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Protocols for Environmental Monitoring of the Aquaculture Development Zone in Saldanha Bay, South Africa

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Contents

1. Background to the Aquaculture Development Zone

The then Department of Agriculture Forestry and Fisheries (DAFF) established an Aquaculture Development Zone (ADZ) in January 2018 in Saldanha Bay with the aim of streamlining the expansion of farming operations and promoting investor confidence in the sector. The ADZ expands on existing aquaculture areas in Small Bay and Big Bay and extends operations into Outer Bay (Figure 1). The authorized species for cultivation include both alien and indigenous species of finfish and shellfish, and seaweeds. The proposal has been subject to a Basic Assessment (BA) process and a final Basic Assessment Report was produced (SRK 2017a). Through stakeholder engagement and in mitigation of the proposed scale of operations, the original area considered for new development in the ADZ was reduced considerably from 1 404 ha to 420 ha giving a total area of 884 ha including existing areas allocated for aquaculture. Most of the total area allocated to aquaculture is for shellfish farming as only 29% of the ADZ is regarded as suitable for finfish (SRK 2017a).

Mitigation and monitoring actions during the design, construction, operation and decommissioning phases are clearly specified in the EMPr and will largely be implemented by the developer/farmer and overseen by the Aquaculture Development Zone Management Committee (AMC) constituted according to Section 13 of the Environmental Authorization (EA). A Consultative Forum (CF) was also established to provide a platform for the public to engage on activities within the ADZ. As the holder of the EA, the Department of Forestry, Fisheries, and the Environment (DFFE, previously known as DAFF and as reflected on the EA) is responsible for implementation of recommendations in the Environmental Management Programme (EMPr) and the present monitoring plan. An Environmental Control Officer (ECO) was appointed during the construction and operational phases to ensure compliance with stipulations given in the Environmental Authorization and the EMPr (SRK 2017b).

As specified in section 7.1 of the EMPr (SRK 2017b), monitoring required during the operational phase must be undertaken by:

- a specialist appointed by the Branch Fisheries Management of the DFFE and approved by AMC, and
- individual operators.

The AMC/DFFE has oversight of environmental monitoring and through an Environmental Representative (condition 19 of the EA) will:

- liaise with the appointed specialist(s) to ensure environmental monitoring actions/methods are performed according to the EMPr and additional sampling plans;
- receive and review environmental monitoring results to ascertain compliance of aquaculture operators with conditions of the EMPr and EA;
- receive and review monthly Farm Monitoring Reports from individual operators;
- notify the AMC co-chairpersons of issues that require immediate attention of the committee;

- notify the AMC Secretariat of issues that require immediate attention of other aquaculture operators within the ADZ; and
- report on environmental aspects at AMC meetings.

The Marine Ecology Specialist Study (Pisces 2017) discusses many of the generic impacts associated with finfish and shellfish aquaculture as well as those specific to the Saldanha Bay/Langebaan Lagoon ecosystem which provide the focus for the required monitoring actions. The significance of these potential impacts both with and without mitigation measures are provided in the final Basic Assessment Report (SRK 2017a). Discussion regarding the different impacts with reference to the scientific literature is provided in the specialist study. Very briefly, the significant impacts can be categorized as:

- modification of seabed by biodeposition;
- modification of water column dissolved oxygen and inorganic nitrogen;
- removal of seston by shellfish;
- creation of habitat by farm structures;
- alteration of behaviour and entanglement of seabirds and marine fauna at finfish sites;
- introduction of aliens and spread of pests;
- transmission of diseases to wild population;
- genetic interaction with wild populations by shellfish and finfish; and
- pollution by therapeutants and trace metals.

Management and mitigation measures that address the above concerns for the different phases of the proposed ADZ development are provided in the final Basic Assessment Report (SRK 2017a) and EMPr (SRK 2017b). A number of environmental mitigation measures proposed for the operation phase in the Basic Assessment Report (BAR) are realistically addressed in the design and planning phase and will not be considered in detail in the present recommendations for monitoring. These are concerned mainly with appropriate siting, buffer zones, production limits through phasing and farm footprints, use of predator nets etc.

The scope of this document encompasses a sampling/monitoring plan to address the concerns related to impacts on the marine ecology of the Saldanha Bay/Langebaan Lagoon system during the operational phase of the ADZ, as specified in Section 7.2 of the Environmental Management Plan (SRK 2017b). Mitigation measures related to ecological concerns raised by potential genetic and biosecurity impacts are outlined in the Environmental Management Plan (EMPr) and referred to briefly in Section 11-13. Monitoring actions are intended to address both farm-scale and far-field impacts (Bay scale) and will be guided by the recommendations in the EMPr and the findings of the hydrodynamic model (PRDW 2017).

2. Introduction to the marine ecology monitoring plan

The primary aim of ecologically focused monitoring is to assess whether an activity or activities are having an unacceptable impact on the environment (Fernandes et al. 2001). Ultimately an area designated for aquaculture may be able to be used indefinitely i.e. in a sustainable manner. Potential use of the environment as a means of disposing waste is included under sustainable use of the environment. However, such use must be localized, short-term and reversible (Fernandes et al. 2001). Important in this context is the assimilative capacity or ability of the environment to absorb and process wastes without damage to the ecosystem. GESAMP (1996) suggest a working definition of monitoring as `*the regular collection, generally under regulatory mandate, of biological, chemical or physical data from predetermined locations such that ecological changes attributable to aquaculture wastes can be quantified and evaluated*'. Monitoring should be informed by valid research such that adequate methodologies and appropriate variables are employed. However, situations may arise where the scientific requirements for monitoring are often tempered by practical limitations such as time scale and finances. Ideally the monitoring programme design should be able to distinguish between natural background variability and real change in the indicator variable. Central to most monitoring programmes is the assessment of environmental status against a control or reference condition as an indication of environmental change.

The objectives (purpose) of any monitoring exercise are (Fernandes et al. 2001): '(i) to provide information on the area where the operation will take place (pre-operational); (ii) to provide information on which management guidelines/recommendations can be based (pre-operational); and (iii) to act as a `temporal and spatial control (operational and post-operational) upon which remedial action might be based'. A key issue is that monitoring actions are not intended solely to document changes as/if they occur but should aim to promote avoidance and mitigation of significant negative impacts on the environment through timely management responses by industry and the regulator (Cranford et al. 2006). A common approach in achieving these goals is the determination of environmental quality objectives so that the environment can be managed in a sustainable manner (Day et al. 2015). Environmental quality standards are then set for specific variables such that the objectives can be attained (GESAMP 1996). Inherent with this approach is the concept of a mixing zone or allowable zone of effect (AZE) where standards may not be met (SEPA 1998, Day et al. 2015).

A monitoring plan should consider the appropriate variables to be sampled for the farming operation and receiving environment. The indicator variables and measurement approach should be able to detect changes over the appropriate temporal and spatial scales. Relevant spatial scales encompass, local (directly at culture structure, lease area, bay (coastal embayment or management area), and regional (broader coastal areas of similar environmental conditions) (Cranford et al. 2006). Temporal conditions should address relevant scales of natural variability and generation times of critical organisms. There are multiple variables that can be sampled, generally falling under three categories: physical (e.g., sediment grain size, wind, currents), chemical (e.g., redox potential, pH, dissolved oxygen), and biological (e.g., species abundance and diversity, productivity). These variables, singly or in combination, form the basis of ecological indicators that aim to provide information on ecosystem status and the impact that aquaculture activities have on ecosystems to regulatory authorities, industry and the public (ICES 2009). Indicators need to be quantifiable and able to detect change over the appropriate temporal and spatial scales, i.e., representative of the system in question. Generally, a suite of environmental impact indicators are considered in industry operational monitoring programmes and the performance of the industry is based on the accumulated evidence ('weight-of-evidence'). Such an approach will require specific thresholds to be predetermined that initiate prompt action on the part of the regulators and operators. Where such thresholds have yet to be determined, an alternative approach (surveillance) attempts to determine if there are detectable differences between aquaculture and control sites, or significant changes over time that cannot be attributed to natural variations (Cranford et al. 2006). Inherent in a recommended monitoring framework is the basic principle that such programmes are reviewed frequently and allow flexibility to add or remove indicators based on the evolution of our state of knowledge.

Certain target areas may require special considerations that could impose specific requirements for variables to be sampled. Sampling design considerations include the number of samples, location and replication. Measurement variation can become a consideration particularly in situations where sampling is difficult to undertake, such as in deep benthic environments. Costly use of time and resources should not be expended on perfecting each measurement, especially where this would lead to a sacrifice in the number of samples taken (Foster et al. 2018). The number and location of sampling sites will depend largely on the hydrography, specific objectives and, as a practical limitation, finances. However, it is essential that important habitats and species are adequately addressed. The number of replicates taken within a site should be sufficient to address natural spatial variability and patchiness such that a degree of reliability can be placed on the results. This is of particular relevance where comparison with a reference or baseline condition is intended. Reference stations need to be defined for each location. Stations of similar depth and substratum type to the sites within the proposed aquaculture operation should be chosen. Reference stations should be positioned away from the probable zone of influence of the operation (e.g., upstream of dominant current direction) but within the same broad vicinity (Noble-James et al. 2017). Under no circumstances should reference stations be located close to the aquaculture site, even if they are believed to be 'upstream' (Fernandes et al. 2001).

Where regular monitoring is intended it is essential that information from each survey is comparable. Methodology and assessment criteria need to be established and, where possible, sampling points/area and in some cases, season, should be fixed. The methodologies to be used including data analyses must be specific for the monitoring design and objectives and allow comparisons to be made. Relatively simple statistics (e.g., regression, analysis of variance) may be employed in combination with specific numerical methods that are standard in environmental data analysis. These encompass univariate summary statistics (e.g., species diversity indices) and multivariate techniques such as cluster analysis and ordination (Fernandes et al. 2001). Power analysis can be used to define how many replicates should be taken in order to assess reliably any potential deviations from the baseline. However, when deciding on the number of replicates practical aspects regarding time and labour demands, as well as the nature of the environment, should also be taken into account. For instance, for benthic subtidal communities it is generally regarded that 3 - 5 replicates are sufficient to provide the necessary information (Fernandes et al. 2001). However, other variables such as water column properties (nutrients, phytoplankton) and physio-chemical parameters could require greater replication to address the inherent short-term spatial and temporal variability that characterizes them.

It should be emphasized that ongoing monitoring programmes need to be responsive to new information concerning environmental impacts and indicators thereof, as well as developments in methodological approaches (Cranford et al. 2006). Some monitoring actions and indicators may prove insensitive to the specific aquaculture impacts they are proposed to target, and as such, a waste of resources. Relatively low-risk practices such as shellfish cultivation in exposed environments may require less frequent monitoring than originally proposed in a monitoring plan. Besides issues of compliance, it is important that regular review of monitoring results is undertaken to facilitate an inclusion or removal of indicators, or amendment of monitoring effort, based on an expanding knowledge base.

3. Sites to be monitored

The ADZ identifies 4 sites within the bay system. The areas allocated for either finfish or shellfish farming in each are shown in Table 1.

Table 1: Areas allocated for the finfish and shellfish farming in the ADZ.

Area	Finfish area (ha)	Bivalve area (ha)
Outer Bay North (OB-N)		217
Outer Bay South (OB-S)	96	
Big Bay (BB)	40	394
Small Bay (SB)		163

The OB and BB sites are largely undeveloped and are earmarked for both finfish and shellfish farming (within OB-N and BB). However, there is little interest by industry in farming finfish in OB-N (A. Bernatzeder, pers. comm.) and as such the area is treated as being allocated for bivalves only (Table 1). Aquaculture in SB is restricted to shellfish farming an activity that has been undertaken for decades. The bivalve industry first developed in Small Bay in the 1980s and research on the impacts of mussel raft culture on benthic macrofauna, water and sediment quality and geochemical processes (dissolved oxygen and nutrient fluxes) was undertaken in the 1990s (Stenton Dozey et al 1999, 2001) and more recently over the period 2009-2013 (Probyn et al. in prep.). This research documented the impacts of bivalve culture on benthic macrofauna in Small Bay and in this plan, the Monitoring actions in SB are addressed separately from the other more recently established ADZ sites.

4. Indicators

Although both benthic and water column effects have been highlighted in the marine ecology specialist study (Pisces 2017), the effect on the benthos will provide the major focus of the monitoring campaign as many studies have shown these to be the more severe. International experience has shown that for both shellfish (review by Keeley et al. 2009) and finfish (review by Forrest et al. 2007) the main environmental impact has been a result of sedimentation of biodeposits. Globally, sea-based aquaculture monitoring has generally focused on organic loading to the benthic habitat in the vicinity of the farm. Sediments generally provide a more stable integrator of near-field past and present activities as well as natural processes that assimilate or disperse particulate wastes than do water column measurements (Cranford et al. 2006, ICES 2009). Understanding of seabed organic enrichment effects are relatively advanced which has resulted in the development of effective environmental indicators and scientifically defensible thresholds. Seabed effects can be particularly pronounced for finfish farming as a result of artificial feed additions. However, a simulation model of finfish farms in Saldanha Bay has shown that none of the sites in OB and BB showed any evidence of organic matter accumulation at the farm site for the specified production tonnages of 1000 - 1500 metric tons (PRDW 2017). The model did identify depositional areas in harbours and along the iron ore and oil jetty, as well near Riet Bay at the entrance to Langebaan Lagoon. Sediment build-up, however, was regarded as negligible in terms of anticipated effects on the benthos (PRDW 2017). Although shellfish feed on naturally occurring plankton populations, the concentration of stock within farm infrastructure results in an 'unnatural' localization of effects on the benthos. However, benthic effects of shellfish are typically of minor influence beyond the boundaries of the farm (NZMPI 2013).

Monitoring of benthic impacts is mandatory in all salmon growing countries (Black et al. 2008) and should be undertaken in Saldanha Bay despite the model predictions of minimal impact. Although there is a wide range of benthic indicators in use by different countries, they all have the primary Environmental Quality Objective of preventing hypoxic or anoxic sediment conditions by maintaining a functional, not necessarily pristine, benthos beneath the culture structures (Black et al. 2008, PNS 2018a). Maintaining functionality is crucial considering the importance of the benthos in promoting organic matter degradation by microbial communities.

Copper (Cu) and zinc (Zn) are two metals that are commonly monitored in finfish growing areas. Copper is the primary active agent in most antifouling products applied to submerged farm structures and Zn is a fish health additive included in feed. Some paint formulations also contain Zn as an antifouling agent (Macleod and Eriksen 2009). Both are ubiquitous in the environment and essential trace nutrients for nearly all organisms. However, toxic effects can occur when they accumulate in high concentrations of bioavailable forms. Copper leaching from antifoulants will primarily be present in the dissolved phase but, as a result of its low solubility, is rapidly partitioned to suspended particulate matter and ultimately incorporated in the sediments. In addition, the actual bioavailable fraction of Cu in the dissolved phase can be orders of magnitude lower than total Cu concentration as a result of binding to naturally occurring organic material (Clement et al. 2010). Zinc in uneaten feed and fish faeces will rapidly settle to the seabed. Thus, sediments are the primary concern in the accumulation of Cu and Zn and both are consistently associated with finfish farming operations at environmentally significant levels beneath and adjacent to fish cages (Clement et al. 2010).

The accumulation of both metals is mediated by settlement processes and as a result may be expected to follow the pattern predicted for organic matter (Keeley et al. 2014). Unlike organic matter, however, metals in sediments are neither broken down over time, nor utilized by biota at appreciable rates. Consequently, they may persist for long periods in environments where physical dispersion is limited. Although model simulations for Saldanha Bay suggest very little accumulation of sediments (and their attendant contaminants) at finfish growing sites (PRDW 2017), Cu and Zn should be monitored until sufficient data are collected to indicate contamination by these two metals within the lease areas is minimal. Generally, studies of metal contamination apply normalization techniques as an aid to interpreting measured concentrations (Ho et al. 2012). Normalization to Aluminium (Al) is common practice and is employed in current monitoring efforts in Saldanha Bay (Clark et al. 2017).

Both finfish and shellfish culture release nutrients to the water column which have putative effects on phytoplankton productivity and community structure. However, numerous studies demonstrate very localized to indiscernible effects of suspended finfish culture on the water column through nutrient enrichment or oxygen depletion (see review by Price et al. 2015). Model simulations of dissolved inorganic Nitrogen (N) release from the proposed finfish farms in Saldanha Bay, support these findings (PRDW 2017). Similarly for shellfish, a meta-analysis of the effects of different aquaculture practices on dissolved nutrient levels has shown no significant effect for bivalve culture (Sarà 2007a). Effects on dissolved oxygen and turbidity have largely been eliminated through better management practices, and near-field nutrient enrichment of the water column is usually not detectable beyond 100 m of the farm. Nutrients as indicators of aquaculture impacts is particularly challenging owing to a high natural variability. A compelling explanation for why increased nutrients are often undetectable around fish farms is that they are being rapidly assimilated by phytoplankton. In fact, measures of chlorophyll or other metrics of phytoplankton production provide useful proxies for hypernutrification (Cranford et al. 2006). Evidence for stimulation of phytoplankton growth around fish cages is variable, though most often a direct causal relationship has not been demonstrated (Price et al. 2015). One possible explanation for the lack of a systematic effect on the water column variables around fish farms, is the presence of strong currents in these areas that promote dilution (Huntington et al. 2006). Alternatively, rapid grazing by microzooplankton could keep stimulation of phytoplankton growth in check (Tett et al. 2003).

Shellfish culture has the added effect of stripping plankton from the water column. Both models (Grant et al. 2008) and field studies (Heasman et al. 1998, Petersen et al. 2008) have shown feeding by intense bivalve culture results in a marked depletion of phytoplankton (and other components of the seston) as water moves through the farmed areas. Larger phytoplankton groups are targeted by mussels and oysters resulting in relative enrichment of smaller size classes with possible ecological costs to other components of the ecosystem 'downstream' of the farm (Cranford et al. 2008). Bivalve filtration could out-compete zooplankton for food potentially redirecting energy flow from pelagic to benthic foodwebs (Cloern 2005). Besides such effects on natural foodwebs, intense shellfish culture may have density-dependent, negative feedback effects within the lease areas (Heasman et al. 1998). Given the potential ecosystem shifts that may arise in areas of intense bivalve culture, indicators of size spectrum changes are perceived as being of high value in monitoring shellfish aquaculture growing areas (Cranford et al. 2006, ICES 2009). Of particular ecological concern is the potential reduction in carrying capacity for other filter feeding organisms in Langebaan Lagoon. The shallow lagoon, exchanges water with the relatively nutrient-poor, upper water column of Big Bay (Monteiro & Largier 1999); a depth range where filtration by cultivated shellfish would be most noticeable. As adequately defined operational, quantitative thresholds are not established for phytoplankton measurements, they fall into the surveillance category of indicators. However, this does not preclude their potential usefulness. Cranford et al. (2006) identifies the possible effects of intensive shellfish feeding on pelagic plankton and foodwebs as of particular concern, particularly at the bay scale.

Based on the above, the ecological indicators chosen for monitoring impacts of aquaculture are:

- benthic macrofaunal community species richness and biomass;
- sediment geochemical variables (total sulfides and/or redox);
- visual and odour characteristics;
- surficial sediment geochemical characteristics (total organic carbon and nitrogen (TOC/N), Al, Cu and Zn);
- sediment geotechnical characteristics (size structure, porosity);
- near-bottom oxygen concentration; and
- upper water column chlorophyll concentration (fluorometer and discrete samples).

Other ecosystem indicators that are presently monitored as part of the State of the Bay programme must also be considered in the context of expansion of aquaculture in the bay. These include:

- fish abundance;
- bird breeding success;
- alien species occurrence.

Together with the ongoing State of the Bay benthic fauna studies in Small Bay, Big Bay and Langebaan Lagoon, these provide useful indicators of the state of the far-field ecosystem relative to aquaculture.

5. Indicators and thresholds

5.1 Benthic oxic-anoxic classification

The primary source of impact on the seabed from finfish and shellfish aquaculture is organic matter input from faeces, pseudofaeces, uneaten artificial feed, and fall-off of culture organisms and fouling organisms (Cranford et al. 2012). Sediment organic enrichment effects are generally less dramatic with bivalve culture than with finfish culture where artificial feed is used. Nevertheless, if organic deposition is sufficiently high, decomposition by sediment bacteria can increase the oxygen demand which, if not balanced by diffusive or advective supply, can lead to anaerobic conditions in the porewaters of the seabed of both finfish and shellfish farms. In severe cases this can lead to oxygen depletion in the water above the sediments that may then have a direct impact on farm operations. Ammonification and sulfate reduction to sulfides follow as typical responses to a lowering of the oxygen reduction (redox) potential. The shift to sulfate reduction is critical because the end-product, sulfide, is toxic (Black et al. 2008). It is important to note that highly organic enriched sediments can occur naturally where inputs from terrestrial or marine sources are large. Periodic oxygen depletion in sediments and overlying water may develop in these areas.

Hargrave (1994) has shown that sediment organic C, redox potential (Eh) and total sulfides (S^{2-}) were effective in describing adverse impacts on the benthos from salmon culture. The two inversely related chemical indicators, Eh and S^{2-}, have been used to classify sediments associated with a salmon farm into 4 organic enrichment groups; normal, oxic, hypoxic and anoxic (Wildish et al. 2001). Subsequently this classification has been expanded into 5 groups, with slight adjustment of the geochemical threshold levels, incorporating two oxic and two hypoxic categories (Cranford et al. 2006, Hargrave et al. 2008a, Hargrave et al. 2008b). The separation of the two hypoxic categories is based on the relative proportion of opportunistic species. Each category has defined Eh and S^{2-} thresholds (Table 2). The maximum S^{2-} threshold level for Oxic B conditions (1500 µM) is slightly higher than previously identified as a maximum S^{2-} concentration for oxic deposits (1300 µM) and the range of Eh potentials characteristic of this enrichment group is slightly broader (+100 to -50 mV) than previously proposed (+100 to 0 mV) in Wildish et al. (2001). This inverse correlation between Eh and S^{2-} has been shown to be similar at both finfish and shellfish 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 at both finfish and shellfish farm sites. In New Brunswick, eastern Canada, an extra category is included by separation into three hypoxic regimes, though the oxic to anoxic transition thresholds remain the same as the 5 category scheme (NBDENV 2012).

Research has shown that the method for classifying the ecological quality status (EQS) of organically enriched marine sediments based on total free sulfide concentrations widely used in monitoring programmes, the ion-selective electrode (ISE) protocol, can produce unreliable data. Therefore, it is suggested that an alternate method proposed by Cranford et al. (2020), is a more suitable approach and should be used preferentially. This method, known as the methylene blue (MB) colorimetric method, provides lower and more consistent readings, and therefore the defined thresholds are lower (Table 2). Monitoring of the Saldanha ADZ to date (2019-2022) in terms of this sampling plan has used the MB colorimetric method and the lower thresholds for assessing sediment health. It is recommended that this is continued, but whichever analytical method is employed to measure suphide concentrations in sediments, it is essential that the appropriate EQS thresholds, as provided in Table 2, are applied.

The statistical program, PRIMER, is widely used to determine benthic macrofaunal community indices, in this program the Shannon-Weiner diversity index (H') is calculated using the follow equation:

H’ = - Σipi(In pi) 1F

The Shannon-Weiner threshold value of 3.0, given in the original Sampling Plan (DAFF 2018), was based on previous papers (Hansen et al. 2001, Warwick et al. 2008 & Borja et al. 2009) which used Log_2 in the above equation not the natural log (In) used by PRIMER. The threshold of 3.0 (log_2) is equivalent to 2.1 (In), the latter threshold value should therefore be used in cases where calculation of the ‘H is conducted using the natural log (In).

Similarly, the threshold values for the AZTI Marine biotic Index (AMBI) have also been revised and values slightly adjusted based on results from Cranford et al. 2020 (Table 2).

Table 2: Ranges of redox potential (Eh) and total sulfides (S^{2-}) in 5 sediment organic enrichment categories Cranford et al. 2006, Hargrave et al. 2008b, Cranford et al. 2020. The Biotic index indicators (Borja et al. 2000, 2003) have been included for comparison.

	Oxic A	Oxic B	Hypoxic A	Hypoxic B	Anoxic
Geochemical:					
Redox (Eh) mV	>100	100 to - 50	-50 to - 100	-100 to – 150	<-150
Sulfides (S^{2-}) µM (ion-selective electrode protocol)	<750	750 to 1500	1500 to 3000	3000 to 6000	>6000
Sulfides (S2-) µM (methylene blue method)	<75	75-250	250-500	500-1100	>1100
Biological:					
Shannon-Wiener (H´)	>4	4 - 3	3 - 2	2 - 1	<1
*Adjusted (Ln) Shannon-Weiner diversity index (H’)	>2.8	2.8 – 2.1	2.1 – 1.4	1.4 -1.7	<0.7
Infaunal Trophic Index (ITI)	>50	50 - 25	<25	<25	<5
AZTI Biotic Index	<1.2	1.2 - 3.3	3.3 - 5	5 - 6	>6
Adjusted AMBI (Cranford et al. 2020)	<1.2	1.2 - 3.0	3.0 - 4.8	4.8 - 6	>6
Effect on sediment	Low effects	Low effects	May be causing adverse effects	Likely causing adverse effects	Causing severe damage

Many countries have chemical thresholds for monitoring aquaculture areas, finfish sites in particular, that are aligned with a classification of sediment state into categories similar to those given in Table 2. For example, in Maine S^{2-} levels of 2500 - 6000 µM (Eh 100 to -100 mV) at any sampling station within a salmon AZE (30 m) is treated as a warning level and levels > 6000 µM as unacceptable impact that will require a mitigation plan and schedule for modification of operations (State of Maine 2008). A similar mitigation plan will be required if subsequent monitoring indicates a deterioration in warning level S^{2-} concentrations within the AZE or S^{2-} concentrations >3000 µM at any station beyond the AZE.

Salmon farms on the Pacific coast in British Columbia employ similar regulatory threshold values of 1300 µM and 4500 µM at 125 m and 30 m from the cages (Fisheries and Oceans Canada 2015). If a single sample exceeds the threshold, then an additional survey will be required before re-stocking for the subsequent production cycle (Seafood Watch 2017).

The Irish finfish monitoring thresholds require Eh values to be < -125mV within the AZE and equivalent to reference station values outside the AZE (Irish DAFF 2008). A breach of the required parameters for either chemical or biological indicators will require the operator to provide a benthic amelioration plan that aims to improve the ecology of the benthos in as short a time as possible. Subsequent surveys of the impacted area serve to assess if the amelioration plan has been successful. In Marlborough Sound, New Zealand, proposed S^{2-} thresholds within the zone of maximum effect, i.e., close to fish cages, is < 1700 µM, and for the outer limit of effect (150 - 600 m) concentrations should remain a conservative <290 µM (Keeley et al. 2014). Scotland also applies different thresholds relative to a mixing zone as given in Annex A, SEPA Fish Farm Procedures Manual but, instead of a fixed AZE (25 m), a less arbitrary approach is allowed whereby a site-specific AZE's are determined according to the dispersing nature of the site (Black et al. 2008). This allows larger benthic footprints in areas of high dispersion with the aim of encouraging development in more physically dynamic environments.

Benthic monitoring of finfish culture in the maritime provinces of Canada, Nova Scotia (PNS, 2018a, 2018b) and New Brunswick (NBDENV 2012), does not implement an AZE but is concentrated in the vicinity of cages - along the outside perimeter of cage arrays. If average S^{2-} is < 1500 µM then no further action is required. If S^{2-} falls between 1500 and 3000 µM (hypoxic A) then a mitigation plan will be required by the operator. In the event that S^{2-} exceeds 3000 µM (hypoxic B/anoxic), extra sampling (their Tier II) will be required for an improved spatial delineation of the impacted area and more effectively define the degree of influence. In their scheme, sulfide concentrations indicative of hypoxic (4500 to 6000 µM) or anoxic conditions (>6000 µM) would likely require special authorization (Gomes Consulting Inc. 2010).

5.2 Benthic geochemical indicators

The Aquaculture Stewardship Council specifies a S^{2-} concentration of <1500 µM (or Eh > 0 mV) as the threshold beyond the AZE for salmon farming (ASC 2017). The benthic AZE is defined as 30 m from a cage array unless a site-specific zone of impact has been established. It is proposed that this threshold is adopted for Saldanha Bay fish farm sites as the threshold outside the AZE. It is suggested that an additional S^{2-} threshold concentration of >3000 µM be applied at the position of the finfish cages as is implemented in the Canadian maritime provinces monitoring programmes. Although Eh is still commonly used as an indicator of sediment organic enrichment there are procedural problems with its measurement that led to high variability in certain sediments (Wildish et al. 2004, ACS 2010). Based on these limitations total sulfide should be the variable used to define oxic status. Redox should strictly only be used as a check on S^{2-} measurements - they should be inversely related (Wildish et al. 2004). In the Aquaculture Stewardship Council's monitoring plan for shellfish, an AZE is not specified; sites for measurement of chemical indicators are limited to beneath the farm (ASC 2012). It is recommended that their threshold S^{2-} concentration of >3000 µM is adopted for annual monitoring of site condition in shellfish growing areas (beneath culture areas). This is the Hypoxic A upper limit when measured using the ion selective electrode (ISE) method and is equivalent to 500 µM when measured using the recommended methylene blue (MB) method, as shown in Table 1.

Failure to meet S^{2-} thresholds for classification as Oxic B or higher at the AZE limit for finfish farms; or Hypoxic A or higher at finfish cages or directly below shellfish longlines, will require management intervention and/or additional sampling (see Table 1 for threshold values dependent on measurement technique (ISE or MB). Non-compliance is dependent on the farm or AZE station being significantly greater than levels measured at the reference stations.

While many countries have established sediment quality guidelines for trace metals very few have aquaculture-specific guidelines and sediment monitoring protocols. The Scottish Environmental Protection Agency (SEPA) has derived sediment standards within the AZE that could potentially cause adverse effects (108 mg/kg for Cu and 270 mg/kg for Zn) and probably will have adverse effects (270 mg/kg for Cu and 410 mg/kg for Zn). Outside the AZE the standards are more stringent: 34 mg/kg for Cu and 150 mg/kg for Zn. This approach acknowledges that impacts will be greatest in the vicinity of the farm and is designed to control both the intensity and spatial extent of the impact (Clement et al. 2010). The Australian and New Zealand Environment and Conservation Council (ANZECC 2000) have derived Interim Sediment Quality Guidelines (ISQGs) that are considered appropriate to apply monitoring of benthic conditions at finfish farms (Keeley et al. 2014). The guidelines specify a ISQG-low of 65 mg/kg for Cu and 200 mg/kg for Zn which represents a 10% probability of adverse effects, and a ISQG-high of 270 mg/kg for Cu and 410 for Zn which is regarded as a 50% probability of significant toxicity. These guidelines are applicable to the worst affected areas in the vicinity of a farm (Keeley et al. 2014)

The critical issue regarding the toxicity of metals in the environment is the fraction that is actually bioavailable to organisms. It is likely that the majority of total Cu and Zn in the sediment under fish farms will be bound to sulfides and organic matter rendering them unavailable for uptake by organisms effectively reducing their subsequent toxicity (MPI 2013). Given the likely reduced bioavailabilty of Cu and Zn in the organic rich sediments it is recommended that the SEPA aquaculture-specific probable effects levels (equal to the ANZECC ISQG-high guidelines) are used as guideline limits within the finfish AZE for the total recoverable metal fraction. This is similar to the criteria recommended by Keeley et al. (2014) for finfish in New Zealand and the US National Oceanic and Atmospheric Administration (NOAA) Effects Range Medium for sediments (Macleod & Eriksen 2007). Metal concentrations outside the AZE should conform to the SEPA limits, i.e. 34 mg/kg for Cu and 150 mg/kg for Zn.

Non-conformance of measured values with the above thresholds will trigger additional sampling to facilitate comparison with between baseline data for reference and farm sites with subsequent data. Failure will require management intervention.

5.3 Benthic community impact indicators

A primary objective with farm sediments is that they should contain a high abundance and biomass of bioturbating macrofaunal animals to enhance aeration and carbon degradation (Black et al. 2008). However, the development of oxygen stress within the benthic environment results in well documented responses by the benthic community that acts to reduce the processing of organic matter. These responses by the benthic community to changes in environmental variables such as oxygen supply are often reflected before they are detectable in some chemical properties (Cranford et al. 2006). They form the basis of indicators of benthic environment status and include (Black et al. 2008, Cranford et al. 2012):

- a decrease in species richness and an increase in the total number of individuals,
- a general reduction in most species biomass,
- a decrease in average body size of species,
- a constriction of that portion of the sediment occupied by infauna, and
- a shift in the relative dominance of trophic groups.

Away from the farm, organic input and oxygen demand decrease, benthic faunal assemblages are typified by increased diversity and functionality (Black et al. 2008, Keeley et al. 2014).

Some of the benthic community indicators that are commonly used are given in Table 3. Biodiversity indices are in common usage to describe the diversity of macrofaunal assemblages. However, they should be interpreted with caution and require a good understanding of what these indicator results actually reveal about community changes (Cranford et al. 2012). They should not be employed in isolation but as part of a suite of indices to interpret changes in benthic fauna and the probable causes. Indicator species or trophic indices have been shown to be extremely useful in situations of high organic loading which result in shifts in community structure from filter feeders to deposit feeders and scavengers or from sensitive to more tolerant opportunistic feeders (McKindsey et al. 2011). Studies conducted by Stenton-Dozey et al. (1999) and Stenton-Dozey et al. (2001) showed that mussel rafts in Small Bay, Saldanha Bay, attracted opportunistic deposit feeders and carnivores while stations away from the aquaculture sites had an increased presence of suspension feeders.

Table 3: Indicators of the intensity of benthic community impacts from organic matter deposition from suspended aquaculture (after Cranford et al. 2012).

Indicator category	Indicators
Biodiversity metrics	Index of the number and abundance of species. Includes the Shannon-Wiener diversity index (*H´*) Pielou's evenness index (*j*), Simpson's dominance index (*c*), and Margalef's species richness (*d*).
Indicator species	Highly enriched marine sediments are generally dominated by a few opportunistic macrofaunal species that are tolerant of high organic enrichment and low oxygen

	conditions. The AZTI Marine Biotic Index (AMBI) is calculated based on the relative proportion of 5 species groups (previously classed as being sensitive to opportunistic).
Trophic indices	In highly organically enriched areas, benthic communities are dominated by deposit feeders and scavengers, at the expense of filter feeders. For example, the Infaunal Trophic Index provides a categorization of overall species abundance within different trophic groups in soft bottom communities.
Benthic similarity	Comparison of community structure using multivariate statistics such as ordination and cluster analyses.
Size structure	Most species that are tolerant to organic enrichment belong to families such as the Spionidae, and have a small size. Differential sieving allows separation of fauna into size categories.

It is proposed that the biodiversity indices such as Shannon-Wiener diversity index (*H´*), Pielou's eveness index (*j*) and Margalef's species richness index (*d*) form part of the suite of biological indicators because of their common usage in assessing benthic community status. All three have shown significant differences between salmon farm sites and reference sites (Wildish et al. 2001). The Infaunal Trophic Index (ITI), which is based on a functional feeding type for benthic fauna, is 'highly recommended' for monitoring benthic community impacts (Cranford et al. 2012) and should be included as a key indicator. The relationship of these indicators with the chemical thresholds discussed previously is shown in Table 1. Application of the AZTI Marine Biotic Index (AMBI), originally Biotic Coefficient) will require allocation of the different benthic species found in Saldanha Bay sediments to different categories of sensitivity to organic loading and hypoxia/anoxia. The index has been shown to be broadly applicable to a range of impact sources, including aquaculture and hypoxia, in European coastal waters and estuaries (Muxika et al. 2005).

Multivariate methods provide additional tools to the univariate indices mentioned above to describe community composition data. The two most common multivariate techniques for analysis of community data are cluster analysis and ordination. Cluster analysis, usually in the form of a dendrogram, simply groups entities in terms of species composition. Ordination groups similar samples or species, or both, close together and dissimilar entities far apart in a low-dimensional space. Relationships between community and environmental data may be investigated using these techniques to provide further insight to the ecology and environmental degradation of an area. Although a very powerful interpretative tool for environmental managers, multivariate analysis should be applied with caution as interpretation is at least partially subjective. It should always be used in conjunction with other methods such as univariate analysis (Telfer and Beveridge 2001).

Whereas the annual geochemical sampling targets near-field effects from organic loading at the culture structures, the benthic survey gives an indication of the overall health of the broader lease area. Should monitoring actions show that the benthic fauna composition in a lease area does not meet the oxic thresholds of Shannon-Wiener ≥2.1and Infaunal Trophic Index ≥25, or other similar metrics, then mitigatory actions will be required to return the area to an oxic classification.

5.4 Water column indicators

Extensive shellfish and finfish cultivation has two potentially countervailing effects on chlorophyll concentrations. Nutrient enrichment from both finfish and shellfish farming could promote phytoplankton production and biomass, whereas filter feeding by bivalves will reduce phytoplankton biomass. Thus, measures of chlorophyll concentration alone may be ambiguous. An increase in the proportion of small-sized picoplankton in the phytoplankton community, however, is a good indicator of excessive feeding by bivalves. The fluorometer time series of chlorophyll concentration and proportion of picoplankton in size-fractionated discrete samples should be analysed for obvious trends that could be correlated with the expansion of aquaculture in Saldanha Bay. Although operational thresholds for these indicators are difficult to fully define, they remain highly relevant indicators of habitat and ecosystem status (Cranford et al. 2006).

6. Monitoring survey sites

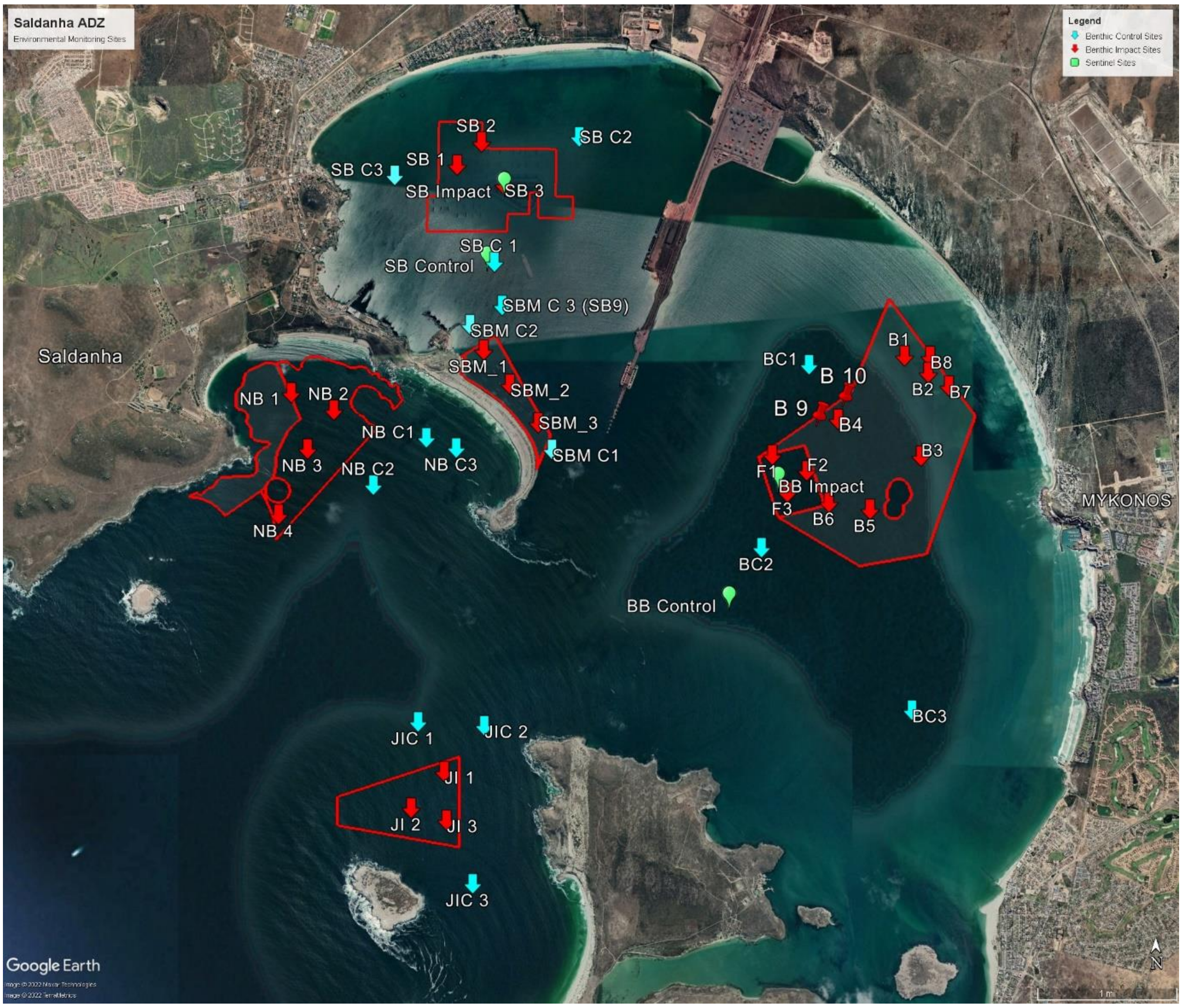

Figure 1: Map of sampling stations for the Saldanha Bay Aquaculture Development Zone showing the benthic chemical (sulphide & redox), sediment (organics, PSD and metals), macrofauna and sentinel (near bottom DO and temperature) sites.

Table 1. Coordinates of the sampling stations for the Saldanha Bay Aquaculture Development Zone.

Area	Site	Latitude	Longitude
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	17.955870
Small Bay (Blue Ocean Mussels)	SBM_1	-33.028207	17.967333
	SBM_2	-33.031754	17.970554
	SBM_3	-33.035732	17.974049
	SBM C1	-33.038483	17.975748
	SBM C2	-33.025602	17.965655
	SB9	-33.023500	17.969833
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	-33.040983	18.013033
	B 8	-33.040497	18.015473
	B9	-33.034919°	18.008291°
	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
Outer Bay North	NB 1	-33.032617	17.943633
	NB 2	-33.034417	17.948867

Area	Site	Latitude	Longitude
	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	-33.038339	17.963950
Outer Bay South	JI 1	-33.071767	17.962450
	JI 2	-33.075517	17.958383
	JI 3	-33.076783	17.962750
	JI C 1	-33.066625	17.959244
	JI C 2	-33.067017	17.967400
	JI C 3	-33.083350	17.965967
Sentinel Sites (Instruments)	SB Control	-33.018900°	17.967750°
	SB Impact	-33.011210°	17.969870°
	BB Impact	-33.041667°	18.003611°
	BB Control	-33.054020°	17.997570°

6.1 Seabed

Although the three-dimensional model simulations for finfish farming in the new areas in BB and OB indicate undetectable accumulation of organic matter at the farm sites (PRDW 2017), these predictions require validation through sampling over the operational phase. An 'ideal' sampling design includes comparison of data from before to after a disturbance that might cause impacts (Underwood & Chapman 2013). A benthic survey should be conducted prior to expansion of aquaculture in OB and BB as a 'before' reference of the area for comparison with the operational or post-operational ('after') phases. Baseline data, if sufficiently detailed and relevant, may be used in place of, or in combination with, reference station data in evaluating results from operational monitoring (State of Maine 2008). Thus, the baseline survey should include replicated, undisturbed control/reference stations that can be used to demonstrate that an impact, if it occurs, is associated with the disturbed area and is not a general phenomenon within the habitat unrelated to the disturbance (Underwood & Chapman 2013). Undisturbed in this instance means isolated from the disturbance being investigated, in the present case organic loading to the seabed from aquaculture, but still exposed to other influences characteristic of the hydrodynamic regime.

Spacing of sampling stations for the baseline study should be stratified random. It is proposed that the number of sample stations should be based on lease area with stratification, i.e. proportional stratification, with a larger sample size (sample unit number) for finfish areas (one per 20 ha) than shellfish areas (one per 50 ha). In this way finfish and shellfish leases are treated as different strata. Currently farmed areas should also be treated as different strata in that at least one station position is located there. If a pre-identified station position is shown to be hard bottomed, the station position should be relocated to the closest point with soft sediment, providing it is within the near vicinity. If large area of rock is found this should be recorded as such. Although confounding the randomness in station positioning, it is deemed necessary as experience has shown that bottom water is often very turbid in the bay precluding reliable photographic/video records - a common means of recording seabed condition in subtidal, hard substratum environments. The rationale for more intensive sampling of finfish sites is based on the reasonable expectation of a potentially greater impact on the benthos from aquaculture in these areas compared to shellfish growing areas. If portions of the lease areas are deemed unsuitable for farming by industry, then sample numbers may be adjusted accordingly. Exclusion of portions of lease areas is most likely for OB where exposure to excessive swell is a practical reality.

The requirement for full macrofauna analyses (both infauna and epifauna), TOC/N, granularity and porosity (Figure 1):

- 10 stations in BB area designated for shellfish farming including at least one in the existing oyster farms (35 ha);
- 3 stations in BB finfish farming area;
- 4 stations in OB-N (based on assumption of only shellfish farming), and
- 3 stations in OB-S. (should this site be developed)
- 6 stations in Small Bay (3 in central area, 3 at rafts along Marcus Island causeway)

Three samples taken from the finfish sites shall also be taken for analysis of the metals Al, Cu and Zn.

Control (reference) stations should be identified; three for each of BB, OB-N,OB-S and six in SB. These should be located in areas where environmental characteristics, such as depth and sediment structure, are as similar as possible to the respective lease areas. They should be located in the same broad vicinity of the farm sites, though at least 100 m from the lease area to minimize potential impact from the farm and at least 100 m apart. Control sites within and/or on the boundary of the marine protected areas around Malgas Island, Jutten Island and Langebaan Lagoon should be considered if they meet the above criteria. Samples must be taken for macrofauna, geochemical (TOC/N, metals) and geotechnical (porosity, granularity) analyses, as above.

Protocols for collecting sediment samples for macrofauna analyses should be maintained within already established monitoring programmes to facilitate comparison with established data-sets (Noble-James et al. 2017). To this end protocols should be aligned with The State of the Bay Programme where 3 replicate samples of 0.08 m^2 and 30 cm deep, where possible, are taken by divers at each station and pooled for subsequent taxonomic analysis of macrofauna in the >1 mm size fraction (Clark et al. 2017). The total sample size is more than double the minimum generally recommended for benthic fauna analysis thereby greatly increasing the likelihood of including rare or sparsely distributed species. Although pooling of replicates for later processing reduces quantification of fine-scale variation, the need to maximize the spatial extent of sampling area in a compliance orientated monitoring programme is prioritized over within station patchiness. This is an acceptable approach (Noble-James et al. 2017) and where resources are adequate should be accompanied by replication at a sub-set of stations (Prezlawski et al. 2018). It is recommended that macrofaunal sampling is replicated at a single station within the three growing areas, BB, OB-N and OB-S. These samples provide information on variability in both small-scale distribution as well as sampling gear performance. While this is not a requirement and depends on practicality, this would equate to three composite samples of approximately 0.2 m^2 at each of the three stations chosen for replication

Sediment geochemical and geotechnical samples should be taken from separate core samples, i.e. they should not be sub-sampled from macrofauna samples. Only the surficial sediment (2 cm) from grabs or diver-collected cores shall be retained for analyses. If multiple samples (replicates) are collected at each station they should be combined and homogenised prior to storing for later analysis (see methods).

This suite of samples is intended to serve as an indication of ecological status at the lease scale prior to major expansion of aquaculture. Significant deterioration at a future date considering both the reference condition and the initial baseline data will initiate a management decision.

In addition to the above, two sentinel stations shall be established one on the boundary of the finfish lease area in Big Bay and one for the intensive shellfish cultivation area in central Small Bay along the main axis of bottom currents. Bottom water oxygen and temperature shall be monitored at sentinel stations and one reference station from each site. Sensors should be moored close to the seabed (<0.5 m off bottom) and programmed for hourly or more frequent readings. Ideally, these stations should be operational prior to development, or as soon thereafter as possible. Significant deterioration in bottom oxygen conditions at either of these sites relative to the reference stations, will initiate further investigation of the extent and magnitude of the effect.

6.2 Water column

As a check on the flow of plankton into Langebaan Lagoon and how it may be affected by aquaculture development (especially shellfish) in the bay, it is proposed that a fluorometer be deployed in the entrance channel. As this is a surveillance approach to monitoring with no specified thresholds, it is important that measures are established prior to major expansion in the bay to assist in interpretation of time series data. These measurements will provide a continuous indicator of phytoplankton abundance (fluorescence) as aquaculture develops within the bay. A long-term commitment to ongoing regular monitoring is critical for establishing time series data which are far superior in identifying directional trends than any combination of independent studies (Noble-James et al. 2017).

It is important that the instrument is readily accessible so that frequent *in situ* calibration samples can be taken at its position for conversion of fluorescence units into chlorophyll concentration. The SAN Parks jetty is a possible option. Calibration will involve regular taking of discrete samples for extracted chlorophyll analyses of both the whole sample and a 2 - 5 µm screened sub-sample. In this way the relative contribution of the small size fraction (picoplankton) to the total phytoplankton community can be measured and the potential far-field effects in the lagoon may be assessed. Sampling should occur during the flood tide to target input to the lagoon.

A minimum of two stations should also be sampled as potential control sites to the SAN Parks jetty site mentioned above for size-fractionated chlorophyll. Frequent water samples (a number of times a week) are currently being taken for phytoplankton identification and enumeration at two sites in the existing shellfish growing areas in the entrance to Small Bay and in North Bay as part of the South African Live Molluscan Shellfish Monitoring and Control Programme. It is recommended that this sampling effort is extended to include discrete samples for size-fractionated chlorophyll analysis. Collection of samples at the three sites should be paired as close as possible in time.

7. Operational surveys

Operational monitoring is to be conducted within the respective lease area following initiation of production. Those areas or sub-areas where farming has not yet started will not need to be surveyed.

7.1 Benthic sampling

a) Chemical investigation (redox (Eh)/sulfides (S^{-2})) at the local scale, three stations; in close proximity to culture structures (0 m), at 30 m which defines the AZE, and at 60 m, along a transect in the direction of prevailing near-bottom currents, i.e., most likely to reflect farm impact. To be conducted annually or during the period of maximum biomass for that production cycle or as close as possible to that time. Additional measurements must be taken at the three reference sites for the respective lease area. A minimum of three replicate grab or core samples are retrieved from each sampling point for S^{-2} (and Eh) measurements in the surficial (upper 2 cm) layer of sediment. Each replicate must be analysed separately, i.e., not composited. Results are tested for significant differences between sample S^{2-} and indicator thresholds and reference station values according to statistical procedures given in the British Columbia, Ministry of Environment, protocols for marine environmental monitoring (BCME 2002).

For finfish stations at 30 m and 60 m determine if there has been a chemical exceedance by a 1-sample t-test:

Redox:	H_0: $\mu \geqslant -50$ mV;	H_A: $\mu < -50$ mV (1-tailed)
ISE:	H_0: $\mu \leqslant 1500$ µM;	H_A: $\mu > 1500$ µM (1-tailed)
MB:	H_0: $\mu \leqslant 250$ µM;	H_A: $\mu > 250$ µM (1-tailed)

For stations at the fish cages (0 m), test the following hypothesis:

Redox:	H_0: $\mu \geqslant -100$ mV;	H_A: $\mu < -100$ mV (1-tailed)
ISE:	H_0: $\mu \leqslant 3000$ µM;	H_A: $\mu > 3000$ µM (1-tailed)
MB:	H_0: $\mu \leqslant 500$ µM;	H_A: $\mu > 500$ µM (1-tailed)

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

H_0: $\mu_F \leq \mu_R$; H_A: $\mu_F > \mu_R$ (1-tailed)

For shellfish, samples are collected under the culture structures and tested as for the finfish 0 m stations:

Redox:	H_0: $\mu \geqslant$ -100 mV;	H_A: $\mu <$ -100 mV (1-tailed)
ISE:	H_0: $\mu \leqslant$ 3000 µM;	H_A: $\mu >$ 3000 µM (1-tailed)
MB:	H_0: $\mu \leqslant$ 500 µM;	H_A: $\mu >$ 500 µM (1-tailed)

In the case of an exceedance perform nested 1-way ANOVA as above.

If a farm station S^{2-} is significantly greater than the reference condition, the exceedance is likely a result of fish farming and management action is required.

b) Sediment characteristics such as colour (e.g., pale/grey, brown/black), visible out-gassing, presence of sulfide oxidising bacteria (e.g., *Beggiatoa* spp.) and smell (e.g., none, medium, strong) must be noted on sample data sheets and photographed. Qualitative assessments are widely employed internationally as a simple, cost-effective means of assessing sediment condition (Keeley et al. 2014). This information should be considered in conjunction with the above statistical analyses in a 'weight-of-evidence' approach to illustrate the status and extent of farm impact. Such assessments could be performed on a regular voluntarily basis by the farmer to complement the annual compliance monitoring.

c) Full macrofaunal analyses shall be conducted at re-randomized sediment station positions with the lease areas every 3 - 5 years. Re-randomization reduces the risk of temporal autocorrelation and is recommended over fixed station positions where the monitoring objective is to assess the overall condition of a habitat (Noble-James et al. 2017). Control positions shall remain fixed providing they comply with the requirements for a control site. Sediment grain size, TOC/N, and porosity analyses shall be performed in parallel with benthic macrofauna sampling. It is more common in environmental monitoring of aquaculture facilities for sampling to be conducted around the period of maximum production. However, it is recommended that it should be aligned with the State of Bay sampling programme to facilitate comparison between the data-sets.

The sampling plan proposed for benthic macrofauna in which data are collected at both farm site stations and reference stations, in both baseline and operational periods is referred to as a beyond BACI (Before After Control Impact) design (Underwood 1992). The rationale for this approach is that organisms may show any pattern of difference between locations (both farm and control sites) before a putative impact (aquaculture). Differences among locations are to be expected but there is no reason to presume that these differences remain constant through time. Thus, there will be statistical interactions between locations (farm and reference) and time of sampling (before and after). An environmental impact is indicated when there is more temporal change in the farm site exposed to a perturbation than is usually the case in similar populations in similar locations where no such disturbance occurs (Underwood 1992). An impact must appear as an interaction between the differences among locations prior to the impact and those differences existing after it begins.

Firstly, determine whether the mean value of the Shannon-Wiener, Infaunal Trophic Indices (ITI) or AZTI Marine biotic Index (AMBI) for a growing area, i.e. BB, OB-N or OB-S, is significantly less than the threshold values by a 1-sample t test:

H_0: $\mu \geq 2.1$; H_A: $\mu < 2.1$ Shannon-Wiener Index (1-tailed)

H_0: $\mu \geq 25$; H_A: $\mu < 25$ Infaunal Trophic Index (1-tailed)

H_0: $\mu \geq 3.0$; H_A: $\mu < 3.0$ AZTI Marine biotic Index (1-tailed)

If there is evidence for exceedance undertake asymmetric ANOVA to test the following hypotheses:

H_0: there is no interaction between farm/reference site and baseline/operational; H_A: there is an interaction (2-tailed).

If a significant interaction is indicated, there is evidence that the exceedance is due to farming activities and management intervention will be required. Alternate metrics may also be used in conjunction with the above indices. Multivariate analyses should also be undertaken to maximize information from the taxonomic data particularly with regard to identifying relationships between different growing areas.

The ITI and AMBI marine biotic indices were originally developed for use in European waters and are therefore not easily applicable to species from South Africa. Presently less than 50% of species found in the ADZ samples can confidently be assigned an ITI group, meaning that the majority of species are not included in the calculations of the biotic coefficients. Similarly, although the number of species on the updated 2020 AMBI species list as increase by 15%, they are still predominantly for species from the Northern Hemisphere. While more Southern Hemisphere species have been included in the list the user still has to change the resolution from species level to genera, or else substitute a South African species with a similar species found in the northern hemisphere. The new AMBI 6.0 version of the software now enables the user to choose which group they wish to assign a specific species to. This allows for a more tailored, regional approach to the process, however, still requires the user to have a good understanding of the feeding modes utilised by the species in their geographical region. Improved applicability of these indices in the South African context would be highly beneficial for marine environmental monitoring in general, and for monitoring impacts of current and future ADZs in particular. Until such time that these indices can be more accurately applied to the majority of species found in the ADZ, it is suggested that results from these indices should be interpreted with caution.

Macrofaunal species often respond to changes in environmental variables before they are chemically detectable (Cranford et al. 2006). These responses include: 1) a reduction in species biomass, 2) a decrease in the average body size of individuals, and 3) a shift in the relative dominance of trophic groups (Black et al. 2008, Cranford et al. 2012). It is therefore suggested that future monitoring surveys include the determination of species biomass, to be used to construct ABC dominance curves (Cumulative abundance-biomass plots) and provide information on the level of disturbance within the aquaculture sites relative to the reference sites. These curves provide an additional tool with which to assess the level of disturbance within the aquaculture sites relative to the reference sites. Protocols should be aligned with the State of the Bay (SOB) Programme in which the blotted wet biomass of all taxa is determined to four decimal places.

Although, monitoring results to date suggest negligible impacts of aquaculture at current production volumes, biotic indices for some sites warrant a precautionary approach. The use of ABC curves on SOB data has shown that much of the bay, including reference sites, are considered moderately disturbed. This is probably a result of cumulative anthropogenic activities in the bay as well as highly variable natural disturbances. Therefore, it is suggested that faunal and metal analyses be undertaken at a more regular interval i.e., every three years not every 3 - 5 years. Thus, ensuring that any impacts associated with the ADZ operations are detected timeously and do not compound the natural/existing disturbance within the bay and that the cumulative impacts within the bay do not push the ecosystem towards detrimental levels of disturbance.

d) As per the biotic indices, asymmetric ANOVA can be performed on TOC/N and geotechnical descriptors (porosity, granularity) to ascertain whether there has been an impact in the growing area. These analyses are provided as supporting information for the above benthic faunal analysis and are not used to initiate management interventions.

e) Samples for metal analyses (Al, Cu and Zn) should be taken at the same time (every 3 years) as the faunal survey at the finfish sites but under cage arrays. Given the dispersive nature of the bottom sediments in the finfish growing areas it is regarded as highly unlikely an exceedance will occur. Should the type of anti-foulants change, this must be updated and considered accordingly. Sampling should be limited to directly under the fish cage structure (the zone of most likely impact) initially and extended should thresholds not be met. Results from replicate cores from each cage group should be tested using a 1-sample t test:

H_0: $\mu \leq 270$ mg/kg; H_A: $\mu > 270$ mg/kg Copper (1-tailed)

H_0: $\mu \leq 410$ mg/kg; H_A: $\mu > 410$ mg/kg Zinc (1-tailed)

Should either metal be shown to exceed the threshold, additional samples will be required incorporating the AZE and reference stations. Three samples should be taken 30 m from cage structures in the direction of residual bottom currents and one at each of the reference stations. Replicate sample stations at the AZE boundary (30 m) should be spaced such that they can be regarded as independent. The AZE boundary samples should be tested for conformance with the beyond AZE thresholds using a 1-sample t test:

H_0: $\mu \leq 34$ mg/kg; H_A: $\mu > 34$ mg/kg Copper (1-tailed)

H_0: μ ≤ 150 mg/kg; H_A: μ > 150 mg/kg Zinc (1-tailed)

Should either metal exceed threshold values perform asymmetrical ANOVA to test the following hypotheses:

H_0: there is no interaction between farm/reference site and baseline/operational; H_A: there is an interaction (2-tailed).

If a significant interaction is indicated, there is evidence that the exceedance is due to farming activities and management intervention will be required.

f) Research done to date (2019-2022), diver observations and difficulties in obtaining grab samples at several stations in Big Bay has revealed the presence of patches of exposed reef within Big Bay, particularly in the finfish precinct of the ADZ but also within the bivalve area. The reef is described as being mostly low profile <1m in height and periodically inundated with sand, however, outcrops of reef >1m in height are also reported. It is suggested that the amount of rocky substratum present in Big Bay is likely significantly more expansive than originally thought and that the full extent of the calcrete platform and the proportion of this habitat type impacted by current and future mariculture activities should be more accurately determined. Updated SANHO bathymetry data is available for much of Big Bay, but not for the ADZ precinct itself, and this should be collected by a dedicated survey.

Previously, the benthic monitoring protocols in this sampling plan were appropriate for soft sediment habitats only, and no data was been collected on the potential impacts of mariculture on reef communities. The sampling plan should therefore be expanded to include monitoring on the epifaunal reef community composition. These surveys should take the form of both video and photographic data capture, with the recommended addition of west coast rock lobster counts to monitor the population status of this commercially important species. An initial, qualitative photographic and video survey and analysis was undertaken during 2022 and this should be repeated at least every three years as part of the benthic monitoring. The survey methodology is described below, and survey site locations are provided in the hard substrate survey report (Dawson et al 2022). Given the limitations placed on taxonomic identification by poor visibility within the water column, reference and impact reef sampling surveys should be conducted on the same day. Should diving conditions allow, it would be desirable for analysis to include quantitative abundance or percentage cover data, not just qualitative data.

g) Frequent servicing/calibration of bottom water oxygen sensors must be undertaken, dependent on the rate of fouling and functioning of sensors (e.g., monthly). Both the correlation between oxygen and temperature and the calculated Apparent Oxygen Utilization (which is largely dependent on temperature) should be assessed as indicators of increased oxygen demand by sediments and the near-bottom water column. The time series should be analysed using the Mann Kendall non-parametric statistical test (or similar) to identify whether there is a significant monotonic trend in the data (Pohlert 2018). The Mann Kendall test is used in place of linear regression analysis as it does not require the assumption of a normal distribution of residuals. Change-point detection (Taylor 2000, Killick & Eckley 2014) can then be employed to identify when the change(s) occurred and the statistical confidence thereof. Evidence in the time series for a developing departure in the sentinel site oxygen and temperature relationship relative to the reference site should trigger additional sampling to verify and determine the extent of sediment deterioration. This could take the form of an initial CTD survey of bottom oxygen/temperature. A chemical investigation of S^{2-} may be required to establish the oxic status of the impacted sediments. Management action will be based on this follow-up investigation.

7.2 Water column

a) Frequent (monthly) servicing of the fluorometer is required to maintain proper functioning and calibration. More frequent (e.g., weekly) sampling is required of size-fractionated chlorophyll at both the entrance to the lagoon and reference control sites. Fixed point sampling (Eulerian) as proposed here integrates temporal and spatial variability that typifies the dynamic water movements associated with tidal cycles and currents. The proposed sampling scheme combines high frequency sampling over short time periods relative to phytoplankton generation times, with longer term low frequency sampling, both of which are required to identify putative farm impacts from natural cycles (see Martin-Platero et al. 2018). The data should be subject to trend and change-point analyses as above. Should the time series record of chlorophyll indicate departures from the apparent natural cycles inherent in the embayment, additional sampling may be required. More importantly, if the proportion of picoplankton entering the lagoon is shown to have increased relative to reference sites and in concert with the expansion of shellfish farming (change-point detection), rapid synoptic surveys must be undertaken to identify the source and magnitude of the depletion in large-celled phytoplankton. Management action may be required based on the findings of these surveys.

b) Annual, non-quantitative samples should be taken of fouling organisms on farm infrastructures, preferably in conjunction with the State of the Bay Programme (Clark et al. 2017).

8. Small Bay

Small Bay comprises an area that has been under cultivation for an extended period, and which is subject to many sources of pollution, including aquaculture (see State of the Bay reports e.g. Clark et al. 2017). Despite obvious indications of habitat degradation in the bay, it is often regarded as a ‘sacrificial zone’ and perhaps even beyond remediation. However, Small Bay is an important nursery ground for finfish, particularly white stumpnose, (Clark et al. 2017) and a popular recreational hub. The bay provides many other crucial ecological services such as assimilative capacity for organic anthropogenic wastes such as fish processing and sewage effluent, as well as a repository for heavy metals and other contaminants in anaerobic sediments. Shellfish aquaculture in the bay has been shown to impact on the ecology of the benthos (Stenton-Dozey et al. 1999, 2001) and the water column (Heasman et al. 1998). Impacts, however, were regarded as very localized and of small concern for the broader bay system. The recent expansion of aquaculture, and scope for further expansion, in the bay does raise new concerns regarding its sustainability, particularly with regard to its assimilative capacity for aquaculture biodeposits.

As an initiation of a monitoring effort in Small Bay it is proposed that a sentinel station is established on the eastern lease boundary of the major longline culture portion in the bay. This position is based on the predominant clockwise circulation within the bay. As with sentinel stations in BB and OB, bottom oxygen and temperature sensors should be moored close to the seabed, and paired with a reference station at North Buoy. North Buoy is currently a monitoring station in the State of the Bay Programme and is outside the putative influence of the shellfish cultivation structures (Stenton-Dozey et al. 2001). Evidence for significant departure from the oxygen temperature relationship indicating oxygen depletion relative to the sentinel site should trigger more extensive sampling. Initially this could be limited to a CTD survey of bottom water oxygen levels over the lease areas. Time series analyses as with the other sentinel stations can be performed to monitor any further deterioration associated with the lease areas.

Irrespective of the sentinel station records, a chemical survey of S^{2-} should be undertaken in the near future to establish the oxic status of the lease areas in Small Bay. The survey should include North Buoy and two other stations as reference conditions. Statistical tests for compliance with S^{2-} thresholds should be performed as outlined earlier and followed up with management interventions should the situation warrant. It is recommended that this survey is preceded by a CTD bottom oxygen survey to map the extent of oxygen depletion associated with the culture area.

9. Sampling procedures

9.1 Macrofauna

Three replicate samples from box-cores, grabs (e.g., Van Veen) or diver-operated suction samples (recommended) are to be taken at each sample site. Sample units should collect at least 0.07 m^2 sediment (diameter 30 cm) and, where possible, to a depth of 30 cm, giving a total surface area of > 0.2 m^2 per site. This is double the minimum recommended area for benthic macrofauna collection by Preslawski et al. (2018). The post-collection procedures given below are based predominantly on Rumohr (1999) and Prezlawski et al. 2018).

a) Gently wash samples with seawater through a 1 mm sieve, avoiding directing flow onto sieve to preserve integrity of fragile organisms. The sieve can be placed in a container into which wash water flows and gently agitated to release light-bodied animals. Continue until all sediment that can pass the sieve is washed through.

b) For grab or box core samples it may be necessary to transfer the sample to the sieve portion by portion as a sediment-water slurry.

c) If re-sieving of samples is carried out a mesh finer than the initial 1 mm must be used.

d) Fragile animals such as some polychaetes, should be picked out by hand during the sieving, to minimize damage. Also stones and large shells should be picked out to avoid a grinding effect during sieving.

e) Once washing is completed remove large-bodied animals that do not float during washing to a sample container.

f) Specimens retained on the sieve are washed off from the underside with a seawater squirt bottle into a funnel and sample container. Minimal amounts of water should be used in this step to ensure adequate preservative concentration.

g) Fix samples with 4 % buffered formaldehyde (1 part 40% formaldehyde plus 9 parts filtered seawater) in tightly sealed containers. Sodium tetraborate at a final concentration of 2 % may be used as a buffering agent. Label both the outside and inside of the container with sample details on durable labels.

h) Staining with e.g., Rose Bengal may be used to facilitate sorting and increase sorting accuracy. The stain can be added to the formaldehyde solution used for fixing.

i) As formalin is toxic and probably carcinogenic it should be handled with great care. Adequate ventilation should be applied for all procedures and thorough rinsing with tap water prior to sorting is mandatory.

j) After sorting into broad taxonomic groups, specimens are identified to the lowest possible taxonomic resolution, weighed, and enumerated. Classification must be undertaken by a qualified professional.

k) After an adequate fixing period samples can be preserved in 70 - 80 % alcohol or saturated propylene phenoxetol for long term storage.

l) It is advisable to store some specimens of each taxon for later taxonomic validation if required.

9.2 Sulfides and redox potential

Sediment samples for chemical measures of oxic status must be taken at three compliance stations: 0 m, 30 m and 60 m along the main axis of residual bottom current flow for finfish areas. For shellfish, samples are limited to directly below the culture structures. Samples must also be taken at three reference stations that were previously established for the particular growing area. 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.

Recommended sample collection and measurement of S^{2-} and Eh is based on Cranford et al. 2020. Three replicate sulphide and three redox samples must be collected by scuba divers at each site, using new 250ml polyethylene plastic jars. Jars must be completely full and tightly sealed underwater with as little exposed to the water column as possible. S^{2-} samples are to be kept on ice before being frozen and delivered to a certified laboratory to be analysed using the methylene blue colorimetric method. Redox samples must be kept on ice before being measured using a redox probe as per (d) below. A photograph of each redox sediment sample should be taken for a colour assessment.

The alternate method of sample collection and measurement for S^{2-} and Eh is based predominantly on Wildish et al (2004) and Fisheries and Oceans Canada (2015b, 2015b). Samples must be collected by either grab or core with a sampling area of at least 200 cm^2. The device must prevent leakage of water or sediment during retrieval. If a grab is used the sample is suitable for analysis if overlying water is clear indicating minimal disturbance of the sediment. The sample device should have penetrated at least 5 cm. If after 3 attempts no sediment is retrieved in grabs then it is likely the bottom substratum is hard and the sample station should be re-positioned by no more than 3 m, maintaining the required distance from the cages. When a sediment sample cannot be taken that position shall be recorded as hard bottom and not suitable for future sampling. Three replicate grabs or cores must be taken from each sample position and each should be treated separately as outlined below.

a) Perform S^{2-} and Eh (if required) analyses within 5 min after sample retrieval. If it is not possible to make these measurements at sea, the samples may be stored on ice and then refrigerated for up to 72 h prior to analysis (see Wildish et al. 2004). Samples can be held in syringes until later analysis provided they are capped and chilled.

b) Sub-samples are taken of the top 2 cm of the sediment surface by means of a cut-off syringe or spatula and transferred to a suitable glass or plastic container and gently homogenized after removing unrepresentative material (e.g., shells, large worms, stones). A minimum of 25 ml of sediment is required for each Eh and S^{2-} analyses.

c) For S^{2-} measurements, place 10 ml of freshly prepared EDTA/NaOH/ascorbic acid buffer in a graduated container and add homogenized sediment up to 20 ml graduation mark (add buffer first).

    i. Briefly stir mixture and insert electrode below the surface of the slurry.

    ii. Gently move electrode until reading stabilizes, typically 1 - 4 mins. If electrode does not stabilize within a reasonable time re-calibration may be necessary.

    iii. Rinse electrode with distilled water to remove any sediment and gently wipe to remove any oily residue prior to further use.

d) After the 10 ml sub-sample has been removed for S^{2-} measurement, place the redox electrode in the remaining sediment.

    i. The electrode should be held stationary in one position.

    ii. Record the Eh value and sample temperature once a relatively stable reading (< 10 mV/min) is achieved, usually within 3 min. Temperature is required for calculation of a correction factor.

    iii. Rinse electrode with distilled water to remove any sediment and gently wipe to remove any oily residue prior to further use.

Details regarding calibration and electrodes used in the measurement of Eh and S^{2-} are provided in Wildish et al. (2004), Fisheries and Oceans Canada (2015a) and PNS (2018b).

Exceedance of sediment quality thresholds at site B4 in the Big Bay precinct is highlighted as an area of concern. The recommended management action is increased spatial coverage of sediment monitoring. Two-three additional stations 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. Two additional benthic stations have therefore been included in this area (Figure 1, Table 1).

9.3 Geotechnical and other geochemical indicators

Collection of sediment for determination of metals (Cu, Zn and Al), TOC/N, porosity and grain size are as described for S^{2-} and Eh. Separate sub-samples of surficial sediment (upper 2 cm) from three replicate cores shall be thoroughly homogenized prior to splitting into separate containers for the different analyses. Caution should be taken to prevent contamination by metallic surfaces. Samples must be kept chilled in the field and stored frozen. The amount of sediment required should be as advised by the analytical laboratories for the respective analyses. Depending on the laboratory, some analyses may be carried out on the same sample, e.g., porosity (water content) and TOC/N. To align with methods currently employed in the State of the Bay Programme, metals should be determined by ICP optical spectrometry after strong acid extraction and microwave digestion (total fraction), TOC/N by elemental analysis (not weight loss on ignition), and sediment particle size distribution by dry sieving (Barry Clark, Anchor Environmental, personal communication). Porosity is calculated from weight loss of wet sediment on drying at 60°C.

9.4 Rapid synoptic surveys

To be undertaken to determine the spatial extent and magnitude of phytoplankton depletion by bivalve cultivation. Depletion of phytoplankton becomes a concern when the scale of cultivation is large enough to remove particles faster than physical re-supply, e.g., through tidal currents, and phytoplankton production (Cranford et al. 2006). This effect has implications for both the production carrying capacity for the cultivated stock and the ecological carrying capacity of other components of the ecosystem (Cranford et al. 2008). A critical additional component of the study is to quantify the biofilter effect of the bivalves on the size structure of the phytoplankton.

The survey involves the rapid, high resolution mapping of chlorophyll with a CTD equipped with a fluorometer. The aim is to collect 3-D data rapidly, within 1 - 2 h, before tidal flushing causes distributional changes (Cranford et al. 2008). Phytoplankton size structure is measured in discrete water samples taken during the survey:

a) Water samples (1 - 2 litre) are collected from 1 m depth and stored chilled, in the dark for processing on return to shore.

b) As soon as possible on return to shore 0.2 litre sub-samples are filtered through a 3 μm Nucleopore (or similar) membrane filter.

c) Screened and unfractionated (100 - 200 ml) sub-samples are then filtered onto Whatman GF/F filters and the filters stored at - 20 °C for later chlorophyll determination by *in vivo* fluorescence or other acceptable method.

9.4 Reef surveys

Epifaunal community surveys of reef habitat within Big Bay should be undertaken during optimal visibility and weather conditions. Three impact sites within the ADZ precinct and three reference sites should be surveyed, with all sites preferably sampled on the same day to minimise confounding weather and visibility effects. A shot-line must be deployed at the identified reef sites and a pair of scientific divers descend to the sea floor. One diver is to slowly swim three 10 m video transects radiating from the shot-line centre with the camera ~50 cm off the seafloor, whilst the other diver conducts at least ten photo-quadrats (0.04 m^2) on reef habitat in the vicinity of the shot-line base. At least 2 - 3 photos must be taken of each quadrat to ensure that the best possible focus is achieved, as well as to account for varying depths of fields of each photograph. Should conditions require the use of the flash or a torch, all photos should be taken using these. Additionally, qualitative collection of biota should be undertaken at all sites to aid in the identification of cryptic biota observed in video transects and photo-quadrats. At a minimum, presence/absence data is to be extracted from the collection, photographs and video footage. However, should conditions and photo quality allow, it is desirable for analysis to include quantitative abundance or percentage cover data. Multivariate statistical analysis should be undertaken to investigate differences between control and impact sites.

Mobile species, such as the economically important west coast rock lobster, are not often captured in photographs as they rapidly retreat when the quadrat is initially dropped and are better represented in video footage. Therefore, it is suggested that lobster counts, to monitor population densities, be included in the reef survey. The diver conducting the video transects should attach a reel to the shot line, holding this reel in front of themselves the diver is to swim the three transects, 10 m in length, counting rock lobsters in the 0.5 m either side of the line (30 m^2 area). The video footage can then be captured as the diver returns to the base of the shot line.

10. Management actions

A breach of the required S^{2-} benthic impact indicators for finfish will require additional sampling to verify and provide greater spatial detail of the magnitude of non-compliance. Sampling should be intensified around the cage/group to accurately map the distribution of non-conformance with the oxic thresholds within the AZE. Based on these findings management actions may include:

a) Extension of the AZE by the AMC. The Scottish EPA allows larger AZE's in dispersive, as opposed to accumulative, environments where supporting information warrants.

b) Reduction in the scale of farming at the site to align with local assimilative capacity. This will involve adjusting on-site stocking levels.

c) Reduction in feed wastage according to a benthic amelioration plan submitted by the operator. Preferable option as it has direct commercial implications for the operator. The plan should include:

i) update of staff training on feeding methods,

ii) installation of submerged video or other device to track feed pellets in real-time to minimize overfeeding (feedback-control).

d) Movement of structures within the lease area to allow recovery on the benthos. Alternatively, investigate whether a re-orientation of cage set-up could take better advantage of dispersive currents. Fallowed sites should be monitored annually to track recovery.

The operator shall be obliged to provide audited (e.g., by certified accountant) information in relation to production and feed input for the implicated entity as deemed necessary by the AMC. If follow-up annual sampling indicates no improvement, further limitation of production levels shall be imposed with attendant reduction in feed. If seabed conditions do not improve in response to production/feed reductions, relocation of the cage structures will be required.

Non-conformance with the shellfish benthic S^{2-} indicator shall require more intensive sampling of an area to verify results and map the zone of negative influence. Based on these findings management actions are limited to:

e) Re-alignment of the culture structures to promote improved dispersion by currents where possible.

f) Reduction in production levels by limiting the number of dropper lines for settlement of mussels or deployment of oyster baskets.

Should these actions prove ineffective at the following annual monitoring campaign, a further decrease in production will be imposed. Additional management actions could require clearing of a site.

Management actions in response to a failure to meet the benthic faunal thresholds are as above but more drastic in that they apply to the whole site, not just ill-performing sub-sets thereof. In the event that the sediment macrofauna oxic thresholds are not achieved, reductions in production feed use/wastage shall be applied as outlined earlier to the whole site. Follow-up benthic fauna surveys shall be carried out on an annual basis until monitoring indicates the benthos has recovered to an oxic status. Continued non-compliance is indicative that the site is unsuitable for aquaculture and it should be de-stocked.

In the event that chlorophyll monitoring and findings of the rapid synoptic survey clearly implicate a bivalve growing area(s) in the depletion and/or alteration of the structure of the phytoplankton community that feeds into Langebaan Lagoon, management action will be required. Bivalve feeding should produce a continuous, press response in the indicators that are clearly distinguishable over the long-term from natural variations. As a first management action, limits on bivalve production should be imposed at certain sites and the level of recovery monitored over time as for the operational phase. As there are no clearly defined thresholds for the phytoplankton indicators (chlorophyll, proportion of picoplankton) it will require a management decision on what level of impact can be regarded as acceptable. Given the sensitivity and ecological importance of the lagoon it would be prudent to apply strict limits such as no significantly detectable change.

Upon establishing that the threshold levels for Cu and/or Zn have been exceeded in potentially bioavailable forms, various management actions shall be imposed that may require (Keeley et al. 2014):

a) Extension of AZE from fixed distance to site specific, e.g. in dispersive environments (SEPA 2000).

b) Reduction of inputs to the system proposed by the operator. Explore alternatives to Cu and Zn based antifoulants and lower levels of nutritional therapeutants such as Zn in feed (Clement et al. 2010).

c) Investigation into the bioavailability of the metal by different extraction protocols, e.g. weak acid extractable fraction as a proxy for bioavailability.

d) Scientific investigation into toxicity of the contamination (see Macleod and Eriksen 2009) and potential adjustment of generic threshold limits with site-specific criteria based on these findings.

Further management imposed reductions in inputs shall be enforced until compliance with thresholds is achieved. Fallowing is a controversial strategy for dealing with metal contaminated sediments as generally much longer time periods are required for metal rehabilitation than for organic enrichment. In addition, there is the potential for metal bioavailability to increase with sediment recovery which in turn might hinder further biological remediation (Clement et al. 2010).

11. Food safety

Farms cultivating molluscan shellfish and finfish are required to comply with the South African Molluscan Shellfish Monitoring and Control Programme (SAMSM&CP), the South African Aquacultured Marine Fish Monitoring and Control Programme (SAAMFM&CP) and the National Residue Control Programme (NRCP) as per the permit conditions contained in the Permit to Engage in Marine Aquaculture. The SAMSM&CP requires that molluscan shellfish farms are classified before marketing their product, a process that could take at least 3 months for a preliminary classification. The SAMSM&CP also requires the testing of Paralytic Shellfish Toxins (PSY), Amnesic Shellfish Toxins (AST), and Diarrhetic Shellfish Toxins (DST) and phytoplankton as well as *E. coli*, *Salmonella* spp., *Vibrio parahaemolyticus* and *V. cholera*. The SAMSM&CP and SAAMFM&CP require that farms furthermore comply with the National Residue Control Programme, which includes the need to test for banned and controlled substances, heavy metals, pesticides and polychlorinated biphenyls (PCBs). Farms exporting product are furthermore required to comply with importing country requirements which could include arsenic, inorganic arsenic, polycyclic aromatic hydrocarbons, dioxins, dioxin-like PCBs and non-dioxin like PCBs. The samples are taken by independent samplers namely the National Regulator for Compulsory Specifications (NRCS) and submitted to the relevant laboratories. Should the concentrations of the contaminants exceed the regulatory limits, the implicated farms are temporarily closed and potentially contaminated products are recalled. The farms remain closed until the regulatory limits are complied with and the reopening protocols are adhered to. The SAMSM&CP and SAAMFM&CP documents are available whereas the National Residue Control Programmes are kept confidential as it deals with banned and controlled veterinary drug residues which are easily manipulated prior to sampling. However, the environmental residue testing requirements are available for distribution.

12. Biosecurity and Aquatic Animal Health Monitoring

The fundamental measures that underpin aquatic animal disease prevention are the application of biosecurity. This paragraph describes the biosecurity principles necessary to mitigate the risks (probability and consequence) associated with the introduction of pathogenic agents into, the spread within or the release from aquaculture facilities in the bay. Biosecurity is a set of physical and management measures which, when used together, cumulatively reduce the risk of infection in aquatic animal populations at an aquaculture facility. Most of the aquaculture activities in the bay can be described as "semi-open systems". In a semi-open aquaculture production system, it is not possible to have control of water entering or exiting the system or of environmental conditions. Some aquatic animals and potential disease vectors may also enter and exit the system. Pathogenic agents can move into, spread within and be released from an aquaculture facility via various transmission pathways. The identification of all potential transmission pathways is essential for the development of an effective biosecurity plan. Since effective isolation of a semi-open system is not possible, the entire bay is regarded as a single epidemiological management unit which will require the coordination of biosecurity measures implemented between different aquaculture operations.

The basic requirements for and effective biosecurity plan are as follows:

Import Control:

1. Only introducing aquatic animals (Seed, juveniles, stock for on-growing) into the aquaculture facility with a known health status, which is of equal or higher status than the animals in the bay (This requires knowing the health or disease status of the aquatic animals in the bay).
2. Ensuring biosecure transport of aquatic animals from the source to the bay to avoid exposure to pathogenic agents.

Early detection and monitoring:

1. Health monitoring of the aquatic animals at the aquaculture facility will include activities like disease surveillance, routine monitoring of stock for important health and production parameters, recording clinical signs of disease, morbidity and mortality rates and their causes.
2. Removing sick or dead aquatic animals from production units as soon as possible and disposing of them in a biosecure manner.

Emergency procedures:

1. Procedures should be developed and implemented to minimize the impact of emergencies, disease events, or unexplained mortality in aquatic animals. These procedures should include clearly defined thresholds that help to identify an emergency incident and activate response protocols, including reporting requirements.

The "Health management procedures for South African bivalves (oysters and mussels) produced for export" document outlines the relevant procedures necessary to support biosecurity and health monitoring in the bay. Similar procedures will need to be developed in conjunction with the department for finfish.

Please also refer to ADZ EMPr for full list of mitigation measures specific to disease management.

13. Genetics

Proposed species for the Saldanha Bay ADZ include the currently cultivated species (*Magallana* Syn *Crassostrea) gigas, Mytilus galloprovincialis* and *Choromytilus meridionalis*); indigenous shellfish (*Haliotis midae* and *Pecten sulcicostatus*) not previously cultured in the bay and finfish species (*Rhabdosargus globiceps, Argyrosomus inodorus* and *Seriola lalandii*); the seaweed *Gracilaria gracilis*; and a number of alien finfish species (*Salmo salar, Oncorhynchus kisutch, O. tshawytscha* and *O. mykiss*).

There are no perceived genetic risks associated with the farming of the proposed alien finfish species. Conversely, farms cultivating indigenous shellfish and finfish. For all farms cultivating indigenous seaweed species in Saldanha ADZ, it is recommended that seed stock are sourced from an area close to where the grow-out will take place. Species should minimize potential genetic impacts on wild populations as much as possible by adhering to the DFFE genetic management guidelines. 'Genetic Best Management Practice Guidelines' already exist for important commercial shellfish species, such as *H. midae*, and for some of the emerging finfish species, including *A. inodorus*, and these guidelines should be strictly adhered to. For all farms cultivating indigenous finfish and shellfish species in the open production systems proposed for the Saldanha ADZ (e.g. rafts, cages and barrels), it is strongly recommended that broodstock or parent fish are sourced from the area in which the grow-out will take place or from the same genetic zone. In order to retain a healthy genetic profile it is recommended that an effective broodstock population size of between 30 and 200 individuals are kept in the hatchery and a rotational breeding program should be implemented. If appropriate broodstock management procedures are used, gene frequencies can be maintained approximating those in the wild stocks.

For additional mitigation measures, please refer to the ADZ EMPr which will be reflected in the site specific EMPr's for individual projects.

Further genetic research between any future farmed indigenous species and wild caught species will be explored when this becomes relevant.

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