Marine demosponge responses to a changing ocean; effects of sedimentation and temperature increases on *Tethya burtoni* metabolism

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"One of the penalties of an ecological education is that one lives alone in a world of wounds […] An ecologist must either harden his shell and make believe that the consequences of science are none of his business, or he must be the doctor who sees the marks of death in a community that believes itself well and does not want to be told otherwise." - Leopold (1966).

Abstract

As atmospheric CO_2 levels currently increase at an unprecedented rate, the effects of climate change are increasingly recognized as a significant threat. Changes to climate are predicted to influence natural systems, including increased storm events, that for an erosion prone country like New Zealand means exacerbated sedimentation in coastal habitats. Additionally, climate change is increasing sea surface temperatures globally and will continue to do so in coming decades. More studies investigating the effects of climate related pressures on marine sponge physiology are needed. These unique invertebrates are a diverse, ubiquitous and functionally important group. Studies examining climate change effects on sponges tend to investigate tropical species and in this regard, limited work has been done in New Zealand.

This research investigates how the pressures of increasing sea surface temperature and sedimentation affects the metabolism of the temperate demosponge, *Tethya burtoni*. Aquaria based experiments were conducted while ancillary *in situ* data investigated current day temperature and sedimentation in various *T. burtoni* habitats. Experiments addressed the immediate respiration response of *T. burtoni* under four sediment concentration treatments (ambient loads; 20 and 100 mg l^{-1}, storm proxy loads; 500 and 1000 mg l^{-1}) and four sediment grain size classes (<500-250 µm, <250-125 µm, <125-63 µm, <63 µm) at 500 mg l^{-1}. An additional experiment investigated the effects of long-term exposure (20-days) to fine sediments (<63 µm) at a storm proxy load (500 mg l^{-1}). Finally, the effects of IPCC projected sea surface temperature increase of low change; 2°C and high change; 4°C were investigated. Temperature treatments were based on the Tauranga mean annual sea surface temperature; 18°C and summer maximum; 23°, while IPCC projections were added to the latter giving treatments of 25°C and 27°C.

High sediment loads at storm proxy concentrations significantly reduced the respiration rate of *T. burtoni*, while ambient concentrations had no significant effect. The two finest sediment grain size classes also reduced respiration rates significantly. These results suggest a protective response to reduce further clogging of the aquiferous system. The long-term experiment did not indicate any differences in respiration in the treatment group. Supplementary observations indicate that this

may have been due to an issue with aquaria conditions. Sedimentation results *in situ* found that *T. burtoni* habitats experience varying amounts of sedimentation, though grain size compositions show similarity across sites. The temperature experiment indicated that 25°C and 27°C had significant impacts on *T. burtoni* survival with significant disease prevalence and morphological changes. The 18°C and 23°C had no significant effect on survival despite some signs of physiological stress present in the latter. Temperature data collected *in situ* confirms experimental ranges used, though with greater variability. These results suggest that a temperature threshold between 23°C and 25°C may exist and that in the absence of adaptation or acclimation, *T. burtoni* may be compromised under future conditions. The loss of sponge populations and even increased reductions in metabolic processes, as they relate to important ecosystem services such as benthic carbon flux, could have a significant effect on coastal trophic dynamics.

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Chapter 1

Introduction

1.1 Sponge diversity

Sponges constitute the phylum Porifera and until recently have been thought to occur in three main phylogenetic classes being the Hexactinellida, Calcarea and Demospongiae. However, recent molecular evidence has suggested that the Homoscleromorpha, a group thought to belong to Demospongiae are in fact phylogenetically well separated and thus constitute a fourth class of sponge (Gazave *et al.*, 2010; 2012). Despite these four classes, Demospongiae certainly dominates the currently described species, comprising 83% of all sponges (Van Soest *et al.*, 2012). These unique invertebrates are among the oldest metazoans extant on Earth (Van Soest *et al.*, 2012) with their evolutionary origins suggested to occur in the late Precambrian era (Li *et al.*, 1998; Sperling *et al.*, 2010). They have been a significant constituent of marine fauna since the early Cambrian (Li *et al.*, 1998) with over 8,500 species currently described, while twice that number are thought to exist (Van Soest *et al.*, 2012). Sponges are typically filter feeders, although carnivorous species have been identified in deep sea environments (Vacelet & Bouryesnault, 1995) and these animals are found in both freshwater and marine environments with the majority found in the latter . In the marine environment they are present in tropical, temperate and polar regions across numerous marine benthic ecosystems from the shallow reefs of the continental shelf through to the abyssal plains (Bergquist, 1978; Maldonado *et al.*, 2012).

1.2 Importance of sponges

Given the widespread diversity, distribution and abundance of sponges it is not surprising that they should play important functional roles in the environment (*see* review by Bell (2008)). Sponges variably provide ecosystem services such as impacting substrate through bioerosion (Goreau & Hartman, 1963) and reef stabilization (Wulff, 1984) while also playing an important role in benthic-pelagic coupling of carbon, silicon, nitrogen and the depletion of oxygen (Richter *et al.*, 2001; Maldonado *et al.*, 2005; Jiménez & Ribes, 2007; Perea-Blazquez *et al.*, 2012). These invertebrates also play important functional roles through the

interactions they have with other organism (*see* review by Wulff (2006)) such as facilitating primary and secondary production, providing habitat, increasing predation protection, enhancing survival of other organisms and some also act as important nursery grounds (Battershill & Bergquist, 1990; Martin *et al.*, 1992; Taylor *et al.*, 2007; Schejter *et al.*, 2012; Costa *et al.*, 2015). In addition to their functional importance, sponges, over the last 50 years have been identified as a significant vector for providing industry with useful compounds due to the diversity of secondary metabolites that many produce (Sipkema *et al.*, 2005) with more than 5300 different products known to be derived from sponges and their associated microorganisms (Laport *et al.*, 2009). This research is globally active (Mayer *et al.*, 2007) with over 200 new metabolites being reported each year (Laport *et al.*, 2009). However, despite the importance of sponges to industry, their widespread distribution and diversity and the numerous functional roles they play in the environment, sponges remain poorly represented in conservation, monitoring and large-scale research efforts as these significant functional roles are still not extensively appreciated outside the research efforts of sponge ecologists (Becerro, 2008; Bell, 2008; Bell *et al.*, 2017) and as a consequence, the resilience of sponges in a changing ocean are not sufficiently understood.

1.3 Factors structuring sponge populations

Various environmental factors have been attributed at least in part to the structuring of communities and individual species of sponges, such as substratum inclination (Preciado & Maldonado, 2005), wave action (Carballo & Ávila, 2004), sedimentation (Abdo *et al.*, 2006), light level (Wilkinson & Trott, 1985), temperature (Duckworth & Battershill, 2001; Leys *et al.*, 2004), depth (Wilkinson & Evans, 1989), salinity (Hopkins, 1962; Longo *et al.*, 2016) and substrate stability, type and orientation (Bell & Barnes, 2000a; Zucht *et al.*, 2008). A recurrent pattern in studies investigating the structuring of sponge distribution and abundance is the difficulty in predicting the community composition at a particular site based solely on the abiotic factors the communities experience (Zea, 2001; Wulff, 2012). Even sites in close geographical proximity may experience significantly different sponge community compositions and abundances (Schlacher *et al.*, 2007) and indeed patterns in distribution often appear stochastic. The factors driving sponge distribution, abundance and community composition are multidimensional and

although abiotic factors are first order filters dictating sponge distribution, biological interactions have also been demonstrated to be an important driver affecting the structuring of sponge populations (Wulff, 2012). Interactions with other organisms that have been shown to affect sponge distribution are numerous and can either enhance or constrain distribution. This can include symbiotic relationships (Carballo & Ávila, 2004; Zucht *et al.*, 2008; Wulff, 2012), predation on sponge spatial competitors (Cebrian & Uriz, 2006), direct predation on sponges (Maldonado & Uriz, 1998), food availability and type (Ribes *et al.*, 1999), direct spatial competition (Bell & Barnes, 2003) and disease (Cervino *et al.*, 2006). Variability in the distribution and abundance of sponges can impact other organisms that rely on these animals or are affected in some way by their presence (Wulff, 2006). It is therefore increasingly important to understand how sponges will respond to anthropogenic pressures and what the consequences are to their own population dynamics, those of other organisms they interact with and the wider ecosystem. The wide distribution of sponges is a consequence of an exceptionally long evolutionary history and adaptive responses to various abiotic and biotic factors. This also means that some may fare better in a changing ocean than others and may consequently become more widely distributed and more abundant while others regress. This also means that assessing functional diversity with diversity classifications based on trophic levels may be inappropriate for sponges. Low functional redundancy of individual species in sponge communities (as a consequence of differential uptake of picoplankton food sources) has been demonstrated (Perea-Blazquez *et al.*, 2013). The loss of some sponge species may have implications in terms of the functional roles they play in the environment that may not be fulfilled by another species. This highlights the concept that even these seemingly passive filter feeders that were for a long time believed to be unselective in their feeding, can in fact be unique players in their ecological interactions and that assessing individual species and how they may fare in a changing ocean could be of greater importance than has been previously recognized.

1.4 Sponge physiology

Despite sponges clearly being widely distributed and playing a plethora of important functional roles in benthic marine environments, studies of their physiology receive relatively little attention with only around 0.7% of published

research on sponges pertaining to this topic (Becerro, 2008). This does vary regionally and Australasia has been identified as an ecoregion giving particularly little focus on studies of sponge physiology (Bell *et al.*, 2015a). In contrast, studies investigating aspects of sponge ecology, systematics and molecular biology dominate the literature, particularly the former (Becerro, 2008); ecology and taxonomy dominate published literature in Australasia (Bell *et al.*, 2015a). Adding to this is the relatively small proportion of research focusing on how sponge physiology is altered and influenced by anthropogenic pressures, particularly interactive effects of multiple pressures and what the consequences of this are. Additional data is needed that assesses the impacts of anthropogenically induced stressors on sponges as this is a critical gap of information in understanding the conservation status of sponges at a global level (Bell *et al.*, 2015a) and is the primary motivation for the research conducted in this thesis. Respiration was selected as the primary response variable under the stressors of sedimentation and increased temperature since it is relatively easy to measure compared to other physiological characteristics such as growth, feeding and reproduction. Since oxygen is required for all energetic processes in the body of aerobic organisms, the consumption of oxygen indicates the total expenditure of energy, allowing respiration rate as a proxy for metabolic demand. There are many physiological aspects of a sponge that contribute to metabolic demand such as feeding, reproduction, growth and chemical or structural defence and these aspects as they relate to sponges are discussed below.

1.4.1 Sponge body plan

In understanding the physiology of sponges, it is first worth discussing their body plan since it is quite unique. Sponge morphology can vary greatly in the degree of internal and external complexity (Bergquist, 1978). Typically, however, sponges are comprised of an outer layer of cells called pinacocytes that form the outer layer of the sponge called the pinacoderm. Imbedded in the pinacoderm are ostia, approximately 200 µm in size (Bergquist, 1978) which are small openings where surrounding water is drawn through by the beating of flagellated collar cells called choanocytes. These line the interior surface of the sponge called the choanoderm and this is the mechanism that allows the pumping of water through the sponges aquiferous system and out of the exhalant osculum (Battershill *et al.*, 2010) (Figure

1.1). Between these two "dermal" layers is an area comprised of collagenous material and polysaccharides that make up the mesohyl. Many mobile cells are located in the mesohyl including archaeocytes; totipotent cells that can change into any other type of specialised cell in the sponge while also playing a role in the transport of food items, repair and defence (Battershill *et al.*, 2010) (Figure 1.1). Sponges can take various body forms, such as thin or thick encrusting, massive, globular or as erect fingers or lobate shapes either branching in a dendritic array or in an anastomose form (Battershill *et al.*, 2010). Morphology can reflect the degree of water movement in the environment (Abraham, 2001) with encrusting forms common at exposed sites while erect forms have been found at more sheltered sites (Roberts *et al.*, 2006a). Since the sponge aquiferous system unselectively takes in water and suspended particulates smaller than ostia openings (though may selectively uptake preferential food particles (Bell *et al.*, 1999; Perea-Blazquez *et al.*, 2013)), it is at the mercy of the water column. Changes to particulates in the water such as increased sediment particles may be problematic for the optimal functioning of this pump like mechanism for many species.

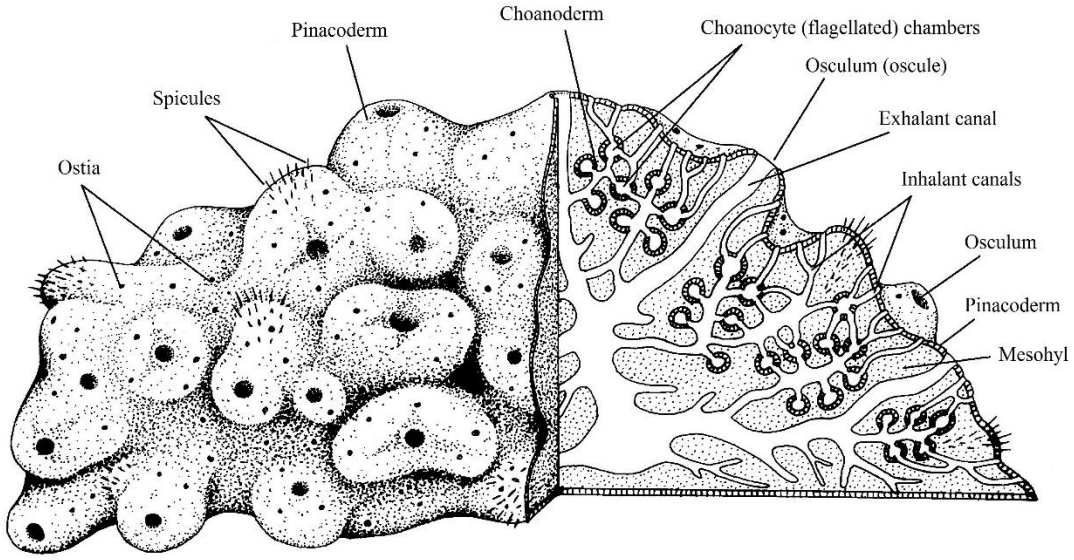

Figure 1.1: Schematic representation of sponge with associated cell type components indicated (after Battershill *et al.* (2010)).

1.4.2 Pumping

The pumping of water through sponges is an important component of their physiology as it is linked directly to important physiological processes such as

feeding, respiration, expulsion of waste products and reproduction (Bergquist, 1978). As mentioned above, the water flow generated through the sponge aquiferous system is due to flagellated choanocyte cells. Sponges have the capacity to pump large volumes of water filtering the equivalent of the overlaying water column in less than one to 56 days depending on the water depth, density and community composition (Reiswig, 1974; Pile *et al.*, 1997; Savarese *et al.*, 1997; Strehlow *et al.*, 2016). Currently <0.01% of described sponges have had their pumping rates assessed (Strehlow *et al.*, 2016). Initial studies investigating pumping in sponges found that rates were essentially constant over time (Jørgensen, 1955; 1966). However, more recent work has found that this is not always the case. In *ex situ* conditions, flow rate of the ambient water has been shown to affect sponge pumping rates with unusual levels of flow either decreasing or increasing filtration capacities (Yahel *et al.*, 2005; Hadas *et al.*, 2008; Maldonado *et al.*, 2012) and *in situ*, ambient flow rate in the environment is correlated with flow rates through the sponge for certain species (Vogel, 1977; 1978). The energetic costs of pumping activities for filter feeders, including sponges, has been considered to be relatively inexpensive with <4% of metabolic expenditure attributed to mechanical pumping (Riisgard & Larsen, 1995). However, for the deep-water sponge *Aphrocallistes vastus* ambient flow rates have been shown to be utilized for passive flow through the aquiferous system and thus reduce the energy expenditure required for mechanical pumping which was calculated to cost around 33% of total metabolic expenditure (Leys *et al.*, 2011). The use of ambient currents for passive flow through this sponge (and likely many others) is not only possible but in fact vital to its success and the authors called for a re-evaluation of the energetic costs of pumping. They called for greater consideration of the mechanisms involved such as the glycocalyx mesh on the collars of sponges that significantly increases water flow resistance (Leys *et al.*, 2011) that, until then, had received little consideration in pumping energy budgets. This may seem a trivial detail at first, however if energy budgets are incorrect, the full extent of how these physiological processes operate under pressure and the subsequent consequences may be grossly underestimated. Pumping in sponges is highly variable between species and pumping rates have been shown to be affected by microbial associations, with sponges having high microbial abundances showing up to 94% slower pumping rates than those with low microbial abundances (Weisz *et al.*, 2008). In addition to ambient flow and

microbial associations, pumping rates and rhythmicity has also been shown to be affected by increasing temperature that may either increase (Riisgard *et al.*, 1993) or decrease (Massaro *et al.*, 2012) pumping activities. Suspended particles have been shown to cause reductions or a complete arrest in pumping activities (Gerrodette & Flechsig, 1979; Leys *et al.*, 1999) and has also been shown to induce a re-start and stop activity in the sponge *Rhabdocalyptus dawsoni* seemingly as if re-testing the water for sediments (Leys *et al.*, 1999). Although normal pumping returned, prolonged exposure to suspended particles may significantly impact other physiological processes in the sponge that are inextricably linked to its pumping activities such as respiration, feeding and reproduction.

1.4.3 Respiration

Respiration is the process by which oxygen is taken in and carbon dioxide is removed from an organism (Abedon *et al.*, 2008) and the rate of respiration is estimated through the consumption of oxygen with this technique having been used for almost a century (Clarke, 1991). In aerobic organisms, oxygen is required for all energetic processes in the body and therefore the consumption of oxygen indicates the total expenditure of energy meaning that respiration rate is a proxy for metabolic demand and can be measured either under normal conditions or under stress (Hadas *et al.*, 2008). The exchange of oxygen and carbon dioxide between a sponge and its environment occurs through diffusion (Bergquist, 1978) since they lack any specialised respiratory surfaces. This occurs along cell surfaces at the exterior of their body, lining of inhalant canals and at cellular surfaces of choanocytes and collar tentacles (Bergquist, 1978). Like pumping rates, the respiration rates of sponges may vary considerably between (Osinga *et al.*, 1999) and within species (pumping versus non pumping individuals) (Schläppy *et al.*, 2007). Respiration can be measured in various ways. A very informative but complex method is the use of radioactive carbon isotopes to assess carbon conversion rates that act as a proxy for respiration (Koopmans *et al.*, 2011) and the fate of this carbon in the sponge tissue (De Goeij *et al.*, 2008). However, if the fate of carbon within the sponge lacks relevance for a given study, a far more time and cost-effective method for assessing respiration may be used by measuring oxygen decline with oxygen meters. The later method has been employed in the research pertaining to this thesis.

1.4.4 Feeding

Small particles both biotic and abiotic are able to remain in suspension in seawater due to physical properties and thus a niche for suspension feeders is created. Micro-organisms and small phytoplankton dominate these communities and suspension feeders consume these (Gili & Coma, 1998). With the exception of carnivorous species, sponges as filter feeders rely on suspended food particles in the water column for energy and nutrition. Sponge filter feeding occurs through the pumping of water through the aquiferous system and generally food items are captured by pseudopodia at the choanocyte and subsequently transferred to amoebocytes to be digested and distributed to other cells in the sponge (Leys & Hill, 2012). Sponge feeding, as with respiration and pumping rates, can be highly variable between species with many factors influencing the ingestion, assimilation and excretion/egestion of organic matter. Factors include but are not limited to size and morphology of the sponge (Vogel, 1978), seasonality and food availability (Ribes *et al.*, 1999) and symbiotic relationships (Vacelet *et al.*, 1996; Van Duyl *et al.*, 2008). The first *in situ* research on sponge feeding ecology was conducted by Reiswig (1971a) on three tropical marine Demosponges with results indicating particulate organic carbon (POC) being an important component of the diet of these sponges. Since then, various studies have contributed to elucidating the feeding ecology of sponges *in situ*. Sponges have been shown variably to take up POC as both living particulate organic matter (LPOC) and detrital particulate organic matter (POC_{det}) (Hadas *et al.*, 2009). Some selectivity in sponge feeding has been observed (Bell *et al.*, 1999), as has resource partitioning between different sponge species inhabiting the same community (Perea-Blazquez *et al.*, 2013). Dissolved organic matter (DOM) contributing to the diet of sponges has been studied to a lesser extent. Evidence of sponges retaining viruses <0.2 μm that fall into the DOM size class has been provided (Hadas *et al.*, 2006), and Yahel *et al.* (2003) provided the first *in situ* quantitative evidence for bulk dissolved organic carbon (DOC) retention by the sponge *Theonella swinhoei* contributing the largest proportion of carbon source for this sponge. De Goeij *et al.* (2008) gave the first direct evidence of DOM being incorporated into the coral reef sponge *Halisarca caerulea* using ^{13}C enriched DOC and POC with DOM also contributing to the majority of the sponge diet. Additionally, retention efficiencies have been observed to drop with an increase in particle size (Duckworth *et al.*, 2006). In a review by Gili and Coma (1998) it was

shown that sponges can ingest between 29-1800mg C m^{-2} sponge day^{-1} demonstrating that rates are variable but that sponges do have the capacity to remove substantial amounts of organic carbon from the ambient water indicating that they play an important role in benthic-pelagic carbon flux. These variations between species and the trophic plasticity seen in some, are synonymous with the large variations in sponge appropriate food sources across temporal and spatial scales. This may be one of the key factors contributing to the global ubiquity of sponges (Maldonado *et al.*, 2012). Fine sediments can fall into the same size ranges of food particles ingested by sponges and thus may clog filtering systems. There is little information available on how the feeding of sponges is affected by sediments but Lohrer *et al.* (2006a) demonstrated that filtration rates of *Aaptos* spp. were significantly reduced under increasing sediment deposition treatments. Filtration capacity has been demonstrated to rise with increasing temperature (Riisgard *et al.*, 1993) and under climate change scenarios, sponge food sources may themselves be altered.

1.4.5 Reproduction

Reproductive strategies among sponges vary significantly and members of the Porifera phylum may reproduce both sexually and asexually. In terms of sexual reproduction, sponge gamete production can arise from both gonochoristic or hermaphroditic sources and development can be either oviparous or viviparous with both methods generally resulting in free-swimming larvae that eventually settle and give rise to sessile juveniles (Maldonado & Riesgo, 2008). Asexual reproductive methods include budding, the formation of gemmules or simply regenerating post fragmentation (Bergquist, 1978). Buds are cell masses that grow at the external surface of a sponge and subsequently separate through the constriction of the tissue joining the bud and paternal body (Maldonado & Riesgo, 2008). Bud formation is common in the *Tethya* genus and incidence has been shown to increase in summer months for some species (Cardone *et al.*, 2010) while becoming compromised under thick sediment layers (Gaino *et al.*, 2006). Gemmules are formed internally and are dormant, highly resistant bodies composed of a dense mass of storage cells and archaeocytes protected by a thick covering (Maldonado & Riesgo, 2008). Gemmule formation is more common among freshwater and brackish water sponges, but still occurs in some marine species as well (Simpson, 1984) and are

thought to provide a means of assurance for population restoration after serious mortality due to unfavourable conditions (Maldonado & Riesgo, 2008). Environmental cues have been suggested to be important for the initiation of reproductive processes in some species and may include light (Elvin, 1976), food availability (Witte, 1996) and wave height (Abdo *et al.*, 2008). However, temperature has been suggested to be one of the most fundamentally important cues for sponge reproduction for both sexual and asexual strategies in environments with variable temperatures (Maldonado & Riesgo, 2008). Temperature cues can either be in the form of dropping (Fromont & Bergquist, 1994) or increasing temperatures (Usher *et al.*, 2004) and under future climate scenarios changing temperatures may pose serious challenges to some sponges that rely on specific temperature cues. Of relevance to the aims and approach in this thesis, is the observation that reproductive state could influence respiration, especially in those species that undergo massive cellular reorganisation in a process of budding. This has implications in selection of sponges for experimental assessments.

1.4.6 Growth and development

Individual species of sponges can have various morphologies and develop into particular forms in response to environmental factors (Kaandorp, 1999; Abraham, 2001). This morphological plasticity of sponges is considered to be a survival strategy (Gaino *et al.*, 1995) with morphology dependant on the environment in which a sponge settles in (Meroz-Fine *et al.*, 2005). This plasticity is true also for their structural components such as spicule size and quantity that have been shown to increase in response to higher levels of wave action (Palumbi, 1986). While sponges can have a diverse morphological plasticity that allows them to adapt to the environment, limitations in their body plan can constrain this ability (Palumbi, 1986). Growth rate can be attributed to various factors. For example biological interactions including spatial competition can constrain growth (González-Rivero *et al.*, 2012), while environmental factors such as temperature can also affect growth rates, resulting in increased growth in summer months when temperatures are warmer (Elvin, 1976; Barthel, 1986). Sponge growth may also be compromised by environmental factors such as suboptimal sediment loading that has been demonstrated to cause weight loss in some species (Abdo *et al.*, 2006; Roberts *et al.*, 2006b). The growth of sponges is generally considered to be a slow process

(Bergquist, 1978). Energy required for growth appears to vary greatly between species, with several studies reporting conflicting results where anywhere between <1-100% of energy from respiration is needed for growth (Riisgard *et al.*, 1993; Thomassen & Riisgard, 1995; Hadas *et al.*, 2008). Since the energetic requirements for growth in sponges appears to vary widely depending on the species, how anthropogenic pressures may affect this component of sponge physiology is also likely to vary considerably.

1.5 Anthropogenic threats to sponges

Anthropogenically derived pressures in the coastal marine environment can occur on local and global scales and can include pollution, nutrient loading, overfishing, facilitating dispersal of invasive marine species, shipwrecks, sedimentation and climate related pressures such as acidification and sea surface temperature increases to name a few (Halpern *et al.*, 2008; Mieszkowska *et al.*, 2014). In a global perspective, sponges, as with most marine organisms, face or will face one or more of these anthropogenic pressures. Despite the widespread distribution of the Porifera phylum, the understanding of these human induced impacts on sponges remains comparatively scarce, in particular how these pressures impact physiological processes (Bell *et al.*, 2015a) and only recently has research begun to really address this issue. Having a greater understanding of how sponges will fare in a changing ocean is becoming increasingly more relevant. This thesis addresses two stressors, namely sedimentation and sea surface temperature change and what the implications of this are for a sea sponge inhabiting New Zealand waters. These two stressors are discussed below to give context to the source, nature of and extent to which these issues are projected to worsen over the coming years and to provide an overview of how sponges have been shown to respond to these stressors.

1.5.1 Sedimentation

Sedimentation is a natural process that occurs in coastal marine environments and is largely due to erosion either coastally or as a result of terrestrial erosion entering riverine systems that subsequently ends up in coastal waters (Airoldi, 2003). Due to this natural process, marine organisms are expected to experience suspended and settling sediments to some degree and this can be a significant factor contributing to the distribution of species (Airoldi, 2003). However, on a global scale there is

growing evidence that the quantity, size and type of sediments that marine organisms may encounter are changing due to a larger amount of sediment from land-based sources entering riverine systems and consequently arriving into coastal marine habitats (Thrush *et al.*, 2004; Walling, 2008; Bell *et al.*, 2015b). The causes of increased sediment loading in coastal environments are various, but mostly relate to anthropogenically induced changes to land use, particularly agricultural intensification and deforestation (Syvitski *et al.*, 2005) while desertification, coastal urbanisation, the alteration of rivers and increasing storm frequency and severity also play a role. Anthropogenic activities in the marine environment such as maintenance dredging and benthic fish trawling can also cause increased sedimentation through re-suspension of sediments and smothering from the dumping of dredge spoil (Airoldi, 2003; McKergow *et al.*, 2005; Syvitski *et al.*, 2005; Fettweis *et al.*, 2010; Schonberg, 2016). It is worth stating that sediments in coastal marine environments may also be reduced due to diversion and damming and this can certainly have a profound impact on coastal ecosystems as well (Thrush *et al.*, 2004). Efforts are made in some areas to reduce the level of sediment entering rivers and coastal habitats through the implementation of storm water systems (Bratieres *et al.*, 2008) and planting of riparian vegetation along streams and rivers to filter out sediments (Richardson *et al.*, 2007). The amplified loading of terrestrial sediments is increasingly recognized as a threat to aquatic coastal environments, yet a solid understanding of what this means for coastal marine ecosystems still requires additional investigation to facilitate informed management practices, particularly at regional scales (Fredston-Hermann *et al.*, 2016).

1.5.1.1 Sedimentation in New Zealand

New Zealand is a country that experiences naturally high levels of erosion and sedimentation (Basher, 2013). The country lies at the Pacific and Australian tectonic plate boundary and thus high rates of uplift, frequent earthquakes and erodible weakly lithified rock are common (Soons & Selby, 1992). Rainfall per annum can range from 500-1000 mm yr^{-1} (Basher, 2013) and frontal storms and extra-tropical cyclones characterize the climate that bring high rainfall and are often the trigger for much of the erosion (Glade, 1998). Erosion in New Zealand sees around 200 megatonnes of soil being delivered into the ocean per annum (Hicks *et al.*, 2011) and while New Zealand only makes up around 0.1% of global land mass,

the country contributes around 1-2% of the annual discharge yield of global sediments into the ocean (Hicks *et al.*, 1996). Due to these properties, erosion in New Zealand is easily amplified through changes to land use such as deforestation and the introduction of grazing animals in large numbers that is further exacerbated by intensive land use in some areas (Page *et al.*, 2000; Glade, 2003). New Zealand coastal waters including harbour environments in ambient conditions may have sediment concentrations at 100 mg l^{-1} or less (Dyer, 1986; Ellis *et al.*, 2002) and during storm events can reach around 1000 mg l^{-1} (Fahey & Coker, 1992). In times where major flooding occurs, sediment plumes can spread over significant areas of coastline (Figure 1.2) and very fine-grained sediments can be transported tens of kilometres off shore and along the coast by tidal currents and wave action (Foster & Carter, 1997; Lohrer *et al.*, 2006a; Tuckey *et al.*, 2006). In the Bay of Plenty, where research for this thesis took place, sedimentation due to land-use is predicted to reduce slightly by 2051 in the Tauranga Harbour due to lower yielding urban land use replacing pasture land use (Hume *et al.*, 2010). However, modelling predictions have suggested that increased storm frequency and severity due to climate change is likely to increase contributions by up to 42.8% by 2051 (Hume *et al.*, 2010). Hume *et al.* (2010) also point out that 95% of fine sediments entering the Tauranga Harbour are moved offshore. These increased levels of fine terrigenous suspended sediments may pose a threat to marine organisms, including sponges, inside and outside the harbour. While estuaries and harbours can experience a greater concentration of settled and suspended sediments than those living in marine environments further afield, Lohrer *et al.* (2006a) have demonstrated that communities and conspecifics further afield from subtidal harbours had a greater sensitivity to sediments of the same concentration. Therefore, while the concentration may be diluted to a greater extent, the effects it has on the organisms inhabiting these areas may be just as great due to a reduced tolerance of these types of sediments. With regard to the anthropogenic threats New Zealand faces in the marine environment, sedimentation is among the top four threats being on par with seabed trawling and surpassed only by ocean acidification and sea surface temperature rise (MacDiarmid *et al.*, 2012) and is certainly an area that requires additional investigation.

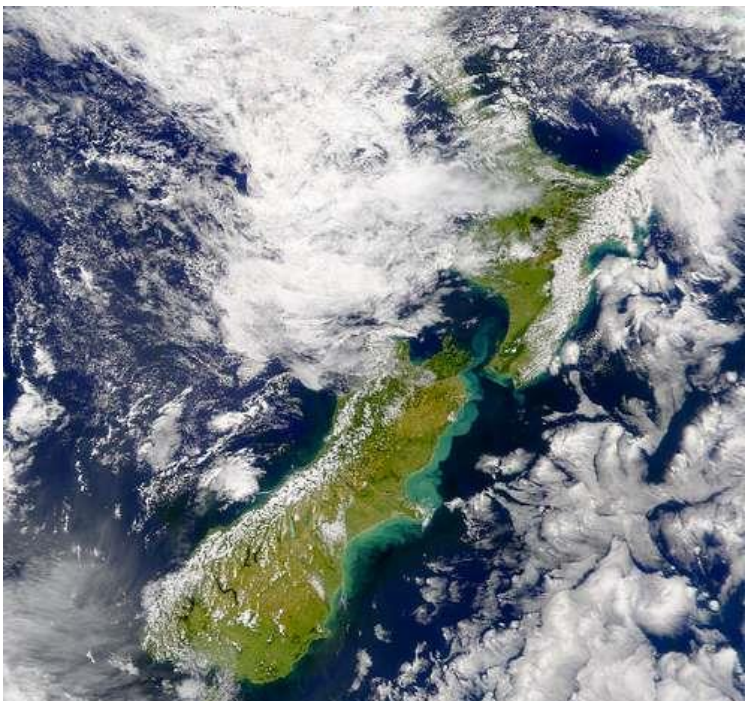

Figure 1.2: Coastal sedimentation here seen as turquoise plumes extending out from the coastline of New Zealand (Courtesy of NASA (*see* http://visibleearth.nasa.gov/)).

1.5.1.2 Marine sponges and sediment

How sedimentation affects sponges has been investigated to some degree and has mostly shown to negatively affect pumping activities (Reiswig, 1971b; Leys *et al.*, 1999), respiration (Lohrer *et al.*, 2006a; Murray, 2009; Bannister *et al.*, 2012; Tjensvoll *et al.*, 2013; Kutti *et al.*, 2015; Biggerstaff *et al.*, 2017), feeding (Lohrer *et al.*, 2006a), reproduction (Roberts *et al.*, 2006b; Whalan *et al.*, 2007), growth (Abdo *et al.*, 2006; Roberts *et al.*, 2006b) and symbiotic relationships (Cheshire *et al.*, 1995; Roberts *et al.*, 2006b). The response of marine sponges to sedimentation is thought to have a generally negative impact (*see* review by Bell *et al.* (2015b)), although some species of sponge do have positive relationships with sediments (Schonberg, 2016). In certain cases, sedimentation has been shown to correlate with increased species diversity (Bell & Barnes, 2000b) and soft bottom specialists have be shown to be very resilient to sedimentation (Ilan & Abelson, 1995). In fact, some formally described species have references to sediments in their naming and it is estimated that around 10% of marine sponges are equipped for sedimentation and turbid conditions (Schonberg, 2016). It may be these species that increase in their distribution and abundance under higher sediment loadings while more sensitive

species regress. With that being said, the mineralogical nature of sediments, even when grain sizes are similar and quantities the same, have been shown to impact sponges differently (Kutti *et al.*, 2015) likely due to the physical properties of the sediments (Bannister *et al.*, 2012). This may mean that even potentially sediment tolerant sponges may not cope with drastic changes in the source of sediments. With regard to respiration, studies have typically focused on the concentrations of sediments and while most report grain size composition, different grain size classes and the effects of respiration have not been explicitly studied. Nevertheless, the consensus is that there is still not a sufficient understanding of tolerance levels and how sponges respond to sedimentation with respect to producing adequate recommendations for environmental monitoring and assessment (Bell *et al.*, 2015b; Schonberg, 2016). The degree of impact and the resilience of sponges to sedimentation can be very species specific and New Zealand sponges have a high level of endemism (Towns & Ballantine, 1993; Hooper & Lévi, 1994), yet few published studies have investigated the implications of sediments on New Zealand sponges but see Lohrer *et al.* (2006a) and Murray (2009). It is therefore important to gain a greater understanding of how sponges inhabiting New Zealand will cope under increasing sedimentation scenarios as this is a particularly relevant issue facing New Zealand, a country that is prone to erosion (Basher, 2013).

1.5.2 Climate change and sea surface temperature rise

Climate change presents a significant threat to natural systems including the ocean and the Intergovernmental Panel on Climate Change (IPCC) confirms that anthropogenic influence on the climate is clear and growing and are 95% confident global warming is due to anthropogenic activities (IPCC, 2013). The global carbon cycle consists of geochemical reservoirs responsible for carbon storage on Earth and also includes the mechanisms by which carbon is transported between them (Heimann, 1993). Through anthropogenic extraction and burning of fossil fuels originating in the lithosphere, carbon dioxide (CO_2) is emitted. These CO_2 emissions deplete the lithosphere of approximately 6.3 Pg C yr^{-1} (Houghton *et al.*, 2001). This is by far in excess of the natural rate of CO_2 that is generated from the lithosphere combined with the global carbon sedimentation rate which is comparably low at around 0.3 Pg C yr^{-1} (Sundquist, 1993; Hedges & Oades, 1997). This has led to a rise in atmospheric CO_2 concentrations that at the beginning of the

Industrial Revolution sat at ~280 ppm, while present day levels sit at ~400 ppm (Rees, 2012). Forecasting suggests a rise to at least 430 ppm by 2030 and 750 ppm by 2100 (Rees, 2012) with a rise to 450 ppm having been suggested as a critical threshold beyond which irreversible and catastrophic change may occur (Hansen *et al.*, 2007). While the climate of Earth has historically had greater CO_2 levels than what is currently experienced (Pearson & Palmer, 2000), concentrations are currently increasing at an unprecedented rate (Siegenthaler *et al.*, 2005) of over 2 ppm per annum that exceed IPCC worst case scenarios (Brierley & Kingsford, 2009). The climate is strongly influenced by the amount of greenhouse gases in the atmosphere (Charlson & Schwartz, 1992) and rising CO_2 levels are responsible for an observed 0.2°C rise in global temperature per decade over the last 30 years alone (Hansen *et al.*, 2006). Rising CO_2 levels indicate an inability of natural systems to capture excess CO_2, yet it is estimated that atmospheric CO_2 concentrations would be 55% greater than the observed changes thus far if it weren't for the oceans having absorbed ~30% of anthropogenically liberated CO_2 emissions (Canadell *et al.*, 2007; Gruber *et al.*, 2009). Despite this buffering capacity of the oceans, increasing CO_2 levels can have various effects in the marine environment including ocean acidification, changes to biogeochemical processing, sea level rise, melting of polar ice caps, changes to ocean currents, potential stratification in certain locations, deoxygenation, amplified severity and frequency of storm events and increased water temperature (Figure 1.3) (Harley *et al.*, 2006; Brierley & Kingsford, 2009; Gruber, 2011). Globally, sea temperature change is considered the most pervasive present day impact on marine ecosystems (Halpern *et al.*, 2008) with a global rise of 0.4°C observed for sea surface water since the 1950's (Levitus *et al.*, 2009) though temperature increases do vary spatially (Burrows *et al.*, 2011; Cheung *et al.*, 2013). The IPCC reports predictions for increases of sea surface temperature under various future emission scenarios by the year 2100. The total range of predicted sea surface temperatures based on these scenarios encompass increasing temperature ranges from 1.1°C to 6.4°C. The best estimate under a low change scenario is a 1.8°C increase and under a high change scenario a best estimate of a 4°C increase is predicted (IPCC, 2007; 2013). These changes in sea surface temperatures pose a significant threat to marine flora and fauna into the future, with large scale effects already becoming evident such as mass bleaching events of corals on the Great Barrier Reef in recent years (Ainsworth *et al.*, 2016).

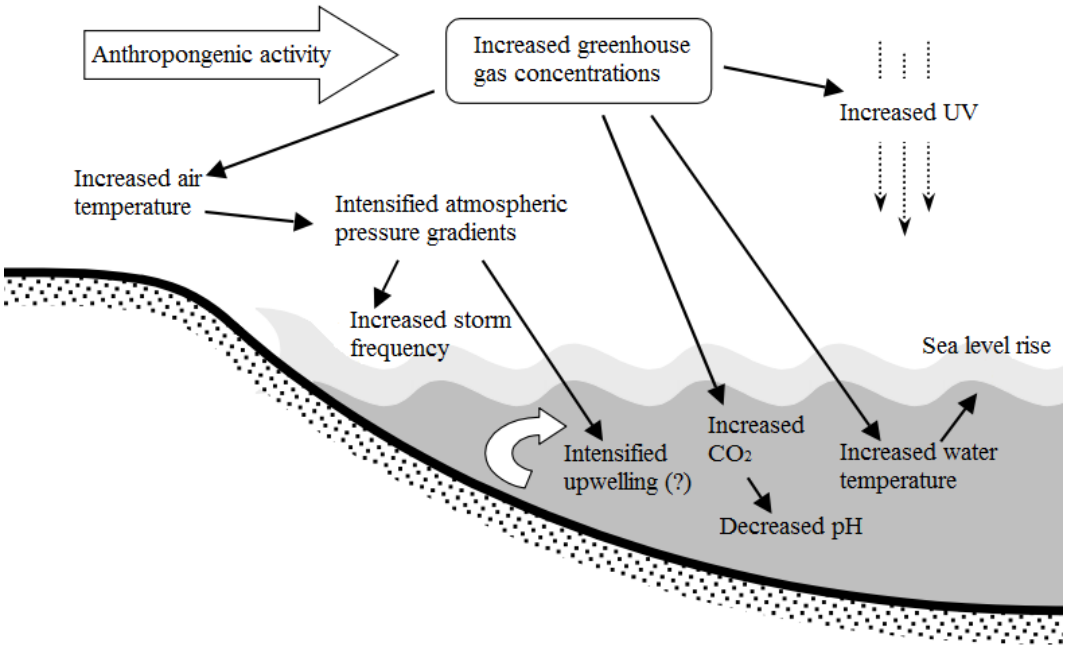

Figure 1.3: Conceptual diagram demonstrating the potential for cascading changes in physical ocean parameters as a result of anthropogenically liberated greenhouse gases (Harley *et al.*, 2006).

1.5.2.1 Sea surface temperature change in New Zealand

New Zealand has the world's fourth largest exclusive economic zone and sea surface temperature increase has been identified as the second highest threat to New Zealand's marine systems, second only to ocean acidification (MacDiarmid *et al.*, 2012). This poses a significant threat to marine organisms, since temperature is regarded as one of the most pertinent abiotic factors influencing the distribution (Marshall *et al.*, 2012), biodiversity (Clark *et al.*, 2017), behaviour (Kidawa *et al.*, 2010) and physiology (Sorte *et al.*, 2011) of marine invertebrates. Sea surface temperatures have increased by 0.6°C around the northern coastlines of New Zealand over the last 30 years (Schiel, 2013) and this rise is consistent with the observed global mean temperature increase (Hansen *et al.*, 2006). Off the coast of Tauranga where research for this thesis took place, sea surface temperatures have an annual mean of 18°C dropping as low as 14°C during winter months and peak to 23°C during summer time (Worldseatemperature.org, 2017). Under worst-case high change IPCC predictions, temperatures could reach 27°C in summer months by the end of the century. Habitats in the intertidal zone may be subject to the

greatest impact (Helmuth & Hofmann, 2000) and this would potentially have serious consequences for ecosystem function (Bracken *et al.*, 2008).

1.5.2.2 Marine sponges and sea surface temperature

How increasing temperature beyond normal ranges affects sponges has been investigated to some degree and has mostly shown to have negative effects. Mass mortality events have been observed to coincide with rapid increases in sea surface temperatures in shallow water Mediterranean ecosystems (Cerrano *et al.*, 2000; 2001; Cebrian *et al.*, 2011) and in deep sea cold water coral reef habitats (Guihen *et al.*, 2012). Increasing sea surface temperatures have been observed to disrupt symbiotic relationships for species with photosynthetic symbionts causing a bleached appearance (Williams *et al.*, 1987; Fromont & Garson, 1999; Hill *et al.*, 2016; Bennett *et al.*, 2017) and also alter the microbiomes for non-photosynthetic species with substantial consequences for the host sponge (Webster *et al.*, 2008; Luter *et al.*, 2012). Additionally, disease has been linked to elevated sea temperatures (Webster, 2007) and greater sensitivity has been shown for larval/juvenile sponges (Byrne *et al.*, 2011), while the opposite is also true for some species, with adult sponges displaying greater sensitivity (Bennett *et al.*, 2017). Metabolic demand has been shown to increase in warmer conditions (Fang *et al.*, 2014), with respiration rates elevated for sponges in higher temperature treatments (Bates, 2015; Bennett *et al.*, 2017; Strand *et al.*, 2017). While many sponges have been shown to be adversely affected by increasing sea surface temperatures predicted for the end of the century, some species have also been shown to be quite thermally tolerant (Whalan *et al.*, 2008; Webster *et al.*, 2011; Duckworth & Peterson, 2012). This is truer for sponges that experience a naturally greater variability in temperature (Duckworth *et al.*, 2012), such as those inhabiting intertidal communities (Halpin *et al.*, 2002). It may be these species that increase in their distribution and abundance under increasing sea surface temperatures while more sensitive species may regress. Much work is focused around tropical species since marine taxa in temperate regions have been shown to have an increased tolerance to thermal stress (Sunday *et al.*, 2011) since these areas typically exhibit a greater natural variation in temperature and organisms must be able to withstand this on daily and season cycles (Muller-Parker & Davy, 2001). Therefore, this environmental attribute may enhance their resilience to temperature fluctuations.

This area of research is gaining traction as the implications for sponges under environmental degradation are increasingly recognized as an important and understudied area of research.

1.6 Thesis Research

The research presented in this thesis focuses on a temperate marine Demosponge found in New Zealand waters and its response to anthropogenically mediated stressors of sea surface temperature rise and sedimentation. The common golf ball sponge, *Tethya burtoni*, Hadromerida, Tethyidae (Sarà & Sarà, 2004) (Figure 1.4) is a spherical orange sponge that can be found on many coastlines around New Zealand, also occurring in harbour environments and has a depth range typically from the intertidal to shallow subtidal around 20 m deep (Kelly, 2015) although specimens have been recorded beyond 100 m depth on the Chatham Rise (Kelly & Buckeridge, 2005). This sponge is common and abundant reaching a size of around 4 cm diameter (Battershill *et al.*, 2010) though may reach sizes up to 6 cm in diameter (Kelly, 2015). *T. burtoni* along with other members of the *Tethya* genus were originally ascribed to the species *Tethya aurantium* (Bergquist & Kelly-Borges, 1991), a very similar sponge but different in skeletal structure. As such, *T. aurantium* only occurs in the Mediterranean Sea, Azores and doubtingly in the Caribbean (Sarà & Sarà, 2004). Sponges have a reputation for being notoriously difficult to classify and overhauls in the systematics of sponges are not uncommon (Hooper & Van Soest, 2002), especially with the recent advances of molecular techniques (Morrow & Cárdenas, 2015). The *Tethya* genus is rich in New Zealand and Australian temperate coastal waters with a high degree of endemism in these regions (Sarà & Sarà, 2004). One third of known *Tethya* species come from temperate waters in these regions and this is surprising since only a few species are known for northern hemisphere temperate seas despite a greater understanding of benthic fauna in these regions. Sarà (1998) suggests this may be linked to evolutionary events and that the genus may in fact originate from south-west Pacific coasts. Little research effort has investigated *Tethya* species in New Zealand waters, with the few published studies of this genus typically focusing on taxonomy (Sarà & Sarà, 2004). The functional roles of these sponges are largely unknown, as is their level of resilience in a changing ocean. Since *T. burtoni* can be found in intertidal habitats and harbour environments, it may be resilient to both increasing

sea surface temperature and sedimentation events and hence is a relevant candidate to experimentally explore a range of physical environmental tolerances.

Figure 1.4: The orange golf ball sponge, *Tethya burtoni* nestled amongst an Ancorinid sponge at Karewa Island.

1.6.1 Aims and objectives

The overarching goal of this thesis was to contribute information on how the anthropogenically induced pressures of increasing sea surface temperature and sedimentation affects *T. burtoni*. Since relatively little literature has been published on the physiology of sponges and even more so, how their physiology is affected by anthropogenic pressures, the primary response variable chosen was respiration as this signifies the metabolic energy demand of aerobic organisms under both normal and stressed conditions. In addition to this, survival and disease metrics were monitored in the temperature experiment since it was conducted over a longer time frame.

As such, this thesis aims to address the following questions through a series of experiments and observations;

1. How does the concentration of sediments affect the immediate respiration response of *T. burtoni*?

2. How do different sediment grain size classes affect the immediate respiration response of *T. burtoni*?
3. How do fine sediments affect the respiration response of *T. burtoni* over a longer period of exposure?
4. How do projected sea surface temperature increases affect *T. burtoni* respiration over a longer time frame and how does this affect disease and survival?
5. What are the sedimentation rates, sediment grain sizes and temperatures currently experienced by *T. burtoni* in differing locations in the Bay of Plenty during summer and how do these *in situ* parameters compare to the *ex situ* aquaria experiments?

1.6.2 Thesis format

This thesis is formatted in such a way that Chapter 1 serves as an introduction, discussing the importance of sponges, aspects of their physiology, the anthropogenic pressure they may face and what gaps remain in the literature. Chapter 2 describes the methods used for the collection of data and subsequent statistical analyses. Chapter 3 presents the results gained from the experiments and recording of *in situ* environmental parameters. Finally, Chapter 4 discusses the findings in the context of current literature, noting limitations of the study and directions for future research in this field.

Chapter 2

Methods

2.1 Methods overview

To address the research questions (see section 1.6.1), a total of four *ex situ* aquaria based experiments took place to address the effects of sedimentation and temperature on *T. burtoni*. Methods described from sections 2.2 to 2.5 were synonymous for at least three of the experiments and as such, have only been described once to avoid unnecessary repetition. These sections are followed by explanations of each of the four experiments. Methods described from sections 2.2 to 2.5 that are used in the experiments are referred to where relevant. Finally, methods employed for the *in situ* collection of sediment and temperature data in various *T. burtoni* habitats are described, followed by a final section outlining the statistical analyses used on all data.

2.2 Specimen collection

For each of the experiments, individual specimens of *T. burtoni* were collected from Rabbit Island (37°37'38.91"S, 176°11'53.37"E) (Figure 2.1) via SCUBA diving at depths between 12-17 m. This site was selected as it was near to the laboratory and supported a large population of *T. burtoni* (personal observation). Individuals approximately 3 cm in diameter with no apparent reproductive buds were carefully removed by means of a hammer and chisel to remove the substrate below without damaging the sponge. Individuals were placed inside a cage and left *in situ* between consecutive collection dives. On the final dive, the remaining sponges were collected and individuals were counted out and placed into durable plastic bags and taken to the surface. The bags were emptied into a large chilly bin containing seawater from the collection site taking care not to expose the sponges to the air. A battery powered transportable air bubbler was placed into the chilly bin to ensure that the water remained oxygenated during transportation. The sponges were immediately transported back to the laboratory where surface associated fouling organisms and surrounding substrate were carefully removed before being allocated randomly to aquaria.

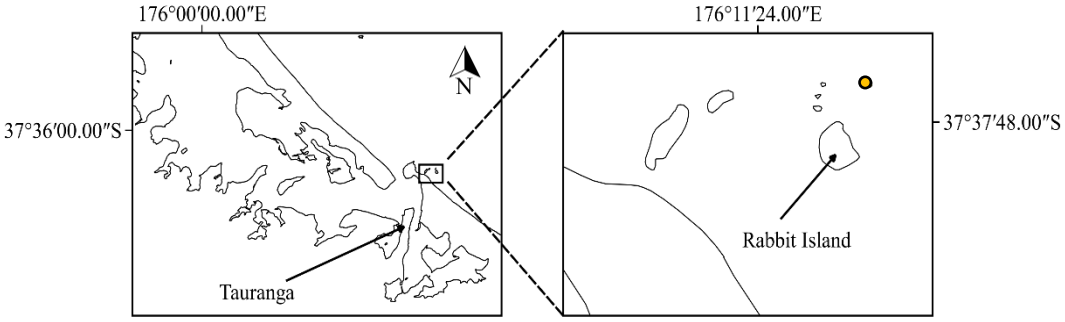

Figure 2.1: Sponge collection site indicated by yellow circle relative to Rabbit Island and the wider city of Tauranga.

2.3 Tank water collection and quality maintenance

Fresh sea water for the aquaria was regularly collected into a 1000 L bin by means of a pump approximately 20 minutes prior to high tide from the Nautilus boat ramp (37°40'14.50"S ,176° 9'52.84"E) that is in close proximity to the laboratory (37°40'16.41"S 176°10'0.19"E) (Figure 2.2). Collected water was not influenced by freshwater runoff at collection times since these were done at times of no rain and salinity was at 35‰ upon collection. The water was filtered through a 250 µm filter sock to remove any large particulates but to retain small bacteria and phytoplankton that sponges feed on. Collected sea water was pumped into an aerated stock tank that was predominantly blacked out to prevent significant algal growth with a slight amount of light let through to maintain a small phytoplankton population. Prior to water changes, water from the stock tank was pumped into aerated tanks not containing sponges in the temperature controlled room where experiments took place to allow the water temperature to reach experimental levels (maintained by Eheim 50 W aquarium heaters) before the water change. Water quality parameters were monitored daily in each of the tanks, both those housing sponges and tanks used for completing water changes. pH was measured with the use of an Oakton pHTestr10 calibrated weekly with a three-point calibration. Oxygen and temperature were measured with the same PreSens PSt3 fibre optic oxygen and temperature probes that were used for respiration measurements (see section 2.5). Salinity was measured in ppt (‰) using a handheld ATC refractometer calibrated

weekly with distilled water and free ammonia was measured using Seachem MultiTest Ammonia test kits.

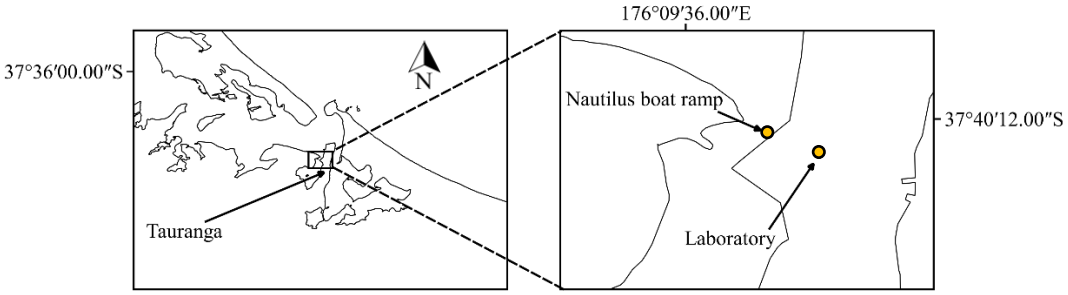

Figure 2.2: Water collection site at the Nautilus Boat ramp relative to the Sulphur Point Coastal Marine Field Station laboratory and the wider city of Tauranga.

2.4 Experimental sediments

2.4.1 Sediment collection

Sediments collected from the sediment traps (see section 2.10.2) were collected at times similar to the laboratory experiments being conducted. It would have been preferable to have the sedimentation rates and grain size compositions established using the sediment traps inform the sediment concentrations and size classes to use in these experiments, however timing did not allow for this and therefore another source of experimental sediments had to be established. It was decided that sediments for the experiments would be collected from a site within the Tauranga Harbour. A site at the southern end of the Waikareao Estuary (37°41'44.5"S 176°09'10.4"E) (Figure 2.3) was selected for the collection of experimental sediments as this area has a high level of fine sediment since it is near the mouth of the Kopurererua Stream and terrigenous sediments are known to deposit in these areas (Hume *et al.*, 2010). The majority of terrigenous sediment leaving the southern Tauranga Harbour entrance is derived from the southern catchments and the Kopurererua catchment is known to contribute large levels of sediments (especially fine grains) into the Tauranga Harbour and coastal waters (Hume *et al.*, 2010). This site was chosen since habitats containing *T. burtoni* are likely to experience sediment in their environment derived from this catchment and likely in increasing concentrations as it is sediments from these terrigenous sources that are predicted to increase under future scenarios (Hume *et al.*, 2010). This is an

important and relevant consideration since mineralogical composition and the source of sediments has previously been shown to affect sponges differently (Bannister *et al.*, 2012; Kutti *et al.*, 2015). Sediments were collected at a low tide with a trowel to around 2 cm depth and wet sieved through a 2000 µm sieve into a bucket to remove any macrofauna and were subsequently taken back to the laboratory for immediate processing.

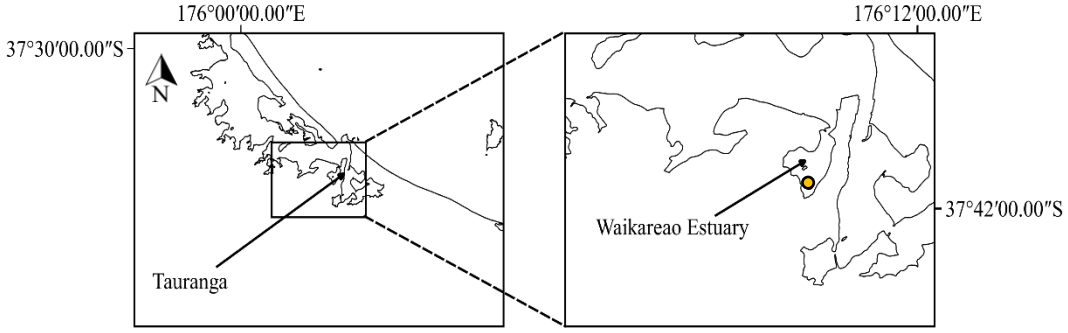

Figure 2.3: Experimental sediment collection site indicated by yellow circle relative to the Waikareao Estuary and the wider city of Tauranga.

2.4.2 Sediment treatment and preparation

The organic fraction and salts in the sediments were removed to eliminate any potential increased microbial activity and alteration to water quality in the respirometer or tanks. The 2000 µm sieved sediments were placed into aluminium baking trays and oven dried at 80°C for 24 hours. Cooled sediments were transferred into an Endecott's EFL 2000 sieve shaker for 20 minutes to separate grains into size classes of <2000 µm -1000 µm, <1000 µm-500 µm, <500-250 µm, <250-125 µm, <125-63 µm and <63 µm. This was done prior to removal of organic components to confirm that an adequate quantity of each of the grain size classes was present for the experiments. The two largest grain size classes were discarded as these were not to be used in the experiments. Each of the remaining grain size classes were then evenly distributed into two 1000 ml beakers not exceeding a combined sediment weight of 300 g per beaker. Under a fume hood, the sediments were covered incrementally with 100 ml of 30% hydrogen peroxide (H_2O_2) for 48 hours. The oxidisation reaction was initially closely monitored as violent reactions can occur if there are high levels of organic matter and distilled water was used to control the reaction as necessary. After 48 hours when the digestion appeared to

have slowed significantly, an additional 100 ml of 30% H_2O_2 was incrementally added to each of the beakers and these were placed into a water bath set to 60°C under the fume hood to finish the oxidisation reaction. When effervescence had ceased, excess H_2O_2 was removed with a syringe being careful not to disturb the sediments. They were then moved to a clean bucket where they were rinsed with 6 L of distilled water and left to stand undisturbed for 48 hours. The excess distilled water was carefully removed with a syringe and the sediments were again rinsed with 6 L of fresh distilled water to remove any remaining H_2O_2 and any salts (Håkanson *et al.*, 1989). The sediments were again left to stand undisturbed for 48 hours before the excess distilled water was removed carefully with a syringe. The sediments were once more placed into aluminium baking trays and oven dried at 80°C for 24 hours and separated into grain size classes of <500-250 µm, <250-125 µm, <125-63 µm and <63 µm using the Endecott's EFL 2000 sieve shaker for 20 minutes. A magnet was run through the sediments thoroughly to remove magnetic components as these would be attracted to the magnetic stir bar in the respirometer and alter concentrations. Grain sizes were kept in their respective grain size classes and transferred to glass specimen jars for later use in experiments.

2.5 Respirometer design

A respirometer was constructed from a 100 ml glass beaker as glass is the preferable material for respirometer construction over other commonly used plastics such as acrylic and PVC as it does not create an oxygen influx bias since it is completely impermeable to oxygen (Wells & Warinner, 1968; Svendsen *et al.*, 2016). The top of the beaker was fitted with a rubber bung that had two holes drilled through it. One hole was used to accommodate a PreSens PSt3 fibre optic oxygen probe and the other a temperature probe. The beaker had a magnetic stir bar placed on the bottom and a PVC cap with drilled holes was put in next, suspended off the stir bar and held in place by the slight tapering of the beaker. This was to act as a platform for the sponge to sit on during respiration measurements. When the bung was fitted the total volume of the respirometer was 80 ml. During experiments the respirometer was placed inside a temperature controlled tank and placed on top of a magnetic stirrer set to 180 rpm. Doing so allowed water flow generated by the magnetic stir bar to pass through the holes of the PVC cap and throughout the beaker, with the effectiveness of this confirmed with fluorescein dye (100 mg l^{-1})

in seawater) to confirm that adequate mixing was occurring throughout the chamber so as to not cause stagnation stress on the sponge when placed in the respirometer. The PreSens PSt3 fibre optic oxygen probe fitted to Fibox 3 transmitter was 0% oxygen saturation calibrated with the temperature probe present using 1 g sodium disulphide to 70 ml water and 100% calibrated using water bubbled with an air stone for 15 minutes. Calibration was completed at the beginning of each day measurements took place. All oxygen and temperature measurements were logged every second via PreSens OxyView software (Fibox 3 for PSt3, version 6.02) and saved as text files.

2.6 Experiment one – sediment concentration

Experiment one aims to address the first experimental question; how does the concentration of sediments affect the immediate respiration response of *T. burtoni*?

2.6.1 Housing tank setup

Thirty specimens of *T. burtoni* (see section 2.2 for collection method) were randomly allocated into one of five tanks (six individuals per tank), each holding 40 litres of 250 µm filtered sea water (see section 2.3 for further detail on water source and maintenance) and were left to acclimatise for a week. The tanks were housed inside a temperature controlled room set to 18°C and each tank was fitted with an Eheim 100 W aquarium heater set to 18°C to reflect the annual mean sea surface temperature experienced in the coastal waters of Tauranga (Worldseatemperature.org, 2017). A 12:12 hour light:dark cycle was maintained by 40 W AquaOne T8 Marine Blue lamps suspended approximately 24 cm above the top of the tanks to reflect *in situ* light levels and each tank contained two air stones (one at each end of the tank) to oxygenate the water and provide water flow throughout the tank. A plastic mesh tray was placed on the bottom of each tank for the sponges to sit on to keep them off the bottom of the tank so water could flow beneath them in case any tissue damage had occurred during collection, transportation or cleaning and to reduce the chances of tissue necrosis. A 20% water change was completed every three days to maintain optimal water quality parameters and a food supply to the sponges. Water quality parameters in the housing tanks remained within acceptable ranges with salinity remaining at a consistent 35‰ throughout, pH ranges between 7.9-8.2 pH units, undetected free

ammonia, temperatures between 17.5-18.9°C and oxygen saturation ranges between 95-99% in the housing tanks.

2.6.2 Sediment treatments

Four sediment treatments were selected based on literature values common for New Zealand harbours in both ambient conditions (20 and 100 mg l^{-1}) (Ellis *et al.*, 2002) and storm conditions (500 and 1000 mg l^{-1}) (Fahey & Coker, 1992). Treatments were made up of equal parts (weight (mg)) of <250-125 µm, <125-63 µm and <63 µm grain size classes (see section 2.4 for detail on the source and treatment of experimental sediments). At the beginning of each day sediment treatments were made up into a 20 ml seawater stock solution (that when added to the 80 ml respirometer would dilute to the relevant treatment concentration), with plain 250 µm filtered seawater for the controls. Stock solutions were made because for the smallest sediment concentrations in pilot experiments, it was found that these were difficult to weigh out accurately in a repeatable and time efficient manner.

2.6.3 Treatment addition and respiration measurements

Due to the restriction of only having one oxygen probe available for making respiration measurements, the measurements for each treatment were made sequentially in a predetermined randomised order over a two-day period to account for any potential respiration differences as a factor of day/time of day. In addition, every sponge in each of the five housing tanks was allocated a number and these were randomly assigned to each treatment (six individuals per treatment) to account for any minor differences in tank condition that could potentially affect the sponges. The respiration measurement protocol was as follows: Inside the housing tank the respective sponge individual was first photographed and was then carefully placed onto the PVC support cap in the respiration vessel with gloved hands to minimise stress. The respiration vessel was wrapped in tinfoil to dark adapt the sponge and cease any activity of potential photosynthetic symbionts to reset sponges to their basal metabolism as best as possible. An air bubbler was placed into the respiration vessel to maintain flow and oxygen saturated water while the sponge was left for 30 minutes to acclimatise and recover from handling. After the acclimation period, the respiration chamber had the bung fitted underwater to ensure no air could enter the chamber. This was done with the temperature probe out of the bung so pressure inside the chamber was not increased as the bung was pushed in. The temperature

probe was replaced underwater and the unit was removed from the tank. To ensure maximal temperature stability within the respirometer during the respiration measurements, the respirometer was kept in a temperature controlled tank filled with seawater and kept at 18°C with an Eheim 50 W aquarium heater. The magnetic stirrer was started and the logging software activated. Oxygen measurements were recorded every second in µmol l^{-1} and this was done undisturbed for a period of 15 minutes. About five minutes into this time the relevant sediment stock solution (or plain seawater for controls) was homogenised by placing the container onto a magnetic stirrer and 2 ml of treatment solution was taken via a 5 ml syringe. The syringe containing the sediment treatment was allowed to sit in the experimental tank to equilibrate the temperature for approximately 10 minutes. When the 15 minutes had elapsed, the temperature probe was carefully removed and 2 ml of the respirometer water was carefully removed with a 5 ml syringe and the 2ml sediment slurry was slowly and carefully added to the respirometer and the temperature probe was replaced. Oxygen concentration measurements were taken for a further 15 minutes and previous trialling had shown that oxygen levels rarely fell below 70% of the starting concentration for a 30 minute measurement with the oxygen consumption curve remaining linear and therefore any potential harmful effects of reduced oxygen on the sponges and subsequent effect on respiration was likely not present. In addition to the respiration measurements of the sponges under differing sediment concentration treatments, one measurement per treatment without a sponge present was also conducted to account for electrode drift (Gatti *et al.*, 2002) and any potential respiration and photosynthesis occurring in the seawater due to microorganisms. Any changes in oxygen levels in these blank measurements were subsequently subtracted from the data pertaining to each of the sponge respiration measurements for the respective treatments and were accordingly adjusted for time. At the cessation of the respiration measurement, the individual sponge was carefully removed from the respirometer, patted dry with a paper towel and photographed again to track any potential changes in surface appearance and size before being wet weighed and returned to a recovery tank. It was decided that wet weights would be used instead of dry weights as preliminary results indicated that wet weights correlated well with that of dry weights (See Appendix, Figure 5.1).

2.7 Experiment two – sediment grain size

Experiment two aims to address the second experimental question; how do different sediment grain size classes affect the immediate respiration response of *T. burtoni*?

2.7.1 Housing tank setup

Forty specimens of *T. burtoni* were collected (see section 2.2 for collection method) allocated, acclimatised and housed in the same manner as for experiment one with water quality parameters remaining stable and within acceptable ranges in the housing tanks as per experiment one. (see section 2.6.1).

2.7.2 Sediment treatments

In addition to a control treatment of 250 µm filtered plain sea water, four grain size classes were selected for the grain size experiments and consisted of <500-250 µm, <250-125 µm, <125-63 µm and <63 µm treatments (see section 2.4 for detail on the source and treatment of experimental sediments). These were added at a 500 mg l^{-1} concentration as this value elicited a statistically significant response in experiment one (but did not depress respiration entirely) and is representative of storm conditions. Unlike the treatments in experiment one, the sediment was not made into stock solutions. This was because it was much simpler to weigh out one measurement opposed to three for each treatment, and also because previous trialling indicated that the largest grain size class of <500-250 µm was not adequately homogenised when stock solutions were created. This is also why it was omitted from experiment one. Prior to sediment addition to the respirometer, 2 ml of water from the housing tank of the respective experimental individual was taken via a 5 ml syringe and the relevant quantity of sediment was added to the syringe that when added to the respirometer would equate to 500 mg l^{-1}.

2.7.3 Treatment addition and respiration measurements

The respiration measurement protocol, treatment temperature acclimation and addition in this experiment followed that of experiment one (see section 2.6.3).

2.8 Experiment three – long-term exposure to fine sediments

Experiment three aims to address the third experimental question; how do fine sediments affect the respiration response of *T. burtoni* over a longer period of exposure?

2.8.1 Housing tank setup

T. burtoni specimens (see section 2.2. for collection method) were initially housed in larger tanks for a week to allow the sponges to recover from collection. After the recovery duration, 20 individuals were randomly allocated to one of 22 small cylindrical plastic tanks containing 1L of plain 250 µm filtered sea water (see section 2.3 for further detail on water source and maintenance) containing a single air stone to oxygenate the water and generate flow. The two tanks not containing sponges were for running blank respiration measurements to account for microbial respiration, photosynthesis and electrode drift (Gatti *et al.*, 2002). The sponges were left to acclimate to the new tanks for five days with a 50% water change of plain 250 µm filtered sea water occurring every two days. Water quality parameters remained stable and within acceptable ranges in the housing tanks as per experiment one (see section 2.6.1) throughout the acclimation and experimental period.

2.8.2 Sediment treatments

Results from experiment two indicated that the <63 µm grain size class elicited a significant response and since it is fine sediments that are predicted to increase in quantity in future sedimentation scenarios, it was decided that the longer-term effects of this grain size class at a concentration of 500 mg l^{-1} would be examined with a control of plain 250 µm filtered sea water.

2.8.3 Treatment addition and respiration measurements

After the acclimation period, the 22 tanks were randomly allocated to either the treatment or control group giving 11 tanks per group; 10 containing sponges and one with just sea water. Baseline respiration measurements were obtained in a pre-determined randomised order for all sponges (and blank measurements from the empty tanks) with the respiration measurement protocol as follows: Inside the housing tank the respective sponge individual was carefully placed onto the PVC support cap in the respiration vessel with gloved hands to minimise stress. The respiration vessel was wrapped in tinfoil to dark adapt the sponge and cease any activity of potential photosynthetic symbionts. An air bubbler was placed into the respiration vessel to maintain flow and oxygen saturated water while the sponge was left for 15 minutes to acclimatise and recover from handling. After the acclimation period, the respiration chamber had the bung fitted underwater to

ensure no air could enter the chamber. This was done with the temperature probe out of the bung so pressure inside the chamber was not increased as the bung was pushed in. The temperature probe was replaced underwater and the unit was removed from the tank. To ensure maximal temperature stability within the respirometer during the respiration measurements, the respirometer was kept in a temperature controlled tank filled with seawater and kept at 18°C with an Eheim 50 W aquarium heater. The magnetic stirrer was started and the logging software activated. Oxygen measurements were recorded every second in µmol l^{-1} and this was done undisturbed for a period of 15 minutes. Any changes in oxygen levels in the blank measurements were subsequently subtracted from the data pertaining to each of the sponge respiration measurements for the respective treatment or control groups and were accordingly adjusted for time. At the cessation of the respiration measurement, the individual sponge was carefully removed from the respirometer, gently patted dry with a paper towel and wet weighed before being returned immediately to its housing tank. That evening, 500mg of <63 µm sediments were added to the treatment tanks. Respiration measurements were taken as per above the following day and at time intervals of 4, 7, 10 and 20 days post baseline measurements. A 50% water change was performed for each of the 22 tanks every two days during this experimental period to maintain adequate water quality parameters and a supply of natural food for the sponges. On the day of a water change 500 ml of water was removed from each of the tanks and replaced with 500 ml of fresh plain 250 µm seawater for controls and 250 mg <63 µm sediment in 500 ml 250 µm filtered seawater for treatment tanks (maintaining a sediment concentration of 500 mg l^{-1}).

2.9 Experiment four – temperature

Experiment four aims to address the fourth experimental question; how do projected sea surface temperature increases affect *T. burtoni* respiration over a longer time frame and how does this affect disease and survival?

2.9.1 Housing tank setup

A total of 120 specimens of *T. burtoni* (see section 2.2 for collection method) were randomly allocated into one of twelve tanks (ten individuals per tank) of 40 litres of 250 µm filtered sea water (see section 2.3 for further detail on water source and maintenance) and were left to acclimatise for ten days. The tanks were housed

inside a temperature controlled room set to 18°C and each tank was fitted with an Eheim 100 W aquarium heater set to 18°C to reflect the mean annual sea surface temperature experienced in the coastal waters of Tauranga for the acclimation period (Worldseatemperature.org, 2017). A 12:12 hour light:dark cycle was maintained by 40 W AquaOne T8 Marine Blue lamps suspended approximately 24 cm above the top of the tanks to reflect *in situ* light levels and each tank contained two air stones (one at each end of the tank) to oxygenate the water and provide water flow throughout the tank. A plastic mesh tray was placed on the bottom of each tank for the sponges to sit on for the duration of the acclimation and experimental period.

2.9.2 Temperature treatments

After the acclimation period, each of the twelve tanks were randomly allocated to one of four temperature treatments so that each treatment contained three replicate tanks. The four temperatures that were selected included; 18°C to reflect the mean annual sea surface temperature experienced in Tauranga coastal waters (the control); 23°C to reflect the summer maximum (Worldseatemperature.org, 2017) and two temperature treatments based on IPCC climate change predictions. The first of these is a low change scenario best estimate for 2100 of a 2°C (rounded up from 1.8°C) increase in sea surface temperature and the second being the high change scenario sea surface temperature increase of 4°C (IPCC, 2007; 2013). These temperature increases were added to the summer maximum of 23°C giving additional temperature treatments of 25°C and 27°C respectively. The treatment temperatures were administered and maintained via the Eheim 100 W aquarium heaters in the tanks that were set to experimental temperature treatments on the day after baseline respiration measurements took place and were at stable experimental treatment temperatures the following day.

2.9.3 Respiration measurements

The respiration rates of nine randomly selected sponges (three per replicate tank) were obtained for each of the four temperature treatments. Measurements took place on the day prior to the treatment temperatures being administered to obtain baseline respiration levels. Respiration measurements were then taken every week for a total of four weeks of temperature treatment to assess respiration of *T. burtoni* over time. In the two warmest temperature treatments, low survival meant

inadequate replicates remained for measurements to be taken for the entire experimental period. As such, in the 27°C treatment only baseline and week one data were obtained and in the 25°C treatment only baseline and week one and two data were obtained. The respiration protocol followed that of experiment three (see section 2.8.3) with experimental temperatures in the respirometer maintained by placing the respirometer in a temperature controlled tank filled with seawater. An Eheim 50 W aquarium heater maintained the water in the tank at the respective temperature treatment. A blank measurement was taken per experimental temperature to account for electrode drift and microbial respiration.

2.9.4 Survival, disease and morphological change assessments

All sponges were photographed inside their tanks the day prior to treatments being administered so they could be referred back to if necessary. Sponge survival, and external indicators of disease and morphological changes were assessed daily at the commencement of the temperature treatments being administered. Since it is difficult to accurately pinpoint a death event of a sponge, criteria were made and sponges were deemed to have died if the entire surface of the sponge had been overcome with bacterial film or if there was more than 50% tissue loss. In cases where both bacterial film and tissue death occurred, the first of the two criteria met was deemed as death of that individual. Individuals that had died were removed from their housing tank and any small remaining pieces of substrate were removed before the sponge was cut though the cross section and photographed with the internal components of the sponge shown. At the end of the full experiment all remaining sponges were removed from their respective tanks and cut and photographed in the manner described above.

2.10 *In situ* sediment and temperature data

The data collected in this section aim to address the questions; what are the sedimentation rates, sediment grain sizes and temperatures currently experienced by *T. burtoni* in differing locations in the Bay of Plenty during summer and how do these *in situ* parameters compare to the *ex situ* aquaria experiments?

2.10.1 Sites

Sites were selected for the collection of sediments, temperature and light readings based on initial reconnaissance SCUBA dives confirming the presence of *T. burtoni*

and were chosen to include those that would predictively see differing rates of sedimentation and possibly grain sizes. These sites included Karewa Island (37°31'51.60"S, 176°7'52.15"E), Motiti Island (37°38'42.16"S, 176°25'4.48"E), Rabbit Island (37°37'38.91"S, 176°11'53.37"E) and Pilot Bay (37°38'11.80"S, 176°10'14.52"E) (Figure 2.4).

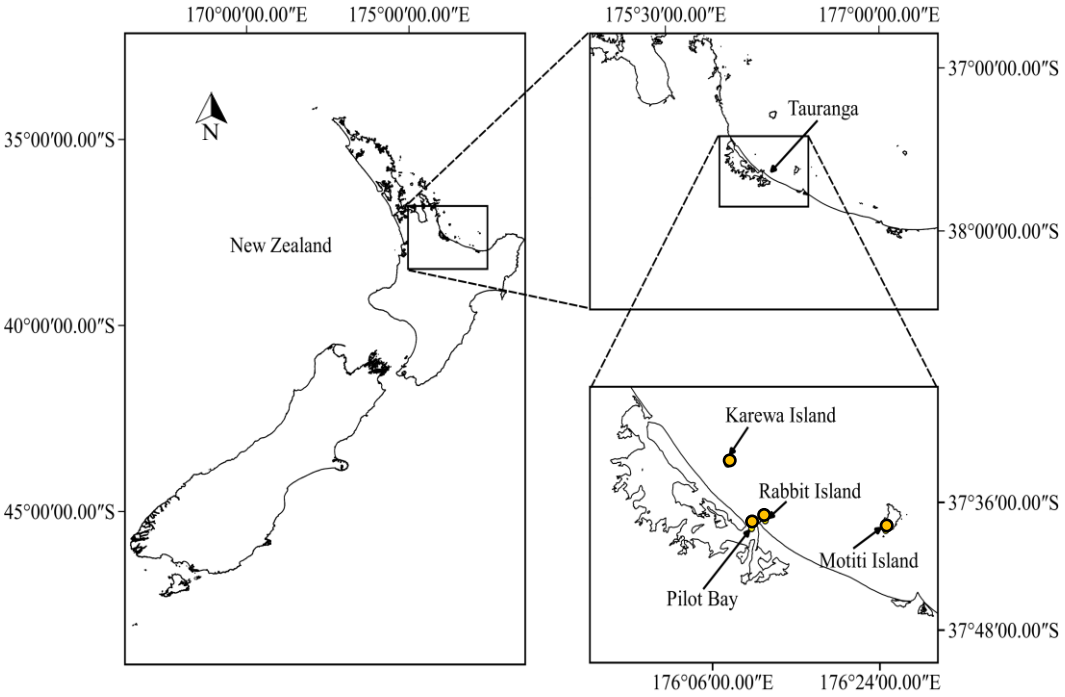

Figure 2.4: Sediment collection sites; Pilot Bay, Rabbit Island, Motiti Island and Karewa Island; indicated by yellow circles relative to the city of Tauranga and wider New Zealand.

2.10.2 Sediment and temperature data collection

Sediments, temperature (°C) and light (Lux) readings were collected during austral summer months of 2017. It was intended that these would consist of three consecutive three-week durations, however due to equipment failure and unfavourable diving conditions this was not always possible. The first sampling period was from the 11th of January - 2nd of February for all locations and all parameters. The second sampling duration spanned from the 2nd of February - 23rd February for both Karewa and Motiti for all parameters bar temperature (°C) and light (Lux) readings at Karewa due to flooding of the logger. Rabbit Island and Pilot Bay could not be accessed this day due to zero visibility conditions at depth nor the following week and thus it was decided that the traps and loggers at Rabbit Island and Pilot Bay would be left for the third and final three-week sampling period. Thus,

for these locations, the sampling duration started on the 2nd of February and finished on the 17th of March. For Karewa and Motiti the final sampling period spanned from the 23rd of February to the 17th of March. For each of these sampling durations, three replicate sediment traps and a single HOBO® Pendant® Temperature/light Data Logger (UA-002-xx) were set out by SCUBA divers at a depth of approximately 15 m at each of the four sampling locations at the interface where the substrate went from rock to sediment. A star picket was hammered into the benthos with the top 1 m above the benthos. The light logger was attached with a cable tie through pre-drilled holes at the top of the picket. Three additional star pickets were hammered into the benthos and the sediment traps were attached to the pickets with a jubilee clip where the trap mouth was elevated 1 m above the benthos (Figure 2.5) (see section 2.10.3 for details on trap design) and 17 cm above the top of the star picket to ensure consistency between replicates at all sites in terms of any hydrodynamic effects that may be created by the trapping configuration itself and to minimise collection of re-suspended sediments. Replicate traps were haphazardly placed near the respective sponge communities and were spaced approximately 2 m apart as it is recommended that traps be spaced at least 10 trap mouth widths apart (Storlazzi *et al.*, 2011) to minimise the hydrodynamic effects of turbulence created from the traps themselves in addition to benthic structures such as rocks. After the passive collection period, the sediment traps were carefully removed from the pickets and capped underwater to avoid any loss of sediments during transportation back to the laboratory. Once arrived, sediments were emptied into containers and any macrofauna such as fish, gastropods, crustaceans, polychaetes and zooplankton present were carefully removed with tweezers. The sediments were left undisturbed to settle for two weeks before excess water was carefully removed with a syringe and the sample was transferred to a 200 ml glass specimen jar. The sample was left for a further two weeks, whereupon excess water was removed before further processing (see section 2.10.4.1). Data from the HOBO® Pendant® Temperature/light Data Loggers (UA-002-xx) was downloaded via HOBOware® Pro Version 3.7.8 and imported into Microsoft Office Excel 2016 for later data analysis.

2.10.3 Sediment trap design

Sediment traps were constructed from white PVC cylindrical piping as this has been found to be the most effective trap shape (Butman, 1986). The trapping area was 19.63 cm^2 with an inner diameter of 5 cm and a height of 35 cm (Figure 2.5). This gives an aspect ratio of 1:7 as an aspect ratio of at least 1:3 but preferably more than 1:5 is recommended as this has the greatest efficiency for capturing sediments and preventing sediment re-suspension within the trap during sampling (Butman, 1986; Wang *et al.*, 2015) while a minimum diameter of 4 cm is recommended (Håkanson *et al.*, 1989). It is recognized here that sediment traps with greater temporal resolution exist and that sediment traps have associated biases and thus may over or under sample sediments and respective grain sizes, however this simplistic design was selected for pragmatic reasons as traps with reduced biases significantly increase in their associated costs and logistical intensity (Thomas & Ridd, 2004).

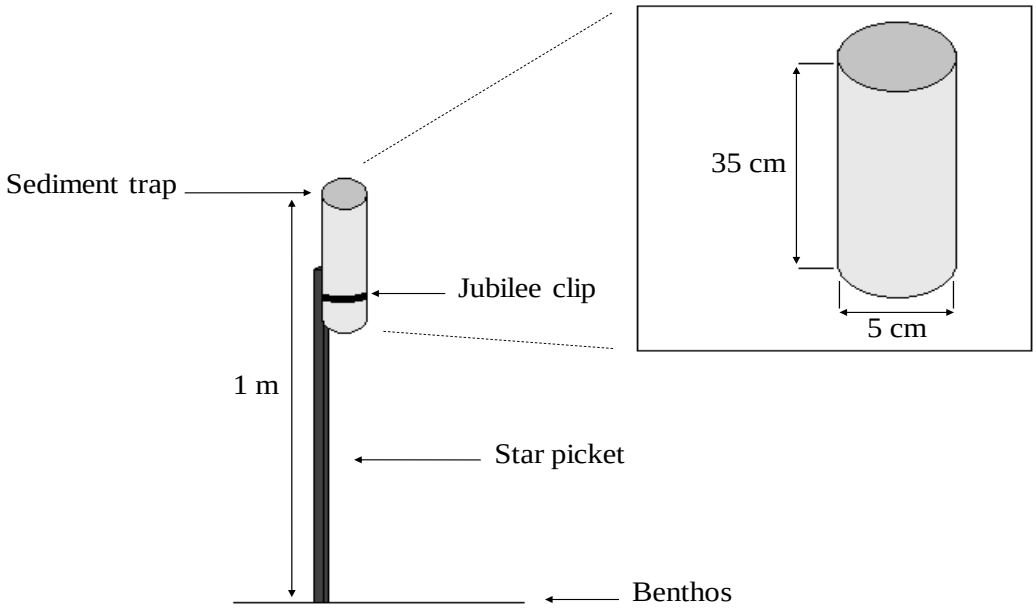

Figure 2.5: Design of sediment trap mounted to star picket relative to the benthos. Insert shows PVC sediment trap with associated length and diameter measurements.

2.10.4 Sediment abundance and grain size

2.10.4.1 Sediment digestion

The organic fraction was removed from the sediment trap samples as these components could have been in various states of decomposition due to their time in

the traps and their coagulating properties could cause inaccuracies with the grain size analysis instrumentation. This was done by initially adding 5 ml of 30% H_2O_2 to each of the samples, with the reaction being carefully monitored and controlled as necessary with distilled water and stirring the sample to remove large bubbles to avoid sample loss. The samples were left to stand at room temperature for 24 hours, before an additional 5 ml of 30% H_2O_2 was added. This process was repeated for an additional three days before the samples were transferred to pre-weighed (to the nearest 0.0001 g) 200 ml beakers that were heated to 200°C on a hot plate to finish the reaction and remove excess H_2O_2 and water. The beakers were then filled with distilled water to rinse the sediments and left to stand for eight days before excess water was carefully removed with a syringe.

2.10.4.2 Sedimentation rate

After removing the organic fraction from the sediment samples and rinsing the sediments to remove salts, the samples were dried in the pre-weighed beakers at 60°C for 40 hours and weighed in the beakers (to the nearest 0.0001 g). The beaker weight was subtracted from that of the sample and beaker combined weight and data recorded. These values were used to determine the sedimentation rate in each trap for each of the three sampling durations at each of the four sites in g m^{-2} d^{-1} using the following equation;

((Sediment weight (g) / days collected) / trapping area (19.63 cm^2) x 1000

2.10.4.3 Grain size analysis

Sediment grain sizes for each of the samples were determined using a Malvern Mastersizer 2000 (Malvern, UK) laser diffractometer with a 0.02 µm – 2000 µm lens. The dried samples were added through a 2000 µm sieve into a Hydro G sample dispersion unit containing water as the dispersant until obscuration reached between 10-20%. The stirrer was set to 750 rpm, the pump set to 1850 rpm and the ultrasound set to 50% to ensure sediment grains were evenly distributed throughout the water medium. Three drops of 10% Calgon was added to the Hydro G unit to act as a chemical dispersant to further separate fine grains that may have bound together during the drying process. Prior to each measurement of the sediment samples, the lasers were aligned and a background reading measurement taken to ensure the dispersant water was not contaminated by any particles. Measurements were run

for 20 s and the unit was cleaned between each measurement. The Mastersizer 2000 works based on Mie theory that predicts spherical particle size based on the way that light passes through or is adsorbed by a particle. The light scattering pattern is captured from a field of particles and then using Mie theory calculates the size of the particles based on this light scattering pattern (Malvern, 2007). Mie theory assumes that there is some known specific information about the particles such as its absorption and refractive index. The particle refractive index was set to 1.5 and the absorption index set to 0.2 and the dispersant refractive index was set to 1.33 for all of the measurements based on professional technician advice for marine samples.

2.11 Data analysis

Data were imported/entered into Microsoft Office Excel 2016 where relevant equations were calculated as necessary. Data were subsequently arranged in a manner that suited data layout requirements for IBM SPSS Statistics (V. 24, 2016) where all data analyses took place.

2.11.1 Experiment one - sediment concentration

The immediate respiration response of *T. burtoni* to different sediment concentrations was examined using a one-way ANCOVA with treatment as the fixed factor, the after-sediment addition respiration measurements as the dependent variable and the before-sediment addition respiration measurements as the covariable. The data were natural log transformed as raw data violated many of the assumptions. The assumptions of ANCOVA (that include both regression and ANOVA assumptions) of linearity, homogeneity of regression lines (interaction term $p>0.05$), normality (Shapiro-Wilk test >0.05) and homoscedasticity (visual inspection of scatter plot) were met for the natural log transformed data with no outliers present as assessed through no cases presenting standardized residuals greater than ±3 standard deviations. However, the homogeneity of variances assumption was not met with the natural log transformed data (Levene's test $p<0.05$). A one-way ANCOVA may be run on rank transformed data in cases where assumptions are violated (Quinn & Keough, 2002) or be run as a one-way ANOVA on the standardized residuals generated through the regression of the rank transformed covariable and dependent variable (e.g. Quade's test (Quade, 1967)).

However, this poses a loss to information as rank scores cannot be back transformed to meaningful values in the way a natural log transformation can despite providing a conservative test. Therefore the one-way ANCOVA analysis was continued on the natural log transformed data despite the homogeneity of variances assumption violation and also because when *n* is equal between groups the procedure is quite robust to heterogeneous variance anyhow ((Hsu, 1983) as cited in Olejnik and Algina (1985)). A significant result initiated a pairwise Bonferroni adjusted *post hoc* test on the natural log covariate adjusted means to understand which sediment concentration treatments elicited a statistically significant respiration response for *T. burtoni*.

2.11.2 Experiment two - sediment grain size

The immediate respiration response of *T. burtoni* to different sediment grain size classes was examined using a one-way ANCOVA with treatment as the fixed factor, the after-sediment addition respiration measurements as the dependent variable and the before-sediment addition respiration measurements as the covariable. The assumptions of ANCOVA (that include both regression and ANOVA assumptions) of linearity, homogeneity of regression lines (interaction term $p>0.05$), normality (Shapiro-Wilk test >0.05), homoscedasticity (visual inspection of scatter plot) and homogeneity of variances (Levene's test $p>0.05$) were met with no outliers present in the data as assessed through no cases presenting standardized residuals greater than ±3 standard deviations. A significant result initiated a pairwise Bonferroni adjusted *post hoc* test on the covariate adjusted means to understand which grain size classes elicited a statistically significant respiration response for *T. burtoni*.

2.11.3 Experiment three – long-term exposure to fine sediments

2.11.3.1 Respiration

A two-way repeated measures ANOVA was run to determine the long-term effects of fine sediments on the respiration rate of *T. burtoni* compared to those exposed to control conditions of plain 250 µm filtered natural sea water. The two within-subject factors are time (six levels) and treatment (two levels) with respiration rate (µmol O_2 g^{-1} FW h^{-1}) as the dependent variable. Shapiro-Wilk tests of normality on the studentized residuals of the raw data indicated a violation of normality in the treatment baseline and treatment day one after exposure groups. As such, a square

root transformation was applied to all data. Shapiro-Wilk tests now confirmed normality in all groups bar the baseline treatment group ($p<0.05$). Harsher transformations did not help and since ANOVA tests are quite robust to deviations from normality the two-way repeated measures ANOVA was continued on the square root transformed data. No outliers were present in the data as assessed through no cases presenting studentized residuals greater than ±3 standard deviations. Mauchly's test of sphericity indicated that the assumption of sphericity had been violated for the two-way interaction term ($\chi^2(14) = 38.215$, $p = 0.001$). In practice, the assumption of sphericity is considered difficult not to violate (Grimm & Yarnold, 2000) and corrections can be made to account for the bias produced when sphericity is violated. This is done by adjusting the degrees of freedom that are used to calculate *p*. This type of correction is called epsilon (ε) and the Greenhouse-Geisser correction is one of the correction methods that has been developed for this and is used here when determining the significance of *p* in the two-way repeated measures ANOVA.

2.11.3.2 Weight

A two-way repeated measures ANOVA was run to determine if the mean weight of *T. burtoni* changed in the control and treatment groups from the start to the end of the experiment. The two within-subject factors are time (two levels) and treatment (two levels) with weight (g) as the dependent variable. Shapiro-Wilk tests of normality on the studentized residuals of the raw data indicated a violation of normality in the control group at the end of the experiment. As such, a square root transformation was applied to all data. Shapiro-Wilk tests now confirmed normality in all groups ($p>0.05$). No outliers were present in the data as assessed through no cases presenting studentized residuals greater than ±3 standard deviations. Mauchly's test of sphericity was not needed for this test as there were only two groups in each of the within-subject factors.

2.11.4 Experiment four - temperature

2.11.4.1 Respiration

A two-way repeated measures ANOVA was run to determine the long-term effects of temperature on the respiration rate of *T. burtoni*. The two warmest temperature treatments of 25°C and 27°C were excluded from the analysis as mortality during

the experimental period precluded respiration measurements in the final two weeks for the 25°C treatment and final three weeks for the 27°C treatment. As such, the two within-subject factors are time (five levels) and treatment (two levels) with respiration rate (μmol O_2 g^{-1} FW h^{-1}) as the dependent variable. Shapiro-Wilk tests of normality on the studentized residuals of the raw data indicated a violation of normality in the 18°C week four and 23°C baseline groups. As such, a natural log transformation was applied to all data. Shapiro-Wilk tests now confirmed normality in all groups bar the 18°C week four group (p<0.05). Since ANOVA tests are quite robust to deviations from normality the two-way repeated measures ANOVA was continued on the natural log transformed data. No outliers were present in the data as assessed through no cases presenting studentized residuals greater than ±3 standard deviations. Mauchly's test of sphericity indicated that the assumption of sphericity had been met for the two-way interaction term ($\chi^2(9) = 11.004$, p = 0.291). A significant interaction term resulted is pairwise comparisons for the simple main effect of treatment.

2.11.4.2 Survival

Survival over time was analysed using the non-parametric Kaplan-Meier method as survival data are typically right skewed and therefore violate parametric statistical assumptions of normality (Kaplan & Meier, 1958). A Mantel-Cox test was performed to test for differences in survival curves with a significant result initiating pairwise Mantel-Cox comparisons to understand which treatment groups' survival curves differed significantly from one another. A Bonferroni correction was applied to α = 0.05 for the pairwise comparisons as this corrects for the increased chance of a family-wise type I error rate that transpires as a result of multiple pair-wise comparisons (Quinn & Keough, 2002).

2.11.5 *In situ* sediment and temperature data

GRADISTAT (Version 8.0) was used to generate percent sediment type composition data and a ternary diagram of the sand-silt-clay composition of the sediments collected in the sediment traps at each of the four locations across the three sampling periods. The relationship of sedimentation rate and mean sediment grain size was tested statistically. Sedimentation rate data violated the normality assumption of the Pearson's product-moment correlation and therefore Spearman's rank-order correlation was run instead.

Chapter 3

Results

3.1 Experiment one – sediment concentration

Experiment one aims to address the first experimental question; how does the concentration of sediments affect the immediate respiration response of *T. burtoni*?

3.1.1 Respiration response

The one-way ANCOVA indicated that there was a significant effect of sediment concentration on the immediate respiration response of *T. burtoni* after controlling for the before-sediment addition respiration measurements, $F_{(4, 24)} = 95.553$, $p < 0.001$. Further *post hoc* pairwise testing revealed the covariate adjusted mean respiration rates of 9.650 µmol O_2 g^{-1} FW h^{-1} in the 20 mg l^{-1} treatment and 9.564 µmol O_2 g^{-1} FW h^{-1} in the 100 mg l^{-1} treatment did not differ significantly compared to the control that had a covariate adjusted mean respiration rate of 12.871 µmol O_2 g^{-1} FW h^{-1} (Table 3.1, Figure 3.1). The two highest sediment concentrations both significantly reduced the respiration rate of *T. burtoni* with covariate adjusted mean respiration rates of 5.663 µmol O_2 g^{-1} FW h^{-1} and 0.607 µmol O_2 g^{-1} FW h^{-1} for the 500 mg l^{-1} and 1000 mg l^{-1} treatments respectively (Table 3.1). The degree to which respiration was reduced was also significantly greater in the 1000 mg l^{-1} treatment with respiration becoming almost completely suppressed compared to the respiration rate observed in the 500 mg l^{-1} treatment (Figure 3.1). Individuals exposed to the 1000 mg l^{-1} treatment also appeared to smooth over in many instances with closed oscula immediately after exposure (Figure 3.2 b) compared to pre-exposure appearances (Figure 3.2 a). After 30 minutes of recovery the sponges returned to a pre-exposure appearance (Figure 3.2 c).

Table 3.1: Bonferroni *post hoc* pairwise comparison test results comparing the natural log transformed respiration response (µmol O_2 g^{-1} FW h^{-1}) of *T. burtoni* between all four sediment concentration treatments and the control (n= 6 individuals per treatment). Significant *p*-values indicated in bold at $\alpha = 0.05$.

	Control	**20 mg l^{-1}**	**100 mg l^{-1}**	**500 mg l^{-1}**
20 mg l^{-1}	1.000			
100 mg l^{-1}	1.000	1.000		
500 mg l^{-1}	**0.001**	0.069	0.132	
1000 mg l^{-1}	**<0.001**	**<0.001**	**<0.001**	**<0.001**

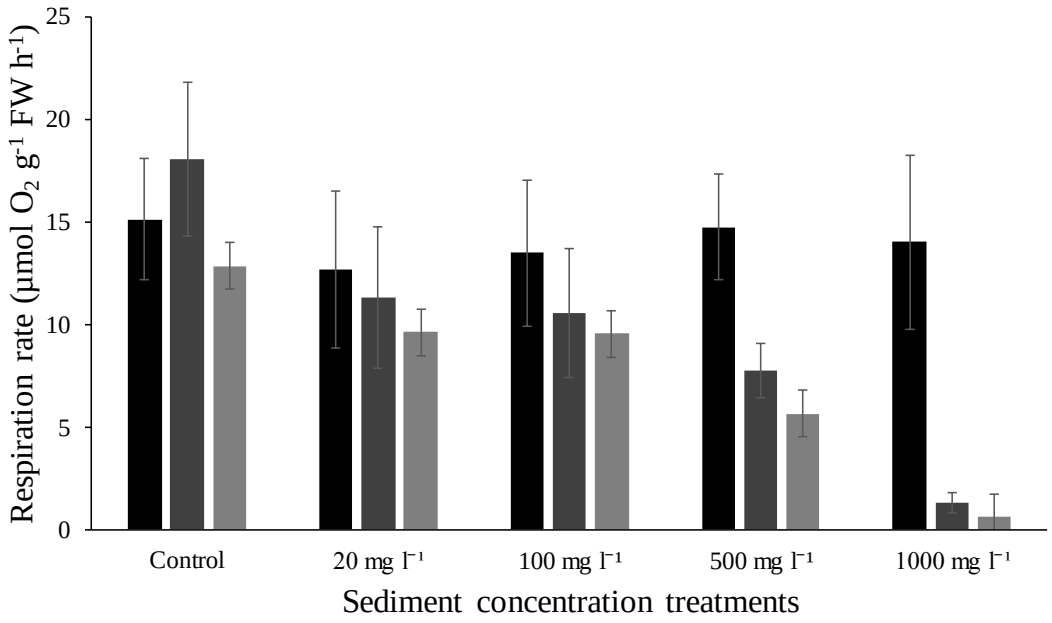

Figure 3.1: Respiration rate (µmol O_2 g^{-1} FW h^{-1}) of *T. burtoni* under four different sediment concentration treatments and the control (n= 6 individuals per treatment). Black bars; mean before-sediment addition respiration rates, dark grey bars; mean after-sediment addition respiration rates and light grey bars; anti-log transformed covariate adjusted mean after-sediment addition respiration rates. Error bars = ± one standard error.

Figure 3.2: Example of an individual *T. burtoni* exposed to the 1000 mg $^{-1}$ treatment **a**: pre-exposure; **b**: immediately after exposure; **c**: 30 minutes of recovery post-exposure.

3.2 Experiment two – sediment grain size

Experiment two aims to address the second experimental question; how do different sediment grain size classes affect the immediate respiration response of *T. burtoni*?

3.2.1 Respiration response

The one-way ANCOVA indicated that there was a significant effect of grain size class on the immediate respiration response of *T. burtoni* after controlling for the before-sediment addition respiration measurements, $F_{(4, 24)} = 10.881$, $p < 0.001$. Further *post hoc* pairwise testing revealed that the <63 µm and <125-63 µm grain size classes both significantly reduced the respiration rate of *T. burtoni* to covariate adjusted mean respiration rates of 7.265 µmol O_2 g^{-1} FW h^{-1} and 9.921 µmol O_2 g^{-1} FW h^{-1} respectively, compared to the control group that had a covariate adjusted mean respiration rate of 14.318 µmol O_2 g^{-1} FW h^{-1} (Table 3.2, Figure 3.3). Despite both the <63 µm and <125-63 µm grain size classes causing a significant reduction in respiration, the degree to which respiration was reduced did not differ significantly between these two treatments although the covariate adjusted mean respiration rate of *T. burtoni* was slightly lower in the <63 µm grain size class (Table 3.2, Figure 3.3). The <250-125 µm and <500-250 µm grain size classes did not elicit a statistically significant response compared to the control with covariate adjusted mean respiration rates of 11.788 µmol O_2 g^{-1} FW h^{-1} and 14.478 µmol O_2 g^{-1} FW h^{-1} observed for the two treatments respectively (Table 3.2, Figure 3.3).

Table 3.2: Bonferroni *post hoc* pairwise comparison test results comparing the respiration response (µmol O_2 g^{-1} FW h^{-1}) of *T. burtoni* between all four sediment grain size treatments and the control (n= 6 individuals per treatment). Significant *p*-values indicated in bold at α = 0.05.

	Control	**<500-250 µm**	**<250-125 µm**	**<125-63 µm**
<500-250 µm	1.000			
<250-125 µm	0.629	0.692		
<125-63 µm	**0.028**	**0.019**	1.000	
<63 µm	**<0.001**	**<0.001**	**0.023**	0.567

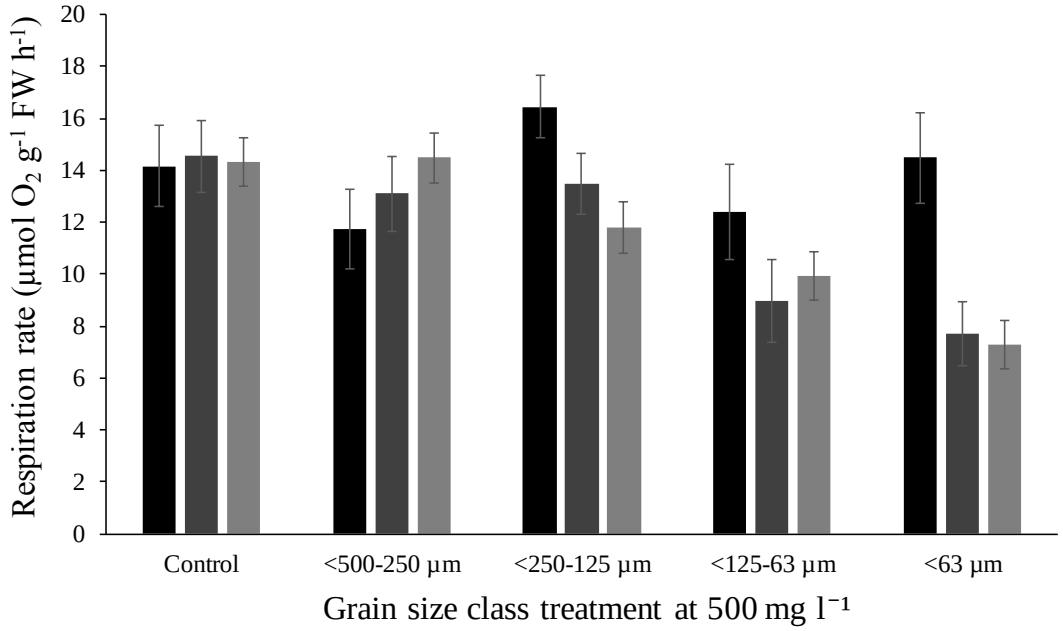

Figure 3.3: Respiration rate (µmol O_2 g^{-1} FW h^{-1}) of *T. burtoni* under four different grain size class treatments and the control (n= 6 individuals per treatment). Black bars; mean before-sediment addition respiration rates, dark grey bars; mean after-sediment addition respiration rates and light grey bars; covariate adjusted mean after-sediment addition respiration rates. Error bars = ± one standard error.

3.3 Experiment three – long-term exposure to fine sediments

Experiment three aims to address the third experimental question; how do fine sediments affect the respiration response of *T. burtoni* over a longer period of exposure?

3.3.1 Respiration response

The two-way repeated measures ANOVA indicated that there was no significant interaction affect between treatment and time on the respiration rate of *T. burtoni* ($F_{(2.688,\ 24.195)} = 0.541$, $p = 0.640$) (Table 3.3). Therefore, main effects were consulted, indicating that neither treatment effect ($F_{(1,\ 9)} = 1.031$, $p = 0.336$) nor time ($F_{(2.793,\ 25.137)} = 2.944$, $p = .056$) affected the respiration rate of *T. burtoni* significantly (Table 3.3). With that being said, the *p*-value for the main effect of time, while not significant at $\alpha = 0.05$, is very close to this value and does provide some weak evidence that time in the small aquaria may have been sub-optimal for *T. burtoni* irrespective of treatment with an apparent decline of 3.246 µmol O_2 g^{-1} FW h^{-1} in the control and 2.178 µmol O_2 g^{-1} FW h^{-1} in the treatment at day 20 post the first sediment addition compared to baseline levels (Figure 3.4). This is further supported by the fact that some individuals in both groups began to develop reproductive buds (Figure 3.6 a) which is a sign of unfavourable conditions and two individuals in the control group and one in the treatment group displayed signs of necrosis in their internal tissues when cross sectioned at the conclusion of the experiment (Figure 3.6 b). Despite this, mean baseline weights of 14.077 g for the control and 14.336 g for the treatment group did not reduce to a statistically significant level, dropping only to mean weights of 14.037 g and 14.182 g for the control and treatment group respectively at the conclusion of the experiment (Table 3.4, Figure 3.5).

Table 3.3: Two- way repeated measures ANOVA comparing the mean respiration rate (µmol O_2 g^{-1} FW h^{-1}) of *T. burtoni* in the control and treatment (<63 µm grain size class at 500 mg l^{-1}) over a 20-day duration based on square root transformed values (n = 10 individuals per group). Significant *p*-values indicated in bold at $\alpha = 0.05$ under a Greenhouse-Geisser correction for violation of sphericity.

	Type III SS	***df***	***MS***	***F***	***p***
Treatment	0.338	1, 9	0.338	1.031	0.336
Time	4.184	2.793, 25.137	1.498	2.944	0.056
Treatment * time	0.966	2.688, 24.195	0.360	0.541	0.640

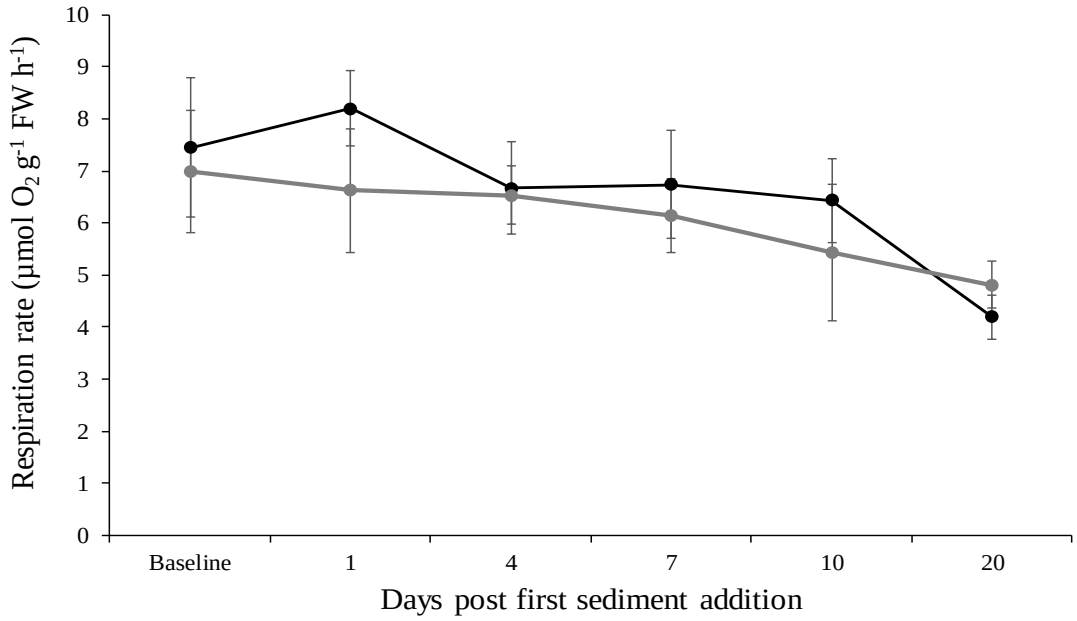

Figure 3.4: Respiration rate (µmol O_2 g^{-1} FW h^{-1}) of *T. burtoni* before treatment addition (baseline) and at five time points post the first day of sediment addition. Error bars = ± one standard error (n = 10 individuals per group). Black line; control, grey line; <63 µm grain size class at 500 mg l^{-1} concentration.

Table 3.4: Two-way repeated measures ANOVA comparing the mean weight (g) of *T. burtoni* in the control and treatment (<63 µm grain size class at 500 mg l^{-1}) at baseline levels and at the conclusion of the 20-day experiment (n = 10 individuals per group). Significant *p*-values indicated in bold at α = 0.05.

	Type III SS	***df***	***MS***	***F***	***p***
Treatment	<0.001	1, 9	<0.001	0.001	0.977
Time	0.003	1, 9	1.498	0.289	0.604
Treatment * time	<0.001	1. 9	0.360	0.001	0.975

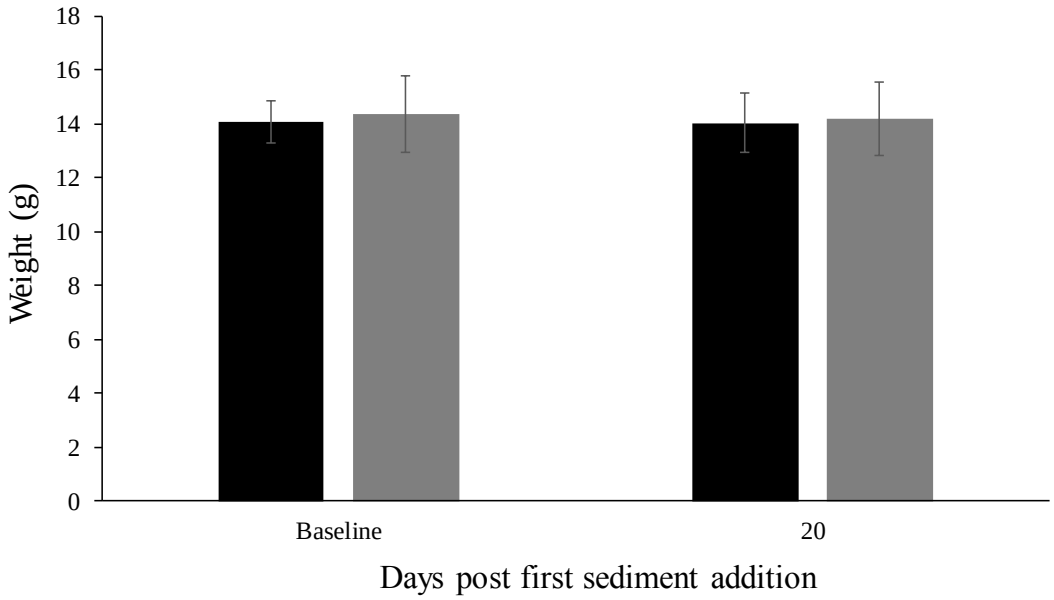

Figure 3.5: Mean weight (g) of *T. burtoni* before treatment addition (baseline) and at the conclusion of the experiment (20 days post first sediment addition). Error bars = ± one standard error (n = 10 individuals per treatment). Black bar; control, grey bar; <63 µm grain size class at 500 mg l^{-1} concentration.

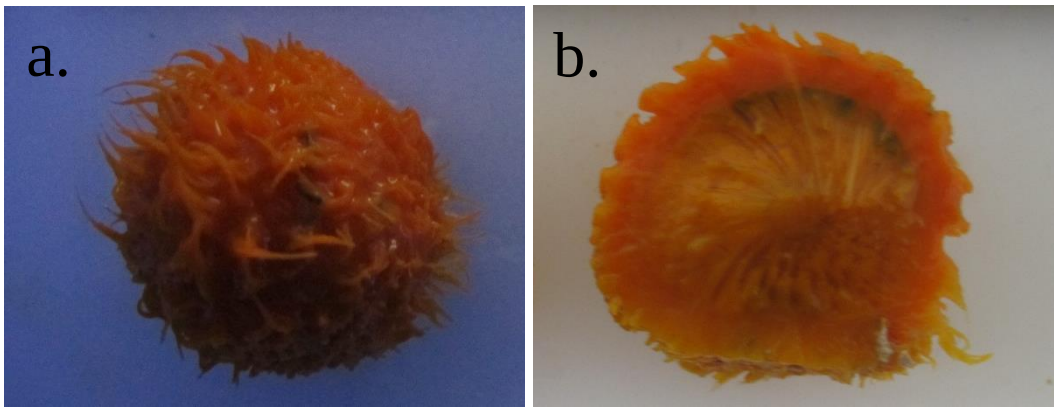

Figure 3.6: Examples of negative responses of *T. burtoni* observed at the conclusion of the long-term 20-day sedimentation experiment. **a**: signs of reproductive buds forming; **b**: internal tissue necrosis just under the dermal cortex layer.

3.4 Experiment four – temperature

Experiment four aims to address the fourth experimental question; how do projected sea surface temperature increases affect *T. burtoni* respiration over a longer time frame and how does this affect disease and survival?

3.4.1 Tank housing conditions

Salinity in the tanks remained at a steady 35‰ throughout the temperature experiment. pH remained steady also, keeping within normal ranges between 8-8.2 pH units in all tanks throughout the experiment and free ammonia was not detected in any tank during the experiment, likely attributable to the regular and consistent water changes. The temperatures fluctuated a small amount within tanks across days due to the error rate of the aquarium heaters (±1°C). There does appear to be a small degree of difference between some of the replicate tanks within temperature treatments, yet all stayed within acceptable experimental ranges (Figure 3.7). Oxygen levels were lower in the warmer temperature treatments dropping to ~80% saturation in the 27°C treatment, ~84% in the 25°C treatment and ~87% in the 23°C treatment compared to ~97% saturation in the 18°C treatment (Figure 3.8). This is likely due to the lowered affinity for oxygen of warmer waters and this may have had an effect on the sponges as a tributary result of the temperature treatment.

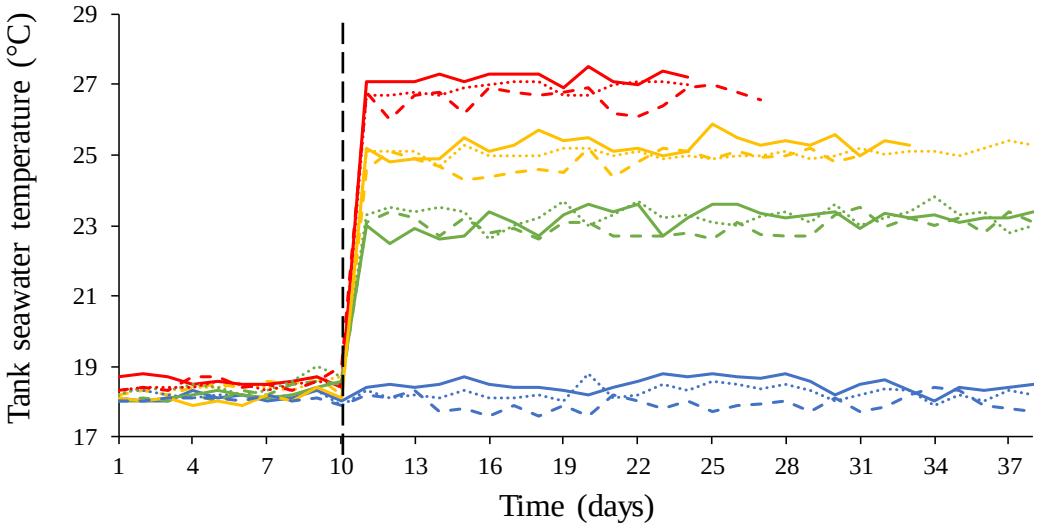

Figure 3.7: Daily temperature readings in each tank where sponges remained alive across the 10-day acclimation period and 28-day experiment. The black vertical dotted line represents the day that temperature treatments were administered with temperature treatments represented by red; 27°C, yellow; 25°C, green; 23°C and blue; 18°C. Small and large dotted lines and the solid line distinguish each of the three replicate tanks in each temperature treatment.

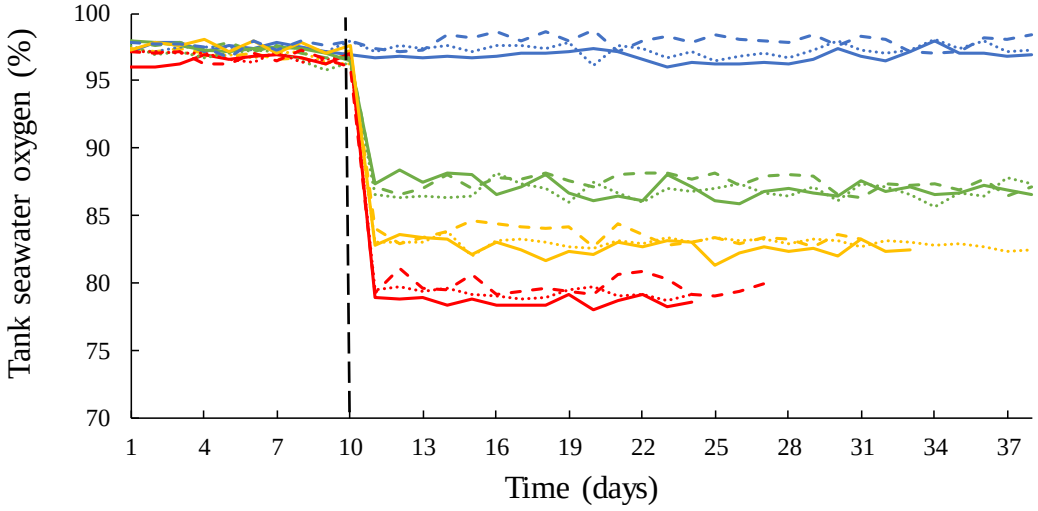

Figure 3.8: Daily oxygen (% saturation) readings in each tank where sponges remained alive across the 10-day acclimation period and 28-day experiment. The black vertical dotted line represents the day that temperature treatments were administered with temperature treatments represented by red; 27°C, yellow; 25°C, green; 23°C and blue; 18°C. Small and large dotted lines and the solid line distinguish each of the three replicate tanks in each temperature treatment.

3.4.2 Respiration response

The two-way repeated measures ANOVA indicated that there was a significant interaction effect of time and temperature treatment on the respiration rate of *T. burtoni* ($F_{(4, 32)} = 4.002$, $p = 0.010$) (Table 3.5). Therefore, the simple main effects of the 18°C and 23°C temperature treatments were compared at each time point. Results from these pairwise comparisons indicated a statistically significant difference in respiration rate for the 18°C and 23°C treatment groups at baseline levels. This is likely an anomaly since there were no extraneous factors that should have influenced respiration at this time point since all sponges were still being housed at 18°C at this time and the order of measurements and tank allocations were entirely randomized. Week one, three and four of temperature treatments indicated no statistically significant difference in respiration rates of *T. burtoni* between the 18°C and 23°C treatment groups, however respiration rate was significantly higher in the 23°C treatment compared to the 18°C treatment in week two of exposure (Table 3.6, Figure 3.9). The respiration rate of *T. burtoni* in the 27°C treatment was elevated after the first week of exposure. It was observed during measurements that individuals with signs of disease appeared to have higher respiration rates than

those that did not. This explains the greater degree of variance in respiration response in this treatment (Figure 3.9). This was also observed in the 25°C treatment in week two, however individuals that did not display signs of disease at this point still has elevated respiration levels and this explains the tighter variance seen for this treatment (Figure 3.9). This makes sense since a significant respiration response was observed in the 23°C treatment at this time also. Respiration rate in diseased individuals could be masked by the respiration of the increased levels of microbe numbers in the sponge and to test whether this could be an explanation for the increased level of respiration a small amount of bacterial film was removed from a sponge in the 25°C treatment group and placed inside the respiration vessel in plain 250 µm filtered sea water. There was a significant oxygen consumption curve observed in this crude test compared to that of a blank plain 250 µm filtered seawater measurement (see Appendix, Figure 5.2) and thus does not discount this assertion. Therefore, the respiration rates observed for those individuals that had signs of disease are not reliable tests of metabolic demand for the sponges themselves as the true respiration rate of the sponge is potentially being masked by increased microbial populations infecting the sponge. The measurements for the 23°C and 18°C treatments are likely to be reliable as individuals that had their respiration rates tested in these groups did not display any signs of disease.

Table 3.5: Two- way repeated measures ANOVA comparing the mean respiration rate (µmol O_2 g^{-1} FW h^{-1}) of *T. burtoni* in the 18°C and 23°C treatments at baseline and weeks one through to four of temperature treatment based on natural log transformed values (n = 9 individuals per treatment). Significant *p*-values indicated in bold at $\alpha = 0.05$.

	Type III SS	***df***	***MS***	***F***	***p***
Treatment	0.464	1, 8	0.464	5.637	**0.045**
Time	1.590	4, 32	0.398	2.512	0.061
Treatment*Time	1.666	4, 32	0.104	4.002	**0.010**

Table 3.6: Multiple *post hoc* pairwise comparisons for the respiration rate (µmol O_2 g^{-1} FW h^{-1}) of *T. burtoni* in the 18°C and 23°C treatments before temperature treatment (baseline) and at weeks one through to four of temperature treatment based on natural log transformed values (n = 9 individuals per treatment). Significant *p*-values indicated in bold at α = 0.05.

	Mean difference	***SE***	***p***	***Lower 95% CI***	***Upper 95% CI***
Baseline	0.301	1.220	**0.039**	0.019	0.582
Week one	0.133	0.212	0.548	-0.623	0.356
Week two	0.555	0.184	**0.017**	-0.979	-0.131
Week three	0.130	0.071	0.105	-2.940	0.034
Week four	0.200	0.109	0.103	-4.520	-0.051

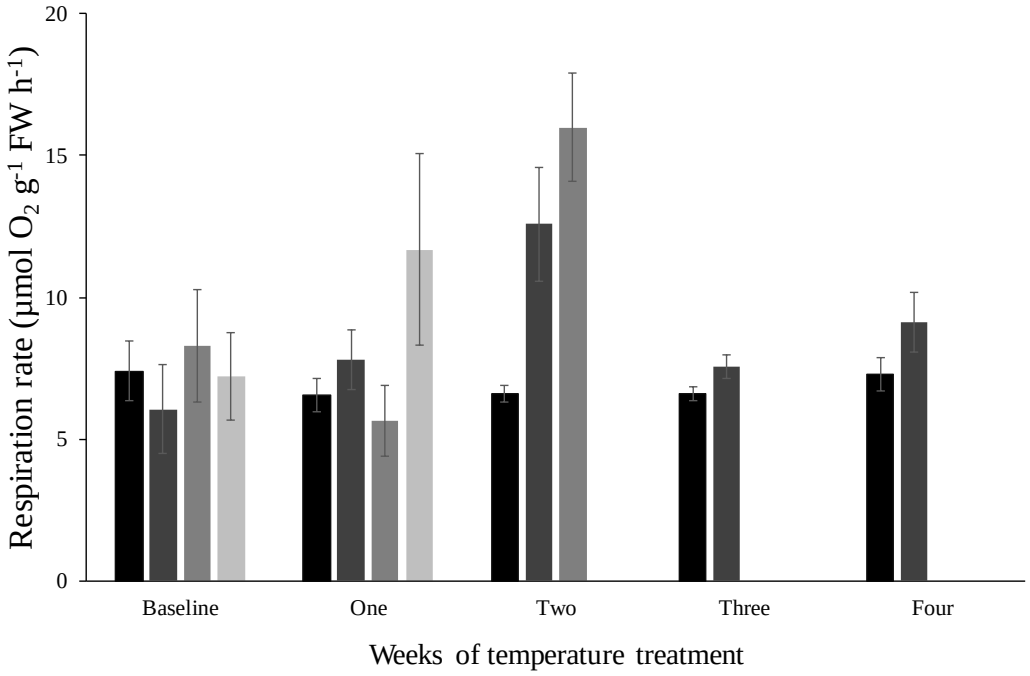

Figure 3.9: Mean respiration rate (µmol O_2 g^{-1} FW h^{-1}) of *T. burtoni* before temperature treatment (baseline) and at four weekly time points post the first day of temperature treatment. Error bars = ± one standard error (n = 9 individuals per treatment). Data displayed are based on raw values. Black bars; 18°C, dark grey bars; 23°C, medium grey bars; 25°C, light grey bars; 27°C.

3.4.3 Survival

Results from the Mantel-Cox test indicated that survival curves differed significantly between the temperature treatments (χ^2 = 185.982, df = 3, p <0.001). Subsequent *post hoc* pairwise Mantel-Cox test comparisons indicated that both the

25°C and 27°C treatments differed significantly in their survival curves compared to that of the control 18°C treatment while the 23°C did not (Figure 3.10, Table 3.7). In addition, the 23°C, 25°C and 27°C treatments were all significantly different from one another in their survival curves (Figure 3.10, Table 3.7). Complete survival was observed for all individuals housed in 18°C sea water while only a single death event occurred in the 23°C treatment on the 20th day of temperature treatment. The 27°C treatment appeared to display three distinctive mass death events on the 9th, 12th and 15th day of the temperature treatment and had a median survival estimate of 12 days while the 25°C treatment had a median survival estimate of 18 days and a distinctive die off event on this day (Figure 3.10, Table 3.8).

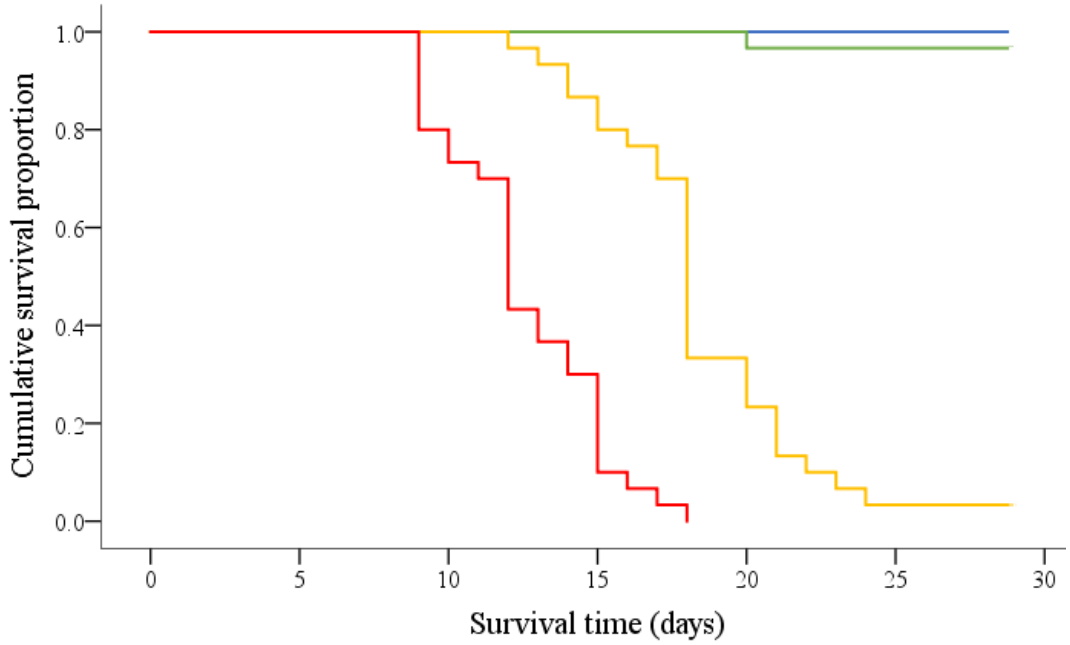

Figure 3.10: Kaplan-Meier cumulative survival proportion curves for *T. burtoni* in the four temperature treatments (n=30 individuals per treatment). Temperature treatments represented by red; 27°C, yellow; 25°C, green; 23°C and blue; 18°C.

Table 3.7: Mantel-Cox pairwise comparison test results comparing survival curves of *T. burtoni* between all four temperature treatments (n=30 individuals per treatment). Significant *p*-values indicated in bold at Bonferroni corrected $\alpha = 0.008$.

	18°C		**23°C**		**25°C**	
	χ^2	*p*	χ^2	*p*	χ^2	*p*
23°C	1.000	0.317				
25°C	62.871	**<0.001**	59.186	**<0.001**		
27°C	68.446	**<0.001**	68.446	**<0.001**	39.802	**<0.001**

Table 3.8: Kaplan-Meier median survival estimates per temperature treatment (n=30 individuals per treatment). Median survival for 18°C and 23°C treatments not present as median survival estimates are based on a cumulative survival proportion of 0.5 or less.

	Total n	**n deceased**	**n survived**	**% survival**	**Median survival (± SE)**
18°C	30	0	30	100	-
23°C	30	1	29	96.7	-
25°C	30	29	1	3.3	18 ± 0.235
27°C	30	30	0	0	12 ± 0.339

3.4.4 Disease and morphological changes

In the 18°C treatment the external appearance of individual *T. burtoni* at the conclusion of the experiment represented healthy individuals (Figure 3.11, Figure 3.12 a, g). Despite significant survival observed in the 23°C treatment, *T. burtoni* in this treatment did experience some changes to their external appearance such as a loss of pigment and very minor dermal cortex loss (Figure 3.11, Figure 3.12 b, f). A few individuals in the 23°C treatment also began to develop what appear to be the formation of reproductive buds (Figure 3.12 c). Disease appeared to dominate individuals within the 27°C treatment, with minimal changes to morphology, though some loss of pigment and minor dermal cortex loss was observed (Figure 3.11, Figure 3.12 d, e). *T. burtoni* individuals in the 25°C treatment underwent the most significant morphological changes with significant loss of dermal cortex common and a loss of internal spongin (Figure 3.12 b, h, i).

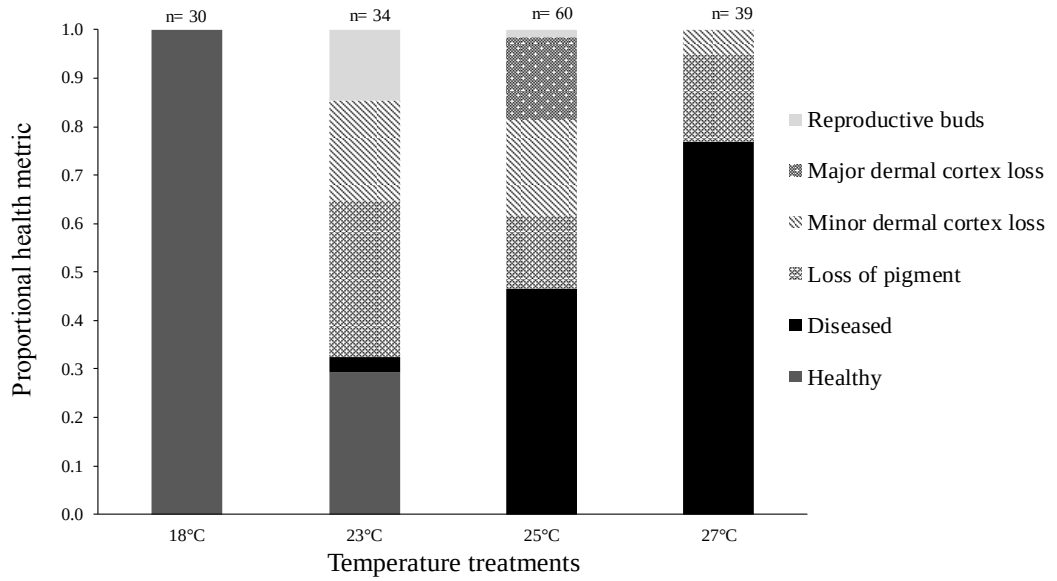

Figure 3.11: Proportional health metrics for all *T. burtoni* individuals at the end of the experimental period or at death in each of the four temperature treatments. Individual sponges displaying more than one of the characterized health metrics were allocated to all health metrics (n per group displayed at top of chart).

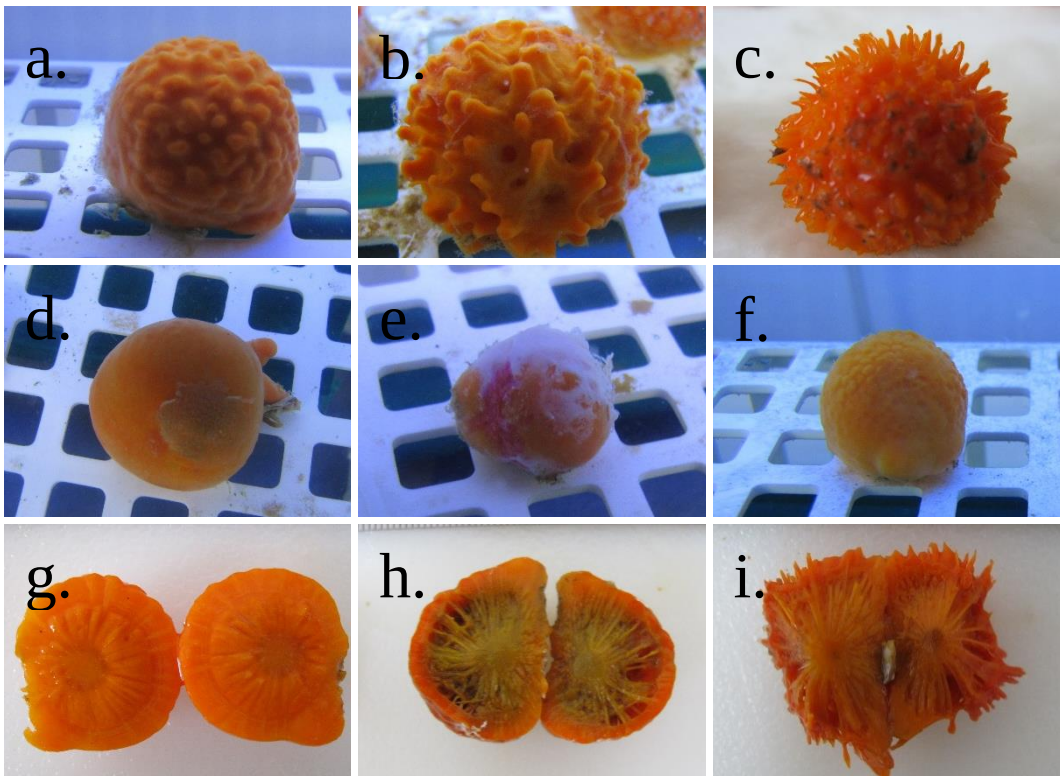

Figure 3.12: Health metric responses of *T. burtoni* observed during the temperature experiment. **a**: healthy; **b**: loss of dermal cortex; **c**: signs of reproductive buds forming; **d**: brown microbial infection; **e**: white and pink microbial infection, **f**; loss of pigment (pale compared to healthy individual); **g**: cross section healthy; **h**: cross section deceased with significant spongin loss; **i**: cross section extensive dermal cortex and internal spongin loss.

3.5 *In situ* environmental results

3.5.1 Sedimentation rates and grain size compositions

Sedimentation rates between each of the four locations where *T. burtoni* was present varied substantially. Karewa Island displayed a relatively consistent and small sedimentation rate over the three sampling periods not exceeding more than 2 g m^{-2} d^{-1} (Figure 3.13). For the first sampling period at Motiti Island, sedimentation rates were virtually identical to that of Karewa Island at 1.2 g m^{-2} d^{-1}. However, sedimentation rates at Motiti Island increased in both the second and third sampling period with rates of 8.3 g m^{-2} d^{-1} and 13.6 g m^{-2} d^{-1} respectively, suggesting greater variability of suspended sediments at this site during the nine weeks of sampling (Figure 3.13). Pilot Bay also experienced relatively consistent sedimentation rates over the first and second sampling period with sedimentation rates of 28.8 g m^{-2} d^{-1} and 29.8 g m^{-2} d^{-1} respectively. Rabbit Island consistently experienced the highest sedimentation rate of all the sites surveyed with 46 g m^{-2} d^{-1} and 52.5 g m^{-2} d^{-1} for the first and second sampling periods respectively (Figure 3.13). Percent volume of grain sizes for each of the sampling locations and times were not as distinct as the sedimentation rates observed (Figure 3.14). Rabbit Island appeared to have the lowest percent volume of fine grain sizes despite having the greatest sedimentation rate and it appears that sedimentation rate and mean grain size are somewhat correlated with an *r* value of 0.482 (Figure 3.15). However, Karewa Island in the first measurement period conformed the least to this relationship and appeared to have lower levels of fine grained sediments than the second and third measurement periods at this site (Figure 3.14). Despite this apparent relationship between mean grain size and sedimentation rate, proportionally speaking Rabbit Island and Pilot Bay still had the greatest amount in terms of g m^2 d^{-1} of all grain sizes (Figure 3.13). All measurements fell into either the sandy-silt or silty-sand categories when plotted onto a ternary diagram (Figure 3.16) with clay components making up < 4% of samples in each of the location and measurement period groups (Table 3.9).

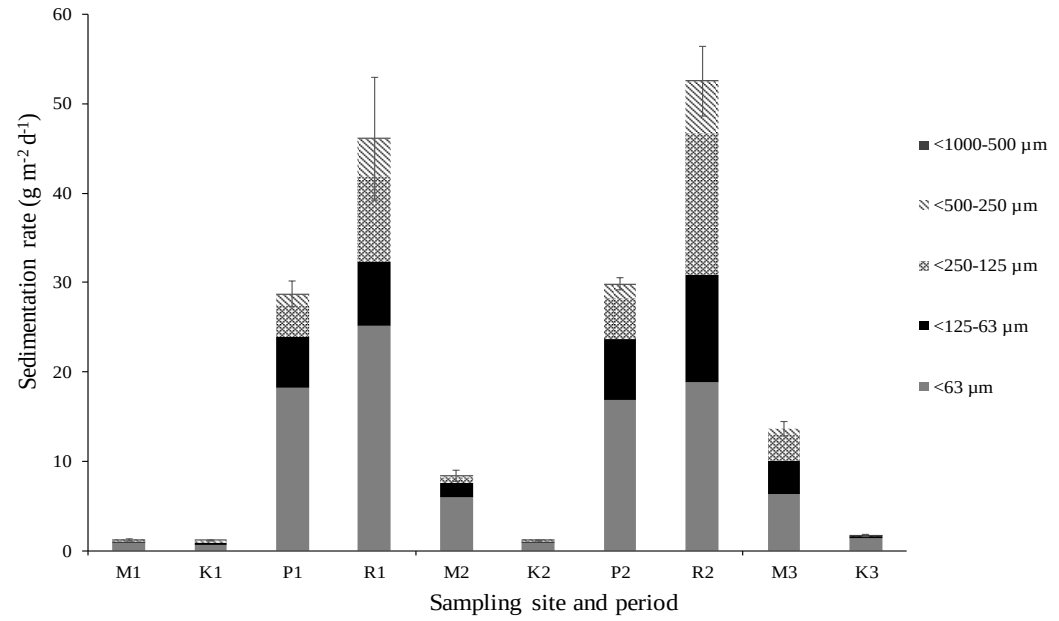

Figure 3.13: Total sedimentation rate (g m^2 d^{-1}) and proportion grain size class at each of the four *T. burtoni* habitat sites. Sampling site: M; Motiti Island, K; Karewa Island, P; Pilot Bay and R; Rabbit Island with numbers 1, 2 and 3 representing the first, second and third sampling periods respectively. (n= 3 sediment traps per measurement period). Error bars = ± one standard error.

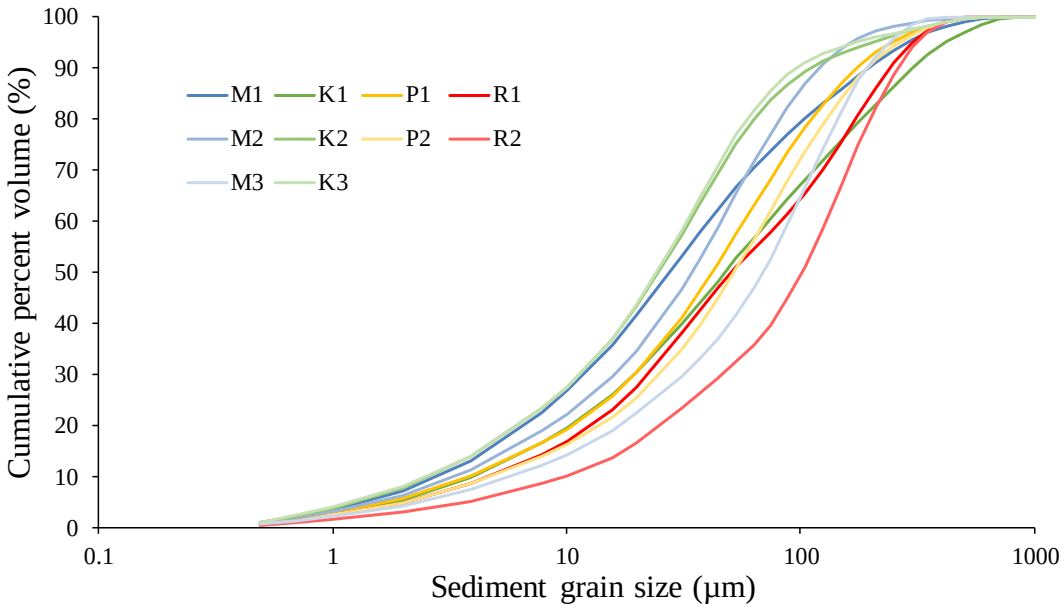

Figure 3.14: Mean cumulative percent volume (%) for the sediment grain sizes (µm) at each of the four sites across the three measured sampling periods. The legend denotes the following: M; Motiti Island, K; Karewa Island, P; Pilot Bay and R; Rabbit Island with the numbers 1, 2 and 3 representing the first, second and third sampling periods respectively. (n= 3 sediment traps per measurement period).

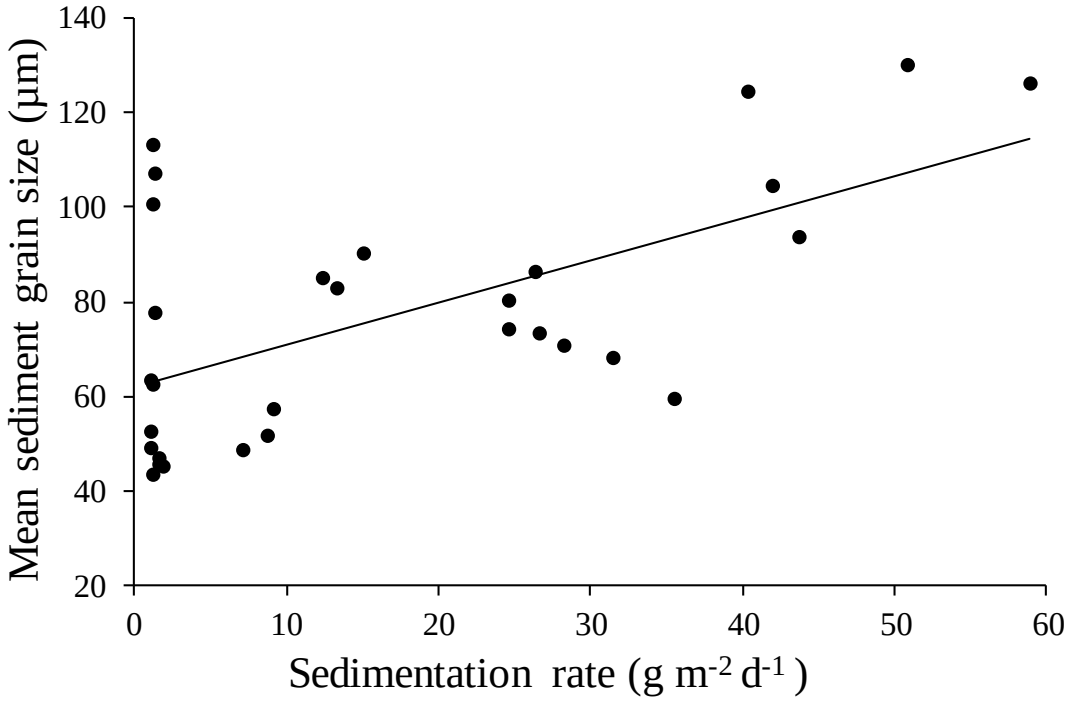

Figure 3.15: Spearman's rank-order correlation of sedimentation rate (g m^{-2} d^{-1}) versus mean sediment grain size (µm) for all sampling sites and measurement periods (n= 30 sediment traps) r = 0.482, p = 0.007 at α = 0.01.

Table 3.9: Percent (%) sediment type composition for each of the locations and sampling periods. M; Motiti Island, K; Karewa Island, P; Pilot Bay and R; Rabbit Island with the numbers 1, 2 and 3 representing the first, second and third sampling periods respectively. (n= 3 sediment traps per measurement period).

	M1	**K1**	**P1**	**R1**	**M2**	**K2**	**P2**	**R2**	**M3**	**K3**
Gravel	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sand	33.5	47.5	42.6	49.0	34.9	25.1	49.6	67.7	58.5	23.4
Mud	66.5	52.5	57.4	51.0	65.1	74.9	50.4	32.3	41.5	76.6
Coarse sand	1.7	4.8	1.0	0.7	0.3	0.8	0.9	0.9	0.0	1.0
Medium sand	7.2	12.2	5.9	13.1	2.4	3.8	7.4	16.7	7.7	3.0
Fine sand	10.9	14.7	14.6	20.7	10.3	6.0	18.1	31.2	25.5	4.9
Very fine sand	13.6	15.7	21.2	14.6	21.8	14.4	23.3	18.9	25.2	14.6
Very coarse silt	24.3	21.6	26.5	22.9	29.9	30.9	24.4	15.3	18.8	32.4
Coarse silt	15.4	11.3	11.5	11.2	13.0	16.4	9.5	6.9	8.4	16.7
Medium silt	13.6	9.8	9.3	8.2	10.8	13.7	7.7	4.8	7.0	13.4
Fine silt	6.0	4.3	4.5	3.9	5.0	6.2	3.8	2.3	3.3	6.1
Very fine silt	3.9	2.8	3.0	2.6	3.3	4.2	2.6	1.5	2.1	4.2
Clay	3.4	2.7	2.7	2.3	3.1	3.6	2.3	1.4	2.1	3.8

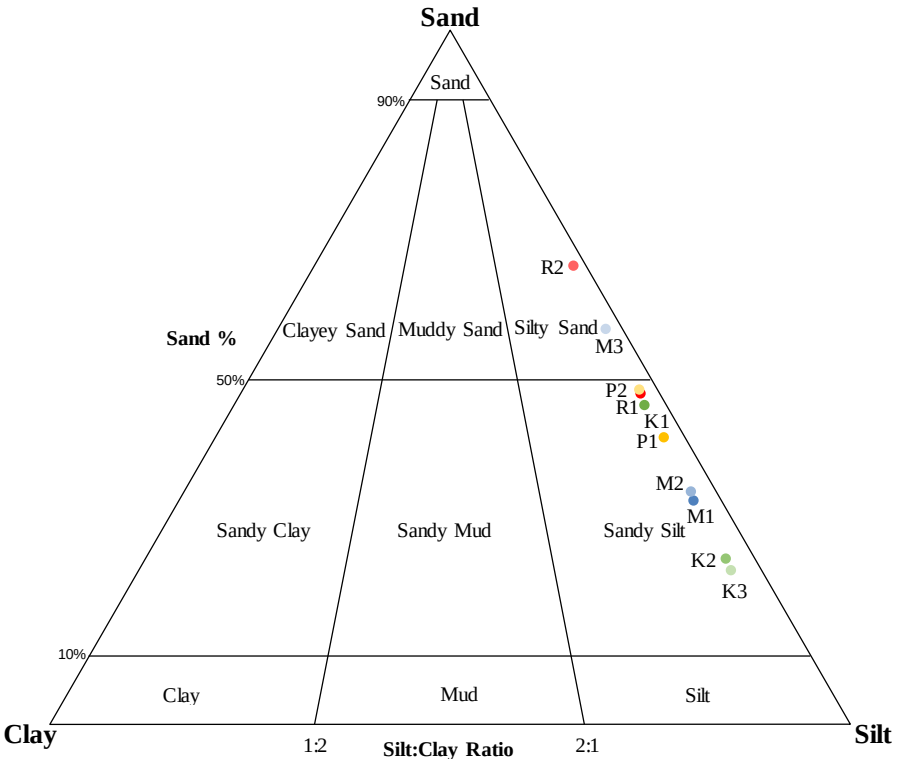

Figure 3.16: Ternary diagram displaying sand-silt-clay composition of the sediments collected in the sediment traps at each of the four locations across the three sampling periods. M; Motiti Island, K; Karewa Island, P; Pilot Bay and R; Rabbit Island with the numbers 1, 2 and 3 representing the first, second and third sampling periods respectively. (n= 3 sediment traps per measurement period).

3.5.2 Temperature

Temperatures at all four of the *in situ* sites surveyed appeared to follow a similar trend and confirmed experimental temperatures used in the temperature experiment were relevant ranges (Figure 3.17). Despite 23°C being reached at the Pilot Bay site at the beginning of March, this peak was only present for a few days and fluctuated to lower temperatures between these days. Warm temperatures of ~21°C did persist for about a two-week period from around the 19th of February till the 5th of March, with temperatures otherwise staying largely below 21°C for the measurement period before and after this peak (Figure 3.17).

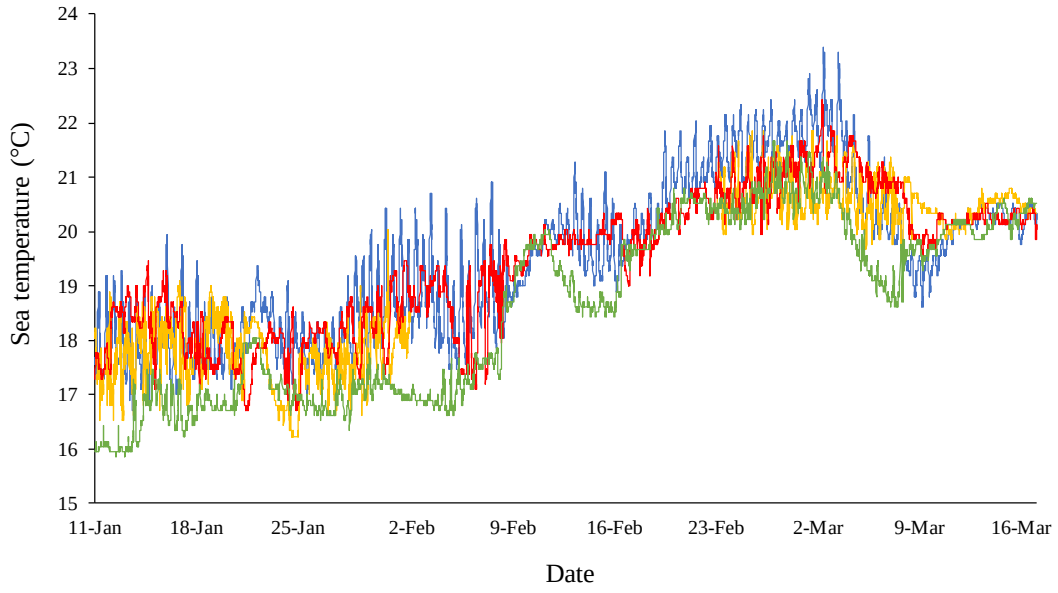

Figure 3.17: *In situ* temperatures (°C) observed over the 2017 summer in the Bay of Plenty at four sites where *T. burtoni* populations exist. Red; Motiti Island, yellow; Karewa Island, green; Rabbit Island and blue; Pilot Bay.

Chapter 4

Discussion

4.1 Sediment

Disruption and damage to sponges from sediments is reported in published research (*see* review by Bell *et al.* (2015b)), however ecological studies also demonstrate that high sediment locations can support sponge communities of great abundance and diversity (*see* review by Schonberg (2016)). Investigations of the metabolic cost to sponges of increased sedimentation and changes to sediment type and grain size have been scarce, with few published investigations addressing the metabolic consequences of this for marine sponges inhabiting temperate southern hemisphere waters. New Zealand is a naturally erosion prone country and climate change modelling suggests that increased storm frequency and severity is predicted to further exacerbate sedimentation problems (Hume *et al.*, 2010). Understanding how sponges may fare in a changing ocean is becoming increasingly important to better consider these unique invertebrates in conservation and management practices (Bell *et al.*, 2015a). The main aim of the first three experiments presented here was to investigate how the common temperate marine golf ball sponge, *T. burtoni*, a relevant neritic zone species characterising a high proportion of New Zealand's inshore reef systems, responds metabolically when presented with varying concentrations and grain size classes of sediments. In addition, how fine sediments affect the respiration rate of *T. burtoni* over a longer period of exposure was also investigated. Understanding the metabolic response of sponges to sediments allows inferences to be made about potential thresholds for health and survivorship and what this may mean for these unique invertebrates and the ecosystem services they provide under future climate scenarios.

4.1.1 Respiration response

Sponges as filter feeders need to maintain water flow through their aquiferous system to utilize particulate and dissolved organic carbon sources for nutrition and to maintain a supply of oxygen for carrying out metabolic processes (Reiswig, 1971b; 1971a; 1974). In this study, a reduction in respiration rate of *T. burtoni* was observed in the storm proxy sediment concentration treatments of 500 mg l^{-1} and

1000 mg l^{-1} in experiment one and the <63 µm and <125-63 µm grain size classes at 500 mg l^{-1} in experiment two. The ambient concentration treatments of 20 mg l^{-1} and 100 mg l^{-1} in experiment one did not elicit a significant immediate change in respiration compared to the control and this suggests that a threshold exists somewhere between 100 mg l^{-1} and 500 mg l^{-1} for *T. burtoni* whereby sediment concentrations may become problematic. While it appears a threshold exists, it is also possible that the effect size in the 20 mg l^{-1} and 100 mg l^{-1} treatments was too small for a significant response to be detected with this particular experiment. This may also be the case for experiment two with respect to grain size classes and differences in respiration rates between them. The long-term experiment did not show any significant differences between the treatment of <63 µm sediments at 500 mg l^{-1} compared to the control over a 20-day exposure period. The reduction in respiration observed in the first two experiments are therefore likely to be short-term responses whereby pumping activities are reduced or completely ceased as a method to prevent further clogging of the aquiferous system. Reductions or a complete arrest in pumping activities have been observed in other species in both experimental (Gerrodette & Flechsig, 1979; Leys *et al.*, 1999) and natural (Reiswig, 1971b) conditions under increased sediment loads. Respiration was virtually supressed in the 1000 mg l^{-1} concentration treatment reaching a rate of 0.607 µmol O_2 g^{-1} FW h^{-1} compared to the pre-treatment rate of 14.039 µmol O_2 g^{-1} FW h^{-1}. This small amount of respiration was likely occurring through diffusion at the surface of the sponge and it is probable that pumping activities were completely arrested for individuals exposed to this treatment. Indeed, the surfaces of the individuals exposed to this treatment did appear to "smooth" over, with exhalant oscula closed after exposure and while not quantitatively measured, it was observed that individuals appeared to inflate after a short period of recovery and return to a warty appearance that is characteristic of this species (Kelly, 2015). Fast recovery rates of less than 30 minutes have been observed for other species as well (Kutti *et al.*, 2015), and while recovery respiration rates were not measured here, these observations suggest that they likely returned to pre-exposure levels after half an hour. For *T. burtoni* individuals exposed to the 500 mg l^{-1} concentration treatment, respiration rates were significantly greater at 5.663 µmol O_2 g^{-1} FW h^{-1} compared to the 1000 mg l^{-1} treatment. This may suggest that pumping was reduced but not completely arrested and *T. burtoni* may still be able to operate somewhat under this

loading. Bannister *et al.* (2012) also noted an apparent closing of oscula and reduced pumping activity of the tropical sponge *Rhopaloeides odorabile* when exposed to sediments, yet conversely demonstrated that respiration rates increased for *R. odorabile* despite an apparent shut down and slowing of pumping. While pumping and respiration in sponges are linked, this demonstrates that using one as a proxy for the other should be done with caution. Interestingly, the increased respiration response seen for *R. odorabile* to sediments (Bannister *et al.*, 2012) has only been observed for one other species, and like *R. odorabile*, is a tropical sponge. Biggerstaff *et al.* (2017) found that *Lamellodysidea herbacea* reduces respiration as an immediate response to increasing amounts of settled sediment as was found in this study, yet over a continued exposure period respiration rate was increased likely due to the increased metabolic cost of producing sediment clearing mucous (Biggerstaff *et al.*, 2017). All other studies investigating the respiration response of sponges to sediments note a reduction (and sometimes no change) to respiration rates after exposure with these studies investigating species from temperate seas (Lohrer *et al.*, 2006a; Murray, 2009; Tjensvoll *et al.*, 2013; Kutti *et al.*, 2015). Despite this predominant trend of a decline in respiration, the concentration of sediments that elicit a response differ, and is likely to be species specific, while sediment type, grain size and exposure duration also play a role. Kutti *et al.* (2015) investigated the response of the deep-water sponge *Geodia barretti* to sediments of differing sources; one from the natural bottom sediment of *G. barretti* habitat and the other crushed granite rock used to simulate mine tailings and drill cuttings. Both sediments were of a similar grain size and concentration (at 10 mg l^{-1} and 50 mg l^{-1} treatments), yet had markedly different effects on *G. barretti*. Individuals exposed to the crushed granite rock had a 60% permanent reduction in oxygen consumption when exposed to sediment concentrations of 50 mg l^{-1} cyclically (12:12 hour treatment: non-treatment exposure cycle) over a 50-day period while natural bottom sediments had no effect (Kutti *et al.*, 2015). This is presumably because *G. barretti* is able to cope with these types of sediment more effectively since they occur naturally in their environment. Interestingly however, in terms of exposure duration, Kutti *et al.* (2015) found that under a single pulse event of crushed granite rock, a significant respiration reduction was only observed at 500 mg l^{-1} whereas *G. barretti* respiration shuts down completely at 100 mg l^{-1} and is even reduced at 50 mg l^{-1} when exposed to a single pulse event of natural sediment (Tjensvoll *et al.*,

2013). *G. barretti* therefore responds sooner to lower concentrations of natural sediments than crushed granite rock and this response may be what allows *G. barretti* to cope with natural sediments over longer periods of exposure. The sponge has thus developed mechanisms to deal with these natural sediments, but not those of extraneous sediments such as crushed granite rock. This may also serve as an explanation as to why there was no observed effect of fine sediments over a long-term exposure at 500 mg l^{-1} in the third experiment conducted here, despite there being a reduction in respiration under an immediate single pulse event of 500 mg l^{-1} of the same sediment type and grain size class (<63 µm) in the second experiment. However, it is noted that there were issues with the long-term experiment (discussed in detail below in section 4.1.3), and therefore this finding remains speculative. While Bannister *et al.* (2012) demonstrated a converse respiration response to that of Kutti *et al.* (2015), they also noted an apparent difference in the respiration response of *R. odorabile* exposed to the same concentration and similar grain sizes of two contrasting mineralogical sediment types. Clay sediments caused a significantly greater affect than carbonate sediments and the authors attributed this to the physical properties of the sediments since clay has adhesive surface properties (Wang & Lee, 1993) while carbonate sediments do not (Milliman *et al.*, 1974). Therefore, *R. odorabile* may need to invest in more metabolically expensive sediment clearance mechanisms such as observed mucous production to remove "stickier" sediments from the aquiferous system (Bannister *et al.*, 2012).

In both the long-term experiments of Bannister *et al.* (2012) and Kutti *et al.* (2015), the sediment types that elicited a more significant response both had smaller mean grain sizes of 3.1 µm and 72 µm respectively compared to the sediment types that had less or no effect with mean grain sizes of 8.2 µm and 139 µm. These smaller grain sizes may have contributed to the observed differences, however the grain size classes used here in the second experiment of < 63 µm and <125-63 µm, while both reducing the respiration rate of *T. burtoni*, did not differ significantly from one another. Hence, sediment type, opposed to grain sizes within these ranges, may be of greater importance with reference to the effects on sponge metabolism. Indeed, sponges appear to be highly sensitive and attuned to sediment mineralogy and some species can actually incorporate sediments into their structure. This ability has been demonstrated in the sponge *Chondrosia reniformis*, that can distinguish between quartz and carbonate particles and depending on the environmental conditions, can

either switch this mechanism on or off (Bavestrello *et al.*, 1998a; 1998b; Cerrano *et al.*, 2007). The observed differences in the respiration response of *T. burtoni* to different grain size classes in the second experiment makes sense mechanically, since the two smallest grain size classes fall within the typical size range of ostia openings that are usually <200 µm in size (Bergquist, 1978). This suggests that these smaller grain size classes may be entering the aquiferous system of *T. burtoni* whereas the larger grain size classes for the most part do not, since they cannot physically enter into the sponge aquiferous system. This is not to say that larger sediment grain sizes do not effect sponges, since it has been demonstrated that sponges may become damaged from the scouring of tissue surfaces from larger grain sizes (Rogers, 1990; Ilan & Abelson, 1995) and continued damage could eventually become metabolically costly due to the need for continual repair efforts.

While most published research investigating the respiration response of sponges to sediments report threshold levels <500 mg l^{-1}, Murray (2009) reported a threshold effect to occur somewhere between 2500 mg l^{-1} and 8500 mg l^{-1} for *Tethya bergquistae*. *T. bergquistae* is also a *Tethya* species that commonly inhabits New Zealand coastlines (similar in latitudinal distribution to *T. burtoni* (Kelly, 2015)), yet appears to have a sediment concentration threshold at least five times that of *T. burtoni*. This difference may be attributed to various factors including experimental sediment type, exposure duration and grain size composition. To date, there has not been any explicit investigation comparing the respiration rates of different species of sponge under identical experimental sediment treatments. With various factors such as grain size, and sediment type having an effect on the respiration response of sponges, further compounded by differences in housing aquaria such as flow rate, water source and a whole host of other factors able to influence sponge physiology and behaviour, comparing results between studies may lack relevance and this is certainly an area for future research effort.

Whether the reduced respiration rates and likely reduced pumping activity in these first two experiments are an active protective response of the sponge, or a passive reduction due to clogging also remains to be resolved. Either way, less sediment is entering the aquiferous system. The response in both cases suggests that this reduction in respiration may be an immediate protective response as opposed to employing more metabolically costly responses such as mucous production (Turon

et al., 1999; Kowalke, 2000), or backwashing (Storr, 1976; Simpson, 1984) that have been observed for sponges exposed to sediments over longer periods of time (Bannister *et al.*, 2012; Biggerstaff *et al.*, 2017). An immediate protective response of a reduction in respiration makes practical sense in both of these experiments due to the transient nature of sediment concentration and grain sizes in the environment. Sponges have been shown to reduce pumping initially and then intermittently increase their pumping activities during exposure to sediments in an apparent re-testing of the water for sediments (Leys *et al.*, 1999); suggesting they are highly responsive to water condition. Lower oxygen consumption may allow organisms to increase survival during periods of environmental stress (Hand & Hardewig, 1996), however, this may also limit energy availability for reproduction and growth over longer periods of suppression. Should sedimentation events become more prevalent and protracted, a further threshold may be reached, one of viability of the sponge even in a somatically reduced form.

4.1.2 *In situ* sedimentation

From the *in situ* data gathered on sedimentation rates in various *T. burtoni* habitat, it was of interest that Rabbit Island, consistently demonstrating the greatest sedimentation rate of all the sites, also had largest *T. burtoni* populations (personal observation). However, Rabbit Island also showed that proportionally, in terms of percent volume, that sediments at this site were generally coarser than at the other sites, yet due to the high level of sedimentation still had the greatest amount in g m^2 d^{-1} of fine sediments falling out of suspension. Since there were healthy and large populations of *T. burtoni* at this site suggests that they are capable of coping with sedimentation in their environment to at least this degree. As the populations of *T. burtoni* and the sedimentation rates at Karewa Island and Motiti Island were much sparser, it is suggested that *T. burtoni* could fare better in environments with some sedimentation since *Tethya* sponges are known to often inhabit high level sediment environments such as harbours (Schonberg, 2016). This conjecture also supports the assertion of an apparent lack of effect of sediment treatment in the long-term experiment. However, the structuring of *T. burtoni* populations are likely multifactorial and previous work has indicated that substrate inclination (which will affect sediment/detritus deposition rates), is more important for *T. burtoni* than other factors including sedimentation (Cárdenas *et al.*, 2012). It is difficult to

accurately attribute the causes of population distributions when it comes to sponges and even studies looking at multiple factors may only be able to explain a minority proportion of how a given sponge population is structured (Wulff, 2012). This is likely because there are so many variables, both abiotic and biotic at work, further compounded by the plasticity and adaptability of many sponges to their environment (Gaino *et al.*, 1995; Meroz-Fine *et al.*, 2005).

Indeed, previous work investigating the effects of sedimentation on conspecifics from a muddier sediment site in a harbour, and a site further afield that contained coarser sediments found that conspecifics of *Aaptos* spp. in the harbour site were more resilient than those from the site further afield (Lohrer *et al.*, 2006a; 2006b). Since Rabbit Island had the highest sedimentation rate of the four sites studied here and the experimental individuals of *T. burtoni* were collected from this site, conspecifics from Karewa Island and Motiti Island that experience very low levels of sedimentation may be more sensitive to sediments compared to conspecifics at the Rabbit Island and Pilot Bay sites. Fine clay sized particles consistently made up <4% of the total volume of sediments at all four sites, despite the majority of sediment volume being made up of particles <125 µm in size. Under future scenarios where fine terrigenous sediments are predicted to increase (Hume *et al.*, 2010), fine clay sized particles may increase in quantity. Since *T. burtoni* only had a notable response to the finer grain size classes in the second experiment, this may indicate that *T. burtoni* could be adversely affected into the future and from the work of Kutti *et al.* (2015) and Bannister *et al.* (2012), changes in the sediment mineralogical composition may also be of fundamental importance. Aquaria based experiments need to be further validated through *in situ* studies and currently only Lohrer *et al.* (2006a) has investigated the respiration rates of sponges (*Aaptos* spp.) *in situ* under settled terrigenous sediments and found that respiration rate as well as feeding and condition were reduced in response to subtidal terrigenous sediment burial event experiments. While field validation is important, dynamic coastlines make subtidal work logistically difficult, hence the attraction of aquaria experiments. The metabolic response of sponges to sedimentation events is dependent on the concentration, grain size distributions, type of particles and exposure period (Maldonado *et al.*, 2008; Bannister *et al.*, 2012; Kutti *et al.*, 2015). How sponges may cope under sedimentation scenarios appears to be species specific and it is difficult to accurately predict due to the innate variable properties

of the sediments and *in situ* conditions which can be highly transient with respect to suspended particles. Under increasing sedimentation scenarios, sediments indeed may affect sponges and other filter feeders in a negative way by compromising filtration systems, while also limiting nutrient availability for phototrophic species through increased turbidity affecting light attenuation and subsequently photosynthetic symbionts (Pineda *et al.*, 2016). Another consideration in this regard is that sediments are not necessarily inert particles in that toxicants can bind to them and may bioaccumulate in the sponge when moved through the aquiferous system becoming potentially lethal (Cebrian *et al.*, 2003; Cebrian *et al.*, 2006).

4.1.3 Conclusions, limitations and future research

Findings from the first two experiments indicate that fine sediments and high sediment loads reduce the respiration rate of *T. burtoni* under abrupt exposure conditions likely as a response to prevent further clogging of the aquiferous system. These results are largely in line with previous research in this field. The third longer term experiment did not indicate any differences in the respiration rate of *T. burtoni* exposed to fine sediments at a high concentration compared to the control. This may mean that *T. burtoni* can cope with fine sediments at high storm equivalent concentrations for an extended period of time. This may be what allows *T. burtoni* to inhabit harbour environments. Additionally, this may also be why they are present in healthy numbers at the Rabbit Island site that experienced the greatest *in situ* sedimentation levels. This finding aligns with observations of other *Tethya* species coping well in high sediment environments (Murray, 2009; Schonberg, 2016).

Observations during the third experiment indicate that the experimental aquaria may have been suboptimal for *T. burtoni* and unfortunately this means that the long-term experiment results may not be reliable. The housing conditions and sediment treatments may have presented issues for the sponges for a number of reasons. Firstly, it was difficult to maintain all of the sediments in suspension in the experimental set up as they did settle out somewhat and this may have contributed to a lack of true experimental effect with regard to suspended sediment. Despite no significant weight loss in the control or treatment groups, both did contain individuals at the termination of the experiment that exhibited evidence of reproductive buds forming and internal tissue necrosis; signs of stress (Saller, 1990;

Cerrano *et al.*, 2001). Additionally, baseline and all subsequent respiration rates were lower in both the control and treatment groups at ~7 µmol O_2 g^{-1} FW h^{-1} compared to the 'before treatment' respiration measures observed in experiments one and two; these were around twice as high ranging between around ~12-16 µmol O_2 g^{-1} FW h^{-1}. Since sponge individuals for these three experiments were collected on the same day, seasonal changes in respiration that has been observed in other species (Burlando *et al.*, 1992; Coma *et al.*, 2002) as an explanation for differences is unlikely and the reduced respiration rate may be masking the true effect of the sediment treatment. It may also be due to higher flow in the tanks where the sponges have reduced pumping activity by utilizing passive flow (Yahel *et al.*, 2005; Hadas *et al.*, 2008), and when in the respirometer pumping remains reduced and therefore less respiration is occurring.

Turbidity may be used as a proxy for suspended particulate concentrations and it would have been desirable to measure this variable at the *in situ* sites to elucidate any between day variation in suspended sediment concentration over the sampling periods and to gain an estimate of this in mg l^{-1} to relate it back to the experiments with greater relevance. However, turbidity sensors at the university were in high demand and the operational costs were not within the funding and time constraint scope of this research. Therefore, a more pragmatic approach was needed to characterise the sedimentation experienced by *T. burtoni* in summer months across various habitats. The sediment traps did provide an advantage in that sediments could be collected and grain sizes analysed. While the sedimentation rates and grain size compositions established did provide some interesting context to the experiments, the units in g m^2 d^{-1} are difficult to relate to mg l^{-1} concentrations and temporal resolution was reduced, especially since sediment trap retrieval was impossible at the Rabbit Island and Pilot Bay sites after the second intended sampling period. This may have also affected the trap biases with regard to the hydrodynamics associated with biofouling and overfilling (Storlazzi *et al.*, 2011); though this did not appear to be extensive upon collection.

Grain size, mineralogy and exposure duration influence the response of sponges to sediments as do artefacts of aquaria systems. A cross species comparison in future research investigating the respiration response of sponges to sediments would be of interest since to date this has not been done under experimental conditions.

Investigating the effects of toxicant bound sediments may also be of interest, particularly in areas that experience coastal pollution. Additionally, further *in situ* work is needed to support aquaria experiments with regard to the cost on metabolism. With regard to *T. burtoni*, a more elegant and reliable long-term experiment is needed that would adequately address the effects of fine sediments at high concentrations over an extended period of time.

4.2 Temperature

Temperature is one of the most important abiotic parameters dictating marine invertebrate population dynamics (Sorte *et al.*, 2011; Marshall *et al.*, 2012). Sea surface temperatures are predicted to increase significantly on a global extent, as well as in New Zealand. Very limited work exists in New Zealand with regard to how increasing sea surface temperatures affect sponges in temperate waters. The aim of the fourth experiment presented here was to investigate how *T. burtoni* may respond metabolically to IPCC projected sea surface temperature increases for the end of the century. Additionally, how survival is affected and to what degree disease is evident was also monitored throughout the experiment. Understanding how sponges may fare in a changing ocean is becoming increasingly important to better consider these unique invertebrates in conservation and management practices and to understand where thresholds may exist and what the consequences are in regard to the ecosystem services they provide should sponge viability be compromised.

4.2.1 Respiration, survival and disease response

In the 18°C treatment that reflects the mean annual sea surface temperature for the Bay of Plenty, the respiration response of *T. burtoni* remained relatively stable from baseline measurements throughout the experimental period of four weeks. Respiration in the 23°C treatment peaked to a mean of 12.576 µmol O_2 g^{-1} FW h^{-1} after two weeks of exposure and was significantly greater than in the 18°C treatment that had a mean respiration rate of 6.590 µmol O_2 g^{-1} FW h^{-1}. However, in the final two weeks of exposure, respiration rates dropped again in the 23°C treatment and while the mean respiration rate was higher, this was not statistically significant. Respiration rates also appeared to peak in the second week of exposure in the 25°C treatment and after the first week of exposure in the 27°C treatment after which respiration measurements were no longer taken in these treatments due to mortality.

In the absence of other physiological measurements, it is difficult to describe what processes may have contributed to the respiration response of *T. burtoni* in this experiment. Metabolic rates typically increase with warming at low temperatures, remain relatively stable at intermediate temperatures and decrease beyond an optimal temperature range (Vohmann *et al.*, 2009). This may have been the case for *T. burtoni* individuals in the 23°C treatment, where the significant increase in respiration in week two may have been attributed to investing in metabolically costly coping strategies while attempting to maintain all physiological processes. Respiration dropped in the third and fourth weeks as sponges entered into a state of metabolic depression as they may have been unable to meet energetic costs related to the maintenance of defence, feeding and tissue repair (Pörtner *et al.*, 2005). A few individuals did appear to begin producing reproductive buds in the 23°C treatment at the cessation of the experiment, likely as a stress response to continual exposure to warm water. Bud formation is common in the *Tethya* genus and incidence has been shown to increase in summer months (Cardone *et al.*, 2010), as a response to injury (Saller, 1990), and in response to temperature increase above summer maxima for *T. bergquistae* (Bates, 2015). In the 25°C treatment in week two and in the 27°C treatment in week one, metabolism may also have been increased to invest in coping strategies including defence from disease. However, increases in microbial respiration rates may have masked the true respiration response of *T. burtoni*. This is particularly likely to be the case in the 27°C treatment where respiration responses appeared to be bimodal. Those with visually evident microbial infection had higher respiration rates than those that did not, with the latter indicating a depression of metabolic activity compared to *T. burtoni* in the 18°C treatment, suggesting a tipping point beyond an optimal range (Vohmann *et al.*, 2009). Increased respiration rates have been observed for other species of sponge, both in respect to natural temperature increases in summer time (Burlando *et al.*, 1992; Coma *et al.*, 2002) and under experimental conditions where temperature treatments above summer maxima have been applied (Bates, 2015; Bennett *et al.*, 2017). Strand *et al.* (2017) demonstrated that *G. barretti* respiration rates doubled in warmer temperature treatments of 11°C and 12°C compared to individuals in ambient control conditions of 7°C with a significant time/treatment interaction and returned to pre-exposure levels during recovery. However, unlike *T. burtoni*, mortality and visible signs of stress such as pigment loss or tissue

necrosis were not evident in any of the treatments though lysosomal destabilisation was two to five times higher. Interestingly, Strand *et al.* (2017) also found that the microbiome of *G. barretti* remained stable, while others have demonstrated the opposite (Lesser *et al.*, 2016) with disruptions to symbiotic relationships with microbes, causing death for some species (Webster *et al.*, 2008). While the microbiome of *T. burtoni* was not expressly investigated here, it may serve as an explanation for the rapid mortality and disease observed in the 25°C and 27°C treatments. A loss of potential symbionts could break down important nutritional pathways for sponges and subsequently increase susceptibly to these stressors. The role of the microbiome of *T. burtoni* under stress requires further investigation.

Little is understood regarding the causes of sponge disease, yet disease has been ascribed to causing rapid mortality in sponges (Cebrian *et al.*, 2011), often attributed to environmental stress. Temperature changes, both low and high, are often implicated in this regard (*see* review by Webster (2007)). In the 27°C treatment, individuals of *T. burtoni* were rapidly overcome by a white film and associated putrefied tissue. Di Camillo *et al.* (2013) reported such an occurrence for *Sarcotragus spinosulus in situ*, where a white cyanobacterial film covered diseased individuals. They also reported specimens with exposed bare skeletons, which was also evident for *T. burtoni* in the 25°C treatment, where individuals suffered from extensive dermal cortex loss. Extensive loss of structure was observed in the 25°C treatment, as well as internal tissue regression upon sectioning. It was rare to see a loss of structure in sponges housed in the 27°C temperature treatment. This lack of structural loss may be because the sponge became inundated with infection before having the opportunity to restructure cells in any manner. Sponges in the 27°C temperature treatment that did not yet present any visual signs of external infection also did not exhibit any structural changes; though did appear to "smooth" over. Perhaps infection was already present internally before any symptoms were observed through visual monitoring and death may have occurred before detection. In the study of Di Camillo *et al.* (2013), calm seas and high temperatures were implicated in the case of *S. spinosulus* and under similar circumstances *T. burtoni* may also suffer. Since *T. burtoni* can be found intertidally (Kelly, 2015) it was hypothesized that these sponges may be tolerant of IPCC projected sea surface temperature increases. Individuals of *T. burtoni* inhabiting intertidal zones may already experience temperature extremes in the ranges of 25°C and 27°C. However,

the dynamic nature of the intertidal zone, coupled with high natural variability in temperatures in these habitats (opposed to consistent temperatures such as those used here experimentally) may allow *T. burtoni* to tolerate these temperatures in these types of environments. In subtidal habitats however, these increases in temperature may be more problematic.

While the size of *T. burtoni* in terms of their resilience to disease and loss of dermal cortex was not expressly measured here, it was observed that smaller individuals appeared to be overcome by disease and loss of structure later than larger conspecifics. Increased sensitivity to ocean warming for older sponge conspecifics has been observed for other species (Bennett *et al.*, 2017), with larvae showing strong resilience to warming (Webster *et al.*, 2011), and also hydrocarbon contamination (Negri *et al.*, 2016). Interestingly, the results from these studies and the observations in this research appears to be in direct contrast to results for other organisms such as corals where larval and juvenile conspecifics are highly sensitive (Przeslawski *et al.*, 2015). With this being said, others have observed instances where smaller sponge conspecifics appear to be more sensitive to suboptimal temperature increase where, *in situ*, larger individuals of *S. spinosulus* were able to recover from a disease outbreak attributed to high temperatures (smaller individuals appeared to be more sensitive) (Di Camillo *et al.*, 2013).

With such significant differences in survival, disease incidence and morphological changes between the 23°C and 25°C treatments, a maximum tolerable temperature range for *T. burtoni* appears to lie between these two temperatures. Maximum tolerable temperature limits have been reported for other sponges such as *R. odorabile*, that has a seemingly very strict limit of 32°C (Webster *et al.*, 2008). Mass mortality events of sponges have been shown to coincide with increases in water temperature (Cerrano *et al.*, 2000; Cebrian *et al.*, 2011) yet, the study of Strand *et al.* (2017) on *G. barretti* indicated increasing temperature did not cause mortality experimentally. The study was initiated since rapid sea water temperature increases coincided with mass mortality of *G. barretti in situ* (Guihen *et al.*, 2012). Strand *et al.* (2017) concluded that other environmental processes either singularly or synergistically with temperature increase, must be responsible for the observed mass mortality events. This certainly highlights the caution needed when

extrapolating from aquaria based experiments to *in situ* conditions and observations, as true sensitivity could be both overestimated and underestimated.

Other environmental conditions and biological interactions may exacerbate or ameliorate the effects of temperature. For example, Bennett *et al.* (2017) found that for four abundant Great Barrier Reef species; two heterotrophic and two phototrophic, ocean acidification exacerbates increased temperature effects for the heterotrophic species while mitigating temperature stress for the phototrophic species. This suggests that under future scenarios of cooccurring increased temperature and ocean acidification, phototrophic species may have an advantage. However, Cebrian *et al.* (2011) found that *Ircinia fasciculate,* a Mediterranean sponge harbouring photosynthetic cyanobacterial symbionts, was significantly compromised *in situ* due to mass die off events. Laboratory experiments confirmed a significant reduction in photosynthetic ability at high temperatures while the heterotrophic sponge *Sarcotragus spinosulum* was uncompromised *in situ* in these abnormal summer warming episodes in the Mediterranean. In the immediate future where summer fluctuations in temperature may become more severe without a decrease in pH, phototrophic sponges may be negatively impacted causing mass die offs such as the ones observed here. Despite an apparent ameliorating effect of ocean acidification for phototrophic species in the study of Bennett *et al.* (2017), all species were compromised in respect to survival and therefore both ocean acidification and sea surface temperature rise may be detrimental to sponges. Conversely, Duckworth *et al.* (2012) found that adult individuals of six common Caribbean coral reef sponges were unaffected with regard to survival, respiration and metabolite production at projected sea surface temperature rise and ocean acidification treatments. Therefore, the effects of climate related pressures may be species and perhaps even location specific.

4.2.2 *In situ* temperature

The *in situ* temperature data collected indicated that the summer maximum temperature of 23°C that was used in the temperature experiment was only present for a few days at the Pilot Bay site and not at the Karewa Island, Motiti Island and Rabbit Island sites. However, temperatures around 21°C did persist for roughly a two-week period at all the sites with diurnal and daily fluctuations of ~1-2°C evident. In the temperature experiment individuals housed in the 25°C treatment

were already undergoing morphological changes in the form of dermal cortex loss within two weeks and some individuals had already begun to die off within this time as well. This 25°C treatment would represent the high change 4°C IPCC prediction when adding to the 21°C that was observed for about a two-week period across sites over the 2017 austral summer. However, the temperature fluctuations and variability observed *in situ* were not replicated well in this experiment. While fluctuations of ±1°C were present in the tanks due to the aquaria heater error rate, these temperature conditions were far more stable and persisted for longer than any time that was recorded *in situ*, though this may vary from year to year. Temperature variability coupled with other factors *in situ* may allow *T. burtoni* to persist in a warming ocean. A more elegant experiment taking natural temperature variability into account would be needed to confirm or refute this assertion. Additionally, understanding how *T. burtoni* may recover from damage caused by increased temperature would give greater insight regarding the resilience level of this species and the ability to overcome abnormal warming events.

4.2.3 Conclusions, limitations and future research

Findings from the temperature experiment indicate that *T. burtoni* survival was significantly compromised under both the IPCC low change (2°C) and high change (4°C) sea surface temperature rise predictions that were added to the summer maxima of 23°C observed in the Bay of Plenty. Median survival estimates equated to 12 and 18 days for the 25°C and 27°C treatments respectively. Only a single individual of *T. burtoni* died during the four weeks of temperature exposure in the 23°C treatment, while all individuals housed in the 18°C treatment survived. This indicates that with regard to this experiment, a temperature threshold for survival exists quite strictly between 23°C and 25°C. This may suggest that in the absence of adaptation, even at a low change, sea surface temperature increase by the year 2100 may have devastating effects for *T. burtoni* populations. While *T. burtoni* survived the 23°C treatment, there was evidence that some individuals may have been stressed physiologically due to the beginnings of reproductive bud formation, minor dermal cortex deterioration and pigment loss. Therefore *T. burtoni* may only be able to tolerate summer maxima temperatures for so long. It is noted that while the extreme temperature experiment was designed to mimic IPCC high change

scenarios, this degree of temperature elevation is experienced now in Tauranga Harbour (BOPRC, 2017), albeit over shorter time periods.

It is recognized here that the degree to which oxygen levels were lowered in the experimental tanks in the warmer temperature treatments may have had a synergistic or cumulative effect on the performance of *T. burtoni* subjected to these temperatures. Reduced oxygen levels in the ocean is also a concerning effect of a higher CO_2 world, though less studied with regard to the effects on organisms (Schmidtko *et al.*, 2017). Previous work has indicated median lethal oxygen concentrations for other marine invertebrates to occur at under 100 µmol O_2 l^{-1} (Keeling *et al.*, 2009), and while aquaria oxygen levels were monitored as percent saturation here, oxygen concentrations at the beginning of respiration measurements indicated starting levels of at least 200 µmol O_2 l^{-1} across treatments. This may indicate that the oxygen levels would not have been lethal to *T. burtoni*, however sensitivity of some sponges to lowered oxygen concentrations has been suggested (Osinga *et al.*, 1999) while historically others have withstood hypoxic events (Lee & Riding, 2016). Therefore, inferences discussed here with regard to the results of the temperature experiments are made with caution due to the potential oxygenation effects. Overall, with regard to the literature discussed and results obtained, responses of sponges to increasing sea surface temperatures appear to be species specific, with multiple abiotic and biotic factors playing either direct or indirect roles.

Increasing atmospheric CO_2 levels may affect the ocean in various ways. Meta-analyses have demonstrated that multiple climate stressors can have more significant impacts on marine organisms whereby effects may be synergistic or additive compared to instances where stressors are administered experimentally in isolation (Wernberg *et al.*, 2012; Kroeker *et al.*, 2013). Far fewer experimental studies exist that address combined impacts compared to those addressing stressors singularly, though the former may be of greater relevance. This issue has also been identified as it relates specifically to sponges (Bell *et al.*, 2015a). In part, this is likely an issue that materializes due to the availability of suitable experimental systems. This is an important area of research to gain a more realistic understanding of how sponges will fare in a changing ocean. Monitoring of sponges is important in this regard and a recent review by Bell *et al.* (2017) highlights the need for greater

consideration of sponges in monitoring programmes since often they are only considered at the phylum level. Given sponges play varying important functional roles in the marine environment and since they appear to show species specific responses to environmental pressures, considering this unique group of invertebrates at a greater resolution is necessary.

4.3 Thesis overview

The overarching goal of this thesis was to contribute information on how the anthropogenically induced pressures of increasing sea surface temperature and sedimentation affects *T. burtoni* through a series of experiments and observations. *T. burtoni* evidently supresses respiration in response to high sediment loads and fine sediments. This points to fine sediments potentially being problematic for this species since sedimentation is transient in nature. Storm severity and frequency are predicted to increase and fluctuations of high sediment concentrations with finer grain sizes may keep the sponge under a perpetual and potentially damaging state of constant attempts at adaptation in some areas. With periods of supressed metabolism likely to increase over time this may have significant effects on the wider ecosystem services that sponges provide, such as their role in benthic-pelagic coupling. The long-term experiment did however suggest that under high sediment loads with fine grain sizes *T. burtoni* was not any worse off with regard to metabolic expenditure than those housed in control conditions. This may not be surprising since *T. burtoni* can be found in harbour environments. However, due to experimental problems potentially confounding results, this experiment should be repeated with more advanced monitoring and aquaria equipment. With regard to temperature, the results suggest that a threshold between 23°C and 25°C may exist for *T. burtoni* in the Bay of Plenty, and that in the absence of adaptation, *T. burtoni* may be compromised under future increases in sea surface temperature. The potential demise of this species is particularly concerning since *T. burtoni* is considered a ‘resilient’ marine sponge. This begs the question of how sponges in New Zealand waters, with greater sensitivity to climate change related stressors may fare. The loss of sponge populations and even a reduction in metabolic processes as it relates to important ecosystem services such as benthic carbon flux could have a significant effect on coastal trophic dynamics.

References

Abdo, D. A., Battershill, C. N., & Harvey, E. S. (2006). Manipulation of environmental variables and the effect on the growth of Haliclona sp.: Implications for open-water aquaculture. *Marine Biology Research, 2*(5), 326-332.

Abdo, D. A., Fromont, J., & McDonald, J. I. (2008). Strategies, patterns and environmental cues for reproduction in two temperate haliclonid sponges. *Aquatic Biology, 1*(3), 291-302.

Abedon, S. T., Breitenberger, C. A., Roden, E. E., & Williams, J. B. (2008). Respiration. In S. E. Jørgensen & B. Fath (Eds.), *Encyclopedia of Ecology* (pp. 3010-3020). Oxford, United Kingdom: Academic Press.

Abraham, E. R. (2001). The fractal branching of an arborescent sponge. *Marine Biology, 138*(3), 503-510.

Ainsworth, T. D., Heron, S. F., Ortiz, J. C., Mumby, P. J., Grech, A., Ogawa, D., Eakin, C. M., & Leggat, W. (2016). Climate change disables coral bleaching protection on the Great Barrier Reef. *Science, 352*(6283), 338-342.

Airoldi, L. (2003). The effects of sedimentation on rocky coast assemblages. *Oceanography and Marine Biology: an annual review, 41*, 161-236.

Bannister, R. J., Battershill, C. N., & de Nys, R. (2012). Suspended sediment grain size and mineralogy across the continental shelf of the Great Barrier Reef: impacts on the physiology of a coral reef sponge. *Continental Shelf Research, 32*, 86-95.

Barthel, D. (1986). On the ecophysiology of the sponge *Halichondria panicea* in Kiel Bight. I. Substrate specificity, growth and reproduction. *Marine Ecology Progress Series, 32*, 291-298.

Basher, L. R. (2013). Erosion processes and their control in New Zealand. In J. R. Dymond (Ed.), *Ecosystem Services in New Zealand - Conditions and Trends* (pp. 363-374). Lincoln, New Zealand: Manaaki Whenua Press.

Bates, T. (2015). *Sponge physiology and function in a changing ocean: responses to ocean acidification and increased sea surface temperature.* Master of Science thesis, Victoria University of Wellington, Wellington.

Battershill, C. N., & Bergquist, P. R. (1990). The influence of storms on asexual reproduction, recruitment, and survivorship of sponges. In K. Rützler (Ed.), *New Perspectives in Sponge Biology* (pp. 397-403). Washington, DC: Smithsonian Institution Press.

Battershill, C. N., Bergquist, P. R., & Cook, S. d. C. (2010). Phylum Porifera. In S. d. C. Cook (Ed.), *New Zealand Coastal Marine Invertebrates* (pp. 57-137). Christchurch, New Zealand: Canterbury University Press.

Bavestrello, G., Arillo, A., Calcinai, B., Cerrano, C., Lanza, S., Sara, M., Cattaneo-Vietti, R., & Gaino, E. (1998a). Siliceous particles incorporation in *Chondrosia reniformis* (Porifera, Demospongiae). *Italian Journal of Zoology, 65*(4), 343-348.

Bavestrello, G., Benatti, U., Calcinai, B., Cattaneo-Vietti, R., Cerrano, C., Favre, A., Giovine, M., Lanza, S., Pronzato, R., & Sara, M. (1998b). Body polarity and mineral selectivity in the demosponge *Chondrosia reniformis*. *The Biological Bulletin, 195*(2), 120-125.

Becerro, M. A. (2008). Quantitative trends in sponge ecology research. *Marine Ecology, 29*(2), 167-177.

Bell, A. H., Bergquist, P. R., & Battershill, C. N. (1999). Feeding biology of *Polymastia croceus*. *Memoirs of the Queensland Museum, 44*(1), 51-56.

Bell, J. J., & Barnes, D. K. A. (2000a). The distribution and prevalence of sponges in relation to environmental gradients within a temperate sea lough: vertical cliff surfaces. *Diversity and Distributions, 6*(6), 283-303.

Bell, J. J., & Barnes, D. K. (2000b). A sponge diversity centre within a marine 'island'. *Hydrobiologia, 440*, 55-64.

Bell, J. J., & Barnes, D. K. A. (2003). The importance of competitor identity, morphology and ranking methodology to outcomes in interference competition between sponges. *Marine Biology, 143*(3), 415-426.

Bell, J. J. (2008). The functional roles of marine sponges. *Estuarine Coastal and Shelf Science, 79*(3), 341-353.

Bell, J. J., McGrath, E., Biggerstaff, A., Bates, T., Cárdenas, C. A., & Bennett, H. (2015a). Global conservation status of sponges. *Conservation Biology, 29*(1), 42-53.

Bell, J. J., McGrath, E., Biggerstaff, A., Bates, T., Bennett, H., Marlow, J., & Shaffer, M. (2015b). Sediment impacts on marine sponges. *Marine Pollution Bulletin, 94*(1), 5-13.

Bell, J. J., Biggerstaff, A., Bates, T., Bennett, H., Marlow, J., McGrath, E., & Shaffer, M. (2017). Sponge monitoring: moving beyond diversity and abundance measures. *Ecological Indicators, 78*, 470-488.

Bennett, H., Altenrath, C., Woods, L., Davy, S. K., Webster, N. S., & Bell, J. J. (2017). Interactive effects of temperature and pCO_2 on sponges: from the cradle to the grave. *Global Change Biology, 23*(5), 2031-2046.

Bergquist, P. R. (1978). *Sponges*. London, United Kingdom: Hutchison and Co Ltd.

Bergquist, P. R., & Kelly-Borges, M. (1991). An evaluation of the genus *Tethya* (Porifera: Demospongiae: Hadromerida) with descriptions of new species

from the Southwest Pacific. *The Beagle, Records of the Northern Territory Museums of Arts and Sciences, 8*(1), 37-72.

Biggerstaff, A., Smith, D. J., Jompa, J., & Bell, J. J. (2017). Metabolic responses of a phototrophic sponge to sedimentation supports transitions to sponge dominated reefs. *Scientific Reports, 7*, 1-11.

BOPRC. (2017). *Tauranga Harbour water temperature*. Retrieved 2017, from http://monitoring.boprc.govt.nz/MonitoredSites/cgi-bin/hydwebserver.cgi/points/details?point=7825.

Bracken, M. E., Friberg, S. E., Gonzalez-Dorantes, C. A., & Williams, S. L. (2008). Functional consequences of realistic biodiversity changes in a marine ecosystem. *Proceedings of the National Academy of Sciences, 105*(3), 924-928.

Bratieres, K., Fletcher, T., Deletic, A., & Zinger, Y. (2008). Nutrient and sediment removal by stormwater biofilters: a large scale design optimisation study. *Water Research, 42*(14), 3930-3940.

Brierley, A. S., & Kingsford, M. J. (2009). Impacts of climate change on marine organisms and ecosystems. *Current Biology, 19*, 602-614.

Burlando, B., Bavestrello, G., & Arillo, A. (1992). Seasonal changes in the metabolism of the calcareous sponge *Clathrina clathrus* (Schmidt). *Comparative Biochemistry and Physiology Part A: Physiology, 101*(2), 341-344.

Burrows, M. T., Schoeman, D. S., Buckley, L. B., Moore, P., Poloczanska, E. S., Brander, K. M., Brown, C., Bruno, J. F., Duarte, C. M., & Halpern, B. S. (2011). The pace of shifting climate in marine and terrestrial ecosystems. *Science, 334*, 652-655.

Butman, C. A. (1986). Sediment trap biases in turbulent flows: results from a laboratory flume study. *Journal of Marine Research, 44*(3), 645-693.

Byrne, M., Gibson, R., Atkinson, R., Gordon, J., Smith, I., & Hughes, D. (2011). Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. *Oceanography and Marine Biology: An Annual Review, 49*, 1-42.

Canadell, J. G., Le Quéré, C., Raupach, M. R., Field, C. B., Buitenhuis, E. T., Ciais, P., Conway, T. J., Gillett, N. P., Houghton, R., & Marland, G. (2007). Contributions to accelerating atmospheric CO_2 growth from economic activity, carbon intensity, and efficiency of natural sinks. *Proceedings of the National Academy of Sciences, 104*(47), 18866-18870.

Carballo, J. L., & Ávila, E. (2004). Population dynamics of a mutualistic interaction between the sponge *Haliclona caerulea* and the red alga *Jania adherens*. *Marine Ecology Progress Series, 279*, 93-104.

Cárdenas, C. A., Davy, S. K., & Bell, J. J. (2012). Correlations between algal abundance, environmental variables and sponge distribution patterns on southern hemisphere temperate rocky reefs. *Aquatic Biology, 16*(3), 229-239.

Cardone, F., Gaino, E., & Corriero, G. (2010). The budding process in *Tethya citrina* Sara and Melone (Porifera, Demospongiae) and the incidence of post buds in sponge population maintenance. *Journal of Experimental Marine Biology and Ecology, 389*(1), 93-100.

Cebrian, E., Martı, R., Uriz, J., & Turon, X. (2003). Sublethal effects of contamination on the Mediterranean sponge *Crambe crambe*: metal accumulation and biological responses. *Marine Pollution Bulletin, 46*(10), 1273-1284.

Cebrian, E., & Uriz, M. J. (2006). Grazing on fleshy seaweeds by sea urchins facilitates sponge *Cliona viridis* growth. *Marine Ecology Progress Series, 323*, 83-89.

Cebrian, E., Agell, G., Martí, R., & Uriz, M. J. (2006). Response of the Mediterranean sponge *Chondrosia reniformis* Nardo to copper pollution. *Environmental Pollution, 141*(3), 452-458.

Cebrian, E., Uriz, M. J., Garrabou, J., & Ballesteros, E. (2011). Sponge mass mortalities in a warming Mediterranean Sea: are cyanobacteria harboring species worse off? *PLoS One, 6*(6), 1-13.

Cerrano, C., Bavestrello, G., Bianchi, C., Cattaneo-Vietti, R., Bava, S., Morganti, C., Morri, C., Picco, P., Sara, G., & Schiaparelli, S. (2000). A catastrophic mass mortality episode of gorgonians and other organisms in the Ligurian Sea (Northwestern Mediterranean), summer 1999. *Ecology Letters, 3*(4), 284-293.

Cerrano, C., Magnino, G., Sarà, A., Bavestrello, G., & Gaino, E. (2001). Necrosis in a population of *Petrosia ficiformis* (Porifera, Demospongiae) in relation with environmental stress. *Italian Journal of Zoology, 68*(2), 131-136.

Cerrano, C., Calcinai, B., Di Camillo, C. G., Valisano, L., & Bavestrello, G. (2007). How and why do sponges incorporate foreign material? Strategies in Porifera. *Porifera Research: Biodiversity, Innovation and Sustainability, 28*, 239-246.

Cervino, J. M., Winiarski-Cervino, K., Polson, S. W., Goreau, T., & Smith, G. W. (2006). Identification of bacteria associated with a disease affecting the marine sponge *Ianthella basta* in New Britain, Papua New Guinea. *Marine Ecology Progress Series, 324*, 139-150.

Charlson, R. J., & Schwartz, S. (1992). Climate forcing by anthropogenic aerosols. *Science, 255*(5043), 423-430.

Cheshire, A. C., Butler, A. J., Westphalen, G., Rowland, B., Stevenson, J., & Wilkinson, C. R. (1995). Preliminary study of the distribution and photophysiology of the temperate phototrophic sponge *Cymbastela* sp. from South Australia. *Marine and Freshwater Research, 46*(8), 1211-1216.

Cheung, W. W., Watson, R., & Pauly, D. (2013). Signature of ocean warming in global fisheries catch. *Nature, 497*(7449), 365-368.

Clark, M. S., Sommer, U., Sihra, J. K., Thorne, M. A., Morley, S. A., King, M., Viant, M. R., & Peck, L. S. (2017). Biodiversity in marine invertebrate responses to acute warming revealed by a comparative multi-omics approach. *Global Change Biology, 23*(1), 318-330.

Clarke, A. (1991). What is cold adaptation and how should we measure it? *American Zoologist, 31*(1), 81-92.

Coma, R., Ribes, M., Gili, J. M., & Zabala, M. (2002). Seasonality of *in situ* respiration rate in three temperate benthic suspension feeders. *Limnology and Oceanography, 47*(1), 324-331.

Costa, M. F. B., Mansur, K. F. R., & Leite, F. P. P. (2015). Temporal variation of the gammaridean fauna (Crustacea, Amphipoda) associated with the sponge *Mycale angulosa* (Porifera, Demospongiae) in southeastern Brazil. *Nauplius, 23*(1), 79-87.

De Goeij, J. M., Moodley, L., Houtekamer, M., Carballeira, N. M., & Van Duyl, F. C. (2008). Tracing ^{13}C enriched dissolved and particulate organic carbon in the bacteria containing coral reef sponge *Halisarca caerulea*: evidence for DOM feeding. *Limnology and Oceanography, 53*(4), 1376-1386.

Di Camillo, C. G., Bartolucci, I., Cerrano, C., & Bavestrello, G. (2013). Sponge disease in the Adriatic Sea. *Marine Ecology an Evolutionary Perspective, 34*(1), 62-71.

Duckworth, A. R., & Battershill, C. N. (2001). Population dynamics and chemical ecology of New Zealand demospongiae *Latrunculia* sp. nov. and *Polymastia croceus* (Poecilosclerida: Latrunculiidae: Polymastiidae). *New Zealand Journal of Marine and Freshwater Research, 35*(5), 935-949.

Duckworth, A. R., Bruck, W. M., Janda, K. E., Pitts, T. P., & McCarthy, P. J. (2006). Retention efficiencies of the coral reef sponges *Aplysina lacunosa*, *Callyspongia vaginalis* and *Niphates digitalis* determined by Coulter counter and plate culture analysis. *Marine Biology Research, 2*(4), 243-248.

Duckworth, A. R., & Peterson, B. J. (2012). Effects of seawater temperature and pH on the boring rates of the sponge *Cliona celata* in scallop shells. *Marine Biology, 160*(1), 27-35.

Duckworth, A. R., West, L., Vansach, T., Stubler, A., & Hardt, M. (2012). Effects of water temperature and pH on growth and metabolite biosynthesis of coral reef sponges. *Marine Ecology Progress Series, 462*, 67-77.

Dyer, K. R. (1986). *Coastal and estuarine sediment dynamics*. Chichester: John Wiley and Sons Inc.

Ellis, J., Cummings, V., Hewitt, J., Thrush, S., & Norkko, A. (2002). Determining effects of suspended sediment on condition of a suspension feeding bivalve (*Atrina zelandica*): results of a survey, a laboratory experiment and a field transplant experiment. *Journal of Experimental Marine Biology and Ecology, 267*(2), 147-174.

Elvin, D. W. (1976). Seasonal growth and reproduction of an intertidal sponge, *Haliclona permollis* (Bowerbank). *Biological Bulletin, 151*(1), 108-125.

Fahey, B. D., & Coker, R. J. (1992). Sediment production from forest roads in Queen Charlotte Forest and potential impact on marine water quality, Marlborough Sounds, New Zealand. *New Zealand Journal of Marine and Freshwater Research, 26*(2), 187-195.

Fang, J. K. H., Schonberg, C. H. L., Mello-Athayde, M. A., Hoegh-Guldberg, O., & Dove, S. (2014). Effects of ocean warming and acidification on the energy budget of an excavating sponge. *Global Change Biology, 20*(4), 1043-1054.

Fettweis, M., Francken, F., Van den Eynde, D., Verwaest, T., Janssens, J., & Van Lancker, V. (2010). Storm influence on SPM concentrations in a coastal turbidity maximum area with high anthropogenic impact (southern North Sea). *Continental Shelf Research, 30*(13), 1417-1427.

Foster, G., & Carter, L. (1997). Mud sedimentation on the continental shelf at an accretionary margin - Poverty Bay, New Zealand. *New Zealand Journal of Geology and Geophysics, 40*(2), 157-173.

Fredston-Hermann, A., Brown, C. J., Albert, S., Klein, C. J., Mangubhai, S., Nelson, J. L., Teneva, L., Wenger, A., Gaines, S. D., & Halpern, B. S. (2016). Where does river runoff matter for coastal marine conservation? *Frontiers in Marine Science, 3*(273), 1-10.

Fromont, J., & Bergquist, P. R. (1994). Reproductive biology of three sponge species of the genus *Xestospongia* (Porifera: Demospongiae: Petrosida) from the Great Barrier Reef. *Coral Reefs, 13*(2), 119-126.

Fromont, J., & Garson, M. (1999). Sponge bleaching on the West and East coasts of Australia. *Coral Reefs, 18*(4), 340-340.

Gaino, E., Manconi, R., & Pronzato, R. (1995). Organizational plasticity as a successful conservative tactics in sponges. *Animal Biology, 4*, 31-43.

Gaino, E., Scalera Liaci, L., Sciscioli, M., & Corriero, G. (2006). Investigation of the budding process in *Tethya citrina* and *Tethya aurantium* (Porifera, Demospongiae). *Zoomorphology, 125*(2), 87-97.

Gatti, S., Brey, T., Müller, W., Heilmayer, O., & Holst, G. (2002). Oxygen microoptodes: a new tool for oxygen measurements in aquatic animal ecology. *Marine Biology, 140*(6), 1075-1085.

Gazave, E., Lapébie, P., Renard, E., Vacelet, J., Rocher, C., & Kolokotronis, S. O. (2010). Molecular phylogeny restores the supra-generic subdivision of Homoscleromorph. *5*(12), 1-15.

Gazave, E., Lapébie, P., Ereskovsky, A. V., Vacelet, J., Renard, E., Cárdenas, P., & Borchiellini, C. (2012). No longer Demospongiae: Homoscleromorpha formal nomination as a fourth class of Porifera. *Hydrobiologia, 687*(1), 3-10.

Gerrodette, T., & Flechsig, A. (1979). Sediment induced reduction in the pumping rate of the tropical sponge *Verongia lacunosa*. *Marine Biology, 55*(2), 103-110.

Gili, J. M., & Coma, R. (1998). Benthic suspension feeders: their paramount role in littoral marine food webs. *Trends in Ecology and Evolution, 13*(8), 316-321.

Glade, T. (1998). Establishing the frequency and magnitude of landslide triggering rainstorm events in New Zealand. *Environmental Geology, 35*(2), 160-174.

Glade, T. (2003). Landslide occurrence as a response to land use change: a review of evidence from New Zealand. *Catena, 51*(3), 297-314.

González-Rivero, M., Ferrari, R., Schönberg, C. H., & Mumby, P. J. (2012). Impacts of macroalgal competition and parrotfish predation on the growth of a common bioeroding sponge. *Marine Ecology Progress Series, 444*, 133-142.

Goreau, T. F., & Hartman, W. D. (1963). Boring sponges as controlling factors in the formation and maintenance of coral reefs. In R. F. Soggnaes (Ed.), *Mechanisms of Hard Tissue Destruction* (Vol. 75, pp. 25-54). American Association for the Advancement of Science.

Grimm, L. G., & Yarnold, P. R. (2000). Reading and understanding more multivariate statistics. In K. P. Weinfurt (Ed.), *Repeated Measures Analysis: ANOVA, MANOVA, and HLM* (pp. 317-361). Washington, DC: American Psychological Association.

Gruber, N., Gloor, M., Mikaloff Fletcher, S. E., Doney, S. C., Dutkiewicz, S., Follows, M. J., Gerber, M., Jacobson, A. R., Joos, F., & Lindsay, K. (2009). Oceanic sources, sinks, and transport of atmospheric CO_2. *Global Biogeochemical Cycles*, *23*(1), 1-21.

Gruber, N. (2011). Warming up, turning sour, losing breath: ocean biogeochemistry under global change. *Philosophical Transactions of the Royal Society A, 369*(1943), 1980-1996.

Guihen, D., White, M., & Lundälv, T. (2012). Temperature shocks and ecological implications at a cold water coral reef. *Marine Biodiversity Records, 5*(68), 1-21.

Hadas, E., Marie, D., Shpigel, M., & Ilan, M. (2006). Virus predation by sponges is a new nutrient flow pathway in coral reef food webs. *Limnology and Oceanography, 51*(3), 1548-1550.

Hadas, E., Ilan, M., & Shpigel, M. (2008). Oxygen consumption by a coral reef sponge. *Journal of Experimental Biology, 211*(13), 2185-2190.

Hadas, E., Shpigel, M., & Ilan, M. (2009). Particulate organic matter as a food source for a coral reef sponge. *Journal of Experimental Biology, 212*(22), 3643-3650.

Håkanson, L., Floderus, S., & Wallin, M. (1989). Sediment trap assemblages: a methodological description. In B. T. Hart & P. G. Sly (Eds.), *Sediment/Water Interactions* (pp. 481-490). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'agrosa, C., Bruno, J. F., Casey, K. S., Ebert, C., & Fox, H. E. (2008). A global map of human impact on marine ecosystems. *Science, 319*(5865), 948-952.

Halpin, P. M., Sorte, C. J., Hofmann, G. E., & Menge, B. A. (2002). Patterns of variation in levels of Hsp70 in natural rocky shore populations from

microscales to mesoscales. *Integrative and Comparative Biology, 42*(4), 815-824.

Hand, S. C., & Hardewig, I. (1996). Downregulation of cellular metabolism during environmental stress: mechanisms and implications. *Annual Review of Physiology, 58*(1), 539-563.

Hansen, J., Sato, M., Ruedy, R., Lo, K., Lea, D. W., & Medina-Elizade, M. (2006). Global temperature change. *Proceedings of the National Academy of Sciences, 103*(39), 14288-14293.

Hansen, J., Sato, M., Ruedy, R., Kharecha, P., Lacis, A., Miller, R., Nazarenko, L., Lo, K., Schmidt, G., & Russell, G. (2007). Dangerous human made interference with climate: a GISS modelE study. *Atmospheric Chemistry and Physics, 7*(9), 2287-2312.

Harley, C. D. G., Hughes, A. R., Hultgren, K. M., Miner, B. G., Sorte, C. J. B., Thornber, C. S., Rodriguez, L. F., Tomanek, L., & Williams, S. L. (2006). The impacts of climate change in coastal marine systems. *Ecology Letters, 9*(2), 228-241.

Hedges, J. I., & Oades, J. M. (1997). Comparative organic geochemistries of soils and marine sediments. *Organic Geochemistry, 27*(7), 319-361.

Heimann, M. (1993). *The global carbon cycle*. Berlin, Germany: Springer-Verlag

Helmuth, B., & Hofmann, G. (2000). Defining thermal stress in the rocky intertidal: linking ecology and physiology through biophysics. *American Zoologist, 40*(6), 1051-1051.

Hicks, D. M., Hill, J., & Shankar, U. (1996). Variation of suspended sediment yields around New Zealand: the relative importance of rainfall and geology. *Water Resources Research, 36*(4), 149-156.

Hicks, D. M., Shankar, U., McKerchar, A. I., Basher, L., Lynn, I., Page, M., & Jessen, M. (2011). Suspended sediment yields from New Zealand rivers. *Journal of Hydrology (New Zealand), 50*(1), 81-142.

Hill, M., Walter, C., & Bartels, E. (2016). A mass bleaching event involving Clionaid sponges. *Coral Reefs, 35*(1), 153-153.

Hooper, J. N., & Lévi, C. (1994). Biogeography of indo-west pacific sponges: Microcionidae, Raspailiidae, Axinellidae. In R. W. Van Soest (Ed.), *Sponges in Time and Space: Biology, Chemistry, Paleontology* (pp. 191-212). Rotterdam, The Netherlands: Balkema.

Hooper, J. N., & Van Soest, R. W. (2002). *Systema Porifera: a guide to the classification of sponges*. New York, United States: Kluwer Academic/Plenum Publishers.

Hopkins, S. H. (1962). Distribution of species of *Cliona* (boring sponge) on the eastern shore of Virginia in relation to salinity. *Chesapeake Science, 3*(2), 121-124.

Houghton, J. T., Ding, Y., Griggs, D. J., Noguer, M., Van der Lindin, P. J., Dai, X., Maskell, K., & Johnson, C. A. (2001). *Climate Change 2001: the scientific basis.* Cambridge, Cambridge University Press.

Hsu, T. (1983) The robustness of ANCOVA to the violations of various assumptions: a review of recent studies. In *Annual meeting of the American Educational Research Association*. Montreal, Canada.

Hume, T. M., Green, M. O., & Elliott, S. (2010). *Tauranga Habour sediment study: assessment of predictions for management.* NIWA, Hamilton, New Zealand. 116p. https://www.boprc.govt.nz/media/32620/NIWA-100721-THSSAssessmentPredictionsManagementAmendedMay2010b.pdf.

Ilan, M., & Abelson, A. (1995). The life of a sponge in a sandy lagoon. *The Biological Bulletin, 189*(3), 363-369.

IPCC. (2007). *Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge, UK and New York, USA: Cambridge University Press.

IPCC. (2013). *Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge, UK and New York, USA: Cambridge University Press.

Jiménez, E., & Ribes, M. (2007). Sponges as a source of dissolved inorganic nitrogen: nitrification mediated by temperate sponges. *Limnology and Oceanography, 52*(3), 948-958.

Jørgensen, C. B. (1955). Quantitative aspects of filter feeding in invertebrates. *Biological Reviews, 30*(4), 391-453.

Jørgenson, C. B. (1966). *Biology of suspension feeding*. (Vol. 27). California: Pergamon Press.

Kaandorp, J. (1999). Morphological analysis of growth forms of branching marine sessile organisms along environmental gradients. *Marine Biology, 134*(2), 295-306.

Kaplan, E. L., & Meier, P. (1958). Nonparametric estimation from incomplete observations. *Journal of the American Statistical Association, 53*(282), 457-481.

Keeling, R. F., Körtzinger, A., & Gruber, N. (2009). Ocean deoxygenation in a warming world. *Annual Review of Marine Science, 2*, 463-493.

Kelly, M., & Buckeridge, J. S. (2005). An early Paleogene sponge fauna, Chatham Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research, 39*(4), 899-914.

Kelly, M. (2015). *Splendid Sponges: A Guide to the Sponges of New Zealand.* NIWA, Auckland, New Zealand. 72p. https://www.niwa.co.nz/coasts-and-oceans/marine-identification-guides-and-fact-sheets/Splendid%20Sponges.

Kidawa, A., Potocka, M., & Janecki, T. (2010). The effects of temperature on the behaviour of the Antarctic sea star *Odontaster validus*. *Polish Polar Research, 31*(3), 273-284.

Koopmans, M., Van Rijswijk, P., Martens, D., Egorova-Zachernyuk, T., Middelburg, J., & Wijffels, R. (2011). Carbon conversion and metabolic rate in two marine sponges. *Marine Biology, 158*(1), 9-20.

Kowalke, J. (2000). Ecology and energetics of two Antarctic sponges. *Journal of Experimental Marine Biology and Ecology*, *247*(1), 85-97.

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M., & Gattuso, J. P. (2013). Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology, 19*(6), 1884-1896.

Kutti, T., Bannister, R. J., Fosså, J. H., Krogness, C. M., Tjensvoll, I., & Søvik, G. (2015). Metabolic responses of the deep water sponge *Geodia barretti* to suspended bottom sediment, simulated mine tailings and drill cuttings. *Journal of Experimental Marine Biology and Ecology, 473*, 64-72.

Laport, M. S., Santos, O. C. S., & Muricy, G. (2009). Marine sponges: potential sources of new antimicrobial drugs. *Current Pharmaceutical Biotechnology, 10*(1), 86-105.

Lee, J., & Riding, R. (2016). *Marine hypoxia, Lithistid sponge microbial communities, and the cause of the Cambrian 'reef gap'*. Presented at the Goldschmidt Conference.

Leopold, A. (1966). *A sand county almanac*. New York: Oxford University Press

Lesser, M. P., Fiore, C., Slattery, M., & Zaneveld, J. (2016). Climate change stressors destabilize the microbiome of the Caribbean barrel sponge, *Xestospongia muta*. *Journal of Experimental Marine Biology and Ecology, 475*, 11-18.

Levitus, S., Antonov, J. I., Boyer, T. P., Locarnini, R. A., Garcia, H. E., & Mishonov, A. V. (2009). Global ocean heat content 1955-2008 in light of recently revealed instrumentation problems. *Geophysical Research Letters, 36*(7), 1-5.

Leys, S. P., Mackie, G. O., & Meech, R. (1999). Impulse conduction in a sponge. *Journal of Experimental Biology, 202*(9), 1139-1150.

Leys, S. P., Wilson, K., Holeton, C., Reiswig, H., Austin, W., & Tunnicliffe, V. (2004). Patterns of glass sponge (Porifera, Hexactinellida) distribution in coastal waters of British Columbia, Canada. *Marine Ecology Progress Series, 283*, 133-149.

Leys, S. P., Yahel, G., Reidenbach, M. A., Tunnicliffe, V., Shavit, U., & Reiswig, H. M. (2011). The sponge pump: the role of current induced flow in the design of the sponge body plan. *Plos One, 6*(12), 1-17.

Leys, S. P., & Hill, A. (2012). The physiology and molecular biology of sponge tissues. *Advances in Marine Biology, 62*, 1-56.

Li, C. W., Chen, J. Y., & Hua, T. E. (1998). Precambrian sponges with cellular structures. *Science, 279*(5352), 879-882.

Lohrer, A. M., Hewitt, J. E., & Thrush, S. F. (2006a). Assessing far-field effects of terrigenous sediment loading in the coastal marine environment. *Marine Ecology Progress Series, 315*, 13-18.

Lohrer, A. M., Thrush, S. F., Lundquist, C. J., Vopel, K., Hewitt, J. E., & Nicholls, P. E. (2006b). Deposition of terrigenous sediment on subtidal marine

macrobenthos: response of two contrasting community types. *Marine Ecology Progress Series, 307,* 115-125.

Longo, C., Cardone, F., Mercurio, M., Marzano, C. N., Pierri, C., & Corriero, G. (2016). Spatial and temporal distributions of the sponge fauna in southern Italian lagoon systems. *Mediterranean Marine Science, 17*(1), 174-189.

Luter, H. M., Whalan, S., & Webster, N. S. (2012). Thermal and sedimentation stress are unlikely causes of brown spot syndrome in the coral reef sponge, *Ianthella basta*. *PloS One, 7*(6), 1-9.

MacDiarmid, A., McKenzie, A., Sturman, J., Beaumont, J., Mikaloff-Fletcher, S., & Dunne, J. (2012). *Assessment of anthropogenic threats to New Zealand marine habitats.* NIWA, Wellington, New Zealand. 255p. http://fs.fish.govt.nz/Doc/22981/AEBR_93.pdf.ashx.

Maldonado, M., & Uriz, M. J. (1998). Microrefuge exploitation by subtidal encrusting sponges: patterns of settlement and post-settlement survival. *Marine Ecology Progress Series, 174*, 141-150.

Maldonado, M., Carmona, M. C., Velásquez, Z., Puig, A., Cruzado, A., López, A., & Young, C. M. (2005). Siliceous sponges as a silicon sink: an overlooked aspect of benthopelagic coupling in the marine silicon cycle. *Limnology and Oceanography, 50*(3), 799-809.

Maldonado, M., Giraud, K., & Carmona, C. (2008). Effects of sediment on the survival of asexually produced sponge recruits. *Marine Biology, 154*(4), 631-641.

Maldonado, M., & Riesgo, A. (2008). Reproduction in the phylum Porifera: a synoptic overview. *Treballs de la Societat Catalana de Biologia, 59,* 29-49.

Maldonado, M., Ribes, M., & van Duyl, F. C. (2012). Nutrient fluxes through sponges: biology, budgets, and ecological implications. In M. A. Becerro, M. J. Uriz, M. Maldonado & X. Turon (Eds.), *Advances in Sponge Science:*

Physiology, Chemical and Microbial Diversity, Biotechnology (Vol. 62, pp. 113-182).

Malvern. (2007). Mastersizer 2000 User Manual. *Worcestershire, United Kingdom: Malvern Instruments*.

Marshall, D. J., Krug, P. J., Kupriyanova, E. K., Byrne, M., & Emlet, R. B. (2012). The biogeography of marine invertebrate life histories. *Annual Review of Ecology, Evolution, and Systematics*, *43*, 97-114.

Martin, D., Rosell, D., & Uriz, M. J. (1992). Harmothöe *Hyalonemae* sp. nov.(Polychaeta, Polynoidae), An exclusive inhabitant of different Atlanto-Mediterranean species of Hyalonema (Porifera, Hexactinellida). *Ophelia*, *35*(3), 169-185.

Massaro, A. J., Weisz, J. B., Hill, M. S., & Webster, N. S. (2012). Behavioral and morphological changes caused by thermal stress in the Great Barrier Reef sponge *Rhopaloeides odorabile*. *Journal of Experimental Marine Biology and Ecology*, *416*, 55-60.

Mayer, A. M. S., Rodriguez, A. D., Berlinck, R. G. S., & Hamann, M. T. (2007). Marine pharmacology in 2003-4: Marine compounds with anthelmintic antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action. *Comparative Biochemistry and Physiology C: Toxicology and Pharmacology*, *145*(4), 553-581.

McKergow, L. A., Prosser, I. P., Hughes, A. O., & Brodie, J. (2005). Sources of sediment to the Great Barrier Reef world heritage area. *Marine Pollution Bulletin*, *51*(1), 200-211.

Meroz-Fine, E., Shefer, S., & Ilan, M. (2005). Changes in morphology and physiology of an East Mediterranean sponge in different habitats. *Marine Biology 147*(1), 243-250.

Mieszkowska, N., Sugden, H., Firth, L. B., & Hawkins, S. J. (2014). The role of sustained observations in tracking impacts of environmental change on marine biodiversity and ecosystems. *Philosophical Transactions of the Royal Society A, 372*, 1-13.

Milliman, J., Müller, G., & Förstner, F. (1974). *Recent sedimentary carbonates part one: marine carbonates*. Berlin, Germany: Springer Verlag.

Morrow, C., & Cárdenas, P. (2015). Proposal for a revised classification of the Demospongiae (Porifera). *Frontiers in Zoology*, *12*(7), 1-27.

Muller-Parker, G., & Davy, S. K. (2001). Temperate and tropical algal-sea anemone symbioses. *Invertebrate Biology, 120*(2), 104-123.

Murray, H. J. R. (2009). *Oxygen consumption rates of sponges and the effect of UV-B radiation and sediment.* Master of Science thesis, Victoria University of Wellington, Wellington.

Negri, A. P., Brinkman, D. L., Flores, F., Botté, E. S., Jones, R. J., & Webster, N. S. (2016). Acute ecotoxicology of natural oil and gas condensate to coral reef larvae. *Scientific Reports, 6*, 1-10.

Olejnik, S. F., & Algina, J. (1985). A review of nonparametric alternatives to analysis of covariance. *Evaluation Review, 9*(1), 51-83.

Osinga, R., Tramper, J., & Wijffels, R. H. (1999). Cultivation of marine sponges. *Marine Biotechnology, 1*(6), 509-532.

Page, M., Trustrum, N., & Gomez, B. (2000). Implications of a century of anthropogenic erosion for future land use in the Gisborne East Coast region of New Zealand. *New Zealand Geographer, 56*(2), 13-24.

Palumbi, S. R. (1986). How body plans limit acclimation: responses of a demosponge to wave force. *Ecology, 67*(1), 208-214.

Pearson, P. N., & Palmer, M. R. (2000). Atmospheric carbon dioxide concentrations over the past 60 million years. *Nature, 406*(6797), 695-699.

Perea-Blazquez, A., Davy, S. K., & Bell, J. J. (2012). Estimates of particulate organic carbon flowing from the pelagic environment to the benthos through sponge assemblages. *Plos One, 7*(1), 1-11.

Perea-Blazquez, A., Davy, S. K., & Bell, J. J. (2013). Low functional redundancy in sponges as a result of differential picoplankton use. *Biological Bulletin, 224*(1), 29-34.

Pile, A. J., Patterson, M. R., Savarese, M., Chernykh, V. I., & Fialkov, V. A. (1997). Trophic effects of sponge feeding within Lake Baikal's littoral zone. 2. Sponge abundance, diet, feeding efficiency, and carbon flux. *Limnology and Oceanography, 42*(1), 178-184.

Pineda, M. C., Strehlow, B., Duckworth, A., Doyle, J., Jones, R., & Webster, N. S. (2016). Effects of light attenuation on the sponge holobiont-implications for dredging management. *Scientific Reports, 6*, 1-13.

Pörtner, H. O., Langenbuch, M., & Michaelidis, B. (2005). Synergistic effects of temperature extremes, hypoxia, and increases in CO_2 on marine animals: from Earth history to global change. *Journal of Geophysical Research, 110*(9), 1-15.

Preciado, I., & Maldonado, M. (2005). Reassessing the spatial relationship between sponges and macroalgae in sublittoral rocky bottoms: a descriptive approach. *Helgoland Marine Research, 59*(2), 141.

Przeslawski, R., Byrne, M., & Mellin, C. (2015). A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. *Global Change Biology, 21*(6), 2122-2140.

Quade, D. (1967). Rank analysis of covariance. *Journal of the American Statistical Association, 62*(320), 1187-1200.

Quinn, G. P., & Keough, M. J. (2002). *Experimental design and data analysis for biologists*. Cambridge: Cambridge University Press.

Rees, A. P. (2012). Pressures on the marine environment and the changing climate of ocean biogeochemistry. *Philosophical Transactions of the Royal Society A, 370*, 5613-5635.

Reiswig, H. M. (1971a). Particle feeding in natural populations of three marine demosponges. *Biological Bulletin, 141*(3), 568-591.

Reiswig, H. M. (1971b). *In situ* pumping activities of tropical demospongiae. *Marine Biology, 9*(1), 38-50.

Reiswig, H. M. (1974). Water transport, respiration and energetics of three tropical marine sponges. *Journal of Experimental Marine Biology and Ecology, 14*(3), 231-249.

Ribes, M., Coma, R., & Gili, J. M. (1999). Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. *Marine Ecology Progress Series, 176*(1), 179-190.

Richardson, D. M., Holmes, P. M., Esler, K. J., Galatowitsch, S. M., Stromberg, J. C., Kirkman, S. P., Pyšek, P., & Hobbs, R. J. (2007). Riparian vegetation: degradation, alien plant invasions, and restoration prospects. *Diversity and Distributions, 13*(1), 126-139.

Richter, C., Wunsch, M., Rasheed, M., KoÈtter, I., & Badran, M. I. (2001). Endoscopic exploration of Red Sea coral reefs reveals dense populations of cavity dwelling sponges. *Nature, 413*(6857), 726-730.

Riisgard, H. U., Thomassen, S., Jakobsen, H., Weeks, J. M., & Larsen, P. S. (1993). Suspension feeding in marine sponges Halichondria panicea and Haliclona urceolus: effects of temperature on filtration rate and energy cost of pumping. *Marine Ecology Progress Series, 96*(2), 177-188.

Riisgard, H. U., & Larsen, P. S. (1995). Filter-feeding in marine macro-invertebrates: pump characteristics, modelling and energy cost. *Biological Reviews of the Cambridge Philosophical Society, 70*(1), 67-106.

Roberts, D., Cummins, S., Davis, A., & Chapman, M. (2006a). Structure and dynamics of sponge dominated assemblages on exposed and sheltered temperate reefs. *Marine Ecology Progress Series, 321*, 19-30.

Roberts, D., Davis, A., & Cummins, S. (2006b). Experimental manipulation of shade, silt, nutrients and salinity on the temperate reef sponge *Cymbastela concentrica*. *Marine Ecology Progress Series, 307*, 143-154.

Rogers, C. S. (1990). Responses of coral reefs and reef organisms to sedimentation. *Marine Ecology Progress Series, 62*(1), 185-202.

Saller, U. (1990). Formation and construction of asexual buds of the freshwater sponge *Radiospongilla cerebellata* (Porifera, Spongillidae). *Zoomorphology, 109*(6), 295-301.

Sarà, M. (1998). A biogeographic and evolutionary survey of the genus *Tethya* (Porifera, Demospongiae) in coral reefs. In Y. Watanabe & N. Fusetani (Eds.), *Sponge Sciences* (pp. 83-94). Tokyo, Japan: Springer.

Sarà, M., & Sarà, A. (2004). A revision of Australian and New Zealand *Tethya* (Porifera: Demospongiae) with a preliminary analysis of species groupings. *Invertebrate Systematics, 18*(2), 117-156.

Savarese, M., Patterson, M. R., Chernykh, V. I., & Fialkov, V. A. (1997). Trophic effects of sponge feeding within Lake Baikal's littoral zone. 1. *In situ* pumping rates. *Limnology and Oceanography, 42*(1), 171-178.

Schejter, L., Chiesa, I. L., Doti, B. L., & Bremec, C. (2012). *Mycale* (*Aegogropila*) *magellanica* (Porifera: Demospongiae) in the southwestern Atlantic Ocean: endobiotic fauna and new distributional information. *Scientia Marina, 76*(4), 753-761.

Schiel, D. (2013). The other 93%: trophic cascades, stressors and managing coastlines in non-marine protected areas. *New Zealand Journal of Marine and Freshwater Research, 47*(3), 374-391.

Schlacher, T. A., Schlacher-Hoenlinger, M. A., Williams, A., Althaus, F., Hooper, J. N., & Kloser, R. (2007). Richness and distribution of sponge megabenthos in continental margin canyons off southeastern Australia. *Marine Ecology Progress Series, 340*, 73-88.

Schläppy, M.-L., Hoffmann, F., Røy, H., Wijffels, R. H., Mendola, D., Sidri, M., & de Beer, D. (2007). Oxygen dynamics and flow patterns of *Dysidea avara* (Porifera: Demospongiae). *Journal of the Marine Biological Association of the United Kingdom, 87*(06), 1677-1682.

Schmidtko, S., Stramma, L., & Visbeck, M. (2017). Decline in global oceanic oxygen content during the past five decades. *Nature, 542*(7641), 335-339.

Schonberg, C. H. L. (2016). Happy relationships between marine sponges and sediments - a review and some observations from Australia. *Journal of the Marine Biological Association of the United Kingdom, 96*(2), 493-514.

Siegenthaler, U., Stocker, T. F., Monnin, E., Lüthi, D., Schwander, J., Stauffer, B., Raynaud, D., Barnola, J.-M., Fischer, H., & Masson-Delmotte, V. (2005). Stable carbon cycle climate relationship during the late Pleistocene. *Science, 310*(5752), 1313-1317.

Simpson, T. L. (1984). *The cell biology of sponges*. New York: Springer Verlag.

Sipkema, D., Franssen, M. C. R., Osinga, R., Tramper, J., & Wijffels, R. H. (2005). Marine sponges as pharmacy. *Marine Biotechnology, 7*(3), 142-162.

Soons, J. M., & Selby, M. J. (1992). *Landforms of New Zealand*. Hong Kong: Longman Paul.

Sorte, C. J. B., Jones, S. J., & Miller, L. P. (2011). Geographic variation in temperature tolerance as an indicator of potential population responses to climate change. *Journal of Experimental Marine Biology and Ecology, 400*(1-2), 209-217.

Sperling, E., Robinson, J., Pisani, D., & Peterson, K. (2010). Where's the glass? Biomarkers, molecular clocks, and microRNAs suggest a 200-Myr missing Precambrian fossil record of siliceous sponge spicules. *Geobiology, 8*(1), 24-36.

Storlazzi, C. D., Field, M. E., & Bothner, M. H. (2011). The use (and misuse) of sediment traps in coral reef environments: theory, observations, and suggested protocols. *Coral Reefs, 30*(1), 23-38.

Storr, J. F. (1976). Field observations of sponge reactions as related to their ecology. In F. W. Harrison & R. R. Cowden (Eds.), *Aspects of Sponge Biology* (pp. 277-282). New York: Academic Press.

Strand, R., Whalan, S., Webster, N. S., Kutti, T., Fang, J. K. H., Luter, H. M., & Bannister, R. J. (2017). The response of a boreal deep sea sponge holobiont to acute thermal stress. *Scientific Reports, 7*, 1-12.

Strehlow, B. W., Jorgensen, D., Webster, N. S., Pineda, M. C., & Duckworth, A. (2016). Using a thermistor flowmeter with attached video camera for monitoring sponge excurrent speed and oscular behaviour. *PeerJ, 4*(2761), 1-21.

Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2011). Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society of London B: Biological Sciences, 278*(1713), 1823-1830.

Sundquist, E. T. (1993). The global carbon dioxide budget. *Science, 259*, 934-941.

Svendsen, M., Bushnell, P., & Steffensen, J. (2016). Design and setup of intermittent-flow respirometry system for aquatic organisms. *Journal of Fish Biology, 88*(1), 26-50.

Syvitski, J. P., Vörösmarty, C. J., Kettner, A. J., & Green, P. (2005). Impact of humans on the flux of terrestrial sediment to the global coastal ocean. *Science, 308*(5720), 376-380.

Taylor, M. W., Radax, R., Steger, D., & Wagner, M. (2007). Sponge associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiology and Molecular Biology Reviews, 71*(2), 295-347.

Thomas, S., & Ridd, P. V. (2004). Review of methods to measure short time scale sediment accumulation. *Marine Geology*, *207*(1-4), 95-114.

Thomassen, S., & Riisgard, H. U. (1995). Growth and energetics of the sponge *Halichondria panicea*. *Marine Ecology Progress Series, 128*(1), 239-246.

Thrush, S., Hewitt, J., Cummings, V., Ellis, J., Hatton, C., Lohrer, A., & Norkko, A. (2004). Muddy waters: elevating sediment input to coastal and estuarine habitats. *Frontiers in Ecology and the Environment, 2*(6), 299-306.

Tjensvoll, I., Kutti, T., Fosså, J. H., & Bannister, R. (2013). Rapid respiratory responses of the deep water sponge *Geodia barretti* exposed to suspended sediments. *Aquatic Biology, 19*, 65-73.

Towns, D. R., & Ballantine, W. (1993). Conservation and restoration of New Zealand island ecosystems. *Trends in Ecology and Evolution, 8*(12), 452-457.

Tuckey, B. J., Gibbs, M. T., Knight, B. R., & Gillespie, P. A. (2006). Tidal circulation in Tasman and Golden Bays: implications for river plume behaviour. *New Zealand Journal of Marine and Freshwater Research, 40*(2), 305-324.

Turon, X., Uriz, M., & Willenz, P. (1999). Cuticular linings and remodelisation processes in *Crambe crambe* (Demospongiae: Poecilosclerida). *Memoirs of the Queensland Museum, 44*, 617-626.

Usher, K. M., Sutton, D. C., Toze, S., Kuo, J., & Fromont, J. (2004). Sexual reproduction in *Chondrilla australiensis* (Porifera: Demospongiae). *Marine and Freshwater Research, 55*(2), 123-134.

Vacelet, J., & Bouryesnault, N. (1995). Carnivorous sponges. *Nature, 373*(6512), 333-335.

Vacelet, J., Fiala-Medioni, A., Fisher, C. R., & BouryEsnault, N. (1996). Symbiosis between methane-oxidizing bacteria and a deep sea carnivorous cladorhizid sponge. *Marine Ecology Progress Series, 145*(1-3), 77-85.

Van Duyl, F. C., Hegeman, J., Hoogstraten, A., & Maier, C. (2008). Dissolved carbon fixation by sponge-microbe consortia of deep water coral mounds in the northeastern Atlantic Ocean. *Marine Ecology Progress Series, 358*, 137-150.

Van Soest, R. W., Boury-Esnault, N., Vacelet, J., Dohrmann, M., Erpenbeck, D., De Voogd, N. J., Santodomingo, N., Vanhoorne, B., Kelly, M., & Hooper, J. N. (2012). Global diversity of sponges (Porifera). *PLoS One, 7*(4), 1-23.

Vogel, S. (1977). Current induced flow through living sponges in nature. *Proceedings of the National Academy of Sciences of the United States of America, 74*(5), 2069-2071.

Vogel, S. (1978). Organisms that capture currents. *Scientific American, 239*(2), 128-139.

Vohmann, A., Mutz, M., Arndt, H., & Weitere, M. (2009). Grazing impact and phenology of the freshwater sponge *Ephydatia muelleri* and the bryozoans *Plumatella emarginata* and *Fredericella sultana* under experimental warming. *Freshwater Biology, 54*(5), 1078-1092.

Walling, D. (2008). The changing sediment loads of the world's rivers. *Annals of Warsaw University of Life Sciences, 39*, 3-20.

Wang, A. J., Liu, J. T., Ye, X., Zheng, B. X., Li, Y. H., & Chen, J. (2015). Settling fluxes of cohesive sediments measured by sediment traps in a semi-enclosed embayment with strong tidal environments. *Continental Shelf Research, 106*, 17-26.

Wang, X. C., & Lee, C. (1993). Adsorption and desorption of aliphatic amines, amino acids and acetate by clay minerals and marine sediments. *Marine Chemistry, 44*(1), 1-23.

Webster, N. S. (2007). Sponge disease: a global threat? *Environmental Microbiology, 9*(6), 1363-1375.

Webster, N. S., Cobb, R. E., & Negri, A. P. (2008). Temperature thresholds for bacterial symbiosis with a sponge. *The ISME Journal, 2*(8), 830-842.

Webster, N. S., Botté, E. S., Soo, R. M., & Whalan, S. (2011). The larval sponge holobiont exhibits high thermal tolerance. *Environmental Microbiology Reports, 3*(6), 756-762.

Weisz, J. B., Lindquist, N., & Martens, C. S. (2008). Do associated microbial abundances impact marine demosponge pumping rates and tissue densities? *Oecologia, 155*(2), 367-376.

Wells, J., & Warinner, J. E. (1968). A recording volumetric respirometer for aquatic animals. *Limnology and Oceanography, 13*(2), 376-378.

Wernberg, T., Smale, D. A., & Thomsen, M. S. (2012). A decade of climate change experiments on marine organisms: procedures, patterns and problems. *Global Change Biology, 18*(5), 1491-1498.

Whalan, S., Battershill, C. N., & de Nys, R. (2007). Variability in reproductive output across a water quality gradient for a tropical marine sponge. *Marine Biology, 153*(2), 163-169.

Whalan, S., Ettinger-Epstein, P., & de Nys, R. (2008). The effect of temperature on larval pre-settlement duration and metamorphosis for the sponge, *Rhopaloeides odorabile*. *Coral Reefs, 27*(4), 783-786.

Wilkinson, C. R., & Trott, L. L. (1985) Light as a factor determining the distribution of sponges across the central Great Barrier Reef. In *Proceedings of the Fifth Internation Coral Reef Congress* (Vol. 5, pp. 125-130). Tahiti.

Wilkinson, C. R., & Evans, E. (1989). Sponge distribution across Davies Reef, Great Barrier Reef, relative to location, depth, and water movement. *Coral Reefs, 8*(1), 1-7.

Williams, E., Goenaga, C., & Vicente, V. (1987). Mass bleachings on Atlantic coral reefs. *Science, 238*(4829), 877-878.

Witte, U. (1996). Seasonal reproduction in deep sea sponges: triggered by vertical particle flux? *Marine Biology, 124*(4), 571-581.

Worldseatemperature.org. (2017). *Tauranga sea temperature*. Retrieved 2017, from https://www.seatemperature.org/australia-pacific/new-zealand/tauranga.htm.

Wulff, J. L. (1984). Sponge mediated coral reef growth and rejuvenation. *Coral Reefs, 3*(3), 157-163.

Wulff, J. L. (2006). Ecological interactions of marine sponges. *Canadian Journal of Zoology, 84*(2), 146-166.

Wulff, J. L. (2012). Ecological interactions and the distribution, abundance, and diversity of sponges. In M. A. Becerro, M. J. Uriz, M. Maldonado & X.

Turon (Eds.), *Advances in Sponge Science: Phylogeny, Systematics, Ecology* (Vol. 61, pp. 273-344). San Diego: Elsevier Academic Press Inc.

Yahel, G., Sharp, J. H., Marie, D., Hase, C., & Genin, A. (2003). *In situ* feeding and element removal in the symbiont bearing sponge *Theonella swinhoei*: Bulk DOC is the major source for carbon. *Limnology and Oceanography, 48*(1), 141-149.

Yahel, G., Marie, D., & Genin, A. (2005). InEx - a direct *in situ* method to measure filtration rates, nutrition, and metabolism of active suspension feeders. *Limnology and Oceanography Methods, 3*, 46-58.

Zea, S. (2001). Patterns of sponge (Porifera, Demospongiae) distribution in remote, oceanic reef complexes of the southwestern Caribbean. *Revista de la Academia Colombiana de Ciencias Exactas, 25*(97), 579-592.

Zucht, W., Sidri, M., Brummer, F., Jaklin, A., & Hamer, B. (2008). Ecology and distribution of the sponge *Aplysina aerophoba* (Porifera, Demospongiae) in the Limski Kanal (northern Adriatic Sea, Croatia). *Fresenius Environmental Bulletin, 17*(7), 890-901.

Appendix

5.1 *T. burtoni* wet weight vs. dry weight

Wet weights were established through gently patting down the sponge individual with a paper towel and weighing. Dry weights were established through placing individual sponges in aluminium foil trays and placing in a drying oven at 80°C for 24 hours. Wet weight and dry weight tested though a Pearson's product-moment correlation (meeting all assumptions) and are highly correlated with a strong *r* value of 0.98 (Figure 5.1).

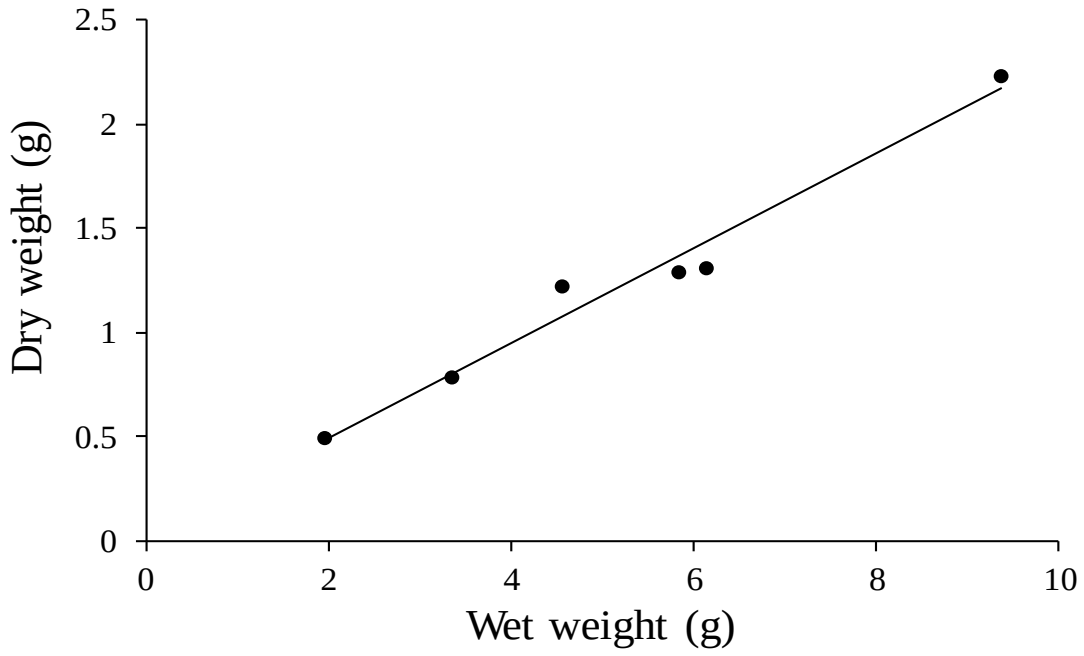

Figure 5.1: Pearson's product-moment correlation of *T. burtoni* wet weight (g) versus dry weight (g) (n= 6 individual *T. burtoni*). $r = 0.986$, $p < 0.001$ at $\alpha = 0.01$.

5.2 Bacterial oxygen consumption

Oxygen measurements were recorded every second in µmol l^{-1} with a PreSens PSt3 fibre optic oxygen probe and temperature probe for an undisturbed for a period of 15 minutes in the respirometer described in section 2.5 of the methods chapter. A single measurement was taken for plain 250 µm filtered seawater at 25°C and another in the same conditions but with the addition of bacterial film removed from an infected *T. burtoni* individual that had been housed for two weeks in the 25°C temperature treatment.

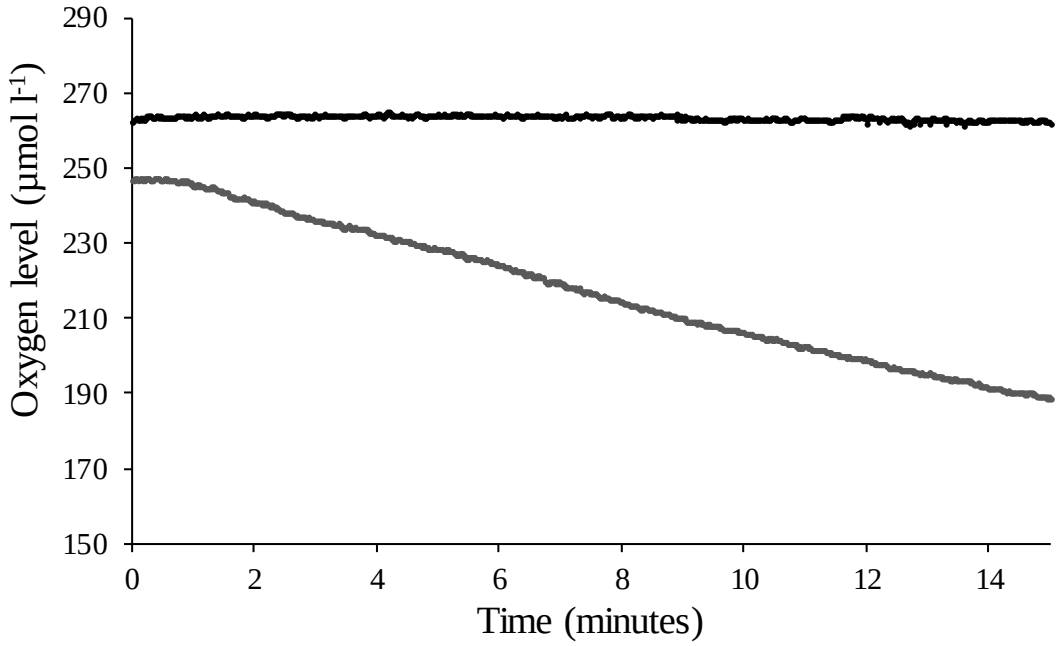

Figure 5.2: Oxygen consumption curve (µmol l^{-1}) at 25°C for plain 250 µm filtered seawater (black line) and for plain 250 µm filtered seawater containing bacterial film removed from a diseased sponge (grey line) over a measurement period of 15 minutes. (n = 1 sample per measurement).