The Storm Bay Observing System

Preliminary review of the sampling parameters and design for assessing the performance of salmon aquaculture

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In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

Contents

Abbreviations

AUV	Autonomous Underwater Vehicles
ANZECC	Australian and New Zealand Environment and Conservation Council
AST	Analytical Services Tasmania
BEMP	Broadscale Environmental Monitoring Program
CATAMI	Collaborative and Automated Tools for Analysis of Marine Imagery
CPCe	Coral Point Count with Excel Extensions
CONNIE	Connectivity Interface; open access online modelling and visualisation tool
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CTD	Conductivity Temperature Depth
DGPS	Differential Global Positioning System
DPIPWE	Department of Primary Industries, Parks, Water and Environment
EAC	East Australian Current
EIS	Environmental Impact Statement
ELs	Environmental Licences
EPA	The Environment Protection Authority Tasmania
FRDC	Fisheries Research and Development Corporation
IMAS	Institute for Marine and Antarctic Studies
IMOS	Integrated Marine Observing System
IP	Internet Protocol
MFDP	Marine Farm Development Plan
ROV	Remote Operated Vehicle
RVA	Rapid Visual Assessment

1. Summary

This research program is designed to provide a comprehensive overview of the potential interactions between salmon farming and the water column, soft-sediment, inshore reefs, deep reefs and seagrass habitats. For each of these different habitats sampling has commenced. The key indicators and sampling designs for the water column, soft-sediment and inshore reef habitats are well developed. Preliminary results from the Storm Bay monitoring and Fisheries Research and Development Corporation (FRDC) project “2015-024: Managing ecosystem interactions across differing environments: building flexibility and risk assurance into environmental management strategies” that is nearing completion, have been used to determine the sensitivity of the sampling designs for assessing the environmental performance of these habitats at different spatial and temporal scales. For the deep reef and seagrass habitats the sampling methods and designs are still being refined; the initial results will be used to identify key indicators species/functional groups and parameters to monitor and to test the power of the sampling design to detect any potential interactions with salmon farming. The report is an initial review synopsis that will be updated as the Storm Bay project progresses. The information will culminate in a full review of the monitoring program, including recommendations for potential refinement in work package four, in the last phase of this project.

2. General background

In Tasmania, farming of Atlantic salmon (*Salmo salar* L) has developed rapidly since the first trials in 1985 and has grown progressively to the current 60,000 tonnes produced in 2020.. Salmon farming in open sea cages produces organic and inorganic wastes which have the potential to impact the receiving environment. The waste products consist of faecal material, uneaten feed pellets and metabolic waste products in dissolved inorganic forms. Dissolved wastes may enhance ambient nutrient levels (Price, Black et al. 2015), influencing primary and secondary production (Price, Black et al. 2015), and when the particulate matter sinks to the seabed it has the potential to change the structure and function of the surrounding benthic communities (Bannister, Valdemarsen et al. 2014, Oh, Edgar et al. 2015).

Hence, the expansion of the Tasmania salmon industry into new growing areas, is contingent on developing a robust science-based environmental monitoring program. This monitoring is central to environmental management, good farm health and maintaining public confidence in the industry. The program must be able to provide the information required to detect ecosystems change and the influence of salmon farming at multiple spatial and temporal scales. Specifically, the program must identify and monitor the relevant ecosystems components that could be affected by salmon farming using an appropriate sampling design. This report will describe the current methods being employed to understand the effects of salmon farming inputs into Storm Bay, and where sufficient information is available, conduct a review of the ecological and statistical sensitivity of the sampling design to inform a future monitoring program. The report is an initial review synopsis that will be updated as the project progresses. The information will culminate in a full review of the project outputs to inform the future monitoring program, including recommendations for potential refinement in work package four in the last phase of this project. This review will also be informed by the biogeochemical model as it becomes available; and model simulations of biomass scenarios will identify hot spots for change and the optimal time and space scales on which to collect observations (e.g. Wild-Allen et al., 2011).

2.1.1. Local-scale

Traditionally, monitoring of salmon leases involves both baseline and ongoing monitoring to determine compliance against relevant legislation, and to assist in the development of an adaptive management based environmental monitoring system (see Woods et al. 2004). For the first two decades of farming in Tasmania, environmental monitoring was focused on the management and regulation of lease scale effects. Initially there is a baseline environmental survey of the lease that includes measurements of currents, bathymetry, habitats and seabed characteristics, and sediment chemistry and faunal communities (Woods, Brain et al. 2004). Ongoing monitoring is then designed to ensure that there are no unacceptable impacts (as defined in the Environmental Licences - see Tables 2 and 4) of salmon farming on the benthos greater than 35 metres beyond the boundary of the lease.

From 1997 to 2003, biannual surveys were undertaken at sites within each farm lease area (internal) and at compliance points 35m from the lease boundary (external) (DPIPWE. 2002, DPIPWE. 2002, DPIPWE. 2005, DPIPWE. 2010, EPA Tasmania. 2013). This included visual assessments of sediment condition via remotely operated vehicle (ROV) with video and spot dives, and measurements of sediment chemistry and faunal communities (Woods, Brain et al. 2004). The biannual visual assessments and quantitative benthic sampling surveys were used to track temporal and spatial changes at and around farm sites to assess environmental performance of the lease and provide information on the recovery of the benthos during fallowing. The performance of these local-scale data is assessed against predefined targets (see sections 2.5 and 3.3 for specific details). From the mid 2000's local scale monitoring moved to annual visual monitoring of sediment condition. The visual assessment methodology is based on the development of visual metrics of different stages of enrichment/sediment health and validation of these against quantitative measurements of sediment chemistry and faunal communities (Macleod and Forbes 2004).

2.1.2. Broad scale

In the mid-late 2000s, environmental monitoring and management expanded to encompass the potential effects on the broader ecosystem (Volkman, Thompson et al. 2009). This culminated in the development of the broadscale environmental monitoring programs (referred to as the BEMP). Initially developed for the D'Entrecasteaux Channel and Huon River Marine Farming Development Plan areas, BEMPs have since been developed for other growing areas (e.g. Tasman Peninsula and Norfolk Bay, Macquarie Harbour, Great Oyster Bay and Mercury Passage). The BEMP program, first developed by Thompson et al. (2008) in the D'Entrecasteaux Channel and Huon estuary, includes a broad suite of environmental performance indicators monitored across multiple sites in order to assess spatial and temporal changes in water column and soft-sediment environments.

In more recent years, the BEMP programs for new Atlantic salmon growing regions has been expanded to include other potential receiving habitats, notably seagrass and inshore rocky reef habitats (Macleod, Ross et al. 2016), but also deeper reef habitats. Whilst the indicators for monitoring the environmental footprint of the salmon industry in the water column and soft sediment environments are now reasonably well established, protocols to monitor the condition of inshore and deep reef habitats or seagrass are limited. The FRDC 2015-024 program was the first to establish performance indicators and monitoring protocols for inshore reef habitats. The information from the BEMP programs are used by the regulatory authority, industry and stakeholders to assess ecological condition in the major growing areas and to develop adaptive management strategies for the salmon industry.

2.2. Overview of the current Storm Bay monitoring

Storm Bay has been identified in the Tasmanian Governments Sustainable Industry Growth Plan for the Salmon Industry as a priority area for the potential expansion of marine salmon farming. Collectively the three major salmonid producers: Tassal Operations Pty Ltd, Huon Aquaculture Company Pty Ltd and Petuna Pty Ltd are aspiring to farm in the Storm Bay region. A combined level of production of 40,000 tonnes per annum was approved. This approval was conditional on a stage development approach, with an initial limit on feed input that would provide for 30,000 tonnes of production, the implementation of a comprehensive environmental monitoring program and the development of a biogeochemical model.

The current FRDC project "2018-131: Storm Bay Observing System: Assessing the Performance of Aquaculture Development" will underpin the development of a robust environmental monitoring program by building on, and refining the indicative BEMP and the local scale lease-specific monitoring program that has been developed by the Environment Protection Authority Tasmania (EPA) and the Tasmanian Planning Commission (Table 1). The objectives of the broad scale components of the research were to provide data to enable key chemical, physical and biological indicators of ecosystem condition to be monitored and the degree of change from background levels of these indicators to be determined, to inform adaptive management responses. This will provide a fully integrated environmental monitoring program that spans all Marine Farm Development Plan (MFDP) areas containing licenced finfish marine farming leases in the Storm Bay region.

The broad scale components of the research are designed to monitor and measure parameters in the water column, soft sediment, seagrass, and surrounding reef systems, across a range of sites and throughout the year. This information will be augmented with local scale lease specific monitoring and measurements of variables in the water column and soft sediment. This report will review the efficacy of the broad and local scale environmental monitoring, being undertaken in the research project, for detecting potential ecosystem changes due to salmon farming.

Table 1: Sampling parameters and frequency

Broad-scale	Matrix	Parameters	Frequency
	Water quality	Nutrients, phytoplankton, environmental parameters	Monthly
	Soft-sediment	Biota, chemistry, visual assessment and particle size	Annually
	Inshore rocky reefs	Fish, invertebrate and macroalgal species richness and abundances	5 years
		Rapid assessment of functional groups and indicator species	Biannually
	Deep rocky reefs	Fish, invertebrate and macroalgal species richness and abundances	Annually
	Seagrass	Seagrass distribution and health; temporal and spatial variance	Annually
Local-scale during peak biomass	Water quality	Nutrients, phytoplankton, environmental parameters	3 months/per year
	Soft sediment	Biota, chemistry, visual assessment and particle size	Annually

3. Water column

3.1. General background

Environmental monitoring programs designed to assess the influence of organic and inorganic nutrients released by salmon farming into the water column have produced varied results both globally (Price and Morris 2013, Price, Black et al. 2015) and in Tasmania (Ross and MacLeod 2013). Some studies have reported enhanced water column nutrient concentrations, chlorophyll *a* and phytoplankton at local-scale distances of up to 200 m in the vicinity of the cage and during peak production while others have reported minimal or no enhancement (see review by Price et al. 2015). Studies at regional or broad-scale, are limited but have similarly, found varied evidence for the influence of salmon farming on the water column (Pitta, Tsapakis et al. 2009, Ross and MacLeod 2013). Collectively these studies have highlighted the need for carefully designed monitoring programs to be able to determine whether any changes in the water column are linked to salmon aquaculture activities and to understand the role of other major sources of nutrients and influences on ecosystem dynamics. For Storm Bay, this includes catchment inflows and the influence of regional oceanography.

3.2. Previous studies

The environmental characterisation of the Storm Bay water column has been reported on between 1985-1989 by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) (Clementson, Harris et al. 1989, Harris, Griffiths et al. 1991), 2009-2015 by the Institute for Marine and Antarctic Studies (IMAS; Swadling, Eriksen et al. 2017), and 2018 by the EPA. These assessments clearly show the high spatial and temporal variability in nutrient concentrations and phytoplankton biomass and composition consistent with intra and inter-annual variability in the influence of the East Australian Current (EAC) and other marine and river influxes (Harris, Griffiths et al. 1991, Swadling, Eriksen et al. 2017). In particular, these data demonstrated that the bottom ammonia concentrations were higher than the surface waters and highly variable, throughout the more recent six-year sampling period (Swadling, Eriksen et al. 2017). The information collected by these sampling programs provides a good baseline for interpreting any potential change in water column nutrient concentrations and phytoplankton biomass and community composition in response to salmon farming or other environmental influences.

3.3. Sampling design

Broad scale – 28 water column sites were chosen to be sampled monthly across Storm Bay. Nutrient dispersion modelling using the CONNIE tool (https://connie.csiro.au/) conducted during the Environmental Impact Statement (EIS) stages was used to estimate the spatial distribution of nutrients from proposed salmon farming activities. The outputs from simulations when farms are operating at a combined 40,000 tonne biomass were used to inform the distribution of monitoring sites. The EPA determined that sampling of sites across and beyond the estimated dispersion range are required to determine the degree of ecosystem condition change attributable to farming activities. More specifically, the sites are distributed across areas that encompass different levels of predicted nutrient exposure and thus response based on the ANZECC (2000) guidelines. The area inside the aqua contour represents 80th percentile and include sites that are more likely to show an environmental effect when farms are operating at 40,000 tonne biomass combined (Figure 1), sites within the dark blue contour line (50th percentile) are where there is less likely to be any observable effect (Figure 1). These sampling sites therefore extend over multiple spatial scales in relation to distance from the leases and include: near scale (within and adjacent to proposed marine farming lease areas), intermediate (<5km) and far-field (>5km). These sites were also chosen to overlap with previous CSIRO, IMAS and EPA studies in Storm Bay (Table 2).

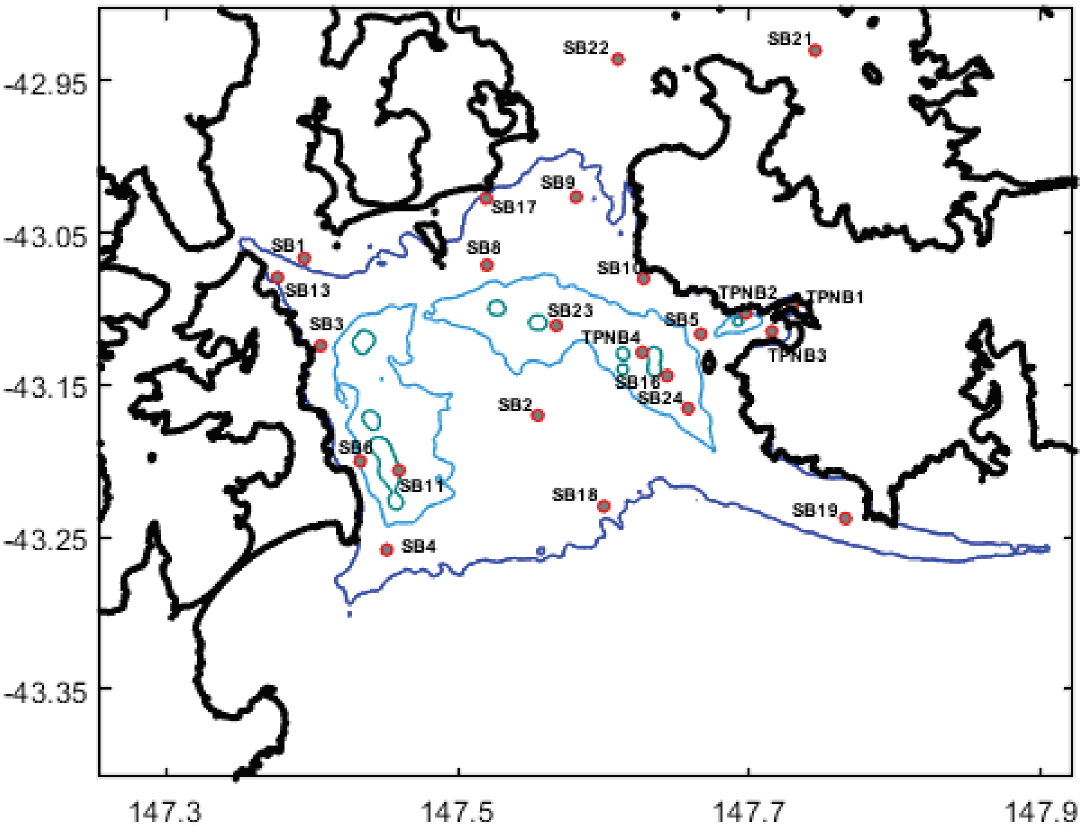

Figure 1: Broad-scale sampling sites for the Storm Bay region and modelled CONNIE3 annualised dispersion output of dissolved nitrogen (ammonium) for surface waters (0-15m depth range) showing the 95th (green), 80th (aqua) and 50th (dark blue) percentiles based on an annual 40,000 tonne biomass. Background concentrations for Storm Bay were supplied by the EPA.

Table 2: The current Storm Bay broad-scale sampling sites, categorisation (near scale, intermediate or far-field) and overlap with previous studies.[1]

Site	**Scale**	**CSIRO (1985-1989)**	**IMAS (2009-2015)**	**EPA (2018)**
BEMP-SB7*	Far-field			
BEMP-SB12*	Intermediate			
BEMP-SB14*	Intermediate			
BEMP-SB15*	Intermediate			
BEMP-SB20	Intermediate			
BEMP-SB11*	Near scale			
BEMP-SB8*	Intermediate			x
BEMP-SB4*	Intermediate			x
BEMP-SB3*	Intermediate			x
BEMP-SB13*	Intermediate			

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[1] * indicates sites that are currently being sampled

BEMP-SB9*	Far-field		x	x
BEMP-SB1*	Far-field		x	x
BEMP-SB5*	Intermediate		x	x
BEMP-SB2*	Far-field	x	x	x
BEMP-SB6*	Intermediate		x	x
BEMP-SB10	Far-field			
BEMP-SB16*	near scale			
BEMP-SB17	Far-field			
BEMP-SB18	Far-field			
BEMP-SB19	Far-field			
BEMP-SB21	Far-field			
BEMP-SB22	Far-field			
BEMP-SB23	Near scale			
BEMP-SB24*	Intermediate			
NUB1*	Near scale			
NUB2*	Near scale			
NUB3*	Intermediate			
NUB4*	Intermediate			

Local scale – The indicative sampling program suggested that local scale sites should be focused on peak production. More specifically, sampling is undertaken monthly for 3[2] months, at the time of peak production, at four sites, along a transect, at distances of 0 m, 10 m, 50 m, and 200 m from the salmon cage in the direction of the dominant current flow. Project FRDC "2015-024: Managing ecosystem interactions across differing environments: building flexibility and risk assurance into environmental management strategies" has highlighted the value of high resolution *in-situ* mapping of the nutrient footprint as a more effective and informative method for evaluating the nutrient footprint from farms (see Box 1). In the Storm Bay project, we will compare this method against the more traditional sampling approach described above to inform recommendations on the monitoring program.

3.4. Water column parameters

Temperature, salinity, fluorescence, pH, dissolved oxygen and turbidity are measured (full water column profile) at each site using a Seabird SBE 19 plus Conductivity Temperature Depth (CTD) or a Yeokal CTD. The CTD is programmed to measure parameters every second on the down and upcasts. At each site, CTD and water samples measurements are reported on at 0.5 – 1 m below the surface, at 10 m depth, and within 1 m of the seabed.

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[2] the transect site adjacent to the lease (200m) will be sampled monthly to provide a more complete temporal signal across the year.

Discrete water samples are collected, using Niskin bottles. Subsamples are taken at the same depth as the CTD for nutrients (unfiltered and filtered), including ammonia, carbon in water, carbon in water dissolved, nitrate, nitrate and nitrite, total nitrogen, nitrogen total kjeldahl, phosphorus dissolved reactive, phosphorus total, and silica molybdate reactive. Integrated water column samples for chlorophyll *a* and phytoplankton analysis are collected from 12 m depth to surface using a weighted Lund tube ("snake").

3.5. Environmental performance assessments

The EPA is responsible for regulating the salmon industry in Storm Bay. For water quality, this includes assessing environmental conditions against investigative trigger levels at a compliance site in the Environmental Licences. The current Environmental Licences for Storm Bay (West of Wedge 10211/1 and Yellow Bluff, 10180/1) provide water quality investigative trigger levels for selected parameters at their respective compliance sites (SB5 and SB3, Table 3). These trigger limits were calculated based on the 20th and 80th percentiles from data previously collecting in areas surrounding the West of Wedge and Yellow Bluff leases. For West of Wedge the trigger levels were derived from data from seven sites (SB5, NUB3, NUB4, SB2, SB7, NUB1, NUB2) collected between February 2014 to June 2019 (see Figure 1). For Yellow Bluff the data from three sites (SB3, SB6 and SB1) collected between January 2018 and June 2019 (see Figure 1). The research program investigated the water column measurements at the compliance site and more broadly across the entire Storm Bay, to assess the suitability of using the current approach for assessing performance.

Performance against the trigger levels is currently assessed using the rolling annual median value (i.e. the middle value of 12 monthly measurements) for each of the parameters measured at the compliance sites. If the rolling annual median of any of the parameters exceed the investigative trigger level at the compliance sites, the Licence holder is required to undertake additional investigations and analysis of other environmental data to determine to what extent the exceedances are caused by marine farming operations.

Considerations for investigative trigger levels and their interpretation

In the first instance, it is important that the monitoring can detect any exceedance of the investigative trigger levels at the compliance sites. Ultimately the monitoring program should also have the ability to determine whether the exceedances are caused by marine farming operations. As such, changes at the compliance site need to be considered in the context of other sources of spatial and temporal variability. Here we describe how

Table 3: Trigger limits for the Storm Bay Environmental Licences (10211/1, 10180/1) compliance sites (West of Wedge SB5 and Yellow Bluff SB3).

Parameter	Yellow Bluff Trigger levels	West of Wedge Trigger levels
Ammonia (surface)	6.0 µg/L	9.0 µg/L
Ammonia (bottom)	10.0 µg/L	17.0 µg/L
Total Nitrogen (surface)	308.0 µg/L	333.0 µg/L
Total Nitrogen (bottom)	330.0 µg/L	340.0 µg/L
Nitrite & Nitrate (surface)	38.6 µg/L	15.0 µg/L
Nitrite & Nitrate (bottom)	41.8 µg/L	35.0 µg/L

Total Phosphorus (surface)	48.0 µg/L	40.0 µg/L
Total Phosphorus (bottom)	40.0 µg/L	40.0 µg/L
Dissolved Reactive Phosphate (surface)	12.8 µg/L	8.0 µg/L
Dissolved Reactive Phosphate (bottom)	14.0 µg/L	12.0 µg/L
Oxygen (surface)	7.7 mg/L (lower limit)	7.8 mg/L (lower limit)
Oxygen (bottom)	7.1 mg/L (lower limit)	7.5 mg/L (lower limit)
Chlorophyll a	1.1 mg/m^3	3.6 mg/m^3

comparisons with data from the other sampling sites will help identify the likely scale of change and thereby determine whether the change is likely due to farming or other sources of variability (see Figures 2 and 3).

The challenge with using a single site for compliance is that it may either miss or detect an exceedance of the trigger values through time due to random chance, and as such comparison with other sites is essential for interpreting any change/exceedance. The first stage of investigation should be to compare the compliance site to other sites in the intermediate area (<5 km) of the lease/s of interest. In the example of the Yellow Bluff lease, we might compare the pattern at SB3 (compliance site) which is ~1.2km from the lease with sites SB12, SB13, SB14 and SB15 that are between 3 and 5km from the lease (Figures 2 and 3). If the pattern is only observed at the closer compliance site, it suggests a localised effect that may be farm related, but given it is only a single site, additional gradient sampling on a transect and/or dispersion modelling (e.g. CONNIE) would be required to identify the likely cause and the spatial extent of change (Figures 2 and 3). Note, the local scale sampling and *in situ* mapping described below will help provide the local scale context when interpreting patterns seen at the compliance site.

If a similar pattern to the compliance site is observed at the other sites, then we suggest the next step would be to compare these sites against the most appropriate far-field (i.e. >5km) reference sites for that region; in this case we might consider SB4, SB7, SB2 and SB9 for comparison (Figures 2 and 3). If the pattern is only observed at the sites in the intermediate area of the lease, additional data and or modelling would be required to understand the likely cause of the change, whereas if the pattern is shared with the far-field sites this would indicate a broader scale system change. In this case other data sources would be used identify whether the change reflects mesoscale oceanography. Long-term data sets (e.g. the Integrated Marine Observing System (IMOS) Maria Island and Storm Bay site SB2), and the more oceanic reference sites for the other BEMP programs in the south east and the Storm Bay BGC model that is nested in the SE Australia regional model will be pivotal in interpreting the drivers and scale of change (Figures 2 and 3).

It is important to recognise that by using a rolling median for compliance only sustained high (or low for parameters like oxygen) values will lead to exceedance of the investigative trigger levels. As Goudey (1999) notes, multiple months can be higher or lower than the trigger level, but the site may still be compliant. This is implicit in deciding to use a rolling annual median and ensures that investigations are not unnecessarily triggered due to occasional low-level spikes in the data, which could be linked to external influences. However, the influence of salmon farming on the water column and surrounding habitats is also likely to vary seasonally depending on the timing of peak production. Trigger limits based on the annual rolling median may not detect these seasonal changes. Hence, the ANZECC recommend the development of seasonal trigger values for indicators that exhibit seasonal variation.

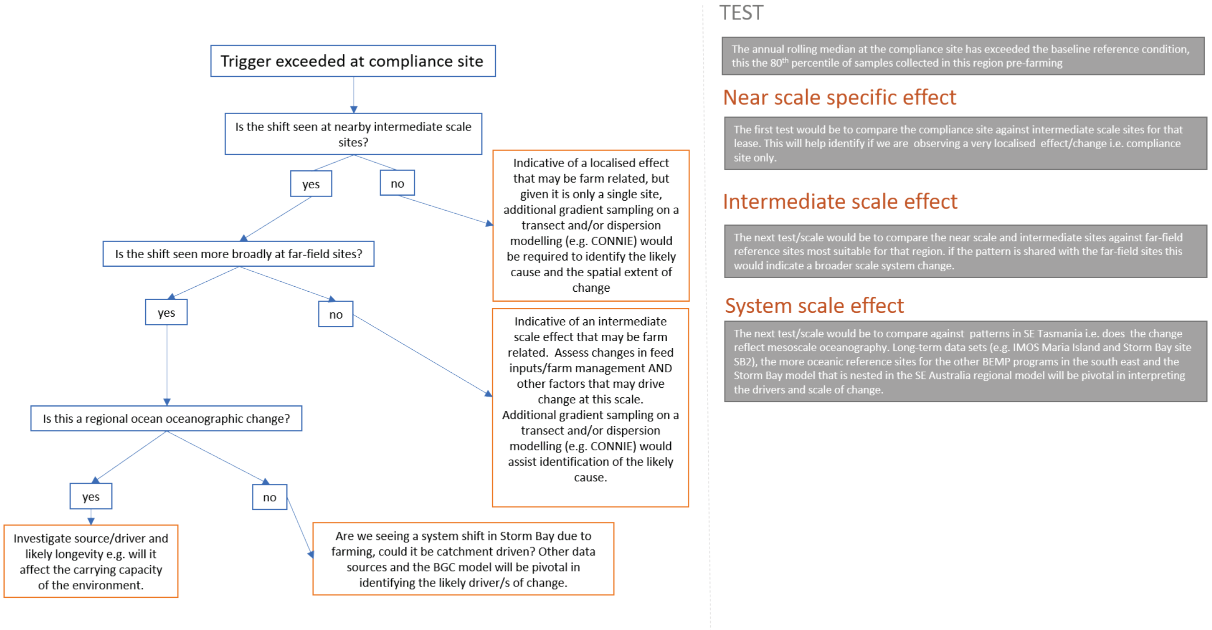


Figure 2: Flow chart showing *proposed* tests of the interactions between salmon farming and the water column at different scales should an investigative trigger be exceeded.

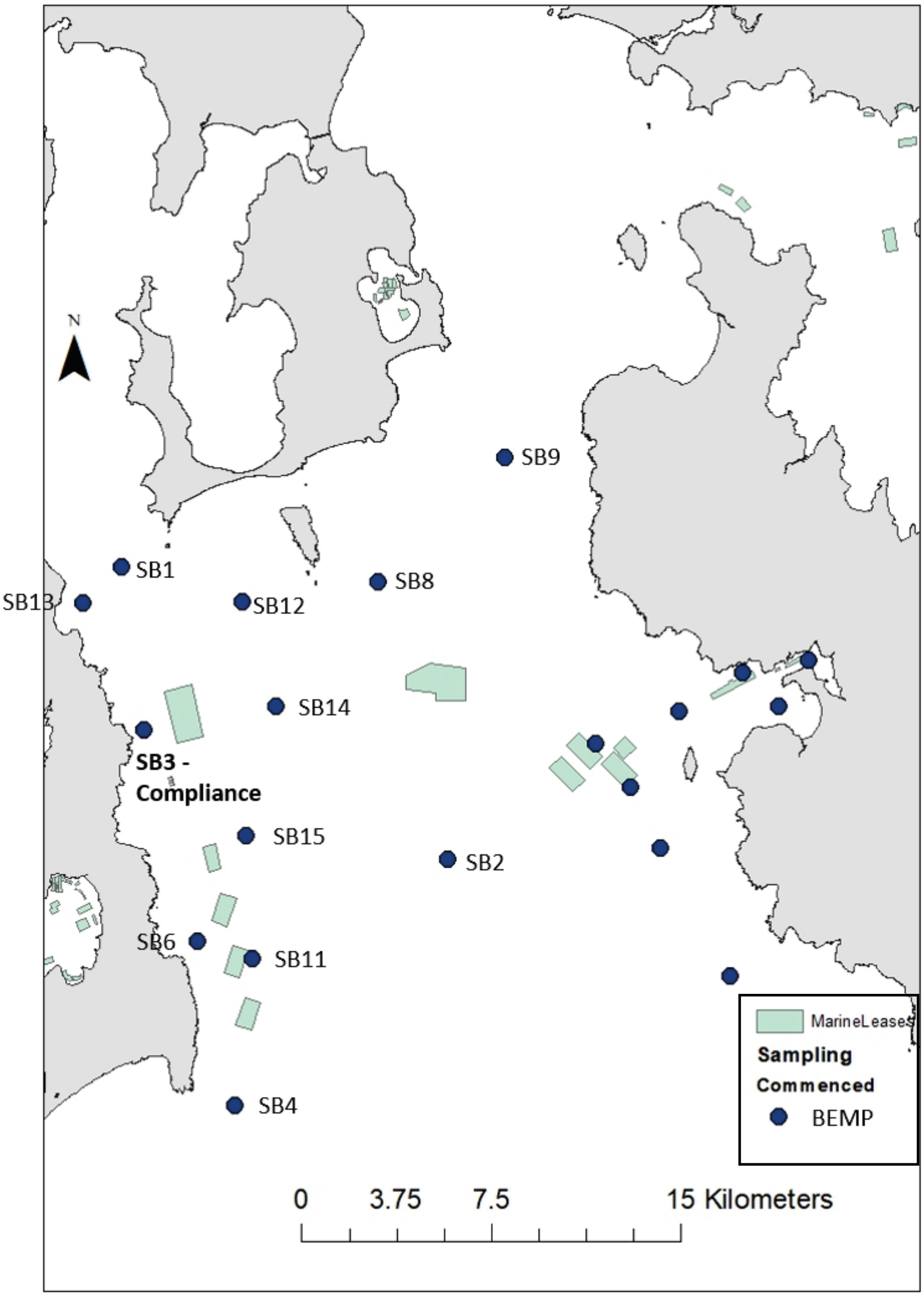


Figure 3: Map of the current sampling highlighting the sites for which the *proposed* tests of the interactions between salmon farming and the water column, should an investigative trigger be exceeded (e.g. at Yellow Bluff compliance site SB3).

BOX 1 Mapping the nutrient footprint

One of the challenges with environmental monitoring programs designed to test the influence of salmon aquaculture activities on the water column has been the absence of accurate *in situ* sensors for measuring nutrient concentrations in seawater (Jansen, Reid et al. 2016). The process of collecting discrete sampling is simple. However, discrete sampling can result in an underestimation of the concentration of key nutrients such as soluble phosphorous and nitrogen, in particular ammonium, which can decline quickly through time due to rapid cycling (Pitta, Tsapakis et al. 2009). Moreover, collecting an appropriate number of discrete samples in time and space to adequately capture the spatial and temporal scale of interest, or to attribute cause and effect relationships to aquaculture, can be difficult. This is because fish husbandry, hydrodynamics and site characteristics are all important sources of variation.

Through FRDC project "2015-024: Managing ecosystem interactions across differing environments: building flexibility and risk assurance into environmental management strategies", it became clear that reducing the time between sample collection and sample analysis was key to achieving more accurate results for ammonium. In-line sampling using two Systea WIZ systems was trialled during FRDC project 2015-024, which allowed for direct processing of samples on the vessel every 15 minutes. With increased sampling effort and faster processing times, the production of nutrient maps in the vicinity of farms was possible (see example Figure B1). Developed in conjunction with CSIRO, the sampling program in this project will use a nutrient auto-analyser adapted for portable use on small vessels and discrete samples to measure the concentrations of surface ammonia and nitrate in-line, with the capacity to take one sample every 3 minutes when fully operational. This instrument can be used to achieve reasonably high spatial resolution and the ability to assess results while on the water. The results will be used to produce high-resolution maps of the nitrate and ammonia footprint around the farms. Note, to ensure compatibility with concentrations measured in the broader monitoring program by Analytical Services Tasmania (AST), we will also include a comparison across laboratories for the discrete samples in the mapping exercise.

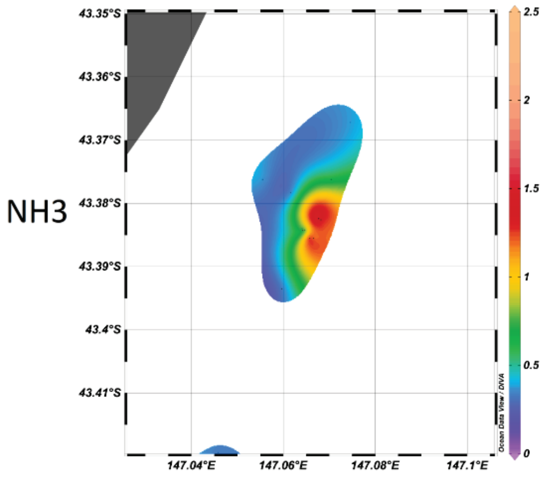


Figure B1: Example of isosurface data plot of NH_3 (range 0-2.5 µM) and NO_x (range 0-2 µM). Samples were collected *in situ* and discretely. through Project FRDC 2015-024.

3.6. Progress to date

We have completed broadscale sampling surveys across Storm Bay at 20 sites in 2019-2020 and three local scale sampling surveys at the Yellow Bluff lease. The local scale sampling was conducted along a transect at distances of 0 m, 10 m, 35 m and 200 m from the pens across three months. Data from the broadscale sampling is used for monthly reporting as required by the respective Environmental Licences. The focus of upcoming analyses will be on testing the effectiveness of the broadscale/local and mapping sampling designs to determine change across key metrics.

4. Soft-sediment

4.1. General background

Salmon farms are mostly situated above soft-sediment (sand, silt and mud) and therefore environmental monitoring programs have primarily focused on assessing any changes in the chemistry and/or benthic fauna of these habitats. Organic enrichment and increased sedimentation derived from salmon farming may alter benthic community assemblage (Wildish, Hargrave et al. 2003, Hargrave 2010), and enhance anaerobic activity, resulting in the accumulation of sulphides with adverse effects on aerobic bacteria, and other organisms due to progressive oxygen depletion (Hamoutene 2014). In addition, salmon farming has the potential to act as a vector for the spread of non-indigenous species into new areas and could alter the distribution and abundances of mobile invertebrate predators and scavengers when they are attracted to waste (Woodcock et al. 2018, Bannister et al. 2019).

An important element of managing the benthic response to farming is to ensure that conditions immediately under cages facilitate the efficient break down and assimilation of waste. In Tasmania, a feature of these conditions is the dominance of opportunistic animals such as capitellid worms and nebaliid crustaceans (Macleod, Moltschaniwskyj et al. 2007). The rotation of cages within fish farm leases and the subsequent fallowing of areas of the seabed is commonly used to allow the recovery of infaunal communities and to ensure that sediments conditions don’t deteriorate to a point that ecological function is significantly impaired thereby threatening the viability of farming operations.

The responses of soft-sediment environments to salmon farming will, however, vary depending on production levels, and under highly different hydrographic and sedimentological conditions (Macleod, Moltschaniwskyj et al. 2006, Macleod, Moltschaniwskyj et al. 2007). The research will focus on assessing the potential interactions of soft-sediment habitats with salmon farming both at the compliance sites and more broadly across Storm Bay.

4.2. Sampling design

To monitor the health and condition of soft sediment habitats in Storm Bay and the spatial extent of the benthic response to farming, the sampling will be conducted at two scales. At the broad (system) scale - 23 sites across Storm Bay have been identified (these are also in the environmental licences) which will be monitored annually (see Table 1); these sites overlap with the water column sampling sites (see Figure 1 and Table 2). To assess the extent of the benthic footprint at the local (lease) scale, sampling will be undertaken at sites that are at varying distances from the cage/lease. More specifically samples will be collected at 35 m from the lease boundary (compliance sites), reference sites (>1 km from the lease) and at four sites 0 m, 10 m, 35 m, and 100 m along replicate transects, in direction of the dominant current and other directions as needed (see Figure 4). The local scale sampling will be focused on peak production as this is the most likely time for maximum effects regardless of season.

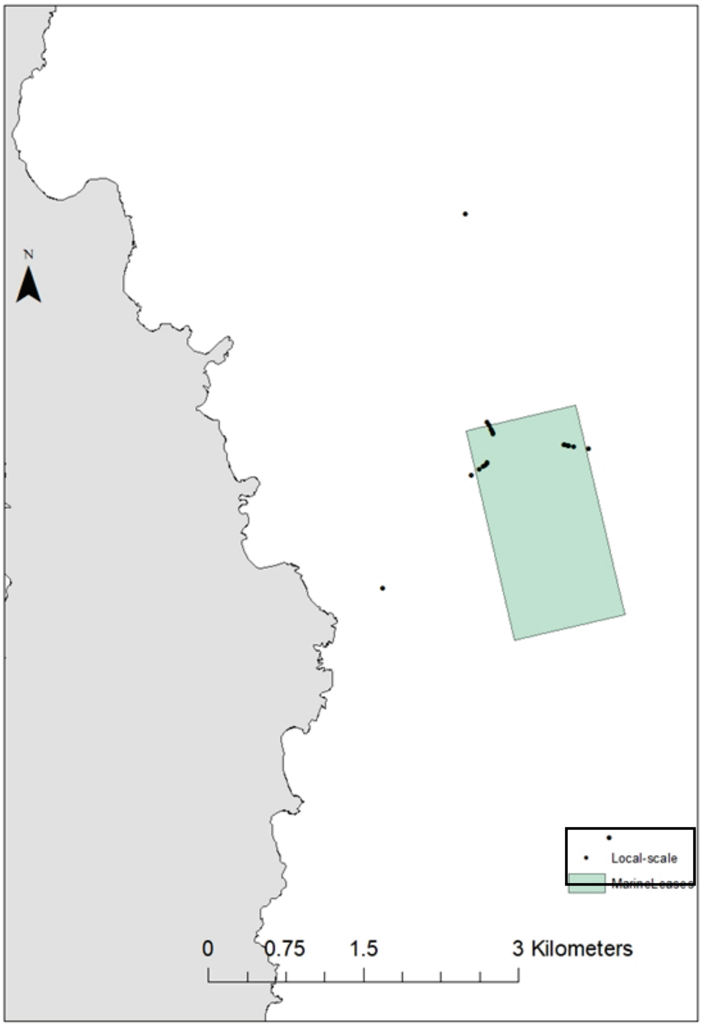


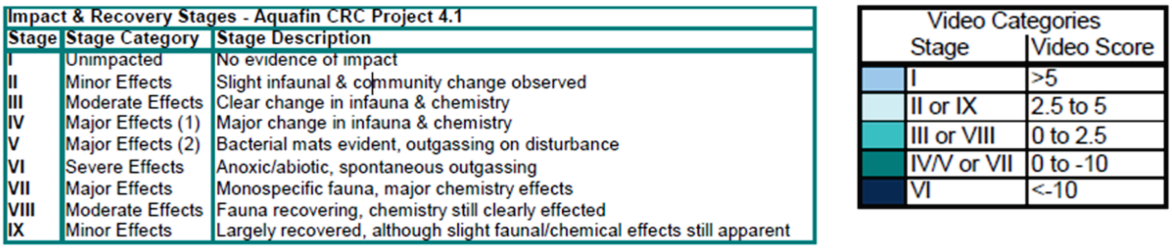
Figure 4: Map of the current local-scale sampling design for testing the interactions between salmon farming (e.g. at the Yellow Bluff lease) and soft-sediment environments.

4.3. Soft-sediment parameters

The health and condition of soft sediments will be assessed using an underwater visual assessment with a ROV and a quantitative benthic survey. These methods have been well established and validated in Tasmania (see Macleod and Forbes 2004). The visual assessment involves the collection of three minutes of footage at each site and sediment condition is assessed following the methods described by MacLeod and Forbes (2004) and outlined in Schedule 3V. The variables measured included numeric categorisation of sediment colour, density of bacterial mats (*Beggiatoa),* presence of gas bubbles coming from the sediment, feed pellets and faeces, farm debris, prevalence of burrows, tracks, worm tubes, and the abundance of key fauna (e.g. molluscs, ophiuroids, annelids, NZ screw shells, seastars). These measures are then used to score the footage for each site according to the key features that are determined to be indicative of impacted or unimpacted conditions as determined by MacLeod and Forbes (2004) (Table 3).

The quantitative benthic surveys include the collection of samples for both biological and physico-chemical characterisation. Benthic macrofauna are sampled in triplicate using a Van Veen Grab (surface area 0.0675 m^2). All samples are sieved to 1mm and the fauna identified to the lowest possible taxonomic resolution possible and counted**.** Sediment cores (250 mm long, 45 mm internal diameter) are collected to evaluate sediment sulphide, redox, particle size, organic carbon and nitrogen content and their isotopic composition ($\delta^{15}N$, $\delta^{13}C$). The methods of collection and analysis are as per those outlined in the environmental licence conditions and MacLeod and Forbes (2004). A visual assessment is also made of each core, including measurement of core length, sediment colour (using a Munsell soil chart), assessment of plant/animal life and assessment for gas vesicles and smell (indicating presence/absence of hydrogen sulphide). At each site, a profile of the physio-chemical properties of the overlying water column (dissolved oxygen, salinity, pH and temperature) is obtained using a water quality sonde, with measurements recorded continuously.

Table 3: The video assessment scoring system from MacLeod and Forbes (2004), showing the impact and recovery stages and corresponding video scores



Impact & Recovery Stages - Aquafin CRC Project 4.1		
Stage	**Stage Category**	**Stage Description**
I	Unimpacted	No evidence of impact
II	Minor Effects	Slight infaunal & community change observed
III	Moderate Effects	Clear change in infauna & chemistry
IV	Major Effects (1)	Major change in infauna & chemistry
V	Major Effects (2)	Bacterial mats evident, outgassing on disturbance
VI	Severe Effects	Anoxic/abiotic, spontaneous outgassing
VII	Major Effects	Monospecific fauna, major chemistry effects
VIII	Moderate Effects	Fauna recovering, chemistry still clearly effected
IX	Minor Effects	Largely recovered, although slight faunal/chemical effects still apparent

Video Categories		
	Stage	Video Score
■	I	>5
■	II or IX	2.5 to 5
■	III or VIII	0 to 2.5
■	IV/V or VII	0 to -10
■	VI	<-10

4.4. Environmental performance assessments

The current soft-sediment environmental indicators included in Environmental Licence are based on the recommendations of Macleod and Forbes (2004). These indicators include visual, physio-chemical and biological impacts (Table 4). Sampling for these indicators is conducted within the farm (visual impacts), compliance sites 35 m outside of the lease and at external reference sites.

Current licencing conditions outline that extensive sediment sampling (including the quantitative benthic sampling of physico-chemical and biological parameters) may be triggered when significant visual impacts are detected (e.g. the presence of fish feed, bacterial mats, gas bubbles or numerous opportunistic polychaetes on the sediment surface) at or extending beyond 35 m from the boundary of the lease.

Power analysis

In Tasmania, the number of sites monitored inside the farm, and outside the lease is not fixed. The current Environmental Licence conditions state that within the farm a minimum of six monitoring stations are required (this number may be increased by the regulator), while outside of the lease the number of compliance/reference monitoring sites are location specific based on the requirements of the EPA and informed by the recommendations of Crawford, Macleod et al. (2002).

Given the inherent variability in background conditions it is important to ensure that the monitoring program can detect meaningful change. The number of compliance/reference sites and replicates collected at each site are key factors that can influence the ability of a sampling design to detect change. Using the data collected in FRDC project 2015-024 we used a power analyses to assess the number of sites and replicates required to detect change at the compliance/reference sites, including the effect size (trigger limits) described in the the environmental licences (Els) (Table 4).

Table 4: Trigger limits for the Storm Bay Environmental Licences (10211/1, 10180/1).

Visual impacts:

- Presence of fish feed pellets.
- Presence of bacterial mats (e.g. *Beggiatoa* spp.).
- Presence of gas bubbling arising from the sediment, either with or without disturbance of the sediment, presence of numerous opportunistic polychaetes (e.g. *Capitella* spp., *Dorvilleid* spp.) on the sediment surface.

Physico-chemical

Redox

- A corrected redox value which differs significantly from the reference site(s) or is < 0 mV at a depth of 3 cm within a core sample.

Sulphide

- A corrected sulphide level which differs significantly from the reference site(s) or is > 250 µM at a depth of 3 cm within a core sample.

Biological:

- A 20 times increase in the total abundance of any individual taxonomic family relative to reference sites.
- An increase at any compliance site of greater than 50 times the total Annelid abundance at reference sites.
- A reduction in the number of families by 50 per cent or more relative to reference sites complete absence of fauna

As natural environmental variation renders some locations more susceptible to significant changes in parameter values, the above thresholds will be considered in addition to baseline environmental information for determining the presence/absence of a significant impact

Methods – Using data from FRDC project 2015-024 we calculated the minimum number of compliance/reference sites and replicates within sites required to detect changes (see below) with at least 80% power[3]. In this project, physio-chemical and benthic samples were collected at 3 compliance and 4 control sites, from 2 leases, at 3 or 4 time points.

The effect sizes tested were:

- 50x, 4x, 1.95x, 1.05x (e.g. 5000, 400, 95 and 5 %) increase in the abundances of Annelids or mean abundances of Annelids increases above the 80th and 95th percentile.
- 95% (-0.95), 50% (-0.5), 5% (-0.05) reduction in the number of families or mean number of families below the 5th and 20th percentile.

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[3] a significant effect is detected 80% of the time with the given effect size(s)

- 20x increase in total abundance of any family (examples included Capitellidae and Spionidae)
- Redox decreases to less than 0mV at 3 m depth
- Sulphide increases to 250 µM at 3 m depth

A mixed effects model was fitted to the data to estimate the means and the variances between sites and replicates within a site for each time point and at each lease. These means and variances were then used to simulate data at the control and compliance sites (with a normal distribution), under various sampling scenarios. One thousand simulations were made for each sampling scenarios such that the number of sites is 3, 5, 10 or 20, and the number of replicates at a site is 2, 3, 5 or 10 for all the different effect size listed above. A mixed effect model was fitted to each of the simulated data, and power was estimated as the proportion of simulations where a significant result was observed.

For the abundances of annelids and capitellids and sulphide concentration, a log transformation was made to fit the normal assumption. For the number of families, a generalised linear mixed model was used with a Poisson error distribution and a log link.

For all parameters (i.e. abundances of annelids, number of families, abundances of capitellids and spionids, and sulphide concentration and redox level), the results indicated that the biggest improvement in power was achieved by increasing the number of sites (x-axis) rather than increasing the number of replicates per site (lines of the same colour), at both leases and for most surveys (see Figures 5 to 8). For most parameters, a sampling design of 3 reference and compliance sites with 2 replicate samples within each site, was enough to detect the trigger limits listed in the EL requirements (see Table 4). However, for redox potential, the analyses indicated that more reference and compliance sites (3-10) were required to achieve 80% power. Similarly, for other smaller effect sizes (e.g. 5%, 95th percentile and 80th percentile increases in the abundances of annelids) a higher number of compliance and reference sites were required to gain 80% power. Overall, the results suggest that it is important to sample a minimum of 3 control and compliance sites, with 2 replicates to detect any potential unacceptable impacts of salmon farming on soft-sediment environments. Current sampling protocols typically specify triplicate samples are collected at a site; this would suggest that duplicates are enough, and the effort better allocated to additional sites.

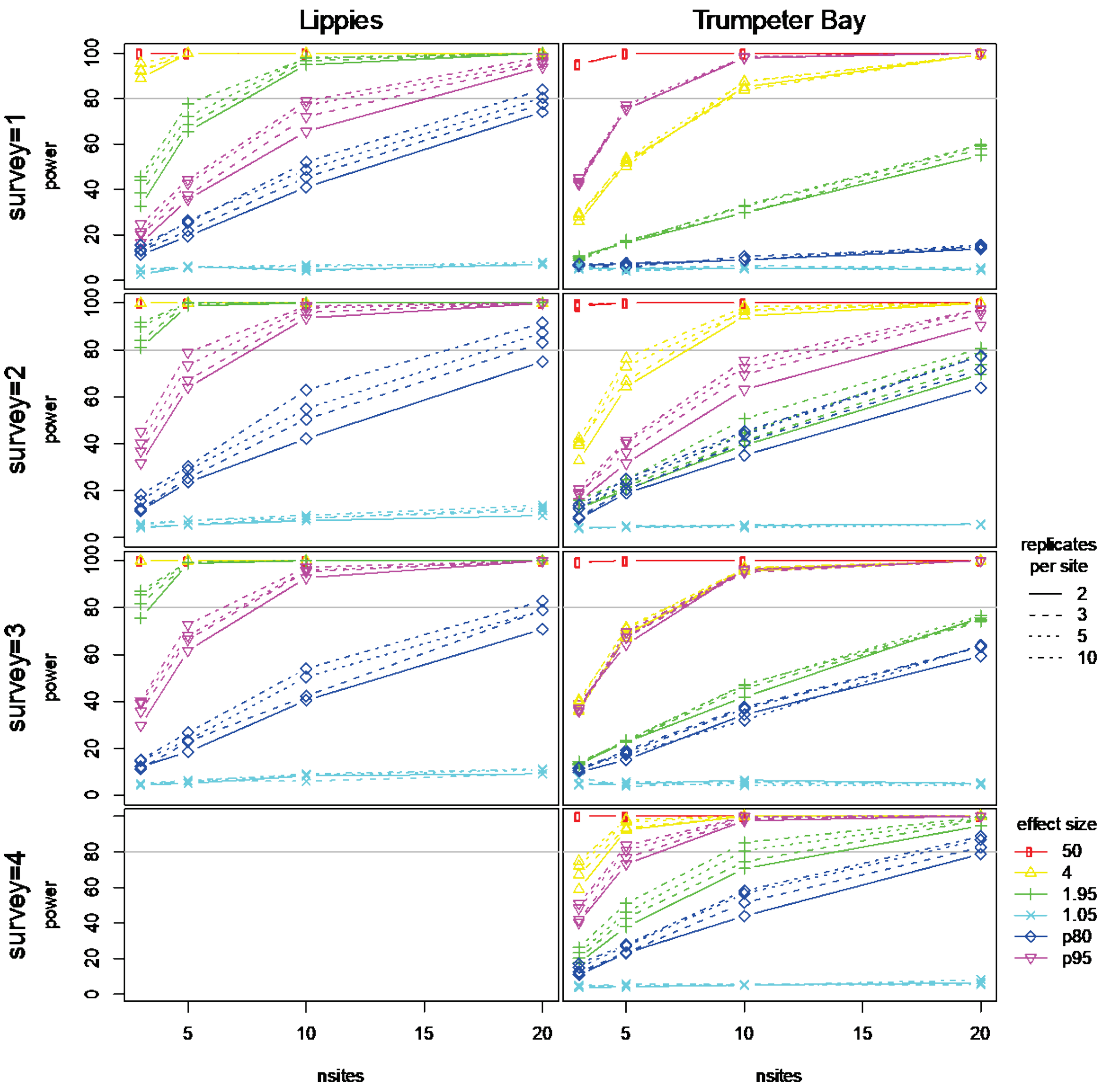

Figure 5: Results from simulation showing the power (y-axis) of increasing replicates (different line styles) and sites (x-axis) for different effect sizes (line colours) on the abundances of annelids. At both leases, more than 80% power for detecting 50x, 4x or 95% increase or greater in the abundances of annelids, was obtained with 3 control and compliance sites and 2 replicates within each site, at all time points.

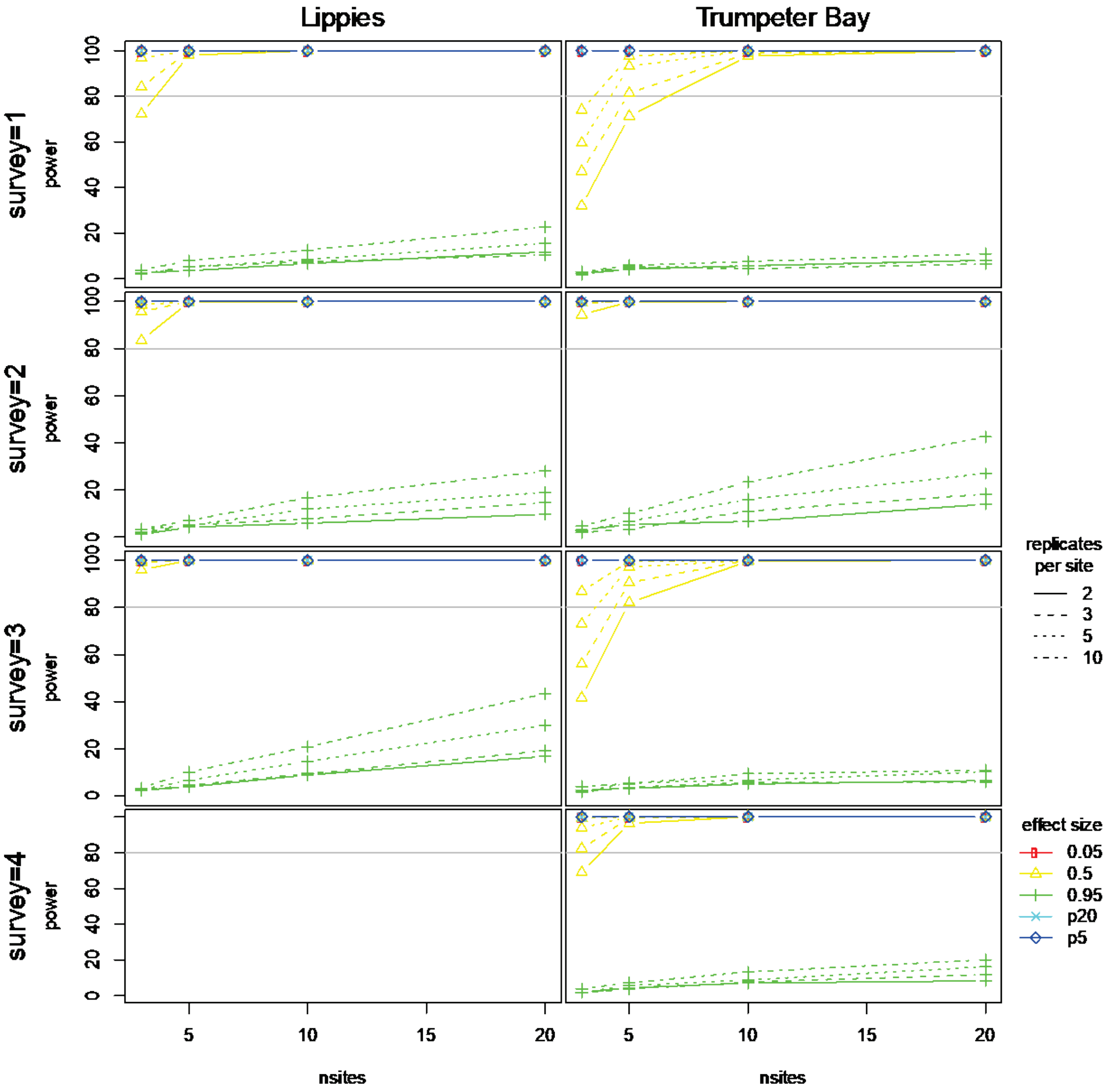

Figure 6: Results from simulation showing the power (y-axis) of increasing the number of replicates (different line styles) and sites (x-axis) for different effect sizes (line colours) on the number of families. At both leases, more than 80% power for detecting a 50% or greater decrease in the number of families was obtained with 3 compliance and control sites and 2 replicates within each site, for most of time points.

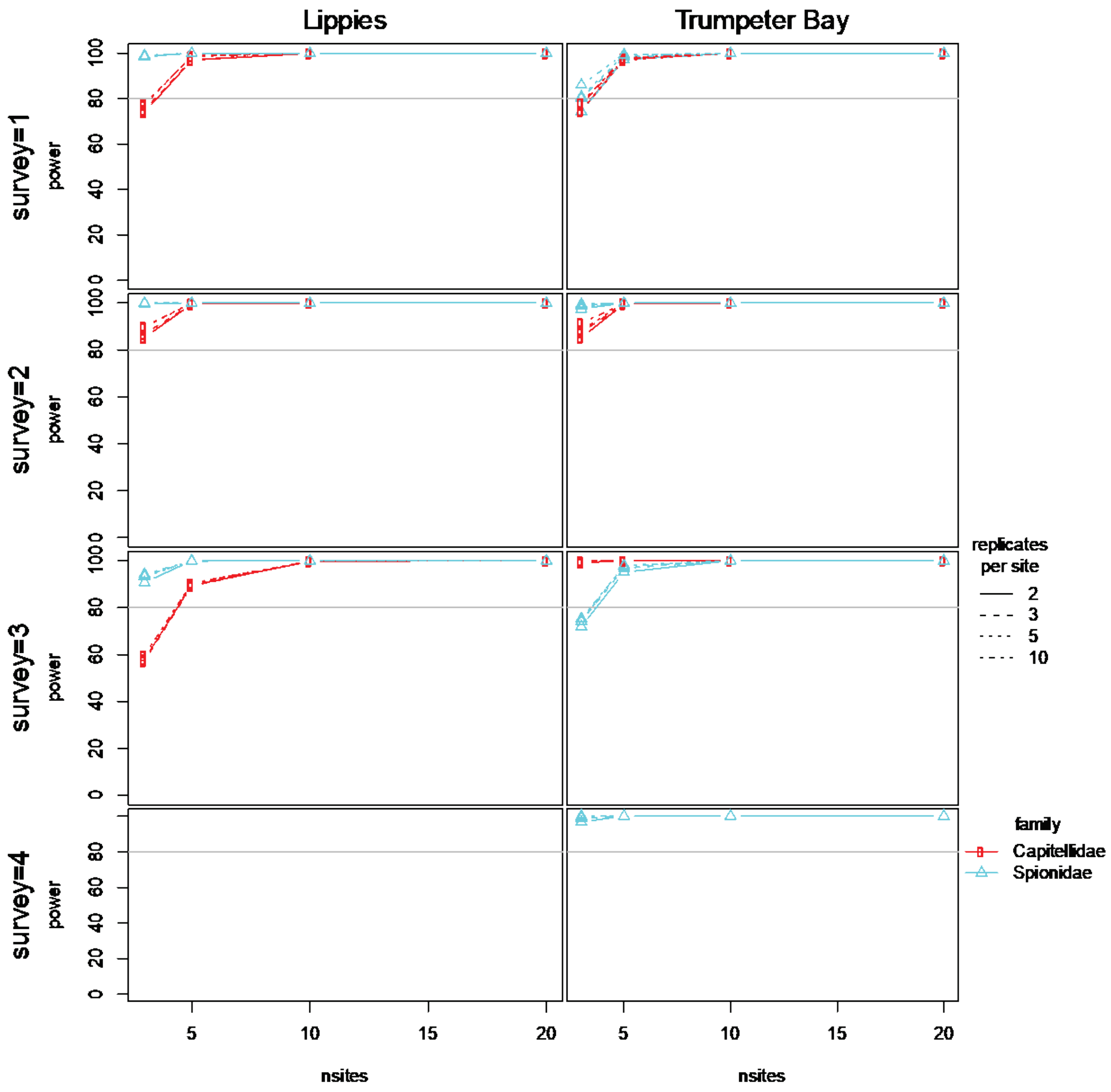

Figure 7: Results from simulation showing the power of increasing replicates (different line styles) and sites for different effect sizes (line colours) on the abundances of Capitellidae and Spionidae. A power analysis was not possible at Trumpeter Bay, survey 4 for capitellids as the model did not fit. At both leases, more than 80% power, for detecting a 20% or greater increase in the abundance of both families, was obtained with 3 compliance and control sites and 2 replicates within each site, for most time points.

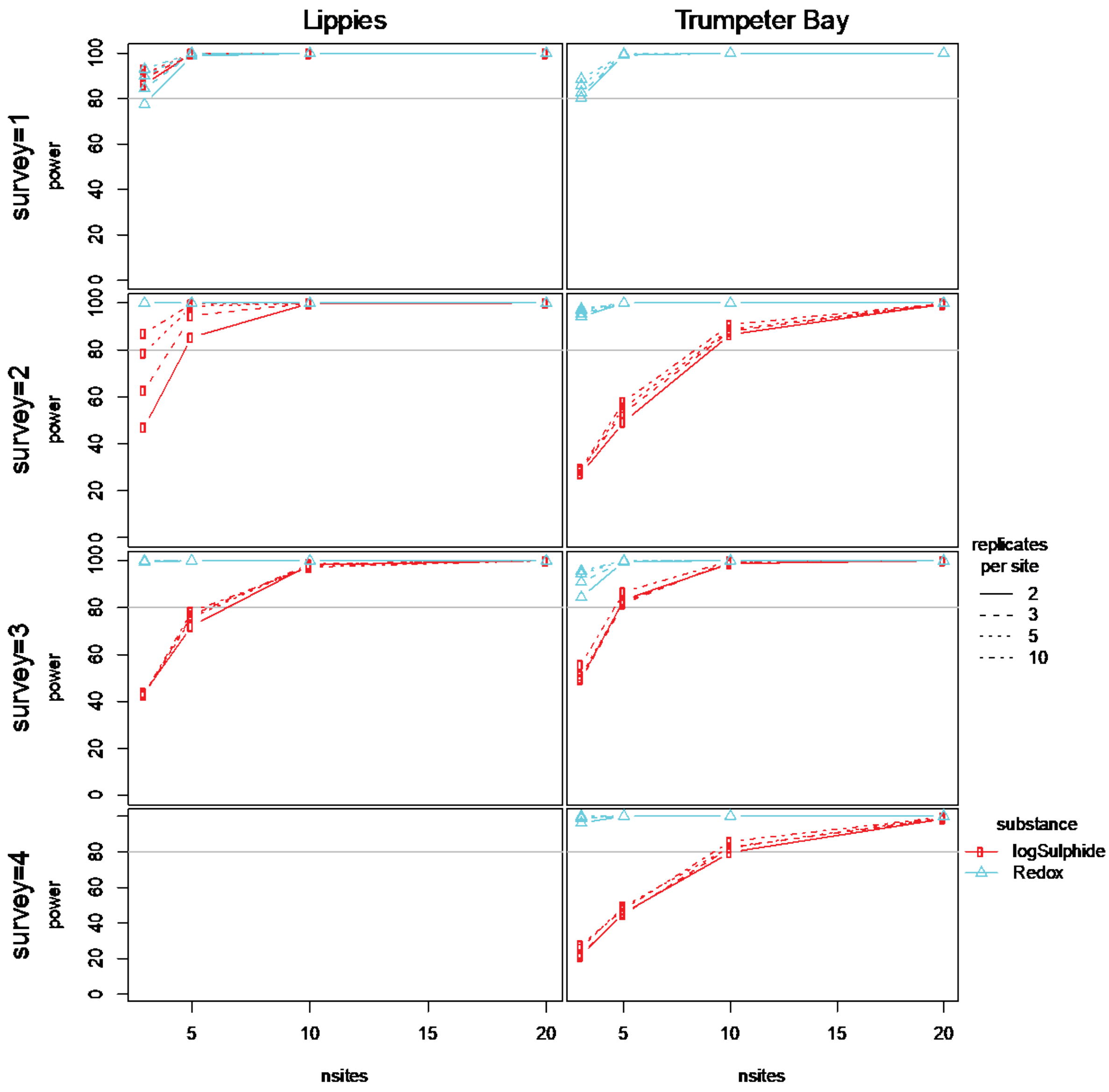

Figure 8: Results from simulation showing the power (y-axis) of increasing replicates (different line styles) and sites (x-axis) for different effect sizes (line colours) on the redox potential (mV) and sulphide (µM). A power analysis was not possible at Trumpeter Bay, survey 1 for sulphides as the model did not fit. At both lease sites, more than 80% power for detecting an increase in sulphides to greater than 250 µM, was obtained with 3 compliance and control sites and 2 replicates within each site. In contrast, between 3 and 10 control and compliance sites with 2 replicates at each site, were needed to obtain 80% power to detect a decrease in redox to below 0mV.

5.6. Progress to date

We have completed the broadscale survey of the 23 sites across Storm Bay in 2019. We have also completed the local scale sampling at peak production of the Yellow Bluff lease. This included four local scale transects at distances of 0 m, 10 m, 35 m and 100 m and eight compliance/four control sites in all directions from the lease. Data is currently being collated with analysis to be undertaken in upcoming months. The focus of analysis will be on testing the number of sites/transects/replicates to determine change across key metrics.

5. Inshore reefs

5.1. Background

Nutrients and organic matter enrichment can affect inshore reefs via several pathways, both direct and indirect (Figure 9). Excess nutrients can be taken up directly by algae but can also lead to increased sedimentation onto the reef through increasing water column productivity. Increased sedimentation can also occur directly through inputs of particulate organic matter (Figure 9). In some cases, sustained organic enrichment can cause a phase-shift in the reef ecosystem, with broadscale loss of the canopy-forming macroalgae and a replacement by turfing or opportunistic species (Eriksson, Johansson et al. 2002, Connell, Russell et al. 2008). Loss of canopy-forming species can potentially have catastrophic impact on reef biodiversity and function. Unfortunately, a management response often only occurs after widespread canopy loss, which is generally too late (Campbell, Marzinelli et al. 2014). Therefore, methods for monitoring the potential effects of salmon farm inputs on inshore temperate reefs need to be sensitive enough to detect a loss of resilience or impact of organic enrichment prior to canopy loss occurring.

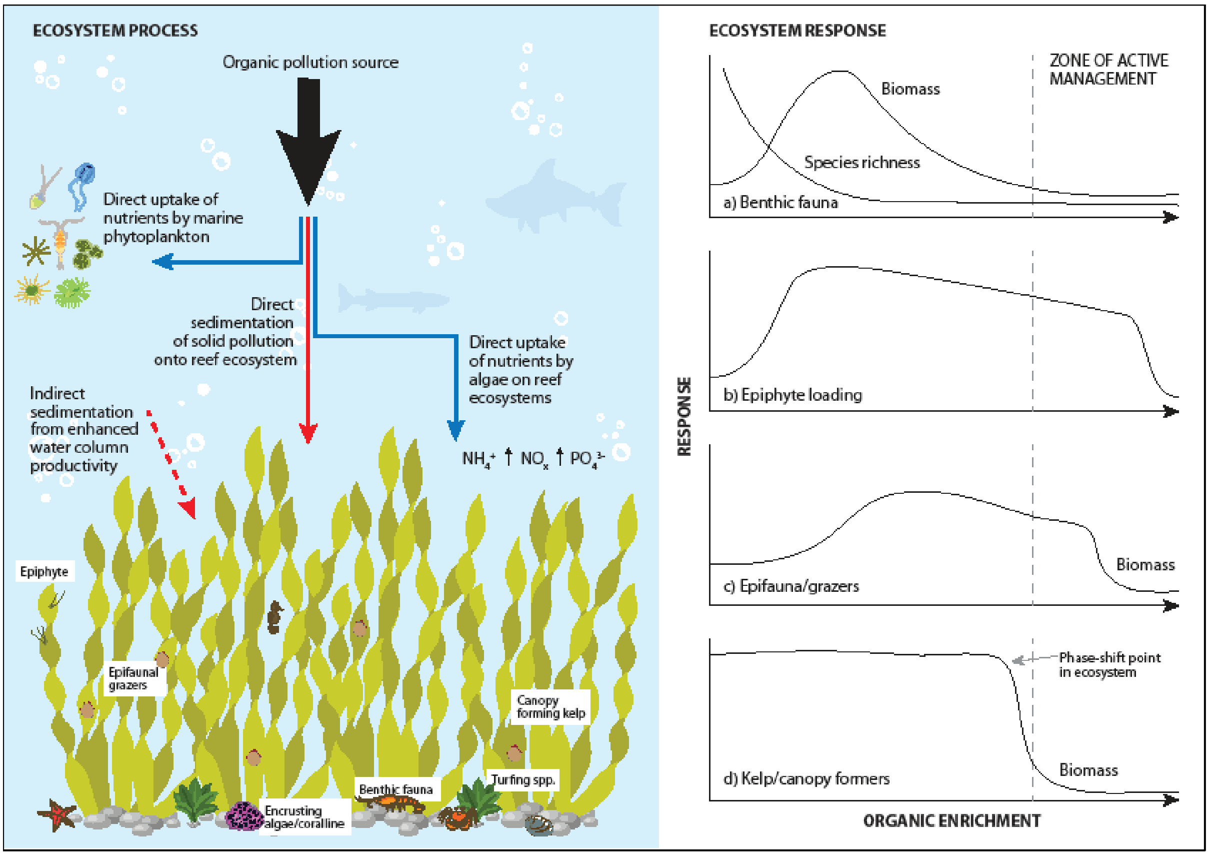

Figure 9: Schematic of potential pathways for impacts on temperate reef ecosystems through organic enrichment (from White et al. 2020a, *In prep*).

5.2. Sampling design and methods

To ensure that the sampling in this project can capture both early warning signs of organic enrichment, but also document broader ecological condition and change of reef ecosystems, two survey techniques will be used. The “Edgar-Barrett” method provides a full census of all fish, invertebrate and algae species at a given site, whilst the Rapid Visual Assessment (RVA) method targets functional groups and indicator species that have relevance to organic enrichment, thereby providing an early indication of organic enrichment on temperate reef ecosystems.

The biodiversity surveys operate primarily as an important baseline for the region documenting the abundance and diversity of all fish, invertebrates and algae. This survey method is designed to maximise the ability to detect i) changes in population numbers and size-structure, ii) cascading ecosystems effects associated with disturbance and iii) long-term change and variability in temperate reef assemblages (Edgar and Barrett 1997, Edgar and Barrett 1999). Briefly, this method involves a comprehensive visual census by diver of along a 200 m transect line, broken into 4 x 50 m sub-sections. All fish species within 5 m of the transect are counted and measured on both sides of the line. Invertebrates and cryptic fish within 1 m of the transect line are counted, with commercially or ecologically important species also measured. Algal diversity is assessed by point-count using a 0.25 m^2 quadrat placed every 10 m along the transect line (n = 20 for 200 m). To track ecosystem shift through time, Edgar-Barrett surveys are generally revisited at sites every 5-10 years.

As this method is consistent and detailed, it allows for unanticipated impacts on inshore reef communities to be examined, offering an invaluable reference to measure future change against, along with broad applicability for broad-scale monitoring programs (Valentine, Jensen et al. 2016). As the method has been consistently implemented across both Tasmania (including at sites in Storm Bay) and southern Australia for over 25 years, it is also possible to compare local results to a broader database and separate out local impacts (e.g. salmon farming) from more regional or broader-scale changes (i.e. climate change). While the value of Edgar-Barrett biodiversity surveys is without question, their ability to detect a loss of resilience or impact of organic enrichment prior to canopy loss is limited. While they will certainly detect when a phase-shift has occurred, they may be less sensitive to a loss in resilience, specifically to organic enrichment from salmon farming. A review of these methods in Valentine et al. (2016) concluded that a more targeted approach would be useful in assessing the impacts of salmon farming on temperate reef ecosystems in south-eastern Tasmania.

To that end, FRDC project 2015-024 developed a RVA survey that would detect change in ecosystem function due to organic enrichment (see White et al. 2020b, *In prep*). The RVA was designed to be complementary to the Edgar-Barrett surveys, and provide the means to rapidly assess functional change and loss of resilience due to organic enrichment. Over three years, this method was developed and tested, with a final scoresheet developed. Broad categories included functional groups such as canopy cover, sub-canopy cover (red, green, brown algae), epiphytic algae cover, filamentous algal cover and nuisance species associated with organic enrichment in the region (i.e. *Chaetomorpha billardierii*, *Asparagopsis armata*).

Sites were assessed using 1 m^2 quadrats at 12 fixed locations at any given site. This technique was designed so that shifts across several functional parameters could be detected simultaneously, providing a more complete picture of enrichment status of a reef than possible through the examination of singular parameters alone. The survey was also designed to be undertaken biannually, so that prolonged growth of enrichment associated species could be separated from acute ecosystem responses to pulse nutrient that occur seasonally, or with rainfall events. This requirement will be reviewed at the end of the project on the basis of 2-3 years of Storm Bay specific data.

5.3. Environmental performance assessment

The RVA method was primarily developed in the south-east D'Entrecasteaux Channel, where the rocky reef community is broadly similar to Storm Bay, to successfully detected and described a broadscale enrichment gradient (White et al. 2020b, *In prep*). Power analysis has highlighted the importance of small-scale variability at the individual site level in determining the ability to detect effects on the key assessment parameters. While FRDC project 2015-024 has demonstrated the utility of the RVA to detect change on key parameters (indicators) relating to organic enrichment, there is still a need to refine the most suitable indicator suite and their relevant thresholds to inform management. A critical element of this refinement will come through the collection of survey data across multiple years and sites in Storm Bay, after which the project will make recommendations for ongoing monitoring.

5.4. Progress to date

We have completed a baseline of Edgar-Barrett surveys (February 2019) and two rounds of RVA surveys in the western region of Storm Bay (August/September 2019 and February/March 2020) across 14 sites from Iron Pot in the north to Cape Queen Elizabeth in the south (Figure 10). In the eastern region, we have completed a baseline of Edgar-Barrett surveys at six sites in February/March 2020. Data is currently being collated with analysis to be undertaken in the coming months. The focus of analysis will be on spatial trend and capacity of the RVA method to determine change across key metrics.

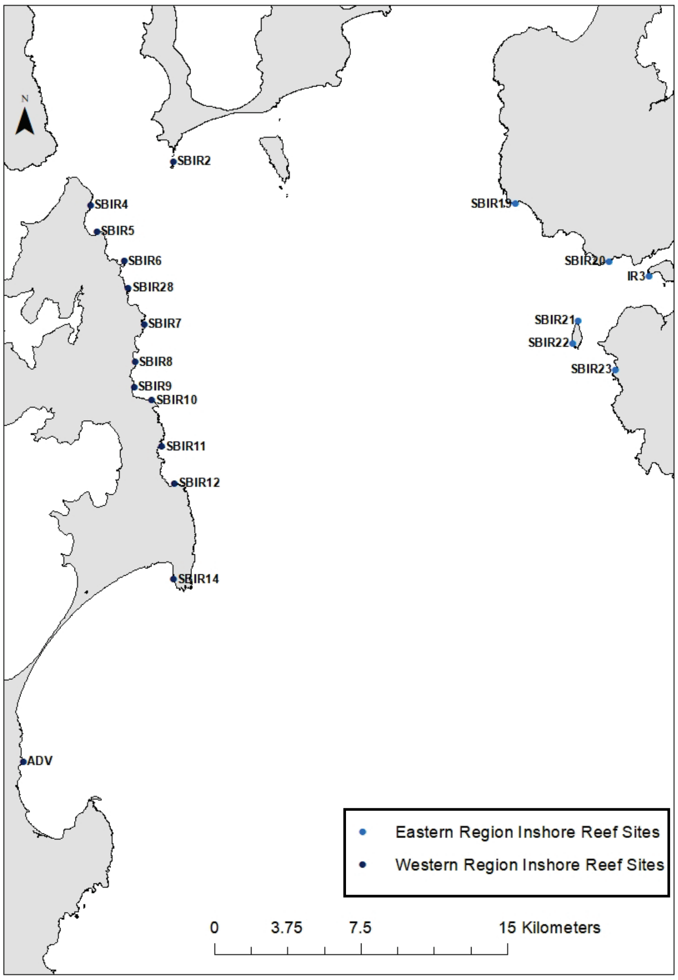

Figure 10: Sites for baseline Edgar-Barret and RVA surveys for monitoring of change in inshore reef ecosystems.

6. Deep reefs

6.1. Background

The release of nutrients and organic matter into the environment from salmon farms could have complex, direct and indirect effects, on the assemblage of the deep reefs. Different taxa can respond positively or negatively to the release of nutrients and organic matter. The direction and magnitude of effects will however, be influenced by quantity of nutrients and organic matter released, and the life stages of the affected organisms. This makes it important to develop a robust set of parameters and monitoring, to detect any changes in the assemblage of the deep reefs associated with salmon farming. In this report, we outline the proposed project sampling for deep reefs (see Figure 11 for overview).

Interactions with salmon farming may occur between the major functional groups and taxa of the deep reefs in Storm Bay through multiple pathways. Directly, the release of nutrients can favour the growth of green algal species (i.e. *Caulerpa*), reds (various species) and turfs (Burfeind and Udy, 2009; Liu et al., 2016). Under prolonged pressure these species may outcompete brown algae for space, particularly in low light conditions found in deeper water (Connell et al., 2008). Given all deep reefs within Storm Bay exist within the photic zone, these systems are likely to be sensitive to changes in the light regime. For example, the combined effects of excess nutrients and organic matter can induce phytoplankton blooms (Downing et al., 1999; Xu et al., 2014) and reduce the availability of light to the benthos further (Motegi et al., 2009) resulting in declines in the growth and survival of algae (Strain, Thomson et al. 2014) and negative impacts on the survival of phototrophic sponges (e.g. cup sponges), (Bell, McGrath et al. 2015).

Increased sedimentation, through the deposition of organic matter will also affect the deep reefs assemblages via a number of mechanisms (Bell, McGrath et al. 2015). At low levels, the deposition of organic matter may increase the supply of food for specific sessile invertebrate groups (e.g. erect and massive sponges, mussels, ascidians etc) (Simpson, 1984). At higher levels, sedimentation can smother the gametophytes or small juveniles of foliose brown algae (Airoldi 2003, Strain, van Belzen et al. 2015) and clog the filtration apparatus of sponges, with potential to inflict at least partial mortality on some sponge taxa (e.g. cup sponges), corals and other sessile invertebrates (Bell, McGrath et al. 2015). Indirectly, the loss of erect sessile invertebrates (e.g. sponges, ascidians and bryozoans) and brown algae could have flow on effects on other trophic groups which include species of commercial value, such as rock lobsters and abalone, which rely on these taxa for habitat and/or food.

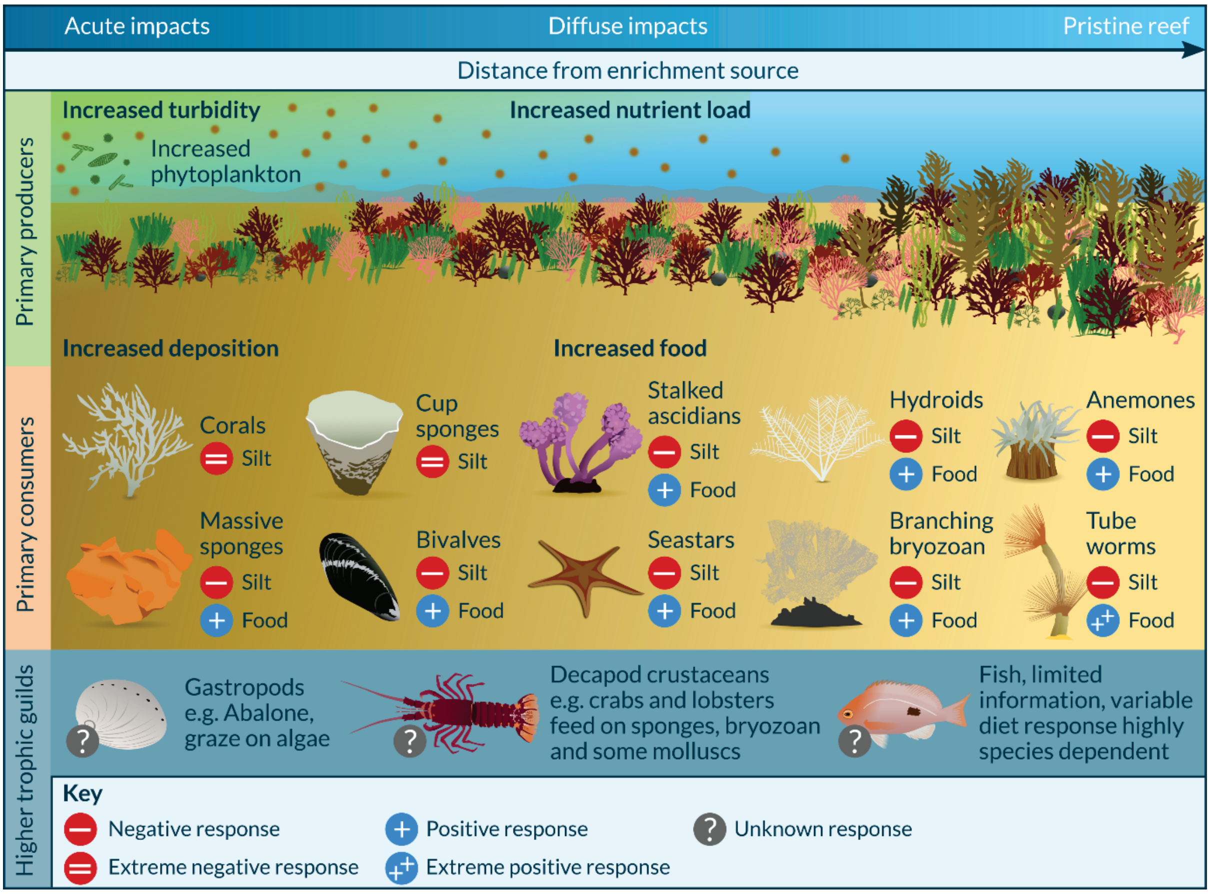

Figure 11: Schematic of potential pathways for impacts on deep reef ecosystems in Storm Bay through organic enrichment.

6.2. Methods for monitoring

Although monitoring techniques for shallow reefs are reasonably well established via scuba-based surveys and manipulative studies, the quantitative assessment of deep reefs is still in its infancy (Perkins, Hosack et al. 2019). Advances in digital imaging technologies are now beginning to address this knowledge gap. This imagery is typically acquired from platforms such as autonomous underwater vehicles (AUVs), ROVs, and imaging sleds that collect large volumes of high-quality benthic imagery over broad temporal and spatial scales (Perkins, Hosack et al. 2019). This imagery, and the ability to make repeated observations over time, will enable any changes to be monitored in deep-water benthic habitats and their associated biological populations (Sward, Monk et al. 2019). With strong baseline and time series data, any change within these ecosystems can be placed within the context of change to the salmon farming regime in Storm Bay and other external drivers.

An AUV is an untethered robotic platform that operates independently to complete pre-determined surveys. An ROV is a robotic platform that sends a live stream of data to a platform located on the surface which allows for remote operation. Overall, AUVs are more suited for survey operations, acquiring sensor data along pre-programmed transects, while ROVs are optimal for high-resolution, highly detailed and interactive work, including high-definition video surveying and physical sampling. The high manoeuvrability makes ROVs suitable for surveying a wide range of habitats (crevices, ledges and high relief). Hence, for this project ROVs will be used as the more suitable method to sample any changes in the diversity, relative abundances (MaxN), and body lengths of fishes along horizontal transects.

In contrast, the AUV is the preferred platform for benthic surveys. The AUV typically collects thousands of images on each deployment. This imagery is then subsampled and scored by overlaying random points on each image. The number of points that fall on a species of interest within each image is recorded which can then be summarized over the site. Images can be scored at a “morphospecies” level, with each distinct morphotype being given a unique classifier or broader morphological groups under the Collaborative and Automated Tools for Analysis of Marine Imagery (CATAMI) classification system (Althaus et al., 2013). Previous work has shown that for the dominant macroalgal taxa e.g. *Ecklonia* or *Caulpera*, scoring 50 points within every 20th image (10-30 m apart) along the transect should be sufficient to provide high precision in within site estimates of cover, for sampling programs being undertaken over multiple years (Perkins, Foster et al. 2016, Perkins, Foster et al. 2017). For rarer species e.g. certain sponge morphotypes or commercially fished mobile invertebrates, sampling using point count approaches are unlikely to provide enough precision and estimates of cover/abundances and length/sizes will thus be taken using the entire transect length.

6.3. Sampling design

Horizontal transects are a well-established survey strategy for providing standardized assessments of fish and benthic assemblages (Sward, Monk et al. 2019). The optimal transect length for Storm Bay will be a trade-off between the detection of enough species or morphological groups to capture an adequate representation of the deep reef communities and the capacity to collect multiple replicate transects where the size of the reef allows.

A horizontal transect length of 200 m has been selected initially, based on ongoing work by IMAS on the east coast of Tasmania. Data here suggests that a 200 m transect will adequately capture fish biodiversity at sites with standard diversity, will detect enough numbers of ecologically important species (e.g. Banded morwong, Boarfish) to be able to assess change in these species over time, and will provide a robust estimate of fish biomass. This transect length is also analogous to the MPA method (i.e. the “Edgar-Barrett” assessment) used to evaluate fish, invertebrate and algal biodiversity on inshore reefs (Edgar and Barrett 1997, Edgar and Barrett 1999). Based on this rationale, a 200 m transect length has been adopted into Environmental Licence requirements for the Storm Bay leases, although this will be reviewed for suitability as part of the Storm Bay project once robust data has been collected for these habitats.

While there is reasonable confidence in the suitability of a 200 m transect length to adequately capture fish communities, the scale needed to capture benthic parameters on deep reef is less well understood. In addition to transect length, the numbers of photo quadrats assessed on the transect line and method used to assess these quadrats (i.e. point count vs percentage abundance) needs to be assessed. Other points for investigation include the number of replicate transects required to monitor change in population or percentage cover through time.

In terms of sampling specifics, IMAS has recently invested in the acquisition of a Boxfish ROV (https://www.boxfish.nz/),, which will be ideal for successfully sampling these habitats. This ROV will have stereo 4K forward facing video cameras for the capture of fish communities (biodiversity and length estimates), with an approximate field of view of 5 m. It also has downward facing stereo 8MP cameras with an approximate footprint of 2 m^2 (depending on height of the ROV from the seabed) that will provide high quality benthic images. We can also capture benthic video for scoring invertebrates by attaching a downward facing video camera. The imagery datasets can be integrated into a USBL and vessel mounted differential GPS feed to obtain a spatial position of ±3 m of each observation. This data can then be linked with high resolution mapping data (also being collected in this project) to explore relationships with environmental factors such as depth or reef rugosity.

For each deep reef site, a 200 m horizontal transect will be captured using the Boxfish ROV. We will aim to collect 1 to 5 replicate transects per site, depending on the size of the reef being surveyed. Reefs to be surveyed include the four known reefs in proximity to the current operations, which are outlined in the Environmental Licences, as well as any additional areas of hard-bottom substrate identified through seafloor mapping undertaken as part of the project. Forward-facing stereo footage will be captured to assess fish communities, with all fish counted and measured along the transect line. Downward facing cameras will continuously capture still images. Analysis of benthic images will initially commence at every 10 m along the transect line (i.e. 20 x images per transect), with this number increased or decreased depending on subsequent analysis of benthic data. As the assessment method for mobile invertebrates is still being refined, data for key species will initially be scored using all three image capture methods (forward-facing video, downward-facing video, downward-facing still-images), with a decision made on the best way forward based on assessment of the data. To assess the natural variability and track any ecosystems shift, the sampling will be undertaken annually, during late summer over 3-5 years.

6.4. Progress to date

Preliminarily AUV and ROV surveys have been conducted by IMAS in Storm Bay. The AUV surveys, were also run through the NESP hub in 2015, data for which the project has access too. The ROV surveys, were completed for this project in March 2020. For the ROV surveys, four sites were surveyed (those currently stipulated in the Environmental Licence) with 1 x 200 m video transect collected at each site (Figure 12). On the Trumpeter Bay deep reef (SB15) we managed to collect an additional 4 x 200 m video transects and at Darts Reef

(SB7) an additional 2 x 200 m video transects. Preliminary observations from these surveys indicated that reef systems with abundant fish communities dominated by barber and butterfly perch were present, with substrate dominated by sponge communities, along with green and red algae, but no brown algae observed. Both sets of data will be used to determine the variation in distribution and abundances of the key taxa, to identify taxa which are likely to have the strongest interactions with salmon farming and to better inform the sampling design.

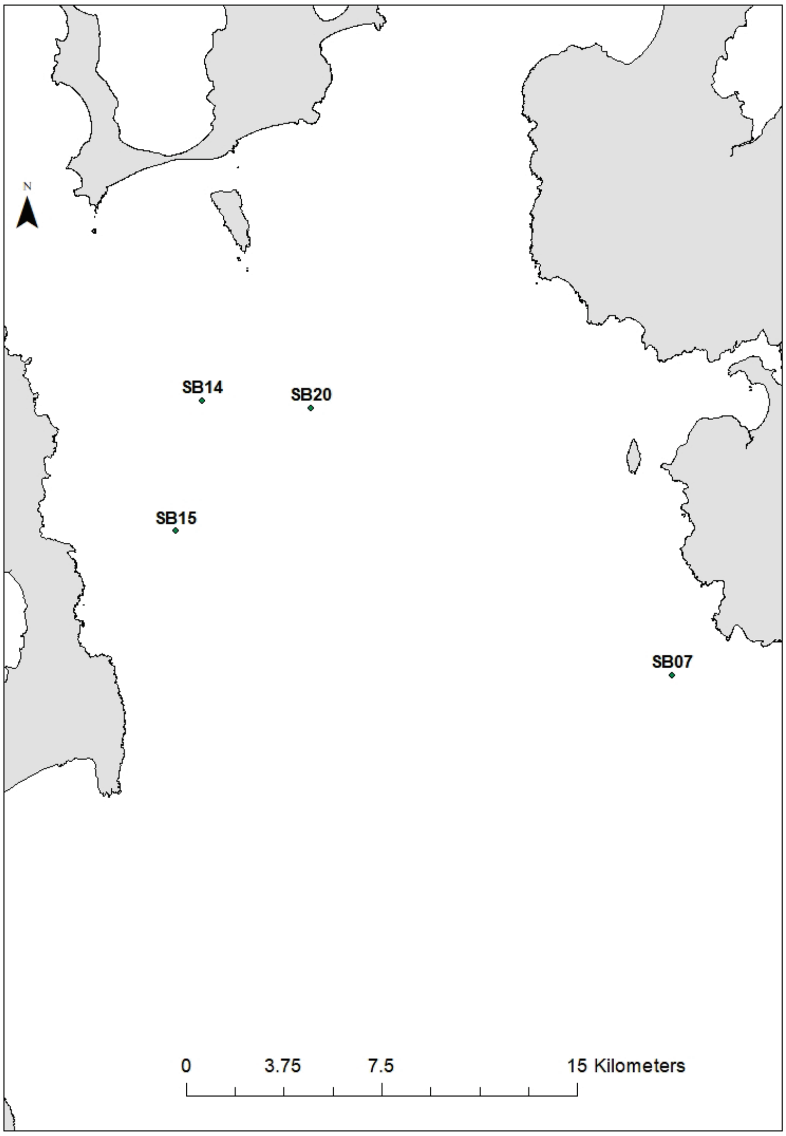

Figure 12: Sites where preliminary ROV transects were undertaken for the Storm Bay project in March 2020.

7. Seagrass

7.1. Background

Seagrass beds are amongst the most highly productive coastal marine ecosystems and are widely considered to be a sink for carbon and nutrients, including nitrogen and phosphorus. They are common in shallow waters, typically down to 20 m depth, where there is sufficient substrate and light to grow. The high primary productivity of seagrass beds in turn supports a large biomass of primary consumers, with these communities providing critical ecosystem services, such as the removal and recycling of nutrients, filtering of the water column and stabilisation of the seabed.

Seagrass beds are known to be susceptible to a range of factors associated with nutrient enrichment, with a general trend for decline globally and across numerous seagrass species in coastal areas subject to increased urbanisation (Burkholder, Tomasko et al. 2007). Decreased light availability is one of the most widely cited causes of seagrass decline, whether through increased shading through enhanced epiphytical algal growth, or increased turbidity of the water column. Understanding the interactions between a potential nutrient source and nearby seagrass beds is critical for management of these systems in the future.

There are numerous potential pathways for interactions with salmon farming on seagrass beds, both acute and diffuse. Alterations to the light regime is one of the better studied pathways and can include direct effects such as shading from cages and farm infrastructure, to more diffuse effects relating to increases of nutrients in the water column from farm outputs. These diffuse effects include the smothering of seagrass and competition for light resources from faster growing epiphytic algal species, as well as lower light penetration through the water column due to increased primary productivity in pelagic systems (Apostolaki, Marbà et al. 2009). There are also potential pathways for interaction between seagrass beds and solid components of fish farm waste, with higher inputs of organic matter into the sediments leading to sulphide invasion in the roots and rhizomes leading to higher mortality in plants (Frederiksen, Holmer et al. 2007).

Most research on the interaction between fish farms and seagrass beds has been undertaken on seagrass beds dominated by *Posidonia oceanica* around sea bream and sea bass cages in the Mediterranean. In contrast, seagrass beds in Storm Bay are dominated by species from the *Zostera tasmanica* complex. While the pathways of potential interaction are the same, the ecology of this species and therefore the potential response may be different. In general, *Posidonia* is slow growing, producing only 1-2 leaves per year have very slow rhizome elongation rates, with individual plants relatively persistent over a large temporal scale. In contrast, species from the *Zostera tasmanica* complex show higher growth rates across leaves, roots and rhizomes, produce seeds in much higher densities and while not ephemeral, turnover of plants occurs at a much higher rate than in *Posidonia* (Waycott, McMahon et al. 2014, Sherman, Smith et al. 2018). These biological and ecological factors may influence the resilience of *Zostera tasmanica* to interactions with fish farm waste, however, this project presents one of the first opportunities to examine these interactions in closer detail.

7.2. Methods for sampling

The seagrass sampling in this project will be broken down into two major components addressing a) the spatial dynamics of the major seagrass beds in Storm Bay, and b) the relative health of the seagrass within those beds. There are no major seagrass beds within the immediate vicinity of any fish farm lease in Storm Bay (i.e. seagrass beds are currently >20 km from an active lease) and therefore the aim of the project will be to assess health and distribution of seagrass beds within the broadscale environmental context.

It is anticipated that spatial extent of seagrass beds in Storm Bay is likely to be quite dynamic. Long-term data on *Zostera nigricaulis* (another species within the *Zostera tasmanica* complex) in Port Phillip Bay indicate wide fluctuations in distribution over 70 years of monitoring, generally without consistent pattern (Ball, Soto-Berelov et al. 2014). Localised change in seagrass within Port Phillip Bay have been attributed to fluctuations in response to nutrients and sedimentation, with moderate nutrient enrichment actually increasing bed size, but higher nutrient levels having detrimental effects (Jenkins, Keough et al. 2015). Even within the one species, seagrass beds in Port Phillip Bay showed high variation in their resilience to organic enrichment. Localised environmental characteristics are thought to influence this, with factors such as hydrodynamics, substrate composition and background nutrient levels all playing a role.

7.3. Sampling design

We anticipate a similar level of variability in seagrass in Storm Bay and thus, each November, in conjunction with sampling and assessment for Environmental Licence conditions, we will undertake a mapping exercise on each of the major seagrass beds (Figure 13). This will allow us to evaluate spatial dynamics of each of the major beds in Storm Bay over a 3-year period. We will use a variety of techniques to achieve this, including drop camera and ROV to thoroughly assess the geographical location of the bed edge every 12 months. We will also use outputs from the seafloor mapping being undertaken to the 5 m contour as part of the project which will allow for identification of any other seagrass beds and their extent.

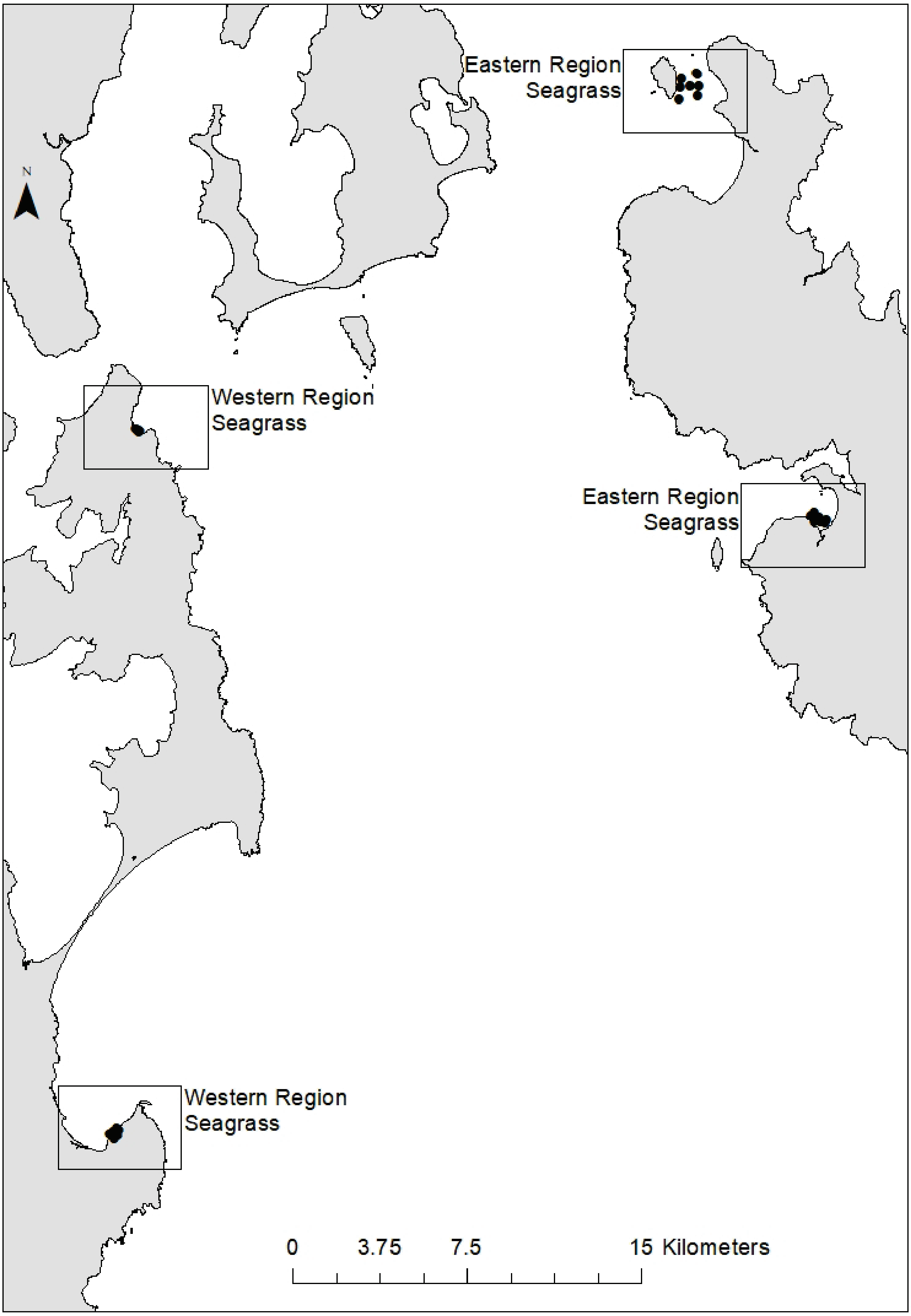

Figure 13: Map of the current sampling design for testing interactions between salmon farming and seagrass habitats.

For bed health assessment, initially we will commence by monitoring variables that reflect pathways of nutrient enrichment. Baseline surveys on seagrass beds in Storm Bay included total seagrass cover, total *Caulerpa* spp. cover and a qualitative epiphyte score. Total seagrass cover provides a measure of bed density, with patchiness and thinning of seagrass a sign of bed decline. In situations of over-stimulation by nutrients, seagrass can be replaced by algal species, such as *Caulerpa* (Burkholder, Tomasko et al. 2007). Therefore, changes in the cover of *Caulerpa* over time can be used as metric for nutrient enrichment. We will continue to monitor for epiphyte growth, quantitatively, rather than qualitatively. In the first year of monitoring, we will also evaluate several other parameters, including the presence of wrack algae, a more detailed breakdown on the types of epiphytic algae observed and the presence or number of any major invertebrate species.

Parameters will be quantified through standardized image analysis using CPCe software (Coral Point Count with Excel extensions) (Kohler and Gill 2006) where 50 random points were distributed on an image and the feature underneath each point identified by the user. This process allows us to score a wide range of parameters at very little extra collection effort. Images are captured using a drop camera set-up, with a downward facing standalone internet protocol (IP) camera (2.7-13.5 zoom lens, field of view 100 degrees, with Full High Definition capture). To quantify the images, the IP camera is attached to a 50 x 50 cm quadrat, which formed part of the drop-camera frame.

Initially, we will undertake the seagrass health assessment in conjunction with the Environmental Licence monitoring in November 2019, however, will look to increase this to quarterly or bimonthly sampling within the second year of the project to assess seasonal variation. On each transect, the drop camera will be deployed every 10 m (geo-referenced through Differential Global Positioning System (DGPS)) and an image captured for subsequent analysis. This will occur across 5-10 transects (including the Environmental Licence transects) on each of the major seagrass beds. After two years of annual sampling, we will examine statistical power on the data we have collected to make recommendations on its ability to detect changes.

Additionally, across the second and third years of sampling, we will look to explore biomarkers as an option for monitoring the health of seagrass beds. Increased tissue nitrogen as a result of nutrient enrichment has commonly been reported in seagrass species, with alternations to the C:N ratio reflecting changes in nutrient regime (Duarte 1990). Changes to δ^{15}N values can also be used to characterise enrichment status of a seagrass bed, particularly when normalised for morphological characteristics, such as leaf mass (Lee, Short et al. 2004). Through the project, we will look to investigate the use of these tools with the aim of linking changes observed in biochemical values with physical parameters collected through image analysis. While it is not expected that these biomarkers will be incorporated into routine monitoring in the future, they will be important in terms of helping us understand how fluctuations in the nitrogen regime may affect seagrass beds. This will help in the interpretation of data from any observational-based monitoring program in the future.

7.4. Progress to date

In November 2019 we undertook sampling in the western region of Storm Bay, corresponding to the location required under the Environmental Licence for the Yellow Bluff lease. Techniques for mapping extent of the seagrass bed were explored, with methods to be refined in the November 2020 sampling effort. Drop camera images were captured from 5 transects, with analysis on these images complete and data analysis underway. An expanded program for the second year of Yellow Bluff sampling is currently being explored.

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