SALDANHA BAY SEA BASED AQUACULTURE DEVELOPMENT ZONE

ANNUAL BENTHIC CHEMICAL SURVEY DRAFT REPORT

June 2023

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Report Prepared by:
Anchor Research & Monitoring
8 Steenberg House, Silverwood Close, Tokai, South Africa
www.anchorenvironmental.co.za

Authors: Jessica Dawson, Yi-Ting Ho, Ken Hutchings and Barry Clark

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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 2023 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.

There are a wide range of benthic indicators in use by different countries, but 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 and monitoring adverse impacts below finfish aquaculture. 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 threshold target 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. An additional S^{2-} threshold concentration of >3000 µM (or Eh < -100 mV) should 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 30 sites within the ADZ during April 2023 (17 Sites in Big Bay, 7 in Outer Bay North and 6 within Small Bay. Triplicate redox and sulphide samples were analysed for each site.

None of the sediment samples exhibited any visual (black colouration) or olfactory (Sulfurous smell) indication of pour sediment quality and samples were predominantly fine with only a few having coarser/shelly material.

Redox potential for the Big Bay Shellfish farm sites were all lower than those of the Big Bay control sites and the average values of the farmed sites did not differ significantly from the prescribed shellfish threshold value of -100 mV. All finfish sites in Big Bay, except one, were not significantly different from the prescribed threshold. While the FF 0 m site was significantly lower (1-sample t-test: p = 0.03) than the specified -50 mV finfish threshold, it was not significantly lower than the -100 mV threshold and did not differ statistically from the Big Bay control redox values, and is therefore not of concern. These values are lower than those recorded in the previous 2022 survey, but similar to those recorded in 2021 and as cage structures are not in place, they still represent baseline conditions. Although values for farmed and reference sites within Big Bay could be categorized as ranging from ‘Poor’ to ‘Bad’, the sulphide values were significantly better ranging from “Good’ to ‘High’.

Redox potential in Outer North Bay varied, with positive and negative values. However, none of the farm sites were significantly lower than the prescribed threshold and the average redox potential at the farm sites was less negative than the average value in the reference/control sites. Similarly, although two high sulphide values were recorded, one each in a farmed and control site, these were outliers and the average sulphide values did not significantly exceed the prescribed thresholds. With average sulphide values in North Bay in 2023 being very similar to those of 2022, and below the prescribed shellfish threshold of 500 µM.

Within Small Bay, the average redox values for all sites exceeded the Anoxic threshold value of -150 mV, however, only two farmed sites were significantly lower/more negative than the threshold and these did not differ significantly from the average values of the reference and control sites. All Sulphide values in Small Bay were low and fell within the ‘High’/Oxic A category. Given these low sulphide results and the fact that all of the average redox values in both farmed and reference sites exceeded the sediment quality threshold (-100mV) (i.e. there were no significant differences between average Control and Impact site Redox values in Small Bay), there is no need for management action.

The Big Bay site, B 4, previously flagged as being of concern due to high Sulphide and Redox values in 2021 and 2022, had significantly lower values in 2023 than in previous years and fell within the Moderate to Good categories. Suggesting that previous high results may have been the result of temporary and variable sediment and organic matter deposition. And no further management action is required. Despite this, it is still advised that sites B 9 and B 10 should still be sampled in the next annual benthic chemical survey to address the spatial gap in sampling sites downwind of mariculture infrastructure.

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 2023 chemical survey:

1. Analytical laboratory measurements of sulphide concentrations in sediments were undertaken during the 2023 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 recent 2021-2023 surveys used the former methodology and thus it is recommended that future ADZ monitoring uses either the ultraviolet spectrometry or the methylene blue methods of sulphide measurement and the revised EQS categories (Cranford *et al.* 2020) 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. It is recommended that, when possible, divers are used in preference to grab sampling for the collection of sediment samples as this carries a lower risk of oxidation.
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 (this was the case with many of the FF stations in 2022 and the little sand/shell grit available was too coarse for analysis). Sediment samples in 2023 were finer and although readings were obtained and were generally low, it is still advised that alternative means for monitoring the health of the benthic environment in these areas (e.g. assessment of visual or photo-quadrats) should be undertaken.
5. The substantial improvement of sediment quality at site B 4 in the Big Bay precinct, suggests that previous concerns regarding this site may have been unnecessary. However, to support this it is still recommended that the two new sampling sites put forward in the revised sampling plan we sampled in the future to ascertain if the previously poor sediment quality at site B 4 was due to the bathymetry, or if there are wider spatial scale benthic impacts occurring downwind of the bivalve infrastructure.

TABLE OF CONTENTS

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 includes approximately to 140 members. 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 published a "Guideline for Bivalve Production Estimates for the Saldanha Bay Aquaculture Development Zone" in 2019, which has since been updated, and new guidelines added in the “Operational Guideline 2023: infrastructure in the Saldanha Bay ADZ”. This document provides specific acceptable mussel and oyster infrastructure densities (mussel lines, oyster lines and/or mussel rafts) per precinct, along with infrastructure guidelines extracted from the ADZs EMPr that operators in the ADZ are required to uphold. Coupled with environmental monitoring, the adherence to the authorised tonnages should facilitate adaptive environmental management of the ADZ as a whole.

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). This plan has since been updated and amended in 2022 following the outcomes of monitoring studies.

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. To ensure the continued annual monitoring within the Bay, WWF appointed Anchor Research and Monitoring (ARM) to undertake the 2023 sampling concurrently with the Annual State of the Bay sampling. This final report presents the findings of the 2023 benthic chemical survey undertaken in April 2023.

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).

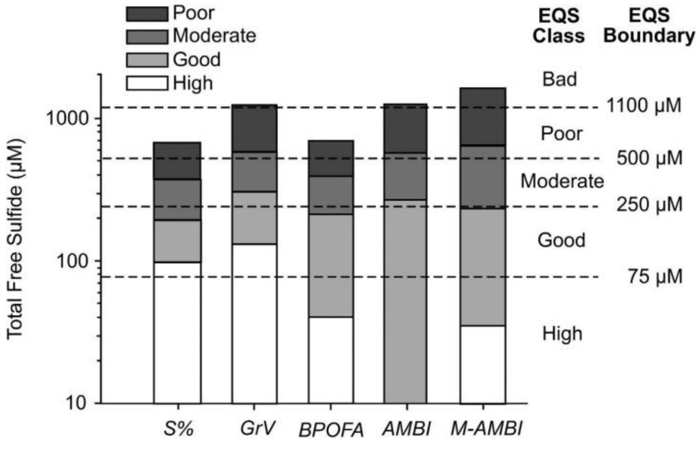

Figure 1 Total free sulphide concentrations and revised ecological quality status (EQS) boundaries for five benthic community indicators (Cranford *et al.* 2020).

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 target 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, DFFE 2022).**

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 (9 – 11th April 2023). Sediment samples for the measurement of redox potential and sulphide (S^{2-}) were collected at 30 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 and the 2021 redox 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 and three samples from within the precinct. 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. The co-ordinates of the sites sampled are included in Table 2 below and shown on the map of Saldanha Bay in Figure 2.

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). It is common practise that when a sample's value is below the laboratory detection limit, a value equal to half the detection limit is reported. In this case, numerous lab results indicated sulphide values were below the detection limit of 60 µM are therefore reported as 30 µM.

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	
	B9	-33.034919°	18.008291°	New sites to be sampled going forward as per 2022 revision of sampling plan. * Not sampled in 2023.
	B10	-33.032928°	18.011506°	
	BC 1	-33.029733	18.007400	
	BC 2	-33.048633	18.001550	
	BC 3	-33.065414	18.020089	
	FF 1	-33.039056	18.002878	
	FF 2	-33.040681	18.007119	
	FF 3	-33.042911	18.004736	
	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

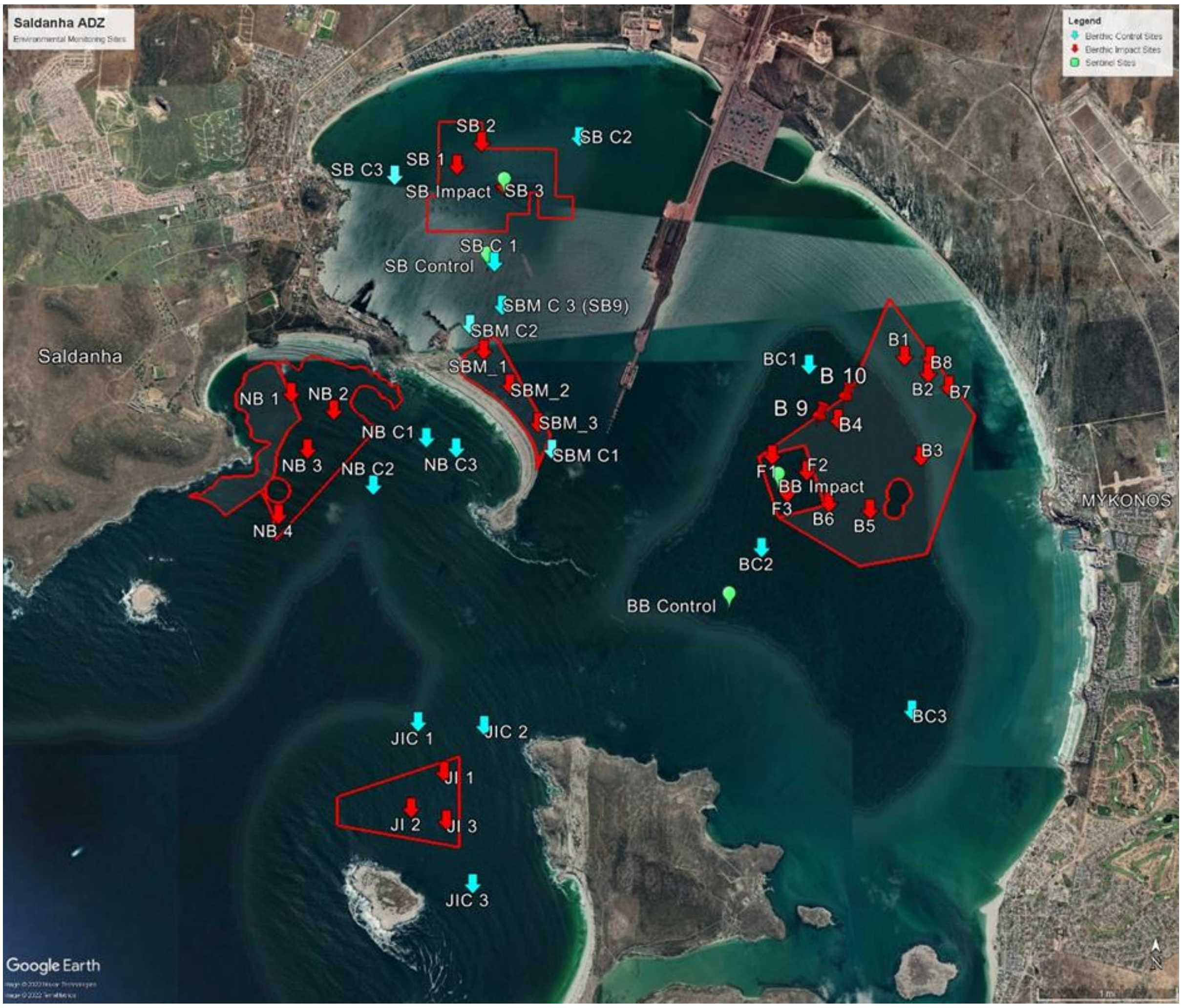

Figure 2 Map of Saldanha Bay showing the stations sampled during the 2023 annual benthic chemical survey of the Saldanha ADZ, control sites are indicated with blue arrows while impact sites are indicated with red arrows. Replacement control sites are indicated with purple arrows.

3.1.3 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)

*Given the no finfish cages are presently located within the Bay – all finfish samples were tested against the -50 mV threshold.

a) If there was evidence for exceedance at a particular station, a non-parametric 1-way Kruskal-Wallis ANOVA was performed to test if the values of farm (F) and reference stations (R) stations differed significantly:

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 non-parametric 1-way Kruskal-Wallis 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). Additionally, no strong sulphur odours were detected emanating from the sediment, yet some samples were course and shelly – see B 5 and BC 2.

4.2 Big Bay

Redox potential for the Big Bay Shellfish farm sites were all lower than those of the Big Bay control sites (Figure 3). Indeed, B 7 and B 8 both had positive redox values, with B 8 having significantly higher (and in this case better) average values than the prescribed threshold, all other sites did not differ significantly from the threshold. Similarly, all Sulphide values for the farm and reference sites were well below the moderate ecological quality standards of 500 µM (Figure 4).

Three sites within the finfish precinct were sampled (FF 1-3) as well as the three samples along a transect 0 m, 30 m and the 60 m, with all yielding negative Redox values. While the FF 0 m site was significantly lower (1-sample t-test: $p = 0.03$) than the specified -50 mV threshold, it was not significantly lower than the -100 mV threshold and did not differ statistically from the Big Bay control redox values and is therefore not of concern. These values are lower than those recorded in the previous 2022 survey, but similar to those recorded in 2021 (Figure 3). Given that fin fish cage structures are not in place, all the data to date represent baseline conditions. Within the finfish precinct, average redox potential was -108.84 mV, which is categorized as a poor/Hypoxic B Ecological Quality standard (Table 1). However, it is worth noting that the average Big Bay Reference site redox potential was even lower, at -221.34 mV, which is considered 'Bad' Quality. This indicates that the low Redox potential in the Finfish precinct is likely to be the result of the natural sediment geochemistry, especially given that no farms are currently operational in this portion of the ADZ. Consequently, sulphide measurements could be expected to range between 500-1100 µM, but were less than detection limits to 102 µM, therefore falling into the Oxic A and B category (Table 1, Figure 4).

In 2022, the redox measurement for site B 4 significantly exceeded the threshold specified for bivalve aquaculture (-100 mV) (1-sample t-test: $t = -29.40$, $p < 0.05$). A 1-way ANOVA was used to compare redox values at B4 to the three reference stations in Big Bay as prescribed in the sample plan (DAFF 2018). ANOVA results indicated significant differences in redox values among these sites ($F_{3,8} = 51.16$, $p < 0.05$). Although no aquaculture infrastructure is located above B 4, it does lie to the north of several longline installations and the prevailing winds during summer are southerly. In 2022 site B4 was therfore flagged as an area of potential concern, with suggestions that an additional 2-3 sites should be positioned along the northern boundary of the Big Bay precinct to ascertain if the poor sediment quality at site B4 is due to the bathymetry, or if there are wider spatial scale benthic impacts occurring downwind of the bivalve infrastructure. Although the revised sampling plan included the collection of samples at two (2) additional sites (B9 and B 10), the 2023 sampling followed the 2018 sampling plan and did not include these two new sites. However, in 2023 both the Redox potential and Sulphide values for B 4 were significantly lower than in previous years and fell within the 'Moderate' to 'Good' categories.

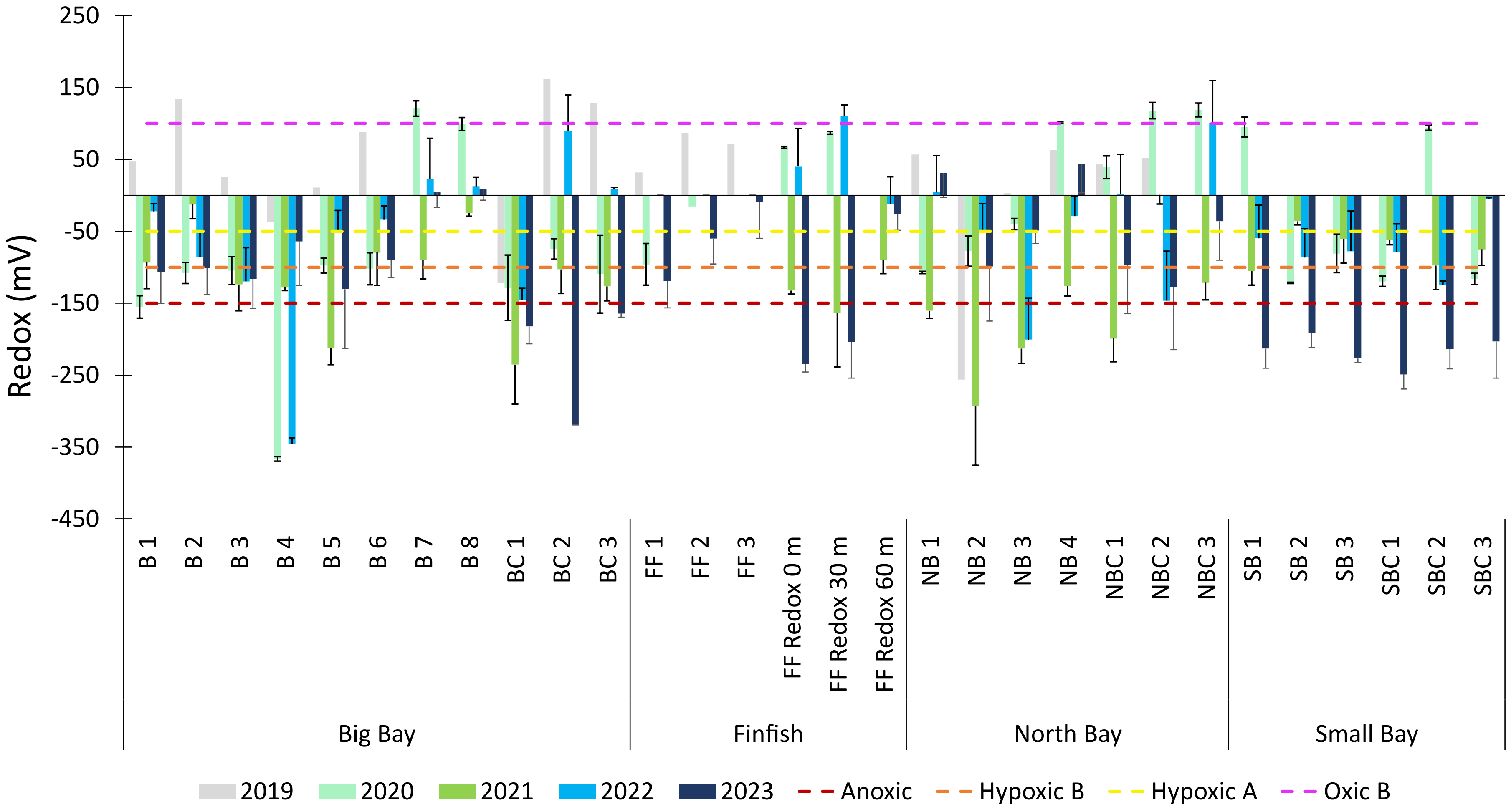

Figure 3. **Redox (mV) measurements recorded during the annual 2023 ADZ monitoring survey (bars ± standard error). Included are historical redox data sampled during the 2019-2022 surveys.**

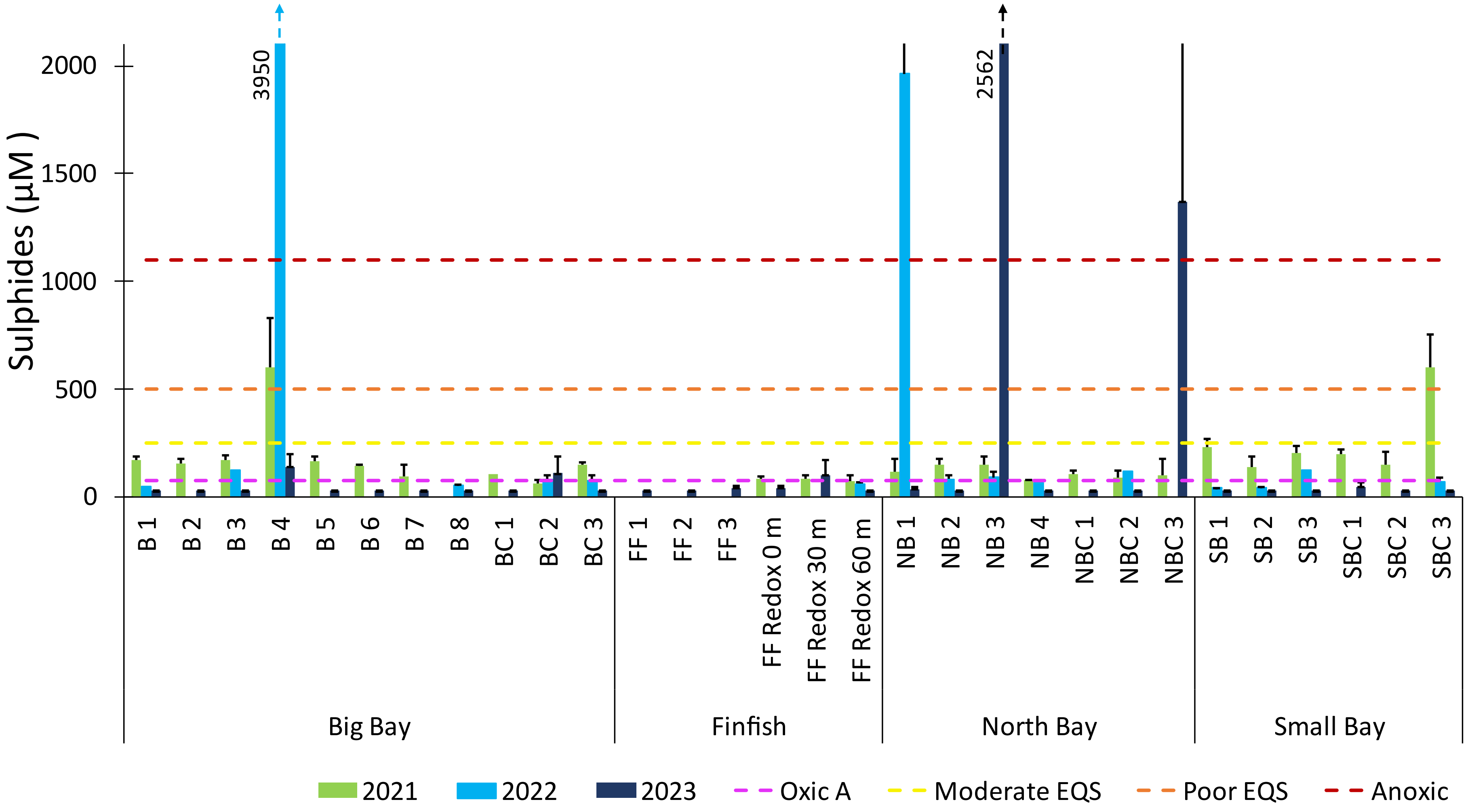

Figure 4. Sulphide (µM) measurements recorded during the annual 2023 ADZ monitoring survey (bars ± standard error). Included are historical data sampled during the 2021-2022 surveys. Arrows next to bars represented continuation irrespective of designated vertical axis scale.

4.3 Outer North Bay

Redox potential in Outer North Bay varied, with positive values recorded at two of the Farm sites, and negative values recorded at the remaining two farm sites and all three reference sites (Figure 3). None of the farm sites were significantly lower than the prescribed threshold and the average redox potential at the Farm sites (-18.05 mV) was less negative than the average value in the reference/control sites (-86.78 mV)(Figure 3). Similarly, the 2023 farm site quality was better than that of the farm sites in 2022, moving from a 'Moderate' category in 2022 to a 'Good'/Oxic B category in 2023 (-18.05 mV in 2023 vs -68.40 mV in 2022).

Conversely the two highest Sulphide values recorded in 2023 were both in North Bay, however, these values were outliers, with all other replicates being below or very close to the laboratory detection limit (Figure 4). The high variance as a result of these outlier values means that these sites did not differ significantly from the prescribed threshold value. In fact, the average sulphide concentration for NB 1 was significantly below the threshold value of 500 µM, and all sites except NB 3 and NBC 3 fell within the High/Oxic A Ecological Quality Standard, making the average sulphide value in North Bay in 2023 very similar to that of 2022 (453 and 464 µM respectively).

4.4 Small Bay

Average redox values recorded within the Small Bay lease area all had negative readings and all exceeded the Anoxic threshold value of -150 mV (Figure 3). However, only the average Redox concentrations of SB 2 and SB 3 were significantly lower than the threshold value (1-sample t-test: $p < 0.05$, for both) they did not differ significantly from the redox values of the reference/control sites within Small Bay. Notably the redox values at all of the Small Bay sites were observed to be lower/more negative than all previous year's results in Small Bay (Figure 3). However, all Sulphide values within Small Bay were low and fell within the 'High'/Oxic A category (Figure 4). Given these low sulphide results and the fact that all of the average redox values in both farmed and reference sites exceeded the sediment quality threshold (-100mV) (i.e. there were no significant differences between average Control and Impact site Redox values in Small Bay), there is no need for management action.

As in all previous years, the correlation between redox readings and measured sulphide concentrations was found to be poor ($R^2 = 0.0565$ (Figure 5). 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, Hamoutene 2014, Cranford *et al*. 2020). Therefore, although it is considerably more expensive, it is still recommended to measure both the Redox potential and then Sulphides within the sediments.

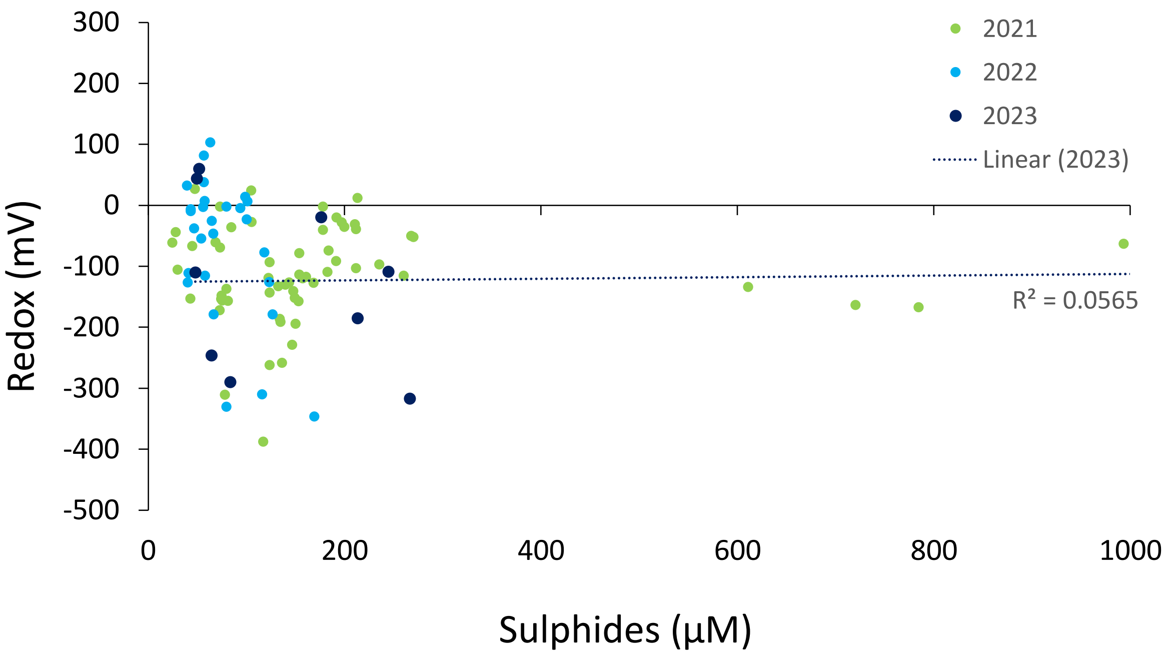

Figure 5. **Relationship between measured redox potential and sulphide concentration in sediment samples collected during the 2021-2023 survey of the Saldanha ADZ.**

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). In 2023, redox values in Small Bay were higher than all previous years. However, all sites were similar with no significant difference between farmed and reference site. Additionally, sulphide concentrations were low and similar 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 with most not significantly different from the prescribed threshold value (-100 mV) as stipulated by the Sampling Plan (DFFE 2022). Any threshold exceedance that did occur were not significantly difference between farmed and reference sites, thus suggesting natural or bay wide impacts as opposed to specific ADZ related impacts. The only sulphide values that exceeded the threshold were outliers (only one out of three replicates in each case) and occurred in both a farmed and a reference site, and are therefore, similarly not of any concern.

The Big Bay site, B 4, previously flagged as being of concern due to high Sulphide and Redox values in 2021 and 2022, had significantly lower values in 2023 than in previous years and fell within the Moderate to Good categories. Suggesting that previous high results may have been the result of temporary and variable sediment and organic matter deposition. And no further management action is required. Despite this, it is still advised that sites B 9 and B 10 should still be sampled in the next annual benthic chemical survey to address the spatial gap in sampling sites downwind of mariculture infrastructure.

The following provides a summary of key findings from the 2023 chemical survey:

1. Analytical laboratory measurements of sulphide concentrations in sediments were undertaken during the 2023 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 recent 2021-2023 surveys used the former methodology and thus it is recommended that future ADZ monitoring uses either the ultraviolet spectrometry or the methylene blue methods of sulphide measurement and the revised EQS categories (Cranford *et al.* 2020) 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. It is recommended that, when possible, divers are used in preference to grab sampling for the collection of sediment samples as this carries a lower risk of oxidation.
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 (this was the case with many of the FF stations in 2022 and the little sand/shell grit available was too coarse for analysis). Sediment samples in 2023 were finer, and although readings were obtained and were generally low, it is still advised that alternative means for monitoring the health of the benthic environment in these areas (e.g. assessment of visual or photo-quadrats) should be undertaken.
5. The substantial improvement of sediment quality at site B 4 in the Big Bay precinct, suggests that previous concerns regarding this site may have been unnecessary. However, to support

this it is still recommended that the two new sampling sites put forward in the revised sampling plan be sampled in the future to ascertain if the previously poor sediment quality at site B 4 was due to the bathymetry, or if there are wider spatial scale benthic impacts occurring downwind of the bivalve infrastructure.

6 APPENDIX 1

Table 3 **Redox (mV) and Sulphides (µM) measured during the 2019 baseline survey and the 2020-2023 annual chemical survey. 2023 and 2023 measurements are continued on the following page.**

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	47.0	-126.5	-158.5	-180.6	-91.5	-31.3	-157.0	191.7	139.9	182.6
	B 2	134.0	-126.1	-78.5	-119.3	-28.2	26.8	-36.6	197.3	134.3	132.7
	B 3	26.0	-99.7	-140.2	-73.5	-157.5	-163.7	-50.5	153.2	213.5	154.0
	B 4	-37.0	-367.9	-360.8	-371.4	-134.3	-130.2	-120.1	611.3	200.0	993.9
	B 5	11.0	-97.0	-115.6	-80.1	-258.6	-186.3	-191.4	136.4	211.9	149.6
	B 6	88.0	-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	-122.0	-37.9	-176.8	-170.7	-167.8	-343.9	-194.5			105.3
	BC 2	162.0	-67.4	-53.9	-102.2	-55.0	-87.0	-167.0	78.2		43.3
	BC 3	128.0	-112.7	-201.8	-14.4	-167.0	-103.0	-109.5	168.7	122.7	154.2
	FF 1	32.0	-49.9	-149.6	-88.4						
	FF 2	87.0	-15.6	ROCK	ROCK						
	FF 3	72.0	ROCK	ROCK	ROCK						
	FF Redox 0 m		64.9	69.6	66.2	-123.0	-141.0	-133.0	75.0	74.4	105.4
	FF Redox 30 m		89.6	87.8	83.5	-311.0	-67.0	-114.0	117.3	73.3	68.8
	FF Redox 60 m		ROCK	ROCK	ROCK	-127.0	-78.0	-63.0	73.1	124.0	24.7
Outer Bay North	NB 1	57.0	-105.1	-106.0	-110.3	-148.0	-182.0	-151.6	30.3	85.0	235.4
	NB 2	-256.0	-35.7	-94.3	-102.5	-388.0	-362.0	-130.0	123.7		178.1
	NB 3	3.0	-55.7	-31.9	-32.8	-172.0	-238.0	-229.0	192.1	183.8	79.8
	NB 4	63.0	102.6	99.0	102.7	-106.0	-119.0	-153.0	79.8	75.6	
	NBC 1	43.0	39.1	11.3	66.5	-262.0	-154.0	-181.0	123.8	73.3	121.4
	NBC 2	52.0	100.7	113.7	139.0	-20.0	-2.0	24.0	28.3	81.3	153.9
	NBC 3		131.2	124.7	100.4	-137.0	-74.0	-153.0	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	-121.0		-44.2	-36.4	-27.2	211.9	157.4	52.5
	SB 3		-120.0	-92.6	-29.7	-2.2	-118.7	-60.5	260.6	135.1	210.5
	SBC 1		-122.9	-130.1	-106.0	-52.0	-74.0	-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		-124.0	-115.8	-69.4	-40.2	720.6	784.7	304.4
Outer Bay South	JI 1	120.0	47.1	65.7	68.4						
	JI 2		107.9	106.4	109.8						
	JI 3	79.0	117.7	117.1	116.9						
	JIC 1	-226.0	75.3	82.6	77.4						
	JIC 2	-15.0	113.1	106.1	100.6						
	JIC 3	8.0	82.7	77.4	91.6						

Area	Site	2022						2023					
		Redox 1	Redox 2	Redox 3	Sulphides 1	Sulphides 2	Sulphides 3	Redox 1	Redox 2	Redox 3	Sulphides 1	Sulphides 2	Sulphides 3
Big Bay	B 1	-38.0	-27.5	-1.4	47.0			-167.0	-20.0	-131.6	<60	<60	<60
	B 2	-17.9	-144.7	-95.1				-134.0	-141.9	-26.9	<60	<60	<60
	B 3	-153.2	-178.7	-27.1		126.9		-172.9	-140.0	-35.5	<60	<60	<60
	B 4	-346.2	-330.7	-359.6	169.2	79.9	11601.5	-19.6	12.7	-185.0	180.0	<60	210.0
	B 5	-25.4	-17.0	-107.3				34.7	-217.7	-208.5	<60	<60	<60
	B 6	-70.9	-23.1	-7.3				-101.9	-125.6	-41.2	<60	<60	<60
	B 7	-35.8	-29.4	135.2				25.7	-17.1		<60	<60	<60
	B 8	-3.1	2.6	37.8	56.1		57.1	13.4	34.9	-20.4	<60	<60	<60
	BC 1	-137.7	-177.0	-122.4				-217.5	-193.2	-136.0	<60	<60	<60
	BC 2	81.4	180.0	6.4	56.6		101.3	-320.9	-315.1	-316.9	<60	<60	270.0
	BC 3	13.6	5.7	7.0	98.9		57.5	-153.3	-171.2	-168.0	<60	<60	<60
	FF 1							-153.6	-159.4	-43.8	<60	<60	<60
	FF 2							6.6	-113.6	-73.1	<60	<60	<60
	FF 3							-109.7	43.9	37.1	<60	<60	<60
	FF Redox 0 m	-66.6	100.4	86.0				-223.7	-245.9		<60	60.0	<60
	FF Redox 30 m	140.5	91.3	100.0				-278.5	-108.9	-224.4	<60	240.0	<60
	FF Redox 60 m	-54.3	-46.3	63.6	54.1	66.6		-30.9	15.8	-62.3	<60	<60	<60
Outer Bay North	NB 1	-38.1	9.3	-115.7	3870.7		58.0	70.9	-37.4	59.9	<60	<60	50.0
	NB 2	103.1	-68.2	-22.9	63.5		100.4	-54.0	-247.4	5.6	<60	<60	<60
	NB 3	-310.0	-179.0	-112.8	116.3	66.8		-14.8	-53.3	-78.0	<60	<60	7630.0
	NB 4	-25.4	-78.9	17.8	64.8			91.7	76.5	-36.3	<60	<60	<60
	NBC 1	81.2	-105.1	28.4				13.1	-221.1	-82.0	<60	<60	<60
	NBC 2		-77.5	-215.1		118.6		-82.0	-295.7	-5.4	<60	<60	<60
	NBC 3	162.9	156.0	-16.2				-5.4	38.8	-141.3	<60	4050.0	<60
Small	SB 1	32.4	-111.1	-100.1	39.8	41.2		-206.8	-263.4	-169.0	<60	<60	<60

Area	**Site**	**2022**						**2023**					
		Redox 1	**Redox 2**	**Redox 3**	**Sulphides 1**	**Sulphides 2**	**Sulphides 3**	**Redox 1**	**Redox 2**	**Redox 3**	**Sulphides 1**	**Sulphides 2**	**Sulphides 3**
	SB 2	-126.8	-6.7	-125.6	40.4	43.3		-188.0	-227.9	-157.6	<60	<60	<60
	SB 3	-126.2	-140.6	33.9	123.6			-222.4	-220.7	-237.4	<60	<60	<60
	SBC 1	-124.7	-1.4	-110.6				-225.8	-231.2	-289.9	<60	<60	84.0
	SBC 2	-119.5	-118.3	-135.2				-218.3	-258.8	-164.4	<60	<60	<60
	SBC 3	-9.4	-4.7	-2.0	43.7	94.1	79.8	-160.1	-304.8	-144.9	<60	<60	<60
Outer Bay South	JI 1												
	JI 2												
	JI 3												
	JIC 1												
	JIC 2												
	JIC 3												

7 APPENDIX 2


SB 2


SB 3


SBC 1


SBC 2


SBC 3


FF 0m


FF 30m


FF 60m


FF 1


FF 2


FF 3


NB1


NB 2


NB 3


NB 4


NBC 1


NBC 2


NBC 3

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