Recruitment, settlement and ontogeny of the serpulid *Spirobranchus cariniferus* (Gray, 1843)

by

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Abstract

Serpulids are a globally represented group of polychaetes and can be found in many habitats from the intertidal fringe to the subtidal environment and even in deep-sea ecosystems. These tube-dwelling worms are often described as pioneer species in new or disturbed habitats. Serpulids secrete a calcareous tube and often occur in aggregations. These patches can range from several centimetres to several metres in diameter and may even form reef systems. Accumulations of tube-dwelling worms provide a new habitat for other species and, therefore, serpulids are considered bioengineers. Serpulid aggregations are known to enhance biodiversity and species abundance and may increase water quality through their filter activity. Despite their ecological importance, their ecology and ontogeny have received little attention.

Spirobranchus cariniferus, a New Zealand endemic intertidal serpulid, is a substantial contributor to intertidal ecosystems. For this and other Serpulidae, the link between larval development and larval settlement is missing. However, this connection is essential to understand recruitment and ecology of tube-dwelling worms. Therefore, in this thesis, I describe the ontogeny of *S. cariniferus* from larval development to recruitment and reproduction.

In the first data chapter, I present my findings on the recruitment of *S. cariniferus* in the field. This serpulid settles aggregatively in the field but not necessarily in response to the presence of adult conspecifics, as has been previously reported. Abiotic factors such as sunlight or wave disturbance have a more substantial effect on recruitment rather than the occurrence of adult individuals of the same or a competing species. Additionally, this chapter provides support for the hypothesis that larvae of *S. cariniferus* may accumulate near the substrate before settlement.

Many sessile marine invertebrate taxa occur in either aggregations or as solitary individuals, with potential benefits and disadvantages associated with each configuration. For *S. cariniferus,* solitary and aggregative individuals can be found in the same habitat. Therefore, the second data chapter compares growth and mortality for individuals living alone or in aggregation. While solitary and aggregative individuals elongate their tubes at a similar rate, further correlations of body to tube sizes lead to the conclusion that solitary worms focus more of their energy on tube length growth rather than body size increment compared to aggregative conspecifics. Mortality is highly variable but does not differ between both configurations. However, individuals living in a patch have a better ability to recover from damage to their tubes.

In the last two decades, the idea that gonochorism is the general reproductive pattern for Serpulidae has been challenged, and instead it has been suggested by some that protandry is the more common trait. Therefore, with my third data chapter, I explore maturation and sex ratio of *S. cariniferus* and whether it changes for individuals living alone vs. in aggregation or based on size. While maturation depends on size, sex does not, and neither maturation nor sex ratio are dependent on whether individuals live in aggregation or not. Further, the ratio of females to males did not favour either sex consistently. For the first time in this species I found evidence of possible hermaphroditism. Through spawning trials and histological sections, I identified nine individuals which simultaneously contained oocytes and sperm cells. I suggest therefore, that *S. cariniferus* has alternating sexes rather than protandry as a reproductive strategy.

In the fourth and final data chapter, I describe the metamorphosis and settlement behaviour of *S. cariniferus* larvae. For this serpulid species, settlement and metamorphosis are separate and distinct steps that involve both behavioural and morphological changes to the larvae. Further, this entire process can be quite prolonged (i.e. over several days), and at some points can be reversed. It is

therefore very important that observations last longer than 24–48 hours, when studying serpulid settlement.

As far as I am aware, this is the first study on a serpulid species to examine aggregative settlement in the field in relation to the presence of adult conspecifics and abiotic factors, and also to explicitly test for consequences of solitary vs. group living on growth and mortality. It is also the first to show evidence of hermaphroditism in this species. I hope my research and this thesis stimulates a more inclusive and holistic investigation of serpulids in the future. Larval development, settlement patterns and ontogeny need to be studied in detail if we want to understand the evolution, ecology, impacts and benefits of these and other sessile marine invertebrates.

In memory of Klaus Martin Wolf,

a poet, activist, friend and father

(* 04/07/1935, † 15/02/2019)

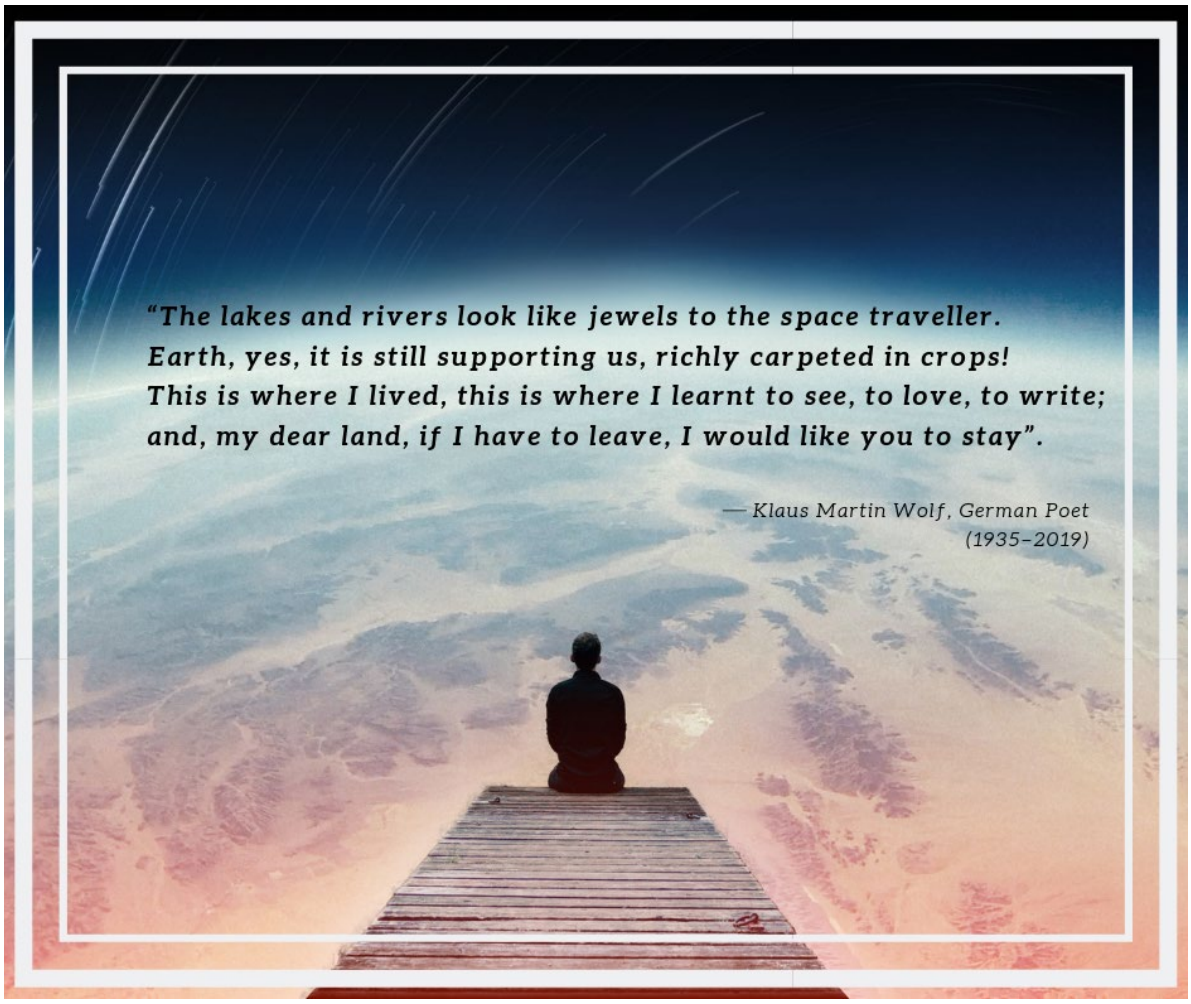
"Der Weltraumfahrer sieht die Seen und Flüsse als Geschmeide.

Die Erde, ja, noch trägt sie uns, reichlich gedeckt mit Korn!

Hier lebte ich, hier lernt' ich sehen, lieben, schreiben;

und wenn ich fort muß, Land, du möchtest bleiben"

A quote from "Abschiedsrede" by K. M. Wolf



Translation of quote from “Abschiedsrede” by K. M. Wolf

Acknowledgements

I left Germany in 2012 for a future in a distant and unknown country. Who would have thought that one milestone of this endeavour will be, bringing these very lines to paper which summarise my work to achieve a degree called Doctor of Philosophy?

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1. General Introduction

Rocky shore environments are among the richest and most diverse habitats (Murray et al., 2006). The wealth of diversity is based on the versatility of ecological niches (Palmer, 1992) and their complex physical environments caused by abiotic factors such as tides, waves and sun exposure (Kostylev et al. 2005; Cifuentes et al. 2007). For example, waves can alter habitat structure, particularly through deposition of sediment or gravel and rocks (Mann, 2000). These circumstances increase the number of microhabitats and ecological niches. Additionally, intertidal species need a high degree of tolerance for salinity and temperature variation, air exposure and other stressors (Menge et al., 1985; Sebens, 1991). In other words, a physical disturbance may prevent the dominance of any taxa in those habitats and allow a greater variety of species (Mann, 2000).

Species living in intertidal habitats often must endure various harsh conditions when the tide is out. Mobile species can hide under rocks, in crevices, or even follow the receding tide, whereas sessile species need other strategies, such as living in shells or tubes for reducing water loss. For sessile species, zonation is a typical pattern in intertidal communities. Commonly, barnacles are found relatively high on the shore, typically followed by mussels or tubeworms at mid to low tide levels, with algae at the subtidal fringe (Knox, 1953, 1949; Little and Kitching, 1996; Morton and Miller, 1973). The upper vertical distribution of sessile intertidal species is mainly limited through abiotic factors as air exposure or temperature fluctuations, whereas the lower edge of the distribution is primarily limited through biotic factors such as competition for space and food, and predators (Connell, 1961; Hidalgo et al., 2007). For example, if barnacles grow in an area lower than their normal distribution, which is dominated by other sessile invertebrates, they are at risk of being overgrown and smothered (Denley and Underwood, 1979; Fischer-Pietter, 1937). Many sessile organisms show zonal patterns in intertidal habitats (Doty, 1966; Little and Kitching, 1996; Stephenson

and Stephenson, 1949), but research about this phenomena is mainly conducted on barnacles and mussels. Particularly underrepresented in this context is the settlement and zonation pattern of tube-dwelling worms.

Serpulids are globally common in many intertidal and subtidal habitats (Knox, 1960; Kupriyanova et al., 2015; Moore et al., 2009; ten Hove and Kupriyanova, 2009; Thomas, 1994; Vanaverbeke et al., 2009). In some areas they occur in dense aggregations, forming subtidal reefs or intertidal patches on rock walls (Klöckner, 1976a; Knox, 1949; Minchinton, 1997; Schwindt et al., 2001; Straughan, 1969). Even in Australia and New Zealand, a few species like *Galeolaria caespitosa*, *Spirobranchus* cf. *krausii* or *Spirobranchus cariniferus* are common components of littoral habitats along these southern coastlines (Knox, 1960). Intertidal and subtidal aggregations of serpulids and sabellarids can increase oxygenation and enrich the intertidal and subtidal habitat complexity (e.g. Bianchi & Morri 1996; Davies et al. 1989; Vanaverbeke et al. 2009), and for that reason they can be considered as bioengineers (sensu Jones et al. 1994). Also, in New Zealand, serpulid aggregations may be an important nursery and habitat for various species (Knox, 1949; Smith et al., 2005). However, despite the potential important ecological role of serpulids, research about settlement dynamics and zonation exists only for a few of the several hundred species. Although the research on Sabellida aggregations has been ongoing for decades with focus on early ontogeny, fouling, reef development and ecological impact (mainly of invasive species and their reefs), there remain many unanswered questions with regard to tubeworms. For example, the origin of worm reefs is still unresolved, as well as the causes and consequences of both aggregative and solitary individuals within the same species and even in the same habitat (Smith et al., 2012; Toonen and Pawlik, 2001a, 2001b, 2001c, 1994).

1.1 Serpulinae (Rafinesque, 1815)

The Polychaeta taxon Serpulinae (Rafinesque, 1815) is a subfamily of the Serpulidae that has a global distribution (Glasby et al., 2000) with around 350 known species (Kupriyanova, 2003; Kupriyanova et al., 2006; Rouse and Pleijel, 2001; ten Hove and Kupriyanova, 2009). Included among these are some of the most problematic invasive species worldwide, *Hydroides elegans* (Haswell, 1883) and *Ficopomatus enigmaticus* (Fauvel, 1923) (GISD, 2008; NIMPIS, 2014). All Serpulinae are sessile as juveniles and adults. They settle solitarily or form aggregations ranging in size from a few centimetres to more than 100 m in diameter (Bianchi and Morri, 2001; Ramos and San Martín, 1999; Schwindt et al., 2004; Smith et al., 2012; ten Hove and van den Hurk, 1993). These worms secrete a calcareous tube, which is commonly attached to rocks but also have been observed on shells of turtles, crabs and various molluscs, algae and various other substrates (e.g. Bailey-Brock, 1976; Bick, 2006; Dittmann et al., 2009; Glasby et al., 2000; Hartmann-Schröder, 1982). The size of individuals in the Serpulinae ranges from 2–100 mm in length, and their lifespans can last between several months and up to 35 years (Glasby et al., 2000). The ability of settled worms to move is mainly limited to a retraction of the individual in its tube in response to danger.

1.1.1 Ecology

Although serpulins are a major component of many marine systems, including intertidal reefs, compared to other sessile organisms (e.g. mussels and barnacles), very little is known about the ecological roles of many tubeworms. Adult individuals of some Serpulinae taxa have a broad tolerance to various stressors such as salinity or temperature (e.g. Knox 1949; Hartmann-Schröder 1996; Dittmann et al. 2009). Some species are characterised as polyhaline to euryhaline. Species of the genus *Ficopomatus* (Southern, 1921) are even able to live in brackish water habitats (ten Hove and Kupriyanova, 2009). These tube

dwellers are non-selective filter feeders. Because of their small size and calcareous tubes, predation from bigger predators, such as fish, is rarely observed. For example, in fish stomach content surveys, only a low number of serpulids have been occasionally recorded (Hiatt and Strasburg, 1960; Randall, 1967). Bosence (1973) describes predation of *Symphodus melops* (Linnaeus,1758) (formerly *Labrus melops*) (a fish) and *Asterias rubens* (Linnaeus,1758) (a starfish) on the relatively large *Serpula vermicularis* (Linnaeus,1767). Otherwise, it has mostly been observed that predators feed only on the tentacular crowns rather than the whole individual (Kupriyanova et al., 2001; Randall, 1967).

A few taxa of the Serpulinae have been referred to as pioneer species as they colonise and recolonise intertidal and subtidal habitats after disturbance (Rasmussen and Brett, 1985). Particular species like *F. enigmaticus* or *S. vermicularis* are capable of building reefs and therefore they are considered bioengineers (McQuaid and Griffiths, 2014). These reefs occur mainly in areas with hard substrates, but serpulid clusters also occur in soft bottom habitats (*S. vermicularis*, *Galeolaria hystrix)* (Mörch, 1863) and mudflats with settlers on small, isolated hard substrate, like rocks and shells, and can grow out to reefs up to several metres in height and width (*F. enigmaticus*) (Fornós et al., 1997; Heiman et al., 2008; Moore et al., 1998; Riedi, 2012; Schwindt and Iribarne, 2000; Smith et al., 2005). Aggregations formed by serpulid worms often have a positive impact on species diversity and abundance and also provide hard substrate and refugia for other species in soft-sediment habitats (Bianchi and Morri, 1996; Chapman et al., 2012; Haanes and Gulliksen, 2011; Smith et al., 2005). They can also lead to a higher diversity in the community, for example through the increased hard substrate in soft bottom communities or provided refuge (Bazterrica et al., 2011; Bruschetti et al., 2011, 2009, 2008; Schwindt et al., 2001). In general, the calcareous structures formed by serpulins increase local habitat complexity (Riedi, 2012), and these worms may improve water quality through their filtering activity (Davies et al., 1989).

Intertidal serpulins like *S. cariniferus* or *G. caespitosa* occur mainly on hard substrate areas and in more patchy aggregations rather than larger and more complex reefs. Nevertheless, they may have substantial impacts on biodiversity due to the provision of shelter for mobile species. For example, mussel and barnacle aggregations provide a variety of small, temporary microhabitats with a more stable climate in which mobile species find protection from desiccation or sunlight (Seed 1996, Hidalgo et al. 2007). Similarly, serpulid aggregations also offer a broad range of habitats for other species. In fact, Heiman et al. (2008) recorded almost ten times more species in intertidal serpulid aggregations compared to an oyster equivalent at the same estuary (Elkhorn Slough, California).

1.1.2 Reproduction

Amongst Serpulinae, most species release their gametes into the open water, although brooding is more common than previously assumed for this taxon (Kupriyanova et al., 2001). Gamete maturation and spawning seem mainly to be regulated through temperature. Maturation of juvenile individuals has been reported within 4–6 weeks post settlement for *Hydroides uncinate, F. ushakovi* (Pillai, 1960) *(formerly Mercierella enigmatica*, Hill, 1967) or within two weeks post settlement for *H. elegans* (Qiu and Qian, 1998). Zuraw and Leone (1972) were able to artificially induce the maturation of *Hydroides dianthus* (Verril 1873) through raising the temperature. Further, various species like *F. enigmaticus* and *H. dianthus* are probably able to undergo multiple spawning events within one season (Dixon, 1981; Kupriyanova et al., 2001; Zuraw and Leone, 1972). In general, it is assumed that serpulins are dioecious. However, based on biased sex ratios and and size differences between both sexes, some researchers have suggested the possibility of protandric sequential hermaphroditism in some if not most species (Dixon, 1981; reviewed by Kupriyanova et al., 2001). The patterns of sexual reproduction for serpulins remain a poorly studied aspect of the biology of this group.

1.1.3 Development from larva to settled juvenile

Currently, larval development has been described to some extent for 48 Serpulinae species. Of these, 12 taxa have lecithotrophic development and seven are likely to be brooding, and thus also have non-feeding development, and 4 have unknown developmental strategies. The remaining 25 serpulin species have planktotrophic development (Giangrande, 1997; Kupriyanova et al., 2001; Tampi, 1960). Planktotrophic larvae can reach competence to metamorphose, and therefore settlement can occur within one to two weeks (Bryan et al., 1997; Gosselin and Sewell, 2012). Metamorphosis and settlement can occur in response to particular cues such as conspecifics eg. *H. dianthus* or microbiological film e.g. *H.elegans* (e.g. Toonen & Pawlik 1994; Bryan et al. 1998; Lau et al. 2002; Hung et al. 2005). For example, under some conditions, if appropriate settlement cues are not provided, or in response to starvation, or the presence of other organisms (e.g. certain copepod species), larvae may delay settlement and maintain planktotrophic stages (Dahms et al., 2004; Dahms and Qian, 2005; Hung et al., 2005). Such delay in ontogeny can also be observed for many other propagules of marine invertebrate taxa like molluscs, echinoderms, ascideans or crustaceans (Eyster and Pechenik, 1987; Forward et al., 1994; Lucas et al., 1979; Olson, 1983; Pechenik, 1990; Wolcott and Devries, 1994).

The terms metamorphosis and settlement have been used in inconsistent ways for marine invertebrates species, including serpulins and other tubeworms, and there is a need to differentiate between these events (Kupriyanova et al., 2001; Rodriguez et al., 1993). Marsden & Anderson (1981) described the metamorphosis of *G. caespitosa* with two processes: the shift from a planktonic to benthic stage (settlement) followed by an attachment and morphological change to the adult form (metamorphosis). It seems that this chronology of developmental processes is generally uniform amongst serpulins and sabellarids.

Reaching competence to metamorphose seems to mainly depend on larval size (Toonen and Pawlik, 2001b) for many Sabellida species. Once the offspring has reached the crucial size for metamorphosis, it will transform to a nectochaeta larva and begin to swim close to, or crawl on, the settlement substrate (Lau et al., 2003; Young & Chia, 1982). It is plausible to assume that larvae “search” for suitable settlement substrates and/or conspecifics (Wilson 1968); consequently, this behaviour could indicate the competence of the larvae to settle. The attachment is accompanied by further morphological changes and the secretion of tube substances; the last steps could be defined as settlement.

The appearance of juvenile morphological characters early in the larval development, well before settlement, has been described as *Galeolaria caespitosa* (Lamarck, 1818), *Spirobranchus triqueter* (formerly *Pomatoceros triqueter*, (Linnaeus, 1758), thus contributing to the muddying of distinctions between these processes (Andrews & Anderson 1962; Groepler 1984; reviewed by Kupriyanova et al. 2001). Further, only a few morphological studies about the metamorphosis of selected serpulins have been published (e.g. Wisely 1958; Grant 1981; Marsden & Anderson 1981; Kupriyanova et al. 2001). However, to date, there has been no effort to compare those crucial steps that occur between planktotrophic and benthic life for various serpulins. It is unknown if the development amongst serpulid species is similar or are if there species-specific differences.

1.1.4 The origin of aggregations

Sessile marine invertebrates often occur as solitary individuals or in aggregation. Gregarious settlement has been described for various species of ascidians, barnacles, mussels, oysters and tubeworms (Crisp, 1967; Hadfield and Paul, 2001; Jensen and Morse, 1975; Keck et al., 1979; Knight-Jones and Stevenson, 1950; Okamoto et al., 1998; Svane et al., 1987; Toonen and Pawlik, 1996). From predominantly laboratory trials, it often seems that propagules settle in response

to cues emitted by biofilm or adult conspecifics (Beckmann et al., 1999; Hidu, 1969; Pawlik, 1990; Wright and Boxshall, 1999). However, settlement has often only been observed in the laboratory under artificial conditions. Cues that are important in the laboratory may be swamped in the field by other biotic and abiotic factors, so it is important to study settlement in natural conditions (Pechenik, 1990; Rius et al., 2010; Sulkin, 1990).

If larvae settle in response to conspecifics, it is challenging to explain how a new aggregation begins in a place with no previous conspecifics. Various ideas have been considered (Toonen and Pawlik, 2001a). Particularly for tubeworms, one suggestion is that larvae, after an extended pelagic stage, lower their threshold for a settlement cue and become more likely to settle isolated (Knight-Jones, 1953; Toonen and Pawlik, 2001a) and in this way form the start of a new aggregation. However, this hypothesis is mainly suggested for species which develop via a lecithotrophic larval stage like *Sabella spallanzanii* (Giangrande et al., 2000) or *Spirobis borealis* (E. W. Knight-Jones, 1953; Kupriyanova et al., 2001), which cannot feed and therefore deplete their energetic reserves until the point they must settle or will die.

An alternative mechanism has recently been suggested for *Hydroides dianthus*. Some larvae of *H. dianthus* seem to be able to settle in response to a biofilm; the attached individual subsequently attracts conspecific larvae (Toonen and Pawlik, 2001c). This hypothesis is referred to as the ‘founder and aggregator hypothesis’. The hypothesis could suggest that larvae at least accumulates near a settlement substrate. In support of this, it has been suggested that larvae could stay in close proximity and settle together (Bryan et al., 1997; Keough, 1983; Marsden, 1991). In this context, solitary individuals could be explained by pre- or post-settlement mortality.

1.1.5 Consequences of living alone vs. in aggregation

Many species that commonly form dense aggregations also have individuals that occur alone. To understand how living in aggregation is beneficial, it is essential to understand the advantages and disadvantages of aggregative and solitary living for sessile invertebrates. In some species, for example, living in aggregation improves probability of survival by mitigating physical stress such as wave action and desiccation (e.g. Barry, 1989; Bianchi and Morri, 1996; Thomas, 1996), and may also increase likelihood of successful reproduction (Hidu, 1971; Kupriyanova, 2006; Qian, 1999; Thomas, 1994). However, living patches also have downsides for the individual, such as higher intra- and interspecific competition for food, space and oxygen (Bertness et al., 1998; Bryan et al., 1997; Woodin, 1976). Increased physiological stress can lead to increased mortality and decreased reproductive success (Fréchette et al., 1992; Hart et al., 2012; Hart and Marshall, 2013; Svane and Ompi, 1993).

For tubeworms, these issues have not been sufficiently addressed. Solitary individuals may have more energy resources for reproduction compared to individuals in an aggregation due to lower competition. However, fertilisation success could be reduced because of a lack of synchronisation and increased distance between males and females (Eckman, 1996; Levitan et al., 1992, 1991). Living alone or in aggregation can influence both the reproductive success of the species and possibly also affect the spatial distribution of males and females. Because males make orders of magnitude more gametes than females (Kupriyanova et al., 2001; Leone, 1970; Levitan and Petersen, 1995), it is plausible that within aggregations the ratio of females to males could be greater compared to groups of solitary individuals, as fewer males would be required (in relation to females) for the fertilisation of eggs. Although hermaphroditism has been reported for some serpulins (e.g. Dixon 1981; Hartmann-Schröder 1982), in general, this group is thought to be dioicous. However, more work is required to test whether hermaphroditism is more common than currently thought and

whether its prevalence varies according to whether individuals are in aggregation or not. For example, some serpulid species may be protandric hermaphrodites (males first, becoming female with age and increasing size) (e.g. Cotter et al., 2003; Kupriyanova et al., 2001). If this was the case, then sex may be affected by the individual growth rate and whether they live in aggregation (if this affects growth rate) (Premoli and Sella, 1995).

1.2 Spirobranchus cariniferus (Gray 1843)

Spirobranchus cariniferus is a member of the Serpulidae order and was first described by Gray in the year 1843 (in “Fauna of New Zealand”) as *Vermetus cariniferus* (Knox, 1949; Read and Fauchald, 2019). Thirty-five years later, Hutton synomised *V. cariniferus* as *Placostegus coeruleus*. In the year 1903, Ehlers described more individuals from the Marlborough Sounds and Auckland Harbour and summarises *P. coeruleus* and *Pomatoceros strigiceps* (Morch 1863) as *Pomatoceros caeruleus,* which was first described by Schmarda (1868) in South Africa. However, in parallel, Ehlers (1907) described *Spirobranchus cariniferus* as a separate species.

Between 1927 and 1928, further specimens were described as *P. coeruleus* from the Cape Maria van Dieman by Augner and Benham. However, Benham already doubted that the serpulids described from South Africa were the same as the one found in New Zealand (reviewed by Knox, 1949). Later, Pacific members of the genus *Pomatoceros* (Philipi, 1844) were placed in the genus *Spirobranchus* (de Blainville, 1818) (Fiege and Ten Hove, 1999; Glasby and Read, 1998; ten Hove and Kupriyanova, 2009). Thereafter, and in agreement with Ehlers (1907), *Spirobranchus cariniferus* was accepted as a species.

This worm is found throughout New Zealand in intertidal areas and rock pools both solitarily and in aggregations. The distribution of *S. cariniferus* is endemic to

New Zealand (Read and Fauchald, 2019; Smith et al., 2012) and is generally limited by the availability of hard substrate. In sheltered areas, this tube-dwelling worm can become the dominating aggregative organism at mid to lower tide levels and exclude other sessile organisms (Morton and Miller, 1973). Nonetheless, despite its endemism, almost nothing is known about the ecology of this species (Gosselin and Sewell, 2012; Knox, 1949; Morton and Miller, 1973).

Knox (1949) was the first to describe early larval stages of *S. cariniferus*, as well as some ecological features of this species. This was followed more than half a century later, by an investigation of the mineralogy of *S. cariniferus* tubes (Riedi, 2012) and a study on reproduction of *S. cariniferus* that includes the larval growth, settlement and metamorphosis of this species (Gosselin and Sewell 2012). Due to the low number of studies on this species, many questions remain, in particular around recruitment ecology. Further, those gaps of knowledge exist for serpulids in general, and therefore research in these areas on *S. cariniferus* can increase our understanding for many other tube-dwelling polychaetes.

1.3 Study area: Wellington Harbour

All of my field studies have been conducted at six different sites around the Miramar Peninsula in Wellington Harbour (Figure 1.1 & Figure 1.2). Study sites were selected based on accessibility and presence of *S. cariniferus* aggregations. Wellington Harbour is located on the southern tip of New Zealand's North Island (Stevens, 2018) and is a natural semi-enclosed embayment that opens in the south into Cook Strait (Figure1.1). In this opening protrudes the Miramar Peninsula, which is connected in the west to the main area of Wellington. The coastline around the peninsula is mainly rocky shore intermittently interrupted by sandy bays and cobble beaches. The salinity in Wellington Harbour ranges between 30 and 35 PSU, and the water temperature

fluctuates in winter between 8°C and 13°C, and in summer between 15°C and 21°C (GWRC, 2019). The tidal movement in the harbour is semidiurnal with a tidal movement around 0.75 m (LINZ, 2018). At all six sites, the rocky substrate consists of more or less hard arkose or greywacke sandstone with a mostly vertical relief (Lachowicz, 2005; Morelissen, 2012), and the high to mid tide levels are dominated by barnacles, mainly the honeycomb barnacle *Chamaesipho columna* (Demello and Phillips, 2011; Stevens, 2018). Subsequently, from the mid to low tidal level, the mussel *Mytilus galloprovincialis* or *S. cariniferus* are the dominant sessile animals (Demello and Phillips, 2011; Stevens, 2018). Further north on the west coast of the greater Wellington region I collected additional specimens at Porirua Harbour and Pukerua Bay (Figure1.3). A further detailed description of all sites, including their geographic coordinates, can be found in the Appendix.

1.4 Conclusion and aims

Despite their broad and prominent distribution, for many serpulids, almost nothing is known about their development, ecology and reproduction. Due to the global distribution of serpulids, their economic and ecological impacts as fouling organisms and invasive species, and their mostly positive effects on biodiversity and water quality, research on tube-dwelling worms is crucial. What research has been done has only been on a very few serpulid species. The origin of aggregations and the appearance of gregarious and solitary individuals remains unclear. More light on the ontogeny of serpulids, as well as the processes of settlement and metamorphosis, will improve our understanding of the dynamics of recruitment of tubeworms, which will help the management of invasive species. The specific aims of my thesis are found below and are the subject of each of the following chapters:

Chapter 2: To examine recruitment of *S. cariniferus* in the field. In particular, I test whether they settle in an aggregated or random pattern, and factors affecting recruitment like conspecifics and abiotic factors (such as light and hydrodynamics).

Chapter 3: To examine potential differences between aggregative and solitary individuals such as growth, mortality and morphology.

Chapter 4: To describe the reproductive cycle and sex expression of *S. cariniferus* and ask whether there is a difference in maturation rate between aggregative and solitary settled individuals, if size affects sex ratio and if *S. cariniferus* are hermaphrodites.

Chapter 5: To describe growth, development settlement and metamorphosis of *S. cariniferus* larvae.

Figure 1.1 Overview of the greater Wellington region, scale: 10 km (Google Earth, 2018).

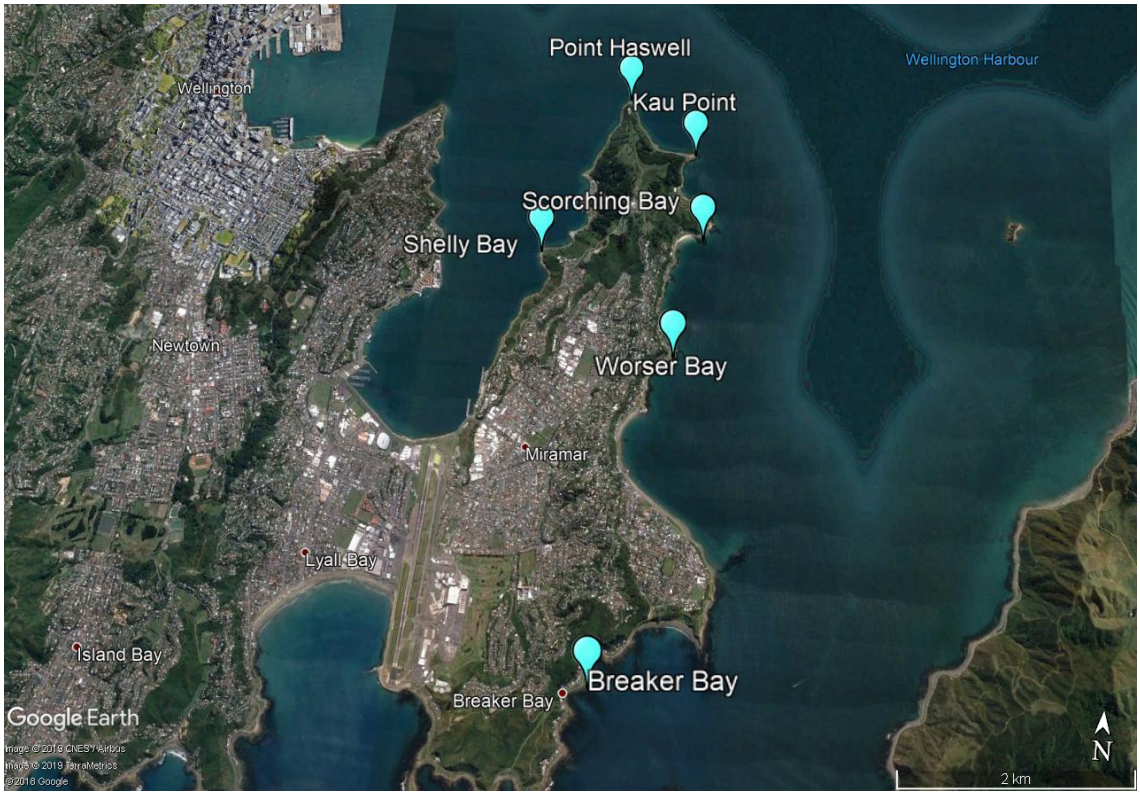

Figure 1.2 Overview of sample sites on the Miramar Peninsula, scale: 2 km (Google Earth, 2018).

Figure 1.3 Overview of sample site location Porirua Harbour and Pukerua Bay, in the north of the greater Wellington region, scale: 4 km (Google Earth, 2018).

2. Recruitment of *S. cariniferus* into various habitats

2.1 Introduction

Aggregative settlement is common amongst subtidal and intertidal marine sessile invertebrates (Keough, 1983), and gregarious marine invertebrates are a ubiquitous and important component of communities. Aggregations have been described all over the world, for example, in mussels (Ackerman et al., 2001; Wildish et al., 1998), oysters (Endean et al. 1956; Luckenbach et al. 2005) and barnacles (Endean et al., 1956; Raimondi, 1988a). Aggregations of tube-dwelling worms, like *S. vermicularis* or *Lanice conchilega* (Pallas, 1766) in particular, have been noted in many regions for their potential as bioengineers (Moore et al., 1998; Vanaverbeke et al., 2009) and because they are often invasive fouling species (Dittmann et al., 2009; Heiman et al., 2008). Worm aggregations known from Europe are, for example, *F. enigmaticus, S. triqueter, L. conchilega* (Bianchi and Morri, 1996; Fornós et al., 1997; Vanaverbeke et al., 2009); examples from Australia and South Africa are *F. enigmaticus, S.* cf. *krausii* (Davies et al., 1989; Glasby et al., 2000; Knox, 1960; McQuaid and Griffiths, 2014) and from North and South America *F. enigmaticus, H. dianthus* and *Phragmatopoma californica* (Fewkes, 1889) (Jensen & Morse 1984; Schwindt & Iribarne 2000).

Aggregations of marine invertebrates on the benthos are often thought to occur as a function of larval recruitment from the plankton (Keough and Downes, 1982; Knight-Jones, 1951; Toonen and Pawlik, 1994) and the settlement behaviour of the larvae in response of the appearance of biofilm (Dobretsov, 2009; Hadfield, 2011; Shimeta et al., 2012; Whalan and Webster, 2014) or conspecifics (Head et al., 2004; Miron et al., 1996; Pawlik, 1988). It is assumed that cues emitted by biofilm, or adult individuals of the same species, induce larval settlement and attachment (Bryan et al., 1998, 1997; Hung et al., 2005; Lau et al., 2002; Toonen and Pawlik, 1996) because they indicate a suitable habitat (Hadfield, 1986; Qian, 1999). In some studies, larvae of barnacles like *Semibalanus balanoides* (former

Balanus balanoides, Linnaeus, 1767) and sessile polychaetes like *Sabellaria alveolata* (Linnaeus, 1767) have been observed searching for conspecifics before attachment to a surface (Knight-Jones, 1953; Wilson, 1968).
The occurrence of aggregative settlement has been tested in the laboratory for a variety of sessile marine invertebrates, for example the mussel *Mytilus edulis* (Linnaeus, 1758) (Eyster and Pechenik, 1987), oyster *Crassostrea virginica* (Gmelin, 1791) (as *Ostrea virginica,* Nelson, 1924), serpulin *Hydroides dianthus* (Toonen and Pawlik, 1996) and barnacle *Balanus amphitrite* (Rittschof et al., 1984). However, laboratory findings are often not applicable to the field where the environment is more complex and comprised of more than the factors utilised in the laboratory (Rius et al., 2010). I could only find seven publications (Bayne, 1964a; Berntsson et al., 2004; Crisp and Meadows, 1962; Diederich, 2005; Hoffman, 1989; Knight-Jones and Stevenson, 1950; Porri et al., 2007; Schmidt, 1982) for barnacles and bivalves which tested for aggregative settlement in the field. For example, for the barnacle *Elminius modestus* a higher rate of recruitment was observed on plates with conspecifics compared to bare plates. This was observed both in the laboratory and in natural habitats, mud flats and areas covered with oyster shells (Knight-Jones and Stevenson, 1950). For *Balanus improvises,* plates treated with extract from conspecifics had a similar rate of recruitment to untreated plates (Berntsson et al., 2004). However, gooseneck barnacle (*Pollicipes polymerus*) larvae settled gregariously during peak season or on the peduncle of adult conspecifics (Hoffman, 1989). Also, larvae of the oyster *Crassostrea gigas* settled in ~3 times larger numbers near conspecifics compared to mussels on the North Sea coast (Diederich, 2005).

Further, although laboratory studies can isolate factors that influence the process of larval settlement and metamorphosis, this does not equate to recruitment to the adult population, which results from a combination of factors from pre- to post- settlement (Connell, 1985; Keough and Downes, 1982; Pineda et al., 2009; Yool et al., 1986).

For example, tidal height modifies biofilm development and other associated cues for settlement through longer exposure to UV radiation and air (Hung et al., 2005). Also, tidal height increases desiccation and decreases the supply of food for the settling larvae (Bayne et al., 1988; Bertness et al., 1999). In contrast, laboratory studies often report settlement in response to only one stressor or cue and limit observations only till settlement. This limited combination of stressors and observations may lead to false interpretations of recruitment. Therefore, while recruitment is important in underpinning the foundation of tubeworm aggregations, it has not been well-studied, as the focus has largely been only on larval settlement.

Some serpulins, like other aggregative sessile marine invertebrates, are of concern due to fouling on infrastructure, for example, *F. enigmaticus*, or *H. elegans* (GISD, 2008; Read and Gordon, 1991; Walters et al., 1997). These tube-dwelling polychaetes are also known as important 'bioengineers' (Jones et al., 1994; Vanaverbeke et al., 2009) because they can alter the local environmental conditions and provide habitat structures for other species, particularly in soft-bottom systems (Fornós et al., 1997; Schwindt and Iribarne, 2000; Smith et al., 2005). Therefore, it is important to understand the recruitment pattern of serpulids in their natural habitat.

Causes of aggregative settlement have been tested in laboratory experiments for a few serpulid species, (e.g. *Hydroides elegans*, *H. dianthus*, *Spirobranchus cariniferus*). One main conclusion of these experiments is that this settlement pattern is caused by the attraction of larvae to conspecific adults or their tubes (Bryan et al., 1997; Gosselin and Sewell, 2012; Toonen and Pawlik, 1994). However, so far, only Minchinton (1997) tried to test for aggregative settlement in the field for serpulins; he used the species *Galeolaria caespitosa.* In field studies, the author inserted gaps in aggregations of adults. When new recruits settled closer to the edge of the gap rather than randomly, Minchinton argued that larvae of *G. caespitosa* settled gregariously in the field. Other authors have argued

against the active, gregarious settlement of larvae of other serpulid species like *Hydroides elegans* in the field, instead suggesting that individuals, due to passive distribution, accumulate in refuges from turbulent water behind artificial and natural structures (Walters et al., 1997). Therefore, there is little consensus on what processes around settlement and recruitment cause aggregations of serpulins and other aggregative sessile polychaetes in the field.

In this chapter, I focus on the recruitment of the native New Zealand serpulid, *S. cariniferus*, in response to biotic cues and abiotic factors. The first aim is to examine if larvae of *S. cariniferus* settle in the field in response to cues from adult conspecifics. If so, I predict that recruitment will be higher in aggregations of established adults compared to bare rocks or mussel beds. The second aim is to examine if larvae settle in response to cues for recently settled conspecific juveniles. If so, then recruitment of new settlers should be aggregated. The third aim is to describe environmental limitations to settlement by examining vertical distribution of recruitment and the effect of shade.

2.2 Methods

2.2.1 Do adult conspecifics mediate recruitment?

To examine recruitment in different microhabitats, I deployed sandstone settlement plates (48 x 48 x 19 mm l/w/h) by bolting them to a rocky substrate. From January 2015 to July 2017, plates were installed at Worser Bay and Scorching Bay on the Wellington coast. From June to December 2016, I installed plates at 3 additional sites: Kau Point, Shelly Bay and Breaker Bay (see in Appendix p. A1–p.A8 for site descriptions). In previous pilot experiments, I trialled various material for settlement plates such as ceramic tiles and PVC slates to which I glued grinded sandstone or conspecific tubes. The recruitment to these plates was limited, and therefore I trialled sandstone plates as described by O'Donnell (1986) as those are closer to the native settlement substrate of

S. cariniferus. Recruitment to these plates was comparable to recuriment studies on other serpulin species; therefore, for all my recruitment studies, I used sandstone plates.

In general, at each site, I installed 6 plates inside *S. cariniferus* patches and 6 onto nearby bare rock outside of patches. Additionally, at four locations (excluding Breaker Bay) I installed 6 plates inside aggregations of the blue mussel *Mytilus galloprovincialis* and 6 on nearby bare rock. If necessary, for all plates attached to bare rock, I removed any visual trace of mussel or tubeworm in a radius of 10 cm around the plate. For these approaches, I have only considered worm or mussel aggregations of at least 30 cm in diameter, and the plate was attached centred in the patch. I attempted to exchange the plates with clean ones monthly during the recruitment period. However, due to environmental (e.g. wave action or erosion) and logistical conditions, it was not always possible. Further, in the winter months, the plates were not exchanged or removed. Plates were brought back to VUCEL (Victoria University Coastal Ecology Lab) where I examined each by using a dissecting microscope and counted all *S. cariniferus* recruits. If recruitment was high, the plates were stained with Methylene Blue to increase the contrast. The recruitment for each plate has been expressed in recruits per cm^2 per day.

I monitored recruitment to the different microhabitats over a period of almost three years. There was a distinct settlement season (summer-early autumn, Figure 2.08); however, the recruitment where for each plate highly variable. Therefore, for the analysis, I only used the data from the peak season, January–May, in 2016 and 2017 because the data collection in 2015 began in March, halfway through the season. I pooled the data for each microhabitat and across 2016 and 2017 in three categories: Mid-Summer = January – February, Late Summer = February – March, Early Autumn = March – April/May. I used an Anderson-Darling test (Stephens, 1974), a Shapiro Wilk test (Ghasemi and Zahediasl, 2012) as well as density- and QQ-plots to explore the distribution of

the recruitment data. It was not normally distributed. The data were also over-dispersed (Cameron and Trivedi, 1990). Therefore, to examine whether there was an effect of microhabitat (e.g. inside mussel or worm aggregations, or bare rock) on recruitment, I fitted a negative binomial model (Gardner et al., 1995; Ver Hoef and Boveng, 2007), with site and year as random factors, and month and microhabitat as fixed factors. The response variable was the count data of recruitment to plates in each month. All statistical analysis have been performed with R (version: 3.5.1 'Feather Spray', 2018, see section 2.2.4).

2.2.2 Do solitary adults mediate the development of new aggregations?

For this approach, I counted recruits around solitary adult *S. cariniferus* on images that were taken every two months, for tube growth measurement (Chapter 3) at Worser Bay (see in Appendix p. A7 for site descriptions) (Figure 2.01 and 2.02), in the summer months of 2015 and 2016. In 2015, I examined the area immediately surrounding each of the 50 solitary individuals from January and March, and around 14 individuals between March and May, 2015. In 2016, I observed the area around 32 individuals between December 2015 and February 2016, as well as 25 individuals between February and May.

Fig. 2.01

Fig. 2.02

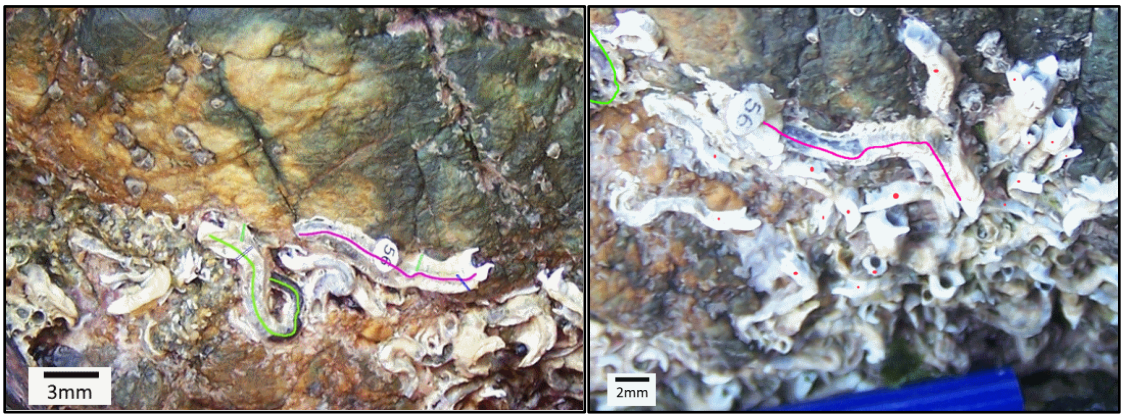

Figure 2.01 Shows several worms that were measured on 30/01/2015. Figure 2.02 Shows the same worms as in Fig. 2.01 marked with lines and new recruits, marked with red dots on 30/09/2015.

2.2.3 Are *S. cariniferus* recruits distributed randomly or in aggregated patterns?

To address whether *S. cariniferus* recruits settle in an aggregated pattern, which would suggest gregarious settlement of larvae, I examined the distribution of new recruits with respect to each other. A subset of 23 randomly selected settlement plates with 8 - 343 recruits from the first experiment were used. The side of the plate which was closest to the rock (i.e. the backs) were photographed using a Canon IXUS digital camera. To test whether settling in aggregation was dependent on the density of settlers, I put plates into three categories according to the number of recruits: low (8–50), medium (51–150) and high (>150). On each plate the distance between each recruit and its nearest neighbour was calculated using ImageJ (1.48v) software (Schnerider et al., 2014) and nearest neighbour analysis based on Clark and Evans (1954). The starting point of each measurement was the posterior end of the tube, as that is where the larva initially settles and begins to grow in length as a juvenile. If the nearest neighbours were touching each other, then I measured to the centre line of the tube (Figure 2.03). I calculated the average distance to the nearest neighbour (d_a) for each individual. If the distance between one individual and its nearest neighbour was longer than 20mm (which is approximately half the length of a plate), then that worm was not further considered because the nearest neighbour for those individuals could have been on the rock surface to which the plate was attached, rather than on the plate itself.

Given the total area of the surface and the count of all recruits on that surface, I calculated the density (ρ) of recruits for each plate: ρ = n/mm^2

I then estimated the expected distance (d_e) to be: $d_e = \frac{1}{2\sqrt{\rho}}$

I followed up with calculating the standard error (sr) for the expected distance to the closest neighbour: $sr = \frac{0.26136}{\sqrt{n\rho}}$

Then the standard nominal deviate (z) was calculated: $z = \frac{(d_a - rd_e)}{sr}$

If z is smaller than 1.96, then tubeworms settle randomly on a plate. I calculated that the ratio (R) of observed to expected distance between two conspecifics as: $R = d_a/d_e$.

If $R \leq 1$ then recruits are distributed randomly on this surface, but if R>1 then these worms are aggregated (Clark & Evans 1954).

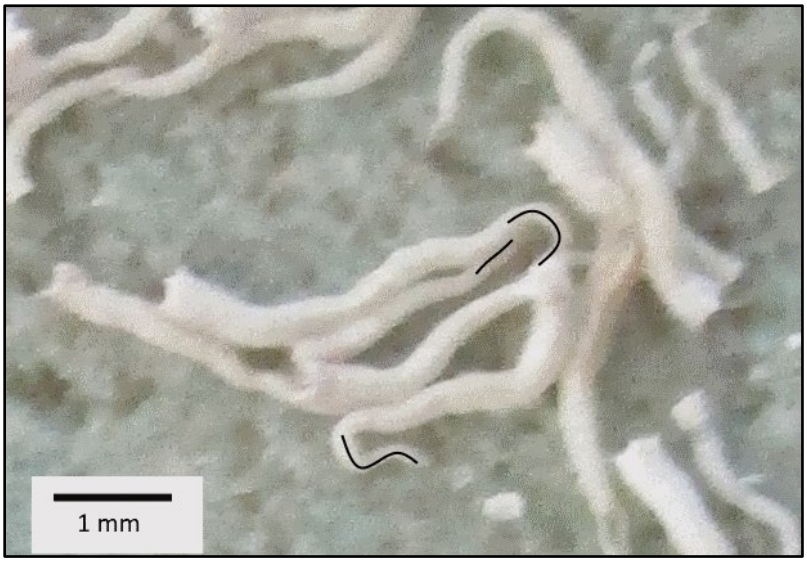

Figure 2.03 Juvenile tube worms on a settlement plate. The start of the centreline is marked with dark lines for three individuals.

2.2.4 Abiotic factors contributing to settlement patterns: Vertical height and shade

Other studies on serpulin species like *G. caespitosa, H. elegans* and other sessile species like *Chthamalus stellatus* (Poli, 1791) or *Mytilus edulis* (Linnaeus, 1758) have found that vertical height and sunlight are dominant abiotic factors which can affect recruitment success particular for intertidal species (e.g. Harley and Helmuth, 2003; O'Donnell, 1986; Shafer et al., 2007; Suchanek, 1981). As the substrate at my sites is predominantly sandstone, I was able to test for these abiotic factors with limited effect of other abiotic factors like different settlement substrate (Raimondi, 1988a).

To test *S. cariniferus* recruitment to various intertidal vertical heights, I placed sandstone settlement plates (80 x 80 x 19 mml/w/h) at three tidal heights: low,

mid and high. Low was defined as the zone from the upper distribution of large-bodied algae (like *Undaria* spec.) to approximately 50 cm above the water level at low tide. In this zone, the plates were installed as low as possible given the conditions of surface and water level. The mid-zone was defined as a 50 cm wide band above the low zone, and plates were installed in the middle of this zone. The high zone was characterised as the area 50 cm above the mid zone. Plates were introduced in the upper third of that zone (Figure 2.06 & 2.07).

In addition to being deployed at three different heights, to examine the role of shade in mediating recruitment, each plate was half covered by a “roof” made of clear acrylic (40x80x3 mm l/w/h) attached 5 mm above the plate (Figure 2.04 & 2.05). Half of each roof was coated with white paint (“shaded roof”), which limited exposure to the sun. The unpainted half (“clear roof”) provided a procedural control for the addition of the structure. The area under the clear roof and the shaded roof was approximately 1,600 mm^2. The non-roofed part of the plate served as a control for the roof and was 80 mm x 40 mm (3,200 mm^2). At Worser Bay, I deployed 30 plates (10 at each height). At Kau Point, I deployed 5 plates at each height, and at Shelly Bay, I used eight plates in the high zone, eight plates in the low and seven plates in the mid zone (see in Appendix p. A2, A5 & A7 for site descriptions).

The number of recruits was counted using a dissecting microscope, as described earlier (see above). Given logistical constraints and environmental conditions, the plates were installed on different dates between December 2016 and February 2017 and were collected between April and May 2017. However, the results of recruitment to mussel and worm patches enabled me to define the time of recruitment for the period of January to March 2017.
Initially, the aim was to analyse and present the results of vertical height and shade in a full factorial 2-way ANOVA-test. However, recruitment to the front site of the plates mainly only occurred in the low zone. At the mid-level, only one plate had settlers to the front side. Because of this recruitment pattern the

decision was made to test both recruitment factors separately. To test the effect of shade, I only included plates from the low zone, and only those with recruitment to the front of the plate. To test the effect of vertical height, I only counted recruits on the back and edge of all plates.

For both experimental datasets in the peak season (January–April 2017), I tested the distribution for normality by visualising density- and QQ-plots. I performed a Shapiro Wilk test and a Levene test with the results of an ANOVA. The data did not fulfil the assumptions of ANOVA, and no transformation improved the fit. The data were also overdispersed (Zuur et al., 2011). Therefore, for each approach, I first used a Kruskal Wallis test to examine whether recruitment differed between the levels of the treatment (low, mid and high tidal heights and shaded roof, no roof, and clear roof for shade treatment). I then used a fitted negative binominal model with site as a random factor and “days of recruitment” as an offset to resolve the bias of different exposure time (Gelman and Hill, 2007). In my model, I used the number of recruits as the response variable. Significant results were further examined with a post-hoc test with Bonferroni adjusted p-values.

For all statistical analysis of recruitment I employed the program R (version: 3.5.1 'Feather Spray', 2018). Therefore, I used the following packages of R: “dplyr”, “lme4”, “multcomp”, “blmeco”, “AER”, “lmtest”, “ggpubr” (Bates et al., 2015; Hothorn et al., 2008; Kassambara, 2018; Kleiber and Zeileis, 2008; Korner-Nievergelt et al., 2015; Wickham et al., 2018; Zeileis and Hothorn, 2002).

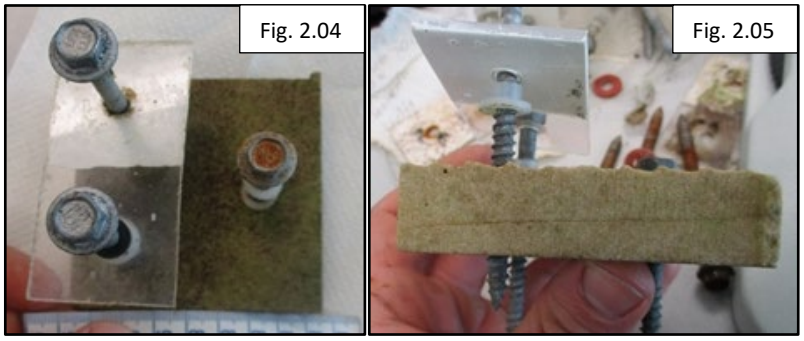

Figure 2.04 & 2.05 Settlement plate with roof in surface (Fig. 2.04) and side view (Fig.2.05).

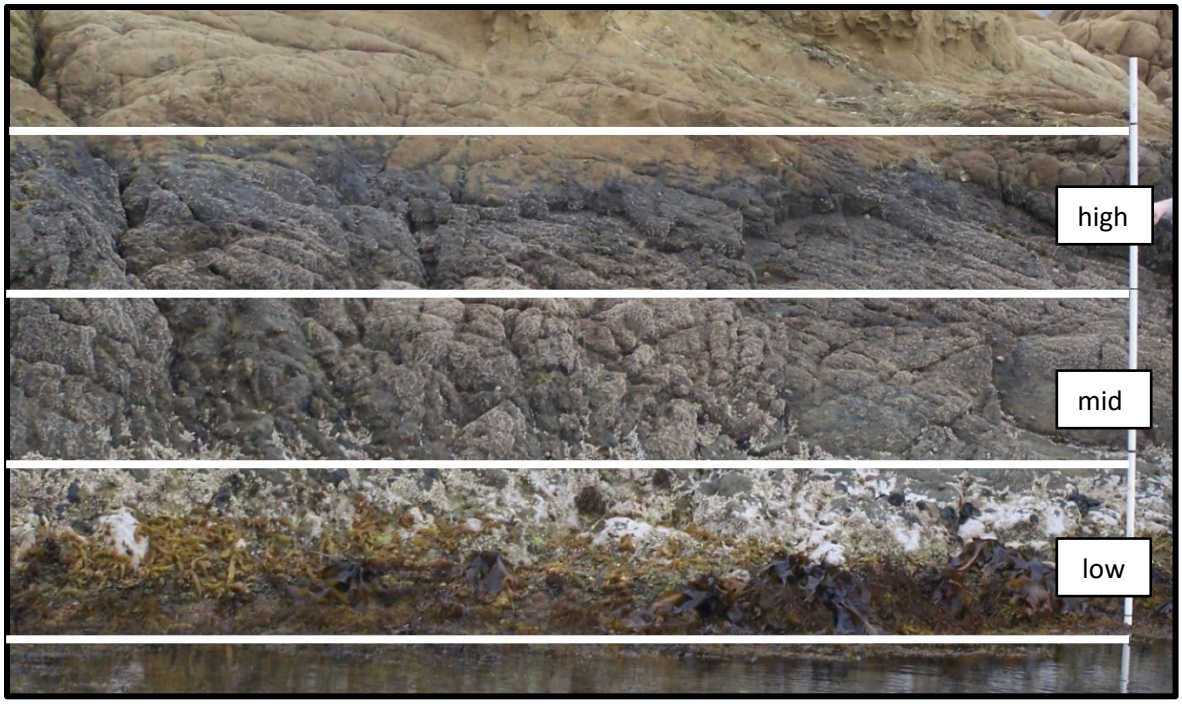

Figure 2.06 A rock wall at Worser Bay. The white bar is approximately 2metres long. The intertidal zonation has been indicated with white lines. Low: from the water level at low tide to 0.5 m above. Mid is the zone from 0.5-1 m above water level at low tide. High is the zone from 1 - 1.5 m above water level at low tide.

Figure 2.07 Settlement plates installed at low, mid and high level at Kau Point.

2.3 Results

2.3.1 Do adult conspecifics mediate recruitment?

The seasonality of recruitment appears clear if we compare the average recruitment every 1–3 months (Figure 2.08). For each microhabitat there is high variability in the average recruitment (Figure 2.08 & in Appendix Table A2.01). Because of the clear seasonality for recruitment, further analysis was performed only on the data for the peak season mid-summer to early autumn (January–March) of the years 2016 and 2017, as my observation in year 2015 began halfway through the recruitment season.

I fitted a negative binominal model with site and year as random factors, and season and the different micro-habitats as fixed factors (Table 2.01). There was no statistically significant difference in recruitment across the microhabitats.

However, the season was significant for recruitment as January to February had the highest recruitment and the recruitment decreased at later times (Table 2.01).

Table 2.01 Recruitment of *Spirobranchus cariniferus* into different microhabitats for the peak Season 2016 & 17 (Jan–April/May), fitted as a function of microhabitat and month (Month-Cat) using a negative binominal regression. Month-Cat: Mid-Summer = January–February; Later Summer = February–March; Early Autumn = March–April/May. Microhabitats: M-in (in mussel aggregation); M-out (bare rock near mussel aggregation); S-in (in Worm aggregation); S-out (in proximity to Worm aggregation).

Source of variation	Coefficient	SE	Z	p-value
M-in & mid-summer	3.54	0.24	14.97	**< 0.01**
M-out	-0.01	0.29	0.04	0.97
S-in	0.48	0.28	1.73	0.08
S-out	0.03	0.28	0.10	0.92
Late summer	-0.25	0.24	-1.06	0.29
Early autumn	-2.5	0.26	-9.63	**< 0.01**

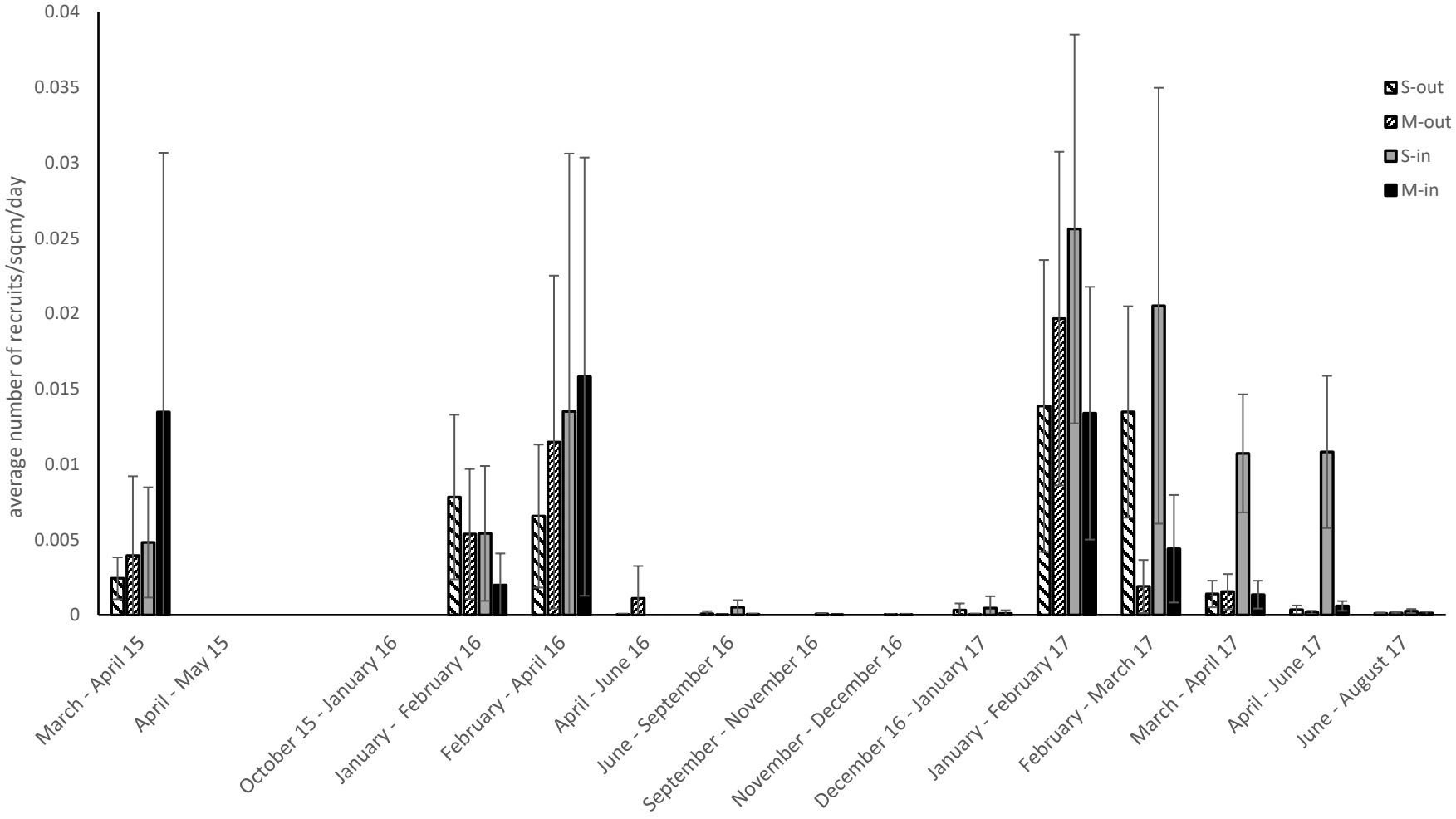

Fig. 2.08 Average recruitment for each microhabitat. Error bars are 95% confidence limits. M-in = plates were inserted into mussel aggregations, S-in = plates were attached to serpulid aggregations, M-out = plates were attached to bare rock in proximity to mussel aggregations, S-out = plates were attached to serpulid free rocks near *S. cariniferus* aggregations.

2.3.2 Do solitary adults mediate the development of new aggregations?

There was little evidence of natural recruitment in close proximity to solitary adult worms. Between January and March 2015, 50 solitary individuals were monitored. For three of these individuals, 11 recruits settled relatively close by (e.g. within 2 mm). Between March and May, 14 solitary adult tubeworms were observed but there were no new settlers (within 10 cm or more distance to the individual) during this time. From December 2015 to February 2016 no recruits were encountered alongside any of the 32 solitary adult worms. Between February 2016 and April of the same year, 10 recruits settled close to each other alongside 2 out of 25 solitary individuals. However, in the following May 2016 one new settler was observed alongside a single solitary individual while a second tubeworm had 6 recruits.

2.3.3 Are *S. cariniferus* recruits distributed randomly or in aggregated patterns?

Of the 23 plates where nearest neighbour distances were measured for recruits to the side of the plate which were closest to the rock, 11 plates were attached to bare rock, seven were in worm aggregations, and five plates were located in mussel aggregations. Eleven of these plates had under 50 recruits, whereas nine plates had more than 50 but less than 150 juvenile tubeworms, and three plates had more than 150 recruits on their reverse side. The nearest neighbour distance between two settled individuals ranged from 0.1 and 17.9 mm. The average distance between two individuals was ~ 0.89 to ~1.39 mm (Table 2.02a & 2.02b). The density (ρ) of recruits on observed plates varied between 0.007 to 0.038 individuals/mm^2 (Table 2.02a and 2.02b). The absolute value of z is greater than 1.96 in all cases, and therefore the recruits are non-randomly distributed. Because the ratio (R) of observed average distance (d_a) between recruits to the expected distances (d_e) given the density of each plate was always smaller than

one, recruits settled in a clumped or aggregated, distribution at all densities and in all microhabitats.

Table 2.02a and 2.02b show the calculated values of the average distance between two settled recruits according to the microhabitat (Table 2.02a) or number of recruits: low 8–50, middle 51–150 and high >150 (Table 2.02b).

Table 2.02b	**bare rock**	**S in**	**M in**
(da) average	1.09	1.07	3.52
Standard deviation	1.34	1.44	4.34
Confidence	0.11	0.13	0.99
De	2.67	2.35	4.90
(ρ) density	0.04	0.05	0.01
$sr = \frac{0.26136}{\sqrt{n^p}}$	0.23	0.23	0.26
R	0.41	0.45	0.72
Z	-6.75	-5.64	-5.42
Total n	587	448	73
Total count	867	658	107
Total mm²	24760.07	14573.92	10292.67
Number of plates	11	7	5

Table 2.02b	**low**	**middle**	**high**
(da) average	3.30	1.06	0.88
Standard deviation	3.79	1.29	0.94
Confidence	0.65	0.11	0.08
De	5.37	2.57	1.59
(ρ) density	0.01	0.04	0.10
$sr = \frac{0.26136}{\sqrt{n^p}}$	0.26	0.23	0.19
R	0.61	0.41	0.55
Z	-8.09	-6.53	-3.68
Total n	130	502	476
Total count	197	760	675
Total mm²	22689.75	20107.36	6829.55
Number of plates	11	9	3

2.3.4 Abiotic factors contributing to settlement patterns: Vertical height

By using a Kruskal Wallis test, I identified the general trend that recruitment to at least one tidal height differs significantly from the others (X^2 = 41.75, df =2, p<0.001). I followed up with a negative binomial model, where site was included as a random factor, the tidal height levels was a fixed factor and the days of recruitment considered as an offset. There were significant differences among the three tidal levels (Table 2.03). A pairwise comparison with Bonferroni-adjusted p-values shows that all three tidal heights are significantly different to each other (Table 2.04). The recruitment to plates at the low height was 24 times higher than those in the middle height, whereas there was virtually no recruitment to plates in the upper tidal height (one individual total) (Figure 2.09).

Table 2.03 Recruitment of *Spirobranchus cariniferus* to different tidal height during summer 2017 (Jan–April), fitted as a function of tidal height using a negative binominal regression.

Source of variation	Coefficient	SE	Z	p-value
Tidal height - high	-8.10	1.43	-5.66	**< 0.01**
Tidal height - low	9.82	1.25	7.87	**< 0.01**
Tidal height - mid	4.87	1.14	4.28	**< 0.01**

Table 2.04 The result of pairwise comparisons with Bonferroni-adjusted p-values for the recruitment to three different altitude levels (January–April

	Coefficient	SE	z	p-values
Low - high	9.82	1.25	7.87	**< 0.01**
Mid - high	4.87	1.14	4.28	**< 0.01**
Mid - low	-4.95	0.66	-7.48	**< 0.01**

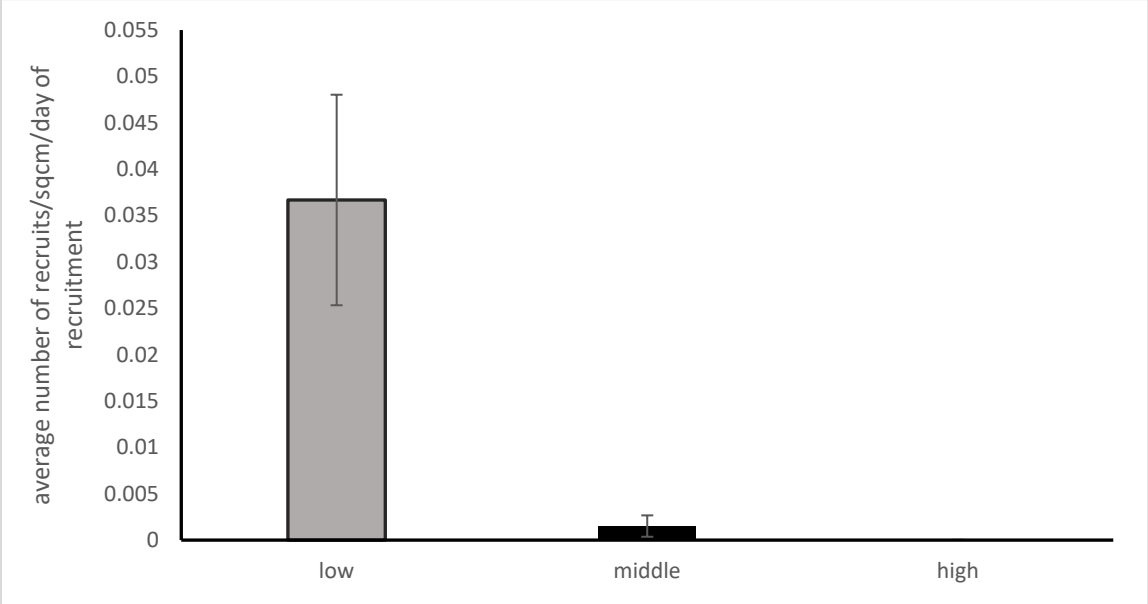

Figure 2.09 Average recruitment for each treatment from January to April 2017. The error bars represent the 95% confidence interval. Total n= 68, 22 plates at low, 23 plate at middle and 23 plates at high tide.

2.3.5 Abiotic factors contributing to settlement patterns: Shade

By using a Kruskal Wallis test, I identified the trend that recruitment to at least one shade treatment differed significantly to the others (X^2= 20.03, df = 2, p < 0.001). Also, here I followed up with a negative binomial model, where site was a random factor, the shade treatments were a fixed factor and the days of recruitment were considered an offset. There was a significant difference among the shade treatments (Table 2.05). A pairwise comparison with Bonferroni-adjusted p-values showed no significant difference in recruitment to surface under the clear roof and no roof, but both were significantly lower compared to under the painted roof (Table 2.06). The average recruitment to the plate under a shaded roof is almost four times higher as the recruitment to the plate under a clear roof (Figure 2.10).

Table 2.05 Recruitment of *Spirobranchus cariniferus* to different shade treatments during summer 2017 (Jan–March), fitted as a function of tidal height using a negative binominal regression.

Source of variation	Coefficient	SE	Z	p-value
No roof	-2.87	0.36	-8.04	**<0.01**
Clear roof	0.34	0.49	0.7	0.49
Painted roof	1.95	0.49	4.03	**<0.01**

Table 2.06 Result of a pairwise comparison with Bonferroni-adjusted p-values for the recruitment to three different altitude levels (January–March 2017).

	Coefficient	SE	z	p-value
No roof - clear roof == 0	0.34	0.49	-0.7	1
Shade - clear roof == 0	1.61	0.47	3.41	**<0.01**
Shade - no roof == 0	1.95	0.49	4.03	**<0.01**

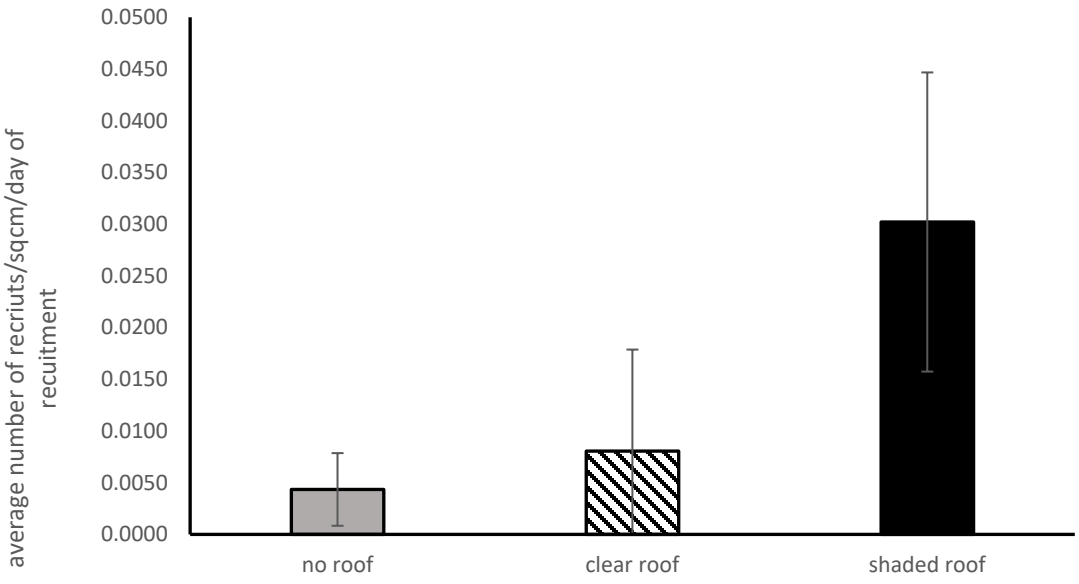

Figure 2.10 Average recruitment for each shade treatment from January to April 2017. The error bars represent the 95% confidence interval. Total n= 54, 18 observations per treatment.

2.5 Discussion

2.5.1 The role of adult conspecifics in mediating recruitment and the formation of aggregations

Contrary to other observations for aggregating invertebrates, including some tubeworm species, I did not observe any effect of established adult *S. cariniferus* aggregations on the recruitment of conspecific larvae in the field. This observation also contradicts the conclusions from a lab-based settlement experiment on *S. cariniferus* (Gosselin and Sewell, 2012). However, the authors in that study recorded very low settlement success after 48 hours, i.e. only 6% of the larvae settled in response to the tubes of adult conspecifics. As far as I am aware, the present study is the first field-based study of *S. cariniferus* recruitment, and there are only three studies on other serpulin species (*G. caespitosa, S. vermicularis*) testing recruitment into patches of conspecifics (Chapman et al., 2007; Minchinton, 1997; O'Donnell, 1986). There are, however,

a number of field-based studies on the recruitment patterns of Serpulinea like *G. caespitosa* (O'Donnell, 1986), *F. enigmaticus* (Dittmann et al., 2009), *H. elegans* (Hurlbut, 1991; Walters et al., 1997)*Spirobranchus* cf*. krausii* (Mohammad, 1975; Straughan, 1969) *Pomatoceros* spp. (Cotter et al., 2003b; Watson and Barnes, 2004) and *S. vermicularis* (Chapman et al., 2007) in competition with other marine sessile invertebrates and to different substrates.

In general, observations about gregarious settlement of serpulins are mainly founded on laboratory-based studies. These studies suggest aggregative settlement occurs mostly in response to adult conspecifics for *H. elegans*, *H. dianthus* (Bryan et al., 1998; Toonen and Pawlik, 2001c, 1996) or biofilm for *H. elegans*, *Spirobranchus lamarckii* (formerly *Pomatoceros lamarckii*, Quatrefages, 1866) (e.g. Unabia & Hadfield 1999; Hamer et al. 2001; Hadfield et al. 2014; Chan et al. 2014). Gosselin and Sewell (2013) observed a weak aggregative response to tubes of adult conspecifics of *S. cariniferus* after 48 hours exposure to the cue in a confined environment. The authors did not consider that larvae of other serpulins like *G. hystrix*, *S. triqueter* and S*. vermicularis* tend to explore the surface for a more extended period of time before final settlement (Fernald et al., 1987; Føyn and Gjøen, 1954; Nelson et al., 2017; Segrove, 1941). Only for the intertidal serpulid *Galeolaria caespitosa* has aggregative settlement been observed in the lab by Marsden and Anderson (1981) and later tested in a field-based study by Minchinton (1997), who made gaps in existing aggregations and observed recruitment into those gaps. He argued that larvae of *G. caespitosa* settled gregariously in the field as new recruits were closer to the edge of the gap and to conspecifics (Minchinton, 1997). It is worth considering the time period and scale over which these studies were conducted, and the time period and scale over which microscopic larvae experience the environment before settling (see also: Pawlik & Mense 1994; Wilson 1968; Marsden & Anderson 1981; Nelson et al. 2017; Knight-Jones & Moyse 1961). For example, it is hard to argue for or against aggregative settlement if larvae settle 2–5 cm from adults of the same species in a 10 x 10 cm gap.

In comparison to other field-based recruitment studies of serpulins (reviewed by O'Donnell, 1986), the highest average rate of recruitment recorded here in peak season was in general similar at 0.026 recruits/cm^2 /day (see Appendix Table A2.01). The average recruitment was lower compared to some subtidal serpulins (Cotter et al., 2003b; Hurlbut, 1991), and this may be because the recruitment for these species is not limited by a tidal cycle. Also, recruitment is lower for *S. cariniferus* compared to that of the intertidal species *G. caespitosa* (O'Donnell, 1986), although it is unclear how the author calculated the average recruitment rate. Chapman et al. (2007) compared the recruitment of three subtidal serpulid species (*S. vermicularis*, *Spirobranchus* spp., *Hydroides* sp.) in field experiments using plates with different structures attached, such as dead and alive *S. vermicularis* aggregations as well as scallop shells. They observed higher recruitment to plates with scallop shells attached. Only *Pomatoceros* sp. seemed to prefer plates with *S. vermicularis* aggregations (Chapman et al., 2007). The higher settlement rate to plates with scallop shells could be caused by active selection by the larvae or passively as the shells could provide a better refuge against turbulent waters compared to the other treatments (Walters et al., 1997).

For *G. caespitosa,* recruitment into various treatments, including artificial tubes and adult conspecifics, has also been tested (O'Donnell, 1986). The highest recruitment was observed to plates with empty conspecific tubes under shade and to plates with live conspecifics. O'Donnell (1986) did not find a significant increase in recruitment to empty conspecific tubes or artificial tubes. She concluded that the appearance of conspecifics is beneficial but not necessary for aggregative recruitment. Therefore, she suggests that the structure itself, regardless whether it is artificial or natural, is sufficient for settlement, an idea similar to that of Walters et al. (1997) about structure providing larvae a refuge from hydrodynamics forces.

However, passive accumulation of larvae behind structures, as observed in various studies (Chapman et al., 2007; O'Donnell, 1986; Walters et al., 1997), is

not necessarily an argument against aggregative settlement (Pawlik, 1992). Marine invertebrate larvae are often discussed as passive particles in the water column (Hannan, 1984; Shanks, 1983). The ciliary locomotory abilities of many larvae is arguably not enough to swim against currents and allows them only to change their position in the water column (Banse, 1986; Mileikovsky, 1973), either to avoid predation, or for feeding (Cronin and Forward, 1986). They may also use different currents to stay close to the shoreline (Cronin and Forward, 1982; Lefèvre and Bourget, 1992) and use the upper water for transport to settlement habitat (Boicourt, 1982; Cronin and Forward, 1986; Shanks, 1983).

Overall, my study on recruitment both inside and outside tubeworm aggregations, and also in proximity to solitary adults, supports the conclusions of O'Donnell (1986) and other studies that adult conspecifics are not an important biological settlement cue for *S. cariniferus* larvae, nor an important starting point in initiating new aggregations.

2.5.2 The role of conspecific larvae in mediating recruitment

Spirobranchus cariniferus larvae settled aggregately in the field regardless of microhabitat and density. Similar patterns have been observed for the barnacle *Tesseropora rosea* (Caffey, 1985). In contrast, the settlement behaviour of *S.* cf. *kraussii* (as *Pomatoleios kraussii,* Straughan 1969), *Ficopomatus uschakovi* (as *Mercierella enigmatica,* Straughan 1972) becomes aggregative as larval density increases (Nishi and Nishihira, 1997; Straughan, 1969, 1972). The discrepancy in the observations for *S. cariniferus* and other serpulins could be due to species-specific responses, or possibly through the use of different, less natural substrata. Other authors measured the distance for individuals settled on glass, asbestos, bakelite and cement surfaces (Nishi and Nishihira, 1997; Straughan, 1969; 1972). Preliminary trials in this study revealed that the material (e.g. PVC or sandstone) has considerable effect on the recruitment success, as not more than 19 individuals of *S. cariniferus* settle to PVC plates (per. Obs.). Other

studies observed recruitment of *G. caespitosa* to sandstone plates in comparable numbers as I found for *S. cariniferus* (O'Donnell, 1986). As far as I am aware, there are no studies of recruitment to natural substrate. Therefore, it is hard to say if the recruitment I observed is reflective of what occurs naturally. However, as rocky surfaces across my study sites consist mostly of sandstone, the plates I used should be similar to natural substrate. The settlement to the backside of plates echos the often observed recruitment to shaded sides of pillars or under rocks (e.g. O'Donnell, 1986; Straughan, 1972).

Studies that focus on the impact of conspecific larvae on the settlement pattern of other larvae of the same species as well as other sessile invertebrates are rare (Aguirre et al., 2013; Caffey, 1985; Clayton and Collins, 1992; Davis and Campbell, 1996; Hurlbut, 1991, 1993; Straughan, 1969; 1972; Wisely, 1960). For the bryozoan *Schizoparella unicornis,* aggregative settlement has been described amongst conspecifics as well as non-conspecific recruits and settlers (Hurlbut 1991). But in the serpulid *Hydroides elegans*, gregarious settlement was observed only in response to conspecific recruits and not to conspecific settlers (Hurlbut, 1991). However, Hurlbut's distinction between recruits and settlers is equivocal because the settlement plates were only submerged for a maximum of 14 days. These findings on *H. elegans* contradict observations of Straughan (1969, 1972) for *F. uschakovi* as well as those in the current research on *S. cariniferus*, where no competition with other sessile species was observed.

However, in all of the above studies, including my own, recruitment under the plate to the rock surface was not considered, which could have an effect on nearest neigbour analysis (Clark and Evans, 1954). In general, it is important with these studies to keep in mind that we observe the recruitment, which is a product of both pre- and post-settlement mortality, and it is not possible to disentangle the two (Gherardi, 1996; Keough and Downes, 1982).

2.5.3 The role of abiotic factors in mediating settlement patterns vertical height and shade

The upper distribution limit of *S. cariniferus* adults is up to 0.5 m above the water level at low tide in the Wellington region (pers. obs.). However, the vertical limit of early recruits was up to 0.5 m above the upper edge of the serpulid zone, or 1 m above the water level at low tide. Knox (1949) observed adult *S. cariniferus* up to 0.6m above the median high-water neaps, which is approximately 1.8 m above the water level at low tide in Christchurch, but the author did not investigate recruitment patterns. The difference in vertical distribution of *S. cariniferus* adults could be explained by differences in the two regions in tidal range or wave exposure, as the sites described by Knox (1949) are likely more sheltered than the sites in Wellington.

Other studies have also found that the distribution of recruits is above that of the adults for other tubeworms, which generally inhabit the lower zones of the intertidal. For example, Straughan (1972) described the vertical limit of *F. uschakovi* adults up to 0.9 m above the water level at low tide at the Brisbane River (Australia), but observed recruitment of this invasive species up to 1.8 m above that. However, this species seems to prefer brackish water as habitat, and therefore increasing salinity decreases the vertical distribution of recruits (Straughan, 1972). O'Donnell (1986) observed recruitment up to 2.14 m (into the "barnacle zone") above the water level at low tide for *Galeolaria caespitosa* at Botany Bay (Sydney, Australia). However, she described the "*G. caespitosa* zone" where adults of this species are dominant above the algae zone at 0.9 m, and up to 1.45 m above the water level at low tide. Further, the author describes low recruitment into the algae zone, which confirms that the lower vertical distribution limit for adults of *G. caespitosa* is determined through the appearance of another more dominant sessile organism (Stephenson and Stephenson, 1949) and possible predation (Paine, 1974). A similar vertical zonation, with a serpulid belt between algae and barnacles, can be described as a feature of the New

Zealand rocky coastline (Morton and Miller, 1973). From my observations and the observations made by O'Donnell (1986), it appears that the upper limit of the zone for intertidal serpulins ends up 0.7 m above the algae zone. However, recruits of both intertidal species appear around 0.5 m above their adult conspecifics.

By contrast, the settlement range of recruits amongst some barnacle species is more similar with the vertical distribution of adults depending on the effects of stressor on the population. Further, it is reported that larvae of two coexisting barnacles (*Balanus glandula* and *B. crenatus*) at Santa Cruz Harbor (California, USA) are dispersed vertically in the water column according to the distribution of adults of the same species (Groseberg, 1982). Larvae of *B. crenatus* tend to swim in -1.5 m to -0.5 m depths under the surface which corresponds to the vertical range of adults. On the other hand, *B. glandula* was mostly found swimming near the water surface. Cyprids of both species settle up to 0.3 m above or below the zone where it was abundant on the shore (Groseberg, 1982).

In other studies at San Juan Island (Washington, USA), the settlement range of *B. glandula* is larger than the tidal height limitation for adults (Connell, 1970), whereas larvae of another barnacle species, *Chthamalus stellatus* in the Gulf of California recruit higher on granite compared to basalt stone because basalt heats up significantly more than granite. In this case, these recruits to basalt are at a higher risk of desiccation (Raimondi, 1988a). However, for barnacles recruitment below, the vertical adult range is often higher compared to the settlement above this range (Connell, 1961; Moyse and Knight-Jones, 1967). It has been suggested that larvae of barnacles can better identify unsuitable substrate due to abiotic stressors rather than unfavourable through conditions such as predation (Strathmann et al., 1981).

Particularly for bivalves, the tidal range and the time submerged in the water determines the vertical distribution. Various studies have shown that the supply

of phytoplankton is limited through tidal movement and mussels only settle to a tidal height where the net energy gain is not negative (reviewed by Bayne et al. 1988). Thus the upper limits for sessile species in their vertical distribution are mostly determined through abiotic stressors such as wave exposure (Harley and Helmuth, 2003), tidal range (Raimondi, 1988b), UV radiation and desiccation (Bertness et al., 1999; Shafer et al., 2007; Stephenson and Stephenson, 1949). All the abiotic stressors have an effect on larvae distribution and attachment, as well as pre- and post-settlement mortality. Because I did not measure the range and effect of each factor, it is not possible to determine which stressor influenced the recruitment of *S. cariniferus* to which limit. However, UV radiation and wave exposure were likely strong factors affecting recruitment of *S. cariniferus* to the exposed side rather than the backside of a plate. The recruitment to any backside of a plate or to the surface under the plate was always higher compared to any exposed side.

Presumably the effects of solar radiation were partially mitigated by the shade provided by the painted roof, given that recruitment was lower and similar under the unpainted roof and unroofed area. In particular, the painted roof likely reduced the effects of UV radiation and desiccation on the settlement surface below and recruits (Bertness et al., 1999; Hung et al., 2005). Similarly for *F. uschakovi* in the Brisbane River (Australia), there was higher settlement on surfaces of pillars orientated away from currents and sunlight (Straughan, 1972). The author postulated that in that case both were important, in that protection from the currents leads to a higher concentration of larvae and the shade allowed more settlers to survive. The idea that refugia against turbulent water promotes the aggregation of settlers is also supported by a study on *Hydroides elegans* at Pearl Harbor (Hawaii) (Walters et al., 1997). Further, for *G. casespitosa,* recruitment under shade is also increased (O'Donnell, 1986). The higher recruitment into shaded places as well as into crevices and pits could be caused through cues emitted by a biofilm that is inhibited by UV-radiation (Hung et al., 2005; O'Donnell, 1986). Although the presence of a structure alone may

accumulate larvae due to hydrodynamic forces, from my study it is clear that the structure alone does not result in higher recruitment. If this had been the case, I would have seen similar recruitment under the clear part as wellas the shaded part of the roof.

There could be multiple reasons why recruitment is lower at mid tidal range and at high tidal range as well as unshaded surfaces. It may be that competent larvae don't reach the highest zones either because of larval distribution in the water column (Connell, 1985) or the larvae show a geotaxis (Bayne, 1964b) or phototaxis response (Marsden, 1988). Also, it is possible that larvae don't settle because of unfavourable conditions (Olson, 1983; Pawlik and Mense, 1994). O'Donnell (1986) argued that the recruitment of *G. caespitosa* to high tidal levels is limited by larval distribution in combination with limited space due to other species. However, in a limited number of cases, recruits of serpulins can overgrow barnacles and other space competitors (Denley & Underwood 1979, pers. obs). Further, larvae may not settle at high or intermediate tidal levels because of missing cues. For example, O'Donnell (1986) suggested that larvae of *G. caespitosa* do not settle to rock surfaces exposed to high UV-radiation because of missing settlement cues such as the presence of a biofilm. This is supported by studies where UV-radiation decreases the metabolic activity of a bacterial film, and this has a significant adverse effect on the settlement of serpulid larvae (e.g. Hung et al. 2005). Finally, another factor contributing to the comparably low recruitment at higher tidal levels could be post-settlement mortality (Hunt and Scheibling, 1997). For example, larvae settling at high tidal levels are more vulnerable to desiccation (Gosselin and Chia, 1995), or possibly an increase mortality through predation by terrestrial species (Drinnan, 1957).

However, observation of recruitment to plates above the vertical range of adult conspecifics is limited to the post settlement survival rate. Larvae could have died after settlement before I was able to observe the plates for recruitment (Straughan, 1972). To really understand the vertical larval distribution and

settlement it would be necessary to observe settlement rates and larvae distribution in the water column (Gherardi, 1996; Groseberg, 1982; Keough and Downes, 1982), and follow post-settlement survival over time.

2.5.4 Conclusion

In conclusion, the results of my study show that larvae of *S. cariniferus* settle aggregatively, and not in response to adult conspecifics. Some authors have proposed that the larvae swim as school and transition to a benthic life together (Marsden, 1991; Marsden and Meeuwig, 1990). This idea has been also suggested for mussels (Keough, 1981) and barnacles (Dobretsov and Miron, 2001), and for copepods naupilii larvae have been described as swimming in "three-dimensional swarms" (p. 260, Barnes & Marshall 1951) (reviewed Cassie 1957; Pineda et al. 2010). It is unknown whether serpulin larvae stay together in the water column. However, some support for this phenomeon comes from lab experiments. For example, larvae of *H. elegans* tend not to settle if they are not at a threshold larval density (Bryan et al., 1997), and propagules of *Spirobranchus giganteus* (Pallas, 1766) and *Spirobranchus polycerus augeneris* (ten Hove, 1970) are more attracted to conspecific larvae rather than to other cues (Marsden, 1991; Marsden and Meeuwig, 1990).

Other studies have tried to find explanations for aggregative settlement. One conundrum is how an aggregation can start if recruits depend on settlement cues from conspecifics (Toonen and Pawlik, 2001a). In this context, one idea for aggregative settlement is the "founder and aggregator hypothesis" (Toonen and Pawlik, 1994). According to this hypothesis, some larvae settle (after reaching competence) in response to biofilm. Those larvae act as "founders". The remaining larvae follow and settle in response to the attachment of the founder. The suggestion that larvae swim together is supportive of the "founder and aggregator hypothesis". Similar aggregative settlement behaviour could also be explained as a settlement of a group of accumulated larvae in response to

certain cues independent of conspecifics (Keough, 1981; O'Donnell, 1986). Solitary individuals could occur through pre- or post-settlement mortality, as conspecific larvae die or become dislodged (Connell, 1985).

Based on my results in this study, I propose that larvae of *S. cariniferus* at least accumulate before settlement near the shoreline. Larvae of the same development stage will settle aggregative regardless of the presence of conspecific adults. Individuals of *S. cariniferus* seem not to settle alone out of necessity as they can prolong their planktotrophic stage like some other serpulins, such as *H. dianthus* (Toonen and Pawlik, 2001b). Therefore, the appearance of a solitary *S.cariniferus* specimen may be explained by events which immediately happen during the settlement process, or between settlement and the observation of the recruit.

3. Trade-off of solitary vs. aggregative occurrence

3.1 Introduction

For organisms that occur in high-density aggregations, there is an important question about the advantages and disadvantages of this settlement strategy. For barnacles, mussels and sessile worms, like serpulins and sabellarians, living side by side with conspecifics can enhance the stability of individuals and community structure (e.g. Bertness & Grosholz 1985; Bertness et al. 1998; Jaubet et al. 2011; Thomas 1996). Higher stability decreases the mortality of individuals, especially for perennial species, and can lead to higher fecundity over the lifespan of an individual as they may live longer (Qian, 1999). Additionally, the aggregative life possibly increases the availability of food through feeding currents and a higher sedimentation of food particles (e.g. Fréchette et al., 1989; Merz, 1984). Further advantages include greater success in reproduction through synchronisation of gamete release and securing suitable habitat for offspring (Qian, 1999; Thomas, 1994). However, the downside is increased competition for food, space and oxygen amongst conspecifics, and with other taxa in context with a larger individual density (Bryan et al., 1997; Woodin, 1976). Further, high density in a population of sessile organisms can enhance physiological stress for these individuals (Fréchette et al., 1992; Okamura, 1986), which, in turn, can lead to a decrease of aggregation size and also reduce the reproductive output of individuals and the population (Hart et al., 2012; Hart and Marshall, 2013; Okamura, 1986; Woodin, 1976). Particularly amongst broadcast spawners, as many serpulins are, a larger number of individuals in a population increases the occurrence of polyspermy which has a negative effect on the reproductive success of the population (Franke et al., 2002; Yund and McCartney, 1994).

Although some of these issues have been studied in other species, they have not been well addressed in tubeworms, like serpulins and sabellarians, which can be

found in high densities in intertidal communities. In sessile polychaetes, tube growth has been considered a form of locomotion to move away from stressors, such as competitors (Fauchald & Jumars 1979). In this case, individuals in aggregations may increase energy allocation to tube growth, and perhaps solitary individuals that do not need to invest as much in avoiding competitors may have more resources for reproduction. However, the possibility of fertilisation success is likely to be reduced in solitary individuals because of a lack of synchronisation and distance between males and females. Further, mortality might be increased due to higher vulnerability of solitary tubes with regard to damage from biotic factors such as predation or crushing by mobile species (e.g. Bosence, 1973; O'Donnell, 1986), and abiotic factors such as wave impact, currents and acidification (e.g. Kaehler, 1999; Welladsen et al., 2010).

Serpulid species are ideal study organisms to evaluate the benefits and costs of both strategies because they commonly occur as both isolated individuals and in dense aggregations. In this chapter, I will focus on the potential trade-offs in growth, and mortality for solitary and aggregative individuals in the native New Zealand tubeworm *Spirobranchus cariniferus*. In the last century, studies of tube-growth of serpulins focused on growth rates of recruits to settlement plates from one to several months (e.g Dew, 1958; Grave, 1933; Paul, 1937, 1942). There are only a few studies that have observed the growth rates of older individuals (e.g. Iyengar 2002; Jacinto et al. 2015). However, those studies were often based on measurements on marked individuals within six months to a year. Therefore, they reflect often only the growth over a longer period for a small number of specimens (Hughes et al., 2008; Iyengar, 2002; Riedi and Smith, 2015). Based on the literature, I expected aggregative individuals to have a higher tube growth rate compared to their solitary counterparts (Knight-Jones and Moyse, 1961; Menge, 1976; Nishi and Nishihira, 1997). Based on the speculative higher tube growth rate and the fact that aggregation increases the ability to retain food particles for filter-feeders (Fréchette et al., 1989; Helms, 2004; Merz, 1984), I predicted a larger and heavier body relative to tube size for

aggregative individuals. Further, I anticipated a higher mortality for solitary worms, particularly after the tube has been damaged, because of the lack of structural support from aggregative conspecifics.

3.2 Methods

3.2.1 Tube growth

To measure tube growth rate, I used bee tags to mark ~100 aggregated and 100 solitary individuals each at Worser Bay and Shelly Bay (see in Appendix p. A5 – p.A7 for site descriptions). Bee tags are small plastic discs used by beekepers to mark their bee stock.The tags have been either attached to their tube or a nearby rock with adhesive, in distance to the anterior end of the tube. Several times over the period of my observations, I tagged additional new individuals to compensate for natural mortality. For this experiment worms were considered aggregative if they appeared in a patch with 10 or more individuals, as there is arguably no or weak competition for space and food between a smaller number of individuals. Every two months I photographed marked individuals with a scale alongside. At first tagging the width and/or height of each tube was measured with ImageJ (version: 1.48v; Schnerider et al., 2014) to sort the worms into initial size categories (<1 mm, 1–2 mm, >2 mm). I used width or height (whichever was more visible in the image) as proxy for initial size because it was not possible to measure the entire length of worms (whether aggregated or solitary) because of the entangled individuals as well as loss of tube through erosion. The measurement of tube width and height in the field revealed no difference between solitary and aggregative worms (see in Appendix Figure A3.01). Also, there was a correlation between tube width and height (see in Appendix Figure A3.01). Subsequently, I measured the length of the tube from anterior along the keel as far I was able to recognise the tube belonging to one individual, using ImageJ. The change of tube length from anterior to a previously defined point was recorded every two months using ImageJ. After the first few months of

measurements, it became clear that worms often lose the posterior and anterior parts of their tube, which made it difficult to determine a tube growth rate. Therefore, I averaged and compared the change in tube length of solitary and aggregative individuals and described this change of tube length as the tube growth rate.

I fit a linear model with change of tube length/day as response to the category of initial size (three levels, based on tube width or height), settlement strategy (solitary or aggregated), season (spring, summer, autumn, winter) and the interaction of settlement strategy and initial size category as fixed factors. For the model, I also included site and year as random factors. Through the analysis of the qq-plot with the residuals of the linear model I identified four outliers and removed them to provide better fitting models; however, this did not affect the outcome of the analysis.

3.2.2 Mortality and recovery

Several individuals tagged for growth died and their tubes were possibly removed from the rock. If I was able to relocate the previous position of the organism in an earlier image, I noted the disappearance of the individual or tube as dead. From this data set of dead individuals, I fitted a binomial logistic regression to test whether aggregated or solitary individuals had greater mortality. For this model, I used the binominal-coded survival data as response to settlement strategy, initial size category, season as fixed factors and year, and site as random factor.

Additionally, I tested the ability of solitary and aggregated individuals to recover from tube damage, and the mortality associated with such damage. In three different trials, solitary and aggregative individuals were marked with bee tags. For half of the marked worms the tube was manipulated. In the first trial (December 2015 to January 2016) at Point Haswell (see in Appendix p.A3 for site descriptions), I manipulated ~30 solitary and ~30 aggregated individuals by

breaking off the anterior portion of the tube (described below). As a control, I marked an additional ~30 solitary and ~30 aggregated individuals. The same set up was repeated in the laboratory with 25 manipulated and 25 control specimens per settlement strategy, to test if the manipulation itself resulted in mortality. In the second and third field trials (June–September 2016 and July–October 2017) at Shelly Bay and Point Haswell, I tagged 20 manipulated and 20 control individuals of both settlement configurations per site. In all trials I used photographs and ImageJ to document the initial tube width and/or height, the initial length (off all specimens) similar to the length of the tubes after manipulation and the measurements for tube growth.

For all trials, the tubes were manipulated by removing 0.1–9 mm of the anterior portion by using forceps without harming the body of the individual or considerably damaging the integrity of the tube. For the first summer field trial, I took images monthly, and in the lab trial (also in summer), I took images weekly to measure growth and mortality. For the two winter field trials, I recorded all individuals with a digital camera before and after manipulation, and then at the end of the test period. All tube lengths were measured with ImageJ. In these experiments, I focused on the stability of a tube rather than the effect of the community on growth, and, therefore, I considered individuals as aggregated if at least two individuals touched each other, as each of the tubes probably provides support and protection for the other. I analysed my measurements with various linear models. For all models I used “the change of tube length” (tube growth) as the response variable, for fixed factors in the model, I used settlement strategy, tube diameter, the length of the anterior tube removed (after manipulation) and the interaction of all three factors. For the field experiments, I also included the season as a fixed factor and site and year as a random factor.

3.2.3 Tube size relative to body dimensions

To examine relationships between body and tube traits, and whether they are different for solitary vs. aggregated individuals, I collected 85 solitary and 105 aggregative individuals from seven sites (Breaker Bay, Kau Point, Point Haswell, Pukerua Bay, Scorching Bay, Shelly Bay, Worser Bay) over the period spring 2016–autumn 2018. In these investigations I tried to remove as much tube as possible for each individual for a better representation of the tube dimensions. This complicated the analysis of dense worm reefs. An individual may experience support for tube stability from another nearby conspecific. Further, all measurements have been made once for each individual in the laboratory. The combination of these factors allowed me to further specify the definition of aggregative individuals for a better differentiation between both settlement types. Therefore, I defined individuals as aggregative if they were next to another specimen and at least a third of their tubes touched the other tube.

In most studies regarding size of sessile worms, the count of abdominal segments is used to differentiate age groups (e.g. Cotter et al. 2003; O'Donnell 1986). For non-invasive measurements, such as tube growth, it is impossible to count the number of abdominal segments. Therefore, I measured the tube width and height in all my experiments to better relate my results to each other. To make my results comparable to investigations by other authors, I established a correlation between the number of abdominal segments and tube width (in results Figure 3.08 and in Appendix Table A3.16).

I took photos of each individual and measured the length and width of tubes with ImageJ. After the picture was taken, I removed each specimen from its tube and collected the tube parts in a dish. The worm was placed on a watch glass with 2 ml filtered (10µm) seawater. The worm length was measured under a microscope with an ocular micrometer, from the tip of the tentacular crown to the pygidium, and width at the widest thorax segment. Body and tube weights were measured

to 0.1 mg after drying both in an oven at 60°C. The dry weight of the tube was recorded after 16 hours. As the used scale was not precise enough to measure sufficiently the dry body weight of these little worms, I noted the weight after the specimen were for 16 and 18 hours in the dry oven; the mean of these two measurements was used. On some occasions the data of the body measurements were not normal distributed; therefore I transformed the body measurement in the relevant data sets with a natural logarithm. With linear models compared tube length, tube width, body length, body width, body dry weight and tube dry weight between both settlement variations. For each model, I included aggregative and solitary settlement as fixed factor and season, and year and site as random factor.

All my statistical calculations in this chapter (tube growth, recovery, size, body dimensions and mortality) were explored with the statistical program R (version 3.5.1 'Feather Spray', 2018). For all linear models of tube growth, recovery as well as body and tube dimensions, I used the following package of R: “psych”, “lme4”, “arm”, “lmtest”, “multcomp”, “lmerTest”, “car”, ggpubr” (Bates et al., 2015; Fox and Weisberg, 2011; Gelman and Su, 2018; Hothorn et al., 2008; Kassambara, 2018; Kuznetsova et al., 2017; Revelle, 2018; Zeileis and Hothorn, 2002). Subsequently, to investigate mortality with logistic regressions, I used additionally the packages: “ggplot2”, “GGally”, “reshape2”, “boot”, “lattice”, (Canty and Ripley, 2017; Sarkar, 2008; Schloerke et al., 2018; Wickham, 2007, 2016).

3.3 Results

3.3.1 Tube growth

There was no difference in tube growth rates between either solitary or aggregative individuals over the various seasons nor according to their initial size class (Figure 3.01a & 3.01b). The linear model revealed that solitary worms grow their tubes slightly but not significantly faster (Table 3.01). All three size

categories differ significantly ($p < 0.01$) in their tube growth rate; the smaller individuals grow faster than mid-sized individuals, and these grow more quickly than the largest conspecifics (Figure 3.02 and pairwise comparisons with Bonferroni-adjusted p-values, $p < 0.05$, in Appendix Table A3.01). Also, the tube-growth rate varied significantly depending on season (Table 3.01). Tube-growth rate was highest in spring followed by winter and lowest in autumn. The growth rate in fall was significantly smaller compared to spring and winter (pairwise comparisons with Bonferroni-adjusted p-values, $p \leq 0.01$). Also, in summer the worms grew significantly slower compared to spring (pairwise comparisons with Bonferroni-adjusted p-values, $p < 0.01$). For each size category is the average and maximum growth rate per season listed in the Appendix (Table A3.02 – A3.13).

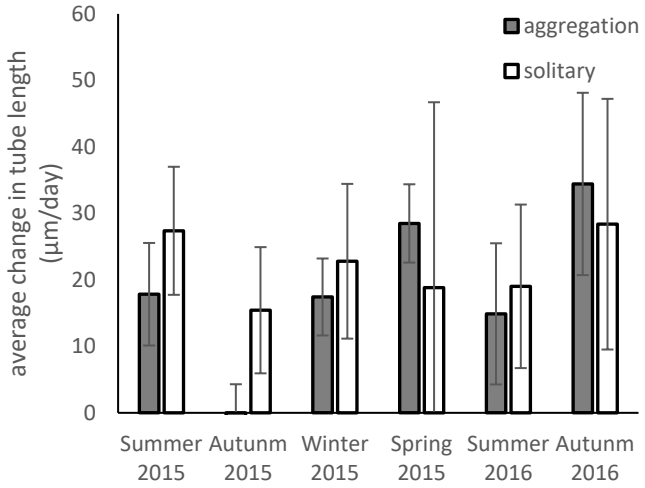

Fig. 3.01b

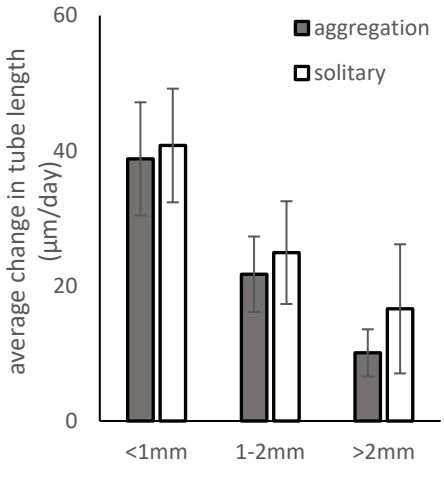

Fig. 3.01b

Fig. 3.01a The change of tube length across all size groups for each season.
Fig 3.01b The change in tube length averaged for all 6 seasons (summer, autumn, winter, spring 2015 & summer, autumn 2016) and plotted for each size group. In both diagrams the error bars show the 95% confidence Interval.

Table 3.01 Change of tube length in µm/day fitted as a function of settlement configuration (Agg/Sol), Season (summer, autumn, winter, spring), size category: <1 mm, 1-2 mm, >2 mm, as well as the interaction between settlement-strategy and size, and Site and Year as random factors.
A pairwise comparison with Bonferroni-adjusted p-values for size category is given in the Appendix Table A3.01.

Source of variation	Estimate	SE	df	t-value	p-value
Intercept	31.92	5.87	5.84	5.44	**<0.01**
size.category>2 mm	-29.48	4.47	982.16	-6.6	**<0.001**
size.category1-2 mm	-19.17	4.56	979.88	-4.21	**<0.001**
Solitary	4.39	5.24	981.75	0.84	0.40
Spring	20.01	3.99	585.11	5.16	**<0.001**
Summer	5.54	2.94	896.52	1.89	0.06
Winter	12.29	4	665.84	3.08	**<0.01**
size.category>2 mm: Solitary	6.52	6.9	983.85	0.95	0.35
size.category1-2 mm: Solitary	-0.36	6.45	983.86	-0.06	0.96

3.3.2 Mortality

The percentage mortality was higher amongst solitary worms across most of the seasons (Figure 3.02). However, in a logistic regression of mortality against season, there was no significant difference in the mortality between solitary and aggregative individuals ($p = 0.72$; see in Appendix Table A3.14). Although the mortality was at the lowest in summer 2016 for all worms (Figure 3.02), this was not statistically significant (logistic regression $p > 0.05$; see in Appendix Table A3.14). On the other hand, the highest mortality is to note for autumn 2016 which is statistically significant (logistic regression $p = 0.03$; see in Appendix Table A3.14).

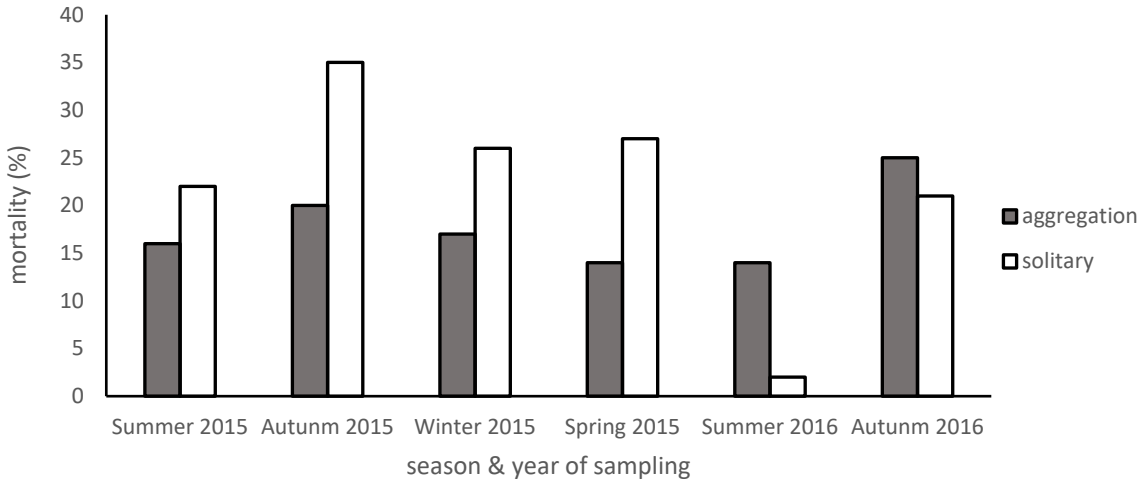

Figure 3.02 Represents the percentage mortality for solitary and aggregative individuals across the six observed seasons. Number of observations (n) for aggregation; **2015**: summer 119; autumn 166; winter 132; spring 129; **2016**: summer 148; autumn 71. Number of observations (n) for solitary; **2015**: summer 88; autumn 51; winter 39; spring 56; **2016**: summer 137; autumn 70.

Surprisingly, the removal of anterior tube material did not increase the mortality of aggregative or solitary settled worms in the lab or field. During the lab trial the mortality rate was 4% amongst the undamaged solitary worms, and there were no deaths amongst the undamaged aggregative worms or all damaged worms. Only in winter 2017 did an undamaged aggregative worm die (3% mortality, in this group). There was no other mortality in any of the field trials regardless of whether the tube was experimentally damaged or not.

3.3.3 Tube recovery

The aggregative individuals kept at the laboratory facilities in summer 2016/17 had a higher growth rate compared to individuals observed in the field at the same time (Figure3.03 and 3.04). However, for some damaged solitary individuals there was a definite negative change of tube length, indicating further loss of tube material after the manipulation. This phenomenon seemed accelerated particularly in the laboratory trial (Figure 3.04).

Figure 3.03 The average growth rates from the field trial of summer 2015/16 of damaged and control individuals which were aggregative (Agg) or solitary (Sol). The error bars represent the confidence interval of 95%. Sample sizes ranged from 20–37 individuals per treatment.

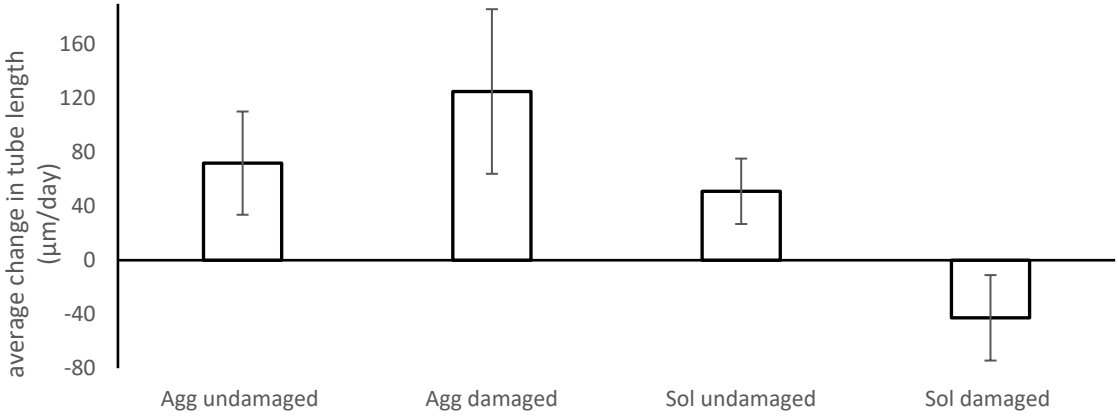

Figure 3.04 The average growth rates from the laboratory trial of summer 2015/16 of damaged and control individuals which were aggregative (Agg) or solitary (Sol). The error bars represent the confidence interval of 95%. Sample sizes ranged from 24–28 individuals per treatment.

In the lab, the interaction between settlement pattern and the length of removed anterior tube material had a significant impact on the tube growth after the manipulation (Table 3.02). With increased damage, solitary worms accumulated further loss of tube-material, whereas aggregative worms boosted their growth rate (Figure 3.05).

Table 3.02 Change of tube length in µm/day fitted as a function of settlement configuration (Agg/Sol) with the continued variable tube width/height and length of the manipulation (manipulation). Also included was the interaction between manipulation and settlement pattern as well as manipulation and width/height. Growth was observed for two weeks in the lab under continuous running filtered (10 µm) seawater n =24-25/treatment.

Source of variation	Estimate	SE	t-value	p-value
(Intercept)	228.03	120.87	1.89	0.07
Width/height	-129.59	68.02	-1.91	0.06
Solitary	-154.55	112.88	-1.37	0.18
Manipulation	57.64	78.29	0.74	0.47
Width/height x Solitary	101.29	80.26	1.26	0.21
Manipulation x Agg/Sol	-131.04	41.37	-3.17	**<0.01**
Width/height x manipulation	14.14	41.51	0.34	0.74

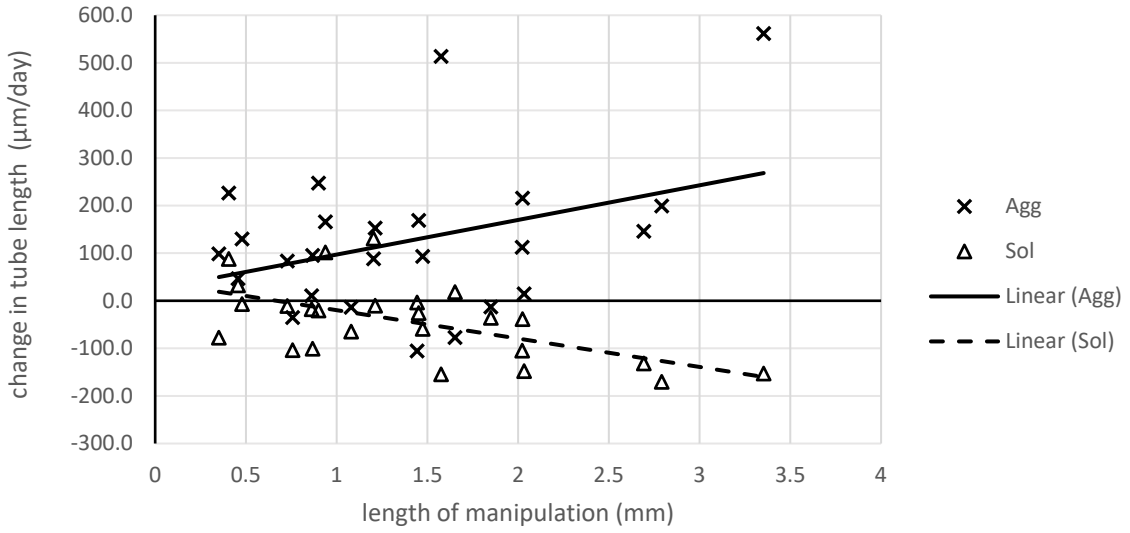

Figure 3.05 The change of tube length plotted against the length of the anterior tube manipulation for each damaged individual in the lab (n= Agg: 25; Sol: 25). **R^2 for Agg** = 0.14 (y = 72.81x + 24.24); **R^2 for Sol** = 0.34 (y = -59.66x + 39.89).

In all field trials, manipulation of the anterior tube had no impact on the growth rate (Figure 3.06). For the purpose of this analysis with a linear model, I included initial size categories (<1 mm, 1-2 mm, >2 mm). Additionally, the length of the removed tube material has been incorporated in the model as three different "length of damage" categories (cut<1mm, cut 1-2mm, and cut >2mm). From the linear model, there was no significant difference in the growth rate between the

different size categories ($p > 0.3$, see in Appendix Table A3.15). Across all the field data, the length of the manipulations had no significant impact on the tube growth regardless of the settlement strategy ($p > 0.05$, see in Appendix Table A3.15). Similar to my laboratory trials, with an increase in the length of the manipulation was the tube growth rate negatively affected for solitary worms (Figure 3.07); however, this observation is statistically not significant ($p > 0.3$, see in Appendix Table A3.15).

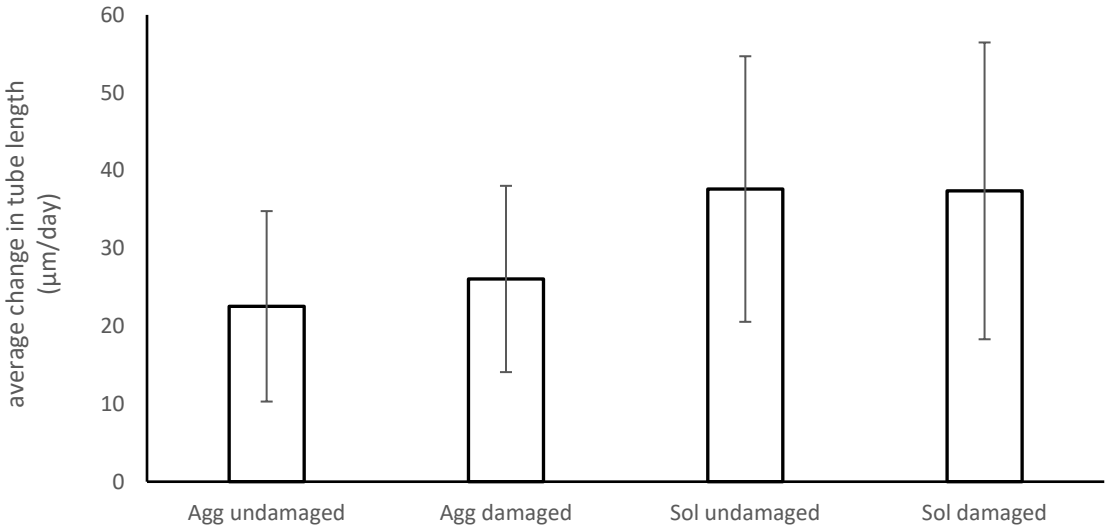

Figure 3.06 Compares the average growth rates of damaged and control individuals which were solitary (Sol) or aggregative (Agg). Error bars represent the confidence interval of 95%. Observations have been made in the field and pooled across summer 2016/17, winter 2017 and winter 2018. Sample size: Agg undamaged 68; Agg damaged 52; Sol undamaged 55; Sol damaged 43.

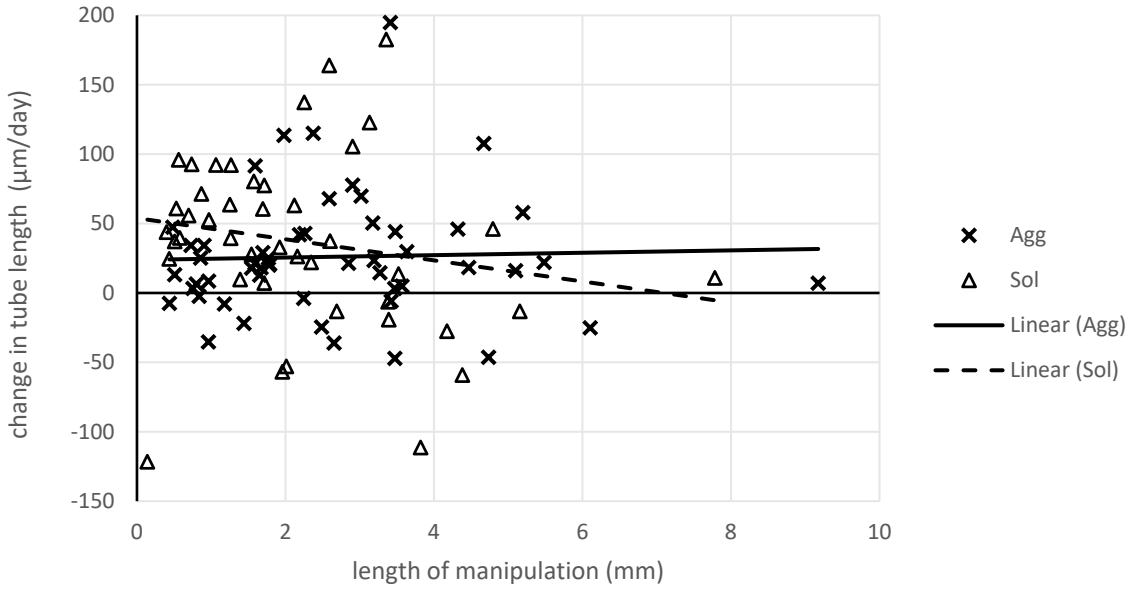

Figure 3.07 The change of tube length plotted against the length of the anterior tube manipulation for each damaged individual in the field from summer 2016/17, winter 2017 & winter 2018 (n = Agg: 52; Sol: 43). **R^2 for Agg** = <0.01 (y = 0.86x + 23.73); **R^2 for Sol** = 0.03 (y = 7.6x + 53.87).

3.3.4 Tube size in relation to body dimensions

The number of abdominal segments increased with increasing tube width (Figure 3.08 & p <0.01, see linear model in Appendix Table A3.16) and body length (Table 3.03). However, the count of abdominal segments is independent to the thorax width (Table 3.03). Solitary worms had fewer abdominal segments compared to aggregative conspecifics; this difference was not significant in correlation with the tube width (linear model p = 0.19; see in Appendix Table A3.16). This difference of lesser abdominal segments became almost significant in correlation with the thorax width and body length (Table 3.03).

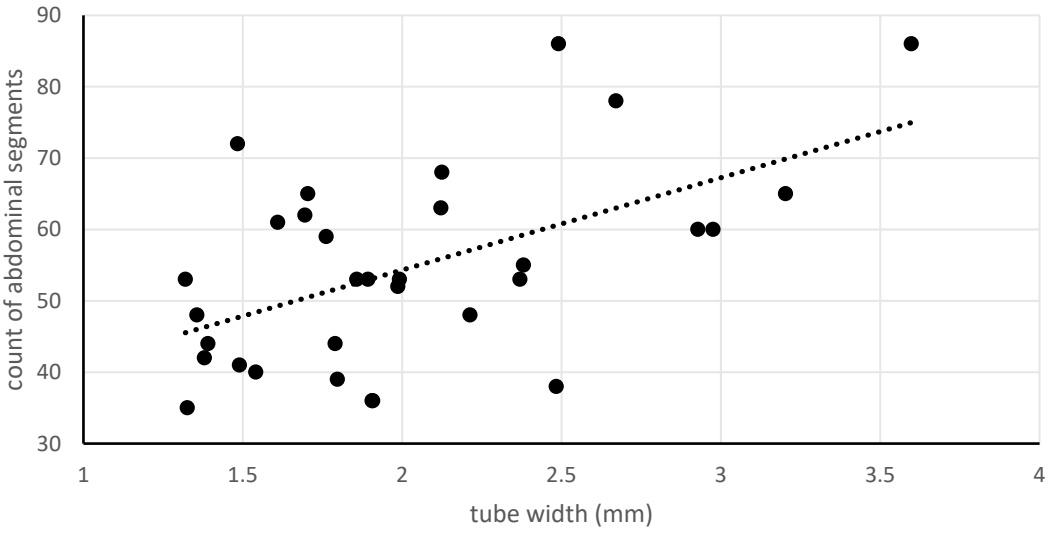

Figure 3.08 The count of abdominal segments of solitary and aggregative individuals (pooled) increases with increasing thorax width (n = 32). **R^2 = 0.29 (y = 12.93x +28.47)**.

Table 3.03 Number of abdominal segments fitted as a function of settlement configuration (Agg/Sol), thorax width, body length. Season, sample site and year have been considered as random factors (n = Agg: 60; Sol: 24).

Source of variation	Estimate	SE	df	t-value	p-value
(Intercept)	33.49	4.23	6.29	7.92	**<0.01**
Solitary	-4.1	2.07	79.14	-1.98	0.05
Body lentgh	2.81	0.44	79.26	6.42	**<0.01**
Thorax width	-1.11	1.69	78.57	-0.66	0.51

If we compare the tube length in response to body length, thorax and tube width it becomes clear that solitary settled worms have significantly longer tubes compared to aggregative individuals ($p<0.01$, Table 3.04 and Fig 3.09). Additionally, solitary worms have a slightly wider tube in relation to thorax width, tube and body length ($p < 0.01$; Table 3.05 and Fig 3.10). On the other hand, aggregative worms have a significantly longer body for a given thorax width, in relation to tube width/height ($p < 0.01$; Table 3.06 and Fig 3.11). However, the thorax width is not significantly larger for aggregative individuals (linear model $p = 0.06$, see in Appendix Table A3.17). In summary, solitary worms have a longer tube for the same body length, body diameter and tube width (Table 3.07). Table 3.07 summarises the linear models of body and tube related measurements and shows for each measured biometric category which settlement pattern is

favoured, and if this difference is significant. The additional linear models can be found in the Appendix (Table A.3.17 – A3.20).

Table 3.04 The natural logarithms of tube length is fitted as a function of settlement configuration (Agg/Sol), tube width/height, thorax width and body length (n = Agg: 88; Sol: 82).

Source of variation	Estimate	SE	df	t-value	p-value
(Intercept)	2.6	0.13	4.31	20.84	**<0.01**
Agg/Sol	0.13	0.04	162.86	3.10	**<0.01**
Tube width/height	0.04	0.02	135.57	1.61	0.11
Thorax width	0.08	0.06	164.27	1.4	0.16
Bodylength.mm	0.04	0.01	123.7	5.07	**<0.01**

Table 3.05 Tube width/height fitted as a function of settlement configuration (Agg/Sol), thorax width, body length and tube length (n = Agg: 88; Sol: 82).

Source of variation	Estimate	SE	df	t-value	p-value
(Intercept)	1.12	0.44	6.48	2.53	**0.04**
Sol/agg S	0.51	0.13	160.92	3.92	**<0.01**
Body diameter	0.3	0.18	163.5	1.60	0.11
Body length.mm	0.06	0.03	163.25	1.80	0.07
Tube length.mm	0.02	0.01	163.89	1.50	0.14

Table 3.06 Body length fitted as a function of settlement configuration (Agg/Sol), tube width/height, thorax width and tube length (n = Agg: 88; Sol: 82).

Source of variation	Estimate	SE	df	t-value	p-value
(Intercept)	0.72	0.13	5.65	5.44	**<0.01**
Sol/agg S	-0.11	0.04	161	-2.8	**<0.01**
Tube width/height	0.07	0.02	157.38	3.29	**<0.01**
Thorax width	0.43	0.04	161.51	10.47	**<0.01**
Tube length.mm	0.01	<0.01	164.55	5.05	**<0.01**

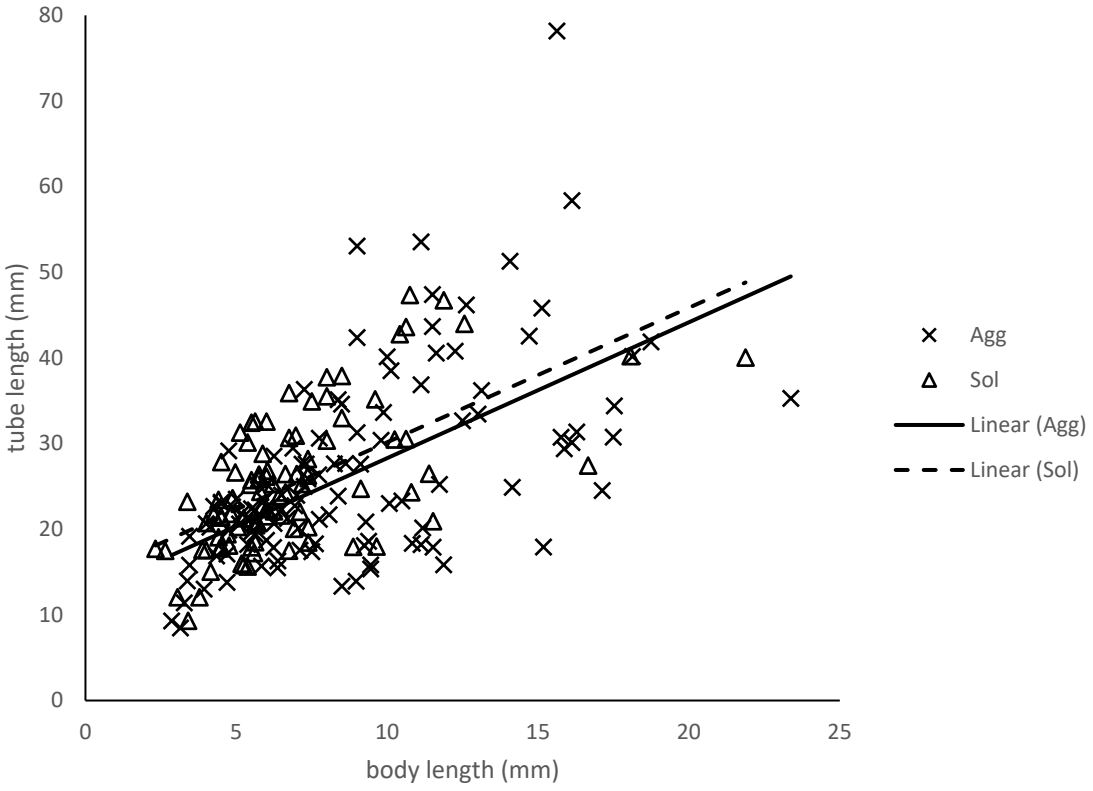

Figure 3.09 Tube length plotted against body length for solitary (Sol) and aggregative (Agg) individuals. Data collected between spring 2016 and spring 2017; solitary n = 93; aggregative n = 130.
R^2 for Agg = 0.34 (y = 1.59x + 12.44); R^2 for Sol = 0.39 (y = 1.57x + 14.45).

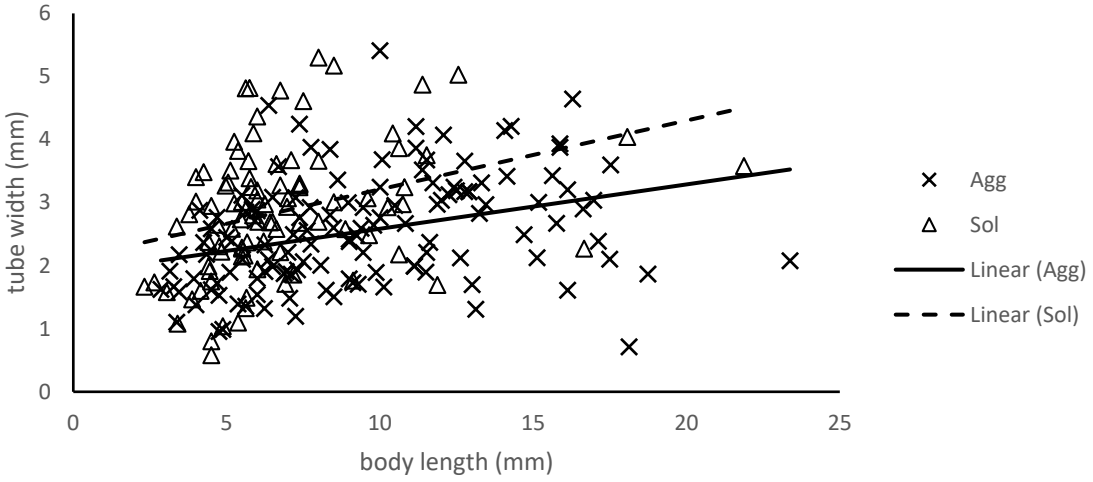

Figure 3.10 Tube width plotted against body length for solitary (Sol) and aggregative (Agg) individuals. Data collected between spring 2016 and spring 2017; solitary n = 93; aggregative n = 130.
R^2 for Agg = 0.11 (y = 0.07x + 1.88); R^2 for Sol = 0.11 (y = 0.11x + 2.12).

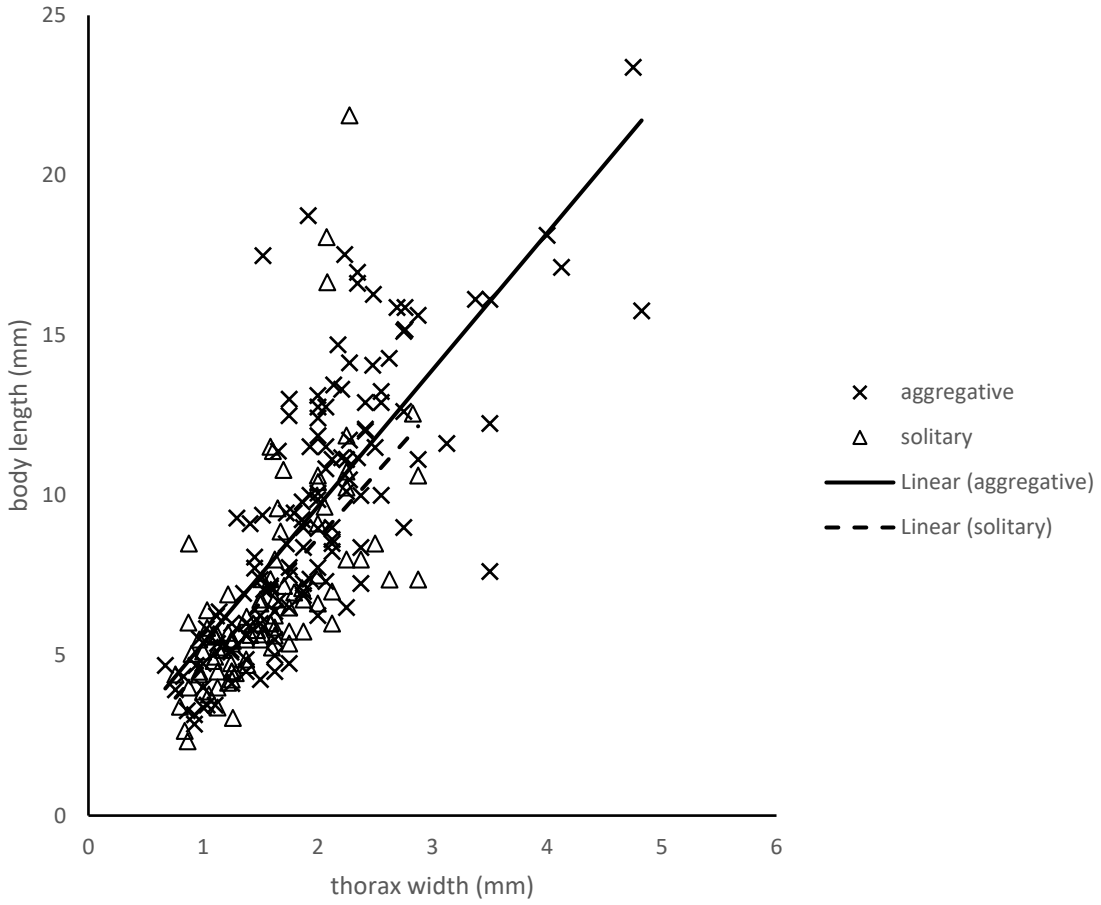

Figure 3.11 Body length plotted against thorax width for solitary (Sol) and aggregative (Agg) individuals. Data collected between spring 2016 and spring 2017; solitary n = 93; aggregative n = 130. R^2 for Agg = 0.59 (4.3x + 1.11); R^2 for Sol = 0.41 (y = 4x + 0.64).

Table 3.07 Summary of the results on the linear models of the size and weight measurements. Additional linear models can be found in the Appendix (Table A3.16 – A3.20).

Abdominal segments	Sol<Agg not significant
Tube length	**Sol>Agg significant**
Tube width/height	**Sol>Agg significant**
Body length	**Sol<Agg significant**
Body diameter	Sol<Agg not significant
Tube dry weight per mm	Sol>Agg not significant
Tube dry weight	Sol<Agg not significant
Worm dry weight	Sol<Agg not significant

3.4 Discussion

3.4.1 Tube growth and recovery

There was no difference in tube growth between solitary and aggregated *S. cariniferus* in the field. In my observations, I did not further investigate intraspecific competition. It could be that individuals in the centre of an aggregation experience a disadvantage through space and food limitation (Okamura, 1986). For example, there is also no significant difference in the overall growth rate between solitary and aggregative individuals of the mussel *Mytilus edulis*; however, individuals in the centre of a large aggregation grew slower than solitary conspecifics and those on the edge (Okamura, 1986). For one gooseneck barnacle species, *Pollicipes polymerus,* recruits that settled on solitary adults grew significantly faster compared to any other conspecific (Helms, 2004). Therefore, the individual's position in the aggregation can have an impact on the individual growth rate (Fréchette et al., 1992; Helms, 2004; Svane and Ompi, 1993). In particular, an increased growth for barnacle individuals living in the middle of a high-density aggregation has been observed. This elevated length growth or “hummocking” occurs to mitigate the limitation of food caused by their centred position in the aggregation (Bertness et al., 1998; Menge, 1976).

Observations of young recruits in high numbers of *Ficopomatus uschakovi* at Brisbane River suggest an adverse effect of intraspecific competition on the individual growth for tube worms (Straughan, 1972). According to the author, recruits in a density of 120 individuals/plate grow up to three times faster compared to recruits in high density (700 individuals/plate) (Straughan, 1972). On the other hand, an elongated vertical tube growth at high densities that also lead to hummock-like or even reef-like structures, has also been described for adults of some serpulin species like *F. enigmaticus*, *G. caespitosa* and *S.* cf. *krausii* (e.g. Schwindt et al. 2001; Straughan 1968; O’Donnell 1986). Also for *S. cariniferus,* similar vertical tube growth at high densities has been observed

(Riedi, 2012; Smith et al., 2012, pers. observations). However, at most field sites around Wellington, such high density was not reached. In this study, the average tube growth rate of *S. cariniferus* was more dependent on season, as in other marine invertebrates (e.g. Bertness and Grosholz, 1985) including intertidal serpulins such as *Galeolaria caespitosa* (O'Donnell, 1986) and subtidal serpulins such as *S. triqueter* (Klöckner, 1976b).

Riedi and Smith (2015) reported for the time from December 2010 to December 2011 a higher average tube growth rate for *S. cariniferus* at Harrington Point (Dunedin) than I found here. However, the authors monitored only a small number of individuals and considered only specimens with newly secreted calcium carbonate at the time of recapture. Therefore, the observations by Riedi and Smith (2015) do not reflect the impact of tube material loss on the growth rate, particularly as the authors measured the growth only once after each six months. Growth rates are reported for adults of one other intertidal species, *G. caespitosa* (O'Donnell 1986), and these are almost identical to my observations on *S. cariniferus*. Because the tidal cycles have less impact on subtidal species, it is clear that those serpulid taxa have a higher tube growth rate compared to intertidal serpulins (Hughes et al., 2008; Iyengar, 2002; Riedi and Smith, 2015). For *Ficopomatus enigmaticus* (Fauvel, 1927), dominant in brackish water, at the Po River Delta (Italy) the water temperature in August is too warm for spawning. Therefore, the individuals pause their reproduction and focus their energy on growth. Consequently, this species has their tube growth peak in August (Bianchi and Morri, 1996). In Wellington Harbour, *S. cariniferus* have their growth peak in spring before spawning begins and the water temperature is possibly too low for reproduction and the survival of the offspring (Anil and Kurian, 1996; Joyce et al., 2013; Thiyagarajan et al., 2003, pers. Obs.). Growth is temperature related and also dependent on food supply, which often increases with warmer temperature in comparison to winter and autumn (Kim et al., 2007; Wu and Levings, 1978).

In general, most reports of serpulid growth focus on new recruits over several months, which is significantly higher than the tube growth of mature and even some juvenile worms over longer periods (e.g. Dew 1958; Grave 1933; Miura & Kajihara 1981; Paul 1937; O'Donnell 1986). However, from all these observations, growth is mainly dependent on initial size, habitat conditions and population density. The recruits of the serpulid *Ficopomatus uschakovi* have the highest growth rate in their salinity optimum (Straughan, 1972). In 2–5 week old recruits of *Hydroides elegans* at Wollongong (Australia), tube growth was almost six times faster in highly polluted water compared to a clean environment (Moran and Grant, 1984). Here, it is important to understand that growth limiting factors like density or competition can become less significant in an eutrophic habitat (Moran and Grant, 1984).

Also, for *S. cariniferus* in this study, the tube growth rate is highly variable in Wellington Harbour but seems mainly to depend on season and initial size. As tube width and height increases, the tube growth rate decreases. The smallest individuals (<1mm in tube width/height) had a higher growth rate throughout the year compared to larger conspecifics (see Table A3.02 - A3.04 and Figure A3.02 in the Appendix). Those individuals likely settled in the previous summer and need to increase their size significantly to improve their ability to survive (Dayton, 1971; Denny, 1995; Denny et al., 1985; Hunt and Scheibling, 2001). For all other individuals (>1mm in tube width/height), the tube growth rate was more variable. However, the higher growth rate in spring (see Table A3.05 - A3.10 and Figure A3.03 in the Appendix) may be associated with a seasonal increase in planktonic food availability at Wellington Harbour due to warmer temperature (Helson et al., 2007; Helson and Gardner, 2007; Pinkerton, 2016). On the other hand, the slowest growth for mid to larger sized individuals occurred, in general, between summer and fall (see Table A3.05 – A3.10 in the Appendix). During this time, mature serpulins concentrate their energetic output on reproduction (Dixon, 1981). However, it is not clear why the growth rate for smaller individuals drops in autumn 2016 whereas larger worms increase tube growth. The accelerated

growth in autumn 2016 for larger individuals can be explained by an extended period of high food supply through warmer temperatures compared to autumn and winter 2015 (GWRC, 2019; Pinkerton, 2016). For *G. caespitosa* the highest growth rate occurred in late summer to autumn; however, the author presents no explanation for the differences (O'Donnell, 1986).

For other serpulins like *G. hystrix*, *Serpula columbiana* (Johnson,1901), and even other taxa of marine invertebrates like *Trichotropis cancellate* (Hinds, 1843) or *Donax hanleyanus* (Philippi, 1847), similar observations have been made (Herrmann et al., 2009; Iyengar, 2002; Riedi and Smith, 2015). However, growth rates are difficult to compare between all those different invertebrate taxa as either only particular parts of the shell were measured (e.g. capitular plates for barnacles Jacinto et al., 2015) or the shell length and width increases differently to tubeworms (e.g. bivalves, Mahé et al., 2010). Further, the energetic investment into tube growth is different for serpulids compared to other invertebrate groups. For *F. enigmaticus* an investment of up to 68% of their total energy balance into tube production has been reported (Dixon, 1977), whereas bivalves and barnacles seem to invest 2–6.6% of their energy into shell growth (Hughes, 1970; Wu and Levings, 1978). A list of published tube and shell growth rates for serpulids, some spirobins as well as a few other species is provided in the Appendix (Table A3.21).

Contrary to my predictions, the rate of tube growth of the worms in the field that were damaged did not differ significantly to the growth rate of undamaged individuals, and responses were similar whether worms were aggregated or solitary. This suggests that solitary worms do not invest more energy in repair than those in aggregation. However, in lab conditions, solitary damaged worms lost more of their tube rather than gained new material. Further, with increased damage, the growth rate decreased for solitary individuals. In the field, this trend was less apparent, potentially because other environmental factors come into play. The only significant finding for the recovery was a higher tube growth rate in

winter compared to summer, which is in agreement with the findings from the tube growth observations. It is possible that results similar to those from the lab trial would have been observed in the field, such as increased tube loss for solitary individuals, if the experiment had continued longer than two months. The elevated growth rate of aggregative individuals in the lab compared to the average growth of undamaged solitary conspecifics in the lab could be caused by a response to intraspecific competition or better ability to retain food (Helms, 2004; Merz, 1984; Okamura, 1986).

3.4.2 Mortality

Mortality was highly variable for the different size categories and solitary as well as aggregative *S. cariniferus* individuals. Known factors for mortality of calcareous tubeworms are predation and physical stressors such as desiccation, heat, and wave exposure. Various fish, crustaceans (Bosence, 1973; Minchin, 1987) and molluscs (Morton and Harper, 2009; Tan and Morton, 1998) prey on serpulids, although for the most part these observations are limited to taxa that are mostly subtidal. There have been no reports on predation on intertidal serpulid species. However, important predators for other intertidal invertebrate taxa, such as mussels, barnacles and limpets, include birds (e.g.Wootton 1992), starfish (e.g. Menge, 1972a, 1972b; Paine, 1969), snails, nudibranchs and crustaceans (e.g. Bertness & Grosholz 1985; Dayton 1971). For these invertebrates, prey species' individual size, tidal height and density are important in determining vulnerability to predation. I did not observe any predation on *S. cariniferus* except in one incident in the lab where I found one individual of the whelk *Haustrum scobina* drilling into a tube of a living worm. This gastropod is known to attack barnacles like *Chamaesipho columna* (Barnett et al., 2010; Novak, 2010). As *C. columna* occur at a higher tidal height than *S. cariniferus,* predation by *H. scobina* is not hindered by the vertical distribution of this serpulid. It is likely that these whelks are not a major predator for *S. cariniferus*, similar to observation that *Tenguella marginalba* (formerly known as *Morula marinalba*) is

not a major predator on *Galeolaria caespitosa* (O'Donnell, 1986). I assume that predation on *S. cariniferus* is probably not a major source of mortality perhaps because of the high energetic cost required and the possible risk of desiccation a predator would experience by attacking the thick calcareous walls of serpulid tubes (Dayton, 1971; O'Donnell, 1986). Further, I observed in the lab that adults of individuals of *S. cariniferus* became more inactive and remained in their tubes if the water temperature reached 19°C, which minimised the ability to exchange gas and feeding (Dill and Fraser, 1997; Poloczanska et al., 2004). This observation would suggest heat as a possible factor for the mortality in *S. cariniferus;* however, if heat and desiccation are the reason for mortality in the field then the mortality should be at its highest around summer, but the opposite was the case.

Therefore, it is more likely that dislodgement from waves and wave-transported debris (e.g. rocks, logs) are the main factors for the mortality for *S. cariniferus* around Wellington Harbour, as they can be for a variety of other intertidal species (Dayton, 1971; Denny, 1995; Hunt and Scheibling, 2001; Shanks and Wright, 1986). This hypothesis is supported by the observations that *S. cariniferus* predominantly appears in sheltered areas or amongst larger sessile organisms (Bianchi and Morri, 2001; Heiman et al., 2008; Hill, 1967; Knox, 1949; Smith et al., 2005). However, there is limited information about sources of mortality for serpulids, with the mortality of new recruits being the most common focus (Klöckner, 1976a; Straughan, 1972). Mortality in new recruits is significantly higher compared to the fatality rates amongst older individuals. To my knowledge mortality estimation for adult serpulins is known only for *Serpula vermicularis* and *Spirobranchus triqueter* . For *S. vemicularis* from the subtidal habitats at Loch Creran (Scotland), 28% mortality was reported over the span of one year and was presumably caused by extreme weather (Hughes et al., 2008). For *P. triqueter* mortality was estimated at Helgoländer Tiefe Rinne (Germany) (Klöckner, 1976b). The ratio of empty tubes to occupied tubes in dredged samples were counted and from this data has been estimated a mortality of 41%

for *S. triqueter* (Klöckner, 1976b). The author suggests that the substrate on which the individual settles has an effect on the survival chance. It was proposed that mobile substrate, like snail shells, could aid the survival of the worm (Klöckner, 1976b). Worms settling onto rocks or other immobile substrate would experience a higher mortality through sedimentation. The mortality of *P. triqueter* is similar to the mortality rate of *S. cariniferus* I found, but mortality for *S. vermicularis* is lower. The difference for the rate between *S.cariniferus* and *S. vermicularis* is perhaps due to a harsher intertidal environment compared to the subtidal habitat where *S. vermicularis* lives.

3.4.3 Tube size relative to body dimensions

From the fitted models on the measurements of body and tube dimensions, it appears that solitary specimens have a significantly longer tube than their aggregative counterparts with the same thorax width, body length and tube width/height. This relationship between tube and body dimension is remarkable because earlier observation of tube growth revealed no difference in growth rate between both settlement cofigurations. Through including the thorax width and body length in the analysis for the tube, it becomes clear that solitary worms have a significantly wider/higher and significantly longer tube than aggregated individuals of similar body size. Conversely, solitary individuals have a smaller and lighter body for similar tube size. This suggests that solitary worms focus their energy on tube length growth rather than increasing their body dimensions. Therefore, solitary worms are more likely to be older than aggregative worms with similar tube width.

The resulting conclusion that solitary worms possibly elongate their tube in a higher rate than same aged aggregative individuals contradicts my expectations that aggregative worms have a higher tube growth rate based on competition and perhaps higher food supply (Bryan et al., 1997; Fauchald and Jumars, 1979; Fréchette et al., 1989; Merz, 1984). Solitary worms make use of the fact that

individuals with a smaller tube width grow significantly faster. Therefore, solitary individuals do not grow faster than aggregative conspecifics of the same tube width but have a higher tube length growth rate compared to the same aged aggregative conspecifics (with a possible wider tube). I speculate that these worms raise their chance to find conspecifics through the increased tube length growth. Support for this speculation is the occasional observation of a several magnitude larger tube growth rates for some solitary worms if new recruits appeared around the individual.

As this study contrasts biometric data of aggregative and solitary settled tube dwelling worms for the first time, to my knowledge, it is difficult to compare these current results with other investigations. Some studies of patch dynamics focused on the intraspecific competition in an aggregation of sessile mussels or barnacles (e.g. Bertness and Grosholz, 1985; Helms, 2004; Okamura, 1986). Particular studies on mussel patches show that on the edge of an aggregation, individuals grow at a similar rate as solitary settled conspecifics, whereas individuals in the centre seem to grow slower (Okamura, 1986). For *Mytilus edulis* in Denmark, individuals in the middle of a patch are significantly smaller in shell length, shell weight and flesh dry weight compared to their conspecifics on the rim of a cluster (Svane and Ompi, 1993). Also, for *F. enigmaticus*, *H. elegans* and *S.* cf. *krausii* it is reported that individuals can adapt their tube growth according to their position in an aggregation or distance to a conspecific (Bianchi and Morri, 2001; Nishi and Nishihira, 1997).

3.4.4 Conclusion

The aim of this chapter was to contrast characteristics in mortality and growth between aggregative and solitary individuals of *S. cariniferus*. Overall, there were no clear differences; both were highly variable and appear to be more dependent on factors other than whether worms are aggregated or not. If there are trade-offs in living alone vs. in aggregation, they are not manifested in these

responses. However, if we look beyond the rates for tube growth, it becomes clear that solitary worms have either a smaller body for the same tube size, or a longer tube for the same body size as those in aggregation, suggesting that worms settled in isolation focus their energy on tube length growth rather than body growth. Therefore, solitary individuals of *S. cariniferus* stay smaller but elongate their tube faster compared to aggregated specimen of the same age.

We can postulate that aggregative immobile species like *S. cariniferus* preserve energy through living in clusters (Ritz, 2000). For example, the structural support of each other can have a positive effect on the energy balance as the aggregative settled organism might need to invest less energy to stabilise their tube or shell, and possibly encounter less destruction through erosion. This energy deficit could also explain why solitary individuals of *S. cariniferus* experience a negative growth rate after their tube has been damaged to a considerable amount. Possibly, the required energy to stabilise and regrow lost anterior tube length is too high for the available food and stored reserves of a solitary individual (Wu and Levings, 1978).

Definitions of aggregative settlement differ throughout this chapter on tube growth, recovery and body metrics regarding how many individuals constituted an aggregation as well as in their distance to each other, as the focus of my observations shifted with the different approaches. For example, it is difficult to postulate that two or three individuals that have settled close to each other will compete for food. On the other hand, it is plausible that even two neighbouring worms can support each other's tube stability. However, by writing this thesis, it occurred to me that this change in definition could become problematic in a conclusive interpretation of results for aggregative and solitary settlement.

In general, more research is needed for the comparison of tube growth, recovery and mortality for aggregative and solitary settled individuals. However, in my opinion, the definition of aggregative settlement needs to reflect the focus of the

experiment. Therefore, we need to systematically narrow at which point the advantages and disadvantages for an aggregative settlement become significant compared to solitary individuals before we can form a uniform definition of aggregative settlement. In general, I propose that with an increase in the number of individuals, the individual tube stability will increase. Therefore, the significance in difference for aggregative and solitary worms could become even clearer for body metric measurements, as well as for tube recovery in field and lab studies.

4. Reproduction: Maturation, sex-ratio and possible hermaphroditism for *S. cariniferus*

4.1 Introduction

One often discussed benefit of living in an aggregation is the possibility to synchronise the reproductive cycles (Pechenik, 1999). A first step to understanding the reproductive cycle of serpulins like *Spirobranchus cariniferus* is to observe the maturation of gametes and the expression of sexes in a population. The ripening of gametes seems to be most likely regulated through water temperature, food and salinity (Gee, 1967; Kupriyanova et al., 2001; Leone, 1970; Qiu and Qian, 1998). However, it is unknown if living in aggregation vs. alone affects maturation rate. Solitary worms appear to focus their energy on tube length growth and maintain a smaller body (see Chapter 2) compared to their aggregative conspecifics. Smaller worms may mature later, possibly with a lower quantity of gametes, or they don't mature at all, perhaps because of an energetic deficit compared to larger individuals (Daly, 1978; Hill, 1967; Kupriyanova et al., 2001; Qiu and Qian, 1998). Therefore, maturation rate may be different for individuals in aggregation compared to solitary and may be mediated by differences in body size.

The distribution of sexes (sex ratio) in population of marine invertebrates is an important component of reproductive ecology. For most marine invertebrate taxa, gonochorism (separated sexes) is the norm (e.g. Juchault, 2002; Kilias, 1982; Schroeder and Hermans, 1975), and reproduction via gonochorism has evolutionarily arisen from common hermaphroditic ancestors (Ghiselin, 1974; Hoagland, 1984; Hodgson, 2009; Juchault, 2002). However, in many invertebrates, including polychaeta, hermaphroditic taxa or species have been observed. It appears that secondary hermaphroditism has arisen independently in multiple taxa (Ghiselin, 1974; Kilias, 1982; Prevedelli et al., 2006).

Amongst hermaphrodites there are two main forms, simultaneous and sequential hermaphroditism. Simultaneous hermaphrodites form and release both type of reproductive products, for example, egg and sperm cells, at the same time (Ghiselin, 1974; Hodgson, 2009). This form of hermaphroditism is favoured when the number of potential mating partners is low (Hodgson, 2009; Puurtinen and Kaitala, 2002). On the other hand, sequential hermaphroditism is where the individuals of a species reproduce first as one sex and subsequently change to the other (e.g. Hoagland, 1984; Premoli and Sella, 1995). The sex that is expressed first amongst sequential hermaphrodites depends on the relative reproductive success of males and females. Consequently, we can differentiate between protogyny (female first) and protandry (male first). Protogyny is most common amongst fish, but also observed in a small number of marine invertebrates (e.g. Ghiselin, 1969; Hoagland, 1984). Protogyny occurs in groups where the reproductive pressure mainly lies on the male individual. In those cases, either females select for larger partners and/or the male needs to defend the territory or offspring (Hoagland, 1984; Premoli and Sella, 1995). Protandric hermaphroditism is more common amongst marine invertebrates and is often explained by the “size advantage model” (Ghiselin, 1969; Hodgson, 2009; Olive, 2006; Premoli and Sella, 1995). According to this theory, the reproductive output for females increases significantly with increased individual size, whereas for males the fertility is not markedly raised through individual growth (Ghiselin, 1969, 1974; Premoli and Sella, 1995). It has been suggested that the sex ratio in an adult population often indicates whether a species is gonochoristic or sequentially hermaphroditic, as in the latter the ratio is mostly skewed towards the earlier sex, possibly because younger, smaller individuals occur in higher frequency than older, larger conspecifics (Cotter et al., 2003a; Obenat et al., 2006; Olive, 2006).

Gonochorism has often been reported in serpulins (e.g. Knox 1949), but there are several examples of individuals where both gamete types have been observed at the same time in the serpulind species: *F. enigmaticus*, *F.*

uschakovia, *H.* elegans, *G. caespitosa*, *S. lamarckii* and *S. triqueter* (Cotter et al., 2003a; Dixon, 1981; Føyn and Gjøen, 1954, 2001; Straughan, 1972; reviewed by Kupriyanova et al.). As some of those hermaphrodites released sperm cells and formed oocytes (which were visible through histological sections), the assumption was made that those individuals changed from male to female (Cotter et al., 2003a; Dixon, 1981). Other evidence comes from spawning, where individuals of *Spirobranchus triqueter* released sperm cells in a first spawning, and then discharged oocytes a week later in a second spawning event (Føyn and Gjøen, 1950).

Serpulins have been generally reported as dioecious with the sporadic observation of hermaphroditic individuals (e.g. Cragg 1939, Dixon 1981, Knox 1949, reviewed Kupriyanova et al., 2001). However, the consensus of current studies suggest for serpulins, sequential hermaphroditism in the form of protandry instead of gonochorism is the rule rather than the exception (Cotter et al., 2003a; Kupriyanova et al., 2001). However, because these tube-dwelling worms have no distinctive gonads and no sex-specific characteristics, it is challenging to prove this hypothesis (Dixon, 1981; Kupriyanova et al., 2001). A biased sex ratio is often referenced as support for protandry (Kupriyanova et al., 2001; Obenat et al., 2006). Further, males tend to be smaller than females (Cotter et al., 2003a) which also supports protandry amongst serpulins (Ghiselin, 1969).

In this chapter, I report the maturation rate of *S. cariniferus* in relation to individual size and whether worms are found in aggregation or alone. This is followed by an evaluation of the sex ratio, also in relation to individual size. If *S. cariniferus* is a protandric species, I expect that the sex ratio is skewed and that the male individuals will be smaller than their female counterparts (Cotter et al., 2003a; Kupriyanova et al., 2001; Obenat et al., 2006). Serpulins form gametes in their abdominal segments and release them through paired ducts in the abdominal segments (Hartmann-Schröder, 1982; Westheide, 1988). Therefore,

through sections of the abdominal segments of female, male, non-spawning and possible hermaphroditic individuals, I will explore the reproductive anatomical characteristics of *S. cariniferus*.

4.2 Methods

4.2.1 Maturation and distribution of male and female individuals

Specimens of various sizes were collected from eight sites in the Wellington region (Breaker Bay, Kau Point, Porirua Harbour, Point Halswell, Pukerua Bay, Scorching Bay, Shelly Bay, Worser Bay) on a total of 14 occasions in summer 2014/15, 2016/17 and 2017/18. To evaluate whether they were mature, and to determine the sex, I attempted to induce gamete release for the collected specimens (81–273 at a time). To do this, for each individual I opened the posterior part of the tube and pushed on the operculum to drive the individual backwards out of its tube. In general, mature specimens released gametes immediately, or after gently squeezing the abdominal segments. Immediately after removing animals from their tubes, males were placed on watch glasses and sperm collected in 2–3 ml of filtered seawater (10µm filter, from here on referenced as FSW). Each female was placed in a separate bowl with 50 to 100 ml FSW. In summer 2016, the body length and thorax width of each individual was measured using a microscope with an ocular micrometer (as in Chapter 2). Spawning individuals were gently squeezed with a probe to release as many gametes as possible. The released gametes were further diluted with FSW. The number of sperm cells was estimated by using a Neubauer hemocytometer and oocytes were counted in a Fuchs-Rosenthal counting chamber to estimate the total number of gametes for each individual.

A binominal logistic regression was used to analyse the maturity in response to size and month. With a second binominal model, I explored the maturation rate in combination to the settlement strategy. For both models the maturity was coded

as “Yes” for mature or “No” for immature individuals. For the investigations described in this chapter, I included individuals from Porirua Harbour. These worms were longer than individuals from other sites. Therefore, to sort the specimen into age groups, I used the thorax width, as this biometric measurement was less variable between sites.The thorax width size categories were <1.5 mm, 1.5-3 mm and >3 mm. The size categories allowed me to compare the maturity ratio for different size groups. For the distribution of both sexes, I used only individuals of November 2016 and 2017, and January and March 2018 from both settlement configurations as data were too limited from collections from other months. This limitation resulted in a lower number of small mature individuals. Therefore, for the analysis of the sex ratio, I changed the thorax width categories to <2 mm, 2–3 mm, >3 mm. For the examination of female and male ratio I again used a logistic regression in response to month and thorax width category. For this model each individual was coded according to its sex. Fecundity was also explored with a logistic regression for each sex separately, in response to thorax width and aggregated or solitary settlement. Additionally, for a comparison of size between both sexes, I fitted a linear model with the body length and thorax width in response to the individual sex.

Data were analysed with the statistical software R (version 3.5.1 'Feather Spray', 2018). In R I utilised the packages “ggplot2”, “GGally”, “reshape2”; “lme4”, “boot”, “lattice”, “psych”, “lme4”, “arm”, “lmtest”, “multcomp”, “lmerTest”, (Bates et al., 2015; Canty and Ripley, 2017; Fox and Weisberg, 2011; Gelman and Su, 2018; Hothorn et al., 2008; Kuznetsova et al., 2017; Revelle, 2018; Sarkar, 2008; Schloerke et al., 2018; Venables and Ripley, 2002; Wickham, 2007, 2016; Zeileis and Hothorn, 2002).

4.2.3 Hermaphroditism

For the histological sections, I selected three female and four male individuals from different sites and of different size. I also sectioned six individuals that were

identified as hermaphrodites from spawning and four individuals of the same or larger size as the hermaphrodites that did not release gametes. Each individual (17 in total) was preserved initially in 5% formaldehyde buffered with borax (1g/L). After 72 hours the formaldehyde was replaced, and after a further week the samples were transferred to 70% ethanol. Before embedding in paraffin, the operculum of each specimen was removed. For larger individuals, I used only the abdominal segments and divided those segments into anterior and posterior abdominal segments if necessary. Each individual was placed in a separate embedding cassette. The samples were dehydrated and infiltrated with paraffin in an automatic tissue processor (Leica TP 1020), the protocol is given in the Appendix (Table A4.01). Subsequently, the samples were embedded at an embedding station (Leica EG1160). A manual microtome (Leica RM 2235) was used to cut 5–7 µm thin sections. The sections were transferred onto glass microscope slides using a warm water bath set to 37°C (Leica HI 1210). The samples were deparaffinised with Histo-Clear (National Diagnostics). Ehrlich - Haematoxylin was used for the regressive staining, and an alcoholic Eosin Y solution was used for counterstaining. The staining protocol, and the recipe for Haematoxylin, Eosin Y and Scotts Tap water, are given in the Appendix (Table A4.02 – A4.04). Sections were mounted on a microscopy slide with Euparal or Entellan mounting medium and photographed using a compound microscope with a digital camera (Canon EOS 550).

4.3 Results

4.3.1 Maturation and fecundity

The percentage of individuals with gametes varied over time from 5–100% and was greatest in mid-summer (December–January, Fig 4.01). However, from a logistic regression and a post hoc comparison (using the Bonferroni procedure) the only significant result was a lower proportion of mature animals in April 2017 compared to November 2016 ($p < 0.01$). The size of the individual had a

significant effect on maturation (Table 4.01), where the smallest individuals (<1.5 mm in thorax width) had significantly lower maturity compared to both of the larger size classes (pairwise comparison with Bonferroni-adjusted p-values; p = 0.02, in Appendix Table A4.05). However, whether individuals were solitary or aggregated did not have an impact on maturation (logistic regression: z= - 0.46; p = 0.64, in Appendix Table A4.06). Further, the fecundity of females or males was also not affected by whether individuals were aggregated or alone (from linear models for males p = 0.38, in Appendix Table A4.07 and for females p = 0.45, Table A4.08). The quantity of released eggs for a sample of 68 females ranged between 1.56 x 10^3 and 2.28 x 10^5 per individual. For 50 males, the quantity of released sperm ranged between 1.56 x 10^3 and 8.31x10^7 cells per specimen. The amount of oocytes was not dependent on female size (linear model p = 0.96, in Appendix Table A4.08) whereas the sperm quantity was dependent on size of the male (linear model p <0.001, in Appendix Table A4.07).

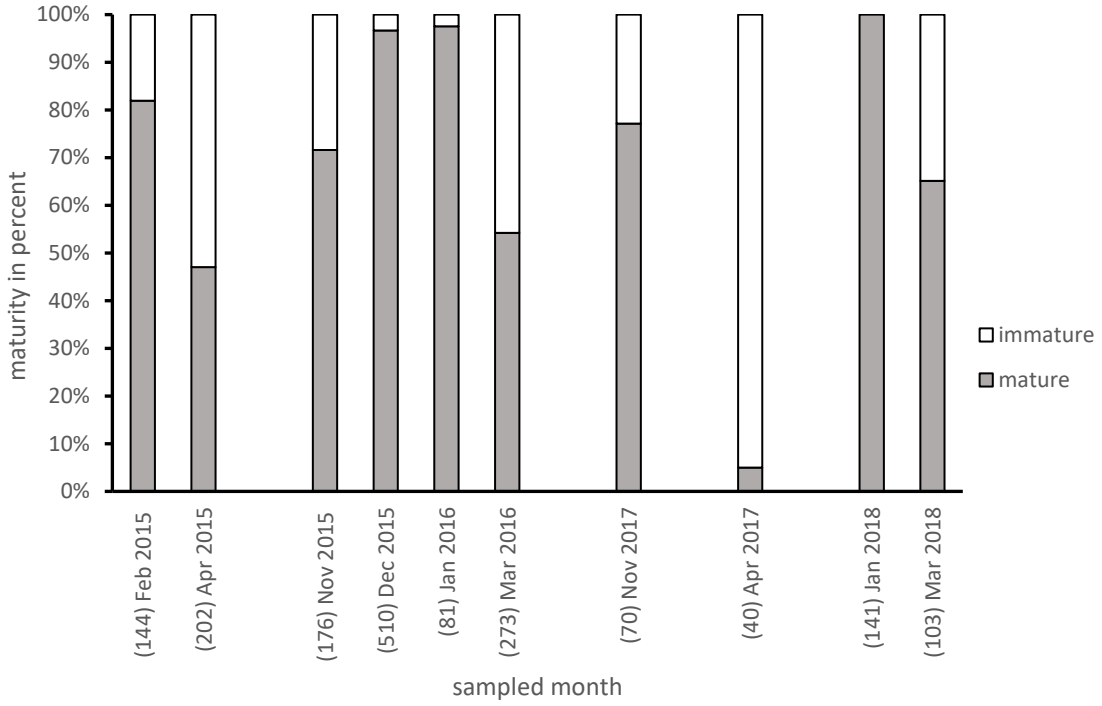

Figure 4.01 Percentage mature and immature individuals for each observed month between February 2015 and March 2018. The number in brackets represents the sample size. Data has been pooled from eight sample sites (Breaker Bay, Kau Point, Porirua Harbour, Point Halswell, Pukerua Bay, Scorching Bay, Shelly Bay, Worser Bay)

Table 4.01 Logistic regression of maturation rate in relation to thorax width in category:
<1.5 mm; 1.5-3 mm; >3 mm. A positive estimate represents an increase in the maturity rate and a negative value reflects a reduction in maturity. Site, Month and Year have been included as random factors. Observations were made in: November, December 2016; February, April, November 2017; January, March 2018. Number of observations is 411.
A pairwise comparison with Bonferroni-adjusted p-values for size category is given in the Appendix Table A4.05.

Source of variation	Estimate	SE	z-value	p-value
(Intercept)	1.13	1.31	0.86	0.39
thorax.cat> 3mm	1.89	0.68	2.78	**< 0.01**
thorax.cat1.5-3 mm	2.81	0.56	0.56	**< 0.01**

4.3.2 Distribution of male and female individuals in *S. cariniferus* populations

The sex ratio was not affected by the settlement strategy (logistic regression p = 0.69, in Appendix Table A4.09). The female to male ratio varied across sampling events from 1 : 0.59 to 1 : 1.3 (F : M). However, during the peak of a spawning season there was a trend to a 1 : 1 distribution of female and male individuals (Figure 4.02 and see in Appendix Table A4.10). Over the complete observation period, the overall sex ratio was 1.1 : 1 (F : M), slightly but significantly in favour of females (exact binomial test, p = 0.02 & $ChiX^{2,}$ p = 0.01,in Appendix Table A4.10). After sorting the females and males into three size categories based on thorax width (<2 mm, 2–3 mm, >3 mm), a logistic regression revealed no significant change in the sex ratio (Table 4.02). Therefore, the size had no significant impact on the distribution of the sexes. Male individuals of *S. cariniferus* seemed to have marginally shorter bodies, but this difference was not significant (p = 0.68, in Appendix Table A4.11). On the other hand, females had a significantly (p < 0.01) wider thorax than the males (in Appendix Table A4.12).

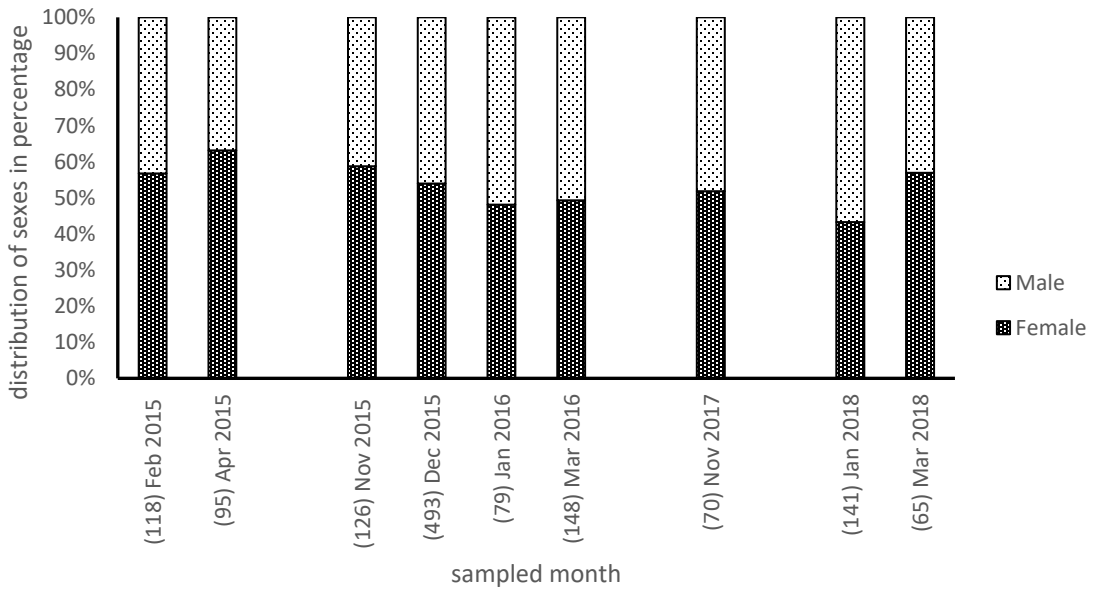

Figure 4.02 The distribution of sexes for all sampled months between February 2015 and March 2018. The number in brackets represent the sample size. Data has been pooled from four sites (Porirua Harbour, Shelly Bay, Worser Bay, Breaker Bay).

Table 4.02 Logistic regression of sex ratio in response to thorax width in category: <2 mm; 2-3 mm;>3 mm. A positive estimate represents an increase in the rate of females. A negative value reflects a higher quota of male individuals. Thorax width, Site and Year have been included as random factor. Observations have been made in: November 2016; November 2017; January, March 2018. Number of observations is 285 (F: 143, M:142).

Source of variation	Estimate	SE	z-value	p-value
(Intercept)	-0.15	0.35	-0.42	0.67
thorax.cat2-3 mm	0.08	0.39	0.2	0.84
thorax.cat>3 mm	-0.63	0.45	-1.4	0.08

4.3.3 Hermaphrodites

Gametes can fill out the whole-body cavity of the abdominal segments (Figure 4.03 A and C, Figure 4.04 A). The testis sits on the septum and appears not to be close to a larger blood vessel (Figure 4.03 D). Ovaries are attached to the dorsal peritoneum supplied by a blind-ending capillary (Figure 4.04 B). This blind-ending capillary was also seen in male-identified individuals without any oocytes (Figure 4.03 B). Presumably, oocytes were found in one specimen that did not release any gametes and in one other apparently male individual (Figure 4.05 A–D). In the latter specimen, the anterior testis was more developed and had most likely spermatogonia (Figure 4.05 A). Whereas the oocytes in the same individual, which seemed to be reduced in size and appeared deformed, were found in the posterior region (Figure 4.05 A). A second as male - identified individual had abdominal segments filled with female and male gametes. Most of the gametes were sperm cells, but there were also distinctive ripe oocytes (Figure 4.05 C and D). In summary, over three spawning seasons, I attempted to spawn 2,725 individuals, of which 2,057 released gametes. Of those 2,057 individuals, six released both eggs and sperm. In addition, I found three individuals with eggs and sperm or possible spermatogonia in their coelom at the same time, out of the 17 histological sectioned specimens. As a result of all observations of gamete release and cross-sections during spawning seasons, at least 0.4% (nine individuals) of all individuals (2,057) appeared to be simultaneous hermaphrodites.

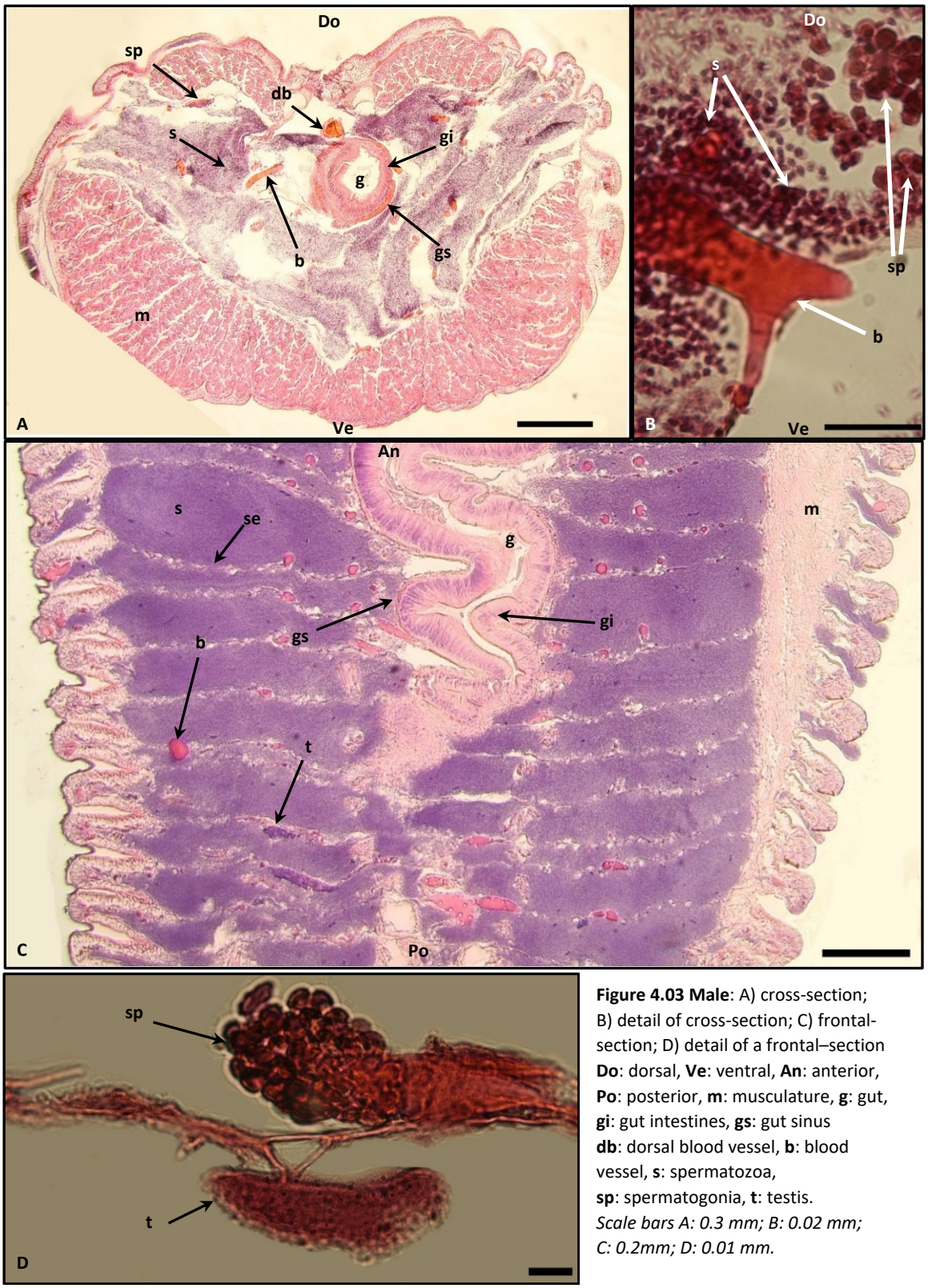

Figure 4.03 Male: A) cross-section; B) detail of cross-section; C) frontal-section; D) detail of a frontal–section
Do: dorsal, **Ve**: ventral, **An**: anterior, **Po**: posterior, **m**: musculature, **g**: gut, **gi**: gut intestines, **gs**: gut sinus **db**: dorsal blood vessel, **b**: blood vessel, **s**: spermatozoa, **sp**: spermatogonia, **t**: testis.
Scale bars A: 0.3 mm; B: 0.02 mm; C: 0.2mm; D: 0.01 mm.

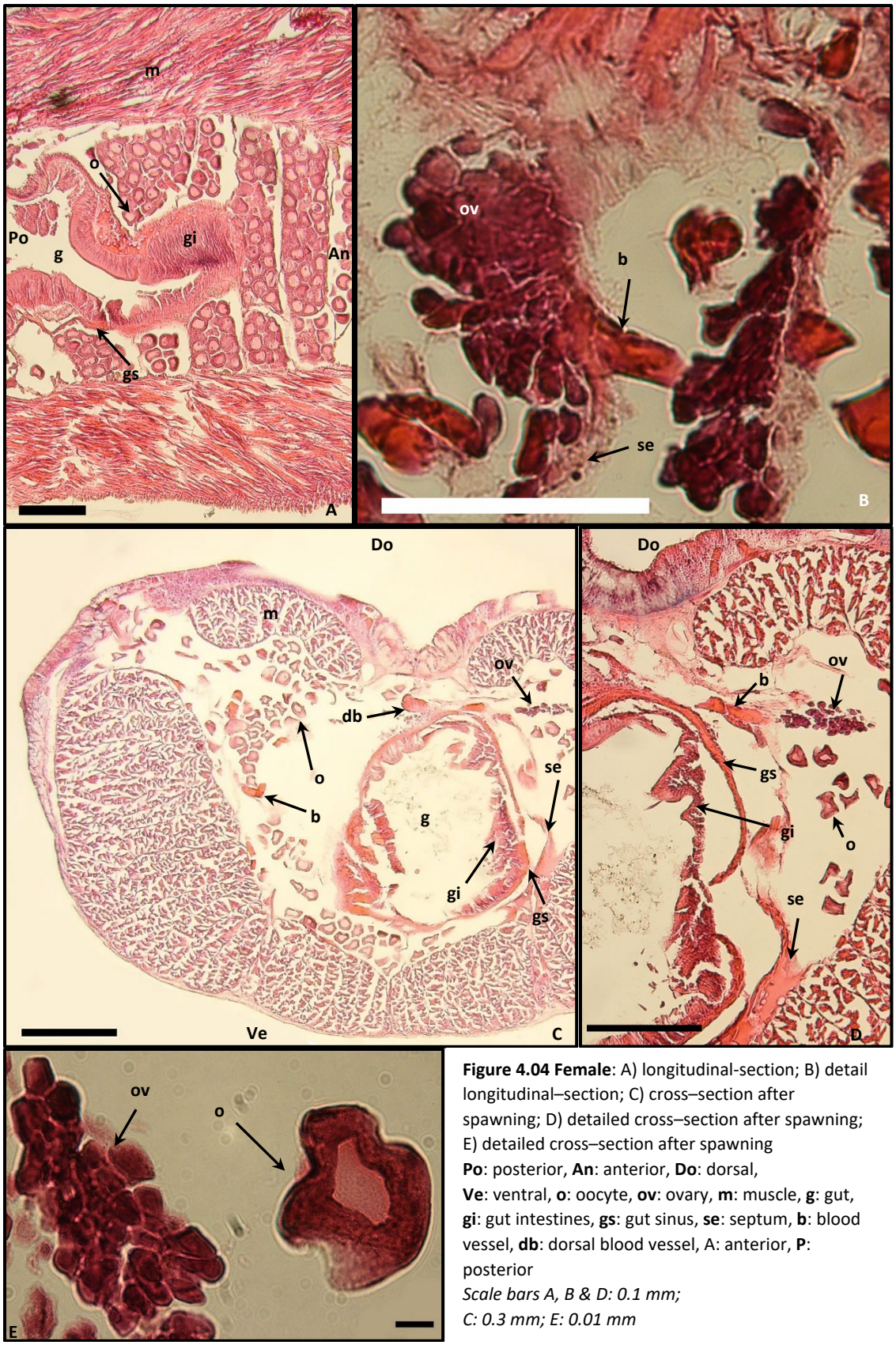

Figure 4.04 Female: A) longitudinal-section; B) detail longitudinal–section; C) cross–section after spawning; D) detailed cross–section after spawning; E) detailed cross–section after spawning
Po: posterior, **An**: anterior, **Do**: dorsal, **Ve**: ventral, **o**: oocyte, **ov**: ovary, **m**: muscle, **g**: gut, **gi**: gut intestines, **gs**: gut sinus, **se**: septum, **b**: blood vessel, **db**: dorsal blood vessel, A: anterior, **P**: posterior
Scale bars A, B & D: 0.1 mm; C: 0.3 mm; E: 0.01 mm

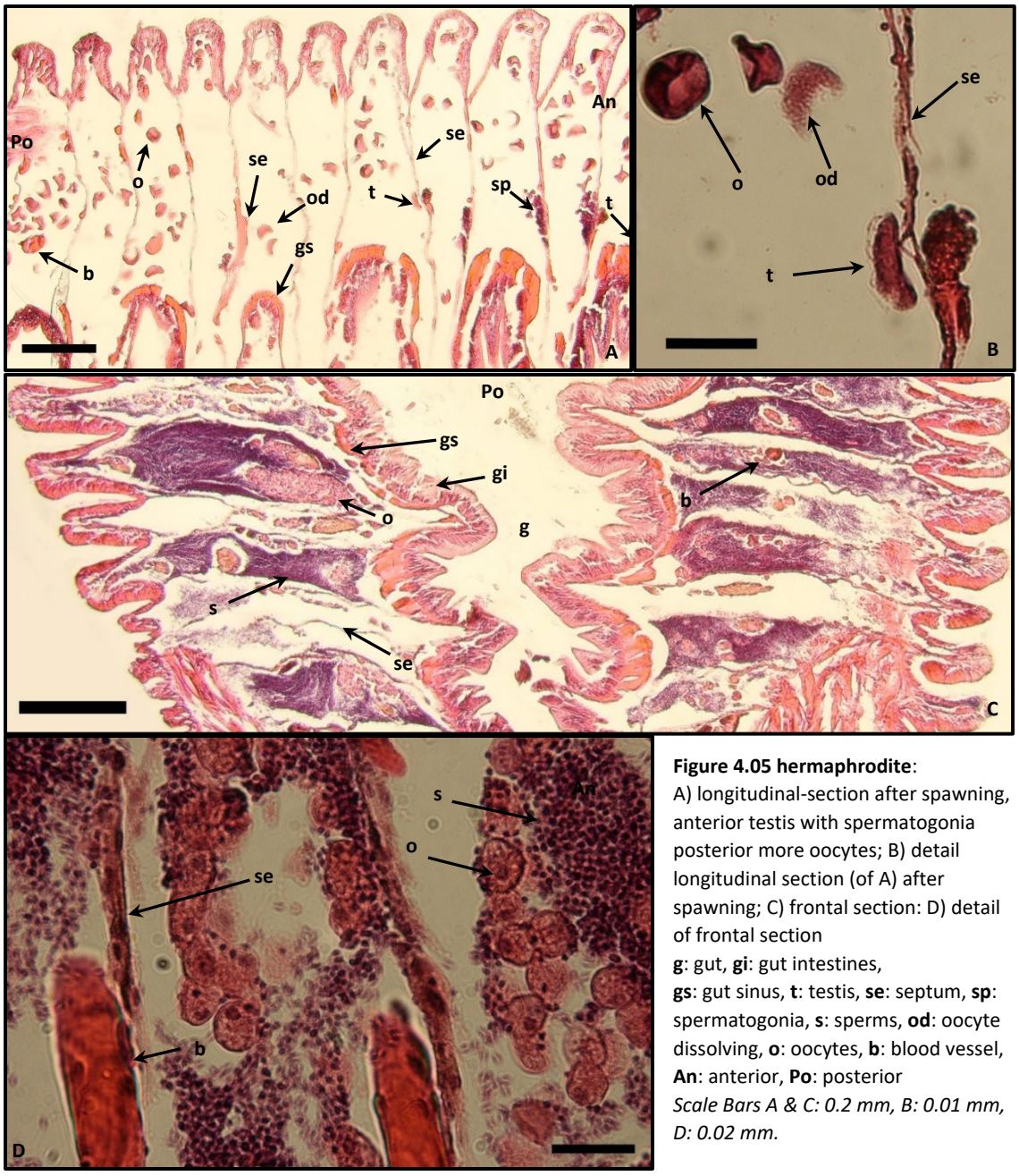

Figure 4.05 hermaphrodite:
A) longitudinal-section after spawning, anterior testis with spermatogonia posterior more oocytes; B) detail longitudinal section (of A) after spawning; C) frontal section: D) detail of frontal section
g: gut, **gi**: gut intestines, **gs**: gut sinus, **t**: testis, **se**: septum, **sp**: spermatogonia, **s**: sperms, **od**: oocyte dissolving, **o**: oocytes, **b**: blood vessel, **An**: anterior, **Po**: posterior
Scale Bars A & C: 0.2 mm, B: 0.01 mm, D: 0.02 mm.

4.4 Discussion

4.4.1 Maturation and fecundity

The maturation of individuals depends on many factors, such as age and/or size (Kupriyanova et al., 2001), nutrition (Leone, 1970), temperature (Dixon, 1981; Klöckner, 1976b; Turner and Hanks, 1960), and salinity (Hill 1967; Leone 1970; Kupriyanova et al. 2001). I observed individuals with gametes from late spring (November) till mid autumn (April), with most individuals mature in summer (December–February), which is comparable to observations made for other serpulins in temperate climate zones (e.g. Dixon, 1981; Klöckner, 1976). However, records about fecundity are sparse for serpulins (Kupriyanova et al., 2001). For *Hydroides dianthus* it has been estimated that one female can release up to 4 x 10^4 oocytes per spawning event (Leone, 1970). *Hydroides elegans* matured 16–25 days after settlement, spawning 1.11 x 10^3 to 9.05 x 10^3 eggs, dependent on salinity and water temperature (Qiu and Qian, 1998). *Ficopomatus enigmaticus* releases between 1 x 10^3 and 1 x 10^5 oocytes per female (reviewed by Kupriyanova et al., 2001). In this study female fecundity for *S. cariniferus* was slightly larger compared to the above listed observations of other authors. However, individuals of *H. dianthus* are smaller than specimens of *S. cariniferus*, and the number of oocytes for *H. elegans* and possibly for *F. enigmaticus* refer to observations made on young adults and not older individuals as in this study. For male fecundity, I only found a reference for *H. dianthus* of up to 7.8 x 10^7 sperm cells (Leone, 1970) per individual, which is similar to the estimated maximum of 8.31 x 10^7 cells per specimen of *S. cariniferus* here.

In general, being solitary or aggregated had no effect on maturation or fecundity. Individual size was important in that more specimens of *S. cariniferus* under 1.5 mm in thorax width were immature compared to the larger conspecifics. However, smaller individuals that didn't release gametes does not imply that they necessarily had no gametes. For example, the overall second smallest specimen

of *S. cariniferus* observed here (0.76 mm in thorax width) released oocytes. Whether these gametes where fertilisable has not been tested; however, the size, shape and observation of a complete vitelin membrane suggests that these oocytes were ripe (Knox, 1949). *Ficopomatus uschakovia*, *H. elegans* and *Hyroides* sp. have been reported to reach maturity within 2–8 weeks after settlement in near ideal conditions (Hill, 1967; Qiu and Qian, 1998; Straughan, 1972). These mature recruits measured a body length of 4–6 mm and a tube length of 9–14 mm (Hill, 1967; Straughan, 1972), which is comparable to a thorax width of 0.67–2.13 mm for *S. cariniferus*. Even individuals of *Ficopomatus ushakovia* which settled late in summer reached maturity before the next spawning season (Straughan, 1972). Also, some mature individuals of *F. enigmaticus* were notably smaller than conspecifics without gametes (Obenat et al., 2006). I only studied individuals larger than 0.65 mm in thorax width. From my observations on growth rates (Chapter 2), these specimens are probably older than two months and likely to have settled at least in the previous season. In general, for *S. cariniferus,* as for other serpulids, size and age are major factors for maturity. Additionally, fecundity was dependent on body size for males, but not females. As the number of oocytes is strongly associated with the energy level of the individual compared to sperm production, perhaps food availability is the controlling factor for the female fecundity (Ghiselin, 1969; Premoli and Sella, 1995). Further investigations are necessary to understand which other factors influence the maturation and fecundity of *S. cariniferus,* particularly for the smaller individuals.

4.4.2 Distribution of male, female individuals and the possibility of hermaphroditism

The ratio of females to males varied between 1 : 0.59 and 1 : 1.3; however, the overall ratio was 1.1 : 1in favour of females. Further, I could find no indication that the sex ratio between solitary and aggregative individuals differed. However,

the female to male ratio does vary considerably for other serpulid species. For example, in laboratory-reared recruits of *Hydroides elegans* the ratio of females to males varied between 1 : 0.2 and 0.33 : 1 (Qiu and Qian, 1998).
For *P. triqueter* there were five females for each male (1 : 0.2) at Cullercoats Bay (north-east coast of England) (Cragg, 1939). For the same species a more even but male dominated proportion of 0.8 : 1 was found in the warmer marine habitat at Bantry Bay (south-west coast of Ireland) (Cotter et al., 2003a). The variability can be caused by various biotic factors like food supply and population structure (Premoli and Sella, 1995). The sex-ratio of serpulins seems to be commonly but not exclusively in favour of females (reviewed by Kupriyanova et al., 2001). A female skewed sex ratio could increase the fecundity of an aggregative population, as the quantity of oocytes could be a limiting factor because of their higher energetic cost compared to sperm cells (Ghiselin, 1969; Kupriyanova et al., 2001; Premoli and Sella, 1995). Other authors have suggested that a male biased sex ratio is supportive for protandry in serpulins (Cotter et al., 2003a; Obenat et al., 2006). This is possibly based on size distribution in populations in combination with the “size advantage theory” (Ghiselin, 1969, 1974). A biased sex ratio is often interpreted as support for sequential hermaphroditism (Cotter et al., 2003a; Kupriyanova et al., 2001; Obenat et al., 2006). However, the sex ratio of a population as well as other factors have influence on the sex and possible sex change of the individual. Therefore, a biased sex ratio cannot be used as a supportive argument for sequential hermaphroditism (Munday et al., 2006; Soong and Chen, 2003; Wright, 1988).

For a small individual it is more efficient to produce smaller sperm cells rather than larger eggs because of their body size and energetic capabilities. Further, the reproductive success of free-spawning males often does not increase equally with individual size (Ghiselin, 1969; Kupriyanova et al., 2001). Protandry has been described for some gastropods (Hoagland, 1984; Orton, 1914; Phillips and Shima, 2009) as well as for some serpulins including *F. enigmaticus* from the Thames Estuary (United Kingdom) (Dixon, 1981) and *S. triqueter* at Drøbak

(Norway) (Føyn & Gjøen 1950; 1954) (reviewed by Kupriyanova et al. 2001). However, more recently at the south-west coast of Ireland *P. triqueter* has been described as gonochoristic (Cotter et al., 2003a). In the same study, four (out 677) individuals of *S. lamarckii* were identified as simultaneous hermaphrodites. In this investigation the females were ~ 1 to 3 (±1) abdominal segments larger than their male counterparts. Further, since the sex ratio for smaller individuals of *S. lamarckii* was skewed towards male the authors argued for protandry in this species (Cotter et al., 2003a).

For *S. cariniferus* in general, there was no significant size difference between males and females, nor was the sex ratio largely skewed in any size class. However, my results revealed that females have a minimal but significantly broader thorax. What causes this difference in size is unclear but perhaps females need a wider body to store the larger eggs compared to the male with smaller sperm cells. I also observed testis and dissolving eggs in one specimen, which suggest a transition from female to male.

Based on anatomical studies, serpulins are characterised by a lack of distinctive reproductive organs (Obenat et al. 2006, Kupriyanova et al. 2001). Ovaries are often described in association with larger blind-ending blood vessels near the dorsal peritoneum (Clark and Olive, 1973; Cotter et al., 2003a; Obenat et al., 2006). In *S. lamarckii,* Cotter et al. (2003) described the testis dorsally associated with the peritoneum and septa; however, in general, the description of the testis is rather sparse. Here, in some hermaphrodites, I could define both reproductive structures at the same time. Whereas the ovaries are clearly obvious (Figure 4.04 B, C & D), other repetitive segmental structures associated with gametocytes are less apparent (Figure 4.05 A&B). I suggest that the testes of *S. cariniferus* individuals are located on the septa between the segments (Figure 4.03 C & Figure 4.05 A). In the case of *S. cariniferus,* the testes were not associated with larger blood vessels (Figure 4.03 D & Figure 4.05 B). For the larger individuals that did not release a huge quantity of gametes during

spawning season, it is possible that they were recovering from their last spawning event. Histological sections of those individuals could confirm that the organism recovers from their last spawning and possibly change their sex between two spawning events.

As others have observed, I found a few hermaphrodites and suggest that the individuals were transiting from one to the other sex (Cotter et al., 2003a; Dixon, 1981). For marine invertebrates other authors differentiate between sequential hermaphroditism, where the individual undergoes one sex change in their ontogeny, and “alternating sexuality”, described for some molluscs (Coe, 1934; Hoagland, 1984), or “sex reversal” (Hodgson, 2009). In the latter the individual alternates their sex according to population structure and energy level of the individual and expresses their sex independently to the previous spawning season (Coe, 1932; Hoagland, 1984; Premoli and Sella, 1995).

Some serpulins in warmer regions are presumably able to spawn at least twice per season (instead of just one event), given how fast some species reach maturity after settlement (Hill, 1967; Qiu and Qian, 1998). In fact, for *F. enigmaticus,* two spawning events were observed at the Po River Delta (Italy) (Bianchi and Morri, 1996). Further, in a laboratory specimen, *F. enigmaticus* spawned a second time, two weeks after the first spawning event (Zuraw & Leone 1968;1972). If *S. cariniferus* and other serpulins release gametes multiple times in a season, then it could be possible that an individual commences a sex-change between these spawning events. The change from male to female or female to male could be more dependent on environmental conditions and perhaps also increases reproductive success (Premoli and Sella, 1995). The production of oocytes is energetically costlier than the production of sperm and the change to the male sex could be seen as a resting period from the female stage (Premoli and Sella, 1995). For serpulins the ratio of female to male is variable between month and coastlines (Cotter et al., 2003a; Føyn and Gjøen, 1954). The lack of larger specific reproductive structures and the ability to produce gametes relatively quickly facilitate alternating sexes rather than just

protandry, where the individual's sex at the start of each spawning season is independent to that of the previous season, (Amemiya, 1929; Coe, 1932; Hodgson, 2009; Premoli and Sella, 1995), which is possibly more common than previously thought in serpulids, or at least in *S. cariniferus*.

4.4.3 Conclusion

There is a clear spawning season for *S. cariniferus* in the Wellington region, where most individuals are mature in the summer, with smaller individuals less likely to be mature than larger ones. There seems to be no difference in maturity, fecundity or sex ratio for solitary vs. aggregated individuals. However, for these matters more investigations are needed as my data set were limited. In general, the sex ratio varies between 1 : 0.59 and 1 : 1.30 (female : male), similar to other serpulins (Cotter et al., 2003a; Qiu and Qian, 1998). Sequential hermaphroditism appears to occur at low levels but is not associated with size, unlike other species (Kupriyanova et al., 2001). As serpulins are possibly able to spawn at least twice per season (Zuraw and Leone, 1968, 1972) it is plausible that individuals change their sex in between two spawning events (Premoli and Sella, 1995). Therefore, there is the possibility of alternating sexes rather than protandric (sequential) hermaphroditism (Hoagland, 1984). The initial sex per season may be determined through energetic reserves and nutrition of the individual as well as the number of males and females in the population. The transition of a specimen to the opposite sex could be observed through a section of individuals which are in recovery from their last spawning event.

5. Larval growth and development of *S. cariniferus*

5.1 Introduction

The recruitment of juveniles into a population is mainly limited by the settlement success of the larvae (Connell, 1985; Keough and Downes, 1982; Yool et al., 1986). Therefore, settlement, the shift from a pelagic life stage to a benthic juvenile, is a crucial point in the life of many marine organisms (Hadfield and Paul, 2001; Pineda, 2000; Pineda et al., 2010). For most marine invertebrates, settlement is closely followed by a metamorphosis when the individual transforms from a pelagic larva to a juvenile with features adapted to a benthic life, and larval structures are ingested or shed (Cataldo et al., 2005; Gros et al., 1997; Kupriyanova et al., 2001; Marsden and Anderson, 1981). To understand larval settlement, it is necessary to recognise and observe larval development and behaviour until the juvenile individual is formed. These processes are not always simple because marine taxa have a variety of primary and secondary larval stages with various specific metamorphosis and settlement processes (Hadfield and Paul, 2001). For example, in barnacles the transition from a pelagic naupilus larva to a benthic cyprid larva permits them to explore and temporarily attach to the substrate with their antennae (Clare et al., 1994; Crisp and Meadows, 1962; Gruner, 1993). Another example is the change in the swimming behaviour of some decapods after the propagule metamorphosis from a pelagic zoea to a pelagic megalopa to allow migration into an estuary for juvenile development (DeVries et al., 1994; Gruner, 1993; Olmi, 1994; Tankersley et al., 1995). Further, particularly for mussels and oysters, the pediveliger larva explores the substrate with a foot before settlement (Ackerman et al., 2008; Cranfield, 1973; Hadfield and Paul, 2001; Petersen, 1984).

Like most marine invertebrates with pelagic larvae, the majority of serpulids for which we know the reproduction, reproduce by broadcast spawning (Giangrande, 1997; Kupriyanova et al., 2001). Initially, larvae develop from a pelagic

trochophore to a pelagic metatrochophore, during which time the individuals rapidly increase in length until they reach a certain size, after which the growth slows or stops (e.g. Hansen, 1999; Toonen and Pawlik, 2001a). The development of larvae from metatrochophora to juvenile is correlated more with specific environmental conditions than growth (Faimali et al., 2002; Hadfield et al., 2001; Hansen, 1999). In general, once the offspring reaches metamorphic competence they become able to metamorphose to a postlarva (or secondary larva), and from that point they can settle and transform to benthic juveniles and develop a calcareous tube (Hadfield et al., 2001). Some serpulin larvae like *H. elegans* or *S. cariniferus* can reach metamorphic competence within one to two weeks, depending on food availability and temperature (e.g. Bryan et al., 1997; Gosselin and Sewell, 2013).

Although this general pattern seems to occur for serpulids where it has been studied, the succession of the ontogenetic progress as well as the terminology is not consistently described in the literature. A variety of publications explore the settlement behaviour of a limited group of serpulin species (reviewed by Kupriyanova et al. 2001). From these published descriptions it becomes clear that events of metamorphosis and settlement can occur parallel or subsequent to one another, and do not always follow the same sequence (e.g. Anderson, 1973; Marsden and Anderson, 1981). The interaction and co-occurrence of metamorphosis and settlement leads to confusion over the developmental processes and inconsistent usage of terminology (Kupriyanova et al., 2001).

Further, the duration of the pre-metamorphic and post-metamorphic stage can vary considerably. For example, the length of time the individual remains a secondary larva is often not recognised in current investigations of the serpulid settlement processes. For example, planktotrophic larvae of many species can slow or stop their development at different stages if they experience unfavourable conditions (Hadfield et al., 2001), such as starvation or the presence of other

organisms (e.g. certain copepod species) (Dahms et al., 2004; Dahms and Qian, 2005; Hung et al., 2005; Pawlik and Mense, 1994; Young and Chia, 1982). Therefore, in this chapter, I will present my findings on the growth, development, metamorphosis and settlement of *S. cariniferus* larvae, which should provide insights into these processes for serpulids in general. Previously, larval growth and settlement has only been reported in one study for this species, and that study was primarily concerned with identifying settlement cues (Gosselin and Sewell, 2012). Here, I explore the relationship between settlement and larval size, and the utility of distinguishing between competence to metamorphose, metamorphosis from one form to another, as well as settlement.

5.2 Methods

Spirobranchus cariniferus individuals were collected from Porirua Harbour, Shelly Bay and Worser Bay in the Wellington region (see Introduction), over the summer periods of 2014/2015–2017/2018. For each larval culture, specimens were individually spawned by removing each worm from its tube (n = 20–100 per site and collection). If the individual released sperm, I placed it on a watch glass with a few drops of FSW (sea water filtered through a 10 µm filter). Each female releasing eggs was placed in a bowl with 50–100 ml of FSW. Both gamete types were gently collected with a glass pipette and mixed in a 500 ml beaker glass to allow fertilisation. Most of the debris was filtered out by pouring the solution gently through a 125 µm mesh. Fertilisation was ended after 90 minutes by filtering the oocytes with a 30 µm mesh. The retained fertilised eggs were poured into a 1,000 ml beaker with ~700 ml FSW and stored in a water bath at ~ 19°C. After 48 hours the hatched larvae were counted in five to six 0.5–1 ml subsamples. Subsequently, the larvae stock was diluted and distributed evenly into six 5 L culture jars with 4 L of FSW, at a concentration of 4–19 larvae/ml. The jars were placed in a water bath with a temperature around 19°C. In the first

week, the filtered seawater was changed every two days. From the start of the second week onwards, FSW and jars were replaced every two days.

In a three week pilot experiment at the beginning of my studies (summer 2014/15), the response of larvae to four different regimes of algal food supplies was observed. For this pilot experiment, larvae were raised in 2 L of FSW at a concentration of 1 larva/ml. If larvae were fed, the total concentration of supplied algae cells were of 2.5 x 10^4 cells/ml. In this trial, two larval cultures were fed with *Isochrysis galbana*, and a further two cultures were supplied with *Pavlova lutheri*. Three additional cultures were given a mixture of both algal species, and finally the larvae in two jars were not supplied any food (starved). As a consequence of this pilot study, further larval cultures were fed solely with *I. galbana* at a concentration of 2–2.5 x10^4 cells/ml.

In total, between summer 2014/15 and summer 2017/18, larvae were raised in cultures to settlement seven times (including the pilot trial). In each experiment, larvae were fed after the jars were cleaned or exchanged and the water replaced. Each culture was subsampled weekly to monitor growth and development until larvae showed settlement behaviour. Once most of the larvae displayed the ability to settle (after approximately three weeks post-hatching), I transferred 50 to 200 larvae into bowls with FSW. Further larval development was observed daily. Larval growth and settlement behaviour were observed by using a dissecting microscope and a compound microscope with an ocular micrometre. For the observations of settlement behaviour, I used larvae that were 21–29 days old. For better contrast, living larvae were stained either with 1 ppm Nile Blue in FSW or 10 ppm Neutral red in FSW for 16–20 minutes.

For the documentation of 21–29-day old larvae and their development, I used a compound microscope (Leica DMLB) with a digital camera (Canon EOS 550) for observation on living larvae and a scanning electron microscope to record preserved individuals (SEM). Propagules for electron microscopy were fixed in

5% formaldehyde with FSW and Borax (~ 1 mg/L). For the Scanning electron microscope (SEM) images, the sample was dropped onto an ash-less filter paper (Watman 41) with several layers of ordinary filter paper beneath, to collect excess liquid. The filter paper was secured to a cryo-holder, which was plunged into a slush freezer (Gatan Alto 2500) with liquid nitrogen, under a partial vacuum. Subsequently, the frozen sample was transferred into the prep-chamber of the microscope where the temperature of the sample was raised from -120°C to -90°C for sublimation of ice to enhance surface detail. In the next step, the sample was cooled to -120°C and coated twice with platinum. Each time the duration of the coating was 120 seconds. Finally, the specimen was transferred into the electron microscope (Jeol JSM 6500F) and kept on the cryo stage at -120°C. For the images, the accelerating voltage was adjusted to 4kV and the probe current was 8V.

5.3 Results

5.3.1 Larval growth and settlement relative to body size

From the pilot experiment, it became clear that the food quality had an effect on the larvae growth. Larvae only fed with *I. galbana* grew faster than larvae that were starved or fed with a mixture of *I. galbana* and *P. lutheri* (Figure 5.01). Larvae supplied with only *P. lutheri* died within the first 10 days of the culture (therefore their growth is not displayed). However, regardless of food supply (except *P. lutheri*), larvae of *S. cariniferus* grew almost linearly over about 13–25 days to 270–300 µm (Figure 5.01). Once this length was reached, the growth rate decreased (Figure 5.01). In the pilot experiment, larvae fed with *I. galbana* or a mixed algae culture were metamorphically competent 12–13 days after hatching, when they had reached an individual length of at least 230 µm, although in all later larval cultures, the smallest settling larvae were 175µm long. In the same jar as the smallest settler, larvae of 230 µm length or longer were found swimming as metatrochophora; therefore larvae could be at more advanced developmental

stages at smaller sizes than siblings in the same conditions. In general, for all further larval cultures, the average growth was similar as shown here for the pilot experiment (Figure 5.01). Most of the settled larvae were approximately 250 µm, but when larvae did not settle, they continued growing up to a maximum of 350 µm (Figure 5.01).

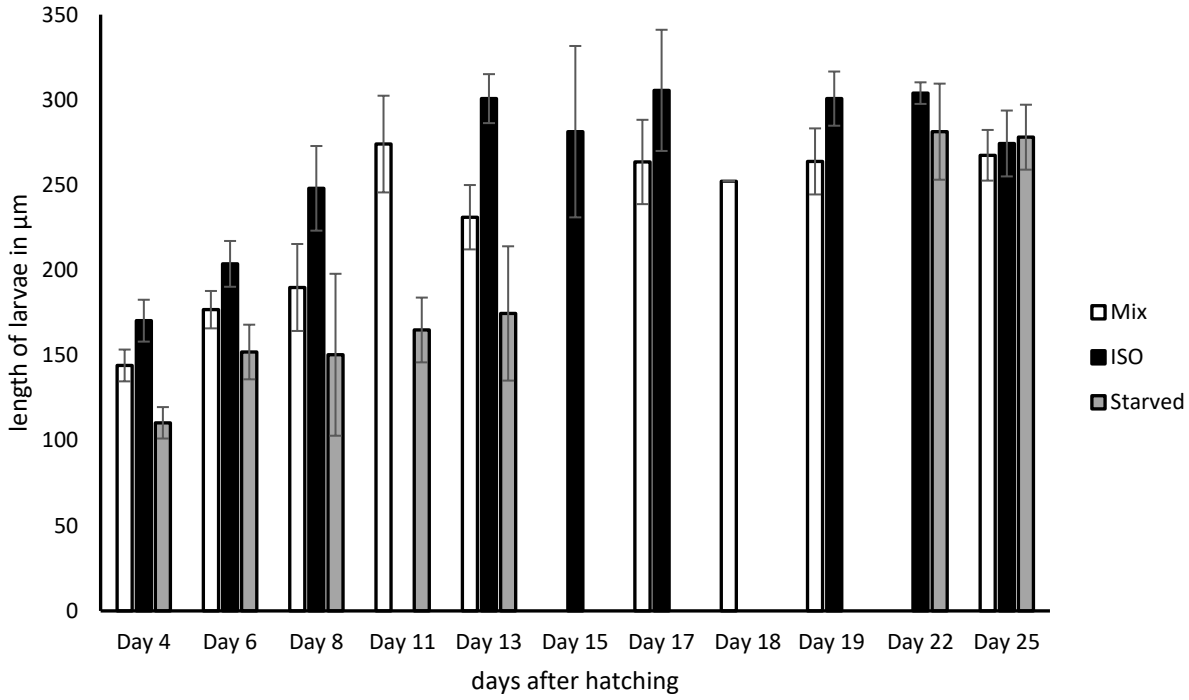

Figure 5.01 Average length of larvae from the first pilot experiment. At day 12–13 larvae of the ISO and Mix treatment were metamorphic competent. On day 19 the first larvae displayed settlement behaviour. On day 22 the first larvae attached to the jar. Abbrevations for Food treatments: **ISO** = Larvae have been fed only with *Isochrysis galbana*; **Mix** = Larvae have been fed with a blend of *I. galbana* and *P. lutheri*; **Starved** = those larvae have not been fed at all. Error bars represent 95% confidence interval.

5.3.2 Larval development with transition from pelagic to benthic habitat and attachment

Larval development steps were observed on larvae of similar age, 25–29 days after hatching. Larva of *S. cariniferus* develop first as trochophores, where the body is divided by a cilial band (the prototroch) into the episphere (anterior of the prototroch) and hyposphere (posterior of the prototroch). In the early days of a trochophore larva, the episphere becomes wider and the hyposphere elongates, then the individual grows into a metatrochophore (Figure 5.02). Towards the end of the metatrochophore stage, the larva forms paired ocelli on their episphere. In their hyposphere, the metatrochophore of *S. cariniferus* starts to develop hair-like chaeta (capillary chaeta) (Figure 5.02 E, F & G). The capillary chaeta support the later developed nectochaeta to move over a surface and possibly aid water circulation for the settled individual (Hartmann-Schröder, 1982; Specht, 1988). At least one specimen at this early stage has already developed uncini (Figure 5.02 C & D). Uncini are particularly short chaeta, often located in abdominal segments. Juvenile and adult worms use these chaeta to hold on to their tube (Hartmann-Schröder, 1996). These uncini have a serrated edge that is genus-specific (Figure 5.02 D) (ten Hove and Kupriyanova, 2009).

Once the first sets of capillary chaeta on the right and left side of the larva are formed (Figure 5.03 D & E), the individual is competent to metamorphose to the secondary larval form. Depending on environmental conditions, metamorphically competent larvae moved to the bottom of the culture jar and began to metamorphose to nectochaeta larvae. This secondary larva can be recognised by three sets of capillary chaeta on both sides. In my experiments larvae began to sink to the bottom of the culture vessel around 14 days after hatching. The benthic metamorphosing larvae then grow lobes on the right and left side, which will merge later to form the collar of the adult worm (Figure 5.03). The episphere becomes the head region (Figure 5.03). Most of the observed individuals lost their prototroch (Figure 5.03. B & C). The developing larvae form the first three

segments (chaetigers) and accomplish the transition to the nectochaeta stage (3-chaetiger larva) (Figure 5.04). The pygidium is posterior to these chaetigers, which originated from the hyposphere and is the growth zone of the adult worm. Subsequently, the parapodium of the most anterior larval chaetiger develops under the collar and can be hidden by it (Figure 5.05 B & C).

As the 3-chaetiger larvae begin the process of settlement, the pygidium flattens and becomes more triangular (Figure 5.05 D & F). The head development continues and becomes more distinguishable from the thorax through a lengthened neck (Figure 5.05 D). The larvae begin a searching behaviour, predominantly by crawling on the surface. Simultaneously, the larvae will start to secrete a mucus, mainly concentrated around the last segment and the pygidium (Figure 5.05 E). The nectochaete larvae was observed on multiple occasions to crawl over the surface pulling a tail formed of mucus (Figure 5.05 A & B). Several times the larva appeared to attach to a substrate via the pygidium or the mucus tail. The final attachment seems to occur once the pygidium or the mucus tail is firmly entangled. For example, larvae were observed settling in an accumulation of algae cells or attaching after the mucus tail became heavy with collected debris. The observed duration of substrate exploration to the final attachment varies between one to eight days. Once the attachment of the larva is completed, a primary tube forms and the metamorphosis continues. The head appears to merge with the thorax and branchial buds appear anterior to the head (Figure 5.06).

The primary tube is secreted as a thin mucus layer (Figure 5.07 A & C). Subsequently, the attached larva begins to form a secondary calcareous tube (Figure 5.07 D & E). Parallel to the tube development, the branchial buds elongate as tentacles and an operculum forms (Figure 5.06). The developing juvenile worm completes the metamorphosis by branching out radioli from those tentacles and the operculum hardens (Figure 5.07 E).

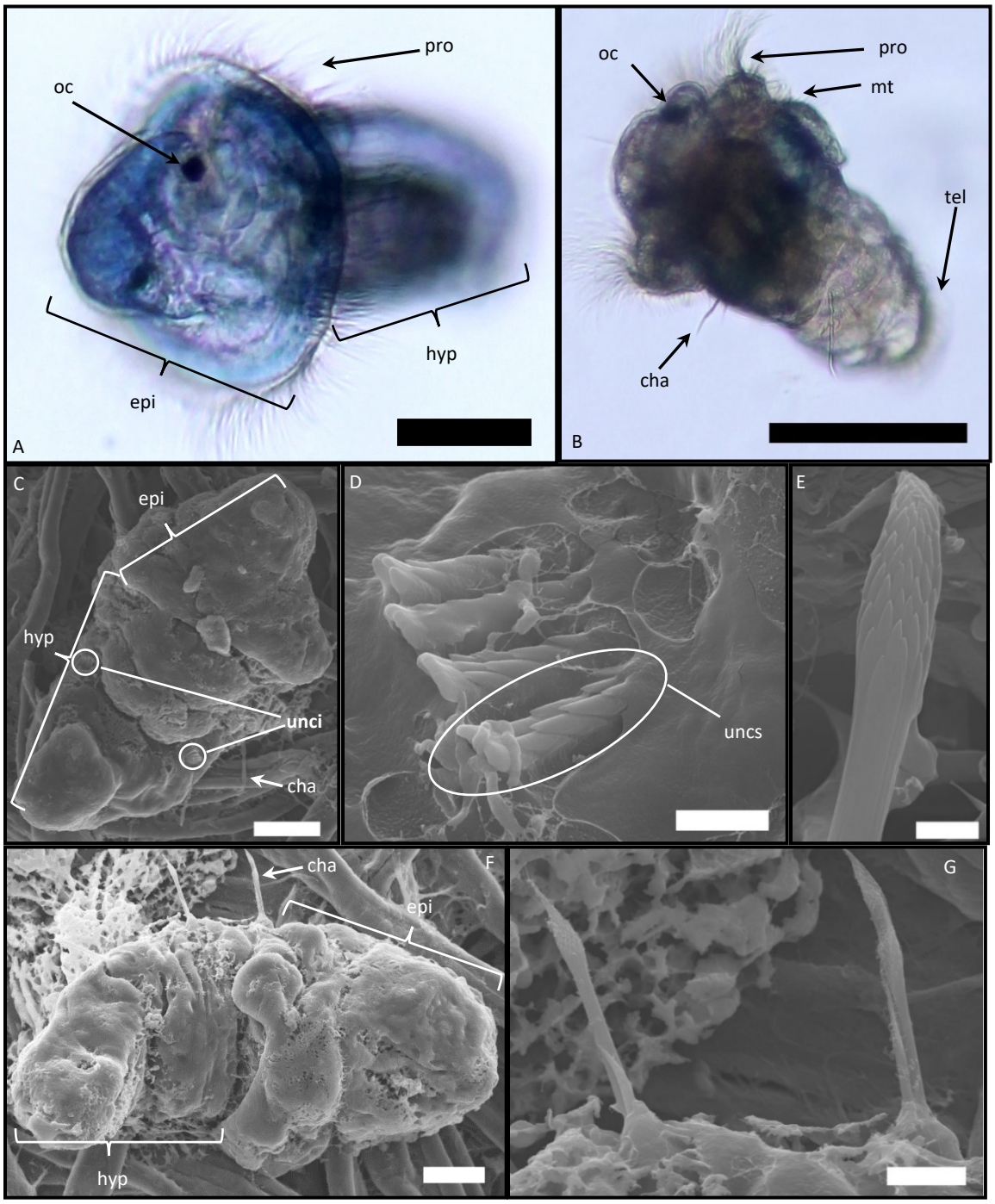

Figure 5.02 Metatrochophora larvae A) 25 d larvae from anterior dorsal stained with Nile blue, B) 25 d larvae form dorsal posterior; C) SEM image of 22 d larvae, ventral view, D) SEM detail image of uncini of a 25 d larvae, E) SEM detail image of capillary chaeta of a 25 d larvae, F) SEM image of 25 d larvae, ventral-lateral view, G) SEM detail image of capillary chaetas of a 25 d larvae.
cha: chaeta; **epi:** episphere; **unci:** uncini; **uncs:** uncinus; **hyp:** hyposphere; **mt:** metatroch; **oc:** ocellus; **pro:** prototroch; **tel:** telotroch *Scales: A 50 µm; B 100 µm; C & F 30 µm; D 3 µm; E 2 µm; G 10 µm*

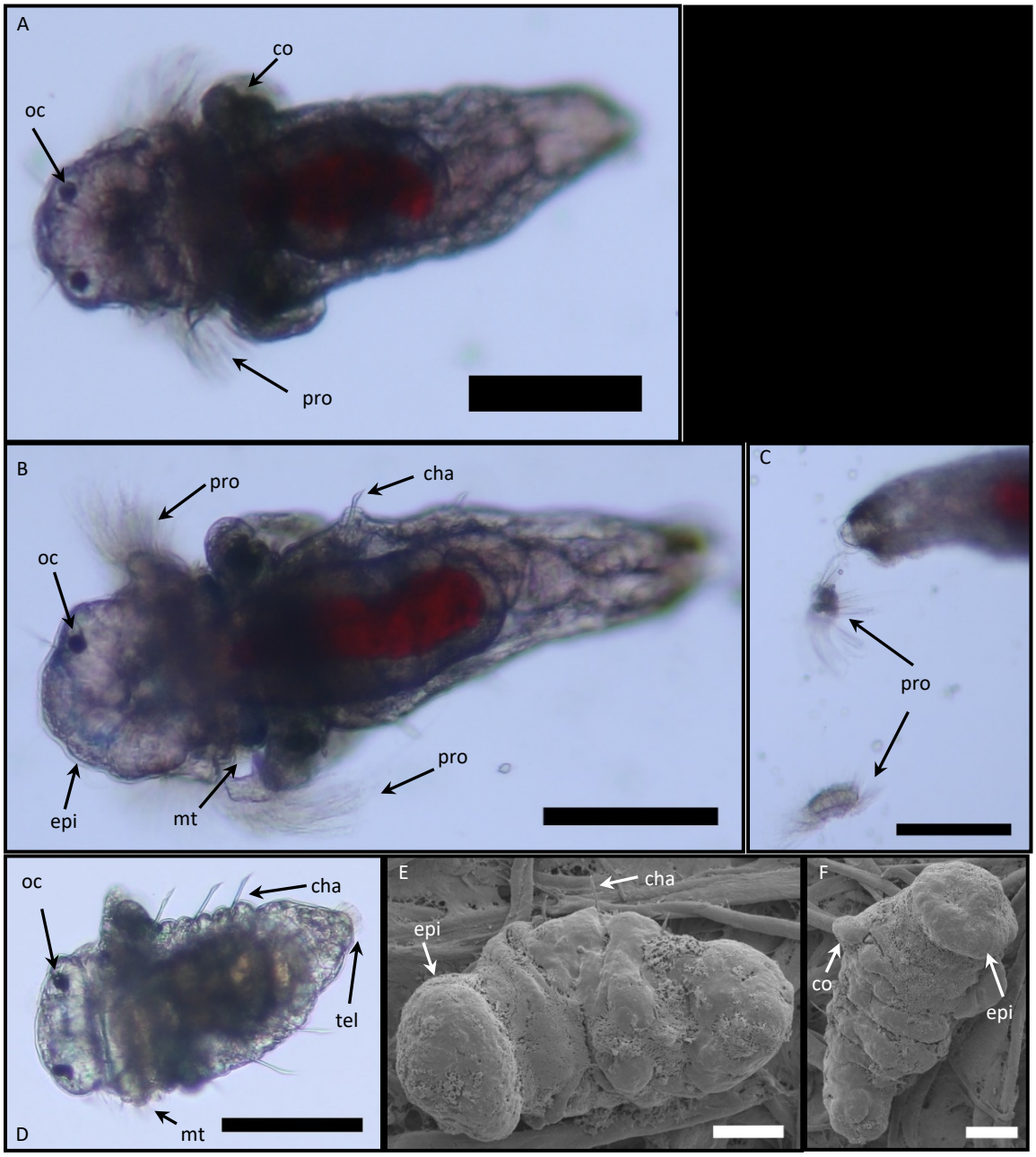

Figure 5.03 Larvae competent to metamorphose **A)** 29 d larvae, stained with neutral red, dorsal view; **B**) 29 d larvae loosing prototroch, stained with neutral red, dorsal view; **C**) parts of removed prototroch next to the hyposphere of a 29 d larvae, stained with neutral red; **D**) 25 d larvae, dorsal view; **E**) SEM image of 26 d larvae, ventral view; **F**) SEM image of 26 d larvae, ventral-lateral view.
cha: chaeta; **co:** collar; **epi:** episphere; **mt:** metatroch; **oc:** ocellus; **pro:** prototroch; **tel:** telotroch
Scales: A – F 50 µm

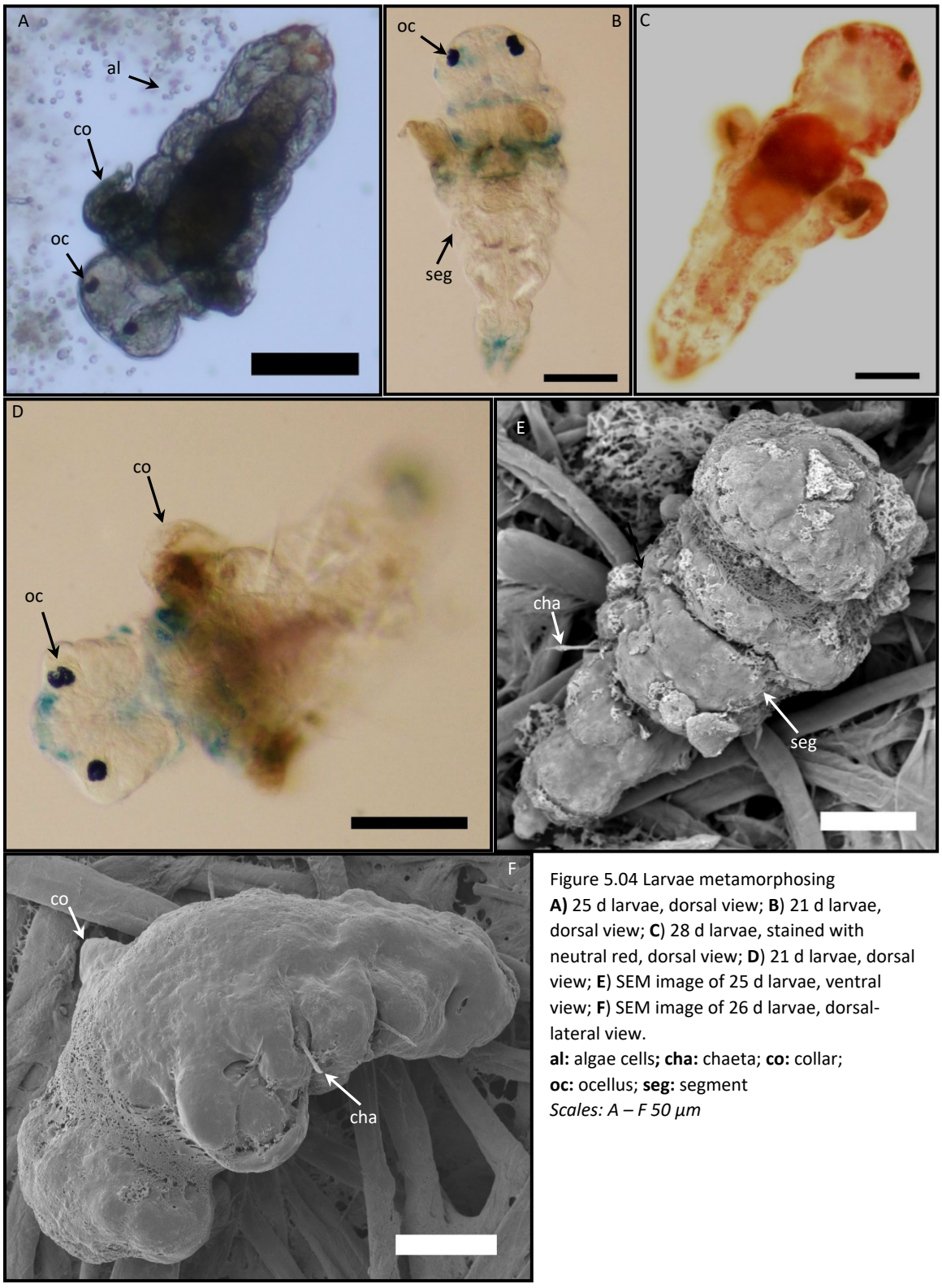

Figure 5.04 Larvae metamorphosing
A) 25 d larvae, dorsal view; **B**) 21 d larvae, dorsal view; **C**) 28 d larvae, stained with neutral red, dorsal view; **D**) 21 d larvae, dorsal view; **E**) SEM image of 25 d larvae, ventral view; **F**) SEM image of 26 d larvae, dorsal-lateral view.
al: algae cells**; cha:** chaeta; **co:** collar; **oc:** ocellus; **seg:** segment
Scales: A – F 50 µm

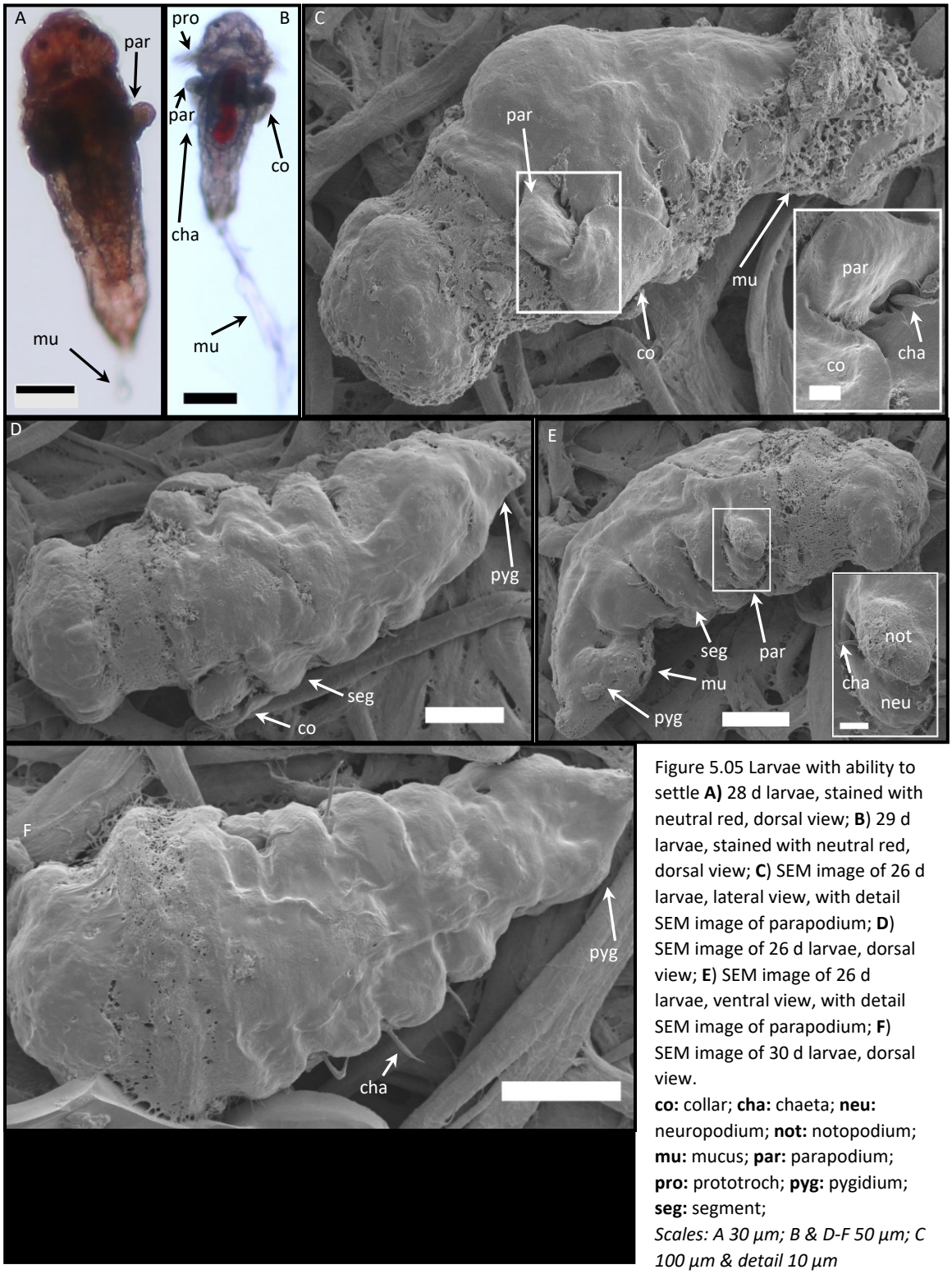

Figure 5.05 Larvae with ability to settle **A)** 28 d larvae, stained with neutral red, dorsal view; **B**) 29 d larvae, stained with neutral red, dorsal view; **C**) SEM image of 26 d larvae, lateral view, with detail SEM image of parapodium; **D**) SEM image of 26 d larvae, dorsal view; **E**) SEM image of 26 d larvae, ventral view, with detail SEM image of parapodium; **F**) SEM image of 30 d larvae, dorsal view.
co: collar; **cha:** chaeta; **neu:** neuropodium; **not:** notopodium; **mu:** mucus; **par:** parapodium; **pro:** prototroch; **pyg:** pygidium; **seg:** segment;
Scales: A 30 µm; B & D-F 50 µm; C 100 µm & detail 10 µm

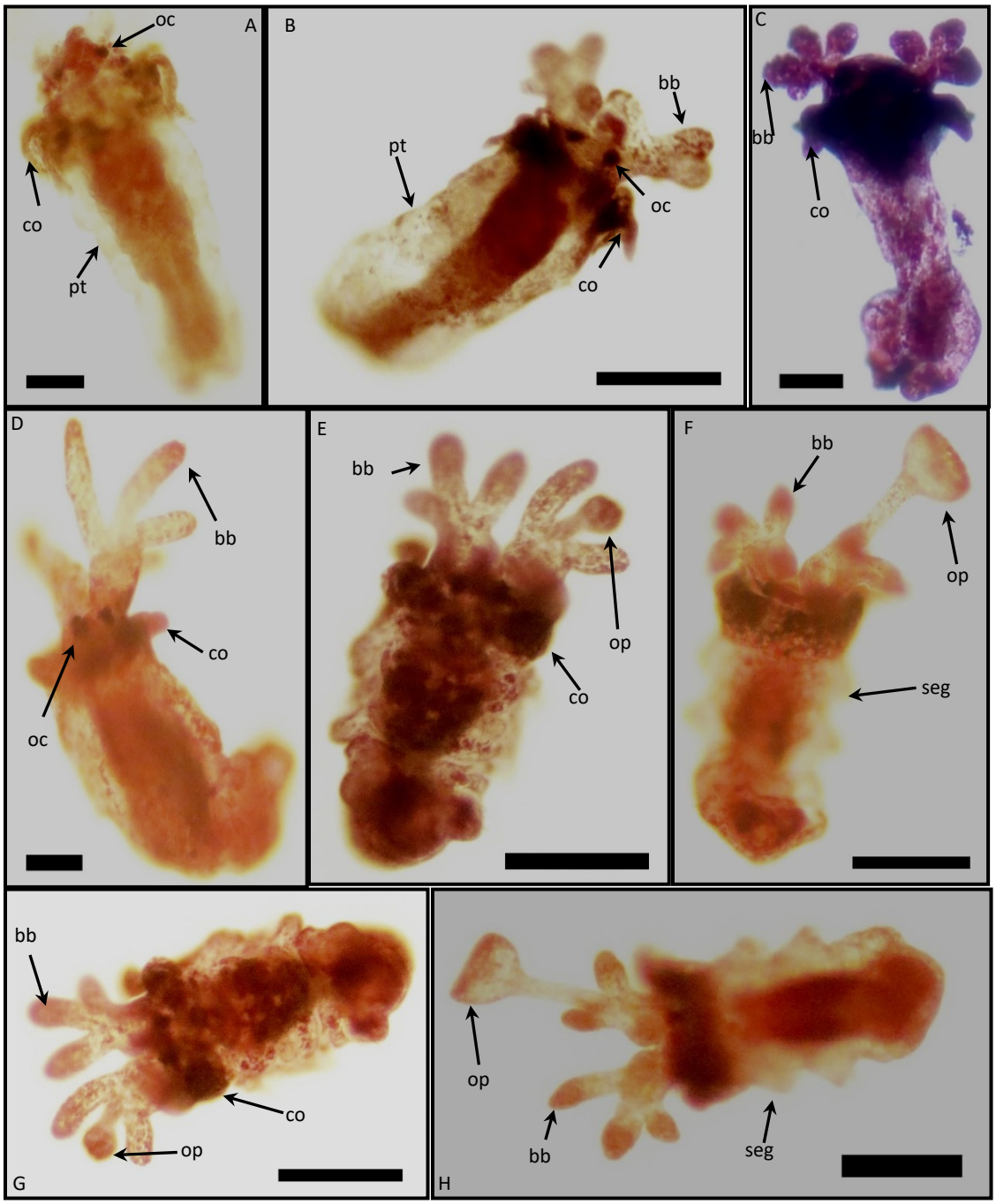

Figure 5.06 Larval development after attachment **A)** 28 d larvae, stained with neutral red, dorsal view; **B)** 28 d larvae, stained with neutral red, dorsal view; **C)** 25 d larvae, stained with nile blue, ventral view; **D)** 28 d larvae, stained with neutral red, dorsal view; **E)** 28 d larvae, stained with neutral red, dorsal view; **F)** 28 d larvae, stained with neutral red, dorsal view; **G)** 28 d larvae, stained with neutral red, dorsal view; **H)** 28 d larvae, stained with neutral red, dorsal view.
bb: branchial buds; **co:** collar; **oc:** ocellus; **op:** operculum; **pt:** primary tube
Scales: A & D 50µm; C 30 µm; B, G, F, H 100 µm

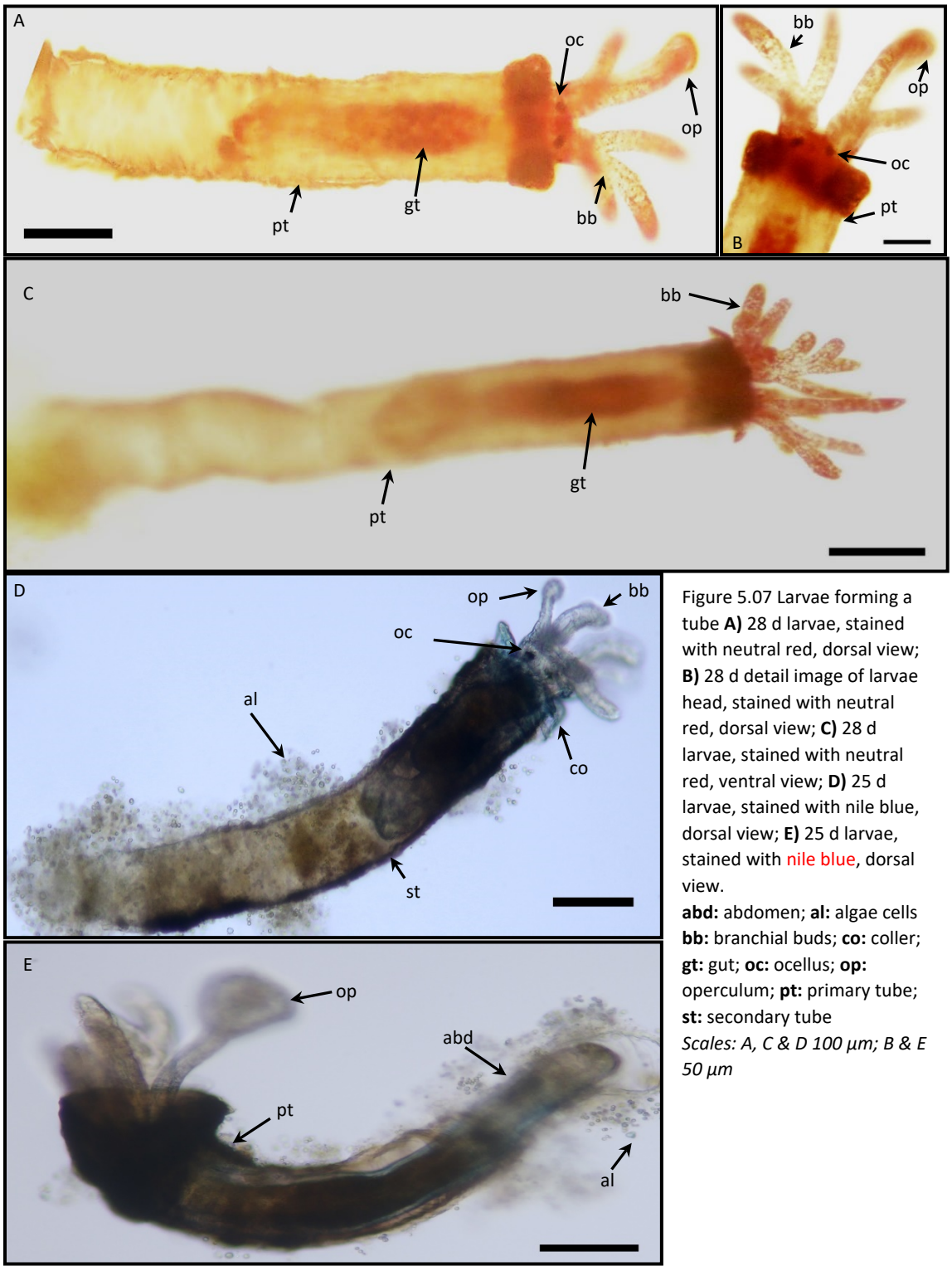

Figure 5.07 Larvae forming a tube **A)** 28 d larvae, stained with neutral red, dorsal view; **B)** 28 d detail image of larvae head, stained with neutral red, dorsal view; **C)** 28 d larvae, stained with neutral red, ventral view; **D)** 25 d larvae, stained with nile blue, dorsal view; **E)** 25 d larvae, stained with nile blue, dorsal view.
abd: abdomen; **al:** algae cells **bb:** branchial buds; **co:** coller; **gt:** gut; **oc:** ocellus; **op:** operculum; **pt:** primary tube; **st:** secondary tube
Scales: A, C & D 100 µm; B & E 50 µm

5.4 Discussion

In general, the growth rate for *S. cariniferus* larvae in this study was slower than the observations made by Gosselin and Sewell (2013). Their best growth responses were to a mixed supply of *I. galbana*, *Dunaliella tertiolecta* and *Chaetoceros muelleri* with a total cell concentration of 0.5–1 x 10^6/ml (Gosselin and Sewell, 2012). Larvae fed solely with *I. galbana* grew faster compared to the larvae in this study. The discrepancy can be explained by a 25–40 times higher algae cell supply (Gosselin and Sewell, 2013). However, similar to their study, growth progressed almost linearly until a certain size (~300µm body length) was reached after which the growth slowed or stagnated (Gosselin and Sewell, 2013, pers.obs.). Similar growth patterns occur for other polychaetes and marine invertebrates, as smaller larval stages are more susceptible to predation (Hansen, 1999; Pechenik, 1990; Pennington and Chia, 1984; Phillips, 2002; Toonen and Pawlik, 2001b). In contrast to other studies, I observed the best larval growth was in response to an algae food supply with only *I. galbana*, as *P. lutheri* seemed to affect the survival negatively, although the reason for this is unclear. The growth of larvae fed with *P. lutheri* was similar to the growth rate of starved larvae, and therefore I assume that the larvae did not feed on this alga. It is possible that the algae or the developing biofilm resulted in a toxic environment for the larvae. Larvae supplied with both algal species developed faster, despite a slower growth rate compared to individuals fed only with *I. galbana*. It is plausible that the biofilm caused by sedimented *P. lutheri* cells aided the development of the larvae. It is also possible that in an unfavourable environment, the offspring increased their chance of survival by metamorphosing faster to nectochaeta or juvenile individuals.

Similar to my observations, larvae of other serpulid species also seem to grow despite starvation (Qian and Pechenik, 1998). Starved larvae feed on their yolk reserves and possibly also on organic matter from dissolving dead conspecifics or from bacteria as described for larvae of other marine invertebrates

(Havenhand, 1995; Manahan, 1983, 1990). Larvae of marine invertebrates like *S. cariniferus* or *Hydroides elegans* may cover a significant part of their energetic needs by absorbing dissolving organic matter (Boidron-Métairon, 1995). However, larvae that were not fed did not develop beyond metamorphically competent individuals. In other studies, starved larvae were able to maintain their metamorphic competence. For example, six day old *H. elegans* larvae, which were able to metamorphose, were first starved for several days and subsequently through adding of 3-isobutyl-1-methylx-anthine (IBMX) to the water the offspring metamorphosed (Pechenik and Qian, 1998; Qian and Pechenik, 1998). IBMX affects the Ca^{2+} transport in cells and interacts with a cyclic nucleotide phosphodiesterase and inhibits the function of this enzyme to hydrolyse the second messengers cAMP and cGMP (Jensen and Morse, 1990; National Center for Biotechnology Information, 2019). Therefore, IBMX directly induces metamorphosis for some marine invertebrates as it acts further downstream in the signal cascade of this development step (Nedved and Hadfield, 2009; Pawlik, 1990). I did not use IBMX or any other chemical to stimulate larvae of *Spirobranchus cariniferus* to metamorphose.

The larval development of *S. cariniferus* is similar to the development of other serpulids. The hyposphere of the trochophora elongates, and the episphere broadens and becomes a metatrochophora within several days (Blake, 2017; Crisp, 1977). Subsequently, the metatrochophora larva will extend capillary chaetae beyond their body wall (Andrews and Anderson, 1962). If enough food is supplied, the metatrochophora larva will form three larval segments and transform into a secondary larva, the nectochaeta (or 3-chaetiger larva) (Heimler, 1988). Under conditions supportive of settlement, the pygidium of the nectochaeta of *S. cariniferus* become flat and triangular shaped, and the larva begins to secrete mucus from their pygidium. As observed for other sessile marine invertebrates, the larva begins to display a searching behaviour by swimming over the surface and crawling on the substratum (Doyle, 1975; Hills et al., 2000; Lagersson and Høeg, 2002; Nelson et al., 2017; Wilson, 1968; Wisely,

1958). During the exploration of the surface, multiple attachments to the substratum can occur (Marsden and Anderson, 1981; Young and Chia, 1982). In most cases the larva attaches via the pygidium and secreted mucus (Andrews and Anderson, 1962; Føyn and Gjøen, 1954). However, on some occasions a mucus tail trailled by the larva becomes entangled, immobilising the propagule and leading to attachment (Føyn and Gjøen, 1954; Wisely, 1958, pers. Obs.). Once the larva is attached, further morphological changes occur such as reduction and merging of head and neck with the thorax (Crisp, 1977; Marsden and Anderson, 1981; Young and Chia, 1982). The prototroch will be reabsorbed or shed (Grant, 1981; Kupriyanova et al., 2001; Marsden and Anderson, 1981). Simultaneously, the attached individual secretes a primary mucus tube. Some larvae still seem to be able to revise their attachment and possibly leave their primary tube and reattach if they get disturbed (Crisp, 1977; Nelson et al., 2017; Wisely, 1958, per. Obs.). The final attachment is accomplished once the individual has formed their secondary calcareous tube.

For many planktotrophic larvae, the commonly used definition of settlement describes the transition from pelagic to benthic habitat, followed by the transformation into a juvenile individuals, summarised as metamorphosis (Bishop et al., 2006). However, this definition is overly simplified and contradicts what we can observe for marine invertebrates. For example, some Decapoda larvae stay planktotrophic after metamorphosis to a megalopa, in order to reach the habitat where they live as juveniles (Anger, 1987; DeVries et al., 1994; Olmi, 1994). Also, some sessile marine invertebrates undergo an extended benthic postlarval stage prior to the final attachment (Ackerman et al., 2008; Costlow and Bookhout, 1957; Cranfield, 1973; Crisp and Meadows, 1962; Hadfield and Paul, 2001; Petersen, 1984; Thorson, 1966). This plasticity in settlement and attachment contributes to the confusion about the sequence of metamorphosis and settlement. Therefore, I propose, at least for *S. cariniferus* and possibly other species of Sabellida, a different interpretation of metamorphosis and settlement

processes which emphasises each stage of larval development and settlement on its own.

There is general agreement that early larval development of serpulins of the genera *Ficopomatus, Galeolaria, Hydroides, Serpula* and *Spirobranchus* progresses via a planktotrophic trochophore into a metatrochophore (Hartmann-Schröder, 1982; Heimler, 1988; Schroeder and Hermans, 1975), which reaches "competence to metamorphose" (Hadfield, 1998; Hadfield et al., 2001). For sessile invertebrates, such as *S. cariniferus,* the competence to metamorphose is particularly important. At this point the larva will move over to a benthic life and subsequently attach to a substrate, which in consequence will affect overall survival and reproductive success. Because of the importance of this step, the success of the larval development past metamorphic competence mainly depends on the availability of food or environmental cues like biofilm (Hadfield et al., 2001; Toonen and Pawlik, 2001b, 2001c). Therefore, metamorphic competence allows the larva to continue a planktotrophic existence until an appropriate cue indicates a suitable settlement substratum nearby. Once the larva has received the external cue, it will proceed with the habitat metamorphosis and further develop into the secondary larval form (postlarva e.g. Jackson et al., 2002; Olmi, 1994; Wolcott and Devries, 1994) as a nectochaete or similar for barnacles as cyripid and transition to a predominantly benthic life (e.g. Gruner, 1993; Hawkins et al., 1999; Marsden and Anderson, 1981; Young and Chia, 1982). This could explain why starved larvae of *S. cariniferus* continued as metatrochophores rather than transitioning to a nectochaeta.

Further, at least for serpulins, the morphological transition from pelagic to benthic organism begins during, or prior to settlement (Hadfield, 2000; Marsden and Anderson, 1981; Schroeder and Hermans, 1975), and occurs over two different metamorphoses in the same individual (sensu Georgiou, Jacobs, Pier, Reitzel in Bishop et al., 2006). The transition from a pelagic to benthic lifestyle and the attachment can be interpreted as happening via different metamorphic

processes, particularly as “habitat metamorphosis” followed later by the “physiological metamorphosis” (sensu Reitzel in Bishop *et al.*, 2006). The habitat metamorphosis entails the transition from pelagic to benthic individual with the change from one larval stage to another e.g. metatrochophora to nectochaeta (in serpulids, Table 5.1) or naupilus to cyripid (in barnacles) (e.g. Carpizo-Ituarte and Hadfield, 1998; Grave, 1933). The physiological metamorphosis (which I will discuss later) describes the transition from mobile larva to a sessile juvenile during and after the attachment of the individual (Table 5.1) (e.g. Maruzzo *et al.*, 2012).

After the habitat metamorphosis, the larva of *S. cariniferus* swims near or crawls on the substratum and makes primary contact for up to eight days. This has often been described as searching behaviour (Hadfield et al., 2014; Marsden and Anderson, 1981; Nelson et al., 2017; Segrove, 1941; Young and Chia, 1982). There are further morphological changes associated with this behaviour, such as the flattening of the pygidium and secretion of mucus. With these changes, we can infer that the larva has reached the ability to settle. Whether these changes and the ability to settle occur as a consequence of the development or have to be initiated through a cue is not completely clear. In some cases it seems that a larva respond by settling after contact with conspecifics or derivate of conspecific adults (e.g. tubes) (Chan and Walker, 1998; Toonen and Pawlik, 1996; Wilson, 1936, 1970). Therefore, the ability to attach to a surface could be initiated through a separate cue. I suggest defining the ability to settle as “competence to settle” which precedes the final attachment (Nelson et al., 2017; Segrove, 1941; Young and Chia, 1982). In fact the larva of the related sabellarid *Phragmatopoma californica* (Fewkes, 1889) (formerly *Phragmatopoma lapidosa californica*) seem to be capable of reversing development from a settlement competent larva back to a pelagic stage if it experiences unfavourable conditions like starvation (Pawlik and Mense, 1994). This plasticity in the larval development may allow the larva to slow or even revise the development into the next larval stage or sessile juvenile if habitat conditions become unfavourable for the

survival of the offspring or later sessile individual (Carpizo-Ituarte and Hadfield, 1998; Chia, 1977; Hadfield et al., 2001; Schroeder and Hermans, 1975).

Table 5.01 Summary of the separate developmental stages with some of their characteristics.

Development step	Characteristic
Metamorphic competent larvae	3 groups of larval chaetae developed on the right and left side of the larvae
Metamorphosis form pelagic metatrochophora to benthic nectochaeta (habitat metamorphosis)	
Nectochaeta	The larva forms 3 chaetiger
Competence to settle	The pygidium becomes triangular shaped and secretion of mucus can be observed.
Settlement	The pygidium secretes mucus, the larva could trail a mucus tail. Larva attaches to the substrata and secretes a primary mucus tube.
Attachment	The larvae form a secondary calcareous tube
Metamorphosis from attached nectochaeta to juvenile (physiological metamorphosis)	
Juvenile	Neck and head reduced, juvenile structures like tentacle and operculum are formed

Because the larval development has been well described for *H. elegans* (e.g. Carpizo-Ituarte and Hadfield, 1998; Hadfield, 1998; Wisely, 1958), this species has often been used as a reference for development of serpulins (Fernald et al., 1987; Nelson et al., 2017; Young and Chia, 1982). Consequently, many of these studies have limited their observation of settlement to a time frame of 24–48 hours (e.g. Chan & Walker 1998; Chan et al. 2014; Pawlik 1986). This limited time for observing settlement often results in reports of a low number of recruits (e.g. Gosselin & Sewell 2013; Okamoto et al. 1998; Watanabe et al. 1998). From earlier investigation it is acknowledged that *H. elegans* has a slightly different larval development compared to other serpulins like *S. triqueter* (Segrove, 1941; Wisely, 1958), which has not been considered in many studies. The larvae of *H. elegans* lack the ability to prolong their pre-attachment stage once they develop to a nectochaeta (Qian and Pechenik, 1998; Wisely, 1958). Once larvae of *H. elegans* have transitioned to a benthic life, they must settle (Qian and Pechenik, 1998). Whereas, other serpulins, like *G. caespitosa*, *S. columbiana, S. triqueter,* and other tubeworms, such as *P. californica,* can delay the attachment and the

further development from nectochaeta to juvenile by at least several days in unfavourable conditions (Føyn and Gjøen, 1954; Marsden and Anderson, 1981; Pawlik and Mense, 1994; Young and Chia, 1982). Additionally, the duration of settlement behaviour until attachment for this *H. elegans* seems to be considerably shorter (~2 hours) compared to other species (Wisely, 1958). For example, under supposedly ideal settlement conditions, the larvae of *S. columbiana* (as *Serpula vermicularis*) explore substrata for up to five days (Fernald et al., 1987). Similarly, *S. caniniferus* probes the substrate for up to eight days before they finally attach (pers. Obs.).

In the laboratory, settlement studies are limited to testing one or a small group of settlement factors. With additional laboratory artefacts, such as small vessel size for settlement, larvae quantity and the limited time over which settlement is observed, it is unlikely that a laboratory test will reflect the settlement behaviour of larvae in a natural environment. A further increase in artificial factors, like the presence of multiple artificial and natural settlement cues, or the use of an incubator benefitting the settlement process (e.g. Chan & Walker 1998), could further inflate the discrepancies between results from laboratory studies and observations in the field. One particular issue in more current studies on serpulid settlement is the use of chemical cues to induce metamorphosis (Marsden and Hassessian, 1986; Pawlik, 1990; Pawlik and Faulkner, 1986; Yool et al., 1986). The supply of these chemical substances can interact and possibly artificially increase the rate of larval development (e.g. Okamoto et al., 1998). Under these circumstances, it appears that metamorphosis follows directly after the larvae reached metamorphic competence and settle almost simultaneously (Gosselin and Sewell, 2012; Qian and Pechenik, 1998).

I have to aknowledge that the larval culture conditions in my experiments could have been suboptimal due to a high initial larval density and relatively low food concentration, which could affect the larval development. High larval mortality, in

my trials and most likely unreported in other studies, could mitigate effects of an initially high larval density. Further, the larval conditions do not necessarily explain the extended benthic larval stages as similar behaviour has been observed for other serpulins such as *S. vermicularis, G. hystrix* (Fernald et al., 1987; Nelson et al., 2017; Young and Chia, 1982) and other tube dwelling polychaetes (e.g. *P. californica:* Pawlik and Mense, 1994) as well as other sessile marine invertebrates like *Amphibalanus amphitrite amphitrite* (Darwin, 1854) (as *Balanus amphitrite amphitrite*) (Clare et al., 1994), *Dreissena polymorpha* (Pallas, 1771) (Ackerman et al., 2008), *M. edulis* (Petersen, 1984) and *Ostrea edulis* (Linnaeus,1758) (Cranfield, 1973). Finally, similar to my experiments, Gosselin and Sewell (2012) found only six percent of *S. cariniferus* larvae settle within the 48 hours after becoming benthic. These authors did not observe the settlement behaviour nor report the survival of the remaining larvae. Therefore, the effects of larval density or food concetration may not have a large effect on the final settlement.

5.4.1 Conclusion

The growth of planktotrophic larvae is primarily dependent on temperature and subsequently on food supply and other factors (Costlow and Bookhout, 1971; Hoegh-Guldberg and Pearse, 1995). However, in laboratory trials we mainly observe the growth rate in response to the availability of particulate food. The growth rate of *S. cariniferus* reported here is slower compared to observations made by other authors on the same species (Gosselin and Sewell, 2012), as the larvae were with only one algal species and in lower quantity. However, the growth rate is similar to larvae of species in other Sabellida. The availability of food particles seems to be the determining factor for the further development of *S. cariniferus* larva. A starved larva grows but does not develop beyond the metrochophora larva (Figure 5.08). The general larval development may be described in five distinctive steps (Figure 5.08): The metatrochophora larva becomes metamorphically competent, it metamorphoses to a nectochaeta, the

post-metamorphic larva becomes competent to attach, it attaches to a substrate, and metamorphoses to a juvenile. Any development beyond the competence to metamorphose is dependent on environmental conditions and one or more possible cues (such as food and biofilm). This allows the larva to prolong the planktotrophic stage and delay further development if it encounters unsuitable conditions. The metamorphically competent larva will continue its pelagic phase until it recognises a cue indicating suitable settlement substrata. Subsequently, the larva transforms to the nectochaeta stage and transitions to a benthic life. This secondary larva displays a searching behaviour, which includes swimming over and crawling on the settlement area with primary contact to the substrate. The larva than reaches competence to settle through further morphological changes and begins to secrete mucus via their pygidium. Possibly in response to another cue, the competent larva will finally attach with the transformed pygidium first (Figure 5.08). The attached propagule will subsequently proceed with the secretion of a primary tube. However, the settlement is only finished once the secondary tube is formed. The individual continues with a second metamorphosis where radioli and other juvenile structures are formed. Simultaneously, eyes and other larval structures become reduced. The development into a juvenile is finally finished by the growth of an operculum. Therefore, development of *S. cariniferus* consists of reaching metamorphophic competence and transitioning to nectochaeta, which can be described as habitat metamorphosis. This first metamorphosis will be followed by reaching settlement competence and the final attachment to a substratum. Subsequently, the individual will follow with a second physiological metamorphosis to the juvenile worm. Those metamorphosis steps correlate and partially depend on the settlement. However, these morphophical changes are observed independently from settlement, as settlement is often finished before metamorphosis is completed.

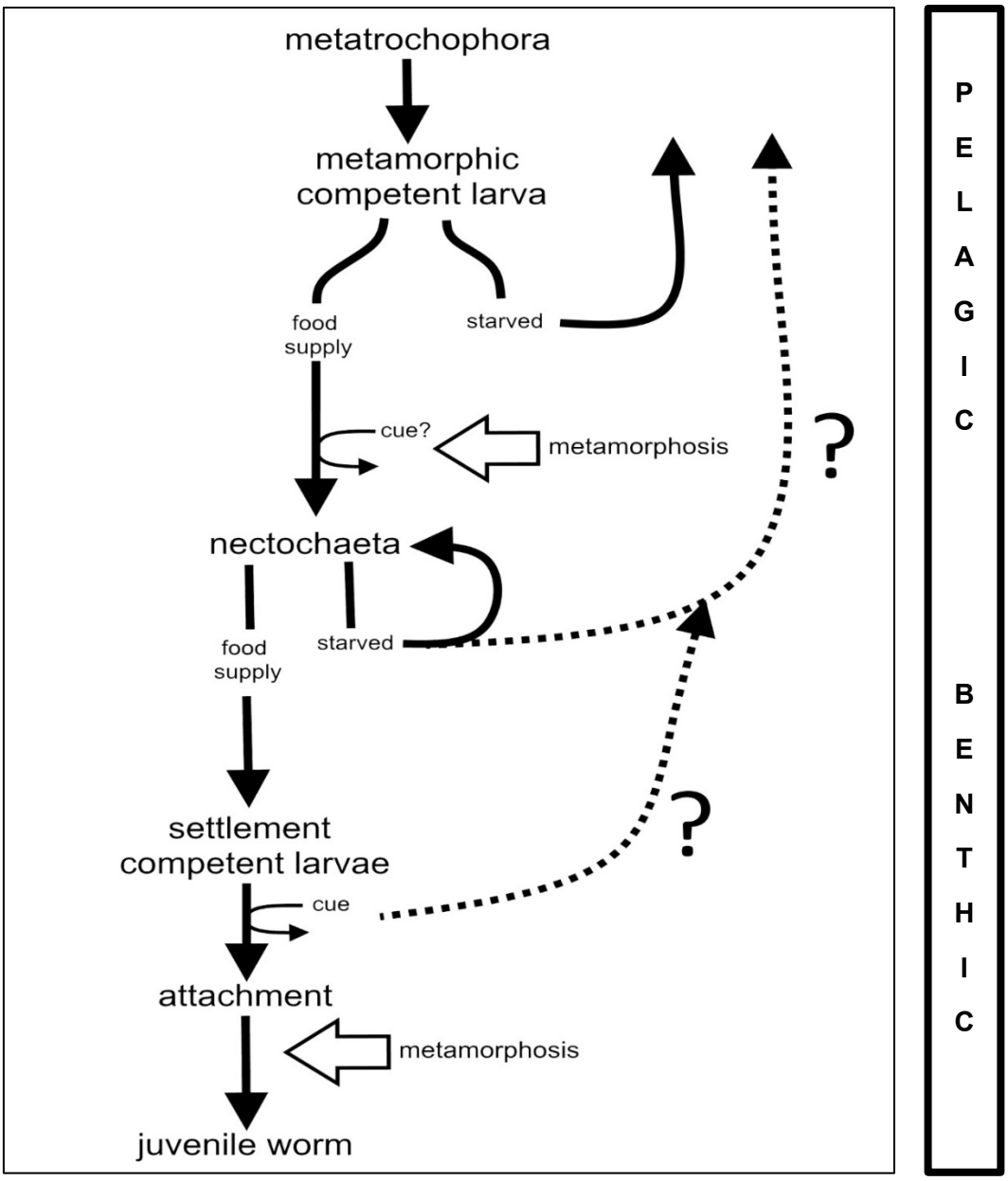

Figure 5.08 Summary of larval development from metatrochophora larva to juvenile worm: The metatrochophora reaches metamorphic competence. If food is supplied the metamorphic competent larva will transit to the benthos and metamorphose to a nectochaeta, possible in response to a cue (emitted by a bacteria film, conspecifics or other taxa). If the propagule is starved, it will continue as pelagic metatrochophora larva. The nectochaeta will reach settlement competence in appropriated conditions, but if the larva becomes starved it will continue as nectochaeta and possibly return to a more pelagic life. The settlement competent larva attaches to a substrate, perhaps in response to a cue (provided by bacteria or conspecifics) and continues with a second metamorphosis to a juvenile worm.

6. General Discussion

In this thesis I have described larval metamorphosis and settlement behaviour for *Spirobranchus cariniferus* in the laboratory. These observations allowed me to interpret recruitment data from the field. I found evidence that larvae of *S. cariniferus* settle aggregatively but not in response to adult conspecifics. On a small scale an interaction between conspecific larvae likely occurs, as otherwise the offspring would appear randomly settled. In addition, from field experiments I found that abiotic factors like tidal height, hydrodynamics forces and sunlight also are important in determining the distribution and recruitment of *S.cariniferus*.

Solitary individuals likely occur due to pre- and post-settlement mortality. Neighbouring larvae could become washed off or die through desiccation prior to the final attachment. After settlement, in addition to the abiotic factors are also biotic factors, like predation or crushing by larger mobile taxa, which are a potential source for mortality of attached individuals (Connell, 1985; Hunt and Scheibling, 1997; Keough and Downes, 1982; O'Donnell, 1986). Another explanation for solitary individuals is that larvae which have drifted away and separated from other larvae may settle solitarily if no conspecifics are around (Thorson, 1966). However, this has not yet been observed for serpulid propagules due to the ability to extend their planktotrophic stage.

Similar to other marine invertebrates, the overall mortality of adult individuals was 18% and was highly variable in regard to season and individual size (e.g. Bertness and Grosholz, 1985) but was not higher for solitary individuals. The main causes for mortality of adult *S. cariniferus* are likely similar to those for other intertidal serpulids and invertebrates: abiotic factors like dislodgment and destruction by waves and debris transported by waves (Denny, 1995; Hunt and Scheibling, 2001; O'Donnell, 1986; Shanks and Wright, 1986). Also tube growth was more dependent on season and individual size rather than settlement pattern. Smaller individuals need to grow faster than larger older individuals, to

increase their chance of survival though mitigating the risk of desiccation, dislodgement, predation and becoming crushed (Bertness and Grosholz, 1985; Dayton, 1971; Denny et al., 1985). The predicted higher tube growth rate for aggregative individuals as a consequence of intraspecific competition and possible higher food availability (Bertness, 1989; Bertness et al., 1998; Fauchald and Jumars, 1979; Fréchette et al., 1992; Merz, 1984) was not supported.

If we conclude that *S. cariniferus* predominantly settle aggregatively and solitary individuals occur by chance through mortality and other factors, the consequences of both configurations are difficult to interpret, as the solitary path may be an unfavourable circumstance for the individual. However, effects on growth, mortality and reproductive output were either small or non-existent. Although tube damage did not directly cause mortality, laboratory experiments showed that damage to the anterior part of the tube can have a severe effect on the tube growth rate of solitary individuals. Solitary individuals seem unable to recover the damaged tube and lose even more of their housing, perhaps due to weakened or unstable tube integrity. A possible explanation could be that solitary individuals have less energetic resources to recover from severe tube damage as perhaps they have a lower availability of food compared to their aggregative conspecifics (Fréchette et al., 1989; Helms, 2004; Ritz, 2000; Wu and Levings, 1978). Long term studies are needed to see if a positive growth rate can be reached before the solitary individual will decease. Additional studies are required to understand at which individual density the increased availability of food in a settled group (Fréchette et al., 1989; Merz, 1984; Ritz, 2000) cannot compensate for the larger number of individuals. This subsequently results in higher competition for food which could have a negative impact on individuals in the aggregation (Bertness et al., 1998; Helms, 2004; Menge and Sutherland, 1976; Woodin, 1976).

Further, although there was no difference in tube growth rate between solitary and aggregative individuals, it seems aggregative individuals with similar tube

width are younger than their solitary counterparts. In turn, individuals living in a patch are likely to have a larger body and wider tube than same aged solitary specimen. A comparison of body size relative to tube size for solitary and aggregative individuals revealed that solitary worms of similar tube sizes have a shorter body, which suggest that these specimens invest more of their energy in tube length growth rather than body growth. One possibility is that solitary individuals may be attempting to increase the opportunity to connect with conspecifics by focusing on tube length growth. Reproductive success is affected by distance to conspecifics and also tube stability and food availability can increase in aggregations (Eckman, 1996).

I found no difference in the sex ratio, reproductive output or maturation between solitary and aggregative worms, although sample sizes for determining reproductive output of solitary worms were low, so these results must be interpreted with caution. Although the number of sperms spawned was dependent on individual size, the quantity of oocytes was not correlated to the size of the female. Besides the lack of a size effect on female fecundity, there was no difference in individual size between both sexes. Evidence of hermaphroditism has been found from spawning trials and histological sections for some serpulins (e.g. Cotter et al., 2003a; Dixon, 1981) as well as for *S. cariniferus* (in this study). However, in contrast to other studies, I found no support for sequential hermaphroditism in the form of protandry with one sex change (from male to female), as has been suggested for other serpulins (Kupriyanova et al., 2001).

Based on my observations from histology in combination with a non-biased sex ratio and lack of relationship between size of individuals and sex, I suggested in Chapter 4 to resurrect the term of “alternating sexes”. This form of sex change was first suggested by Coe (e.g. 1934, 1936, 1941, 1943) and has been broadly discussed for various mollusc species for decades (e.g. Amemiya, 1929; Loosanoff, 1942). For oysters and some other molluscs, alternating sexuality is

widely accepted as a reproductive trait (Ghazala and Muzammil, 2002; Leonard, 1968; Runham, 1992; Saucedo and Southgate, 2008). This pattern of sex change has also been suggested for some polychaete species (Premoli and Sella, 1995; Sella and Ramella, 1999) but not further investigated. The theory of alternating sexes describes the ability to change the sex, possibly prior to each spawning event, in accordance to population structure and energetic reserves of the individual (Coe, 1934; Hoagland, 1984; Runham, 1992). In contrast, sequential hermaphroditism is a single sex change in the ontogeny of the individuals, either from male to female (protandry) or from female to male (protogeny) (Hoagland, 1984; Hodgson, 2009).

In general, the variability in reproductive strategies amongst polychaetes seems to exceed that of other marine invertebrates (Wilson, 1991). This diversity and plasticity in polychaete reproduction is a consequence of the often simple reproductive structures of these worms (Giangrande, 1997; Hartmann-Schröder, 1982; 1996, Schroeder and Hermans, 1975). Even though a wide variety of publications exist, to date only a limited number of polychaetes species have been investigated for their reproductive traits. Currently, of all known polychaetes, reproduction has been studied in less than 350 species (~3% of all polychaetes) (Giangrande, 1997). Amongst these, some kind of brooding seems to be the most common reproductive trait and is probably plesiomorphic in this group (Giangrande et al., 1994; Levin, 1984; Wilson, 1991, reviewed by Giangrande, 1997). However, hermaphroditism has been described for at least 67 Polychaeta species, with simultaneous and sequential hermaphroditism nearly equally represented. Hermaphroditism is particularly common for tube dwelling worms like Sabellidae and Serpulidae (Giangrande, 1997; Kupriyanova et al., 2001; Schroeder and Hermans, 1975). However, reproduction has been described in only ~ 48 taxa in the Serpulinae (~ 13% of all serpulid species) (Giangrande, 1997). Within these serpulid taxa, hermaphroditism has been identified through the occasional observation of individuals with sperms and oocytes, or presumed as a consequence of a biased sex ratio in a population.

Confusingly, both male biased and female biased sex ratios have been interpreted as support for sequential hermaphroditism in the form of protandry (Cotter et al., 2003a; Ghiselin, 1969; Kupriyanova et al., 2001; Obenat et al., 2006; Obenat and Pezzani, 1994). These studies have generally not differentiated sequential hermaphroditism from alternating sexuality (Hoagland, 1984; Premoli and Sella, 1995).

Sequential hermaphroditism is identified by one sex change in the ontogeny of the individual and is thought to be derived from simultaneous hermaphroditism as an evolutionary transition to gonochorism (Ghiselin, 1974; Hoagland, 1984). On the other hand, alternating sexuality is possibly derived from gonochorism and has been reported for marine invertebrates that may change their sex each season or after each spawning event (Ghazala and Muzammil, 2002; Premoli and Sella, 1995; Runham, 1992; Saucedo and Southgate, 2008; Sella and Ramella, 1999). Alternating sexuality seems to develop parallel to secondary broadcast spawning in different invertebrate taxa. Various reports suggest that oysters as well as serpulins developed from brooding ancestors (Andrews, 1979; Bhaud et al., 1995; Giangrande, 1997; Rouse and Fitzhugh, 1994; Strathmann, 1978). Therefore, alternating sexuality as well as broadcast spawning could be understood as adaption to a perennial sessile life style (Giangrande et al., 1994; Heller, 1993; Juchault, 2002; Prevedelli et al., 2006; Strathmann, 1990).

The ecology of sessile marine invertebrates such as *S. cariniferus* is underpinned by recruitment, which in turn depends on larval development and settlement; therefore, it is crucial to understand these processes (Eckman, 1996; Levin, 2006; Pineda, 2000; Pineda et al., 2010, 2009). There is a rich literature describing the varied development and settlement of marine sessile invertebrates like barnacles, bivalves and oysters (e.g. Ackerman et al., 2008; Cole and Knight-Jones, 1949; Durante, 1991; Gravely, 1909; Vye et al., 2017), including serpulins, such as, for example, *G. caespitose, H. dianthus, S. lamarckii, S. triquetter* and *S.* cf. *krausii* (e.g. Chan et al., 2014; Cotter et al., 2003b; Grant,

1981; Hamer et al., 2001; Zeleny, 1911, 1905), although these studies have focused on a small number of species. For serpulins we can differentiate two trends from these studies. Earlier work (i.e. in the last century) aimed to understand and observe settlement behaviour and associated development steps of mostly native species (e.g. Føyn and Gjøen, 1954; Lacalli, 1977; Marsden and Anderson, 1981; Segrove, 1941). More recently (i.e. in the last 30 years), studies have concentrated on three genra of serpulins (*Ficopomatus, Hydorides* and *Spirobranchus)* which include invasive species to understand aggregative settlement in response to biological and chemical cues (e.g. Bryan et al., 1998; Harder et al., 2002; Lau et al., 2002; Toonen and Pawlik, 2001a). Unfortunately, some of these more recent investigations on settlement have missed the connection to earlier information on larval development. As a consequence, recent observations of larval settlement have been interpreted without the context of larval developmental processes, leading to misinterpretation of the factors important for recruitment and aggregative settlement. Several studies conducted on other marine invertebrates have concluded that larval behaviour has a crucial effect on settlement, and therefore larval development and settlement should be studied together (e.g. Eckman et al., 1994; Tamburri et al., 1992; reviewed in Eckman, 1996).

From all reviewed studies there is overwhelming evidence that the process of settlement can be quite protracted, and that there is high plasticity in settlement and metamorphosis. Therefore, studies that only focus on larval settlement within a limited time window (i.e. less than 48 hrs) and with low larval density (e.g. 10 individuals per settlement trial) may be too limited in scope to provide a holistic view. Larval development for *S. cariniferus* and other serpulins is variable in time, as different steps can be paused, extended or even possibly reversed, but follows a particular pattern of development sequences that may depend on various cues and environmental conditions (Hadfield and Paul, 2001; Pawlik and Mense, 1994; Toonen and Pawlik, 1994). The way settlement is studied in the laboratory is often highly artificial, and it is difficult to compare what happens in

those studies with what happens in the field (Hadfield and Strathmann, 1996). Chemical cues which act higher up in the signal cascade (e.g. IBMX, fatty acids, L-DOPA) are often supplied in laboratory trials (Bryan et al., 1997; Jensen et al., 1990; Kupriyanova et al., 2001; Okamoto et al., 1998, 1995; Pawlik, 1990; Yool et al., 1986) and can force the larvae to settle, but contact with naturally occurring cues often does not result inevitably in metamorphosis or settlement (e.g. Butman, 1987; Pawlik, 1992; Woodin, 1986, 1991; reviewed in Eckman, 1996). Observed responses of these induced behavioural changes increase the inconsistency in the terminology and interpretation of development and settlement for *S. cariniferus* and other serpulids. Therefore, in accordance with publication for serpulins and other sessile marine invertebrates, I suggest differentiating the sequences of larval development and settlement into the following steps: metamorphic competence (e.g. Hadfield et al., 2001), habitat metamorphosis (sensu Reitzel in Bishop et al., 2006), competence to attach (Coon et al., 1990), attachment (Nelson et al., 2017), and physiological metamorphosis (sensu Reitzel in Bishop et al., 2006).

Larvae which settle in patches regardless of the occurrence of adult conspecifics cannot exclusively be explained by passive larval distribution (for example, due to hydrodynamic forces that collect them together) (Pawlik, 1992). In my study I demonstrated that larvae of *S. cariniferus* settle aggregatively regardless of the presence of nearby adult conspecifics. Therefore, we possibly have to differentiate between “gregarious settlement” where larvae preferentially settle near adults, for example some barnacles (Hadfield and Paul, 2001; Jeffery, 2002; Larman and Gabbott, 1975; Scheltema et al., 1981) versus “aggregative settlement” where larvae settle together, such as is possible for serpulins (Hadfield and Paul, 2001; Marsden and Meeuwig, 1990; Toonen and Pawlik, 2001c). Both occur more or less through “choice” by larvae or “communication” of the propagules with their environment and/or conspecifics via cues (Barnes and Marshall, 1951; Dobretsov and Miron, 2001; Hadfield and Paul, 2001).

This report is, to my knowledge, the first study to demonstrate aggregative settlement of a serpulid in the field. *Spirobranchus cariniferus*, and possibly other serpulids, likely settle aggregatively through interactions with other conspecific larvae. As a consequence, new serpulid aggregations can form on any suitable substrate. If the environmental conditions allow, serpulid aggregations can form on little rocks, mussel shells or other hard substrata, or even blades of algae, and grow out to develop reef-like structures (Fornós et al., 1997; Moore et al., 1998; Riedi, 2012; Schwindt et al., 2004). Serpulins are pioneer organisms able to colonise empty subtidal and intertidal habitats (Manoudis et al., 2005; Nicoletti et al., 2007; Rasmussen and Brett, 1985). Further, subtidal and intertidal serpulins and other tube dwelling worms are recognised as bioengineers as their structures provide refuges and nurseries for other species (Jones et al., 1994; McQuaid and Griffiths, 2014; Smith et al., 2005; Vanaverbeke et al., 2009) and can therefore alternate composition of flora and fauna (Bazterrica et al., 2011; Bruschetti et al., 2009; Chapman et al., 2012; Dittmann et al., 2009; Haanes and Gulliksen, 2011; Heiman et al., 2008; Knox, 1949; Schwindt et al., 2001).

Aggregations of tube dwelling polychaetes can be found almost all around the globe from deep sea vents to temperated intertidal habitats (Bergquist et al., 2000; Fisher et al., 1997; Jaubet et al., 2011; Vanaverbeke et al., 2009) Aggregations of serpulins in particular are prominent in tropical habitats like Caribbean waters (Hill, 1967; ten Hove, 1979, 1970); sub tropical areas along coastlines of Brazil, Hawaii, South Africa or Australia (Bailey-Brock, 1976, 1972; Knox, 1960; Qiu and Qian, 1998; Schwan et al., 2015; Straughan, 1967; Walters et al., 1997), on temperate coasts of New Zealand, Europe and North America (Bastida-Zavala et al., 2017; Klöckner, 1976b; Riedi and Smith, 2015; Ruiz et al., 2000; ten Hove, 1979), and even in Antarctic regions (Ramos and San Martín, 1999). There is even a freshwater species, *Marifugia cavatica* (Absolon & Hrabě, 1930), that exists exclusively in caves in southern Europe (Kupriyanova et al., 2009). The global distribution of the serpulin taxa is due to geological drift and other historical topographic events. However, the successful worldwide spread of

invasive serpulid species, like members of the genera *Ficopomatus* or *Hydroides,* is due to human-mediated transport of pelagic larval stages and ship hull fouling recruits (Carlton, 1996a, 1996b; 2000, Link et al., 2009; Palumbi, 1995; Shanks, 1995; Thorson, 1966; Toonen and Pawlik, 2001c). The relatively fast development to a mature individual after settlement (Hill, 1967; Kupriyanova et al., 2001; Qiu and Qian, 1998) and their survival and resilience to various environmental conditions like eutrophication and various salinity and temperature ranges enable serpulins to settle in various habitats and contribute to rapid establishment of populations of these invasive species (Bianchi and Morri, 2001; Dittmann et al., 2009; Heiman et al., 2008; Hill, 1967; Jackson, 1977; Knox, 1949; Straughan, 1972).

By understanding the relationship between settlement cues, larval development and settlement behaviour, we can explore recruitment and the ecological consequences for both intertidal and subtidal serpulins. For example, native species could be encouraged to develop aggregations to restore ecosystems, and invasive species need to be discouraged from settlement on infrastructure. More work is also needed on larval development and settlement to form a more general model of principal processes for marine invertebrates. This is especially true for the interaction of larvae with their environment and with conspecifics, where more holistic studies are required. Further laboratory and field-based studies are required to recognise the effects of abiotic factors like currents, sunlight and biotic factors like predation, interspecific competition and crushing on different life stages from recruits to adult serpulins. Future work on the reproductive traits of sessile species like *S. cariniferus*, for example, through sectioning of larger individuals which aren't releasing gametes, is vital. Such studies will give us a better picture of the possibility of hermaphroditism and possible ecological requirements and consequences as well as the evolution of these traits.

7. References

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Appendix

Appendix to Chapter 1 - Sample Sites

Breaker Bay

(Latitude: 41°19’58.84” S; Longitude: 174°49’28.21” E)

Off all my field stations, the most southern site is Breaker Bay, a land mass projection between Eve Bay and Flax Bay. The rocky substrate at Breaker Bay has an almost vertical relief (Figure A1.01 - A1.03) and consists of greywacke, a more solid sandstone variety compared to my other sides around the peninsula (Lachowicz 2005; Morelissen et al. 2016: 108). As this site is almost on the southern end of the Miramar Peninsula, this site experiences the greatest wave exposure and strongest currents. In general, sessile organisms in the intertidal are sparse at this site. Barnacle and serpulid aggregations are relatively rare (Figure A1.01 – A1.04), and mussels are absent.

Figure A1.01 – A1.04 Sample site Breaker Bay, with a limited number of smaller serpulid aggregations (Fig. A1.04).

Kau Point

(Latitude: 41°17’20.33” S; Longitude: 174°50’3.46” E)

At the cape between Mahanga Bay and Kau Bay on the northeastern end of the peninsula, is where the sample station ‘Kau Point’ is located. This sample site is a relatively exposed land protrusion and therefore particularly susceptible to wave impact from the open area of Wellington Harbour (Figure A1.05 – A1.07) as well as waves and currents from the south (Lachowicz 2005). Barnacles and mussels mostly covered the rocks on the northern side of this cape (e.g. Figure A10.7). Whereas at the southern rocks the mussels were often replaced by serpulids.

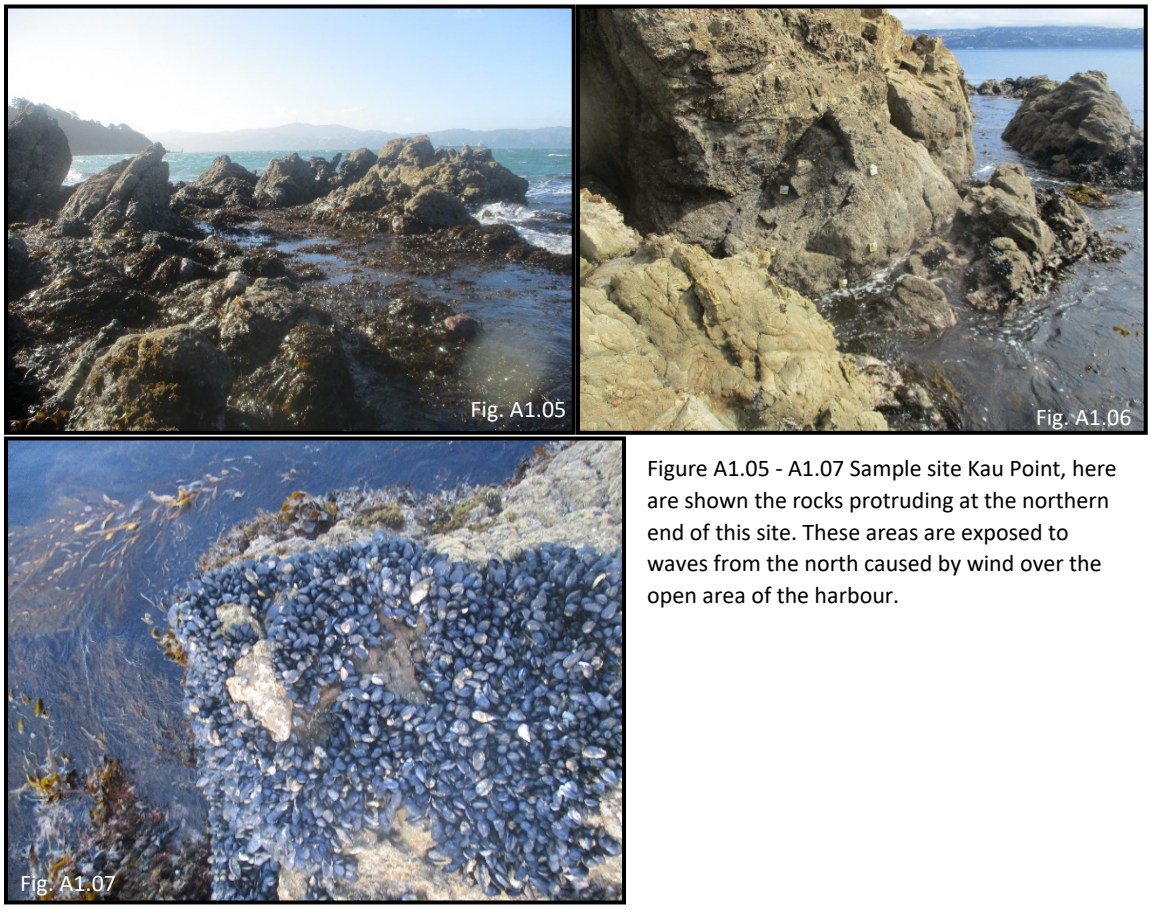

Figure A1.05 - A1.07 Sample site Kau Point, here are shown the rocks protruding at the northern end of this site. These areas are exposed to waves from the north caused by wind over the open area of the harbour.

Point Haswell

(Latitude: 41°17’2.14” S; Longitude: 174°49’34.14” E)

At the northern land protrusion of the peninsula is the sample site Point Haswell. This site is probably the longest land projection of the peninsula. Despite this extension, it seems to be more sheltered than the sites on the east coast of the peninsula, as it is averted from the harbour opening and therefore more sheltered from the impact of currents (Robertson and Stevens 2007). However, because the rocks are smaller this area is flatter than other sites. As a consequence of this, low profile waves caused by northerly winds may have a large effect on flora and fauna at this site. The distribution of mussels is limited at this site; however, barnacles and serpulids are present in high numbers (Figure A1.08). Because of the softer relief and the flatter habitat, the vertical separation of sessile species is not as strongly pronounced as at other sides. The borders of barnacles and serpulid patches appear to be more entangled with each other and interrupted by leafy algae (Figure A1.09).

Figure A1.08 & A1.09 shows the patchy distribution of barnacles and serpulids, partially interrupted by algae at Point Haswell.

Porirua Harbour

(Latitude: 41°7’1.23” S; Longitude: 174°50’26.66” E)

Porirua Harbour is located at the north-western coast of the greater region of Wellington. For the spawning experiments, I collected additional specimens from the Onepoto Arm of Porirua Harbour. The Onepoto Arm is the smaller of the two estuaries which form Porirua Harbour (Oliver and Milne 2012; Mawer and Arona 2015). This harbour is affected by eutrophication and toxic pollution through stormwater inlets and high pollution in the last century (Discovery Marine Ltd 2015; Mawer and Arona 2015; Stevens 2017). The harbour is rich in sediment and mud through low water turbidity. The substrate of the intertidal habitat of in the Onepoto estuary consists mainly of hard mud and gravel (Figure A1.10 & A1.11) (Stevens and Robertson 2013). Barnacles and mussel are rare, and the dominating sessile species seems to be *Spirobranchus cariniferus* (Figures 1.11 & 1.12). The salinity at Porirua Harbour ranges from 21 to 32 PSU (Read 1984: 402). The tidal movement is semidiurnal, with tidal neap around 0.4m (Read 1984: 402). The water temperature in this estuary ranges in winter between 9°C and 12°C, whereas in summer the range is from 15°C to 24°C (GWRC 2019).

Figure A1.10 Example of the gravel substrate seen in the Onepoto Arm of Porirua Harbour, with serpulids aggregatively settled on the large rocks.

Figure A1.10 shows the view onto the Onepoto Arm from the shoreline, with many serpulid aggregation attached to rocks. In figure A1.12 can we see an aggregation of *S. cariniferus* on a smaller rock in muddy sediment.

Pukerua Bay

(Latitude: 41° 1'43.54"S; Longitude: 174°53'26.59"E)

Along the coastline, further north of Porirua Harbour, is my most northern sample station, Pukerua Bay. I collected specimen of *S. cariniferus* from this site occasionally. The shoreline consists of a sand and gravel beach with small rocky areas. Samples were taken from a flat plateau with a few uprising rocks; the substrate here was also the harder sandstone greywacke (Robertson and Stevens 2007). Serpulis and barnacle were at this site in low abundance, and mussels were absent. The semidiurnal tidal neap is around 1.1 m, and the salinity is close to 35 PSU (Paul et al. 1983; Johnson et al. 2007). The water temperature at Pukerua Bay ranges in winter between 11°–15°C and in summer between 15° to 23°C (GWRC 2019).

Shelly Bay

(Latitude: 41°17’57.53” S; Longitude: 174°49’1.77” E)

On the western coastline of the Miramar Peninsula is a rock plateau between Shelly Bay and Sharks Bay where I installed the sample site Shelly Bay. This station is a rock plateau with several smaller bays and smaller protrusions (Figure A1.13). This site is the most sheltered of my study sites because of the

connection between Miramar and Wellington main area (Robertson and Stevens 2007). Thus, Shelly Bay is mostly exposed to northerly or southerly wind-driven waves but not to currents. As at most other sample stations around the peninsula, the rocky substrate here is probably an arkose sandstone which is softer than the greywacke at Breaker Bay (Figure A1.14). The mid-low to low-low tidal level at this site is dominated by *S. cariniferus* aggregations (Fig. A1.15).

Figure A1.13 shows the view over the plateau to the northern harbour at Shelly Bay. Figure A1.14 shows rocks heavily encrusted by barnacles. Figure A1.15 shows a dense serpulid patch at Shelly Bay.

Scorching Bay

(Latitude: 41°17’49.66” S; Longitude: 174°50’8.52” E)

The sample site Scorching Bay is a long stretch of rocky reef between two bays. To the south of this site is the sandy Sorching Bay, and to the north is the more rocky Mahanga Bay. The more southern area of this sample site seems to experience a higher disturbance by waves and currents compared to the more northern parts of this area (Figure A1.16; pers. Obs.). In general, the intertidal

habitat is mainly dominated by barnacle and mussel aggregation (Figure A1.17 & A1.18). However, on a rock surface with less exposure to current and waves can be found large patches of *S. cariniferus* beneath the barnacle band (Figure A1.19).

Figures A1.16 – A1.19 show the sample site Scorching Bay with the more exposed rocks on the southern end of this station (A1.16 – A1.19). In some areas, the rocks were nearly covered by a blanket of barnacles (Figure A1.18). Serpulid aggregations were rare but some surfaces were covered in high densities of *S. cariniferus* (Figure A1.19).

Worser Bay

(Latitude: 41°18’28.81” S; Longitude: 174°49’59.09” E)

The station Worser Bay is a cape at the northern end of Worser Bay. This sample site consists more or less high rock walls and outcrops in multiple rows separated by small sand areas (Stevens 2018) (Figure A1.20 & A1.21). Sedimentation at this site seems higher compared to my other sides around the

Miramar Peninsula, which causes a more variable habitat. The more sheltered rock walls are covered with barnacles and serpulid patches (Figure A1.22). At the south facing end of this station were *S. cariniferus* and barnacles, the dominating sessile species. A higher hydrodynamic disturbance can be observed, particularly at the northern end of this station. Barnacle and mussel patches were dominat in the area with stronger disturbance; however, in between mussel patches and on mussels were smaller aggregations of *Spirobranchus cariniferus* (Fig. A1.23).

Figures A1.20 – A1.23 Sample site Worser Bay with high rock walls and rock outcrops separated by sandy areas. Serpulid aggregations could reach a high density in the south of this site (Figure A1.22). I particularly observed *S. cariniferus* individuals settled on mussel shells at Worser Bay (Figure A1.23).

Appendix to Chapter 2 - Recruitment

Table A2.01 Compares the average recruitment/cm^2/day and the highest individual count to a plate per microhabitat and month in the peak season. M-in = plates were inserted into mussel aggregations, S-in = plates were attached to serpulid aggregations, M-out = plates were attached to bare rock in proximity to mussel aggregations,
S-out = plates were attached to serpulid free rocks near *S. cariniferus* aggregations

	S-in	M-in	S-out	M-out
average recruitment/cm^2/day March – April 2015	0.0048	0.0135	0.0017	0.0039
standard error of recruitment/cm^2/day March – April 2015	0.0019	0.0088	0.0007	0.0027
the highest count on a single plate during March – April 2015	34	30	11	54
average recruitment/cm^2/day January – February 2016	0.0054	0.002	0.0078	0.0053
standard error of recruitment/cm^2/day January – February 2016	0.0023	0.0011	0.0028	0.0022
the highest count on a single plate during January – February 2016	57	33	66	37
average recruitment/cm^2/day February – April 2016	0.0135	0.0158	0.0065	0.0114
standard error of recruitment/cm^2/day February – April 2016	0.0087	0.0074	0.0024	0.0056
the highest count on a single plate during February – April 2016	457	186	98	219
average recruitment/cm^2/day January – February 2017	0.0256	0.0134	0.0139	0.02
standard error of recruitment/cm^2/day January – February 2017	0.0066	0.0043	0.0049	0.0056
the highest count on a single plate during January – February 2017	594	381	353	348
average recruitment/cm^2/day February – April 2017	0.0205	0.0044	0.0135	0.002
standard error of recruitment /cm^2/day February – April 2017	0.0074	0.0018	0.0036	0.0009
the highest count on a single plate during February – April 2017	34	49	172	415
average recruitment/cm^2/day April – May 2017	0.0107	0.0013	0.0014	0.0016
standard error of recruitment/cm^2/day April – May 2017	0.002	0.0004	0.0004	0.0006
the highest count on a single plate during April - May 2017	48	21	35	13

Appendix to Chapter 3 - Trade-off

Tube growth

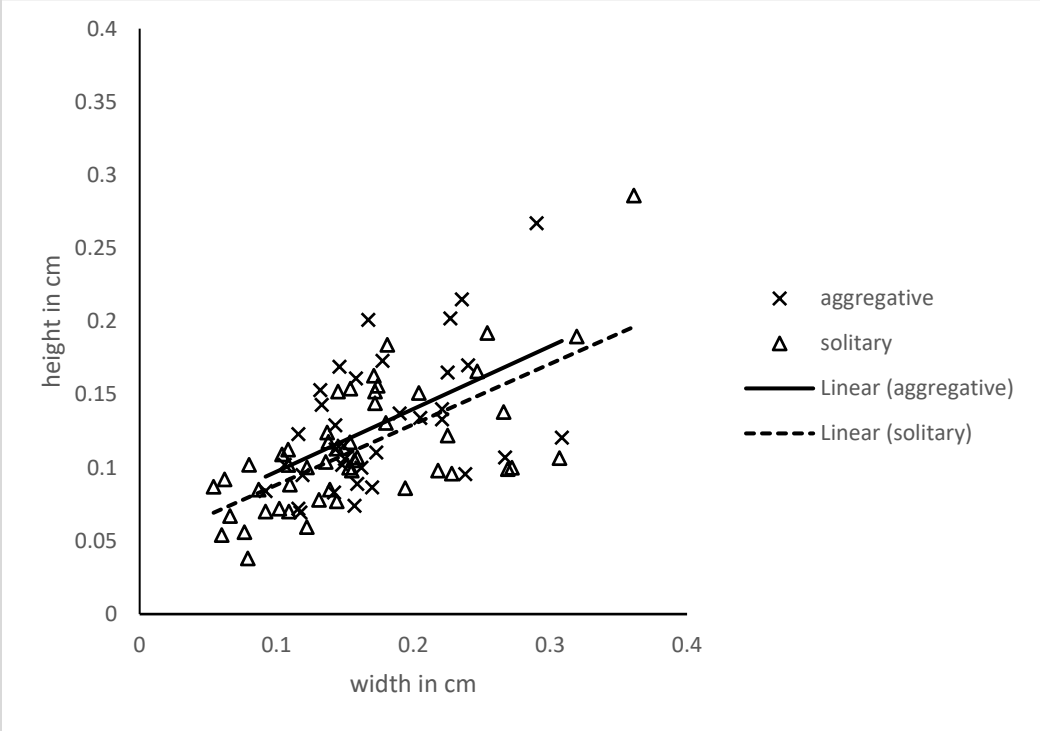

Figure A3.01 Compares the tube width and height of aggregative (X) and solitary (Δ) individuals. The linear regression shows that with expansion in tube width the tube height increases as well.
***R²* for Agg** = 0.26 (y = 0.43x + 0.05)
***R²* for Sol** = 0.46 (y = 0.41x + 0.05)

Table A3.01 Results of a pairwise comparison, with Bonferroni-adjusted p-values for the different size categories, of linear model "change of tube length" (Table 3.01).

Source of variation	Estimate	SE	z-value	p-value
>2 mm - <1 mm	-29.48	4.47	-6.6	**<0.01**
1-2 mm - <1 mm	-19.17	4.56	-4.21	**<0.01**
1-2 mm - >2 mm	10.31	3.21	3.21	**<0.01**

Table A3.02 – A3.04 List the average and maximum growth rate for all individuals smaller than 1 mm in tube width/height.

<1 mm Agg	Summer 2015	Autumn 2015	Winter 2015	Spring 2015	Summer 2016	Autumn 2016
average (µm/d)	37.33	23.38	56.54	56.44	38.06	24.06
Stdev (µm/d)	41.05	34.46	25.9	44.74	41.33	49.55
n	15	12	10	13	32	12
Max (µm/d)	129.3	110	99.6	152.5	100	131.4

<1 mm Sol	Summer 2015	Autumn 2015	Winter 2015	Spring 2015	Summer 2016	Autumn 2016
average (µm/d)	33.55	46.21	46.57	35.43	52.37	12.26
Stdev (µm/d)	32.32	75.81	27.21	38.51	38.99	65.1
n	11	5	7	17	58	20
Max (µm/d)	74.1	152.5	74.2	99.6	182.9	93.5

<1 mm All	Summer 2015	Autumn 2015	Winter 2015	Spring 2015	Summer 2016	Autumn 2016
average (µm/d)	35.73	30.1	52.44	44.54	47.28	16.69
Stdev (µm/d)	14.2	48.66	26.09	41.93	40.2	59.17
n	26	17	17	30	90	32
Max (µm/d)	129.3	152.5	99.6	152.5	182.9	131.4

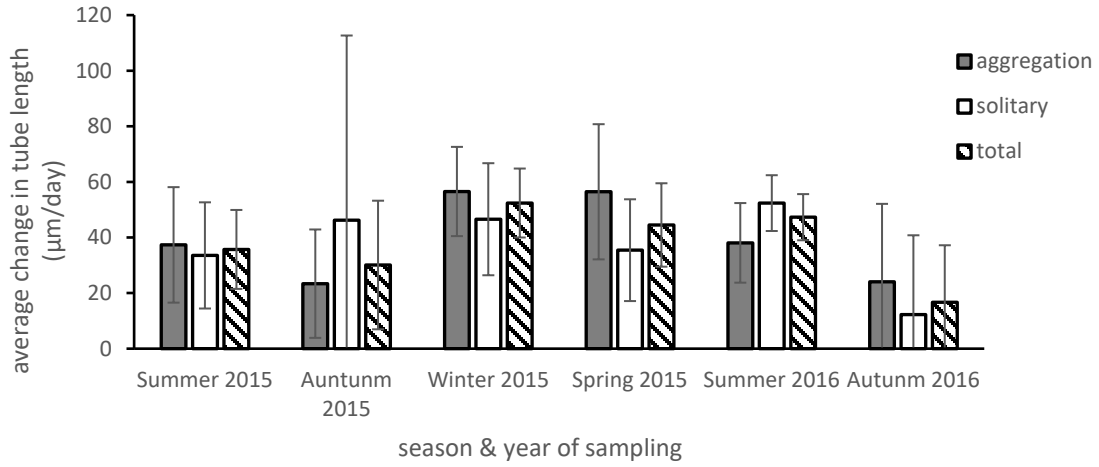

Fig. A3.02 The average change of tube length for individuals <1 mm tube width/height for each season. The error bars show the 95% confidence interval.

Table A3.05 – A3.07 List the average and maximum growth rate for all Individuals of 1 – 2 mm in tube width/height, "n" represents the number of observations.

1-2 mm Agg	Summer 2015	Autumn 2015	Winter 2015	Spring 2015	Summer 2016	Autumn 2016
average (µm/d)	22.74	4.42	22.4	33.57	25.08	42.11
Stdev (µm/d)	42.72	32.2	32.85	32.65	74.86	44.32
n	41	66	45	45	48	18
Max (µm/d)	158.7	91.5	103.1	114.7	460.4	111.7

1-2 mm Sol	Summer 2015	Autumn 2015	Winter 2015	Spring 2015	Summer 2016	Autumn 2016
average (µm/d)	29.36	17.99	20.17	43.73	24.28	14.81
Stdev (µm/d)	36.07	29.15	33.31	50.68	65.34	57.36
n	42	21	14	14	54	21
Max (µm/d)	106.9	84.7	104	95.6	395	121.4

1-2 mm Sol & Agg combined	Summer 2015	Autumn 2015	Winter 2015	Spring 2015	Summer 2016	Autumn 2016
average (µm/d)	28.37	7.7	21.87	35.98	25.07	27.41
Stdev (µm/d)	35.89	31.87	32.68	37.46	69.91	52.92
n	83	87	59	59	102	39
Max (µm/d)	158.7	91.5	104	114.7	460.4	121.4

Table A3.08 – A3.10 List the average and maximum growth rate for all individuals larger than 2 mm in tube width/height, "n" represents the number of observations.

>2 mm Agg	Summer 2015	Autumn 2015	Winter 2015	Spring 2015	Summer 2016	Autumn 2016
average (µm/d)	11.54	-7.16	13.42	24.25	3.83	28.41
Stdev (µm/d)	30.02	29.88	26.49	26.97	21.5	45.22
n	48	62	56	54	54	23
Max (µm/d)	95.6	57.1	123.2	96.7	78.1	152.1

>2 mm Sol	Summer 2015	Autumn 2015	Winter 2015	Spring 2015	Summer 2016	Autumn 2016
average (µm/d)	19.91	10.05	27.39	-12.84	7.67	48.71
Stdev (µm/d)	46.08	22.04	15.11	82.6	21.92	51.59
n	23	10	8	11	25	15
Max (µm/d)	146.7	45.1	52.5	59.4	59.2	128.6

>2 mm Sol & Agg combined	Summer 2015	Autumn 2015	Winter 2015	Spring 2015	Summer 2016	Autumn 2016
average (µm/d)	14.25	-4.75	15.17	17.97	5.88	36.09
Stdev (µm/d)	35.89	29.61	25.68	43.18	21.3	48.06
n	71	72	64	65	79	38
Max (µm/d)	146.7	57.1	123.2	96.7	78.1	152.1

Table A3.11 – A3.13 List the average and maximum growth rate for all individuals all, "n" represents the number of observations.

All Agg combined	Summer 2015	Autumn 2015	Winter 2015	Spring 2015	Summer 2016	Autumn 2016
average (µm/d)	20.65	0.87	20.95	31.73	20.42	32.08
Stdev (µm/d)	38.13	32.6	31.34	32.96	52.61	45.63
n	104	140	111	112	134	53
Max (µm/d)	158.7	110	123.2	152.5	460.4	152.1

All Sol combined	Summer 2015	Autumn 2015	Winter 2015	Spring 2015	Summer 2016	Autumn 2016
average (µm/d)	28.27	19.7	28.53	25.55	33.14	22.51
Stdev (µm/d)	38.51	37.37	29.11	60.05	51.9	59.91
n	76	36	29	42	137	56
Max (µm/d)	146.7	152.5	104	99.6	395	128.6

All Sol & Agg combined	Summer 2015	Autumn 2015	Winter 2015	Spring 2015	Summer 2016	Autumn 2016
average (µm/d)	23.87	4.77	22.52	30.04	26.85	27.2
Stdev (µm/d)	38.37	34.28	30.94	41.98	52.54	53.35
n	180	176	140	154	271	109
Max (µm/d)	158.7	152.5	123.2	152.5	460.4	152.1

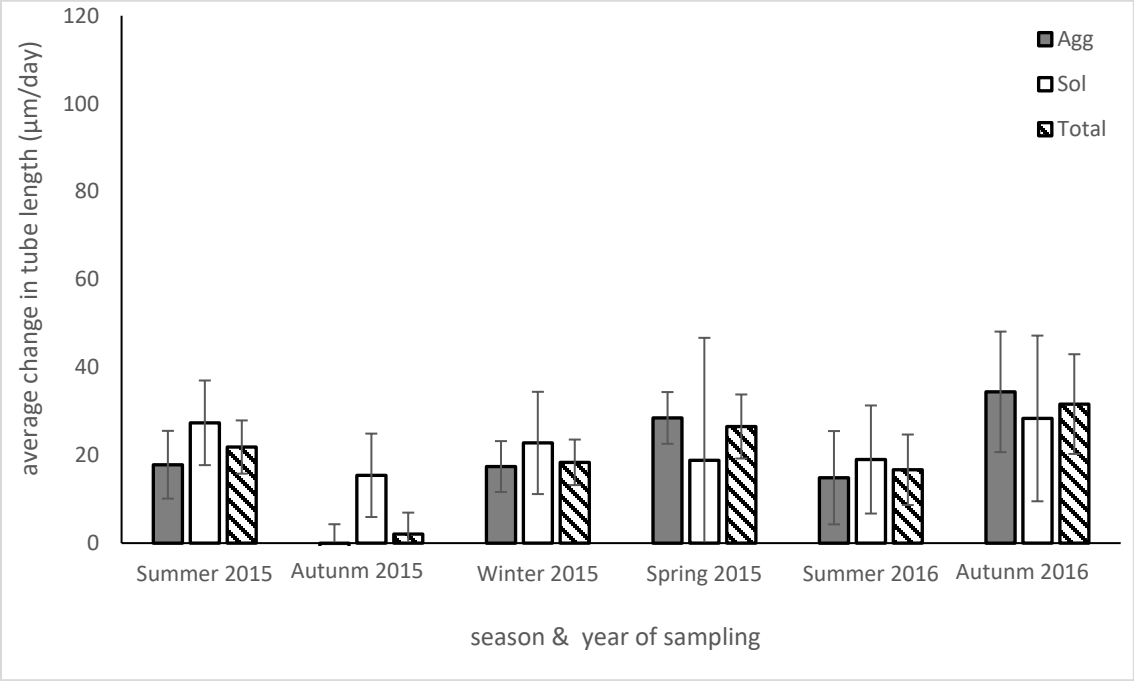

Fig. A3.03 The average change of tube length for individuals >1 mm tube width/height for each season. The error bars show the 95% confidence interval.

Mortality

Table A3.14 Logistic regression of mortality in response to Season, size category (<1mm, 1-2mm, >2mm), settlement configuration (Agg/Sol), interaction of settlement pattern and size category. Dead individuals have been coded with "0" and alive individuals with "1". Therefore, a positive estimate represents an increase in survival rate whereas a negative estimate means an increase in mortality. The fatality rates have been calculated for the population I observed for the tube growth between summer 2015 and autumn 2016.

Source of variation	Estimate	SE	z-value	p-value
(Intercept)	6.56	4.46	1.47	0.14
size.category>2mm	0.42	0.6	0.70	0.48
size.category1-2mm	-0.43	0.55	-0.78	0.44
Solitary	0.23	0.65	0.36	0.72
Summer 2015	-0.43	0.50	-0.85	0.4
Winter 2015	-0.05	0.6	-0.09	0.93
Spring 2015	-0.40	0.51	-0.78	0.43
Summer 2016	1.28	0.67	1.93	0.05
Autumn 2016	-1.08	0.51	-2.13	0.03
size.category>2mm:Solitary	-0.68	0.86	-0.8	0.43
size.category1-2mm:Solitary	-0.63	0.75	-0.84	0.4

Tube recovery

Table A3.15 Change of tube length in µm/day fitted as a function of settlement configuration (Agg/Sol), season, size category: <1 mm, 1–2 mm, >2 mm, length of the manipulation as category: cut<1 mm, cut 1–2 mm, cut >2 mm. Also included in the model are the interactions between settlement-pattern & size category, settlement-pattern & length category of manipulation and size category & length category of manipulation (n = Agg: 120; Sol: 98).

Source of variation	Estimate	SE	df	t-value	p-value
(Intercept)	1.12	37.04	5.92	0.03	0.98
Solitary	19.80	30.50	198.1	0.65	0.52
SeasonWinter	45.14	15.76	58.68	2.87	**<0.01**
size.category >2 mm	-13.76	30.98	198.26	-0.44	0.66
size.category 1-2 mm	-0.70	28.84	198.12	-0.02	0.98
cut <1 mm	-4.5	45.79	198.06	-0.1	0.92
cut >2 mm	13.13	38.66	198.71	0.34	0.74
cut 1-2 mm	64.72	65.85	198.66	0.98	0.33
size.category>2 mm:cut<1 mm	13.7	61.81	198.01	0.22	0.83
size.category1-2 mm:cut<1 mm	-10.07	45.64	198.00	-0.22	0.83
size.category<2 mm:cut>2 mm	-0.81	43.14	198.73	-0.02	0.99
size.category1-2 mm:cut>2 mm	-6.64	38.13	198.77	-0.17	0.86
size.category<2 mm:cut1-2 mm	-61.58	68.24	198.7	-0.90	0.37
size.category1-2 mm:cut1-2 mm	-37.05	61.77	198.37	0.60	0.55
Solitary:size.category>2 mm	-27.7	37.77	198.19	-0.73	0.46
Solitary:size.category1-2 mm	-6.15	31.41	198.06	-0.2	0.85
Solitary:cut<1 mm	18.15	27.6	198.05	0.66	0.51
Solitary:cut>2 mm	-10.41	19.92	198.34	-0.52	0.60
Solitary:cut1-2 mm	-30.08	29.94	198.99	-1.01	0.32

Tube size in relation to body dimensions

Table A3.16 Count of abdominal segments fitted as a function of settlement configuration (Agg/Sol), tube width/height (n = Agg: 46; Sol: 24)

Source of variation	Estimate	SE	t-value	p-value
(Intercept)	27.52	8.35	3.3	<0.01
Solitary	-6.76	4.96	-1.37	0.19
tube width	14.37	4.13	3.48	<0.01

Table A3.17 The natural logarithms of thorax width fitted as a function of settlement configuration (Agg/Sol), tube width, tube length and body length; Year & sample location have been included as random factors. n = Agg: 88; Sol: 82.

Source of variation	Estimate	SE	df	t-value	p-value
(Intercept)	-0.20	0.10	6.5	-1.96	0.09
Solitary	-0.06	0.03	160.38	-1.89	0.06
tube width	0.04	0.02	161.01	2.14	0.03
body length.mm	0.06	<0.01	162.29	10.13	<0.001
tube length.mm	<0.01	<0.01	164.66	1.32	0.19

Table A3.18 Tube density (tube dry weight /mm) fitted as a function of individual dry weight, tube dry weight, settlement configuration (Agg/Sol), tube width, tube length, thorax width and body length; Season, Year & sample location have been included as random factors. n = Agg: 88; Sol: 82.

Source of variation	Estimate	SE	df	t-value	p-value
(Intercept)	2.14	0.29	112	7.35	<0.001
individual dry weight	-0.05	0.03	112	-1.58	0.12
tube dry weight	0.01	<0.01	112	9.38	<0.001
Agg/Sol: Sol	-0.09	0.14	112	-0.64	0.53
tube width	-0.04	0.07	112	-0.57	0.57
thorax width	0.47	0.18	112	2.6	0.01
body length.mm	-0.02	0.04	112	-0.45	0.65
tube length.mm	-0.08	0.01	112	-8.85	<0.001

Table A3.19 The natural logarithms of tube dry weight fitted as a function of individual dry weight, settlement configuration (Agg/Sol), tube width, tube length, thorax width and body length; Season, Year & sample location have been included as random factors. n = Agg: 88; Sol: 82.

Source of variation	Estimate	SE	df	t-value	p-value
(Intercept)	1.86	0.24	11.42	7.78	<0.001
individual dry weight	0.04	0.02	98.69	2.08	0.04
Agg/Sol: Sol	-0.09	0.11	112.31	-0.77	0.45
tube width	0.06	0.06	80.6	1.12	0.27
thorax width	0.44	0.14	112.62	3.21	<0.01
body length.mm	0.13	0.03	98.59	4.12	<0.001
tube length.mm	0.01	0.01	103.60	1.46	0.15

Table A3.20 The natural logarithms of individual dry weight fitted as a function of tube dry weight, settlement configuration (Agg/Sol), tube width, tube length, thorax width and body length; Season, Year & sample location have been included as random factors. n = Agg: 88; Sol: 82.

Source of variation	Estimate	SE	df	t-value	p-value
(Intercept)	<0.001	3.68	1.59	-0.48	0.69
tube dry weight	<0.001	<0.001	0.01	1	0.32
Agg/Sol: Sol	-0.1	0.08	0.97	-1.26	0.21
tube width	0.12	0.05	0.01	2.6	0.01
thorax width	0.29	0.1	0.01	2.74	0.01
body length.mm	0.16	0.03	0.99	5.8	<0.001
tube length.mm	<-0.01	0.01	0.01	-0.17	0.86

Table A3.21 List of tube and shell growth rate for Serpulidsae and some other marine invertebrates (in red letters)

Species	**habitat**	**tube growth rate mm/d**	**notes**	**Reference**
Ficopomatus uschakovi (as Mercierella enigmatica)	intertidal & subtidal (dominantly brackish water)	average 0.82	Growth dependent on salinity used only juvenile individuals	(Straughan 1972)
F.enigmaticus (as *Mercierella enigmatica*)	dominantly brackish water	average: 0.18-0.5	Growth dependent on salinity used only juvenile individuals	(Hill 1967)
Galeolaria caespitosa	intertidal	average: 0.037 – 0.054	Growth rate depends on the season	(O'Donnell 1986)
Galeolaria hystrix	subtidal	juvenile 0.2 – 0.3 adults 0.03 – 0.06	Growth rate depends on initial size and season	(Riedi and Smith 2015)
Hydroides elegans (as *H. norvegica*)	intertidal & subtidal	1.56	used only juvenile individuals	(Paul 1937)
Hydroides elegans (as *H. norvegica*)	intertidal & subtidal	max. 1.87 – 2.44 (~ 23d after settlement) max. 0.375 (120 d after settlement)	Used only juvenile individuals. Measurements made only on the largest individuals	(Paul 1942)
Hydroides elegans (*H. norvegica*)	intertidal & subtidal	0.28 – 0.64	Measured new recruits to a sea wall 8-12 months after settlement	(Dew 1958)
Hydroides elegans	intertidal & subtidal	0.27 – 1.73	Measured only the 10 biggest juvenile individuals. Growth depended on pollution/eutrophication	(Moran and Grant 1984)
Hydroides dianthus (as *H. hexagonis*)	intertidal & subtidal (brackish water)	0.16 – 0.92	observed only 16 days to 2 years after settlement	(Grave 1933)
Hydroides eozensis	intertidal & subtidal	0.15 – 0.21	used only juvenile individuals	(Miura and Kajihara 1981)
Hydroides uncinata	subtidal	0.18 – 0.79	used only juvenile individuals	(Hill 1967)
Janua heterostropha (as *Spirobis pagentstecheri)*	intertidal & subtidal	average: 0.019	used only juvenile individuals settled & grown in Lab	(de Silva 1967)

Continuation of Table A3.21 List of tube and shell growth rate for Serpulids and some other marine invertebrates

Species	habitat	tube growth rate mm/d	notes	Reference
Lamellibrachia sp.	deep sea (hydrothermal vents)	0.02 – 0.03	adults	(Bergquist et al. 2000)
Spirobranchus triqueter (as *Pomatoceros triqueter)*	intertidal & subtidal	0.12 – 0.23	used only juvenile individuals	(Føyn and Gjøen 1954)
S.triqueter (as P. triqueter)	intertidal & subtidal	0.02 – 0.49	growth rate depends on season used only juvenile individuals	(Klöckner 1976)
Serpula vermicularis	subtidal	0.29	used only juvenile individuals	(Bosence 1973)
Serpula vermicularis	subtidal	0.09 – 0.22	adults	(Hughes et al. 2008)
Serpula columbiana	subtidal	~0.3 without parasite (*T. cancellate*) 0.1 – 0.25 (with parasite)	adults	(Iyengar 2002)
Spirobranchus cariniferus	intertidal	juvenile 0.1 adults 0.01	Growth depends on the initial size. A small number of observations	(Riedi and Smith 2015)
Spirobranchus krausii (as *Pomatoleios krausii*)		0.13 -0.05	Juvenile in the lab 3 months after settlement	(Crisp 1977)
Spirobis (*Spirobis*) *corallinae*	intertidal	0.012 – 0.024 (average 0.018)	Used only juvenile individuals settled & grown in Lab	(de Silva 1967)
Spirobis (*Spirobis*) *rupestris*	lower intertidal	Summer: 0.017 -0.023 Winter: 0.007	Used only juvenile individuals only 1year generation time	(Gee 1967)
Spirobis (*Spirobis*) *spirobis*	intertidal	in Lab: average 0.018 In field: Summer average 0.018 Winter average 0.0051	In lab used only juvenile individuals. In field used juvenile and adult individuals.	(de Silva 1967)
Spirobis (*Spirobis*) *tridentatus*	subtidal	average 0.012	Used only juvenile individuals settled & grown in Lab.	(de Silva 1967)
Cerastoderma edule (Bivalve)	intertidal (brackish water)	0.012 – 0.03	Small to large individuals (growth depends on initial size). Tidal movement is the determining factor in shell growth.	(Mahé et al. 2010)
Donax hanleyanus (Bivalve)	intertidal	recruits: 0.032 juveniles: 0.009 Adults: <0.001	Reared in Lab (growth depends on initial size).	(Herrmann et al. 2009)
Nucella ostrina (Gastropoda)	intertidal	0.007	Juvenile 6 days after settlement reared in the lab. Not a sessile specices.	(Moran 2000)
Pollicipes pollicipes (Barnacle)	Intertidal - subtidal	0 – 0.075 average juvenile: 0.042 adult: 0.019 large individuals: <0.01	Measured the growth of the capitular plates, growth depends on initial size.	(Jacinto et al. 2015)
Trichotropis cancellate (Gastropoda)	subtidal	0.017 – 0.022	Small to large individuals. Not a sessile specices.	(Iyengar 2002)

Appendix to Chapter 4 - Sex ratio & possible hermaphroditism

Protocols for sectioning

Table A4.01 Dehydration & infiltration protocol used for the Leica TP 1020

1	60	70% ethanol
2	60	70% ethanol
3	60	95% ethanol
4	60	95% ethanol
5	60	100% ethanol
6	60	100% ethanol
7	60	100% ethanol
8	80	50:50 xylene : ethanol 100%
9	45	xylene
10	45	xylene vacuum
11	80	paraffin vacuum
12	80	paraffin vacuum

Table A4.02 Scotts Tap Water (Carleton & Drury 1957)

2 gram Potassium bicarbonate
10 gram Magnesium Sulphate
dissolve both in 1 L distilled Water

Table A4.03 Mixing protocol for Haematoxylin (after Ehrlich) and mixing protocol for Eosin Y (Cold Spring Harbor Laboratory 2008, 2014)

Haematoxylin (after Ehrlich)	**Eosin Y**
100 ml Water	stock solution
100 ml ethanol 100%	dissolve 2 gram of Eosin Y in 40ml distilled water
100 ml glycerol	add 160 ml of ethanol 96%
10 ml (glacial) acetic acid	
2 g haematoxylin	solution for staining
Mix all ingredients	dilute 200 ml of stock solution with 600ml ethanol 80%
add Aluminum potassium sulfate to excess (saturated solution with undissolved material at the vessel ground)	add 4 ml glacial acetic acid and mix
Mix again	

Table A4.04 Staining protocol with Haematoxylin and Eosin Y in ehtanol

1	5 (min)	Deparaffinizing in Histo-Clear
2	5 (min)	Deparaffinizing in Histo-Clear
3	5 (min)	Remove the Histo-Clear with ethanol 100%
4	5 (min)	Remove the Histo-Clear with ethanol 100%
5	5 (min)	Rehydrating the section in ethanol 90 %
6	5 (min)	Rehydrating the section in ethanol 70 %
7	5 (min)	Rehydrating the section in ethanol 50%
8	5 (min)	Rehydrating the section in tap water
9	5 (min)	Stain with Haematoxylin
10	10 dips	Wash off excess stain in tap water
11	6 dips	Rinse in acid-ethanol (1%HCL in 70% EtOH) for differentiation and discoloration
12	2 (min)	Rinse in tap water
13	5 (min)	Bluing in Scotts tap water
14	2 (min)	bath in tap water
15	1 (min)	counterstaining in Eosin Y solution
16	10 dips	clean excess stain off in tap water
17	5 dips	clean excess stain off and dehydrate in ethanol 70%
18	5 dips	clean excess stain off and dehydrate in ethanol 90%
19	5 (min)	dehydrate in ethanol 100%
20	5 (min)	dehydrate in Histo-Clear
21	5 (min)	dehydrate in Histo-Clear
22	10 dips	remove excess Histo-Clear in ethanol 100%
23		mount with Entellan or Euparal

Maturation rate

Table A4.05 Results of a pairwise comparison with Bonferroni-adjusted p-values, for the maturity rate of the different size categories of the logistic regression (Table 4.01). Number of observations is 411.

	Estimate	SE	z- value	p-value
> 3 mm - < 1.5 mm	1.89	0.68	2.78	**0.02**
1.5-3 mm - < 1.5 mm	2.81	0.56	5.00	**< 0.01**
1.5-3 mm - >3 mm	0.91	0.40	2.27	0.07

Table A4.06 Logistic regression of maturation rate in response to settlement configuration (Agg/Sol). Maturity has been coded with "Yes" or "No". Therefore, a positive estimate represents an increase in the maturity rate. A negative value reflects a higher quota in immaturity. Site Month and Year have been included as random factor. Observation have been made in: November, December 2016; February, April, November 2017; January, March 2018. Number of observations is aggregative 68, Solitary 59

Source of variation	Estimate	SE	z-value	p-value
(Intercept)	0.67	1.9	0.35	0.72
Agg/Sol: Sol	-0.23	0.50	-0.46	0.64

Table A4.07 The natural logarithm of male fecundity as function of settlement configuration (Agg/Sol), and thorax width. The sample site has been included as a random factor. n = Agg: 25; Sol: 13.

Source of variation	Estimate	SE	df	t-value	p-value
(Intercept)	10.29	1.52	21.94	6.79	< 0.001
Thorax width	2.9	0.69	34.09	4.23	< 0.001
Agg/Sol: Sol	0.63	0.7	33.65	0.9	0.38

Table A4.08 The female fecundity as function of settlement configuration (Agg/Sol), and thorax width. The sample site has been included as a random factor. n = Agg: 25; Sol: 13.

Source of variation	Estimate	SE	df	t-value	p-value
(Intercept)	15664.3	14108.74	11.72	1.11	0.29
Thorax width	337.49	6430.08	24.94	0.05	0.96
Agg/Sol: Sol	6575.51	8676	51	0.76	0.45

Table A4.09 Logistic regression of sex ratio in response to settlement configuration (Agg/Sol). A positive estimate represents an increase in the rate of females. A negative value reflects a higher quota of male individuals. Thorax width, Site and Year have been included as random factor. Observation have been made in: November 2016; November 2017; January, March 2018. Number of observations is 101 (F: 25 solitary & 36 aggregative, M:18 solitary & 22aggregative).

Source of variation	Estimate	SE	z-value	p-value
(Intercept)	-0.54	0.77	-0.7	0.49
solitary	0.17	0.44	0.39	0.69
thorax width	0.02	0.35	0.06	0.95

Table A4.10 Sex ratio for each observed month

Month	Female	Male	ratio (female : male)	exact binomial test (p-value)	Chi X^2
February 2015	67	51	1 : 0.77	0.17	0.14
April 2015	60	35	1 : 0.59	0.01	0.01
November 2015	74	52	1 : 0.71	0.06	0.05
December2015	266	227	1 : 0.83	0.09	0.08
January 2016	38	41	1 : 1.1	0.82	0.74
March 2016	73	75	1 : 1	0.94	0.87
November 2017	28	26	1 : 0.91	0.89	0.79
January 2018	61	80	1 : 1.3	0.13	0.11
March 2018	37	28	1 : 0.76	0.32	0.26
total	704	615	1.1 : 1	0.02	0.01

Table A4.11 Length of the body off all worms have been fitted as a function of sex (F = female; M = male), month. Site, settlement configuration, Month and Year have been included as random factor. Observation have been made in: November 2016; November 2017; January, March 2018. Number of observations is 285 (F: 143, M:142)

Source of variation	Estimate	SE	df	t-value	p-value
(Intercept)	9.07	1.29	8.01	7.05	**< 0.01**
Male	-0.16	0.39	271.86	-0.42	0.68

Table A4.12 Thorax width off all worms have been fitted as a function of sex (F = female; M = male), month. Site, settlement strategy, and Year have been included as random factor. Observation have been made in: November 2016; November 2017; January, March 2018. Number of observations is 285 (F: 143, M:142)

Source of variation	Estimate	SE	df	t-value	p-value
(Intercept)	2.20	0.26	6.36	8.63	**< 0.01**
Male/Female: M	-0.21	0.08	276.88	-2.76	**< 0.01**

Reference to the Appendix

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