Factors that influence growth in colonial bryozoans, Otago Harbour, Aotearoa New Zealand.

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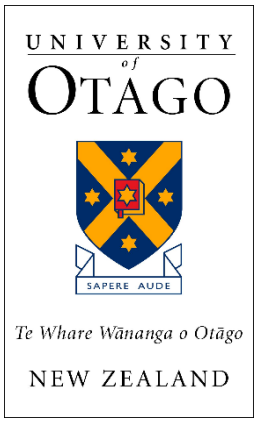
Submitted in partial fulfilment of the requirements of the degree of Master of Science

Marine Science

University of Otago

March 2024



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Acknowledgments

I would like to thank Abby Smith, for her guidance and supervision during this project, the University of Otago Marine Science Department for providing a conducive atmosphere to work in, the Portobello Marine Laboratory Staff, particularly Linda for instrumental support in my various experiments during this project, Jess Moffit for granting me access to the bryozoan colonies that made Chapter Four possible, the University of Otago for the many resources they provide, the Smith Lab Group for furthering my obsession for bryozoology, the International Bryozoology Association for enabling me to present research from this project at the IBA 2022 Conference at Trinity College in Dublin, Ireland, and of course my many friends and family members that continue to encourage me to follow my dreams.

Abstract

Understanding growth rates and the factors that influence growth is important for any organism, and is especially complicated in colonial organisms such as bryozoans. The aim for this is study was to investigate the factors that influence colony growth in bryozoans, with a focus on the Otago Harbour. Ideally one could do so by developing culture techniques and baseline data for suitable model species and then assessing how their growth rates are influenced by environmental factors (e.g., season, temperature, food). I started out by conducting surveys of Otago Harbour to find out what bryozoans live there (Chapter 2). Of the twelve bryozoan taxa found in Otago Harbour, eight of these taxa had not been recorded in a prior survey. Conversely, nineteen species previously noted as present were not found in our surveys. A long-term dataset for an abundant local bryozoan (*Watersipora subatra*) was then developed to obtain baseline growth and development data (Chapter 3). Wild colony growth of *W. subatra* populations was recorded as high as 111 mm/y during summer 2022, and as low as 27 mm/y during winter 2021, with a realistic annual growth rate lying somewhere between the two values. Then, manipulative experiments were conducted to investigate the effect of raised temperature on growth (Chapter 4). Wild Bryozoa found in Otago Harbour exhibited both a susceptibility and tolerances to increases in heat; significance was found between increases of +1 or +2 degrees and the colony and zooid characteristics of *Beania* sp., and in the composition of *Caberea zelandica,* no significance was found in any parameter of *Bugulina flabellata.* Then the effect of food availability on growth rate was investigated, using *Watersipora subatra* (Chapter 5). The highest growth rate was recorded when feeding the colonies a mixed diet, and there was a clear preference when comparing the results of single plankton feeds (mixed treatments yielded a growth rate nearly four times that of ambient wild growth). Finally, I collated the results of these

experiments within the context of the wider bryozoan growth literature (Chapter 6), allowing for the foundation of a framework to understand the factors that influence growth in Bryozoa within and indeed outside of Otago Harbour.

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CHAPTER ONE: INTRODUCTION

1.1 Bryozoans

1.1.1 General Ecology

Bryozoans are a phylum of largely colonial sessile attached benthic marine invertebrates, though there are freshwater species (e.g., Ko 2019), and even a few unattached non-sessile Bryozoa (Hageman et al. 1997, Hageman et al. 1998, Hermansen et al. 2001). Bryozoans are lophotrochozoans, meaning they collect suspended particles with tentacles on a muscular ring, called a lophophore (Sun et al. 2009, Okamura et al. 2011). This structure generates a feeding current that draws food in.

Most bryozoans calcify, growing a biomineralized skeleton for protection and support (Hageman et al. 1998, Steger & Smith 2005, Smith et al. 2006, Loxton et al. 2018). Bryozoan colonies are made up of individual animals called zooids (Lidgard & Jackson 1989, Hageman et al. 1998). Autozooids are the most abundant of these, and are responsible for feeding (Okamura et al. 2011); in contrast heterozooids are non-feeding zooids that may play a role in reproduction, defence, or motility (Serova et al. 2017, Schack et al. 2019, Schack et al. 2020).

Bryozoans usually grow on hard surfaces, including rocks, macroalgae, hard-shelled animals, boats, wood, and anthropogenic objects, preferring to grow on undersides and in darkness (Mackie et al. 2014, Li et al. 2016). Bryozoans can be epiphytic (occurring on plants), epialgal (occurring on algae), or epizootic (occurring on animals) across many organisms including: macroalgae, sponges, molluscs, and even crayfish (Piazzi et al. 2015). Bryozoan predators include nudibranchs, pycnogonids, and fish (Seed 1976, Lidgard 2008, Linneman et al. 2014).

Bryozoans are important components of biodiversity, particularly in the temperate Southern Hemisphere (Smith 2014) (Figure 1.1). They provide both habitat stabilisation by encrusting over loose substrate (Hageman et al. 1998), and habitat space for other invertebrates and small fish in the form of bryozoan 'reefs' or thickets (Wood et al. 2013). In addition, bryozoans sequester carbonate in their skeletons, which can contribute to the formation of sediment after death (Smith 2014). Our capacity to manage impacts and understand resiliency of these creatures is complicated by their colonial lifestyle. Even such basic questions as "how old can they get?" and "how fast do they grow?" are both unknown and surprisingly difficult to ascertain.

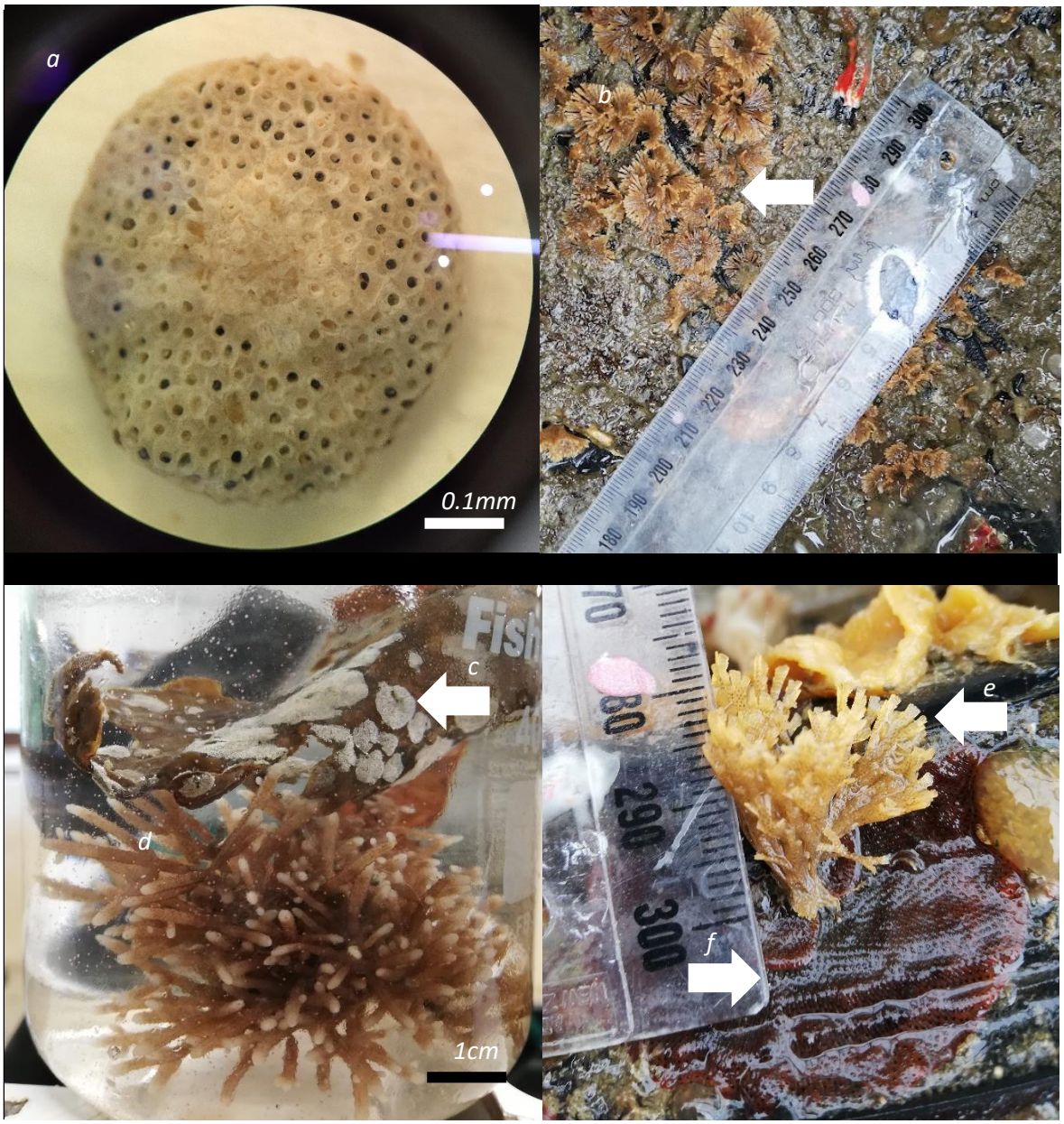


Figure 1.1. Photographs showing the diversity of local Bryozoa – (a) *Otionellina* sp., a free-living motile species found on sandy substrates; (b) colonies of *Caberea zelandica;* (c) *Membranipora membranacea* living on a kelp blade; (d) *Elzerina binderi*; (e) *Bugulina flabellata* on a dark red encrusting colony of (f) *Watersipora subatra*.

1.1.2 Taxonomy & Evolution

Bryozoans were first formally categorized as 'corallines' in the plant kingdom; the idea they could be animals arose in 1599 but wasn't formally recognised until 1755 (Ryland 1970). The

term Bryozoa emerged in 1831, one year after the term Ectoproct was used to describe the same group. In 1869 the phylum was found to contain two distinct groups of animals leading to the Entoprocts being removed from Bryozoa and placed in their own sister phylum (Ryland 1970). As bryozoans are lophotrochozoans, they are most closely related to brachiopods and phoronids (Ryland 1970, Sun et al. 2009) (Figure 1.2).

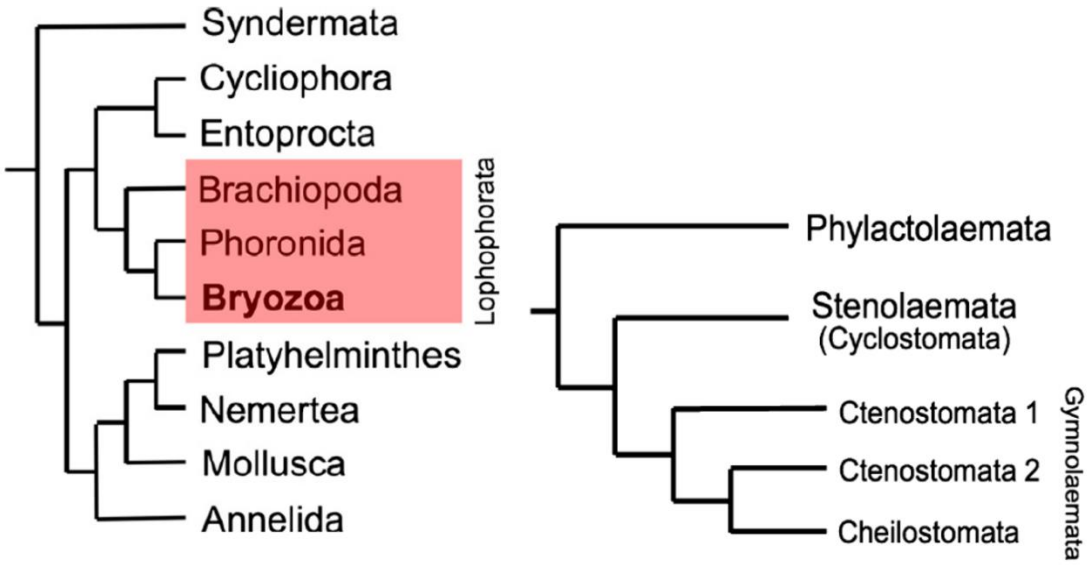


Figure 1.2. Tree diagram showing the classification of the phylum (Taylor and Waeschenbach, 2015).

Over 5800 known species of bryozoan exist today, and of these, only two genera are solitary. Bryozoa are typically divided into three classes: Phylactolaemata, Stenolaemata, and Gymnolaemata (Ryland 1970). Phylactolaemata are entirely freshwater, and are unusual compared to other Bryozoa in that they possess a gelatinous exoskeleton. Stenolaemata are mostly extinct, but about 600 marine species remain today. Gymnolaemata includes both freshwater and marine taxa, and the majority of extant Bryozoa are in this class (Ryland 1970, Pagès-Escolà et al. 2020).

1.1.3 Bryozoan Colonial Growth Forms

Bryozoan colonies have a variety of growth forms (Figure 1.3), both within and between species and individuals (Hageman et al. 1998). As colonial organisms, bryozoans grow by the iterative budding of new zooids, adding individual organisms to the colony. The growth of bryozoan colonies involves both vertical and horizontal expansion, allowing them to occupy and colonize various substrates in marine and freshwater environments (Ryland 1970, Hageman et al. 1998).

Vertical growth in bryozoans refers to the extension of the colony in a perpendicular direction from the substrate or attachment point. It occurs through the process of budding, where new zooids are generated by asexual reproduction. The newly formed zooids develop from specialized buds, known as ancestrulae, which undergo growth and morphological changes, ultimately becoming functional zooids capable of feeding (Ryland 1970, Hageman et al. 1998).

The vertical growth of bryozoan colonies can result in the formation of elongated structures such as branches, tubes, or erect fan-like shapes. The growth rate and pattern can vary among species and are influenced by environmental factors such as nutrient availability, water flow, and competition with other organisms (Hageman et al. 1998).

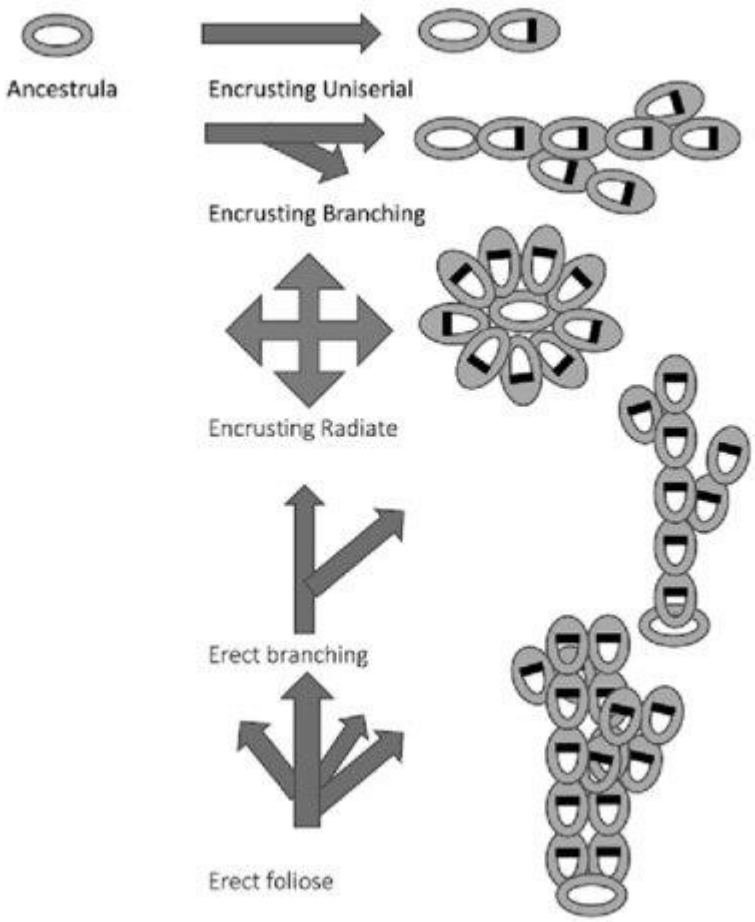


Figure 1.3. Growth form diversity of Bryozoa achieved by individual zooids combining in different orientations (Sourced from Smith & Key 2020).

In addition to vertical growth, bryozoans also exhibit horizontal growth, which involves the expansion of the colony across the substrate or attachment surface. Horizontal growth occurs through the process of encrustation, where the colony spreads laterally by budding new zooids along the edges of the existing colony (Hageman et al. 1998).

Encrusting bryozoans form thin sheets or crusts that gradually extend over surfaces such as rocks, shells, seaweed, or other substrates. As new zooids are added, the colony increases in size and coverage. Encrusting colonies can fuse with neighbouring colonies, creating larger

interconnected networks or forming intricate patterns on the substrate (Ryland 1970, Hageman et al. 1998).

1.1.4 Bryozoan Growth Rates

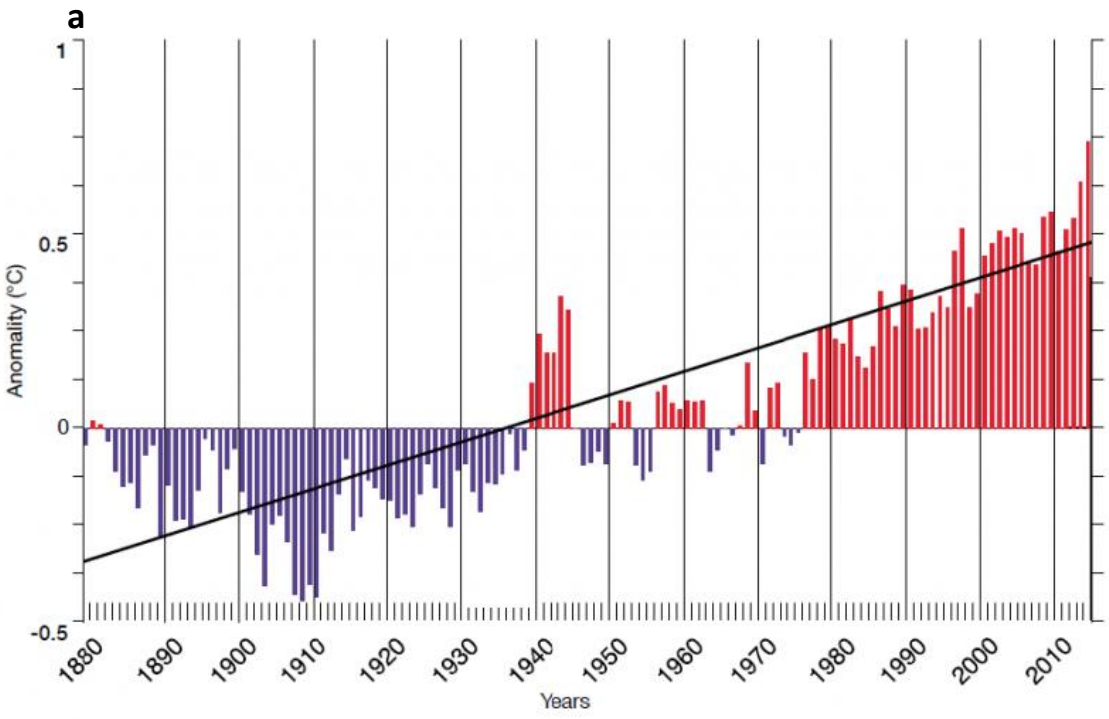
Bryozoans generally are thought to grow slowly. More recent work shows that growth rates also vary greatly, and a number of factors that can influence them (Marshall & Keough 2009, Sams & Keough 2012, Smith & Key 2020, Marshall et al. 2003, Hartikainen et al. 2009).

The growth of bryozoan colonies is influenced by various factors, including environmental conditions, resource availability, and interactions with other organisms. Some of the studied factors affecting bryozoan growth include nutrient availability, water flow, competition and predation, substrate availability, temperature, and acidification (O'Dea & Jackson 2002, Smith 2014). Bryozoans require an adequate supply of nutrients, including dissolved organic matter and planktonic food particles, to support their growth. Nutrient availability can vary based on factors such as water temperature, currents, and local ecosystem productivity (O'Dea & Jackson 2002, Hartikainen et al. 2009). Water flow plays a crucial role in bryozoan growth. Moderate water currents help bring food particles to the feeding structures, enhancing feeding efficiency and providing a continuous supply of nutrients. Excessive water flow, on the other hand, may inhibit growth or dislodge fragile colonies, or there may be no observed effect (Hermansen et al. 2001). Bryozoans can face competition from other organisms, such as algae or other colonial invertebrates, for space and resources. Interactions with predators, such as grazing organisms or fouling organisms, can also influence bryozoan growth and survival (Allen et al. 2008, Marshall & Keough 2009, Sams & Keough 2012, Linneman et al. 2014). The availability and suitability of the substrate or attachment surface can affect bryozoan growth. Some species prefer specific types of

substrates, while others can adapt to a wide range of surfaces (Li et al. 2016). Temperature has been recorded to have an influence of bryozoan growth, typically growth in stimulated by increased temperature until a critical point is reach and temperatures beyond that point have a negative effect on bryozoan growth (Smith 2014). Acidification has been observed to influence the growth of bryozoans, particularly when interacting with the effect of increased temperature (Lombardi et al. 2011a, Smith 2014, Pecquet et al. 2017).

1.2 Ocean Warming

Global warming is rapidly influencing the world's ecosystems, and our projections for the future are not encouraging (Figure 1.4). Marine heatwaves are happening more often and lasting longer than they used to, and are projected to be even more so. Such high temperatures, even over a short time, pose a potential threat to many marine ecosystems (Marzonie et al, 2022).



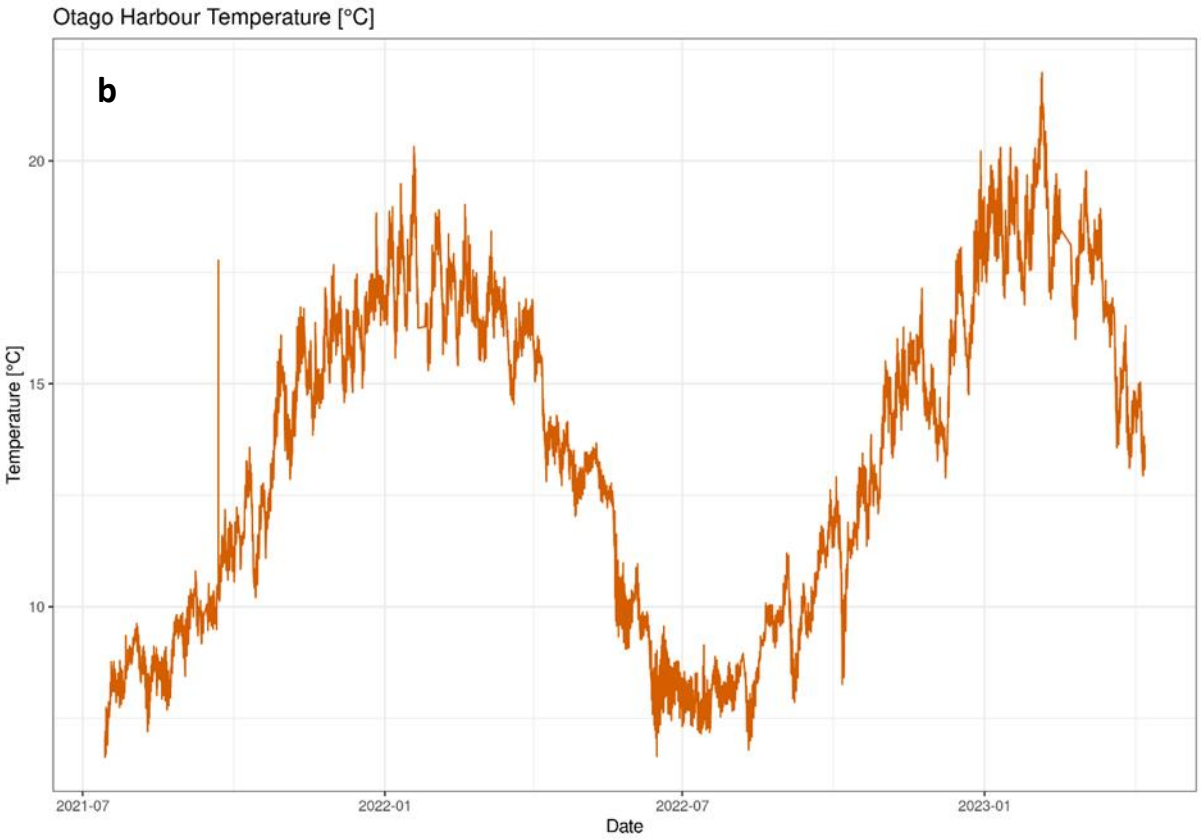


Figure 1.4. (A) Annual global sea surface temperature anomalies relative to the average from 1880 to 2015 with superimposed linear trend (Base period 1951–1980), red positive, blue negative (IUCN, 2017), with the Otago Harbour Temperature record measured at Portobello Marine Laboratory of late 2021 to early 2023 shown below (B) accessed from (https://harbourconditions.otago.ac.nz/)).

Understanding how organisms respond to increases in temperature is an essential tool for studying the world's changing climate, and attempting to prevent biodiversity loss. Experiments into the heat tolerance of organisms have a long history, and have been performed on a wide variety of organisms (Jones & Berkelmans 2011; Marshall & McQuaid 2020; Molina et al 2023). Increased heat temperature has been observed to cause thermal stress on organisms, and lead to a decline in the health and survival of both individuals and populations. Antarctic taxa in particular are vulnerable to temperature increases both in

terms of habitat loss and food availability declining, as well as direct negative physical impacts from heat stress (Molina et al 2023). Some taxa do possess a thermal tolerance, however. Scleractinian corals in particular are also widely observed to be vulnerable to temperature increases, though tolerances seem to vary between taxa (Jones & Berkelmans 2011; Marzonie et al, 2022). Species with a greater geographic range are believed to have a greater thermal tolerance, enabling them to survive and potentially invade into warmed ecosystems (Bates et al, 2013). Heat tolerance of marine and freshwater taxa has been reported to decline with latitude (Sunday et al, 2019).

1.3 Bryozoans and Ocean warming

Bryozoans are ectothermic organisms, meaning that their body temperature is influenced by the surrounding environment. As global temperatures rise, bryozoans may experience changes in their physiological processes. Higher temperatures, and a reduced pH can affect their growth rates, reproductive cycles, metabolism, with larvae being particularly vulnerable (Rodolpho-Metalpa et al, 2010; Durrant et al. 2013; Pages-Escola et al, 2018). Some studies have shown that increased temperatures can lead to earlier reproduction and accelerated growth in bryozoans (Amui-Vedel et al. 2007; Lord 2017). However, extreme temperature events, such as heatwaves, can be detrimental and lead to mortality or reduced reproductive success (Pages-Escola et al. 2018).

Additionally, rising atmospheric carbon dioxide (CO_2) levels leads to an increased absorption of CO_2 by the oceans, and thus ocean acidification. Reduced pH conditions can adversely affect bryozoans' ability to calcify and construct their skeletal structures (Rodolpho-Metalpa et al. 2010; Durrant et al. 2013; Pages-Escola et al. 2018). Bryozoans rely on calcium carbonate to build their colonies, and lowered pH waters can inhibit the formation and

maintenance of their skeletons, leading to reduced growth rates, weakened colony structures, and increased vulnerability to predation.

Climate change can alter ocean currents and circulation patterns, affecting the transport of bryozoan larvae and dispersal of colonies. Bryozoans generally have a planktonic larval stage that relies on water currents for dispersal and colonization of new habitats (Marshall & Keough 2007, Allen et al. 2008), and changing water patterns could influence the movement of rafting bryozoan taxa (Li et al. 2016).

Climate change can also disrupt ecological interactions bryozoans have with other organisms. For instance, changes in temperature and ocean chemistry may affect the availability and quality of food sources for bryozoans, potentially influencing their growth and reproductive success. Additionally, changes in predator-prey relationships or shifts in the abundance of competing species can indirectly impact bryozoan populations. It is important to note that the effects of climate change on bryozoans can vary among species and geographic regions. Some bryozoan species may exhibit adaptive capacities, while others may be more vulnerable to environmental changes (Smith 2014). Further research is needed to fully understand the interactions between climate change and bryozoans, as well as the long-term implications for marine and freshwater ecosystems.

1.4 Aims and Hypotheses

The aim for this is study is to investigate some of the factors that influence colony growth in bryozoans, with particular focus on those within Otago Harbour. To achieve this requires development of culture techniques and background information for suitable model species and assessing how they respond to environmental factors (e.g., season, temperature, food).

My first objective, then, was to find out what bryozoans are in Otago Harbour (Chapter 2).

I then developed a long-term dataset for an abundant local bryozoan (*Watersipora subatra*) to obtain baseline growth and development data (Chapter 3) (Figure 1.5).

Once we had a clear understanding about how local bryozoans grow in the wild, I was able to conduct manipulative experiments to investigate how increased temperatures affect growth of bryozoans (Chapter 4). Then I investigated how food availability affected growth of *Watersipora subatra* while also allowing for future experimentation by providing data on its suitability as a model organism and achieving near-wild growth rates in a laboratory environment (Chapter 5) (Figure 1.5).

Chapter six is a synthesis that considers at the implications of these studies for bryozoans in Otago Harbour and beyond.

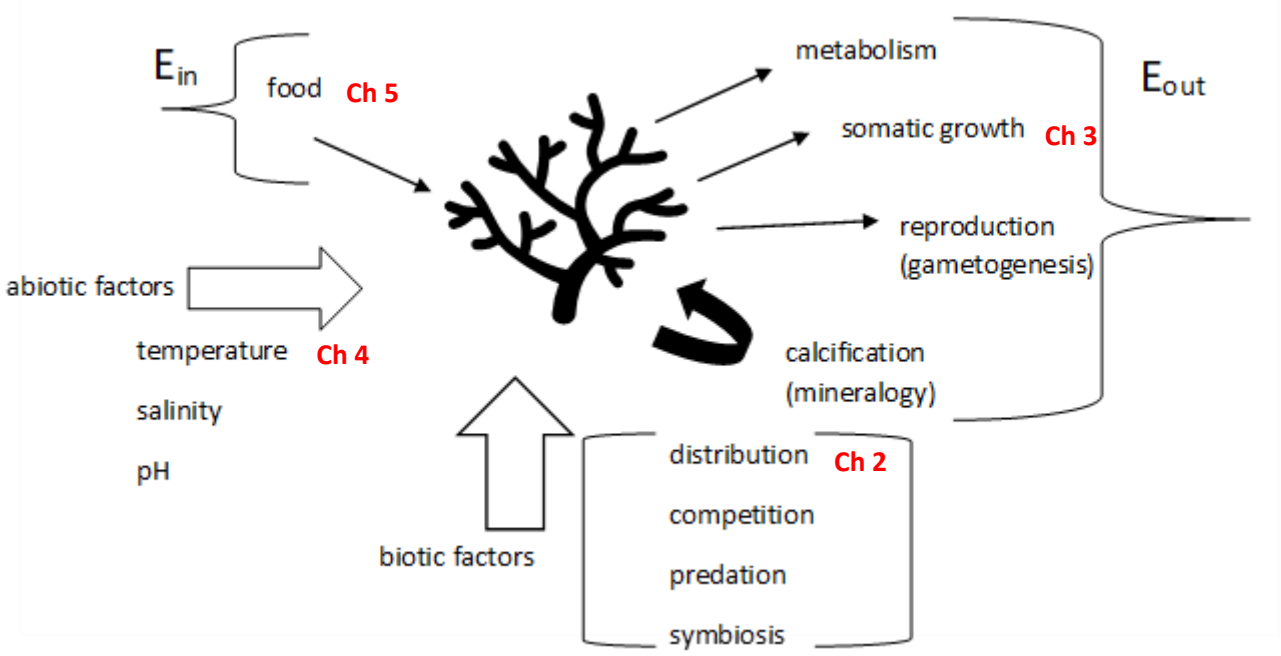
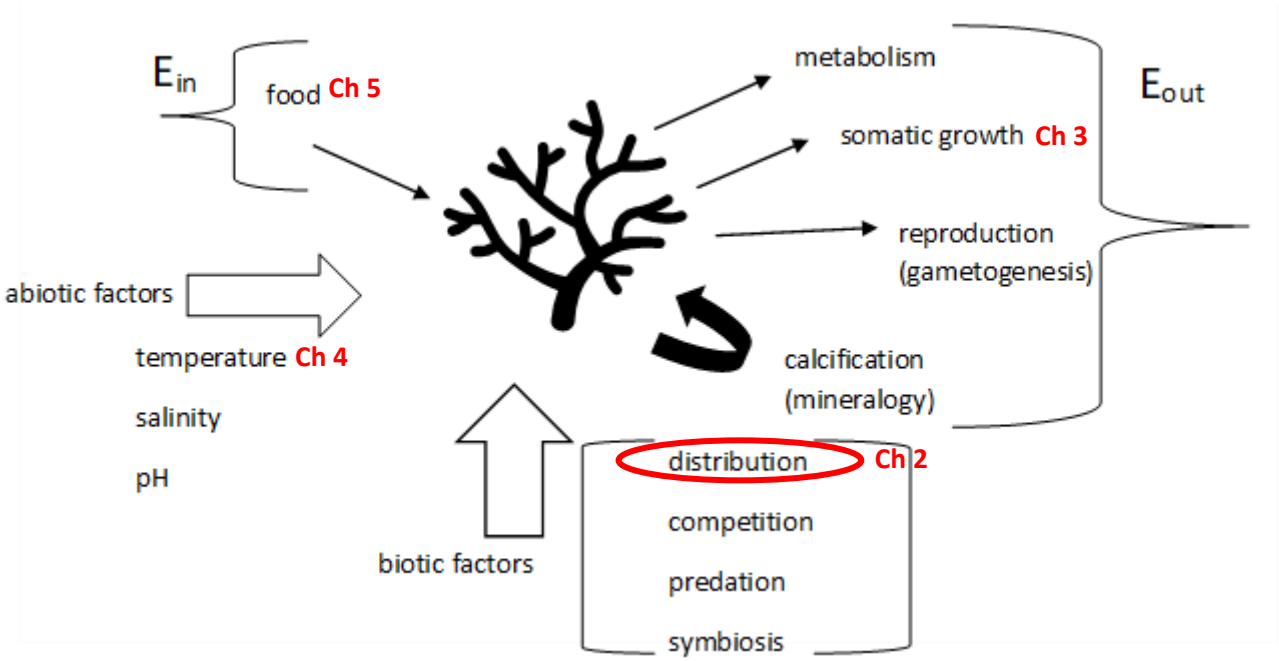


Figure 1.5. Graphical abstract showing energy flow into and out of a bryozoan, as well as the biotic and abiotic factors that may influence this pathway. Chapter two focuses on the distribution aspect of this framework, chapter three the somatic growth aspect, chapter four the temperature aspect, and chapter five the food aspect.

CHAPTER TWO: BRYOZOAN SURVEYS OF OTAGO HARBOUR



2.1 Introduction

2.1.1 Aims

This chapter provides a survey of the marine Bryozoa around Otago Harbour, Dunedin, New Zealand, supplementing published information with new surveys carried out in 2021-2022. The purpose of this work is to provide a comprehensive list of the current bryozoan fauna in Otago Harbour, and to investigate if it has changed over time. The data will allow analysis of bryozoan functional groups as well as trends in distribution. Alongside providing distribution information about Bryozoa, the surveys will enable description of the biota associated with bryozoans.

2.1.2 Otago Harbour

Otago Harbour (Wai Ōtākou) is the port of Dunedin City, located on the east coast of the South Island between Bluff and Banks Peninsula (Figure 2.1). As the only naturally sheltered anchorage for a considerable distance, it has significant ecological and historical importance (Carter, 2012) (Figure 2.1). Otago Harbour (45° 50'S 170° 38'E) is roughly 23 km long, with an average width of 2 km. It lies on a NE-SW axis, and the harbour mouth is roughly 0.4 km wide (Grove & Probert 1999). Additionally, it is relatively shallow and well-mixed, with an average depth of 4.5m, though a shipping channel with a minimum depth of 12m dominates tidal flow in the area, and the harbour channel is >30m deep between Kamau Taurua (Quarantine Island) and Rakiriri (Goat Island) (Croot & Hunter 2000; Smith et al, 2010). It is classified as a tidal inlet, though some parts are estuary or mudflat, and has an area of approximately 46 km^2 (Croot & Hunter 2000; Smith et al, 2010). The area has been inhabited since approximately 1250 AD due to its plentiful marine life. Sealing, whaling, and extensive anthropogenic modification has occurred in the last 800 years. European development of

the city Ōtepoti Dunedin included dredging, shoreline construction, pollution, and land reclamation – over 340 hectares in the last 150 years (Smith et al, 2010, Carter 2012). There are also 43 shipwrecks present in the harbour (Carter 2012). Otago Harbour has one main input of freshwater, the Water of Leith (Croot & Hunter, 2000).

The harbour is divided in the inner and outer harbour by two peninsulas at Port Chalmers and Portobello. The inner harbour is shallow and muddy with a water residence time of 15-30 days (Croot & Hunter 2000; Smith et al, 2010), whereas the outer harbour is deeper, has a water residence time of 12-24 hours, with sea-grass ecosystems, and coarser sediments (Croot & Hunter 2000; Mills & Berkenbusch, 2009; Smith et al, 2010). Salinities in the outer harbour range from 31 to 34, decreasing towards the inner harbour, typically 29-34 (Grove & Probert 1999; Croot & Hunter 2000). The tidal range is around 2.1m.

The harbour is well-studied due to its closeness to the University of Otago and Portobello Marine Laboratory (e.g., McClatchie et al, 1991; Leung et al, 2009; Smith et al, 2010; Housiaux et al, 2018). Benthic environments of the harbour have been separated into four types – mud communities, fine sand communities, stable shell communities, and unstable sand communities (Grove & Probert 1999).

Many previous studies have investigated the biodiversity of Otago Harbour. There are at least 11 known polychaete species that occur within Otago Harbour, a major component of local benthic communities (Peoples et al, 2012). At least six intertidal copepod species have been found in Otago Harbour (Stringer et al, 2012). Other groups including molluscs, echinoderms, barnacles, brachiopods, crustaceans, cnidarians, fish, seabirds, and marine mammals are commonly found within the Harbour. There is also an effect of seasonality on the more motile biota of Otago Harbour. Sevengill shark, for example, have been recorded in

the harbour, but only in summer months (Housiaux et al, 2018). Additionally, marine parasites haven been studied in Otago Harbour, there are at least 20 different trematode species that target molluscs, crustaceans, polychaetes, fish, and seabirds (Martorelli et al, 2004; Leung et al, 2009).

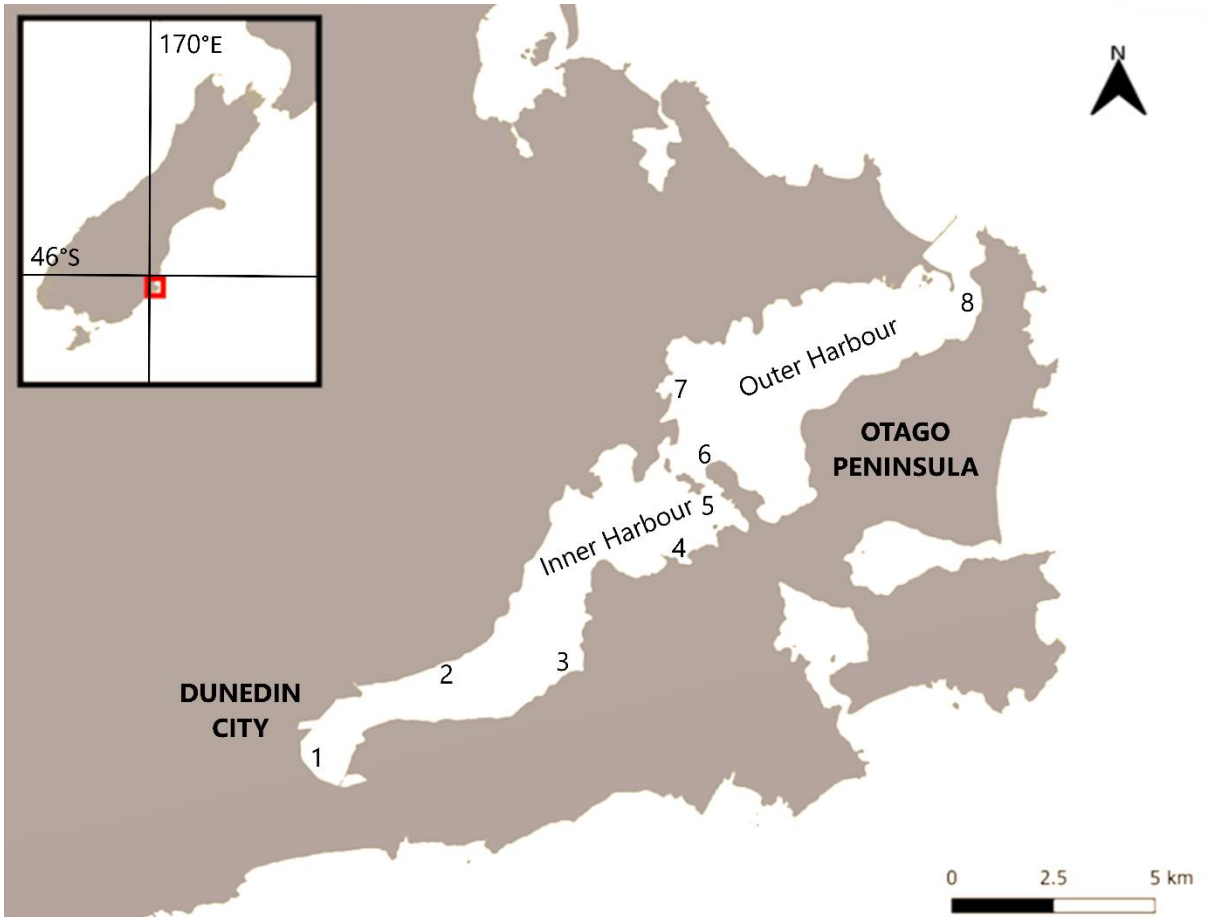


Figure 2.1. Satellite map of New Zealand showing location of Dunedin at the bottom of the South Island (Google Maps 2023). Locations of interest are indicated with the following numbers: City Wharves (1). Ravensbourne (2), Macandrew Bay (3), Broad Bay (4), Quarantine Island (5), Portobello Marine Laboratory (6), Port Chalmers (7), Harrington Point (8).

The Otago coast has a complex hydrology, including three main water masses – neritic water, subtropical water, and subantarctic water (Sutton, 2003; Takagaki, 2016). These water

masses have been observed to hold consistent characteristics, even during periods of strong stratification (Takagaki, 2016). Subtropical water moves from the Tasman Sea and extends northeast following the Otago coast, becoming part of the Southland Current, a feature composed of 10% Subtropical water and 90% subantarctic water (Sutton, 2003). The convergence between these water masses is referred to as the subtropical front (Currie & Hunter, 1999). Also of note is a region of upwelling at and south of Cape Saunders (Russell & Vennell, 2017), due to secondary flow caused by the deflection of the Southland Current at the headland (Russell & Vennell, 2017). Additionally, there is a prominent counterclockwise eddy near Blueskin Bay caused by asymmetric flow around the Otago Peninsula (Russell & Vennell, 2017) and several inputs of freshwater and terrigenous material through the river mouths along the coast and the Otago Harbour entrance (Takagaki, 2016).

2.2 Methods

Monthly surveys were carried out in Otago Harbour beginning in early 2021. Particular interest was given to the floating pontoon at Portobello Marine Laboratory (-45.8278, 170.6405), as well as a set of tyres that have been hanging off the wharf in the water for several years, known to host bryozoan communities in the past. These areas were surveyed for bryozoans at least monthly for 22 months from February 2021 to October 2022. More ad hoc surveys occurred, when possible, around the Harbour. Quarantine Island (-45.8284, 170.6365) was surveyed in March of 2021; Harrington Point beach (-45.7930, 170.7254) was surveyed in November 2021. The harbour piles along the main shipping lane in Otago Harbour were surveyed in October 2022. Port Chalmers (-45.8098, 170.6270) was surveyed in late 2021 and late 2022. The accessible shoreline of the inner harbour was surveyed in early 2022 (Table 2.1).

Table 2.1. Location and month of each survey.

Survey ID	Date	Location
1	Feb-21	Portobello
2	Mar-21	Quarantine Island
3	Mar-21	Portobello
4	Apr-21	Portobello
5	May-21	Portobello
6	Jun-21	Portobello
7	Jul-21	Portobello
8	Aug-21	Port Chalmers
9	Aug-21	Portobello
10	Sep-21	Portobello
11	Oct-21	Portobello
12	Nov-21	Harrington Point
13	Nov-21	Portobello
14	Dec-21	Portobello
15	Jan-22	Inner Harbour
16	Jan-22	Portobello
17	Feb-22	Portobello
18	Mar-22	Portobello
19	Apr-22	Portobello
20	May-22	Portobello
21	Jun-22	Portobello
22	Jul-22	Portobello
23	Aug-22	Portobello
24	Sep-22	Portobello
25	Oct-22	Port Chalmers
26	Oct-22	Harbour Shipping Piles
27	Oct-22	Portobello

Once a bryozoan colony was located, it was photographed *in-situ*, and the information for each location recorded. A small subsample was then collected from each colony for photomicrography. Samples were bleached in a 10% bleach solution, left to dry and photographed again under a microscope. The colonies were identified and labelled. For larger specimens, material was split, and half was bleached and dried, and half preserved in 90% ethanol. A series of labelled slides of dried bryozoan material was also made for easily

comparing newly collected material with older material. All this material was stored at Portobello Marine Laboratory, Portobello.

2.3 Results

In total, six sites were surveyed over the 21-month period from February 2021 to October 2022. Fifty-nine bryozoan colonies from 12 species were collected, identified, and preserved. Table 2.2 shows the overall distribution of these species at these sites.

Watersipora subatra (Ortmann, 1890) was observed to encrust on the greatest number of substrates, it was seen on tyres, oyster and clam shells, and rock. *Elzerina binderi* (Busk, 1861), on the other hand, exclusively grew on the stalks of the ascidian *Pyura pachydermatina* (Herdman, 1881). *Membranipora membranacea* was observed only encrusting on macroalgae. *Bugulina flabellata* (Thompson in Gray, 1848) and *Beania* sp. were observed growing on both tyres and shells. *Celleporina proximalis* (Uttley & Bullivant, 1972) was observed only encrusted on macroalgae. *Caberea zelandica* (Gray, 1843) was observed encrusted on tyres and shells. *Hippomenella vellicata* (Hutton, 1873) was found only as a fragment washed into the intertidal. *Telopora lobata* (Tenison-Woods, 1880) was encrusted on the *Hippomenella vellicata* (Hutton, 1873) fragment. *Smittoidea maunganuiensis* (Waters, 1906) was observed encrusting on tyres and shells. *Electra scuticifera* (Nikulina, 2008) was found encrusting only on macroalgae. *Parasmittina delicatula* (Busk, 1884) was observed encrusting only on tyres.

Watersipora subatra. Membranipora membranacea, Caberea zelandica, and *Smittoidea maunganuiensis* were observed consistently from early 2021 to late 2022 (Table 2.2; Figure 2.2). *Elzerina binderi* was observed only in late 2021 and late 2022. *Bugulina flabellata* and *Beania* sp. were observed only in early 2022. *Celleporina proximalis* was observed only

during early 2021, whereas *Electra scuticifera* was observed only during late 2021. *Hippomenella vellicata Parasmittina delicatula* and *Telopora lobata* were observed in early 2022.

Populations of *Watersipora subatra* and *Caberea zelandica* were observed in groups of up to 10+ colonies. *Beania* sp., *Elzerina binderi, Parasmittina delicatula and Smittoidea maunganuiensis* populations were observed in groups of up to 5 colonies. Populations of *Membranipora membranacea* and *Bugulina flabellata* were observed in groups of between 2 and 10+ colonies. *Celleporina proximalis* and *Electra scuticifera* were each observed only as single colonies, and *Hippomenella vellicata* was only a fragment of a colony, with a single colony of *Telopora lobata* encrusted on it. populations were observed as having 1-5 colonies (Table 2.2).

Most species were observed either as being solitary, or occurring within the presence of up to 6+ other bryozoan taxa. *Membranipora membranacea, Electra scuticifera* and *Celleporina proximalis* were observed only as solitary colonies. (Table 2.2).

Table 2.2. Table showing survey results for each taxon.

	Substrate					When found				Sites located						Specimen Info				
Species	Tyre	Shell	Macroalgae	Rock	Ascidian	Early 2021	Late 2021	Early 2022	Late 2022	Quarantine Island	Inner Harbour	Harrington Point	Portobello	Outer Harbour	Port Chalmers	Single colony	2-5 colonies	5-10 colonies	10+ colonies	Fragment

Celleporina proximalis	*Beania*	*Bugulina*	*Membranipora*	*Elzerina binderi*	*Watersipora subatra*
	X	X			X
	X	X			X
X			X		
					X
				X	
X			X		X
			X	X	X
	X	X	X		X
		X	X	X	X
			X	X	
X	X	X	X	X	X
			X	X	X
			X	X	X
X	X			X	X
	X	X	X	X	X
		X	X		X
			X		X

Electra scuticifera	*Smittoidea maunganuiensis*	*Telopora lobata*	*Hippomenella vellicata*	*Caberea zelandica*
	X			X
	X			X
X				
	X			X
X	X			X
	X	X	X	X
	X			X
	X	X	X	X
X				
X	X	X		X
	X			X
				X
				X
			X	

Parasmittina delicatula	×	×	×	× ×

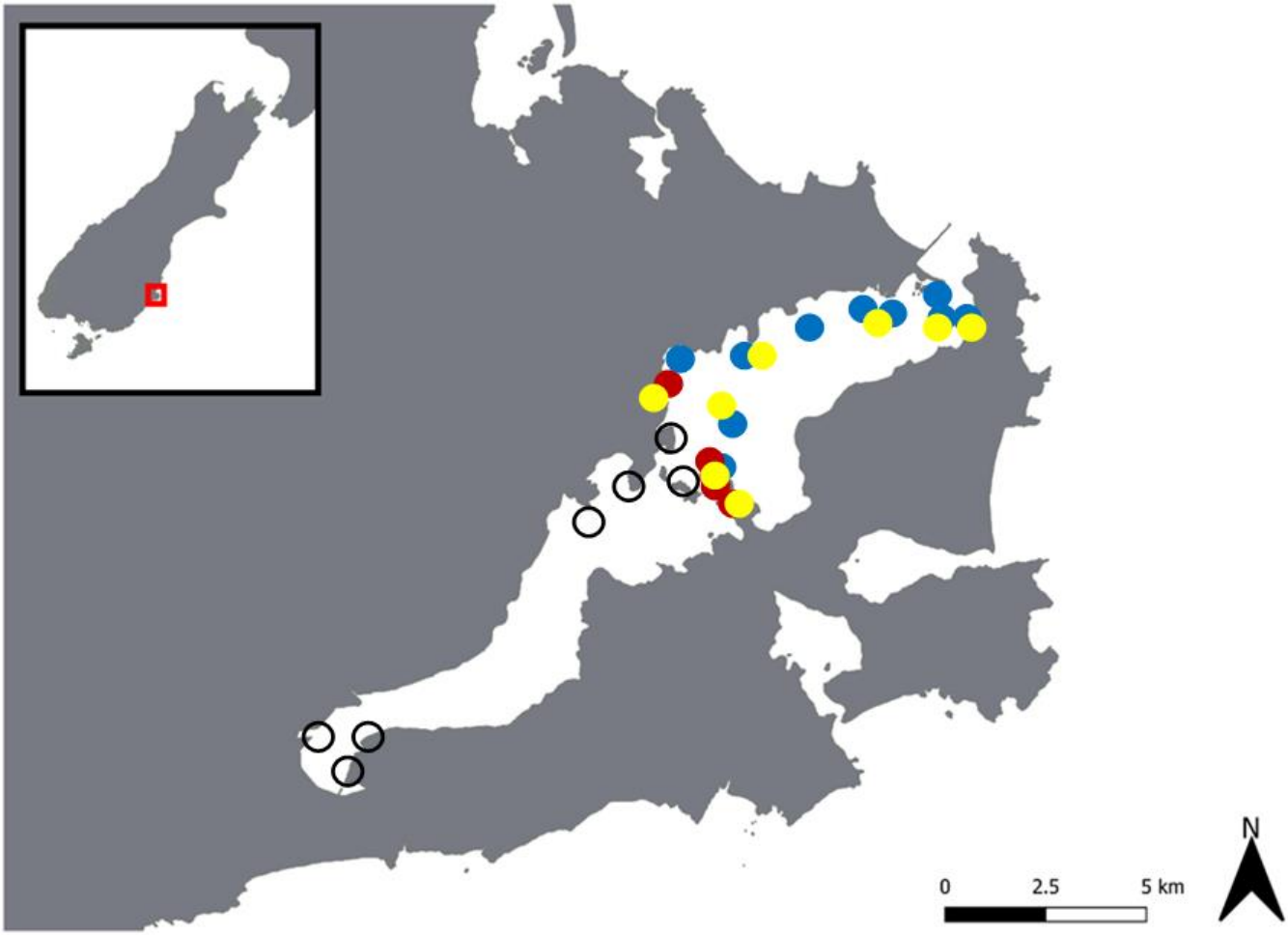


Figure 2.2. Map of Otago Harbour, Dunedin showing distribution of *Elzerina binderi* (blue), *Watersipora subatra* (red), and *Membranipora membranacea* (yellow). Locations surveyed but where the species were not found are indicated by open circles.

2.4 Discussion

2.4.1 Prior Surveys

This is one of only three bryozoan-specific surveys performed in Otago Harbour; the other two were conducted by Hamilton (1897) and Gordon & Mawatari (1992). We recorded 12 species, of which two were invasive and ten were native to NZ.

The earliest known published survey is from 1897, and was of the littoral bryozoans of the Dunedin area. The specimens were collected by Augustus Hamilton (1853-1913), and identified through correspondence with Eliza Catherine Jelly (1829-1914) at the Natural History Museum, UK (Table 2.3).

Table 2.3. Results of a survey of Bryozoa in the Dunedin area, 1897 (Hamilton, 1897).

Recorded taxa (Hamilton, 1897)	Currently accepted name (WORMS 2023)
Amathia swainsoni (Hutton, 1873)	*Amathia biseriata* (Krauss, 1837)
Beania swainsoni (Hutton, 1873)	*Amathia biseriata* (Krauss, 1837)
Catenicella cribraria (Busk, 1852)	*Paracrobricellina cribaria* (Busk, 1852)
Catenicella hastata (Busk, 1852)	*Costaticella bicuspis* (Gray, 1843)
Catenicella perforata (Busk, 1852)	*Cornuticella perforata* (Busk, 1852)
Catenicella ventricosa (Busk, 1852)	*Orthoscuticella ventricosa* (Busk, 1852)
Chorizopora brongniartii (Audouin, 1826)	*Chorizopora bronguiartii* (Audouin, 1826)
Cribrilina monoceros (Busk, 1884)	*Arachnopusia unicornis* (Hutton, 1873)
Flustrella binderi (Harvey n.d.)	*Elzerina binderi* (Busk, 1861)
Lepralia pallasiana (Moll, 1803)	*Cryptosula pallasiana* (Moll, 1803)
Membranipora hians var. *occultata* (Waters n.d.)	*Membranipora cyclops* (Busk, 1854)
Membranipora solidula (Hincks, 1860)	*Crassimarginatella solidula* (Hinks, 1860)
Monoporella crassatina (Waters, 1882)	*Macropora levinseni* (Brown, 1952)
Smittina trispinosa (Johnston, 1838)	*Parasmittina aotea* (Brown, 1952)

The results of the 1897 survey required cross-referencing with current taxonomy (WoRMS 2023; Gordon & Taylor 2008; Smith et al. 2022)) to identify many of the taxa described, as much of it had changed since it was published.

Gordon and Mawatari (1992) found ten fouling bryozoan species in Otago Harbour (Table 2.4), though they excluded non-fouling bryozoan taxa from this study. The species recorded are quite a different list from that of Hamilton.

Table 2.4. Results of the 1992 survey of Otago Harbour (Gordon & Mawatari 1992). *W. subtorquata* is now identified as *Watersipora subatra* (Vieira et al. 2014).

Recorded taxa (Gordon & Mawatari 1992)
Arachnopusia unicornis (Hutton, 1873) (encrusting)
Bugulina flabellata (erect, bushy)
Bugula neritina (Linnaeus, 1758) (erect, bushy)
Calloporina angustipora (Hincks, 1885) (encrusting)
Antarctothoa bathamae (Ryland & Gordon, 1977) (encrusting)
Celleporina proximalis (encrusting, nodular)
Chaperiopsis cervicornis (Busk, 1854) (encrusting, calcareous*)*
Cryptosula pallasiana (Moll, 1803) (encrusting)
S*chizosmittina cinctipora* (Hincks, 1883) (encrusting)
Watersipora subtorquata (d'Orbigny, 1852)

Of the fourteen taxa reported by Hamilton (1897), only *Elzerina binderi* was found in Otago Harbour in 2023, 106 years later. Three bryozoan taxa were found by both this study and Gordon and Mawatari (1992): *Bugulina flabellata*, *Celleporina proximalis*, and *Watersipora subatra*. The other bryozoan taxa observed earlier have either disappeared from Otago Harbour during the last 30 years, or were missed in our surveys. Meanwhile we encountered eight additional species hitherto not recorded here.

2.4.2 Species Distributions

The 12 species found across the harbour varied in their distributions, both spatially and temporally. The species with the widest distribution was *Watersipora subatra*, followed by

Membranipora membranacea, and *Elzerina binderi*. Most other taxa were limited to a single site.

The invasive species *Watersipora subatra* was present at the Portobello and Port Chalmers sites, which are both active ports. The population at Portobello was observed over 2020 – 2022, and persisted over this time. Several populations were found encrusting on fist-sized rocks along beaches around Portobello Marine Laboratory. The genus *Watersipora* is known for its widespread distribution (Ferrario et al. 2015, Reverter-Gil & Souto 2019).

Membranipora membranacea is a globally-distributed bryozoan known to encrust on various macroalgae. As expected, every observation of this species was of colonies encrusted on several different macroalgae. The most common was *Macrocystis*, though *M. membranacea* was also frequently seen on smaller, rhodophyte alga. It was also seen encrusting on sea grass at a single site on the sandbar in the middle of Otago Harbour. It was never seen encrusting on *Durvillaea* or *Undaria*.

Elzerina binderi was observed at numerous sites across the outer harbour, and a single observation of it having rafted to a beach in the outer harbour was recorded as well. The rafted individual likely came from one of the populations present on the piles in the middle of the outer harbour. *E. binderi* was also observed at Portobello in 2021, though this population consisted of several small colonies that disappeared after several months, and re-colonised the following year.

2.4.3 Influences on Distribution

Substrate appears to have an influence on bryozoan distributions within Otago Harbour. *Watersipora subatra, Bugulina flabellata, Beania* sp.*, Caberea zelandica,* and *Smittoidea*

maunganuiensis were all observed to encrust on submerged car tyres and mollusc shells, suggesting they are restricted to environments within Otago Harbour that possess these substrates. Curiously, *Parasmittina delicatula* was only observed on tyres, indicating it may prefer artificial objects. *Membranipora membranacea, Celleporina proximalis,* and *Electra scuticifera* were only observed on macroalgae, either rooted or rafting. Apparently certain bryozoans are only found on this type of substrate. *Watersipora subatra* was observed across the widest range of substrates as it was found encrusting on intertidal rocks, tyres, and shells, indicating that it has a high tolerance of substrate type. *Elzerina binderi* was observed often on the stalks of ascidians, indicating it may have a preference for substrate.

Watersipora subatra, Membranipora membranacea, Caberea zelandica, and *Smittoidea maunganuiensis* were observed consistently from early 2021 to late 2022 suggesting there is no effect of seasonality on their presence in Otago Harbour. *Elzerina binderi* was only observed in late 2021 and late 2022, the explanation for this is unclear, though they may be a seasonal species. The time period in which the greatest number of bryozoan taxa was observed was early 2022, this could be due to a particularly warm summer period encouraging colony growth throughout Otago Harbour.

The presence of other bryozoan taxa seems to have little effect on the distribution of observed bryozoan taxa in Otago Harbour. *Watersipora subatra, Elzerina binderi* were observed both solitary and in the presence of other bryozoan taxa. *Bugulina flabellata, Beania* sp, *Caberea zelandica, Smittoidea maunganuiensis,* and *Parasmittina delicatula* were observed only in communities with other bryozoan taxa. *Membranipora membranacea, Celleporina proximalis*, and *Electra scuticifera* were all observed without any other bryozoan taxa present. It is possible these observations are due to environments suitable for

bryozoans having many bryozoan taxa present regardless of whether or not they have an effect on each other. In contrast, bryozoan taxa observed to be exclusively solitary have adapted to a niche few other bryozoan taxa have been able to colonise.

It also appears that there is little to no adverse intra-specific effect influencing the distribution of bryozoan taxa observed in Otago Harbour as most taxa were observed to have populations ranging between 2-10+ colonies. Only a few taxa were observed to have populations of up to 10+ colonies, indicating either these are highly successful taxa, or that there is an upper limit that the populations of other taxa can reach before a negative effect is experienced by having a large population of all the same taxa competing for the same resources.

The effect of temperature, pH, and salinity differences throughout the Otago Harbour sampling sites likely had little effect on the differences in bryozoan fauna collected. Otago Harbour is well-mixed with a reasonably short water residence time, particularly in the outer harbour where bryozoans were more abundant. Seasonality may have an influence on bryozoan fauna as bryozoans typically grow fastest in the summer months, meaning they are more likely to be observed during this time. Many colonies were collected during the winter at Portobello but some of the fieldwork associated with these surveys were specifically designed to take place during the spring and summer of 2021/2022 in order to maximise the effectiveness of sampling. It would still however, be an excellent idea to survey the harbour with greater effort in winter months in the future to further understand the impact of seasonality on the resident bryozoan fauna.

2.4.4 Where would you go to find bryozoans?

If you really needed to collect some bryozoans, Portobello is the place to go. It showed the highest diversity of species, probably due to intense sampling effort and ease of access. Portobello has a rocky beach with significant anthropogenic influence on it due to the presence of Portobello Marine Laboratory and consequent vessel traffic. There are numerous objects in the water available for colonisation by Bryozoa.

Quarantine Island curiously showed no species of Bryozoa, which was unexpected due to its close proximity to the Portobello sampling sites. No bryozoans could be found along the coastlines, encrusting on any shells, shipwrecks, or wharves. It is possible that the presence of the two shipwrecks deters colonisation due to their presumed input of metals into the water, but this seems unlikely. It is also possible cryptic species were missed, or that bryozoans can be found in the deeper subtidal around the island. During surveys of the harbour the following year, piles near Quarantine Island were surveyed and also showed no bryozoan colonies; they were covered in sea tulips, mussels, and macroalgae. Competition may thus be a factor.

Harrington Point yielded two species of Bryozoa: *Elzerina binderi*, and *Membranipora membranacea*. Both of them had drifted onto the shore. The *M. membranacea* was encrusting on rafting macroalgae, and the *E. binderi* had seemingly become unattached and floated shore-ward. This beach was a sandy beach with little typical bryozoan habitat aside from a derelict boat mooring station absent of any bryozoans. Surveys of the harbour found *E. binderi* growing abundantly across many of the piles in the harbour; it is likely that one these is the origin for the *E. binderi* collected at this location. Additionally, this suggests the distribution of *E. binderi* may be influenced by its ability to raft without need of an encrusting surface (e.g., buoys, or macroalgae) a characteristic displayed by relatively few

Bryozoa, as the collected colony was alive and healthy. *E. binderi* may be able to raft to different locations and establish new populations.

Port Chalmers is an active port, and as *W. subatra* is a fouling species, the presence of this taxon on buoys is not surprising. *Caberea zelandica* was also observed, as was *Membranipora membranacea* encrusted on macroalgae tangled around a buoy. Several bryozoan taxa were observed on trawling equipment at Port Chalmers; however, the origin of these specimens was unknown.

2.4.5 Invasive Species

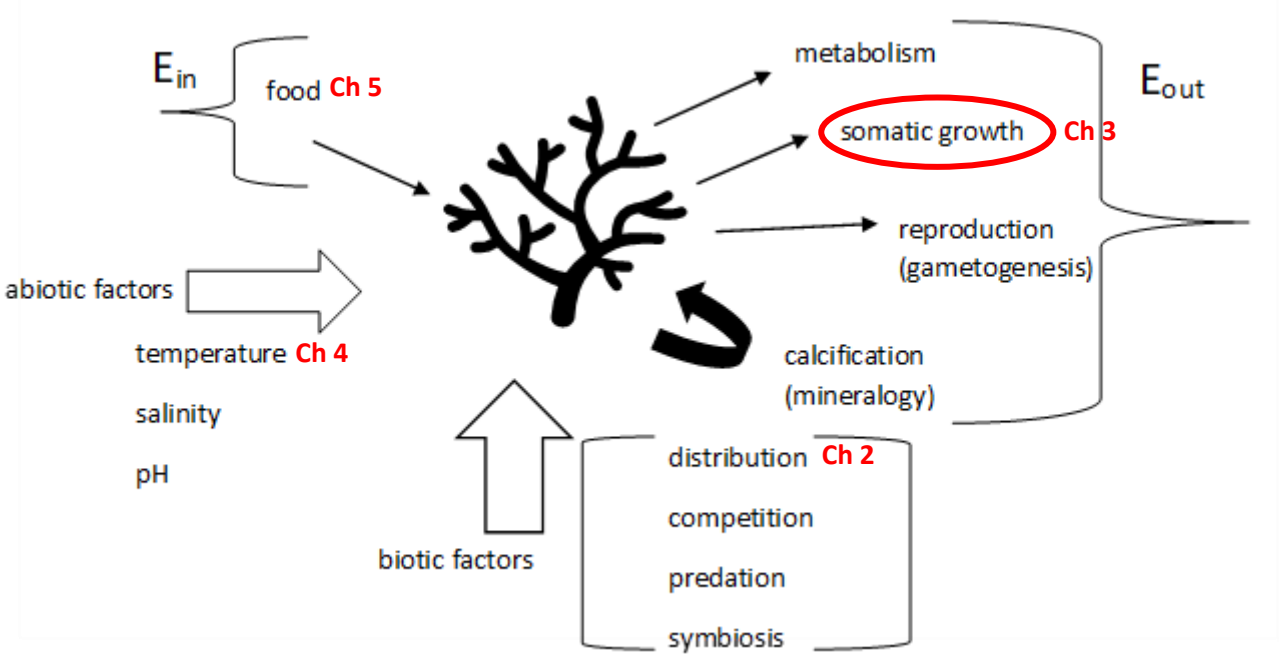
Two widespread invasive bryozoan taxa were observed in Otago Harbour, *Watersipora subatra* and *Bugulina flabellata*. Both of these species were previously recorded in Otago Harbour in 1992 (Gordon & Mawatari 1992). No new invasive taxa have been recorded suggesting there have been no successful invasions of new species in the last 30 years.

Of the ten fouling species Gordon and Mawatari (1992) found in Otago Harbour, seven were not re-recorded, meaning they may no longer be present in the Harbour - *Arachnopusia unicornis*, *Bugula neritina*, *Calloporina angustipora*, *Chaperiopsis cervicornis, Cryptosula pallasiana*, and S*chizosmittina cinctipora*.

2.5 Conclusions

While simple distribution data is neither romantic nor exciting, it is the bedrock on which conservation exists. We cannot conserve populations we don't know about; we can't argue that populations are damaged without a time series of data. Here we have shown that a single survey of Otago Harbour may miss many of the bryozoans present, but also that the species present have changed over the years.

CHAPTER THREE: WILD GROWTH OF *WATERSIPORA SUBATRA*



NOTE: Chapters three and five combined have been published as:

Feary, T, Smith, A.M. 2023. Food for thought: Investigating the impacts of feeding regime on the growth and survival of a locally invasive cheilostome bryozoan. Bryozoan Studies 2022 (Proceedings of the 19th International Conference on Bryozoa, 2022).

Feary carried out field & lab work, ran experiments, analysed data, and wrote drafts.

Smith commented on drafts and provided financial support.

3.1 Introduction

Different methods have been used for studying the growth rate of Bryozoa including calcein staining (Moran 2000, Linard et al. 2011, Smith 2014) photography, and growth checks, but methods used are not comparable across the phylum, so measured growth rates are also not easily compared. Nevertheless, a basic understanding of natural growth is essential background to understand the effects of climate change and ocean acidification on the growth and survival of bryozoans (Smith 2009, Lombardi et al. 2011b, Seroy & Grünbaum 2018).

Lab and field experiments aim to imitate and extrapolate wild growth rates for marine organisms that are hard to culture. Once a reliable culture method is established, it allows experimentation investigating how growth rates may be affected by different stressors, the effect of changed growth rate on survival. We propose to work on the ubiquitous, robust, and easily recognisable intertidal bryozoan *Watersipora subatra*.

Watersipora subatra is a cheilostome bryozoan belonging to the family Watersiporidae. Colonies are typically dark-red or black, with an orange or crimson band around the colony

edge, known as the growing edge. *Watersipora is a widespread genus, and some species are considered to be a fouling pest species in almost all of its range.* A native range is currently unknown for *Watersipora subatra,* but it is theorised to have originated from somewhere in coastal Asia (Vieira et al. 2017; Reverter-Gil & Souto 2019) (Figure 3.1).

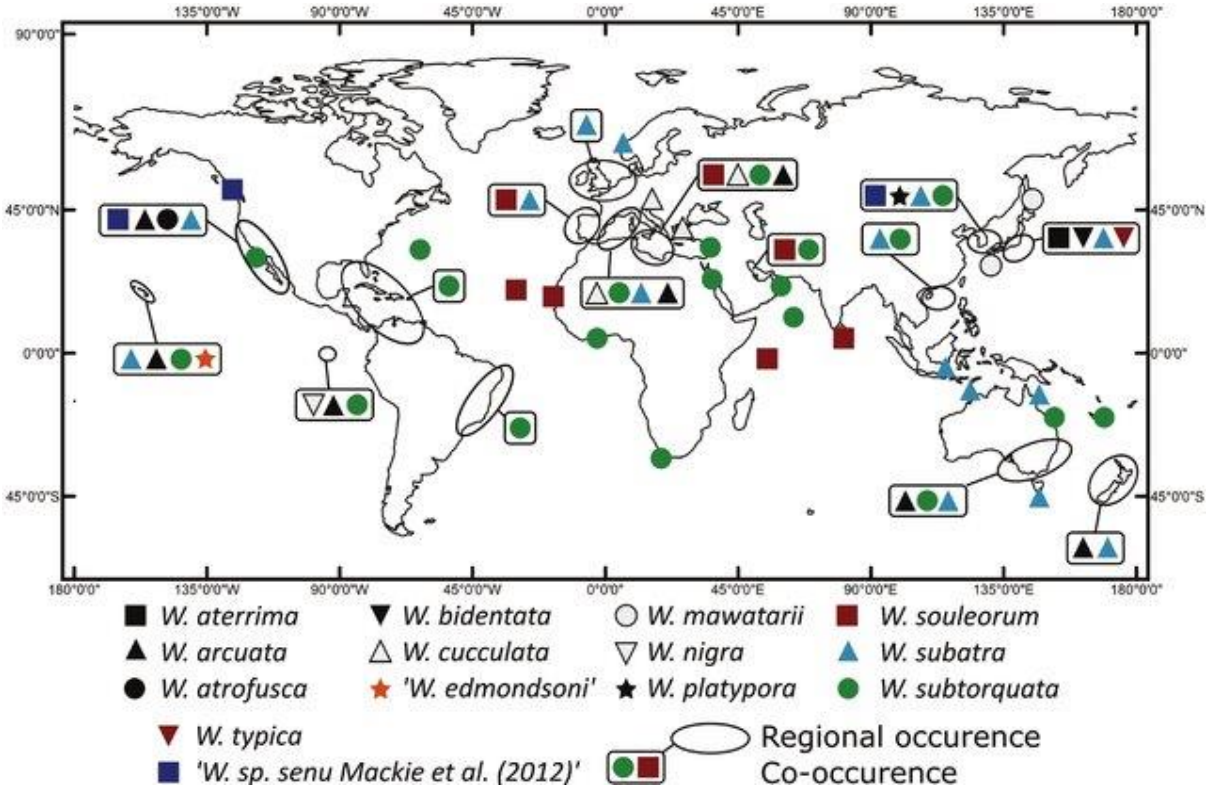


Figure 3.1. Global range of *Watersipora* (Gauff et al. 2023).

W. subatra will grow readily on sheltered intertidal rocks and submerged artificial substrates such as buoys or wharf pilings. *Watersipora* spp. also haves a history of being used in laboratory research across the wider literature, making it an excellent candidate for a model species.

The aim for this study is to obtain a long-term dataset for wild growth in *Watersipora subatra*. Understanding how the wild growth of *W. subatra* varies seasonally will provide essential baseline data for future research.

3.2 Methods

Portobello Marine Laboratory has a population of *Watersipora subatra* on a set of tyres hanging of the end of its pier; these colonies were used to find a wild growth rate for *W. subatra*, as well observing how other colony characteristics vary seasonally.

Observation of the tyres began in early 2021 and ended in late 2022. Colonies were observed at least monthly for this period, and fortnightly in the summer, when coastal bryozoan growth tends to be highest. During each observation, the four tyres were pulled out of the water onto the pier. Each side of each tyre was photographed, and then individual colonies were photographed with a scale. Notes were taken of colony coverage, colour, shape, and co-existing biota on the tyres. The tyres were then returned to the water.

To estimate the wild growth rate of *W. subatra*, 33 colonies on two tyres at Portobello Marine Laboratory were labelled and digitally photographed monthly (Figure 3.2). Of these 33 colonies, seven were successfully rephotographed; the other colonies died or were overgrown. Photographs were analysed using the imaging software ImageJ to find the change in colony diameter and area over time. The results were standardised to mm/day to make them comparable. Datasets were obtained for the wild growth of *W. subatra* during Winter 2021, and January to October of 2022 (Figure 3.3, Table 3.2, Table 3.3). The results will be used later to compare with growth rate in laboratory feeding experiments (Chapter 5).

Temperature, pH, and other environmental variables of the water at Portobello Marine Lab were monitored and collected hourly over the time period of this study by a sensor attached to the end of the pier.

3.3 Results

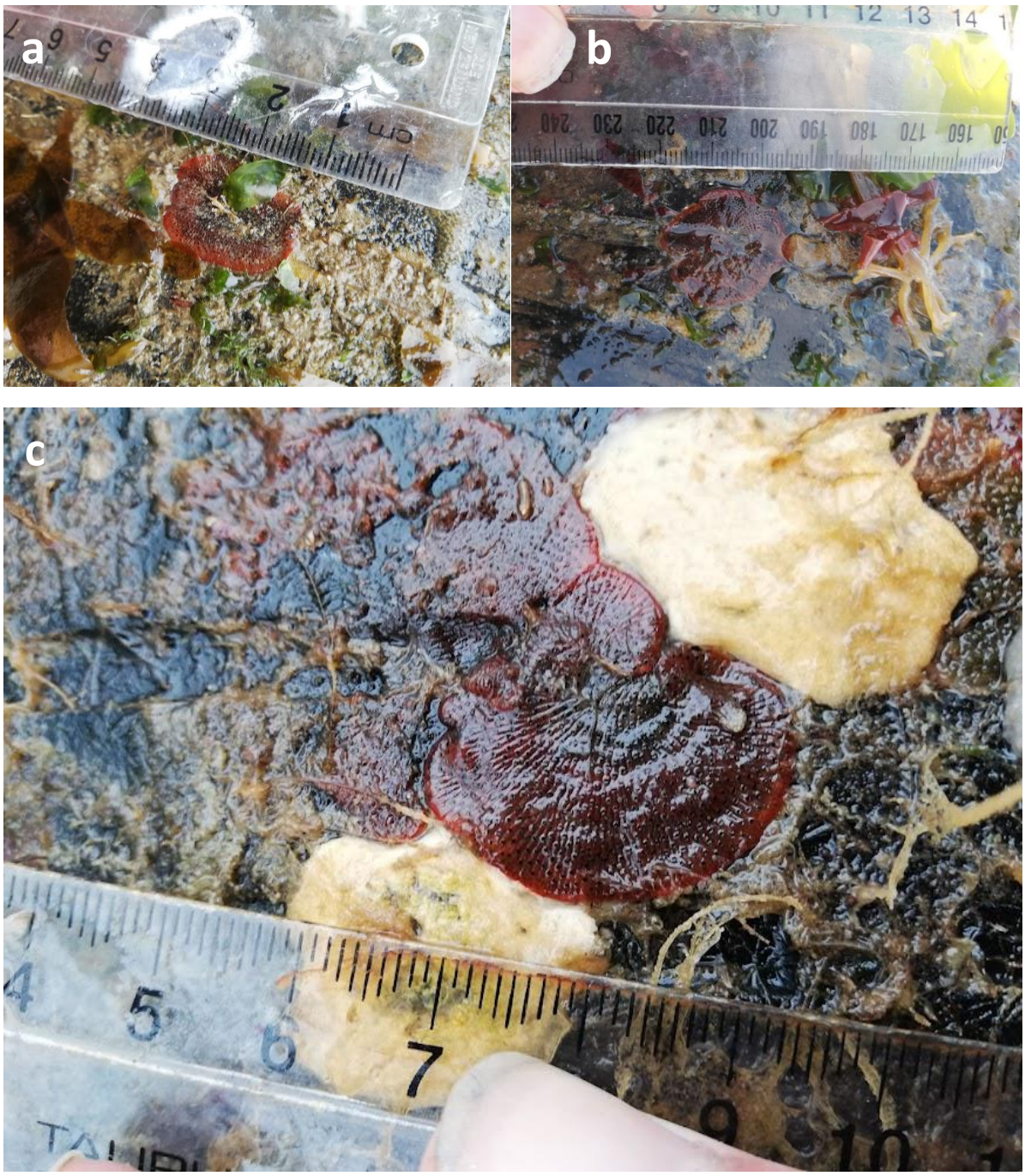
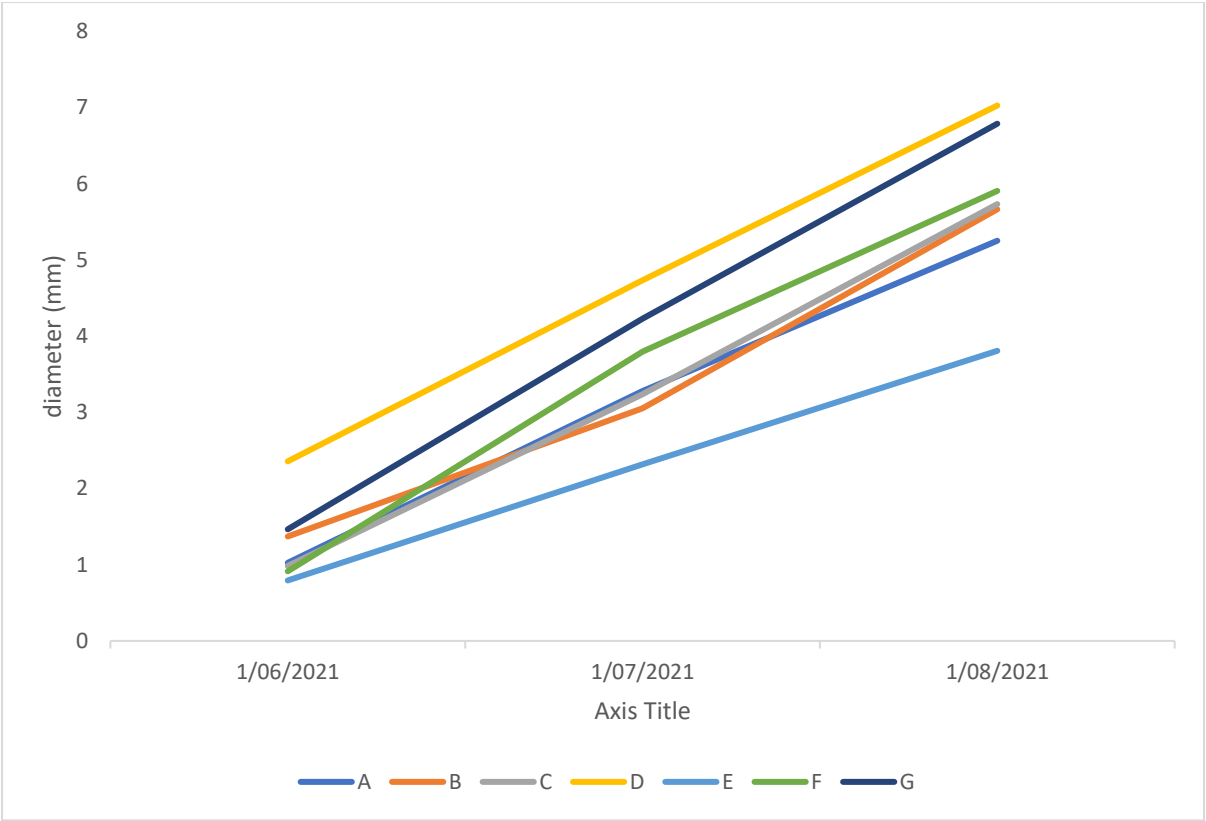


Figure 3.2. Photographic progression of a colony of *Watersipora subatra* during February (a) May (b) and October (c).

a



b

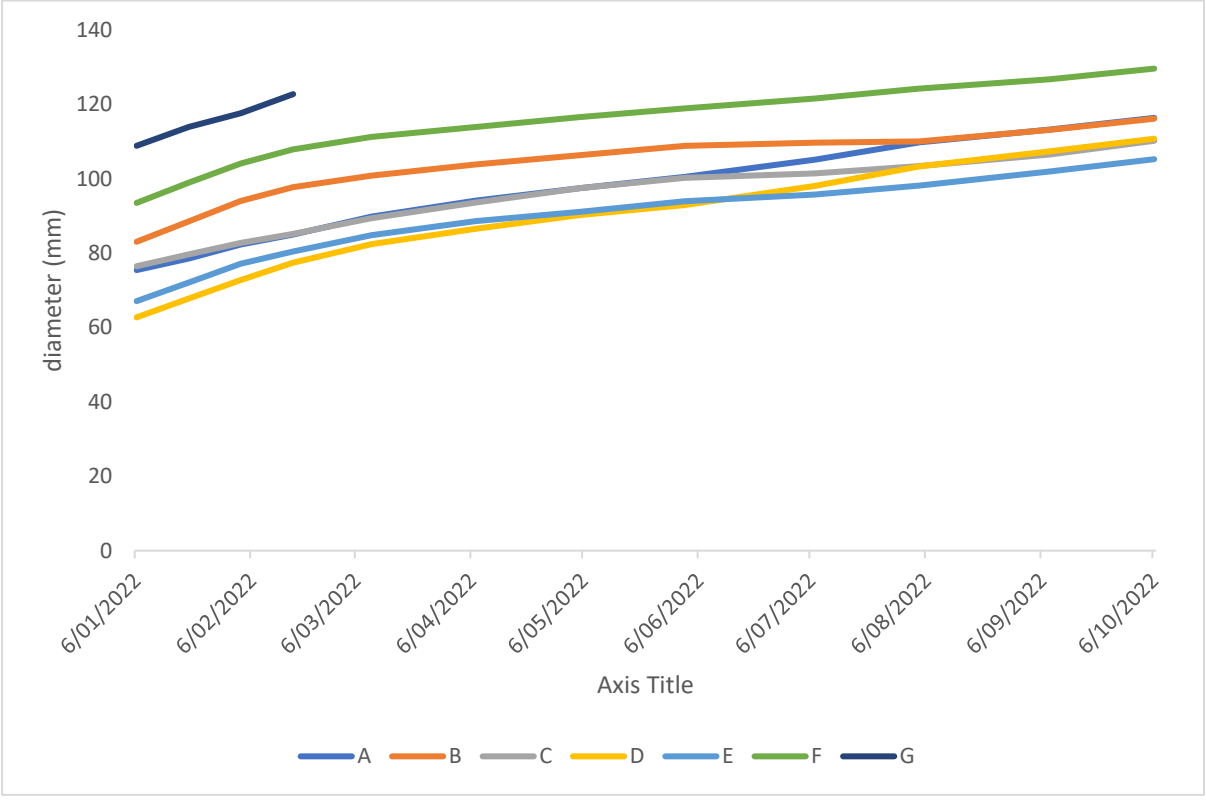


Figure 3.3. Diameters of wild *Watersipora subatra* colonies taken from photographs monthly at Portobello Marine Laboratory, 2021-2022.

Table 3.2. Total growth between time points, with rate per day given in brackets for wild colonies photographed at Portobello Marine Laboratory, 2021-2022. Calculated from change in diameter over time period between samples.

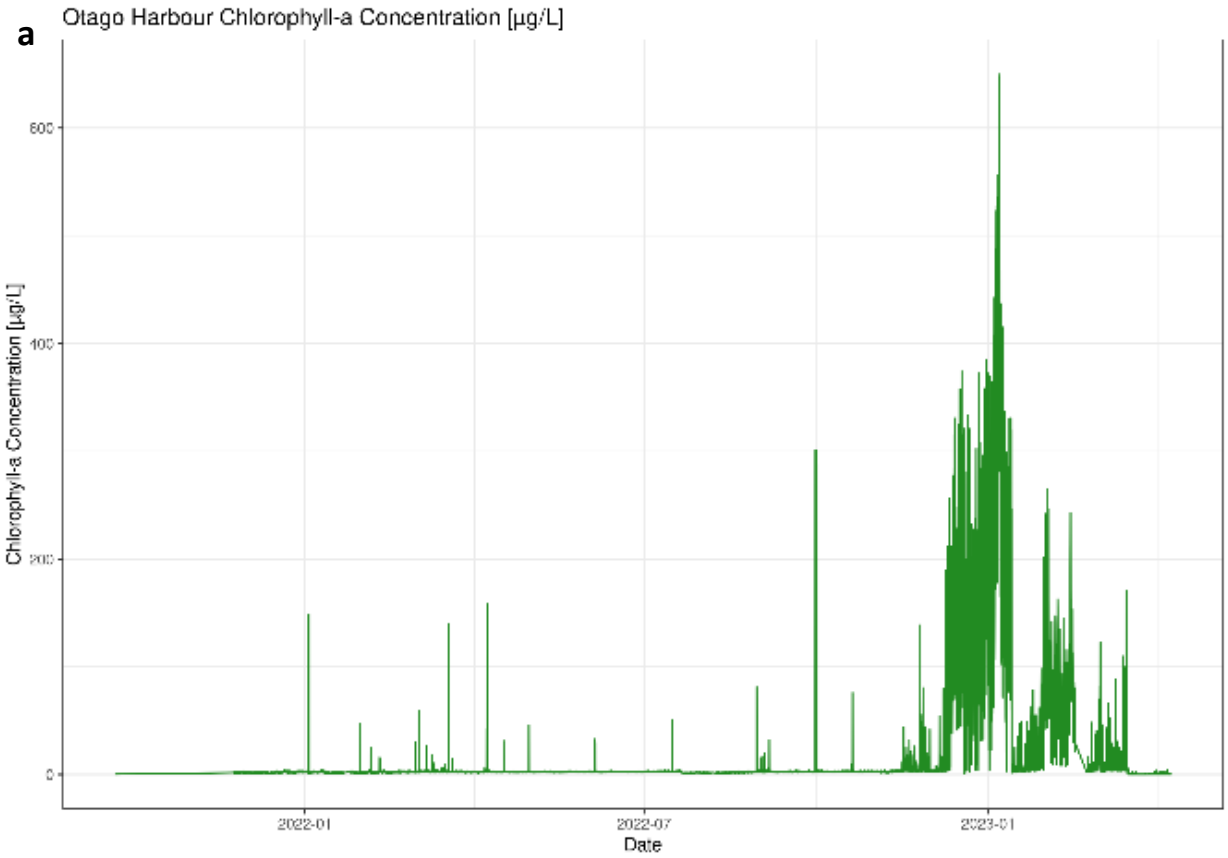
Date	A	B	C	D	E	F	G
7-Jun-21	2.25 (0.08)	1.68 (0.06)	2.25 (0.08)	2.38(0.08)	1.52 (0.05)	2.88 (0.10)	2.76 (0.09)
4-Aug-21	1.97 (0.07)	2.61 (0.09)	2.49 (0.08)	2.29(0.08)	1.49 (0.05)	2.11 (0.07)	2.56 (0.09)
Date	A	B	C	D	E	F	G
20-Jan-22	3.14 (0.22)	5.44 (0.39)	3.21 (0.23)	5.06 (0.36)	5.02 (0.36)	5.46 (0.39)	5.1 (0.36)
3-Feb-22	3.78 (0.27)	5.5 (0.39)	3.08 (0.22)	5.02 (0.36	5.05 (0.36)	5.11 (0.37)	3.68 (0.26)
17-Feb-22	2.66 (0.19)	3.77 (0.27)	2.38 (0.17)	4.64 (0.33)	3.29 (0.23)	3.82 (0.27)	5.11 (0.37)
10-Mar-22	4.82 (0.23)	3.05 (0.15)	4.26 (0.2)	5 (0.24)	4.34 (0.21)	3.36 (0.16)	
7-Apr-22	4.26 (0.15)	3.02 (0.11)	4.23 (0.15)	4.14 (0.15	3.78 (0.14)	2.69 (0.1)	
5-May-22	3.37 (0.12)	2.51 (0.09)	3.83 (0.14)	3.68 (0.13)	2.53 (0.09)	2.67 (0.1)	
2-Jun-22	3 (0.1)	2.45 (0.08)	2.72 (0.09)	2.66 (0.09)	2.82 (0.1)	2.31 (0.08)	
7-Jul-22	4.68 (0.14)	0.94 (0.03)	1.28 (0.04)	5.18 (0.15)	1.79 (0.05)	2.64 (0.08)	
4-Aug-22	4.66 (0.15)	0.34 (0.01)	1.91 (0.06)	5.25 (0.17)	2.46 (0.08)	2.67 (0.09)	
8-Sep-22	3.36 (0.09)	3.1 (0.09)	3.17 (0.09)	4.01 (0.11)	3.81 (0.11)	2.54 (0.07)	
6-Oct-22	3.18 (0.12)	2.96 (0.11)	3.65 (0.14)	3.4 (0.13)	3.27 (0.12)	2.85 (0.11)	

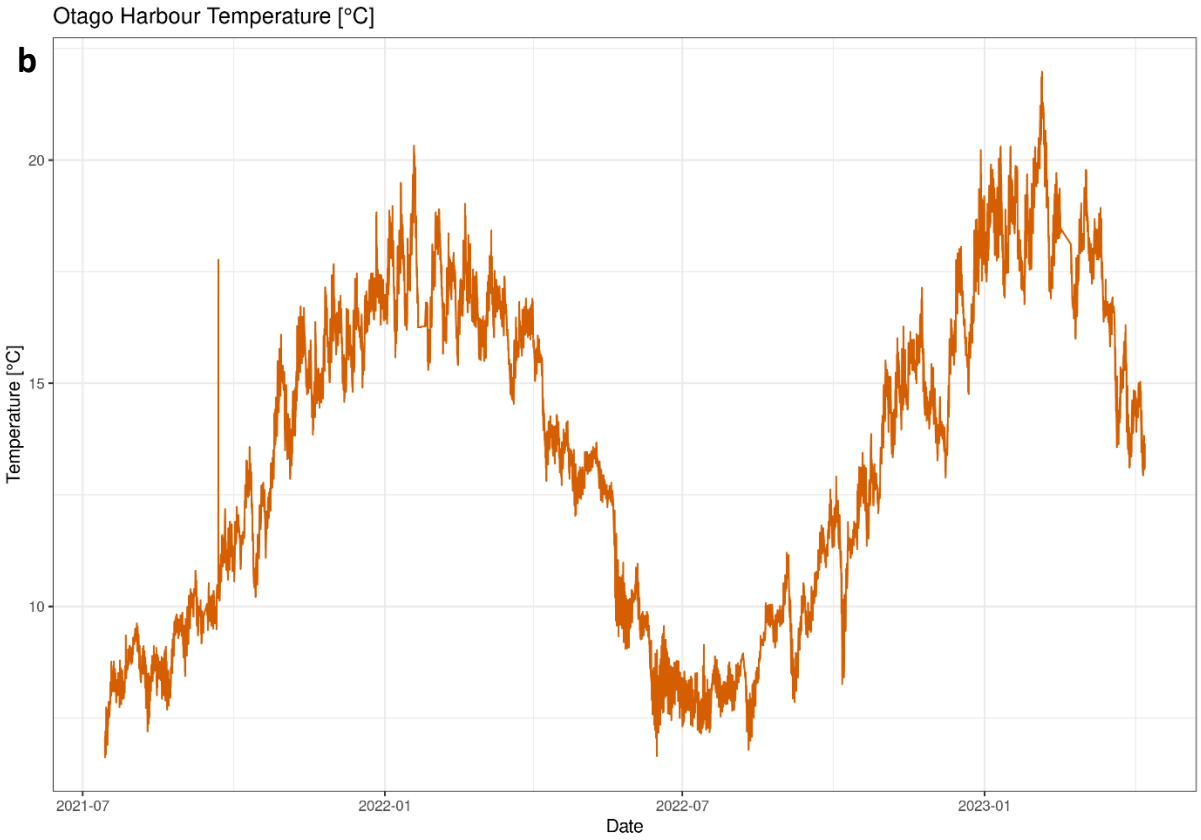
Winter 2021 (June – August) showed an average growth of 0.074 mm/day (SD = 0.014, N = 7). Summer 2022 (January - February) showed an average growth of 0.303 mm/day (SD = 0.072, N = 7). Autumn 2022 (March - May) showed an average growth of 0.146 mm/day (SD = 0.046, N = 6). Winter 2022 (June - August) showed an average growth of 0.08 mm/day (SD = 0.043, N = 6). Spring 2022 (September - October) showed an average growth of 0.105 mm/day (SD = 0.018, N = 6). Average growth was lowest in winter 2021 (0.074 mm/day), and at its highest in summer 2022 (0.303 mm/day) (Table 3.3; Table 3.4). Approximate calculations indicate that the annual growth rate for these colonies are 5.11 mm/y (Winter 2021), 110.59 mm/y (Summer 2022), 53.29 mm/y (Autumn 2022), 29.2 mm/y (Winter 2022), and 38.33 mm/y (Spring 2022).

Table 3.3. Summary of results from wild growth during winter 2021, and January to October 2022,

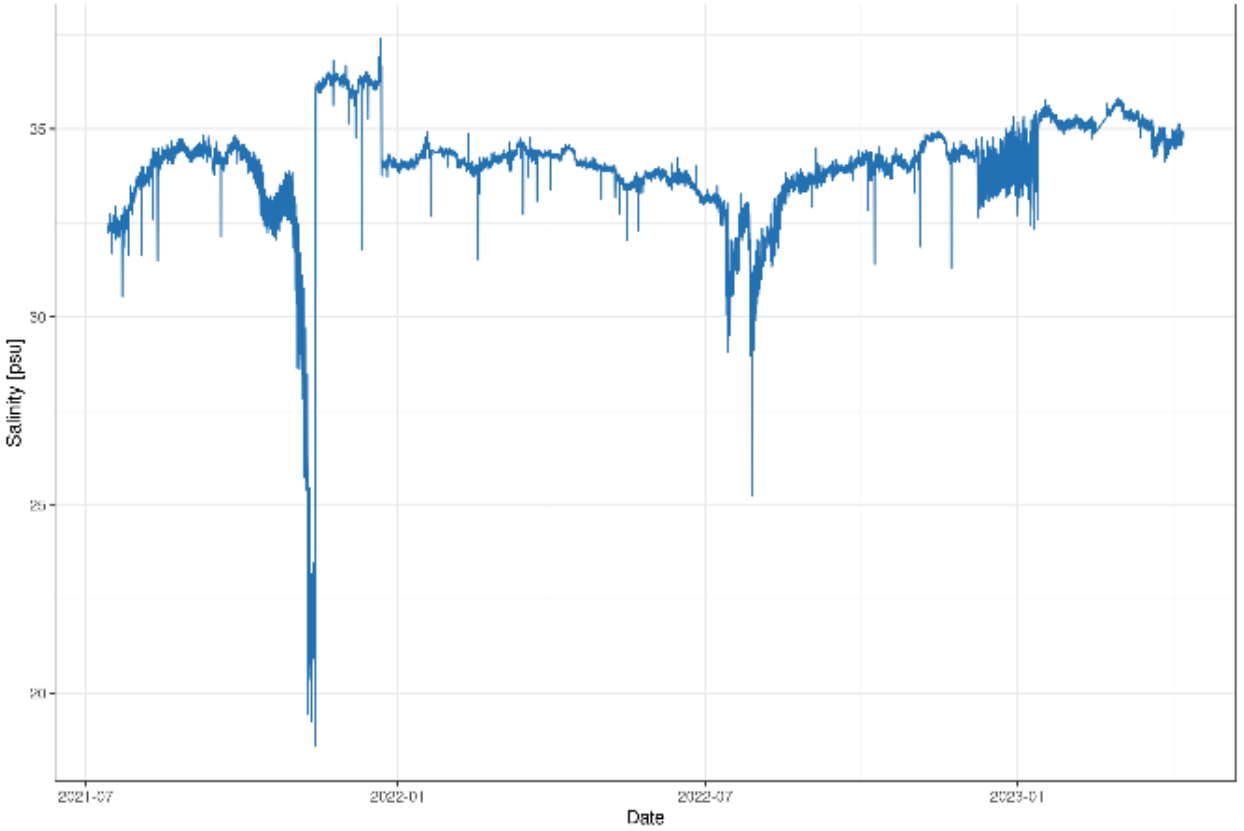
2021	AVERAGE	STDEV	N
WINTER	0.074454	0.014595	7
2022	AVERAGE	STDEV	N
SUMMER	0.303822	0.072955	7
AUTUMN	0.146463	0.046375	6
WINTER	0.088555	0.043274	6
SPRING	0.105854	0.018561	6

Seawater characteristics showed expected trends. Temperature was highest in the summer months (likely encouraging growth of *W. subatra* colonies), and lowest in the winter. Dissolved oxygen decreased towards the peak of summer (correlating with an increase in *W. subatra* growth as this decrease is caused by an increase in phytoplankton activity), whereas salinity was highest in the summer. Both chlorophyll *a* concentration and pH showed a spike at the start of 2023 (the chlorophyll spike in particular having a positive effect on colony growth if *W. subatra*) (Figure 3.4).


a
Otago Harbour Chlorophyll-a Concentration [µg/L]
Chlorophyll-a Concentration [µg/L]
600
400
200
0
2022-01
2022-07
2023-01
Date

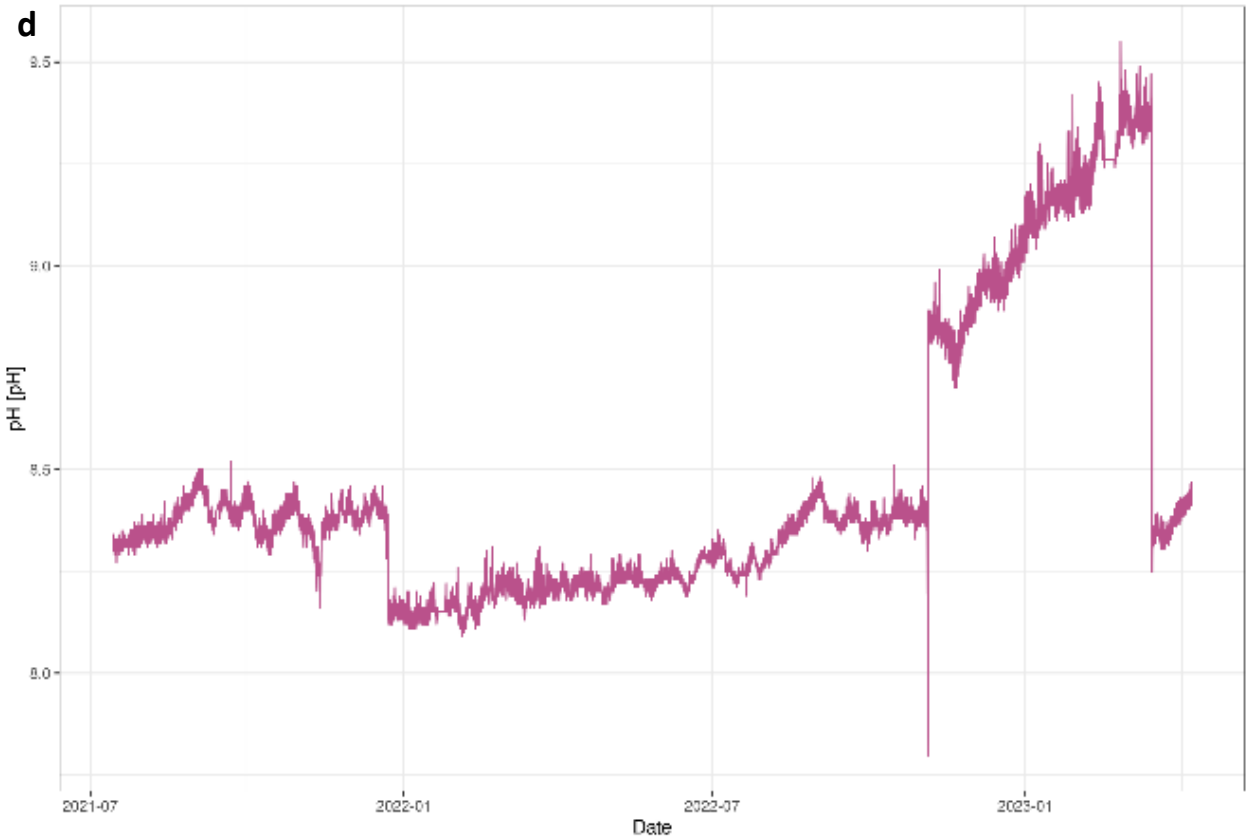

b
Otago Harbour Temperature [°C]
Temperature [°C]
20
15
10
2021-07
2022-01
2022-07
2023-01
Date

c Otago Harbour Salinity [psu]



Otago Harbour pH [pH]

d



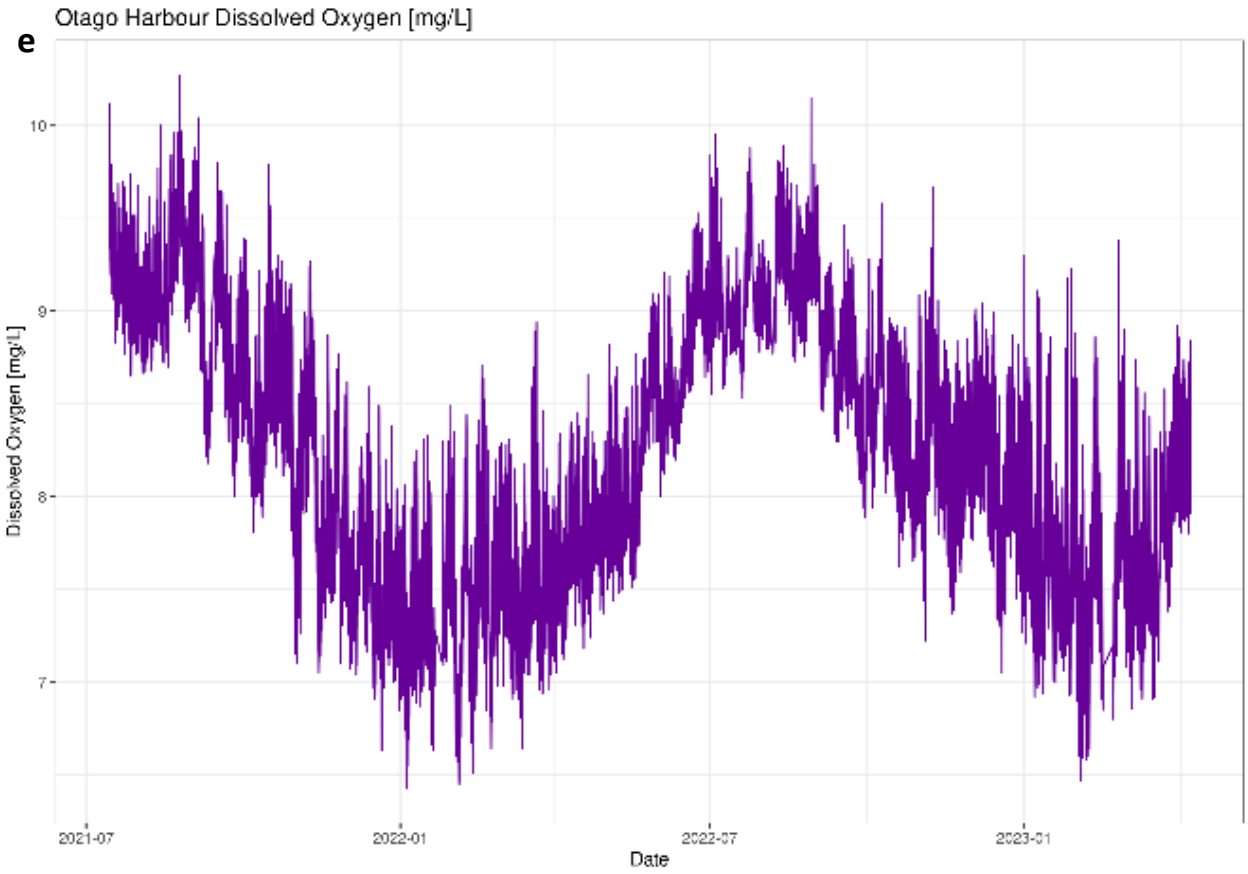


Figure 3.4. Time-series data of physical characteristics of the water at Portobello Marine Laboratory mid 2022 – early 2023 – Chlorophyll-*a* concentration (a), temperature (b), salinity (c), pH (d), dissolved oxygen (e), (Accessed from (https://harbourconditions.otago.ac.nz/)).

3.4 Discussion

3.4.1 Growth Rates

Overall, *Watersipora subatra* grows quite well in Otago Harbour. Taken together, the annual growth rate for these colonies was, on average: 0.143 mm/day (SD = 0.093, N = 5). Approximate calculations indicate that the annual growth rate for these colonies are 5.11 mm/y (Winter 2021), 110.59 mm/y (Summer 2022), 53.29 mm/y (Autumn 2022), 29.2 mm/y (Winter 2022), and 38.33 mm/y (Spring 2022).

Growth is often recorded as being lowest during winter for bryozoans and other encrusting and colonial organisms, and was an expected observation for the tyre colonies. Similarly, growth is recorded as being higher in summer months, as this period typically aligns with an abundance of resources and breeding seasons.

Compared to other bryozoans, *Watersipora subatra* has a relatively fast growth rate (Smith 2014), as can be expected from an invasive species. Only two other species have a comparable growth rate, *Membranipora membranacea* (37 to 4380 mm/y (Saunders & Metaxas 2009)), and *Einhornia crustulenta* (77.1 mm/y (Kuklinski et al. 2013)).

3.4.2 Population Characteristics

Presence of *Watersipora subatra* colonies on each of the four tyres varied over time. Tyre A never showed any populations of *W. subatra* over the 2021-2022 period. Tyre B possessed a population of *W. subatra* from January 2021 to June 2021. Tyre C showed populations of *W. subatra* from January 2021 to December 2021. Tyre D showed populations of *W. subatra* from January 2021 to December 2022. Tyres were at suspended in the water at different depths, tyre A was the shallowest, floating just below the surface, and regularly exposed during high tide, and tyre D was the deepest at roughly 5m below the surface. The lack of any *Watersipora subatra* on tyre A is interesting as the species is intertidal. It is possible they were outcompeted by more desiccation-tolerant fauna such as barnacles, which it was covered in during the 2021-2022 monitoring period. Tyre B showed the presence of *W. subatra* colonies for a short time, suggesting the colonies were able to encrust and establish before they were wiped out, either due to the shallowness of the tyre having a direct negative effect on colony survival, or being outcompeted by other fauna. Tyre C having colonies for a longer period than tyre B supports the effect of depth having a positive effect

on the survival of *W. subatra* colonies, as does tyre D having colonies over the entire 2021-2022 monitoring period, indicating this depth may be the most suitable.

Presence of colonies on each part of the tyres varied among yearly quarters. The sides of the tyres bore colonies for the entirety of the 2021-2022 monitoring period. The road-face section of the tyres only showed colonies during April-June 2021, and January-September 2022. The inside section of the tyres was colonised from July 2021 to June 2022. The outside surface of the tyres showed colonies for the entire 2021-2022 monitoring period, indicating that this region of the tyres is the most suitable for *Watersipora subatra*. The road-side face was colonised for shorter periods before they died out, for no clear reason. The inside section of the tyres was colonised for a short time. The inside section is more sheltered than other sections of the tyre, but the disappearance of these colonies may be attributed to being outcompeted or grazed upon by other fauna. Additionally, there did not seem to be any obvious patterns in the distribution on the tyre related to the shaded versus top surface.

Colouration of the *Watersipora subatra* colonies showed a small amount of variation. Colonies were a bright orange-red colour aside from during April-June 2021, when black colonies were observed. Variation in colony colouration can be explained by the health of the colonies as the time during which black colonies were observed also coincided with the mass die-off of the colonies from tyre B, as they eventually became grey-black and fell off. After this period, colonies became brighter, and the phenomenon was not observed again over the remainder of the 2021-2022 monitoring period.

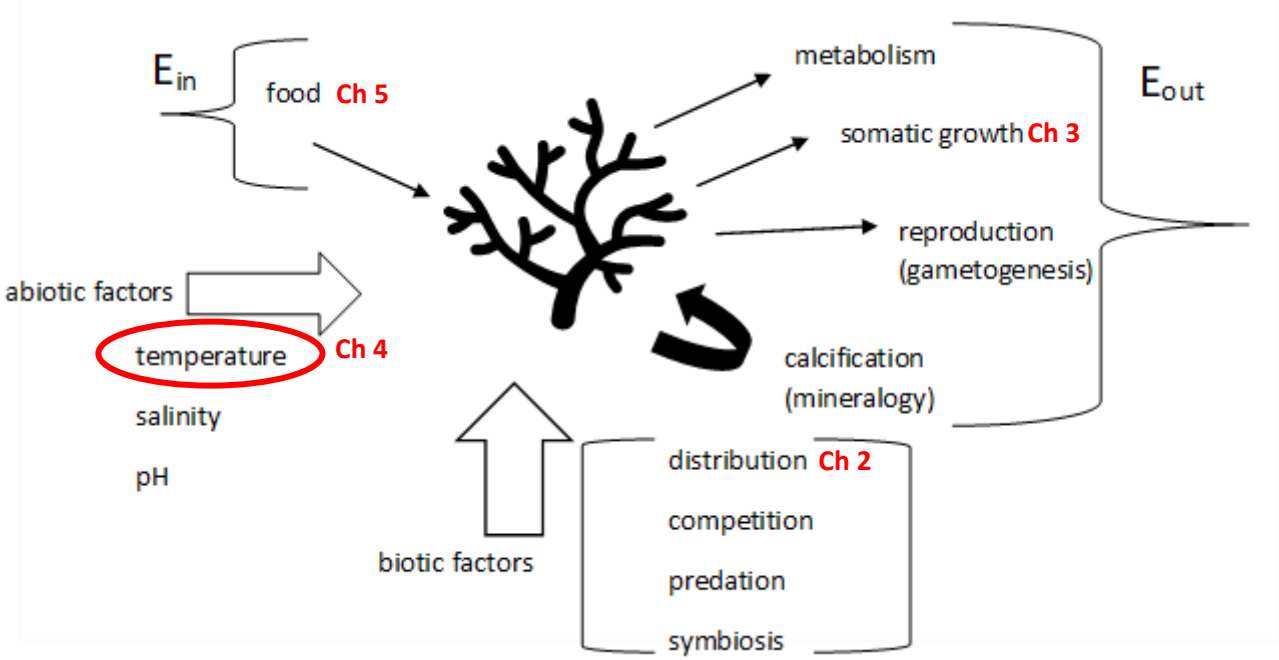
Shape of the *Watersipora subatra* colonies did not show any variation; for the entirety of the monitored period (January 2021 to December 2022) all colonies were round and laminar. There was no difference in colony shape among the tyres either. Colonies of bryozoans are

often observed to have different growth forms for various reasons. However, no variation was seen in the growth forms of the colonies observed over the 2021-2022 monitoring period.

3.5 Conclusion

Overall *Watersipora subatra* grows about 53 mm/y on average in Otago Harbour. Wild growth was higher in the summer months, as expected. Breeding in *W. subatra* also occurs around this time. Observed slower growth in the winter months is also expected. *W. subatra* has a relatively high growth rate among bryozoans. As they are an invasive species, this makes sense as they are capable of outcompeting other encrusting taxa. Our measurements of *W. subatra* growth overlap with other studies reporting similar growth rates. The collection of this long-term dataset detailing the growth of *W. subatra* across multiple seasons is valuable as it allows for a deeper understanding of their ecology, and how seasonality influences their growth, while also providing baseline data for future experiments involving *W. subatra* as a model organism.

CHAPTER FOUR: HEAT TOLERANCE TRIALS ON LOCAL BRYOZOA



4.1 Introduction and Aims

Of the many abiotic factors that influence growth in bryozoans, temperature tends to be one of the most commonly studied. Past research has shown Bryozoa having both positive and negative responses to increased heat (Menon 1972, Rodolpho-Metalpa et al. 2010, Ashton et al. 2017). Small increases in temperature have been observed to encourage growth in some bryozoan taxa, though large increases in temperature can push colonies beyond a critical limit and causes zooid mortality. *In-situ* experimentation has also shown that increased temperature can have a community-wide impact, with increased temperatures encouraging less-diverse bryozoan assemblages as the more robust, heat-tolerant taxa outcompete other species (Aston et al. 2017).

There is a benefit to performing *in-situ* experiments when exploring the influence of temperature as it allows for organisms to be studied in their natural environment without elaborately laboratory setup or disturbance (even more so for habitat-forming organisms such as Bryozoa), though there is the downside of much less control over other environmental variables that may influence the results. Nonetheless, Otago Harbour provides an excellent opportunity for this type of experimentation on local Bryozoa.

The aim for this chapter is to understand the effect of increased water temperature on growth of bryozoans found locally at Portobello. These results will contribute to the wider understanding of the effects of environmental change on bryozoan growth, something that is poorly studied when compared to other phyla. Specifically, we will examine the effect on temperature on growth rate, zooid morphology, and skeletal mineralogy.

4.2 Methods

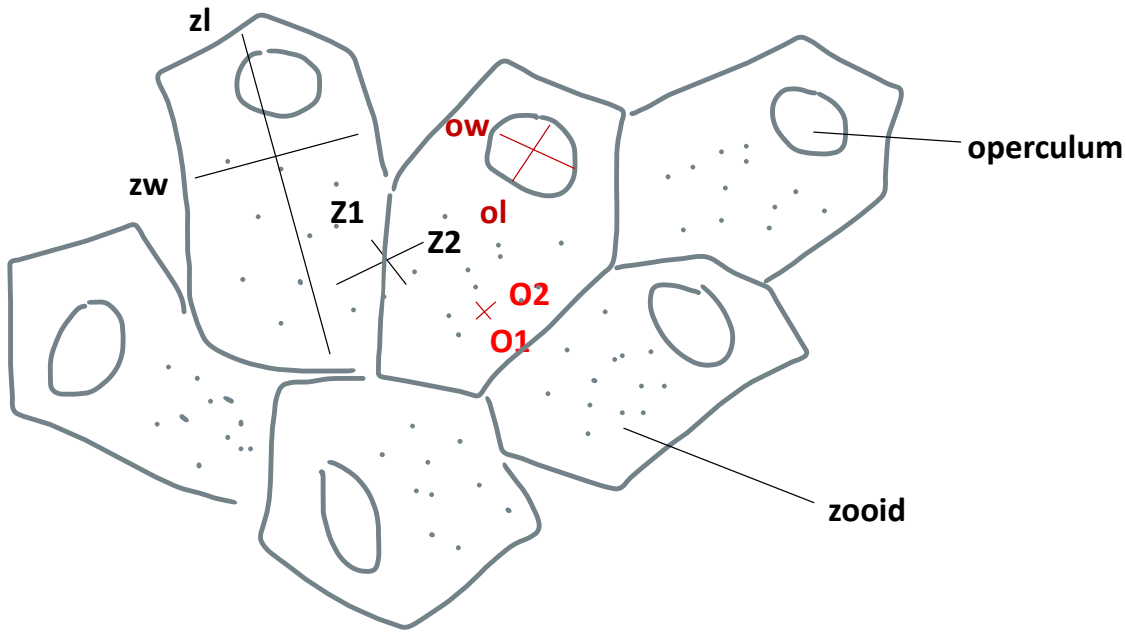
In order to examine the effect of temperature on growth rate in marine invertebrates of Otago Harbour, twelve 15cm^2 heat-able settling plates (as in Ashton et al. 2017) were cleaned and then bolted to the wharf at Portobello Marine Laboratory (-45.8278377, 170.6405168, 5m water depth). The plates were positioned away from direct sunlight and oriented to allow colonies to grow on either face. Three different temperature regimes were set: +1 degree, +2 degree, and a control, each with four plates assigned to the treatment. The plates were in the water for three months (January – March 2022) and monitored remotely to ensure they continued to operate during that time. The temperature treatments were chosen as they matched some climate projections of the time for near-future water temperature increases for the years 2050 (+1 degree), and 2100 (+2 degrees) (IPCC 2019), however the current rate of environmental change has rendered these predictions outdated, as the oceans appear to be reaching these increases sooner. Water temperatures of Otago Harbour were recorded at Portobello Marine Laboratory for the duration of these three months (Chapter 3, Figure 3.2).

Plates were photographed shortly after removal, and bryozoans were removed and photographed individually for identification. Species present and settlement density form part of another study and will not be considered here (Moffit, in prep).

At least 15 bryozoan colonies were collected from each treatment for growth rate analysis. Bryozoan colonies were weighed and measured before being placed in a 10% bleach solution for two hours to remove excess biological matter, and re-photographed under a microscope to analyse skeletal characteristics and identify species. Characteristics measured included zooid length, zooid width, operculum length, and operculum width (Figure 4.1).

Beania sp. presented a unique issue in that each zooid is connected to two neighbouring zooids with short tubes, these were not included in zooid measurements.

a



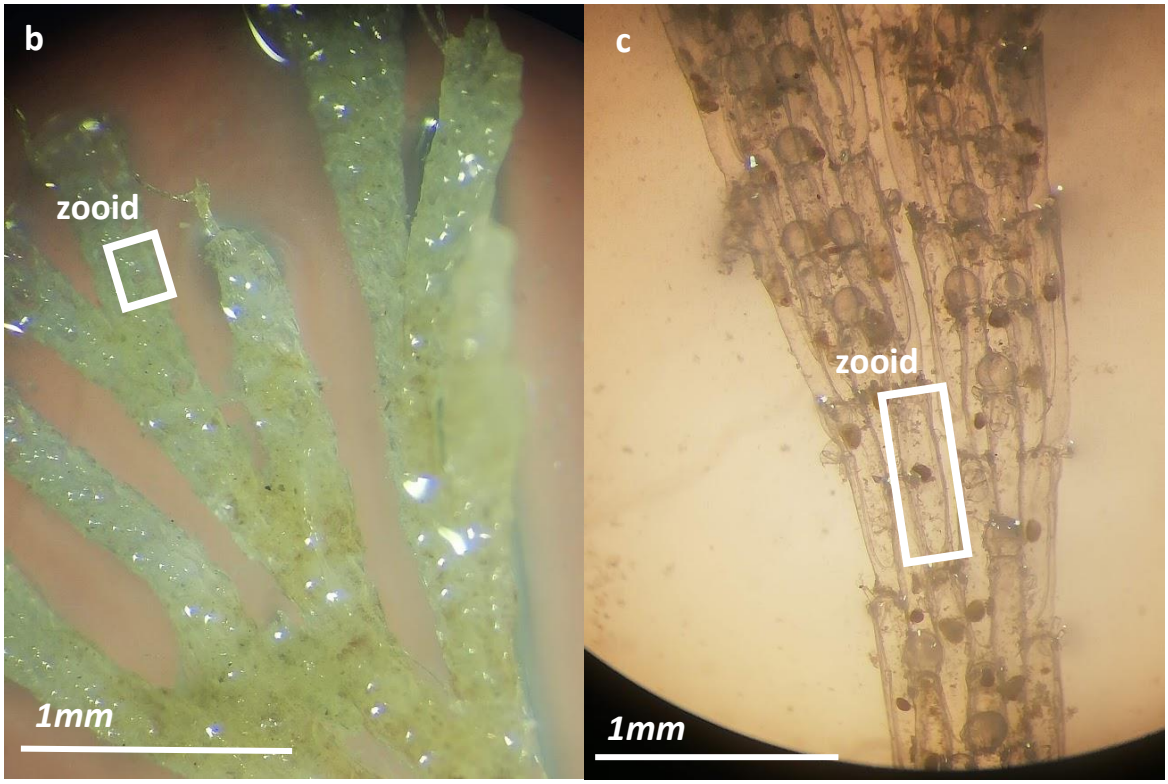
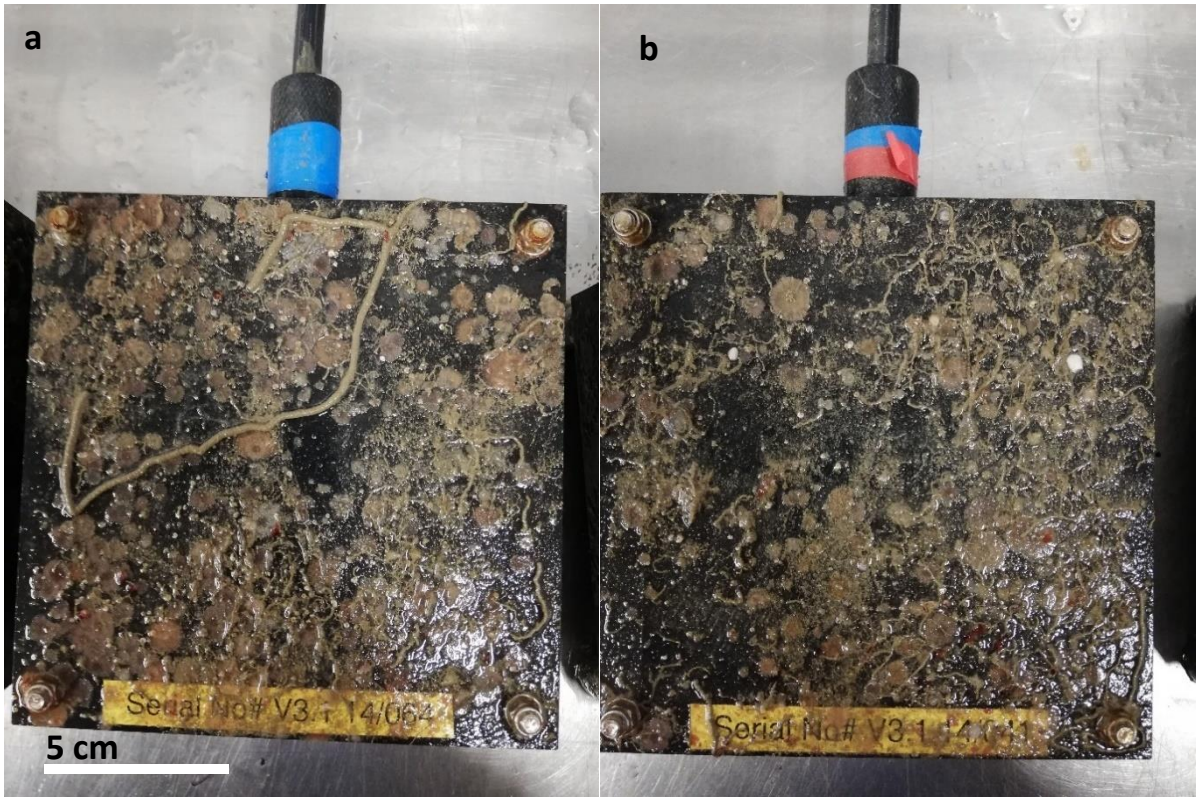


Figure 4.1. Line drawing showing (a) measurements of zooid (zl, zw) and operculum (ol, ow) lengths and widths on a generalised representation of a bryozoan colony, (b) a magnified colony of *Caberea zelandica*, and (c) a magnified colony of *Bugulina flabellata*.

From these samples (Figure 4.2), bleached colonies with enough skeletal material, and controls taken from the tyre colonies from Portobello Marine Lab were ground to a fine powder with a small amount of pure mineral halite in an agate mortar and pestle. The ground samples were then analysed at the University of Auckland's Chemistry Department PANalytical Empyrean X-Ray diffractometer (XRD) (Figure 4.3). Eighteen samples were run in total, seventeen long scans, and one short scan focused from 25 – 32 °2Θ (in this context theta symbolises the angle of diffraction). Fifteen were controls in the species *Bugulina flabellata* (N=7) and *Caberea zelandica* (N=8). One sample of *C. zelandica* from the heated +1 plates, and two samples of *C. zelandica* from the heated +2 plates made up the remainder of the samples. Material of *Beania* sp. and *Bugulina flabellata* from the heated

plates was of an insufficient volume to read correctly in the XRD. Calcite and halite peaks were used to find the wt% $MgCO_3$ in calcite.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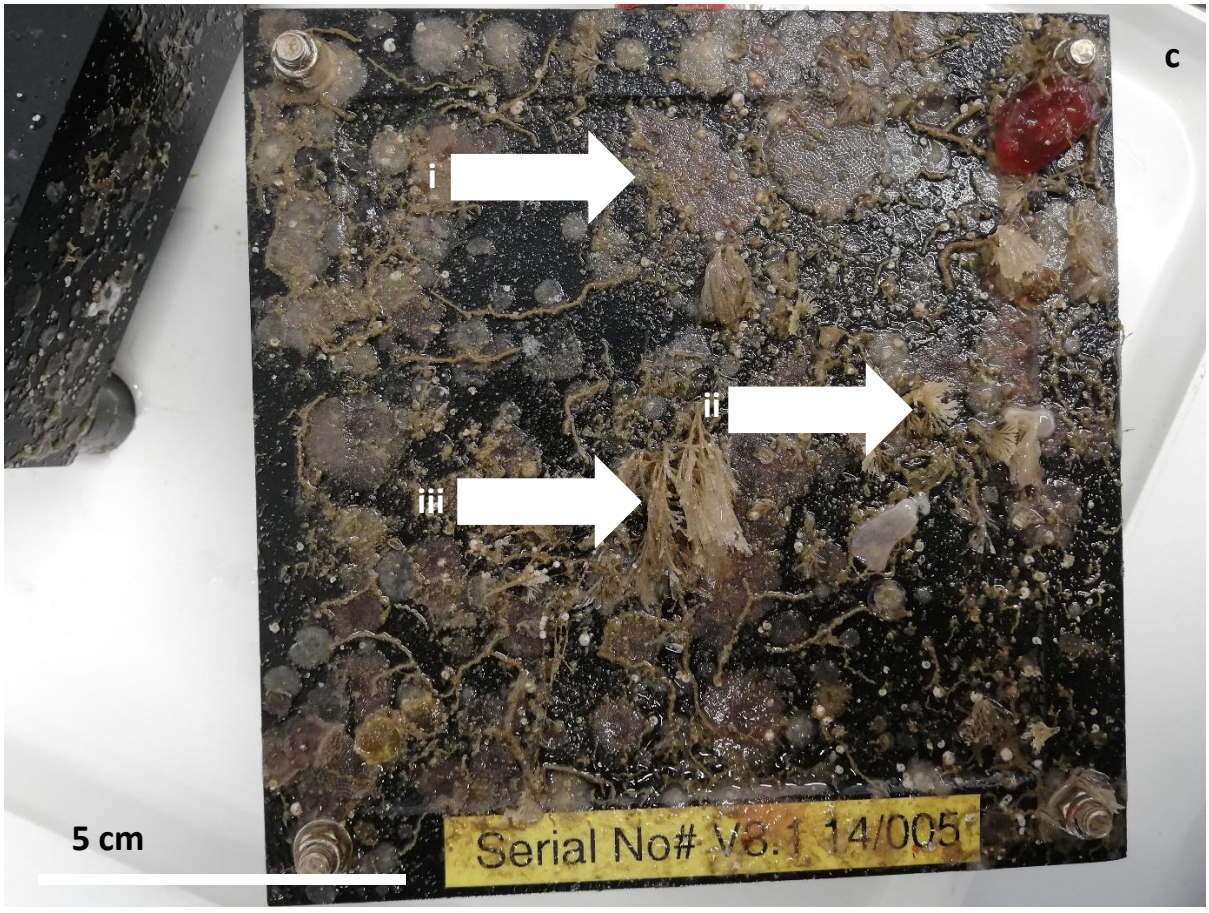

c
i
ii
iii
5 cm
Serial No# V3.1 14/005

Figure 4.2. Experimental settling plates showing colonies prior to sampling. (a) control plate at ambient temperature; (b) a plate held at +2° above ambient temperature for three months; (c) a plate showing *Beania* sp (i), *Caberea zelandica* (ii), and *Bugulina flabellata* (iii).

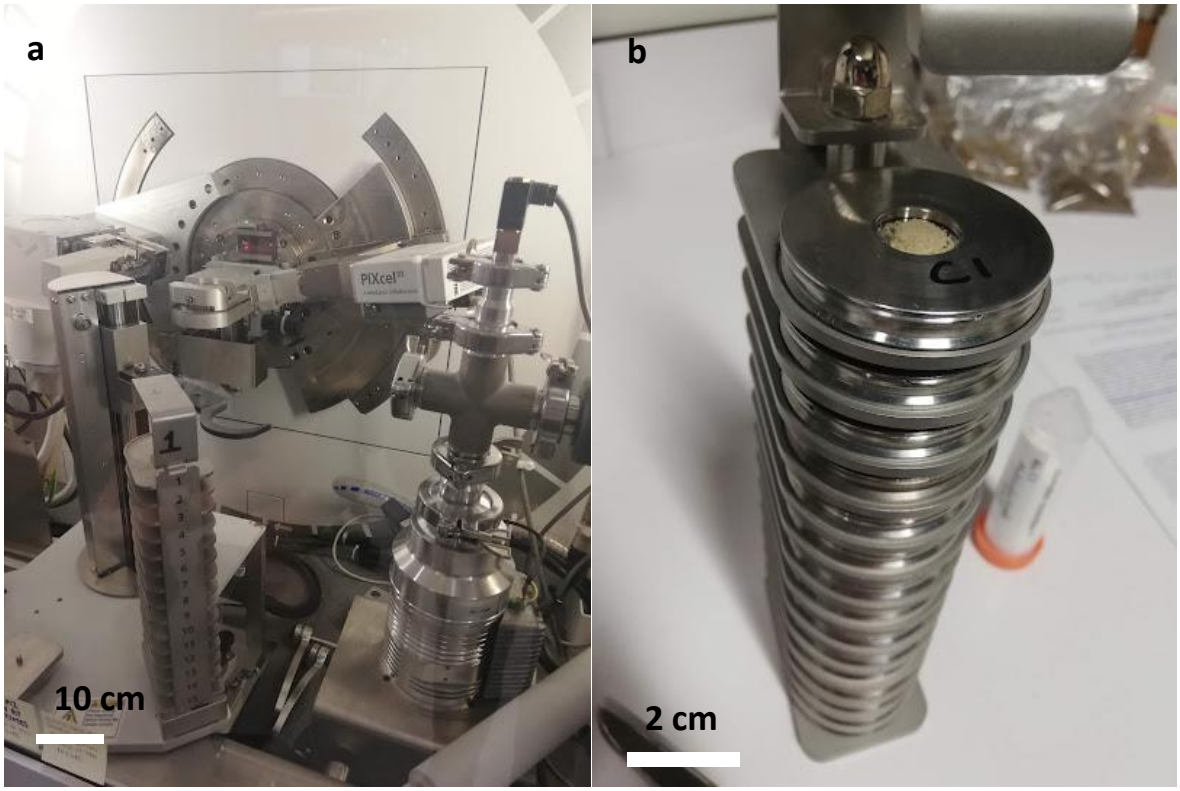


Figure 4.3. X-Ray diffractometry (XRD) of ground samples (a) the instrument and (b) powdered samples lined up prior to analysis. Powdered samples of bryozoan material were placed into the discs (b) and arranged in stacks before being inserted into the apparatus (a) and analysed.

4.3 Results

4.3.1 Effect of temperature on bryozoan colony characteristics

Results of the ANOVA run on the data revealed the area of *Beania* sp. colonies ranged from 2.795 mm^2 in the control treatment to 30.816 mm^2 in the +2° treatment. Dry weight varied

from 0.002 g in the control treatment to 0.021 g in the +2 degrees treatment. Wet weight ranged from 0.004 g in the control treatment to 0.040 g in the +2 degrees treatment. Diameter varied from 1.77 mm in the control treatment to 19.61 mm in the +2 degrees treatment. A significant difference was found when comparing means of *Beania* sp. dry weight, wet weight, area, and diameter among heat treatments (Table 4.1, Figure 4.4).

Area of *Bugulina flabellata* colonies ranged from 13.23 mm^2 in the +2 degrees treatment to 27.02 mm^2 also in the +2 degrees treatment, there was no significant difference among means from any of the treatments. Wet weight varied from 0.014 g in the +2 degrees treatment to 0.033 g in the +2 degrees treatment, there was also no significant difference among means from any of the treatments. Dry weight ranged from 0.002 g in the +2 degrees treatment to 0.06 g also in the +2 degrees treatment, there was no significant difference among means from any of the treatments. Diameter varied from 8.4 mm in the +2 degrees treatment to 13.1 mm also in the +2 degrees treatment, though there was also no significant difference among means from any of the treatments (Table 4.1, Figure 4.4).

Similarly to *B.* flabellata, there was no significant difference among means from any of the *Caberea zelandica* treatments for area, weight, or diameter. (Table 4.1, Figure 4.4).

Table 4.1. P-values of statistical analyses (ANOVA) testing for relationships among the means of different colony parameters, zooid parameters, and composition with temperature for three species of bryozoan grown under different heat treatments over three months at Portobello Marine Laboratory, Dunedin, New Zealand. Significant results are highlighted blue, and nearly statistically significant results are highlighted in yellow. (Raw data in Appendix 1) As *B. flabellata* and *C. zelandica* are erect, branching species, colony area and diameter are a coarse measure due to potential overlap (highlighted in green).

	Beania sp.	*Bugulina flabellata*	*Caberea zelandica*
Colony Parameters			
dry weight	0.023 (2df)	0.739 (2df)	0.640 (2df)
wet weight	0.012 (2df)	0.830 (2df)	0.380 (2df)
area	0.017 (2df)	0.820 (2df)	0.540 (2df)
diameter	0.021 (2df)	0.739 (2df)	0.640 (2df)
Zooid Parameters			
zooid length	0.082 (2df)	0.735 (2df)	n/a
zooid width	0.111 (2df)	0.510 (2df)	n/a
zooid area	0.080 (2df)	0.652 (2df)	n/a
operculum length	0.001 (2df)	n/a	n/a
operculum width	0.002 (2df)	n/a	n/a
operculum area	0.001 (2df)	n/a	n/a
Composition			
Wt% $MgCO_3$ in calcite (y = 30x - 882)	n/a	n/a	0.001 (2df)

Table 4.2. Results of post-hoc testing on results of ANOVA testing for relationships among the means of different colony parameters, zooid parameters, and composition with temperature for three species of bryozoan grown under different heat treatments over three months at Portobello Marine Laboratory, Dunedin, New Zealand. Significant results are highlighted blue.

	Group Pairs	Absolute Difference	Standard Error	q Score
***Beania* sp. area**				
	Control vs Heated +1°C	14.76	4.73	3.12
	Control vs Heated +2°C	22.54	4.73	4.76
	Heated +1°C vs Heated +2°C	7.78	4.73	1.64
***Beania* sp. dry weight**				
	Control vs Heated +1°C	0.02	0.01	2.02
	Control vs Heated +2°C	0.05	0.01	5.46
	Heated +1°C vs Heated +2°C	0.03	0.01	3.44
***Beania* sp. wet weight**				
	Control vs Heated +1°C	0.01	0.00	3.04
	Control vs Heated +2°C	0.02	0.00	5.16
	Heated +1°C vs Heated +2°C	0.01	0.00	2.13
***Beania* sp. diameter**				
	Control vs Heated +1°C	9.17	2.94	3.12
	Control vs Heated +2°C	14.35	2.94	4.87
	Heated +1°C vs Heated +2°C	5.17	2.94	1.76

***Beania* sp. operculum length**	Control vs Heated +1°C	0.04	0.01	5.81
	Control vs Heated +2°C	0.05	0.01	8.17
	Heated +1°C vs Heated +2°C	0.02	0.01	2.36
***Beania* sp. operculum width**	Control vs Heated +1°C	0.03	0.01	3.65
	Control vs Heated +2°C	0.06	0.01	6.44
	Heated +1°C vs Heated +2°C	0.02	0.01	2.78
***Beania* sp. operculum area**	Control vs Heated +1°C	0.01	0.00	7.32
	Control vs Heated +2°C	0.01	0.00	9.42
	Heated +1°C vs Heated +2°C	0.00	0.00	2.09
***Caberea zelandica* Wt% MgCO3 in calcite (y = 30x - 882)**	Control vs Heated +1°C	4.05	0.48	8.50
	Control vs Heated +2°C	2.30	0.48	4.83
	Heated +1°C vs Heated +2°C	1.75	0.48	3.67

4.3.2 Effect of temperature on bryozoan zooid characteristics

Operculum length showed a significant difference among the three treatments in *Beania* sp. (Table 4.1). Operculum length of *Beania* in the control treatment was 0.09 mm, and the lowest of the three treatments. Operculum length of *Beania* in the +1 degree treatments was approximately 0.13 mm. Operculum length of *Beania* in the +2 degree treatment ranged from below 0.12mm to just over 0.16mm, and had the greatest range of the three treatments. Similarly, operculum width also showed a significant difference among the three treatments in *Beania* sp. (Table 4.1). Operculum width was lowest in the control treatment at around 0.12mm. In the +1 degree treatment operculum width varied between roughly 0.14mm to over 0.18mm. The +2 degree treatment also showed variation among zooids, with operculum widths ranging from approximately 0.16mm to almost 0.19mm. Operculum area was lowest in the control treatment (0.010 mm^2), and also showed a significant

difference (Table 4.1). The +1 degree treatment showed variation in operculum area, ranging from 0.017 to 0.024 mm^2. The +2 degree treatment showed wider variation in operculum area, ranging from 0.021 to almost 0.030 mm^2 (Table 4.1,

Figure 4.5).

In the control treatment average zooid length was approximately 0.61 mm. The +1 degree treatment showed variation in zooid length, ranging from just over 0.48 to 0.59 mm, and an average length of 0.535 mm. The +2 degree treatment also displayed variation in zooid length, as measurements ranged from 0.57 to 0.635 mm, with an average of 0.61 – the same as the control treatment. Similarly, zooid width also showed no significant difference among the three treatments in *Beania*. The control treatment had an average zooid width of 0.41mm. The +1 degree treatment ranged from 0.30 to 0.41mm, with an average width of 0.355mm. The +2 degree treatment ranged from 0.39 to 0.455 mm, and had an average of 0.415mm. Zooid area in the control treatment had an average of 0.245 mm^2. In the +1 degree treatment it ranged from 0.15 to just below 0.245 mm^2. The +2 degree treatment also varied, ranging between 0.23 and 0.27 mm^2, with an average area of approximately 0.245 mm^2 (Table 4.1, Figure 4.5).

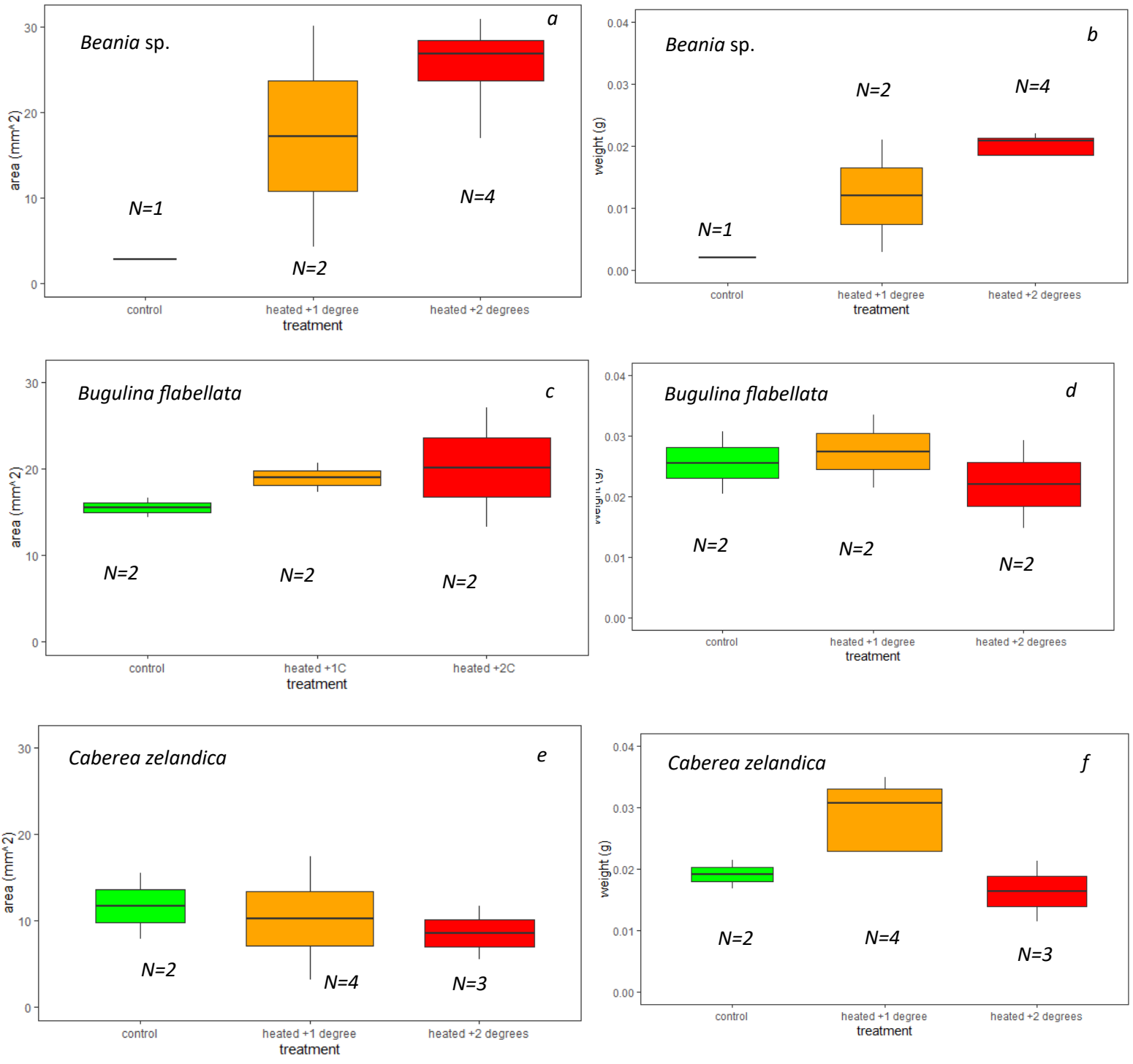
Zooid length showed no significant difference among the three treatments in *Bugulina flabellata.* The control treatment varied from 0.6 to 0.9 mm. The +1 degree treatment varied between 0.71 and 1.12 mm. Similarly, zooid width also showed no significant difference among the three treatments in *Bugulina flabellata*. Zooid width of *Bugulina flabellata* varied among the three treatments. The control treatment varied between 0.18 and 0.216 mm. The +1 degree treatment ranged from 0.19 to 0.25 mm. The +2 degree treatment ranged from 0.19 to 0.199 mm. Zooid area showed no significant difference among the three treatments

in *Bugulina flabellata*. Zooid area was also seen to vary among the three treatments in *Bugulina flabellata*. The control treatment ranged from just over 0.110 to 0.195 mm^2. The +1 degree treatment ranged from 0.149 to 0.290 mm^2. The +2 degree treatment was clustered around 0.16 mm^2 (Table 4.1, Figure 4.5).

4.3.3 Effect of temperature on bryozoan mineralogy

Magnesium content in calcite varied from 2.7 to as high as 6.2 wt% $MgCO_3$ in calcite in *Bugulina flabellata*, and varied from 1.1 to 7.1 wt% $MgCO_3$ in calcite in *Caberea zelandica*. Skeletal mineralogy in *C. zelandica* varied across the three treatments, and was found to have a significant difference (Table 4.1). In the control treatment it ranged from 2.7 to 7.1 wt% $MgCO_3$ in calcite, with an average of 5.5 wt% $MgCO_3$ in calcite. In the +1 degree treatment it had an average of 1.1 wt% $MgCO_3$ in calcite. In the +2 degree treatment it ranged from 2.0 to 3.7 wt% $MgCO_3$ in calcite (Figure 4.6, Table 4.2).

Water temperature ranged from 20.1 to 14.8 degrees Celsius from January to March of 2022, it was highest in January and decreased over time, reaching its lowest of the three months in March (Figure 4.7).



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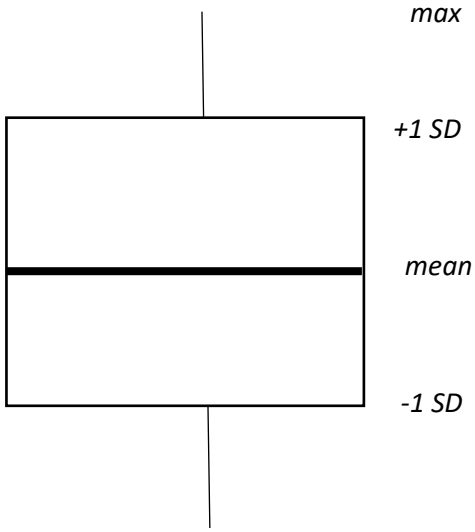


Figure 4.4. Plots showing area (a) and weight (b) of *Beania* sp colonies grown under different heat treatments over three months, area (c) and weight (d) of *Bugulina flabellata* colonies grown under different heat treatments over three months, area (e) and weight (f) of *Caberea zelandica* colonies grown under different heat treatments over three months at Portobello Marine Laboratory, New Zealand.

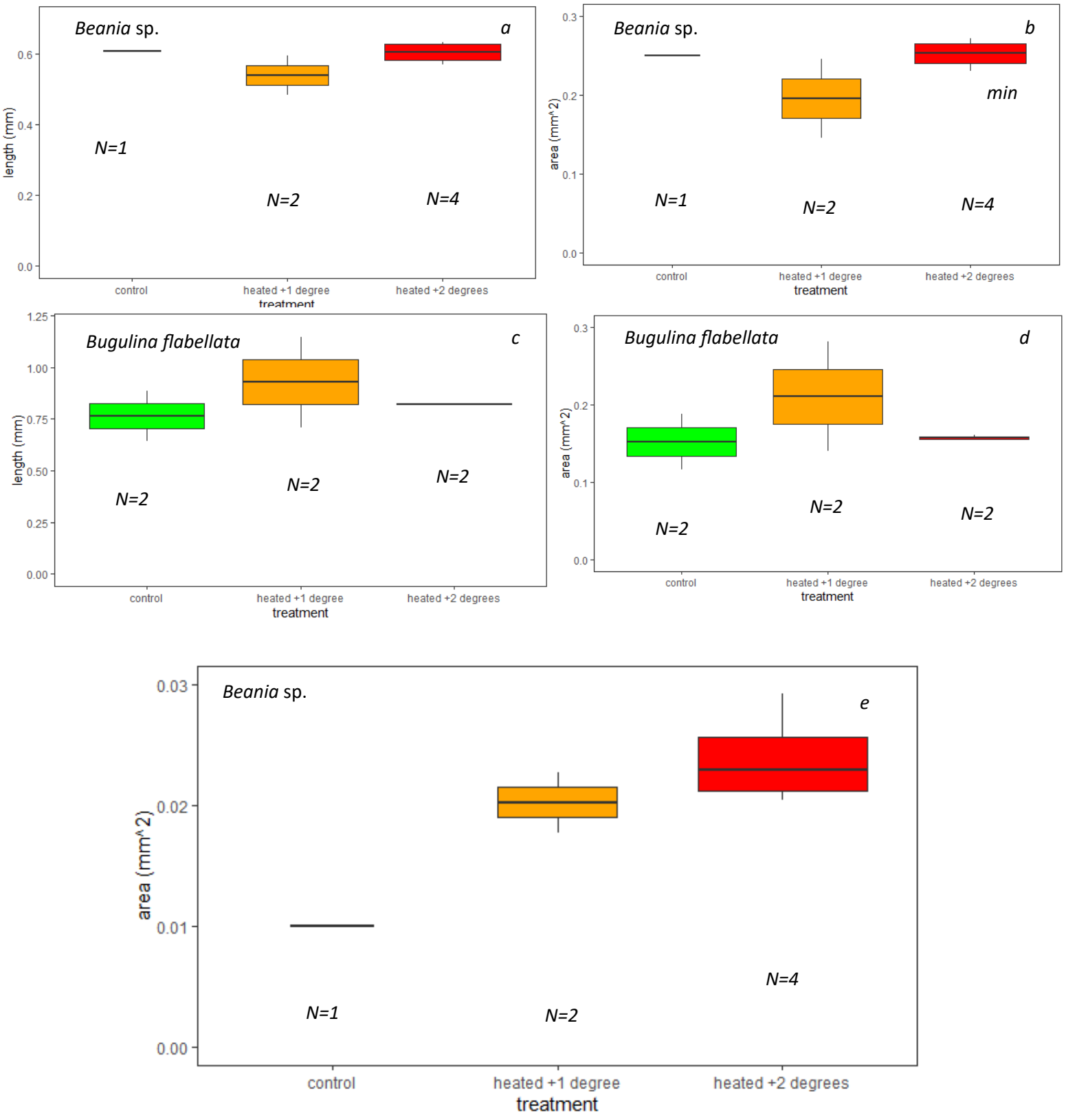


Figure 4.5. Plots showing zooid length (a) zooid area (b) of *Beania* sp. colonies grown under different heat treatments over three months, zooid length (c) and area (d) of *Bugulina flabellata* colonies grown under different heat treatments over three months, operculum area of *Beania* sp. colonies grown under different heat treatments over three months is also shown (e).

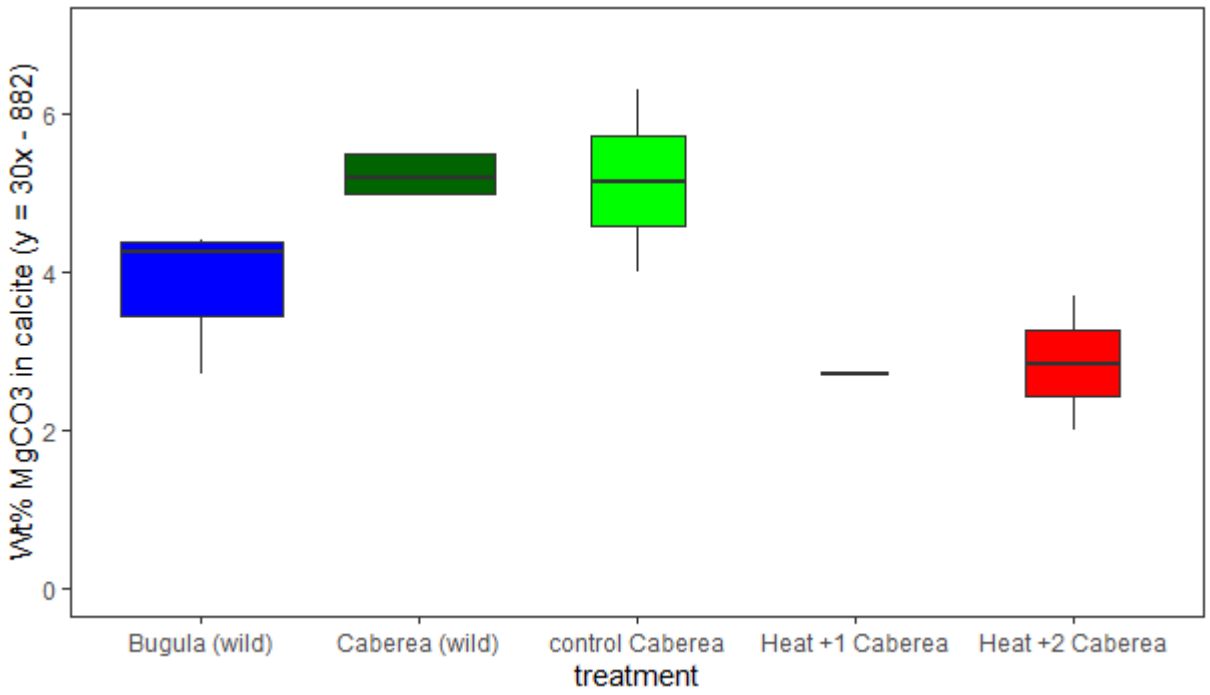


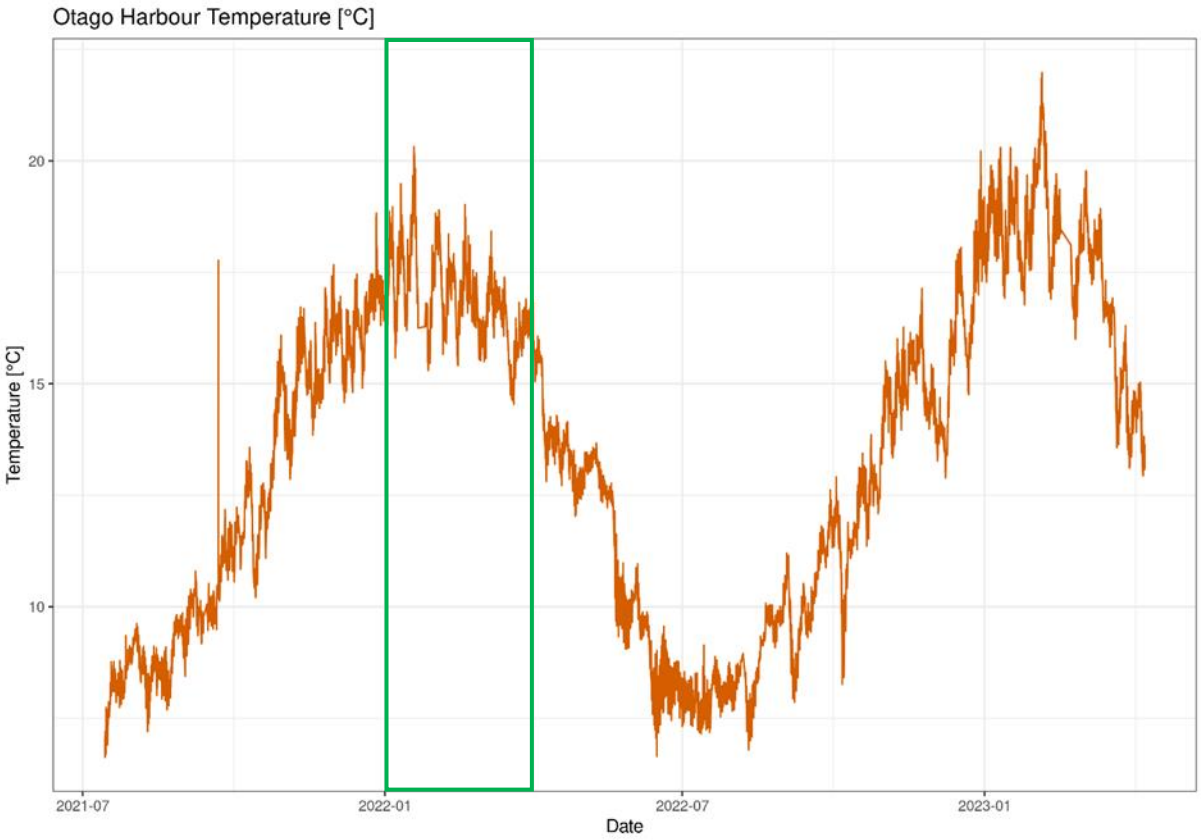
Figure 4.6. Plot showing composition of *Caberea zelandica* colonies grown under different heat treatments over three months at Portobello Marine Laboratory and composition of wild *Bugulina flabellata* and *Caberea zelandica* colonies collected at Portobello Marine Laboratory, New Zealand in Summer 2022

Table 4.2. Skeletal carbonate analysis by XRD in *Bugulina flabellata* and *Caberea zelandica*, both wild collections and colonies grown under different temperatures over three months at Portobello Marine Laboratory, Dunedin, New Zealand.

Species	Colony ID	Sample origin	Mineralogy	Wt% $MgCO_3$ in calcite (y = 30x - 882)
Bugulina flabellata	1	PML Wharf	IMC	4.2
Bugulina flabellata	2	PML Wharf	LMC	3.2
Bugulina flabellata	3	PML Wharf	IMC	4.3
Bugulina flabellata	4	PML Wharf	LMC	2.7
Bugulina flabellata	5	PML Wharf	IMC	6.2
Bugulina flabellata	6	PML Wharf	IMC	4.4
Caberea zelandica	7	PML Wharf	IMC	4.0
Caberea zelandica	8	PML Wharf	IMC	6.3
Caberea zelandica	9	PML Wharf	IMC	5.5
Caberea zelandica	10	PML Wharf	IMC	5.0

Caberea zelandica	11	PML Wharf	IMC	5.2
Caberea zelandica	12	PML Wharf	IMC	7.1
Caberea zelandica	13	PML Wharf	LMC	3.1
Caberea zelandica	14	PML Wharf	LMC	2.7
Caberea zelandica	15	Heated Plate +1 degree	LMC	1.1
Caberea zelandica	16	Heated Plate +2 degrees	LMC	3.7
Caberea zelandica	17	Heated Plate +2 degrees	LMC	2.0

Figure 4.7. Temperature record of Otago Harbour at Portobello Marine Laboratory from late 2021 to early 2023, the time period of this chapter's experiment is highlighted in green (January – March 2022).



4.4 Discussion

4.4.1 Effect of Temperature on Growth and Calcification

Beania sp. showed a significant difference among mean weight, area, and diameter (Table 4.1). *Bugulina flabellata*, and *Caberea zelandica* showed no significant changes in colony weight and size when exposed to increased temperatures. There was no significant relationship found among colony characteristics and temperature treatment. It is possible that small increases of temperature (+1 degree, +2 degrees) don't reaching the upper thermal limit in either species, so that there was no response at the colony level.

Other studies have found that colony growth in bryozoans can be both positively and negatively affected by temperature, though there appears to be a trend with invasive species showing either a greater resistance to temperature increase, or a benefit from it (Barnes et al. 2006; Amui-Vedel et al. 2007; Lord 2017).

However, there are also many reports of bryozoans being negatively impacted by increased temperatures. *Cellaporella hyalina* reportedly forms smaller zooids at higher temperatures (Hunter & Hughes 1994), *Celleporaria nodulosa* displays reduced growth at high temperatures (Durrant et al. 2013), *Myriapora truncata* displays necrosis at high temperatures (Rodolpho-Metalpa et al. 2010), and *Pentapora fascialis* also experiences necrosis at high temperatures (Pagès-Escolà et al. 2018).

Given that the size of colonies from the plates was recorded, and that at most they grew for three months, growth rate can be calculated for these colonies and compared to other growth rates across the literature (Table 4.3). Assuming a growing period of three months, the three taxa possess a very fast growth rate when compared to other bryozoans (Smith 2014), though there seems to be little variation among the three taxa, or among the heat treatments (Table 4.3). *Bugulina flabellata* is an invasive species and will tend to have a high growth rate (Wang et al. 2015; Souto et al 2018).

Table 4.3. Growth rates of colonies found on the plates calculated over a growing period of three months.

treatment	taxon	growth rate (mm/day)	growth rate (mm/month)
Control	*Beania* sp.	0.02	0.64
Heated +1°C	*Beania* sp.	0.22	6.61
Heated +2°C	*Beania* sp.	0.03	0.79
Heated +2°C	*Beania* sp.	0.05	1.57
Heated +2°C	*Beania* sp.	0.07	2.12
Heated +2°C	*Beania* sp.	0.06	1.94
Control	*Bugulina flabellata*	0.05	1.56
Control	*Bugulina flabellata*	0.05	1.45
Heated +1°C	*Bugulina flabellata*	0.05	1.58
Heated +1°C	*Bugulina flabellata*	0.06	1.73
Heated +2°C	*Bugulina flabellata*	0.07	1.98
Heated +2°C	*Bugulina flabellata*	0.05	1.39
Control	*Caberea zelandica*	0.05	1.50
Control	*Caberea zelandica*	0.04	1.07
Heated +1°C	*Caberea zelandica*	0.04	1.32
Heated +1°C	*Caberea zelandica*	0.04	1.11
Heated +1°C	*Caberea zelandica*	0.02	0.67
Heated +1°C	*Caberea zelandica*	0.05	1.59
Heated +2°C	*Caberea zelandica*	0.03	0.90
Heated +2°C	*Caberea zelandica*	0.04	1.30
Heated +2°C	*Caberea zelandica*	0.04	1.11

Similarly, as the weights of colonies was recorded, the calcification rate can be calculated for several colonies from the plates as well (Table 4.4). These results are unusual compared to other New Zealand Bryozoa, as the calcification rate appears to be very slow (Smith & Nelson 1994).

Table 4.4. Calcification rates of *Caberea zelandica* samples from the heated plates.

treatment	taxon	dry weight	Wt% $MgCO_3$ in calcite (y = 30x - 882)	calcification rate (mg $CaCO_3$ y^{-1})
Heated +1°C	*Caberea zelandica*	0.011794657	1.1	0.002133923
Heated +2°C	*Caberea zelandica*	0.008143414	3.7	0.004955745
Heated +2°C	*Caberea zelandica*	0.006137791	2	0.00201903

4.4.2 Effect of Temperature on Zooid morphology in bryozoans

Significant morphological changes were observed in the zooid length, zooid area, operculum length, operculum width, and operculum area in *Beania* sp (Table 4.1). No significance was found in either *Bugulina flabellata* or *Caberea zelandica* (Table 4.1). The lack of morphological change indicates that these species may tolerate the increases in temperature experienced in this experiment, and that a greater temperature increase is required to induce changes in zooid morphology.

Zooid morphology has previously been used to predict or indicate temperature, particularly in fossil Cheilostomes as the size of individual zooids within the colony has been thought to be dependent on the ambient temperature during formation. Colonies with a low variation in zooid morphology suggest low levels of seasonality, whereas a high seasonality is suggested by colonies with a high level of zooidal variation, this is known as MART (Mean Annual Range of Temperature) analysis (Ishimura et al. 2008; Okamura et al. 2011; McClelland et al. 2014).

Observations of this phenomenon have been documented across the literature (Menon 1972; Hunter & Hughes 1994; O'Dea & Okamura 1999; Lombardi et al. 2006), though there is some criticism of MART analysis (O'Dea & Okamura 1999; Okamura et al. 2011; McClelland et al. 2014), specifically that other factors can influence zooid morphology, and the analysis having relatively low predictive power (McClelland et al. 2014). Assuming MART analysis worked consistently in the study species, then some greater variation in zooid morphology would presumably have been observed across the three taxa from the heated plates in this study.

4.4.3 Effect of temperature on skeletal carbonate mineralogy in bryozoans

A negative relationship between temperature and wt% $MgCO_3$ in calcite in *Caberea zelandica* was observed, with statistical analyses concluding there was a significant difference among the means of the different treatments (Table 4.2). When analysing wild colonies collected at Portobello, wt% $MgCO_3$ in calcite appeared to be higher in *Caberea zelandica* than *Bugulina flabellata*.

There is a considerable literature proposing that Mg in bryozoan calcite is strongly affected by water temperature (Rodolpho-Metalpa et al. 2010; Swezey et al. 2017; Pagès-Escolà et al. 2018), our observations support this as *Caberea zelandica* showed significant variation in Mg when exposed to raised temperatures. It is possible that both *Beania* sp. and *Bugulina flabellata* are relatively robust to temperature changes, and that the increase in temperature (+1°C, +2°C) was not high enough to elicit a response from those taxa. It should also be mentioned that previous studies have reported *C. zelandica* is an LMC species, with about 3 wt% $MgCO_3$ on average (Smith et al. 2006). Additionally, *Bugulina* species are typically reported as IMC species with around 6-8 wt% $MgCO_3$ (Table 4.5).

Table 4.5. Summary table of MgCO3 for bryozoan taxa, modified from Smith (2014). Taxa of note to this study are in bold.

Taxon	Mean Wt% MgCO3 in Calcite (Dominant)	Reference(s)
Adeonellopsis wetherelli	2.9	Taylor et al. 2009
Arachnopusia columnaris	3.9	Taylor et al. 2009
Bugula californica	**6**	**Rucker & Carver 1969**
Bugula dentata	**6.3**	**Smith et al. 1998**
Bugula pacifica	**4**	**Borisenko & Gontar 1991**
Bugula simplex	**6.5**	**Rucker & Carver 1969, Schopf & Manheim 1967**
Bugula spicata	**6.3**	**Poluzzi & Sartori 1974**
Bugula turrita	**10.2**	**Clarke & Wheeler 1922**
Caberea boryi	**5.9**	**Poluzzi & Sartori 1973, 1974**
Caberea ellisi	**6**	**Borisenko & Gontar 1991, Rucker & Carver 1969**
Caberea rostrata	**6**	**Crowley & Taylor 2000**
Caberea zelandica	**2.7**	**Smith et al. 1998**
Callopora aurita	4.8	Taylor et al. 2009
Calpensia cellairoides	3	Taylor et al. 2009
Cellaria immersa	2.1	Smith et al. 1998
Celleporaria agglutinans	5.2	Smith et al. 1998
Chondriovelum adeliensis	4.5	Taylor et al. 2009
Electra pilosa	8.6	Rucker & Carver 1969, Taylor et al. 2009
Hornera foliacea	2.7	Smith et al. 1998
Membranipora hastingsae	6	Rucker & Carver 1969
Microporella umbonata	6	Rucker & Carver 1969
Onchoporella buskii	7.9	Taylor et al. 2009
Onychocella alveolata	2.2	Taylor et al. 2009
Parasmittina trispinosa	5.7	Taylor et al. 2009
Reteporella beaniana	4.9	Taylor et al. 2009
Reteporellina denticulata	7.8	Taylor et al. 2009
Telopora buskii	2.8	Smith et al. 1998
Turritigera cribrata	5.9	Taylor et al. 2009
Umbonula littoralis	5.6	Taylor et al. 2009
Volviflustrellaria volvox	3	Taylor et al. 2009
Watersipora cucullata	**3.5**	**Taylor et al. 2009**
Watersipora subovoidea	**2**	**Rucker & Carver 1969**
Watersipora subtorquata*	**3.7**	**Unpublished (Smith)**

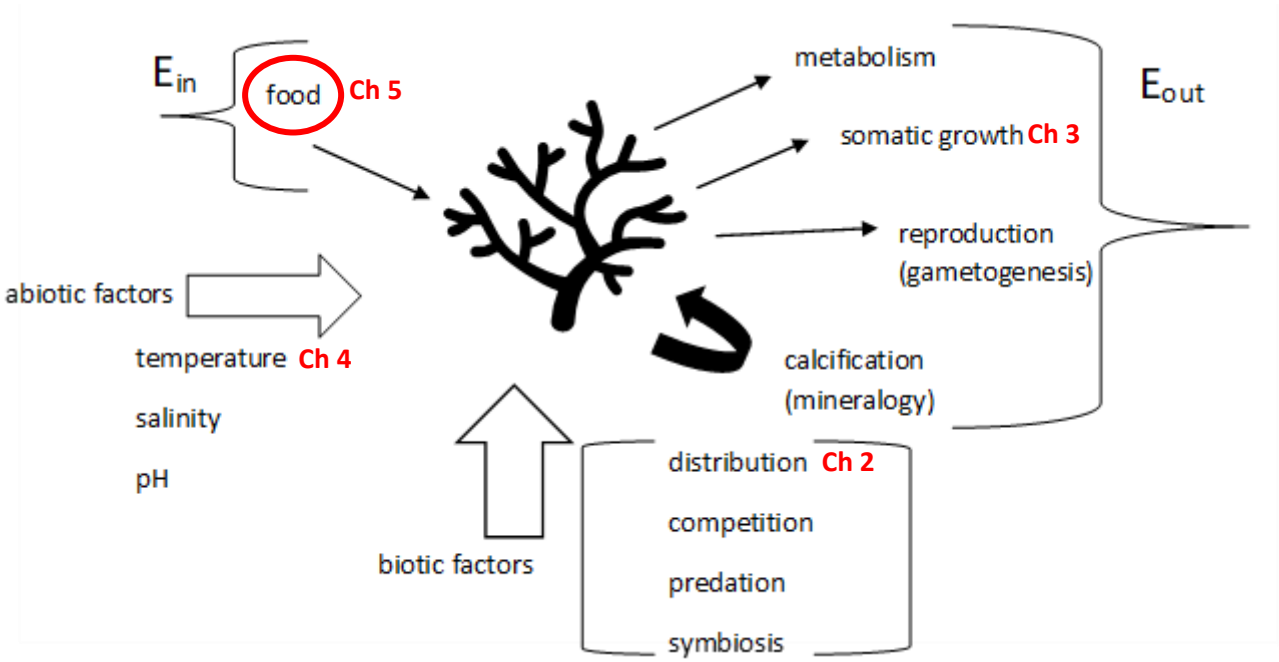
4.5 Importance

Here I have reported on a few bryozoans that were subjected to artificial *in-situ* temperature increases. The temperature increases were through the medium of the plate, but also probably included water-temperature increases, at least close to the plate. Significant differences were observed in *Beania* sp (weight, colony area, colony diameter, zooid length, zooid area, operculum length, operculum width, and operculum area), and *Caberea zelandica* (composition). No significant difference was observed in *Bugulina flabellata*, whether colony-wide, zooid-morphology, or skeletal mineralogy. The discrepancy between *Beania* sp. and *B. flabellata* may be explained by the colony surface of *Beania* sp. being close to the heated surface, whereas *B. flabellata* is only basally attached with the majority of the colony potentially being cooler than the base. However, *C. zelandica* is also an erect species and responded differently from *B. flabellata*. Additionally, a more in-depth multivariate analysis of colony and zooid characteristics could also shed some light on these questions, and allow for the analysis of colony morphometrics. While small sample sizes limited the robustness of the data set, it appears that we could conclude that these bryozoan species are robust to small but persistent temperature increases. This is not perhaps surprising because they are the weedy coastal type of species that can manage a wide range of environmental parameters.

It is nevertheless likely a future much-warmed ocean could still challenge these bryozoans, and the communities that rely on them for niche generation and substrate stabilisation would become more vulnerable to high-temperature events. Bryozoans could also be vulnerable to storm events, something predicted to increase in both magnitude and occurrence in the future.

Any future studies of thermal tolerance in coastal bryozoans should consider more species, increase sample size, and probably increase the temperature ranges included.

CHAPTER FIVE: FEEDING AND GROWTH



NOTE: Chapters three and five combined have been published as:

Feary, T, Smith, A.M. 2023. Food for thought: Investigating the impacts of feeding regime on the growth and survival of a locally invasive cheilostome bryozoan. Bryozoan Studies 2022 (Proceedings of the 19th International Conference on Bryozoa, 2022).

Feary carried out field & lab work, ran experiments, analysed data, and wrote drafts.

Smith commented on drafts and provided financial support.

5.1 Introduction

Bryozoans are lophophorates, meaning they collect suspended particles using tentacles anchored to a muscular ring, called the lophophore (Sun et al. 2009, McClelland et al. 2014). They have a stomach, and possess a U-shaped gut. The energy derived from collected and digested food is used for metabolic activity, reproduction, and somatic growth.

But how fast do bryozoans actually grow? Published bryozoan growth rates vary greatly (Barnes et al. 2006, Amui-Vedel et al. 2007). Coastal crustose Bryozoa can grow very quickly and are typically annual or perennial, with some encrusting species living for 3-4 seasons. For example, *Membranipora membranacea* can grow as much as 12mm in one day (Smith 2014). Measuring bryozoan growth rate is difficult, not least because they are colonial, small, cryptic, and tend to be found at depth.

Different methods have been used for studying the growth rate of Bryozoa including photogrammetry (Førde et al. 2016), isotope chemistry (Key et al. 2018), and staining (Moran 2000, Linard et al. 2011, Smith 2014), but the growth rates measured by different methods are not easily comparable. Nevertheless, a basic understanding of growth is essential background to understand these marine colonies, and the potential effects of

climate change and ocean acidification on their growth and survival (Smith 2009, Lombardi et al. 2011a, Seroy & Grünbaum 2018).

Watersipora subatra, the red-ripple bryozoan (Figure 5.1), has the potential to be an effective "lab rat" model organism. It is invasive, fast-growing, and abundant (Viola et al. 2018, Reverter-Gil & Souto 2019, Culver et al. 2021). The area of origin of *W. subatra* is yet to be determined, although other members of the genus are thought to have originated in Asia, and have since invaded parts of Europe and the Pacific (Ferrario et al. 2015, Reverter-Gil & Souto 2019). The first record of *W. subatra* in Aotearoa New Zealand was in Dunedin in 1982, though it was initially misidentified as *Watersipora subtorquata* (Gordon & Mawatari 1992, Vieira et al. 2014). A population of *W. subatra* remains nearby, conveniently located on a set of tyres dangling off the pier at Portobello Marine Laboratory, Dunedin, South Island, New Zealand (Figure 5.2).

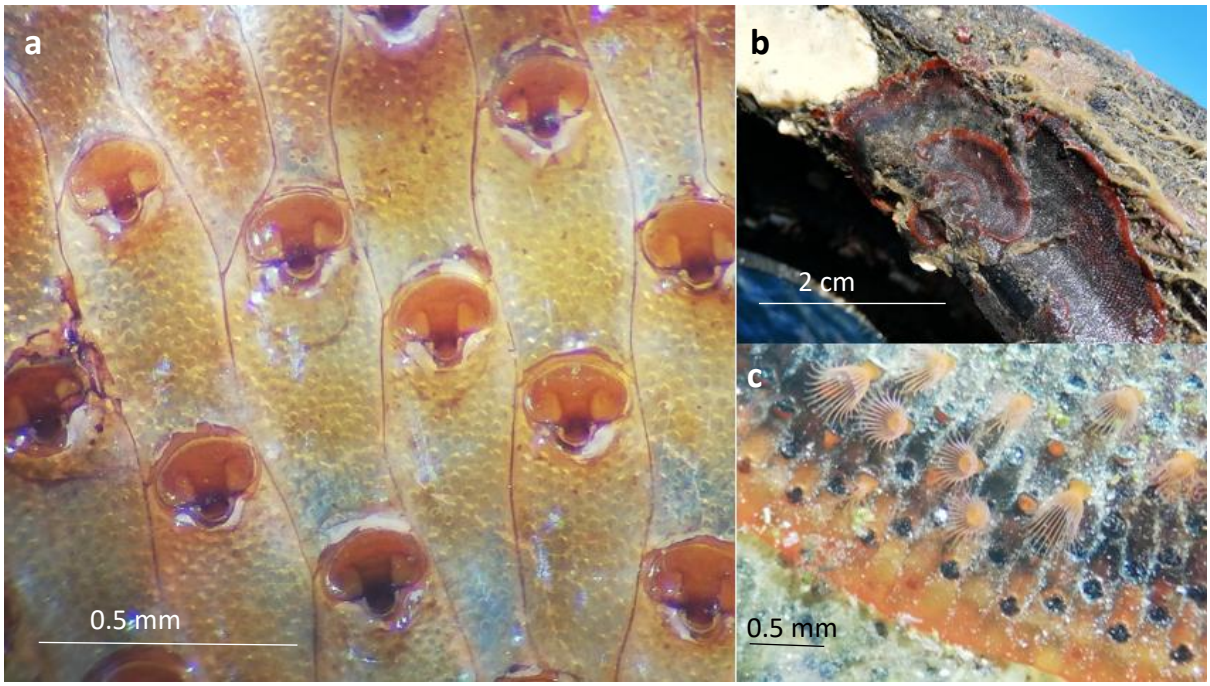


Figure 5.1. *Watersipora subatra* zooids under (a) magnification, (b) encrusted on a tyre, and (c) magnified with lophophores extended during feeding.

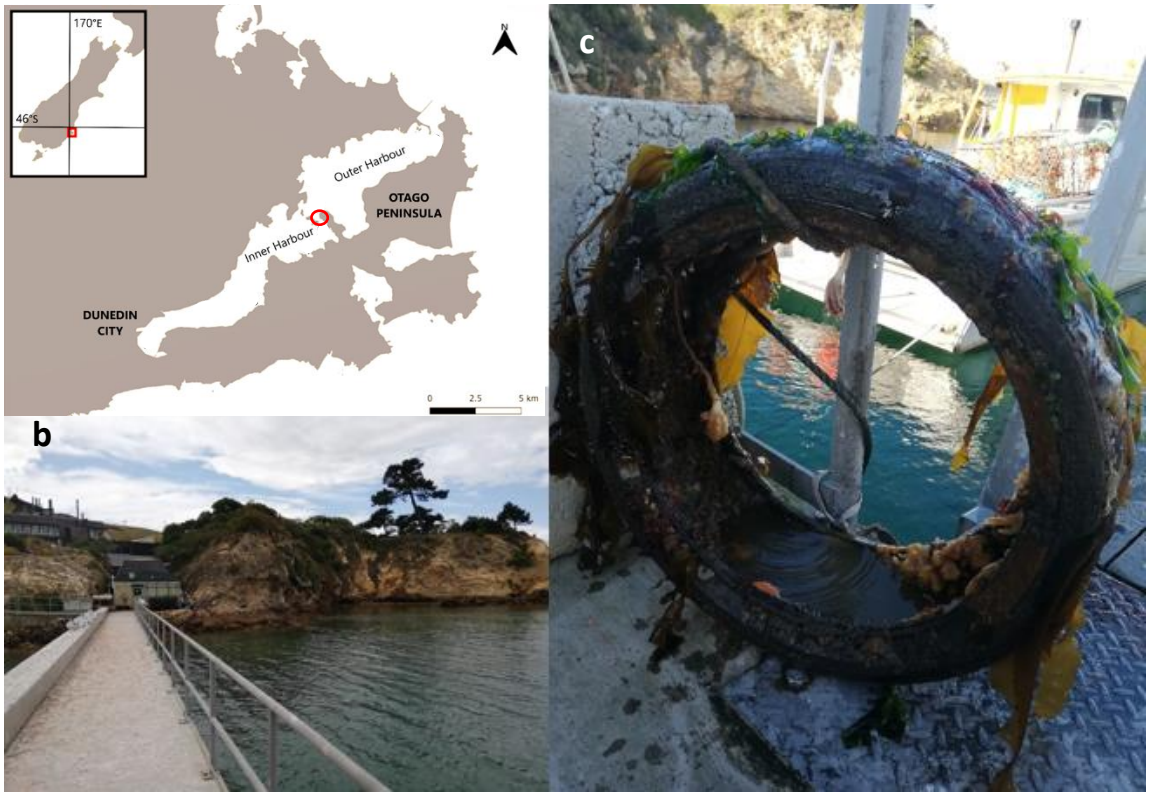


Figure 5.2. Location of the study population of *Watersipora subatra*. (a) map highlighting Portobello Marine Laboratory in Otago Harbour, New Zealand; (b) the pier at Portobello Marine Lab; and (c) the colonized tyre.

The opportunities offered by *Watersipora subatra*, and the genus *Watersipora* in general, have been recognised such that it appears frequently in the literature (e.g., Ng & Keough 2003, Mackie et al. 2006, Láruson et al. 2012, Mackie et al. 2012, Mackie et al. 2014, Sun et al. 2009, Kuhlenkamp & Kind 2013, Ferrario et al. 2015, Sams et al. 2015). A thorough review, however, reveals gaps in laboratory culture protocols. In particular, what do we feed it, what is its wild growth rate, and how does laboratory feeding regime affect growth?

5.2 Methods

As a pilot study in winter 2021, bryozoan fragments were maintained in the lab and subjected to each of four feeding regimes. Eight *Watersipora subatra* colonies were

collected with a serrated knife from the tyres at Portobello Marine Laboratory and cut into 48 fragments with diameters of approximately 0.5 – 0.9 cm. Each fragment had live zooids and a defined growing edge. These fragments were glued to microscope slides in randomly assorted lines of three colonies with cyanoacrylate marine glue, yielding a total of sixteen microscope slides each with three colony fragments on them.

Feeding treatments consisted of the diatom *Chaetoceros mulleri* (3-20 µm), *Tetraselmis chuii*, a green flagellate (12-14 µm), haptophyte *Pavlova lutea* (approximately 5 µm), and a mixture of roughly 1/3 of each, to investigate the effect of food type and size on survival and growth in *W. subatra*. For each treatment, a 500ml beaker with two slides (i.e., six colonies) was kept in a flow-through dark-covered water bath. Input of 10ml of food occurred every three days for two weeks. Water was drained from the bath during feeding to prevent contamination among feeding regimes. Diameter across the widest point of each of the colony fragments was measured at the beginning of the experiment and at the end of each week, using calipers.

Methodology was improved and refined for the summer feeding trial. Colonies of *W. subatra* were again collected from several tyres at Portobello Marine Laboratory and divided into 72 fragments with a scalpel. Four fragments with diameters of approximately 0.5 – 0.9 cm were glued to each of 18 6-cm^2 settlement plates using cyanoacrylate glue (Figure 5.3). They were then divided into each of six feeding treatments – *Chaetoceros mulleri*, *Tetraselmis chuii*, *Pavlova lutea*, a mixture of 1/3 each of the three, kelp flakes, and plain seawater. Seawater was collected locally at Portobello Marine Lab. Kelp flakes were cut from local *Macrocystis* sp. and minced small by knife. Colony plates were photographed weekly for three weeks. Fragments were photographed individually at the end of the

experiment, and were subsequently bleached in a 10% solution and re-photographed to investigate zooid characteristics (Fig 4.3C). The characters measured included zooidal length, zooid width, zooid morphology, operculum length, and operculum width. Fragment growth was defined as change in total area over time in mm/d, as measured with ImageJ.

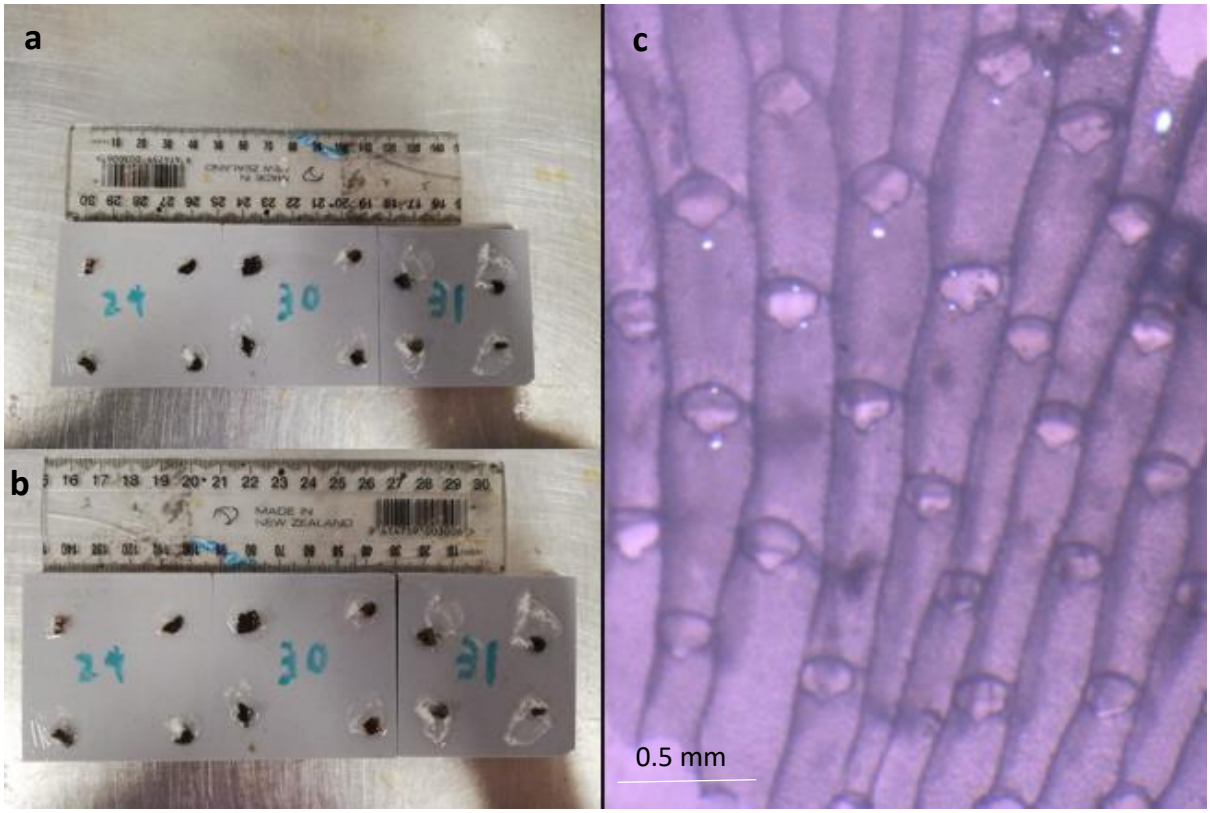


Figure 5.3. *Watersipora subatra* (a, b) colony fragments glued to slides; (c) magnified after bleaching showing zooid morphology.

5.3 Results

Laboratory-grown colonies in winter did not grow much, and in some cases, they shrank (that is, material from the colony died and fell off); diameter change ranged from -0.190 to 0.095 mm/day (mean of 0.008 mm/day, SD = 0.06, n = 48) (Table 4.1).

The effect of feeding regime on *W. subatra* colonies varied among treatments during the winter trials (Fig 4.4). Change in colony diameter (mm/day) was observed to be greatest in

colonies fed *Chaetoceros*, and the mixed treatment (equal parts *Chaetoceros*, *Pavlova*, and *Tetraselmis*). Change in colony diameter was observed to be either low or to show no change in colonies fed *Pavlova.* Colonies fed *Tetraselmis* showed substantial variation in response to the treatment with two showing an increase in colony diameter, and the remainder showing either no change, or a negative change.

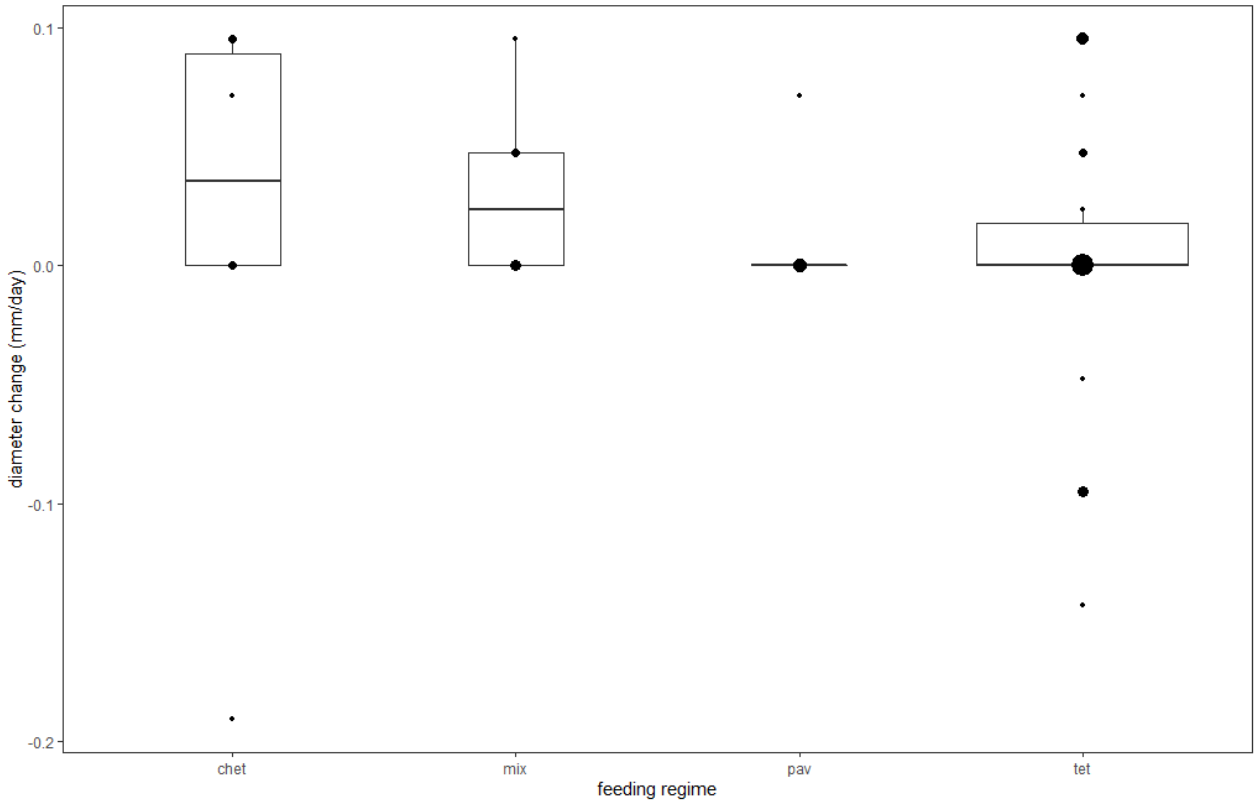


Figure 5.4. Graph comparing feeding regime with change in fragment diameter during winter 2021. Chet = *Chaetoceros*, mix = mixed treatment, pav = *Pavlova*, tet = *Tetraselmis*.

In the summer trials, colonies in the laboratory grew more. Total change in diameter ranged from -0.448 to 0.479 mm/day (mean of 0.12 mm/day, SD = 0.251, n = 72) (Table 5.4). Growth in summer is equivalent to a maximum annual growth rate of 30.4 cm/year. Of course, annual growth would be much less in real life, or similar.

The effect of feeding regime on *W. subatra* colonies varied among treatments during the summer trials (Figure 5.5). Change in colony diameter (mm/day) was greatest in colonies fed *Chaetoceros*, the mixed treatment, and *Pavlova*. Change in colony diameter was observed to be negative in the kelp flake and seawater treatments. ANOVA and post-hoc Tukey's tests showed there was a significant effect of feeding regime on change in colony diameter (Figure 5.6).

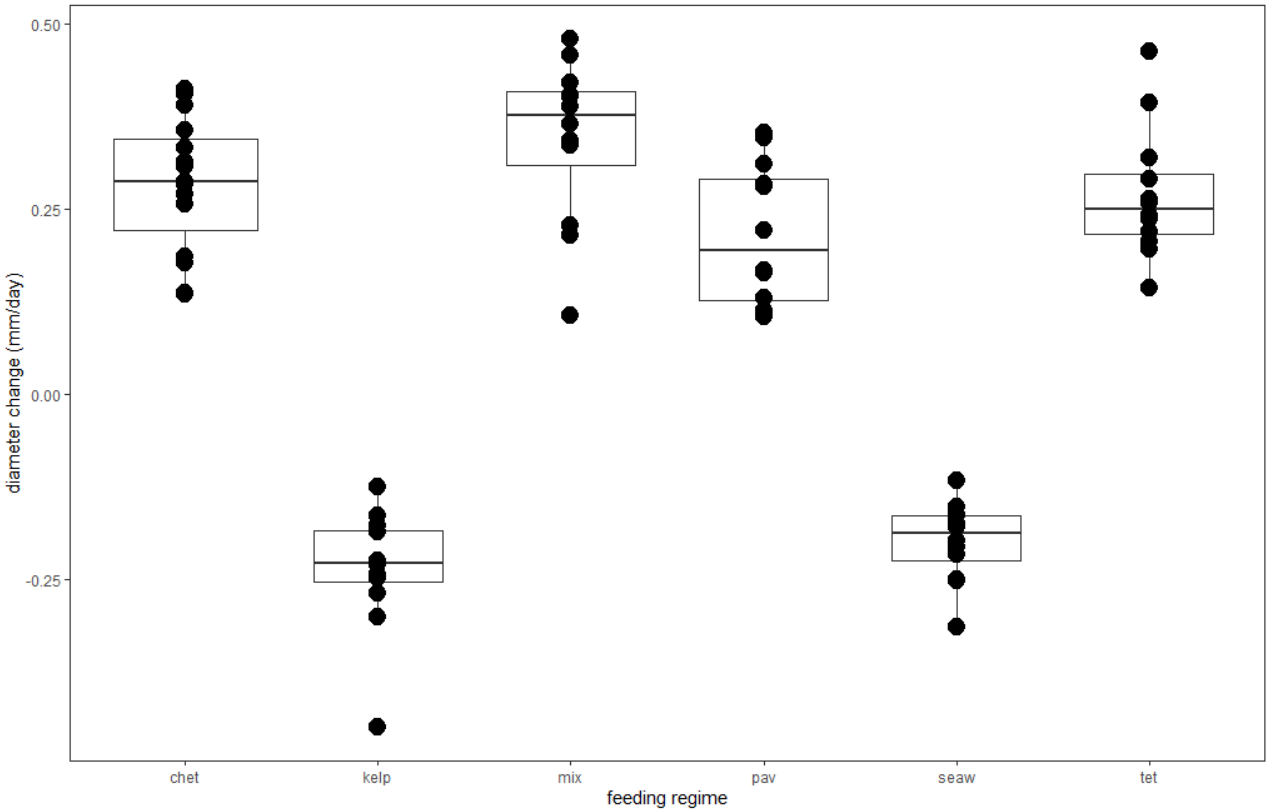


Figure 5.5. Graph comparing feeding regime with change in fragment diameter after three weeks during summer 2022. Chet = *Chaetoceros*, kelp = kelp flakes, mix = mixed treatment, pav = *Pavlova*, seaw = seawater treatment, tet = *Tetraselmis*.

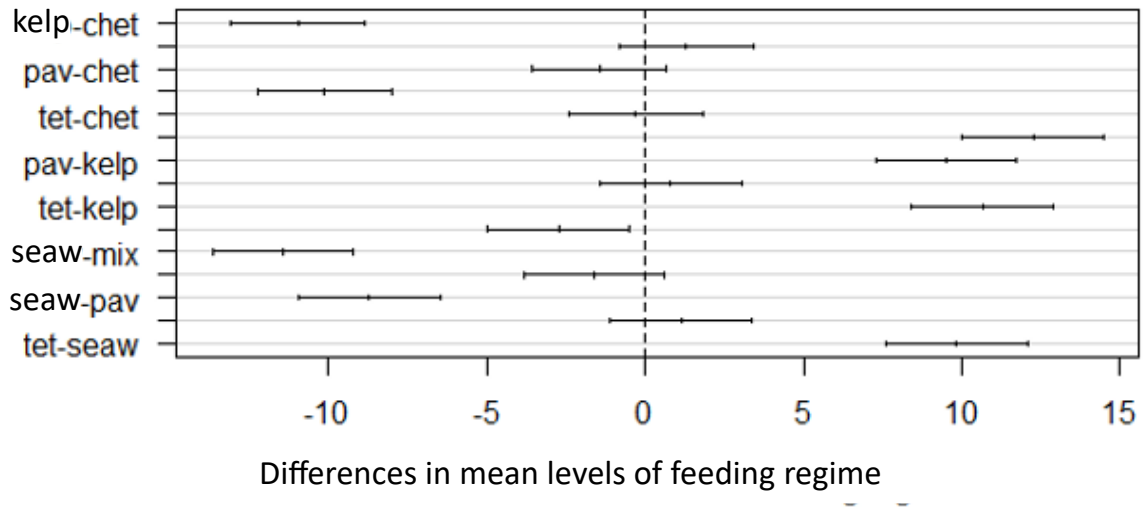


Figure 5.6. Graph showing the difference in means of feeding regime with 95% confidence intervals. Chet = *Chaetoceros*, kelp = kelp flakes, mix = mixed treatment, pav = *Pavlova*, seaw = seawater treatment, tet = *Tetraselmis*.

5.4 Discussion

5.4.1 What does Watersipora like to eat?

In winter, growth was observed in colonies fed *Chaetoceros, Pavlova, Tetraselmis,* and an even mixture of the three, suggesting *W. subatra* is able to consume a wide range of foods with different sizes. The highest growth rate was seen in the mixed treatment, suggesting that for *W. subatra* a mixed diet is optimal.

In summer, growth was observed in colonies fed *Chaetoceros, Tetraselmis,* and the mixed treatment. As with the winter results, *W. subatra* likes a mixed diet, but also supports the use of *Chaetoceros* and *Tetraselmis* as feeds. In the winter trials, no growth was observed in the colonies fed *Pavlova*, possibly due to colony preference or a flaw in the initial study

design as replication was less. It may also be a case of seasonality, such that *W. subatra* exhibits greater diet selection in summer when growth is typically greatest. Presumably it is beneficial to eat everything offered in summer in order to utilize optimal conditions for growth. It has been observed that the freshwater phylactolaemate bryozoan *Fredericella sultana* also employs a generalist feeding strategy (Raddum & Johnsen 1983), because there is a benefit to feeding on a range of foods in interspecific competition among Bryozoa (Best & Thorpe 1986, Allen et al. 2008, Svensson & Marshall 2015, Comerford et al. 2020). Furthermore, seasonal changes in feeding preferences have also been previously recorded in Bryozoa as *Plumatella geimermassardi* has been observed to have a higher feeding rate in June and July, than during August (Todini et al. 2018).

Chaetoceros mulleri is a diatom with an average length of 3-20 µm, *Tetraselmis chuii* is a green flagellate with an average length of 12-14 µm, and *Pavlova lutea* is a haptophyte with an average length of approximately 5 µm. The mixture of the three therefore contains a size range of 3-20 µm. The results of the feeding trials indicate that *W. subatra* can ingest particles of at least up to 20 µm, and shows little discrimination among phytoplankton groups.

Next steps worth trying include both larger and smaller food, ciliates, and marine invertebrate food available in pet stores. Other foods successfully fed to Bryozoa include *Dunaliella, Cryptomonas, Isochrysis* (Kahle et al. 2003, McKenzie et al. 2012), *Thalassiosira, Nannochloropsis,* and *Brachionus* (Svensson & Marshall 2015). *Synechoccus, Colpidium, Tetrahymena* have been fed to freshwater Bryozoa (Wood 2020), as have fish food and algal pellets, with varying results (Table 5.1).

Table 5.1. Table showing bryozoan feeding regimes across the literature.

Bryozoan food	Size	Comments	Reference(s)
Dunaliella	5 to 25 µm	Unicellular algae	Kahle et al. 2003; McKenzie et al. 2012
Cryptomonas	40 µm	Flagellated	Kahle et al. 2003; McKenzie et al. 2012
Isochrysis	3 to 7 µm	Haptophyte	Kahle et al. 2003; McKenzie et al. 2012
Thalassiosira	4 to 32 µm	Centric diatom	Svensson & Marshall 2015
Nannocloropsis	2 to 5 µm	Microalgae	Svensson & Marshall 2015
Brachionus	0.2 to 0.6 µm	Rotifer	Svensson & Marshall 2015
Synechoccus	0.8 to 1.5 µm	Cyanobacterium	Wood 2020
Colpidium	50 to 150 µm	Ovular or kidney-shaped ciliate	Wood 2020
Tetrahymena	35 µm	Ovular ciliate	Wood 2020
Tetraselmis	5–10 µm	Green unicellular flagellate	Smith et al. 2019
Rhodomonas	6 µm	Red cryptomonad	Hermansen et al. 2001
Oxyrrhis	20-30 um	Heterotrophic dinoflagellate	Jebram & Rummert 1978

A final recommendation for feeding *Watersipora subatra* would be to maintain a mixed diet of *Chaetoceros, Pavlova,* and *Tetraselmis,* or similar phytoplankton taxa.

5.4.2 How fast does Watersipora grow?

Colonies in our lab trials grew at rates between -0.448 to 0.479 mm/day, with slower growth in winter (mean = 0.008 mm/day, SD = 0.06, N = 48) and faster in summer (mean = 0.12 mm/day, SD = 0.251, N = 72). These rates fall well within the normal range for encrusting bryozoans (Smith 2014). These rates are similar to what we observed in wild *Watersipora* growing nearby on tyres at Portobello (Chapter 3).

When comparing between winter and summer trials, both wild and cultured growth was, as expected, higher during the summer (Table 5.2). Similar trends in seasonality have been observed in *Watersipora* previously (Ng & Keough 2003, Sams et al. 2015).

Comparing the results of Chapter 3 with these experiments show an overlap in growth rates between laboratory colonies, and wild colonies (Table 3.1), indicating that a growth rate comparable to that of wild colonies has been achieved in a laboratory environment when feeding colonies a mixed diet.

Table 5.2. Data table for annual growth of laboratory colonies under different feeding regimes.

Feeding regime	winter growth mm/y	summer growth mm/y
Tetraselmis	1.8	98.4
Chaetoceros	7.01	103.5
Pavlova	n/a	78.7
Mix	18.7	126.1

5.4.3 How does diet affect growth in Watersipora?

Feeding regime was observed to have an effect on growth in *W. subatra* colonies. Change in colony diameter (mm/day) was highest in colonies fed *Chaetoceros*, one of the *Tetraselmis* treatments, and the mixed treatment (equal parts *Chaetoceros*, *Pavlova*, and *Tetraselmis*). Change in colony diameter was observed to be low or show no change in colonies fed *Pavlova* and *Tetraselmis*. Feeding *Chaetoceros, Pavlova, Tetraselmis,* and a mixture of the three promoted growth during summer, but during winter only *Chaetoceros, Tetraselmis,* and the mixture promoted growth. Feeding kelp flakes or seawater did not promote growth and yielded a negative change in colony diameter. Other studies have noted that changes in diet may affect particle capture rates as well as growth (Riisgard & Manriquez 1997, Okamura 1985).

We show here that colonies of *Watersipora subatra* will grow in culture when fed *Chaetoceros* or a mixture of phytoplankton, suggesting that *W. subatra* is a generalist feeder rather than specializing in a single type of phytoplankton. It may also be that they grow better on a varied diet. In either case they appear to dislike or be unable to feed on *Pavlova*, at least in summer. The effects of feeding *Tetraselmis* varied among individuals. One difference between the two experiments is the inclusion of kelp flakes and filtered seawater

as a control. *W. subatra* showed no growth when fed kelp flakes indicating they are unable to be sustained with this food source. The filtered seawater treatment acted as a control as expected there was no growth in these treatments. Since growth in the wild is similar to maximum growth in the lab, our results support the feeding of *W. subatra* a varied mixed diet, but not including *Pavlova* or kelp flakes.

5.4.4 Summary and conclusions

Feeding *W. subatra* a variety of cultured plankton enabled lab-based growth that was similar to wild growth nearby in several colonies. *W. subatra* thus demonstrates considerable potential as a "lab-rat" model species for experiments designed to explore environmental effects on growth.

CHAPTER SIX: SYNTHESIS & REVIEW OF GROWTH IN BRYOZOANS

6.1 Introduction & Aims

The aim for this study was to investigate the factors that influence colony growth in bryozoans, focusing on Otago Harbour as an accessible example. Achieving this goal required the development of culture techniques and background information for suitable model species and to assess how their growth rates respond to environmental factors (e.g., season, temperature, food).

My first objective was to find out what bryozoans live in Otago Harbour (Chapter 2). I then developed a long-term dataset for an abundant local bryozoan (*Watersipora subatra*) to obtain baseline growth and development data (Chapter 3). Once we had a clear understanding about how local bryozoans grow in the wild, I was able to conduct manipulative experiments to investigate how increased temperatures affect growth of bryozoans (Chapter 4). Then I investigated how food availability affected growth of *Watersipora subatra* while also allowing for future experimentation by providing data on its suitability as a model organism and instructions for achieving near-wild growth rates in a laboratory environment (Chapter 5).

In this chapter I will discuss the most significant findings from each chapter, and then show how these new findings help us to understand growth in bryozoans.

Of the twelve bryozoan taxa found in Otago Harbour, eight of these taxa had not been recorded in a prior survey. Equally, nineteen species previously noted as present were not found in our surveys. There were no new invasive bryozoans recorded since a previous survey in 1992, indicating there have may have been no new exotic invasions. Adding up all three surveys suggests that, at various times, Otago Harbour may have hosted up to thirty species of bryozoans.

Wild growth of *Watersipora subatra* populations over nearly two years at Portobello Marine Lab were 111 mm/y during summer, and 27 mm/y during winter (chapter 3), a realistic annual growth rate for *W. subatra* will lie somewhere between these two values. When compared to other measured growth rates from the literature, *W. subatra* seems to grow faster than many bryozoans.

Wild Bryozoa found in Otago Harbour exhibited both susceptibilities and tolerances to increases in heat, though it would be wise to investigate the interaction of a heat increase with a pH decrease. Significance was found between controls and increases of +1 or +2 degrees in the colony and zooid characteristics of *Beania* sp., and in the composition of *Caberea zelandica. Bugulina flabellata* showed no significance in any parameter indicating that it, like many coastal species, can tolerate small increases in temperature.

In the lab, feeding experiments showed a significant influence of food type on the growth of the bryozoan *Watersipora subatra*. The highest growth rate was recorded when feeding the colonies a mixed diet, and there was a clear preference when comparing the results of single plankton feeds. Kelp flakes were found to be unsuitable for feeding *W. subatra*.

We were able to grow *W. subatra* in the lab at nearly the same rate as in wild populations, particularly when a preferred food source was offered. *W. subatra* is thus a candidate for an effective model organism to study bryozoan growth in future experiments.

6.2 Growth rates matter

Understanding growth is essential to understanding organisms and being able to answer other questions about them. Energy goes into bryozoans via food and is used to fuel metabolism, somatic growth, reproduction (both reproductive structures and actual gametogenesis), and/or calcification (of whatever mineralogy) (Fig 6.1). This relationship can be affected by abiotic factors (temperature, salinity, pH), and biotic factors (distribution, competition, predation, symbiosis). The aspects of this framework that the previous chapters have focused on are food, somatic growth, temperature, and distribution. There is of course ample opportunity for further research in the future.

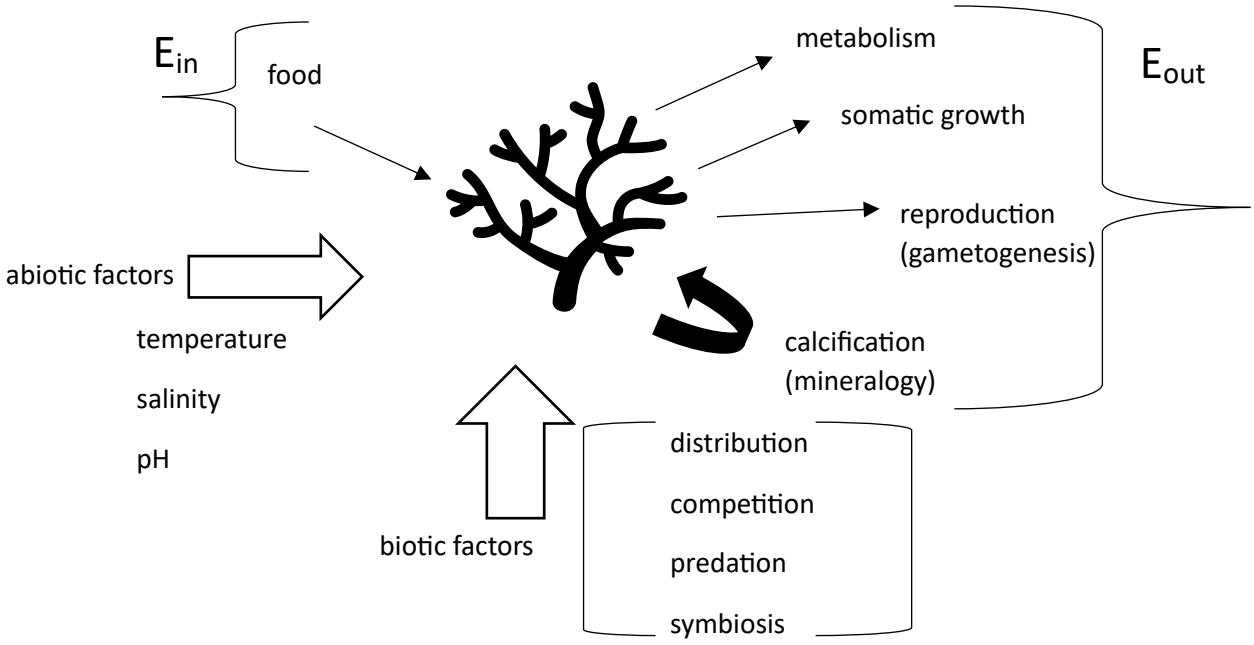


Figure 6.1. Conceptual framework figure of energy flow into and out of a bryozoan, as well as the biotic and abiotic factors that may influence this pathway.

Somatic growth is the increase in size and development of an organism's body throughout its life. While it is governed by genetic and hormonal factors, the environment has a considerable influence. Somatic growth is important throughout an organism's life cycle and is a dynamic process that allows organisms to adapt to a changing environment (Bartke

2017; Le Bourg & Le Bourg 2020). In the case of colonial organisms, the individual structural units (zooids) only grow briefly, but the colony also grows by the addition of new units. This is what we measured in relation to food and temperature.

Adequate nutrition is essential for optimal growth. Animals need sufficient energy and nutrients to support growth processes, including tissue development and reproduction. The same is true for a colony.

Understanding and managing the factors that influence growth rates in animals are critical for various applications, including agriculture, conservation, and wildlife management. Especially for laboratory culture, nutrition and environment are essential for promoting healthy growth minimizing negative impacts. Colonial animals, operating on two levels, increase the complexity for those who wish to keep them in culture.

6.3 Growth rates vary.

Genetic factors play a substantial role in determining an animal's growth potential. Different species have evolved with specific growth patterns and rates tailored to their ecological niches (Le Bourg & Le Bourg 2020). Nevertheless, environmental factors such as temperature, food availability, water quality, and habitat suitability can significantly influence an animal's growth rate (Le Bourg & Le Bourg 2020). For example, warmer temperatures often accelerate metabolic processes, leading to faster growth. Bryozoan growth has been observed to vary when exposed to different factors throughout the wider literature (Table 6.1).

Table 6.1. Literature review of different studies investigating the influence of various factors on the growth of different bryozoan taxa.

Bryozoan taxa	Factor(s) investigated	Reference(s)
Bicellarina alderi	Depth	Stępień et al, 2017
Bugula neritina	Seasonality	Keough 1986
Bugula neritina	Temperature	Lord, 2017
Bugula neritina	Acidification	Pecquet et al, 2017
Bugula spp.	Food availability	Svensson & Marshall 2015
Calpensia nobilis	Acidification	Lombardi et al, 2015
Cellarinella nutti	Environmental change	Barnes et al, 2006
Celleporaria nodulosa	Temperature	Durrant et al, 2013
Celleporaria nodulosa	Acidification	Durrant et al, 2013
Celleporella cornuta	Acidification	Swezey et al, 2017
Celleporella hyalina	Seasonality	Hunter & Hughes 1994
Celleporella hyalina	Water flow	Hermansen et al, 2001
Chartella barleei	Depth	Stępień et al, 2017
Conopeum reticulum	Seasonality	Menon 1972
Conopeum seurati	Temperature	O'Dea & Okamura 1999
Conopeum seurati	Chlorophyll concentrations	O'Dea & Okamura 1999
Cryptosula pallasiana	Temperature	Amui-Vedel et al, 2007
Cryptosula pallasiana	Acidification	Lombardi et al, 2011a
Cupuladria exfragminis	Seasonality	Okamura et al, 2011
Cupuladriidae	Seasonality	O'Dea & Jackson 2002
Electra	Colony size	Lutaud 1983 in Lidgard & Jackson 1989
Electra pilosa	Water flow	Hermansen et al, 2001
Electra pilosa	Seasonality	Menon 1972
Lophopus crystallinus	Nutrient concentrations	Hartikainen et al, 2009
Membranipora	Colony size	Lutaud 1983 in Lidgard & Jackson 1989
Membranipora membranacea	Temperature	Pratt et al, 2022
Myriapora truncata	Acidification	Lombardi et al, 2011b
Myriapora truncata	Temperature	Pages-Escola et al, 2018
Myriapora truncata	Temperature	Rodolpho-Metalpa et al, 2010
Pentapora fascialis	Seasonality	Lombardi et al 2006
Pentapora fascialis	Temperature	Pages-Escola et al, 2018
Plumatella sp.	Nutrient concentrations	Hartikainen et al, 2009
Sarsiflustra abyssicola	Depth	Stępień et al, 2017
Watersipora subtorquata	Temperature	Lord, 2017
Watersipora subtorquata	Planktonic duration	Sams et al, 2015
Watersipora subtorquata	Competition	Sams et al, 2015
Watersipora subtorquata	Food availability	Svensson & Marshall 2015

Bryozoan growth is affected by season, with greater growth in spring and summer in several species (Keough 1986; Hunter & Hughes 1994; Svensson & Marshall 2015; Lord 2017;

Pecquet et al. 2017; Stępień et al, 2017). Part of the seasonal effect is bound to be due to the influence of temperature (Rodolpho-Metalpa et al, 2010; Pages-Escola et al, 2018).

Another important factor that has been observed to influence bryozoan growth is pH. *Celleporaria nodulosa* displays reduced growth at high temperatures, its maximum tolerance being reported as 27 degrees Celsius, additionally a lower pH also negatively affects colony growth and interacts with temperature (Durrant et al. 2013). *Celleporella cornuta* colonies when exposed to a heightened pH grew faster and possessed thinner skeletons (Sweezey et al. 2017). *Cryptosula pallasiana* colony growth rates increase with temperature, along with morphology changes, though decreased pH reduces the number of active zooids, and negatively impacts regeneration (Amui-Vedel et al. 2007; Lombardi et al. 2011a). *Culpensia nobilis* also shows a negative response to lowered pH – a slower growth rate and longer zooids (Lombardi et al. 2015). *Myriapora truncata* yields a thickened skeleton and inhibited growth under low pH conditions (Lombardi et al. 2011b).

Depth has also been observed to have an influence on growth. *Chartella barleei* and *Bicellarina alderi* are influenced by increasing depth, it grows zooids with a greater length and surface area (Stępień et al, 2017). *Sarsiflustra abyssicola* shows a greater zooid length and surface area in deep water colonies (Stępień et al, 2017).

The importance of food availability on growth has also been studied. *Conopeum seurati* colony growth rate increases with heightened chlorophyll concentrations (O'Dea & Okamura 1999). *Watersipora subtorquata* colony growth rate also increases with an increase in food availability (Svensson & Marshall 2015).

Also notable is the observation that colony size can affect growth rate as well. Studies of the genus *Electra* have shown growth rate increases with colony size (Lutaud 1983 in Lidgard &

Jackson 1989), and *Membranipora* has also shown an increased growth rate when colony size is larger (Lutaud 1983 in Lidgard & Jackson 1989).

Other, less-studied factors that can influence growth include competition, and larval duration. In *Watersipora subtorquata* colony growth is reduced after an extended planktonic duration, which also results in a higher mortality (Sams et al. 2015), competition from ascidian colonies can reduce colony growth (Sams et al. 2015).

Bryozoan growth rates also vary naturally among species. We have shown that *Watersipora subatra* is a relatively fast-growing species (wild colonies grew between 27 and 111 mm/y), typical of an invasive species that successfully outcompetes other slower-growing taxa. The only bryozoan observed to have a growth rate substantially higher than *W. subatra* is *Membranipora membranacea* (37 to 4380 mm/y), though *Einhornia crustulenta* has a comparatively high growth rate as well (77.1 mm/y) (Table 6.2).

Table 6.2. Literature review of different studies measuring the annual growth rates of different bryozoan taxa as in Smith (2014), growth data for Watersipora subatra from this study has been added.

Taxon	Growth rate (mm/y)	Reference(s)
Adeonellopsis sp.	6.9	Smith et al., 2001
Arachnopusia inchoata	5.6	Bowden et al., 2006
Callopora dumerilii	5.5	Kuklinski et al., 2013
Cellaria incula	8	Brey et al., 1999
Cellarinella foveolata	5	Winston, 1983
Cellarinella margueritae	3.4 - 5.4	Winston, 1983; Barnes et al., 2007
Cellarinella njegovanae	4.4	Winston, 1983
Cellarinella rogickae	4.6	Barnes et al., 2007
Cellarinella rossi	4.3	Winston, 1983
Cellarinella watersi	4.1	Barnes, 1995; Barnes et al., 2007
Chaperiopsis protecta	4	Bowden et al., 2006
Cribrilina annulata	1.3	Kuklinski et al., 2013
Diploselen cf *obelium*	3.7	Kuklinski et al., 2013

Diplosolen arctica	4.3	Kuklinski et al., 2013
Einhornia crustulenta	77.1	Kuklinski et al., 2013
Escharella immersa	2.5	Kuklinski et al., 2013
Fenestrulina rugula	4	Bowden et al., 2006
Harmeria scutulata	2.5	Kuklinski & Taylor, 2006
Membranipora membranacea	37 to 4380	Saunders & Metaxas, 2009
Membraniporella nitida	1.4	Kuklinski et al., 2013
Microporella arctica	2.2	Kuklinski et al., 2013
Patinella sp.	1.3	Kuklinski et al., 2013
Pennipora anomalopora	2.9	Taylor & Voigt, 1999
Pentapora foliacea	20	Pätzold et al., 1987
Puellina hincksi	3.8	Kuklinski et al., 2013
Stomhypselosaria watersi	4.5	Barnes et al., 2007
Swanomia belgica	0.6 to 9.7	Smith, 2007
Tegella arctica	4.2	Kuklinski et al., 2013
Watersipora subatra	**27 to 111**	**This study**

6.4 Culturing and Model Organisms

Once an organism's growth rate and factors that influence it are sufficiently understood, it is possible to culture it in a laboratory environment. This opportunity enables a range of useful experiments that would otherwise be difficult in the field. If several other criteria are met, a particular organism might serve as a model for others of its kind.

Model organisms are specific taxa that are extensively studied in scientific research because they serve as representative examples of broader biological groups or processes. These organisms are chosen because they are relatively easy to work with, have short generation times, and often share genetic and physiological traits with other species (Parades 2016; Hatchett et al. 2022). The study of model organisms has advanced the understanding of many biological principles and has had significant implications in assorted scientific fields (Hatchett et al. 2022; Nadir et al. 2023).

Model organisms typically have well-characterized and relatively simple genomes (Matranga & Corsi 2012; Hatchett et al. 2022), simplifying genetic analysis and allowing researchers to

identify and manipulate specific genes with greater ease (Matranga & Corsi 2012; Nilsson et al. 2018). Short generation times enable researchers to observe multiple generations in a relatively short period (Nadir et al. 2023). Rapid generations are particularly valuable for studying genetic changes, selection, and inheritability (Matranga & Corsi 2012). Model organisms tend to produce a large number of offspring in each generation, increasing the statistical power of experiments and analyses and allowing for larger experiments. Model organisms are typically easy to collect and are accessible to researchers (Matranga & Corsi 2012; Hatchett et al. 2022; Nadir et al. 2023). They must also readily grow and be maintained in a laboratory environment (Nilsson et al. 2018; Nadir et al. 2023). A wide accessibility enables multiple research groups to work with the same organism, facilitating the reproducibility of experiments (Matranga & Corsi 2012). Ethical concerns and regulations play a role in the selection of model organisms as they must be non-endangered and non-protected (Canesi et al. 2008; Matranga & Corsi 2012; Hatchett et al. 2022).

The cheilostome bryozoan *Watersipora subatra* displays many of the characteristics required to be used as a model organism in future studies focused on understanding the effect of environmental change on sessile colonial organisms. *Watersipora subatra* meets many of the requirements for a model organism, as it is a common, invasive bryozoan (Table 6.3). The genus *Watersipora* has also been used widely across the literature in a number of different experiments (Sams et al. 2015; Svensson & Marshall 2015; Lord 2017).

Table 6.3. Framework assessing the suitability of *Watersipora subatra* as a model organism.

Requirements to be considered as a model organism	Does *Watersipora subatra* meet them?
Grows fast, reaches maturity quickly	Yes
Ease of manipulation	Yes
Relatively short life span	Yes
Produces many offspring	Yes

Measurable response to stimuli	Yes
Ease of collection - local, non-endangered	Yes
Non-charismatic	Yes
Ease of growth in a restricted area	Yes
Well-characterised, simple genome	Unknown
Relatively easy to feed and maintain	Yes
Reasonably well-understood life cycle	Yes

6.5 Knowledge Gaps

Energy goes into bryozoans via food and is used to fuel their metabolism, somatic growth, reproduction (gametogenesis), or calcification (mineralogy). This relationship can be affected by both abiotic factors (temperature, salinity, pH), and biotic factors (distribution, competition, predation, symbiosis). The aspects of this framework that the previous chapters have focused on are food, somatic growth, temperature, and distribution, though there is still an opportunity for further research in the future.

After studying *Beania* sp., *Bugulina flabellata*, *Caberea zelandica*, and *Watersipora subatra*, seasonality and food type were found to have a significant influence on colony growth rate in *W. subatra*, and small increases in temperature were found to have a significant influence on the growth of *Beania* sp. and *C. zelandica*, but not *B. flabellata*. The results are interesting, but there is still room for speculation and future experimentation regarding other aspects of the growth framework (Figure 6.1), namely pH, salinity, large increases in temperature, competition, predation, and symbiosis.

The majority of bryozoans are calcifying organisms, so it is expected that reduced seawater pH could have a significant influence on colony growth rate and survival. The literature also supports this view as some bryozoan taxa a have been observed to grow poorly in lower-pH conditions. Also of note is the relationship between pH and temperature change, as these

factors may have an interactive effect and have a significantly higher negative impact on colony growth when bryozoans are exposed to both, rather than if they were exposed to either one alone. Alternatively, in some taxa they cancel each other out.

Our results suggest that *Beania* sp. and *Caberea zelandica* growth in influenced by temperature, but *Bugulina flabellata* is tolerant of small increases in temperature, though there is still likely to be a thermal upper limit for them and *W. subatra*. Low temperatures during winter appear to have slowed the growth of wild colonies. As *W. subatra* is an invasive species it will likely be able to tolerate fluctuations in temperature and other environmental factors, though it will possess a temperature limit beyond which colonies will experience necrosis, likely in the range of 26-29 °C as has been documented in other bryozoans. Other bryozoan taxa in the harbour could presumably possess a lower thermal limit than *W. subatra* and *Bugulina flabellata*.

In many studies, it has been found that larvae are more susceptible than adults to the effects of different factors. Both vertebrate and invertebrate eggs and larvae have shown a heightened susceptibility to different environmental factors, the damage of which can persist into adulthood and impact colony growth (Kleypas et al. 2006; Sams et al. 2015).

Competition likely also affects the growth of Bryozoa in Otago Harbour, both interspecific and intraspecific. Invasive bryozoan taxa are likely less impacted by this factor when compared to non-invasive Bryozoa, as invasive species tend to be highly competitive (Davis 2017). Invasive bryozoans will however exert competition on non-invasive bryozoans as well (Davis 2017). Non-bryozoan competitors would include other encrusting taxa including ascidians, sponges, barnacles, and certain types of algae.

Predation presumably has a minor effect on bryozoan growth in Otago Harbour as there are relatively few known predators of bryozoans in the harbour though, of course, there could be unrecorded pycnogonids or nudibranchs (Lidgard 2008).

Symbiosis would likely only influence growth in bryozoans that have a symbiotic relationship with another organism. There are at least two bryozoan species found in Otago Harbour that grow largely on macroalgae.

Exploring the effects of biotic and abiotic factors, as well as the interactions between them is an important next step for further understanding the framework of growth in bryozoans. Investigating the influence of pH, salinity, extreme temperature changes, competition, and predation and their interactions on somatic growth in bryozoans is the logical next move, though it could be taken further and the influences of these factors on reproduction, metabolism, and skeletal mineralogy could be studied as well.

In order to achieve this successfully and obtain an understanding of the growth framework across most Bryozoa, many different bryozoan species would need to be studied, though this could be made easier by highlighting a few model bryozoan species and studying them in great detail, whilst also performing some less extensive experimentation on other species.

Watersipora subatra shows promise as a model organism, and the further refining of laboratory techniques for the keeping of *Watersipora subatra* as a model organism should be enacted, as well as finding other taxa suitable for use as a model organism for this phylum. We should refine the currently accepted method for spawning *W. subatra* – a more concise and robust method for spawning and raising larvae year-round could be developed and used across the literature, as currently there are several different published methods for

spawning *Watersipora* spp. that have varying degrees of success and larval mortality in *W. subatra*.

6.6 Summary

Although this study has focused on a few bryozoan taxa in a single harbour in New Zealand, our findings have wider implications and signal pathways to future research. Overall, food type, temperature, and seasonality were observed to affect colony growth in the studied Bryozoa, along with pH, turbidity, water flow, competition, and other environmental factors. The results from this study also support the potential of *Watersipora subatra* as a model organism and provide data to facilitate this. Additionally, the results from this study have also provided the first bryozoan-focused survey of Otago Harbour since 1992, something long overdue.

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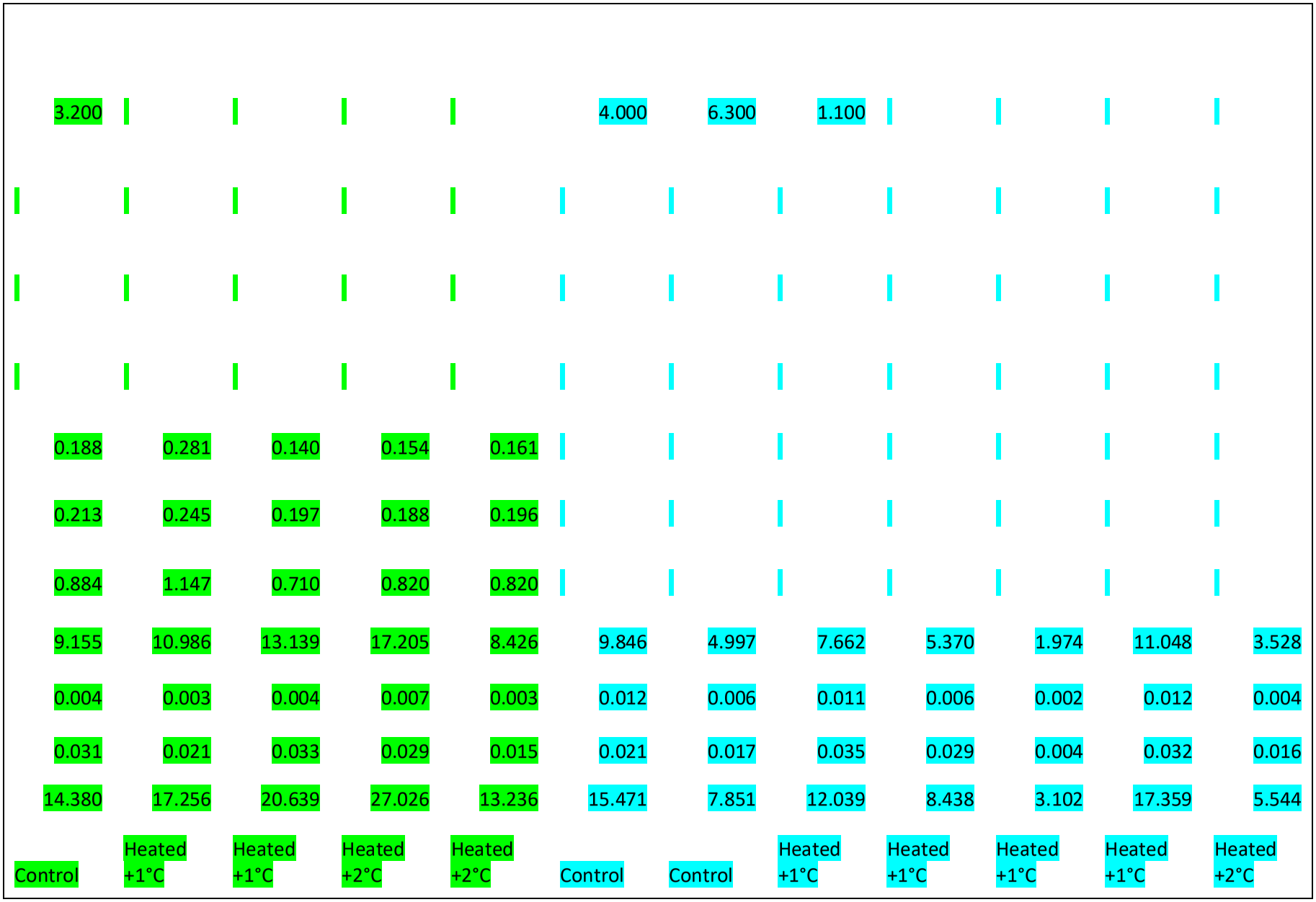
Appendix 1

Appendix 1. Table of parameter data for colonies removed from heated plates. *Beania* sp. is highlighted in yellow, *Bugula flabellata* in green, and *Caberea zelandica* in blue.

Treatment	area (mm^2)	dry weight (g)	wet weight (g)	diameter (mm)	zooid length (mm)	zooid width (mm)	zooid area (mm^2)	operculum length (mm)	operculum width (mm)	operculum area (mm^2)	Wt% MgCO3 in calcite (y = 30x - 882)
Heated +2°C	16.908	0.039	0.012	10.764	0.585	0.450	0.263	0.112	0.191	0.021	
Heated +2°C	30.816	0.035	0.022	19.618	0.633	0.385	0.244	0.149	0.164	0.024	
Heated +2°C	27.620	0.098	0.021	17.583	0.571	0.403	0.230	0.162	0.180	0.029	
Heated +2°C	25.979	0.060	0.021	16.539	0.627	0.433	0.271	0.129	0.158	0.020	
Heated +1°C	30.784	0.040	0.021	19.149	0.485	0.299	0.145	0.124	0.143	0.018	
Heated +1°C	4.327	0.008	0.003	2.755	0.596	0.411	0.245	0.122	0.186	0.023	
Control	2.795	0.004	0.002	1.779	0.607	0.411	0.249	0.086	0.116	0.010	
Control	16.625	0.020	0.004	10.584	0.641	0.181	0.116				4.200


3.200
4.000
6.300
1.100
0.188
0.281
0.140
0.154
0.161
0.213
0.245
0.197
0.188
0.196
0.884
1.147
0.710
0.820
0.820
9.155
10.986
13.139
17.205
8.426
9.846
4.997
7.662
5.370
1.974
11.048
3.528
0.004
0.003
0.004
0.007
0.003
0.012
0.006
0.011
0.006
0.002
0.012
0.004
0.031
0.021
0.033
0.029
0.015
0.021
0.017
0.035
0.029
0.004
0.032
0.016
14.380
17.256
20.639
27.026
13.236
15.471
7.851
12.039
8.438
3.102
17.359
5.544
Control
Heated +1°C
Heated +1°C
Heated +2°C
Heated +2°C
Control
Control
Heated +1°C
Heated +1°C
Heated +1°C
Heated +1°C
Heated +2°C

Heated +2°C	11.651	0.021	0.008	7.415	—	—	—	—	—	—	3.700
Heated +2°C	8.484	0.011	0.006	5.399	—	—	—	—	—	—	2.000