ECOLOGY OF BIOFOULING AND IMPACTS ON MUSSEL AQUACULTURE: A CASE STUDY WITH *DIDEMNUM VEXILLUM*

by

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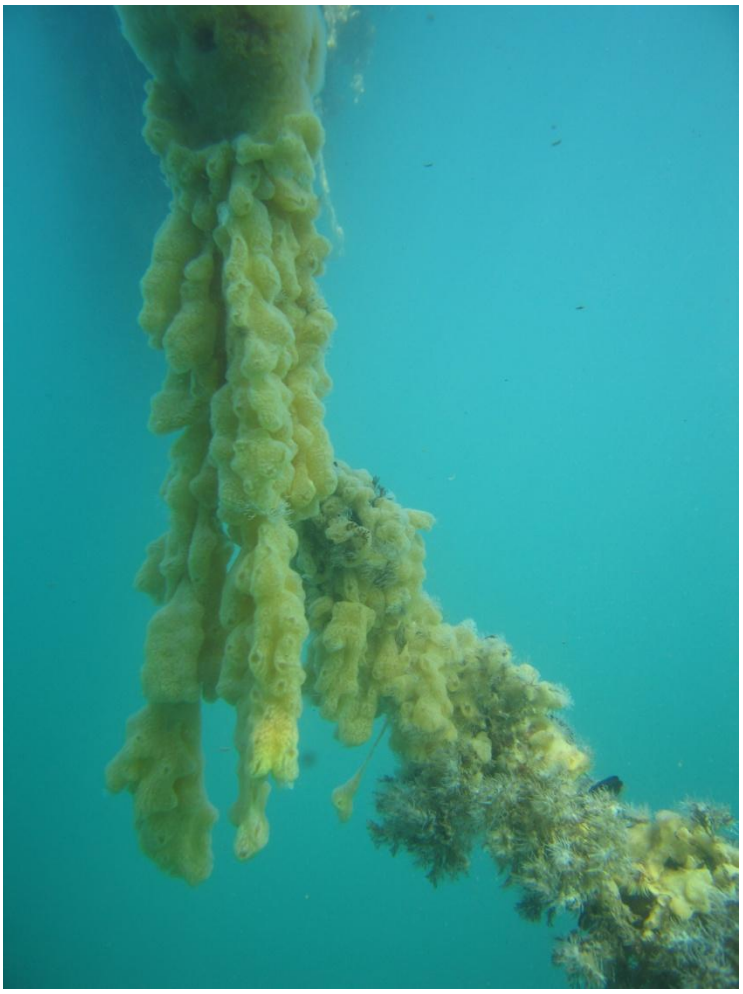

For Hannah

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GENERAL ABSTRACT

Over the past decade, several species of non-indigenous ascidian have had adverse effects in the marine environment and on associated industries. The colonial ascidian *Didemnum vexillum* is a recent successful invader in temperate marine communities worldwide, proving problematic to mussel aquaculture in New Zealand. At the inception of this thesis, control strategies to manage the threat from *Didemnum* to mussel aquaculture were implemented in the absence of information on the biological processes underpinning the species' invasion success. Background information on *Didemnum* presented in Chapter 2 recognises this paucity of information on several key biological attributes as well as negative impacts of this species. The ability to obtain larvae and culture colonies in the laboratory was a crucial first step. Thus, Chapter 3 presents laboratory experiments that describe the first successful methods to induce spawning in adult *Didemnum* colonies, as well as techniques for the successful settlement and metamorphosis of the larvae produced, and for laboratory culture of juveniles.

Chapters 4 to 6 address key aspects of the biological characteristics of *Didemnum* that relate to its invasiveness and spread. The recruitment and reproductive development of *Didemnum* were assessed over a 20-month period at two locations in central New Zealand. Results indicated that the reproductive season for *Didemnum* in New Zealand (at least 9 months) is considerably longer than previously believed, with recruitment patterns strongly correlated with seasonal water temperature fluctuations at each location. Secondly, the natural dispersal ability of *Didemnum* was assessed using a weight-of-evidence approach that combined laboratory and field studies. Larval competency trials revealed that > 70 % of larvae were able to settle and undergo metamorphosis following an artificial settlement delay of 2 hours. Larval viability decreased with increasing delay duration; however 10 % of larvae remained viable following a 36 hour delay. These findings were supported by a field-based study documenting larval recruitment at distances up to 250 m from source populations.

Exponential decay models indicated that, given favourable conditions, larval dispersal distances greater than 1 km were theoretically possible, which is a much greater distance than previously assumed. Lastly, the impacts of *Didemnum* on cultured New Zealand green-lipped mussels (*Perna canaliculus*) were investigated. At the level of invasiveness experienced in a field experiment, only small mussel size classes were vulnerable to direct *Didemnum* impacts, with negative effects restricted to fouling-related displacement of mussels as opposed to reduced size or condition. However, at the greater levels of invasiveness evident at other places and times, *Didemnum* impacts have the potential to be considerably larger. As such, the ability to predict invasiveness, and hence impacts, is critical for stakeholders. However, for reasons discussed in the thesis, making reliable specific predictions of invasiveness is difficult.

Despite such limitations, it is clear that an understanding of a species' basic biological attributes can still greatly assist with management decisions, as highlighted throughout the chapters in this thesis. My research findings have led to a better awareness of commercial impacts and potential spread of this species. Simultaneously, my research also highlights the limitations inherent in inferring invasiveness from other situations (e.g. places, times, and related species); there is a need to specifically evaluate a species' biological attributes and invasive behaviour when introduced into a novel environment.

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CHAPTER 1

GENERAL INTRODUCTION, OVERVIEW AND THESIS STRUCTURE

1.1 Background

One of the major human-associated threats to ecosystems and biodiversity is biotic invasion (Ruiz et al. 1997; Lockwood et al. 2005), with invasive species having been shown to have considerable ecological and economic impacts on a global scale (Pimental et al. 2000; Colautti et al. 2006). While government agencies and associated stakeholders have been working for decades to control exotic species on land and freshwater, recognition of non-indigenous marine species as an important threat to coastal ecosystems and their associated values is relatively recent, and as such the development of approaches for control of these species is ongoing (Bax et al. 2001; Hewitt et al. 2009). With the vast increase and changing patterns of international maritime trade, the introduction and establishment of exotic marine species has become progressively more widespread (Carlton and Geller 1993), and the number of non-indigenous species introduced into new marine locations continues to increase (Ruiz et al. 1999; Harris and Tyrrell 2001; Hewitt et al. 2004).

To date almost 200 non-indigenous marine species have been introduced into New Zealand, the majority into international ports via shipping-related mechanisms, such as ballast water and hull fouling (Cranfield et al. 1998; Nelson 1999; Hayden et al. 2009; Gordon et al. 2010). Once introduced to New Zealand these species often continue to spread domestically, both by natural dispersal mechanisms and through anthropogenic transport vectors such as vessel movements and aquaculture transfers between regions (Dodgshun et al. 2007). Although recorded non-indigenous marine species in New Zealand are numerous, only a few are demonstrated to cause economic and ecological harm and are thus recognised as marine 'pest' species (Falk-Petersen et al. 2006), and it is these which typically receive public attention. This is particularly the case for conspicuous organisms that affect areas of high conservation value or economically important sectors such as aquaculture. At present, New Zealand's

marine environment appears to be free of some of the world's most notorious invasive marine species (e.g. the European shore crab *Carcinus maenas*, the northern Pacific sea star *Asterias amurensis*, and the green seaweed *Caulerpa taxifolia*). Nonetheless, the last 10-15 years has seen an increased prevalence of invasions and adverse effects from non-indigenous biofouling species, both in New Zealand and globally (Cohen and Carlton 1998; Cranfield et al. 1998; Ruiz et al. 2000; Hewitt et al. 2004).

1.2 Invasive marine biofouling species

Biofouling refers to the accumulation of organisms and biogenic structures on ship hulls and other submerged surfaces (Railkin 2004; Zardus et al. 2008). Marine fouling communities are characterised by the presence of a variety of sessile marine invertebrates including ascidians, sponges, bryozoans and cnidarians, as well as an array of macroalgae. Organisms such as barnacles, mussels and tube building polychaetes are also often present and produce shells or other rigid structures as they grow, allowing for attachment of additional organisms after the primary substratum has been colonised, resulting in multilayered fouling communities (Scheer 1945). Many common biofouling species are recognised as non-indigenous to affected areas, often sharing common characteristics of 'invasiveness' including high rates of growth and reproduction, limited natural predators, and the ability to thrive in a range of environments (Erlich 1989).

Biofouling species or assemblages have proved particularly problematic for commercial activities in marine environments, affecting a range of industries such as commercial fishing, the global shipping trade, offshore oil and gas production, and aquaculture. For example, biofouling reduces the speed of vessels through increased frictional drag, smothers oceanographic equipment,

adds weight to floating structures, and promotes structural deterioration through surface corrosion (Gitlitz 1981; Abarzua and Jakubowski 1995). For the global shipping industry alone, biofouling costs billions of dollars per year in prevention, maintenance, and fuel consumption (Townsin 2003). Increased global shipping, recreational boating and aquaculture activities during the last few decades, and the associated creation of extensive areas of artificial habitat, has led to a substantial rise in the amount of substrate available to fouling species (Glasby and Connell 1999; Floerl and Inglis 2003; Minchin et al. 2006). This creation of novel habitat is only likely to escalate, with anthropogenic structures continuing to be added to the marine environment as coastal regions become increasingly urbanised (Bulleri and Chapman 2009).

Marine aquaculture is particularly vulnerable to biofouling impacts, as fouling organisms are often strong spatial competitors that are able to reach a very high density or biomass in relatively short time frames (Nandakumar et al. 1993; Dealteris et al. 2004; Blum et al. 2007). Shellfish crops appear to be particularly at risk from biofouling, as shellfish farms are often heavily colonised through the creation of complex novel substrates due to the high density of bivalve shells present within relatively small areas (McKindsey et al. 2007; Fitridge et al. 2012). In addition, farms are often situated in locations that are protected from strong wave action (e.g. harbours and embayments), providing optimal conditions for fouling organisms (Osman and Whitlatch 1999; Davis and Davis 2007), and farm structures are usually suspended thus providing protection from predation by other benthic invertebrates (Rocha et al. 2009).

The solitary ascidians *Ciona intestinalis* and *Styela clava* have emerged as particularly problematic species that have had considerable impacts on cultured mussel and oyster industries around the world. Impacts include increased costs for production and processing (e.g. Carver et al. 2003; Thompson and MacNair 2004; Gretchis 2005; Ramsay et al. 2008; Fitridge et al. 2012) and negative

impacts on growth rates and meat yields due to competition for space and food resources (e.g. Le Blanc et al. 2003; Daigle and Herbinger 2009). Several reasons for lower growth rates have been suggested, including obstruction of water flow (Uribe and Etchepare 2002) and competition for food at a local-scale (Lesser et al. 1992). When present in large numbers, the high filtration rate of ascidians has also been shown to cause a dramatic reduction in the availability of phytoplankton and suspended organic matter at a larger scale (Hily 1991; Peterson and Riisgård 1992; Coma et al. 2000; Riisgård and Larsen 2000); potentially altering the local carrying capacity for bivalve culture. Because of these effects on culture species, as well as impacts on the surrounding ecosystem, biofouling is now widely considered one of the most important problems facing the marine aquaculture industry (Lambert 2007; Adams et al. 2011).

New Zealand, even though geographically isolated from other problem areas, is not immune to these difficulties. Invasive biofouling organisms have become prevalent over the last 15 years resulting in actual or perceived economic and ecological impacts at a local scale, although adverse effects across broad spatial scales are yet to be documented (e.g. Forrest and Taylor 2002; Coutts and Forrest 2005, 2007). As is the situation globally, biofouling organisms have been particularly detrimental to commercial shellfish operations. Currently several introduced ascidians threaten New Zealand's highly valued shellfish aquaculture industry, and as such efforts to control and manage these species are ongoing. In addition to the solitary ascidians *Styela clava* and *Ciona intestinalis* referred to above, the colonial ascidian *Didemnum vexillum* (Kott 2002) is a relatively recent arrival to New Zealand, and has been regarded with concern primarily because of its perceived potential to adversely affect subtidal aquaculture operations. Of these, the green-lipped mussel (*Perna canaliculus*) industry is the most vulnerable, as well as being the most important aquaculture sector in New Zealand in terms of earnings (AQNZ 2012).

1.3 Biological knowledge and biofouling risk management

Underpinning the effective management of species such as *Didemnum vexillum* is the need for biological knowledge that relates to invasion potential, spread and impacts. The ability to predict whether new exotic species will successfully establish, propagate and spread is an imprecise science (e.g. Williamson and Fitter 1996; Kolar and Lodge 2001), particularly in the marine environment (e.g. Carlton 1996; Nyberg and Wallentinus 2005; Locke 2009). Numerous studies across a wide range of taxonomic groups have attempted to define what characteristics determine whether a species is likely to be invasive, as well as what characteristics determine whether a community is vulnerable or resistant to invasion (e.g. Lodge 1993; Carlton 1996; Williamson and Fitter 1996; Ruiz et al. 1997; Sakai et al. 2001; Kolar and Lodge 2002; Stachowicz et al. 2002; Colautti et al. 2006). Species traits believed to contribute to successful establishment include a lack of natural enemies, ability for habitat modification, association with human activities, genetic variability and phenotypic plasticity, and a high degree of competitiveness (Cuddington and Hastings 2004; Liu and Stiling 2006; Troost 2010). More generally, successful colonists are often species with high reproduction rates, a broad tolerance to a wide range of environmental conditions, as well as an ability to colonise a variety of habitat types (Troost 2010).

Acquisition of species specific biological knowledge that supports such predictions and enables informed management decisions is essential. For example, understanding dispersal mechanisms and natural dispersal potential is critical to decisions regarding the minimum spatial scales over which to focus management of anthropogenic vectors such as fouled vessels (Forrest et al. 2009). As many biofouling species have a limited natural dispersal ability, vessels and other anthropogenic vectors can play an important role in greatly extending the spatial scale and rate of species spread. Simultaneously, knowledge of actual

and potential impacts provides a critical context for understanding the importance of management, and for optimising management approaches (e.g. by providing insight into effort required to reduce invasion levels to a point where density-dependent effects are mitigated).

1.4 Scope and structure of this thesis

1.4.1 Background

This thesis uses *Didemnum vexillum* (hereafter referred to as *Didemnum*), as a case study to research key areas of biological knowledge that support management, and to more broadly explore key issues around biofouling management in New Zealand. *Didemnum* is a useful case study species as it has a range of effects in different environments, and is easily manipulated in the laboratory or field. This is strengthened by the fact that although it is a high-profile invasive organism, it is not regulated by regional or central government legislation, allowing for relative ease of utilisation of the species for field-based trials. *Didemnum* is also an effective model species as it displays all the characteristics typical of a fouling pest with the potential to be managed; it is benthic, conspicuous, has a limited depth range, is thought to have a relatively short dispersal phase, and can be highly invasive on artificial structures (especially marine farms). Because of this, the lessons learned and knowledge gained from the study of *Didemnum* can be used to provide insights into the management of marine pest organisms more generally. Perhaps most importantly, there are still several key areas of knowledge that have not been investigated for *Didemnum* that have a considerable bearing on the successful management of this species.

1.4.2 Objectives and aims of the study

The overall aim of this study was to evaluate the ecology and impacts of *Didemnum* biofouling with specific reference to the New Zealand green-lipped mussel aquaculture industry. This is achieved through field and laboratory based experimental investigations with *Didemnum*. Specific aims were:

1. Develop a reliable method for inducing spawning of *Didemnum* colonies and for laboratory culture of juvenile recruits, to facilitate laboratory-based investigations of juvenile life-stages.

2. Describe patterns of field recruitment and tissue reproductive state in relation to seawater temperature, including the development of statistical models to predict critical temperature thresholds for the seasonal onset and cessation of *Didemnum* recruitment.

3. Evaluate the relative sensitivity, utility and practicality of directly measuring recruitment in the field compared with inferring recruitment potential based on the reproductive maturity of *Didemnum* tissue samples.

4. Assess the natural dispersal capacity of *Didemnum*, including determining the larval competency period under laboratory conditions and measuring the dispersal distance of larval recruits from isolated field populations.

5. Estimate the theoretical post-dispersal recruitment of *Didemnum* larvae from a discrete source population and provide a conceptual model of dispersal and spread in this species.

6. Experimentally investigate the impacts of *Didemnum* biofouling on the commercial culture of New Zealand green-lipped mussels.

1.4.3 Content of thesis chapters

This thesis is presented as a collection of individual chapters (published articles or manuscripts submitted or to be submitted for publication). As each chapter represents a discrete piece of work, there may be some degree of overlap in terms of common background material. A preface is presented at the beginning of each chapter, to highlight the linkages and continuity among chapters. The preface also describes where the work has been submitted or published and, for multi-authored publications, the contribution made by co-authors.

Chapter 2 provides background information on *Didemnum*, including key biological attributes and life-cycle characteristics, current geographic distribution, and management history, providing context for the following chapters. Chapter 3 forms the basis of a guide to experimental protocols developed for the induced spawning and laboratory culture of *Didemnum.* This chapter includes a detailed description of the methodology surrounding optimum spawning procedures, techniques for delaying larval metamorphosis, preferred larval settlement conditions, and feeding regimes for juvenile colonies. The incentive to include this chapter in the thesis stemmed from difficulties in the initial stages of this project, when attempting to spawn *Didemnum* under laboratory conditions for research into the competency period (hence dispersal potential) of the larvae. At the time, no literature was available in this area for *Didemnum* and as such protocols were developed through comprehensive laboratory spawning trials.

The themes for technical chapters 4 to 6 relate to several aspects of the biological characteristics of *Didemnum* and seek to examine the reasons behind

this species' demonstrated invasiveness and success as a fouling organism. These chapters explore the natural progression of this species' life history (reproduction and dispersal mechanisms) as well as the associated impacts; however the findings are applicable in a broader context when using this case study to predict the outcomes of future invasions. This focus reflects the fact that *Didemnum* is recognised as a significant threat to the New Zealand aquaculture industry, yet work on this species has primarily focused on the development of management strategies and eradication attempts in the absence of the biological attributes underpinning the species' success (Coutts and Forrest 2007).

In Chapter 4 the reproductive biology of *Didemnum* is evaluated, using field-based studies to describe the reproductive seasonality of this species (through assessment of both settlement plate counts and colony tissue analysis). At the time of writing, there had been no research on the reproductive patterns of this species in New Zealand although comparative work has been carried out on United States populations (Auker 2006; Osman and Whitlatch 2007; Auker and Oviatt 2008; Valentine et al. 2009). To aid future management decisions, statistical models predicting critical temperature thresholds for the onset and cessation of *Didemnum* recruitment are presented.

Leading on from larval release from the parent population, Chapter 5 evaluates the natural dispersal capacity of *Didemnum* populations. This is achieved through a combination of field experiments measuring larval settlement at a range of depths and distances from established populations, lab based experiments determining the species' larval competency period, and analysis of systematic surveillance records for the Marlborough Sounds region. Predictions of post-dispersal recruitment of *Didemnum* larvae from discrete source populations as well as a conceptual model of dispersal and spread in this species are presented.

Chapter 6 assesses the impacts of *Didemnum* biofouling on commercial culture of the New Zealand green-lipped mussel. This impact is gauged through a field experiment that aimed to establish the effect of this species to the mussel industry across multiple stages of production. Negative impacts of biofouling on shellfish aquaculture have been widely documented, however these reports are primarily observation based and there has been no experimental investigation into these impacts and the associated costs to the New Zealand aquaculture industry.

Finally, the General Discussion in Chapter 7 is used to expand on the main findings of this research, and compare observed patterns within the context of marine invasion ecological theory and pest management principles. The findings of the thesis are discussed more broadly in terms of insights provided into the management of marine pest organisms, and the contribution of scientific knowledge, ultimately providing enhanced information for risk analysis procedures and decision making by managers and industry members alike.

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CHAPTER 2

BACKGROUND TO *DIDEMNUM VEXILLUM* AND MANAGEMENT HISTORY IN NEW ZEALAND

Preface

This chapter provides background information on *Didemnum*, including key biological attributes and life-cycle characteristics, current geographic distribution, and management history, providing context for the following chapters. Note that the reproductive seasonality, natural dispersal potential, and commercial impacts of *Didemnum* are not comprehensively reviewed, as these are separate themes addressed in Chapters 4, 5 and 6, respectively. An overview of the management history for this species in New Zealand is included, since this background information is relevant to the implications of this research, as considered in the General Discussion in Chapter 7.

2.1 Background

Issues surrounding biofouling pest species and the New Zealand aquaculture industry first emerged in the late 1990's with a population explosion of the non-indigenous solitary ascidian *Ciona intestinalis*. This species had been present in New Zealand for several decades but had never demonstrated such a prolific level of population growth before (Forrest et al. 2011). Several bays within the Marlborough Sounds were affected by the population explosion, with the associated increase in species biomass resulting in substantial loss of mussel crops on farms in these areas and cost the mussel industry an estimated NZ$10 million in lost production (Cole 2002; Coutts 2002a). More recently, the 'clubbed tunicate' *Styela clava,* another solitary ascidian, was discovered in Auckland's Viaduct Basin in 2005 (Davis and Davis 2006). This discovery initiated a rapid delimitation survey, and trials of several mitigation and eradication tools (e.g. Coutts and Forrest 2005; Gust et al. 2008), as the species was known to have severely impacted the mussel industry in Prince Edward Island, Canada (Thompson and MacNair 2004; Clarke and Therriault 2007). The tremendous density and abundance of *S. clava* in Prince Edward Island is still regarded as a serious threat to the long-term economic viability of the regions' shellfish industry (Clarke and Therriault 2007). In New Zealand, *S. clava* has subsequently spread to another important mussel growing area in the Coromandel area, and is causing pronounced adverse impacts on cultured mussels and farming operations (McFadden et al. 2007). Together with these solitary ascidians and other species, *Didemnum vexillum* (hereafter referred to as *Didemnum*) forms part of a suite of fouling organisms that potentially threatens shellfish aquaculture in New Zealand.

The efficacy of management of any marine pest species requires a thorough understanding of the biology of that species. However, an extensive understanding of the biological attributes and ecology of the specific pest

species to be managed, as well as the ecosystems or resources at risk, is rarely available. In the case of *Didemnum*, the demonstrated invasiveness of this species on mussel crop lines the Marlborough Sounds resulted in it being perceived as a significant threat to mussel culture in this country. Efforts to manage *Didemnum* are ongoing, although they have been considerably hampered by a lack of fundamental knowledge surrounding the invasion mechanisms and establishment processes of this species. Although present in New Zealand since 2001 there are still considerable knowledge gaps surrounding the key biological attributes and ecology of *Didemnum*, in particular how these relate to its presence in the New Zealand environment. The species' invasiveness in a range of regions globally has led to increased research overseas on *Didemnum*'s taxonomy, ecology, possible impacts and mitigation strategies, and what is known is summarised in this chapter. Together with specific information on *Didemnum*'s management history in New Zealand, this background knowledge provides context for subsequent chapters.

2.2 Biological attributes and life-cycle characteristics

2.2.1 Growth, reproduction and morphology

Colonial ascidians are made up of numerous individual animals, termed zooids, embedded in a tough outer covering or tunic and sharing a circulatory system. *Didemnum* zooids are relatively small, at approximately 1 mm in length, with colonies comprised of thousands of individuals. Each zooid possesses an individual incurrent siphon but shares a common cloacal chamber and excurrent siphon with other zooids in the colony (Kott 2002). Like most colonial ascidians, *Didemnum* colonies are capable of both sexual and asexual reproduction. Embryos are brooded within the tunic of the colony and are believed to take several weeks to fully develop into 'tadpole' larvae about 1.4 mm in length

(Lambert 2009). Colonies generally mature in late spring, releasing large numbers of non-feeding larvae as sea temperatures increase (Auker and Oviatt 2008; Valentine et al. 2009). These larvae are thought to have a short, free-swimming stage after which they settle on suitable substrate and undergo metamorphosis into a juvenile (Lambert 2005). Ascidian metamorphosis involves a series of steps to transform the motile, non-feeding larva into a sessile, suspension feeding individual (Cloney 1978).

Asexual reproduction takes places by means of budding and is used to expand the size of a colony. In this way colonies are composed of genetically identical individuals, however fusion between colonies will often occur (Smith et al. 2012). In addition, fragments of colonies can readily reattach and grow under suitable environmental conditions, and this may aid in the spread of this species to new locations (Bullard et al. 2007b; Hopkins et al. 2011; Morris and Carman 2012). *Didemnum* colonies have been observed to undergo seasonal regression and regrowth cycles during the colder winter months, with colonies becoming smaller and more two-dimensional in morphology (Valentine et al. 2007a).

As a colonial organism, *Didemnum* can form extensive sheets that overgrow other parts of the colony, as well as other epibionts (Kott 2002). The species' morphology will vary depending on the substrate. In quiet, sheltered waters large colonies will often develop long lobes or tendrils (Figure 2.1a), sometimes over a metre in length (Coutts and Forrest 2007). These tendrils often possess constrictions separating thicker portions at the base of the extension (Kott 2002), which may increase the probability of detachment. Small colonies and those growing on flat horizontal or exposed substrates tend to exhibit an encrusting growth pattern (Figure 2.1b). Colonies can also be a combination of these two morphologies and form encrusting mats with numerous short lobes (Figure 2.1c). *Didemnum* colonies are generally a yellowish cream colour; however, colony colour will often vary among colonies or colony location

(including beige, pale orange and pale pink variations; see Lambert 2009), possibly due to local environmental conditions or available trace elements. Tunic surface patterns include clearly visible aggregations of zooids into groups with dark spicule-free bands present between zooid groups (Kott 2002; Lambert 2009; Figure 2.1d).

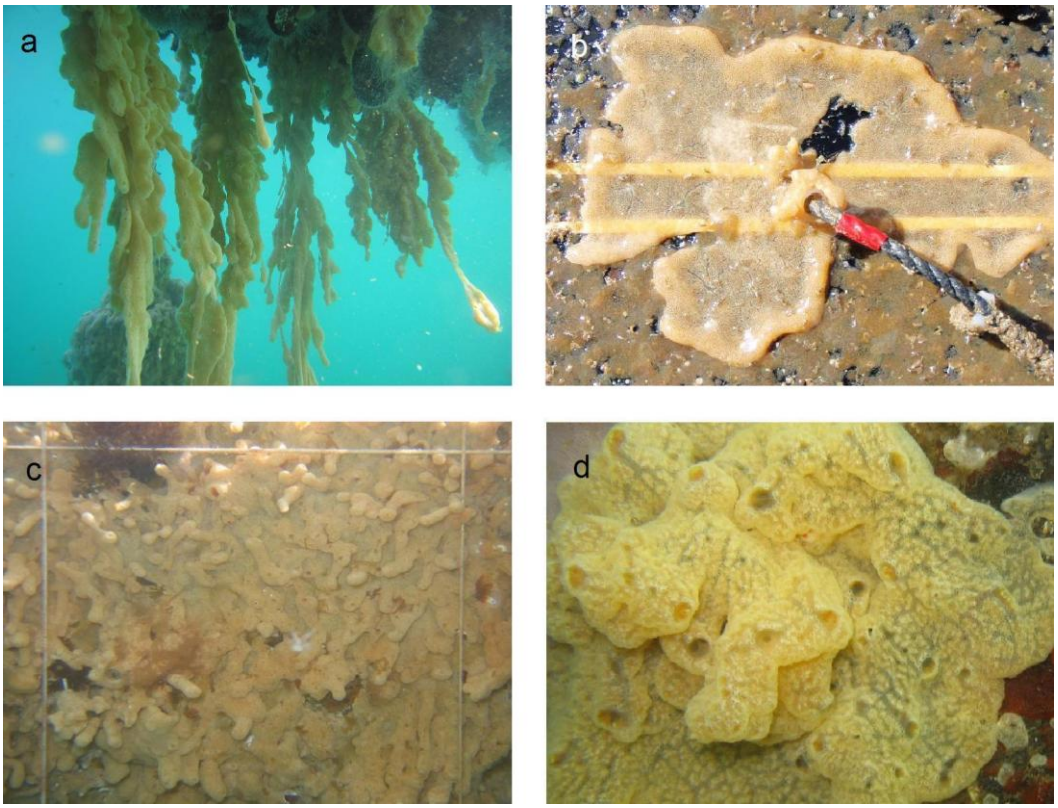

Figure 2.1 Varying morphology of *Didemnum vexillum*: **a.** long tendril-like lobes in sheltered environments; **b.** encrusting colonies growing on flat horizontal surfaces; **c.** encrusting mat with numerous short lobes present; and **d.** tunic surface pattern with aggregations of zooids visible.

2.2.2 Environmental tolerances and habitat preferences

Populations of *Didemnum* have been shown to tolerate a wide range of environmental conditions including considerable variations in temperature, salinity, and water quality. *Didemnum* has a wide thermal tolerance ranging from < 0 to > 24 °C (Bullard et al. 2007a; Valentine et al. 2007b; Cohen 2011), although water temperatures in the upper range have been shown to reduce the growth rate of colonies (McCarthy et al. 2007). The species also appears to tolerate a wide salinity range with colonies reported from brackish systems with salinities levels as low as 26 psu (Osman and Whitlatch 2007; Lambert 2009; Cohen 2011). Under experimental conditions, *Didemnum* colonies have been shown to tolerate short-term but severe declines in salinity, although extended low-salinity stress leads to moderate mortality in this species (Bullard and Whitlatch 2009; Gröner et al. 2011). Similar to most ascidians, *Didemnum* survives best in locations that are protected from strong wave action and those without high levels of sedimentation (Osman and Whitlatch 1999). High levels of suspended particulate matter will often lead ascidians to close their oral siphons to prevent clogging of the siphon and branchial filtering wall, thus ceasing respiration to avoid suffocation (Monniot et al. 1991; Tyree 2001).

This species has been shown to thrive on artificial substrates such as floating docks, pilings and buoys (Bullard et al. 2007a; Coutts and Forrest 2007). *Didemnum* has also been demonstrated to overgrow high value biogenic habitats such as macroalgal beds and seagrass (Carman and Grunden 2010), as well as erect fauna such as horse mussels (*Atrina zelandica*), finger sponges and hydroid trees in New Zealand. Overseas populations have invaded shallow-water natural habitats such as tide pools, estuaries and lagoons (e.g. Valentine et al. 2007a; Mercer et al. 2009; Carman and Grunden 2010), along with a large area of cobble, pebble and gravel habitat on Georges Bank, in north eastern United States. Surveys of this region have indicated a 230 km² area has been

affected, with up to 75 % coverage of the seafloor in the four years since discovery (Valentine et al. 2007b; Lengyel et al. 2009; Morris et al. 2009). In contrast, *Didemnum* colonies in New Zealand do not appear to have colonised natural habitats (with the exception of the upright substrata described above), and have demonstrated poor survival when transplanted to the seabed (e.g. Hopkins et al. 2011). Survivorship of transplanted colonies is likely to be affected by a number of factors; with survival shown to be higher in areas with low sedimentation rates, as well as where predation was excluded (Hopkins et al. 2011).

2.2.3 Feeding preferences and predation

Nearly all ascidians are ciliary-mucus filter feeders, consuming primarily phytoplankton, suspended particulate matter, diatoms and suspended bacteria (Millar 1971; Monniot et al. 1991; Lambert 2005). Colonial ascidians such as *Didemnum* utilise very small particulate matter, primarily in the 0.5-2 µm range (Coma et al. 2001; Bone et al. 2003), although they do ingest larger particles including their own gametes (Young 1988; Lambert 2005). The filtering capacity of colonial ascidians is difficult to determine and has been shown to vary; estimates for other didemnid species have ranged from 2-3.6 $L\ h^{-1}\ g_{dw}^{-1}$ (Koike and Suzuki 1996). As such, the high filtration rate of large aggregations of ascidians can have a dramatic effect on available plankton and suspended organic matter at local scales (reviewed by Riisgård and Larsen 2000).

Few predators have been reported for *Didemnum*, although there have been suggestions of predation by sea stars, chitons, and green and red sea urchins (Bullard et al. 2007a; Valentine et al. 2007b; Epelbaum et al. 2009). There have been reports of predation of this species by the periwinkle *Littorina littorea*; however this was predominantly on unhealthy and dying colony tissue (Carman et al. 2009). Although many didemnid ascidian species have been shown to

possess cytotoxic organic compounds which may reduce predation (e.g. Vervoort et al. 1998), recent analysis determined that *Didemnum* does not contain potent anti-predator secondary metabolites (Lambert 2009). Instead, it is believed that generalist fish predators are deterred by the highly acidic tunic (pH 2-3) (Bullard et al. 2007a). The newly metamorphosed juveniles are likely to be at the most risk, on the basis that a number of studies show high levels of predation on recruits of other colonial ascidian species (Osman and Whitlatch 2004; Whitlatch and Osman 2009).

2.3 Geographic distribution

2.3.1 Global distribution

The global recorded distribution of *Didemnum* at present includes the northeast and west coasts of the United States (including southern Alaska), British Columbia, Ireland, Wales, Scotland, England, northern France, the Netherlands, Japan and New Zealand (Griffith et al. 2009; Lambert 2009; Bishop et al. 2010; Beveridge et al. 2011; Cohen et al. 2011). The most recent confirmed new occurrence was of a population in the Lagoon of Venice, in northern Italy, detected during an environmental survey in July 2012 (Tagliapietra et al. 2012). The native range of *Didemnum* has not been unequivocally identified; however, recent genetic analysis suggests the species originated from Japanese waters (Lambert 2009; Stefaniak et al. 2009; Stefaniak et al. 2012). From its native range, it is believed *Didemnum* was translocated to a number of temperate regions worldwide as epifaunal growth on cultured Pacific oysters, *Crassostrea gigas*. This scenario is supported by numerous countries with past *C. gigas* imports now reporting established populations of *Didemnum* (e.g. France, the United States, Canada, and New Zealand; Lambert 2009).

2.3.2 Recorded distribution within New Zealand

Within New Zealand, *Didemnum* has been recorded extensively within the Marlborough Sounds and Nelson regions, with additional isolated populations recorded within Tauranga, Whangamata, Auckland, Wellington and Lyttelton harbours (Figure 2.2). The first recorded New Zealand occurrence of *Didemnum* was in Whangamata Harbour during October 2001, when observed growing on wharf piles and mooring lines throughout the port area (Kott 2002). Two months later, during a routine biosecurity survey of Shakespeare Bay, near Picton, it was discovered smothering the hull of a heavily fouled barge which had previously been moored in Whangamata Harbour (Coutts 2002a). Following its initial incursion in Shakespeare Bay, *Didemnum* was translocated to a number of bays within the Marlborough Sounds region (Pannell 2008; see Figure 2.3). This regional spread was primarily through the transfer of infected mussel seed-stock and equipment, as well as the movement of vessels within the region (Coutts and Forrest 2007).

The formal designation for *Didemnum* in New Zealand is 'cryptogenic', a term that describes a species whose geographic origin (i.e. whether native or non-indigenous) is uncertain. Although now widely regarded as an invasive species (Lambert 2009), *Didemnum* was never formally listed as an 'unwanted organism' under the Biosecurity Act 1993, although this has not prevented central government from providing assistance to the Marlborough Sounds based aquaculture industry and local authority group with management efforts (see Section 2.5).

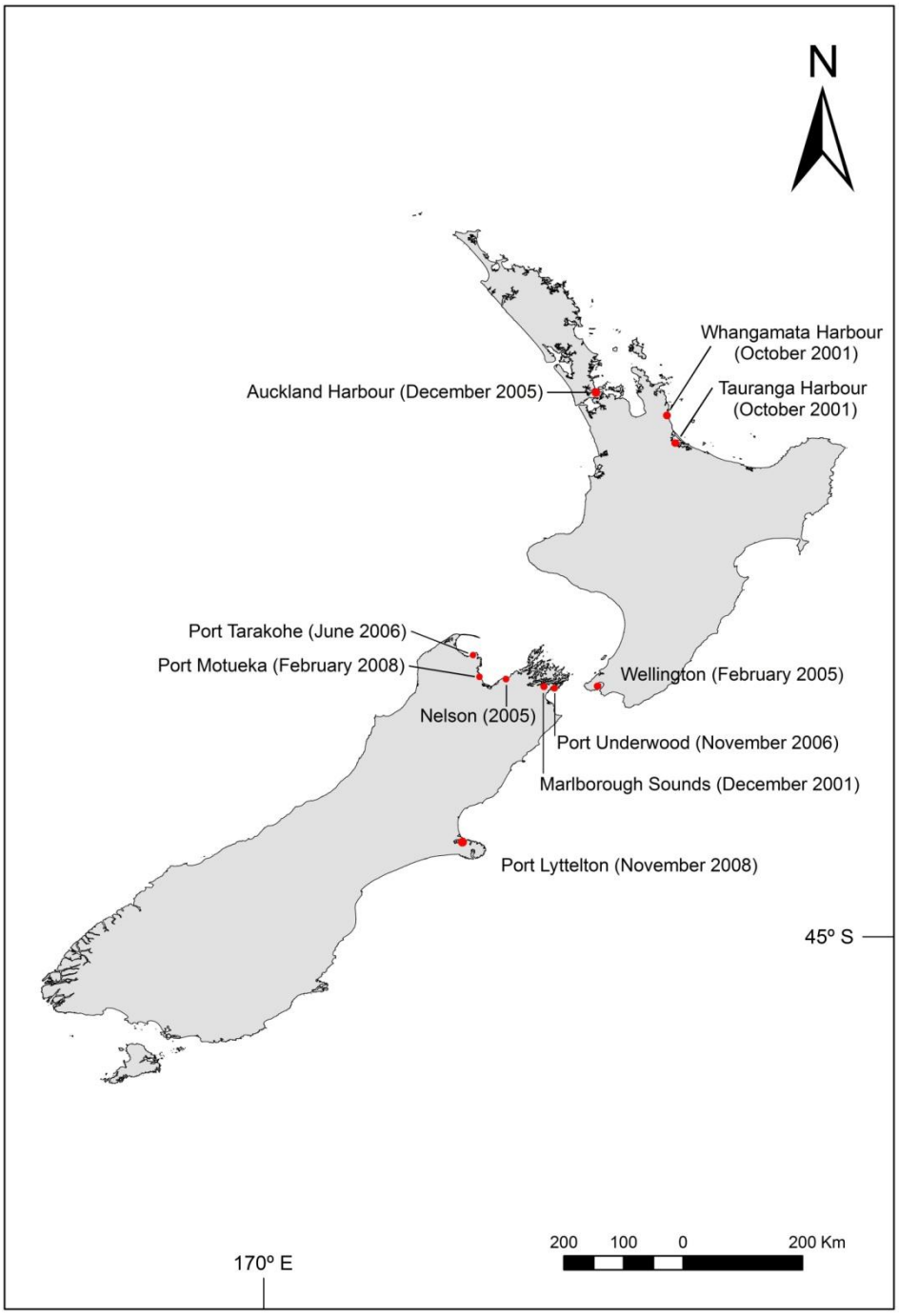

Figure 2.2 Regional distribution of *Didemnum vexillum* in New Zealand. The date *Didemnum* populations are believed to have been first recorded in each location is indicated (A. Pannell, pers. comm.; Pannell and Coutts 2007; Ansell and Coates 2008).

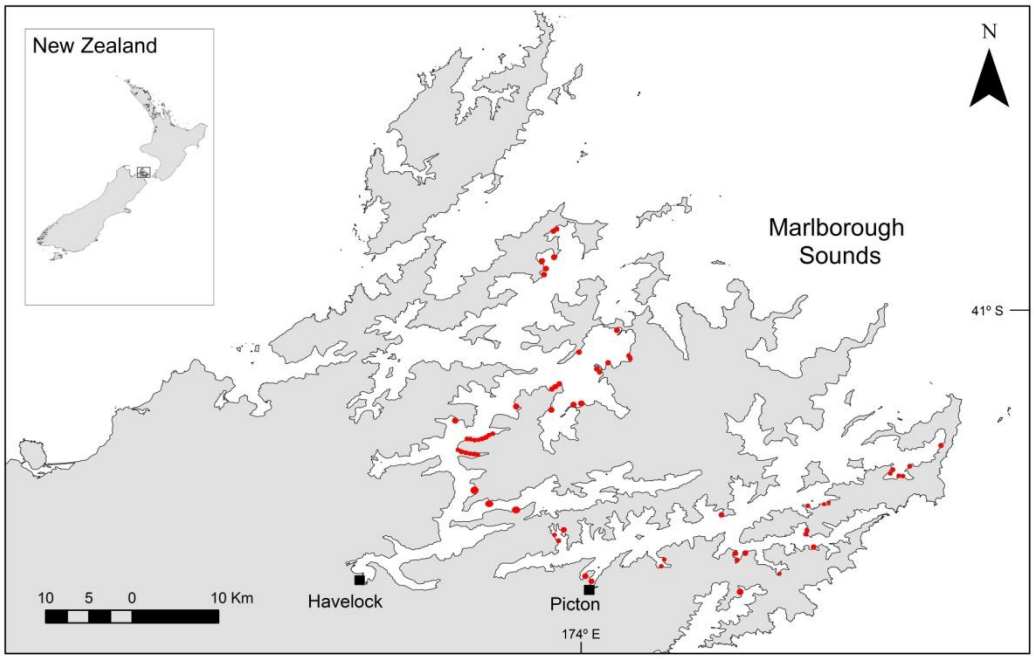

Figure 2.3 Distribution of *Didemnum vexillum* within the Marlborough Sounds region. Population locations were determined on the basis on a regional survey, conducted by a regional working group formed to manage the species, carried out in early 2008 (Pannell 2008). Additional populations are likely to have become established in the five years following.

2.4 *Didemnum vexillum* and the New Zealand aquaculture industry

The presence of *Didemnum* in the Marlborough Sounds poses a considerable threat to the New Zealand aquaculture industry. The Marlborough Sounds region has the largest number of mussel farms in New Zealand, currently encompassing 2,800 hectares, and accounting for approximately 70 % of total New Zealand green-lipped mussel (*Perna canaliculus*) production by volume in 2011 (AQNZ 2012). Mussel farms are at particular risk due to the species demonstrated invasiveness on artificial structures (Coutts and Forrest 2007; Auker and Oviatt 2008) and the ascidians' ability to overgrow and smother

mussels on crop lines (Figure 2.4). The increased biomass present also leads to the destabilisation of mussel crops and added pressure on equipment, such as backbone lines and floats, often leading to substantial mitigation and control costs and occasionally resulting in the loss of mussel crops.

Figure 2.4 *Didemnum vexillum* colonies smothering New Zealand green-lipped mussel crop lines in the Marlborough Sounds, New Zealand.

2.5 Management history of *Didemnum vexillum* in New Zealand

Even though initially described as an indigenous species to New Zealand following its discovery (Kott 2002), the New Zealand Mussel Industry Council considered *Didemnum* to be a serious biosecurity risk. Particular industry concern was expressed around potential economic effects if *Didemnum* became widespread in the Marlborough Sounds region, especially because of expected impacts on mussel spat and seed-stock survival (Coutts 2002b). After consideration of a benefit-cost analysis (Sinner and Coutts 2003), the local port company (Port Marlborough New Zealand Limited) and the regional regulatory

agency (Marlborough District Council) instigated an extensive management programme during August 2003, with the aim of eradicating the species from the region (Coutts 2005; Coutts 2006; Coutts and Forrest 2007).

The 2003 management programme consisted of cleaning the infected barge on which the species had been discovered, then towing the barge into deeper water within Cook Strait where it was subsequently scuttled (Coutts 2005). Eradication of *Didemnum* was then attempted within Shakespeare Bay, as *Didemnum* had spread considerably throughout this bay since its first discovery in 2001 (Coutts 2002b; Forrest 2003). Infected moorings and vessels in Shakespeare Bay were slipped, cleaned, antifouled (in the case of vessels) and returned to the water. Infected barges were removed from the water or treated *in situ* through a combination of wrapping in polyethylene sheet and treatment with granulised chlorine. The area of seabed directly beneath the mooring site of the barge was covered in dredge spoil, and the area beneath the wharf covered in geotextile fabric, to smother any *Didemnum* colonies present. The piles at the wharf within Shakespeare Bay were also treated by wrapping in polyethylene sheets. The total cost of this eradication attempt was c. NZ$350K (Coutts 2005; Coutts 2006; Coutts and Forrest 2007).

Although many of the treatment methods utilised showed positive results (e.g. large population reduction), overall the eradication attempt was unsuccessful, hence *Didemnum* again began to proliferate in Shakespeare Bay. Subsequently, anthropogenic transport vectors aided the spread of *Didemnum* from the confines of Shakespeare Bay, in particular the movement of an infected salmon farm sea-cage to East Bay in outer Queen Charlotte Sound (Coutts 2005). In April 2006, *Didemnum* was found to have spread from the affected salmon cages at East Bay to an adjacent mussel farm, with subsequent crop losses reported at this location (Pannell 2008). This occurrence renewed concerns about the potential impacts on the local mussel industry and prompted the establishment

of a *Didemnum* Working Group (DWG), representing various stakeholders, to manage the threat of *Didemnum* to mussel farming interests within the Marlborough Sounds region.

The DWG instigated a further eradication and control programme of *Didemnum* throughout the top of the South Island, supported by Marlborough District Council and MAF Biosecurity New Zealand (Ansell and Coates 2008). A sustained regional management programme was implemented over 2006 to 2008, including attempts to eradicate established populations as well as control high risk spread vectors such as recreational vessels (see Pannell and Coutts 2007). Simultaneous with these management efforts, the research described in this thesis was initiated, being driven by the need for biological knowledge to inform and support the management programme. During the course of the research the second management programme was terminated (in July 2008) due to insufficient funding and a perception that *Didemnum* was spreading beyond practical control (Pannell 2008). At that point over NZ$1 million had been spent on *Didemnum* management within the region (Ansell and Coates 2008). Despite the termination of formal eradication efforts, there nonetheless remains interest in reducing the rate of regional-scale spread, and protecting specific high value areas that are perceived to be at risk (e.g. bays used for mussel industry spat holding) (Pannell 2008).

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CHAPTER 3

INDUCED SPAWNING AND CULTURE TECHNIQUES FOR THE INVASIVE ASCIDIAN *DIDEMNUM VEXILLUM*

Preface

This chapter forms the basis of a guide to experimental protocols developed for the induced spawning and laboratory culture of *Didemnum*. The development of these procedures stemmed from difficulties in the initial stages of this project, when attempting to spawn *Didemnum* under laboratory conditions to carry out experiments needed for the research presented in Chapter 5. At the time, no literature was available in this area for *Didemnum* and as such protocols were developed through comprehensive laboratory spawning trials.

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Author contributions:

The author designed the experiments, performed the laboratory trials, and prepared the manuscript. My co-author and thesis supervisor assisted with experimental design and reviewed an earlier version of the manuscript.

3.1 Abstract

The colonial ascidian *Didemnum vexillum* (hereafter referred to as *Didemnum*) has become relatively widespread in New Zealand since its initial discovery in 2001. Despite the potential economic and ecological impacts of *Didemnum*, there are still considerable knowledge gaps surrounding its key biological attributes. The ability to obtain larvae and culture colonies in the laboratory is crucial to research into larval longevity and dispersal potential, and the factors affecting colony survivorship and growth. The following paper presents methods for spawning and culture of *Didemnum* under laboratory conditions. A 'light shocking without cycles' technique was used to stimulate larval release in adult colonies, with > 500 larvae being produced from ~ 100 g of tissue at the peak of the reproductive season. Following release, the larvae were allowed to metamorphose and the juveniles were cultured under controlled conditions for four weeks. Recruit survival during the four weeks of culture was > 85 % with the majority having formed small colonies of 4 to 6 zooids with a dense cover of white spicules throughout the tunic. The most effective laboratory spawning conditions are described with respect to light and temperature. The ability to obtain *Didemnum* larvae on demand will enable increased research into several aspects of this species' reproductive biology and ecology.

3.2 Introduction

The ongoing human-mediated spread of non-indigenous ascidian species is causing growing concern globally (Lambert 2001; Carver et al. 2003; G. Lambert 2005; Le Blanc et al. 2007). In particular, ascidians pose a significant threat for aquaculture industries, as they are often strong spatial competitors that are able to reach a very high density or biomass in relatively short time-frames (Stachowicz et al. 2002; Blum et al. 2007; Arsenault et al. 2009). The potential for adverse effects on natural ecosystems has also been documented for some species, including the colonial ascidian *Didemnum vexillum* (Kott, 2002) (Valentine et al. 2007; Lengyel et al. 2009). Despite the potential impacts of *Didemnum*, there are still considerable knowledge gaps surrounding its key biological attributes, information that underpins successful management. In particular, research into the factors affecting colony survivorship, growth and the potential spread of this species is still relatively scarce (but see Daniel and Therriault 2007). For other ascidian species, a range of abiotic and biotic factors that affect both the larval and post-settlement life-stages have been shown to dramatically influence subsequent adult populations (Grosberg 1981; Young and Chia 1984; Stoner 1990; Osman and Whitlatch 1995, 1996, 2004; reviewed by Bates 2005 and C.C. Lambert 2005). Hence, in order to better understand the factors controlling the distribution and persistence of *Didemnum*, increased research on the early life-stages (i.e., larvae and newly settled juveniles) is required. For example, research on larval longevity will aid our understanding of ascidian dispersal potential, as this is heavily dependent on the initial planktonic phase of the life history. Furthermore, knowledge of the environmental conditions that favour ascidian larvae and juveniles may be applied when predicting areas susceptible to new invasions or range expansions (Epelbaum et al. 2009a).

The reproductive biology of many colonial ascidians has been well-documented (Millar 1971; Harvell and Grosberg 1988; Svane and Young 1989; C.C. Lambert

2005; G. Lambert 2005). Colonies are made up of morphologically identical individuals, termed zooids, which are enclosed in a common tunic and share a circulatory system. Colonies undergo both asexual and sexual reproduction. Colonial ascidian embryos are brooded within the colony, and develop for one to several weeks until they are mature and tadpole larvae are released. Brooding in *Didemnum* occurs within the tunic of the colony (Figure 3.1) and the embryos are believed to take several weeks to fully develop into free-swimming larvae about 1.4 mm in length (Lambert 2009). Previous research with other colonial ascidian species indicates that colonies release larvae in response to light stimulation, often spawning at dawn under natural conditions (Whittingham 1966; Lambert and Brandt 1967; Watanabe and Lambert 1973; West and Lambert 1976; Grosberg 1988; Svane and Havenhand 1993; Bingham 1997; Forward et al. 2000).

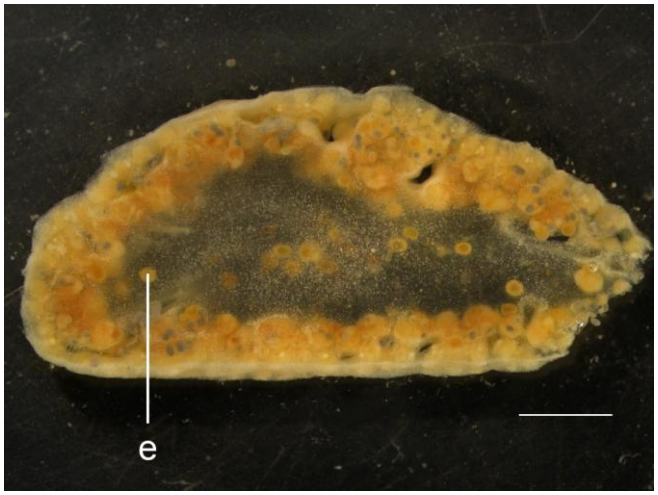

Figure 3.1 Cross section of a *Didemnum vexillum* colony. Numerous individual zooids are visible directly beneath the outer layer of the tunic, and several brooded embryos are visible in the central region between these layers. The location of one such embryo (e) is indicated. Scale bar 2 mm.

Several studies have documented laboratory procedures to induce spawning and to culture ascidians (Berrill 1937; Grave 1937; Costello and Henley 1971; Cloney 1987); however, there has been no assessment of these methods for *Didemnum*. Controlled research on the dispersal and growth of this species has been hampered by difficulties in obtaining sufficient larvae on demand. Previous work on colonial ascidians has shown that the time of larval release can be manipulated through maintaining the colonies in complete darkness for several hours prior to light exposure (termed the dark adaptation period) (Costello and Henley 1971; Watanabe and Lambert 1973; Svane and Young 1989; Forward et al. 2000). It has also been suggested that increasing this dark adaptation period will result in a shorter duration between light exposure and larval release (termed the latency period), as well as increasing the number of larvae that are released (Watanabe and Lambert 1973). The present study documents procedures that were recently developed to successfully induce spawning in *Didemnum* colonies, as well as techniques for the successful settlement and metamorphosis of the larvae, and culture of the juvenile recruits. A range of methods were assessed over the course of this study, with those found to be the most successful for this species presented here.

3.3 Collection and maintenance of laboratory colonies

The *Didemnum* colonies used during this study were collected from beneath a floating pontoon in Port Nelson, New Zealand, between December 2008 and June 2009. Small (< 20 cm^2 area, ~ 30 g) lobe-shaped colonies were found to be most suitable for the trials as they generally had less detritus associated with them. Also, as the larvae are brooded within the tunic beneath the zooids in *Didemnum* species (Lambert and Lambert 2010), damage to these larvae is less likely to occur when using lobe or tendril-shaped specimens than from removing encrusting or two-dimensional colonies from the substratum. Following

collection, the colonies were immediately transported to the nearby laboratory in 2 L plastic containers filled with natural seawater. Upon arrival at the laboratory, the colonies were rinsed in filtered seawater (0.35 µm) and any debris or associated epibionts removed. The colonies collected were used immediately for induced spawning trials, following the procedures outlined below. The initiation of spawning trials as soon as possible after field collection was vital, as earlier attempts to induce larval release in colonies collected from distant locations (several hours from the laboratory) had consistently failed.

3.4 Induced spawning of adult colonies

3.4.1 Dark adaptation period

The *Didemnum* larvae produced in this study were obtained using a 'light shocking' technique (Bullard and Whitlatch 2004) that stimulates and enables control of the time of larval release through the manipulation of a dark adaptation period. The colonies need to be maintained in constant darkness for a duration that is long enough to induce sufficient larval release, but not so long as to lead to deterioration of colony health. Based on multiple observations of colony health and spawning success in relation to dark adaptation, a period of 48 hours is recommended for this species. To start this dark adaptation period, five colonies were placed into two 50 L black PVC bins (2-3 colonies per bin) with secure lids that excluded light. Each bin was filled with ambient temperature seawater (~ 16 °C, salinity ~ 35 psu, filtered to 0.35 µm and UV treated). The colony fragments were supported by 60 mm² plastic mesh, suspended horizontally to raise them approximately 10 cm off the base of the bin and allow adequate water flow to all zooids within the colony. If placed directly on the base of the bin the underside of the colony did not receive adequate water flow and the health of the tissue deteriorated. The colonies often grew around, and

naturally attached to, the mesh during the time held in the bins (Figure 3.2), suggesting they were not overly stressed during this period. During the dark adaptation phase each bin contained a single air stone that provided a constant stream. The water temperature was maintained between 18 and 20 °C. Before the lids were sealed each bin had 50 ml of concentrated *Isochrysis* sp. algal solution added to the filtered seawater to provide a food source for the colonies (1.5×10^7 cells.L^{-1} algal concentrations in the bins).

3.4.2 Light exposure to induce larval release

Following the dark adaptation period the colonies were removed from the bins and suspended individually within five 1 L glass beakers of filtered (0.35 µm) seawater. If the colonies had attached themselves to the plastic mesh, the mesh was cut adjacent to the tissue to prevent damage to the zooids (Figure 3.2). The beakers were placed in an area receiving natural sunlight and were also exposed to bright artificial light, to stimulate larval release (Cloney 1987). The artificial light was provided by two standard lamps, each containing a single Osram 18W warm-white fluorescent bulb (producing 1200 lumen), positioned 20 cm from the beakers. There was no need for aeration of the beakers. The water temperature increased due to the heat produced by the lights, although this was kept below 22 °C by placing the beakers in a cold water bath when necessary. The colonies are likely to become stressed at higher temperatures as growth of this species has been shown to be adversely affected by water temperatures above ~ 23 °C (McCarthy et al. 2007).

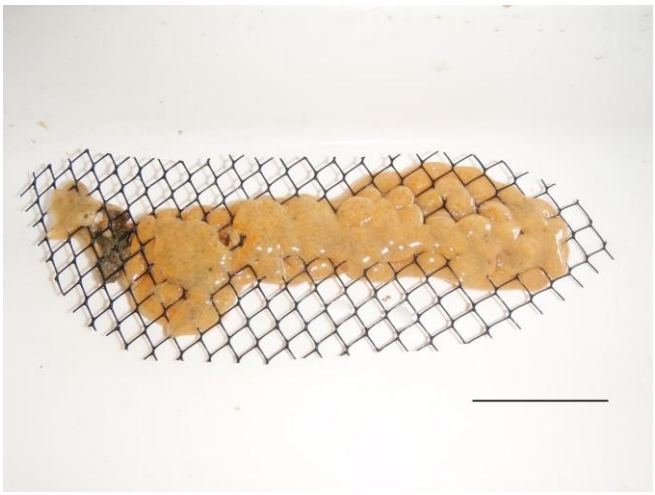

Figure 3.2 *Didemnum vexillum* colony overgrowing plastic mesh netting, following containment in the laboratory for 48 hours. Scale bar 3 cm.

3.4.3 Patterns of larval release

Larvae appeared to be released from the common cloacal apertures that were spread across the surface of the tunic. The larvae were easily visible and often aggregated at the sides of the beakers nearest to the light source, as they are positively phototactic at this stage (Grave 1937). When the water was maintained between 20 and 22 °C, the first larvae were consistently released within 3 to 4 hours following initial light exposure; this latency period was the shortest recorded in all trials. The frequency of larval release was initially slow but increased steadily over the following hour until release was at intervals of approximately 10-20 seconds. The colonies continued to release larvae for approximately 5 hours, although the frequency decreased considerably after the initial couple of hours.

3.4.4 Patterns of larval settlement

As the *Didemnum* larvae were visible to the naked eye, they could be collected using a standard pipette, after which they could be examined directly or allowed to settle onto a hard substratum and metamorphose into juveniles. Pilot studies suggested less settlement occurred when larvae were exposed to well-lit or completely dark environments, supporting the recommendation for shaded conditions to facilitate metamorphosis in ascidian larvae (Lambert and Lambert 2010). The duration of swimming activity and timing of larval settlement varied considerably; some larvae only swam for a few minutes while others took several hours to initiate the metamorphosis process. In a study examining larval settlement rates of 193 larvae, 56.0 % (on average) had successfully settled onto the substratum and metamorphosed into juveniles 24 hours after release from the colony (Figure 3.3). On average, 6.1 % of the larvae examined were recorded actively swimming at this point. When assessed again a further 24 hours later, a small proportion (~ 18 %) of these larvae had subsequently metamorphosed into juveniles, although most had become immobile and unresponsive to light or tactile stimuli. Consequently, to ensure sufficient time for completion of all metamorphosis events, larvae are best left undisturbed for at least 48 hours after release from the parent colony.

Even under ideal conditions, a consistent 10-15 % of the larvae metamorphosed at the water surface and are subsequently suspended, inverted, within this layer (e.g., 14.4 % in Figure 3.3). This phenomenon has been found in many ascidian species, and may be normal for a proportion of ascidian larvae (Millar 1971). These recruits, and others that have attached to non-target substrata, can be carefully removed and repositioned on more suitable substrata if desired. This method has been previously used to position newly settled juveniles of other colonial ascidian species in precise locations (Berrill 1937; Boyd et al. 1986; Epelbaum et al. 2009b).

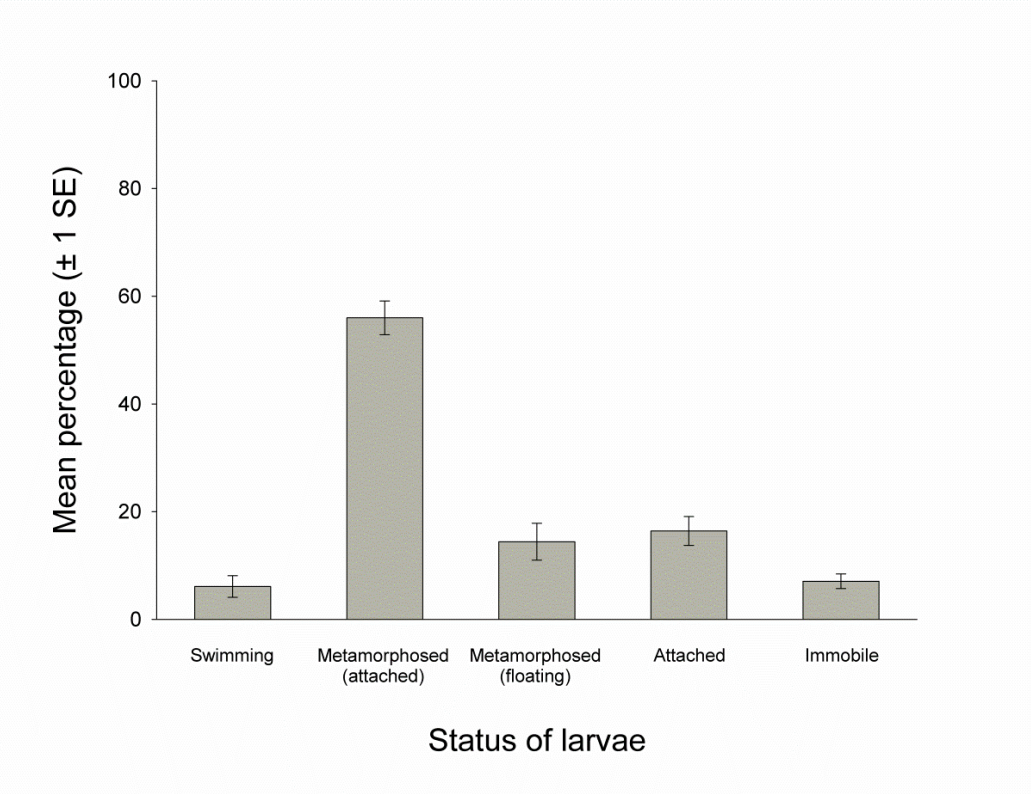

Figure 3.3 Status of *Didemnum vexillum* larvae, 24 hours after release from the parent colony. Larvae were randomly allocated across 10 tissue culture plates (total larvae = 193, sourced from five separate colonies) and classified as either: swimming; attached to the substrate and metamorphosed; metamorphosed within the water column (floating); attached to the substrate but still a larva; or immobile.

3.4.5 Techniques to delay metamorphosis

Research into the competency period and energetic reserves of ascidian larvae require techniques to delay metamorphosis. A range of methods were investigated to achieve this delay, including the use of aeration, mechanical agitation, and varying light regimes. The most successful delay method was found to be a combination of exposure to a white surface (the larvae were in

transparent containers and placed on the white surface) and continuous bright light. Once exposed to this regime the larvae became dormant and remained inactive for the period of light exposure, as also described for the colonial ascidian *Diplosoma listerianum* (Marshall et al. 2003; Bennett and Marshall 2005). *Didemnum* larvae were able to successfully metamorphose into juveniles following artificial delays of up to 36 hours using this method (Fletcher et al. 2013; see Chapter 5).

3.5 Culture of juvenile recruits

3.5.1 Culturing methods

In a separate study, larvae that had successfully metamorphosed into recruits (*n* = 174) were cultured under laboratory conditions for four weeks, with colony survivorship and development documented (Fletcher et al. 2013; see Chapter 5). Recruits were situated within individual wells (6 ml) of sterile 12-well Falcon™ tissue culture plates and were maintained at 18 °C in a 12 : 12 hour light : dark regime. Ascidian recruits quickly develop atrial and branchial siphons, and begin feeding within one to two days (Takeuchi 1980; Epelbaum et al. 2009b). The recruits in the current study were fed an algal solution of *Isochrysis* sp. diluted in filtered seawater (5×10^7 cells.L^{-1}) every second day. The containers were not aerated, as pilot studies had shown replacing the water every second day was sufficient to enable colony growth. Aeration of the culture containers has sometimes been shown to negatively affect feeding efficiency through the suspension of fecal pellets and detritus, leading to a reflex cessation of pumping (Milkman 1967). There was often considerable fecal pellet build up over the two days, so the area around the recruit was gently cleaned with a soft paintbrush to dislodge fecal matter before the water was changed as required.

3.5.2 Description of juvenile colonies

Following settlement and metamorphosis of the tadpole larva, the juvenile recruits were initially transparent, with pale yellow digestive organs often visible within the posterior region. In addition, black 'eyespots' (the ocellus and statolith within the tadpole larva) were often visible near the anterior region of the recruit (Figure 3.4a). Paired yellow-brown coloured lateral organs of the thorax were generally visible two days following settlement, and these subsequently turned white following the production of calcium carbonate spicules from within these structures (as documented in Valentine et al. 2009). The spicules were generally visible throughout the surface of the tunic, four days post-settlement. The production of fecal matter by the recruits over this time indicated that they were successfully feeding (Valentine et al. 2009). Visible contractions in the recruits, occurring during water inhalation and exhalation, were witnessed after approximately one week.

Two weeks after settlement and metamorphosis, the majority of recruits had undergone asexual budding and divided into a two-zooid colony. Each zooid had its own inhalant oral siphon and a shared cloacal aperture out of which the filtered water was exhaled (Figure 3.4b). At the four week assessment, there was > 85 % recruit survival (Fletcher et al. 2013; see Chapter 5), with the majority of these recruits having formed small colonies of four to six zooids having a dense cover of white spicules throughout the tunic. The numbers of zooids observed in these colonies are similar to those found in other laboratory-raised colonial ascidian species (Milkman 1967; Boyd et al. 1986; Epelbaum et al. 2009b).

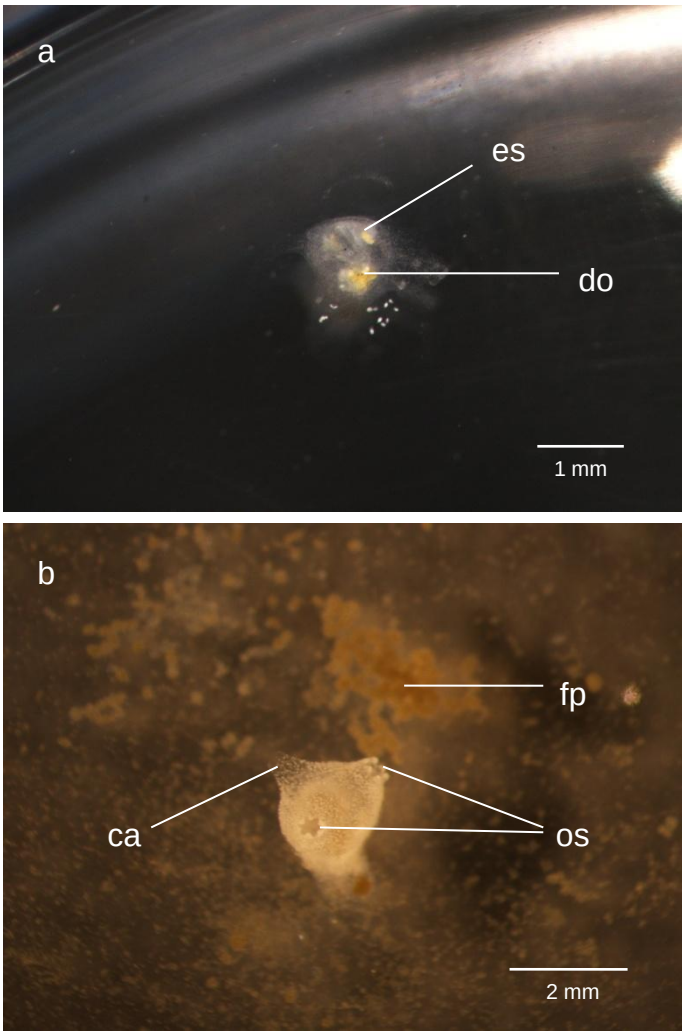

Figure 3.4 a. Newly settled *Didemnum vexillum* recruit. The location of the eyespot (es) in the anterior region and the digestive organs (do) in the posterior region of the zooid are indicated. Spicules have not yet been produced at this early stage. Scale bar 1 mm. **b.** Juvenile *Didemnum* colony two weeks after initial settlement of the larva. The colony has undergone asexual reproduction and consists of two zooids (one has budded from the oozooid). The location of the two oral siphons (os), shared cloacal aperture (ca) and fecal pellets (fp) are indicated. White calcium carbonate spicules are distributed throughout the tunic. Scale bar 2.0 mm. Labels correspond to those used by Valentine et al. (2009).

3.6 Discussion

The techniques described in this paper can be applied to research into several aspects of the reproductive biology and ecology of *Didemnum*. The ability to obtain larvae on demand has already enabled research into the larval competency period of this species, which has indicated some larvae are capable of successful settlement and colony growth following 36 hours of delayed metamorphosis (Fletcher et al. 2013; see Chapter 5). This information is currently being used to predict the spread of *Didemnum* within the Marlborough Sounds aquaculture region of New Zealand. These techniques can also be applied to determine factors such as the preferred settling time of *Didemnum* larvae and the potential carry-over effects of delayed settlement of the recruits in terms of decreased fitness and growth rates. Similarly, the ability to culture juvenile colonies with precise control of environmental variables and adequate replication of trials enables greater isolation of various factors affecting colony survivorship, growth and reproduction. Using these methods, it is hoped to further investigate the growth rates of *Didemnum* colonies in order to determine the length of time to reproductive maturity, and hence the number of larvae potentially released over a spawning season. Together with information on larval longevity, this knowledge will enable predictions of the potential annual spread of this species by natural dispersal.

Although laboratory culture of juvenile colonies can have several advantages, an awareness of differences between laboratory and natural environments is required. Life-history traits exhibited under laboratory conditions may not accurately reflect those occurring in nature where considerably more factors influence natural populations (e.g., predation and physical disturbance). For example, previous work on the larval duration of ascidians is mainly based on laboratory studies; however, as laboratory culture conditions are inherently different from nature, it has been suggested that larval swimming time may be

over-estimated by experiments conducted in closed systems (Shanks 2009). This phenomenon is also illustrated by the current research with reference to the morphology of the early one and two-zooid *Didemnum* colonies that were quite different from those observed growing on settlement plates in the field. The laboratory-raised colonies were very erect in appearance, while those observed in the field are generally quite flat and dome-shaped (see Valentine et al. 2009 for a description). It is proposed that the laboratory-raised colonies exhibit this morphology as they are growing in static water with no forces to prevent upright growth. Although results of experiments using laboratory-raised colonies should perhaps be interpreted with caution, cultured colonies still provide a valuable means for investigating aspects of a species' biology under controlled and replicated conditions.

3.7 Conclusions and future directions

The methods for induced spawning and culture of *Didemnum* colonies presented in this work were adequate for current research purposes, which required the controlled release of moderate numbers of *Didemnum* larvae in order to determine their competency period and make estimates of the dispersal potential of this species. However, some applications of this method, such as large-scale studies into the interactions between *Didemnum* early life-stages and other species within fouling communities, may call for more larvae than was produced in the present study. Hence, continued efforts to improve and refine methods to induce larval release in this species would be desirable. Similarly, additional research on techniques for maintenance of adult laboratory cultures is essential, as these individuals can be used as brood stock cultures, which will enable long-term experiments year round.

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CHAPTER 4

REPRODUCTIVE SEASONALITY OF THE INVASIVE ASCIDIAN *DIDEMNUM VEXILLUM* AND IMPLICATIONS FOR SHELLFISH AQUACULTURE

Preface

Information on the reproductive seasonality of marine pests is vital as it can be applied to the successful management of these species through appropriate timing of eradication and control attempts, as well as effective risk management for industry practices. This chapter contains results of research into the reproductive patterns of *Didemnum* under New Zealand environmental conditions.

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Author contributions:

The author co-designed the experiments, carried out field and laboratory work, analysed the data and prepared the manuscript. My co-authors assisted with experimental design and aspects of fieldwork, as well as providing assistance with data analysis and feedback on earlier versions of the manuscript.

4.1 Abstract

The global spread of invasive fouling species poses a significant barrier to the development of shellfish aquaculture, which has led to a need to understand the biological characteristics of fouling species that underpin management. One such fouling species, the colonial ascidian *Didemnum vexillum* (hereafter referred to as *Didemnum*), has become a very successful invader in temperate marine communities worldwide, and is proving problematic in a number of aquaculture regions. To evaluate the scope for managing risks to shellfish aquaculture around seasonal reproductive patterns, recruitment patterns and larval development of *Didemnum* in relation to water temperature were assessed over a 20-month period at two locations in central New Zealand. The findings indicated that the reproductive season for *Didemnum* in New Zealand is considerably longer than comparable northern hemisphere populations (at least 9 months of the year compared with 3 to 5 months in the United States). Reproductive patterns were strongly correlated with water temperature, with a 3 month period during the winter months (surface water temperature < 12 °C) when larval recruitment was not detected at the study sites. However, during that period, late-stage larvae were often present in tissue sections, suggesting that the species has the potential to recruit year-round, albeit at very low levels during winter. Information on the duration of the reproductive season as well as critical temperature thresholds for spawning will enable more effective risk management in relation to aquaculture industry practices (e.g. timing of seed-stock deployment), as well as assisting in the wider management of this species.

4.2 Introduction

The global spread of invasive species poses a significant practical and economic barrier to the development of shellfish aquaculture (McKindsey et al. 2007). Biofouling in particular, poses a considerable threat to this industry, as fouling organisms are often strong spatial competitors that are able to reach a very high density or biomass in relatively short time frames (Dealteris et al. 2004; Blum et al. 2007; Vance et al. 2009). Ascidians are among the most prolific and devastating biofoulers to shellfish aquaculture operations globally (Lambert 2007; Valentine et al. 2007b; Ramsay et al. 2008; Daigle and Herbinger 2009; Adams et al. 2011). Impacts caused by ascidian fouling include increased costs of production and processing, as well as negative effects on growth rates and meat yields of cultured species due to competition for space and food resources (reviewed by Fitridge et al. 2012). The proliferation of fouling organisms and their associated impacts on the aquaculture industry has led to an increased demand for tools to mitigate effects, and a need to understand the biological characteristics of fouling species that underpin management.

Knowledge of the seasonal development and recruitment success of marine fouling organisms is of particular importance in formulating effective management strategies (Ramsay et al. 2009). For example, understanding seasonal recruitment variation and its environmental drivers will help to define circumstances (e.g. time periods) when fouling of seed-stock, crop or aquaculture structures may be problematic or, alternatively, when it may be of little consequence for industry operations. The reproductive seasonality of ascidians can vary widely (e.g. Turon 1988, 1992) and is poorly understood for many species (Perez-Portela et al. 2007), but it is apparent that water temperature is a dominant controlling factor (reviewed by Bates 2005; CC. Lambert 2005; G. Lambert 2005). For temperate ascidian species, spawning typically occurs during the summer months (G. Lambert 2005), with a

subsequent decline, and in some instances a temporary halt, in growth and reproduction over the colder months (Coma et al. 2000). By contrast, ascidian populations in warmer waters, such as many tropical regions, have been shown to release gametes continuously year-round (Goodbody 1961; Van Duyl et al. 1981; Stoner 1990).

The reproductive seasonality of the colonial ascidian *Didemnum vexillum* (Kott 2002) is of increasing international interest, as this species has become a very successful invader in temperate marine communities worldwide; its alien range includes both coasts of North America, north-western Europe, the United Kingdom, Ireland and New Zealand (Stefaniak et al. 2012). *Didemnum* colonies are capable of rapid growth and expansion through both sexual and asexual reproduction, and are able to quickly foul large areas of any suitable substratum. In New Zealand, the rapid growth and high biomass of *Didemnum* on subtidal mussel farms can lead to the destabilisation of crops and added pressure on equipment, which has in some instances led to substantial management costs (e.g. Coutts and Forrest 2007; Pannell and Coutts 2007). Understanding the species' reproductive seasonality and recruitment patterns, and the utility of seawater temperature as an indicator of recruitment potential, is integral to such management efforts.

The influence of seawater temperature on reproductive patterns in *Didemnum* has previously been inferred from observations made in a number of studies from both the northeast and west coasts of the United States. The length of the reproductive season has been shown to vary, from 3.5 to 5 months, depending on local conditions (Auker 2006; Osman and Whitlatch 2007; Auker and Oviatt 2008; Valentine et al. 2009; Sorte and Stachowicz 2011). *Didemnum* embryos are brooded within the tunic of the colony and are believed to take several weeks to fully develop into 'tadpole' larvae about 1.4 mm in length (Lambert 2009). Once mature, larvae are released from the parent colony via shared

excurrent siphons (see Kott 2002 for a description of colony morphology). Larval release is believed to be dependent on seawater temperatures achieving a critical threshold, with larval recruitment thought to occur when sea surface temperatures exceed 14 °C (Valentine et al. 2009). Recruitment of *Didemnum* is believed to be restricted by very low water temperatures in some areas, with colonies generally undergoing periods of degeneration during colder winter months (Valentine et al. 2007a). Despite progress towards understanding the importance of temperature on reproductive seasonality, it remains unclear whether temperature-related recruitment patterns are consistent among different geographic locations. Moreover, it is unclear whether temperature-related changes within a location are consistent and predictable over time.

The aim of the present study was to better elucidate the reproductive seasonality of *Didemnum* in relation to seasonal changes in seawater temperature. Patterns of field recruitment and tissue reproductive state are described, as well as the development of statistical models to predict critical temperature thresholds for the onset and cessation of *Didemnum* recruitment. The relative sensitivity, utility and practicality of directly measuring recruitment in the field compared with inferring recruitment potential based on the reproductive maturity of *Didemnum* tissue samples is also considered. Such knowledge will assist the aquaculture industry, as well as other stakeholders, in monitoring risk and developing appropriate management responses for this species.

4.3 Methods

4.3.1 Recruitment studies

The temporal recruitment patterns of *Didemnum* larvae were investigated in field studies at two sites (Nelson, NN; Ruakaka Bay, RK) over a 20-month period

between 7 November 2007 and 29 July 2009. Sampling at the RK study site was carried out on a large marine farm located in Queen Charlotte Sound (c. 41°13'S, 174°7'E), within New Zealand's Marlborough Sounds region, whereas the NN site was situated approximately 80 km west within the commercial port at Nelson (c. 41°15.4'S, 173°16.6'E) (Figure 4.1).

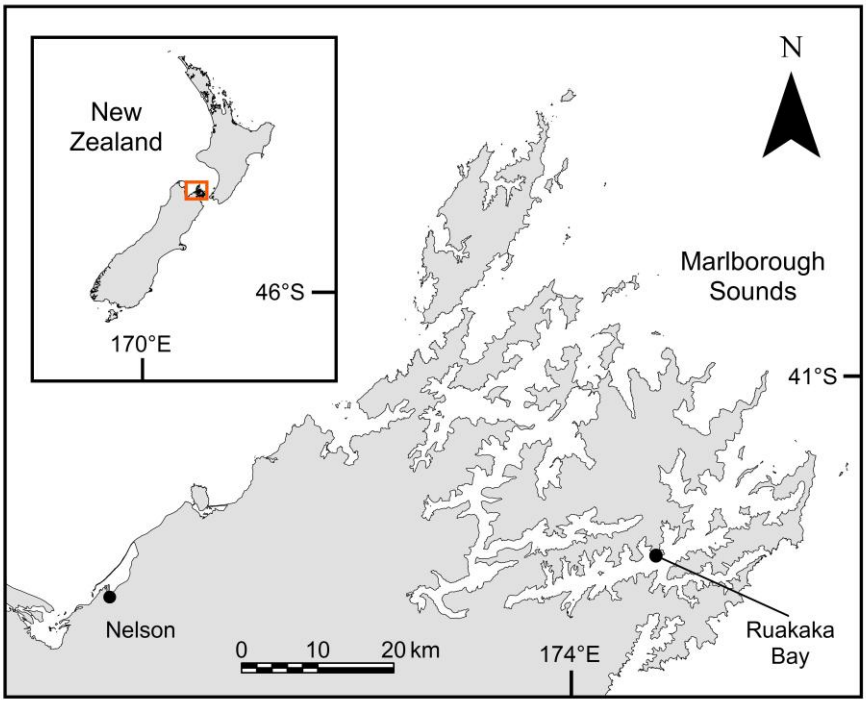

Figure 4.1 Map of the Nelson and Marlborough Sounds regions showing an inset of the location within New Zealand. Filled circles indicate the two study sites used in the *Didemnum vexillum* recruitment study, Nelson (NN) and Ruakaka Bay (RK).

Sites were within or adjacent to important shellfish aquaculture zones and had substantial populations of *Didemnum* present; however, the population at RK was considerably larger (c. 30 tonnes wet weight) than the NN population (c. 2 tonnes wet weight) at the time of sampling. Source population weight

estimates were calculated based on a preliminary survey of *Didemnum* biomass present on the predominant substrata available at each location (e.g. RK: steel pontoon, exterior netting; NN: wharf piles, marina berths) (L. Fletcher, unpubl. data). The RK site was in relatively deep water (~ 30 m depth), while the NN site was in a shallow (~ 5 m depth), predominantly estuarine environment with a large tidal exchange. It was expected that differences between sites in terms of their environmental characteristics and the spatial distribution of *Didemnum* would influence the magnitude of recruitment; however, the study was primarily focused on the seasonal patterns in the occurrence of recruitment in relation to seawater temperature.

To measure *Didemnum* recruitment, five arrays, each with three settlement plates attached, were deployed from floating structures at each site (n = 15 per sampling period), with arrays spaced approximately 5 m apart. The settlement plates were made from roughened black Perspex (20 x 20 cm) and were positioned horizontally within each array and configured to ensure they remained at a constant depth (0.5-1.5 m) with respect to the water surface. The plates were retrieved and replaced weekly from November 2007 through to the beginning of May 2008. Due to logistic constraints, the sampling period was subsequently changed to fortnightly from 7 May 2008 until the end of sampling in July 2009. At the end of each sampling period (week or fortnight), the arrays were retrieved and immediately returned to the laboratory for analysis. Using a dissecting microscope, the numbers of *Didemnum* recruits were counted on the downward-facing surface of each plate, reflecting the orientation where maximum recruitment occurs (Valentine et al. 2009). A 2 cm buffer zone around the edge of each plate was excluded to account for edge effects (Hurlbert 1984); hence data were recorded as counts of individuals recruiting to the central 16 x 16 cm (256 cm^2) of each plate. The term recruitment is used here as a measure of the number of newly settled individuals that survive between settlement and the time a census is taken (Keough and Downes 1982).

4.3.2 Tissue section analysis

At the time of the recruitment study, tissue section analysis of *Didemnum* for different larval developmental life-stages was also conducted. Compared with recruitment studies, tissue analysis is more straightforward and gives immediate results. The goal of this work was to evaluate whether tissue analysis could provide a sensitive indicator of the potential for larval recruitment, which would serve as a useful proxy variable for monitoring purposes. Due to logistic constraints, this study was carried out for the RK site only. From that site, five lobe-shaped *Didemnum* colony tissue samples (< 30 cm^2 area, ~ 50 g) were collected every fortnight over the 20-month sampling period. Lobe-shaped colonies were preferred as they generally had less detritus and epibionts associated with them, and could be sampled without damaging the embryos. As embryos are brooded within the tunic beneath the zooids in *Didemnum* species, removal of encrusting or two-dimensional colonies from the substratum can damage them. Samples were collected from distinct colonies spaced > 5 m apart to ensure independence. Each sample was immediately preserved in fixative (70 % ethanol, 5 % glyoxal) and transported back to the laboratory.

In the laboratory, 10 cross-sections were excised from each sample, with a thickness of 1 mm that ensured the central test region (where the embryos are brooded) remained intact (Figure 4.2a, b). Sections were taken from different parts of the colony to ensure a range of tissue was sampled. In total, 45 sampling dates were analysed with 50 sections examined for each date (i.e. 5 samples each with 10 subsamples per occasion), yielding a total of 2250 cross-sections. Each section was mounted on a glass slide and examined for reproductive condition under a dissecting microscope.

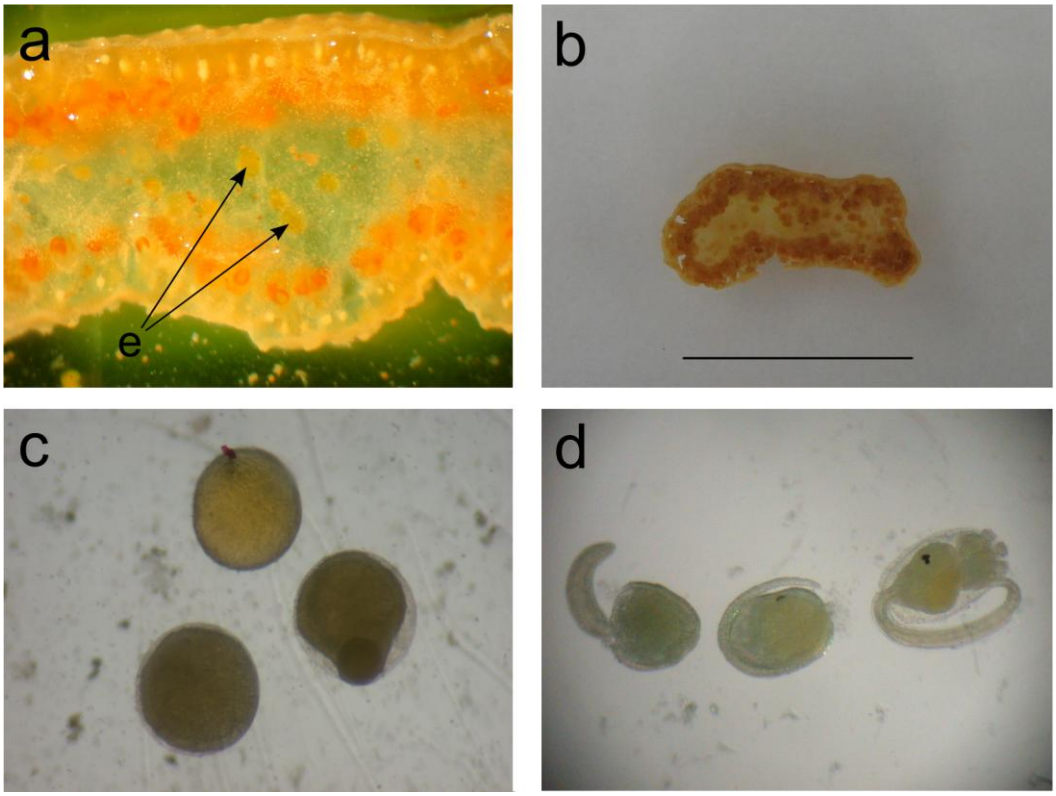

Figure 4.2 *Didemnum vexillum* colony tissue section photographs: **a.** close-up of a tendril cross section highlighting individual zooids surrounding colony perimeter and brooded embryos (e) in the central test region; **b.** illustration tissue section photograph, scale bar = 20 mm; **c.** *Didemnum* eggs; and **d.** development stages of *Didemnum* larvae: early tail bud, late tail bud and mature larvae (from left to right). Photographs a, c and d courtesy of A. Coutts.

All embryos present within the central test region were assigned to one of four reproductive stages (e.g. Figure 4.2c-d): egg; early tail bud (small tail budding off from main body, no eyespot); late tail bud (lengthening of tail, formation of dark eyespot, possible presence of papillae); and mature larvae (three papillae used for attachment to substratum, defined eyespot visible), and counts of each stage recorded. A digital photo was taken of each section with the camera positioned

at a standard distance from the slide, and image analysis software (ImageJ; Abramoff et al. 2004) was used to calculate its total cross-sectional area. The reproductive stage counts were then standardised to 1 cm² of colony tissue, to allow comparison between sampling dates and prevent bias due to differences in tissue section size (and thus capacity to produce and brood embryos). Sections were also scored to one of the following five categories according to the highest reproductive stage present: (1) no embryos present, (2) presence of eggs, (3) presence of early tail bud larvae, (4) presence of late tail bud larvae, and (5) presence of mature larvae. A fortnightly maturity index (MI) was calculated using the formula of Yoshida (1952):

$$MI = \frac{n\,F}{N}$$

where *F* is the reproductive stage (1-5 in *Didemnum*), *n* is the number of sections in stage *F* and *N* is the total number of sections examined.

4.3.3 Environmental data

Water temperature was measured hourly for the duration of the study using a TidbiT v2 Temperature Logger positioned at 1 m depth at each site. Water temperature data are reported as either weekly or fortnightly averages depending on the sampling period involved. Maximum and minimum temperatures during each sampling period, and temperature change between periods were also calculated. In addition, day length data for both sites was obtained from the US Naval Meteorology and Oceanography Command (USNO 2012).

4.3.4 Statistical analyses

The recruitment count data contained a disproportionately high number of zero values (448 of the 1710 observations = 26.2 %), as well as overdispersion (variance higher than the mean) in the non-zero count data. As such, recruitment data at both sites were analysed using a zero-inflated negative binomial (ZINB) regression model, which assumes that the population consists of two different types of observation: zero counts, and non-zero counts, which are modelled using a negative binomial distribution (Zuur et al. 2009). A range of explanatory variables were initially examined (temperature, day length, plate exposure time, Julian day, month, season, year, site), and highly co-linear variables were omitted. The Akaike information criterion (AIC) was used for model selection; the final model was validated through inspection of the residuals, and consisted of recruitment counts as the dependent variable, with temperature, site (NN or RK), and year (Year 1 or Year 2) as predictor variables.

Critical temperatures associated with the onset of *Didemnum* recruitment, as well as the subsequent cessation as water temperatures declined, were also investigated. Data were split into eight sections based on site, year and pre-summer or post-summer phase. The pre-summer phase was represented by the data sub-group from the onset of recruitment to its summer maximum, whereas the post-summer phase extended from this summer peak to the mid-winter cessation of recruitment. The need to analyse these two different phases separately arose from their distinctively different recruitment versus temperature responses. For each site, season and pre- or post-summer combination, either linear or negative binomial regression models were fitted to the data depending on which best described the dataset structure, as determined through AIC and residual analysis. These models were used to calculate the water temperatures at which the predicted recruitment was < 1 (reflecting the presence of a single recruit on one settlement plate). A regression

model was not fitted for the pre-summer phase at NN during Year 1, as recruitment was already well underway when sampling was started at that site.

Differences in the abundances of larval development stages were tested using a distance-based permutational analysis (PERMANOVA, Anderson 2001) based on Bray-Curtis similarity matrices of the square-root transformed data. The experimental design comprised two factors: Year (random with two levels) and Season (with four levels nested in Year), with water temperature included as a covariate. Fortnightly sampling events were assigned to seasons based on the meteorological calendar, and each contained data from three complete months. Each term in the analyses was tested using 9999 random permutations of the appropriate units (Anderson 2001). Similarity percentage analysis (SIMPER, Clarke 1993) was used to identify the percentage contribution of each stage to observed differences between factors.

The fortnightly MI was related to fortnightly average water temperatures using the Pearson product-moment correlation coefficient. Additionally, cross-correlation analyses were used to compare the temporal trend of MI with recruitment levels measured in *Didemnum* over the course of the study. In cross-correlation, two series of data are lagged with respect to one another (time lags were in fortnights), and the usual Pearson correlation coefficient is computed on the transformed series. Correlations at time lag *t* measure relationships of the values of the first series with values of the second series *t* fortnights earlier (negative lags) or later (positive lags). Multivariate analyses were performed using the software PRIMER 6 and PERMANOVA (Clarke and Gorley 2006; Anderson and Gorley 2007). All other analyses were carried out using software package R version 2.13.2 (R Development Core Team 2011), as implemented in the zeroinfl and MASS libraries.

4.4 Results

4.4.1 Environmental data

The average water temperature data showed a regular cyclic annual pattern, although seasonal ranges differed appreciably between the sites (Figure 4.3). Water temperatures at NN showed a wider seasonal range, with temperatures higher in the austral summer and lower over the winter months than at RK. The lowest average temperature at NN was recorded during the last month of the study (16 - 29 July 2009; min. 9.1 °C; Figure 4.3), while the lowest at RK was recorded during the preceding fortnight (2 - 15 July 2009; min. 10.4 °C; Figure 4.3). The highest average water temperatures at both sites were recorded at the beginning of February 2008, with temperatures reaching a maximum of 22.7 °C at NN and 18.7 °C at RK (30 January - 6 February 2008; Figure 4.3). Daylight hours followed a regular cyclic annual pattern; the shortest days were recorded in mid-June each year (9:11 h; Figure 4.3), increasing to a maximum length during late December each year (15:09 h; Figure 4.3). Generally, the change in seawater temperature lagged 1-2 months behind the change in day length.

4.4.2 Recruitment patterns in relation to seawater temperature

A well-defined seasonal pattern of recruitment was evident for *Didemnum* over the course of the study. Recruitment occurred at both sites over two distinct reproductive seasons: November 2007 to July 2008 (Year 1), and October 2008 to July 2009 (Year 2). At NN in Year 1, recruits were detected from commencement of sampling (28 November 2007), when the water temperature had already reached a weekly average of 19.7 °C. In Year 2, recruitment commenced on 5 November 2008 when the average water temperature for the previous week was 15.2 °C (Figure 4.4a). Recruits were first detected in Year 1 at RK on 28 November 2007, when the average water temperature for the preceding week was 15.9 °C. The Year 2 recruitment at RK commenced

approximately a month earlier on 22 October 2008, when the average water temperature for the previous fortnight was 13.6 °C (Figure 4.4b). Recruitment levels at RK were considerably higher than NN over the entire sampling period. The highest recruitment recorded at NN was in Year 2 on 28 January 2009 with an average of 125.1 ± 2.6 (mean ± SE) recruits per plate (Figure 4.4a). The highest recruitment recorded at RK coincided with this peak at NN (28 January 2009) with an average of 431.9 ± 59.3 recruits per plate (Figure 4.4b). At RK, the peak in *Didemnum* recruitment was concurrent with the peak water temperature in both years (Figure 4.4b). In contrast, peak recruitment at NN occurred prior to the peaks in water temperature across both years (three and two weeks prior for Years 1 and 2, respectively; Figure 4.4a).

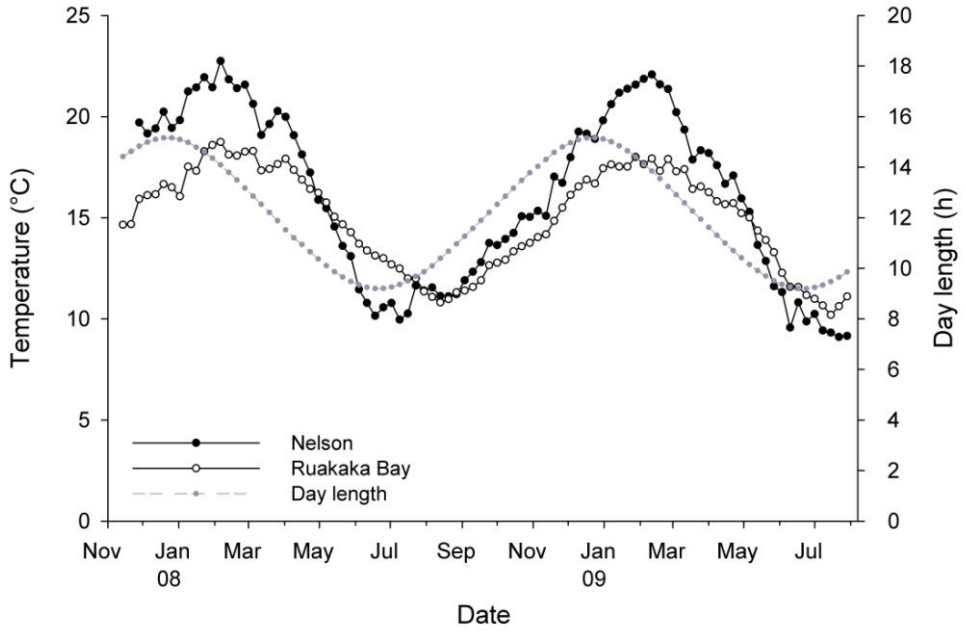

Figure 4.3 Weekly average sea surface temperature profiles from study sites at Nelson and Ruakaka Bay, and recorded day length in Nelson, New Zealand. Filled circles = Nelson; unfilled circles = Ruakaka Bay. Grey dashed line = day length.

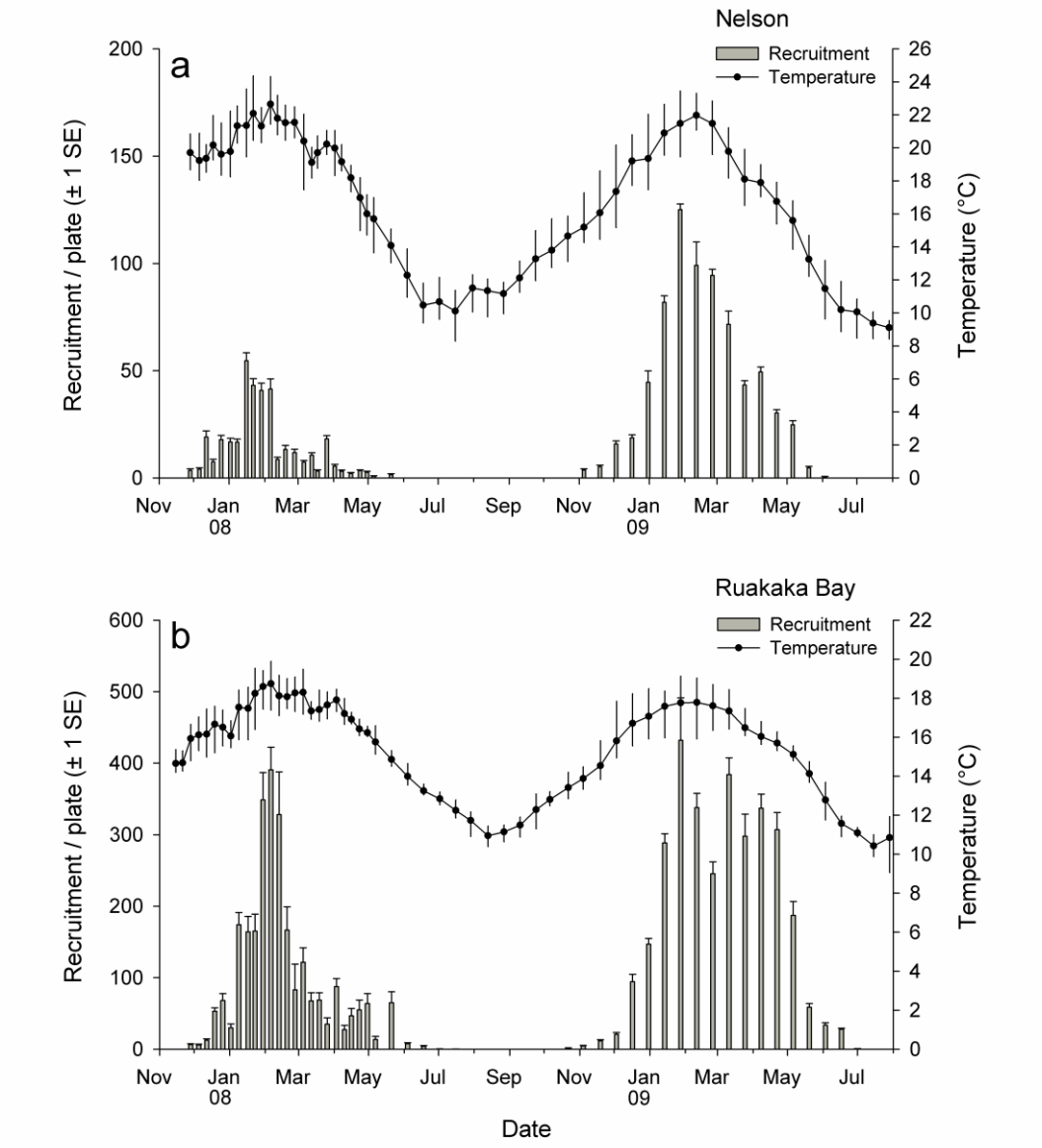

Figure 4.4 Mean number (± SE; *n* = 15, except for Ruakaka Bay on 21 May 2008, which was *n* = 12) of *Didemnum vexillum* recruits per 256 cm² and water temperature profile at both the **a.** Nelson and **b.** Ruakaka Bay sites. Sampling frequency changed from weekly to fortnightly on 21 May 2008. Error bars on the temperature profile represent the range of water temperatures (maximum and minimum values) recorded during that sampling period. Note the differences in scale on the two y-axes.

Higher water temperatures were associated with higher levels of recruitment (Figure 4.5), and the ZINB regression model predicting *Didemnum* recruitment levels from average water temperature, location and reproductive season was highly significant (chi-squared test, $X^2_6 = 2547.7$, $p < 0.001$). All three explanatory variables included were statistically significant in both parts of the model ($p < 0.05$; Table 4.1). Based on the negative binominal model estimates, the expected number of recruits per plate was predicted to be 1.64 ($= \exp^{0.495}$) times higher following a one degree rise in the water temperature, while holding all other variables in the model constant. The expected recruitment at RK was 20.63 ($= \exp^{3.027}$) times higher than the expected recruitment at NN, assuming constant water temperatures and within the same reproductive season (Year). Similarly, the expected recruitment in Year 2 was 4.53 ($= \exp^{1.510}$) times higher than the expected recruitment in Year 1, assuming constant water temperatures and location (Table 4.1).

Table 4.1 Maximum likelihood parameter estimates of the multiple zero-inflated negative binomial (ZINB) regression model fitted to the *Didemnum vexillum* recruitment count data. Reference factor levels and the dispersion parameter are indicated.

	Negative binomial portion			Zero-inflated portion		
Parameter	Estimate (SE)	z value	*p*-value	Estimate (SE)	z value	*p*-value
Intercept	-7.248 (0.289)	-25.063	**< 0.001**	16.783 (1.190)	14.099	**< 0.001**
Temperature	0.495 (0.014)	34.414	**< 0.001**	-1.179 (0.078)	-15.205	**< 0.001**
Site						
Nelson	Reference			Reference		
Ruakaka Bay	3.027 (0.063)	48.410	**< 0.001**	-0.800 (0.326)	-2.450	**0.014**
Year						
Year 1	Reference			Reference		
Year 2	1.510 (0.058)	26.234	**< 0.001**	-1.369 (0.276)	-4.961	**< 0.001**
Log (theta)	0.302 (0.041)	7.335	**< 0.001**			

Significant values ($p < 0.05$) are indicated by bold type.

The regression curves fitted to pre- and post-summer phases accurately modelled the recruitment data at both sites and years, evident from the relatively narrow range (i.e. 95 % confidence interval) of predicted recruitment levels with changes in water temperature (Figure 4.6). Water temperatures where predicted recruitment levels were < 1 were higher when modelling the onset of recruitment, as opposed to the cessation, in all instances. Recruitment was predicted to begin at NN when water temperatures reached 13.4 ± 0.4 °C (Figure 4.6a). This was similar to RK, where the critical thresholds for recruitment were predicted to be 13.9 ± 0.2 °C and 12.8 ± 0.2 °C for Year 1 and Year 2, respectively (Figure 4.6b). By contrast, there was greater site and year variation in the predicted temperature thresholds for the cessation of recruitment at the end of the post-summer phase. The threshold water temperatures for the post-summer phase were 14.9 ± 0.5 °C and 12.0 ± 0.6 °C at NN for Year 1 and Year 2, respectively (Figure 4.6a). Recruitment was predicted to extend to even lower temperature at RK, with threshold water temperatures of 11.5 ± 0.1 °C and 11.1 ± 0.5 °C in Year 1 and Year 2, respectively (Figure 4.6b). Despite the difference between NN and RK, it is evident that within each location, the water temperature threshold in Year 2 was cooler than in Year 1.

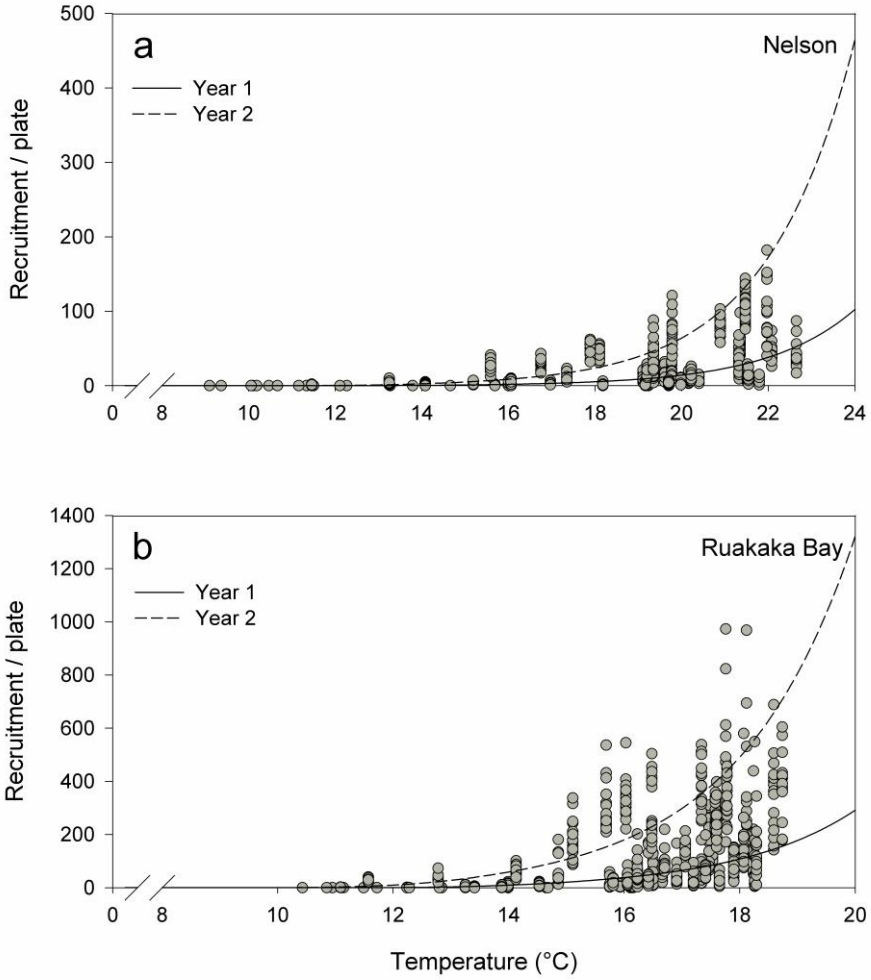

Figure 4.5 Relationship between *Didemnum vexillum* recruitment and water temperature at both the **a.** Nelson and **b.** Ruakaka Bay sites during the 2007-2008 and 2008-2009 reproductive seasons (Year 1 and Year 2, respectively). Note the difference in scale on both the x- and y-axes.

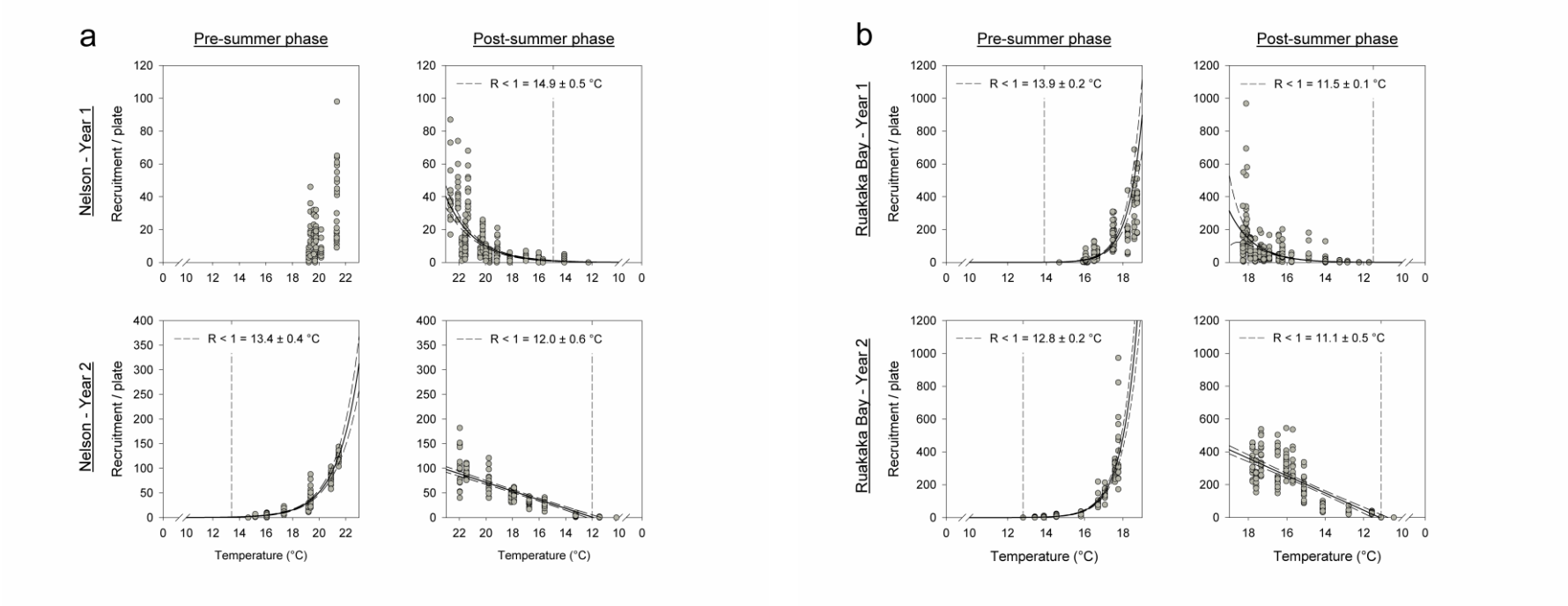

Figure 4.6 Predicted *Didemnum vexillum* recruitment with varying water temperatures at both the **a.** Nelson and **b.** Ruakaka Bay sites. Data were split into eight sections based on site, year and pre-summer or post-summer phase. The pre-summer phase was represented by the data sub-group from the onset of recruitment to its summer maximum, whereas the post-summer phase extended from this summer peak to the mid-winter cessation of recruitment. Solid lines represent regression models fitted to the data to predict recruitment at increasing or decreasing water temperatures. Dashed lines represent 95 % confidence intervals of the recruitment levels and were derived from the standard error of the estimated parameters. Water temperatures where the predicted recruitment is less than 1 (R < 1) are indicated (vertical dashed line) where possible. Note the difference in scale on the y-axis.

4.4.3 Tissue section analyses

A distinct overall pattern of seasonality in tissue reproductive state was shown for all four reproductive stages over the 20 months of study. Abundances of all four larval stages (Figure 4.7a) and MI values (Figure 4.7b) were at their lowest levels during the winter months (July and August) each year, and peaked in summer. Despite this general pattern, the different metrics showed marked variation on shorter time-scales. PERMANOVA revealed highly significant temperature and season (nested in year) effects on the proportion of different larval development stages present within tissue cross-sections ($p < 0.01$; Table 4.2). No significant differences were found between the two years ($p = 0.761$; Table 4.2).

The SIMPER procedure revealed that significant differences between seasons were primarily due to fluctuations in the proportion of eggs (% contribution ranged from 83.6 - 92.1 %). The average abundance of eggs was high during summer (22.16 ± 2.20 SE), autumn (29.88 ± 3.63 SE) and spring (27.87 ± 4.21 SE), with a considerable drop in average abundance over the winter season (7.56 ± 1.88 SE). Accordingly, MI values also exhibited noticeable decreases during late May to early July each year, although there were also distinct short-term declines during the relatively consistent summer peak of recruitment (compare Figure 4.4b and Figure 4.7b). Nonetheless, there was a significant positive correlation between fortnightly MI and water temperature during the course of the study ($r = 0.735$, $df = 43$, $p < 0.001$). Comparing the time course of MI values to that of larval recruitment through cross-correlation analysis revealed a cyclic annual pattern over the course of both variables (Figure 4.8a). The maximum correlation value was found at time lag +1, indicating that peaks in MI values preceded, by two to four weeks, the periods of highest recruitment.

Examination of patterns in the seasonal presence/absence of the different reproductive stages reveals important patterns that are less obvious in the temporal variability of the count data (Figure 4.8b). It is evident that early reproductive stages (eggs, early tail bud, and late tail bud) are present year-round. Mature larvae were detected on 90 % of sampling dates, being sporadically absent during winter months. However, there was no extended period of larval absence. By contrast, recruits were detected on settlement plates at RK on 80 % of sampling events, with a distinct and continuous winter period of some 3 months without any being recorded (Figure 4.8b).

Table 4.2 Results of the permutational ANOVA testing for temporal differences in the abundances of larval development stages at scales of season and year, and with water temperature as a covariate. Analyses based on Bray-Curtis similarity matrices from the square-root transformed data. Each term was tested using 9999 random permutations of appropriate units. Estimates of multivariate variation are given for each temporal scale.

Source	*df*	SS	MS	Pseudo-F	*p* (perm)
Temperature	1	8621.20	8621.20	10.494	**0.002**
Year	1	463.78	463.78	0.503	0.761
Season (Year)	5	4039.00	807.81	4.294	**< 0.001**
Residual	36	6773.20	188.14		
Total	43	19897.00			

Significant values ($p < 0.05$) are indicated by bold type.

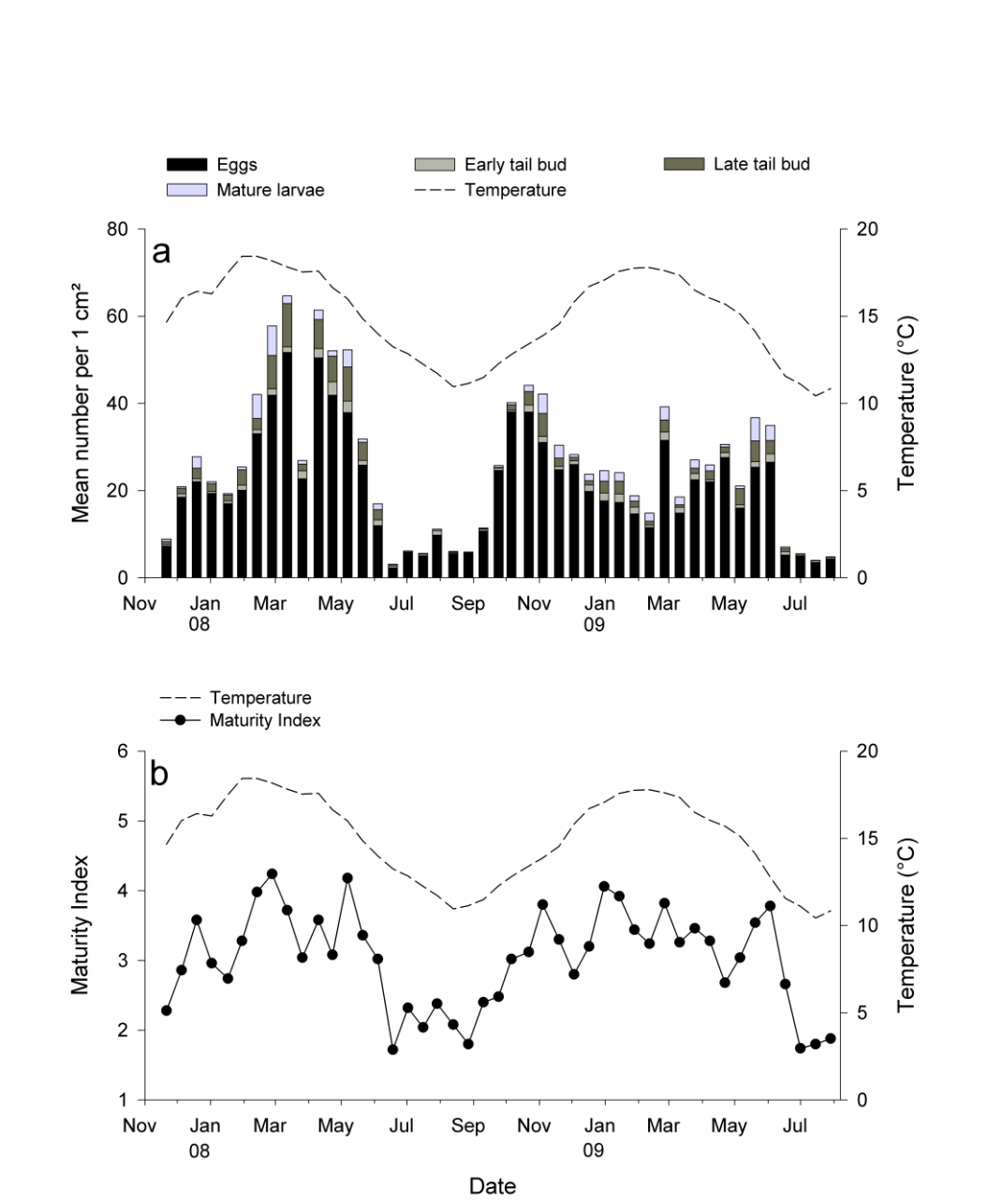

Figure 4.7 a. Reproductive cycle of *Didemnum vexillum* inferred from counts of the four different reproductive stages indicated. Tissue sections were taken from colony samples collected fortnightly from Ruakaka Bay between 21 November 2007 and 29 July 2009 ($n = 50$ per sampling date). **b.** Maturity index of *Didemnum* colony samples collected fortnightly from Ruakaka Bay between 21 November 2007 and 29 July 2009 ($n = 50$ per sampling date). Average water temperature values during the period are indicated.

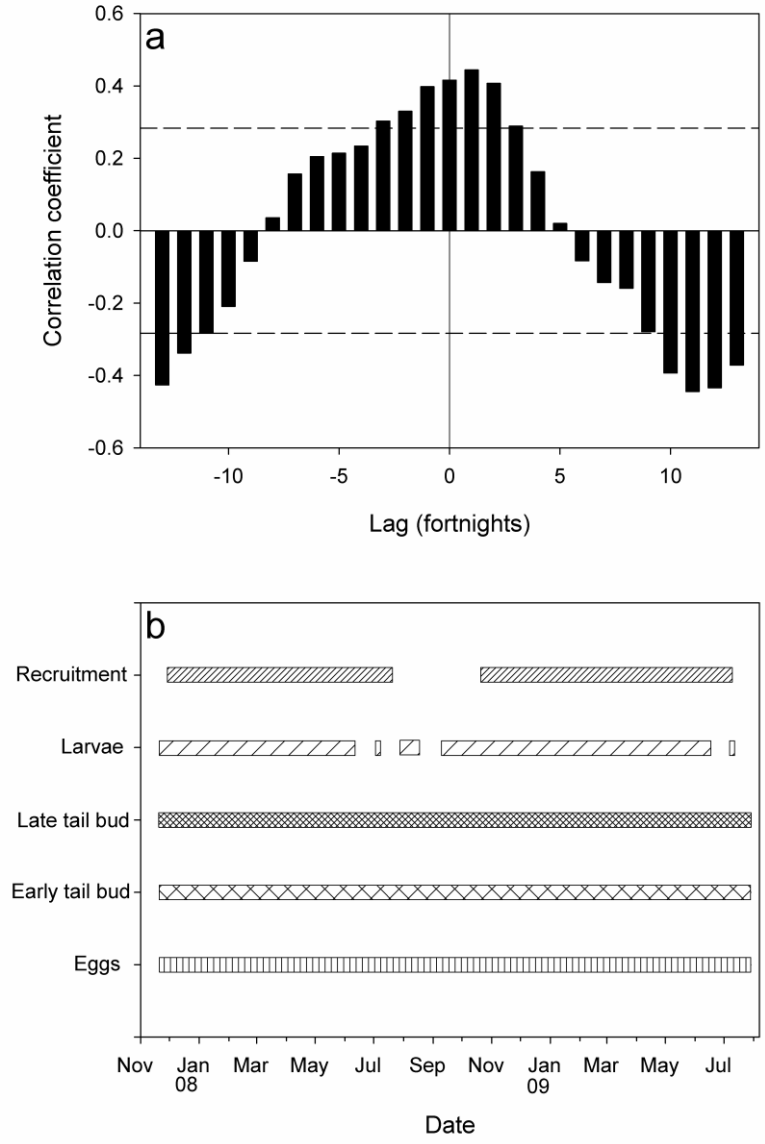

Figure 4.8 a. Results of the cross-correlation analysis of *Didemnum vexillum* maturity index (MI) versus larval recruitment for the corresponding sampling period. Data series were lagged with respect to one another (time lags = fortnights), and the correlation coefficient computed for each transformed series. Dashed lines represent 95 % confidence intervals of the correlation coefficient. **b.** Patterns in the seasonal occurrence of *Didemnum* larval recruitment as well as the presence/absence of the different *Didemnum* reproductive stages.

4.5 Discussion

4.5.1 Temporal variation in recruitment at study sites

The *Didemnum* populations at the study sites showed clear seasonality in their reproductive cycles, with recruitment occurring from late spring right through to mid-winter each year. This pattern of reproductive seasonality and peaks in larval recruitment were strongly correlated with water temperature fluctuations at each location. At RK there was a peak in *Didemnum* recruitment concurrent with the peak of surface water temperature in both years, supporting the previously recognised influence of water temperature on recruitment patterns (Auker 2006; Osman and Whitlatch 2007; Auker and Oviatt 2008; Valentine et al. 2009; Sorte and Stachowicz 2011). The results indicate the recruitment season for *Didemnum* in central New Zealand is at least 9 months of the year, considerably longer than comparable northern hemisphere populations (3-5 months). The short period during winter months when no recruitment was detected corresponded to surface water temperatures of approximately 12 °C or less.

The greater recruitment in Year 2 than Year 1 is most likely a reflection of the change from a weekly to fortnightly sampling at the end of the first reproductive season. The approximate doubling of recruitment at the NN site from Year 1 to 2 is consistent with the doubling of settlement plate exposure time. By contrast, recruitment at RK was fairly consistent between years. It is hypothesised that the extensive fouling community immediately adjacent to the experimental arrays at RK led to the settlement plates becoming ‘saturated’ with recruits during the two week deployment. The marine farm structure at RK contains a considerable surface area of artificial substrata (e.g. steel pontoon, netting, rope) within a relatively condensed area (0.9 hectare), with available surfaces heavily colonised by a range of fouling organisms. In particular, the didemnid

ascidian *Diplosoma listerianum* recruited in very high numbers to the plates at RK, and grew in a sheet-like morphology that often covered much of the available surface. Thus, it is conceivable that the plates became space-limited over the course of each two-week deployment.

4.5.2 Onset and cessation of recruitment as a function of water temperature

The onset of *Didemnum* recruitment in this study was associated with slightly lower temperatures than previously assumed from other studies, with recruits detected when water temperatures reached ~ 13 °C at one of the study sites. In the Gulf of Maine, Valentine et al. (2009) first observed *Didemnum* recruits at water temperatures in the range of 14.1 to 19.4 °C. In nearby Narragansett Bay, Rhode Island, recruitment was detected at the considerably higher temperatures of 18.4, 19.7 and 22.5 °C at three different sites (Auker and Oviatt 2008). It has been suggested that fluctuations in water temperature may limit the onset, but not the decline, of reproduction in ascidians (Millar 1971). In the post-summer period of this study, larvae consistently continued to recruit at temperatures below the onset temperature, with 11.1 °C being the lowest average temperature where recruits were detected. Larvae in the Gulf of Maine study (Valentine et al. 2009) also continued to recruit at temperatures below the temperature of initial appearance, with recruitment ceasing at temperatures in the range of 9.3 to 11.3 °C; slightly lower than the current study findings of 11.1 to 11.5 °C.

The length of a species' reproductive season can be affected by preceding periods of unusually cool or warm water temperatures (Cerrano et al. 2000; Coma et al. 2000). Previous studies have indicated that warmer winter temperatures can result in earlier recruitment of *Didemnum* as well as overall higher summer abundances (Gittenberger 2007; Valentine et al. 2007a). *Didemnum* colonies generally undergo periods of degeneration during the

colder winter months, with colonies becoming smaller and more two-dimensional in morphology. This winter hibernation or dormancy is characterised by a marked decrease in investment in secondary production (i.e. growth and reproduction) over this period (Coma et al. 2000). Valentine et al. (2009) hypothesised that the degree to which colonies degrade in the cool season influences the length of time they require to regenerate, reproduce sexually, and brood and release larvae. They proposed that owing to this, larvae will be released at the end of a developmental period as water temperatures warm, not necessarily when a particular water temperature is reached.

In the Netherlands, unusually warm winter temperatures were thought to be the main reason for a dramatic population increase in *Didemnum* in 1998 (Daniel and Therriault 2007). In 1997, the minimum water temperature was -2 °C, while in the following year it only dropped to 4 °C. Thus, those authors suggested that colder winter temperatures could have been limiting colony success. A similar result has been documented for *Diplosoma listerianum*, which fails to recruit at all if mean water temperatures for the preceding winter drop below ~ 4 °C (Osman et al. 2010). Based on population observations in the Netherlands and the United States, it has been suggested that 4 °C may be a critical lower temperature that limits the spread or growth of *Didemnum* colonies (Daniel and Therriault 2007). Similarly, increased water temperatures have also been shown to reduce the growth of *Didemnum* colonies (Gittenberger 2007; McCarthy et al. 2007), thus higher than optimal summer temperatures could also restrict development and reproduction of this species. Sea surface water temperatures in central New Zealand show less annual variation than those documented for the northern-hemisphere populations of *Didemnum* (c. 10-23 °C in the present study). It is possible that the reduced seasonal range of temperature, and the lack of associated regulation of reproductive output, is a key factor that explains the extended reproductive season of this species in this study.

It is apparent from the current study, and others cited above, that critical temperature thresholds for recruitment of *Didemnum* are inconsistent both within adjacent regions and among different locations globally. Nonetheless, overall patterns in the seasonal relationship between water temperature and recruitment in this species are similar in all cases. Although the duration of the recruitment period varies considerably, seasonal peaks in relation to increased water temperatures are generally consistent among locations. In a similar manner, critical temperature thresholds for the onset and cessation of *Didemnum* recruitment vary to some extent between locations globally. However, the general pattern is consistent with higher critical temperatures required at the onset of recruitment as opposed to the cessation at the end of the reproductive season. In the present study, the variation in the predicted temperature thresholds for this cessation of recruitment (between sites and years) suggests that, while seawater temperature is clearly important, additional factors may also influence recruitment patterns in *Didemnum*.

4.5.3 Additional factors driving recruitment variation

Strong site differences in recruitment between NN and RK were consistent across both years. Considerably lower recruitment at NN is consistent with the fact that source population biomass at that location was considerably less than at RK, and was spread over a wider geographic area. Such findings are in line with expectations regarding the role of propagule pressure in invasion success (Leung and Mandrak 2007; Lockwood et al. 2009); with sites containing source populations of a greater biomass likely to have proportionately greater recruitment success. Additionally, the NN site is in a predominantly estuarine environment with localised salinity variations potentially influencing recruitment success at this site. Colonial ascidian colonies can often tolerate wide fluctuations in salinity; however, they are rarely found in salinities < 25 psu (Millar 1971; Vázquez and Young 2000; G. Lambert 2005). The NN site is located

approximately 1.5 km from the mouth of a river. Although typical dry weather flows in the river are low (< 1 $m^3.s^{-1}$) and surface salinity adjacent to the NN site is typically 32-33 psu, flood flows (mean 85 $m^3.s^{-1}$) can reduce surface salinity to as low as 20 psu (B. Forrest, unpubl. data). Under experimental conditions, *Didemnum* colonies have been shown to tolerate short-term but severe declines in salinity, although extended low-salinity stress leads to moderate mortality in this species (Gröner et al. 2011). Conceivably, such events could reduce recruitment success at the study site, although they clearly do not prevent it. By contrast, the RK site was unlikely to have been affected by salinity variation, as it has no significant freshwater source nearby.

Fluctuations in other environmental factors such as nutrients, dissolved oxygen and pollutant levels may also influence marine invertebrate reproduction processes (Thorson 1950; Giese 1959; Rivkin 1991), and it can be difficult to solely attribute changes in biological responses to a single variable (Coma et al. 2000; Brockington and Clarke 2001). For example, seasonal fluctuation of food availability (primarily phytoplankton and suspended organic matter), in conjunction with temperature, are considered crucial to the dynamics of benthic suspension feeders in many temperate coastal communities (Coma et al. 2000). Reproduction investment per zooid is high in species with small zooid size (e.g. didemnids) within the colonial ascidians (Tarjuelo and Turon 2004) and, as such, seasonal energetic constraints may affect sexual reproduction in this group. Fluctuations in chlorophyll *a* were found to be correlated with *Didemnum* recruitment patterns at one of three sites examined by Auker and Oviatt (2008); nonetheless, in that study, water temperature was shown to be the most important environmental variable. Hence, although a range of factors may be important in the reproductive seasonality of *Didemnum*, it is suggested that seawater temperature provides both a useful and practical indicator for monitoring and assessing recruitment risk in an aquaculture context.

4.5.4 Seasonality of larval development

The larval development of *Didemnum* at RK followed a distinct seasonal pattern that, like recruitment, was correlated with water temperature. There was a marked decrease in egg abundance over the colder winter months, with abundances of the remaining three stages also exhibiting smaller fluctuations in line with seasonal changes. While no previous studies have examined the relationship between water temperature and larval production in *Didemnum*, the results presented are supported by similar research on other colonial ascidian species. A strong relationship between water temperature profiles and peak embryo production was found for the brooded larvae of three species of *Aplidium* from the west coast of Scotland (Millar 1958, 1971). Similarly, the colonial ascidian *Metandrocarpa taylori* showed strong seasonal peaks in the occurrence of brooded larvae on the California coast (Haven 1971). Interestingly, in the present study, there was no extended period of larval absence over the entire sampling period, with mature larvae detected on 90 % of sampling dates at RK. By contrast, recruits on settlement plates at RK were detected on only 80 % of sampling occasions, with a distinct and continuous absence over the winter period. As such, it appears that despite the potential for recruitment being indicated by the presence of mature larvae, recruits are not being detected in the field.

4.5.5 Implications for shellfish aquaculture risk management

Patterns of seasonal reproduction in *Didemnum* illustrated here have implications for the commercial culture of green-lipped mussels within the study region. Accumulated biofouling on mussel lines often presents a serious operational challenge to farm managers and can negatively affect farm productivity (Gretchis 2005; McKindsey et al. 2007; Daigle and Herbinger 2009; Davis and Davis 2010). Patterns of biomass accumulation for *Didemnum* in this

region closely follow the recruitment patterns described in the present study (L. Fletcher, unpubl. data); following settlement, individual recruits can undergo rapid expansion through asexual budding of zooids to form substantial colonies. Population biomass of this species is highest over the summer period, with colonies typically undergoing a winter regression, as observed in other locations globally (e.g. Valentine et al. 2007a). Periods of increased recruitment and growth of *Didemnum* will influence the level of connectivity and potential spread of established populations, with higher biomass leading to increased propagule pressure. Information on reproductive seasonality and colony development of *Didemnum* is therefore essential for effective timing of mitigation techniques, as well as for developing vector management plans (e.g. movement of boats and seed-stock) to prevent secondary spread from infected locations.

Both the settlement plate and colony tissue section sampling methods used in the present study provided valuable information on the reproductive seasonality of *Didemnum*. Both methods could be easily applied in an aquaculture industry setting; however, the preferred method would depend on whether managers were interested in solely detecting recruitment potential or documenting the magnitude involved. The possibility of detection issues with the settlement plate method, with perhaps very low levels of recruitment being missed during the winter months, means tissue section analysis may provide more reliable results for less effort, or at least a more conservative estimate of recruitment potential. Although tissue section analysis would not reliably reflect the magnitude of recruitment during the reproductive season, the cross-correlation analysis showed a two to four week lag in peaks in recruitment levels when compared to MI values. As such, tissue analysis could provide an early warning of the onset of spawning at the beginning of the season.

Monitoring using either method would provide relatively simple means for the shellfish industry to track the risk of their seed-stock and crops becoming infected by *Didemnum*. Such knowledge would enable the industry to mitigate the adverse effects of *Didemnum* fouling through management of farm operations around seasonal windows of risk. In the current study area, which is New Zealand's most important region for mussel culture, shellfish crops are vulnerable to *Didemnum* infestation for at least 9 months of the year, and probably longer based on the results of tissue section analysis. This duration is considerably longer than was previously believed for the region, meaning that opportunities for management are less than once envisaged. However, the same management principles can be applied to other locations and other fouling species. For example, future mussel industry plans in New Zealand involve holding hatchery-reared spat in the sea for short-term grow-out (3-4 months) before distribution to different growing regions nationally. Knowledge of seasonal fouling recruitment windows could be used to minimise risk to the spat, which is a relatively vulnerable stage in the industry production chain. Furthermore, by reducing fouling risk during short-term grow-out, there will be a concomitant reduction in the likelihood that high-risk species will be inadvertently spread among growing regions as a result of inter-regional spat transfers.

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CHAPTER 5

NATURAL DISPERSAL MECHANISMS AND DISPERSAL POTENTIAL OF THE INVASIVE ASCIDIAN *DIDEMNUM VEXILLUM*

Preface

Information on the natural dispersal potential of marine pests is critical to decisions regarding the minimum spatial scales over which to focus management of anthropogenic vectors such as fouled vessels. As many biofouling species have limited natural dispersal ability, these vectors can play an important role in extending the spatial scale and rate of species spread. This chapter follows on from larval release from the parent population and the subsequent recruitment described in Chapter 4, and aims to quantify the natural dispersal capacity of *Didemnum* populations.

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Author contributions:

The author co-designed the experiments, carried out field and laboratory work, analysed the data and prepared the manuscript. My thesis supervisors assisted with experimental design and aspects of fieldwork, as well as providing useful comments on earlier versions of the manuscript.

5.1 Abstract

Over the past decade, several species of non-indigenous ascidians have had adverse effects on a range of coastal ecosystems, and associated industries like aquaculture. One such species, the colonial ascidian *Didemnum vexillum* (hereafter referred to as *Didemnum*), poses a threat to the highly-valued New Zealand green-lipped mussel industry, and there is interest in whether and to what extent its spread can be managed at a regional scale (< 100 km). An important component in the decision-making process for managing human-mediated pathways of spread is an understanding of *Didemnum's* natural dispersal potential. This study uses a weight-of-evidence approach, combining laboratory and field studies, to assess the role of natural dispersal mechanisms in the spread of *Didemnum*. Under laboratory conditions, > 70 % of *Didemnum* larvae remained viable and able to settle and undergo metamorphosis successfully following an artificial delay of 2 hours. Larval viability decreased with increasing delay duration, although 10 % of larvae remained viable following a 36 hour delay. A field-based study documented larval dispersal from two discrete source populations, with recruitment consistently detected on settlement plates at 250 m from source populations at one experimental site. Exponential decay models used to predict maximum larval dispersal distances at this site indicated that dispersal greater than 250 m is theoretically possible (> 1 km in some situations). That being so, the successful establishment and persistence of populations will depend on a wide range of processes not taken into account here. Research findings are supported by surveillance of *Didemnum* spread in the wider study region; there are a number of instances where the species established on artificial structures that were several kilometres from known source populations, at a time when intensive regional-scale management of anthropogenic vectors was underway. Collectively, these findings indicate that *Didemnum* has the ability to spread further by natural dispersal than previously assumed; probably hundreds of metres to kilometres depending on the local hydrological conditions, which has important implications for the ongoing management of this pest species world-wide.

5.2 Introduction

The spread of non-indigenous species in the marine environment can have widespread economic and ecological impacts (Pimental et al. 2000; Colautti et al. 2006; Molnar et al. 2008). The recent introductions of several high-profile pest species to a number of regions world-wide has led to increased attention on the physical and biological processes underpinning population spread and invasion in the marine environment (e.g. Carlton 1996; Floerl and Inglis 2005; Verling et al. 2005; Johnston et al. 2009). Propagule supply, dispersal vectors, dispersal distances, diversity of resident communities, interactions between species, disturbance regimes, predator-prey relationships, and environmental conditions encountered, are all fundamental for understanding patterns of invasion in the field (Rilov and Crooks 2008). For invasive species, an understanding of natural dispersal potential is of particular importance as this underpins a number of common management needs, such as identification of the spatial scales at which control of anthropogenic transport vectors is desirable, as well as identification of delimitation zones for surveillance (Sakai et al. 2001; Puth and Post 2005; Forrest et al. 2009).

The majority of benthic marine invertebrates have limited adult movement, and as such the dispersal of pelagic early life-stages often represents a key process in their lifecycle (Thorson 1950; Gaines et al. 2007; Pineda et al. 2007). The dispersal of planktonic propagules (e.g. larvae, spores) has been shown to play a crucial role in the connectivity of geographically separated marine populations, along with the persistence and spread of local populations, re-colonisation of disturbed areas and definition of species' range limits (Levin 2006; Gaines et al. 2007; Cowen and Sponaugle 2009). Planktonic dispersal is dependent on a complex interaction of physical and biological processes acting at different temporal and spatial scales, and at both the individual and population level (Huret et al. 2010). Factors influencing dispersal can include propagule duration,

behaviour (e.g. vertical migration within the water column), food resources, predators encountered, and the influence of currents and other oceanographic processes (Fiksen et al. 2007; Pineda et al. 2007; Shanks 2009). Human activities in the marine environment (e.g. vessel movements) provide an added dimension to dispersal; they can increase species ranges by transporting them across barriers to their natural dispersal, and in the case of invasive species may greatly accelerate rates of spread (Carlton 1996; Ruiz et al. 2000; Floerl and Inglis 2005).

Although it is well recognised that an understanding of natural dispersal potential is critical for long-term successful management (Myers et al. 2000), there is still a lack of empirical data around dispersal capacity for the majority of marine species. Measuring natural dispersal in marine systems is often problematic, for example, because propagules are often numerous and small, making *in situ* tracking over large distances impractical. Furthermore, the relative roles of natural and anthropogenic dispersal can be confounded. Previous efforts to characterise dispersal patterns of invasive and non-invasive species have drawn on a number of techniques including field-based studies, genetic analysis of populations, chemical tracking such as trace elemental fingerprinting, as well as a range of modelling approaches (Palumbi 2003; Kinlan and Gaines 2003; Cowen et al. 2006; Levin 2006; Cowen and Sponaugle 2009). The duration of the planktonic period has often been used as a proxy for propagule dispersal potential in the marine environment (Shanks et al. 2003; Kinlan et al. 2005; Shanks 2009); however, application of this approach for larval durations of more than a few hours requires knowledge of complex local currents. Recent estimates utilising advection/diffusion models, incorporating realistic mortality terms, as well as vertical positioning behaviour, have provided a more realistic depiction of propagule movement at local scales (e.g. Cowen et al. 2006).

Ascidians as a group are represented by a number of globally cosmopolitan invasive species whose dispersal potential has received increased interest in recent years due to their actual or potential impacts (Castilla et al. 2004; Blum et al. 2007; Lambert 2007; Valentine et al. 2007; Ramsay et al. 2008; Adams et al. 2011). One such species, the colonial ascidian *Didemnum vexillum* (Kott 2002), has become a very successful invader in temperate marine communities worldwide (Lambert 2009; Stefaniak et al. 2009). In New Zealand, *Didemnum* was first detected in 2001, and has since been inadvertently spread by anthropogenic transport mechanisms to a number of geographically distant regions (Coutts and Forrest 2007). Subsequent intra-regional spread has been facilitated by vessel movements, movements of aquaculture equipment and seed-stock, as well as natural 'stepping-stone' dispersal among artificial structures (Forrest et al. 2009).

The free-swimming, lecithotrophic tadpole larvae of colonial ascidians are generally considered to be competent for minutes to an hour or two at most, such that populations are maintained by highly localised larval recruitment (Berrill 1950; Ayre et al. 1997; Lambert 2002). In the case of *Didemnum*, the actual natural dispersal ability is unknown, but is considered to be limited, based on past observations of spread over scales of hundreds of metres (e.g. Coutts and Forrest 2007), and reported short-duration (< 2 hrs) larval competency periods for other species in the same genus (Berrill 1950; Olson 1985). However, surveillance of *Didemnum* spread in the Marlborough Sounds region (c. 41°13'S,174°7'E) of central New Zealand has revealed a number of instances where the species has established on artificial structures that were several kilometres from known source populations, at a time when intensive regional-scale management of anthropogenic vectors was underway. As the new incursions could not be attributed to known vector movements, the cause was hypothesised to be natural larval dispersal to an extent that was far greater than previously assumed. Hence, the aims of this study were to better elucidate larval

duration and the role of natural dispersal mechanisms in the spread of *Didemnum*. A laboratory-based experiment to determine how long *Didemnum* larvae remained competent and able to settle when metamorphosis was artificially delayed was conducted, as well as field-based studies that measured and predicted larval dispersal from discrete source populations. Based on the research findings, a conceptual model of dispersal and spread in *Didemnum* is presented.

5.3 Methods

5.3.1 Larval competency period

Four small (< 20 cm² area, ~ 30 g), lobe-shaped sections of *Didemnum* colony were collected from beneath a floating pontoon in Port Nelson, New Zealand (c. 41°15.4'S, 173°16.6'E), in April 2009. After transportation back to the laboratory the colonies were maintained in complete darkness for 48 hours, an interval long enough to induce sufficient larval release, but not so long as to lead to deterioration of colony health (Fletcher and Forrest 2011). The colonies were placed into two 50 L black PVC bins (2 colonies per bin) with secure lids that excluded light. Each bin was filled with seawater sourced from a nearby aquaculture facility (~ 16 °C, ~ 35 psu, filtered to 0.35 µm and UV treated), with oxygenation maintained using a single air stone. At completion of the dark adaptation phase, the release of mature larvae was stimulated using a 'light shocking' technique described by Fletcher and Forrest (2011) (see Chapter 3).

Following release from the parent colony, individual larvae were collected with a 5 ml pipette and transferred to a 200 ml glass rinsing bowl. The seawater (c. 18 °C) in which the colony sections were held was replaced at 15 min intervals to ensure larvae being sampled were collected within 15 min of release. To prevent settlement and metamorphosis of pipetted larvae, each

bowl was placed on a white surface and exposed to continuous bright light provided by four Osram 18 watt cool-white fluorescent bulbs. Pilot studies investigating a range of delay methods (including the use of aeration, mechanical agitation, and varying light regimes) indicated the method used to be the most successful (Fletcher and Forrest 2011; see Chapter 3). Once exposed to this regime the larvae became dormant and remained inactive for the period of light exposure, as previously described for the colonial ascidian *Diplosoma listerianum* (Marshall et al. 2003; Bennett and Marshall 2005) To investigate how delayed settlement and metamorphosis as a result of exposure to bright light affected larval competency, treatments involving different light exposure durations (2, 6, 12, 18, 24, 36 and 48 hours) were applied. To apply the time delay treatments, glass bowls with individual larvae were randomly allocated to one of five replicates (each with 20 larvae) in each of the treatments. Allocation of larvae to delay treatments was randomised to prevent any potential bias through changes in fitness based on time of larval release. Larvae released at the onset of spawning may be in better metabolic condition than those released at the tail end of spawning, and as such the allocation to delay treatments was randomised. Control larvae were not exposed to bright light; larvae in this treatment were immediately transferred to sterile tissue culture dishes (as outlined below) and allowed to settle and metamorphose into a juvenile recruit without delay.

Following completion of the delay treatments, larvae were stimulated to swim by initiating a 'shadow response', a phenomenon in which larvae exhibit an increase in swimming activity in response to a sudden decrease in light intensity (Svane and Young 1989). Twelve larvae were collected from each bowl and transferred to separate wells (6 ml volume) in a sterile 12-well Falcon™ tissue culture dish. Although in some instances the use of pre-conditioned substrates is preferred, sterile containers were used for these trials to limit the introduction of micro-organisms to the wells. Previous studies describing similar research

have used sterile systems without any negative effects (e.g. Marshall et al. 2003; Bennett and Marshall 2005). Larvae in the undelayed control and the five shortest delay treatments (2 to 24 hours) swam actively in response to the shadow effect. Larvae in the 36 and 48 hour treatments could not be induced to swim, but were not attached to the bowls so were included in the experiment and subsequent analysis. The culture dishes were placed in a shaded container and left undisturbed for three days. Pilot studies revealed that these conditions provided the best settlement success and enabled competent larvae to settle and complete metamorphosis into recruits (Fletcher and Forrest 2011; see Chapter 3).

After three days, each well was checked using a dissecting microscope and the viability of each larva recorded according to whether it was: attached and metamorphosed; metamorphosed within the water column (floating); attached but still in larval form; swimming; or immobile (unresponsive to light or touch). Following this assessment, the culture dishes were maintained for a further three weeks at 18 °C, in a 12 : 12 hour light : dark regime. During this period, *Didemnum* colonies were fed a solution of *Isochrysis* sp. diluted in filtered seawater (5×10^7 cells.L^{-1}), which was replaced every second day. At the end of the three week period, each colony was assessed for survival, and developmental stage was recorded. The presence of both calcium carbonate spicules within the tunic and fecal pellets around the colony were used as criteria for survival, as these indicate development and feeding (Valentine et al. 2009).

Larval viability (%) after three days and colony survival after three weeks in culture, were both calculated as a proportion of the initial 12 larvae present in the dishes. Treatment effects were assessed using one-way analysis of variance (ANOVA) with Tukey's HSD (STATISTICA 8.0, StatSoft Inc. 2008) to test for differences among delay treatments in these two response measures. One-way

ANOVA was also used to test for *Didemnum* mortality (percent change) during the three week culture period. Brown-Forsythe's tests were used to check the assumption of homogeneity of variance, which was met by all three datasets ($p > 0.05$). Results of Shapiro-Wilk's W test for normality indicated both the colony survival data and the mortality data were not normally distributed. Examination of normal probability plots after various transformations indicated the untransformed data had the closest to normal distribution. Thus, as ANOVA has been shown to be fairly robust to violations of the normality assumption (Quinn and Keough 2002), and both data sets had adequate ($n > 30$) and balanced replication amongst levels, analysis was carried out on the untransformed data.

5.3.2 Larval dispersal experiment

The dispersal of *Didemnum* larvae was investigated in field studies at two locations (Ruakaka Bay, RK; Blackwood Bay, BB) within the Marlborough Sounds (Figure 5.1). Each location had a discrete *Didemnum* population associated with an artificial structure; hence dispersal could be measured without confounding effects from other larval sources. The structures were a marine farm at RK having c. 30 tonnes *Didemnum* (wet weight), and a small (3 x 5 m) floating research pontoon at BB with c. 0.35 tonnes wet weight. Source population weight estimates for the RK structure were calculated based on a preliminary survey of *Didemnum* biomass present on the predominant substrata available at the farm (e.g. steel pontoon, exterior netting) (L. Fletcher, unpubl. data). Weight estimates for the BB site were based on the weight of a representative sample of fouled mussel culture ropes hanging beneath the structure (B. Forrest, unpubl. data). Larval dispersal was measured by deploying floating arrays of settlement plates at increasing distances up to 250 m from each source, and dispersal distance inferred from the recruitment of larvae to the plates after three deployment periods of four weeks each. The term recruitment is used

here as a measure of the number of newly settled individuals that survive between settlement and the time a census is taken (Keough and Downes 1982). Plates were made from roughened black Perspex (20 x 20 cm) and were positioned horizontally within each array on a mooring system configured to ensure they remained at a constant depth with respect to the water surface. The three deployments (Trials 1-3) occurred over the Austral summer and early autumn (January 17 to April 10, 2008), coinciding with the seasonal peak in *Didemnum* reproduction (Fletcher et al. 2013; see Chapter 4). At the end of each four week trial, the arrays were retrieved and returned to the laboratory, where the numbers of *Didemnum* recruits were counted on the downward-facing surface of each plate, reflecting the orientation where maximum recruitment occurs (Valentine et al. 2009). A 2 cm buffer zone around the edge of each plate was excluded to account for handling effects; hence data were recorded as counts of individuals recruiting to the central 16 x 16 cm (256 cm^2) of each plate. In some instances, recruits had undergone asexual reproduction and divided into small (~ 4 zooids) colonies by the time of census. These colonies were still counted as single recruits, as they had originated from recruitment of a single larva (see Valentine et al. 2009 for a detailed description of colony development).

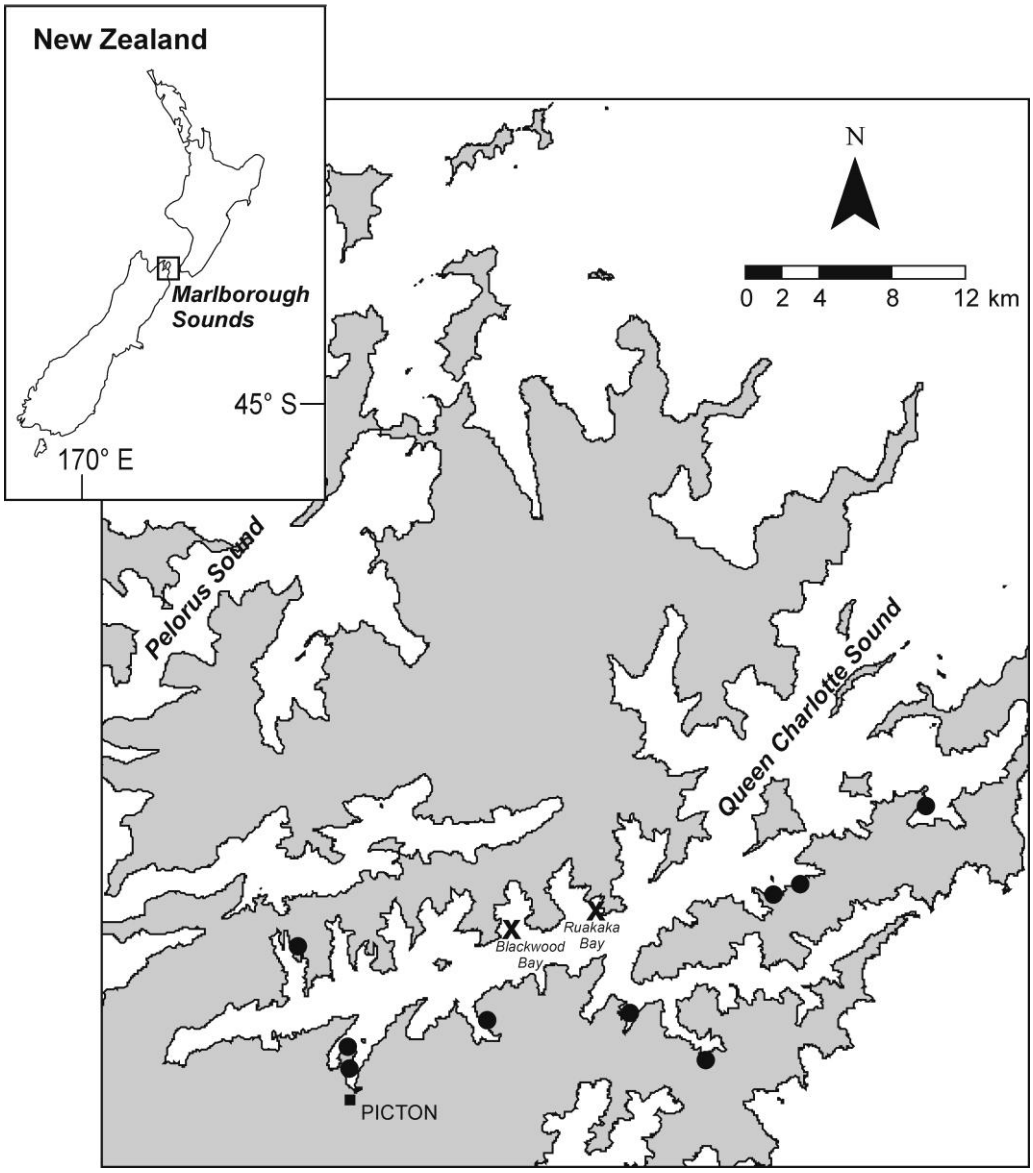

Figure 5.1 Map of the Marlborough Sounds region showing an inset of the location within New Zealand. Black crosses indicate the two sites used in the *Didemnum vexillum* dispersal field study, Blackwood Bay (BB) and Ruakaka Bay (RK). Filled circles indicate sites used in the industry-led monitoring programme. Ruakaka Bay was also used as a site in this programme.

At RK, settlement plates were positioned on the arrays at three depths (1, 5 and 10 m below the water surface), for seven distances from the source population (0, 50, 150, 175, 200, 225 and 250 m) in the direction of the prevailing tidal flow (*n* = 3 per distance/depth combination). Three depths were included to evaluate whether larvae sank to progressively greater depths with increasing distance from the source, as has been suggested for other colonial ascidians (e.g. Davis and Butler 1989; Bingham and Young 1991). The BB arrays were arranged at similar distances (0, 20, 60, 100, 140 and 250 m down-current of the *Didemnum* source) and trials conducted at the same time as RK. However, owing to the shallow nature of the BB site, only the 1 m depth interval was used (*n* = 3 per array). At both locations, array spacing and the maximum distance of 250 m were constrained by navigational safety issues.

The direction and speed of surface water currents at both sites were measured every four weeks using modified 'holey sock' drogues (Sombardier and Niiler 1994). Drogues were released in batches of up to four at a time and were left to drift for between 1 and 3 hours, while continuously recording position and time. Water currents below the surface were also measured at both sites. At RK, an acoustic Doppler current profiler (ADP) recorded water current speed and direction (averaged over 4 m depth bands) between the sea floor and the water surface c. 32 m above the instrument. Data from the three upper bands (0-4, 4-8, and 8-12 m) were used in this analysis. The meter was programmed to take measurements every second over a five minute period, which were then averaged, every 45 minutes of a 40-day deployment (January 17 to February 27, 2008). At BB, sub-surface water current data were collected using a 2-dimensional acoustic current meter (ACM) stationed at 4 m depth. Readings (revolutions per minute) were taken every 15 min and these were averaged over an 84-day deployment (January 17 to April 10, 2008). Across the two locations, the water temperature 1 m below the surface was comparable among sites, ranging from 16.4 °C to 19.9 °C over the course of the experiment.

At RK the marine farm operator removed the heavily infested exterior netting at the end of Trial 1, leading to a substantial decline in *Didemnum* biomass at the site (c. 60 % decrease in wet weight) between Trial 1 and 2. Hence, statistical analysis of RK data involved a two-way ANOVA to test for the effects of distance and depth on recruit counts within each trial, as opposed to including Trial as a fixed factor in the analysis. At BB there was an unexpected die-back of the *Didemnum* colonies hanging beneath the pontoon, which could not be explained by seasonal variation alone. In addition, the 0 m plate array was lost from BB during Trial 2, resulting in an unbalanced design between trials. Hence, analysis of the 1 m depth data at BB consisted of a one-way ANOVA for distance within each trial separately as well. Recruit density data at both locations were log10 (x + 1) transformed to meet the assumptions of normality and homogeneity of variance of error terms. All analyses of the field trial results were performed using STATISTICA 8.0 (StatSoft Inc. 2008).

The theoretical post-dispersal recruitment of *Didemnum* larvae was modeled for each location, trial and depth combination using a one-phase exponential decay equation (Weidner and Sells 1973), to reflect the advection and dilution of larvae as a function of distance. Exponential decay models have previously been applied to a number of studies describing propagule dispersal from a point source, including juvenile bivalves and kelp zoospores (Norkko et al. 2001; Cie and Edwards 2011, respectively). In the current study, the level of recruitment was described using the formula:

$$R_d = R_0 e^{-\alpha d}$$

where R_d is the number of recruits at distance d from the larval source, R_0 is the number of recruits at the source (i.e. where d = 0), and α is a constant describing how quickly the level of recruitment decreases with distance d. Other non-linear approaches were considered; however, an exponential decay model was

selected as this gave the best model fit and as such appeared to best describe the underlying behavior of the system. The maximum theoretical dispersal distance (d_{max}) was calculated using the error structure of the fitted model and represents the distance from the source population at which the probability of detecting recruitment at a level < 0.333 (reflecting the presence of a single recruit on one of the three settlement plates) was less than 1 %. A decay curve was unable to be fitted for BB during Trial 2 as there were no 0 m data. Prediction curves were developed using the software package R version 2.13.2 (R Development Core Team 2011).

5.4 Results

5.4.1 Larval competency period

Approximately 73 % (SE 5.5 %) of the larvae in the undelayed control treatment had successfully settled three days after larval release (Figure 5.2a). Although not statistically significant, there was slightly higher viability in the two hour delay treatment than the control (76.7 % ± 5.5 %). This was followed by a gradual decline in viability with increasing time, to a point where there were no viable larvae in the 48 hour delay treatment. Hence, the delay treatment effect was highly significant in the ANOVA (Table 5.1a); with pairwise comparisons indicating significant differences between the three shortest delay times and those with a delay of 12 hours or more (Tukey's HSD, $p < 0.05$, Figure 5.2a). After three weeks in culture, the majority of the settled *Didemnum* had undergone asexual reproduction to form small colonies; hence, the pattern of colony survival (Figure 5.2b) was similar to the pattern of larval viability. Accordingly, there was a highly significant delay treatment effect in the ANOVA (Table 5.1b). There were no significant differences among delay treatments in mortality between three days and three weeks in culture (Table 5.1c).

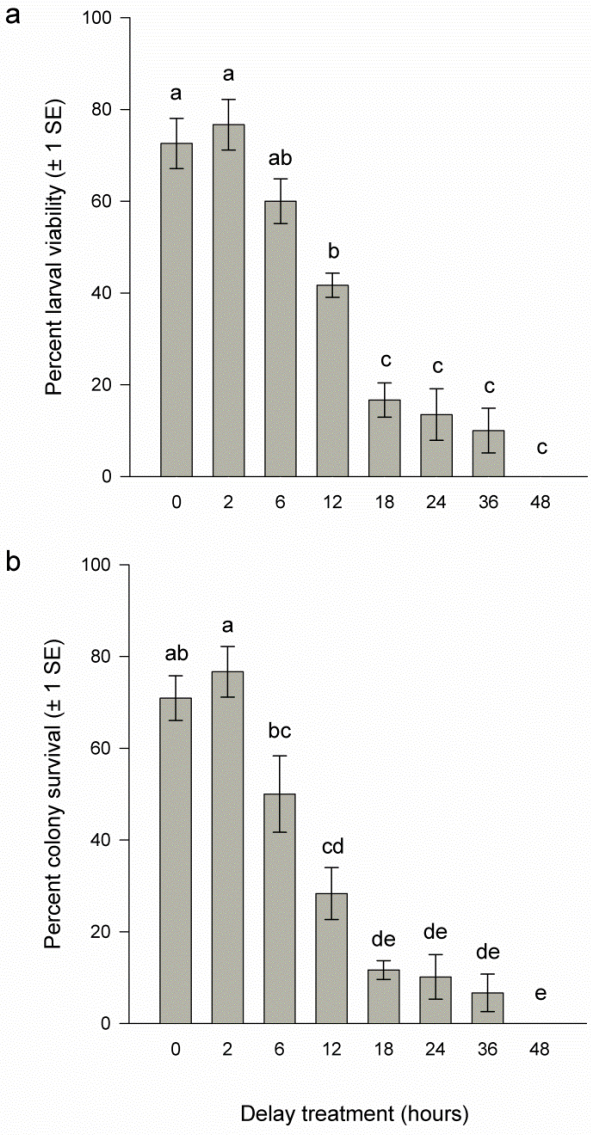

Figure 5.2 Mean percentages (± SE, $n = 5$) of: **a.** initial *Didemnum vexillum* larvae recruitment following delay of settlement and metamorphosis for up to 48 hours after larval release; and **b.** *Didemnum* colony survival three weeks after delayed settlement and metamorphosis treatments. Columns sharing a letter indicate groupings from the ANOVA that were not significantly different from each other (Tukey's HSD test, $p > 0.05$).

Table 5.1 One-way analysis of variance (ANOVA) testing the effect of settlement and metamorphosis delay duration on the level of: **a.** larval viability after 3 days; **b.** colony survival after 3 weeks; and **c.** mortality (percent change) during the 3 week culture period.

Source of variation	*df*	a. Larval viability		b. Colony survival		c. Mortality	
		MS	*F* (*p*-value)	MS	*F* (*p*-value)	MS	*F* (*p*-value)
Delay	7	4608.010	46.061 (**< 0.01**)	4573.378	36.593 (**< 0.01**)	114.087	2.150 (0.066)
Error	32	100.042		124.978		52.951	

Significant values ($p < 0.05$) are indicated by bold type.

Table 5.2 Two-way analysis of variance (ANOVA) testing the effects of distance from source population, water depth, and their interaction on the level of *Didemnum vexillum* larval recruitment on settlement plates within Ruakaka Bay, Marlborough Sounds.

Source of variation	*df*	a. Trial 1		b. Trial 2		c. Trial 3	
		MS	*F* (*p*-value)	MS	*F* (*p*-value)	MS	*F* (*p*-value)
Distance	6	1.837	139.367 (**< 0.01**)	3.103	76.824 (**< 0.01**)	2.037	74.212 (**< 0.01**)
Depth	2	7.889	598.472 (**< 0.01**)	1.188	29.421 (**< 0.01**)	0.950	34.598 (**< 0.01**)
Distance x Depth	12	0.167	12.685 (**< 0.01**)	0.127	3.143 (**< 0.01**)	0.057	2.087 (**0.039**)
Error	42	0.013		0.040		0.027	

Significant values ($p < 0.05$) are indicated by bold type.

Table 5.3 Results from three one-way analysis of variance (ANOVA) testing the effect of distance from the source population on the level of *Didemnum vexillum* larval recruitment on settlement plates within Blackwood Bay, Marlborough Sounds.

Source of variation	a. Trial 1			b. Trial 2			c. Trial 3		
	df	MS	*F* (*p*-value)	*df*	MS	*F* (*p*-value)	*df*	MS	*F* (*p*-value)
Distance	5	2.894	92.300 (**< 0.01**)	4	0.016	0.774 (0.566)	5	3.130	80.668 (**< 0.01**)
Error	12	0.031		10	0.021		12	0.039	

Significant values ($p < 0.05$) are indicated by bold type.

5.4.2 Larval dispersal experiment

There were distinct differences in current speed and direction between the upper and lower depth bands examined at RK. Current speeds within the upper layer (0 - 4 m) averaged 9.01 $cm.s^{-1}$ (± 7.83 $cm.s^{-1}$) over the course of the 40-day deployment; more than twice that measured within the middle (4 - 8 m) and lower (8 - 12 m) depth layers (4.38 ± 3.39 $cm.s^{-1}$ and 4.07 ± 2.82 $cm.s^{-1}$, respectively). However, whereas currents within the middle and lower bands flowed predominantly southeast along the alignment of the settlement plate arrays, surface currents unexpectedly flowed primarily to the north and therefore away from the arrays. ADP measurements were supported by drogue studies that indicated a strong influence of local wind on surface water currents. Drogue speeds varied across the three deployments, with a mean value of 9.56 $cm.s^{-1}$ (±1.94 $cm.s^{-1}$).

The magnitude of *Didemnum* dispersal at RK site greatly declined after Trial 1 when the marine farm operator removed the *Didemnum*-infested netting (Figure 5.3). Nonetheless, the pattern among trials remained similar in that there was generally an initial sharp decline in recruitment to the settlement plate arrays within tens of metres from the RK source, followed by a long tail of relatively low recruitment out to 250 m. Within each trial at RK, recruitment was generally lowest at the shallow depth where the predominant water current movement was away from the alignment of the settlement plate arrays. However, within each trial the level of recruitment varied considerably between depths with increasing distance, and as such significant distance x depth interactions were present in all cases (Table 5.2a-c).

The magnitude and distance of larval dispersal from the smaller *Didemnum* population at BB was considerably less than recorded at RK (Figure 5.4). High larval recruitment was detected at the source (distance = 0 m) where the

settlement plate arrays were immediately adjacent to (< 1 m from) reproductive colonies. The maximum dispersal distance recorded was 140 m; however, only a few recruits were detected beyond the 60 m array. The effect of distance from the larval source population was highly significant in the ANOVA for both Trial 1 (Table 5.3a) and Trial 3 (Table 5.3c); however, due to the loss of the 0 m plate array during Trial 2 the effect of distance was not significant (Table 5.3b). Sub-surface current speeds at BB averaged 3.13 $cm.s^{-1}$ (± 1.81 $cm.s^{-1}$) over the course of the 84-day ACM deployment. Drogue speeds varied across the six deployments, with a mean value of 7.09 $cm.s^{-1}$ (± 0.84 $cm.s^{-1}$), largely indicating a predominant surface flow at BB along the alignment of the plate arrays.

Exponential decay curves accurately modelled the recruitment data at both locations, evident from the relatively narrow range (i.e. 99 % CI) of predicted recruitment levels at increasing distances from the source populations (Figure 5.5). At RK, the majority of predicted dispersal distances were considerably greater than the outermost distance examined by the field experiments. The maximum theoretical dispersal distance of *Didemnum* larvae at this site was at a depth of c. 10 m (d_{max} = 1029 m), at the time of Trial 1 when source population biomass and reproductive output (i.e. recruitment at distance = 0) were greatest. However, during the same period, a smaller dispersal distance than was actually measured was predicted for the shallowest 1 m depth (d_{max} = 220 m, recruitment was detected at 250 m). At BB, a maximum dispersal distance could only be calculated for Trial 3 (d_{max} = 94 m, compared with measured = 140 m), as the error component of the model for Trial 1 was larger than the constant describing the rate that recruitment decreased with distance.

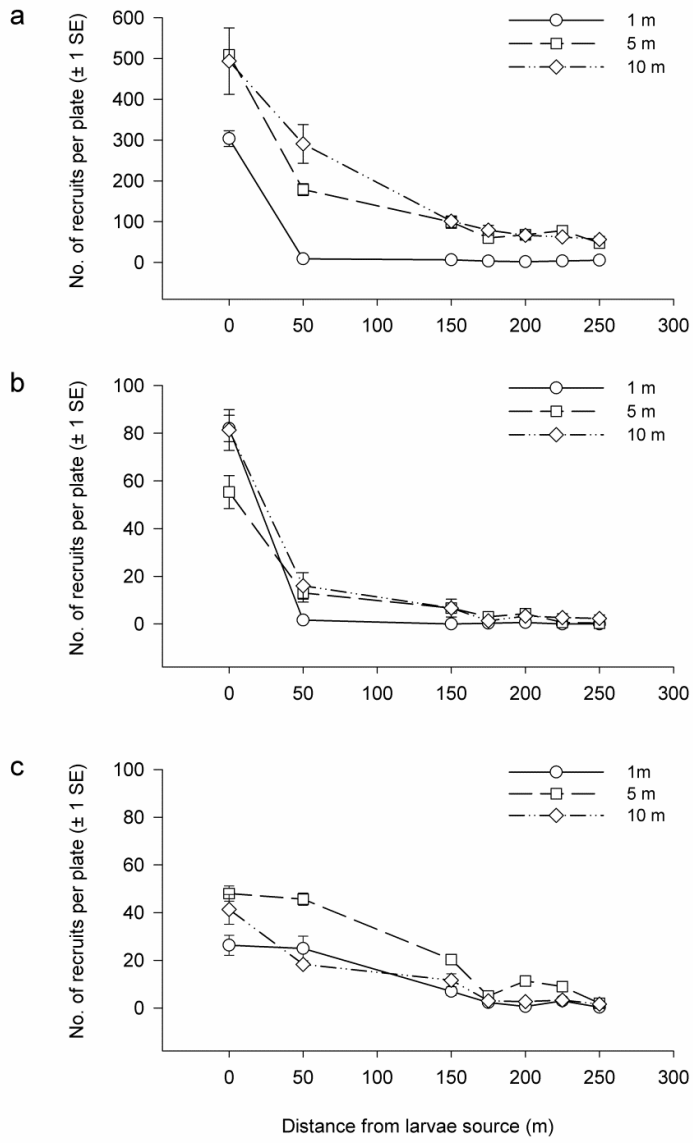

Figure 5.3 Mean number (± SE, *n* = 3) of *Didemnum vexillum* recruits on settlement plates at seven distances from the Ruakaka Bay source population and at three depths in the water column. Figures represent three separate four week trials: **a.** Trial 1, 17 January 2008 - 13 February 2008; **b.** Trial 2, 13 February 2008 - 12 March 2008; and **c.** Trial 3, 12 March 2008 - 10 April 2008. Note the differences in scale on the y-axis.

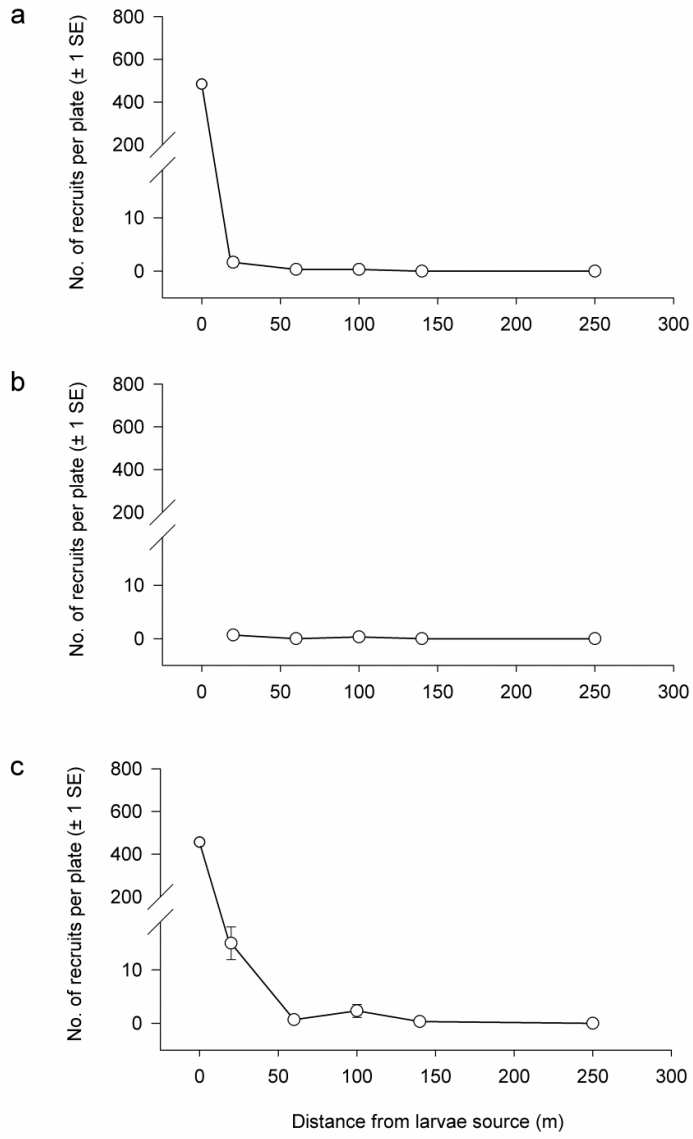

Figure 5.4 Mean number (± SE, *n* = 3) of *Didemnum vexillum* recruits on settlement plates at six distances from the Blackwood Bay source population. Plates were situated at a depth of one metre below the water surface. Figures represent three separate four week trials: **a**. Trial 1, 17 January 2008 - 13 February 2008; **b.** Trial 2, 13 February 2008 - 12 March 2008; and **c.** Trial 3, 12 March 2008 - 10 April 2008.

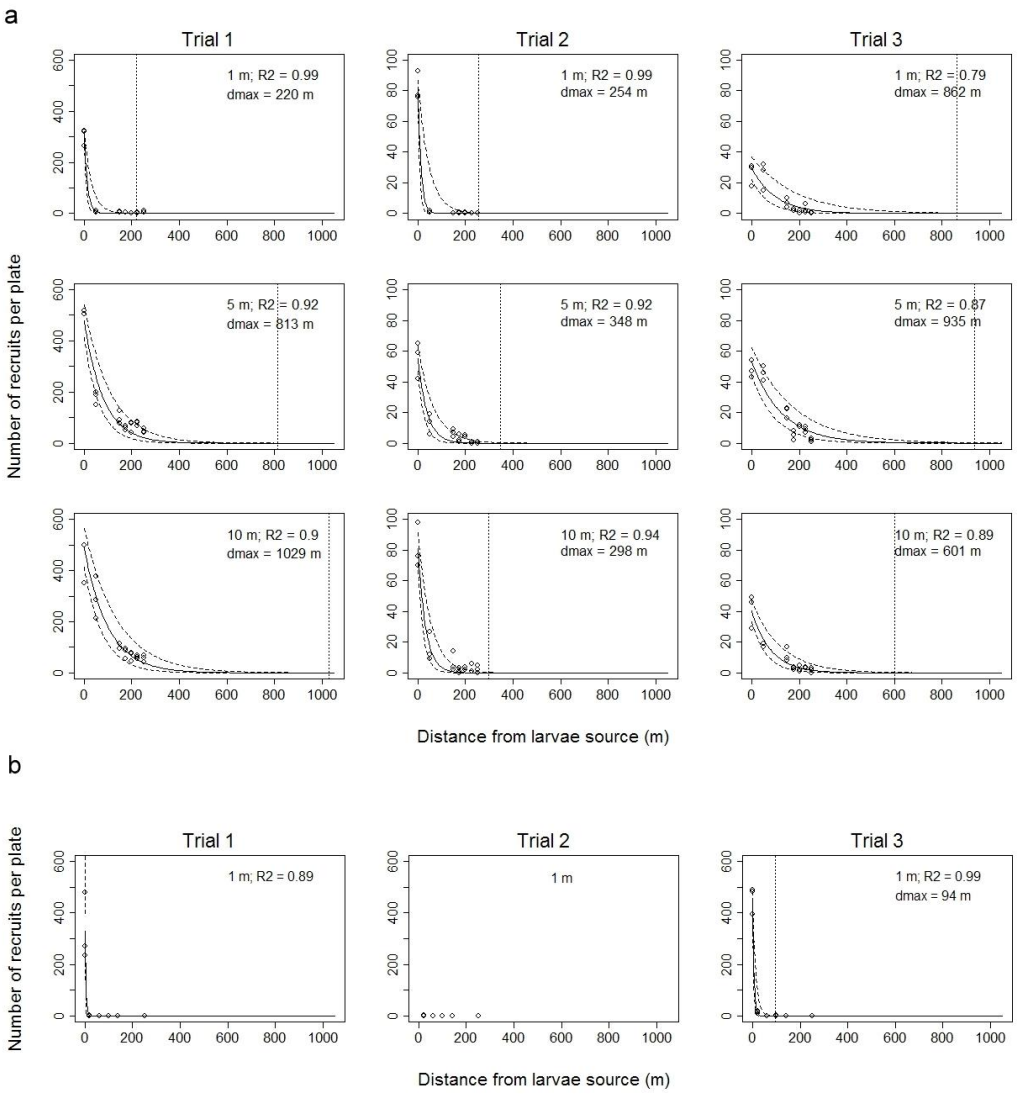

Figure 5.5 Exponential decay curves showing predicted dispersal of *Didemnum vexillum* larvae at both the **a.** Ruakaka Bay and **b.** Blackwood Bay sites. Dispersal curves are shown for three four week periods and three water depths. Recruitment data at the source (0 m) was unavailable for Trial 2 at Blackwood Bay and as such a dispersal curve was unable to be fitted. Dashed lines represent 99 % confidence intervals of the recruitment levels and were derived from the standard error of the estimated parameters. Maximum theoretical dispersal distances (d_{max} = recruitment < 0.333) are indicated where possible (vertical line). Note the differences in scale on the y-axis.

5.5 Discussion

Colonial ascidian populations have long been assumed to be maintained by highly localised larval recruitment and hence are believed to have a relatively restricted natural dispersal potential. The larval competency periods for species within the *Didemnum* genus have traditionally been reported as being of relatively short duration (< 2 hrs) (Berrill 1950; Olson 1985). By contrast, larvae of *Didemnum vexillum* were able to remain competent and viable within the water column for at least an order of magnitude longer under lab conditions. Although not reflecting the wide range of processes involved in successful population range expansion, these findings, together with predictions from field dispersal studies, provide compelling reasons to believe the larval swimming time of *Didemnum*, and hence larval dispersal, may be longer than previously assumed.

5.5.1 Factors affecting larval competency

Although *Didemnum* larvae were viable for up to 36 hours, viability steadily declined as delay time increased beyond 2 hours. The marked decline in viability by 36 hours, and absence of viable larvae at 48 hours, is consistent with the inability to stimulate larval swimming at the end of these two delay treatments. These findings are consistent with the results of a similar study on another didemnid ascidian (*Diplosoma listerianum*), which found the majority (> 70 %) of larvae could successfully settle and undergo metamorphosis following an experimental delay of 15 hours (Marshall et al. 2003).

By contrast, within the *Didemnum* genus, the few studies conducted to date reveal a larval competency period of 2 hours or less (Berill 1950; Olson 1985); an order of magnitude less than described here for *Didemnum vexillum*. Ascidian larvae are non-feeding and as such have finite energetic reserves with which to

perform metabolic functions and carry out metamorphosis (Svane and Young 1989; Marshall et al. 2003). Consequently, the observed reduction in settlement success with increasing duration of the delay treatment may be attributed to depletion of these nutritional resources through artificial extension of the larval stage. Although the non-feeding larval stages of several other marine invertebrates have been shown to utilise dissolved organic matter (DOM) directly from the surrounding seawater (Jaeckle and Manahan 1989; Jaeckle 1994; Wendt and Johnson 2006), it is believed that this method of energy acquisition has not been assessed for ascidian larvae. The use of DOM by lecithotrophic larvae may provide an advantage in terms of survival and dispersal ability, particularly with reference to the detrimental latent effects caused by prolonged periods of swimming (e.g. Woollacott et al. 1989; Pechenik et al. 1993; Pechenik et al. 1998). Interestingly, for *Didemnum* there was no significant difference among delay treatments in survival from newly settled recruits to three week old colonies, indicating that delayed settlement and metamorphosis resulted in no significant 'carry-over' effects on adult survival and growth; a possibility that has been previously suggested (see Marshall et al. 2003).

Although no difference in overall colony survival between delay treatments was detected, the limitations of the experiments being carried out in a laboratory environment are recognised, and it is appreciated that a range of additional factors would influence the long-term performance of these recruits under natural conditions. Recent studies assessing juvenile survival of other ascidian species have shown delaying the onset of metamorphosis can have severe consequences for adults, with the strongest effects on initial growth rates (Marshall et al. 2003). It has also been suggested that delayed metamorphosis effects will be more prevalent in the field for ascidians than for other organisms where larval locomotion is less expensive (Bennett and Marshall 2005); although this remains to be tested. Further research with *Didemnum* is required to

understand the effects of delayed settlement and metamorphosis on longer term post-metamorphic performance under field conditions.

5.5.2 Dispersal under field conditions

The overall trend of decreasing recruit numbers with increasing distances as far as 250 m from the source population will reflect a combination of factors in addition to simple physical dilution. For example, mortality from predation is known to be high during the pelagic phase of marine invertebrate life histories (Morgan 1995); hence, additional time spent in the water column will result in increased exposure of larvae to planktonic predation (Rumrill 1990; Pechenik 1999). Estimates of ascidian larval mortality based on *in situ* observations have ranged from 29 % (Stoner 1990) to 87 % mortality (Olson and McPherson 1987), and been attributed to factors such as predation by fish and invertebrates. The exponential decline in *Didemnum* with distance from the source populations is also consistent with observations from other field studies indicating that ascidian larvae can settle very soon after release, hence given suitable habitat may be retained close to their natal site (e.g. Olson 1985; Davis and Butler 1989; Stoner 1990; Bingham and Young 1991). Retention to the parent population may also in part be due to the gregarious nature of ascidians, whereby the presence of conspecific individuals (other larvae, juveniles or established adults) encourages further settlement of larvae within an area (Petersen and Svane 1995; Rius et al. 2010). This localised retention may have important consequences in terms of the ability of populations to adapt to local habitat change and is likely to play a key role in the persistence of populations (Strathmann et al. 2002; Kawecki and Ebert 2004).

The lower recruitment at the surface compared with deeper plate arrays at RK was unexpected, as observations from elsewhere in the region indicated that infestation levels on artificial structures were relatively uniform with depth. The

results at RK are most likely explained by the fact that the majority of water flow within the upper 4 m was perpendicular to the plate arrays; hence the majority of larvae would have been advected away from the plates. Currents in the surface layer were also faster moving due to wind effects, and it is possible that greater boundary layer water movement may have reduced settlement success (Crimaldi et al. 2002; Knights et al. 2006). A variety of other mechanisms may also have played some role in the vertical patterns observed; including the influence of physical and chemical habitat cues (e.g. Degnan and Johnson 1999; Green et al. 2002), the presence or absence of conspecifics (e.g. Young and Braithwaite 1980; Manriquez and Castilla 2007; Rius et al. 2010), as well as the particular light conditions encountered (e.g. Svane and Dolmer 1995; McHenry and Strother 2003; Rius et al. 2010). In the current study, it is possible that larvae actively avoided the surface layer, as indicated for a variety of coastal invertebrates (Shanks and Shearman 2009), or that ontogenetic changes in orientation behaviour may have influenced larval position within the water column. For ascidians generally, this behaviour usually follows a characteristic pattern of positive phototropism and negative geotropism (resulting in upward swimming) throughout the swimming period, with a switch to the opposite responses (resulting in downward swimming or sinking) just before settlement (Svane and Young 1989). Previous work with colonial ascidians has demonstrated that larvae are capable of controlling their vertical position in the water column. However, this phenomenon can be quite variable, as the larvae of some species occur closer to the seabed as distance from the larval source increases (Davis and Butler 1989; Bingham and Young 1991), while others can spend the majority of their time passively drifting or swimming upwards (Young 1986).

The decrease in recruitment observed after Trial 1 at RK (i.e. RK at distance = 0 m) was generally accompanied by a simultaneous reduction in recruitment density for each given distance from the source, and a reduction in

the dispersal distance estimated by the exponential decay model. The decreased recruitment may in part reflect reduced reproductive output at RK over Trials 2 and 3 compared to Trial 1 due to seasonal patterns (Fletcher et al. 2013; see Chapter 4), but is probably more a reflection of the considerable reduction in *Didemnum* biomass that occurred due to marine farm operations. In the same way that recruitment levels were reduced at RK with the biomass reduction, the overall lower recruitment measured at BB is consistent with the fact that source population biomass at that location was some two orders of magnitude less than at RK. Such findings are in line with expectations regarding the role of propagule pressure in invasion success (Leung and Mandrak 2007; Johnston et al. 2009; Lockwood et al. 2009); hence, it stands to reason that sites containing source populations of a greater biomass could lead to proportionately greater realised dispersal.

During the field experiments, the regional spread of *Didemnum* from ten discrete source populations within Queen Charlotte Sound (Figure 5.1) was being simultaneously monitored as part of a regional surveillance programme, the results of which support the findings of the field study (Pannell 2007). The surveillance programme yielded data from 68 monitoring arrays located up to 688 m from source populations (generally located on isolated wharves, jetties, moorings, or marine farms). The results indicated highly variable patterns of recruitment in relation to distance from the source (Table 5.4). Thirty one (c. 46 %) of the stations were found to have at least one settlement plate with *Didemnum* colonies present while the remaining 37 stations had no visible colonies present. The infected stations covered a range of distances from the source populations with the furthest infected station located 350 m from its closest source. This station was located in Ruakaka Bay, adjacent to the RK site used for the dispersal trials. Interestingly, several stations located less than 50 m from source populations were clear of *Didemnum*, highlighting the considerable degree of variability in the successful spread of this species.

Table 5.4 Results of an industry-led surveillance programme documenting the natural dispersal of *Didemnum vexillum* larvae at 68 stations adjacent to ten discrete source populations within Queen Charlotte Sound, New Zealand. Infected stations had *Didemnum* colonies present on at least one of the three settlement plates examined.

Distance from source (m)	Status of *Didemnum* colonies on monitoring stations		
	Didemnum present	*Didemnum* absent	Stations examined
0 - 50 m	18	13	31
51 - 100 m	3	11	14
101 - 150 m	2	2	4
151 - 200 m	2	3	5
201 - 250 m	3	1	4
251 - 300 m	1	0	1
301 - 350 m	2	1	3
351 - 400 m	0	2	2
401 - 450 m	0	1	1
451 - 500 m	0	1	1
> 500 m	0	2	2

Exponential decay models predicted a maximum dispersal of > 1000 m at RK, hence greater dispersal might have been measured at that location had it been feasible to deploy settlement plates at distances beyond 250 m. The larvae of this species will almost certainly be advected across greater distances than indicated by the settlement plate results, as the laboratory trials described above indicate larval viability was clearly unlikely to be limiting at the scale of these trials. It is possible larvae may also be advected beyond the maxima predicted by the exponential decay curves, as these predictions were based on the settlement plate detection method, which may underestimate true dispersal. For example, at average water current speeds of 10 $cm.s^{-1}$, larvae that

were still competent after c. 1 day could theoretically have been advected on the order of 10 km, given a unidirectional current. The extent to which such conditions arise will be situation-specific, and in the current study region would only occur during episodic events such as strong and persistent winds.

5.5.3 Dispersal potential in Didemnum vexillum and a conceptual model for spread

It is also recognised that relatively long-distance advection may be less likely when the many factors that limit true dispersal and subsequent establishment in nature are considered. In addition to factors limiting dispersal distance, such as predation in the water column, the successful establishment and persistence of populations will depend on a wide range of post-settlement processes not taken into account here (e.g. Booth and Brosnan 1995). The majority of invasive invertebrates have annual spread rates that can be similar to, but are often less than, their predicted average dispersal distance (Kinlan et al. 2005), often because of processes leading to propagule retention (McQuaid and Phillips 2000; Kinlan and Gaines 2003; Levin 2006). Nonetheless, the possibility that larvae of *Didemnum* may be competent after dispersal over scales of a few kilometres is a plausible explanation for the extended range expansions of this species in the study region in situations where anthropogenic transport vectors were not implicated.

Didemnum colonies are capable of both sexual and asexual reproduction and are potentially able to utilise both mechanisms in their dispersal strategies (Lengyel et al. 2009). *Didemnum* colonies often exhibit a long tendril-like morphology that leads to the generation of fragments. Small fragments and those associated with some algal species or floating debris were noted drifting within the water column within the study area, which could enhance dispersal. Sinking rates for detached fragments range from 3.11 $cm.s^{-1}$ to 8.68 $cm.s^{-1}$,

depending on fragment size (L. Fletcher, unpubl. data). For example, given water currents of 10 $cm.s^{-1}$ and a depth of 30 m, fragments with lowest sinking rates could spread perhaps 100 m from their release point. While this is at least an order of magnitude less that potential larval dispersal, it may nonetheless be an important additional mechanism of local spread, as colony fragmentation appears an important mode of establishment in *Didemnum* (Coutts and Forrest 2007; Bullard et al. 2007), as it is for other colonial ascidians (Worcester 1994; Agius 2007). In addition, despite larvae being important for longer range dispersal, juvenile ascidians can experience high levels of post-settlement mortality (Stoner 1990; Gosselin and Qian 1997; Osman and Whitlatch 2004), whereas fragmentation reattachment success is generally high in *Didemnum* (Hopkins et al. 2011; Morris and Carman 2012).

A conceptual model of spread for *Didemnum* therefore consists of at least three key elements, similar to that described for the spread of the invasive kelp *Undaria pinnatifida* (Forrest et al. 2000): human-mediated transport at regional scales or greater; a combination of anthropogenic and larval dispersal that results in range expansion and the development of new populations within regions (e.g. across scales of kilometres); and fragmentation of established colonies, which in combination with larval dispersal is likely to lead to local intensification in establishment over scales of tens to hundreds of metres (Figure 5.6). Whereas the longer range processes involving larval dispersal and anthropogenic transport may be most important in establishing founding populations, local infilling may be driven by fragmentation processes. However, there are clearly a range of factors that are independent of propagule supply, such as suitable habitat availability and local environmental conditions, which will play key roles in determining whether a species becomes established in a new environment (Caley et al. 1996).

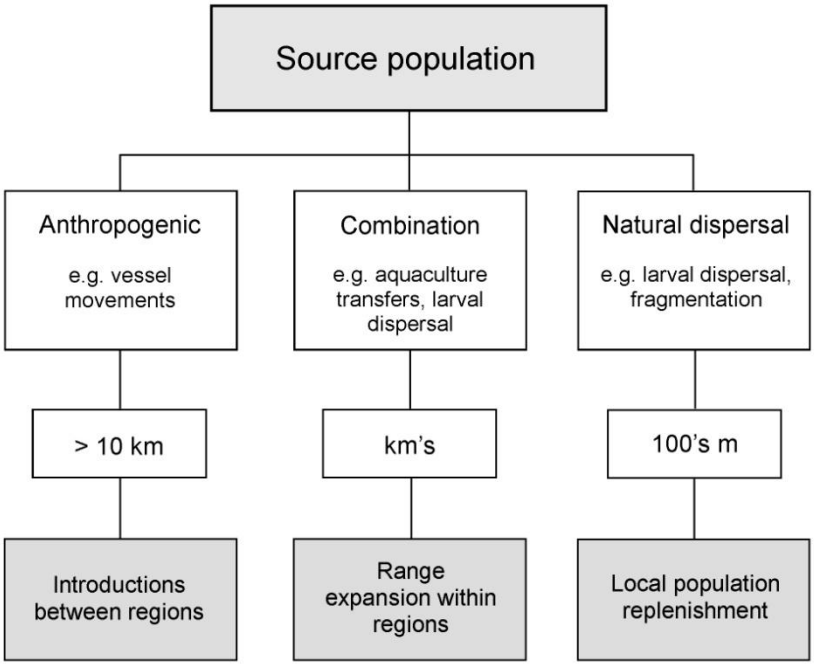

Figure 5.6 Conceptual model of pathways of spread from established *Didemnum vexillum* populations. Pathways are represented by three vector types: anthropogenic (human-mediated spread); a combination of anthropogenic and natural dispersal mechanisms; and purely natural dispersal mechanisms. Vector type will influence the scale of spread (between regions, within regions and local) and thus the resulting type of population establishment.

5.5.4 Implications for management of Didemnum vexillum

A management programme for *Didemnum* in the current study region was based on the premise that the competency period for this species was of a similar order to that for other species in the same genus (i.e. < 2 hours). The fact that it may be at least an order of magnitude longer has significant implications for management, and highlights the need for species-specific information to underpin decision-making. The recognition of an unexpectedly long larval

competency period for *Didemnum*, and the possibility that occasionally the species may be capable of natural range extensions over scales of a few kilometres, led stakeholders to re-evaluate their attempts to manage the spread of this species in New Zealand. Essentially, efforts to manage anthropogenic vectors and control established populations were potentially being undermined by the dispersal of *Didemnum* beyond zones of regular surveillance and delimitation. Although eradication is no longer regarded as feasible in the study region, effective control to reduce the risk of spread to certain high value sub-regions (e.g. important areas for shellfish aquaculture seed-stock) may still be possible, and the increased understanding of natural dispersal potential provided here is critical to such efforts.

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CHAPTER 6

IMPACTS OF THE INVASIVE ASCIDIAN *DIDEMNUM VEXILLUM* ON GREEN-LIPPED MUSSEL (*PERNA CANALICULUS*) AQUACULTURE IN NEW ZEALAND

Preface

Empirical knowledge of actual and potential impacts of marine pests provides a necessary context for understanding the importance of management and for optimising the management approaches utilised. This chapter leads on from the biological attributes investigated in earlier chapters, and explores the associated impacts of *Didemnum* to the New Zealand green-lipped mussel industry.

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Author contributions:

The author co-designed the experiments, carried out field and laboratory work, analysed the data and prepared the manuscript. My co-authors assisted with experimental design and aspects of field work, as well as providing feedback on earlier versions of the manuscript.

6.1 Abstract

Biofouling can pose a significant threat to shellfish aquaculture, as fouling organisms are often strong spatial competitors that are able to reach high densities or biomass in relatively short time frames. This study investigated the potential impacts of the colonial ascidian *Didemnum vexillum* on cultured New Zealand green-lipped mussels, *Perna canaliculus* at one farm in the Marlborough Sounds region. Three size classes of mussels were examined: small (20-40 mm shell length at deployment); medium (40-60 mm); and large (60-70 mm). Replicate 4 m lines were assigned to one of three treatments: (a) ambient fouling; (b) fouling enhanced by *Didemnum* fragment inoculation (in addition to ambient fouling); and (c) control lines which were kept free of *Didemnum*. After 15 months, subsections of lines (0.5 m length) were processed to determine the effects of fouling cover on mussel density within lines, as well as on individual mussel size and condition. A highly significant negative relationship was shown between *Didemnum* biomass and mussel density for small mussels, and to a lesser extent for medium mussels. Values of mussel condition indices were similar across size classes, and across fouling treatments within each size class. These results indicate mussels may only be vulnerable to direct *Didemnum* fouling impacts at early stages of production, and that impacts may be restricted to displacement of mussels as opposed to reduced size and condition. This information will assist in the implementation of management procedures through increased understanding of *Didemnum* effects at different stages of mussel production.

6.2 Introduction

Marine aquaculture is the world's fastest growing primary sector, providing an essential food resource to a rising world population. An inherent issue in the commercial culture of marine species is that submerged surfaces are colonised by a range of organisms, a process termed biofouling (Railkin 2004). Biofouling accumulation can pose a significant threat to marine aquaculture industries, with many biofouling species being strong spatial competitors that can reach very high densities or biomass over relatively short periods (Dealteris et al. 2004; Blum et al. 2007). Negative effects of biofouling in aquaculture can include impacts to cultured species directly, impacts to infrastructure (immersed structures such as cages, netting and pontoons), as well as associated environmental impacts on local ecosystems. The costs associated with biofouling are believed to be substantial, but are often difficult to quantify. In addition to revenue lost through effects on crop (e.g. Jeffs and Stanley 2010), there are a number of direct costs associated with control and mitigation efforts (Adams et al. 2011). Conservative estimates of direct economic losses attributed to biofouling within the European aquaculture industry have been appraised at 5 10 % of the industry value (Lane and Willemsen 2004), and more specifically up to 20 % and 30 % of final market price for oysters (Enright 1993) and scallops (Claereboudt et al. 1994), respectively.

Direct impacts of biofouling on marine aquaculture can vary considerably depending on the species cultured and the method of culture (reviewed by Fitridge et al. 2012), the geographic location and local environmental conditions. Commercial shellfish culture appears to be particularly prone to biofouling due to the creation of complex novel substrates that arise through the combination of bivalve shells and artificial structures (McKindsey et al. 2007). In addition, farm structures are usually suspended and may be protected from predation, thereby providing a refuge for biofouling organisms (Rocha et al. 2009). The overall composition and biomass of biofouling communities is spatially and

temporally variable (Woods et al. 2012). Communities are generally characterised by the presence of a variety of sessile, suspension-feeding invertebrates including: ascidians, bivalves, hydroids, bryozoans and cnidarians, as well as an array of macro algae (Scheer 1945), and may include non-indigenous species. While there are instances where biofouling presence has not been found to adversely affect shellfish culture (e.g. Arens et al. 2011), the presence of fouling species, particularly large aggregations, is in most cases seen as detrimental to these activities.

Ascidians are among the most prolific and devastating biofoulers to shellfish aquaculture operations globally (Lambert 2007; Adams et al. 2011). Negative effects on production caused by ascidian fouling can include crop losses through additional weight placed on shellfish species (Boothroyd et al. 2002; Thompson and MacNair 2004; Ramsay et al. 2008), as well as impacts on general operations within farms as high ascidian cover can impede the efficiency of processing equipment (Davis and Davis 2010). Cultured shellfish are also particularly vulnerable to interference competition from overgrowth; colonial ascidians often create a physical barrier, compromising the opening of shellfish valves and reducing the availability of food to the shellfish underneath (Lesser et al. 1992; Lodeiros and Himmelman 1996). Furthermore, direct competition for food resources has been demonstrated between ascidians and cultivated shellfish, including several species of oysters (Riisgård et al. 1995), mussels (Le Blanc et al. 2003; Daigle and Herbinger 2009), and scallops (Ross et al. 2004; Su et al. 2008).

Currently, several introduced ascidians threaten New Zealand's highly valued green-lipped mussel (*Perna canaliculus*) industry, and efforts to control and manage these species are ongoing. Relatively low profit margins in this industry mean the impacts of biofouling and expenditures associated with control can be very significant at the individual farm level. The recent human-mediated

introduction of the invasive colonial ascidian *Didemnum vexillum* (Kott 2002) has led to negative effects on mussel culture within the Marlborough Sounds region (Figure 6.1), which is New Zealand's most important growing area for green-lipped mussels. Since first being detected in 2001, *Didemnum* has been inadvertently spread within the region by anthropogenic transport mechanisms (Coutts and Forrest 2007). *Didemnum* colonies are capable of rapid growth and expansion through both sexual and asexual reproduction, and as such are able to quickly colonise large areas of artificial and natural substrata (e.g. Coutts and Forrest 2007; Valentine et al. 2007b). This increased biomass can lead to the destabilisation of mussel crops and added weight on infrastructure, which has led to substantial mitigation and control costs (Pannell and Coutts 2007).

In order to determine the cost effectiveness of possible mitigation strategies, it is necessary to quantify actual impacts of biofouling species on shellfish culture and the causative factors. Blue mussels (*Mytilus edulis*) fouled by *Didemnum* in the north-east United States have been shown to have smaller shell lengths and lower condition values than those free of fouling within experimental systems (Auker 2010). However, there remains a lack of empirical data regarding the impacts of *Didemnum* fouling within an industry setting, as previous work is primarily anecdotal. As long as evidence of impacts remains largely speculative, rational and fully informed management decisions cannot be made. This study investigated the impacts of *Didemnum* biofouling on the commercial culture of New Zealand green-lipped mussels. The effect of *Didemnum* fouling on mussel survival was evaluated experimentally through assessment of mussel density on mussel line sections with varying levels of fouling cover. In addition, individual mussels were evaluated for effects of fouling on size and condition through measurement of a range of morphometric indices. Effects were quantified between three size classes of mussels, in order to determine whether some mussel life-stages or stages of the production cycle are more vulnerable to negative effects of *Didemnum* overgrowth.

6.3 Methods

6.3.1 Study site

The experiment was carried out on a mussel farm located on the northern side of Fairy Bay (41°07'S, 173°52'E), situated within Pelorus Sound, which is part of the larger Marlborough Sounds system (Figure 6.1). Pelorus Sound is a relatively deep (average depth ~ 40 m), narrow, and highly indented system which is subject to freshwater input at its head from two rivers. Fairy Bay is located approximately 25 km from the head of the Sound. The mussel farm used for the experiment had previously been identified as having a well-established *Didemnum* population.

6.3.2 Set-up of experimental mussel lines

Mussels free of *Didemnum* fouling were collected on 21 November 2008 from nearby farms within Pelorus Sound. A continuous length of culture rope (~ 60 m) was transplanted for each of the three mussel size classes: small (20-40 mm shell length); medium (40-60 mm); and large (60-70 mm) (Table 6.1). Following collection, each length of mussel rope was cut into 15 replicate 4 m lines (45 lines in total). There were approximately 670 mussels per metre on each line within the small size class, and 140 to 150 mussels per metre on the lines with medium and large mussels, respectively (Table 6.1). The medium and large mussels had been reseeded to reduce crowding as part of standard industry practice. Five replicate vertical lines of each of the three size classes were randomly assigned to each of three *Didemnum* fouling treatments: (1) ambient fouling, in which lines were left to get naturally fouled by *Didemnum* and other species; (2) ambient fouling enhanced with fragment inoculation, in which lines were artificially inoculated with *Didemnum* colonies (hereafter referred to as 'enhanced fouling') in an attempt to simulate 'worst-case' fouling; and (3) control lines on which fouling was maintained at a very low level by removal of

Didemnum. Fragment inoculation was used as an enhanced fouling treatment method as *Didemnum* fragments have been demonstrated to attach to substrata and grow extremely rapidly (Valentine et al. 2007a; Morris and Carman 2012). Replicate vertical lines of each treatment were randomly allocated along the two horizontal backbone ropes, with each line spaced approximately 25 cm apart.

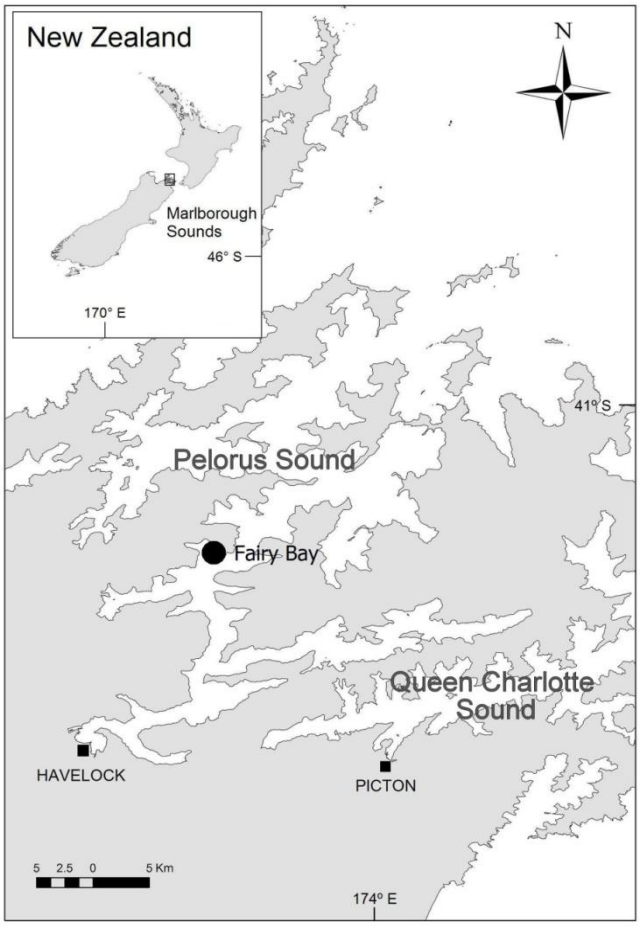

Figure 6.1 Map of the Marlborough Sounds region showing an inset of the location within New Zealand. A filled circle indicates the study site at Fairy Bay.

Table 6.1 Average shell length, meat weight, and stocking density of mussels (*Perna canaliculus*) within each size class at the start of the experiment.

Size class	Shell length (mm ± SE)	Meat weight (g ± SE)	Density (ind./ m)
Small	30.32 ± 0.41	2.16 ± 0.08	~ 670
Medium	53.91 ± 0.49	12.38 ± 0.30	~ 140
Large	64.28 ± 0.48	19.90 ± 0.39	~ 150

The enhanced fouling treatment lines were inoculated on 28 November 2008. Medium sized *Didemnum* fragments (~ 30 cm², ~ 50 g) were attached to the mussel lines using industry-supplied continuous tubular cotton mesh stocking. The stocking encased the mussel line with colony fragments inserted into the stocking approximately every 30 cm. The stocking facilitated establishment of *Didemnum* colonies by holding them in place on the mussels, and the cotton biodegraded after several weeks. Only the enhanced fouling treatment lines were encased in the cotton mesh stocking at this stage. Due to the large size of the mesh and temporary nature of the material, detrimental effects of this encasement on mussel growth and survival were not expected and were not controlled for. The *Didemnum* colonies established from fragments had reached a considerable level of cover six weeks following initial inoculation of the lines (Figure 6.2a).

Figure 6.2 Set-up of experimental mussel (*Perna canaliculus*) lines showing: **a.** enhanced fouling treatment lines 6 weeks after inoculation with *Didemnum vexillum* fragments; and **b.** experimental lines on 29 January 2010, ~ 14 months after deployment. The two lines in the centre are from the control group and the lines on the far left and far right are from the enhanced fouling group, highlighting the differences in *Didemnum* biomass present between treatments.

The experiment ran for 15 months, a period that included two Austral summers (December - March), corresponding with periods of increased *Didemnum* growth and reproduction within this region (Fletcher et al. 2013; see Chapter 4). Water temperatures ranged from 8.3 °C during winter months (June 2009) to 20.2 °C in summer (February 2010). Control lines were treated to eliminate fouling three times over the course of the experiment; on 6 March 2009, 4 June 2009 and 29 January 2010. Treatment involved a combination of freshwater immersion and manual removal of colonies. Large *Didemnum* colonies were removed by hand, after which the lines were completely immersed in freshwater (~ 3 psu) for 3 hours within a 600 L plastic bin then returned to their original position on the backbone rope. Ideally, additional lines subjected to manual removal alone (as well as immersed in sea water for the same duration) would have been incorporated as a procedural control for the freshwater treatment; however, logistically this was not possible. In addition, short duration freshwater immersion has been shown to be an effective method of *Didemnum* removal (Denny 2008) that has no long-term detrimental effects on the survival or growth of *P. canaliculus* (Forrest and Blakemore 2006). The control lines were consistently free of appreciable fouling over the course of the experiment (Figure 6.2b).

6.3.3 Processing of mussel lines

Collection and sample processing at the end of the 15 month deployment was carried out during autumn (2 March 2010 and 25 March 2010) at a time when *Didemnum* colony biomass was still at a seasonal high, and prior to mussel spawning and the loss of condition that was expected to occur during the June to July period (Fox 2003). Two replicate 4 m lines were lost during the experiment (large mussel control and large mussel ambient fouling), so final processing was carried out for 43 lines in total. One 0.5 m section was randomly selected from each line, and all mussels and *Didemnum* colonies present within

each section were removed. The upper parts of the lines, in the 0-1 m depth interval, were not sampled as these mussels had been exposed to low salinity following several major storm events. The number and weight of mussels and *Didemnum* colonies was recorded for each 0.5 m section sampled; and a random subsample of 20 mussels was collected and returned to the laboratory for analysis of mussel size and condition. To investigate effects of *Didemnum* biomass on mussel density, additional 0.5 m sections were processed as described above for the 4 m lines that received ambient or enhanced fouling treatments.

6.3.4 Mussel size and condition measurements

A total of 860 mussels (20 mussels from 43 sections) were processed in randomly ordered batches over a 2 day period. A range of morphometric characteristics were measured, including shell length, whole wet weight, whole (live) volume, shell wet weight, half-shell weight of the cooked mussel, tissue cooked weight, and tissue dry weight. Measurements of shell length, whole wet weight, and live volume were completed initially, after which mussels were frozen and later thawed for further analysis. Once thawed, mussels were cooked in water at 95 °C for 5 minutes, then immersed in cold water for 2 minutes and left standing for a further 2 minutes to drain any excess water. Shell wet weight, half-shell weight and tissue cooked were measured separately using pre-weighed aluminium dishes. Dry tissue weights were recorded after drying at 80 °C to a constant weight (~ 48 hours). All weights were recorded to the nearest ± 0.01 g and length measurements to the nearest ± 0.01 mm. Mussel volume was recorded to the nearest 1.0 ml (using a 250 ml graduated measuring cylinder).

Condition index (CI, Hickman and Illingworth 1980), was calculated using the formula:

$$CI = \frac{dry\ tissue\ weight}{whole\ \ live\ \ weight - shell\ wet\ weight} \times 100$$

In addition, an industry applied green weight index (GWI, Fox 2003) was calculated using the formula:

$$GWI = \frac{cooked\ meat\ weight}{whole\ \ live\ \ weight} \times 100$$

6.3.5 Statistical analysis

Fouling effects on mussel density

Mussel loss for each 0.5 m section (expressed as a percentage of initial stocking density; see Table 6.1) was calculated to enable comparisons between size classes. Differences in mussel density, mussel weight and mussel loss among treatments were tested using a distance-based univariate permutational analysis of variance (PERMANOVA, Anderson 2001) based on Euclidean similarity matrices of the data. The experimental design comprised two factors: mussel size (fixed with three levels) and fouling treatment (fixed with three levels). The distribution of each individual variable was first examined for departures from normality and homogeneity of variance. Data were transformed, if necessary, to achieve approximate unimodal symmetry, to avoid right skewness and to eliminate intrinsic mean–variance relationships. Each

term in the analyses was tested using 4999 random permutations of the appropriate units. Significant terms were investigated using *a posteriori* pairwise comparisons with the PERMANOVA *t*-statistic and 999 permutations. For the ambient and enhanced fouling treatments, the effect of *Didemnum* biomass on mussel density within each size class was further investigated using linear regression analysis. *Didemnum* biomass was treated as a continuous predictor variable, irrespective of fouling treatment, and the relationship with mussel density modelled. Due to the loss of lines during the experimental phase, both the PERMANOVA and regression analyses were run unbalanced.

Fouling effects on size and condition

Differences in mussel shell length, wet weight, and condition (CI and GWI) between experimental treatments were tested using univariate PERMANOVA as described above. The experimental design comprised three factors: size (fixed with three levels), fouling treatment (fixed with three levels), and line (random and nested in the size x fouling treatment interaction). Due to the loss of lines during the experimental phase, only the data from four randomly chosen replicate lines were analysed. The effect of *Didemnum* biomass on mussel condition (CI and GWI) was examined through linear regression analysis as described above for the effect on mussel density. As CI and GWI account for variations in mussel size, data were pooled across size classes for these regression analyses (n = 29 sections). Permutational analyses of variance were performed using the software PRIMER 6 and PERMANOVA (Clarke and Gorley 2006; Anderson and Gorley 2007). All other analyses were carried out using the software package STATISTICA version 9.1 (StatSoft Inc. 2010).

6.4 Results

The level of *Didemnum* fouling present within the sections processed varied considerably between treatment lines. The average biomass present across all ambient fouling and enhanced fouling lines, irrespective of mussel size class, was comparable at the conclusion of the study (0.67 ± 0.10 kg per section and 0.59 ± 0.07 kg per section, respectively). Control lines were kept consistently free of *Didemnum* fouling, and all sections had no colonies present at the time of sampling.

6.4.1 Effects of Didemnum vexillum fouling on mussel density and weight

There was a significant size class x fouling treatment interaction effect on both mean mussel density and mean mussel weight (Figure 6.3; Table 6.2a,b), driven by variation in fouling treatment effects between size classes. Pairwise comparisons indicated a significant effect of fouling treatment on both variables in the small size class, but not in the medium or large ($p < 0.05$; Figure 6.3). On average, the density of small size class mussels was between 83 and 121 % higher in the control treatment (247.8 ± SE 24.2 mussels per section) compared to the enhanced fouling and ambient fouling treatments (135.2 ± 17.9 and 111.7 ± 19.2 mussels per section, respectively). Mussel weight followed a similar pattern, with the mean weight of small size mussels in the controls between 42 and 77 % higher (7.11 ± 0.31 kg) than the enhanced fouling and ambient fouling treatments (5.02 ± 0.44 and 4.01 ± 0.58 kg, respectively).

Percentage mussel loss varied significantly between size classes (Figure 6.4; Table 6.2c). Pairwise comparisons indicated significant differences in mussel loss between the small size class compared to both other size classes ($p < 0.01$); however, no overall effect of fouling treatment on mussel loss was detected ($p > 0.05$; Table 6.2c). Although not significant ($p = 0.08$), the interaction effect indicated a variation in fouling effects between size classes, due to a greater loss of small mussels in the ambient fouling and enhanced fouling treatments (66.8 ± 5.7 % and 59.8 ± 5.3 %, respectively) compared with the small mussel controls (26.3 ± 7.2 %).

Linear regression analysis showed a strong and highly significant negative relationship between *Didemnum* biomass and mussel density within the small mussels ($r^2 = 0.631$, $p < 0.001$, Figure 6.5a). The model predicted ~ 40 % mussel loss for an increase of 1 kg of *Didemnum* fouling per 0.5 m section. A similar but weaker relationship was evident in the medium size class ($r^2 = 0.253$, $p = 0.024$, Figure 6.5b). In contrast, there was no relationship between *Didemnum* biomass and mussel density for large mussels ($r^2 = 0.130$, $p = 0.141$, Figure 6.5c).

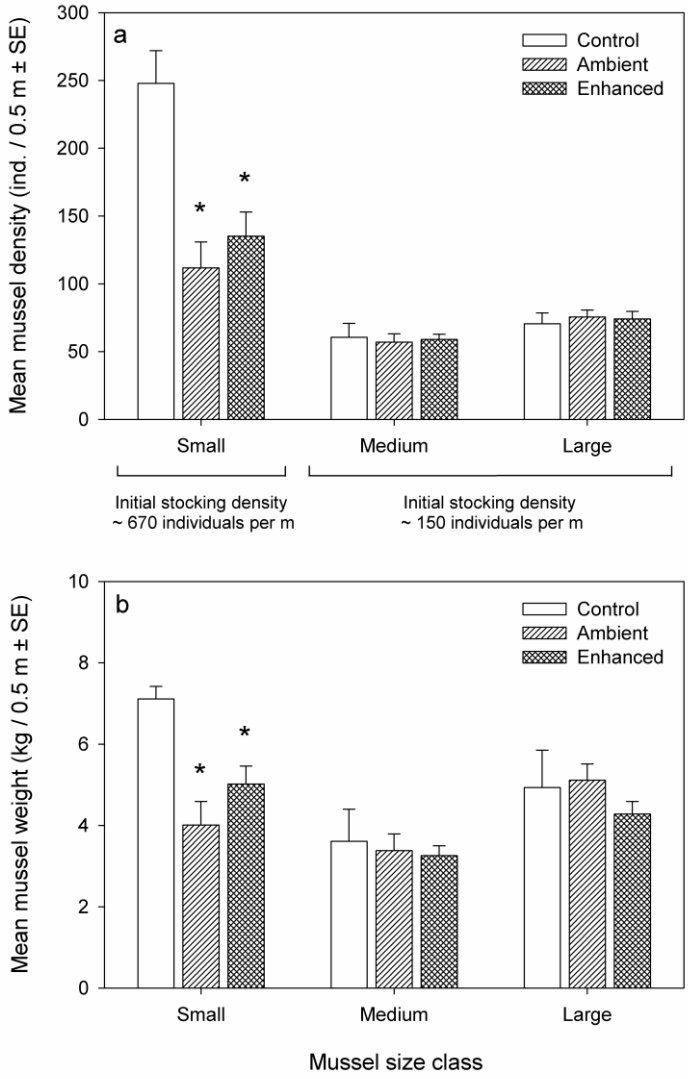

Figure 6.3 a. Mean mussel density and **b.** mean mussel combined weight (± SE, *n* = 10 for ambient and enhanced and *n* = 5 for controls, except large ambient *n* = 8 and large control *n* = 4) of *Perna canaliculus* present per 0.5 m section of line for three size classes of mussels (small: 20-40 mm shell length at deployment; medium: 40-60 mm; and large: 60-70 mm) and three fouling treatments (controls, ambient fouling, and enhanced *Didemnum vexillum* fouling). Initial stocking densities of the size classes per metre of experimental line are indicated. Stars denote significant fouling treatment effects (within mussel size class) in relation to control lines as indicated by the pairwise comparisons (*t*-statistic, $p > 0.05$).

Table 6.2 Results of permutational ANOVAs testing for mussel size class and fouling treatment effects on: **a.** mean mussel density; **b.** mean combined mussel weight; and **c.** percentage of mussel loss over the 15 month deployment. Analyses were based on Euclidean distances of the data and each term was tested using 4999 random permutations of appropriate units.

Source of variation	*df*	a. density			b. weight			c. mussel loss		
		MS	Pseudo-F	*p* (perm)	MS	Pseudo-F	*p* (perm)	MS	Pseudo-F	*p* (perm)
Size	2	0.912	23.823	**< 0.01**	22.584	12.894	**< 0.01**	12636.00	28.112	**< 0.01**
Fouling	2	0.095	2.476	0.089	6.037	3.447	**0.038**	802.08	1.785	0.177
Size x Fouling	4	0.114	2.985	**0.027**	5.476	3.127	**0.024**	983.61	2.188	0.081
Residual	63	0.038			1.751			449.47		
Total	71									
Transformation			Log_{10}			None			None	

Significant values ($p < 0.05$) are indicated by bold type.

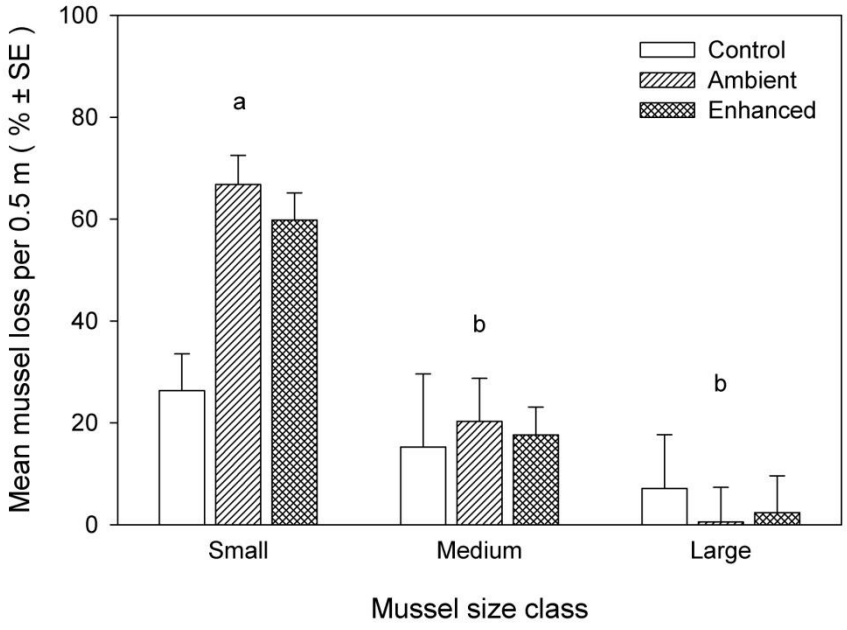

Figure 6.4 Mean mussel (*Perna canaliculus*) loss (% ± SE, $n = 10$ for ambient and enhanced and $n = 5$ for controls, except large ambient $n = 8$ and large control $n = 4$) per 0.5 m section of line for three size classes of mussels (small: 20-40 mm shell length at deployment; medium: 40-60 mm; and large: 60-70 mm) and three fouling treatments (controls, ambient fouling, and enhanced *Didemnum vexillum* fouling). Size classes sharing a letter indicate groupings from the pairwise comparisons that were not significantly different from each other (*t*-statistic, $p > 0.05$).

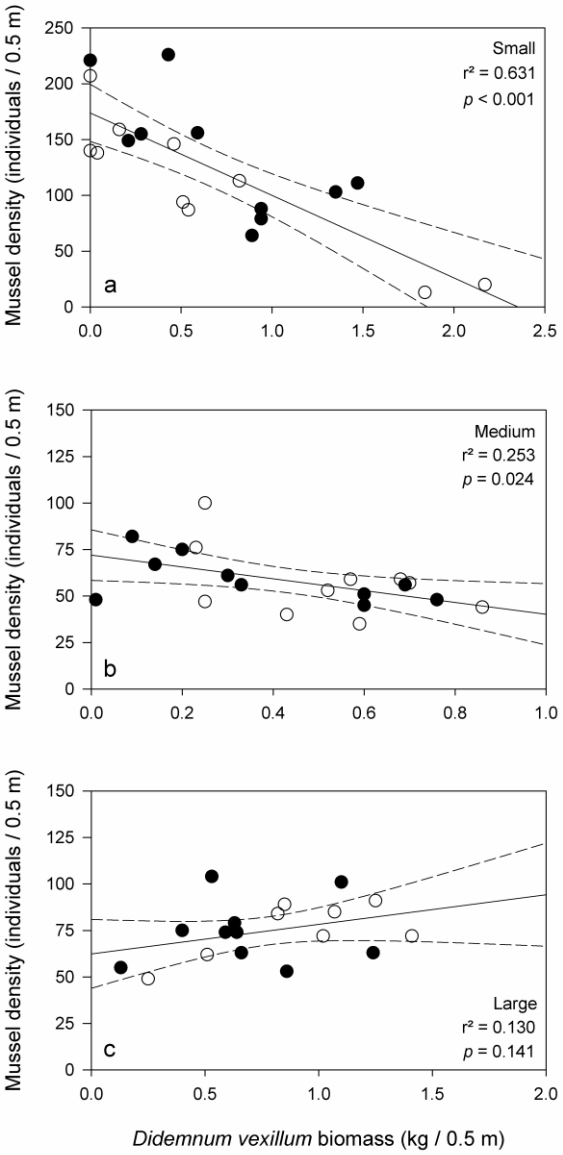

Figure 6.5 Relationship between mussel (*Perna canaliculus*) density and *Didemnum vexillum* biomass per 0.5 m section. Results are presented for the: **a.** small size class (20-40 mm shell length at deployment); **b.** medium size class (40-60 mm); and **c.** large size class (60-70 mm) of mussels. Unfilled circles = ambient fouling. Filled circles = enhanced *Didemnum* fouling. Dashed lines represent 95 % confidence intervals. Note the differences in scales on the x- and y-axes.

6.4.2 Effects of Didemnum vexillum fouling on mussel size and condition

After 15 months, mussels within the small size class were significantly smaller than the other two size classes. By contrast, mussels within the medium and large size classes were a similar size at the end of the experiment (Figure 6.6). PERMANOVA analyses revealed highly significant effects of mussel size and replicate line (indicating spatial variability) on both the shell length and live weight of individual mussels ($p < 0.01$; Table 6.3). Pairwise comparisons indicated significant differences in both shell length and live weight between mussels in the small size class compared to both other size classes ($p < 0.01$), but not between the medium and large size classes for both shell length ($p = 0.447$) and live weight ($p = 0.640$). No significant effect of fouling treatment was detected for either shell length ($p = 0.078$; Table 6.3) or live weight ($p = 0.359$; Table 6.3). Although marginally non-significant, the interaction effects for both shell length and live weight ($p = 0.057$ and $p = 0.071$, respectively) indicated a variation in fouling effects between size classes. In particular, within the small size class, the shell length and live weight of individual mussels within the ambient fouling (82.85 ± 1.04 mm shell length and 34.41 ± 1.10 g live weight) and enhanced fouling treatments (81.53 ± 0.89 mm shell length and 31.97 ± 0.93 g live weight), were greater than in the controls (71.95 ± 0.66 mm shell length and 21.79 ± 0.64 g live weight).

There was no apparent effect of fouling on mussel condition. Within each of the CI and GWI indices, condition values were similar across the three size classes of mussels, and across fouling treatments within each size class (Figure 6.7). PERMANOVA analyses revealed highly significant spatial variability in both indices (Line, $p < 0.01$; Table 6.4), but no significant effect of fouling treatment was detected for either CI ($p = 0.230$; Table 6.4) or GWI ($p = 0.189$; Table 6.4). There was a significant effect of size class on CI ($p = 0.042$; Table 6.4), but no significant difference in GWI ($p = 0.173$; Table 6.4). Pairwise comparisons indicated significant differences in CI between the medium and large mussels only ($p < 0.01$). Linear regression analyses indicated no significant relationship between *Didemnum* biomass and both CI ($r^2 = 0.110$, $p = 0.079$; Figure 6.8a) and GWI ($r^2 = 0.023$, $p = 0.431$; Figure 6.8b).

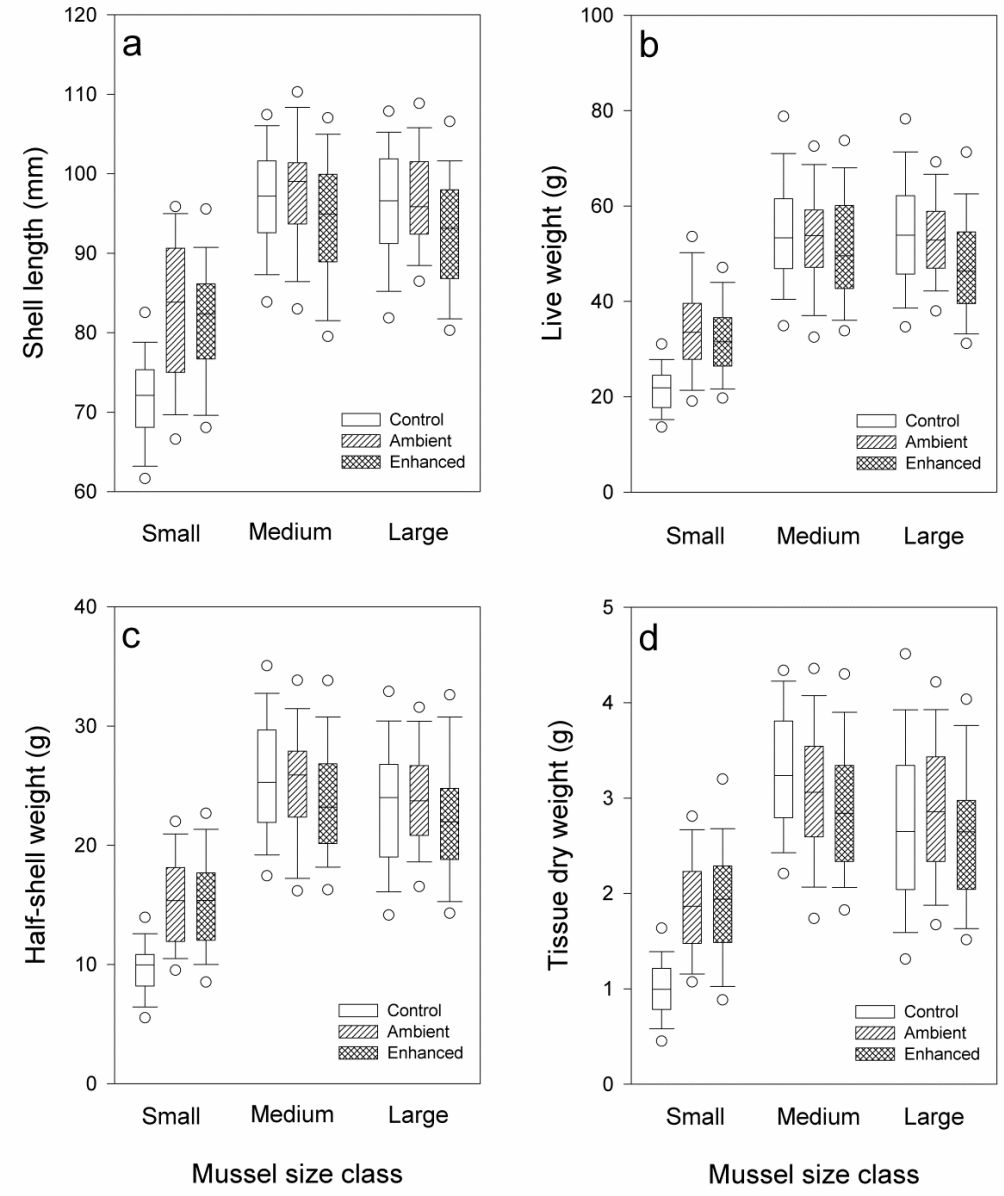

Figure 6.6 Various indices of individual mussel (*Perna canaliculus*) size: **a.** shell length; **b.** live weight; **c.** half-shell weight; and **d.** tissue dry weight, for the varying mussel size classes (small: 20-40 mm shell length at deployment; medium: 40-60 mm; and large: 60-70 mm) and fouling treatments (controls, ambient fouling, and enhanced *Didemnum vexillum* fouling). The crossbar inside each box indicates the median, bottom and top whiskers represent the 10th and 90th percentile, respectively, while lower and upper circles represent the 5th and 95th percentile, respectively.

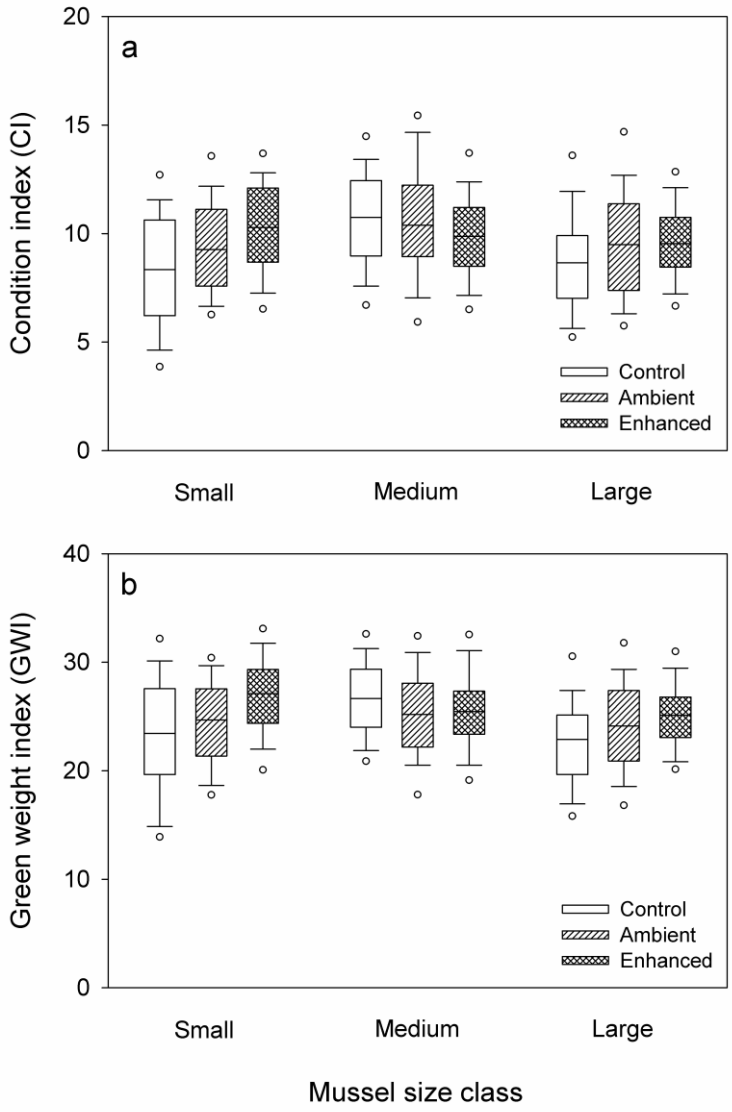

Figure 6.7 Individual mussel (*Perna canaliculus*) **a.** condition index (CI) and **b.** green weight index (GWI) for the mussel size classes (small: 20-40 mm shell length at deployment; medium: 40-60 mm; and large: 60-70 mm) and three fouling treatments (controls, ambient fouling, and enhanced *Didemnum vexillum* fouling). The crossbar inside each box indicates the median, bottom and top whiskers represent the 10th and 90th percentile, respectively, while lower and upper circles represent the 5th and 95th percentile, respectively.

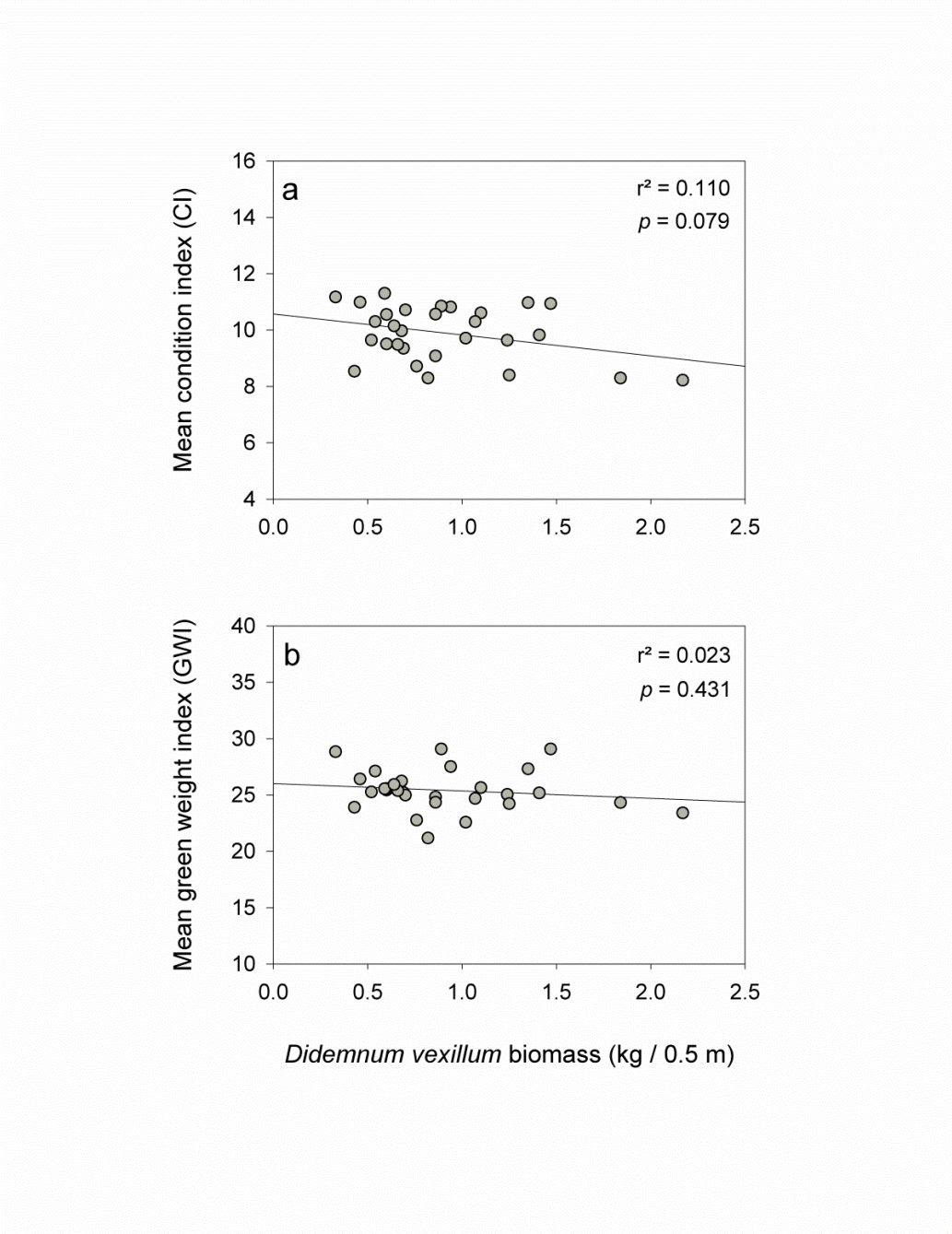

Figure 6.8 Relationship between **a.** mean mussel (*Perna canaliculus*) condition index (CI) and **b.** mean mussel green weight index (GWI) per 0.5 m section of mussel line and *Didemnum vexillum* biomass present for each section. Results are pooled across mussel size classes (*n* = 29 sections).

Table 6.3 Results of permutational ANOVAs testing for mussel size class and fouling treatment effects on: **a.** shell length and **b.** live weight. Analyses based on Euclidean distances of the untransformed data and each term was tested using 999 random permutations of appropriate units.

Source	*df*	a. shell length			b. live weight		
		MS	Pseudo-F	*p* (perm)	MS	Pseudo-F	*p* (perm)
Fouling	2	1190.20	2.671	0.078	827.80	1.056	0.359
Size	2	23411.00	52.540	**0.001**	42654.00	54.390	**0.001**
Fouling x Size	4	1166.10	2.617	0.057	1975.00	2.518	0.071
Line (Fouling x Size)	27	445.60	10.453	**0.001**	784.21	9.219	**0.001**
Residual	684	42.60			85.07		
Total	719						

Significant values ($p < 0.05$) are indicated by bold type.

Table 6.4 Results of permutational ANOVAs testing for mussel size class and fouling treatment effects on: **a.** condition index (CI) and **b.** green weight index (GWI). Analyses based on Euclidean distances of the square root transformed data and each term was tested using 999 random permutations of appropriate units.

Source	*df*	a. condition index			b. green weight index		
		MS	Pseudo-F	*p* (perm)	MS	Pseudo-F	*p* (perm)
Fouling	2	1.238	1.678	0.230	2.131	1.761	0.189
Size	2	2.505	3.396	**0.042**	2.212	1.829	0.173
Fouling x Size	4	0.988	1.340	0.277	1.358	1.123	0.357
Line (Fouling x Size)	27	0.738	6.030	**0.001**	1.210	8.703	**0.001**
Residual	684	0.122			0.139		
Total	719						

Significant values ($p < 0.05$) are indicated by bold type.

6.5 Discussion

6.5.1 Fouling impacts on cultured mussels

Despite the recognised threat of *Didemnum* biofouling to commercial shellfish industries globally (Daniel and Therriault 2007; Valentine et al. 2007b; Cohen et al. 2011), there is a distinct lack of empirical data for direct impacts of *Didemnum* fouling on cultured bivalves under industry conditions. Although only reflecting one study site, the results suggest that New Zealand green-lipped mussels are most vulnerable to direct *Didemnum* impacts at early stages of mussel production. Additionally, negative impacts within the study system appear restricted to fouling-related displacement of smaller mussels as opposed to reduced growth and condition. The mussels remaining in the presence of *Didemnum* and other fouling species in this study appear to have not been adversely affected by the overgrowth that occurred. These findings support the limited literature investigating direct impacts of colonial ascidians on cultured mussel species; impacts are often restricted to fouling related crop losses and production costs, with little demonstrated impact on mussel productivity (e.g. Arens et al. 2011; Cordell et al. 2012).

Significant reductions in mussel density due to increased fouling biomass were only recorded between treatments within the small mussel size class. Control lines within the small size class had significantly higher mussel density when compared to both the enhanced and ambient fouling treatments. The combined mussel weight within sections followed a similar pattern; however, the increased mussel density within the small control sections was not reflected in the combined weights for this treatment, indicating that although there were more mussels present overall they were individually smaller. The effect of *Didemnum* on cultured mussel density and product weight has not been investigated elsewhere. However, a similar relationship was reported between

the invasive ascidian *Ciona intestinalis* and the cultured blue mussel *Mytilus edulis* (Daigle and Herbinger 2009). In that study, approximately two thirds fewer live mussels (> 45 mm) were recorded in highly fouled sections (100 % coverage) compared to those with low levels of fouling (0-10 % coverage), although such comparisons have the potential to be confounded for reasons described below (see Section 6.5.2).

Even once standardised for initial stocking density, the number of mussels lost was considerably greater from the small size class, and decreased with increasing mussel size. A higher loss recorded in the small size class is supported by the strong negative relationship between *Didemnum* biomass and small mussel density within individual sections, suggesting that small mussels at the study site were being displaced by *Didemnum* and may be at more risk from overgrowth by this species. This relationship was also evident, although to a lesser extent, within the medium mussels. On the other hand, the apparent displacement by *Didemnum* appears to be less of a threat to larger mussels (> 40 mm), based on the levels of *Didemnum* fouling described in the current study. The impact of fouling by the solitary ascidian *Ciona intestinalis* on blue mussel (*Mytilus galloprovinciallis*) culture in southern Australia has also been shown to be size specific, with mussel condition significantly lower in small mussels only (Sievers et al. 2013). Higher filtration rates and superior competition for resources in the larger mussels were hypothesised as the reason for the less pronounced reductions in flesh weights, and thus condition in the larger mussels (Sievers et al. 2013).

Due to the initial high stocking density of lines within the small mussel size class in the current study, it is possible the mussels underwent a process of self-thinning over the course of their deployment. Current industry practice involves stripping and reseeding mussels onto new ropes at lower densities once they reach ~ 40-50 mm shell length. This reseeding serves a dual purpose as it not

only thins the mussels out to a more productive stocking density, but also removes or reduces the effects of biofouling (Woods et al. 2012). This reseeding step was not incorporated into the present study because it would have caused the disruption and removal of fouling organisms. During the experiment, as mussels grew and available space became limited, byssal attachments may have been stressed by increased weight from fouling overgrowth. This may explain the lower level of mussel loss recorded for the control treatment within the small mussels, as the periodic removal of fouling present through freshwater treatments will have interrupted this process.

High levels of fouling on lines did not appear to directly affect individual mussel size or condition in the current study. Mussels within the medium and large size classes had comparable shell lengths and live weights across fouling treatments at the conclusion of the study. However, control mussels within the small mussel size class were smaller in both shell length and live weight measurements, possibly due to increased intra-specific resource competition. That being so, the small control mussels did not appear to exhibit any negative effects on condition when compared to other treatment groups. The absence of a measurable effect on growth and condition of *P. canaliculus* suggests that *Didemnum* is not adversely affecting the nature or quantity of food available to the mussels within this study system. This is most likely explained by differences in particle filtration capabilities of the two species and subsequent resource partitioning. Green-lipped mussels have a clearance particle range of 5-20 µm (Safi and Gibbs 2003), whereas colonial ascidians such as *Didemnum* utilise very small particulate matter, primarily in the 0.5-2 µm range (Bone et al. 2003).

In contrast to the current study, Auker (2010) reported unfouled blue mussels (*Mytilus edulis*) to have greater shell lengths and a higher tissue index (volume-based condition index) than those fouled by *Didemnum* in the north-east United States. Similarly, at the conclusion of a five month study in British Columbia,

Canada, cultured Pacific oysters (*Crassostrea gigas*) fouled by *Didemnum* were also shown to have a lower condition index than those kept free of fouling through manual cleaning with soft-wire brushes (Switzer et al. 2011). However, the experimental set-up of both studies differed considerably from the research presented here; groups of mussels were held in plastic-wire mesh envelopes (as opposed to being attached to industry culture ropes as in the current study), and oysters were grown in plastic trays. *Didemnum* colonies in the Auker (2010) study were observed to overgrow the mesh of the envelopes as well as the mussels themselves, thus possibly obstructing water flow into the cages and thereby contributing to the lower growth rates observed (e.g. Uribe and Etchepare 2002). Similarly, the plastic trays containing the fouled oysters in the Switzer et al. (2011) study were left undisturbed over the course of the deployment, with the exterior of the trays becoming fouled. The possibility that water flow obstruction was driving the differences in mussel and oyster condition is supported by data from an 'industry control' treatment in the Switzer et al. (2011) study, in which the plastic trays were replaced with clean trays mid-way through the experiment (mimicking standard industry practice). Despite being heavily fouled with *Didemnum*, oysters in this treatment were not found to have significantly different condition index values than oysters that were manually kept clean (Switzer et al. 2011).

6.5.2 Broader considerations for inferring and assessing fouling impacts

The results presented showed no direct effects of *Didemnum* fouling on mussel size and condition but did indicate negative effects on the density of small mussels. However, additional factors need to be considered when assessing the wider implications of these findings. Mussel farms within the study region are known to accumulate a diverse range of biofouling organisms which can account for a considerable biomass on mussel ropes (Woods et al. 2012). Because the level of *Didemnum* biomass on lines at the conclusion of the study was

comparable between ambient and enhanced fouling treatments, no difference in the effects between these treatments was observed. Hence, despite fragment inoculation quickly leading to a considerable *Didemnum* cover (see Figure 6.2a), ambient inoculation by *Didemnum* larvae in the water column led to a similar level of *Didemnum* fouling in the longer term (i.e. over the 15 month experimental duration). Similarly, while this study showed a negative relationship on small mussel density with increased *Didemnum* biomass, the estimate of a 40 % reduction in mussel density per kilogram of *Didemnum* (based on the 0.5 m sections) should be treated with caution, as the biomass measurement reflected a point in time at the end of the experiment. The amount of *Didemnum* would have differed greatly between seasons, and possibly inter-annually. More frequent assessment of fouling effects during the course of the study may have better elucidated direct impacts, and this approach is recommended for future research in this area.

In addition, although *Didemnum* was the dominant fouling species present on the ambient and enhanced treatment lines, particularly during summer months when this species experiences considerable colony growth (Valentine et al. 2007a), other fouling species were also commonly observed (e.g. the blue mussel *Mytilus galloprovincialis* and the seaweed *Undaria pinnatifida*). As such, the observed effects on mussel density and loss may not be exclusively attributable to *Didemnum* overgrowth. By periodically treating the control lines with freshwater, all fouling was effectively removed. Ideally, a treatment involving manual removal of *Didemnum* colonies alone (i.e. without freshwater) would have been incorporated, to investigate its importance relative to background fouling. However, given the rapid growth of *Didemnum* this step would need to have been undertaken frequently, and would have been prohibitively labour intensive at the scale of the experiment.

Lastly, while fouling observed on the ambient and enhancement treatment lines was reasonably high, it was far less than some *Didemnum* infestations evident in nearby locations and at other times, as this species has been observed to completely smother mussels elsewhere in the region (Pannell and Coutts 2007). Thus, it can be difficult to experimentally mimic the worst-case invasiveness, even at adjacent locations. However, as the worst case is generally of most interest, many studies of biofouling attempt to infer potential impacts from a comparison of areas of high versus low levels of fouling (e.g. de Sa et al. 2007; Daigle and Herbinger 2009; Fitridge 2011). Such assessment approaches can lead to ambiguous results, especially when the spatial and temporal variation of infestation and stochastic nature of the invasion process are considered (Forrest and Taylor 2002; Stachowicz et al. 2002; Padilla 2010). A recent review of the marine invasion literature found the majority of field-based impact experiments are presence-absence designs, where it is unclear if impacts are caused by universal or causal agents (Thomsen et al. 2011). For instance, on mussel farms in the current study region, it is not certain that low mussel density in the presence of high *Didemnum* biomass automatically reflects a displacement effect by *Didemnum*; it could simply reflect that *Didemnum* invaded bare space created by mussel drop off or dislodgement (e.g. during industry handling of culture lines). In this study, the fact that the control lines remained as an intact continuous column of mussels across all size classes provides confidence that the *Didemnum* effect measured was real.

6.5.3 Wider industry implications of fouling

Impacts of biofouling on cultured shellfish operations are often varied, including negative effects on growth rates and meat yields, as well as lost revenue through stock mortality, crop losses, and increased costs of production and processing (Fitridge et al. 2012 and references therein). The level of fouling present within a marine farm can be substantial, with a recent assessment of

two farms within the Marlborough Sounds region indicating that a significant proportion (c. 15 %) of total biomass present on mussel lines comprised biofouling species, even following reseeding of ropes between crop stages (Woods et al. 2012). A range of species-dependent effects of fouling in New Zealand culture regions have been reported. In the Marlborough Sounds, it has been estimated that mussel farms lose up to 15 % of their seed stock through biofouling (Hembry 2008). Across New Zealand, impacts include direct effects on mussels during grow-out, physical effects on harvesting and product processing, as well as effects on product value through disfiguration of the mussel shell (e.g. Sinner et al. 2000; Heasman and de Zwart 2004; Jeffs and Stanley 2010).

Based on the observation that smaller mussel size classes were the most vulnerable to the negative effects of fouling, it is hypothesised that the *Didemnum* overgrowth would have had an even greater impact on very small mussel spat. A greater impact of fouling on spat and very small mussels is consistent with industry observations from growing areas. Among the problematic fouling species in addition to *Didemnum* and other ascidians, are indigenous blue mussels, *Mytilus galloprovincialis*, and macroalgae such as *Colpomenia* spp. As part of the current study, it was attempted to quantify the effects of *Didemnum* fouling on spat (< 5 mm shell length). However, this treatment was not successful due to the natural loss of some spat from the experimental lines, reflecting a wider industry problem with spat retention during the early stages of mussel grow-out. The loss of spat created gaps in the otherwise uniform 'sock' of mussels, which could have been readily colonised by *Didemnum*, leading to inferred treatment effects where none existed.

Biofouling within shellfish aquaculture operations is an important management issue, and expenditure for control can be significant even at the individual farm level. An understanding of the impacts of fouling species, particularly across different stages of the production chain, is necessary for the implementation of

successful management procedures. In the Marlborough Sounds aquaculture region, adoption of new industry practices, coupled with an apparent decline in the level of invasiveness of *Didemnum* over recent years, has led to adequate means of control at present. Management of *Didemnum* biomass on crop lines through intermediate reseeding of lines has prevented the substantial crop losses observed in this region previously. However, further research on cost effective treatment methods is required, as well as management of vector movements such as transfers of infected stock and equipment, in order to reduce the risk of spread to areas important for growing shellfish aquaculture seed-stock.

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

7.1 Synthesis of research findings

While recognised as a significant threat to New Zealand's highly valued aquaculture industry at the inception of this PhD, work on *Didemnum vexillum* (hereafter referred to as *Didemnum*) at that stage had focused on the development of management strategies and eradication attempts largely in the absence of information on biological attributes underpinning invasion success. Collectively, the research described in this thesis fills a number of previous knowledge gaps, including information on the reproductive patterns, spread potential and commercial impacts of *Didemnum* and how these relate to the New Zealand marine environment. The thesis explores several key biological characteristics of *Didemnum* and aims to better elucidate the reasons behind this species' demonstrated invasiveness and success as a fouling organism.

Early research on the natural dispersal of *Didemnum* was hampered by difficulties in obtaining sufficient larvae on demand. Comprehensive laboratory spawning trials described in Chapter 3 resulted in the first documented methods to successfully induce spawning in adult *Didemnum* colonies, as well as techniques for the successful settlement and metamorphosis of the larvae produced, and laboratory culture of the juvenile recruits. These techniques will enable future research into several aspects of *Didemnum* biology and ecology, in particular the factors affecting colony survivorship, growth and reproductive output. The ability to obtain larvae on demand has already facilitated research into the larval competency period of this species (as described in Chapter 5), with this information currently being used to predict the spread of *Didemnum*, and other pest species, within the Marlborough Sounds region using a hydrodynamic model.

Prior to this thesis, the reproductive patterns of *Didemnum* in New Zealand had been inferred from several studies from the north eastern United States. The

length of the reproductive season at these locations had been shown to vary, from 3.5 to 5 months, depending on local conditions (Auker 2006; Osman and Whitlatch 2007; Auker and Oviatt 2008; Valentine et al. 2009). As such, it remained unclear whether the environmental effects driving reproductive patterns were consistent between geographic locations, as well as whether effects within a location were consistent and predictable over time. This thesis has shown that *Didemnum* populations in central New Zealand exhibit clear seasonality in their reproductive cycles, and that recruitment patterns are strongly correlated with site-specific water temperature fluctuations (Chapter 4). Results show the recruitment season for *Didemnum* in central New Zealand is at least 9 months of the year, considerably longer than previously believed.

Chapter 5 provided the first experimental assessment of the natural dispersal ability of *Didemnum.* Larvae were shown to remain competent and viable within the water column following considerable metamorphosis delays (up to 36 hours). Although not reflecting all of the processes involved in successful population range expansion, these findings suggest the larval swimming time of *Didemnum*, and hence the larval dispersal potential of this species, is far longer than previously assumed. These findings were supported by documented larval recruitment at distances up to 250 m from source populations. Furthermore, exponential decay models indicated that, given favourable conditions, larval dispersal distances greater than 1 km are theoretically possible within the study region.

Despite the recognised threat of *Didemnum* to commercial shellfish industries globally (Daniel and Therriault 2007; Valentine et al. 2007b; Cohen et al. 2011), this thesis presents the first study to quantify effects on a cultured bivalve under industry conditions (Chapter 6). Experimental results suggested that the negative impacts of *Didemnum* fouling on the commercial culture of New Zealand green-lipped mussels were of most concern for smaller mussel size

classes, and restricted to fouling-related displacement of mussels rather than effects on individual mussel size or condition. That being so, as noted in Chapter 6 infestation levels and associated impacts of *Didemnum* have been shown to vary considerably between locations as well as between years. Thus, at the greater levels of invasiveness than the experimental situation described in this thesis, *Didemnum* impacts have the potential to be considerably greater. An understanding of the reasons for such variable invasiveness, and the ability to predict future invasiveness (and consequently impacts), are of vital importance to managers. Hence, species traits underpinning *Didemnum*'s success as a fouling organism as well as the rapid proliferation and spread of this species are discussed below.

7.2 What contributes to *Didemnum vexillum*'s success as an invader?

Previously published reports have identified *Didemnum* as a very successful invader in a number of regions and habitat types globally. However, the research presented here investigating the impact of this species on commercial mussel culture provides an example of a situation where the effects of this invasiveness were more limited than anticipated. Similarly, although identified as a competitively dominant species in a range of natural habitats overseas (e.g. Georges Bank fisheries region in the north east United States; Valentine et al. 2007b), *Didemnum* has shown very limited invasiveness in natural habitats within New Zealand (Coutts and Forrest 2007). This variability in the level of invasiveness demonstrated, both at local and global scales, raises a number of questions around the potential factors contributing to *Didemnum*'s success as an invader.

7.2.1 Marine invasion ecology and characterising invasion success

The past two decades have seen a considerable increase in research devoted to identifying general rules in marine invasion ecology. Numerous studies across a wide range of taxonomic groups have attempted to define what characteristics determine whether a species is likely to be invasive, as well as what characteristics determine whether a community is vulnerable or resistant to invasion (e.g. Lodge 1993; Carlton 1996; Williamson and Fitter 1996; Ruiz et al. 1997; Sakai et al. 2001; Kolar and Lodge 2002; Stachowicz et al. 2002; Colautti et al. 2006). Following introduction to a new habitat, species encounter a range of abiotic and biotic barriers to establishment, with successful introductions reliant on the species being able to live in, or adapt to, these new conditions (Colautti et al. 2006). Failure to establish successfully is often due to several common causes, including unsuitable environmental conditions, high predation, competition with resident species, and disease (Troost 2010). Species traits believed to contribute to successful establishment include a lack of natural enemies, ability for habitat modification, association with human activities, genetic variability and phenotypic plasticity, and a high degree of competitiveness (Cuddington and Hastings 2004; Liu and Stiling 2006; Troost 2010). More generally, successful colonists are often species with high reproduction rates, a broad tolerance to a wide range of environmental conditions, as well as an ability to colonise a variety of habitat types (Troost 2010). While it is clear that *Didemnum* colonies possess a number of these invasive characteristics, the recent high-profile proliferation of *Didemnum* populations in a number of temperate regions worldwide (see Lambert 2009) suggests particular idiosyncrasies of this species may be driving invasion success, more so than general traits shared with a wider suite of species.

7.2.2 Specific life-history traits underpinning success

Didemnum populations possess several unique characteristics that may facilitate the establishment and success of this species. Life history theory predicts a trade-off between fast reproductive rates that facilitate colonisation, and competitive ability that facilitates establishment (Pianka 1970). However, in some invaders, such as *Didemnum*, both strategies are represented (e.g. Troost 2010). Being a colonial organism, *Didemnum* populations are able to undergo rapid asexual growth, allowing for efficient monopolisation of available resources (e.g. Valentine et al. 2007a). In addition, during periods of suitable environmental conditions, populations are able to undergo rapid sexual reproduction through the production of large numbers of free-swimming larvae (e.g. Valentine et al. 2009; see Chapter 4). The ability for dispersal and reattachment of colony fragments, as well as the ability to exploit transport vectors (e.g. attachment to a ship hull), will also aid in the rapid establishment of populations. The transport of fecund adults is likely to be a more efficient means of population establishment than development from a single settled larva (Morris and Carman 2012; see Chapter 5). *Didemnum* populations have also been shown to demonstrate a wide physiological tolerance to environmental conditions such as temperature and salinity (e.g. McCarthy et al. 2007; Gröner et al. 2011; Lenz et al. 2011); a species trait that often confers a competitive advantage and will likely assist in invasion success, particularly with reference to future climate-change induced shifts in local temperature and salinity regimes.

In addition to life-history traits, there is growing evidence that for some organisms the genetic characteristics associated with isolated invasion events may also be facilitating invasion success (e.g. Puillandre et al. 2008; Zhan et al. 2010). Population genetic theory predicts that founding populations of invasive species will only contain a small subset of the total genetic variation present in the source population, and that further loss of genetic diversity is likely (Nei et

al. 1975; Sakai et al. 2001; Roman and Darling 2007). Despite these theoretical predictions, reduced genetic diversity may actually enhance invasiveness in colonial organisms, at least in the short-term, by reducing inter-colony conflict and thus enabling the occupation of more physical space (e.g. Ben-Shlomo et al. 2008; Westerman et al. 2009). *Didemnum* populations in New Zealand have been shown to display greatly reduced genetic diversity when compared to populations from Japan, which is believed to be within the likely region of origin for this species (Smith et al. 2012). Due to this reduced genetic diversity, colonies in New Zealand are much more likely to fuse and form large competitively dominant colonies, a phenomenon that may explain the extreme propensity for biofouling that *Didemnum* displays outside its putative native range (Coutts and Forrest 2007; Smith et al. 2012). In contrast, while a common member of the fouling community in several regions of Japan (Lambert 2009), the genetically diverse *Didemnum* populations in these areas generally exhibit quite different morphologies and life-history traits to those within the introduced range; in Japan colonies remain predominantly two-dimensional in structure and do not form the large three-dimensional drooping colonies demonstrated elsewhere (e.g. Coutts and Forrest 2007). Based on the characteristics of this species shown in Japan, one would not predict that *Didemnum* would become so invasive in a range of environments globally. In fact, prior to its almost simultaneous proliferation in several temperate regions worldwide, *Didemnum* had no reported invasion history. This situation is not unusual, a number of high-profile invasive species have remained essentially dormant for decades within their native regions, before the proper combination of conditions and circumstances facilitated successful introduction to a recipient region (Carlton 1996) Examples of this scenario include the introduction of the Asian shore crab *Hemigrapsus sanguineus* into Atlantic North America (McDermott 1991), as well as more locally, the recent introduction and rapid spread of the colonial ascidian *Eudistoma elongatum* in northern New Zealand (Smith et al. 2007; Morrisey et al. 2009). Consequently, it is clear difficulties exist

surrounding the timely and accurate identification of potential pest species, as discussed further below.

7.3 Predicting impacts and prioritising management efforts

The high variability in the level of *Didemnum* invasiveness across small spatial scales, as well as year to year, is a feature of many invasive species, and makes reliable predictions of invasive potential challenging. The impacts of invasive species in marine ecosystems have been shown to vary within particular locations (e.g. Ceccherelli and Campo 2002; Thomsen and McGlathery 2006), within geographical regions (e.g. Bulleri et al. 2010; Thomsen et al. 2006; Heiman and Micheli 2010), as well as across years (e.g. Staehr et al. 2000; Forrest and Taylor 2002). Populations of some introduced organisms proliferate rapidly only to taper off a few years later, possibly due to overexploitation of resources or other mechanisms still not fully understood. Other well-established species can appear harmless for decades before their populations suddenly explode. Examples of this invasion lag phase in New Zealand include population explosions on mussel farms of the ascidian *Ciona intestinalis* within the Marlborough Sounds region (Cole 2002; Coutts 2002), and of the barnacle *Balanus trigonis* in the Coromandel region (e.g. Jeffs and Stanley 2010). Potential high-risk marine pest species are often identified based on their invasiveness or demonstrated impacts elsewhere (e.g. Hayes and Sliwa 2003), and their potential distribution assessed through evaluation of the 'match' between natural tolerances and environmental conditions present in the recipient area (e.g. Smith et al. 1999; Epelbaum et al. 2009; Somero 2010). Hence, a question often asked by invasion ecologists since the emergence of this research area in the mid 1990's, is whether and to what extent a detailed understanding of species biology would aid in the prediction of invasiveness? The general consensus is that even with complete knowledge of a species'

biological characteristics, as well as characteristics of the community at risk, the outcome of introductions is often quite random even at a localised scale (e.g. Stachowicz et al. 2002). Although there is awareness of many of the general characteristics of invasions, and the attributes of species and recipient environment that may increase the likelihood of a successful invasion, the accurate prediction of whether a particular species will invade a particular locality, and to what extent, is often still very difficult (Carlton 1996; Kolar and Lodge 2001).

Despite these limitations, predictions surrounding the potential impacts of invasive species are crucial to enabling informed management decisions. Without this information, chosen management options are likely to be disputed, ranging from no management of a species, to the application of a precautionary approach that assumes worst-case impacts and leads to comprehensive management efforts. In all cases, a robust evaluation of the feasibility, costs, and benefits of management options is crucial, especially with limited funding available and competing priorities for invasive species management (Molnar et al. 2008). Successful invasive species management in the marine environment is generally reliant on the species having a limited natural dispersal potential, low fecundity and specific habitat requirements, as well as individuals being conspicuous and easily visible. Eradication or control has been shown to be easier, cheaper and more effective very soon after detection (Simberloff 2003). However, no intervention may still be a valid approach for some introduced species; for example, where the costs of intervention outweigh the benefits, where risks are negligible, or where a species is essentially unmanageable (Forrest et al. 2006). As illustrated by the recent introduction of the Mediterranean fanworm *Sabella spallanzanii*, to two harbours in New Zealand, difficulties often arise in defining outer boundaries for surveillance and vector control when introduced species do not meet the general criteria above, and as

such eradication programmes are often not undertaken or soon discontinued (e.g. Read et al. 2011).

Management may be possible without comprehensive knowledge of a species' population biology; however, successful eradication and control methods can often be tailored to the idiosyncrasies of a particular species when this information is available (Simberloff 2003). Similarly, detailed information of a species' biology will in some instances influence the level of management instigated. For example, in the case of *Didemnum* in New Zealand, a working group formed to manage the species in the Marlborough Sounds indicated that they would likely have not considered management at the scale that was attempted, had detailed information on aspects of the species biology been available at the start of their programme. The management programme was based on the premise that the species was capable of very limited natural dispersal, and as such it was assumed larvae released would be largely contained within localised areas (e.g. within embayments). Recognition that the larval competency period for *Didemnum* was longer than previously believed, and that natural range extensions over scales of a few kilometres were possible, ultimately led stakeholders to re-evaluate their attempts to manage the spread of this species within the region. Efforts to manage anthropogenic vectors and control established populations were likely being undermined by the dispersal of *Didemnum* larvae beyond zones of regular surveillance and delimitation.

The above findings illustrate that decisions around whether and to what extent management is attempted should be made on a case by case basis, with consideration of any new information being a crucial component. For example, since undertaking the dispersal studies in Chapter 5, it has been more clearly established that multiple generations per year are possible in *Didemnum* (Morris and Carman 2012). Combined with information on the natural dispersal potential of larvae from Chapter 5, such knowledge suggests that natural

dispersal over scales of several kilometres may be possible in a given reproductive season.

7.4 Application of research findings to biofouling management

The proliferation of fouling organisms and the associated impacts on aquaculture industries globally has led to an increased demand for tools to mitigate the effects of biofouling pests. Presently, there are several options for managing potential and established threat species and this management involves a sequence of intervention points depending on the stage of invasion. The ideal situation for aquaculture management is total prevention of spread to aquaculture areas, through effective management of international and domestic transport vectors. The feasibility and efficacy of vector management is often limited however, especially when the pest species has a large natural dispersal range. In the case of *Didemnum*, although a substantial natural dispersal potential was present, the regional spread of this species was exacerbated by human-mediated activities, primarily the transfer of infected aquaculture seed-stock. Consequently, aquaculture management would ideally attempt to avoid infection of seed stock, crops or equipment through management of farm operations around biological characteristics of specific high-risk species. Once a pest has become well established on a marine farm, management actions must take on a direct control approach, so as to mitigate the impacts on crops and equipment. Control options generally involve treatments for the reduction or removal of biomass and have had varying levels of success. Following on from control options, industry can attempt to contain the incursion, so as to limit the repeated infection of treated structures and crops, and to prevent secondary spread. A more specific description of vector management, treatment options, and secondary spread prevention, with particular reference to the implications of the thesis findings, is given below.

7.4.1 Incursion prevention and vector management

Control and eradication of pest species in the marine environment is often technically and financially difficult (McEnnulty et al. 2001; Meyerson and Reaser 2002). Very few efforts to eradicate a marine species have ever been successful, with key exceptions being instances where arguably novel circumstances (e.g. the ability to close off an environment for treatment) have contributed to these successful management outcomes (e.g. Culver and Kuris 2000; Bax et al. 2001; McEnnulty et al. 2001; Wotton et al. 2004; Hopkins et al. 2011). Due to the difficulties in managing established marine pests, incursion prevention and the management of high-risk vectors for introduction is a critical aspect of both biofouling and invasive species management. Increased emphasis in this area is evident in the development of regulatory frameworks for assessing marine pest risks (e.g. Hewitt et al. 2009), comprehensive border surveillance programmes, and global efforts to manage risks associated with ballast water and hull fouling on international vessels (e.g. development of standards). Simultaneously, in New Zealand there is increasing emphasis on the management of domestic transport pathways for marine pests.

The efficacy of domestic pathway management in relation to the natural spread potential of a species is an especially important consideration, in particular the identification and management of internal borders (natural barriers to dispersal and establishment) once a pest species has been introduced to a country (Forrest et al. 2009). The importance of information on species dispersal potential for the long term success of eradication and control programmes is well recognised (Myers et al. 2000; Wotton and Hewitt 2004). Information on species natural dispersal potential is especially critical to decisions regarding the minimum spatial scales over which to focus management of human-mediated vectors of spread. A study by Darbyson et al. (2009) found that adults of the invasive solitary ascidian *Styela clava* can withstand over 48 hours of air

exposure with only 10 – 11 % mortality after this time. This ability to survive 48 hours out of the water greatly increases the potential dispersal of this species through the movement of boats transported on trailers (potential range of 1600-2000 km), a vector that had not been considered previously for this species. As *S. clava* has a limited planktonic larval duration (generally less than 24 hours) and thus its natural dispersal capacity is relatively low, estimates such as this highlight the importance of efficient vector identification and control.

In the case of *Didemnum*, the natural dispersal capacity of larvae is clearly important for the regional scale spread of this species; however, longer range processes such as anthropogenic transport are likely to be more important in establishing founding populations at greater scales. Activities associated with the aquaculture industry often lead to the inadvertent transport of fouling species across regional scales and, as such, management of these activities is often needed (Naylor et al. 2001; Dodgshun et al. 2007). This may include regulations around vessel movements and aquaculture transfers, as well as sterilisation of contaminated aquaculture equipment or seed-stock (e.g. Forrest et al. 2007). A number of countries, including New Zealand, have adopted industry Codes of Practice for the translocation of equipment and stock to reduce the risk of such spread at regional scales where bivalves are regularly transported among sites for grow-out (Wasson et al. 2001; Forrest et al. 2007). Management of other high-risk vectors such as recreational vessel movements may involve anti-fouling technologies in the case of vessel hulls, as well as increased levels of surveillance, regulation and vessel maintenance to prevent fouling accumulation in 'niche areas' (e.g. sea chests; Coutts and Dodgshun 2007).

7.4.2 Mitigation of fouling effects and treatment of infected structures or crops

If incursion prevention fails and a pest species becomes established on a marine farm, management of the species can involve a number of treatments to mitigate the impacts on crops and equipment. An understanding of the biological characteristics of fouling species and how these relate to local environmental conditions can optimise management approaches applied. For example, research into the reproductive seasonality of pest species can aid in the mitigation of adverse effects of fouling through management of farm operations around seasonal windows of risk. With reference to *Didemnum* fouling and the New Zealand aquaculture industry, Chapter 4 revealed that the duration of the *Didemnum* recruitment season in the Marlborough Sounds region was considerably longer than previously assumed, meaning that opportunities for management were less than once envisaged. However, the same management principles can be applied to other locations and fouling species more generally. Most pest species have a fixed reproductive season, often regulated by water temperature, during which eggs or larvae are released and dispersed. Industry can therefore manage their activities to avoid deploying vulnerable life-stages (such as mussel or oyster spat) during high risk periods. This approach has been suggested for commercial scallop culture in Québec, Canada, whereby weekly monitoring of spat collection enables the prediction of the optimal collector deployment period to reduce the abundance of undesirable species while maintaining a high collection of scallop spat (Cyr et al. 2007). In New Zealand, future mussel industry plans involve holding hatchery-reared spat in the sea for short-term grow-out (3-4 months) before distribution to different growing regions nationally. Knowledge of seasonal fouling recruitment windows could be used to minimise risk to the spat, which appears to be a vulnerable stage in the industry production chain. In addition, by reducing fouling risk during this short-term grow-out, there will be a simultaneous reduction in the likelihood that high risk species will be

inadvertently spread among growing regions as a result of inter-regional spat transfers.

The need for straightforward and cost-effective tools to directly control biofouling pest species once established is also recognised. Although there has been increased recent interest in the development of treatment solutions, the New Zealand aquaculture industry is still lacking effective and affordable management tools. Traditional methods for fouling control have involved mechanical cleaning or the use of antifouling coatings, however neither option has proved ideal. Mechanical cleaning (scraping, brushing or cleaning with water jets) is both labour intensive and costly (Hodson et al. 1997), while the use of antifouling coatings has become increasingly restricted due to negative environmental effects (Stewart et al. 1992). This has motivated a search for control methods that are environmentally benign, inexpensive and logistically feasible (Locke et al. 2009), a process which is still at a relatively early stage in the marine environment. Current natural antifouling methods include biological control using grazers (e.g. Lafferty and Kuris 1996; Lodeiros and Garcia 2004; Messing and Wright 2006; Atalah and Forrest 2011; Switzer et al. 2011), smothering or encapsulation techniques using dredge spoil, geotextile fabrics and polythene wrappings (e.g. Coutts and Forrest 2005, 2007), and the application of physical stressors such as air drying, ultraviolet light, steam, hot water, freshwater immersion, electricity and pressure washing (e.g. Carver et al. 2003; Forrest and Blakemore 2006; Le Blanc et al. 2007; Denny 2008; Paetzold and Davidson 2011; Arens et al. 2011).

A range of chemical treatments have also been trialled overseas and in New Zealand with relative success; however these methods are still too labour intensive, and as such prohibitively expensive, to apply on a large scale in New Zealand. Chemical treatments present additional difficulties as they must not have significant detrimental effects on the cultured species or the environment,

with their use often requiring prior regulatory approval. Research has been undertaken into the antifouling efficacy of chemical treatments using sodium hydroxide, chlorine, acetic acid, citric acid, brine, and hydrated lime solutions (e.g. MacNair and Smith 2000; Carver et al. 2003; Rajagopal et al. 2005; Forrest et al. 2007; Switzer et al. 2011; Rolheiser et al. 2012). More recent research has focused on the development of control methods that focus on key stages of a fouling organism's life cycle, such as larval settlement and metamorphosis (e.g. Willis and Woods 2011; Cahill et al. 2012). The requirement for knowledge of local reproductive and spawning behaviour for successful application of these mitigation methods has been noted (Willis and Woods 2011). With reference to research presented in this thesis, information on *Didemnum* reproductive patterns as well as techniques for spawning adult colonies will have potential relevance in this field (Chapters 3 and 4). The development of novel antifouling agents may represent an efficient and environmentally sustainable solution to the biofouling problem. Increased collaboration among aquaculture farm types and regions, including international research partnerships such as the Collective Research on Aquaculture Biofouling (CRAB) consortium in Europe, will also aid in this process.

7.4.3 Prevention of secondary spread

Treatments to reduce pest species' biomass on infected aquaculture structures will not only mitigate direct effects such as smothering of crops, but will also reduce propagule supply and therefore the likelihood of secondary spread. Similar to initial incursions, marine pests can undergo secondary spread through either natural mechanisms (e.g. passive transport of propagules in water currents) or by association with human-mediated transport vectors (Hulme et al. 2008). As discussed in Chapter 5, the propagule dispersal capacity of coastal invertebrates and macroalgae (the main problem groups for biofouling) is likely to be limited, typically in the order of hundreds of metres to tens of kilometres

per year (Forrest et al. 2000; Shanks et al. 2003; Siegel et al. 2003; Miller and Ayre 2008). Dispersal capacity can have profound implications on the level of connectivity among local populations of marine organisms. As this exchange drives population replenishment, the degree to which populations self-recruit or receive subsidies from other populations impacts heavily on the persistence and spread of invasive species (Roughgarden et al. 1988; Botsford et al. 2001; Palumbi 2003; Cowen and Sponaugle 2009).

Even following establishment, the containment of pest species through active management and control efforts may be a worthwhile management approach. This may include limiting repeat infections of treated structures and crops, as well as preventing the infection of nearby uninfected farms. This is particularly important in concentrated aquaculture regions, such as the Marlborough Sounds, where species' spread can be facilitated by the large number of artificial structures in close proximity. Farms or structures with established populations are likely to acts as reservoirs for further spread, often acting as 'stepping stones' for the spread of a species in a way analogous to that described for artificial structures along the Adriatic coast of Italy (e.g. Bulleri and Airoldi 2005). The transfer of stock or equipment among sites may also be an important factor in the spread of established pest species, even if the target species is found within the broad geographic region (Bourque et al. 2003; McKindsey et al. 2007). However, the recent development of tools (e.g. codes of practice) by stakeholder groups such as aquaculture companies and their national agencies (e.g. New Zealand Mussel Industry Council) is likely to reduce the risk of inadvertent transfer of pest species during the course of their operations. Although eradication of *Didemnum* is no longer regarded as feasible within the Marlborough Sounds, effective management to reduce the risk of secondary spread to certain high value sub-regions (e.g. important areas for shellfish aquaculture seed-stock) may still be possible. As such, population control to reduce the biomass present on structures and marine farms may play an

important role in limiting the instances of secondary spread and establishment. That being so, the efficacy of any control or mitigation strategies initiated will depend on the ongoing long-term commitment of resources.

7.5 Concluding remarks

The efficacy of management of any marine pest species requires a thorough understanding of the biology of that species. An extensive understanding of the biological attributes and ecology of the specific pest species to be managed, as well as the ecosystems or resources at risk, is rarely available however (Simberloff 2003). This thesis demonstrates the importance of an integrated approach to research in that it considers several key aspects of *Didemnum* biology and ecology, as well as the related impacts of this species on aquaculture. Ultimately such knowledge provides enhanced information for risk analysis procedures (e.g. Sinner and Coutts 2003) and decision-making by managers and aquaculture industry members alike. The findings of this research have led to a better awareness of commercial impacts and spread potential for *Didemnum*, as well as how its biological attributes and behaviour in the New Zealand environment differ to that previously assumed and described from overseas studies. Simultaneously, the research presented highlights the limitations inherent in inferring invasiveness from other situations (e.g. places, times, and related species), and the need to specifically evaluate a species' biological attributes and invasive behaviour following introduction to a novel environment. Despite these limitations, it is clear that underpinning biological knowledge can still greatly assist with management decisions, as highlighted throughout the chapters in this thesis. An increased appreciation of potential impacts is likely to lead to greater stakeholder involvement and support for management of marine pest species in general, as well as enabling managers to prioritise which species and what areas receive management efforts.

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