10.71
	Other BA 1	7.14	1.43	0.00	0.00	0.00	0.00	0.00	7.14	0.00	4.29	0.00
	Other BA 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Other BA 3 (c.f. *Dictyota sp.*)	0.00	5.71	0.00	0.00	1.43	0.00	1.43	3.57	1.43	2.14	0.00
Total Phaeophyta		45.00	36.43	70.71	76.43	65.00	58.57	50.71	56.43	47.14	42.14	37.86
Red algae	*Asparagopsis sp.*	0.00	0.00	0.00	5.00	0.71	6.43	3.57	2.86	1.43	0.71	0.71
	Other Red Algae	8.57	15.71	0.00	0.71	1.43	3.57	0.00	9.29	0.00	2.14	1.43
	Foliose coralline algae	0.00	7.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Hypnea sp.	5.00	21.43	28.57	15.71	32.14	27.86	39.29	8.57	38.57	25.71	9.29
	c.f. *Martensia sp.*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.71	0.00	0.00	0.00
	Acanthophora sp.	0.00	0.00	0.00	0.00	0.71	0.00	1.43	1.43	0.00	3.57	0.00
	Crustose Coralline Algae	1.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.71	0.00	5.71
Total Rhodophyta		15.00	44.29	28.57	21.43	35.00	37.86	44.29	22.86	40.71	32.14	17.14
Green algae	*Bryopsis indica*	1.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Caulerpa	0.00	3.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.57
Other	Turfing Algae	4.29	0.00	0.00	0.00	0.00	2.86	0.71	0.00	7.14	10.00	0.71
Total Algae		65.71	84.29	99.29	97.86	100.00	99.29	95.71	79.29	95.00	84.29	64.29
Cyanobacteria	Blue Green Algae	0.00	0.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Rock (Rock)	4.29	0.71	0.00	0.00	0.00	0.00	2.14	3.57	1.43	7.86	2.86
	Sand (Sand)	0.00	5.71	0.00	0.00	0.00	0.00	0.00	4.29	0.00	0.71	16.43
	Silt (Silt)	0.00	0.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.43
	Fissure	8.57	2.14	0.71	2.14	0.00	0.71	0.71	4.29	3.57	5.71	7.86
	Flotsam/seagrass wrack (CO)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Bare Substrate		12.86	9.29	0.71	2.14	0.00	0.71	2.86	12.14	5.00	14.29	28.57

Not found 2019 Newly identified 2019

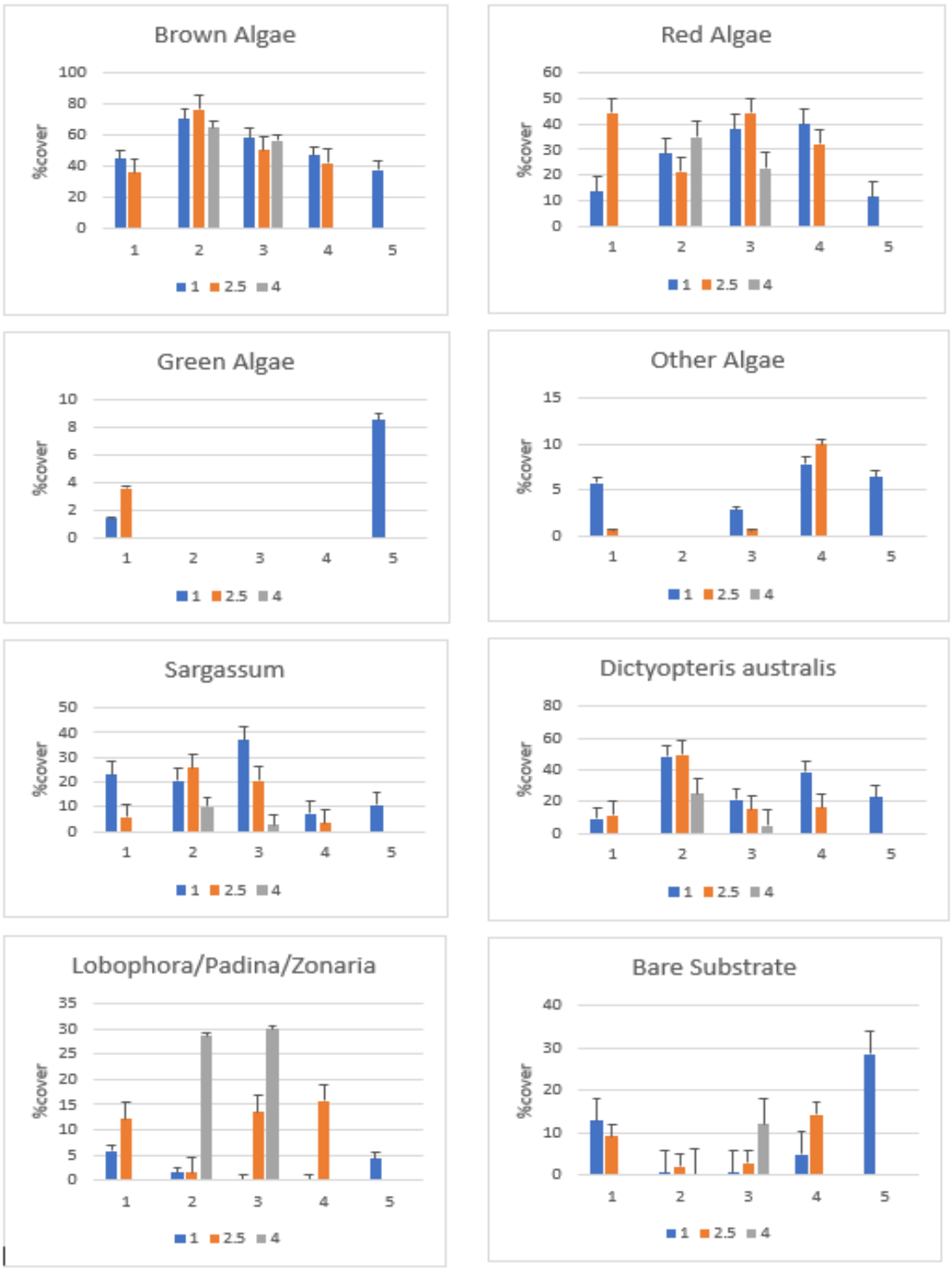


Figure 3-2 Mean percentage cover of the major algal groups, some common species and bare substrate at each site and depth category (1 m, 2.5 m, 4 m)

3.1.2 Marine Fauna

Sessile fauna taxa represented on the FPE seawall included several sponge taxa, stinging hydroids, soft and hard corals, ascidians, fan worms (Sabellidae), barnacles and oysters. Mobile benthic taxa were also recorded including nudibranchs (*Tritoniopsis alba)*, painted crayfish (*Panulirus versicolor)*, sea urchins (*Temnopleurus alexandri)* and holothurians. The most abundant taxa groups were molluscs, soft corals and crustaceans. Hard coral from the family Faviidae was recorded at sites 3 and 5 in the deepest strata.

Benthic fauna represented a small proportion of the FPE seawall assemblage, consistent with the 2014 survey. Site 1 had the highest fauna cover (14%) whereas no fauna were recorded on transects at site 2. The deep-water (4 m) transects across all sites had the highest percentage cover of marine fauna comprising of 4.3%.

Other fauna incidentally observed on and adjacent to the FPE seawall included wobbegong shark (*Orectolobus maculatus*), green turtles (*Chelonia mydas*), schools of striped catfish (*Plotosus lineatus*), bream (*Acanthopagrus australis*) and other various small-bodied fish species.

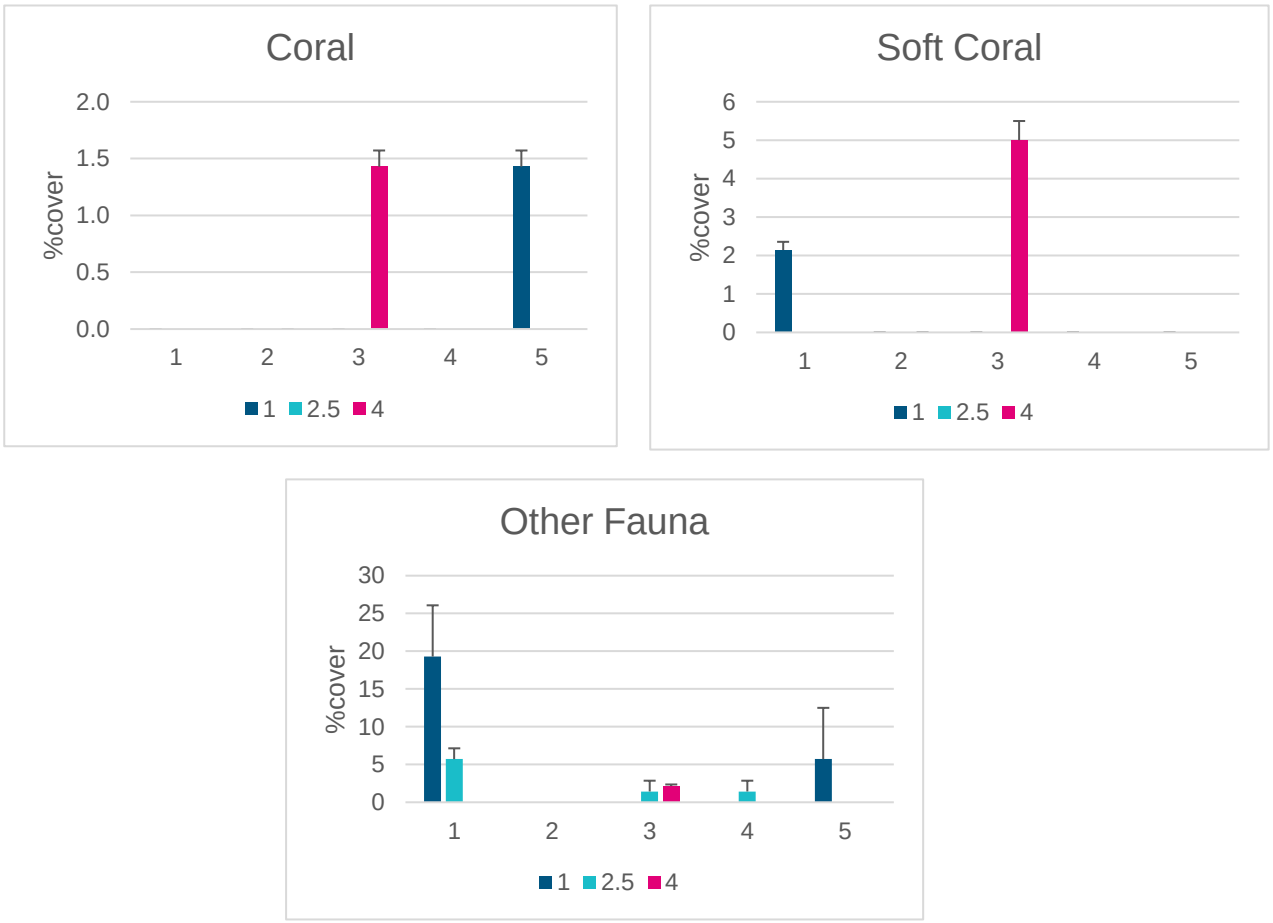


Figure 3-3 Mean percentage cover of macro-fauna groups at each site and depth category (1 m, 2.5 m, 4 m)

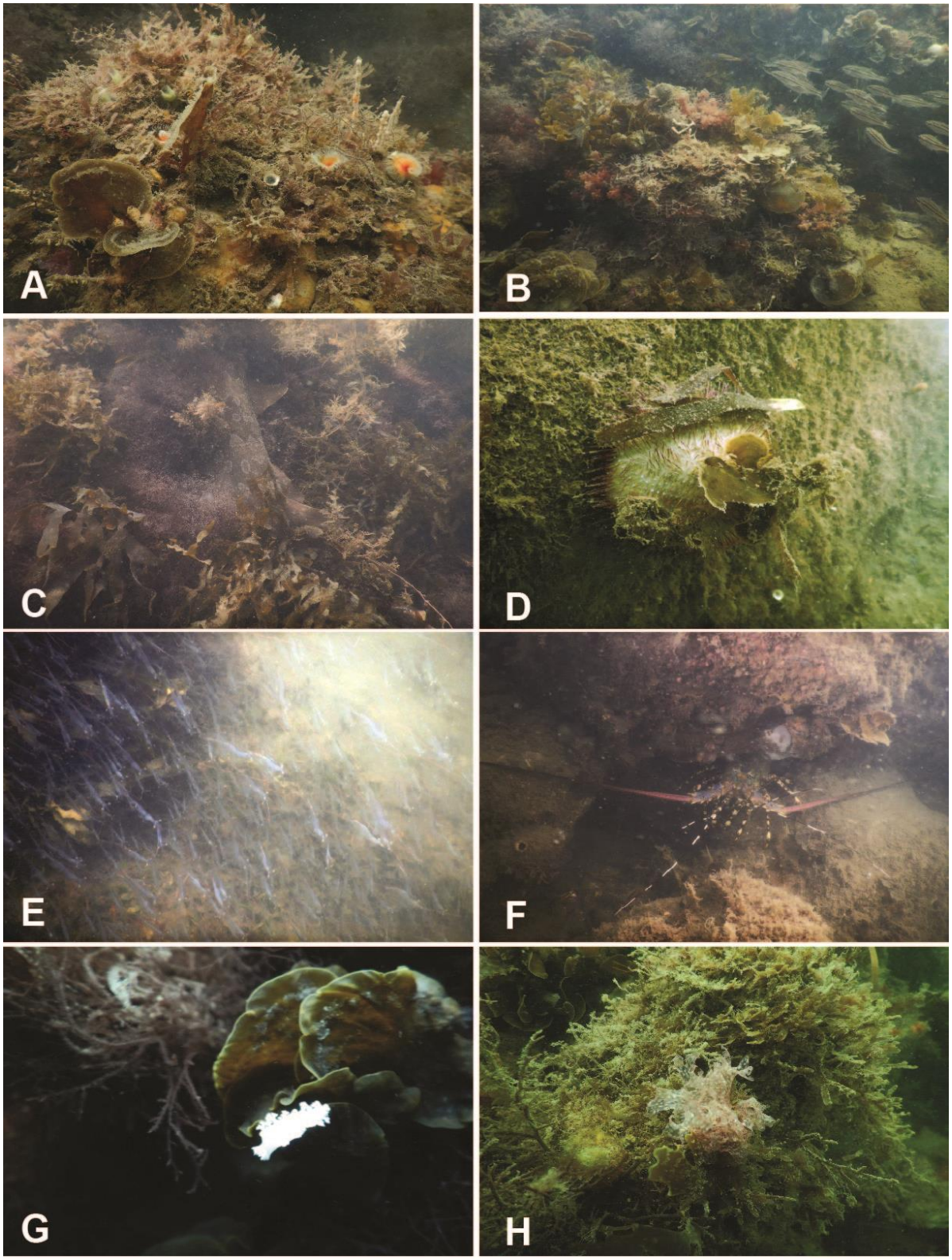


Figure 3-4 Examples of fauna encountered in the 2019 survey: Christmas tree worms (A); school of cardinal fish (B); spotted wobbegong, *Orectolobus maculatus* (C); sea urchin *Temnopleurus alexandri* (D); mysid shrimps (E); painted crayfish *Panulirus versicolor* (F); nudibranchs (G, H).

3.1.3 Taxonomic Richness

Figure 3-5 shows the total number of taxa groups recorded at each site and depth stratum in 2019. There was a trend of increasing taxa richness with depth at all sites. There was some variation in the number of macroalgae taxa among sites and depth strata, generally ranging from two to eight brown algae taxa and one to six red macroalgae taxa. These being the two most common taxa groups across all sites and strata.

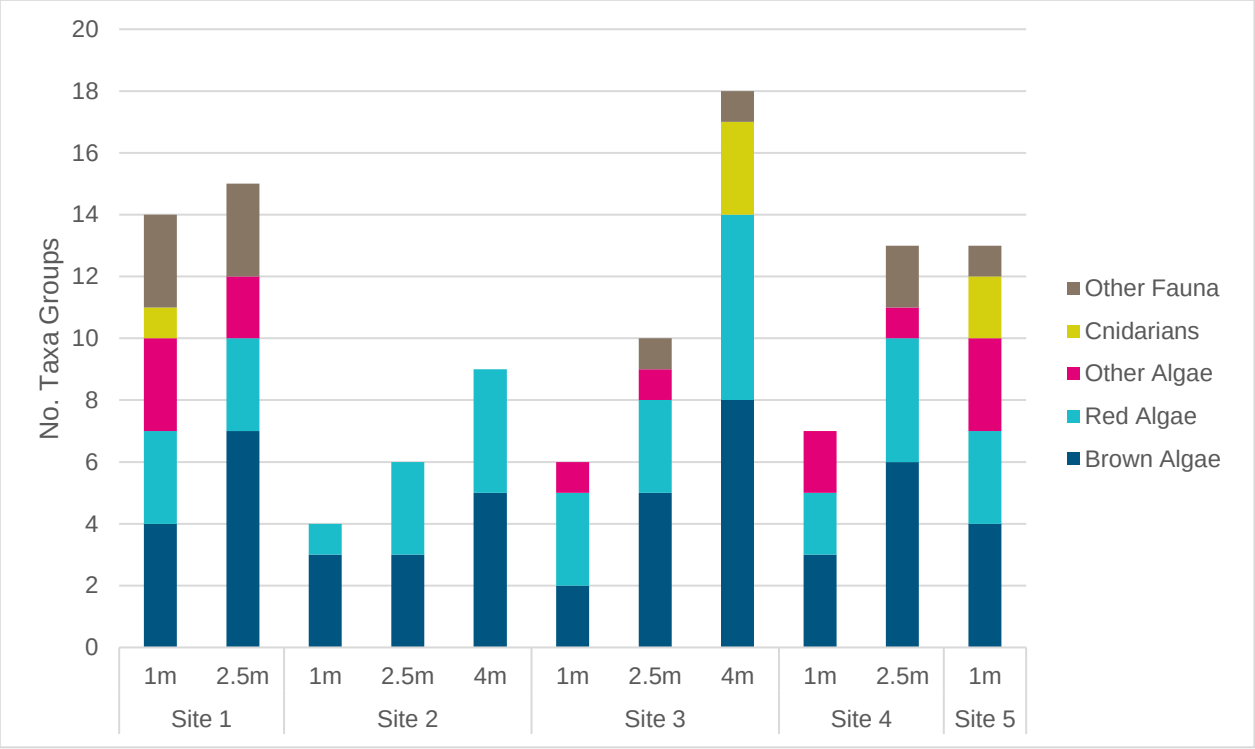
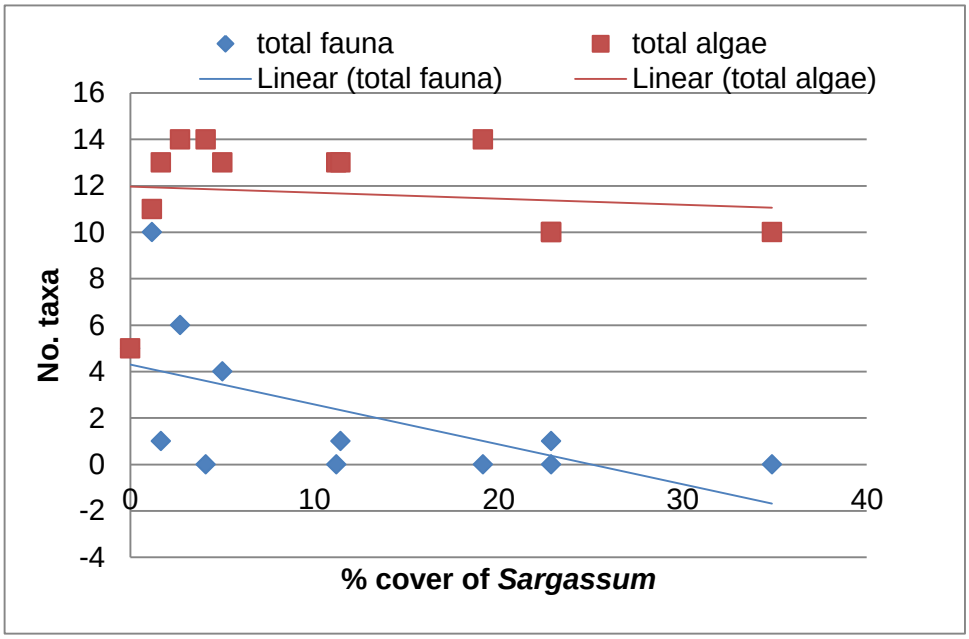


Figure 3-5 Number of taxa groups recorded at each site and depth stratum (May 2019)

Figure 3-6 shows that the number of benthic fauna and flora taxa were (weakly) negatively associated with the percentage cover of the canopy forming brown alga *Sargassum*. This pattern was consistent between the 2014 and 2019 studies (Figure 3-6).



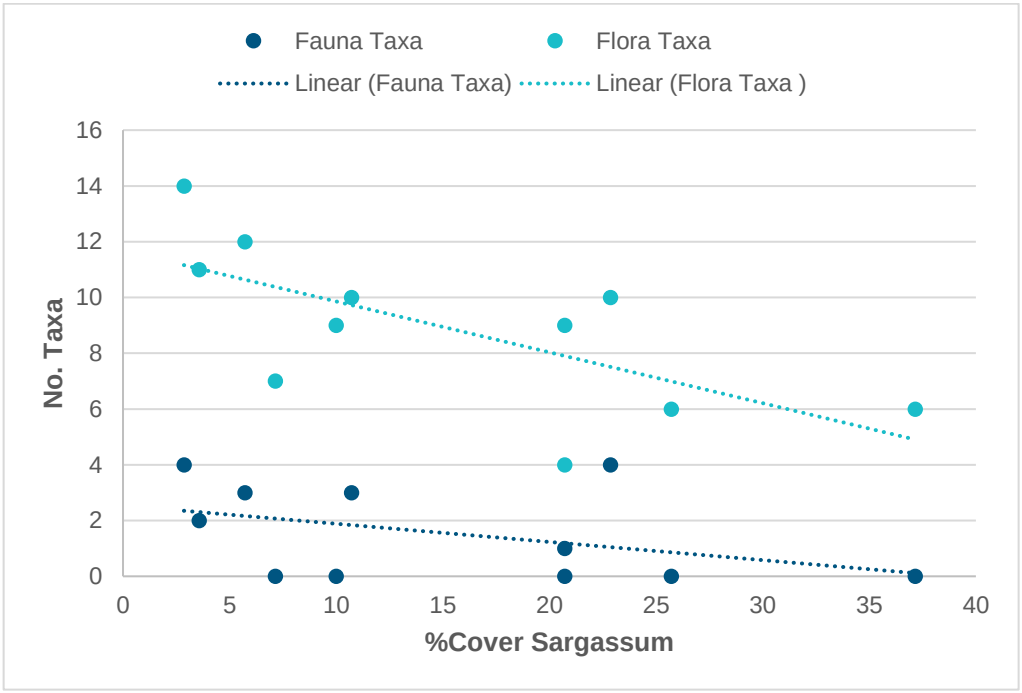


Figure 3-6 Relationship between number of fauna and flora taxa and percentage cover of *Sargassum* algae on each transect in 2014 (top) and 2019 (bottom)

3.1.4 Multivariate Patterns in Benthic Assemblage Structure

Figure 3-7 is a shade plot that shows that relative abundance of the 15 most abundant taxa at each site, strata and year. The shade plot also shows sample groupings produced by cluster analysis.

The plot shows that a core suite of taxa numerically dominated across all sites, times and strata, especially *Sargassum, Asparagopsis* and typically turfing algae.

Patterns in assemblage structure over time and space were explored using non-metric multi-dimensional scaling (Figure 3-8). Samples with similar taxonomic composition and abundance will group together, samples that are distant from each other are dissimilar. The stress value of 0.15 indicates that the ordination was a reliable approximation of patterns in higher dimensional space.

The ordination shows that benthic assemblages in 2009 were dissimilar to those in 2014 and 2019, separating at a Bray-Curtis similarity level of ~40%. In 2009, shallow water assemblages at site 2 and 3, located on the north-eastern section of the FPE, were dissimilar to all other sites and strata. The shade plot presented in Figure 3-7 shows that *Sargassum* and *Lobophora* were more abundant, and *Dictyopteris* and *Hypnea* were less abundant, in the shallow strata of sites 2 and 3 compared to other sites and times. The other 2009 samples also had high abundance of *Sargassum, Lobophora* and turfing algae, and unlike other sites and times, *Caulerpa* was also abundant.

Benthic assemblages were similar to each other during 2014 and 2019, grouping at the ~45% Bray Curtis similarity level. These assemblages were numerically co-dominated by *Sargassum*, *Dictyopteris*, *Hypnea* and a variety of other algae taxa (Figure 3-7).

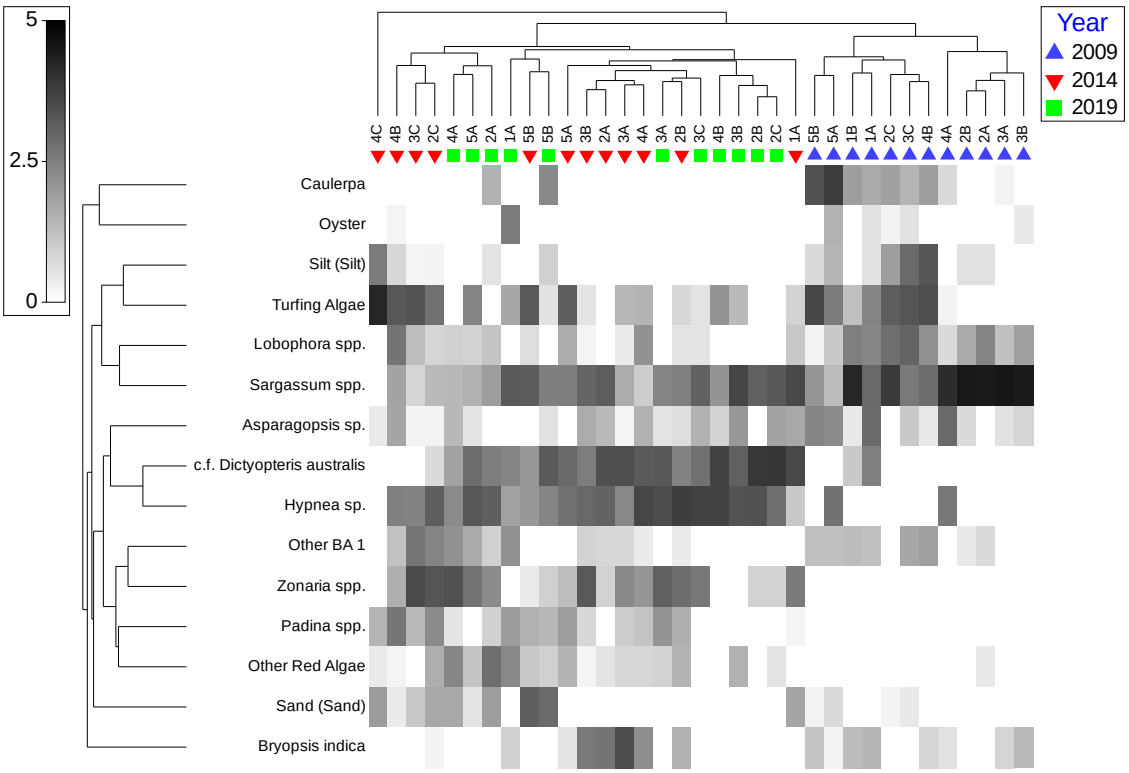


Figure 3-7 Shade plot on fourth-root transformed Bray Curtis similarities of benthic community data from 2009, 2014 and 2019

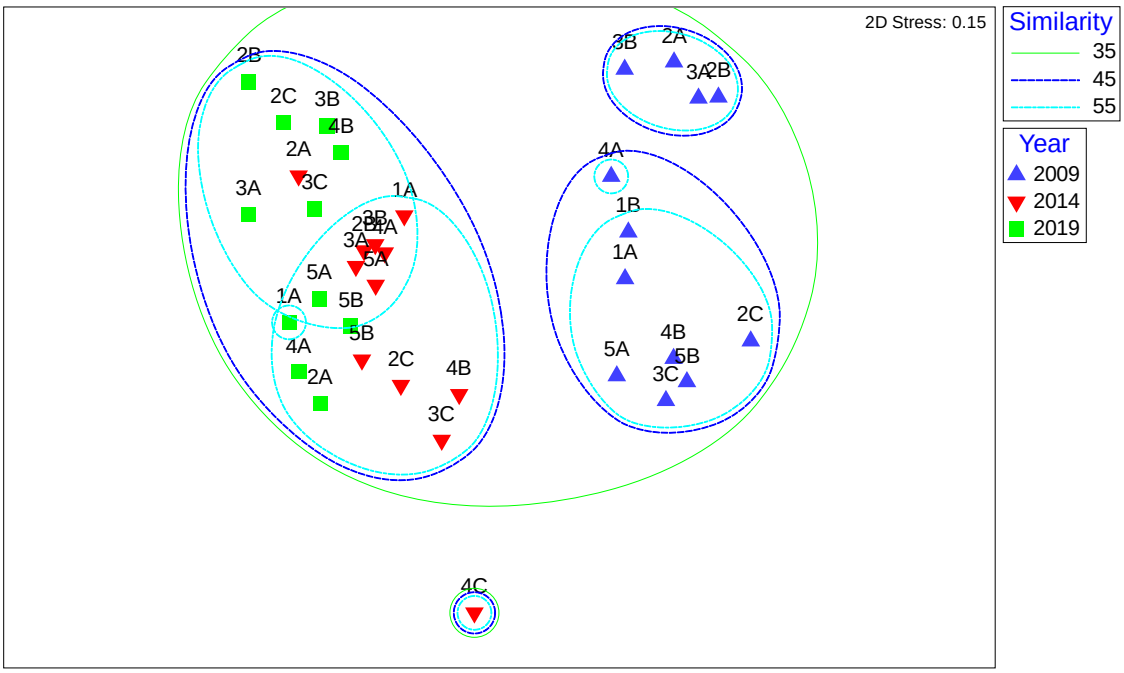


Figure 3-8 n-MDS ordination on fourth-root transformed Bray Curtis similarities of benthic community data from 2009, 2014 and 2019

3.1.5 Qualitative Dive Survey Sites

Summaries of the taxa observed at each of the qualitative dive sites (Figure 2-2) are provided below and in Figure 3-9.

Site 6 – General Purpose Wharf

This site was much more extensively colonised than when previously visited in 2014. The upper intertidal wall was covered in oysters and the upper subtidal and intertidal parts of the wall were densely covered in macroalgae, including *Sargassum* and *Asparagopsis.* Below this macroalgal layer, rocks were coated in turfing algae, stinging hydroids (Figure 3-9 H) and the brown algae (*Hincksia sordida*). Encrusting sponges and an unidentified species of sea urchin were also present and occasional schools of caridean shrimp were encountered close to the base of the seawall. The seawall at this site was also characterised by the presence of fine silts across all depth strata and poor water clarity.

Site 7 – Fisherman Islands No. 4

This site included three depth strata on pylons beneath the wharf at Fisherman Islands No. 4. The site was characterised by high diversity of a variety of species which are commonly found on pylons and floating pontoons throughout western Moreton Bay. The community included soft corals from the genus *Dendronephthya* (Figure 3-9 F) and the family Clavulariidae (Figure 3-9 G) and a variety of fouling organisms including barnacles (Figure 3-9 D), oysters, bryozoans in both encrusting and erect growth forms, colonial ascidians, solitary ascidians several species of sponges and stinging hydroids.

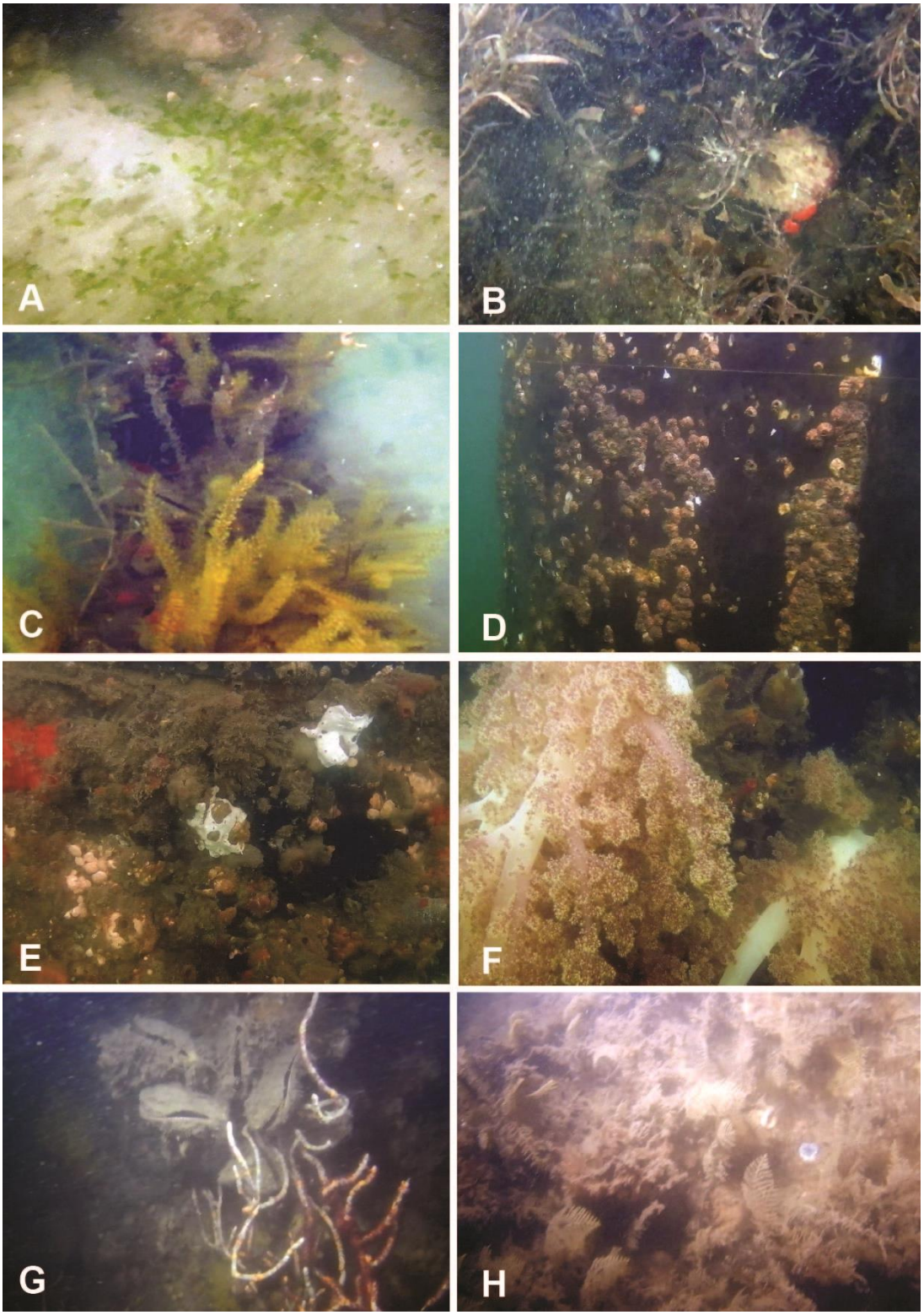


Figure 3-9 Images representative of qualitative sites: *Halophila* sp. at the seawall base at site 8 (A) *Sargassum* and urchins on the mid-wall at site 8 (B) *Echinomuracea* sp. at the base of the wall at Site 8 (C) barnacleson the intertidal pylon at site 7 (D); sponges and ascidians at site 7 (E); *Dendronephthya* at site 7 (F); large bivalves and plexaurid soft corals on pylons at Site 7 (G); and small stinging hydroids and algae at site 6 (H).

Site 8 – Fisherman Islands No. 11

This site was comprised of mixed substrates of rocky outcrops interspersed with bare sand. In the shallowest strata, rocky areas hosted algal communities comprised of *Sargassum* spp., *Dictyopteris australis*, *Padina sp.*, *Asparagopsis sp.*, turfing algae and a variety of other species (Figure 3-9 B). Immediately at the base of the wall was a *Halophila* seagrass meadow (Figure 3-9 A). Macroalgae were less common at deeper strata where two species of soft coral (*Echinomuricea*) were dominant and limited other fauna or flora was observed (Figure 3-9 C).

3.2 Baited Remote Underwater Video

A total of 11 fish species were recorded by BRUVS in 2019, five of which are of recreational and/or commercial fisheries importance (Table 3-2). In 2014, 12 fish species were recorded, eight of which are of recreational and/or commercial importance.

Yellowfin bream (*Acanthopagrus australis*) were the most abundant and widespread species in both years, and was recorded at all five sites. Yellowfin bream were also typically the first species to be recorded. Other abundant species were the silver biddy (*Gerres subfasciatusa)* and mackerel (*Scomberomorus* sp.)

Several species observed in 2014 were not observed in 2019 and vice versa. Wobbegong (*Orectolobus maculatus*), jewfish (*Argyrosomus japonicus*), black-spot tuskfish (*Choerodon schoenleinii*), Moses perch (*Lutjanus russelli)* and sea mullet were observed in 2014 but not 2019. Species new in 2019 were mackerel (*Scomberomorus* sp. Figure 3-11 G, H) tailor (*Pomatomus saltatrix*), snapper (*Chrysophrys auratus* Figure 3-11 D), and mask-rays (*Neotrygon kuhlii*).

In 2014, site 4 had both the highest species richness and abundance, although high abundance at this location was mostly driven by a very large Max N for *A. australis* recorded here (45) (Figure 3-10). In contrast, sites 1 and 2 had the highest richness and abundance in 2019, which were among the least rich and abundant sites in 2019.

Table 3-2 Time of first arrival (*t1st*) in minutes for all species recorded at each BRUVS site, commercial and recreational species are underlined - 2019

Species name	Common name	Site				
		1	2	3	4	5
Acanthopagrus australis	Yellowfin bream	01:18	00:01	02:00	00:01	16:49
Rhabdosargus sarba	Tarwhine	02:12	00:57	-	-	-
Pseudolabrus guentheri	Gunther's wrasse	-	00:22	-	06:12	-
Pomatomus saltatrix	Tailor	-	-	02:44	-	-
Caranx sexfasciatus	Bigeye trevally	13:52	04:01	-	-	-
Gerres subfasciatus	Silver biddy	00:12	02:40	03:40	-	-
Pentapodus paradiseus	Paradise threadfin bream	-	-	-	02:38	-
Monodactylus argenteus	Butter bream	-	-	-	12:22	-
Neotrygon kuhlii	Blue-spotted mask ray	-	-	08:46	-	-
Scomberomorus sp.	Mackerel	04:00	11:16	05:48	-	-
Overall Max N		13	16	13	3	2

Blue shaded – known or likely fisheries species

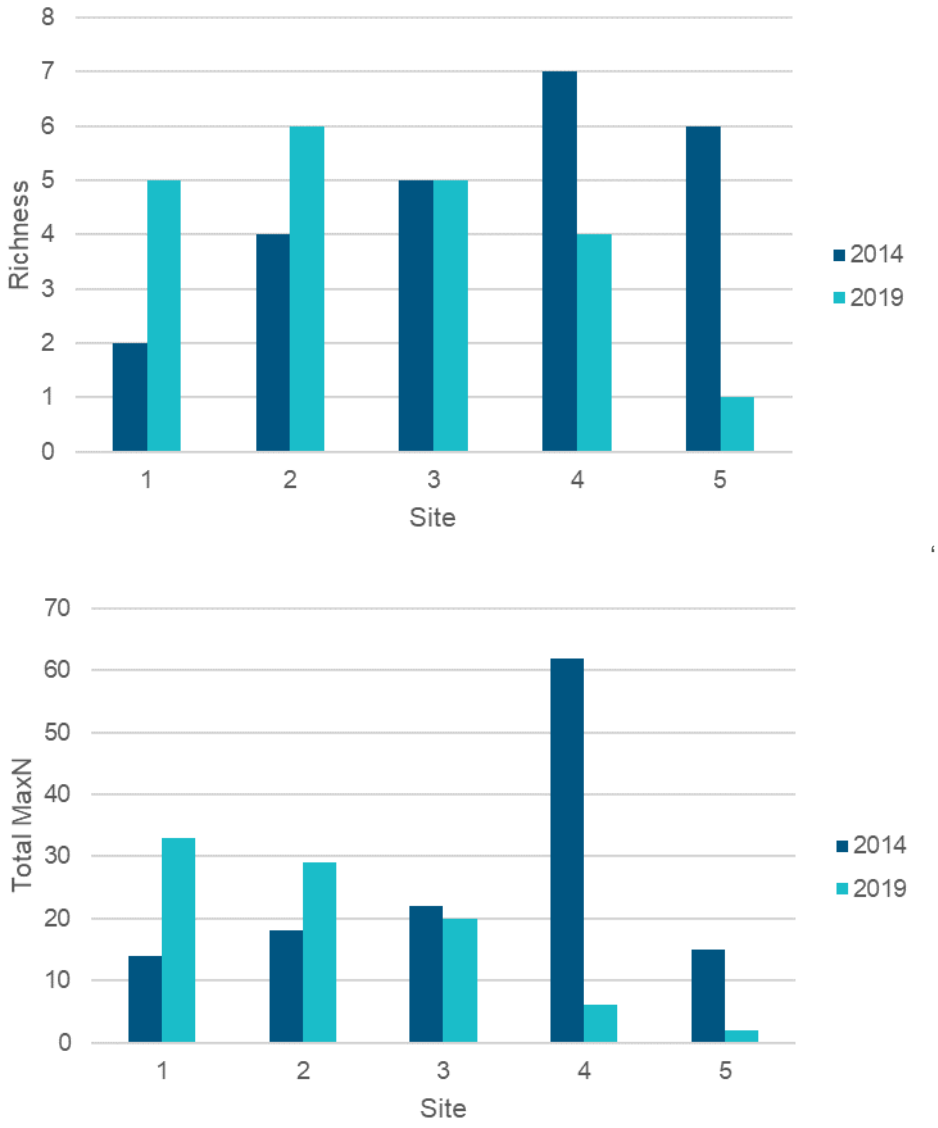


Figure 3-10 Species richness (upper plot) and abundance (lower plot) from BRUVS deployed at the FPE Seawall in 2014 and 2019.

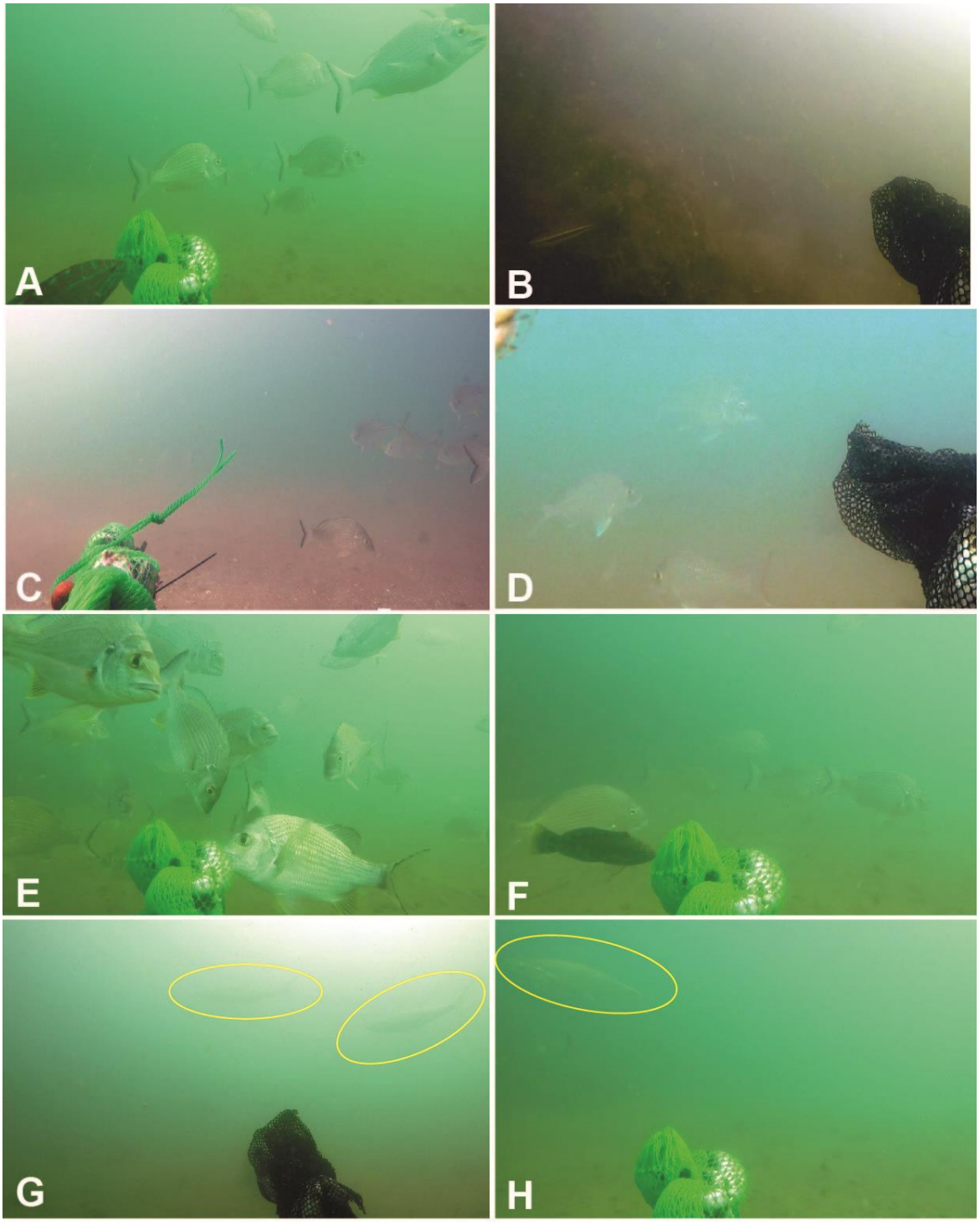


Figure 3-11 Screen captures of some species captured by BRUVS at the FPE seawall including Gunther's wrasse, *P. guentheri* and yellow-fin bream, *A. australis* (A); paradise threadfin bream, *P. paradiseus* juvenile (B); big-eye trevally, *Caranx sexfasciatus* and bream (C) juvenile snapper *Chrysophrys auratus* (D); abundant yellow-fin bream (E); *P. guentheri* and tarwhine, *Rhabdosargus sarba* (F); mackerel, *Scomberomorus* sp. (G) and (H).

3.3 e-DNA

A list of marine vertebrate taxa detected in e-DNA samples is provided Table 3-3. A total of four classes, 16 orders, 21 families and at least 23 species were detected. Bony fish (*Actinopteri*) was the most frequently detected taxa with 18 species, 11 of which are considered to have fisheries values. Three elasmobranchs were detected: whaler shark (site 2), bamboo shark (site 5) and blue spotted stingray (both sites). Cormorant was also detected at both sites and green turtle was detected in one sample.

Most bony fish species (11 of the 18 species) recorded in e-DNA samples occur in association with reef habitats. The remaining species inhabit mangrove, soft sediment and/or pelagic habitats.

Four of the 10 fish species recorded in BRUVS were recorded in e-DNA samples: Gunther's wrasse, tailor, tarwhine and blue-spotted mask ray. An additional seven species recorded in e-DNA samples were incidentally observed: blackfish, garfish, sea mullet, bamboo shark, green turtle, cormorant and silversides.

These results indicate relatively good agreement between e-DNA and BRUVS/incidental observations. Several widespread and abundant species recorded in BRUVS were not detected in e-DNA samples, most notably yellow-finned bream, silver biddy and mackerel.

3.4 Soft Sediment Habitats

3.4.1 Side-scan Sonar

The seawall, dense seagrass meadows and small hard features (pipes and debris) were detected in the sides-scan sonar mosaic (Figure 3-12 and Figure 3-13). Close-ups of the side-scan mosaic (Figure 3-13) also show large fauna (shark or dolphin) and schools of fish. Mobile fauna observed on side-scan sonar could not be ground-truthed, but permanent habitat features were subsequently filmed with drop cameras.

Dense patches of seagrass were detected on the southern extent of the side-scan mosaic. Based on ground-truthing, the observable extent is consistent with meadows of >30 % cover, comprising species with high biomass including *Zostera muelleri* and *Halophila spinulosa*. It is important to note that lower density seagrass cover occurs beyond this extent, but this was not visible on the side-scan sonar.

Other features of interest included a series of pipes at ground-truthing site 16 (Figure 3-13D, Figure 3-14C) and large metal debris at ground-truthing site 1 (Figure 3-13B, Figure 3-14B). Still images from additional ground-truthing imagery show a variety of sea-floor morphologies including fine sand with small bedforms (Figure 3-14 E, F), bioturbated muddy sand (Figure 3-14 A), and high plasticity mud with polychaete castings (Figure 3-14 G).

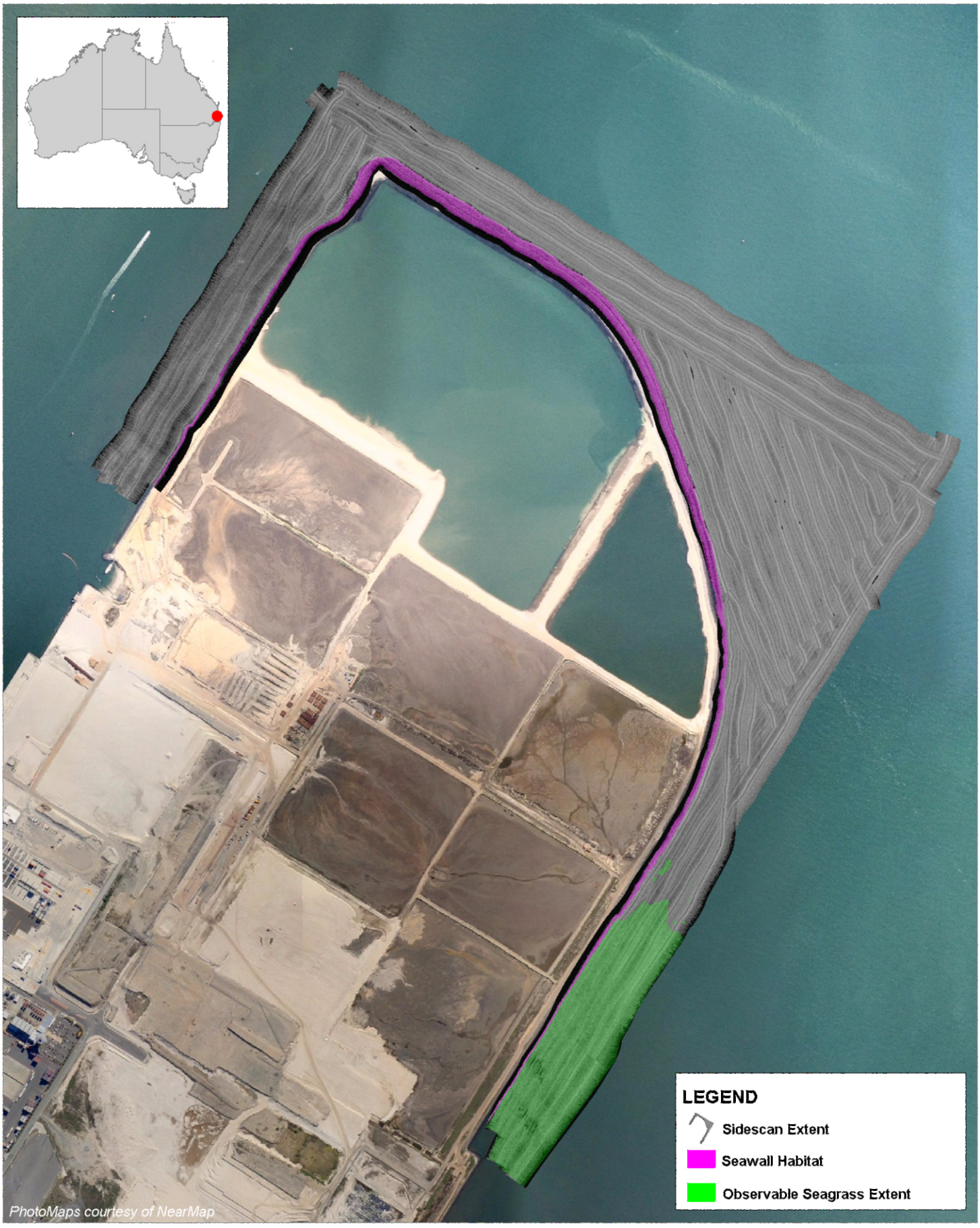
Table 3-3 Species recorded in e-DNA metabarcoding samples from site 2 and 5

Class	Order	Family	Genus	Common name	5-1	5-2	5-3	5-4	2-1	2-2	2-3	2-4	BRUVS/ Other	Habitat*
Actinopteri	Anguilliformes	Anguillidae	*Anguilla reinhardtii*	Longfinned eel	X								No	R, P, M
Actinopteri	Atheriniformes	Atherinidae	*Atherinomorus* sp.	Silversides	X	X	X	X				X	No/Yes	R
Actinopteri	Beloniformes	Hemiramphidae	*Hyporhamphus quoyi*	Quoy's garfish		X	X	X					No/Yes	P
Actinopteri	Centrarchiformes	Kyphosidae	*Girella tricuspidata*	Blackfish			X						No/Yes	R
Actinopteri	Centrarchiformes	Terapontidae	*Pelates quadrilineatus*	Fourlines terapon				X					No	R
Actinopteri	Gobiiformes	Gobiidae	*Mugilogobius wilsoni*	Wilson's goby		X							No	M
Actinopteri	Labriformes	Labridae	*Pseudolabrus guentheri*	Gunthers wrasse			X		X			X	Yes	R
Actinopteri	Labriformes	Labridae	*Scarus* sp.	Parrotfish					X				No	R
Actinopteri	Mugiliformes	Mugilidae	*Mugil cephalus*	Mullet	X	X	X	X	X			X	No/Yes	P, M
Actinopteri	Perciformes	Platycephalidae	*Platycephalus australis*	Flathead		X							No	S, M
Actinopteri	Perciformes	Platycephalidae	*Platycephalus fuscus*	Dusky flathead		X	X						No/Yes	S, M
Actinopteri	Perciformes	Serranidae	*Epinephelus malabaricus*	Malabar groper						X			No	R, M
Actinopteri	Perciformes	Siganidae	*Siganus* sp.	Rabbitfish	X			X	X				No	R
Actinopteri	Perciformes	Sillaginidae	*Sillago aeolus*	Western trumpeter	X	X		X				X	No	S
Actinopteri	Priacanthiformes	Priacanthidae	*Priacanthus macracanthus*	Red bigeye					X				No	R
Actinopteri	Scombriformes	Pomatomidae	*Pomatomus saltatrix*	Tailor	X			X					Yes	R, P
Actinopteri	Spariformes	Sparidae	*Rhabdosargus sarba*	Tarwhine	X		X	X					Yes	R
Aves	Pelecaniformes	Phalacrocoracidae	*Phalacrocorax sulcirostris*	Cormorant		X	X	X		X	X		No/Yes	P, R
Chondrichthyes	Carcharhiniformes	Carcharhinidae	*Carcharhinus* sp.	Whaler shark						X	X	X	No	P, R, M
Chondrichthyes	Myliobatiformes	Dasyatidae	*Neotrygon kuhlii*	Blue-spotted mask ray							X		Yes	S
Chondrichthyes	Orectolobiformes	Hemiscylliidae	*Chiloscyllium punctatum*	Bamboo shark (NT IUCN)	X								No/Yes	S, R, M
Reptilia	Testudines	Cheloniidae	*Chelonia mydas*	Green turtle (V EPBC, NCA)							X		No/Yes	R, P

*R= reef; P = pelagic/open water; M = mangrove creeks; S = soft sediments

NT = near threatened under IUCN; V = Vulnerable under EPBC Act and NC Act

Blue shaded – known or likely fisheries species



Title:

Side-scan Mosaic and Habitats

Figure: 3-11

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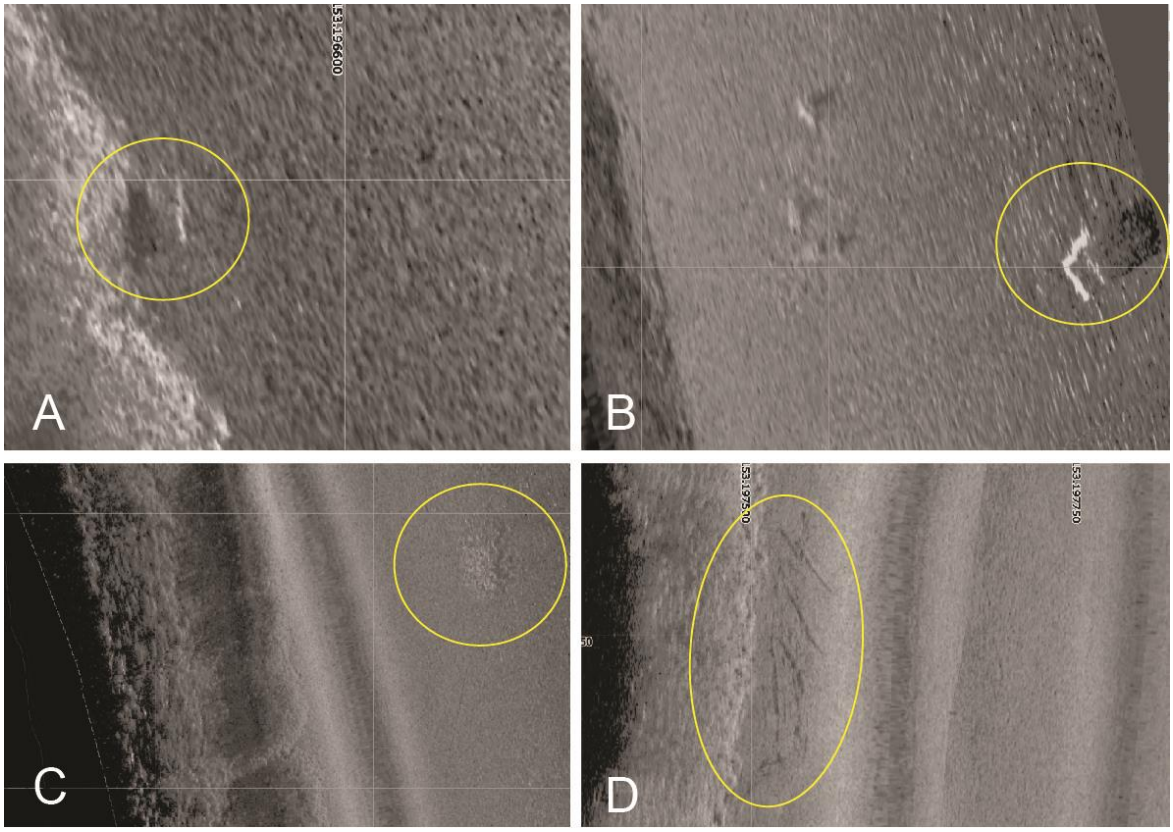

Figure 3-13 Side-scan points of interest: shark or dolphin near the base of the wall (A); large reflective debris near ground truthing site 1 (B); school of fish (C); piping debris at ground truthing site 16 (D).

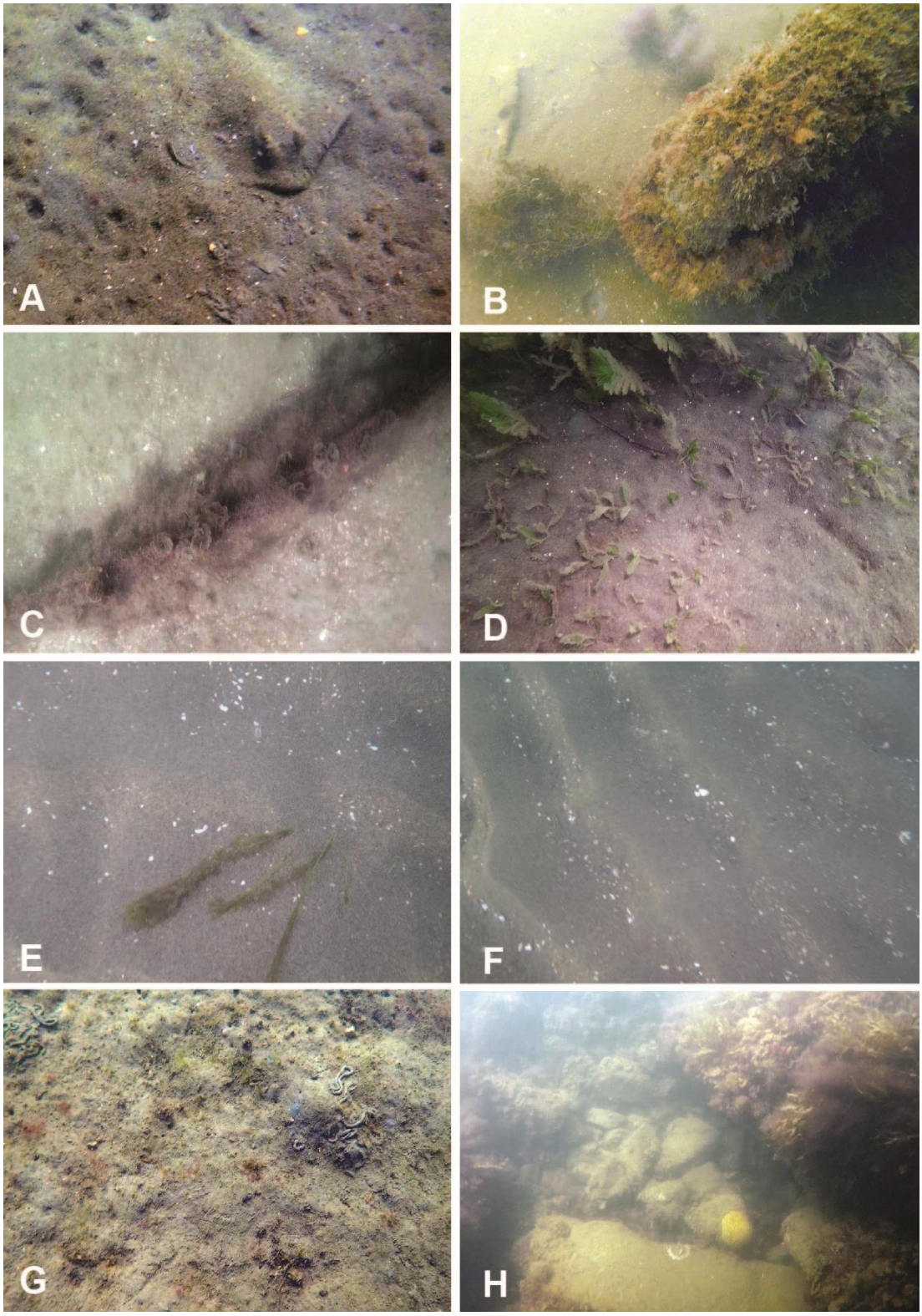

Figure 3-14 Screen grabs of ground-truthing videos: blue-spotted maskray buried in muddy sand at site 1 (A); large debris at site 2 (B); pipe sections at site 3 (C): *Halophila decipiens* and *H. spinulosa* over sandy mud at site 5 (D); *Halodule spinulosa* with filamentous epiphytes at site 8 (E); fine sand, site 10 (F); mud at site 12 (G); small *Goniastrea* coral at site 13 (H).

3.4.2 Soft Sediment Habitats

3.4.2.1 Acoustic Classes

A total of 54,693 acoustic records were collected at the 200 kHz frequency (Table 3-4). Five classes of sediment were selected based on interrogation of prospective sediment types observed in side-scan and down-scan backscatter intensity. Fine separation in decibel range was used in transitional areas to show more resolution in the transition between fine sand and mud-dominated substrates. The five acoustic classes were grouped into sand-dominated, mud-dominated, mixed mud and sand classes in **Error! Reference source not found.**.

A map showing the spatial distribution of the 200 kHz acoustic classes in 2009 (using QTC methodologies) and in 2014 (using backscatter intensity) within the study area is shown in Figure 3-15**Error! Reference source not found.**. The 2019 results Figure 3-15 and Figure 3-16**Error! Reference source not found.** show that the south-eastern and north-western faces of the seawall are surrounded by fine sands with muddier sediments located north and east of the seawall. A series of large sediment waves can be seen east of the seawall, with the long axis of these sediment waves running approximately north-south.

It is possible that many of the differences in the 2014 and 2019 datasets are methodological rather than being related to changes in sediment type. In 2014 the main pattern identified was the dominance of silty sands across most of the study area with a mixture of silt and coarse sandy sediments located within a slight depression near the northern tip of the seawall (Figure 3-15**Error! Reference source not found.**). In contrast, the 2019 data showed that fine sands dominated the north-western and south-eastern faces of the seawall, with sandy mud to mud dominant in the eastern parts of the study area. Soft sediment habitat mapping using 2019 methodologies showed good agreement with grab and video data, side-scan imagery and patterns in bathymetry.

Table 3-4 Summary of acoustic classification results for 200kHz frequency with seabed class characterisation inferred from sediment analyses

Class	Number of Acoustic Records	% of Total Acoustic Records	Characterisation of Seabed Class	Location
1	16,463	30.1%	Seawall or fine sand	Occurs where acoustic lines traverse seawall and in shallowest areas, northwest and south of study area extent
2	4,133	7.6%	Muddy sand	Transition between sand and muddy areas
3	11,331	20.7%	Sandy mud	Common in eastern part of the study area
4	5,556	10.2%	Sandy Mud (higher mud content than class 3)	Mud dominant with sand, common in deeper areas
5	17,210	31.5%	Mud	Common in deepest areas, north-east and south

2014

2019

LEGEND

2014

- Class 1 (Rock)
- Class 2 (Medium to Coarse Sand)
- Class 3 (Silt to Fine Sand)
- Class 4 (Silt and Coarse Sand)

2019

Backscatter Intensity (dB range, habitat)

187	to	237	Sand / Rock
186	to	187	Muddy Sand
184	to	186	Muddy Sand
183	to	184	Sandy Mud
-1	to	183	Mud

Title:

Distribution of 200kHz Acoustic Seabed Classes in 2014 and 2019

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Habitat Classes

Interpolated Backscatter

LEGEND

Backscatter Intensity
(dB range, habitat)

222

187

56

LEGEND

- Seawall
- Class 1 Sand
- Class 2, 3, 4 Muddy Sand to Sandy Mud
- Class 5 Mud with Silt
- Seagrass

Title:

Habitat Classes (Left) and Interpolated 200kHz Backscatter Intensity (Right)

Figure: 3-15

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Approx. Scale

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3.4.2.2 Data Validation Using Video Assessment Data

Ground-truthing (video and benthic grab) surveys were conducted at representative areas based on initial characterisation of the data. Figure 3-17 shows imagery of sediments collected in May 2019. As mentioned previously, video ground truthing assessments (Figure 2-3) were located in areas of interest based on the side-scan mosaic and initial classification of 200 kHz backscatter intensity. Physical seabed attributes observed for each video transect are detailed in Table 3-4. Dominant epibiota and any other observations for each substrate class are described below and in Table 3-5:

- Seawall: mapped primarily based on extent mapped in the side-scan sonar mosaic, consists of rock covered in macroalgae;
- Class 1 – Fine sand: located along the north-western and south-eastern seawall faces, consisting of fine sand with shell grit and small bedforms (Figure 3-14 G) along the north-western seawall, typically in shallow waters with higher bed shear forces. Along the south-eastern face bedforms were absent but seagrass cover was extensive. Sparse seagrass cover along the north-western seawall (Figure 3-14 H). Occasional overlap with seawall rock was included in this acoustic class.
- Class 2 – muddy sand: transitional habitat located on the deeper margins of fine sand habitats (Class 1). Often dimpled in appearance and containing shell fragments. This class was relatively minor in extent.
- Classes 3 and 4 – sandy mud: mud with sand and shell grit, often dimpled and bioturbated. Common to the east of the seawall and forming ridges with long axes running north-south. Support numerous rays and tubeworms and occasional macroalgae (Figure 3-14 A). Small patches of *Halophila spinulosa* were found in parts of this habitat (Figure 3-14 D).
- Class 5 – Mud with sand and silt layer: high-plasticity mud with sand and shell grit, often with a silt layer. This class was located in the deepest parts of the study area, near the north-eastern and northern survey extents and between the north-eastern and southern survey limits. Supports occasional seagrass in shallower waters, ascidians, deposit feeding tubeworms and is often highly bioturbated with tubeworm castings (Figure 3-14 G).

3.4.3 Bathymetry

A digital elevation model (DEM) produced by reducing soundings to AHD using RTK GPS then adjusted to LAT using Port of Brisbane tidal planes. This is shown in **Error! Reference source not found.**, alongside the DEM produced for 2014. The data were collected for habitat mapping purposes and are not to be used for engineering or navigation purposes.

The two DEMs were similar with most differences related to interpolation noise rather than changes in depth. Changes in seafloor elevation appear to have occurred along the north-western survey margin, at the northern extremity of the seawall and at the southern extremity of the survey extent. The north-western survey area boundary appears to have become deeper, and the large sandbank near the tip of the FPE appears to have shifted slightly south. At the southern survey limits, a similar process may have occurred, where the position of a sandbank has moved slightly, appearing to have drifted slightly north-east since 2014. Furthermore, detailed bathymetry survey would be required to confirm these patterns.

Table 3-5 Summary of benthic habitat attributes for each video transect

Wapoint no.	Dominant acoustic class	Video assessment seabed attributes:	
		Substrate type	Dominant epibiota and other observations
1	3/4	Sandy mud with shell fragments; slight dimples in sand, some bioturbation	Bare substrate with shell pieces, several *Cnemidiocarpa* ascidians, crinoids, stingrays and macroalgae. *Halophila spinulosa* in one small patch
2	1/2	Muddy sand with shell fragments; slight dimples in sand	Bare substrate with shell pieces, large metal debris covered in fouling. Small colony of *Echinomuracea* soft coralseveral *Cnemidiocarpa* ascidians, crinoids, stingrays and macroalgae. *Halophila spinulosa* in one small patch
3	4/5	Sandy mud with slightly silty upper layer	Primarily bare substrate with detritus, *Zostera* fragments and pipe fragments near seawall
4	4	Sandy mud with shell grit and seagrass	Sandy mud with *Halophila spinulosa, H. decipiens,* and macroalgae
5	4	Sandy mud with shell grit and seagrass	Sandy mud with *Halophila spinulosa, H. decipiens,* and macroalgae
6	1	Hard rocky substrate (e.g. seawall)	Dense macroalgae, dominated by *Sargassum Bryopsis, Hypnea* and *Lobophora* spp.
7	1	Fine sand with seagrass	Seagrass meadow with *Zostera muelleri, H spinulosa, H. decipiens,* and macroalgae
8	1	Fine sand with shell grit and bedforms	Open substrate with some *Halodule uninervis* eavy coating of epiphytes) and sea-pens
9	1/2	Fine sand with mud, shell grit and bedforms	Open substrate
10	1	Fine sand with shell grit and bedforms	Open substrate
11	seawall (1)	Hard rocky substrate (e.g. seawall) with silty sand at its base	Dense macroalgae, dominated by *Sargassum, Asparagopsis, Hypnea, Dictyota* and *Lobophora* spp.
12	5	Silty mud, shell girt and occasional rubble	Open substrate with stalked tubeworms, *Cnemidiocarpa* ascidians
13	seawall (1)	Hard rocky substrate (e.g. seawall) with silty sand at its base	Dense macroalgae, dominated by *Sargassum, Hypnea, Dictyota* and *Lobophora* spp. Bream and wrasses (labridae) observed, as well as *Goniastrea* coral
14	4/5	Silty mud, shell girt and occasional rubble	Open substrate with bivalves, holothurians, tubeworms, and *Cnemidiocarpa* ascidians
15	5	Bioturbated mud and silt	Bare silty and plastic mud with occasional large burrows, some *Halophila spinulosa*
16	4/5	Sandy mud with slightly silty upper layer	Primarily bare substrate with detritus, *Zostera* fragments and pipe fragments near seawall

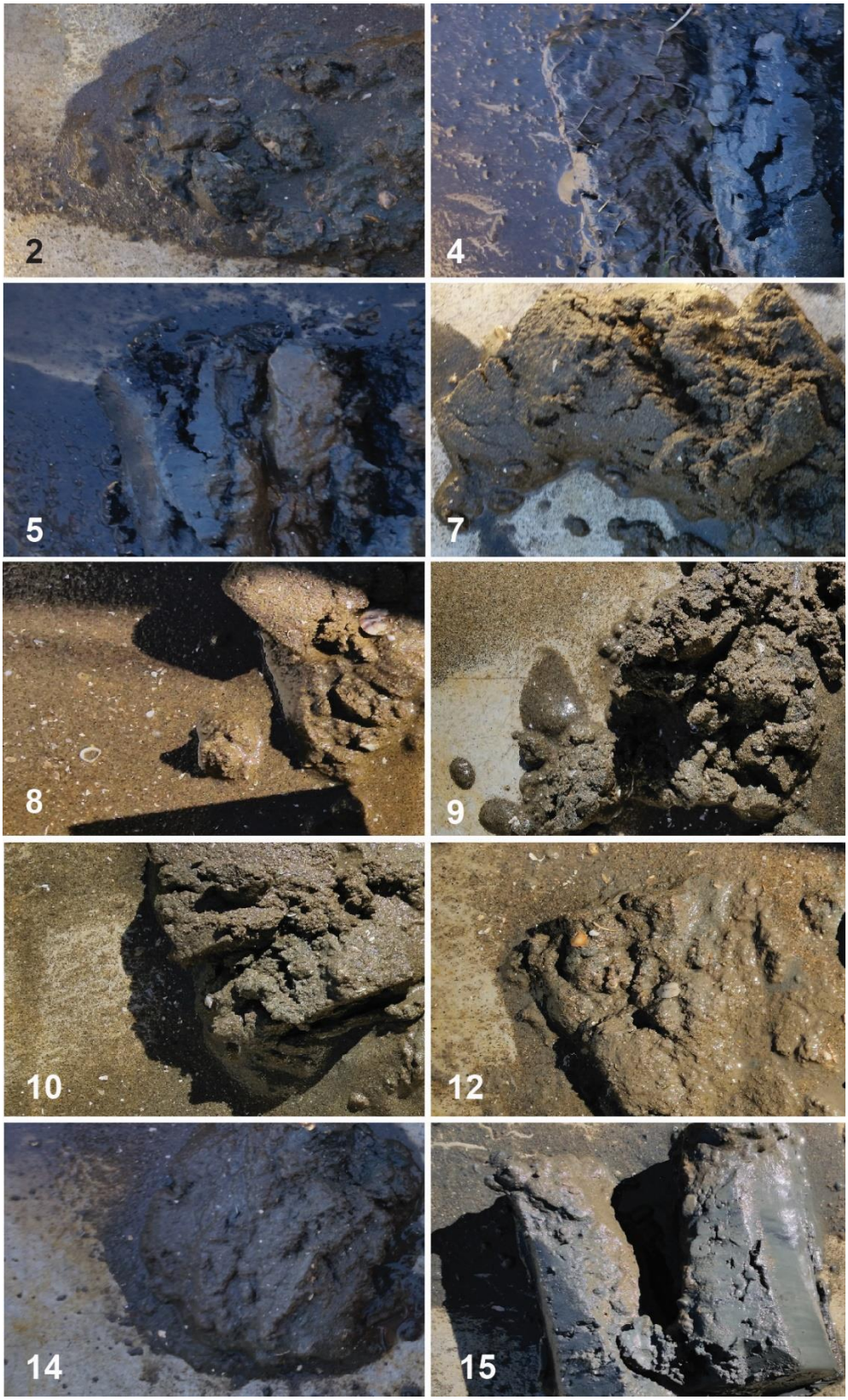
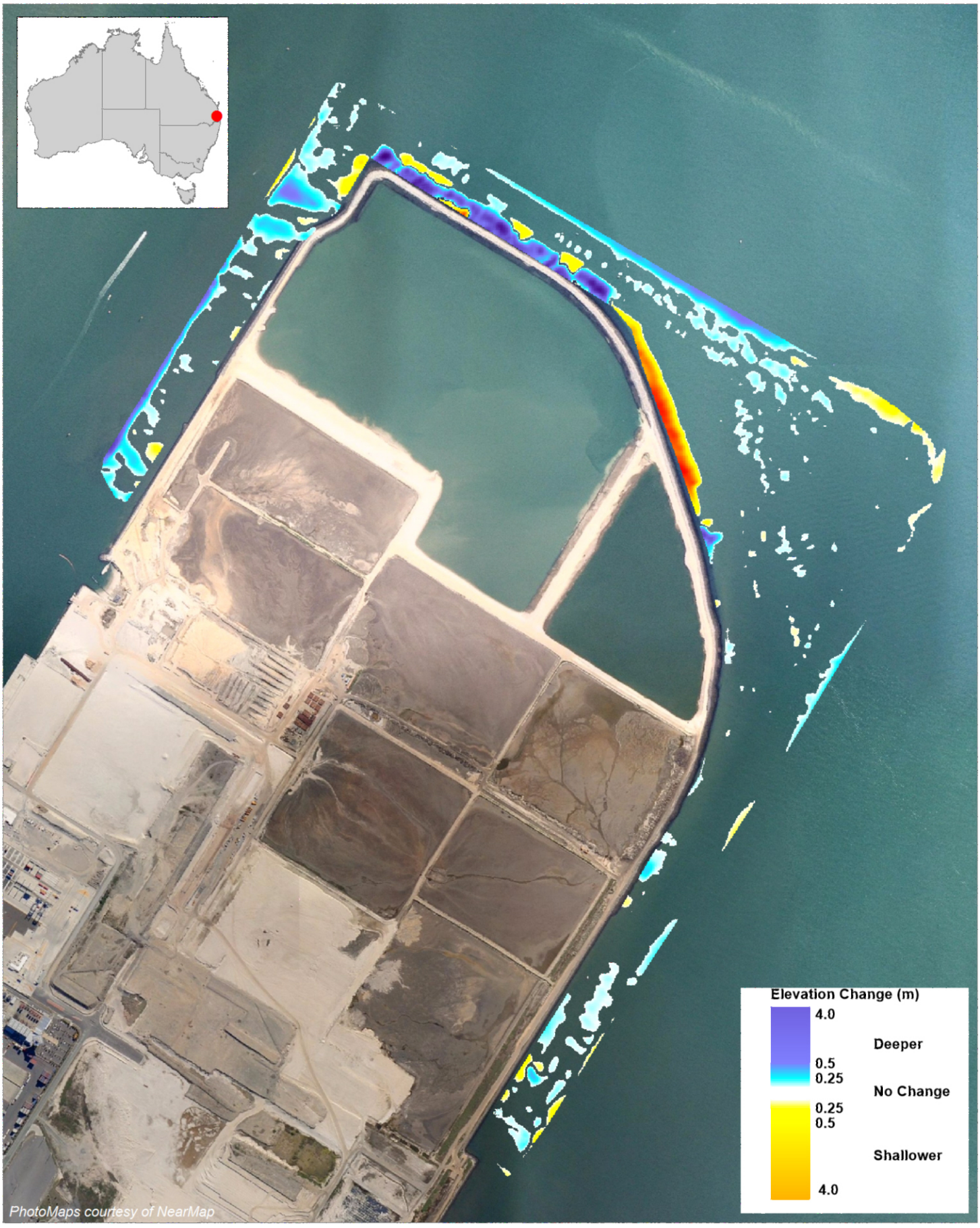


Figure 3-17 Photos of sediments from ground-truthing locations: mud at sites 4, 5, and 15; sand at sites 7 and 8, and 10; muddy sand at sites 9 and 12; sandy mud at sites 2 and 14.



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Differences in Bathymetry between 2014 and 2019

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4 Discussion

4.1 Seawall Benthos

The FPE seawall benthic communities comprised of a range of brown (predominantly *Sargassum* spp., *Dictyopteris australis*, *Lobophora* and *Padina*), green (*Bryopsis*) and red (commonly *Asparagopsis* and *Hypnea*) macroalgae species, as well as sponges, hard corals, soft corals, sea anemones, echinoderms and ascidians. The types of taxa recorded in the present study were consistent with those recorded at the same sites by BMT WBM (2009; 2014).

4.1.1 Temporal Patterns

Temporal patterns in the abundance of broad taxonomic groups displayed complex patterns (Figure 4-1). Brown algae (predominantly *Sargassum* and *Dictyopteris australis*) was the most abundant taxa over time, and showed no clear temporal trend, with cover ranging from 46% (2014) to 60% (2009) (Figure 4-1). Red algae cover showed the largest change over time, increasing from 8% in 2009, to 23% in 2014 and 30% in 2019 (Figure 4-1). By contrast, turfing algae and green algae cover had a corresponding decline over time, especially between 2014 and 2019.

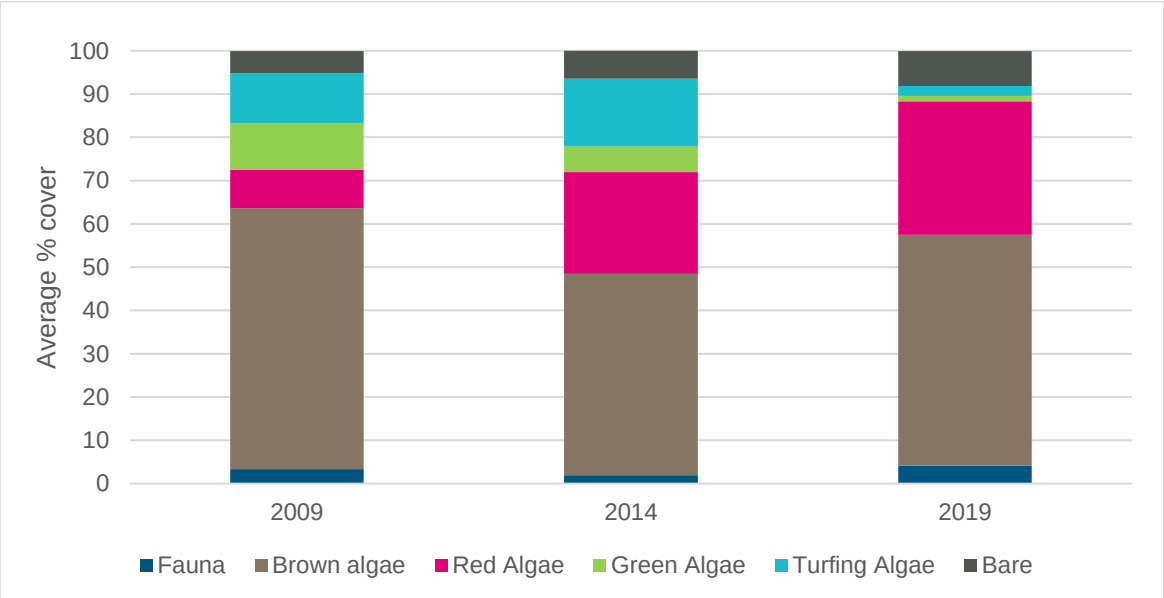

Figure 4-1 Mean percentage cover of major benthic cover groups in 2009, 2014 (BMT WBM 2009, 2014) and 2019 (present study)

At finer taxonomic levels, there were distinct changes in assemblage structure over time. Multivariate analysis identified a shift in community structure between 2009 and 2014, but similar community structure in 2014 and 2019. This was mainly a driven by declines in *Caulerpa*, *Sargassum* and silt cover between 2009 and 2014/19, and increased cover of red and green algae species over this period.

The temporal changes in benthic community structure are likely a response to multiple processes:

- Flood disturbance – reductions in salinity and increased suspended sediments (and nutrient) concentrations during flood events stressful conditions for sessile marine species. Major flood events occurred in 2011 and 2013, coincident with the period when the largest shift in benthic community structure was observed. The flood events resulted in the loss of *Caulerpa taxifolia* in adjacent soft sediment habitats (BMT WBM 2006; 2014), similar to trends observed on the FPE rock wall. Similar major changes in reef benthos were reported in western Moreton Bay following the 1974 flood (Lovell 1989).
- Dry periods – salinity in western Moreton Bay is near sea-water concentrations during dry periods. This provides more favourable conditions for marine species that can only live in a narrow range of salinities (i.e. stenohaline species). Shifts in community structure occur as new species colonise and also in response to biological interactions (grazing, competition, predation etc.).

4.1.2 Comparison to Other Reef Communities

Patterns in numerical dominance of FPE seawall assemblages were broadly consistent with those on natural substrates in western Moreton Bay. Relict coral reef communities in western Moreton Bay have a high percentage of macroalgae (approximately 50%) and bare substrate (approximately 30-40%) (Harrison et al. 1991; 1995; EHMP 2006), similar to that recorded on the FPE seawall.

In these reefs, hard coral cover was consistently low (<10%, but typically >5%), and was usually specifically dominated by *Favia* sp., a species that is known to be a relatively stress tolerant massive coral (EHMP 2006). EHMP (2007) data indicates that while hard coral cover on natural relict coral reefs was typically <10%, it is rarely <5%. In the present study, hard coral cover was <0.5% at sites where it was recorded. In time, hard coral cover may increase as colonies grow larger and more corals recruit onto the wall.

Brown algae cover was consistently greater in water depths of <2 m (except at site 2). Low light levels in deeper waters may be the key factor limiting brown algae cover in the deeper waters, as is often the case on natural reef systems in Moreton Bay (EHMP 2006; see below). In terms of depth related changes in community structure, terrigenous and relict Holocene reefs in western Moreton Bay typically display the following broad community structure patterns:

- Brown algae such as *Sargassum* tends to form an over-storey canopy from the lower intertidal zone down to water depths of ~3 m below LAT (Lovell 1989); and
- Below these depths bare substrate tends to be the most conspicuous feature, with a sparse cover of hard and soft corals, colonial ascidians, sponges and small bryozoans present in places. Living corals form a thin veneer over predominantly unconsolidated Holocene carbonate deposits that are interspersed with patches of soft sediment and seagrass. The seaward edge of hard corals is delineated by the edge of hard substrate (Harrison et al. 1991), which typically occurs in water depths <3 m (Lovell 1989). The upper limit of corals typically occurs in the upper subtidal zone, but may occasionally extend into the lower intertidal zone (Johnson and Neil 1998a;b).

Overall, the results of the present study demonstrate that the FPE seawall continues to support an abundant macroalgae cover, similar to patterns in shallow natural reef systems elsewhere in western Moreton Bay. Inter-annual variations occur in numerically dominant macroalgae cover, possibly reflecting changes in water quality conditions and biological interactions. It is also possible that

seasonal changes in macroalgae assemblages also occur, although this has not been examined to date.

4.2 Fish and Other Marine Vertebrates

4.2.1 Fish Habitat Values

The impact assessment study carried out for the FPE seawall (WBM 2001) predicted that this area would represent a locally important fisheries habitat and that the FPE seawall would provide shelter and an aggregation device for fish and shellfish of fisheries significance. Based monitoring in 2014 and 2019 and the pilot e-DNA survey demonstrated that most fish species at and adjacent to the FPE seawall are of direct commercial and recreational importance, consistent with IAS predictions.

BRUV surveys found that yellow-fin bream was the most abundant fish species recorded in 2014 and 2019 surveys at all sites. This suggests that the FPE represents a locally important habitat for this species. Most other fish species that use the rock wall are not site attached species, and therefore display great variability in the in time and space. A greater level of temporal replication would be required to characterise these communities.

The pilot e-DNA survey found that most fish (and other marine vertebrate) fauna in the vicinity of the FPE are reef-associated species, but many also utilise other habitat types that occur nearby to the FPE. This is consistent with BMT WBM's (2014) suggestion that the position of the FPE seawall proximal to important fisheries habitats (seagrass, mangroves, saltmarsh, reefs) exerts a strong influence on fish habitat values and functions, as has been found by other workers (Clynick and Chapman 2002). It is also possible that e-DNA detected near the seawall may have been transported from adjacent habitats elsewhere by currents, as is certainly the case for the small number of soft-sediment (i.e. flathead, trumpeter whiting) and mangrove (Wilson's goby) specialist species.

4.2.2 Sampling Methodologies

The e-DNA pilot study detected more than 23 marine vertebrate species, most of which were reef-associated species. The e-DNA samples did not detect several species that are abundant in the area, most notably yellow-finned bream. This could be due to limitations with the e-DNA library bank (e.g. confusing bream with the closely related tarwhine), or a function of the low levels of replication. Advice has been sought from Curtin University which will be incorporated into the final report.

Approximately half of the species recorded by BRUVs were detected in e-DNA samples, which is remarkedly consistent given the very small sample size used in the e-DNA study (eight litres of water). Species accumulation curves shown in Figure 4-2 have a steep trajectory at a sample size of eight, suggesting that with greater replication a much wider number of species would be detected.

Overall, the e-DNA and BRUV methodologies provide complementary data on the vertebrate fauna values of the FPE and surrounding areas. Further sampling effort would be required should these tools be considered for more detailed investigations of community structure and biodiversity values in time and space.

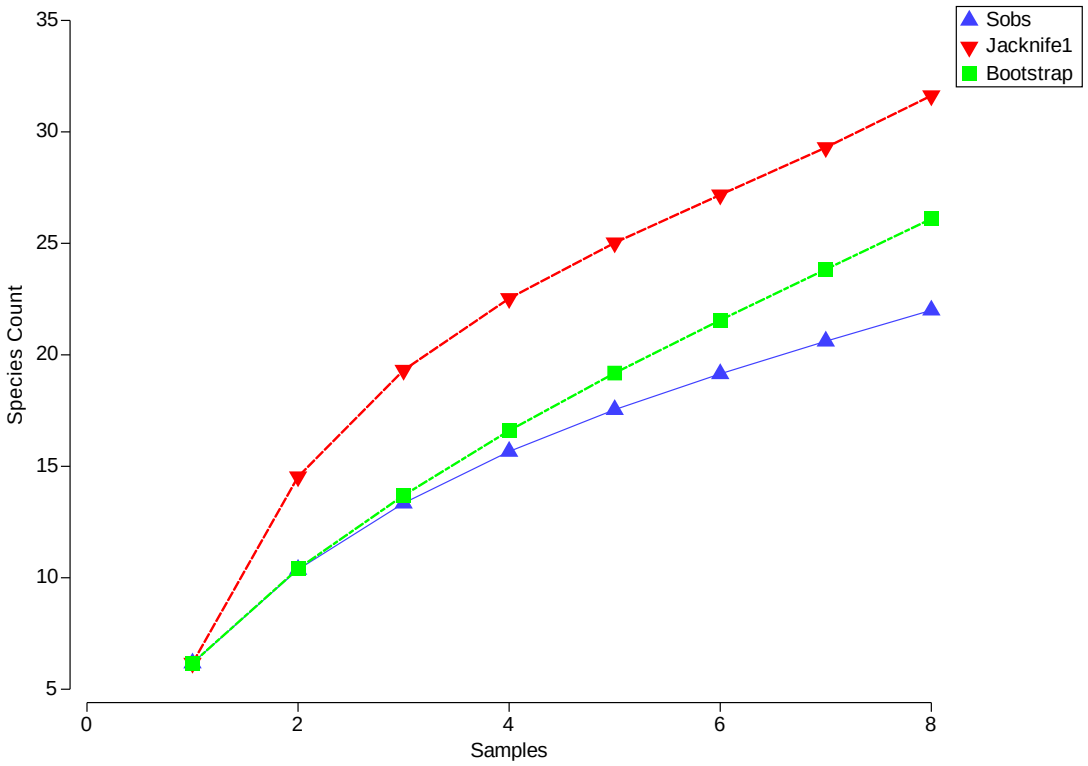

Figure 4-2 Species accumulation curve for e-DNA samples based on Sobs, Jack-knife and boot strap methods

4.3 Soft Sediment Habitats

Surface seabed habitats surrounding the FPE seawall are largely comprised of sand- to mud-dominated soft sediment classes. These were distributed as two broad classes, with three transitional mixtures of sand and mud between these two extremes. Single-beam acoustic backscatter intensity could not reliably separate fine sand from seawall substrates; however, side-scan sonar was used to determine the extent of seawall. Sides-scan sonar was also useful for defining many small features such as debris, pipes, schools of fish, and large fauna such as sharks.

Backscatter intensity provided a different distribution of soft sediments than previously observed using QTC methodologies. While the 2019 hardness estimation had a higher degree of subjectivity, in that decibel range separation was determined after interrogating broad areas of apparently similar seabed, the spatial patterns produced through this workflow were highly representative of observed conditions and are consistent with coastal processes operating in the area.

Fine sandy sediments dominated the shallowest areas on the north-western and southern faces of the seawall, where bed shear stresses from wave energy are sufficient to winnow fine sediment out of the seafloor, leaving behind heavier sands. Once depths have become sufficient to remove the influence of short-period wind waves generated across Moreton Bay, fine sediments are no longer mobilised under the majority of wave and wind conditions, and deposited fines begin to remain as a part of the sediment matrix. This occurs in waters deeper than approximately 1.75 m below LAT over much of the site.

Several large sediment ridges east of the seawall were arranged in an approximately north-south direction suggesting that sediment transport is occurring from an east to east-south-east weather pattern. Sediment s on the crest of the shallowest sediment wave appear to have more sand content, with muddier sediments present in troughs between the sand ridges.

Both the QTC methodology and backscatter intensity approach used in 2019 were unable to effectively separate fine sands from seawall habitat. This may be partially due to the shallow waters that both occur in, preventing the use of the second echo, which is typically more effective for understanding hardness. Nevertheless, the full extent of seawall habitat could be mapped using side-scan sonar, to differentiate any seawall mis-classified as sand.

The dominant sediment type in the vicinity of the seawall (within the extent of the current study area) was medium to coarse sand. Areas dominated by finer sediments (i.e. silt to fine sand) also occur along the mid south-eastern wall, at the eastern extent of the study area (offshore from the wall), as well as along the north-eastern wall. Just as PSD investigations found that there was more coarse sand within sediment fractions, the 2014 acoustic dataset contained a much larger proportion of class 2 sediments (96%, Table 3-4) in 2009 (90%).

The present survey and the 2009 survey both identified an isolated patch of finer sediments located adjacent to the south-eastern section of the FPE seawall, as shown in **Error! Reference source not found.**. In the absence of pre-FPE seawall sediment data form these areas, it is uncertain whether these fine sediments have always been present in this area. Data from the surrounding areas suggests that these areas were predominantly comprised of sands (WBM 1992), suggesting that there may have been a change in sediment types in these areas.

The soft sediment seabeds are largely bare substrate, occasionally containing organic debris and/or surface periphyton layer. However, patches of sparse seagrass (*Zostera muelleri*, *Halodule uninervis*) and macroalgae were occasionally observed over sandy substrates. Both bare and vegetated soft sediments provide habitat for marine invertebrate communities, such as macroinvertebrates (e.g. worms, molluscs etc.). These soft sediment habitats and their associated communities provide ecological functions that are important to the maintenance of local ecosystem processes, including nutrient cycling processes, primary production, provision of food resources, and a linkage between littoral wetland areas (i.e. mangroves, saltmarsh), seagrass beds and deeper nearshore soft sediment habitats. Soft sediment macroinvertebrate communities also represent a significant food resource for larger animals, including fish and crustaceans of fisheries significance that occur in the vicinity of the seawall and wider area (e.g. bream, whiting, flathead, sand crabs).

5 Conclusions

The present study demonstrated that:

- Consistent with the results of the 2009 and 2014 surveys, the FPE seawall continues to support an abundant macroalgae assemblage numerically dominated by two brown algae species: *Sargassum* and *Dictyopteris australis.*
- Also consistent with the 2009 and 2014 surveys, benthic fauna cover was low across the study area. This is likely due to: (i) competition with macroalgae and (ii) where *Sargassum* formed a dense over-storey, benthic fauna in the under-storey were not observable using video-based assessment methods.
- There was a shift in community structure between 2009 and 2014/19 surveys. Successive major flood events in 2011 and 2013 are likely key drivers of change, as also observed in adjacent seagrass meadows.
- Patterns in benthic community structure on the FPE seawall were similar to that observed on natural reef systems in western Moreton Bay. The main exception to this was that hard coral cover on the FPE seawall was generally lower (<0.5%) than observed on similar shallow water reefs (typically 5-10%).
- A baited remote underwater video (BRUV) system was successful in quantifying the relative abundance of large (>5 cm) fish within the water column directly above the FPE seawall. The FPE seawall provides a locally important fisheries habitat. The crevices and high macroalgae cover provide shelter for small reef dwelling species, and large schools of fish of direct fisheries significance (particularly bream and mackerel) aggregate around the rock wall.
- A pilot study using e-DNA meta-coding methods detected more than 23 marine vertebrate species, most of which were reef-associated species. Approximately half of the species recorded by BRUVs were detected in e-DNA samples, which is remarkable given the small sample size adopted in this pilot. The two methodologies provide complementary data on the vertebrate fauna values of the FPE and surrounding areas, however further sampling effort would be required should these tools be considered for more detailed investigations of community structure and biodiversity values in time and space.
- The substrates adjacent to the FPE seawall were comprised of sand, with small areas of fine sediment detected in places.
- No gross changes in sediment types were recorded over time in areas immediately adjacent to FPE seawall, except perhaps a small area of silts located immediately adjacent to the south-east section of the wall.

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Appendix A Curtin University e-DNA report

An eDNA biodiversity survey of marine species present at a marine seawall site in Queensland

Prepared for

BMT Eastern Australia

Prepared by
eDNA frontiers
Curtin University

Report Authors:
Dr. Rose Lines and Dr. Tiffany Simpson

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Executive Summary

Study Scope:

Using environmental DNA (eDNA) metabarcoding methods, eDNA frontiers laboratory was tasked with analysing the marine biodiversity present at the Seawall at the Port of Brisbane. BMT provided a total of 8 samples consisting of 4 seawater samples suspended on filter membranes collected at 2 locations at the Seawall. The objective of the study was to use eDNA metabarcoding to test the water samples for the presence of marine reef fish, sharks, and dugongs.

Samples and Data:

Samples delivered to eDNA frontiers for analysis comprised 8 filtered water samples from two sites at the Port of Brisbane Seawall.

Filter membranes were processed to extract eDNA and three metabarcoding methods were used to detect marine vertebrates. These assays were selected as collectively they target a wide range of fish, mammals and elasmobranchs.

Results and Conclusions:

eDNA testing recorded a variety of marine vertebrates from all 8 samples in this survey. Analysis of samples detected 4 classes, 16 orders, 21 families, and at least 23 species.

Fish (Class Actinopteri) were abundant with at least 18 species detected across both sites. Elasmobranchs were also detected at both sites. Dugongs were not detected in this study. Green turtle DNA was detected in one of the samples.

This report documents the taxa detected at each site of the Port of Brisbane Seawall and makes recommendations for future work.

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1.0 INTRODUCTION

1.1 eDNA Background

Environmental DNA (eDNA) refers to a complex mixture of genomic DNA present in biological samples as a by-product of metabolic processes – it can be derived from multiple sources such as: faeces, urine, skin, hair, saliva and whole microorganisms (e.g. bulk samples), which can be extracted and analysed from the environment non-invasively (Taberlet *et al.* 2012a, Taberlet *et al.* 2018).

Traditionally, genetic approaches to characterise organisms used DNA barcoding; a technique where genetic markers (barcodes) consisting of higher interspecific than intraspecific variation were analysed, resulting in species identification (Hebert *et al.* 2003). However, this method was not feasible when a biological sample contained many species. With the development of Next Generation Sequencing technologies, it is now more favourable to utilise DNA "metabarcoding", which utilises the amplification of multiple taxonomic groups simultaneously, allowing for the ability to generate complete biotic surveys from complex (multi species) samples. The method is a powerful tool that can used to identify taxa from bacteria to mammals, importantly in most instances it decreases the cost and laboratory time associated with species identification (Thomsen *et al.* 2012; Bohmann *et al.* 2014; Gardner *et al.* 2016; Stat *et al.* 2017). In recent years there has been a growing amount of literature reporting the use of these methods for biodiversity analyses (Thomsen & Willerslev, 2015) due to the wide applicability of metabarcoding and eDNA methods (Figure 1-1).

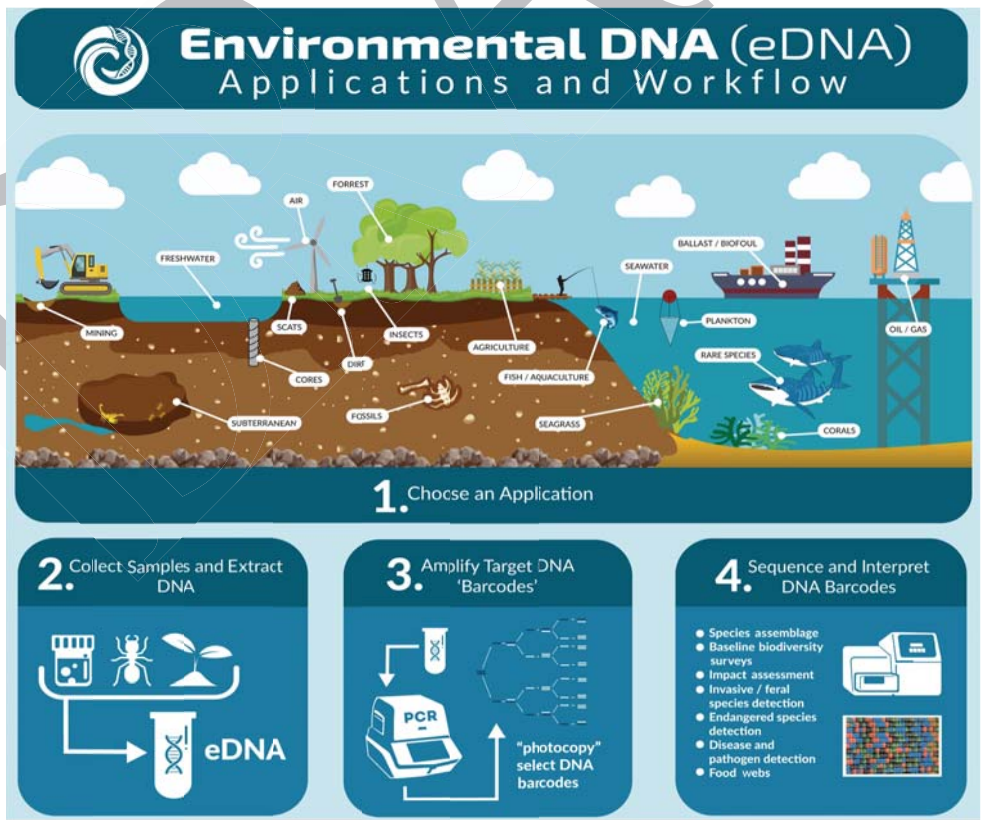

Figure 1-1. Environmental DNA (eDNA) Applications and Workflow

eDNA based methods have been used as a novel technique for both total biodiversity assessment (Stat *et al.* 2017), detection of fish (Miya *et al*. 2015, Stat *et al.* 2018) and alien invasive species (AIS) (Pochon *et al.* 2013). eDNA can be detected in a range of substrates such as water, sediment and biofoul (Koziol *et al.* 2018). Collection of samples is rapid and a wide range of taxa can be detected in a single sample, without observing the individual organisms (Ficetola *et al.* 2008).

Studies comparing the efficacy of eDNA metabarcoding with traditional techniques for detection of AIS have demonstrated that molecular based techniques can outperform the detection efficacy of traditional methods, while also reducing detection time (Valentini *et al.* 2016). This is paramount for biosecurity purposes, as the early detection of first colonisers, which may be initially low in abundance, is critical in the successful eradication of AIS before they become established and are difficult or logistically unfeasible to eradicate (Simberloff, 2003).

Koziol *et al.* (2018) report that the biological substrate used in eDNA studies heavily influences the biotic profiles obtained, accordingly, there are often advantages to processing more than a single biological substrate when employing eDNA metabarcoding. Depending on the question being addressed it may be advisable to collect samples from a range of different substrate types. Another important consideration in experimental design is selection of appropriate eDNA assay. Stat *et al.* (2017) discussed the importance of using multiple metabarcoding assays to detect a greater diversity of taxa. Hence, assay selection will depend on the aims of the study. For this study four assays were selected that target a wide diversity of fish, marine mammals, elasmobranchs and marine invertebrates.

2.0 OBJECTIVES

Using environmental DNA (eDNA) metabarcoding, eDNA frontiers was tasked with analysing the marine vertebrate biodiversity present at the Port of Brisbane Seawall. BMT Eastern Australia provided eight seawater samples suspended on filter membranes. The objective of the study was to use eDNA metabarcoding to test the water samples for the presence of fish, sharks and dugongs.

3.0 METHODS

3.1 Sampling Locations

Water samples were collected by BMT staff on 30th May 2019 at the Port of Brisbane Seawall. Four replicates were taken at each site (Table 3-1) totalling 8 water samples. BMT did not provide a water control for either site.

Table 3-1. Water Sample Collection Details from the Port of Brisbane Seawall

Sample	Date	Time	Sample Type	Sample Volume
FI5-1	5/30/19	13:00	Water filtering	1 L
FI5-2	5/30/19	13:00	Water filtering	1 L
FI5-3	5/30/19	13:00	Water filtering	1 L
FI5-4	5/30/19	13:00	Water filtering	1 L
FI2-1	5/30/19	12:00	Water filtering	1 L
FI2-2	5/30/19	12:00	Water filtering	1 L
FI2-3	5/30/19	12:00	Water filtering	1 L
FI2-4	5/30/19	12:00	Water filtering	1 L

3.2 Sample Collection

Water samples consisting of 1L were collected and filtered using 0.45µm mixed cellulose esters with a peristaltic Sentino pump to capture eDNA present in the water. All filtering was carried out by BMT staff. Water membranes were frozen prior to shipping. All samples were shipped frozen to eDNA frontiers and on arrival, samples were stored at -20°C.

3.3 Laboratory Methods

3.3.1 eDNA Extraction and Analysis

DNA was extracted from half a filter paper using a Qiagen DNeasy blood and tissue kit, following the eDNA frontiers lab's SOPs and detailed in Koziol *et al.* (2018), Stat *et al.* (2017), Stat *et al.* (2018). Each water sample was assigned an individual barcode tag and amplified by PCR using three assays: (i) a proprietary mtDNA 16S assay termed '16S NEST' (ii) a mtDNA COI assay termed 'COI elasmo' (Ward *et al.* 2005, Fields *et al.* 2015) and (iii) a 16S mammal assay (Taylor 1996). A library was generated and sequenced using the Illumina MiSeq® platform. Laboratory extraction controls were included to test for contamination.

3.3.2 Bioinformatics and Taxonomic Assignments

Bioinformatic tools were used to analyse raw sequence data. Results were demultiplexed and trimmed using ObiTools and quality filtered with Usearch v11 for sequencing errors (maxee=1) and minimum length (COI minlength=100, 16S NEST minlength=50, 16S Mammal minlength=90). Sequences were then dereplicated and unique sequences were transformed into zero radius operational taxonomic units (ZOTUs) to provide sensitive taxonomic resolution (Usearch v11) (Edgar 2018). ZOTUs, in contrast to OTUs are a more exact sequence variant, clustering at 99% to improve taxonomic resolution. Generated ZOTUs were queried against the nucleotide database NCBI (Genbank) and assigned to the species level. Taxonomic

assignments were based on an in-house Python script which does further filtering of Blast results (evalue <= $1e^{-5}$, %identity >= 94 and qCov >= 100), combines it with ZOTU table results and produces a table containing the taxonomic information available from Blast taxonomy database (accessed August 2019).

It is important to note that barcodes recovered are converted to the lowest possible taxon based on similarities and differences to a DNA database (NCBI's Genbank). This database, and the taxonomic framework that underpins it may contain errors. Accordingly, the DNA taxon identifications should be interpreted as the best available assignment based on currently available information and that errors at species level are possible.

4.0 RESULTS AND DISCUSSION

A range of taxa were detected in all samples. The dominant Class of marine vertebrate taxa detected at both sites was Actinopteri (fish). A list of marine vertebrate taxa identified are detailed in Table 4-1. Analysis of samples detected 4 classes, 16 orders, 21 families, and at least 23 species.

The objective of the study was to use eDNA metabarcoding to test the water samples for the presence of fish, sharks, and dugongs. Dugongs were not detected in this study. The failure to detect dugong DNA in these samples may indicate that dugongs were not present in the immediate area at the time of sampling, or low level of replication may be impacting on species detection rates.

The Class Actinopteri was the most detected taxa with 18 species detected across both locations sampled at the Port of Brisbane Seawall. Requiem sharks were detected at site FI2, but not site F15. Bamboo shark (*Chiloscyllium punctatum)* was detected at site F15 but not F12. Blue spotted stingray (*Neotrygon kuhlii)* was detected at both sites. The Cormorant marine bird *(Phalacrocorax sulcirostris*) was also detected at both sites. Interestingly Green turtle (*Chelonia mydas*) was detected in one sample. Our lab has previously reported that detection of reptile DNA from environmental samples is problematic. Adams *et al*. 2019 propose that detection of reptiles is difficult from environmental samples due to low shedding rates. If turtle faeces were present in the water this would account for the detection of turtle DNA in this sample. However, another possibility exists. The sample that contained turtle DNA also tested positive for *Carcharinhus sp*. (shark). It is therefore possible that turtle tissue may have been consumed by the shark and excreted into the water, facilitating detection of the turtle DNA.

Table 4-1. Vertebrate Taxa detected at the Port of Brisbane Seawall, using eDNA metabarcoding. Presence of the species at each site is indicated by the symbol ●. Fish common names as per FishBase.org (accessed Aug 2019)

Phylum	Class	Order	Family	*Genus species*	Common name	FI5-1	FI5-2	FI5-3	FI5-4	FI2-1	FI2-2	FI2-3	FI2-4
Chordata	Actinopteri	Anguilliformes	Anguillidae	*Anguilla reinhardtii*	Speckled long-finned eel	●							
Chordata	Actinopteri	Atheriniformes	Atherinidae	*Atherinomorus sp.*	Silversides	●	●	●	●				●
Chordata	Actinopteri	Beloniformes	Hemiramphidae	*Hyporhamphus quoyi*	Quoy's garfish		●	●	●				
Chordata	Actinopteri	Centrarchiformes	Kyphosidae	*Girella tricuspidata*	Parore			●					
Chordata	Actinopteri	Centrarchiformes	Terapontidae	*Pelates quadrilineatus*	Fourlines terapon				●				
Chordata	Actinopteri	Gobiiformes	Gobiidae	*Mugilogobius wilsoni*	Wilson's Goby		●						
Chordata	Actinopteri	Labriformes	Labridae	*Pseudolabrus guentheri*	Gunthers wrasse			●		●			●
Chordata	Actinopteri	Labriformes	Labridae	*Scarus sp.*	Parrotfish					●			
Chordata	Actinopteri	Mugiliformes	Mugilidae	*Mugil cephalus*	Mullet	●	●	●	●	●			●
Chordata	Actinopteri	Perciformes	Platycephalidae	*Platycephalus australis*			●						
Chordata	Actinopteri	Perciformes	Platycephalidae	*Platycephalus fuscus*	Dusky flathead		●	●					
Chordata	Actinopteri	Perciformes	Serranidae	*Epinephelus malabaricus*	Malabar grouper						●		
Chordata	Actinopteri	Perciformes	Siganidae	*Siganus sp.*	Rabbitfish	●			●	●			
Chordata	Actinopteri	Perciformes	Sillaginidae	*Sillago aeolus*	Western trumpeter	●	●		●				●
Chordata	Actinopteri	Priacanthiformes	Priacanthidae	*Priacanthus macracanthus*	Red Bigeye					●			
Chordata	Actinopteri	Scombriformes	Pomatomidae	*Pomatomus saltatrix*	Bluefish	●			●				
Chordata	Actinopteri	Spariformes	Sparidae	*Rhabdosargus sarba*	Goldlined Seabream	●		●	●				
Chordata	Aves	Pelecaniformes	Phalacrocoracidae	*Phalacrocorax sulcirostris*	Cormorant		●	●	●		●	●	
Chordata	Chondrichthyes	Carcharhiniformes	Carcharhinidae	*Carcharhinus sp.*	Requiem shark						●	●	●
Chordata	Chondrichthyes	Myliobatiformes	Dasyatidae	*Neotrygon kuhlii*	Blue-spotted Stingray			●				●	
Chordata	Chondrichthyes	Orectolobiformes	Hemiscylliidae	*Chiloscyllium punctatum*	Bamboo shark	●							
Chordata	Reptilia	Testudines	Cheloniidae	*Chelonia mydas*	Green Turtle							●	

5.0 CONCLUSIONS AND RECOMMENDATIONS

This report documents the taxa recovered by an eDNA survey from 8 samples collected from the Port of Brisbane Seawall. A range of taxa including fish, elasmobranchs, a bird and a turtle were detected. Dugongs were not detected in this study.

As dugongs occur in lower abundance, a recommendation for future surveys would be to; (i) increase the number of replicates at each site (ii) to include more sites so an ordination analysis can be executed and (iii) possibly expand sampling to include replicate plankton net tows and/or sediments to see if some of the target DNA are more abundant in these substrates (see Koziol *et al.* 2018).

It is also recommended that in future surveys, control samples be collected and included in the analysis. The samples and DNA extracts derived from this study will be stored within eDNA frontiers premises for a period of 12 months.

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Appendix 1 - Glossary

Term	Definition
% value in data	Represents the percentage similarity of a DNA sequence recovered from a sample compared to reference sequences in a database (e.g. compared to DNA databases such as GenBank or references generated in-house)
(x) value in data	Represents the frequency the % value was recorded in the dataset
16S rRNA	The 16S rRNA refers to a conserved gene region of mitochondrial DNA, which codes for a subunit of the ribosome. 16S rRNA is found in all eukaryotes making it a good candidate for DNA barcoding
18S rRNA	The 18S rRNA refers to a conserved gene region of nuclear DNA, which codes for a subunit of the ribosome. 18S rRNA is found in all eukaryotes making it a good candidate for DNA barcoding
18S AIS reference database	Reference 18S rRNA sequences of invasive marine species that are available in DNA databases
AIS	Alien Invasive Species
Assay	In the context of eDNA metabarcoding an assay refers to a PCR 'test' that selectively targets a subset of biota from an environmental DNA sample. The use of multiple assay when combined will always detect a wider diversity of taxa than a single assay. eDNA assays should be selected to address the question relevant to the study.
Barcode	Refers to a region of DNA sequenced for many species that is able to (through variation in the DNA sequence) is able to differentiate species. DNA barcodes are the most common targets of eDNA studies that seek to explore taxon assemblages.
COI	The gene region that is being used as the standard barcode for almost all animal groups is a 648 base-pair region of the mitochondrial cytochrome c oxidase 1 gene ("CO1"). COI is proving highly effective in identifying birds, butterflies, fish, flies and many other animal groups. COI is not an effective barcode region in plants because it evolves too slowly, but two gene regions in the chloroplast, matK and rbcL, have been approved as the barcode regions for plants
CO1 AIS reference database	Reference COI sequences of invasive marine species that are available in DNA databases
DNA	Deoxyribonucleic Acid (DNA) is the hereditary material that contains the genetic information of an organism
DNA metabarcoding	Is a genetic technique that simultaneously amplifies and sequences barcode regions (e.g. COI, 18S, 16S) of many different species in parallel
eDNA	Environmental DNA (eDNA) refers to genetic material that is recovered from an environmental substrate (e.g. water, sediment, air)

Term	Definition
Eukaryotes	An organism where cells contain a nucleus surrounded by a membrane and has the DNA bound together by proteins (histones) into chromosomes. The cells of eukaryotes also contain an endoplasmic reticulum and numerous specialised organelles not present in prokaryotes, especially mitochondria, golgi bodies, and lysosomes
Fisheries	Department of Primary Industries and Regional Development, Fisheries Division, Aquatic Biosecurity Section
GenBank	Publicly available repository of genetic information. Contains the barcode information of genes that have previously been sequenced
Genome	A genome is all the genetic material of an organism. It consists of DNA (or RNA in RNA viruses). The genome includes both the genes (the coding regions) and the noncoding DNA. In eukaryotes it refers to the genomes of the nucleus, mitochondria and chloroplasts. In prokaryotes, there is a single genome (as they do not contain mitochondria or chloroplasts)
Illumina MiSeq	Next generation sequencing platform developed by the company Illumina
IMP	Introduced marine pests
Low abundance	Low abundance reads have been defined as those that constitute <0.1% of total reads for a particular sample
Metabarcoding assay	A PCR reaction using a specific set of primers that simultaneously amplifies the same gene target from multiple species. Also see definition of 'assay'.
Mitochondrial DNA (mtDNA)	The mitochondrion (plural mitochondria) is a double membrane-bound organelle found in all eukaryotic organisms. mtDNA markers (e.g. 16S or COI) are common DNA barcodes.
Mitogenomes	Refers to the mitochondrial genome
NGS	Next generation sequencing or second generation sequencing refers to massively parallel sequencing technology, as opposed to first generation sequencing or sanger sequencing where only a single template is sequenced at one time
Nucleotide	A compound consisting of a nucleotide linked to a phosphate group. Nucleotides form the basic structural unit of nucleic acids such as DNA
PCR	Polymerase chain reaction (PCR) is the technique that is used to amplify (akin to photocopying DNA) specific regions of the genome from specific groups of taxa
Primer	A short DNA strand (≈20bp in size) used in PCR to target particular groups of organisms and genes. Two of them are required for PCR (a forward and a reverse)
Primer binding site	A primer-binding site is the target region of a genome where the primer attaches to start replication. The primer binding site is on one of the two complementary strands of a double-stranded nucleotide polymer, in the strand which is to be copied, or is within a single-stranded nucleotide polymer sequence

Term	Definition
Prokaryote	Any of the typically unicellular microorganisms that lack a distinct nucleus and membrane-bound organelles and that are classified as a kingdom (Prokaryotae syn. Monera) or into two domains (Bacteria and Archaea)
RNA	Ribonucleic acid (RNA) is a polymeric molecule implicated in various biological roles in coding, decoding, regulation, and expression of genes
rRNA	ribosomal ribonucleic acid is the RNA component of the ribosome, and is essential for protein synthesis in all living organisms
Sequence	DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases—adenine, guanine, cytosine, and thymine—in a strand of DNA
Shotgun sequencing	Refers to randomly sequencing short pieces of DNA (≈150bp in size) after shearing or cutting DNA (e.g. fragmenting a genome)
EDNA frontiers	Trace and Environmental DNA laboratory, Curtin University
OTU	Operational Taxonomic Unit is a molecular biology term that describes unique DNA barcode clusters and how they are different from one another. It is usually defined by a % cut-off based on DNA sequence similarity. The value of OTUs is that biodiversity can be compared without the need to assign each sequence into a taxonomic framework and is most appropriate when there are large deficiencies in the underpinning taxonomic framework. OTU are very similar in function to ZOTUs (see below).
ZOTU	Zero-radius Operational Taxonomic Unit is a molecular biology terms that describes unique DNA barcode clusters and how they are different from one another. It is usually defined by a % cut-off based on DNA sequence similarity. The value of ZOTUs is that biodiversity can be compared without the need to assign each sequence into a taxonomic framework and is most appropriate when there are large deficiencies in the underpinning taxonomic framework. ZOTU are very similar in function to OTUs (see above) but describe more exact sequence variants

BMT has a proven record in addressing today's engineering and environmental issues.

Our dedication to developing innovative approaches and solutions enhances our ability to meet our client's most challenging needs.

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