Benthic Infauna Assessment

CAMPS BAY OUTFALL

Prepared for:

CITY OF CAPE TOWN
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1 Introduction

The City of Cape Town (CCT) treats most household and industrial liquid waste at 17 wastewater treatment works (WWTW) and six smaller facilities (CCT, 2018). At the WWTWs wastewater undergoes secondary treatment processes, including chemical or ultraviolet disinfection, before being discharged into rivers, canals, vleis, aquifers or the sea. However, as in other coastal cities in South Africa and elsewhere in the world, the CCT's wastewater management strategy includes discharging preliminary treated wastewater into the marine environment via three marine outfalls located in Green Point, Camps Bay and Hout Bay.

Preliminarily treatment includes sand and grit removal followed by screening to remove plastic, paper and larger foreign materials. No chemicals are used in this process. The screened effluent is then pumped and discharged through diffusers at the end of these underwater pipelines. Diffusers have multiple ports which discharge effluent in alternating horizontal directions to aid dispersion and dilution. While preliminary treatment reduces the suspended solids load and removes objects such as plastic, rags and paper, there is inefficient removal of other contaminants related to wastewater. However, well-designed, maintained, and effective outfalls with preliminary treatment processes are considered to pose a low human health risk (WHO, 2003).

Camps Bay was chosen for this initial benthic assessment as of the three sites it is least affected by other major sources of pollution. At Hout Bay there is substantial ongoing nearshore wastewater pollution from the Disa and Baviaans Rivers, and pollution from the harbour. Similarly, Green Point is close to the Port of Cape Town, as well as Black River and the Diep River, the latter both heavily polluted and discharging substantial daily volumes of contaminated water into Table Bay.

As part of the CCT's environmental monitoring efforts, CLS Southern Africa (CLS SA) was contracted to conduct a once off survey of benthic macrofauna distributions in soft sediments in the vicinity of the Camps Bay outfall. The outfall was commissioned in 1977 and is the oldest of the three systems. The outfall itself is 1.5 km long and is licenced to discharges 5.5 million litres (ML) of effluent per day at a depth of 23 m (Figure 1.1). The objective of this initial assessment was to investigate whether there were any measurable effects on benthic macrofauna distributions attributable to the effluent discharged through the outfall. This document provides the methods, results and discussion related to this assessment.

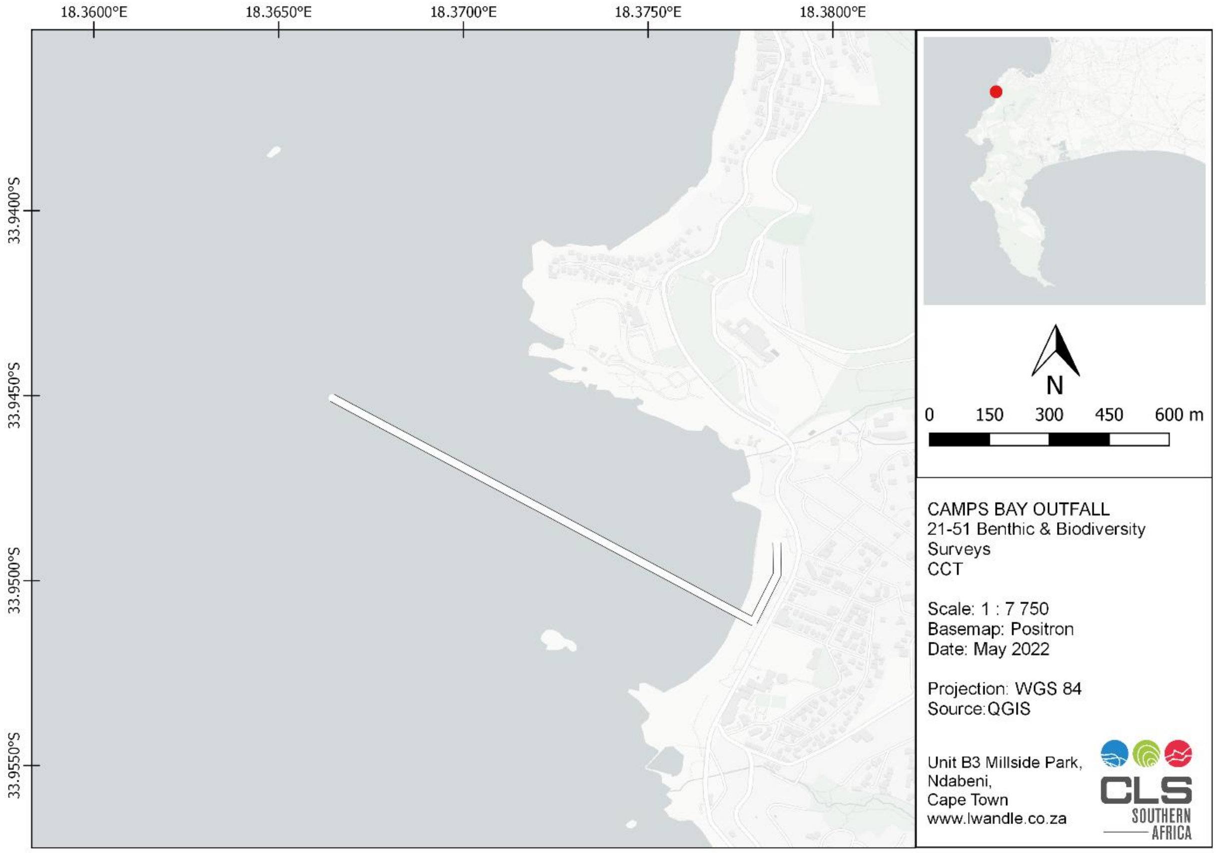

Figure 1.1: Map of the Camps Bay outfall.

2 Approach

Gradient survey designs are well suited to assess impacts in the vicinity of a point source discharge, especially when the plume has been investigated and assessed by hydrodynamic modelling studies, as is the case with respect to the Camps Bay outfall (PRDW, 2020; Ellis & Schneider, 1996). However, the benthic environment in the vicinity of the outfall comprises large granite boulders and reef areas, with sparse sand patches in-between. Implementing a gradient survey design with sampling sites at predetermined distances away from the outfall would therefore be challenging. This was confirmed by an investigative drop camera survey along a gradient design at the outfall. As a result, a comparative control-impact survey design was implemented. In the impact area, nearfield sites within a 0-500 m distance from the outfall and far field sites located 500-1000 m from the outfall were sampled. An equal number of sites were sampled in a geographically removed control area. It is important that control areas represent an environment that is comparable to the impact area in terms of habitat, geomorphology, seabed topography and oceanographic parameters, but is also geographically distant from, in this case, the discharge being investigated. Additionally, all sites were selected within a similar depth range to ensure that differences identified in benthos community composition are not due to varying depths.

Sand patches suitable for sediment sampling were identified in the field using previously collected drop camera footage and the vessel's echosounder. The sandy areas in Camps Bay are characterised by medium to coarse grained sand and shell fragments. This environment is not conducive to grab sampling as the grab cannot effectively penetrate the sand and the granules get stuck in the jaws causing sample loss and failed grabs. Therefore, benthic macrofauna samples were collected using a diver operated manual hand corer. Samples for particle size analyses were also collected by divers. The sections below provide details of the site selection, survey design and statistical analyses approach. Samples were analysed by reputable laboratories and specialists. Field work and data analyses protocols are detailed in Section 5.

3 Site Description

Camps Bay is a small embayment (~850 m wide) located on the west coast of the Cape Peninsula. The surrounding coastline is characterised by rocky headlands and shores with sandy pocket beaches in-between. Camps Bay beach itself is bounded by rocky headlands with Maidens Cove to the north and the rocky shores below Camps Bay drive to the south. Nearshore subtidal rocky substrate at these headlands is dominated by kelp (*Ecklonia maxima & Laminaria pallida*) and related biological communities. The seafloor inshore of the diffusers is mainly medium to coarse grained sand, however at the diffuser, and to the north and south of the diffuser, there are extensive underwater granite boulders and exposed bedrock (Eagle *et al.,* 1977; CSIR, 2017). Between these reef structures there are pockets of sand which range in size. These reported characteristics were confirmed by drop camera footage collected by CLS SA during a site visit prior to field work (Figure 3.1).

Figure 3.1: Drop camera images recorded at Camps Bay showing the reef structures with pockets of sand.

Wave action is strong within Camps Bay, with waves generally propagating from the south-west direction throughout the year (PRDW, 2020). The southern corner of the bay is more protected from this than the northern edge of the bay. Water column measurements recorded during summer and winter in 2021 show seawater temperatures ranging from 9.6 °C to 15.1 °C within Camps Bay (CLS SA, 2021). This is within the expected range for the greater Table Bay region (Quick & Roberts, 1993; Lwandle, 2007).

4 Survey Design

This assessment included survey activities at the outfall (Impact area) and a comparable control area south of Camps Bay. Drop camera imagery, bathymetry data, marine charts, and satellite imagery, were used to select a suitable control area along the coastline adjacent to Camps Bay. The areal cover of the selected location is similar to that of the corresponding impact area (Figure 4.1). Ecoregions, Marine Protected Area (MPA) zonation and other anthropogenic disturbances were also considered when selecting locations to ensure comparability between surveyed areas. Additionally, the study area is urbanised, and the surrounding coastal waters are exposed to anthropogenic disturbances from multiple sources and activities. This includes substantial urban runoff including but not limited to stormwater drain outlets as shown in Figure 4.1.

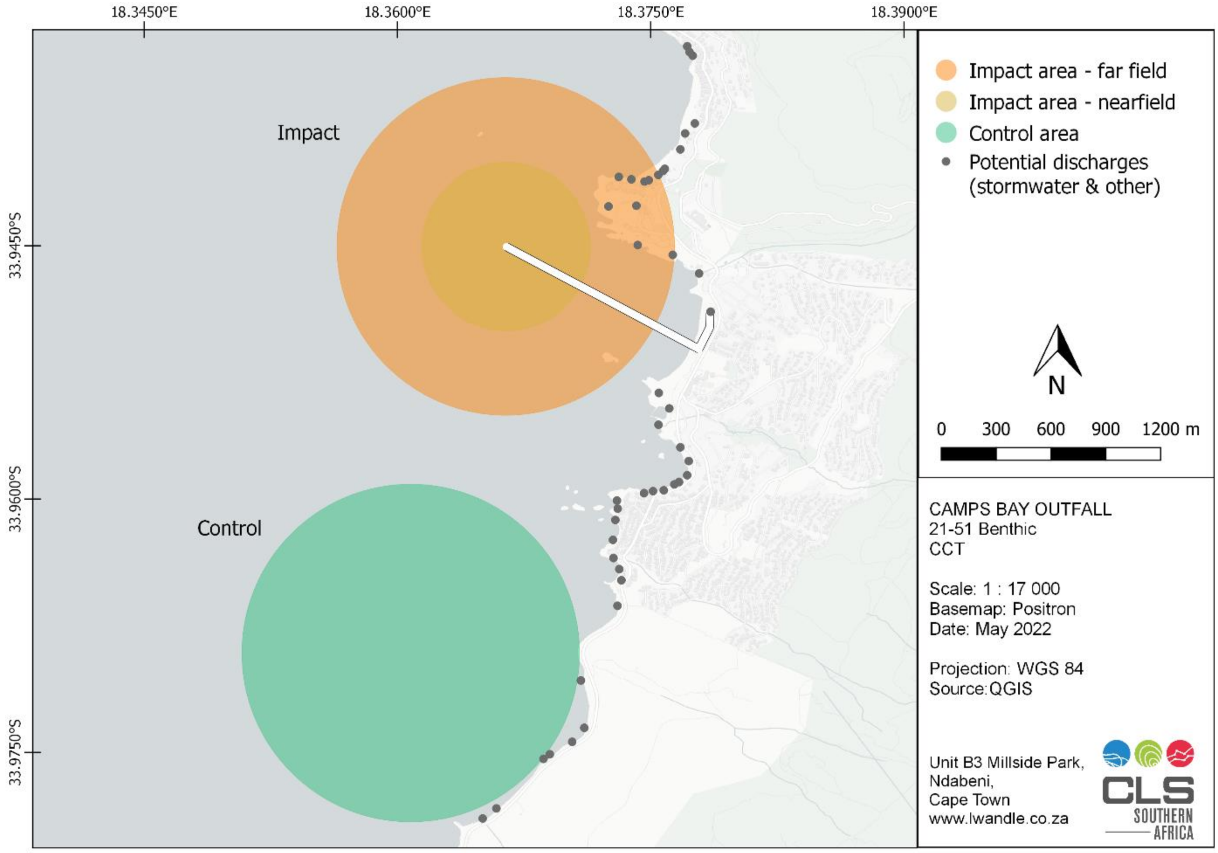

Figure 4.1: Map showing the Impact and Control sampling areas included in this assessment, in relation to other potential discharge sources (stormwater drains etc., provided by CCT).

A total of 12 sites were sampled for this assessment. In the impact area, three nearfield (NF, 0-500 m from outfall) and three far field sites (FF, 500-1000 m) were selected. In the control area, six sites were randomly distributed. At each of these sites, divers collected one 500 g sediment sample for particle size analyses (PSA), and three hand corer replicates which were filtered through 1 mm aperture sieve bags for benthic macrofauna analyses.

The resulting metrics from this survey for each sampling area (Impact & Control) are thus:

PSA

- Particle size distribution per site

Benthic macrofauna

- Species abundance per replicate
- Species biomass per replicate
- Species diversity per sampling area

5 Methods

This section details the field work and data analyses protocols that were followed for each of the survey components. The sampling plan for this survey is included in Appendix A.

The benthic survey was conducted over two days (5th & 11th April 2022) by a team of scuba divers from University of Cape Town's Research Dive Unit (UCT RDU). CCT provided the vessels and skippers for this work. A commercial dive supervisor was present at all times to record individual dive times and ensure the recommended no decompression limits were adhered to.

On arrival at each sampling area, six suitable sand patches were selected and marked by shot lines with surface marker buoys. Figure 5.1 shows the locations of Impact and Control sampling sites, and Table 5.1 lists the site depths and distances away from the discharge. The predominantly rocky benthic habitat in both areas resulted in benthic sites being clustered in areas with sufficient sand. Replicate sampling sites were generally ~50 m from each other except for one case where spacing was ~20 m.

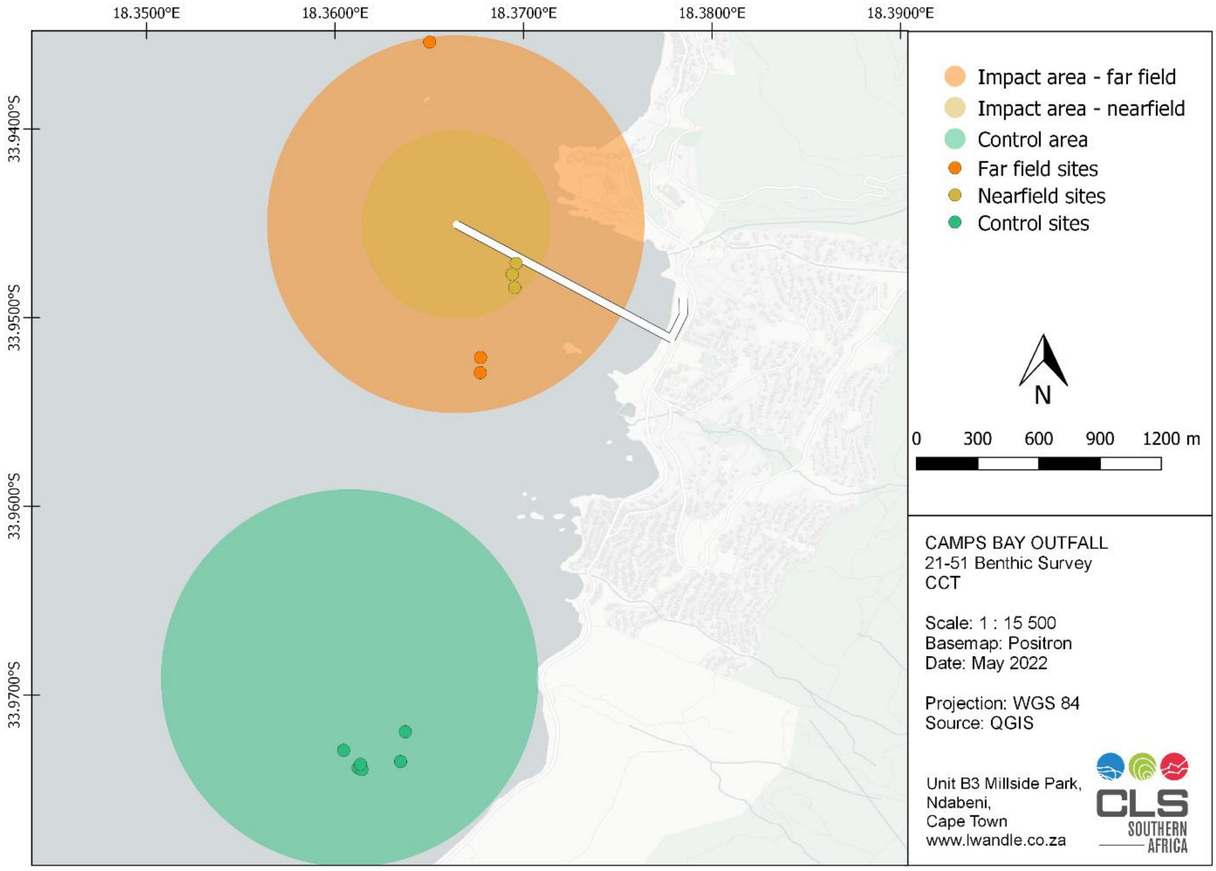

Figure 5.1: Map showing the locations of the benthic sampling sites within the Impact and Control sampling areas.

Table 5.1: Site depths and approximate distances away from the outfall discharge for the Impact and Control areas.

Impact Area			Control Area		
Site name	Depth (m)	Distance from discharge (m)	Site name	Depth (m)	Distance from discharge (m)
NF-1	16	367	**C-1**	20	3230
NF-2	18	404	**C-2**	21	3236
NF-3	18	470	**C-3**	21	3139
FF-1	14	790	**C-4**	21	3206
FF-2	25	880	**C-5**	20	3168
FF-3	28	1075	**C-6**	20	2990

5.1 Field Work

After descending at the shot line, divers collected samples using a hand corer, which sampled an area of 0.08 m^2 to a depth of 30 cm. The sample was transferred into 1 mm aperture sieve bags and shaken underwater to discard the fine sand and fauna less than 1 mm. This was repeated at three locations around the shot line so that three replicate samples were collected for benthic macrofauna analyses per site. At each site, divers also filled a sample jar with ~500 g of surficial sediment for particle size analyses. On completion at each site, divers returned to the surface with three filled sieve bags and one sample jar. The sediment was predominantly medium to coarse grained sand (> 1 mm) and a large volume of sediment was retained in each sieve bag. As a result, a 500 g subsample of this retained sediment was extracted for benthic macrofauna analyses by randomly scooping from the top, middle and bottom of each sieve bag, resulting in three 500 g replicate subsamples per site. The samples were preserved with ~5% formalin before transfer to benthic macrofauna specialists for sorting, identification and enumeration. PSA samples were transferred into Ziplock bags before transfer to the laboratory (Control Geoscience).

5.2 Data Analyses

5.2.1 Benthic Macrofauna

In the laboratory, the preserved macrofaunal samples were rinsed with freshwater to remove all traces of the formalin, and hand-sorted to extract the preserved fauna from the sediment with the aid of a stereomicroscope. The organisms were then transferred to a 1% phenoxyethanol (ethylenglycolmonophenyl-ether) solution for preservation. Any organisms considered dead at the time of collection (for example empty shells, decapitated polychaetes) were excluded from the analysis. Specimens were identified to the lowest taxonomic level possible and counted. Densities were calculated and expressed as number of specimens per sample. Pycnogonida (sea spiders), Platyhelminthes (flat worms), Nematoda (round worms) and Oligochaeta (segmented worms) were not identified to lower taxonomic levels. Wet biomass was estimated by blot-drying the specimens on absorbent tissue for a standard time and then weighing the specimens to the nearest 0.0001 g on an analytical precision balance. Weights were recorded per species/taxa as g/sample. Molluscs were weighed in their shells. All taxonomic names were verified against WoRMS (World Register of Marine Species, www.marinespecies.org).

The species richness, and Shannon-Wiener indices were calculated for each site, and the averages and standard deviations per sampling area were calculated. Non-parametric statistical analysis methods were applied due to departures from normal distributions. Kruskal-Wallis tests were used to test the null hypothesis that species richness and diversity, according to the Shannon-Wiener indices, are equal across sampling areas. Dunn's test was used post-hoc following the Kruskal-Wallis tests to conduct multiple pairwise comparisons and identify the significantly different sample means.

Data required square root transformation to reduce the effect of outliers. A Bray-Curtis dissimilarity matrix was used to determine the relationship between species composition at each site, and a Non-metric Multi-dimensional Scaling (NMDS) plot was generated using this matrix. A Shepard's plot was used to determine whether the ordination was appropriate. An ANOSIM, followed by a SIMPER analysis were used to determine the dissimilarity within and between sampling areas and identify species that contributed to the most dissimilarity.

Distributional analysis (Abundance/Biomass Comparison (ABC)) curves were used to represent patterns of species abundance and biomass in an area. The underlying assumption for the interpretation of the ABC curves is that when communities are undisturbed, the biomass is dominated by individuals of a few species, which grow to a large size. The biomass curve for the community therefore lies above the abundance curve on the ABC plot. Where sites have undergone moderate disturbance, the larger, longerlived species may be eliminated, reducing the difference between the abundance and biomass curves. The curves may therefore lie close to one another on the graph and may cross each other one or more times. At severely disturbed sites, opportunistic, small, short-lived species dominate the samples, and the abundance curve lies above the biomass curve. The difference in area under the abundance and biomass curves is summarised by the W-statistic. W tends towards +1 when the abundance is even across species, but the biomass is dominated by a single species (undisturbed), and W tends towards -1 in disturbed communities (although neither limit is likely to be attained in practice). W around 0 indicates moderately disturbed communities. A Kruskal-Wallis test was used to determine whether there was a significant difference in these W-statistics.

5.2.2 Particle Size Analyses

Particle Size Analyses (PSA) were analysed by the Control Geosciences laboratory in Cape Town. The laboratory applied standard geotechnical techniques (Test Reference: ASTM D6913-04 (2009)), which involved the use of 20 different sieve sizes (75 mm - 0.075 mm). Results were grouped into the particle size categories listed in Table 5.2 and plotted as stacked histograms.

Table 5.2: Particle size categories used for this assessment.

Category name	Particle size range (mm)
Fine sand	0.075 mm – 0.25 mm
Medium sand	0.25 mm – 0.425 mm
Coarse sand	0.425 m – 2.0 mm
Fine gravel	2.0 mm – 6.7 mm
Medium gravel	6.7 mm – 19 mm
Coarse gravel	19 mm – 75 mm

6 Results

6.1 Benthic Macrofauna

A total of 43 taxa were identified in this study. A full list is included in Appendix B. The average total abundance of benthic macrofauna was highest at the nearfield impact sites, and lowest at the control sites. This was largely due to the high abundance of the isopod *Eurydice longicornis* at the nearfield impact sites, and its absence from the far field impact sites and control sites. There were also several taxa that were present in the far field impact sites and control sites but absent in the nearfield impact sites, such as Oligochaetae, Nematoda and the phyllodocid polychaete *Prosphaerosyllis sublaevis*.

Average species richness and Shannon-Wiener indices for each site are listed in Table 6.1. Figure 6.1 shows the average Shannon-Wiener indices for each area. According to the Kruskal-Wallis test, there was a significant difference in species richness between sampling areas (nearfield impact, far field impact and control) (χ^2 = 6.129, df = 2, p = 0.047). Dunn's tests revealed that there was a significant difference in the calculated species richness between the far field impact sites and the control sites (Z = 2.441, p = 0.044). The species richness at the near-field impact sites were however not significantly different to either the control (Z = 1.201, p = 0.460) or the far-field impact sites (Z = 1.074, p = 0.283). The Shannon-Wiener diversity indices did not differ significantly between any of the areas (χ^2 = 4.199, df = 2, p = 0.105).

Table 6.1: Average species richness and Shannon-Wiener indices for all sites.

Impact			Control		
Site	Average species richness	Average Shannon-Wiener indices	Site	Average species richness	Average Shannon-Wiener indices
NF-1	4.67 (1.53)	0.44 (0.19)	**C-1**	3.67 (0.58)	1.2 (0.29)
NF-2	5.33 (2.08)	1.49 (0.34)	**C-2**	4.33 (1.15)	1.44 (0.29)
NF-3	4 (1.00)	0.38 (0.11)	**C-3**	4.33 (2.08)	1.19 (0.48)
FF-1	4 (1.73)	1.09 (0.6)	**C-4**	4.33 (0.58)	1.38 (0.23)
FF-2	7 (4.36)	1.39 (0.18)	**C-5**	3.33 (1.15)	1.06 (0.32)
FF-3	7.33 (1.15)	1.28 (0.22)	**C-6**	3.33 (1.15)	1.12 (0.37)

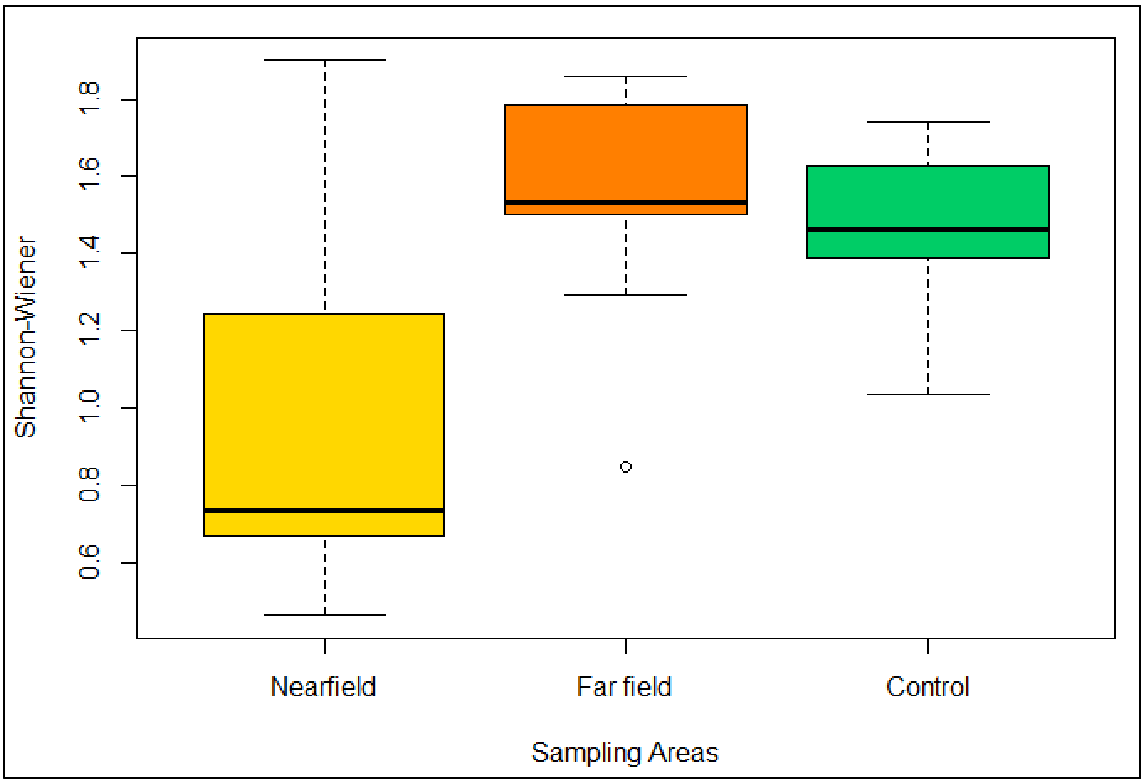

Figure 6.1: Box plots of Shannon-Wiener diversity indices for macrobenthos in each sampling area.

According to the NDMS plot, species composition at the near field impact sites is dissimilar to that of the far field impact sites and control sites, and these sites are grouped (Figure 6.2). The ANOSIM results supported this with a significant R statistic of 0.610 ($p < 0.001$). The nearfield impact sites were dissimilar to the far field and control sites, with significant R statistics of over 0.8 (Table 6.2). The far field impact sites and control site comparison had a lower level of dissimilarity with significant R-statistic of less than 0.3 (Table 6.2).

Table 6.2: R-statistics with corresponding p-values from the various ANOSIM computations.

Comparison	R-statistic	p-value
Nearfield impact – far field impact	0.986	0.0001
Nearfield impact – control	0.824	0.0001
Control – far field impact	0.266	0.0026

The SIMPER analysis revealed that three species were responsible for most y of the dissimilarity observed between the near field sites and the far field and control sites (Table 6.3). *E. longicornis* accounted for 58.55 % of the dissimilarity between the species composition at the nearfield impact sites and the control sites, and 45.00 % of the dissimilarity between the species composition at the nearfield impact sites and far field impact sites. However, the dissimilarity between the sampling areas remains >77% even when *E. longicornis* was removed from the analysis due to other species that were specific to sampling areas in low abundances.

The cumulative abundance-biomass dominance comparisons revealed that all sites were moderately disturbed, with W-statistics close to 0. There was no significant difference in the W-statistics between each replicate sample (χ^2 = 20.599, df = 35, p = 0.802). Abundance-Biomass curves for each sampling area are shown in Appendix C.

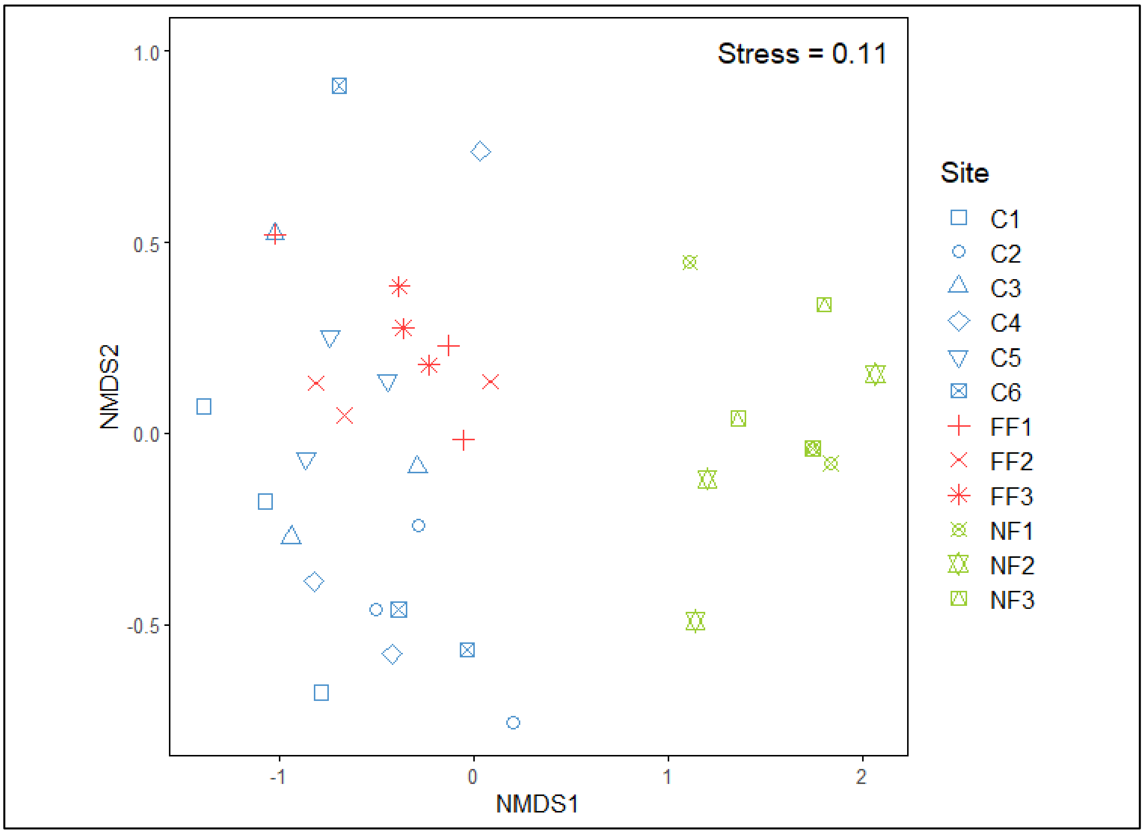

Figure 6.2: NDMS ordination plot of benthic macrofauna abundance from samples collected at the nearfield impact, far field impact and control sites.

Table 6.3: Result of the SIMPER analyses. Table listing species that contributed up to 70% of the observed dissimilarity between sites that were significantly dissimilar.

Comparison	Species	Average contribution to dissimilarity (%)	Cumulative contribution (%)
Nearfield Impact - Control	*Eurydice longicornis*	58.55	58.55
	Prionospio saldanha	6.07	64.62
	Prophaerosyllis sublaevis	4.96	69.58
	Oligochaeta	4.59	74.17
Nearfield Impact – Far field Impact	*Eurydice longicornis*	45.00	45.00
	Oligochaeta	22.37	67.37
	Nematoda	5.72	73.09

6.2 Particle Size Analyses

PSA results show that the nearfield sampling sites (~367-470 m from the discharge) comprised sand, mainly medium grain sized (0.25 - 0.425 mm). Sediment grain size at the far field sampling sites was coarser, comprising mainly coarse sand (0.425 m – 2.0 mm) and fine to medium gravel (2.0 mm – 19 mm). Coarse gravel (19 mm – 75 mm) contributed to 8% of the sample collected at site FF-2, which was likely a single shell fragment or small rock (Figure 6.3). Particle size distribution recorded at the control sites was similar to that of the far field impact sites, with the majority being classified as coarse sand and fine gravel (Figure 6.4). The samples contained less medium sized gravel, however, very small percentages of fine and medium sand were recorded at all control sites (<2%).

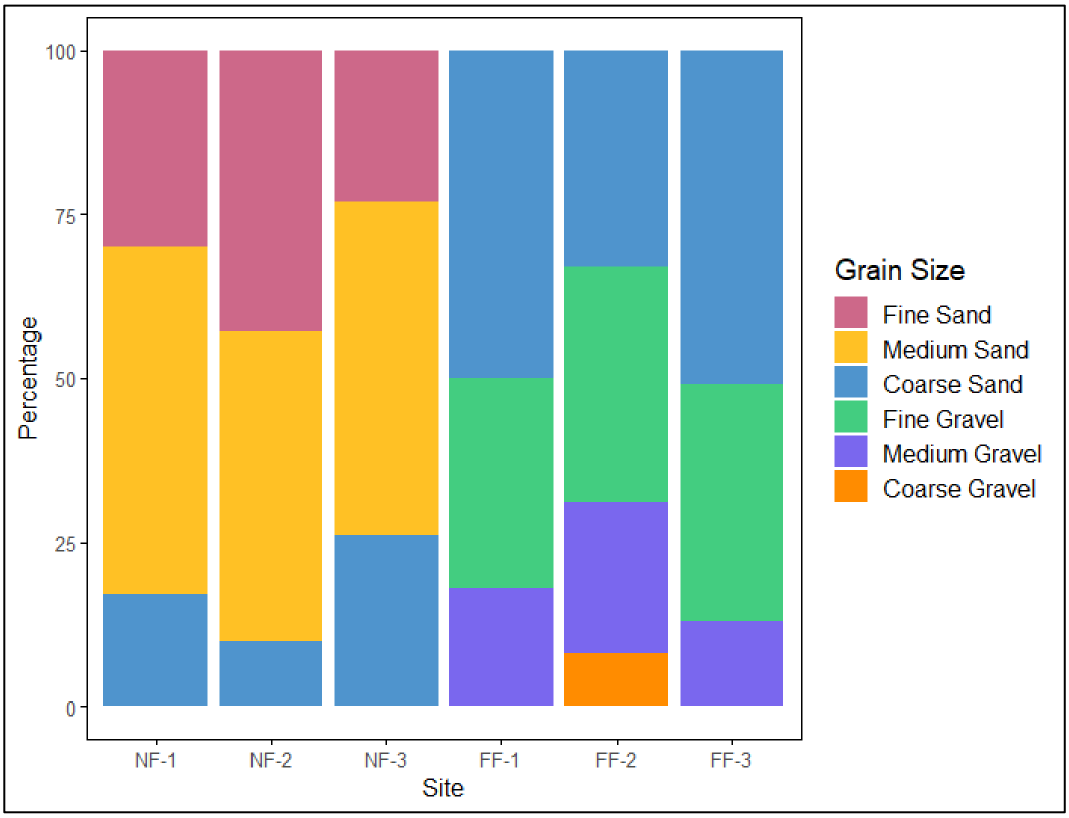

Figure 6.3: Particle size distribution at each benthic sampling site in the impact area. NF refers to nearfield and FF refers to far field.

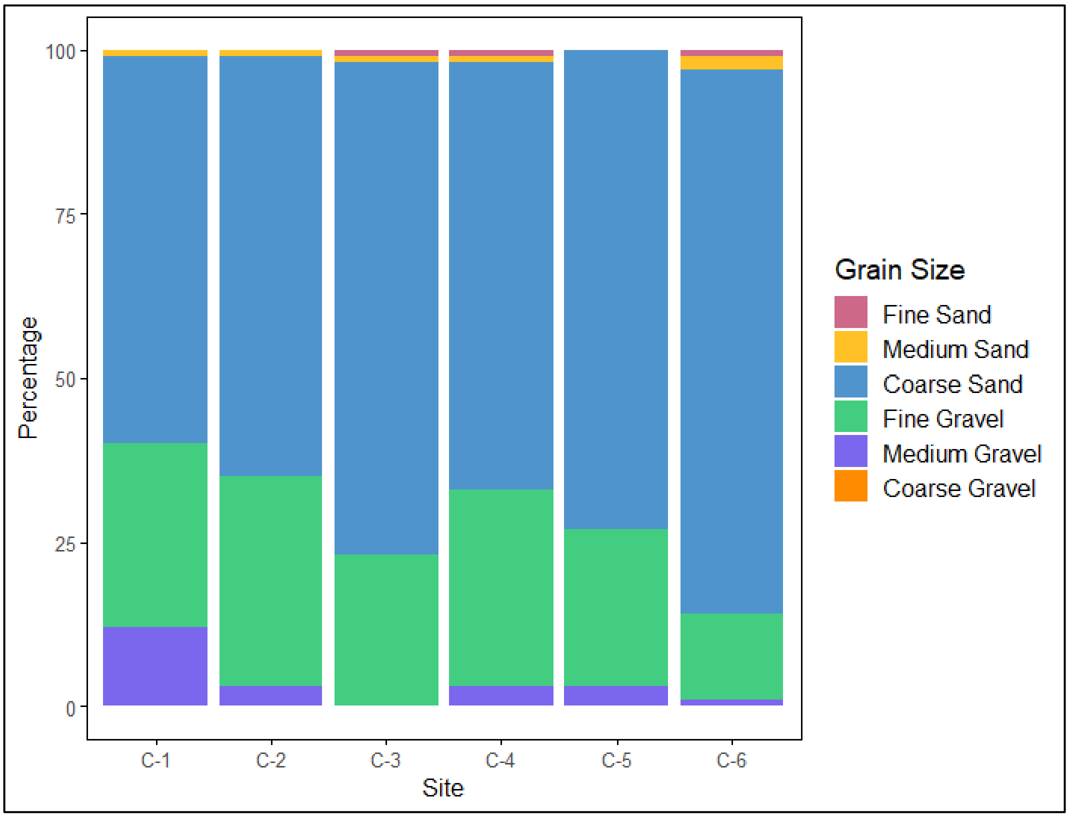

Figure 6.4: Particle size distribution at each benthic sampling site in the control area. C refers to control.

7 Discussion

Marine sediment particle size generally decreases with an increase in depth, as higher energy processes occur in shallower waters closer to the coast (Romano *et al.,* 2017). Camps Bay and the surrounding coastline is exposed and experiences moderate to high wave action. It was therefore expected that most samples collected comprised majority coarse sand (0.425 mm-2 mm). The particle size distribution recorded during this study aligns with previous surveys in the area (Quick & Roberts, 1993; CSIR, 2017). Sites in the nearfield impact area displayed slightly finer particle size distribution, with over 70% of samples collected here comprising fine to medium sand (0.075 mm-0.425 mm), compared to sites in the far field and control areas. Effluent discharge has been shown to alter grain size composition through input of significant amounts of fine-grained particulate matter (Roper *et al.,* 1989; Arvai *et al.,* 2002). Results suggest that the constant supply of finer grained particles in the effluent discharge is contributing to the variation observed in particle size around the outfall. However, particle size recorded at the nearfield sites, although slightly finer than that in the far field sites, is still classified as sand which is unlikely to accumulate contaminants and organics, and materially alter the benthic ecology. Accumulation of metals, contaminants and particulate organic material is usually a risk in sediments that are less than 0.063 mm (fine mud). Spatial variation in particle size at Camps Bay was also observed by CSIR (2017), who suggested that irregular benthic topography in this area could be affecting local currents.

A total of 42 taxa were identified over the 36 samples that were sorted and processed to determine benthic macrofauna abundance and biomass. There was no significant difference in species diversity between sampling areas (nearfield impact, far field impact and control), as indicated by Shannon-Wiener indices. However, according to the ANOSIM, species composition at the nearfield impact sampling area was dissimilar to both the far field and control areas. This was largely due to the higher abundance of the isopod *Eurydice longicornis*, which contributed to approximately half of the dissimilarity observed. However, the dissimilarity between areas was still apparent when this species was removed from the analysis, as there are several other species that were unique to the nearfield sites but occur in low numbers.

E. longicornis is a carnivorous isopod common on most beaches that are exposed to moderate to high wave action along the Cape Peninsula and has been recorded at Camps Bay before (Brown, 1973). These isopods scavenge and prey on decaying and living animal matter (Brown, 1973). They should therefore have minimal food sources related to the outfall discharge. Sediment particle size distribution strongly conditions benthic macrofauna species composition (Magno *et al.,* 2012; Romano *et al.,* 2017). It is therefore a reasonable assumption that the differences observed in the survey data can be controlled by this too. Laboratory experiments conducted in the 1960s showed that *E. longicornis* prefers sand grain sizes of 0.178-0.500 mm over coarser grained sand. The majority of the sediment collected at the nearfield impact sites, where *E. longicornis* was abundant, falls within this preferred range. Sediment particle size at the far field impact sites and control sites was coarser than this (mostly >0.425 mm).

The differences in particle size distribution and benthic macrofauna species composition between nearfield sites and those further afield can likely be attributed to a combination of the natural (wave exposure) and anthropogenic factors (outfall discharge). Despite this, monitoring should be implemented to ensure that these do not worsen over time. Anoxic (black), sludge-like sediment, absence of benthic organisms, or presence of few invasive species are characteristics of a system that is highly disturbed and polluted. None of these characteristics were evident in this study.

8 Limitations

One of the predominant limitations to this assessment is the lack of comparable data from the study area over time. As a result, comparisons were made with existing data and literature from the greater Table Bay and West Coast regions. Additionally, as this was a once off survey, it presents a snapshot in time and no seasonal or interannual inferences on benthic macrofauna patterns could be made.

Another challenge was identifying suitable control areas with comparable benthic habitat and oceanographic conditions to the impact area, but that is also geographically distant enough to not be affected by the disturbance. This challenge is not unique to this assessment but does increase the risk of confounding results. There are many sources of urban runoff and anthropogenic disturbances across the entire coastline included in this assessment (Figure 4.1). It is impossible to quantify the impact of this and differentiate this from impacts directly related to the Camps Bay outfall.

9 Conclusion

In conclusion, benthic macrofauna species composition at the nearfield impact sites varied from that at the far field impact and control sites. This is likely being conditioned by differences in particle size distribution, as these sites were characterised by finer sand. The input of particulate matter from the effluent is likely altering the particle size distribution close to the outfall discharge. However, the benthic ecology is not materially altered and characteristics that signal extreme environmental degradation such as anoxic sediment and 'dead zones' were not evident in this study.

A once off survey can only provide a snapshot in time. Further monitoring of the benthic macrofauna and sediment properties in these environments by repeatable and comparable surveys would be ideal to increase sample numbers and improve statistical robustness. However, abiotic conditions are unlikely to change and the probability for impacts to the benthic community are thus unlikely.

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Appendix A - Sampling Plan

Sampling Plan

Camps Bay Outfall: Benthic Survey

Prepared for:

CITY OF CAPE TOWN
ISIXEKO SASEKAPA
STAD KAAPSTAD

Reference: CLS-SA-21-70 SAMPLING PLAN

V1.0 – 28/03/2022

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CHRONOLOGY ISSUES

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CCT	Electronic	G. Oelofse, J. Du Toit

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1 INTRODUCTION

The City of Cape Town (CCT) operates three marine outfalls that discharge wastewater offshore of Camps Bay, Green Point and Hout Bay. At all outfalls, the effluent moves through a 3 mm mesh which removes solids and general litter from the waste stream before being discharged. At the end of the outfalls, diffusers rapidly dilute the effluent as it is discharged. The Camps Bay outfall was commissioned in 1977 and is the oldest of the three systems. The outfall itself is 1.5 km long and discharges 5.5 million litres (Ml) of effluent per day at a depth of 23 m (Figure 1.1).

As part of the CCT's environmental monitoring efforts, CLS Southern Africa (CLS SA) has been contracted to conduct a once off benthic survey at the Camps Bay outfall to determine whether the discharge is affecting macrofauna diversity, relative abundance and community composition to an important degree. This document provides a detailed sampling plan that will be followed to complete the scope of work for the survey.

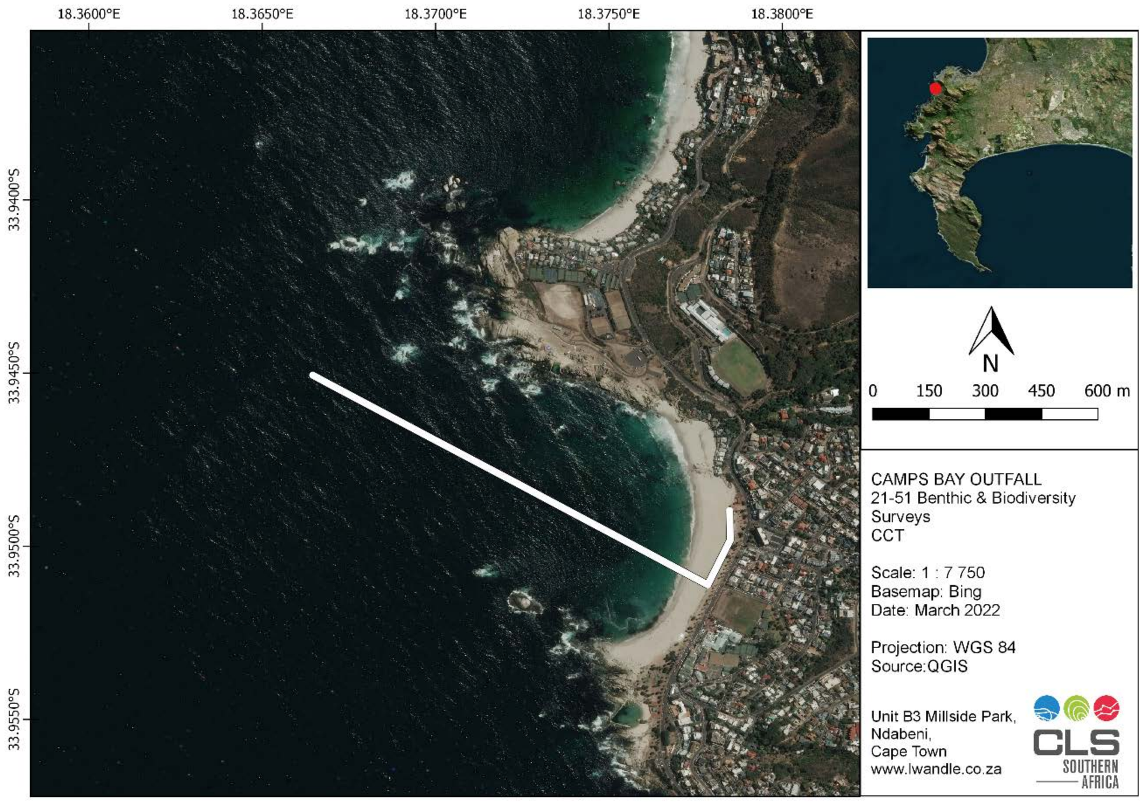

Figure 1.1: Map of the Camps Bay outfall.

2 SURVEY DESIGN

Gradient survey designs are well suited to assess impacts in the vicinity of a point source discharge, especially when the plume has been investigated and assessed by hydrodynamic modelling studies, as is the case with respect to the Camps Bay outfall (PRDW, 2020; Ellis & Schneider, 1996). However, drop camera images of the seafloor that were recorded along a gradient design at the outfall show that the seafloor at most sites is hard substrate. Implementing a gradient survey design with sites at predetermined logarithmic distances away from the outfall would therefore be challenging.

In view of the above for this survey, a comparative control-impact survey design will be implemented. In the impact area, there will be nearfield sites within a 0-500 m distance from the outfall with far field sites located 500-1000 m from the outfall. An equal number of sites will be sampled in a geographically removed control area. It is important that this control area represents an environment that is comparable to the impact area in terms of habitat, geomorphology, and oceanographic parameters, but is also geographically distant. Additionally, all sites should fall within a similar depth range to ensure that differences identified in benthos community composition are not due to varying depths. The Camps Bay nearshore is characterised by large boulders and interspersed rocky areas. As a result, divers will need to identify sand patches that are suitable for sediment sampling. The sandy areas in Camps Bay are characterised by coarse grained sand and shell fragments. This environment is not conducive to grab sampling as the grab cannot effectively penetrate the sand and the granules get stuck in the jaws causing sample loss and failed grabs. Therefore, macrobenthos sampling will be conducted using a diver operated manual hand corerer. The sections below provide details of the site selection, survey design and statistical analyses approach.

2.1 Site selection

Drop camera imagery, bathymetry data, marine charts and satellite imagery, were used to select a suitable control area along the coastline adjacent to Camps Bay. Ecoregions, Marine Protected Area (MPA) zonation and other anthropogenic disturbances were also considered when selecting this location to ensure comparability. Any differences between the control and impact areas will be explicitly discussed when interpreting survey results and all limitations to this study will be stated. The control area covers a similar area to the corresponding impact area. One control area, containing randomly distributed sites, is deemed sufficient since the benthic habitats and wave action along the peninsula are broadly similar.

In the impact area, sampling sites have been selected in the nearfield (within 500 m) and far field (500-1000 m) regions. The sites have been randomly distributed within a similar depth range but may need to be shifted in the field to areas that are sandy and therefore suitable for sediment sampling. Additionally, all sites are less than 30 m deep to remain within non-decompression scuba diving limits.

Figure 2.1 displays the planned sampling sites.

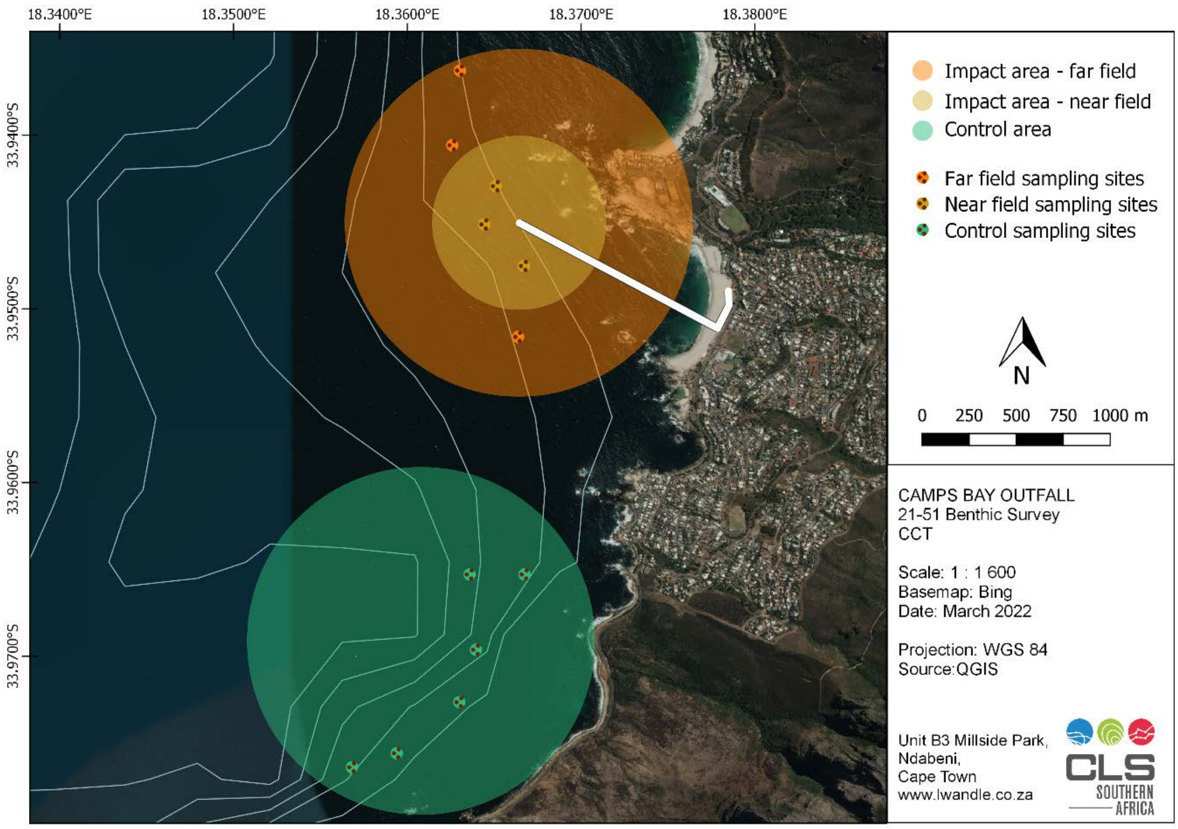

Figure 2.1: Map showing the planned impact and control areas with sampling sites. Note that sampling sites map be shifted to areas with sand bottom in the field.

2.2 Survey design

There are 6 sites within each of the sampling areas (impact and control areas), therefore 12 sites in total. Three replicate samples will be collected at each site giving 18 samples within the impact area (nearfield and far field) and 18 samples within the control area (Table 2.1).

Table 2.1: Summary of the number of sites within each sampling area.

Sampling Area	Description	No. of sites	No. of replicates	Total samples
Impact	Nearfield and far field sites	6	3	18
Control	Randomly placed sites in control area	6	3	18
			Total	**36**

At each site, a diver-operated manual hand corer will be used to collect sediment samples and divers will collect a particle size analysis (PSA) sample in a 500 ml jar. The sample pot covers a surface area of 0.08 m^2 and penetrates to a depth of ~30 cm, therefore the total volume of sediment collected at each site is ~0.024 m^3. Sediment vacuumed from the sample pot is passed through a 1 mm sieve bag. The contents of the bag are then returned to the surface for processing and macrobenthos sample collection onboard the vessel. Due to the coarse characteristic of the sediment in Camps Bay, three subsamples will be taken from each sieve bag to resolve the entire sample.

The resulting metrics from this survey for each sampling area (impact, nearfield control and far field control) will be:

- Species abundance per replicate
- Species biomass per replicate
- Species diversity per sampling area

Appendix A lists all site IDs.

2.3 Statistical analyses

Statistical analyses will be computed using R version 4.1.1.

Species richness and Shannon-Wiener diversity indices will be computed. The Shannon-Wiener diversity index (H') is commonly used in ecology, it is a measurement of the biodiversity within a sample, considering both the abundance and evenness of each species.

The initial composition and spatial changes in composition of macrobenthos will be assessed by Non-Metric Multidimensional Scaling (NDMS) based on a Bray-Curtis dissimilarity matrix using appropriately transformed data. NDMS ordination plots will be generated which are non-parametric and two-dimensional. Both Analysis of Similarity (ANOSIM) and Similarity Percentage (SIMPER) analyses will be calculated as supplementary analyses to the ordination to quantify the relationships between and among the groupings. Cumulative dominance (Abundance-Biomass Comparison – ABC) curves will be generated for the impact, nearfield control and far field control areas to indicate the level of disturbance present in the communities. The assumption of using ABC curves to show disturbance is that when undisturbed, the community biomass is dominated by a few species which grow to a larger size with the opposite in disturbed areas.

3 EQUIPMENT

The following equipment is required to conduct this survey:

- Scuba dive gear for six divers
- Diver-operated manual hand corer
- PSA jars
- 1 mm sieve bags
- Sample containers
- Forceps
- 10% formalin solution
- Personal protective equipment

4 PERSONNEL

The survey team will consist of the following personnel:

- CLS SA survey lead
- Skipper/dive supervisor
- Dive team including standby diver

5 SURVEY PROTOCOLS

The following protocols and work procedures will be followed by the survey team. A Toolbox Talk meeting and diver briefing will be conducted by the CLS SA survey lead and dive supervisor to discuss the planned operations, related risks and mitigation measures. The dive supervisor will keep a log of the dive times and manage the dive operations throughout the survey.

5.1 Benthic survey

At each of the confirmed sampling positions, the following steps must be followed:

1. Deploy a shot line and surface marker buoy to indicate the site.
2. Record a waypoint on the handheld GPS.
3. Once dive buddy gear checks have been completed, the divers should enter the water on instruction from the dive supervisor.
4. Descend at the shot line with the manual hand corer and jar for the PSA sample.
5. Push the corer into the sediment three times and empty contents into 1mm sieve bags.
6. Repeat the above step to complete three replicates per site.
7. Fill the jar for the PSA sample.
8. Divers to secure sieve bags to the shotline to be pulled up by personnel on board the vessel and ascend to surface.
9. On the vessel, collect subsamples per sieve bag
10. Place retained macrobenthos in prelabelled jar and cover with ~1 cm of seawater.
11. Store both macrobenthos and PSA samples in a cooler box.
12. Conduct steps 5 to 11 at three locations around the shot line.
13. Record any observations on sediment texture and benthic characteristics.

5.2 Sample curation

The retained macrobenthos organisms from each site will be collected into prelabelled jars and covered with seawater (1-2 cm). On return to the office on the same day as sample collection, an approximate equal volume of 10% formalin will be added to the sample for preservation. This will result in a final formalin concentration of 4-5%. Samples should be gently shaken to ensure thorough mixing with the formalin solution. Personnel must wear gloves and masks, and work in a well-ventilated area when handling formalin. The plastic jars must be tightly sealed and stored in a cool area away from direct sunlight before transfer to the laboratory for sorting.

6 SCHEDULE

The benthic survey is expected to take 3 days and will be scheduled according to the most suitable weather conditions.

7 REFERENCES

Ellis, J.I. and Schneider, D.C., 1997. Evaluation of a gradient sampling design for environmental impact assessment. Environmental Monitoring and Assessment, 48(2), pp.157-172.

PRDW., 2020 Final Report on Dispersion Model Calibration and Results for Camps Bay. S2101-RP-CE-008-R0.

Appendix A - Sample IDs

Sampling Area	Site No.	Activity	Sample ID	Sampling Area	Site No.	Activity	Sample ID
Impact Nearfield	1	Replicate 1	NF-1R1	Impact Far field	1	Replicate 1	FF-1R1
		Replicate 2	NF-1R2			Replicate 2	FF-1R2
		Replicate 3	NF-1R3			Replicate 3	FF-1R3
	2	Replicate 1	NF-2R1		2	Replicate 1	FF-2R1
		Replicate 2	NF-2R2			Replicate 2	FF-2R2
		Replicate 3	NF-2R3			Replicate 3	FF-2R3
	3	Replicate 1	NF-3R1		3	Replicate 1	FF-3R1
		Replicate 2	NF-3R2			Replicate 2	FF-3R2
		Replicate 3	NF-3R3			Replicate 3	FF-3R3

Sampling Area	Site No.	Activity	Sample ID	Sampling Area	Site No.	Activity	Sample ID
Control	1	Replicate 1	C-1R1	Control	4	Replicate 1	C-4R1
		Replicate 2	C-1R2			Replicate 2	C-4R2
		Replicate 3	C-1R3			Replicate 3	C-4R3
	2	Replicate 1	C-2R1		5	Replicate 1	C-5R1
		Replicate 2	C-2R2			Replicate 2	C-5R2
		Replicate 3	C-2R3			Replicate 3	C-5R3
	3	Replicate 1	C-3R1		6	Replicate 1	C-6R1
		Replicate 2	C-3R2			Replicate 2	C-6R2
		Replicate 3	C-3R3			Replicate 3	C-6R3

Appendix B - Species List

Phylum/Order	Family	Lowest taxonomic ID
Polychaeta	*Aphroditidae*	*Aphroditidae*
	Aphroditidae	*Harmothoe sp.*
	Capitellidae	*Notomastus latericeus*
	Cirratulidae	*Cirratulidae*
	Dorvilleidae	*Protodorvillea biarticulata*
	Glyceridae	*Glycera benguellana*
	Glyceridae	*Glycera papillosa*
	Hesionidae	*Syllidia armata*
	Lumbrineridae	*Scoletoma (=Lumbrineris) tetraura*
	Maldanidae	*Isocirrus glandularis*
	Nephtyidae	*Nephtys capensis*
	Oenonidae	*Drilonereis monroi*
	Onuphidae	*Onuphis holobranchiata*
	Orbiniidae	*Phylo capensis*
	Paraonidae	*Paraonidae*
	Paraonidae	*Paradoneis lyra capensis*
	Phyllodocidae	*Phyllodocidae*
	Spionidae	*Rhynchospio mzansi*
	Spionidae	*Prionospio saldanha*
	Spionidae	*Scolelepis (Scolelepis) squamata*
	Spionidae	*Scolelepis (Parascolelepis) gilchristi*
	Syllidae	*Prosphaerosyllis sublaevis*
	Syllidae	*Exogone heterosetosa*
	Syllidae	*Syllis armillaris*
	Syllidae	*Syllis prolifera*
	Syllidae	*Syllis vittata*
	Syllidae	*Syllidae (smooth dorsal cirri)*
	Terebellidae	*Terebellidae*
Amphipoda	*Leucothoidae*	*Leucothoe spinicarpa*
	Lysianassidae	*Lysianassa ceratina*
	Phoxocephalidae	*Heterophoxus opus*
	Pontogeniidae	*Paramoera capensis*
	Pontogeniidae	*Pontogeniidae*
	Tryphosidae	*Hippomedon longimanus*
	Tryphosidae	*Hippomedon normalis*

	Urothoidae	*Urothoe pinnata*
Isopoda	*Cirolanidae*	*Natatolana sp.*
	Cirolanidae	*Natatolana pilula*
	Cirolanidae	*Eurydice longicornis*
Pycnogonida		*Pycnogonida*
Platyhelminthes		*Platyhelminthes*
Nematoda		*Nematoda*
Oligochaeta		*Oligochaeta*

Appendix C - ABC Plots

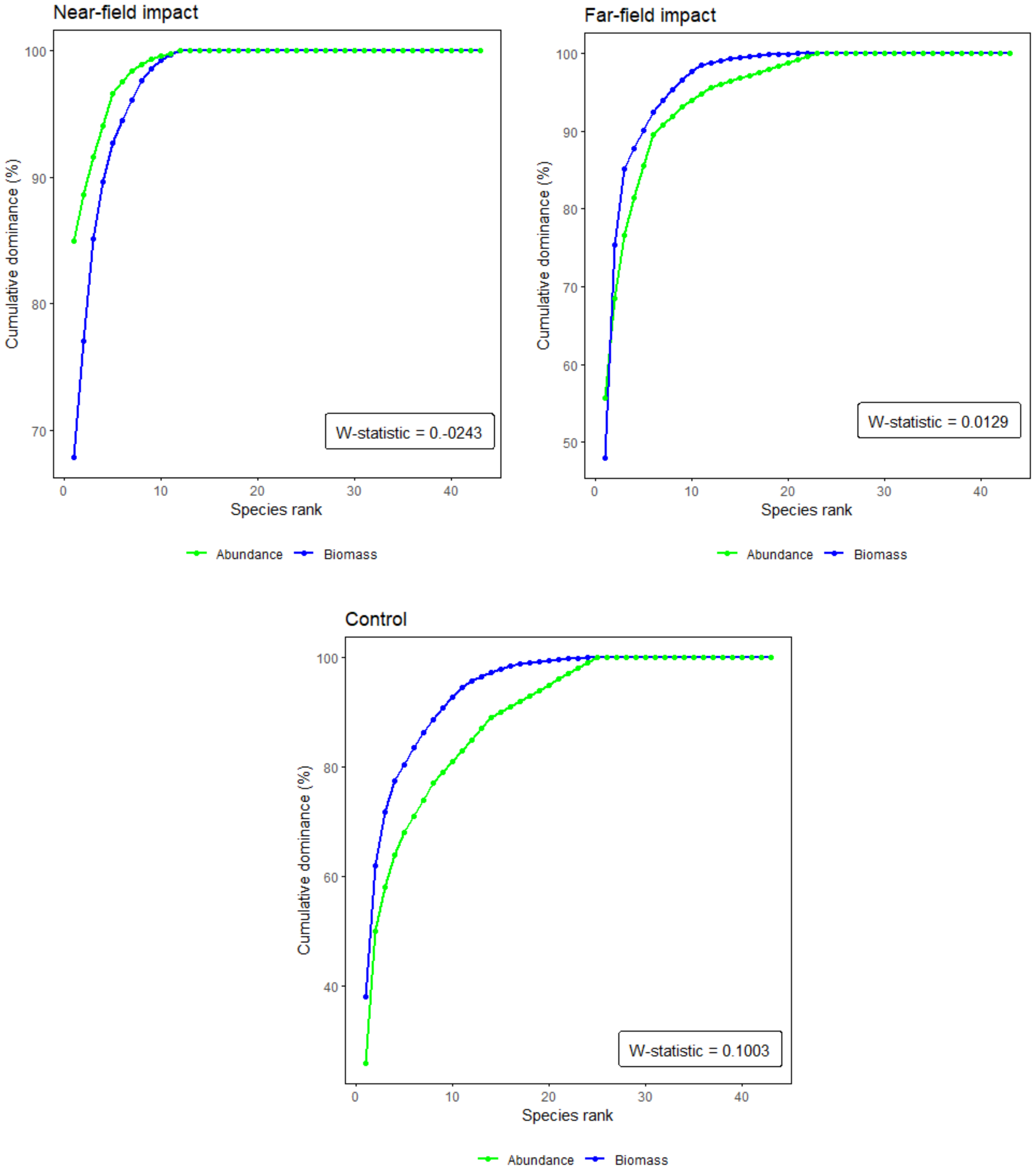

Figure 10.1: Abundance-Biomass curves for each sampling area. Top left: Nearfield impact, top right: Far field impact, bottom: Control.