An integrated larval development and population genetics approach for predicting the establishment and dispersal potential of a recently introduced polychaete (Annelida: Spionidae) in southern Africa

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Some of the contents contained in this thesis (Chapters 2–4) are taken directly from manuscripts submitted or drafted for publication in the primary scientific literature. This resulted in some overlap in content between the chapters.

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Abstract

Boccardia proboscidea is a recently introduced polychaete in South Africa (SA) where it is a notorious pest of commercially reared abalone. The species was restricted to abalone farms distributed in three biogeographic regions up until 2011, when the first wild population was detected in the southern part of the country. If *Boccardia proboscidea* becomes invasive, it could pose a threat to the intertidal marine ecosystem of SA. The overarching aim of this thesis was therefore to predict the establishment and dispersal potential of *B. proboscidea*. The first objective was to assess the feasibility of using a closely related species to ground truth in the predictions. In Chapter 2, reproductive experiments were integrated with molecular studies to show that the non-indigenous oyster pest *Polydora hoplura*, like *B. proboscidea* can produce both planktotrophic and adelphophagic larvae (poecilogonous development). Due to a similar reproductive strategy along with its status as an aquaculture pest, *P. hoplura* was chosen as the "predictor" species. In Chapter 3 I investigated the effect of temperature on larval development of *P. hoplura* and *B. proboscidea* using temperature regimes reflective of the SA coast to determine establishment potential. Results showed that temperature significantly affected survivorship and developmental rate of planktotrophic and adelphophagic larvae for both species. For *P. hoplura,* survivorship of both larval types was highest at the intermediate to high temperature treatments (21 and 24°C) and was generally lower at the lower temperatures (12 and 17°C). *Boccardia proboscidea* exhibited a difference in survival optima where low temperatures favoured high planktotroph survival but low adelphophagic larval survival. Conversely, increased temperatures favoured high adelphophagic larval survival but low planktotroph survival and this was most likely driven by increased rates of sibling cannibalism. There was also a positive relationship between temperature and developmental rate for both larval

types of both species. *Polydora hoplura*'s response to experimental temperatures is congruent with its present distribution. Based on this I predicted that *B. proboscidea* should become established along a large section of the SA coast and differences in survival optima may also facilitate its establishment in colder waters where *P. hoplura* appears to be absent. In Chapter 4, I investigated the phylogeography of *P. hoplura* using mtDNA (Cyt *b*) and nDNA (*ATPSα*) gene fragments. Results showed genetic connectivity among all sampling sites distributed across two biogeographic regions. I hypothesized that the low genetic structure observed was likely due to anthropogenic dispersal mechanisms rather than natural dispersal. Finally in Chapter 5, I discussed the potential for natural dispersal of *B. proboscidea.* Based on temperature-specific planktonic larval duration and current velocities along the SA coast, *B. proboscidea* could potentially cover hundreds of kilometres in a single generation from each of its three point sources. However once the discrepancy between potential and effective dispersal was accounted for based on the literature, planktotrophic larvae would be expected to cover considerably shorter distances. When compared to the historical movement of other introduced marine invertebrates in the region, these adjusted distances appear to better reflect the reality of larval dispersal along the SA coast. *Boccardia proboscidea* benefits from a versatile reproductive strategy which may aid the worm in its attempt to invade the SA coast but anthropogenic dispersal could be a critical factor facilitating its widespread dispersal.

Opsomming

Boccardia proboscidea het onlangs in Suid-Afrika (SA) begin voorkom, waar die polychaete ‘n bekende plaag in kommersiële perlemoen is. Die spesie was aanvanklik beperk tot perlemoen plase van drie biogeografiese streke, maar in 2011 is die eerste wilde populasie aan die suidelike kus van die land gevind. Indien *B. proboscidea* ‘n indringer word, kan dit ‘n bedreiging inhou vir die mariene ekosisteme van Suid-Afrika. Die algehele doel van hierdie tesis was dus om die potensiele verspreiding en vestigings vermoë van *B. proboscidea* in Suid-Afrika te voorpel. Die eerste objektief was om die moontlikheid te ondersoek om ‘n naverwante kandidaat spesie te gebruik wat die voorspellings rondom *B. proboscidea* in die werkliheid te kan toets. In Hoofstuk 2, is voorplantings eksperimente met molekulêre studies geintigreer om te wys dat die uitheemse oester plaag, *Polydora hoplura,* net soos *B. proboscidea,* beide planktotrofiese en adelfofagiese larwes (poekilogeen ontwikkeling) kan produseer. Danksy die feit dat *P. hoplura* ‘n soortgelykie voortplantings strategie en status as akwatiese pes het, is dit as ‘n “voorspeller” spesie gekies. In Hoofstuk 3 ondersoek ek die effek van temperatuur op larvi ontwikkeling van *B. proboscidea* en *P. hoplura* deur temperatuur toestande te gebruik wat verteenwoordigend van die Suid-Afrikaanse kus is, om hierdeur die vestigings potensiaal te bepaal. Die resultate het getoon dat temperatuur oorlewing en die groei tempo van planktotrofiese en adelphofagiese larwes van albei spesies aansienlik affekteer. Vir *P. hoplura* was die oorlewing van albei larvi tipes die hoogste vir intermediêre tot hoë temperatuur behandelinge (21 en 24°C) en meestal laer teen laer temperature (12 en 17°C). *B. proboscidea* het verskillende oorlewings optima getoon, waar laer temperature hoër planktotrofiese oorlewing bevorder maar laer adelphofagiese larwes oorlewing veroorsaak. Inteendeel, verhoogte temperature het hoër adelphofagiese larwes oorlewing bevorder maar laer

planktotrofiese oorlewing: dit was moontlik as gevolg van verhoogte tempo's van kannibalisme. *P. hoplura* se reaksie op eksperimentiële temperature is in ooreenstemming met die spesie se huidige verspreiding. Gebasseer op die bogenoemde het ek voorspel dat *B. proboscidea* gevestig sal raak langs groot dele van die Suid-Afrikaanse kus en dat verskille in oorlewings optima die vestiging in kouer waters kan aanhelp waar *P. hoplura* bleik om afwesig te wees. In Hoofstuk 4, ondersoek ek die pylogeografie van *P. hoplura* deur gebruik te maak van mtDNA (Cyt *b*) en nDNA (*ATPSα*) geen fragmente. Resultate toon 'n gekonekteerde genetika tussen al die studie areas van twee biogeografiese streke. Ek stel die hipotese dat die lae genetiese struktuur moontlik deur antropogeniese verspreidings meganismes eerder as deur natuurlike verspreiding veroorsaak word. Laastens, in Hoofstuk 5, het ek die potensiaal vir natuurlike verspreiding van *B. proboscidea* bespreek. Gebasseer op die temperatuur-spesifieke planktoniese larvi duur en stroom snelhede langs die Suid-Afrikaanse kus, kan *B. proboscidea* moontlik honderde kilometres dek in 'n enkelle generasie vanaf slegs drie puntbronne van die spesie. Waaneer die verskil tussen potensiële en effektiewe verspreiding in ag geneem word, volgens die literatuur, kan daar van planktoniese larvi verwag word om aansienlike korter afstande te beweeg. Indien geskiedkundige bewegings van uitheemse mariene invertebraat spesies in die area ondersoek word, blyk dit dat die aangepaste afstande 'n beter voorstelling van werklike larvi verspreiding langs die Suid-Afrikaanse kus skep. *Boccardia proboscidea* het die voordeel van 'n aanpasbare voortplantings strategie wat die wurm moontlik kan aanhelp om 'n indringer aan die Suid-Afrikaanse kus te word. Antropogeniese verspreiding kan ook wel 'n belangrike faktor wees wat 'n wye verspreiding aanspoor.

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Chapter 1

General Introduction

1. Global and regional distribution of marine alien invasives

The science of predicting the establishment and spread of marine alien species is relatively young and remains one of the biggest challenges of invasion biology (Carlton and Gellar 1993). In less than 30 years, a considerable volume of work has shown that marine bioinvasions pose one of the biggest threats to global biodiversity (Bax *et al.* 2003; Hewitt and Campbell 2007; Rilov and Crooks 2009). Most marine species introduced outside of their native range never actually become invasive, with many either failing to complete the final three steps of the invasion process (survival in the recipient region, initial establishment and long term establishment), or for those that do become established, are simply integrated into the community without altering ecosystem dynamics (Bax *et al.* 2003; Haydar 2010). However, the few species that do become invasive can have profound effects on community structure, significant economic impacts and may even pose a danger to human health (Bax *et al.* 2003). Once an invasive species is established it is almost impossible to eradicate and therefore intercepting the earliest stages of the invasion process, ideally vector transport, is the only proven effective strategy that can avert a successful invasion event (Thresher and Kuris 2004). The most common pathways for the introduction of marine aliens are shipping (including ballast water and hull fouling) and aquaculture but the expansion of trade and tourism in the 21st century have created new pathways for dispersal such as the pet and aquarium trade, live seafood trade and the construction of superstructures above the waterline (Molnar *et al.* 2008; Strecker *et al.* 2011).

A survey by Molnar *et al*. (2008) identified northern California, the Hawaiian Islands, the North Sea and the eastern Mediterranean as the top four regions of the world with the highest levels of invasion. One classic example is the introduction of the European

shore crab, *Carcinus maenas,* to the United States of America. This species has significantly altered rocky shore communities, via predation on native shellfish and other invertebrates (Leonard *et al.*1999; Grosholz *et al.* 2000). *Carcinus maenas* was first introduced to the east coast of North America and, through a secondary introduction, eventually made its way to the west coast (California) where it has expanded its range northwards along the Pacific and is now established as far north as British Columbia in Canada (de Rivera *et al.* 2007). Reproductive studies by Hines *et al.* (2004) and de Rivera *et al.* (2007) predicted that *C. maenas* could potentially extend its range as far north as Alaska, since temperatures at multiple sites in that region are able to support larval development. In a separate but equally infamous invasion, the ctenophore, *Mnemiopsis leydi,* was single-handedly responsible for the collapse of an entire fishery, along with sharply reducing species richness of mesozooplankton, after being inadvertently introduced to the Black Sea, presumably via ballast water (Ivanov *et al.* 2000; Shiganova *et al.* 2001; Oguz *et al.* 2008). It was suggested that temperature was the most crucial factor followed by salinity in influencing the dispersal and range expansion of this species (Shiganova 1998).

In recent years, multiple studies conducted in the southern hemisphere have found that the problem of marine introductions also extends to this region, especially in Australia, where more than 250 species are confirmed as introduced (Hayes and Sliwa 2003; Hewitt *et al.* 2004). A noted case example is in Tasmania where the screwshell, *Maoricolpus roseus,* was introduced in the 1920s with oysters from New Zealand. The species, which is known for its wide thermal tolerance, proliferated rapidly to the point where it was able to outcompete native screwshells and attain densities exceeding 1000 ind.m^{-2}, higher than any other benthic invertebrate in the areas that were sampled (Bax

and Williams 2001; Bax *et al.* 2003; Gunasekera *et al.* 2005). In contrast to Australia, research on marine introductions in South Africa is still considered to be in its infancy due to a lack of taxonomic expertise for certain groups and limited surveys along the country's vast coastline (Robinson *et al.* 2005; Mead *et al.* 2011). Early work on the southern African coast first identified 15 introduced species in the region (Griffiths et al. 1992) and since then more than 80 introduced and 39 cryptogenic species (not demonstrably native or introduced- Carlton, 1987) have been confirmed (Mead *et al.* 2011). Of these, the Mediterranean mussel, *Mytilus galloprovincialis,* is regarded as the most invasive based on the definition of Vermeij (1996) in that it has spread beyond its source population and is a serious threat to indigenous species. *Mytilus galloprovincialis* was first detected at a port on the west coast of South Africa (Saldanha Bay) in the mid-1970s (Grant *et al.* 1984). Since then it had expanded its range northwards at a rate of 115 $km.y^{-1}$, colonizing rocky shore habitats on the entire west coast of South Africa, with movement south being considerably slower (25 $km.y^{-1}$), possibly due to the warmer water temperatures which attests to its antitropical distribution (Hockey and van Erkom Schurink 1992; Bownes and McQuaid 2006; Zardi *et al.* 2007). The species has successfully out-competed the native mussel *Aulacomaya ater* on the west coast while also displacing and reducing the overall size of native limpet species (Hockey and van Erkom Schurink 1992; Robinson *et al.* 2005; Zardi *et al.* 2007). On the south coast, *M. galloprovincialis* also exhibits habitat segregation with another native mussel *Perna perna* (Bownes and McQuaid 2006). To compound matters further, *M. galloprovincialis* was also anthropogenically transported to Port Elizabeth on the east coast as a transplantation experiment but this isolated stock was subsequently removed and propagules that spawned from the stock eventually died out (Robinson *et al.* 2005). Through natural dispersal, *M. galloprovincialis*, characterized by high fecundity and

recruitment rates (Harris *et al.* 1998) now occupies a large section of the southern African coast from central Namibia to East London (>2000 km) (Mead *et al.* 2011). Additional candidates in South Africa that could potentially become invasive includes the Pacific barnacle, *Balanus glandula,* and the notorious *C. maenas,* both of which have already caused significant changes to community structure at sites where they have been recorded thus far (Griffiths *et al.* 2011).

While the majority of studies in South Africa have addressed the introduction of free-living organisms, detailed studies on the spread of aquaculture pests are rare (Simon and Booth 2007; Simon *et al.* 2009). This is probably because of their inconspicuous nature and the fact that, for the most part, they are restricted to farmed environments. However, considering South Africa's vibrant aquaculture industry where oyster spat is imported from international suppliers and is also transported among oyster farms within the country, there is the potential for the introduction of aquaculture pests that could pose a threat to the marine ecosystem (Wolff and Reise 2002; Haupt *et al*. 2010a). This is especially true for polychaetes, which are known pests of commercial molluscs (Blake 1969; Radashevsky et al. 2006; Simon *et al.* 2006; Walker 2011) and are invasive in many parts the world (Currie *et al.* 2000; Leppakoski *et al.* 2002; Schwindt *et al.* 2004; Tovar-Hernandez *et al.* 2011; Giangrande *et al.* 2012).

2. General introduction to the Spionidae and the *Polydora*-complex

Polychaetes are a diverse group of annelids, mostly marine (>98%), with more than 8,000 species described thus far (Beesley *et al.* 2000). Within the polychaetes, the Spionidae is among the best-studied families and the most ecologically dynamic,

capable of being free-living, obligate or facultative symbionts (Rouse and Pleijel 2001; Blake 2006; Walker 2011). Free living spionids are known for creating and residing in tubes, produced by binding sand grains and detritus material, from which they feed by extending a pair of tentaculate feeding appendages known as “palps” (Fauchuld and Jumars 1979). Some individuals can also burrow into the calcareous structures of other invertebrates, such as molluscs and sponges, and these have been listed in the literature as commensals (Martin and Britayev 1998). However, the term “commensal” is used broadly since many of these organisms do in fact cause considerable damage to their hosts, including commercially important hosts (Simon *et al.* 2006; Simon and Booth 2007; Zapalski 2011; Walker 2011).

Within the Spionidae, the *Polydora* complex has been implicated in many shell infestation cases in both commercial and non-commercial species (Martin 1996; Lewis 1998; Ruellet 2004; Radashevsky and Olivares 2005; Simon *et al.* 2006; Simon and Booth 2007; Sato-Okoshi *et al.* 2008; Simon *et al.* 2009). The group, commonly known as “polydorids”, consists of nine genera and is distinguished from other members of the Spionidae by an enlarged fifth segment with large modified spines (Blake 2006; Walker 2011). The majority of polydorids belong to two genera, *Polydora* Bosc 1802 and *Dipolydora* Verrill 1879, with considerably fewer species belonging to the seven other genera (*Boccardia, Boccardiella, Polydorella, Pseudopolydora, Amphipolydora, Tripolydora, Caraziella*) (Delgado-Blas 2008).

3. Global distribution of polydorids

Polydorids have a nearly worldwide distribution and this is emphasized by the fact that incidents of shell infestations by these worms have been confirmed in virtually all

continents of the world: North America (both Canada and USA) where polydorids infest oysters and scallops (Owen 1957; Bower *et al.* 1992); South and Central America (Mexico, Chile, Argentina and Brazil) where they infest edible mussels, clams, scallops and abalone (Tinoco-Orta and Caceres-Martinez 2003; Vargas *et al.* 2005; Diez *et al.* 2011), Africa (South Africa and Namibia) where worms infest farmed abalone and oysters (Nel *et al.* 1996; Simon *et al.* 2006; Simon and Booth 2007; Simon *et al.* 2009; de Lange unpubl data), Asia (India, China and Japan) where members of the genera *Polydora, Dipolydora, Boccardia* and *Boccardiella* are pests of scallops, pearl oysters and abalone (Kojima and Imajima 1982; Ghode and Kripa 2001; Sato-Okoshi and Abe 2012), Europe (the Mediterranean, Portugal, France and the United Kingdom) where worms from the genus *Polydora* infest wild and cultured oysters (Almeida *et al.* 1996; Royer *et al.* 2006) and Australia and New Zealand where they infest cultured abalone and both cultured and wild oysters (Handley and Bergquist 1997; Dunphy and Wells 2001; Lleonart *et al.* 2003; Walker 2011). Understanding the extent of polydorid infestation with respects to specific problem species is complicated by the fact that many of these have been introduced by anthropogenic pathways (Walker 2011). At least 20 polydorid polychaetes have been clearly documented as introduced worldwide with many more cryptogenic (Carlton 1987; Radashevsky and Olivares 2005; Radashevsky *et al.* 2006)

Vectors for introduction of polydorids include the taking up and releasing of ballast water (which may contain larvae and adults) in bays, estuaries and inland waters in different parts of the world (Carlton 1987; Carlton and Gellar 1993), movement of aquaculture products such as commercial molluscs that may already be harbouring worms (Culver and Kuris 2000) and hull fouling of ships by encrusting organisms such as sponges that can also harbour different species of worms (Carlton and Geller 1993; David and

Williams 2012a). Like all sessile marine invertebrates, once these polydorids arrive in their new environment, their eventual establishment and natural dispersal patterns will be influenced by their reproductive strategies (Kinlan and Gaines 2003).

4. Reproductive biology of polydorids

Sexual reproduction is the dominant mode of reproduction in polydorids with asexual reproduction found in only eight species thus far (Blake and Arnofsky 1999; David and Williams 2012b). In sexual reproduction, females brood their offspring in egg capsules that are attached to the maternal tube by single or double filaments. All polydorids are intratubular brooders but the extent of yolk provisioning by the female may differ significantly among species resulting in the production of planktotrophic, lecithotrophic or adelphophagic larvae (reviewed by Blake and Arnofsky 1999).

Free-swimming planktotrophic larvae develop from eggs that are nutritionally poor. After hatching, the larvae spend considerably more time in the water column than any other larval type where they derive nutrition by actively feeding on a variety of phytoplankton. In contrast, lecithotrophic larvae are typically non-feeding and develop from eggs that are provisioned with adequate yolk to reach an advanced stage of development. As a consequence these larvae tend to settle soon after hatching (Blake 1969; Blake and Arnofsky 1999). In adelphophagic development, the larvae feed on nurse eggs provided by the female while still residing within the egg capsule. The larva consumes the nurse eggs, subsisting on this yolk source until it has been exhausted, then leaves the capsules and either directly settles or spends a short time in a planktonic state prior to settlement (Gibson 1997; Blake and Arnofsky 1999). Adelphophagy is often considered a variation of lecithotrophy (exo-lecithotrophy), since it is also yolk provisioning but in

the form of nurse eggs rather than direct provisioning (Radashevsky 1994; Sato-Okoshi *et al.* 2008).

In addition to the aforementioned reproductive strategies, some polydorids also exhibit a rare ability where the female is capable of producing more than one type of larva (poecilogony) (Giard 1905; Knott and McHugh 2012). Poecilogonous development was once considered a common phenomenon among marine invertebrates. However, a review by Hoagland and Robertson (1988) reported that many of the species thought to be poecilogonous actually consisted of sibling species producing different larval types. Poecilogony can exist as a developmental polymorphism, where two reproductive strategies are genetically fixed in a population, or it may exist as an adaptive polyphenism where environmental variables trigger a switch between reproductive modes (Knott and McHugh 2012). For example, in the free-living spionid, *Streblospio benedicti,* some females are capable of producing only planktotrophic larvae whereas other females can only produce lecithotrophic larvae. Experimental manipulation from the early 1980s to present has shown that *S. benedicti* cannnot switch between planktotrophy and lecithotrophy, even when cultured under a variety of environmental conditions (Levin and Creed1986; Chu and Levin 1989; Bridges *et al.* 1994). In contrast, Rice and Rice (2009) showed that in *Polydora cornuta*, the production of either planktotrophic or adelphophagic larvae depends on the amount of stored sperm available. Females usually producing planktotrophic larvae, when cultured in isolation, eventually deplete their stored sperm. As such, the number of unfertilized eggs in the capsules increases and these eggs then serve as extra embryonic nutrition for the developing embryos that were successfully fertilized. More recently, a preliminary study by Gibson *et al.* (2012) found that histone modifications facilitate the activation of genes

that regulate early development and differentiation in *P. cornuta* embryos, indicating that epigenetic processes could also be involved in the production of different larval types.

5. Larval dispersal and genetic connectivity

Understanding the extent of larval dispersal makes it possible to assess levels of genetic connectivity among different populations and vice versa. This becomes important when dealing with the ability of a non-indigenous species to spread and become invasive since it directly influences long-term persistence of the population (Bradbury *et al.* 2008). Species that produce planktotrophic larvae that spend a long time in the water column would be expected to disperse further from its natal site and show less genetic structuring than species that exhibit abbreviated larval development such as in lecithotrophy or adelphophagy (Todd 1998; Kyle and Boulding 2000).

However, recent examples in the literature have shown that the dispersal potential of a species and its actual dispersal is not necessarily congruent and knowledge of a species' larval biology alone may be insufficient for predicting range expansion (Cowen *et al.* 2000; Taylor and Hellberg 2003; Tepolt *et al.* 2009). For example, some studies have shown that local recruitment in species that produce larvae with a long planktonic phase can result in lower levels of gene flow among populations (Hellberg 2009, and references therein) whereas larvae that exhibit abbreviated development could maintain gene flow among spatially separated populations via passive dispersal or unorthodox vectors such as biofouling or rafting (Havenhand 1995; Pettengill *et al.* 2007). The inherent physiological plasticity of larvae under variable environmental conditions is equally important. In particular, temperature was identified as the most important abiotic factor affecting marine invertebrate larvae as it delimits the areas that can support

development (Hoegh-Guldberg and Pearse 1995; O'Connor *et al.* 2007; Cowen and Sponaugle 2009). The marine environment also possesses an array of oceanographic forces (upwelling cells, currents and gyres, to name a few) that could restrict larval movement regardless of the planktonic nature of the larva (Cowen and Sponaugle 2009; Hellberg 2009). Larval dispersal in the marine environment can therefore be considered as a dynamic process where larval life history interacts with the biogeographical features of the region.

6. Marine biogeography of southern Africa

The South African coast is an ideal region for conducting dispersal studies due to the presence of different biogeographic regions (Figure 1). The coast is bordered by two major currents: on the west coast is the north-flowing, cold Benguela Current system where the temperature regime on that coast allows for nutrient upwelling and hence large scale productivity, while on the east coast, there is the south-westerly-flowing warm Agulhas Current system with less productivity (Branch and Branch 1988; Emanuel *et al.* 1992; Branch *et al.* 2010). The southern coast is characterized by intermediate temperatures and eddies that form where the Agulhas Current deflects away from the coast in the Agulhas Bank region. These eddies are capable of transporting small bodies of water along with its associated organisms, around Cape Point where it becomes integrated with the northward-flowing Benguela system (Shannon 1985; Reason *et al.* 2006). The coastal biogeographic provinces have been defined by a variety of factors, the most important being temperature (Emmanuel *et al.* 1992; Teske *et al.* 2014). Within these bioregions, phylogeographic studies have identified even more differentiation where distinct genetic biogeographic breaks (phylogeographic barriers) exist within conspecific taxa (reviewed by Teske *et al.* 2011). Of these breaks, Cape

Point has been shown to be the most prominent barrier, separating the cool-temperate west coast from the warm-temperate south coast (Teske *et al.* 2007a; von der Heyden *et al.* 2008). On the south coast, Cape Agulhas has also been identified as a significant but weaker phylogeographic break than Cape Point (Evans *et al.* 2004; Teske *et al.* 2011) while on the southeast coast, additional breaks have been identified near Algoa Bay where a disjunction between the warm-temperature and subtropical biota exists (Emmanuel *et al.* 1992; Teske *et al.* 2007; von der Heyden *et al.* 2008). Finally, breaks have also been reported on the east coast, specifically the Wild Coast and further north near St. Lucia (Teske *et al.* 2006, 2007b, 2008). Since these breaks were found to have a significant impact on the dispersal capabilities of a variety of marine organisms in South Africa (Teske *et al*. 2011, and references therein), they could potentially influence the spread of alien species depending on where the species becomes established.

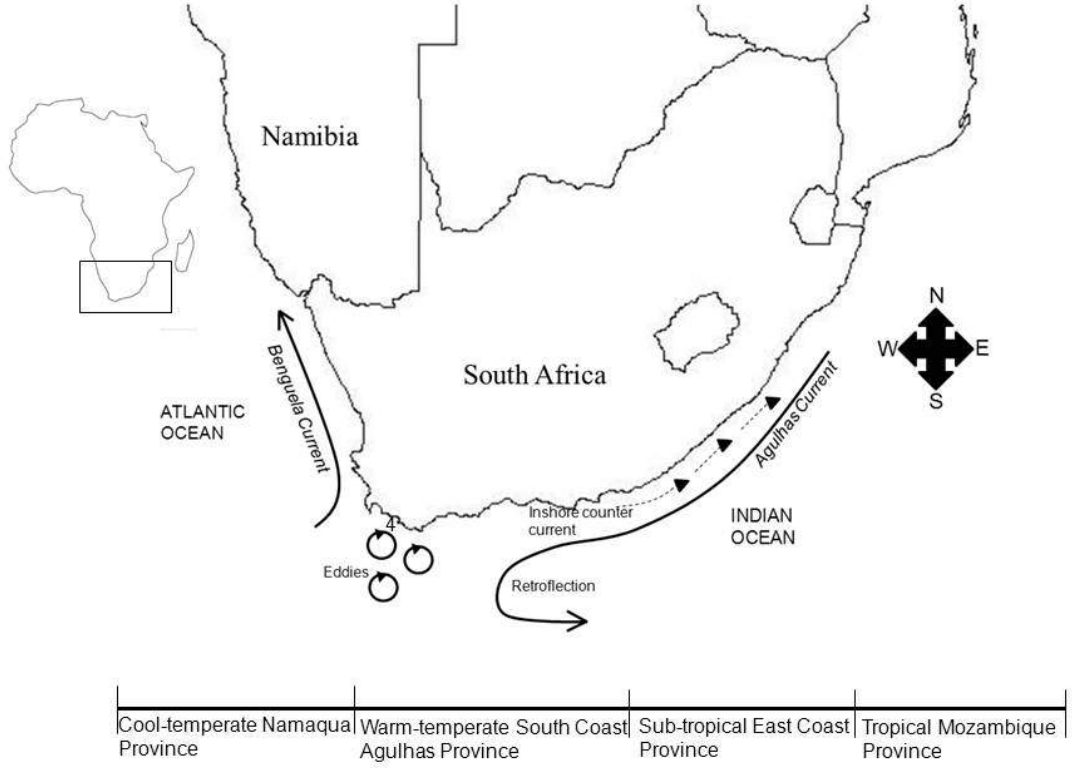

Figure 1: Map showing major oceanographical features along the southern African coastline along with the main biogeographical provinces. Map redrawn from von der Heyden *et al.* (2008)

7. **Problem** Statement

In South Africa, abalone farming is a lucrative part of the aquaculture industry and is done in on-shore culture facilities with farms located mainly in the Western Cape Province (Hauck and Sweijd 1999; Steinberg 2005). In recent years, there have been increasing reports of infestation of the commercially reared abalone, *Haliotis midae* Linnaeus 1758, by the introduced polydorid, *Boccardia proboscidea* Hartman 1940 (Simon *et al.* 2006; Simon and Booth 2007; Simon *et al.* 2010a; Boonzaaier *et al.* 2014). The native range of *B. proboscidea* includes the western coast of North America, extending as for north as British Columbia and as far south as southern California where it assumes a primarily free living existence in soft sediment, although it is often

associated with molluscs as a secondary borer (Hartman 1940; Woodwick 1963; Oyarzun *et al.* 2011). *Boccardia proboscidea* was also introduced to Australia and Spain, presumably via ballast water (Blake and Kudenov 1978; Martinez *et al.* 2006), and Hawaii with the transportation of cultured oysters (Bailey-Brock 2000). Furthermore, it has also been recorded from Japan (Sato-Okoshi 2000), New Zealand (Read 2004), Argentina (Jaubet *et al.* 2011) and the United Kingdom (Hatton and Pearce 2013), though details concerning its introduction to these regions are unclear. In South Africa *B. proboscidea* was first found in 2004 on an abalone farm at Hermanus in the southern part of the country (Simon *et al.* 2009, 2010b). The species was later detected at abalone farms on the west and east coasts (Jakobsbaai and Haga Haga respectively). The infestation of *B. proboscidea* at all three farms was attributed to the deliberate movement of infested abalone among the different farms (Simon *et al.* 2009). While phylogeographic breaks on the South African coast could influence natural dispersal of *B. proboscidea*, the anthropogenic movement of infested abalone among farms has now created point sources for the species in three different biogeographic regions. As a result, *B. proboscidea* has an opportunity to colonize a large segment of the South African coast more rapidly and extensively than it would have from a restricted point source. The original source population of the species is unknown though genetic studies found that farmed populations of *B. proboscidea* from South Africa shared a single haplotype with those from North America (Simon *et al.* 2009). In addition, populations on the south coast had the highest haplotype diversity (Simon *et al.* 2009), which was most likely the result of multiple introductory events considering that the probable timeline since *B. proboscidea* 's first introduction would have been too short for new haplotypes to arise by mutations. This high diversity also indicates that it may be the oldest and largest population in the region (Simon *et al.* 2009). In 2011, *B. proboscidea* was

recorded in high densities in sediment at the outflow path of an abalone farm in Gansbaai, located in the southern part of the country- the first record of a wild population (Simon and van Niekerk, unpubl data). This means that its movement into the wild has begun and its establishment as a potentially invasive species in South Africa is imminent.

The short and long term effects of *B. proboscidea* on marine communities in South Africa, should it become established in the wild is difficult to predict since marine ecosystems are dynamic. However, field surveys conducted in areas where the worm has been introduced may offer some insights into its potential impacts in South Africa. In Argentina, *B. proboscidea* was first detected in 2008 in the city of Mar del Plata near an area of sewage outfall (Jaubet *et al.* 2011). These high nutrient conditions resulted in rapid proliferation of the worm (656250 ind.m^{-2}) that facilitated the formation of large biogenic reefs in the impacted area. Five years after the detection of these reefs, worm density has more than doubled (approximately 1.4 to 2.3 million ind.m^{-2}) and the reefs have now displaced every other native intertidal invertebrate in the area, including important ecosystem engineering mussels (Garaffo *et al.* 2012; Jaubet *et al.* 2013). In Australia, *B. proboscidea* was also found at multiple sites subjected to high nutrient discharge but their densities were considerably lower (350000 ind.m^{-2}) (Petch 1989). While the worms were capable of consolidating sediment to form thick layers of “tube mats” (Dorsey 1982), biogenic reefs have never appeared and the species is not considered a serious threat to marine communities in Australia (Hayes *et al.* 2005; Walker 2009). In South Africa, the outflow path of shellfish farms (particularly abalone farms) is also subjected to high nutrient conditions and thus may be prime environment for the proliferation of *B. proboscidea*.

8. Study rationale and objectives

The overarching aim of this study was to predict the establishment, dispersal and range expansion potential of *B. proboscidea* in South Africa through an integrated approach that combines larval developmental studies with population genetics. This unique framework evaluates larval behaviour and ecology and their response to environmental variables, specifically temperature within the context of the dynamic coastal biome of southern Africa. Since *B. proboscidea* is in the incipient stages of a potential invasion, I used an additional "predictor species" as a means to ground truth in my predictions. This study is the first to use such an approach to predict the spread of a recent invader. While there are many characteristics that define a suitable predictor species, some important ones include a close relationship (taxonomically) with the problem species, the predictor must also be well-established in the recipient environment and, most importantly, share a similar reproductive strategy to the problem species. *Boccardia proboscidea* exhibits poecilogonous development where it produces both planktotrophic and adelphophagic larvae in the same egg capsule (Gibson 1997). In order to predict *B. proboscidea*'s spread I originally intended to use two polydorid predictor species: *Dipolydora capensis,* which is a native pest of abalone that produces planktotrophic larvae (Simon 2011) and *Polydora hoplura*, which is a non-indigenous pest of oysters and abalone that was thought to produce only adelphophagic larvae (Wilson 1928; Blake and Arnofsky 1999). I chose these two species since they were both well-established aquaculture pests in South Africa (Simon 2011; Boonzaaier *et al.* 2014), and each species exhibits one of the two larval developmental modes exhibited by *B. proboscidea*. However, surprising preliminary laboratory observations on *P.hoplura* found that some females were producing planktotrophic larvae while others were producing adelphophagic larvae. This indicated either the presence of sibling species or

a case of poecilogonous development. In either instance, *P. hoplura* could still be used as a predictor since both reproductive strategies are expressed in the species or complex.

Polydora hoplura was first recorded in South Africa in the 1940s and, like *B. proboscidea,* it was first recorded in the Western Cape Province (Millard 1952). It is therefore possible that *P. hoplura* could have dispersed naturally along the southern African coast prior to the establishment of the country's commercial aquaculture industry 20 years later. Since then, it has been suggested that the movement of oysters may have facilitated the spread of the species (Haupt *et al.* 2010a; Simon 2011). *Polydora hoplura* is now distributed as far west as Saldanha Bay and as far east as Haga Haga. Interestingly, the worm was found infesting oysters on an onshore oyster farm in Kleinzee (~600 km north of Saldanha Bay) though it has never been recorded in the wild in that region (Simon 2011). Both *P. hoplura* and *B. proboscidea* are now the two most pestiferous species of shellfish in South Africa (Simon *et al.* 2006; Simon and Booth 2007).

Based on the similarity of *B. proboscidea* and *P. hoplura* in terms of their non-indigenous nature, their status as aquaculture pests and the fact that they are both *Polydora*-type spionids (Blake 2006), it appeared that *P. hoplura* alone could be a suitable candidate for predicting the spread of *B. proboscidea*. However, since preliminary laboratory studies found that the reproductive strategy of the species in South Africa is different from conspecifics in other parts of the world, I first needed to elucidate its reproductive biology. Therefore, this project was divided into four objectives:

1. In Chapter 2, I determined whether the different females of *P. hoplura* producing different larval types were sibling species or whether it represented a true case of poecilogony (i.e. the same species). Moreover, I also developed a new laboratory culture protocol for polydorid worms and used it to re-describe reproduction and development in *P.hoplura.*

2. In Chapter 3, I used the culture protocol developed in Chapter 2 to investigate the effect of temperature on larval development of *P. hoplura* and *B. proboscidea*. In particular I evaluated brood size, larval survival, larval developmental time and developmental rate under temperatures representative of those found on the South African coast. I then compared the results for the predictor species with its actual distribution and used this information to determine which sites along the coast could support viable populations of *B. proboscidea*.

3. In Chapter 4, I used mitochondrial and nuclear DNA markers to determine the population structure of *P. hoplura,* which would aid in elucidating the dispersal potential of the closely related, *B. proboscidea.*

4. Finally in Chapter 5, I presented a synthesis of *B. proboscidea* dispersal potential in South Africa.

CHAPTER 2

Poecilogony in *Polydora hoplura* (Polychaeta: Spionidae) from commercially important molluscs in South Africa.

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1. Introduction

Developmental strategies play a crucial role in shaping the demographic patterns of sessile species in the marine environment. It can influence larval recruitment and dispersal, which can in turn influence gene flow and population divergences, leading to speciation (Jablonski and Lutz 1983; Levin *et al.* 1991; Palumbi 1994; Cowen and Sponaugle 2009). Developmental modes in marine invertebrates have traditionally been classified based on both trophic and dispersal mode. In general, species that produce actively feeding larvae that spend an extended time in the water column are categorized as planktotrophic and are derived from small eggs that are nutritionally poor. Alternatively, lecithotrophic larvae proceed from larger eggs that are maternally provisioned and are usually nutritionally rich and consequently spend relatively less time in the water column compared to planktotrophic larvae (Thorson 1950; Vance 1973). Some marine invertebrates also exhibit adelphophagy, where females produce nurse eggs, which the developing larva feed on. The provisioning of this extra-embryonic nutrition allows the larva to develop to an advanced stage and settle soon after hatching. This type of development is considered a variation of lecithotrophy without an increase in egg size since the yolk is simply packaged externally rather than into normally developing oocytes (Radashevsky 1994; Blake and Arnofsky 1999).

The advantages and disadvantages associated with the different developmental modes can be linked to their dispersal capabilities (Levin 2006, and references therein). Planktotrophy can facilitate range expansion since larvae spend a relatively long time in the water column and hence can disperse beyond its natal habitat. Additionally, movement and settlement away from the parent means avoidance of competition between parent and offspring for resources (Pechenik 1999). However, larvae would

most likely suffer high mortality in the plankton due to predation or the inability to encounter a suitable substratum for metamorphosis (Mileikovsky 1971; Palmer and Strathmann 1981). In lecithotrophic development, the larvae spend a shorter time in the plankton and therefore have a higher chance of survival compared to planktotrophic larvae (Thorson 1950). This type of development can be advantageous in unstable environments, such as those where strong current systems can carry larvae away from the natal population to sites that are not conducive towards settlement (Wray and Raff 1991).

Spioniform polychaetes exhibit all of the aforementioned modes of development and some are also capable of producing more than one type of larva, the so-called "poecilogonous" worms (Giard 1905). Poecilogony was previously considered to be a widespread phenomenon among marine invertebrates until Hoagland and Robertson (1988) demonstrated that most of the species that were reported as poecilogonous actually consisted of sibling species. Poecilogony has been confirmed in only six spionid worms thus far: *Pygospio elegans, Streblospio benedicti* and four members of the *Polydora*-complex, *Boccardia polybranchia, Boccardia proboscidea*, *Polydora cornuta*, and *Polydora* cf. *websteri* (recently found infesting local farmed oyster, *Crassostrea gigas*) (Rasmussen 1973; Levin 1984; Gibson 1997; McKay and Gibson 1999; Schulze *et al.* 2000; Duchene 2000 ; Simon and Williams unpubl data). In general, individuals of a species can produce broods with only one specific type of larva while some individuals may produce broods consisting of both larval types but these broods tend to be "fixed" in a female. This can be considered as an individual-specific polymorphism (ISP) since individuals are unable to switch between brood types (Knott and McHugh 2012). In contrast, if individuals of a species are capable of switching brood type as a result of

some external trigger, it can be considered as an adaptive polyphenism (AP) (Knott and McHugh 2012). However, these categories should be utilized with caution; the inability to demonstrate that an individual can switch from one developmental mode to another does not necessarily mean it cannot switch but that the appropriate trigger has not yet been identified.

Of all the poecilogonous spionids described thus far, only *P. cornuta* has demonstrated adaptive polyphenism (Rice and Rice 2009). In this species, the percentage of fertilized eggs decreases as sperm becomes limited and there is a gradual transition to adelphophagic development, which is accompanied by an increase in larval size at hatching (Rice and Rice 2009). The other five species possess individuals that produce one specific type of brood but different individuals producing different types of broods can occur sympatrically. More recently, *Polydora* cf. *websteri* was found to produce mixed broods consisting of both planktotrophic and adelphophagic larvae (Simon unpubl data). However mixed broods in this species have only been found in South Africa while it is known to only produce planktotrophic larvae in other parts of world where its reproduction has been reported (Simon unpubl data).

Individual-specific polymorphisms can also be highly variable. For example, in *B. proboscidea*, individual worms are capable of producing either broods of only planktotrophic larvae (type I), broods with mainly adelphophagic larvae along with nurse eggs (type II), and mixed broods of planktotrophic and adelphophagic larvae and nurse eggs (type III) (Gibson 1997). In type III broods, both types of larvae occur in the same egg capsule and the larger adelphophagic larvae are capable of cannibalizing their smaller planktotrophic siblings (Gibson 1997). In *B. polybranchia*, both adelphophagic

and planktotrophic larvae are also found in the same egg capsule but the planktotrophic larvae form eight days after the appearance of adelphophagic larvae (Duchêne 2000).

The pest polychaete, *Polydora hoplura,* is a primary borer of commercial abalone and oysters in South Africa and was believed to be introduced to the region (Mead *et al.* 2011; Simon 2011). The species has also been found boring into shellfish in many regions, including Europe, New Zealand and Australia (Walker 2011, and references therein). *Polydora hoplura* was first recorded producing adelphophagic larvae in southwest England, where it was associated with oysters (Wilson 1928). This developmental mode was later confirmed in specimens from New Zealand (Read 1975) and from onshore culture facilities in South Africa (CA Simon pers obs). However, in November 2012, specimens collected from oysters from offshore culture facilities in Saldanha Bay, South Africa, were found producing mainly broods of planktotrophic larvae alongside broods of adelphophagic larvae in the same shell. Based on these preliminary observations, it was hypothesized that *P. hoplura* may consist of sibling species (Hoagland and Robertson 1988) or, alternatively, represent a true case of poecilogony. The purpose of this study was therefore to elucidate the reproductive biology of *P. hoplura* by (1) evaluating whether worms producing different types of larvae genetically conform to a single taxonomic unit, and (2) describing and comparing the brood structure and larval development of the two reproductive morphs.

2. Materials and Methods

2.1 Specimen collection and culture protocols

Thirty farmed specimens of the oyster, *Crassostrea gigas,* were obtained from Saldanha Bay, South Africa in November 2012. Specimens were transported to the laboratory and

placed in an aquarium in a climate control room with artificial seawater (Seachem Georgia, USA). Pilot experiments found that females consistently produced broods at a salinity of 33 and a temperature of 21ºC and worms were also easily reared under these conditions. As such these conditions were used for the reproductive experiments. A 12h light: 12h dark photoperiod was chosen as it corresponds to neutral daylength (Fong and Pearse 1992). . Shells were carefully broken with pliers and examined for worms and egg strings. Any egg strings found with accompanying females were isolated and observed under a dissecting microscope (Leica MZ75 Heerbrugg, Switzerland) and typed as either “planktotrophic” or “adelphophagic” based on the presence or absence of nurse eggs. Egg strings were ruptured and individual eggs in one capsule counted. Fecundity was calculated by multiplying the number of eggs per capsule by the number of egg capsules per string. Worms that were not brooding were isolated and then fitted into 1.2–1.5 mm open-ended diameter glass capillary tubes (Hirschmann Laborgerate Eberstadt, Germany). The capillary tubes were shaved off to match the length of the worm’s body. Capillary tubes were placed in 6 cm diameter, 1.5 cm deep petri dishes with seawater (one tube per dish) and worms were fed a mixed diet of ground fish feed (Tetramin Melle, Germany) suspended in seawater (1 ml suspension) and 1 ml of algae cultured in the laboratory (*Isochrysis galbana* and *Nitzschia closterium*)- stock cultures obtained from the Department of Agriculture, Forestry and Fisheries, South Africa. Worms were unpaired and only data obtained from the first brood produced were used in this study. Water was changed and worms were fed every two days with diet alternating between Tetramin fish feed and algae at each feeding period. The environmental conditions were as described above.

2.2. Genetic studies

To test the hypothesis that the different reproductive morphs may represent cryptic speciation, a molecular analysis was carried out on both morphs. Worms that produced planktotrophic and adelphophagic larvae were stored in 99% EtOH. Genomic DNA of 15 females that produced adelphophagic larvae and 15 females that produced planktotrophic larvae was extracted using a tissue extraction kit (Macherey-Nagel Germany), according to the manufacturer's instructions. A section of the mitochondrial Cyt *b* gene (~500-bp fragment) was amplified using polymerase chain reaction (PCR) and the primers of Boore and Brown (2000): Cyt *b* 424F' and Oyarzun *et al.* (2011): Cyt *b* 876R with cycling conditions: initial denaturation 95°C, 5 min; 40 cycles of 95°C for 30 s, annealing 48°C, 30 s, extension 72°C, 30 s; final extension 72°C, 10 min. In addition, a fragment of the nuclear gene that codes for the alpha subunit of the ATP synthetase nuclear-encoded protein complex (*ATPSα*) was amplified via PCR using the primers of Jarman *et al.* (2002): AtpSaF'and AtpSaR' with cycling conditions: initial denaturation 95°C, 5 min; 35 cycles of 95°C for 30 s, annealing 60°C, 30 s, extension 72°C, 30 s; final extension 72°C, 10 min. It should be noted that the primers used to amplify *ATPSα* flank a single intron in the gene and therefore bp-length can be variable (~140 bp – ~550 bp) depending on presence or absence of the intron in the species (Jarman *et al.* 2002). All PCR products were verified by 1% agarose gel electrophoresis and gel fragments were excised and purified using a gel extraction kit (Bioflux Tokyo, Japan). Purified PCR products for the Cyt *b* marker were sequenced with the forward primer (Cyt *b* 424F') and a species- specific internal forward primer (AtpINTF) was designed and used to sequence *ATPSα* PCR products. Products were sequenced using BigDye chemistry (ABI, Foster City, CA) and analyzed on an Applied Biosystems 3100 genetic analyser by the Central Analytical Facility at Stellenbosch University. Cyt *b* and *ATPSα*

sequences were verified using the BLASTN tool in GenBank and Cyt *b* sequences were translated to amino acids to ensure gene functionality. All sequences were deposited into GenBank (Accession nos: KF482868-KF482897).

2.3 Larval development

Worms in culture were monitored from oviposition, which was designated as Day 1 of development. The number of eggs per capsule in each brood was counted, without disturbing the brooding female, and fecundity was calculated by the number of eggs per capsules multiplied by the number of capsules per string. In broods of planktotrophic larvae, the 3-chaetiger larvae were separated by brood upon release transferred to glass finger bowls and at this point was fed only algae. Water was changed every two days to prevent rapid proliferation of protozoans; in spite of this about 50% mortality still occurred. A screen was constructed with fine wire meshing (200- µm pore size) and used for regular water changes. There was an observable reduction in ciliate contamination during each water change; however, some ciliates remained attached to the provisional chaetae of early stage larvae. Oyster shells were broken into smaller fragments, sterilized by heating at 50ºC for 10-15 min and then placed at the bottom of the finger bowls as a metamorphic cue for competent larvae. Larval growth was tracked based on the addition of segments and melanophores. In broods of adelphophagic larvae, larval development was monitored throughout the intracapsular stage, without removing broods from the maternal burrow. Egg diameter and larval sizes were determined using a dissecting scope (Leica L2, Switzerland) with a camera attachment (Leica EC3, Switzerland) and the Leica Application Suite measurement software (Leica Microsystems Ltd., Switzerland). For both developmental modes, developmental time is defined as the time elapsed between oviposition and settlement of the advanced larvae.

Additional data collected included egg diameter in µm, the number of nurse eggs in broods of adelphophagic larvae, size of larvae at hatching in µm and size of larvae at settlement in µm.

2.4 Data analysis

Mitochondrial and nuclear DNA sequences were manually edited in BIOEDIT (Hall 1999) and aligned using the CLUSTAL W alignment tool (Thompson *et al.* 1994). A parsimony network was constructed using TCS ver. 1.21 (Clement *et al.* 2000) with a connection limit of 95%. The variation between and within reproductive morphs was determined using an AMOVA in ARLEQUIN ver. 3.5 (Excoffier and Lischer 2010).

A Kolmogorov-Smirnov test indicated that data on brood traits were not normally distributed and hence the non-parametric Spearman's Rank Correlation and Mann-Whitney U test were used to compare the brood traits and developmental time of the two reproductive morphs. A size comparison of 3-chaetiger larvae that emerged from broods of adelphophagic larvae (referred from here on as intermediate-stage larvae) along with their advanced adelphophagic siblings and larvae from broods of planktotrophic larvae were carried out using a Kruskal-Wallis H test. An Independent Samples Kruskal-Wallis H Test was used for a pairwise comparison among these different larval types to determine where significant differences lay. A Kruskal-Wallis H test was also used to determine if there were significant differences in the size at settlement of the different larval types. All statistical analyses were completed using SPSS ver.20.0 (IBM Corp. 2011).

3. Results

3.1. Genetic comparisons of reproductive types

All 30 individuals were successfully amplified for the Cyt *b* marker and 11 individuals (six females producing adelphopahgic larvae and 5 females producing planktotrophic larvae) for the *ATPSα* marker. After editing sequences, a 375-bp fragment for Cyt b and a 225-bp fragment for *ATPSα* remained for analysis. A total of 21 haplotypes was recovered for the Cyt *b* marker. AMOVA results for Cyt *b* (Table 2.1) attributed 95.8% of the variation to differences within morphs and 4.2% of the variation to differences between morphs. A parsimony network showed no genetic differentiation with both reproductive morphs sharing haplotypes (Figure 2.1). There was one disconnected haplotype that was shared by an individual that produced planktotrophic larvae and two individuals that produced adelphophagic larvae. Results from the *ATPα* gene also showed no genetic differentiation (Figure 2.2). The *ATPα* gene yielded a total of five haplotypes of which three were unique and two were shared between the individuals of both reproductive morphs.

Table 2.1: AMOVA results for *Polydora hoplura* reproductive morphs based on Cyt *b* sequences*

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among morphs	1	8.933	0.23540 V_A	4.18
Within morphs	28	151.267	5.40238 V_B	95.82
Total	29	160.200	5.63778	

*significance: among morphs: $P = 0.27$, within morphs: $P = 0.002$

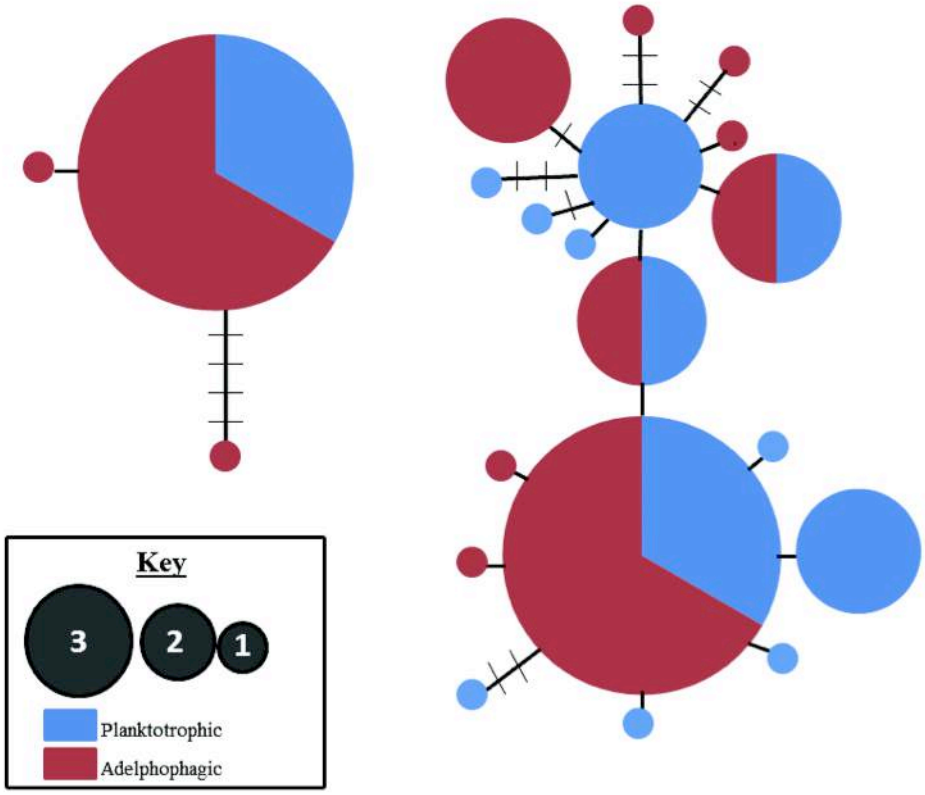

Figure 2.1: Haplotype network of *Polydora hoplura* reproductive morphs. Network based on mtDNA marker Cyt *b* representing 30 individuals, 21 haplotypes: 14 unique, 4 shared by 2 individuals each and 2 shared by 3 individuals each. Each connecting line represents one mutational step with each perpendicular line representing an additional mutational change. Size of circles proportionate to the number of indivudals represented.

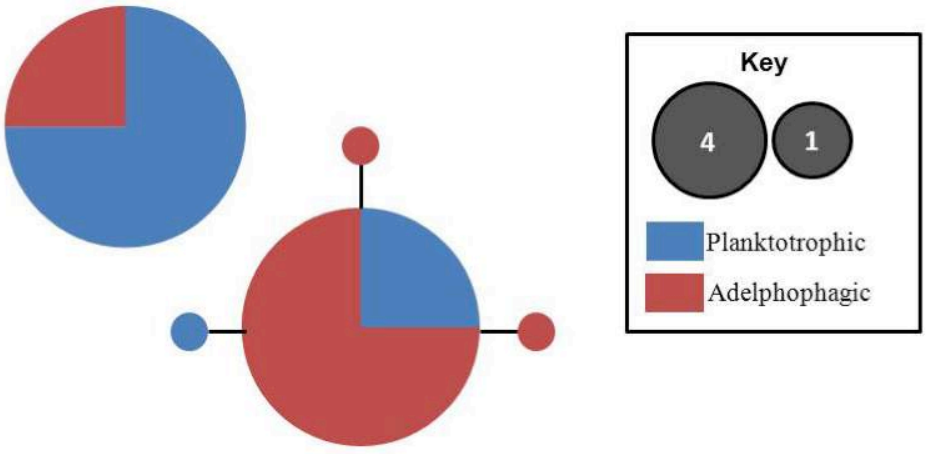

Figure 2.2: Haplotype network of *Polydora hoplura* reproductive morphs. Network based on nDNA marker, *ATPSα* representing 11 individuals, 5 haplotypes: 3 unique and 2 shared by 4 individuals each. Each connecting line represents one mutational step. Size of circles proportionate to number of individuals represented

3.2. Brood structure

Females that produced planktotrophic larvae ranged from 47–127 chaetigers in length and produced 14–50 egg capsules per string, while females that produced adelphophagic larvae ranged from 48–120 chaetigers and produced 14–44 egg capsules per string. Females of each developmental mode showed a strong positive correlation between length (number of chaetigers) and the number of egg capsules produced (Figure 2.3). All eggs from broods of planktotrophic larvae hatched, while less than 5% of the eggs from broods of adelphophagic larvae hatched, with most of the eggs being consumed by developing larvae. Table 2.2 summarizes the brood characteristics of both reproductive morphs. Broods with adelphophagic larvae had significantly larger eggs (Mann-Whitney U test, U = 0, P <0.001) and were significantly fewer than eggs from broods of planktotrophic larvae (Mann-Whitney U test, U = 167.5, P <0.01). Broods of adelphophagic larvae also had significantly fewer larvae compared to broods of planktotrophic larvae (Mann-Whitney U test, U = 0, P <0.001). Advanced adelphophagic larvae were more than twice the size of the intermediate stage larvae and the planktotrophic larvae at hatching whereas the intermediate stage larvae were significantly larger at hatching than planktotrophs (Kruskal-Wallis test, H_2 = 82, P <0.01). However, there were no significant differences in the size at settlement among the three larval types (Kruskal-Wallis test, H_2 = 0.3, P = 0.86). Females of both morphs produced up to three consecutive broods over a two-month period and no single female was observed to “switch” between modes in this study.

Females deposited egg strings, which were attached to the inside of its tube via double filaments. The egg string was continuous and divided into capsules, which were separated by a thin layer with 1–2 filaments per capsule. In broods of planktotrophic

larvae, females deposited eggs evenly into each capsule, all of which developed into larvae that were restricted to individual capsules. In broods of adelphophagic larvae, some females deposited fertilized and nurse eggs in an uneven arrangement, where six to eight adjoining capsules only contained nurse eggs. Additionally, larger adelphophagic larvae were capable of moving between capsules after exhausting nurse eggs in their own capsules.

Table 2.2: Brood characteristics of planktotrophic and adephophagic morphs of *Polydora hoplura*. Values represent mean and standard deviation with *N* = number of broods, ad = advanced larvae (16-18 chaetigers), i = intermediate larvae (3 chaetigers).

Brood traits	Reproductive morphs		
	Planktotrophic	Adelphophagic	
Egg diameter (µm)	70.9 ± 1 *N*= 30	127.4 ± 6 *N*= 27	
# Eggs/brood	1564.4 ± 637.9 *N*= 30	1144.1 ± 395 *N*= 27	
#Larvae/brood	1544.3 ± 645.9 *N*= 30	19.9 ± 10.5 *N*= 27	
Size at hatching (µm)	210 ± 20.3 *N*= 30	567.7 ± 184.7 (ad) *N*= 20	271.3 ± 22.6 (i) *N*= 3
Size at settlement (µm)	1046.6 ± 40.8 *N* = 16	1040.1 ± 37.2 (ad) *N* = 11	1043 ± 92.8 (i) *N* = 3

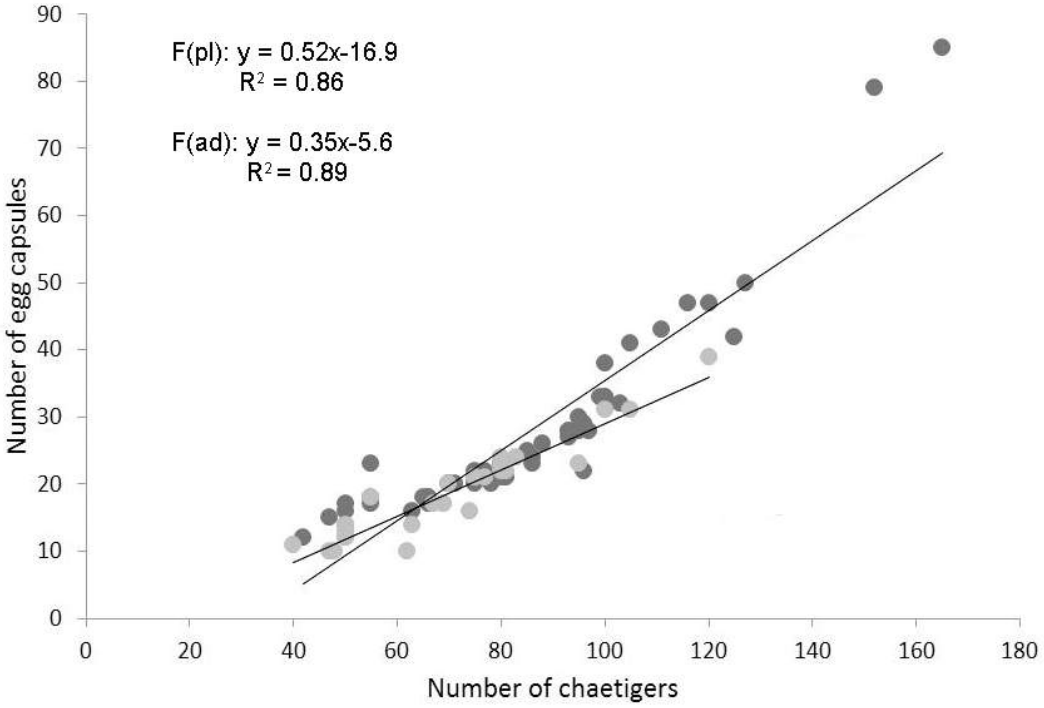

Figure 2.3: Correlation analysis (Spearman rank correlation) for number of chaetigers and number of egg capsules string $^{-1}$ in broods of planktotrophic larvae (dark circles) and adelphophagic larvae (light circles) of *Polydora hoplura*. Each data point represents a single brood produced by a female. F(pl): females producing planktotrophic larvae (r_s= 0.96, *N* = 50 broods, p<0.05); F(ad): females producing adelphophagic larvae (r_s= 0.95, *N* = 27 broods, p<0.05).

3.3. Planktotrophic development

Females deposited egg strings containing eggs that were round, and white to pale yellow (Day 1), which began dividing within 48 h of oviposition. Achaetigerous larvae with their developing prototrochs and distinct eyespots, became visible two days later. Day 8 was characterized by development of the intracapsular 3-chaetiger stage where the mouth was well developed and four kidney shaped eyespots (two lateral and two medial) had formed. At this point, the egg string was crowded with 3-chaetiger larvae that had developed ciliary bands and swimming chaetae. The brooding female ruptured the egg string by first tearing at the capsule with her mouth, pushing her head through one end of the string and moving through the entire length of the string; strong

contractions by the female's body funneled the larvae out at both ends of the tube. Upon release, planktotrophic larvae swam rapidly throughout the water column feeding in different patches of algae, but slowed down at later stages possibly due to a reduction in swimming chaetae accompanied by an increase in body size.

At hatching, 3-chaetiger larvae possessed swimming chaetae that were long and slender with fine serrations and extend past the posterior region of the body (Figure 2.4A). In its planktonic state (Day 11), the 3-chaetiger larvae actively fed in the water column and had a single row of melanophores accompanied by a single row of nototrochs (individual cilia arranged horizontally below the melanophores) on the dorsal region of the body (Figure 2.4B). On Day 17, some larvae had added a chaetiger, and by Day 24, most larvae had 5–7 chaetigers with 3 or 4 rows of melanophores (Figure 2.4C, D). Development to the 8- and 9- chaetiger stages occurred within the next 48 h. On day 32, some larvae had reached the 10 and11- chaetiger stages with 3–5 rows of melanophores present (Figure 2.4E). By Day 38 larvae were approximately 12–14 chaetigers long and had developed rudimentary palps. By Day 41, all surviving planktotrophic larvae were either at the 16- or 18-chaetiger stage with elongated palps (Figure 2.4F). This larval form was competent to settle and had already developed a modified fifth chaetiger with at least two modified spines. The competent larvae still retained early larval features, including four distinct eyespots, ramified melanophores, nototrochs and a reduced telotroch. Developmental time for planktotrophic larvae from oviposition to settlement was 40.2 ± 2.2 days for N = 20 broods.

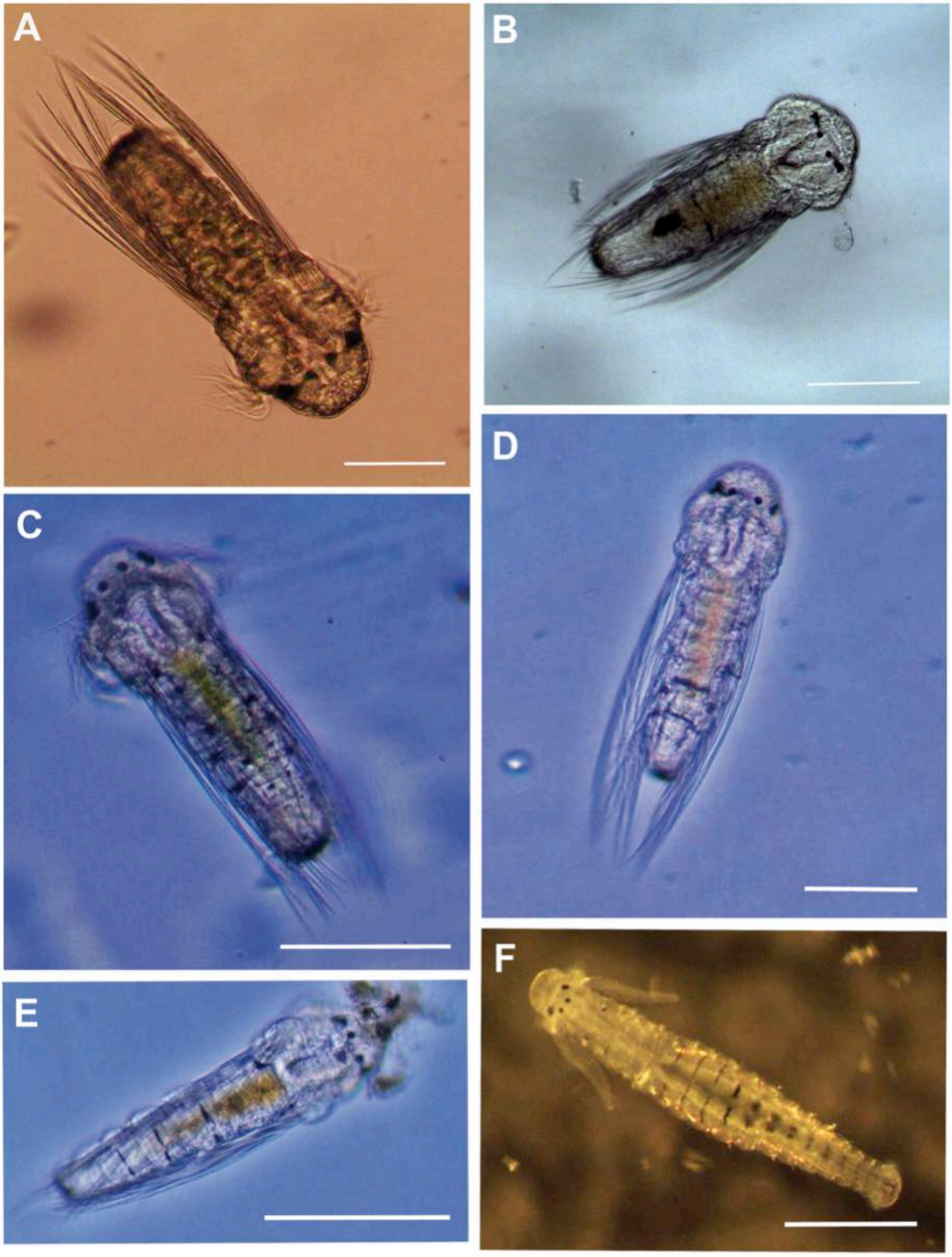

Figure 2.4: Planktotrophic development of *Polydora hoplura*. A. Three chaetiger larvae with long swimming chaetae. B. Later three chaetiger larvae with single row of dorsal melanophores. C, D & E. Intermediate larval stages showing increase in size and addition of ramified melanophores. F. Larvae at settlement with juvenile palps and row of continuous ramified melanophores. Scale bars: A - 50 µm, B - 100 µm, C, D - 150 µm, E - 300 µm, F - 300 µm.

3.4. Adelphophagic development

At 21°C, females deposited egg strings that were divided into capsules in a bead-like structure (Figure 2.5A). Eggs were round, and yellower than those that gave rise to planktotrophic larvae. Egg division became apparent approximately seven days after oviposition with the achaetigerous larvae forming 24 hours later. The 3-chaetiger adelphophagic larva possessed relatively long chaetae but not as long as those of the 3-chaetiger planktotroph. By Day 9 most larvae had already developed a maximum of 4 or 5 chaetigers with 2 rows of melanophores (Figure 2.5B). On Day 10, larvae had reached the 6–8 chaetiger stage, while some specimens (< 20%) had remained at the 3 and 4 chaetiger stages; by this time, ramified melanophores began developing on the posterior segments of the 8-chaetiger larvae. By Day 12, larvae had reached the 12–14 chaetiger stage (Figure 2.5C, D) and more than 80% of nurse eggs had been consumed; rudimentary palps also became visible at this stage. By Day 13, all nurse eggs had been exhausted and the larger adelphophagic larvae were moving between capsules.

All larvae, including those that remained at earlier stages (9–11 chaetigers) evacuated the egg string once the female had ruptured it. Advanced larvae (16–18 chaetigers) spent a maximum of 4 days in the water column before settling on oyster shells (Figure 2.5E). The developmental time for advanced adelphophagic larvae was 16.6 ± 2.4 days for N = 16 broods, which was significantly faster than planktotrophic development (Mann-Whitney U test, U = 0, $P < 0.01$). Broods with intermediate stage larvae (3–5 chaetigers) possessed swimming chaetae that appeared to be shorter than in their planktotrophic counterparts (Figure 2.6). These larvae were larger than 3-chaetiger planktotrophs, and were capable of feeding in a similar manner. The developmental

time for intermediate larvae was approximately 35 days. The presence of yolk in the gut of the intermediate larvae indicated that it may also have fed on nurse eggs though adelphophagia was not directly observed. Only four broods were observed to have both intermediate and advanced adelphophagic larvae and most broods (*N* = 20) consisted of larvae that emerged at an advanced stage (16–18 chaetigers).

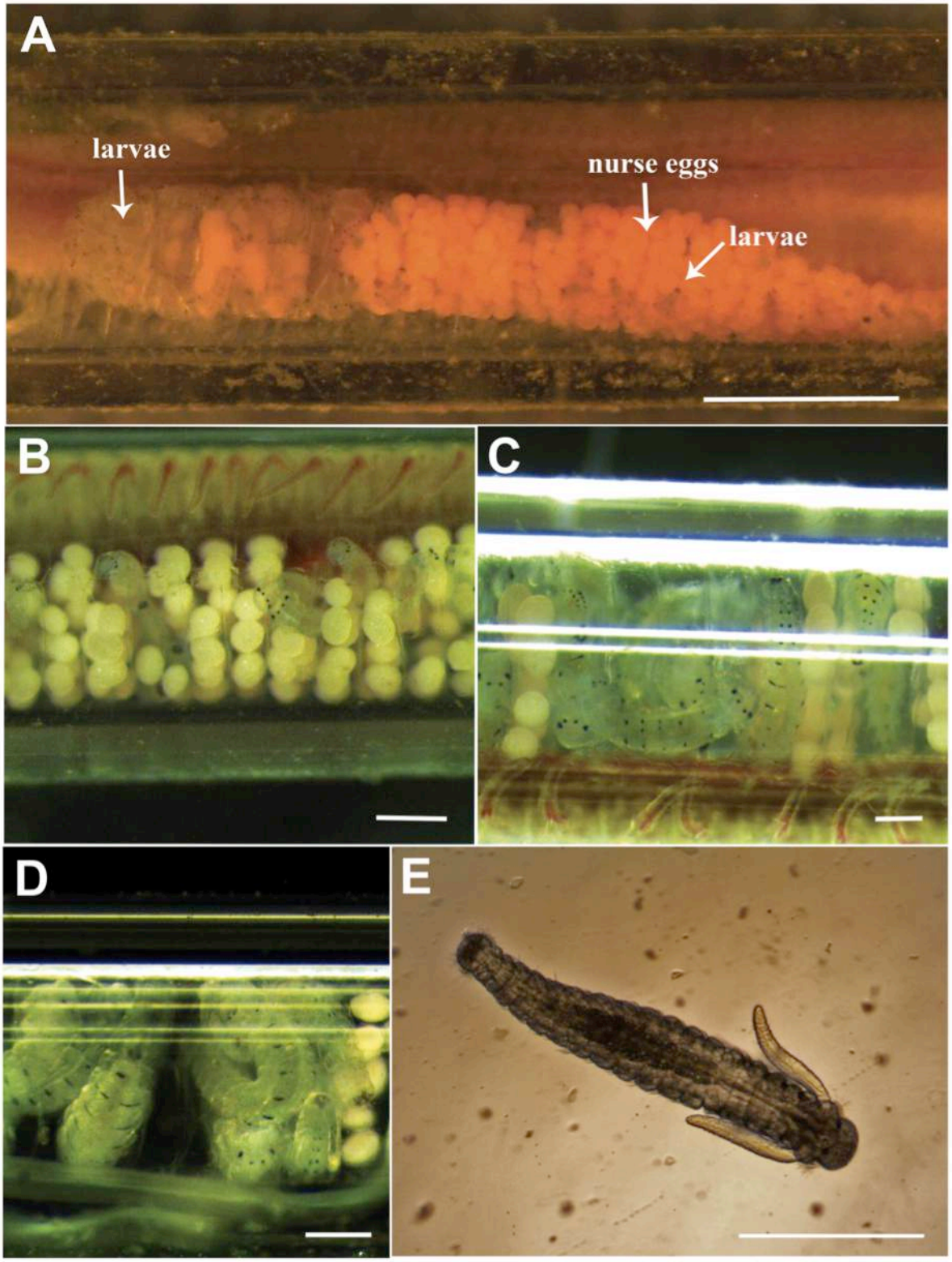

Figure 2.5: Adelphophagic development in *Polydora hoplura*. A. Egg string showing uneven distribution of larvae and nurse eggs. B. Nurse egg consumption by 3–5 chaetiger larvae. C, D. Intermediate larval stages and decrease in nurse egg supply. E. Larvae at settlement stage with juvenile palps approximately 2 days after settling on oyster shells (gut filled with residual yolk from adelphophagy at earlier larval stages.). Scale bars: A - 750 µm, B - 300 µm, C - 150 µm, D - 200 µm, E - 450 µm.

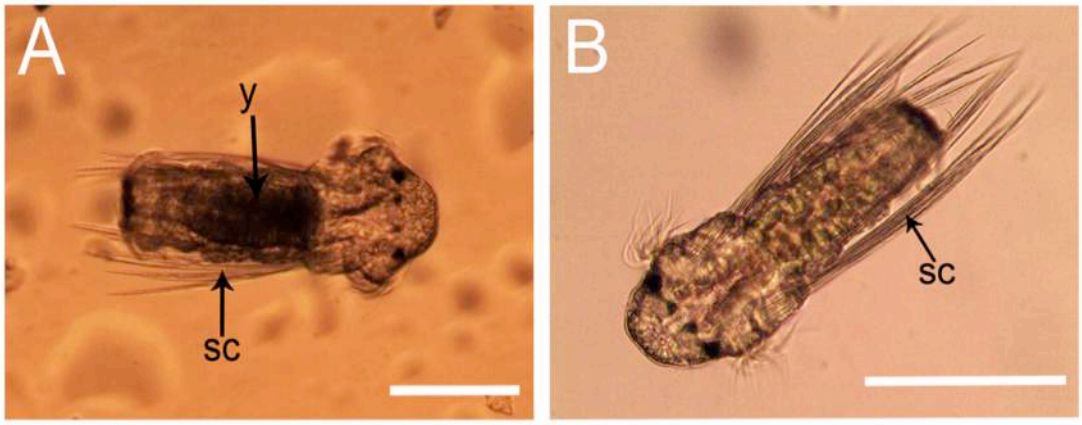

Figure 2.6: Comparison of early larval stages of *Polydora hoplura* reproductive morphs. A. Intermediate stage larvae from broods of adelphophagic larvae (3 chaetigers). B. Planktotrophic 3-chaetiger larvae at hatching. y - yolk from nurse egg ingestion, sc - swimming chaetae. Scale: A, B - 100 µm.

4. Discussion

The purpose of the present study was to determine whether different individuals of *P.hoplura* producing planktotrophic and adephophagic larvae represented sibling species or poecilogony, and to describe the brood structure and larval development of the two reproductive morphs. The results from the molecular study indicate a single, poecilogonous species. The AMOVA results from the mtDNA marker Cyt *b* showed that most of the variation in sequences (> 95%) lay within the morphs rather than between. Although there is no information on the level of variation necessary to constitute sibling species in the Spionidae, the AMOVA results are supported by the parsimony network that showed a large number of different haplotypes (21), and from a taxonomic point of view, also a large amount of haplotype sharing between the two reproductive morphs. A disconnected haplogroup was retrieved which may have represented recently introduced individuals. This isolated haplogroup did in fact connect with the main network but only when the fixed connection limit was adjusted to 25 mutational steps. The mtDNA results were also supported by preliminary results from the nDNA marker

ATPSα which also showed shared haplotypes between the two reproductive morphs. Similar to the mtDNA network, the isolated haplogroup in the nuclear network did connect but only after the fixed connection limit was adjusted to 11 mutational steps. The local oyster industry in South Africa depends on the importation of spat and the movement of oysters from nursery to grow-out facilities. As such, the disconnected haplotypes could have been carried by by worms that arrived with introduced oysters that were part of my study sample. However, the source of these introduced specimens is unknown though further genetic studies could compare mtDNA sequences collected in the present study with *P. hoplura* sequences from other regions of the world where *C. gigas* is regularly farmed to determine potential sources of introduction. In addition to aquaculture, the worms could have arrived via ballast water, as the Saldanha Bay harbor has one of the busiest ports within South Africa (Griffiths *et al.* 2009b). Pertinent to the focus of the present study, these disconnected haplogroups had haplotypes shared between the two reproductive morphs. My finding is broadly congruent with four of five other spionids showing true poecilogonous development (Gibson *et al.* 1999; Schulze *et al.* 2000; Rice *et al.* 2008; Kesaniemi *et al.* 2012). Here, we confirm true poecilogony in a fifth spionid worm using molecular methods, since there is no molecular evidence to suggest cryptic speciation.

The brood structure of *P. hoplura* showed that there was low intrabrood variation in egg sizes within broods of planktotrophic larvae and broods of adelphophagic larvae, but that there was a significant dimorphism in egg size between them. In general, for poecilogonous species that are polymorphic for different larval types, differences in egg size are rare and possibly attributed to constraints on oogenesis (Knott and McHugh 2012). Also, for females that produce adelphophagic larvae, the provisioning of extra

embryonic yolk is thought to be an alternative to increasing the size of eggs (Gibson and Carver 2013). In *P. cornuta,* for example, females produced broods of planktotrophic and adelphophagic larvae that emerge from eggs of similar sizes (McKay and Gibson 1999). One exception is in *B. proboscidea,* where Gibson (1997) found that type I and type II females produced larger eggs than females that deposited mixed broods (type III). Additionally, planktotrophic and adelphophagic larvae from type I and II broods were smaller at hatching compared to their respective counterparts in type III broods.

There was smaller variation observed in the size at hatching in planktotrophs compared to advanced adelphophagic larvae. This may be due to the fact that all planktotrophic larvae are supplied with only a limited amount of yolk that would enable them to reach the 3-chaetiger stage, thus constraining larval size. In contrast, advanced adelphophagic larvae exhibited more variability in the size at hatching. The factors that can drive this variation in adelphophagic broods include sibling competition (Kamel *et al.* 2010), the unequal distribution of nurse eggs in capsules (Collin and Spangler 2012) or variation in temperature, the latter of which has been observed in type III broods of *B. proboscidea* (Oyarzun and Strathmann 2011). In *P. hoplura,* there was an uneven distribution of nurse eggs in some broods and some larvae were capable of moving among capsules to exploit additional nurse eggs. This uneven distribution of nurse eggs most likely facilitated sibling competition, which may be responsible for the observed variation in hatching size. Sibling competition was also suggested as the main reason for the size variability in the adelphophagic larvae of *P. cornuta* and type II broods of *B. proboscidea* (Gibson 1997; McKay and Gibson 1999; Kamel *et al.* 2010). This variation in offspring size can be advantageous in unpredictable environments, where it can act as a bet-hedging strategy; the larger offspring are capable of settling soon after hatching

(betting on increased chances of survival via recruitment to the natal population), whereas the smaller offspring could spend a longer time in the water column which increases its chances for dispersal. Alternatively, adelphophagy may simply be an alternative mechanism to produce large offspring and the production of different sized offspring is simply a “by-product” of this developmental strategy (Collin and Spangler 2012).

In addition to poecilogonous development, which in itself confers ample variability in larval traits, the emergence of intermediate-type adelphophagic larvae in some broods mean that even a monomorphic population that only exhibits adelphophagy can show a high degree of variability in the stage and size at hatching. This intermediate 3-chaetiger larva appears to be morphologically and ecologically similar to its 3-chaetiger planktotrophic counterpart. However, the presence of yolk material in the gut of the intermediate larvae and the absence of it in the planktotrophic larvae suggests that the former may be capable of feeding on nurse eggs. Interestingly, Gibson (1997) found that the 3-chaetiger larvae that suspended development in type III broods of *B. proboscidea* did not exhibit adelphophagy, though it is morphologically equipped for ingesting nurse eggs (Gibson and Carver 2013). The reason behind the absence of adelphophagia in type III larvae of *B. proboscidea* and its presence in the intermediate larvae of *P. hoplura* is unknown and might be species-specific. However, it should be noted that adelphophagia in the intermediate larvae was not directly observed and the yolk material in the gut of these larvae may simply represent left over yolk from the egg. Further studies, incorporating a detailed morphological and histological study along with a larger sample size of broods of adelphophagic larvae, are needed to test this hypothesis.

The faster developmental time in adelphophagic larvae compared to planktotrophic larvae was undoubtedly due to nurse egg provisioning. One hypothesis is that in adelphophagic development, nurse eggs represent a food source that is more consistently available and may be of higher energy content than food planktotrophs would usually encounter in the water column, though no conclusive study that addresses the energy content of nurse eggs has been conducted (but see Moran and McAlister 2009). Additionally, planktotrophic larvae must expend energy swimming during development, whereas with adelphophagic larvae, most of the energy can be directed towards growth since development is mostly intracapsular. The faster developmental time of adelphophagic larvae can be advantageous since it may allow for faster colonization of a suitable substratum. This is especially the case if individuals are capable of producing successive broods within a short period of time, which incidentally is a common trait of polydorid worms (Blake and Arnofsky 1999). .

Despite these novel findings of *P. hoplura*, the mechanism underlying poecilogony in this species is unknown. I did not observe individuals to switch their developmental mode in this study, which would imply that, like *S. benedicti, P. hoplura*'s developmental mode is genetically determined (individual-specific polymorphism). However, the "absence of evidence is not evidence for absence" and there is still a host of environmental factors that can be tested to determine if individuals can switch, e.g. food availability, temperature and photoperiod, to name a few. Sperm limitation experiments also constitute a separate study that can be conducted to determine if sperm depletion causes individuals to switch as has been shown in *P. cornuta* (Rice and Rice 2009). Additionally, Gibson *et al.* (2013) recently found that *P. cornuta* was capable of adjusting its brood structure via an epigenetic response when exposed to folate and bisphenol A.

Hence, there is the possibility that these chemicals may play a role in influencing developmental modes in some poecilogonous species. It is interesting to note that both *P. hoplura* and *P.* cf. *websteri* that are now considered poecilogonous in South Africa are monomorphic for a single reproductive mode in every other region of the world (Wilson 1928; Blake 1969; Blake and Arnofsky 1999). Since oyster culture methods in other countries are broadly similar to those implemented on South African farms, it is unlikely that factors on the farm itself (e.g. water movement and host availability) would drive reproductive shifts in these species. Based on the current study, *P. hoplura*'s developmental mode appears to be genetically determined but I also acknowledge that further work on the species is necessary to support this hypothesis.

In conclusion, poecilogony may have facilitated *P. hoplura*'s establishment on the South African coast and in other areas of the world. The ability to express both developmental modes means that a single population can expand its range through planktotrophy and at the same time maintain a robust population through adelphophagic development. A stable polymorphism may also buffer events such as bottlenecks; if some females that survive are capable of producing planktotrophic larvae, these larvae could disperse away from the population thereby avoiding the depressive costs associated with a small population size (Pechenik 1999). Poecilogony may have also aided *P. hoplura*'s proliferation on oyster farms in South Africa. The reasons for successful proliferation in the wild is amplified on an onshore farm setting since the larvae have a reliable supply of substrata and food and lack predators that they would otherwise have encountered in the wild. Despite the rarity of poecilogony in marine invertebrates (Knott and McHugh 2012), all the major non-indigenous polychaete pests in South Africa now exhibit this mode of reproduction (Simon *et al.* 2010a; current study), which means that it may

serve as a good predictor for identifying potential pests of commercially-important bivalves.

In this chapter, I have shown that *Polydora hoplura* exhibits a similar reproductive strategy (poecilogony) to the problem species *B. proboscidea*. In addition, due to its non-indigenous nature and status as an aquaculture pest, *P. hoplura* should be an appropriate species for predicting the spread of *B. proboscidea* in South Africa.

CHAPTER 3

The effect of temperature on larval development of two non-indigenous poecilogonous polychaetes (Annelida: Spionidae) with implications for life history theory, establishment and range expansion

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1. Introduction

One aspect of invasion biology studies is predicting how a species will spread, which in turn can facilitate surveying efforts to mitigate the damage inflicted by these species (Kolar and Lodge 2001). Evaluating the life history patterns of marine invertebrates, specifically their reproductive strategies, has therefore been useful in understanding their potential to spread to new ranges (Levin 2006, and references therein). Life history patterns, especially developmental strategies, have particular importance to sedentary animals such as bivalves and some polychaetes, where they play a major role in governing the spatial distribution of adults. In general, species that exhibit planktotrophic development where the larvae spend an extended period in the water column would be expected to have higher dispersal capabilities when compared to species that exhibit abbreviated larval development (such as lecithotrophy) (Jablonski and Lutz 1983). Lecithotrophic larvae, for example are directly provisioned with yolk from the mother while adelphophagic larvae are provisioned with yolk in the form of nurse eggs (Jablonski and Lutz 1983; Blake and Arnofsky 1999). As a consequence of this maternal provisioning, the adelphophagic larvae tend to emerge at an advanced stage and settle soon (sometimes within hours) after hatching. In adelphophagic development, there is also the potential for high variability in offspring size because egg size and larval size are uncoupled, which may facilitate sibling competition among larvae (Kamel *et al.* 2010; Collin and Spangler 2012). As such, emerging adelphophagic larvae that are smaller than their larger siblings may have better dispersal capabilities, which in turn could influence dispersal patterns.

Temperature and food availability have long been considered as the most important factors affecting larval development in marine invertebrates (Hoegh-Guldberg and

Pearse 1995). In particular, temperature governs the duration of the planktonic phase of pelagic larvae and, as a consequence, can strongly influence range expansion and biogeographical patterns (O'Conner *et al.* 2007; de Rivera *et al.* 2007). To date, few temperature-dependent developmental studies have been conducted on species that exhibit poecilogonous development:- the rare ability to produce more than one type of larvae (see review by Knott and McHugh 2012). The purpose of this study was to investigate the effect of temperature on larval development of two polychaetes *Polydora hoplura* Claparede 1869 and *Boccardia proboscidea* Hartman 1940 that are polymorphic for planktotrophy and adelphophagy. These results in turn may be useful to aid in predicting the potential establishment and range expansion of the recently introduced polychaete species, *B. proboscidea* in South Africa (SA).

Boccardia proboscidea has a worldwide distribution and has been introduced to many regions via aquaculture and ballast water (Chapter 1; Simon *et al.* 2009). The species was first recorded on an abalone farm in the southern part of the country in 2004 (Hermanus) and, since then, has been found on abalone farms on the western and eastern coasts of the country, but never on wild molluscs (Simon *et al.* 2006, 2009, 2010a; Boonzaaier *et al.* 2014). In 2011, the species was found in high densities at the outflow of an abalone farm in Gansbaai, South Africa (Simon and van Niekerk, unpubl data), highlighting the potential of this species to become established as a potentially invasive species in the region. The establishment and range expansion of *B. proboscidea* are of particular concern since this species has been found to create large biogenic reefs in a sewage impacted area of Argentina where they have displaced other native intertidal fauna (Jaubet *et al.* 2011, 2013).

A major factor contributing to the success of *B. proboscidea* in its introduced range is probably the fact that the species is poecilogonous, i.e. females are capable of producing more than one type of larva (Gibson 1997; Knott and McHugh 2012). The reproductive biology of *B. proboscidea* was first described in detail by Blake and Kudenov (1981), based on Australian populations; the authors found that females produced broods containing both planktotrophic and adelphophagic larvae and nurse eggs in the same egg capsule. Later studies by Gibson (1997) and, most recently, by Jaubet *et al.* (2014), working on North American (California) and South American (Argentina) populations, respectively, found three different types of broods occurring in the same population:- type I broods with only planktotrophic larvae, type II broods with adelphophagic larvae and nurse eggs, and type III broods with planktotrophic and adelphophagic larvae and nurse eggs. In SA, type II broods were misidentified and subsequent observations revealed that only type III broods were present in the region (Simon *et al.* 2010a). In type III broods, larger adelphophagic larvae are capable of feeding on their smaller planktotrophic siblings and or their less competitive adelphophagic siblings (sibling cannibalism), thus the eventual fate of the planktotrophs depends on when the brooding female ruptures her egg capsules (Oyarzun and Strathmann 2011). Sibling cannibalism could therefore influence the dispersal capabilities of this species by influencing the number of dispersive larvae that enters the water column. This degree of flexibility allows a species to capitalize on the advantages that accompany both larval types and have undoubtedly contributed to their success in their introduced range.

South Africa is flanked by the cold waters of the Atlantic Ocean on the west coast, where the north-ward flowing Benguela Current dominates, and the warm waters of the

Indian Ocean on the east coast, where the southwesterly-flowing Agulhas Current dominates.The south coast is considered to be a mixing zone characterized by intermediate temperatures (Shannon 1985; Reason *et al.* 2006; Chapter 1). To date, *B. proboscidea* has been found mainly on onshore abalone farms on both the west and south coast of the country and an abalone farm on the east coast (Chapter 1).

My main goal in this study was to determine whether *B. proboscidea* could successfully produce broods, and how effectively they could complete development under temperatures representative of the SA coast and therefore potentially expand its range beyond its present distribution. For comparison, I also investigated temperature-dependent development in a second, closely related species, *Polydora hoplura*. The latter is non-indigenous to SA (first recorded here in the 1940s), distributed widely along the SA coast, extending as far east as Haga Haga and as far west as Saldanha Bay and is also a pest of farmed shellfish (Millard 1952; Nel *et al.* 1996; Simon *et al.* 2006; Simon 2011). *Polydora hoplura* also exhibits poecilogonous development; however, unlike *B. proboscidea*, a female can only produce one of the two larval types but females of the two reproductive morphs usually occur sympatrically (Chapter 2). By comparing the outcomes of different temperature treatments on the development of these two species, it should be possible to make some inferences as to the effect of temperature on the establishment and range expansion potential of *B. proboscidea*.

2. Materials and Methods

2.1. Distribution of *Polydora hoplura* and *Boccardia proboscidea*

To document the current distribution of *P. hoplura* and *B. proboscidea*, surveys were done in 2012 and 2013 at 12 different sites along the South African coast (Figure 3.1). A

variety of substrata were sampled, including sediment, sponge, coralline algae and different types of molluscs (such as oysters, abalone and limpets). Due to permit limits, a maximum of five abalone and 10 oysters could be sampled per site, but neither mollusc was consistently present at all sites. Substrata were broken using pliers and worms were extracted with tweezers. Substrata were then placed in a vermifuge (0.05% phenol solution), which agitated any remaining worms in their tubes, allowing for easier extraction. Worms were then identified live under a dissecting microscope (Leica MZ75 Heerbrugg, Switzerland).

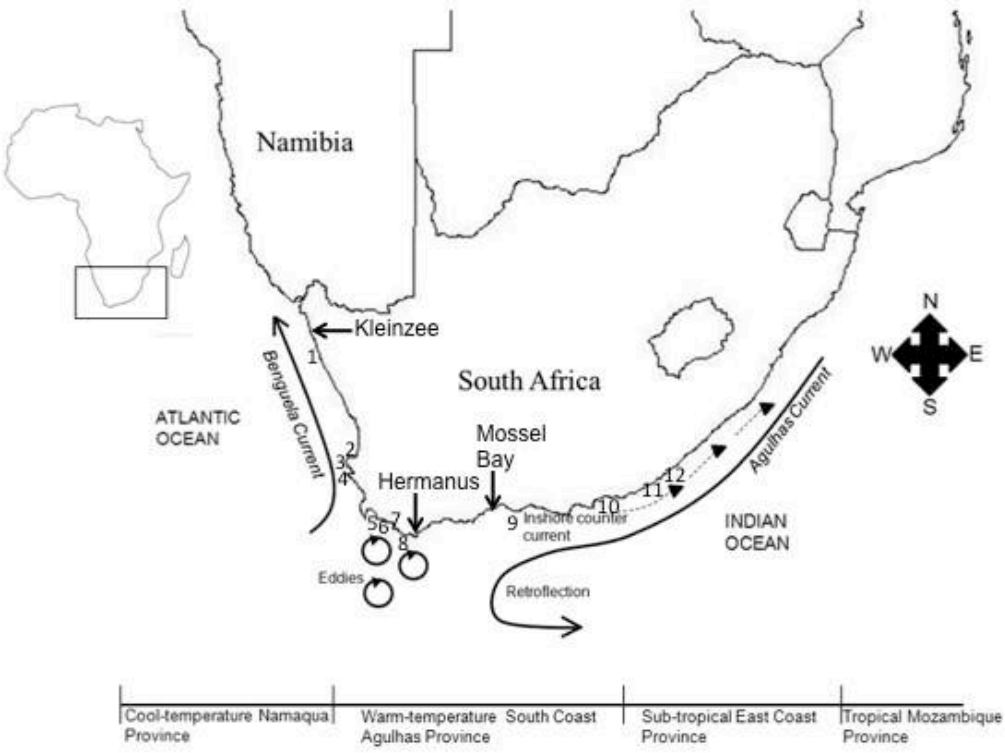

Figure 3.1: Map of South Africa showing sites sampled for *Polydora hoplura* and *Boccardia proboscidea* (numbers) along with major current systems and marine biogeographic regions. 1=Kleinzee, 2=Paternoster, 3=Jacobsbaai, 4=Saldanha Bay, 5=Strand, 6=Bettysbaai, 7=Kleimond, 8=Gansbaai, 9=Knysna, 10=Port Elizabeth, 11=Haga Haga, 12= Glen Gariff.

2.2. Collection and culture protocol

Specimens of *P. hoplura* were collected from farmed oysters (*Crassostrea gigas*) from Saldanha Bay, South Africa in November 2012. Oysters were placed in an aquarium filled with artificial seawater (Seachem, Georgia USA), kept at a temperature of 21°C and a salinity of 33. Oysters were fed a mixture of ground fish feed (Tetramin, Melle Germany) and cultured algae (*Isochrysis galbana* and *Nitzschia closterium*), obtained from the Department of Agriculture Forestry and Fisheries (DAFF), South Africa. After an acclimatization period of 24 hours, some oysters were shucked and shells were broken with pliers to extract worms from their burrows. Worms were identified using the dissecting microscope, fitted into 1.2–1.4 mm diameter glass capillary tubes and placed in a 60 X 15 mm petri dish (one worm/dish) where they were fed every other day. Seawater was changed every two days and Petri dishes were covered to prevented significant evaporation of seawater. Specimens of *B. proboscidea* were collected in February 2012 and August 2013 from sediment in the outflow path of an abalone farm in Gansbaai, South Africa. Worms were transported to the laboratory in plastic containers sealed with parafilm and identified using the dissecting microscope. Worms were then placed in a 21°C aquarium with sediment taken from the worm's habitat. Prior to use, the sediment was sieved using a 500-µm filter, washed with freshwater and frozen overnight to kill any fauna and to remove any dead organic material that could produce unwanted products of decomposition. Pilot experiments found that *B. proboscidea* suffered high mortalities when cultured in 100% artificial seawater. Therefore, filtered seawater was obtained from the abalone farm and adjusted with Seachem sea salts to a salinity of 33 when necessary. All other conditions (feeding, temperature regime, etc) were similar to those outlined above for *P. hoplura*.

2.3. Constant temperature treatments

I obtained sea temperature records for several sites along the South African coast (Figure 3.2). I obtained *in situ* water temperature records from the South African Weather Service (SAWS) for sites on the west (Port Nolloth, Saldanha Bay), southwest (Betty's Bay), the south (Mossel Bay) and the south-east (Port Elizabeth) coasts. Additional *in situ* water temperature records for a second east coast site (Haga Haga) were obtained from records kept by an abalone farm at that site. After reviewing the temperature data, a range of representative temperatures were chosen for the temperature treatments: 12°C, 17°C, 21°C, 24°C, and 28°C (Table 3.1).

A Buchi B-480 (Buchi Labortechnik, Switzerland) water bath was used to culture worms and larvae at 24°C and 28°C and a temperature-adjusted climate control room was used for 12°C, 17°C and 21°C treatments. The initial temperature for all specimens was 21°C and water temperature was increased or decreased by 1°C every 24 hours until the desired temperature was reached to allow ample time for acclimatization.

Worms were monitored until oviposition, which was designated as Day 1 of development. To determine if the different temperatures affected the worms' reproductive output, brood size was evaluated for each temperature treatment for each species. For females of *P. hoplura* that produced broods of adelphophagic larvae, brood size was determined by calculating the number of larvae present throughout the egg string after hatching. For females that produce broods of planktotrophic larvae, all eggs undergo division to become larvae, hence brood size was determined by multiplying the number of eggs per capsule by the number of capsules per string. In *B. proboscidea*, brood size was calculated by determining the mean number of larvae (both

planktotrophic and adelphophagic) per capsule per brood per temperature treatment. It should be noted here that brood sizes between species are not comparable since they were quantified differently. Sibling cannibalism was assessed by tracking the number of planktotrophic larvae (3-8 chaetigers) throughout the intracapsular developmental stage from the beginning to the end of nurse egg feeding and then calculating the percentage survival in a maximum of three capsules per string per brood per temperature treatment. The size of planktotrophic and adelphophagic larvae at hatching was determined by using a calibrated microscope camera attachment (Leica EC3, Switzerland) and the Leica Application Suite measurement software (Leica Microsystems Ltd, Switzerland).

After females ruptured their egg capsules, the planktotrophic and adelphophagic larvae of both species were separated by brood and transferred to glass finger bowls and at this point were fed only algae (*I. galbana* and *N. closterium*). In both *B. proboscidea* and *P. hoplura,* larval development was monitored by tracking the number of chaetigers added until settlement (~16-18 chaetigers). Settlement was induced by placing sieved sand from the worm's habitat or broken oyster shells that were sterilized by heating, (~50°C) at the bottom of the finger bowls. Only data from the first brood produced by a female were used for this observation.

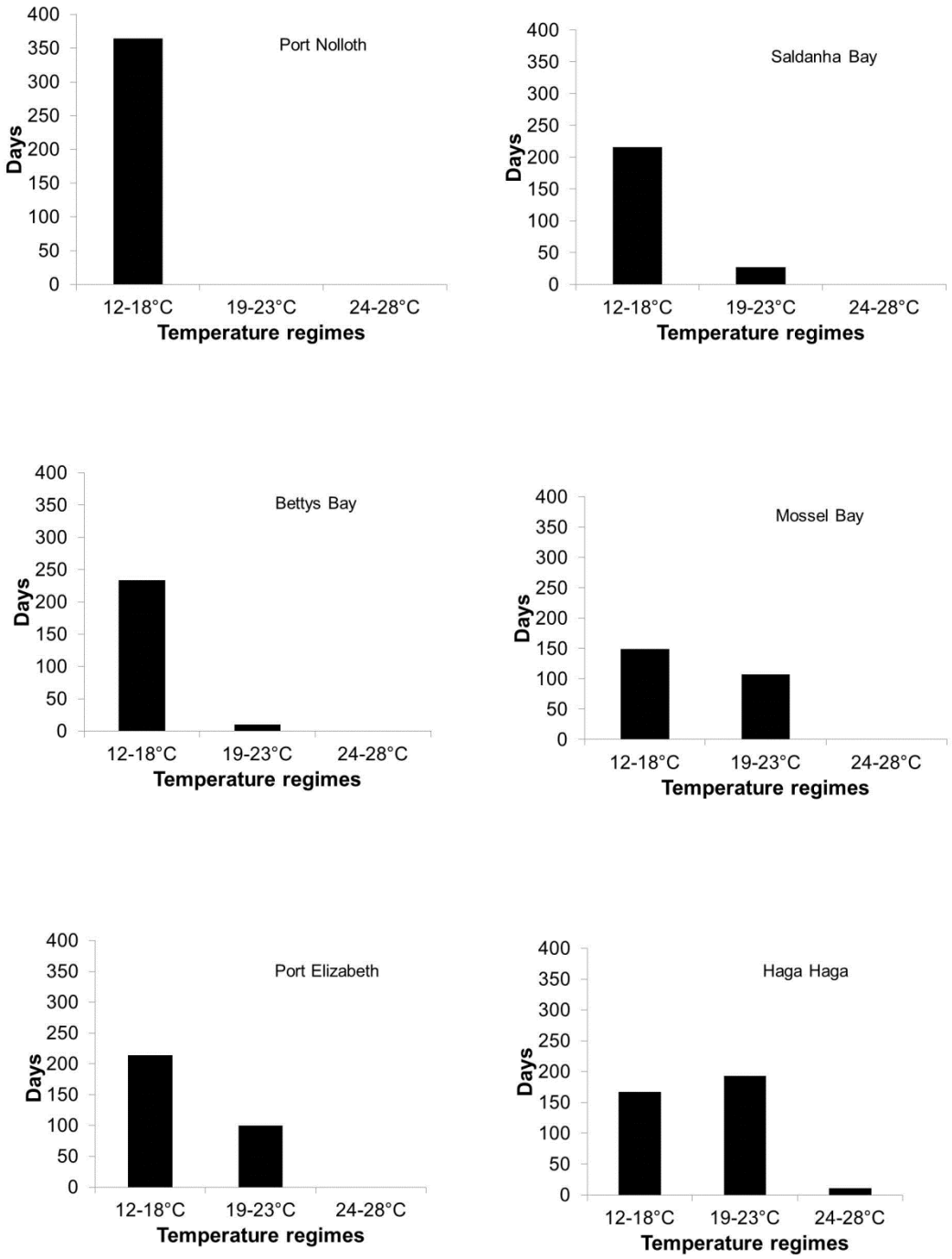

Figure 3.2: Number of days in 2011 with water temperature at different regimes; low (12–18°C, intermediate (21–23°C) and high (24–28°C) at six sites representing west (Port Nolloth, Saldanha Bay), south west (Bettys Bay), south (Mossel Bay), south east (Port Elizabeth) and east (Haga Haga) coasts.

Table 3.1: Number of females of *Polydora hoplura* and *Boccardia proboscidea* cultured at five different temperature treatments and the number of individual larvae followed (one brood followed per female). Number of larvae followed is reflective of the total number of larvae that survived to settlement.

	Polydora hoplura			
Temperature treatment	Number of females		Number of larvae tracked	
	Planktotrophic	Adelphophagic	Planktotrophic	Adelphophagic
12.0 °C	16	14	420	98
17.0 °C	18	15	589	132
21.0 °C	20	16	1515	258
24.0 °C	16	16	1674	274
28.0 °C	19	15	462	171

	Boccardia proboscidea		
Temperature treatment	Number of females	Number of larvae tracked	
		Planktotrophic	Adelphophagic
12.0 °C	19	278	248
17.0 °C	17	276	407
21.0 °C	21	260	799
24.0 °C	21	104	463
28.0 °C	16	17	360

2.4. Data analysis

2.4.1. Brood size and larval size at emergence

A Kolmogorov-Smirnov test indicated that the data for brood size were normally distributed. A one-way ANOVA was used to determine whether different temperatures had a significant influence on brood size for both species. To determine if there were significant differences in the size of both larval types at hatching at each temperature treatment, the Kruskal-Wallis H test was used (since data were not normally distributed).

An independent samples Kruskal-Wallis test was used to determine where significant differences lay.

2.4.2. Larval survivorship

Larval survivorship was assessed as the proportion of larvae from each female at each temperature treatment that survived to settlement. It should be noted that larval survivorship data are based on overall survival and with the exception of sibling cannibalism in *B. proboscidea* I did not distinguish between intracapsular and planktonic mortality. The raw data for larval survivorship were transformed using an arc-sin square root transformation and the transformed data were evaluated using a second order polynomial regression (de Rivera *et al.* 2007) for both larval types of *P. hoplura* and adelphophagic larvae of *B. proboscidea.* A third order polynomial regression was used to evaluate survival of planktotrophic larvae of *B. proboscidea* since it provided a better fit to the data. A one-way ANOVA was used on the transformed values to determine if there were significant differences in larval survival at the different temperature treatments and a Tukey HSD *post hoc* test was used to determine where significant differences lay. Both types of data (raw percentage survivorship and transformed survivorship) are reported. For *B. proboscidea,* a Kruskal-Wallis H test was used to determine whether temperature had a significant effect on sibling cannibalism (i.e. percentage of planktotrophic larvae surviving prior to their liberation from the egg capsule) and an independent-samples Kruskal-Wallis test was used to determine where significant differences lay.

2.4.3. Developmental time and rate

Total developmental time (D_t) was defined as the time elapsed between oviposition and settlement. In order to determine the effect of temperature on developmental rate for both larval types, developmental time was converted to developmental rate ($r = 1/D_t$), which was plotted using a simple linear regression. Brooding time for both species was plotted using a linear regression and R^2 values were calculated for *B. proboscidea* and for females of *P. hoplura* producing planktotrophic and adelphophagic larvae respectively. A one-way ANCOVA was implemented in order to determine if there was a significant difference in developmental rate between larval types. All statistics were completed using SPSS ver. 20.0 (IBM Corp. 2010).

3. Results

3.1. Distribution of *Polydora hoplura* and *Boccardia proboscidea*

Polydora hoplura and *B. proboscidea* were present at six of the 12 sites sampled and of these six sites they were both found at three of the same sites (Table 3.2). *Boccardia proboscidea* was present in very low numbers at most of the sites where the species was found. However, at Gansbaai, at the outflow path of an abalone farm, the worms were recorded in such high numbers that the sediment appeared to be consolidated by the tube building activities of the worm. Although *B. proboscidea* was not recorded in the wild at Haga Haga, it was found in moderate numbers (>25 worms/shell) at the abalone farm near the sampling site.

Table 3.2: Distribution and abundance of *Polydora hoplura* and *Boccardia proboscidea* along with their substrata along the South African coast. Substrata: S - sediment, SP – Sponge (*Haliclona* sp.), C - coralline algae (*Mesophyllum engelhartii*), L – Limpet (*Scutellastra* sp.), O – oyster (*Striostrea margaritacea* and *Saccostrea cucullata*), AB – abalone (*Haliotis midae*), (-) substratum unavailable.

Sites	**Species**											
	Polydora hoplura						***Boccardia proboscidea***					
West Coast	**S**	**SP**	**C**	**L**	**O**	**A**	**S**	**SP**	**C**	**L**	**O**	**A**
Kleinzee[a]	-	-	0	0	-	-	-	-	0	0	-	-
Saldanha Bay	-	16	5	0	-	-	-	0	0	0	-	-
Jacobsbaai	0	15	8	0	-	-	0	3	4	-	-	-
Paternoster	-	17	0	0	-	-	-	1	0	0	-	-
South-west coast												
Strand	-	0	0	-	-	-	-	1	0	-	-	-
Kleimond	-	0	-	0	-	-	-	1	-	0	-	-
Bettysbaai	-	0	6	-	-	28	-	0	16	-	-	0
Gansbaai	0	-	-	-	-	-	>100[*]	-	-	-	-	-
Knysna	0	4	0	0	11	-	-	-	-`	-	-	-
South-east coast												
Port-Elizabeth	-	0	7	-	16	-	-	0	0	-	0	-
East coast												
Glengariff	-	0	0	0	0	0	-	0	0	0	0	0
Haga Haga[b]	0	0	0	0	0	0	0	0	0	0	0	0

[a]*Polydora hoplura* was found infesting farmed oysters near sampling site
[b]both *Polydora hoplura* and *Boccardia proboscidea* were found infesting farmed abalone near sampling sites
[*]specimens sampled at the outflow of an abalone farm

3.2. Effect of temperature on development of *Polydora hoplura*

All females of *P. hoplura* cultured at the different temperature treatments survived and successfully brooded and ruptured their egg strings. During intracapsular development, planktotrophic larvae attained a maximum of three chaetigers, while adelphophagic

larvae attained a maximum of 18 chaetigers. Temperature had no significant effect on the brood size of planktotrophic larvae while there was a significant decrease in brood size of adelphophagic larvae at 28°C (Table 3.3).

Table 3.3: Brood size of *Polydora hoplura* and *Boccardia proboscidea* cultured at five different temperature treatments. P = planktotrophic, A = adelphophagic, C= average number of egg capsules/brood. Values for P represents the mean number of eggs/larvae per brood ± SD, values for A represent the mean number of larvae per brood ± SD and values for type III broods represent the mean number of larvae per capsule per brood ± SD. Values below means represent ranges of individual counts.

Temperature treatment (°C)	***Polydora hoplura***		***Boccardia proboscida***
	P(C)	A	Type III (C)
12	1324.8 ± 235.7 (32.8) 920–1720	21.6 ± 5 12–30	16.5 ± 2 (37.9) 12–20
17	1268.3 ± 228.9 (30.5) 880–1640	23 ± 5 15–30	15.6 ± 2.5 (40) 8–20
21	1256.7 ± 184.2 (30.9) 1040–1800	21.4 ± 1.2 10–21	15.8 ± 2 (37) 12–20
24	1241.9 ± 172.4 (31.8) 924–1600	22.3 ± 5.4 14–30	15.4 ± 1.9 (38.2) 12–19
28	1254.1 ± 213 (30.8) 840–1638	[a]14.2 ± 2.4 9–21	15.2 ± 1.4 (39.5) 14–19
ANOVA	$F_{4,69}$ = 1.9, *P* >0.05	$F_{4,77}$ = 13.5, *P* <0.05	$F_{4,90}$ = 1.1, *P*>0.05

[a]significant, Tukey's HSD *post hoc* test

There was a strong negative relationship between temperature and brooding time for females producing both larval types (Figure 3.3). In females producing adelphophagic larvae, the mean brooding time was longer at 28°C than at 24°C, however, the difference was not statistically significant (Mann-Whitney U, U = 80, *P* = 0.44). There were no significant differences in hatching sizes for planktotrophic larvae at the different temperature treatments (Kruskall-Wallis H = 5.1, *P* = 0.19) (Figure 3.4). However,

emerging adelphophagic larvae exhibited a bimodal distribution in their sizes in relation to temperature. Larvae emerging at 12°C and 17°C were smaller than larvae emerging at 21°C, 24°C and 28°C (Kruskal-Wallis H = 74, $P < 0.05$) (Figure 3.4). Adelphophagic larvae also exhibited a high degree of variability in their hatching sizes at higher temperatures. Qualitative observations found that, on rare occasions, some emerging adelphophagic larvae from the 21°C, 24°C and 28°C temperature treatments hatched as early as the 3-chaetiger stage with an observable amount of yolk in their gut. In addition, at 12°C, eight out of 14 broods examined had uneaten nurse eggs after larvae evacuated their capsules.

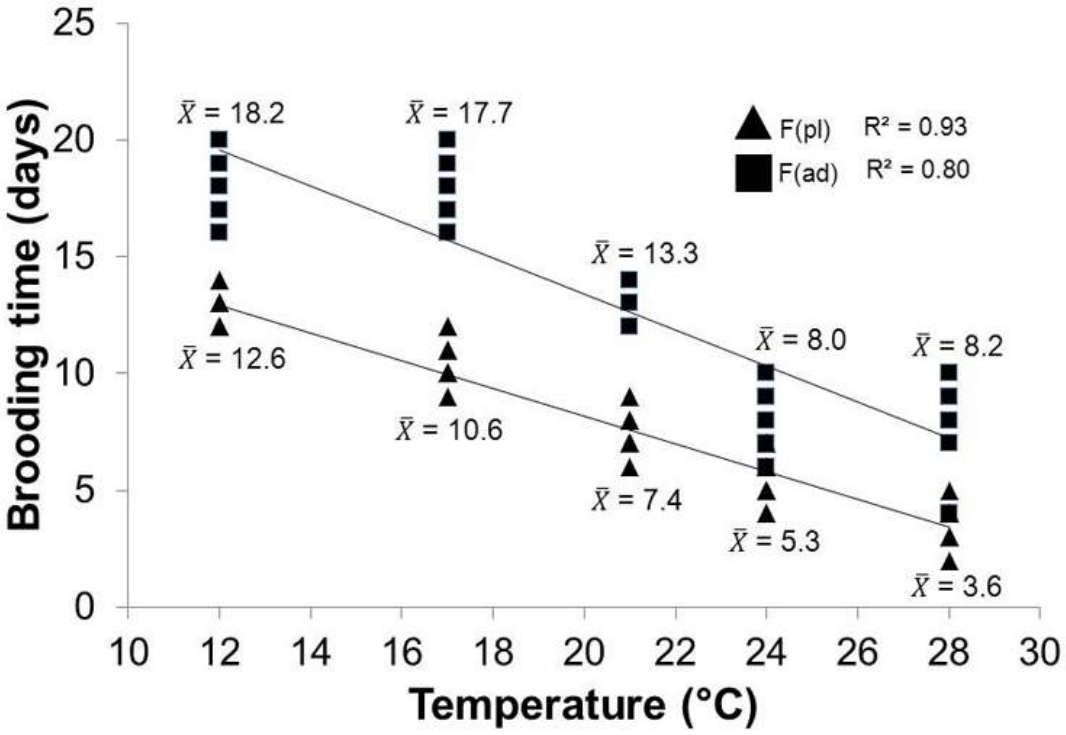

Figure 3.3: Mean brooding time of *Polydora hoplura* producing planktotrophic larvae, F(pl) and adelphophagic larvae, F(ad) at five different temperature treatments.

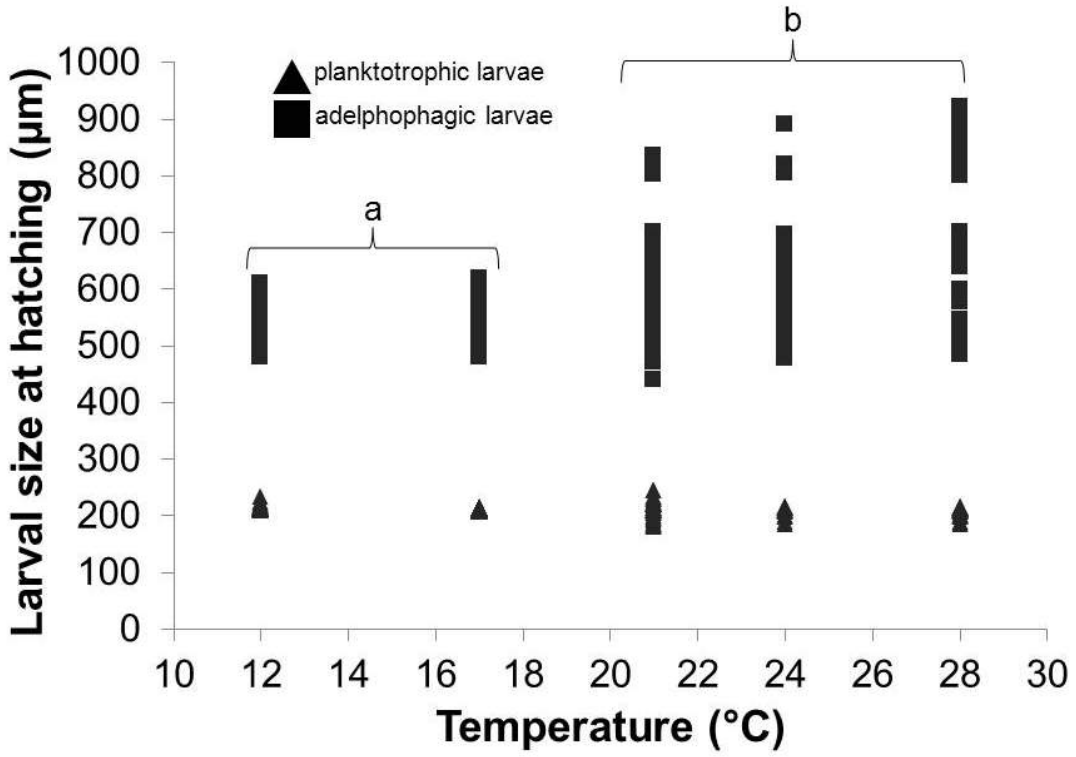

Figure 3.4: Effect of temperature on larval size at hatching in *Polydora hoplura* for planktotrophic and adelphophagic larvae. Letters denote significant differences obtained from an independent samples Kruskal-Wallis H test.

Temperature strongly affected survivorship of both planktotrophic and adelphophagic larvae (planktotrophic larvae; ANOVA: $F = 185.9$, $P < 0.05$; adelphophagic larvae, ANOVA: $F = 49.6$, $P < 0.05$). For both larval types, survival peaked at 24°C and was the lowest at the extreme low temperature (12°C) though a Tukey's post hoc test detected no significant difference in survival of adelphophagic larvae at 12 and 17°C (Figure 3.5). Overall survival was highest at 24°C for planktotrophic larvae (8.6% ± 1.5) and for adelphophagic larvae (72.9% ± 2.7) surviving to settlement. However, survival was lowest at 28°C for planktotrophic larvae (2.4% ± 1.4) and at 12°C for adelphophagic larvae (32.4% ± 6.6).

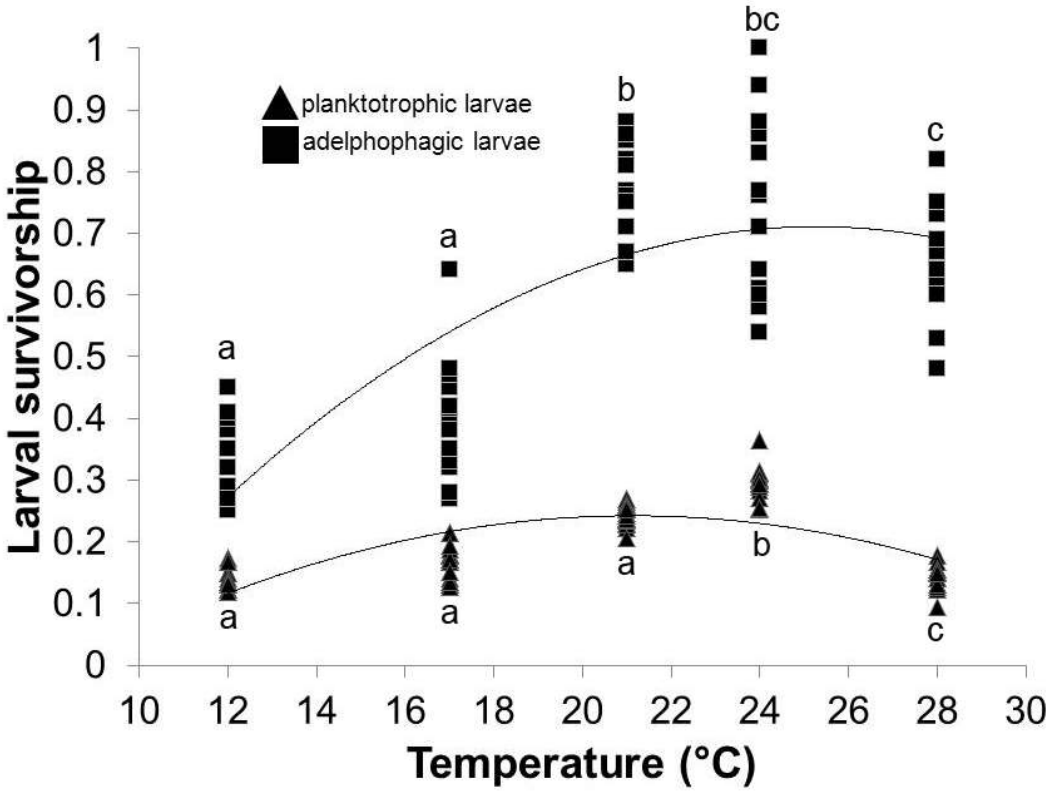

Figure 3.5: Effect of temperature on larval survival in *Polydora hoplura*. Polynomial regression of proportion of larvae that survived to settlement per female versus temperature for planktotrophic larvae (R^2 = 0.51) and adelphophagic larvae (R^2 = 0.56). Letters denote results from a Tukey's HSD post-hoc test.

There was an inverse relationship between larval developmental time from oviposition to settlement and temperature for both larval types. Developmental time decreased as temperature increased from 12°C (planktotrophic larvae: Kruskal-Wallis H = 807.6, $P < 0.05$; adelphophagic larvae: Kruskal-Wallis H = 4066.2, $P < 0.05$). The actual developmental time at the lowest temperature treatment (12°C) for planktotrophic and adelphophagic larvae was 60 ± 0.4 days and 21.6 ± 1.7 days, respectively, which was more than twice the time it took for larvae to settle at the highest temperature (28°C) (20.6 ± 1.3 days for planktotrophic larvae and 9.8 ± 1.7 days for adelphophagic larvae). There was a positive relationship between temperature and developmental rate for both larval types with adelphophagic larvae having a higher developmental rate than

planktotrophic larvae at each temperature treatment (Figure 3.6). The developmental rate of adelphophagic larvae also had a slope three times larger than planktotrophic larvae (ANCOVA: F = 359, $P < 0.05$).

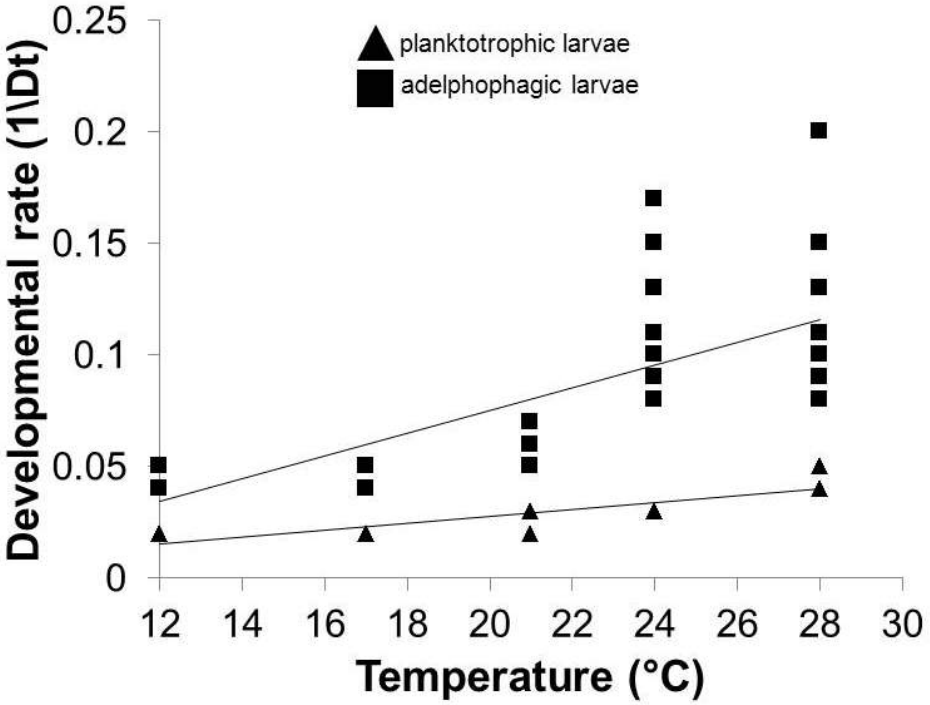

Figure 3.6: Linear regression of developmental rate as a function of temperature for *Polydora hoplura*. Planktotrophic larvae: y = 0.001x - 0.003, Adelphophagic larvae: y = 0.005x - 0.265.

3.3. Effect of temperature on development of *Boccardia proboscidea*

All females of *B. proboscidea* successfully brooded and ruptured their egg capsules at the different temperature treatments. During intracapsular development, planktotrophic larvae attained a maximum of eight chaetigers, while adelphophagic larvae attained a maximum of 18 chaetigers. Worms cultured at different temperature treatments did not significantly adjust their brood size (Table 3.3). There was an inverse relationship between temperature and brooding time (Figure 3.7). At lower temperatures (12°C and

17°C), females were observed to liberate planktotrophic and adelphophagic larvae at an earlier developmental stage (4-6 chaetigers and 11-14 chaetigers for planktotrophic and adelphophagic larvae, respectively). This was reflected in significant differences in the sizes of planktotrophic and adelphophagic larvae at hatching at the different temperature treatments (planktotrophic larvae: Kruskal-Wallis H = 159.1, $P < 0.05$; adelphophagic larvae: Kruskal-Wallis H = 255.5, $P < 0.05$) (Figure 3.8). In addition, 13 out of 19 broods at 12°C and 11 out of 17 broods at 17°C still had left over nurse eggs after the larvae evacuated their capsules.

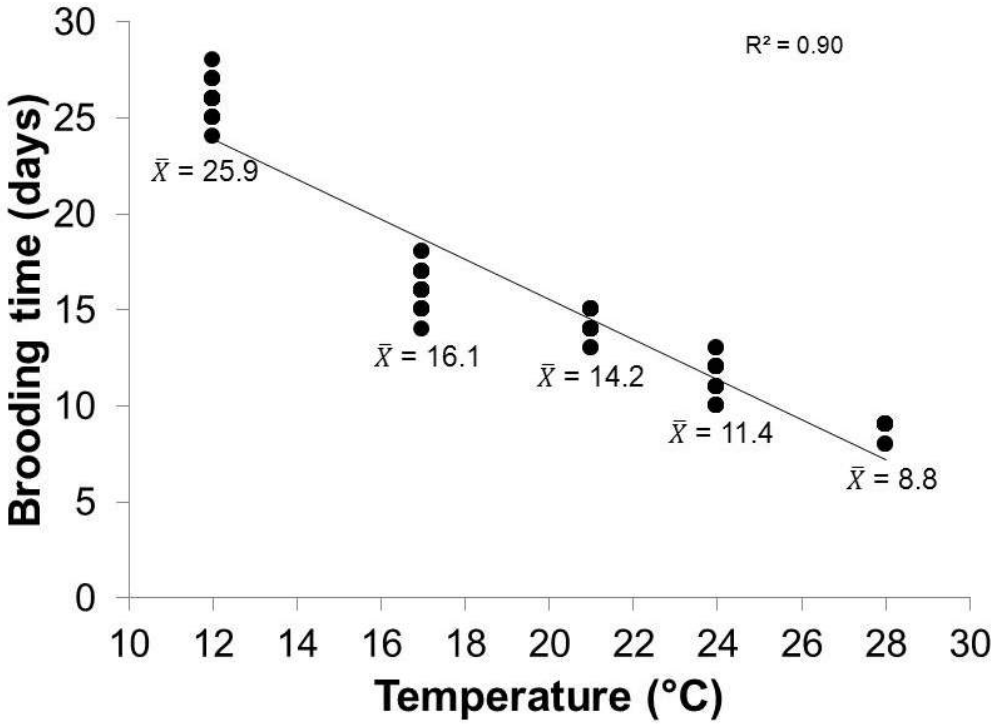

Figure 3.7: Mean brooding time of *Boccardia proboscidea* at five different temperature treatments.

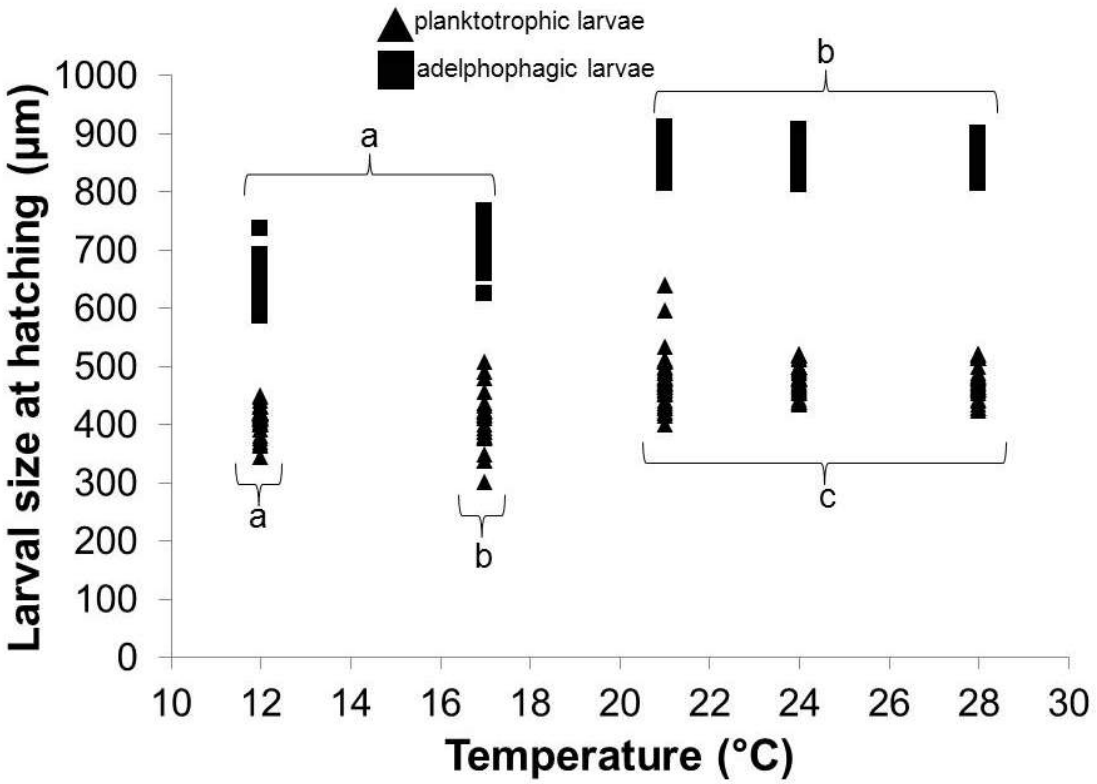

Figure 3.8: Effect of temperature on larval size at hatching in *Boccardia proboscidea* for planktotrophic and adelphophagic larvae. Letters denote significant differences obtained from an independent samples Kruskal-Wallis H test.

Temperature significantly influenced the percentage of planktotrophic larvae that survived cannibalism (Kruskal-Wallis H = 67.4, $P < 0.05$). At 12°C, 59% ± 0.1 of planktotrophic larvae remained after adelphophagia and this decreased as temperature increased, with only 30.1% ± 0.1 of larvae remaining at the 28°C temperature treatment. Temperature also significantly affected survival to settlement of both planktotrophic and adelphophagic larvae (planktotrophic larvae, ANOVA: F= 206.1, $P < 0.05$; adelphophagic larvae, ANOVA: F = 32.9, $P < 0.05$) (Figure 3.9). Planktotrophic larval survival was highest at 12°C (4.8% ± 0.01) and survival remained constant up to 21°C. Survival dropped significantly at 24°C and again at 28°C which was the lowest recorded survival for this study (< 1% larval survivorship). Adelphophagic larval survivorship was

highest at 21°C (14.7% ± 0.03), while a Tukey's *post hoc* test found no statistical difference between survival of adelphophagic larvae at 17°C and 24°C. The lowest survivorship was found at the extreme temperatures (12°C and 28°C).

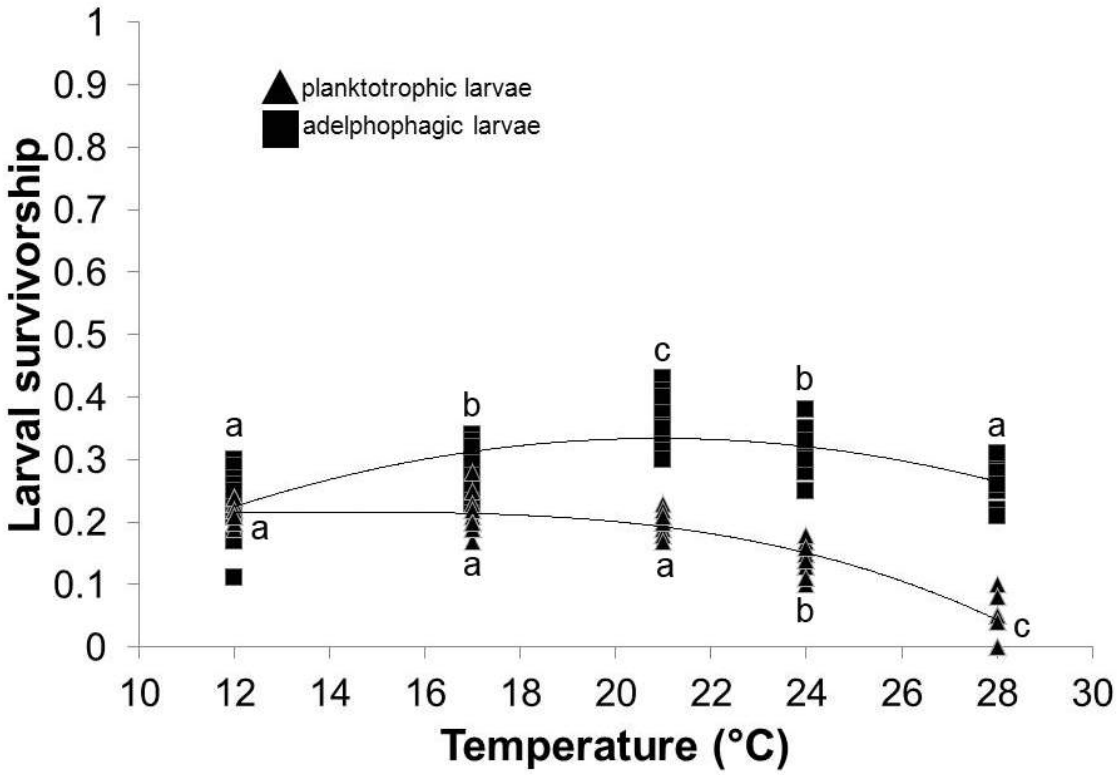

Figure 3.9: Effect of temperature on larval survival in *Boccardia proboscidea*. Polynomial regression of proportion of larvae that survived to settlement per female versus temperature for planktotrophic larvae ($R^2 = 0.89$) and adelphophagic larvae ($R^2 = 0.50$). Letters denote results from a Tukey's HSD post hoc test.

There was an inverse relationship between temperature and developmental time for planktotrophic and adelphophagic larvae of *B. proboscidea* (planktotrophic larvae; Kruskal-Wallis H = 898.7, $P < 0.05$; adelphophagic larvae: Kruskal-Wallis H = 2124.3, $P < 0.05$). As a consequence, there was a positive relationship between developmental rate and temperature (Figure 3.10). Developmental rate also increased with a rate of change (slope) that was approximately twice as high for adelphophagic larvae as for planktotrophic larvae (ANCOVA: F = 450.2, $P < 0.05$).

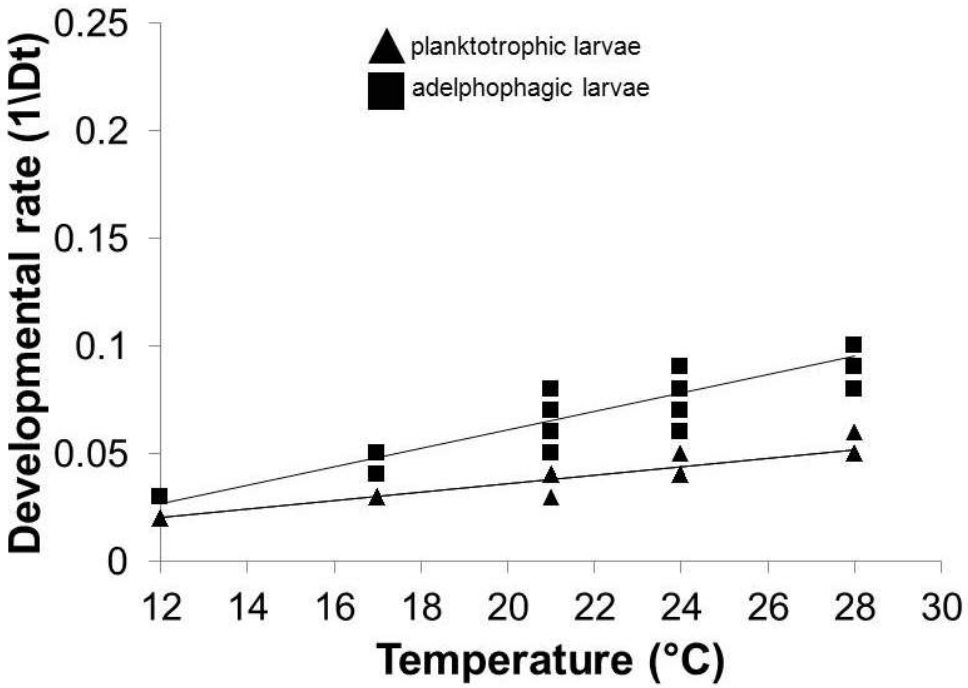

Figure 3.10: Linear regression of developmental rate as a function of temperature for *Boccardia proboscidea*. Planktotrophic larvae: y = 0.002x - 0.003, Adelphophagic larvae: y = 0.004x - 0.024.

4. Discussion

The current study investigated the effect of temperature on reproduction and development of the recently introduced polychaete *Boccardia proboscidea* in an attempt to determine if it possesses the capacity to reproduce and survive to settlement in temperatures it could encounter at different points along the South African coast. This was carried out by investigating the effect of temperature on brood size and larval size, larval survivorship, developmental time and developmental rate, of planktotrophic and adelphophagic larvae, of *B. proboscidea*. I used the distribution and reproductive data gathered from *B. proboscidea,* together with similar data from a closely-related

"predictor" species, *Polydora hoplura,* that is already established in South Africa to make my predictions.

In marine invertebrates, studies that have investigated the effect of temperature on brood size have yielded conflicting results. For example, Levin and Creed (1986) reported an increase in brood size with increasing temperature for the polychaete *Streblospio benedicti*. By contrast, Kamps (1978) and Collin and Spangler (2012) found that an increase in temperature resulted in a decrease in brood size in the copepod *Diaptomus pallidus* and the gastropod *Crepidula* cf. *onyx*. For both species examined in this study, it seems that temperature only affects the brood size of adelphophagic larvae of *P. hoplura* cultured at 28°C. It is possible that these females may have simply adjusted their nurse egg to larvae ratio. In producing fewer larvae and more nurse eggs, the female may be mitigating sibling competition, which I found to be more intense at higher temperatures, presumably due to faster growth rates and faster rates of nurse egg consumption by some individuals. Unfortunately, we did not measure nurse egg to larvae ratio in the present study and so cannot confirm this hypothesis. There are, however, other factors, such as food availability and quality, that may influence brood size (Qian and Chia 1991; Qian 1994). However, in this study all worms were fed in a similar manner and cultured individually. Interestingly, reproductive studies on fish species such as *Tinca tinca* found that its initial brood size also remained unchanged at different temperature treatments, but over time cumulative brood size (i.e., mean brood size per female per season) differed significantly (Morawska 1984). My results are based on initial brood size and hence tracking the brood size of each worm over successive broods may yield different results.

Adelphophagic larvae of *P. hoplura* and *B. proboscidea* emerged at a larger size at higher temperatures compared to low temperature conspecifics. Similar studies on gastropods also found that adelphophagic larvae hatched at a larger size at temperatures above 21°C (Collin and Spangler 2012). The most probable explanation is that the physiological effects (such as faster growth rate and metabolism) on larvae cultured at higher temperatures facilitated intense sibling interactions. In *P. hoplura*, adelphophagic larvae are not confined to a single capsule and are able to move between adjacent capsules after exploiting the nurse eggs allocated to them (Chapter 2). As such, these larvae were able to rapidly accelerate their growth during the intracapsular phase and in extreme cases, deprive their siblings of nurse eggs. This may explain why, on rare occasions, 3–4 chaetiger larvae emerged at higher temperature treatments. In *B. proboscidea,* similar factors were probably responsible for the increased hatching size of adelphophagic larvae at higher temperatures. Furthermore, in this species, the intensity of sibling interactions was likely amplified by sibling cannibalism.

My finding that the non-feeding planktotrophic larvae of *B. proboscidea* showed a significant increase in hatching size at higher temperatures, while the planktotrophic larvae of *P. hoplura* did not was interesting, but puzzling. Traditional life history theory proposes that planktotrophic larvae are supplied with just enough reserves to form the necessary feeding and locomotive structures for a planktonic lifestyle. When this pre-feeding stage ends, the larvae must feed in order to grow and complete metamorphosis (Vance 1973; Herrera *et al.* 1996) (Figure 3.11a). In almost all *Polydora*-type worms studied thus far, these structures usually form by the 3-chaetiger stage, which then emerges from the egg capsules, thus terminating the pre-feeding stage (e.g. in *P.*

hoplura). In *B. proboscidea,* however, it appears as though females supply the planktotrophic larvae with extra reserves that extend the pre-feeding stage to as late as eight chaetigers. This extended pre-feeding stage can therefore act as a “growth window” that allows for a high degree of variability in size and or stage at hatching in response to external factors, which in this study was temperature (Figure 3.11B). It is interesting to note that the planktotrophic larvae of *B. proboscidea* from North America develop to the maximum size of only three chaetigers (Gibson, 1997). Preliminary investigation of brood traits found that eggs of *B. proboscidea* from South Africa were larger than type I and type III eggs from North America (David unpubl. data). It is therefore possible that the extra reserves needed for an extended pre-feeding stage most likely originated from the egg itself. In fact, recent studies by Jaubet *et al.* (2014) found that some planktotrophic larvae of *B. proboscidea* from Argentina were capable of reaching a maximum of six chaetigers and also emerged from eggs that were larger than those reported from North America. Planktotrophs of *B. proboscidea* from Australia are also capable of exceeding three chaetigers during the pre-feeding stage (Blake and Kudenov 1978); however, there are no published data on egg sizes from that region for comparison. An alternative, though less likely, explanation for the variability in hatching size is that the planktotrophic larvae may be feeding inside its capsule but to a lesser extent than their adelphophagic siblings, since they do in fact possess the appropriate digestive structures necessary for nurse egg consumption (Gibson and Carver 2013). However, in this study I did not directly observe feeding in planktotrophic larvae. From an evolutionary perspective, it is difficult to elucidate the advantages of size variability in planktotrophic larvae, especially in a polymorphic system where adelphophagic larvae already confer variability in hatching size. One explanation is that planktotrophic larvae that are capable of reaching a relatively large size in the capsule, would less likely be

eaten by its adelphophagic siblings. These advanced planktotrophic larvae would then spend a shorter time in the water column than their 3-chaetiger counterparts, which may increase their survival without greatly diminishing dispersal capabilities (but see Pechenik 1999).

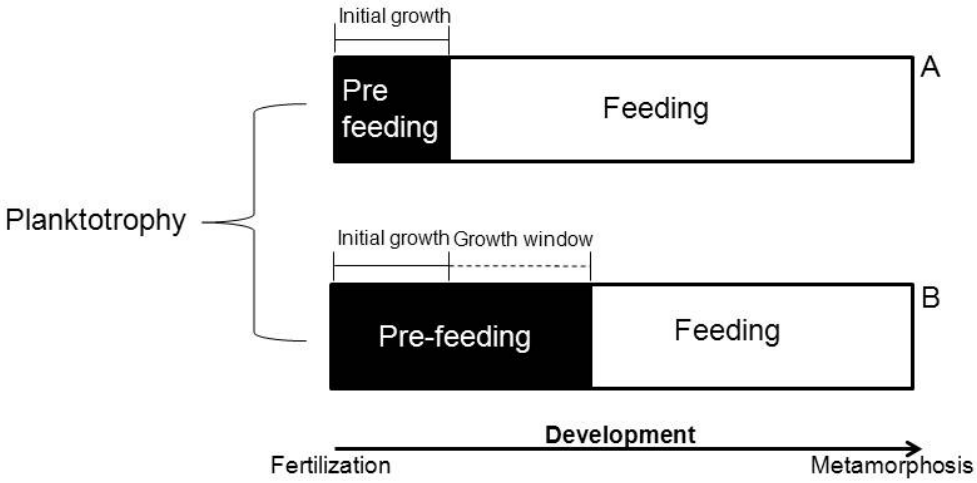

Figure 3.11: Modified diagram from Herrera *et al.* (1996) showing two variations of planktotrophic development in marine invertebrates. A) Typical planktotrophic development where short pre-feeding stage is followed by lengthy feeding (planktonic) phase. B) Second type of planktotrophic development with extended pre-feeding stage comprising of a "growth window" followed by the obligate planktonic phase.

My survivorship results for both larval types of *P. hoplura* and adelphophagic larvae of *B. proboscidea* is consistent with previous studies on decapods (de Rivera *et al.* 2007) and bivalves (Verween *et al.* 2007) that found high larval mortalities at temperatures as low as 11°C and as high has 30°C. In *B. proboscidea*, the extremely low survivorship to settlement of planktotrophic larvae at the highest temperatures was undoubtedly exacerbated by sibling cannibalism. At the lower temperatures, females ruptured their capsules when both types of larvae were at a relatively early stage of development with nurse eggs still present. As such, some planktotrophic larvae were spared from cannibalism by their adelphophagic siblings. Conversely, the higher temperatures

accelerated intracapsular development of adelphophagic larvae, which in turn greatly accelerated the rate at which planktotrophic larvae were eaten by their siblings. Based on these results, it appears as though females play a large role in determining which larval type dominates by altering the brooding period. In fact, in the absence of a brooding female, no planktotrophic larvae were found to survive to hatching (David unpubl. data). This variance in hatching may be a direct response to the duration of brooding time. When development is inhibited by low temperature, a female's reproductive success over her lifespan may be compromised if a longer time is spent investing maternal care in a single brood. In this case she may opt to hatch her brood regardless of her offspring's current developmental stage. My findings are congruent with a similar study by Oyarzun and Strathmann (2011) who found that *B. proboscidea* from North American populations also hatched their capsules early in development at low temperatures, which resulted in higher survival of planktotrophic larvae.

In terms of developmental rate, the adelphophagic larvae of both species had a slope two to three times greater than that of their planktotrophic siblings indicating an increase in performance of a similar magnitude regardless of temperature. The hypothesis that abbreviated larval development proceeds at a higher developmental rate than planktotrophic development has been confirmed in other marine invertebrates, such as echinoderms and crustaceans (Pearse 1969; Hoegh-Guldberg and Pearse 1995). However, this is the first documentation of such a trend within a polymorphic system. My data suggest that with adelphophagy, a reliable supply of food (nurse eggs) provided by the female allows the larvae to invest most of their energy in growth and development since the larvae do not need to seek out food, whereas planktotrophic larvae need to

partition their energy budget for both growth/development and swimming (to seek out food) and will therefore always “lag” behind adelphophagic development.

The results from this study serve as a first step in understanding *B. proboscidea*’s potential spread by identifying key areas along the South African coast where the species could become established. Based on the results obtained, my predictor species *P. hoplura* appears capable of producing broods across the broad temperature regimes that reflect a large portion of the SA coast (from Port Nolloth to Haga Haga). Indeed, the current study and the most recent survey by Simon (2011) found the species is distributed from Saldanha Bay in the west to Haga Haga in the east. However, differences in responses to temperature may influence the sizes of the populations along the coast. For example, on the west and south west coast, the mainly cold-water temperatures could result in low survivorship of both larval types and consequentially low recruitment year round. I therefore predict that although self-sustaining populations can be established in this region, numbers should be low due to temperature-induced limitations on larval survival. In fact, this was found to be the case in my surveys that yielded relatively low numbers of *P. hoplura* at Saldanha Bay, Paternoster and Jacobsbaai with the species absent further north at Kleinzee. At sites further east annual temperatures follow a broader trend and population sizes may vary based on seasonal changes in water temperature. I predict that at these sites seasonal highs should result in larger sizes at hatching, faster developmental rates (therefore faster recruitment rates) and ultimately larger population sizes while the opposite should occur during seasonal lows. My predictions are in concordance with reality, where large populations of *P. hoplura* (>100 individuals/abalone) were found at Mossel Bay during

late spring (van Niekerk unpubl. data) and low numbers of worms were found inhabiting oysters at Port Elizabeth during winter (Table 2).

Based on the information gathered for *P. hoplura* and the developmental results for *B. proboscidea*, I predict that *B. proboscidea* should be capable of establishing and maintaining self-sustaining populations in its current range. In addition, it could become established at sites where *P. hoplura* has also been recorded, specifically in regions on the west and south west coast. The fact that *B. proboscidea* did not adjust its brood size suggests that its reproductive output should remain constant regardless of whether the worm establishes itself in the colder waters of the west coast or the warmer subtropical waters of the east coast of the country. One critical difference between the larval types of *P. hoplura* and *B. proboscidea* is that the planktotrophic and adelphophagic larvae of *B. proboscidea* exhibit different survival optima in response to temperature. I predict that *B. proboscidea* should become established at Saldanha Bay and surrounding areas where the cold water temperatures would support high planktotrophic larval survivorship. In regions north of Saldanha Bay, such as Kleinzee and Port Nolloth, the consistently low temperatures throughout the year would also favor high planktotroph survival but this might be cancelled out by increased predation due to increased time spent in the water column. This means that dispersal north may well occur but the rate of population development may be slower. The establishment of *B. proboscidea* on the south west coast seems almost inevitable with populations already recorded at four different sites in this region since 2011 (Table 2). The temperature regimes here are very similar to those found at Saldanha Bay, therefore I predict a similar scenario to the west coast sites. At sites further east, the difference in survival optima of the two larval types may confer an advantage to *B. proboscidea,* where it would be capable of

maintaining robust populations since variability in water temperature can facilitate survival of either planktotrophic or adelphophagic larvae. At Haga Haga on the east coast, the presence of *B. proboscidea* on farmed abalone and its absence on wild abalone is puzzling. One possible reason for this is that undisclosed farm management practices or environmental conditions may prevent the establishment of consistent populations on the farm itself (Boonzaaier *et al.* 2014). This in turn may prevent the larvae from escaping in numbers large enough to maintain viable populations in the wild. However, if temperature is considered, and sufficient larvae are capable of arriving here, I predict that *B. proboscidea* would become established and maintain viable populations in the region. Higher water temperatures due to global climate change are expected to facilitate range expansion in many marine invertebrates (Hoegh-Guldberg and Bruno 2010). In *B. proboscidea,* increases in water temperatures would result in denser and more structured populations on the west coast since temperatures would favour higher adelphophagic larval survivorship along with faster developmental rates. However, increases in temperatures would be detrimental to planktotrophic larvae and consistently high temperatures may even trigger females to adjust their brood characteristics over time to allow for the survivorship of planktotrophs.

While larval development and survivorship strongly influence establishment and range expansion dynamics, post-settlement mortality of juveniles may affect recruitment rates and subsequently, population sizes in many marine invertebrates (Hunt and Scheibling 1997). Unfortunately, there are currently no data available on early post settlement of *B. proboscidea* in the wild. In fact, this remains one of the least explored aspects of larval life history in marine invertebrates, possibly due to the difficulty in tracking a single cohort of recruits over time in the wild (Penin *et al.* 2011). The studies that have been

conducted are limited to mainly molluscs (specifically, mussels, barnacles and gastropods), ascidians, and more recently, corals. These studies report significant mortality rates of more than 50% for newly settled recruits, driven primarily by competition with established adults and predation by other organisms (Gosselin and Qian 1997; Penin *et al.* 2011; Bohn *et al.* 2013). Despite the fact that *B. proboscidea* reproduces year round and can produce up to eight broods throughout its reproductive period (Gibson 1997), if such high mortalities occur in *B. proboscidea* juveniles after each settlement event, this could result in a slow rate of population establishment and expansion. It may even manifest itself as a lag phase, which could delay or even prevent a potential invasion. Lag phases are common phenomena of introductory events, however, considering that *B. proboscidea* populations appear to be well established on abalone farms, these worms could in theory provide a consistent source of new recruits at wild sites near the farms, thereby actually shortening a potential lag phase for the species.

The establishment of an introduced species in its new environment is a crucial phase in the invasion process (Haydar 2010) and my current study has shown that *B. proboscidea* is capable of producing viable populations at different points along the SA coast. In summary, I expect that on the west and south west coast, *B. proboscidea* will maintain small, dispersed populations, while on the south and east coast we predict the establishment of confined, dense populations. In addition to the establishment of viable populations, the ability to disperse to different regions to maintain genetic connectivity is also important since it could influence the long term persistence of a population once the species becomes established (Johst *et al.* 2002; Bradbury *et al.* 2008). In SA, the dispersal of many marine invertebrates can be influenced by oceanographic features,

which are manifested in the form of phylogeographic breaks (Teske *et al.* 2011, and references therein). These breaks may hinder larval dispersal of *B. proboscidea* and may even isolate populations, which could lead to the formation of distinct lineages in certain regions. The following chapter will investigate the population structure of the predictor species, *P. hoplura,* which would give some insights into the extent of *B. proboscidea*'s dispersal capabilities.

CHAPTER 4

Genetic connectivity in the non-indigenous polychaete *Polydora hoplura* (Annelida: Spionidae) in southern Africa

1. Introduction

Marine invasions are recognized as one of the leading threats to global biodiversity (Carlton and Geller 1993; Carlton 1996; Chapin *et al.* 2000; Bax *et al.* 2003; Molnar *et al.* 2008; Haydar 2010). Some of the negative effects of invasive species that contribute to the loss of biodiversity include the alteration of habitat in the introduced range, the displacement of native species and, more fundamentally, the disturbance of ecosystem processes, such as nutrient cycling and primary and secondary production (Mack *et al.* 2000; Branch and Steffani 2004). A successful invasion is largely dependent on whether the introduced species is capable of completing the final three phases of the invasion process: (1) survival (the ability to tolerate environmental variables in the recipient environment), (2) initial establishment and (3) long term establishment and spread (Haydar 2010).

In sessile marine invertebrates, natural dispersal can only occur via larval movement and consequentially a species' reproductive mode becomes a critical factor in its ability to spread to different regions (Sol *et al.* 2012). In marine invertebrates that actively brood their young, planktotrophic larvae are derived from eggs that are nutritionally poor and hence the larvae must feed in the water column to reach the appropriate stage for metamorphosis. As a consequence, these larvae have a lengthy dispersive phase which has the advantage of expanding the range of the species and reducing competition between parent and offspring due to movement away from the natal habitat (Pechenik 1999). In contrast, some species can produce lecithotrophic or adelphophagic larvae that are provisioned with yolk from the parent and these larval types consequently spend less time in the water column prior to metamorphosis (Blake and Arnofsky 1999). As a result, retention rates can be high and this strategy is advantageous in

unpredictable environments where strong currents can carry larvae away from suitable habitats to ones without an appropriate substratum for metamorphosis (Wray and Raff 1991).

Some species of polychaetes and gastropods also exhibit poecilogonous development; a rare strategy where the species is capable of producing more than one type of larva (Giard 1905; Knott and McHugh 2012). Poecilogony can exist as either an individual specific polymorphism, where different females in a population can be monomorphic for a single reproductive strategy (e.g. some females can produce planktotrophic larvae only while others can only, produce lecithotrophic or adelphophagic larvae) or as an adaptive polyphenism, where a single female can switch between reproductive modes (Chapter 1; Knott and McHugh 2012). In Chapter 2, I suggested that poecilogony could greatly aid in the success of introduced species that exhibit this developmental mode since the planktotrophic larvae can disperse and expand the range of the species from its point source while larvae with abbreviated development can recruit and maintain a robust population size in areas where it has become established.

Genetic markers have served as powerful tools for measuring dispersal in the marine environment by assessing genetic structure and gene flow among spatially-separated populations (Levin 2006; Cowen and Sponaugle 2009). Interestingly, these studies have shown that the relationship between predicted dispersal based on reproductive mode and actual dispersal over time is not necessarily congruent. For example, many species with planktonic developmental modes have shown marked genetic structure and low levels of connectivity among populations (Bowen *et al.* 2006; Teske *et al.* 2006; Miller and Ayre 2008), while some species that exhibit abbreviated larval development (such

as lecithotrophy) have shown high levels of population connectivity across different sampling sites (Pettingill *et al.* 2007; Hellberg 2009). These discrepancies can be attributed to a variety of factors, including larval life history (e.g. retention of planktotrophic larvae in the natal habitat) and unorthodox dispersal vectors for species with abbreviated larval development (e.g. rafting and biofouling of encrusting organisms) (Teske *et al.* 2007a; David and Williams 2012b). Furthermore, the dynamic nature of the marine environment could influence larval movement via oceanographic features such as currents, eddies and upwelling cells (to name a few). In such cases, these features can act as gene flow barriers that can hinder larval migration and ultimately result in the establishment of biogeographic provinces (Hellberg 2009). Genetic connectivity strongly influences the long-term persistence of a population and, as such, it plays a major role in the preservation of evolutionary potential. This, however, is not necessarily a requirement for a successful invasion event (Bradbury *et al.* 2008; Haydar 2011). For example, studies on the invasive green crab, *Carcinus maenas,* found high levels of genetic connectivity among 21 populations distributed along the entire Pacific Coast of North America despite putative breaks on this coast (Tepolt *et al.* 2009). It was hypothesized that larval exchange among these populations was facilitated by the prevailing oceanographic conditions in the region along with the wide thermal tolerance of *C. maenas* larvae (Tepolt *et al.* 2009).

The SA coast is a unique environment to study phylogeography, since it possesses a variety of coastal features that could influence structure in a population (Figure 4.1). The coastal regime comprises the cold waters of the Atlantic Ocean on the west coast, where the northward-flowing Benguela Current dominates, and the warm waters of the Indian Ocean on the east coast, where the southward-flowing Agulhas Current

dominates. The South African coast can be divided into at least four biogeographic zones (cool temperature, warm temperate, sub-tropical and tropical) (Teske *et al.* 2009). Within these zones, up to five phylogeographic breaks have been reported for a variety of marine invertebrates and fishes, with the one at Cape Point being the most prominent (Teske *et al.* 2011). The exact locations of some of these breaks also differ according to species (Teske *et al.* 2008, 2011).

The aim of this study was to determine the population structure of the well-established predictor species, *Polydora hoplura*. *Polydora hoplura* is used as a predictor species due to its similarities to *B. proboscidea* both in terms of life history (both species are poecilogonous) and status as an aquaculture pest in South Africa (Chapters 1 and 2). *Polydora hoplura* was first recorded in the 1940s at Table Bay on the south coast (Millard 1952) and subsequent surveys in the 1950s found the worm inhabiting intertidal stations at Saldanha Bay on the west coast (Day 1955). By the 1960s, *P. hoplura* had been recorded near Mossel Bay in the southern part of the country (Day 1967). After its first introduction, the worm could have dispersed naturally along the South African coast up until the 1970s when the first commercial scale oyster venture was established in the country (Haupt *et al.* 2010b). At this point, the importation of oysters from international hatcheries, along with the establishment of farms and movement of oysters among them could have resulted in the frequent movement of worms across different biogeographic regions. *Polydora hoplura* now boasts a relatively wide distribution; as far west as Saldanha Bay and as far east as Haga Haga (Simon 2011). By elucidating the genetic differences among populations of *P. hoplura,* it will be possible to make some initial predictions regarding *B. proboscidea*'s future success in maintaining genetic connectivity among its spatially-separated populations.

2. Materials and Methods

2.1. Sampling protocol and laboratory procedures

A variety of potential substrata that harboured *Polydora hoplura* were collected in 2012 and 2013 from seven localities covering a wide distribution of the species (Figure 4.1). These substrata include, sponge (*Haliclona* sp.), coralline algae (*Mesophyllum engelhartii*), oysters (*Striostrea margaritacea*), abalone (*Haliotis midae*), hermit crabs (unconfirmed species), barnacles (unconfirmed species) and limpets (*Scutellastra* sp.). Sampling was carried out while strictly adhering to permit conditions. Despite intensive sampling I was unable to obtain *P. hoplura* from Haga Haga, one of only three sites north east of Port Elizabeth (the others being Port Alfred and East London) where the species has been recorded (Simon 2011). Substrata were immersed in a vermifuge (0.05% phenol solution), which facilitated the evacuation of worms from their tubes. Worms were identified under a dissection microscope and placed in 99% EtOH for DNA extractions. Genomic DNA was extracted from whole worms using a tissue extraction kit (MACHEREY-NAGEL, GmbH & Co. Germany), following the instruction manual. The mitochondrial DNA marker Cyt *b* (~500 bp fragment) was amplified using the polymerase chain reaction (PCR) and the primers Cyt *b* 424F' (Boore and Brown 2000) and Cyt *b* 876R' (Oyarzun *et al.* 2011). Cycling conditions included: initial denaturation at 95°C for 5 min, followed by: 40 cycles of 95°C for 30 s, annealing 48°C for 30 s, extension 72°C for 30 s; final extension 72°C for 7 min. Preliminary trials using several nDNA markers found that the intron of the α subunit of the ATP-synthetase nuclear encoded protein complex (*ATPSα*, ~250 bp) gave the most consistent results. The fragment was amplified using a primer of Jarman *et al.* (2002): *ATPSα*F' and a new reverse primer, (*ATPSα*R1) (5'-CATGAAAAAGGCACAATCCC-3') (Williams, unpubl, data). Conditions for amplification of the *ATPSα* gene were the same for Cyt *b,* except

for the annealing temperature, which was increased to 55°C for 30 s in order to increase specificity of the primer to the target region. All PCR products were verified via 1% agarose gel electrophoresis, and gel bands were excised and purified using a gel extraction kit (BIOFLUX Tokyo, Japan). Purified PCR products for both markers were sequenced with the forward primers using BigDye chemistry (ABI, Foster City, CA) and analyzed on an Applied Biosystems 3730xl genetic analyzer at the Central Analytical Facility at Stellenbosch University. All sequences were verified using the BLASTN tool on Genbank and mtDNA sequences were translated to amino acids to check for gene functionality using the ExPASy translation tool (Gasteiger *et al.* 2003). Sequences were deposited into Genbank (accession numbers KJ858577 - KJ858680).

2.2. Data analysis

Mitochondrial and nuclear DNA sequences were aligned using the Clustal W alignment tool (Thompson *et al.* 1994) and edited by eye in Bioedit ver 5.0.6 (Hall 1999). For the *ATPSα* data set, alleles were identified using the PHASE algorithm (Stephens *et al.* 2001) in DnaSp ver 5.10 (Librado and Rozas 2009) with the following parameters: 100 iterations, 1 thinning interval and 100 burn-in iterations. After editing, a 360 bp and 186 bp fragment remained for analysis for the Cyt *b* and *ATPSα* markers respectively. In order to determine the evolutionary relationships among haplotypes sampled at the various sites, parsimony networks were constructed for both genes using TCS ver. 1.2.1 (Clement *et al.* 2000), with the fixed connection limit set to a 95% confidence interval. To assess levels of connectivity between the cool-temperate and warm-temperate biogeographic provinces, populations were separated into two clusters (Table 4.1) and a hierarchical AMOVA (Analysis of Molecular Variance) along with Φ_{ST} calculations were

carried out in Arlequin 3.5 (Excoffier and Lischer 2010). To determine if the breaks at Cape Agulhas and Algoa Bay influenced genetic connectivity on the south coast, pairwise Φ_{ST} values for a combination of sites in this biogeographic province were also calculated.

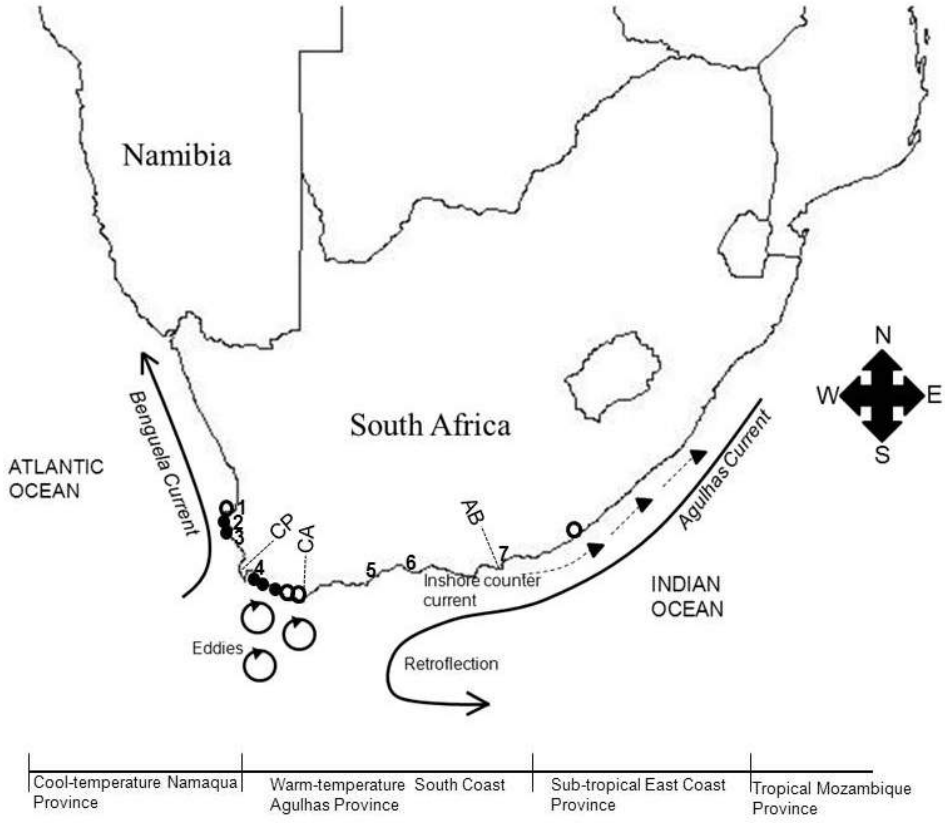

Figure 4.1: Map of South Africa showing major oceanographic features along with biogeographic provinces and major phylogeographic breaks within the sampling range: CP- Cape Point, CA- Cape Agulhas, AB- Algoa Bay. Closed circles and open circles represent known wild and farmed populations of *Boccardia proboscidea* while numbers represent sampling localities for *Polydora hoplura*: 1-Paternoster, 2- Saldanha Bay, 3- Jakobsbaai, 4- Bettysbaai, 5- Mossel Bay, 6- Knysna, 7- Port Elizabeth.

Table 4.1: Sample sizes for *Polydora hoplura* collected from seven localities representing the cool temperature Namaqua biogeographic province (Bustamante and Branch 1996) and the warm temperate south coast Agulhas biogeographic province along the coast of South Africa.

Site no.	Biogeographic region	Locality	Genetic marker	
			Cyt *b*	*ATPSα*
1	Cool-temperate	Paternoster	15	11
2		Jakobsbaai	15	12
3		Saldanha Bay	15	12
4	Warm-temperate	Bettysbaai	14	12
5		Mossel Bay	15	10
6		Knysna	15	10
7		Port Elizabeth	15	12

3. Results

A 360 bp and 186 bp fragment with 76 and 18 variable positions were obtained for Cyt *b* and *ATPSα* markers respectively. For the Cyt *b* gene, a total of 42 haplotypes (7 shared, 35 unique) was recovered which were shared among sampling sites without any clear geographic pattern (Figure 4.2). Three of the seven haplotypes were shared by individuals from populations that spanned the three different phylogeographic barriers (Fig. 4.1).

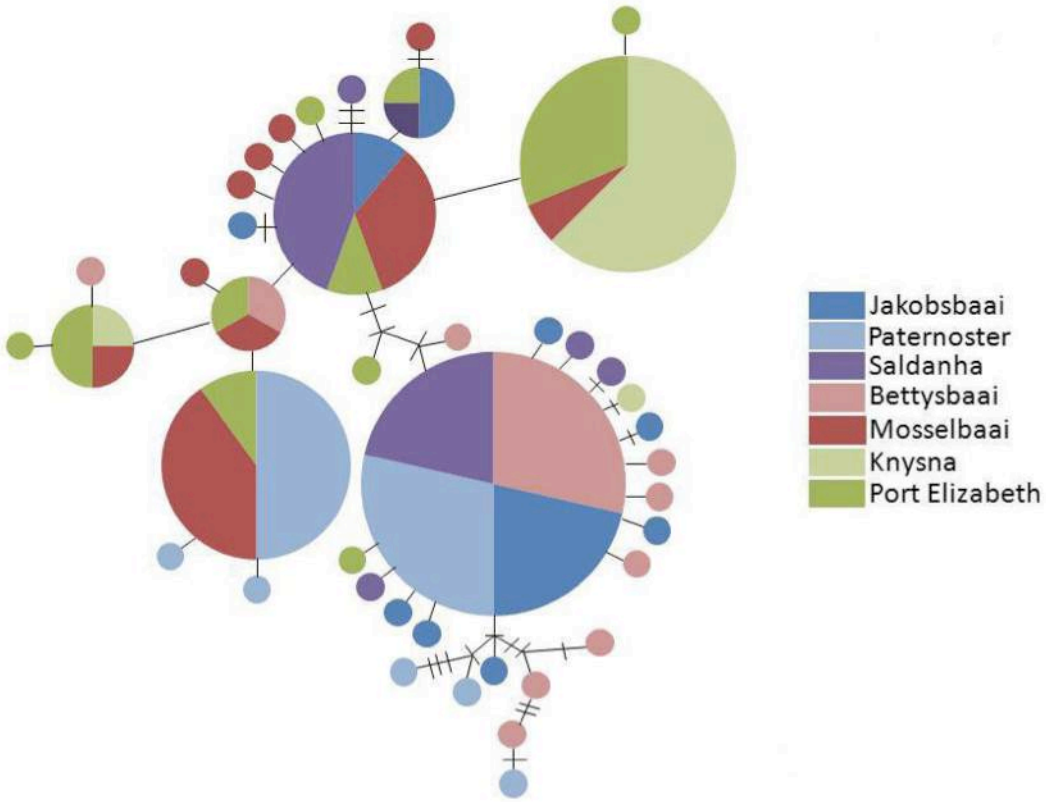

Figure 4.2: Haplotype network for *Polydora hoplura* based on mtDNA *Cyt b* sequences. Size of the circles is representative of individuals with that haplotype. The smallest circles represent a haplotype frequency of one. Each connecting line between haplotypes represent one mutational step and perpendicular lines represent an additonal mutational change.

When sites were clustered by biogeographic region, a hierarchical AMOVA test found that approximately 96% ($P < 0.05$) and 4% ($P < 0.05$) of variation in sequences was due to differences within and among clusters respectively (Table 4.1) with an overall Φ_{ST} value of 0.044 ($P = 0.01$) between biogeographic regions indicating shallow but significant genetic structuring. On the south coast, there was weak genetic differentiation across all sites with the Bettysbaai (site 4) and Port Elizabeth (site 7) pairing exhibiting the highest levels of genetic differention ($\Phi_{ST} = 0.236$). For the *ATPSα* gene, a total of 44 haplotypes (14 shared, 30 unique) was recovered which exhibited weak genetic structuring. In particular, the most common haplotype was shared among

all populations sampled (Figure 4.3). Additionally, there was a single haplotype shared by only Paternoster individuals that could not be connected to the main network.

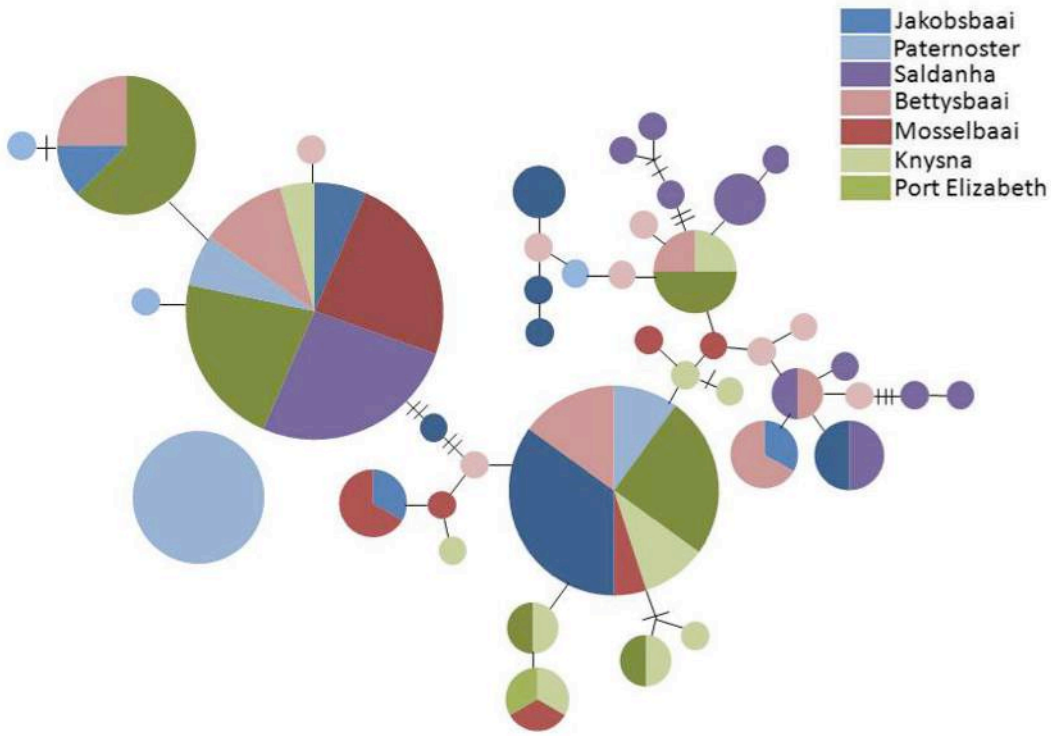

Figure 4.3: Haplotype network for *Polydora hoplura* based on nDNA *ATPSα* sequences. Size of the circles is representative of the number of individuals with that haplotype. The smallest circles represent a haplotype frequency of one. Each connecting line between haplotypes represents one mutational step and perpendicular lines represent and additional mutational change.

A hierarchical AMOVA for *ATPSα* detected shallow but significant genetic structuring with approximately 96% ($P < 0.05$) and 4% ($P < 0.05$) of variation in sequences due to differences within and between clusters respectively (Table 4.2) and an overall Φ_{ST} value of 0.042 ($P = 0.01$) between the two biogeographic regions. Pairwise Φ_{ST} values among south coast sites were also generally low and similar to the mtDNA dataset, Knysna and Bettysbaai showing the most differentiation ($\Phi_{ST} = 0.151$) (Table 4.3).

Table 4.2: Hierarchical AMOVA for *Polydora hoplura* populations based on Cyt *b* and *ATPSα* DNA sequences. Populations grouped into clusters based on biogeographic region.

Source of variation	df	Sum of squares	Variance components	Percentage of variation
Cyt *b*				
Between clusters	1	11.10	0.15 V_A	4.42
Within clusters	102	337.08	3.30 V_B	95.58
Total	103	348.18	3.46	
ATPSα				
Between clusters	1	12.98	0.14 V_A	4.25
Within clusters	142	441.65	3.11 V_B	95.75
Total	143	454.63	3.25	

Table 4.3: Results of AMOVA analyses of Cyt *b* and *ATPSα* intron sequences of *Polydora hoplura* with pairwise comparisons of south coast sites. Statistically significant values *(P* <0.05) are indicated in bold.

Locus	Combinations	Φ_{ST} value
Cyt *b*	(4)(5)	0.149
	(4)(6)	**0.189**
	(4)(7)	**0.236**
	(5)(7)	0.018
	(5)(6)	0.087
	(6)(7)	**0.051**
ATPSα	(4)(5)	0.081
	(4)(6)	**0.151**
	(4)(7)	**0.089**
	(5)(7)	0.000
	(5)(6)	0.024
	(6)(7)	0.100

4. Discussion

The purpose of the present study was to evaluate the population structure of the non-indigenous polychaete *P. hoplura* along the South African coast. This information will then be used to help predict the dispersal potential of the more recent invader, *B. proboscidea.* In general, for both markers multiple haplotypes were shared among distant populations, but Φ_{ST} values along with a hierarchial AMOVA found shallow but significant structure between the two biogeographic regions. The haplotype networks

exhibited some discordance, where more geographic structure was detected for Cyt *b* compared to *ATPSα* which could be due to differences in coalescence times since nuclear alleles take a longer time to be fixed in a population (Pavlova *et al*. 2013). There is also the possibility that differences in sensitivity and variability between both markers may be contributing to the observed discordance in genetic structure (Colbeck *et al.* 2008; DiBattista *et al.* 2012). Despite the discordance, both markers complemented each other by exhibiting a large amount of haplotype sharing across the sampling area. The disconnected group of Paternoster individuals in the nuclear network was unexpected and it is unlikely that it represents spatial structure in the region considering that all specimens were sampled from a single area and a similar disconnected group was not detected by mtDNA. Rather, the disconnected group could be due to a very recent introduction of worms carrying a unique haplotype of the *ATPSα* marker, a phenomenon already reported in similar studies (Cameron *et al.* 2008). Alternatively, it could represent diversity from a secondary introduction, though, in previous studies such a finding is usually detected in the mitochondrial genome (Bracco *et al.* 2007)

In sessile marine invertebrates, phylogeographic patterns can be shaped by historical (ancient allopatric divergences and climate shifts) and/or contemporary processes (biogeographic barriers and introductions) (Duran *et al.* 2004; Selkoe *et al.* 2008). Based on the results from this study, it appears that certain contemporary processes, particularly introductory events, could be having a pronounced effect on the observed genetic pattern of *P. hoplura*. Along the South African coast, genetic studies have shown that Cape Point is a prominent barrier separating the cool temperate west coast biota from the warm temperate south coast biota (Teske *et al.* 2007a, 2014; von der Heyden *et al.* 2008). However, I found weak structuring between these biogeographic

regions (Φ_{ST} = 0.044 and 0.042 for mtDNA and nDNA, respectively). Despite the fact that this weak genetic differentiation was significant for both markers *(P* < 0.05), the lack of any clear geographic patterning and the absence of distinct clades in the parsimony networks indicate that larval exchange and frequent movement are probably occurring among all localities. Alternatively, the significant structure between some localities, but shared haplotypes between them could indicate "ancestral" polymorphisms, which could obscure the detection of geneflow (Hare 2001). Further studies using microsatellite markers may help elucidate this problem.

Within the biogeographic provinces, the region between Cape Point and Cape Agulhas is considered to be a transition zone with both abalone (*Haliotis midae*) and mudprawn (*Upogebia africana*) populations west of Cape Agulhas showing marked genetic differentiation from populations east of this break (Evans *et al.* 2004; Teske *et al.* 2006). However, the results in this study detected weak structure for *P. hoplura* between Bettysbaai and sites east of the Cape Agulhas break (Table 4.3). Similarly, on the south east coast, the Algoa Bay break, characterized by isolated upwelling events, along with temperature differences, has been hypothesized as the cause for genetic disjunction in the local fish species *Clinus cottoides* and the snail *Nassarius kraussianus* (von der Heyden *et al.* 2008; Teske *et al.* 2008). In contrast my results found that *P. hoplura* populations from Port Elizabeth shared haplotypes with populations as geographically distant as Saldanha Bay and Jacobsbaai (>800 km). Marine biogeography is shaped primarily by temperature (Murawski 1993) and the South African biogeographic provinces are no different (Teske *et al.* 2011). As shown in Chapter 3, *P. hoplura* larvae are capable of surviving and settling at the thermal regimes representative of the these biogeographic regions and hence based on temperature

responses only, the genetic homogeneity observed across the biogeographic provinces is congruent with the species' physiological capabilities.

In many marine phylogeographic studies conducted in South Africa thus far, genetic structure or lack thereof has often been credited to the organism's developmental mode and/or the prevailing hydrographic and environmental conditions of its environment (Tolley *et al.* 2005; Neethling *et al.* 2008; Teske *et al.* 2011, 2014; Reynolds *et al.* 2014). However, I hypothesize that much of the genetic connectivity observed in *P. hoplura* is most likely due to anthropogenic dispersal mechanisms rather than natural dispersal. Firstly, *P.hoplura* is a notorious pest of commercial oysters, and to a lesser extent abalone in South Africa (Simon *et al.* 2006) and these animals are frequently transported among onshore and offshore farms located in different biogeographic provinces in the country (Griffiths *et al.* 2009a; Simon 2009; Haupt *et al.* 2012). The movement of larvae via the inflow and outflow path of the farms, combined with shellfish transplantations could in theory provide a mechanism that allows larvae to cover large distances with the farm serving as an intermediary. For example, larvae could be introduced with the inflow into the farms where they can settle on molluscs, which could then be transported to another locality and there larvae can exit through the outflow, successfully settle in the region and interbreed with local populations. In fact, recent studies by Williams (unpubl data), using the same genetic markers found conclusive evidence for larval exchange between farmed and wild populations. This could explain why a large number of shared haplotypes were found among populations as geographically distant as Saldanha Bay and Port Elizabeth and why, despite significant p values in the AMOVA results, Cape Point is not an effective genetic barrier for this species (possibly due to multiple bidirectional introductions). In support of this

hypothesis, a recent study by Woodin *et al.* (2014) found that transregional transport of aquaculture materials had allowed the polychaete *Diopatra biscayensis* to breach an important biogeographic boundary in the Bay of Biscay in France. In addition, studies have found that shellfish translocations can deliver large propagule pools of hitchikers (Roman and Darling 2007), meaning that even an isolated oyster transplantation event could supply enough adults and larvae to alter the genetic makeup of a population.

In retrospect, it is tempting to hypothesize that *P. hoplura* could have also dispersed naturally along the coast after its establishment in the 1950s, prior to commercial oyster movement. This may be the case for local expansion via larval diffusion near points of entry. However, there are additional vectors that could have facilitated the spread of the species over wider spatial scales after establishment. For example, Walker (2013) suggested that *P. hoplura* was most likely distributed worldwide by early colonizers and explorers via the hull fouling of ships. This vector could also be responsible for contemporary movement of the species, considering that *P. hoplura* has been found boring into encrusting sponge (Chapter 3), which can attach to ship hulls and facilitate dispersal (David and Williams 2012b). In fact, a study by Pettingil *et al.* (2007) found that hull fouling alone was responsible for maintaining genetic connectivity among global subpopulations of the serpulid polychaete, *Hydroides elegans.* Global surveys of transoceanic shipping also have found polychaetes to be one of the most abundant taxa introduced through ballast water (Carlton 1985; Carlton and Geller 1993; Chu *et al.* 1997; Levings *et al.* 2004) and South Africa is known to have one of the highest levels of shipping traffic in the world (Zachail and Heidelhoff 1999). Since *P. hoplura* can produce planktotrophic larvae, which could spend up to four weeks in the water column (Chapter 2), there is the possibility that larvae could be taken up into ballast water and

transported among various ports in the country. One major implication of multiple introductory events across biogeographic regions is the reluctance of populations to adapt to local environmental conditions due to an influx of individuals from localities in a different biogeographic region. This would prevent formation of distinct lineages as clearly reflected by the absence of geographic patterning in the mtDNA haplotypes of *P. hoplura*.

Estimates of geneflow and migration rates were not conducted in this study since one of the major assumptions of these analyses is that the system conforms to specific models of migration, all of which are based on natural movement of migrants (Wright 1969; Slatkin 1981; reviewed by Marko and Hart 2011). Since anthropogenic mechanisms may be strongly influencing dispersal in *P. hoplura*, the underlying assumptions of these analyses would be violated. In particular, it could erode strong genetic signals, which would render gene flow estimates uninformative. While it is acknowledged that F-statistics and its analogues are also routinely used as proxies for gene flow (Whitlock and McCauley 1999), here these statistics are strictly used to show genetic differentiation among populations. A caveat of this chapter is the absence of sampling localities northeast of Port Elizabeth for the sub-tropical and tropical biogeographic provinces. As such, it is difficult to ascertain whether the strong flowing Agulhas Current could act as a mechanical barrier separating populations in this region from the south and east coast or whether my hypothesis of human mediated transport would similarly be supported by genetic connectivity with sites that extend to these biogeographic provinces.

In conclusion, I have shown that populations of *P. hoplura* in South Africa are genetically connected, but the observed phylogeographic pattern paints a complex picture of dispersal from its first record at Saldanha Bay to its widespread distribution along the coast of the country. While natural dispersal could, in theory, have contributed to this genetic connectivity, at least on a local scale, anthropogenic dispersal mechanisms are much more likely to be the main contributors to the observed connectivity, across larger geographical distances.

Chapter 5: Meta-analysis and Synthesis

1. Progress thus far

The anthropogenic movement of infested abalone was implicated in the spread of the polychaete *Boccardia proboscidea* to three different biogeographic regions of South Africa (Simon *et al.* 2009). This means that the worm has the opportunity to colonize a large section of the South African coast and, should it become invasive, could pose a serious threat to the intertidal marine ecosystem, as has been shown in Argentina (Jaubet *et al.* 2013). The overarching aim throughout this thesis was to evaluate the establishment and dispersal potential of *Boccardia proboscidea* in South Africa. In order to accomplish this I first assessed the feasibility of using the non-indigenous oyster pest *Polydora hoplura* as a predictor species that could be used to help ground-truth the subsequent predictions (Chapter 2). In Chapter 3, I showed that *P. hoplura*'s physiological capabilities are congruent with its current distribution and, therefore, based on *B. proboscidea*'s physiological capabilities, it should also be capable of establishing itself at multiple sites along the South African coast, including expanding its range beyond that of *P. hoplura* on the west coast. Finally, in Chapter 4, I showed that there was weak genetic structuring and shared diversity among *P. hoplura* populations distributed between two biogeographic provinces. This could be due to ancestral polymorphims or to a lesser extent, founder and bottleneck events. However, considering the vibrant aquaculture trade in South Africa coupled with the propensity of *P. hoplura*'s larvae to settle on fouling organisms and be taken up into ballast water, I concluded that the observed genetic patterns are most likely being driven by anthropogenic dispersal. Based on that study, there is the strong possibility that *B. proboscidea* could also be dispersed anthropogenically since its planktotrophic larvae can be taken up into ballast water and/or can settle on encrusting organisms such as sponges, that can foul ship hulls (Chapter 3). This would increase the speed of a

potential invasion. However, there is also the possibility that *B. proboscidea* may disperse naturally from its point sources through larval diffusion. In this chapter, I focus specifically on the natural movement of larvae to predict the potential dispersal distances and trajectories that *B. proboscidea* may cover as it makes its way along the South African coast. I then provide a final discussion regarding the presence of *B. proboscidea* in South Africa. While *P. hoplura* was a good predictor for estimating establishment potential of *B. proboscidea*, the lack of geographic patterning due to anthropogenic dispersal observed in Chapter 4 makes it a less than an ideal candidate for estimating natural dispersal distances. Therefore, I focus specifically on the problem species.

2. Potential dispersal distances of *Boccardia proboscidea*

In marine invertebrates, planktonic larval duration (PLD) is strongly correlated with dispersal distance and is therefore a good predictor for estimating the extent to which larvae can travel (Shanks *et al.* 2003, Shanks 2009). In addition, for larvae that act as passive particles, oceanographic flow patterns strongly influence not only distance but also the trajectory of dispersing larvae. In order to estimate the potential dispersal distances that *B. proboscidea* larvae could cover from its point sources (Haga Haga, Hermanus and Jakobsbaai), a simple equation was used that incorporated both factors: $C_v = d/PLD$, where C_v = current velocity, d = distance travelled, PLD = planktonic larval duration. In this equation, PLD data (in days) was obtained from the Chapter 3 dataset, according to the prevailing temperature regimes at the different point sources, and was then converted to hours. Published C_v values were obtained from Boyd *et al.* (1992) and converted from $c.ms^{-1}$ to $k.mh^{-1}$. Additionally, two assumptions were made prior to

applying the equation. Firstly, it was assumed that planktotrophic larvae are mainly responsible for effective dispersal of the species and hence only developmental data from this larval type was used. Also, it was assumed that larval movement will be dictated by the prevailing surface current regimes in accordance with the null hypothesis of larval transport (Shanks 2009). Since C_v can fluctuate at different points along the coast, I used the lower value of the given ranges.

Table 5.1 shows the potential distances that larvae would be expected to cover based on current velocity and PLD only. On the east coast, the coastal current near Haga Haga flows at a velocity of 1.8–5.4 km.h^{-1} southwards and the velocity decreases by about 0.9 km.h^{-1} at approximately 68 km west of Port Elizabeth, prior to its divergence from the continental shelf (Boyd *et al.* 1992). An irregular countercurrent also runs closer inshore at a velocity of 0.9–1.8 km h^{-1}. For planktotrophic larvae of *B. proboscidea* dispersing from Haga Haga, bidirectional dispersal is expected and the potential dispersal distance that planktotrophic larvae can cover based on current velocity and PLD are 450.5 ± 45 km southwards and 225.3 ± 22.6 km northwards. On the west coast, the Benguela current flows northwards as a “jet” with speeds of around 1.8–2.7 km h^{-1} and is generally much weaker than the Agulhas Current, especially the region of flow between the Cape Peninsula and Cape Columbine. In addition, this current overlies a southward flowing current with speeds of 0–0.9 km.h^{-1}. The direction of gene flow for many marine invertebrates studied in South Africa has been predominantly northwards following the Benguela Current. However, once *Polydora*-type planktotrophic larvae approach the end of their development, the increase in larval size often results in sinking of the larvae below the surface, presumably as a result of negative phototaxis and hydrodynamic constraints (Wilson 1928; Hansen *et al.* 2010).

Since horizantal swimming speed is actually slower for advanced larva compared to early larval stages (Hansen *et al.* 2010), they can still act as passive particles below the surface, hence the undercurrent in the Benguela region may also facilitate southward dispersal. Therefore, planktotrophic larvae would be expected to disperse both north and south from their point sources on the west coast. Based on PLD and current velocity near Jakobsbaai, I would expect larvae to cover an average distance of 623 km north and its movement south would be considerably slower (346.2 km). On the south coast near Hermanus, flow velocities are around 0–1.4 $km.h^{-1}$ westwards and eastwards, while the current velocity slightly changes east near Mossel Bay, to 0.4–1.4 $km.h^{-1}$. Larvae could potentially cover distances of 138.5 km in both directions.

Table 5.1: Potential dispersal distances of planktotrophic larvae of *Boccardia proboscidea* based on current velocity (C_v) obtained from Boyd *et al.* (1992) and planktonic larval duration (PLD) obtained from Chapter 3.

Site	Mean temperature (°C)	Current velocity[a] (km h^{-1})	Average PLD (h)	Distance (km)
Haga Haga	21.0 ± 1.3	0.9 N 1.8 S	250.3 ± 23.5	225.3 N 450.5 S
Hermanus	14.8 ± 0.9	0.4 W 0.4 E	346.2 ± 31.8	138.5 W 138.5 E
Jakobsbaai	14.9 ± 2.8	1.8 N 0 S	346.2 ± 31.8	623 N 346.2 S

[a]only values in the lower range was used.

Based on PLD and current velocity, planktotrophic larvae of *B. proboscidea* would be expected to cover very long distances, ranging from 138.5–623 km. Effective dispersal distances spanning hundreds of kilometers are not uncommon among marine species

that produce planktonic larvae. For example, planktonic larvae of the gastropod *Philine* spp. and the crustacean *Cancer magister* can cover estimated distances of 260 km and 500 km, respectively (Cadien and Ranasinghe 2003; Shanks 2009). In contrast, other invertebrates such as the polychaete *Maranzelleria viridis* and the echinoderm *Acanthaster planci* both have maximum dispersal distances of less than 120 km (Moran *et al.* 1992; Bochert 1997). Despite the strong correlation between PLD, current velocity and dispersal distance, many recent studies incorporating different methods of distance estimates (including genetic and biophysical approaches), have shown large discrepancies with the distances predicted based on current velocities and PLD only. In the majority of these studies, current velocities and PLD often greatly overestimate dispersal distances and therefore supports a “closed ocean” view as opposed to the traditional view that the ocean is “open” (Levin 2006). For example, in the Caribbean, a genetic study found that effective dispersal distances of goby fishes was less than 30 km, despite the fact that potential distance was estimated to be 500 km based on current velocity and PLD (Taylor and Hellberg 2003). In South Africa, McQuaid and Phillips (2000), found that the larvae of *M. gallprovincialis* on the south east coast of South Africa could potentially cover distances of 200 km, however net displacement of individuals on plankton grids (effective dispersal) was less than 20 km. Similarly, *M. galloprovincialis* was found to have dispersed along 115 km of the west coast in a year (Hockey and van Erkom Schurink 1992), however if the PLD of this species (~4 weeks, Seed 1969; Jorgensen 1981) and current velocity of the Benguela near Saldanha Bay are considered, the species should have been able to cover up to 470 km in less than a year! Prior to 2012, the Pacific barnacle, *B. glandula* was only found on the west coast of South Africa. However, a recent study by Pope *et al.* (unpubl. data) found that in the last five years the species has extended its distribution from Misty Cliffs on the west

coast to St. James on the south west coast (approximately 60 km of coastline). However, if PLD (~3 weeks, Schwindt 2007) and current velocity are considered then the species should have been able to disperse to distances of up to 505 km. It is interesting to note that in all these studies, larvae appear to cover around 10% or less of the predicted dispersal estimates. If the potential dispersal distances of *B. proboscidea* are arbitrarily adjusted based on this discrepancy, larvae would be expected to disperse to considerably shorter distances (Table 5.2).

Table 5.2: Adjusted dispersal distances for *Boccardia proboscidea* based on discrepancies between potential (based on current velocity and PLD) and effective dispersal distances of marine species

Site	Potential dispersal distance (km)	Adjusted dispersal distance (km)
Haga Haga	225.3 N 450.5 S	22.5 N 45.1 S
Hermanus	138.5 W 138.5 E	13.9 W 13.9 E
Jakobsbaai	623 N 346.2 S	62.3 N 34.6 S

These adjusted dispersal estimates appear to better reflect the reality of larval dispersal on the South African coast. For example, on the west coast, using the adjusted dispersal distance from Jakobsbaai and a maturation time of four months for planktotrophic larvae (Gibson 1997), *B. proboscidea* would be expected to extend its range at a rate of 186 $km.y^{-1}$ northwards and 103.8 $km.y^{-1}$ southwards, which is faster than the rate of spread reported for *M. galloprovincialis* - 115$km.y^{-1}$ north, 25 $km.y^{-1}$ south (Hockey and van Erkom Schurink 1992). In addition, using the adjusted values on the south coast, planktotrophic larvae are expected to have a dispersal rate of approximately 42 $km.y^{-1}$ both eastwards and westwards. Pertinent to this prediction, the

species has extended its distributional range on this coast to three sites, all within 76 km west of Hermanus. A fourth population was found at Kommetjie earlier in 2014 (Simon unpubl, data), which is located about 90 km from Hermanus and 28 km north of the biogeographic break at Cape Point (Fig. 5.1). However, it is not known whether this population originated from Hermanus (which would indicate movement of the larvae around Cape Point) or if they have travelled south from Jakobsbaai. The question eventually arises: why such large discrepancy between potential and effective dispersal distances? Biogeographic boundaries can significantly influence the extent of dispersal, as was discussed in Chapters 3 and 4, and may explain *B. glandula*'s slow movement around Cape Point. However, even in the absence of these boundaries, other factors such as lack of suitable habitat and larval behavior (particularly sinking, diel vertical migrations and local retention) could shorten the potential dispersal distances (Cowen and Sponaugle 2009; Shanks 2009). In the case of *B. proboscidea*, larvae are generalists in terms of habitat preferences and are capable of settling on a variety of substrata present along the South African coast (Chapter 3). In addition, planktotrophic larvae of polydorids are not known to exhibit complex larval behavior that would facilitate high retention rates (Blake 1969; Blake and Arnofsky 1999). However, two important factors that could shorten the potential dispersal distances of *B. proboscidea* are the dilution of larvae by offshore advection and predation (White *et al.* 2014). On the east coast once the Agulhas Current diverges from the continental shelf and enters the retroflection zone, some larvae could be carried with this current offshore and would be lost at sea. In addition, predation in the plankton is believed to be one of the most important sources of larval mortality in marine invertebrates (Morgan *et al.* 1995). Laboratory experiments in Chapter 3 did not include predators, hence the survivorship rates calculated could be considered conservative compared to actual survivorship that

would occur in the wild. However, in this regard *B. proboscidea*'s benefits from its developmental mode since post-settlement success is not dependent on planktotrophic larvae and could even be driven solely by adelphophagic larvae (Chapter 3).

Figure 5.1: Distribution map (as of 2014) of farmed and wild populations of *Boccardia proboscidea* in South Africa. Large bordered circles represent point sources, smaller circles represent wild populations. Yellow circle represent a recently recorded population at Kommetjie (Simon unpubl data). *Satellite image reproduced with permission from NASA Earth Observatory.*

4. Dispersal trajectory of *Boccardia proboscidea*

In Chapter 3, I showed that *B. proboscidea* is capable of surviving and completing development at temperatures reflecting the coastal stretch from Kleinzee to Haga Haga. However, north of Haga Haga, in the tropical Mozambique province (Griffiths *et al.* 2010), water temperatures can exceed 28°C (Carrasco and Perissinotto 2011). Although I did not include higher temperatures in the reproductive experiments, my survivorship data showed a clear trend of decreasing survival as temperature increased to 28°C for

both larval types (Chapter 3). This indicates that larvae would probably not be able to survive in large enough numbers in this region and, as such, *B. proboscidea* may reach its biogeographic limit tropical Mozambique province. Incidentally, *M. galloprovincialis* is also believed to have reached its limit on the east coast due to the higher temperatures there (Zardi *et al.* 2007). South of Haga Haga, there are few barriers that could prevent natural movement of planktotrophic larvae. The coastal dunefields of Alexandria (one of the largest in the world) are located approximately 250 km south of Haga Haga and have been hypothesized as a weak barrier to gene flow for the cumacean, *Iphinoe truncata,* presumably due to a lack of suitable habitat (Teske *et al.* 2006). However, *B. proboscidea* frequently creates tubes in sand and has been found on sandy beaches (Jaubet *et al.* 2014), so it is unlikely that these dunefields would serve as a strong barrier for dispersal.

On the south west coast, larvae moving eastwards from Hermanus are expected to disperse unimpeded until their arrival at the eastern edge of the coast near Port Elizabeth. Considering that the coastal stretch from Hermanus to Port Elizabeth is approximately 620 km and larvae are predicted to only move 42 km.y^{-1} (based on adjusted dispersal estimates) with movement presumably beginning since its first record in the wild in 2011, it will take quite some time (~10 years) for the species to extend its range to the south east, unless it is aided by anthropogenic dispersal mechanisms. At this point, the Agulhas Current flowing southwards could obstruct northeastward migration. Alternatively, the larvae could be caught in the counter current prior to approaching Port Elizabeth. Unfortunately I was unable to obtain the predictor species, *P. hoplura* from sites north of Port Elizabeth (the worm has been found at Port Alfred and East London) and so could not confirm genetic connectivity among populations in

that region. This could have allowed me to ascertain whether the current could act as a mechanical barrier separating populations in this region from the rest of the coast.

The west coast exhibits the strongest signal of asymmetrical geneflow (Von der Heyden *et al.* 2009; Teske *et al.* 2011) and, as such, larval movement will be predominantly northward though southward movement is possible either by larvae utilizing the southward flowing undercurrent (Boyd *et al.* 1992), or via anthropogenic dispersal mechanisms, specifically hull fouling and/or ballast water. Pertinent to my predictions, a small number of worms were found in the wild at Jakobsbaai and approximately 19 km north at Paternoster. There are no prominent features detected that could obstruct dispersal, so I expect *B. proboscidea* to become established along a large section of this coast, as has been found for other introduced species, such as *M. galloprovincialis* and *B. glandula* (Robinson *et al.* 2005; Laird and Griffiths 2008).

The predictions regarding dispersal distances and trajectories presented in this chapter are informative but simple. Further studies using a biophysical modelling approach that incorporates the reproductive data presented in this thesis could be used to test these predictions. Biophysical modelling couples complex oceanographic circulation models with particle tracking models and has been used extensively to predict dispersal distances and trajectories of a variety of species (reviewed by Metaxas and Saunders 2009). However, many of the parameters needed to construct a high-resolution 3D model for the entire South African coast were unavailable at the beginning of this project. Additionally, biophysical modelling is often considered a doctoral project in itself (Nicolette Chang, CSIR, pers comm.) and therefore was not feasible given the time frame for this thesis. Genetic distances are also often used to estimate effective

dispersal (Levin 2006). However, since *B. proboscidea* is still in its incipient stages of spread, such as an approach was not possible. Also, the use of the predictor species in this regard would not have been appropriate considering that the current genetic profile of *P. hoplura* appears to be shaped primarily by anthropogenic rather than natural dispersal (Chapter 4).

5. Future of *Boccardia proboscidea* in South Africa

The ability to produce large biogenic reefs in the intertidal zone (Jaubet *et al.* 2011, 2013, 2014) means that *B. proboscidea* poses a very real threat to the marine ecosystem of South Africa. This threat is maximized by the fact that the reefs were found in an area of high nutrient discharge in Argentina. If larvae were to encounter and settle at sites with these conditions in South Africa, it could form similar structures, which could displace other native intertidal fauna. Even large tracts of consolidated sediment caused by the tube building activities of the worms at high densities may cause geophysical alterations in the environment that could negatively affect other organisms (Wallentinus and Nyberg 2007). At Gansbaai, an astoundingly large population is already established at the nutrient rich outflow of an abalone farm (Chapter 1 and 3). However, since the discovery of this population in 2011, no reefs have been produced and this could be due to the population's lower growth rate compared to their conspecifics in Argentina. The intertidal zone, where *B. proboscidea* is most often found in the wild, can be subjected to varying degrees of salinity fluctuations as a result of factors such as rainfall, proximity to freshwater outflow and tidal changes (Jansson 1967; Johnson 1967). As such, salinity could also be an additional factor influencing the dispersal and eventual distribution of the worm in South Africa. However, I did not investigate salinity tolerance in this species though a multi-taxa study on polychaetes

found that members of the Phyllodocidae, Capitellidae and Neredidae were capable of surving at salinites below 24 with mortalities only significantly occurring below 15 (Lyster 1965).

In terms of biotic effects on native fauna, after arriving in South Africa in the 1970s, *M. galloprovincialis* was capable of out-competing the indigenous mussel *Aulacomya ater* on the west coast and competes for space with *Perna perna* on the south coast (Van Erkom Schurink and Griffiths 1990; Bownes and McQuaid 2006; Zardi *et al.* 2007). *Boccardia proboscidea* may compete with the native polychaete *Nainearis laevigata* since both species have been found together in sediment. However, recent studies by Mjindi and Simon (unpubl, data) found that *B. proboscidea* is found in higher densities in fine sediment, while *N. laevigata* appears to dominate coarser sediment, meaning that habitat segregation for both species could be the most likely scenario.

With respects to mitigating the spread of *B. proboscidea*, it seems almost impossible on the west and southwest coasts where the worm has already begun its spread. Culver and Kuris (2000) successfully eradicated another polychaete pest, *Terabrasabella heterouncinata,* from California by disposing of the most preferred hosts of the worm within the vicinity of the point source, along with installing screens of specific pore size that would prevent larvae and adults from escaping the abalone farm via the outflow. The establishment of three geographically distant point sources, along with the lack of host specificity in *B. proboscidea* makes it difficult to apply similar methods. However, it may prove helpful for farms to install screens with pore sizes of approximately 0.25 mm, which could prevent both adult worms and larvae from escaping into the wild. This would dramatically reduce the founder population outside the farms and slow a potential invasion. At the outflow of the abalone farm at Gansbaai, where the worm was found in

high densities, sediment (along with the worms) could be excavated and moved to the high water mark for desiccation, though this method would be very labour intensive. At Haga Haga on the east coast, it appears that undisclosed “farm management practices” has minimized the predominance by *B. proboscidea* that has occurred at other farms (Boonzaaier *et al.* 2014). These practices may be preventing the larvae from accumulating in the wild since I did not detect populations at the outflow nor at any sampled location on the east coast (Chapter 3). However, as previously indicated, propagules dispersing from Hermanus could still migrate to this region by utilizing the countercurrents to move eastwards. In addition, anthropogenic dispersal mechanisms could inadvertently transport the worms to the east coast through a variety of vectors.

While traditional presence/absence surveys could also be used to validate my predictions by tracking the spread of the species, surveys of *B. proboscidea* are extremely difficult to carry out. Thus far, all the introduced marine invertebrates surveyed in South Africa can be easily tracked on rocky shores and sheltered bays (see Robinson *et al.* 2005). However, *B. proboscidea,* like many polydorids, can occupy a variety of habitats, which includes taking up the role as a facultative symbiont of an inconspicuous substratum, such as coralline algae or an isolated sponge colony (Chapter 3). Interestingly, the species has yet to be found inhabiting wild shellfish (Boonzaaier *et al.* 2014; Chapter 3). Nevertheless, for such surveys to be done accurately, a variety of substrata in the intertidal (and preferably subtidal) along with sediment (if present) would need to be intensively sampled and carefully processed (see Simon 2011). The promise of genetic sampling techniques such as eDNA analyses could also significantly reduce the workload in carrying out such surveys (Ficetola *et al.* 2014; Pedersen *et al.* 2015).

In conclusion, the results obtained throughout this project have shown that *Boccardia proboscidea* can disperse and become established along a large section of the South African coast irrespective of biogeographic boundaries and phylogeographic breaks. Its success will be due in part to its versatile reproductive strategy and wide tolerance of environmental variables (specifically temperature). In addition, planktotrophic larvae could potentially cover relatively large distances due to the prevailing current regimes of the coast, along with the movement of larvae via anthropogenic dispersal mechanisms. However, it is unclear which of the two dispersal mechanisms will play the biggest role in the spread of the worm. Finally, due to the stochastic nature of the marine environment (which is often underestimated in these types of studies), and the only recent exodus of *B. proboscidea* into the wild from abalone farms, it is premature at this stage to predict with confidence whether the species will become invasive in South Africa.

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