

Predicting the dispersal potential of an invasive polychaete pest along a complex coastal biome

Journal:	<i>Integrative and Comparative Biology</i>
Manuscript ID	ICB-2016-0011
Manuscript Type:	Symposium article
Date Submitted by the Author:	27-Jan-2016
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Keywords:	poecilogony, agulhas, phylogeography, spionid

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Predicting the dispersal potential of an invasive polychaete pest along a complex coastal biome

Running title: Dispersal of a shellfish parasite

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Word count: 6400

Abstract

Boccardia proboscidea is a recently introduced polychaete in South Africa (SA) where it is a notorious pest of commercially reared abalone. Populations were originally restricted to abalone farms but a recent exodus into the wild at some localities has raised conservation concerns due to the species' invasive status in other parts of the world. Here, we assessed the dispersal potential of *B. proboscidea* by using a population genetic and oceanographic modeling approach. Since the worm is in its incipient stages of a potential invasion, we used the closely related *Polydora hoplura* as a proxy due its similar reproductive strategy and its status as a pest of commercially reared oysters in the country. Populations of *P. hoplura* were sampled from seven different localities and a section of the mtDNA gene, *Cyt b* and the intron *ATPSa* was amplified. A high resolution model of the coastal waters around southern Africa was constructed using the Regional Ocean Modeling System (ROMS). Larvae were represented by passive drifters that were deployed at specific points along the coast and dispersal was quantified after a 12-month integration period. Our results showed a discordance between the genetic and modeling data. There was low genetic structure ($\Phi = 0.04$ for both markers) and no geographic patterning of mtDNA and nDNA haplotypes. However, the dispersal model found limited connectivity around Cape Point – a major phylogeographic barrier on the southern African coast. This discordance was attributed to anthropogenic movement of larvae and adult worms due to vectors such as aquaculture and shipping. As such, we hypothesized that cryptic dispersal could be overestimating genetic connectivity. Though wild populations of *B. proboscidea* could become isolated due to the Cape Point barrier, anthropogenic movement may play the critical role in facilitating the dispersal and spread of this species on the southern African coast.

Introduction

Marine invasions and climate change are recognized as the two most important threats facing global marine biodiversity (Stachowicz et al. 2002; Molnar et al. 2008; Cheung et al. 2009; Doney et al. 2012). Some of the negative effects of invasive species that contribute to the loss of diversity 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 (Bax et al. 2003). In terms of climate change, distributional models predict latitudinal shifts in species ranges most likely due to the elimination of thermal barriers that currently serve as biogeographic obstacles (Doney et al. 2012). When taken together synergistically, climate change and marine invasions have the potential to reduce biodiversity by displacing native species through local extinctions (Stachowicz et al. 2002; Brierley and Kingsford 2009).

In sessile and sedentary marine organisms, natural dispersal can only occur via larval movement and consequently, a species' reproductive mode becomes a critical factor in its ability to spread to different regions (Levin 2006). Species that produce larvae with long pelagic larval duration (PLD) phases (e.g. planktotrophic larvae) are expected to disperse longer distances while species that produce larvae with abbreviated pelagic phases, such as direct developers and those producing lecithotrophic or adelphophagic larvae, are expected to disperse shorter distances (Jablonski and Lutz 1983; Pechenik 1999). A few species are able to produce more than one type of larvae with differing dispersal capabilities; a rare developmental strategy known as 'poecilogony' (reviewed by Knott and McHugh 2012). The underlying mechanism governing this polymorphic condition is unknown for most taxa that exhibit it. However, it has been suggested that poecilogony could greatly aid in the success of

recently introduced alien species where the planktonic larvae would facilitate range expansion while their siblings, with lower dispersal capabilities, could build and maintain a robust population size in the natal habitat (David et al. 2014).

The South African (SA) coast is one of the most complex coastal systems in the world (Durgadoo et al. 2008). As such, it is an ideal region for investigating the role that larval dispersal plays in the invasion process since dispersal dynamics would depend on where on the coast they are released. The SA coast is flanked by two major coastal systems that are divided into four different biogeographic regions (Teske et al. 2009). The nutrient rich Atlantic coast to the west is characterized by a cold northward flowing Benguela Current (Shannon 1985). On the east coast, the warm and fast southward flowing Agulhas Current is the dominant system (Fig. 1). The Agulhas current undergoes a retroflexion downstream of the Agulhas Bank region, spinning off warm eddies into the South Atlantic (Gordon et al. 1987; Lutjeharms and Van Ballegooyen 1988) and closer to the coastline there is an inshore countercurrent that is predominant (Heydorn et al. 1978; Lutjeharms and Roberts 1988). The biogeographic regions are often supported by distinct intraspecific phylogeographic assemblages that can be attributed to limited dispersal between the regions (e.g., Teske et al. 2011, 2014).

The heterogeneous nature of the SA coastal system has for a long time supported a vibrant aquaculture industry with both onshore and offshore farms distributed along the country's ~2000 km coastline, the majority of which are aggregated in the Cape regions (Haupt et al. 2010; DAFF 2012). However, in recent years, aquaculture farms have been plagued by polychaete pests that burrow into the shells of farmed oysters and abalone, creating tubes in which adult worms reside and assume a sedentary existence (Simon et al. 2006; Boonzaaier

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et al. 2014; Simon 2015). These tubes lead to the formation of mud blisters in the nacreous layer of the shell, along with reduced growth rate of the shellfish, which taken together, reduces their commercial viability (Simon et al. 2010)

The purpose of the current study is to predict the dispersal and invasion potential of the recently introduced aquaculture pest, *Boccardia proboscidea* Hartman 1940 along the SA coast. In Argentina, *B. proboscidea* is an invasive auto-ecosystem engineer where it produces massive biogenic reefs that have displaced the native intertidal fauna in that region (Jaubet et al. 2013). *Boccardia proboscidea* was first discovered in SA in 2004, infesting abalone on an onshore farm on the south west coast of the country (Hermanus). Due to anthropogenic movement of its abalone host, the worm has now established itself on onshore farms on the west (Jacobs Bay) and east coast (Haga Haga) of the country, a distribution that spans all four biogeographic zones (Simon et al. 2009; Boonzaaier et al. 2014) (Fig 2). The establishment of founder populations in these different regions mean that larvae escaping the farms would be exposed to different currents, which may enhance their movement around the country compared to if they had spread from a single source.

Boccardia proboscidea is poecilogonous (Gibson 1997), and a recent study found that its larvae are capable of adapting and surviving under the broad thermal regimes of the SA coast, indicating high establishment potential (David and Simon 2014). In fact, the worm was recently found at seven wild sites on the west and south-west coasts in low densities, indicating that its spread into the wild has begun (David 2015) (Fig 2). While *B. proboscidea*'s broad thermal tolerance and its opportunistic placement in different biogeographic regions has increased its invasive potential, dispersal along the SA coast will be a critical factor governing the worm's long term establishment. By understanding how *B. proboscidea* larvae will disperse

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3 along the SA coast, it should be possible to make accurate predictions that would aid in future
4 management strategies for this species. Genetic markers are often regarded as powerful tools
5 for measuring dispersal and connectivity in the marine realm (Levin 2006). However, its power
6 is known to decrease in less than ideal scenarios where stochastic and unpredictable factors
7 are brought into play (Selkoe et al. 2008). As such, there has been a concerted effort to
8 introduce a certain level of 'oceanographic realism' into the field to accurately predict larval
9 transport (Selkoe et al. 2008). In this study we used a population genetic approach combined
10 with a high-resolution ocean model to determine the dispersal potential of *B. proboscidea*.
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22 Since *B. proboscidea* is in its incipient stages of a potential invasion, we assessed the
23 genetic structure of the closely related polychaete, *Polydora hoplura* which is used here as a
24 'predictor' for estimating dispersal. *Polydora hoplura* is also an invasive aquaculture pest in
25 SA and is notorious for being a prolific pest of commercially reared oysters, and to a lesser
26 extent, abalone in the country. The species enjoys a relatively wide distribution along the SA
27 coast and occurs at sites that coincide with the location of farms where *B. proboscidea* has
28 been reported. *P. hoplura* is also poecilogonous and was previously used as a 'predictor' for
29 assessing the establishment potential of *B. proboscidea* (David and Simon 2014). The aims of
30 this study were therefore to (1) determine the genetic structure of *P. hoplura*, (2) quantify larval
31 transport by using a particle tracking model integrated into a high-resolution ocean model that
32 spans the SA coast and (3) make predictions regarding the dispersal potential of *B.*
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Materials and Methods

Sampling protocol

Ten potential localities encompassing the entire distributional range of *Polydora hoplura* were sampled (Fig. 3). A variety of substrata known to harbor the worm were collected in 2012 and 2013. These substrata included; 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. After intensive sampling we were able to obtain appropriate sample sizes (minimum of 10 specimens per site) from seven of the localities (Table 1). We were 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 in the wild (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 dissecting microscope and placed in 99% EtOH for DNA extractions.

Genetic studies

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 and 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 (*ATPS α* , ~250 bp) gave the most suitable results. The fragment was amplified using the primers of Jarman et al. (2002): *ATPS α F'* and *ATPS α R1*. Conditions for amplification of the *ATPS α* 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. Sequences were deposited into Genbank (accession numbers KJ858577 - KJ858680).

Mitochondrial and nuclear DNA sequences were aligned using the Clustal W alignment tool and edited in Bioedit ver 5.0.6. For the *ATPS α* data set, alleles were identified using the PHASE algorithm 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 *ATPS α* 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 that are separated by the Cape Point break, populations were separated into two clusters and a

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hierarchical AMOVA (Analysis of Molecular Variance) along with Φ_{ST} calculations were carried out in Arlequin 3.5 (Excoffier and Lischer 2010).

Dispersal model

To determine the level of statistical likelihood of an oceanographically driven connection between our sites of interest, we assumed a null model of larval dispersal by seeding Lagrangian virtual drifters at specified localities in a high-resolution model of the waters around South Africa (Table 2). The regional model used, SAFR12, is a $1/12^\circ$, Agulhas focused, eddy-resolving implementation of the Regional Ocean Modeling System (ROMS) (Shchepetkin and McWilliams 2005), which, using an AGRIF approach (Debreu et al. 2008), is embedded in a $1/4^\circ$ parent domain that spans the wider South West Indian Ocean region. In the vertical, SAFR has 42-depth levels, discretized using a terrain-following, sigma-coordinate. These levels are stretched toward the free-surface, allowing for maximum vertical resolution on the shelf, and high concentration of layers above the thermocline in the deeper ocean. Fine spatial resolution, and the use of higher order numerics, allows the model to appropriately capture the behavior of the Agulhas Current at the western boundary and the strong mesoscale variability associated with the Agulhas retroflexion and leakage. Additionally, the AGRIF connection to the $1/4^\circ$ parent domain allows us to preserve the variability associated with the Agulhas source regions and its effect on western boundary variability (Penven et al. 2006).

To map the connectivity between South African coastal sites, we use the ARIANE Lagrangian package (Blanke and Raynaud 1997) to seed virtual drifters in the model at specific sites that covered an extensive stretch of the southern African coast (Table 2). Drifter trajectories are subsequently calculated from the advected pathway that each takes through

the daily, 4-dimensional velocity fields, extracted from SAFR12 for the 1991-2010 period. For each month between January 1991 and December 2009, drifters were released at a rate of 1 per depth level per day for each location and tracked for a year. To show the statistical likelihood that a drifter would reach a particular location based on a specific release point, probability density plots were constructed by spatially binning the drifter trajectories onto the model grid and normalizing the distribution by the total number of floats that were released.

Results

Population genetic study

A 360 bp and a 186 bp fragment with 76 and 18 variable positions were obtained for *Cyt b* and *ATPS α* 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 (Fig. 4a). Individuals from populations that spanned three different phylogeographic barriers shared three of the seven haplotypes. 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 3). An overall Φ_{ST} value of 0.044 ($P = 0.01$) between the cool temperate and warm temperate biogeographic regions indicated shallow but significant genetic structuring. On the south coast, there was weak genetic differentiation across all sites with the Betty's Bay (site 5) and Port Elizabeth (site 9) populations exhibiting the highest levels of genetic differentiation ($\Phi_{ST} = 0.236$). For the *ATPS α* 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 (Fig. 4b). Additionally, there was a single haplotype

shared by only Paternoster individuals that could not be connected to the main network. A hierarchical AMOVA for *ATPS α* 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 3) 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, with Knysna and Betty's Bay showing the highest level of differentiation ($\Phi_{ST} = 0.151$).

Dispersal model

After 20-year dispersal simulations were completed, a total of 289,798 trajectories were recovered for each site. In general, the model showed limited connectivity between eastern and western boundaries (Figs. 5 and 6). Drifters deployed at Jacobs Bay followed a predominantly northward trajectory along the path of the Benguela Current (Fig. 4a, b). While some southward dispersal was observed for drifters at this site, after one year there was zero connectivity with all sites east of Cape Point when drifters were released from west coast localities (Fig. 6). While some drifters deployed at east coast sites like Port Elizabeth and Haga Haga were able to reach west coast sites, overall connectivity was low ($< 40\%$). At Hermanus (50 km east of Betty's Bay), drifters were capable of traversing the Cape Point barrier and integrating itself into the Benguela system. There was no connectivity between Hermanus and sites on the east coast (Port Elizabeth and Haga Haga) while connectivity in the opposite direction was low ($\sim 50\%$) (Fig. 5c, 6). At Haga Haga on the east coast, the trajectory of drifters was predominantly southwards following the Agulhas Current though connectivity with Port

Elizabeth was strongly unidirectional (up to 100% connectivity from Haga Haga to Port Elizabeth but less than 10% from Port Elizabeth to Haga Haga).

Discussion

The purpose of the current study was to estimate the dispersal potential of the recently introduced oyster pest, *Boccardia proboscidea* in southern Africa. Our results showed discordance between the connectivity patterns estimated by the population genetic study and the ocean modeling approach.

Discordant connectivity between genetic and modeling approach

In our dispersal model, Cape Point was shown to be a prominent barrier preventing strong bidirectional dispersal; connectivity from west to east was non-existent while east to west connectivity was less than 50%. This is in contrast to the genetic results that showed low genetic structure and high levels of connectivity between the cool-temperate and warm-temperate biogeographic regions ($\Phi_{ST} = 0.04$ for both *Cyt b* and *ATPS α*). For the genetic study, both markers complemented each other by exhibiting high frequencies of haplotype sharing across the sampling area. The disconnected group of Paternoster individuals in the *ATPS α* 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 the *Cyt b* marker. Rather, the disconnected group could be due to a very recent introduction of worms carrying a unique haplotype for that particular marker, a phenomenon previously reported in a similar study focusing on invasive earthworms (Cameron et al. 2008).

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On the South African coast, studies have shown that Cape Point is the prominent break separating the west and east coast biota (von der Heyden et al. 2008; Teske et al. 2009, 2011, 2014,). The dispersal model supported this, showing zero connectivity around Cape Point when drifters are deployed from west coast sites (Jacobs Bay and Saldanha Bay). While the dispersal model showed some leakage from east to west coast, overall connectivity was low in that direction (< 50%) possibly due to the majority of drifters being swept offshore by the Agulhas eddies. One caveat of this study is resolution constraints where our dispersal model may not have fully captured the fine-scale coastal processes (e.g. waves and some inshore coastal currents). Consequently, it is likely that the simulated floats will a) more quickly and easily penetrate the open ocean domain than larvae would in reality, and b) that these floats would subsequently be more subject to offshore circulation patterns during their lifetime. As such, the probability densities shown are likely to be biased toward east to west connectivity, and against any spread against the prevailing offshore flow, e.g. west to east action against the Agulhas Current and coastal retention at the retroflexion.

In contrast to the dispersal model, our genetic study found weak structuring between these two biogeographic regions (Φ_{ST} = 0.044 and 0.042 for mtDNA and nDNA, respectively). The lacks of any clear geographic patterning in haplotypes indicate that frequent larval exchanges are probably occurring among most of the localities. With respect to the less prominent breaks on the south and southeast coasts, low Φ_{ST} values indicated limited genetic connectivity, which was further corroborated by the modeling data that showed 0% connectivity between Hermanus and Port Elizabeth and ~50% connectivity in the opposite direction (Fig. 6).

In marine organisms, phylogeographic patterns can be shaped by historical (ancient allopatric divergences and climate shifts) and or contemporary processes (biogeographic

barriers and introductions) (Pettingill et al. 2007; Selkoe et al. 2008). Based on the results from this study, it appears that contemporary processes, particularly introductory events, could be responsible for the discordance between connectivity patterns observed between the virtual drifters and the sequence data. It is possible that genetic analyses may have presented an exaggerated idea of connectivity in *P. hoplura* due to continuous anthropogenic dispersal. Firstly, *P. hoplura* is a notorious pest of commercial oysters, and to a lesser extent, abalone in South Africa. These shellfish, along with their polychaete pests, are frequently transported among onshore and offshore farms located in different biogeographic provinces in the country (Haupt 2009). Shellfish transplantations combined with the movement of larvae between the intertidal and farms could in theory provide a mechanism that allows larvae to cover large distances, with the farms serving as intermediaries. Shellfish translocations are capable of delivering large propagule pools of 'hitchhikers' meaning that even an isolated oyster transplantation event could in theory supply enough adults and larvae to significantly alter the genetic composition of a population (Roman and Darling 2007). This hypothesis is also supported by a recent study that found conclusive evidence for larval exchange between farmed and wild populations of *P. hoplura* using similar genetic markers (Williams 2015). The results highlight an important limit to population genetics, where the inability of molecular markers to conclusively detect 'cryptic dispersal' (here defined as *the consistent movement of individuals via anthropogenic pathways*) may obscure our understanding of connectivity patterns. Considering that anthropogenic transport was responsible for establishing *B. proboscidea*'s point sources in SA (Simon et al. 2009), it is likely that this same movement may have also bolstered the genetic diversity of these populations, thereby increasing the invasion potential of the species.

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Reconstructing Polydora hoplura's introduction history

Taking a historical perspective, *Polydora hoplura* was first recorded in South Africa in the 1950s near Table Bay, on the west coast, approximately 145 km south of Jacobs Bay. Based on our dispersal model, oceanographic conditions could have facilitated the spread of the species naturally along this coast as was the case for the invasive mussel *Mytilus galloprovincialis* in SA (Branch and Steffani 2004). However, the colder water temperatures further north may have decelerated the rate of spread or altogether impeded establishment of the species at some sites (David and Simon 2014), which may explain the absence of *P. hoplura* populations from Kleinsee (~600 km north of Jacobs Bay) during sampling. Natural movement and settlement around Cape Point after establishment on the west coast would have been unlikely based on our model. However, ships could have facilitated movement from the west coast to the south and east coasts. This is supported by the fact that planktotrophic larvae can be easily taken up into ballast water and *P. hoplura* has been found burrowing into sponge and barnacles, which frequently encrust on the hulls of ships (Walker 2013). While these vectors could have facilitated some 'leakage' of specimens around Cape Point, populations would have still been highly structured, registering a strong phylogeographic signal. By the 1970s when commercial oyster farming became established in the country (Haupt et al. 2010), frequent movement of oysters would have enhanced the spread of the worm and begun homogenizing populations, eventually extinguishing any geographic patterning of haplotypes, as reflected in our genetic study.

Predicting dispersal potential of Boccardia proboscidea

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3 A temperature-dependent study found that *B. proboscidea* has the potential to become
4 established at various sites that encompass a large swath of the SA coast (David and Simon
5 2014). Based on the current study it appears that these populations could become isolated
6 geographically with Cape Point being the major barrier separating populations. However, the
7 proposed anthropogenic dispersal mechanism that could be responsible for *P. hoplura*'s
8 genetic homogeneity may also be applied to *B. proboscidea*. Indeed, the movement of infested
9 abalone among onshore farms across southern Africa was primarily responsible for the
10 establishment of the three original source populations of *B. proboscidea* (Simon *et al.* 2009).
11 The good news is that the movement of farmed abalone is now more strictly controlled by the
12 aquaculture industry (WWF 2010) but the bad news is that *B. proboscidea* is a generalist in
13 terms of habitat preference, and can occupy a variety of substrates besides shellfish, such as
14 encrusting sponges and coralline algae, both of which can attach to the hulls of ships (David
15 and Simon 2014). As a consequence, anthropogenic transport via less conspicuous vectors is
16 still a strong possibility. Previous studies by Pettingil *et al.* (2007) found that hull fouling alone
17 was responsible for spreading and maintaining genetic connectivity among global
18 subpopulations of the serpulid polychaete, *Hydroides elegans* while Woodin *et al.* (2014) found
19 that anthropogenic movement via aquaculture had allowed the polychaete *Diopatra*
20 *biscayensis* to breach an important biogeographic barrier in France.
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46 Currently, *B. proboscidea* has established multiple founder populations in the wild
47 outside abalone farms at Gans Bay, across the bay from Hermanus (Simon and Mjindi
48 unpublished data) and appears to be moving west towards the Cape Point barrier (David
49 2015). Based on our results for *P. hoplura*, larval movement around Cape Point from this
50 direction should be minimal (less than 50% connectivity from the model estimates).
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Climate change in the marine realm is expected to cause long term alterations of global ocean circulation patterns while also affecting abiotic factors such as temperature, salinity and pH, and may thus be expected to also influence *B. proboscidea*'s distribution and spread. Due to the sheer complexity of the SA coast, discerning clear long term regional trends is difficult and is still being explored (De Young et al. 2011). Interestingly, global climate change models have predicted future increases in sea surface temperatures (SSTs) for the Indian Ocean (Luo et al. 2012). At Haga Haga, one of two sites sampled on this coast, daily water temperatures can exceed 24°C. Temperatures above 24°C was found to be lethal for pre-hatched planktotrophic larvae of *B. proboscidea* (David and Simon 2014). A warmer Indian Ocean could therefore result in less planktotrophic larvae being released into the water column, which in turn, may restrict the potential range of *B. proboscidea*.

In conclusion, the results obtained from our genetic study and dispersal simulations have shown that *Boccardia proboscidea* can disperse and become established along a large section of the South African coast irrespective of biogeographic boundaries. Its success will be due in part to its versatile reproductive strategy and wide thermal tolerance. However, it appears that anthropogenic movement will most likely be the critical factor governing both the spread and long term establishment of this species in southern Africa.

Funding

This work was supported by the National Research Foundation of South Africa (NRF) through the Thuthuka Program (Grant #: TTK20100726000013413) awarded to Carol A. Simon.

Acknowledgements

We thank the Marine Research Group and the Evolutionary Genomics Group at Stellenbosch University for helpful comments with regards to the genetic aspect of the study. Kevin Ruck, Matthias Wessels, Mike Gray and various oyster and abalone farms in South Africa allowed us access to our sampling sites. We also thank the Climate Systems Analysis Group at the University of Cape Town for the resources to run the numerical model, and the South African Environmental Observation Network (SAEON) Egagasini node for funding its development through an NRF PDP Program.

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Figure legends

Fig. 1. Map of southern Africa showing the major biogeographic provinces along with key oceanographic features.

Fig. 2. Distribution map of *Boccardia proboscidea* in South Africa – large bordered circles represent point sources (abalone farms), smaller circles represent wild populations.

Fig. 3. Sampling localities for *Polydora hoplura* with three known phylogeographic breaks within the sampling areas; circled numbers represent sites where an adequate sample size was retrieved and asterisks represent sites with at least one commercial-scale shellfish farm in the area.

Fig. 4. Haplotype network for *Polydora hoplura* based on A) mtDNA - *Cyt b* and B) nDNA - *ATPSa* sequence data. Size of 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 additional change.

Fig. 5. Oceanographically driven dispersal of planktonic drifters on the South African coast showing: A) trajectory of drifters released at five different localities, B-F) probability distribution of drifters seeded from different starting points, with mean surface velocity overlaid where distribution > 0 (i.e. everywhere a float reaches).

Fig. 6. Connectivity of drifters among selected localities. Plots separated to illustrate drifter connectivity between west coast to east coast sites and vice versa. Blank plots refer to 0 connectivity within the 1 year integration period.

For Peer Review

Tables

Table 1: Sample sizes for *Polydora hoplura* collected from seven localities representing the cool temperature Namaqua biogeographic province and the warm temperate south coast Agulhas biogeographic province along the coast of South Africa.

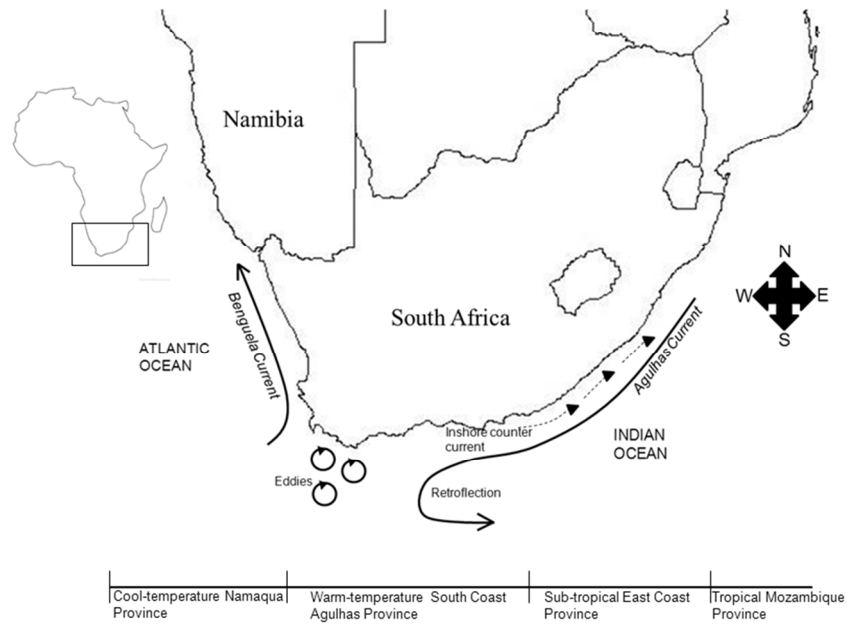
Site no.	Biogeographic region	Locality	Genetic marker	
			Cyt <i>b</i>	<i>ATPSα</i>
2	Cool-temperate	Paternoster	15	11
3		Saldanha Bay	15	12
4		Jacobsbaai	15	12
5	Warm-temperate	Bettysbaai	14	12
7		Mossel Bay	15	10
8		Knysna	15	10
9		Port Elizabeth	15	12

Table 2: GPS co-ordinates of localities chosen for Lagrangian drifter release.

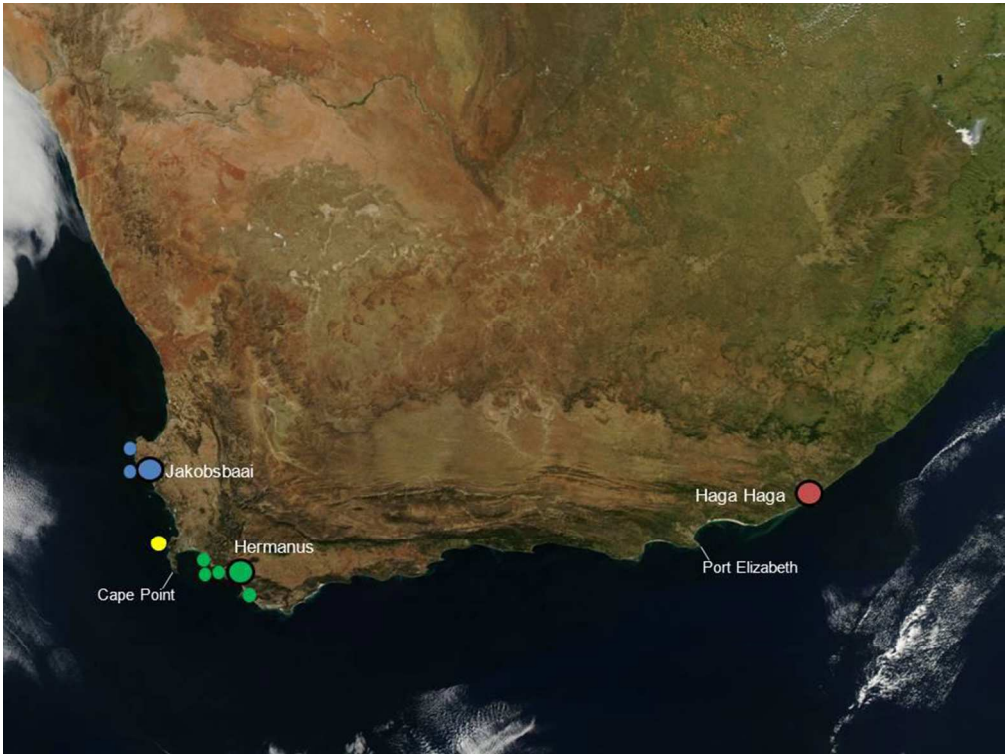
Locality	Latitude	Longitude
Jacobs Bay	32°58'8" S	17°52'51" E
Saldanha Bay	33°2'20" S	17°59'19" E
Hermanus	34°24'55" S	19°15'20" E
Port Elizabeth	33°49'33" S	25°44'44" E
Haga Haga	32°45'46" S	28°15'20" E

Table 3: Hierarchical AMOVA for *Polydora hoplura* populations based on Cyt *b* and *ATPS α* DNA sequences. Populations grouped into clusters based on biogeographic region.

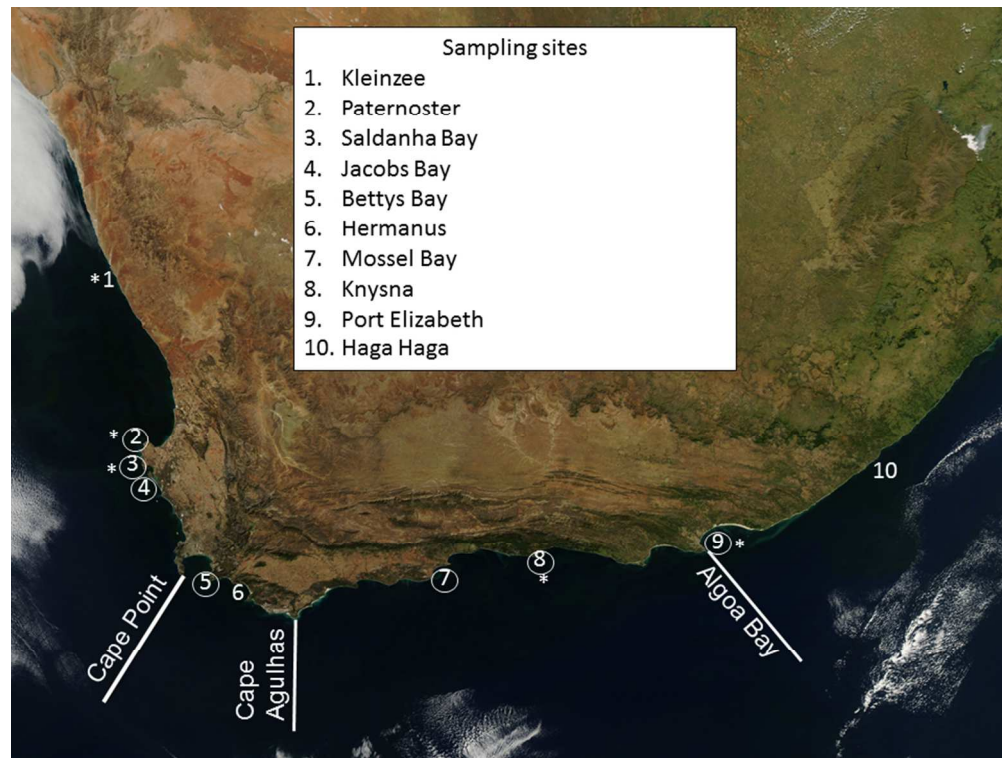
Source of variation	df	Sum of squares	Variance components	Percentage of variation
Cyt <i>b</i>				
Between clusters	1	11.10	0.15 V _A	4.42
Within clusters	102	337.08	3.30 V _B	95.58
Total	103	348.18	3.46	
<i>ATPSα</i>				
Between clusters	1	12.98	0.14 V _A	4.25
Within clusters	142	441.65	3.11 V _B	95.75
Total	143	454.63	3.25	



Map of southern Africa showing the major biogeographic provinces along with key oceanographic features.
254x189mm (96 x 96 DPI)

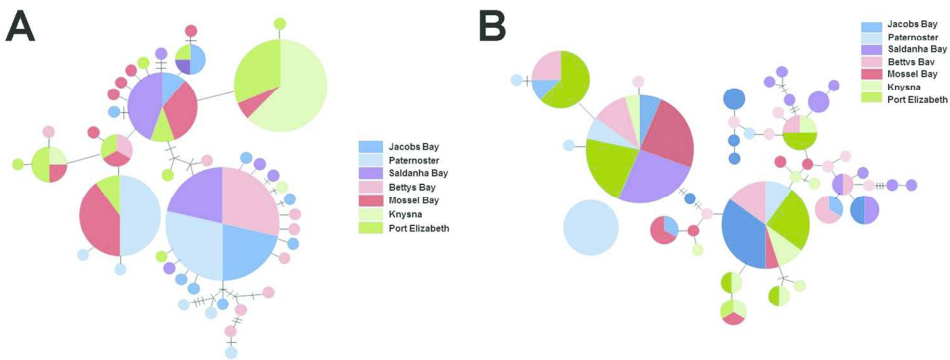


Distribution map of *Boccardia proboscidea* in South Africa – large bordered circles represent point sources (abalone farms), smaller circles represent wild populations.
254x190mm (96 x 96 DPI)



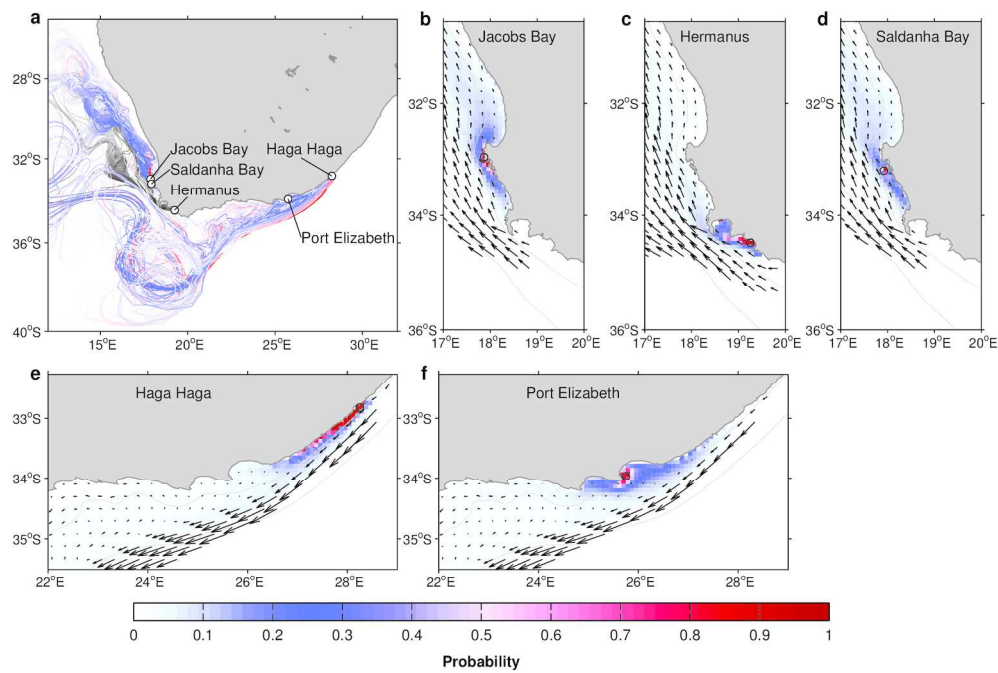
Sampling localities for *Polydora hoplura* with three known phylogeographic breaks within the sampling areas; circled numbers represent sites where an adequate sample size was retrieved and asterisks represent sites with at least one commercial-scale shellfish farm in the area.

254x190mm (96 x 96 DPI)

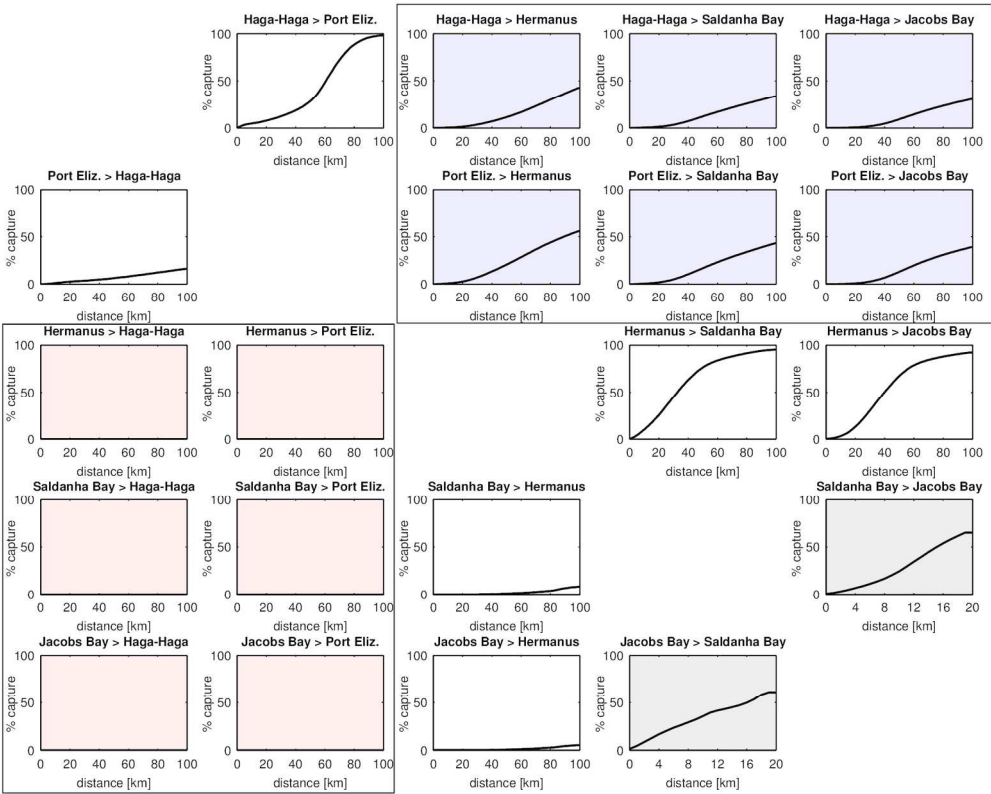


Haplotype network for *Polydora hoplura* based on A) mtDNA - Cyt b and B) nDNA - ATPSa sequence data. Size of circles is representative of individuals with that haplotype. The smallest circles represent a haplotype frequency of one. Each connecting line between haplotypes represent one mutational step and perpendicular lines represent an additional change.

149x63mm (300 x 300 DPI)



Oceanographically driven dispersal of planktonic drifters on the South African coast showing: A) trajectory of drifters released at five different localities, B-F) probability distribution of drifters seeded from different starting points, with mean surface velocity overlaid where distribution > 0 (i.e. everywhere a float reaches). 186x127mm (300 x 300 DPI)



Connectivity of drifters among selected localities. Plots separated to illustrate drifter connectivity between west coast to east coast sites and vice versa. Blank plots refer to 0 connectivity within the 1 year integration period.
168x134mm (300 x 300 DPI)