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TEMPORAL CONTINGENCIES ASSOCIATED WITH MULTIPLE ANTHROPOGENIC DISTURBANCES IN SHALLOW MARINE ASSEMBLAGES

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY IN SCIENCE
THE UNIVERSITY OF SYDNEY

JANUARY 2014

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22nd January, 2014

ACKNOWLEDGEMENTS

Ross Coleman and Dieter Hochuli, who have provided the right balance of intelligent commentary, friendship and sport-related distraction. The large group of people who have taken on a supervisor-esque role at some point in the last three years: Elizabeth May, Mark Emmerson, Martina

Doblin, Michael Harris and Sue Briggs. The collaborators who have contributed to the research presented in this thesis: Eoin O'Gorman, Florian Schneider, Hanne Hetjens and Prune le Merrer and Richard Murphy. Tom Savage, as without his time and talent much of these data would still be stuck in gooey-sample form, and Marti Anderson, Matt Day, Jung-Ho Lee, Rattan Bhandari, Henk van Rein and Brendan McNamara for having the poise and finesse not expected from people dealing with someone who... well, someone such as me. My peers in CMEG, particularly Kiran Liversage, Clarissa Fraser and Ana Bugnot. Their willingness to be distracted by my whims and fancies, and not subsequently withhold academic support or professional respect, is quite extraordinary. A great number of volunteer-companions helped on various parts of this project. Noteworthy of these are Kiran (again), Robert Blackburn, Les Edwards, Danya Rose and Deb Rich, who gave up far too much of their time for a measly return of terrible sandwiches and awkwardly-gushing gratitude. The various institutions who have invested resources in me and my research including the University of Sydney, Queen's University Belfast, the Australian Marine Sciences Association, the British Ecological Society, the Ecological Society of Australia, King's College London, the Linnean Society of NSW, and the Marine Institute, Ireland. I would like to thank Paris Goodsell and Joy Waddington, without whose memory and generosity I would not have been able to do half the things I have done. And of course my friends, my family and Keturah (who falls into both categories), you are the people for whom it is all worthwhile.

And of course, my examiners: Jeffrey Levinton, Dave Raffaelli and Alistair Poore. This thesis and associated manuscripts look a lot better now thanks to their thoughtful and constructive comments.

TABLE OF CONTENTS 4

ABSTRACT

Many coastal systems are experiencing severe anthropogenic pressure from climate change, overexploitation, habitat degradation and pollution. Research designed to comprehend the direct effects of these disturbances on marine systems is increasingly common, yet more novel work needs to be done to characterise the more nuanced roles of indirect effects and temporal contingencies, which are events that have happened at some point in time and lead to one of many possible outcomes, before ecosystem level response to multiple potentially-interacting stressors can be understood and predicted. The original contribution to knowledge made by the research in this thesis is the examination of the interactive effects of anthropogenic stressors in the context of indirect effects and temporal contingencies using two series of manipulative experiments. In the first series of experiments, the null hypothesis of no assemblage response from a semi-natural food web to the interactive effects of warming, eutrophication and changing body size of a top predator (European shore crab, *Carcinus maenas*) was tested. It was found that while the effects of minor nutrient enrichment are negligible and warming produces subtle responses, the manipulation of top predator body size evokes a strong trophic cascade. This suggests that the long-term decreased body size effect of warming will be of greater ecological consequence than any short-term direct effects of higher temperature that may be experienced by individuals. The complex size relationship between *C. maenas* and its amphipod prey was examined and it was discovered that relative body size can explain much of the trophic interaction between these species. It was also found that the absence of refugia down-shifts the size-structuring of the predator-prey relationship so that larger *C. maenas* exert greater predation pressure on smaller amphipods. This implies there is size-based selection pressure on prey species in simplified habitats. In the second set of experiments, the effect of copper

contamination on two successional stages of subtidal benthic community assembly, particularly biofilms and settling macro-invertebrates, was examined. It was found that: (1) the amount of copper sequestered by marine biofilms depends on the degree and length of exposure to copper; (2) microphycological assemblages contained within marine biofilms are sensitive to changes in copper concentration in the surrounding water column, irrespective of whether the changes involve exposure to or depuration from copper, and not just exposure to elevated copper concentrations *per se*, and; (3) invertebrate assemblages respond to increased exposure to copper rather than any directionless change in the copper status of the surrounding water column. This suggests that benthic invertebrate settlement is robust to the indirect effect of copper-induced changes in biofilm communities, but not to the direct toxic effect of copper itself. This has positive implications for the capability of biofilms to sequester contaminants without compromising other ecological functions. Given that ecological experiments are more easily interpreted when examining multiple food web compartments or using a variety of technical methods and instruments over a range of spatial and temporal scales it is clear that complex natural systems are best understood when observed at multiple scales and from several perspectives. The work described in chapter two and four highlight the importance of a host of temporal contingencies including legacy effects, ecological history and the coincidence of random events such as rainfall for identification and interpretation of the underling mechanisms in community assembly. Research focused on the indirect effects of anthropogenic disturbance, as these effects are likely to be larger than their direct concomitants and, if they are of comparable magnitude across type of disturbance, might be analogous in their ultimate ecological impact, is recommended. Management designed to minimise negative ecological effects in the face of human-induced change should focus on amelioration of those anthropogenic stressors over which humanity has the most control.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 TYPES AND CONSEQUENCES OF ANTHROPOGENIC DISTURBANCE

Natural systems supply society with a range of ecosystem services that include the provision of food and materials, and the efficient regulation of air quality and water purity, as well as non-tangible benefits such as aesthetics and cultural significance (MA 2005; Worm et al. 2006; Cardinale et al. 2012). The economic value of these free services is now recognized and increasingly being integrated with traditional assessments of natural capital (Costanza et al. 1997; Poloczanska et al. 2007; TEEB 2009; Barbier et al. 2011; Raffaelli and White 2013). In the Anthropocene (Crutzen 2006), coastal systems and the value they provide to society are under pressure from a variety of anthropogenic disturbances including overexploitation and pollution, a situation which is likely to be exacerbated over coming decades by global changes in climate that will warm most ecosystems, change the size structuring of populations and increase the input of terrigenous pollutants and the simplification of habitats (MA 2005; Pall et al. 2011; Binzer et al. 2012; IPCC 2013). The aim of this thesis is to understand the way the following disturbances might modify the natural function of marine systems.

1.1.1 Anthropogenic Global Warming

Pre-industrial era concentrations of atmospheric carbon dioxide (CO_2) were approximately 280 parts per million (ppm) and global concentrations are projected to reach 400ppm before the end of 2014 (IPCC 2013; Tan and Keeling 2014). This change is largely attributed to the combustion of fossil fuel and deforestation (Doney and Schimel 2007) and predicted to result in a global increase in mean surface temperature of between 1.5-6°C by

2100 (IPCC 2013). Historically, degrees of warming similar to those expected in the near future were last observed during the Palaeocene-Eocene Thermal Maximum (PETM) where 35-50% of benthic foraminifera went extinct (Aubry et al. 1998). On the basis of future climate scenarios and given that the pace of CO_2 rise is sixteen times faster today than during the PETM (Gibbs et al. 2006; Widdicombe and Spicer 2008), it is predicted that 15-37% of all species will become extinct by 2050 (Thomas et al. 2004). This is because when an organism is outside its usual range of tolerance, warming accelerates metabolism and the ageing process, thereby reducing the ability of individuals to avoid the myriad ecological causes of death (Brown et al. 2004) though this might be mitigated by behaviour or range shifts (Parmesan and Yohe 2003). At shorter time scales, temperature determines biochemical reaction rates (Hochachka and Somero 2002b; Clarke 2003; Guderley 2004), thereby modifying organism metabolism, and hence demand for food and energy (Brown et al. 2004; Ehnes et al. 2011; McElroy et al. 2012). This leaves less energy available for growth and reproduction and the avoidance of disease and parasitism (Dobson and Carper 1992; Poulin 2006; Christensen et al. 2011; Levinton et al. 2011). Many marine organisms are not able to ingest prey at the rate required to meet these elevated metabolic demands (Rall et al. 2010; Twomey et al. 2012). This constraint may be termed a ‘feeding bottleneck’ and which could place energetic restrictions on components of an assemblage and with subsequent starvation of individuals at higher trophic levels (Vucic-Pestic et al. 2010b; Vucic-Pestic et al. 2011; Brose et al. 2012; Lang et al. 2012; Rall et al. 2012; Wiescher et al. 2012). The phenomenon of a feeding bottleneck may explain warming-induced decreases in population size (Meehan 2006) and further rises in temperature are expected to shift communities toward having smaller–bodied organisms that die younger (Brown et al. 2004) and require less food (Daufresne et al. 2009; Yvon-Durocher et al. 2011; Binzer et al. 2012). Temperature may explain latitudinal differences in body length seen within taxa such as copepods and phytoplankton (Lonsdale and Levinton 1985; Morán et al. 2010). Furthermore, these energy restrictions are likely to encourage intra-guild and cannibalistic predation, as upper trophic levels consume each other, thus weakening top-down control (Brose et al. 2012; Schneider and Brose 2012). Ultimately, in the face of temperature change, the relative success of species will be mediated by their ability to resist or manage the documented effects of warming on reproduction, growth, and mortality (Olive 1995; Brown et al. 2004; Lawrence and Soame 2004; Savage et al. 2004). Chapter two of this thesis asks which species comprising an assemblage of intertidal taxa might be more or less sensitive to warming and whether the longer term effects of warming (decreasing body size) have greater ecological consequences than the short term effects of warming (increasing temperature).

1.1.2 Forced Decreases in Body Size

Many marine systems are already subject to size-selective fishing practices, which have led to the direct removal of the oldest and largest top predators at rates of up to 300% above natural background mortality (Halliday et al. 1992; Pauly et al. 1998; Olsen et al. 2004; Shin et al. 2005; Greenstreet and Rogers 2006; Swain et al. 2007; Darimont et al. 2009) and reductions in the average body size of species (Bianchi et al. 2000; Shackell and Frank 2007; Audzijonyte et al. 2013). Organism body size is a functional trait that governs many processes such as physiology (Brown et al. 2004), population dynamics (Stouffer and Bascompte 2010; Heckmann et al. 2012), and the strength of trophic interactions (Jennings et al. 2001), which can be used to explain their trophic position (O'Gorman and Emmerson 2010; Thierry et al. 2011) and many other effects that are usually attributed to the identity of a species rather than its functional traits (Menezes et al. 2010; Schneider and Brose 2012; Wiescher et al. 2012). These size-driven changes to predator-prey interaction strengths (Berlow et al. 2009) occur due to the effect body size has on predator size preferences

(Stouffer et al. 2011; Zook et al. 2011; Schneider and Brose 2012), ingestion rates (Wahlström et al. 2000; Sørnes and Aksnes 2004; Vonesh and Bolker 2005; Brose et al. 2008; Vucic-Pestic et al. 2010b; Rall et al. 2011; Schneider et al. 2012), attack rates (Vucic-Pestic et al. 2011) attack success (Rall et al. 2010; Vucic-Pestic et al. 2010b; Stouffer et al. 2011; Zook et al. 2011; Kalinkat et al. 2013) and access to refugia (Lima and Dill 1990; Rudolf 2006). Recent work has established that changes to the size distribution of the upper trophic levels of a system can alter the predation pressures experienced by lower levels resulting in allometric trophic cascades (Heithaus et al. 2008; Jochum et al. 2012), which can involve changes in community composition (Mills et al. 1993; Power et al. 1996), restructuring of the relative biomass of populations (Borer et al. 2005), and secondary extinctions (Eklöf and Ebenman 2006). As it is unknown how communities will respond to changes in the size distribution of upper trophic levels in the context of a direct warming effect, chapter two of this thesis asks whether these allometric cascades will be more or less intense when observed in the context of increased temperature.

1.1.3 Pollutants

The contaminations of contaminants in coastal waters has risen greatly over the last two centuries (Smith et al. 1999; MA 2005), due largely to increased terrigenous inputs from agriculture, aquaculture, and industrial, mining and municipal wastewaters/effluents (Mance 1987; Pitt 1995; Bonsdorff et al. 1997; Paerl 1997; Zhou et al. 2008). The eutrophication and pollution of marine waters by terrestrial contaminants will be exacerbated by climate change-induced increases in the intensity of episodic rainfall events over the coming decades (Christensen and Christensen 2003; Pall et al. 2011).

1.1.3.1 Eutrophication

The productivity of many systems and autotrophic species is limited by nutrient availability (Smith 1979; Pace and Funke 1991; Suberkropp and Chauvet 1995; Pedersen and Borum 1996). Thus, increasing the nutrient status of a nutrient-limited ecosystem provides lower trophic levels with an energy supplement that can enhance primary production (Bonsdorff et al. 1997; Harding Jr and Perry 1997; Raffaelli 1999; Nixon and Buckley 2002; McGlathery et al. 2007; Carreiro-Silva et al. 2009; Shurin et al. 2012), which might then passed on to higher trophic levels, which usually increase in abundance and diversity (Rossi and Underwood 2002; Gamfeldt et al. 2005; Borer et al. 2006; Rall et al. 2008; Jochum et al. 2012; O'Gorman et al. 2012a). These bottom-up subsidies can lead to systems with high levels of variability in relative predator-prey biomass (Fussmann et al. 2000; Rip and McCann 2011; Binzer et al. 2012). While it is suspected that by increasing primary production, nutrient loading may subsidize primary productivity to meet warming-driven increases to top-down demand (Shurin et al. 2012), the experiments contained within chapter two of this thesis will be the first study to empirically measure the structure of a food web in response to manipulations of all three of these factors.

1.1.3.2 Copper Contamination

In addition to nutrient pollution, many marine systems also face contamination from metals such as copper (MA, 2005). At extremely-low concentrations copper is an essential micronutrient for the natural function of organisms (Gledhill et al. 1997; Andrade et al. 2004), though it becomes toxic at higher doses (Fernandes and Henriques 1991; Hall et al. 1998). Thus it is widely used as a biocide in many anti-fouling paints applied to the hulls of ships (Johnston and Webb 2000; Schiff et al. 2004; Warnken et al. 2004) to interfere with many of the basal processes of life including photosynthesis, respiration and reproduction

(Shioi et al. 1978; Fernandes and Henriques 1991; Garman et al. 1994; Cid et al. 1995). There is a large body of work to show the differential tolerances of species to the toxicity of copper and other metals (e.g. Jelic-Mrcelic et al. 2006) by looking at the effect of copper on early colonists and the subsequent effect these changes can have on secondarily-arriving species, the work presented in chapter four of this thesis is the first to examine the underlying community assembly processes leading to copper tolerant assemblages.

1.2 A LACK OF FOCUS ON TEMPORAL CONTINGENCIES

Temporal contingencies are defined here as events that are dependent on some condition, state or occurrence that has happened in the past or might happen in the future, potentially by chance or accident, and leading to one of numerous possible outcomes. Examples of temporal contingencies include longer-term versus shorter-term effects of disturbance, ecological history (past ecology of a system such as species arrival order and previous species interactions), legacy effects (lingering environmental disturbances such as sediment contamination) and experimental timing relative to ecological or environmental events such as extreme weather, change in season or recruitment of larvae (Farrell 1991; Underwood and Chapman 2006; Lindenmayer and Likens 2010). Despite it being widely acknowledged that natural systems are in continuous flux and thus extremely variable over time (Myers and Southgate 1980; Morrisey et al. 1992; Hay et al. 1993; Serôdio et al. 1997; White and Walker 1997; Law 1999), compared to the work done to understand the role of spatial variation in ecological outcomes (Chase 2003), relatively little work has been done to understand the role temporal contingencies in ecological systems (but see, Chase 2003; Underwood and Chapman 2006; Coleman et al. 2007; Raffaelli and White 2013). This is particularly true in restoration ecology, where less than 5% of studies consider temporal contingencies and the majority of these studies confirm the influence of ecological history and timing and persistence of disturbance on biodiversity outcomes (Vaughn and Young 2010; Brudvig 2011). Experiments designed to unravel these influences will need to have complex manipulations of start time, end time and experiment duration (Underwood and Chapman 2006), run for longer time periods to detect effects (O'Gorman et al. 2012a) or look to side-step these logistical complications by the use of novel techniques such as the manipulation of predator body size to represent the gradual decrease in average organism size expected in response to sustained warming (Jochum et al. 2012). While failure to run experiments over the longer term prevents ecologists from observing the diffuse role of indirect effects (Paine 1980; Barkai and McQuaid 1988; Menge 1997; Yodzis 2000) and limit our ability to predict long-term changes to community structure (O'Connor and Crowe 2005; Smith et al. 2007; Stachowicz et al. 2008), the experiment done in chapter two provides glimpses of the longer-term effects of warming by manipulating both temperature and decreasing body size over a six week experiment. In an example related to the temporal contingency of legacy effects and disturbance timing, given that successional change is a complex process dependent on many competing factors (Underwood and Chapman 2006), researchers trying to understand the effect of a disturbance on the development of benthic communities must account for disturbance history (Dayton 1971; Connell 1975; Sousa 1979b; Sutherland 1980), starting condition (Grubb 1977; Samuels and Drake 1997; Stampfli and Zeiter 2004; MacDougall et al. 2008), recruitment timing (Underwood and Denley 1984) and seasonal effects (Jenkins et al. 2001; Murphy et al. 2009; Jackson et al. 2010). For example, given the composition of the initial biofilm is critical in determining which species of macro-algae and invertebrates recruit (Meadows and Williams 1963; Mihm et al. 1981; Morse et al. 1984; Wahl 1989; Rodriguez et al. 1993; Keough and Raimondi 1996; Wieczorek and Todd 1998; Lau et al. 2005; Tung and Alfaro 2011), it is not known whether the documented effect of contaminants such as copper on marine benthic invertebrate assemblages (Correa et al. 1999; Johnston and Keough 2000; Johnston and Webb 2000; Johnston and Keough 2002; Johnston et al. 2003; Piola and Johnston 2006a; Piola and Johnston 2006b; Piola et al. 2009) is primarily due to a direct toxic effect on the organisms measured (Hall et al. 1998) or an indirect legacy effect delivered through biofilms impacted directly (Teitzel and Parsek 2003; Massieux et al. 2004; Sun et al. 2013). Is copper sequestered by marine biofilms and if so, does that alter the settlement preferences of recruiting invertebrates?

1.3 STUDY SYSTEMS

The experiments described in this thesis use a crab-amphipod-algae system and an assemblage of biofilms and sessile invertebrates to examine trophic interactions and community assembly respectively. It is widely acknowledged that ecologists need to shift away from the laboratory and towards field or enclosed mesocosm studies where we can examine the assemblage level response of multiple species interacting at different spatial scales (Johnston and Keough 2002; Harley et al. 2006; Rost et al. 2008). This is necessary for three reasons: (1) the wide variety of physiology and strategies employed by species ensures that responses are species specific and generalization is difficult (Blidberg et al. 2000; Przeslawski et al. 2008); (2) simple systems capture little of the physical variability inherent in nature (Connell 1974; Levine 1989) and do not characterize the direct and indirect influences that species have on their neighbours (DeAngelis 1996) and; (3) spatial scales larger than can be studied in the laboratory often determine patterns of abundance, even at smaller scales (Carpenter et al. 1995). This is particularly relevant to the study of biofilms and environmental change where the logistical realities of monitoring and manipulating microorganisms and water chemistry in a natural setting renders laboratory study appealing.

1.4 RESEARCH OBJECTIVES

The aims of the research presented in this thesis are to understand the interactive impacts of warming, decreased body size, nutrients, habitat simplification and copper on marine communities in natural, whole ecosystem contexts while bringing focus to the understudied areas of indirect effects, temporal contingencies and ecological history. To achieve these goals the thesis is split into two distinct parts which address aspects of these human-impact stressors and knowledge gaps. Specifically, the first part (chapters two and three) will: (1) examine the effects of warming, nutrient status and predator-prey size structure and habitat complexity on the trophic interactions within a model marine intertidal system; (2) characterize the direct and indirect effects of multiple disturbances as they are propogated throughout a model food web (McCann 2000); (3) provide the first comparison of the relative effects of exposing a whole assemblage of species to warmed conditions over a short time period, with the long-term consequences for a model community coping with anthropogenically-forced changes in top-predator size structure. The second part, chapter four, is primarily made of two large experiments that will: (1) consider the direct toxic effects copper can have on a temperate microbial and macro-invertebrate community; (2) characterize the ability of marine biofilms to sequester aqueous copper and the indirect effect this may have on colonisation by marine invertebrates in the same model community; (3) draws attention to the concept of direct and indirect effects over time, that is, how historical disturbance can impact future assemblages as both a land-use legacy and how interference with early stages of assembly can potentially set a model community onto alternate successional trajectories (Sousa 1979a; Sousa 1979b; Chase 2003); and (4) by using various technical methods, illustrate how choice of method used is central to the result observed, thus building awareness of how the materials and methods used can influence the interpretation of experiments.

CHAPTER 2: COMMUNITY REORGANISATION IN RESPONSE TO PREDATOR SIZE RESTRUCTURING AND OCEAN WARMING, BUT NOT NUTRIENT ENRICHMENT

ABSTRACT

How multiple stressors in marine systems might interact to affect ecological communities is a topic of ongoing inquiry. It is also not clear whether the shorter-term direct effect of warming will be of greater ecological consequence than a major longer-term effect of increased temperature: decreased average body size of individuals, which is a phenomenon already seen for many overexploited marine predator populations. Using an outdoor array of semi-natural mesocosms, a food web based on a predator (*Carcinus maenas*), an assemblage of amphipods and six species of algae was exposed to manipulations of warming, reductions in predator body size and low doses of eutrophication. Warming affected moulting rates in *C. maenas* and restructured the amphipod assemblage, but this effect was not passed onto to other compartments of the food web. Conversely, decreasing crab size caused an allometrically-mediated trophic cascade that led to a reduced abundance of the alga, *Fucus serratus*. This suggests that in other ecological systems the longer-term decreased body size effect of warming could be of greater ecological consequence than any shorter-term direct effects of higher temperature that may be experienced by individuals. There was no observed effect of eutrophication on any compartment of this food web. The potential for indirect interactions is identified, whereby stressors may interact by impacting different species that share in a trophic relationship.

2.1 INTRODUCTION

Distorting the pre-disturbance structure of food webs can lead to increased uncertainty over the delivery of economically-valuable ecosystem services that are the higher-level products of fine-scale ecosystem and food web processes (Worm et al. 2006; Shackell et al. 2010). The way in which species contribute to ecosystem processes is constrained by specific biological and environmental conditions (Montoya et al. 2006), which are subject to change strongly associated with anthropogenic drivers such as overexploitation, habitat degradation, the burning of fossil fuel, and the over-use of agricultural fertilizers (MA 2005).

2.1.2 Effects of Anthropogenic Warming on Food Webs

Anthropogenically-increased atmospheric CO_2 is a major contributing factor in the predicted global increase in mean surface temperature of at least 1.5-2°C by 2100 (IPCC 2013). Temperature directly modifies rates of metabolism (Rall et al. 2010; McElroy et al. 2012), growth (O'Gorman et al. 2012b), reproduction (Olive 1995; Ramofafia et al. 2003), and interactions between species (Montoya and Raffaelli 2010; Harley 2011), and the effects of anthropogenic warming have already been noted at all levels of biological organisation (Twomey et al. 2012). Many food webs are acutely sensitive to changes in environmental temperature regime (Petchey et al. 1999; Yvon-Durocher et al. 2011; O'Gorman et al. 2012b). This happens as warming can disrupt the total energy of a system by altering the rate (Nemani et al. 2003; Yvon-Durocher et al. 2010; Demars et al. 2011a) and type of primary production, for example, temperature stimulates blooms of cyanobacteria (Jöhnk et al. 2008), whilst simultaneously modifying the ability of consumers to regulate these blooms (Sanford 2002; O'Connor 2009; Kratina et al. 2012; Lemoine and Burkepile 2012).

Future climate scenarios involving increased temperature are likely to favour smaller-bodied organisms (James 1970; Gardner et al. 2011). This has already been observed across gradients in space (Lonsdale and Levinton 1985; Moran et al. 2010) and time (Finkel et al. 2005; Sheridan and Bickford 2011) and is potentially due to the competitive exclusion of larger organisms (Daufresne et al. 2009; Yvon-Durocher et al. 2011) in favour of smaller individuals and species that grow faster and reproduce earlier (Lonsdale and Levinton 1985; Atkinson et al. 2003). These changes may result in a thermal cascade (Brose et al. 2012), whereby warming-induced changes to size structure within a community may be apparent across multiple trophic levels (Jochum et al. 2012). In many systems, higher-trophic levels have already experienced disproportionate rates of extinction due to their relatively slow rates of population growth, and their sensitivity to overexploitation and/or habitat loss (Tracy and George 1992; McKinney 1997; Terborgh et al. 2001; Duffy 2003; Cardillo et al. 2005; Collette et al. 2011), yet less is known how changes to the size-structuring of the upper trophic levels of food webs will impact the rest of the assemblage (but see below).

2.1.3 Top-Down Effects in Size-structured Communities

A general reduction in top predator biomass (Frank et al. 2006; Shackell and Frank 2007) and trophic efficiency (Shackell et al. 2010) has led to systems with little capacity for top-down control (Kordas et al. 2011). In addition to the extinction of species, increasing evidence suggests that populations of many predator species are experiencing a disproportionate decline in average body size (Bianchi et al. 2000; Shackell and Frank 2007; Audzijonyte et al. 2013). In marine systems this is primarily due to size-selective fishing practices, which lead to the direct removal of the oldest and largest top predators and results in a contemporary evolutionary pressure that selects for rapid growth and early reproduction (Jorgensen et al. 2007; Andersen and Brander 2009; Sharpe and Hendry 2009; Palkovacs

2011; Enberg et al. 2012). Trophic cascades are suggested to be a consistent phenomenon in many systems subject to either dramatic reductions in abundance or loss of top predator species (Strong 1992; Heithaus et al. 2008; Reynolds and Bruno 2012) and can result in secondary extinctions (Eklöf and Ebenman 2006).

The strength of trophic interactions is also governed by the relative body size of participants (Jennings et al. 2001). Subsequently, many food webs are size-structured, that is, they are characterized by predators eating prey that are within a certain range of optimal prey body sizes (Hall et al. 1976; Wahlström et al. 2000; Loeuille and Loreau 2005; Brose et al. 2008; Petchey et al. 2008a; Troost et al. 2008; Vucic-Pestic et al. 2010b; Zook et al. 2011; Twomey et al. 2012). It is becoming increasingly apparent that changes to the size structure of communities can have far-reaching effects that are comparable to the removal of entire species (Heckmann et al. 2012; Audzijonyte et al. 2013). Such body-size-mediated cascades can shift the flow of energy and nutrients throughout a system (Economo et al. 2005; White et al. 2007; Ehnes et al. 2011), leading to the destabilization of the community (Loeuille and Loreau 2005; Eklöf and Ebenman 2006; Rall et al. 2008) resulting in increased rates of extinction (Otto et al. 2007; Heckmann et al. 2012). The research described in this chapter will determine whether the shorter-term effects of increased temperature are of greater ecological consequence than the effects of decreasing average body size of upper trophic levels species as a result of long-term warming and overexploitation.

2.1.4 Eutrophication and Interactive Effects

The species that make up food webs will also be subject to other environmental and ecological factors (Raffaelli 2000; Halpern et al. 2007; Hobday and Lough 2011), such as eutrophication (Raffaelli 1999; Vinebrooke et al. 2004; Christensen et al. 2006). The nutrient status of coastal waters has risen greatly over the last two centuries (Smith et al. 1999) and many will be exacerbated by climate-change-induced increases in the intensity of episodic rainfall events over the coming decades, particularly in the United Kingdom (Christensen and Christensen 2003; Pall et al. 2011). Increasing the nutrient status of an oligotrophic ecosystem enhances primary production (Bonsdorff et al. 1997; Nixon and Buckley 2002; McGlathery et al. 2007; Carreiro-Silva et al. 2009; Shurin et al. 2012), which is then passed on to higher trophic levels that increase in abundance and diversity over the shorter-term (Rossi and Underwood 2002; Gamfeldt et al. 2005; Borer et al. 2006; Rall et al. 2008; Jochum et al. 2012; O'Gorman et al. 2012a) and resulting in a longer-term ecosystem that is characterized by the dominance of a few tolerant taxa (Pearson and Rosenberg 1978). Ecological responses to the manipulation of multiple factors can be synergistic, additive, or antagonistic (Crain et al. 2008; Darling and Côté 2008). For instance, in aquatic systems warming accelerates growth and as fast-growing individuals forage with greater risk they are more vulnerable to fishing (Biro and Post 2008). Similarly, nutrients may increase primary production (McGlathery et al. 2007), but since respiration is more sensitive to warming than photosynthesis (Gillooly et al. 2001; Yvon-Durocher et al. 2010), rises in temperature might counter enrichment through increased demand for resources (Rall et al. 2010; Vucic-Pestic et al. 2011). While warming might favour smaller-bodied organisms, this effect is likely lost with increasing nutrient enrichment. that is, the relative effects of temperature and eutrophication are contingent on the size structuring of the community (Binzer et al. 2012). Not considering multiple effects makes prediction much more difficult (Folt et al. 1999) and, while reviews of multi-stressor effects are accumulating (Crain et al. 2008; Darling and Côté 2008), greater care needs to be taken to ensure that studies of the effect of any given disturbance are done from a more realistic multi-stressor perspective (Power et al. 2000). This is particularly true for complex multi species systems where co-tolerance is important for maintaining community stability (D Vinebrooke et al. 2004). Understanding the interplay between the direct and indirect effects of stressors such as warming, body size and nutrient enrichment is critical for predicting ecological responses to the challenges that organisms used in experiments already face on a daily basis and global changes these species might encounter in the near future (Vinebrooke et al. 2004; Christensen et al. 2006; Dolbeth et al. 2011). As introduced in the next section, understanding the effects of numerous anthropogenic stressors is made more complicated by the reticulated and intricate structure of ecological systems.

2.1.5 Consequences of Disturbance for Food Webs

Food webs in ecosystems are governed by many fine-scale processes and map the flow of energy and nutrients between species (DeAngelis 1980; Hall and Raffaelli 1991; Raffaelli 2000; Borer et al. 2006). Distorting the structure of such food webs can impact productivity (O'Gorman et al. 2008; O'Gorman and Emmerson 2009; O'Gorman and Emmerson 2011), destabilise communities (Loeuille and Loreau, 2005; Eklöf and Ebenman, 2006; Rall et al., 2008), increase rates of extinction (Emmerson and Raffaelli, 2004; Otto et al., 2007; Brose, 2008; Heckmann et al., 2012) and create new communities, which might well have altered function (Petchey et al. 1999) or productivity (Cheung et al. 2013). In food webs, 95% of species tend to be no more than three links apart (Williams et al. 2002), and seemingly-small perturbations to any species can be swiftly propagated to the entire food web (O'Gorman and Emmerson 2010), potentially leading to catastrophic phase shifts as seen in cases where the depletion of top predators has led to systems dominated by comb jellies or primary producers (Borer et al. 2006; Daskalov et al. 2007). Impacts to species beyond the original subject species are called indirect effects (Wootton 1993; Raffaelli and Hall 1996). Whilst typified by predictable trophic cascades in simple food chains, indirect effects in reticulated food webs such as the one studied here can produce unexpected consequences that may overwhelm direct effects on physiology, demographics and species interactions (Bothwell et al. 1994; Abrams 1995; Menge 1997; Yodzis 2000; Ives and Cardinale 2004; Suttle et al. 2007; Connell et al. 2011). The presence of indirect effects makes it difficult to assess the direct impact of a given stressor on species and communities (Sih et al. 1998; Wootton and Emmerson 2005; Reiss et al. 2009; Reiss et al. 2011). It is not currently known how the direct effects of warming, decreasing body size and eutrophication may interact through indirect effects that are communicated through food webs. The work presented in this chapter will unravel the direct and indirect effects of these stressors by examining the assemblage responses of multiple trophic levels in an intertidal food web to changes in temperature, nutrient status and predator body size.

2.1.6 Chapter Aims

Using the logical framework proposed by Underwood (1990), these experiments address the following models (M) by testing their associated hypotheses (H):

M1) Predators with a decreased average body size consume less prey than larger predators.

    H1) Assemblages of amphipods will exhibit larger populations in the presence of smaller crabs compared assemblages exposed to larger crabs.

M2) Trophic cascades occur in response to changes in the average body size of predators.

    H2) Larger assemblages of amphipods found in the presence of smaller crabs will lead to reductions in the biomass of some algal species as compared to smaller assemblages of amphipods found in the presence of larger crabs.

M3) Warming increases growth rates.

    H3) Greater rates of moulting will be observed for *C. maenas* exposed to high temperatures as compared to those of crabs observed at lower temperatures.

M4) Warming increases metabolic demand leading to starvation-associated mortality at higher trophic levels.

  H4) Greater rates of mortality will be observed for *C. maenas* exposed to high temperatures as compared to those animals exposed to lower temperatures.

M5) Tolerances to increased warming are species specific.

  H5) Exposure of a complex group of primary producers and consumers to different temperatures will lead to different species dominating the warmer mesocosm assemblages as compared to those dominating the ambient temperature mesocosm assemblages.

M6) Nutrient enrichment increases bottom-up productivity.

  H6) Nutrient enrichment will lead to greater abundances of algae and amphipods.

M7) If warming decreases producer and primary consumer biomass by increasing metabolic demand, smaller crabs exert reduced top-down control and there is increased productivity associated with nutrient enrichment community, responses to all three stressors will be interactive, with the effects of warming and nutrient enrichment largely cancelling each other out.

  H7) The composition of the amphipod and algae assemblages exposed to orthogonal comparisons of ambient and warmed temperature, background and enriched nutrients and five treatments of decreasing crab body size will differ between treatments.

2.2 METHODS

2.2.1 Experimental Set-Up

The experiment was done in the mesocosm facility at Queen's University Marine Laboratory in Portaferry, Northern Ireland. Here, 110 mesocosms (dimensions L: 60 cm, W: 40 cm, H: 23.5 cm, 45L capacity) were supplied with fresh, gravel-filtered seawater, pumped from the adjacent Strangford Lough. This coarse grade of filter allows the passage of meiofauna, microfauna and algal spores, ensuring that each mesocosm experiences a semi-natural degree of connectivity to the Lough. This facilitates the import of species, particularly the opportunistic filamentous brown algae *Ectocarpus* spp. This facility is outside and unsheltered, experiencing fluctuations in temperature and salinity representative of nearby intertidal systems. Water was delivered to the mesocosms via overhead dump buckets, simulating turbulence characteristics of the natural shore and providing an aerated water supply at a continuous rate of approximately 1.5 $L.min^{-1}$. Mesocosms are a useful tool for examining future scenarios and the effects of contaminants (Gray 1992; Benton et al. 2007), and benefit from the control of laboratory studies while also capturing the natural environmental variability of field experiments (Yvon-Durocher et al. 2011). It bears noting that organisms in mesocosms have limited mobility compared to organisms in nature and it is possible that this could impact interaction strengths.

All experimental specimens were collected near Portaferry, Northern Ireland, at the entrance to Strangford Lough (52.3809° N, 5.5486° W). Five types of living algae were added to all mesocosms in relative quantities similar to those found on the shoreline where they were collected Vye et al (2013 unpublished data): *Cladophora* spp. (4g), *Corallina* spp. (16g), *Fucus serratus* (21g), *Mastocarpus stellatus* (3g), and *Ulva lactuca* (1g). Biomass, as wet weight of alga, was determined after excess seawater had been removed in a salad spinner. Algae were secured to an extruded mesh inlay (20 mm mesh size) using commercial garden wire, with the exception of *Ulva,* which was allowed to float freely to mimic natural conditions (Bulnheim 1979). All algal species were given 48 hours to acclimate after addition to the tanks before introducing mobile invertebrates. To reduce the effect of imported epifauna, the algae and mesh were washed in a 10 g.L^{-1} pyrethrum-based pesticide bath (*Vitax Py* Spray Insect Killer Concentrate) before being thoroughly rinsed in seawater and placed in the experimental mesocosms.

Shore crabs (*Carcinus maenas,* considered in this experiment to be an omnivorous top predator) and amphipods (generalist primary consumers, grazers and detritivores including at least eight species of *Chaetogammarus, Gammarus, Orchestria* and *Talitrus* amphipod) were also collected from intertidal areas near Portaferry. A random mix of 125 amphipods of the various species was added to all tanks on six occasions over the course of the experiment. All *C. maenas* specimens were kept in separate holding tanks and acclimated to control conditions until the experiment commenced and treatments were applied over the following six weeks.

2.2.2 Experimental Design

The experiment had one independent factor (nutrient enrichment) and two continuous predictor variables (allometric manipulation of top predator body size and mean temperature). Continuous predictor variables were chosen to avoid uneven sample sizes, maximise replication, and capture more of the variability inherent to the manipulation of average crab mass and mean temperature. All treatments were allocated at random to the 100 mesocosms in the facility.

Temperature was manipulated to reflect the current spring time ambient of 8.6°C and an increase up to 14.8°C by the addition of aquarium heaters (Elite$^{\text{TM}}$ 300W thermostats) to half of the mesocosms. This lead to an increase in mean temperature (+3.5°C) and temperature variability (±0.4°C) in those treatments, thereby imitating nearly the full range of warming of up to +4.8°C expected for global oceanic waters by the end of the century as predicted by the IPCC business-as-usual scenario model RCP8.5 (IPCC 2013). Throughout the experiment, between seven and twelve measurements of temperature (one decimal place|) were made with an in-tank aquarium thermometer for each mesocosm (DX, UK). The averages of these data were used as covariates for each replicate.

Body sizes of the experimental top predator *C. maenas* were altered to represent a skew towards smaller top predator body masses reflecting overexploitation of large individuals in harvested systems (Pauly et al. 1998; Audzijonyte et al. 2013) and climate-driven changes in size structure (Daufresne et al. 2009; Gardner et al. 2011; Sheridan and Bickford 2011). The experiment began with four treatment levels of average top predator body mass (aiming at equidistant values on log scale: M = 1,2,4,8 g) with 20 replicates for each level. Individual crabs were selected to reproduce log normal size distributions as observed in nature (log μ = M, log σ = 0.5; O'Gorman and Emmerson 2010). Individual crab body masses [g] were derived from measures of carapace width, W [mm] (M = 2.67x10-4, W2.95; O'Gorman et al. 2010) and an allometric power law specified the number of individuals, N, for each body mass level ($N = 7\ M^{-0.25}$; yielding N = 7,6,5,4 individuals). The exponent of -0.25 corresponds to mass-abundance observations in comparable field experiments (O'Gorman and Emmerson 2011). This allometric design balances the confounding effects of the number of individuals and total biomass (Figure 2.1) that are largely unavoidable in body-size experiments and produced distributions of predators that reflect naturally-size-structured populations of decreasing average body size (Jochum et al. 2012; Schneider et al. 2012). It is also possible that moult and reproductive timing might vary between ontogenetic stages, though this has not been accounted for in this experiment.

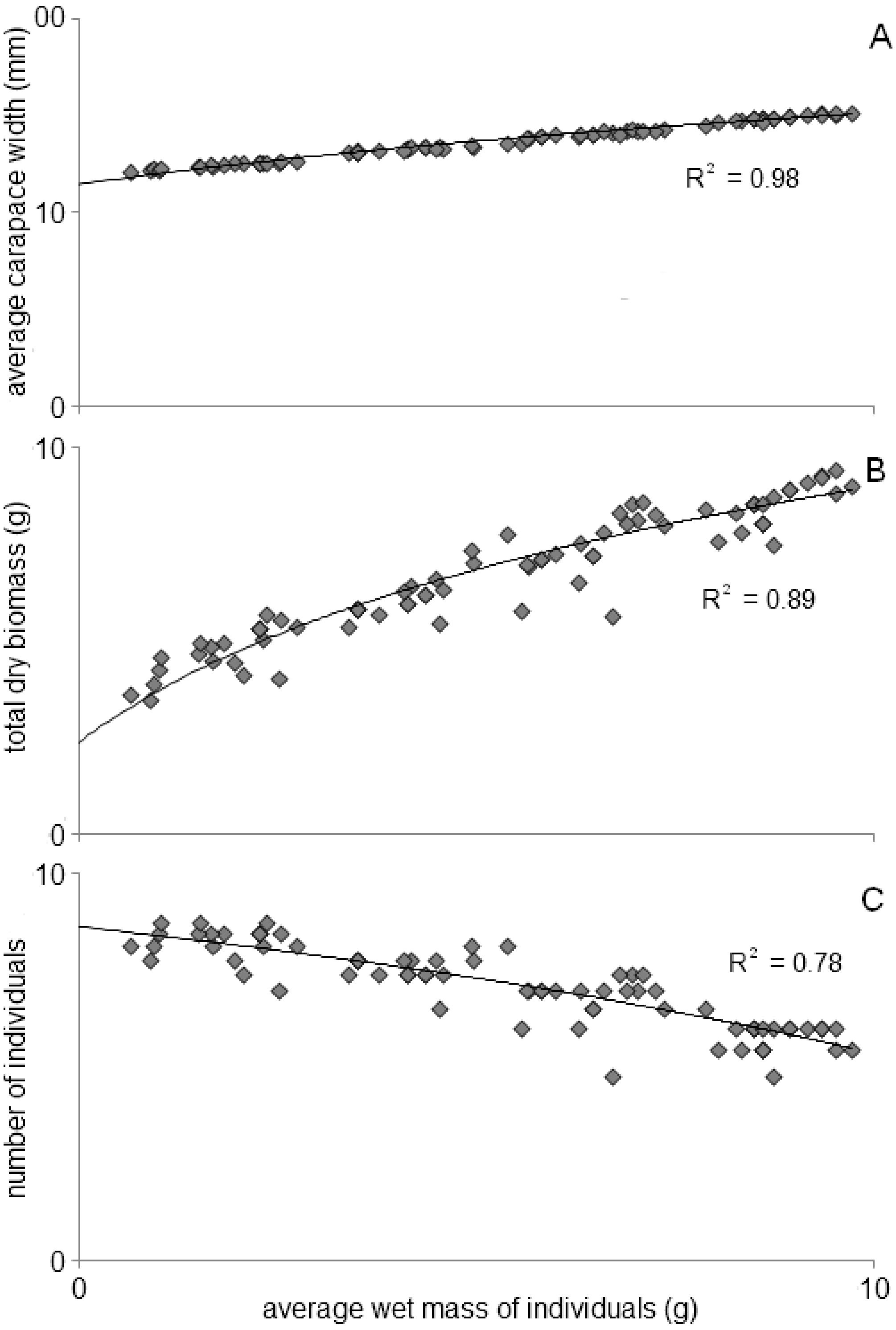


Figure 2.1: Average wet mass of individuals strongly-correlates positively with average carapace width (A, y = 8.18*log(x) + 14.04) total biomass (B, y = 2.70*log(x) + 1.74) and negatively with number of individuals (y = -1.68*log(x) + 7.30, C). As the x-axis is plotted on a log scale, log trend lines were used.

To maintain the body-mass treatment, crabs that had died were replaced upon detection with animals held in reserve and maintained under similar temperature conditions. As part of the natural process of growth, the average body size of *C. maenas* individuals gradually increased over the course of the experiment. To account for these changes in predator size over the duration of the experiment, the initial and final body mass distribution of the top predators was used to produce an integrated estimate of the average predator body mass throughout the experiment to be used as a covariate in subsequent analyses, henceforth referred to as crab size (Figure 2.2; Schneider et al. 2012).

Nutrient treatments were chosen to reflect the ambient concentrations found in Strangford Lough and a representative level of eutrophication consistent with changes observed elsewhere (Smith et al. 1999). Enrichment was achieved by addition of 140g of Osmocote Pro (3-4M), contained in four perforated 50 ml tubes (Fredriksen et al. 2005). The tubes were secured to the extruded mesh in each mesocosm with cable ties. Identical tubes containing gravel were included in control mesocosms. Osmocote Pro (3-4M) is a slow-release fertiliser that is pelletized with an external coating so that nutrients are released at a set rate over the life of the pellet. This product contains 17% N, 5% P and 8% K as well as 1.2% Mg, 0.3% Fe and 0.04% Cu. Previous studies for the mesocosm array used here have shown that this quantity of Osmocote elevates inorganic nitrogen content by 1.112 ± 0.112 $\mu m.L^{-1}$, phosphate content by 0.376 ± 0.024 $\mu m.L^{-1}$ and ammonium content by 1.007 ± 0.091 $\mu m.L^{-1}$, approximately doubling the background concentrations found in Strangford Lough Vye et al (2013 unpublished data).

Twenty six mesocosms were excluded due to overheating from thermostats, container leakages and nutrient spillages, leaving a total sample size of 74, depending on the analysis.

2.2.3 Response Variables collected to test Hypotheses

Univariate general linear models and multivariate PERMANOVA with a maximum of 9999 permutations for each analysis were used to test the null hypotheses that relative amphipod species abundances, relative change in algal biomass, rates of moulting in *C. maenas* and rates of *C. maenas* mortality are not affected by (1) nutrient enrichment, a fixed factor with two levels; (2) mean temperature, a covariate; and (3) crab size, a covariate (Anderson 2001a). Covariates were log(x+1) transformed to increase data collinearity between dependent and independent variables, which is an assumption of ANCOVA and essential for increasing linearity in crab size, which was designed on a log-normal scale (Appendix 1; Quinn and Keough 2002; Sokal and Rohlf 2012). In all cases, the data were fitted using a TYPE I SS to account for the increased lack of independence associated with uneven sample sizes that remained in the nutrient factor after some samples were excluded for the various reasons detailed above (Hector et al. 2010). The order of the fit was permutated to ensure that this did not affect results: only results that were consistently significant across all model variants were accepted, enabling rejection of the relevant null hypotheses (Hector et al. 2010). Shepard diagrams were used to visually determine the spread of distortion stress in all resemblance matrices created from the dependent data. For all analyses in PERMANOVA, interaction terms with a p value equal to or greater than 0.25 were pooled with the residual in a stepwise fashion, which greatly reduces the rate of Type II errors (Underwood, 1997).

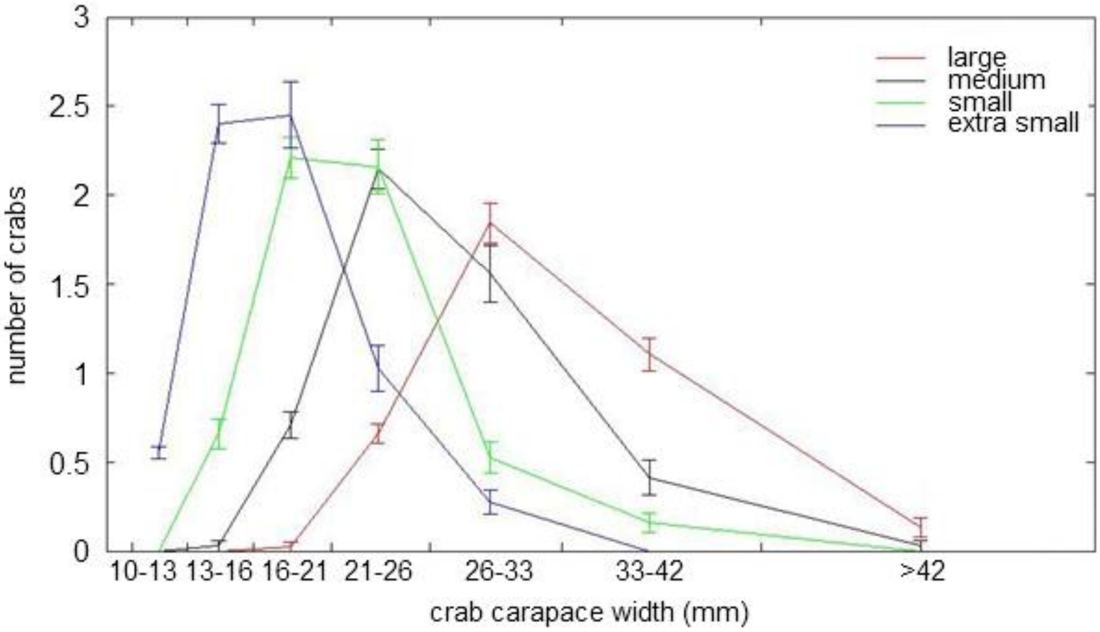


Figure 2.2: Average number of *C. maenas* of varying body size included in the top predator body size treatments. The control treatment had no crabs and is not illustrated here. The x-axis is scaled according to the log normal distribution used in the allometric model.

To test the models that warming increases rates of growth and starvation, the total number of moults and all mortalities of *C. maenas* were recorded and standardised for numbers of individuals for each mesocosm. The data for each replicate were divided by the number of live crabs present to standardise for the inflated rates of moulting and mortality inherent in treatments with larger numbers of crabs. A log(x+1) transformation was applied to increase data homoscedasticity. As *Carcinus maenas* moult and death rates are univariate data, Euclidean distances were used to create the resemblance matrix.

At the end of the experiment, each mesocosm was censused for amphipods. These data were used to test the hypotheses that greater populations of amphipods will be found in mesocosms with smaller crabs, that tolerances to warming are species specific, nutrient enrichment increases bottom up productivity and that the effects of warming and eutrophication will largely cancel each other out. Counts were differentiated to genus or species level (8 different species). Juvenile amphipods that were too small to identify reliably were pooled together as ‘small amphipod spp.’ for each mesocosm. A log(x+1) transformation was applied to down-weight the effect of species that are hyper-abundant compared to others in the assemblage. Amphipod species abundances were compared between treatments using separate Bray-Curtis dissimilarity matrices, which are ideal for multivariate data sets where the dependent variables are vaguely defined or not in standard units of measurement, for instance, where species are identified to varying taxonomic resolution or units alternate between abundance and percentage cover (Bray and Curtis 1957). Bray-Curtis similarities do not classify shared zeros as an indication of likeness, making them ideal for data sets containing rare species (Clarke et al. 2006). A dummy variable was added to the assemblage (+1) to decrease distortion stress (Clarke et al. 2006).

The wet and dry masses of the five algal species added at the outset of the experiment were measured and their respective wet-dry relationships determined to test the hypotheses that changes in top predator size cause trophic cascades, tolerances to warming are species specific, nutrient enrichment increases bottom up productivity and that the effects of warming and eutrophication will largely cancel each other out. Initial dry weights were estimated by applying these equations to the original wet weight data for each species. The raw change in the biomass of each species was then calculated. The dry weight of the ephemeral filamentous brown alga *Ectocarpus* spp., which grew in the tanks during the experiment, was measured. No transformations were applied to algal assemblage data. Algal dry weight data were analysed using a resemblance matrix created from Euclidean distances between algal dry weights, which should be used in situations where a zero (zero change in this case) is relevant to the hypothesis.

To characterize the food web and quantify the strength and reliability of the trophic cascade tested by hypothesis one, a model was developed using Pathways Analysis in the AMOS 5 software (Wootton 1994; Arbuckle 2003). Here, regression weights and multiple correlations were calculated for the effects of crab size and warming on the abundance of amphipod prey, and the subsequent effects they had on change of biomass in the various algal species. Nutrients were excluded on the basis of their lack of a significant effect at any trophic level when analysed using PERMANOVA. Acceptability of the fit of the model was determined by comparing the data with our model using a chi square analysis so that no significant differences were observed.

2.3 RESULTS

2.3.1 *Carcinus maenas* Moults and Mortality

Temperature had no effect on moulting rates, thereby rejecting the hypothesis that warming increases crab growth rates. Rather, warming significantly increased the probability of any given individual dying during the six weeks of the experiment, thus supporting the hypothesis that increased temperature leads to greater top down control and higher rates of starvation associated mortality (Table 2.1). There was no effect of crab size or eutrophication on moulting or mortality rates in crabs, nor any interactive effects of the experimental factors (Table 2.1), suggesting that the effect of warming was consistent across changes in predator body size and the nutrient status of the experimental water.

2.3.2 Amphipod Species Abundances

Consistent with the hypotheses that amphipod populations are larger in the presence of smaller crabs and that response to warming are species specific, but in contrast to the hypothesis that eutrophication increases bottom up productivity, amphipod assemblages were altered by decreasing crab size and warming, but not nutrients (Table 2.3). The hypothesis that stressors would largely cancel each other out was not supported as there was no interaction between warming, nutrients and crab size. Crab size had the strongest effect, impacting all species, while the effects of warming were less obvious, and did not affect all species equally (Figure 2.4A). While decreasing crab size positively affected all species of amphipod except the less-abundant species *Gammarus zaddachii*, this effect was entirely driven by the presence and absence of crabs rather than size *per se* (Figure 2.4B). Warming clearly and continuously reduced the abundance of *C. marinus* and *C. storensis*, and at the highest temperatures *G. finmarchius* and the rare species were not present at all (Figure 2.4C). Small amphipods did not appear to be affected by warming (Figure 2.4).

Table 1: Single-factor analyses with two covariates testing the null hypotheses that rates of moulting and mortality in the crab *Carcinus maenas*, and assemblages of amphipods and algae are not affected by: (1) warming, a continuous covariate; (2) crab size, a continuous covariate; and (3) nutrients, a fixed factor with two levels (background and enriched). Interaction terms with a $p > 0.25$ were pooled with the residual in a stepwise fashion so that those terms that explain the least variation are pooled first. This was done to increase the power of tests (Underwood 1997) and pooled terms are marked as such. This led to a total of three, three, three and four degrees of freedom being pooled with the residual for moulting, mortality, amphipod and algae analyses respectively. Significant values are in bold.

	GLM crab moulting			GLM with binomial link crab mortality			
Source	MS (df)	F	*P*	estimate (df)	se	z	*p*
Warming	0.17 (1)	0.71	0.40	0.21(1)	0.11	2.00	**0.049**
Crab size	0.37 (1)	1.57	0.22	-0.30 (1)	0.35	-0.86	0.39
Nutrients	0.01 (1)	0.02	0.89	-0.24 (1)	0.41	-0.58	0.56
w x cs	0.69 (1)	2.90	0.10	pooled			
w x n	Pooled			pooled			
cs x n	Pooled			pooled			
w x cs x n	Pooled			pooled			
Pooled residual	0.24 (51)			52.50 (52)			

Table 2.2: Multivariate single-factor PERMANOVA with two covariates testing the null hypothesis that an assemblage of gammarid amphipods is not affected by: (1) warming, a continuous covariate; (2) crab size, a continuous covariate; and (3) nutrients, a fixed factor with two levels (background and enriched). Data were log(x+1) transformed. The resemblance matrix was calculated using Bray-Curtis similarities. Interaction terms with a *p* value greater than 0.25 were pooled with the residual (Underwood 1990) and are marked as such. A total of three degrees of freedom were pooled with the residual. Significant values are highlighted bold.

Source	df	MS	Pseudo-*F*	*p*
warming (w)	1	3616.5	3.77	**0.01**
crab size (cs)	1	18912	19.70	<**0.001**
nutrients (n)	1	467.07	0.49	0.70
w x cs	1	POOLED		
w x n	1	POOLED		
cs x n	1	1610.7	1.68	0.18
w x n x cs	1	POOLED		
pooled residual	67	960.08		
total	71			

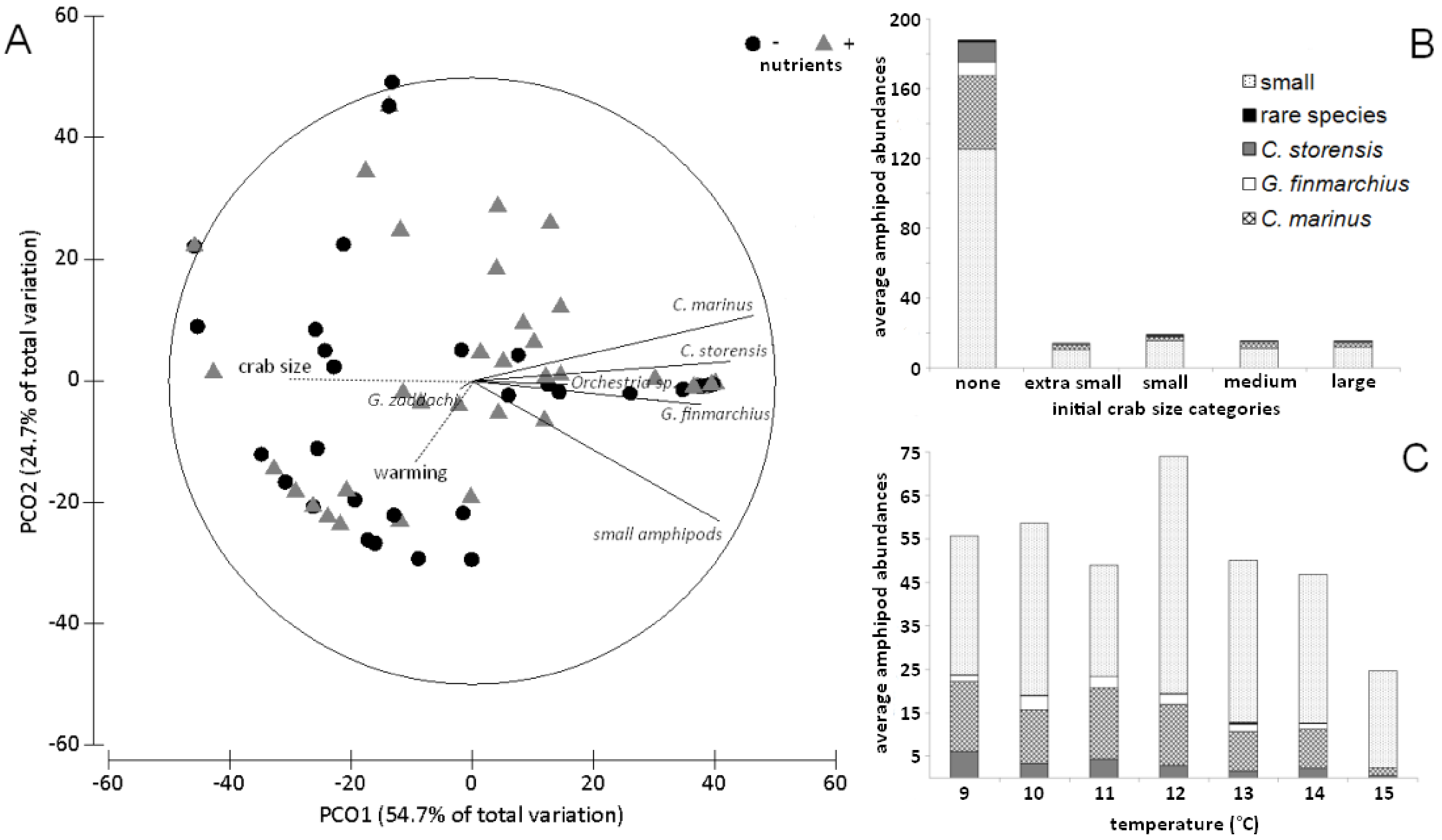


Figure 2.3: A) Euclidean ordination of mesocosms based on the resemblance matrix created from the amphipod assemblages and as analysed with PERMANOVA. This plot allows comparison of amphipod species (solid vectors) with crab size and warming covariates (dotted lines) and the nutrient factor (symbols) in multivariate space. B) Effect of crab size on individual species. C) Effect of warming on individual species. For ease of illustration in panels B and C, crab size and warming have been plotted as bins and less abundant amphipod species have been grouped as ‘rare species’.

2.3.3 Changes in Algal Biomass

Algal assemblages were altered by decreasing crab size, but not warming or nutrients (Table 2.4). The magnitude and direction of the effect differed between species (Figure 2.5A). This supported the hypothesis that trophic cascades occur in response to changes in predator size, but did not support the hypotheses that tolerances to warming are species specific or that eutrophication increases bottom up productivity. As there was no interaction between warming, crab size and nutrients, H7 was not supported. *Fucus serratus* linearly decreased in abundance with decreasing crab size while *Corallina* and *Ectocarpus* spp. exhibited much weaker opposing trends, decreasing when exposed to the largest crabs and increasing in the absence of crabs respectively (Figure 2.5B).

2.3.4 Pathways Analysis

A trophic cascade explains the negative effect of decreasing crab size on *F. serratus* (Figure 2.6). Here, the negative direct effect of larger crabs on the abundance of *C. marinus* released *F. serratus* from grazing pressure. This cascade was not found for the other abundant species of amphipod in the tanks, for example, *G. finmarchius*, which had no significant effects on any of the algal species. Warming reduced the abundance of *C. marinus*, though this effect did not appear to trickle down to lower trophic levels. This finding provides joint support for the hypotheses that changes to the top predator size impact amphipod populations and cause trophic cascades, but provides further evidence against H6 and 7, that the addition of nutrients causes bottom up productivity that would cancel out the effects of warming and decreasing crab size. The effect of warming also supports the notion that responses to warming are species specific, but only for the amphipods and not the algae.

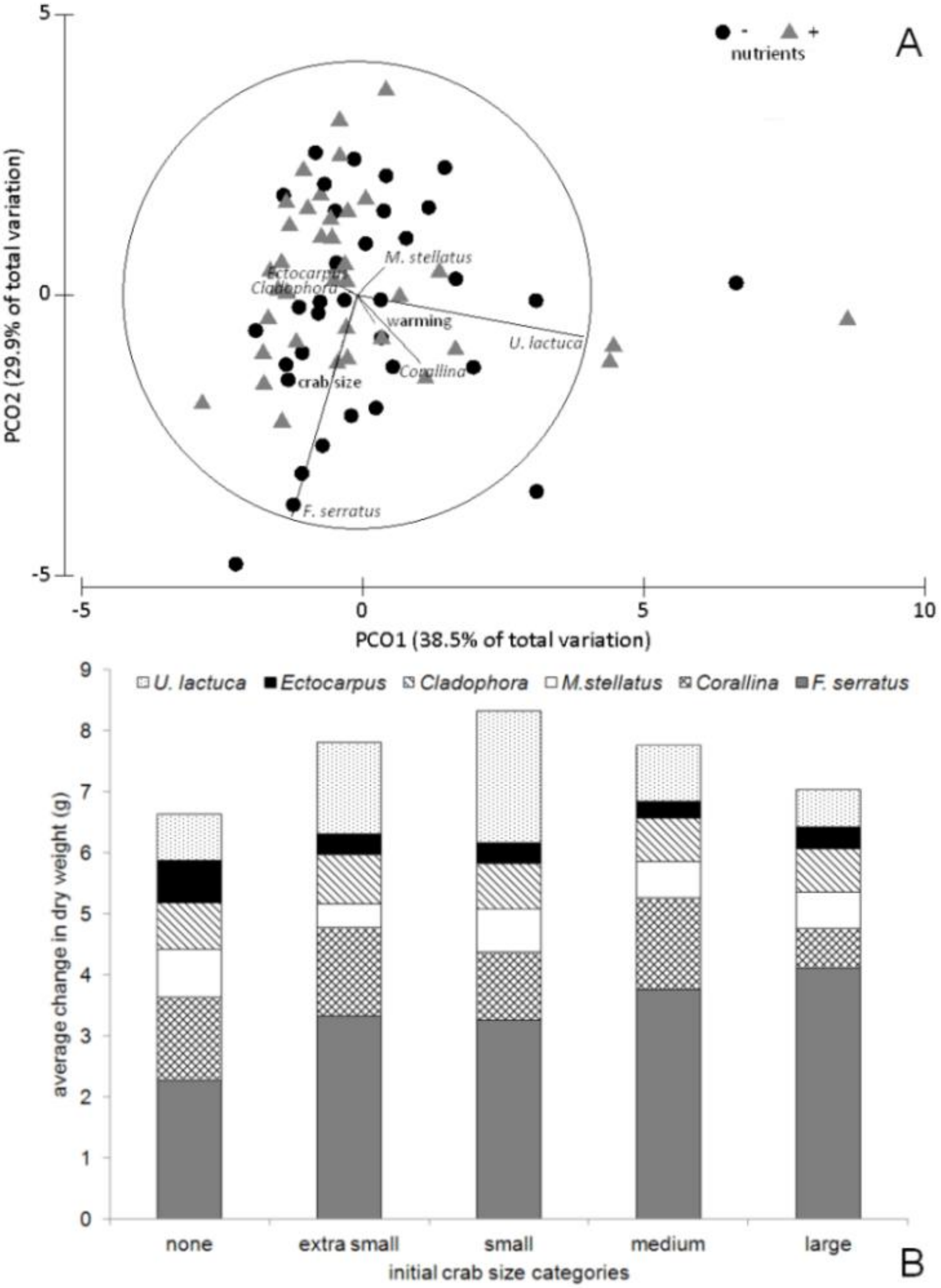


Figure 2.4: A) Euclidean ordination of mesocosms based on the resemblance matrix created from the algal assemblages and as analysed with PERMANOVA. This plot allows comparison of algal species (solid vectors) with crab size and warming covariates (dotted lines) and the nutrient factor (symbols) in multivariate space. B) Effect of crab size on individual species. C) Effect of warming on individual species. For ease of illustration in panels B and C, crab size and warming have been plotted as bins and less abundant amphipod species have been grouped as ‘rare species’.

Table 2.3: Multivariate single-factor PERMANOVA with two covariates testing the null hypothesis that the relative amounts of biomass within an assemblage of algal species are not affected by: (1) warming, a continuous covariate; (2) crab size, a continuous covariate; and (3) nutrients, a fixed factor with two levels (background and enriched). As data are a change in biomass, rather than absolute values, and there were no zeros in the data set, Euclidean distances were used to calculate the resemblance matrix. Interaction terms with a *p* value greater than 0.25 were pooled with the residual (Underwood 1990) and are marked as such. A total of four degrees of freedom were pooled with the residual. Significant values are highlighted bold.

Source	df	MS	Pseudo-*F*	*p*
Warming	1	9.56	1.08	0.36
Crab size	1	33.84	3.81	**0.01**
Nutrients	1	9.99	1.12	0.33
w x cs	1	POOLED		
w x n	1	POOLED		
n x cs	1	POOLED		
w x cs x n	1	POOLED		
Pooled Residual	69	8.94		
Total	72			

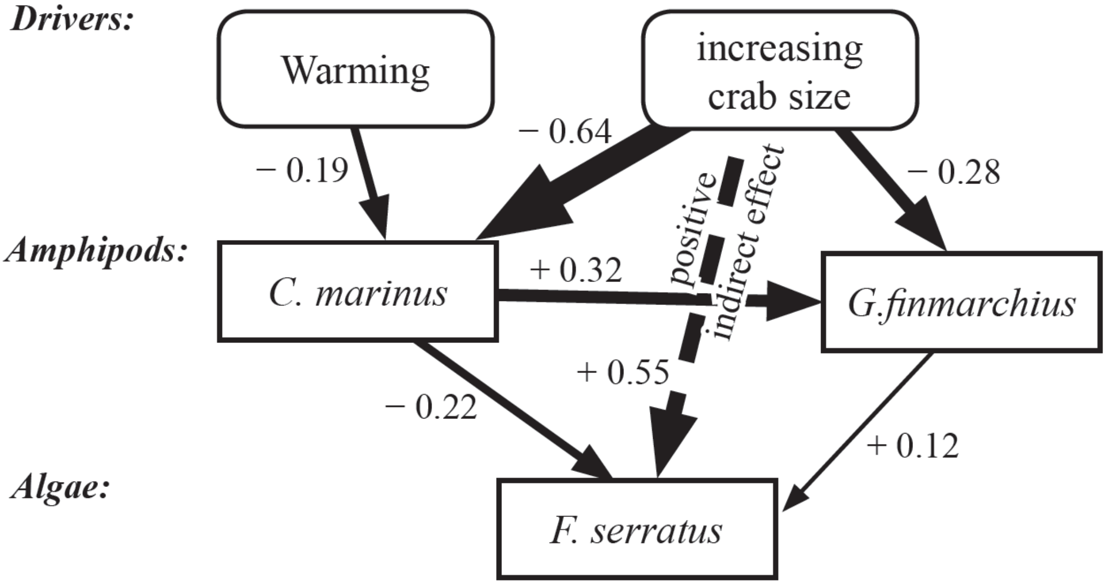


Figure 2.5: Pathways analysis quantifying the strength and direction of trophic relationships between common species in the mesocosms. For the above model, there were no discrepancies between the data and the model; comparison of the data and model yielded χ^2 = 0.6, 3 d.f., *p* = 0.90. Standardised regression weights (the direction and strength of the correlation) are given next to their respective relationships (arrows).

2.4 DISCUSSION

Here the response of an assemblage of crabs, amphipods and various algal species to manipulations of temperature, top-predator body size and nutrient enrichment was observed. Warming increased *C. maenas* mortality, but had no effect on rates of *C. maenas* moulting thus supporting the hypothesis that warming leads to higher rates of mortality, but rejecting the hypothesis that warming increases moulting rates. Warming altered the relative abundances of amphipods, but not the relative amount of algae, providing support for the hypothesis that responses to warming are species specific in terms of amphipod assemblages but not algal assemblages. While decreasing crab size had no effect on the abundances of various amphipod species, thus rejecting the hypothesis that larger populations of amphipods would be found in mesocosms with smaller crabs, there were large numbers of amphipods present in those mesocosms characterized by a complete absence of crabs and an effect of decreasing crab size was observed in the brown algal species, *F. serratus*, supporting the hypothesis that smaller crabs would result in a trophic cascade. There was no effect of nutrient enrichment on any compartment of the food web, thus rejecting the hypotheses that eutrophication increases primary productivity such that it would cancel out the top down effects of warming and crab size.

2.4.1 Effects of Warming

It is generally believed that ectothermic organisms respond to warming with increased growth (Reitzel et al. 2004; O'Connor et al. 2007) and moulting (Fowler et al. 1971; Poleck and Denys 1982) and this effect declines as individuals age and increase in size (Sutcliffe et al. 1981; Pöckl 1992; Angilletta Jr and Dunham 2003). Additionally, warming can make organisms more susceptible to disease (Altstatt et al. 1996; Friedman et al. 1997; Lester et al. 2007) and parasitism (Poulin 2006), both of which lead to increased mortality rates and may have played a role here. While warming singularly increased rates of mortality in *C. maenas*, no interactive effect of temperature or body size on moulting rates was observed (Table 2.1). While the potential for a moulting response to warming and body size may have been restricted by food availability in the mesocosms (Klein Breteler 1975), it appears that warming does not influence population size structure over the time scales examined here.

Changes to the relative abundances of amphipod species may have occurred here due to a direct effect of warming on the amphipods themselves, which is consistent with the hypothesis that responses to warming are species specific. All organisms live within a discrete window of temperatures, outside of which functional and physiological limitations occur (Hochachka and Somero 2002a; Poloczanska et al. 2007; Pörtner and Farrell 2008). In this experiment, some species (for example, *G. finmarchius*) were less affected by warming, while others (such as *C. marinus* and *C. storensis*) responded strongly, with higher abundances observed in the cooler mesocosms (Figure 2.4). The ability to adapt physiologically to changing temperature is species specific (Bulnheim 1979; Peck 1989; Pörtner et al. 1999; Tomanek and Somero 1999; Somero 2002; Stillman 2003; Bailey et al. 2005; Somero 2005; Helmuth et al. 2006; Tomanek 2010) with responses likely due to pre-adaptation to certain conditions, via phenotypic plasticity (Sinclair et al. 2006) and acclimatization potential (Hochachka and Somero 2002a).

2.4.2 Effect of Decreasing Predator Body Size

For amphipod species abundances, effects were observed only between the presence and absence of the top predator (Figure 2.3), but it is apparent from the algal assemblage (Figure 2.4) and the pathways analysis (Figure 2.5) that there was an allometric cascade whereby the negative effect of increasing average top predator body mass on the most abundant adult amphipod species (*C. marinus*) translated to a positive effect on the brown alga *F. serratus*, which is a food species that has been associated with gammarid amphipods (Hagerman 1966; Fredriksen et al. 2005). It is likely that other amphipod species were not abundant enough to have a cascading effect. An allometric effect was detected on the algal assemblage, but not the amphipod assemblage and this might be explained by timing. A fixed number of individual amphipods were added to the mesocosms over six occasions up until and including the last week of the experiment. Thus, the amphipod community was constantly renewed and in a state of flux, whereas as the algal communities were not manipulated other than via the original deployment during the six weeks. Consequently, algal biomass responses probably represent a more integrated longer-term measure of the effects of the primary treatments. While it is possible that the omnivorous crabs were consuming the algae, the fact that the analysis did not detect a direct pathway between crab body size and *F. serratus* change in biomass suggests this is unlikely. Thus, the allometric cascade was likely driven by changes in the body size ratio of predators and their prey, with consequences for attack success rates (Rall et al. 2010; Vucic-Pestic et al. 2010b; Stouffer et al. 2011; Zook et al. 2011; Kalinkat et al. 2013), thereby altering the arrangement and strength of trophic interactions (Warren and Lawton 1987; Cohen et al. 1993; Jonsson and Ebenman 1998; Emmerson and Raffaelli 2004; Brose et al. 2006a; Berlow et al. 2009). It should be noted that the trophic cascade described here is phenomenological only, that is Pathways Analysis only provides correlative evidence that the algae are being eaten by the amphipods, which are being eaten by the crabs; there is no causative evidence. Based on previous gut content analysis of *C. maenas* been used in a manipulative field experiment it is likely that this scenario is accurate (Jochum et al. 2012). To maintain the crab size treatment for the duration of the experiment, those crabs that had died were replaced. While mortality rates were generally low, the policy of maintaining top predator populations may have led to a top-heavy trophic structure with no capacity to correct itself through a crash in predator numbers.

These findings provide strong support for the hypothesis that having smaller top predators increases prey abundances and leads to trophic cascades. These findings have interesting implications when applied to ecological case studies in North Pacific and Tasmanian kelp forests. The addition of sea otters to eastern North Pacific Kelp forests produced a cascading reduction of sea urchins and increased in kelp biomass (Wilmers et al. 2012). Similarly, when fishery activity depleted lobster stocks in Tasmanian kelp forests there were less predators to cap invasive sea-urchin populations and urchins barrens became more common (Ling et al. 2009). Given that the removal of predators and the reduction of average body size of predators can have similarly cascading effects, longer-term reductions the body sizes of lobsters and otters could reduce the capacity of kelp systems to sustain fisheries and sequester carbon.

Experimental manipulations for decreasing crab size are inextricably correlated with reduced total biomass and increased numbers of individual predators (Schneider et al. 2012). The allometric design used here reflects the way the size structure of natural populations will remain self-governed in response to decreasing body mass, though partitioning the individual effects of body mass, number of individuals and total biomass is unnecessary for understanding the ecological consequences of manipulating these strongly auto-correlated measures (Figure 2.1). Thus, this chapter adds to a growing body of evidence that altering the natural size structure of predator populations can create trophic cascades without removal of entire species (Jennings et al. 2001; Heckmann et al. 2012; Jochum et al. 2012; Audzijonyte et al. 2013). This chapter is also the first evidence to suggest that the indirect, long-term, ecological effects of warming-induced changes to size-structured food webs may be as strong if not stronger than the direct, short-term, physiological effects of warming, which would be of particular substantive significance if longer-term reductions in average body size from increased temperature were to synergistically-interact with present day reductions in organism size due to overexploitation. It would be of interest to test whether this theory holds true for organisms living at their upper thermal limits.

2.2.3 Nutrient Enrichment and Indirect Interactions

The level of nutrient enrichment used here did not significantly affect the relative species abundances in either the macro-algae or amphipod assemblages, nor did it affect mortality or growth rates of the top predator, *C. maenas*, thus rejecting the hypothesis that nutrient enrichment boosts bottom up productivity. Previous studies have shown nutrient enrichment to increase primary production (Nixon 1995; Bonsdorff et al. 1997; Harding Jr and Perry 1997), which is passed on to higher trophic levels and culminates with increased top predator mortality and extinction via a paradox of enrichment (Rosenzweig et al. 2008). The presence of indirect pathways may explain the lack of a consistent or strong effect of nutrients. Indirect effects from abiotic changes can overshadow direct effects (Abrams 1995; Menge 1995; Yodzis 2000; Connell et al. 2011) and obscure the individual effects of single species (Sih et al. 1998; Reiss et al. 2009; Reiss et al. 2011), which greatly reduces the ability of managers to predict whole community responses to relatively simple environmental changes (Berlow et al. 2004; Wootton and Emmerson 2005). While the design of this experiment allows for detection of indirect effects arising from interactions with warming or top predator body mass, it is difficult to determine whether the mode of compensation varies across different treatments or if there are indirect effects that are associated with the ecology of the mesocosms. Indeed, warming can increase primary productivity (Nemani et al. 2003; Yvon-Durocher et al. 2010; Demars et al. 2011b), while also increasing rates of consumption (Mulholland et al. 1983; Steinman et al. 1991; Mulholland et al. 1994; Shurin et al. 2012). Another possibility is that there have been changes in the palatability of algae under warming conditions (Poore et al. 2013) which would influence the grazers' preferences. Either way, the effects of warming and nutrients should, therefore, cancel each other out, though in the experiments presented here they do not. It may simply be that the dose of nutrients used was not sufficiently strong to produce a clear response, particularly given that intertidal organisms are often subjected to large amounts of environmental variability (Dayton 1971). That said, this was not the case in Vye et al (2013 unpublished data) and thus the hypothesis of stressors cancelling each other out has been rejected.

The constant input of microorganisms from Strangford Lough might provide an energy subsidy for the mesocosm systems, so quantification of zooplankton, phytoplankton, and biofilm communities in future experiments may provide a clearer picture, particularly given that these organisms play an important role in marine communities (Montagna et al. 1995; Carman et al. 1997; Olafsson et al. 1999), and may be acting as a voracious buffer against eutrophication effects as seen with mesograzers in seagrass communities (Reynolds et al. 2014) and communities with dramatically altered foodwebs (Heck and Valentine 2007).

2.2.4 Conclusion

In conclusion, here it is shown that under future warming scenarios it is expected that some species will be able to exploit their new environment, while other species will not be as competitive and decline possibly to extinction (Somero 2010). In nature, such an effect could result in the evolution of novel communities (Lurgi et al. 2012; Moya-Laraño et al. 2012). Similarly, body size-driven changes to predator-prey interaction strengths (Berlow et al., 2009) can result in trophic cascades (Heithaus et al., 2008; Jochum et al., 2012), which shift the flow of energy and nutrients throughout a system (Economo et al., 2005; White et al., 2007; Ehnes et al., 2011) and may overshadow the direct physiological effects of warming. Thus, the next chapter further investigates the strong role of relative predator-prey body size in the trophic relationship shared by *C. maenas* and amphipods. Additionally, this system exhibits potential for indirect interactions: warming-impacted mortality rates in *C. maenas* may have played a role in the allometrically-mediated trophic cascade where more *F. serratus* was found in the absence of larger crabs. Due to the high connectivity of natural systems (Williams et al. 2002), indirect perturbations and feedback loops within food webs are likely to drive many of the observed consequences of environmental change (McCann 2000; Borer et al. 2006; Connell et al. 2011; Audzijonyte et al. 2013). It is proposed that the quantification and understanding of indirect interaction effects is of high importance for predicting the ecosystem consequences of environmental change. This is particularly relevant for experiments that wish to examine the dual effect of warming on the present-time biology of individuals and the long-term size restructuring of their populations.

CHAPTER THREE: CHANGING BODY SIZE AND THE ABSENCE OF REFUGIA ALTERS TROPHIC INTERACTIONS BETWEEN THE SHORE CRAB *CARCINUS MAENAS* AND AMPHIPODS

ABSTRACT

In a changing world where the average body sizes of species' populations are declining and habitat simplification is leading to losses in prey refugia, examination of the fundamental trophic interaction between individual species will help interpretation of complex food webs responding to these disturbances. While it is recognized that interaction strengths between trophically-adjacent species can be altered by changes in their relative body size and the access that prey species have to refugia, less is known about how these phenomena might interact for many species pairs that are experiencing such conditions. The research in this chapter aims to characterise the trophic relationship between the shore crab, *Carcinus maenas,* and assemblages of amphipod prey in response to variation in relative predator-prey body size ratio and the level of access prey have to refuge from predation. To achieve this aim *C. maenas* and amphipods of varying relative body size were placed in mesocosms with or without refugia. Rates of predation were documented after one night. It was found that changing body size ratio consistently perturbed predator-prey interactions, whilst the effect of removing a prey refuge depended on the size of the refuge relative to the size of the prey. It is clear that removal of refugia caused a downshift in the optimal body size ratio for strong interaction strengths. This implies there would be size-based selection pressure on prey species in simplified habitats. Further work is recommended to determine whether these predicted shifts in optimal body size ratio will actually manifest in prey populations of species over the longer term and whether other anthropogenic stressors such as warming and ocean acidification will accelerate the onset of this potential phenomenon.

3.1 INTRODUCTION

3.1.1 Quantifying Trophic Interactions in a Changing World

In a world where organisms are getting smaller (Gardner et al. 2011; Audzijonyte et al. 2013), the likelihood of species populations crashing is increasing and habitat simplification is common (MA 2005), many aspects of conservation have moved away from single species conservation towards holistic styles of ecosystem management. Restoration effort is being done at the ecosystem grade of organisation (Comín 2010), while the International Union for the Conservation of Nature is establishing a list of threatened ecosystems (Rodríguez et al. 2011).

Given that in natural systems species are generally no more than two trophic links apart (Williams et al. 2002), the impacts of anthropogenically-forced changes to these complex multispecies systems are notoriously difficult to predict (Woodward et al. 2010). While the speed and extent to which a perturbation can propagate through a food web is determined by the number and strength of trophic interactions (May 1974; Borrvall et al. 2000; McCann 2000; Dunne et al. 2002; Neutel et al. 2002), this interconnectedness means that even small changes in the abundance of a species can have surprisingly large effects (Emmerson and Raffaelli 2004; Borer et al. 2006; Berlow et al. 2009). These unexpectedly large effects can be partially attributed to indirect effects (Berlow et al. 2004; Wootton and Emmerson 2005), whereby the effect of a single species can be masked by strong trophic interactions with and between adjacent species (Sih et al. 1998; Connell et al. 2011; Reiss et al. 2011) or cancelled out by feedback loops (Montoya et al. 2009).

The strength of interactions between species (hereafter referred to as ‘interaction strengths’) can be measured by various indices that compare prey populations in the presence and absence of predators (Connell 1961; Paine 1992; Laska and Wootton 1998).

Development of these indices can allow estimation of true interaction strengths across systems and species (Berlow et al. 1999). These interaction strengths are mediated by species traits such as relative body size (Jennings et al. 2001; Berlow et al. 2009), prey density (Berlow et al. 2004) and the characteristics of the domain in which the interaction takes place (Schmitz 2007), all three are the focus of the work presented in this chapter. First, interaction strengths may be mediated by size-based niche differentiation (Sinclair et al. 2003; Dial et al. 2008; Kohda et al. 2008) when there is decreased feeding on prey that are simply too large or too small for any given predator (Brose et al. 2008; Brose 2010). This is exemplified by hump-shaped relationships that are repeatedly observed for rates of attack and consumption when scaled with body mass or size (Wahlström et al. 2000; Brose et al. 2008; Rall et al. 2010; Vucic-Pestic et al. 2010b; Vucic-Pestic et al. 2011; Rall et al. 2012; Twomey et al. 2012; Kalinkat et al. 2013).

Rates of predation are constrained by movement, detectability and encounter rates at small body size ratios, and detectability, attack success and predator disinterest at large body size ratios (Frank 1982; Bosman et al. 1989; Brose et al. 2008; Petchey et al. 2008a; Vucic-Pestic et al. 2010b). Thus, particularly large and small prey individuals are able to use size-based refugia to avoid predation (Paine et al. 1985; Castilla et al. 1994; Rudolf and Armstrong 2008). An increasingly-disproportionate amount of the global human population lives on narrow strips of coastline (MA 2005). Use of marine resources and development of coastal spaces (for example, trawling and the building of seawalls) has led to the simplification and homogenization of local habitats, which has negative implications for the reliability of ecosystem functions including the provision of prey refuges (Jones et al. 1994; Dobson et al. 2006). The loss of prey refugia may lead to subsequent declines in local biodiversity (Lotze et al. 2006; Thrush et al. 2006; Airoldi et al. 2008). Changes in refuge availability could disrupt natural predator-prey interaction by altering encounter rates and modifying prey behaviour as prey spend more time in shelter so that predation tends to be highest at intermediate levels of complexity, where prey feel safe enough to forage, yet habitat structure is not extensive enough to protect them completely (Poole 1961; Real 1979; Crowder and Cooper 1982; Lima 1998; Rypstra et al. 2007). This chapter asks whether an absence of prey refugia will influence the effects of decreasing body size on trophic relationships as while it is recognized that prey size is related to the efficacy of a refuge (Rudolf 2006), it is not known how a loss of refugia will impact the optimal predator-prey body size ratio for maximum rates of predation.

Although this is likely to be dependent on feeding mode (Kalinkat et al. 2013), handling times tend to display a U-shaped relationship, whereby times are increased at smaller and larger body size ratios (Elner and Hughes 1978; Goss-Custard et al. 2006; Vucic-Pestic et al. 2011; Rall et al. 2012). While predicting which aspects of structure drive ecological responses to the presence of refugia (Warfe et al. 2008), environmental shelters have been observed to reduce predation rates by decreasing the likelihood of encounters (Sih 2005), providing prey with sanctuary (Real 1979; Frank 1982; Bosman et al. 1989; Muirhead and Sprules 2003; Gergs and Ratte 2009; Kalinkat et al. 2013) and leading to patchiness in predator/prey distributions (Poole 1961; Materna 2004; Rypstra et al. 2007; Coleman 2008). Accessibility to refugia (Vucic-Pestic et al. 2010a) and altered prey behaviour can change the efficacy of any given refuge, which is in turn dependent on the body sizes of the predator and prey (Werner and Hall 1988; Lima and Dill 1990; Lima 1998; Byström et al. 2003; Rudolf 2006; Rudolf 2008). This is particularly complicated when the refuge also functions as a resource, such as leaf litter in detritus communities (Kalinkat et al. 2012).

Organism density is also important to consider when examining predator-prey interactions (Vucic-Pestic et al. 2010a), particularly in the interpretation of interaction strength indices (Berlow et al. 1999), because the per capita consumption rates of predators change with varying prey density, usually creating non-linear functional response curves that are hyperbolic (Type II), sigmoid (Type III) or a combination of the two (Holling 1959; Murdoch and Oaten 1975; Kalinkat et al. 2013). Functional responses can vary in shape and magnitude, depending on the size of their predator-prey components (Brose et al. 2008) such that having concomitant body-size data increases the predictive power these curves add to ecological models such as those of population dynamics (Kalinkat et al. 2013). For example, in a system using beetle predators, smaller prey were consumed following a Type II curve, while larger prey were consumed following a Type III functional response (Vucic-Pestic et al. 2010b), though it must be noted that this is not a generalised pattern as that study confounded identity with prey size and the outcome can also depend on the size of the predator (Kalinkat et al. 2013). The effectiveness of refuge varies with the density of prey, which can alter the use of shelter (Kaiser 1983; Hunsicker et al. 2011) and is related to the average body size of populations and the arrangement of food webs (Reuman et al. 2008; Reuman et al. 2009). Similarly, the presence and extent of a refuge also impacts the functional response (Vucic-Pestic et al. 2010a), often shifting the curve from Type II to Type III (Murdoch and Oaten 1975; Hassell et al. 1977; Real 1977; Crawley 1992; Anderson 2001b; Vucic-Pestic et al. 2010a; Alexander et al. 2012). This is because refugia reduce searching success, particularly at low prey densities (Scheffer and De Boer 1995; Rickers and Scheu 2005). Fundamental aspects of functional response curves contribute to the trophic structure of food webs (Petchey et al. 2008b), and changing the type of these curves can have detrimental effects on the ecological stability of systems (Oaten and Murdoch 1975; Williams and Martinez 2004; Fussmann and Blasius 2005; Brose et al. 2006b; Rall et al. 2008).

Finally, palatability is an important predictor for whether a grazer consumes a certain algal or not and this is often related to the grazers mouthparts (Steneck and Watling 1982). Understanding which species of algae are most palatable to amphipods is be important information for any future studies that examine the effects of body size and refugia on food chains and trophic cascades.

3.1.2 Chapter Aims

The strongest effects observed in the experiment described in chapter two were those in response to changes in predator presence and body size. While food web responses are often more than the sum of trophic interactions (Cohen et al. 2009), knowledge of fundamental aspects that underpin species interaction strengths can help researchers understand the mechanisms that determine how perturbations propagate through food webs. Accordingly, three experiments were done to examine the relationship between the green shore crab, *Carcinus maenas*, and an assemblage of intertidal amphipod prey from the Irish Sea in response to changes in predator-prey relative body size, prey density and refuge extent, which is, in this case, the rock debris that is a conspicuous component of intertidal habitats that are commonly associated with these taxa (for example, Lee 2004). A pilot study was also done to determine preliminary feeding behaviour of these amphipod assemblages for use in future experiments considering the dual role of algae as a resource and a refuge. These experiments address the following models (M) by testing their associated hypotheses (H):

M1) prey consumption rates are optimised within a certain window of predator-prey body size ratio

  H1) lower interaction strengths are observed between larger *C. maenas* and smaller amphipods and vice versa leading to a hump-shaped relationship between body size ratio and interaction strength

M2) attack rates are influenced by predator-prey body size ratio

  - H2) for *C. maenas* and an assemblage of amphipod prey a hump-shaped relationship will be demonstrated between body size ratio and attack rates

M3) handling times are greater in mobile predator-prey relationships that have smaller and larger body size ratios

  - H3) if *C. maenas* and amphipod prey body size ratio is manipulated a U-shaped relationship between handling time and body mass ratio will emerge

M4) predator/prey interactions are stronger in the absence of refugia

  - H4) the removal of rock refugia will lead to stronger interaction strengths between *C. maenas* and amphipods

M5) predator/prey interactions weaken as refugia decrease in size

  - H5) interaction strengths between *C. maenas* and amphipods will gradually weaken as rock refugia are added to the arena

M6) refugia are most effective at low prey densities

  - H6) addition of a rock refuge will induce Type III functional response curves between *C. maenas* and amphipod prey

M7) absence of refugia increases predator attack rates

  - H7) the rate at which *C. maenas* attacks amphipods will be greater in the absence of rock refugia

M8) refugia have no effect on time spent handling prey.

  - H8) the time *C. maenas* spends handling amphipods will remain the same irrespective of the presence of absence of refugia

M9) the effectiveness of a refuge is mediated by the relative body size of predator/prey species

  - H9) relative predator-prey body size will interact with refuge presence and absence to alter the relationship between *C. maenas* and their amphipod prey

M10) the effectiveness of a refuge is mediated by the relative body size of predator/prey species, particularly at greater prey densities.

  H10) relative predator-prey body size will interact with refuge presence and absence and prey density to alter the relationship between *C. maenas* and its amphipod prey

M11) amphipods graze upon some algal species more than other algal species

  H11) if amphipods are presented with various species of algae, some pairings will exhibit higher interaction strengths than others

3.2 METHODS

3.2.1 General Methods

All specimens were collected by hand from the intertidal near Portaferry, Northern Ireland (54.3809° N, 5.5486° W). Unless stated otherwise, all experiments were done overnight in a non-climate-controlled laboratory using 10L mesocosms filled with fresh gravel-filtered seawater pumped from Strangford Lough, Northern Ireland. Refugia, where used, were created by the addition of between three and seven pebbles (Wentworth scale of grain classification; Wentworth 1922) randomly selected from a stockpile with a mean rock volume of 24.5±1.8 mL (n =30). This led to an increase in habitat surface area at the expense of a relatively small proportion of the total habitat volume held within the mesocosms (Warfe et al. 2008). Use of this method allowed manipulation of the size of the refuge while holding the type of the refuge (rocks) constant (Stoner and Lewis III 1985).

Throughout this series of trials crabs and amphipods in various size categories were used (Table 3.1). Crabs were sorted into three stratified bins that were based on a log scale of carapace widths. Due to the difficulty of handling small, highly-mobile specimens, amphipods were sorted coarsely by eye. While it was acknowledged that having a mixed species assemblage has the potential to increase the variance of the response if crabs are not equally likely to consume the various species it was expected that the random selection of a large number of amphipods would distribute this variation evenly between treatments. Amphipod assemblages were comprised of randomly-mixed species and consisted primarily of *Gammarus finmarchius, Chaetogammarus marinus* and an *Orchestria* species.

Of the various measures of interaction strength, dynamic indices that measure per capita interaction strength (Wootton 1997) were the most appropriate for this short-term experiment as it had no opportunity to reach equilibrium (Berlow et al. 1999). Based on the Lotka–Volterra competition equations, per capita interaction strength (where used) was calculated using Equation 1:

$$(1)\ \alpha_{ij} = \ln(P/C)/T$$

where α_{ij} is the effect of species j on species i, P is the abundance of prey in the treatment where predators are present, C is the abundance of prey where the predators are absent and T is the time period over which the experiment is carried out (Laska and Wootton 1998).

Table 3.1: The various size categories of *Carcinus maenas* and amphipods used in these experiments are given. Mean dry weight (g) crab of amphipod body size categories is documented, while the range of carapace widths (mm) is also shown for each crab size category.

	SMALL		MEDIUM		LARGE	
	dry mass	carapace	dry mass	carapace	dry mass	carapace
C. maenas	0.24	13-16	0.78	20-26	3.156	33-42
amphipod assemblage	0.002	-	0.011	-	0.021	-

3.2.2 Experiment One: Influence of Relative Body Size and the Presence of Refugia on Predator-Prey Interaction Strengths

Predator-prey feeding trials were done to establish the role of relative body size (a log ratio of individual predator to individual prey dry mass) on interaction strength in the presence and absence of simple refugia (H1 and 4). The relationship between interaction strength and predator-prey body size ratio, which is calculated by taking the log of the ratio between individual crab dry mass and individual amphipod dry mass (Table 3.1; nine levels: 2.12, 2.77, 3.58, 4.23, 4.47, 5.04, 5.69, 5.93, 7.40), was compared in the presence and absence of refugia (two levels: three rocks and no rocks). Each mesocosm contained 40 amphipods of the small, medium or large size category (three levels) as well as the presence or absence of refugia as required (two levels). A single crab of small, medium or large body size was added to each mesocosm and the system was left overnight for 18 hours. After this time crabs were removed from the mesocosms and the density of surviving amphipods was assessed to determine the interaction strength according to Equation 1. Interaction strengths were analysed as a two-factor PERMANOVA based on a Euclidean distances similarity matrix (n=3, N=54, max permutations=9999) done in the PERMANOVA+ add-in for PRIMER6 (Primer-E, Plymouth). A no-crab control was used to calculate interaction strength. To increase the power of terms higher up in the table, interaction terms with a *p* value greater than 0.25 were pooled with the residual (Underwood 1997). Permutational pairwise tests were done to determine differences between the levels of the factor body size ratio.

3.2.3 Experiment Two: Effect of Refuge Extent and Predator Body Size on Predator-Prey Interaction Strengths

Additional trials were done to determine whether the effect of refuge is stronger in the presence of more extensive shelter (H4 and 5) and whether this varies depending on the body size of the predator (H1 and 9). Refuge extent (four levels: three, five and seven rocks and a no rock control) was compared orthogonally with *Carcinus maenas* body size (four levels: small, medium, large and a no-crab control). Each mesocosm was furnished with five small, ten medium and five large amphipods, one crab of relevant body size and the required number of rocks designated to each treatment. After 21 hours the mesocosms were assessed for the total density of amphipods. Rates of amphipod attrition in the absence of crabs were again used to calculate interaction strengths (Equation 1) and compared in a Bray-Curtis similarity matrix analysed as a two-way fixed factor multivariate PERMANOVA (n=3, N=36, max permutations=9999) as above, which tested the null hypothesis that refuge size has no effect on interaction strength, irrespective of the size of the predator (H4). Pairwise tests were done as above to determine, for each sized predator, what extent of refuge is required to elicit a reduction in interaction strength.

3.2.4 Experiment Three: Role of Relative Predator-Prey Body Size and Refuge in the Determination of Functional Response Curve

The functional response of single crabs to varying densities and sizes of amphipods was tested. This tested the hypotheses that attack rates and handling times is influenced by prey density and the predator-prey size ratio (H2 and 3). It also tested the hypotheses that the presence of a refugia affects predator attack rates and prey handling times and that this phenomenon is mediated by prey density and predator-prey body size ratio (H6, 7, 8, 9 and 10). This experiment had three replicates in each level of three factors: (1) body size ratio using log ratios calculated as above, but using only the medium and large crab size categories (Table 3.1; 6 levels: 3.58, 4.23, 5.04, 5.69, 5.93, 7.4); (2) extent of refuge (two levels: present and absent); and (3) density of amphipods (seven levels: 2, 4, 6, 8, 12, 16 and 32). Factors were compared orthogonally (n=3, N=252, max permutations=9999). Five rocks were randomly selected to create refugia. A no-crab control was used to determine amphipod attrition in the absence of the predator. Given the virtual lack of amphipod attrition in the controls, the remaining number of amphipods in each mesocosm was assumed to represent the number not consumed and used to calculate rates of predation, which was then standardized to the nearest whole number of amphipods consumed per 12 hour block. As above, PERMANOVA was used to test the null hypothesis that consumption rates of amphipods irrespective of size do not vary between crab size, amphipod size, refuge presence and amphipod density. As the data were univariate, they were compared using a resemblance matrix based on Euclidean distances. To account for heterogeneous variances resulting from uneven sample sizes, the model was fitted using a more conservative Type I sum of squares. Interaction terms with a p value greater than 0.25 were again pooled with the residual. Logistic regression was used to determine the shape of the relationship between proportions of prey consumed and prey density for each dataset. If the proportion of prey consumed decreases with increasing prey density, then the logistic regression yields a significant negative first order term, and the functional response is appropriately described by a Type II model (Juliano 2001). Conversely, if the proportion of prey consumed first increases then decreases, the logistic regression yields a significant positive first order term followed by a significant negative second order term, thus describing a Type III functional response curve. Where logistic regression models lacked significant negative and positive first order terms in the correct direction, the appropriate functional response type was determined by fitting both Type II and Type III models to the data and selection of the curve with the lowest Akaike Information Criterion (AIC) values (Akaike 1974). Those data describing a Type II curve (no data set described a Type III curve) were subsequently modelled using maximum likelihood estimation (MLE; Bolker 2010) with the random predator equation, which assumes a Type II shape and accounts for the non-replacement of prey as they are consumed (Equation 2):

$$N_e = N_0(1^{-a(N_{eh}-T)})$$

where N_e is the number of prey eaten, N_0 is the initial density of prey minus the attrition/escape rates as measured in the controls, a is the attack rate (encounters per unit of food density), *h* is the handling time (average time spent handling an item of food) and *T* is the total time available (Rogers 1972).

Raw consumption data were non-parametrically bootstrapped (n=2000), then equation (1) was applied to each dataset to construct 95 % confidence intervals around the mean functional response curve of each predator treatment. MLE starting values of *a* and *h* for each bootstrapped dataset were the same as those derived from the original data for each predator treatment. Derived values for *a* and *h* were used to test hypotheses that predator-prey body size ratio and the presence/absence of refugia affects attack rates and handling times.

3.2.5 A Pilot Study on Interaction Strengths between an Amphipod Assemblage and 9 Species of Intertidal Algae

To test H11, consumer-producer relationships between an amphipod assemblage and 9 species of intertidal algae (*Ascophyllum nodosum*, *Cladophora rupestris*, *Corallina* spp., *Fucus serratus*, *F. vesiculosus*, *Laminaria digitata*, *Mastocarpus stellatus*, *Saccharina latissima* and *Ulva lactuca*) were measured in microcosm experiments. Algae were spun to remove excess water before 0.5 gram wet weight of one species was added to separate microcosms containing 150mL of seawater and five amphipods (one small, three medium and one large). Similar microcosms without amphipods were used as controls. After four days the dry weight was recorded. Change in biomass was determined using the equations in Table 2 to calculate initial dry weight. These data were used to calculate interaction strengths using Equation 1. To test the null hypothesis of no differences between levels, interaction strengths were analyzed as a single fixed factor PERMANOVA with unrestricted permutation of the raw data (n=4-5, N=44) followed by pair-wise tests also done using PERMANOVA based on a Euclidean distance resemblance matrix.

3.3 RESULTS

3.3.1 Experiment One: Influence of Relative Body Size, nut no Effect of the Presence of Refugia on Predator-Prey Interaction Strengths

Interaction strength increased with increasing predator/prey body size ratio until reaching a plateau at a ratio of 4.47 with a small peak at ratio 5.93 irrespective of the absence or presence of a refuge (Figure 3.1; Table 3.2). These findings support the hypothesis that lower interaction strengths are observed between larger *C. maenas* and smaller amphipods and vice versa leading to a hump-shaped relationship between body size ratio and interaction strength, but reject the hypothesis that the removal of rock refugia will lead to stronger interaction strengths between *C. maenas* and amphipods.

3.3.2 Experiment Two: Effect of Refuge Extent and Predator Body Size on Predator-Prey Interaction Strengths

Interaction strengths between large crabs and amphipod prey was 20% greater ($\bar{x}$=1.73±0.09SE) than between those between their small and medium conspecifics ($\bar{x}$=1.44±0.10 and $\bar{x}$=1.44±0.08), whose effect was not significantly different from each other (large crabs > medium crabs = small crabs). Interaction strengths in those mesocosms without any refuge at all ($\bar{x}$=1.71±0.12SE) were 30% greater than in those with a refuge comprised of seven pebbles ($\bar{x}$=1.31±0.09SE). No differences were observed among mesocosms containing refuges comprised of three or five pebbles (no refuge=3=5 > 3=5=7). These results were supported by significant main effects of predator size and refuge extent on the interaction strength between variously-sized *Carcinus maenas* and an assemblage of amphipods (Table 3.3). These data provide further support for the hypothesis of a hump-shaped relationship between body size ratio and interaction strength. They also provide support for the hypothesis that stronger interaction strength are observed in the absence of refugia, contradicting the patterns seen in the first experiment (Table 3.2). Hypothesis 5 is retained as interaction strengths between *C. maenas* and amphipods will gradually weaken as rock refugia are added to the arena; however, as there is no interaction between crab size and refuge extent, H9 is rejected.

Table 3.2: 2-way univariate PERMANOVA that tests the null hypothesis of no difference in interaction strength between *C. maenas* and amphipod prey for (1) body size ratio, which is a fixed factor with nine levels of increasing body size ratio; and (2) refuge presence, which is also a fixed factor with two levels (refuge presence and refuge absence). Euclidean distances were used to create the resemblance matrix. Interactions terms with a *p* value >0.25 were pooled with the residual and are marked as such (Underwood 1997). One degree of freedom was pooled with the residual. Significant terms are highlighted bold.

Source	df	MS	F	*P*
Body size ratio (bsr)	8	1.39	3.81	**<0.001**
Refuge presence (rp)	1	0.77	2.10	0.16
bsr x rp	1	POOLED		
Pooled Residual	44	16.03		
Total	53			

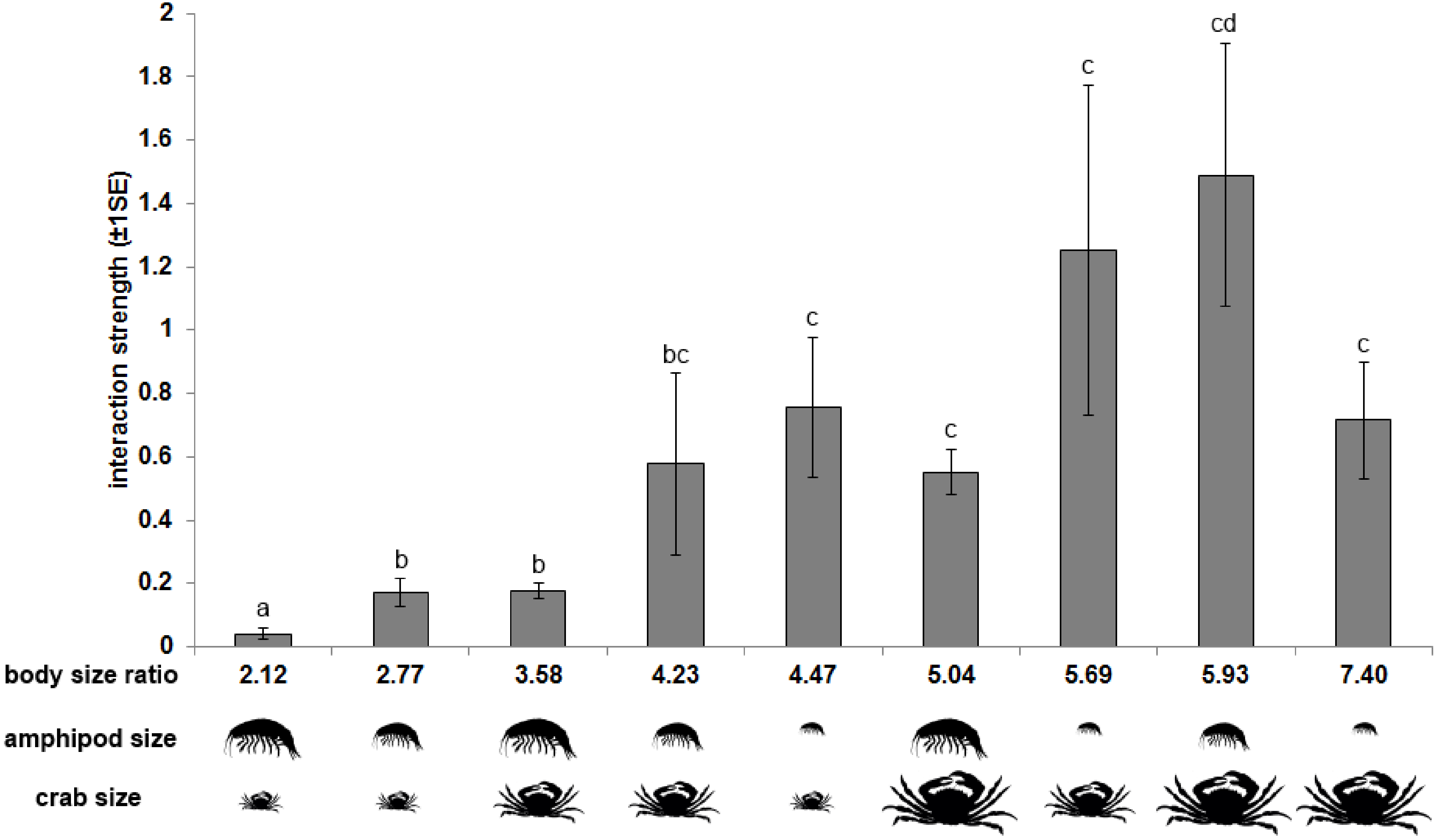


Figure 3.1: Interaction strength between amphipods and the crab *Carcinus maenas* as a function of relative body size. Silhouettes denote the relative sizes (small, medium and large) within predator and prey species and are not to scale. Letters are used to group treatments that were not dissimilar according to pairwise tests. Interaction strength was a function of body size ratio exhibiting a hyperbolic relationship with a small peak at ratio 5.93 thereby supporting the hypothesis of lower interaction strengths between larger *C. maenas* and smaller amphipods and vice versa leading to a hump-shaped relationship between body size ratio and interaction strength. There was no effect of refuge presence, rejecting H4.

Table 3.3: A 2-way multivariate PERMANOVA was used to test the null hypothesis of no difference in interaction strength between *C. maenas* and an assemblage of prey containing amphipods of three size categories (1) crab size, which is a fixed factor with three levels of increasing crab size (small, medium and large); and (2) refuge extent, which is also a fixed factor with four levels (refuge absence and refuges comprised of 3, 5 and 7 pebbles size rocks (Wentworth 1922). Data were log(x) transformed and Bray Curtis similarities were used to create the resemblance matrix. Significant terms are highlighted bold.

Source	df	MS	Pseudo-*F*	*p*
Crab size (cs)	2	0.15	4.08	**0.03**
Refuge extent (re)	3	0.11	3.07	**0.04**
cs x re	6	0.05	1.47	0.22
Residual	24	0.04		
Total	35			

3.3.3 Experiment Three: Relative Body Sizes of Species, Availability of Refugia and Prey Density all play a role in producing Type II Functional Response Curves.

All functional responses were deemed to be Type II (Table 3.4). Only two curves were ambiguous: medium crabs and small amphipods in the presence of a refuge (Figure 3.2D) and large crabs and small amphipods in the absence of a refuge (Figure 3.2F). This finding rejects the model that refugia are most effective at low prey densities. While the type of the functional response is not affected, magnitude and fine-scale shape of curves is mediated by two strong effects (Table 3.5): small amphipods are better than larger amphipods at finding refuge from crabs (Figure 3.2DF vs. ABCE) and the phenomenon that crabs consume more amphipods at higher prey densities (Figure 3.2). This result supports the hypothesis that relative predator-prey body size interacts with refuge presence and absence to alter the relationship between *C. maenas* and their amphipod prey, but not the hypothesis that relative predator-prey body size interacts with refuge presence and absence and prey density to alter the relationship between *C. maenas* and its amphipod prey

Variability in interaction strength was greater in those treatments with a higher density of amphipods (F: $23.90_{(6,248)}$, $p < 0.001$), though there were no differences in dispersion for body size ratio (F: $3.01_{(5,249)}$, $p = 0.10$) and refuge presence (F: $4.35_{(1,253)}$, $p = 0.09$).

With regard to attack rates and handling time, it is clear that the absence of a refuge alters the size structuring of predator-prey relationships (Figure 3.3). In the presence of a refuge, medium crabs direct their focus away from small amphipods and large crabs exhibit reduced attack rates for both medium and small amphipods, while in the absence of refugia, medium crabs are free to attack all sizes of amphipod equally and large crabs are now feeding on more medium-sized amphipods (Figure 3.3A). Where there was no refuge, relative body size of predator and prey has no effect on handling times (Figure 3.3B). Conversely, the presence of refugia induces a U-shaped relationship between increasing body size ratio and handling time in these taxa (Figure 3.3B). These findings support H3, 7 and 9, that the relationship between body size ratio and handling time is U-shaped, attack rates are increased in the absence of refugia and the relative body size of predators and prey will determine the effectiveness of a refuge respectively. These data reject H2 and 8, that attack rates are influenced by predator prey body size ratio and that refugia have no effect on the time spent handling prey.

3.3.4 A Pilot on Study Interaction Strengths between an Amphipod Assemblage and 9 Species of Intertidal Algae

Interaction strength with amphipods varies across algal species (Table 3.6). Pairwise comparisons revealed that some species are strongly-negatively affected by amphipods, while others are not affected at all (Figure 3.4). *Fucus vesiculosus* and *Ascophyllum nodosum* experienced the strongest negative effects, while other species such as *Laminaria digitata* and both species of red algae (*Mastocarpus* and *Corallina*) did not appear to be significantly different from zero (Figure 3.4).

Table 3.4: Model selection of functional response type using logistic regression and Akaike Information Criterion (AIC) values calculated from Rogers' and Hassell's equations. If the logistic regression yields a significant negative first order term, the functional response is described as a Type II model. Conversely, if the logistic regression yields a significant positive first order term followed by a significant negative second order term, it is describing a TYPE III functional response curve. Where logistic regressions are inconclusive, the functional response type is selected as the curve with the lowest AIC value.

treatment		**Logistic regression Type II FR**			**Logistic regression Type III FR**					**Rogers' Equation Type II FR**	**Hassell's Equation Type III FR**
body size ratio	**refuge**	**1st order**	***p***	**AIC**	**1st order**	***p***	**2nd order**	***p***	**AIC**	**AIC**	**AIC**
3.58	+	-0.07	<0.001	68.21	-0.11	0.16	0.00	0.64	69.99	NA	NA
3.58	-	-0.03	0.04	110.25	-0.05	0.54	0.00	0.78	112.18	NA	NA
4.23	+	-0.04	0.00	78.11	0.02	0.80	0.00	0.40	79.41	NA	NA
4.23	-	-0.08	<0.001	73.62	-0.02	0.75	0.00	0.47	75.10	NA	NA
5.04	+	-0.04	0.00	90.55	-0.22	0.01	0.00	0.02	86.91	NA	NA
5.04	-	-0.09	<0.001	98.45	0.11	0.13	-0.01	0.01	92.68	110.16	113.09
5.69	+	0.00	0.95	84.17	-0.23	0.01	0.01	0.02	79.84	84.17	no convergence
5.69	-	-0.02	0.05	132.79	0.02	0.74	0.00	0.52	134.36	NA	NA
5.93	+	-0.02	0.05	83.28	0.05	0.53	0.00	0.33	84.34	83.46	89.19
5.93	-	-0.08	<0.001	84.58	-0.02	0.76	0.00	0.45	86.01	NA	NA
7.40	+	-0.05	0.02	72.42	0.02	0.82	0.00	0.46	73.86	NA	NA
7.40	-	-0.02	0.11	75.17	0.06	0.39	0.00	0.25	75.83	75.35	80.84

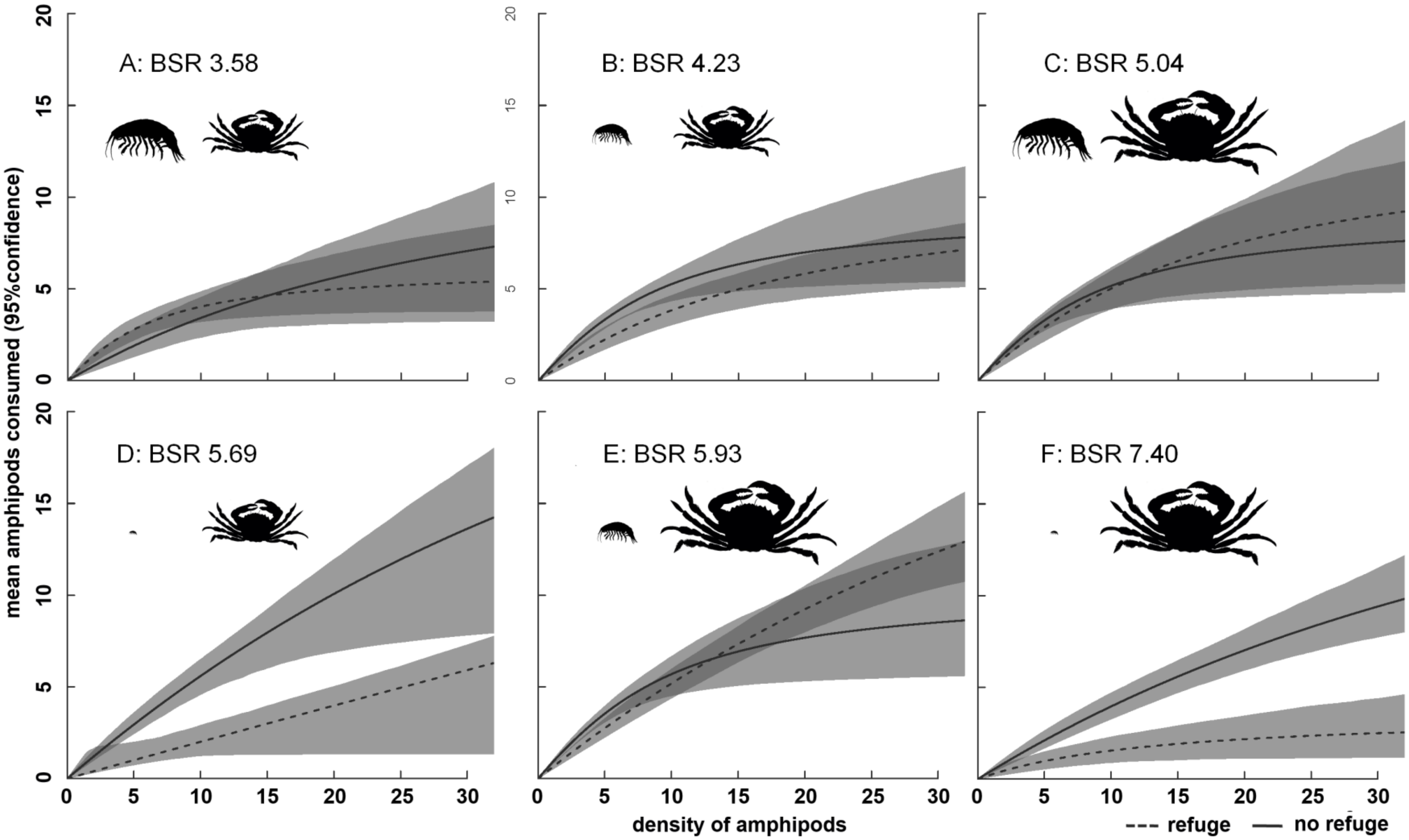


Figure 3.2: Twelve-hour functional response curves in response to increasing predator-prey body size ratio (panels A to F) and refuge availability. Silhouettes represent amphipod and crab size categories and are not to scale. These data support the model that the effectiveness of a refuge is mediated by the relative body size of predator/prey species, but reject the models that refugia are most effectives at low prey densities and that the effectiveness of a refuge is determined by the predator-prey body size ratio.

Table 3.5: 3-way univariate PERMANOVA that tests the null hypothesis of no difference in interaction strength between *C. maenas* and amphipod prey for (1) body size ratio, which is a fixed factor with six levels of increasing body size ratio; (2) refuge presence, which is also a fixed factor with two levels (refuge presence and refuge absence); and (3) density, which is a fixed factor comprised of seven levels of increasing prey density (2, 4, 6, 8, 12, 16, 32 and 64). Euclidean distances were used to create the resemblance matrix. Interactions terms with a *p* value >0.25 were pooled with the residual and are marked as such (Underwood 1997). A total of two degrees of freedom were pooled with the residual. Significant terms are highlighted bold.

	df	MS	F	*p*
Body size ratio (bsr)	5	32.18	3.75	**<0.001**
Refuge presence (rp)	1	120.13	17.69	**<0.001**
Density (d)	6	212.45	31.14	**<0.001**
bsr x rp	5	32.36	1.51	**<0.001**
bsr x d	1 POOLED			
rp x d	1 POOLED			
bsr x rp x d	30	9.46	2.08	0.11
Pooled Residual	207	6.91		
Total	254			

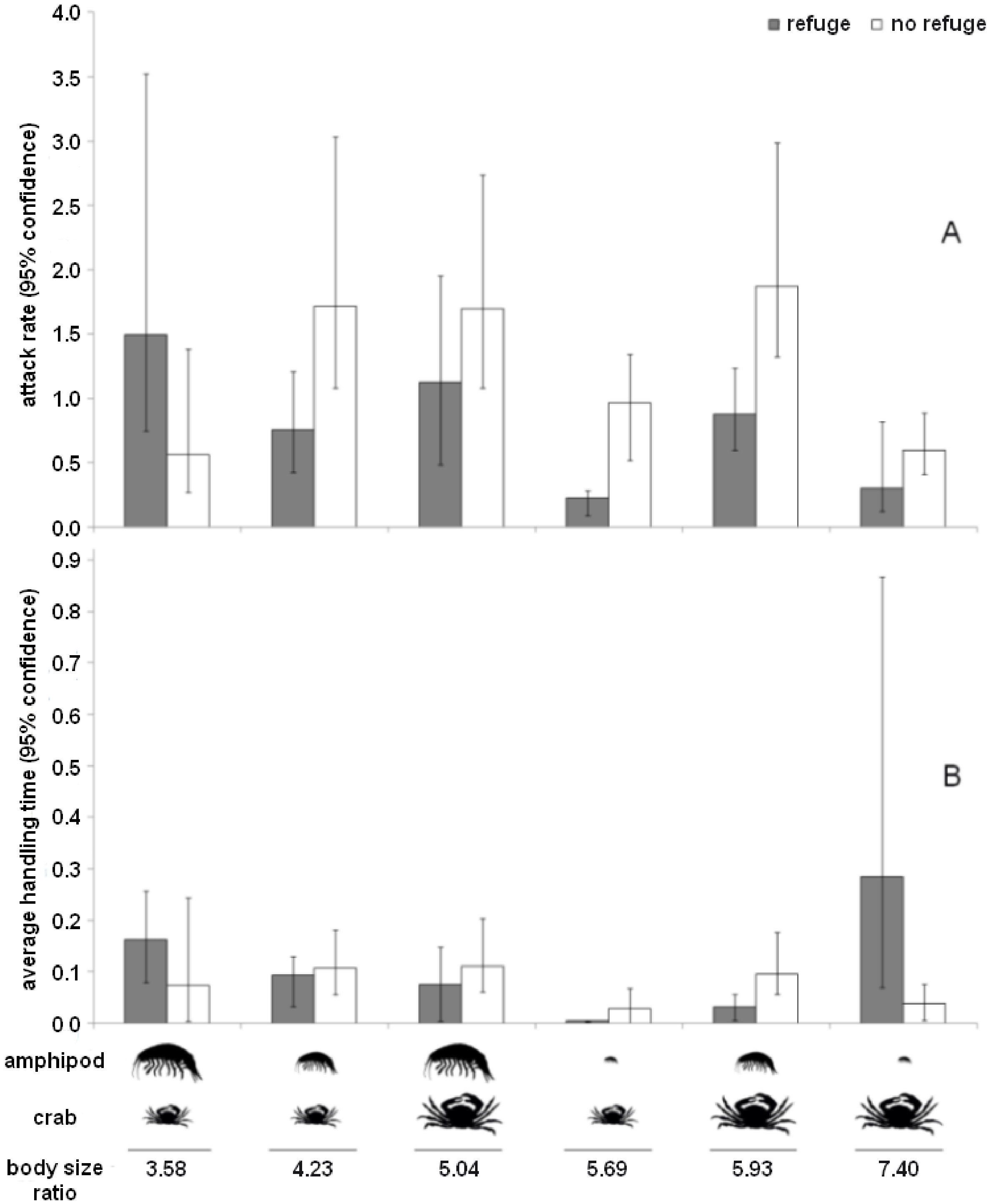


Figure 3.3: Bootstrapped estimates of (A) attack rate (encounters per unit of food density) and (B) handling time (average time spent handling an item of food) extracted from the Rogers II equation in the presence and absence of refugia. These results support H9, which predicted that relative body size will determine the effectiveness of a refuge and suggest an alternate hypothesis: that refuges and body size interact to increase size selectivity in predator-prey relationships.

Table 3.6: 1-way univariate PERMANOVA that tests the null hypothesis of no difference in interaction strength between an assemblage of amphipods and nine species of intertidal algae. Significant terms are highlighted bold.

Source	df	MS	F	*p*
Algae type	8	0.31	3.93	**0.002**
Residual	35	0.08		
Total	43			

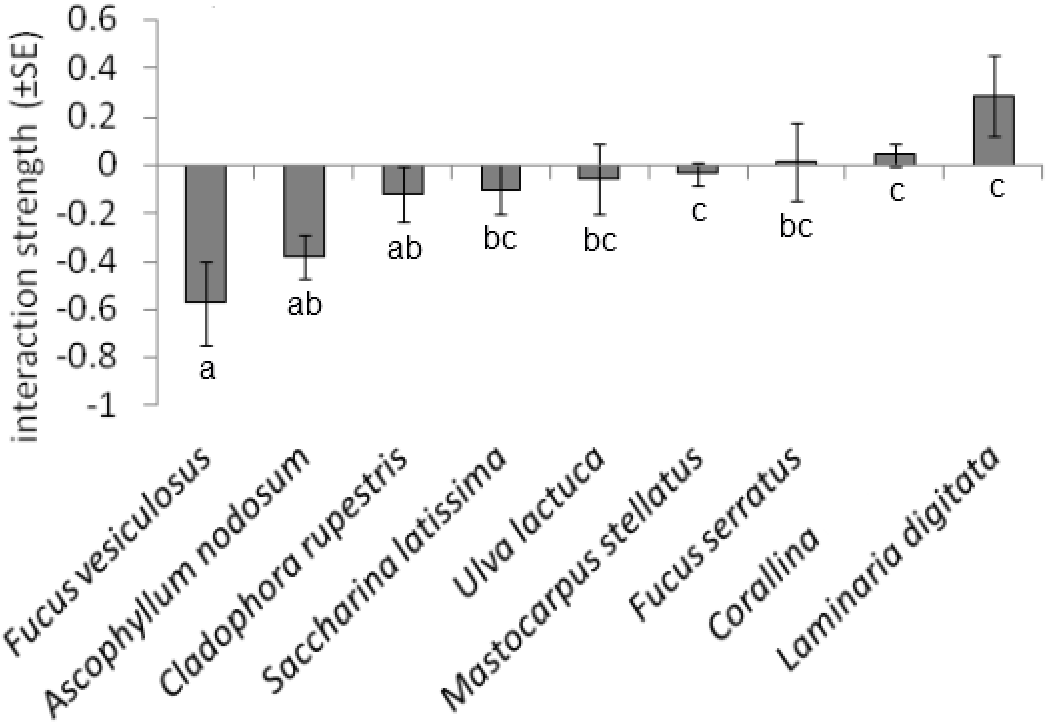


Figure 3.4: Interaction strength between amphipods and 9 species of algae. Interaction strength varied greatly between algal types with the strongest negative effect of amphipods observed in the brown alga *Fucus vesiculosus* ($F_{(8,35)}$: 3.93, $p < 0.001$). Negligible effects were observed in all species of red and green alga observed here. Letters are used to group treatments that were not dissimilar according to pairwise tests. The group on the top row represents those species whose biomass was more-strongly reduced in the presence of amphipods. The group on the middle row is made of those species that were negatively affected by the presence of amphipods but the response was either weak or highly variable. The group on the bottom row is comprised of those species that experienced a neutral or highly-variable positive effect.

3.4 DISCUSSION

Here the short-term predator-prey relationship between *C. maenas* and assemblages of its gammarid amphipod prey was characterised in three separate experiments. In summary, it was found: (1) that changing body size in this relationship alters predator-prey interaction strengths, attack rates and handling time supporting H1, 2 and 3 respectively; (2) refugia play a role in mediating crab-amphipod interaction strengths, attack rates and handling times, but not the shape of functional response curves respectively, supporting H4, 5 and 7, but rejecting H6 and 8; (3) the optimal crab-amphipod body size ratio was mediated by refugia presence, but not prey density, which supports H9, but rejects H10; and (4) herbivory by amphipods was strongest on *F. vesiculosus* and *A. nodosum* , supporting H11.

3.4.1 Changes in Body Size

By manipulating predator-prey body size ratio it was found that changes in the relative body size of *C. maenas* and its amphipod prey can alter interaction strengths, functional response curves and the access that amphipods have to the sanctuary of rock refugia. The hump-shaped relationship between body size ratio and interaction strength observed in the first experiment has been thoroughly characterised between coleopterans and arachnids (Brose 2008; Rall et al. 2010; Vucic-Pestic et al. 2010b), in freshwater plankton (Wahlström et al. 2000), in predatory asteroids (Twomey et al. 2012) and between *C. maenas* and edible mussels, *Mytilus edulis* (Elner and Hughes 1978)**.** These hump-shaped relationships are usually driven at one end by small prey being of too little energy content to motivate predation (Elner and Hughes 1978; Sih 1984; Petchey et al. 2008a) and by prey being too large to consume at the other (Elner and Hughes 1978; Rudolf and Armstrong 2008).

3.4.2 Presence/Absence of Refugia

These experiments recorded mixed results with regard to the effect of refugia on the predator-prey interactions between *C. maenas* and amphipods. In experiment one, a smaller refuge comprised of just three pebbles had no effect on the interaction. It was found that a refuge of at least seven pebbles (a two-fold increase in the availability of refugia) was the threshold required to evoke a predator-prey relationship that was different from the pebble-free control (Gotceitas and Colgan 1989). There was no clear evidence to suggest that the presence of refugia induced Type III functional response curves as expected (Murdoch and Oaten 1975; Crawley 1992; Vucic-Pestic et al. 2010a). There were, however, two curves that were neither Type II nor Type III, those associated with the smallest amphipods that produced the largest and third largest body size ratios, that may exhibit subtle Type III inflections which promote stability (Williams and Martinez 2004) . This suggests that prey size has a more deterministic role than refuge presence, predator size (Vucic-Pestic et al. 2010b) or the body size ratio between predators and prey as seen for various terrestrial arthropods (Kalinkat et al. 2013). While some simple experiments have found Type III functional response curves in single predator and single prey systems (for example, Vucic-Pestic et al. 2010a; Vucic-Pestic et al. 2010b), many do not (Kaiser 1983; Munyaneza and Obrycki 1997; Hoddle 2003) and the results presented here add to a growing body of evidence that suggests Type III functional responses curves are more commonly observed in environmentally- and ecologically-complex systems such as those found in nature (Murdoch et al. 1975; Sarnelle and Wilson 2008; Kalinkat et al. 2013).

3.4.3 Interactive Effects

A factorial analysis of the data from experiment three showed that smaller amphipods were better at utilising the rock refugia than their larger conspecifics. An interaction between body size and refuge was not been observed in experiments one or two. This contradiction in results between experiment three and experiments one and two may be because the three pebbles used in experiment one were not enough to constitute a refuge, as supposed for the lack of effect of a refuge found in experiments with leaf litter communities (Kalinkat et al. 2013). It may also be the interactive effect detected in experiment three was not driven by predator size, but rather by prey size, which can determine whether prey has access to a shelter or not (Lima and Dill 1990; Vucic-Pestic et al. 2010b). In this case, interactive effects of body size and refuge presence would not be detected in experiment two as it included only manipulations of crab size and not amphipod size. Finally, it might simply be that experiments one and two were not powerful enough to detect interactive effects that seem clear in illustrations of the data and, in experiment one, are obfuscated by variability associated with treatments that have larger crabs and smaller amphipods (Figure 3.1; Figure 3.2). In experiment three, an interaction between body size ratio and presence of refuge impacted attack rates. In the absence of refugia, the size structuring of the relationship between *C. maenas* and its prey is less strict, so that larger crabs are able to feed upon a wider range of prey sizes. As strong hump-shaped relationships between attack rates of predators and body size ratio are a commonly-observed result of changes in detectability, encounter rates and attack success over varying body size ratio (Hassell 1978; Real 1979; Mittelbach 1981; Brose et al. 2008; Vucic-Pestic et al. 2011), and access to refugia can decrease predation success, particularly for smaller prey (Rickers and Scheu 2005), it is unsurprising that removing refugia has led to a down-shift in the position of the optimal body size ratio, thereby allowing predators to apply greater top-down pressure on smaller prey. Similarly, the commonly-observed U-shaped relationship between body size ratio and handling time (Rall et al. 2012) was here flattened out by the absence of refugia, that is, size structuring with regard to handling times appears dependent on the presence of refugia. The lack of an effect of body size ratio in the absence of refugia potentially reflects the competing influence of various factors such as prey being too small to catch or too large to eat quickly (Hyatt 1979; Aljetlawi et al. 2004). Conversely, the U-shaped relationship observed in the presence of refugia might be due to the role refugia play in reducing encounter rates by providing sanctuaries at large body size ratios and obstacles at smaller body size ratios while increasing the greater escape efficiency recorded for relatively-smaller prey (Brose et al. 2008; Hauzy et al. 2010). These possibilities could be tested experimentally by measuring rates of predation and amphipod escape rates as a proportion of the number of encounters with crabs in response to (1) a covariate ratio of time spent hiding and motionless with time spent active; and (2) response to varied body size ratio. Extrapolating from the results of this small-scale experiment, the broad-scale simplification of habitats seen around the world (MA 2005) may have already led to greater predation on smaller individuals, in terms of interaction strengths and attack rates, and on both smaller and larger individuals in terms of handing time, which has long-term implications for the size structure of populations. A downward shift in the optimal body size ratio for predator-prey relationships (Hall et al. 1976) would translate to a size-based bottle-neck, whereby increased selection pressure is exerted on larger prey and leading to future populations comprised of smaller individuals, as already seen in over-exploited fish populations (Audzijonyte et al. 2013) and expected under future warming scenarios (Daufresne et al. 2009). Alternatively, reduced pressure on larger individuals relative to smaller individuals may lead to strong body-size-based refuges (Rudolf and Armstrong 2008) and populations with a larger average individual body size .

3.4.4 Density

While increased prey density led to unsurprisingly greater and more variable rates of predation, density did not interact with refuge presence to influence rates of predation by crabs on amphipods. This contradicts the Given that density is a factor of species abundance and the size and structure of the habitat available (Real 1977; Crawley 1992), it might be that the rock refugia used here did not approach their maximum capacity, that is, the density above which additional prey cannot find sanctuary (Scheffer and De Boer 1995). For the system used here, the threshold might be found at greater densities of amphipod. The interaction strengths observed here might be inflated due to the restricted mobility of the participants.

3.4.5 Future Work

Given the importance of intra-guild predation and competition in predator-prey interactions (Sih et al. 1998; Buckel and Stoner 2004; Griffin et al. 2008) and the sensitivity of the interaction strength index used here to predator abundance (Berlow 1999), these experiments would benefit from the inclusion of additional predators, be they conspecifics or predators from another species with an entirely different mode of feeding, particularly as *C. maenas* exerts stronger predation pressure at greater densities (Raffaelli et al. 1989). With regard to herbivory trials, it was found that brown algae with vesicles (*A. nodosum* and *F. vesiculosus*) suffer greater herbivory from the amphipod assemblage than do all other species of algae. I suggest that this is related to their being deposited higher on the strandline due to their vesicles and thus are more commonly associated with high densities of amphipod (Ingólfsson and Agnarsson 2003; Viejo and Åberg 2003). In this chapter the herbivory effects on *F. serratus* are not strong, yet in the second chapter of this thesis a strong trophic cascade of amphipod herbivory on this species was recorded (Figure 2.4) and an even stronger effect might have been found if *A. nodosum* or *F. vesiculosus* had been used in that experiment. It is proposed that future work is undertaken to unravel the dual role of these species as both resource and refugia for amphipods (Kalinkat et al. 2012). A test of the null hypothesis of no difference in interaction strength between *C. maenas* and amphipods in response to refugia presence, a factor with three levels: (1) no refuge, (2) presence of live *A. nodosum*/*F. vesiculosus*; (3) presence of a synthesised algal dummy made of rubber using plaster moulds of *A. nodosum* and/or *F. vesiculosus*, would determine the importance of these algal species as a refuge. A census of all species after a longer-term experiment done to test the null hypothesis of no difference in species abundance and biomass in response to the same factor with three levels would allow determination of the importance of these algal species as a resource. Some organisms alter their use of refugia depending on the presence and absence of food, as seen for urchins in kelp forests (Harrold and Reed 1985), and these experiments would further investigate that phenomenon. Furthermore, trophic cascades have been shown to be robust to changes in habitat complexity that disrupt predator prey interactions, with the authors suggesting that the strength of the cascade might be due to whether habitat complexity provides actual refuge for prey (Grabowski 2004). Experiments that examine the role of body size, and the presence/absence of food source refugia would also allow examination of the interactive effects of body size and refugia presence on the trophic cascade between crabs, amphipods and algae, thereby supporting or refuting the suspicion in Grabowski (2004).

Finally, as predator-prey interactions are strongly affected by the physical features of their domain (Schmitz 2007), it is important that these relationships are examined under various environmental contexts, particularly warming and pCO_2, both of which impact trophic interactions (Orr et al. 2005; Lang et al. 2012; Russell et al. 2013) and are likely to change markedly over coming decades (IPCC 2013). All of these observations can be tested relatively easily using the *C. maenas*/amphipod predator-prey system because of the baseline trophic information documented here.

3.4.6 Conclusion

Here it was observed that size-related changes in predator-prey relationships are driven primarily by prey size rather than predator size, that the presence of refugia increases size selectivity in predator-prey relationships, and that refugia and body size interact to create U-shaped predator-prey handling time relationships. These ecological observations of a model systemconfirm the intimate relationship between organism body size and the availability of refugia and suggest that habitat simplification should be added to warming, overexploitation and eutrophication (Beukema 1991; Gardner et al. 2011; Audzijonyte et al. 2013) as anthropogenic activities that might cause reductions in the average body size of species.

CHAPTER FOUR: ROLE OF HISTORICAL DISTURBANCE TO BIOFILMS IN THE DEVELOPMENT OF A FOULING ASSEMBLAGE

ABSTRACT

In the assembly of a marine community, succession begins with new surfaces being covered by a biofilms, which prepares the substrate for settlement by various invertebrate species. While the trajectory of this process is clearly affected by the degree and timing of disturbance, relatively little work has been done to understand the role of temporal contingencies in community assembly compared to the research done to understand the role of spatial factors in successional processes. The research described in this chapter was done to understand the relative roles of present and historical disturbance on the development of a benthic marine community using copper contamination as a typical anthropogenic disturbance. The experiments described herein were done using a delivery system of PVC settlement plates wearing collars coated in copper-based anti-fouling paint. This study found that (1) the amount of copper sequestered by marine biofilms depends on the degree and length of exposure to copper; (2) microphycological assemblages contained within marine biofilms alter their composition in response to changes in copper concentration in the surrounding water column, irrespective of whether the changes involve exposure to or depuration from copper, and not just exposure to elevated copper concentrations *per se*; (3) fouling invertebrate assemblages respond with altered species abundances to increased exposure to copper and (4) invertebrate assemblage development proceeds in a consistent manner irrespective of the copper exposure history of the biofilm. This suggests that benthic invertebrate settlement is robust to the indirect effect of copper-induced changes in biofilm communities, but not to the direct toxic effect of copper itself. This has positive implications for the capability of biofilms to sequester contaminants without compromising other

ecological functions such as preparing hard substrata for settlement by invertebrates. This work highlights the importance of accounting for a host of temporal contingencies including biofilm age and the coincidence of random events such as rainfall by designing experiments that feature proper replication on spatio-temporal axes. Without accounting for these temporal factors the capacity of researchers to interpret the effects of any given disturbance is extremely limited, particularly for understanding the mechanics of intrinsically temporally-contingent ecological processes such as community assembly.

4.1 INTRODUCTION

4.1.1 Community Assembly in Stochastic Systems

In all natural aquatic systems, community assembly invariably begins with new or disturbed surfaces being swiftly covered by a biological film of largely self-propagating, close-knit communities of bacteria, protists, viruses and microalgae living in an extracellular polymeric substance (EPS; Dayton 1971; MacLulich 1986; Murray et al. 1986; Battin et al. 1999; O'Toole et al. 2000; Larsen et al. 2001). Formation of the biofilm EPS greatly improves cell attachment (Costerton et al. 1978) and prepares the substrata for subsequent colonization by macroalgae and invertebrates (Meadows and Williams 1963; Wahl 1989; Thompson et al. 1998; Wieczorek and Todd 1998). As described earlier (chapter 1.3), temporal contingencies are events that are dependent on some condition, state or occurrence that has happened in the past or might happen in the future, potentially by chance or accident, and leading to one of numerous possible outcomes. The outcomes of community assembly are, among other things such as species arrival order, mediated by the timing and history of disturbance (Dayton 1971; Sousa 1979b; Sousa 1979a; Sutherland 1980; Underwood and Chapman 2006). Thus, the exact disturbance and ecological history of a community plays a role in assemblage observed at any given time, which might produce any number of potential climax communities or stable states (Gleason 1927; Lewontin 1969; Sutherland and Karlson 1977; Dean and Hurd 1980; Underwood et al. 1983; Palmer et al. 1997). Despite these dependencies, compared to the role of spatial factors, temporal contingencies such as the legacy effect of historical disturbance remain critically understudied in many aspects of ecology (Chase 2003; Brudvig 2011) with few exceptions (for example, Underwood and Chapman 2006; Coleman et al. 2007). To understand the role of disturbance history and the legacy effects such disturbances might have on subsequent communities and community assembly, researchers need to monitor the development of assemblages in nature in response to manipulation of a disturbance. This requires use of a consistently-effective disturbance vector by which interference to the study system is virtually guaranteed. Copper was chosen as the disturbance vector for the experiments contained within this chapter due to its toxicity at elevated concentrations (Bryan 1971; Hall et al. 1998; Dewez et al. 2005).

4.1.2 Disturbance of Marine Systems by Copper

Elevated concentrations of the metal copper is a common anthropogenic disturbance found in many aquatic environments around the world (e.g. Breault et al. 1996; Webb and Keough 2000). Sources of copper discharge include waste from agricultural, industrial, and mining activity, the corrosion of pipes, urban runoff and as a leachate from modern antifouling paints (Mance 1987; Pitt 1995; Apte and Day 1998; Stauber et al. 2000; Schiff et al. 2004; Warnken et al. 2004; Zhou et al. 2008). At trace amounts copper is an essential micronutrient for various molecular processes (Gledhill et al. 1997; Andrade et al. 2004) but at higher concentrations copper is often toxic and is thus widely-used as a biocide (Fernandes and Henriques 1991; Hall et al. 1998). The roles of timing and frequency of exposure to copper in assemblage structure has seen much consideration (for example, Johnston and Keough 2000; Johnston et al. 2003), however, there is less research concerned with the copper eco-toxicology of assembly rules, where the literature tends to focus on the individual compartments of succession such as early-colonising biofilms (Barranguet et al. 2000) or secondarily-arriving microalgae (Gustavson and Wängberg 1995) and falls short of capturing the response of each stage of the assembly process.

4.1.3 Effect of Copper on Biofilms

Biofilms exhibit reduced biomass and photosynthesis, and changes in algal and bacterial community structure in response to copper exposure (Barranguet et al. 2003; Massieux et al. 2004; Perales-Vela et al. 2007; Sun et al. 2013). As biofilms are mostly anionic in nature they have the capacity to bind metals such as copper from the water column (McLean et al. 1996; Huang et al. 2000). Thus, biofilms have been suggested for use as potential bio-remediation tools in the restoration of polluted environments (von Canstein et al. 1999; White and Gadd 2000). Metals are absorbed by the polysaccharide components of the EPS where they are complexed and neutralised away from metabolic processes (Christensen et al. 1985; Mittelman and Geesey 1985; Harrison et al. 2007). Furthermore, the microorganisms in biofilms produce more EPS in response to copper exposure (White and Gadd 2000; Sheng et al. 2005). This limits penetration and diffusion of toxicants (Liehr et al. 1994; Anderl et al. 2000; Barranguet et al. 2002), providing shelter for the healthy organisms that live close to the substratum and away from the exterior surface of the biofilm (Teitzel and Parsek 2003). The concentration to which copper can accumulate in biofilms is a function of the degree of the exposure (White and Gadd 2000; Barranguet et al. 2002). With exposure to lower levels of copper, concentrations of copper in the biofilm rise rapidly before reaching a plateau, the height of which is dependent on which species of bacteria are present (Bitton and Freihofer 1977; Kim et al. 1996; Massieux et al. 2004). Laboratory studies show that copper is retained by biofilms for up to four weeks after the original source of copper is removed and the system restored to natural concentrations (Boivin et al. 2006). This legacy of copper, hereafter referred to as ‘copper legacy’, retained with the biofilm matrix may explain persistent copper-induced changes to resource use and metabolic profile of microbial communities that can still be observed after copper exposure has ceased (Griffiths et al. 2000; Boivin et al. 2006), though retention of copper does not explain extended changes to the fatty acid profiles of other microbial biofilm communities (Kiikkilä et al. 2001). It is currently unknown whether a legacy of bound copper ions or biofilm community historically altered by copper compromises the functional role of biofilms in preparing substrata for settlement by sessile benthic invertebrates (Meadows and Williams 1963; Geesey and Jang 1989).

4.1.4 Effect of Copper on Settling Marine Invertebrates

Differential tolerances to copper exposure can lead to increased abundances of some taxa at the expense of others (Gustavson et al. 1999; Jelic-Mrcelic et al. 2006). Many studies examined the responses of marine invertebrate assemblages to copper exposure have sampled from more mature communities finding that exposure of settling macro-invertebrates to copper has negative effects on ascidians and barnacles, leading to competitive advantages for some bryozoan and serpulid species (Johnston and Keough 2000; Johnston and Webb 2000; Johnston and Keough 2002; Johnston and Keough 2005; Vinot and Pihan 2005). Differential effects of elevated copper on settling fauna are also apparent at lower taxonomic resolutions, such as between families and species of bryozoans, often dependent on their status as native or invasive species that may have arrived into an ecosystem via ship hulls coated with antifouling paint (Floerl et al. 2004; Piola and Johnston 2006a; Piola and Johnston 2006b; Piola and Johnston 2008; Piola et al. 2009). While there is a wealth of data on each component of the assembly process, it technically remains unknown which, if not all, stages of benthic marine succession leading to these mature assemblages are the most sensitive to copper toxicity and thus most responsible for driving the observed results.

4.1.5 Chapter Aims

While it is well-established that the structure and composition of complex biofilms is sensitive to copper disturbance (Massieux et al. 2004; Sun et al. 2013) and that macro-invertebrate settlement is mediated by the biological and chemical characteristics of biofilm substrata (Henschel and Cook 1990; Keough and Raimondi 1995; Keough and Raimondi 1996; Wieczorek and Todd 1998), it is unknown whether the observed ecological effect of copper on benthic marine invertebrates (Johnston and Keough 2000; Johnston and Webb 2000; Johnston and Keough 2002; Johnston and Keough 2005; Piola and Johnston 2006a; Piola and Johnston 2006b) is due solely to a direct effect on the physiology of the invertebrates themselves, or as an indirect effect delivered as a copper legacy through the biofilm or a combination of the two.

Using the logical framework proposed by Underwood (1990), these experiments address the following models (M) by testing their associated hypotheses (H). Note that for these models and hypotheses ‘exposure to copper’ refers to delivery of a low enrichment that is much higher than background concentrations (Birch and Taylor 1999), but well below maximum concentrations given in Australian water quality guidelines (ANZECC/ARMCANZ 2000).

M1) exposure to levels of copper retards growth of biofilms

    H1) if developing biofilms are exposed to copper contamination they will produce less total biofilm mass than those biofilms not exposed to elevated concentrations of copper

M2) exposure to copper induces the production of organic material by the biofilm

- H2) biofilms exposed to copper will be comprised of a greater proportion of organic (EPS) material than those biofilms not exposed to elevated copper concentrations

M3) copper in the surrounding water column is absorbed by biofilms

- H3) copper exposure will increase total copper amount and concentration in the biofilms compared to those biofilms not exposed to copper

M4) sequestered copper is retained by biofilms for long enough to affect settling invertebrate settlement after the surrounding water column is restored to a state of lower copper concentration

- H4) for nine days after the source of copper has been removed concentrations of copper in biofilms will remain as high as those biofilms still exposed to copper

M5) algal communities are altered by exposure to elevated levels of copper

- H5) exposure to copper will lead to changes in the relative amounts of photosynthetic pigments and associated degraded pigments (phaeopigments) compared to those algal communities not exposed to elevated levels of copper

M6) copper interferes with the photosynthetic apparatus of the phototrophs within biofilms

- H6) biofilms exposed to elevated amounts of copper will exhibit reduced photosynthetic efficiency of Photosystem II compared to those biofilms not exposed to elevated concentrations of copper

M7) settling invertebrate communities are sensitive to both direct exposure to copper and changes in biofilm substrata as induced by previous exposure to copper

- H7) exposure of developing benthic communities to varying time series of copper contamination will influence the species abundances of subsequent macro-invertebrate assemblages.

To address these hypotheses, two experiments unravelling the way copper disturbance impacts the consecutive roles of biofilms and settling invertebrates in the development of benthic marine communities are done to explore the concept of a copper legacy impacting the structure, biology and chemistry of natural biofilms after depuration and how this affects the successional responses of settling macro-invertebrate.

4.2 METHODS

4.2.1 Study Site

Experiments were done over sub-tidal sandy sediments in Spring Cove, Manly, which lies in a sheltered bay near the entrance to Sydney Harbour, NSW, Australia (33.808°S, 151.291°E; Figure 4.1). Spring Cove is largely surrounded by national park and is only sparsely used as anchorage. Almost all (86%) of the catchment of Sydney Harbour has been urbanised (Birch et al. 1996; Birch et al. 1999). While concentrations of pollutants in estuaries such as Sydney Harbour have declined due to dredging, reductions in industry and more stringent regulation of discharges and effluents, elevated loads of metals such copper are still able to enter estuaries via storm water runoff containing road-dust and historically-contaminated soil and river sediments (Scollens 1998; Mackie et al. 2007; Birch and McCready 2009; Birch et al. 2013). Near the mouth of the estuary metal concentrations are generally low and well below values that would cause adverse biological effects (Birch and Taylor 1999), making it an ideal location for experimental manipulation of copper concentration. The use of other antifouling paints such as tributyltin has been phased out since January 1st (IMO 2003).

4.2.2 Artificial Units of Habitat

These experiments were carried out using artificial units of habitat (AUH), in this case, settlement plates (Johnston and Keough 2000; Johnston and Keough 2002; Mayer-Pinto et al. 2011). These AUH are the most time-effective method to provide new surfaces, which are essential for examining the early successional ecology of biofilms (Bender et al. 1984; Gee and Warwick 1996; Sabater et al. 2007). Uniform in size, complexity and history (Gee and Warwick 1996), AUH ensure that colonists in different sampling units are affected by treatment rather than nuances in their small-scale environment (Chapman et al. 2008), although some artificial structures favour certain types of taxa, as seen in their benefit to non-indigenous species (Dafforn et al. 2012), and this bias must be considered when interpreting results. Each AUH consisted of a PVC backing plate (140x140x3mm), a round, coarsely-sanded PVC settlement plate (radius 50mm, 1 mm thick) and a close-fitting PVC collar (140x140x1mm) all held together using stainless-steel flathead bolts and cable ties (Figure 4.2). The use of interchangeable collars allowed for manipulation to proceed with minimal disturbance to the experimental subjects. In all experiments, the underside of the settlement plate, i.e. the side facing the substratum, was used as the primary experimental surface. Each AUH was maintained at a constant orientation, distance and position relative to the sediment by being affixed to the top of a pointed, plastic tomato stake (900 x 23 x 23 mm, Plastic Recyclers, Australia) which was pushed approximately 600mm into the sediment. Each AUH was placed no closer than 420mm from any other unit, a distance that is three times the width of a single unit.

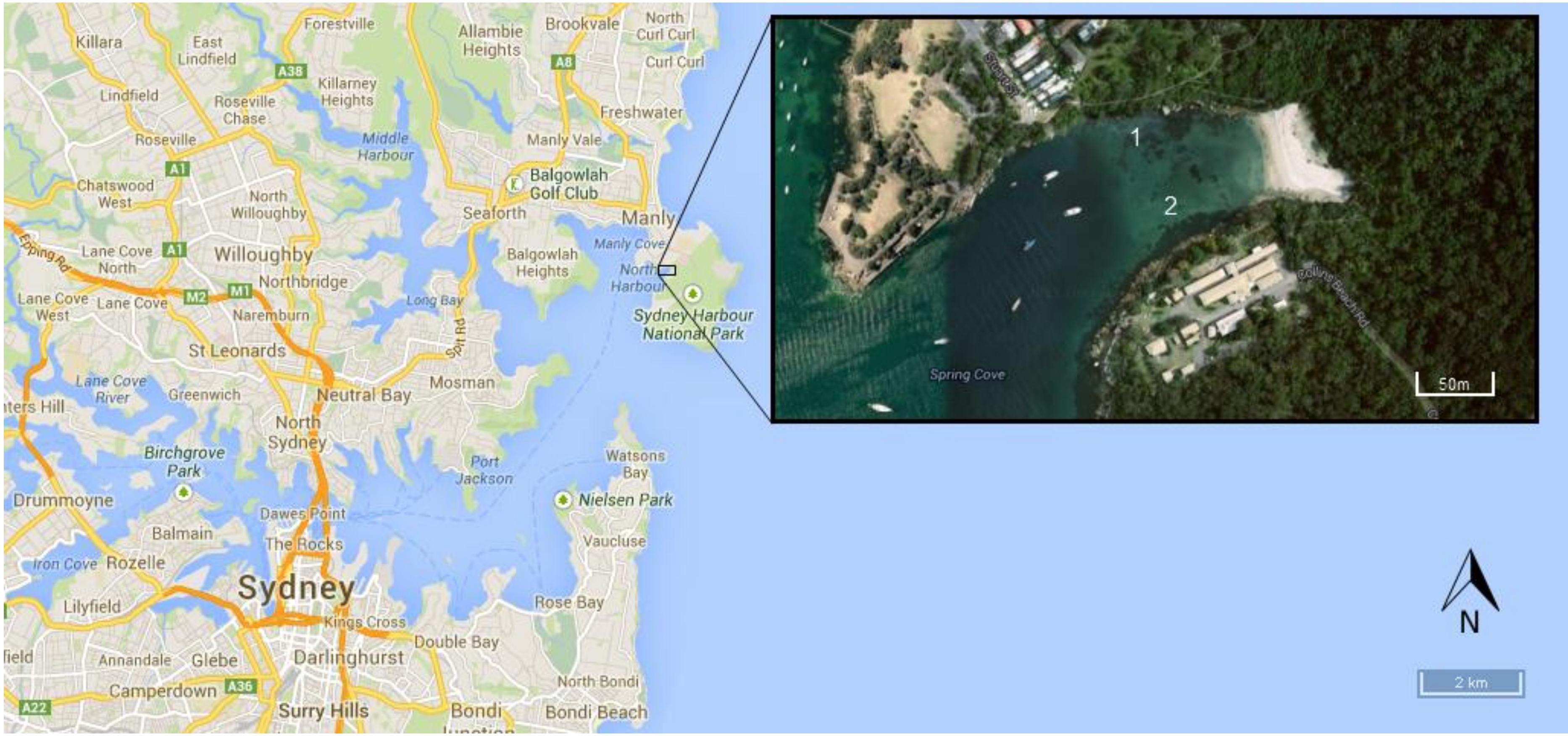


Figure 4.1: Location of field site in northern Sydney Harbour (33.808°S, 151.291°E) used for both experiments in this chapter. The insert focuses on Collins Beach in Spring Cove, and illustrates the relative positions of sites one and two.

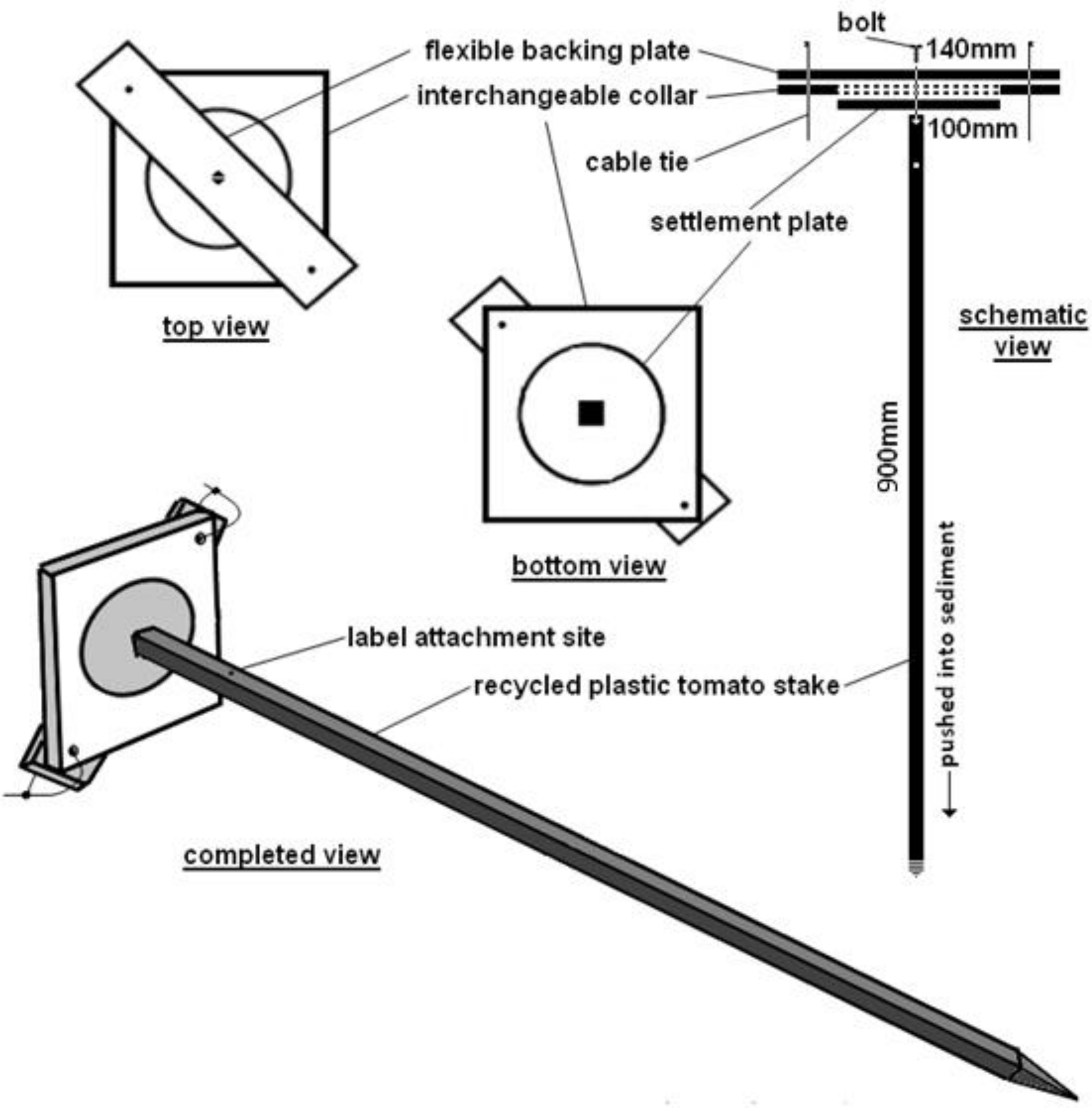


Figure 4.2: Artificial units of habitat used to create uniform substrata for sampling.

4.2.3 Copper Delivery Protocol

The AUH collars were used to deliver copper treatments to settlement plates. This was done by painting interchangeable PVC collars with two coats of non-toxic primer-undercoat followed by four coats of anti-fouling paint (Norglass Laboratories Pty Ltd 'Shipshape Primer' and 'Topflight Anti Fouling Paint' Punchbowl, Australia). Topflight antifouling paint functions by leaching toxins constantly. Control collars were painted with just two coats of the primer-undercoat. While this method has been used repeatedly in the literature (for example, Canning-Clode et al. 2011), its efficacy in delivering a local dose of copper using a different brand of anti-fouling paint to nearby settlement plates was tested here. Fifty-six collars painted with just primer or primer then two coats of anti-fouling paint were randomly suspended from separate tomato stakes that were deployed sub-tidally at site two in Spring Cove (Figure 4.1). These collars were left in the field for two, four, six or eight weeks, after which they were removed from the harbour and taken to the laboratory for analysis. Collars were placed individually for two hours in two litres of unfiltered seawater that had been collected that day from a boat ramp at Little Manly, a small embayment west of Collins Beach. Unfiltered seawater was used to examine temporal variability in background copper concentration. Water samples were kept refrigerated and 10mL samples were acidified with a drop of ultrapure HNO_3 prior to analysis for copper concentration using an inductively-coupled plasma optical emission spectrometer (ICP-OES, Varian, USA). The null hypothesis of no difference in copper concentration over time was tested using a univariate PERMANOVA with two fixed factors: copper (copper and no copper) and weeks from deployment (0, 2, 4, 6, and 8).

4.2.4 General Spectrometry Methods

All settlement plates sampled from both experiments were analysed using a field spectrometer. While detail particular to each experiment is provided below, a general method is presented here to reduce repetition. Field spectrometers such as the one used here (Analytical Spectral Devices, Boulder, Colorado, USA) take measurements of the visible and near-visible wavelengths (400-750nm) and are capable of recording large amounts of data over very short time scales (Paerl et al. 2003). These data are at great spectral resolution and absorption at specific wavelengths can be used to determine the relative abundance of various photosynthetic pigments in the surface layer of the biofilm (Figure 4.3; Bidigare et al. 1989; Murphy et al. 2005a; Murphy et al. 2005c), including the important biliproteins, phycoerythrin and phycocyanin, which, due to their water solubility (Glazer 1994), cannot be fingerprinted using solvent-based methods such as High Performance Liquid Chromatography (HPLC). Reflectance data were optimized and calibrated against a white reference (~99% reflective Spectralon® panel, Labsphere, USA) every two minutes during use. Four readings were taken, one from each quadrant of each settlement plate. These were used to derive an average spectrum per plate. To further reduce noise and emphasize pigment absorption, reflectance data were filtered using a 26nm smoothing interval (Savitzky and Golay 1964; Steinier et al. 1972; Murphy et al. 2011). To account for changes in profile y-intercept associated with analysing microbenthos after multiple calibrations of the spectrometer, smoothed spectra were normalised by dividing all values by the value at 750 nm (Kromkamp et al. 2006). Second order derivatives were also calculated from smoothed, un-normalised data to reduce background effects (Murray et al. 1981; Demetriades-Shah et al. 1990; Murphy et al. 2005a) and enhance subtle changes associated with overlapping pigments (Murphy et al. 2005b; Kuhl and Polerecky 2008). These data were then normalised to chlorophyll-a by dividing all values by the maximum value in the spectra (approximately

674 nm), which represents this pigment (Jackson et al. 2010). The pigments were then used as a proxy for assemblage composition (Table 4.1; Underwood 1984; Stauber and Jeffrey 1988; Millie et al. 1993; Brotas et al. 1995; Kelly et al. 2001; Millie et al. 2002).

4.2.5 General Statistical Methods

All data were analysed using PRIMER 6 and PERMANOVA+ software. Unless stated otherwise, analyses were done with a maximum of 9999 permutations and Euclidean distances were used to calculate all resemblance matrices. These were chosen as these data sets generally lacked zeroes and were in the same unit of measurement. Shepard diagrams (PRIMER 6) were used to visually determine the extent of distortion stress in all resemblance matrices created from the dependent data. To account for the increased lack of independence associated with uneven sample sizes, data were fitted using a TYPE I Sum of Squares (Anderson 2001a; Hector et al. 2010). The order of the fit was permutated to ensure that this did not affect results (Hector et al. 2010); however, this was ultimately proven unnecessary as results were consistently significant across all model variants. Alpha (type I error) was set at $p = 0.05$. Interaction terms with a p value equal to or greater than 0.25 were pooled with the residual in a stepwise fashion. This greatly reduces the rate of Type II errors (Underwood, 1997). Monte Carlo p values were used where there were too few unique permutations possible to calculate permutational probability values.

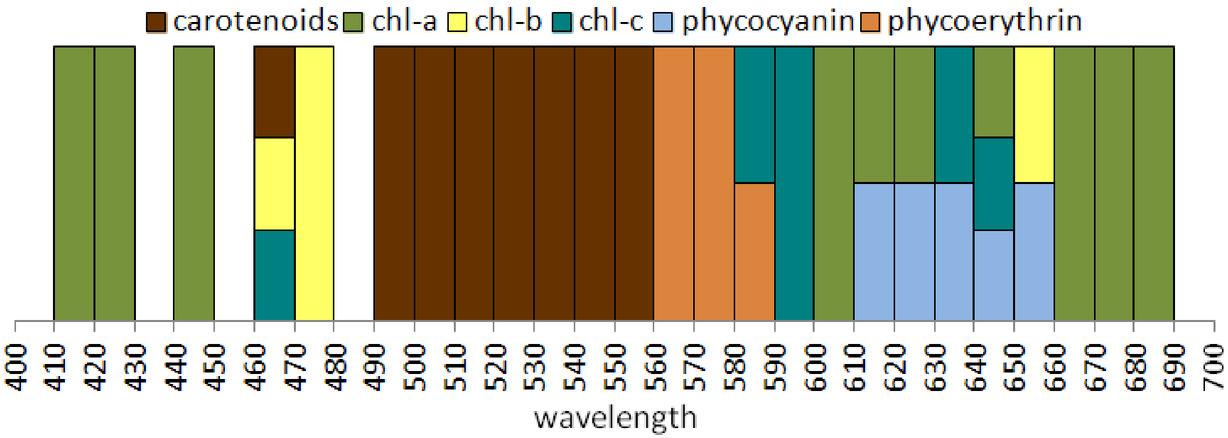
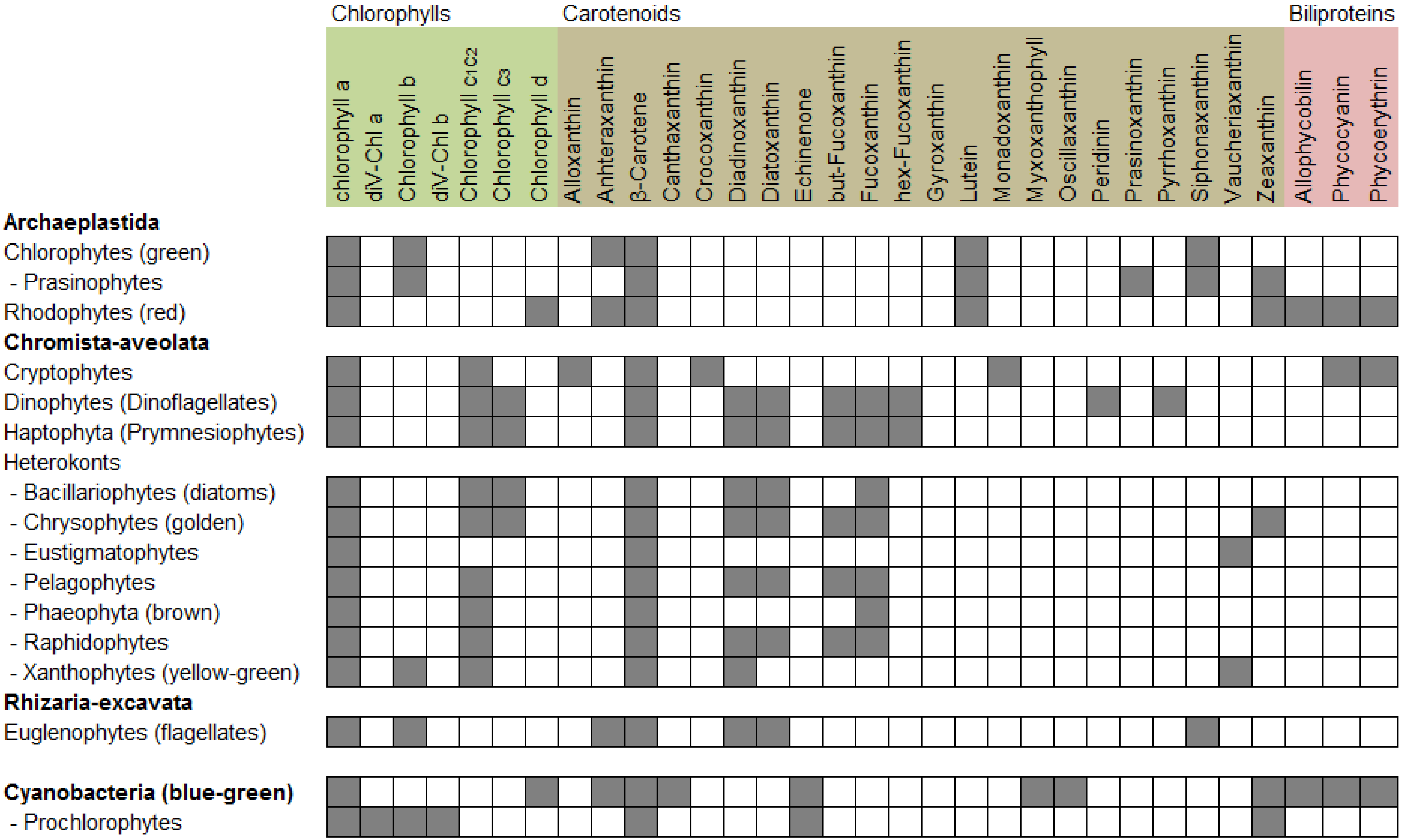


Figure 4.3: Absorption wavelengths documented in the literature for various photosynthetic pigments are shown for 10nm wavelengths bins (Murphy et al. 2005c; Simis et al. 2007b; Murphy and Tolhurst 2009; Murphy et al. 2009; Murphy et al. 2011) Bins with single-colour bars denote wavelengths that give a clear spectral signal. Bins with multiple colours are characterised by several pigments, and are more easily resolved using derivative analysis (Murphy et al. 2005a).



	Chlorophylls							Carotenoids																						Biliproteins		
	chlorophyll a	dV-Chl a	Chlorophyll b	dV-Chl b	Chlorophyll c_1c_2	Chlorophyll c_3	Chlorophyll d	Alloxanthin	Anhteraxanthin	β-Carotene	Canthaxanthin	Crocoxanthin	Diadinoxanthin	Diatoxanthin	Echinenone	but-Fucoxanthin	Fucoxanthin	hex-Fucoxanthin	Gyroxanthin	Lutein	Monadoxanthin	Myxoxanthophyll	Oscillaxanthin	Peridinin	Prasinoxanthin	Pyrrhoxanthin	Siphonaxanthin	Vaucheriaxanthin	Zeaxanthin	Allophycobilin	Phycocyanin	Phycoerythrin
Archaeplastida																																
Chlorophytes (green)	■		■						■	■										■							■					
- Prasinophytes	■		■							■										■					■		■		■			
Rhodophytes (red)	■						■		■	■										■									■	■	■	■
Chromista-aveolata																																
Cryptophytes	■				■			■		■		■									■										■	■
Dinophytes (Dinoflagellates)	■				■	■				■			■	■		■	■	■						■		■						
Haptophyta (Prymnesiophytes)	■				■	■				■			■	■		■	■	■														
Heterokonts																																
- Bacillariophytes (diatoms)	■				■	■				■			■	■			■															
- Chrysophytes (golden)	■				■	■				■			■	■		■	■												■			
- Eustigmatophytes	■									■																		■				
- Pelagophytes	■				■					■			■	■		■	■															
- Phaeophyta (brown)	■				■					■							■															
- Raphidophytes	■				■					■			■	■		■	■															
- Xanthophytes (yellow-green)	■		■		■					■			■															■				
Rhizaria-excavata																																
Euglenophytes (flagellates)	■		■						■	■			■	■													■					
Cyanobacteria (blue-green)	■						■		■	■	■				■							■	■						■	■	■	■
- Prochlorophytes	■	■	■	■						■					■														■			

Table 4.1: The pigments found in various types of phototroph (Rowan 1989; Millie 1993; Jeffrey et al. 1997; Paerl et al. 2003; Simis et al. 2007a). Grey cells denote characteristic pigments. It should be noted that the Cyanobacteria are a polyphyletic grouping.

4.2.6 Experiment One: Sequestration and Retention of Copper by Marine Biofilms and their Effect on Resident Microphytes

This experiment tested models one to five: (1) that exposure to levels of copper retards growth of biofilms, (2) exposure to copper induces the production of organic material by the biofilm, (3) copper in the surrounding water column is absorbed by biofilms, (4) sequestered copper is retained by biofilms for long enough to affect settling invertebrate settlement after the surrounding water column is restored to a state of lower copper concentration and (5) algal communities are altered by exposure to elevated levels of copper.

Sixty four (n=4 per treatment) settlement plates (AUH) were randomly deployed sub-tidally at Collins Beach (Site 2; Figure 4.1 *insert*) and exposed to one of four regimes of copper contamination (Figure 4.4A). Copper contamination and the respective control were delivered by interchangeable collars as described above. Beginning in mid-summer (early January 2013), biofilms were allowed to develop under their relevant copper dose before their collars were changed to the dose required for the second stage of their regime (Figure 4.4A). Manipulation of collars occurred after 30 days and settlement plates were destructively sampled on four occasions: 24, 27, 34 and 39 days from initialisation. This sampling was designed to increase replication in time and allow for comparison of biofilms at times ‘near’ and ‘far’ from the disturbance. This timing was chosen as appropriate for experiments on biofilms (Blanck and Dahl 1996; Morin et al. 2007), allowing for manipulation before second-order succession is likely to have begun (Glasby 1999). Approximately one third of the sampling units were lost due to interference by the public, with the number lost somewhat balanced across treatments but resulting in fewer replicates than originally planned.

Field spectrometry readings were taken from each settlement plate immediately after return from the field and separation of the settlement plate from their treatment collars (see method above). The two halves of the encrusting assemblage on each settlement plate were then scraped into separate centrifuge tubes using a razor blade. Half of the sample was set aside for metals analysis, while the other half was retained for pigment extraction. Samples for metals analysis were first freeze-dried to determine the dry weight of the sample. The organic component of the samples was then dissolved in 5mL of ultra-pure nitric acid (15.8M) in a microwave-assisted digestion. Samples were then made up to 10mL, centrifuged and the decanted liquid analysed for copper concentration using ICP-OES (Varian, USA). The remaining sludge was then dried to determine the remaining inorganic content of the sample. Pigment extraction was achieved by adding 900µL of 90% acetone to the sample before the sample was vortexed and stored for 24 hours at -20°C. Chlorophyll-a (chl-a) concentration in extracts was estimated using a Turner Designs laboratory fluorometer (Sunnyvale, USA) calibrated with pure chlorophyll-a standard (DHI, Denmark). The chl-a acidification module was used in order to estimate phaeopigment after acidification of the extract with HCl according to EPA Method 445.0 (Arar and Collins 1997). Concentrations were calculated by dividing pigment amounts by the dry weight determined from the other half of the sample. Pigment concentration in the extract was then expressed as the amount of chlorophyll-a and phaeopigment per cm^2.

To characterize the surrounding water column, ambient phytoplankton abundance and background water quality were assessed using seawater samples that were also collected from the site on every sampling occasion. These were taken from the water column surrounding the AUH, without being particularly close to any specific unit. On each occasion, three 2L samples were collected and filtered through GF/C glass fibre filter (nominal pore size 1.2µm; Whatman UK) and the chlorophyll-a and phaeopigment concentrations were determined as above. Three 10mL water samples were also collected on each sampling day, acidified with a drop of ultrapure HNO_3 (15.8M), and assessed for copper concentration.

For most analyses, PERMANOVA was used to test for no differences in the total dry weight of the biofilm (testing H1), relative amounts of organic and inorganic matter (testing H2), total amount of copper in the biofilm (testing H3), concentration of copper in the biofilm (also testing H3), length of time up to 9 days this copper is retained within the biofilm (testing H4), total amount, concentrations and relative abundance of chlorophyll-a and phaeopigment in the biofilm (testing H5), and both smoothed and 2nd order derivative reflectance data (testing H5) in response to four fixed factors that each had two levels of treatment (Figure 4.4A): (1) presence of ‘disturbance’, which comprised samples from regimes that were undisturbed (++/--) versus those that were disturbed (-+/+-); (2) ‘copper origin’, which was divided into samples that were (++/+-) or were not (--/-+) exposed to copper at the start of the experiment; (3) ‘before’ (day 24 and 27) or ‘after’ (day 34 and 39) disturbance; and (4) temporal ‘proximity of sampling to disturbance’, levels of which are hereafter known as ‘near’ (day 27 and 34) and ‘far’ (day 27 and 39). This powerful design is similar to a BACI (before-after/control-impact) design in that interpretation of interaction terms allows determination of the temporally-variable effects of copper regime ‘before’ and ‘after’ the disturbance (Underwood 1992; Underwood 1994). Where necessary for interpretation, pair-wise tests were done within PERMANOVA. To test the null hypotheses that the concentrations of copper, chlorophyll-a and phaeopigment are at consistent levels in ambient concentrations in the water column throughout the course of the experiment, seawater sample data were analysed as two PERMANOVA with sampling date as a fixed factor.

4.2.7 Experiment Two: The Interplay of the Relative Effects of Copper on: (1) the Physiology and Composition of Biofilms; and (2) the Composition of Settling Macro-Invertebrates.

This experiment tested model 5 to 7: (5) algal communities are altered by exposure to elevated levels of copper; (6) copper interferes with the photosynthetic apparatus of the phototrophs within biofilms and (7) settling invertebrate communities are sensitive to both direct exposure to copper and changes in biofilm substrata as induced by previous exposure to copper.

Artificial units of habitat (Figure 4.2) were deployed at two sites in the northern region of Spring Cove, Collins Beach, during early October, 2012 (austral spring; Figure 4.1). These settlement plates were furnished with interchangeable collars that exposed them to one of four copper regimes (Figure 4.4B): (1) where settlement plates experienced no exposure to copper (--); (2) where assemblages were exposed to copper for both time periods (++); (3) where control conditions were followed by copper exposure (-+); and (4) where initial exposure to copper was followed by control conditions (+-). Half of the plates from each treatment were returned to the laboratory for analysis 14 days after deployment. Sampling at fourteen days avoids most secondary settlement, which begins to dominate hard surfaces after approximately two weeks (Wieczorek and Todd 1997). All remaining plates were given freshly-painted collars consistent with their relevant treatments. After a total of 16 weeks, settlement plates were brought back to the laboratory for analysis. To maintain copper dosage during this time, AUH were given fresh collars at 9 weeks. 20 and 24 sampling units were lost to non-demonic intrusions by bad weather and interference ‘before’ and ‘after’ the manipulation respectively (Hurlbert 1984).

For the ‘before’ round of sampling, AUH were removed from the water and the settlement plates rapidly sealed into small plastic bags with some seawater taken straight from the field experiment site at Spring Cove. Samples were placed in closed insulated boxes for dark adaptation (Mallick and Mohn 2003; Kromkamp et al. 2006) and driven straight to the laboratory where they were first analysed for chlorophyll-a fluorescence using a DIVING PAM underwater fluorometer (Walz GmbH, Germany) and a field spectrometer as above. On each plate, four replicate measures each of maximum quantum yield of Photosystem II (PAM) and the visible wavelengths of light (field spectrometer) were taken. PAM analysis was done in a darkened room with the fibre-optic positioned at a 60° angle 2 cm from the settlement plate. Maximum effective quantum yield (F_V/F_M) was recorded and used to test H6 (Schreiber and Bilger 1993). Spectral analysis was done as specified in the general methods above. Biofilms were then scraped off using razor blades and stored in a -80°C freezer for future pigment analysis, which was done by HPLC and used to test H5, that algal communities are influenced by exposure to copper. Samples were placed in 3mL of 4°C chilled HPLC-grade 100% acetone and vortexed for 30 seconds. Then samples were placed in a sonicator for 15 minutes in the dark and on ice and left overnight in a darkened cold room at 4°C. Milli-Q water (200µL) was added and the mixture sonicated for a further 15 minutes and vortexed for another 30 seconds. The solution was then passed through a 0.2µm syringe filter before analysis using an HPLC pump system (Waters Australia Pty Ltd, Australia). Peaks in chromatograms were identified by eye and integrated using Empower Pro software (Water Australia Pty Ltd, Australia). Two separate datasets were derived from the output: one containing all the identified peaks and another containing just chlorophyll-a, which can be used as a proxy for total phototrophic biomass (Underwood 1984; Jeffrey et al. 1997; Brotas and Plante-Cuny 1998; Kelly et al. 2001; Paerl et al. 2003). Both datasets were used to test H5 (that algal communities are affected by exposure to copper). Biofilm samples used to test H5 and 6 (that copper interferes with the photosynthetic apparatus of phototrophs within biofilms) were analysed with a design that had one fixed factor, copper exposure (yes or no), and one random factor, spatial variation (Site 1 and Site 2).

For sampling of invertebrates, settlement plates were removed to the laboratory in small plastic bags before being stored in 7% buffered-formalin made with seawater. Prior to assessment, samples were rinsed and lightly brushed free of debris, loose epifauna and macroalgae. Using a dissecting microscope (maximum x40 resolution; Olympus, Japan), attached invertebrates were identified to species where possible and a reference set in 70% ethanol was used to maintain consistency in identification. Invertebrate samples used to test the hypothesis that exposure of developing benthic communities to varying time series of copper contamination will influence the species abundances of subsequent macro-invertebrate assemblages were analysed with a design that had two fixed factors, (1) 'copper origin' (--/-+ versus ++/+-) and (2) 'disturbance' (--/++ versus -+/+-), and one random factor, spatial variation (Site 1 versus Site 2). Invertebrate samples were compared using a Bray-Curtis similarity matrix as resemblance matrices calculated in Bray-Curtis space do not assess joint absences as evidence of similarity (Bray and Curtis 1957; Clarke et al. 2006). An overall log(x+1) transformation was applied to down-weigh highly-abundant species and reduce distortion stress in the resemblance matrix.

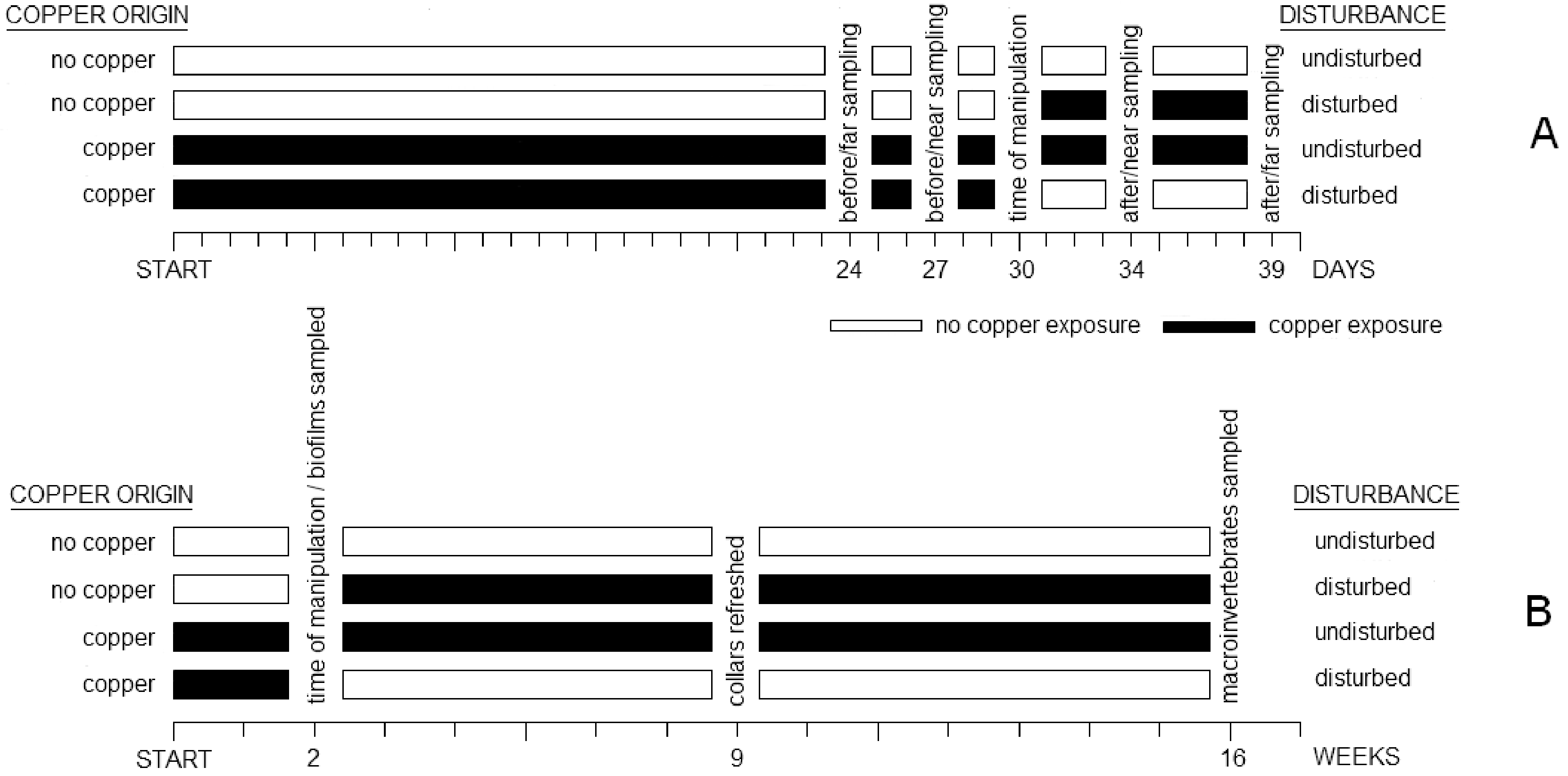


Figure 4.4: Copper exposure regime and sampling times in two experiments. Experiment one **(A)** used orthogonal comparisons of 'copper origin' (starting with and without copper), copper 'disturbance' (change or no change in levels of exposure to copper) and two factors associated with the timing of sampling relative to experimental manipulation of copper exposure: 'before/after' and 'near/far'. This experiment ran for 39 days during which biofilms were destructively sampled before/far, before/near, after/near and after/far relative to the manipulation on days 24, 27, 34 and 39 respectively. In experiment two **(B)** settlement plates were exposed to one of four treatments derived from orthogonal comparisons of 'copper origin' (starting with and without copper), copper 'disturbance' (change or no change in levels of exposure to copper). Biofilms were sampled on one occasion after two weeks, while macrofaunal assemblages were sampled at 16 weeks, which is 14 weeks after the manipulation. This procedure was carried out at two randomly selected sites.

4.3 RESULTS

4.3.1 Copper Leaching Experiment

For eight weeks, water parcels surrounding copper collars exhibited greater concentrations of copper ($\bar{x}$ = 2.37 ± 0.34SE µg.L^{-1}, n = 21), than those found in background seawater ($\bar{x}$ = 0.19 ± 0.06SE µg.L^{-1}, n = 20) collected from near Collins Beach. PERMANOVA detected these differences between copper treatments which did not change over time (Table 4.2).

4.3.2 Experiment One: Sequestration and Retention of Copper by Marine Biofilms and their Effect on Resident Microphytes

While there were no main effects of either ‘copper origin’ or ‘disturbance’ on the total dry weight of biofilm, the significant interaction between ‘proximity to disturbance’ and ‘before/after’ suggests that biofilm amounts increased steadily over time until reaching a plateau at 34 days, which was not significantly different from 39 days ($\bar{x}$ = 3.82 ± 0.76SE and $\bar{x}$ = 4.84 ± 0.51SE mg.cm^{-1} respectively). This finding rejects the hypothesis that biofilms exposed to copper will have less total mass (Table 4.3).

The ratio between organic and inorganic content within the biofilms was not affected by the factor ‘copper origin’ or ‘disturbance’ and did not vary over time (Table 4.4). This result rejects the hypothesis that exposure to copper increases the production of organic material within the biofilm.

Table 4.2: 2-factor univariate PERMANOVA to test the null hypotheses that there is no variation in copper leached from collars in response to paint type, which comprised of collars painted with a copper-based paints and collars that were painted with just paint primer and that the amount of copper leached is constant over time. No transformations were applied. Significant differences are shown in bold. Interaction terms with a *p* > 0.25 were pooled with the residual to increase the power of tests (Underwood 1997) and are marked as such. This led to one degree of freedom being pooled with the residual.

source	df	MS	F	*p*
Paint type	1	0.49	37.79	**< 0.001**
Time since deployment	4	< 0.01	0.27	0.92
pt x td	1	POOLED		
Pooled Residual	35	0.01		
Total	40			

Table 4.3: 4-factor univariate PERMANOVA to test the null hypotheses that there is no variation in the total dry weight of biofilms for (1) presence of ‘disturbance’, which comprised samples from regimes that were undisturbed (all copper exposure ‘++’ or no copper exposure ‘--’) versus those that were disturbed (experiencing a change in copper exposure ‘-+’ or ‘+-’); (2) ‘copper origin’, which was divided into samples that were (++/+-) or were not (--/-+) exposed to copper at the start of the experiment; (3) ‘before’ or ‘after’ disturbance; and (4) temporal ‘proximity of sampling to disturbance’, levels of which are ‘near’ and ‘far’. All factors are fixed. Euclidean distances were used to create the resemblance matrix. No transformations were applied. Significant differences are shown in bold. Interaction terms with a $p > 0.25$ were pooled with the residual to increase the power of tests (Underwood 1997) and are marked as such. A total of nine degrees of freedom were pooled with the residual. Pairwise tests were done to determine differences between levels of the factor ‘proximity to disturbance’ within levels of the factor ‘before/after’.

source	df	MS	F	*p*
Copper origin (co)	1	0.00	0.47	0.50
Disturbance (d)	1	0.00	0.36	0.56
Before/after (ba)	1	0.17	42.46	<**0.001**
Proximity to disturbance (pd)	1	0.00	0.11	0.75
co x d	1	POOLED		
co x ba	1	POOLED		
co x pd	1	POOLED		
d x ba	1	POOLED		
d x pd	1	POOLED		
ba x pd	1	0.02	4.57	**0.04**
co x d x ba	1	0.00	1.40	0.25
co x d x pd	1	POOLED		
co x ba x pd	1	POOLED		
d x ba x pd	1	POOLED		
co x d x ba x pd	1	POOLED		
Pooled residual	44	0.00		
Total	50			
		pairwise test		
pairwise test (ba x pd)		before	near > far	
		after	near = far	

Table 4.4: 4-factor univariate PERMANOVA to test the null hypotheses that there is no variation in the relative amounts of organic and inorganic material in biofilms for (1) presence of ‘disturbance’, which comprised samples from regimes that were undisturbed (all copper exposure ‘++’ or no copper exposure ‘--’) versus those that were disturbed (experiencing a change in copper exposure ‘-+’ or ‘+-’); (2) ‘copper origin’, which was divided into samples that were (++/+-) or were not (--/-+) exposed to copper at the start of the experiment; (3) ‘before’ or ‘after’ disturbance; and (4) temporal ‘proximity of sampling to disturbance’, levels of which are ‘near’ and ‘far’. All factors are fixed. Euclidean distances were used to create the resemblance matrix. No transformations were applied. Significant differences are shown in bold. Interaction terms with a $p > 0.25$ were pooled with the residual to increase the power of tests (Underwood 1997) and are marked as such. A total of ten degrees of freedom were pooled with the residual.

source	df	MS	F	*p*
Copper origin (co)	1	0.01	0.05	0.84
Disturbance (d)	1	0.33	2.34	0.14
Before/after (ba)	1	0.07	0.48	0.51
Proximity to disturbance (pd)	1	0.08	0.56	0.48
co x d	1	POOLED		
co x ba	1	POOLED		
co x pd	1	POOLED		
d x ba	1	POOLED		
d x pd	1	0.27	1.93	0.18
ba x pd	1	POOLED		
co x d x ba	1	POOLED		
co x d x pd	1	POOLED		
co x ba x pd	1	POOLED		
d x ba x pd	1	POOLED		
co x d x ba x pd	1	POOLED		
Pooled residual	45	0.14		
Total	51			

There was no difference in copper concentrations between the '++' and '+-' regimes of copper exposure as tested by the interaction term between the factors 'copper origin' and 'disturbance', hence supporting the model that sequestered copper is retained by biofilms for long enough to affect settling invertebrate settlement after the surrounding water column is restored to a state of lower copper concentration (Table 4.5). It is clear that the copper-painted collars successfully deliver copper to the settlement plates, as in the factor 'copper origin', the average amount of copper contained within biofilms from the '++' and '+-' regimes was more than twice the mean amount found in those from the '--' and '-+' regimes ($\bar{x}$ = 1.54 ± 0.44SE µg and $\bar{x}$ = 0.66 ± 0.12SE µg /settlement plate respectively; Table 4.6). Within the two-level factor of copper origin, copper concentrations were significantly greater in biofilms that had been exposed to '++' and '+-' regimes of copper as opposed to the '--' and '-+' regimes of copper (Figure 4.5; Table 4. 6). These results support H3, that copper exposure will increase total copper amount and concentration in the biofilms compared to those biofilms not exposed to copper. Copper concentration varied over time (Figure 4.5; Table 4. 5). Concentrations were highest at the first (day 24) sampling event and drop off significantly on the other three occasions (days 27, 34 and 39; Table 4.5).

Seawater samples were taken to determine background concentrations of copper during the experiment. While some samples contained elevated amounts of copper, background concentrations were generally low throughout the experiment ($\bar{x}$ = 0.039 ± 0.017SE $\mu g.L^{-1}$, n = 20; $F_{(6,13)}$:0.86, p = 0.44). Thus, it is clear that the experimental collars leached enough copper to raise local concentrations above background levels.

Table 4.5:Univariate 4-factor PERMANOVA analysis testing the null hypotheses that the total amount of copper contained in biofilms are not affected by (1) presence of ‘disturbance’, which comprised samples from regimes that were undisturbed (all copper exposure ‘++’ or no copper exposure ‘--’) versus those that were disturbed (experiencing a change in copper exposure ‘-+’ or ‘+-’); (2) ‘copper origin’, which was divided into samples that were (++/+-) or were not (--/-+) exposed to copper at the start of the experiment; (3) ‘before’ or ‘after’ disturbance; and (4) temporal ‘proximity of sampling to disturbance’, levels of which are ‘near’ and ‘far’. All factors are fixed. Euclidean distances were used to create the resemblance matrix. No transformations were applied. Significant differences are shown in bold. Interaction terms with a $p > 0.25$ were pooled with the residual to increase the power of tests (Underwood 1997) and are marked as such. A total of nine degrees of freedom were pooled with the residual.

Source	df	MS	F	*p*
Copper origin (co)	1	0.51	5.61	**0.02**
Disturbance (d)	1	0.00	0.05	0.84
Before/after (ba)	1	0.11	1.18	0.30
Proximity to disturbance (pd)	1	0.06	0.65	0.44
co x d	1	POOLED		
co x ba	1	POOLED		
co x pd	1	POOLED		
d x ba	1	POOLED		
d x pd	1	POOLED		
ba x pd	1	POOLED		
co x d x ba	1	POOLED		
co x d x pd	1	POOLED		
co x ba x pd	1	POOLED		
d x ba x pd	1	0.15	1.63	0.22
co x d x ba x pd	1	0.29	3.20	0.08
Pooled Residual	41	0.09		
Total	47			

Table 4.6:Univariate 4-factor PERMANOVA analysis testing the null hypotheses that the concentration of copper contained in biofilms are not affected by (1) presence of 'disturbance', which comprised samples from regimes that were undisturbed (all copper exposure '++' or no copper exposure '--') versus those that were disturbed (experiencing a change in copper exposure '-+' or '+-'); (2) 'copper origin', which was divided into samples that were (++/+-) or were not (--/-+) exposed to copper at the start of the experiment; (3) 'before' or 'after' disturbance; and (4) temporal 'proximity of sampling to disturbance', levels of which are 'near' and 'far'. All factors are fixed. The resemblance matrix was calculated in Euclidean space. No transformations were applied. Significant differences are shown in bold. Interaction terms with a *p* > 0.25 were pooled with the residual to increase the power of tests (Underwood 1997) and are marked as such. A total of nine degrees of freedom were pooled with the residual. Pairwise tests were done to determine differences between levels of the factor 'proximity to disturbance' within levels of the factor 'before/after' and vice versa.

Source	df	MS	F	*p*
copper origin (co)	1	7.98	9.17	**<0.001**
disturbance (d)	1	0.48	0.55	0.46
before/after (ba)	1	13.93	16.00	**<0.001**
proximity to disturbance (pd)	1	0.00	0.01	0.94
co x d	1	POOLED		
co x ba	1	POOLED		
co x pd	1	POOLED		
d x ba	1	1.81	2.08	0.16
d x pd	1	POOLED		
ba x pd	1	7.73	8.88	**0.01**
co x d x ba	1	POOLED		
co x d x pd	1	POOLED		
co x ba x pd	1	POOLED		
d x ba x pd	1	POOLED		
co x d x ba x pd	1	POOLED		
pooled residual	41	0.87		
total	47			

pairwise tests		
ba x pd	before	far > near
	after	near = far
	near	before = after
	far	before > after

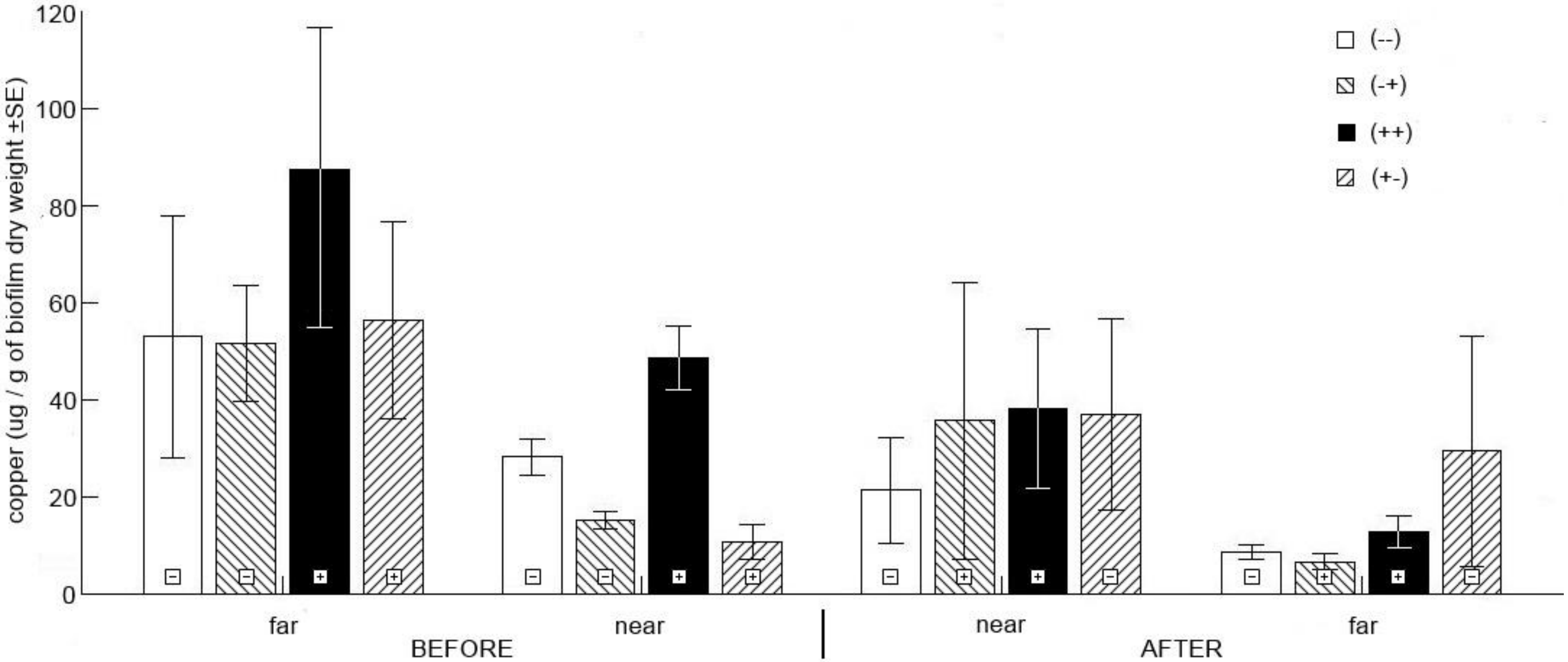


Figure 4.5: Copper concentrations found in biofilms from all treatments. '+' and '-' symbols denote exposure to copper and control conditions respectively. Copper concentrations were higher in biofilms exposed to '++' and '+-' regimes of copper pollution as opposed to those found in biofilms exposed to '--' and '-+' regimes of copper ($F_{(1,41)} = 9.17$, $p < 0.01$). Copper concentration varied over time with the highest concentration observed at the first sampling event ($F_{(1,41)} = 8.88$, $p = 0.01$).

While the total amount of chlorophyll a and associated phaeopigments within biofilms increased over time until reaching a plateau of $\bar{x} = 0.48 \pm 0.06$SE and $\bar{x} = 0.14 \pm 0.02$SE µg/cm^2 respectively at 34 days, there was no effect of any factor on their concentration, which was similar irrespective of treatment ($\bar{x} = 316.40 \pm 32.18$SE and $\bar{x} = 91.96 \pm 7.52$SE µg.g^{-1} biofilm dry weight respectively; Tables 4.7 and 4.8). Conversely, in the presence of a disturbance, irrespective of whether it is the addition or removal of copper, the ratio of chlorophyll-a to phaeopigment was 41% lower in those samples that were taken 'after' they had experienced the perturbation compared to those samples taken 'before' the disturbance (Table 4.9). This effect was not observed in the absence of a disturbance. Similarly, for the smoothed reflectance data, and using chlorophyll-a as a proxy for photosynthetic biomass, the relative reflectance at the chlorophyll-a absorption at 674nm suggests that quantities of pigment were generally 14% higher in biofilms sampled 'after' the manipulation, that is, they increased over time (Table 4.10). Pigments were 16% more abundant 'near' to copper disturbance events, irrespective of whether the disturbance involved exposure to or depuration from the pollutant (Figure 4.6). These findings were supported by a significant main effect of the 'before/after' factor, a significant interaction term between 'disturbance' and 'proximity to disturbance', and subsequent pairwise tests (Table 4.10). From the 2nd derivative data it is further apparent that the relative amounts of different pigments varied during the experiment (Figure 4.7, Table 4.11): initially an increase in the relative proportion of carotenoids and phycocyanin was observed and while not illustrated by Figure 4.7, a decrease in phycocyanin was apparent between the 'before-near' and 'after-near' sampling times. Again, the comparative amounts of various pigments were affected by exposure to or depuration from copper disturbance and that effect was mediated by the timing of sampling relative to the timing of disturbance (Figure 4.8, Table 4.11). In the absence of a disturbance, small differences in carotenoids such as fucoxanthin were seen between ‘near’ and ‘far’ samples, that is, in samples temporally close to the disturbance, rather than temporally far from the disturbance (Figure 4.8). Conversely, the presence of a copper disturbance, whether the removal or addition of copper, resulted in greater shifts in the relative amounts of phycoerythrin, phycocyanin and chlorophyll-c. Chlorophyll-c was found in greater abundance in ‘far’ samples, while ‘near’ samples were associated with phycoerythrin and phycocyanin (Figure 4.8). In the absence of a clear effect of copper origin, these data do not support H5, that exposure to copper leads to changes in the relative amounts of photosynthetic pigments and associated degraded pigments (phaeopigments) compared to those algal communities not exposed to elevated levels of copper.

Amounts of chlorophyll-a and phaeopigment in the surrounding water column were characterised by low concentrations on days 4 and 39, moderate concentrations on days 0, 27, 30 and 34, and a disproportionately-large pulse of pigments at day 24 (Pseudo-$F_{(6,14)}$: 6.22, $p < 0.001$; n = 21; Figure 4.9). The pulse of pigments on day 24 may be related to a 48.6mm precipitation event on day 23, though given that another rainfall event of 30.4mm occurred on day 38, a day before the low concentrations observed on day 39, this is not a reliable inference (BOM, 2013). The loss of samples from day 24 precluded use of the event in interpretation of the role of phytoplankton in determining the concentration of chlorophyll-a in biofilms. The relative amounts of chlorophyll-a and phaeopigment also varied during the experiment, with higher proportions of phaeopigment observed on days 0, 27, 30 and 39 (Figure 4.9).

Table 4.7: Multivariate 4-factor PERMANOVA analysis testing the null hypotheses that the total amounts of chlorophyll-a and phaeopigments in biofilms are not affected by (1) presence of ‘disturbance’, which comprised samples from regimes that were undisturbed (all copper exposure ‘++’ or no copper exposure ‘--’) versus those that were disturbed (experiencing a change in copper exposure ‘-+’ or ‘+-’); (2) ‘copper origin’, which was divided into samples that were (++/+-) or were not (--/-+) exposed to copper at the start of the experiment; (3) ‘before’ or ‘after’ disturbance; and (4) temporal ‘proximity of sampling to disturbance’, levels of which are ‘near’ and ‘far’. All factors are fixed. The resemblance matrix was calculated in Euclidean space. Significant differences are shown in bold. Interaction terms with a *p* > 0.25 were pooled with the residual to increase the power of tests (Underwood 1997) and are marked as such. A total of ten degrees of freedom were pooled with the residual.

source	df	MS	Pseudo-*F*	*p*
Copper origin (co)	1	0.00	0.44	0.53
Disturbance (d)	1	0.00	0.35	0.58
Before/after (ba)	1	0.09	12.54	**<0.001**
Proximity to disturbance (pd)	1	0.02	2.24	0.14
co x d	1	POOLED		
co x ba	1	POOLED		
co x pd	1	POOLED		
d x ba	1	POOLED		
d x pa	1	POOLED		
ba X pd	1	0.00	1.37	0.25
co x d x ba	1	POOLED		
co x d x pd	1	POOLED		
co x ba x pd	1	POOLED		
d x ba x pd	1	POOLED		
co x d x ba x pd	1	POOLED		
Pooled Residual	43	0.00		
Total				

Table 4.8:Multivariate 4-factor PERMANOVA analysis testing the null hypotheses that the concentration of chlorophyll-a and phaeopigment contained in biofilms are not affected by (1) presence of ‘disturbance’, which comprised samples from regimes that were undisturbed (all copper exposure ‘++’ or no copper exposure ‘--’) versus those that were disturbed (experiencing a change in copper exposure ‘-+’ or ‘+-’); (2) ‘copper origin’, which was divided into samples that were (++/+-) or were not (--/-+) exposed to copper at the start of the experiment; (3) ‘before’ or ‘after’ disturbance; and (4) temporal ‘proximity of sampling to disturbance’, levels of which are ‘near’ and ‘far’. All factors are fixed. The resemblance matrix was calculated in Euclidean space. Significant differences are shown in bold. Interaction terms with a $p > 0.25$ were pooled with the residual to increase the power of tests (Underwood 1997) and are marked as such. A total of ten degrees of freedom were pooled with the residual.

Source	df	MS	Pseudo-*F*	*p*
Copper origin (co)	1	39245	3.13	0.08
Disturbance (d)	1	4039.4	0.32	0.59
Before/after (ba)	1	6137.1	0.49	0.50
Proximity to disturbance (pd)	1	11450	0.91	0.35
co x d	1	POOLED		
co x ba	1	POOLED		
co x pd	1	POOLED		
d x ba	1	POOLED		
d x pd	1	POOLED		
ba x pd	1	28424	2.27	0.14
co x d x ba	1	POOLED		
co x d x pd	1	POOLED		
co x ba x pd	1	POOLED		
d x ba x pd	1	POOLED		
co x d x ba x pd	1	POOLED		
Pooled Residual	42	12533		
Total	47			

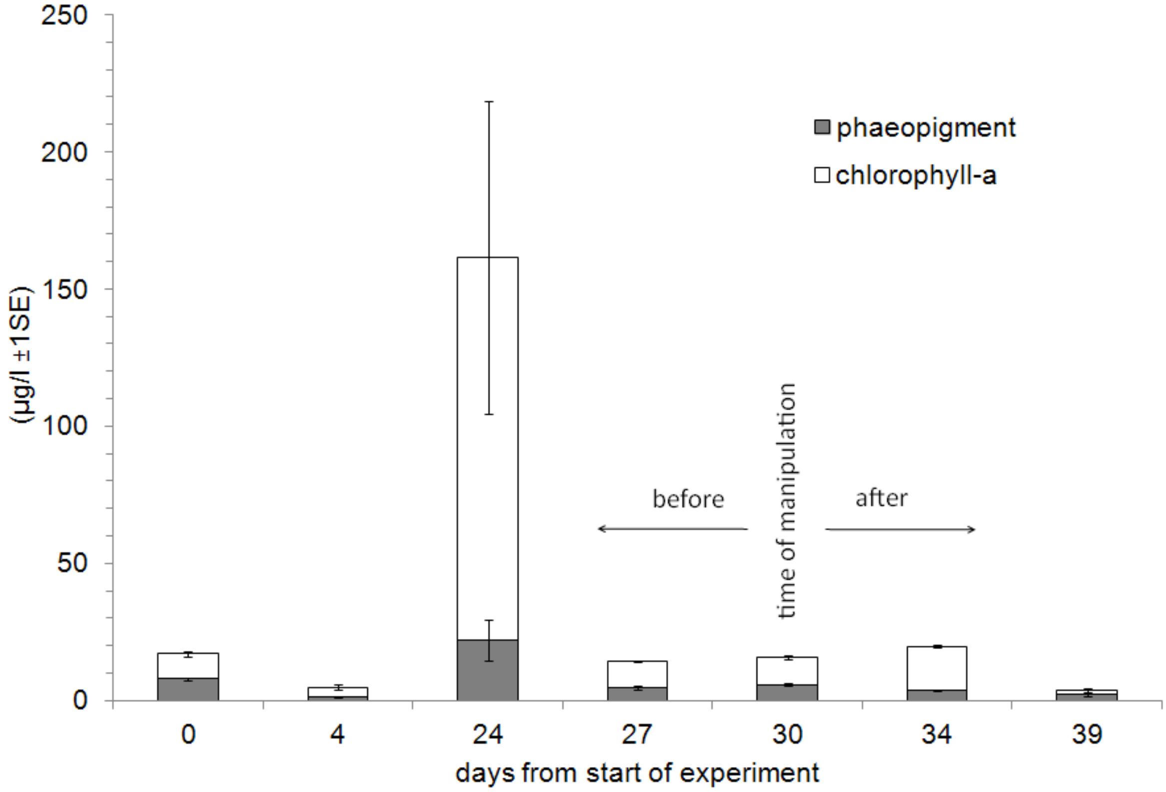


Figure 4.6 Relative concentrations of chlorophyll-a and phaeopigment in the surrounding water column throughout the experiment (Pseudo-$F_{(6,14)}$: 6.22, $p < 0.001$; n = 21). Samples were taken within metres of the AUH without being particularly close to any one unit.

Table 4.9:Univariate 4-factor PERMANOVA analysis testing the null hypotheses that the relative amounts of chlorophyll-a and phaeopigment contained in biofilms are not affected by (1) presence of 'disturbance', which comprised samples from regimes that were undisturbed (all copper exposure '++' or no copper exposure '--') versus those that were disturbed (experiencing a change in copper exposure '-+' or '+-'); (2) 'copper origin', which was divided into samples that were (++/+-) or were not (--/-+) exposed to copper at the start of the experiment; (3) 'before' or 'after' disturbance; and (4) temporal 'proximity of sampling to disturbance', levels of which are 'near' and 'far'. All factors are fixed. The resemblance matrix was calculated in Euclidean space. Significant differences are shown in bold. Interaction terms with a *p* > 0.25 were pooled with the residual to increase the power of tests (Underwood 1997) and are marked as such. A total of eight degrees of freedom were pooled with the residual.

Source	df	MS	*F*	*p*
Copper origin (co)	1	0.00	0.04	0.84
Disturbance (d)	1	0.02	0.28	0.60
Before/after (ba)	1	0.54	7.20	**0.01**
Proximity to disturbance (pd)	1	0.02	0.25	0.62
co x d	1	0.13	1.80	0.19
co x ba	1	POOLED		
co x pd	1	POOLED		
d x ba	1	0.51	6.83	**0.01**
d x pd	1	POOLED		
ba x pd	1	POOLED		
co x d x ba	1	0.12	1.60	0.22
co x d x pd	1	POOLED		
co x ba x pd	1	POOLED		
d x ba x pd	1	POOLED		
co x d x ba x pd	1	POOLED		
Pooled Residual	40	0.07		
Total	47			

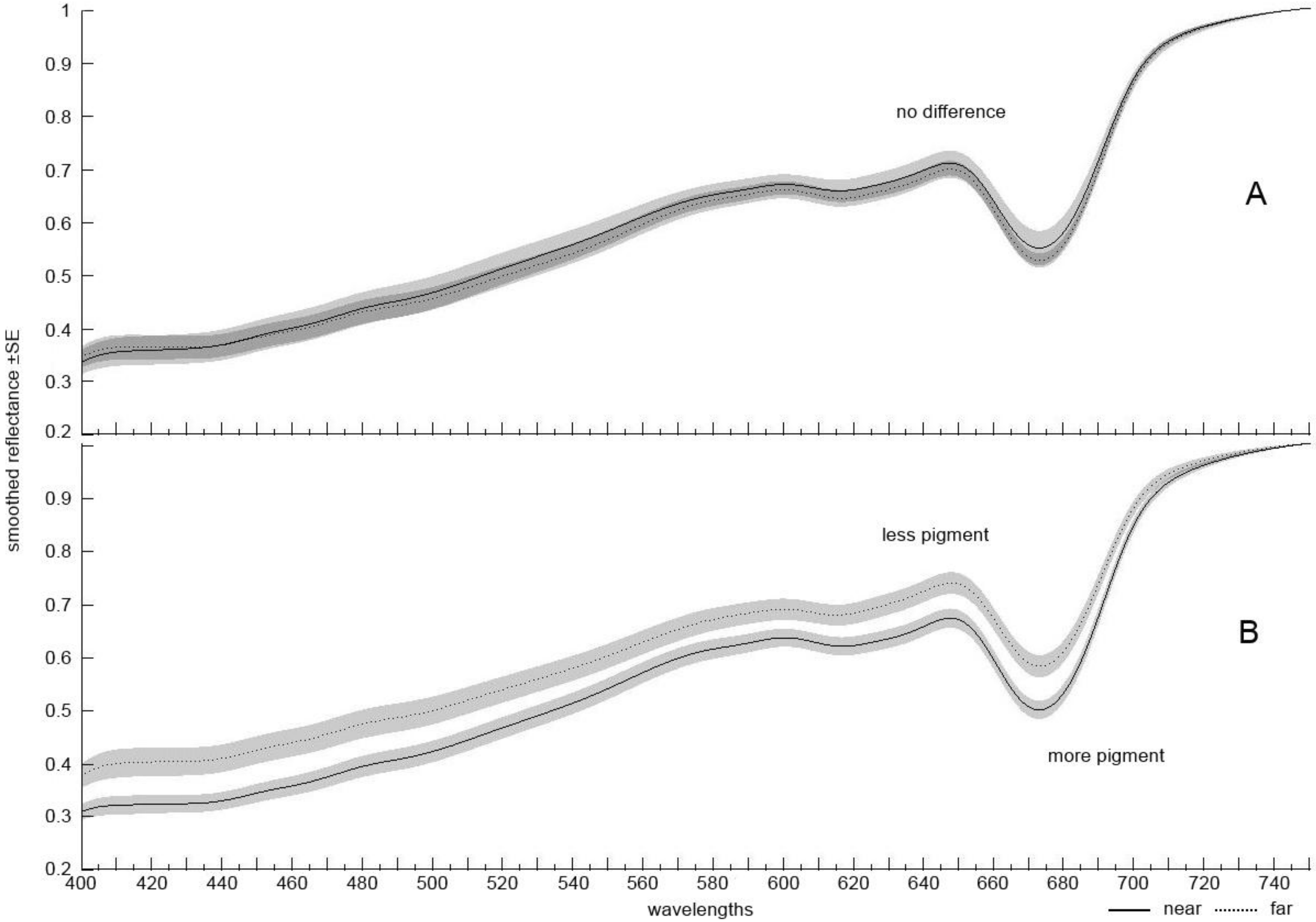


Figure 4.7: Smoothed reflectance data near and far in the absence (A) and presence (B) of a disturbance. Before/after and management factor data were pooled for this illustration. In the absence of disturbance (A) there is no effect of sample timing. When biofilms are subjected to a copper disturbance (B) less reflection and hence more absorption and thus more pigments are found 'near' to, not 'far' from, the disturbance (Pseudo-F(1,43): 5.71, $p < 0.02$). This pattern is consistent for both the before and after sides of the manipulation and irrespective of whether it involves the depuration or addition of copper.

Table 4.10: Comparison using 4-factor multivariate PERMANOVA to test the null hypotheses that there is no variation in spectral profiles (wavelengths 400-750 smoothed through a 26nm smoothing interval) of biofilms for (1) presence of ‘disturbance’, which comprised samples from regimes that were undisturbed (all copper exposure ‘++’ or no copper exposure ‘--’) versus those that were disturbed (experiencing a change in copper exposure ‘-+’ or ‘+-’); (2) ‘copper origin’, which was divided into samples that were (++/+-) or were not (--/-+) exposed to copper at the start of the experiment; (3) ‘before’ or ‘after’ disturbance; and (4) temporal ‘proximity of sampling to disturbance’, levels of which are ‘near’ and ‘far’. All factors are fixed. Euclidean distances were used to create the resemblance matrix. Significant differences are shown in bold. Interaction terms with a *p* > 0.25 were pooled with the residual to increase the power of tests (Underwood 1997) and are marked as such. A total of ten degrees of freedom were pooled with the residual. Pairwise tests were done to determine differences between levels of the factor ‘proximity to disturbance’ within levels of the factor ‘disturbance’.

source	df	MS	Pseudo-*F*	*p*
Copper origin (co)	1	1.19	1.28	0.28
Disturbance (d)	1	0.07	0.03	0.89
Before/after (ba)	1	41.74	44.72	**<0.001**
Proximity to disturbance (pd)	1	1.32	1.41	0.25
co x d	1	POOLED		
co x ba	1	POOLED		
co x pd	1	POOLED		
d x ba	1	POOLED		
d x pd	1	5.33	5.71	**0.02**
ba x pd	1	POOLED		
co x d x ba	1	POOLED		
co x d x pd	1	POOLED		
co x ba x pd	1	POOLED		
d x ba x pd	1	POOLED		
co x d x ba x pd	1	POOLED		
Pooled Residual	43	0.93		
Total	48			
	pairwise tests			
d x pd		yes	near > far	
		no	near = far	

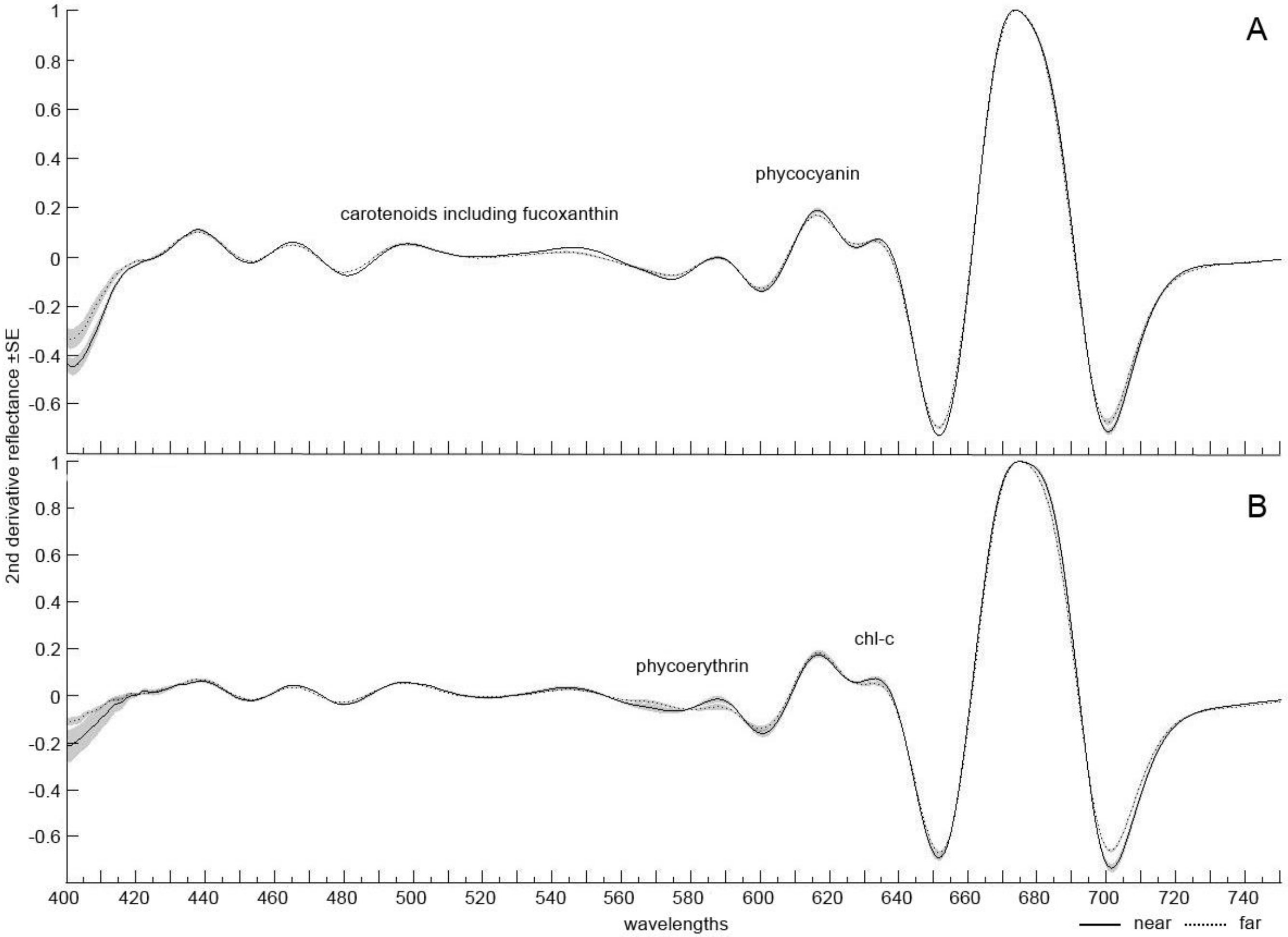


Figure 4.8: 2nd derivative spectra near and far, before (A) and after (B) the disturbance. Once normalised to the chlorophyll-a peak centred near 674nm, the relative amounts of different pigments varied over the course of the experiment (Pseudo-$F_{(1,41)} = 5.33$, $p < 0.01$). 'Before 'the manipulation (A) carotenoids and phycocyanin increased over time. 'After' the manipulation (B) a subtle decrease in phycoerythrin and chlorophyll-c was observed between 'near' and 'far' sampling times.

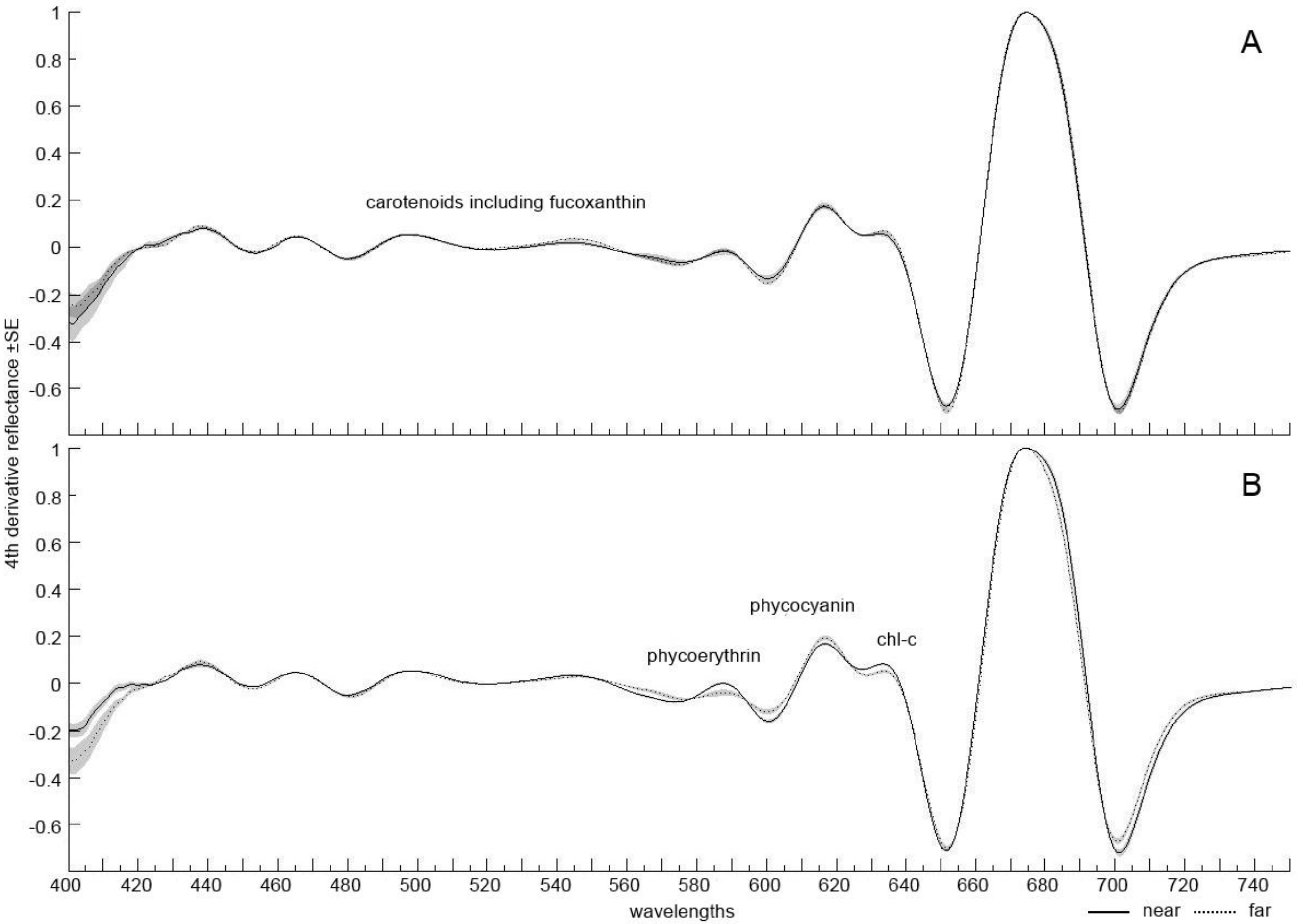


Figure 4.9: 2nd derivative spectra of the factors ‘proximity to disturbance’ and ‘disturbance’. Differences in the relative amount of various pigments between ‘near’ and ‘far’ depends on the absence (A) or presence (B) of a copper disturbance (Pseudo-$F_{(1,41)} = 4.54$, $p < 0.01$).

Table 4.11: Comparison using 4-factor multivariate PERMANOVA to test the null hypotheses that there was no variation in spectral profiles (2nd derivative of wavelengths 400-750 normalized to the chlorophyll peak at ~674nm) of biofilms for (1) presence of ‘disturbance’, which comprised samples from regimes that were undisturbed (all copper exposure ‘++’ or no copper exposure ‘--’) versus those that were disturbed (experiencing a change in copper exposure ‘-+’ or ‘+-’); (2) ‘copper origin’, which was divided into samples that were (++/+-) or were not (--/-+) exposed to copper at the start of the experiment; (3) ‘before’ or ‘after’ disturbance; and (4) temporal ‘proximity of sampling to disturbance’, levels of which are ‘near’ and ‘far’. All factors are fixed. Euclidean distances were used to create the resemblance matrix. Significant differences are shown in bold. Interaction terms with a $p > 0.25$ were pooled with the residual to increase the power of tests (Underwood 1997) and are marked as such. A total of eight degrees of freedom were pooled with the residual. Pairwise tests were done to determine differences between levels of the factor ‘proximity to disturbance’ within levels of the factor ‘disturbance’ and between levels of the factor ‘proximity to disturbance’ within levels of the factor ‘before/after’.

Source	df	MS	Pseudo-*F*	*p*
Copper origin (co)	1	0.79	1.81	0.14
Disturbance (d)	1	0.12	0.28	0.89
Before/after (ba)	1	10.97	25.21	<**0.001**
Proximity to disturbance (pd)	1	0.31	0.70	0.51
co x d	1	POOLED		
co x ba	1	POOLED		
co x pd	1	0.64	1.47	0.20
d x ba	1	POOLED		
d x pd	1	1.97	4.54	**0.01**
ba x pd	1	2.32	5.33	<**0.001**
co x d x ba	1	POOLED		
co x d x pd	1	POOLED		
co x ba x pd	1	POOLED		
d x ba x pd	1	POOLED		
co x d x ba x pd	1	POOLED		
Pooled Residual	41	0.44		
Total	48			

pairwise tests

d x pd	yes	near ≠ far
	no	near = far
ba x pd	before	near ≠ far
	after	near = far

4.3.3 Experiment Two: The Interplay of the Relative Effects of Copper on: (1) the Physiology and Composition of Biofilms; and (2) the Composition of Settling Macro-Invertebrates.

Beta-carotene, chlorophyll-a, chlorophyll-b, chlorophyll-c, diadinoxanthin, fucoxanthin and violaxanthin concentrations were reliably identified using Empower Pro chromatograms. These data largely reject H5, with the only support for the model that algal communities are altered by exposure to elevated levels of copper being the apparent space dependent changes in chlorophyll-a concentration as detected by the HPLC at Site 2. There was no effect of copper or site on the whole assemblage of pigments (Table 4.12); however, there was an interactive effect of copper exposure and site when chlorophyll-a was isolated as a proxy for total algal biomass (n = 48). This interaction suggested that copper exposure limited chlorophyll-a, but only at Site 2 where chlorophyll-a concentrations were 27% higher in control-treated biofilms compared to those from all other treatments, which were not different from one another (Table 4.13). There was no apparent effect of copper exposure or site on the smoothed or 2nd derivative spectrometry data sets (n = 46; Table 4.14, 4.15).

Supporting H6, exposure to copper significantly decreased the photosynthetic health (F_V/F_M) of the biofilm assemblage by an average of 60% ($\bar{x} = 0.40 \pm 0.06$SE to $\bar{x} = 0.16 \pm 0.07$SE; n = 29) and this effect was consistent across both sites (Table 4.16).

Table 4.12: Multivariate 2-factor PERMANOVA analysis of HPLC chromatograms testing the null hypothesis that relative amounts of beta-carotene, chlorophyll-a, chlorophyll-b, chlorophyll-c, diadinoxanthin, fucoxanthin and violaxanthin in biofilms does not vary with: (1) exposure to copper, a fixed factor with two levels (copper and no copper); and (2) site, a random factor with two levels. Resemblance matrices were based on Euclidean distances because response variables contained no zeroes and were in the same unit of measurement (µg).

source	df	MS	Pseudo-*F*	*p*
Copper (c)	1	2787.9	0.86	0.50
Spatial variation (sv)	1	1096	1.36	0.25
c X sv	1	3218.6	3.98	>0.05
Residual	44	808.61		
Total	47			

Table 4.13: Univariate 2-way PERMANOVA analysis of chlorophyll-a amount determined by HPLC chromatograms testing the null hypothesis that the amount of chlorophyll-a in biofilms does not vary with: (1) exposure to copper, a fixed factor with two levels (copper and no copper); and (2) site, a random factor with two levels. Resemblance matrices were based on Euclidean distances.

source	df	MS	*F*	*p*
Copper (c)	1	2167.2	0.74	0.66
Spatial variation (sv)	1	1085.5	1.59	0.21
c X sv	1	2913.5	4.27	**0.04**
Residual	44	681.72		
Total	47			

Table 4.14: Comparison using 2-factor multivariate PERMANOVA to test the null hypotheses that there is no variation in spectral profiles (wavelengths 400-750 smoothed through a 26nm smoothing interval) of biofilms for (1) exposure to copper, a fixed factor with two levels (copper and no copper); and (2) site, a random factor with two levels. Resemblance matrices were based on Euclidean distances. Significant differences are shown in bold. Interaction terms with a *p* > 0.25 were pooled with the residual to increase the power of tests (Underwood 1997) and are marked as such. One degree of freedom was pooled with the residual.

source	df	MS	Pseudo-*F*	*p*
Copper (c)	1	0.31	0.62	0.44
Spatial variation (sv)	1	0.95	1.93	0.17
c x sv	1	POOLED		
Pooled Residual	41	0.49		
Total	43			

Table 4.15: Comparison using 4-factor multivariate PERMANOVA to test the null hypotheses that there is no variation in spectral profiles (2^{nd} derivative of wavelengths 400-750 normalized to the chlorophyll peak at ~674nm) of biofilms for (1) exposure to copper, a fixed factor with two levels (copper and no copper); and (2) site, a random factor with two levels. Resemblance matrices were based on Euclidean distances. Significant differences are shown in bold. Interaction terms with a $p > 0.25$ were pooled with the residual to increase the power of tests (Underwood 1997) and are marked as such. A single degree of freedom was pooled with the residual.

source	df	MS	Pseudo-F	p
Copper (c)	1	0.11	0.23	0.76
Spatial variation (sv)	1	1.29	2.63	0.09
c x sv	1	POOLED		
Pooled Residual	41	0.49		
Total	43			

Table 4.16: Univariate 2-way PERMANOVA analysis of PAM fluorometry data to test the null hypothesis that chlorophyll fluorescence of biofilms does not vary in response to (1) exposure to copper, a fixed factor with two levels (copper and no copper); and (2) across sites, a random factor with two levels. Resemblance matrices were based on Euclidean distances. Significant differences are shown in bold. Interaction terms with a $p > 0.25$ were pooled with the residual to increase the power of tests (Underwood 1997) and are marked as such. One degree of freedom was pooled with the residual.

source	df	MS	*F*	*p*
Copper (c)	1	0.41	7.9811	**0.0104**
Spatial variation (sv)	1	0.02	0.3224	0.5788
c x sv	1	POOLED		
Pooled Residual	26	0.05		
Total	27			

Settling invertebrate species were strongly influenced by main effects of site and ‘copper origin’ (that is, whether copper was applied for the first stage of the experiment) but not ‘disturbance’ (Table 4.17). No interactions were observed between any factors (n=56). Spirobid and serpulid species as well as the common bryozoan *Watersipora subtorquata* were generally associated with Site 1, while barnacle species such as *Balanus trigonus* were associated with Site 2 (Figure 4.10). ‘--' and ‘-+’ samples were populated with the invasive *Cryptosula pallasiana* and a species of green sabellid, while ‘++’ and ‘+-’ samples were associated with a species of *Celleporina* bryozoan, a small entangling bivalve *Hiatella australis* and an unidentified white calcareous sponge (Figure 4.10). In the absence of an interaction between ‘copper origin’ and ‘disturbance’ these data reject H7 and thus the model that settling invertebrate communities are sensitive to both direct exposure to copper and changes in biofilm substrata as induced by previous exposure to copper.

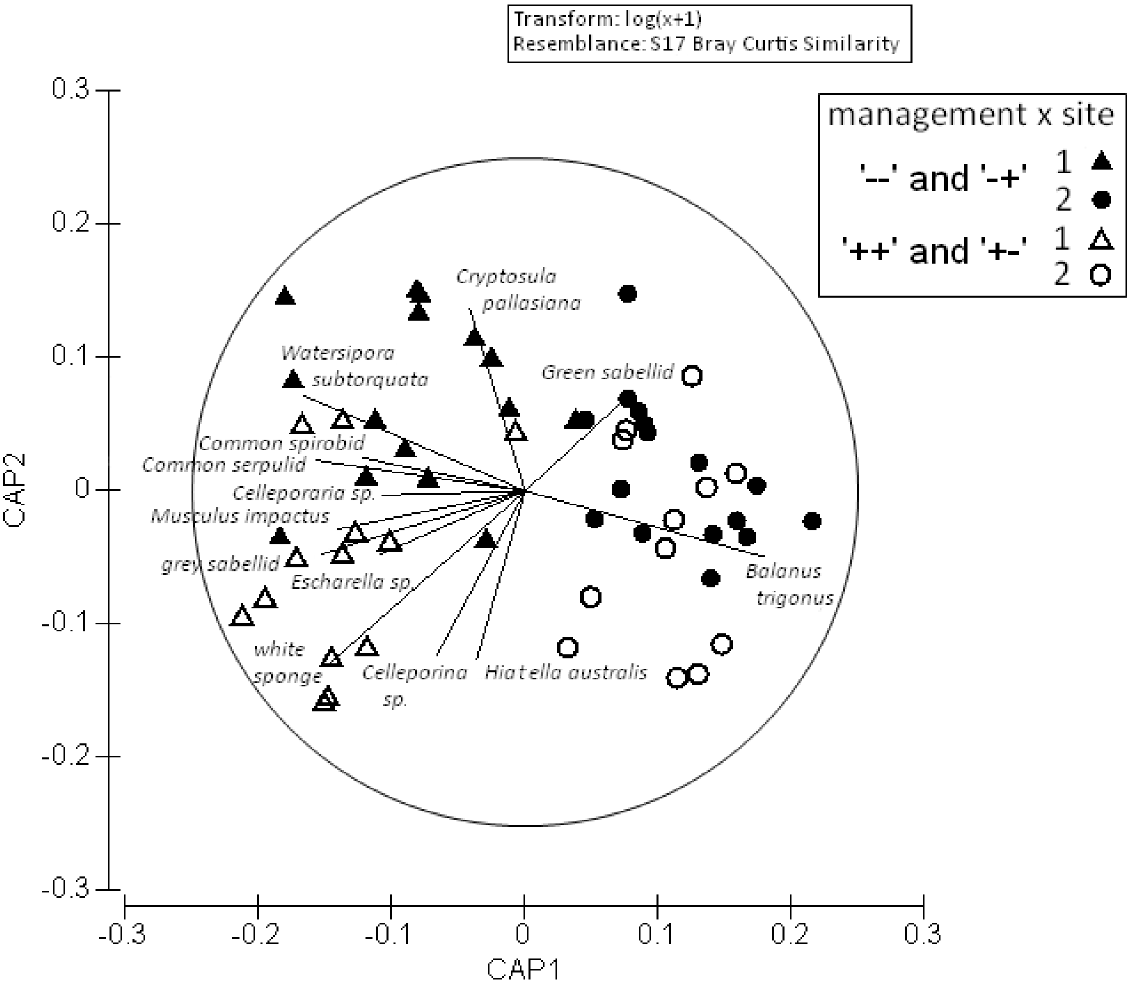


Figure 4.10: Settling invertebrate species were influenced by main effects of site (Pseudo-$F_{(1,52)}$: 16.02, $p < 0 .001$) and 'copper origin' (Pseudo-$F_{(1,52)}$: 2.53, $p < 0.01$), but not 'disturbance' (Pseudo-$F_{(1,52)}$: 0.68, $p = 0.74$). Canonical analysis of principle coordinates (CAP) ordination of invertebrate assemblages was used to illustrate the main effects of 'copper origin' and 'site'. Principal species differences between Sites 1 (triangles) and 2 (circles) is largely captured by the x-axis (CAP1), while assemblage differences between '--' and '-+' (closed symbols) and '++' and '+-' (open symbols) levels of the factor 'copper origin' are illustrated by the y-axis (CAP2). The vector overlay tells the strength (length of the line toward the circular boundary, which represents a 100% correlation) and direction of associations between individual species and certain samples. To simplify the plot, only species that correlated with a CAP by more than an arbitrary value of 0.4 were included.

Table 4.17: 2-way multivariate PERMANOVA to test the null hypotheses that there is no variation in settling macrofaunal assemblage for (1) exposure to copper, a fixed factor with two levels (copper and no copper); and (2) site, a random factor with two levels. Resemblance matrices were based on Bray Curtis similarities (Bray and Curtis 1957; Clarke et al. 2006). A log(x+1) transformation was applied to down weigh super-abundant species and decrease distortion stress in the resemblance matrix. Significant differences are shown in bold. Interaction terms with a $p > 0.25$ were pooled with the residual to increase the power of tests (Underwood 1997) and are marked as such. A total of three degrees of freedom were pooled with the residual.

source	df	MS	Pseudo-*F*	*p*
Copper (c)	1	1158.6	2.53	**0.01**
Disturbance (d)	1	327.04	0.68	0.74
Spatial variation (sv)	1	7189.6	16.02	**<0.001**
c x d	1	POOLED		
c x sv	1	POOLED		
d x sv	1	POOLED		
Pooled Residual	52	448.68		
Total	55			

4.4 DISCUSSION

Here the impact of copper exposure on the development of benthic marine communities was examined in terms of the legacy effect of copper on the structure, chemistry and biology of natural biofilms and settling macro-invertebrate assemblages. In summary it was found that: (1) exposure to copper had no impact on the total amount of biofilm or the relative amounts of organic and inorganic material contained within the biofilm thus rejecting H1 and 2; (2) the amount of copper sequestered by biofilms was proportional to the degree and length of exposure thereby supporting H3 and 4; (3) while a directionless change in the copper exposure regime altered biofilm microphyte composition, algal assemblages contained within biofilms showed no clear response to copper origin thus rejecting H5; (6) exposure to copper decreased the photosynthetic health of the biofilms supporting H6 and; (7) while copper exposure altered the composition of macroinvertebrate assemblages there was no effect of changing copper state in the first two weeks of assemblage development thus rejecting H7.

4.4.1 Temporal Contingencies in Disturbance Ecology

These findings reinforce the importance of timing and history of disturbance in community assembly (Dayton 1971; Sousa 1979a; Sousa 1979b; Sutherland 1980; Underwood and Chapman 2006). In experiment one it was found that copper concentrations decreased over time from the greatest concentration in the youngest biofilms to a low plateau in the older biofilms. It might be that age is an important determinant in biofilm responses to copper, as seen for other metals (Ivorra et al. 2000). Younger biofilm structures are dominated by the proteins, polysaccharides and lipids of the EPS (Sabater et al. 2007), while older films contain larger amounts of algal and bacterial biomass (Barranguet et al. 2003).

The polysaccharide component of the EPS has been to shown to be effective at binding copper (Mittelman and Geesey 1985), thus potentially forming an outer layer of dead cells that buffer the living biofilm from further contamination (Teitzel and Parsek 2003). It is likely that the higher concentrations seen here in the younger biofilms reflect these early community-level defence mechanisms at work. Biofilms that experienced an increase in copper exposure (-+) did not increase in copper concentration 'after' the manipulation (Figure 4.5). It may be that the biofilms had aged to a point that the various defence mechanisms found in mature films (Harrison et al. 2007) had been established and sudden exposure to copper was subsequently ineffectual. Alternatively, confounding by temporal factors may have played a role, that is, some random factor associated with specific sampling times is responsible for the observed effect (Underwood and Chapman 2006). For example, concentrations of copper found in the biofilms may have been driven by the background concentrations of copper found in the water column, although given that ambient levels throughout the entire experiment were generally far below values expected to negatively impact upon even small numbers (<1%) of marine fauna (ANZECC/ARMCANZ 2000; 0.3µg.L-1 for copper) this is unlikely to be the cause. Many experiments designed to unravel the factors associated with time find starting time, end time and experiment duration to be unavoidably correlated and thus difficult to define and classify for a robust experimental design (Chapman 2002). Differences in any of these may account for differences in observations in experiments one and two, particularly those associated with the spectral, HPLC and chlorophyll-a extraction data that test H5. Confounds such as these are further complicated when they coincide with random events, such as the rainfall events documented here for experiment one. To avoid confounds associated with random events researchers must ensure there is proper replication within levels of the experiment as is done here (e.g. multiple before and multiple after sampling; Underwood 1992; Underwood 1994; Kelaher et al. 2003). This is particularly relevant to community assembly ecology where successional and seasonal changes can be difficult to distinguish (Underwood and Chapman 2006). To improve generality those experiments need to be replicated in time as well as space (Vaughn and Young 2010), although this is difficult, particularly in longer term research programs (Lindenmayer and Likens 2010).

4.4.2 Direct and Historical Effects of Copper on stages of Community Assembly

4.4.2.1 Physical Structure of Biofilms

There was no evidence that exposure to elevated concentrations of copper reduced total mass and greater amounts of organic matter relative to inorganic matter in the biofilms leading to a rejection of hypotheses 1 and 2. Previous studies have shown exposure to copper to decrease the production of biomass and alter the balance between organic and inorganic matter in biofilms in concentrations as low as 1µM (Soldo and Behra 2000; Barranguet et al. 2003; Massieux et al. 2004). At two magnitudes less than 1µM, perhaps the concentrations used here are too low to elicit changes in the total and relative amounts of organic and inorganic matter in biofilms.

4.4.2.2 Chemistry of Biofilms

Exposure to copper increased the total amount and concentration of copper found in the marine biofilms observed here (Table 4.5; 4.6). This response is similar to that observed for freshwater biofilms, where accumulation of copper is clearly proportional to the degree of exposure (Ferris et al. 1989; Barranguet et al. 2002; Massieux et al. 2004; Bhaskar and Bhosle 2006). The amount and concentration of copper is greater in those biofilms from the ‘++’ and ‘+-’ regimens of copper exposure than those in the ‘--’ and ‘-+’ regimes of exposure

(‘++’ is equal to ‘+-’ which is greater than ‘--’ which is equal to ‘-+’). Previous studies have shown that even after 28 days from depuration, concentrations of copper remain up to 25 times greater in treated biofilms than in controls (Boivin et al. 2006). During the recovery phase after the source of copper is removed, biological impacts, such as altered biochemistry, metabolism and resource use, can be ongoing (Griffiths et al. 2000; Kiikkilä et al. 2001; Boivin et al. 2006). Given that the concentrations of copper observed here were up to three orders of magnitude smaller than the maximal adsorptions of copper observed in freshwater monospecific (Bitton and Freihofer 1977; Norberg and Persson 1984; Kim et al. 1996) and community biofilms (Massieux et al. 2004), it may be that the copper sequestered by the biofilms in this experiment may be rendered inert to the degree that they are unable to impact settling invertebrates (Harrison et al. 2007).

4.4.2.3 Algal Biology of Biofilms

While there was no effect of exposure to copper on the total amounts or concentrations of chlorophyll-a and phaeopigment, exposure to copper led to more phaeopigment relative to live chlorophyll-a. Degradation of chlorophyll in response to 3mg.L^{-1} copper has been observed in the chlorophyte genus *Scenedesmus*, although that concentration was much higher than that used here. Further chlorophyll-a data were provided by HPLC analysis in experiment two and the smoothed data field spectrometry analysis of both experiments. The HPLC data suggested that chlorophyll-a was apparently sensitive to exposure to copper but only at Site 2. Thus, increased copper exposure has reduced biomass. This notion is complicated by the spectral data from both experiments. The smoothed spectral data from experiment one showed reduced amounts of pigments (photosynthetic biomass; Murphy et al. 2009) in response to the factor ‘disturbance’, that is, rather than exposure to elevated amounts of copper, a change in copper state, irrespective of direction, was correlated with the observed effect (Table 4.10), while the smoothed data from experiment two indicated no effect of copper on the amount of pigment at all. The observed differences may be explained by methodological disparities, which are discussed below.

Previous studies have shown exposure to lower levels of copper limits growth in many phototroph species including diatoms and cyanobacteria (Gibson 1972; Rachlin et al. 1983; Les and Walker 1984). In the HPLC and 2nd derivative spectral data from experiment two there was no effect of copper beyond the HPLC-detected changes in chlorophyll-a apparent at Site 2 (Table 4.13). Conversely, the 2nd derivative spectral data of experiment one show that the relative abundances of pigments changed over time and that ‘disturbance’, explains some of the observed changes in assemblage composition. This result is counter-intuitive, as the interaction between ‘disturbance’ and ‘proximity to disturbance’ suggests that assemblages sampled on day 24 are similar to those sampled on day 39 and that assemblages sampled on day 27 are similar to those sampled on day 34. It is possible that biofilm microphytes of days 24 and 39 are similar because they were both sampled the day after large rainfall events of 49 and 39mm respectively (BOM, 2013), and that changes in microphytic communities from the nutrient and fresh-water pulse (Paerl et al. 1990) have masked any of the subtler differences that may exist between assemblages sampled on day 27 and day 34; however, this does not explain differences between assemblages that have and have not experienced a disturbance. While changes in assemblage composition ‘after’ the disturbance are potentially attributable to opportunistic species capitalising on the change in copper state, changes in those biofilms sampled ‘before’ the disturbance can hardly be explained as driven by their proximity to the disturbance. It might be that a completely different force such as reduced biofilm maturity at day 27 (Yallop et al. 2000) may be responsible for the apparent similarity of these assemblages to those recovering from the change of copper state at day 34.

Irrespective of the driver 'before' the disturbance, changes in assemblage composition have not been attributed to exposure to copper ('copper origin') *per se*.

In the literature, observations as to which underlying species are responsible for the repeated observed effects of copper on community structure are inconsistent: copper exposure in biofilms has been reported to select for diatoms over cyanobacteria (Brand et al. 1986; Havens 1994; Miao et al. 2005), *vice-versa* (Barranguet et al. 2000; Barranguet et al. 2003; Massieux et al. 2004), and in some instances to provide no selective advantage to either taxon (Igic 1982; Fernandes and Henriques 1991). Variation in findings between studies could be explained by differential responses between algal species (Medley and Clements 1998; Genter and Lehman 2000), changes in the bacterial community (Massieux et al. 2004; Boivin et al. 2006), and/or the roles of uncontrolled environmental factors such as temperature (Barranguet et al. 2003), water chemistry (Baccini 1985), the presence of sediments (Shi et al. 1998; Barranguet 2000; Gillan 2004) and hemisphere-level spatial differences in communities and ecology (as noted by Inglis 1996; Chapman and Tolhurst 2004; Murphy et al. 2009). Disparities in results may also be dependent on the methods used. For example, while HPLC and chlorophyll-a extraction fluorometry are able to assess entire biofilms, field spectrometry and PAM only assess that which forms the top layer of the sample, which may be comprised of dead cells causing the detail of living cells hugging the substrata to be obfuscated (Teitzel and Parsek 2003; Kromkamp et al. 2006). Chlorophyll-a extraction fluorometry, HPLC and PAM are primarily sensitive to photosynthetic pigments, while reflectance profiles record information about whatever they are pointed at and may record changes associated with the non-pigment components of the biofilm (Murphy et al. 2005a). Irrespective of the mechanisms, while the changes in the relative amounts of chlorophyll-a and phaeopigment and the spatially-dependent changes in chlorophyll-a detected by HPLC in experiment two provide weak support for H5, that is, that copper exposure affects phototroph assemblage composition, the remaining chlorophyll-extraction, HPLC and spectrometry data do not.

The chlorophyll-a:phaeopigment ratio and 2nd derivative spectral data from experiment one suggest an alternate hypothesis: that a directionless change in copper status, be it exposure-to or depuration-from copper does affect algal assemblage composition. This notion would explain the lack of an effect of copper found in the spectral data of experiment two, where there was press exposure to copper, but no change in copper state (Table 4.14; 4.14). Thus, it is proposed that such a change in copper state could facilitate positive flux in community structure without threatening community structure, sensu the intermediate disturbance hypothesis (Connell 1978; Flöder and Sommer 1999). This may function as an alternative hypothesis for those studies that examined pulse exposures of copper (for example, Barranguet et al. 2000; Barranguet et al. 2003; Massieux et al. 2004; Miao et al. 2005), but cannot be used for those where exposure to copper was chronic (for example, Barranguet et al. 2002; Sun et al. 2013). Other differences in communities' response between this study and those mentioned above may be due to disparities between responses by marine and freshwater assemblages or the degree of exposure used, which can be unrealistically high in many eco-toxicological studies (Calow 1998). It bears clarifying here that given the use of interchangeable collars is designed to minimise procedural disturbance and that all assemblages, including the control, also had their collars changed it is unlikely that the 'disturbance' effect was a procedural artefact and was associated with the physical change of the disturbance rather than the change in copper state.

Exposure to even small concentrations of copper reduced photosynthetic health, as measured by PAM fluorometry (Table 4.16). This finding is supported in the literature (Barranguet et al. 2002; Mallick and Mohn 2003; Massieux et al. 2004), and has been attributed to the disruptive influence of copper on electron transport in Photosystem II (Shioi et al. 1978; Küpper et al. 2002), the process to which PAM fluorometers are sensitive (Schreiber 2004). This finding confirms that copper had a detrimental effect on the function of the biofilms in the lead up to settlement by macroinvertebrates, which was tested by H7.

4.4.2.5 Subsequent Development of Settling Macroinvertebrate Assemblages

There was no interaction between 'copper origin' and 'disturbance' in experiment two; only main effects of copper exposure and site were detected (Table 4.17). Assuming that there had been no invertebrate settlement by the 24 day mark, this suggests that the composition of macroinvertebrate assemblages that have been exposed to copper was likely mediated by direct toxicity to the macroinvertebrates rather than indirectly through settlement on biofilms containing a copper legacy, which, as shown here, vary in chemistry and ecological structure in response to copper exposure and copper state disturbance. That said, given that it is impossible to know exactly when invertebrate settlement began, or to observe whether the settling invertebrates are taking up copper that has been bound by the biofilm, this insight remains untested. If copper did alter these biofilms, this alteration might have impacted invertebrate settlement as seen for numerous aquaculture species in response to films dominated by diatoms (Fitt et al. 1989; Rahim et al. 2004; Tung and Alfaro 2011) This may be driven by the duration of exposure for each stage of the copper history regime; the first stage lasted only two weeks before collars were changed and the settlement plates exposed to the opposing copper treatment for the remaining 14 weeks. An interaction may have appeared if the first stage were extended for longer, though then the researcher runs the risk of a secondary settlement pulse occurring before the collars are changed. The result may also have been affected by the timing of the collar change relative to recruitment events as the timing of recruitment can affect rates of succession (Farrell 1991); perhaps the collar change occurred at a time not proximal to settlement by invertebrates and the biofilms may have recovered from the copper exposure before any major recruitment events, however, given the retention times of copper found in the biofilms here, this recovery is unlikely to have happened before settlement had occurred. Irrespective of the cause, a solution to this would be to sample biofilms over numerous occasions in anticipation of recruitment events; this would require considerable luck in timing and a great number of AUH. Previous studies on the effect of copper exposure on the settling invertebrate communities of Sydney Harbour have found serpulids and some bryozoan species, including *Bugula nerita* and *W. subtorquata,* to increase in abundance at the expense of less-tolerant species such as solitary ascidians, *Balanus* species of barnacles and other bryozoan species including *Schizoporella spp*, *Microporella lunifera, Beania megellanica,* and *Celleporaria nodulosa* (Johnston and Keough 2000; Johnston and Keough 2002; Johnston and Keough 2005; Piola and Johnston 2006a; Dafforn et al. 2008; Piola and Johnston 2008; Piola and Johnston 2009). In a recent study, while assemblages found inside an Australian marina were dominated by bryozoans, spirobids and sponges while barnacles, ascidians and non-spirobid polychaetes were found in nearby Batesmans Bay, it is difficult to tell whether these differences were driven by copper status or other environmental variables including lead, warming or higher turbidity that were also associated with the marina (Rivero et al. 2013). Here it was found that polychaetes and barnacles were more closely associated with site rather than copper status; spirobids and serpulids were found in abundance at Site 1, while *B. trigonus* was strongly correlated with Site 2 (Figure 4.10). These seemingly-random patterns have been observed before, where site and season have been used to explain variation in species and assemblage-level responses to copper exposure (Johnston and Keough 2005; Piola and Johnston 2006b). This is particularly true for polychaetes, which may be benefiting from factors such as enhanced nutrient status which coincides with severe sediment contamination in highly-modified estuaries (Dafforn et al. 2013). Here differences between copper exposures were attributed to a species of *Celleporina*, an entangling bivalve *H. australis* and a calcareous sponge, which were found on the control plates, and the invasive *Cryptosula pallasiana* and a species of green sabellid, which were found in the copper-exposed plates. The notion that invasive bryozoan species such as *C. pallasiana* are found in disproportionate abundance in copper-contaminated sites may be due to their having been transported on ship hulls coated in antifouling paint; their innate resistance to copper is what allows them to successfully arrive in new locations beyond their native range (Piola et al. 2009; Piola and Johnston 2009).

4.4.3 Conclusion

In conclusion, while the exact mechanisms driving macrofaunal composition in response to copper exposure are complicated it appears that invertebrate assemblages are more sensitive to the direct effects of copper, rather than indirect effects as delivered through biofilms that have been impacted by copper. This is a positive result for the use of biofilms as a management tool in the remediation of polluted sediments or the sequestration of contaminants from the water column (Gadd and White 1993; Barnes et al. 1994; von Canstein et al. 1999) as the biofilms appear capable of neutralising copper without compromising their role in preparing substrata for settlement by invertebrate taxa (Dayton 1971), which may be of economic-relevance to benthic aquaculture species. The research done in this chapter highlights the potential for temporal confounding to impact the outcomes of community assembly and dictates that the impact of a disturbance cannot be properly interpreted without accounting for possible temporal contingencies. It is recommended that in future experiments a more intense sampling regime is used to identify any fractal dimensions in composition the biofilms may exhibit in response to copper over time as this would give greater resolution on the relative sizes of the direct and indirect effects of copper on benthic invertebrate communities. Additional work should also observe what happens to the copper once is has been bound by the biofilm. Does the copper remain in the biofilm indefinitely or is it release back in to the water column once the surrounding water is less contaminated? Is the copper ingested by protozoa, snails, and other grazers and detritivores or is it absorbed by settling organisms? Answers to these questions would give certainty to the inference that settling organisms are more vulnerable to copper in the water column that copper that is bound in biofilms.

CHAPTER FIVE: GENERAL DISCUSSION

5.1 SUMMARY OF EXPERIMENTAL OUTCOMES

5.1.1 Chapter Two

The longer-term effect of warming (decreased body size) will be of greater ecological consequence than any shorter-term direct effects of higher temperature that may be experienced by individuals. There was no observed effect of eutrophication on any compartment of this food web, which is counter to large sections of the literature and might be explained by the relatively low doses of nutrients that were used. The potential for indirect interactions is identified, whereby stressors may interact by impacting different species that share in a trophic relationship.

5.1.2 Chapter Three

Removal of refugia caused a downshift in the optimal body size ratio for strong interaction strengths between predators and prey. This implies that losses of habitat complexity will place selection pressure on larger prey individuals and should be added to the list of anthropogenic stressors expected to cause gradual reductions in the average body size of species populations over coming decades.

5.1.3 Chapter Four

The extent of the legacy effect of copper contamination, that is, the amount of copper sequestered and retained in marine biofilms, depends on the degree and length of exposure to copper. Microphytic assemblages contained within these biofilms are sensitive to directionless changes in copper concentration in the surrounding water column. As the effect of copper on invertebrate assemblages was similar irrespective of copper legacy or microalgal community in the biofilm, it appears that biofilms have inbuilt compensatory mechanisms to ensure their function in preparing surfaces for recruitment by fauna are not perturbed by copper exposure. This work highlights the importance of accounting for a host of temporal contingencies including legacy effects, ecological history and the coincidence of random events such as rainfall for identification and interpretation of the underling mechanisms in community assembly.

5.2 CHOICE OF METHOD AND SYSTEM INFLUENCES RESULT

The use of multiple technical methods and instruments to monitor different compartments of ecological assemblages exemplifies how results of experiments are tempered by the lens through which they are observed. For example, in any experiment it is possible that the results observed are a function of those species chosen as focal organisms, particularly in laboratory studies where the experimenter usually has complete control over environmental and ecological factors (Connell 1974; Lawton 1999). For instance, in chapter three interaction strength between amphipods and *F. vesiculosus* was much higher than between amphipods and *F. serratus*. If *F. vesiculosus* had been used instead of *F. serratus* in the mesocosm experiment in chapter two the trophic cascade may have been much stronger.

While it is widely accepted that the only true tests of ecological theory are done using empirical field data (Connell 1974; Shrader-Frechette and McCoy 1993; Underwood 2000), use of mesocosm arrays such as those described in chapter two is justified as they strike a balance between the need to manipulate environmental factors while still allowing for natural variability (Yvon-Durocher et al. 2011). The array at Queen’s University Belfast allowed the warming of experimental assemblages above ambient, while its supply of fresh seawater straight from Strangford Lough still allowed for diurnal and seasonal cycles of temperature and colonisation by a diverse range of species including planktonic microalgae, macroalgal spores and microfauna such as copepods.

In chapter two examination of the amphipod communities revealed only an effect of the presence and absence of crabs and it was not until *F. serratus* biomass was also quantified that an effect of decreasing crab size was detected (Figure 4.4). Failure to examine the sessile primary producer compartment of assemblages, thereby capturing the effect of predation on mobile grazers, might explain the conflicting results observed in experiments attempting to understand the importance of large predators in marine sub-littoral assemblages (Hall et al. 1990).

In chapter four, if fewer means of observation had been used to examine the characteristics of biofilms, it would have been difficult to understand the various phenomena taking place. That is, by looking at the physical and chemical structure of biofilms it was possible to discern that biofilms were able to absorb copper while maintaining constant concentrations of copper, but this strategy was unable to determine any biological response to copper sequestration (Table 4.4; 4.5; 4.6). However, analysis of the spectral profile did detect changes in the biology of the films in response to a copper disturbance; there were changes in the relative amounts of various pigments (Table 4.10; 4.11). Interpretation of both indicates compensatory mechanisms, rather than simple resistance or resilience, are used in response to copper exposure, which is a conclusion that is more descriptive than would be possible with less information. It is clear that complex natural systems are best understood when observed from multiple vantage points.

Finally, it bears mentioning that when running multiple statistical analyses the observer runs the risk of an inflated family-wide error rate, whereby the probability of making at least one type I error during the entire series of tests exceeds 0.05 (Sokal and Rohlf

2012). Thus, each view point should be carefully considered and associated with clear hypotheses (Underwood 1991; Underwood 1999; Underwood et al. 2000).

5.3 TEMPORAL CONTINGENCIES

In chapter two, the effects of decreasing body size as a longer term effect of warming exhibited the potential to cause more changes to a system than the direct short term effects of increased temperature. Other research across a variety of ecological systems has also demonstrated that changes in food web size structure can have a larger effect on community metabolism and biogeochemical fluxes than does warming (Yvon-Durocher et al. 2012). It is also likely that community responses to reductions in the average body size of populations will be further complicated by other longer-term effects such as the creation of novel communities due to alterations in the ranges of species as facilitated by warming (Byers and Pringle 2006), and the experience of unusual environmental conditions that are characterised by extremes that are more intense than currently found in nature (McElroy et al. 2012).

While many studies discussed elsewhere in this thesis have examined the effect of copper contamination on marine communities, few have considered the legacy effect of copper retained within biofilms after the original source of copper has been removed or the potential negative effect copper might have on the ability of biofilm to prepare the surface for subsequent settlement. In chapter four it was found that the amount of copper sequestered by biofilms is directly related to the degree and length of exposure, the assemblage response observed is mediated by timing of sampling and recruitment events, and that the micro-algal assemblages within these biofilms are sensitive to changes in copper state, irrespective of whether the changes involve exposure to or depuration from copper, than exposure to elevated copper per se. It is possible that in previous studies where assemblage change has been attributed to an increase in copper toxicity may be also explained to some degree by a directionless change in copper status that has benefitted opportunistic species. Conversely, we detected no effects of disturbance history on the composition of the settling macro-invertebrate assemblage. It appears that any legacy effect of the observed changes in copper concentration and micro-algal assemblage in the biofilm on the settlement preferences of macro-invertebrates is swamped by differences driven by large natural variation and exposure to increased levels of copper. This was unexpected given the widely-acknowledged importance of biofilm composition and structure in the recruitment preferences of macro-invertebrates (Scheltema 1961; Wieczorek and Todd 1997; Wieczorek and Todd 1998; Tung and Alfaro 2011). Biofilms offer protection to their inhabitants through a variety of defence mechanisms including absorption and immobilization of toxicants, precipitation of metal complexes, and utilisation of usually-dormant persister cells that are able to multiply and replace their less-tolerant conspecifics (Harrison et al. 2007). It may be that even in the presence of a persistent copper legacy, biofilms in nature are able to compensate for the toxicity and maintain biofilm functionality in the preparation of substrata for settlement (Harrison et al. 2007).

By focusing on the shorter and longer term effects of warming, the research presented in chapter two will help us to better predict community grade responses to near-future anthropogenic disturbance. Rather than just describing the ultimate effects of copper disturbance on marine benthic systems, the focus on the legacy effect of copper and multiple stages of marine benthic succession has given further insight into the mechanisms driving the commonly-observed effects (for example, Barranguet et al. 2000; Johnston and Keough 2002). The results of both experimental chapters reiterate the need for greater focus on temporal contingencies in ecology.

5.4 EFFECTS OF ANTHROPOGENIC DISTURBANCE

5.4.1 Are all disturbances equal?

The experiments described in chapter two examine two of the direct (warming) and indirect (decreasing body size) effects of climate change. Though indirect effects are difficult to unravel experimentally when compared to direct effects (Kordas et al. 2011), the mesocosm experiments reported in this thesis have done so. Here I have shown that stressors such as warming, overexploitation and habitat simplification directly interact to impact a single species or indirectly interact by affecting separate species that share a trophic relationship (Binzer et al. 2012; Jochum et al. 2012). In other studies indirect effects can be of greater ecological consequence than their direct counterparts (Connell et al. 2011). This raises the following question: given that subsequent to any disturbance some species will be ‘winners’ and others ‘losers’ (Somero 2010) and that these impacts will be passed on as indirect effects depending on the strength and number of trophic interactions associated with the ‘winners’ and ‘losers’ (Pimm 1991; McCann 2000; Emmerson and Raffaelli 2004), are all disturbances equal? While the results from the experiments shown in chapters two to four would suggest that disturbances are not equal with regard to direct effects as different species have responded to different stressors, once indirect effects are considered, nuances between direct effects of disturbances become more trivial. For example, in chapter two the effects of warming and decreasing crab size are blurred when considering the indirect effect of each stressor on amphipod herbivory of *F. serratus.* It may be that difference would be more indistinct if the magnitude of disturbance were held constant across stressors, if such a notion were possible to quantify. Certainly, this is the case for those biofilms experiencing a change in copper state in chapter four; the microalgae assemblages responded similarly to increases and decreases of copper of diametrically-opposed magnitude. Exploration of this concept would require development of a metric for magnitude of disturbance. This could be done via preliminary trials that determine and compare the direct lethal (or sub-lethal) effects of various stressors on individual specimens of numerous focal species to create a disturbance coefficient similar to the Q_{10} temperature coefficient, but for mortality rate (or some other sub-lethal response variable) over units of disturbance. While this would require a large body of data, this data could be collected rapidly in a mesocosm setting whereby direct effects would not be masked by indirect effects or ecological noise. The population (or functional trait level) changes in focal species' characteristics in response to these stressors could then be the subject of experiments in nature that would measure the manifestation of indirect effects. Comparison of these data sets might allow researchers to make general predictions about system-wide responses to disturbance, which has been a long standing aim of ecology (Arbuckle 2003).

5.4.2 Effects of multiple anthropogenic disturbances

In the first experimental chapter, I examined the response of a simple marine food web to simultaneous exposure to warming, nutrients and reductions in the average body size of the top predator in the system. Interestingly, I found strong patterns associated with the main effects of warming and manipulation of top predator size, but no effect of eutrophication and no interactive effects between any of the factors. These effects were manifested as an increase in mortality rates for *C. maenas* and some species of amphipod in response to warming and a trophic cascade whereby the reduced top-down predation pressure exerted by smaller *C. maenas* on their amphipod prey translated to a negative effect on *F. serratus*, which is associated with intertidal amphipods (Ingólfsson and Agnarsson 2003). *Chaetogammarus maenas* is likely choosing to feed on *F. serratus* ahead of the other species of algae for reasons of palatability (Steneck and Watling 1982), that *C. marinus*' mouthparts (mandibles particularly) are potentially suited to processing leathery macrophytes such as *F. serratus*. A lack of interactive effects on marine assemblage structure were also observed between eutrophication and decreasing *C. maenas* body size in a field experiment in Lough Hyne, Ireland (Jochum et al. 2012). Similarly, main effects of warming and nutrient enrichment, but no interactive effects, have been observed to affect size structure and density in an alpine grassland food web (de Sassi et al. 2012). Conversely, interactive effects between warming, nutrients and size structuring are common in the literature: for instance, by strengthening top down effects (Barton et al. 2009; Kratina et al. 2012) as predators become more generalist (O'Gorman et al. 2012b), and increase consumption rates to meet the demands of accelerated metabolism (Lang et al. 2012), warming has been shown to diminish increased bottom-up productivity and counteract the paradox of enrichment (Rall et al. 2010; Vucic-Pestic et al. 2011; Kratina et al. 2012; Shurin et al. 2012). The relative effects of warming and nutrients are also dependant on the size structuring of the community, where small body size is advantageous at warmer temperatures, but this benefit is lost with increasing eutrophication (Binzer et al. 2012). Here I observed warming- and body-size-driven alterations in top down control with no apparent changes to bottom-up productivity. Changes in the relative importance of top-down versus bottom-up controls (Power 1992; Hoekman 2010) can lead to unbalanced populations of coexisting species (Berlow et al. 2009) with severe consequences such as increased rates of predator starvation (Rall et al. 2010; Twomey et al. 2012), reduced species richness (O'Gorman et al. 2012b), and hence decreased ecosystem stability (Emmerson et al. 2005). It may be, however, that the stressors examined here will interact more obviously with other anthropogenic stressors. For example, communities responding to the global effects of ocean warming face additional pressure from ocean acidification, increased $p\text{CO}_2$ and micro-plastic debris (Derraik 2002; Halpern et al. 2008; Thompson et al. 2009; IPCC 2013). In isolation, acidification may decrease species’ defence capability through shell dissolution (Orr et al. 2005), and marine filter feeders have shown adverse effects to the ingestion of plastic debris (Thompson et al. 2004; Browne et al. 2008), though the ecological consequences of simultaneous exposure to all three is still largely unknown, particularly in the context of warming.

Thus, the experiments described in chapter three found that changing body size ratio consistently disrupts trophic interactions between *C. maenas* and its amphipod prey, while the effect of removing a prey refuge is contingent on the size of the refuge relative to the size of the prey. Interactions between body size ratio and access to refuge have implications for a down shift in the optimal body size ratio for predation which might lead to prey populations with smaller average body sizes in a simplified and homogenized world. Given the role habitat complexity plays in maintaining high diversity as seen in kelp holdfasts and on rocky shores (Hauser et al. 2006; Matias et al. 2010), future work is planned to examine the roles species diversity, warming and habitat structure play in predator-prey interactions. I predict that warming will interact constructively with greater predator diversity and removal of refugia to increase rates of predation and predator generalism on assemblages of mussels, oysters, gastropods and amphipods. For experiments including *C. maenas*, this effect will be more pronounced outside of its native range. This would support the notion that climate change and losses of biodiversity will disturb food webs and that this effect can be exacerbated by some invasive species.

This thesis has greatly advanced the aquatic copper ecotoxicology literature by moving from simpler studies of the effect of copper on ecosystem structure to looking at how copper interferes with the function of marine systems, in this case the preparation of surfaces and the mechanisms underlying the development of mature benthic marine communities. Logistical limitations meant that the scope of the experiments in chapter four was bounded to a single stressor focus, which is common for much of the copper ecotoxicology literature (for example, Barranguet 2000; Johnston and Keough 2002; Piola and Johnston 2006a). A better understanding of the role of copper as a disturbance would be gained if studies were done in a multi-stressor context , similar to the multiple environmental gradients considered in Sun et al. (2013), but using manipulative experiments. For example, temperature may determine tolerance of certain taxa to copper (Bouvy et al. 2000) and given that levels of sedimentation and water chemistry can affect the bioavailability of copper (Fernandes and Henriques 1991; Teitzel and Parsek 2003) experimental manipulation of these and other physical aspects of the environment would test the hypothesis that copper toxicity is mediated by other anthropogenic stressors and allow development of a more holistic picture of community responses to copper. This approach could and should be extended to studies of other metal contaminants thereby going a long way to addressing the criticisms made by Mayer-Pinto et al. (2010) that ecologists do not have a clear understanding of what metals actually do in ecosystems.

While exposure of assemblages to warming, changes in food web size-structure, habitat simplification and contamination by nutrients and copper has led to strong system-altering effects in the experiments described in this thesis, these types of changes are often associated with in ultimate increase in community tolerance in polluted sites as sensitive species are replaced with tolerant ones (Bååth et al. 1998; Rutgers et al. 1998; Muñoz et al. 2001; Van Beelen et al. 2001). The negative implications of these adjustments on the structure and functioning of communities are potentially grim (Sala et al. 2000; Tylianakis et al. 2008), though in some systems functionally-similar species have undergone compensatory increases, leading to a maintenance of ecological functions (Shackell et al. 2010) In the least, given anthropogenic stressors are forces over which we have fundamental control (Poloczanska et al. 2007; Ghedini et al. 2013), better management of terrestrial runoff (Power et al. 1999), reversal of fishery-induced size changes (Conover et al. 2009) and the reduction of greenhouse gas emissions to conform to the IPCC Representative Concentration Pathway 2.6 “best-case” scenario present positive opportunities for management of the potential and ongoing impacts of these disturbances.

5.5 SUMMARY OF KNOWLEDGE ADVANCEMENTS

Experiments described in this thesis have advanced our knowledge of general ecology by highlighting the importance of accounting for temporal contingencies by showing that the shorter- and longer-term effects of global change can have disparate effects, the timing of sampling and ecological events can influence observations and examination of all stages of succession are required to properly interpret assemblages responses to disturbance. Our comprehension of the effects of anthropogenic disturbance has been improved for: (1) the discipline of intertidal food web ecology, where it was suggested that longer-term reductions in population body size in response to warming might exacerbate trophic cascades that are already occurring due to the present day overexploitation of marine predators; (2) predator-prey allometry, where it is now apparent that trophic relationships exhibit an interactive response to simultaneous changes in the relative predator/prey body size and the domain of the interaction and not just to alterations of one factor or the other; and (3) the benthic community assembly literature by suggesting that the presence of a disturbance is sometimes more important than the type of disturbance; and that marine biofilms are able to compensate for exposure to copper such that their role in preparing substrate for settlement by marine biofilms is maintained.

I have integrated experiments that use two ecological systems and different anthropogenic stressors to show how disturbance can have varied direct impacts on assemblages and individuals, but once indirect effects are taken into account, the identity of the impact can become less influential than the magnitude of the impact. Rather than continuing in a piecemeal fashion, whereby ecologists look at the effects of particular stressors on particular systems, the disturbance ecology community would greatly benefit from a concerted effort to develop an index of disturbance, whereby the magnitude of various types of disturbance is quantified in a way that is comparable across stressors and can be easily tailored for the species and systems of interest.

INDEX OF FIGURES AND TABLES

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