

**Demography and Population Projections of the Invasive  
Tunicate *Styela clava* in southern New Zealand**

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There is pleasure in the pathless  
woods;

There is rapture on the lonely shore;

There is society, where none intrudes,

By the deep sea, and music in its roar:

I love not man the less,

But nature more...

— Lord Byron

To Mum and Dad



## Abstract

This thesis is about the demography of the tunicate *Styela clava*, a species of some notoriety because of its invasiveness and impacts in many parts of the world. Species assemblages have continuously changed throughout evolutionary history, but the rate of today's anthropogenically facilitated dispersal is unparalleled in history. Non-indigenous species (NIS) are now considered one of the most important risks to native biodiversity. NIS become invasive by becoming both widespread and locally dominant. This requires that a species becomes established, spreads locally, and increases in abundance. In the early stages of invasion, its demography and life history characteristics are of crucial importance. In New Zealand, *Styela* has established populations in several places, but none of these populations has yet reached the high densities found in other countries. In Lyttelton Port, where this study was located, *Styela* was first noticed in 2005. It therefore presented an ideal situation to study an invasive species in its early stages of establishment and provided a potentially good model for understanding how invasive species get local traction and spread from initial infestation points. Therefore, I set out to determine demographic features of *Styela* to understand the numbers game of population dynamics.

This study used empirical data on growth rates, size-frequencies through time, and size and age to maturity to test several models, including von Bertalanffy, Logistic dose-response, Ricker and power models of individual growth. The most useful proved to be the von Bertalanffy model. *Styela* individuals shrink frequently, so average growth rates were often quite low, even though some individuals reached 160 mm or more in total length. Mortality was greatest in summer, presumably after reproduction, and lowest in winter. Fewer than 5% of individuals survived 12 months, and most or all of these died soon afterwards. Populations were, therefore, essentially annual. Recruitment was difficult to determine because of the cryptic nature of small juveniles. However, size-frequency, abundance and mortality data indicated that recruitment most likely occurred in early spring (late-October), and then again in late summer, with growth to maturity (at c. 50 mm total length) within < 5 months.

Several manipulative experiments showed that *Styela* did not readily capitalise on provision of free space but the other non-native ascidian, *Ciona intestinalis*, rapidly recruited. Transplants of *Styela* were greatly affected by *C. intestinalis*, which overgrew them, similar to a localised replacement of *Styela* by *Ciona* seen overseas.

Lefkovitch modelling was used to test whether *Styela* had an "Achilles heel" in its life history, whereby managed removal could impact future populations. This showed that under several scenarios intervention would most likely be ineffectual.

Overall, this study showed that the original populations in Lyttelton Port are either static or in decline, somewhat contrary to original expectations. Nevertheless, it appears that these small populations may be acting as stepping stones for spread of this species outside of the port.

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# Chapter 1

## General Introduction

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### 1.1 Background

Historically, mountains and oceans represented formidable natural barriers to many of earth's species and ecosystems evolved in relative isolation. The commencement of international travel by oceanic ships inaugurated the breakdown of many biogeographic barriers for an array of marine species. Our ancestors inadvertently carried a variety of marine species as passengers on these ships. These species encrusted hulls, dry ballast and anchor chains (Carlton 1999) and were then transported throughout the world. As ships moored in various international locations they often released propagules or individuals, sometimes resulting in local establishment of species that did not naturally occur there. Carlton & Hodder (1995) effectively demonstrate the dispersive capabilities of species from a bygone day in a study where they followed a replica of a 16th-century sailing vessel along the coast of North America, checking experimental fouling panels at each port it visited. Their work showed excellent survival of all common fouling species between ports, suggesting these slow-moving vessels likely significantly altered the distributions of marine organisms along continental margins and around the world (Carlton & Hodder 1995). Despite efforts to reduce the spread of such species in recent times, a multitude of anthropogenic vectors, such as biofouling, ballast water, aquaculture, and live bait, continue to transport many potential invaders around the globe on a daily basis (Mack *et al.* 2000, Ruiz *et al.* 2000, Steneck & Carlton 2001). Furthermore, the rate of new introductions has increased exponentially over the last 100 years, showing little sign of easing (Levings *et al.* 2002). Although species assemblages have continuously changed throughout evolutionary history via species migrations, shifting and forming continents, and changes in environmental conditions (e.g., climate), the rate of today's anthropogenically facilitated dispersal is unparalleled in history.

Non-indigenous species (NIS) in the marine environment have become a hot topic, having both ecological and social ramifications, and are considered one of the two most important risks to native biodiversity, second only to habitat loss (Elton 1958, Carlton 1989, Sala 2000). NIS differ from native colonisers in that they have different evolutionary histories to the communities in which they are introduced, and their spread has been aided by anthropogenic activity (Colautti & MacIsaac 2004). Through the processes involved in establishment and local spread, followed by an increased abundance, a NIS can become an invasive species, that is one that is both widespread and locally dominant (Richardson *et al.* 2000, Colautti & MacIsaac 2004).

There are many examples in the literature of invasive species affecting native biodiversity, the functioning of ecosystems, and human values such as fisheries, aquaculture, water cooling systems, navigation, tourism and human health worldwide (e.g., Walker & Kendrick 1998, Simberloff & von Holle 1999, Ruiz *et al.* 2000, Grosholz 2002, Bax *et al.* 2003, Hayes *et al.* 2005, Forrest & Blakemore 2006). Some dramatic examples highlighting the significance of such invasions in the marine environment include: the introduction of the comb jelly *Mnemiopsis leidyi* leading to the collapse of fisheries in the Black Sea (Shushkina & Musayeva 1990, Shiganova 1998), the invasion of the Mediterranean by the seaweed *Caulerpa taxifolia* smothering vast areas of native habitat (Meinesz *et al.* 2001, Boudouresque *et al.* 1995), and the North Pacific seastar *Astersias amurensis* that has invaded Tasmania and Port Phillip Bay, Australia becoming a dominant invertebrate predator affecting the commercial bivalve fishery (Grannum *et al.* 1996, Ross *et al.* 2002). Fortunately, most potentially invasive species die in transit due to an intolerance to conditions during the voyage (Bax *et al.* 2003). Of the species that do survive, most fail to establish because of unsuitable environmental conditions or too few founding individuals. Furthermore, of those species that do establish, very few actually become invasive (Bax *et al.* 2003, Jeschke & Strayer 2005). But, despite the multitude of obstacles, innumerable species have managed to become invasive worldwide. Cranfield *et al.* (1998) explain that in the past 200 years, more than 130 NIS have become established in New Zealand's coastal waters alone. This report is currently being updated and since 1998 this number has significantly increased (Seaward, personal communication).

While many case histories of biological invasions can be found in the literature, work investigating the mechanisms by which NIS succeed in gaining traction and becoming established within novel environments is limited (Bulleri & Airoidi 2005). Life-history traits of the invader and local conditions in new environments, including physical and biological

attributes, have been advocated as major determinants of successful establishment in new areas (Stachowicz *et al.* 1999, Byers 2002a, Meiners *et al.* 2004, Locke *et al.* 2007). In the marine environment, man-made structures provide excellent physical and biological conditions for nurturing recent arrivals and supporting invasion success. Unfortunately, they are also the areas that receive the highest propagule pressure from both international and domestic vessels.

### 1.1.1 Invasions onto Man-Made Structures

The first point of potential contact in a new environment for marine invaders is often ports and marinas (Floerl *et al.* 2009). Today's extensive global trade and passenger movements provide frequent vector opportunities to these areas (Jenkins 1996). For example, Carlton (1999) predicts that at any given time some 10,000 species are being transported around the globe in the ballast tanks of ships alone. What's more, ports and marinas provide ample artificial habitat for NIS species to settle on. Pier pilings, pontoons, jetties, breakwaters, retaining walls and other harbour infrastructure are all common artificial substrata in ports and marinas and can comprise several square kilometres of settlement space per facility (Glasby & Connell 1999). It has been suggested that artificial surfaces may grant a more "level playing field" for NIS in environments for which they otherwise may not be as well adapted compared to native species (Tyrrell & Byers 2007). Indeed, in some areas, NIS are more common on artificial structures, such as pontoons and pilings, than are species native to the area (Glasby *et al.* 2007). Not only do artificial structures support NIS (Holloway & Keough 2002, Lambert & Lambert 2003, Thornber *et al.* 2004), they also function as corridors for expansion (Bulleri & Airoidi 2005, Floerl *et al.* 2009), giving them a place to gain a "foothold" in new environments before population expansion and further spread. Because artificial habitats have become so common along vast stretches of coastline and within estuaries (Connell & Glasby 1999, Chapman & Bulleri 2003, Bulleri & Chapman 2004), they can function as "stepping stones" for invasion, allowing propagules to spread to and colonise areas that they normally would not reach (Floerl & Inglis 2005, Bulleri *et al.* 2006). Furthermore, as well as providing a demographic conduit for spread, artificial structures can become vectors themselves as they are moved around ports and marinas as required.

Aquaculture, the farming of fish, shellfish and aquatic plants, is another major gateway for NIS (Naylor *et al.* 2001). Again, aquaculture facilities provide abundant artificial hard surfaces for NIS (e.g., mussel socks, lines, buoys, the shellfish themselves). For example, the longline mussel aquaculture industry in Prince Edward Island, Canada, has been overwhelmed by extremely high

abundances of the invasive ascidian *Styela clava*, causing both production and processing problems as they foul equipment and attach to mussel shells (LeBlanc *et al.* 2007). Aquaculture can also serve as a vector of NIS species through the intentional release of species in new areas for aquaculture purposes, accidental escapes of cultured organisms from aquaculture facilities, and the transport and culture of shellfish and their associated organisms (Siguan 2003, Ruiz *et al.* 1997). Good examples are, the highly invasive kelp *Undaria pinnatifida*, that was deliberately introduced for farming to the French Atlantic coasts (Siguan 2003), the Japanese or Pacific oyster *Crassostrea gigas*, which is widely cultured and has spread from farmed areas to much of the temperate coastline of the world (Shatkin *et al.* 1997), and the invasive alga *Codium fragile tomentosoides*, which is believed to have been spread along the eastern coast of North America on shells of commercial oysters (Malinowski & Ramus 1973).

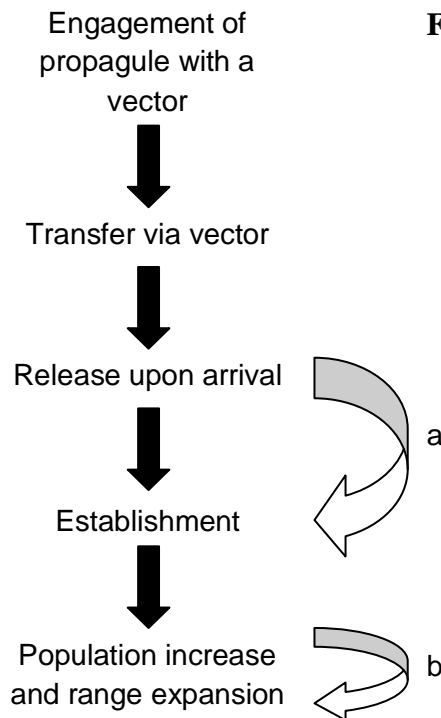
Nevertheless, suitable conditions in new environments, such as available man-made substratum, are not the only determinants of invasion success. It is well known that demography and life-history features of NIS play a key role in invasion success, and mounting evidence implicates propagule pressure as a major determinant (Simberloff 2009).

### 1.1.2 The Role of Demography in Invasion Success

Interactions between man-made substrata and NIS can culminate in individuals coalescing into populations. As these populations form, there can be an exponential increase in dispersive propagules leading to increasing population size and spreading along a coastline (Lockwood *et al.* 2005, Colautti *et al.* 2006b, Simberloff 2009). The majority of literature uses the term “propagule pressure” in the context of initial establishment of a NIS, often being defined as a product of frequency (e.g., how often a ship arrives with a species) and intensity (number of propagules arriving each time) of propagules arriving in a new area. However, the term can also be used to describe the frequency and intensity of propagules released from a source population (Lockwood *et al.* 2005, Figure 1.1); this is how this term will be used in this thesis.

There are many ways for an invasive species to achieve high propagule production. One is through massive reproductive output by a few individuals, or multiple spawning events in a season. Another is by having relatively large seed populations that together release large numbers of propagules. Therefore, in the early stages of species invasion, demographic characteristics of the individuals within the populations are of critical importance. How fast do they grow, how long to maturity, how long do they live, how many times do they reproduce, how

many larvae do they produce? Answering these questions underpins much of the work in invasion biology and can lead to conclusions on likely rates and patterns of spread that are important to management of invasive species.



**Figure 1.1:** The process of species invasion, beginning with the engagement of a propagule in its native range with a vector, followed by transport to a new environment by that vector and subsequent release upon arrival, through to establishment followed by population increase and range expansion. Propagule pressure to a new environment will increase the chance that a species establishes in a new environment (arrow a) and propagule pressure in that new environment will increase the chance of further spread leading to range expansion (arrow b) (figure adapted from Lockwood *et al.* 2005).

Baker (1965, 1974) discussed several characteristics which may be expected in “the ideal weed” and proposed that plants that have none or very few of these characteristics are unlikely to be “weedy”. Some of these characteristics included rapid growth to sexual maturity, the ability to reproduce sexually and asexually, and tolerance to a wide range of environmental stressors and conditions (also see Rejmánek & Richardson 1996). Many of these characteristics repeatedly appear in the literature on invasive species. For example, successful invasion of birds in Britain has been associated with dispersal ability, capacity to compete for habitat and resources with native species, high rate of population increase due to larger clutches and multiple clutches per season (propagule pressure), and proximity of the source population resulting in multiple incursions (O’Connor 1986). Freshwater fish invaders have been characterized as possessing tolerance to a broad range of environmental conditions, rapid dispersal and colonization, aggressiveness, and competitiveness (Moyle 1986). A wide look at the literature shows several characteristics common to successful invaders emerging, supporting the contention that relationships exist between demographic and life-history characteristics and the potential for

invasiveness (Sakai *et al.* 2001). Therefore, any insight into what aspects improve the invasiveness of species must be based on a complete knowledge of the population biology of the species under investigation.

However, not all successful invaders exhibit the classic invasive traits outlined above. High abundance, broad distribution, and an association with humans may also increase the chances of successful invasion (Elton 1958). There remains much uncertainty about which traits make sessile marine species successful invaders. *Styela clava* is likely a successful invader because of its ability to form massive populations as a result of high propagule pressure (and consequently further enhancing propagule pressure), thus increasing its chances of further spread. It has clearly been vectored internationally by shipping, but transports locally on structures, both via demographic means and anthropogenic activity. In eastern Canada *Styela* has keyed into aquaculture structures and formed enormous populations, sometimes thousands of individuals per m<sup>2</sup> (Osman & Whitlatch 1999, Bourque *et al.* 2005). From these populations it has then been able to spread rapidly among facilities and along the coastline.

In New Zealand, *Styela clava* has established populations in many places, but none yet at the densities seen elsewhere. Therefore, *Styela clava* presents an ideal situation to study a notoriously invasive species in its early stages of establishment after arrival but before spreading along the coast. I will use *Styela* as a model organism in this thesis to understand how and which demographic parameters can contribute to population growth, propagule pressure and further spread.

## 1.2 Study Organism

### Taxonomic status

Phylum: Chordata

Subphylum: Tunicata

Class: Ascidiacea

Order: Pleurogona

Suborder: Stolidobranchia

Family: Styelidae

Species: *Styela clava* (Herdman 1882)

Ascidians appear to be one of the most successful groups of organisms among fouling communities and continue to increase in abundance in ports worldwide (Mack *et al.* 2000, Lambert 2007). Styelidae is a large family with many species found worldwide (Brewin 1946). One causing considerable disruption in many countries is *Styela clava* (hereafter referred to as *Styela*, Figure 1.2). It is commonly known as the clubbed or Asian tunicate and is an invader that has successfully established populations in temperate waters worldwide. *Styela* is native to the northwest Pacific Ocean. Its native range extends from the Sea of Okhotsk, through southern Siberia, Japan, Korea and northern China, south to Shanghai (Abbott & Johnson 1972, Figure 1.3). *Styela* was first described by Herdman (1882) from samples dredged from the sea of Okhotsk (Abbott & Johnson 1972). Little is known about *Styela*'s distribution and ecology in its native range. It is considered a delicacy in southern Korea, often prepared by steaming, in a dish called "mideuduck" (Figure 1.4e). However, in its native range it expanded readily onto man-made structures, becoming a nuisance species in some areas. For example, it fouls fish cages (Cao *et al.* 1998) and encumbers the hanging culture of oysters (Kang *et al.* 1980) and edible sea squirts (Rho *et al.* 1993) in Korean waters. It may also cause an asthmatic condition in Japanese workers removing the species from shellfish products (Morris *et al.* 1980, Clarke & Therriault 2007). Thus, by occupying natural as well as artificial structures in its native range, *Styela* has increased its population size, and therefore, potential reproductive output contributing to the range expansion seen today.

*Styela* was first discovered outside its native range in North America on the Californian coast in 1932. It is now found in temperate marine waters worldwide including the western and eastern coasts of North America (Wonham & Carlton 2005), Canada (Lambert 2003), throughout much of Europe (Charlisle 1954, Christiansen & Thomsen 1981, Davis & Davis 2005, Minchin *et al.*

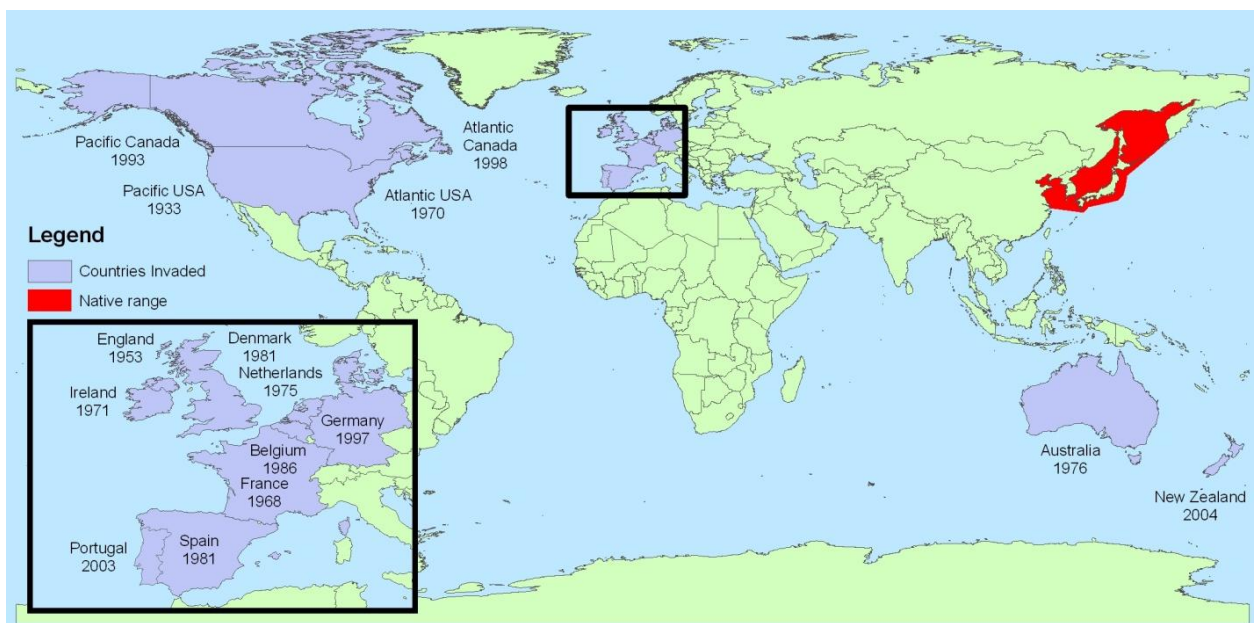


2006, Nunn & Minchen 2009), southern Australia (Hewitt *et al.* 1999), and more recently, New Zealand (Davis & Davis 2006, Figure 1.3). In its invasive range, *Styela* poses a potential threat to native biodiversity and aquaculture production. In several invaded locations, *Styela* has proliferated to reach very high densities, up to 500-1500 individuals per m<sup>2</sup> (Holmes 1976, Lützen 1999, Minchin & Duggan 1988, Osman & Whitlatch 1999). In Canada, it has reduced production in cultivated shellfish including oysters, scallops, clams and mussels by fouling and overgrowing shellfish and aquaculture equipment which can smother cultured animals (Bourque *et al.* 2005, Figure 1.4c, d, f), and in some cases has resulted in up to a 50% loss of shellfish production (Colautti *et al.* 2006a). On mussel longlines, for instance, *Styela* can foul mussel shells so much that when the mussel ropes are pulled up to be harvested, the weight of the ascidians can cause the mussels to fall off the rope (Gittenberger 2009). Anti-fouling treatments have been tested against *Styela* in aquaculture, including air exposure and acetic acid treatments, but to no avail (LeBlanc *et al.* 2007). But, even if appropriate antifouling treatments are found, they will be of little use if the transport vectors are not also addressed.

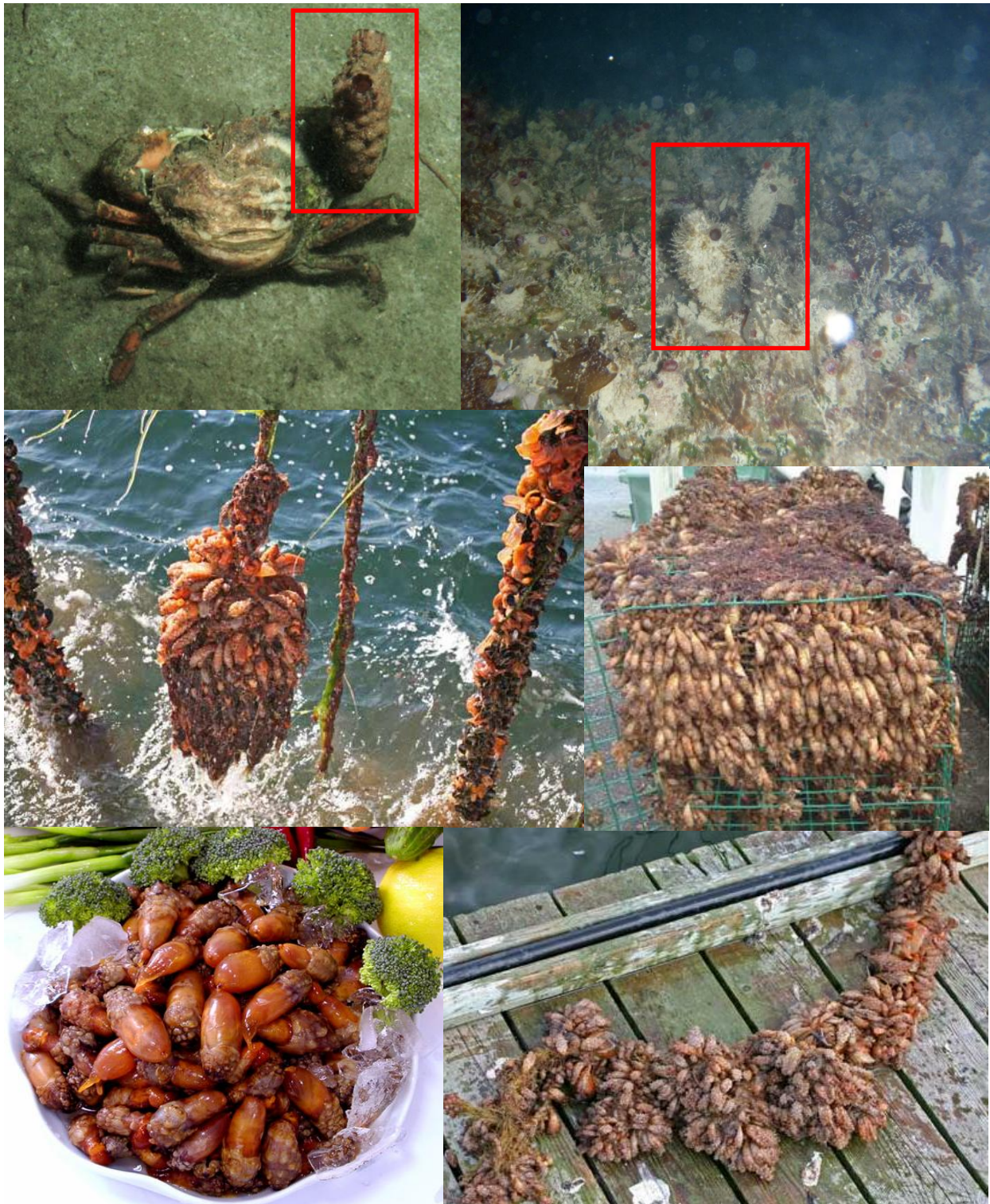
Global spread of *Styela* has most likely been on the hulls of international vessels and the transportation of aquaculture equipment. *Styela* readily attaches itself to boat hulls (Darbyson *et al.* 2009). Ballast water is considered an unlikely vector due to *Styela*'s short larval time. There have also been anecdotal reports of tunicates occurring on the carapaces of crabs and lobsters (Bernier *et al.* 2009). Bernier *et al.* (2009) investigated this and found several ascidian species (but no *Styela*), suggesting that lobster and crabs may present a vector for the spread of invasive tunicates regionally. Surprisingly, an internet search found an image of *Styela* attached to a crab carapace (*Necora puber*) in Port Zélande, Netherlands (Junne 2007, Figure 1.4a).



**Figure 1.2:** View of an adult *Styela clava* in Lyttelton Port on a vertical pontoon face showing the distinctive lateral banding on siphons, stalk attaching to substratum, and covering of epibionts and silt. Shown actual size. Photograph courtesy of Dr. Chris Woods, NIWA, Christchurch.



**Figure 1.3:** Map highlighting the native range of *Styela clava* (in red), extending from the Sea of Okhotsk south to Shanghai. Map also illustrates the invasive range of *Styela clava* (in purple) along with year of first detection in those countries.



**Figure 1.4:** Clockwise from top. (a) *Styela clava* attached to a crab (*Necora puber*) carapace in Port Zélande, Netherlands (<http://www.junne-diving.eu/images/zelande2007.htm>). (b) Photograph of *Styela clava* amongst the matrix of other fouling organisms at one of my sites in Lyttelton Port. (c) An oyster cage from Canadian waters overgrown by *Styela clava* (see <http://www.dfo-mpo.gc.ca/science/Publications/annualreport-rapportannuel/ar-ra0607/sect1-eng.html>). (d) *Styela clava* covering Christmas tree rope (used in aquaculture), Prince Edward Island, Canada (<http://www.ascidians.com>). (e) Medeodok-chim, a Korean dish made from steamed *Styela clava*. (f) *Styela clava* infestation on mussel ropes, Prince Edward Island (<http://www.ascidians.com>).

### 1.2.1 *Styela clava* in New Zealand

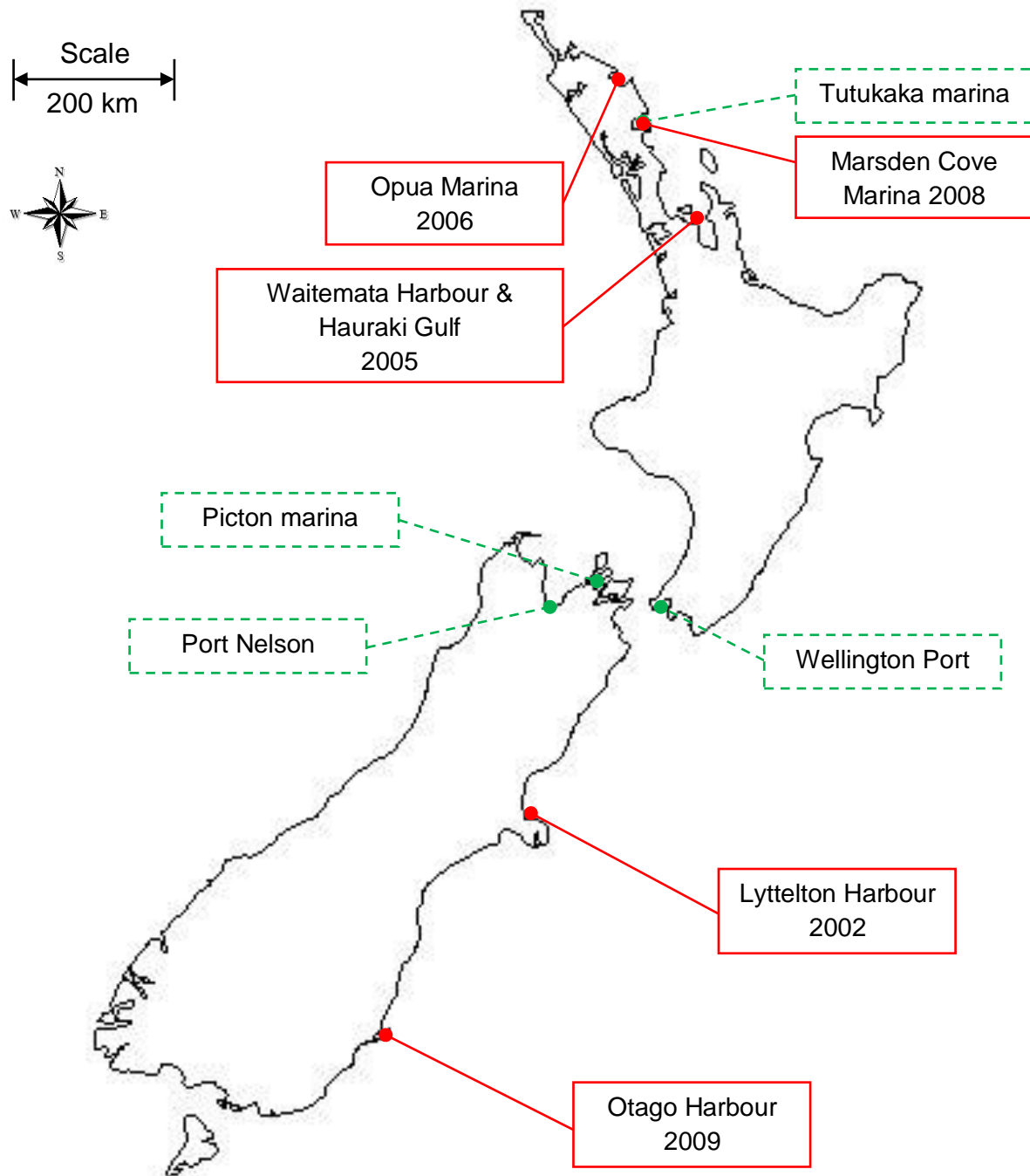
There are several established non-indigenous ascidians known in the marine waters of New Zealand. Those classified as pests on the MAF Biosecurity New Zealand website ([www.biosecurity.govt.nz](http://www.biosecurity.govt.nz)) include the colonial *Didemnum vexillum*, and the solitary ascidians *Pyura stonifera prueputialis* and *Styela clava*. *Styela* is the only one of these classified as an unwanted organism. *Ciona intestinalis* (Linnaeus 1767) is also non-native to New Zealand. It was first recorded in Lyttelton Port in 1948 (Brewin 1950), but has been recorded throughout much of the country (Inglis *et al.* 2006a). Although *C. intestinalis* has become a major problem in some areas overseas and is now considered a greater threat (than *Styela*) in Prince Edward Island, Canada (Ramsey *et al.* 2008), it has (thus far) not been a pest in New Zealand, even though it is widely spread throughout the country.

*Styela* was first detected in New Zealand in the Viaduct Basin, Waitamata Harbour (Auckland) in August 2005 (Davis & Davis 2006). A second specimen was later identified from samples taken as part of a baseline survey of Lyttelton Port (Christchurch) in 2004 (Gust *et al.* 2008). It was also found in hull fouling samples taken off a Lyttelton Port tug boat in May 2002, but was incorrectly identified as *Styela plicata* at the time (Gust *et al.* 2008). It has been suggested that *Styela* has been present in New Zealand since at least 2002, based on analysis of specimens from Waitemata Harbour and nearby areas of the Hauraki Gulf (Kluza *et al.* 2006). It has since been detected in Tutukaka Marina, the Hauraki Gulf (Waiheke Island, Mahurangi, and Wilson Bay), Port Otago, and recently in Port Nelson (Figure 1.5).

Genetic evidence indicates there has been multiple incursions of *Styela* into New Zealand waters (Goldstien *et al.* 2010). This is shown by the distribution of genotypes among sites and regions within New Zealand. Not only are there significant genetic differences between northern (Auckland area) and southern (Lyttelton Port) populations. Most of the Auckland area populations are probably related, but those from Lyttelton Port seem to have high genetic diversity, even though most sites were < 1 km apart.

It is dubious that aquaculture is a conduit for *Styela* into New Zealand waters due to our lack of importation of stock from infested areas. The most plausible vector of *Styela* into New Zealand is via commercial ships and recreational boats from overseas ports. Arrival via plankton in ballast water is unlikely because *Styela* has such a short larval life compared to the long transport

time needed to get to New Zealand from overseas source populations. Therefore, transport on hulls seems most likely based on knowledge of the life history of this species.



**Figure 1.5:** Map of New Zealand indicating areas *Styela clava* has been detected and dates it was first detected (or dates when previous samples were taken in which *Styela clava* has since been identified). Areas indicated in red (solid lines) are known established populations. Areas in green (dashed lines) are areas where a few individuals were found and have not established because of successful eradication (information from MAF BNZ, <http://www.biosecurity.govt.nz/seasquirt> and Gust *et al.* 2008).

Within New Zealand *Styela* may broaden its range via several vectors. Although New Zealand regulates ballast water discharge for international vessels entering the country, there is no such regulation for vessels within New Zealand. Therefore, any ships that take on ballast water in infested areas and discharge this water within a short time frame (1-3 days) are potential domestic vectors of *Styela*. *Styela* will also settle on a variety of surfaces that are potential transport vectors, including vessel hulls, aquaculture equipment, and mobile port infrastructure. Subsequent translocation of such equipment presents opportunities for further spread. For example, floating concrete pontoons are commonly found within ports and marinas in New Zealand and provide excellent habitat for *Styela*. These structures are often moved around ports and harbours and provide an excellent conduit for further spread. Furthermore, the processing and movement of fouled shellfish and other aquaculture equipment within New Zealand may provide opportunities for further spread.

Fouling problems by *Styela* in New Zealand have not been as extensive as overseas. However, anecdotal evidence is that *Styela* is fouling many mussel and oyster aquaculture operations on Waiheke Island (Hauraki Gulf), potentially causing some production and processing problems (Goldstien, personal communication). *Styela* may therefore pose a further threat to the rest of New Zealand's aquaculture industry, particularly our Greenshell™ mussel (*Perna canaliculus*) industry worth hundreds of millions per year (NZ\$204 million in 2008, Aquaculture New Zealand 2009). Greenshell™ mussels, like their Canadian counterparts, grow suspended in the water column and therefore, they may be particularly susceptible to fouling by *Styela*. Stopping the spread of *Styela* to our major mussel growing areas is considered to be a priority.

### **1.2.2 Description and General Biology of *Styela clava***

*Styela clava* is a solitary tunicate. Its body is covered by a tough, brown, warty tunic that tapers to a stalk and attaches to the substratum by a membranous plate or peduncle. It is a relatively large ascidian that can attain a length of 200 mm or more (Minchin *et al.* 2006, this study). Larger specimens are often covered by epibionts such as corals, algae, hydroids, sponges, barnacles and other conspecifics (Lützen 1999, personal observation), which can make *Styela* fairly cryptic among the matrix of other fouling species. Small individuals have no distinct peduncle and the body is attached directly to the substratum (Lützen 1999, Cohen 2005, personal observation). Post-settlement *Styela* are sessile, and all natural dispersal takes place at the gamete and larval stages (Lambert 2005). *Styela* attaches to hard substrata such as rocks, stones and shells of bivalves (Lützen 1999), but can also attach on timber, rope and other conspecifics

(personal observation). It is typically found in shallow water (0-200 cm depth) in areas without tides, or attached to floating objects (Lützen 1999), although it is commonly found deeper (Abbot & Johnston 1972, Buizer 1980), and has been found as deep as 40 m (Dauvin *et al.* 1991).

*Styela* is hermaphroditic and oviparous (Holmes 1969). McClary *et al.* (2009) suggest that *Styela* may potentially be self-fertile, and some other ascidian species are capable self-fertilization (Ruppert & Barnes 1994), although most solitary ascidians reproduce sexually and possess mechanisms to prevent self-fertilization (Cloney 1992). The release of gametes appears to be synchronous and limited to a short interval (3 hours) in the afternoon in eastern Canada (Bourque *et al.* 2007). Larvae hatch after ~12 hours and are planktonic for a further 12-36 hours (Holmes 1969, Svane 1984, Minchin *et al.* 2006). An individual produces relatively few, lecithotrophic, medium-sized, snake-like larvae (Bullard & Whitlatch 2004) that are negatively buoyant (sink), negatively geotactic (swim up), positively phototactic (move towards light) (Davis 1997), and do not seem capable of spreading far as they seldom travel more than a few centimetres by sustained swimming activity (Minchin *et al.* 2006). Little is known about egg and larval ability to disperse, but a working hypothesis is that dispersal is short-range and that the species leap-frogs, in relatively small steps, from highly localised small populations to expand.

Juvenile *Styela* grow at an average rate of 10-15 mm in length per month in a California population (Morris *et al.* 1980). Growth slows as animals reach sexual maturity (at ~ 85-90 mm in length), typically within five to six months in California (Parker *et al.* 1999) and 10 months in Denmark (Lützen 1999). The observed regional differences in growth and age to sexual maturity are probably related to differences in water temperature or food supply. Growth is reduced over winter when water temperatures are lower (Lützen 1999). There have been no published estimates for mortality rates of *Styela*, but the maximum life span has been estimated to be three years (Morris *et al.* 1980, Lambert & Lambert 1998).

*Styela* can tolerate a wide range of environmental conditions, which is a recurrent trait of invasive species. It is able to persist in temperatures ranging from -2 to 23°C (Buizer 1980, Lützen 1999), but it may not be able to reproduce at temperatures below 15°C (Eno *et al.* 1997). *Styela* is also able to endure hyposalinity as low as 8‰ due to its ability to close its siphons for extended periods of time (Sims 1984, Lützen 1999). However, it may not be able to persevere in an area where salinity is consistently below 20‰ (Davis & Davis 2004).

As *Styela* can be an aggressive invader, it may be a good competitor for space and food. Experimental work by Osman *et al.* (1989) illustrates how *Styela* is capable of greatly reducing localised settlement rate of oysters by filtering their planktonic larvae. Because it grows to a relatively large size it can stand above many co-occurring sessile species, *Styela* may compete well for food and prey on propagules of its competitors. Factors that enable *Styela* to build traction and expand populations include the availability of hard substrate for attachment (Lützen 1999) combined with sufficient phytoplankton as a food source (Locke *et al.* 2007). In Lyttelton Port, there are ample hard substrates including floating concrete pontoons, pilings, rock walls and other concrete structures.

### 1.3 Study Outline and Aims

Investigating demographic and life history features of invasive species allows a determination of how they gain traction in a new environment and build up the numbers required for population expansion and further spread. There is a paucity of work investigating the demography and life history of *Styela* and few studies have been done on this species in New Zealand waters. This study describes various demographic and population features of *Styela* in local populations in Lyttelton Harbour. A fellow student, Sarah Nutsford, worked on a collaborative but separate thesis focusing on reproduction and early life history of *Styela* in Lyttelton Port and much of her work is used as parameters in models and to aid with the interpretation of results, and *vice versa*.

Chapter Two, **General Demography**, describes basic demographic features of *Styela* in Lyttelton Port including distribution and abundance, population size structure, recruitment, and survivorship. I focus my observations of abundance on *Styela* on aggregations of the species at two sites within Lyttelton Port on two types of artificial surface, floating concrete pontoons and wharf pilings. I then compare my findings with other work describing the abundance of *Styela* in these areas to determine how much the population may have grown over the last few years. Population size structure through time is illustrated as length-frequency distributions which are used to explore the populations dynamics through time and the timing of recruitment events (and growth rates in the next chapter). Recruitment is further investigated experimentally using clearance plots and the deployment of artificial surfaces. Finally, patterns of survivorship are investigated using a mark-recapture style experiment.



Chapter Three, **Growth**, scrutinizes observed rates of growth in *Styela* and describes size-specific, spatial and temporal patterns in growth rates. A mark-recapture style experiment provides the monthly growth increment data necessary. These data are then used to model age-structured growth curves, and as parameter for inclusion in the following chapter.

Chapter Four, **Lefkovich Matrix Models**, applies the data collected above, uniting growth, survival and recruitment data in several deterministic size-structured matrix models. These models are then used to investigate which demographic characteristics or life history stages contribute the most towards population growth, and thus invasion success.

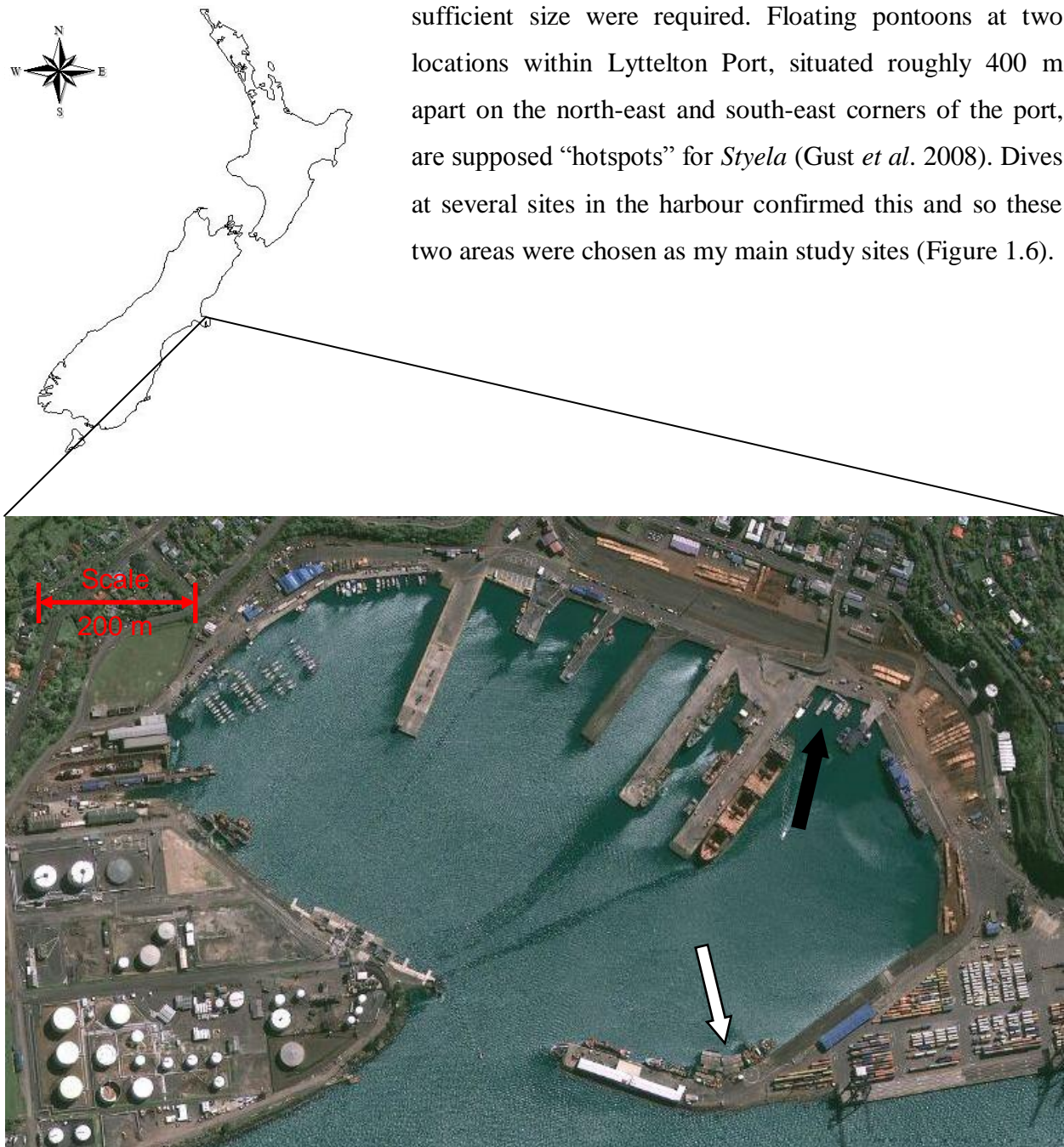
Chapter Five, **Experimental Studies**, aims to test hypotheses about density-dependence in demographic responses and how these responses vary spatially (i.e., by depth, site, in light or dark habitats). This chapter also investigates the rates of recruitment of other commonly occurring ascidian species in Lyttelton Port as a comparison to the recruitment patterns of *Styela*.

The final chapter presents a general discussion of the demographic and life history features investigated, how this work relates to *Styela*'s ability as an invasive species, and how the models described herein can aid in the management and decision-making associated with invasive species.

## 1.4 Study Sites

Lyttelton Harbour is situated on the east coast of the South Island of New Zealand (Figure 1.6). It contains New Zealand's main South Island shipping port and has a recreational marina. Lyttelton Port is one of New Zealand's busiest ports and is connected directly via shipping to all other New Zealand ports. It also accommodates substantial import and export to and from several international ports (Inglis 2001). To gain the data necessary for this study, populations of

sufficient size were required. Floating pontoons at two locations within Lyttelton Port, situated roughly 400 m apart on the north-east and south-east corners of the port, are supposed "hotspots" for *Styela* (Gust *et al.* 2008). Dives at several sites in the harbour confirmed this and so these two areas were chosen as my main study sites (Figure 1.6).



**Figure 1.6:** Map of Lyttelton Port showing my two study sites. A&B pontoons (indicated by the black arrow) and Z wharf pontoons (indicated by the white arrow).

### *Site A&B*

A and B pontoons (hereafter referred to as site A&B) are situated at the north-east corner of Lyttelton Port (Figure 1.6, Figure 1.7). This site is characterised as having heavy boating traffic from public ferries that come and go on a regular basis. This site is comprised of 7 concrete pontoons close to concrete and wooden pilings. The depth at this site is about 2 m at low tide. These pontoons originally came from site Z, but were moved here for use as the public ferry terminal (Lyttelton Port Company, personal communication).

### *Site Z*

Z wharf pontoons (hereafter referred to as site Z) are situated at the south-east corner of the port (Figure 1.6, Figure 1.8). This site is made up of 52 concrete pontoons close to a mixture of concrete and wooden pilings. The depth under these pontoons is considerably greater about 8 m and shipping traffic is much lower. Z pontoons are also much more sheltered than A&B, consistently having better water clarity.

At both sites, *Styela* is restricted to artificial structures such as floating concrete pontoons and ropes. It is also found in much lower densities on groyne rocks and pier pilings (personal observation). The temperature at these sites was monitored for the duration of the study using 4 StowAway Tidbit temperature loggers (Anset Computer Corp., Bourne, MA, USA). Two loggers, at 1 and 2 m depth, were used per site. Temperature was no different between sites, ranging between ~ 7°C during winter and ~ 20°C in summer (Figure 1.9). Two contrasting predictions for the minimum water temperatures required for *Styela* to successfully spawn have been published. Nimpis (2002) and Cohen (2005) both agree on 15°C, while Parker *et al.* (1999) suggest it may be able to spawn in waters as low as 10°C. A study of the *Styela* population in Lyttelton Port by Nutsford (unpublished data) agrees, more-or-less, with the estimate of around 15°C.

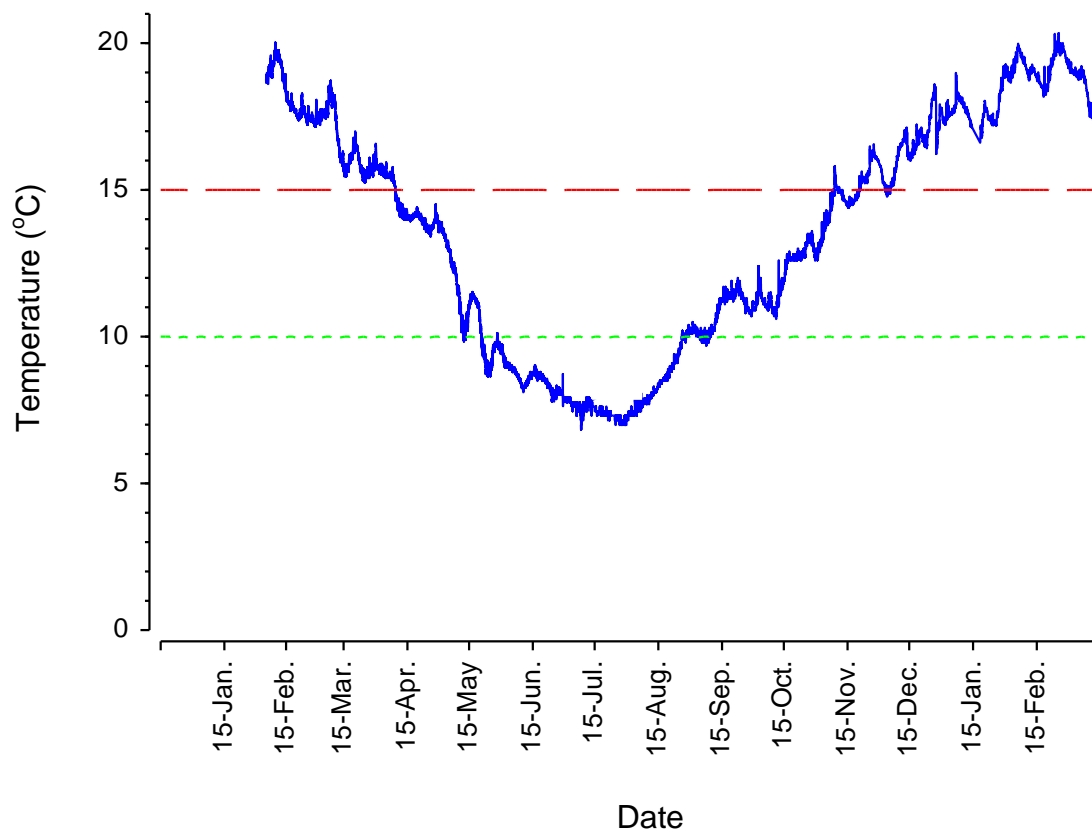
The sediment throughout the port is dominated by mud (< 1% sand) (Curtis 1985), and visual inspection below each of the sites confirmed this. Although both sites are relatively sheltered year-round with little wave action, pontoon faces (particularly the vertical faces) are covered in high levels of this fine mud/silt (Figure 1.4b), likely stirred by vessel movement within the port. Consequently, water clarity was low (generally < 1 m, although this was not quantified. See Gust *et al.* (2008) for further details).



**Figure 1.7:** View of A&B pontoons site, Lyttelton Port, facing west.



**Figure 1.8:** View of Z wharf pontoons site, Lyttelton Port, facing west.



**Figure 1.9:** Raw temperature data, from both sites A&B and Z combined and the two contrasting published predictions on the minimum water temperatures required for successful spawning in *Styela clava*. The hatched line refers to the 15°C threshold suggested by Nimpis (2002), Cohen (2005) and Nutsford (unpublished data). The dotted line refers to the 10°C threshold suggested by Parker *et al.* (1999).

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## Chapter 2

### General Demography

“If a man will begin with certainties, he shall end in doubts, but if he will be content to begin with doubts, he shall end in certainties.” – Francis Bacon

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#### 2.1 Introduction

To establish in new locations and become invasive, species must overcome a multitude of geographic, environmental and biological hurdles. Undoubtedly, life history characteristics are important in invasion success (Baker 1974, Sakai *et al.* 2001, Thompson 2004), yet many different life history strategies are represented in invasive species (Moyle 1986, O’Connor 1986). Consequently, there are many ways to be invasive, each of which involves the interaction of various life history stages with new environments.

The success of ascidians as invasive species is probably due to a suite of demographic characteristics such as rapid growth to maturity, high reproductive output, repeated larval release, advanced larvae, making use of habitat not generally used by other species, broad tolerances to environmental stressors, cryptic nature (especially as juveniles) and few, if any, predators (Lambert & Lambert 1998). For example, *Ciona intestinalis* is particularly successful due to its huge reproductive output (Carver *et al.* 2003), coupled with a broad tolerance to temperature (Dybern 1965) and salinity (Dybern 1967).

While ascidian life histories are generally well known (Brusca & Brusca 1990), the invasive characteristics of the cryptic invader *Styela clava* are largely unknown. Previous studies have shown that *Styela* reaches maturity quickly (Lützen 1999, Parker *et al.* 1999), and although it has a relatively low reproductive output (Bullard & Whitlatch 2004), by forming dense populations it can exert relatively high propagule pressure (Bourque *et al.* 2007). In estuaries off Prince Edward Island, *Styela* exhibits a distinct annual cycle with recruitment beginning at the end of

June and ending in late October (Bourque *et al.* 2007). It is also known to have a broad tolerance to temperature and salinity (Buizer 1980, Sims 1984, Lützen 1999). However, little is known about rates of mortality and growth, basic biology that is crucial to understanding the mechanisms behind *Styela*'s success as an invader.

Various methods have been employed by ecologists to determine demographic features of marine species. Simply sampling lengths (or weights) of individuals within a population and compiling these data as size-frequency distributions can aid in understanding the dynamics of populations because they reflect an interaction of rates of reproduction, recruitment, growth and mortality of the age groups sampled. For instance, total mortality is often estimated from the sequential decline observed in cohorts of fish (Chapman & Robson 1960). Data of this type are widely used in fisheries in the analysis of catch curves (Krebs 1985). However, there are more direct, and arguably more accurate, methods for determining these parameters. Measurements of tagged individuals offer more resolute information on mortality rates, and can also produce size-specific mortality rates. However, tag-recapture experiments are much more time-consuming.

Summarizing how mortality is occurring within a population can answer a multitude of important questions. How long does a species live? Is mortality high among juvenile and/or older individuals? Does mortality vary spatially or temporally? Quantifying mortality and recruitment are essential to understanding the life histories and population dynamics of any organism and is important to know about the demography of *Styela* to gain some insight into what characteristics contribute most towards its success as an invasive species. This may lead to conclusions regarding the fate of the population in Lyttelton Port and the potential for spread from established populations, potentially providing important information for its management.

In this chapter I examine several aspects of the demographics of *Styela clava*, particularly, patterns of distribution and abundance, population size structure, recruitment and survivorship of the species. I specifically address the following questions:

- (i) How abundant is *Styela* at my sites in Lyttelton Port?
- (ii) How abundant are other ascidians in Lyttelton Port?
- (iii) What are the temporal patterns of recruitment and survivorship in *Styela*?
- (iv) Does survivorship differ spatially between sites and depths?
- (v) Is survival size-dependent in *Styela*?

## 2.2 Materials and Methods

### 2.2.1 Distribution and Abundance

The abundance of *Styela* was examined at two sites in Lyttelton Harbour, A&B and Z (Figure 1.6, Chapter 1). At each site, the abundance of *Styela* was sampled on pontoons and adjacent pilings. Density was recorded in one of two ways. On pontoons, 1 m<sup>2</sup> quadrats were placed randomly along the vertical pontoon faces between 0 and 1.5 m depth. On pilings, a 1 m long rope was placed vertically and *Styela* were counted around the pilings (which were ~1 m in circumference). This was replicated 15 times on each sampling occasion and was done bimonthly. All visible *Styela* within quadrats were counted. Abundance was recorded as the number of individuals per m<sup>2</sup>. The density of *Styela* per m<sup>2</sup> is graphed seasonally by site and substratum. A non-parametric analysis of variance (ANOVA) on ranked data was done in the statistical package R (version 2.9.0); the non-parametric test was used because data were unbalanced (due to sampling bimonthly but pooling data into seasonal groupings) and did not meet the assumptions of ANOVA.

In addition, the abundance of the invasive ascidian *Ciona intestinalis* and native ascidian *Cnemidocarpa* sp. was determined at Z pontoons using randomly placed quadrats, but on only two sampling occasions (6 August and 14 December 2009). A plot was made of the mean numbers of *Styela*, *Ciona intestinalis* and *Cnemidocarpa* sp. per m<sup>2</sup> to illustrate differences in density between these three ascidian species.

### 2.2.2 Population Size Structure

Random quadrats also yielded bimonthly data on population size structure. All visible *Styela* within each quadrat on pontoons were measured to the nearest millimetre using Vernier calipers. Initially I aimed to measure approximately 30 individuals at each site (this was done for December 2008 and February 2009). It became apparent that these sample sizes were too small to adequately quantify patterns in population dynamics. Sample size was increased to around 100 individuals at each of the sites for the remainder of my sampling period (April 2009 to February 2010). Because I did not obtain a sample size big enough during density measures, further quadrats were sampled until the desired sample size was reached.

Length-frequency data for each site were compiled into 16 10-mm size classes (0-10 mm to 150+ mm). Each length-frequency observation consisted of the actual number of *Styela* measured.



These data were plotted as histograms to illustrate the size structure at each site and give an indication of the arrival of new recruits to pontoon faces. A December 2009 sample at site A&B (completing 1 year) is missing due to limited sample size (i.e.,  $n < 20$ ) because of persistent lack of water clarity during this month. Because length-frequency distributions, growth and mortality data were similar at both sites, data were pooled (aiming to increase the resolution of length-frequency data) and all other available length-frequency data (from mark-recapture experiment) were included to increase sample size.

### 2.2.3 Recruitment

Bourque *et al.* (2007) investigated recruitment in *Styela* using 10 cm × 10 cm PVC plates suspended vertically ~ 2 m below the water surface and observed recruitment densities of up to 0.95 individuals per cm<sup>2</sup> per week off the eastern coast of Prince Edward Island, Canada. In New Zealand (Bayswater Marina, Auckland), McClary *et al.* (2009) tried a similar experimental design (10 cm × 10 cm roughened Perspex™ plates set at 1-2 m depth), but no *Styela* were detected on plates for the duration of the investigation (October 2006 to April 2007). Given this wide discrepancy, I examined *Styela* recruitment in two ways: (1) by deploying settlement substrata and (2) using clearance plots. These methods were used to gain some insight into the rates and timing of recruitment in *Styela*. For the purposes of this study recruitment was defined as any animal that had reached a visible size.

#### *Deployed substrata*

Two different substrata were assessed for their suitability as recruitment surfaces: (1) settlement panels and (2) ropes. Settlement panels were deployed at three depths (0, 0.5 and 1.0 m below the low tide mark). These consisted of 15 cm × 15 cm Hardieflex™ panels bolted to lengths of 4 × 2 inch timber. Five of these arrays were attached to wooden pilings adjacent to the A&B pontoons on 22 January 2009. Hardieflex™ was used as it is a smooth concrete-based material (made up of cellulose fiber, cement and sand), thus providing a standard substrate on which recruits could be distinguished and a surface similar in composition to the floating concrete pontoons. Hardieflex™ panels have successfully been used in algal settlement experiments in the past (e.g., Taylor & Schiel 2003, Dunmore 2006). The panels were checked (by visual census) *in situ* on a weekly basis between January 2009 and March 2009 for any *Styela* recruits.

The second substrata used were thin nylon ropes (3 mm diameter × 3 m long). Fifteen ropes were deployed on 6 August 2009 between and at the edges of floating concrete pontoons at site Z,

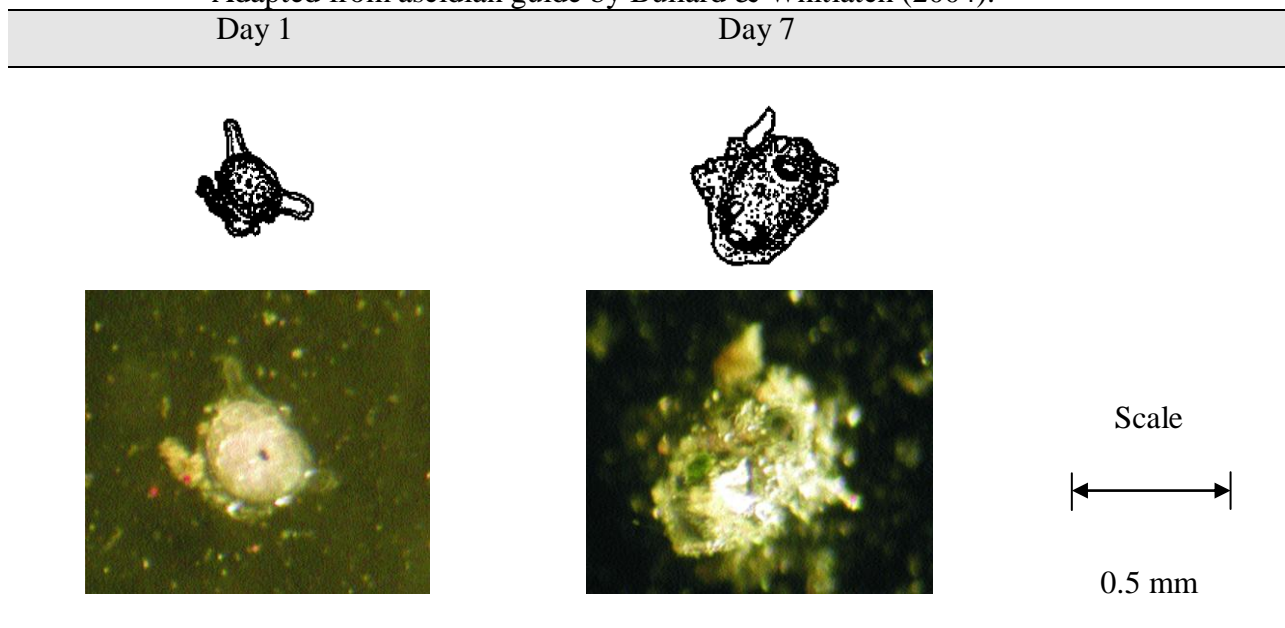
because *Styela* were observed attached to ropes in close proximity to these pontoons. The ropes were checked weekly for *Styela* recruits by simply lifting the ropes and checking them using a dissection microscope (20× optical zoom). Ropes were checked until February 2010.

### Clearance plots

A clearance experiment, consisting of  $n = 5$  plots, was established on vertical faces of pontoons at site Z during October 2009 and monitored until the end of February 2010, covering the majority of *Styela*'s major spawning period in Lyttelton Port (Nutsford, unpublished data). The 5 plots investigated here are part of a bigger experimental design covered thoroughly in Chapter 5. Plots were 0.5 m × 0.5 m and marked in corners by Selleys® Aqua Knead-it putty. Plots were cleared of all organisms using a hammer to aid removal of the larger hard-bodied organisms (e.g., oysters), then scraped using a metal paint scraper to remove the majority of organisms remaining, and finally scraped using iron wool to remove any smaller organisms. Plots were only cleared once.

Within clearance plots recruitment of *Styela* was monitored monthly. The guide by Bullard & Whitlatch (2004) was used to help in identification of recently recruited *Styela* (Figure 2.1). It was hoped that this part of the clearance experiment would make detection of smaller individuals easier and allow some insight into the temporal patterns of recruitment in *Styela*.

**Figure 2.1:** One-day old and seven-day old juvenile *Styela clava*, as drawing and colour plate. Adapted from ascidian guide by Bullard & Whitlatch (2004).



### 2.2.4 Survival

Individual *Styela* were mapped *in situ* on vertical pontoon faces at both sites beginning in December 2008. An alphanumerically labeled quadrat placed over marked permanent quadrat areas (using Selleys<sup>®</sup> Aqua Knead-it putty) was used to locate individuals. Thus, it was essentially a mark-recapture experiment without the need for tags. The addition of individuals was staggered throughout the experiment (i.e., additional individuals were mapped each month to increase the sample size as individuals died). The timing of re-visits was opportunistic depending on weather conditions and water clarity, and so the time at liberty was not always exactly one month. Individuals over a wide size range were included to be representative of the natural population size structure (and to include all sizes for use in growth analysis, Chapter 3). However, data are lacking for the smaller sizes due to their cryptic nature and problems associated with finding them when < 30 mm in length.

By recording individuals through time, encounter histories were obtained for 133 individuals over 16 monthly sampling occasions (December 2008 to December 2010) describing growth (Chapter 3) and survival rates (for individual encounter histories see Appendix 2.1). Often when an individual *Styela* died it left behind a “husk” or part of its tunic (Figure 2.2). If an individual was absent from its original location it was assumed dead.

Survival was analyzed using the program MARK (White & Burnham 1999). MARK was used because the data are staggered (i.e., individuals were added to the data each month to offset mortality and increase the sample size). This makes calculations of monthly mortality rates tricky. However, MARK can readily deal with this using Pollock’s staggered entry design (Pollock *et al.* 1989). A known-fates model was used to investigate site, depth, and time-dependence in survival rates. The known-fates model was selected because *Styela* cannot move once settled, and thus its fate is known (i.e., it is either present and alive at the location it was initially observed, or it is not, and therefore assumed to be dead). The known-fate model type is a product of simple binomial likelihood and is exactly the same as logistic regression. The advantage of using MARK for known-fate analysis is the convenience of model selection, capabilities to model average survival estimates easily and ability to easily compare the differences between models. Assumptions of the survival models implemented in MARK include: (1) marked animals have the same probability of recapture; (2) marked animals have the same probability of surviving; (3) marks are not lost or missed; (4) all samples are instantaneous (Pollock *et al.* 1990).

The relative fit of the models investigated was determined by ranking the Bayesian Information Criterion (BIC) values (Schwarz 1978). The Akaike Information Criterion (AIC) values (Akaike 1974) and model deviances were also considered as alternative methods for determining the best-fit models. Deviance is a quality of fit statistic produced for a model and may be used to compare the fit of two or more nested models. BIC and AIC describe the trade-off between accuracy and model complexity. For example, when estimating model parameters it is possible to reduce model deviance by adding additional parameters, which can result in over-fitting. BIC and AIC resolve this problem by penalising additional parameters in a model. The model with the lowest BIC or AIC is considered the most parsimonious. The difference between the two methods is that BIC places greater penalty on additional parameters than AIC. Figures illustrating survival estimates were produced as necessary.

The finite annual mortality rate was calculated by converting monthly survival estimates into instantaneous mortality rates (Equation 2.1). In this form, monthly mortality rates can be added directly (Krebs 1985). Instantaneous mortality rates were added for the months January 2009 to December 2009 and separately for March 2009 to February 2010. The average of these two annual instantaneous mortality rates was then calculated to take into account differences in survival rates between January and February 2009 and the same months in 2010. The mean annual instantaneous mortality rate was then converted back to the mean annual finite mortality rate (Equation 2.2). A derived survivorship curve was also made by plotting the cumulative monthly mortality rate (i.e., cumulative sum of monthly instantaneous mortality rates back-calculated into finite mortality rate) from February 2009 to February 2010.

**Equation 2.1:**  $\log_e(\text{finite survival rate}) = \text{instantaneous mortality rate}$

**Equation 2.2:**  $\text{Finite mortality rate} = 1.0 - e^{\text{instantaneous mortality rate}}$

### *Survival – size-specific*

A separate analysis was used to investigate size-specific differences in survival rates because many of the individual encounter histories used in the previous models were missing associated length covariate values (i.e., the length the month before the individual died). This reduced data set consisted of 92 individual encounter histories over 13 encounter occasions (February 2009 to February 2010) (for individual encounter histories see Appendix 2.2). Again, the known-fates

model type was implemented in MARK and included total length, body length and body diameter (see Figure 3.1, Chapter 3 for the definition of these measurements) the month before the individual was determined dead as covariates in the model structure. Model fits were assessed using BIC, AIC and model deviance and figures illustrating size-specific survival estimates were produced as necessary.

The size-specific model was further investigated for any normalizing selection on size (i.e., is survival lower in relatively big or small individuals). This is accomplished by including an additional parameter allowing for curvature in model structure (Equation 2.3).

**Equation 2.3:** 
$$\text{Survival} = \beta_1 + \beta_2 \times \text{size} + \beta_3 \times \text{size}^2$$

*Note: The structure of the model that does not include the additional normalizing parameter is  $\text{Survival} = \beta_1 + \beta_2 \times \text{size}$*

Finally, a complex model of size-specific survival through time was investigated in this analysis. A figure including the three variables was produced to illustrate the patterns in survival. The first two months (February and March 2009) were dropped from this figure as the data were too sparse for these months and produced unreasonable estimates of survival.



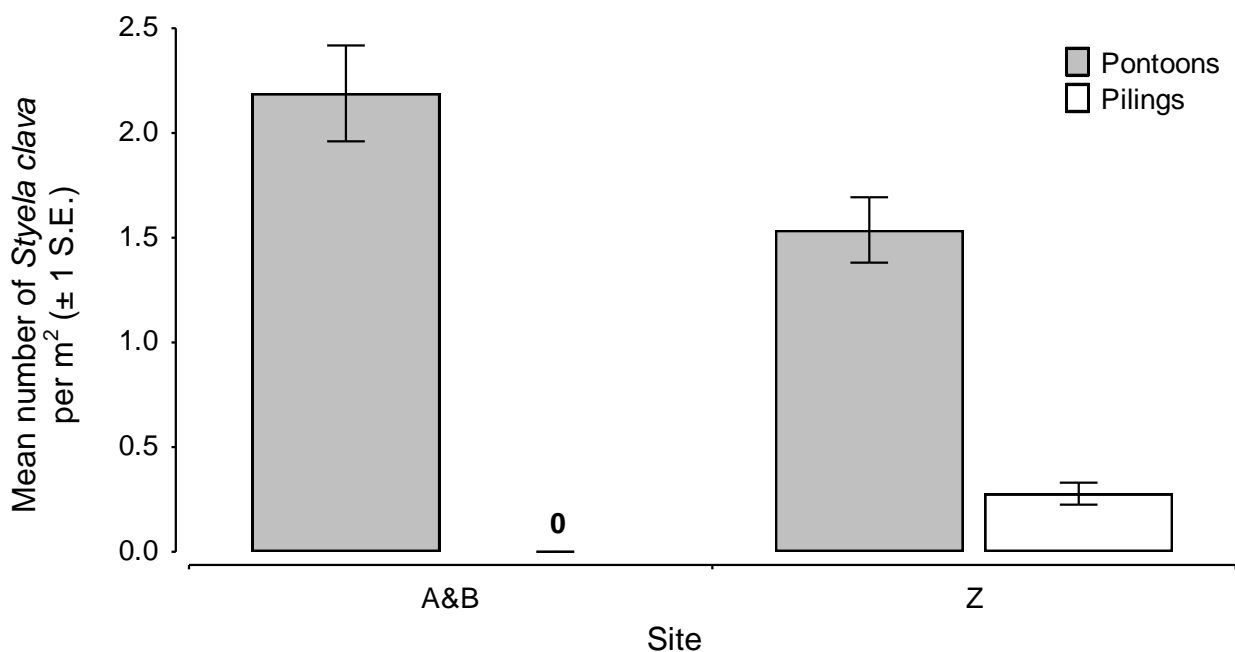
**Figure 2.2:** A deceased *Styela clava*, illustrating the remnants of its tunic and lack of internal organs. This individual was collected from a vertical pontoon face at site Z, February 2010.

## 2.3 Results

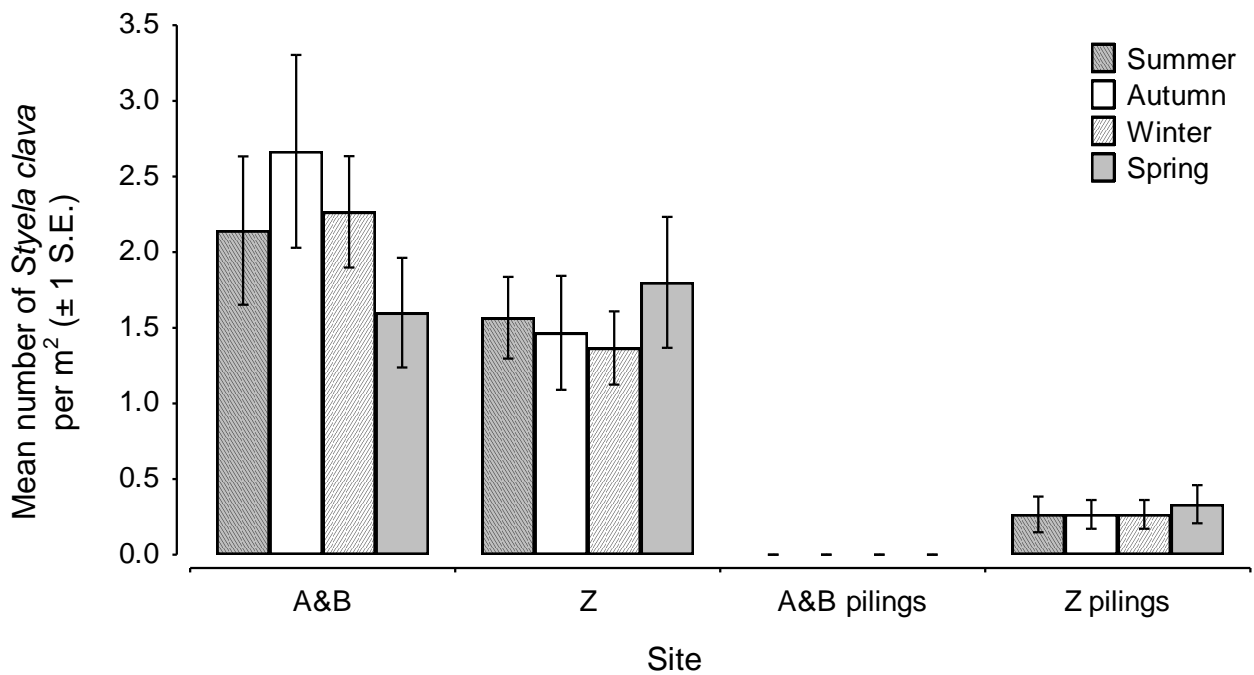
### 2.3.1 Distribution and Abundance

*Styela* displayed a relatively patchy distribution at the two sites examined, with densities ranging between 0 and 10 individuals per m<sup>2</sup>. Fewer individuals were found on pilings compared with pontoons ( $F_{1,343} = 260.12$ ,  $p < 0.001$ ) (Figure 2.3) with none being found on the A&B pilings for the duration of the study. There was no significant difference between numbers at the A&B pontoons and the Z pontoons ( $F_{1,343} = 0.39$ ,  $p = 0.535$ ). The average density of *Styela* changed slightly seasonally, particularly at A&B pontoons (Figure 2.4), but differences were not significant ( $F_{3,343} = 2.45$ ,  $p = 0.0639$ ). This relatively constant density reflected the extreme patchiness in abundance, and therefore high spatial variance.

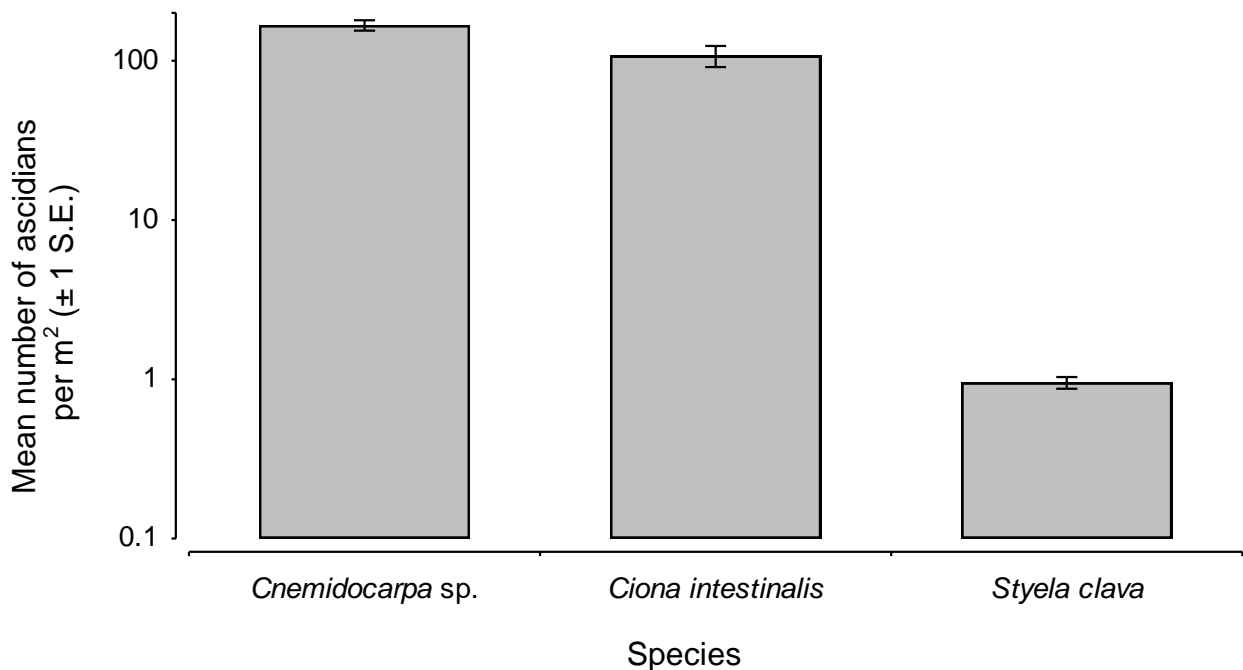
*Styela*'s abundance was also compared to that of other ascidian species to get a feel for how abundant it is in Lyttelton port (this is discussed further in Chapter 5). Densities of the other two common ascidians in Lyttelton Port were much higher than that of *Styela* (Figure 2.5). Densities of *Cnemidocarpa* sp. and *C. intestinalis* ranged between 128 to 200 per m<sup>2</sup> and 36 to 192 per m<sup>2</sup>, respectively.



**Figure 2.3:** Mean numbers ( $\pm 1$  S.E.) of *Styela clava* per m<sup>2</sup> at two sites (A&B and Z) on two substrate types (vertical concrete pontoon faces and wooden wharf pilings), across all depths (maximum depth of ~2 m).



**Figure 2.4:** Quarterly mean numbers ( $\pm 1$  S.E.) of *Styela clava* per m<sup>2</sup> at two sites (A&B and Z) on two substrate types (vertical concrete pontoon faces and wooden wharf pilings), across all depths (maximum depth of ~2 m).



**Figure 2.5:** Mean numbers on logarithmic scale ( $\pm 1$  S.E.) of the ascidians *Cnemidocarpa* sp., *Ciona intestinalis* and *Styela clava*, per m<sup>2</sup>, at both sites (A&B and Z), across all depths (maximum depth of ~2 m).

### 2.3.2 Population Size Structure

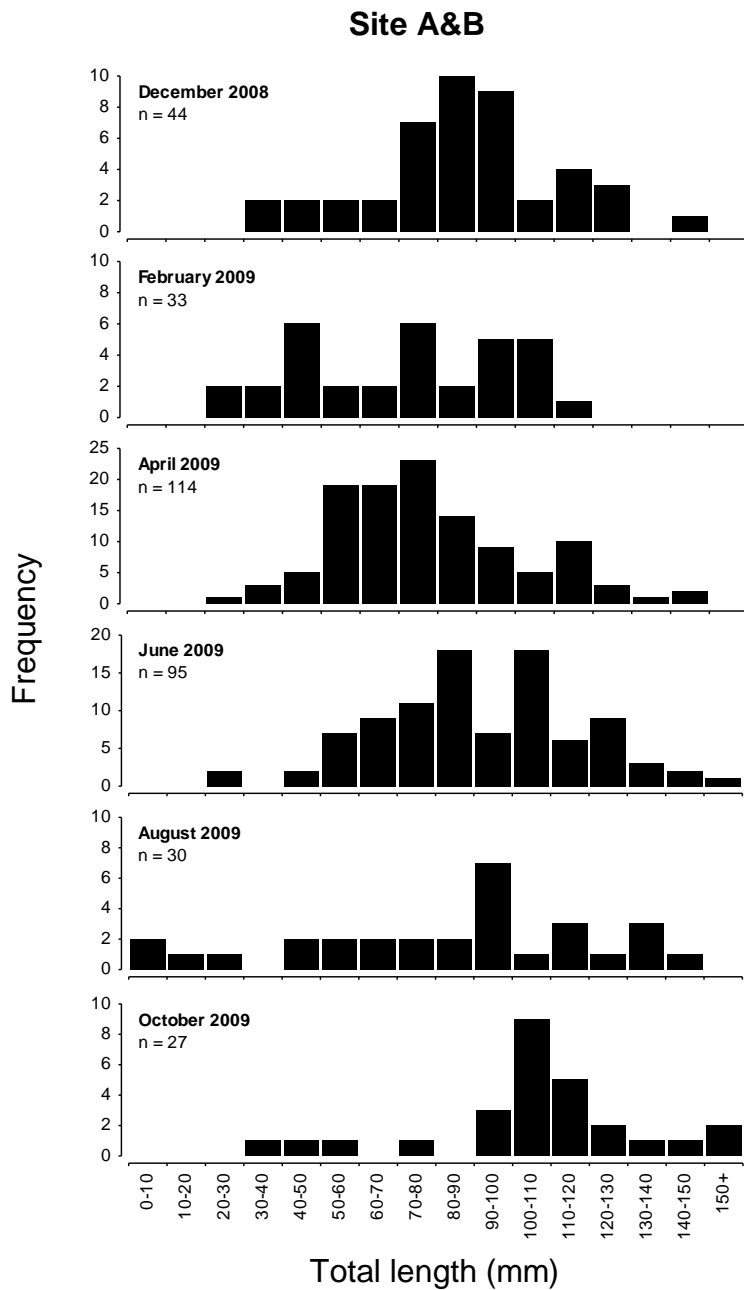
The largest individual *Styela* recorded during the course of the study was 168 mm total length at site Z (November 2009) and the smallest individual detected was 7 mm total length at site A&B (August 2009). Detecting smaller individuals (< 30 mm) at the two sites was extremely difficult because they were invariably covered in sediment and encrusting organisms such as hydroids, and therefore highly cryptic. This is likely to introduce considerable bias towards under-estimating primary recruitment.

Population size-structure changed considerably through time, showing movement of often ill-defined modes at both sites (Figure 2.6, Figure 2.7). The largest individuals progressively disappeared throughout the study, and size-frequencies for similar months in different years were remarkably similar (e.g., February of 2009 and 2010) (Figure 2.8). These modes are further analyzed for growth in Chapter 3.

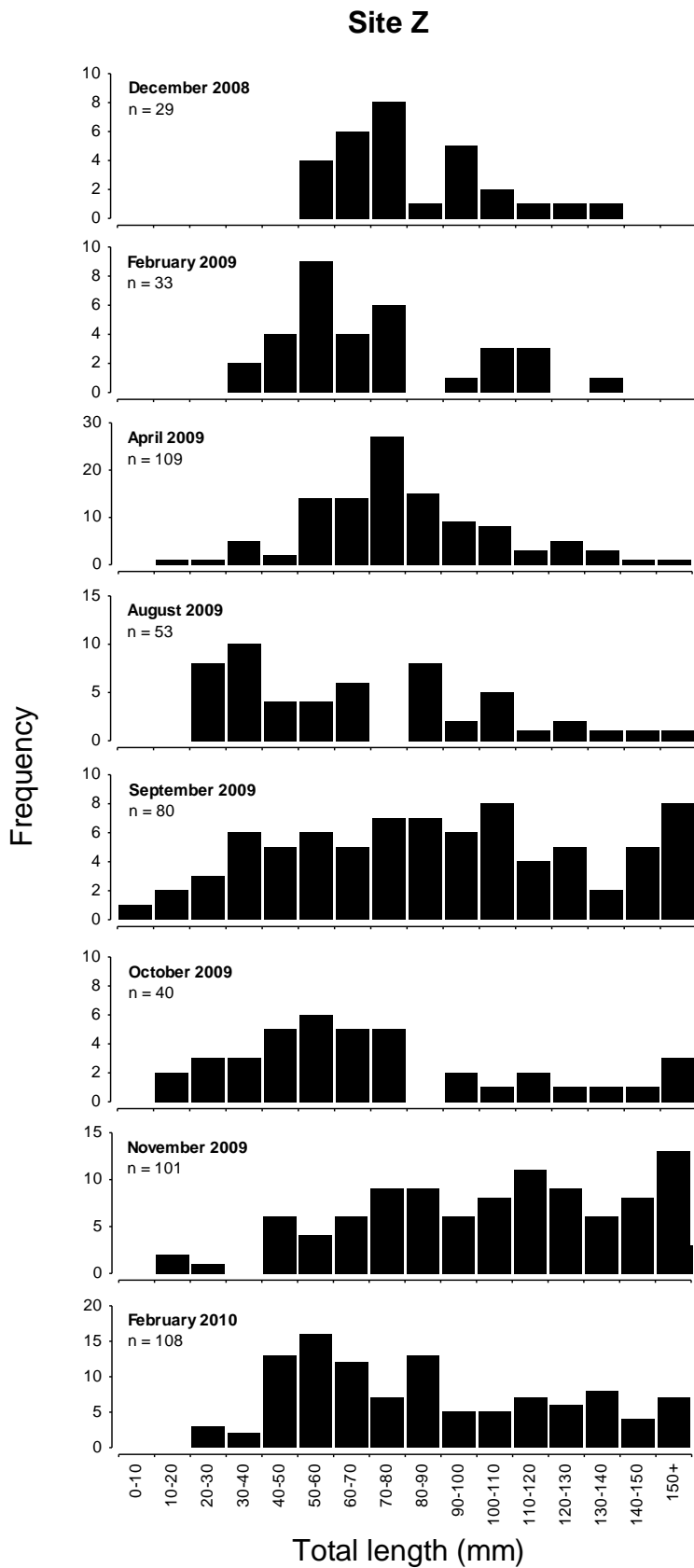
Before my interpretation of recruitment from the size-frequency samples the reproductive periodicity of *Styela* in Lyttelton Harbour must first be made clear. Work by Nutsford (unpublished data) showed that *Styela* was reproductively active from October 2008 to March 2009 then again from October 2009 to February 2010 (see side notes to Figure 2.8).

Few “recruits” (i.e., in the 0-30 mm length classes) were evident until August 2009 (Figure 2.8). Because reproduction ceased by late summer (i.e., March), these represent a new age-class or cohort appearing and reflect reproduction some months earlier. Furthermore, during February of both years a mode also seems to occur around the 40-50 and 50-60 mm size-classes that was absent 2-3 months prior. This anomaly may represent another cohort (actually the first cohort for each year) produced by reproduction some months earlier. It is difficult to know if these two modes reflect pulses of recruits several months after a reproductive event or if trickle recruitment is occurring and these modes reflect coalescing trickle recruits.

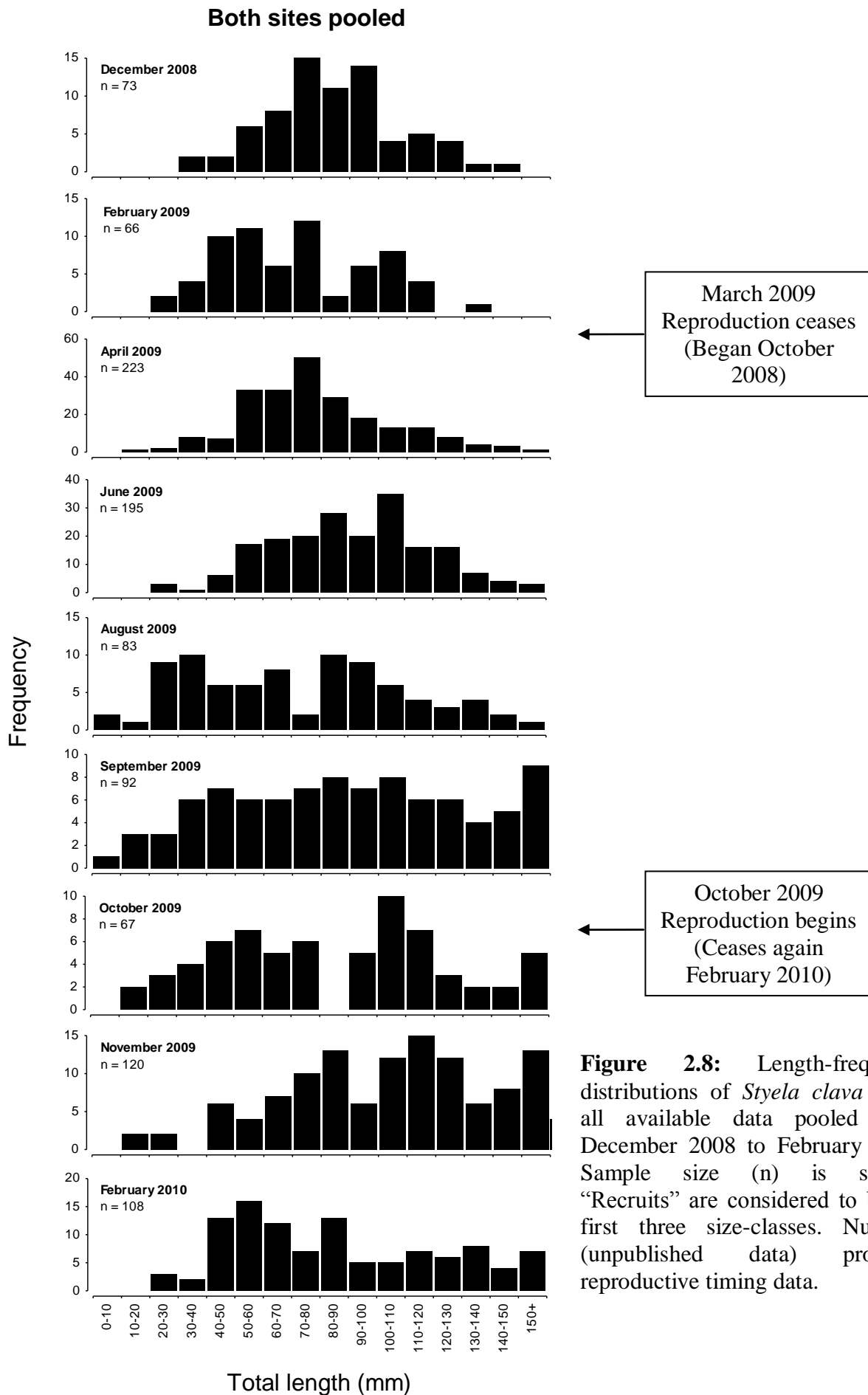




**Figure 2.6:** Length-frequency distributions of *Styela clava* at A&B pontoons from December 2008 to October 2009 (Note: missing December 2009 sample). Sample sizes (n) are shown. “Recruits” are considered to be the first three size-classes.



**Figure 2.7:** Length-frequency distributions of *Styela clava* at Z pontoons from December 2008 to February 2010. Sample sizes (n) are shown. “Recruits” are considered to be the first three size-classes.



**Figure 2.8:** Length-frequency distributions of *Styela clava* using all available data pooled from December 2008 to February 2010. Sample size (n) is shown. “Recruits” are considered to be the first three size-classes. Nutsford (unpublished data) provided reproductive timing data.

### 2.3.3 Recruitment

#### *Deployed substrata*

No *Styela* were detected on Hardieflex™ panels or ropes for the duration of the study (November 2008 to March 2009). The failure to achieve recruitment on the Hardieflex™ panels may have been due to the high levels of siltation on these panels at A&B. The ropes deployed at Z were covered in ascidians (predominantly *Ciona intestinalis*) by the end of the study (February 2010), but again, no *Styela* were detected on ropes.

#### *Clearance plots*

During the course of the clearance experiment only a single *Styela* individual recruited into one of the clearance plots. This individual was discovered in mid-January 2010 and was ~ 35 mm total length and reached ~ 70 mm by mid-February 2010. However, *Ciona intestinalis* and *Cnemidocarpa* sp. did recruit into clearance plots (discussed in Chapter 5).

### 2.3.4 Survival

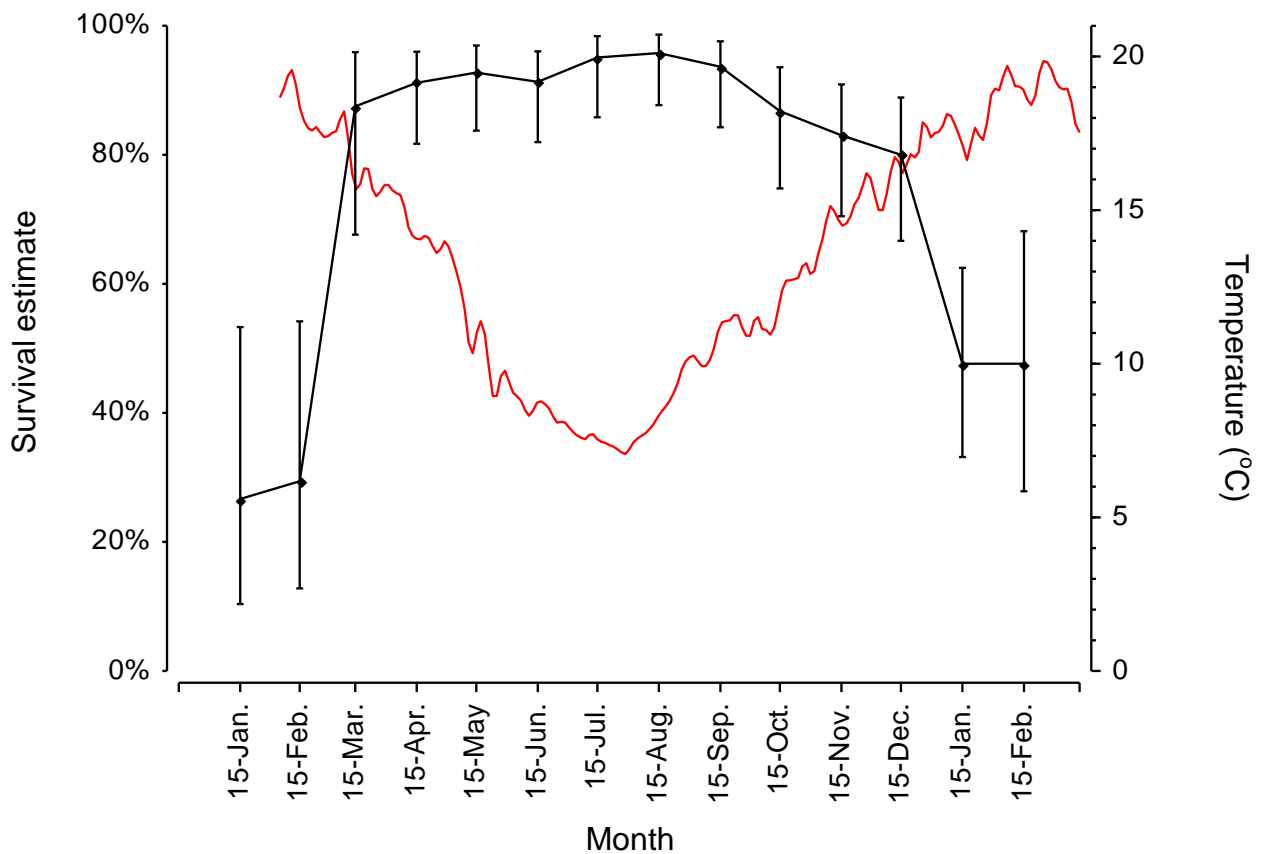
#### *Survival through time*

Model selection showed that survival estimates were time-dependent, as the general and more parameterised time-dependent model ( $\hat{S}_t$ ) was more parsimonious (reflected by its lower BIC and AIC value) than the constant survival model ( $\hat{S}_c$ ) (Table 2.1). The time-dependent model displays abrupt drops in monthly survival occurred during January and February of both 2009 and 2010 (Figure 2.9). Overall, there was a strong negative correlation between survival and temperature (Spearman's rank correlation coefficient  $\rho = -0.93$ ,  $p < 0.001$ ). In particular, the abrupt increase in survival coincides with the temperature falling below ~ 16°C and the decline again coincides with temperatures above ~ 17°C. The drop in survival during January and February 2010 is not as pronounced as it was a year earlier.

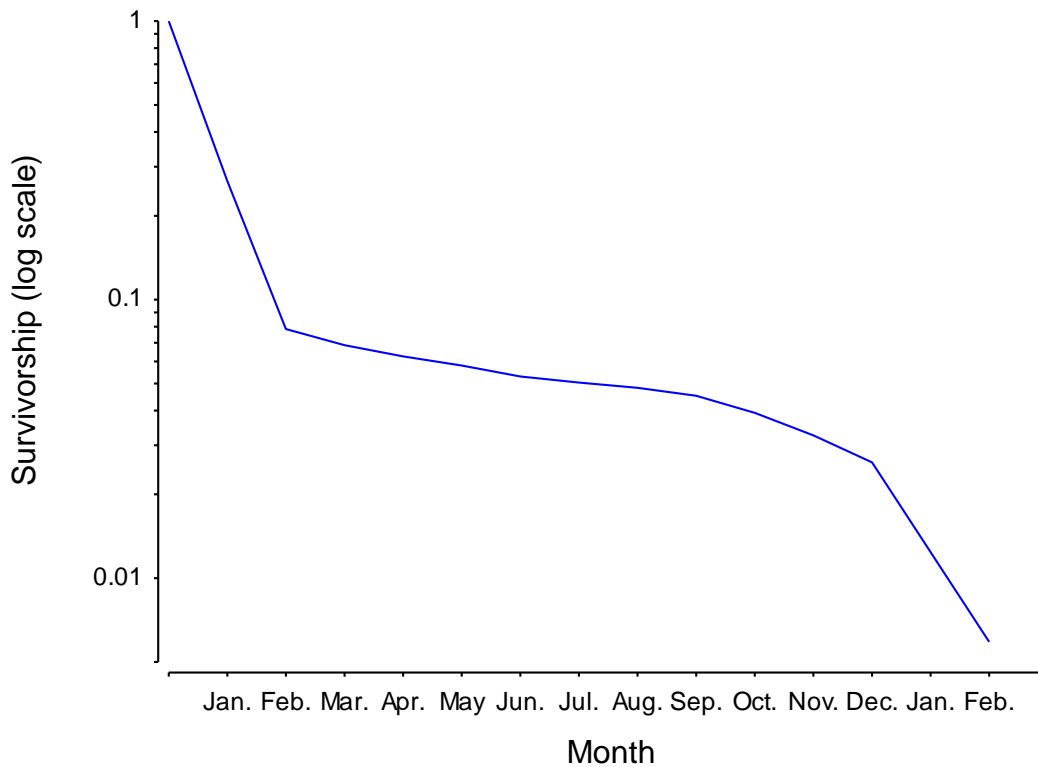
The derived finite annual mortality rate of this species was 95.57% (1 S.E.  $\pm$  2.46%), indicating that *Styela* in Lyttelton Harbour is likely an annual with very few individuals living for longer than about 1 year. The derived survivorship curve (Figure 2.10) describes initial high mortality during summer, specifically January and February, followed by a relatively constant rate of mortality from March to December. Mortality is again high at the onset of the next summer.

**Table 2.1:** Summary of fits to spatial and temporal models of *Styela clava* survival examined in MARK.  $K$  = the number of parameters in the model; BIC = Bayesian Information Criterion; AIC = Akaike Information Criterion. Survival models are ordered according to BIC.

Survival model	Model abbreviation	$K$	Deviance	BIC	AIC
Time	$\hat{S}_t$	16	479	584	512
Constant	$\hat{S}_c$	1	619	625	621
Site	$\hat{S}_{site}$	2	615	628	619
Depth	$\hat{S}_{depth}$	2	617	630	621



**Figure 2.9:** Monthly percentage survival estimates ( $\pm$  95% C.I.) of *Styela clava* for both sites combined, from January 2009 to February 2010 (i.e., model  $\hat{S}_t$ ). Data are from tag-recapture style experiment. Temperature data (red line) are also plotted.



**Figure 2.10:** *Styela clava* survivorship curve (derived using monthly survival estimates) for both sites combined, from January 2009 to February 2010.

#### *Survival by site and depth*

Model selection indicated that survival estimates were not site-dependent because the BIC value was larger for the site-structured model ( $\hat{S}_{site}$ ) than the constant survival model ( $\hat{S}_c$ ) (Table 2.1). However, model selection differed between BIC and AIC for  $\hat{S}_{site}$  (i.e., AIC selected  $\hat{S}_{site}$  as the more parsimonious model over  $\hat{S}_c$ ). This is because BIC places a heavier penalty on the number of parameters used in the model. Despite this discrepancy, the difference in survival estimates between sites was minimal (survival rates at A&B and Z were 82.2% and 87.5%, respectively) and, therefore,  $\hat{S}_{site}$  was rejected. Similarly, survival rates were deemed not to differ across a depth range of one meter, as the depth-dependent survival model ( $\hat{S}_{depth}$ ) displayed a similar trend to  $\hat{S}_{site}$  (Table 2.1) and was rejected for the same reason.

#### *Size-specific survival*

Model selection suggests that survival rates may be size-dependent. The most parsimonious model according to the BIC was the body diameter model ( $\hat{S}_{diameter}$ ) (Table 2.2). The  $\hat{S}_{diameter}$  model (Figure 2.11) indicates a trend of higher monthly survival rates with increasing body

diameter. Although the total length ( $\hat{S}_{length}$ ) and body length ( $\hat{S}_{body}$ ) models were not considered as parsimonious model choices according to BIC, they do exhibit a similar trend (increasing survivorship with increasing length) to  $\hat{S}_{diameter}$  (Figure 2.12). Furthermore, AIC values were the same for the models  $\hat{S}_{length}$ ,  $\hat{S}_{body}$  and  $\hat{S}_c$ , and the deviance was lower for  $\hat{S}_{length}$  and  $\hat{S}_{body}$  than  $\hat{S}_c$ .

When a curvature parameter is introduced to  $\hat{S}_{length}$  (Equation 2.3) a reduction in the model deviance is observed (model deviance of 414 *cf.* 416). BIC does not select for this model, and the AIC values are the same for  $\hat{S}_{length}$  and the  $\hat{S}_c$ . Because of little support for this model, I chose to reject it, thus concluding that there is no normalizing selection on survival rates.

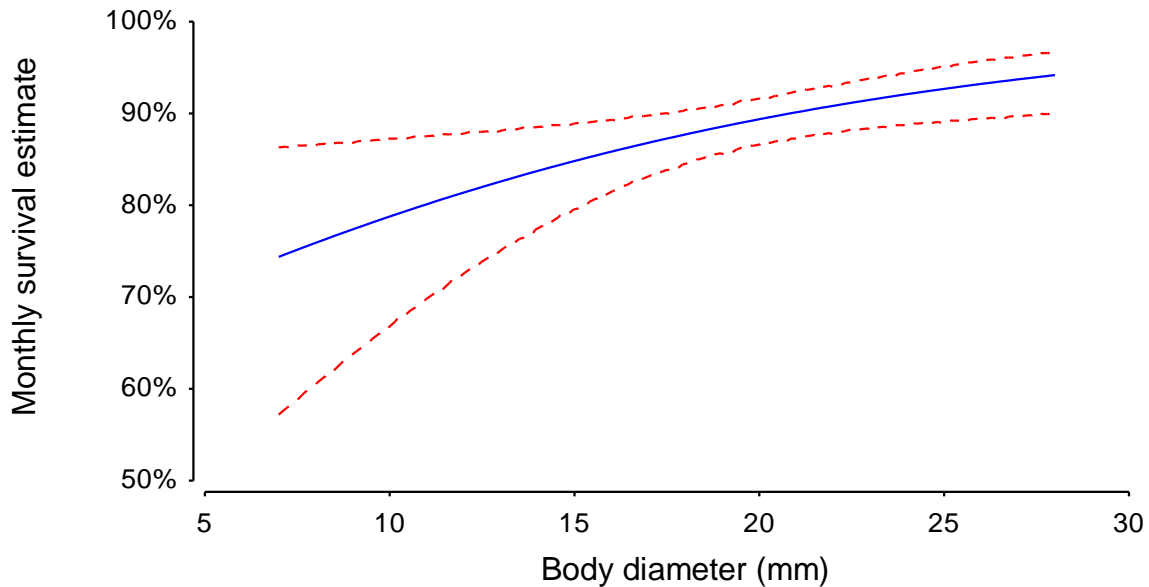
**Table 2.2:** Summary of fits to size-specific models of *Styela clava* survival examined in MARK.  $K$  = the number of parameters in the model; BIC = Bayesian Information Criterion; AIC = Akaike Information Criterion. Survival models are ordered according to BIC.

Survival	Model abbreviation	$K$	Deviance	BIC	AIC
Diameter	$\hat{S}_{diameter}$	2	411	424	415
Constant	$\hat{S}_c$	1	418	425	420
Total length	$\hat{S}_{length}$	2	416	429	420
Body length	$\hat{S}_{body}$	2	416	429	420
Multi-metric	$\hat{S}_{multi}$	2	417	430	421
Total length <sup>2</sup>	$\hat{S}_{length^2}$	3	414	434	420
Time $\times$ Length	$\hat{S}_{t \times length}$	22	345	486	391

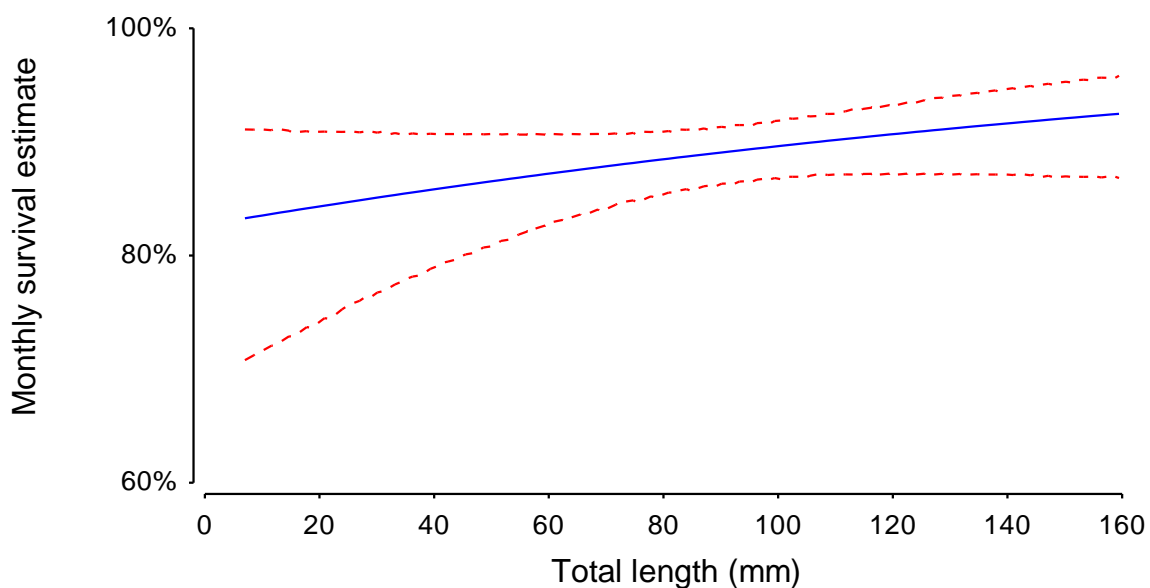
Finally, the complex time and length (total length) model of survival ( $\hat{S}_{t \times length}$ ) was the least parsimonious model according to BIC, the most parsimonious according to AIC, and had the lowest model deviance (Table 2.2). The  $\hat{S}_{t \times length}$  model (Figure 2.13) describes low survival of the largest sizes ( $\sim 100$ -160 mm) during December 2009 and January 2010 and low survival of the smaller individuals ( $< 100$  mm) during January and February 2010. Low survival is also observed in smaller individuals ( $< 60$  mm) during June and November 2009 (illustrated by the red patches for the months in Figure 2.13).

Although untested, the low survival of the larger individuals during December 2009 and January 2010 may be mortality occurring after reproduction. The mortality of the smaller individuals the following month (February 2010) may have been recently recruited individuals dying off; however, the standard error surrounding this period seems rather high (Figure 2.14). It is unclear if the periods of low survival in the smaller individuals during June and November 2009

represent actual mortality at these times or if they are artefacts of limited data in these areas. The standard error surrounding these two periods does not seem unreasonably high (Figure 2.14), but, sampling errors may arise when too few animals around that size are sampled. This stems from the realisation that if an individual is lost from a smaller sample size, then this is going to have a greater influence on dragging down survival estimates.

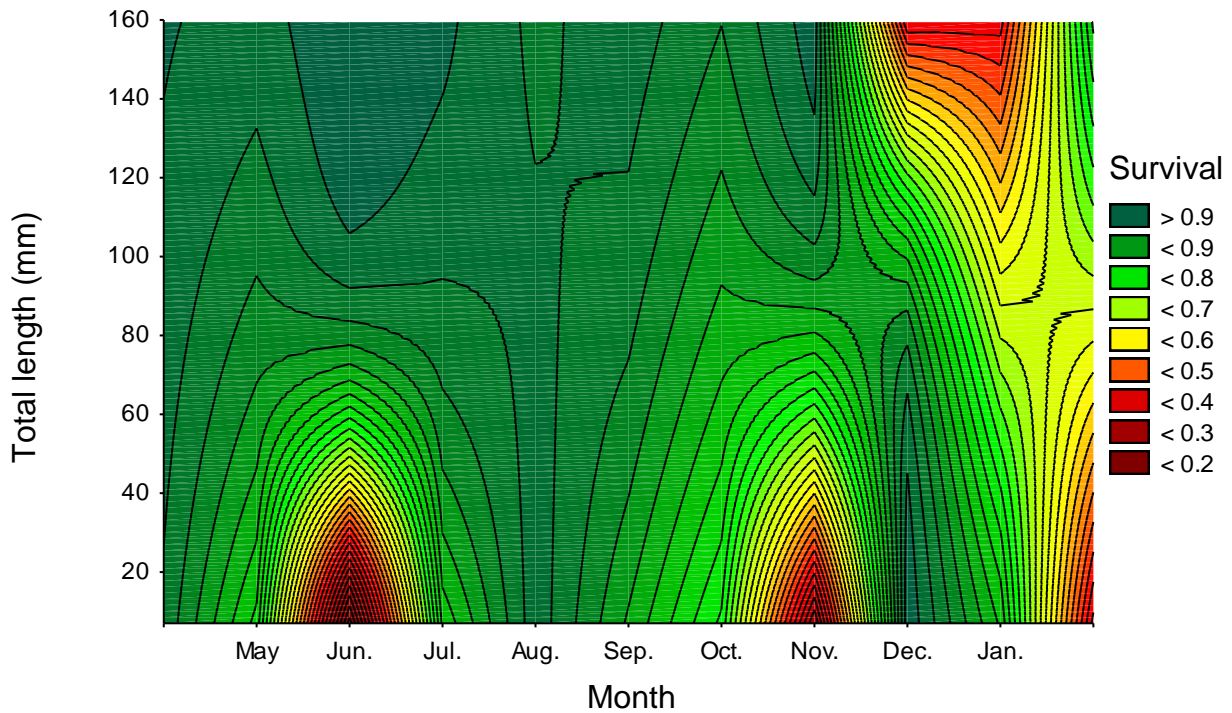


**Figure 2.11:** Size-specific (body diameter) monthly survival estimates ( $\pm$  95% C.I.) of *Styela clava* for both sites pooled. Data are from mapped ascidians.

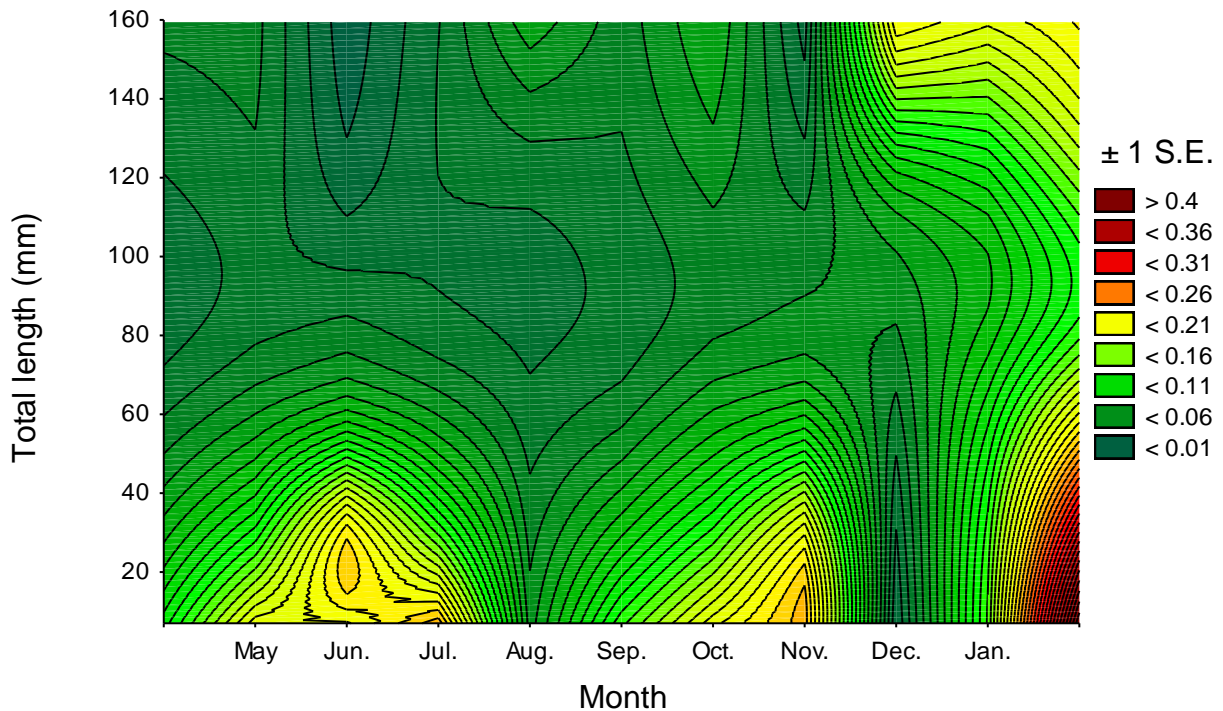


**Figure 2.12:** Size-specific (total length) monthly survival estimates ( $\pm$  95% C.I.) of *Styela clava* for both sites pooled. Data are from mapped ascidians.





**Figure 2.13:** Size-specific (total length) survival through time (monthly from April 2009 to February 2010) of *Styela clava* for both sites pooled. The colours correspond to proportionate survival rates according to the key to the right of the figure.



**Figure 2.14:** Standard error ( $\pm 1$  S.E.) of size-specific survival through time model (Figure 2.13). The colours correspond to S.E. rates according to the key to the right of the figure.

## 2.4 Discussion

The distribution and abundance of invasive ascidians can be highly variable both spatially and temporally and temperature is known to play a crucial role in demographic patterns and structuring ascidian communities (Yamaguchi 1975, Dijkstra 2007, McCarthy *et al.* 2007). At my sites in Lyttelton Port, *Styela* occurred in relatively low numbers (0-10 individuals per m<sup>2</sup>) and displayed a very patchy/clumped distribution. In 2006 Gust *et al.* (2008) carried out comprehensive surveys of *Styela*'s distribution and abundance in Lyttelton Port, finding it widely distributed throughout the inner port with maximum densities of 1-10 individuals per m<sup>2</sup> at most sites in which it was detected. However, higher densities (> 10 individuals per m<sup>2</sup>) were observed at my two sites back in 2006 (Gust *et al.* 2008). Similarly, an investigation in Viaduct Harbour and Freeman's Bay (Auckland) showed comparable densities (1-10 individuals per m<sup>2</sup>) to those observed in Lyttelton Port (Gust *et al.* 2005). Nevertheless, the Lyttelton population does not appear to be attaining the densities observed in some North Island populations, such as Waiheke Island (Goldstien, personal communication), and is certainly not attaining the extreme densities of up to 500-1500 individuals per m<sup>2</sup> seen in other invaded places such as Prince Edward Island (PEI), Canada (Minchin & Duggan 1988, Osman & Whitlatch 1999). Despite the contrasting densities, it has been suggested that past performance of exotic species is a poor indicator of potential for invasion success (Crooks & Soulé 1999).

The abundances recorded by Gust *et al.* (2008) in 2006 compared with the present study suggest that the original populations I investigated are either static or in decline. This was unexpected, given the quick expansion of *Styela* seen elsewhere. For example, *Styela* arrived in PEI in 1997, established rapidly, spread, invaded, and became a nuisance in several estuaries of PEI, reaching peak abundance between 2003 and 2005 (Ramsay *et al.* 2008). In contrast, the invasive ascidian *Ciona intestinalis* and native *Cnemidocarpa* sp. are much more abundant than *Styela* on floating pontoons and other artificial structures at my sites in Lyttelton Port. The difference in densities between these species is most likely reflected in the number and density of propagules that each produce (this will be discussed further in Chapter 5). The reasons for the apparent lack of population growth will be considered in Chapter 6.

### *Recruitment*

Reproduction, larval settlement and recruitment in ascidians are well studied processes (e.g., Svane & Young 1989, Stoner 1990) and have been closely linked to temperature (Yamaguchi 1975, Bourque *et al.* 2007). Studies have shown *Styela* become reproductively active at around

15°C both overseas (Bourque *et al.* 2007) and here in New Zealand (McClary *et al.* 2009). In Lyttelton Port, average water temperatures after winter reached 15°C for the first time in November 2009, and according to Nutsford (unpublished data) this coincided, more or less, with the onset of spawning. The clearance experiment and deployed substrata were set up to cover *Styela*'s reproductive season and aimed to quantify levels and timing of recruitment events. Apart from one individual, *Styela* failed to recruit to any of these surfaces. McClary *et al.* (2009) also failed to achieve recruitment using slightly different techniques. Many reasons may account for the lack of recruitment to settlement plates and clearance plots. A few of these are low adult population densities, incompatible substratum surface chemistry, and sedimentation. For example, given the low adult population densities in Lyttelton Port of around 1 per m<sup>2</sup>, it might be expected that recruitment at a similar density (or higher, factoring in post-settlement juvenile mortality) might be possible. If all else is equal, and given that the surface area of all my settlement panels and clearance plots combined is just 1.3625 m<sup>2</sup>, then we might expect just one individual to settle. Substratum surface chemistry can affect larval settlement by affecting larval behavior, larval adhesive strength, and post-settlement processes such as metamorphosis and mortality (Roberts *et al.* 1991). Lastly, some of the plates became coated in a layer of silt which may have interfered with larval attachment.

Fortunately, length-frequency analysis provided considerable insight into timing of recruitment. It is difficult to know if the two new modes appearing in February (2009 and 2010) and August (2009) (Figure 2.8) represent pulses of recruits several months after a reproductive event or if trickle recruitment is occurring and these modes reflect coalescing trickle recruits. This depends on the reproductive behavior of *Styela* and whether it reproduces continuously or in batches. Most ascidians reproduce in one or two discrete peaks (Svane & Young 1989). For instance, Kazihara (1964) reports two spawning peaks (early summer and fall) in *Styela plicata* in Japan; he related these peaks to a supposed optimum temperature for breeding. In New Zealand, McClary *et al.* (2009) report a spawning event in *Styela clava* occurring between 24 August and 8 September 2006, followed by a brief period (about a month) of gonad development and inactivity, before another spawning event. This lends credit to the hypothesis that recruitment may be pulsed to some extent and that the two new modes appearing may be new cohorts.

My ability to detect individuals < 30 mm length is limited, due to their cryptic nature when small and therefore, these modes may very well represent reproduction some months prior. If so, then the February cohort of each year must stem from reproduction occurring no earlier than October

of the previous year (as reproduction begins October, Nutsford, unpublished data). This begs the question; can an individual grow from settlement to 40-50 or 50-60 mm in length in just 4 months? The clearance experiment (section 2.3.3) showed this is plausible because the single individual grew from settlement after October 2009, when the clearance plot was initiated, to about 70 mm length by mid-February 2010 (growth is covered in more detail in the succeeding chapter). This means that individuals within the cohort appearing after reproduction around October may potentially be reaching maturity. Consequently, some individuals within the population may have the opportunity to reproduce twice (i.e., as soon as they reach maturity and again the following season).

### *Survival*

This study indicates that *Styela* in Lyttelton is a facultative annual and less than 5% of the population survives longer than one year (i.e., 95.57% annual mortality rate). It seems unlikely that these few individuals survive long enough to breed again. Senescence is highest during the summer months of January and February, which coincides with its second major spawning period (Nutsford, unpublished data). Seasonal variations of water temperature appear to have an important influence on the reproductive cycles and survival of *Styela* and are likely to be playing a pivotal role in structuring this population. Survivorship was very similar between sites. This is not surprising, given the proximity of the sites and that the pontoons that constitute site A&B originally came from site Z (Lyttelton Port Company, personal communication). There was an almost negligible difference in survival by depth; however, this was only investigated across a one metre range.

The annual survivorship observed in *Styela* was unexpected given that individuals in populations in Limfjord, Denmark, were surviving up to 21-24 months (Lützen 1999) and in Southampton, on the south coast of England, they survived for 15 months or more (Holmes 1969). One account by Morris *et al.* (1980) found that individuals lived for 12 to 18 months in a Californian population. Other studies have shown considerable mortality, especially of smaller individuals, throughout winter (Lützen 1999). In contrast, this study suggested reasonably high survivorship over the winter months and high mortality in mid-summer.

*Summary*

*Styela* has a relatively short life expectancy of around a year and the population in Lyttelton Port appears to be either static or in decline. Population expansion may therefore depend on high recruitment, such that population growth exceeds mortality. *Styela* appears to have two reproductive episodes, one in late spring and again in late summer. Population size structure suggests that recruits are able to reach reproductive maturity quickly and some individuals may be able to reproduce more than once within their lifetime. This, coupled with *Styela*'s hermaphroditic nature, means that the propagule pressure exerted by a relatively small population like this could be reasonably high. The proceeding chapters aim to clarify the growth patterns in *Styela*.

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## Chapter 3

### Growth

“An organism is so complex a thing, and growth so complex a phenomenon, that for growth to be so uniform and constant in all the parts as to keep the whole shape unchanged would indeed be an unlikely and an unusual circumstance. Rates vary, proportions change, and the whole configuration alters accordingly”

– D’Arcy Wentworth Thompson, *On Growth and Form* (1961)

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#### 3.1 Introduction

Determining the growth rate of an organism provides basic biological data and can be an important and reliable indicator of an organism’s health, habitat quality, and contribution to its population. Furthermore, once determined it may be used for many other purposes. For example, in fisheries, growth rates linked with recruitment and mortality data may be used to estimate sustainable yields from fish populations (Beverton & Holt 1957, Ricker 1958) or growth coupled with mortality rates has been used to construct population models such as matrix models (e.g., Rogers-Bennett & Leaf 2006, Dudas *et al.* 2007).

Two primary methods have been employed by ecologists to determine growth rates of organisms: mark-recapture experiments and size-frequency analysis. Mark-recapture experiments may use tags, notches, paint markers, fluorescent stains or elemental markers to identify individuals or cohorts (e.g., Heald 1978, Stewart & Deacon 1995, Takada 1995, Fujikura *et al.* 2003, Laudien *et al.* 2003). Size-frequency analysis involves large samples of individual lengths and subsequent analysis of shifting modes through time to determine growth rates (e.g., Schnute & Fournier 1980). Other methods include growth ring analysis (e.g., Chambers & Miller 1995); analysis of stable isotopes (e.g., Pätzold 1984); and analysis of lipofuscin (an autofluorescent age pigment) (e.g., Lomovasky *et al.* 2002).

Growth rates may be used to develop growth curves. Among other things, growth curves can be used to predict age-at-length, the time required for species to enter a fishery or how long it takes a species to reach maturity. There are many different ways to model growth. Perhaps the most commonly used growth model in biology is that proposed by von Bertalanffy (von Bertalanffy 1934). This is often used in fisheries biology, whereby an organism follows a growth rate ( $K$ ) and has an asymptotic size ( $L_{\infty}$ ). However, this growth function may not be the best curve to describe the growth of a particular species. There are many other growth models that may be more suitable (e.g., Richards, Gompertz, Tanaka, logistic, power, exponential etc). Of course, organisms are not constrained to grow according to any growth models and any of these may fit any given data set. Therefore, the problem is not in finding a growth model to use but in selecting the one that adequately describes the growth of an organism.

In the case of solitary tunicates, there is no *a priori* reason to select a particular growth model. All growth models are based on there being accurate measures of size and that these measurements remain a relatively constant indication of size through time. Thus, a metric needs to be worked out for tunicates because they may expand and shrink as gonads develop or as they take on or lose water. Therefore, I developed a metric for size in *Styela clava* that would minimize variations in body shape.

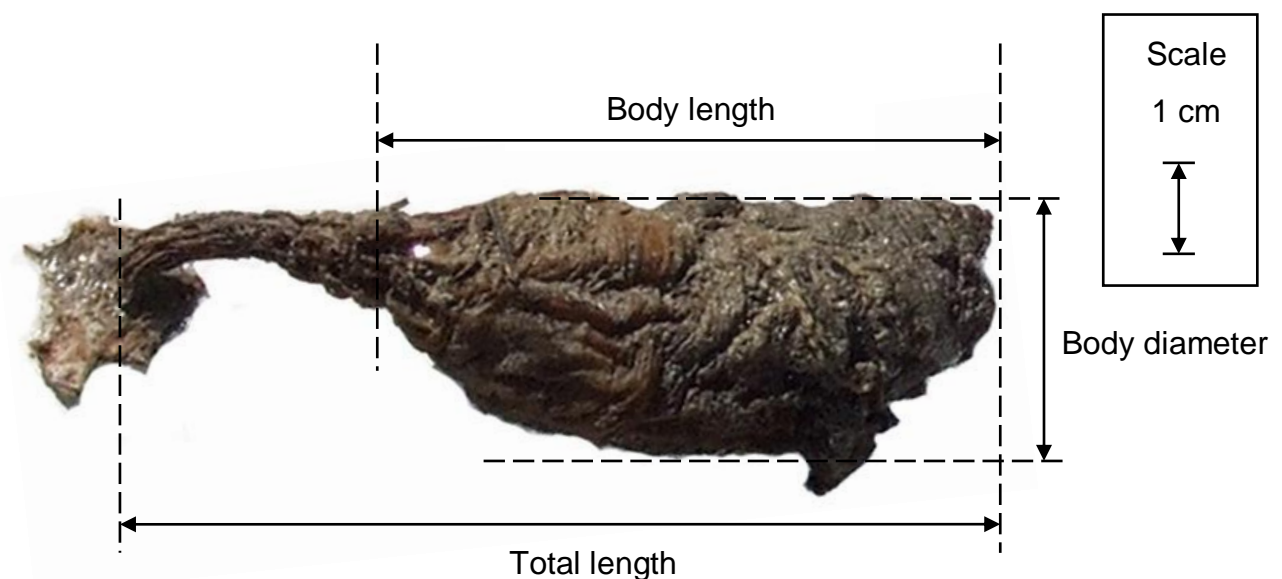
Not only is it important to develop appropriate metrics so that we can accurately determine the growth of invasive species as basic ecological data and for inclusion in more complex models that may be used to aid management, it is also important to examine spatial and temporal patterns of growth. If we do not then it is impossible to determine whether estimates represent an average growth rate or some extreme. Therefore, the aim of this chapter was to describe patterns of growth in *Styela clava*. I specifically address the following questions:

- (i) Does the rate of growth in *Styela clava* vary temporally/seasonally?
- (ii) Does the rate of growth in *Styela clava* vary spatially (i.e., by site or depth)?
- (iii) Are growth rates size-specific?
- (iv) Can *Styela*'s growth be adequately described using an age-at-length model?

## 3.2 Materials and Methods

### 3.2.1 Allometry

*Styela clava* is a soft-bodied organism with lateral muscles that allow it to expand and contract. Consequently, an individual may vary in size considerably in a relatively short space of time because its body can change shape as it feeds or is disturbed. This complicates measuring the length or growth of an individual. Therefore, a preliminary allometric investigation was done to determine the best *in situ* measurements of *Styela* that minimize variations in body shape. Approximately 100 *Styela* were collected from two sites. The total length, body length and body diameter (Figure 3.1) of these individuals were measured to the nearest millimeter using Vernier calipers. The body diameter was measured at the cross section of greatest diameter (not including encrusting organisms) because *Styela* is not uniformly cylindrical. Individuals were squeezed to expel water retained in the tunic and their wet weights were recorded to the nearest 0.1 of a gram.



**Figure 3.1:** Adult *Styela clava* specimen showing the dimensions recorded for animals measured *in situ*.

The length-weight relationship is defined by Equation 3.1. The lengths of all individuals were plotted against their weights and the values of  $a$  and  $b$  determined using the curve fitting program TableCurve 2D v5.01 (Jandel Scientific, Corte Madera, California). TableCurve uses the Levenburg-Marquardt procedure for finding the minimum of the squared sum of deviations (essentially a log-log regression). Several other curves were tested, e.g., log-linear, but Equation



3.1 provided the best fit. As well as investigating each of the three measures individually, a multi-metric ( $M$ ) was derived (Equation 3.2).  $M$  aimed to reduce the variation due to fluctuations in body dimensions.

**Equation 3.1:**

$$W = aL^b$$

where  $W$  is the wet weight in grams,  $L$  is the length in millimeters, and  $a$  and  $b$  are estimated parameters.

**Equation 3.2:**

$$M = (\text{total length} \times \text{body length} \times \text{body diameter})^{\frac{1}{3}}$$

where  $M$  is the calculated multi-metric parameter with units mm.

### 3.2.2 Growth Increments

Individual *Styela* were mapped and measured for *in situ* growth studies on vertical pontoon faces at both sites beginning in March 2009. A 1 × 1 m alphanumerically labelled quadrat placed over marked permanent quadrat areas was used to relocate individuals. Thus it was essentially a mark-recapture style experiment without the need for tags and removed any effect of the presence of tags on animals. New individuals were mapped throughout the experiment to increase the sample size as individuals died. The timing of re-sampling was opportunistic, depending on weather conditions and water clarity, and therefore time at liberty was not consistently equal to one month. Individuals over a wide size range were included for use in growth analysis, but data are lacking for individuals < 30 mm due to their cryptic nature and problems associated with finding them when this small. The total length, body length and body diameter (Figure 3.1) of all individuals were measured to the nearest millimeter. Contact with individuals was kept to a minimum to reduce any handling effects. Their depth was also recorded. Sampling was done approximately monthly from March 2009 to February 2010. Growth of individuals was measured from discovery to death or the end of my sampling regime.

To estimate the size-specific growth rate of individuals in the two populations, all growth increment data were standardized to daily increments ( $\Delta L_t$ ) (Equation 3.3).  $\Delta L_t$  was then plotted against initial length ( $L_t$ ) for each month. Four size-classes were chosen based on length-at-maturity and size-specific differences in growth rates (total length data set only) (Table 3.1) to further illustrate the difference in growth rates between individuals at different stages of their life

history (i.e., pre-breeding, mature individuals, individuals approaching  $L_\infty$ , individuals larger than  $L_\infty$ ). A plot of mean growth by month, and mean growth for each size-class was produced.

**Equation 3.3:**

$$\Delta L_t = \frac{L_2 - L_1}{t_2 - t_1} = \frac{\Delta L}{\Delta t}$$

where  $\Delta L_t$  is the daily growth increment with units  $\text{mm d}^{-1}$ ,  $L_1$  is the initial length in mm,  $L_2$  the final length in mm,  $t_1$  the date at which  $L_1$  was recorded and  $t_2$  the date at which  $L_2$  was recorded.

**Table 3.1:** The size-classes used for illustration and analysis of growth rates in *Styela clava* individuals at different life history stages and justification for choosing these boundaries. The  $L_\infty$  term is described shortly.

Size-class	Size (mm)	Reason for size-class boundary
1	< 45	Estimated length-at-maturity (Nutsford, unpublished data).
2	45-85	Size-classes 2 and 3 were determined as the remaining length-range divided by 2.
3	85-125	
4	> 125	$\sim L_\infty$ according to length-frequency analysis.

Standardized growth increments using both the total length and multi-length metrics were analyzed in R (version 2.9.0) using a general linear model to determine which variables best described growth rates. Variables included month, site, depth, size-class (for total length data set only) and initial length. All non-significant interaction terms were removed from the simplified models. Model fits were checked for normality and variances were checked by plotting fitted values against residuals.

An *ad-hoc* analysis was done on the proportion of individuals shrinking each month using a generalized linear model using the binomial distribution in R. This aimed test the null hypothesis that the proportion of individuals shrinking does not differ temporally.

### 3.2.3 Growth Models

Four growth functions were investigated: von Bertalanffy, logistic-dose response, Ricker, and power models of *Styela* growth. These were fitted to initial length versus standardized growth increments. Only individuals with  $t > 2$  months (60 days) were included in this data set to avoid

extrapolating the daily growth rates from short time periods and avoid excessive shrinkage data in analyses. In cases of multiple encounters, individual growth rates were calculated for the longest time at liberty. The average time interval was 188 days and the maximum interval was 330 days. The use of growth rates of many different sized individuals over several months removes the effects of environmental and seasonal variation from the observed growth pattern (this was acceptable because growth rates did not appear to follow any discernible temporal pattern). These models were input as user-defined programs in TableCurve. The Akaike information criterion (AIC) (Akaike 1974) and the Schwartz-Bayesian criterion (BIC) (Schwarz 1978) were calculated (using Equation 3.4 and Equation 3.5, respectively). Models were then ranked according to the residual sum of squares (RSS), the BIC and the AIC. BIC and AIC are essentially goodness-of-fit values that describe the trade-off between accuracy and model complexity. When estimating model parameters it is possible to reduce model deviance by adding additional parameters, which can result in over-fitting. BIC and AIC resolve this problem by penalising additional parameters in a model. The model with the lowest BIC or AIC is considered the most parsimonious. The two methods differ in that BIC places greater penalty on additional parameters than AIC. The structure of the growth models used is described below.

**Equation 3.4:** 
$$AIC = k \ln(RSS) - k \ln(k) + 2m$$

**Equation 3.5:** 
$$BIC = k \ln(RSS) - (k - m) \ln(k)$$

where  $k$  is the number of data points,  $m$  is the number of parameters and RSS is the residual sum of squares.

#### *von Bertalanffy Growth Model*

The incremental growth rate of many organisms decreases as size (or age) increases. This function is often modelled using the von Bertalanffy mathematical model (von Bertalanffy 1934). The length-at-age form of the model expresses the length ( $L_t$ ) as a function of the age ( $t$ ) of the organism (Equation 3.6).

**Equation 3.6:** 
$$L_t = L_\infty [1 - e^{-K(t-t_0)}]$$

**Equation 3.7:** 
$$f(L_t) = L_\infty (1 - e^{-K}) - L_t (1 - e^{-K})$$

where  $f(L_t)$  is a function of daily growth,  $L_t$  is the length at time  $t$ . The remaining parameters are described below.

The model includes the parameter  $L_\infty$ , which is the asymptotic length or the mean length of very old organisms. This does not imply that  $L_\infty$  is the greatest length attained by all individuals, but it indicates the average length of mature animals, independent of short-term fluctuations in length due to temporary environmental effects.  $K$  is a curvature parameter which determines how fast the organism approaches  $L_\infty$ . A high value of  $K$  indicates a short-lived species that reaches its  $L_\infty$  relatively quickly. Finally,  $t_0$  determines the hypothetical point in time when the organism has zero length. Changing  $t_0$  does not affect the shape or asymptote of the curve. Alternatively, the von Bertalanffy function expressed by Equation 3.7 describes a linear decrease in growth rate as a function of size and is the form that can be fitted to the *Styela* data set.

#### *Logistic-Dose Response Model*

The logistic-dose response model (Equation 3.8) incorporates a transition between a fast-growing group of smaller individuals that maintain a constant growth rate, and larger individuals that grow slowly at a diminishing rate. While this transition may seem strange in a biological sense, it has been successfully used to describe the growth of marine invertebrates such as sea urchins (e.g., Rogers-Bennett *et al.* 2003).

#### **Equation 3.8:**

$$f(L_t) = a / \left( 1 + \left( \frac{L_t}{b} \right)^c \right)$$

where  $f(L_t)$  is a function of daily growth,  $L_t$  is the length at time  $t$ , and  $a$ ,  $b$ , and  $c$  are estimated parameters.

#### *Ricker Model*

The Ricker function is often used for population growth (Hastings 1997), but may be translated to *Styela* growth (Equation 3.9). The Ricker function describes increasing growth rate with increasing size to a maximum growth rate followed by an asymptotic decrease in growth rate towards 0 as the animal matures.

#### **Equation 3.9:**

$$f(L_t) = BL_t e^{-KL}$$

where  $f(L_t)$  is a function of daily growth,  $L_t$  is the length at time  $t$ , and  $B$ ,  $K$  and  $L$  are estimated parameters.

*Power Model*

Finally, the power curve translated to *Styela* growth (Equation 3.10) (Kaufmann 1981) describes a very high growth rate initially. The growth rate decreases rapidly (at first) with increasing size and then approaches zero growth as size increases.

**Equation 3.10:**

$$f(L_t) = e^{-alnL_t+lnb}$$

where  $f(L_t)$  is a function of daily growth,  $L_t$  is the length at time  $t$ , and  $a$  and  $b$  are estimated parameters. Note: This is the differential form of the traditional power curve  $L_t = [ab(t + t_0)]^{\frac{1}{a}}$ .

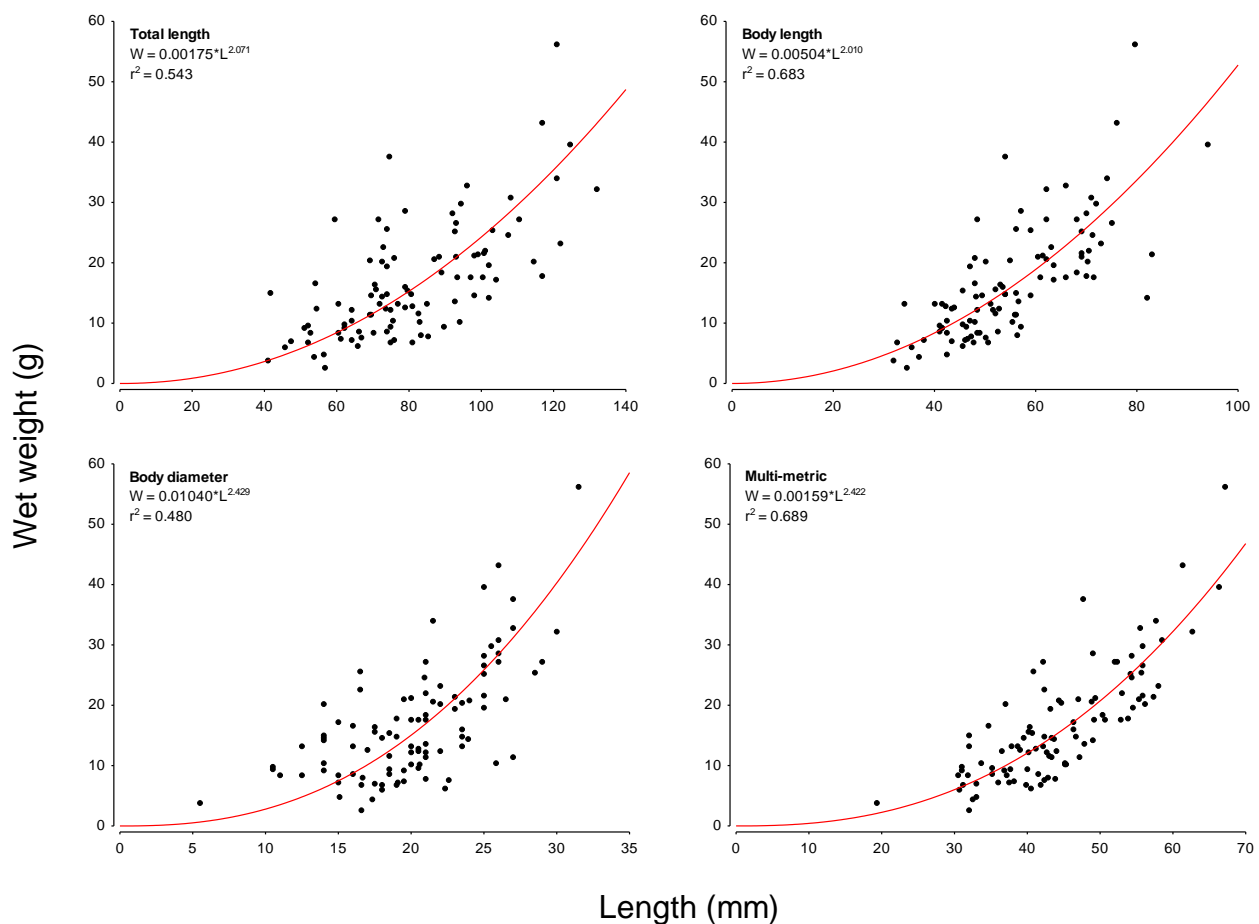
**3.2.4 Length-Frequency Analysis**

Length-frequency distributions may be used to determine growth rates by tracking shifting modes through time. Here, both data sets (total length and multi-length,  $M$ ) were analyzed separately. The individual components in size-frequency samples were approximated using Battacharya's method (Battacharya 1967). These components were assumed to represent groups of *Styela* with similar ages (cohorts). These components were then refined using the "NORMSEP" algorithm (written in FORTRAN by Tomlinson 1971) within the program FAO-ICLARM fisheries stock assessment program FiSAT II. This method applies maximum likelihood theory to optimize the fit of normally distributed components from size-frequency samples. NORMSEP produces a mean, standard deviation and the number of individuals for each normally distributed component of the mixture distributions for each month sampled. Modes representing what were assumed to be the same cohorts were then linked visually and the associated growth increments determined. Finally, growth increment data were used to estimate the von Bertalanffy growth parameters  $L_\infty$  and  $K$  by means of Munro plots (Munro 1982), using FiSAT II.

### 3.3 Results

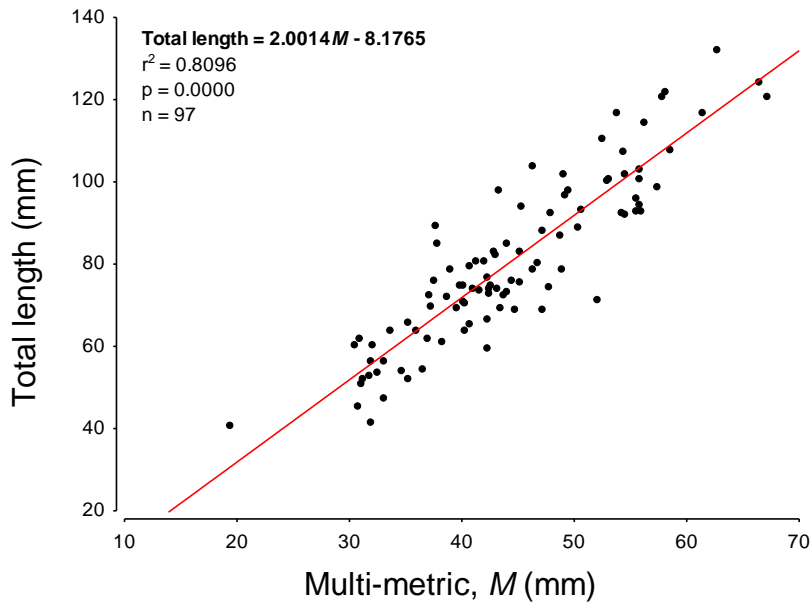
#### 3.3.1 Allometry

Of the four length metrics investigated, the multi-metric measurement correlated best with wet weight ( $r^2 = 0.689$ ), closely followed by the body length ( $r^2 = 0.683$ ) (Figure 3.2). The multi-metric and total length measures were both chosen for use in growth analyses. These two metrics were chosen for further use because the multi-metric may provide more accurate results, but total length is much easier to visualize and comprehend.



**Figure 3.2:** Scatterplots of lengths (including total length, body length, body diameter and the calculated multi-metric) versus wet weight of *Styela clava*. The best fit of Equation 3.1 and associated  $r^2$  for each of the lines fits are shown.

A regression of the multi-metric,  $M$ , versus total length (Figure 3.3) yielded Equation 3.11. This was derived for comparing later analyses that use the different length metrics (i.e., the  $L_{\infty}$  derived from the total length and  $M$  data sets provided by length-frequency analysis).



**Figure 3.3:** The relationship between the calculated multi-metric,  $M$ , and total length. Associated  $r^2$  and  $p$  values shown. Sample size,  $n$ , shown.

**Equation 3.11:** 
$$\text{Total length} = 2M - 8.18$$

where  $M$  is the calculated multi-metric.

### 3.3.2 Growth Increments

Individuals included in the mark-recapture experiment ranged in size from 8 to 161 mm in total length. Growth data for individuals less than 30 mm in length are sparse and data for individuals less than 8 mm are missing altogether. The significance level of the same effects was similar in the general linear models using the total length and multi-metric,  $M$ , data sets (Table 3.2 and Table 3.3).

#### *Temporal and Size-Specific Growth*

Although growth rates in *Styela* differed significantly through time using both length metrics ( $p = 0.010$  and  $0.002$ , for total length and  $M$  data sets, respectively, Table 3.2 and Table 3.3), there was no clear seasonal pattern to the changes, but during the colder months (May to October)

mean daily growth rates varied greatly between months (Figure 3.4). The smaller length-classes, for the total length data set, consistently had higher mean growth increments at both sites; however, at site A&B the largest individuals ( $> 123$  mm total length) had a negative mean monthly growth increment (Figure 3.5). This implies that these individuals were growing to this size-class, then fluctuating in size. Monthly growth increments showed great variability between individuals (Figure 3.6 and Figure 3.7) with negative growth (shrinkage) observed in many individuals.

#### *Growth by Site and Depth*

Growth rates differed significantly between sites using both length metrics ( $p < 0.05$ , Table 3.2 and Table 3.3) with site Z displaying slightly higher growth rates on average than A&B (mean  $\Delta L_t$  for total length data set at site A&B =  $0.114 \text{ mm d}^{-1}$  and at Z =  $0.158 \text{ mm d}^{-1}$ /mean  $\Delta L_t$  for *M* data set at site A&B =  $0.0613 \text{ mm d}^{-1}$  and at Z =  $0.0787 \text{ mm d}^{-1}$ ). Growth rates did not differ significantly across a depth range of 1 m ( $p > 0.1$ ).

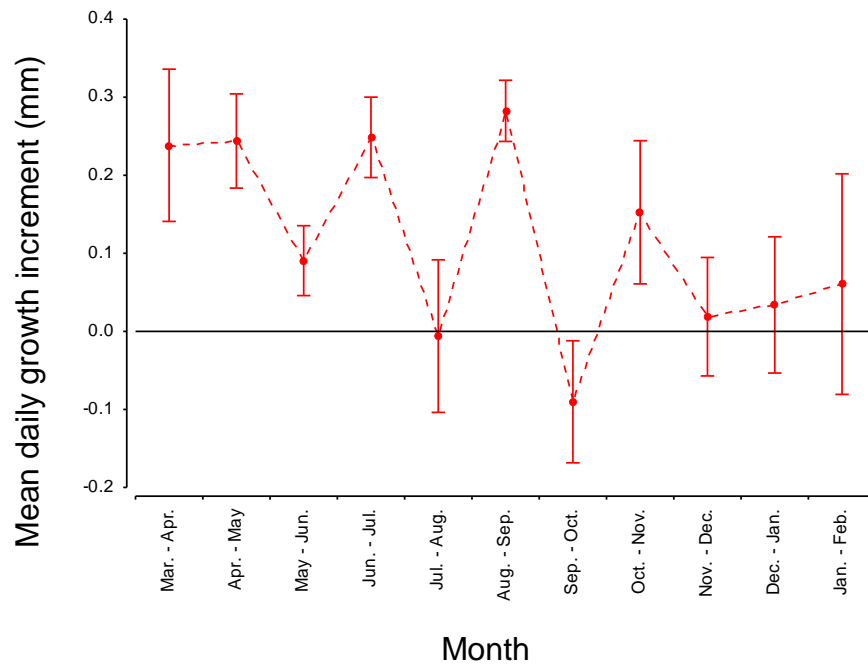
**Table 3.2:** Results of general linear model performed on the standardized daily growth increment of *Styela clava* using total length data. Month, depth and initial length were included as covariates. Site and size-class were included as factors. The interaction between site and size-class was significant (for residual vs. fitted values see Appendix 3.1).

Effect	df	MS	F	p values
Month	1	1.302	6.7927	<b>0.010</b>
Site	1	1.229	6.4122	<b>0.012</b>
Depth	1	0.336	1.7549	0.186
Size-class	3	1.414	9.3742	<b>0.000</b>
Initial length	1	1.088	5.6745	<b>0.018</b>
Site $\times$ size-class	3	0.881	4.5944	<b>0.004</b>
Residuals	382	0.192		

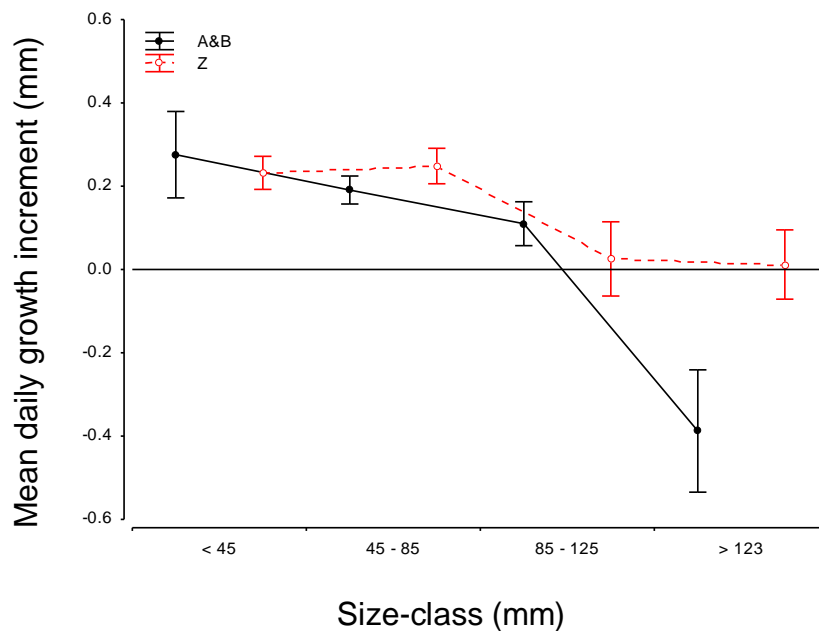
**Table 3.3:** Results of general linear model performed on the standardized daily growth increment of *Styela clava* using multi-length (*M*) data. Month, depth and initial length were included as covariates. Site and size-class were included as factors (for residual vs. fitted values see Appendix 3.1).

Effect	df	MS	F	p values
Month	1	0.3086	9.6838	<b>0.002</b>
Site	1	0.2423	7.6055	<b>0.006</b>
Depth	1	0.0628	1.9712	0.161
Initial length	1	0.5934	18.6222	<b>0.000</b>
Residuals	389	0.0319		





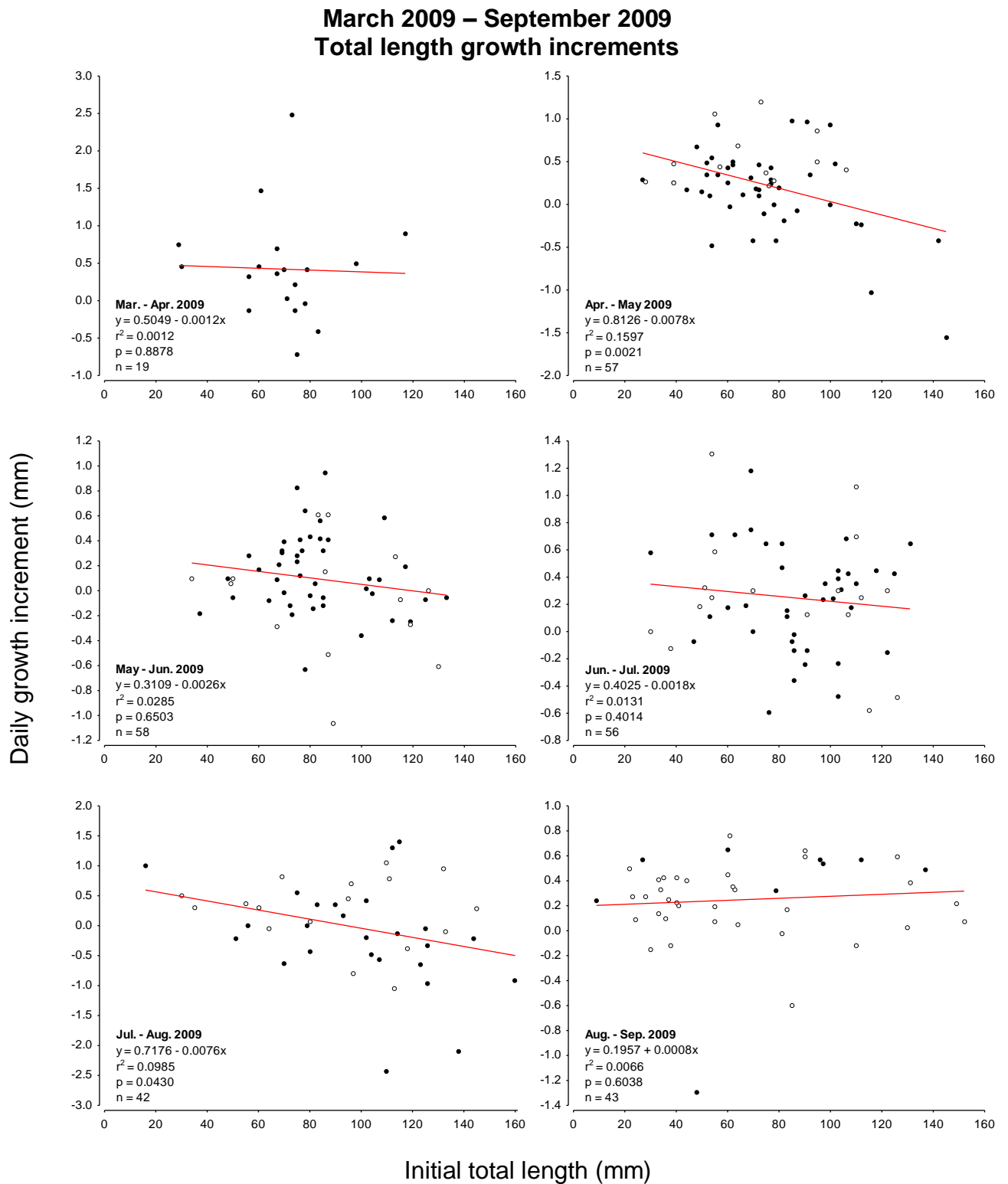
**Figure 3.4:** *Styela clava* mean daily growth increments ( $\pm 1$  S.E.) across all depths, sites and sizes combined, from March 2009 to February 2010.



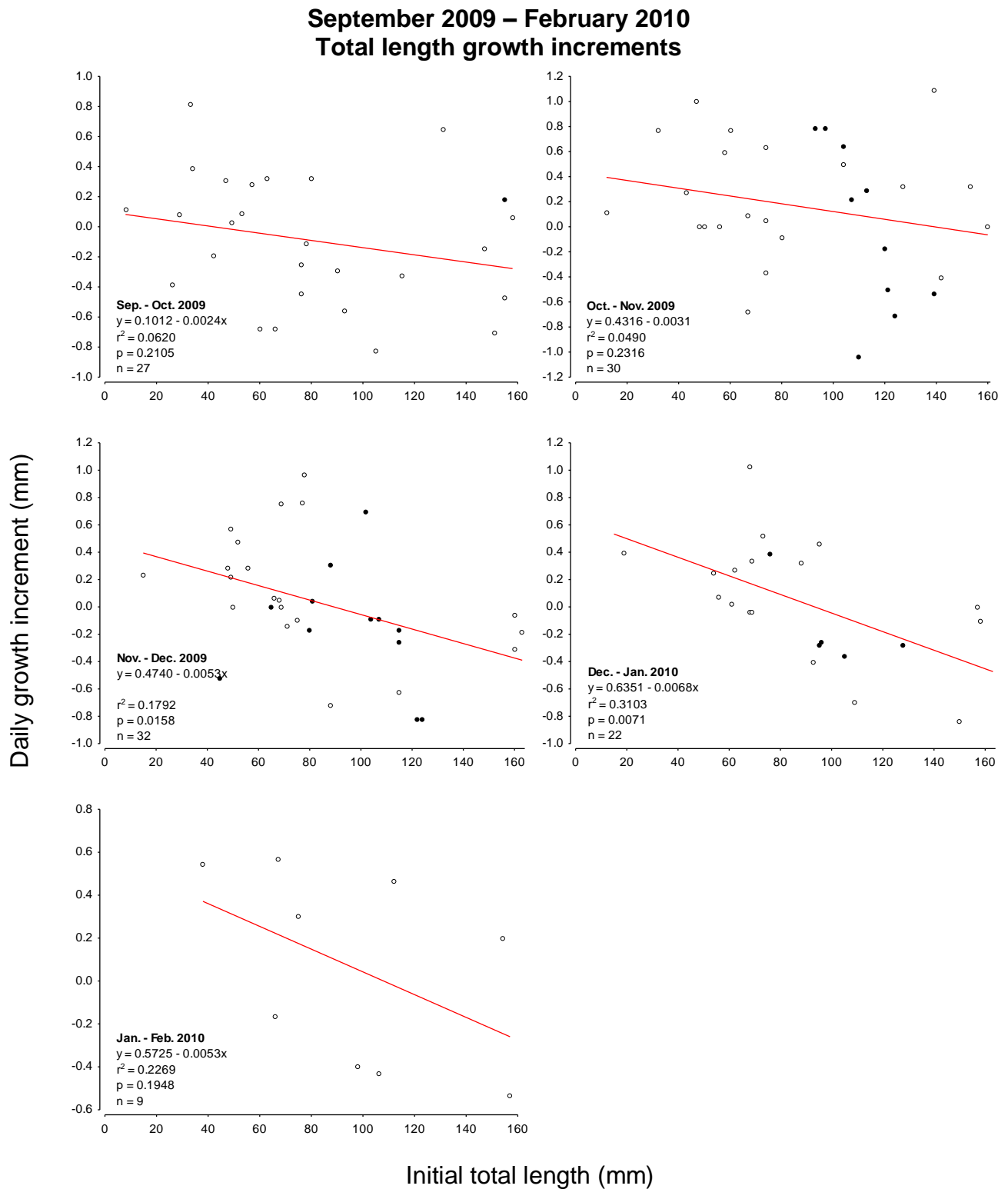
**Figure 3.5:** *Styela clava* mean daily growth increments ( $\pm 1$  S.E.) across all depths combined, between sites for the four chosen size-classes.

### Shrinkage

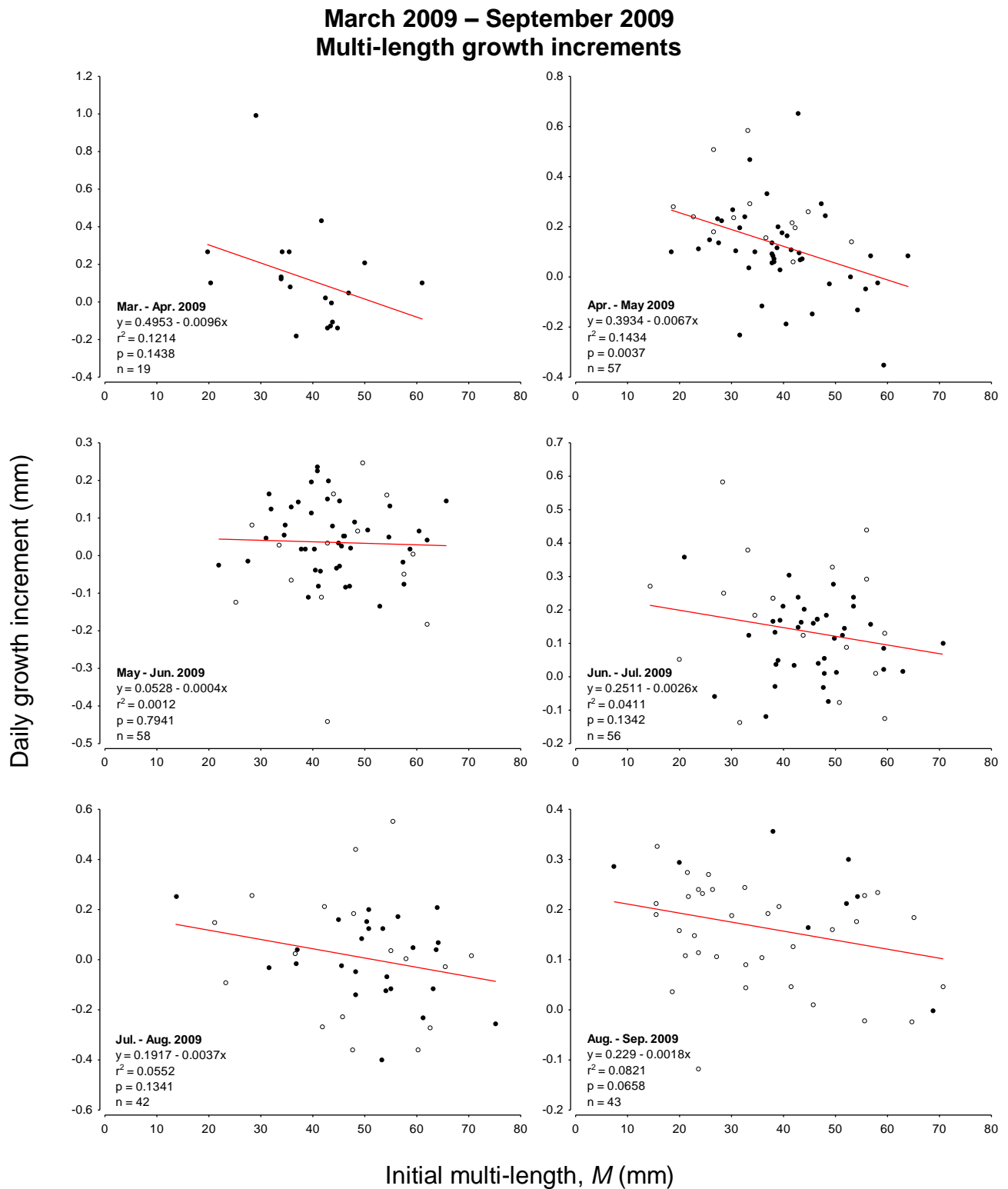
The proportion of individuals shrinking each month ranged between 11.6% and 51.9% but was not significantly different between months ( $z_{1,11} = 1.577$ ,  $p = 0.115$ ).



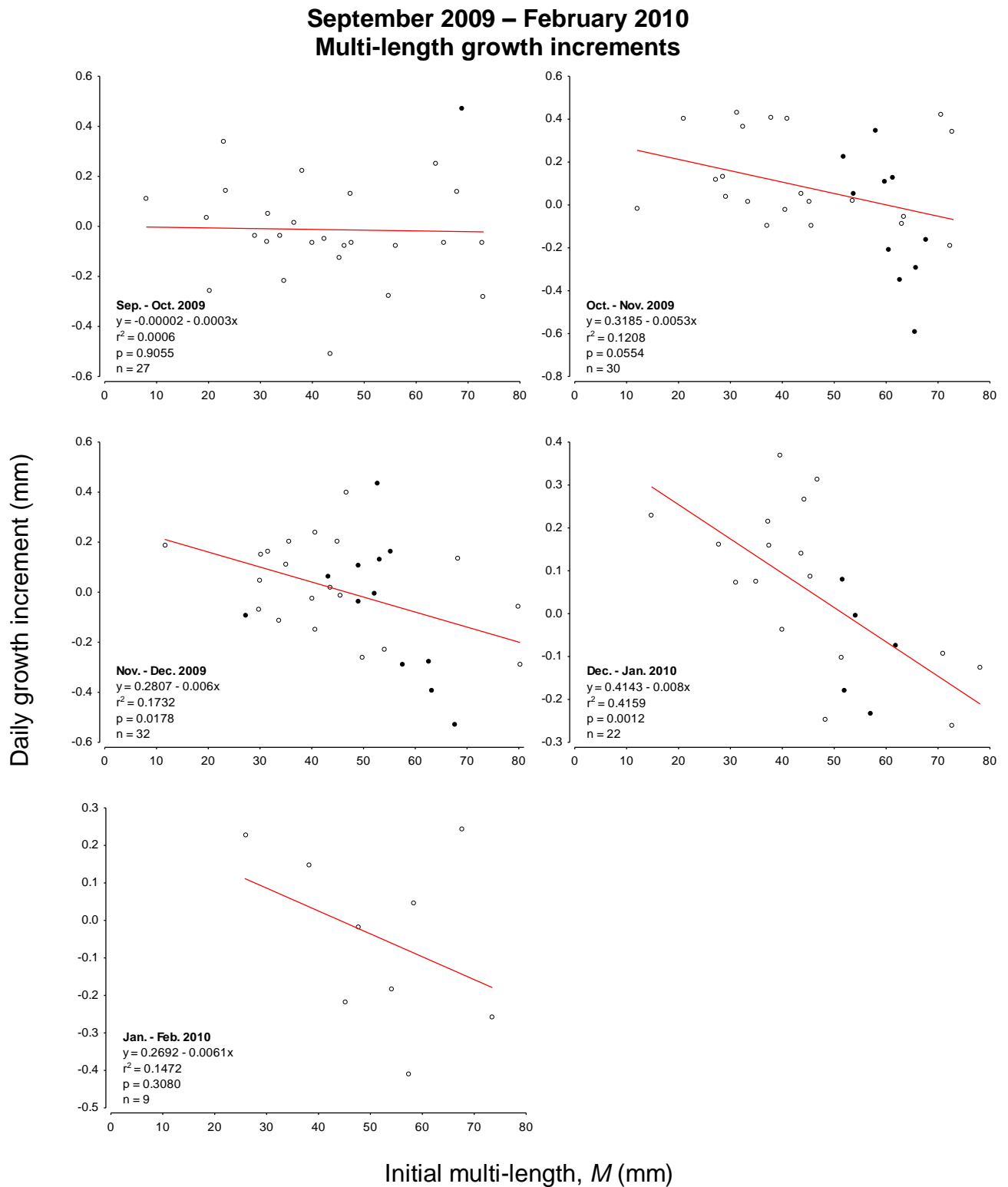
**Figure 3.6:** Scatterplots of daily growth increments versus initial length using total length data by month at A&B pontoons (solid circles) and Z-wharf pontoons (open circles). Line represents a regression through both sites data collapsed;  $r^2$  and p value for this line is shown. Sample size, n, for both sites is shown. Continued on next page.



**Figure 3.6:** Continued. Scatterplots of daily growth increments versus initial length using total length data by month at A&B pontoons (solid circles) and Z-wharf pontoons (open circles). Line represents a regression through both sites data collapsed;  $r^2$  and p value for this line is shown. Sample size, n, for both sites is shown.



**Figure 3.7:** Scatterplots of daily growth increments versus initial length using multi-length,  $M$ , data by month at A&B pontoons (solid circles) and Z-wharf pontoons (open circles). Line represents a regression through both sites data collapsed;  $r^2$  and  $p$  value for this line is shown. Sample size,  $n$ , for both sites is shown. Continued on next page.



**Figure 3.7:** Continued. Scatterplots of daily growth increments versus initial length using multi-length,  $M$ , data by month at A&B pontoons (solid circles) and Z-wharf pontoons (open circles). Line represents a regression through both sites data collapsed;  $r^2$  and p value for this line is shown. Sample size,  $n$ , for both sites is shown.

### 3.3.3 Growth Models

Due to the extremely high variation in growth in *Styela* and its inherent ability to shrink (even over long time intervals), growth models based on monthly growth increment data are likely to be of limited use. All models investigated offer a very poor fit to the data set (e.g., the best fit obtained was using the logistic dose response model on multi-metric data,  $r^2 = 0.2744$ ). No shrinkage occurred in individuals until they reached around 30 mm in length. Maximum growth rates appear to be achieved by individuals approximately 60 mm in length and this reaches a plateau as individuals get larger. The models and their fits in terms of initial length against daily growth rate are displayed for total length (Figure 3.8, Table 3.4) and multi-length (Figure 3.9, Table 3.5). The models translated into age-at-length curves (Figure 3.10), and the parameter estimates and time-to-maturity (TTM) for each model (Table 3.6 and Table 3.7) are displayed below these.

#### *von Bertalanffy Growth Model*

The von Bertalanffy model (see Figure 3.8, 3.9, 3.10) provided the best fit to the data set according to BIC for total length (Table 3.4) and the second best fit for the multi-metric (Table 3.5). This model predicts that the smallest individuals have the fastest growth and yields the shortest time to maturity using the total length data (Table 3.6) and second shortest using the multi-length data (Table 3.7).

#### *Logistic Dose-Response Model*

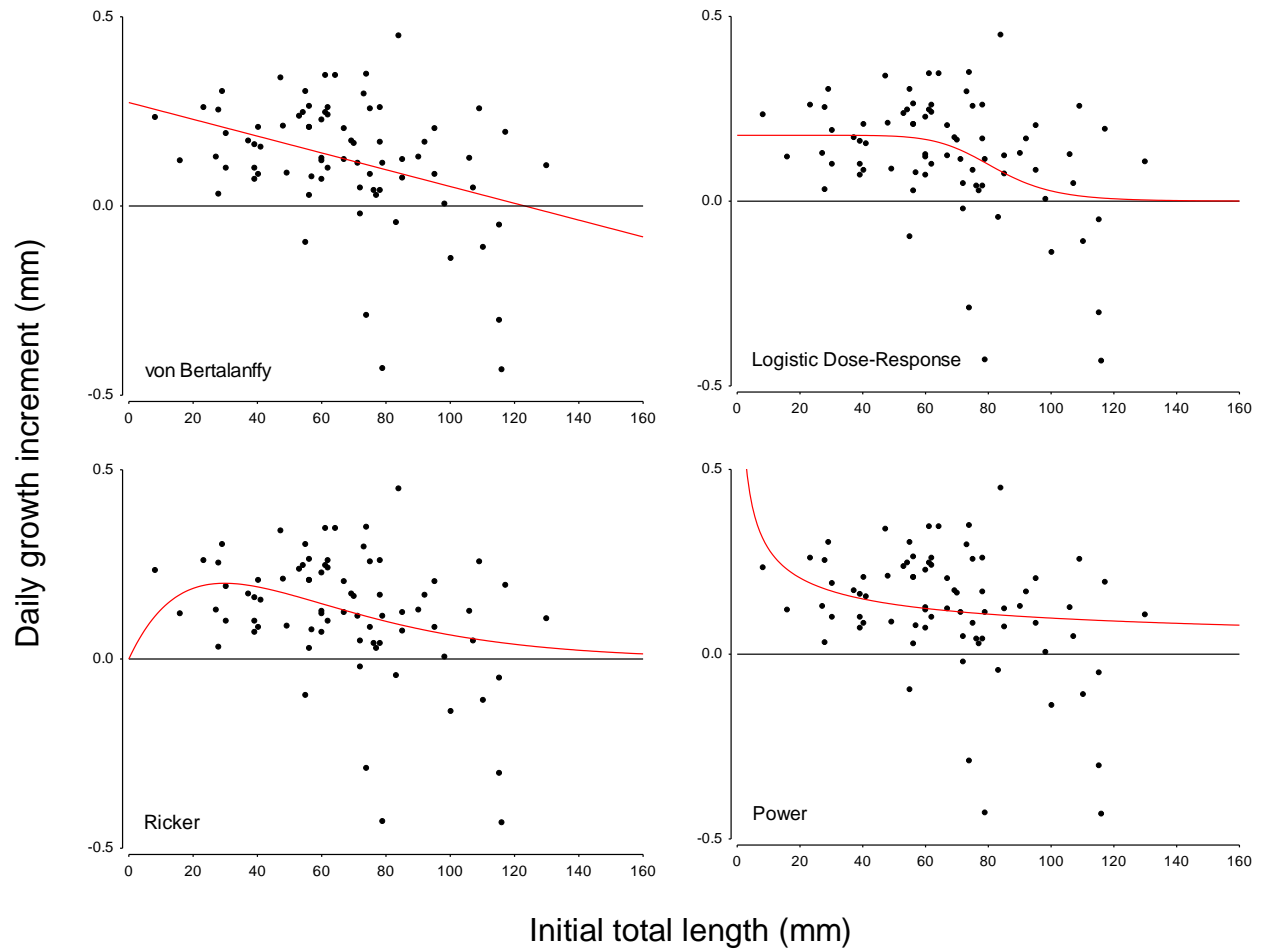
The logistic dose-response curve incorporates a transition between a fast-growing group of younger ascidians, which maintain a constant growth rate, to ascidians growing slowly at a rate that diminishes as size increases. According to  $r^2$  and RSS, the logistic dose-response model fits the data better than the other three models examined here (Table 3.4 and Table 3.5). However, when the extra parameter is taken into account using BIC, the model is ranked third using total length data but remains the best fit using the multi-length data. AIC ranks it as the most parsimonious model using both data sets (as its penalty on extra parameters is less than that of BIC).

*Ricker Model*

The Ricker function fits the data about as well as the von Bertalanffy model (Table 3.4 and Table 3.5), but it produced unrealistic age-at-length curves (Figure 3.10) suggesting *Styela* will only grow to around 45 mm in 1 year. Given the ~ 95% annual mortality rate of *Styela* (see Chapter 2) this is unlikely because growth would need to be achieved well into a second year. Therefore, the Ricker curve was rejected.

*Power Model*

The power curve provided the worst fit to both the total length and multi-metric data according to all goodness-of-fit measures (Table 3.4 and Table 3.5). However, due to the scarcity in the data for lengths < 30 mm (total length), a high growth rate rapidly diminishing to a much lower growth rate as described by power growth (Figure 3.8 and Figure 3.9) remains plausible.

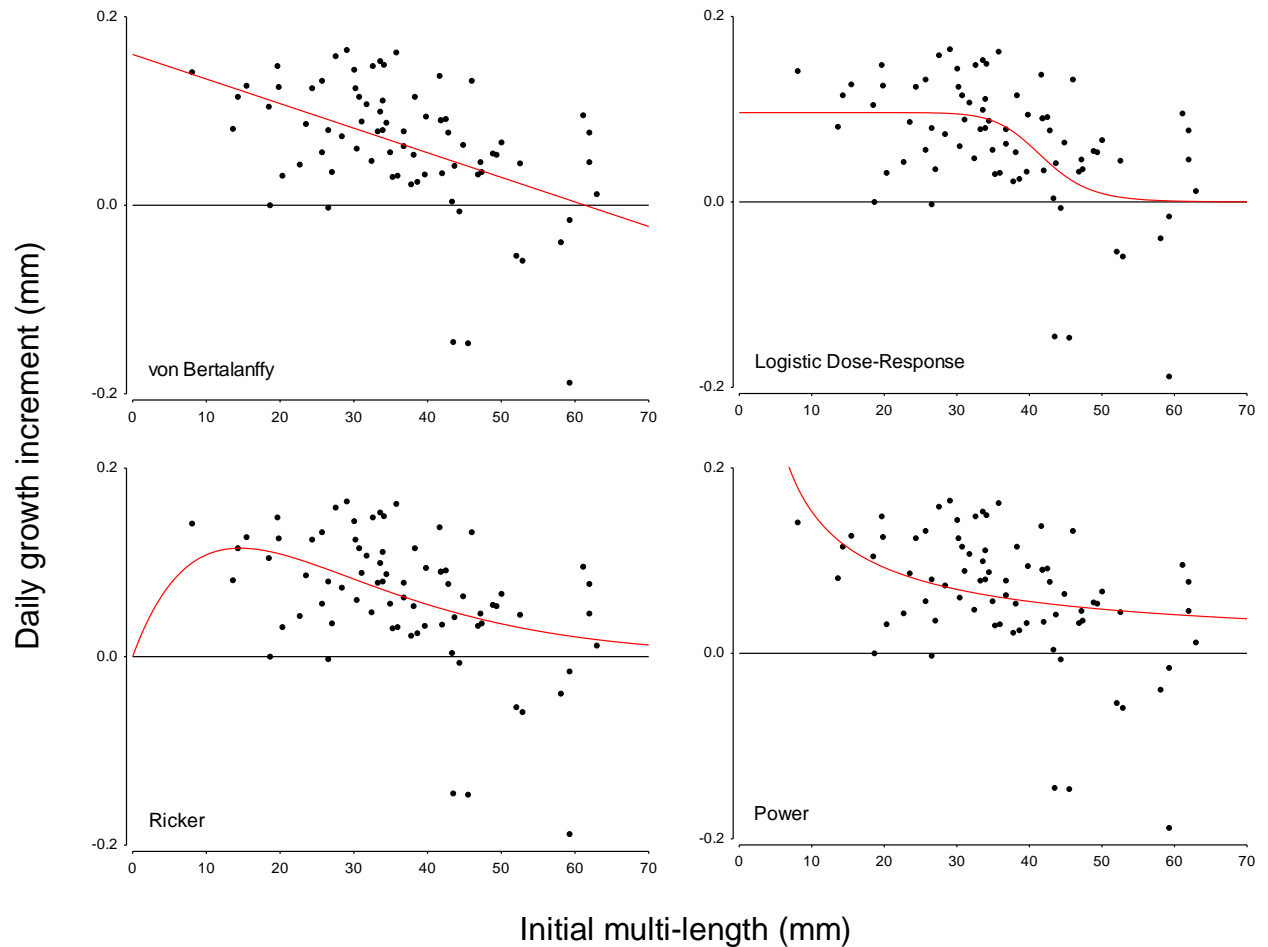


**Figure 3.8:** *Styela clava* growth using all available data illustrating change in total length as a function of the initial total length (at first observation). Four growth models are fitted to the same data – von Bertalanffy, Logistic dose-response, Ricker, and power growth functions ( $n = 76$  individuals).

**Table 3.4:** Summary of fits to total length data of the four growth functions used to model *Styela clava* size-specific growth.  $r^2$  = R-squared value; RSS = residual sum of squares; BIC = Bayesian Information Criterion; AIC = Akaike Information Criterion,  $K$  = number of parameters.

Model	$r^2$	RSS	BIC	AIC	$K$
von Bertalanffy	0.1304	1.7157	-279	-284	2
Ricker	0.1209	1.7345	-279	-283	2
Logistic dose-response	0.1522	1.6727	-277	-284	3
Power	0.0628	1.8490	-274	-278	2

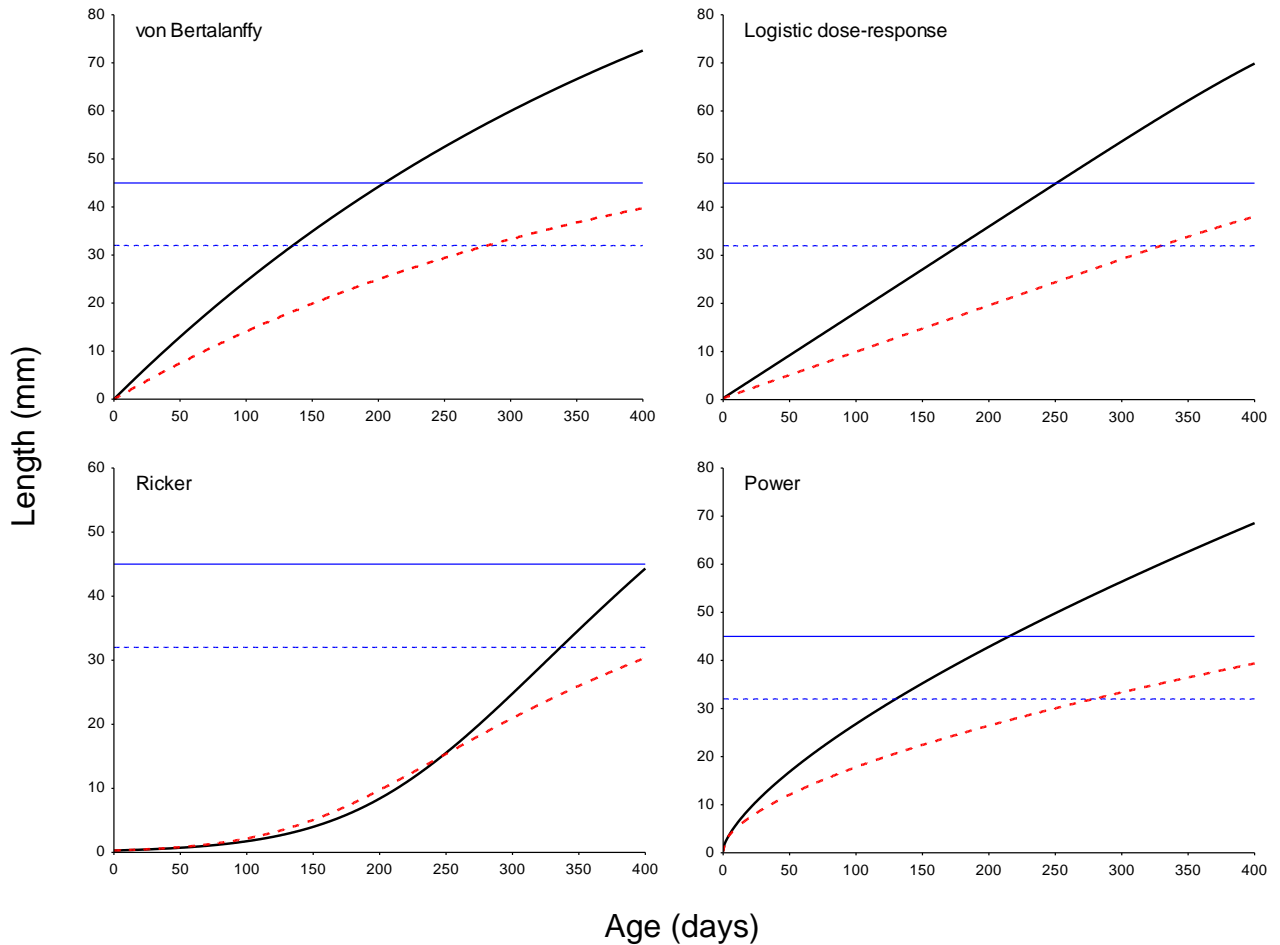




**Figure 3.9:** *Styela clava* growth using all available data illustrating change in multi-metric ( $M$ ) as a function of the initial multi-length,  $M$  (at first observation). Four growth models are fitted to the same data – von Bertalanffy, logistic dose-response, Ricker and power growth functions ( $n = 76$  individuals).

**Table 3.5:** Summary of fits to multi-metric ( $M$ ) data of the four growth functions used to model *Styela clava* size-specific growth.  $r^2$  = R-squared value; RSS = residual sum of squares; BIC = Bayesian Information Criterion; AIC = Akaike Information Criterion,  $K$  = number of parameters.

Model	$r^2$	RSS	BIC	AIC	$K$
Logistic dose-response	0.2744	0.2562	-420	-427	3
von Bertalanffy	0.2237	0.2741	-419	-423	2
Ricker	0.2088	0.2794	-417	-422	2
Power	0.1371	0.3047	-411	-415	2



**Figure 3.10:** Length-at-age models based on fitted length growth models using total length (solid curve) and multi-length,  $M$  (dashed curve). The solid horizontal line is the estimated average size-at-maturity for the total length curve and the dashed horizontal line is the average size-at-maturity for  $M$ .

**Table 3.6:** Parameter values for *Styela clava* total length versus time growth models and time-to-maturity (TTM) in days.

Functions	Parameters	TTM
von Bertalanffy	$L_{\infty} = 123.255 \text{ mm}$ $K = 0.00222 \text{ day}^{-1}$	205 days
Logistic dose-response	$a = 0.178$ $b = 82.214$ $c = 8.592$	252 days
Ricker	$B = 0.0183 \text{ mm}$ $K = 0.0336 \text{ day}^{-1}$	> 400 days
Power	$a = 0.465$ $b = 0.831$	224 days

**Table 3.7:** Parameter values for *Styela clava* multi-metric ( $M$ ) versus time growth models and time-to-maturity (TTM) in days.

Functions	Parameters	TTM
von Bertalanffy	$L_{\infty} = 61.380$ mm $K = 0.00261$ day <sup>-1</sup>	282 days
Logistic dose-response	$a = 0.0964$ $b = 41.855$ $c = 12.645$	331 days
Ricker	$B = 0.0214$ mm $K = 0.0685$ day <sup>-1</sup>	> 400 days
Power	$a = 0.724$ $b = 0.810$	279 days

### 3.3.4 Length-Frequency Analysis

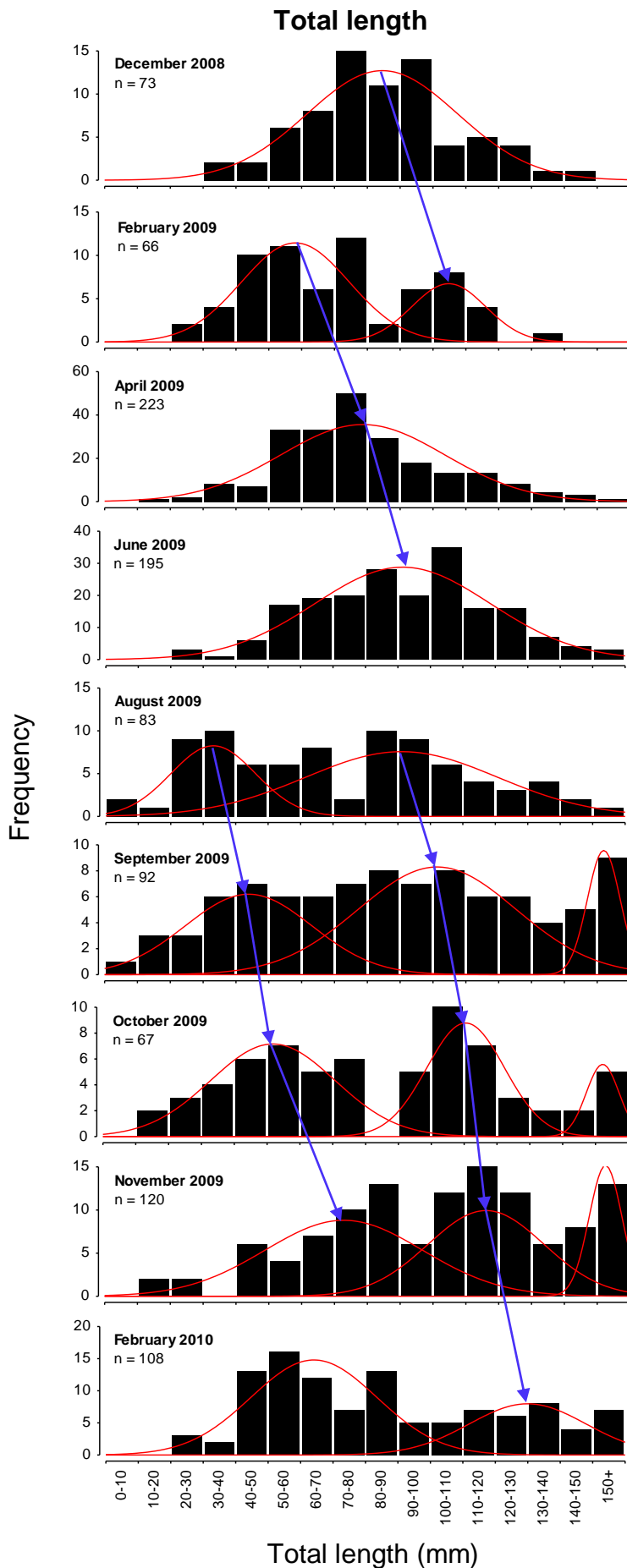
Length-frequency distributions changed considerably through time, showing movement of often ill-defined modes (Figure 3.11 and Figure 3.12). Multiple major spawning events and/or trickle recruitment over its reproductive period may account for the wide range of length-classes observed year-round in *Styela*. Frequent shrinkage or individuals staying within the same length-class over several months would also contribute to the range of length-classes observed.

In some samples there are probably more cohorts present (e.g., in February 2010 there may be a third cohort present between the two that have been identified) but they could not always be clearly defined. Furthermore, sometimes a mode was not linked to another mode the next month (e.g., the mode in June 2009 was not linked to August 2009) either because the mode in the next month appeared to shrink (e.g., June to August 2009) or two modes may have merged in the following month (e.g., November 2009 to February 2010). Despite this, 1 to 3 individual components for each month were teased from the mixture distributions (Figure 3.11 and Figure 3.12, for individual components see Appendix 3.2 and 3.3) and linked according to Figure 3.11 and Figure 3.12. The new cohorts appearing in February and August 2009 assume that *Styela* had two major spawning events some time earlier.

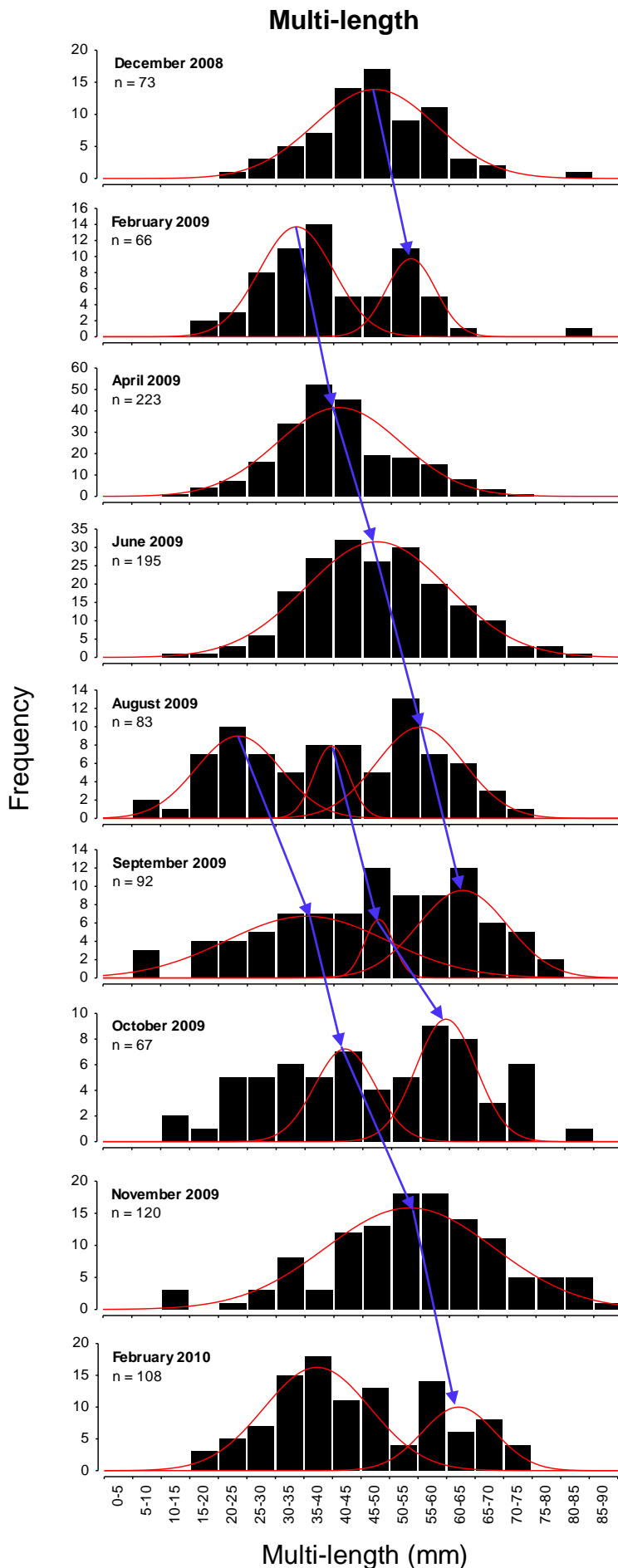
The first major spawning event must have taken place around October 2008. The cohort produced would need to have grown rapidly from settlement to the 40-50 and 50-60 mm length-bins in February (i.e., growing between 40 and 60 mm in 4 months). Judging by direct observation of some individuals (e.g., Chapter 2, section 2.3.3) such growth rates are reasonable.

Length at maturity is known to be ~ 45 mm (Nutsford, unpublished data), and therefore some of these individuals would be reproductive and may have the opportunity to reproduce before water temperatures dropped below ~ 15°C (April 2009). The second reproductive episode, occurring sometime in mid-summer to early autumn, would have produced the new cohort that appeared in August 2009. Individuals from two different cohorts most likely comprised the breeders. The new cohort appears to have then grown for the remainder of winter and early spring, making up much of the reproductive population at the end of spring (October 2009).

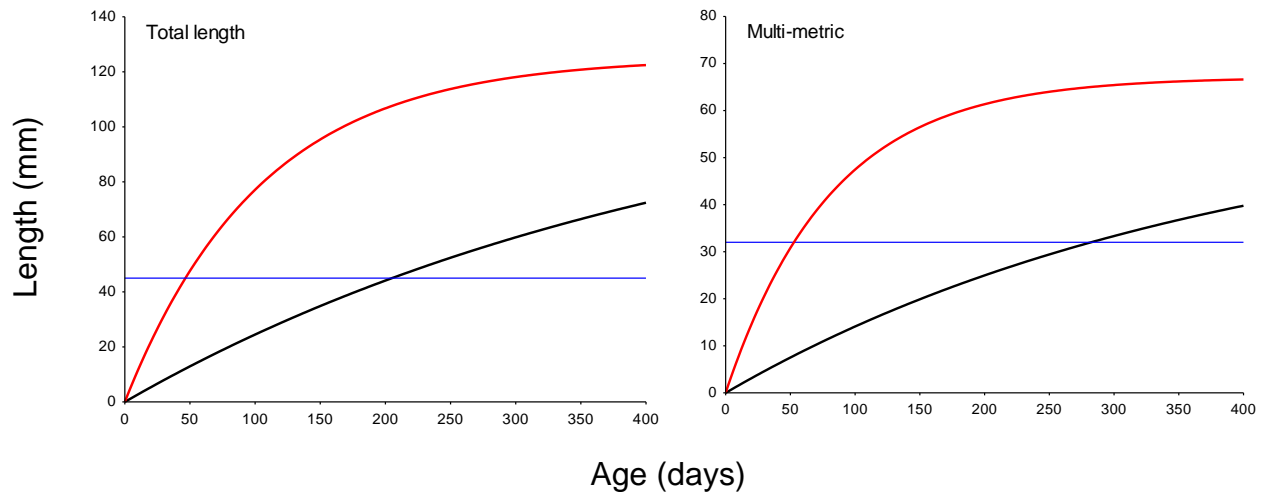
The Munro plot produced in FiSAT II yielded  $L_{\infty} = 125.18$  mm,  $K = 0.009575$  d<sup>-1</sup> and time to maturity of 47 days, using the total length data, and  $L_{\infty} = 67.11$  mm,  $K = 0.012282$  d<sup>-1</sup> and time to maturity 53 days using the multi-length data (Figure 3.13). Using Equation 3.11 to convert the multi-length  $L_{\infty}$  into total length units yields  $L_{\infty} = 126.04$  (*cf.* 125.18), the size-frequency analyses using the two data sets produce essentially the same growth curve and TTM. These estimates of the von Bertalanffy growth function are much higher than those produced by the mark-recapture data (i.e.,  $L_{\infty}$  is bigger and individuals reach their  $L_{\infty}$  much faster). However, the growth curves produced by length-frequency analysis were deemed a much better representation of *Styela*'s growth. Overlaying case profiles of total length through time from mark-recapture individuals on the total length model produced by length frequency analysis lends support to this model (Figure 3.14).



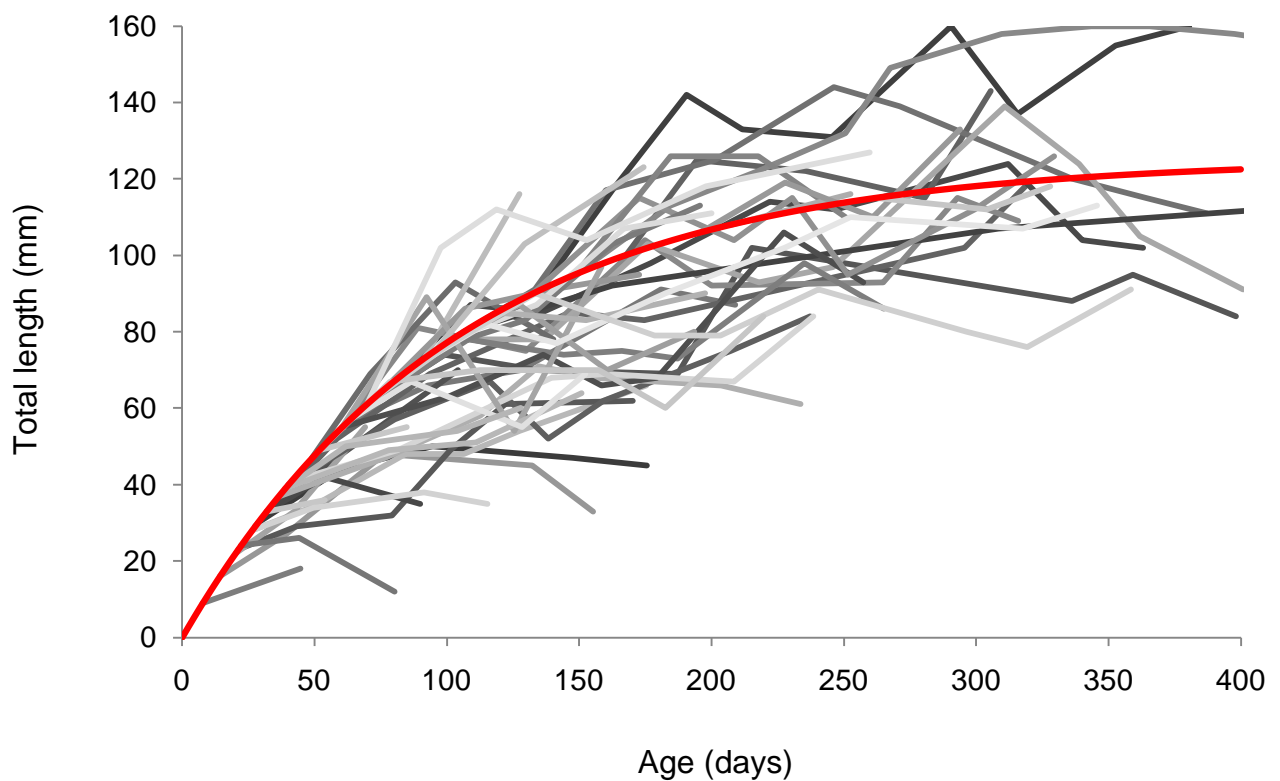
**Figure 3.11:** Length-frequency distributions of total length for *Styela clava* from December 2008 to February 2010. Red lines represent normal distributions assumed to be cohorts. Blue arrows represent how the normal distributions were linked (N.B.: cohorts could not be clearly separated sometimes; see text for explanation). Sample size (n) is shown. For individual component means, standard deviations and component sizes see Appendix 3.2.



**Figure 3.12:** Length-frequency distributions of multi-length for *Styela clava* from December 2008 to February 2010. Red lines represent normal distributions assumed to be cohorts. Blue arrows represent how the normal distributions were linked (N.B.: cohorts could not be clearly separated sometimes; see text for explanation). Sample size (n) is shown. For individual component means, standard deviations and component sizes see Appendix 3.3.



**Figure 3.13:** Length-at-age models based on length-frequency analysis (red line) and growth increment analysis (black line) using both total length and multi-metric data. The horizontal blue line is the size at maturity estimate.



**Figure 3.14:** Length-at-age model based on length-frequency analysis (red line) overlaid with case profiles of individuals followed through time in the mark-recapture study.

### 3.4 Discussion

*Styela clava* growth was highly variable between individuals. *Styela* also had the frustrating ability to shrink, even over several months. While shrinkage is unusual, it is not unheard of in ascidians (Svane and Lundälv 1981) or even among other organisms (e.g., Thomas & Ikeda 1987, Rogers-Bennett *et al.* 2007). Svane and Lundälv (1981) recorded shrinkage in the ascidian *Ascidia mentula* and noted that it occurred more frequently in older individuals during warmer periods. My data show that larger individuals shrink more frequently than smaller individuals, but there seems to be no seasonal pattern to this. The high variation and negative growth made developing an appropriate age-at-length curve difficult, as traditional growth models were of limited use (i.e., usually fish and shellfish do not shrink). Both mark-recapture and length-frequency data were used to produce models of *Styela* growth and both methods yielded quite different models. In the following discussion each of these methods is considered.

Mark-recapture experiments are often considered a more accurate, albeit time consuming, approach to measuring the growth of an organism due to their higher resolution compared with length-frequency analysis. However, the high resolution in growth increments derived from mark-recapture appears to be its downfall in the present study. The mark-recapture derived age-at-length curves underestimated the potential growth of *Styela*, suggesting that the species would only grow to ~ 70 mm (total length) in 1 year. This made no sense, given a life expectancy of ~ 1 year (see Chapter 2) and the high proportion of individuals over 70 mm total length within a year (see Figure 3.11). Thus, the models produced from the mark-recapture individuals do not adequately represent the growth of *Styela*. The severe underestimates of growth that each of these models produce stems from the high variation in growth rates and the species inherent ability to shrink, thus influencing the curve by dragging down growth estimates. Therefore, on the basis of the sizes actually observed within the population coupled with survival estimates leading to the conclusion that this species lives for little more than a year, I choose to reject all of these models and conclude that mark-recapture, while a valuable tool, cannot be used effectively without size-frequency analysis for measuring growth in *Styela*. This probably applies to all solitary ascidians.

Length-frequency distributions reflect an interaction of rates of reproduction, recruitment, growth and mortality of the age groups sampled. Because of the relatively small population size, short life span and apparently continuous breeding throughout several months of the year, size-frequency analysis was unable to discern size and age modes unequivocally. Despite this,



sensible estimates of cohorts were estimated. These translated into reasonable estimates of age-at-length curves using the two data sets (i.e., total length and multi-length). Furthermore, the two data sets produced almost identical outcomes with very similar values for  $L_{\infty}$  (when translated using Equation 3.11) and similar values of time to maturity, lending support to the models produced. Therefore, the von Bertalanffy growth model produced using length-frequency analysis for total length was considered an adequate model for describing the growth of *Styela* in Lyttelton Port. This technique is likely to be broadly applicable to modeling the growth of any solitary ascidian.

The age-at-length model selected describes rapid growth of juvenile *Styela*, reaching maturity in ~ 50 days. Growth slows from maturity and begins to asymptote towards  $L_{\infty} \approx 125$  mm length. Yamaguchi (1975) found *Styela plicata* to become mature at 40-60 mm and grow from settlement to maturity in less than one year in a population in Japan. Similarly, Nutsford (unpublished data) found that *Styela clava* in Lyttelton Harbour reached maturity at ~ 45 mm total length. This means that those individuals that settle in mid-Spring and reach 40-60 mm in length are likely becoming mature (i.e., growing from settlement to maturity in 5 months or less). In a Californian population, juvenile *Styela* grew rapidly at an average rate of 10-15 mm per month (Morris *et al.* 1980) which is within the bounds of *Styela clava* growth in my study.

Growth rates did vary temporally, but no clear seasonal signal was apparent. While growth rates were similar across all depths on pontoon faces, growth rates did differ between the two sites studied. The difference in growth rates between these two sites is minimal, but may be caused by differences in environmental conditions between the two sites. Individuals at site Z had a slightly higher daily growth rate than those at A&B; coincidentally Z pontoons generally had better water clarity (see section 1.4, Chapter 1). Although speculative, this is a plausible cause.

### *Summary*

These growth patterns suggest that recruits are able to reach reproductive maturity very quickly (< 5 months). This means that some individuals may be able to reproduce more than once within its lifetime. In the following chapter, growth data is amalgamated with survival data from the preceding chapter to form a model that explores the life history stages of *Styela* that are most important to population growth and simulate some simple biological, environmental and management scenarios.

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## Chapter 4

### Lefkovitch Matrix Models

“Everything should be made as simple as possible, but not simpler.” – Albert Einstein

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#### 4.1 Introduction

Biological invasions are considered to be one of the most important risks to biodiversity (Elton 1958, Carlton 1989, Sala 2000) and the literature is full of examples highlighting the significance of such invasions (e.g., Shushkina & Musayeva 1990, Shiganova 1998, Walker & Kendrick 1998, Ruiz *et al.* 2000, Bax *et al.* 2003, Hayes *et al.* 2005, Forrest & Blakemore 2006). However, little work has been done linking quantitative field data, such as fecundity, growth and survival of individuals within the population, and theory on invasiveness, invasibility, and rates of spread (Parker 2000).

Identifying which life-cycle stages or life history characteristics contribute the most to invasion success is crucial to understanding why only some species succeed in becoming invasive or why some species are more successful invaders than others. Matrix demographic models provide a valuable framework from which to investigate the life history characteristics that contribute most to population growth, and therefore invasion success, by translating demographic information from the individual to the population level. Matrix models may also be used to determine the finite rate of increase of populations. This can be useful in determining if a species will spread, and, if so, give an indication of its rate of spread (Caswell 2001, Neubert & Parker 2004, Jongejans *et al.* 2008, Shea *et al.* 2010). This has practical implications for management or eradication planning of invasive species.

The simplest matrix demographic model is the Leslie matrix (Leslie 1945), which is based on age-specific growth, survival, and fecundity rates. However, it can be difficult or impossible to age some organisms accurately. Furthermore, in some organisms, survivorship and fecundity are

more closely related to size than age. Lefkovitch (1965) realized this and extended the Leslie model, replacing the age groups with stage groups.

In a Lefkovitch matrix, the importance of different life history stages or matrix elements (i.e., reproduction, growth, survival) toward population growth can be investigated using sensitivity or elasticity analysis. These analyses determine the total (sensitivity) or proportional (elasticity) contributions of each life history stage or matrix element towards population growth (de Kroon *et al.* 1986, Dudas *et al.* 2007). The use of such analyses applied to biological systems has proved very useful. For example, matrix models and elasticity analysis of loggerhead sea turtles has led to a redirection of conservation efforts away from enhancing the survival of eggs in nests to reducing adult mortality through the use of turtle exclusion devices in fishing nets (Crouse *et al.* 1987, Crowder *et al.* 1994, Heppel & Crowder 1996). In invasion biology, determining a particularly sensitive life history stage can provide managers with an “Achilles heel” that management or eradication programs can target (Parker 2000). For example, Dudas *et al.* (2007) showed that adult survival is most crucial for population growth in two populations of an invasive clam, and therefore any process that reduces the survival of these larger clams will affect population growth (e.g., harvesting).

Understanding the life history characteristics and vital rates that are most influential to *Styela*'s population growth is crucial in determining its invasion success. Therefore, the aim of this chapter was to amalgamate growth and survival data from the previous two chapters in the form of a matrix demographic model and use this to further investigate what life history characteristics are most important to population growth. I wanted to further this by investigating how the population might respond to management action or environmental changes through simulation. I specifically address the following questions:

- (i) What life history characteristics are most important for population growth in *Styela clava*?
- (ii) Does *Styela clava* have an “Achilles heel” (i.e., a vulnerable life history stage or vital rate)?
- (iii) What management actions, environmental changes, or biological changes could potentially result in population growth or decline?

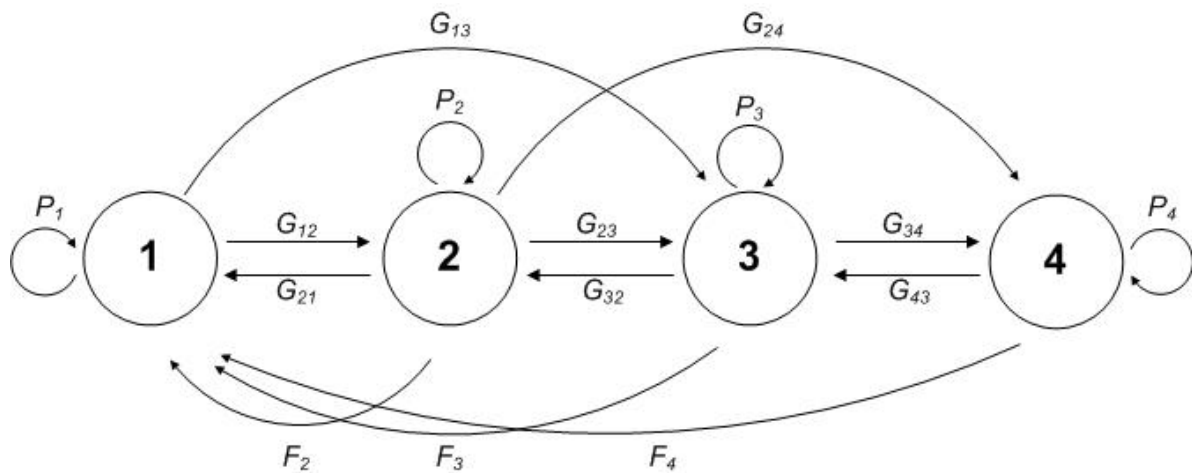
## 4.2 Materials and Methods

### 4.2.1 Matrix Demographic Model

A size-structured matrix model (Lefkovitch 1965) was developed using growth and survival data from Chapters 2 and 3. Four size-classes were chosen, based on length-at-maturity and size-specific differences in growth rates (Table 4.1). Transitions between size-classes were described in a transition matrix,  $A$ .  $A$  is a matrix of  $a_{ij}$ 's that describes how each stage contributes to the number of individuals in all other stages at the next time step, and the dominant eigenvalue ( $\lambda_1$ ) of  $A$  represents the asymptotic rate of population increase. The  $a_{ij}$ 's of the matrix (including fecundity, growth and survival) are shown as a life-cycle graph and in matrix form (Figure 4.1).

**Table 4.1:** The size-classes used in the *Styela clava* matrix demographic model and reasons for choosing these boundaries.

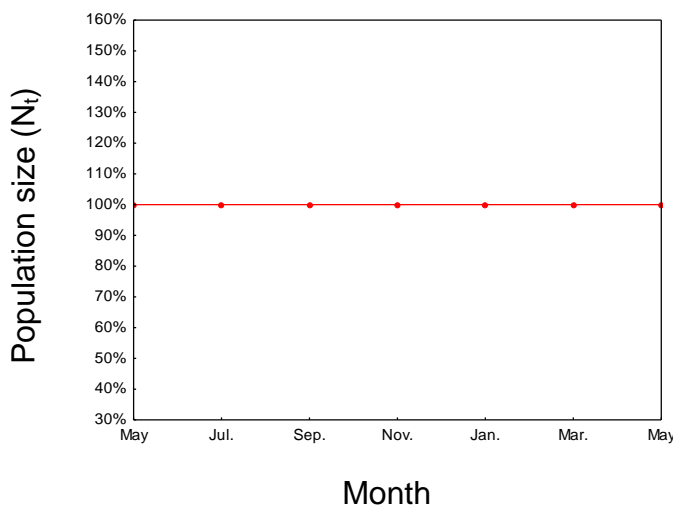
Size-class	Size (mm)	Reason for size-class boundary
1	< 45	Estimated length-at-maturity (Nutsford, unpublished data).
2	45-85	Size-classes 2 and 3 were determined as the remaining length-range divided by 2.
3	85-125	
4	> 125	$\sim L_\infty$ according to length-frequency analysis (Chapter 3).



**Figure 4.1:** Life cycle graph and structure of the size-based transition matrix used in this study of *Styela clava*. The nodes represent the chosen size-classes.  $G_{ij}$  is the probability of surviving and growing (or shrinking) from size-class  $i$  to size-class  $j$  in two months;  $P_i$  is the probability of surviving and remaining in size-class  $i$  in two months;  $F_i$  is the reproductive contribution of each size-class  $i$  to the first size-class.

$$\begin{pmatrix} P_1 & F_2 & F_3 & F_4 \\ G_{12} & P_2 & G_{32} & 0 \\ G_{13} & G_{23} & P_3 & G_{43} \\ 0 & G_{24} & G_{34} & P_4 \end{pmatrix}$$

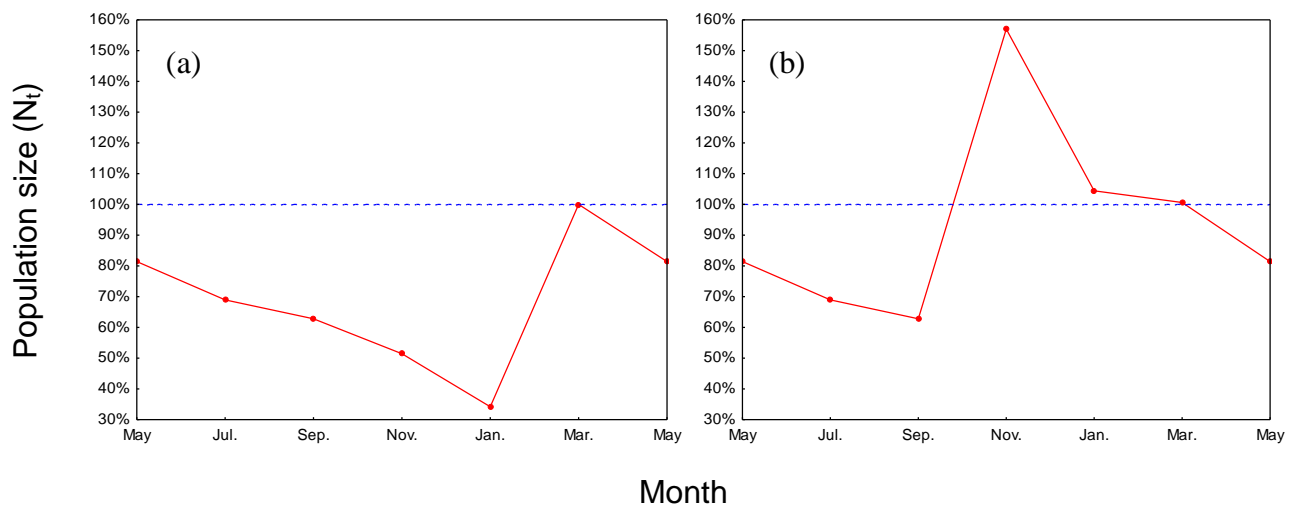
The time step chosen for this model was two months. This was necessary because if a time-step of one year was used, for example, almost all individuals would grow from any size-class to the largest size-class in that year and, as shown in Chapter 3, the majority of individuals would die by the end of the year, resulting in a matrix full of zeros and of little use (i.e., this model would focus on between-year dynamics and ignore the majority of within-year processes). However, if annual fecundity is partitioned between each bimonthly period (i.e., the fecundity estimate used in the matrix was the total annual fecundity divided by six) and this deterministic model was used to generalize over a year, it would describe a system with a static population size over the course of that year, in which reproduction and recruitment exactly balance mortality every two months (assuming population census occurs after reproduction) (Figure 4.2). Obviously this model would not represent the real-world population well because *Styela* is not reproductively active year-round (Nutsford, unpublished data) and mortality is acting upon the population during periods of reproductive inactivity (i.e., take a population of initial size,  $N_t$  during a non-reproductive month. The number of individuals alive a month later,  $N_{t+1}$ , will be a product of  $N_t$  and the bimonthly survival probability,  $\hat{S}$ ).



**Figure 4.2:** Hypothetical population size over a year described by a bimonthly matrix model that uses fecundity estimate,  $F_i$ , equal to the total annual fecundity divided by six. Although this model assumes that the population is static on a year-to-year basis, it should not be static on a monthly basis because of mortality and reproduction.

In this case, a better model would describe a fluctuating population that declines during periods of reproductive inactivity and grows after reproduction. Periodic matrix models (Caswell 2001) provide such a framework for modeling the demography of annuals. Therefore, I propose two potential population models that focus on within-year processes and ignore between-year dynamics. Each incorporates temporal variability in survival and fecundity parameters by constructing bimonthly matrices specific to each two-month period. The time series of data collected (Chapters 2 and 3) allowed six bimonthly size-structured matrices for the population (i.e., a year). These models assume that the population in Lyttelton Port is static on a year-to-

year basis, as discussed in Chapter 2, providing another reason for choosing to focus on within-year dynamics. In Chapter 2 I showed that  $\sim 95\%$  of individuals senesce within 1 year. The model assumes that throughout the year, deaths of older individuals are equaled by addition of new recruits (achieving a static population). I had to assume that the population was static because there is an unknown parameter in the model (i.e., the settlement/recruitment parameter, explained shortly). To illustrate the dynamics of the two models explored, I produced hypothetical trajectories of population size ( $N_t$ ) over discrete time steps (instead of continuous growth) (Figure 4.3). Population census is assumed to occur after reproduction; that is, a reproductive episode occurring during January/February would lead to a population increase in the population at census in March.



**Figure 4.3:** Two hypothetical population models describing the population size,  $N_t$ , of *Styela* over one year. The first model (a) describes a population with a single reproductive episode. The second model (b) describes a population with two reproductive episodes.

The first model is based on the assumption that *Styela* reproduces once a year during January and February, the peak reproductive period (Nutsford, unpublished data) (Figure 4.3a). This model describes a population trajectory characterized by a steady decline, due to mortality, from May to January, followed by a sharp rise in population size after January back to the original population size in the same month as the previous year, due to reproduction and because the model assumes  $\lambda_1 = 1$  over a year. For the population to increase from the size in January (34%) back to that observed in March the previous year (100%) would require population growth,  $\lambda_1 = 2.9233$  (using Equation 4.1 and rearranging for  $\lambda_1 = N_{t+1}/N_t$ ). The second model assumes *Styela* reproduces twice a year (October and again January/February, October being the second largest

peak in reproductive activity according to Nutsford, unpublished data). Here, I assume that reproductive output during each peak is the same and, therefore, that the total annual reproductive output is twice that of the first model (Figure 4.3b). The two peaks in this model require  $\lambda_1 = 2.4999$  and  $0.9637$ , respectively. These two models were produced because there is some uncertainty about how often *Styela* reproduces (Nutsford, unpublished data), but, evidence suggests that there may be two peaks of reproductive activity (see Discussion of Chapter 2). A matrix is produced for each two-month period within each of these models. The periods when no reproduction occurs resulting in  $\lambda_1 < 1$  (i.e., population decline) and the periods where reproduction occurs resulting in  $\lambda_1 > 1$  (i.e., population growth equal to the increase required to reset the population size,  $N_t$ , back to 100%).

**Equation 4.1:**

$$N_{t+1} = N_t \lambda$$

where  $N_t$  is the population size at time  $t$ ;  $N_{t+1}$  is the population size at time  $t+1$ ; and  $\lambda$  is the finite rate of population increase for that time period; for the purposes of this model it is assumed to be equal to  $\lambda_t$ . This is the simple exponential form of population growth (Skalski et al. 2005).

*Growth transitions ( $G_{ij}$  and  $P_i$ )*

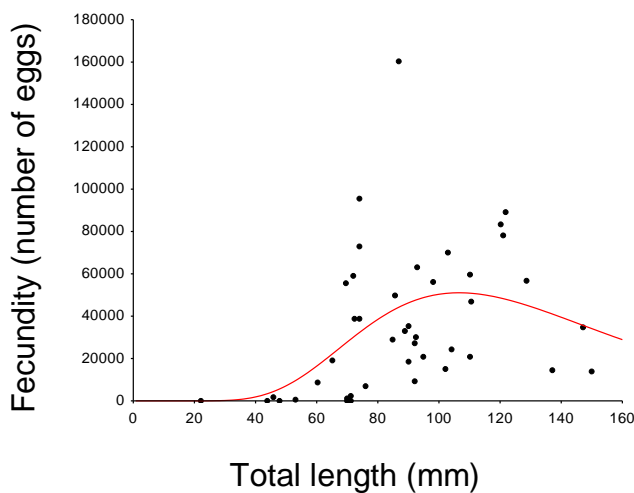
Growth transition probabilities between size-classes were determined using the observed changes in lengths of those individuals in the mark-recapture study (Chapter 3) every two months. Transition probabilities were calculated without time-dependence (i.e., no temporal changes in growth rates were taken into account). This was deemed appropriate because there was little evidence for seasonality in growth rates (Chapter 3).

*Survival probabilities ( $\hat{S}$ )*

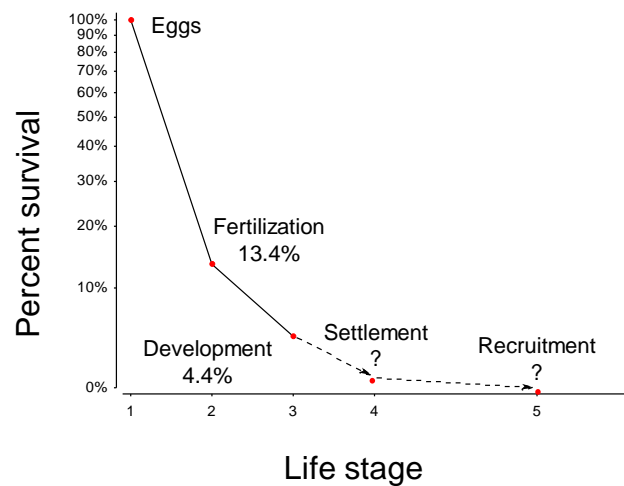
Demographic data from the mark-recapture study were used to generate bimonthly survival estimates (Chapter 2). A new MARK model was produced to generate these estimates and associated confidence intervals ( $\pm 95\%$  C.I.). The model chosen for inclusion in matrices was the time-dependent model,  $\hat{S}_t$ . The model including size-specificity in survival estimates,  $\hat{S}_{t \times size}$  (from Chapter 2), was not used because of its inherent complexity and concern that sampling error may be interfering with survival estimates. Sampling errors arise when too few animals are sampled within a size-class. Growth transitions were multiplied by survival estimates.

*Fecundity ( $F_i$ )*

Size-specific fecundity (number of eggs per individual) data were provided by Nutsford (unpublished data) (Figure 4.4). A log normal function was fitted to the data (Figure 4.4, Equation 4.2) using the curve fitting program TableCurve 2D v5.01 (Jandel Scientific, Corte Madera, California). The fecundity of each size-class was determined by taking the mean of the size-class boundaries and calculating the fecundity for each length using Equation 4.2. Confidence intervals ( $\pm 95\%$  C.I.) were produced about each of these values using the evaluation function in TableCurve. Fecundity estimates were converted into the reproductive contribution of each size-class,  $F_i$ , using Equation 4.3. The fertilization rate and development rate (i.e., survival from fertilized egg to larvae) estimates were provided by Nutsford (unpublished data) and are summarized in Figure 4.5.



**Figure 4.4:** Scatterplot of fecundity (number of eggs) against total length for  $n = 43$  individual *Styela clava*. The fit to this data is a log normal curve (Equation 4.2),  $r^2 = 0.21$ ,  $p < 0.05$ . Data from Nutsford (unpublished data).



**Figure 4.5:** Life stage graph for *Styela clava* eggs through to developed larvae. The percentages represent the proportion of the total number of eggs produced estimated to remain at each juvenile stage. Settlement and recruitment rates are unknown. Data from Nutsford (unpublished data).

**Equation 4.2:**

$$fecundity = 51055.197 \exp \left[ -\frac{1}{2} \left( \frac{\ln \left( \frac{length}{106.550} \right)}{0.381} \right)^2 \right]$$

**Equation 4.3:**

$$F_i = (fecundity \times fertilization\ rate \times development\ rate)$$



Settlement and recruitment rates could not be determined for *Styela* (i.e., the probability of survival from development to a size large enough to be detected in population surveys, which was  $\sim 30$  mm) (Figure 4.5) due to the cryptic nature of small individuals. Therefore, these parameters were entered into the matrix model as an unknown,  $R$ , and thus the matrix model becomes:

$$\begin{pmatrix} P_1 \times \hat{S} & G_{21} \times \hat{S} + F_2 \times R & F_3 \times R & F_4 \times R \\ G_{12} \times \hat{S} & P_2 \times \hat{S} & G_{32} \times \hat{S} & 0 \\ G_{13} \times \hat{S} & G_{23} \times \hat{S} & P_3 \times \hat{S} & G_{43} \times \hat{S} \\ 0 & G_{24} \times \hat{S} & G_{34} \times \hat{S} & P_4 \times \hat{S} \end{pmatrix}$$

Because of the unknown  $R$ , the model cannot be used to make population projections or predictions on population growth (or decline). However, by assuming that the population is static over the course of a year, which seems a reasonable assumption (see Discussion of Chapter 2);  $R$  can be solved iteratively (Vaughn & Saila 1976) for any given value of  $\lambda_1$ . The values of  $\lambda_1$  used here are the population growths required during each reproductive episode in the two models to bring the population size back to 100%. Finally, the assumptions of each of these models are summarized (Table 4.2).

**Table 4.2:** Summary assumptions included in each of the models used to describe this population of *Styela clava*.

Model 1 (single reproductive episode)	Model 2 (two reproductive episodes)
Population is static over a year	Population is static over a year
Growth rates are size-specific	Growth rates are size-specific
Survival is time-dependent	Survival is time-dependent
Fecundity is size-specific	Fecundity is size-specific
<i>Styela</i> reproduces once a year	<i>Styela</i> reproduces twice a year
	Reproductive output for each peak is the same
	The settlement/recruitment, $R$ , rate during each of these peaks is the same

### 4.2.2 Elasticity Analysis

Determining how much various life-history stage transitions affect population dynamics can be done by examining how changes to matrix elements affect the dominant eigenvalue,  $\lambda_1$  (i.e., population growth). This process is called sensitivity or elasticity analysis. Sensitivity is defined as the partial derivative of a population's finite growth rate ( $\lambda_1$ ) to changes in matrix elements (Caswell 1978, Caswell 2001, Equation 4.4). High sensitivity of a matrix element implies that even a small change in its value has a large effect on the population growth rate (Horvitz & Schemske 1995). Elasticity is the sensitivity scaled to take into account the magnitude of population growth and the matrix element (Equation 4.5) and indicates the relative contributions of the matrix elements to population growth rate (de Kroon *et al.* 1986). Elasticity is used because matrix elements are often measured at different scales (i.e., growth transitions and fecundity). This is the case with my data so elasticity was used instead of sensitivity. Elasticities were calculated using the PopTools add-in for Microsoft Excel (available online, [www.cse.csiro.au/poptools/](http://www.cse.csiro.au/poptools/)).

**Equation 4.4:**

$$s_{ij} = \frac{\partial \lambda}{\partial a_{ij}}$$

**Equation 4.5:**

$$e_{ij} = \frac{a_{ij}}{\lambda} \frac{\partial \lambda}{\partial a_{ij}}$$

To examine how varying matrix elements contributed to changes in elasticities, matrix simulations were done using minimum and maximum parameter estimates (Mills *et al.* 1999, Hunter *et al.* 2000). Fecundity and survival parameters were varied  $\pm$  the 95% confidence intervals of the estimates. Growth transitions were varied  $\pm$  5% (i.e., 5% more transitioning into the next size-class or 5% more remaining in a size-class) (Rogers-Bennett & Leaf 2006). Elasticity analysis was also done on matrices after removal of shrinkage transitions and individuals skipping size-classes to determine their influence on  $\lambda_1$ .

### 4.2.3 Simulations

Two theoretical scenarios were explored by modifying matrix elements imitating potential biological/environmental or management alterations and observing subsequent changes in the population growth rate resulting from the new matrix. Here, I did two simulations. (1) An attempt at control of *Styela* numbers by removing 50% of the three largest size-classes, a purely hypothetical quantity that could result from intensive removal by divers. This was expressed in

the matrix as a 50% reduction in the reproductive contribution of all size-classes; (2) to imitate the effects of a particularly good season for *Styela* through some environmental or biological change, I doubled settlement (or fecundity, both giving the same matrix), resulting in a 50% increase in the reproductive contribution of all size-classes.

## 4.3 Results

### 4.3.1 Matrix Demographic Model

#### *Growth transitions*

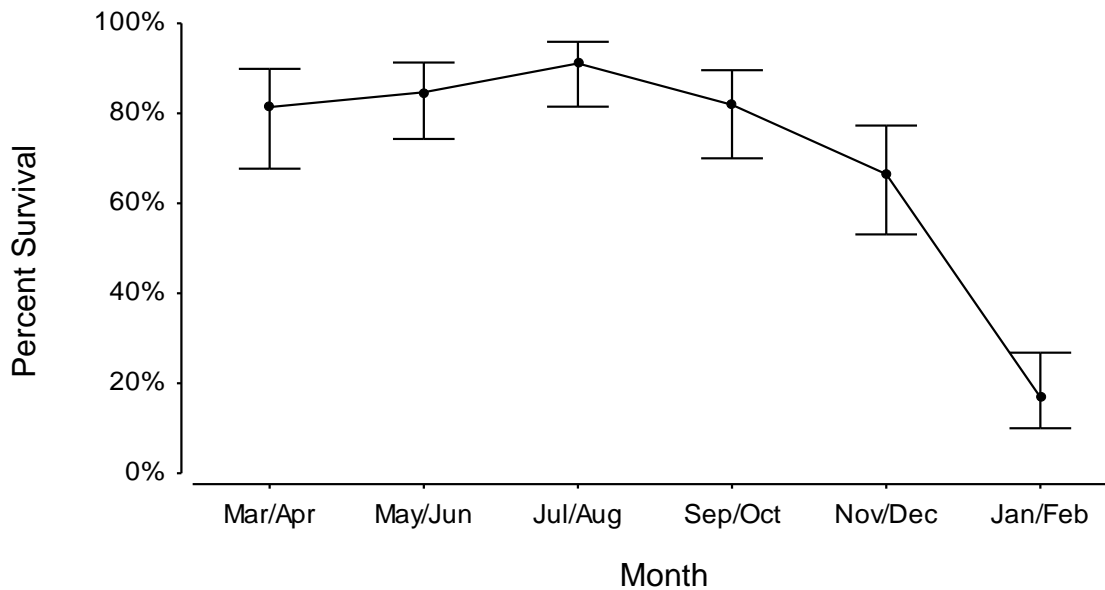
The growth transition probabilities of individuals are presented in matrix form below:

$$\begin{pmatrix} 0.383 & 0.043 & 0 & 0 \\ 0.577 & 0.603 & 0.091 & 0 \\ 0.039 & 0.311 & 0.655 & 0.152 \\ 0 & 0.043 & 0.255 & 0.848 \end{pmatrix}$$

The diagonal elements represent the probability of an individual remaining in that size-class after two months ( $P_i$ ). For example, the first value of the matrix ( $P_{11} = 0.383$ ) represents a 38% chance that an individual will remain in the first size-class after two months. Elements in the subdiagonal above are the probabilities of individuals shrinking a size-class in two months. Backward growth transitions occurred for 9% ( $G_{32} = 0.091$ ) and 15% ( $G_{43} = 0.152$ ) of the two largest size-classes. There was also a small backward growth transition ( $\sim 4\%$ ) from the second size-class to the first ( $G_{21} = 0.043$ ); this was simply added to the fecundity estimate for this size-class ( $F_2$ ). Elements in the subdiagonal below are the probabilities of individuals growing into the next size-class in two months ( $G_{ij}$ ). For example, there is a 58% chance that an individual in the first size-class will grow to the second size-class in two months ( $G_{12} = 0.577$ ). Elements below this represent the probability of skipping a size-class; for example, there is a 4% chance that an individual in the first size-class will grow to the third size-class in two months ( $G_{13} = 0.039$ ).

#### *Survival probabilities*

Bimonthly survival probabilities,  $\hat{S}$ , produced by MARK are summarized in Figure 4.6. These were discussed in depth in Chapter 2.



**Figure 4.6:** Survival estimates,  $\hat{S}$ , for each bimonthly period, and  $\pm 95\%$  confidence intervals of *Styela clava* produced in MARK from mark-recapture data.

### Fecundity

The estimated fecundity and reproductive contributions of each size-class are presented in Table 4.3.

**Table 4.3:** Size-specific fecundity estimates for *Styela clava* as fecundity (i.e., the number of eggs estimated to be produced by each size-class each reproductive event) and reproductive contribution to each size-class  $F_i$ .

Size-class	Size (mm)	Fecundity	$F_i$	- 95% C.I.	+ 95 % C.I.
2	45-85	22032	969	195	1744
3	85-125	51017	2245	1569	2921
4	> 125	38172	1680	451	2909

### Matrices

Because not all elements of the matrix demographic model were known, the population growth rate could not be determined. However, setting population growth at  $\lambda_1 = 1$  and iteratively solving the missing settlement/recruitment estimate in the matrix allowed for exploration of the elasticities of the matrix elements, thus giving an idea of what size-class transitions contributed most towards population growth. Matrices for all bimonthly periods in both models were developed. Here I provide matrices for two periods of reproductive inactivity (July/August and November/December). I only provide these two due to the similarities between matrix structure and elasticities for all non-reproductive months. These two months had the highest and lowest

bimonthly survival estimates ( $\hat{S} = 0.911$  and  $0.665$ , respectively). I also display matrices for the reproductive periods of both models. The non-reproductive months July/August had the highest bimonthly survival estimate ( $\hat{S} = 0.911$ , left matrix) resulting in equivalent population decline (i.e.,  $\lambda_1 = 0.911$ ). November/December had the lowest survival estimate for a non-reproductive month ( $\hat{S} = \lambda_1 = 0.665$ , right matrix):

$$\begin{pmatrix} 0.349 & 0.039 & 0 & 0 \\ 0.526 & 0.549 & 0.083 & 0 \\ 0.036 & 0.283 & 0.597 & 0.138 \\ 0 & 0.039 & 0.232 & 0.773 \end{pmatrix} \quad \begin{pmatrix} 0.255 & 0.029 & 0 & 0 \\ 0.384 & 0.401 & 0.061 & 0 \\ 0.026 & 0.207 & 0.436 & 0.101 \\ 0 & 0.029 & 0.170 & 0.564 \end{pmatrix}$$

The matrix for the model describing a single reproductive episode during January/February had survival  $\hat{S} = 0.169$  and required  $\lambda_1 = 2.9233$ . Solving iteratively for the settlement/recruitment rate yielded,  $R = 0.07066$ :

$$\begin{pmatrix} 0.00120 & 68.477 & 158.632 & 118.709 \\ 0.09751 & 0.102 & 0.015 & 0 \\ 0.00659 & 0.053 & 0.111 & 0.026 \\ 0 & 0.007 & 0.043 & 0.143 \end{pmatrix}$$

The matrices for the model describing two reproductive pulses during October ( $\hat{S} = 0.819$ , set  $\lambda_1 = 2.4999$ , left matrix) and January/February ( $\hat{S} = 0.169$ , set  $\lambda_1 = 0.9637$ , right matrix). For both models  $R = 0.00615$ :

$$\begin{pmatrix} 0.314 & 5.995 & 13.807 & 10.332 \\ 0.473 & 0.494 & 0.075 & 0 \\ 0.032 & 0.255 & 0.536 & 0.124 \\ 0 & 0.035 & 0.209 & 0.695 \end{pmatrix} \quad \begin{pmatrix} 0.065 & 5.967 & 13.807 & 10.332 \\ 0.098 & 0.102 & 0.015 & 0 \\ 0.007 & 0.053 & 0.111 & 0.026 \\ 0 & 0.007 & 0.043 & 0.143 \end{pmatrix}$$

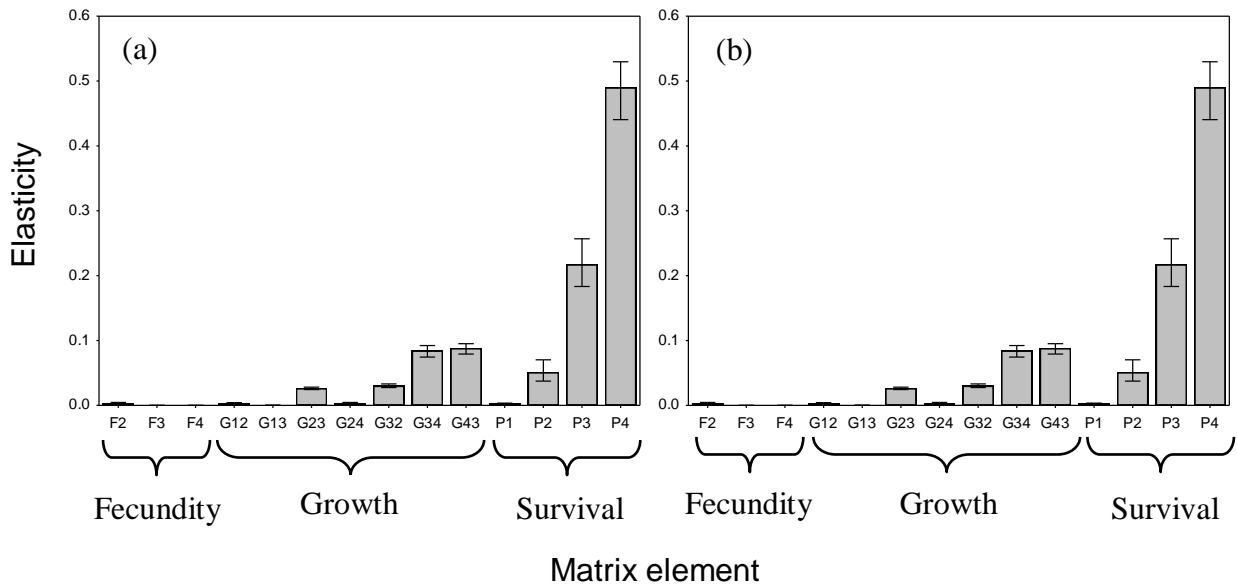
Note that the settlement/recruitment rate,  $R$ , is an order of magnitude less than the previous model ( $R = 0.00615$ , *cf.*  $0.07066$ ). This is because less recruitment is required to recover the population size, given that the total annual fecundity is double the previous model. The fecundity,  $F_i$ , and the settlement/recruitment rate,  $R$ , during these two months are set exactly the same, yet population growth values,  $\lambda_1$ , are different. This is due to the differences in observed survival estimates during these two months.

### 4.3.2 Elasticity Analysis

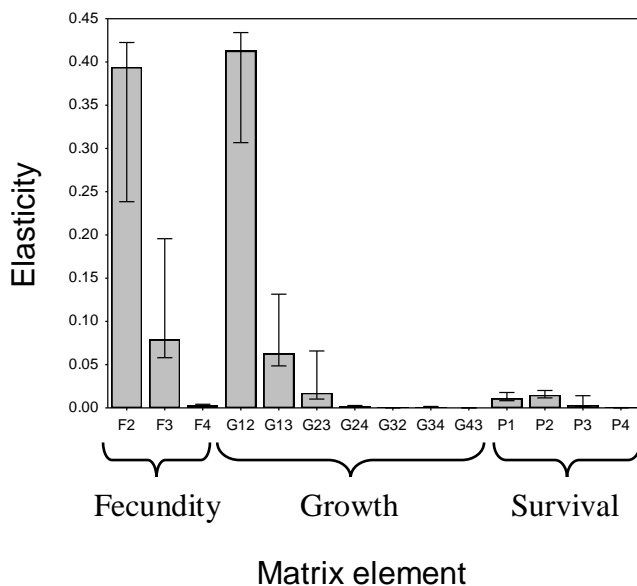
During periods of reproductive inactivity, the factor that contributes most towards population growth is survival of the largest size-class, followed by survival of the second largest size-class (Figure 4.7). Changing matrix elements from their mean to the maximum and minimum values of fecundity, survival and growth or removing backward growth transitions and skipping of size-classes did not alter the relative ranking of the elasticities (N.B. the  $\pm$  whiskers on Figure 4.7, 4.8, and 4.9 represent the maximum and minimum elasticity value from all matrix element perturbations). This suggests that survivorship in the two largest size-classes are the most important stages in *Styela*'s life history during periods of reproductive inactivity. This is obvious given that the population is not being topped up through reproduction; of course, survival will be important during these months.

However, during the period of reproduction in the first model (single peak of reproduction), the factors that contribute most towards population growth are growth from the first size-class to the second ( $G_{12}$ ), followed by reproductive contribution from the first ( $F_1$ ) and second ( $F_2$ ) size-classes (Figure 4.8). Changing matrix elements from their mean to maximum and minimum values was far more variable, but it still did not change the relative ranking of the elasticities. This switch is likely because of the high mortality occurring across all size-classes during these two months, and reproductive output is essentially replacing the population. A very similar elasticity pattern was observed during both reproductive months in the second model (Figure 4.9). The only difference was a slightly higher relative elasticity for survival and slightly lower elasticity for growth of the smallest size-class and reproductive contributions of the two smallest size-classes. Once again, perturbation analysis did little to affect the relative ranking of elasticities.

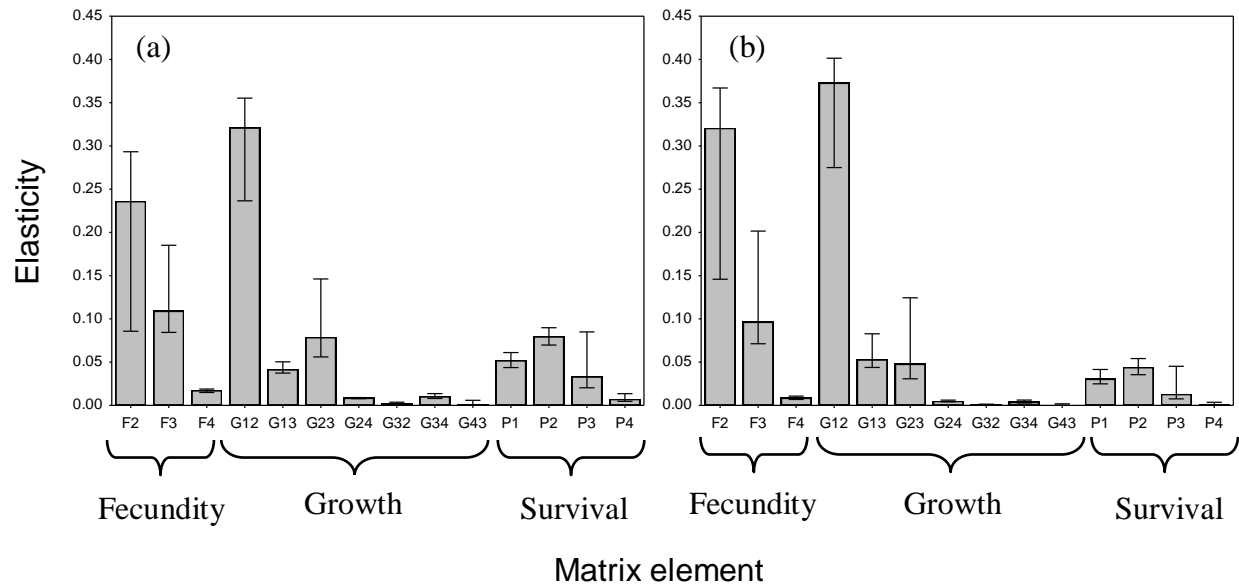
By combining the matrix elements for each vital rate (fecundity, growth, survival) into their respective size-classes or life-cycle stages (Figure 4.10), a general trend appears. During months of reproductive inactivity, the two largest size-classes, representing individuals of  $\geq 85$  mm total length, are the most important contributors towards population growth (really population "preservation"). However, as the water warms and reproduction begins, the two smallest size-classes become more important, reflected by reproductive contribution and growth into the larger size-classes.



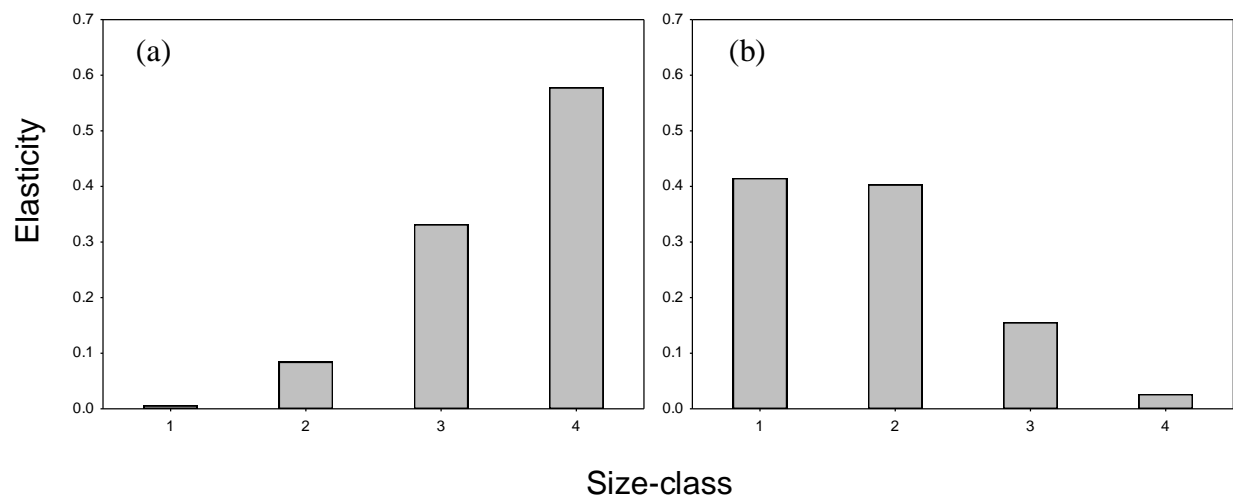
**Figure 4.7:** Matrix element elasticity values  $\pm$  maximum and minimum matrix element perturbation elasticity value for (a) July/August and (b) November/December. Only these two elasticity graphs have been displayed because all of the months when there is no reproduction occurring have similar elasticity values for each of the matrix elements. These two months were presented as they had the highest and lowest survival estimates ( $\hat{S} = 0.911$  and  $0.665$ , respectively).  $F_i$  is the reproductive rate for size-class  $i$ ,  $P_i$  is the survival rate for size-class  $i$ , and  $G_i$  is the growth rate from size-class  $i$  to  $j$ . Note: the elasticity value for  $F_2$  is not equal to 0 because of the small backwards growth transition from size-class 2 to size-class 1.



**Figure 4.8** Matrix element elasticity values for January/February.  $F_i$  is the reproductive rate for size-class  $i$ ,  $P_i$  is the survival rate for size-class  $i$ , and  $G_i$  is the growth rate from size-class  $i$  to  $j$ .



**Figure 4.9** Matrix element elasticity values for (a) September/October and (b) January/February.  $F_i$  is the reproductive rate for size-class  $i$ ,  $P_i$  is the survival rate for size-class  $i$ , and  $G_i$  is the growth rate from size-class  $i$  to  $j$ .



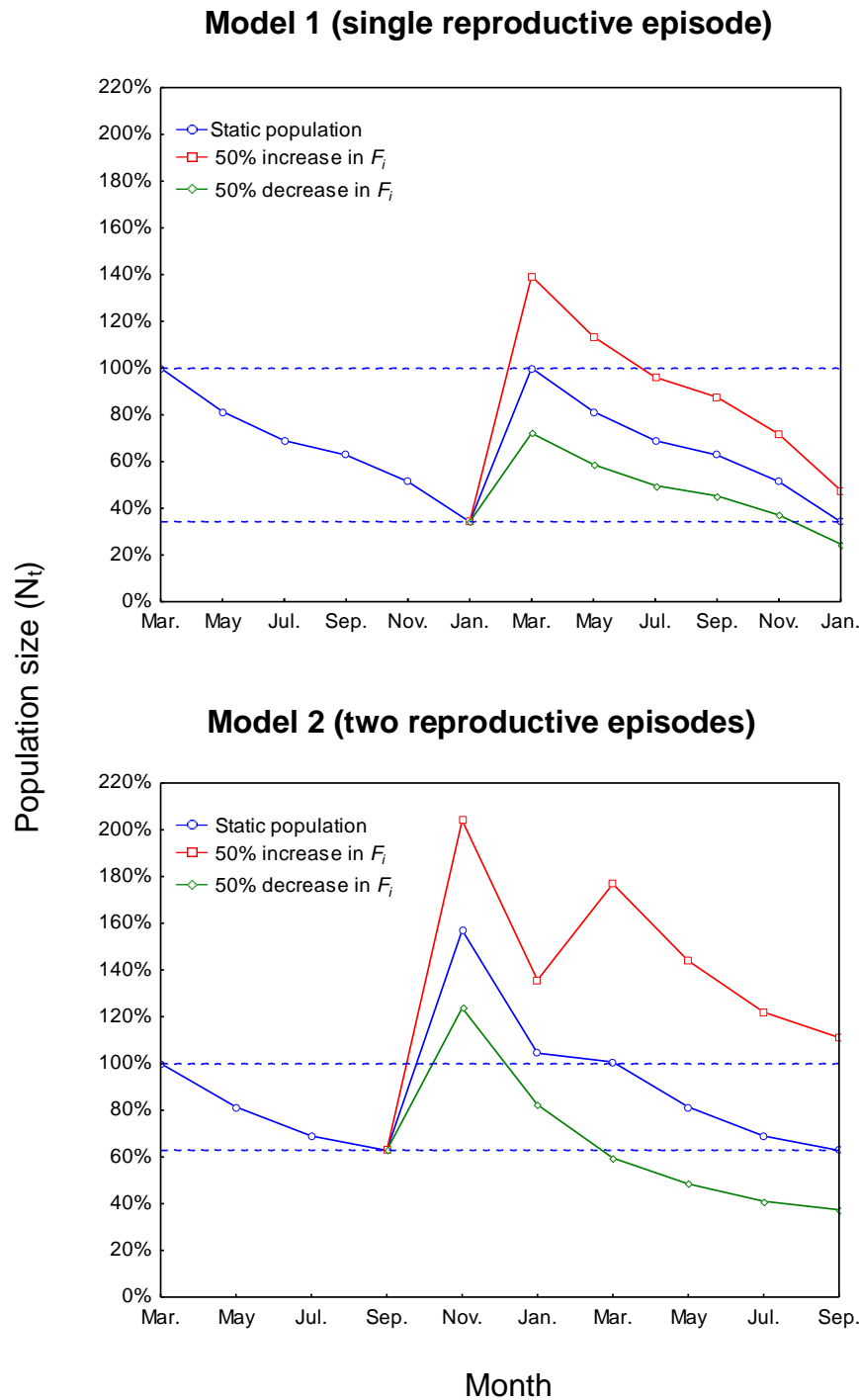
**Figure 4.10:** Total size-class elasticity values for (a) July/August (non-reproductive period) and (b) September/October of the second model (reproductive period). I have chosen to present only for these two months because of the similarities between all non-reproductive and all reproductive months.

### 4.3.3 Simulations

First, I simulated the effects of removing 50% of the three largest size-classes. This was reflected in the matrix model as a 50% decrease in the reproductive contribution,  $F_i$ , of these size-classes and resulted in population increase of  $\lambda_1 = 2.1092$  during the reproductive episode in the first model (N.B.:  $\lambda_1 = 2.9233$  was required to reset the population to 100%). If projected out for the



rest of the year, it results in a net population loss of only about 10% in the single reproductive episode model, and about 20% loss in the second model (Figure 4.11). Alternatively, a 50% increase in  $F_i$  resulted in a 10% net population growth in the first model after a year, and a 50% increase in population for the second model (Figure 4.11).



**Figure 4.11:** Hypothetical population trajectories through time for both models (single reproductive event or two reproductive peaks) that may arise from intensive control of *Styela* (50% decrease in  $F_i$ ) or an improvement in environmental or biological conditions leading to doubled settlement or fecundity (50% increase in  $F_i$ ). The population trajectories of a static population are also shown.

## 4.4 Discussion

Identifying which life history features are most important to the invasion success of a species can assist the development of effective population management strategies. Characteristics including high fecundity, fast growth and tolerances to a wide range of environmental stressors are but a few of the features often possessed by successful invasive species (Ehrlich 1986). While all stages of an invader's life history must be successful for it to persist in a new environment, some stages contribute more to population growth than others. Using elasticity analysis to identify the life-history features that contribute most towards population growth and invasion success for an invasive species can allow managers to target the transitions in the life-history that have particularly large effects on  $\lambda_1$  (McEvoy & Coombs 1999). Once identified, these stages or transitions might be considered a species' "Achilles heel". Unfortunately, some species do not have such a weakness (e.g., Parker 2000). Furthermore, little or no work has been done investigating temporal patterns, or switches, in the importance of life-history stages towards  $\lambda_1$  at shorter time scales (< 1 year). The majority of matrix models produced choose the convenient time-step of a year. This choice can obscure patterns in elasticities at shorter temporal scales (within-year), having important implications from theoretical, biological, and management perspectives.

Analysing annual species such as *Styela* using matrix demographic models is complicated because two time scales must be considered; within-year (including reproduction, growth and mortality) and between-year (changes in population growth and size between different years) (see Caswell 2001). The necessity to describe the year using six bimonthly matrices for *Styela* allowed detailed insight into within-year population dynamics and temporal patterns in elasticity. This revealed a switch from larger individuals being the most important contributors to population growth during non-reproductive periods of the year, to the smaller individuals being far more important at the onset of reproduction.

This temporal switch in importance of the smallest individuals to the biggest complicates answering the question "does *Styela clava* have an Achilles heel (i.e., a vulnerable life history stage or vital rate)?" The answer is "it depends". During the months when *Styela* is reproductively active, the smaller individuals contribute most towards population growth. However, when they are small, *Styela* is quite hard to find due to their cryptic nature and the abundance of other fouling species on most surfaces within a port. If managers tried controlling the population at this time of year, it is likely to have little impact on the population overall as

many individuals will be missed. Thus, this life stage during this time of year could hardly be considered an Achilles heel. However, during the remainder of the year, the importance of the larger individuals is greater, and this is also when they are easiest to find. Therefore, the larger individuals, during non-reproductive months only, might be considered *Styela*'s Achilles heel. Likewise, Schaffelke *et al.* (2005) investigate the size-specific fecundity of *Undaria pinnatifida* in Australia and conclude that management efforts should target larger individuals, potentially reducing management costs.

It can be asked, however, if an intensive management regime targeting larger individuals at the appropriate time would really have an appreciable effect on population growth. Through a simple simulation I attempted to answer this question. The models indicate that removal of 50% of the adult population before reproduction would only result in a net loss to the population after the reproductive event(s) of 10-20%. Similarly, through simulation of a population of the exotic shrub *Cytisus scoparius*, Parker (2000) showed that 99.9% of seeds in prairies, and 70% of seeds in urban populations would have to be destroyed to suppress its invasion. On the contrary, if, for example, biological or environmental conditions improved and the reproductive contribution of each size-class doubled, this could result in a net gain to the population 10-50%. In other words, a large attempt at controlling the population is likely to have a relatively small impact on the overall population size over a few years. Conversely, a small improvement in reproductive contribution could lead to significant population growth in only one year. This increase in reproductive contribution, or propagule pressure, is a likely mechanism for population expansion and further spread in *Styela*.

While these models are based on real data, like all models they should be treated with caution. No matter how carefully it is constructed, a model always leaves things out, and the data for estimated parameters are always imprecise (Shea & Kelly 1998). Regardless of which of the two models is better, and whether or not the parameters are precise, the better model in the end is the one that answers your questions with some degree of confidence. Perturbation analysis (i.e., varying confidence intervals of model parameters) suggested that elasticity estimates were fairly robust to parameter changes, as the relative ranking of matrix element elasticities did not change. Perturbations also suggest that even if some parameters changed further than the maximum or minimum confidence intervals tested here, it is unlikely to greatly affect the overall ranking of elasticities, and hence should not affect conclusions.

*Summary*

This chapter suggests that *Styela* may have an Achilles heel, but that this changes temporally. The best management strategy for this species would likely be one that targets the larger size-classes during the time of year that matters, before reproduction yet when the general population is large enough to detect easily. Nevertheless, the chances of success are slim, relative to the chances of further population expansion.

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## Chapter 5

### Experimental Studies

“If your experiment needs statistics, you ought to have done a better experiment.”

– Ernest Rutherford

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#### 5.1 Introduction

Non-indigenous species (NIS) that establish in new environments can potentially achieve extremely high population densities. These populations can form dense monocultures that may displace native species, reduce species richness, and dominate a site (Sheley & Petroff 1999). For example, in North America, the invasive zebra mussel *Dreissena polymorpha* has achieved densities of up to 750,000 animals per square metre in some power plant pipelines (O’Neill & MacNeill 1991) and are costing each infested plant up to US\$3 million annually (Leung *et al.* 2002). In Prince Edward Island (PEI), Canada, *Styela* has achieved densities of 500-1500 individuals per m<sup>2</sup> in some places (Minchin & Duggan 1988, Osman & Whitlatch 1999), which can accelerate population growth and increase the chances of range expansion by greatly increasing propagule pressure.

In contrast, density-dependent competition among the individuals in a population is common, caused by limited availability of a necessary resource (e.g., a food source, space) (Nybakken & Bertness 2005). At low density, individuals do not interfere with each other, but as density increases, resources may become less available, each individual acquires less of a resource. The population responds with a drop in one or more vital rates, usually growth and survivorship, but also fecundity and recruitment (e.g., Forrester 1995).

Orientation and position of substrata also play important roles in the demographic responses and structuring of sessile marine invertebrate communities (Glasby & Connell 2001). Dark areas such as the undersides of subtidal rocks, corals formations and man-made surfaces often support

communities of sessile invertebrates at higher density and diversity than upward-facing surfaces nearby (Pomerat & Reiner 1942). This pattern has been attributed to larval behaviour because the larvae of most subtidal invertebrates demonstrate negative phototaxis before settlement (Thornson 1964). Alternatively, Young & Chia (1984) suggest that the pattern may be due partly to negative phototaxis of larvae and partly to high post-settlement mortality of juveniles on more exposed substrates.

Transplant experiments are frequently used to test hypotheses concerning density-dependence and spatial variations in species abundance, including orientation and position of substrata, in demographic responses including survival, growth, and recruitment (Keough & Downes 1986, Dalby & Young 1992). For example, Keough & Downes (1986) transplanted the colonial ascidian *Trididemnum opacum* colonies onto clay tiles to test adult mortality and recruitment patterns and found that when transplanted to unprotected open habitats, they were preyed upon intensively, probably by fish and urchins.

Populations of invasive species can also influence the propagule pressure of native species in invaded environments. In some areas of southern Australia, the invasive polychaete *Sabella spallanzanii* reached such high densities that it formed a canopy of feeding fans which strongly influenced recruitment and larval abundance of sessile invertebrates (Holloway & Keough 2002). In contrast, Ross *et al.* (2007) investigated the impacts of *Sabella spallanzanii* and *Styela* on soft sediment assemblages in Port Phillip Bay, Australia, through experimental manipulation of densities and showed their effects on other species were likely to be negligible.

In this chapter, I describe a series of experiments done to examine whether some important demographic parameters in the *Styela* population in Lyttelton Port are density-dependent. If this were the case, it may provide a possible explanation for *Styela*'s limited density in Lyttelton compared to other invaded locations (e.g., Prince Edward Island). Transplant experiments in which the density of *Styela* was modified were used to examine if density affects survival, growth and recruitment of *Styela* and if any of these vital rates differed spatially. I used a clearance experiment to examine whether species surrounding *Styela* on pontoons might be influencing its vital rates through competition for limited resources (e.g., food in the water column, settlement space etc). I also wished to test if other species, specifically *Ciona intestinalis* and *Cnemidocarpa* sp., were recruiting before *Styela*. My experiments were designed to address the following questions:

- (i) Is survival and growth of *Styela clava* density-dependent?
- (ii) Do these demographic responses vary spatially (depth/site)?
- (iii) Is position of substrata important (light/dark)?
- (iv) Do the species surrounding *Styela* affect its survivorship or growth?
- (v) What other species are recruiting before *Styela*?

## 5.2 Materials and Methods

### 5.2.1 Transplant Experiments

#### *Initial Trial*

An initial trial was done to determine if *Styela* could be transplanted successfully using super-glue. The trial consisted of nine individuals attached using Selleys<sup>®</sup> gel super-glue to six small Hardieflex<sup>™</sup> panels. Individuals of varying length were collected at random from vertical and horizontal surfaces at site Z and kept under a moist towel until they could be glued to a panel; the time taken from removal from the pontoon to deployment was < 10 minutes. A scalpel was used to slice away the rough end of the peduncle that was attached to the concrete pontoon face, leaving an even surface to apply glue. Individuals were attached to panels haphazardly and these panels were randomly attached to a larger panel in an array suitable for deployment (Figure 5.1). The array was deployed on 25 November 2008 between two pontoons, orientated vertically, at site Z. This array was checked weekly for 2 months and survivorship of *Styela* was ascertained by visual inspection.



**Figure 5.1:** *Styela clava* transplant trial array. Individuals are super-glued to small Hardieflex<sup>™</sup> panels which are then bolted to a larger panel before being deployed vertically in the water column.



### *Transplant Experiment A*

The first transplant experiment was designed to test the null hypotheses that survivorship and growth rates do not differ with depth (down to the maximum depth of the pontoons of 1.5 m) and density of *Styela*. To examine the generality of the results I replicated the experiment at two sites. This experiment was deployed on 17 March 2009. Individual animals were attached to Hardieflex™ panels using Selleys® gel super-glue using the same methods described above (Figure 5.2). Initial lengths ranged from 53 - 121 mm in total length. The panels were 150 × 150 mm and were attached to 2 m lengths of 75 × 50 mm treated timber using stainless steel fasteners. These arrays were then attached to pontoons at sites A&B and Z using stainless steel fasteners. Within each of the two sites, four arrays of panels were deployed. Each array consisted of 3 panels set at 0.5, 1.0, and 1.5 m depth (Figure 5.3). The two densities of individuals tested were 3 and 6 *Styela* per panel. The design was balanced so that two replicates of each density at each depth per site were deployed. Panels were checked *in situ* monthly until June 2009 for survival and growth of the transplanted individuals. Individuals were recorded as present or absent and lengths (total length, body length, and body diameter) were measured to the nearest millimeter using Vernier calipers.

The difference in presence/absence (assumed to be survivorship) between site, depth, density and through time was analyzed using a generalized linear mixed effects model (GLMER) with time and array as the random effects, specifying binomial distribution (i.e., 1 = alive, 0 = dead), in R (Crawley 2007). The difference in growth increments between treatments and through time was analyzed using a linear mixed effects model (LMER), again with time and array as the random effects (Crawley 2007). Mixed effects models were required for these analyses because different factors were applied at different spatial and temporal scales (i.e., multiple measurements of the response of each experimental unit, within blocks (arrays), through time), in order to test for time-dependence of the response to any treatment that has been applied.

### *Transplant Experiment B*

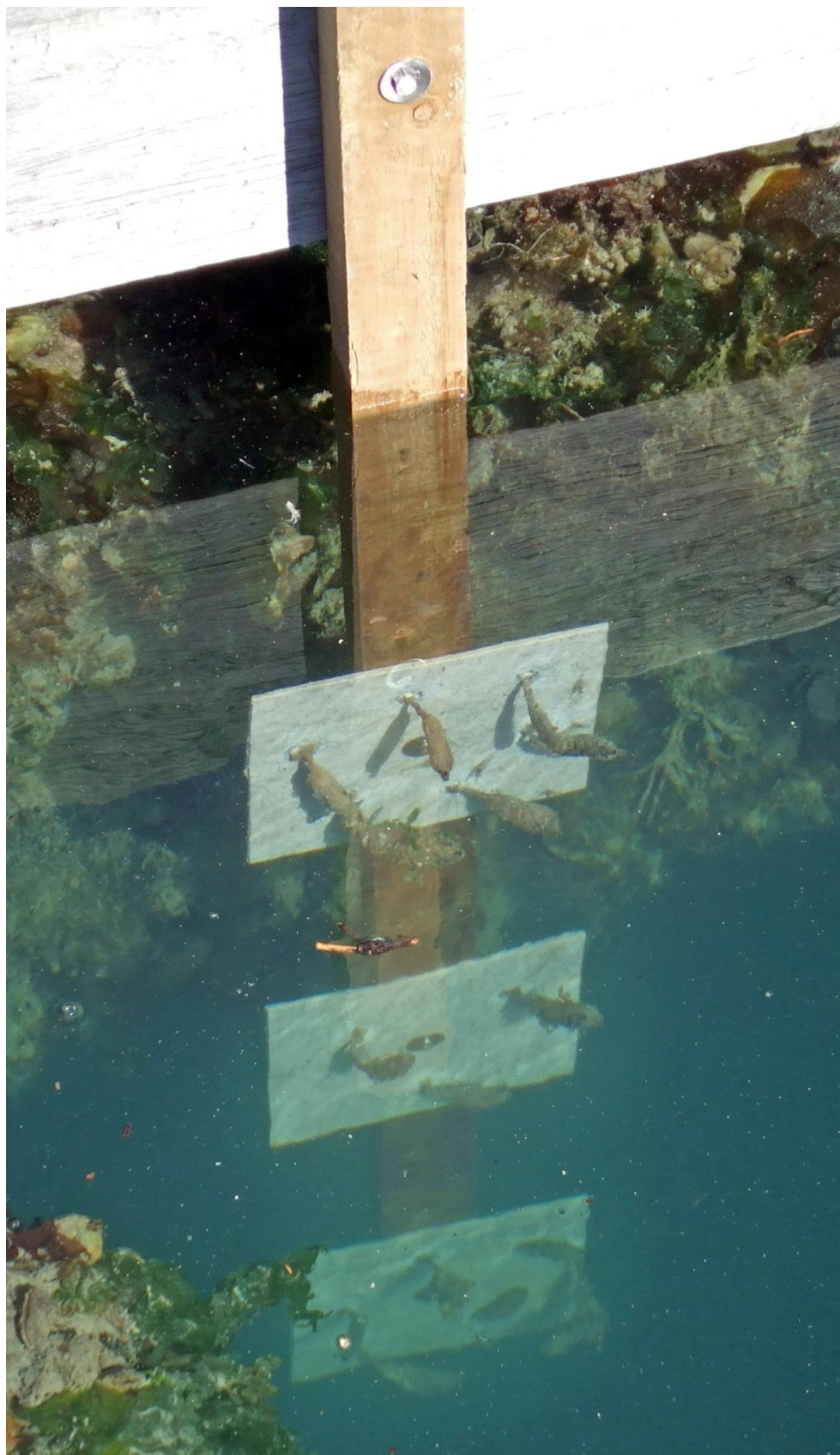
A pattern often observed in ascidians is a preference for floating structures over fixed structures (e.g., pilings) (Connell 2000, Holloway & Connell 2002). Holloway & Connell (2002) suggest one of the reasons for this may be because floating structures are exposed to a higher and more constant light intensity and that this may affect survival of attached ascidians. Therefore, a second transplant experiment was designed to test the null hypotheses that survivorship and growth rates do not differ on surfaces in light and dark areas. The experiment consisted of single

*Styela* individuals attached to 250 × 250 mm Hardieflex™ panels (n = 6 for each treatment). Those in the dark treatment were suspended under pontoons using rope. Those in the light treatment were suspended from the sides of pontoons at the same depth using rope. Initial dive surveys had confirmed that below the pontoons was relatively dark and shaded, while panels suspended from the sides of pontoons were exposed to light. This experiment was deployed on 25 November 2009 at Z wharf pontoons.

The difference in survivorship between treatments (light/dark) and through time (November 2009 to February 2010) was analyzed using a generalized linear mixed effects model with time as the random effect and specifying binomial distribution (i.e., 1 = alive, 0 = dead) in R (Crawley 2007). The difference in growth increments between treatments and through time was analyzed using a linear mixed effects model with time as the random effect (Crawley 2007).



**Figure 5.2:** Six *Styela clava* individuals, transplanted to a Hardieflex™ panel (150 × 150 mm) using super-glue, before deployment. This was part of Transplant Experiment A.



**Figure 5.3:** Deployed *Styela clava* transplants attached to the side of a concrete pontoon at site Z, behind this is the pontoon face. This was part of Transplant Experiment A. This shows an array with a density of six at 0.5 m depth, three at 1.0 m and six again at 1.5m.

### 5.2.2 Clearance Experiments

The clearance experiment was designed to test two different sets of questions. The first part of the clearance experiment aimed to gain insight into the rates and timing of recruitment of *Styela*, and to determine if other commonly occurring ascidian species were recruiting at similar rates and times as *Styela*. Because only one *Styela* ever recruited to clearance plots (Chapter 2), this section focuses on the recruitment of *Ciona intestinalis* (hereafter referred to as *Ciona*) and *Cnemidocarpa* sp. and tests whether they recruit before *Styela*, and if *Styela* may be out-competed for available space. The second part of the clearance experiment examined how clearances around adult *Styela* affect demographic responses (specifically survivorship and growth) through time.

The initial clearance experiment was established on vertical faces of pontoons at site Z during October 2009 and monitored until February 2010, covering *Styela*'s major spawning period in Lyttelton Port (Nutsford, unpublished data). Plots were 0.5 m × 0.5 m and marked using Selleys® Aqua Knead-it putty set against the pontoon face in the corners of each plot so a quadrat could be set over these. Plots were cleared using a hammer to aid removal of the larger hard-bodied organisms (e.g., oysters), then scraped using a metal paint scraper to remove the majority of organisms remaining, and finally scraped using iron wool to remove any smaller organisms. The treatments and their controls were as follows:

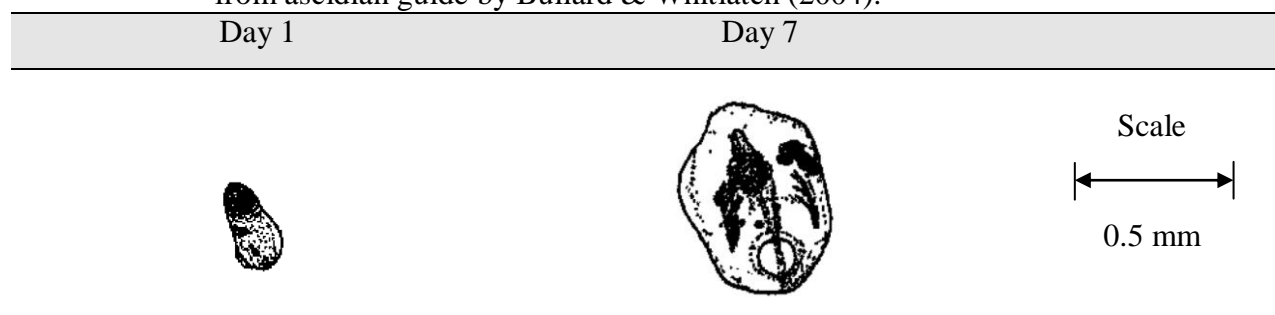
- (i) Clearance plot (*Styela* absent) – all organisms removed from plots (n = 5 replicates).
  - (ii) Clearance plot (*Styela* present) – all fouling organisms apart from *Styela* were removed from plots. Plot location was selected over an area where two *Styela* were already attached to the concrete pontoon face (n = 5 replicates).
  - (iii) Control plot (*Styela* present) – control plots selected over an area where two *Styela* were already attached to the concrete pontoon face (n = 5 replicates).
  - (iv) Control plot (*Styela* absent) – control plots (i.e., no organisms removed) selected over an area containing no *Styela* (n = 5 replicates).
- } Recruitment questions
- } Growth and survival questions

The last set of control plots, (iv), along with the plots described in (iii) above, were initially going to be controls for recruitment questions, but this never came to fruition because high levels of siltation meant searching for recruits in these plots was extremely difficult without disturbing the experiment. Therefore (iv) plots were discarded and the plots described in (iii) were only used to answer growth and survival questions. Within clearance plots and controls, (i) and (ii), recruitment of *Ciona*, and *Cnemidocarpa* sp. was monitored monthly. The guide by Bullard & Whitlatch (2004) was used to help in identification of recently recruited *Ciona* (Table 5.1). Recent recruits of *Cnemidocarpa* sp. were easily identifiable because juveniles looked similar to adult forms. By counting the number of ascidians arriving in clearance plots and controls each month, the recruitment patterns of *Ciona*, and *Cnemidocarpa* sp. were determined.

Recruitment was analyzed between treatments (*Styela* present and absent) and through time using a generalized linear mixed effects model specifying Poisson distribution as the response was count data. Here I tested the null hypothesis that recruitment of *Ciona* and *Cnemidocarpa* sp. is equal in plots with and without *Styela* present. If disproved, and ascidians recruited more into plots with *Styela*, then it may be indicative of some need for structural complexity.

The second part to this experiment involved monitoring the lengths and survival of the *Styela* remaining within clearance plots (ii) and controls (iii), to test the null hypotheses that removal of potential competitors from around *Styela* would not affect demographic responses and therefore that *Styela* adults are not affected by other sessile species. The difference in survivorship between treatments and through time (October 2009 to February 2010) was analyzed using a generalized linear mixed effects model with time as the random effect and specifying binomial distribution (Crawley 2007). The difference in growth increments between treatments and through time was analyzed using a linear mixed effects model with time as the random effect (Crawley 2007).

**Table 5.1:** Drawings of one-day old and seven-day old juvenile *Ciona intestinalis*. Drawings from ascidian guide by Bullard & Whitlatch (2004).



## 5.3 Results

### 5.3.1 Transplants

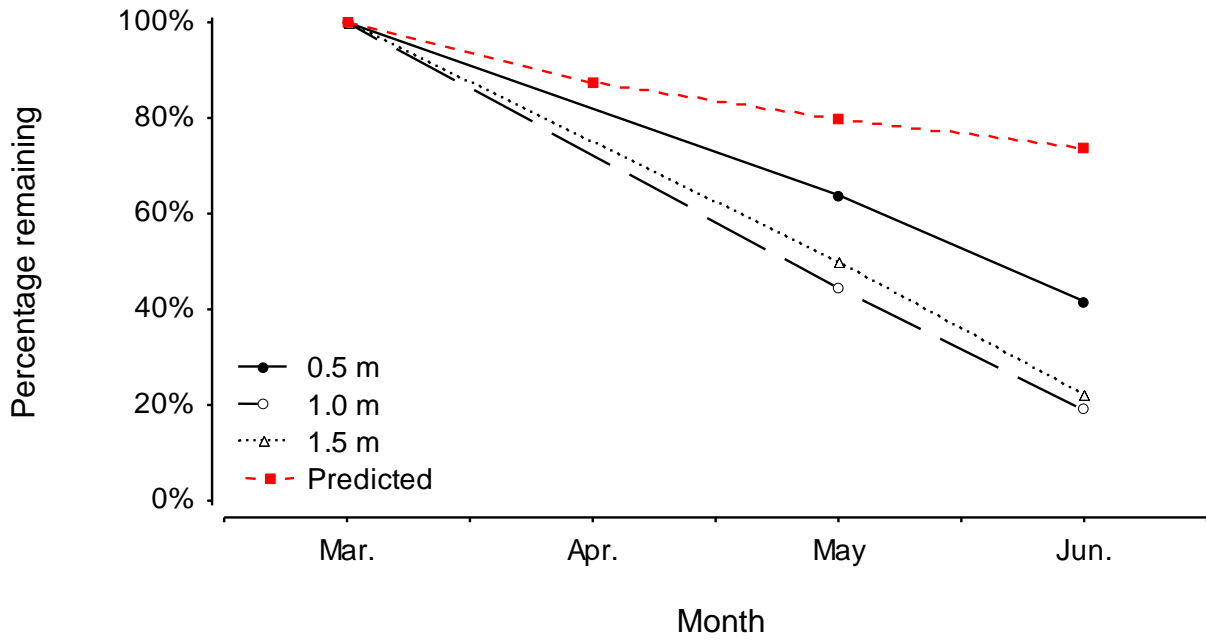
#### *Initial Trial*

Most individuals survived several weeks after gluing and deployment and, therefore, a full transplant experiment was considered to be feasible.

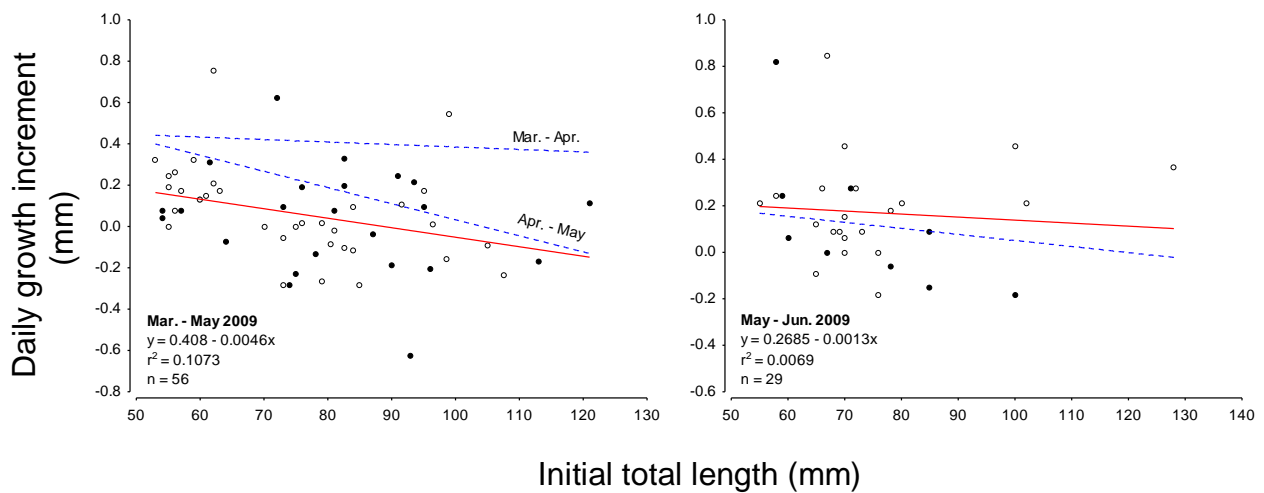
#### *Transplant Experiment A*

Survival of the transplanted individuals was slightly lower across all depths than that predicted by survival estimates produced from mark-recapture data in Chapter 2 (Figure 5.4), but care must be taken in the interpretation of this as the predicted line represents the survival of all sizes across all depths. Nevertheless, the individuals selected for transplanting were from a wide size range and interspersed randomly between depths. Surprisingly over such a narrow depth range, survival between depths was significantly different according to the generalized linear model (GLMER) ( $z_{1,324} = -2.235$ ,  $p = 0.026$ ) (Figure 5.4). While significant, the differences were quite small, likely to be of little biological importance, and could be an artifact of randomly assigning individuals of different lengths between depths. There was no significant difference in survivorship between individuals transplanted at different sites ( $z_{1,324} = -1.396$ ,  $p = 0.163$ ) or different densities ( $z_{1,324} = 0.657$ ,  $p = 0.511$ ). Survival through time was significantly different ( $z_{3,324} = -4.408$ ,  $p = 0.000$ ).

Best-fit lines of initial length against daily growth increment for transplanted individuals were similar to those observed in mark-recapture experiments (Figure 5.5). The general linear model (LMER) revealed that daily growth increments did not differ significantly between site ( $t_{1,87} = 0.791$ ,  $p = 0.574$ ), depths ( $t_{1,87} = 0.383$ ,  $p = 0.767$ ), densities ( $t_{1,87} = 0.791$ ,  $p = 0.444$ ), or months ( $t_{3,87} = 1.244$ ,  $p = 0.431$ ).



**Figure 5.4:** The percentage of transplanted individuals at three different depths (0.5 m, 1.0 m and 1.5 m) remaining over the four months that the first transplant experiment was monitored (November 2009 to February 2010). Also shown is the predicted percentage remaining based on survival estimates of naturally occurring individuals across all depths in the mark-recapture study from Chapter 2.

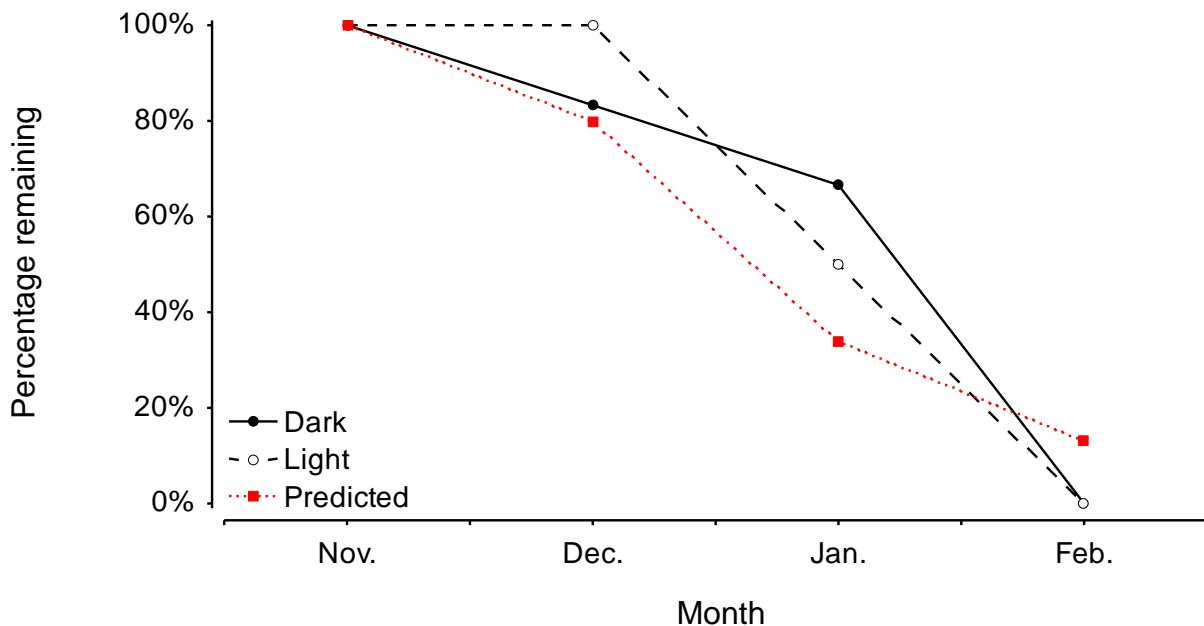


**Figure 5.5:** Scatterplots of daily growth increments versus initial total length for transplanted *Styela clava* individuals at A&B pontoons (solid circles) and Z-wharf pontoons (open circles) across all depths and densities combined. The solid line represents a regression through both sites data combined;  $r^2$  for this line is shown. Sample size ( $n$ ) is also shown. The hatched lines are the regressions from the mark-recapture data from Chapter 2.

*Transplant Experiment B*

Survival did not differ significantly between transplanted individuals in dark and light areas (GLMER yielded  $z_{1,48} = 0.000$ ,  $p = 0.999$ ), but percent survival varied between treatments through time ( $z_{3,48} = -3.183$ ,  $p = 0.001$ ) (Figure 5.6). All individuals had died or fallen off panels by February 2010, three months after the experiment began. This was most likely because of the overwhelming recruitment and growth of *C. intestinalis* that smothered *Styela* (Figure 5.7). When plotted next to the percent remaining predicted by the mark-recapture data (Chapter 2), there is little difference in survival through time (Figure 5.6).

The LMER model showed that daily growth increments were not significantly different in light and dark areas ( $t_{1,18} = -1.600$ ,  $p = 0.356$ ) or between months ( $t_{3,18} = -6.411$ ,  $p = 0.099$ ) (Figure 5.8).



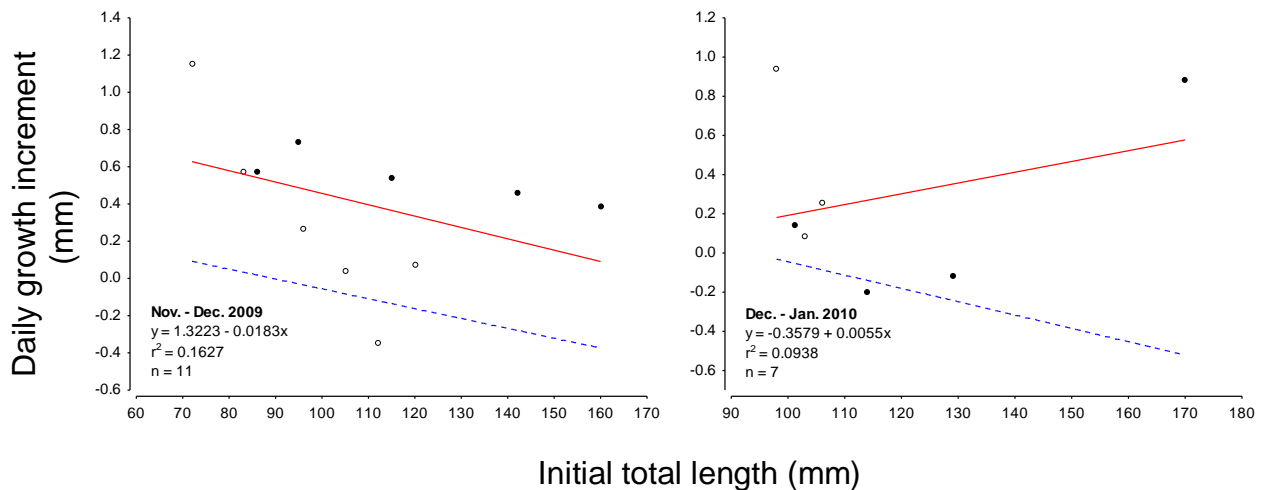
**Figure 5.6:** The percentage of transplanted individuals deployed under pontoons (dark,  $n = 6$ ) and beside pontoons (light,  $n = 6$ ) remaining over the four months that the light/dark experiment was monitored (November 2009 to February 2010). Also shown is the predicted percentage remaining based on survival estimates of naturally occurring individuals in the mark-recapture study from Chapter 2.





**Figure 5.7:**

Transplant experiment panels after three months at liberty. Top row and bottom row panels are dark and light treatment panels, respectively. The translucent-yellow blobs prominent on the dark treatment panels are *Ciona intestinalis*. No *Styela clava* remain on these panels.



**Figure 5.8:** Scatterplots of daily growth increments versus initial total length for *Styela clava* individuals transplanted to dark areas (solid circles) and light areas (open circles). The solid line represents a regression through both sites data collapsed;  $r^2$  for this line is shown. Sample size ( $n$ ) is also shown. The hatched lines are the regressions through the mark-recapture data from Chapter 2.

### 5.3.2 Clearance Experiments

#### *Recruitment into Clearance Plots*

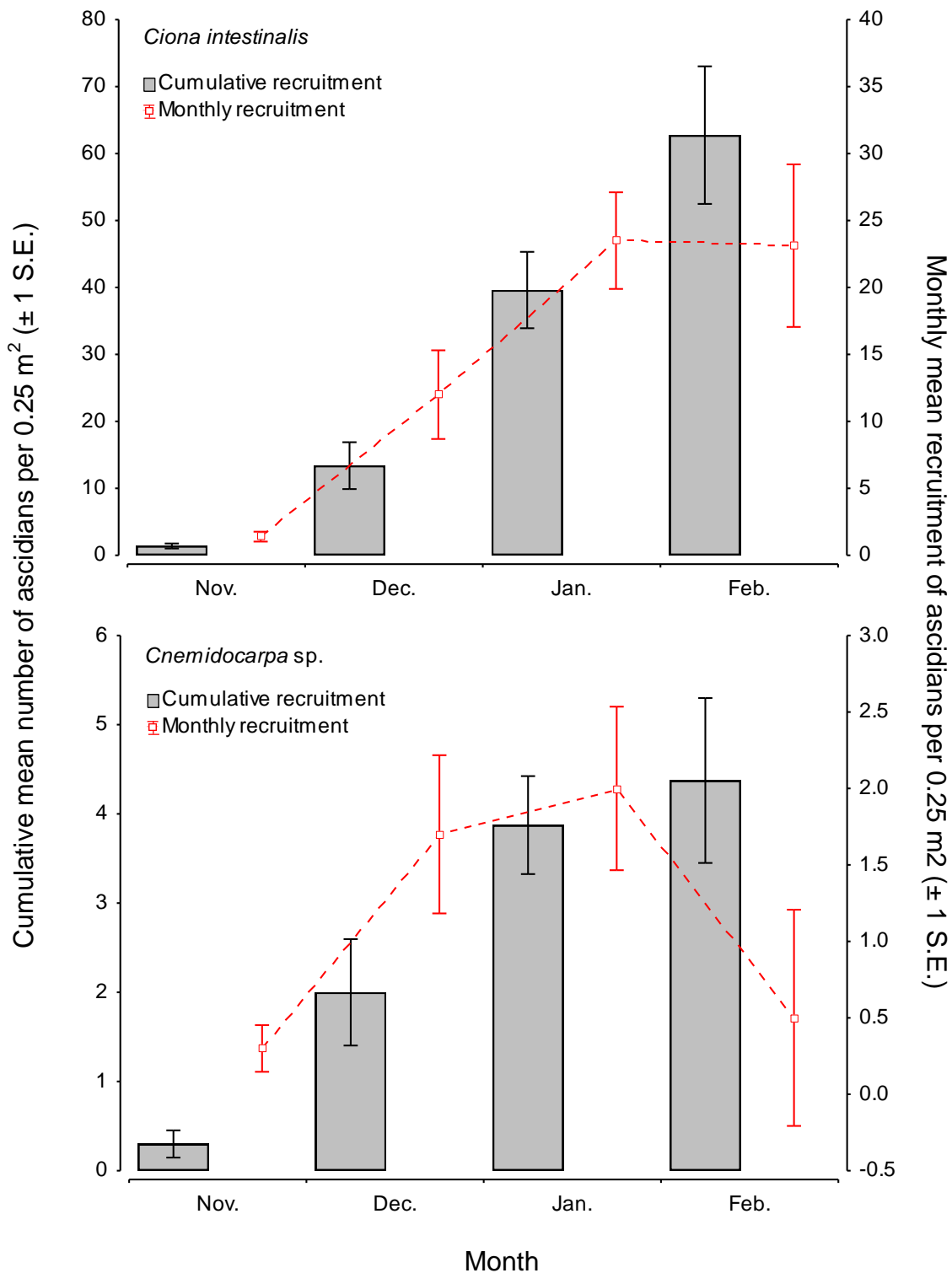
It was initially expected that the control plots (i.e., those plots that had not been cleared of organisms) could also be used for observing levels of recruitment in the three ascidians investigated. However, the high levels of siltation and the difficulty in gathering data for each of these plots made them unfeasible, so they were dropped from the analysis. Although only a single *Styela* recruited into one of the clearance plots during this clearance experiment (Chapter 2), *Ciona intestinalis* and *Cnemidocarpa* sp. recruitment occurred (Figure 5.9).

Recruitment of both *Ciona intestinalis* and *Cnemidocarpa* sp. was not significantly different between plots containing *Styela* and plots without ( $z_{1,36} = -0.948$ ,  $p = 0.343$  and  $z_{1,36} = -1.563$ ,  $p = 0.118$ , for *Ciona intestinalis* and *Cnemidocarpa* sp., respectively). However, recruitment was significantly different through time ( $z_{3,36} = 6.806$ ,  $p < 0.001$  and  $z_{3,36} = 4.276$ ,  $p < 0.001$  for *Ciona intestinalis* and *Cnemidocarpa* sp., respectively). *Ciona intestinalis* was the first species to recruit into clearance plots. Its numbers rose steadily over the four months the clearance plots were monitored. *Cnemidocarpa* sp. recruited later and with fewer numbers than *Ciona intestinalis* (Figure 5.9).

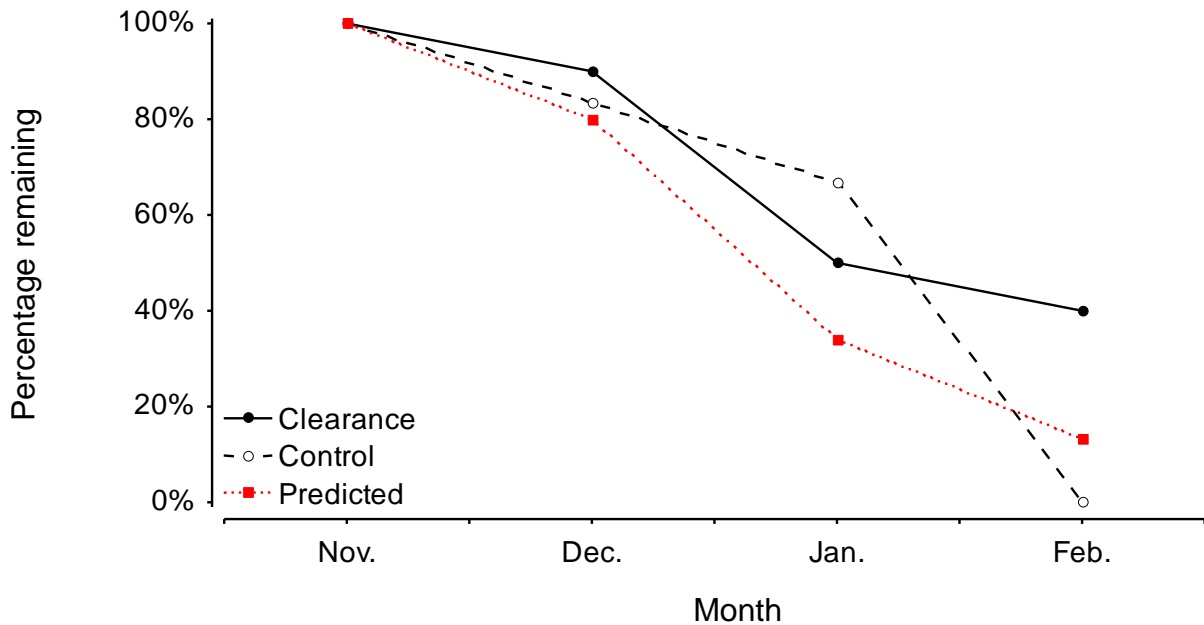
#### *Mortality and Growth of Styela in Clearance Plots and Controls*

Survival of the individuals remaining in clearance and control plots was not significantly different between treatments ( $z_{1,64} = -0.792$ ,  $p = 0.428$ ), but was significantly different through time ( $z_{3,64} = -4.002$ ,  $p < 0.001$ ) (Figure 5.10). The expectation was that control and predicted plots should be the same, because the treatments were identical. The difference between these two lines illustrates the variable nature of survival rates.

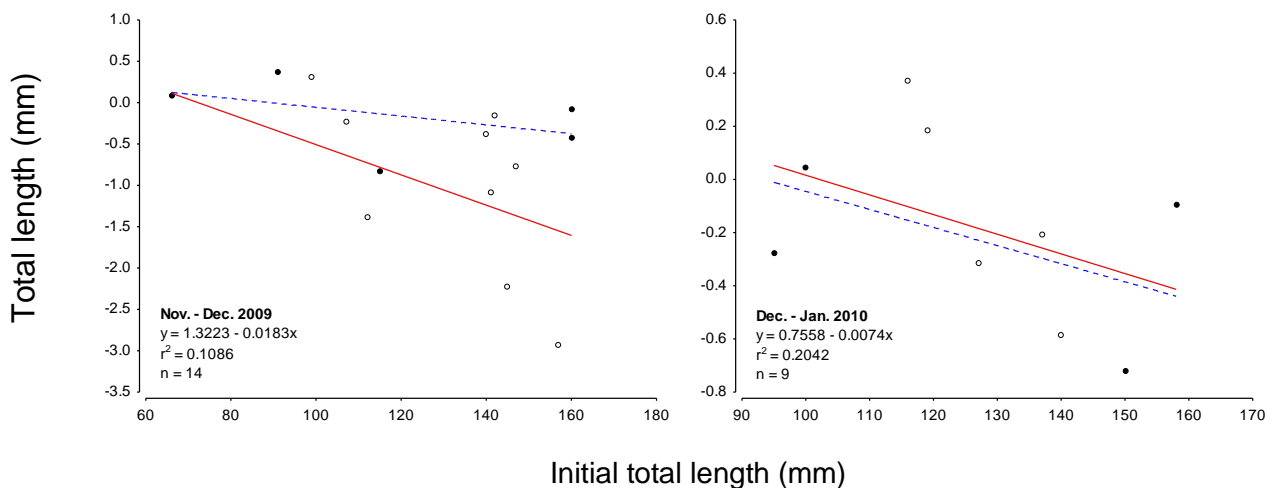
Negative growth (shrinkage) occurred in most individuals in treatment and control plots (Figure 5.11). According to LMER, daily growth increments were not significantly different between treatments ( $t_{1,23} = 1.261$ ,  $p = 0.427$ ) or months ( $t_{3,23} = 0.777$ ,  $p = 0.580$ ).



**Figure 5.9:** Monthly mean recruitment rate ( $\pm 1$  S.E.) and cumulative mean ( $\pm 1$  S.E.) number of *Ciona intestinalis* and *Cnemidocarpa* sp. per 0.25 m<sup>2</sup> in clearance plots at Z pontoons from November 2009 to February 2010, across all depths (maximum depth of 2 m). Plots were cleared October 2009 (n = 5).



**Figure 5.10:** The percentage of individuals remaining in the clearance plots and control plots over the four months that the clearances were monitored (November 2009 to February 2010). Also shown is the predicted percentage remaining based on survival estimates of naturally occurring individuals in the mark-recapture study from Chapter 2.



**Figure 5.11:** Scatterplots of daily growth increments versus initial total length of *Styela clava* individuals in clearance plots (open circles) and control plots (closed circles). The solid line represents a regression through these growth increments; equation and  $r^2$  for this line is shown. Sample size (n) is also shown. The hatched lines are the regressions through growth increment data from the mark-recapture data from Chapter 2.

## 5.4 Discussion

Transplanting solitary ascidians like *Styela* using super-glue was a viable approach for testing demographic responses. While there was some inconsistency between vital rates (survivorship and growth rates) of transplanted individuals and those individuals sampled in the mark-recapture work (Chapters 2 and 3), these differences were quite small. Ascidians have been successfully transplanted in other studies using monofilament fishing line to lash them to substrates (Young 1985), and by settling them on hard substrates first then moving them around (Young & Chia 1984). Dalby & Young (1992) also used glue to transplant *Styela plicata*. They found that while some individuals fell off because of glue dissolution, the method was reasonably successful. Few individuals actually fell off my panels, those that senesced generally left behind a “husk” or part of its tunic (Figure 2.2, Chapter 2) and so it was easy to tell whether an individual died or fell off.

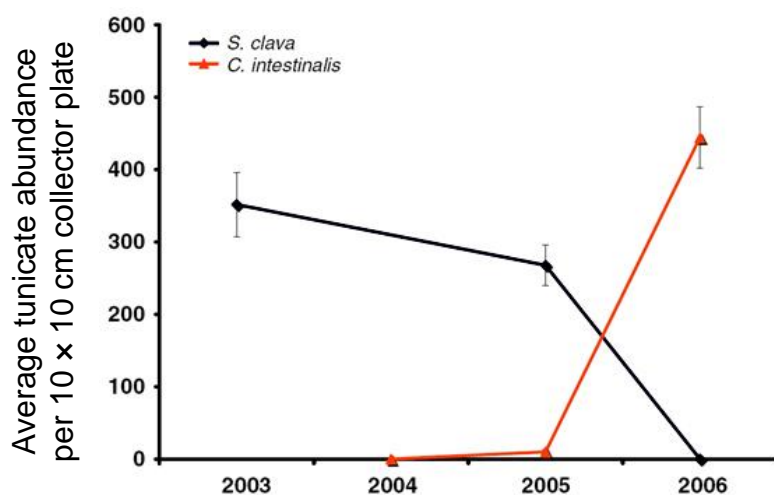
The two transplant experiments aimed to test hypotheses on density-dependence and spatial variation in vital rates and recruitment of *Styela*; and the effects of habitat in light and dark areas, on vital rates of transplanted individuals. In this study, increased animal density did not affect vital rates in *Styela*. This is hardly surprising given the densities tested (3 and 6 individuals per  $150 \times 150$  mm panel), and that *Styela* has been recorded, in some places, at densities of over 100 per  $m^2$  (Holmes 1976, Lützen 1999, Minchin & Duggan 1988, Osman & Whitlatch 1999). I was not able to achieve such high densities in my experiments because of the relatively low densities at which *Styela* occurs throughout the port (approximately 1 per  $m^2$ ) and my limitation in accessing individuals using SCUBA. However, I achieved a considerable sample size of 108 individuals in my experiment. Although this experiment showed no differences at the densities tested, density-dependence may well become important at higher densities. While demographic responses did not differ between sites in this experiment, survival did differ between depths. However, the signals were weak and could potentially be due to the chance sizes of individuals transplanted at different depths (i.e., an experimental artifact). Survivorship and growth of transplanted individuals on artificial substrata deployed in light and dark areas did not differ between treatments or months. However, after just three months at liberty, panels were overwhelmed by massive recruitment of *Ciona*, particularly those panels in dark areas, to the extent that they smothered the *Styela* transplants. This suggests that *Ciona* has the ability to smother *Styela*, and that *Ciona* seems to be keying into darker areas.

While individuals monitored in the clearance experiment did display some differences in growth after 1 month compared to those seen in the mark-recapture work of Chapter 2, these responses were no different the following month suggesting that there was some initial shock to the removal of surrounding organisms. Survivorship of the individuals within clearance plots was analogous to those individuals from mark-recapture work (Chapter 2).

It is recognized that residents within any community can affect the recruitment dynamics of their own and other species by preying on settling larvae, removing or adding space for larvae to colonize and/or stimulating or prohibiting larval settlement on available substratum nearby (Osman & Whitlatch 1995). *Styela* grows higher in the water column than many other sessile species in Lyttelton Port, which may offer an advantage in filtering food and preying on larvae of potential competitors (Lützen 1999). Osman *et al.* (1989) showed that *Styela* is capable of greatly reducing the local settlement rate of oysters by feeding on their planktonic larvae. Furthermore, the presence of *Styela* reduced the settlement rates of most species in a study by Osman & Whitlatch (1999). However, the densities observed at Lyttelton Port are unlikely to have much impact on the recruitment rates of other species, especially the other ascidians, such as *Ciona* and *Cnemidocarpa* sp., which occur in such high numbers. Indeed, in my experiments the most successful recruiters were *Ciona* and *Cnemidocarpa* sp., rather than *Styela*. This pattern was also observed by Nutsford (unpublished data), who used a variety of surfaces to investigate recruitment of *Styela* and other ascidians in Lyttelton Port. NIWA also monitored recruitment of a variety of species within the port over 8 months and again, no *Styela* recruited (Floerl, personal communication). The difference in densities between *Ciona* and *Cnemidocarpa* sp. (Chapter 2) is most likely reflected in the number and density of propagules that each produce. It may well be that these other species out-compete *Styela*.

As a result of numerous successful invasions by different species, we are now observing overlapping distributions in invasive species (Leppäkoski *et al.* 2002, Armistead *et al.* 2009). This may lead to several different scenarios. In some cases, interactions between NIS may accelerate the rate at which they establish and spread, a paradigm that has been coined an “invasional meltdown” by Simberloff & von Holle (1999). This occurs via some facilitative interaction between a pair or group of species, specifically mutualisms between plants and animals that disperse or pollinate them or modification of habitat by animals or plants. For example, the zebra mussel *Dreissena polymorpha* enhanced populations of the invasive Eurasian faucet snail *Bithynia tenaculata* on the Saint Lawrence River by increasing surface area and

spatial heterogeneity, providing refuge and trapping sediments and biodeposits on hard substrates (Ricciardi *et al.* 1997, Simberloff & von Holle 1999). Floerl *et al.* (2004) show that the introduced bryozoan *Watersipora subtorquata* can facilitate the transport of species on ship hulls in Queensland, Australia. *W. subtorquata* is tolerant to several antifouling biocides, and thus can “piggy-back” species that could otherwise not settle on treated hulls (Floerl *et al.* 2004). Conversely, if two NIS are competing for the same resource, one species can reduce the abundance or population size of the other. For example, in PEI Ramsay *et al.* (2008) observed patterns of settlement of *Styela clava* and *Ciona* on collector plates (10 × 10 cm PVC) from 2003 to 2006. *Styela* was the first of the two to arrive in PEI in 1997 (Locke *et al.* 2007) and remained the only exotic nuisance tunicate until 2003 when *Ciona* was reported in low abundance. Following this, the abundance of *Ciona* rapidly increased; while the abundance of *Styela* declined considerably (Ramsay *et al.* 2008, Figure 5.12). This is a form of competitive interference between the two invasive ascidians (Nybakken & Bertness 2005).



**Figure 5.12:** Mean abundance of *Styela clava* and *Ciona intestinalis* from 2003 to 2006 in the Brundenell estuary, Prince Edward Island, Canada (figure from Ramsey *et al.* 2008).

*Ciona* was first recorded in New Zealand in about 1948, in Lyttelton Harbour (Brewin 1950). Like *Styela*, it is a sessile, solitary sea squirt that lives mainly on hard substrates including man-made structures such as pilings and floating concrete pontoons. The work of Ramsey *et al.* (2008) suggests it is highly likely that *Ciona* is a competitive dominant. Indeed, this study has shown that it is much more abundant on concrete pontoons in Lyttelton Port than its counterpart, *Styela*. Furthermore, *Ciona* appears to be far more proficient at recruiting to available bare space than *Styela*. *Ciona* might not just be over-growing *Styela*, it may well be filter-feeding eggs and larvae from the water column before they have a chance to settle. Such high densities could also be sequestering available space, essentially out-competing *Styela* for space and inhibiting its

ability to gain traction. However, while bare space was provided in the clearance experiment, only one *Styela* settled. This suggests that space is not the limiting factor for *Styela*. The hypothesis that *Ciona* is out-competing *Styela* in Lyttelton port is only speculative and further work would be necessary to determine whether this is actually the case.

### *Summary*

Free space does not seem to be the limiting factor in *Styela*'s apparent lack of population growth in Lyttelton Port. The presence of *Ciona intestinalis* or the well developed fouling community may be inhibiting *Styela*'s capabilities as a highly successful invader, perhaps by out-competing it for some other limiting resource such as food. Although untested, the high abundance of other ascidians may be affecting the number of *Styela* gametes in the water column.



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## Chapter 6

### General Discussion

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#### 6.1 Overview

This study investigated demographic characteristics of the invasive tunicate *Styela clava* in Lyttelton Port with the aim of relating these to its ability to expand its introduced populations. *Styela* is a notorious invader that has spread from its native range in Siberia, Japan, Korea and China to temperate marine areas worldwide. In its wake, *Styela* has caused problems for aquaculture by fouling farmed shellfish and associated infrastructure (Bourque *et al.* 2005, Colautti *et al.* 2006a). The majority of research on *Styela* to date has focused on documenting its distribution and spread (e.g., Davis & Davis 2005, Minchen *et al.* 2006, Davis & Davis 2007, Krone 2007, Nunn & Minchen 2009). A major pattern of spread exhibited by *Styela* is the localised expansion of populations once it arrives in a new area and, at times, its exponential population growth to very high densities. *Styela*'s establishment in new areas is undoubtedly aided by man-made surfaces, such as pier pilings, pontoons, and other harbour infrastructure, that are common in ports and marinas worldwide and structures that support other commercial activities such as aquaculture (Holloway & Keough 2002, Lambert & Lambert 2003, Thornber *et al.* 2004). Locke *et al.* (2007) suggest that the provision of such artificial structures is most likely the key factor in the successful establishment of non-indigenous tunicates including *Styela* in Prince Edward Island. Artificial habitats not only facilitate incursion but they also may serve as an epicenter for further spread (Bulleri & Airoidi 2005, Floerl *et al.* 2009). Thus, it may well be the case that *Styela* essentially "leap frogs" from place-to-place on artificial surfaces along stretches of coastline or within estuaries, with each new population or location acting as a stepping stone to spread further. However, underpinning population expansion is the requirement that the founding individuals grow, survive and reproduce, gaining local traction and building propagule pressure. Therefore, *Styela*'s invasion success results from a complex interplay between its demography, life history, artificial structures, natural habitats and ecological factors.

From an evolutionary perspective, native species should have inherent advantages over non-indigenous species (NIS) because they should be better adapted to local conditions (Byers 2002b). However, man-made substrata have been introduced to native ecosystems only relatively recently, in an evolutionary sense. Tyrrell & Byers (2007) suggest that artificial surfaces may grant a more “level playing field” for NIS in new environments by providing a novel context for competitive interactions. Regardless, NIS excel in establishing on artificial substrata and thus have a place to gain a foothold in new environments. In New Zealand, *Styela* has also keyed into man-made surfaces, particularly floating structures such as concrete pontoons. This pattern has been shown in tunicates before (Connell 2000, Holloway & Connell 2002). Suggested reasons for this preference of floating structures over fixed structures (e.g., pilings) include greater and more constant light intensity, recruitment by depth-stratified larvae (Holloway & Connell 2002), and availability in areas frequented by primary vectors such as ships. Although my study in a port showed that natural light levels did not affect growth and mortality of transplanted adult *Styela* individuals, light is known to affect larval behavior and the larvae of most subtidal invertebrates demonstrate negative phototaxis before settlement (Thornson 1964). Locke *et al.* (2007) also speculate that perhaps floating structures are more difficult for benthic predators to access, although little evidence exists supporting this contention. The following discussion aims to identify which characteristics *Styela clava* possess that have contributed to its success as an invasive species, and to assess these in the context of potential expansion from source populations.

## 6.2 Demography and invasiveness

Following establishment in a new environment, a NIS must increase its population size and expand its range if it is to become invasive. Propagule pressure is required for both to occur, yet there are several ways for invasive species to achieve critical propagule densities. One is through massive reproductive output by a few individuals; another is by having relatively large seed populations that together release large numbers of propagules. *Styela* is one of the latter species. It has clearly been vectored internationally by shipping, but through demographic means and transport on man-made structures it is able to vector domestically. In Prince Edward Island it became enormously abundant on aquaculture structures and can entirely overgrow mussel socks and gear (Arsenault *et al.* 2009). From these populations it has then been able to spread rapidly among facilities and along the coastline. In New Zealand, populations have established

themselves in several locations throughout the country, but none of these populations has yet reached the densities observed elsewhere.

In the early stages of species invasion, demographic characteristics of the individuals within the population are of critical importance. For instance, consider the implications of determining how fast a species grows and its length-at-maturity. Using some well-established mathematical techniques, age-at-length can be derived (e.g., Schnute & Fournier 1980, Savard *et al.* 1994, Herrmann *et al.* 2009) and, therefore, so can age-to-maturity. The faster a species reaches maturity, the sooner it can begin contributing towards population growth. As well, by measuring rates of mortality, maximum age and average age can be derived (e.g., Harriott 1985), enabling an assessment of whether there is one or several reproductive episodes. By measuring size-specific gamete output and amalgamating the above parameters, finite population growth (e.g., Caswell 2001, Dudas *et al.* 2007) and potential rates of spread can be calculated (e.g., Neubert & Parker 2004, Jongejans *et al.* 2008, Shea *et al.* 2010). Thus, through a more complete knowledge of an organism's demography, we can begin to understand the underlying mechanisms and numbers behind invasion success and use these to derive adaptive management strategies.

My study demonstrated that growth rates were highly variable between individual ascidians, particularly during the colder months. This resulted in considerable plasticity in the length-at-age of this species. Nevertheless, individuals had the capacity to grow rapidly and reach maturity in less than 2 months after their presumed settlement date. For example, individuals that settled in mid-Spring could reach 40-60 mm by February, at which time most had reached sexual maturity. Consequently, these individuals had the capacity to reproduce early within their first year, and some may have survived to reproduce a year later. This has great implications for propagule production and is a likely key attribute contributing towards *Styela*'s invasion success. For example, depending on how many large individuals reproduce over two seasons, there is a potential to double reproductive output of a population before all initial individuals die.

My study also shows that the populations of *Styela* in Lyttelton Port are most likely annual, with < 5% of individuals living slightly longer than 1 year. This was unexpected, given that individuals in populations studied elsewhere appear to live longer. In Limfjord, Denmark, the maximum age was 1.75 – 2 years (Lützen 1999) and along the coasts of Southampton, on the south coast of England, *Styela* lived for up to 15 months (Holmes 1969). This suggests that there is no physiological constraint causing them to senesce after a year. Variability of this nature

usually reflects habitat quality, but genetic differences among overseas source populations cannot be ruled out (Goldstien *et al.* 2010). Other studies have shown considerable mortality, especially of smaller individuals, throughout winter (Lützen 1999). In contrast, my study showed the highest survival over the winter months and the greatest mortality during summer, coinciding more or less with the onset of reproduction. Potentially, unfavourable conditions within the port coupled with reproduction could be placing excess stress on individuals and the majority of individuals in the populations simply may not be able to endure this.

Harbours and marinas are exposed to frequent anthropogenic disturbance, which is likely to alter water circulation and sedimentation patterns. These areas will undoubtedly accumulate pollution such as agricultural and industrial wastewater, and urban run-off containing both organic and inorganic contaminants. Experimental work by Johnston & Keough (2002) subjecting hard-substrate assemblages to copper pollution showed that a single pollution event could affect the assemblage for at least 8 weeks after it occurred. In New Zealand, experiments showed that ascidian-dominated assemblages were significantly altered after 3 to 6 months when exposed to a gradient of contaminant levels (Turner *et al.* 1997). Ports, therefore, may not be ideal environments for many invasive species. They may simply be literally the first port of call for a new species when it arrives. However, in most cases little is known about port and marina habitats, including food supply population dynamics, larval survival and settlement success. In contrast, the spread of species such as *Styela* to aquaculture areas, offer suitable artificial habitats, good water quality and dynamic flow regimes, and food, which are requirements of commercial production, especially for filter-feeders. Coincidentally, these may well be the ideal environment for filter-feeding ascidians such as *Styela* (Jiang *et al.* 2008).

### 6.3 Demographic Model Approach

The approach I used in my study provided good demographic data that highlight some of the features of *Styela* that contribute to its ability to invade new areas. My goal was to use these data in an appropriate model-based approach. There is a long history of this sort of approach, not least of which is in all forms of fisheries management, in which models are a fundamental tool. Fisheries models are used extensively to identify the characteristics of fish species that influence population growth, including growth rate, egg production, recruitment and mortality (Ricker 1958). This allows an assessment to be made of the effects of different exploitation levels and, when they work, management strategies for sustainability (e.g., Beverton & Holt 1957, Ricker 1958, Berkeley *et al.* 2004, Anderson *et al.* 2008). All such models are based on a combination of empirical data and derived attributes because a full knowledge of all parameters is virtually impossible to attain (Cadrin *et al.* 2005). Furthermore, all projections from such models are really “forecasts”, based on a series of assumptions and calculations, and so are usually provided as probabilistic outcomes. The hallmarks of my study are similar in kind. Unlike fisheries, however, with the goal of sustainability, the goal of invasion biology is quite the opposite. Invasive species management aims to control or eradicate the species in question. Despite this, the underlying principals are the same in determining which characteristics of organisms are important for invasion success and adaptively targeting the species weaknesses.

The matrix demographic model done in this study showed that larger individuals in the populations contributed most towards population growth because of their fast growth, high potential reproductive output and their over-representation in the population. However, simulation showed that although eradication efforts could target these individuals, removal would need to be both time-specific and substantial, so that the majority of the population is removed before reproduction. This would not be trivial because smaller individuals are difficult or impossible to detect, and larger individuals can be numerous and widely spread. Eradication would, therefore, require a robust sampling design that detects the majority of individuals and results in few missed individuals. Because Lyttelton Port, like many ports and marinas, is characterized by low visibility and high levels of siltation coating the fouling communities, cryptic species such as *Styela* will never be easy to detect across their size distribution. Invasive populations are often sparse, spatially and temporally patchy in their distribution, and tend to be aggregated; hence, they can easily be missed by traditional simple random or stratified sampling designs (Inglis *et al.* 2006b). In PEI, for example, Canary *et al.* (2010) assessed the effectiveness of divers searching for the invasive tunicate *Ciona intestinalis* on mussel longlines using

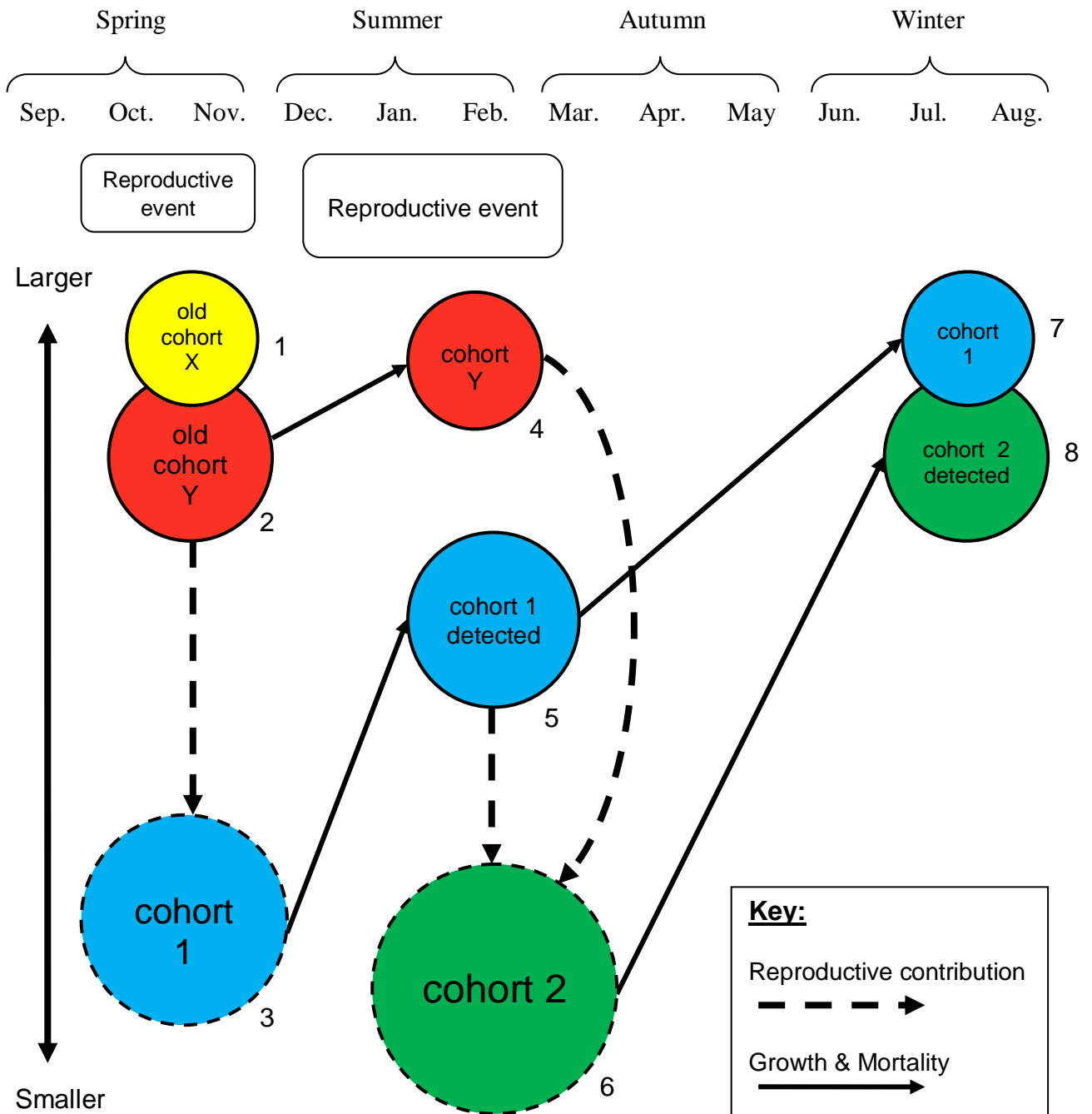
artificial tunicates (small white balloons) arranged separately or in clusters of three. Their experiment showed that divers detected about 80% of single decoys and 94% of clusters in water visibility of about 2.75 m. Even in these relatively propitious circumstances, this level of detection is probably insufficient to allow effective eradication of such a reproductively prolific species. This level of detection would be impossible to achieve in ports and harbours, such as those occupied by *Styela* in New Zealand. The results of my modelling, therefore, indicate that eradication of this species would be highly difficult, if not impossible.

Eradication efforts have succeeded for some species. For example, a small localized population of the green alga *Caulerpa taxifolia* was successfully eradicated in California (Anderson & Keppner 2001), at huge expense (Anderson 2005, also see [www.sccat.net](http://www.sccat.net), date of access 29/6/2010). My models show, however, that despite the fact that the study populations do not seem to have grown much in Lyttleton Port in the last few years, it is unlikely to die off and may well have spread to areas outside the port. In fact, there are recent reports of its presence in the wider confines of Lyttleton Harbour (Gust *et al.* 2008, personal observation). Unfortunately, therefore, a combination of *Styela*'s cryptic nature, especially when small, rapid growth from settlement to maturity, hermaphroditic nature, and reproductive output will help ensure its eventual spread.

## 6.4 Status of the Lyttelton Population

The process model below (Figure 6.1) encapsulates and summarizes the conclusions of this study in terms of population dynamics. This is shown in terms of the life cycle of *Styela clava* in the Lyttelton Port populations. The model includes three major assumptions, based on empirical data and figures derived from my modelling work: (1) That reproductive episodes occur around October and again from late December to February, in accordance with data collected by Nutsford (unpublished data); (2) individuals in the population are annual; and (3) the growth rates outlined in Chapter 3 are appropriate. The model illustrates how the two major reproductive episodes may produce two cohorts of individuals within a year, when these cohorts are large enough to be detected, and when each of the cohorts reproduces. The following summary relates to the numbers on Figure 6.1.

1. Old cohort X – the older and presumably larger cohort, born between 2 and 4 months before cohort Y. The individuals within the cohort will likely die off in the next few months.
2. Old cohort Y – the second cohort, born of the previous year.
3. Cohort 1 – the new cohort produced during the reproductive episode in October, consisting of the reproductive contribution of cohort X and cohort Y.
4. Cohort Y – after 3 months, many of the individuals within cohort Y have senesced, but some may still remain. The individuals within the cohort will likely die off in the next few months.
5. Cohort 1 – 3 months later, this cohort has reached a size that can be detected by diver survey. Many of the individuals within this cohort will already be mature.
6. Cohort 2 – the new cohort produced during the reproductive episode in late-December to February, consisting of the reproductive contribution of the individuals that remain in cohort Y and the new cohort born a few months ago.
7. Cohort 1 – the following season, is now acting as cohort X.
8. Cohort 2 – the following season, this cohort can now be detected by diver survey, is now acting as cohort Y.



**Figure 6.1:** Proposed life cycle of *Styela clava* within Lyttelton Port. The circles with solid outlines represent the detectable portion of the population at any given time. The circles with dashed outlines represent portions of the population that are not detected because of their small size, but must be present given the population dynamics observed in this study. The sizes of circles represent the relative proportion of the population within each of the cohorts at any given time (not drawn to scale). The original population is made up of two cohorts that were born the previous year (old cohort X and Y), similar to the new population that is made up of the two cohorts produced in the model. The scale on the right (larger and smaller) pertains to the relative size of the individuals within each of the cohorts. The numbering of each component is referred to in the text.



Given this life cycle relating to the results of my study, the status of the Lyttelton Port can be compared the work of Gust *et al.* (2008). My work shows that the population in Lyttelton port may be static or in decline. This is somewhat surprising given the rapid expansion of *Styela* elsewhere (e.g., Ramsay *et al.* 2008). It is not unusual for invasive species to establish themselves in an area and then die out. In fact, this may be a common fate of species that arrive to a new area but do not spread (Williamson 1996). Several hypotheses may account for *Styela*'s apparent lack of population growth, a few of these being: (a) the population may be in a lag-phase before population expansion; (b) the populations studied may be dying out at the spatial/temporal scale investigated; (c) my sampling regime was only a snapshot of the population in time and the years of the study were not favourable to *Styela* expansion. In the following part of the discussion, the first two of these will be considered.

Some invasive populations expand rapidly; others have a lag-phase before population expansion (Kowarik 1995, Crooks & Soulé 1999). Crooks & Soulé (1999) propose three reasons for lags in population expansion of invasive species: inherent, environmental and genetic. Inherent lags are those caused by the life history features of a species, such as specific settlement cues or reproductive requirements that prevent or delay its spread. Environmental lags occur when some environmental variable suppresses population growth, potentially affecting population densities and sufficient numbers of propagules to expand. Subsequent environmental change may provide suitable windows of opportunity for spread by positively affecting habitat and food resources, dispersal vectors, and interspecific and intraspecific interactions (Crooks & Soulé 1999), resulting in population growth and expansion. Genetic factors, related to the lack of fitness of the invader in the new environment, can suppress a population until such time as the population is given a genetic boost by further incursions. For example, the European green crab *Carcinus maenas* was first recorded in New York, USA in 1817 (Say 1817), yet took almost 100 years to increase its range to the Gulf of Maine (Roman 2006). However, following its spread to the Gulf, its range expansion rapidly accelerated and populations have exploded in eastern Canadian waters. Roman (2006) suggests that this acceleration of range expansion may be due to "cryptic" incursions of the species introducing genetic variation and resulting in a dilution of founder effects.

A genetic study of *Styela* in Lyttelton Port by Goldstien *et al.* (2010) indicates that multiple incursions of the species probably occurred. In fact, the Lyttelton population appears to have higher genetic diversity than the populations sampled in the known parts of its native range.

Therefore, population growth in the port is unlikely to be restricted by a lack of genetic diversity. It seems much more likely, therefore, that the population in the port is being restricted by environmental conditions.

Population decline following rapid population expansion is a well-documented phenomenon in NIS (e.g., Simpson 1984, Freeland 1986, Freeland *et al.* 1986, Boyd & Barbour 1993), but the causes are often ambiguous. Suggested reasons for such population declines include competition with other introduced species, parasitism by newly introduced species, and exhaustion of resources (Simberloff & Gibbons 2004). Alternatively, metapopulation models may explain some of the patterns in *Styela*'s population dynamics. The metapopulation concept was initially proposed by Levins (1969, 1970) and focused on the extinction and re-colonization of populations. In Levins' classic paradigm, species form regional ensembles of transient populations, and persist through dispersal and re-colonization of vacant habitats. However, the importance placed on extinction limited the use of this concept. This was realized and metapopulation models incorporating spatio-temporal variability in population dynamics arose, where the models assumed that such a high degree of dispersal between populations occurs that extinction is unlikely (e.g., Iwasa & Roughgarden 1986), or the likes of the mainland-island paradigm was formed (Boorman & Levitt 1973), in which a central population, immune to extinction, supplied propagules to transient populations. It is unknown and untested if any of these processes acted on the populations in my study, but it is possible that they may be transient or "stepping stone" populations. Local dispersal outside of ports is common as vessels or equipment move about the country, providing opportunities for NIS to spread from their initial point of incursion and form new populations. While these new populations may proliferate, the original population can decline and may even go locally extinct. In this fashion, the population as a whole can remain at the same size, or be growing, but particular transient (sub-) populations may be in decline at any given time. One potential realization of this is that, at some point, all of these smaller populations could consolidate into a single large population, resulting in a crescendo of propagule pressure and the type of exponential spread that *Styela* is known for.

Whatever the combination of mechanisms and processes involved in these local populations, *Styela* is still managing to spread around New Zealand at an alarming rate. It was only discovered in New Zealand in 2005 (Davis & Davis 2006) and has since been found in major ports in the North and South Islands. All indicators are that species is here to stay and will incorporate into the natural benthic fauna.

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## Literature Cited

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- Abbott D.P. & Johnson J.V.** (1972). The ascidians *Styela barnharti*, *S. plicata*, *S. clava*, and *S. montereyensis* in Californian waters. *Bulletin of the Southern California Academy of Sciences* **71**, 95-105.
- Akaike H.** (1974). A new look at the statistical model identification. *Transactions on Automatic Control* **19**, 716-723.
- Anderson C.N.K.; Hsieh C.; Sandin S.A.; Hewitt R.; Hollowed A.; Beddington J.; May R.M. & Sugihara G.** (2008). Why fishing magnifies fluctuations in fish abundance. *Nature* **452**, 835-839.
- Anderson L.W.J.** (2005). California's reaction to *Caulerpa taxifolia*: a model for invasive species rapid response. *Biological Invasions* **7**, 1003-1016.
- Anderson L.W.J. & Keppner S.** (2001). *Caulerpa taxifolia*: marine algal invader provokes quick response in US waters. *Aquatic Nuisance Species Digest* **4**, 21-23.
- Aquaculture New Zealand** (2009). New Zealand aquaculture: farm facts. (<http://www.aquaculture.org.nz>). Cited 25 June 2010.
- Armistead J.S.; Arias J.R.; Nishimura N. & Lounibos L.P.** (2009). Interspecific larval competition between *Aedes albopictus* and *Aedes japonicus* (Diptera: Culicidae) in Northern Virginia. *Journal of Medical Entomology* **45**, 629-637.
- Arsenault G.; Davidson J. & Ramsay A.** (2009). Temporal and spatial development of an infestation of *Styela clava* on mussel farms in Malpeque Bay, Prince Edward Island, Canada. *Aquatic Invasions* **4**, 189-194.
- Baker H.G.** (1965). Characteristics and modes of origin of weeds. In: Baker H.G. & Stebbins G.L. (Eds.). *The Genetics of Colonizing Species*. New York Academic, New York, USA.
- Baker H.G.** (1974). The evolution of weeds. *Annual Review of Ecological Systems* **5**, 1-24.
- Battacharya C.G.** (1967). A simple method of resolution of a distribution into Gaussian components. *Biometrics* **23**, 115-135.
- Bax N.; Williamson A.; Agüero M.; Gonzalez E. & Greeves W.** (2003). Marine invasive alien species: a threat to global biodiversity. *Marine Policy* **27**, 323-323.

- Berkeley S.A.; Hixon M.A.; Larson R.J. & Love M.S.** (2004). Fisheries sustainability via protection of age structure and spatial distribution of fish populations. *Fisheries* **29**, 23-32.
- Bernier R.Y.; Locke A. & Hanson J.M.** (2009). Lobsters and crabs as potential vectors for tunicate dispersal in the southern Gulf of St. Lawrence, Canada. *Aquatic Invasions* **4**, 105-110.
- Beverton R.J.H & Holt S.J.** (1957). On the dynamics of exploited fish populations. Fishery Investigations Series II Marine Fisheries Great Britain Ministry of Agriculture Fisheries and Food 19.
- Boorman S.A. & Levitt P.R.** (1973). Group selection on the boundary of a stable population. *Theoretical Population Biology* **4**, 85-128.
- Boudouresque C.F.; Meinesz A.; Ribera M.A. & Ballesteros E.** (1995). Spread of the green alga *Caulerpa taxifolia* (Caulerpales, Chlorophyta) in the Mediterranean: possible consequences of a major ecological event. *Scientia Marina* **59**, 21-29.
- Bourque D.; Davidson J.; MacNair N.G.; Arsenault G.; LeBlanc A.R.; Landry T. & Miron G.** (2007). Reproduction and early life history of an invasive ascidian *Styela clava* Herdman in Prince Edward Island, Canada. *Journal of Experimental Marine Biology and Ecology* **342**, 78-84.
- Bourque D.; MacNair N.; LeBlanc A.; Landry T. & Miron G.** (2005). Preliminary study of the diel variation of ascidian larvae concentrations in Prince Edward Island. Canadian Technical Report of Fisheries and Aquatic Science No. 2571.
- Boyd R.S. & Barbour M.G.** (1993). Replacement of *Cakile edentula* by *C. maritima* in the strand habitat of California. *American Midland Naturalist* **130**, 209-228.
- Brewin B.I.** (1946). Ascidiens in the vicinity of the Portobello Marine Biological Station, Otago Harbour. *Transactions of the Royal Society of New Zealand* **76**, 87-131.
- Brewin B.I.** (1950). Ascidiens of New Zealand. Part IV. Ascidiens in the vicinity of Christchurch. *Transactions of the Royal Society of New Zealand* **78**, 344-353.
- Brusca R.C. & Brusca G.J.** (1990). Invertebrates. Sinauer Associates, Sunderland, Massachusetts, USA.
- Buizer D.A.G.** (1980). Explosive development of *Styela clava* Herdman 1882, in the Netherlands after its introduction (Tunicata: Ascidiacea). *Bulletin of the Zoological Museum of the University of Amsterdam* **7**, 181-187.
- Bullard S.G. & Whitlatch R.B.** (2004). A guide to the larval and juvenile stages of common Long Island Sounds ascidians and bryozoans. Department of Marine Sciences, University of Connecticut, Groton, USA.
- Bulleri F.; Abbiati M. & Airoidi L.** (2006). The colonisation of human-made structures by the invasive alga *Codium fragile* ssp. *tomentosoides* in the north Adriatic Sea (NE Mediterranean). *Hydrobiologia* **555**, 263-269.

- Bulleri F. & Airoidi L.** (2005). Artificial marine structures facilitate the spread of a non-indigenous green alga, *Codium fragil* ssp. *tomentosoides*, in the north Adriatic Sea. *Journal of Applied Ecology* **42**, 1063-1072.
- Bulleri F. & Chapman M.G.** (2004). Intertidal assemblages on artificial and natural habitats in marinas on the north-west coast of Italy. *Marine Biology* **145**, 381-391.
- Byers J.E.** (2002a). Impact of non-indigenous species on natives enhanced by anthropogenic alteration of selection regimes. *Oikos* **97**, 449-458.
- Byers J.E.** (2002b). Physical habitat attribute mediates biotic resistance to non-indigenous species invasion. *Oecologia* **130**, 146-156.
- Cadrin S.X.; Friedland K.D. & Waldman J.R.** (2005). Stock Identification Methods: Applications in Fishery Science. Elsevier Academic Press, California, USA.
- Cao S.; Zhang C.; Zhang G. & Wu Y.** (1998). Source study on species composition of fouling organisms on mariculture cages. *Journal of Dalian Fish* **13**, 15-21.
- Carlisle D.B.** (1954). *Styela mammiculata* n. sp. a new species of ascidian from the Plymouth area. *Journal of the Marine Biological Association of the United Kingdom* **33**, 329-334.
- Carlton J.T.** (1989). Man's role in changing the face of the ocean: biological invasions and implications for conservation of nearshore environments. *Conservation Biology* **3**, 265-273.
- Carlton J.T.** (1999). The scale and ecological consequences of biological invasions in the world's oceans. In: Sandlund O.T., Schei P.J., Viken Å. (Eds.). Invasive species and biodiversity management. Kluwer Academic Publishers, Dordrecht, Netherlands.
- Carlton J.T. & Hodder J.** (1995). Biogeography and dispersal of coastal marine organisms: experimental studies on a replica of a 16th-century sailing vessel. *Marine Biology* **121**, 721-730.
- Carver C.E.; Chrisholm A. & Mallet A.L.** (2003). Strategies to mitigate the impact of *Ciona intestinalis* L. biofouling on shellfish production. *Journal of Shellfish Research* **22**, 621-631.
- Caswell H.** (1978). A general formula for the sensitivity of population growth rate to changes in life history parameters. *Theoretical Population Biology* **14**, 215-230.
- Caswell H.** (2001). Matrix population models: construction, analysis, and interpretation, Second edition. Sinauer, Sunderland, Massachusetts, USA.
- Chambers R.C. & Miller T.J.** (1995). Recent Developments in Otolith Research. In: Secor D.H.; Dean J.M. & Campana S.E. (Eds.). University of South Carolina Press, Columbia, SC.
- Chapman D.G. & Robson D.S.** (1960). The analysis of a catch curve. *Biometrics* **16**, 354-368.
- Chapman M.G. & Bulleri F.** (2003). Intertidal seawalls: new features of landscape in intertidal environments. *Landscape and Urban Planning* **62**, 159-172.

- Christiansen J. & Thomsen J.C.** (1981). *Styela clava* Herdman 1882, a species new to the Danish fauna (Tunicata Ascidiacea). *Steenstrupia* **7**, 15-24.
- Clarke C.L. & Therriault T.W.** (2007). Biological synopsis of the invasive tunicate *Styela clava* (Herdman 1881). Canadian Manuscript Report of Fisheries and Aquatic Sciences 2807.
- Cloney R.A.** (1992). Phylum Urochordata, Class Ascidiacea. *In*: Strathmann M.F. (Ed.). Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast. University of Washington Press, Seattle, USA.
- Cohen A.N.** (2005). Guide to Exotic Species in San Francisco Bay. San Francisco Estuary Institute, Oakland, USA, ([www.exoticsguide.org](http://www.exoticsguide.org)).
- Colautti R.I.; Bailey S.A.; van Overdijk C.D.A.; Amundsen K. & MacIsaac H.J.** (2006a). Characterised and projected costs of nonindigenous species in Canada. *Biological Invasions* **8**, 45-59
- Colautti R.I.; Grigorovich I.A. & MacIsaac H.J.** (2006b). Propagule pressure: a null model for biological invasions. *Biological Invasions* **8**, 1023-1037.
- Colautti R.I. & MacIsaac H.J.** (2004). A neutral terminology to define 'invasive species'. *Diversity and Distributions* **10**, 135-141.
- Connell S.D.** (2000). Floating pontoons create novel habitats for subtidal epibiota. *Journal of Experimental Marine Biology and Ecology* **247**, 183-194.
- Connell S.D. & Glasby T.M.** (1999). Do urban structures influence local abundance and diversity of subtidal epibiota? A case study from Sydney Harbor, Australia. *Marine Environmental Research* **47**, 373-387.
- Cranfield H.J.; Gordon D.P.; Willan R.C.; Marshall B.A.; Battershill C.N.; Francis M.P.; Nelson W.A.; Glasby C.J. & Read G.B.** (1998). Adventive marine species in New Zealand. Technical Report 34. National Institute of Water and Atmospheric Research, Wellington, New Zealand.
- Crawley M.J.** (2007). The R book. Wiley & Sons, Chichester, England.
- Crooks J.A. & Soulé M.E.** (1999). Lag times in population explosions of invasive species: causes and implications. *In*: Sandlund O.T.; Schei P.J. & Viken Å. (Eds.). Invasive Species and Biodiversity Management. Kluwer Academic Publishers, Dordrecht.
- Crouse D.T.; Crowder L.B. & Caswell H.** (1987). A stage-based population model for loggerhead sea turtles and implications for conservation. *Ecology* **68**, 1412-1423.
- Crowder L.B.; Crouse D.T.; Heppell S.S. & Martin T.H.** (1994). Predicting the impact of turtle excluder devices on loggerhead sea turtle populations. *Ecological Applications* **4**, 437-445.
- Curtis R.J.** (1985). Sedimentation in a rock walled inlet, Lyttelton Harbour, New Zealand. Ph.D. thesis (Geography), University of Canterbury, Christchurch, New Zealand.

- Dalby J.E. & Young C.M.** (1992). Role of early post-settlement mortality in setting the upper depth limit of ascidians in Florida epifaunal communities. *Marine Ecology Progress Series* **80**, 221-228.
- Darbyson E.A.; Hanson J.M.; Locke A. & Willison J.H.M.** (2009). Settlement and potential for transport of clubbed tunicate (*Styela clava*) on boat hulls. *Aquatic Invasions* **4**, 95-103.
- Dauvin J.C.; Iglesias A. & Gentil F.** (1991). Nouvelles especes pour l'inventaire de la faune marine de Roscoff - crustaces amphipodes, cumacs et decapodes, mollusques gastropodes et ascidies. *Les Cahiers de Biologie Marine* **32**, 121-128.
- Davis M.H.** (1997). Physiological factors influencing larval behaviour in three species of solitary ascidian. Ph.D. thesis (Department of Biology), Open University, UK.
- Davis M.H. & Davis M.E.** (2004). New records of *Styela clava* Herdman, 1882 (Tunicata, Ascidiacea) in Europe. *Newsletter of the Porcupine Marine and Natural History Society* **14**, 24-28.
- Davis M.H. & Davis M.E.** (2005). *Styela clava* (Tunicata: Ascidiacea) a new edition to the fauna of the Portuguese coast. *Journal of the Marine Biological Association of the United Kingdom* **85**, 403-404.
- Davis M.H. & Davis M.E.** (2006). *Styela clava* (Tunicata: Ascidiacea) a new edition to the fauna of New Zealand. *Porcupine Marine Natural History Society Newsletter* **20**, 23-28.
- Davis M.H. & Davis M.E.** (2007). The distribution of *Styela clava* (Tunicata, Ascidiacea) in European waters. *Journal of Experimental Marine Biology and Ecology* **342**, 182-184.
- de Kroon H.; Plaisier A.; van Groenendael J.M. & Caswell H.** (1986). Elasticity: the relative contribution of demographic parameters to population growth rate. *Ecology* **67**, 1427-1431.
- Dijkstra J.; Harris L.G. & Westerman E.** (2007). Distribution and long-term temporal patterns of four invasive colonial ascidians in the Gulf of Maine. *Journal of Experimental Marine Biology and Ecology* **342**, 61-68.
- Dudas S.E.; Dower J.F. & Anholt B.R.** (2007). Invasion of the varnish clam (*Nuttallia obscurata*): a matrix demographic modeling approach. *Ecology* **88**, 2084-2093.
- Dunmore R.A.** (2006). Demography of early life stages of habitat-forming intertidal fucoid algae. Ph.D. thesis (Biological Sciences), University of Canterbury, Christchurch, New Zealand.
- Dybern B.I.** (1965). The life cycle of *Ciona intestinalis* (L.) f. *typica* in relation to the environmental temperature. *Oikos* **16**, 109-131.
- Dybern B.I.** (1967). The distribution and salinity tolerance of *Ciona intestinalis* (L.) f. *typica* with special reference to the waters around Southern Scandinavia. *Ophelia* **4**, 207-226.
- Ehrlich P.R.** (1986). Which animal will invade? In: Drake J.A. (Ed.). Ecology of biological invasions of North America and Hawaii. Springer-Verlag, New York, USA.
- Elton C.S.** (1958). The ecology of invasions by animals and plants. The University of Chicago Press, Chicago, USA.

- Eno N.C.; Clark R.A. & Sanderson W.G.** (1997). Non-native marine species in British waters: a review and directory. Peterborough, UK, Joint Nature Conservation Committee, 133-134.
- Floerl O. & Inglis G.J.** (2005). Starting the invasion pathway: the interaction between source populations and human transport vectors. *Biological Invasions* **7**, 589-606.
- Floerl O.; Inglis G.J.; Dey K. & Smith A.** (2009). The importance of transport hubs in stepping-stone invasions. *Journal of Applied Ecology* **46**, 37-45.
- Floerl O.; Pool T.K. & Inglis G.J.** (2004). Positive interactions between nonindigenous species facilitate transport by human vectors. *Ecological Applications* **14**, 1724-1736.
- Forrest B.M. & Blakemore K.A.** (2006). Evaluation of treatments to reduce the spread of a marine plant pest with aquaculture transfers. *Aquaculture* **257**, 333-345.
- Forrester G.E.** (1995). Strong density-dependent survival and recruitment regulate the abundance of a coral reef fish. *Oecologia* **103**, 275-282.
- Freeland W.J.** (1986). Populations of cane toad, *Bufo marinus*, in relation to time since colonization. *Australian Wildlife Research* **13**, 321-329.
- Freeland W.J.; Delvinqueir B.L.J. & Bonnin B.** (1986). Food and parasitism of the cane toad, *Bufo marinus*, in relation to time since colonization. *Australian Wildlife Research* **13**, 489-499.
- Fujikura K.; Okoshi K. & Naganuma T.** (2003). Strontium as a marker for estimation of microscopic growth rates in a bivalve. *Marine Ecology Progress Series* **257**, 295-301.
- Gittenberger A.** (2009). Invasive tunicates on Zeeland and Prince Edward Island mussels, and management practices in the Netherlands. *Aquatic Invasions* **4**, 279-281.
- Glasby T.M. & Connell S.D.** (1999). Urban structures as marine habitats. *Ambio* **28**, 595-598.
- Glasby T.M. & Connell S.D.** (2001). Orientation and position of substrata have large effects on epibiotic assemblages. *Marine Ecology Progress Series* **214**, 127-135.
- Glasby T.M.; Connell S.D.; Holloway M.G. & Hewitt C.L.** (2007). Nonindigenous biota on artificial structures: could habitat creation facilitate biological invasions? *Marine Biology* **151**, 887-895.
- Goldstien S.J.; Schiel D.R. & Gemmell N.J.** (2010). Regional connectivity and coastal expansion: differentiating pre-boarder and post-boarder vectors for the invasive tunicate *Styela clava*. *Molecular Ecology* **19**, 874-885.
- Grannum R.K.; Murfet N.B.; Ritz D.A. & Turner E.** (1996). The distribution and impact of the exotic seastar, *Asterias amurensis* (Lütken), in Tasmania. *In: The introduced northern Pacific seastar, Asterias amurensis* (Lütken), in Tasmania. Australian Nature Conservation Agency, Canberra, Australia.
- Grosholz E.** (2002). Ecological and evolutionary consequences of coastal invasions. *Trends in Ecology and Evolution* **171**, 22-27.



- Gust N.; Floerl O.; Inglis G.; Miller S.; Fitridge I. & Hurren H.** (2005). Rapid delimitation survey of *Styela clava* in the Viaduct Harbour and Freemans Bay, Auckland. NIWA client report CHC2005-147.
- Gust N.; Inglis G.; Floerl O.; Peacock L.; Denny C. & Forrest B.** (2008). Assessment of population management options for *Styela clava*. MAF Biosecurity New Zealand Technical Paper No: 2009/04.
- Harriott V.J.** (1985). Mortality rates of scleractinian corals before and during a mass bleaching event. *Marine Ecology Progress Series* **21**, 81-88.
- Hastings A.** (1997). Population biology concepts and models. Springer-Verlag, New York, USA.
- Hayes K.R.; Cannon R.; Neil K.; Inglis G.** (2005). Sensitivity and cost considerations for the detection and eradication of marine pests in ports. *Marine Pollution Bulletin* **50**, 823-834.
- Heald D.** (1978). A successful marking method for the saucer scallop *Amusium balloti* (Bernadi). *Australian Journal of Freshwater and Marine Research* **29**, 845-851.
- Heppell S.S. & Crowder L.B.** (1996). Models to evaluate headstarting as a management tool for long-lived turtles. *Ecological Applications* **6**, 556-565.
- Herrmann M.; Lepore M.L.; Laudian J.; Arntz W.E. & Penchaszadeh P.E.** (2009). Growth estimations of the Argentinean wedge clam *Donax haneyanus*: a comparison between length-frequency and size increment analysis. *Journal of Experimental Marine Biology and Ecology* **379**, 8-15.
- Hewitt C.; Campbell M.L.; Thresher R.E. & Martin R.B.** (1999). Marine biological invasions of Port Phillip Bay, Victoria. Centre for Research on Introduced Marine Pests CSIRO Technical Report No. 20.
- Holloway M.G. & Connell S.D.** (2002). Why do floating structures create novel habitats for subtidal epibiota? *Marine Ecology Progress Series* **235**, 43-52.
- Holloway M.G. & Keough M.J.** (2002). An introduced polychaete affects recruitment and larval abundance of sessile invertebrates. *Ecological Applications* **12**, 1803-1823.
- Holmes N.J.** (1969). Aspects of the biology of *Styela clava* Herdman. Thesis, Southampton University, UK.
- Holmes N.J.** (1976). Occurrence of the ascidian *Styela clava* Herdman in Hobsons Bay, Victoria: a new record for the southern hemisphere. *Proceedings of the Royal Society of Victoria* **88**, 115-116.
- Horvitz C.C. & Schemske D.W.** (1995). Spatiotemporal variation in demographic transitions of a tropical understory herb: projection matrix analysis. *Ecology* **65**, 155-192.
- Hunter C.M.; Moller H. & Fletcher D.** (2000). Parameter uncertainty and elasticity analyses of a population model: setting research priorities for shearwaters. *Ecological Modelling* **134**, 299-323.

- Inglis G.** (2001). Criteria for selecting New Zealand ports and other points of entry that have a high risk of invasion by new exotic marine organisms. Final research report for Ministry of Fisheries research project ZBS2000/01A.
- Inglis G.; Gust N.; Fitredge I.; Floerl O.; Woods C.; Hayden B. & Fenwick G.** (2006a). Port of Lyttelton baseline survey for non-indigenous marine species. MAF Biosecurity New Zealand Technical Paper No. 2005/01.
- Inglis G.; Hurren H.; Oldman J. & Haskew R.** (2006b). Using habitat suitability index and particle dispersion models for early detection of marine invaders. *Ecological Applications* **16**, 1377-1390.
- Iwasa Y. & Roughgarden J.** (1986). Interspecific competition in metapopulations with space-limited subpopulations. *Theoretical Population Biology* **30**, 194-214.
- Jenkins P.T.** (1996). Free trade and exotic species introductions. *Conservation Biology* **10**, 300-302.
- Jeschke J.M. & Strayer D.L.** (2005). Invasion success of vertebrates in Europe and North America. *Proceedings of the National Academy of Science USA* **102**, 7198-7202.
- Jiang A.; Lin J. & Wang C.** (2008). Physiological energetics of the ascidian *Styela clava* in relation to body size and temperature. *Comparative Biochemistry and Physiology, Part A* **149**, 129-136.
- Johnston E.L. & Keough M.J.** (2002). Direct and indirect effects of repeated pollution events on marine hard-substrate assemblages. *Ecological Applications* **12**, 1212-1228.
- Jongejans E.; Shea K.; Skarpaas O.; Kelly D.; Sheppard A.W. & Woodburn T.L.** (2008). Dispersal and demography contributions to population spread of *Carduus nutans* in its native and invaded ranges. *Journal of Ecology* **96**, 687-697.
- Junne A.S.** (2007). <http://www.junne-diving.eu/images/zelande2007.htm>, Photos sous-marines, Zelande 2007. Web publication, date of access 15/6/2010.
- Kanary L.; Locke A. & Watmough J.** (2010). Evaluating the effectiveness of SCUBA-based visual searches for an invasive tunicate, *Ciona intestinalis*, in A Prince Edward Island estuary. *Aquatic Invasions* **5**, 41-47.
- Kang P.A.; Kim Y. & Yoon D.S.** (1980). Studies on the hanging culture of oyster, *Crassostrea gigas*, in the Korean coastal waters. On the fouling organisms associated with culturing oysters at the oyster culture farms in Chungmu. *Bulletin of the Fisheries Research and Development Agency, Busan* **25**, 29-34.
- Kaufmann K.W.** (1981). Fitting and using growth curves. *Oecologia* **49**, 293-299.
- Kazihara T.** (1964). Ecological studies on marine fouling animals. *Bulletin of the Faculty of Fisheries, Nagasaki University* **16**, 1-138.
- Keough M.J. & Downes B.J.** (1986). Effects of settlement and post-settlement mortality on the distribution of the ascidian *Trididemnum opacum*. *Marine Ecology Progress Series* **33**, 279-285.

- Kluza D.; Ridgway I.; Kleeman S. & Gould B.** (2006). BNZ organism impact assessment. *Styela clava* (clubbed tunicate).
- Kowarik I.** (1995). Time lags in biological invasions with regard to the success and failure of alien species. *In*: Pysek P.; Prach K.; Rejmánek M. & Wade M. (Eds.). *Plant Invasions: General Aspects and Special Problems*. SPB Academic Publishing, Amsterdam.
- Krebs C.J.** (1985). *Ecology: the experimental analysis of distribution and abundance*, Third Edition. Harper & Row Publishers, New York, USA.
- Krone R.; Wanke C. & Schröder A.** (2007). A new record of *Styela clava* Herdman, 1882 (Urochordata, Asidiacea) from the central German Bight. *Aquatic Invasions* **2**, 442-444.
- Lambert G.** (2003). New records of ascidians from the NE Pacific: a new species of *Trididemnum*, range extension and redescription of *Aplidiopsis pannosum* (Ritter, 1899) including its larva, and several non-indigenous species. *Zoosystema* **25**, 665-679.
- Lambert G.** (2005). Ecology and natural history of the protochordates. *Canadian Journal of Zoology* **83**, 34-50.
- Lambert G.** (2007). Invasive sea squirts: a growing global problem. *Journal of Experimental Marine Biology and Ecology* **342**, 3-4.
- Lambert C.C. & Lambert G.** (1998). Non-indigenous ascidians in southern California harbors and marinas. *Marine Biology* **130**, 675-688.
- Lambert C.C. & Lambert G.** (2003). Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. *Marine Ecology Progress Series* **259**, 145-161.
- Laudien J.; Brey T. & Arntz W.E.** (2003). Population structure, growth and production of the surf clam *Donax serra* (Bivalva, Donacidae) on two Namibian sandy beaches. *Estuarine and Coastal Shelf Science* **58S**, 105-115.
- LeBlanc N.; Davidson J.; Tremblay R.; McNiven M. & Landry T.** (2007). The effect of anti-fouling treatments for the clubbed tunicate on the blue mussel, *Mytilus edulis*. *Aquaculture* **264**, 205-213.
- Lefkovich L.P.** (1965). The study of population growth in organisms grouped by stages. *Biometrics* **21**, 1-18.
- Leppäkoski E.; Gollasch S. & Olenin S.** (2002). Alien species of European waters. *In*: *Invasive Aquatic Species of Europe: Distribution, Impacts, and Management*. Kluwer Academic Publishers, Dordrecht, Netherlands.
- Leslie P.H.** (1945). On the use of matrices in certain population mathematics. *Biometrika* **33**, 183-212.
- Leung B.; Lodge D.M.; Finnoff D.; Shogren J.F.; Lewis M.A. & Lamberti G.** (2002). An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. *Proceedings of the Royal Society of London Series B* **269**, 2407-2413.

- Levings C.; Kieser D.; Jamieson G.S. & Dudas S.** (2002). Marine and estuarine alien species in the Strait of Georgia, British Columbia. Alien Invaders in Canada's waters, wetlands, and forests. Natural Resources Canada 111-131.
- Levins R.** (1969). Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bulletin of the Entomological Society of America* **15**, 237-240.
- Levins R.** (1970). Extinction. *In: Some Mathematical Problems in Biology*. Desternhaber M. (Ed.). American Mathematical Society, Providence, USA.
- Locke A.; Hanson J.M.; Ellis K.M.; Thompson J. & Rochette R.** (2007). Invasion of the southern Gulf of St. Lawrence by the clubbed tunicate (*Styela clava* Herdman): Potential mechanisms for invasions of Prince Edward Island estuaries. *Journal of Experimental Marine Biology and Ecology* **342**, 69-77.
- Lockwood J.L.; Cassey P. & Blackburn T.** (2005). The role of propagule pressure in explaining species invasions. *Trends in Ecology and Evolution* **20**, 223-228.
- Lomovasky B.J.; Morriconi E.; Brey T. & Calvo J.** (2002). Individual age and connective tissue lipofuscin in the hard clam *Euromalea exalbida*. *Journal of Experimental Marine Biology and Ecology* **276**, 83-94.
- Lützen J.** (1999). *Styela clava* Herdman (Urochordata, Ascidiacea), a successful immigrant to north west Europe: ecology, propagation and chronology of spread. *Helgoländer Meeresuntersuchungen* **52**, 383-391.
- Mack R.N.; Simberloff D.; Lonsdale W.M.; Evans H.; Clout M. & Bazzaz F.A.** (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* **10**, 689-710.
- Malinowski K.C. & Ramus J.** (1973). Growth of the green alga *Codium fragile* in a Connecticut estuary. *Journal of Phycology* **9**, 102-110.
- McCarthy A.; Osman R.W. & Whitlatch R.B.** (2007). Effects of temperature on growth rates of colonial ascidians: a comparison of *Didemnum* sp. to *Botryllus schlosseri* and *Botrylloides violaceus*. *Journal of Experimental Marine Biology and Ecology* **342**, 172-174.
- McClary D.; Phipps C. & Hinni S.** (2009). Reproductive behavior of the clubbed tunicate, *Styela clava*, in northern New Zealand waters. MAF Biosecurity New Zealand Technical Paper No: 2009/01.
- McEvoy P.B. & Coombs E.M.** (1999). Biological control of plant invaders: regional patterns, field experiments, and structured population models. *Ecological Applications* **9**, 387-401.
- Meiners S.J.; Cadenasso M.L. & Pickett S.T.A.** (2004). Beyond biodiversity: individualistic controls of invasion in a self-assembled community. *Ecology Letters* **7**, 121-126.
- Meinesz A.; Belsher T.; Thibaut T.; Antolic B.; Mustapha K.B.; Boudouresque C.F.; Chiaverini D.; Cinelli F.; Cottalorda J.M.; Djellouli A.; Abed A.E.; Orestano C.; Grau A.M.; Ivesa L.; Jaklin A.; Langer H.; Massuti-Pascual E.; Peirano A. Tunesi L.; Vaugelas J.; Zavodnik N. & Zuljevic A.** (2001). The introduced green alga *Caulerpa taxifolia* continues to spread in the Mediterranean. *Biological Invasions* **3**, 201-210.

- Mills L.S.; Doak D.F. & Wisdom M.J.** (1999). The reliability of conservation actions based on elasticity analysis of matrix models. *Conservation Biology* **13**, 815-829.
- Minchen D. & Duggan C.B.** (1988). The distribution of the exotic ascidian, *Styela clava* Herdman, in Cork Harbour. *Irish Naturalists Journal* **22**, 388-393.
- Minchen D.; Davis M. & Davis M.** (2006). Spread of the Asian tunicate *Styela clava* Herdman, 1882 to the east and south-west coasts of Ireland. *Aquatic Invasions* **1**, 91-96.
- Molye P.B.** (1986). Fish introductions into North America: patterns and ecological impact. In: Mooney H.A. & Drake J.A. (Eds.). *Ecology of Biological Invasions of North America and Hawaii*. Springer-Verlag, New York, USA.
- Morris R.H.; Abbott D.P. & Harderlie E.C.** (1980). *Intertidal invertebrates of California*. Stanford University Press, Stanford, California, USA.
- Munro J.L.** (1982). Estimation of the parameters of the von Bertalanffy growth equation from recapture at different time intervals. *Journal du Conseil* **40**, 199-200.
- Naylor R.L.; Williams S.L. & Strong D.R.** (2001). Aquaculture – a gateway for exotic species. *Science* **294**, 1655-1656.
- Neubert M.G. & Parker I.M.** (2004). Projecting rates of spread for invasive species. *Risk Analysis* **24**, 817-831.
- Nimpis.** (2002). <http://crimp.marine.csiro.au/nimpis>, *Styela clava* reproduction & life cycle. National Introduced Marine Pest Information System. Hewitt C.L.; Martin R.B.; Sliwa C.; McEnnulty F.R.; Murphy N.E.; Jones T. & Cooper S. Web publication, date of access 9/2/2010.
- Nunn J.D. & Minchin D.** (2009). Further expansions of the Asian tunicate *Styela clava* Herdman 1882 in Ireland. *Aquatic Invasions* **4**, 591-596.
- Nybakken J.W. & Bertness M.D.** (2005). *Marine Biology: an Ecological Approach*, sixth edition. Pearson Education Publishing, San Francisco, USA.
- O'Connor R.J.** (1986). Biological characteristics of invaders among bird species in Britain. *Philosophical Transactions of the Royal Society of London B* **314**, 583-598.
- O'Neill C.R. Jr. & MacNeill D.B.** (1991). The zebra mussel (*Dreissena polymorpha*): an unwelcome North American invader. Sea Grant Coastal Resources Fact Sheet.
- Osman R.W. & Whitlatch R.B.** (1995). The influence of resident adults on larval settlement: experiments with four species of ascidians. *Journal of Experimental Marine Biology and Ecology* **190**, 199-220.
- Osman R.W. & Whitlatch R.B.** (1999). Ecological interactions of invading ascidians within epifaunal communities of southern New England. In: Pederson J. (Ed.), *Marine Bioinvasions: Proceedings of conference, January 24-27, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA*.

- Osman R.W.; Whitlatch R.B. & Zajac R.N.** (1989). Effects of resident species on recruitment into a community: larval settlement versus post-settlement mortality in the oyster *Crassostrea virginica*. *Marine Ecology Progress Series* **54**, 61-73.
- Parker I.M.** (2000). Invasion dynamics of *Cytisus scoparius*: a matrix model approach. *Ecological Applications* **10**, 726-743.
- Parker L.E.; Culloty S.; O’Riordan R.M.; Kelleher B.; Steele S. & Van der Velde G.** (1999). Preliminary study on the gonad development of the exotic ascidian *Styela clava* in Cork Harbour, Ireland. *Journal of the Marine Biological Association of the United Kingdom* **79**, 1141-1142.
- Pätzold J.** (1984). Growth rhythms recorded in stable isotopes and density bands in the reef coral *Porites lobata* (Cebu, Philippines). *Coral Reefs* **3**, 87-90.
- Pollock K.H.; Nichols J.D.; Brownie C. & Hines J.E.** (1990). Statistical inferences for capture-recapture experiments. *Wildlife Monographs* **107**, 1-97.
- Pollock K.H.; Winterstein S.R.; Bunck C.M. & Curtis P.D.** (1989). Survival analysis in telemetry studies: the staggered entry design. *Journal of Wildlife Management* **53**, 7-15.
- Pomerat C.M. & Reiner E.R.** (1942). The influence of surface angle and of light in the attachment of barnacles and of other sedentary organisms. *Biological Bulletin, Marine Biology Lab, Woods Hole* **82**, 14-25.
- Ramsay A.; Davidson J.; Landry T. & Arsenault G.** (2008). Process of invasiveness among exotic tunicates in Prince Edward Island, Canada. *Biological Invasions* **10**, 1311-1316.
- Rejmánek M. & Richardson D.M.** (1996). What attributes make some plant species more invasive? *Ecology* **77**, 1655-1661.
- Rho Y.G.; Lee Y.H. & Park M.W.** (1993). The environmental factors affecting mortality of cultured sea squirt, *Halocynthia roretzi* (Drasche). Bulletin of National Fisheries Research and Development Agency (Korea). Yangsan Korea. No 47.
- Ricciardi A.; Whoriskey F.G. & Rasmussen J.B.** (1997). The role of zebra mussel (*Dreissena polymorpha*) in structuring macroinvertebrate communities on hard substrata. *Canadian Journal of Fisheries and Aquatic Sciences* **54**: 2596–2608
- Richardson D.M.; Pysek P.; Rejmanek M.; Barbour M.G.; Panetta F.D. & West C.J.** (2000). Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions* **6**, 93-107.
- Ricker W.E.** (1958). Handbook of computations for biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada 191, 382.
- Roberts D.; Rittschoff D.; Holm E. & Schmidt A.R.** (1991). Factors influencing initial larval settlement: temporal, spatial and surface molecular components. *Journal of Experimental Marine Biology and Ecology* **150**, 203-211.
- Rogers-Bennett L. & Leaf R.** (2006). Elasticity analysis of size-based red and white abalone matrix models: management and conservation. *Ecological Applications* **16**, 213-224.

- Rogers-Bennett L.; Rogers D.W.; Bennett W.A. & Ebert T.A.** (2003). Modeling red sea urchin (*Strongylocentrotus franciscanus*) growth using six growth functions. *Fishery Bulletin* **101**, 614-626.
- Rogers-Bennett L.; Rogers D.W. & Schultz S.A.** (2007). Modeling growth and mortality of red abalone (*Haliotis rufescens*) in northern California. *Journal of Shellfish Research* **26**, 719-727.
- Roman J.** (2006). Diluting the founder effect: cryptic invasions expand a marine invader's range. *Proceeding of the Royal Society B* **273**, 2453-2459.
- Ross D.J.; Johnson C.R. & Hewitt C.L.** (2002). Impact of introduced seastars *Asterias amurensis* on survivorship of juvenile commercial bivalves *Fulvia tenuicostata*. *Marine Ecology Progress Series* **241**, 99-112.
- Ross D.J.; Keough M.J.; Longmore A.R. & Knott N.A.** (2007). Impacts of two introduced suspension feeders in Port Phillip Bay, Australia. *Marine Ecology Progress Series* **340**, 41-53.
- Ruiz G.M.; Carlton J.T.; Grosholz E.D. & Hines A.H.** (1997). Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. *American Zoologist* **37**, 621-623.
- Ruiz G.M.; Rawlings T.K.; Dobbs F.C.; Drake L.A.; Mullady T.; Huq A. & Colwell R.R.** (2000). Global spread of microorganisms by ships. *Nature* **208**, 49-50.
- Ruppert E.E. & Barnes R.D.** (1994). *Invertebrate Zoology*, sixth edition. Saunders Publishing, San Diego, USA.
- Sakai A.K.; Allendorf F.W.; Holt J.S.; Lodge D.M.; Molofsky J.; With K.A.; Baughman S.; Cabin R.J.; Cohen J.E.; Ellstrand N.C.; McCauley D.E.; O'Neil P.; Parker I.M.; Thompson J.N. & Weller S.G.** (2001). The population biology of invasive species. *Annual Review of Ecological Systems* **32**, 305-332.
- Sala O.E.** (2000). Biodiversity – global biodiversity scenarios for the year 2100. *Science* **287**, 1770-1774.
- Savard L.; Parsons D.G. & Carlsson D.M.** (1994). Estimation of age and growth of Northern shrimp (*Pandalus borealis*) in Davis Strait (NAFO subareas 0+1) using cluster and modal analysis. *Journal of Northwestern Atlantic Fisheries Science* **16**: 63-74.
- Say T.** (1817). An account of the Crustacea of the United States. *Journal of the Academy of Natural Sciences of Philadelphia* **1**, 57-63.
- Schaffelke B.; Cambell M.L. & Hewitt C.L.** (2005). Reproductive phenology of the introduced kelp *Undaria pinnatifida* (Phaeophyceae, Laminariales) in Tasmania, Australia. *Phycologia* **44**, 84-94.
- Schnute J. & Fournier D.** (1980). A new approach to length-frequency analysis: growth structure. *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 1337-1351.
- Schwarz G.** (1978). Estimating the dimension of a model. *The Annals of Statistics* **6**, 461-464.

- Shatkin G.; Shumway S.E. & Hawes R.** (1997). Considerations regarding the possible introduction of the Pacific oyster (*Crassostrea gigas*) to the Gulf of Maine: a review of global experience. *Journal of Shellfish Research* **16**, 463-477.
- Shea K.; Jongejans E.; Skarpaas O.; Kelly D. & Sheppard A.W.** (2010). Optimal management strategies to control local population growth or population spread may not be the same. *Ecological Applications* **20**, 1148-1161.
- Shea K. & Kelly D.** (1998). Estimating biocontrol agent impact with matrix models: *Carduus nutans* in New Zealand. *Ecological Applications* **8**, 824-832.
- Sheley R.L. & Petroff J.K.** (1999). Biology and Management of Noxious Rangeland Weeds. Corvallis O.R. (Ed.). Oregon State University Press, Oregon, USA.
- Shiganova T.A.** (1998). Invasion of the Black Sea by the ctenophore *Mnemiopsis leidyi* and recent changes in the pelagic community structure. *Fisheries Oceanography* **7**, 305-310.
- Shushkina E. & Musayeva E.** (1990). Structure of the plankton community from the Black Sea and its changes as a result of the introduction of a ctenophore species. *Oceanography* **30**, 225-228.
- Siguan M.A.R.** (2003). Pathways of biological invasions of marine plants. In: Ruiz G.M. & Carlton J.T. (Eds.). *Invasive Species: Vectors and Management Strategies*. Island Press, Washington, USA.
- Simberloff D.** (2009). The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics* **40**, 81-102.
- Simberloff D. & Gibbons L.** (2004). Now you see them, now you don't! – population crashes of established introduced species. *Biological Invasions* **6**, 161-172.
- Simberloff D. & von Holle B.** (1999). Positive interactions of non-indigenous species: invasional meltdown? *Biological Invasions* **1**, 21-32.
- Simpson D.A.** (1984). A short history of the introduction and spread of *Elodea Michx* in the British Isles. *Watsonia* **15**, 1-9.
- Sims L.L.** (1984). Osmoregulatory capabilities of three macrosympatric Stolidobranch ascidians, *Styela clava* Herdman, *S. plicata* (Lesueur), and *S. montereyensis* (Dall). *Journal of Experimental Marine Biology and Ecology* **82**, 117-129.
- Skalski J.R.; Ryding K.E. & Millsaugh J.J.** (2005). Primer on wildlife population dynamics. In: *Wildlife Demography: Analysis of Sex, Age, and Count Data*. Elsevier Academic Press, Oxford, UK.
- Stachowicz J.J.; Whitlatch R.B. & Osman R.W.** (1999). Species diversity and invasion resistance in a marine ecosystem. *Science* **286**, 1577-1579.
- Steneck R.S. & Carlton J.T.** (2001). Human alterations of marine communities. In: Bertness M.D.; Gaines S.D. & Hay M.E. (Eds.). *Marine Community Ecology*. Sinauer Associates, Sunderland, Massachusetts, USA.



- Stewart A. & Deacon J.W.** (1995). Vital fluorochromes as tracers for fungal growth studies. *Biotechnic and Histochemistry* **70**, 57-65.
- Stoner D.S.** (1990). Recruitment of a tropical colonial ascidian: relative importance of pre-settlement vs. post-settlement processes. *Ecological Society of America* **71**, 1682-1690.
- Svane I.** (1984). Observations on the long-term population dynamics of the perennial ascidian, *Ascidia mentula* O.F. Muller, on the Swedish west coast. *Biological Bulletin* **167**, 630-646.
- Svane I. & Lundalv T.** (1981). Reproductive patterns and population dynamics of *Ascidia Mentula* O.F. Müller on the Swedish west coast. *Journal of Experimental Marine Biology and Ecology* **50**, 163-182.
- Svane I. & Young C.M.** (1989). The ecology and behaviour of ascidian larvae. *Oceanographic Marine Biology Annual Review* **27**, 45-90.
- Takada Y.** (1995). Variation of growth rate with tidal level in the gastropod *Momodonta labio* on a boulder shore. *Marine Ecology Progress Series* **117**, 103-110.
- Taylor D.I. & Schiel D.R.** (2003). Wave-related mortality in zygotes of habitat-forming algae from different exposures in southern New Zealand: the importance of 'stickability'. *Journal of Experimental Marine Biology and Ecology* **290**, 229-245.
- Thomas P.G. & Ikeda T.** (1987). Sexual regression, shrinkage, re-maturation and growth of spent female *Euphausia superba* in the laboratory. *Marine Biology* **95**, 357-363.
- Thompson G.A.** (2004). Mechanisms of invasion and persistence of the invasive kelp *Undaria pinnatifida* (Harvey) Suringar within intertidal areas of southern New Zealand. Ph.D. thesis (Biological Sciences), University of Canterbury, Christchurch, New Zealand.
- Thorner C.; Kinlan B.P.; Graham M.H. & Stachowicz J.J.** (2004). Population ecology of the invasive kelp *Undaria pinnatifida* in California: environmental and biological controls on demography. *Marine Ecology Progress Series* **268**, 69-80.
- Thornson G.L.** (1964). Light as an ecological factor in the dispersal and settlement of larvae of marine bottom invertebrates. *Ophelia* **1**, 167-208.
- Tomlinson P.K.** (1971). NORMSEP: normal distribution separation. In: Abramson N.J. (Ed.) Computer programs for fish stock assessment. FAO Fisheries Technical Paper 101 (FIRD/T101). FAO, Rome.
- Turner S.J.; Thrush S.F.; Cummings V.J.; Hewitt J.E.; Wilkinson M.R.; Williamson R.B. & Lee D.J.** (1997). Changes in epifaunal assemblages in response to marina operations and boating activities. *Marine Environmental Research* **43**, 181-199.
- Tyrrell M. & Byers J.E.** (2007). Do artificial substrates favour nonindigenous fouling species over native species? *Journal of Experimental Marine Biology and Ecology* **342**, 54-60.
- Vaughan D.S. & Sails S.B.** (1976). A method for determining mortality rates using the Leslie matrix. *Transactions of the American Fisheries Society* **3**, 380-383.
- von Bertalanffy L.** (1934). Untersuchungen ueber die Gesetzmäßigkeit des Wachstums. *Wilhelm Roux' Arch Entwickl Organ* **131**, 613-652.

- Walker D.I. & Kendrick G.A.** (1998). Threats to macroalgal diversity: marine habitat destruction a fragmentation, pollution and introduced species. *Botanica Marina* **41**, 105-112.
- White G.C. & Burnham K.P.** (1999). Program MARK: survival estimation from populations of marked animals. *Bird Study* **46S**, 120-138.
- Williamson M.** (1996). Invasions. *Ecography* **22**, 5-12.
- Wonham M.J. & Carlton J.T.** (2005). Trends in marine biological invasions at local and regional scales: the Northeast Pacific Ocean as a model system. *Biological Invasions* **7**, 369-392.
- Yamaguchi M.** (1975). Growth and reproductive cycles of the marine fouling ascidians *Ciona intestinalis*, *Styela plicata*, *Botrylloides violaceus*, and *Leptoclinum mitsukurii* at Aburatsubo-Moroiso Inlet (central Japan). *Marine Biology* **29**, 253-259.
- Young C.M.** (1985). Abundance patterns of subtidal solitary ascidians in the San Juan Islands, Washington, as influenced by food preferences for the predatory snail *Fusitriton oregonensis*. *Marine Biology* **84**, 309-321.
- Young C.M. & Chia F.S.** (1984). Microhabitat-associated variability in survival and growth of subtidal ascidians during the first 21 days after settlement. *Marine Biology* **81**, 61-68.

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## Appendices

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### Appendix I

**Appendix 2.1:** All individual encounter histories used in temporal, site and depth-specific survival analysis. “/\*###\*/” = the individuals specific code; “101010101010101000110000” = an example of an individuals encounter history (10 = the individual was encountered alive, 11 = the individual was encountered dead, 00 = the individual was either missed on the sampling occasion or has died on a previous sampling occasion); the remaining string of numbers denote the occurrence of that encounter history (always 1); site (0 = site A&B, 1 = site Z); the depth in cm.

Code	Encounter history	Occurrence, Site, depth
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/*002*/	00000000101010110000000000000000	1 0 30;
/*003*/	00000000101100000000000000000000	1 0 40;
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/*005*/	00000000001010100000000000000000	1 0 60;
/*006*/	00000000001010101000000000000000	1 0 100;
/*007*/	00000000001010101000000000000000	1 0 90;
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/*013*/	000000001010101010101010101100	1 0 10;
/*014*/	00000000101010110000000000000000	1 0 90;
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/*019*/	00000000101010101010101011000000	1 0 100;
/*020*/	000000001010101010101010001100	1 0 100;
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/*022*/	00000000001010101010001100000000	1 0 70;
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/*024*/	00000000101010101010001010101000	1 0 30;
/*025*/	00000000001010101010001100000000	1 0 100;
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```

**Appendix 2.2:** All individual encounter histories used in size-specific survival analysis. “/\*####\*/” = the individuals specific code; “101010101010101000110000” = an example of an individuals encounter history (10 = the individual was encountered alive, 11 = the individual was encountered dead, 00 = the individual was either missed on the sampling occasion or has died on a previous sampling occasion); the remaining string of numbers denote the occurrence of that encounter history (always 1); site (0 = site A&B, 1 = site Z); the depth in cm; TL = total length (mm); BL = body length (mm); BD = body diameter (mm).

Code	Encounter history	Occurrence, Site, depth, TL, BL, BD
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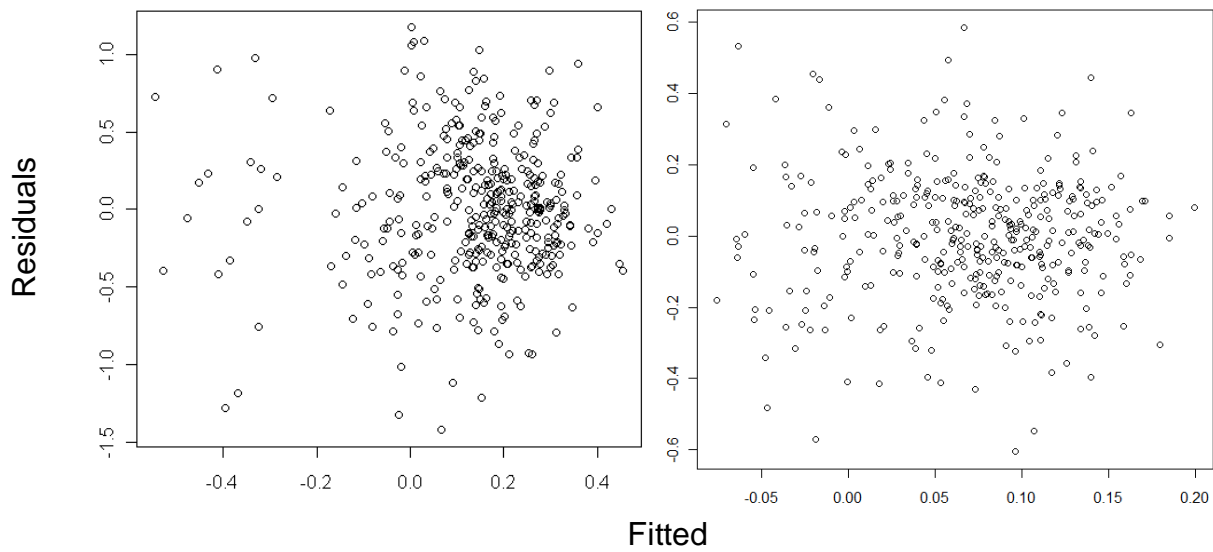
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---



**Appendix II**



**Appendix 3.1:** Scatterplots of residual versus fitted values for the general linear models on growth data using total length (left), multi-metric, *M* (right).

**Appendix 3.2:** Individual component means, standard deviations and component sizes for each normally distributed component of the length-frequency analysis using total length data.

Month	Component	Mean	S.D.	Component size
December 2008	1	84.59	22.90	73
February 2009	1	58.10	16.39	47
	2	104.80	11.26	19
April 2009	1	78.63	25.00	223
June 2009	1	91.46	27.01	195
August 2009	1	33.10	13.05	27
	2	91.14	29.51	56
September 2009	1	43.85	19.31	30
	2	101.27	24.04	50
	3	152.01	5.00	12
October 2009	1	51.75	18.94	34
	2	110.46	11.81	26
	3	152.19	5.00	7
November 2009	1	72.84	24.00	53
	2	116.16	13.66	44
	3	151.55	5.00	19
February 2010	1	59.05	11.38	52
	2	122.91	19.42	38

**Appendix 3.3:** Individual component means, standard deviations and component sizes for each normally distributed component of the length-frequency analysis using the multi-metric, *M*, data.

Month	Component	Mean	S.D.	Component size
December 2008	1	47.16	10.48	73
February 2009	1	33.55	6.40	44
	2	53.41	4.30	21
April 2009	1	40.95	10.71	223
June 2009	1	47.5	12.33	195
August 2009	1	23.47	7.32	33
	2	39.61	3.02	12
	3	54.99	7.63	38
September 2009	1	35.31	13.91	47
	2	47.81	2.50	8
	3	62.30	7.72	37
October 2009	1	25.00	3.94	12
	2	41.92	5.24	19
November 2009	1	53.15	14.60	116
February 2010	1	37.31	9.31	76
	2	62.11	6.38	32