SALDANHA BAY SEA BASED AQUACULTURE DEVELOPMENT ZONE

ANNUAL BENTHIC CHEMICAL SURVEY FINAL REPORT

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ANNUAL BENTHIC CHEMICAL SURVEY

July 2021

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EXECUTIVE SUMMARY

Monitoring of benthic impacts below mariculture installations is international best practice and is being undertaken in Saldanha Bay to validate dispersion model predictions of minimal impact. The WWF South Africa through its Fish for Good initiative is currently implementing a Fisheries Improvement Project with the Saldanha Bay mussel sector (which is designated as a "catch and grow" fishery by the Marine Stewardship Council). WWF (SA) appointed Anchor Research and Monitoring (ARM), to undertake the 2021 benthic chemical monitoring survey linked to the Saldanha Bay Aquaculture Development Zone (ADZ) as per the requirements of the Sampling Plan for prescribed environmental monitoring.

Although there is a wide range of benthic indicators in use by different countries, they all have primary Environmental Quality Objectives of preventing hypoxic or anoxic sediment conditions by maintaining a functional benthos beneath the culture structures. Organic deposition and the subsequent decomposition by sediment bacteria increases oxygen demand which can lead to anaerobic conditions in the porewaters of the seabed beneath both finfish and shellfish farms. In severe cases this can lead to oxygen depletion in the water above the sediments, which may have direct impacts on farm operations. Ammonification and sulphate reduction to sulphides occur as typical responses to lowering of the oxygen reduction (Redox) potential. Sediment organic carbon, redox potential (Eh) and total sulphides (S^{2-}) have effectively been used in describing adverse impacts below finfish aquaculture. Furthermore, the inversely related chemical indicators Eh and S^{2-} have been used to classify sediments associated with fish farming into five organic enrichment groups: two oxic, two hypoxic and one anoxic. The Aquaculture Stewardship Council (ASC 2017) specifies a S^{2-} thresholds of < 1500 µM (or Eh > -50 mV) as the acceptable threshold beyond the Acceptable Zone of Effect (AZE). The benthic AZE is defined as 30 m from a fish cage array unless a site-specific zone of impact has been established. The Saldanha Bay ADZ Protocols for Environmental monitoring (commonly referred to as the Sampling Plan) proposed that this threshold is adopted for Saldanha Bay fish farm sites as the threshold outside the AZE. It has been suggested that that an additional S^{2-} threshold concentration of >3000 µM (or Eh < -100 mV) be applied at the position of the finfish cages (DAFF 2018). For shellfish aquaculture sites the Sampling plan recommended that S^{2-} threshold concentration of >3000 µM (or Eh < -100 mV) be adopted for annual monitoring of site condition in the shellfish aquaculture zones (ASC 2012). Failure to meet S^{2-} thresholds of 1500 µM (Eh of -50 mV) at the AZE limit for finfish farms or 3000 µM (Eh of -100 mV) at finfish cages or directly below shellfish longlines will require management intervention and/or additional sampling (DAFF 2018). Non-compliance is dependent on the farm or AZE station being significantly greater than levels measured at the reference stations.

There has, however, been some recent research on the measurement of total dissolved sulphides in organically enriched marine sediments below aquaculture infrastructure. Two studies demonstrated that the commonly used ion-selective electrode method for determination of free sulphides in a sediment slurry can lead to significant positive bias (Brown *et al.* 2011, Cranford *et al.* 2020). Brown *et al.* (2011) reported orders of magnitude higher sulphide concentration detected in the buffered sediment–porewater slurry using the ion-selective electrode method than in porewater samples isolated and analysed separately using the methylene blue method (as used in this study). Cranford *et al.* (2020) compared three methods of measuring sulphide in marine sediments (methylene blue colorimetric, direct ultraviolet spectrophotometry and ion selective electrode) and found good agreement between the former two methods and the same positive bias with the latter method.

These authors empirically compared the relationships between total free sulphide in marine sediment (measured using direct ultraviolet spectrophotometry) with several macrofauna indicators and developed a set of revised Ecological Quality Status (EQS) categories. For this study, sulphide concentration was determined by the Council for Scientific and Industrial Research (CSIR) using the methylene blue colorimetric method and we have applied the revised EQS categories to the interpretation of sulphide results, rather than the equivalent Hargrave *et al.* (2008b) geochemical categories.

Sediment was successfully collected by divers at 27 sites within the ADZ during March and April 2021 (14 Sites in Big Bay, 7 in Outer Bay North and 6 within Small Bay; Figure 2 and Table 2). Triplicate redox and sulphide measurements were quantified at each site.

Redox potential for all three sample points along the finfish transect were all negative, but only the 0m monitoring site was significantly lower than the specified threshold (-100 mV). These values are noticeably different to those recorded in the previous survey, despite cage structures not being place (i.e. still represent baseline conditions). Although, the monitoring site at 0m was found to be not significantly different to the reference sites in Big Bay. Within the finfish precinct, average redox potential was -128.56 mV, placing it within the Hypoxic B category. Consequently, sulphide measurements are expected to range between 500 and 1100 µM, however redox measurements and sulphide concentrations were not consistently correlated, and this was not the case with the average sulphide concentration measured in FF samples being 82 µM equivalent to a “Good” EQS or Oxic B category. Monitoring sites within the finfish area all had significantly lower sulphide values in comparison to the specified thresholds (i.e. 250 and 500 µM).

Sites within the Big Bay lease area all had negative redox values of which majority fell into the Hypoxic A category (sites B1, B2 and B6-B8) and the rest placed into the Hypoxic B category (i.e. B3-B5). Only sites B4 and B5 recorded values that significantly exceeded the threshold specified for bivalve aquaculture (-100 mV). However, ANOVA indicated no significant differences in redox values among these sites. All sites within Big Bay had significantly low sulphide measurements compared to the “Moderate” EQS or Hypoxic A category threshold (250 µM). With the exception of site B4, all other Big Bay sites were placed into the High EQS (Oxic A) or “Good” EQS (Oxic B) categories. The average sulphide concentration at the B4 site (600 µM) places the site in the “Poor” EQS (Hypoxic B) category, corroborating the redox result. As there is no farming near those sites, and the redox and sulphide values recorded during the survey did not exceed any thresholds no management actions are currently triggered.

Redox measurements from Outer Bay North were all consistent with negative values being recorded across all impact and reference sites with the exception at NB C2 yielding a positive value (average of 0.67 mV). From these, only sites NB1 and NB3 were significantly below the threshold specified for bivalve aquaculture (-100 mV). Additionally, ANOVA results indicated a significant difference in redox values among sites. Post-hoc results indicated both NB1 and NB3 yielded significantly lower redox values compared to reference station NB C2, but not to reference stations NB C1 and NB C3. With the exception of NB C2, all sites were placed into the Hypoxic B category. The average sulphide concentrations measured in the sediments at all control and impact North Bay sites were below the 250 µM threshold and fell within the “Good” sediment EQS.

Redox values surveyed within the Small Bay lease area all had negative recordings. Site SB 1 was the only site to have exceeded the threshold value, but this was not significant; while the rest, along with the reference stations had redox values below -100 mV and were placed in the Hypoxic A or Hypoxic B geochemical categories. Average sulphide concentrations were notably higher than those measured in sediments collected form Big Bay or Outer Bay, but with the exception of the deeper (14m CF~8m) SB C3 site, were classified as "Good' EQS. Interestingly, in the previous 2020 survey, redox values exhibited high variability with site SB1 recording positive redox values and SB2 and SB3 recording negative values (Mostert *et al.* 2020a). Given that none of the average redox or sulphide values measured at the Small Bay impact sites during the 2021 survey significantly exceeded the sediment quality targets target (-100mV or >500 µM for redox and sulphide respectively), there is no need for management action.

Overall, the redox values were consistent across the established ADZ lease areas and fell within -100 mV redox threshold as stipulated by the Sampling plan (DAFF 2018). In instances where thresholds were exceeded (e.g. FF 0m, B4 and B5, NB1 and NB3), redox measurements were not consistently, significantly different from those measured at control sites, whilst sulphide measurements did not exceed equivalent EQS thresholds. Therefore, no management actions are required at the present time but recommendations for future monitoring are provided below. The following provides a summary of key findings from the 2021 chemical survey:

1. Analytical laboratory measurements of sulphide concentrations in sediments were undertaken during the 2021 survey. Recent research indicates that the methylene blue method employed by the contracted laboratory (CSIR) results in sulphide measurements that are considerably lower (and more accurate) than those obtained using and ion-selective electrode protocol (upon which the DAFF Sampling Plan (2018) and Hargrave *et al.* (2008b) Geochemical categories are based). The 2021 sulphide measurements were therefore evaluated against the revised sediment Ecological Quality Standards (EQS) developed by Cranford *et al.* (2020). It is recommended that future ADZ monitoring uses either the ultraviolet spectrometry or the methylene blue methods of sulphide measurement and these revised EQS categories to assess sediment health below mariculture facilities.
2. Redox potential measurements are relatively inexpensive and easy obtain and should continue to be collected alongside sulphide measurements to provide additional information on the state of the benthic environment and allow for comparisons with redox measurements taken to date.
3. Redox potential average measurements in 2021 were consistently negative throughout the sites surveyed and it is suspected that this is largely due to the natural, organically enriched nature of Saldanha Bay and its location within and upwelling region. Collection of sediment samples by divers as opposed to grab sampling (higher risk of oxidation exposure); which yielded highly variable readings in the previous chemical surveys, may have also played a role in the more consistent, negative redox potential readings obtained during the 2021 survey compared to the 2019 and 2020 grab sampling surveys. It is recommended that, when possible, divers are used in preference to grab sampling for the collection of sediment samples.
4. In instances where farming structures fall over hard substrata, redox and sulphide measurements are not considered suitable tools for monitoring the health of the benthic environment as sediment cannot be collected from hard substrata. Alternative means for

monitoring the health of the benthic environment in these areas (e.g. assessment of visual or photo-quadrats) need to be identified and implemented in the future.

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1 BACKGROUND

The Branch Fisheries Management in the then Department of Agriculture, Forestry and Fisheries (now Department of Forestry, Fisheries and the Environment; DFFE), obtained Environmental Authorisation (EA) on 8 January 2018 to establish a sea-based Aquaculture Development Zone (ADZ) in Saldanha Bay. An ADZ is an area that has been earmarked specifically for aquaculture activities with the purpose of encouraging investor and consumer confidence, creating incentives for industry development, to provide marine aquaculture services, manage the risks associated with aquaculture, as well as to provide skills development and employment for coastal communities. The development of ADZs supports the Policy for the Development of a Sustainable Marine Aquaculture sector in South Africa (2007) objective aimed at creating an enabling environment that will promote growth and sustainability of the marine aquaculture sector in South Africa, as well as to enhance the industry's contribution to economic growth. The Branch Fisheries Management has created an enabling environment for the sustainable expansion within the ADZ operations in the existing aquaculture areas in Small Bay, Big Bay and outer Bay North and will further extend operations into Outer Bay South/Entrance Channel. The authorized species for cultivation include both alien and indigenous species of finfish and shellfish, and seaweeds.

Saldanha Bay is the primary area for bivalve production in South Africa, with the majority of national oyster and mussel production to date originating here. As a result of improved opportunities for local mussel import substitution, the opening up of export markets for oysters, and improved access to water and land space through Operation Phakisa Oceans Economy, there is a renewed interest in expanding and fully utilizing the bay for further oyster and mussel production, as well as exploring potential finfish production in the outer, more exposed parts of the bay.

The then DAFF (now DFFE) appointed an Environmental Assessment Practitioner (EAP) to undertake an Environmental Impact Assessment for the establishment of an Aquaculture Development Zone in Saldanha Bay in 2016/2017. Appeals against the authorisation were lodged to the then Minister of Environmental Affairs and the authorisation was upheld as per the letter dated 7th June 2018. As required in terms of the EA, the Branch Fisheries Management appointed an Environmental Control Officer in 2018 and set up a Consultative Forum (CF – a public and industry forum), which has grown to 140 members thus far [1]. The Aquaculture Management Committee (AMC – a government committee) meets every two months to ensure that the implementation of the ADZ occurs in line with the requirements specified in the EA and Environmental Management Programme (EMPr). The Branch Fisheries Management recently published a "Guideline for Bivalve Production Estimates for the Saldanha Bay Aquaculture Development Zone". This document ensures that the production per annum as specified in the EA are upheld by the operators in the ADZ for the first two years after which this will be reviewed and amended based on environmental monitoring. Coupled with environmental monitoring, the adherence to the authorised tonnages should facilitate adaptive environmental management of the ADZ as a whole.

[1] Clark BM, Massie V, Hutchings K, Biccard A, Brown E, Laird M, Gihwala K, Swart C, Makhosonke A, Sedick S, Turpie J. and Vermaak N. 2019. The State of Saldanha Bay and Langebaan Lagoon 2019, Technical Report. Report No. AEC 1841/1 prepared by Anchor Environmental Consultants (Pty) Ltd for the Saldanha Bay Water Quality Forum Trust, September 2019.

The Branch Fisheries Management appointed an independent specialist to compile a Sampling Plan for the ADZ which was reviewed by local and international stakeholders and experts (DAFF 2018). Further work conducted for the ADZ by independent specialists include, Dispersion modelling completed by PRDW, baseline macrofauna sampling done by Capricorn Fisheries Monitoring and macrofauna and physicochemical properties of the sediment analysed by Steffani Marine Environmental Consultant. In 2020, the Branch Fisheries Management appointed Anchor Research and Monitoring (ARM) to compile the ADZ baseline benthic survey report (Mostert *et al.* 2020a) and to conduct the annual redox survey and compile the resulting report (Mostert *et al.* 2020b). The WWF South Africa through its Fish for Good initiative is currently implementing a Fisheries Improvement Project with the Saldanha Bay mussel sector (which is designated as a "catch and grow" fishery by the Marine Stewardship Council). WWF (SA) appointed ARM to undertake the 2021 benthic monitoring survey and conduct the annual benthic chemical surveys of the Saldanha Bay ADZ in 2021 and 2022 in an effort to support the development of the ADZ by fulfilling the requirements as per the Sampling Plan. This report presents the findings of the 2021 benthic chemical survey that was completed at the end of March 2021.

2 INTRODUCTION

Monitoring of benthic impacts below mariculture installations is international best practice and is mandatory in all Salmon growing countries (Black *et al.* 2008). Benthic monitoring is being undertaken in Saldanha Bay to validate dispersion model predictions of minimal impact (PRDW 2017, DAFF 2018). Although there is a wide range of benthic indicators in use by different countries, they all have primary Environmental Quality Objectives of preventing hypoxic or anoxic sediment conditions by maintaining a functional benthos beneath the culture structures (Black *et al.* 2008, PNS 2018). Maintaining functionality is crucial considering the importance of the benthos in promoting organic matter degradation by microbial communities.

Organic matter input from faeces, pseudo-faeces, uneaten feed and fall-off of culture organisms and fouling organisms is the primary source of impact on the seabed by aquaculture (Cranford *et al.* 2012, DAFF 2018). Shellfish feed on naturally occurring plankton populations which may result in an unnatural concentration of organic matter under farm infrastructure, however, this is typically of minor influence beyond the boundaries of the farm (NZMPI 2013). Generally, organic enrichment associated with bivalve aquaculture is less severe compared to finfish culture where artificial feed is used. Nevertheless, organic deposition and the subsequent decomposition by sediment bacteria increases oxygen demand which can lead to anaerobic conditions in the porewaters of the seabed beneath both finfish and shellfish farms (DAFF 2018). In severe cases this can lead to oxygen depletion in the water above the sediments, which may have direct impacts of farm operations as well as impacts on the benthic organisms. Ammonification and sulphate reduction to sulphides occur as typical responses to lowering of the oxygen reduction (Redox) potential (DAFF 2018). The production of sulphide by sulphate reduction is problematic, as sulphide is toxic (Black *et al.* 2008). However, it must be noted that highly organic enriched sediments can occur naturally where inputs from terrestrial or marine sources may be large, resulting in periodic oxygen depletion in sediments and overlying waters in these areas (DAFF 2018).

Sediment organic carbon, redox potential (Eh) and total sulphides (S^{2-}) have effectively been used in describing adverse impacts below finfish aquaculture (Hargrave 1994). Furthermore, the inversely related chemical indicators Eh and S^{2-} have been used to classify sediments associated with fish farming into four organic enrichment groups; normal, oxic, hypoxic and anoxic (Wildish *et al.* 2001). Oxic sediment typically has a high concentration of oxygen allowing aerobic respiration to occur, while in hypoxic conditions the amount of dissolved oxygen is limited but aerobic respiration continues although in a limited capacity (Diaz and Rosenberg 1995, Gray *et al.* 2002). Under anoxic conditions there is little to no oxygen available for aerobic respiration and anaerobic respiration takes over (Diaz and Breitberg 2009). Subsequently the classification was expanded into five groups with slight adjustments of the geochemical threshold levels, incorporating two oxic and two hypoxic categories as well as the anoxic category (Cranford *et al.* 2006, Hargrave *et al.* 2008a, Hargrave *et al.* 2008b). Each of the five defined categories has defined Eh and S^{2-} thresholds (Table 1). The inverse relationship between Eh and S^{2-} has proven to be comparable between both finfish and bivalve aquaculture sites (Cranford *et al.* 2006). Consequently, these chemical indicators provide an effective means of determining organic matter enrichment and oxic status of seabed deposits for both finfish and shellfish aquaculture operations.

There has, however, been some recent research on the measurement of total dissolved sulphides in organically enriched marine sediments below aquaculture infrastructure. Two studies demonstrated that the commonly used ion-selective electrode method for determination of free sulphides in a sediment slurry can lead to significant positive bias (Brown *et al.* 2011, Cranford *et al.* 2020). Brown *et al.* (2011) reported orders of magnitude higher sulphide concentration detected in the buffered sediment–porewater slurry using the ion-selective electrode method than in porewater samples isolated and analysed separately using the methylene blue method (as used in this study). Cranford *et al.* (2020) compared three methods of measuring sulphide in marine sediments (methylene blue colorimetric, direct ultraviolet spectrophotometry and ion selective electrode) and found good agreement between the former two methods and the same positive bias with the latter method. These authors empirically compared the relationships between total free sulphide in marine sediment (measured using direct ultraviolet spectrophotometry) with several macrofauna indicators and developed a set of revised Ecological Quality Status (EQS) categories (Figure 1).

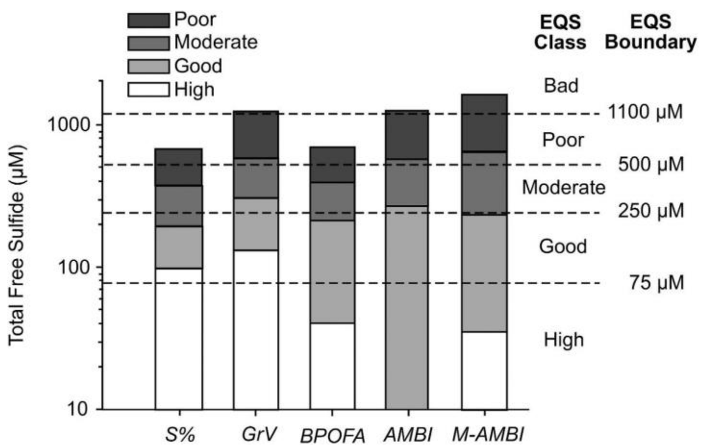


Figure 1 Total free sulphide concentrations and revised ecological quality status (EQS) boundaries for five benthic community indicators (Cranford *et al.* 2020).

The Sampling Plan identified the Aquaculture Stewardship Council's (ASC) thresholds as suitable for monitoring the impacts of finfish aquaculture in Saldanha Bay (ASC 2017, DAFF 2018). The ASC specifies a S^{2-} thresholds of < 1 500 µM (or Eh > -50 mV) as the acceptable threshold beyond the Acceptable Zone of Effect (AZE). The benthic AZE is defined as 30 m from a fish cage array unless a site-specific zone of impact has been established. It has been proposed that this threshold is adopted for Saldanha Bay fish farm sites as the threshold outside the AZE. It has been suggested that that an additional S^{2-} threshold concentration of >3 000 µM (or Eh < -100 mV) be applied at the position of the finfish cages (DAFF 2018). For shellfish aquaculture sites it is recommended that S^{2-} threshold concentration of >3 000 µM (or Eh < -100 mV) be adopted for annual monitoring of site condition in the shellfish aquaculture zones (ASC 2012). Failure to meet S^{2-} thresholds of 1 500 µM (Eh of -50 mV) at the AZE limit for finfish farms or 3000 µM (Eh of -100 mV) at finfish cages or directly below shellfish longlines will require management intervention and/or additional sampling (DAFF 2018). Non-compliance is dependent on the farm or AZE station being significantly greater than levels measured at the reference stations. For this study, sulphide concentration was determined by the Council for Scientific and Industrial Research (CSIR) using the methylene blue colorimetric method and we have applied the revised EQS categories to the interpretation of sulphide results, rather than the equivalent Hargrave *et al.* (2008b) geochemical categories (Table 1).

Table 1 Ranges of redox potential (Eh) and total sulphides (S^{2-}) in five sediment organic enrichment categories as indicated in the Sampling Plan (Cranford *et al.* 2006, Hargrave *et al.* 2008b, DAFF 2018) and recommended revised ecological quality standards (Cranford *et al*. 2020).

Geochemical	Oxic A	Oxic B	Hypoxic A	Hypoxic B	Anoxic
Ecological Quality Standard	**High**	**Good**	**Moderate**	**Poor**	**Bad**
Redox (Eh) mV	>100	100 to -50	-50 to -100	-100 to -150	<-150
Sulfides (S^{2-}) µM (Hargrave *et al*. 2008b)	<750	750 to 1500	1500 to 3000	3000 to 6000	>6000
Sulfides (S^{2-}) µM (Cranford *et al*. 2020)	<75	75-250	250-500	500-1100	>1100

3 METHODS

3.1.1 Sample collection

The annual redox survey of the Saldanha Bay ADZ was conducted during the annual Saldanha State of the Bay survey (21st March – 1st April 2021). Sediment samples for the measurement of redox potential and sulphide (S^{2-}) were collected at 27 stations in Big Bay, Small Bay and Outer Bay North. Scientific divers collected triplicate sediment samples at each of the 18 stations where macrofauna were sampled in Big Bay and Outer Bay North. Additionally, triplicate samples were collected at control and impact sites in Small Bay as was previously done in the 2020 rapid synoptic survey (Mostert *et al.* 2020a, Figure 1). In the finfish precinct in Big Bay, three sediment samples were collected at 0m, 30m and 60m along a transect from the edge of the proposed finfish cage location. Sediment samples were collected by the divers in new, 250ml polyethylene plastic jars which were sealed on the seafloor and then placed on ice until aboard the survey vessel. Redox potential was measured using a Hach HQ 40 D portable meter equipped with an IntelliCAL® MTC101 ORP/redox probe from the undisturbed top layer of sediment. Measurements were conducted on the evening of the sample collection day immediately upon opening the sample jars. Photographs of the sediment samples were taken, and the sediment was observed for colour, visible out-gassing and smell. The sulphide samples were placed on ice until they were transferred to shore where they were frozen at -18°C until submission to the CSIR for sulphide (S^{2-}) analysis. During the survey, two new control sample sites at a similar depth to aquaculture infrastructure and impact sites were selected in Small Bay – SB C3 and Outer Bay North – NB C3. The co-ordinates of the sites sampled are included in Table 2 below and shown on the map of Saldanha Bay in Figure 2.

Table 2 Co-ordinates of the chemical survey sites from Big Bay, Small Bay and Outer Bay North, replaced sites are highlighted in red.

Area	Site	Latitude°	Longitude°	Comments
Big Bay	B 1	-33.028808	18.019161	
	B 2	-33.030550	18.022083	
	B 3	-33.039167	18.021183	
	B 4	-33.035367	18.010983	
	B 5	-33.044667	18.014917	
	B 6	-33.043950	18.009850	
	B 7	-33.031920	18.024640	
	B 8	-33.028870	18.022320	
	BC 1	-33.029733	18.007400	
	BC 2	-33.048633	18.001550	
	BC 3	-33.065414	18.020089	
	FF Transect 0m	-33.042419	18.004349	
	FF Transect 30m	-33.042670	18.004450	
	FF Transect 60m	-33.042926	18.004562	
Outer Bay North	NB 1	-33.032617	17.943633	
	NB 2	-33.034417	17.948867	
	NB 3	-33.038433	17.945633	
	NB 4	-33.045200	17.942067	
	NB C 1	-33.037283	17.960267	
	NB C 2	-33.042167	17.953733	
	NB C 3	-33.03834	17.96395	New site selected – 30th March 2021
Small Bay	SB 1	-33.009100	17.964067	
	SB 2	-33.006717	17.967067	
	SB 3	-33.011133	17.969850	
	SB C1 (North Buoy)	-33.019128	17.968656	
	SB C2	-33.006194	17.979093	
	SB C3	-33.010171	17.95587	New site selected – 28th March 2021

Figure 2 **Map of Saldanha Bay showing the stations sampled during the 2021 annual benthic chemical survey of the Saldanha ADZ, control sites are indicated with blue arrows while impact sites are indicated with red arrows. Replacement control sites are indicated with purple arrows.**

3.1.2 Laboratory analyses

Measurements of sulphide (S^{2-}) were undertaken by CSIR in Cape Town with reference to the Standard Methods for Examination of Water and Wastewater (4500-S2−SULFIDE, Methylene Blue Method). Pre-weighed wet sediment is acidified with Nitric acid (HNO_3) in an enclosed reaction vessel in the presence of continuous Nitrogen gas carrier. The liberated Hydrogen sulphide (H_2S) generated during the acidification is carried into receiving Zinc Acetate solution which converts H_2S into insoluble Zinc sulphide (ZnS) precipitate. The Sulphide is then quantified via iodometric titration and final result is based on the original mass of sample used (mg/kg and mmol/kg). There is a concern that the acid volatile sulphide methodology used would result in measurements of total sedimentary sulphide, including the chemically bound component (e.g. FeS) that is not bioavailable, rather than just free sulphide in pore water that is the ecotoxic component (Brown *et al.* 2011). However, this would result in overestimates of the free sulphur in samples and hence is a conservative approach (i.e. sulphide concentration results are likely to indicate poorer sediment quality than in reality).

3.1.3 Statistical analyses

Survey results were tested for significant differences between chemical (redox and sulphides) sample and indicator thresholds (Table 1) and reference station average values according to statistical procedures given in the British Columbia Ministry of Environment protocols for marine environmental monitoring (BCME 2002). Univariate data were analysed using the software package, Dell STATISTICA v.13.

For finfish stations at 30m and 60m from the cages, samples were tested for chemical exceedance by a 1-sample t-test:

Redox: H_0: $\mu \geq -50$ mV; H_A: $\mu < -50$ mV (1-tailed)
Sulphide: H_0: $\mu \leq 250$ µM; H_A: $\mu > 250$ µM (1-tailed)

1. For stations at the fish cages (0 m) samples were tested for chemical exceedance by a 1-sample t-test:

   Redox: H_0: $\mu \geq -100$ mV; H_A: $\mu < -100$ mV (1-tailed)
   Sulphide: H_0: $\mu \leq 500$ µM; H_A: $\mu > 500$ µM (1-tailed)

   a) If there was evidence for exceedance at a particular station, a nested 1-way ANOVA was performed to test for farm (F) and reference stations (R) stations:

      H_0: $\mu F \leq \mu R$; H_A: $\mu F > \mu R$ (1-tailed)

2. Samples collected at the shellfish farm site were tested for chemical exceedance by a 1-sample t-test:

   Redox: H_0: $\mu \geq -100$ mV; H_A: $\mu < -100$ mV (1-tailed)
   Sulphide: H_0: $\mu \leq 500$ µM; H_A: $\mu > 500$ µM (1-tailed)

   a) In the case of an exceedance, a nested 1-way ANOVA was performed as above.

The redox and sulphide measurements are included in Appendix 1. Photographs of the sediment were taken and are included in Appendix 2.

4 RESULTS AND DISCUSSION

4.1 Nature of sediment

On visual inspection none of the sediment was black in colour (Appendix 2). The sediment composition consisted predominately of sand (Dawson *et al.* 2021). Additionally, no strong sulphur odours were detected emanating from the sediment.

4.2 Big Bay

Redox potential for the three sample points along the finfish transect were all negative in the recent survey (Figure 3), but only the 0 m monitoring site was significantly lower (1-sample t-test: $t = -6.21$, $p < 0.05$) than the specified threshold (-100 mV). These values are noticeably lower than those recorded in the previous survey, despite cage structures not being place (i.e. still represent baseline conditions). The average redox measurement at the 0 m monitoring site was, however, not significantly different to the average measurement for the reference sites in Big Bay (1-way ANOVA: $F_{3,8} = 3.04$, $p > 0.05$). Within the finfish precinct, average redox potential was -128.56 mV, placing it within the Hypoxic B category (Table 1). Consequently, sulphide measurements are expected to range between 500 and 1100 µM, however redox measurements and sulphide concentrations were not consistently correlated and this was not the case with the average sulphide concentration measured in FF samples being 82 µM equivalent to a "Good" EQS or Oxic B category (Table 1, Figure 3). Monitoring sites within the finfish area all had significantly lower sulphide values (1-sample t-test, $p < 0.05$) in comparison to the specified thresholds (i.e. 250 and 500 µM).

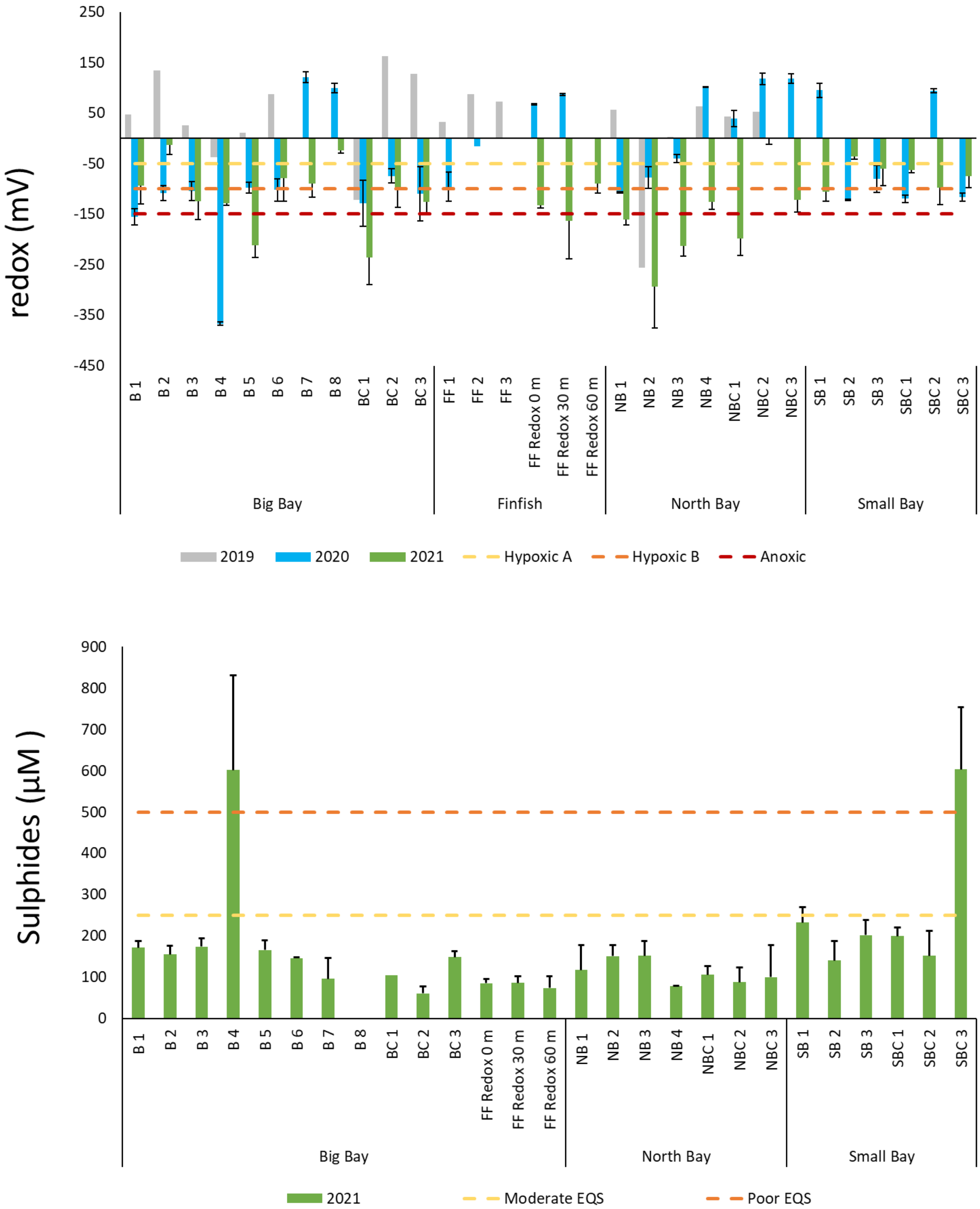


Figure 3 Redox (mV) and sulphide (µM) measurements recorded during the annual 2021 ADZ monitoring survey (bars ± standard error). Included are redox values sampled during the 2019 and 2020 surveys.

The remaining sites within the Big Bay lease area all had negative redox values of which majority fell into the Hypoxic A category (sites B1, B2 and B6-B8) and the rest placed into the Hypoxic B category (i.e B3-B5). Redox measurements for sites B4 (1-sample t-test: $t = -6.68$, $p < 0.05$) and B5 (1-sample t-test: $t = -4.81$, $p < 0.05$) significantly exceeded the threshold specified for bivalve aquaculture (-100 mV). A 1-way ANOVA was used to compare redox values at B4 and B5 to the three reference stations in Big Bay as prescribed in the sample plan (DAFF 2018). However, ANOVA indicated no significant differences in redox values among these sites ($F_{4,10} = 3.39$, $p > 0.05$). Interestingly, site B4 had significantly more negative redox value compared to all the reference stations in the previous 2020 chemical survey (Mostert *et al.* 2020a, Figure 3). Sites B4 and B5 appear to fall in a sandy area among the abrasion platform (calcrete reef) and this area could be a depression in the platform where organic matter may accumulate resulting in high organic loading (see Dawson *et al.* 2021), hence the observed low redox values. Despite the redox values recorded at these sites been below the threshold for bivalve mariculture (-100 mV) the values were not significantly different from the average recorded at control stations and no management actions are currently triggered.

All sites within Big Bay had significantly low sulphide measurements (1-sample t-test, $p < 0.05$) compared to the “Moderate” EQS or Hypoxic A category threshold (250 µM). With the exception of site B4, all other Big Bay sites were placed into the High EQS (Oxic A) or “Good” EQS (Oxic B) categories (Figure 3). The average sulphide concentration at the B4 site (600 µM) places the site in the “Poor” EQS (Hypoxic B) category, corroborating the redox result. However as mentioned above the redox measurement was not significantly different from that recorded at Big Bay control sites and due to high variability in the three sulphide readings for this site, the average concentration was not significantly different from the “moderate” (Hypoxic A) threshold. The other site (B5), with a redox measurement significantly lower than the Hypoxic A threshold (-100mV), had an average sulphide concentration of 166 µM, placing it within the “Good” EQS (Oxic B) category (Figure 3).

4.3 Outer North Bay

Redox measurements from Outer Bay North were all consistent with negative values recorded across all impact and reference sites (Figure 3), with the exception at NB C2 yielding a positive value (average of 0.67 mV). Only sites NB1 (1-sample t-test: $t = -5.61$, $p < 0.05$) and NB3 (1-sample t-test: $t = -5.47$, $p < 0.05$) were significantly below the threshold specified for bivalve aquaculture (-100 mV). Additionally, ANOVA results indicated a significant difference in redox values among sites ($F_{4,10} = 16.68$, $p < 0.05$). Post-hoc results indicated both NB1 and NB3 yielded significantly lower redox values compared to reference station NB C2, but not to reference stations NB C1 and NB C3. In fact, site NB C2 differed significantly to all the sites within the area due to positive redox measurements being recorded at this site. With the exception of NB C2, all sites were placed into the Hypoxic B category (Table 1). Interestingly, in the previous chemical surveys, all the reference stations in the Outer North Bay had positive redox values (Figure 3).

The position of the site NB1 is relatively sheltered with the current directions likely resulting in the deposition of organic matter from the Outer Bay North ADZ in this area (PISCES 2017). It should be noted that the average value recorded at NB1 during the 2019-2021 surveys was 43, -107.13 and -160.53 mV, respectively, suggesting a worsening trend. However, although the redox values recorded at NB1 and NB3 significantly exceed the -100 mV threshold, the sulphide values for these sites averaged 116 µM and 152 µM respectively, near the lower end of the "Good" sediment EQS (Figure 3). Indeed, the average sulphide concentrations measured in the sediments at all control and impact North Bay sites were below the 250 µM threshold and fell within the "Good" sediment EQS (Figure 3). Furthermore, the average redox value at the NB1 and NB3 sites were only significantly different from the NB C2 reference station (which was the outlier), but not from the other two reference sites and hence no management interventions are considered necessary at this stage.

The disparity in redox potential readings at North Bay control sites in the recent survey (negative) compared to the previous years (positive) may be a result of temporal or spatial heterogeneity in marine sediments but is possibly also a result of the different sampling techniques. During the 2019 and 2020 surveys, sample collection and measurement of redox was predominately based on Wildish *et al.* (2004) and Fisheries and Oceans Canada (2015a, b) with the use of a Van Veen grab. During the grab retrieval time from each sampling point to the vessel, there is the risk of sediment being oxidized during its passage through the water column or even the loss of very fine, muddy sediment from the grab (resulting in higher redox measurements); whereas the collection of sediment samples with divers in the 2021 survey, with samples sealed on the seafloor, alleviates any immediate risk of oxidation (and results in more negative readings). Risk of oxidation exposure was only present while measuring redox potential with the handheld probe onshore, but this appears to be minimal given the negative recordings observed.

4.4 Small Bay

Redox values recorded from samples collected within the Small Bay lease area in 2021 all had negative values and fell mostly within the Hypoxic A or Hypoxic B geochemical categories (Figure 3). Average sulphide concentrations were notably higher than those measured in sediments collected form Big Bay or Outer Bay North, but with the exception of the deeper (14m CF~8m) SB C3 site, were classified as "Good' EQS (Figure 3). In 2021, SB 1 was the only site to have exceeded the threshold value for shell fish (-100 mV) , but this was not significant (1-sample t-test: $t = -0.26$, $p < 0.05$); whilst all other Small Bay impact and reference stations had redox values below -100 mV and were placed in the Hypoxic A category (Figure 3, Table 1). Interestingly, in the previous 2020 survey, redox values exhibited high variability with sites SB1 and SB C2 recording positive redox values and the remaining sites recording negative values (Mostert *et al.* 2020a, Figure 3). Given that none of the average redox or sulphide values measured at the Small Bay impact sites during the 2021 survey significantly exceeded the sediment quality targets target (-100mV or >500 µM for redox and sulphide respectively), there is no need for management action.

The high sulphide concentration measured in samples collected at the SB C3 site exceeded 500 µM and places this sediment into the "Poor" EQS category. This is probably a result of it being considerably deeper than the other sites, and Small Bay in particular, is known to experience regular, seasonal hypoxia of near bottom water due to upwelling linked water movements, organic loading and relatively high retention times (Clark *et al.* 2020). The high sulphide concentration recorded at this site is, however, not reflected in the redox readings, which despite being negative was not significantly different from the other Small Bay sites. Indeed, as mentioned above the correlation between redox readings and measured sulphide concentrations was poor (Figure 4). Other studies have also presented figures showing a particularly poor relationship for data in the negative redox potential range between sulphide concentration and redox protentional (e.g. Brown *et al.* 2011, Cranford *et al*. 2020).

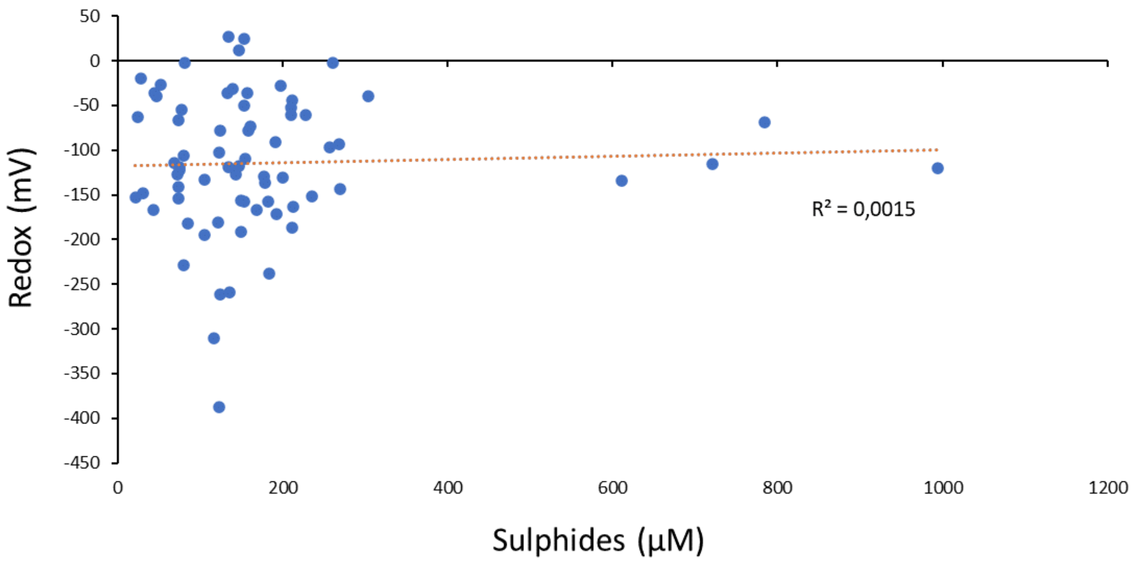


Figure 4 Relationship between measured redox potential and sulphide concentration in sediment samples collected during the 2021 survey of the Saldanha ADZ.

It was previously reported that sources of organic carbon and nitrogen in Small Bay; which include fish factory wastes, biogenic waste from mussel and oyster culture as well as sewage effluent from the wastewater treatment works, in conjunction with the sheltered nature of Small Bay have the potential to influence redox and sulphide readings and should be taken into account when assessing the future redox and sulphide measurements in this precinct (Mostert *et al.* (2020a). This appears to be the case with sulphide concentrations being elevated (albeit not above threshold levels) in Small Bay compared to the other lease areas in Big Bay and Outer Bay North. The entire Saldanha Bay, however, is a highly productive environment with considerable natural enrichment due to the advection of nutrient rich upwelled waters into the sun-warmed and relatively shallow bay. Seasonal (summer and autumn) natural hypoxia of deeper water is associated with upwelling processes and the decay of phytoplankton blooms, and this is reflected in the widespread negative redox values that were observed across all three lease areas within Saldanha Bay.

5 FINDINGS SUMMARY & MANAGEMENT RECOMMENDATIONS

Overall, the redox values were consistent across the established ADZ lease areas and fell within -100 mV redox threshold as stipulated by the Sampling Plan (DAFF 2018). In instances where thresholds were exceeded (e.g. FF 0m, B4 and B5, NB1 and NB3), redox measurements were not consistently, significantly different from those measured at control sites, whilst sulphide measurements did not exceed equivalent EQS thresholds. Therefore, no management actions are required at the present time but recommendations for future monitoring are provided below and should be incorporated into amendments/ updates to the Sampling Plan. The following provides a summary of key findings from the 2021 chemical survey:

1. Analytical laboratory measurements of sulphide concentrations in sediments were undertaken during the 2021 survey. Recent research indicates that the methylene blue method employed by the contracted laboratory (CSIR) results in sulphide measurements that are considerably lower (and more accurate) than those obtained using and ion-selective electrode protocol (upon which the Sampling Plan (2018) and Hargrave *et al.* (2008b) Geochemical categories are based). The 2021 sulphide measurements were therefore evaluated against the revised sediment Ecological Quality Standards (EQS) developed by Cranford *et al.* (2020). It is recommended that future ADZ monitoring uses either the ultraviolet spectrometry or the methylene blue methods of sulphide measurement and these revised EQS categories to assess sediment health below mariculture facilities.
2. Redox potential measurements are relatively inexpensive and easy obtain and should continue to be collected alongside sulphide measurements to provide additional information on the state of the benthic environment and allow for comparisons with redox measurements taken to date.
3. Redox potential average measurements in 2021 were consistently negative throughout the sites surveyed and it is suspected that this is largely due to the natural, organically enriched nature of Saldanha Bay and its location within and upwelling region. Collection of sediment samples by divers as opposed to grab sampling (higher risk of oxidation exposure); which yielded highly variable readings in the previous chemical surveys, may have also played a role in the more consistent, negative redox potential readings obtained during the 2021 survey compared to the 2019 and 2020 grab sampling surveys. It is recommended that, when possible, divers are used in preference to grab sampling for the collection of sediment samples.
4. In instances where farming structures fall over hard substrata, redox and sulphide measurements are not considered suitable tools for monitoring the health of the benthic environment as sediment cannot be collected from hard substrata. Alternative means for monitoring the health of the benthic environment in these areas (e.g. assessment of visual or photo-quadrats) need to be identified and implemented in the future.

6 APPENDIX 1

Table 3 Redox (mV) and Sulphides (µM) measured during the 2019 baseline survey and the 2020-2021 annual chemical survey.

Area	Site	2019	2020			2021					
		Redox	Redox 1	Redox 2	Redox 3	Redox 1	Redox 2	Redox 3	Sulphides 1	Sulphides 2	Sulphides 3
Big Bay	B 1	47	-126.5	-158.5	-180.6	-91.5	-31.3	-157	191.7	139.9	182.6
	B 2	134	-126.1	-78.5	-119.3	-28.2	26.8	-36.6	197.3	134.3	132.7
	B 3	26	-99.7	-140.2	-73.5	-157.5	-163.7	-50.5	153.2	213.5	154.0
	B 4	-37	-367.9	-360.8	-371.4	-134.3	-130.2	-120.1	611.3	200.0	993.9
	B 5	11	-97	-115.6	-80.1	-258.6	-186.3	-191.4	136.4	211.9	149.6
	B 6	88	-123.5	-124.9	-57.9	-126.9	12.1	-123.5	143.6	147.8	
	B 7		137.3	124.7	100.4	-115.4	-35.6	-117.6		45.2	146.7
	B 8		117.1	91.9	88.4	-29.5	-15.8	-28.5			
	BC 1	-122	-37.9	-176.8	-170.7	-167.8	-343.9	-194.5			105.3
	BC 2	162	-67.4	-53.9	-102.2	-55	-87	-167	78.2		43.3
	BC 3	128	-112.7	-201.8	-14.4	-167	-103	-109.5	168.7	122.7	154.2
	FF 1	32	-49.9	-149.6	-88.4						
	FF 2	87	-15.6	ROCK	ROCK						
	FF 3	72	ROCK	ROCK	ROCK						
	FF Redox 0 m		64.9	69.6	66.2	-123	-141	-133	75.0	74.4	105.4
	FF Redox 30 m		89.6	87.8	83.5	-311	-67	-114	117.3	73.3	68.8
	FF Redox 60 m		ROCK	ROCK	ROCK	-127	-78	-63	73.1	124.0	24.7
Outer Bay North	NB 1	57	-105.1	-106	-110.3	-148	-182	-151.6	30.3	85.0	235.4
	NB 2	-256	-35.7	-94.3	-102.5	-388	-362	-130	123.7		178.1
	NB 3	3	-55.7	-31.9	-32.8	-172	-238	-229	192.1	183.8	79.8
	NB 4	63	102.6	99	102.7	-106	-119	-153	79.8	75.6	
	NBC 1	43	39.1	11.3	66.5	-262	-154	-181	123.8	73.3	121.4
	NBC 2	52	100.7	113.7	139	-20	-2	24	28.3	81.3	153.9
	NBC 3		131.2	124.7	100.4	-137	-74	-153	178.3		21.5
Small Bay	SB 1		67.4	108.6	108.7	-143.3	-93.5	-78.7	270.3	268.3	159.0
	SB 2		-122.7	-121		-44.2	-36.4	-27.2	211.9	157.4	52.5
	SB 3		-120	-92.6	-29.7	-2.2	-118.7	-60.5	260.6	135.1	210.5
	SBC 1		-122.9	-130.1	-106	-52	-74	-61.2	210.8	160.8	227.5
	SBC 2		86.7	99.8	96.4	-39.3	-156.3	-96.8	47.6	150.2	256.7
	SBC 3		-108.2		-124	-115.8	-69.4	-40.2	720.6	784.7	304.4
Outer Bay South	JI 1	120	47.1	65.7	68.4						
	JI 2		107.9	106.4	109.8						
	JI 3	79	117.7	117.1	116.9						
	JIC 1	-226	75.3	82.6	77.4						
	JIC 2	-15	113.1	106.1	100.6						
	JIC 3	8	82.7	77.4	91.6						

7 APPENDIX 2


BC 1


BC 2


BC 3


FF 0m


FF 30m


FF 60m


NB 1


NB 2


NB 3


NB 4


NB C1


NB C2

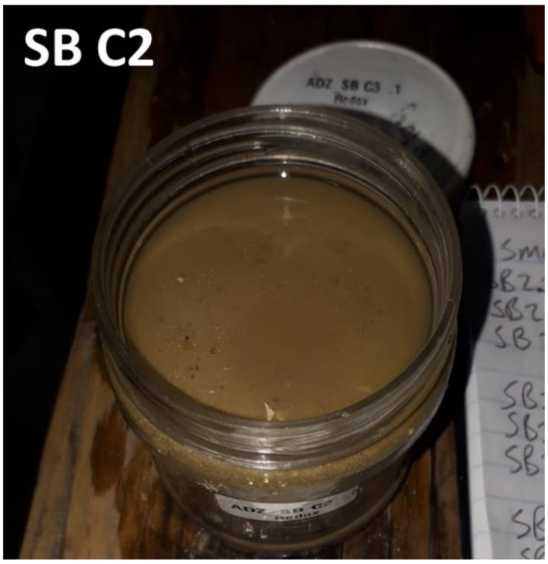

NB C3

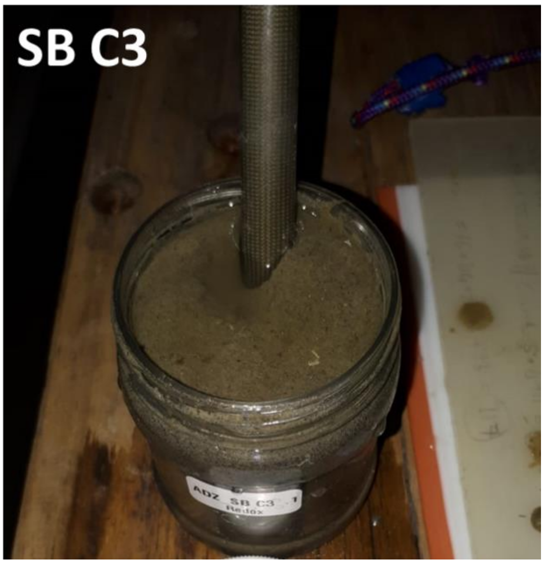

SB 1


SB 2
ADZ_SB2


SB 3
MTC101


SB C1


SB C2


SB C3

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