Understanding and Mitigating Kelp Forest Losses Caused by Environmental Change in *Aotearoa* New Zealand

Bren School of Environmental Science & Management

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Signature Page

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The Group Project is required of all students in the Master of Environmental Science and Management (MESM) Program. The project is a year-long activity in which small groups of students conduct focused, interdisciplinary research on the scientific, management, and policy dimensions of a specific environmental issue. This Group Project Final Report is authored by MESM students and has been reviewed and approved by:

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Introduction

Kelp forests are “extensive underwater habitats” defined by large seaweeds that form canopies over the seafloor (Wernberg et al., 2019). Like trees in a forest, kelps provide food, shelter, nursery ground, and habitat for many organisms, such as the commercially important rock lobster and abalone (Cornwall et al., 2023). Kelp forests form crucial and biodiverse ecosystems, mitigate ocean acidification effects at local scales, reduce marine pollution via nitrogen and carbon sequestration, and boost local economies through eco-tourism (Cornwall et al., 2023).

Kelp forests are showing global declines and are under pressure from the direct and indirect effects of anthropogenic activities (Wernberg et al., 2019). Here, we focus on ocean warming and marine heatwaves (MHW), which can be correlated with reduced nutrient availability (Cavanaugh et al., 2021; Edwards & Estes, 2006; Zimmerman & Kremer, 1984). Ocean warming happens because the ocean absorbs vast quantities of heat due to increased concentrations of greenhouse gases in the atmosphere, mainly from fossil fuel consumption (Laffoley & Grimsditch, 2009; NOAA, 2023). The ocean has absorbed more than 93% of the excess heat from greenhouse gas emissions since the 1970s, causing ocean temperatures to rise (Laffoley & Grimsditch, 2009). MHWs are anomalous warm seawater events that can “substantially affect marine ecosystems” (Oliver et al., 2021). They can be caused by a combination of local oceanic and atmospheric processes such as “air-sea heat flux” and “horizontal temperature advection” (Oliver et al., 2021). The global count of MHW days per year has risen between 1925 and 2016 due to increases in MHW duration and frequency (Oliver et al., 2018). Researchers have found that global average marine heatwave frequency and duration increased by 34% and 17%, respectively, resulting in a 54% increase in annual marine heatwave days globally” (Oliver et al., 2021). This trend is projected to increase further under climate change as a “consequence of long-term ocean warming” (Oliver et al., 2021).

Marine heatwaves can cause mass mortality of marine species and economic damages totaling billions of dollars (Smith et al., 2023). At the individual level, elevated temperatures have also been shown to impact the physiological functions of kelp negatively (Cornwall et al., 2023). However, a more fundamental mechanism driving kelp forest loss, which has been demonstrated in southern California, is the limited nutrient availability associated with warmer water (Cavanaugh et al., 2021; Edwards & Estes, 2006; Zimmerman & Kremer, 1984). California kelp species, such as *Macrocystis pyrifera* (giant kelp), depend on reliable nitrogen input to survive (Edwards & Estes, 2006; Gerard, 1982). MHW events are defined by deeper thermoclines, which interfere with the upwelling of cold, nutrient-rich waters to areas where kelp forests grow (Edwards & Estes, 2006)), thereby affecting kelp forest survival.

In response to threats, the scientific community is working to restore kelp forests and counteract declines (Eger et al., 2022). Many passive restoration measures have failed to re-establish lost kelp populations (Wernberg et al., 2019). Additionally, kelp restoration efforts “have been largely disconnected, with varying methodologies trialed by different actors in different countries” (Eger et al., 2022). Therefore, it is crucial to determine and employ consistent methods of active restoration to restore kelp.

The socioeconomic importance of kelp worldwide dates back 10,000–70,000 years (Erlandson et al., 2007, Volman, 1978). For example, the *Māori* people of New Zealand

(*Aotearoa* in their language) have consumed highly nutritious red and green seaweeds and used the inflatable blades of bull kelp (or *rimurapa)* to create food storage bags (Te Ara: The Encyclopedia of New Zealand, n.d.). In pre-European times, a primary food source came from "gathering seafood during low tide in the sand or on rocky shores," and these food types are still an essential component of the *Māori* diet (Fox, 2010). Harvesting seafood (or *kaimoana*) is an integral part of the *Māori* culture, where various techniques and locations for acquiring seafood are passed down through generations (Fox, 2010).

Research in New Zealand has shown marked declines in the distribution of kelp, particularly on the east coast of New Zealand's South Island (Tait et al., 2021). In response to degrading kelp habitat, New Zealand researchers are studying kelp restoration and propagation methods. There have been studies on the effect of MHWs on kelp life cycles in a lab setting, specifically for *Macrocystis pyrifera* (Le et al., 2022b). Applying effective restoration strategies in New Zealand could one day benefit other regions worldwide where kelp is also declining.

Any kelp distribution and management assessment in New Zealand would be incomplete without considering the perspective of Indigenous *Māori*. Recognizing New Zealand's co-governance system and the *Māori kaitiakitanga* (guardianship) philosophy is critical for a holistic understanding of kelp forest loss and its effects. Embracing *Māori* perspective alongside scientific innovation is crucial for safeguarding and revitalizing the health of marine ecosystems.

Project Objectives

This project examined how kelp forest communities are changing in *Aotearoa* New Zealand. To achieve this, we structured the project around three pillars. First, we sought to identify the trends and understand the environmental and geographic factors influencing kelp forests at three different sites across *Aotearoa* New Zealand using historical dive survey data. Second, we evaluated the effects of increased temperatures on the development of the early life stage of native kelp species and investigated the importance of cleaning methods during the sporulation phase to increase the final output and cultivation success of native kelp. Finally, to recognize the *Māori* people's deep and enduring relationship with coastal resources, we assessed the social and cultural dynamics surrounding how the *Māori* people value coastal resources and how they perceive the impact of status quo marine resource management through an online survey.

A. In-situ Dive Surveys

Introduction

Kelp forests are declining globally at an annual rate of ~2% (Wernberg et al., 2019), a trend attributed mainly to increased sea surface temperature (SST) (Hollarsmith et al., 2020). The significant threat of heat-related disturbance events to kelp forest ecosystems has been documented across various regions, including New Zealand and California (Cornwall et al., 2023; Rogers-Bennett & Catton, 2019; Tait et al., 2021). Elevated SST associated with marine heatwaves (MHW) is strongly linked to decreased nutrient availability on the northeastern Pacific coast, which has translated to declines in habitat-forming kelp such as *Macrocystis pyrifera* (Cavanaugh et al., 2021; Edwards & Estes, 2006; Zimmerman & Kremer, 1984). There is evidence of a similar mechanism contributing to the decline of *M. pyrifera* in New Zealand (Tait, 2019).

MHWs, often driven by climate change, are associated with extensive losses of kelp forest ecosystems, notably in California in 2014–2017 and New Zealand from 2017-2018 (Rogers-Bennett & Catton, 2019; Tait et al., 2021). The destructive impacts observed in New Zealand after the 2017-2018 MHW mirrored those in California, where an MHW from 2014–2017 elevated SST by over 2.5°C above the average for an unprecedented 226 days (Rogers-Bennett & Catton, 2019). The parallels between the impacts of MHWs in New Zealand and California underscore the need for further interpretation and analysis of monitoring data to understand how these critical ecosystems respond to climate and ocean dynamics changes.

The community composition data provided by the Cawthron Institute is sourced from areas that extend beyond those studied in the existing literature and encompasses a representative range of New Zealand's coastline, providing broad spatial coverage for ecological assessment. Analysis of this data, combined with SST and SST anomaly data provided by NOAA Coral Reef Watch, will help understand the impacts of MHW on kelp forest communities across a wider geographic range. Furthermore, it will allow for comparisons between different regions of New Zealand. This will provide critical evidence of the extent of impacts associated with warming events on kelp abundance and community structure, addressing gaps in the literature on regional variability in climate change effects on coastal ecosystems in New Zealand.

Research Questions

1. How does the community composition of kelp forests vary across different coastal locations in *Aotearoa* New Zealand?
2. Do SST anomalies impact the abundance of different functional groups of seaweed?
3. Are the effects of SST anomalies on seaweed consistent across different locations in *Aotearoa* New Zealand?

Methods

Data Collection

Figure 1-A. Dive Survey Sites. *Map of the North and South Islands of New Zealand. The red points indicate locations where scientific divers at the Cawthron Institute conducted intertidal and subtidal surveys to collect the data used in the analysis.*

Survey sites

Dive surveys were performed by scientific divers from the Cawthron Institute across three locations in coastal New Zealand: Port of Napier (Pania Reef), Lyttelton Harbour, and Fiordland National Park (Figure 1-A). Port of Napier is on the east coast of the North Island in the Hawke's Bay Region, Lyttelton Harbour is on the east coast of the South Island in the Canterbury Region, and Fiordland National Park is on the southwestern coast of the South Island in the Southland Region. The Fiordland National Park location includes two fiords (Milford and Doubtful Sounds), which both receive glacial freshwater input. Additionally, Doubtful Sound has an industrial freshwater input from a hydroelectric plant. This selection of sites allows for a comprehensive evaluation of temperate rocky reef ecosystems across diverse environmental conditions, which contributes to the understanding of nationwide habitat trends.

Port of Napier (Pania Reef)

Pania Reef is the prominent seabed feature in southern Hawke's Bay (Duffy, 1992). The reef extends north-easterly, beginning approximately 800 m from the Port of Napier (Figure 2-A). It is widest at the southwestern end (~400 m), approximately 1 km northeast of the leading port breakwater, where the boulder and rock substrate emerge gradually from a 15 m deep sand bottom. Toward the seaward end, the topography becomes progressively steeper with large rocks, fissured with crevices, protruding from a sandy seabed at 18 m water depth. At its closest points, the reef is approximately 0.9 km southeast of the capital dredging footprint and 3.3 km northwest of an offshore spoil disposal area. Eight dive surveys were conducted at Pania Reef between April 2016 and October 2023 to monitor marine ecosystems, establish baseline conditions, and document ecosystem changes resulting from this dredging project.

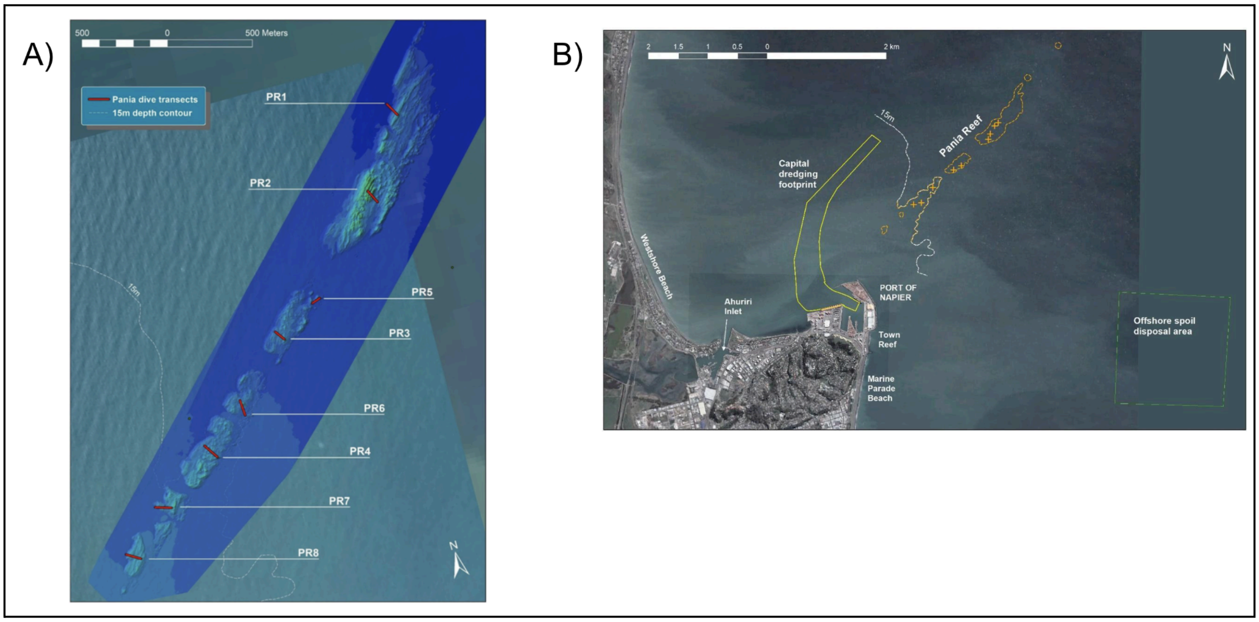

Figure 2-A: Location of significant features in the Port of Napier/Hawke's Bay. *Pania Reef extends NE from the 2023 dredging project area. The surveys conducted as part of the monitoring efforts comprised eight 100 m dive transects spaced out along the length of the reef (PR1–PR8).*

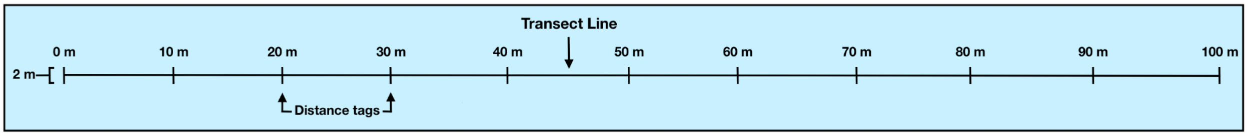

Figure 3-A: Pania Reef transect intervals. *Each transect laid along Pania Reef was 100 m long and divided into ten 10 m intervals by distance tags. Data was recorded at each interval of each transect.*

For each survey, eight 100 m transects were run along Pania Reef and tagged at 10 m intervals within a 2 m band, 1 m on each transect line (Figures 2-A and 3-A). Each survey recorded water depth in meters, habitat/substrate type (bedrock, boulders, cobble, shell hash, silt, sand/gravel, or bare), and relative abundance of algal and faunal species, including fish and invertebrates, using the ordinal scale described in Table 1-A. Five photo-quadrats were taken at each intersection with a 0.25 m^2 rectangular quadrat frame: one at the transect line distance tag and the remaining at the four compass sectors around the distance tag at a radial distance of about 1 meter. Video footage was taken with a GoPro between each of the 10m distance tags. In addition to video footage, both divers had hand-held compact cameras to photograph species. Video and photo media were used to complement the data compiled in the field.

Table 1-A. *Description of Ordinal Kelp Forest Abundance Scale (from Sneddon & Dunmore, 2021).*

Category	Rank Value	Description
Absent	**0**	Not observed
Rare	**1**	1-2 individuals, or a single cluster or patch of individuals in one small area (e.g., small patch of sponge or algae)
Occasional	**2**	3-10 individuals throughout the (2 m x 10 m) area of assessment
Common	**3**	> 10 individuals throughout the (2 m x 10 m) area of assessment
Abundant	**4**	Individuals abundant enough to form a distinct zone or habitat (e.g., mussels, barnacles and some algae), or hundreds to thousands of individuals per m^2

Lyttelton Harbour

Known as *Whakaraupō* in *Māori*, Lyttelton Harbour is a natural harbor on the Banks Peninsula in the Canterbury region of New Zealand's South Island. It is Christchurch's main port and is vital in maritime trade, hosting cargo shipping, fishing, and recreational boating activities. It supports diverse marine habitats, including rocky reefs and soft sediment environments. The area is also a focal point for conservation and ecological restoration efforts, as it holds cultural and ecological significance. This is particularly true regarding the *Ngāi Tahu iwi* (tribe).

Thirteen dive surveys were conducted in Lyttelton Harbour between February 2016 and September 2024 to establish baseline conditions in benthic habitats and monitor changes as Lyttelton Port Company Ltd. implemented its dredging project. For each survey conducted at

Lyttelton Harbour, eleven 30 m subtidal transects were laid at 6 locations (Figure 4-A). At each location, a 100 m offshore transect was positioned, and two 30 m transects — a deep (6-8 m) and a shallow (3-5 m) — were laid perpendicular to the 100 m transect line, roughly parallel to the shore (Figure 4-A, panel A). Two locations, LH07 and BP13, were unsuitable for a deep (6-8 m) transect line, so only shallow transect lines were surveyed. Along each of the 30 m transects, eight 1 m^2 quadrats were haphazardly placed (Figure 4-A, Panel A). Water depth (meters) was measured from wrist-mounted dive computers at each quadrat. Additionally, the estimate of percentage cover of substrate type (bedrock, boulders, cobble, shell hash, silt, sand/gravel, or bares), canopy-forming and understory algae, and encrusting invertebrates were assessed along with counts of solitary epifauna (Sneddon & Dunmore, 2021).

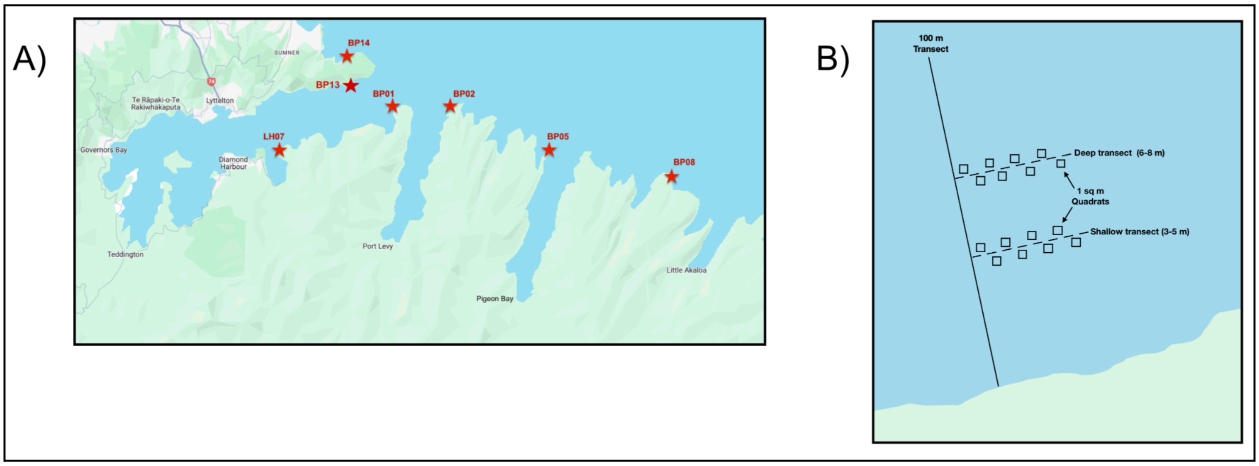

Figure 4-A. Dive survey transects locations and layout in Lyttelton Harbour. *Panel A shows the locations of each dive survey in Lyttelton Harbour. All locations contained a deep and shallow transect, except sites LH07 and BP13, which only had shallow transects. Panel B shows the design layout for deep (6-8 m) and shallow (3-5 m) transects established at each dive survey location in Lyttelton Harbour. (Details from Sneddon & Dunmore, 2021).*

Fiordland National Park (Meridian)

Eight intertidal and subtidal sites have been surveyed in Doubtful Sound since 2006, and three in Milford Sound since 2007 (Figure 5-A). Both fiords are part of Fiordland National Park and *Te Wahipounamu*, a UNESCO World Heritage site (UNESCO, n.d.). Milford Sound serves as a reference point for comparison with Doubtful Sound. Manapōuri Power Station, in the inner fiord, releases discharge into Doubtful Sound and is operated by Meridian Energy. Cawthron Institute was contracted to survey the Fiordland National Park sites, which are called ‘Meridian’ after the company (Crossett et al., 2024). These two fiords will collectively be referred to as Fiordland National Park for the remainder of the section.

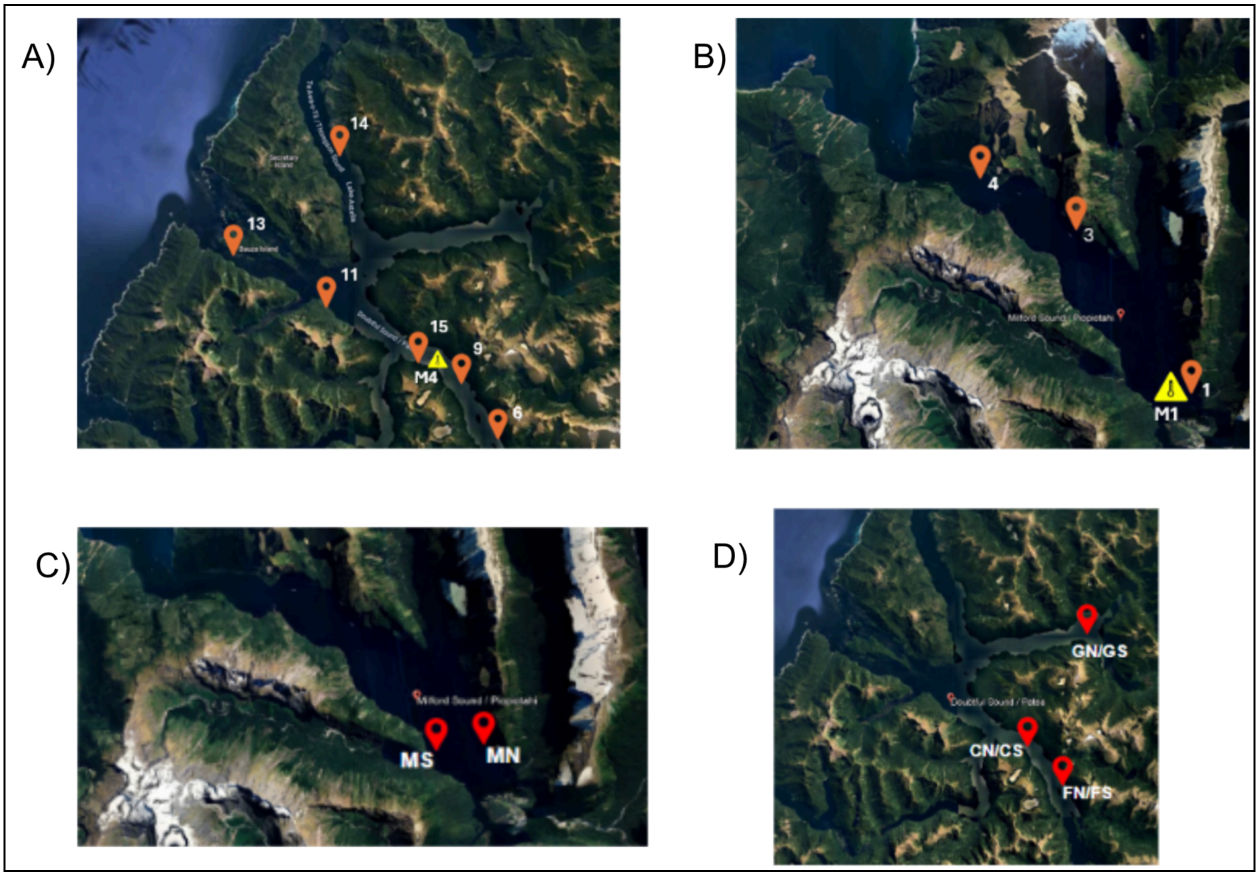

Figure 5-A. Core sites and transitional sites of Milford and Doubtful Sound. *The yellow triangles represent moorings, and the red dots represent the various sites. Panel A shows the approximate locations of core sites of Doubtful Sound, which covers intertidal and subtidal datasets. Panel B shows all approximate locations of core sites in Milford Sound. Panel C and D show the approximate location of all transitional sites across both fiords. MS and MN represent Milford South and North, and GN/GS, CN/CS, and FN/FS represent various bays within Doubtful Sound.*

Fiordland National Park includes core sites, which are continuously surveyed and non-core sites, which were surveyed at pilot stages (Figure 5-A). Only core sites are evaluated in this project because of their greater temporal consistency, and core sites included three different data types: intertidal, transitional, and subtidal. Intertidal data were collected between low and high tide zones at each site and included numbered sites shown in panel A and B in Figure 5-A. Transitional data included surveying intertidal areas from inner to outer fiords, representing different zones where intertidal communities transition from one group to another (i.e., barnacles to mussels) (Panel C and D of Figure 5-A). Lastly, Subtidal data were collected at two, four, seven, ten, and sixteen m at various points across the fiords, covering the same numbered locations as the Intertidal data. All data were collected with an underwater camera and associated standardized photo-quadrat of 0.25 m^2. Images were analyzed by Cawthron scientists to assess percentage cover of sessile species, such as kelp, and counts of mobile species, such as gastropods and urchins.

Additionally, temperature was monitored from two mooring buoys, M1 and M4, within each fiord as illustrated by the yellow triangle in Figure 5-A, panel B. Temperature mooring buoys, which have been running since 2005, collected data every 30 minutes as a 25 m depth profile (0.5, 1, 1.5, 2, 3, 5, 7, 9. 11, 14, 19, and 25 m). Small gaps in the mooring temperature exist due to technical errors, maintenance, or extreme weather conditions.

Data Wrangling

Species were categorized based on taxonomic group definitions provided by Cawthron scientists along with combined phyla and class-level classifications. Seaweed species were further classified into functional groups—Canopy, Subcanopy (red, green, brown), Floor Species, and Epiphytes—using *New Zealand Seaweeds: An Illustrated Guide* by Wendy Nelson and expert insights from our collaborators at Cawthron (Table 2-A). Functional groups are defined by their structural and ecological roles in a kelp forest ecosystem. The canopy group consists of large, surface-reaching kelps, such as *Macrocystis pyrifera* and *Ecklonia radiata*, which provide habitat complexity and primary productivity. The subcanopy includes smaller understory seaweed that grow beneath the canopy, contributing to vertical structure and shade. Turfing algae form dense, low-growing mats. Encrusting species grow as thin layers over hard substrates. Lastly, epiphytic organisms, including small algae and invertebrates, grow on the surfaces of larger seaweed or other substrates.

Table 2-A. Seaweed functional group and subgroup classifications. *Seaweed taxa from all surveyed sites were categorized into functional groups and subgroups. (Not all species listed in this table were present in all three sites).*

Functional Group	Subgroup	Species
Canopy		*Ecklonia radiata*, *Macrocystis pyrifera*, *Carpophyllum maschalocarpum*, *Carpophyllum flexuosum*, *Landsburgia quercifolia*, *Undaria pinnatifida*, brown blade recruits
Subcanopy	**Rhodophyta**	red branching *Rhodophyllis*, red filamentous algae, feather red *Plocamium*, red feathery *Ballia*, red feathery *Euptiloda*, foliose red algae, *Rhodophyta* sp, red fine algae, *Ceramium, Plocamium cirrhosum, Pterocladia capillacea*
	Chlorophyta	*Codium, Chlorophyt*a ball, fine green filamentous, *Ulva lactuca*, *Chlorophyta*
	Phaeophyta	*Halopteris*, brown alga filamentous, *Dictyota ocellata*, *Desmarestia*, *Zonaria*, *Carpomitra costata, Dictyota*
Floor Species	**Turfing**	fine green moss-like alga, Coralline turf, fine tufty fluffy red, brown tufted algae
	Encrusting	*Microzonia*, brown encrusting algae, Coralline paint, *Rhizopogonia* red prostrate blades, *Microzonia velutina*, corallinales, red encrusting algae

Seaweed abundance was measured differently across sites. Surveys in Lyttelton Harbour and Fiordland National Park used percent cover, while an ordinal scale was used in surveys at the Port of Napier (Table 1-A). To enable cross-site comparisons between the surveys conducted in Lyttelton Harbour and the Port of Napier, percent cover data were converted to the ordinal scale using definitions provided by our collaborators at the Cawthron Institute (Table 3-A). Fiordland National Park sites had six different depths of data collection across multiple survey types. Converting these surveys to ordinal data would greatly dilute the specificity that could be achieved with percent occurrence data from both fiords of Fiordland National Park, so the ordinal scale conversion was not applied to data from these sites.

Like most ecological survey data, the observations are zero-inflated, i.e., many taxa are often not observed at a site. To address the zero-inflated nature of the data, we created two versions for each dataset. The first, 'presence-only,' excluded all zero values, retaining only instances where observations were recorded. The second, termed 'presence-absence,' converted abundance data into a binary format. With this binary method, values greater than zero were recorded as present (1), and zero values were recorded as absent (0).

Table 3-A. Guide for converting percent cover to ordinal abundance.

Lyttelton Harbour (Percent Cover)	Port of Napier (Ordinal Abundance)	Description
0%	**0**	**Absent**
1-5%	**1**	**Rare (1-2 individuals)**
6-25%	**2**	**Occasional (3-10 individuals)**
26-50%	**3**	**Common (> 10 individuals)**
51-100%	**4**	**Abundant (forms distinct zone)**

SST data from NOAA's Coral Reef Watch Daily Global 5 km Satellite Sea Surface Temperature Anomaly product (Version 3.1, released August 1, 2018) was used to characterize temperature change that might determine differences in seaweed abundance across sites and time. This dataset contains SST (°C) values and SST anomalies (the difference between present-day SST and historical average, ranging from -5 °C to + 5°C) for a 0.05° satellite grid. The spatial extents of each site were cropped to specific bounding coordinates to prepare the SST data. In Lyttelton Harbour, the extent was defined as the minimum enclosing rectangle between (43.7°S, 172.7°E) and (43.5°S, 173.0°E). Mean SST and SST anomalies were calculated for each month between 2000 to the present. The Port of Napier's extent was set between (-39.0°S, 176.0°E) and (-40.0°S, 177.0°E), with the same SST metrics calculated monthly for the same time range. For Fiordland National Park, the extent covered (-47.5°S, 166.5°E) to (-34.5°S, 178.5°E), and annual anomalies were calculated since survey dates were not recorded at this site. Therefore, a monthly SST from 2006 to 2024 was created to capture all possible survey dates. The dive survey datasets were integrated with SST data obtained from NOAA Coral Reef Watch, using the survey dates to ensure that each survey record included corresponding SST and SST anomaly values for the specific date and location (Figure 7-A). The SST anomaly was then categorized into a factor, grouping anomalies by their first digit to create bins of similar magnitude.

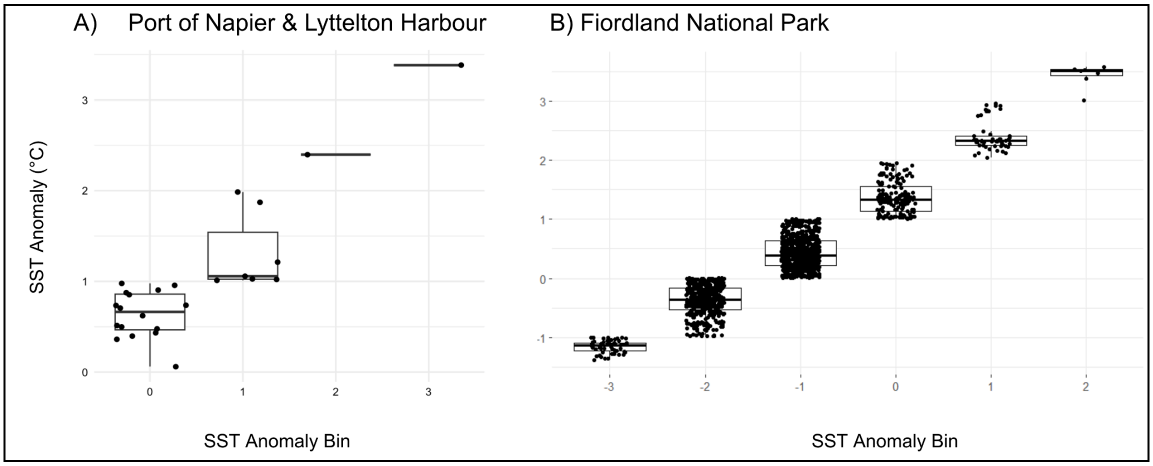

Figure 6-A: *SST data, provided by NOAA Coral Reef Watch, were grouped into bins in preparation for statistical analyses based on the leading integer of the anomaly in °C. Panel A shows SST anomaly data for all sites in the Port of Napier and Lyttelton Harbour. Panel B shows SST anomaly data for all sites in Fiordland National Park.*

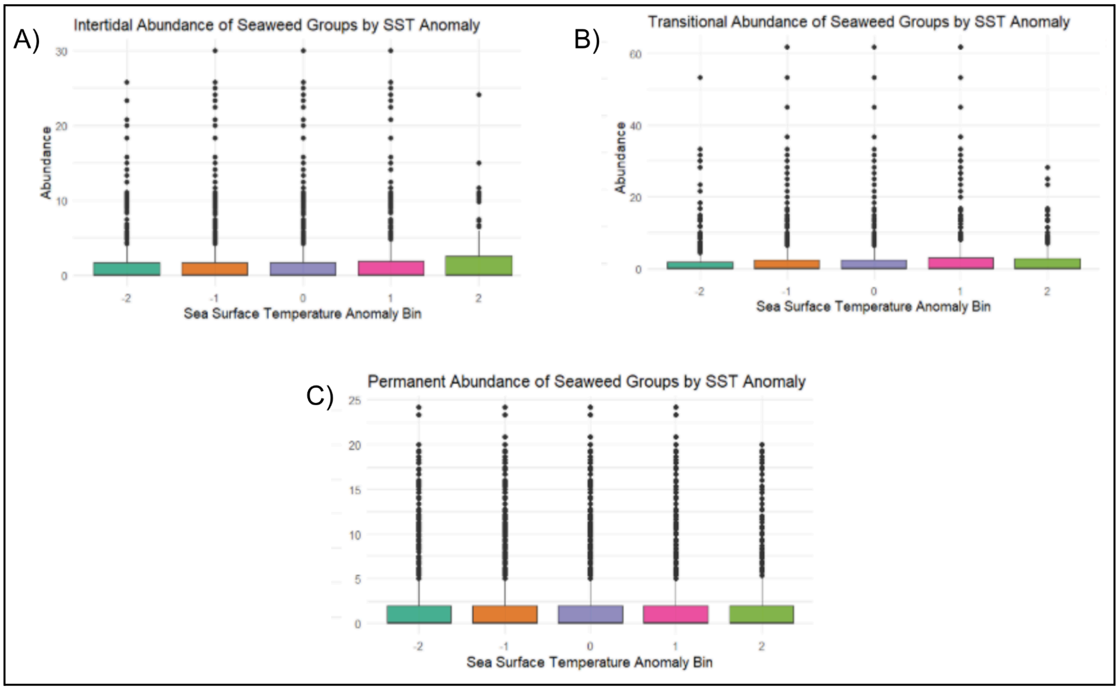

Figure 7-A: *Boxplots of percent cover abundance within each of the SST Anomaly bins for sites at Doubtful Sound and Milford Sound within the Fiordland National Park sites. Intertidal data occurrences are displayed in panel A, transitional data in panel B, and permanent data in panel C.*

Methods for Research Question 1

- How does the community composition of kelp forests vary across different coastal locations in *Aotearoa* New Zealand?

Non-metric multidimensional scaling (nMDS) was performed using the Bray-Curtis dissimilarity index to investigate how the abundance of different functional seaweed groups varied across coastal locations in New Zealand. The dataset included median ordinal abundance (Table 3-A) for six functional groups—Canopy, *Chlorophyta* Subcanopy, *Rhodophyta* Subcanopy, *Phaeophyta* Subcanopy, Encrusting, and Turfing—across transects from Lyttelton Harbour (3-5 m and 6-8 m) and Port of Napier. For this analysis only, missing values were replaced with zeros to ensure a complete dissimilarity matrix. The analysis was performed in two dimensions with twenty random starts to ensure a stable solution. The resulting ordination was rotated and centered for interpretability, and sites were plotted in ordination space to visualize differences in community composition across locations. Convex hulls were added to highlight patterns of community differentiation.

The percent cover data from the Fiordland National Park underwent non-metric multidimensional scaling (nMDS) using the Bray-Curtis dissimilarity index to assess how the abundance of different functional groups of seaweed varied between the two fiord locations in New Zealand. The dataset included the mean percent cover of six functional groups across three data types (intertidal, transitional, and subtidal) and two fiords (Doubtful and Milford), spanning depths from above the tide line to 16 m. Missing values were excluded to ensure a complete dissimilarity matrix. The initial analysis was conducted with two dimensions, but the model was later adjusted to a three-dimensional ordination space ($k = 3$) due to a lack of a stable solution. The model was iterated up to 200 times to optimize the solution, with data retained on their original scale. Scores were extracted and visualized to highlight the differences in community composition between the two fiords, with hulls added to emphasize the communities' distinctiveness across the sites.

To further investigate which functional groups contributed most to compositional differences between locations, we conducted a SIMPER (Similarity Percentage) analysis. This analysis decomposed the Bray-Curtis dissimilarities into contributions from each functional group, identifying taxa that drove differences between pairs of locations. Permutation tests calculated significance values, and only groups with an $\alpha < 0.05$ were considered strong contributors to dissimilarity.

Methods for Research Questions 2 & 3

- Do SST anomalies impact the abundance of different functional groups of seaweeds?
- Are the effects of SST anomalies on seaweed consistent across different locations across *Aotearoa* New Zealand?

For all three versions of datasets (original data, presence-only, and presence-absence) for each of the sites (Lyttelton 4 m, Lyttelton 7 m, Port of Napier, and Fiordland National Park), Kruskal-Wallis tests were conducted to identify which algal functional groups had statistically significant changes in abundance in response to SST anomalies for each transect at each site. Functional groups were first split into Canopy, Subcanopy, and Floor Species. The Subcanopy functional group was broken down further into subgroups with discrete phyla, including *Phaeophyta* (Subcanopy Brown), *Rhodophyta* (Subcanopy Red), and *Chlorophyta* (Subcanopy Green). Floor species subgroups, Encrusting and Turfing, were also created. The Subcanopy subgroups, *Phaeophyta* (Subcanopy Brown), *Rhodophyta* (Subcanopy Red), and *Chlorophyta* (Subcanopy Green), were tested both together and separately. Similarly, the Floor species subgroups, Encrusting and Turfing algae, were also tested both together and separately (Table 2-A). The results of each Kruskal-Wallis test were considered significant if $\alpha < 0.05$. The combinations that yielded significant results were then used for further analysis to determine the threshold of SST anomaly, resulting in a response in seaweed abundance for the various functional groups. Wilcoxon or Dunn tests were conducted for each combination of algae functional group and subgroup, site, and transect across each SST anomaly bin appropriate for a given site.

Results of Wilcoxon or Dunn tests were considered significant if $\alpha < 0.05$. After all statistical tests were performed, the percentage of tests that showed significant results was calculated for each location, site, functional group and subgroup to determine which locations and taxa had the strongest response to changes in SST.

Additional analyses were taken for Fiordland National Park sites, as more than two depths were surveyed compared to Lyttelton Harbour and Port of Napier. To standardize the process, each data type—intertidal, transitional, and subtidal —was analyzed separately using Spearman's rank correlations and linear mixed-effects models (LMMs) for both fiords combined and for each Milford Sound and Doubtful Sound individually.

To assess temporal trends in the mean abundance of seaweed functional groups and subgroups at Milford and Doubtful Sound, we developed a series of LMMs for each species group and subgroup. Specifically, we fit separate models for each group and subgroup using the *lme4* package in R, where mean percent cover was modeled as a function of the year while accounting for variation among sites and replicates. The models were specified as follows:

$$\text{mean\_count} \sim \text{year} + (1|\text{site}) + (1|\text{rep})$$

Site and replicate were included as random intercepts to account for spatial and sampling variability, respectively. Data were grouped by species functional group and subgroup (Table 2-A) before model fitting, ensuring that each model was estimated independently for each seaweed group and subgroup. These models allow us to evaluate whether there are significant temporal trends in algal abundance while controlling for site-level and replicate-level variation.

To investigate the role of depth on the subtidal data, an additional model was run:

mean_count ~ depth + sea_surface_temperature_anomaly + (1|site) + (1|rep)

Results

SST Time Series

New Zealand experienced above-average SST anomalies and MHWs nationwide during 2016–2019 and 2022–2023 (Figure 8-A). These SST anomalies and marine heatwave events were observed at Lyttelton Harbour and Port of Napier (Figure 9-A). During the survey, Lyttelton Harbour experienced SST anomalies within bins 0°C, 1°C, and 3°C. The survey dates at Port of Napier coincided with SST anomalies within bins 0°C, 1°C, and 2°C (Figure 9-A). The survey dates for Fiordland National Park spanned 1°C and 2°C, as well as cold anomalies from -1°C, -2°C, and -3°C during the February months when photo surveys took place (Figure 10-A).

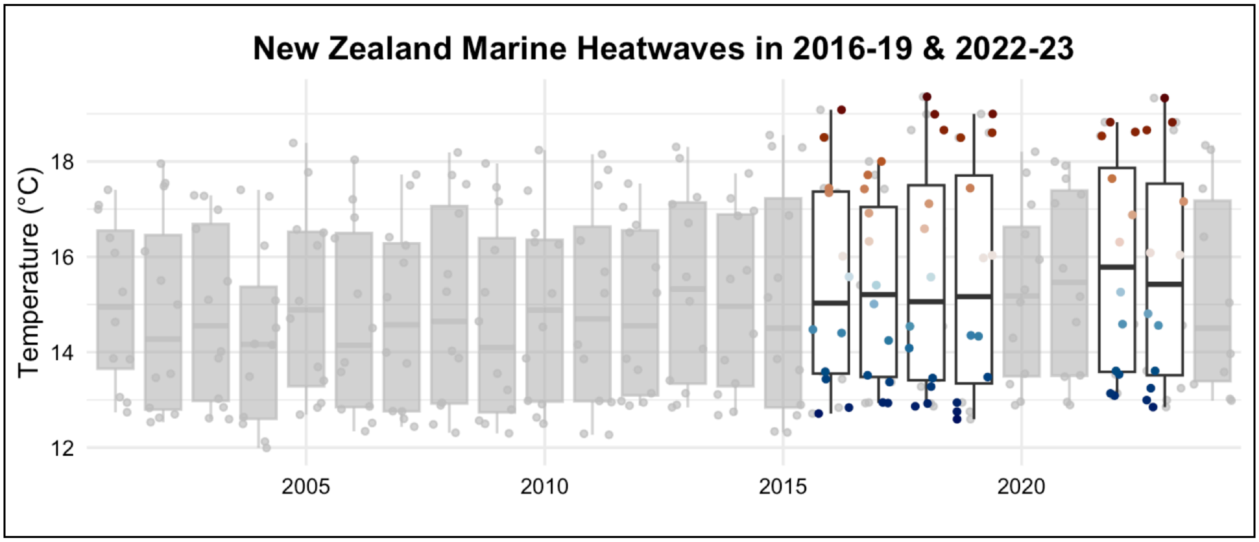

Figure 8-A: *Annual average sea surface temperature (°C) distribution in New Zealand. Each point represents the monthly average sea surface temperature across the extent of New Zealand's coastal waters. Data provided by NOAA Coral Reef Watch Satellite Data for New Zealand between 2000-2024 shows anomalous SSTs during marine heatwave events in 2016-19 and 2022-23.*

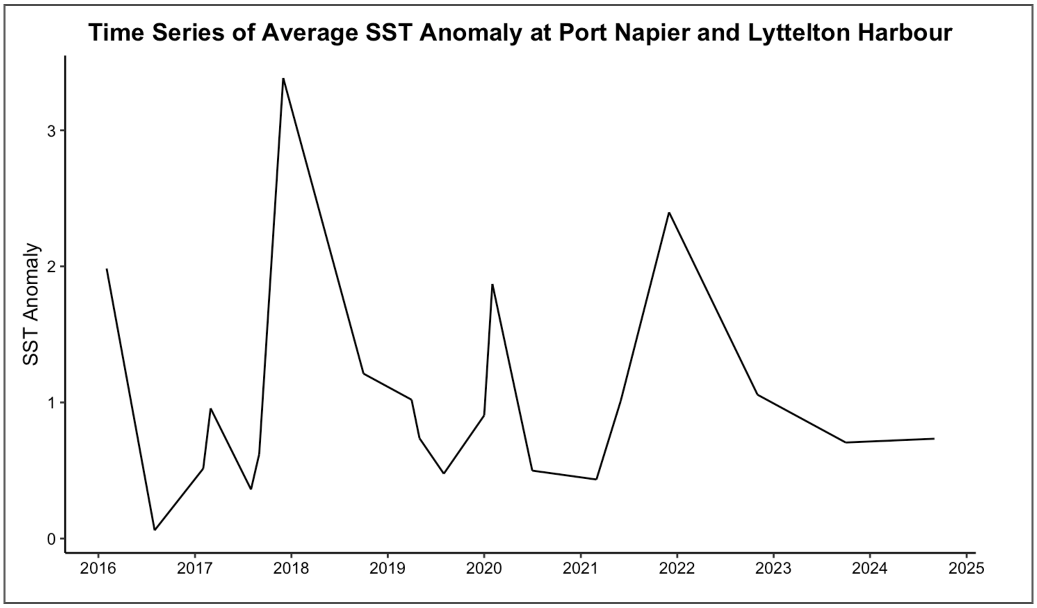

Figure 9-A: *SST anomaly data provided by NOAA Coral Reef Watch. Average SST anomalies (°C) at Port of Napier and Lyttelton Harbour show local marine heatwave events around 2018 and 2022.*

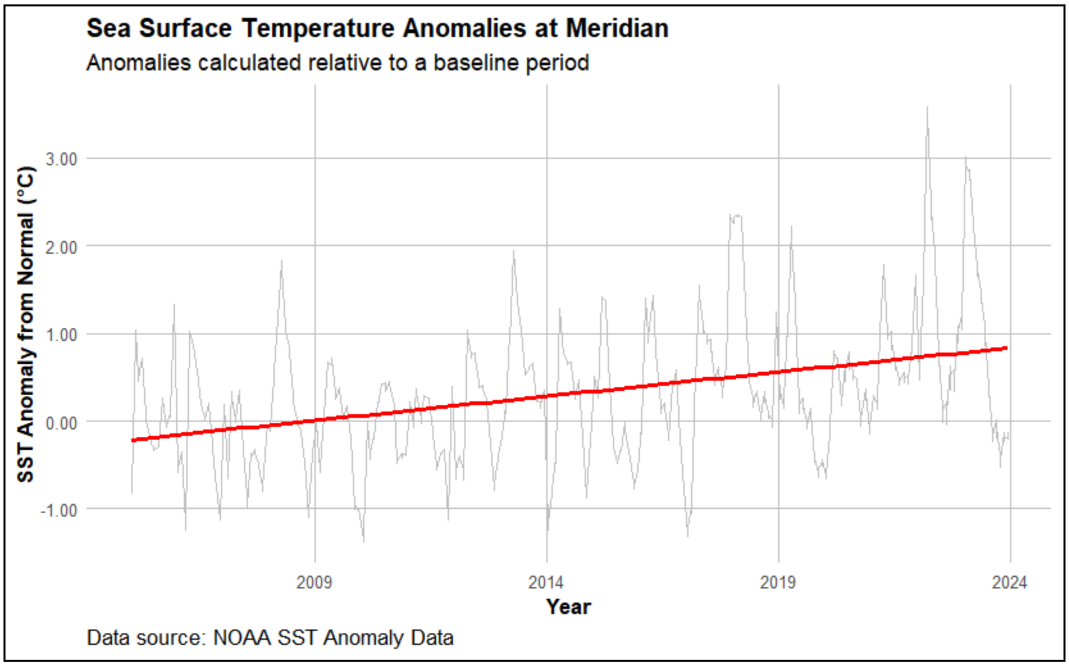

Figure 10-A: *SST anomaly data provided by NOAA Coral Reef Watch. This data plot demonstrates average SST anomalies (°C) at Fiordland National Park site (Milford and Doubtful Sound) from 2005 to 2024 with the historical average shown in red.*

Research Question 1: Community Composition of Dive Survey Sites

Percent occurrence charts indicated that the data is zero-inflated, particularly for transects surveyed in Lyttelton Harbour and most photo quadrat surveys for Intertidal, Transitional, and Subtidal sites across Fiordland National Park.

In the shallow transects of Lyttelton Harbour (3-5 m), encrusting seaweeds were present in nearly half of the surveys, while the rest of the groups and subgroups were present in less than a quarter (Figure 11-A, Panel A).

In the deep transects in Lyttelton Harbour (6-8 m), encrusting seaweeds were present in nearly one-third of the surveys, while the rest of the groups and subgroups were present in less than a quarter (Figure 11-A, Panel B).

At Port of Napier, over a quarter of transects contained canopy and/or *Rhodophyta* subcanopy, while all other functional groups were present in fewer than a quarter of the surveys (Figure 11-A, Panel C).

In the Fiordland National Park sites, the canopy functional group was only seen in the subtidal data at the depths of 4 and 7 m for half of the sites across the two fiords (Figure 11-A, Panel D and 11-A, Panel E). All data types at both fiords had encrusting, turfing, and brown and red algae subcanopy species. *Chlorophyta* species were sparse across all sites.

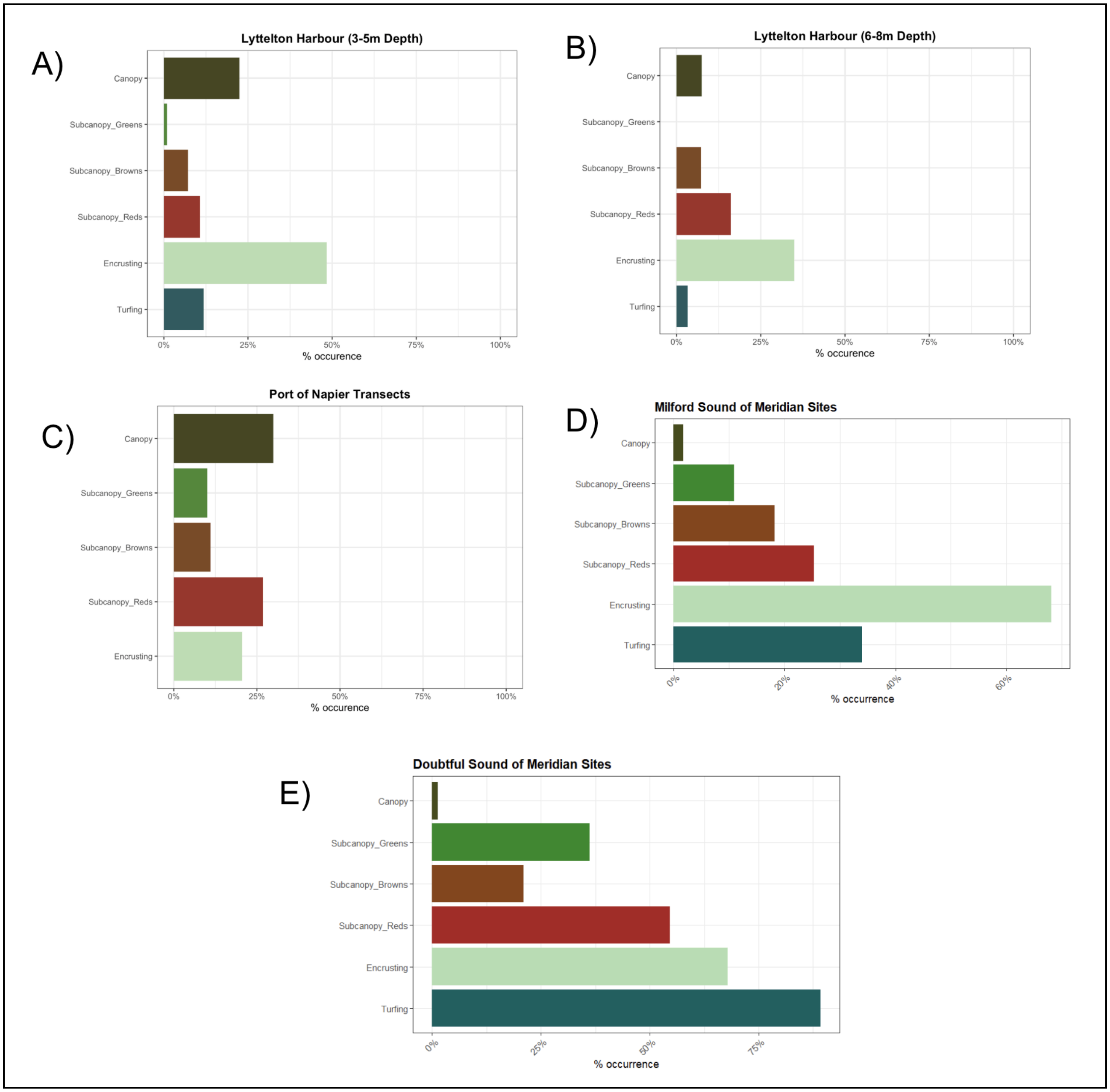

Figure 11-A: *The percent occurrence of functional groups and subgroups across all dive survey locations shows that much of the abundance data are zero-inflated, particularly for sub-canopy. Total counts for both Milford and Doubtful Sound for all functional groups of seaweed from 2006-2011, 2013, 2014, 2016, 2019, and 2024.*

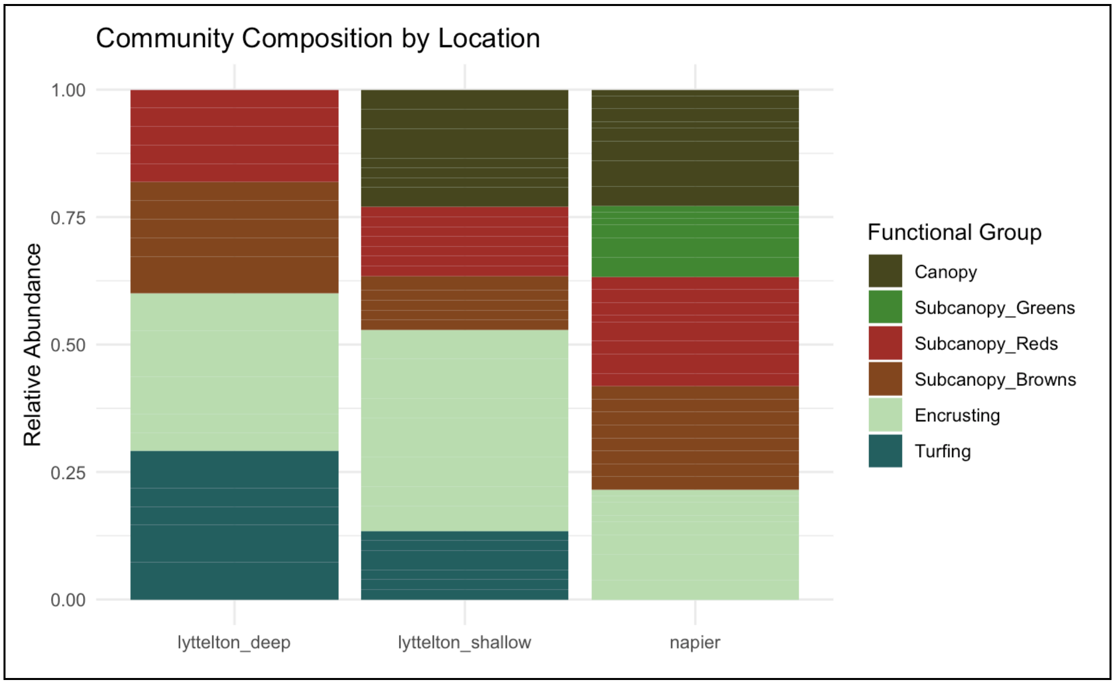

Figure 12-A: *Each color represents a different seaweed functional group. The bars show the relative abundance of each functional group in sites at the Port of Napier and Lyttelton Harbour. Lyttelton Deep refers to the 6-8 m depth transects. Lyttelton Shallow refers to the 3-5 m depth transects.*

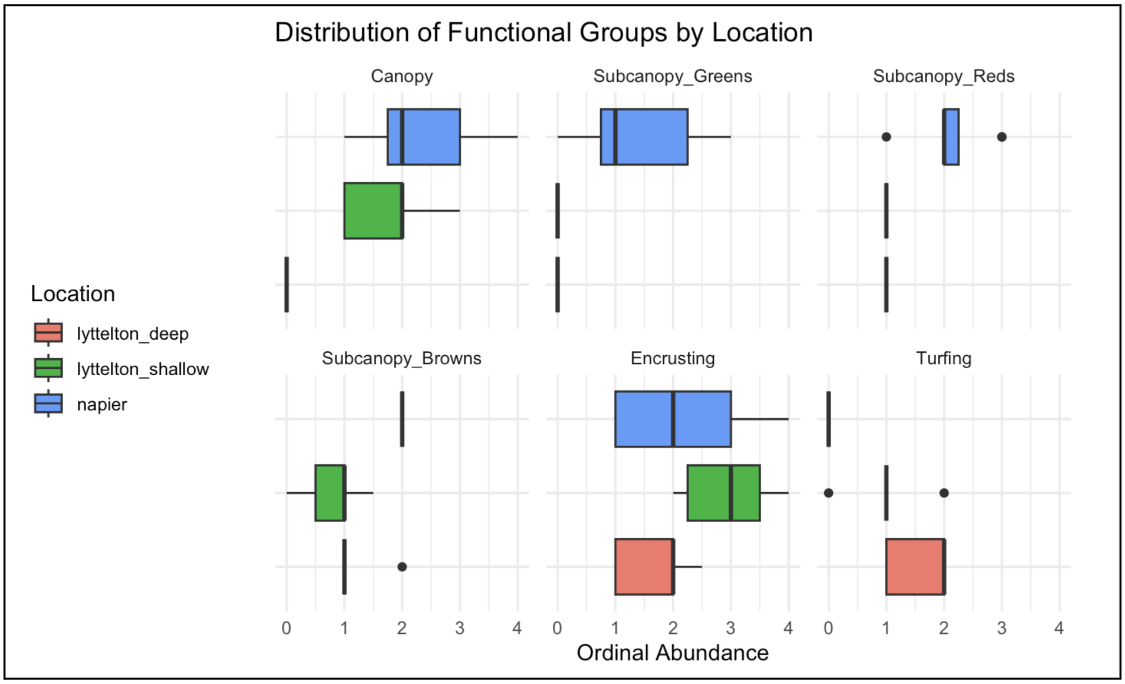

Figure 13-A: *The spread of the relative abundance of functional seaweed groups varies across the three sites in Lyttelton Harbour and Port of Napier. Shallow transects contained more canopy species, and subcanopy species were more common at Port of Napier.*

Non-metric multidimensional scaling (nMDS) for Port of Napier and Lyttelton Harbour reveals distinct community compositions among the three sites (Figure 14-A). The axes represent variation in different functional groups (Table 2-A). Along axis 1, Turfing and *Chlorophyta* subcanopy exhibit the highest scores and contribute most to the separation, with an inverse relationship where Turfing groups increase as *Chlorophyta* subcanopy decreases (Figure 14-A). Axis 2 is primarily driven by differences in *Phaeophyta* subcanopy, followed by Canopy and Encrusting groups, which decline as *Phaeophyta* subcanopy increases (Figure 14-A).

A separate nMDS was conducted for all Fiordland National Park sites and data types (Figure 15-A). Figure 17-A shows a large degree of overlap across all data types from both fiords in Fiordland National Park, as shown by the large overlap of the hulls.

Two SIMPER analyses were conducted. The first highlights the average community composition across Lyttelton Harbour shallow transects, Lyttelton Harbour deep transects, and Port of Napier transects. The second compares average community composition across intertidal, transitional, and subtidal data types of Milford and Doubtful Sound in the Fiordland National Park. Lyttelton deep is characterized by a higher abundance of turfing species and lower canopy cover. In contrast, both Napier and Lyttelton shallow contain canopy species, though Napier has fewer turfing species (Figures 14-A, 15-A). The highest contributors to differences between the fiords in the Fiordland National Park sites were Encrusting algae species. Additionally, brown Subcanopy species were much higher in Milford Sound, whereas Turfing algae species determined the community composition in Doubtful Sound. Canopy algae species contributed minimally to the difference between the two fiords.

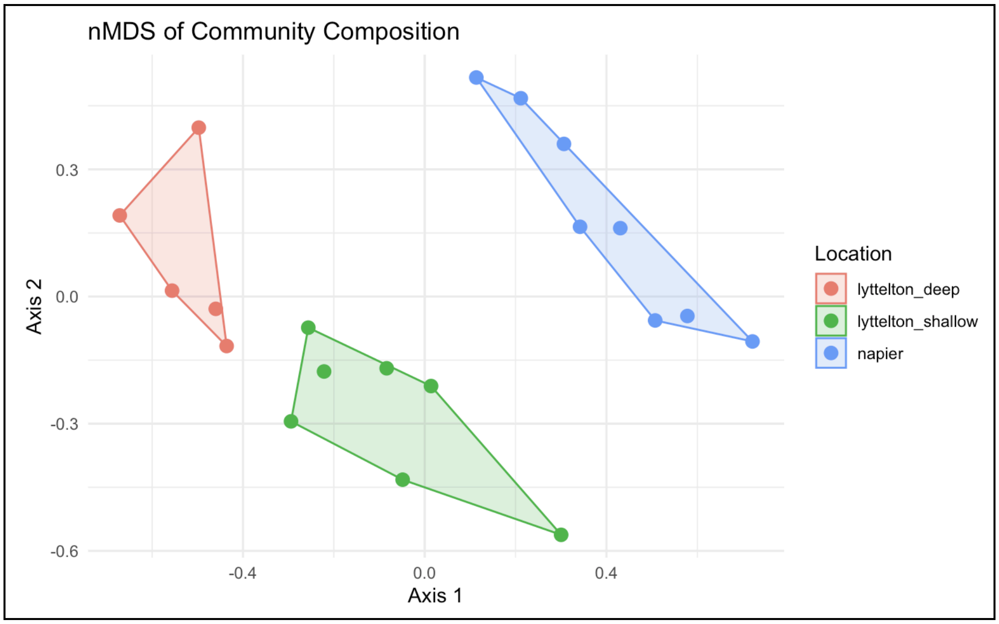

Figure 14-A: *Non-metric multidimensional scaling (nMDS) creates a two-dimensional visualization of the compositional differences for communities in three dive survey locations: Port of Napier, Lyttelton Harbour (Deep, 6-8 m), and Lyttelton Harbour (Shallow, 3-5 m).*

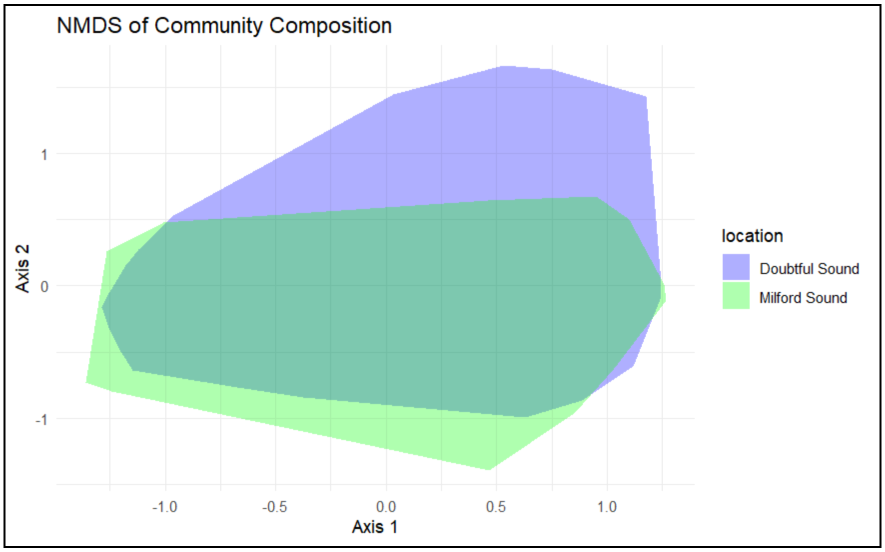

Figure 15-A: *Non-metric multidimensional scaling (nMDS) creates a two-dimensional visualization of the compositional differences for communities in three data types (Intertidal, Transitional, and Subtidal) across the two fiords, Doubtful and Milford Sound, of Fiordland National Park.*

Time series plots for shallow transects in Lyttelton Harbour show relatively stable levels of seaweed functional groups between 2016 and 2024 (Figure 16-A). During this time period, there was a slight increase in Canopy species, relatively consistent levels of Floor species (with a notable spike in 2017), and a consistently low relative abundance of subcanopy species (Figure 16-A).

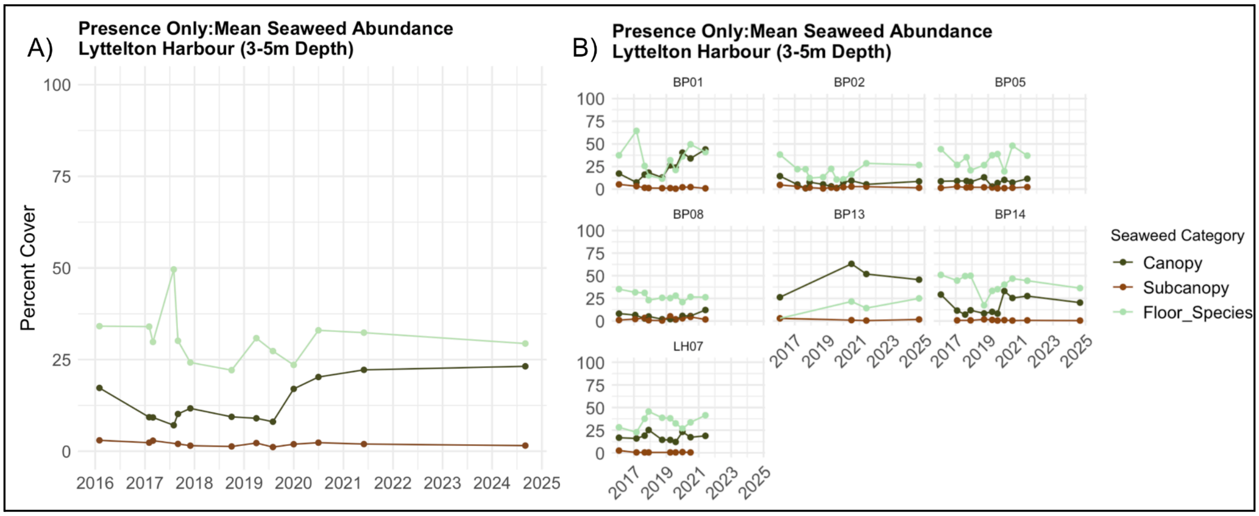

Figure 16-A: Time series for seaweed functional groups in Lyttelton Harbour (3-5 m). *The colored lines represent the different seaweed functional groups: Canopy, Subcanopy, and Floor species. Each point represents the mean percent cover of species in each group for each survey. Variability was suppressed for clarity.*

Between 2016 and 2024, deep sites at Lyttelton Harbour saw a relatively low abundance of Canopy, Subcanopy, and Flooring seaweed species, with a notable drop in 2017 (Figure 17-A). There is some site-specific variation, particularly at BP01 and BP14. Time series plots indicate consistently low abundance levels of canopy and subcanopy functional groups (Figure 17-A). Slight increases in Floor species were observed at sites BP05 and BP08.

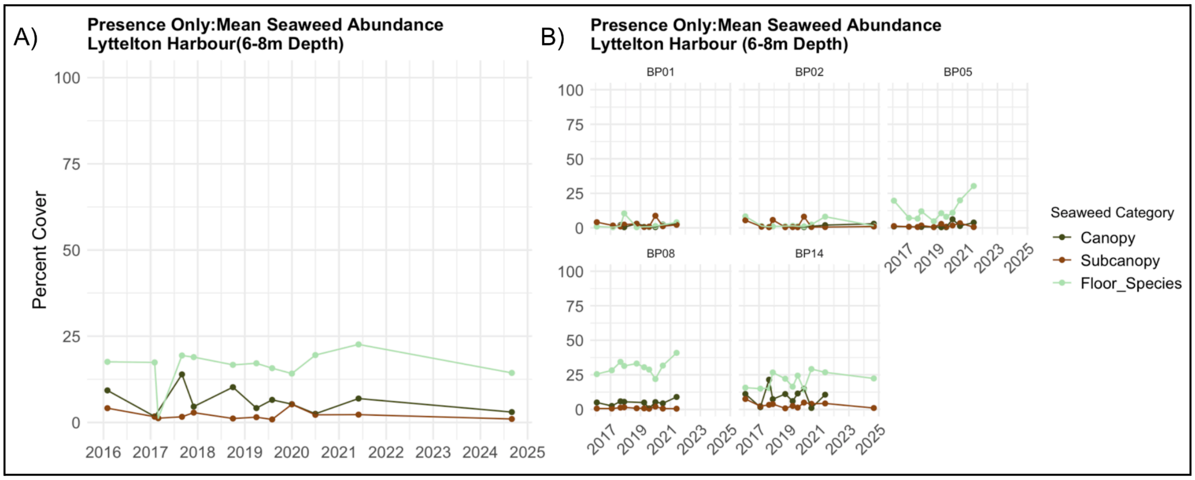

Figure 17-A: Time series for seaweed functional groups in Lyttelton Harbour (6-8 m). *The different colored lines represent the different seaweed functional groups: Canopy, Subcanopy, and Floor species. Each point represents the mean percent cover of species in each group for each survey. Variability was suppressed for clarity.*

At Port of Napier, where abundance was recorded using an ordinal scale (Table 1-A), the ordinal abundance of the canopy and subcanopy species remained relatively stable between 2016-2023, with a spike in subcanopy in 2020. There was a slight decline in Floor species after 2020 (Figure 18-A).

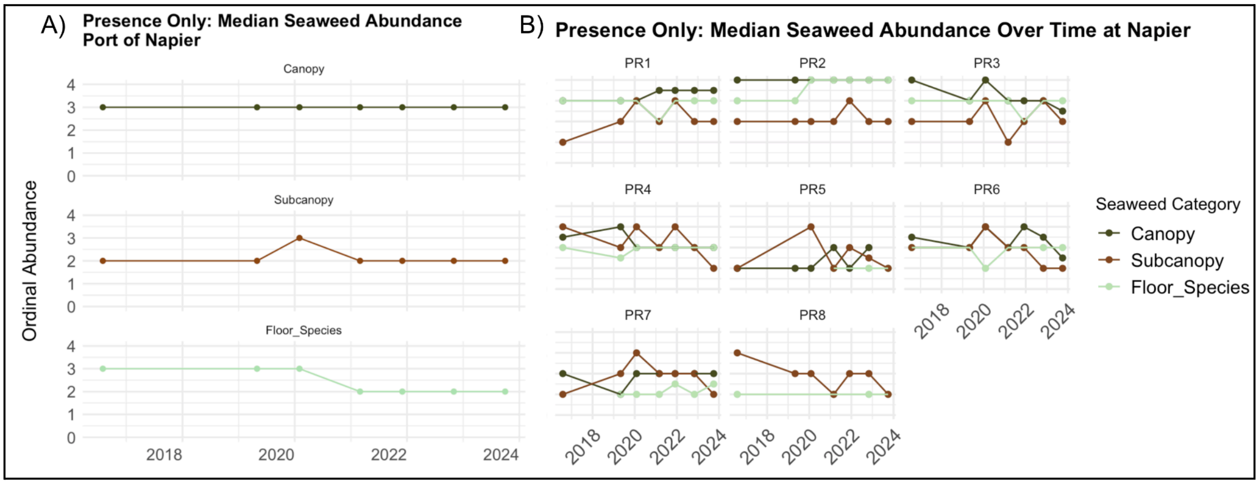

Figure 18-A: Time series for seaweed functional groups in the Port of Napier. *The different colored lines represent the different seaweed functional groups: Canopy, Subcanopy, and Floor species. Each point represents the median ordinal abundance of species in each group for each survey. Variability was suppressed for clarity.*

Time series for Fiordland National Park sites were tracked based on the data type, as it was assumed that intertidal, transitional, and subtidal surveys had different communities. Total occurrence was calculated by totaling the average percent cover across replicates throughout all years. Throughout the surveys, most seaweed species decreased over time, with the red Subcanopy having the most significant decrease. Only Floor species increased in percent cover (Figure 19-A). Across the transitional sites, all seaweed species experienced a sharp decline around 2016, and there is only evidence of red Subcanopy species starting to increase in percent cover (Figure 19-A). In the subtidal photo surveys, Floor species saw the sharpest decrease in percent cover while green Subcanopy species increased (Figure 19-A).

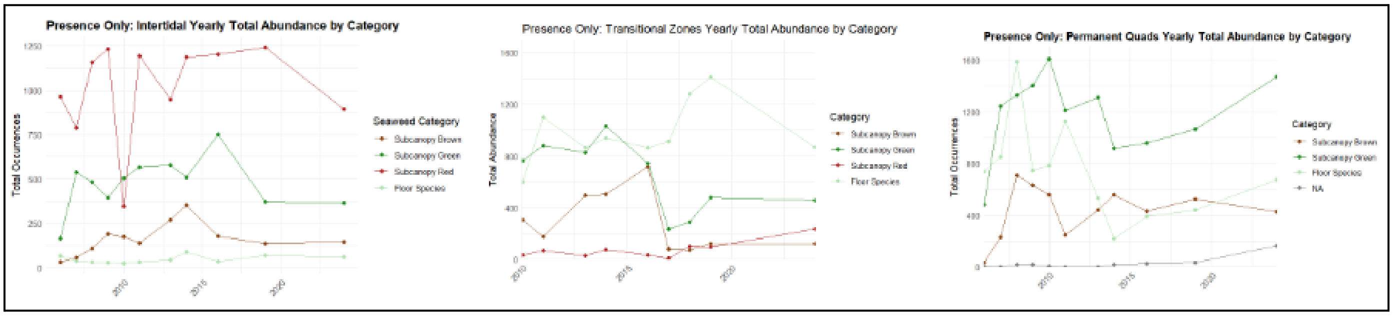

Figure 19-A: *Panel of time series plots of seaweed functional groups for all three data types of Fiordland National Park, from left to right: intertidal, transitional, and subtidal.*

Research Question 2: Functional taxa groups response to SST anomalies

Kruskal-Wallis tests indicated that subcanopy functional groups exhibited the strongest response in seaweed abundance to SST anomalies between Lyttelton Harbour and the Port of Napier, with *Rhodophyta* (53.5%), *Phaeophyta* (45.5%), and *Chlorophyta* (40.9%) showing the highest variation. In contrast, Canopy (15.5%) and Encrusting (13.8%) seaweeds demonstrated the lowest response (Figure 20-A).

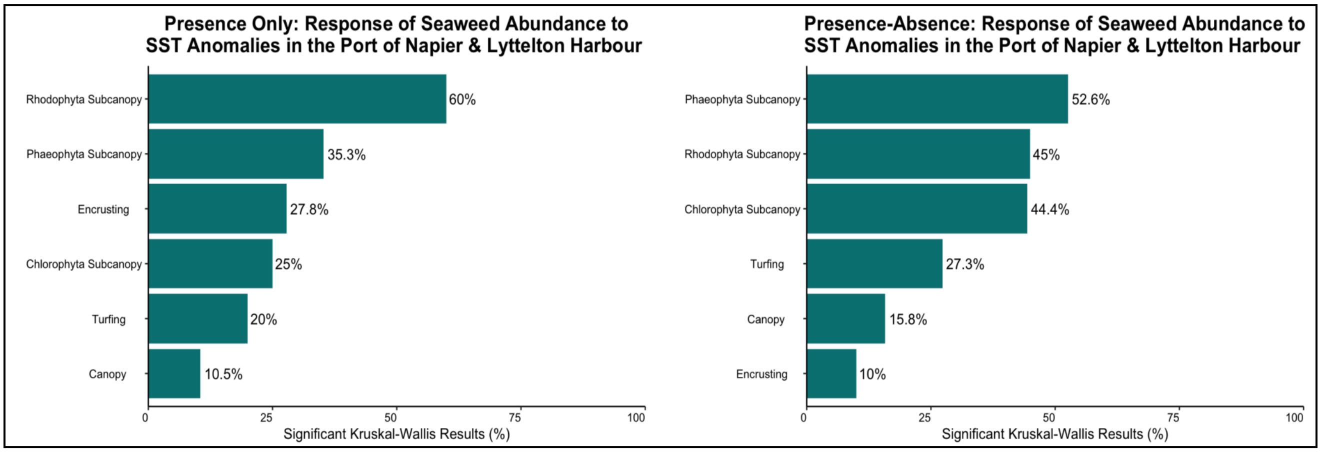

Figure 20-A: Significant results for functional groups in the Port of Napier & Lyttelton Harbour. *Each bar represents the percentage of significant results (α < 0.05) for functional groups and subgroups across all of the survey sites on the east coast of NZ (Lyttelton Harbour (3-5 m), Lyttelton Harbour (6-8 m), and the Port of Napier).*

Across the Fiordland National Park sites, the range of responses were much more narrow, ranging from 30-50% across all functional groups. However, encrusting species showed the strongest response to SST anomalies at 60%, with *Rhodophyta* (48%), *Chlorophyta* (40%), and *Phaeophyta* (37.7%) following close behind. These values were calculated by testing all data type ranges against SST anomalies and then summed (Figure 21-A). Turfing species showed no statistically significant response to SST anomalies across all the Fiordland National Park sites and therefore, it is not pictured in Figure 21-A. Wilcoxon and Dunn tests revealed significant differences in median seaweed abundance across various SST anomaly thresholds. Comparisons between 0°C–1°C, 0°C–2°C, 0°C–3°C, and 1°C–3°C showed significant results in more than 60% of tests, whereas the 1°C-2°C threshold yielded significant differences in only ~30% of cases.

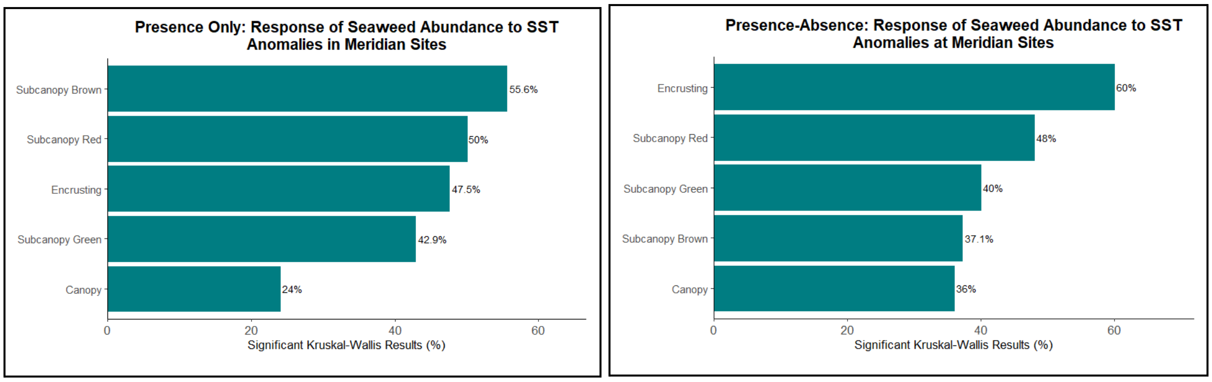

Figure 21-A: *Kruskal-Wallis tests evaluating the differences in abundance for seaweed functional groups across all Fiordland National Park sites, including intertidal, transitional and subtidal (1,3, 4, MN, MS, CN, CS, FS, FN, GN, GS, 6, 9, 11, 13, 14, 15) for different SST anomalies yielded significant results for all groups for all sites.*

Wilcoxon tests were run across all sites in the Port of Napier and Lyttelton Harbour to evaluate the difference in abundance between different SST anomaly bins, which yielded significant results for all combinations of SST anomalies. There was a notably smaller difference in abundance between SST anomalies 1°C and 2°C compared to other combinations, which resulted in significant results of 63.5-73%. The highest was reported for 1–3, followed by 0–3 and 0–1 (Figure 22-A).

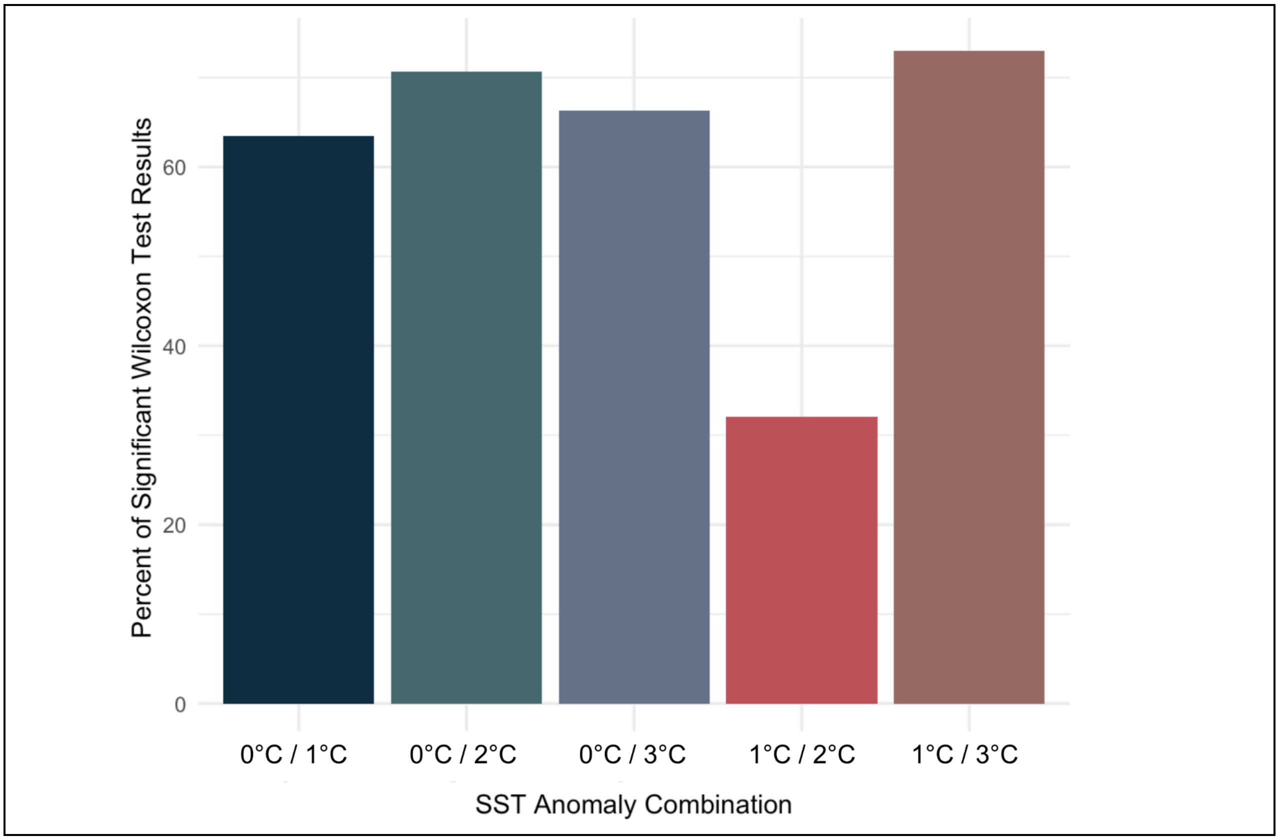

Figure 22-A: Percentage of significant results between different combinations of SST anomalies*. Each bar represents the percentage of tests that yielded significant results (α < 0.05) between the combination of SST bins specified along the x-axis.*

Dunn and Wilcoxon tests run across transitional sites (MN, MS, CN, CS, FS, FN, GN, GS), intertidal sites (1, 3, 4, 6, 9, 11, 13, 14, and 15), and subtidal sites (same as intertidal, but also all the sites at meter depths of 2, 4, 7, 10, and 16. No combination reached 60% significance, but -1:1, -2:1, and -2:2 anomaly bins showed the most significant change in abundance in correlation with sea surface temperature anomalies (Figure 23-A).

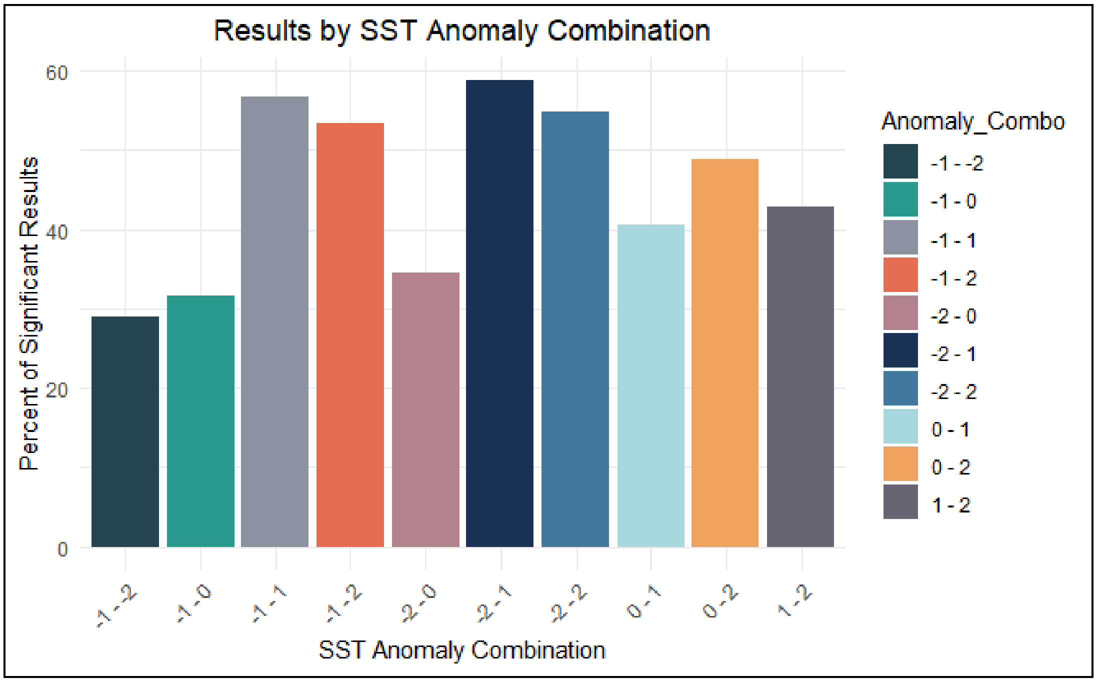

Figure 23-A: *Each bar represents the percentage of tests that yielded significant results (α < 0.05) between the combination of SST bins specified along the x-axis.*

Research Question 3: Response to SST anomalies across coastal locations in New Zealand

At the Port of Napier, sites PR3 and PR7 exhibited significant results in 40% of Kruskal-Wallis tests. PR5 showed the lowest response, with significance in only 20% of tests. No clear trend was observed concerning depth or proximity to shore (Figure 24-A).

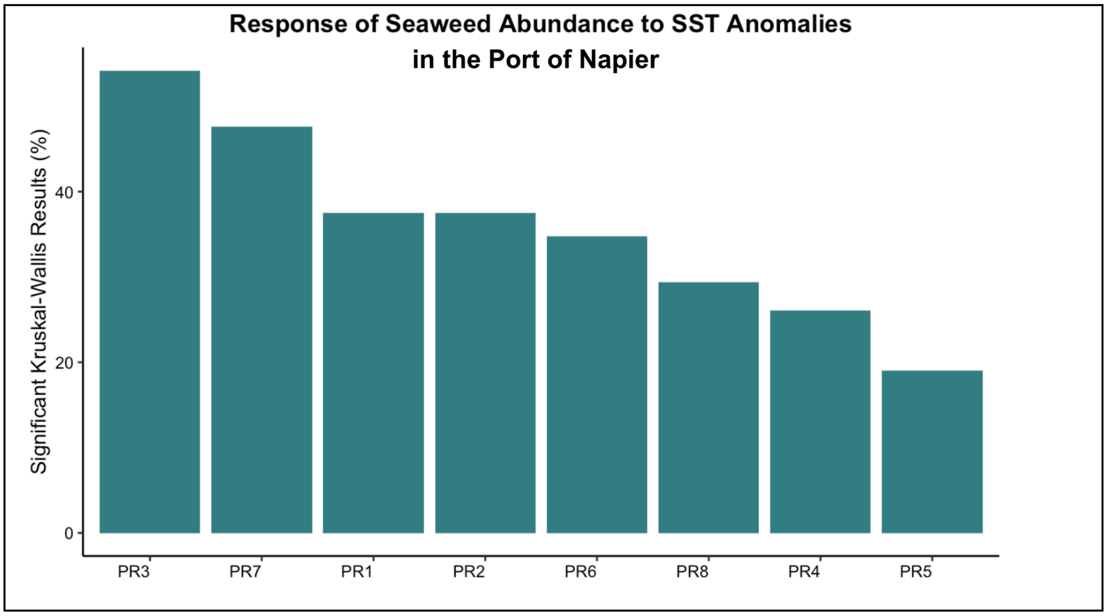

Figure 24-A: *Each bar represents the percentage of significant results from Kruskal-Wallis tests for each of the sites surveyed in the Port of Napier for all combined seaweed functional groups.*

In Lyttelton Harbour, responses were more pronounced in shallower transects, particularly at BP13, which had significant results in 87.5% of tests (Figure 25-A).

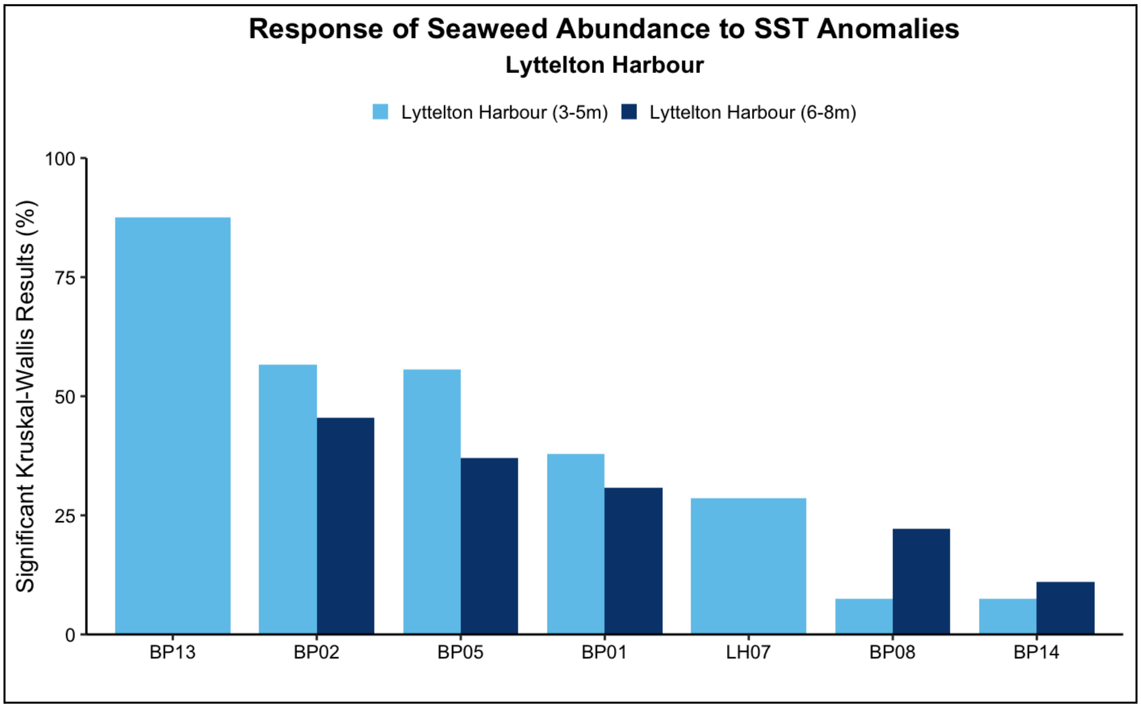

Figure 25-A: *Each bar represents the percentage of significant results from Kruskal-Wallis tests for each of the sites surveyed at Lyttelton Harbour for all combined seaweed functional groups. The lighter blue bars represent shallower sites (3-5m) and the darker blue represents deeper sites (6-8m).*

Site Comparison for Fiordland National Park Locations

Since Fiordland National Park had depth and replicate components, instead of transects like Port of Napier and Lyttelton Harbour, a different approach was taken to account for those variables across the two fiords. Spearman's Ranking was conducted for all Fiordland National Park data types (intertidal, transitional, and subtidal). Positive correlation or relationship mean abundances are increasing with sea surface temperature while negative correlations or relationships mean an abundance decrease. This method examines the correlation between the mean occurrence of kelp and SST anomalies.

Similarly, linear mixed model effects were conducted for all Fiordland National Park data types. This model examined the relationship between SST anomalies and mean occurrence data. This model also considered replicates and site impact on the relationship between the other two variables to understand the impact these variables had on SST anomalies and seaweed occurrence data. As shown in Tables 4-A through 6-A, there was a wide range of results for the different functional groups of seaweed.

Table 4-A. Significant results for Spearman's Ranking and Linear Mixed Model Effects (LMME) for Intertidal sites

	Milford Sound	**Doubtful Sound**
Spearman's Ranking	Extremely weak negative correlation (-0.025 rho)	Very weak positive correlation (0.018 rho)
LMME	*Brown subcanopy* (+) relationship abundance & SST *Encrusting, green subcanopy,* and *red subcanopy* showed (-) relationship *Encrusting* species showed the strongest (-) relationship Site variation is low, except for *brown subcanopy* and *turfing* algae.	All groups are increasing (+) relationship in abundance Moderate variance among sites (*brown & floor species*) or high replicate variance (*green* species). *Red subcanopy* species had the strongest (+) relationship with SST with little variation overall.

In the Intertidal data**,** Milford Sound showed very weak negative correlations with a correlations coefficient (rho) of -0.025 and a < 0.0001 p-value, while Doubtful Sound occurrence data consistently showed a weak positive correlation with sea surface temperature anomalies with a rho value of 0.018 and < 0.0001 p-value. (Table 4-A).

Table 5-A. Significant Results for Spearman's Ranking and Linear Mixed Model Effects for Transitional Sites

	Milford Sound	**Doubtful Sound**
Spearman's Ranking	Barely statistically significant (0.0185 rho & 0.02 p-value)	Not statistically significant (p-value: 0.673)
LMME	Not enough data for the random effects component to run the model	All groups but *brown subcanopy* species (+) relationship *Green subcanopy* had the strongest positive trend. Site-level variation was the largest for *brown* subcanopy and turfing species. *Red subcanopy* and encrusting species have low residual variation, indicating relatively stable trends.

In the Transitional data, there was a weak positive correlation between SST and mean occurrences for Milford Sound with 0.185 rho and 0.021 p-value (Table 5-A). However, Doubtful Sound showed no statistical significant correlation (p-value: 0.67). Running the LMME model for Doubtful Sound showed that all species groups increased with the exception of brown Subcanopy species. However, brown Subcanopy species had the highest site-to-site variation, which could explain the decreasing pattern. In Milford Sound, all species groups showed a negative correlation between SST anomalies and species occurrence, aside from brown Subcanopy. There was little difference from site to site for all functional groups besides Turfing and brown Subcanopy species.

Table 6-A. Significant results for Spearman's Ranking and Linear Mixed Model Effects for Subtidal sites

	Milford Sound	Doubtful Sound	Depth-Focus
Spearman's Ranking	Statistically significant but extremely weak positive correlation (rho: 0.0079, p-value: 0.0005)	Statistically significant but extremely weak positive correlation (rho: 0.0105, p-value: 1.02e-11)	Mean occurrences are statistically different depending on the depth of the survey from 2-16 m (p-value: 2.2e-16)
LMME	*Canopy* and *red subcanopy* show a (+) relationship b/w mean_count and SST *Brown* and *green subcanopy* (-) relationship *Encrusting* species groups had the strongest (-) relationship Sites differences led to large variation of mean occurrences of *encrusting species*.	*Canopy species* have a weak (-) relationship over time *Encrusting* species showing a moderate (-) relationship b/w mean_count & SST over time **All Subcanopy species had a (+) relationship over time** *Red subcanopy* had the strongest (+) relationship Site difference didn't impact abundance.	Deeper areas had less diversity and fewer mean occurrences. SST anomalies had a very weak (+) effect on mean count as depth decreased Little correlations between depth and SST Differences in site were not the cause for difference in mean count

Across the Subtidal data, there appeared to be an extremely weak positive correlation (0.0079 and 0.0105 rho values for each fiord) between SST anomalies and mean occurrences per Spearman's Ranking. At Milford Sound, there was not enough occurrence data to run the model. At Doubtful Sound, all subcanopy species had a positive relationship with SST, while brown subcanopy algae species observed a negative relationship. Site-to-site differences were minimal but did impact encrusting and brown Subcanopy species.

The linear mixed-effects model revealed that depth is the strongest predictor of species abundance, with deeper areas exhibiting significantly lower mean counts. SST anomaly had a minor positive effect (estimate coefficient at 0.0233), suggesting a weak temperature-related response; the model also indicated moderate site-level variability ($\sigma = 0.334 \pm 0.578$). However, much of the total variation in species abundance remains unexplained, highlighting the potential influence of other environmental factors as seen in residual values (24.93 ± 4.99) (Table 6-A).

Discussion

This analysis highlights variations in the community composition of kelp forests across different New Zealand coastal locations. These findings demonstrate the role of environmental variables, such as depth, latitude, and SST, in the structure of kelp forest ecosystems. The observed differences in functional group distribution suggest that local site conditions mediate the effects of temperature fluctuations, which has implications for ecosystem resilience.

The strong influence of SST anomalies on subcanopy species, particularly within the *Rhodophyta* and *Phaeophyta* taxa, indicates that temperature shifts affect community composition and could drive changes in the future (Figure 20-A). The pronounced responses at the shallow sites in Lyttelton Harbour highlight the vulnerability of nearshore ecosystems to warming events (Figure 25-A). In contrast, surveys in the Port of Napier exhibited more variable responses across transects, with no clear trend emerging. This suggests that site-specific factors influence the degree of sensitivity to temperature changes. The results of the Wilcoxon tests suggest that even moderate temperature anomalies are enough to elicit substantial shifts in the abundance of seaweed (Figure 22-A).

The weak correlations between SST anomalies and functional group occurrences at the Fiordland National Park sites suggest that temperature fluctuations may affect deeper, fiord-based ecosystems less directly. The observed increase in most functional groups—except for floor species, *Chlorophyta* subcanopy, and *Phaeophyta* subcanopy—suggests that while deeper water communities may be buffered against temperature anomalies, other environmental factors such as depth and site-to-site variability likely play a strong role in shaping distributions. The overall decrease in abundance with depth aligns with the known light requirements of many seaweed species, reinforcing the need to consider depth-related habitat constraints in kelp forest management strategies.

B. Aquaculture Experiment

Introduction

The threat of climate change has prompted researchers to explore innovative methods of kelp restoration. One method to boost the productivity of an aquaculture facility is to implement a cost-effective cleaning treatment to reduce contamination and guarantee successful biomass production for out-planting activities. Contamination, unwanted organisms in a seaweed culture, can dramatically impact the growth and quality of kelp during the indoor nursery period (Visch et al., 2024). Optimizing juvenile kelp production during this nursery period is crucial for the success of any aquaculture operation before the at-sea grow-out period (Visch, 2024), whether the biomass is used for commercial application or restoration practices. Methods of addressing contamination include mechanical removal and chemical disinfection (Visch, 2024). Mechanical removal can involve scraping the reproductive sorus tissue with a razor blade or scalpel (Richmond, 2014). Chemical disinfection of seaweed samples can be done with disinfectants such as Betadine and ethanol (Richmond, 2014; Nelson, 2005). Although these alternatives have been trialed on various species of kelps, it has not been determined if a specific method is significantly better at limiting contamination while simultaneously increasing seaweed production in kelp native to New Zealand.

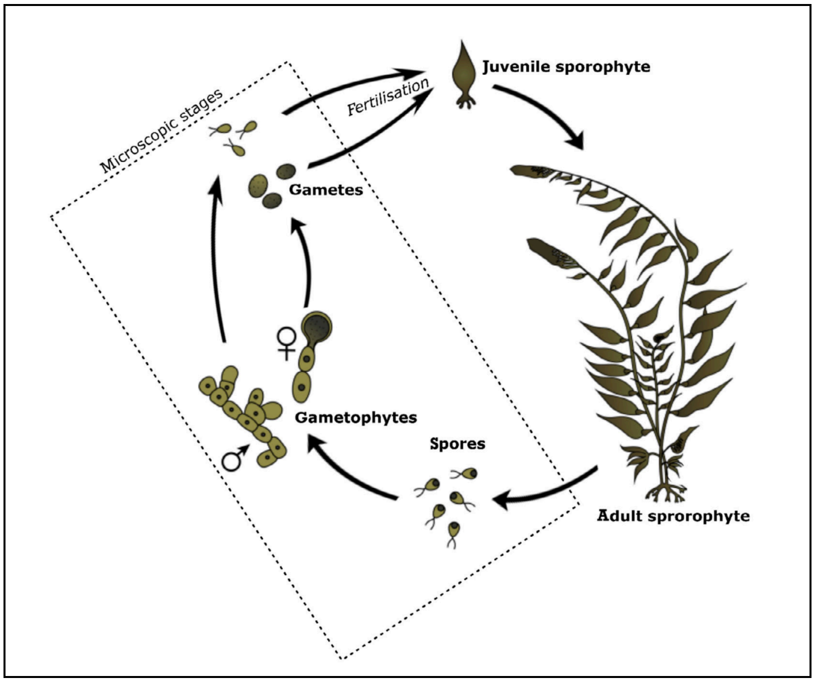

***Figure 1-B*. Overview of kelp life cycle** *(Suebsanguan et al., 2021)*

Beyond the need to improve and optimize sporulation operations, research has focused on understanding the impact of heat stress (e.g. climate change and marine heatwaves) on kelp, both worldwide (Arafeh-Dalmau et al., 2019, 2024; Duarte et al., 2018; Filbee-Dexter et al., 2020; Hollarsmith et al., 2020; Oliver et al., 2020; Rogers-Bennett & Catton, 2019) and in New Zealand specifically (Cornwall et al., 2023; Hay, 1990; Kajtar et al., 2022; Tait et al., 2021; Thomsen et al., 2019). This research can lead to the identification of species-specific responses to heat stress in order to develop heat-resistant strains of seaweeds. Cultivation of heat-resistant strains of kelp can be applied to aquaculture and restoration practices, better equipping and future-proofing susceptible ecosystems to the impacts of disturbances that occur at much longer time scales, such as ocean warming. Young kelp propagules are especially susceptible to short, intense disturbance events such as MHWs (Crossett et al., 2023; Umanzor et al., 2021). However, different kelp species have varying levels of tolerance to elevated temperatures (Hay, 1990; Nelson, 2005; North et al., 1986; Praeger et al., 2022; Vadillo Gonzalez et al., 2024; Visch et al., 2024). *Ecklonia radiata* has been shown to have the highest rate of sporophyte formation when kept under 17 °C, but is also known to tolerate a range of temperatures from 11-22°C (Visch et al., 2024). This broad range of temperature tolerance aligns with *Ecklonia radiata*'s distribution from New Zealand's subantarctic islands to subtropical Africa (Nelson, 2020). In contrast, *Lessonia variegata* is confined to a small band of habitat in the coastal area of the Cook Strait (D'Archino & Zuccarello, 2021; Nelson, 2020). Research on a similar species in the same genus, *Lessonia corrugata*, demonstrated a narrow preferred temperature range and the highest success rate for cultures maintained at 12°C (Visch et al., 2024). *Macrocystis pyrifera*, also known as Giant kelp, is another species with a known sensitivity to temperature (Le et al., 2022a). Specifically, research on this globally distributed species has shown the highest success rate for cultures maintained at 12°C, and that developmental stages such as spore settlement and germination were negatively impacted when temperature was increased to levels above 19.8°C (Le et al., 2022a).

New Zealand has growing interest in seaweed farming due to its low environmental impact, sustainable production, and the possibility to diversify the current aquaculture sector in alignment with the country's broader environmental and sustainability goals. The New Zealand government is committed to developing the nation's aquaculture to generate NZD 3 billion annually in value by 2035 (New Zealand Government, 2021). Beyond food provisioning, seaweed farming can provide critical ecosystem services such as nutrient extraction and carbon sequestration (Racine et al., 2021).

Research Questions

1. Does the cleaning process of reproductive sorus tissue before sporulation affect spore release, sporophyte survival, and contaminant levels of native New Zealand kelp?
2. Does temperature affect the spore release, sporophyte survival, and contaminant levels of native New Zealand kelp?

Methods

Sample Collection and Treatment

Ecklonia radiata, *Lessonia variegata*, and *Macrocystis pyrifera* kelp reproductive blades (*L. variegata* and *M. pyrifera*: n = 40 blades; *Ecklonia radiata*: n = 12 blades) were collected by divers in Queen Charlotte Sound in the South Island of New Zealand in July 2024. Samples were transported in sampling bags without water to the stock intake facility at Cawthron Aquaculture Park for treatment exposure.

Blades from each species were divided evenly among the treatments based on the number of available samples (*M. pyrifera* and *L. variegata*: n = 10 blades/treatment; *Ecklonia radiata*: n = 3 blades/treatment). Sample preparation for treatment exposure began by excising and discarding the non-reproductive tissue from the blade samples. The remaining sorus tissue strips were rinsed in filtered (0.35µm) and autoclaved seawater. From here, the tissue in replicate was exposed to one of four cleaning treatments: 1) Control, which consisted of dipping the samples twice in filtered and autoclaved seawater; 2) Betadine solution, which consisted of dipping the blade in a 1% Betadine solution and subsequently rinsing twice in filtered and autoclaved seawater; 3) Ethanol, which consisted of dabbing each portion of tissue with a paper towel sprayed with 70% ethanol solution and then rinsing the tissue twice in filtered and autoclaved seawater; 4) Scraping, which consisted of scraping the surface of the sorus tissue (by abrasion) with a razor blade and then rinsing it twice in filtered and autoclaved seawater.

Following the treatment exposure, the tissue portions per each treatment were sandwiched between two paper towels moistened with filtered and autoclaved seawater in separate trays per species and treatment to avoid contamination. The holding trays were stored overnight in an industrial refrigerator at 10°C in the dark. After overnight storage, each blade was prepared for spore release by rehydrating it in a 200 ml beaker with filtered and autoclaved seawater for 40 minutes and stirring the solution occasionally. The resulting sporulated solution from each replicate was then filtered through a 50-micron filter and poured into a new 200 ml beaker. All replicates for each species and treatment were kept in separate beakers.

Experiment data collection began at this point by documenting the concentration of spores/milliliter in the spore solution using an inverted Olympus CKX53 microscope at 200x magnification. A portion of 50µL of the sporulated fluid was pipetted onto a Neubauer-improved haemocytometer (Marienfeld, Germany), and the concentration of spores was calculated and expressed as spores × mL^{-1} . This step was repeated for each species-treatment combination (e.g., *L. variegata* Control, etc.). Following initial spore count calculations, ten mL of the sporulated fluid was pipetted into 6-multi well plates. Each species-treatment combination was represented by ten replicates (except for *Ecklonia radiata*, which had three replicates per treatment) and incubated at 15°C, 12:12 photoperiod, and 10-15 µmol m^{-2} s^{-1} LED white light (n = 92 total replicates). The Control samples only of each species were further divided between 15°C (n=10 for *L. variegata* and *M. pyrifera*, n=3 for *Ecklonia radiata*) and 21°C (n=10 for *L. variegata* and *M. pyrifera*, n=3 for *Ecklonia radiata*) culture cabinets with 12:12 photoperiod and 10-15 µmol m^{-2} s^{-1} LED white light (n = 23 total replicates).

Post-Treatment Assessment and Data Collection

Each well was placed under a microscope at 200x magnification, and a digital camera (Olympus DP22) mounted on the microscope was used to take photos of the well from fields of view at the four poles and one in the center of the well (Figure 2-B). The well plates were not marked, so the exact same area of the well was not photographed at every monitoring period. Pictures were taken at various time intervals during the incubation period (1 day, 4 days, 10 days, 21 days, 28 days, 35 days, 42 days, 49 days, and 56 days) to document the effects of temperature and treatment on the different developmental stages.

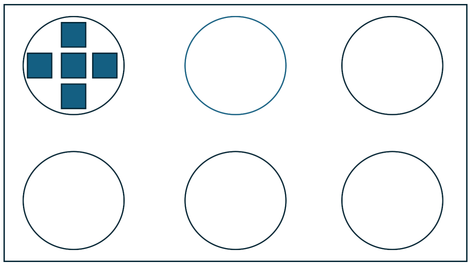

Figure 2-B. Method for photo documentation of sample wells. *Modified by Biancacci from (Le et al., 2022)*

Photos were analyzed using ImageJ image analysis software to manually assist with counting either spores, gametophytes or sporophytes and to assess contaminant levels (Figure 3-B). Each photo was analyzed for specific variables based on the time elapsed since sporulation:

- Day 1: settled spores counted and contamination level assessed
- Day 4: germinated spores counted
- Day 10: gametophytes counted and divided by sex
- Day 21 - 56: sporophytes and blades counted, contamination level assessed
    - Once a sporophyte was detected in a sample (ex: ecklonia_control_1 on day 21), that sample was no longer monitored for the duration of the experiment

Exact counting was used to determine values for settled spores, germinated spores, gametophytes (male and female), and sporophytes. Contaminants were evaluated with a Likert ordinal scale (0 = none, 1 = rare, 2 = uncommon, 3 = common, 4 = abundant).

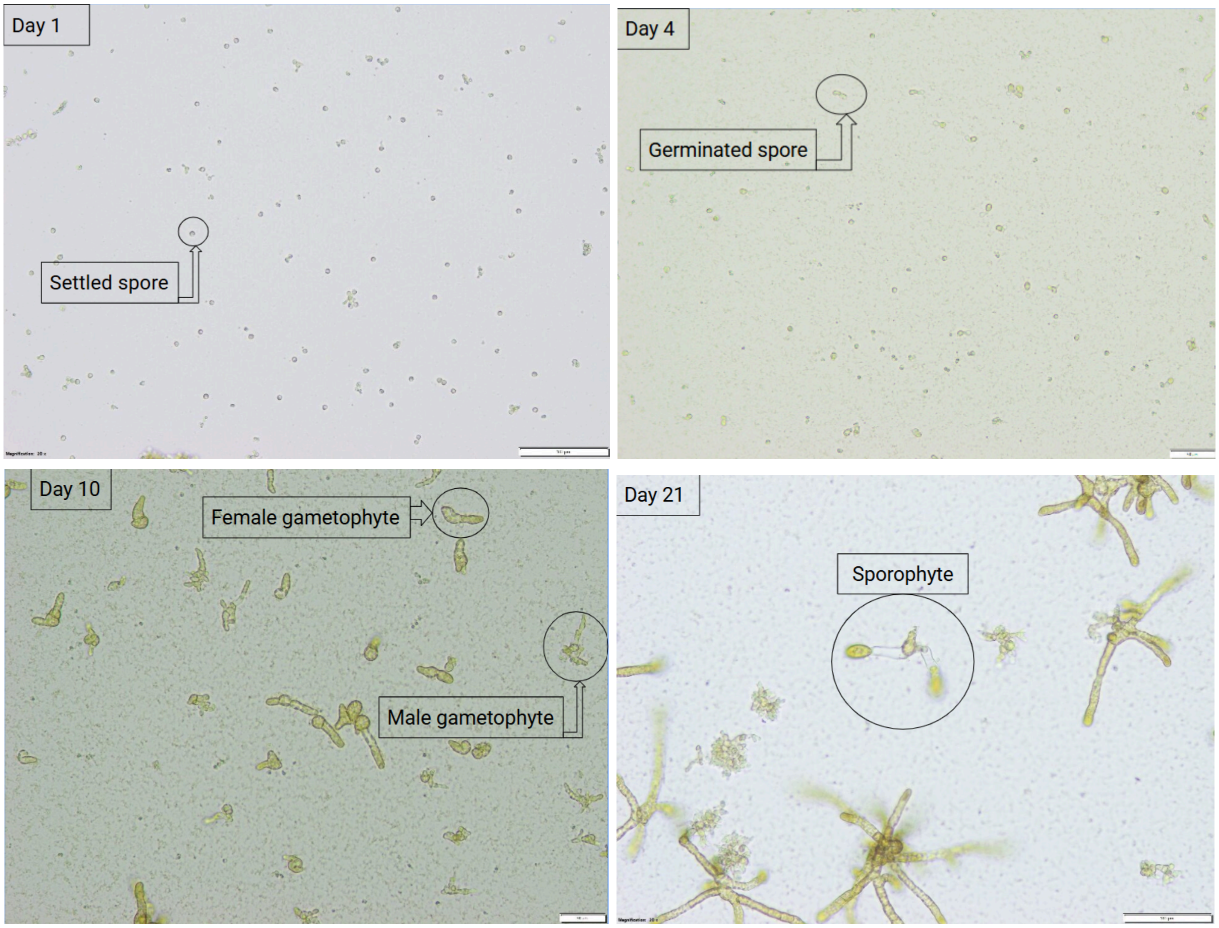

Figure 3-B. Example of the development stages of Lessonia variegata (from Control treatment at 15°C). *Day 1) Example of a settled spore highlighted in the circle; Day 4) Example of a germinated spore highlighted in the circle; Day 10) Examples of female and male gametophytes highlighted in separate circles; Day 21) Example of a sporophyte with two blades highlighted in the circle*

To obtain "per well" values, the five images associated with each sample (ex: ecklonia_control_1) were averaged to represent the 0.36 mm^2 area sampled at 200x magnification. This average was then multiplied by 260 (for all variables except average contamination and percent male gametophytes per well) to reflect the total area of the well (96.21 mm^2).

Data Analysis

Group members worked both independently and alongside Cawthron staff to analyze the data generated during the experiment. Data analysis methods aimed to determine the effects of treatment and temperature on the kelp developmental stages and variation in response between species. Program R was used to create plots and conduct statistical analyses.

1. *Spore settlement (%):* This was calculated by dividing the number of settled spores from Day 1 by the initial number of spores that were released on day 0, then multiplying by 100.
2. *Germination (%):* This analysis focused on observed germinated spores/well from day 4. The percentage of germinated spores was calculated by dividing the total number of germinated spores by the total number of spores that had settled on day one, multiplied by 100.
3. *Percent male gametophytes (sex ratio)*: This analysis focused on the percentage of male gametophytes/well observed on Day 10.
4. *Sporophyte development*: This analysis focused on observations of sporophytes/well from days 21-56. The total number of sporophytes was estimated by multiplying the average value of sporophytes per photographed area (n = 5 per well at 200x magnification) by the total area of the well (96.21 mm^2) and expressed as sporophytes mm^2.
5. *Contamination*: This analysis focused on average contamination level/well observed on Day 1 and Day 21-56.

All data were analyzed visually for normality of distribution and equal variances (QQ-plots) and using statistical tests (Shapiro-Wilks, Levene's). The appropriate statistical test was chosen based on the data's distribution and the number of groups being compared. An α value of 0.05 was chosen as the threshold for significance. For parametric data, ANOVA (standard and Welch's) was used for comparisons between the four cleaning treatments and species, and the Independent T-test was used for comparisons between the two temperature groups. For nonparametric data, the Kruskal-Wallis test was applied for comparisons between the four cleaning treatments and species, while the Mann-Whitney U test was used for two-group temperature comparisons (15°C and 21°C). Statistical analysis aimed to test the hypotheses that cleaning processes applied to reproductive sorus tissue before sporulation does affect spore release, sporophyte survival, and contaminant levels, and that temperature does affect the spore release, sporophyte survival, and contaminant levels of native New Zealand kelp.

Results

Cleaning Treatment Comparison

1. ***Spore Settlement (%)****- All species at 15° on day 1*

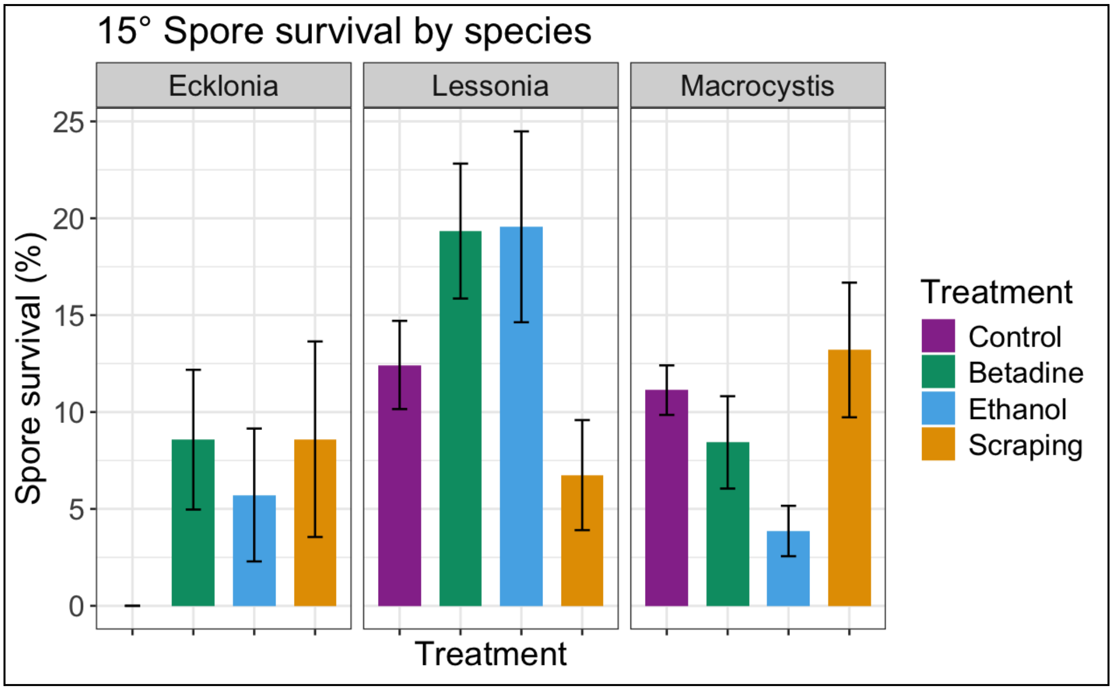

***Figure 4-B**. **Comparison of the percent of surviving spores observed in samples exposed to different treatments at 15°C.** The percent of surviving spores for each treatment were compared within each species.*

For *Ecklonia radiata*, the Kruskal-Wallis rank sum test determined there was no significant difference in the percent of surviving spores based on treatment (P-value: 0.217833170).

For *Lessonia variegata,* the Kruskal-Wallis rank sum test determined that there was a difference in the percent of surviving spores based on treatment (p-value: 0.01407946). A Dunn test with a Bon Ferroni correction determined Betadine had significantly higher spore survival than Scraping (p-value: 0.0051), and Ethanol had a significantly higher spore survival than Scraping (p-value: 0.0120).

For *Macrocystis*, a one-way ANOVA determined there was a significant difference in the percent of surviving spores based on treatment (F-value: 3.089; p-value: 0.0392). A Tukey's Honestly Significant Difference post-hoc test determined that Scraping had a significantly higher percent of surviving spores than the Ethanol treatment (p-value: 0.0324337). There were no significant differences for the other treatment comparisons (Betadine–Control p-value: 0.8397, Ethanol–Control p-value: 0.1322; Scraping–Control p-value: 0.9187; Ethanol–Betadine p-value: 0.5024; Scraping–Betadine p-value: 0.4661).

2. ***Spore settlement (%) by treatment-*** *Control on day 1*

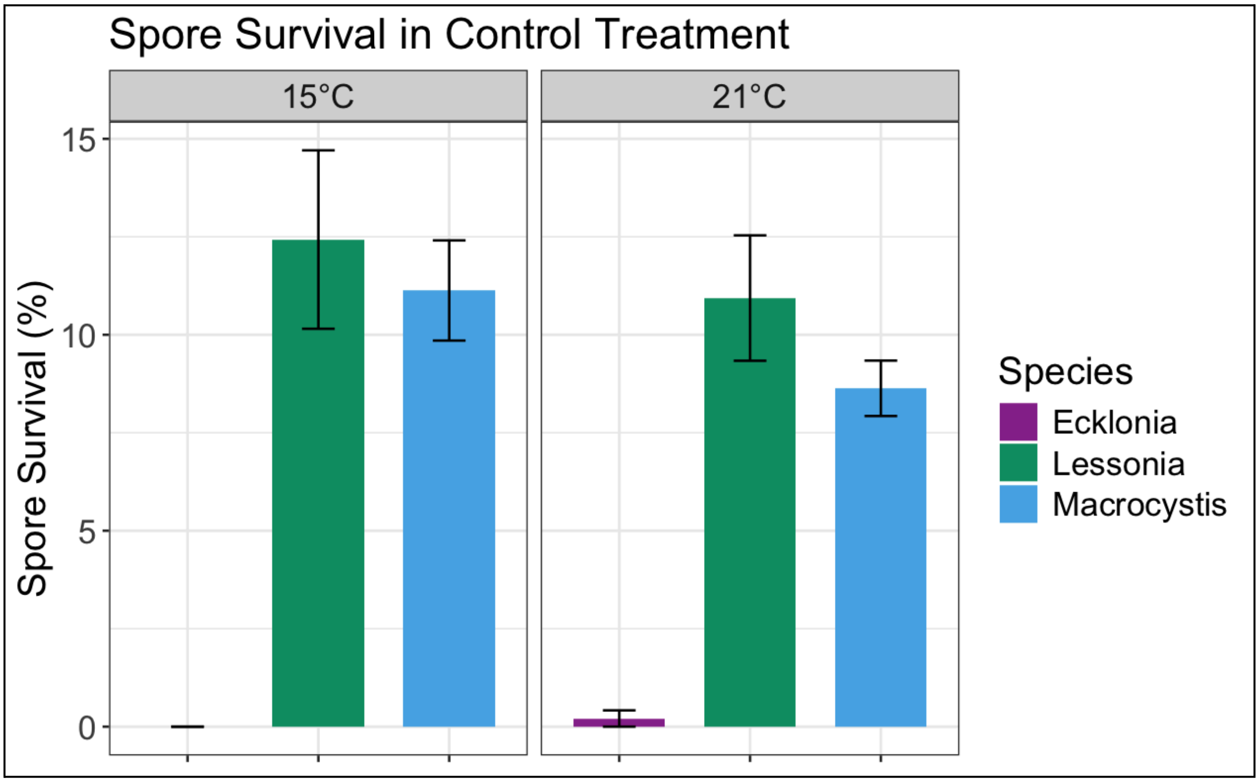

***Figure 5-B*. *Comparison of the percent of surviving spores observed in samples exposed to the Control treatment at 15°C and 21°C*.** *The percent of surviving spores for each species were compared within each temperature exposure. The Control treatment was the only treatment with samples exposed to 15°C and 21°C.*

At 15°C, a one-way ANOVA determined there was a difference in the percent of surviving spores by species exposed to the Control treatment (F-value: 6.039, p-value: <0.01). A Tukey's Honestly Significant Difference post-hoc test determined that *Ecklonia radiata* had a significantly lower spore survival compared to both *Lessonia variegata (*p-value: <0.01) and *Macrocystis pyrifera* (p = 0.0165339). There was no significant difference between *Lessonia variegata* and *Macrocystis pyrifera* (p-value: 0.8601986).

At 21°C, a one-way ANOVA determined there was a difference in the percent of surviving spores by species exposed to the Control treatment (F-value = 9.644, p-value: <0.01). A Tukey's Honestly Significant Difference post-hoc test determined that *Ecklonia radiata* had a significantly lower spore survival compared to both *Lessonia variegata* (p-value: <0.001) and *Macrocystis pyrifera* (p-value: <0.01). There was no significant difference between *Lessonia variegata* and *Macrocystis pyrifera* (p-value: 0.3661983).

3. ***Spore settlement (%) by treatment-*** *Betadine on day 1*

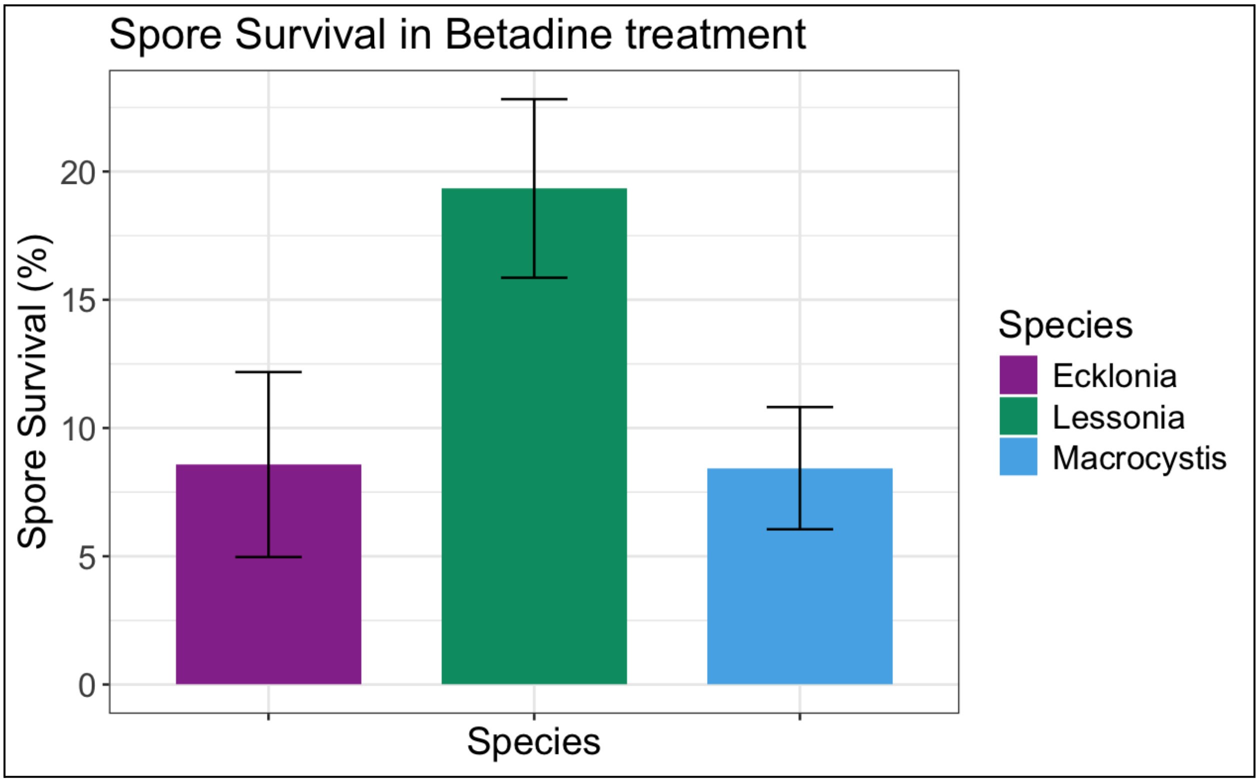

***Figure 6-B**. **Comparison of the percent of surviving spores observed in samples exposed to the Betadine treatment at 15°C**.*

On day 1, the Kruskal-Wallis rank sum determined there was a difference in the percent of surviving spores by species exposed to the Betadine treatment (p-value: 0.02856). A Dunn test with a Bon Ferroni correction determined *Lessonia variegata* had a significantly higher spore survival percent than *Macrocystis* pyrifera (p-value: <0.01). There was no significant difference in the percent of surviving spores between *Ecklonia radiata* and *Macrocystis pyrifera* (p-value: 0.4970), or *Lessonia variegata* and *Ecklonia radiata* (p-value: 0.0437).

4. ***Spore settlement (%) by treatment-*** *Ethanol on day 1*

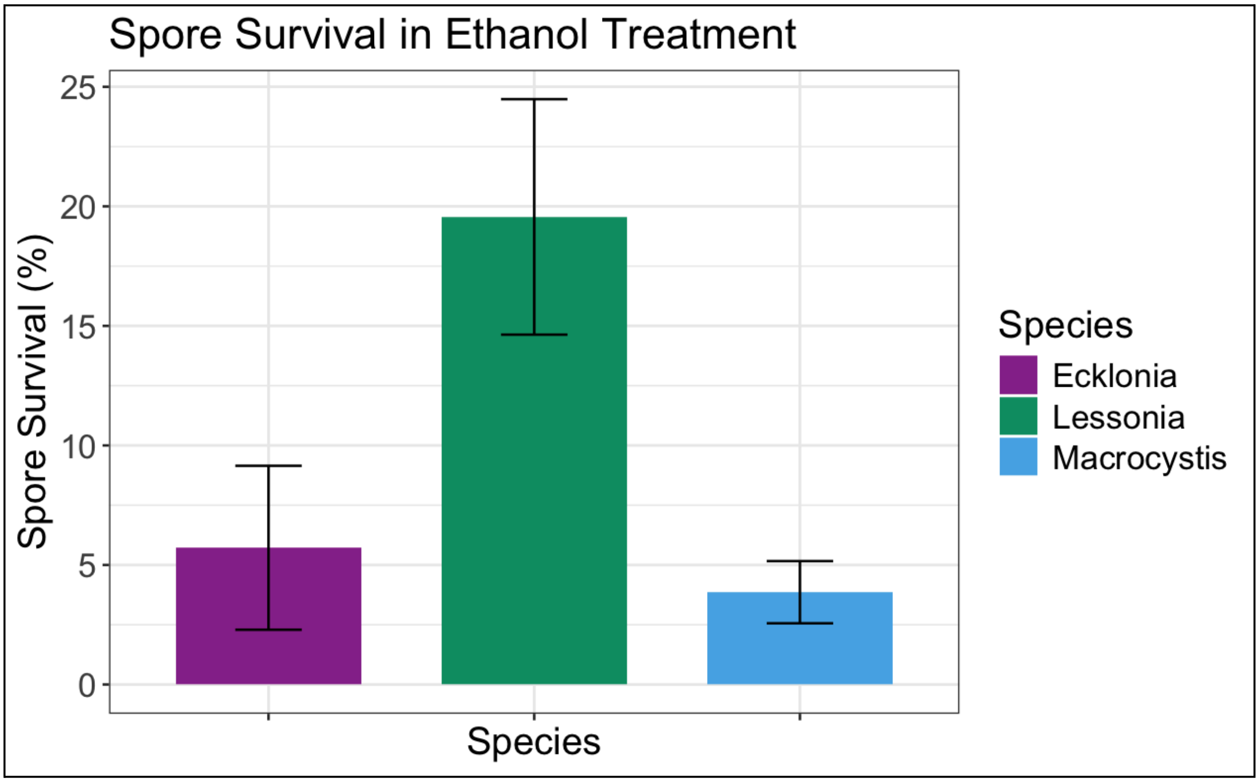

***Figure 7-B**. **Comparison of the percent of surviving spores observed in samples exposed to the Ethanol treatment at 15°C**.*

The Kruskal-Wallis rank sum determined there was a difference in the percent of surviving spores by species exposed to the Ethanol treatment (p-value: <0.01). A Dunn test with a Bon Ferroni correction determined *Lessonia variegata* had a significantly higher spore survival than *Ecklonia radiata* (p-value: <0.001) and *Macrocystis pyrifera* (p-value: 0.1052). There was no significant difference between *Ecklonia radiata* and *Macrocystis pyrifera* (p-value: 0.4465).

5. ***Spore settlement (%) by treatment-*** *Scraping on day 1*

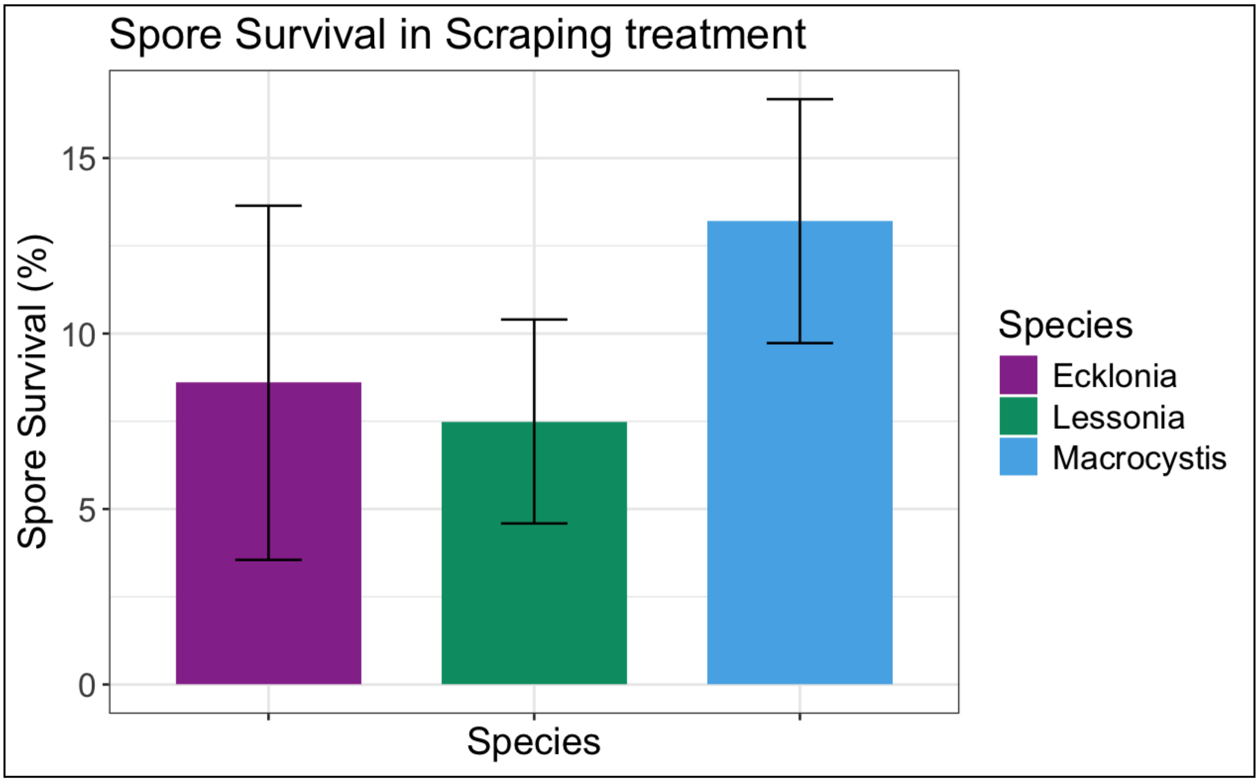

***Figure 8-B**. **Comparison of the percent of surviving spores observed in samples exposed to the Scraping treatment at 15°C**.*

The Kruskal-Wallis rank sum determined there was no difference in the percent of surviving spores by species exposed to the Scraping treatment (p-value: 0.2741).

6. *Contamination assessment- Ecklonia radiata*

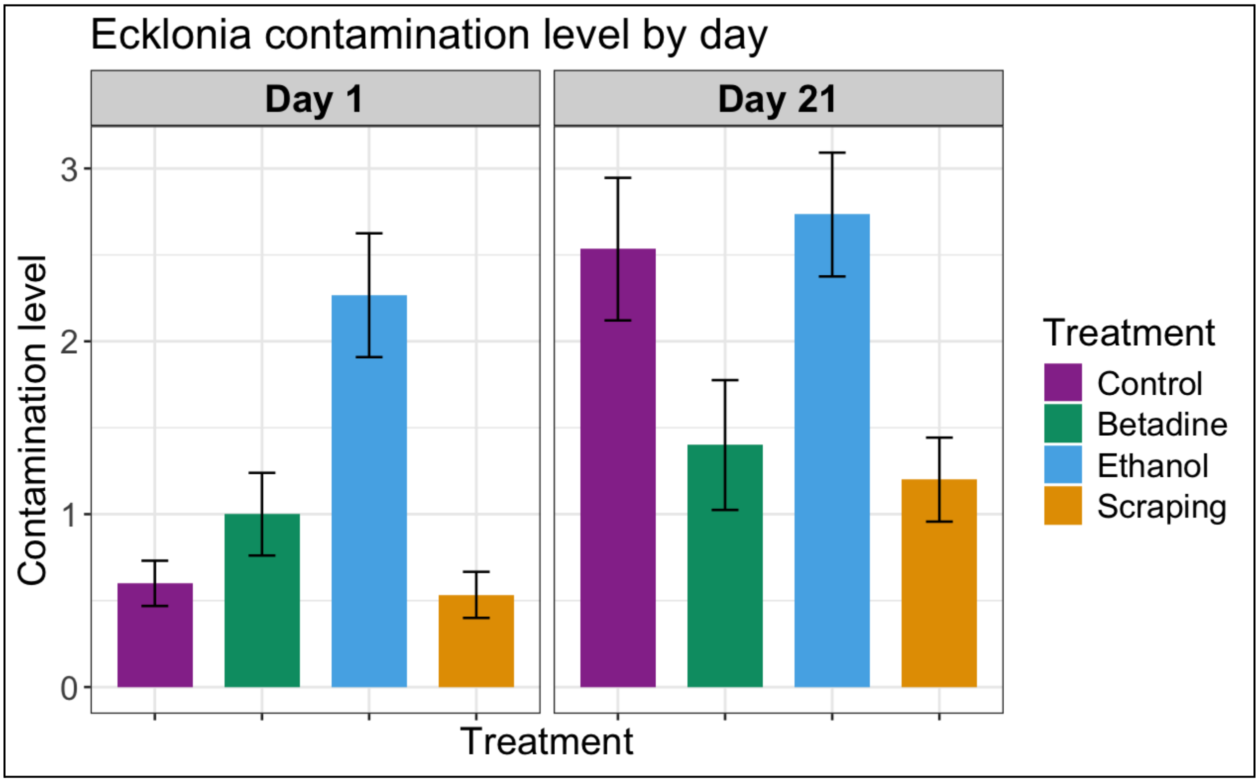

***Figure 9-B**. **Comparison of the Ecklonia radiata contamination level observed in samples exposed to different cleaning treatments at 15° temperature on day 1 and day 21**. The contamination level for each treatment was compared within each day. Contamination level is the grand average of the mean contamination level calculated from each sample's 5 well photos.*

On day 1, the Kruskal-Wallis rank sum determined that there was a significant difference in contamination level based on treatment (p-value: <0.001). A Dunn test with a Bon Ferroni correction determined the Ethanol treatment had significantly higher contamination than the Control (p-value: <0.001) and Scraping (p-value: <0.001) treatments. There was no significant difference in contamination for the other group comparisons (Betadine–Control p-value: 0.8271; Betadine–Ethanol p-value: 0.0304; Betadine–Scraping p-value: 0.5338; Control–Scraping p-value: 1).

On day 21, the Kruskal-Wallis rank sum determined that there was a significant difference in contamination level based on treatment (p-value: <0.01). A Dunn test determined the Ethanol treatment had more contamination than the Scraping treatment (p-value: 0.0172). There was no significant difference in contamination for the other group comparisons (Betadine–Control p-value: 0.1191; Betadine–Ethanol p-value: 0.0455; Betadine–Scraping p-value: 1; Control–Ethanol p-value: 1; Control–Scraping p-value: 0.0516).

7. *Contamination assessment- Lessonia variegata*

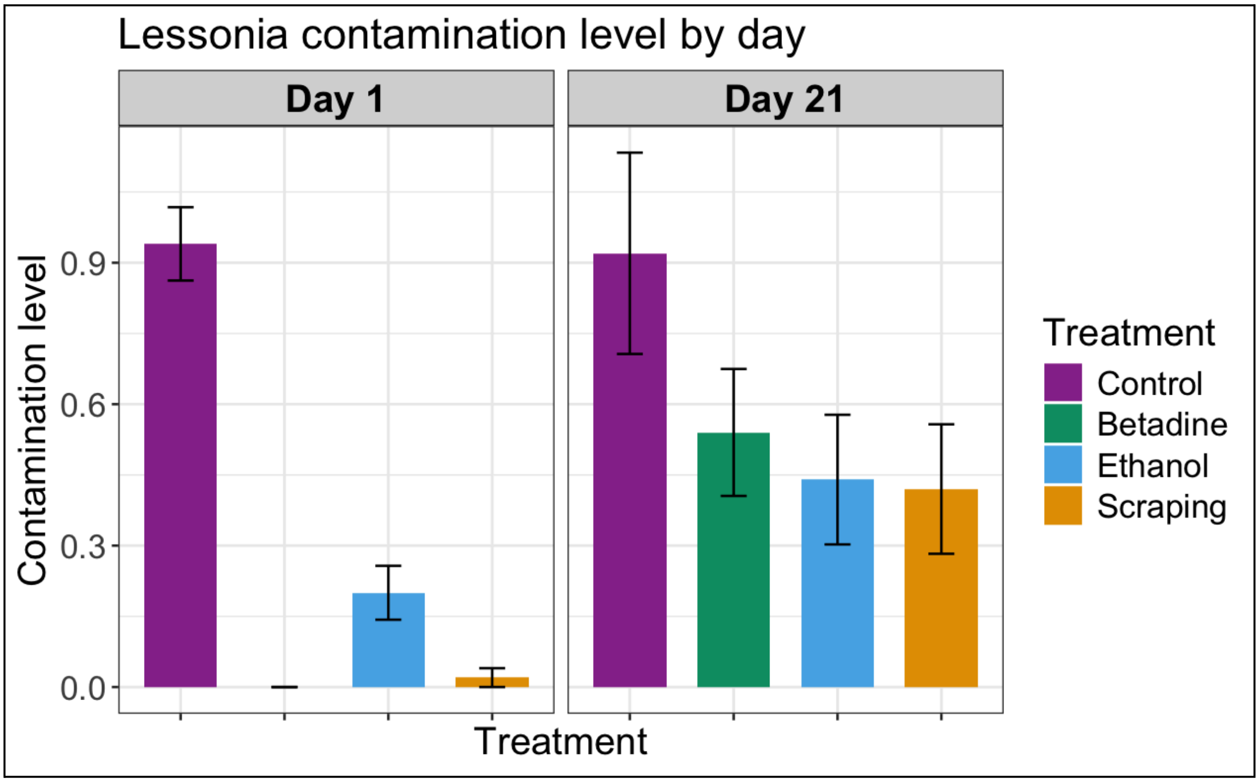

***Figure 10-B*. *Comparison of the Lessonia variegata contamination level observed in samples exposed to different treatments at 15° temperature on day 1 and day 21*.** *The contamination level for each treatment was compared within each day. Contamination level is the grand average of the mean contamination level calculated from each sample's 5 well photos.*

On day 1, the Kruskal-Wallis rank sum determined that there was a significant difference in contamination level based on treatment (p-value: <0.001). A Dunn test determined Betadine had significantly less contamination than Control (p-value = 0.0); Ethanol had significantly less contamination than Control (p-value: 0.0); and Scraping had significantly less contamination than Control (p-value: 0.0). There was no significant difference in contamination for the other group comparisons (Betadine–Ethanol p-value: 0.0843; Betadine–Scraping p-value: 1; Ethanol–Scraping p-value: 0.144).

On day 21, the Kruskal-Wallis rank sum determined there was no significant difference in contamination levels between treatments (p-value = 0.2875).

8. *Contamination assessment – Macrocystis pyrifera*

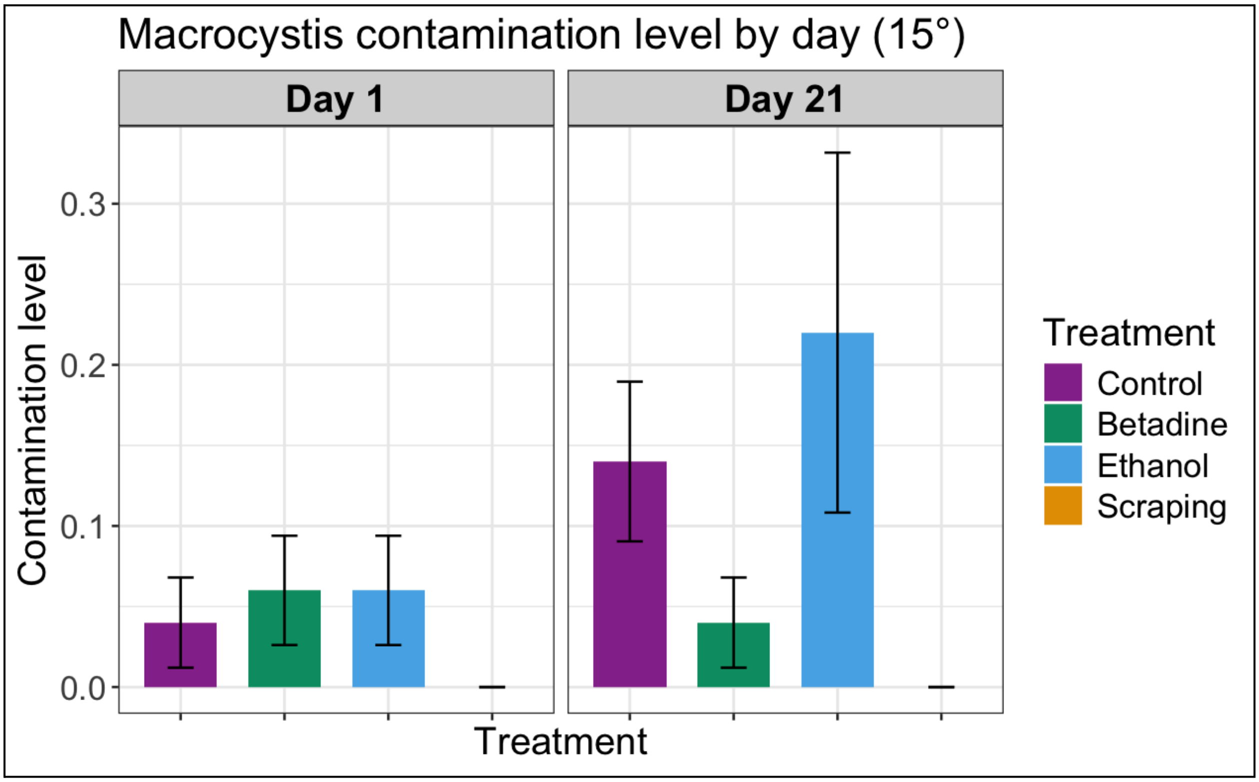

***Figure 11-B**. **Comparison of the Macrocystis pyrifera contamination level observed in samples exposed to different treatments in the 15° temperature exposure on day 1 and day 21**. The contamination level for each treatment was compared within each day. Contamination level is the grand average of the mean contamination level calculated from each sample's 5 well photos.*

On day 1, the Kruskal-Wallis rank sum determined there was no significant difference in contamination level between treatments (p-value = 0.3751).

On day 21, the Kruskal-Wallis rank sum determined there were significant differences in contamination level between treatments (p-value = 0.03599). A Dunn test with a Bon Ferroni correction determined the Control treatment had a significantly higher contamination level than the Scraping treatment (p-value: 0.0169). There was no significant difference in contamination for the other group comparisons (Betadine–Control p-value: 0.1426; Betadine–Ethanol p-value: 1; Betadine–Scraping p-value: 1; Control–Ethanol p-value: 0.8353; Ethanol–Scraping p-value: 0.2728).

9. ***Germination (%)***

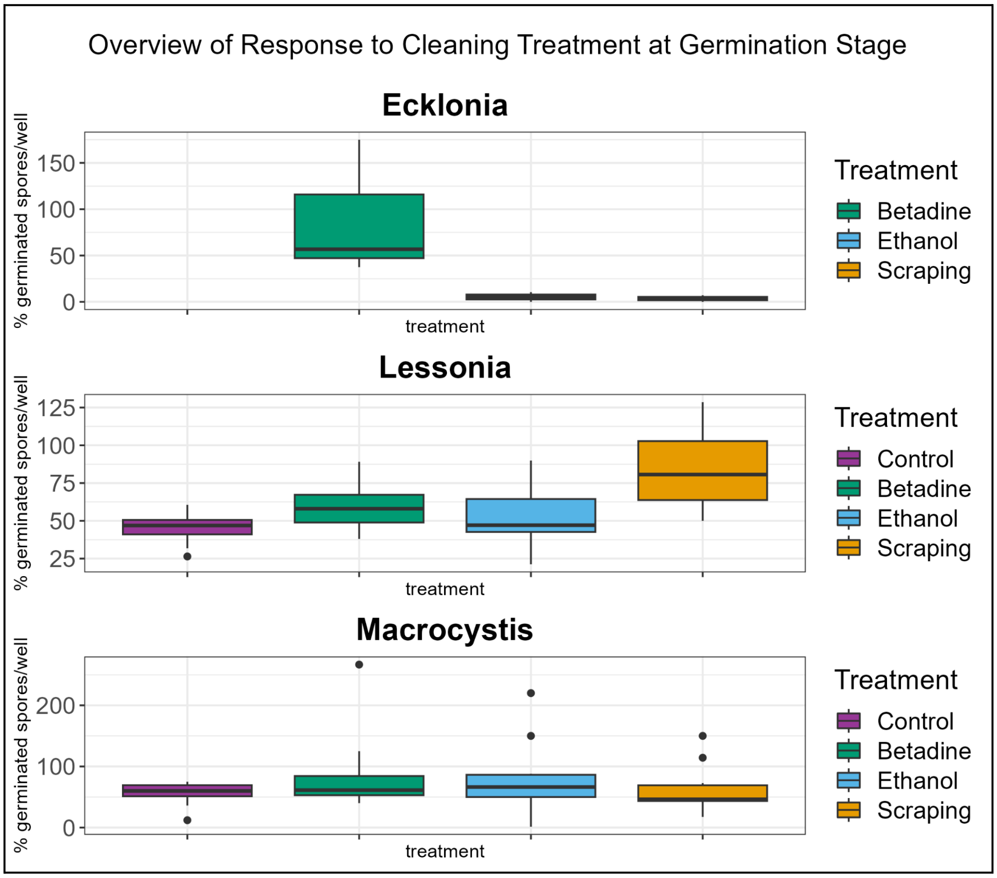

***Figure 12-B*. *Overview of Response to Cleaning Treatment at Germination Stage*.** *This demonstrates the percent of settled spores which germinated on day 4 that were observed in samples (n = 3 for E. radiata, n = 10 for L. variegata and M. pyrifera) exposed to the different cleaning treatments.*

E. radiata did not show differences in germination percentage between the treatments tested (ANOVA, F = 2.238, p = 0.223). There were no germinated spores observed in the Control samples.

L. variegata demonstrated different germination percentages depending on the cleaning treatment (Welch's ANOVA, F = 5.7214, p = 0.006768). Specifically, the Scraping treatment had a higher percentage of germinated spores when compared to the Control treatment (Games-Howell, p = 0.019). Aside from those two treatments, there were no other significant differences observed.

M. pyrifera did not show differences in germination between the treatments that were tested (Kruskal-Wallis, p = 0.6048).

*10. **Percent male gametophytes (sex ratio)***

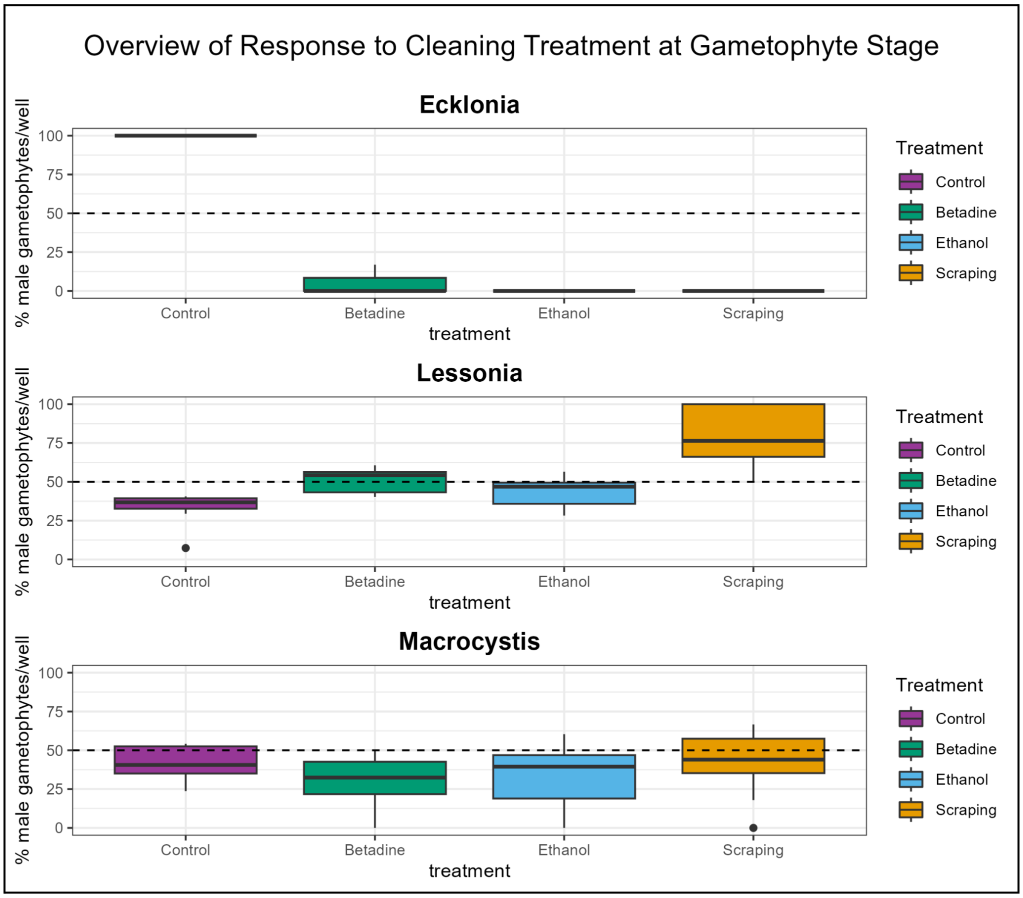

***Figure 13-B. Overview of Response to Cleaning Treatment at Gametophyte Stage**. This demonstrates the percent of male gametophytes in samples (n = 3 for E. radiata, n = 10 for L. variegata and M. pyrifera) exposed to the different cleaning treatments.*

There were no significant differences reported for the percentage of male gametophytes across the cleaning treatment in cultures of *E. radiata* (Kruskal-Wallis test, p = 0.343).

L. variegata showed a higher percentage of male gametophytes in cultures obtained from the Betadine treatment compared to the Control (Dunn test, p = 0.0087). Additionally, *L. variegata* treated with the Scraping method resulted in a significantly higher percentage of male gametophytes when compared to both the Control (p = <0.001) and Ethanol (p = 0.0025) treatments.

There were no significant differences reported for the percentage of male gametophytes across the cleaning treatment in cultures of *M. pyrifera* (Kruskal-Wallis test, p = 0.2515).

11. ***Sporophyte development***

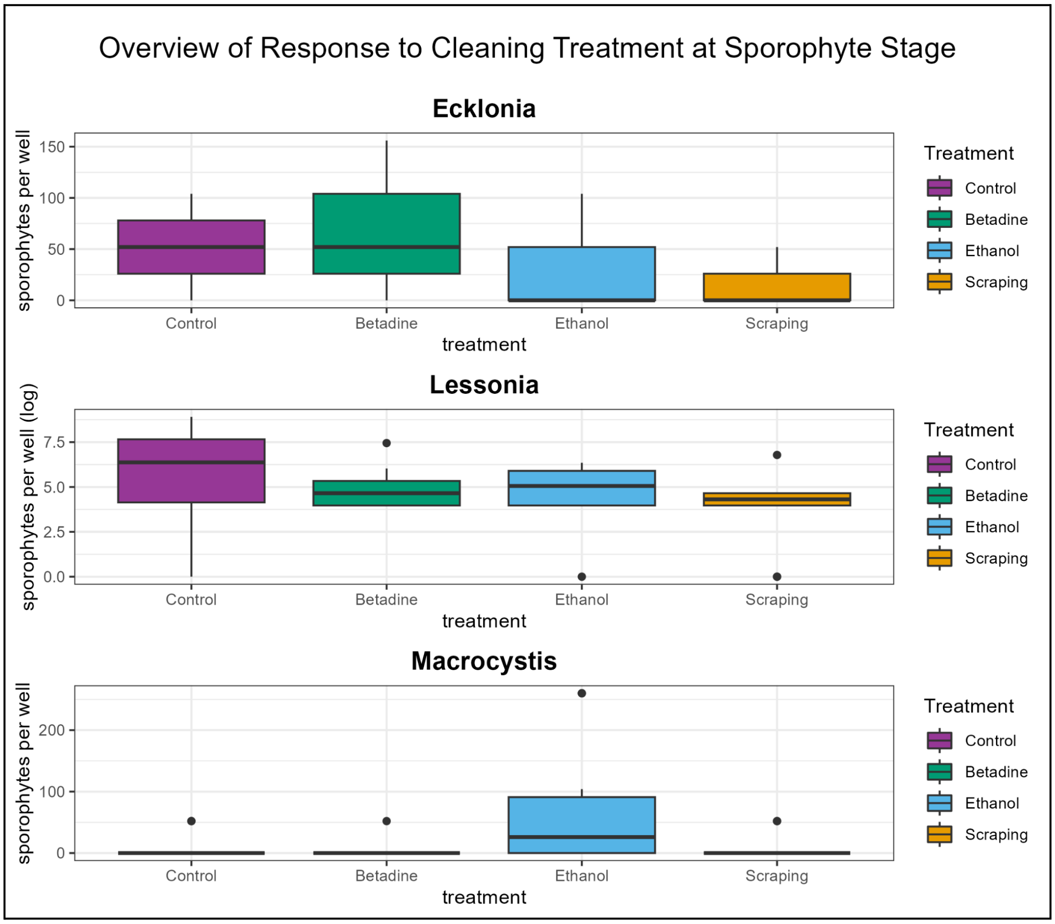

***Figure 14-B*. *Overview of Response to Cleaning Treatment at Sporophyte stage (15°)*.** *This demonstrates the amount of sporophytes observed in samples (n = 3 for E. radiata, n = 10 for L. variegata and M. pyrifera) exposed to the different cleaning treatments. The sporophyte count of L. variegata was log-transformed.*

There were no significant difference in sporophyte output across treatments for any of the species tested (Kruskal-Wallis, *E. radiata* (p = 0.7088), *L. variegata* (p = 0.1979), *M. pyrifera* (0.1155).

1. ***Spore Settlement (%) -** day 1*

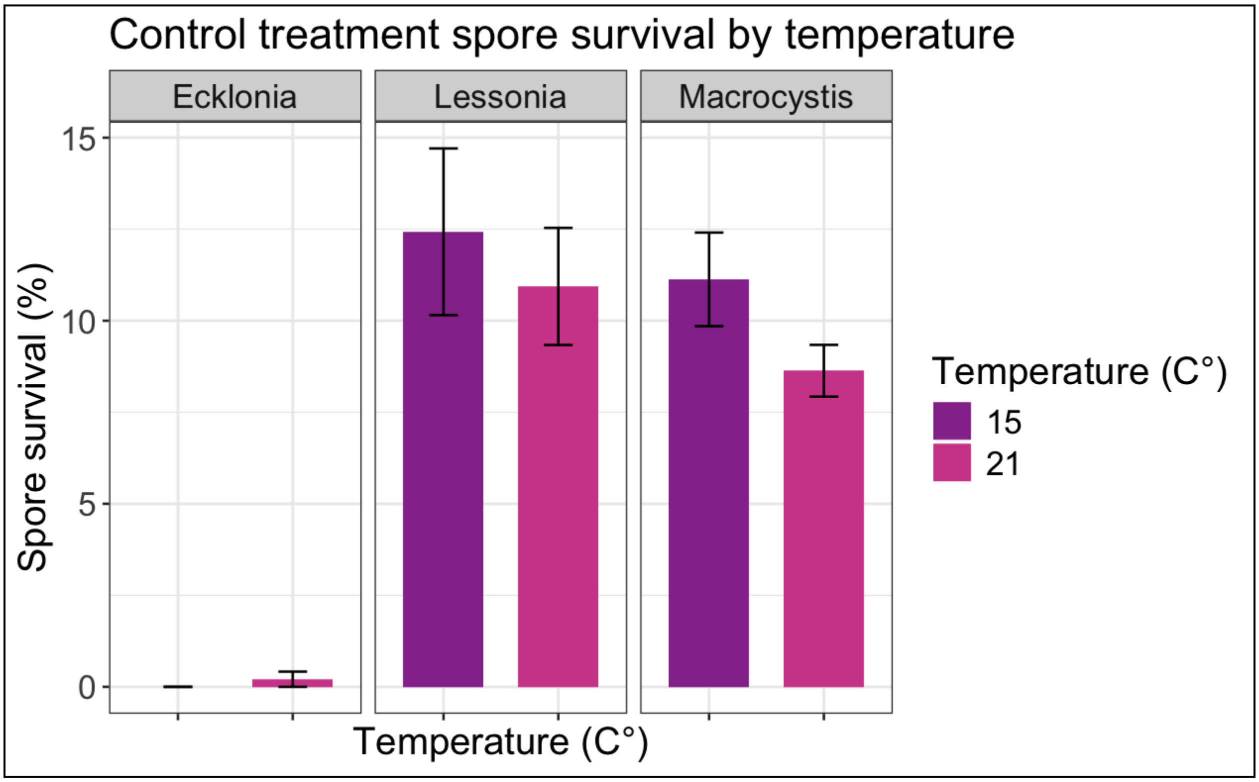

***Figure 15-B**. **Comparison of the percent of surviving spores observed in samples exposed exclusively to the Control treatment at 15°C and 21°C.** The spore survival for each temperature was compared within species.*

On day 1, a Mann-Whitney U test determined there was no significant difference in the percentage of surviving spores between the 15°C and 21°C groups for *Ecklonia radiata* (p-value = 0.6171).

On day 1, a Mann-Whitney U test determined there was no significant difference in the percentage of surviving spores between the 15°C and 21°C groups for *Lessonia variegata* (p-value: 0.7959).

On day 1, a Mann-Whitney U test determined there was no significant difference in the percentage of surviving spores between the 15°C and 21°C groups for *Macrocystis pyrifera* (p-value: 0.3256).

Macrocystis pyrifera and *Lessonia variegata* reported higher percentages at 15 °C compared to 21°C, while an opposite trend was recorded for *Ecklonia radiata* (Figure 15-B).

2. ***Contamination assessment in Ecklonia radiata cultures under two temperatures, 15°C and 21°C***

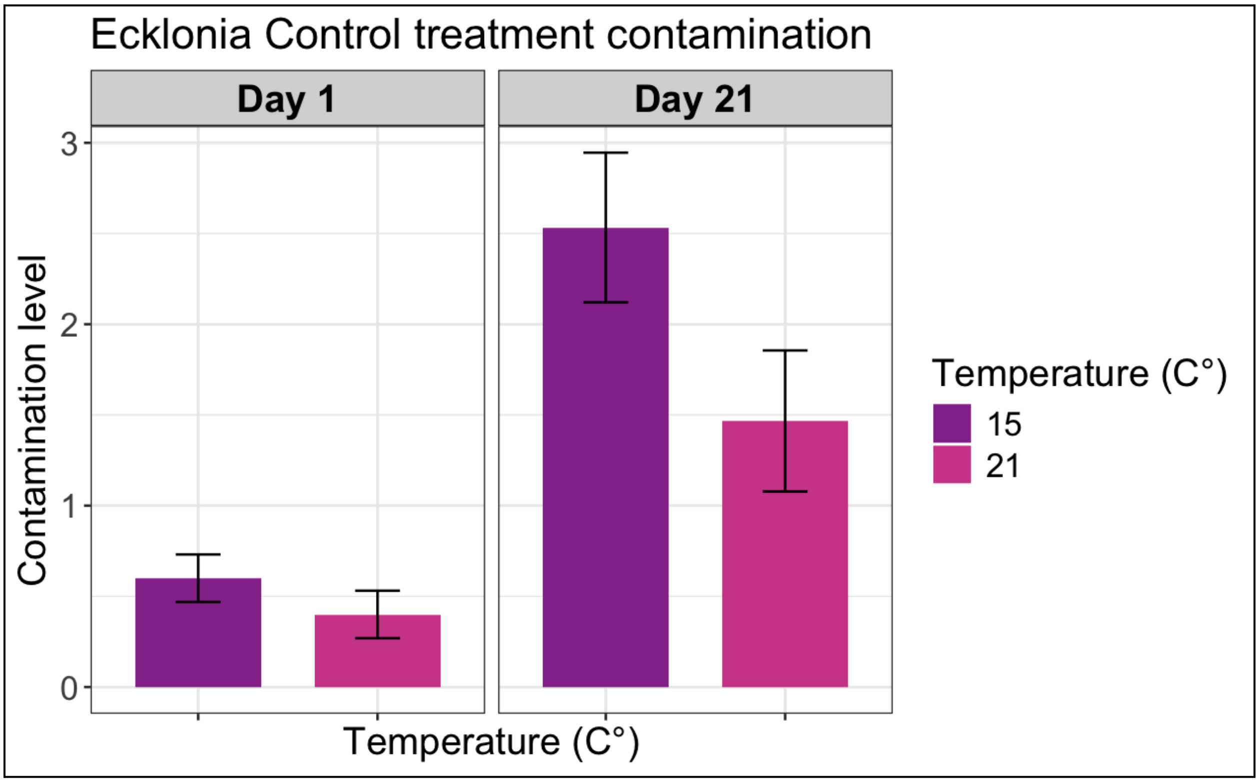

***Figure 16-B**. **Comparison of the Ecklonia radiata contamination levels observed in samples exposed to only the Control treatment cultured at two different temperatures, 15°C and 21°C, on day 1 and day 21**. The contamination level for each temperature was compared within the day.*

A Mann-Whitney U test determined there was no significant difference in the contamination levels between the 15°C and 21°C groups for the Control treatment on day 1(p-value: 0.2923), or on day 21 (p-value: 0.0731). Contamination levels trended higher on day 21.

3. *Contamination assessment in Lessonia variegata cultures under two temperatures, 15°C and 21°C*

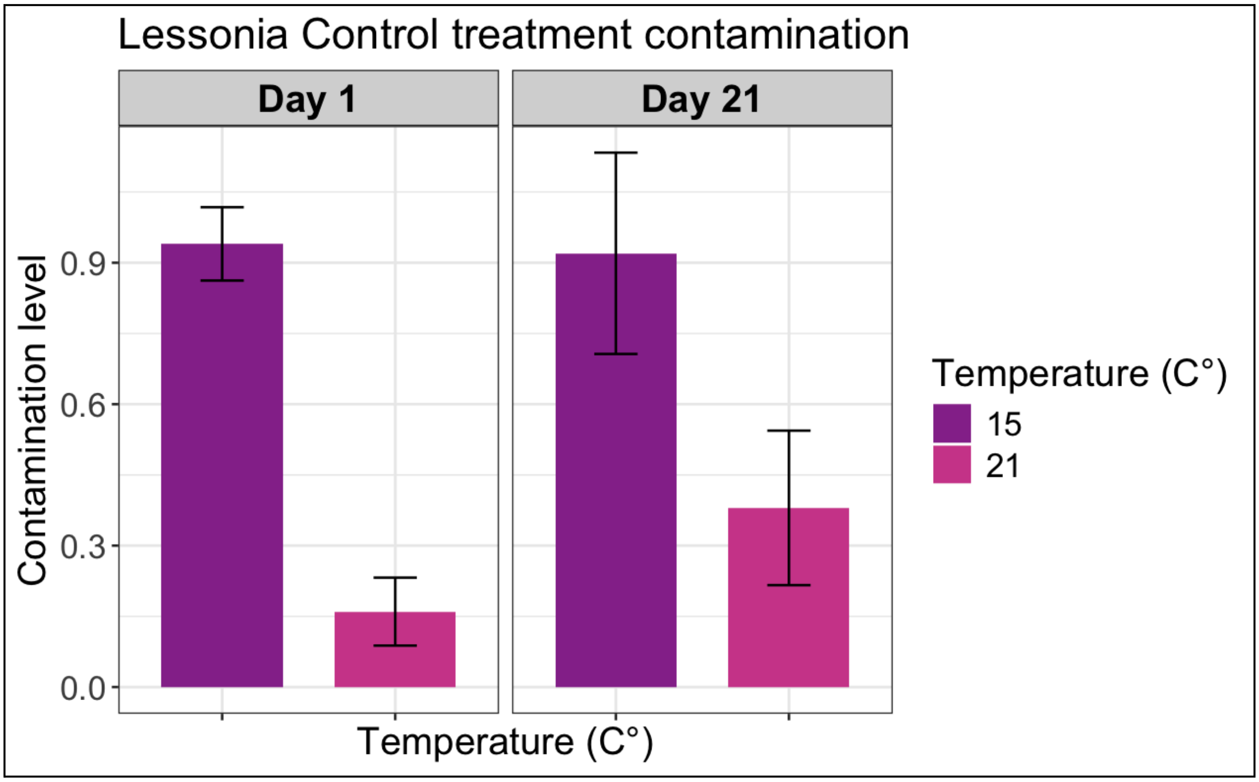

Figure 17-B*. *Comparison of the Lessonia variegata contamination levels observed in samples exposed to only the Control treatment cultured at two different temperatures, 15°C and 21°C on day 1 and day 21. *The contamination level for each temperature was compared within the day.*

On day 1, a Mann-Whitney U test determined the contamination level for the 15°C group was significantly higher than the 21°C group for the Control treatment (p-value: <0.001). On day 21, a Mann-Whitney U test determined the contamination level for the 15°C group was significantly higher than the 21°C group for the Control treatment (p-value: 0.02243).

4. Contamination assessment in Macrocystis pyrifera cultures under two temperatures, 15°C and 21°C

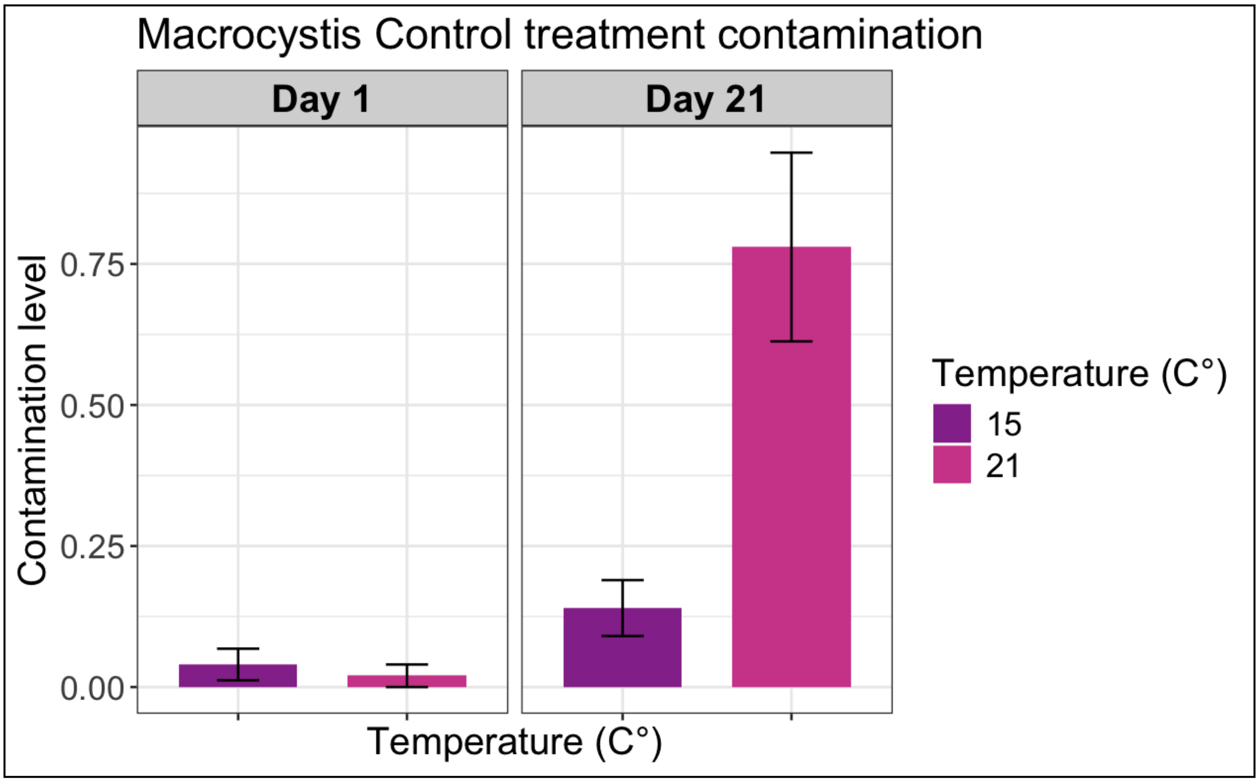

Figure 18-B*. *Comparison of the Macrocystis pyrifera contamination levels observed in samples exposed to only the Control treatment cultured at two different temperatures, 15°C and 21°C, on day 1 and day 21. *The contamination level for each temperature was compared within the day.*

On day 1, a Mann-Whitney U test comparing the contamination levels between the 15°C and 21°C groups showed no significant difference between contamination levels based on temperature for the Control treatment (p-value: 0.5676). On day 21, a Mann-Whitney U test determined the contamination level for the 21°C group was significantly higher than the 15°C group for the Control treatment (p-value: <0.001).

5. Germination (%)

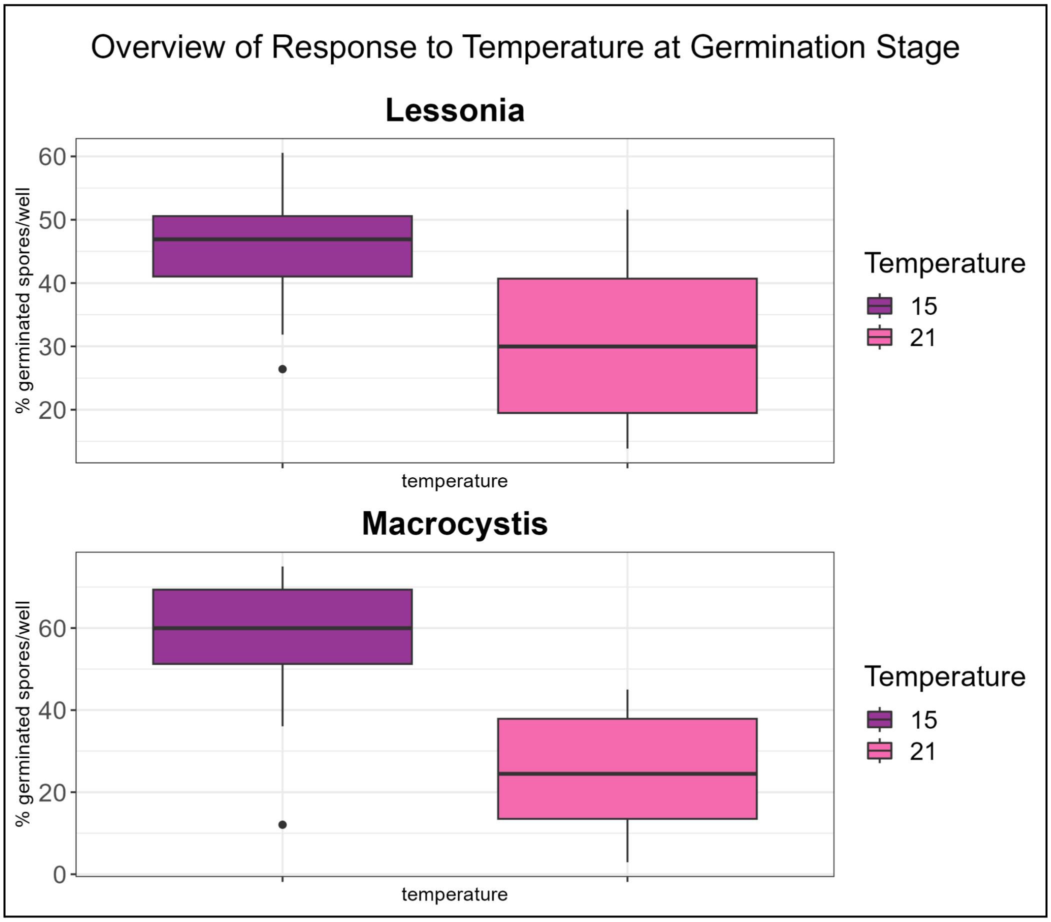

Figure 19-B*. *Overview of Response to Temperature at Germination Stage*. *This demonstrates the percent of settled spores which germinated on day 4 that were observed in samples (n = 3 for E. radiata, n = 10 for L. variegata and M. pyrifera) exposed to the two different temperatures.

Both *L. variegata* and *M. pyrifera* demonstrated a higher percent of germinated spores in the 15°C treatment when compared to the 21°C treatment (Independent T-test; *L. variegata* p = 0.01571, *M. pyrifera* p = 0.001033).

No settled or germinated spores were observed in any *E. radiata* samples stored at 15°C. At 21°C, two *E. radiata* samples had valid percent germinated values (0% and 100%). The other sample contained germinated spores but did not have any settled spores observed on day 1, which led to an invalid output.

6. Percent male gametophytes (sex ratio)

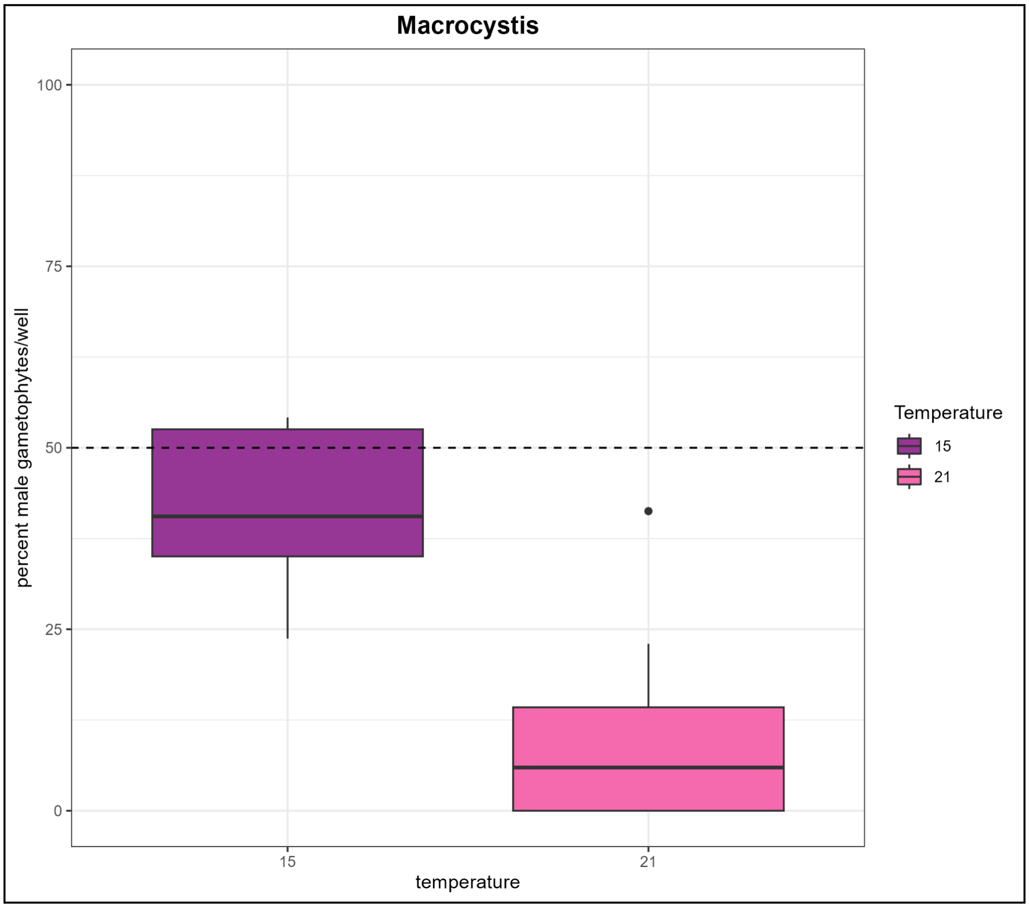

***Figure 20-B**. Comparison of the percent of male gametophytes that developed in samples (n = 10) of M. pyrifera exposed to the two different temperatures tested.*

A significant difference was reported for the sex ratio in *M. pyrifera* (Mann-Whitney U test, p = 0.0007339), where a significantly higher percentage of male gametophytes was recorded at 15°C compared to 21 °C (Figure 20-B).

Only two *E. radiata* replicates out of six (n = 1 at 15°C, n = 1 at 21°C) developed any gametophytes, and both were composed entirely of male gametophytes.

None of the samples of *L. variegata* exposed to 21°C developed any gametophytes.

7. Sporophyte development

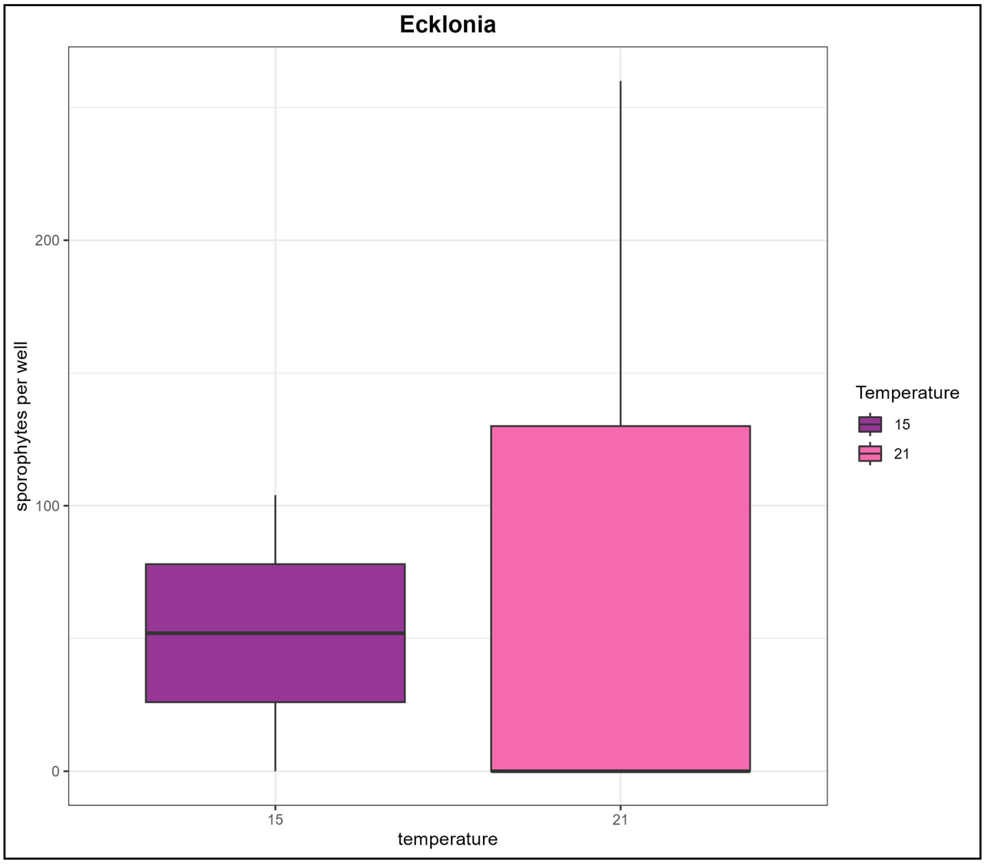

Figure 21-B. Comparison of the sporophyte development in samples (n = 3) of E. radiata exposed to the two different temperatures.

There was no significant difference in sporophyte output between the temperatures tested for *E. radiata* cultures (Mann-Whitney U test resulted in p = 1).

Sporophytes did not develop in any samples of either *L. variegata* or *M. pyrifera* when exposed to 21°C.

<u>Discussion</u>

This experiment evaluated the effects of cleaning treatments and temperature on the early life stages of three native New Zealand kelp species: *E. radiata*, *Lessonia variegata*, and *Macrocystis pyrifera*. Specifically, we examined separately differences between cleaning treatments and temperatures in spore release, germination, sex ratio, sporophyte output, and contamination levels to answer our research questions:

1. Does the cleaning process of reproductive sorus tissue before sporulation affect spore release, sporophyte survival, and contaminant levels of native New Zealand kelp?
2. Does temperature affect the spore release, sporophyte survival, and contaminant levels of native New Zealand kelp?

1. *Effects of cleaning treatment on developmental stages*

Our findings revealed that responses to cleaning treatments were species-specific. For *E. radiata*, no significant differences in spore survival were observed between treatments, suggesting that cleaning treatments did not significantly affect spore viability for this species. In contrast, *L. variegata* showed higher spore survival when exposed to the Ethanol and Betadine treatments compared to Scraping, indicating that chemical treatments were more effective in preserving spore viability than the mechanical ones. In *M. pyrifera*, the Control treatment resulted in higher spore survival than the Ethanol treatment, suggesting Ethanol may be too harsh for this species.

L. variegata was the only species that demonstrated a significant response to germination based on the cleaning treatment. It was determined that the Scraping treatment had a higher germination rate compared to the Control treatment.

At the gametophyte stage, which is crucial for sex differentiation and fertilization (Visch et al., 2024), *L. variegata* was the only species significantly impacted by cleaning treatments. The Betadine and Scraping treatments resulted in a higher percentage of male gametophytes compared to the Control treatment. This finding is important for understanding what factors might impact sex ratios in gametophyte cultures, which can influence fertilization success and subsequent sporophyte output (Visch et al., 2024). On the other hand, the different cleaning treatments didn't have an impact on the final sporophyte output for any of the species investigated.

2. *Effects of temperature on developmental stages*

There was no statistically significant difference in the percent of surviving spores between the temperatures for the three species. There were inverted trends in terms of spore survival, with *M. pyrifera* and *L. variegata* reporting higher percentages at 15 °C compared to 21°C, while *E. radiata* had higher percentages at 21 °C compared to 15°C, suggesting different species-specific temperature niche preferences. For germinated spores, *Lessonia variegata* and *Macrocystis pyrifera* had a higher percent of germinated spores at 15°C compared to 21°C. s. This supports Visch et al.'s finding in 2024 that *E. radiata* tolerates higher temperatures than *M. pyrifera*.

The temperature comparison demonstrated a significant difference in germination rate for both *L. variegata* and *M. pyrifera*, with the 15°C treatment demonstrating higher percentages of germinated spores in both species (Figure 19-B). This is consistent with the observed declines in germination rate at higher temperatures (above 19.8˚C) for *M. pyrifera* reported in other studies (Le et al., 2022a). It was difficult to formally assess the differences in germination rate for *E. radiata* at the different temperatures, as the 21°C treatment was the only group to contain any germinated spores. Praeger et al., 2022 found *E. radiata* spore germination was highest at 17°C compared to 14°C and 20°C, so the 21°C treatment having germinated spores and the 15°C treatment having none is notable.

Research into the optimal male-to-female gametophyte ratio is a priority in order to enhance gametogenesis and fertilization (Visch et al., 2024). The current consensus is that having a surplus of males increases fertilization, but polyspermy (when multiple sperm fertilize a single egg) is a concern due to a potential increase in mortality (Visch et al., 2024). Elevated temperatures had a notable effect on *L. variegata*, as no gametophytes were observed at 21°C (Figure 20-B). However, Nelson, 2005 found for *Lessonia variegata* that as temperature increases, the male-to-female ratio decreases, and at higher temperatures (12°C and 15°C compared to 10°C), there are more females produced. *M. pyrifera* was also substantially impacted by elevated temperature at this stage. Although gametophytes persisted at 21°C, consistent with existing research (tom Dieck, 1993), the percentage of males was significantly lower than what was observed at 15°C. There was limited data for the samples of *E. radiata*, as only two replicates out of six (n = 1 at 15°C and n = 1 at 21°C) developed gametophytes, making it impossible to evaluate the impact of temperature on sex ratio. However, Praeger et al., 2022 found that *E. radiata* maintained a 1:1 sex ratio regardless of temperature exposure. These results highlight the sensitivity of *L. variegata* to elevated temperatures and indicate a potential mechanism for decreased sporophyte output for *M. pyrifera* when exposed to higher temperatures. Higher temperatures may prevent optimal fertilization rates if a surplus in male gametophytes is beneficial. These results suggest that the three species have differing susceptibility to temperature changes, which can influence the species' sex ratio and, therefore, reproductive success.

Sporophyte development was influenced by temperature and showed distinct species-specific responses. Both *L. variegata* and *M. pyrifera* failed to produce sporophytes at 21°C, suggesting these species are more susceptible than *E. radiata* to elevated temperatures. The absence of sporophyte development in *M. pyrifera* at 21°C aligns with previous research indicating that sporophytes develop up to 19°C (Deysher & Dean, 1986) and that the gametophyte stage has a higher thermal tolerance than the sporophyte stage (Mabin et al., 2019; Schiel & Foster, 2006; tom Dieck, 1993). The higher temperature may be impeding production of lamoxirene by female *M. pyrifera*, which is a pheromone that attracts sperm (Le et al., 2022b). In contrast, *E. radiata* developed sporophytes at 21°C, showing a potentially higher heat tolerance than the other species investigated, although no significant difference in sporophyte production was observed between 15°C and 21°C. This lack of difference conflicts with Praeger et al. (2022), who found that sporophyte formation was favored at temperatures under 17°C. These findings highlight the importance of understanding species-specific thermal limits when considering their use in aquaculture and restoration efforts.

3. *Effects of cleaning treatment on contamination levels (15°C only)*

Cleaning with autoclaved seawater is a common cleaning treatment for kelp blades (Alsuwaiyan et al., 2019; Le et al., 2022a; Le et al., 2022b; Vadillo Gonzalez et al., 2024; Visch et al., 2024), so any treatment with a significant difference from this treatment is of note. For *E. radiata*, the Ethanol treatment resulted in higher contamination compared to the Control treatment on day 1 and the Scraping treatment on both days 1 and 21, suggesting that the Ethanol treatment might not be the preferred cleaning treatment for this species. For *Lessonia variegata*, the Betadine, Ethanol, and Scraping treatments resulted in significantly lower contamination levels compared to the Control treatment on day 1, indicating autoclaved seawater alone may not be sufficient in providing clean cultures for this species. However, by day 21, no significant differences were observed, suggesting that the cleaning treatments have a short-term impact but may not be effective over the long term for this species. For *M. pyrifera*, there were no differences in contamination levels on day 1, but by day 21, the Scraping treatment had a significantly lower contamination level compared to the Control treatment, suggesting that mechanical treatments may be effective at reducing contamination over time for this species.

4. *Effects of temperature on contamination levels (Control only)*

Contamination levels also varied across temperature and time. *E. radiata* showed no significant difference in contamination level based on temperature, though day 21 did have higher average contamination levels than day 1. Visch et al. (2024) found that contamination levels for *E. radiata* were lower at 12°C compared to 18°C, however the researchers used a Betadine solution as their treatment. *Lessonia variegata* showed a significant difference between contamination levels based on temperature only, as the 15°C groups had higher contamination levels on both day 1 and day 21. This may indicate that *Lessonia variegata* contaminants do not survive at higher temperatures, and further research into higher-temperature storage in early life stages may help reduce contamination for this species. For *Macrocystis pyrifera,* there was a significant difference in contamination level on day 21, as the 21°C group had a significantly higher contamination level. This finding seems to align with the trend found by Visch et al. (2024) of *Macrocystis pyrifera* contamination level decreasing as temperature decreases (12°C relative to 15°C and 18°C in their study), though they used a Betadine solution cleaning treatment. It is possible that elevated temperatures promote contamination regardless of cleaning treatment for this species.

C. Engagement with *Māori*

<u>Introduction</u>

The *Māori* worldview is deeply connected to the environment, representing a holistic approach to ecological management. *Mātauranga Māori* (*Māori* knowledge) shaped by exercising *kaitiakitanga* (environmental guardianship), *mauri* (life force)*, mana* (spiritual power)*,* and *tapu* (spiritual restrictions)*,* guides their environmental practices (Love, 2018; Taikato, 2021). *Māori* participation in ecosystem management is required in *Aoteroa* New Zealand, through laws such as the Marine and Coastal Area (Takutai Moana) Act 2011, which integrates *Mātauranga Māori* and recognizes that *Māori* have rights to use resources in specific ways (Mackill & Rennie, 2011). Additionally, the Fisheries (Kaimoana Customary Fishing) Regulations of 1998 and Fisheries (South Island Customary Fishing) Regulations 1999, allow *tangata whenua* having *manawhenua manamoana* to establish reserves for sustainable non-commercial fishing (Fisheries (Kaimoana Customary Fishing) Regulations 1998, 1998).

Legislation and regulations are necessary due to the shortcomings of New Zealand's original foundational document, the Treaty of Waitangi/*Te Tiriti o Waitangi*, signed on February 6, 1840 (Stokes, 1992). The Treaty has both an English and a *Māori* version, which has led to conflicting interpretations (Kawharu, 1989) (I. H. Kawharu, 1989; M. Kawharu, 2000; Stokes, 1992). The English version asserts sovereignty, while the *Māori* text suggests governance rights interpreted as a partnership. Debates continue about the true intent and meaning of the Treaty (New Zealand Government, n.d.).

McCarthy et al. (2014) illustrate how *Māori* perspectives can inform coastal management project objectives. Researchers assessed the decline in *kaimoana* (seafood) by engaging 62 *Ngāi Tahu* members (the principal *iwi* of the South Island). Interviewees cited overfishing and changes in fisheries management as key factors in environmental degradation. The depletion of *taonga* (revered species) such as *pāua* (abalone) and other seafood is not simply a lost food resource but also a loss of cultural identity, tradition, and community cohesion (Mccarthy et al., 2014). By integrating *mātauranga Māori* and concerns with modern ecological practices, coastal managers can forge a path toward sustainable environmental stewardship and ensure the resilience and vitality of New Zealand's precious marine ecosystems for future generations.

Objective:

1. Gather information through engagement and online surveys to understand if *tangata kaitiaki* (guardians) and *mana whenua* (Indigenous peoples of New Zealand) living in *Te Tauihu* (Top of the South Island, NZ) have experienced a change in *rimurimu* (seaweed) forest ecosystems and if a change occurred, how this impacts them.
2. Determine to what extent this issue overlaps with the cultural identity for *Māori* in *Te Tauihu*.

Rationale: *Māori* have an intimate spiritual relationship with seaweed and many living organisms in these habitats. Specifically, many *taonga* (revered organisms) species live within kelp forests, such as *pāua* (abalone) and *kina* (sea urchins). Seaweed forests are also crucial to *māori* culture through *kaimoana* and associated economies, such as fishing. Moreover, *Māori* have acted as stewards of kelp forests and local coastal ecosystems for hundreds of years. Understanding the opinions, experiences, and values of *mana whenua* concerning the environment is essential to New Zealand's political system. The cultural understandings gleaned from surveys will be paired with the natural science portion of this research project to guide policy recommendations.

Methods

We documented the opinions, experiences, and values of *mana whenua* in *Te Taihu* using a survey questionnaire that focused on questions about personal experiences, policy, natural resource management, and the natural environment. The surveys were conducted using the Qualtrics survey tool. Survey participants were asked to set aside approximately 5-10 minutes to complete the survey and answer eighteen questions (including cultural identity, economics, ecology, and demographics). No prior preparation was necessary since participants were expected to respond based on their everyday activities, direct experience, and personal knowledge of impacts on the environment in New Zealand. Confidentiality was preserved, as no information identifying participants will be kept past UCSB's Institutional Review Board (IRB) timeline, and all data will be identified only with a code number. Data is stored on a secure electronic server and will be destroyed within the IRB-required time frame. No incentives were provided for survey participants. Participants could give their consent at the start of the survey and withdraw their consent at any time during or after the survey. They were informed that they agreed to participate in a survey of *mana whenua*'s opinions and experiences related to the environment. The subject population was contacted through the Cawthron *Māori* Research & Development team, *Te Kāhui Āio.* The IRB has approved all methods of this study.

Cawthron distributed the survey to *Māori* stakeholders and *iwi* leaders who are local to *Te Tauihu* (Top of the South Island, NZ), as well as Pou *Taiao* (environmental) managers and *iwi whanau* who Cawthron knew spent time in or on the *moana* (ocean). Cawthron specifically asked each person to forward the survey to others in each email. Survey pass 1 ended on January 6th, 2025. As of February 10, 2025, Cawthron sent emails with the survey to 31 recipients, of which 19 responded. Although 19 responses were recorded, not all respondents answered every question. Once the survey was closed to responses, we interpreted the results qualitatively through the Qualtrics interface and in Excel. Ultimately, Cawthron plans to use the survey results as part of a larger internal peer-reviewed report and will also refer to our results for future projects.

Hui:

On September 17, 2024, Cawthron organized a *hui* with many *Māori* community members. A *hui* is a central ritual in *Māori* culture where a group is called together for a specific purpose. Although this particular *hui* was not analyzed for this project, the topics discussed in this *hui* informed many of the survey questions. A major theme that influenced the creation of at least one survey question was the impact of kina (urchins) on kelp forests and the role kina plays as an ecological and cultural component in reef communities. One *hui* participant mentioned that kina often receives too much blame for negative impacts on kelp forests. The participant stated that since kelp and kina have existed together for at least thousands of years, the problem is likely very complex. They also discussed water quality issues and the desire to be included in kelp forest restoration activities.

Results

We received 19 responses in total. Although there were 19 responses, not all respondents answered all questions. Some questions were skipped by the respondents, not completed, or for the write-in questions had responses like “N/A” or “?” added in. We did not statistically analyze the results given the limited response rate, but the data was put into graphs and visualized for qualitative analysis.

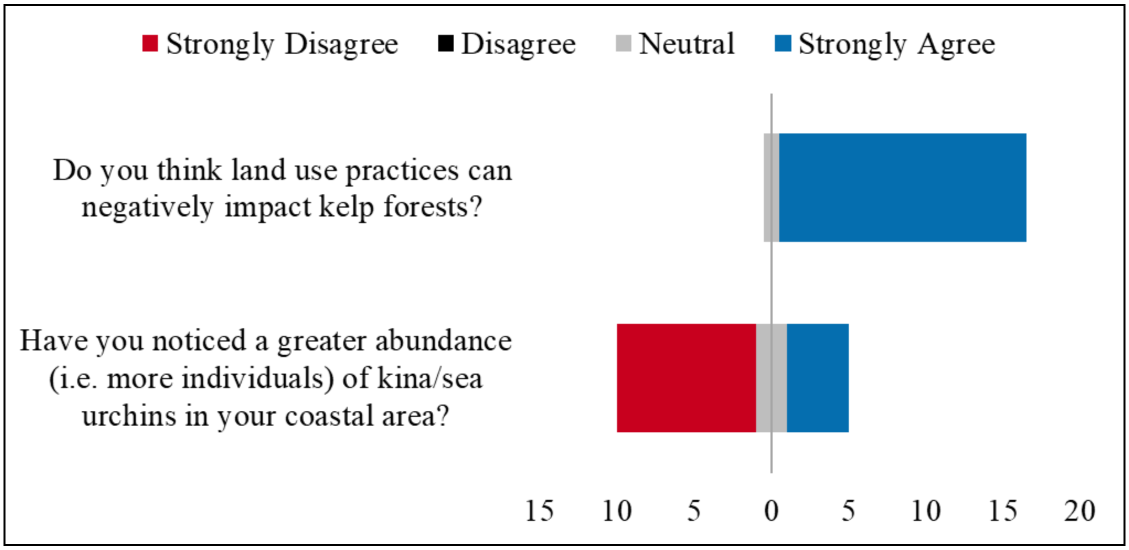

***Figure 1-C*. *Responses to two separate questions regarding kina and land use impacts*.** *This figure shows the number of responses for each question, with disagreement on the left and agreement on the right.*

Most respondents (n=9) answered that they had not seen an increase in abundance, while four had. Two were unsure. Most respondents (n=16) answered that they thought land use practices could negatively impact kelp forests, while one respondent was unsure (**Figure 1-C**).

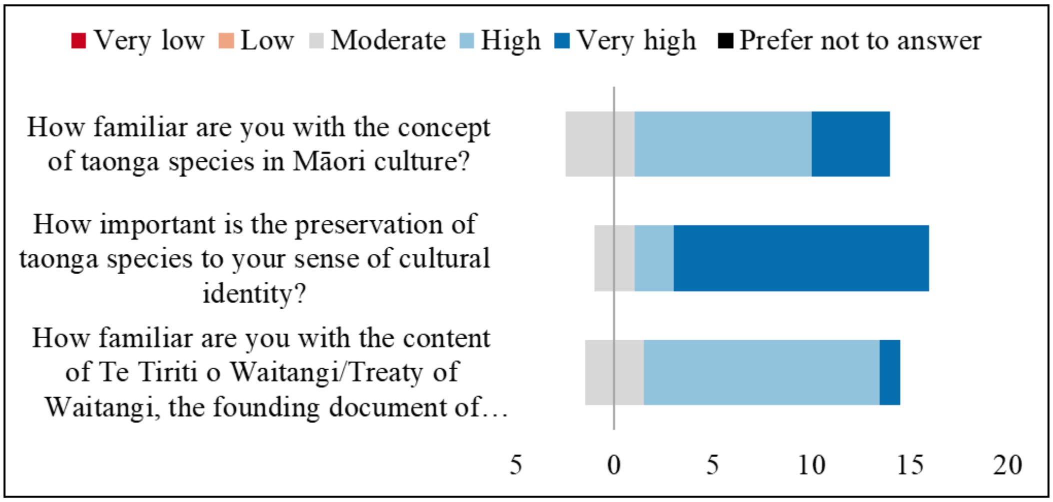

***Figure 2-C*. *Importance of taonga and Treaty of Waitangi to cultural identity*.** *This figure shows the number of responses for each question, with lower familiarity on the left and higher familiarity on the right.*

Most respondents felt that they were highly familiar with the Treaty of Waitangi (n=12), while only one was “highly familiar” and four were “moderately familiar.” All respondents said they value *taonga* of moderate to very high importance, with the majority (n=13) selecting “very high importance.” Most respondents also had a high level of familiarity with the concept of *taonga* species (n=9). Specifically, four had “very high familiarity,” and five had “some familiarity.”

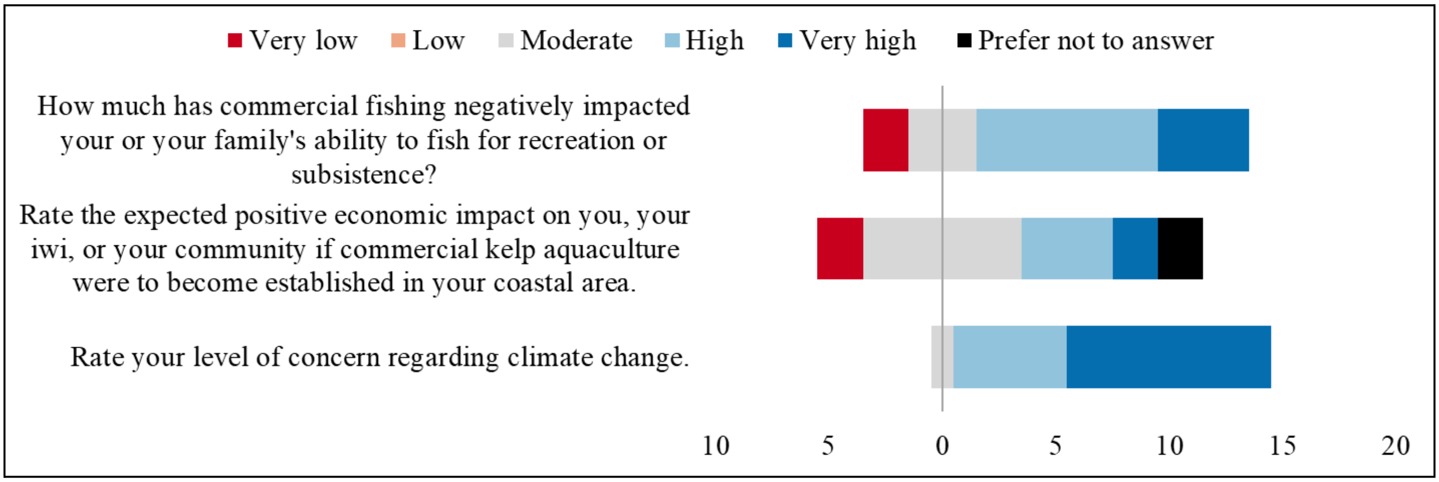

***Figure 3-C*. Concern surrounding climate change, kelp aquaculture, and fishing.** *This figure shows the number of responses for each question, with low levels on the left and high levels on the right.*

All respondents were either moderately, highly, or very highly concerned about climate change. Most respondents expected a moderate positive impact on their community if kelp aquaculture were to be established (n=7). The second most common response was high positive impact (n=4). Very high, very low, and prefer not to answer were all tied with two responses each. When asked how commercial fishing has negatively impacted them, the majority of respondents selected "high" impact (n=8).

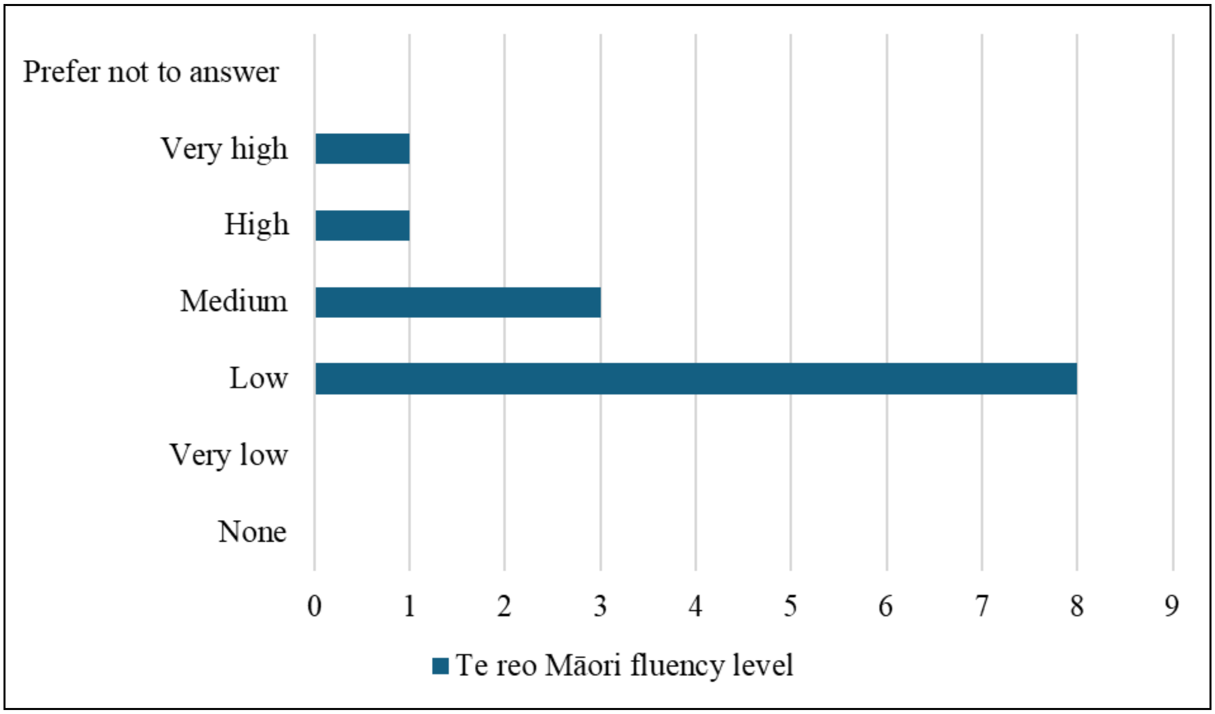

Figure 4-C. Te reo Māori fluency level. *This figure shows the number of responses for each level of fluency from very low to very high.*

Most respondents (n=8) answered they had low *Te Reo Māori* fluency. Only three responded that they had medium-level fluency, one answered high fluency, and one answered very high. None answered that they had very low or no fluency.

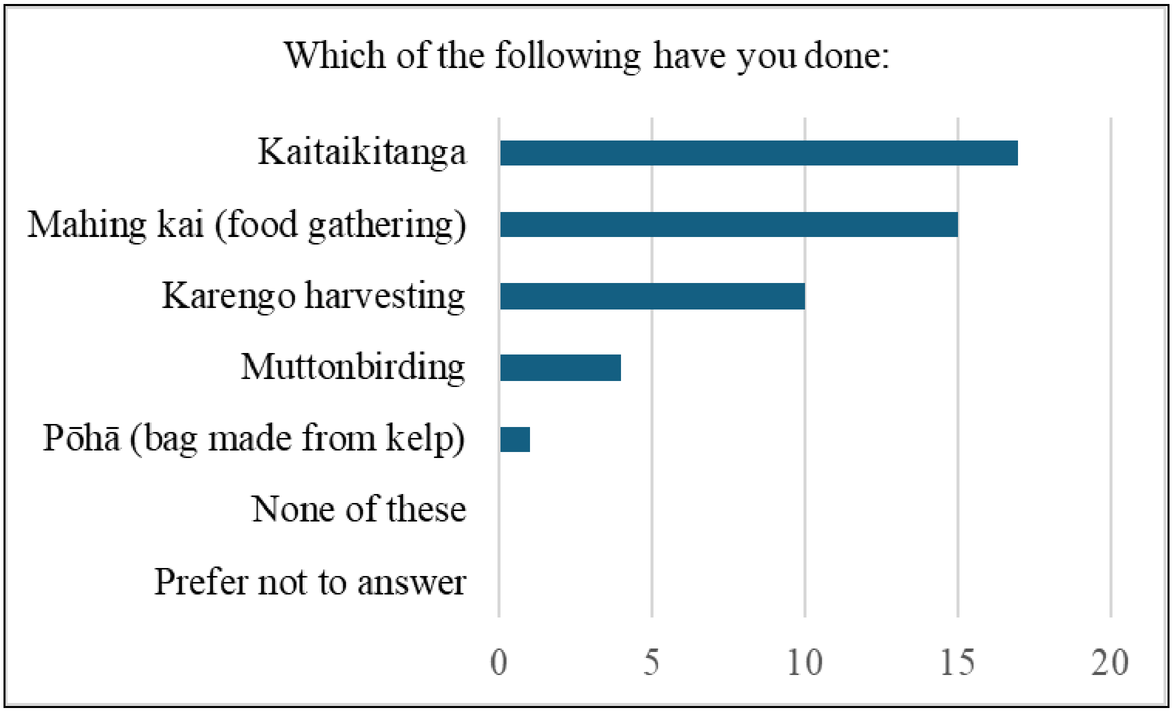

Figure 5-C*. *Māori traditional activities. *This figure shows the number of activities each respondent had participated in. Respondents could select as many activities as they have participated in.*

Seventeen of the nineteen survey respondents had participated in *kaitiakitanga*, fifteen in *mahinga kai*, and ten in *karengo* harvesting. Only four had participated in muttonbirding and only one had participated in making or using *pōhā* bags. None of the respondents answered "none" of the mentioned activities.

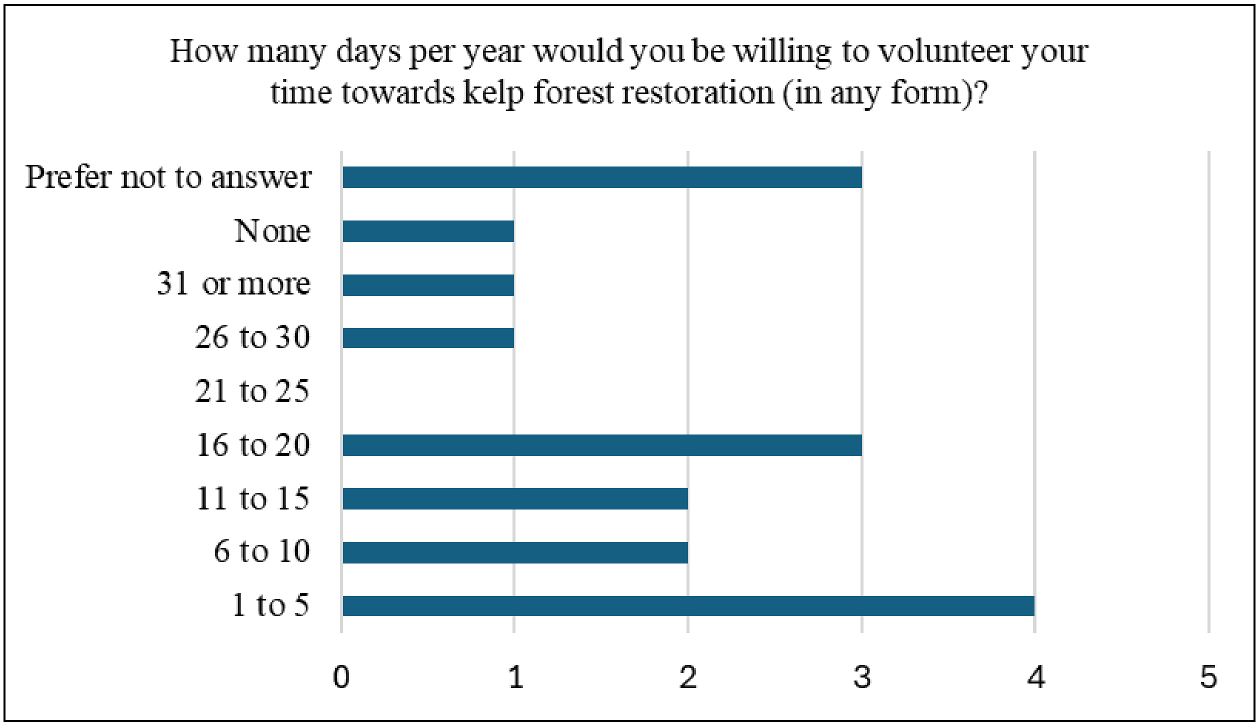

***Figure 6-C**. **Volunteering time towards kelp restoration.** This figure shows the number of hours each respondent would be willing to participate in per year. "*

The largest number of selected responses was "1 to 5" days per year (n=4), but the remainder varied in number. "Prefer not to answer" was chosen by three of the respondents, as was "16 to 20" hours. One person answered that they would be willing to volunteer no time at all.

Discussion

The majority of the respondents seemed to be highly engaged with *Māori* culture and to value the environment highly. Most were concerned or highly concerned about climate change. All respondents stated that they value *taonga* of moderate to very high importance, with the majority selecting "very high importance." When asked how much of a negative impact commercial fishing has on their family's ability to fish, the majority of respondents stated that it has a high impact (n = 8) or very high impact (n = 4). The remaining two respondents selected very little impact. This seems to imply that community members are optimistic that kelp aquaculture could benefit them if they are involved early on. An additional surprising result is that although increasing numbers of urchins (kina) are often blamed for decreasing kelp forests, the respondents do not notice increased kina levels. The respondents left many suggestions of who they thought would be good contacts to consult in the future.

Lastly, most respondents answered that they thought land use practices have negatively impacted kelp forests. This is a striking result, as poor land management has been identified as a significant stressor to kelp forests in New Zealand and worldwide. Generally, the understanding is that poor land management results in high coastal sedimentation, which can smooth kelp and reduce their photosynthetic efficiency. This also highlights the high complexity (brought up in the *hui*) around factors contributing to *rimurimu* change and the importance of engaging everyone within a community to ensure the current and future management of this critical but declining habitat.

It is also essential to understand the context of questionnaires such as this, precisely how participants may interpret a survey question. For example, Figure 3-C asks participants to express their concerns about climate change, kelp aquaculture, and commercial fishing. Commercial fishing can come in many different forms, and in *Aotearoa*, New Zealand, this includes trawling (generally for finfish species), and *pāua* and kina diving. We suspect that most participants considered commercial fishing to be the taking of finfish but can only speculate. We suggest a future survey could inquire about the impact of specific fisheries on people and places. Furthermore, and this was an oversight from all parties in this project, it is important to keep clauses consistent in such surveys. Again, in Figure 3-C, participants are asked if kelp aquaculture would have a 'positive' impact, while in the next question, participants are asked if commercial fishing has had a 'negative' impact on them. We cannot say if this inconsistency had an effect on the results, but we will endeavor to keep the clauses consistent in future surveys.

Conclusions and Recommendations

Recommendations for Kelp Forest Restoration

Effective kelp forest conservation and restoration can follow two distinct strategies, each with different levels of risk and potential benefits. One conservative approach involves focusing on deeper, more stable habitats that have shown lower sensitivity to fluctuations in SST. These areas may serve as climate refugia, providing long-term habitat stability for kelp species. *Ecklonia radiata* demonstrated resilience to increased water temperature in a controlled aquaculture environment, making this species a potential candidate for further investigation towards efforts to conserve and restore canopy-forming kelp. Additionally, floor species (encrusting and turfing) exhibited a minimal response to temperature variations. These species could be leveraged to maintain foundational ecosystem structure in these stable habitats. By prioritizing deeper sites and more temperature-resilient seaweed species, managers can enhance the persistence of kelp forests with a lower risk of failure due to climate variability.

In contrast, a more proactive but higher-risk approach would involve targeting sites most vulnerable to warming. This includes shallow water environments with thermally sensitive subcanopy species. Although these areas face greater exposure to MHWs, restoration efforts could yield valuable insights into ecosystem adaptation. Potential strategies to encourage successful restoration could involve managing herbivore populations to minimize additional pressures (Miller et al., 2024; Miller & Shears, 2023). While this approach is more likely to fail in the short term, it could provide critical data for developing long-term resilience strategies and mitigating future climate impacts.

Aquaculture

We recommend further implementation and research on Betadine as a cleaning agent since our results showed that it maximizes spore survival without simultaneously reducing sporophyte development. Using Betadine for the three species (*Ecklonia radiata*, *Lessonia variegata*, and *Macrocystis pyrifera*) can support researchers in identifying species-tailored cleaning protocols, resulting in cleaner stock and seeding cultures for successful outcomes in cultivation. Additionally, since *E. radiata* has the greatest capacity to develop sporophytes at higher temperatures (21°C), this species could be further trialed as a candidate to be applied to restoration projects even in areas that experience MHWs more frequently.

Recommendations for Community Involvement

We recommend that Cawthron continue to look into partnerships with *Māori* people, especially for aquaculture projects, as they may feel that aquaculture will positively impact themselves and their communities. Since respondents expressed substantial interest in kelp restoration (most respondents expressed willingness to volunteer their time anywhere between 1-20 days per year), involving the community whenever possible will be essential. We recommend further investigation into what *Māori* think about other *taonga* species. This could involve a more detailed survey or interviews, especially since our survey only directly addressed *kina*. It is worth considering efforts to encourage the monitoring of coastal areas through citizen science by providing training and equipment to *mana whenua*, as many respondents stated a deep concern and commitment to protecting the environment. We recommend following up with individuals and groups as suggested by the survey respondents.

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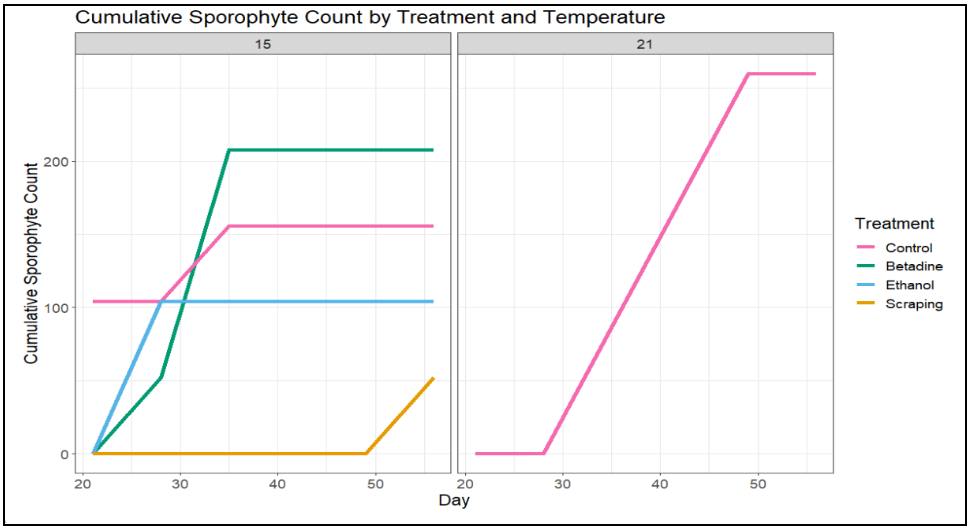
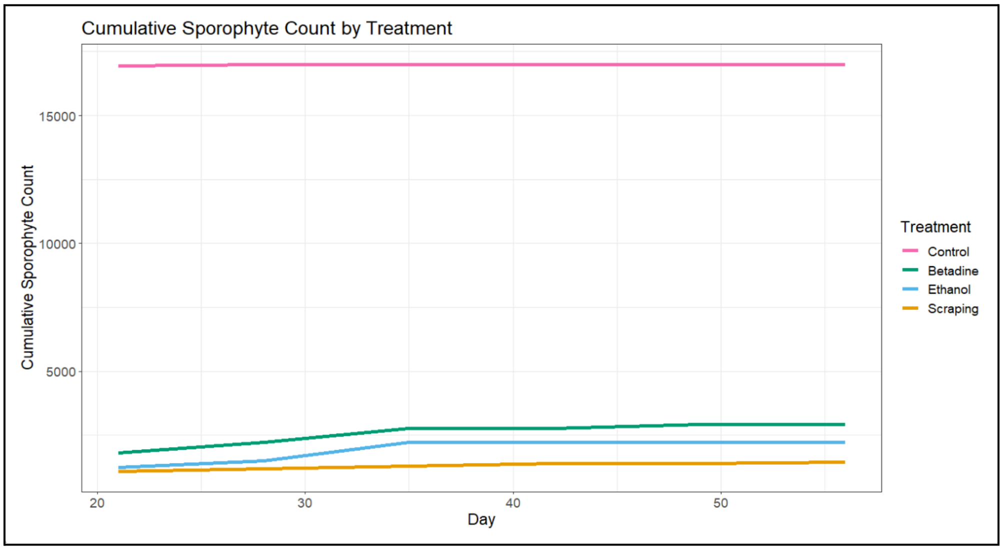
Appendix

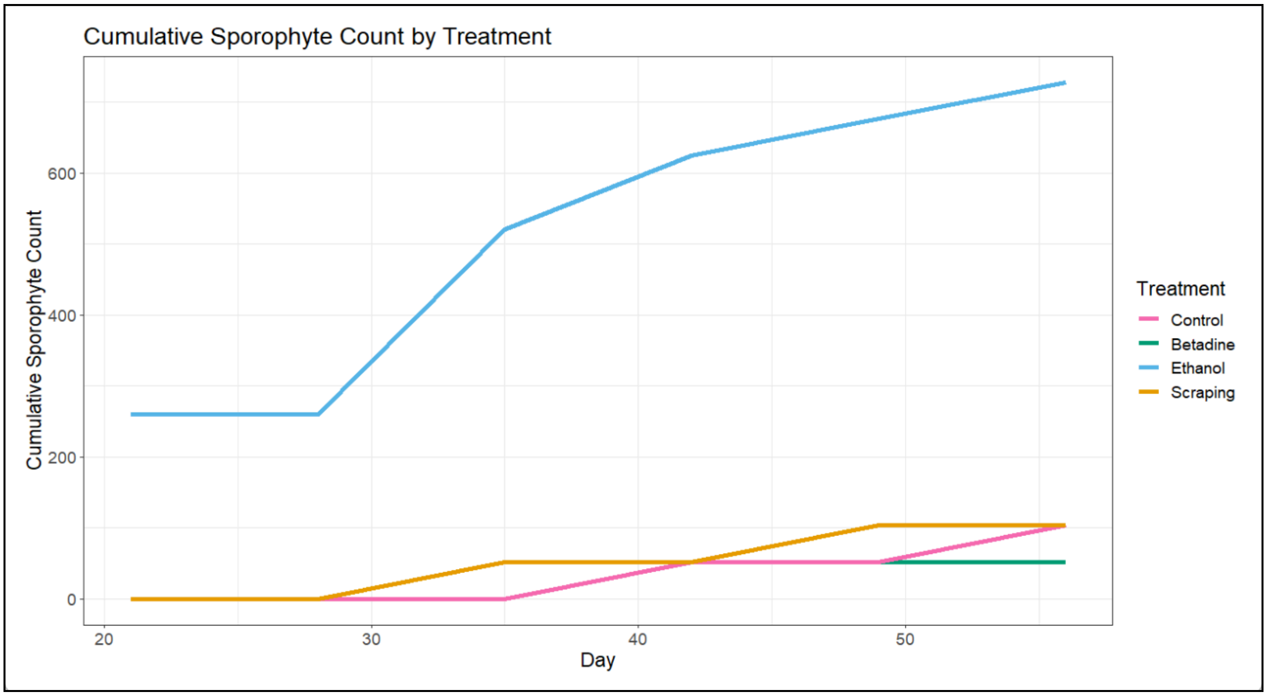
Section 1 (Aquaculture Experiment):



Cumulative sum of E. radiata sporophytes observed in each cleaning treatment/temperature group over time*. At 21°C, the Control treatment had the highest overall sporophyte output. At 15°C, the samples exposed to Betadine had the highest sporophyte output out of samples.*



Cumulative sum of L. variegata sporophytes observed in each cleaning treatment group over time. *No sporophytes were observed in the samples stored at 21°C. The samples exposed to the Control (autoclaved seawater) treatment had the highest sporophyte output.*



Cumulative sum of M. pyrifera sporophytes observed in each cleaning treatment group over time*. No sporophytes were observed in the samples stored at 21°C. The samples exposed to the Ethanol treatment had the highest sporophyte output.*