OceanEcology

BACI Study on the Impact of Otter Trawling on Mud Habitat in the Torbay MCZ

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Prepared for

Devon & Severn IFCA
Inshore Fisheries and Conservation Authority

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LIST OF ABBREVIATIONS

AMBI	AZTI Marine Biotic Index
ANOVA	Analysis of Variance
BACI	Before-After-Control-Impact
C	Control
D&SIFCA	Devon and Severn Inshore Fisheries and Conservation Authority
EEC	European Economic Community
EU	European Union
EUNIS	European Nature Information System
ID	Identification
IDA	Industrial Denatured Alcohol
IQI	Infaunal Quality Index
L	Litres
LOI	Loss on Ignition
MCAA	Marine and Coastal Access Act
MCZ	Marine Conservation Zone
MDS	Multidimensional Scaling
MPA	Marine Protected Area
N	Total Number of Individuals
NMBAQC	NE Atlantic Marine Biological Quality Control
OEL	Ocean Ecology Limited
P	Present
PCA	Principal Components Analysis
PERMANOVA	Permutational Analysis of Variance
PERMDISP	Permutational Test for Homogeneity of Multivariate Dispersions
PRIMER	Plymouth Routines in Multivariate Ecological Research
PropD	Proportion of Damaged Taxa
PSaS	Particle Size Analysis Software
PSD	Particle Size Distribution
S	Total Diversity
SAC	Special Area of Conservation
SE	Standard Error
SIMPER	Similarity Percentages
SIMPROF	Similarity Profile
SOP	Standard Operating Procedures
T	Treatment
TaxD	Taxonomic Distinctness
WoRMS	World Register of Marine Species

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1. NON-TECHNICAL SUMMARY

Ocean Ecology Limited (OEL) was commissioned by Devon & Severn Inshore Fisheries and Conservation Authority (IFCA) to undertake a before-after-control-impact (BACI) study on the impact of otter trawling on mud habitats in the Torbay Marine Conservation Zone (MCZ). This study involved the acquisition of grab samples within two locations, Location A and Location B, within the 'subtidal mud' feature of the MCZ, one day before and one day after experimental trawling in October 2017. Different trawl gears were used in each location to assess the impacts associated with the use of each gear type separately. In Location A a box trawl was used while in Location B a lighter wing trawl was used. Location A, where the heavy box trawl gear was used, was sampled six months following trawling disturbance, to assess recovery of the mud habitats and macrobenthic communities. Results of the study will inform management of the otter trawl fishery in the Torbay MCZ and the use of similar gears in other Marine Protected Areas (MPAs).

The sampling design was developed by Devon & Severn IFCA and Eastern IFCA in conjunction with Matt Witt at Exeter University. Benthic sampling was carried out using a 0.1m^2 Day grab and was undertaken across each location, within which were three trawled sites and two control sites. Six grab samples were taken within each site for sediment and macrobenthic analysis. Macrobenthic samples were sieved over a 1 mm mesh sieve with all taxa retained and identified to species level where possible at OEL's NMBAQC participating laboratory. Data indicate that the study area across both locations is characterised by muddy sand habitats and communities typical of such habitats; macrobenthic assemblages are dominated by the bivalves *Spisula subtruncata* and *Fabulina fabula* and to a lesser extent the polychaetes *Euclymene oerstedii*, *Melinna palmata* and *Ampharete lindstroemi*. *Nephtys* spp. and *Magelona filiformis* are also present in both locations although in smaller numbers. The biotope that best characterises conditions across both locations therefore appears to fall somewhere between EUNIS Habitat A5.244 *Spisula subtruncata* and *Nepthys hombergii* in shallow muddy sand and EUNIS Habitat A5.242 *Fabulina fabula* and *Magelona mirabilis* with venerid bivalves and amphipods in infralittoral compacted fine sand.

Natural spatial and temporal variation was evident in traditional diversity indices and ecological quality indicators, as well as faunal assemblages and individual species abundance, although results show no impact of trawling on any of these responses. PERMANOVA results suggest no detectable impact of either of the trawl gears used on either sediment composition or the macrobenthic community assemblage across BACI groups. While temporal differences in a number of responses are evident in April 2018 compared to October 2017, this is most likely as a result of natural seasonal variation in benthic communities. Despite no statistical difference, ecological quality as indicated by mean Infaunal Quality Index (IQI) values dropped in April 2018 in trawled sites from "high" to "good" status, while no change was evident in control areas. The lack of any clear signal of trawl impacts should be caveated by the short-term nature of the fishing disturbance and sampling so soon after the cessation of trawling. Future monitoring may seek to assess impacts of trawling at intensities more representative of the operational fishery, by utilising the same sampling design before and after the three-month trawl season within the Torbay MCZ. Collection of biomass and/or size data may also help elucidate trawling impacts as abundance data alone may not accurately reflect changing dominance structures within macrobenthic communities.

2. INTRODUCTION

This report has been prepared by Ocean Ecology Limited (OEL) on behalf of Devon and Severn Inshore Fisheries and Conservation Authority (D&SIFCA) to present the results of a focused study on the impacts to and recovery of protected subtidal mud habitats subject to otter trawling within the Torbay Marine Conservation Zone (MCZ). Bottom-towed fishing may elicit various physical and biological impacts to designated features of marine protected areas (MPAs) through physical interaction with the seabed. Such impacts may include reductions in benthic habitat complexity and a loss of finer sediments (Martín et al. 2014, Palanques et al. 2014), disruptions to benthic food webs (Hiddink et al. 2017), reductions in productivity, diversity and species richness (Kaiser et al. 2006), and changes in community dominance patterns (Borja & Franco 2000, Collie et al. 2000, Kaiser et al. 2006). In MPAs, the use of mobile and demersal fishing gears must therefore be managed to minimise impacts on designated features and to ensure that management and conservation objectives within a site are met.

2.1. Study Site

Torbay Marine Conservation Zone (MCZ) is located on the South Devon coast, UK, and is an inshore site that covers an area of approximately 20 km^2 extending from Oddicombe Beach in the north to Sharkham Point in the south (Figure 1). The site boundary extends 1 - 2.5 km offshore and to depths of 30 m[1] and falls within the Devon and Severn Inshore Fisheries and Conservation Authority (IFCA) district. Under the Marine and Coastal Access Act (MCAA) 2009, D&S IFCA have a responsibility to manage fisheries within MCZs in their district and to ensure that the conservation objectives of these sites are met. Torbay MCZ includes a variety of intertidal and subtidal habitats exposed to varying levels of environmental disturbance, supporting high levels of biodiversity. The site received designation as part of the first tranche of MCZs in the UK in order to protect a number of these intertidal and subtidal habitat features (Table 1).

Table 1. Designated features and management and conservation objectives for the Torbay MCZ and Lyme Bay and Torbay SAC.

Site	Feature	Management/Conservation Objective
Torbay MCZ	Intertidal coarse sediments	Maintain in favourable condition
	Intertidal mixed sediments	Maintain in favourable condition
	Intertidal mud	Maintain in favourable condition
	Intertidal sand and muddy sand	Maintain in favourable condition
	Subtidal mud	Recover to favourable condition
	Low energy intertidal rock	Maintain in favourable condition
	Moderate energy intertidal rock	Maintain in favourable condition
	Intertidal underboulder communities	Maintain in favourable condition
	Seagrass beds	Recover to favourable condition
	Long-snouted seahorse *Hippocampus guttulatus*	Recover to favourable condition
	Native oyster *Ostrea edulis*	Maintain in favourable condition
	Peat and clay exposures	Maintain in favourable condition
Lyme Bay and Torbay SAC	Reefs	Subject to natural change, maintain or restore in/to favourable condition
	Submerged or partially submerged seacaves	Subject to natural change, maintain in favourable condition

[1]https://designatedsites.naturalengland.org.uk/Marine/MarineSiteDetail.aspx?SiteCode=UKMCZ0019&SiteNameDisplay=Torbay+MCZ

The Torbay MCZ site also falls within the Torbay section of the Lyme Bay and Torbay Special Area of Conservation (SAC) (Figure 1), designated under the European Union (EU) Habitats Directive (92/43/EEC). The area was designated as an SAC in September 2017. The Torbay and Lyme Bay SAC covers a total area of approximately 312.5 km^2 and receives designation to protect its reef and submerged sea cave features. Table 1 summarises the designated features and management objectives of each protected site within the study area.

2.2. Project Background and Objectives

On 1st January 2014 a new Mobile Fishing Permit Byelaw came into force within the D&SIFCA district. Under this byelaw the use of bottom-towed fishing gear is prohibited in a number of areas throughout the district to protect sensitive habitats from the impacts of mobile demersal gears, including Lyme Bay and Torbay SAC. These management measures also provide protection for 75 % of the total MCZ area, encompassing a number of protected features. Of the 6.91 km^2 extent of the 'subtidal mud' feature within the MCZ site, which management measures for both the Torbay MCZ and the Lyme Bay and Torbay SAC SAC seek to recover to favourable condition, 54 % is now protected from demersal fishing gear. Particle size analysis carried out as part of an MSc study into the impact of scallop dredging in the Torbay MCZ, however, identified that the area of subtidal mud habitat within the site is in fact largely composed of muddy sand (IFCA 2017). Screening assessments have been undertaken for the remaining 46% (3.21 km^2) of this feature within the site, and together with public consultations, the results of this process have led to the prohibition of scallop dredging within the area of what was considered to be subtidal mud, although has since been found to be muddy sand.

Otter trawling for the common cuttlefish, *Sepia officinalis*, is still permitted within the MCZ. This fishery generally operates from April to June each year, depending on cuttlefish migration into the bay for spawning, and is regulated by D&SIFCA under the Mobile Fishing Permit Byelaw. Following advice from Natural England, the fishery is subject to a Monitoring and Control Plan that requires information on the following:

- Spatial distribution of fishing;
- The level of fishing effort;
- The area of impact of the trawl gear on the mud feature;
- Before-after-control-impact (BACI) studies into such impacts; and
- Information on the potential for technical modification of the gear to be collated.

While some of this information can be gathered from fishers, detailed information on the area of impact and the nature of trawling impacts on the designated mud habitat of the MCZ is unknown and has therefore required the design and implementation of a targeted research programme to fulfil the requirements of the Monitoring and Control Plan.

D&SIFCA have therefore collaborated with SeaFish and OEL to conduct a research project funded by the Department for Environment, Food & Rural Affairs (DEFRA) consisting of a four research strands aimed at gathering information on the area of impact and the nature of trawling impacts on the designated mud habitat of the MCZ. The aims of these research strands were to:

1. To evaluate the area of impact of the fishing vessel's trawl doors and trawl net on the mud habitat of the MCZ;
2. **Undertake a BACI study of the impact of the heavy box trawl on the mud habitat;**
3. **Undertake a 'gear modification' BACI study of the impact of the regular trawl gear used in the cuttlefish fishery on the mud habitat; and**
4. **Undertake a study to assess recovery of benthic habitats, six months after experimental trawling with the heavy box trawl.**

This report presents the results of the BACI and recovery studies listed in strands 2, 3, and 4 of the project (highlighted in bold) undertaken by OEL on behalf of D&SIFCA. Results of the project are expected to be transferrable to other MPAs in which trawling occurs in mud habitats and requires management, particularly MCZs that will be designated during the third tranche of designation in 2018. Recovery of subtidal mud features are likely to be included as management objectives for Tranche 3 designated MCZs and the outputs of the project will inform assessments of demersal trawling impacts in such areas.

The objectives of this study were therefore:

- To undertake a BACI study of the impact of the heavy box trawl gear on the mud habitat within Torbay MCZ;
- To undertake a BACI study of the impact of the regular trawl gear on the mud habitat within Torbay MCZ; and
- To assess recovery of the mud habitat within Torbay MCZ from any impacts associated with the heavy box trawl gear prior to the reopening of the fishery.

2.2.1. Hypotheses

In order to meet the project objectives and to effectively identify any impacts of the two trawling gears on subtidal mud habitats within the Torbay MCZ, and recovery following trawling with the heavy box trawl, the following null and alternative research hypotheses were tested:

Sediments

H0(a): For each gear type, there is no change in sediment particle size distribution between trawled areas and un-trawled control areas and immediately before and after trawling, and/or where a change is observed, the magnitude of change does not differ between trawled and un-trawled areas.

H0(b): In areas subject to heavy box trawling, there is no change in sediment particle size distribution throughout the recovery period and/or where a change is observed, the magnitude of change during the recovery period does not differ between trawled and un-trawled areas.

Infauna

H0(c): For each gear type, there is no change in infaunal diversity and/or community composition between trawled areas and un-trawled control areas and immediately before and after trawling, and/or where a change is observed, the magnitude of change does not differ between trawled and un-trawled areas.

H0(d): In areas subject to heavy box trawling, there is no change in infaunal diversity and/or community composition throughout the recovery period and/or where a change is observed, the magnitude of change during the recovery period does not differ between trawled and un-trawled areas.

These research questions were addressed using the methods outlined in the following sections.

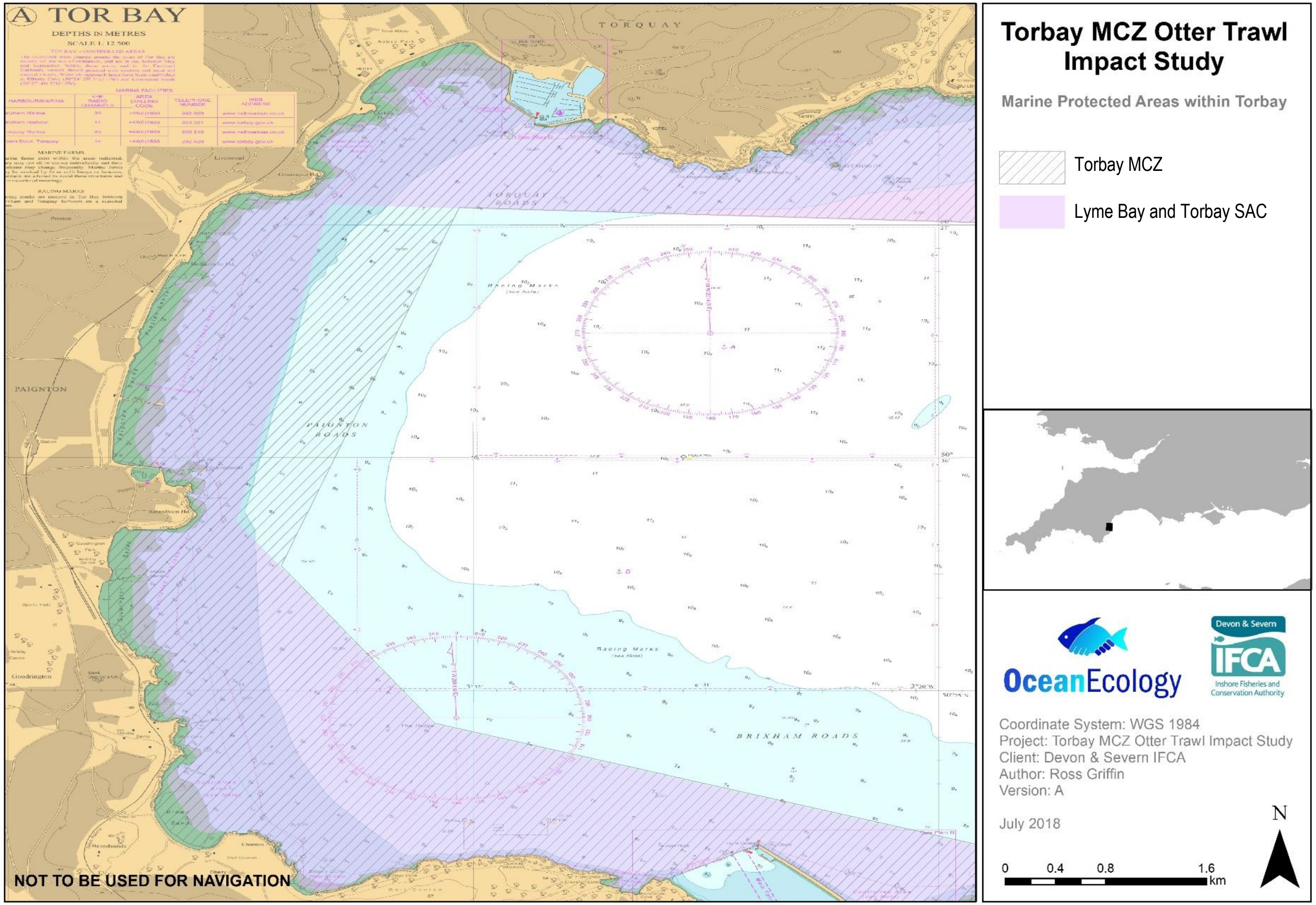

Figure 1 Map illustrating the location of the Torbay Marine Conservation Zone (MCZ) and the Lyme Bay and Torbay Special Area of Conservation (SAC).

3. METHODS

3.1. Sampling Design

3.1.1. BACI Study

To accurately address the research hypotheses H0(a) and H0(c) benthic samples were taken before and after trawling in two locations of subtidal mud (Location A and Location B) within the Torbay MCZ. Trawling in Location A utilised a heavy box trawl, while the regular trawl used in the cuttle fishery was used in Location B, to assess impacts of each gear on benthic habitats and their infaunal communities.

In Location A a 7 fathom (12.8 m) box trawl was utilised, whereby the trawl footrope measures 12.8 m and a side panel of netting is fitted between the top and lower panels of netting. This side netting provides more height to the trawl headline and allows the centre section of the footrope to have better contact with the seabed, reducing the chance of fish escaping below the gear. The box trawl has mini hoppers along the length of the ground gear and chain “ticklers” along the footrope. The headline measures 11 m, allowing the top panel of netting to be towed ahead of the ground gear (Caslake & Montgomerie 2017).

In Location B a 9 fathom (16.45 m) wing trawl was used. This trawl is a simple two panel design with a 16.45 m footrope and is a lighter trawl than that used in Location A. The ground gear of this trawl includes a number of 10 cm long rubbers 2.5-4 cm thick that are located every 45 cm, and 11 mm long link chains at the centre and wing ends of the footrope. The headline is 13.3 m and again allows the top netting to be towed above and ahead of the ground gear. The same otter doors were used on each trawl gear (Caslake & Montgomerie 2017).

The sampling protocol was designed by D&SIFCA and Eastern IFCA in discussion with Dr Matthew Witt at Exeter University, and was identical in each location. In each of the two locations three impact (trawled) sites and two control (un-trawled) sites 1200 m in length and >75 m apart were sampled. Five experimental trawl tows of the full 1200 m were carried out in each of the impact sites to replicate fishing effort during the cuttlefish season, while control sites were unimpacted by trawling.

Each site was sub-divided into three 400 m sections and two samples taken at random within each section (site: n = 6; impact: n = 18; control: n = 12), with a total of 30 samples obtained in each location before and after trawling. All sampling stations were sampled for Particle Size Distribution (PSD) and macrobenthic analysis. Sampling was carried out from the 10th to the 13th October 2017, with samples obtained within 24-48 hours before and 24-48 hours after experimental trawling occurred. The sampling design is presented in Figure 2 and Table 2 and displayed geographically in Figure 3.

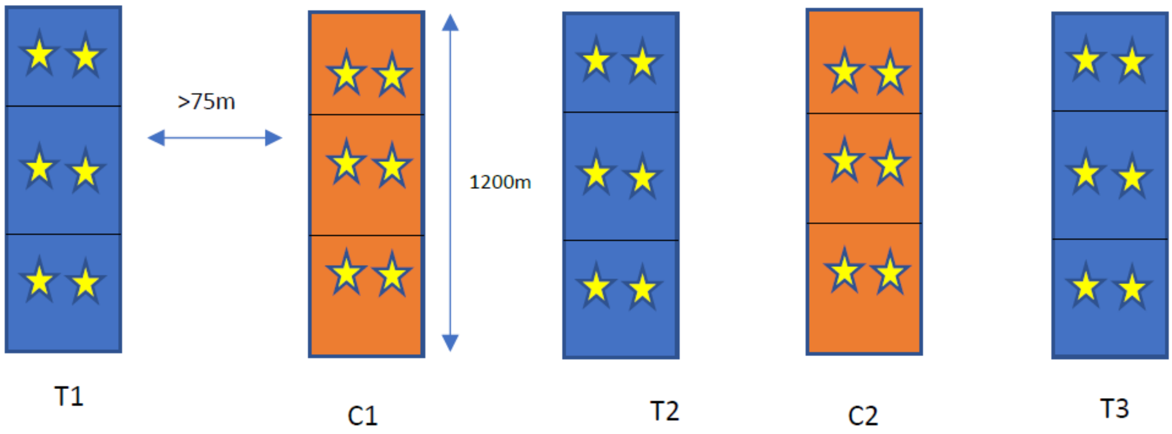

Figure 2. BACI sampling design utilised to monitor trawling impacts in the Torbay MCZ (T = tows/trawled sites; C = control sites).

Table 2 Number of sample stations in each location targeted during the BACI study.

Location	Treatment	Tows			Controls	
		T1	T2	T3	C1	C2
A	Before	6	6	6	6	6
	After	6	6	6	6	6
B	Before	6	6	6	6	6
	After	6	6	6	6	6

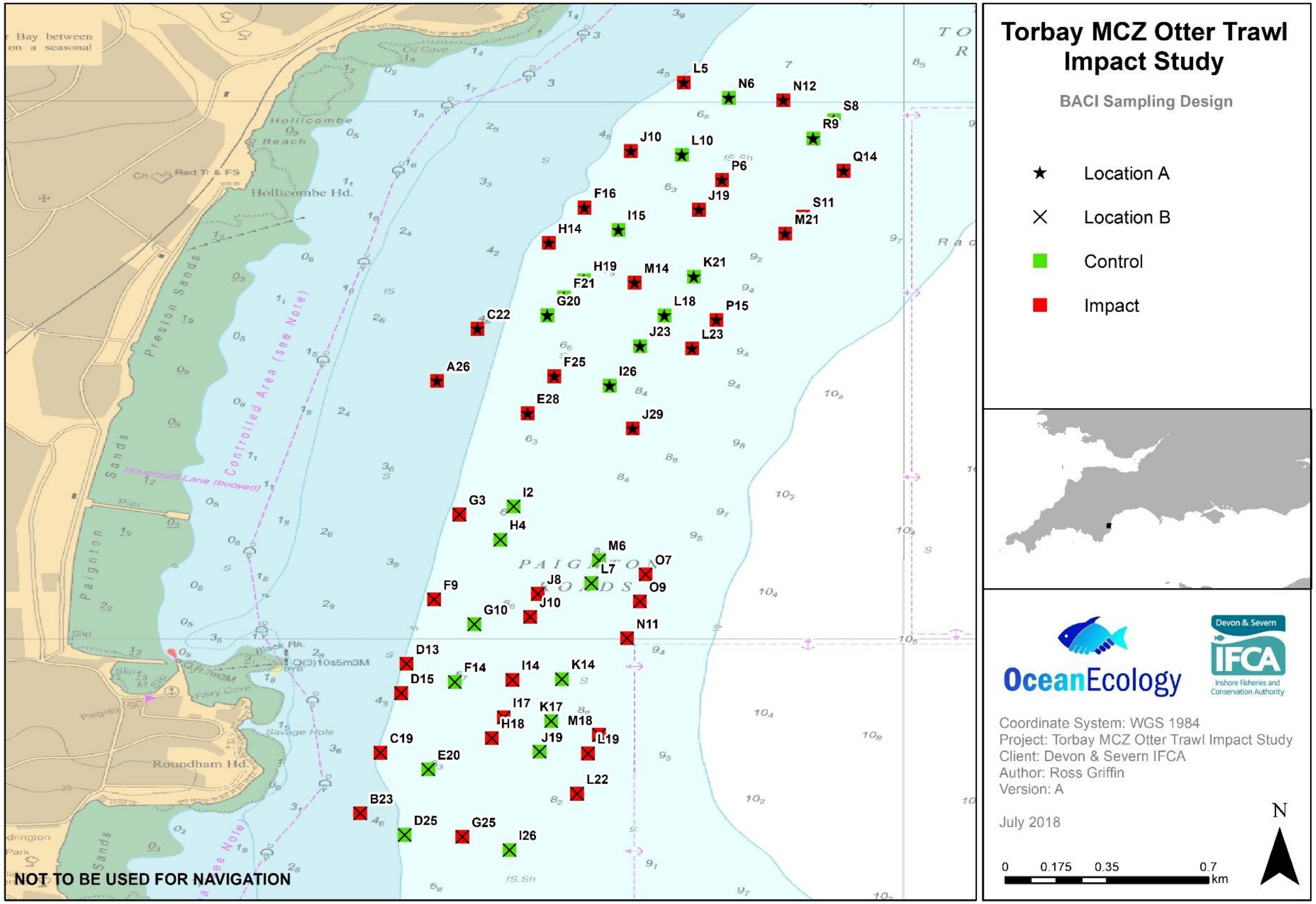

Figure 3 Grab sampling stations targeted during the BACI study on the impact of otter trawling on mud habitat in the Torbay MCZ.

3.1.2. Recovery Study

To assess recovery between the cessation of trawling and the reopening of the fishery in 2018 and to address research hypotheses H0(b) and H0(d), sites in Location A were revisited on the 12th April 2018. Sampling was undertaken in an identical design to that in October 2017, with a total of 30 samples obtained for PSD and macrobenthic analyses. The project scope only sought to assess recovery following use of the heavy trawling gear, therefore no samples were obtained from Location B in 2018.

3.2. Field Methods

3.2.1. Survey Vessel

The Marine and Coastal Agency (MCA) Category 2, 10 m dedicated survey vessel 'Seren Las' (Plate 1), operated by OEL, was utilised to undertake all grab sampling operating out of Brixham Marina in 2017 and Torbay Marina in 2018. 'Seren Las' has been specifically designed for the collection of benthic grab samples and due to its shallow draft, it is an ideal platform for shallow subtidal surveying. 'Seren Las' can accommodate up to five survey personnel and is a comfortable and stable platform with ample space on the back deck for the processing of grab samples.

Plate 1 Dedicated survey vessel, *Seren Las*, utilised for grab sampling during the BACI study on the impact of otter Trawling on mud habitat in the Torbay MCZ.

3.2.2. Survey Equipment

The vessel was equipped with a Hemisphere V104s GPS compass system that provided a highly accurate offset position of the sampling equipment when deployed from the stern. All sampling was undertaken using OEL's 0.1 m^2 Day grab.

3.2.3. Benthic Grab Sampling

3.2.2.1 Sample Collection

To assure consistency in sampling, grab samples were screened by the lead marine ecologist and considered unacceptable if:

- The sample was less than 5 L. i.e. the sample represented less than half the 10 L capacity of the Day grab used or 2.5 L on hard-packed sands.
- The jaws failed to close completely or were jammed open by an obstruction, allowing fines to pass through (washout or partial washout).
- The sample was taken at an unacceptable distance from the target location.
- There was obvious contamination of the sample from sampling equipment, paint chips etc.

The station was to be abandoned in the event of three failed attempts at obtaining an acceptable sample, however, all stations were sampled successfully with a minimum sample volume of 5 L obtained at all stations.

3.2.2.2 Sample Processing

All field sample processing methods were undertaken in line with the *Guidelines for the Conduct of Benthic Studies at Marine Aggregate Exaction Sites* (Ware et al. 2011), in-house Standard Operating Procedures (SOPs) and OEL's Quality Management System (QMS).

Initial sample processing was undertaken aboard the survey vessel in line with the following methodology:

- Assessment of sample size and acceptability made.
- Photograph of the sample with station details and scale bar was taken.
- 10% of sample removed for subsequent PSD analysis and transferred to labelled foil tray.
- Sample emptied onto 1 mm sieve net laid over 4 mm sieve table and washed through using gentle rinsing with seawater hose.
- Remaining sample for faunal sorting and identification backwashed into a suitably sized sample container using seawater and diluted 10 % formalin solution added to fix sample prior to laboratory analysis.
- Sample containers clearly labelled internally and externally with date, sample ID and project name.

3.3. Laboratory Methods

On arrival to the laboratory, all faunal and PSD samples were logged in and entered into the project database created in OEL's web-based data management application ABACUS in line with in-house SOPs and OEL's QMS.

3.3.1. Particle Size Distribution (PSD) Analysis

PSD analysis of samples was undertaken by in-house laboratory technicians at OEL's NMBAQC participating laboratory. All PSD analysis was undertaken in line with OEL's SOP for PSD analysis corresponding to NMBAQC Best Practice Guidance (Mason 2016) .

3.2.2.3 Sample Preparation

Frozen sediment samples were first transferred to a drying oven and thawed at 80°C for at least 6 hours prior to visual assessment of sediment type and wet sieving over a 1 mm sieve. Before any further processing (e.g. sieving or sub-sample removal), samples were mixed thoroughly with a spatula and all conspicuous fauna (>1 mm) which appeared to have been alive at the time of sampling were removed from the sample.

3.2.2.4 Dry Sieving

The >1 mm fraction was then returned to a drying oven and dried at 80°C for at least 24 hours prior to dry sieving.

Once dry, the sediment sample was run through a series of Endecott BS 410 test sieves (nested at 0.5 φ intervals) using a Retsch AS200 sieve shaker to fractionate the samples into particle size classes. The dry sieve mesh apertures used are given in Table 3.

Table 3 Sieve series employed for Particle Size Distribution (PSD) analysis by dry sieving (mesh size in mm).

Sieve aperture (mm)												
63	45	32	22.5	16	11.2	8	5.6	4	2.8	2	1.4	1

The sample was transferred onto the coarsest sieve at the top of the sieve stack, which was then shaken for a standardised period of 20 minutes. The sieve stack was then checked to ensure the components of the sample had been fractioned as far down the sieve stack as their diameter would allow. A further 10 minutes of shaking was undertaken if there was evidence that particles had not been properly sorted (e.g. veneers of silt overlying coarse fractions).

3.2.2.5 Laser Diffraction

The fine fraction residue (<1mm sediments) was transferred to a suitable container and allowed to settle for 24 hours before excess water was syphoned from above the sediment surface. The fine fraction was analysed by laser diffraction using a Beckman Coulter LS13 320. Due to the silty nature of the sediments, ultrasound was used to agitate particles and prevent aggregation of fines.

The dry sieve and laser data were then merged for each sample with the results expressed as a percentage of the whole sample. Once the data was merged, PSD statistics and sediment classifications were generated from the percentages of the sediment determined for each sediment fraction using the Gradistat v7 software.

Sediment descriptions were defined by their size class based on the Wentworth classification (Wentworth 1922) (Table 4). Statistics such as mean and median grain size, sorting coefficient, skewness and bulk sediment classes (percentage silt, sand and gravel) were also derived in accordance with the Folk classification (Folk 1954).

Table 4 Classification used for defining sediment type based on the Wentworth Classification System (Wentworth 1922).

Wentworth Scale (mm)	Phi units (φ)	Sediment Types
>256 mm	<-8	Boulders
64 - 256 mm	-8 to -6	Cobble
4 - 64 mm	-6 to -2	Pebble
2 - 4 mm	-2 to -1	Granule
1 - 2 mm	-1 to 0	Very coarse sand
0.5 - 1 mm	0 - 1	Coarse sand
250 - 500 µm	1 - 2	Medium sand
125 - 250 µm	2 - 3	Fine sand
63 - 125 µm	3 - 4	Very fine sand
31.25 – 63 µm	4 - 5	Very coarse silt
15.63 – 31.25 µm	5 - 6	Coarse silt
7.813 – 15.63 µm	6 - 7	Medium silt
3.91 – 7.81 µm	7 - 8	Fine silt
1.95 – 3.91 µm	8 - 9	Very fine silt
<1.95 µm	>9	Clay

3.3.2. Macrobenthic Analysis

For each macrobenthic sample, the excess formalin was drained off into a labelled container over a 1 mm mesh sieve in a well-ventilated area. The sample was then re-sieved over a 1 mm mesh sieve to remove all remaining fine sediment and fixative. Low-density biota was then separated from the sediment by elutriation with fresh water poured over a 1 mm mesh sieve and transferred into a Nalgene and preserved in 70 % Industrial Denatured Alcohol (IDA). The remaining sediment was subsequently separated into 1 mm, 4 mm and 8 mm fractions and sorted under a stereomicroscope to extract any remaining biota (e.g. high-density bivalves not 'floated' off during elutriation). The residual sediment fractions were then transferred into labelled containers and preserved in IDA.

All biota present was identified to species level, where possible, and enumerated by trained benthic taxonomists using the most up to date taxonomic literature and checks against existing reference collections. All identifications were recorded directly into ABACUS utilising the most up to date taxonomic classifications provided through a live link to the World Register of Marine Species (WoRMS)[2]. Colonial taxa (e.g. hydroids and bryozoans) were identified to species level where possible and recorded as present (P).

Prior to further analysis of the macrobenthic data, an initial rationalisation of the faunal list and associated abundance data was carried out. This primarily involved the removal and/or combination of taxa to avoid potential misrepresentation of numerical abundance. Abundances for individuals identified as juveniles were combined with abundances for adults and taxa identified from eggs removed. Taxa recorded as P were given the numerical value of 1.

A full reference collection was retained including at least one example specimen of each taxon.

[2] http://www.marinespecies.org/index.php

3.4. Data Truncation & Standardisation

3.4.1. Species Nomenclature Checks

As the macrobenthic data may be used for comparison with future studies, it was imperative that the species nomenclature was standardised and updated. The macrobenthic species lists were therefore checked using the WoRMS match taxon tool.

3.4.2. Data Truncation

Once the species nomenclature had been standardised in accordance with WoRMS accepted species names, the species lists were examined carefully to truncate the data, excluding incidental recordings that might have skewed the data analysis or combining taxa with differing levels of identification. During this process a number of datasets were produced and analysed using the statistical methods outlined below. These included separate datasets with the inclusion and exclusion of fragments and juveniles. This was done in order to assess the effects of physical interaction with the trawl gear on faunal communities as well as any potential juvenile settlement following reduced competition due to the removal of adults by trawling.

3.5. Data Analysis

The PRIMER v7 software package (Clarke & Gorley 2015) was utilised under OEL's user licence to undertake the multivariate statistical analysis on both the biotic (macrobenthic) and abiotic (PSD) datasets. Univariate tests were undertaken in R Studio (v1.1447) (R Studio Team 2015). The statistical methods applied to the data were chosen to address each of the research hypotheses and to ensure that project objectives were met.

3.5.1. Analysis of Sediment Data

Patterns in the abiotic data were investigated using principal components analysis (PCA) and PERMANOVA. PCA was utilised to assess the spread of the abiotic data and PERMANOVA was performed to formally assess any significant changes in the sediment data in relation to the BACI design.

3.5.2. Univariate Statistics

Univariate analysis of individual responses (e.g. species richness, Shannon-Wiener, TaxD, AMBI/IQI, species abundances) were tested within a linear model framework including treatment (fixed: impact vs control), sampling time (fixed: before vs after) and site (random: nested within treatment) as factors and an interaction between treatment and time as indicative of a trawling impact. Diagnostic plots of model residuals were used to determine whether model assumptions were violated. Where appropriate, generalised linear models (GLMs) were used to analyse overdispersed count data. A number of univariate indices were calculated from the data, as summarised in Table 5. In addition to traditional measures of diversity and ecological status, the proportion of damaged taxa in each sample was calculated (PropD), in order to assess potential impacts on infauna of physical interaction with the fishing gear.

Table 5. Biodiversity indices used to test for changes in the infaunal communities as a result of trawling and subsequent recovery.

Diversity Index	Description
Number of Species (S)	The number of species present in a sample, with no indication of relative abundances.
Number of Individuals (N)	Total number of individuals counted in a sample.
Shannon Weiner's Diversity (H' Loge)	Shannon Weiner's diversity index (H' loge) is derived from the number of species present as well as the relative abundance of each species. A high Shannon Weiner's diversity index (approaching 1) indicates a high number of species and an even spread of the abundance between those species (evenness). A low diversity index (approaching zero) indicates a low number of species or an uneven spread of the abundance between the species present.
Average Taxonomic Distinctness (AvTD, Δ+)	The average taxonomic path length through the phylogeny of all the species in a data-set between any two randomly chosen species.
AMBI	The AMBI (Borja & Franco 2000) index is commonly used as an element in multimetric indices. The index establishes a disturbance classification according to 5 ecological groups (EG) of species (Puente & Diaz 2008). - Group I "Species very sensitive to organic enrichment and present under polluted conditions (Initial state)" - Group II "Species indifferent to organic enrichment, always present in low densities with non-significant variations over time (from initial state to slight unbalanced)". - Group III "Species tolerant to excess organic matter enrichment. These species may occur under normal conditions, but their

	populations are stimulated by organic enrichment (slight unbalanced situations)." - Group IV "Second order opportunistic species (slight to pronounced unbalanced situations), mainly small sized polychaetes." - Group V "First-order opportunistic species (pronounced unbalanced situation)" (Borja et al. 2000).
Infaunal Quality Index (IQI)	The IQI is a multimetric index that expresses the ecological health of benthic macroinvertebrate (infauna) assemblages in accordance with the normative definitions of the Water Framework Directive (WFD) as an Ecological Quality Ratio (EQR). The index incorporates taxa number, the AZTI Marine Biotic Index (AMBI, a measure of sensitivity to disturbance) and Simpson's evenness (a measure of the distribution of individuals across the different taxa). To fulfil the requirements of the WFD, the IQI also incorporates each metric as a ratio of the observed value to that expected under reference conditions.
Proportion of Damaged Individuals (PropD)	Proportion of damaged taxa within each sample. Calculated as: $$PropD = \left(\frac{D}{N + D}\right)$$ Where: *D* = number of damaged taxa in a sample and *N* = number of non-damaged taxa in a sample.

3.5.3. Multivariate Staistics

Multivariate analysis was undertaken on a Bray-Curtis similarity matrix derived from square-root transformed species abundance data. A summary of the multivariate techniques utilised is presented in Table 6. SIMPROF and nMDS routines were performed on the data to assess patterns in the multivariate community data, and SIMPER analysis was undertaken to identify those groups contributing most to dissimilarity across the experimental design (i.e. between treatments and sampling times). A PERMDISP (Clarke & Gorley 2015) routine was utilised to characterise dispersion patterns in the dataset, as a potential indicator of stress (i.e. as a result of trawling disturbance) (Warwick & Clarke 1995). Permutational analysis of variance (PERMANOVA) was then performed on the multivariate data using the same design as described above for the ANOVA routine.

Analytical routines were performed on data from each location separately (i.e. each gear was tested separately) rather than construct an overcomplicated and unbalanced PERMANOVA model to formally compare gear types. Analyses were first performed on adult and juvenile taxonomic data merged, before being re-run with juveniles removed to assess any potential impacts of trawling on changing patterns in the proportion of adults and juveniles within the dataset.

Table 6. Statistical routines employed in PRIMER v7 to assess changes in PSD and macrobenthic data as a result of trawling in Torbay MCZ.

Routine/Technique	Description
Distributional Techniques	
Ranked species abundance (k dominance) curves	Provide a means of visually representing species richness and evenness within a sample or series of pooled samples.
Ordination Techniques	
Multi-dimensional Scaling (MDS)	MDS ordination plots can be used to represent the similarity of samples based on their multivariate structure by arranging them graphically in a multidimensional plot. This plot can be configured to display the sample points in two dimensions and provides a stress value that indicates the degree to which the plot is providing a representative interpretation of the similarity between the samples (see Clarke et al. 1993).
Principal Components Analysis (PCA)	PCA is primarily used to explore variance within datasets based on sample dissimilarity, to highlight relationships between groups of variables, and to reduce large numbers of variables into a smaller number (principal components) by combining those that are highly correlated. This ordination method uses Euclidean distance, and is more suited to analysis of normalised environmental data than to biological community data. The two-dimensional plot displays relative sample dissimilarity along the primary and secondary principal component axes, and eigenvectors which indicate the direction and strength of correlations between variables.
Exploratory Techniques	
Similarity Percentages (SIMPER)	Using the Bray-Curtis measure of similarity (Bray & Curtis 1957) the SIMPER routine identifies the variables primarily providing the discrimination between two observed sample clusters. This analysis breaks down the contribution of each variable to the observed similarity between samples effectively meaning the key characterising variables of identified groups can be identified.
Hypothesis Testing Techniques	
Permutational Multivariate Analysis of Variance (PERMANOVA)	PERMANOVA (permutation-based MANOVA) has a similar function to the ANOSIM test, however, PERMANOVA uses distance measures (Bray-Curtis coefficients or Euclidean distance) rather than ranking to preserve information. This versatile test can handle complex, unbalanced designs including those with multiple factors, fixed factors (where all categories of the factor have been sampled) and random factors (where the levels of the factor have been randomly sampled from a wider ‘population’), interaction terms and covariates. When used with multivariate data, the test uses permutations to make it distribution-free (Anderson 2005).
PERMDISP	PERMDISP tests the homogeneity of multivariate dispersions within groups, on the basis of any resemblance measure and can be used to help interpret the results of a PERMANOVA analysis, which makes the implicit assumption (as for ANOVA and ANOSIM) that dispersions are roughly constant across groups (Anderson 2006).

4. RESULTS

4.1. Sediments

In total, all 150 sediment samples obtained were analysed for full particle size classification. Sediment types based on site-averaged PSD data were classified using the Folk Triangle (Folk 1954) and each Folk classification was converted to BSH type (EUNIS Level 3) using the adapted Folk triangle (Long 2006). Results indicate that in Location A, sediments are generally characterised by poorly sorted very fine sands and very coarse silts. In April 2018 sediment sorting across control sites had increased from poorly sorted to moderately sorted. Broad-scale habitats across both experimental trawl locations are predominantly made up of muddy sand (mS), although in April 2018 sediments within Location A had changed to slightly gravelly muddy sand ((g)mS) in both impact and control sites (Table 7). This shift in broad habitat type was characterised by an increase in the percentage volume of sandy sediments and a loss of muddy fractions.

Table 7. Sediment characteristics across BACI groups, based on site-averaged sediment PSD data from October 2017 and April 2018.

Location A							
Treatment	**Time**	**Habitat**	**Folk and Ward**	**Sorting**	**Gravel**	**Sand**	**Mud**
Impact	One Day Before	Muddy Sand (mS)	Very Fine Sand	Poorly Sorted	0.26%	68.59%	31.15%
	One Day After	Muddy Sand (mS)	Very Coarse Silt	Poorly Sorted	0.12%	66.29%	33.59%
	Six Months After	Slightly Gravelly Muddy Sand ((g)mS)	Very Fine Sand	Poorly Sorted	3.12%	81.41%	15.47%
Control	One Day Before	Muddy Sand (mS)	Very Coarse Silt	Poorly Sorted	0.11%	62.49%	37.40%
	One Day After	Muddy Sand (mS)	Very Coarse Silt	Poorly Sorted	0.24%	58.93%	40.83%
	Six Months After	Slightly Gravelly Muddy Sand ((g)mS)	Very Fine Sand	Moderately Sorted	1.43%	84.91%	13.66%
Location B							
Treatment	**Time**	**Habitat**	**Folk and Ward**	**Sorting**	**Gravel**	**Sand**	**Mud**
Impact	One Day Before	Muddy Sand (mS)	Very Coarse Silt	Poorly Sorted	0.06%	69.14%	30.80%
	One Day After	Muddy Sand (mS)	Very Fine Sand	Poorly Sorted	0.17%	65.86%	33.97%
Control	One Day Before	Muddy Sand (mS)	Very Coarse Silt	Poorly Sorted	0.13%	82.25%	17.62%
	One Day After	Muddy Sand (mS)	Very Fine Sand	Poorly Sorted	0.16%	76.35%	23.49%

Results of the PERMANOVA routine performed on the multivariate sediment data indicate a significant effect of sampling time and a significant random effect of site on sediment composition in both Location A and Location B, with no significant interaction term evident for the use of either gear type (Table 8). Pairwise comparisons (Appendix 1) indicate significant changes in sediment composition six months following trawling at both control and impact sites in Location A, and a significant change in Location B one day after trawling compared to before trawling was undertaken.

Table 8. Results of PERMANOVA performed on the multivariate sediment data across BACI groups within each study location.

Location A					
Source	**d.f.**	**S.S.**	**M.S.**	**Pseudo-F**	**Probability**
Time	2	3640.2	1820.1	13.762	**0.001**
Treatment	1	811.75	811.75	1.7455	0.183
Site(Treatment)	7	7401.7	1057.4	7.9952	**0.001**
Time*Treatment	2	348	174	1.3157	0.28
Residuals	77	10183	132.25		
Total	89	22418			
Location B					
Source	**d.f.**	**S.S.**	**M.S.**	**Pseudo-F**	**Probability**
Time	1	152.34	152.34	13.861	**0.001**
Treatment	1	857.82	857.82	1.2159	0.419
Site(Treatment)	3	2116.5	705.5	64.19	**0.001**
Time*Treatment	1	26.28	26.28	2.3911	0.085
Residuals	53	582.51	10.991		
Total	59	3730.3			

Principal coordinate analysis of particle size distribution data from each location shows close grouping of samples across BACI groups (Figure 4 and Figure 5). Samples taken from both impact and control treatments in Location A in April 2018 are closely grouped and generally appear as outliers in Figure 4 compared to samples in October 2017. This is in agreement with the results of PERMANOVA that demonstrate sampling time as a significant factor in determining multivariate sediment composition, and overlaid Pearson rank correlated sediment vectors demonstrate that these samples are generally characterised by coarser grain sizes than other samples.

Outliers are also evident in data from Location B, although these are samples from both impact and control sites and are evident as outliers consistently before and after trawling disturbance, suggesting no temporal change at these stations and that sediment composition at these sites differs from other samples (Figure 5). There is some grouping of samples from before and after trawling evident, consistent with PERMANOVA results (Table 8).

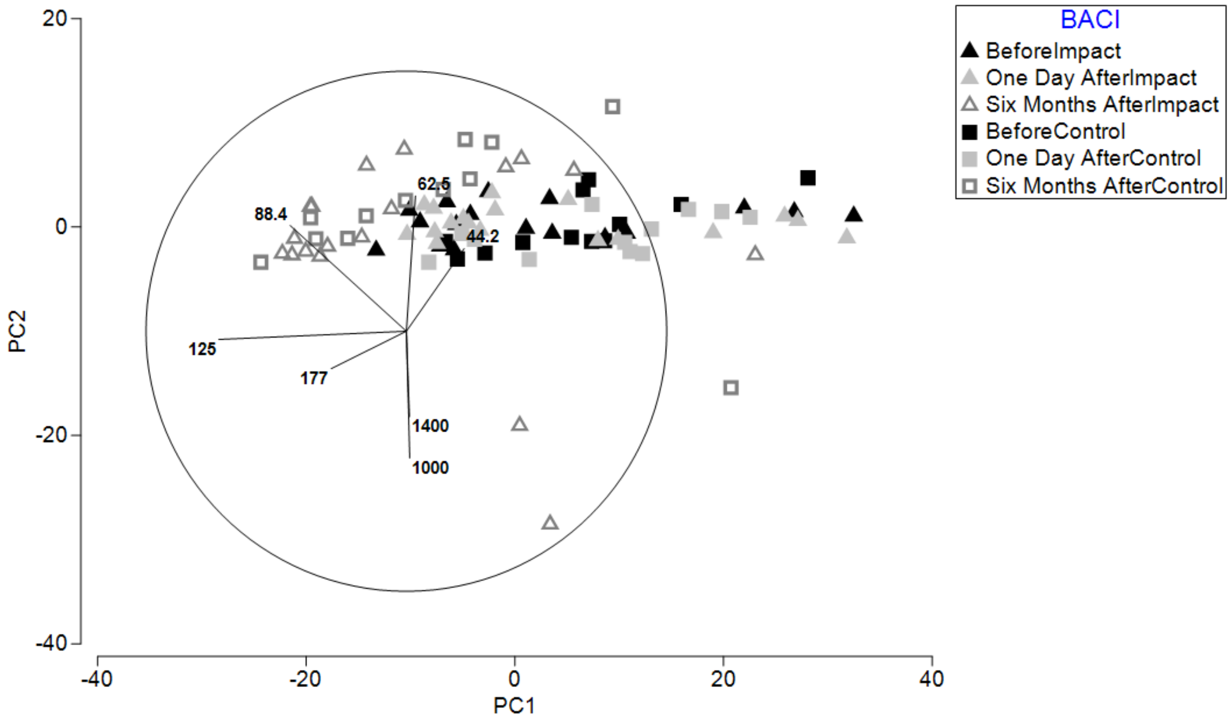

Figure 4. PCA ordination of sediment PSD from Location A across treatment groups and sampling times, with overlaid Pearson rank correlated sediment vectors.

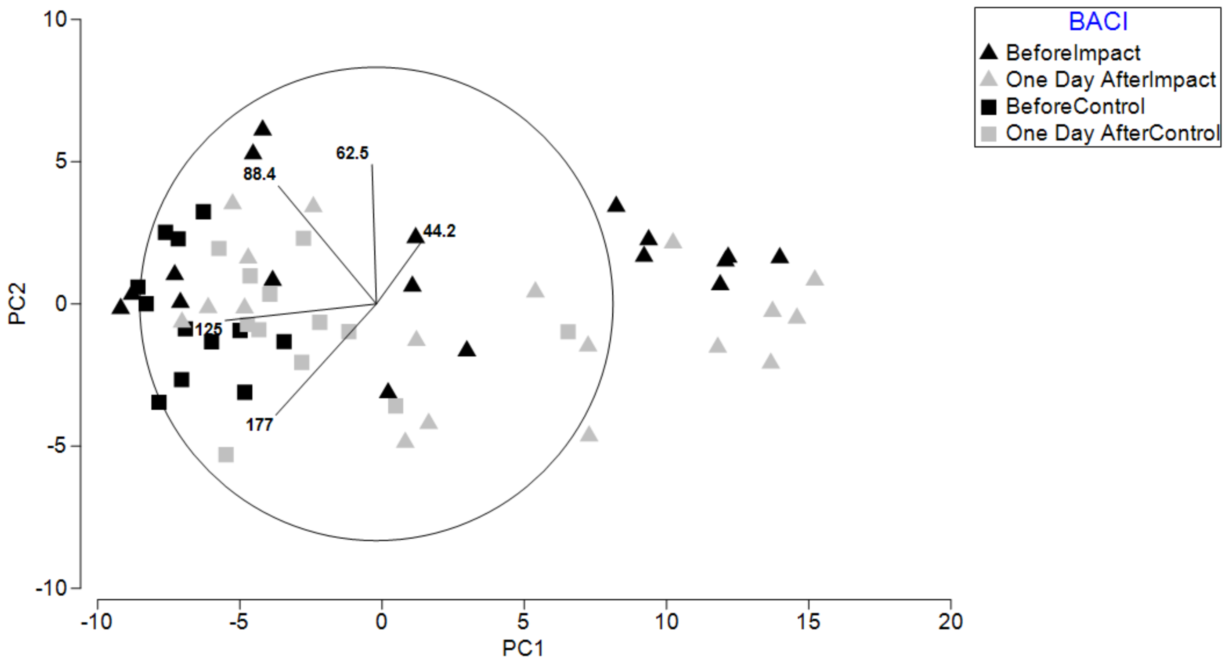

Figure 5. PCA ordination of sediment PSD from Location B across treatment groups and sampling times, with overlaid Pearson rank correlated sediment vectors.

4.2. Diversity Indices and Ecological Quality

A total of 200 taxa (Appendix 2) were identified in samples across all sampling periods and trawl locations. While some variation is evident, diversity indices and measures of ecological quality were broadly similar between treatments and across sampling events (Figure 6). Values appear similar across treatments, likely due to the relative locality of different treatment sites. Seasonal declines in species richness (Figure 6a) and total number of individuals (Figure 6b) are apparent in April 2018 compared to samples from October 2017, with a similar trend observed in mean IQI scores, albeit of a smaller magnitude (Figure 6g).

Results of linear mixed models fitted to the diversity and ecological quality data in Location A and B indicate that while taking into account the variation at the site level (i.e. included as a random effect in models), all diversity indices and ecological quality scores were similar across trawled and un-trawled sites, with no significant effect of treatment observed in any response variable (Table 9 and Table 10).

The change across sampling times evident in Location A in Figure 6 is demonstrated by a significant main effect of sampling time on species richness, total number of individuals, Shannon-Wiener, taxonomic distinctness and IQI values (Table 9), with a reduction in these values in April 2018 when compared to October 2017. In Location B, where a lighter trawl gear was used, a significant main effect of sampling time is evident for species richness and IQI values (Table 10). The interaction term for all response variables is non-significant however, suggesting no impact of trawling on diversity or ecological quality measures in either location (i.e. the magnitude of change between sampling events did not differ significantly between trawled and un-trawled sites). Despite non-significance of the interaction term, it is noteworthy that mean IQI scores in Location A dropped from “high” ecological quality on average to “good” in trawled areas six months after trawling disturbance (indicated by the shading in Figure 6g). The lack of data following this period in Location B does not allow a comparison of this trend between gear types however.

The proportion of damaged individuals in the data is variable between BACI groups. Fewer damaged individuals were present in control sites than in impact sites in both locations following trawling, although no significant interaction term is observed for either trawl gear. A significant main effect of time is evident however in Location A, with the proportion of damaged individuals decreasing six months following trawling disturbance (Table 9).

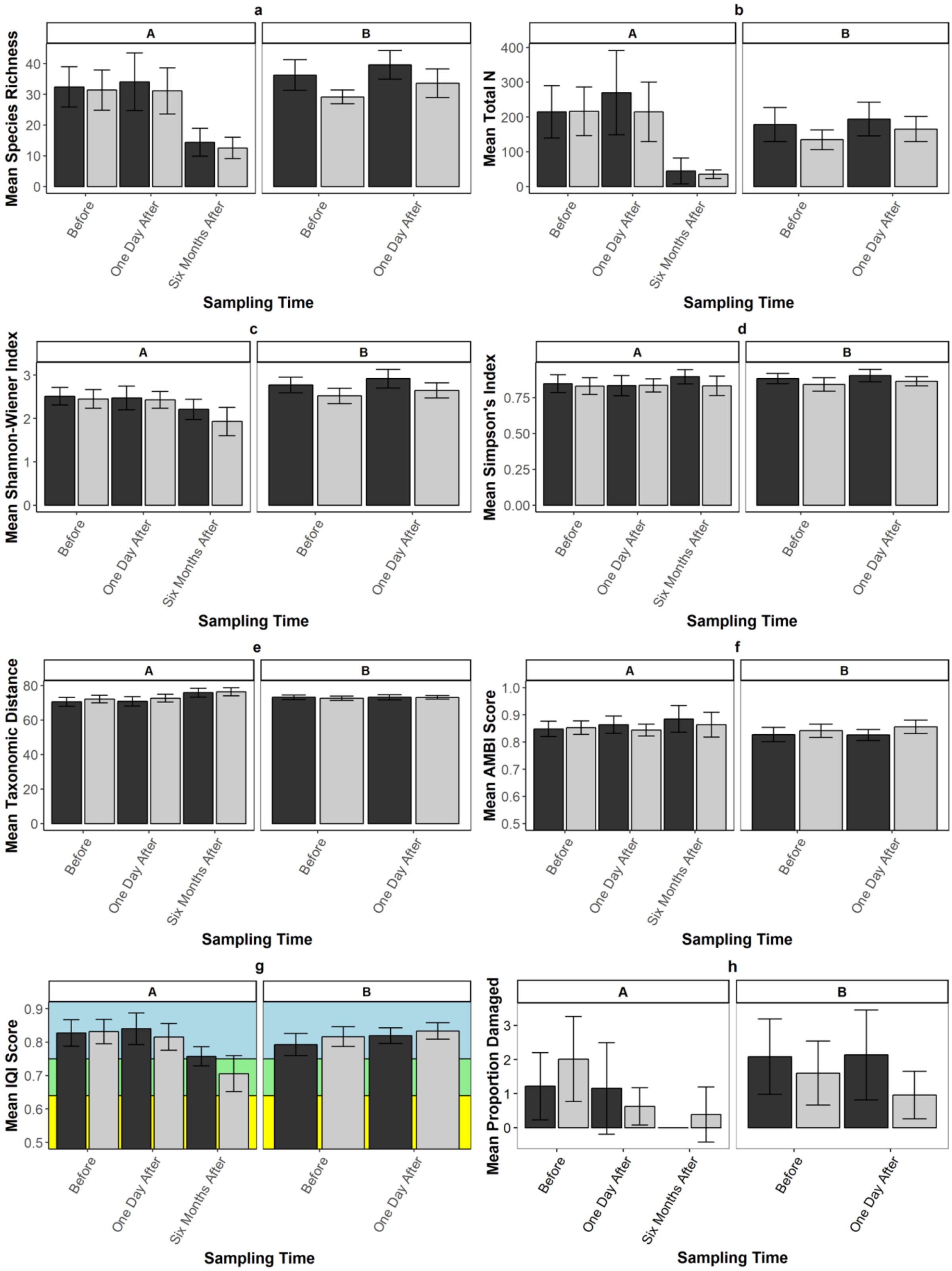

Figure 6. Mean (± S.E.) diversity and ecological quality indices across control (black) and trawled (grey) sites and sampling time in Location A (heavy trawl gear) and Location B (regular trawl gear). a) species richness, b) total number of individuals, c) Shannon-Wiener values, d) Simpson's index, e) Average Taxonomic Distinctness, f) AMBI score, g) Infaunal Quality Index, h) proportion of damaged taxa. IQI classifications are indicated by coloured shading in Figure 1g (yellow = moderate; green = good; blue = high).

Table 9. Results of linear mixed models performed on diversity and ecological quality indices across sampling times, treatments and sites in Location A (heavy trawl gear).

	Species Richness		Total N		Shannon		Simpson's	
Fixed Effects	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**
Time	31.25	**< 0.001**	33.45	**< 0.001**	7.83	**< 0.001**	0.72	0.49
Treatment	0.08	0.791	0.09	0.78	1.49	0.28	0.52	0.5
Time*Treatment	0.06	0.939	0.64	0.53	0.64	0.53	0.82	0.44
Random Effects	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**
Site(Treatment)	24.69	4.97	2898	53.83	0.002	0.05	0.0004	0.02
	TaxDist		**AMBI**		**IQI**		**PropD**	
Fixed Effects	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**
Time	12.13	**< 0.001**	1.3	0.28	20.5	**< 0.001**	5.15	**< 0.01**
Treatment	0.86	0.4	0.89	0.35	0.42	0.54	0.16	0.71
Time*Treatment	0.2	0.82	0.41	0.66	1.28	0.28	1.17	0.31
Random Effects	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**
Site(Treatment)	1.05	1.02	0	0	0.0003	0.02	0.193	0.44

Table 10. Results of linear mixed models performed on diversity and ecological quality indices across sampling times, treatments and sites in Location B (regular trawl gear).

	Species Richness		Total N		Shannon		Simpson's	
Fixed Effects	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**
Time	6.51	**< 0.01**	2.39	0.13	2.85	0.1	1.54	0.22
Treatment	3.21	0.11	0.65	0.45	5.28	0.05	3.28	0.11
Time*Treatment	0.13	0.72	0.24	0.62	0.02	0.9	0.001	0.97
Random Effects	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**
Site(Treatment)	19.84	4.45	516.8	22.73	2E-05	0.005	0.0002	0.01
	TaxDist		**AMBI**		**IQI**		**PropD**	
Fixed Effects	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**
Time	0.2	0.66	0.51	0.48	7.16	**< 0.01**	0.4	0.53
Treatment	0.29	0.61	0.74	0.42	0.001	0.98	2.72	0.16
Time*Treatment	0.22	0.64	0.83	0.37	0.33	0.57	0.56	0.46
Random Effects	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**
Site(Treatment)	0.001	0.03	0.0001	0.01	0.001	0.03	0.04	0.19

4.2.1. K-Dominance Curves

K-dominance curves demonstrate relatively unperturbed conditions at both locations, with little variability across BACI group-averaged data from sites subject to either trawl gear. There is almost complete overlap in the curve from one day before and one day after trawling disturbance at both locations (Figures 7 and 8), suggesting high similarity in the levels of disturbance across BACI groups in each location, although there is evidence of temporal variability at Location A, with dominance curves based on April 2018 data appearing slightly more elevated, indicating slightly more perturbed conditions at this site at this time (Figure 7).

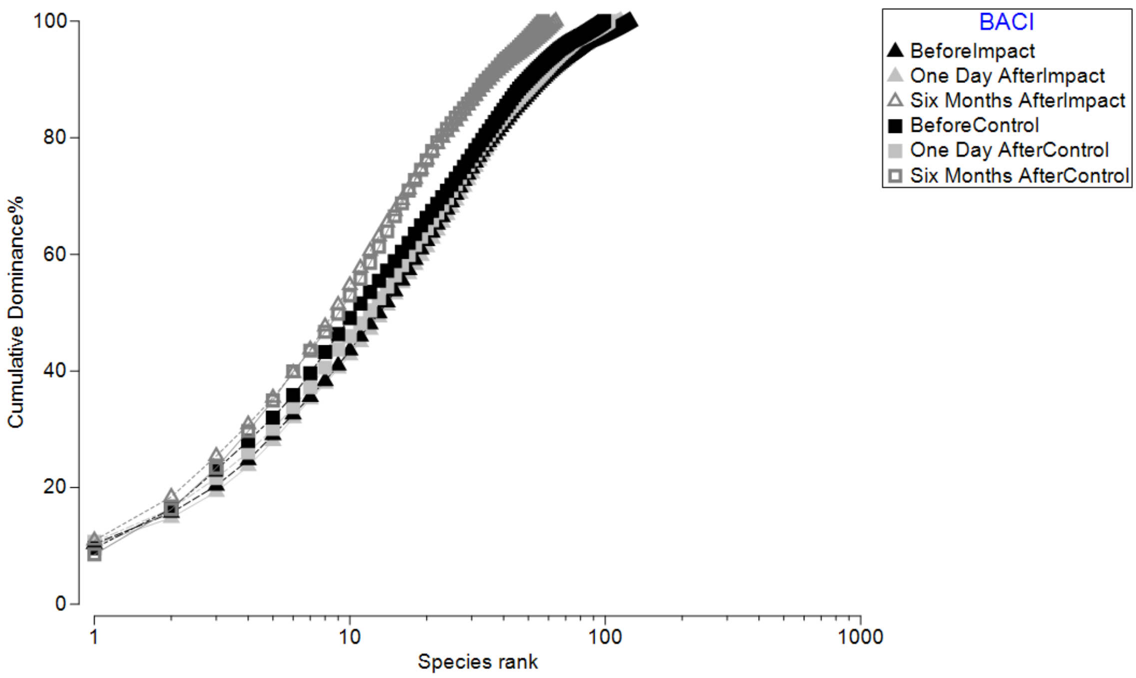

Figure 7. *K*-dominance curves based on square-root transformed species abundance data from Location A in October 2017 and April 2018, one day before, one day after and six months after experimental trawling.

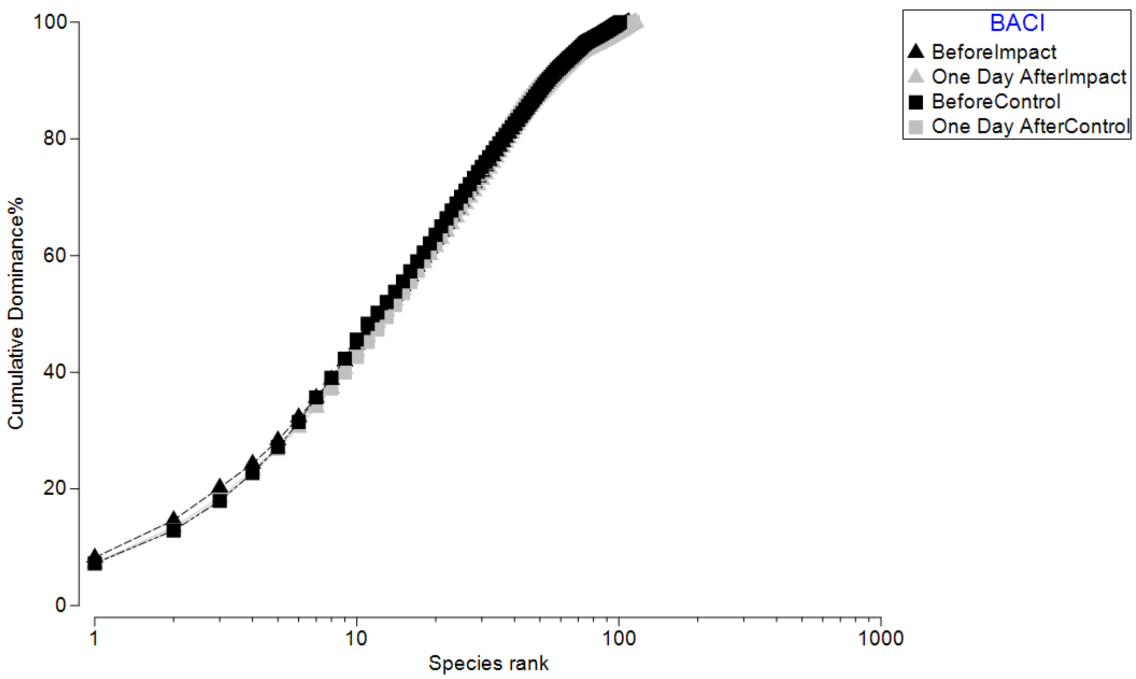

Figure 8. *K*-dominance curves based on square-root transformed species abundance data from Location A in October 2017, one day before and one day after experimental trawling disturbance.

4.2.2. Contribution of AMBI Groups

While no significant trends are evident in overall AMBI levels due to trawling, with samples across all factor levels classified as "undisturbed" (Figure 6f), Figure 9 allows examination of the trends in the proportional contribution of each individual AMBI group to overall abundance. Overall, samples in both treatment areas before and after trawling are dominated by taxa in AMBI Groups I and II, suggesting good ecological status. While the relative proportion of Group I taxa in trawled and un-trawled areas does show a small reduction one day after trawling compared to one day before, no significant effect of sampling time, treatment, or interaction term is evident. Significant differences in the mean contribution of each group are evident however ($F(4, 450) = 430.63$, $p < 0.001$). The same trend is present for Location B, in which there is again no significant difference in the main effects of sampling time and treatment or the interaction term, but significant differences between the contribution of each group ($F(4, 292) = 193.83$, $p < 0.001$).

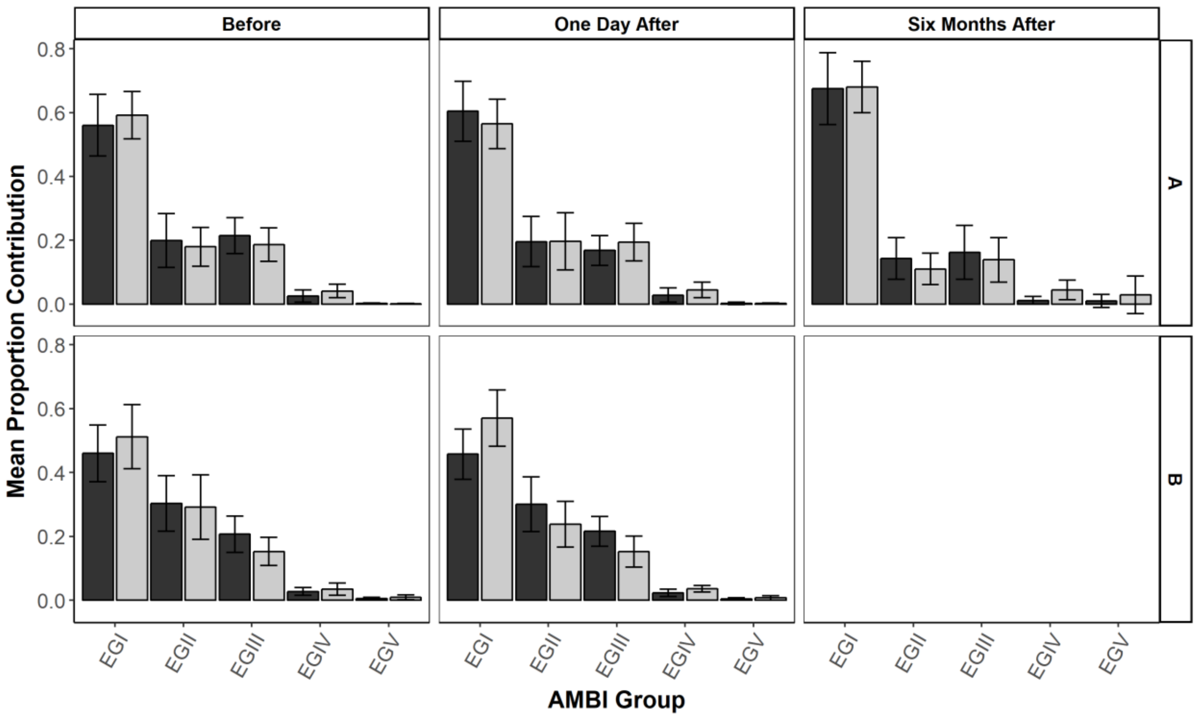

Figure 9. Mean proportional contribution of AMBI groups to overall abundance across control (black) and trawled (grey) sites at each sampling time. No data are available for Location B six months after experimental trawling.

4.3. Macrobenthic Community Structure

The assemblage across Locations A and B is typical of shallow circalittoral muddy sand habitats, and SIMPER analysis performed across all data indicates that in both locations macrobenthic communities were dominated by the bivalves *Spisula subtruncata* and *Fabulina fabula* and to a lesser extent the polychaetes *Euclymene oerstedii*, *Melinna palmata* and *Ampharete lindstroemi*. *Nephtys* spp. and *Magelona filiformis* are also present in both locations although in smaller numbers (Table 11). The broad-scale biotope that best characterises conditions across both locations therefore appears to fall somewhere between EUNIS Habitat A5.244 *Spisula subtruncata* and *Nepthys hombergii* in shallow muddy sand and EUNIS Habitat A5.242 *Fabulina fabula* and *Magelona mirabilis* with venerid bivalves and amphipods in infralittoral compacted fine sand.

Table 11. Results of SIMPER analysis to show the species contributing most to similarity between samples in each experimental trawl location.

Location A					
Species	Mean Abundance (per m^2)	Mean Similarity	S.D.	% Contribution	Cumulative %
Euclymene oerstedii	540.44	4.4	0.42	20.18	20.18
Fabulina fabula	64	2.94	0.55	13.48	33.66
Spisula subtruncata	77.11	2.07	0.54	9.5	43.16
Melinna palmata	131.22	1.71	0.58	7.84	51
*Ampharete lindstroemi*_Aggregate	120.78	1.17	0.5	5.35	56.35
Nucula nitidosa	34.78	1.15	0.59	5.29	61.64
*Nephtys*_Juvenile	26.78	1.11	0.63	5.08	66.72
Chamelea striatula	22.22	0.79	0.65	3.63	70.35
Location B					
Species	**Mean Abundance (per m2)**	**Mean Similarity**	**S.D.**	**% Contribution**	**Cumulative %**
Spisula subtruncata	264.83	7.02	0.95	20.35	20.35
Euclymene oerstedii	222.5	3.13	0.49	9.07	29.41
*Ampharete lindstroemi*_Aggregate	130.83	2.85	0.85	8.25	37.66
Fabulina fabula	84.83	2.66	0.6	7.72	45.39
Melinna palmata	122.33	2.54	0.63	7.37	52.76
Nucula nitidosa	56.33	1.86	1.11	5.38	58.13
Abra alba	80.33	1.8	0.74	5.23	63.36
Chamelea striatula	40.17	1.6	1.59	4.62	67.99
Corbula gibba	36.17	1.23	1.04	3.58	71.56

4.3.1. Impacts of Experimental Trawling on Macrobenthic Communities

3.2.2.6 Location A

An MDS ordination of samples taken from Location A indicates close grouping of samples, demonstrating the high similarity in macrobenthic communities across the site. Samples taken six months after experimental trawling, in both control and impact sites, appear as outliers compared to the majority of samples (Figure 10).

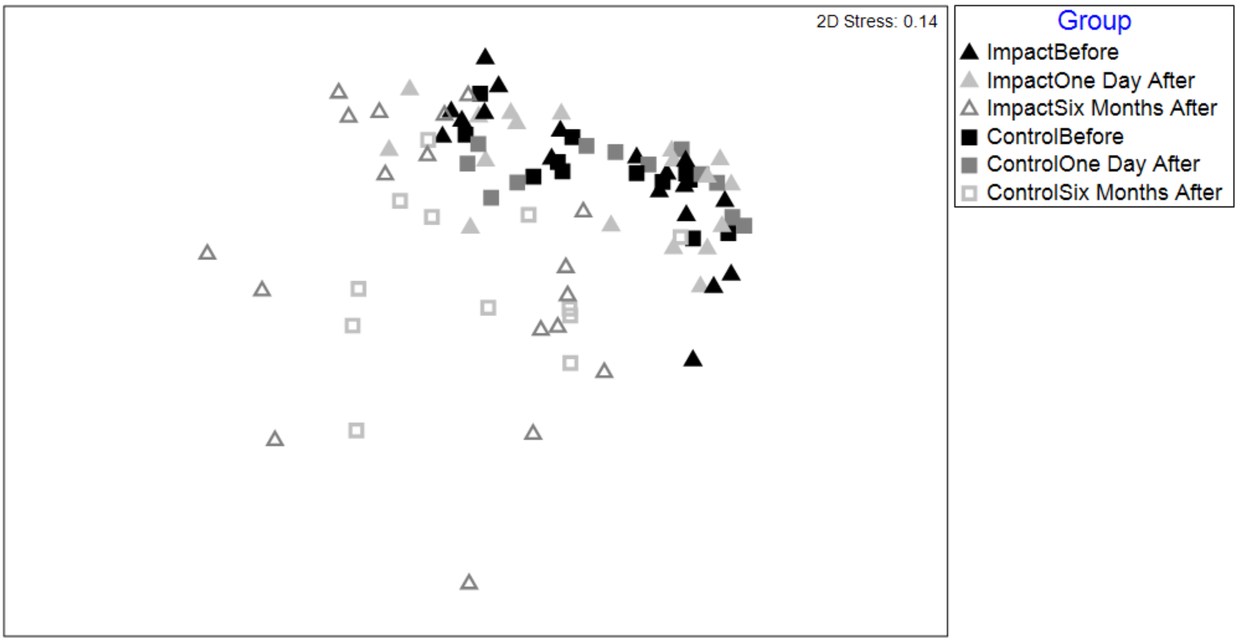

Figure 10. nMDS ordination of samples from Location A in October 2017 and April 2018, based on a Bray-Curtis similarity matrix derived from square-root transformed abundance data (Impact: triangles; Control: squares. Before: black symbols, One Day After Trawling: grey symbols; Six Months After Trawling: hollow grey symbols).

Results of the PERMDISP routine indicate significant heterogeneity in the multivariate dispersions between BACI groups ($F(1, 5) = 5.95$, $p = 0.001$). Pairwise comparisons of the variance in the multivariate data are presented in Appendix 3, although results generally indicate that the largest differences in dispersion within the data exist between samples taken in October 2017 and those obtained in April 2018, further demonstrating temporal differences in the structure of the data. PERMANOVA results demonstrate a significant main effect of time and a significant random effect of site, nested within treatment (Table 13). No significant difference in the macrobenthic assemblage is evident between treatments, nor a significant treatment/time interaction term. Pairwise comparisons indicate that across both control and impact sites no change is evident between the day before experimental trawling and the day after, although significant changes in the macrobenthic assemblages occurred over the next six months.

The four main groups present in the macrobenthic dataset from Location A at the Class level comprise Bivalvia, Polychaeta, Ophiuroidea and Malacostraca. The mean densities of each group across sampling times and treatment sites are presented in Figure 11. Mean densities of these groups indicate little variability in the distribution between treatment sites, and two-factorial ANOVA demonstrates no significant interaction term, although again a main effect of sampling time shows significantly reduced densities six months following trawling disturbance for Bivalvia, Polychaeta and Ophiuroidea. No effect over time is evident for Malacostraca (Table 12).

SIMPER analysis between sampling times for sites across each treatment reveals little difference in the abundance of key species before and one day after trawling (Appendix 4). The abundance of a number of species demonstrates a notable decline between one day after trawling and six months after however, and this trend was largest in those species most abundant in samples, notably *E. oerstedii*, *S. subtruncata*, *F. fabula* and *M. palmata.* These species comprise 20 % of the dissimilarity between these time points at trawled sites. The same trend in species abundance between sampling times is evident at control sites, although it is worth noting that mean dissimilarity between sampling times across all species and all samples is lower at control sites than it is at trawled sites in Location A; dissimilarity values of 55.51 and 62.81 are evident between one day before and one day after trawling at trawled and control sites respectively. Mean densities of the top six species identified as contributing to dissimilarity between before trawling and one day after trawling are presented in Figure 12. Linear mixed models indicate a significant main effect of sampling time on densities of all species except *F. fabula.* No significant effect of treatment or the interaction term is evident for any group (Table 12).

Table 12. Results of two-factorial linear mixed models undertaken on taxonomic class and species density data from Location A across sampling times.

	Bivalvia		Polychaeta		Ophiuroidea		Malacostraca		*E. oerstedii*	
Fixed Effects	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**
Time	23.09	**< 0.001**	31.26	**< 0.001**	7.68	**< 0.001**	2.70	0.07	15.77	**< 0.001**
Treatment	0.64	0.48	0.08	0.79	0.03	0.88	0.50	0.53	0.05	0.85
Time*Treatment	0.64	0.53	0.29	0.75	0.07	0.99	0.04	0.96	0.39	0.68
Random Effects	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**
Site(Treatment)	35.01	5.92	1970.00	44.38	5.81	2.41	2.48	1.58	1.98	1.41
	A. lindstroemi		***M. palmata***		**Philinidae**		***F. fabula***		***C. gibber***	
Fixed Effects	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**
Time	19.55	**< 0.001**	21.11	**< 0.001**	6.22	**< 0.01**	1.68	0.19	25.84	**< 0.001**
Treatment	0.34	0.60	0.29	0.63	0.07	0.80	0.01	0.94	0.02	0.90
Time*Treatment	0.78	0.46	0.17	0.85	0.54	0.59	2.79	0.07	0.21	0.82
Random Effects	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**
Site(Treatment)	21.23	4.61	40.35	6.35	4.86	2.21	11.32	3.37	4.86	2.21

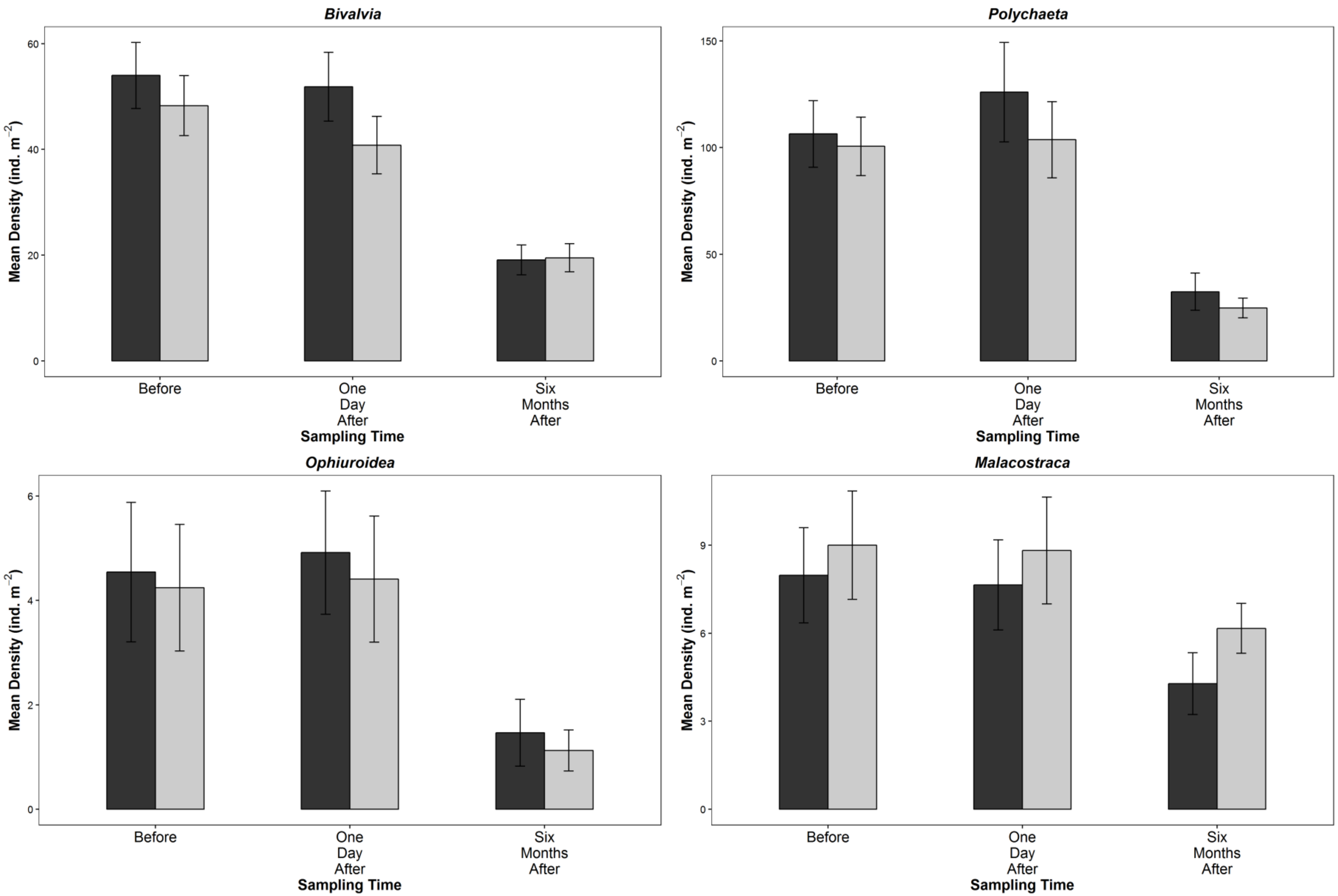

Figure 11. Mean (± S.E.) densities of the four main class groups present in Location A (Bivalvia, Polychaeta, Ophiuroidea, Malacostraca) across sampling times in control (black bars) and impact (light grey bars) sites.

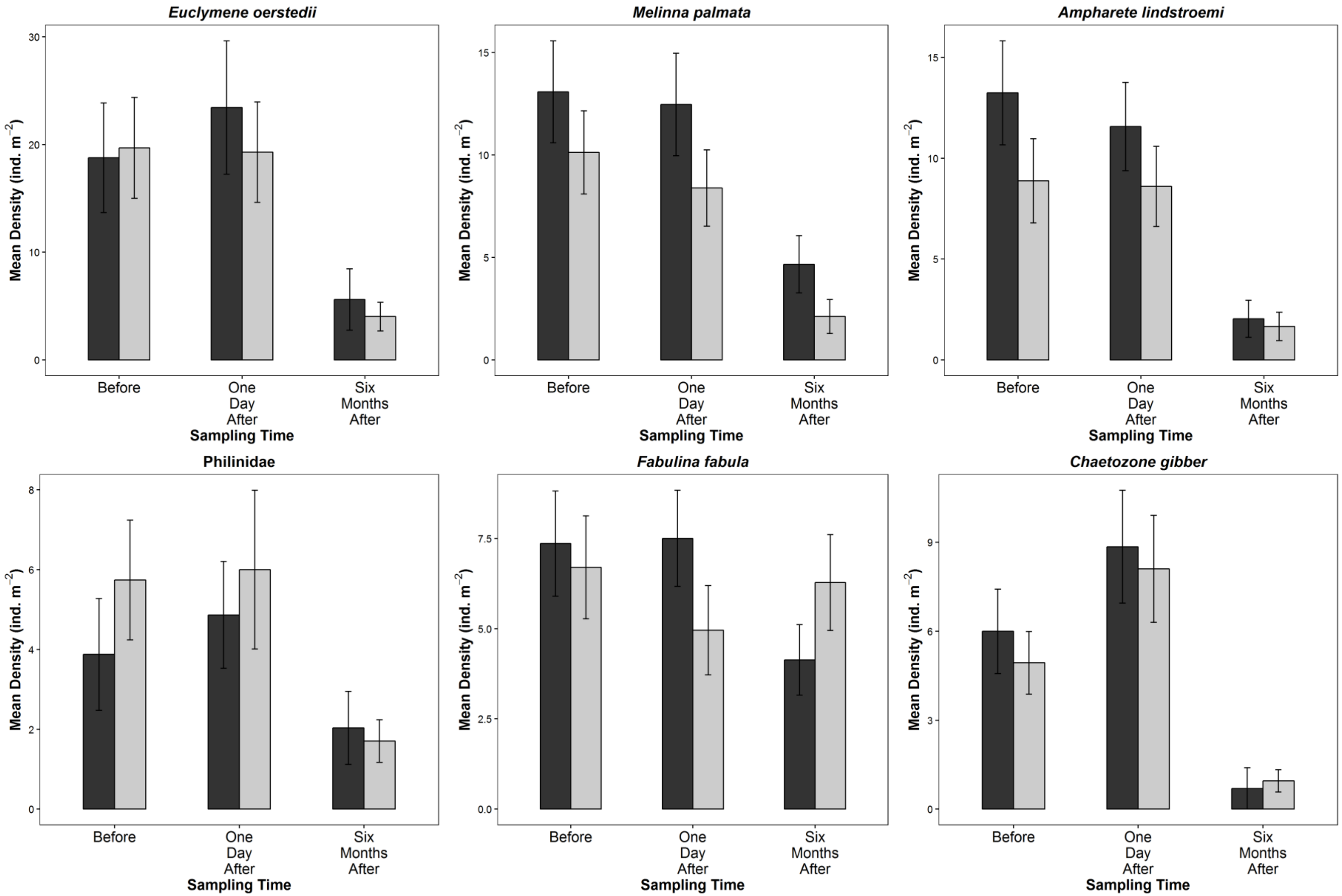

Figure 12. Mean (± S.E.) densities of the key species present in Location A as identified through SIMPER analysis across sampling times and in control (black bars) and impact (light grey bars) sites.

Table 13. PERMANOVA results performed on Bray-Curtis similarity matrices derived from square-root transformed abundance data from Location A and Location B.

Location A					
Source	**d.f.**	**S.S.**	**M.S.**	**Pseudo-F**	**Probability**
Time	2	25337	12668	7.3825	**0.001**
Treatment	1	2418.1	2418.1	0.14161	1
Site(Treatment)	3	51228	17076	9.9511	**0.001**
Time*Treatment	2	2580.6	1290.3	0.75193	0.869
Residuals	81	1.39E+05	1716		
Total	89	2.21E+05			
Location B					
Source	**d.f.**	**S.S.**	**M.S.**	**Pseudo-F**	**Probability**
Time	1	1453.6	1453.6	1.5309	**0.048**
Treatment	1	1658.4	1658.4	0.34741	0.939
Site(Treatment)	6	43564	7260.7	7.6469	**0.001**
Time*Treatment	1	488.01	488.01	0.51397	0.963
Residuals	50	4.75E+04	949.49		
Total	59	9.59E+04			

3.2.2.7 Location B

An MDS ordination of square-root transformed abundance data from Location B indicates no clear grouping of samples in relation to the BACI design, yet a number of samples from the trawled sites comprise relative outliers, demonstrating grouping of both before and after samples (Figure 13).

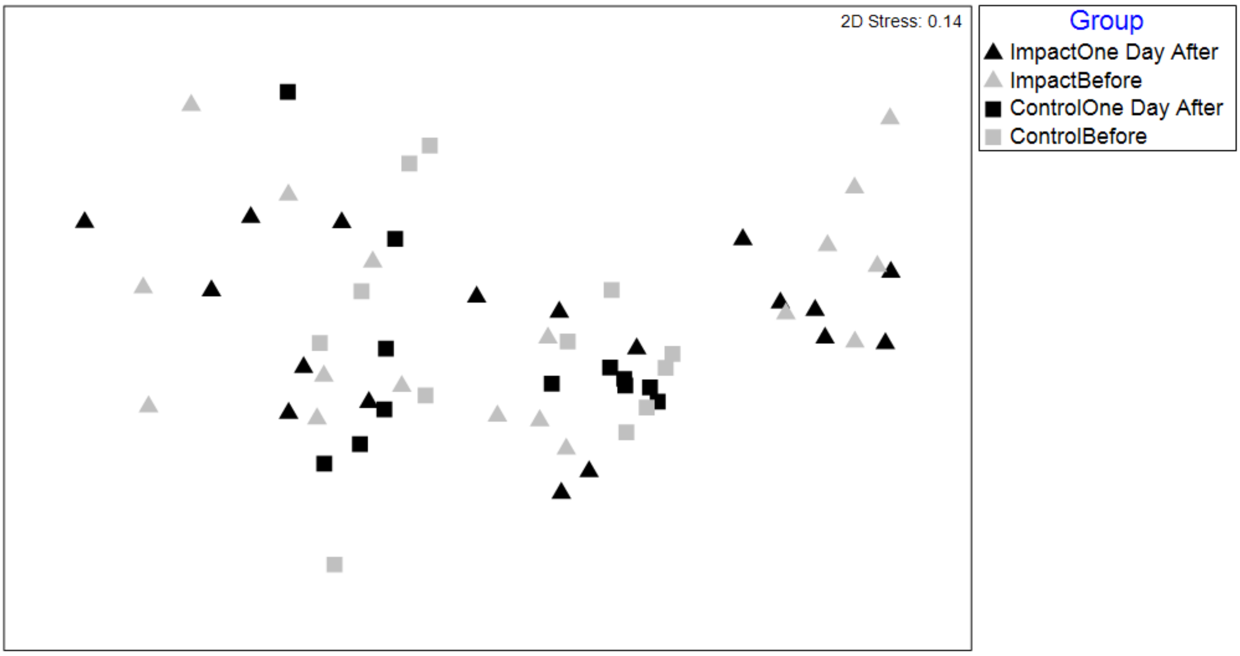

Figure 13. nMDS ordination of samples taken one day before and one day after experimental trawling using modified gear in Location B in October 2017, based on a Bray-Curtis similarity matrix derived from square-root transformed abundance data (Impact: triangles; Control: squares. Before: black symbols, One Day After Trawling: grey symbols).

The multivariate dispersions demonstrate significant heterogeneity across BACI groups at Location B (F (3,56) = 9.07, p = 0.001), and pairwise comparisons indicate that these differences occur between treatment groups rather than across sampling times. No change in the spread of the data is evident between sampling times in each treatment. PERMANOVA reveal a small yet significant main effect of sampling time and a significant random effect of site on the macrobenthic assemblage within Location B. No significant interaction between treatment and sampling time is evident (Table 13), and pairwise comparisons demonstrate no significant differences between BACI groups (Appendix 3).

The same four groups as in Location A dominate the data from Location B, given the relative locality of the two areas to each other. In Location B, where sampling was only undertaken one day before and one day after trawling, a significant effect of time is only evident for Polychaeta, which demonstrate a significant increase in densities following experimental trawling disturbance. No significant effect of treatment or the interaction term is evident for any group (Table 14).

SIMPER analysis performed on data from Location B indicates that species abundances are generally comparable in both control and trawled sites before and after experimental trawling, although mean dissimilarity across all samples was slightly larger in trawled (58.66) than control (48.34) sites. No further samples were collected from this location and an assessment of recovery after six months as in Location A is therefore not possible. Results of linear mixed models performed on density data for the main class groups and top six species present in Location B (Figure 14 and Figure 15) indicate a significant effect of sampling time on densities of *A. lindstroemi* and *M. johnstoni*. Results for only one species, *Melinna palmata*, show a significant interaction between sampling time and treatment (Table 14).

Table 14. Results of two-factorial linear mixed models performed on class and species density data from Location B across sampling times.

Fixed Effects	Bivalvia		Polychaeta		Ophiuroidea		Malacostraca		*E. oerstedii*	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Time	1.19	0.28	6.62	**< 0.05**	2.72	0.11	0.05	0.83	0.27	0.61
Treatment	1.70	0.24	0.87	0.38	0.16	0.70	0.08	0.79	0.19	0.68
Time*Treatment	0.34	0.56	1.17	0.28	1.05	0.31	1.27	0.26	0.30	0.59
Random Effects	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**
Site(Treatment)	333.80	18.27	16.39	4.05	1.21	1.10	3.00	1.73	43.74	6.61
Fixed Effects	***A. lindstroemi***		***M. palmata***		***S. subtruncata***		***F. fabula***		***M. johnstoni***	
	F-value	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**
Time	4.76	**< 0.05**	1.40	0.24	0.24	0.63	0.41	0.52	15.08	**< 0.001**
Treatment	0.94	0.37	0.05	0.83	0.14	0.72	0.03	0.87	1.47	0.26
Time*Treatment	0.26	0.61	4.26	**< 0.05**	0.02	0.88	0.43	0.51	2.30	0.14
Random Effects	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**	**Var**	**S.D.**
Site(Treatment)	15.61	3.95	17.14	4.14	0.03	0.16	7.41	2.72	7.15	2.67

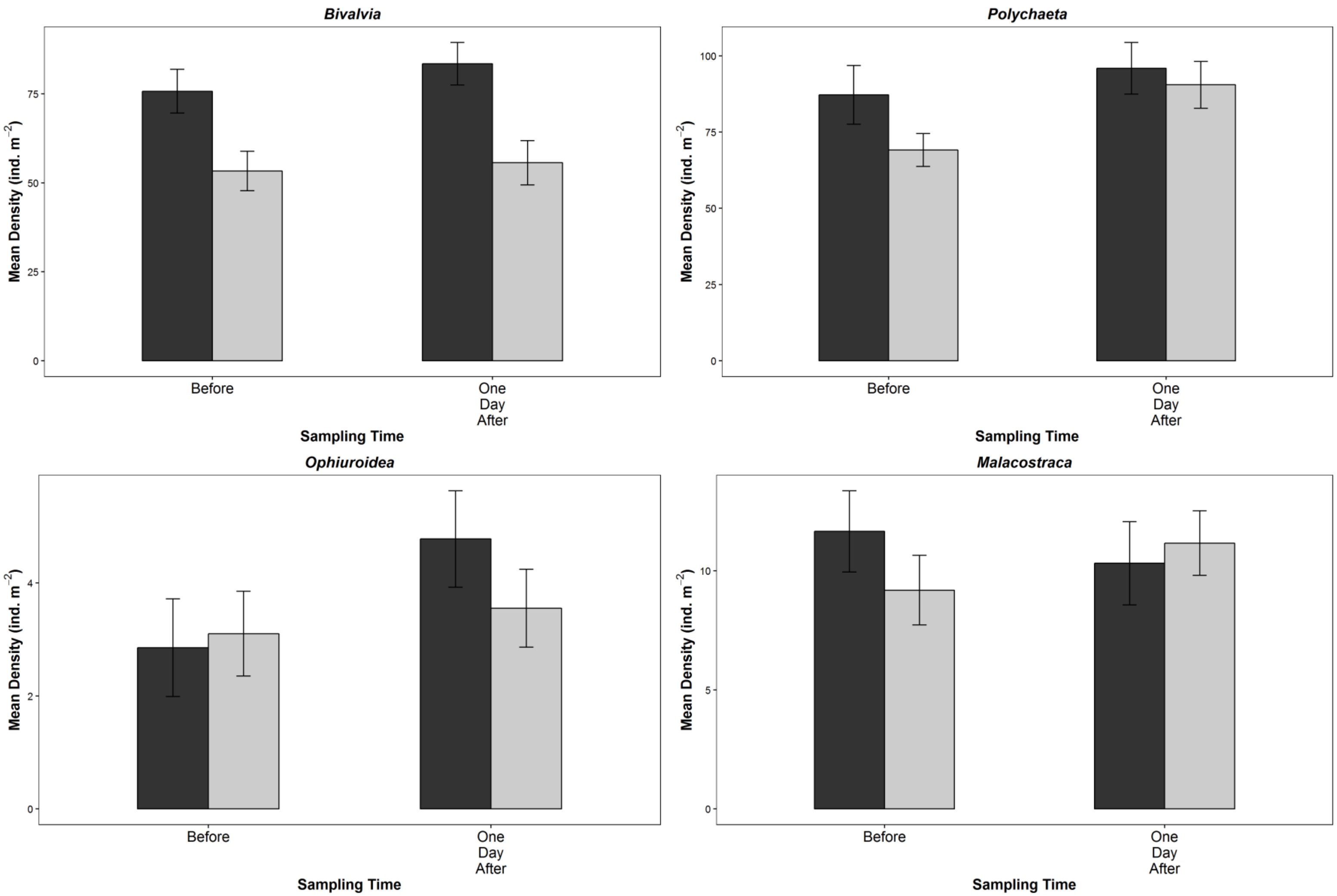

Figure 14. Mean (± S.E.) densities of the four main class groups present in Location B (Bivalvia, Polychaeta, Ophiuroidea, Malacostraca) across sampling times in control (black bars) and impact (light grey bars) sites.

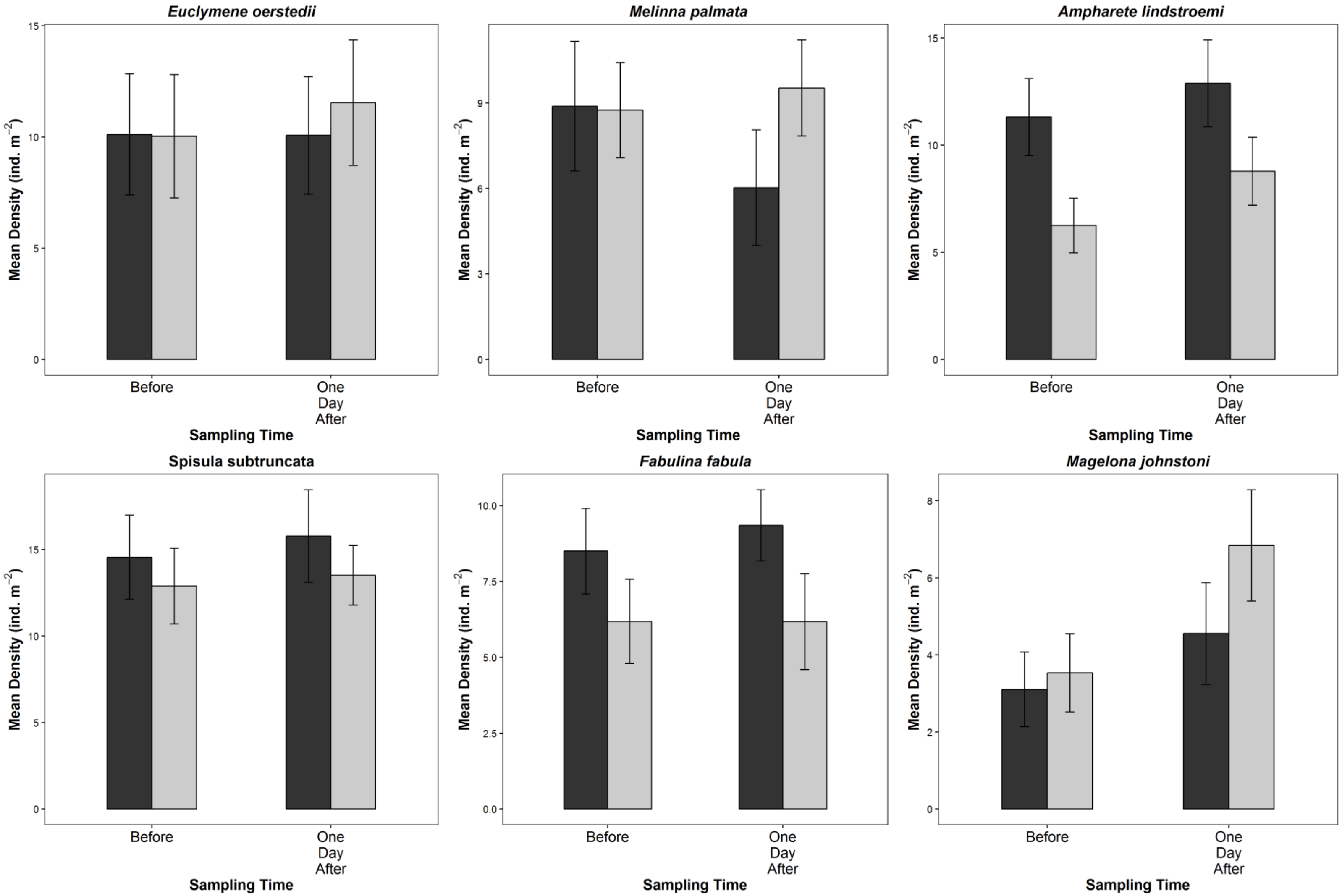

Figure 15. Mean (± S.E.) densities of the key species present in Location B as identified through SIMPER analysis across sampling times and in control (black bars) and impact (light grey bars) sites.

5. DISCUSSION

This report summarises results of BACI monitoring of two trawl gears used within the Torbay MCZ aimed at assessing the impacts of these gears on the ‘subtidal mud’ feature of the site. The results of this work will help inform management of the *Sepia officianalis* fishery within Torbay as well as the use of these gears in similar habitats within other designated sites.

5.1. Trawling Impacts

Results suggest little detectable impact of either gear type on sediment composition or macrobenthic communities within the study areas following five experimental passes of either trawl gear. There are strong signals of temporal change at Location A, where the heavy trawl gear was used, although these are considered to be clear seasonal effects on sediment properties, overall diversity indices and ecological quality as well as overall community structure. Much of the variation within the data from Location A is between samples obtained in October 2017 and in April 2018, with lower values observed for most biological responses in April 2018. Overwinter mortality causes reductions in overall abundance, density and biomass of benthic taxa as a result of lower temperatures and increased storm disturbance (Buchanan et al. 1978, Zwarts & Wanink 1993), before a subsequent increase following spawning and juvenile recruitment from spring into autumn for many taxa (George 1964, Rossi & Lardicci 2002, Scaps 2002). The significant effect of sampling time on many responses is therefore indicative of these natural seasonal changes. In Location B samples were not taken six months after trawling and a comparison of recovery or longer-term impacts between the two gear types is not possible.

The broad-scale habitat types represented by sediment particle size distribution data remained constant, classified as muddy sand across all BACI groups one day before and one day after trawling, with changes only evident in Location A after six months following experimental trawling using the heavier trawl gear. In April 2018 the broad scale habitat in this location had changed from muddy sand to slightly gravelly muddy sand, with a loss of finer sediment fractions and a corresponding increase in gravel and sand content. While a loss of muddy sediments often results from trawling-induced sediment resuspension and dispersal (Madron et al. 2005, Ferré et al. 2008, Palanques et al. 2014), this effect is observed across both control and impact sites and is likely a result of increased physical disturbance throughout the winter months; storm disturbance has similar effects to trawling on sediment resuspension and the release of particulate organic matter (Pusceddu et al. 2005).

Overall diversity analyses show that ecological conditions and benthic diversity are generally good throughout the study period in both locations, with communities generally dominated by disturbance-sensitive species. Significant differences in a number of these community-level measures are evident across sampling times, although none demonstrate a significant interaction and the response over time is similar between both control and impact sites, for both gear types used. Although the reduction in mean IQI values at trawled sites subject to heavy trawl gear use after six months is non-significant compared to control sites, average values reduce from “high” ecological status to “good”, and this may be of note or concern to managers. These high-level ecological indicators are valuable tools for managers when assessing habitat condition with limited resources. This change in status at trawled sites may indicate impacts of trawling on long-term recruitment patterns or community structure. While a similar reduction is apparent after six months across control sites, ecological status as indicated by IQI values remains “high”. The proportional contribution of AMBI groups to each allows insight into this change in overall IQI status, and shows that in Location A, six months following the use of the heavier trawl gear the relative proportion of AMBI Group V, first order opportunistic species (Borja & Franco 2000), increases. This may suggest changing dominance patterns in macrobenthic communities within samples from trawled sites that becomes apparent following overwinter reductions in overall abundance and diversity, although results need to be interpreted with caution given the lack of any statistical significance.

Significance of the interaction term would indicate a potential impact of trawling on the reported responses, demonstrating that the magnitude of the change in response between sampling times varies between treatments, with a change at trawled sites relative to that observed at control sites. Yet a significant interaction is only evident for the abundance of a single species, the terebellid polychaete *Melinna palmata* in Location B. This tube-dwelling worm lives up to five years in a mucus-lined tube that projects above the sediment surface (Retiere 1979, Fauchald & Jumars 1979) and is classified as an AMBI Group III species, classed as 'tolerant' of disturbance. This species demonstrated a significant increase in abundance one day after experimental trawling relative to control sites, at which a decrease in abundance is observed (Figure 15). However, given that this species is relatively long-lived, and the lack of any significant interaction for any other species abundance, or overall community composition as indicated by PERMANOVA, it is unlikely that this increase in impact sites after trawling is as a result of an influx of this species in response to trawling. As this species inhabits tubes just below the sediment surface, trawling may have caused a loss of sediment surface layers resulting in increased numbers of individuals at the sediment surface that have been retained in samples a day after trawling.

The changes in the test statistic in the pairwise comparison of the PERMANOVA results at Location A should be noted, with an increase in the t-value six months after trawling, indicating a further separation between the macrobenthic community in samples from control and impact sites. This also coincides with the increase in opportunistic taxa at trawled sites. While no significant interaction (and hence statistical evidence of a trawling impact) is evident in PERMANOVA results, this increased difference following the winter may further demonstrate the potential differences in dominance patterns between control and impact sites after winter. Physical disturbance associated with bottom-contact fishing gears may elicit such changes, which may be driven by altered sediment composition as a result of fishing disturbance (Martín et al. 2014, Palanques et al. 2014, Clarke et al. 2018), and the trends in the sediment data are consistent with those observed in the macrobenthic data. Similarly, an increase in the test statistic after use of the lighter trawl gear in Location B is also evident (Appendix 3), and is greater than that observed over the same period in Location A. While these test statistics may demonstrate a shift in conditions at impact sites away from those similar to control sites, the lack of a significant interaction at either study location means assigning causality to this is difficult. The significant random effect of site highlights the natural heterogeneity in marine habitats (Morrisey et al. 1992, Underwood 1992), and indicates that between-site variability in the macrobenthic assemblages is greater than that between treatments. Therefore the impact of trawling cannot easily be isolated from natural spatial variability.

5.2. Limitations and Further Research

Further information on the trawl gears used in each study location would provide further context to the results. While the available information presented in the introduction of this report allows some insight into the gear differences, no information on the penetration depth of each gear is available. Penetration depth of bottom-contact fishing gear correlates significantly with the magnitude of the reduction in benthic biota (Hiddink et al. 2017) and is therefore a key consideration when interpreting results. Generally, the heavier the gear used, the greater the physical interaction with the seabed and the greater the impacts. The box trawl utilised in Location A is the heavier of the two gears, yet no detectable impact of either gear is evident in the results presented here. Despite the different size and weight of the two gears, the same otter doors were used with each. Trawl doors can cause significant furrows in the seabed, with depths from 2 cm (Gilkinson et al. 1998) up to 30 cm reported (Krost et al. 1990), depending on the angle of the tow and the sediment characteristics. While the penetration of the footrope may differ between gears due to the different weights and ground gear used (miniature rockhoppers compared to rubber bobbins), the tracks from otter boards may be the only discernible evidence of trawling on the seabed (Krost et al. 1990). Recent work (Szostek et al. 2017) has produced an online resource to allow predictions of mean penetration depths of different gear types based on a review of existing evidence, and outputs suggest that despite the differences in the two trawls utilised in this study, penetration of the sediment from a single pass of the gear may be comparable and within the region of 0.7 cm, consistent with the lack of any detectable impact of either gear (Szostek et al. 2017). Furthermore, given that the door spread of the wing trawl and otter trawl used was around 33 m (Caslake & Montgomerie 2017), sampling in the middle of each site may well have missed any areas affected by the trawl doors, meaning only the impact of the footrope and ground gear are reflected in the data.

The sampling design utilised in this study is statistically robust with regards to replication of treatments, although is not without limitation. Samples were obtained one day prior to and one day following experimental trawling, with potential confounding effects that should be considered when interpreting results. The generalities of bottom-contact fishing gears have been reported in a number of reviews and meta-analyses (Collie et al. 2000, Kaiser et al. 2006, Clarke et al. 2017, Hiddink et al. 2017, Sciberras et al. 2018), with well-documented reductions in benthic abundance, diversity and biomass as a result of chronic fishing disturbance. However, most macrobenthic species within the present dataset are likely to pass through the trawl gear and hence not be physically removed from the seabed. In the days immediately following short-term fishing disturbance, therefore, many damaged or dead individuals may still be present in the area after physical interaction with the trawl gear, and sampling within the immediate hours following trawling may capture and preserve these individuals which will be represented in the data. No increase in the proportion of damaged taxa is observed in results, however. In fact, in the days immediately following fishing many scavengers may move into an area to predate dead and dying individuals or those that have been brought to the surface, temporarily increasing overall abundance and biomass within an area (Cesar & Frid 2009). This does not however appear to be the case in the present study.

It should be noted that this study presents analysis of trends in sediment composition and macrobenthic abundance only, and the absence of detailed biomass data is potentially important. Biomass data can help elucidate fishing impacts that may not be evident in abundance data alone. For example, where overall abundance may demonstrate an apparent recovery, biomass data may indicate that such trends are in fact characterised by rapid colonisation of small-bodied individuals resulting in an overall decrease in biomass (Jennings et al. 2001, Duplisea et al. 2002). Recovery in abundance is heavily determined by recruitment from surrounding, unimpacted areas, whereas recovery of biomass is largely influenced by changes in the population structure (e.g. size and age). In a recent meta-analysis of bottom-trawling disturbance, Hiddink et al. (2017) recommend using recovery rates based on biomass data to more effectively assess and model trawling impacts and subsequent trends in benthic communities. This would give appropriate consideration to recovery of body size and age structure within macrobenthic communities rather than abundance alone, and allows insight into other ecosystem processes that managers may consider within an ecosystem-based management framework, such as energy flow and benthic-pelagic coupling (Hiddink et al. 2017).

Additionally, it has been widely discussed that the impacts reported by studies of short-term, or 'pulse' fishing disturbance are likely to be markedly different to those of chronic, long-term fishing disturbance that is more representative of actual fishing effort over a season (Kaiser et al. 2006). The cuttle fishery in Torbay runs for three months a year from April to June, and a single instance of experimental trawling based on five passes of the gear will not be representative of fishing effort over a whole season. Depending on effort throughout these three months, fishing pressure and consequent impacts on macrobenthic communities and subtidal mud habitats may be more severe than those documented here. Reductions in biomass and abundance have been shown to significantly correlate with trawling frequency (Hiddink et al. 2017) and impacts over the course of a three month season, where trawl frequency may be significantly higher than that used in this study, may differ from those reported.

The reduction in mean IQI scores and ecological status after six months following use of the heavier trawl gear, along with the described limitations of the study may warrant further investigation into the impacts of either gear type under more realistic trawling intensities. To ensure a robust and accurate assessment of the impacts of either trawl gear on subtidal mud habitats within the Torbay MCZ, it is considered that future monitoring would benefit from undertaking a similar sampling design before and after the entire trawling season, using detailed effort data from Vessel Monitoring System (VMS) or logbook data and discussions with local fishermen to inform sampling. Furthermore, collecting both size and biomass data in addition to abundance data alone would allow more accurate elucidation of any impacts and recovery in macrobenthic communities resulting from trawling, contributing to effective conservation of subtidal mud habitats within the Torbay MCZ and other MPAs around the UK.

5.3. Summary and Hypotheses Testing

In summary, results show no detectable impact of experimental trawling on subtidal habitats and their associated macrobenthic assemblages within the Torbay MCZ, using either normal trawl gear or the lighter, modified gear. The research hypotheses presented in the introduction are individually addressed below in light of the results presented.

Sediments

H0(a): For each gear type, there is no change in sediment particle size distribution between trawled areas and un-trawled control areas and immediately before and after trawling, and/or where a change is observed, the magnitude of change does not differ between trawled and un-trawled areas.

This null hypothesis of no change in sediment composition can be **accepted** as results indicate sediment characteristics to be similar across both treatments and in samples obtained one day before and one day after an experimental pass of the trawl gear.

H0(b): In areas subject to heavy box trawling, there is no change in sediment particle size distribution throughout the recovery period and/or where a change is observed, the magnitude of change during the recovery period does not differ between trawled and un-trawled areas.

In the context of the results presented, this null hypothesis can also be **accepted**. Whilst a change in sediment composition was observed six months after experimental trawling, the patterns in the data are representative of natural temporal variation, likely due to increased storm disturbance over winter. The trends over time are consistent in trawled and control sites, indicating no difference in these trends as a result of trawling.

Infauna

H0(c): For each gear type, there is no change in infaunal diversity and/or community composition between trawled areas and un-trawled control areas and immediately before and after trawling, and/or where a change is observed, the magnitude of change does not differ between trawled and un-trawled areas.

Results indicate that this null hypothesis of no change in the macrobenthic communities in the study areas can be **accepted**, as no change in the overall assemblage, or diversity indices, is evident in the data. Temporal changes immediately following trawling are evident in IQI values and species richness in Location B, where the lighter trawl gear was used, although these trends are consistent across both trawled and control sites and likely due to broader changes or sampling error across the study site rather than any trawling impact.

H0(d): In areas subject to heavy box trawling, there is no change in infaunal diversity and/or community composition throughout the recovery period and/or where a change is observed, the magnitude of change during the recovery period does not differ between trawled and un-trawled areas.

This hypothesis of no change during the recovery period can also be **accepted**. The macrobenthic assemblage in Location A, where the heavier gear was used, significantly changed during the six month recovery period. However this again is indicative of natural temporal variation, particularly due to natural overwinter mortality and reductions in the abundance and diversity of macrobenthic communities, and is consistent across both trawled and control sites.

Overall, whilst the results presented show no detectable impact of either gear type on the sediment composition or macrobenthic communities associated with the ‘subtidal mud’ feature of Torbay MCZ (more accurately described as ‘subtidal muddy sand’), it is considered that there remains potential for the trawl fishery to negatively impact upon the subtidal habitats within the site, given the described limitations of the study. It would therefore be prudent for D&SIFCA to remain vigilant to such adverse effects and ensure adequate monitoring is undertaken and/or further work is conducted to assess potential cumulative impacts of repeated trawling of areas during each cuttle fishery season and over multiple seasons.

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