The importance of selective filters on vessel biofouling invasion processes

by

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Abstract

The spread of exotic species is considered to be one of the most significant threats to ecosystems and emphasises the need for appropriate management interventions. The majority of marine non-indigenous species (NIS) are believed to have been introduced via ship biofouling and their domestic spread continues to take place via this mechanism. In some countries, biosecurity systems have been developed to prevent the introduction of NIS through biofouling. However, implementing biosecurity strategies is difficult due to the challenges around identifying high-risk vectors. Reliable predictors of risk have remained elusive, in part due to a lack of scientific knowledge. Nonetheless, invasion ecology is an active scientific field that aims to build this knowledge. Propagule pressure is of particular interest in invasion ecology as it describes the quantity and quality of the propagules introduced into a recipient region and is considered to be an important determinant in the successful establishment of NIS. Environmental history affects health and reproductive output of an organism and, therefore, it is beneficial to examine this experimentally in the context of biofouling and propagule pressure. The aim of this thesis was to examine how voyage characteristics influence biofouling recruitment, survivorship, growth, reproduction and offspring performance through the ship invasion pathway. This was to provide fundamental knowledge to assist managers with identifying high-risk vessels that are likely to facilitate the introduction or domestic spread of NIS, and to understand the processes affecting biofouling organisms during long-distance dispersal events. Chapter One provides an introduction to the issues addressed in this thesis. Each data chapter (Chapters Two – Five) then focused on a stage of the invasion process and included field experiments using a model organism, *Bugula neritina*. Finally, Chapter Six provides a summary of key findings, discussion and the implications to biosecurity management. Throughout this thesis, the effect of donor port residency period on the success of recruits was highlighted. Chapter Two focused on recruitment in the donor region. As expected, recruitment increased with residency period. Importantly, recruitment occurred every day on vulnerable surfaces, therefore, periods as short as only a few days are able to entrain recruits to a vessel hull. The study presented in Chapter Three showed that there was high survivorship of *B. neritina* recruits during 12 translocation scenarios tested. In particular, the juvenile short-residency recruits (1-8 days) survived voyages of 8 days at a speed of 18 knots; the longest and fastest voyage simulated. Interestingly, variation in voyage speed and voyage duration had no effect on the survivorship of recruits, but did have legacy effects on post-voyage growth. Again, *B.*

neritina which recruited over very short residency periods of 1 day continued to perform well after translocation and had the highest level of reproductive output after the voyage scenarios (Chapter Four). Recruits that were older (32-days) and reproductively mature at the commencement of the scenarios failed to release any propagules. Even though the number of 'at sea' and 'port residency' days were equal, reproductive output was higher after short and frequent voyages than after long and infrequent voyages. Finally, the study presented in Chapter Five examined transgenerational effects of *B. nertina*. Results showed that although the environmental history of the parent colony had a carry-over effect on offspring performance, it was the offspring environment that was a stronger determinant of success (measured by reproductive output and growth). Although cross-vector spread is possible (i.e. parent and offspring both fouling an active vessel), offspring released from a hull fouling parent into a recipient environment will perform better. In combination, these studies have provided new insights into NIS transport via vessel biofouling. Although shipping pathways are dynamic and complex, these results suggest that juvenile stages that recruit over short residency periods and are then translocated on short voyages, may pose a higher risk for NIS introduction than originally assumed. This has implications for marine biosecurity management as short residency periods are common and short, frequent voyages are typical of domestic vessel movements which are largely unmanaged.

Contents

Chapter One: General Introduction

Over the centuries, human activities associated with trade and travel have resulted in the introduction and establishment of non-indigenous species (NIS) to most marine ecosystems around the world (Molnar et al. 2008). Although many NIS introductions are relatively recent and it may take decades or centuries to determine their ecological and evolutionary effects (Crooks and Rilov 2009), there are many examples where NIS have had a range of significant ecological and economic impacts (Pimentel et al. 2000, Bax et al. 2003, Colautti et al. 2006). Due to future changes in climate, technology, trade and a growing human population, NIS introductions are forecast to increase and their spread is considered to be one of the most significant threats to marine ecosystems (Carlton and Geller 1993, Stachowicz et al. 2002, Bax et al. 2003, Occhipinti-Ambrogi 2007, Ricciardi 2007, Hulme 2009). Unlike habitat loss which can potentially be reduced or reversed, NIS are rarely eradicated from a region once established (Lonhart 2009).

NIS in the marine environment

NIS are introduced into coastal marine environments deliberately for recreational and aquaculture purposes or, more frequently, accidentally through vessel movement (commercial and recreational), fisheries and the aquarium trade (Ruiz et al. 2000). Vessels can transport NIS as spores or larval stages in ballast water, or as biofouling, which is the accumulation of unwanted organisms on hulls and other submerged parts of a vessel (Bax et al. 2003, Coutts and Dodgshun 2007, Inglis et al. 2010). After the initial introduction of a NIS into a region, secondary spread can occur by natural dispersal and human-assisted transport, such as domestic shipping, boating and aquaculture (Carlton 1996, Forrest et al. 2009). Ports and marinas are considered hot spots for NIS around the globe and are often where new incursions first establish. This is due to the high levels of disturbance, an abundance of artificial habitat, such as pilings, pontoons and seawalls (Dafforn et al. 2009), and the high supply of NIS shipping provides (Carlton 1996, Drake and Lodge 2004, Hayes et al. 2005, Sylvester et al. 2011).

Biofouling is thought to have been the transport mechanism responsible for the majority of species introductions to coastal systems worldwide. For example, in New Zealand greater

than 69% of introductions are attributed to biofouling (Cranfield et al. 1998); 78% in Port Philip Bay, Australia (Hewitt et al. 2004a), 90% in Hawaii (Godwin 2003), 62% in Croatia (Gollasch 2007), and 50% in Italy (Gollasch 2007). The invasion pathway via ship biofouling involves multiple successive stages (Floerl 2002, Lewis and Coutts 2009): (1) recruitment to a vector at a donor location, (2) translocation to a recipient destination, (3) transfer from the hull to the recipient environment (introduction), (4) colonisation of the local habitat/s and establishment of a self-sustaining population, and (5) spread and associated impacts (Figure 1.1). Like other pathways, various selective filters act during each stage and dictate which organisms move through to the next step in the sequence (Kocak et al. 1999, Coutts and Taylor 2004, Floerl and Inglis 2005, Dafforn et al. 2008, Clark and Johnston 2009, Piola et al. 2009, Coutts et al. 2010a, Hopkins and Forrest 2010). Selective filters may severely restrict the number of biofouling species that are translocated on vessels; however, observational studies show that a large number of species are still arriving into new regions through this process (Gollasch 2002, Davidson et al. 2010, Inglis et al. 2010, Clarke Murray et al. 2011, Sylvester et al. 2011, Zabin et al. 2014). For example, 187 species were recorded on 508 international vessels arriving into New Zealand between 2004 and 2007 (Inglis et al. 2010). Additionally, 170 fouling taxa were recorded on the hulls of 40 merchant vessels in the Ports of Vancouver and Halifax (Sylvester et al. 2011). Although the viability and reproductive status of these organisms was not established, their presence is indicative of species-specific responses to the filters, including tolerance to toxic antifouling treatments, and also highlights untreated or ineffectual areas on the hull which are left open to colonisation (Coutts and Taylor 2004, Dafforn et al. 2008, Piola and Johnston 2009, McKenzie et al. 2012).

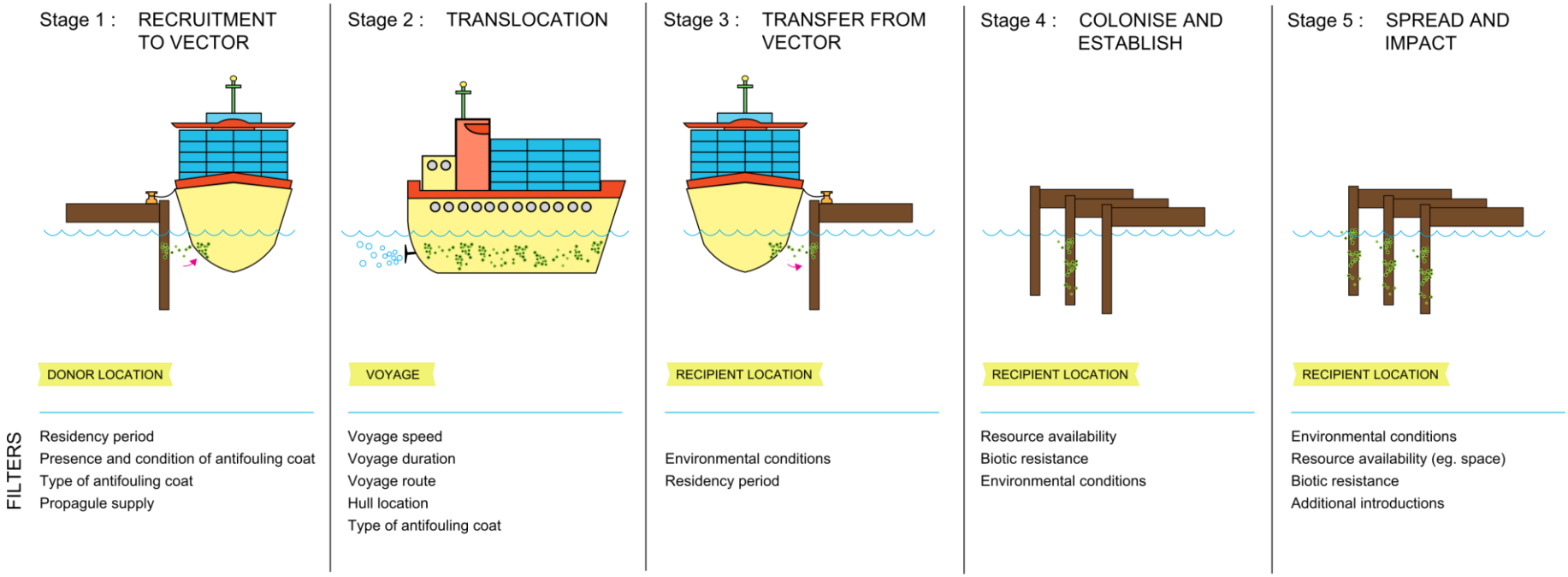


Figure 1.1: Five stages of the transfer and establishment of non-indigenous species by vessel biofouling and example filters relevant to each stage.

Marine Biosecurity and the challenge of identifying high-risk vessels, with a focus on New Zealand

To address the pressing issue of unintentional NIS introductions, some coastal nations have established 'marine biosecurity systems'. These systems aim to exclude, eradicate or effectively manage economic, environmental and human health risks posed by NIS through risk identification, prevention, surveillance, and targeted responses (Biosecurity Council 2003). New Zealand is one of only a handful of countries (others include Australia and the United States (Dahlstrom et al. 2011)) that have marine biosecurity anchored in national policy. There are currently around 339 marine NIS recorded in New Zealand (K. Seaward, *personal communication*), although many of them are yet to be seen as harmful (Cranfield et al. 1998, Hayden et al. 2009). NIS that have spread beyond their first point of discovery include the Mediterranean fan worm *Sabella spallanzanii*, the alga *Undaria pinnatifida*, the club ascidian *Styela clava* and the colonial ascidian *Didemnum vexillum* (Coutts and Forrest 2007, Russell et al. 2008, Morrisey et al. 2014), all of which are likely to have arrived via biofouling. It is probable that these species will have some impact on the New Zealand environment, as they have in other parts of the world. For example, in Port Philip Bay (Australia), *Sabella spallanzanii* has impacted scallop fisheries and demersal fish populations (Currie et al. 2000) and recruitment, survival and growth of sessile taxa (Holloway and Keough 2002a, b). Currently, there are a number of high-impact invaders not present in New Zealand, such as the northern Pacific sea star *Asterias amurensis*, the aquarium weed *Caulerpa taxifolia*, the European shore crab *Carcinus maenas*, the Chinese mitten crab *Eriocheir sinensis* and the Asian clam *Potamocorbula amurensis*. There is a need to maintain effective marine biosecurity to prevent the introduction of these or other potentially high-impact NIS.

Biosecurity is included in New Zealand's national legislation, primarily under the Biosecurity Act (1993). Policy framework and intervention is established and led by the Ministry for Primary Industries (MPI) and focuses on pre-border, border and post-border stages of the invasion process. New Zealand has some good management systems including: (1) Port Biological Baseline Surveys to document which species are present (Inglis et al. 2008, Seaward et al. 2015), (2) ballast water management (IMO 2005, Ministry for Primary Industries 2005), (3) a Marine High Risk Site Surveillance programme carried out at 11 main domestic shipping ports and marinas that aims to detect new incursions of high-risk target

species early, when abundances are low and successful control may be more likely (Morrisey et al. 2014), (4) incursion response protocol (Biosecurity Council 2003) and (5) public outreach programmes and stakeholder partnerships (Biosecurity Council 2003).

Domestic pathways, such as domestic shipping, boating and aquaculture operations, are largely unmanaged despite management being important for containing incursions and preventing spread to areas of high value. Additionally, strategies in New Zealand are heavily end-stage focused and new incursions continue to occur (Morrisey et al. 2014). Although the end-stages are a vital and natural starting point for biosecurity systems to progress, it is also important to focus on improving preventive measures (Mack et al. 2000). Prevention is difficult to implement due to a lack of resources, the practicality of implementation without impacting trade and a lack of fundamental understanding of the invasion pathway and processes. Recently in 2014, MPI introduced a Craft Risk Management Standard (CRMS) that requires vessels arriving into New Zealand for a period of 21 days or more ("long-stay vessels") to arrive with a 'clean' hull that has no more than a slime layer and goose barnacles (Ministry for Primary Industries 2014). If staying for a period of 20 days or less ("short-stay vessels"), then a slime layer, goose barnacles, macroalgae and low coverage of one type of organism (either tubeworms, bryozoans or barnacles) is acceptable. Post-border surveys provide evidence that many international vessels arriving into New Zealand do not meet the CRMS (Inglis et al. 2010). As it is not feasible to check all vessels that do arrive, the challenge is how to administer the CRMS in a cost-effective manner. To do this, high-risk vessels must be identified, thereby allowing resources to be focused strategically within the constraints of economic and technological limitations.

A high-risk vessel may be defined as one that has a relatively high likelihood of facilitating a NIS introduction or establishment event. Such a vessel may be expected to have an abundance of biofouling organisms and/or organisms that have a high level of fitness, environmental tolerance and ability to produce healthy offspring. Identifying high-risk vessels, however, is difficult due to the substantial variation in the occurrence and abundance of biofouling organisms between vessels. Also, the fitness of the biofouling organisms is unknown – will they survive and reproduce, and will their propagules cope in the new environment? Essentially, there is a severe lack of underpinning knowledge regarding determinants of biofouling extent and fitness (Inglis et al. 2010). Consequently, it is unclear which vessels should be prioritised as high-risk and inspected or otherwise managed.

Currently, MPI requires 48-hours notice pre-arrival through an Advanced Notice of Arrival form that provides information including length of intended stay in New Zealand and previous locations of extended stationary periods (Ministry for Primary Industries 2010). Utilising this information could allow high-risk vessels to be identified, yet how risk changes with variation in these factors is largely untested.

Taking the focus away from the recipient environment and how underpinning ecological theory can help

Management decisions regarding NIS should be guided by underpinning scientific evidence. Although a number of invasion ecological theories have been proposed, the development and testing of new theories has tended to focus on recipient environments and traits of invasive species, such as space limitation, environmental mismatch, competition from native fauna, fast growth, early maturity, and high fecundity (Mack et al. 2000, Ruiz et al. 2000, Berezina 2007, Johnston et al. 2009). Invasibility may be influenced by these factors, but their explanatory importance fluctuates from negligible to highly important (Clark and Johnston 2009). Recently there has been a realisation that the arrival of individuals, known as propagule pressure, is vital to understanding invasion success (Lockwood et al. 2005). Propagule pressure is multifaceted and is a measure of: (1) the number of individuals released into a region (propagule size), (2) the number of release events (propagule number), and (3) the quality and the genetic diversity of the individuals released (Lockwood et al. 2005, Hedge et al. 2012).

There is strong evidence in terrestrial systems of a positive relationship between propagule pressure and population establishment for historic introductions, as seen for insects, birds and mammals (Green 1997, Wolf et al. 1998, Ahlroth et al. 2003). The significance of propagule pressure is also emerging as paramount to marine invasion likelihood (Johnston et al. 2009). For example, it is likely that many of the NIS present on the reefs of southeast Florida are due to the high number of aquarium releases (Semmens et al. 2004). Results from novel manipulative field experiments on algae (Valentine and Johnson 2003, Britton-Simmons and Abbott 2008) and bryozoans (Clark and Johnston 2005, Clark and Johnston 2009) provide further evidence of the importance of propagule pressure on introduction success. More recent research has teased apart the importance of each intrinsic parameter (propagule size, number, quality and genetic diversity) of propagule pressure on the recruitment of some

Australian NIS (Hedge and Johnston 2012, Hedge et al. 2014). Although pivotal in demonstrating the importance of propagule pressure in the marine introductions, these studies have again focused on the final stages of the invasion pathway; once the propagules have been released in the recipient location. It is now important to determine which pre-recipient environment processes result in some organisms having high quality or low quality when they arrive in a new environment. Identifying those determinants could perhaps help to identify effective ways of mitigating risk. It has been suggested that establishing the determinants of propagule pressure, particularly in the marine environment where control at the border is difficult, is one of the few realistic management options to prevent or control marine invasions (Johnston et al. 2009). It could also be used to assist in the identification of high-risk vessels (i.e., those more likely to apply high propagule pressure).

Propagule pressure at the recipient location (Stage Three, Figure 1.1), which occurs both at international borders and through domestic pathways, relies on propagules being supplied to the recipient location following successful completion of early pathway stages: (1) recruitment to a vector in the donor location, and (2) propagule translocation (Figure 1.1). Vessels can transport species over far greater distances and shorter timeframes than what is capable through natural dispersal alone by overcoming dispersal barriers (Godwin 2003). In classical marine ecology studies, dispersal is often the first stage that must be examined to understand and predict recruitment patterns and community assembly. However, in biofouling invasions, propagule translocation, which is akin to dispersal, and propagule pressure will not occur unless there is recruitment to the vector first. Recruitment to a vector marks the initiation of the invasion pathway and is a foundational stage that will influence eventual propagule pressure at the recipient location. Understanding recruitment and survival during translocation will help to elucidate the determinants of propagule pressure at the border.

What do we know about recruitment, translocation survivorship and propagule pressure determinants?

Post-voyage observational studies have identified a number of risk factors, but many of these studies do not always have consistent findings. For example, some studies have shown that the age of the anti-fouling coat, are reliable predicators of biofouling abundance (Coutts 1999, Floerl and Inglis 2005, Davidson et al. 2009, Sylvester et al. 2011), but others have

shown no relationship with abundance (Davidson et al. 2010, Ashton et al. 2014). At times, data obtained through observational studies do not have strong predictive power when used in risk modelling. For example, in a study by Inglis et al. (2010), 24 variables were selected to determine their power to predict biofouling extent (measured by level of fouling scores). Some of the variables did influence biofouling, such as voyage history for yachts and maintenance and vessel design on commercial vessels, but the predictive power was relatively low. The presence or abundance of NIS on a vessel may not be the only useful predictor of risk but also their condition, including viability, ability to produce healthy offspring and survive could also matter.

Previous studies have used the number of ship arrivals as a proxy for propagule pressure (e.g., Drake and Lodge 2004), but relationships were not strong and the models have been criticised for their simplicity. For example, Verling et al. (2005) and Briski et al. (2013a) examined variation in propagule pressure in ballast water and found that it varied between vessels and is not a simple function of total ship arrivals. This is because not all vectors and voyage characteristics are the same. Voyage characteristics are variations in the filters that influence arrival of species (Figure 1.1); including length of residency period, vessel speed, voyage duration, condition of antifouling coating and voyage frequency. Variation in these will determine the environment that the hull biofouling organism experiences. As the environment influences an organism's survival and fitness, it is likely that biofouling-mediated propagule pressure will be altered by voyage characteristics. The influence of pre-recipient environment factors on the physiological condition of biofouling organisms, and their ability to produce strong propagules that are going to be able to establish in a new environment, will be important in propagule pressure and colonisation success. This, however, has not yet been studied.

Thesis overview

Thesis outline

The hypotheses tested in this thesis were designed to elucidate determinants of recruitment, propagule survivorship and reproductive output through the biofouling invasion process. This was done to provide knowledge which may underpin the identification of high-risk vectors, which is a vector that has high probability of introducing a new species due to the presence of

high quality recruits that are likely to release viable, robust propagules into the new region (i.e., mediate high propagule pressure). This may help to implement the biosecurity strategies designed to reduce NIS introduction and spread. Each chapter of this thesis examines one of the sequential steps along the invasion pathway and selected associated voyage characteristics (i.e., the filters that influence arrival (Figure 1.1)) that may impact recruitment, propagule survivorship and reproductive output. Chapter Two specifically investigates the relationship between vessel residency period in port and recruitment of resident biofouling organisms to their hulls (Stage One: recruitment to the vector). *In situ* recruitment was measured to determine the likelihood and extent of exporting organisms from a donor location. To further understand the importance of residency period, the survivorship (i.e., supply[1]) of organisms that recruited over different residency periods was measured during voyage scenarios that differed in speed and duration in Chapter Three (Stage Two: translocation). As post-voyage survivorship and growth are also important indicators of future propagule potential, these were measured after each scenario. Chapter Four tests how residency periods in the donor location and the voyage pattern influenced propagule pressure to the recipient location (Stage Three: transfer from hull of vector in the recipient location). This stage, and Stage Four (colonise and establish), are investigated further using transgenerational plasticity as an underlying mechanism in Chapter Five. Finally, the main findings are summarised and synthesised in Chapter Six.

Model organism: Bugula neritina

The experiments conducted as part of Chapters Three, Four and Five of this thesis used a model organism, *Bugula neritina* (hereafter referred to as *Bugula*), to examine some of the research questions outlined above. *Bugula,* a cheilostome bryozoan (Class Gymnolomata; Family Bugulidae), was selected for a number of reasons. Firstly, *Bugula* displays life-history characteristics common to invasive species, such as rapid growth and maturation, high reproductive output (both sexual and asexual) and a wide environmental tolerance (Keough and Chernoff 1987, Floerl et al. 2004, Piola and Johnston 2006). *Bugula* is a colonial invertebrate that grows asexually through the addition of new zooids in an upright, bushy form (Figure 1.2). Reproductive maturity is generally reached when colonies have bifurcated approximately five times which can occur in as little as 2 weeks (Keough and Chernoff

[1] In this thesis the term propagule "survivorship" during translocation is used interchangeably with the term propagule "supply". A propagule that survives the translocation stage has essentially been supplied to the recipient location, conversely, for a propagule to be supplied to a location it must survive the translocation stage.

1987), although the local *Bugula* observed during this study reached maturity at around four bifurcations after 28 days post-settlement. Each reproductive zooid is hermaphroditic, and only one larva is brooded at a time for a period thought to be around 1 week (Woollacott and Zimmer 1975). Colonies can tolerate elevated levels of copper pollution and are frequently recorded in port environments (Piola and Johnston 2006).

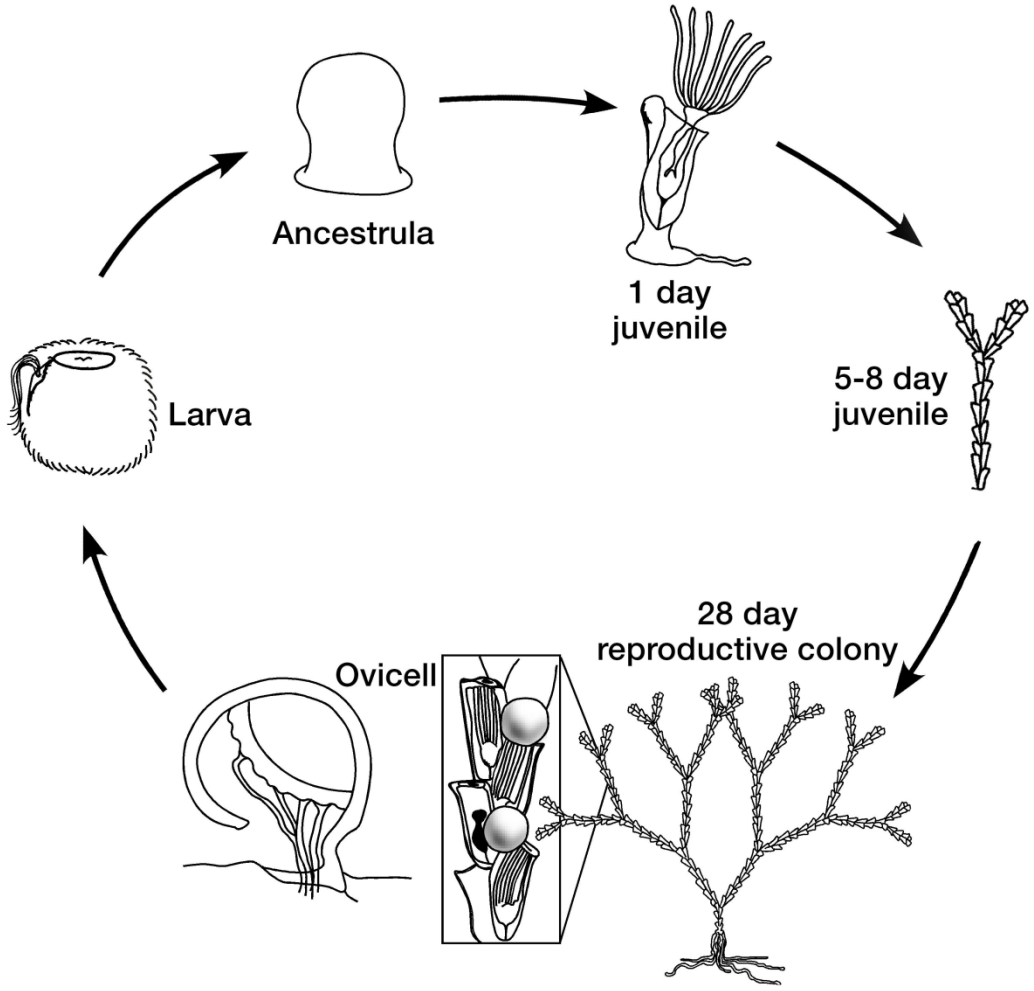


Figure 1.2: Life cycle of *Bugula neritina*. Based on Figure 1 Sharp et al. (2007) with permission from original illustrator, Kelyn Davidson.

In addition, *Bugula* is a filter feeder and this feeding mechanism is likely to be compromised during biofouling translocation. Zooids create feeding currents to filter food using the lophophore, a crown of ciliated tentacles, which likely selectively filters phytoplankton (but not armoured phytoplankton, such as diatoms <50 µm in size (Kitamura and Hirayama 1984)). They may also be able to feed on bacteria (Gosselin and Qian 2000). Consequently, food intake will likely be compromised during a voyage due to hydrodynamic forces inhibiting the feeding process (e.g., if the recruit is attached to the laminar flow region) or

due to travelling through food-poor environments (e.g., if the recruit is attached in a protected niche area). Food levels can, therefore, be used as a proxy for voyage duration.

Further, *Bugula* is a ubiquitous biofouling organism commonly recorded on boat hulls (and other structures) in temperate to sub-tropical coastal waters. Although it is not on the New Zealand Register of Unwanted Organisms, an Australian CSIRO hazard analysis of marine pests has classified it as having moderate-to-high impact potential in Australia (Hayes et al. 2005). In New Zealand, *Bugula* is established in all ports (Gordon and Mawatari 1992), and is thought to have been introduced in 1949 through vessel biofouling (Gordon and Mawatari 1992, Cranfield et al. 1998). An MPI survey of international vessels arriving in New Zealand showed that *Bugula* was recorded on 95 of the 508 vessels sampled (Inglis et al. 2010). Interestingly, *Bugula* was recorded on 50% of all yachts sampled, as well as a single container/general cargo vessel and a single passenger vessel. Results of this survey also showed that *Bugula* occurred most frequently in niche areas and on dry-dock support strips than on other hull surfaces. *Bugula* has a NIS status in New Zealand, yet is not regulated by legislation and so can be used in field and laboratory experiments.

Finally, *Bugula* has been used successfully in many other laboratory and field studies (Wendt 1996, 1998, Bone and Keough 2005, Piola and Johnston 2006, Burgess and Marshall 2011a, McKenzie et al. 2012). Colonies regularly bifurcate approximately every four zooids (Keough and Chernoff 1987) providing a measurement tool for size. This is often referred to as the branching score (Keough and Chernoff 1987, Bone and Keough 2005, Piola and Johnston 2006). Larvae are brooded in large visible chambers called ovicells (Woollacott and Zimmer 1972), which allow reproductive maturity to be easily determined. At spawning a portion of larvae are released. These lecithotrophic larvae are relatively large (c.300 – 400 µm) and have a short dispersal period, mostly settling within a few hours of release (Woollacott and Zimmer 1975, Keough and Chernoff 1987). *Bugula* larvae are easily obtained in the lab by light-shocking reproductively mature colonies. Peak reproduction and growth occurs over the summer months, like many biofouling organisms. Colonies regress back to dormant basal stolons over the cooler winter period (Bone and Keough 2005). All of these attributes make *Bugula* an apt model organism that can provide insights into the biofouling invasion process and its management.

Study sites

The experiment presented in Chapter Two was conducted at the Port of Lyttelton and the experiments in Chapters Four and Five were conducted at the Naval Point Yacht Club Psych Jetty in Magazine Bay (Figure 1.3). The Port of Lyttelton and Magazine Bay are both located on the northern side of Lyttelton Harbour, south of Christchurch city on the eastern coast of the South Island, New Zealand. Lyttelton Harbour is a 15 km long narrow embayment shaped by volcanic activity with rocky basalt, mud and sand beaches forming the shoreline. Coarse sand and crushed shell sediment is distributed through the middle of the harbour and mud covers most other areas. The hills surrounding Lyttelton Harbour are a source of fine loess sediment into the harbour and there is high sedimentation at these experimental sites. The high tide range in Lyttelton Harbour is 1.64 m (neap tides) to 1.92 m (spring tides) (Heath 1975).

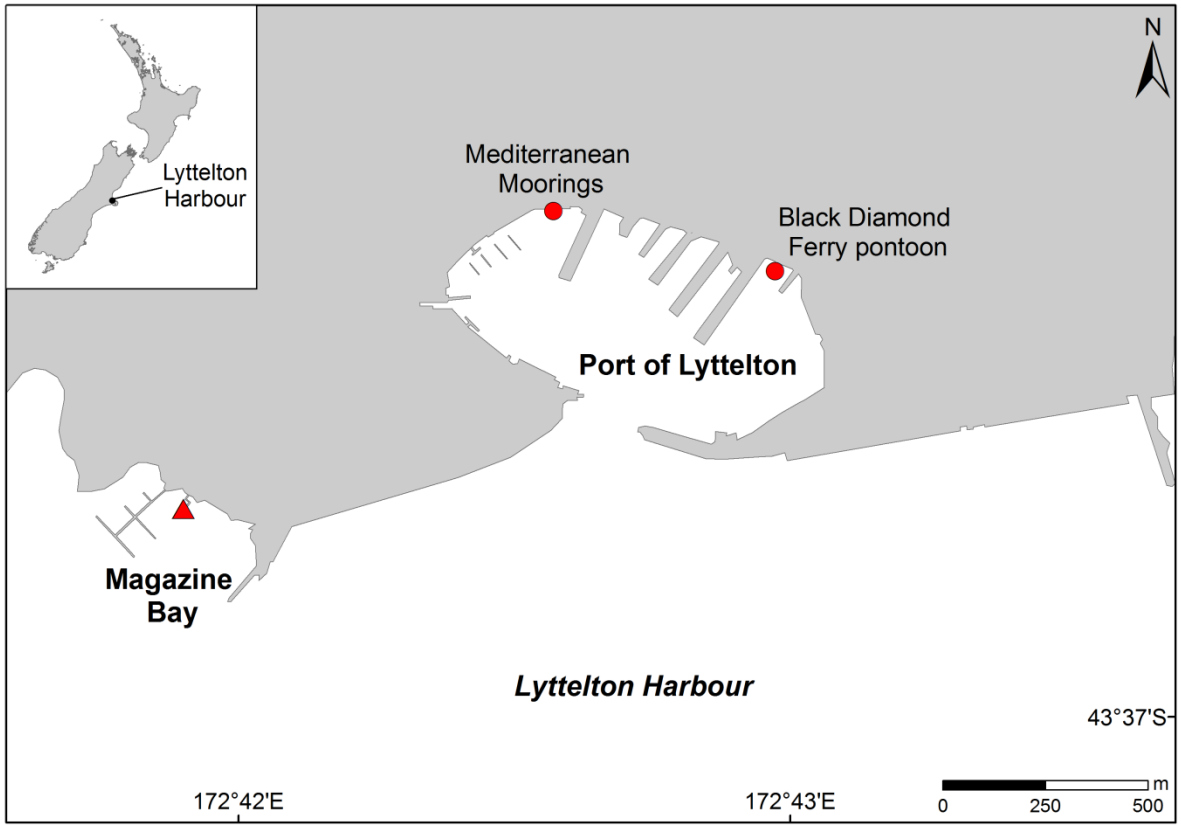


Figure 1.3: Port of Lyttelton and Magazine Bay. Circles depict study sites in the port and a triangle represents the marina study site.

The Port of Lyttelton is the third largest deep water port in New Zealand and the busiest port in the South Island (Lyttelton Port of Christchurch 2013). Commercial, recreational, domestic and international vessels use the Port of Lyttelton. A 2004 Port of Lyttelton baseline invertebrate survey identified 269 species, 23 species with non-indigenous status and two on the New Zealand register of unwanted organisms (Inglis et al. 2008). Eighteen of these 23 species are thought to have been introduced via vessel biofouling. Magazine Bay Marina is located approximately 1 km from the Port of Lyttelton and has high recreational yacht traffic. The Psych Jetty is used for temporary moorings and permanent moorings are located c. 20 m away at the main Magazine Bay Marina complex. There are no documented biodiversity surveys of the Psych Jetty, however, through personal observation, it is suggested that the fauna are similar to those found in the adjacent Port of Lyttelton. The sessile temperate marine community is dominated by bryozoans, ascidians (both colonial and solitary), algae, molluscs and crustaceans. A number of NIS have been observed at the jetty including *Undaria pinnatifida, Watersipora subtorquata, Bugula neritina, Styela clava, Caprella mutica* and *Ciona intestinalis*.

The Chapter Three study was at BAE Systems ship yard, Williamstown, Australia (Figure 1.4). Here I ran a mesocosm experiment on a shipping wharf. I used Hobson Bay to grow pre-experimental *Bugula* and as a water source for the experiment. Hobson Bay is located within Port Philip Bay which has high shipping traffic and around 160 introduced and cryptogenic species (Hewitt et al. 2004b). These sites were chosen as this is a hot spot for NIS, high vessel movement and ease of site access.

Figure 1.4: Location of BAE Systems ship yard (black circle), Hobson Bay, Australia.

Specific Aims

Utilising *Bugula* and these study sites, in this thesis I specifically tested:

- Chapter Two: the abundance and species richness of biofouling community in the Port of Lyttelton over 1, 5 and 15 day residency periods.
- Chapter Three: the survival and growth of *Bugula* colonies on experimental plates during and after voyage scenarios that varied in donor location residency period (1, 8 or 29 days), speed (6 or 18 knots) and duration (2 or 8 days).
- Chapter Four: the reproductive output of *Bugula* colonies after voyage scenarios that varied in duration and frequency (short-frequent or long-infrequent) and donor port residency period (1, 8 or 32 days).
- Chapter Five: The effect of the *Bugula* parent colony experience (‘at sea’ or ‘port residency’) on the survivorship, growth and reproductive output of the offspring colonies in a similar, or different, environment to that of the parent.

Chapter Two: Temporal variation in biofouling vector recruitment: How risky are short vessel residency periods?

ABSTRACT

Recruitment to a ship hull in the donor location marks the initiation of a translocation event for a known or potential invasive species and is, therefore, a key stage where interventions can take place. There is substantial variation in the occurrence and abundance of biofouling organisms between vessels, including vessels of the same type, despite the use of antifouling coatings. Despite recent efforts better predictors of biofouling are needed to reduce the risk of new introductions and the spread of established non-indigenous species (NIS). There is a perception that vessels undergoing very short port residency periods are unlikely to pose a significant risk for the spread of NIS. However, an empirical investigation of risk estimates in relation to residency periods is so far lacking. The aim of this chapter was to examine the relationship between vessel residency periods in ports (ranging from very short to relatively long) and recruitment of resident biofouling organisms to their hulls. Settlement plates mimicking highly susceptible (not protected by antifouling) hull surfaces were deployed in the Port of Lyttelton, New Zealand, over three residency periods: 1, 5 and 15 days, and then inspected for recruitment. An experimental design involving continuous plate replacement allowed the determination of total and daily recruit abundance and richness, as well as variation in these variables. Recruitment occurred on a daily basis throughout the 30-day experimental period. There was a significant effect of residency period on mean abundance of total recruits (1 day mean (± S.E.) = 20 ± 1; 5 day = 61 ± 7; 15 day = 300 ± 39), but not on average daily recruitment. Total taxonomic richness also increased (1 day mean (± S.E.) = 3.6 ± 0.06; 5 day = 4.9 ± 0.144; 15 day = 7.1 ± 0.17), but the average daily richness decreased

with residency period. Community structure differed between residency periods; trends were driven by turnover of algae, ascidians, bryozoans, ciliophorates, crustaceans, molluscs and polychaetes. These results indicate that vessel hulls with unprotected surfaces can be at risk of entraining recruits from the local larval pool during residency periods as short as 1 day. However, recruitment will vary on a daily basis and repeating the experiment during a range of seasons would be beneficial. Many commercial and merchant vessels (e.g. container and passenger vessels) have short residency periods, including international vessels and domestic vessels. Short residency recruits are small and, using current inspection methods, will be indistinguishable from the slime layer which is an allowable level of biofouling on international vessels arriving into New Zealand. The success of recruits that settle and grow over different residency periods will be examined in the following chapters of this thesis to investigate the biosecurity risks posed during subsequent invasion stages.

INTRODUCTION

Recruitment to a vessel hull marks the beginning of the invasion process for many biofouling species (Chapter One: Figure 1.1) and is a prerequisite to successful translocation by marine vessels (Prendergast et al. 2010). Despite the strong, economically-driven increase in antifouling technologies, the transfer of marine organisms via vessel biofouling continues to occur. This is due to a number of reasons, including: use of inappropriate antifouling coats (Floerl et al. 2005, Dafforn et al. 2011), failure to maintain coats (Davidson et al. 2008), cleaning of coats (Ralston and Swain 2014), damage to the antifouling surface, (Otani et al. 2007, Piola and Johnston 2008), untreated or ineffectual areas (Minchin and Gollasch 2003, Coutts and Taylor 2004, Coutts and Dodgshun 2007) and tolerance to antifouled surfaces by organisms (Hoare et al. 1995a, Hoare et al. 1995b, Floerl et al. 2004, Cassé and Swain 2006, Dafforn et al. 2008, Piola and Johnston 2009, McKenzie et al. 2012).

As antifouling paints are not a panacea to preventing vessel biofouling recruitment, managers need meaningful variables that can be used to help identify vessels that are of a high risk of transporting biofouling species, including NIS. Vessel residency period in previous destination ports is a potential risk factor. It may be assumed that little or no recruitment occurs during short residency periods (i.e., low risk), whereas the opportunity and encounter rate of competent larvae with hull surfaces increases with longer residency periods. However, examining residency period from a larval ecological perspective indicates that there can be larval recruitment in a single day and that the production of larvae does not occur at a

constant rate but in pulses (Blythe and Pineda 2009, Giménez 2010). Post-larval processes then reduce the number of propagules that make it to recruitment. This is an example of where an applied management issue needs to be underpinned by science. Although a relationship between time spent in port and abundance of biofouling organisms has been shown in observational studies by Sylvester et al. (2010, 2011), recruitment during short residency periods may not always be insubstantial. Many vessels have tight time schedules and residency periods less than 14 days. For example, 73% of passenger vessels, 58% of container vessels and 72% of car carriers arriving into New Zealand between 2000-2005 resided at individual ports for less than 1 day; 87% of bulker vessels, 78% of heavy lift and 60% of Roll-on-Roll-off vessels resided for between 1 and 14 days. In contrast, 97% of recreational vessels resided for more than 14 days during the same period (Inglis et al. 2012). Currently, there is little understanding of whether such vessels are equally or unequally likely to 'pick up' resident species and facilitate their dispersal to future destinations.

A better understanding of whether short residency periods can affect the 'biosecurity risk' of vessels is relevant for both international and domestic shipping pathways. Domestic vessels travelling from high-risk localities, such as port environments with high numbers of NIS, to high value areas are of particular concern. In New Zealand these areas include aquaculture regions (e.g., Marlborough Sounds), fisheries areas (e.g., Chatham Islands), marine reserves (e.g., Fiordland or the Poor Knight Islands) and other special regions (e.g., sub-Antarctic Islands). Domestic pathways are largely unmanaged and there is currently no science based guidance on the likelihood of vessels to 'pick up' NIS as a function of the time they spend in donor ports or anchorage. Strategies are currently being developed to protect Fiordland (Fiordland Marine Guardians 2014) and the sub-Antarctic Islands (New Zealand Department of Conservation 2011) from NIS. However, a lack of information regarding the relative risk posed by vessels with different voyage and 'behaviour' profiles is a challenge to the development of effective management frameworks.

The risk of international vessels that spend short residency periods in ports overseas before arriving into New Zealand is also unknown. The recent introduction of the Craft Risk Management Standard (CRMS) in New Zealand, which is currently in a 4-year voluntary period before it becomes mandatory in 2018, states that international arriving vessels staying in New Zealand for a period of 21 days or more cannot arrive with more than a slime layer and goose barnacles fouling the vessel. The slime layer is defined as "A layer of microscopic

organisms, such as bacteria and diatoms, and the slimy substances that they produce" (MPI 2014). Given the difficultly of identifying marine organisms *in-situ* and the need to clear vessels at the border rapidly, any macro-organism found on an international vessel arriving in New Zealand waters is treated as a risk organism (Bell et al. 2011). An understanding of which organisms are likely or capable of recruiting over short residency periods will be beneficial in helping to determine the risk of the 'slime layer' of a seemingly clean vessel arriving into New Zealand, or any of its high-value areas.

Research on recruitment and community succession on hard substrates in natural marine environments can help us to understand the process of biofouling of artificial structures (Jenkins and Martins 2009), but to a limited extent. For example, the observed daily recruitment in natural marine environments provides evidence of the sporadic nature of recruitment (both temporally and spatially). However, many of these studies focus on recruitment of one species group, for example barnacles (Jarrett 1997, Blythe and Pineda 2009), scallops (Arnold et al. 1998), crabs (Giménez 2010), and oysters (Michener and Kenny 1991), although Hurlbut (1991) did examine daily community recruitment of seven co-occurring sessile invertebrates. It is useful to examine recruitment at the assemblage level with particular focus on port biota as this often contains NIS (Inglis et al. 2010).

Recruitment is a complex interaction between the supply of competent larvae and substrate availability (Anderson and Underwood 1994). These two factors differ between port and natural environments. Larval supply is largely determined by the abundance and composition of larvae and hydrographic characteristics that influence delivery to a surface (Eckman 1983, Shanks 1983, Farrell et al. 1991). Local abundance of larvae is likely to be high in shipping ports because there are many artificial structures providing space, an often limiting resource for broadcast spawning biofouling organisms. Additionally, artificial structures have higher diversity and abundance of biofouling organisms and different community composition than natural rocky reefs (Connell and Glasby 1999). Ports are also NIS hot spots and thus NIS are likely to comprise a larger proportion of the larval community in ports compared to the natural environment. Furthermore, port environments are often semi-enclosed environments due to the use of protective structures, such as breakwalls. These features influence hydrology leading to retention of the already abundant propagules (Floerl and Inglis 2003). Finally, ports are often resource-rich environments, which possibly reduces competition for food, thus leading to higher fecundity and survivorship.

Studies that have examined recruitment and colonisation to artificial structures in ports or harbours are often focused on community processes and do not include the enumeration and identification of recruits over periods of one or a few days (Glasby 2001, Lin and Shao 2002, Berntsson and Jonsson 2003, Bram et al. 2005, Dafforn et al. 2008, Dafforn et al. 2009, Dziubińska and Szaniawska 2010). A small number of studies have provided evidence that recruitment occurs in shipping ports over short periods, such as 40 hrs (McKenzie et al. 2012) or one week (Otsuka and Dauer 1982, Bullard et al. 2004, Dziubińska and Janas 2007), but not in a manner that compared recruitment densities and diversity over different periods of time (Otsuka and Dauer 1982, Henrikson and Pawlik 1995, Bullard et al. 2004, Dziubińska and Janas 2007, McKenzie et al. 2012). Because these studies often used percentage biofouling cover as the main metric of interest, the completeness of recruit detection, particularly of microscopic stages, cannot be ascertained. Dziubińska and Janas (2007) stated that recruits >1 mm were recorded which suggests that some young juveniles will have been missed. A recent review on the development of fouling assemblages to non-toxic surfaces in both temperate and tropical environments for a period of 1 - 4 weeks found that recruitment could occur within a week of submergence (Floerl et al. 2010). None of the 19 studies reviewed specifically compared recruitment over short residency periods even though this temporal variability is likely to be highly relevant to biosecurity management, and no study to date has examined rates of recruitment to visiting vessel hulls during a range of residency periods at a coastal shipping port.

The aim of this study was to examine the influence of temporal variation in residency period on the likelihood of vessels to export propagules that have recruited to an unprotected hull surface. The hypotheses were:

1. The risk of exporting a propagule from a source location's larval pool is influenced by residency period
2. There is daily variation in the likelihood of propagule entrainment and export

METHODS

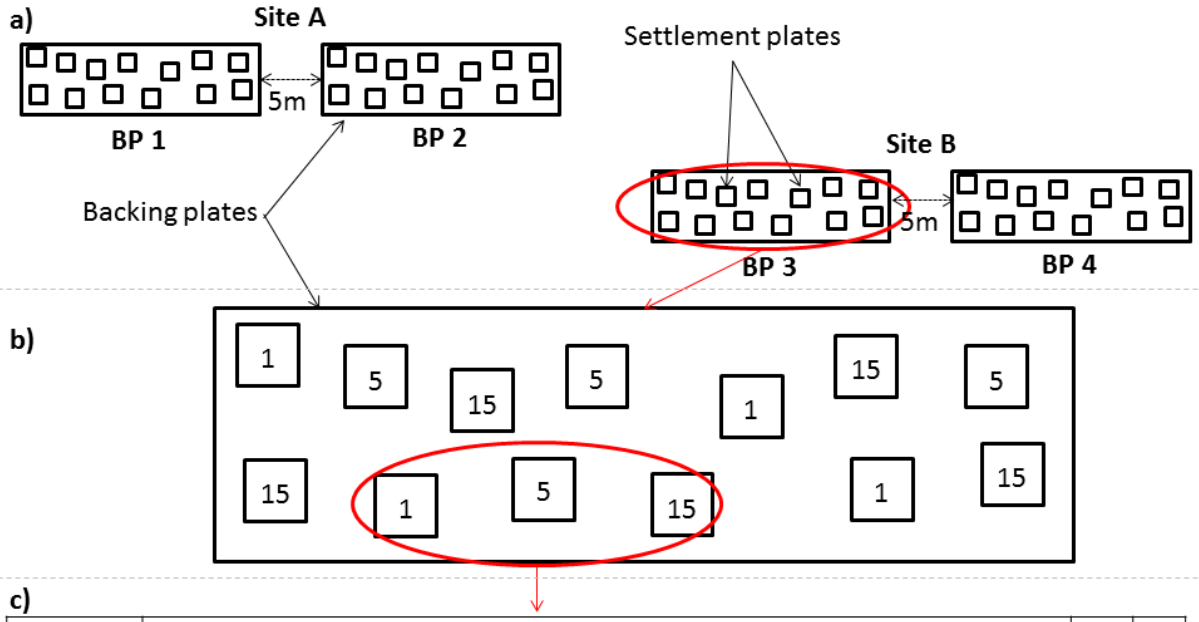
Field methods

Recruitment of fouling organisms to settlement plates was investigated over three residency periods: 1 day, 5 days and 15 days at the Port of Lyttelton, South Island, New Zealand

(Figure 1.3). Each experiment lasted 16 days. There were two rounds (2^{nd} – 17^{th} of January, and 11^{th} – 26^{th} of February 2013) spaced 25 days apart during the summer period, when recruitment is highest. Settlement plates (150 × 150 mm) were made from light grey, 4 mm thick PVC sheets. These plates were attached with a galvanised bolt to a larger PVC backing plate (1220 × 600 mm) (Figure 2.1a). Each backing plate was suspended vertically from either a floating pontoon or a buoy to ensure that they remained at a constant depth of 0.5 – 1.0 m below the sea surface; a pilot study had shown that recruitment was higher at this depth than at 2.0 – 2.5 m below sea level (Appendix 2). The larger backing plates were used to represent a section of vessel hull that would have similar hydrodynamic flows acting on it. Two anchor lines and weights attached to the lower side of the backing plate were used to minimise movement and maintain the position of the backing plate in the water, although both sampling sites were sheltered from wave exposure and did not have strong currents. Two backing plates were deployed 5 m apart at two sites within the Port. In a port environment biofouling spatial patterns are likely to reflect propagule pressure from the surrounding environment (Hedge and Johnston 2012) which will vary according to flow diversity (Palardy and Witman 2014) and the characteristics of the port, such as open or semi-enclosed due to permanent breakwalls (which may retain propagules), shading, and disturbance, such as pollution, freshwater input, and mechanical forces. The Port of Lyttelton is semi-enclosed and 4.4 - 8% of water is exchanged depending on tidal amplitude (D. Goring, *personal communication*). This semi-enclosed environment may contribute to low variation between sites, therefore, two replicates, rather than independent samples, were taken in the Port of Lyttelton (Chapter One: Figure 1.3).

The settlement plates were prepared as appropriate surfaces for recruitment (Qian et al. 2000, Dobretsov and Qian 2006) by sanding on one side, soaking for 24 hrs in seawater then rinsing in fresh seawater daily for a period of 2 days prior to placement in the Port. Settlement plates (n=4) assigned to the three residency periods (1 day, 5 days, 15 days) were haphazardly allocated to a position on the backing plate (Figure 2.1b). Backing plates were briefly removed from the water at the same time each day to allow for plate collection and replacement (Figure 2.1c). A study by Hurlbut (1991), who examined the recruitment of seven target species in Pearl Harbour, Hawaii, showed that daily removal of plates had no significant effect on recruit abundance compared to undisturbed plates over a 14 day period. Once removed, each plate was placed into a sealed plastic bag and transported back to the laboratory (approximately 20 min) in an insulated container filled with seawater. On day 16,

all plates were removed, concluding that experimental round. Two settlement-backing plate configurations were deployed for a further month to allow recruits to mature, which were then used to assist with taxonomic identification.



Residency	Plate collection (C) & Replacement (R)															n	n
1-day		C&R	C&R	C&R	C&R	C&R	C&R	C&R	C&R	C&R	C&R	C&R	C&R	C&R	C&R	C	15
5-day						C&R					C&R					C	3
15-day																C	1
Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	

Figure 2.1: Experimental design of recruitment study. a) Four backing plates (BP) each containing 12 experimental plates were placed at two sites within the Port of Lyttelton. b) Each experimental plate was haphazardly allocated one of the three residency periods. c) The continuous plate collection and replacement schedule for one replicate – 1-day plates were collected and replaced daily, 5-day plates collected and replaced every 5th day and 15-day plates were collected on the final day of the experiment.

Laboratory procedure

Three of the four replicates were processed typically within 4 hrs of collection. In the laboratory, each plate was submerged in seawater and examined using a Nikon SMZ-IB stereo microscope with a swing-arm stand at ×35 magnification. Examining the plates fresh and submerged in seawater allowed identifying features to be viewed, including siphon movement by ascidians, protrusion of worms from tubes and the lophophore of bryozoans. A thin black plastic mesh grid with a hole size of 10 mm × 12 mm was overlaid on the plate to

facilitate the systematic scanning of the plate. The outer 3 mm of each plate were excluded from analyses to avoid edge effects. For each plate, recruit abundance was determined. A recruit is defined here as an individual or colony that was present between settlement and the time the census was taken (Keough and Downes 1982), thus colonies made up of more than one zooid were recorded as a single recruit.

Organisms were photographed using a Leica M205C stereo microscope with a DFC 295 (3MP) digital camera and identified to the lowest taxonomic level possible. Taxonomic experts were used to confirm the identity of some taxa; however, due to the difficulty of identifying early stage juveniles some recruits were assessed as 'unknown'. Given this limitation, analyses were carried out at the phylum level to prevent bias towards older recruits where taxonomic knowledge or ease of identification was greatest. On five occasions, not all of the plates were able to be processed on the same day. When this occurred, unprocessed plates were placed in a -20°C freezer for examination at a later date. An earlier trial determined that the examination of frozen plates, compared with fresh plates, was unlikely to provide a different estimate of the abundance or identity of taxa (Schimanski, *unpublished data*).

Experimental design and statistical analyses

Generalised Linear Mixed Effects Models (GLMM) were used to test for effects of residency period on four response variables: total abundance (number of recruits per plate), total richness (number of taxa per plate), average abundance per day (total abundance/residency period) and average richness per day (total number of taxa/residency period). The experimental design consisted of four factors: residency period (fixed, with three levels: 1 day, 5 days and 15 days), experimental round (fixed, with two levels), site (random, with two levels) and backing plate (random, with four levels).

The continuous plate replacement design was chosen as it was considered unreasonable to compare one randomly chosen 1-day or 5-day plate to a 15-day plate. This is due to the inherent temporal variability in the recruitment of marine organisms. Variability, such as recruit pulses, over the 15 day period would be captured on the 15-day plates, but would not be captured on the 1-day or 5-day plates if only one sample was taken during that period. Therefore, one 1-day or one 5-day plate is unlikely to be a fair representative of *daily*

recruitment over the entire 15 day experimental period. The continuous plate replacement design also allowed multiple hypotheses relating to recruitment risk to be examined (see *Introduction*). This continuous plate replacement design, however, also meant the design was unbalanced. Many statistical models, including the GLMMs presented here, can deal with an unbalanced design by modelling the between and within variation, giving 'weighted' variance estimates for each residency period (Zuur et al. 2009). Nevertheless, *P*-values need to be interpreted with caution.

A saturated model was initially fitted to the data with all explanatory variables and interactions. Model selection using AIC was conducted to obtain the most parsimonious model. Models were then validated by inspecting the deviance residuals (Zuur et al. 2009). All models were run using R v.3.1.1 (R Core Team 2014). Negative binomial errors were used for the over-dispersed abundance (count) data and Poisson errors for species richness. To compensate for over-dispersion, a scale parameter θ was used to inflate standard errors (Zuur et al. 2009). Significant terms were investigated using Tukey's post-hoc tests to determine the significant differences between each level. The *lsmeans* R package (Lenth and HervÃ 2015) was used to run the Tukey's post-hoc tests due to the differences in standard errors (Searle et al. 1980). Offsets were also included in the model when examining average daily recruitment between the three residency periods. Offsets were appropriate as they hold constant an observational unit that differs in some dimension, while the remaining explanatory variables are evaluated (Crawley 2007). In this instance the experimental day was held constant. Offset analyses were checked using a GLMM on daily abundance (abundance divided by day), which produced the same significant effects as the offset model. Here, the offset model is presented as it is a more efficient method that used the entire unmanipulated dataset. GLMMs were run using the *glmmadmb* function in the *glmmADMB* package in R (Fournier et al. 2012).

A model-based analysis of multivariate abundance, using the *manyglm* function in the *mvabund* R package (Wang et al. 2012), was used to assess community composition between the three residency periods. *Manyglm* fits a univariate generalised linear model based on the explanatory variables to the abundance of each taxonomic group, thus creating a multivariate analysis across taxa (Wang et al. 2012). The multivariate test statistic is based on the likelihood ratio and a step-down Monte Carlo resampling algorithm with 500 resamples which calculates adjusted *P*-values for each taxonomic group. The *manyglm* test does not

allow for random effects, but because they were previously shown to contribute only a small amount of variation to the response, this was still considered an appropriate method to use. A negative-binomial distribution was specified due to over-dispersed count data as described above. Again, models were validated through visual inspection of residuals. An adjusted univariate test was then conducted to highlight the taxa which drove the observed patterns. One plate had 1826 recruits with high numbers of red algae and barnacles present. This was a clear outlier in the data. Models were run with and without this outlier; inclusion of the outlier did not change the statistical significance of any of the factors tested. The data point was removed from all graphs to improve visualisation of the data.

RESULTS

Total accumulation

Recruits were present on all of the 456 settlement plates examined. Abundance ranged from 1 – 1826 recruits per settlement plate and was lowest and least variable for the 1 day residency period (mean (± S.E.) = 19.5 ± 0.6; Figure 2.2). In comparison, 5-day plates had an approximately 3-fold increase in abundance (mean = 61.0 ± 6.6). The 15-day plates had a 15-fold increase, and were the highest and most variable of all residency periods (mean = 300.1 ± 39.3). The positive relationship between total recruitment and residency length was consistent over the 2 experimental rounds (Figure 2.2), although there was an interactive effect of residency period and experimental round (Table 2.1) with no significant difference in recruitment between 5-day and 15-day plates in round 2 (Figure 2.2, Table A2.1). There was a higher abundance of 5-day recruits in round 1 than in round 2 (Table A2.1). Site and backing plate accounted for a small amount of variability for all response variables (Table 2.1). The abundance and richness of recruits differed between experimental days and round (Table 2.2). Ascidians, bryozoans, crustaceans, molluscs and unidentified taxa were the primary drivers of the observed patterns. Residency period also had an effect on taxonomic richness, which ranged from 1-9 taxonomic groups per settlement plate (Table 2.1; Figure 2.3). Richness was lower on 1-day plates (mean = 3.6 ± 0.06) compared to 5-day (mean = 4.9 ± 0.144) and 15-day (mean = 7.1 ± 0.17) plates. There was no statistical difference in richness between 5-day and 15-day plates (Table A2.2). Experimental round also had an effect; taxonomic richness was higher during round 2 than during round 1. This was particularly evident on 1-day and 5-day plates (Figure 2.3).

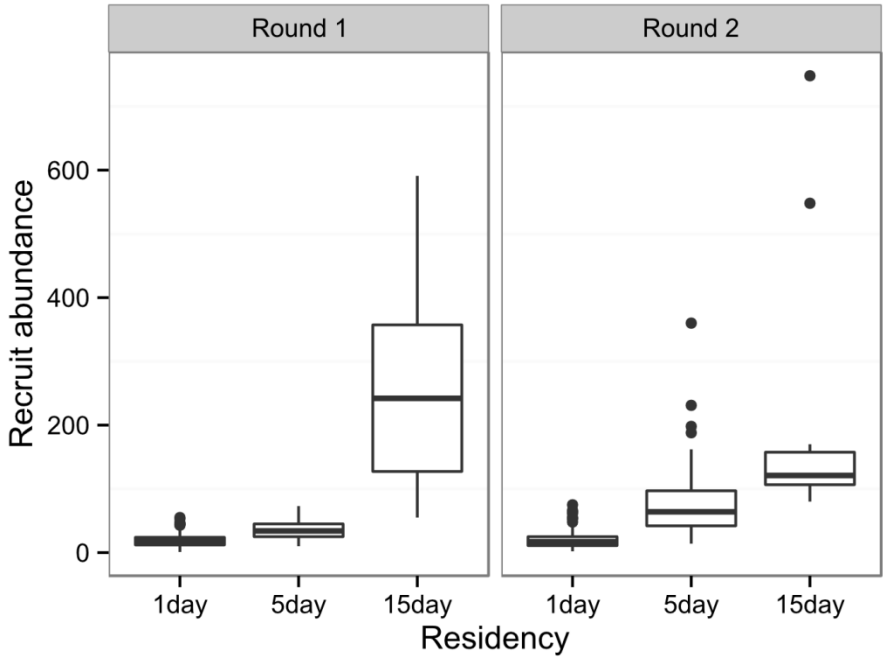


Figure 2.2: Total abundance of biofouling recruits (individual or colony) on settlement plates set mimicking different residency periods (1, 5, 15 days of stationary mooring in the donor port) in the Port of Lyttelton between January (first round) and February 2013 (second round).

Table 2.1: Estimates from Generalised Linear Mixed Modelling (GLMM) on the effect of residency period and experimental round on total and daily recruit abundance and taxonomic richness. Variation due to the random effects of site and backing plate(site) are also presented.

	Abundance (total)			**Richness (total)**			**Abundance (daily)**			**Richness (daily)**		
Source	**Estimate**	***s.e.***	***Z*-value (*P*-value)**	**Estimate**	***s.e.***	***Z*-value (*P*-value)**	**Estimate**	***s.e.***	***Z*-value (*P*-value)**	**Estimate**	***s.e.***	***Z*-value (*P*-value)**
Intercept	2.96	0.1	30.88 (<0.001)	1.11	0.04	26.47 (<0.001)	2.96	0.1	30.88 (<0.001)	1.12	0.04	27.08 (<0.001)
5 day	0.6	0.1	6.01 (<0.001)	0.37	0.09	4.12 (<0.001)	-1.00	0.10	-10.00 (<0.001)	-2.9	0.12	-24.06 (<0.001)
15 day	2.49	0.16	15.60 (<0.001)	0.84	0.12	7.23 (<0.001)	-0.22	0.16	-1.36 (0.17)	-4.73	0.3	-15.86 (<0.001)
Round 2	-0.002	0.06	-0.04 (0.97)	0.29	0.06	5.21 (<0.001)	-0.002	0.06	-0.04 (0.97)	0.28	0.05	5.25 (<0.001)
5 day × Round 2	0.86	0.14	6.14 (<0.001)	-0.09	0.12	-0.73 (0.466)	0.86	0.14	6.14 (<0.001)		Pooled	
15 day × Round 2	-0.14	0.23	-0.61 (0.54)	-0.29	0.16	-1.79 (0.074)	-0.14	0.23	-0.61 (0.54)		Pooled	
Site SD		4.54^{-05}			7.06^{-05}			1.21^{-04}			9.83^{-05}	
Back Plate SD		0.17			8.44^{-05}			0.17			6.1^{-05}	
Distribution	Negative binomial			Poisson			Negative binomial			Poisson		

Table 2.2. Estimates from Generalised Linear Mixed Modelling (GLMM) on the effect of experimental day and experimental round on recruit abundance and taxonomic richness in the Port of Lyttelton, New Zealand. Variation due to the random effects of site and backing plate (within site) are also presented.

	Abundance			**Richness**		
Source	**Estimate**	***s.e.***	***Z* (*P*-value)**	**Estimate**	***s.e.***	***Z* (*P*-value)**
Intercept	2.8	0.1	27.48 (<0.001)	0.95	0.09	10.3 (<0.001)
Day	0.02	0.01	4.27 (<0.001)	0.02	0.01	2.04 (0.041)
Round 2	-1.0	0.11	-9.21 (<0.001)	0.44	0.22	2.02 (0.044)
Day:Round2	0.03	0.01	5.22 (<0.001)	-0.02	0.01	-1.5 (0.135)
Site SD	6.07^{-05}			1.03^{-04}		
Backing Plate SD	0.19			1.06^{-04}		
Distribution	Poisson			Poisson		

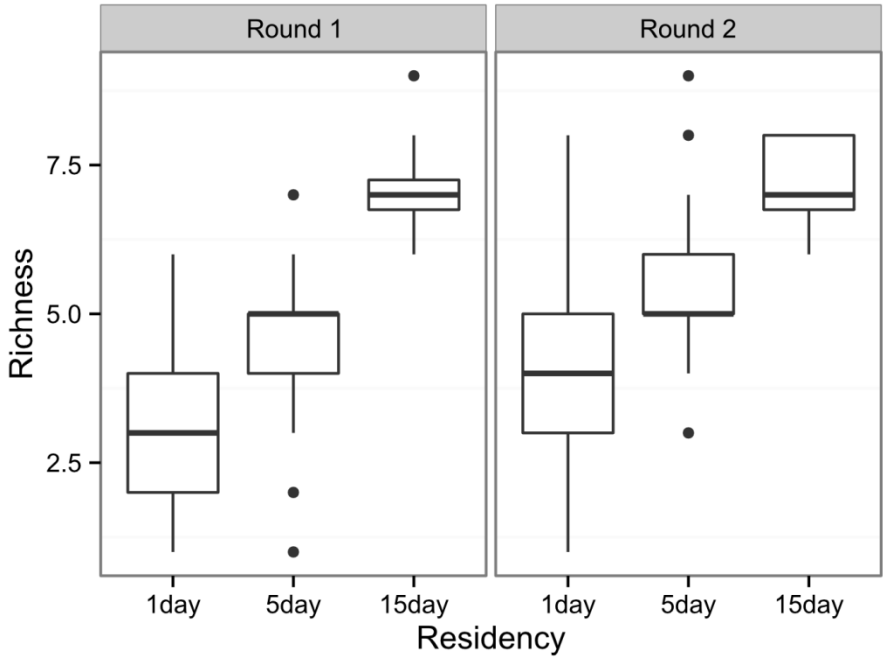


Figure 2.3: Taxonomic richness (number of taxa) of biofouling recruits on settlement plates set mimicking different residency periods (1, 5, 15 days of stationary mooring in the donor port) in the Port of Lyttelton between January (first round) and February 2013 (second round).

Daily rate of accumulation

Recruitment per plate per day was similar across all residency periods: 1 day mean = 19.53 ± 0.62 S.E.; 5 day mean = 12.19 ± 1.32, and 15 day mean = 15.59 ± 2.617 (Figure 2.4), although there was a residency period - round interaction (Table 2.1). Tukey comparisons showed that only the 5-day-round 1 plates differed from the other groups, all other comparisons were not significantly different (Table A2.3)

Average daily richness accumulation decreased with residency period (Table 2.1). Daily richness accumulation was greatest for 1-day plates (3.57 ± 0.06 S.E.), followed by 5-day (0.98 ± 0.03) and 15-day (0.47 ± 0.01) plates (Figure 2.5, Table A2.2). There was also an effect of experimental round, with higher average daily richness accumulation in round 2 compared to round 1.

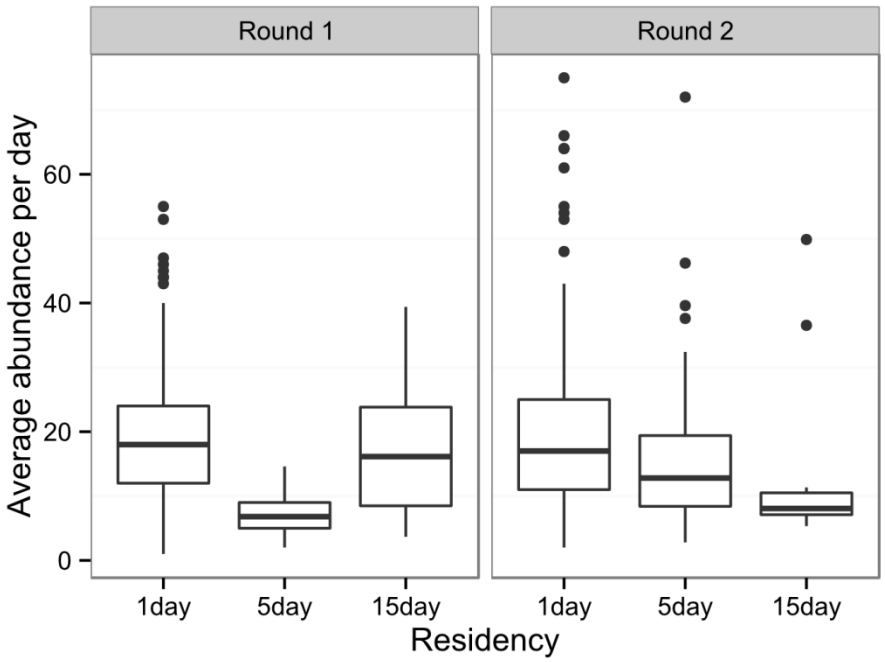


Figure 2.4: Average daily rate of abundance accumulation of biofouling recruits (individual or colony) on settlement plates set mimicking different residency periods (1, 5, 15 days of stationary mooring in the donor port) in the Port of Lyttelton between January (first round) and February 2013 (second round).

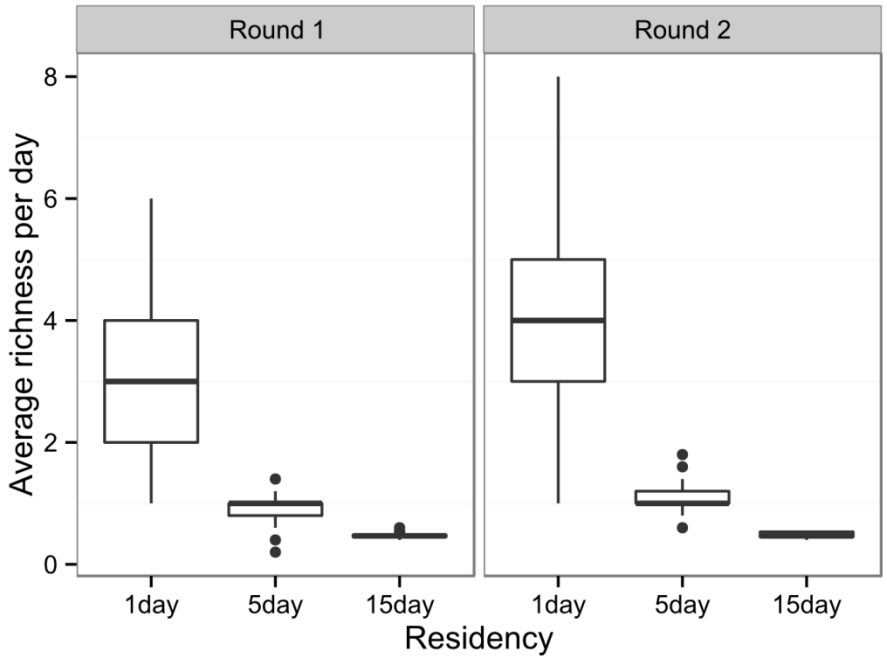


Figure 2.5: Average daily rate of richness accumulation (number of taxa) of biofouling recruits on settlement plates set mimicking different residency periods (1, 5, 15 days of stationary mooring in the donor port) in the Port of Lyttelton between January (first round) and February 2013 (second round).

Community composition

In descending order of abundance, the following taxonomic groups were recorded on the settlement plates: ascidians, crustaceans, bryozoans, algae, unidentified recruits, ciliophora, polychaetes, foraminifera, molluscs and hydroids. All of these groups were recorded at least once in each level of residency period. Therefore, although richness per plate decreased with residency period, 1-day plates entrained the same number of taxonomic groups as the 5-day and 15-day plates (Figure 2.6). Community structure (i.e., abundance and richness) differed between 1-day and 15-day, and 5-day and 15-day plates, although there was no difference between 1-day and 5-day plates (Table 2.3). Univariate GLMs indicated that algae, ascidians, bryozoans, ciliophorates, crustaceans, molluscs, and polychaetes drove these differences with higher abundance on 15-day plates compared to 1-day and 5-day plates (Figure 2.6). Some taxonomic groups were very common across all residency periods and abundance steadily increased with residency period (Figure 2.6). For example, ascidians were found on 92% of 1-day plates (mean recruit abundance per plate = 7 ± 1 S.E.), 100% of 5-day (mean abundance = 24 ± 2 S.E.) and 15-day plates (mean = 94 ± 20 S.E.). Bryozoans were also common and recorded on every plate examined, but the increase in mean abundance per plate

with residency period was not as great (1 day mean = 8 ± 1 S.E., 5 day mean = 11 ± 0.8 S.E., and 15 day mean = 22 ± 3 S.E.). Both algae and crustaceans were recorded on fewer than half the 1-day plates (33% and 42% respectively), compared to 5-day (62% and 78%) and 15-day plates (96% and 100%). Of the 18843 recruits recorded, 705 were unidentified (<4%); 74% of these unidentified recruits were on 1-day plates, 16% on 5-day plates and 10% on 15-day plates.

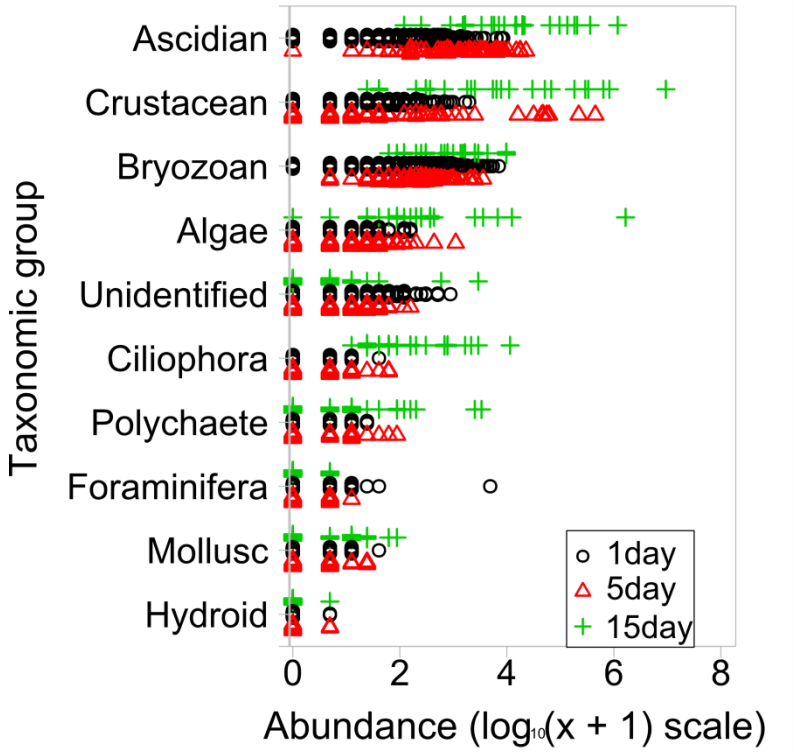


Figure 2.6: Community composition of biofouling recruits on settlement plates that were set to mimic different residency periods (1, 5, 15 days of stationary mooring in the donor port) in the Port of Lyttelton between January (first round) and February 2013 (second round). Each data point represents the abundance of individuals from a specific taxonomic group on one plate. Abundance is displayed on a $\log_{10}(x+1)$ scale to improve visualisation of data.

Table 2.3: Estimates from multivariate Generalised Linear Model (GLM) on the effect of residency period and experimental round on recruit community composition in the Port of Lyttelton, New Zealand.

Source	**Wald value (*P*-value)**
Intercept	34.27 (0.001)
Residency 5 day	6.68 (0.275)
Residency 15 day	13.01 (0.001)
Residency 5 day – 15 day	9.08 (0.003)
Round	18.50 (0.001)
Distribution	Negative binomial

DISCUSSION

This study demonstrates that recruitment of resident biofouling species can occur on settlement surfaces exposed to the local larval pool for durations as short as a single day. On average, 20 individuals from four taxonomic groups recruited to a 150 × 150 mm settlement plate over a 24 hr period. Bryozoans, ascidians, crustaceans, algae, ciliophora, polychaetes, foraminifera, molluscs and hydroids successfully recruited to plates over a 24 hr period. Vessels whose hulls are unprotected, lacking antifouling coatings, or where these coatings are no longer effectual are thus able to export locally-established species even when arriving and departing within 24 hrs.

Short residency periods can occur before international vessels arrive at a recipient border. For example, an international yacht may complete an in-water clean prior to leaving their last port from which they make the crossing to New Zealand. Following the cleaning, they may remain at the port for one or two days before departing for New Zealand and arrive at the border with a seemingly clean hull. Oil rigs are another example of structures that will reside for short periods after in-water cleaning before translocation. This study suggests, however, that recruitment is capable over such short residency periods and (if the recruits survive the translocation) these juveniles will be microscopic, difficult to detect from the slime layer, and likely to be missed upon visual inspection. As any macrofouling organism present on the hull of a vessel arriving into New Zealand waters is treated as a potential risk species by MPI (Bell et al. 2011), the slime layer may be a potential risk for the entry of NIS. This risk of short residency periods also applies to vulnerable surfaces on domestic vessels residing in ports before travelling to further domestic ports or high-value areas. In both cases, however, it is only an invasion risk if the juvenile recruits survive the translocation event and develop to reach reproductive maturity. The subsequent chapters within this thesis examine the success of short residency recruits during ensuing stages of the invasion process.

In this study, it was found that although recruitment did occur every day, there was temporal variability in recruitment between days and experimental rounds. This suggests that the chance of recruitment will not be the same on any given day. Such temporal variance is common in natural environments (Hurlbut 1991, Pineda 2000, Palardy and Witman 2011) due to many factors, such as reproductive periods of adult stock (Geraci and Romairone 1982), winds and currents (Hawkins and Hartnoll 1982), tides and tidally-generated internal waves (Shanks 1983), stratification of larvae in the water column (Grosberg 1982) and spatial

variation in the abundance of larvae (Gaines et al. 1985). In ports noise (McDonald et al. 2014), hydrodynamics created by vessels (Koehl 2007) and artificial structures (Dafforn et al. 2009) are additional factors likely to affect temporal variability in recruitment. Furthermore, as there is distinct seasonality in recruitment in temperate environments, with peak recruitment often occurring in summer (when this study took place). The timing of substrate availability in relation to reproductive period will also be important for recruitment risk. If recruitment does occur over other seasons, the communities are likely to differ (Kocak et al. 1999). Due to the inherent nature of temporal variability in the marine environment (Gaines et al. 1985) it would be beneficial to repeat this study on different seasons to test the generality of these results (Dafforn et al. 2009).

Recruit abundance increased over the three residency periods (1 day, 5 days and 15 days), with the greatest abundance on the 15-day plates. Similar patterns have been shown in other studies; for example, Raimondi (1990) found that recruitment of the intertidal barnacle, *Chthamalus anisopoma,* increased for approximately the first 2 weeks before it plateaued. Hurlbut (1991) found that percent cover of seven common fouling species on settlement plates deployed in Pearl Harbour, Hawaii increased until approximately day 10, after which mortality occurred. The exact mechanisms operating behind the observed patterns were not examined in my study, but variation in propagule supply and substrate availability are known to be two major determinants of recruitment likelihood (Connell 1985). Moreover, previous studies have shown that propagule supply can be more limiting than substrate availability (Lee and Bruno 2009, Palardy and Witman 2014). Supply of propagules will be particularly important during short residency periods, where there is likely an abundance of available space on a vessel hull (Jackson 1977, Petraitis 1995). All types of vessels are likely to feature areas susceptible to biofouling development. Some vessels feature old AF coatings that are no longer able to deter recruits. Even vessels with recent AF coatings often have areas that are unprotected by a lack of coatings, such as dry-docking support strips, some sea chests, or where AF coatings have failed prematurely due to excessive or insufficient drag, such as sonar domes, bilge keels and rudder stocks.

Pinpointing the supply of propagules as the underlying driving force behind increased abundance with residency period, however, assumes that recruitment correlates with supply (Pineda et al. 2009). This may not be the case, as juvenile mortality is widespread and variable among benthic marine invertebrates (Wethey 1984, Hunt and Scheibling 1997). In a

review by Gosselin and Qian (1997), 20 of 30 studies found greater than 90% juvenile mortality, with high mortality in the early stages (e.g., exceeding 30% in the first day). However, juvenile mortality is often studied in intertidal regions (e.g., Underwood 1979, Gosselin and Chia 1995), where the relative importance of factors will likely differ from a port environment. For example, desiccation will be more important to organisms in the intertidal zone than on a floating submerged hull. Results do need to be interpreted carefully as they do not indicate mortality. Chapters Three, Four and Five of this thesis do examine survivorship and mortality of different aged recruits during subsequent stages of the invasion pathway.

Interestingly, the average daily recruitment did not differ between 1 and 15 day residency periods. This suggests that frequently departing vessels, with short residency periods, may have a pool of propagules similar in abundance to longer residing vessels. As larval selection is influenced both positively and negatively by existing microbial films and other organisms, it was expected that the daily rate would be dissimilar (see Prendergast et al. (2010) for review). A settlement study in Hawaii examined settlement on 1-day submerged and 14-day submerged plates and found that daily settlement was higher on 14-day plates compared to 1-day plates for five of the seven species examined, suggesting that recruits were attracted to more developed fouling communities (Hurlbut 1991). Organisms recorded in my study in the Port of Lyttelton, such as barnacles, have shown habitat selection behaviours including territoriality (Crisp 1961) and gregariousness (Knight-Jones 1953). Nonetheless, a positive relationship between abundance and mortality can also occur (e.g., Hedge et al. 2012), which may mask any attraction. Essentially, presenting average accumulation assumes events occur uniformly throughout the sample period, and consequently underlying mechanisms such as, attraction and mortality, cannot be determined.

The settlement plates in this study represented unprotected areas of a boat hull. However, the ineffective surfaces on hulls may be much greater than the settlement plate surface area (150 × 150 mm); for example 20% of the total wetted surface of a vessel can be untreated due to dry docking support strips alone (Coutts 1999). A larger patch or new substrate is likely to sample a greater number of propagules than a smaller patch, and a recent survey found that total wetted surface area explained some variation in fouling biomass of commercial vessels (Inglis et al. 2010). Other factors such as complexity of substrate, niche areas, and generator

noise may also promote greater recruitment to AF defunct surfaces (McDonald et al. 2014). Therefore, recruitment may have been underestimated in this study.

It is likely that coastal environments will continue to be severely modified in the future due to an increase in urban development and sea level rise. The addition of artificial habitats, such as pilings and pontoons, or modification of existing habitats, such as the extension of seawalls, will increase novel habitats, which tend to be colonised by greater proportions of NIS compared to native taxa (Glasby et al. 2007, Tyrrell and Byers 2007, Bulleri and Chapman 2010, Airoldi and Bulleri 2011). This in turn can result in a build-up of local resident populations and an increase in local propagule production, possibly resulting in even greater abundance and diversity of recruits to susceptible surfaces of short residency vessels. Such situations will likely be exacerbated as tolerance to antifouling coats evolve, toxic treatments are banned and shipping escalates. The results from this study, particularly combined with these additional factors, suggest that recruitment of biofouling organisms over short residency periods is likely to be a future risk for NIS introduction and spread.

Conclusions

This study provides evidence that vessel residency periods as short as 1 and 5 days are able to entrain recruits – a process that could initiate the invasion pathway for a prospective invader or facilitate domestic spread of established NIS. Although recruitment did vary between days, it did occur daily. Risk, as indicated by recruit abundance and richness, increased with length of residency period tested; 15 day recruitment was greater than 5 day, which was greater than 1 day. It is unknown which, if any, of these settlers will go on to reproduce in a recipient location if translocated from the donor location ("effective dispersal"), and subsequent chapters of this thesis will explore survivorship, growth and reproduction of these different-aged recruits.

Chapter Three: The effect of selected filters on the translocation and post-translocation growth of a ship biofouling species.

ABSTRACT

The likelihood that NIS are translocated on any given vessel is not equal. Some vessels will lose fouling organisms *en route*, or host organisms with compromised health on arrival in the recipient environment (low risk vessels). Other vessels will arrive with healthy propagules that will survive in the recipient environment (high risk). Therefore, robust screening tools are needed to effectively predict and manage the risk associated with each vessel entering a port or harbour. These tools can be developed through understanding how different voyage factors, such as speed and voyage history, influence the survivorship and health of hull-fouling propagules. In this study, a dynamic flow device was used to run a multi-factorial experiment testing the effect of three voyage characteristics on the survivorship and growth of the bryozoan *Bugula neritina*. The characteristics tested were: vessel residency period in donor location, vessel speed, and voyage duration. Residency period was tested by manipulating the age of the recruit. *En route* survivorship was high under all scenarios. However, there was an effect of residency period on survivorship; 8-day recruits had higher survivorship (100%) than 1-day (75%) or 29-day (92%) recruits. Speed (6 and 18 knots) and duration (2 and 8 days) had no significant effect on survivorship. The voyage scenario had legacy effects on the colonies, and survivorship declined 7 days post-voyage (ranging from 58 – 91%). There was also an interactive effect of speed, duration and age on growth during this period; 1-day recruits on the 18 knot, 8 day voyage scenario had the lowest growth rate. Some colonies, particularly the large 29-day recruits, decreased in size through loss of branches and branch tips during the experimental period. The results from this study indicate that residency period is an important indicator of survivorship and post-voyage growth. Mid-residency periods (8 days) appear to be the highest risk, but importantly, short residency recruits (1 day) also survived even after fast and long voyages. This finding supports previous

work suggesting that short residency periods of approximately a week or less may present a high risk of transferring NIS.

INTRODUCTION

Propagule supply to a region through ballast water and biofouling is one of the most important factors influencing NIS colonisation and establishment in a recipient location (Lockwood et al. 2005, Johnston et al. 2009, Simberloff 2009). Therefore, identifying the factors that increase or decrease propagule supply is particularly important in order to make predictions of vector risk. However, models that use frequency of vessel movement as a proxy for propagule pressure have been criticised because all vectors are not equal in the quality and quantity of propagules they carry, and therefore, the propagule pressure they apply (Verling et al. 2005, Johnston et al. 2009). Numerous post-voyage surveys have provided clear evidence of this variation for both ballast water (Smith et al. 1999, Briski et al. 2013b) and biofouling (Inglis et al. 2010, Clarke Murray et al. 2011). The unequal propagule 'load' on each vector is partly because various selective filters (i.e., factors that affect the survivorship and health of organisms throughout the shipping pathway (Chapter One: Figure 1.1)), act to decrease propagule supply (Floerl 2002, Lewis and Coutts 2009).

To accurately predict which vessels pose the highest and lowest risk of NIS invasion, it is necessary to identify which filters affect propagule survivorship and health during the translocation stage. This level of detail in the literature is lacking. Selective filters that may be important have been identified (Floerl 2002, Lewis and Coutts 2009). However, few of these filters have been rigorously tested through manipulative experiments and the actual role of each remains unknown. This lack of knowledge leads to difficulties in developing predictive models.

Hydrodynamic forces, antifouling (AF) coatings and economic incentives to minimise drag have resulted in relatively lower levels of biofouling on the laminar flow areas of a vessel compared to niche areas, such as sea chests, where prevention of biofouling is difficult. Niche areas are often considered a greater biosecurity risk (Coutts et al. 2003, Coutts and Taylor 2004, Coutts and Dodgshun 2007). Nonetheless, observational studies have shown that some species can tolerate high drag forces and that the main hull areas also transport organisms to new regions (Coutts and Taylor 2004, Davidson et al. 2010, Inglis et al. 2010). For example,

a study conducted in New Zealand showed that 29% of species richness on yachts and launches was from laminar flow areas (Inglis et al. 2010). Antifouling coats are also not a panacea for preventing the attachment of biofouling organisms. In fact, some NIS, such as *Watersipora subtorquata* selectively recruit to AF surfaces over non-AF surfaces (McKenzie et al. 2012) which then facilitates recruitment of other species (Floerl et al. 2004). Additionally, small scrapes (< 1cm) in AF surfaces can enhance recruitment compared to control non-AF surfaces (Piola and Johnston 2008). Moreover, due to practical limitations some areas of the hull are often not coated in effective AF coats, such as dry-docking support strips, resulting in high fouling (Coutts and Taylor 2004). Hull-fouling species may have wide environmental tolerances and physiological traits which allow them to survive strong selection pressures (e.g., hydrodynamic force and toxicity tolerance) during transportation, both of which may be advantageous upon arrival to the new recipient environment (Crooks and Rilov 2009). Although management focus may be directed to niche areas, fouling on laminar flow areas is still a significant risk.

Hull-fouling translocation survivorship is often examined post-voyage *in situ* or once the vessel is in dry-dock (e.g., Coutts and Dodgshun 2007, Inglis et al. 2010, Hopkins and Forrest 2010). While such inspections provide important evidence of the porosity of marine borders, they can also provide contradictory information on underpinning mechanisms, thus creating a 'black box' for managers. For example, a biofouling observational study in Australia showed a relationship between age of AF coat and fouling extent (Floerl and Inglis 2005), whereas other studies in the USA have shown no relationship (Davidson et al. 2010, Ashton et al. 2014, Zabin et al. 2014). A large observational study between 2004 and 2007 inspected 508 international vessel arrivals into New Zealand and found that some of the 24 predictor variables examined influenced the biofouling characteristics (i.e., the extent of biofouling and number of NIS), but the predictive power of these was relatively low (Inglis et al. 2010). Importantly, these studies did not provide information on failed translocations; that is those organisms that had colonised the vessel but failed to arrive. Furthermore, they did not provide information on whether the organisms on the various types of vessels had high fitness and were likely to survive, grow, reproduce and establish a successful founder population in the new environment.

A few studies have examined *en route* hull-fouling survivorship. For example, Carlton and Hodder (1995) examined biofouling on a 16th century replica sailing ship over an 800 km voyage and did not detect any change in biofouling abundance and diversity. However, the vessel travelled at slow speeds, averaging 4 knots. Brock et al. (1999) surveyed biofouling on a decommissioned battleship in Washington (USA) before it was towed on a transoceanic transfer to Pearl Harbour, Hawaii via a 9 day period in the Columbia River, Oregon. This vessel had high levels of fouling (up to 30 cm thick) that had developed over a 5 year period. Post-voyage sampling showed that all temperate marine fouling had been eliminated, likely due to the freshwater river stopover period and the increase in temperature throughout the voyage. Similarly, Davidson et al. (2008) examined changes in the hull-fouling communities of two obsolete vessels transferred from California to Texas over a 43 day period. During this translocation, salinity varied from 0 - 37 ppt and temperatures ranged from 9.9 - 31.6°C. Interestingly, although biofouling abundance decreased during the voyage, species richness increased. These opportunistic observational studies all provide useful information on translocation survivorship but significant knowledge gaps remain.

The complexity of the shipping pathway emphasises the need for controlled experimental work to elucidate factors that influence translocation likelihood. Indeed, controlled manipulative studies have been used to fill management knowledge gaps present in other invasion pathways. For example, *Styela clava* was a problematic NIS around Prince Edward Island, Canada, and the risk of translocating it overland to other ports through trailer transport was unknown (Darbyson 2009). Using air exposure experiments to mimic overland transport of boats, Darbyson (2009) examined survival of *S. clava* during 48 hrs of air exposure, and found there was only 10 - 11% mortality. This suggests that *S. clava* could be spread 1600-2000 km from infected areas, an area encompassing the entire Atlantic seaboard of Canada. A similar study by Hillock and Costello (2013) found that it could be up to 11 days for 99% mortality of the *S. clava* population after air exposure and that larger individuals took longer to die than smaller individuals. Experimental work is an essential next step beyond observational and opportunistic studies (Ruiz et al. 2000, Kolar and Lodge 2001, Johnston et al. 2009), as controlled experiments: (i) allow specific conditions to be controlled enabling variables of interest to be tested; (ii) allow organisms to be observed *in situ* without having to remove and damage them, and (iii) are generally less costly and time consuming.

Only two previous studies have experimentally controlled and tested *en route* survivorship of hull-fouling communities. In one study Coutts et al. (2010a) attached pre-fouled plates to a custom-made keel to test the effect of voyage speed on biofouling. In a second study, Coutts et al. (2010b) used magnetic settlement plates attached to a vessel to test the effect of speed and hull location on biofouling survivorship. Both studies found a decrease in biofouling percent cover and species richness with increasing vessel speed. These studies only examined speed, but factorial experiments that allow other variables to be simultaneously tested are necessary to investigate interactions between the factors in this complex pathway.

Study aims and hypotheses

The importance of residency period

Some vessel types, such as container vessels, arrive frequently and have short port residency periods and voyage times between ports. Other vessel types, such as barges, have long residency periods and long voyage times between ports. Whether a species has a higher chance of surviving translocation and establishing if it arrives on one vessel type over another is unknown. Chapter Two introduced the notion that there is inadequate scientific understanding with regard to the risk of donor port residency periods, in particular, the threat of short residency periods that are often assumed to be low risk. The results presented in Chapter Two showed that recruitment occurs over residency periods as short as 1 day and that the total number of recruits on 15 1-day experimental panels was not different to one 15-day experimental panel. The next logical question is 'will these different aged recruits survive an ensuing voyage'? It important to know not only what survives a voyage, but also if organism fitness is high enough for continued survival and subsequent reproduction in the new environment thus achieving effective dispersal. Consequently, legacy effects of the voyage are also important to determine. In this study the survivorship and growth of different aged recruits were examined during and after translocation. A fully crossed experimental design was used to also test the effects of voyage speed and voyage duration.

The hypotheses tested were:

1) Residency period, vessel speed and voyage duration influence survivorship and growth during translocation of hull-fouling recruits.
2) Residency period, vessel speed and voyage duration during translocation has legacy effects on survivorship and growth of hull-fouling recruits after translocation.

METHODS

The bryozoan *Bugula neritina* (Linnaeus 1758) was selected as the study organism. Brood stock were collected from Anchorage Marina, Williamstown, Australia (37° 51' 14.1"S 144° 54' 05.3"E) and Sandringham Yacht Club, Sandringham, Melbourne, Australia (37° 56' 44.6"S 144° 59' 48.5"E). Colonies were collected from a range of localities to obtain genetic variability in the stock. Colonies were placed into a lightproof insulated container filled with ambient water and transported back to the Defence Science and Technology Organisation (DSTO) laboratory. Colonies were cleaned of extraneous fouling and placed inside seawater-filled aerated tubs. Tubs were covered with black plastic and left in a temperature controlled laboratory for 48 hrs at a temperature of 19.6 - 22.0°C. To induce spawning, colonies were exposed to a fluorescent light. Single larvae, within a drop of seawater, were pipetted onto 120 × 120 mm, dark grey PVC settlement plates, and left to settle overnight in a dark environment. To minimise water evaporation, a soaked paper cloth was placed around the perimeter of each settlement plate and plastic containers were placed over each plate. Settlement was examined the following morning using a dissecting microscope. Gregarious settlers were thinned so that colonies were spaced no closer than 40 mm. Recruits that did not show normal upright settlement were also removed. Location of the recruits was marked using a pencil to draw around the recruit. Four recruits were randomly labelled on the plate to be used in the study. Plates were then placed on a rack and into a seawater-filled insulated container, and transported out to the study site at the BAE Systems facility in Williamstown, Melbourne (Chapter One: Figure 1.4). At the site, plates were attached to a large backing plate and hung below a floating pontoon in Hobson Bay (sea temperature ranging from 21.2 - 22.9°C) until the start of the experimental treatment.

Experiments were conducted using a dynamic flow device (DFD) to examine the effects of voyage characteristics on translocation survivorship and growth. The DFD comprised a large rotating cylinder (the rotor) contained within a circular seawater holding tank (Figure 3.1). There was an octagonal baffle between the rotor and the holding tank wall. The rotor spun at adjustable speeds (between 0 - 20 knots) creating a flow of water between the spinning rotor and the octagonal baffle wall. The water between the outside of the octagonal baffle wall and the holding tank wall was not exposed to this flow and was used as a control. Test settlement plates, with attached *Bugula*, were placed on holding racks inside the octagonal baffle wall in the high-flow environment ("spin" colonies) and control plates were placed onto the holding

tank wall in the low-flow environment ("no spin control" colonies) allowing the effect of speed and duration to be tested. The DFD was located on a wharf directly adjacent to Hobson Bay, allowing ambient seawater[2] to be continually pumped from Hobson Bay through the tank using the existing pump and two additional pumps (Grundfos FP5 Jet pumps). The total water exchange rate was 120 L/min. The volume of the DFD is 6240 L and therefore, the entire volume of the water in the system was being replaced every hour. Throughout the experimental period temperature, turbidity, salinity, dissolved oxygen and pH were also recorded in the DFD and at the floating pontoon (where colonies were placed when not in the flow device) throughout the experimental period. For further details on the DFD see Bishop (1982), though the drive motor and control system have been replaced due to wear in intervening years.

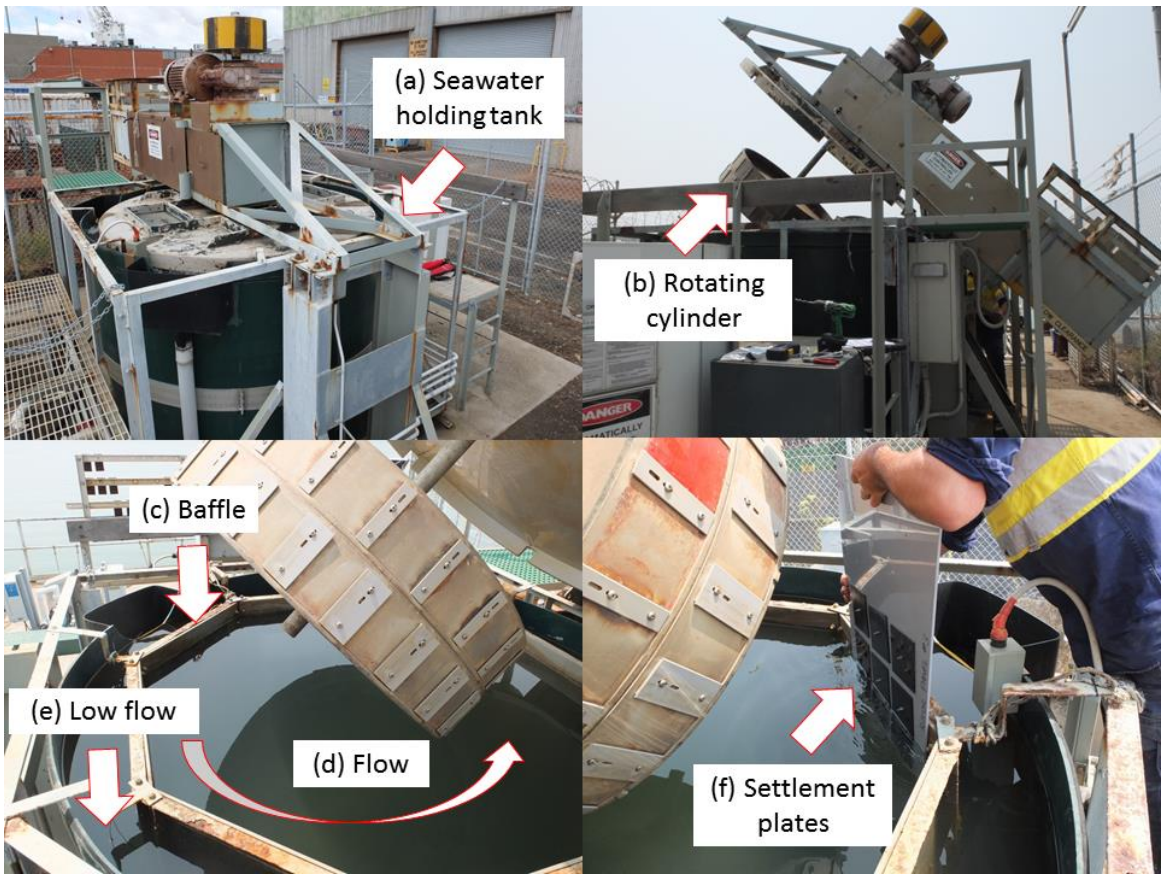


Figure 3.1: Experiments were run using a dynamic flow device (DFD) which consisted of: (a) a circular seawater holding tank; (b) an internal rotor cylinder, which sat inside a baffle (c), creating flow inside the baffle (d), but protected water outside the baffle in a low-flow environment (e). *Bugula* recruits on settlement plates (f) were placed in the flow and low-flow areas.

[2] Hobson Bay seawater during the experimental period: temperature mean = 22.1°C ± 0.23 S.E., salinity mean = 36.4 ppt ± 0.27 S.E., dissolve oxygen mean = 6.3g/L ± 0.3 S.E., pH mean = 8.1 ± 0.02 S.E., turbidity mean = 8.1 NTU ± 0.7 S.E.

Experimental design

This experiment had four factors: age (fixed, with three levels: 1-day (Age 1), 8-days (Age 2), and 29-days (Age 3)), speed, (fixed, with two levels: 6 knots and 18 knots), duration, (fixed, with two levels: 2 days and 8 days) and plate (random, with 72 levels). The effect of these factors on survivorship and growth were tested in a fully crossed design. These selections were made based on common vessel voyage profiles and to correspond with parameters tested in other chapters of this thesis. As only one speed could be tested at one time, two consecutive 8 day runs were conducted; one at 6 knots and another at 18 knots. Similarly, only one duration could be tested at one time; after 48 hrs of spin time all 2 day duration plates were removed from the DFD and then the spin continued with the remaining 8 day duration plates. Removing plates and resuming the rotor spin was done quickly (the device was stopped for <1 min). A pilot study testing the effect of starting and stopping the rotor on day 2 of a 4-day spin showed no significant effect on survivorship of the colonies (ANOVA, $F_{1, 46} = 0.1608$, $P = 0.6902$).

Residency period was tested by manipulating the age of the recruit. Recruit age starts from when that organism settles on a substrate, therefore, the age of the recruit exported on a vessel will be equal to, or less than, that vessel's residency period. Although residency period and age of recruit may not match precisely, it is impossible for a vessel to have a recruit from a location that is older than that particular residency period. Three age groups were selected based on short, mid and long residency periods and different life-history stages of *Bugula* (Chapter One: Figure 1.2):

- An *Age 1* recruit is 1 day post-settlement. It is a small, unbranched colony comprising 1 - 2 zooids and reproductively immature. It represents a short residency period.
- An *Age 2* recruit is 8 days post-settlement. It is a larger colony that has bifurcated once or twice, but is still reproductively immature. It represents a mid-length residency period.
- An *Age 3* recruit is 29 days post-settlement. It is a large colony that has bifurcated, on average, 8 times (range 5 - 10 bifurcations) and is reproductively mature. It represents a long residency period.

The time before the start of the experiment that brood stock colonies were spawned was dependent on the pre-determined age group. For example, the Age 2 group were settled and deployed into the field 8 days before the experiment. During one settlement event a parasitic worm contaminated the larval stock which led to high mortality. The brood stock and larvae

were discarded and the spawning had to be repeated resulting in the 18 knot-8 day group being 6 days old. This was considered acceptable because 6 and 8 day colonies would be at the same life history stage (immature colony with one or two bifurcations), and different from the other two age groups. Therefore, the hypotheses regarding short, mid and long residency periods could still be tested.

Once each voyage scenario was complete, plates were removed from the DFD and submerged in seawater. Each settlement plate was then examined under a dissecting microscope. The response variables: presence/absence, size, appearance and ovicell presence were recorded. A colony was considered reproductively mature if ≥1 ovicell was visible. A colony was considered to have survived if it was still attached to the settlement plate; this included colonies that had decreased in size through loss of peripheral zooids but were still attached. A previous study found that *Bugula* could survive 8 day periods (the maximum time period tested in this experiment) in a low food environment (see Chapter Four). In addition, degeneration and regeneration of polypides (the organs and tissues inside the exoskeleton) is a common strategy that may allow an organism to survive through periods of physiological stress (Gordon 1977). Therefore, if a colony remained attached after the 8 day experimental period it was considered to have survived. As *Bugula* commonly bifurcates after each four zooids (Keough and Chernoff 1987), size was measured by counting the number of bifurcations along the longest branch (Marshall 2008). After the last bifurcation each zooid was given a value of 0.25 to obtain a branching score for each colony. Growth was standardised by calculating the change in bifurcation per day using the following equation:

$$\frac{Size_{After} - Size_{Before}}{Time_{After} - Time_{Before}} = \frac{\Delta S}{\Delta T} = Growth\ per\ day$$

Due to incomplete measurements where only the number of bifurcations was recorded, growth could not be analysed during the 6 knot spin, and the effect of speed on growth during the voyage could not be determined.

Once inspected, settlement plates were then attached to a large PVC backing plate and suspended 1 m from the floating pontoon in Hobson Bay. To examine spin legacy effects[3],

[3] A legacy effect is the impact of an event that persists after that event has occurred.

colonies were re-inspected at 7 days post-spin and 28 days post-spin. Upon completion of the study, colonies were removed from the backing plates and preserved in ethanol. There was high mortality across all groups (spin, no-spin control and field control, *see below*) between the 7 and 28 day post-spin measurements, suggesting that an uncontrolled and untested factor in the environment caused the observed widespread mortality rather than legacy effects of the experimental factors. Consequently, it was decided that the 28 day measurements were unreliable and the experiment would be concluded at 7 days post-spin. Due to this high mortality, change in reproductive status was not able to be determined as the full 29 day post-spin period was needed to examine this.

Two controls were used in this experiment; the first involved placing 36 plates in the low-flow area of the DFD behind the octagonal baffle to control for the effect of the spin. The second was a field control where 36 plates were placed in Hobson Bay. In addition to this, two pilot studies were run prior to the experiment to test if *Bugula* could survive and grow in the DFD and if some colonies could survive the slow spin speed (6 knots). Pilot studies showed that *Bugula* had similar survivorship, but lower growth in the DFD compared to in the field (Hobson Bay). As *Bugula* growth is very responsive to food (Schimanski, *unpublished data*), two extra seawater pumps were added to the system to increase water flow and the supply of phytoplankton to the colonies.

Analyses

To investigate the effects of each factor on the survivorship and growth of *Bugula* a univariate permutational analysis of variance (PERMANOVA) (Anderson 2001) using distance-based Euclidean distances for a single response variable was conducted in PRIMER v6 (Clarke and Gorley 2006) with the add-on PERMANOVA+ (Anderson et al. 2008) package. This analysis allows partitioning of the variability of the full design with both fixed and random factors, as well as interactions between factors. PERMANOVA can also account for unbalanced designs. Separate PERMANOVA analyses were performed on survivorship and growth data taken immediately after the spin and 7 days post-spin. Type I sums of squares (SS) were used for the balanced binary data and Type III SS were used to analyse the unbalanced growth data. Each of the analyses used 4999 permutations. To increase power, non-significant terms with $P > 0.25$ were pooled and the analyses were repeated until the simplest model was reached. Significant terms were further investigated using *a posteriori*

pairwise comparisons with the PERMANOVA *t* statistic (999 permutations). PERMDISP was used to test the homogeneity of variance, as PERMANOVA detects differences in either means or variance (Anderson 2006). Due to an insufficient number of permutable units, *P*-values were calculated using a Monte Carlo random sample (Anderson and Robinson 2003). As the hypotheses addressed the effect of speed, duration and age on survivorship and growth, the no-spin control was analysed separately to the spin colonies. However, no-spin control results were plotted on all graphs with spin colonies, to help visualise the results in context. The field control was excluded from analyses.

RESULTS

Statistical tables of PERMANOVA results, pairwise comparisons and PERMDISP results are presented in Appendices A3.1-A3.6. Survivorship was greater in the no-spin control group (99%) compared with the recruits exposed to the spin (89%, $F_{1, 286} = 10.89$, $P = 0.0014$; Figure 3.2). The factor age had an effect on survivorship of the spin colonies ($F_{2, 115} = 4.41$, $P = 0.0228$), with 100% of Age 2 recruits surviving, more than either Age 1 (79%, t = 2.99, $P = 0.004$) or Age 3 (90%, t = 2.71, $P = 0.009$) recruits. There was however, no effect of speed ($F_{1, 115} = 0.059$, $P = 0.81$) or duration ($F_{1, 115} = 0.53$, $P = 0.47$) on survivorship during the spin. There were also no random effects of plate on recruit survivorship, or any other response measured in this study. Even 7 days after the treatment period, survivorship was lower for spin recruits (83%) than no-spin recruits (92%; $F_{1, 269} = 5.55$, $P = 0.02$; Figure 3.3), although there was no effect of the factors (age, duration or speed) on post-spin survivorship ($P > 0.05$).

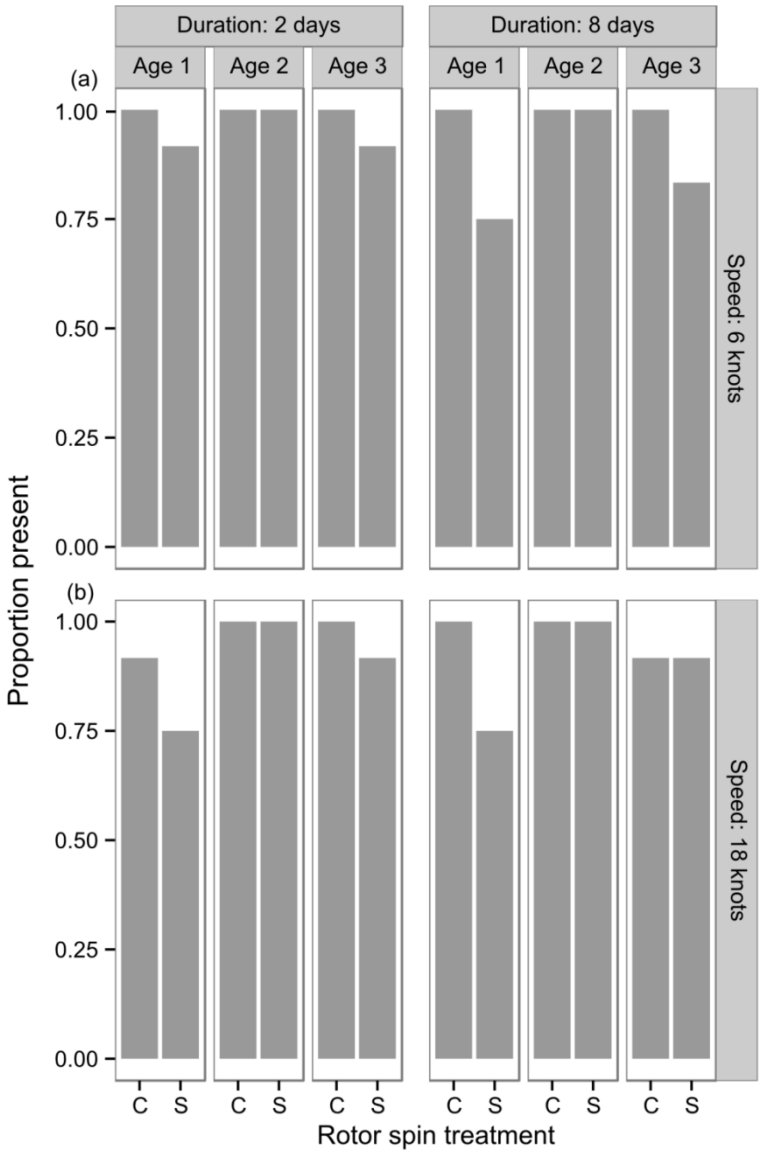


Figure 3.2: Proportion of recruits that were present in the dynamic flow device after each voyage scenario. Rotor spin treatment was spin (S) or no-spin control (C), and is represented along the x-axis. The proportion of colonies that were present after each scenario is represented along the y-axis. Speed tested was (a) 6 knots or (b) 18 knots. Each separate panel shows the spin duration (2 days or 8 days) for each age group (Age 1 = 1-day, Age 2 = 8-days, Age 3 = 29-days).

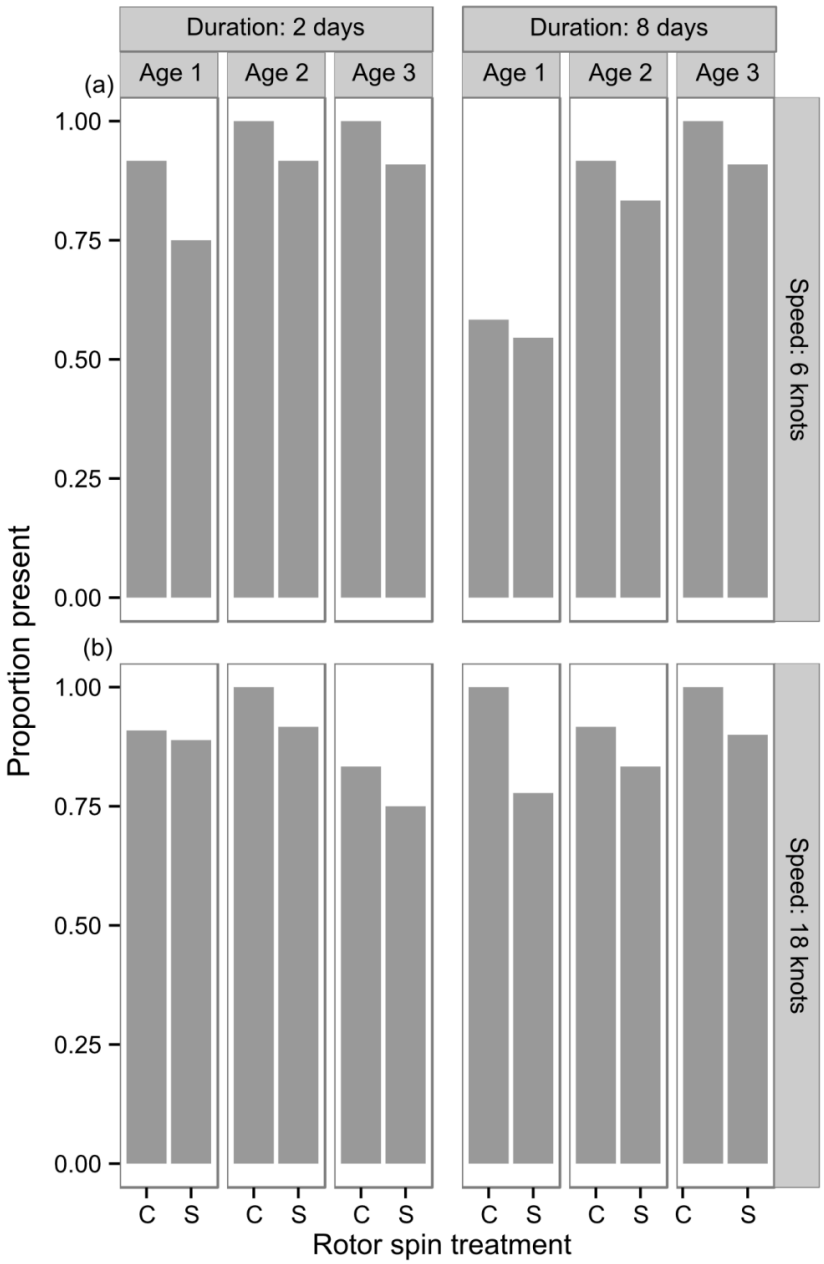


Figure 3.3: The proportion of *Bugula* recruits that were present on settlement plates deployed in Hobson Bay, Melbourne 7 days after the dynamic flow device spin (i.e., the legacy effects of the voyage scenario). Rotor spin treatment was spin (S) or no-spin control (C), and is represented along the x-axis. The proportion of colonies that were present after the scenario is represented along the y-axis. Speed tested was (a) 6 knots or (b) 18 knots. Each separate panel shows the spin duration (2 days or 8 days) for each age group (Age 1 = 1-day, Age 2 = 8-days, Age 3 = 29-days).

Recruits that were exposed to the spin also had lower growth and a greater decrease in size (due to loss of zooids) than the no-spin control recruits during both the treatment ($F_{1, 132} = 42.31$, $P = 0.0002$) and post-spin period ($F_{1, 268} = 14.99$, $P = 0.0006$). The factors duration and age did not affect growth rates during the spin, although the largest reduction in size occurred in the Age 3 group (-1.125 bifurcations/day) during the 18 knot – 8 day spin (Figure

3.4). All factors (age, speed, duration) did however, interactively affect growth 7 days post-spin ($F_{1, 119} = 7.45$, $P = 0.0012$), where there was both an increase and decrease in colony size (Figure 3.5). The highest average growth rates (bifurcation/day) were in the Age 2 and Age 3 groups after the 6 knot-2 day spin (mean = 0.31 ± 0.14 S.E.; 0.30 ± 0.18 S.E., respectively). However, the largest decrease in size was also in the Age 2 and Age 3 groups, particularly after exposure to the 18 knot-8 day spin (Figure 3.5). Growth rate was low for Age 1 colonies (mean = 0.08 ± 0.03 S.E.) and although it did not differ between the two speeds tested it was less after the 2 day duration than the 8 day duration (Figure 3.5). Variability in daily growth differed between Age 1, Age 2 and Age 3 recruits (Figure 3.5). This was expected as Age 3 colonies were larger, and therefore, had the potential to lose more zooids than the Age 1 or Age 2 recruits.

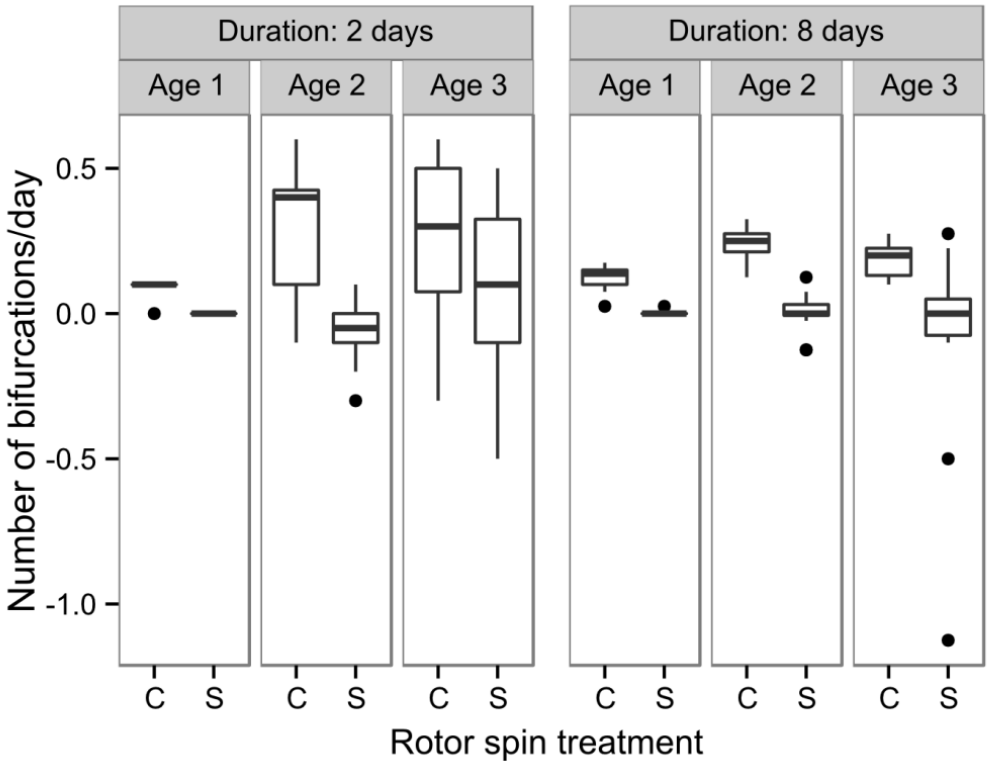


Figure 3.4: Daily growth rate (number of bifurcations/day) of *Bugula* attached to settlement plates in the dynamic flow device during the voyage scenario. Due to missing data growth could only be examined for the 18 knot treatment and, therefore, a comparison between speeds could not be made. Rotor spin treatment was spin (S) or no-spin control (C), and is represented along the x-axis. Growth per day is represented along the y-axis. Each separate panel shows the spin duration (2 days or 8 days) for each age group (Age 1 = 1-day, Age 2 = 8-days, Age 3 = 29-days).

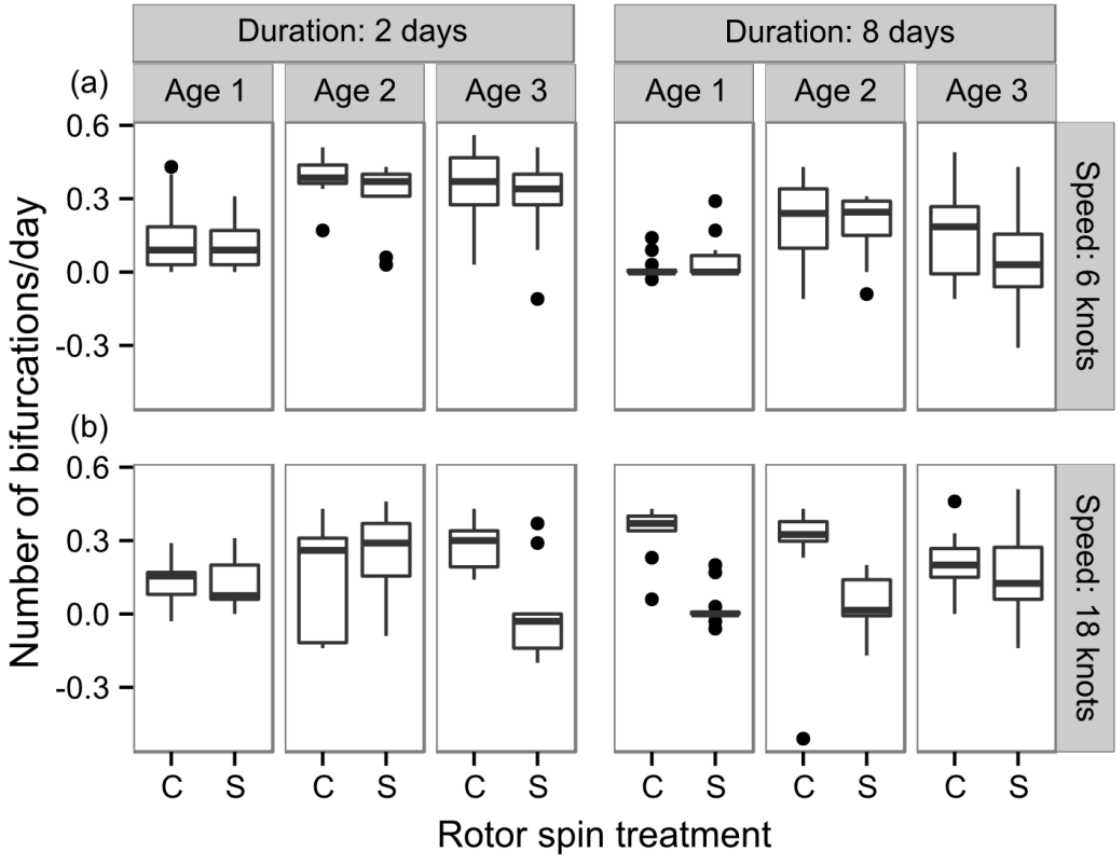


Figure 3.5: Daily growth rate (number of bifurcations/day) of *Bugula* attached to settlement plates deployed in Hobson Bay, Melbourne over the 7 day period after the dynamic flow device treatment (i.e., legacy effects of the voyage scenario). Rotor spin treatment was spin (S) or no-spin control (C), and is represented along the x-axis. Growth per day is represented along the y-axis. Speed tested was (a) 6 knots or (b) 18 knots. Each separate panel shows the spin duration (2 days or 8 days) for each age group (Age 1 = 1-day, Age 2 = 8-days, Age 3 = 29-days).

Colony appearance also altered after some voyage scenarios. All Age 3 recruits conformed to the hydrodynamic flow (state of anisotropy) resulting in a low, compact and streamlined appearance with branches positioned away from the flow (Figure 3.6). The younger Age 1 and Age 2 recruits did not show this configuration. Additionally, transparent zooids were observed at the tips of 27% (6 of the 22) of Age 3 spin recruits, although they were not observed on any of the Age 3 control recruits (Figure 3.7). Asymmetrical growth was also observed post-spin. Twenty-three percent of the Age 2 spin recruits did not bud new zooids on the outer branches of the colony (compared to 5% of no-spin controls), but did develop new zooids as expected on the inner branches. Size and sampling constraints meant that this asymmetrical growth could not be examined for the Age 1 or Age 3 recruits.

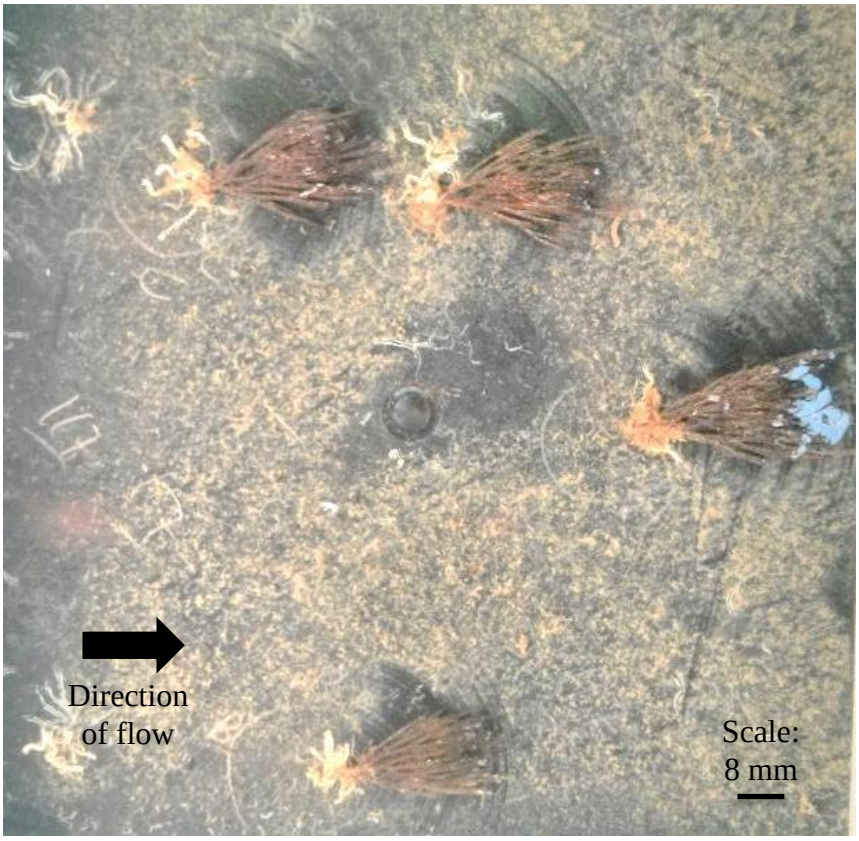


Figure 3.6: Age 3 colonies post-spin. Note that colonies are re-orientated to a streamlined position close to the plate with branches bunched close together.

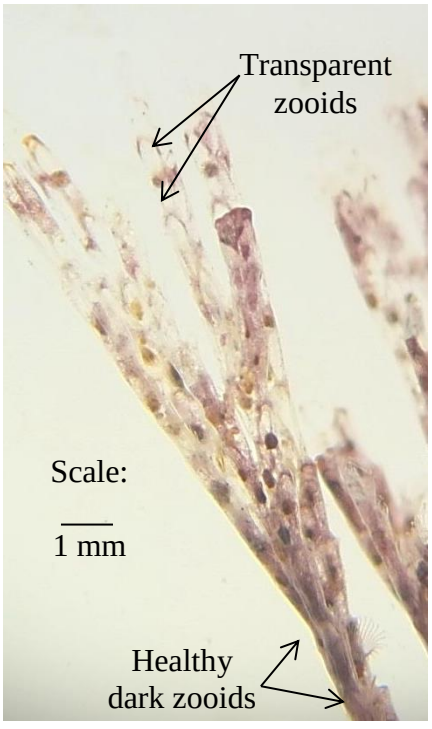


Figure 3.7: An example of the transparent zooids observed around branch tips of some Age 3 colonies post-spin.

DISCUSSION

This study is the first to examine how vessel residency period may influence translocation success, and results indicate that it is a significant factor. Survivorship was high across all groups but it was the Age 2 recruits that had 100% survivorship after all voyage scenarios. Importantly, Age 1 recruits could also survive and grow post-voyage, even after an 8 day voyage at 18 knots. Neither speed nor duration influenced *en route* survivorship, but these factors did affect post-voyage growth, with recruits on the fastest and longest voyage scenarios having the slowest post-voyage growth. These results suggest that many vessel types, including those that reside for short residency periods in the donor location, are capable of transporting biofouling organisms. This is supported by an observational study that found a weak relationship, with low predictive power, between residency period and biofouling characteristics (Inglis et al. 2010).

The age of an organism often relates to size, particularly in colonial organisms, and other studies have found a link between size and survivorship during stressful events. For example, air exposure experiments on marine *Styela clava* (Hillock and Costello 2013) and freshwater zebra mussels (*Dreissena polymorpha)* showed that larger individuals had higher survivorship than smaller individuals (Ricciardi et al. 1995). Larger colonies may be expected to have increased drag, however, it was observed during the present study that Age 3 colonies bent with the flow, which may have allowed this species to conform within the boundary layer of the plate and survive at speed (Koehl 1984, Denny et al. 1985, Denny et al. 1998). Additionally, the outer branches closest to the direction of flow of larger colonies may disrupt flow so that the inner zooids are protected. This was shown in a study by Okamura (1984), where feeding by larger *Bugula* colonies (2.5 - 3 cm tall) was not disrupted at the highest flows measured but was disrupted in smaller colonies (1.5 - 2 cm tall). Admittedly flows examined in this study were much lower (maximum of 0.23 knots) than even the slowest speed in the present experiment.

Attachment strength may also vary with age of the recruit. Bryozoans generally attach to a substrate through a bioadhesive substance which hardens (Soule and Soule 1977). However, as research in this area is dominated by antifouling technology development, little is known beyond initial recruitment and how attachment strength of *Bugula* changes with the age; this is an area that warrants further study. Indeed, little is known about the attachment strength of

most biofouling species, except mussels and barnacles (Clarke Murray et al. 2012). Clarke Murray et al. (2012) found *Styela clava* attachment strength increased with height, but there was no relationship between height and attachment strength for other solitary ascidians examined. Results of this study may be the consequence of an interaction between attachment strength and drag force. In the present study, the Age 2 colonies were small, with only one or two bifurcations, and may have had a lower drag profile than the Age 3 colonies, but with higher attachment strength than the Age 1 recruits.

Interestingly, speed and duration did not significantly affect survivorship. Like many other filter-feeders, *Bugula* live in environments exposed to hydrodynamic flows, such as tidal flow, unidirectional currents and wave exposure and have also been recorded on the laminar flow surfaces of vessels (Inglis et al. 2010). Therefore, it is expected that colonies can cope with flow. The non-significant effect of speed on survivorship however, is different to two previous experimental studies on *en route* hull-fouling survivorship, which both showed a decrease in survivorship with increasing speed (Coutts et al. 2010a, Coutts et al. 2010b). Coutts et al. (2010b) found an 11% reduction in cover of fouling organisms at 5 knots, a 24% reduction at 10 knots, and an 85% decrease at 18 knots. Coutts et al. (2010a) found a significant decrease in percentage cover ranging from 23 - 37% across all speed treatments: slow (mean = 4.6 knots), medium (mean = 8.4 knots) and fast (mean = 17.9 knots) in one trial. However, in a second trial there was a 44% decrease in cover in the fast speed treatment but no decrease in the slow and medium speed treatments. There were a number of differences between these two studies and the study presented here. In particular, this study focused on the survivorship of early life stages whereas both studies by Coutts et al. (2010a, b) used older biofouling communities that were 2-5 months old. Additionally, they used naturally-recruited biofouling assemblages as opposed to independently spaced and manipulated recruits as used here. Different forces will be important depending on fouling composition. For example, lift forces will be more important for the survivorship of older, more developed communities and drag forces more likely to influence the survivorship of individual recruits (Clarke Murray et al. 2012). In this study, recruits were spaced independent of each other. It would also be of interest to know how the results would be influenced if the recruits were among a biofouling community. Recruitment is often facilitated by other species and many sessile organisms, including *Bugula*, are gregarious settlers. Other fouling organisms are likely to provide protection from hydrodynamic drag, as

shown in high-density seaweed communities (Johnson 2001) and consequently, even higher survivorship post-voyage might occur, if individual colonies were found within a community.

A number of different taxa were tested in the Coutts et al. (2010a) and Coutts et al. (2010b) studies (34 and 13 respectively) and they noted that survivorship related to the different morphological characteristics of the taxa. Higher survivorship was demonstrated by solitary/encrusting/hard, colonial/encrusting/soft and colonial/erect/flexible organisms. *Bugula* has a colonial/erect/flexible morphology that allows colonies to reconfigure with flow, a strategy used by other taxa, such as algae in high flow systems (e.g., Denny and Gaylord 2002). Similarly, Clarke Murray et al. (2012) tested attachment strength and drag coefficient of eight common hull-fouling species and found that dislodgement varied between species. Indeed, fundamental zonation studies provide additional evidence that some species can tolerate higher water velocity than others (Schneider et al. 2005). For example, several studies have shown a positive relationship between wave exposure and the abundance of *Mytilus galloprovincialis* (Gosling and Wilkins 1981, Skibinski and Roderick 1991). Finally, Coutts et al. (2010a) used randomly selected vessels to attach pre-fouled settlement plates and test the effect of voyage speed. Because of the random nature of the vessel selection, components of the voyage profile, such as voyage conditions (calm/choppy/very choppy), voyage duration and residency period between voyages, likely varied between vessels. The plates attached to the fastest moving vessels had the greatest reduction in fouling, but these were also the vessels that spent the most time at sea and the shortest time in port residency. In this study, the mesocosm experiments allowed factors of interest to be tested and other variables to be controlled.

Voyage duration is often-overlooked and untested in other studies. For example, Clarke Murray et al. (2012) measured attachment strength that represents an instantaneous force required to dislodge an organism. The authors noted that fatigue fracture; that is, the accumulated effect of force over time (as may occur during a long voyage), is not accounted for in the model they used, and results may be a conservative estimate. Coutts et al. (2010b) tested survivorship over very short durations (20 minutes). The vessels used in the Coutts et al. (2010a) study did differ in voyage duration; however, the authors did not incorporate this variable into the analyses. The *en route* observational studies by Carlton and Hodder (1995) and Davidson et al. (2008) were over long durations, but due to the opportunistic nature of the studies they could not make comparisons between vessels and therefore, could not test the

effect of voyage length. Inglis et al. (2010) used a modelling approach to identify predictors of vessel biosecurity risk, measured by biofouling extent and presence of NIS. They found that voyage history, which included mean time at sea (voyage duration), had the greatest influence on model fit for recreational vessels but not commercial. However, overall the model's predictive power was low, suggesting a lot of within-vessel class variation. The present study suggests that voyage duration is not important for translocation survivorship, but effects post-voyage growth.

Adverse post-voyage effects may derive from starvation during oceanic transit. *Bugula* uses a cilia-lined lophophore to divert water, create feeding currents and to pass food particles into the mouth (Winston 1977). The lophophore retracts when exposed to high flow (*personal observation*), making it more difficult to feed at high velocities (Okamura 1984). The gut passage time of *Bugula stolonifera* is estimated to be around 27 mins and feeding occurs frequently (Winston 1977). If flows simulated were large enough to disrupt feeding the health of the colony is likely to be effected. *Bugula* has a tough exoskeleton that may remain attached for a period of time regardless of nutritional stress and condition of the polypide. Therefore, this species may remain attached and be recorded as present, but have reduced functional capacity. Subsequent chapters of this thesis will examine health, including reproductive output, post-voyage.

Growth, although reduced during the spin, was not significantly affected by age or duration[4]. Some colonies, particularly the larger Age 3 colonies, decreased in size through loss of branch sections. Fragmentation is an important survival mechanism for some colonial species, as it may prevent dislodgement of the entire colony (Winston 2010). It can also be an important dispersal mechanism if fragments can reattach (Bullard et al. 2007), which has been demonstrated by *Bugula* under experimental conditions (Hopkins et al. 2011). Some colonies displayed asymmetrical growth where new zooids budded on the central, not the peripheral, branches. Bryozoan growth is sensitive to flow, as demonstrated by different growth forms under various flows (Jebram 1970). Phenotypic plasticity, that is the ability of an individual organism to modify its phenotype within its lifetime, is an adaptive strategy that can enhance fitness relatively quickly in a new environment (Sakai et al. 2001, Smith 2009). Phenotypic plasticity has been demonstrated as a response to hydrodynamic flow by sponges,

[4] Only results from the 18 knot run were analysed, therefore, the effect of speed on voyage growth could not be examined.

which changed the strength and stiffness of the body wall (Palumbi 1984), snails, which increased foot size (Etter 1996, Trussell 1997), and barnacles, which altered the length of their cirri (Marchinko and Palmer 2003, Marchinko 2007). Although not tested in this study, the observed asymmetrical growth could potentially be a form of phenotypic plasticity. Phenotypic plasticity is investigated further in Chapter Five.

Translocation is not only a dispersal mechanism, but also a disturbance. How colonies 'deal' with this disturbance contributes to how successful they will be as NIS. Importantly, the legacy results showed that not only did the colonies survive the voyage, but many continued to survive and grow 7 days post-voyage indicating that they may reproduce in the recipient location in the future. None of the factors individually influenced survivorship 7 days post-voyage, however, all three factors interactively affected post-voyage growth. Recruits that were Age 1 on an 8 day-18 knot voyage tended to have the slowest growth rates. Some colonies increased and others decreased in size. Although a reduction in size is likely to reduce reproductive output, in all cases, base zooids remained attached. Because *Bugula* is a colonial animal, no specific unit is essential to survival and regeneration and recovery is possible after colony sections are lost (Chadwick and Loya 1990, Smith and Hughes 1999, Bone and Keough 2005).

If colonies cannot prevent the loss of zooids, then the ability to recover from this damage becomes paramount. *Bugula* has been shown to recover well after damage. For example, Bone and Keough (2005) experimentally damaged *Bugula* by removing sections of the colony over three locations and found that regeneration of the lost area was rapid and did not alter future growth rate. However, it was predicted that reproductive onset was delayed by 7 - 20 days and the colony could have up to 70% lower reproductive output (Bone and Keough 2005). Bone and Keough (2005) also found that the location of damage is important – there will be less reproductive impact for colonies that lost entire branches compared with those that lost the growing ends of multiple branches. This study showed that larger Age 3 colonies often had transparent zooids on all of the branch tips which may affect reproduction. Regrowth at the site of breakage has also been observed in many other taxonomic groups (Bely and Nyberg 2010). Coutts et al. (2010a) recorded legacy effects in fouling communities by measuring a change in percentage cover 7 days post-voyage. Because no new recruitment was observed over this period, a change in percentage cover could be interpreted as growth of existing organisms. They found that communities from slow and medium speed vessels had a

greater increase in percentage cover than those from the fast vessel during one trial. There was also a decline in species cover for the fast vessel communities in a second trial. Results here support this research, but also suggest that it is the age of the recruit that may be a more influential factor than speed and voyage duration.

Limitations of the study

This study did have a bias in recruit selection, as only recruits that survived to the start of the spin period were used in the experiment. Recruits used in the Age 3 group had already survived 29 days and may have had higher fitness than Age 1 recruits that only needed to survive for 1 day. However, due to high survivorship (100%) of Age 1 recruits in previous experimental work (see Chapter Four), this was not considered to have a strong influence on the results, but is a caveat in interpreting the age factor results.

Conclusions and implications for vessel biofouling management

Although survivorship was high across all groups, age was still a significant factor. Age 2, mid-residency, recruits had the highest *en route* survivorship (100%) and relatively high post-voyage growth. Short residency recruits had lower survivorship and growth, but importantly, 79% of these did survive and grow. Speed and duration of voyage did not influence translocation or post-voyage survivorship, but did influence post-voyage growth; with faster, longer voyages having the greatest impact. Although survivorship was high, effective translocation only occurs when an organism reproduces. The next step is to determine if different-aged recruits will reproduce, releasing propagules into the recipient environment, which is the focus of Chapter Four.

Distances travelled under each voyage scenario would be substantial – a vessel travelling: 6 knots for 2 days = 288 nautical miles (NM); 6 knots for 8 days = 1152 NM; 18 knots for 2 days = 864 NM; and 18 knots for 8 days = 3456 NM. Although other important filters, such as latitudinal change in abiotic variables are not included in this study, fast vessels have quick transit times minimising exposure to unsuitable conditions. Therefore, the ability of biofouling to remain attached in high hydrodynamic flow, and the legacy effects of the voyage, are important components of NIS translocation. There are numerous examples of long-distance transfers of hull-fouling species around the world where NIS are found great distances from their native geographical location, such as *Undaria pinnatifida*. But, we have

little empirical evidence about what contributes to a successful transfer. With predicted climate change, ocean conditions are likely to alter and potentially reduce environmental barriers further. As speed alone will not remove recruits, focusing management effort, such as mandatory maintenance regimes on high-risk vessels categories, is likely to return biosecurity benefits.

Integrating the supply of individuals to a location (i.e., propagule dispersal) is an important part of marine ecology. It should be treated with equal importance in the study of invasion ecology, due to its links to propagule pressure in the recipient location. Marine communities are not saturated with species. Human assisted pathways work in opposition to natural dispersal limitation and it is likely NIS will continue to be transported along the hull-fouling pathway. Consequently, there is a need for further robust, empirically-derived data to understand interactions between filters during the important pre-arrival stages.

Chapter Four: Pre-arrival processes, reproduction and transfer from the vector

ABSTRACT

Propagule pressure is a critical determinant of establishment success. One feasible angle for understanding and controlling human-assisted spread of non-indigenous species is to understand how early stages of the invasion process, such as propagule entrainment or transport, influence levels of propagule pressure to recipient environments. Using hull-fouling as a model invasion pathway, and *Bugula neritina* as a model organism, the effect of two voyage characteristics; donor port residency period and subsequent voyage pattern, on propagule pressure was examined. Voyage scenarios were created by manipulating food levels in a series of field experiments. Ambient food represented vessels residing in a port where *Bugula* would be able to feed, and limited food represented vessels underway at sea where *Bugula* would not be able to feed. Short, moderate and long port residency periods (1 day, 8 days and 32 days, respectively) were simulated to examine the performance of recruits that are exported from a port at different ages and stages of development (1-day = Age 1, 8-days = Age 2, and 32-days = Age 3). The effect of short voyages interspersed by short port residency periods (referred to as Short Pulse voyages (SP)) or long voyages interspersed by long port residency periods (referred to as Long Pulse voyages (LP)) was also examined. Spawning success, number of larvae and larval size were measured over multiple spawning events after each voyage scenario. All colonies survived the voyage scenarios, but depending on their pre-recipient environment experience many failed to release propagules. Colonies that had been exposed to an SP scenario had higher reproductive output than those that had been exposed to an LP scenario in the Age 1 and Age 2 groups. However, Age 3 colonies did not release larvae under any voyage scenario. Propagule output of voyage colonies (SP and LP) did not decline over multiple spawning events as it did for constant food control colonies. SP larvae were significantly smaller and more variable than the

constant food control larvae for the first spawning only. Variably sized larvae and an increase in propagule output with spawning may be evidence of bet-hedging strategies. Results suggest that vectors with biofouling organisms on short intra-coastal (domestic) voyages may facilitate higher propagule pressure than those that spend longer periods underway (e.g., slow-movers on international pathways).

INTRODUCTION

Propagule pressure is regarded as a key component of invasion success (Johnston et al. 2009). It comprises several dimensions, in particular: the number of individuals released into a location in a single event (propagule size), the number of release events (propagule number) and the quality and diversity of the propagules released (Lockwood et al. 2005, Hedge and Johnston 2012; for definitions see Box 4.1 *A note on terminology*). These are all important aspects to consider as populations with larger propagule size, number and diversity have greater potential to overcome environmental and demographic stochasticity, a main cause of small population failure (Sakai et al. 2001, Frankham et al. 2002, Allendorf and Lundquist 2003, Kolbe et al. 2004, Simberloff 2009). Propagule quality may influence future fitness of individuals over multiple life-history stages (McGinley et al. 1987, Pechenik et al. 1998, Marshall and Keough 2004, Dias and Marshall 2010).

Recent studies have demonstrated the positive relationship between propagule pressure and colonisation success, particularly when there is disturbance in the recipient environment (Valentine and Johnson 2003, Clark and Johnston 2005, Dethier and Hacker 2005, Valentine and Johnson 2005, Britton-Simmons and Abbott 2008, Hedge and Johnston 2012, Hedge et al. 2014). The focus of these studies has been on the role of propagule pressure after arrival in the recipient location. However, by identifying which vessels are most likely to transport high-quality (fitness) recruits it may be possible to develop risk-reduction strategies to lower propagule pressure and establishment likelihood, both in the context of new NIS and the spread of existing ones.

To understand biological invasions, it is important to understand how organisms interact with their environment. Although constrained within the limits of its genotype, the interactions of an individual with its environment can modify its life history (Begon et al. 2006). For example, low food availability can lead to longer dispersal periods in gastropod veligers

(Pechenik 1984, Bell 1993) and low quality food can lead to the development of larger mandibles in a grasshopper (Thompson 1992). Adult marine sessile organisms are generally unable to escape an unfavourable environment. Consequently, phenotypic plasticity is a common phenomenon (Smith 2009). Reproductive output is strongly intertwined with the environment. For example, the bryozoan *Bugula neritina* produced smaller and more variably sized larvae when grown at 25ºC than at 19ºC (Burgess and Marshall 2011a). Similarly, the tubeworm *Hydroides diramphus* produced larvae that performed better (greater fertilisation success and survival) in the same salinity levels that their parents experienced (26 ‰ or 35 ‰) (Jensen et al. 2014), and a larger number of gametes were produced by the bivalve *Crassostrea gigas* when reared in an environment with increased food abundance (from 6.1 µg L-1 to 33.0 µg L-1) (Ernande et al. 2004). Propagule pressure to a new recipient habitat will, therefore, be influenced by conditions an organism experienced before arrival to that environment, including the conditions experienced during the translocation stage.

Hull-fouling is particularly effective at increasing propagule pressure to a location, which occurs at the third stage of the invasion pathway (Chapter One: Figure 1.1). Selective filters may severely restrict the number of biofouling species that are translocated on vessels. However, surveys in New Zealand show that a large number (187) of species are still arriving into the region. Interestingly, 73% of the 128 NIS recorded from a sample of 508 international vessel arrivals are not yet established in New Zealand (Inglis et al. 2010). The need to obtain more information on failed invaders has been recognised (Carlton 1996, Miller et al. 2007, Miller and Ruiz 2009) and it is thought that the failure of some introductions may be due to the compromised health of the organisms on arrival leading to low propagule pressure (Carlton 1996, Wonham et al. 2001, Verling et al. 2005, Lodge et al. 2006). Proxies, such as the number of ship arrivals used to estimate propagule pressure (e.g., Drake and Lodge 2004), are approached with caution as all vessels are not an equal risk due to variety in diversity, number and quality of organisms transported (Verling et al. 2005). Pineda et al. (2006) used the term “effective dispersal” to describe how many settlers go on to reproduce. Similarly, it is useful to think of “effective translocation” as the number of hull-fouling organisms that arrive and go on to reproduce, the product of which will comprise propagule pressure to the recipient environment.

Voyage characteristics dictate what conditions a hull-fouling organism experiences. Vessel residency period in the donor location and voyage pattern are two characteristics that will be

examined in this chapter. Vessel residency period refers to the time a ship is moored in port. These residency periods can be highly variable. For example, many merchant vessels reside for one day or less but some recreational vessels will reside for several weeks or more.

Depending on residency period, vessels leaving a donor port can transport hull-fouling organisms that range from one day to several months in age and vary in their state of development and overall condition. Aside from residency periods, vessels also display different types of voyage patterns. For example, some vessels undertake frequent short voyages, such as domestic cargo vessels travelling between ports along the same coast. Other vessels can embark on long infrequent voyages, such as work barges that are contracted for construction or maintenance projects requiring them to undertake long, slow voyages infrequently every few months. It is unknown whether and how voyage characteristics influence propagule pressure to a recipient environment. For example, it is not clear if the conditions experienced *en route* by a hull-fouling organism that was exported from a source port only 8 days after it settled to the hull location will affect its future fecundity in the recipient habitat. Similarly, the question of how a 30-day old reproductively mature organism, or 1-day old recruit will perform after exposure to long voyages compared to short voyages has not been addressed. An understanding of these effects could be used in risk assessment models that aim to detect high-risk vectors. This chapter examines the influence of (i) vessel residency period in the donor location and (ii) voyage pattern on propagule pressure to the recipient location. The following hypothesis was tested: Propagule size, quality and diversity are influenced by residency period, voyage pattern and spawning event.

Box 4.1: A note on terminology:

Propagule pressure terminology is not intuitive and can be confusing. For example, *propagule number* refers to the number of introduction events, but could be confused with the number of individuals released. *Propagule size* refers to the number of individuals released, however, could be confused with size of an individual. The term *propagule* itself can have multiple meanings including, a larval or adult stage. Here the chapter is written using terms that aim to be less ambiguous to a reader who may, or may not, have a background in propagule pressure. Below is a list of terms used in propagule pressure literature, along with their definition and how they are referred to in this chapter. Terms not specific to propagule pressure literature are also included in the list.

Propagule pressure term	**Definition**	**Term used in this chapter**
Propagule	"An ecologically relevant unit of dispersal, defined as a colonising organism or vegetative structure capable of establishing a self-sustaining population" (Lee and Bruno 2009, p. 7052). It can refer to many stages including an adult or a larva. Here a propagule is the larva released from a colony.	Propagule
Propagule size	Number of larvae released from a colony	Number of larvae
Propagule quality	Size of larvae is used as a measure of quality	Size of larvae
Propagule diversity	Normally a measure of genetic diversity. Here the size of the reproductive population, that is, the number of colonies that spawned, or *spawning success*, is used as a measure of diversity.	Spawning success
Propagule number	The number of introduction events. In this study a spawning event is considered an introduction event.	Spawning event
	The time a ship is moored in port	Residency period
	The frequency and duration that a vessel spends at port and at sea. Here two voyage patterns are examined: Short Pulse (SP) profiles which consist of 2 days at sea followed by 2 days at port, and Long Pulse (LP) voyages which consist of 8 days at sea followed by 8 days at port.	Voyage pattern

METHODS

Experiments were conducted at the Naval Point Yacht Club Psych Jetty in Magazine Bay, Lyttelton Harbour, South Island, New Zealand (Figure 1.3).

Collection of brood stock and spawning.

Reproductively mature *Bugula* colonies were collected from pilings in Magazine Bay. Care was taken to collect colonies from locations separated by approximately 20 m to capture genetic variability. Colonies were transported to the lab in ambient seawater in an insulated light-proof container to prevent spawning. In the laboratory, adults were cleaned of sediment and extraneous fouling organisms, such as ascidians, and rinsed in 45 µm filtered seawater. Cleaned adults were transferred into aerated tubs containing filtered seawater at 18°C (the same temperature as ambient seawater). Tubs were covered in black plastic to create a dark environment and left in a dark laboratory for a period of 48 hrs. After 48 hrs, under red-light conditions, colonies were randomly selected and transferred into 3 L beakers filled with seawater filtered to 1 µm. Fluorescent lights were used to shock the colonies and induce spawning. Larvae released from the colonies were visible after 10 mins. To avoid selection bias, spawning was allowed to continue for 30 mins to allow a large pool of larvae to accumulate before commencing larval transfer to settlement containers. *Bugula* can settle within an hour of light shock (Wendt 1996), but settlement of larvae in glass beakers was not observed during this period.

Prior to spawning, 48 × 2 L white plastic High-Density Polyethylene (HDPE) square containers with tight sealing lids and filled with filtered seawater were prepared for larval settlement (Figure 4.1). A hole (ca. 10 mm) was drilled into each lid allowing an 8 mm galvanised bolt to attach a light grey PVC settlement plate (150 mm × 150 mm, 4 mm thick) to the underside of the lid. PVC plates were selected for settlement due to their successful use in other *Bugula* studies (Bone and Keough 2005), their durability and ease to work with. The plates were sanded on one side and left in seawater to develop a biofilm which promotes settlement (Qian et al. 2000, Dobretsov and Qian 2006). Approximately 30 larvae were randomly selected and pipetted into the settlement containers. Care was taken not to disturb the containers during larvae transfer, including limiting light fluctuations, as this had the potential to disrupt the settlement process (Wendt and Woollacott 1999). An earlier trial of the settlement containers showed high recruitment (93%), although only 40% of larvae

recruited to the target area (underneath side of plate) and others settled on the sides of the container, edge, or top-side of the plate (unpublished data). Larvae were left in the temperature controlled lab for 24 hrs to settle. The following day, each plate was examined for settlement.

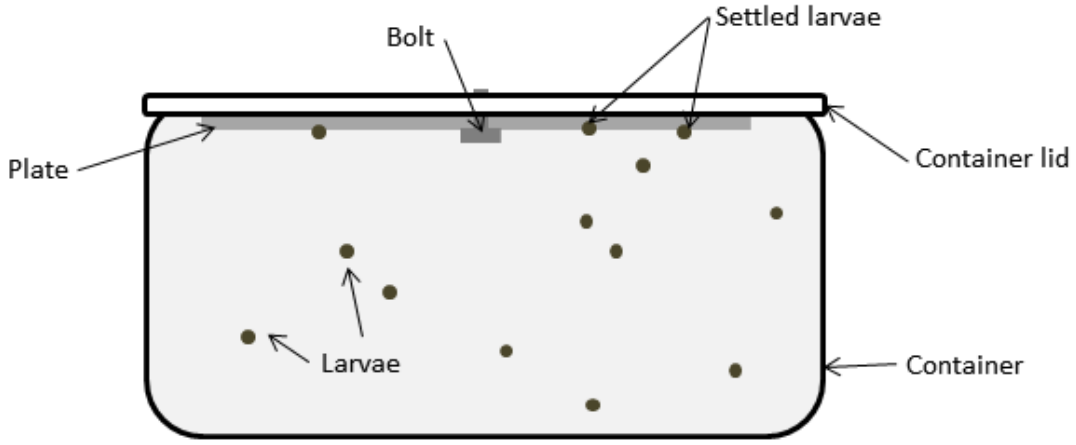


Figure 4. 1: Larval settlement containers. PVC settlement plates were attached to the underside of the removable container lid. *Bugula* larvae introduced into the seawater-filled container were left to settle for 24 hrs. The container was secured to the lid to contain settled larvae.

Based on laboratory trials and field observations it was estimated that the Age 3 (*see definition below*) colonies would branch up to 40 mm in width, so recruits were thinned (> 40 mm) apart using forceps up to a maximum of five colonies per plate (150 mm^2), to minimise competition between the colonies. Recruits within 10 mm of the edge of the plate were also removed. Three of these recruits on each plate were haphazardly selected, circled and labelled as experimental colonies to increase representation.

Container lids were then replaced and containers were transported to the study site (approximately 20 mins). Settlement plates (with attached lids) were removed from the settlement containers and assigned haphazard positions to 1.8 m wooden frames (six in total). Plates were spaced 130 mm apart and positioned horizontally on the under–side of the wooden frame to prevent sediment build-up (Wendt and Woollacott 1999). Food control boxes (as described below) were attached to the lid so as each plate was contained within the food control box. Frames were then suspended 1m below sea level from a floating pontoon (Figure 4.2).

Experimental design

Thirty-six plates were deployed from the Naval Point Yacht Club Psych Jetty. Three experimental colonies were present on each plate giving a sample size of three or nine depending on the effect of each plate.

Food limitation

Food limitation is likely to be a selective filter that occurs on all voyages as a vessel moves away from nutrient-rich port water, particularly when travelling through food-poor oceanic water (Murphy et al. 2001), or when the recruit is unable to feed due to hydrodynamic forces (Lewis and Coutts 2009). Food availability is important for the regulation of reproductive activity (Boltovskoy et al. 2009), therefore food limitation during a voyage is likely to influence the number of propagules released into a recipient location. To test how reproductive output of a hull-fouling species in the recipient location is influenced by donor residency period and subsequent voyage pattern, food limitation was used as a proxy for vessel movement. Food was limited using Food Control Boxes (FCBs), based on a design used by Clark and Johnston (2005). FCBs were settlement containers with rectangular openings (110 mm × 40 mm) cut into each of the four sides (Figure 4.2). Openings were either left open to allow ambient water to pass through, mimicking port residency, or covered in 63 µm mesh, to mimic time at sea. The mesh restricted phytoplankton and water flow (see Appendix 4 for flow measurements) and consequently food. Although phytoplankton is a main food source for *Bugula* (see Chapter One: *Model organism: Bugula neritina*), this bryozoan may also feed on bacteria (Gosselin and Qian 2000). Consequently, I chose to use the term 'food limited' rather than 'no food' to account for this potential supplementary food source. Dissolved oxygen was reduced in mesh containers compared to open containers (84 ± 4% and 93 ± 3% respectively; Appendix 4), but was not considered biologically significant as *Bugula* has been recorded growing in locations with lower dissolved oxygen, for example, 66% at Port Kembla, Australia (Piola and Johnston 2006). In addition, to control for higher recruitment rates within the open FCBs, extraneous fouling was removed (by toothbrush and scalpel) from the plates each week. The base portion of the FCB was sealed with the lid, which was attached between the plate and the frame, allowing the open and mesh portion of the FCB to be easily alternated according to voyage pattern. To control the effect of removing the FCB, all FCBs were removed and replaced, even if a change was not required. This also

provided an opportunity to remove any sediment that had accumulated in the bottom of the FCB.

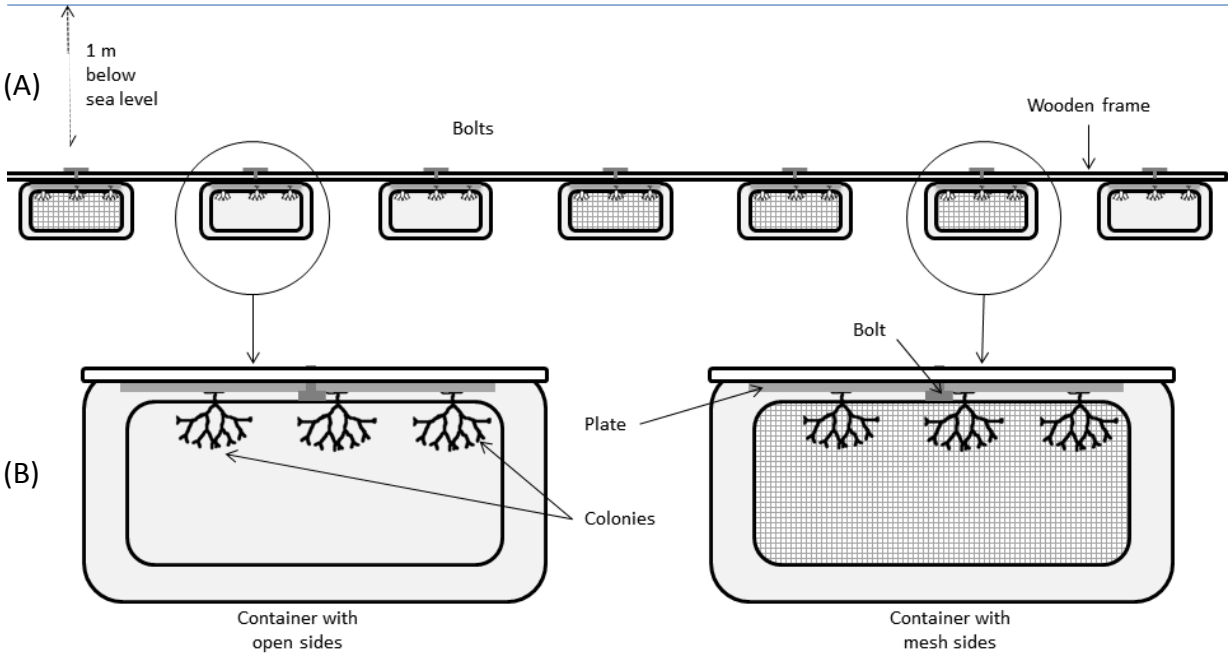


Figure 4. 2: Food control boxes (FCBs) based on a design by Clark and Johnston (2005) were used to manipulate food levels in the field. FCBs were attached to a frame and suspended 1m below sea level (A). Plates with recruits were attached to the underside of the lid. The container base, which had either open or mesh-covered sides, was attached to the fastened lid (B).

Factor One: Residency period

The age of a hull-fouling organism at the time of voyage will be less than or equal to the vessel residency period where that organism recruited. To test if residency period in the donor port did influence propagule pressure at the recipient location by affecting fecundity or offspring fitness of the transported organisms, the age of the recruit was manipulated. Age had three levels: 1-day = Age 1, 8-day = Age 2, and 32-day = Age 3 recruits. These ages were selected based on two criteria:

1) They represent short, mid and long port residency periods for vessels in New Zealand ports (Inglis et al. 2010).

2) They provide a range of size and life history stages: a 1-day recruit comprises 1-2 zooids and is approximately 2 mm in length (with no branching); an 8-day recruit is a reproductively

immature colony likely to have bifurcated once; and a 32-day recruit is reproductively mature, highly branched and bushy in form.

Colony size was very similar within each age group (Age 1: 0 bifurcations; Age 2: 1–3 bifurcations; Age 3: 8–10 bifurcations). All colonies were derived from the same spawning event and were grown in the field until the required age. Once each age was reached, the colonies went through one of the four experimental levels (see *Factor 2: Voyage pattern*). The experimental period for each age group was temporally staggered (Figure 4.3). Temporal variation was unavoidable – to work with different ages it was necessary to either stagger the experiment, or stagger the initial spawning using different batches of brood stock. It was considered preferable to do the former to minimise confounding variables that would be introduced by using different brood stock (e.g., legacy effects due to variation in maternal experience (Marshall and Keough 2004, Burgess and Marshall 2011a)) and due to logistical constraints when collecting response data.

Factor Two: Voyage pattern

The effect of voyage pattern on propagule pressure was tested by manipulating food delivery patterns. Voyage pattern had four levels (including two controls): short pulse (SP), long pulse (LP), constant starvation control (CS) and constant full food control (FF). The SP pattern alternated between 2 days in port (open container, food available) followed by 2 days at sea (mesh container, limited food available). This represented a frequently moving vessel, such as a domestic cargo vessel that travels between domestic ports around New Zealand. The LP pattern alternated between 8 days in port followed by 8 days at sea. This represented a slow moving vessel that travels between New Zealand and Australia (Inglis et al. 2010). CS colonies were in mesh containers throughout the experiment, and FF colonies were in open containers throughout the experiment. Colonies were subjected to the voyage pattern for a period of 48 days which was when ovicells were visible on the youngest colonies (the Age 1 group).

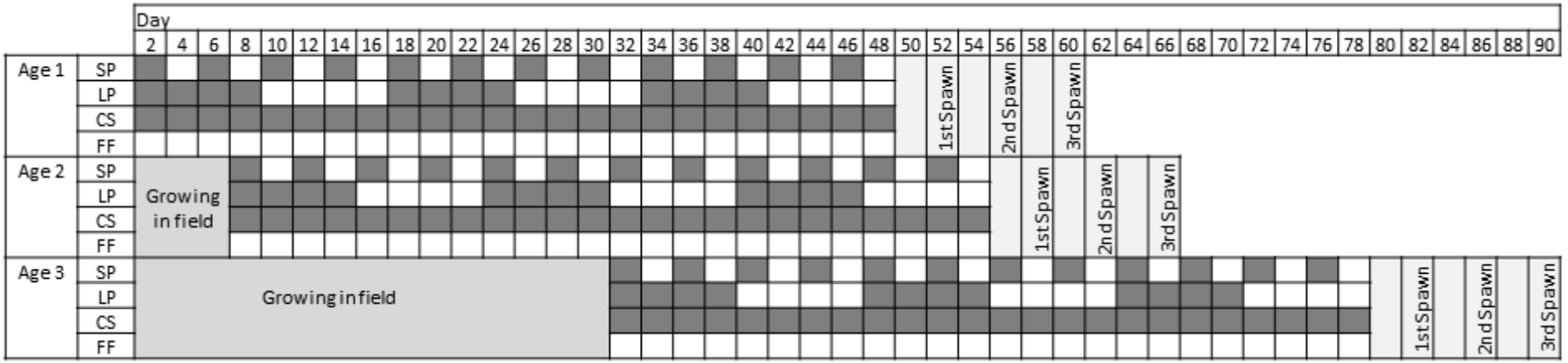


Figure 4. 3: Schematic of the experimental design. Each age was crossed with each voyage pattern (short pulse (SP), long pulse (LP), constant starvation control (CS) and full feed control (FF)). Dark grey boxes indicate a low food environment (mesh FCB's), and the white boxes indicate ambient food environment (open FCB's). "Growing in the field" indicates colonies growing until the required age was met. Three spawning events were induced 2 days after completion of each treatment.

Factor Three: Spawning event

On completion of the experimental run each plate was removed from the frame and transported back to the laboratory individually in the 2 L settlement containers filled with ambient sea water. Plates were then transferred into an aerated aquarium at 20°C (ambient seawater) and left in the dark for 48 hrs in preparation for spawning. Prior to spawning, under red light conditions, each colony was placed into a separate 600-mL beaker containing 1 µm-filtered seawater. Colonies were then exposed to bright light to induce spawning. Each colony was spawned three times over a 6 day period (48 hrs apart), thus spawning event had three levels (1^{st}, 2^{nd} and 3^{rd} spawning) (Figure 4.3).

Quantifying propagule pressure

The number of larvae released from each colony during the three spawning events was recorded. In colonial animals reproductive output is related to colony size and age, however, by selecting three ages colony size was controlled *a priori* in this study. A colony was considered to have spawned if it had released ≥1 larva and the number of colonies that spawned was recorded as spawning success, a measure of reproductive population size and diversity. The size of individual larva, a reliable indicator of propagule quality and future fitness (Marshall et al. 2003), was also recorded. Larvae were measured based on techniques developed by Marshall et al. (2003), which involved the haphazard selection of 10 larvae from each colony. However, all larvae were measured when a colony released less than 10 larvae. Each selected larva was pipetted onto a concave microscope slide, along with a drop of seawater, which allowed the larva to swim. *Bugula* larvae are slightly oval in shape and have one ciliated grove that runs along the larva. To ensure size measurements were comparable, larvae were only measured when this ciliated grove faced upwards. The swimming larva was recorded under a dissecting microscope (×30 magnification) until the correct orientation was displayed; this typically took < 10 seconds. Still images of the larva in the desired orientation were then taken from the video using *Microsoft movie maker* (Microsoft 2011) and the maximum diameter of the larva was measured using *ImageJ* (Schneider et al. 2012).

Data analysis

Permutational Analysis of Variance (PERMANOVA) (Anderson 2001) was used to examine the influence of five experimental factors on each of three response variables. The five factors were: Age (fixed, with three levels: Age 1, Age 2, and Age 3); Pattern (fixed, with four levels: short pulse (SP), long pulse (LP), full feed control (FF) and constant starvation control (CS)); Spawning event (fixed, with three levels: 1st, 2nd and 3rd); Plate (random, with 36 levels, nested in Age × Pattern) and Colony (random, with 3 levels, nested in Plate). The three response variables were: number of larvae released (count data), spawning success (binary data), and larvae size (continuous variable). All PERMANOVA analyses were undertaken in *PRIMER v6* (Clarke and Gorley 2006) with *PERMANOVA+* (Anderson et al. 2008) using similarity matrices based on Euclidean distance. When this distance measure is used in combination with a single response variable, the pseudo-*F* and pseudo-*P* values returned by PERMANOVA are identical to those from a traditional non-permutational ANOVA, but are not constrained by its assumptions. Since each individual colony was spawned three times 'colony' was included as a random factor in the model to account for the repeated measures design. There was no replication at the lowest level as there was only one measurement per colony per spawning event and therefore, it was not possible to estimate the highest-order interaction, which was excluded from the analysis. However, this does not have consequences for the analyses of the higher-level terms in the model (Anderson et al. 2008). Type I sums of squares were used for the balanced hierarchical binary and count data, whereas the more conservative type III sums of squares were used to analyse the unbalanced size data. Each term was tested using 9999 permutations. Significant terms were then investigated using *post hoc* pairwise comparisons with the PERMANOVA *t* statistic and 999 permutations. *P*-values for the pairwise analyses were obtained using a Monte Carlo random sample (Anderson and Robinson 2003) due to insufficient permutable units. Non-significant terms at a significance level of $P > 0.25$ were pooled to increase the power of the analysis (Winer et al. 1991, Underwood 1997). To separate the effects on the mean and dispersion of the response variables, a test for homogeneity of dispersions (PERMDISP) (Anderson 2006) was done in combination with the main PERMANOVA test. As some groups released comparatively low numbers of larvae (LP and CS produced a total of 30 larvae, whereas FF and SP produced a total of 2,924 larvae) and other groups did not spawn (e.g., Age 3-SP), larvae size data were only analysed for the SP and FF treatment at Age 1 and Age 2. This was to avoid asymmetry in the model and a large imbalance in the design. Asymmetry can lead to

incorrect *F*-Ratios and *P*-values and highly unbalanced design can result in the loss of independence during permutation when using PERMANVOA (Anderson et al. 2008).

RESULTS

Spawning success

Voyage pattern, age and spawning event interactively affected the spawning success of *Bugula* colonies (Table 4.1). Overall, colonies under FF treatment had the highest proportion of spawning success (ranging from 0.4 - 1.0), which, except for the Age 3 group, was not different to the SP colony spawning success (0.55 - 1.0, Figure 4.4). *Bugula* colonies under the LP and CS treatments generally had a low proportion of spawning success (ranging from 0 - 0.4) and did not differ across age or spawning events (all pairwise comparisons $P > 0.34$; Table A4.1). Only FF colonies in the Age 3 group spawned and no spawning was recorded for the Age 3 SP, LP and CS colonies (Figure 4.4). Age 1 and Age 2 recruits had higher spawning success when exposed to the short pulse (SP) treatment than when exposed to the long pulse (LP) treatment (Figure 4.4). Age 2 FF colonies had higher spawning success in the 1st spawning than in the 2nd and 3rd spawning events ($P = 0.04$). SP, LP and CS colonies did not show this trend, with the greatest proportion of spawning recorded during the 2nd spawning event (Figure 4.4), although these trends were not statistically significant (pairwise comparisons, $P > 0.181$; Table A4.1).

Table 4. 1: Results of univariate PERMANOVAs, based on Euclidean distances, for spawning success of *Bugula neritina* colonies and number of larvae released in response to the experimental treatments in Magazine Bay 2013.

		Spawning success			Number of larvae		
Source	*df*	MS	F	*P*	MS	F	*P*
Age	2	1.6	8.2	0.004	3.9	1.9	0.1613
Pattern	3	9.0	45.4	0.001	96.3	47.9	0.0001
Spawning	2	0.2	2.3	0.141	6.3	23.5	0.0001
Age × Pattern	6	1.5	7.8	0.001	7.0	3.5	0.0102
Age × Spawning	4	0.1	1.0	0.409	0.5	1.8	0.1202
Pattern × Spawning	6	0.2	2.7	0.02	10.3	38.0	0.0001
Plate (Age × Pattern)	24	0.2	1.0	0.465	2.0	1.0	0.4217
Age × Pattern × Spawning	12	0.2	2.1	0.042	1.8	6.7	0.0001
Colony (Plate (Age × Pattern))	72	0.2	3.5	0.001	1.9	7.1	0.0001
Spawning × Plate (Age × Pattern)	48	0.1	1.5	0.039	Pooled		
Residual	144	0.1			0.3		
Transformation		presence/absence			log(x +1)		

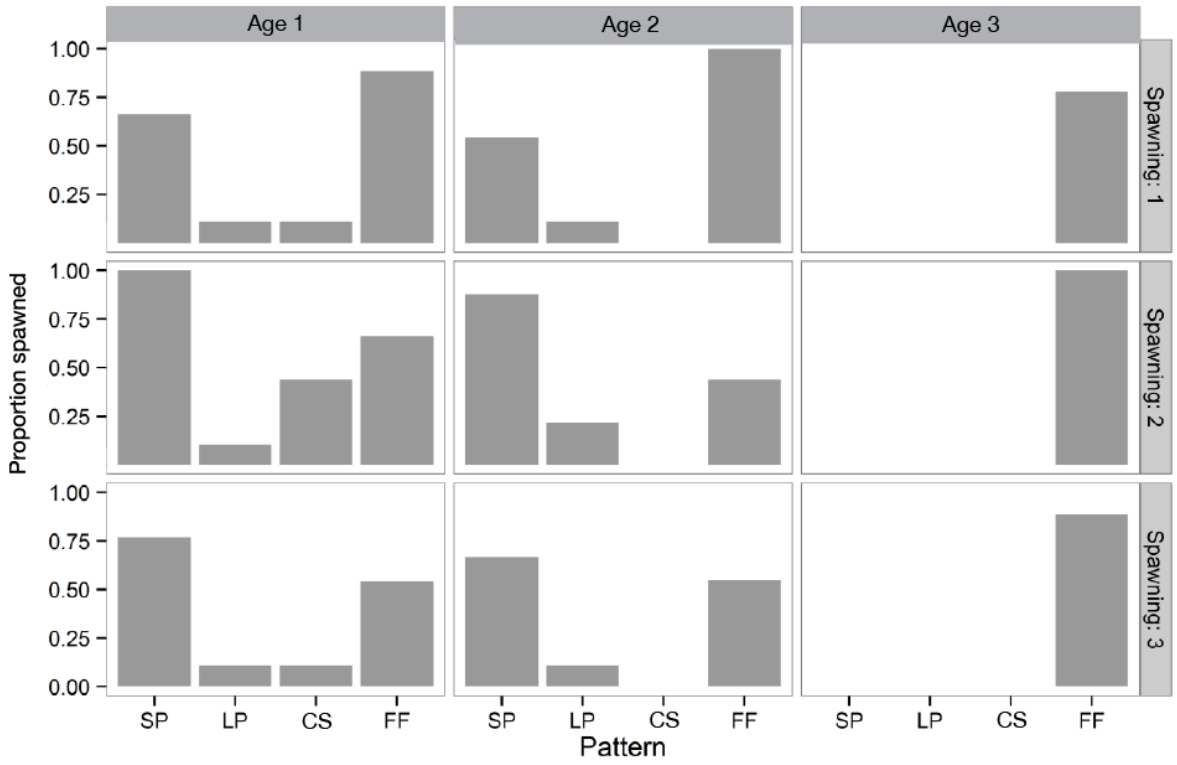


Figure 4.4: A 9-panel depiction of the proportion of *Bugula* colonies that spawned after exposure to simulated voyages at the Naval Point Yacht Club Psych Jetty, Magazine Bay. Voyage patterns were short pulse (SP), long pulse (LP), constant starvation control (CS) and constant food control (FF). Age (1 = 1-day, 2 = 8-days, 3 = 29-days) is shown left to right and the three spawning event are stacked vertically.

Number of larvae

There was a significant three-way interaction between voyage pattern, age and spawning (Table 4.1) on the number of larvae released. SP colonies generally released more larvae (mean = 4.8 ± 1.3 S.E.) than LP colonies (0.1 ± 0.07 S.E.), except in the Age 3 group, where neither group released larvae (Figure 4.5; Table A4.2). Age 2 recruits subject to SP feeding regimes produced as many larvae (mean = 13.4 ± 5.1 S.E.) as recruits exposed to the constant food control (FF) treatments (10.3 ± 3.7 S.E.), except for one of the spawning events (Figure 4.5). Overall, LP and CS colonies released the least number of larvae and were not different to each other (Table A4.2). The Age 3 SP, LP and CS colonies did not release any larvae, but the Age 3 FF control did (Figure 4.5). There was an effect of age on the number of larvae released by the SP colonies; (Age 1: mean = 3.7 ± 0.8 S.E., Age 2 = 10.6 ± 3.6 S.E., Age 3 = no larvae released), but there was no effect of age on the number of larvae released by the LP or CS colonies, which was consistently low, or FF colonies, which was consistently high. In

relation to larval output over subsequent spawning events, the number of larvae released by the FF control colonies significantly declined from the 1st to the 2nd event. However, the other three voyage patterns (SP, LP, CS) did not show this trend.

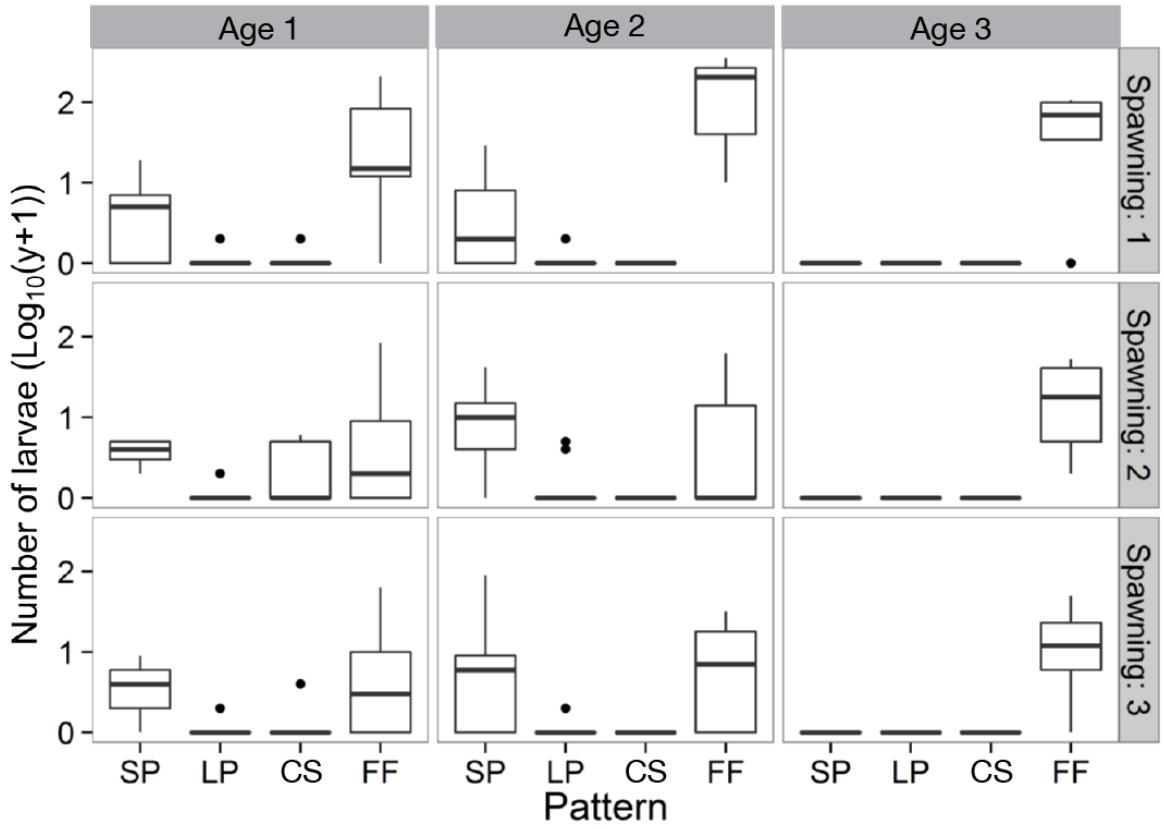


Figure 4.5: A 9-panel depiction of the number of larvae released by *Bugula* colonies grown under simulated voyage patterns at the Naval Point Yacht Club Psych Jetty, Magazine Bay. Voyage patterns were short pulse (SP), long pulse (LP), constant starvation control (CS) and constant food control (FF). Age (1 = 1-day, 2 = 8-days, 3 = 29-days) is shown left to right and the three spawning event are stacked vertically. Number of larvae is presented on a $\log_{10}(y+1)$ to improve visualisation.

Size of larvae

There were significant interactions between pattern and spawning, and age and spawning on larval size (Table 4.2). SP larvae were smaller (mean = 333.9 µm ± 8.81 S.E.) than FF larvae (389.0 ± 4.0 S.E.) in the 1st spawning event, but not in the 2nd or 3rd events (Table A4.3, Figure 4.6). Age 1 larvae size increased from the 1st to the 2nd spawning (mean = 294.09 µm ± 9.1 S.E.; 382.6 ± 8.6 S.E., respectively), but there was no size difference between the other larvae released (Table A4.4). Age 2 larvae were larger (mean = 408.5 µm ± 5.4 S.E.) than Age 1 larvae (338.1 ± 4.9 S.E.) in 1st spawning, but were significantly smaller in 3rd spawning (407.6 ± 4.2 S.E.; 366.41 ± 3.7 S.E., respectively) (Table A4.4). PERMDISP

showed that the SP larvae size was more dispersed than the FF larvae in the 1st spawning, but not in the other two spawning events (Table A4.5). The 3rd spawning larvae size was more dispersed in both ages compared to the previous spawning events (Table A4.6).

Table 4. 2: Results of Univariate PERMANOVA, based on Euclidean distances, for the larvae size after age, pattern and spawning factorial manipulations.

		Larvae size		
Source	*df*	MS	F	*P*
Age	1	2910	1.38	0.259
Pattern	1	40641	19.34	0.001
Spawning	2	21596	8.87	0.003
Age × Pattern	1	197	0.094	0.768
Age × Spawning	2	57266	23.53	0.001
Pattern × Spawning	2	12806	5.26	0.013
Spawning × Colony (Plate (Age × Pattern))	18	1854	1.45	0.111
Pooled(1)	30	2748	2.15	**
Pooled(2)	18	2926	2.13	**
Residual	420	1278		
Transformation		none		

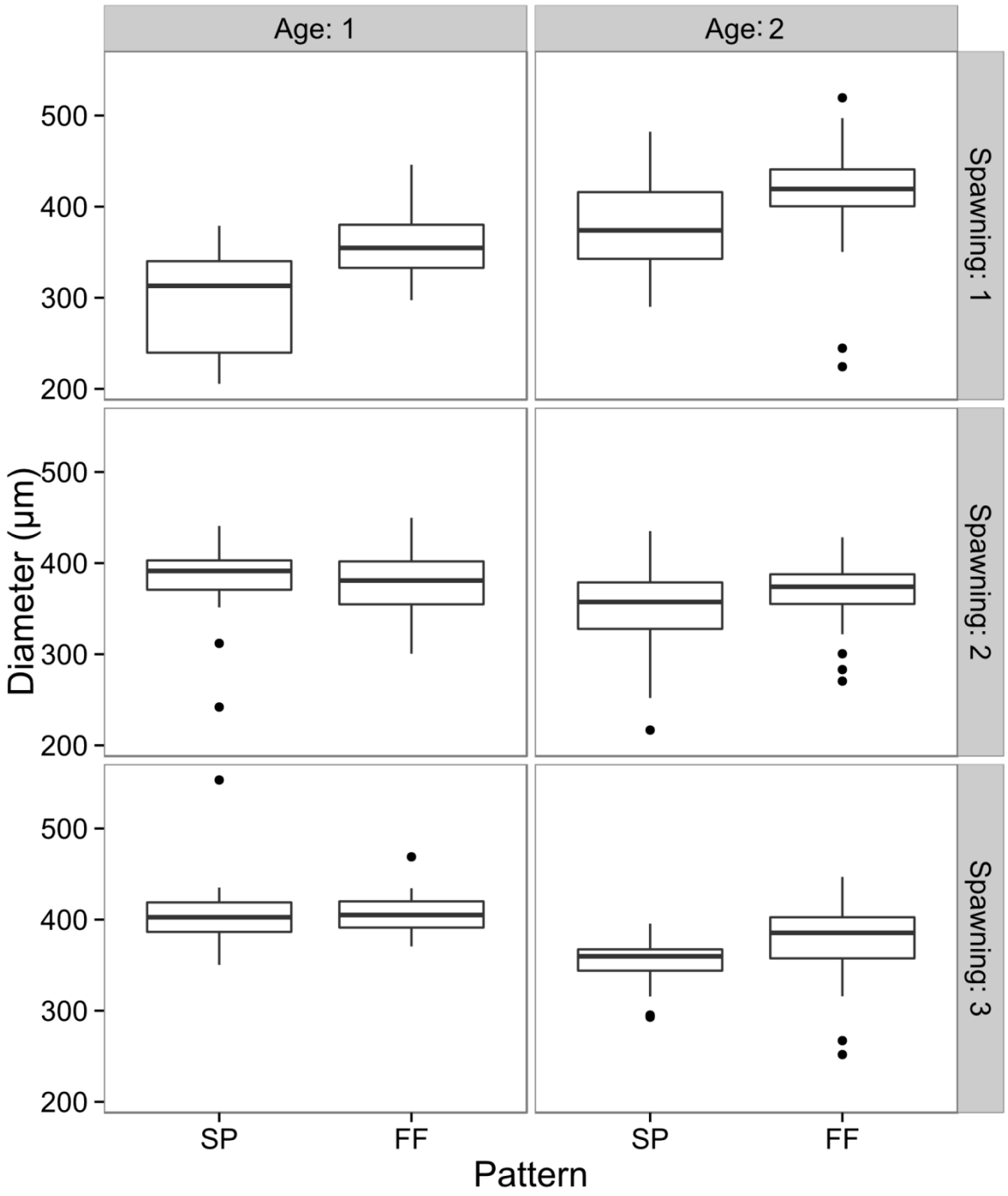


Figure 4. 6: Diameter (µm) of larvae released by experimental colonies reared under simulated voyage patterns at the Naval Point Yacht Club Psych Jetty, Magazine Bay. Voyage patterns were short pulse (SP) and constant food control (FF). Age (1 = 1-day and 2 = 8-day) is shown left to right and the three spawning event are stacked vertically. Due to the very low number of larvae released from CS, LP and Age 3 colonies (see Figure 4.5) these groups were excluded from the analysis to prevent statistical complications resulting from asymmetrical and highly unbalanced data.

DISCUSSION

The results of this study suggest that the delivery of propagules to a recipient environment (propagule pressure) can be influenced by the interaction between (i) the residency period of a vessel in a previous port of call and the time at which its hull became colonised, and (ii) the voyage pattern between source and the recipient environment. *Bugula* colonies that were 'exported' from a source environment at Age 1 or Age 2 released a greater number of larvae if subjected to a short-pulse voyage pattern (SP) than if subjected to a long-pulse voyage pattern (LP). SP colonies also had higher spawning success and, therefore, a larger breeding population compared to LP colonies. Large propagule sizes and greater genetic diversity among propagules in a population can enhance colonisation success (Ahlroth et al. 2003, Hedge et al. 2014), as the potential to successfully adapt to the new selection pressures in the recipient environment is improved (Lockwood et al. 2005, Pineda et al. 2012). These results suggest that vessels undergoing frequent short voyages may represent a relatively high-risk of translocating biofouling organisms that are capable of producing high-quality propagules.

Bugula is a voracious feeder (Dahms et al. 2007) and even though low-food days were kept constant between the SP and LP levels, colony reproduction in this study appears vulnerable to patterns of food availability. The low number of larvae released in the LP level, which was similar to that of the constant starvation control (CS), suggests that resources were mostly used for survival and maintenance rather than reproduction in the LP group. Colonies were not able to store enough resources during longer periods of abundant food to maintain reproduction over long periods of food deprivation. Short periods of food deprivation, however, were more tolerable. It appears that it is the period without food that influences the number of larvae released (i.e. the journey length, rather than the residency in between).

The terminal investment hypothesis states that there is a shift of 'current reproduction' with age; young organisms should invest in growth to increase future reproduction and older organisms, due to higher probability of death, should invest in current reproduction (Williams 1966, Velando et al. 2006). For example, greater brood sizes are produced by burying beetles at a later age compared to young beetles (Trumbo 2009). *Bugula* colonies can live for over a year, but abundance fluctuates seasonally with peak reproduction and growth occurring through the summer months and regression back to dormant basal stolons over the cooler

winter period (Bone and Keough 2005). Additionally, as *Bugula* is a colonial animal that grows through asexual reproduction, the older the colony is the greater number of genets (zooids) and the greater its reproductive potential. It seems reasonable to expect then that the older colonies would show the highest reproduction. This was shown by the FF control colonies (Age 1-FF output < Age 2-FF output < Age 3-FF output), however the voyage colonies (i.e., SP, LP, CS) did not show this trend. The Age 3 colonies, which were reproductively mature at the start of the experiment, did not release any larvae after the simulated voyages, although those that were Age 1 and Age 2 and reproductively immature at the start of the experiment, did reproduce. Here the voyage-like physiological stress may override colonial and life-history predictions.

Chapter Three showed that growth occurred post-voyage suggesting that *Bugula* can recover from stress. The present study showed that the number of larvae released (and spawning success) did not decline with successive spawning event in the voyage colonies as it did in the constant food control (FF) treatment. In fact, at times larvae number and spawning success from the same colony increased over time post-voyage. In combination, these results suggest that reproductive output from the same colony will increase with time post-voyage when the vessel is in the recipient location. Recovery of a colony is an important concept to consider as this will determine the future reproductive output by that introduced organism. It is not just the size of the initial introduction event that is important but also the number of introduction events (measured here through consecutive spawning events) that can be a stronger determinant of colonisation success (Hedge et al. 2014).

Quality (as indicated here by larval size) may be as important as quantity for propagules entering a new environment (Marshall and Keough 2008). Various factors can cause offspring size variation, with stress, maternal size and nutrition, and habitat quality regarded as the major drivers (Marshall and Keough 2008). Smaller larvae, such as those produced by SP colonies in the first spawning, are expected to have decreased future fitness (e.g., reduced embryonic development, growth, survivorship and later reproduction (McGinley et al. 1987, Marshall et al. 2003, Dias and Marshall 2010)) than the larger larvae released by FF control colonies. Interestingly, there was greater size variability in SP larvae than in FF control larvae. The size inconsistency seen in the SP larvae may be beneficial as variability in offspring size has been suggested as an adaptive life-history strategy in marine invertebrates for dealing with unpredictably variable environments (Marshall et al. 2008). It is thought that

this is a bet-hedging strategy, which increases the chance that at least some offspring will be suitable for the unpredictable future environment (Cohen 1966, Koops et al. 2003, Marshall et al. 2008). Alternatively, variability in offspring size may also be a result of the cost involved with producing uniformly sized offspring (Fox and Czesak 2000). However, when SP larvae number increased over subsequent spawnings, larval size also increased, was less variable and not different to the FF larvae. This suggests that more effort was required to produce variable offspring resulting in the reduced number of larvae. *Bugula* has been shown to produce various sized offspring according to environment (e.g., Burgess and Marshall 2011b). SP larvae size variation was largest in the first spawning event, not the subsequent 2nd and 3rd spawning events. There could be methodological reasons behind this, as SP colonies were maintained in a temperature controlled lab without environmental variability throughout the spawning events and, therefore, the first spawning event occurred closest to when the SP colonies were in the pulsed environment.

Management implications

Short duration voyages (e.g. 1 day) are representative of domestic vessel traffic, such as cargo vessels, ferries and fishing vessels. The findings presented here suggest that hull-fouling organisms transported along these pathways can arrive at recipient ports in good physiological condition and are able to produce healthy offspring. Reproductive output after a series of short voyage durations was greater than that produced by recruits on scenarios representing vessels that spend longer periods underway, such as international heavy lift vessel which can take weeks to move between locations. Currently in New Zealand marine biosecurity management is focused on international vessel arrivals to prevent new incursions. My results are highly relevant for efforts to limit the domestic spread of NIS already present in domestic ports.

Visual inspections are sometimes used to check vessel hulls for high-risk organisms (Bell et al. 2011), but very small early life stages that survived a journey may be indistinguishable in a slime layer and missed during a visual inspection. This work has shown that early stages, picked up during a short residency, could go on to reproduce. Tools to detect even microscopic stages of marine taxa would be beneficial for preventing both new introductions and domestic translocations of potentially viable organisms (Wood et al. 2013).

Conclusion

This study demonstrated that propagule pressure was influenced by important pre-recipient environment variables. Residency period, voyage pattern and spawning event interactively affected number of larvae released (propagule size), spawning success (propagule diversity) and size of larvae (propagule quality). All colonies survived, but depending on their previous experience, many failed to release larvae when spawning was attempted (i.e., no propagule pressure) and were not "effective translocations" (See definition in Chapter Four: Introduction). Although restricted to the bryozoan *Bugula*, the results suggest that vessels whose voyage profiles are characterised by successive short intra-coastal voyages punctuated by short residency periods may deliver a greater number of propagules than vessels that spend longer periods underway during which biofouling organisms are unable to filter-feed. Plasticity of juvenile stages may have contributed to their success and is investigated further in Chapter Five of this thesis. To predict propagule pressure via ship biofouling it is imperative to examine the biofouling organism within the context of its translocation environment.

Chapter Five: Can adaptive transgenerational plasticity contribute to hull-fouling colonisation, establishment and spread?

ABSTRACT

Transgenerational plasticity (TGP) is when the environment of the parent influences (both positively and negatively) the phenotype of the offspring. It is an important determinant of offspring performance and likely to play a significant role in the spread of non-indigenous species in the marine environment. Here I investigate if transgenerational plasticity enhances the fitness of the common hull-fouling bryozoan *Bugula neritina* under different vessel voyage scenarios. Parent and offspring environments were manipulated by adjusting food levels; limited food represented an *en route* voyage environment and ambient food represented a port residency environment. Experiments were designed to simulate four scenarios encountered by hull-fouling organisms: (1) parent resides in full feed port environment, its offspring recruits to a vessel hull and is transported on short, frequent pulse-like voyages (SP); (2) parent resides in port, reproduces and its offspring also resides in port; (3) parent recruits to a vector and is transported on SP voyages, its offspring resides in port; (4) parent transported on SP voyages, its offspring are also transported on SP voyages. Important invasion characteristics (size of colony, spawning success, number of larvae and quality of larvae) were measured after each scenario and the effects of both offspring and parent environment were determined. Transgenerational plasticity was indicated if the parent environment affected offspring performance. Parent environment did influence offspring fitness, although the effect was not as strong as the effect of offspring environment. Offspring that were exposed to the SP voyage scenario were smaller (mean bifurcation = 6 ± 1.3 S.E.), had lower spawning success (33%) and released fewer larvae (mean = 1 ± 4.6 S.E.) than those offspring that remained in port (mean bifurcation = 8 ± 0.2 S.E., spawning success =

67%, mean larvae number = 27 ± 9.4 S.E.). The effect was stronger if parents had been exposed to the same environment as the offspring. Reproductive output results were not consistent across the two parent ages; this was most likely due to a rapid change in weather compromising the health of one age group. Larval size (F4 generation) was not significantly different between groups. Results indicate that TGP is evident through carry-over effects of the parent environment. The influence of other types of TGP is unclear and methodological limitations, such as uncontrolled paternal effects, may have impeded results. The offspring environment also had an impact on offspring fitness. Based on these findings, I suggest that vessels that reside in port post-voyage are higher risk than to those that reside in port and then embark on a voyage. With the determinants of successful and unsuccessful establishment of non-indigenous species not completely understood, investigating the role of TGP is an important topic for future research on invasive species' spread.

INTRODUCTION

The final stages of the invasion pathway: population colonisation, establishment, spread and impact in the recipient environment (Chapter One: Figure 1.1), can be divided into two phases (Dietz and Edwards 2006). The first phase is the initial introduction, when factors such as propagule pressure, disturbance, and resource availability are important for establishment of the founding population. The second phase of further expansion is when evolutionary factors, such as genetic drift and phenotypic plasticity, are important due to the biotic and abiotic constraints experienced by the introduced organisms (Dyer et al. 2010). Many introductions fail, and of those that succeed there is large variation in the rate of population growth and the extent of range expansion of the introduced organisms (Crooks and Soule 1999). Some populations experience long lag phases – the period between initial introduction and population growth and range expansion, while others do not (Mack 1985). Determinants of this variation are largely unknown (Crooks and Soule 1999). Typically, a non-indigenous species (NIS) introduced to a new environment will experience strong population bottlenecks, often with related low genetic diversity (Dyer et al. 2010). As a consequence, the potential for adaptation to the new environment is considered to be restricted (Barrett and Kohn 1991, Ghalambor et al. 2007). Although this restriction is often counteracted by multiple incursions from a range of source populations, evolutionary change may be an important primary driver for continuing the colonisation process (Sakai et al. 2001).

Phenotypic plasticity, which is an individual's ability to modify life-history, morphological, physiological and behavioural traits in response to environmental cues, is regarded as playing an important role in adaptation to a new environment (Smith 2009). Phenotypic plasticity has been identified as a trait of invasive species (Rejmánek and Richardson 1996, Clements et al. 2004, Richards et al. 2006), particularly when a founding population has low genetic diversity (Dyer et al. 2010). When an NIS is introduced to an environment or environmental variable for which they have no prior adaptation, within-generation plasticity may give them a competitive edge by expression of an alternative phenotype. For example, the invasive alga *Caulerpa racemosa* is known to rearrange its photosynthetic apparatus in response to changes in light and temperature which may contribute to its colonisation success in seagrass beds in the Mediterranean Sea (Raniello et al. 2004). At this early stage, immediately post-introduction, phenotypic plasticity may be a particularly important mechanism that allows the invader extra time to adapt and integrate into the new environment (Sexton et al. 2002, Fordyce 2006).

Phenotypic plasticity can also occur across generations when the environment or phenotype of the parent influences the phenotype of the offspring (Badyaev and Uller 2009, Bonduriansky and Day 2009, Wolf and Wade 2009). This is known by a number of different names, including transgenerational plasticity, maternal effects (Mousseau and Fox 1998, Wade 1998, Marshall and Uller 2007, Marshall 2008, Burgess and Marshall 2011a), and parental effects (Jensen et al. 2014). Here I use the term transgenerational plasticity (TGP). In some species TGP is one of the most influential factors acting on offspring phenotype and fitness (Wade 1998). TGP links one generation to the environment of the previous generation (Marshall 2008), and is very important for successive generations beyond the initial arriving individual. Unlike phenotypic plasticity, which requires time for the individual to respond (Weinig 2000), TGP can minimise the stress for the offspring based on the maternal experience of that stress (Donohue and Schmitt 1998). During the initial phase of introduction, phenotypic plasticity and TGP will be important for survival and establishment of the founder population (Dyer et al. 2010). In the second phase of expansion, TGP will be pivotal to the speed with which this stage progresses, as it assists in adapting to the new conditions and, therefore, the potential for competitive dominance and rapid expansion.

TGP can take many forms that increase or decrease offspring fitness (Figure 5.1). Parents that respond to their environment by increasing offspring fitness through adjustment of offspring phenotype, is one type of maternal effect called adaptive transgenerational plasticity (ATGP) (Van Dam and Baldwin 2001, Galloway and Etterson 2007), also known as anticipatory parental effects and adaptive parental programming (Horton and Stetson 1990). For example, after maternal exposure to a predator, *Daphnia cucullata* produced offspring with a morphological change (increased helmet length) which reduces predation (Agrawal et al. 1999). However, as selection acts on maximising maternal fitness (Smith and Fretwell 1974, Bernardo 1996), TGP can increase maternal fitness at the cost of offspring success at that particular time if it leads to higher maternal survivorship and future maternal reproduction. For example, when the Chinese quail mates with a lower quality male it produces smaller (lower quality) eggs than when the female mates with a higher quality male (Uller et al. 2005). This is known as selfish maternal effects (SME) (Marshall and Uller 2007). Finally, offspring fitness may reflect the resources available to the parents. For example, offspring weight in *Daphnia* was reduced after the parents were exposed to low-resource environments, suggesting a carry-over effect between parents and offspring (Tessier and Consolatti 1991).

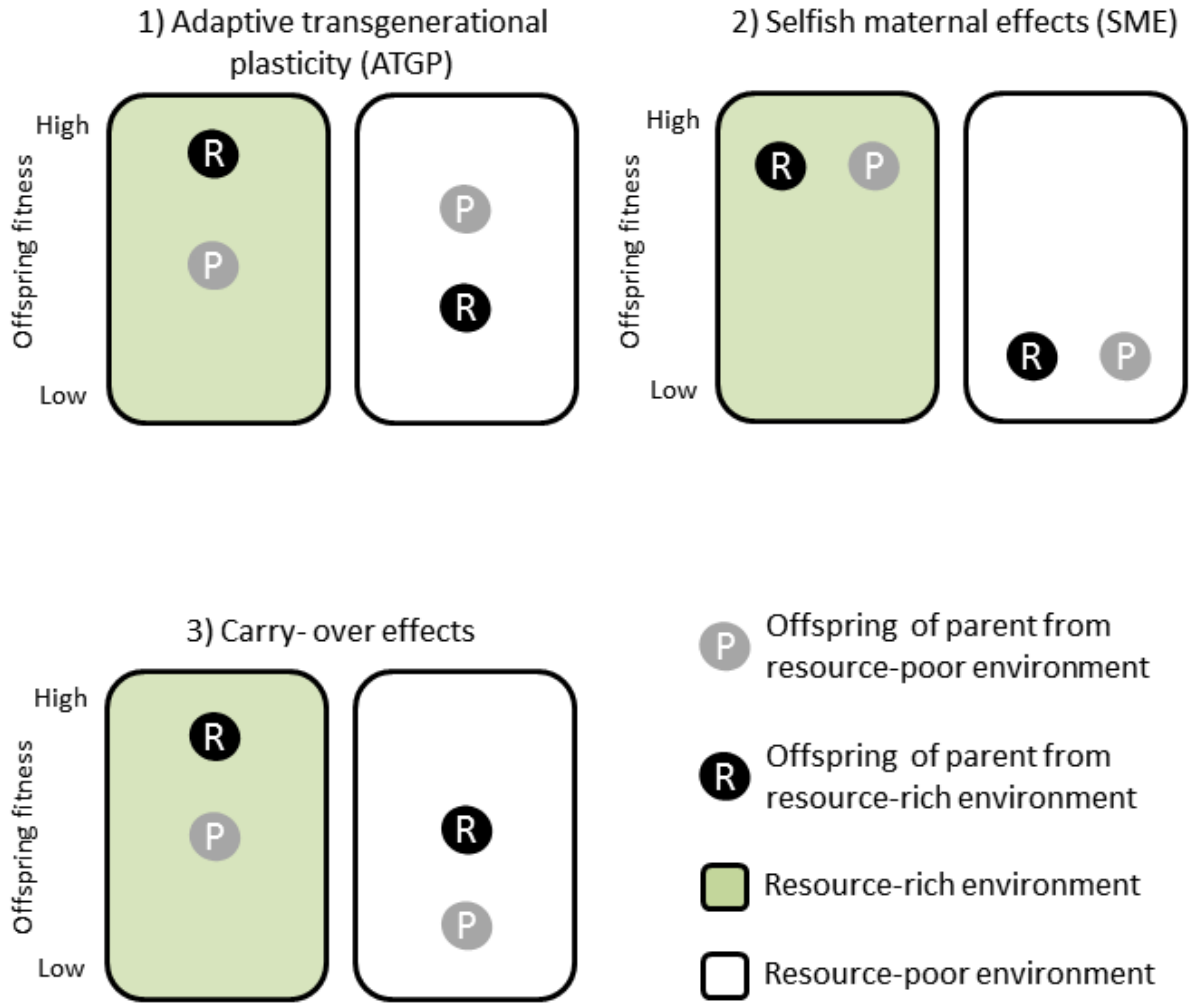


Figure 5.1: Three types of transgenerational plasticity: (1) adaptive transgenerational plasticity; (2) selfish maternal effects, (3) carry-over effects. Location of the circles along the high-low gradient (y-axis), represent offspring fitness in resource-rich (green-shaded box) or resource-poor (white box) environments. The grey circle with "P" indicates an offspring whose parent was in a resource-poor environment. The black circle with the letter "R" represent an offspring from a parent that was in a resource-rich environment. Schematic based on Figure 1, Uller et al. (2013).

TGP is a flexible mechanism allowing sessile organisms to cope in heterogeneous environments (Galloway and Etterson 2007), therefore it could be expected to be an important strategy for many marine organisms. It has been suggested that TGP in the marine environment is likely to play an important role in connectivity between marine populations (Marshall et al. 2010, Jensen et al. 2014) and, therefore, in fisheries management (Marshall et al. 2008), climate change responses (Burgess and Marshall 2011b, Munday et al. 2012, Clarke Murray et al. 2014), aquaculture (Marshall et al. 2008) and pollution resistance (Piola and Johnston 2006, Marshall 2008). It is also likely to play a role in range expansion and

marine invasions (Marshall et al. 2008), since the ability of the introduced species to respond and adapt to the new environment largely determines the success of that introduction (Smith 2009).

The importance of TGP has recently been identified in plant invasions. For example, when the invasive grass *Aegilop triuncialis* was grown in poor soil conditions they produced offspring that had greater stress tolerance through higher photosynthetic efficiency (Dyer et al. 2010). When the invasive grass *Cyperus esculentus* were grown in nutrient-poor patches they adjusted seed dispersal so more propagules were placed into nutrient rich patches (Dyer et al. 2010). The invasive thistle, *Carduus nutans*, had a higher proportion of germination and shorter mean germination time when parent plants were grown in warmer temperatures (Zhang et al. 2012). Similarly, Fenesi et al. (2014) compared the response of two invasive and two congeneric introduced, but non-invasive, species and showed that adaptive responses were expressed in the invasive species only and that TGP was mainly displayed in resource-rich conditions, suggesting it could allow high reproduction in the first generation in a resource rich environment.

Can TGP play a role in the performance of marine organisms transported on vessel hulls?

When a reproductively mature parent arrives on the hull of a vessel into a new environment, propagules may be released during the vessel's residence period. The success of these propagules has been viewed in light of external factors, such as parasites, predators, competition and disturbance in this new environment (Torchin et al. 2002, DeRivera et al. 2005, Altman and Whitlatch 2007). However, simple explanatory relationships, for example, between enemy-release and introduction, may not be appropriate due to the complexity underlying the invasion process (Colautti et al. 2004). As TGP can increase offspring fitness, particularly in high-resource sites (Fenesi et al. 2014), it may also be an important factor in the fitness of the introduced propagules to the port environment.

ATGP is likely to be most common in species with naturally short dispersal periods, as offspring and parent are more likely to share the same environment (Marshall et al. 2008). Many hull-fouling organisms have relatively short natural dispersal periods (e.g., bryozoans, ascidians, hydroids). Propagules released from a parent on a ship's hull may either recruit to the same hull, to another adjacent vessel or to natural (e.g., reefs) and artificial habitats

(pontoons or pilings) in the surrounding environment. Consequently, TGP may also play a role in the success of a propagule that is released from a hull-fouling parent. Through TGP, a parent may prepare the offspring for life on a vessel hull and the environmental conditions it is likely to face (e.g., food limitation, drag forces), and thereby increasing its fitness during the ensuing voyage. Thus, when examining TGP in regard to hull-fouling, not only is it important to determine performance once released from the hull into the resource-rich port environment, where both individual phenotypic plasticity and TGP may be important, but also the ability for the next generation to survive another voyage and spread. If TGP makes progeny fitter under some voyage scenarios then this information could identify high-risk vessels that deserve management prioritisation to decrease likelihood of infection of new vessels or local population expansion. If TGP increases fitness of vector-to-recipient environment recruits, risk of establishment in the new environment is increased. If TGP increases fitness of vector-to-vector recruits, the spread of the organism in the new recipient location will be enhanced.

Here a fully-crossed factorial experiment, manipulating both the parent and offspring environments, was performed to investigate if TGP enhances the fitness of the common hull-fouling organism *Bugula neritina* under voyage-like scenarios. If ATGP occurs it would be expected that offspring fitness would increase when parent and offspring environments match. Selfish maternal effects (SMEs) are likely if offspring fitness in a resource-poor environment will always be low regardless of parent environment. Carry-over effects will be evident if the parent from a low-quality environment has offspring with lower fitness in both the low and high-quality environments (Figure 5.1). If there is no effect of parent environment then there would be no evidence of TGP in this experiment.

Bugula was selected as a model organism as TGP plasticity has been demonstrated in early life stages of this species exposed to copper-rich environments (Marshall 2008), and to link with and expand on other research of this thesis. The null hypothesis was: there will not be any effect of parent or offspring environment on offspring fitness.

Box 5.1: A note on terminology

Transgenerational plasticity (TGP)	When the environment or phenotype of the parent influences the phenotype of the offspring (Badyaev and Uller 2009, Bonduriansky and Day 2009, Wolf and Wade 2009). Also known as maternal effects. There are different types of TGP including *adaptive transgenerational plasticity*, *selfish maternal effects*, and *carry-over effects* as described below.
Adaptive transgenerational plasticity (ATGP)	A parental adjustment of offspring phenotype according to local environment that maximise offspring fitness (Galloway and Etterson 2007).
Selfish maternal effects (SME)	A parental adjustment in offspring phenotype according to local environment that decreases current offspring fitness, but likely increases future maternal fitness (Marshall and Uller 2007).
Carry-over effects	A conduit between parent and offspring that reflects the resources available to the parent. This can be beneficial or detrimental to offspring. Also known as legacy effects (Marshall 2008, Uller et al. 2013).
Fitness	Increases positively with the size of colony, colony spawning success, number of larvae released and size of larvae.

METHODS

Experimental design

To examine TGP it is necessary to have a fully crossed design that manipulates both parent and offspring environments (Doughty and Reznick 2004, Uller et al. 2013, Burgess and Marshall 2014); therefore an orthogonal approach was adopted in this study (Figure 5.2). Here Factor 1, *parent environment*, had two levels: short pulse voyage (SP) and full feed port residency (FF). These levels were chosen because long pulse voyage colonies did not released a sufficient number of propagules (Chapter 4). Factor 2, *offspring environment*, had the same two levels: SP and FF. As in Chapter Four, SP represents repeating short (2 day)

voyages separated by short (2 day) vessel residency periods. FF represents a vessel that remains in port. Combinations of these parent and offspring environment factors represent possible scenarios for two generations of a hull-fouling organism (Figure 5.2):

1. Parent colony resides in port (FF), reproduces and its offspring recruits to a vector and is transported on short, frequent voyages (SP);
2. Parent resides in port, reproduces and its offspring also resides in port;
3. Parent recruits to a vector (as a larva) and is transported on short, frequent voyages (SP). The parent reproduces and its offspring are released into and reside in port (FF);
4. Parent recruits to a vector (as a larva) and is transported on a SP voyage, it reproduces and its offspring also recruits to a vector and is transported on a SP voyage

As age was identified as an important factor influencing recruit survivorship and propagule pressure (Chapters Three and Four), a third factor, *parent age*, was included in this study. Parent age was the age of the parent colony when it was exposed to the treatment (SP or FF) and had two levels: Age 1, where the parents were exposed to the SP 1 day post-settlement, and Age 2, where parent colonies were exposed to the SP 8 days post-settlement. There were two experimental runs (one for each age) spaced 8 days apart. Three colonies were grown on each settlement plate, as in Chapter Four, and there were three replicate settlement plates. Settlement plate was included as a random factor in the statistical model.

Port residency period and voyage time were simulated in the field by manipulating food levels using Food Control Boxes (FCBs). Colonies were grown within a FCB, with or without 63 µm mesh; the mesh restricted water flow and food and was used to mimic *en route* voyage environment when hull-fouling organisms have limited access to food. Open containers allowed ambient food levels inside the container and was used to mimic port residency period. Full details of the FCBs are presented in Chapter Four (Figure 4.2).

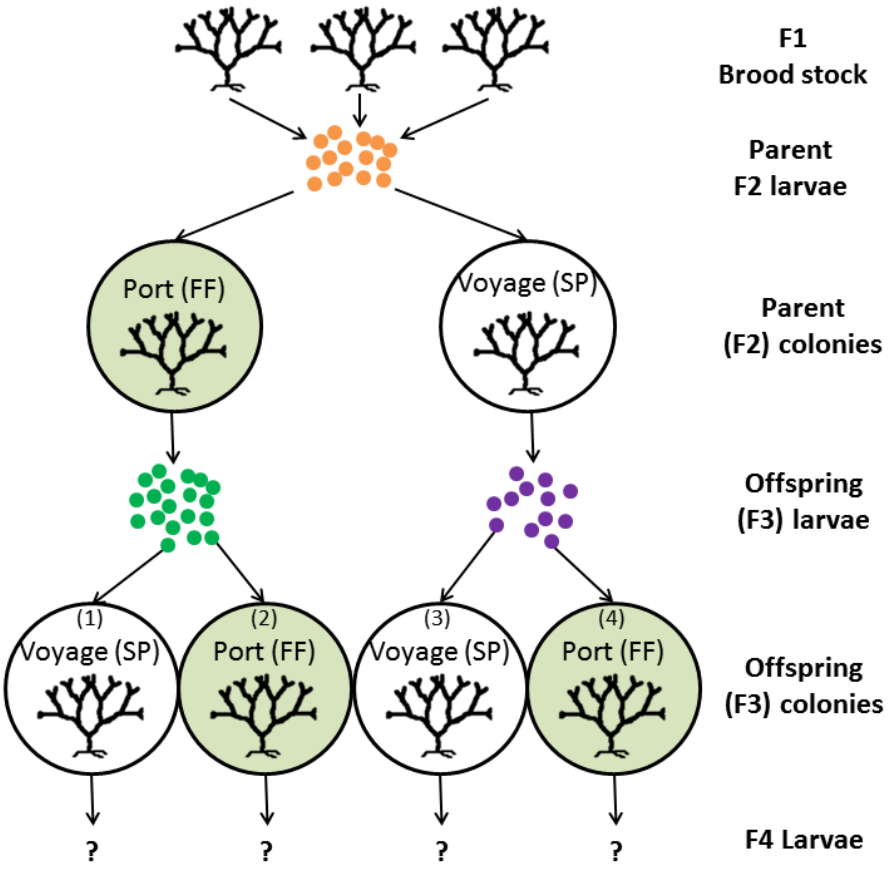


Figure 5.2: Orthogonal design used to examine transgenerational plasticity. Age 1 parent (F2) colonies grown under short pulse voyage conditions (SP) or full feed port residency conditions (FF) were spawned. Their offspring (F3 generation) were grown under either the same or different conditions from their parent. Offspring (F3) were spawned and reproductive output (F4 generation) was measured. This experiment was repeated using Age 2 parent colonies.

ATGP will only occur if the parent environment is a reliable predictor of the offspring environment, indicating that environmental predictability must be considered in relation to the organism under study (Burgess and Marshall 2014). Here the variable environment (SP) was a repeating pattern of 2 days of food followed by 2 days of low food. This short pulse pattern could be considered a reliable predictor to *Bugula* as there were 12 repeats of this pattern during the 48-day period the parent colonies (and also the F3 offspring colonies) were grown. Additionally, *Bugula* broods larvae for approximately 1-2 weeks (Silen 1972, Woollacott and Zimmer 1975), and therefore it is likely there were at least two repeats of the SP cycle during the brooding period.

Often only one life history stage is examined for TGP (e.g., gametes (Jensen et al. 2014), early stage recruits (Marshall 2008)), but here offspring were grown through to reproductive

maturity and spawned. This allowed a range of important population parameters to be measured: size of offspring (F3) colony, number of offspring (F3) colonies spawned and number and size of larvae (F4) produced by the offspring colonies. The early life-history stages (larvae size and number) were measured because it is at this phase that TGP may be particularly prevalent due the limited ability for early life stages to assess their own environment (Uller 2008).

Experimental procedure

Experiments were conducted at the Naval Point Yacht Club Psych Jetty (43°36′42″ S, 172°42′20″ E) in Magazine Bay, Lyttelton Harbour (Chapter One: Figure 1.3) and at the University of Canterbury, South Island, New Zealand. *Bugula* brood stock was collected from Magazine Bay and transported to the lab in an insulated light-proof container. Colonies were cleaned of sediment and extraneous fouling, and kept in the dark in 18ºC aerated water for 48 hrs. Colonies were then exposed to fluorescent lights to induce spawning.

Larvae were randomly pipetted into settlement containers filled with filtered seawater. PVC settlement plates (150 mm × 150 mm) that had been lightly sanded and conditioned in seawater to encourage larval recruitment were attached to the underside of the lids. Larvae were left undisturbed for 24 hrs to settle. The following day, settlement plates were removed from the settlement containers and recruits were examined. Three recruits on each plate were used in the experiment. They were haphazardly selected and labelled using a pencil to circle each recruit on the plate. To minimise competition effects recruits were thinned so that no recruit was closer than 40 mm to another recruit (see Chapter Four for further details). In addition, recruits within 10 mm of the edge of the plate were removed to avoid the influence of edge effects. The lid, with attached settlement plate and recruits, was then replaced back on the settlement containers and transported out to the field site. In the field, settlement plates were haphazardly attached to the underside of 1.8 m wooden frames using a galvanised bolt. To prevent siltation effects on the colonies (Wendt 1998), plates were hung horizontally with colonies on the underside of the plate. These recruits were on-grown to become the *parents* (or *F2 generation*).

The parents were grown under either SP or FF environments for 48 days; a time period in which it was expected that reproductive maturity would be reached. After 48 days, plates

were transported back to the laboratory, placed into aerated aquarium and left in the dark for 48 hrs in preparation for spawning. On the morning of spawning, working under red-light conditions, each colony was removed from the settlement plate and placed in a separate beaker. Florescent lights were then used to induce spawning. Larvae released are the o*ffspring*, or *F3 generation.*

The offspring (F3 generation) were settled onto settlement plates, thinned, transported, deployed into the field and exposed to the same environments (SP or FF) for 48 days using the same techniques as described above for the parent colonies. There was, however, a series of cold fronts that occurred a few days before spawning of the Age 1 colonies was scheduled. This change in weather resulted in a decrease in seawater temperature from 14.8 to 12.5ºC over a 4-day period. Continued exposure to low temperature is likely to affect reproduction, and the Age 2 colonies still had another 8 experimental days remaining to complete the full experiment. To minimise the impact of temperature change, the Age 2 recruits were relocated from the field to a controlled laboratory environment at the University of Canterbury for the final 8 days of the experiment. Colonies, still in their food control boxes, were placed in a 2000L re-circulating seawater system. Fresh seawater was collected from Magazine Bay and used in the system to provide similar conditions, including the same food. Temperature was maintained at 15ºC. Age 1 colonies were spawned in the laboratories without weather interruptions. The size of offspring colonies, number of colonies that spawned, number of larvae released from each colony and size of 10 randomly selected larva were recorded. Number of bifurcations was used to measure colony size. Larvae were filmed using a microscope camera under X30 magnification. Snapshots from the video were taken when the larva was orientated with the ciliated groove facing outward. From the snapshot the maximum diameter of the larva perpendicular to the ciliated groove was measured using ImageJ (Schneider et al. 2012). For full details on measuring larvae see Chapter Four. Colonies were spawned on three occasions over a 6-day period (i.e., every 2 days), however, only data from the first spawning event was used in the analyses due to a consistent decrease in the number of larvae across all groups, and to reduce the number of factors in the analyses. The larvae released from the offspring colonies were the *F4 generation*.

Data analysis

Data was analysed using a univariate permutational analysis of variance (PERMANOVA) based on Euclidean distances (Anderson 2001) in PRIMER v6 (Clarke and Gorley 2006) with the PERMANOVA+ add on (Anderson et al. 2008). PERMANOVA was selected for these analyses to deal with the over-dispersed count data (with many zeros), a nested design, mixed and random effects, and in one case, an unbalanced design. PERMANOVA uses permutations to generate 'pseudo-*P*-values,' rather than the tabled *P*-values used in ANOVA, which do not require normally distributed response data. Four factors were included in the saturated model: parent environment (fixed), offspring environment (fixed), parent age at stress (fixed) and plate (random). Four response variables were analysed: colony size (count data), number of larvae released (count data), spawning success (binary data) and larva size (continuous data). A transformation was performed on number of larvae data ($\log_{10}$ (x + 1)) to improve normality.

For each analysis 4999 permutations were used. The binary and count data were balanced, therefore Type I sums of squares were used in these analyses. However, the size data were unbalanced, therefore the more conservative type III sums of squares were used. Terms with a significance level of $P > 0.25$ were pooled, in a step-wise fashion, to increase the power of the test (Winer et al. 1991, Underwood 1997). *Posteriori* pairwise comparisons were made for significant terms using 999 permutations. Monte Carlo random sampling (Anderson and Robinson 2003) was used to obtain *P*-values if there were insufficient permutable units. As PERMANOVA detects differences in either means or variances, but does not distinguish between the two, a test for homogeneity of dispersions (PERMDISP, Anderson 2006) was also conducted to separate the effects of these. In one analysis where there was significant variation in dispersion, the main PERMANOVA test results were checked using a Generalised Linear Mixed Model (GLMM) with negative binomial errors in R v.3.0.2 (R Core Team 2013) as GLMM does not assume homogeneity of variances.

There was a comparatively low number of larvae released from the Age 1 group and not all levels of each factor had larvae (i.e., data points) leading to an asymmetrical data set. F4 larvae size data, therefore, was only analysed for the Age 2 group. Asymmetry can be problematic as it may cause incorrect *F*-Ratios and *P*-values, and a highly unbalanced design can lead to a loss of independence during permutations (Anderson et al. 2008).

RESULTS

Colony size

The size of the colony was significantly affected by offspring environment ($F_{1, 48} = 55.8$, $P = 0.0002$; Figure 5.3), with larger sized colonies in the FF environment (mean number of bifurcations = 8 ± 0.2 S.E.) compared to the SP environment (mean = 6 ± 1.3). Although the effect was not as strong ($F_{1, 48} = 4.4$, $P = 0.05$), parent environment also affected growth; offspring from FF parents reached a greater size (mean = 8 ± 0.2 S.E.) than offspring from SP parents (mean = 7 ± 1.4). The random factor plate also had a significant effect on growth ($F_{16, 48} = 3.2$, $P = 0.0012$). Parent age at the time of experiment had no significant effect on colony size ($F_{1, 48} = 2.9$, $P = 0.104$; Table A5.1).

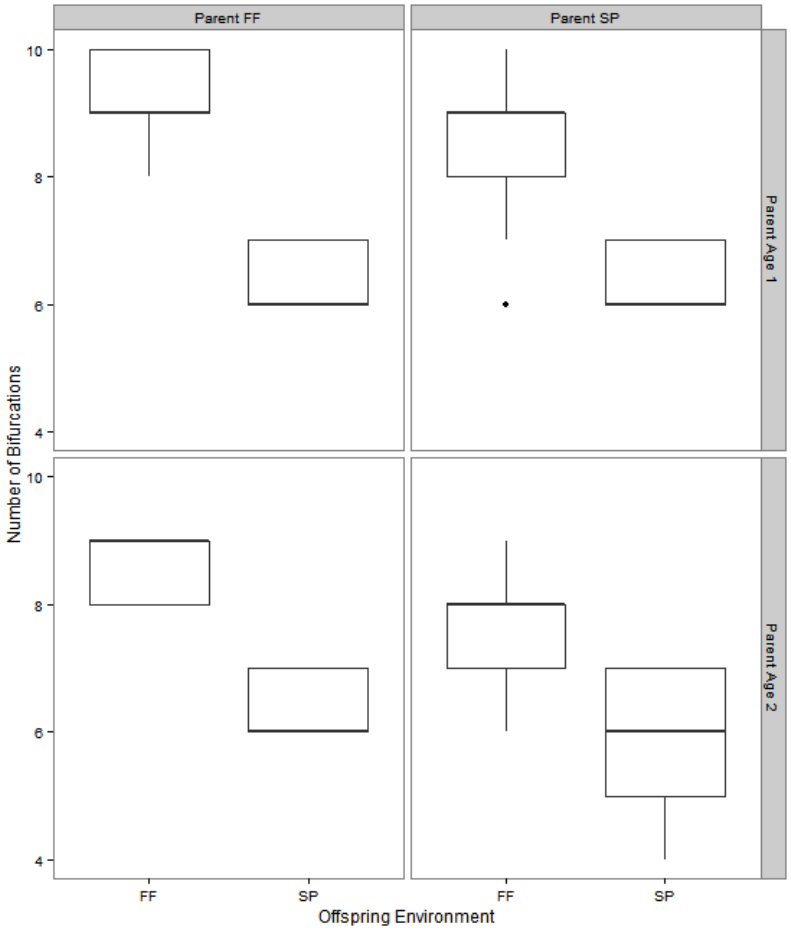


Figure 5.3: Size (number of bifurcations) of offspring (F3 generation) colonies at time of spawning after different hull-fouling scenarios at the Naval Point Yacht Club Psych Jetty, Magazine Bay. Age that parent was exposed to voyage scenario is stacked vertically (Parent Age 1 = 1 day, Parent Age 2 = 8 days), type of scenario that parent was exposed to is shown left to right and offspring environment is displayed along the x-axis. Scenarios were residency period in port with full feed (FF) or short frequent voyage periods (SP).

Spawning success of offspring (F3 generation)

The significant effect of offspring environment was also reflected in spawning success ($F_{1, 48}$ = 10.32, P = 0.0068; Table A5.1), with higher spawning success in offspring exposed to FF environment (SP parents = 33%, FF parents = 100%) than offspring exposed to the SP environment (SP parents = 0%, FF parents = 66%; Figure 5.4). There was also an interactive effect of parent environment and parent age on spawning success ($F_{1, 48}$ = 7.58, P = 0.0142, Figure 5.4). Pairwise comparisons showed that there was no effect of parent environment on spawning success in the Age 1 group, although there was a difference in the Age 2 group (t = 2.3, P = 0.05; Table A5.2), where again FF parent colonies had the highest spawning success. Overall, spawning success was higher in the Age 2 group than the Age 1 group (Figure 5.4). There were slightly significant random effects of plate ($F_{16, 48}$ = 1.9, P = 0.047), but no difference in dispersion of the data (Tables A5.3 and A5.4).

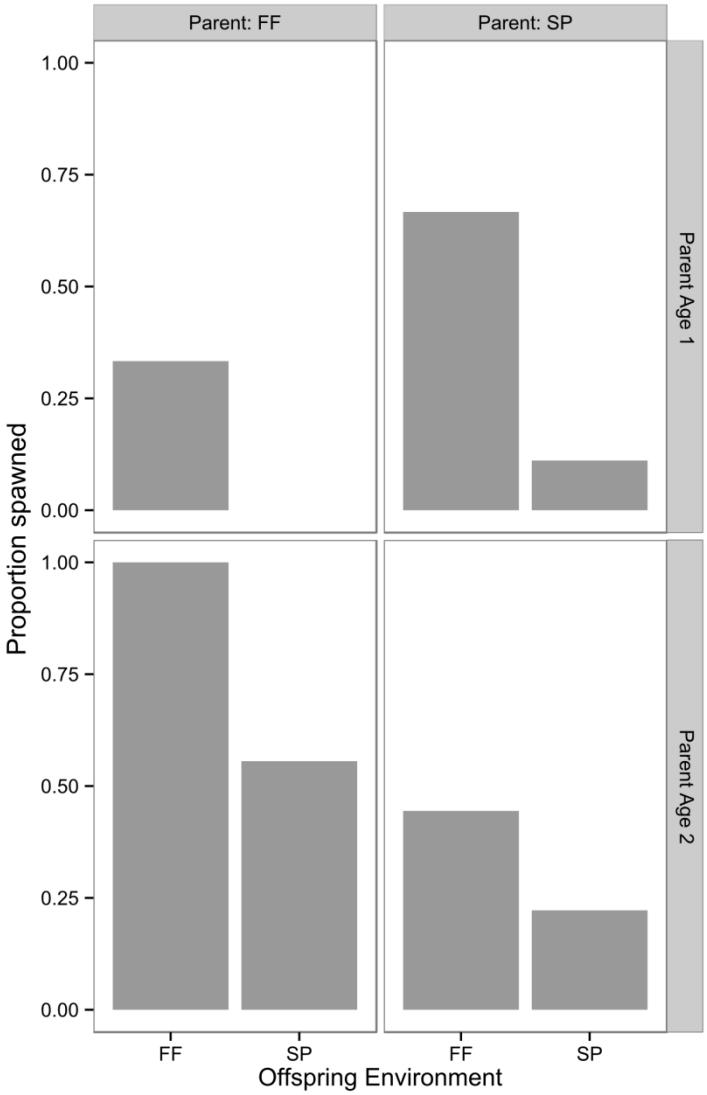


Figure 5.4: Proportion of offspring (F3 generation) colonies that spawned after different hull-fouling scenarios at the Naval Point Yacht Club Psych Jetty, Magazine Bay. Parent environment is shown left to right, age that parent was exposed to voyage scenario is stacked vertically (Parent Age 1 = 1 day, Parent Age 2 = 8 days) and offspring environment is displayed along the x-axis. Scenarios were residency period in port with full feed (FF) or short frequent voyage periods (SP).

Larvae number

A total of 985 larvae were released from the 72 offspring colonies. As with spawning success, there was a trend for higher output from FF offspring (total = 963) than SP offspring (total = 22), FF parents (total = 774) than SP parents (total = 211), and Age 2 parents (total = 899) than Age 1 parents (total = 86; Figure 5.5). However, the effect of parent environment, offspring environment and parent age on larvae number was interactive ($F_{1, 48}$ = 8.8, P = 0.0092; Table A5.1). There was an overlap of data in some groups, particularly in the SP

parent, SP offspring, and Age 1 groups. Pairwise tests (Table A5.5) showed that comparisons only within the FF parent, FF offspring, or Age 2 groups were statistically significant as listed:

- FF offspring significantly different to SP offspring in the FF parent–Age 2 group (t = 3.571, P = 0.002)
- FF parent significantly different to SP parent in the FF offspring–Age 2 group (t = 2.9575, P = 0.0056)
- Age 1 significantly different to Age 2 in both FF parent groups (t = 3.6043, P = 0.001; t = 2.4079, P = 0.023).

PERMDISP analyses showed significant heterogeneity of variances between groups ($F_{7, 64}$ = 2.86, P = 0.008), however, the pairwise comparisons revealed that none of comparisons relevant to the hypothesis of this study were significantly different, except for any comparison made with the FF parent–SP offspring–Age 1 colonies, which did not release any larvae (Table A5.6). The GLMM used to investigate this heterogeneity of variance further showed a significant 2-way interaction between parent environment and parent age (Z = -3.99, P = <0.0001) and a significant effect of offspring environment (Z = -6.98, P = <0.0001; Table A5.7), suggesting that the significant 3-way interaction, as presented above, was driven by the unequal variances. Both of these analyses have their advantages and disadvantages. PERMANOVA results were chosen because of its general robustness when dealing with complex designs, such as adopted here. There were no significant random effects of plate ($F_{16, 48}$ = 1.7195, P = 0.0758).

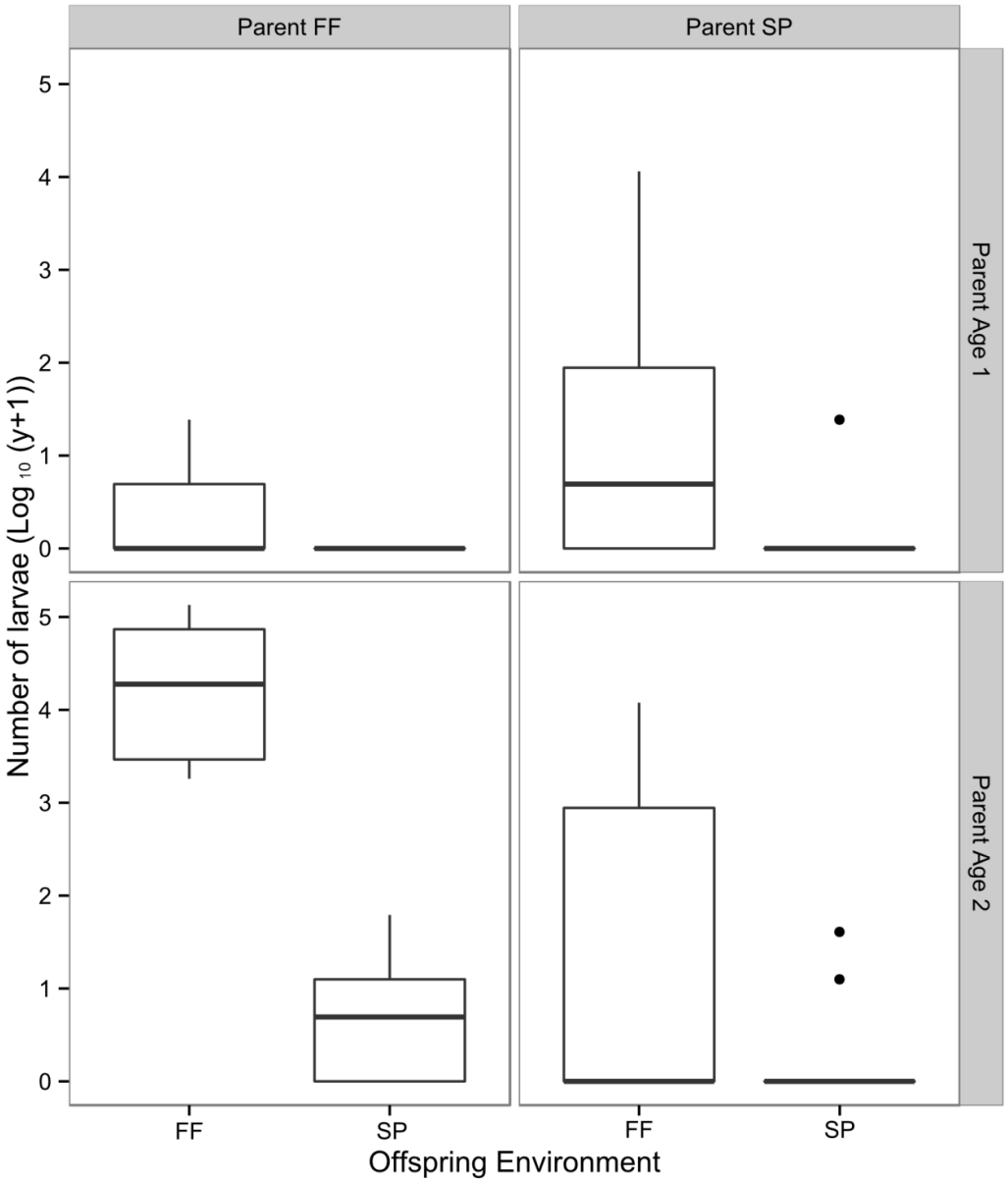


Figure 5.5: Number of (F4 generation) larvae (log_{10} (y+1) transformed) released by offspring (F3 generation) colonies after different hull-fouling scenarios at Naval Point Yacht Club Psych Jetty, Magazine Bay. The age that the parent was exposed to a voyage scenario is stacked vertically (Parent Age 1 = 1 day, Parent Age 2 = 8 days), type of scenario that parent was exposed to is shown left to right and offspring environment is displayed along the x-axis. Voyage scenarios were residency period in port with full feed (FF) or short frequent voyage periods (SP).

Larval size

In contrast to other response variables, there was no significant effect of parent environment ($F_{1, 205}$ = 0.8, P = 0.378) or offspring environment ($F_{1, 205}$ = 1.2, P = 0.2792) on larval size (Figure 5.6, Table A5.8). Additionally, PERMDISP analyses showed no significant difference in variance between the groups.

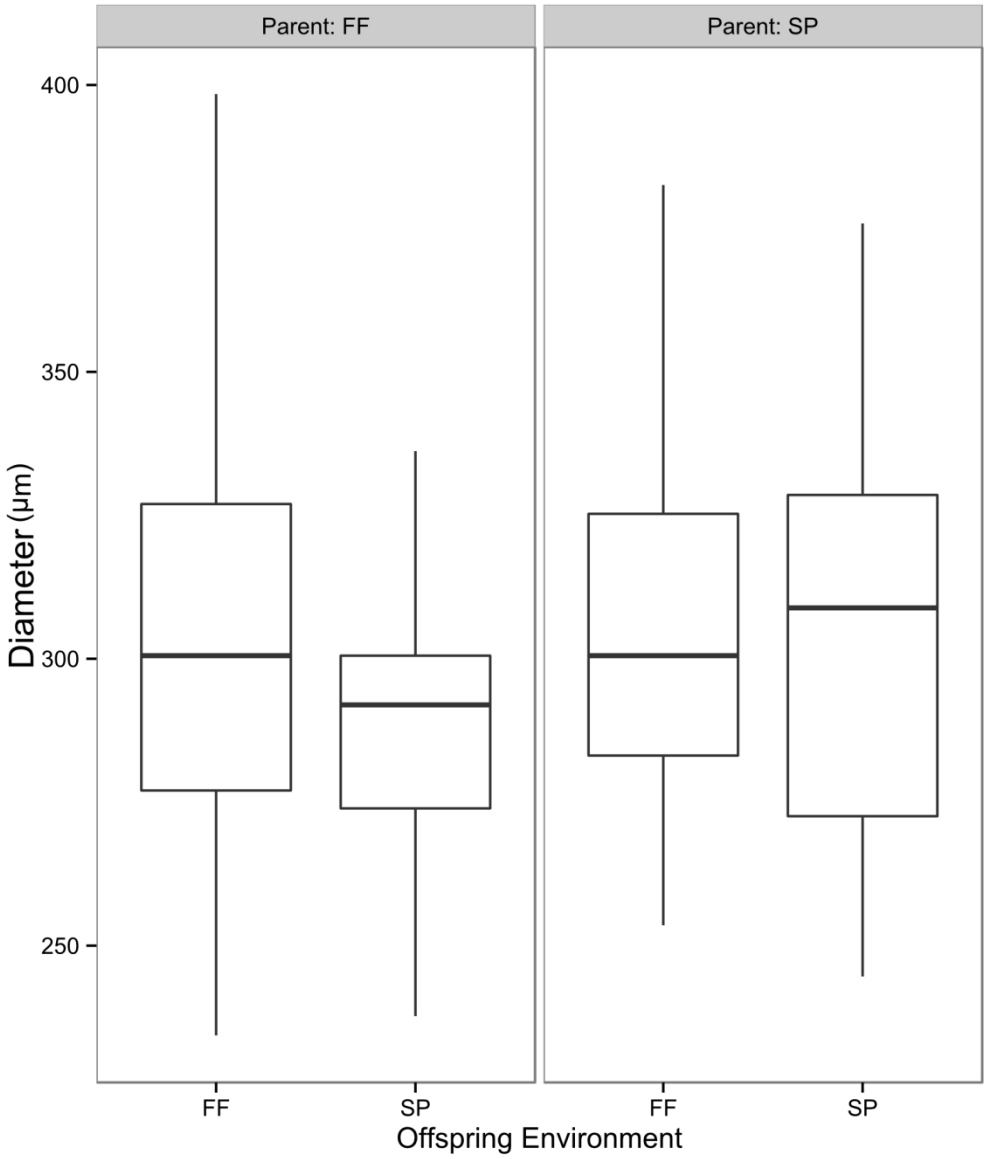


Figure 5.6: Size (µm) of F4 generation larvae released by offspring (F3 generation) colonies after different hull-fouling scenarios at Naval Point Yacht Club Psych Jetty, Magazine Bay. Parent environment is shown left to right and offspring environment is displayed along the x-axis. Environments were residency period in port with full feed (FF) or short frequent voyage periods (SP).

DISCUSSION

The outcome of species introductions partly depends on how the introduced individuals respond to the new environment and how historical events have shaped the species and/or individual. Filters during a translocation can remove NIS but they may also modify those that survive and prepare their offspring for the future environment. Therefore, transgenerational

plasticity (TGP) may be an important factor in marine invasions. This study used TGP as underlying rationale to elucidate important factors in hull-fouling transport.

The results showed that out of the four scenarios, offspring fitness (size, spawning success and reproductive output) was highest when the parent organism resided in port, reproduced, and its offspring also resided in port. This higher fitness with matching environment (both FF) may either be evidence of ATGP or a combination of a carry-over effect of the high-resource parent environment and an effect of offspring environment. The high fitness demonstrated is expected to enhance establishment in the recipient environment.

Offspring fitness remained high, although lower than the previously described FF parent – FF offspring scenario, when the parent was transported on a voyage (SP), reproduced, and then the offspring resided in port (FF). ATGP and/or carry-over effects could again be underlying mechanisms. This vector-to-port scenario describes the first introduction event after arrival to a recipient location, and results suggest that the offspring of the arriving individuals may only be slightly compromised by the parent voyage.

When the parent resided in port (FF), reproduced, and then its offspring were transported on a voyage (SP), offspring had low fitness. This suggests that the offspring environment had a stronger effect than the parent environment on offspring fitness, although parent environment was still a significant influence. Alternatively, this result could also be an indication of SMEs where reproduction was reduced while in resource-poor environment. Propagule health was compromised on arrival in the recipient location although, importantly, reproduction did occur. In this scenario the number of arrivals (i.e., vectors or adults on the vessel) may be important for introduction success.

Finally, offspring fitness was lowest when the parent was transported on a voyage (SP), reproduced, and then its offspring were also transported on a voyage (SP). This result does not suggest ATGP as an underlying mechanism, even though parent and offspring environments matched. The results could be due to carry-over effects of the resource-poor parent environment, SMEs and the immediate effect of offspring environment. Importantly, offspring still reproduced after both parent and offspring went through a voyage, and although the risk is lower compared to other scenarios, spread via a vector-to-vector pathway

could still occur. Again, it will be the number of arrivals (i.e., propagule number) that will be important for the introduction success of vector-to-vector recruits.

The results from these scenarios suggest that offspring fitness was affected by its parent's environment, but the effect of the offspring's environment was stronger. The role of ATGP and SMEs is unclear, as highest fitness was not always displayed when parent and offspring environments matched. The results suggest that carry-over effects are also important determinants of fitness. If a vessel resides in port post-voyage it is likely to be a higher risk of delivering propagules to that port than if it is frequently moving or if it resides in port before going on a voyage. Long residency periods in a given port pose a higher risk of delivering propagules to that port than

ATGP predicts that offspring performance will be higher when offspring environment matches parent environment (Uller et al 2013). Although our FF offspring performed better when their parent environment matched (i.e., was also FF), this was not the case for SP offspring, which had the lowest fitness when environments matched. This has implications for 'climate matching,' which is the comparison of environmental conditions between different geographical locations, an approach often used to predict invasion likelihood (Floerl et al. 2013). These results suggest that climate matching will only be a good predictor of success if the two environments are high resource habitats. Introductions may still be successful if the environments don't match, and it is more likely if the organism moves to a higher quality environment. The importance of environment quality is supported by a study where ATGP was mainly manifested under resource-rich, rather than stressful, conditions by two invasive plants (Fenesi et al. 2014). ATGP has been demonstrated in marine organisms, (Crean and Marshall 2008, Marshall and Keough 2009) and also in low food environments (Stahlschmidt and Adamo 2014). However, ATGP is complex and may be expressed more explicitly under some environments and not others (Fenesi et al. 2014).

A recent meta-analyses of 58 plant and animal studies by Uller et al. (2013) found overall weak experimental evidence for ATGP. It was suggested in this meta-analysis, and in a follow-up forum by Burgess and Marshall (2014) that methodological reasons could have resulted in the limited association, emphasising the need to consider the assumptions of ATGP. One assumption of ATGP is that selection acts to maximise maternal fitness, not offspring fitness. In poor quality and/or patchy habitats resources may be low and

competition high, in which case it may be beneficial for parent colonies to produce fewer offspring at that time if there are future opportunities to reproduce. Indeed, a recent study by Hedge et al. (2012) showed that the introduction of high numbers of larvae of the invasive oyster *Crassostrea gigas* enabled greater colonisation, compared to the introduction of low numbers of larvae, but this was offset with increased competition and a larger proportion of post-settlement mortality compared to when fewer larvae were introduced. Guided by this example, the low number of larvae produced by SP parent - SP offspring colonies in the present study may have had higher survivorship in the low food environment due to reduced competition for the limited resource. Reducing immediate reproductive fitness until there are opportunities to reproduce again under better environmental conditions is termed selfish maternal effects (SMEs). SMEs were demonstrated in a study by Marshall and Keough (2004) where *Bugula* colonies damaged by predation produced smaller offspring, allowing resources to be directed toward recovery rather than reproduction, which will in turn increase future reproduction as *Bugula* is colonial. Therefore, it is important to consider life history strategy and lifetime reproduction when quantifying maternal fitness (Marshall and Uller 2007). *Bugula* spawns larvae on a number of occasions throughout the summer season. Ideally spawning throughout the reproductive season would have been recorded, but was not in this study due to seasonal constraints. Initially colonies were induced to spawn on three occasions over a 6 day period. However, due to very low numbers of larvae released in the second and third spawning events, only the first spawning data was used in this study. It may have been possible that reproductive output would have changed over the lifetime.

Although the role of ATGP is unclear, the significant effect of the parent environment on offspring fitness suggests a carry-over effect. Carry-over effects have been described as one of the most influential factors on offspring fitness (Wade 1998). The higher quality FF parent environment enhanced the reproductive output of offspring (‘silver spoon effect’) and the poorer quality SP parent environment decreased offspring performance. The direct effect of offspring environment was stronger than the effect of parent environment. Colony size, spawning success and larvae number were less in the SP offspring environment compared to the FF offspring environment. This is not a surprising result due to the close relationship between resource abundance and fitness (resource allocation theory), particularly for *Bugula*, which has rapid growth that is sensitive to food levels and a short time to reproductive maturity. Ambient food availability has been shown to have a strong impact on fitness and reproduction in many other organisms as well. For example, a study by Ernande et al. (2004)

showed that as food abundance increased, the Pacific oyster, *Crassostrea gigas*, shifted resource allocation from growth to reproduction.

Maternal nutrition and other stresses often have a strong effect on offspring size in many species (Qian and Chia 1991, Steer et al. 2004), although here no significant difference in mean larva size or variance between groups was detected. Interestingly, offspring colony size varied between groups; SP colonies were smaller than FF colonies. The size of a *Bugula* colony has been previously reported to relate to the size of larvae produced (Marshall and Keough 2004). Despite SP colonies being significantly smaller, they produced larvae that were not significantly different to FF colonies. This suggests that SP colonies were producing offspring that were higher quality than expected from their size. However, the SP colonies in this study were on average 73% the size of FF colonies, whereas the small colonies studied by Marshall and Keough (2004) were proportionally smaller (50% the size) than the large colonies, although still equal in age. Other studies have shown that fewer, higher quality (i.e., larger) offspring are produced in patchy habitats. For example, the seed beetle *Stator limbatus* laid larger, but fewer, eggs on a poorer quality host plant (Fox and Mousseau 1996). Under competition, the bryozoan *Watersipora subtorquata* produced larger larvae than parents without competition, which allowed higher dispersal potential (Marshall and Keough 2009). Lastly, female field crickets decreased fecundity, but increased offspring quality after exposure to chronic low-food environments (Stahlschmidt and Adamo 2014).

Size of Age 1 and Age 2 colonies was not significantly different, yet there was significantly lower spawning success and fewer larvae released by the Age 1 colonies. Six colonies in the Age 1 group had grown a small extra branch from the base, whereas only one colony in the Age 2 group had done this. This may indicate a redirection of resources and physiological stress in the Age 1 group, as extra growths have been observed on other experimental colonies grown in low quality conditions (*pers. obs.*) and may have led to the unexpectedly low reproductive output in the Age 1 group. Although it is not known what caused stress to the Age 1 group, it may have been a legacy effect of being exposed to stress at a younger age (1 day post-settlement compared to 8 days post-settlement for the Age 2 group). However, if this was the case, FF parent - FF offspring would have performed similarly in both age groups as they were never placed under stress. Another possibility is that the difference could be due to the sudden change in weather and sharp decrease in temperature, (14.8ºC - 12.5ºC over a 4-day period) just prior to the Age 1 group spawning. Although the Age 2 group were

also exposed to the temperature decline, they were placed into a controlled temperature aquarium for 8 days after the storm and prior to spawning, which may have reduced the impact on their reproduction.

TGP may not have been identified due to methodological reasons (Uller et al. 2013, Burgess and Marshall 2014). For instance, I did not know the history of the brood stock prior to the experiment and historical events may have unknowingly influenced results. Another limitation to the study is that I did not know the history of the father to the offspring. *Bugula* colonies are hermaphroditic, however, self-fertilisation is understood to be uncommon and cross-fertilisation is the main method of reproduction (Silen 1966, 1972). These colonies were grown in the field and eggs may have been fertilised by sperm from existing resident 'wild' colonies, or other experimental colonies. It is possible that a SP parent may have been fertilised by a FF parent (or 'wild' colony) and vice-versa. Paternal effects also influence offspring phenotype (Uller 2008, Bonduriansky and Day 2009, Crean and Bonduriansky 2014, Jensen et al. 2014) and may have influenced results in this study.

Conclusion

This novel study demonstrates that both parent and offspring environments influence offspring fitness and carry-over effects are evident. Reproduction occurred after all voyage scenarios but recruits on vectors that reside in port post-voyage had higher reproductive output compared to those that continued on voyages. TGP may enhance establishment in high resource port environments. However, spread is still possible through vector-to-vector transport. The outcome of species introductions partly depends on how the introduced individuals respond to the new environment and how historical events have shaped the species and/or individual. Identifying the mechanisms behind successful and unsuccessful hull-fouling translocations and establishment are needed, not only in light of species spread via the hull-fouling pathway but also to understand population growth in both high-resource (port) and resource-poor (voyage and recipient environment expansion) environments. TGP may explain why we have many arrivals but few establishments. Bridging together management and the underpinning science can be challenging in the field of marine invasions, but understanding the role of TGP and phenotypic plasticity may prove a fruitful avenue that allows a match to be made.

Chapter Six: General Discussion

Colonisation success of non-indigenous species (NIS) in a recipient location will increase with the number and quality of propagules released into that region. Therefore, identifying the factors that enhance propagule pressure to the recipient region is likely to help manage NIS introductions (Johnston et al. 2009), but there is still a lack of understanding on this issue. To understand propagule pressure it is of critical importance to examine not just the causative factors leading to the arrival of individuals, but also the determinants of the physiological condition of these organisms and their ability to produce viable propagules that can establish in a new environment. Using ship biofouling as a model invasion pathway, the aim of this thesis was to elucidate the importance of selective filters on the vessel biofouling invasion process.

Repeatedly during the course of this thesis, the importance of residency period on the biofouling invasion process was highlighted. The period a [simulated] vessel spent in a donor location influenced recruitment of *Bugula*, the survivorship of colonies during translocation, and post-voyage reproduction and growth in the recipient location (Figure 6.1a). Recruitment occurred over periods as short as 24 hrs. Juvenile recruits that may be present on vessels following short and mid-length residency periods (1-8 days), survived the simulated translocation stage and were capable of reproducing post-voyage. In comparison, recruits that were older (29 - 32 days), and reproductively mature when leaving the donor location, survived the mimicked translocation but failed to reproduce post-voyage. This finding indicates that short residency periods, which are typical of many voyage profiles, may be associated with a relatively high risk for the ability of a vessel to spread NIS. The risk of short residency periods was further emphasised by the finding that recruits exposed to short and frequent voyages had higher reproductive output than those exposed to long but infrequent voyages. Other filters tested, including voyage speed, duration and frequency, had no significant effect on propagule survivorship during translocation, which was consistently high (Figure 6.1b, c, d). These filters, however, did have some impact on post-voyage growth, which is an indicator of future reproductive potential. The findings of this thesis are important because the risk of short resident vessels, and other filters tested, were largely unknown and a significant gap for biosecurity managers. In combination, these results

suggest that vessels with short residency periods and short voyage profiles (e.g., intra-coastal voyages) may also be high-risk for NIS transfer. The risk of intra-coastal voyages has previously been highlighted through observations studies (Ashton et al. 2014, Zabin et al. 2014), yet this is the first empirically derived evidence. This thesis empirically links the invasion ecological theory of propagule pressure to applied management, by providing information that may support the identification of high-risk vectors. It emphasises the need to examine NIS in the context of relevant transport pathway characteristics as has previously been highlighted (Sylvester et al. 2013). The general discussion presented here is divided into sections based on the stages of the invasion process and provides evidence for my conclusions. The implications of my findings for biofouling management are then presented.

Stage One: Recruitment to a vector in the donor location

Recruitment to a vector in the donor location marks the initiation of the invasion pathway. At this stage the length of the vector residency period was highlighted as a significant factor in biosecurity risk. Interestingly, donor location residency period also influenced risk during subsequent stages of the invasion process (Figure 6.1a). The abundance and richness of recruits increased with the three residency periods tested but, critically, there was frequent and abundant recruitment to vulnerable surfaces over short (1 day) and medium (5 day) residency periods (Chapter Two: *Temporal variation in biofouling vector recruitment: How risky are short vessel residency periods?*). This is an important finding as it may be assumed that short residency periods pose a relatively low risk, but my results indicate this is not the case. Indeed, marine larval ecology literature provides evidence of frequent recruitment over short periods by many taxa, including barnacles, crabs and ascidians (Hurlbut 1991, Blythe and Pineda 2009, Jarrett 1997). Additionally, recruitment will be particularly high in port environments due to large populations of broadcast spawning biofouling organisms and physical features of the port that retain the abundant propagules (Floerl and Inglis 2003). Understanding recruitment dynamics over short residency periods is particularly important as short residency periods are more common than long residency periods. For example, more than 50% of international container vessels, passenger vessels and car carriers in New Zealand spend less than a day in port (Inglis et al. 2012). Furthermore, structures such as oil rigs typically reside around ports and harbours for short periods after in-water cleaning and before translocation to further deployment areas (Hopkins and Forrest 2010). The results presented in Chapter Two indicate that these vectors may accumulate biofouling communities comprising a diverse range of species during these short residency periods.

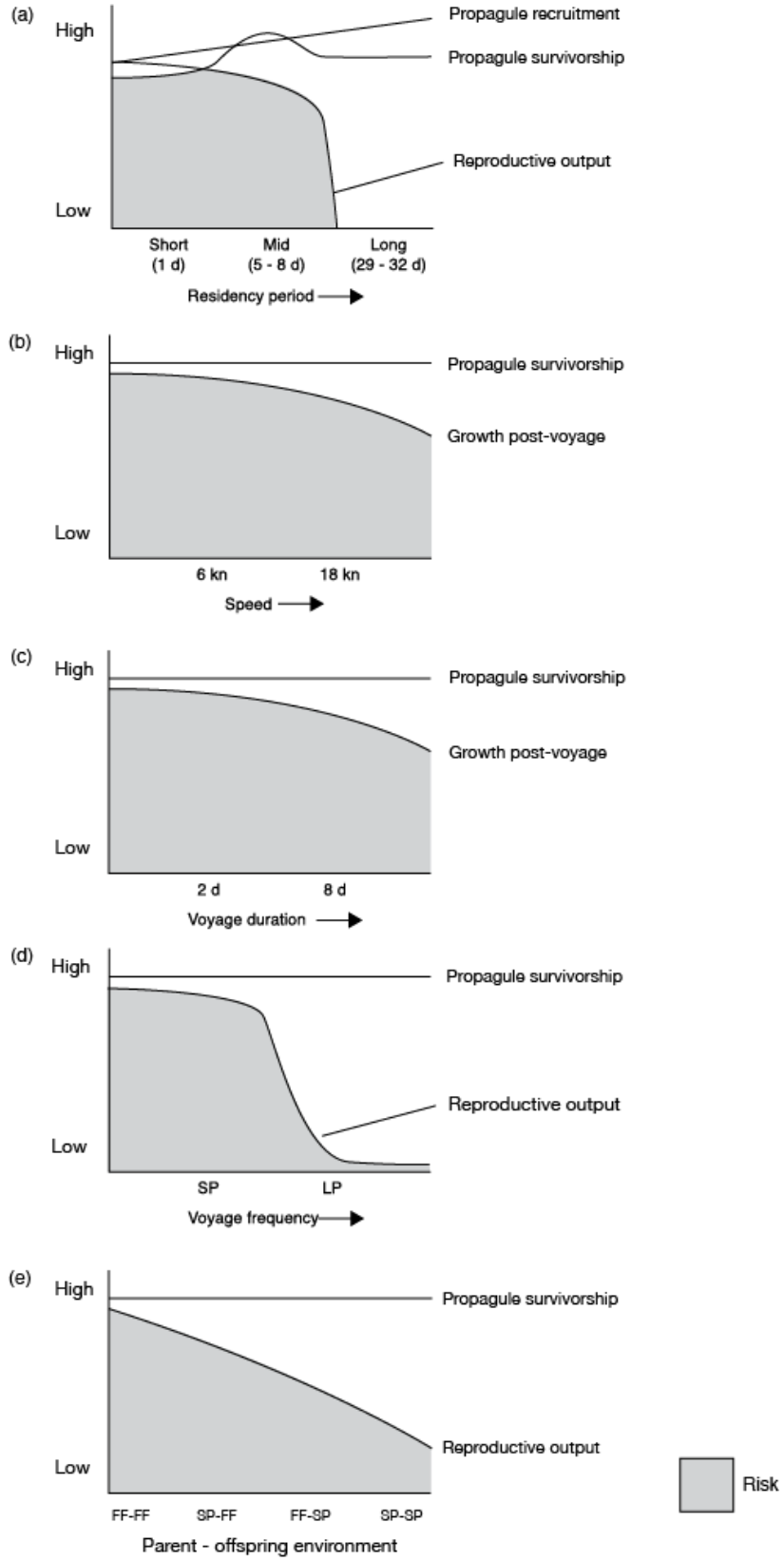


Figure 6.1: Summary of main results indicating high-risk scenarios. Filters examined were: a) residency period measured in days (d); b) voyage speed measured in knots (kn); c) voyage duration measured in days (d); d) voyage frequency (SP = short pulse, LP = long pulse); and transgenerational effects measured through manipulating parent and offspring environments (SP = short pulse, or FF = long pulse) (e). Each response to the filter examined is represented by a labelled line. Risk, indicated by the grey shading, is where all responses measured overlap.

Stage Two: Translocation

The translocation stage links the donor and recipient locations. Building from the results of Chapter Two, I examined the survivorship of three different ages of recruits, representative of recruitment events that took place during short, moderate and long residency periods of vessels after a series of translocation scenarios. Scenarios ranged from a voyage at 6 knots travelling for 2 days (a distance travelled of 288 NM) to a voyage at 18 knots travelling for 8 days (a distance of 3456 NM) (Chapter Three: *The effect of selected filters on the translocation and post-translocation growth of a ship biofouling species*). Again, the age of recruit was identified as an influential factor on successful translocation. Overall, at least 75% of recruits, including the juvenile stages, survived all the voyage scenarios. The 8 day-old juveniles had 100% survivorship, the highest of all age groups. Reproductively mature colonies often lost fragments and decreased in size during translocation. Interestingly, voyage speed, duration and frequency did not influence translocation survivorship (Figure 6.1b, c, d). The high translocation survivorship of *Bugula* recruits demonstrated by my studies indicate high human-mediated dispersal via ship biofouling of this species and is supported by the paradox of *Bugula's* global cosmopolitan distribution, yet low natural dispersal potential (Perez-Portela et al. 2013). Larval ecology highlights the role of dispersal limitation to help understand current distributional patterns, forecast future patterns, particularly with climate change, and to manage areas of significant value, including marine reserves. A better understanding of dispersal limitation via human-mediated vectors through additional empirical tests would be equally beneficial to help understand NIS spread and develop predictive models.

Stage Three: Transfer from the vector

The release of propagules into the recipient location, occurring at Stage Three of the invasion pathway, is considered one of the most important determinants of invasion success (Johnston et al. 2009, Lockwood et al. 2005). The results presented in Chapter Four: *Pre-arrival processes, reproduction and transfer from the vector* demonstrate that pre-arrival factors can affect the outcome of species introductions. Colonies that were 1-day old at the time of the simulated voyage onset had the greatest reproductive output post-scenario. This was followed by juvenile recruits that were 8-days old at the time of voyage onset. It was the colonies that were 32-days old and reproductively mature when they were exported from the donor

location that failed to release any propagules (Figure 6.1a). The finding that juveniles have higher success than mature individuals after exposure to an environmental change is supported by climate change studies. For example, juvenile stages of four Antarctic invertebrates were found to be more resistant to warming than adult stages (Peck et al. 2013). Sexually immature Antarctic clams were more resistant to hypoxic conditions than mature clams (Clark et al. 2013). The immune responses of starved and injured Antarctic molluscs were generally lower in older clams than in younger clams (Husmann et al. 2011). Juvenile King George whiting had higher survivorship during temperature increases than mature whiting (Meakin et al. 2014). The robustness of juveniles has also been demonstrated by *Bugula* after a change in temperature; 1-day juvenile recruits that were transplanted to a new location survived a cold 'freeze' event, while mature colonies did not (Keough and Chernoff 1987). These findings imply that juveniles may also be more tolerant to temperature changes that are associated with voyages spanning latitudes. The increased tolerance of juvenile organisms to stress also occurs in the terrestrial environment, as shown by insects, mammals and fish (Sørensen and Loeschcke 2002, Murtha and Keller 2003, Ső and Csermely 2003). The high survivorship across all life stages yet low reproductive output by mature recruits shown in this thesis may partially explain the observation that many biofouling NIS arrive, but few establish (Inglis et al. 2010, Sylvester et al. 2011).

Recruits from treatments mimicking short voyages followed by short port residency periods released a greater number of larvae than recruits from treatments mimicking long voyages followed by long residency periods (Figure 6.1d). Slow-moving vessels with long residency periods (e.g., barges) are often regarded as posing a particularly high risk of NIS transfer (Hopkins and Forrest 2008). However, in Chapter Four I demonstrated that vessels with voyage profiles featuring short and frequent voyages (e.g., merchant vessels) are also highly likely to facilitate the transfer of viable and reproductively successful recruits. Additionally, as the frequency of arrival events is a very strong determinant of colonisation likelihood (Hedge et al. 2012), short and frequent voyages may result in a higher cumulative risk than long but infrequent voyages. Short and frequent voyages are typical of domestic travel and other studies have also highlighted these pathways as potentially high-risk (e.g., Floerl 2002, Clarke Murray et al. 2011, Ashton et al. 2014, Zabin et al. 2014). In New Zealand, domestic marine transport pathways are currently largely unmanaged.

Stage Four: Colonise and establish in recipient environment

How the introduced organism responds to the new recipient environment and the carry-over effects of the translocation event will be important factors influencing recipient environment colonisation and establishment success. *Bugula* is a colonial organism and consequently, increasing colony size through growth is an important measure of future reproductive potential. The study presented in Chapter Three examined post-voyage growth of translocated recruits. Voyage speed, voyage duration and the age of recruit at the time of translocation interactively affected post-voyage growth in the recipient environment. One-day recruits, or those that were on an 18 knot and/or 8 day voyage scenario, had the lowest growth rates (Figure 6.1b, c). Importantly, however, growth still occurred after all scenarios indicating that both fast and long voyages pose a translocation risk, as well as short and slow voyages.

The study presented in Chapter Five (*Can adaptive transgenerational plasticity contribute to biofouling colonisation, establishment and spread*) took a step further beyond the initial propagule introduction and examined the survivorship, growth and reproduction of the offspring released by the translocated organism. In this study, the parent experience (i.e., translocated compared to not translocated) did have some carry-over effect on offspring success, but it was the offspring experience that was more influential on offspring growth and reproduction (Figure 6.1d). The results demonstrate that *Bugula* translocated to a high resource environment (e.g., a port) can produce viable offspring that will also go on to reproduce. The successful reproduction of the succeeding generation will increase the likelihood of establishment success in the recipient location. This finding is important as successful reproduction reduces the dependency on repeat inoculation events for establishment success. Carry-over effects have been described as a major influence on offspring fitness (Wade 1998). However, the results from my study indicate that the offspring of the translocated individual will only be slightly compromised by the parent experience. Nonetheless, carry-over effects may also partially explain the observation that there are many more NIS arrivals than establishments (Inglis et al. 2010, Sylvester et al. 2011). This is a complex mechanism that should receive further attention.

Stage Five: Spread and impact

Finally, in Chapter Five, transgenerational plasticity (TGP) was used to examine ongoing cross-vector (i.e., parent and offspring both fouling an active vessel) spread post-

translocation. This study showed no evidence to suggest a translocated parent colony could prepare its offspring for a similar translocation event. However, although reproductive output of the offspring was lower if both parent and offspring had been exposed to a translocation scenario than if either generation had not been exposed to the scenario, reproduction did still occur indicating that cross-vector spread is possible (Figure 6.1e). Understanding the importance of TGP in the marine environment is in its infancy and this is the first study I am aware of to address the role of TGP in biofouling invasions. The importance of TGP in plant invasions has recently been identified (Dyer et al. 2010, Zhang et al. 2012, Fenesi et al. 2014). As plant ecology has many parallels to marine invertebrate ecology, for example due to the largely sessile adult stage and limited dispersal via an early life-stage, it can provide us with useful guidance and fruitful avenues for marine invasion research.

Wider implications for biosecurity management

The results of this thesis have implications for biosecurity management and identifying high-risk vectors. A slime layer is an allowable level of fouling on an international vessel arriving into New Zealand under the Craft Risk Management Strategy (CRMS). Juvenile recruits, entrained over short residency periods, may be present in a vessel's slime layer and, due to their small size, difficult to detect during a visual inspection. Given that the recruits that entrained to a vector over short to mid residency periods were more tolerant to voyage scenarios, and develop to release more propagules than large mature recruits, the slime layer may be a potential risk for the exportation of viable organisms from a donor location and also the importation of viable organisms to a recipient location. This has both international and domestic pathway management implications.

Molecular tools to detect microscopic stages of NIS in the slime layer (e.g., Pochon et al. 2015) would be beneficial to identify unwanted species both before and after translocation. For example, if a vector was travelling domestically from a high-risk locality, such as a port, to a high-value area, such as a marine reserve, molecular detection tools could be used to determine exportation risk from that locality before translocation. Similarly, at a border the molecular tool could be used to test the slime layer of an international vessel upon arrival to a new region post-translocation. Additional interventions to reduce the colonisation risk during short residency periods in the donor location would be to manage local resident populations of high-risk species (Forrest and Hopkins, 2013), vessel inspections and treatment of biofilm using hull cleaning technology or similar tools.

The results presented here could feed into probabilistic models allowing the entire invasion process to be addressed holistically. Proxies for propagule pressure, such as the number of ship arrivals, are appealing for estimating invasion risk because obtaining actual biological census data for all vessels is logistically impossible (Wonham et al. 2013). Although these proxy models are useful the surrogate measures assume all propagules have equal probability of arrival and assume no interaction occurs with environment (Leung et al. 2012). This study, and others examining ballast water translocations (Verling et al. 2005, Briski et al. 2013a), have shown that this is not true. Some models, such as Leung's TEASI model, partition the invasion process into different components to gain better insight: TEASI components = transport, establishment, abundance, spread and impact. Dependencies (specific factors that affect the outcome of each component) that are used in the model need to be parameterized and validated to help accurately predict propagule pressure. Using results from this thesis, residency period, voyage duration, voyage speed and voyage frequency could be used as dependencies that influence uptake of propagules, survivorship and propagule pressure. Further manipulative studies that isolate and test specific filters are needed to provide strong, robust predictive models for risk assessment.

This thesis used ship-mediated biofouling invasions as a model pathway, although results will also apply to other biofouling vectors that follow the same sequence of events. These include vectors that may be cleaned and then reside in a location for short periods before translocating to a new location, such as towed marine farm cages and other aquaculture equipment, aquaculture seed stock transfers, pontoons, and oil rigs (Floerl et al. 2005, Hopkins and Forrest 2008). Furthermore, these vectors are particularly at risk of transporting NIS to new locations as they are often towed at slow speeds, over short distances and between similar environments (Hopkins et al. 2010). Intra-coastal voyages have also been highlighted as high risk. Management focus is often on international arrivals but this work is supported by other studies that emphasise the risk of NIS spread via intra-coastal pathways.

Conclusions

Through novel experimentation, this thesis offers new insights into NIS transport via vessel biofouling. Propagule pressure at a recipient location was clearly impacted by all voyage characteristics, yet propagule translocation was consistently high after all scenarios. In particular, the results showed that juvenile stages that recruit over short residency periods and

are then translocated on short voyages, may pose a high risk. Investigating the links between generations of organisms may also provide insights into NIS establishment success and failure.

Invasions are forecasted to increase and predictions and management decisions still need to be made with the best information available. Investigating management issues using underpinning ecological theory will provide information to feed into and improve predictive risk models, such as TEASI, allowing management to advance. As research on pre-arrival stages and determinants of propagule pressure are further explored, the fundamental goal of predicting and mitigating the next invasion may be a little more attainable.

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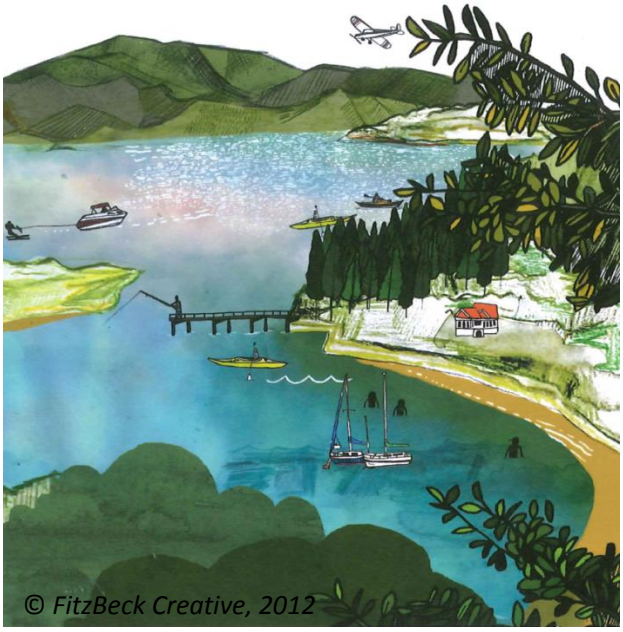

This thesis is dedicated to my Mum and Dad

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Appendices

Appendix 1

Chapter Two post-hoc tests

Table A2.1: Tukey pairwise comparisons of total abundance for residency period and the experimental round in the Port of Lyttelton.

Groups (residency period, round)	Estimate	*s.e.*	*Z*-value (*P*-value)
1day,1 - 5day,1	-0.6	0.1	-6.01 (<0.001)
1day,1 - 15day,1	-2.49	0.16	-15.6 (<0.001)
1day,1 - 1day,2	0.01	0.06	0.04 (1.0)
1day,1 - 5day,2	-1.47	0.18	-8.17 (<0.001)
1day,1 - 15day,2	-2.35	0.29	-8.22 (<0.001)
5day,1 - 15day,1	-1.89	0.19	-10.08 (<0.001)
5day,1 - 1day,2	0.61	0.12	5.16 (<0.001)
5day,1 - 5day,2	-0.86	0.15	-5.70 (<0.001)
5day,1 - 15day,2	-1.74	0.3	-5.77 (<0.001)
15day,1 - 1day,2	2.49	0.17	14.61 (<0.001)
15day,1 - 5day,2	1.02	0.24	4.29 (0.003)
15day,1 - 15day,2	0.14	0.24	0.61 (0.9904)
1day,2 - 5day,2	-1.47	0.17	-8.58 (<0.001)
1day,2 - 15day,2	-2.35	0.28	-8.37 (<0.001)
5day,2 - 15day,2	-0.88	0.33	-2.7 (0.0754)
1 = experimental round 1; 2 = experimental round 2			

Table A2.2 Tukey pairwise comparisons for total and average daily richness of recruits in the Port of Lyttelton over the three residency periods tested.

	Total richness			Mean richness per day		
Groups	**Estimate**	***s.e.***	***Z*-value (P-value)**	**Estimate**	***s.e.***	***Z*-value (*P*-value)**
1 day – 5 day	-0.32	0.1	-3.18 (0.0042)	2.9	0.12	24.06 (<0.0001)
1 day – 15 day	-0.70	0.14	-4.93 (<0.0001)	4.73	0.299	15.86 (<0.0001)
5 day – 15 day	-0.37	0.17	-2.21 (0.0698)	1.83	0.34	5.45 (<0.0001)

Table A2.3: Tukey pairwise comparisons of average daily recruit abundance for residency period and experimental round in the Port of Lyttelton.

Groups (residency period, round)	**Estimate**	***s.e.***	***Z*-value (*P*-value)**
1,1 - 5,1	1.00	0.11	8.90 (<0.0001)
1,1 - 15,1	0.20	0.17	1.19 (0.84)
1,1 - 1,2	0.00	0.06	0.04 (1.00)
1,1 - 5,2	0.14	0.19	0.74 (0.98)
1,1 - 15,2	0.35	0.31	1.12 (0.87)
5,1 - 15,1	-0.80	0.20	-3.92 (<0.0001)
5,1 - 1,2	-1.00	0.13	-7.73 (<0.0001)
5,1 - 5,2	-0.86	0.17	-5.19 (<0.0001)
5,1 - 15,2	-0.66	0.33	-1.99 (0.35)
15,1 - 1,2	-0.20	0.18	-1.12 (0.88)
15,1 - 5,2	-0.06	0.26	-0.24 (1.00)
15,1 - 15,2	0.14	0.26	0.56 (0.99)
1,2 - 5,2	0.14	0.18	0.77 (0.97)
1,2 - 15,2	0.34	0.30	1.13 (0.87)
5,2 - 15,2	0.20	0.35	0.58 (0.99)

Appendix 2

Chapter Two pilot study

Prior to commencing the main settlement plate study a pilot study was carried out to help determine the best experimental design. The aims were to determine:

1) Depth to deploy plates
2) Ability to detect 1-day recruits
3) Time to process plates
4) Suitable experimental set-up and handling
5) Number of replicate plates to use

In November 2012 lightly sanded, 150 × 150 mm, grey PVC settlement plates were attached to a PVC backing plate and deployed in the Port of Lyttelton off the Z-Berth pontoons. A total of 76 plates were deployed. Two factors were tested: depth (fixed with two levels; 0.5-1m and 2-2.5m below sea level), and residency period (fixed with two levels; 1-day and 15-days). Eighteen plates were assigned to each factor combination. Plates were collected, individually placed into a sealed plastic bag and transported back to the laboratory in an insulated container filled with ambient sea water. At the laboratory plates were inspected under the dissecting microscope and the abundance and taxonomic group were recorded.

Results

Due to the low number of recruits on the 1-day plates, only 15-day plate data was used to analyse depth and replicate plate data. Recruit abundance was greater ($Z_{1, 34}$ = -12.99, P < 0.001 (GLM with Poisson distribution) at 0.5 - 1m (mean = 40 ± 3.7) compared to 2.0 - 2.5m (mean = 16 ± 1.4, Figure A2.1). Taxonomic richness was also higher at the shallower depth (mean = 10 ± 0.37) compared to the deeper plates (mean = 7 ± 0.36, $F_{1, 34}$ = 26.9, P < 0.001, ANOVA). Therefore the shallower depth was selected to use in the main study. Recruits were able to be detected on 1-day plates under ×35 magnification. A plastic grid was efficient in guiding the recorder to ensure all parts of the plate were inspected.

Based on the abundance and richness cumulative means (Figures A2.3 and A2.4) and the time to process plates, eight settlement plates (separated between two backing plates) were assigned to each residency period in the main study. Results from the analyses of six of these

plates will be presented in Chapter Two, the remaining plates were kept for future genetic analysis (not presented in this thesis).

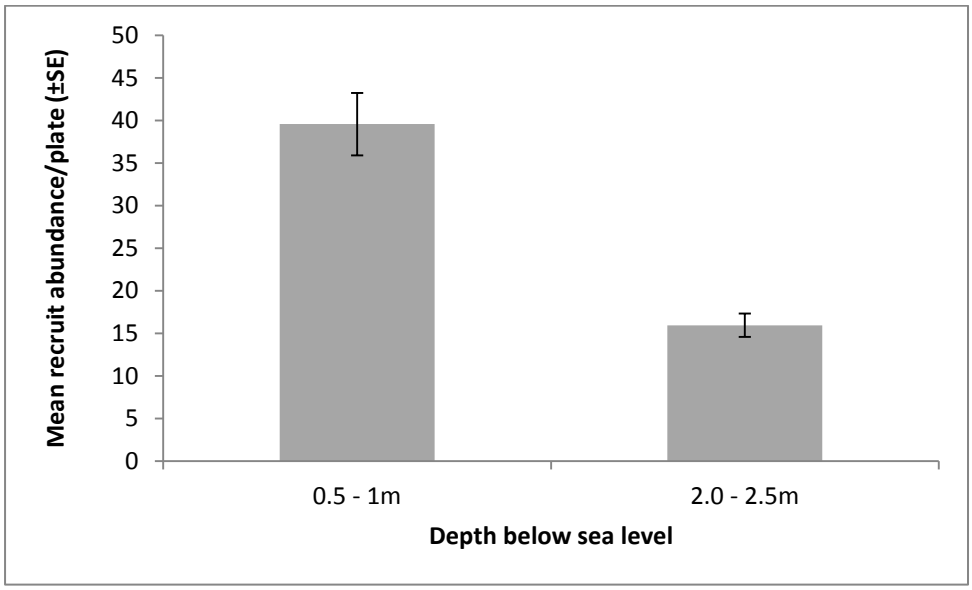

Figure A2.1 Average abundance of recruits (± S.E.) per settlement plate (150 × 150 mm) recorded at two depths (0.5 - 1 m and 2.0 - 2.5 m) in the Port of Lyttelton.

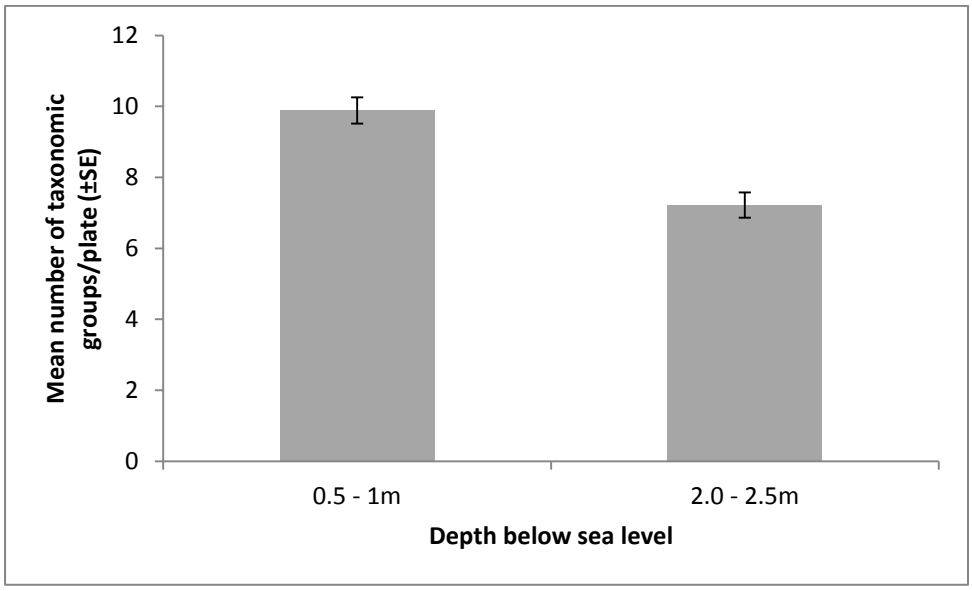

Figure A2.2 Average number of taxonomic groups (± S.E.) per settlement plate (150 × 150 mm) recorded at two depths (0.5 - 1 m and 2.0 - 2.5 m) in the Port of Lyttelton.

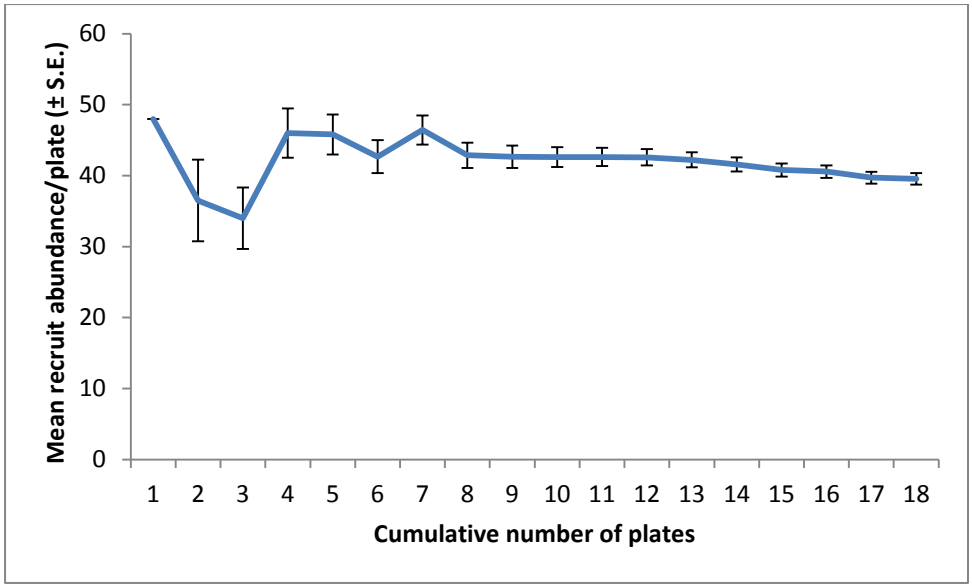

Figure A2.3 Cumulative means of recruit abundance (± S.E.) per settlement plate (150 × 150 mm) recorded in the Port of Lyttelton.

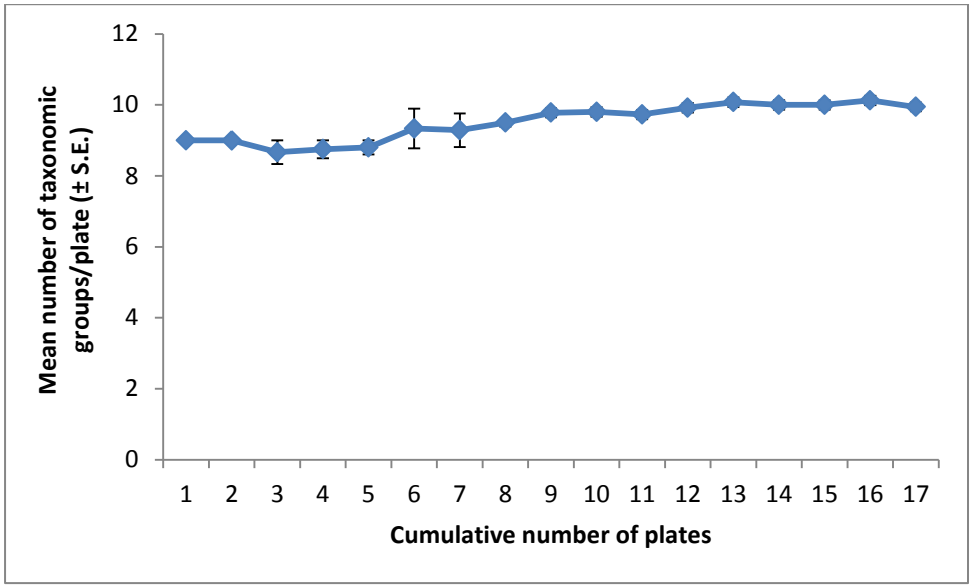

Figure A2.4 Cumulative means of number of taxonomic groups (± S.E.) per settlement plate (150 × 150 mm) recorded in the Port of Lyttelton.

Appendix 3

Chapter Three statistical tables

Table A3.1: Results of univariate PERMANOVA, based on Euclidean distances, for the effect of the spin compared to no-spin control on survivorship and growth of *Bugula* colonies immediately after the spin (T_0) and 7 days post-spin (T_7).

	Survivorship T_0				**Survivorship T_7**			
Source	***df***	**MS**	**F**	***P*(MC)**	***df***	**MS**	**F**	***P*(MC)**
Spin vs. no-spin control	1	0.59	10.89	**	1	0.59	5.55	*
Residual	286	0.05			269	0.11		
Transformation	presence				presence			

	Growth T_0[1]				**Growth T_7**			
Source	***df***	**MS**	**F**	***P*(MC)**	***df***	**MS**	**F**	***P*(MC)**
Spin vs. no-spin control	1	1.56	42.31	***	1	0.47	14.99	***
Residual	132	0.04			268	0.03		
Transformation	none				none			

* $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$

[1] Measurements for 18 knots only

Table A3.2: Results of univariate PERMANOVAs, based on Euclidean distances, for survivorship of *Bugula* colonies immediately after the spin (T_0) and 7 days post-spin (T_7).

	Survivorship T_0				**Survivorship T_7**			
Source	***df***	***MS***	***F***	***P*(MC)**	***df***	**MS**	**F**	***P*(MC)**
Age	*2*	*0.52*	*4.41*	*	2	0.45	2.27	0.13
Speed	*1*	*0.01*	*0.06*	*0.81*	1	0.03	0.13	0.72
Duration	*1*	*0.06*	*0.53*	*0.47*	1	0.05	0.25	0.62
Plate (Speed × Duration × Age)	*24*	*0.12*	*1.43*	*0.11*	24	0.20	1.55	0.06
Res	*115*	*0.08*			100	0.13		

* $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$

Table A3.3: Pairwise comparisons for the significant effect of age on survivorship immediately after the spin (T_0) using Euclidean distance, Type I sums of squares and 999 permutations of residuals under a reduced model.

Age groups	***t***	***P*(MC)**
1, 8	2.9881	0.004
1, 29	1.3086	0.203
8, 29	2.7116	0.009

Table A3.4: Results of univariate PERMANOVAs, based on Euclidean distances, for growth of *Bugula* colonies immediately after the spin (T_0) and 7 days post-spin (T_7).

	Growth T_0[1]					**Growth T_7**			
Source of variation	***df***	**MS**	**F**	***P*(MC)**	**Source of variation**	***df***	**MS**	**F**	***P*(MC)**
Age	2	0.01	0.11	0.90	Age	2	0.14	6.06	**
Duration	1	0.02	0.30	0.59	Speed	1	0.17	7.67	**
Plate (Duration × Age)	12	0.07	1.51	0.16	Duration	1	0.30	13.05	***
Residual	48	0.04			Age×Speed	2	0.03	1.42	0.24
					Age×Duration	2	0.04	1.86	0.17
					Speed×Duration	1	0.07	2.88	0.09
					Age×Speed×Duration	2	0.17	7.45	**
					Residual	119	0.02		

* $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$

[1] Measurements for 18 knots only

Table A3.5: Pairwise comparisons for significant 3-way growth interaction 7 days post-spin.

Groups (speed, duration, age)	***t***	***P*(MC)**
1-18-2, 1-18-8	2.3848	0.034
6-2-1, 6-8-1	1.47	0.168
18-2-8, 18-8-8	3.362	0.005
6-2-8, 6-8-8	2.2004	0.027
18-2-29, 18-8-29	1.73	0.105
6-2-29, 6-8-29	2.8104	0.014
18-2-1, 6-2,1	0.14265	0.907
18-2-1, 6-2-1	0.76288	0.462
18-2-8, 6-2-8	1.0638	0.279
18-8-8, 6-8-8	2.8233	0.015
18-2-29, 6-2-29	3.5294	0.008
18-8-29, 6-8-29	1.0067	0.32
18-2-1,18-2-8	1.6235	0.122
18-2-1, 18-2-29	1.54	0.141
18-2-8, 18-2-29	2.8834	0.011
18-8-1, 18-8-8	0.42565	0.68
18-8-1, 18-8-29	2.1361	0.049
18-8-8, 18-8-29	1.736	0.111
6-2-1, 6-2-8	3.2191	0.008
6-2-1, 6-2-29	2.5871	0.014
6-2-8, 6-2-29	0.15297	0.89
6-8-1, 6-8-8	2.839	0.013
6-8-1, 6-8-29	0.28691	0.762
6-8-8, 6-8-29	1.5763	0.115

Table A3.6: PERMDISP for significant 3-way growth interaction 7 days post-spin.

Groups (speed, duration, age)	***t***	***P*(perm)**
(18-2-1,18-2-8)	1.0291	0.302
(18-2-1,18-2-29)	0.98842	0.451
(18-2-1,18-8-1)	3.0333	3.9E-2
(18-2-1,18-8-8)	0.84803	0.474
(18-2-1,18-8-29)	0.89841	0.419
(18-2-1,6-2-1)	4.04E-2	0.972
(18-2-1,6-2-8)	4.2978E-2	0.969
(18-2-1,6-2-29)	0.65188	0.598
(18-2-1,6-8-1)	1.1892	0.468
(18-2-1,6-8-8)	0.3518	0.776
(18-2-1,6-8-29)	1.219	0.335
(18-2-8,18-2-29)	0.18703	0.862
(18-2-8,18-8-1)	4.0501	1E-3
(18-2-8,18-8-8)	0.71875	0.477
(18-2-8,18-8-29)	0.48153	0.764
(18-2-8,6-2-1)	0.99376	0.33
(18-2-8,6-2-8)	0.91148	0.547
(18-2-8,6-2-29)	0.18701	0.906
(18-2-8,6-8-1)	2.1395	4.7E-2
(18-2-8,6-8-8)	0.76297	0.627
(18-2-8,6-8-29)	0.47534	0.632
(18-2-29,18-8-1)	3.525	2.4E-2
(18-2-29,18-8-8)	0.9143	0.43
(18-2-29,18-8-29)	0.31212	0.779
(18-2-29,6-2-1)	0.97647	0.503
(18-2-29,6-2-8)	0.93204	0.358
(18-2-29,6-2-29)	0.32308	0.753
(18-2-29,6-8-1)	1.9169	0.239
(18-2-29,6-8-8)	0.82335	0.426
(18-2-29,6-8-29)	0.24364	0.846
(18-8-1,18-8-8)	5.183	1E-3
(18-8-1,18-8-29)	2.7185	4E-3
(18-8-1,6-2-1)	2.9628	5.7E-2
(18-8-1,6-2-8)	2.5601	1.8E-2
(18-8-1,6-2-29)	3.1223	2E-3
(18-8-1,6-8-1)	1.6388	0.316
(18-8-1,6-8-8)	3.4004	2E-3
(18-8-1,6-8-29)	3.8525	3E-3
(18-8-8,18-8-29)	1.3238	0.273
(18-8-8,6-2-1)	0.81045	0.442
(18-8-8,6-2-8)	0.76868	0.465
(18-8-8,6-2-29)	0.33299	0.749
(18-8-8,6-8-1)	2.4854	2.8E-2
(18-8-8,6-8-8)	0.42619	0.706
(18-8-8,6-8-29)	1.4261	0.183
(18-8-29,6-2-1)	0.93318	0.421
(18-8-29,6-2-8)	1.0105	0.499
(18-8-29,6-2-29)	0.59589	0.68
(18-8-29,6-8-1)	1.5763	0.154

Groups (speed, duration, age)	***t***	***P*(perm)**
(18-8-29,6-8-8)	0.91994	0.561
(18-8-29,6-8-29)	0.14819	0.907
(6-2-1,6-2-8)	1.0809E-2	0.992
(6-2-1,6-2-29)	0.63664	0.62
(6-2-1,6-8-1)	1.174	0.423
(6-2-1,6-8-8)	0.30747	0.807
(6-2-1,6-8-29)	1.2295	0.302
(6-2-8,6-2-29)	0.611	0.665
(6-2-8,6-8-1)	1.0068	0.325
(6-2-8,6-8-8)	0.26417	0.907
(6-2-8,6-8-29)	1.223	0.241
(6-2-29,6-8-1)	1.5701	0.13
(6-2-29,6-8-8)	0.44871	0.76
(6-2-29,6-8-29)	0.59667	0.566
(6-8-1,6-8-8)	1.5089	0.155
(6-8-1,6-8-29)	2.1837	9.4E-2
(6-8-8,6-8-29)	1.127	0.257

Appendix 4

Food control boxes

The level of food *Bugula* colonies were exposed to was used as a proxy for vessel movement. Food control boxes (FCBs) were used to manipulate food: *open* sided containers, allowing ambient water into the box represented a vessel in port, *mesh* containers with 63 µm mesh covered sides reduced water flow and, therefore, food levels inside the box and represented vessel time at sea. Water flow and dissolved oxygen within each type of FCB was quantified.

Methods

Water exchange inside each type of FCB was quantified using the Fluorescent Dye Rhodamine WT (Sunnyvale, CA) to trace flow. Six FCBs (3 open and 3 mesh) were deployed in the field. The containers were submerged, except for a small section which allowed dye to be sampled without disrupting water exchange. While *in situ* each FCB was sealed using a plastic cover to contain water within the FCB. Using a syringe, 1ml of dye was injected into each container and mixed evenly. The plastic covers were removed and 10 ml water samples from inside the FCB were taken every 2 minutes for 6 minutes using a sterile syringe. Samples were immediately placed in falcon tubes, labelled, covered with tin foil (to prevent light degradation) and placed into a temperature controlled box for transport back to the laboratory for analysis. Dye concentrations within each FCB were quantified using a Turner Designs Trilogy ® Laboratory Fluorometer. Each sample was run six times and the average fluorescence was calculated. Difference between each fluorescence sample concentration and the standard was calculated using the following equation:

$$C_{difference} = C_{Standard} - C_{sample}$$

Data was then plotted (on natural log scale) and a regression model fitted. The turnover time was calculated using the following equation:

$$- \quad Turnover\ time = \frac{1}{\beta}$$

Where, β is the slope of the regression model (or the effect of time on Fluorescence).

Dissolved Oxygen (DO)

Three open and 3 mesh FCBs were deployed in the field for 2 days. DO was then measured by slowly bringing the FCB to the surface so that the container was submerged, except for a small section (as above) which allowed the DO probe to be inserted. DO was measured using the YSI 550A Handheld Dissolved Oxygen Meter (Yellow Springs, OH, USA).

Results:

Water exchange was slower in the mesh containers (2.04 min) compared to the open containers (0.57 min; Figure A4.1) indicating reduced water flow and potentially reduced food (phytoplankton) availability to the filter-feeding bryozoans in the mesh FCBs. DO was lower in the mesh containers (84 ± 4%) compared to the open containers (93 ± 3%; Figure A4.2), however, this reduced level was not considered biologically significant as *Bugula* has been recorded growing at lower levels of DO (e.g., 66% at Port Kembla and 74% Botany Bay, Australia (Piola and Johnston 2006)).

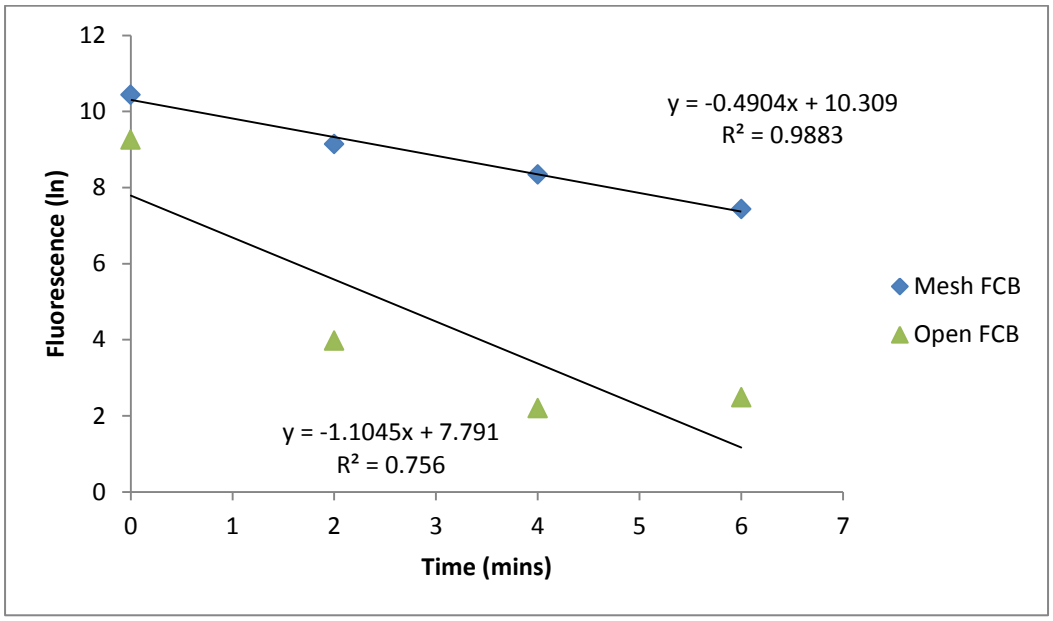

Figure A4.1 Concentration of fluorescence over a six minute period inside open and mesh FCBs.

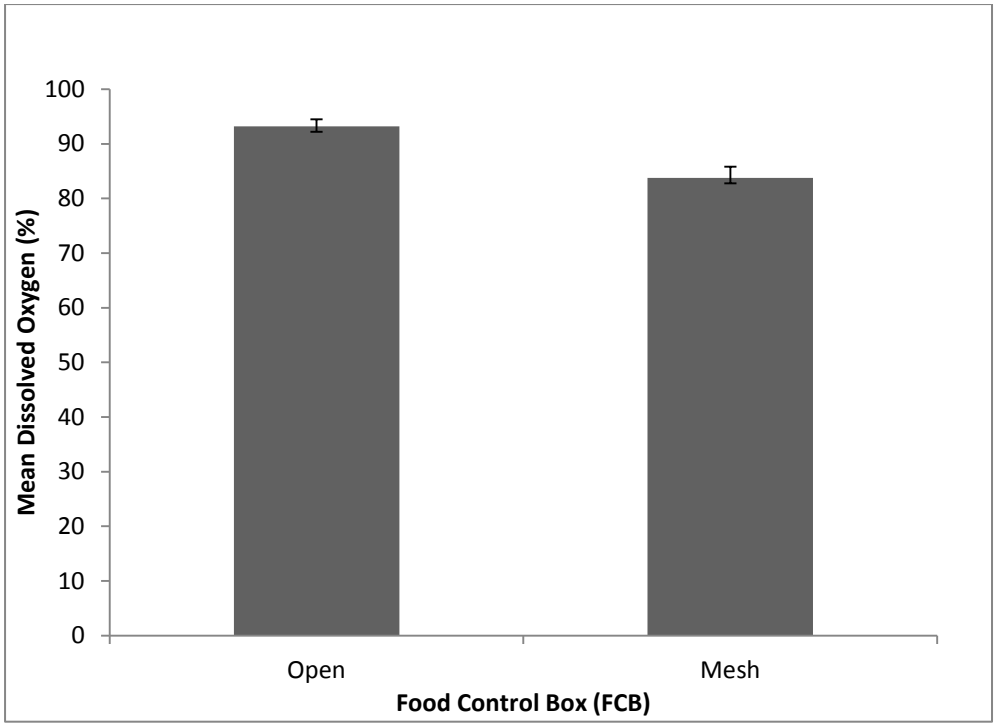

Figure A4.2: Mean dissolved oxygen inside an open and mesh FCB after a 2 day deployment in the field.

Appendix 5

Chapter Four post-hoc tests

Table A4.1: Pairwise comparisons for voyage pattern × spawning event × age interactive effect on spawning success.

Pattern	Spawning	Age	*t*	*P*(MC)
SP	Spn 1	A1, A2	0.378	0.712
SP	Spn 1	A1, A3	3.464	0.029
SP	Spn 1	A2, A3	2.500	0.066
SP	Spn2	A1, A2	1.000	0.397
SP	Spn2	A1, A3	0.000	NA
SP	Spn2	A2, A3	8.000	0.002
SP	Spn3	A1, A2	0.500	0.662
SP	Spn3	A1, A3	7.000	0.005
SP	Spn3	A2, A3	3.464	0.025
LP	Spn 1	A1, A2	negative	NA
LP	Spn 1	A1, A3	1.000	0.373
LP	Spn 1	A2, A3	1.000	0.359
LP	Spn2	A1, A2	0.447	0.673
LP	Spn2	A1, A3	1.000	0.360
LP	Spn2	A2, A3	1.000	0.380
LP	Spn3	A1, A2	negative	NA
LP	Spn3	A1, A3	1.000	0.381
LP	Spn3	A2, A3	1.000	0.353
CS	Spn 1	A1, A2	1.000	0.373
CS	Spn 1	A1, A3	1.000	0.378
CS	Spn 1	A2, A3	NA	NA
CS	Spn2	A1, A2	1.512	0.193
CS	Spn2	A1, A3	1.512	0.223
CS	Spn2	A2, A3	NA	NA
CS	Spn3	A1, A2	1.000	0.385
CS	Spn3	A1, A3	1.000	0.362
CS	Spn3	A2, A3	NA	NA
FF	Spn 1	A1, A2	1.000	0.377
FF	Spn 1	A1, A3	0.707	0.496
FF	Spn 1	A2, A3	2.000	0.122
FF	Spn2	A1, A2	1.000	0.367
FF	Spn2	A1, A3	1.732	0.147
FF	Spn2	A2, A3	5.000	0.006
FF	Spn3	A1, A2	<0.0001	1.000
FF	Spn3	A1, A3	2.212	0.110
FF	Spn3	A2, A3	2.121	0.103
SP, LP	Spn 1	A1	2.500	0.079
SP, CS	Spn 1	A1	2.500	0.073
SP, FF	Spn 1	A1	1.000	0.387
LP, FF	Spn 1	A1	4.950	0.004
CS, FF	Spn 1	A1	4.950	0.014

Pattern	Spawning	Age	*t*	*P*(MC)
SP, LP	Spn2	A1	8.000	0.002
SP, CS	Spn2	A1	1.890	0.131
SP, FF	Spn2	A1	1.732	0.156
LP, CS	Spn2	A1	1.061	0.356
LP, FF	Spn2	A1	2.500	0.056
CS, FF	Spn2	A1	0.632	0.579
SP, LP	Spn3	A1	0.109	0.012
SP, CS	Spn3	A1	0.094	0.018
SP, FF	Spn3	A1	0.620	0.224
LP, CS	Spn3	A1	1.000	1.000
LP, FF	Spn3	A1	0.199	0.040
CS, FF	Spn3	A1	0.202	0.055
SP, LP	Spn 1	A2	1.789	0.134
SP, CS	Spn 1	A2	2.500	0.067
SP, FF	Spn 1	A2	2.000	0.122
LP, CS	Spn 1	A2	1.000	0.371
LP, FF	Spn 1	A2	8.000	0.002
CS, FF	Spn 1	A2	0.000	NA
SP, LP	Spn2	A2	2.683	0.054
SP, CS	Spn2	A2	8.000	0.002
SP, FF	Spn2	A2	2.828	0.046
LP, CS	Spn2	A2	1.000	0.384
LP, FF	Spn2	A2	0.894	0.441
CS, FF	Spn2	A2	4.000	0.012
SP, LP	Spn3	A2	2.500	0.065
SP, CS	Spn3	A2	3.464	0.029
SP, FF	Spn3	A2	0.500	0.656
LP, CS	Spn3	A2	1.000	0.378
LP, FF	Spn3	A2	2.828	0.050
CS, FF	Spn3	A2	5.000	0.013
SP, LP	Spn 1	A3	0.000	0.002
SP, CS	Spn 1	A3	0.000	NA
SP, FF	Spn 1	A3	7.000	0.002
LP, CS	Spn 1	A3	0.000	NA
LP, FF	Spn 1	A3	7.000	0.003
CS, FF	Spn 1	A3	7.000	0.003
SP, FF	Spn3	A3	8.000	0.001
LP, FF	Spn3	A3	8.000	0.005
CS, FF	Spn3	A3	8.000	0.001
SP	Spn 1, 2	A1	1.732	0.235
SP	Spn 1, 3	A1	0.378	0.763
SP	Spn 2, 3	A1	2.000	0.185
LP	Spn 1, 2	A1	negative	NA
LP	Spn 1, 3	A1	negative	NA
LP	Spn 2, 3	A1	NA	NA
CS	Spn 1, 2	A1	1.000	0.408
CS	Spn 1, 3	A1	negative	NA

Pattern	Spawning	Age	*t*	*P*(MC)
CS	Spn 2, 3	A1	1.732	0.227
FF	Spn 1, 2	A1	1.000	0.407
FF	Spn 1, 3	A1	1.732	0.229
FF	Spn 2, 3	A1	1.000	0.423
SP	Spn 1, 2	A2	1.732	0.217
SP	Spn 1, 3	A2	1.000	0.421
SP	Spn 2, 3	A2	2.000	0.169
LP	Spn 1, 2	A2	1.000	0.422
LP	Spn 1, 3	A2	0.000	NA
LP	Spn 2, 3	A2	1.000	0.441
CS	Spn 1, 2	A2	0.000	NA
CS	Spn 1, 3	A2	0.000	NA
CS	Spn 2, 3	A2	0.000	NA
FF	Spn 1, 2	A2	5.000	0.040
FF	Spn 1, 3	A2	4.000	0.062
FF	Spn 2, 3	A2	1.000	0.403
SP	Spn 1, 2	A3	0.000	NA
SP	Spn 1, 3	A3	0.000	NA
SP	Spn 2, 3	A3	0.000	NA
LP	Spn 1, 2	A3	0.000	NA
LP	Spn 1, 3	A3	0.000	NA
LP	Spn 2, 3	A3	0.000	NA
CS	Spn 1, 2	A3	0.000	NA
CS	Spn 1, 3	A3	0.000	NA
CS	Spn 2, 3	A3	0.000	NA
FF	Spn 1, 2	A3	2.000	0.201
FF	Spn 1, 3	A3	1.000	0.427
FF	Spn 2, 3	A3	1.000	0.397

Table A4.2: Pairwise comparisons for voyage pattern × spawning event × age interactive effect on number of larvae.

Pattern	Spawning	Age	*t*	P(MC)
SP	Spn 1	A1, A2	0.478	0.660
SP	Spn 1	A1, A3	2.764	0.042
SP	Spn 1	A2, A3	2.319	0.073
SP	Spn2	A1, A2	1.297	0.283
SP	Spn2	A1, A3	11.314	0.001
SP	Spn2	A2, A3	3.397	0.018
SP	Spn3	A1, A2	0.952	0.384
SP	Spn3	A1, A3	6.951	0.002
SP	Spn3	A2, A3	3.162	0.033
LP	Spn 1	A1, A2	<0.001	1.000
LP	Spn 1	A1, A3	1.000	0.381
LP	Spn 1	A2, A3	1.000	0.368
LP	Spn2	A1, A2	0.749	0.495
LP	Spn2	A1, A3	1.000	0.365

Pattern	Spawning	Age	*t*	P(MC)
LP	Spn2	A2, A3	1.000	0.366
LP	Spn3	A1, A2	<0.001	1.000
LP	Spn3	A1, A3	1.000	0.357
LP	Spn3	A2, A3	1.000	0.388
CS	Spn 1	A1, A2	1.000	0.382
CS	Spn 1	A1, A3	1.000	0.379
CS	Spn 1	A2, A3	NA	NA
CS	Spn2	A1, A2	1.597	0.176
CS	Spn2	A1, A3	1.597	0.181
CS	Spn2	A2, A3	NA	NA
CS	Spn3	A1, A2	1.000	0.000
CS	Spn3	A1, A3	1.000	0.356
CS	Spn3	A2, A3	NA	0.403
FF	Spn 1	A1, A2	1.802	0.153
FF	Spn 1	A1, A3	0.106	0.922
FF	Spn 1	A2, A3	2.421	0.075
FF	Spn2	A1, A2	<0.001	0.936
FF	Spn2	A1, A3	1.675	0.169
FF	Spn2	A2, A3	2.372	0.077
FF	Spn3	A1, A2	0.466	0.677
FF	Spn3	A1, A3	1.818	0.144
FF	Spn3	A2, A3	1.878	0.137
SP, LP	Spn 1	A1	2.578	0.056
SP, CS	Spn 1	A1	2.578	0.052
SP, FF	Spn 1	A1	2.061	0.106
LP, CS	Spn 1	A1	<0.001	1.000
LP, FF	Spn 1	A1	4.211	0.013
CS, FF	Spn 1	A1	4.211	0.009
SP, LP	Spn2	A1	8.690	0.003
SP, CS	Spn2	A1	1.479	0.226
SP, FF	Spn2	A1	<0.001	0.974
LP, CS	Spn2	A1	1.377	0.231
LP, FF	Spn2	A1	1.752	0.151
CS, FF	Spn2	A1	0.810	0.475
SP, LP	Spn3	A1	5.875	0.005
SP, CS	Spn3	A1	4.397	0.013
SP, FF	Spn3	A1	0.320	0.761
LP, CS	Spn3	A1	0.447	0.682
LP, FF	Spn3	A1	2.795	0.032
CS, FF	Spn3	A1	2.503	0.067
SP, LP	Spn 1	A2	2.118	0.100
SP, CS	Spn 1	A2	2.319	0.086
SP, FF	Spn 1	A2	6.011	0.006
LP, CS	Spn 1	A2	1.000	0.396
LP, FF	Spn 1	A2	11.109	0.003
CS, FF	Spn 1	A2	11.490	0.002
SP, LP	Spn2	A2	2.482	0.057

Pattern	Spawning	Age	*t*	P(MC)
SP, CS	Spn2	A2	3.397	0.034
SP, FF	Spn2	A2	0.987	0.386
LP, CS	Spn2	A2	1.000	0.353
LP, FF	Spn2	A2	2.043	0.104
CS, FF	Spn2	A2	3.683	0.021
SP, LP	Spn3	A2	2.985	0.029
SP, CS	Spn3	A2	3.162	0.035
SP, FF	Spn3	A2	0.293	0.782
LP, CS	Spn3	A2	1.000	0.397
LP, FF	Spn3	A2	7.229	0.005
CS, FF	Spn3	A2	8.268	0.003
SP, LP	Spn 1	A3	NA	NA
SP, CS	Spn 1	A3	NA	NA
SP, FF	Spn 1	A3	7.738	0.004
LP, CS	Spn 1	A3	NA	NA
LP, FF	Spn 1	A3	7.738	0.001
CS, FF	Spn 1	A3	7.738	0.003
SP, LP	Spn2	A3	NA	NA
SP, CS	Spn2	A3	NA	NA
SP, FF	Spn2	A3	7.165	0.002
LP, CS	Spn2	A3	NA	NA
LP, FF	Spn2	A3	7.165	0.004
CS, FF	Spn2	A3	7.165	0.003
SP, LP	Spn3	A3	NA	NA
SP, CS	Spn3	A3	NA	NA
SP, FF	Spn3	A3	5.381	0.007
LP, CS	Spn3	A3	NA	NA
LP, FF	Spn3	A3	5.381	0.010
CS, FF	Spn3	A3	5.381	0.004
SP	Spn 1, 2	A1	0.322	0.762
SP	Spn 1, 3	A1	0.594	0.536
SP	Spn 2, 3	A1	0.364	0.753
LP	Spn 1, 2	A1	<0.001	1.000
LP	Spn 1, 3	A1	<0.001	1.000
LP	Spn 2, 3	A1	NA	NA
CS	Spn 1, 2	A1	2.270	0.054
CS	Spn 1, 3	A1	0.426	0.668
CS	Spn 2, 3	A1	1.972	0.086
FF	Spn 1, 2	A1	4.900	0.002
FF	Spn 1, 3	A1	4.750	0.001
FF	Spn 2, 3	A1	0.156	0.897
SP	Spn 1, 2	A2	3.664	0.009
SP	Spn 1, 3	A2	2.156	0.067
SP	Spn 2, 3	A2	1.030	0.303
LP	Spn 1, 2	A2	1.473	0.163
LP	Spn 1, 3	A2	NA	NA
LP	Spn 2, 3	A2	1.473	0.181

Pattern	Spawning	Age	*t*	P(MC)
CS	Spn 1, 2	A2	NA	NA
CS	Spn 1, 3	A2	NA	NA
CS	Spn 2, 3	A2	NA	NA
FF	Spn 1, 2	A2	7.104	0.001
FF	Spn 1, 3	A2	6.566	0.001
FF	Spn 2, 3	A2	0.657	0.524
SP	Spn 1, 2	A3	NA	NA
SP	Spn 1, 3	A3	NA	NA
SP	Spn 2, 3	A3	NA	NA
LP	Spn 1, 2	A3	NA	NA
LP	Spn 1, 3	A3	NA	NA
LP	Spn 2, 3	A3	NA	NA
CS	Spn 1, 2	A3	NA	NA
CS	Spn 1, 3	A3	NA	NA
CS	Spn 2, 3	A3	NA	NA
FF	Spn 1, 2	A3	1.655	0.143
FF	Spn 1, 3	A3	1.976	0.088
FF	Spn 2, 3	A3	0.974	0.363

Table A4.3: Pairwise comparisons for voyage pattern × spawning event interactive effect on size of larvae.

Pattern	Spawning	*t*	P(MC)
SP, FF	Spn 1	5.289	0.001
SP, FF	Spn 2	0.96	0.342
SP, FF	Spn 3	1.726	0.105
SP	Spn1,Spn2	2.499	0.049
SP	Spn1,Spn3	NA	NA
SP	Spn2,Spn3	1.132	0.279
FF	Spn1,Spn2	0.191	0.989
FF	Spn1,Spn3	1.470	0.19
FF	Spn2,Spn3	2.119	0.073

Table A4.4: Pairwise comparisons for age × spawning event interactive effect on size of larvae.

Age	Spawning	*t*	P(MC)
1,2	Spn 1	5.745	0.001
1,2	Spn 2	2.016	0.047
1,2	Spn 3	4.75	0.002
1	Spn1,Spn2	3.709	0.022
1	Spn1,Spn3	NA	NA
1	Spn2,Spn3	2.388	0.059
2	Spn1,Spn2	2.532	0.021
2	Spn1,Spn3	1.966	0.084
2	Spn2,Spn3	0.41	0.696

Table A4.5: Permdisp pairwise comparisons for pattern × spawning event interactive effect on size of larvae.

Pattern	Spawning	*t*	P(perm)
SP,FF	Spn1,Spn1	2.427	0.021
SP,SP	Spn1,Spn2	2.877	0.011
SP,FF	Spn1,Spn2	4.259	0.001
SP,SP	Spn1,Spn3	3.859	0.001
SP,FF	Spn1,Spn3	4.319	0.001
FF,SP	Spn1,Spn2	1.17	0.254
FF,FF	Spn1,Spn2	3.115	0.002
FF,SP	Spn1,Spn3	2.742	0.007
FF,FF	Spn1,Spn3	3.205	0.004
SP,FF	Spn2,Spn2	1.796	0.109
SP,SP	Spn2,Spn3	1.495	0.134
SP,FF	Spn2,Spn3	1.836	0.09
FF,SP	Spn2,Spn3	0.188	0.858
FF,FF	Spn2,Spn3	0.048	0.967
SP,FF	Spn3,Spn3	0.231	0.825

Table A4.6: Permdisp pairwise comparisons for age × spawning event interactive effect on size of larvae.

Age	Spawning	*t*	P(perm)
1	Spn1,Spn2	1.435	0.178
1	Spn1,Spn3	3.301	0.004
1,2	Spn1,Spn1	0.516	0.648
1,2	Spn1,Spn2	1.262	0.239
1,2	Spn1,Spn3	2.582	0.008
1	Spn2,Spn3	2.022	0.039
1,2	Spn2,Spn1	1.004	0.363
1,2	Spn2,Spn2	0.438	0.711
1,2	Spn2,Spn3	0.796	0.451
1,2	Spn3,Spn1	2.91	0.004
1,2	Spn3,Spn2	1.611	0.12
2,2	Spn1,Spn2	0.734	0.494
2,2	Spn1,Spn3	2.059	0.034
2,2	Spn2,Spn3	1.431	0.173

Appendix 6

Chapter Five statistical tables

Table A 5.1**:** Results of univariate PERMANOVAs, based on Euclidean distances for *Bugula neritina* colony size, spawning success and number of larvae released in response to the experimental treatments.

		Size of colony			Spawning success			Spawning success		
Source	*df*	MS	Pseduo-F	*P*(MC)	MS	Pseduo-F	*P*(MC)	MS	Pseduo-F	*P*(MC)
Parent Environment (PE)	1	6.7	4.4	.	0.22	0.84	NS	5.4	4.3	*
Offspring Environment (OE)	1	84.5	55.8	***	2.722	10.32	**	42.2	33.3	***
Parent Age (PA)	1	4.5	2.9	NS	1.39	5.26	*	26.3	20.7	***
PE × OE	1	2	1.3	NS	<-0.001	Negative		3.5	2.7	NS
PE × PA	1	0.9	0.6	NS	2	7.58	*	19.9	1.8	**
OE × PA	1	0.9	0.6	NS	0.005	0.21	NS	11.5	9.0	**
PE × OE × PA	1	0.06	0.04	NS	0.22	0.84	NS	11.2	8.8	**
Plate (PE × OE × PA)	16	1.5	3.2	**	0.26	1.9	*	1.3	1.7	NS
Residual	48	0.5			0.14			0.7		
Transformation		Nil			Nil (presence/absence data)			log(x +1)		

'NS' P > 0.05; '.' *P* = 0.05; * *P* < 0.05; ** *P* < 0.01 and *** *P* < 0.001

Table A 5.2: Pairwise test results for parent environment (PE) × parent age (PA) spawning success interaction.

Groups	*t*	*P*(MC)
PA-1, PE-SP, PE-FF	1.5119	0.166
PA-2, PE-SP, PE-FF	2.3094	0.05
PA-1, PA-2, PE-SP	0.2582	0.815
PA-1, PA-2, PE-FF	5.5	0.001

Table A 5.3: PERMDISP results for offspring environment (OE) spawning success.

Groups	*t*	*P*(perm)
OE-SP,OE-FF	1.539	0.199

Table A 5.4: PERMDISP results for parent environment (PE) × parent age (PA) spawning success interaction.

Groups	*t*	*P*(perm)
PA-1,PE-SP,PA-1,PE-FF	1.4932	0.299
PA-1,PE-SP,PA-2,PE-SP	0.33778	1
PA-1,PE-SP,PA-2,PE-FF	1.0726	0.47
PA-1,PE-FF,PA-2,PE-SP	1.1435	0.434
PA-1,PE-FF,PA-2,PE-FF	0.41026	1
PA-2,PE-SP,PA-2,PE-FF	0.72887	0.703

Table A 5.5: Pairwise results for the parent environment (PE) × offspring environment (OE) × parent age (PA) interactive effect on the number of larvae.

Groups	*t*	*P*(MC)
OE-SP, PA-1, PE-SP, PE-FF	1.412	0.1696
OE-SP, PA-2, PE-SP, PE-FF	0.98017	0.3314
OE-FF, PA-1, PE-SP, PE-FF	1.6226	0.1134
OE-FF, PA-2, PE-SP, PE-FF	2.9575	0.0056
OE-SP, OE-FF, PE-SP, PA-1	1.6731	0.072
OE-SP, OE-FF, PE-SP, PA-2	1.7519	0.079
OE-SP, OE-FF, PE-FF, PA-1	1.8697	0.074
OE-SP, OE-FF, PE-FF, PA-2	3.571	0.002
OE-SP, PE-SP, PA-1, PA-2	0.50709	0.611
OE-FF, PE-SP, PA-1, PA-2	0.30535	0.769
OE-SP, PE-FF, PA-1, PA-2	2.4079	0.023
OE-FF, PE-FF, PA-1, PA-2	3.6043	0.001

Table A 5.6: Results of number of larvae PERMDISP tests for parent environment (PE) × offspring environment (OE) × parent age (PA)

Groups	*t*	*P*(perm)
PA-1,PE-SP,OE-SP,PA-1,PE-SP,OE-FF	2.8441	1.00E-03
PA-1,PE-SP,OE-SP,PA-1,PE-FF,OE-SP	3.0459	1.00E-03
PA-1,PE-SP,OE-SP,PA-1,PE-FF,OE-FF	0.96753	0.649
PA-1,PE-SP,OE-SP,PA-1,PE-SP,OE-SP	1.0147	0.542
PA-1,PE-SP,OE-SP,PA-2,PE-SP,OE-FF	3.5222	1.00E-03
PA-1,PE-SP,OE-SP,PA-2,PE-FF,OE-SP	2.8743	0.146
PA-1,PE-SP,OE-SP,PA-2,PE-FF,OE-FF	5.7691	1.00E-03
PA-1,PE-SP,OE-FF,PA-2,PE-FF,OE-SP	3.0258	1.00E-03
PA-1,PE-SP,OE-FF,PA-2,PE-FF,OE-FF	2.7598	1.00E-03
PA-1,PE-SP,OE-FF,PA-2,PE-SP,OE-SP	2.7467	1.00E-03
PA-1,PE-SP,OE-FF,PA-2,PE-SP,OE-FF	0.75157	0.696
PA-1,PE-SP,OE-FF,PA-2,PE-FF,OE-SP	2.5409	1.00E-03
PA-1,PE-SP,OE-FF,PA-2,PE-FF,OE-FF	4.6151	1.00E-03
PA-1,PE-FF,OE-SP,PA-1,PE-FF,OE-FF	4.0794	1.00E-03
PA-1,PE-FF,OE-SP,PA-2,PE-SP,OE-SP	3.7117	1.00E-03
PA-1,PE-FF,OE-SP,PA-2,PE-SP,OE-FF	3.6836	1.00E-03
PA-1,PE-FF,OE-SP,PA-2,PE-FF,OE-SP	5.5202	1.00E-03
PA-1,PE-FF,OE-SP,PA-2,PE-FF,OE-FF	5.8273	1.00E-03
PA-1,PE-FF,OE-FF,PA-2,PE-SP,OE-SP	0.11657	1
PA-1,PE-FF,OE-FF,PA-2,PE-SP,OE-FF	3.4474	1.00E-03
PA-1,PE-FF,OE-FF,PA-2,PE-FF,OE-SP	2.0064	0.31
PA-1,PE-FF,OE-FF,PA-2,PE-FF,OE-FF	5.7419	1.00E-03
PA-2,PE-SP,OE-SP,PA-2,PE-SP,OE-FF	3.4357	1.00E-03
PA-2,PE-SP,OE-SP,PA-2,PE-FF,OE-SP	1.8027	0.371
PA-2,PE-SP,OE-SP,PA-2,PE-FF,OE-FF	5.7379	1.00E-03
PA-2,PE-SP,OE-FF,PA-2,PE-FF,OE-SP	3.253	1.00E-03
PA-2,PE-SP,OE-FF,PA-2,PE-FF,OE-FF	4.2123	1.30E-02
PA-2,PE-FF,OE-SP,PA-2,PE-FF,OE-FF	5.6715	1.00E-03

Table A 5.7: Results of generalised linear mixed model (GLMM) for number of larvae

	Z value	*P*(>|z|)
Intercept	-0.52	0.6025
PE-SP	3.22	0.0013
OE-SP	-6.98	<0.0001
PA-2	6.02	<0.0001
PE-SP × PA-2	-3.99	<0.0001
Plate (random)	StdDev	<0.0001

Table A 5.8**:** Results of univariate PERMANOVAs, based on Euclidean distances, for larvae size in response to the experimental treatments.

		Spawning success		
Source	*df*	MS	F	*P*
Parent Environment (PE)	1	1237.3	0.8	NS
Offspring Environment (OE)	1	1882.2	1.2	NS
Residual (+pooled)	205			
Transformation		nil		

'NS' $P > 0.05$