C•I•B
DST-NRF Centre of
Excellence for
Invasion Biology

Marine alien species in Western Cape harbours, South Africa: A tool for strategically focusing monitoring efforts

Koebraa Peters

This thesis is presented as a partial fulfilment of the Master of Science (Conservation Biology) Degree in the Percy FitzPatrick Institute of African Ornithology, Department of Biological Sciences, University of Cape Town.

Supervisors: Prof. Charles Griffiths and Dr. Tamara Robinson

May 2013

DEDICATION

To Iesrafeel, for keeping me going through this year and to my Mother for allowing me to be just who I am today…

Cover Photo: Koebraa Peters

Plagiarism Declaration

I, the undersigned, know the meaning of plagiarism and declare that all of the work in this minor dissertation, save for that which is properly acknowledged, is my own, carried out in the Percy FitzPatrick Institute of African Ornithology in the Department of Biological Sciences, University of Cape Town. It has not previously in its entirety or in part been submitted to any University. Any other sources of information are fully acknowledged.

....

Koebraa Peters

....

Date

ACKNOWLEDGEMENTS

First of all, an enormous Thank You to Andrea Plos, because without her this study would not have been possible. Then also a special thank you to Pieter Truter and Steve (as well as the two student divers) who, along with Andrea, made my data collection possible.

Thank you to my supervisors, Charles Griffiths, who was always present to give me a helping hand when needed and to Tammy Robinson, for her dedication and for always making time for me in her busy schedule.

To all my friends who helped me through this year, thank you so much, this CB class is the best class with the best group of friends I could ever have imagined. For the late nights, frantically typing away reports and the early mornings working on our theses, you guys are the best. Thanks to Vera Liebau, Lea Cohen, Caz Sanguinetti, Kirsten Retief, Jessica Greenston and Chrissie Madden for driving me around and opening their homes to me.

To my family for their constant support and encouragement, I cannot thank you enough. Also, to my fiancé Iesrafeel Abbas for always being there, encouraging me to take breaks and waking me up at 12:00 PM to continue with my work. Words cannot describe what your support means to me.

To my friends who are not in my CB class, but were always there to help me, especially Saachi Sadcha, thank you so much for helping with identification and thanks to you, Kirsty McQuaid and Vera for keeping me sane during data processing. Also, thank you to Jonathan Jonker and Tammy for ensuring that my harbour data were available to me. Thank you to Marc Rius for helping me to identify some ascidian species. Thank you to Peter Ryan for advising all of us on our projects and to Megan Laird for helping me to identify anemones.

A huge thank you goes to the National Research Foundation for assisting in funding my MSc degree. Thank you so much to the Centre for Invasion Biology, the Percy FitzPatrick Institute and everyone in the Department of Zoology (University of Cape Town) who offered their help.

If I have forgotten to mention anyone, I do apologise. I therefore, thank each and everyone for helping me get through this year.

Table of Contents

ABSTRACT

Alien species are the second most important cause for the loss in biodiversity globally, after habitat destruction. Marine alien species are transferred across the globe through various vectors, including ballast water, hull fouling, aquaculture facilities and the aquarium and pet trade. Ballast water has previously been considered as the primary vector of alien species transfer. However, fouling is becoming widely recognised as an important vector for the transfer of marine alien species both internationally, as well as in South Africa, where it has been reported to contribute 48% of marine species introductions. The objectives of this study were to document alien species from fouling assemblages in six South African harbours (St Helena Bay, Saldanha Bay, Table Bay, Hout Bay, Gansbaai and Mossel Bay) and to use the data collected to identify factors (such as vectors and other harbour characteristics and activities), that could be used by management authorities to target harbours upon which to focus monitoring efforts. This was done by taking subtidal scrape samples and visual samples from harbour walls and pillars. The prioritisation of harbours was obtained through the use of regression tree models utilising CART (Classification and Regression Trees).

Of the 86 known marine and estuarine alien species, 19 were detected in this study. These included species from the taxonomic groups Ascidiacea, Bivalvia, Brachiopoda, Bryozoa, Crustacea, Hydrozoa, Polychaeta and Porifera. Table Bay and Hout Bay had the highest number of alien species (13 species each, from scrape samples). Table Bay, Hout Bay and Mossel Bay had significantly more species than St Helena Bay (Kruskal Wallis: H (5, 47) = 29.35, $p<0.05$; Multiple comparisons: $p<0.05$). Unexpectedly, Hout Bay, a small local fishing harbour, had the most alien species in visual (mean 4.4 ±0.6 SE) and scrape (mean 5.1 ±0.74 SE) samples. Table Bay and Hout Bay harbours were similar in alien species number, composition and biomass. This could be due to a combination of their fairly enclosed nature, their proximity to one another (therefore experiencing similar climatic and biogeographic conditions), as well as intra-regional vessel movement. For the percentage cover of alien species per m^2, there was a change in space occupancy by different taxonomic groups. In the most western harbour (St Helena Bay) the dominant space occupiers were Bivalvia (contributing 67.5% ±7.32 SE) and in the eastern-most harbour (Mossel Bay), the dominant space occupiers were Ascidiacea (contributing 70.1% ±4.46).

The Mediterranean mussel *Mytilus galloprovincialis* was the only species detected in all the harbours and contributed most towards the biomass of alien species in four of them (St Helena Bay, Saldanha Bay, Gansbaai and Mossel Bay). Low biomass values detected in Table Bay and Hout Bay were thought to be due to the presence of the invasive European crab *Carcinus maenus*. The Disc lamp shell *Discinisca tenuis* appeared in both St Helena Bay and Saldanha Bay harbours. This is the first known record for the spread of this brachiopod species from aquaculture facilities, where it was previously thought to be restricted. This finding suggests the potential of intra-regional transfer of marine alien species in the region. The regression tree models for both the visual and scrape samples indicated that harbours that contain yachts and are smaller than 6.27 km^2 had the most alien species. Given the limited sample size of this study, this information can be used as a baseline study which should be expanded by increasing the sample size in order to make robust recommendations. The outcomes of this study does however show that the use of regression tree models and rapid port surveys can be useful for prioritising harbours for monitoring. This is specifically directed at fouling, since management in this sector is lacking intra-regionally.

Keywords: Marine alien species, Vectors, Fouling, Regression trees, Monitoring

Chapter 1: MARINE ALIEN SPECIES – CURRENT KNOWLEDGE

1.1. Introduction

Alien species are recognised as the second most important threat to biodiversity, following habitat destruction (Wilcove et al., 1998). The introduction and transfer of marine species across the globe has been occurring since humans began navigating the oceans (Carlton 1987, 1999). Alien species are those that have been anthropogenically introduced (intentionally or unintentionally) into an area in which they do not naturally occur (Richardson et al., 2000). The terms non-indigenous, alien, and invasive species are used interchangeably throughout the literature (see Mineur et al., 2012) which may result in confusion, thus for the purpose of this paper I will refer to a species that has been moved, through anthropogenic activity into a novel environment, as an alien species and those specifically related to the marine environment will be termed marine alien species. Marine alien species are globally recognised as a threat to native marine species and are an important driver of environmental change (Bax et al. 2003). Traditionally, alien species that impose an impact on native species or their environment, or that have economic impacts, are termed invasive (IUCN, 1999). However, recently there has been recognition that impact alone is not sufficient to define an invasive species, thus the spreading of a species into a novel environment is now considered as the defining characteristic of an invasive species, regardless of whether it has a quantifiable impact or not (Blackburn et al., 2011).

The overall impact of an invasive alien species has three dimensions, namely the total area occupied by that species, its abundance (either in number of individuals or biomass) and the effect it has per individual, or per biomass unit (Parker et al. 1999). Impacts of invasive species manifest at five different levels (Parker et al. 1999). Firstly, at the level of individuals which includes changes in variables such as growth rate and mortality rate, for example the alien alga *Acanthophora spicifera* reducing the growth rate of native algae in Pearl Harbour (Russel, 1992). The second level is involved with genetic effects such as hybridisation, for example the hybridisation of *Crassostrea* species (Gaffney and Allen, 1993). Thirdly, impacts can be observed at the level of the population, where effects on population dynamics, such as population growth and abundance, can occur. An example of this includes the negative effects of the invasive invertebrate predator *Bythotrephes longimanus* on zooplankton populations (Pangle et al., 2007). The fourth level can be observed in communities, such as alterations in species richness, diversity and trophic structure. An example of this has been shown by the invasive ctenophore *Mnemiopsis leidyi* in the Black Sea, which had negative impacts on the pelagic community structure influencing mesozooplankton, ichtyoplankton fish resources (Shiganova, 1998). The last level involves effects on ecosystem processes, such as nutrient availability. An example of this is the presence of the invasive broccoli weed *Codium fragile* spp. *tomentosoides* in Nova Scotia which has caused changes in sedimentation rates and nutrient cycling (Trowbridge, 1998).

Invasive alien species are able to cause social, economic and human health impacts by compromising biodiversity services (Molnar et al., 2008). The most serious social and economic impacts caused by invasive alien marine species are impacts that negatively affect human health, or those that cause decreases in economic production of marine-based activities, such as fisheries, aquaculture and tourism (Shiganova, 1998; Bax et al., 2002; Bax et al., 2003; Sephton et al., 2011). These impacts may feed back into social impacts through decreases in employment, in economic activities that are directly impacted by invasive alien species (Bax et al., 2003).

The concept of invasional meltdown is a model which holds that, as the cumulative number of species introductions increases, the ecosystem becomes more susceptible to additional invasions, due to alien species facilitating one another (Simberloff & Von Holle, 1999). Invasional meltdown highlights the serious conservation implications that may develop as a result of the spread of alien species (Parker et al., 1999). However, the premise of invasional meltdown is controversial as it has been acknowledged that there is a lack of evidence for this model (Simberloff, 2006). Even so, there is some evidence for the facilitation of alien species by other alien species (e.g. O'Dowd et al., 2003; Relva et al., 2010). Thus it has been suggested that even a partial reduction in the pressure exerted by mechanisms of introduction for marine alien species could produce major benefits (Parker et al., 1999). This suggests that efforts to prevent invasions, particularly vectors of alien species transfer, should be the focus of management actions.

1.2.Vectors of marine alien species

For centuries, marine species have been transported and introduced between distant areas and through this extensive time period, the vectors of introduction have changed (Griffiths et al., 2009). According to Lockwood et al. (2007), a vector is the way in which a species is transported along a pathway and that pathway is the route that exists between the source and the region of release. Historical vectors of marine alien species include wooden vessels and dry ballast (Griffiths et al., 2009), while modern vectors include external hull fouling (Carlton, 1999), ballast water (Carlton, 1987; Coles et al., 1999; Hewitt et al., 2009), aquaculture (Eldredge, 1994; Mead et al., 2011a; Haupt et al., 2012) and the aquarium and pet trade (Ruiz et al., 1997, Hayes, 2002, Bax et al., 2003).

Wooden vessels and dry ballasts are no longer in common use, suggesting that a different suite of species would have been transferred historically, compared to the ones being transferred currently (Griffiths et al., 2009). Dry ballast was used in early wooden vessels

(Carlton, 1996), where solid materials, such as coastal sand and rocks, were used to regulate buoyancy and to stabilise the vessels (Carlton, 1996; Griffiths et al., 2009). This resulted in several intertidal species being added to the vessel, along with the solid material and several accidental additions, such as coastal plants, seeds and insects (Carlton, 1985; Griffiths et al., 2009). Due to the semi-dry environment that was created, meiofauna and infauna of sandy and cobble habitats were able to survive and were offloaded along with the dry ballast at the arrival port termed the 'ballast point' (Carlton, 1996). Dry ballast, however, was replaced with ballast water in the late 1800s (Hewitt et al., 2009). This resulted in a change from the translocation of species that usually attach themselves to rocks and that burrow into sand, to those species that are floating or swimming in the water profile, such as planktonic organisms (Hewitt et al., 2009).

Ballast water as a vector of marine alien species has been given significant attention in the scientific literature (Coles et al., 1999; Ruiz et al., 2000; Awad et al., 2003; Hewitt et al., 2009) and by the late 1990's, was thought to be the primary vector responsible for alien invasive species transfer (Coles et al., 1999). This resulted in the International Maritime Organisation (IMO) introducing voluntary ballast water guidelines in 1997 (GESAMP, 2002). This was followed by the IMO's International Convention for the Control and Management of Ships' Ballast Water and Sediments, which was adopted during a Diplomatic Conference in 2004 (IMO, 2004). The aim of the Convention is to prevent, minimise and eliminate risks associated with the transfer of harmful ballast water organisms, to the environment, human health, property and resources (IMO, 2004). The IMO has also developed a demonstration port survey, as well as a management plan, which aims to reduce the risk of marine alien species transfer in ballast water (GESAMP, 2002). Because ballast tanks are able to retain water and sediment, planktonic species and those associated with sediments may be transported via this vector (Hewitt et al., 2009).

Previously, ballast water has been recognised as the primary pathway for the introduction of marine alien species globally (Coles et al., 1999; Ruiz et al. 2000). However, it is becoming more apparent that hull fouling is responsible for a large proportion of alien introductions (e.g., Thresher, 1999; Sink et al., 2012; Godwin, 2005). Historically, wooden ships were intensely encrusted with fouling organisms (Carlton 1999). It has been estimated that a wooden vessel in the 1700s was able to carry about 120 marine fouling species, either boring into, or nestling on the hull, plus 30 species associated with dry ballast and the anchor chain (Carlton 1999). The problem with wood-boring species is that not only do they damage the vessels, but they also start damaging the infrastructure (wooden piers and pilings) in the areas to which they have been introduced (Griffiths et al., 2009). Presently, most vessels are made of steel, thus a completely different suite of fouling organisms is being transferred across the globe (Griffiths et al., 2009). However, small local wooden vessels (e.g. fishing vessels) still occur in ports in South Africa (personal observation). It is also important to consider internal plumbing of ships, which are susceptible to fouling (Bax et al., 2003). More than half of the recognised marine alien species in the UK have been associated with shipping, with the main mechanism of transport being fouling (Eno, 1996). Following the same trend, in Australia hull fouling has been shown to be the primary mode of introduction of marine alien species historically (Thresher, 1999). Shipping, therefore plays an important role in the transfer of marine alien species across the globe (Hewitt et al., 2009; Minchin et al., 2009), resulting from both ballast water and hull fouling.

Fouling impacts on shipping by reducing the speed of the vessels, and in turn results in additional use of fuel to maintain speed (Hewitt et al., 2009). Fouling assemblages are able to establish and accumulate when vessels have long port layover times (Lee & Chown, 2007) and travel at slow speeds (Hewitt et al., 2009). The manufacturing and use of anti-fouling paints, as well as increase in speed of modern vessels, has decreased the extent of hull fouling; however, fouling is still apparent on many vessels, especially smaller ones (Bax et al., 2003) which are likely neglected in terms of maintaining hull anti-fouling measures. Additionally, studies on the South African National Antarctic Supply (SANAP) vessel, the SA Agulhas have shown that the antifouling technology used on such vessels have limited success in controlling fouling, since fouling organisms are still detected on hulls (Lee & Chown, 2009) and sea chests (Lee & Chown, 2007).

The use of tributyltin (TBT)-based paints was used extensively to prevent fouling on the hulls of vessels (both small boats and commercial vessels) since the 1960s (Smith et al., 2008). However, due to the negative impacts on organisms, the use thereof was banned during 1987 in the UK, but only for fish farm equipment and vessels less than 25 m long (Great Britain-Parliament, 1985), since larger vessels were difficult to enforce management upon (Matthiessen et al., 1995). In Brazil its use on vessels was only banned in 2003 (Fernandez et al., 2005). Thus, the use of TBT on vessels has progressively been banned in countries from 1987 onward (IMO, 1999). The International Convention on the Control of Harmful Anti-fouling Systems on Ships was adopted in 2001 and came into force during 2008 (IMO, 2011). This Convention prohibits the use of harmful organotins (such as TBT) in anti-fouling paints and it also formed a mechanism that will prevent the potential future use of other harmful substances in anti-fouling systems (IMO, 2011). Annex I of the Convention states that no ships are to apply or reapply organotin compounds that act as biocides in anti-fouling systems. This includes application to fixed and floating platforms, as well as floating storage units, floating production storage and offtake units (IMO, 2011). The reason that hull fouling may therefore be responsible for a large proportion of alien introductions could be linked to the fact that TBT-based anti-fouling paints were effective and their banning could arguably have exacerbated the problem of fouling. Therefore, although the problem of using harmful substances has been addressed, there is no convention that deals with fouling as a problem.

Aquaculture poses several risks for the introduction of alien species. Firstly, the culture species can escape and establish self-sustaining populations in the wild (Robinson et al., 2005b, Haupt et al., 2012), or the target species may carry associated epifauna, parasites or diseases, which could establish as alien species (Minchin et al., 2009). As an example, several aquaculture studies have focussed on the consequences of farming oysters, in the context of alien species introductions (e.g. Eldredge, 1994; Minchin, 1996; Robinson et al., 2005b). These studies have shown that species associated with oysters, such as polychaetes that burrow into the shells of oysters, or bivalves that attach themselves to oysters, as well as algae and other organisms, all have the potential to be transported with aquaculture species. A European study has reported the introduction of oyster predators (the gastropod *Ocinebrellus inornatus*) that were transported with American oysters in the 1880s (Minchin et al., 1995), which could have detrimental impacts on naturally-occurring shellfish in the region. These studies clearly demonstrate the importance of aquaculture as a vector for alien species transfer and it should therefore be considered as a target for management.

Developed countries have become well-advanced in terms of research and monitoring programmes in the field of alien and invasive species (Olenin et al., 2007). A country that has a particularly good monitoring system with regards to marine alien species and alien species in general is Australia, with several published studies having monitored and reported the eradication of marine alien species in harbours (Connell & Glasby, 1999; Connell, 2001; Hewitt & Martin, 2001; Sliwa et al., 2009; Cribb et al., 2010). These eradication programmes were organised and implemented by the government to ensure the removal of the alien species (e.g. Bax et al., 2002; Bax et al., 2003). One such programme was implemented in the Darwin Harbour Estuary, where routine monitoring for alien species allowed for the detection of an unidentified mussel species (later identified as *Mytilopsis* sp.) in one of the marinas (Bax et al., 2002). Consequently, rapid response by the Northern Territory

Government and Australian national agencies allowed for a risk assessment to assess the situation, as well as to identify how the problem would be solved. The marinas were then quarantined through the use of their double lock gates in order to proceed with chemical treatment to eradicate the alien mussel species. After 12 months the eradication of the species was confirmed (Bax et al., 2002), establishing this as the first successful eradication of a marine alien species. Thus, early detection of marine alien species is important and subsequently, rapid response should follow (Hulme, 2006). In California, response to the detection of the invasive marine alga *Caulerpa taxifolia*, proceeded 17 days after its discovery, when containment and eradication treatments were executed and resulted in exceptional progress toward its eradication (Anderson, 2005). Contrastingly, in the Mediterranean Sea, although *C. taxifolia* was discovered in 1984, the impacts of its presence were only realised five years later, after which it had already become well established and colonised more than 100 km^2 of benthos in the next decade (Meinesz, 1999). These examples indicate that it is easier to manage or eradicate a species if an invasion is identified early on, which is why regular monitoring of areas that are susceptible to invasions are important.

1.3. Harbours – Centres for marine invasions

The constant increase in the human population and the expansion in the tourism industry (Glasby, 1999), as well as the growing network of shipping traffic on a global scale (Drake & Lodge, 2004), have played an important role in the development of coastal infrastructure such as harbours and marinas required for services of economic, recreational, residential and aesthetic value (Glasby, 1999). These structures are also built as defence mechanisms (e.g. breakwaters and seawalls) against harsh weather conditions and the need for these structures is predicted to increase, due to intensification of storms and sea level rise as a result of climate change (Bulleri & Airoldi, 2005). As with any form of urban infrastructure, the development may be displacing previous natural habitat, or having other impacts on the environment. Harbours play a particular role in creating novel marine habitats (Bax et al., 2002) that may contribute to the homogenisation of biota in coastal areas (Bulleri & Chapman, 2010) by acting as colonisation corridors (Bax et al., 2002). These harbours and marinas are permanent, sheltered and shallow subtidal habitats (Arenas et al., 2006) influenced by frequent disturbances (such as boat traffic and maintenance) and shifts in environmental conditions such as tides, salinity stress (Bax et al., 2003) and large changes in temperature (Arenas et al., 2006). Due to the physical structure of harbours, the areas usually have reduced water flow, elevated turbidity and abrasion by sediments (Bulleri & Chapman, 2010).

In relation to marine alien species the most important feature of these harbours is, however, the constant vessel activity. This allows for the transportation of the organisms that establish on artificial structures. As a result, harbours become areas with a high influx of alien organisms (Bax et al., 2003). The combination of a sheltered environment and a means of dispersal via shipping, results in an increase in opportunity for alien species to establish in harbours (Bax et al., 2003). Because harbours are scattered along coastal regions, they form fragmented patches of habitat that not only provide a suitable environment for alien species to invade, but also facilitate the spread of alien species, by functioning as corridors across areas of unsuitable habitat (Bulleri & Airoldi, 2005). Some studies have shown that alien species occur more commonly on floating docks and other artificial substrates, compared to adjacent natural substrates (Glasby & Connell, 2001, Glasby et al., 2006, Bulleri & Airoldi, 2005), further highlighting the fact that harbours are susceptible to invasion. Another reason why alien species are more likely to occur in harbours is due to the areas from which they came. Since most species are picked up in ports, they are already exposed to a shallow, sheltered environment and therefore the new port likely presents similar conditions for species to establish in. Ascidians (such as *Ascidiella aspersa*, *Ciona intestinalis, Botryllus schlosseri, Styela clava*) are a common group that establishes on artificial structures and dominates the area in terms of biomass (Arenas et al., 2006). Furthermore, certain species (such as the black striped mussel *Mytilopsis sallei*) become excellent invaders, often modifying the artificial environment in such a way that they act as system engineers (eg, Simberloff & Von Holle, 1999). These are examples of species that were introduced to harbours or marinas and were able to establish themselves and spread rapidly, demonstrating the usefulness of artificial structures or an artificial environment to such species. Similarly, Vaselli et al. (2008) demonstrated that coastal-defence structures, such as breakwaters in normally soft-bottomed environments, allowed for the establishment of native and alien species usually associated with rocky environments. The mussel *Mytilus galloprovincialis* was abundant on the seaward side of breakwaters, whereas filamentous algae dominated on the landward side. The artificial environment provided suitable habitat for the presence of the alien alga *Caulerpa racemosa* and allowed it to persist, by providing hard substrata and high rates of sediment, which this species is able to tolerate (Piazzi et al., 2007). This type of environment was therefore likely to allow the species to become more abundant, which was said to eventually enhance its long distance dispersal (Vaselli et al., 2008). In cases like these the presence of an artificial environment can exacerbate the spread of alien species.

1.4. Marine alien species in South Africa

Marine alien species had not been considered as a topic in South Africa until 1992, when the first review took place and listed 15 introduced species (Griffiths et al., 1992). Since this time much effort has been focused on documenting recent invasions and their impacts (Branch &

Steffani, 2004; Robinson et al., 2005a; Bownes & McQuaid, 2006; Rius & McQuaid, 2006; Zardi et al., 2006; Hampton & Griffiths, 2007, Xavier et al., 2007; Hanekom, 2008; Laird & Griffiths, 2008; Branch et al., 2008; Branch et al., 2010; Rius et al., 2011; Haupt et al., 2012) with the most recent publication on the status of alien and cryptogenic marine and estuarine species moving on to consider historical invasions as well. This latest work listed 86 introduced and 39 cryptogenic species along the South African coast (Mead et al., 2011a). These species represent 17 major taxonomic groups and consist of both plant and animal species (Mead et al., 2011a). While the introduction history of marine alien species and their associated vectors, distribution patterns and systematics are now well documented, there is still a lack of data quantifying the impacts of many of the listed species and therefore, no indication of how they may have impacted the environment, or other species (Mead et al., 2011a).

In terms of the vectors responsible for the introduction of marine alien species in South Africa, hull fouling contributes 48% of the introductions, whereas ballast water only contributes 38% (Mead et al., 2011b). These vectors are followed by aquaculture and petroleum infrastructure (Sink et al., 2012). In South Africa, there are several mechanisms that pose a risk for transferring alien species intra-regionally (Sink et al., 2012). These include aquaculture (Robinson et al., 2005b, Rius et al., 2011; Haupt et al., 2012), the movement of recreational vessels, such as yachts (Floerl & Inglis, 2005; Jurk, 2011) and regional shipping, which includes fishing vessels (Sink et al., 2012). For example, vessels in the small pelagic fishery operate without any spatial management regulations in place and therefore vessels are able to move intra-regionally to follow the sardine and anchovy stocks (see Fairweather et al., 2006; Pichegru et al., 2009). Due to this lack of managing the fishery and thereby vessel movement, the likelihood of marine alien species introductions is high. Additionally, in the Western Cape 62% of sampled recreational vessels were shown to have secondary levels of fouling on their hulls and alien species were present in these fouling communities (Jurk, 2011). Furthermore aquaculture, such as the farming of oysters, also plays a role in the intra-regional transfer of alien species, due to the transportation of other species associated with the cultured species (Robinson et al., 2005b, Haupt et al., 2012). A recent experimental study (Haupt et al., 2012) indicated that the translocation of *Crassostrea gigas* (oysters), even after undergoing the usual cleaning processes for the commercial industry, still maintains a host of species either burrowing into the shells, or attaching themselves to the shells. Although in low numbers, these species still have the potential for being introduced into the area that the oysters have been translocated to. Species that survived various levels of treatment, as well as the translocation, included the invasive polychaete *Polydura hoplura*, the invasive mussel *Mytilus galloprovincialis* and *Ascidia* species. Several additional species occurred on uncleansed oysters (Haupt et al., 2012). The oyster operation in the Knysna Estuary operates by purchasing juveniles in Jeffreys Bay (where spat are imported from Chile and France). These are then grown in the estuary for four months and then translocated to an oyster farm in Algoa Bay, where they are left to grow to market size and returned to the Estuary. Although oysters are manually cleaned, the consignments are not inspected for associated species and therefore, the potential of transferring species in both directions is high (Haupt et al., 2012).

Previously, it has been shown that six of the known alien species in South Africa have become invasive by having negative impacts on indigenous species and spreading rapidly. These species are *Balanus glandula* (Pacific barnacle), *Carcinus maenas* (European green crab), *Ciona intestinalis* (Sea vase ascidian), *Crassostrea gigas* (Pacific oyster), *Mytilus galloprovincialis* (Mediterranean mussel) and *Semimytilus algosus* (Bisexual mussel) (Sink et al., 2012). As an example of the impacts that invasive species have had in South Africa, the Mediterranean mussel *Mytilus galloprovincialis* was first detected in 1979 along the west coast (Branch et al., 2008) and since then, has spread extensively along rocky shores, competitively displacing indigenous species and transforming the ecosystem by allowing the establishment of several other species (Robinson et al., 2005a; Bownes & McQuaid, 2006; Branch et al., 2010). Additionally, *M. galloprovincialis* became invasive to such an extent that it was even able to spread into a sandbank habitat in Langebaan Lagoon (Robinson & Griffiths, 2002) where it eventually died off, however (Robinson et al., 2007). *M. galloprovincialis* was also deliberately introduced from the west coast to the south coast for mariculture purposes (Branch & Steffani, 2004). However, they have been shown to grow slower in the southern regions (Steffani & Branch, 2003). Furthermore, Steffani & Branch (2003) reported that *M. galloprovincialis* was scarcer and grew slower in sheltered sites compared to wave-exposed sites, suggesting that its competitive ability with other species was likely minimal. On exposed shores, this invasive mussel has also been reported to potentially displace the indigenous limpet *Scutellastra argenvillei* completely (Steffani & Branch, 2005). Branch et al. (2008; 2010) demonstrated the changes that occurred with the subsequent invasion of *M. galloprovincialis* by reporting significant declines in the tube-building polychaete *Gunnaria capensis* with the expansion of *M. galloprovincialis* on semi-exposed and exposed shores. A negative relationship was found between *M. galloprovincialis* and adults and recruits of the indigenous limpet *Scutellastra granularis* occupying most of the space, which intensified with wave exposure (Branch et al., 2008; 2010). These studies have all taken place along rocky shores of South Africa and they report extensive findings on the negative impacts of *M. galloprovincialis*.

Other alien species with the potential of impacting on the natural environment and biodiversity are three species that were unintentionally imported with oyster spat (*Ostrea edulis*). These are the Chilean sea urchin *Tetrapygus niger*, the European crab *Xantho incisus*, and the Namibian brachiopod *Discinisca tenuis* (Haupt et al., 2010). Although these species are currently localised they have the potential of becoming problematic. For example, *T. niger* is a well-known economic and ecological pest in its original distribution (northern Chile), where it completely destroys kelp beds by grazing on them (Rodriguez, 2003; Vega et al., 2005). This is important because there are several commercially valuable kelp-bed ecosystems on the west coast of South Africa (Branch & Griffiths, 1988) which could be an enormous loss if *T. niger* is able to invade these ecosystems. Additionally, although information on the impacts of *X. incisus* is not precisely known, the fact that it is a predator could mean that it could damage shellfish populations in the region (Haupt et al., 2010), whether affecting native species and thereby biodiversity, or aquaculture species and thereby affecting the economy. As with the European crab, information on the impacts of the brachiopod *D. tenuis* are lacking, although because it is a filter feeder, Haupt et al. (2010) suggested that it may compete with native fauna for both food and space and may also have a negative impact on the consumers of shellfish, since it attaches to oysters and other shellfish. Whether these impacts will arise is not known, but their potential requires action on South Africa's part to be able to avoid problematic situations, such as invasion and its subsequent impacts.

Three port surveys have been completed in South Africa (in Saldanha Bay, Port Elizabeth and Richards Bay), documenting the species occurring in the harbours, as well as highlighting the presence of alien species (Awad et al., 2003; Hutchings et al., 2006). Only two of these (Awad et al., 2003; Hutchings et al., 2006) are discussed in detail, since the information regarding the survey in Richards Bay has not been released to the public. The first survey was conducted in Saldanha Bay in 2002 and was a large-scale project which focussed on assessing the risks associated with ballast water (Awad et al., 2003). The survey involved data collection with the use of the CRIMP protocols (GloBallast, 2001). Subtidal scrape samples from hard substrates, bottom transects to visually sample soft substrates, tubular cores to sample benthic infauna and dinoflagellate cysts, rotenone samples for fish species, plankton tows, crustacean traps and beach seine nets, as well as intertidal samples, were all used to collect data for the port survey (GloBallast, 2001). This survey recorded 14 marine alien species and three cryptogenic species present in the harbour (Awad et al., 2003).

The survey of Port Elizabeth harbour took place in 2005 and sampling occurred during spring (September) at three depth zones. Scrape samples and visual samples were collected from hard substrata (Hutchings et al., 2006). Additionally, gill nets and trek nets were used to sample fish species and sediment cores to sample benthic organisms. Phytoplankton and zooplankton were also sampled. Only five alien species were reported in the Port Elizabeth survey. Four of these were bryozoans (*Bugula dentata*, *Bugula neritina*, *Steginoporella buskii* and *Watersipora subovoidea*) and one was the amphipod *Monocorophium ascherusicum* (Hutchings et al., 2006).

All of these surveys, however, focussed on large harbours (Port Elizabeth, Saldanha, and Richards Bay), with no studies explicitly considering alien species in smaller harbours. This is problematic, since port surveys are especially important when researching marine alien species, as they are known sites of introduction (e.g. Coles et al., 1999; Glasby, 1999; Connell, 2001; Bax et al., 2002; Bulleri and Airoldi, 2005).

The present study therefore recognises this gap and aims to firstly, document alien fouling species in six harbours along the western and southern Cape coasts. This is particularly important since these harbours include four that have never been previously surveyed for alien species. Secondly, it aims to use the data collected, to identify factors that can be used by management authorities to decide which harbours should be prioritised for monitoring. This study offers a baseline assessment of marine alien species in Western Cape harbours, which could be expanded to the rest of the coast.

Chapter 2: MARINE ALIEN SPECIES IN SIX SOUTH AFRICAN HARBOURS

2.1. INTRODUCTION

2.1.1. Artificial environments with vectors of alien species transfer

Alien species are a global problem and have caused immense conservation concern (Parker et al., 1999; Bax et al., 2003). These species are concentrated in areas along the coast associated with anthropogenic activity, particularly harbours. Harbours are artificial environments that have produced a platform for the establishment of alien species through their sheltered nature, which provides both subtidal and intertidal habitats (Arenas et al., 2006). The infrastructure associated with harbours provides several substrata onto which species establish. These can include harbour walls, pillars (made either of concrete or wood), pontoons, pilings, buoys and ropes (Lambert and Lambert, 2003; Floerl & Inglis, 2003; Cohen et al., 2005). Alien species occur more often on artificial substrata than natural substrata within the same proximity (Glasby et al., 2006, Bulleri & Airoldi, 2005). Additionally, the design of a harbour can exacerbate the extent of fouling (Floerl & Inglis, 2003). Harbours that were partially enclosed (compared to unenclosed harbours) supported a larger number of organisms recruiting to artificial surfaces (including harbour infrastructure and vessel hulls) (Floerl & Inglis, 2003). The level of recruitment by fouling organisms was also influenced by the local tidal amplitude, the volume of the harbour basin relative to the size of the entrance channel (Floerl & Inglis, 2003), as well as the volume of freshwater input into the harbour, since this is likely to affect larval retention and survival of certain species (Bax et al., 2003; Floerl & Inglis, 2003).

In South Africa, there are several large harbours (such as Table Bay, Saldanha Bay and Richards Bay) which are major international ports, supporting regional commercial and international shipping activity, yachting, petroleum infrastructure and aquaculture facilities (Ports and Ships, 2012). There are also small, local harbours which focus on regional shipping, yachting and fishing activity, such as Hout Bay and Mossel Bay. All of these activities can act as vectors or pathways for marine alien species (e.g. Floerl et al., 2005; Robinson et al., 2005b; Haupt et al., 2012). This highlights the fact that harbours, whether large or small, have the potential for having multiple vectors or pathways and the more of these a harbour has, the higher the chance for the arrival of alien species. Williamson (1996) and Mack et al. (2000) suggested that the probability of an alien species occupying an available niche on a vector in a source location is related to (i) the abundance and selectivity of the vector, and (ii) the local supply of colonising propagules (the abundance of larvae). Thus if vectors are not monitored and managed efficiently, then the impacts associated with alien species are likely to be exacerbated (Sink et al., 2012).

This emphasises the risks associated with multiple vectors, since certain species are only able to be transported with specific vectors. For example wood-boring organisms with wooden vessels (see Griffiths et al., 2009), as well as oyster predators (such as the gastropod *Ocinebrellus inornatus*) with oyster translocations (Minchin et al., 1995) and other oyster-associated species (Haupt et al., 2012). Similarly, planktonic species can only be transported by ballast water (Hewitt et al., 2009). Due to the presence of alien species from various vectors in one location, multiple vectors are likely to increase the risk of introducing marine alien species into an environment. For example, the dinoflagellate *Alexandrium minutum* (via ballast water), the cnidarian *Metridium senile* (via ship fouling) and the bivalve *Ostrea edulis* (through aquaculture), all occur on the West Coast due to several vectors (Mead et al. 2011a). This further poses a problem in terms of management, since the presence of several vectors, suggests that different management strategies need to be developed in order to regulate the problem (Sink et al., 2012). This would require lots of manpower, financial input, planning and monitoring, as seen in the South African port surveys in Saldanha and Port Elizabeth (Awad et al., 2003; Hutchings et al., 2006). Since developing countries often lack either one or all of these facilities, the risks imposed by multiple vectors is of serious concern (Sink et al., 2012, Mead et al., 2011b). Although the habitat (harbour infrastructure) and the vectors contribute towards the presence of alien species in harbours, these are not the only factors influencing the establishment and survival of alien species. Environmental factors play a large role in the survival of alien species in the introduced area (e.g. Epelbaum et al., 2009).

The South African coastline is divided into six marine ecoregions; the Southern Benguela, Southeast Atlantic, Agulhas, Natal, Delagoa and Southwest Indian ecoregions (Sink et al., 2012). The focus of this study is in the Southern Benguela and Agulhas ecoregions, but only focuses on the coastal areas where harbours occur. Within the six designated ecoregions, developed during the 2011 National Biodiversity Assessment for South Africa, there are 22 ecozones (Sink et al., 2012) of which four (Southwestern Cape inshore, Southwestern Cape inner shelf, Namaqua inshore and Agulhas inshore) are the focus of this study. Due to the nature of these ecoregions and ecozones in terms of biogeography, depth patterns, distinct species assemblages, climate and resources (Sink et al., 2012), the ecozones may offer different physical environments to arriving alien species. The Southern Benguela ecoregion is a high productivity region due to the cold Benguela Current resulting in a nutrient-rich upwelling system (Cushing, 1971). Due to this high productivity, it is therefore also the region in which most of the fish-processing factories occur (Sink et al., 2012), which is linked to the small pelagic fishery (Fairweather et al., 2006). In contrast, the Agulhas ecoregion experiences warm water intrusions from the Agulhas Current (Shillington & Harris, 1978) that consists of nutrient-poor, tropical water (Griffiths et al., 2010). It also experiences upwelling of cold South Atlantic central water, which is driven by wind (Shillington & Harris, 1978); however the region is not as productive as the Benguela ecoregion (Sink et al., 2012).

2.1.2. Lack of marine alien species monitoring in South Africa

In South Africa, we have very limited resources, both financial and manpower, for appropriate planning and coordination of marine alien species management (Sink et al., 2012). Due to this lack of resources and uneven sampling found along the South African coast, the true spread of invasions is likely to be obscured (Sink et al., 2012). Monitoring of marine alien species in South Africa is lacking, partly due to limited taxonomic expertise (Griffiths et al., 2009) and baseline data in the form of port surveys (Parker et al., 1999). According to the National Biodiversity Assessment, there is a need for surveys along the south and east coasts (Sink et al., 2012). Furthermore, even though the need for prevention mechanisms (such as management interventions) of future marine invasions was highlighted in the National Spatial Biodiversity Assessment (Lombard et al., 2004), this issue has still received minimal attention in South Africa (Sink et al., 2012).

Australia is a good example to follow, since they are able to detect and manage marine alien species rapidly, through continuous monitoring implemented by government and national agencies (Bax et al., 2002). In contrast, research and monitoring in South Africa is much needed, specifically focussing on understanding the historical and current processes that shape marine invasive alien populations (Sink et al., 2012). Due to the poor monitoring system and lack of available data in South Africa, we therefore need a way of focussing monitoring/surveillance in order to target harbours that can act as a rapid response method for marine alien species occurrences.

2.1.3. Aims of the study

This study focuses on fouling organisms in six harbours on the west (St. Helena Bay, Saldanha Bay Table Bay, Hout Bay) and south (Gansbaai and Mossel Bay) coasts of South Africa. Due to the nature of harbours, being shallow, sheltered habitats with a high flux of boat traffic and associated activities, as well as aquaculture facilities, all of these factors influence the species composition occurring on artificial structures that make up the harbours. The aims of the study are therefore to (1) document alien fouling species in the six harbours and (2) use the data collected to identify factors (such as vectors and other harbour characteristics and activities) that can be used by management authorities to target harbours upon which to focus monitoring efforts.

The specific objectives of this study were therefore to:

1) Determine the relative number of alien species in the six selected harbours in South Africa
2) Determine the percentage cover and biomass per m^2 of each alien species per harbour
3) Compare the alien species occurrences and abundances among the six harbours
4) Determine the contribution of various taxonomic groups towards the total number and biomass, as well as percentage cover of alien species
5) Determine whether the number of vectors and/or pathways present in a harbour is correlated to the number of alien species present
6) Determine the factors that potentially influence the presence of alien species in harbours in this region

2.2. MATERIALS AND METHODS

2.2.1. Study sites

The fieldwork for this study took place in six harbours along the Western Cape coastline. These were St. Helena Bay, Saldanha Bay, Table Bay, Hout Bay, Gaansbaai and Mossel Bay harbours (Figure 1). Sampling occurred during September, October and November 2012. The first four of the harbours occur in the Southern Benguela ecoregion and the remaining two in the Agulhas ecoregion (Figure 1 and Table 1). All of the harbours are marine and an attempt was made to spread sampling throughout the harbours.

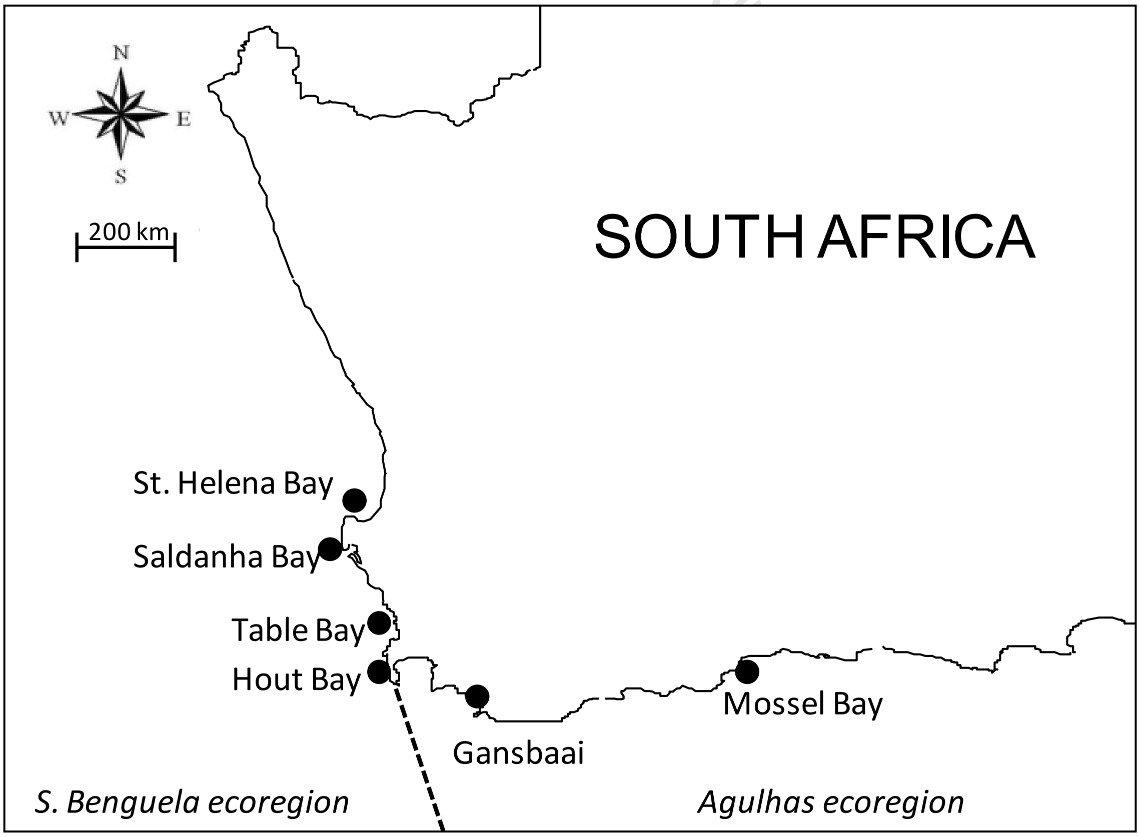

Figure 1. Locations of the six harbours sampled on the South African coastline. The dashed line separates the Southern Benguela and Agulhas ecoregions.

2.2.2. Data collection

In order to obtain data on the presence of alien species in harbours, 10 visual samples (1x1m quadrat) were taken subtidally to detect large, mobile species, which could be missed in smaller scrape samples (as in the port survey from Port Elizabeth; Hutchings et al., 2006). The dominant substrate sampled was concrete harbour walls and in Hout Bay and St Helena Bay, approximately 25% of the samples were collected from wooden pillars. Unfortunately, visibility in Gansbaai and Hout Bay harbours was poor and only two and five visual samples could be collected at these harbours, respectively. Species counts were obtained for alien species from a target list (Appendix 1) and percentage cover of each of those species were recorded by divers. Samples were collected to detect the relative number of alien species and the wet biomass of these species were recorded, thus scrape samples were taken from the bottom right hand corner of each 1x1 m visual sample for consistency. Ten subtidal scrape samples (15x15 cm quadrat) were collected by divers per harbour, at depths between 1 – 5 m. These were taken at random locations in the harbour, with samples separated by at least 1 m. However, for St Helena Bay only nine samples were adequate for identification since the 10th sample decomposed. Samples were collected by divers and were immediately preserved in 10% formalin in the field. Samples were removed with the use of metal (15x15 cm) quadrats and the biological material was scraped off using a paint scraper. The sample was collected in a mesh net and brought to the surface to be preserved. Divers were qualified scientific commercial divers that are often involved in this type of biological surveys for various companies and institutions. Diving was managed by a qualified diving supervisor from the University of Cape Town.

Species in visual and scrape samples were identified to the lowest possible taxonomic level. Species known to be alien to South African shores (obtained from Mead et al. 2011a) were specifically targeted (Appendix 1). Unknown species were retained, but due to the time limitations of this project, could not be pursued. The guides used to identify species were Day (1967), Day (1969), Griffiths (1976), Branch et al. (2010) and Paige et al. (2012). When uncertainty of species identification occurred, the relevant experts were contacted.

Data were collected regarding activity in the harbours, as well as the sizes of the harbours (Table 1). The data for the large harbours (i.e. Table Bay, Saldanha Bay and Mossel Bay) were collected from the website http://www.ports.co.za. The information for the smaller harbours was obtained from the harbour masters and yacht clubs. Photographs of each harbour were extracted from Google Earth and harbour area was calculated using Digimize image software (Digimizer 4.2.2.0, 2012). Information about the presence of aquaculture was obtained from Dr Sue Jackson (Stellenbosch University).

2.2.3. Data analysis

All the data were analysed in STATISTICA 10. Prior to univariate analyses, normality and homogeneity of variances were considered. As data were not normal, Kruskal-Wallis tests were used to analyse differences in the relative number of alien species recorded per sample among harbours, as well as the percentage cover and the biomass of alien species in various taxonomic groups. Significant differences were further explored using Multiple Comparisons tests. The relative number of alien species recorded in each harbour was compared using a Chi-squared Goodness of fit test. A Correlation analysis was run to detect whether the number of vectors and pathways present in a harbour influenced the total number of alien species recorded in each harbour. The Classification and Regression Tree analysis (CART) was used to produce regression tree models in order to determine which factors were the best predictors for the presence of alien species recorded in the various harbours (Floerl et al., 2005). The potential predictors used were ecoregion, aquaculture presence, harbour area, number of commercial vessels, international shipping, regional commercial shipping, ship repair and hull cleaning, petroleum infrastructure and yachts (Table 1). The number of commercial vessels in 2011/12 refers to the number of vessels visiting the port from March 2011 – February 2012 (Table 1). Yachting included both international and regional yachts and all of the harbours had regional fishing vessels, as well as regional repair and hull cleaning of fishing vessels. CART analysis does not recognise the six harbours as the sample size (i.e. it does not group the numbers of alien species based on the harbours). Each sample collected is seen as a separate entity and therefore the number of predictor variables used (listed in Table 1) does not exceed the number of samples in the CART analysis. The "best" tree was selected through the use of V-fold cross-validation, due to the relatively small sample size. This method of pruning runs the analysis multiple times with different randomly drawn samples from the data set. Each time a subsample of the dataset is left out from the computations and that subsample is used as a test sample for cross-validation. The "best" tree is selected as the tree with the best average accuracy for cross-validated predicted values (StatSoft Inc., 2011). The cost associated with selecting the "best" model or "right-sized" tree is computed as a CV (cross-validation) cost. The CV cost was chosen by following Breiman et al. (1984) since this chooses the "right-sized" tree as the smallest tree whose costs do not exceed the minimum CV cost (i.e. the tree with the lowest cost) plus 1 times the standard error of the CV costs for that minimum CV cost tree. Following Breiman et al. (1984), this method of selecting the appropriate tree was chosen, as it helps to avoid "over fitting" and "under fitting" of data and is a powerful method for pruning trees when working with small datasets (StatSoft Inc., 2011)

Table 1. Factors considered as potential predictors for the number of alien species, and their units of measurement as used to construct regression tree models. *Petroleum infrastructure refers to oil rigs and vessels (associated with oil and gas) that spend time in harbours.

			Harbours					
	Predictor Variables	**Levels**	**St Helena Bay**	**Saldanha Bay**	**Table Bay**	**Hout Bay**	**Gansbaai**	**Mossel bay**
1	Ecoregion	Benguela/Agulhas	Benguela	Benguela	Benguela	Benguela	Agulhas	Agulhas
2	Aquaculture	Yes/No	No	Yes	No	No	No	No
3	Harbour area	km²	0.32	9.03	3.51	0.25	0.23	0.14
4	Commercial vessels in 2011/12	Number of vessels	0	528	2775	0	0	1567
5	International shipping	Yes/No	No	Yes	Yes	No	No	No
6	Regional commercial shipping	Yes/No	No	No	Yes	No	No	No
7	Ship repair and hull cleaning	Yes/No	Yes	Yes	Yes	No	No	Yes
8	Petroleum infrastructure*	Yes/No	No	Yes	Yes	No	No	Yes
9	Yachts (International & Regional)	Yes/No	No	Yes	Yes	Yes	No	Yes

2.3.RESULTS

2.3.1. Visual samples

Seven alien species were visually recorded from a target list consisting of nine species (see Appendix 1). The seven species found were the sponge *Suberites ficus,* the bivalve *Mytilus galloprovincialis,* crustaceans *Balanus galandula* and *Carcinus maenus,* and the ascidians *Ciona intestinalis, Clavellina lepadiformis and Styela plicata.* The total number of alien species detected in each harbour varied from seven in Table Bay to two in St Helena Bay and Gansbaai (Figure 2). These differences were not statistically significant (Chi-Square Goodness of Fit: $\chi^2 = 9.17$, df = 5, p>0.05).

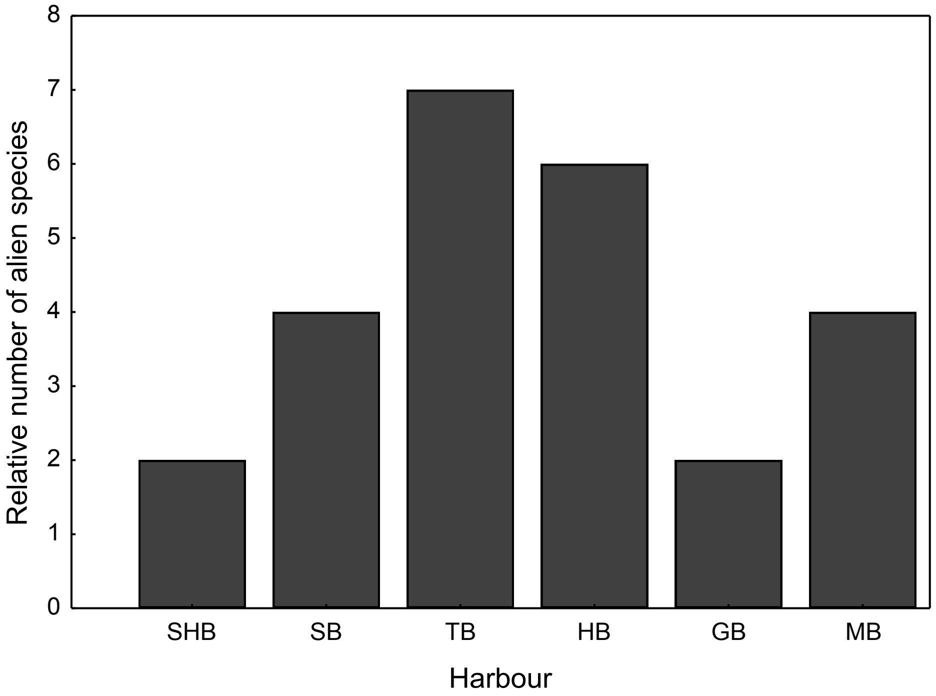

Figure 2. The relative numbers of alien species recorded visually in St Helena Bay (SHB), Saldanha Bay (SB), Table Bay (TB), Hout Bay (HB), Gansbaai (GB) and Mossel Bay (MB) harbours.

There was a significant difference in the number of alien species detected per sample among the six harbours (Kruskal-wallis; $H_{(5, 47)} = 29.35$, $p<0.05$). The number of alien species in St Helena Bay (1.1 ±0.1 SE) was significantly lower than that found in Table Bay, Hout Bay and Mossel Bay (Multiple comparisons; $p<0.05$) (Figure 3).

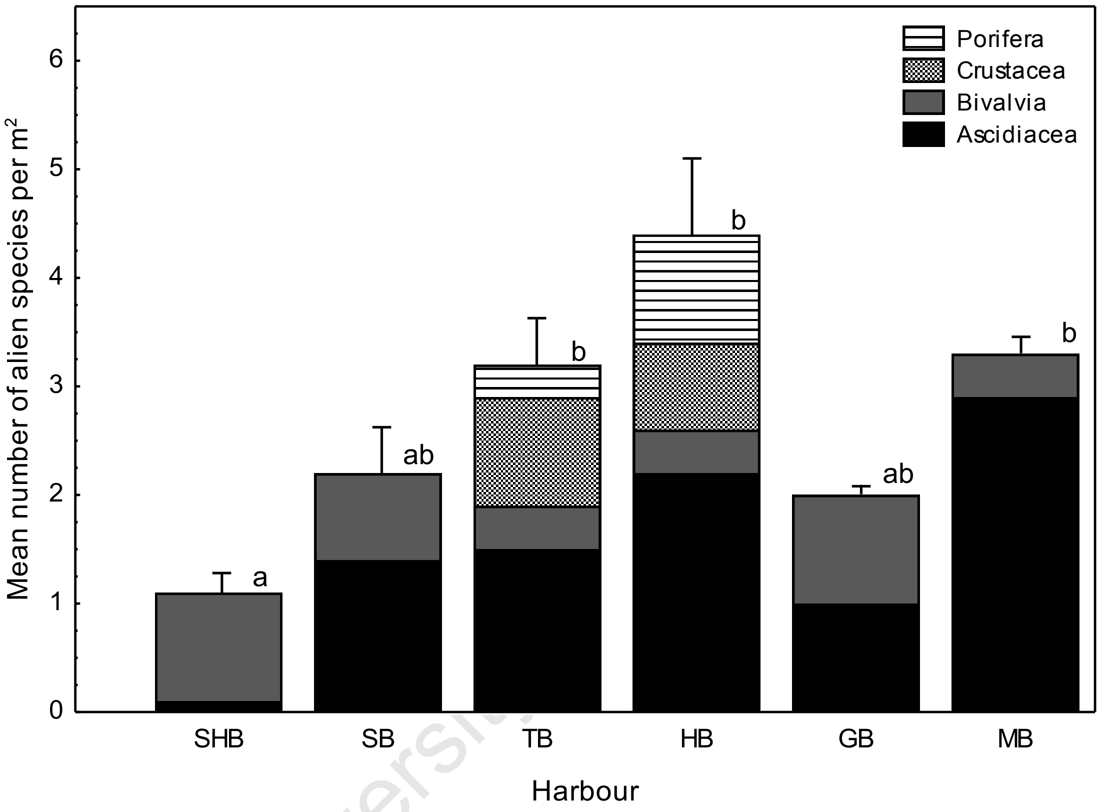

Figure 3. The mean number (+SE) of alien species per sample, in each taxonomic group recorded visually in St Helena Bay (SHB), Saldanha Bay (SB), Table Bay (TB), Hout Bay (HB), Gansbaai (GB) and Mossel Bay (MB) harbours. Letters differ where harbours supported a significantly different number of alien species (all taxa combined).

Significant differences were detected in the number of alien species in the various taxonomic groups among the harbours (Table 2). Alien Ascidiacea and Bivalvia were found in all of the harbours. In the west, St Helena Bay was dominated by Bivalvia while the most easterly port, Mossel Bay, was dominated by Ascidiacea. Alien Crustacea and one Porifera species were detected only in Table Bay and Hout Bay (Figure 3).

Table 2. The results of Kruskal-Wallis considering the number of alien species recorded per m^2 in the visual quadrats. Significant differences are denoted by p-values <0.001.

Taxonomic Group	**Kruskall-Wallis H-value**	**df**	**N**	**p-value**
Ascidiacea	33.38	5	47	<0.001
Bivalvia	24.05	5	47	<0.001
Crustacea	36.56	5	47	<0.001
Porifera	29.17	5	47	<0.001

There was a significant difference in the percentage cover of alien species among harbours (Kruskal-Wallis; $H_{(5,\ 47)} = 23.29$, $p<0.05$). The percentage cover of alien species was significantly lower in Saldanha Bay (30.2 ±4.82 SE) compared to St Helena Bay (67.5 ±6.47 SE), Hout Bay (75.2 ±10.84) and Mossel Bay (85.9 ±4.6) (Multiple comparsions: p<0.05) (Figure 4). Significant differences were also detected in the percentage cover of the various taxonomic groups among harbours (Table 3). Alien ascidians were dominant space occupiers in Mossel Bay, Hout Bay, Table Bay and Gansbaai (Figure 4). In Mossel Bay, the Light-bulb sea squirt *Clavellina lepadiformis* was the largest contributor (42.5% ±5.54 SE). The invasive Vase tunicate *Ciona intestinalis* was the largest contributor towards percentage cover in Hout Bay (18% ±3.74) and Table Bay (18% ±4.16 SE) (Table 4). Additionally, *C. intestinalis* was detected in all of the harbours except Gansbaai. The Mediterranean mussel *Mytilus galloprovincialis* was the only bivalve species detected visually, and was found in all of the harbours. Although it was found in each harbour, it was a dominant space occupier only in St

Helena Bay, Gansbaai and Saldanha Bay with mean percentage cover ranging from 17.6% ±4.82 (SE) to 65.5% ±7.32 (SE) (Table 4). The invasive European crab *Carcinus maenus* was detected only in Table Bay in the surveys of percentage cover (1.8% ±0.59 SE).

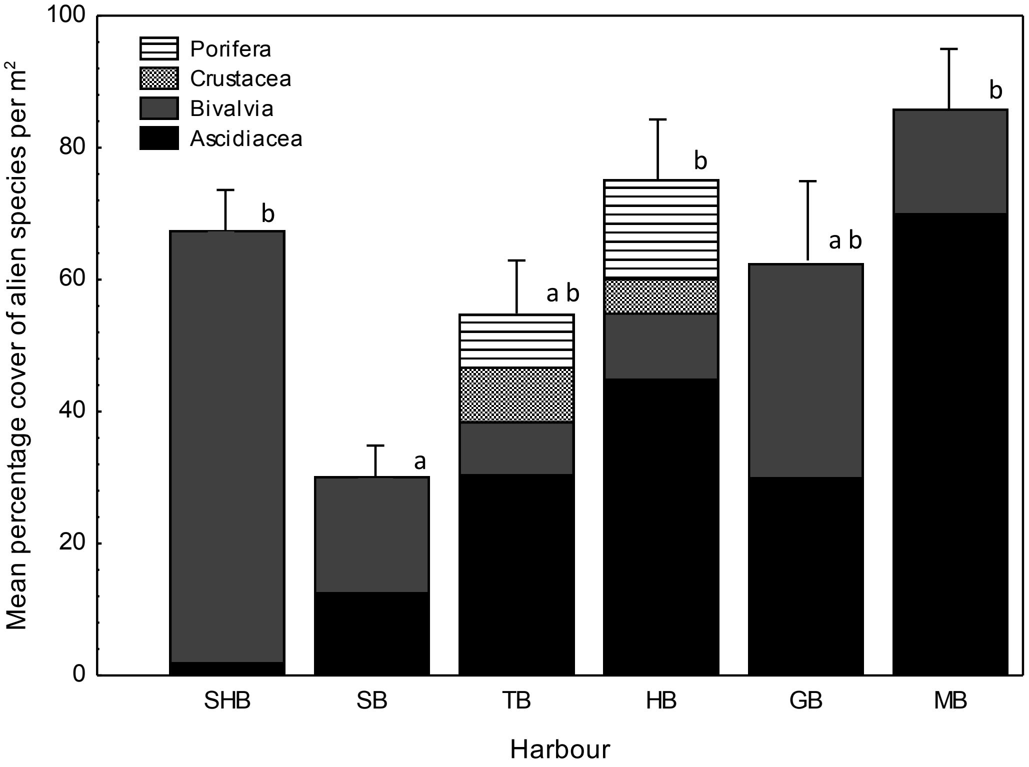

Figure 4. The mean percentage cover (+SE) of alien species per sample, in each taxonomic group recorded visually in St Helena Bay (SHB), Saldanha Bay (SB), Table Bay (TB), Hout Bay (HB), Gansbaai (GB) and Mossel Bay (MB) harbours. Letters differ where harbours supported a significantly different number of alien species (all taxa combined).

Table 3. The results of Kruskal-Wallis considering the percentage cover of alien species recorded per m^2 in the visual quadrats. Significant differences are denoted by p-values <0.001.

Taxonomic Group	**Kruskall-Wallis H-value**	**df**	**N**	**p-value**
Ascidiacea	30.65	5	47	<0.001
Bivalvia	13.78	5	47	<0.001
Crustacea	36.48	5	47	<0.001
Porifera	31.45	5	47	<0.001

Table 4. Mean percentage cover (±SE) of the alien species recorded per m^2 in the visual quadrats.

Taxonomic Group	**Species Name**	**Mean % Cover (per m^2) ±SE**					
		SHB	**SB**	**TB**	**HB**	**GB**	**MB**
Ascidiacea	*Ciona intestinalis*	2 ± 2	6 ± 2.08	18 ± 4.16	18 ± 3.74	0	18.1 ± 5.45
	Clavellina lepadiformis	0	4 ± 1.63	9.5 ± 2.83	18 ± 6.63	30 ± 0	42.5 ± 5.54
	Styela plicata	0	2.6 ± 1.33	3 ± 3	9 ±5.57	0	9.5
Bivalvia	*Mytilus galloprovincialis*	65.5 ± 7.32	17.6 ± 4.82	8 ± 4.23	10 ± 6.32	32.5 ± 12.5	15.8 ± 6.95
Crustacea	*Balanus glandula*	0	0	6.5 ± 2.59	5.2 ± 2.13	0	0
	Carcinus maenus	0	0	1.8 ± 0.59	0	0	0
Porifera	*Suberites ficus*	0	0	8 ± 4.16	15 ± 4.47	0	0

The regression tree model produced by the CART analysis indicated three terminal nodes and two non-terminal nodes. This model had a CV error of 1.03 ±0.24 (SE). The presence or absence of yachts was the factor that explained the largest relative proportion of variation in the data (Figure 5). The first split in the regression tree model indicated that harbours that had yachts, had more alien species (3.17 ± 0.3 SE) than those that did not (1.25 ± 0.01 SE). Harbour area was the next most important factor in predicting the number of alien species. Those harbours that had yachts and were smaller than 6.27 km^2 supported the highest number of alien species (3.56 ± 0.16 SE).

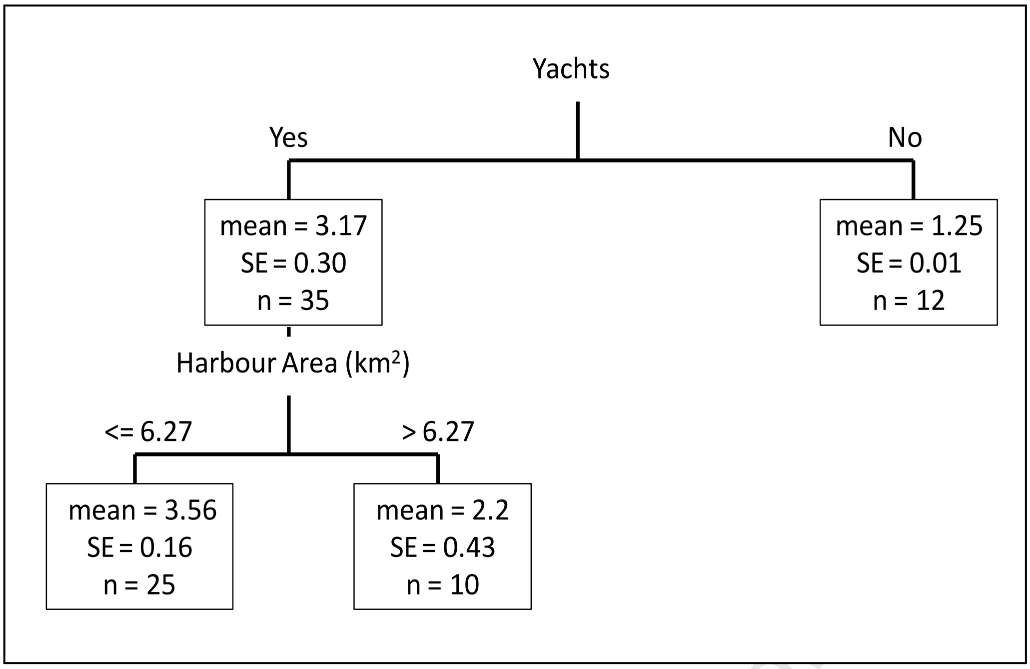

Figure 5. Regression tree for predicting the number of alien species (from visual samples) in harbour fouling communities. The mean, standard error (SE) and the sample size (n) are reported for each node. CV= 1.03 ±0.24 (SE).

2.3.2. Scrape samples

A total of 18 alien species from the taxonomic groups Ascidiacea, Bivalvia, Brachiopoda, Bryozoa, Crustacea, Hydrozoa, Polychaeta and Porifera were recorded in the scrape samples. The number of alien species recorded in each harbour varied from 13 in Table Bay and Hout Bay to four in St Helena Bay (Figure 6). These differences were not statistically significant (Chi-Square Goodness of Fit: $\chi^2 = 9.16$, df = 5, $p>0.05$). There was a significant difference in the number of alien species detected per m^2 among the six harbours (Kruskal-Wallis; $H_{(5, 59)} = 24.709$, $p<0.05$). Hout Bay had the highest number of alien species (5.1 ±0.74 SE) whereas Gansbaai had the least number of alien species (1.7 ±0.3 SE) (Figure 7). Significant differences were detected among the harbours but these were not systematic.

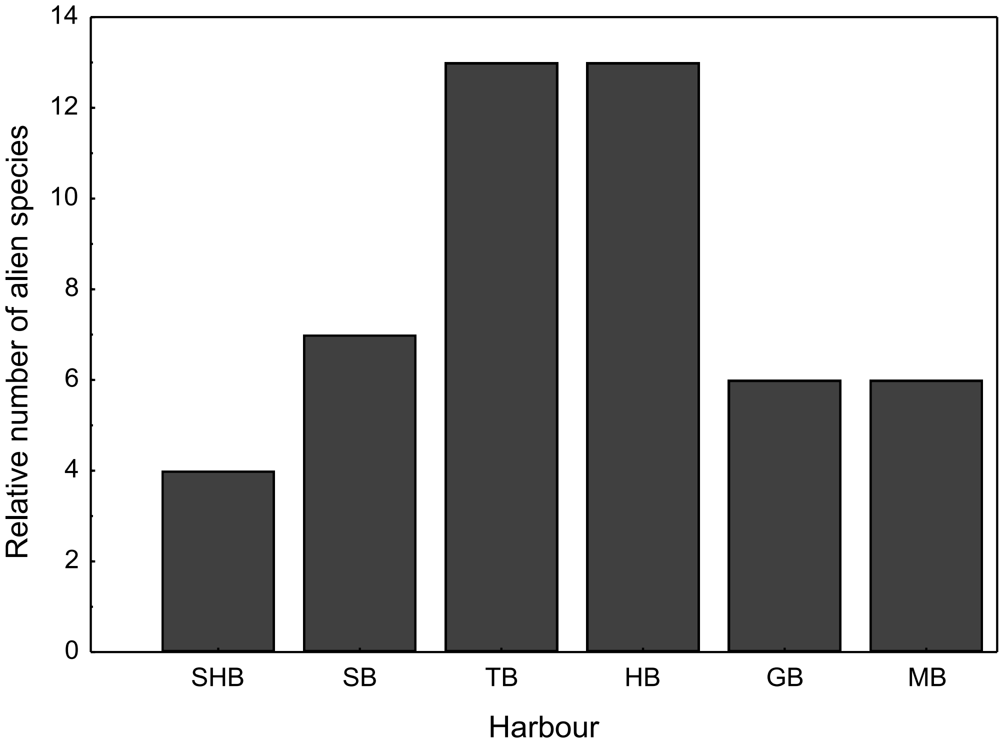

Figure 6. The relative numbers of alien species recorded from scrape samples in St Helena Bay (SHB), Saldanha Bay (SB), Table Bay (TB), Hout Bay (HB), Gansbaai (GB) and Mossel Bay (MB) harbours.

Significant differences were detected in the number of alien Ascidiacea, Bivalvia, Brachiopoda, Crustacea, Polychaeta and Porifera among the harbours (Table 5). There were no significant differences detected among harbours for Bryozoa and Hydrozoa. Bivalvia were detected in each harbour whereas ascidians were detected only in four harbours (Figure 7). The largest number of Ascidiacea species was detected in Hout Bay harbour which was also the harbour in which the largest number of taxonomic groups was detected (Figure 7).

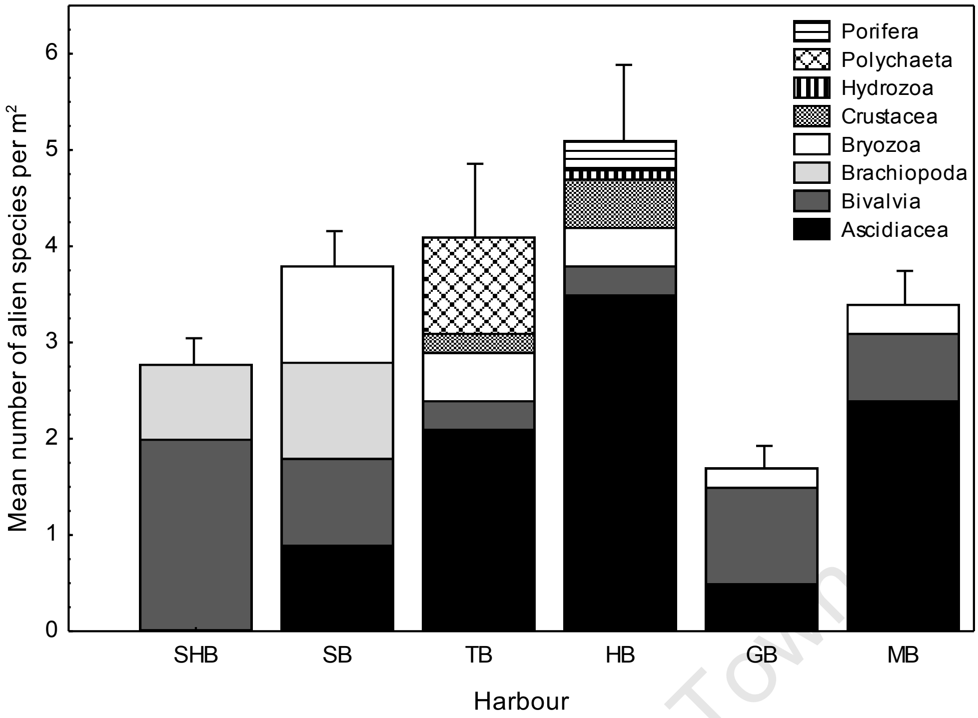

Figure 7. Mean number (+SE) of alien species per sample, in each taxonomic group, recorded from scrape samples in St Helena Bay (SHB), Saldanha Bay (SB), Table Bay (TB), Hout Bay (HB), Gansbaai (GB) and Mossel Bay (MB) harbours. The only significant statistical differences found was that HB differed from SB and GB, and TB differed from GB.

Table 5. The results of Kruskal-Wallis considering the number of alien species recorded per m^2 in the scrape quadrats. Significant differences are denoted by p-values <0.001 and <0.05.

Taxonomic Group	**Kruskal-wallis H-value**	**df**	**N**	**p-value**
Ascidiacea	33.94	5	59	p<0.001
Bivalvia	36.39	5	59	p<0.001
Brachiopoda	37.4	5	59	p<0.001
Bryozoa	5.96	5	59	p>0.05
Crustacea	15.08	5	59	p<0.05
Hydrozoa	4.9	5	59	p>0.05
Polychaeta	58	5	59	p<0.001
Porifera	15.22	5	59	p<0.05

There was a significant difference in the biomass of alien species among harbours (Kruskal-Wallis: H $_{(5,\ 59)}$ = 13.39, p<0.05). Gansbaai had a significantly lower biomass (2829.23g ±3989.27 SE) of alien species than Saldanha Bay (12 114.96g ±14 626.5 SE) (Multiple comparisons; p<0.05) (Figure 8).

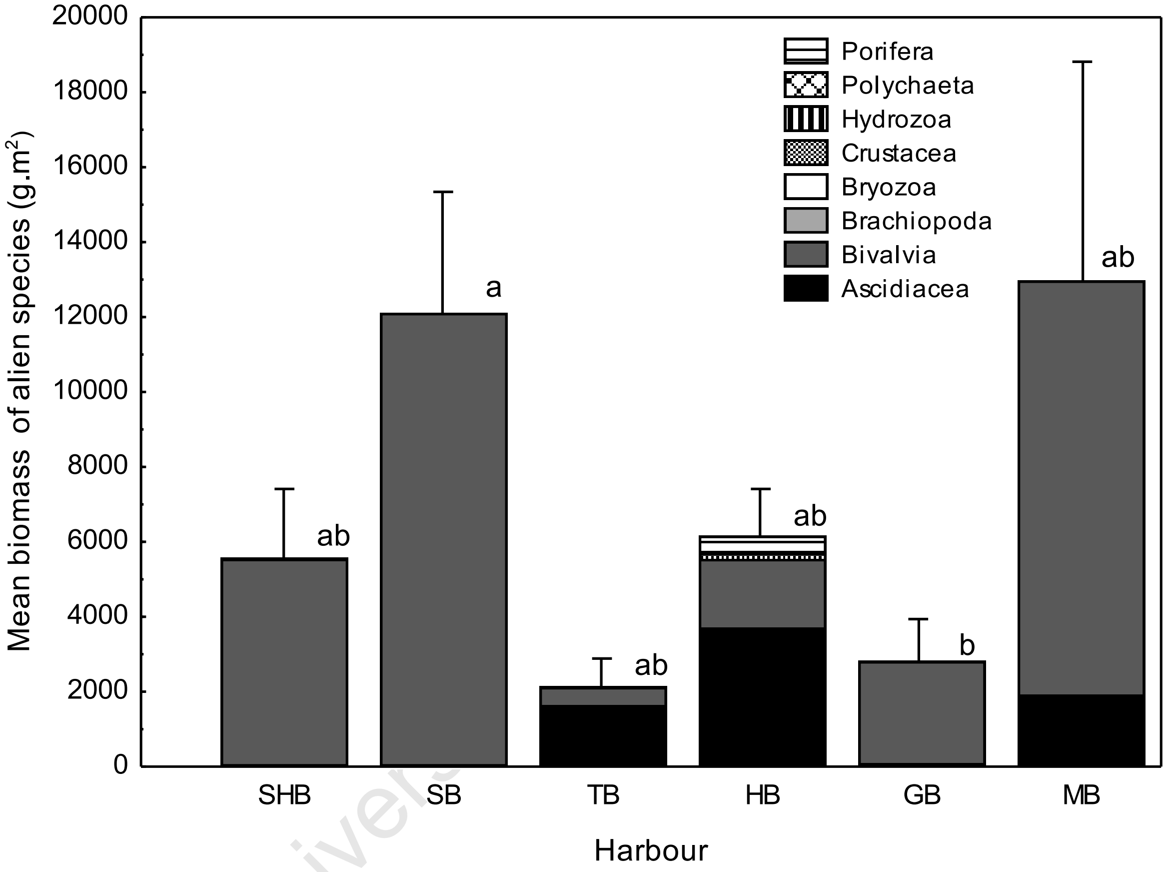

Figure 8. The mean biomass (+SE) of alien species per sample, in each taxonomic group recorded, from scrape samples in St Helena Bay (SHB), Saldanha Bay (SB), Table Bay (TB), Hout Bay (HB), Gansbaai (GB) and Mossel Bay (MB) harbours. Letters differ where harbours supported a significantly different number of alien species (all taxa combined).

Significant differences in the biomass of alien species were detected for Ascidiacea, Bivalvia, Brachiopoda, Crustacea, Polychaeta and Porifera among harbours. Bryozoa and Hydrozoa showed no significant difference in the biomass of alien species (Table 6). These two groups had biomass values less than 9.5 g.m^2 (Table 7). Bivalvia consisted of *M. galloprovincialis* and the Bisexual mussel *Semimytilus algosus*. This group contributed the largest proportion of biomass in four of the harbours predominantly due to the presence of *M. galloprovincialis*. However, in Table Bay and Hout Bay the largest contributors were Ascidiacea (Figure 8 and Table 7). In Table Bay, the Dirty sea squirt *Ascidiella aspersa* supported the highest biomass (1 512.38 g ±560.43 SE) and in Hout Bay, the Vase tunicate *Ciona intestinalis* dominated (2 331.14 g ±1 077.84 SE) (Table 7).

Table 6. The results of Kruskal-Wallis considering the biomass of alien species recorded per m^2 in the scrape quadrats. Significant differences are denoted by p-values <0.001 and <0.05.

Taxonomic Group	**Kruskal-wallis H-value**	**df**	**N**	**p-value**
Ascidiacea	40.83	5	59	p<0.001
Bivalvia	23.81	5	59	p<0.001
Brachiopoda	37.72	5	59	p<0.001
Bryozoa	5.66	5	59	p>0.05
Crustacea	14.76	5	59	p<0.05
Hydrozoa	4.9	5	59	p>0.05
Polychaeta	57.34	5	59	p<0.001
Porifera	15.21	5	59	p<0.05

No other species, besides *M. galloprovincialis,* was detected in all of the harbours. However, the ascidian *Botryllus schlosseri* occurred in five of the six harbours with the highest biomass in Hout Bay harbour (263.88 g ±246.85 SE) (Table 7). Similarly, the ascidian *C. lepadiformis* was also abundant in Hout Bay harbour (631.28 g ±195.19 (SE)). The Black coral worm *Dodecaceria fewkesi* was detected only in Table Bay harbour with a biomass of 1 150 g ±623.05 (SE) (Mutliple comparisons; $p<0.05$). The Disc lamp shell *Discinisca tenuis,* previously only recorded in an aquaculture facility in Saldanha Bay was abundant in St. Helena Bay harbour (33.93 g ±19.63 SE) and less so in Saldanha Bay harbour (1.08 g ±1.08 SE) (Table 7). The invasive European crab *Carcinus maenus* was detected only in Hout Bay in the biomass survey (150.05 g ±150.05 SE).

Twelve species were detected in the scrape quadrats but not in the visual quadrats. These were the ascidians *Ascidiella aspersa, Botryllus schlosseri, Cnemidocarpa humilis, Corella eumyota, Diplosoma listerianum,* the amphipod *Monocorophium ascherusicum,* the polychaete *Dodecaceria fewkesi*, the brachiopod *Discinisca tenuis*, the bivalve *Semimytilus algosus,* the bryozoans *Bugula neritina* and *Watersipora subtorquata* and the hydrozoans *Obelia dichotoma*. In contrast, only one of the species detected visually (the Pacific barnacle *Balanus glandula*) was not detected in the scrape samples.

Table 7. Mean biomass (±SE) of the alien species recorded per m^2 in the scrape quadrats.

Taxonomic Group	Species Name	MEAN biomass (g.m^{-2}) ± SE					
		SHB	SB	TB	HB	GB	MB
Ascidiacea	*Ascidiella aspersa*	0	0.24 ±0.78	1 512.38 ±560.43	148.08 ±79.88	0	0
	Botryllus schlosseri	0	2.07 ±1.96	5.48 ±4.02	263.88 ±246.85	1.59 ±1.59	1.16 ±0.55
	Ciona intestinalis	0	0	0.46 ±0.46	2 331.14 ±1 077.84	0	86.58 ±81.49
	Clavellina lepadiformis	0	0	4.66 ±3.45	631.28 ±195.19	86.70 ±63.44	579.74 ±145.29
	Cnemidocarpa humilis	0	9.59 ±6.19	77.43 ±77.43	295.98 ±182.13	1.64 ±1.64	0
	Corella eumyota	0	0	0.38 ±0.38	30.02 ±24.36	0	0
	Diplosoma listerianum	0	0.39 ±0.34	43.22 ±23.05	16.67 ±5.52	2.51 ±2.51	0
	Styela plicata	0	0	0	0	0	1 253.20 ±480.45
Crustacea (Amphipoda)	*Monocorophium ascherusicum*	0	0	0.10 ±0.08	0.1 ±0.06	0	0
Crustacea (Decapoda)	*Carcinus maenas*	0	0	0	150.05 ±150.05	0	0
Polychaeta	*Dodecaceria fewkesi*	0	0	1 150 ±623.05	0	0	0
Brachiopoda	*Discinisca tenuis*	33.93 ±19.63	1.08 ±1.08	0	0	0	0
Bivalvia	*Mytilus galloprovincialis*	5 119.35 ±2 329.55	12 101 ±2 512	484.11 ±304.67	1 830.16 ±1 607.7	2 727.36 ±1 126.08	11 056.5 ±5 521.18
	Semimytilus algosus	426.41 ±273.93	0	0	0	0	0
Bryozoa	*Bugula neritina*	0	0.06 ±0.06	3.98 ±2.97	0.96 ±0.73	0	0.6 ±0.56
	Watersipora subtorquata	0	0	0.19 ±0.13	0	9.42 ±6.76	0
Hydrozoa	*Obelia dichotoma*	0	0	0	0.36 ±0.36	0	0
Porifera	*Suberites ficus*	0	0	0	466.04 ±273.95	0	0

The regression tree model produced by the CART analysis indicated three terminal nodes and two non-terminal nodes. This model had a CV error of 3.03 ±0.62 (SE). As with the visual data, the presence or absence of yachts was the factor that explained the largest relative proportion of variation in the data (Figure 9). The first split in the regression tree model indicated that harbours that had yachts, had more alien species (3.72 ±3.13 SE) than those that did not (2.16 ±0.27 SE). Harbour area was the next most important factor predicting the number of alien species. Those harbours that had yachts and were smaller than 6.27 km^2 supported the highest number of alien species (4.3 ±2.84 SE).

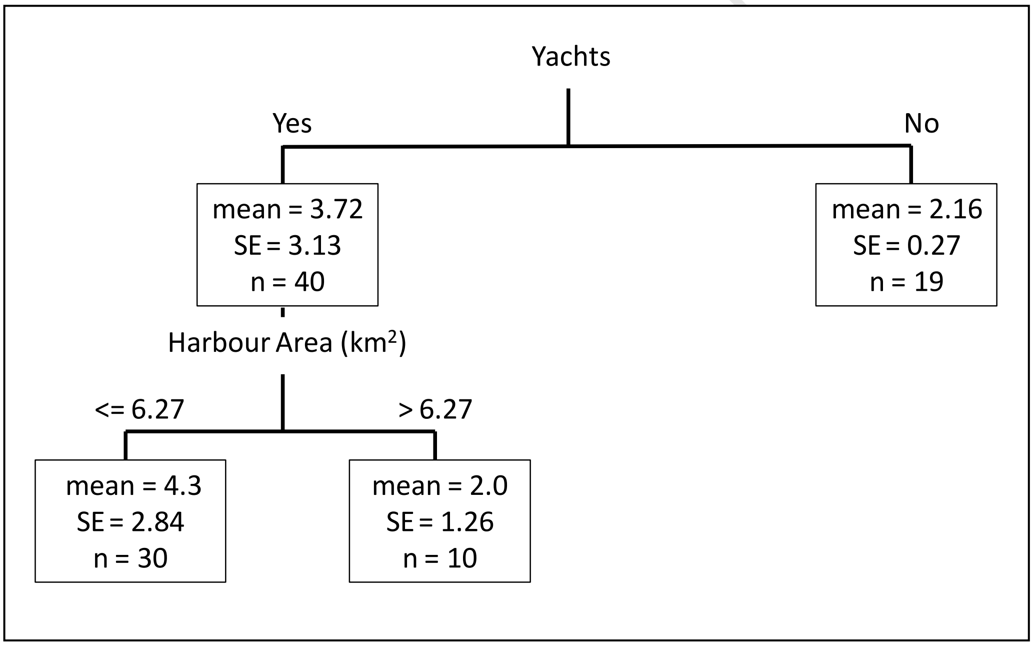

Figure 9. Regression tree for predicting the number of alien species (from scrape samples) in harbour fouling communities. The mean, standard error (SE) and the sample size (n) are reported for each node. CV= 3.03 ±0.62 (SE).

Overall, 19 alien species were detected. There was no relationship between the number of vectors and pathways present in the harbours and the relative number of alien species (including those from visual and scrape quadrats) per harbour (r^2 = 0.19, N = 5, $p<0.05$) (Figure 10).

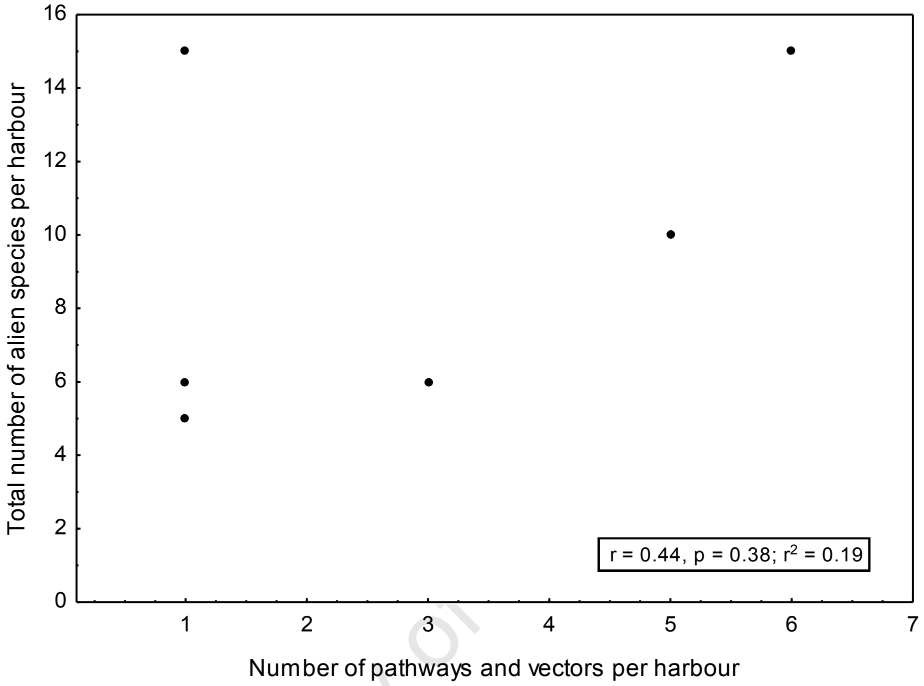

Figure 10. The relationship between the number of pathways and vectors per harbour, and the total number of alien species per harbour.

2.4. DISCUSSION

Harbours play an important role in the spread of marine alien species by acting as colonisation corridors across areas of unsuitable habitat (Bax et al., 2003; Bulleri & Airoldi, 2005). The infrastructure that constitutes harbours provides hard substrates in an environment that does not naturally have these habitats (e.g. Floerl & Inglis, 2003; Cohen et al., 2005). This has allowed for the occurrence of fouling organisms to establish in harbours worldwide, including both native and alien species (e.g. Connell & Glasby, 1999; Bax et al., 2002, 2003; Awad et al., 2003; Arenas et al., 2006). The aim of this study was firstly to document alien fouling species in six Western Cape harbours, and secondly to use the data collected to identify factors (such as vectors and other harbour characteristics and activities such as pathways) that can be used by management authorities to prioritise harbours, upon which to focus monitoring efforts.

2.4.1. Harbours influencing high alien species numbers

In total, 19 alien species were detected and the visual samples had less alien species (seven) than the scrape samples (18) because fewer species are large enough to be identified with certainty. In the visual samples Table Bay, Hout Bay and Mossel Bay had significantly more species than St Helena Bay (Multiple comparisons; $p<0.05$). Unexpectedly, Hout Bay, a small local fishing harbour, had the most alien species (4.4 ±0.6 SE). Hout Bay and Table Bay represented the same taxonomic groups, with species from Ascidiacea, Bivalvia, Crustacea and Porifera occurring in both of the harbours.

In the scrape samples, a similar pattern occurred, as the highest number of alien species was reported from Hout Bay (5.1 ±0.74 SE) and Table Bay (4.1 ±0.69 SE). Additionally, the taxonomic groups in both Hout Bay and Table Bay were fairly similar (Figure 7). Hout Bay is a small local harbour, and the fact that it had the highest number of alien species was therefore unexpected. Floerl and Inglis (2003) reported that the design of harbours can exacerbate hull fouling because enclosed harbours are known to have higher recruitment of several organisms in partially enclosed and enclosed marinas, due to the flow of water. This suggests that the trapping of water in enclosed harbours could limit the natural dispersal of planktonic propagules through advective current movement. This will increase propagule pressure relative to the availability of surfaces such as harbour infrastructure and boat hulls (Floerl & Inglis, 2003). This may explain why Hout Bay had the highest number of alien species present, compared to the other harbours, since it is fairly enclosed and may therefore accumulate a higher proportion of propagules. The notion that enclosed harbours accumulate a higher propagule pressure should therefore be further investigated in the Western Cape and the rest of South Africa as this may be important for the management of marine alien species.

Table Bay and Hout Bay were similar in both species number and composition for both visual and scrape samples (Figures 3 and 7). Between the two harbours only three species (the invasive European crab *Carcinus maenus*, the hydrozoan *Obelia dichotoma* and the sponge *Suberites ficus*) were unique to Hout Bay harbour and two species (the polychaete *Dodecaceria fewkesi* and bryozoan *Watersipora subtorquata*) were unique to Table Bay. Table Bay is a large harbour that received the highest number of commercial vessels (2775) and has both international and regional commercial shipping, as well as international and regional yachts. It also has ship repair and hull cleaning, as well as petroleum infrastructure (Table 1) which are all factors that can contribute to the presence of alien species in a harbour. The act of vessels moving among harbours in the Cape Town region therefore influences the transfer of alien species, since it was previously found that yachts in the Table Bay harbour had various levels of fouling and that these vessels travelled regionally (Jurk, 2011). Additionally, other commercial vessels in the harbour and harbour infrastructure are susceptible to alien species transfer. Thus the reason for these two harbours being so similar in the composition of alien species, even though there are clear differences in the characteristics of the harbours, could be due to a combination of their enclosed nature (Floerl & Inglis, 2003), their proximity to one another (therefore experiencing similar climatic and biogeographic conditions), as well as intra-regional vessel movement. This is plausible since harbour infrastructure and the transfer of species through vectors are known to contribute towards the homogenisation of organisms in coastal regions (Bulleri & Chapman, 2010).

Additionally, the pattern observed for the taxonomic composition for both visual and scrape samples across the harbours was the same (Figures 3 & 7) in that Table Bay and Hout Bay were fairly similar, the same for St Helena Bay and Saldanha Bay and finally Gansbaai and Mossel Bay. This could be a reflection of intra-regional movement of vessels within the three pairs of harbours, resulting in a similar composition of species. Since all the harbours have regional fishing vessels, as well as regional repair and hull cleaning of fishing vessels, this may account for the similarity in the alien species found across the harbours. This stresses the importance of sampling and monitoring vessels as a potential vector for alien species transfer regionally. This type of monitoring is particularly important because once alien species have entered a new region; there are few legal or economic restrictions that prevent their spread within that region (Miller et al., 2001).

2.4.2 Significance of percentage cover and biomass of alien species in harbours

As with the number of alien species per m^2, the percentage cover of alien species in the harbours showed a similar pattern. However, Saldanha Bay supported a significantly lower cover of alien species than St Helena Bay, Hout Bay and Mossel Bay (Multiple comparisons: $p<0.05$). In St Helena Bay, the most western port in the study, a high percentage cover of alien species per m^2 was due to the space being occupied predominantly by mussels (67.5% ±6.47 SE), largely due to *Mytilus galloprovincialis* (contributing 65.5% ±7.32 SE). In Hout Bay, situated in the middle of the sample site distribution, the high percentage cover was due to a number of taxonomic groups but the most contributing group was Ascidiacea. This may be a result of the enclosed and sheltered nature of the harbour. The dominant space occupiers in Mossel Bay, the eastern-most port were Ascidiacea (contributing 70.1% ±4.46). This is an interesting pattern to observe (i.e. the change in space occupiers per m^2 from the western-most and eastern-most ports). It may be that the nutrient rich water (Cushing, 1971) experienced on the west coast has allowed the filter feeding Meditteranean mussel, *M. galloprovincialis,* to dominate in this harbour, in terms of space. Contrastingly, Ascidiacea may prefer the warmer, nutrient poor waters (Griffiths et al., 2010) provided further east, allowing them to dominate in terms of percentage cover. Although it is not known whether all of the alien Ascidiacea species in the present study prefer warmer waters, it has been suggested that some of the species observed here such as *Diplosoma listerianum*, *Styela plicata* and *Ascidiella aspersa* are able to recruit earlier in warmer waters (Stachowicsz, 2002) which may aid the invasion process in warmer waters. Additionally, Lambert and Lambert (2003) reported that several other non-native ascidians increased in abundance when experiencing warmer temperatures.

The biomass of alien species recorded per m^2 indicated a completely different pattern. The harbour that was shown to have the lowest percentage cover (Saldanha Bay) in Figure 4 had one of the highest biomass values (Figure 8, 12 114.96 g ±2511.54 SE) predominantly due to *M. galloprovincialis* (contributing 12 101 g ±2 512 SE). The explanation provided for the percentage cover in terms of biogeography supports this. Although Mossel Bay appears to have the same pattern, the variation in this harbour was very high and it is therefore likely that the result has been inflated by a few samples with larger mussels that may have occurred in certain areas that were dominated more by mussels than ascidians. Another interesting pattern is that shown by the biomass of alien species in Table Bay and Hout Bay. Although these two harbours experienced high alien species numbers, they supported fairly low biomass of alien species (Figure 8). As observed in Figure 8, the biomass among the harbours was driven by Bivalvia (in this case *M. galloprovincialis*). *M. galloprovincialis* was detected in each harbour but dominated in terms of biomass only in St Helena Bay, Saldanha Bay, Gansbaai and Mossel Bay. Since *Mytilus* has a long history of spreading along the South African coasts (Robinson et al., 2005a; Branch et al., 2008, 2010), its invasion may be contributing towards a shift to an alternative stable state, or the system reaching a state of equilibrium (Parker et al., 1999), by increasing steadily to a stable equilibrium density, such as that seen for the zebra mussel *Dreissena polymorpha* (Ramcharan et al., 1992). Beisner et al. (2003) reported that when alternate stable states occur, the state variables (such as temporally or spatially averaged abundances of species or guilds, spatial coverage and age or stage population components) will persist in one of various possible configurations, or different equilibrium points that are locally stable. The affected community can respond in one of two ways, either returning to the same equilibrium after a small perturbation, or it can shift to a different equilibrium after a large perturbation (Beisner et al., 2003). Additionally, its fast growth and reproductive output (Branch & Steffani, 2004) could be playing an important role in its dominance.

In Table Bay and Hout Bay, the contribution of *M. galloprovincialis* towards the biomass was minimal, explaining why the overall biomass of alien species was low in these two harbours. The invasive European crab *Carcinus maenus* was detected only in Table Bay and Hout Bay, which may explain the low biomass of *M. galloprovincialis* since *C. maenus* may be predating upon the mussels, thereby potentially controlling the density of the mussel population in these two harbours**.** *Carcinus maenus* is known to decimate shellfish populations (Robinson et al., 2005a) and this species is currently restricted to sheltered, coastal sites (Hampton & Griffiths, 2007; Mead et al., 2011a). Robinson et al. (2005a) reported high numbers of *C. maenus* in Table Bay harbour (133 568 individuals) and Hout Bay harbour (9 180 individuals). However, low abundances were detected in the present study which may be a reflection of population decline and thus requires further investigation.

2.4.3. Monitoring implications

The CART analysis for both visual and scrape samples showed the same pattern. Harbours with yachts, and that were smaller than 6.27 km^2 had the largest number of alien species. The reason for this could be that recreational vessels have long layover times in ports (Floerl, 2002, Hewitt et al., 2009) compared to other vessels such as commercial ships (Lewis et al., 2003). Due to the long periods of time that yachts spend in harbours, they may be contributing to the presence of more alien species, by not only transferring species, but also providing a sufficient amount of time, for these species to establish on the hulls and spread to the available surrounding habitat (e.g. Bax et al., 2002; Floerl et al., 2005; Jurk, 2011). Yachts also travel at slower speeds, adding to the factors that could increase hull fouling (Hewitt et al., 2009). It is important to take into account that the sample size for the visual samples was reduced, since 50% of the samples in Hout Bay and 80% for the samples in Gansbaai could not be taken due to visibility difficulties. In other instances, this may have resulted in an underrepresentation of the data which may have affected the final outcome of the analyses. However, the data collected from the scrape samples showed the same pattern in the regression tree model. Unexpectedly, smaller harbours were included in the harbours that had the most species which may be explained by the higher propagule pressure being trapped in small, but particularly enclosed harbours (Floerl & Inglis, 2003).

In this study, first indications, based on the sample size considered, are that hull fouling of yachts are important vectors of alien species transfer in the Western Cape. Jurk (2011) reported that ascidians comprised a large percentage (71%) of fouling on yachts sampled in Table Bay harbour. Furthermore, it has often been stated that recreational vessels have been underestimated as a vector for the transfer of marine alien species (e.g. Jurk, 2011; Ashton et al., 2006). However, based on the sample size of the present study, this does not conclusively indicate that yachts have to be prioritised for management. Although 10 samples were sufficient for several of the harbours, in some of the larger harbours, such as Table Bay, a sample size of 10 may not have been representative of the number and densities of alien species occurring in the harbour per m^2. Additionally, areas that were inaccessible at the time may also influence the results differently. Thus the results of this study should be used as a baseline study. With an increase in the number of harbours being sampled, as well as the number of quadrats sampled in those harbours, more robust recommendations could be achieved.

Although the CART analysis for both the scrape and visual samples were the same (differing only in the abundance of alien species), we need both of these sampling methods for monitoring harbours for alien species. Visual sampling is quick and does not require a large amount of data processing. It is also inexpensive and the results of which species were present can be obtained quickly. However, visual samples are only useful for detecting large species and when visibility is good. Scrape sampling detects more species, especially those that are inconspicuous. They also allow for the quantification of biomass of these species. However, this method requires greater taxonomic expertise and is more costly in terms of the materials required (e.g. preservation solutions, storage units), as well as the amount of time required to process and identify species. The combination of the two sampling methods is therefore more useful than only one of them.

2.4.4. Multiple vectors and pathways and alien species abundances

Mead et al. (2011b) reported that 51% of alien species are potentially arriving at the South African coastline through multiple vectors. Similarly, the San Francisco Bay and Delta, possibly the most invaded estuary, is known to host several alien species, with invasions increasing at a rapid rate. The extent of invasions is due to several factors, with one of these being the influence of multiple transport vectors (Cohen & Carlton, 1998). Therefore it is likely that multiple vectors would result in the transfer of more alien species that could potentially establish in harbours. In the present study there was no relationship between the total number of alien species present per harbour and the number of vectors and pathways (such as shipping) that occurred within those harbours. Thus, the initial prediction that multiple vectors would result in higher alien species presence was not supported. This suggests that it is not necessarily the number of vectors or pathways that is related to the number of alien species present, but more likely the type of vectors and/or and their magnitude (Williamson, 1996; Mack et al., 2000). Additionally, the establishment and survival or persistence of an alien species in a harbour is also dependent on the environmental conditions, such as temperature and salinity for example (Epelbaum, 2009). As previously mentioned, the design of the harbour itself, is also thought to play a role in the number of alien species, as well as their abundances (Floerl & Inglis, 2003).

2.4.5. Species-specific information

An important discovery in this study was the detection of the brachiopod *Discinisca tenuis* in both St Helena Bay and Saldanha Bay, because this species was previously thought to occur only in an aquaculture facility in the vicinity of the Saldanha Bay harbour (Haupt et al., 2010; Mead et al., 2011a). Since the spread of *D. tenuis* is evident in this study, this species should be carefully monitored since it may be an indication of intra-regional transfer between the harbours which could result in further spread. This also has implications for the potential transfer of other alien species which warrants investigation.

As in previous studies (Mead et al., 2011a, 2011b), the Black coral worm *Dodecaceria fewkesi*, was detected only in Table Bay harbour. *D. fewkesi* forms hard coral-like colonies (Blake, 1996; Picker & Griffiths, 2011) that resemble gravel and these colonies were found to transform the subtidal habitat at certain sample sites, as it formed masses of hard material that covered large sections of the wall and were difficult to remove. There is therefore, a possibility that these colonies can cause physical changes in the habitat, as do various other alien species (e.g. *M. galloprovincialis*) (Robinson et al., 2005a, Branch et al., 2008). The calcareous colonies that these polychaetes produce may have the ability of doing something similar and should therefore be investigated. Since *D. fewkesi* was detected only in Table Bay harbour (given the previous record in the same harbour), it could be an indication that it has not yet spread to other harbours, which makes this a crucial opportunity for action to be taken towards its eradication in order to prevent further spread.

Given the importance of biological invasions and the results highlighted herein, the failure to address the issue of biotic invasions could result in severe global consequences, such as the disruption of ecological processes that supply ecosystem services, and the creation of homogenous, impoverished ecosystems composed of cosmopolitan species (Mack et al., 2000).

Chapter 3: CONCLUSIONS AND FUTURE RECOMMENDATIONS

Conclusions

Detecting alien species from fouling assemblages in harbours is an important requirement for the management and conservation of native marine species. With the use of rapid port surveys and the prioritisation of sites for monitoring, specific harbours could eventually be prioritised for alien species management. This is particularly relevant in South Africa, where resources in terms of financial aid, capacity and manpower are limited. The use of the regression tree model in this study could aid further monitoring and inform subsequent management interventions.

This study has provided first indications, based on the give sample size, that the smaller, more enclosed harbours situated in Cape Town had the most alien species. This will become important when harbours need to be selected for monitoring of marine alien species in order to prevent further spread into natural habitats. Yachts have been shown to support various levels of fouling internationally and in South Africa (e.g. Floerl et al., 2005; Jurk, 2011) and therefore play an important role in the potential transfer of alien species across the globe. Although yachts were shown to be important factors for predicting the number of alien species in harbours, the present study merely provides a baseline and therefore it cannot emphatically be concluded that harbours with yachts have to be prioritised for monitoring. However, since any information on marine alien species associated with fouling in South African harbours is lacking, this study has provided significant progress in the field.

Additionally, it has revealed the first record for the spread of the brachiopod *Discinisca tenuis* from an aquaculture facility to St Helena Bay and Saldanha Bay harbours, highlighting the potential of intra-regional species transfer of marine alien species in the region. Along with the possibility that harbours situated closely to one another are acting as recipient ports for alien species via intra-regional vessel movement, these findings highlight the importance of fouling in the region. Taxonomic groups that are important for the monitoring of alien species are Ascidiacea and Bivalvia, since these groups contained the most species with the highest biomass, respectively. However, this should not negate the monitoring and consequent management of more inconspicuous species, since overlooking these species may prove to be more threatening than one would assume.

A positive outcome of this study was that four of the six harbours had not been sampled before and unexpectedly, one of the smallest harbours (Hout Bay) supported the most alien species (13), along with Table Bay. For such a small-scale study, this has been a good improvement in the available data on the presence and abundance of alien species in harbours. Furthermore, a method for prioritising harbours for monitoring of alien species could be the first step towards addressing fouling as a problem.

Further Recommendations

Extending the study area to harbours along the east coast will result in a more robust analysis which could better prioritise monitoring efforts for alien species along the coast of South Africa. This will also reveal the scale of alien species introductions in South African harbours and could indicate whether the composition of alien species has changed in harbours, such as

Port Elizabeth, that have been surveyed previously. This harbour is particularly important, since only five alien species were reported in the previous port survey (Hutchings et al., 2006). Along with a larger number of harbours being sampled, an increased sample size within harbours will likely improve the number of important factors simulated by the regression tree models.

Another important recommendation for future research is the sampling of vessels by taking visual and scrape samples from the hulls of yachts and commercial vessels. This can later be extended to the sampling of other vessels found in harbours, as well as other artificial structures such as buoys and other floating structures, which have been shown to contain different species assemblages in comparison to natural substrates (Glasby, 2001). This may be relevant for detecting certain species that establish more readily on other available structures. The investigation of yachts particularly, may reveal information relevant to intra-regional transfer of marine alien species. This information would be important for management authorities, since yachts are known to travel intra-regionally and internationally (e.g. Bax et al., 2002, 2003; Wasson et al., 2001; Jurk, 2011). The potential for intra-regional transfer of alien species is evident in the present study, which stresses the importance of incorporating the sampling of vessels.

The incorporation of hull samples will allow the investigation of additional factors relating to the presence of alien species, such as the travel history associated with vessels, as well as the maintenance of vessels, such as the use of anti-fouling paints and frequency of cleaning the hulls (Floerl et al., 2005). This will reveal whether vessels indeed move between ports and how frequently this is done. More importantly, it will highlight which ports are being visited, which will shed light on the presence of certain alien species and will aid the analysis of the regression trees.

Furthermore, since a known target list was used in this study, there were still a large number of unidentified species. Even though many of these may be native species and some were recognised as such, there were some species that could not be identified. Therefore, it is recommended to have such species identified by relevant experts, as it may result in the identification of currently unknown alien species in South Africa. However, this will be time-consuming and therefore the first step to improve the research would be to make use of the target lists and samples should be preserved for later investigation, or it could be sent to experts to identify simultaneously.

In summation, rapid port surveys can be used for the prioritisation of harbours for monitoring marine alien species. This is an important finding for South Africa and other countries where resources are limited, since it provides a component of input required for the optimisation of monitoring effort, ultimately aiding the prevention of potential invasions.

REFERENCES

Anderson, L. W. J. (2005). California's reaction to *Caulerpa taxifolia*: A model for invasive species rapid response. *Biological Invasions*, *7*, 1573 – 1564.

Arenas, F., Bishop, J. D. ., Carlton, J. T., Dyrynda, P. J., Farnham, W. F., Gonzalez, D. J., Jacobs, M. ., et al. (2006). Alien species and other notable records from a rapid assessment survey of marinas on the south coast of England. *Journal of the Marine Biological Association of the UK*, *86*(06), 1329 – 1337.

Ashton, G., Boos, K., Shucksmith, R., & Cook, E. (2006). Risk assessment of hull fouling as a vector for marine non-natives in Scotland. *Aquatic Invasions*, *1*(4), 214–218.

Awad, A., Clarke, C., Greyling, L., Hilliard, R., Polglaze, J., & Raaymakers, S. (2003). *Ballast water risk assessment. Port of Saldanha Bay. Republic of South Africa*. GloBallast Monograph Series no. 13.

Bax, N, Hayes, K., Marshall, A., Parry, D., & Thresher, R. (2002). *Man-made marinas as sheltered islands for alien marine organisms : Establishment and eradication of an alien invasive marine species.* In Veitch, C. R. & Clout, M. N. (eds.). Turning the tide: the eradication of invasive species. IUCN SSC Invasive Species Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK. (pp. 26–39).

Bax, N, Williamson, A., Aguero, M., Gonzalez, E., & Geeves, W. (2003). Marine invasive alien species: a threat to global biodiversity. *Marine Policy*, *27*(4), 313–323.

Beisner, B. E., Haydon, D. T., & Cuddington, K. (2003). Alternative stable states in ecology. *Frontiers in Ecology and the Environment*, *1*(7), 376–382.

Blackburn, T. M., Pysek, P., Bacher, S., Carlton, J. T., Duncan, R. P., Jarosik, V., Wilson, J. R. U., et al. (2011). A proposed unified framework for biological invasions. *Trends in Ecology and Evoolution*, *26*(7), 333–339.

Blake, J. A. (1996). Family Cirratulidae. In: Blake, J. A., Hilbig, B. & Scott, P. H. (eds). *Taxonomic Atlas of the Santa Maria Basin and Western Santa Barbara Channel, Vol 6. Annelida, Part 3*. Santa Barbara Museum of Natural History (pp. 263-384).

Bownes, S. J., & McQuaid, C. D. (2006). Will the invasive mussel *Mytilus galloprovincialis* Lamarck replace the indigenous *Perna perna* L . on the south coast of South Africa? *Journal of Experimental Marine Biology and Ecology*, *338*, 140–151

Branch, G.M. & Griffiths, C.L. (1988). The Benguela Ecosystem: PartV. The coastal zone. *Oceanography and Marine Biology: an Annual Review*, *26*, 395–486.

Branch, G. M., & Steffani, C. N. (2004). Can we predict the effects of alien species ? A case-history of the invasion of South Africa by *Mytilus galloprovincialis* (Lamarck). *Journal of Experimental Marine Biology and Ecology*, *300*, 189–215.

Branch, G. M., Odendaal, F., & Robinson, T. B. (2008). Long-term monitoring of the arrival , expansion and effects of the alien mussel *Mytilus galloprovincialis* relative to wave action. *Marine Ecology Progress Series, 370*, 171–183.

Branch, G. M., Odendaal, F., & Robinson, T. B. (2010). Competition and facilitation between the alien mussel *Mytilus galloprovincialis* and indigenous species : Moderation by wave action. *Journal of Experimental Marine Biology and Ecology*, *383*, 65 – 78.

Branch, G. M., Griffiths, C. L., Branch, M. L. & Beckley, L. E. (2010). Two Oceans: A guide to the marine life of southern Africa. Struik Publishers. Cape Town, South Africa. (pp. 456).

Breiman, L., Friedman, J. H., Olshen, R. A., & Stone, C. J. (1984). *Classification and regression trees.* Monterey, CA: Wadsworth & Brooks/Cole Advanced Books & Software.

Bulleri, F., & Airoldi, L. (2005). Artificial marine structures facilitate the spread of a non-indigenous green alga, *Codium fragile* ssp. *tomentosoides* in the North Adriatic Sea. *Journal of Applied Ecology*, *42*(6), 1063–1072.

Bulleri, F., & Chapman, M. G. (2010). The introduction of coastal infrastructure as a driver of change in marine environments. *Journal of Applied Ecology*, *47*(1), 26–35.

Carlton, J. T. (1985). Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water. *Oceanography and Marine Biology: An Annual Review*, 23, 313-371.

Carlton, J.T. (1987). Patterns of transoceanic marine biological invasions in the Pacific Ocean. *Bulletin of Marine Science*, *41*, 452- 465.

Carlton, J. T. (1996). Biological invasions and cryptogenic species. *Ecology, 77*, 1653-1655.

Carlton, J. T. (1999). The scale and ecological consequences of biological invasions in the world's oceans. In: Sandlund OT, Schei PJ, Viken A, (eds). *Invasive Species and Biodiversity Management*. Dordrecht: Kluwer Academic Publishers. (pp. 195–212).

Clark, B. M., Tunley, K., Hutchings, K., Steffani, N., Turpie, J., Jurk, C. & Gericke, J. (2012). *Saldanha Bay and Langebaan Lagoon. State of the Bay 2011 Technical Report*. Anchor Environmental Consultants. Saldanha Bay Water Quality Trust.

Cohen, A. N. and Carlton, J. T. (1998). Accelerating invasion rate in a highly invaded estuary. *Science*, *279*, 555–558.

Cohen, A. N., Harris, L. H., Bingham, B.L., et al. (2005) Rapid assessment survey for exotic organisms in southern california bays and harbors, and abundance in port and non-port areas. *Biological Invasions, 7*, 995–1002.

Coles, S. L., DeFelice, R. C., Eldredge, L. & Carlton, J. T. (1999). Historical and recent introductions of non-indigenous marine species into Pearl Harbor, Oahu, Hawaiian Islands. *Marine Biology*, *135*, 147–158.

Connell, S. D. (2001). Urban structures as marine habitats: an experimental comparison of the composition and abundance of subtidal epibiota among pilings, pontoons and rocky reefs. *Marine Environmental Research 52*, 115–125.

Connell, S. D. & Glasby, T. M. (1999). Do urban structures influence local abundance and diversity of subtidal epibiota? A case study from Sydney Harbour, Australia. *Marine Environmental Research 47*, 373–387.

Cribb, H., Maher, J. & Beatty, A. (2010). *Monitoring for Marine Pests*. 2008 -2009 Report. Darwin Harbour. Northern Territory Government. Australia (pp. 35).

Cushing. D. H. (1971). Upwelling and the production of fish. *Advances in Marine Biology, 9*, 255-334.

Day, J. H. (1967). A Monograph on the Polychaeta of Southern Africa. Part I: Errantia. Trustees of the British Museum (Natural History). London. (pp. 877).

Day, J. H. (1967). A Monograph on the Polychaeta of Southern Africa. Part II: Sedantaria. Trustees of the British Museum (Natural History). London. (pp. 877).

Day, J. H. (1969). A Guide to the Marine Life of South African Shores. University of Cape Town. Cape Town. (pp. 300).

Drake, J. M. & Lodge, D. M. (2004). Global hot spots of biological invasions: evaluating options for ballast water management. *Proceedings of the Royal Society London B Biological Sciences, 271*, 575–580.

Eldredge, L. G. (1994) *Perspective in aquatic exotic species management in the Pacific Islands.* Volume 1: Introductions of commercially significant aquatic organisms to the Pacific Islands, South Pacific Commission, South Pacific Regional Environment Programme. New Caledonia.

Eno, N. C. (1996). Non-native marine species in British waters: effects and controls. *Aquatic Conservation: Marine and Freshwater Ecosystems, 6*, 215–28.

Epelbaum, A., Herborg, L. M., Therriault, T. W., & Pearce, C. M. (2009). Temperature and salinity effects on growth, survival, reproduction, and potential distribution of two non-indigenous botryllid ascidians in British Columbia. *Journal of Experimental Marine Biology and Ecology*, *369*(1), 43–52.

Fairweather, T. P., Hara, M., Lingen, C. D. Van Der, Raakjær, J., Shannon, L. J., Louw, G. G., Degnbol, P., et al. (2006). A knowledge base for management of the capital-intensive fishery for small pelagic fish off South Africa. *American Journal of Marine Science*, *28*, 645–660.

Fernandez, M. A. S., Wagener, A. L. R., Limaverde, A. C., Scofield, A. L., Pinheiro, F. M., & Rodrigues, E. (2005). Imposex and surface sediment speciation: A combined approach to evaluate organotin contamination in Guanabara Bay, Rio de Janeiro, Brazil. *Marine Environmental Research*, *59*, 435 – 452.

Floerl, O. (2002). *Intracoastal spread of fouling organisms by recreational vessels*. PhD thesis. James Cook University, Townsville, Australia.

Floerl, O., & Inglis, G. J. (2003). Boat harbour design can exacerbate hull fouling. *Austral Ecology*, *28*(2), 116–127.

Floerl, O., & Inglis, G. J. (2005). Starting the invasion pathway: the interaction between source populations and human transport vectors. *Biological Invasions*, *7*(4), 589–606.

Floerl, O., Inglis, G. J., & Hayden, B. J. (2005). A risk-based predictive tool to prevent accidental introductions of nonindigenous marine species. *Environmental Management*, *35*(6), 765–778.

Gaffney, P. M. & Allen, S. K. (1993). Hybridization among *Crassostrea* species: a review. *Aquaculture, 116*, 1–13.

GESAMP. (2002). *'Treatment and management of ships' ballast water to control introductions of non-indigenous species'*. Report of the GESAMP Ballast Water Correspondence Group to Marine Environment and Protection Committee of the International Maritime Organization. MEPC 48/2.

Glasby, T. M. (1999). Differences between subtidal epibiota on pier pilings and rocky reefs at marinas in Sydney, Australia. *Estuarine, Coastal and Shelf Science, 48,* 281–290.

Glasby, T.M. (2001). Development of sessile marine assemblages on fixed versus moving substrata. M*arine Ecology Progress Series*, *215*, 37–47.

Glasby,T.M. & Connell, S.D. (2001). Orientation and position of substrata have large effects on epibiotic assemblages. *Marine Ecology Progress Series,* 214, 127-135.

Glasby, T.M., Connell, S.D., Holloway, M.G. & Hewitt, C.L. (2006) Non-indigenous biota on artificial structures: could habitat creation facilitate biological invasions? Marine Biology, 151, 887–895.

GloBallast. (2001). Global Ballast Water Management Programme. Port Surveys. Issue 4.

Godwin, L. S. (2005). *Hull Fouling As A Mechanism For Marine Invasive Species Introductions.* Proceedings of a workshop on current issues and potential management strategies. Honolulu, Hawaii. (pp. 54).

Great Britain-Parliament. (1985). Food and Environment Protection Act, 1985. Her Majesty's Stationary Office, London, Chapter 48 (pp. 38). In Smith et al., (2008). Macrofaunal recovery following TBT ban. Long-term recovery of subtidal macrofaunal communities in relation to declining levels of TBT contamination. E*nvironmental Monitoring and Assessments* , *136*, 245-256.

Griffiths, C. L. (1976). Guide to the benthic marine amphipods of Southern Africa. South African Museum. Cape Town. (pp. 106).

Griffiths, C. L., Hockey, P. A. R., Van Erkom Schurink, C., & Le Roux, P. J. (1992). Marine invasive aliens on South African shores: implications for community structure and trophic functioning. *South African Journal of Marine Science*, *12*, 713 – 722.

Griffiths, C. L., Mead, A. & Robinson, T. B. (2009). A brief history of marine bio-invasions in South Africa. *African Zoology, 44*, 241–247.

Griffiths, C. L., Robinson, T. B., Lange, L., & Mead, A. (2010). Marine biodiversity in South Africa: an evaluation of current states of knowledge. *PLoS ONE*, *5*(8), 1 – 13.

Hampton, S. L., & Griffiths, C. L. (2007). Why *Carcinus maenas* cannot get a grip on South Africa's wave-exposed coastline. *African Journal of Marine Science*, *29*(1), 123–126.

Hanekom, N. (2008). Invasion of an indigenous *Perna perna* mussel bed on the south coast of South Africa by an alien mussel *Mytilus galloprovincialis* and its effect on the associated fauna. *Biological Invasions*, *10*, 233–244.

Haupt, T. M., Griffiths, C. L., & Robinson, T. B. (2010). Oysters as vectors of marine aliens, with notes on four introduced species associated with oyster farming in South Africa. *African Zoology*, *45*, 52–62.

Haupt, T. M., Griffiths, C. L., & Robinson, T. B. (2012). Intra-regional translocations of epifaunal and infaunal species associated with cultured Pacific oysters *Crassostrea* gigas. *African Journal of Marine Science*, *34*(2), 187–194.

Hayes, K. R. (2002). Identifying hazards in complex ecological systems — Part 2: Infections modes and effects analysis for biological invasions. *Biological Invasions*, 4, 251–261.

Hewitt, C. L., & Martin, R. B. (2001). *Revised protocols for baseline port surveys for introduced marine species : survey design sampling protocols and specimen handling.* Centre for Research on Introduced Marine Pests. Technical Report No.. 22. CSIRO Marine Research, Hobart. (pp. 46).

Hewitt, C. L., Gollasch, S. & Minchin, D. (2009). The vessel as a vector - biofouling ballast water and sediments. In: *Biological Invasions in Marine Ecosystems*, (eds) G. Rilov & J.A. Crooks. Springer-Verlag, Berlin. (pp. 117-131)

Hulme, P. E. (2006). Beyond control : wider implications for the management of biological invasions. *Journal of Applied Ecology*, *43*(5), 835–847.

Hutchings, K., Atkinson, L. & Clark, B. (2006). *Sampling and Identification of Marine Fauna and Flora in The Port of Port Elizabeth.* Anchor Environmental Consultants (pp. 10).

IMO. (1999). *Anti-fouling systems moving towards the non-toxic solution*. Focus on IMO. International Maritime Organization (pp. 24).

IMO. (2004). *International convention for the control and management of ships' ballast water and sediments*. BWM/CONF/36, London, UK.

IMO. (2011). *International Convention on the Control of Harmful Anti-fouling Systems on Ships*. Available online at www.imo.org. Accessed 18 January 2013.

International Union for the Conservation of Nature (IUCN). (1999). IUCN guidelines for the prevention of biodiversity loss due to biological invasion. Species 31/32, 28–42.

Jurk, C. (2011). *Fouled Yachts: A Vector For Marine Invasive Alien Species To South African Shores?* MSc Thesis. University of Cape Town, South Africa.

Laird, M. C., & Griffiths, C. L. (2010). Present distribution and abundance of the introduced barnacle *Balanus glandula* Darwin in South Africa. *Afrian Journal of Marine Science, 30*(1), 93 – 100.

Lambert, C. C., & Lambert, G. (2003). Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. *Marine Ecology Progress Series, 259*, 145–161.

Lee, J. E., & Chown, S. L. (2007). *Mytilus* on the move: transport of an invasive bivalve to the Antarctic. *Marine Ecology Progress Series, 339,* 307–310.

Lee, J. E., & Chown, S. L. (2009). Temporal development of hull-fouling assemblages associated with an Antarctic supply vessel. *Marine Ecology Progress Series*, *386*, 97–105.

Lewis, P. N., Hewitt, C. L., Riddle, M., & McMinn, A. (2003). Marine introductions in the Southern Ocean: an unrecognised hazard to biodiversity. *Marine Pollution Bulletin, 46*(2), 213–223.

Lockwood, J. L., Hoopes, M. F. & Marchetti, M. P. (2007). *Invasion Ecology*. Oxford, UK: Blackwell.

Lombard, A. T., Strauss, T., Harris, J., Sink, K., Attwood, C., & Hutchings, L. (2004). *South African National Spatial Biodiversity Assessment 2004: Technical Report. Volume 4: Marine Component*. Pretoria: South African National Biodiversity Institute.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. & Bazzaz, EA. (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications, 10, 689-710.*

Matthiessen, P., Waldock, R., Thain, J. E., Waite, M. E., & Scrope-Howe, S. (1995). Changes in periwinkle *(Littorina littorea)* populations following the ban on TBT-based antifoulings on small boats in the United Kingdom. *Ecotoxicology and Environmental Safety, 30,* 180–194.

Mead, A., Carlton, J. T., Griffiths, C. L., & Rius, M. (2011a). Introduced and cryptogenic marine and estuarine species of South Africa. *Journal of Natural History, 45*(39-40), 2463–2524.

Mead, A., Carlton, J. T., Griffiths, C. L., & Rius, M. (2011b). Revealing the scale of marine bioinvasions in developing regions: a South African re-assessment. *Biological Invasions, 13*(9), 1991–2008.

Meinesz, A. (1999). Killer algae. Chicago, USA: University of Chicago Press. In Bax et al. (2003). Marine invasive alien species: a threat to global biodiversity. *Marine Policy*, *27*(4), 313–323.

Miller, T. W., Chapman, J. W. & Coan, E. V. (2001). Live seafood: a recipe for biological and regulatory concern? In: Paust BC, Rice AA (eds), *Marketing and Shipping Live Aquatic Products*. Seattle, Washington: University of Alaska Sea Grant Program. (pp. 249–256).

Minchin, D., McGrath, D., Duggan, C. B. (1995). The distribution of the slipper limpet, *Credipula fornicata* (L.)(Gastropoda: Mollusca) in the North-Eastern Atlantic and notes of records from Irish waters. *Journal of Conchology, 35*, 247–54.

Minchin, D. (1996). Management of the introduction and transfer of marine mollusks. Aquatic Conservation. *Marine and Freshwater Ecosystems, 6*, 229–44.

Minchin, D., Gollasch, S., Cohen, A. N., Hewitt, C. L. & Olenin, S. (2009). Characterizing vectors of marine bioinvasions. In: *Biological Invasions in Marine Ecosystems*, (eds) G. Rilov & J.A. Crooks. Springer-Verlag, Berlin. (pp. 109-116).

Mineur, F., Cook, E. J., Minchin, D. A. N., Macleod, A., & Maggs, C. A. (2012). Changing coasts: marine aliens and artificial structures. *Oceanography and Marine Biology: An Annual Review*, *50,* 189–234.

Molnar, J. L., Gamboa, R. L., Revenga, C., Spalding, M. D. (2008). Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and Environment, 6,* 485-492.

O'Dowd, D. J., Green, P. T., & Lake, P. S. (2003). Invasional meltdown on an oceanic island. *Ecological Letters, 6*, 812–817.

Olenin S., Minchin, D. & Daunys, D. (2007). Assessment of biopollution in aquatic ecosystems. *Marine Pollution Bulletin, 55,* 379-394.

Paige, M., Kelly, M. & Herr, B. (2012). Seasquirts common to the ports and harbours of New Zealand. Marine identification guides and fact sheets. NIWA. (pp. 41). Available online at www.niwa.co.nz.

Pangle, K. L., Peacor, S. D. & Johannsson, O. E. (2007). Large non-lethal effects of invasive invertebrate predator on zooplankton population growth rate. *Ecology, 88,* 402 – 412.

Parker, I. M., Simberloff, D., Lonsdale, W. M., Goodell, K., Wonham, M., Kareiva, P. M., Williamson, M. H., et al. (1999). Impact : toward a framework for understanding the ecological effects of invaders. *Biological Invasions, 1,* 3–19.

Piazzi, L., Balata, D., Foresi, L., Cristaudo, C. & Cinelli, F. (2007). Sediment as a constituent of Mediterranean benthic communities dominated by *Caulerpa racemosa var. cylindracea. Scientia Marina,* 71, 129–135.

Pichegru, L., Ryan, P., Le Bohec, C., Van der Lingen, C., Navarro, R., Petersen, S., Lewis, S., et al. (2009). Overlap between vulnerable top predators and fisheries in the Benguela upwelling system: implications for marine protected areas. *Marine Ecology Progress Series, 391,* 199–208. doi:10.3354/meps08283

Picker, M., & Griffiths, C. L. (2011). *Alien & Invasive Animals*. Cape Town: Struik Publishers. (p. 240).

Ports and Ships (2012). Ports of Southern Africa. Available online at www.ports.co.za. Accessed on 28 November 2012.

Ramcharan, C. W., Padilla, D. K., & Dodson, S. I. (1992). Models to predict potential occurrence and density of the Zebra mussel *Dreissena polymorpha*. *Canadian Journal of Fisheries and Aquatic Science, 49,* 2611 – 2620.

Relva, M. A., Nunez, M. A., & Simberloff, D. (2010). Introduced deer reduce native plant cover and facilitate invasion of non-native tree species: evidence for invasional meltdown. *Biological Invasions, 12,* 303 – 311.

Richardson, D. M., Pysek, P., Rejmanek, M., Barbour, M. G., Dane, F., & West, C. J. (2000). Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions, 6*(2), 93–107.

Rius, M., & McQuaid, C. D. (2006). Wave action and competitive interaction between the invasive mussel *Mytilus galloprovincialis* and the indigenous *Perna perna* in South Africa. *Marine Biology, 150,* 69 – 78. doi:10.1007/s00227-006-0322-4

Rius, M., Heasman, K. G., & McQuaid, C. D. (2011). Long-term coexistence of non-indigenous species in aquaculture facilities. *Marine Pollution Bulletin, 62,* 2395–2403. doi:10.1016/j.marpolbul.2011.08.030

Robinson, T. B. and Griffiths, C. L. (2002). Invasion of Langebaan Lagoon, South Africa, by *Mytilus galloprovincialis* - effects on natural communities. *African Zoology, 37,* 151–158.

Robinson, T., Griffiths, C., McQuaid, C., & Rius, M. (2005a). Marine alien species of South Africa - status and impacts. *African Journal of Marine Science, 27*(1), 297–306.

Robinson, T. B., Griffiths, C. L., Tonin, A., Bloomer, P & Hare, M. P. (2005b). Naturalized populations of *Crassostrea gigas* along the South African coast: distribution, abundance and population structure. *Journal of Shellfish Research,* 24: 443-450.

Robinson, T. B., Griffiths, C. L., Branch, G. M., & Govender, A. (2007). The invasion and subsequent die-off of *Mytilus galloprovincialis* in Langebaan Lagoon, South Africa: effects on natural communities. *Marine Biology, 152*(2), 225–232.

Rodriguez, S.R. (2003). Consumption of drift kelp by intertidal populations of the sea urchin *Tetrapygus niger* on the central Chilean coast: possible consequences at different ecological levels. *Marine Ecology Progress Series, 251*, 141–151.

Ruiz, G. M., Carlton, J. T., Grosholz, E. D. & Hines, A. H. (1997). Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent and consequences. *American Journal of Zoology*, 37, 621 – 632.

Ruiz, G. M., Fofonoff, P. W., Carlton, J. T., Wonham, M. J. & Hines, A. H. (2000). Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. *Annual Review of Ecology and Systematics, 31*, 481–531.

Russel, D. J. (1992). The ecological invasion of Hawaiian reefs by two marine red algae, *Acanthophora spicifera* (Vahl) Boerg and *Hypnea musciformis* (Wulfen) J. Ag., and their association with two native species, *Laurencia nidifica* J. Ag. and *Hypnea cervicomis* J. Ag. *ICES Marine Scientific Symposium* (Act Symp), 194, 110-125.

Sephton, D., Vercaemer, B., Nicolas, J. M., & Keays, J. (2011). Monitoring for invasive tunicates in Nova Scotia, Canada (2006-2009). *Aquatic Invasions*, *6*(4), 391–403.

Shiganova, T. A. (1998). Invasion of the Black Sea by the ctenophore *Mnemiopsis leidy*i and recent changes in pelagic community structure. *Fisheries Oceanography*, *7*, 305–310.

Shillington, F. A. & Harris, T. F. W. (1978). Surface waves near Cape Town and their associated atmospheric pressure distributions over the South Atlantic. *Ocean Dynamics, 31*, 67–82.

Simberloff, D. (2006). Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both? *Ecology Letters, 9*, 912 – 919.

Simberloff, D., & Von Holle, B. (1999). Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions*, *1*, 21–32.

Sink, K., Holness, S., Harris, L., Majiedt, P., Atkinson, L., Robinson, T., Kirkman, S., et al. (2012). *Technical Report Volume 4: Marine and Coastal Component Volume 4 : Marine and Coastal Component* (pp. 329).

Sliwa, C., Migus, S., Mcennulty, F., & Hayes, K. R. (2009). Marine Bioinvasions in Australia. Chapter 25. G. Rilov, J. A. Crooks (eds) *Biological Invasions in Marine Ecosystems.* (pp. 425–437).

Smith, R., Bolam, S. G., Rees, H. L., & Mason, C. (2008). Macrofaunal recovery following TBT ban: Long term recovery of subtidal macrofaunal communities in relation to declining levels of TBT contamination. *Environmental Monitoring and Assessment*, *136*, 245–256.

Stachowicz, J. J., Terwin, J. R., Whitlatch, R. B., & Osman, R. W. (2002). Linking climate change and biological invasions : Ocean warming facilitates nonindigenous species invasions. *PNAS*, *99*, 15497 – 15500.

StatSoft, Inc. (2011). STATISTICA (data analysis software system), version 10. www.statsoft.com.

Steffani, C. N., & Branch, G. M. (2003). Growth rate, condition, and shell shape of *Mytilus galloprovincialis*: responses to wave exposure. *Marine Ecology Progress Series*, *246*, 197–209.

Steffani, C. N., & Branch, G. M. (2005). Mechanisms and consequences of competition between an alien mussel, *Mytilus galloprovincialis*, and an indigenous limpet, *Scutellastra argenvillei*. *Journal of Experimental Marine Biology and Ecology*, *317*, 127–142.

Thresher, R. E. (1999). Diversity, impacts and options for managing invasive marine species in Australian waters. *Australian Journal of Environmental Management*, *6*, 164–74.

Trowbridge, C. D. (1998). Ecology of the green macroalga *Codium fragile* (Suringar) Hariot 1889: invasive and non-invasive subspecies. *Oceanography and Marine Biology Annual Reviews, 36*, 1-64.

Vaselli, S., Bulleri, F., & Benedetti-cecchi, L. (2008). Hard coastal-defence structures as habitats for native and exotic rocky-bottom species. *Marine Environmental Research*, *66*(4), 395–403.

Vega, J. M. A., Vasquez, J. A. & Buschmann, A. H. (2005). Population biology of the subtidal kelps *Macrocystis integrifolia* and *Lessonia trabeculata* (Laminariales, Phaeophyceae) in an upwelling ecosystem of northern Chile: interannual variability and El Niño 1997–1998. *Revista Chilena de Historia Natural, 78*, 33–50.

Wasson, K., Zabin, C. J., Bedinger, L., Cristina Diaz, M., & Pearse, J. S. (2001). Biological invasions of estuaries without international shipping: the importance of intraregional transport. *Biological Conservation*, *102*, 143–153.

Wilcove, D. S., Rothstein, D., Dubow, J., Phillips, A. & Losos, E. (1998). Quantifying threats to imperiled species in the United States. *BioScience*, *48*, 607–615.

Williamson, M. (1996). *Biological Invasions*. Chapman & Hall, London.

Xavier, B. M., Branch, G. M., & Wieters, E. (2007). Abundance , growth and recruitment of *Mytilus galloprovincialis* on the west coast of South Africa in relation to upwelling. *Marine Ecology Progress Series, 346*, 189–201.

Zaitsev, Y., & Marnaev, V. (1997). *Biological Diversity in the Black Sea: A Study of Change and Decline*. New York: United Nations Publications.

Zardi, G. I., Nicastro, K. R., Porri, F., & McQuaid, C. D. (2006). Sand stress as a non-determinant of habitat segregation of indigenous (*Perna perna*) and invasive (*Mytilus galloprovincialis*) mussels in South Africa. *Marine Biology*, *148*, 1031–1038.

APPENDIX 1. Target list of known marine alien species, their origin and their South African distribution. Information obtained from Mead et al., 2011a and Picker and Griffiths, 2011. Note that not all species on this list occur in the biogeographic zones sampled in this study but the potential of these species being detected in harbours was considered.

Taxonomic Group & Scientific Name	**Origin**	**SA Distribution**
PORIFERA		
*Suberites ficus**	NE Atlantic and Meditteranean	W coast
CNIDARIA		
Anthozoa		
*Metridium senile**	N Atlantic	Table Bay, Agulhas Bank
Sagartia ornata	Europe, Meditteranean	Saldanha Bay
Hydrozoa		
Coryne eximia	N Atlantic, Pacific	Table Bay harbour
Gonothyraea loveni	N Atlantic	Table Bay harbour
Laomedea calceolifera	N Atlantic	N Atlantic
Moerisia maeotica	Black Sea region	KZN lagoons
Obelia bidentata	Uknown	Table Bay harbour, Durban
Obelia dichotoma	Uknown	Lambert's Bay to Algoa Bay
Obelia geniculata	Europe, Meditteranean	Entire coastline
Pachycordyle navis	Europe, Meditteranean	Table Bay harbour
Pennaria disticha	Uknown	Durban northwards
Pinauay larynx	N Atlantic	Cape Peninsula
Pinauay ralphi	N Atlantic	Table Bay harbour, Durban
ANNELIDA		
Polychaeta		
Boccardia proboscidea	N Pacific	Abalone aquaculture, W Cape
Dodecaceria fewkesi	Pacific N America	Table Bay docks
Ficopomatus enigmaticus	Australia	Cape Town to Kosi Bay
Hydroides elegans	Indo-Pacific	False Bay
Janua pagenstecheri	Europe	Cape Town to Durban
Neanthes succinea	Europe	Mossel Bay to Durban
Neodexiospira brasiliensis	West Indies, Brazil	Cape Town to Port Elizabeth
Polydora hoplura	Europe, Meditteranean	Saldanha Bay to Plettenberg Bay

CRUSTACEA		
Cirripedia		
Amphibalanus venustus	Tropical N Atlantic	Hermanus to Mozambique
*Balanus glandula**	N American Pacific	W coast
Isopoda		
Dynamene bidentata	Europe	Port Elizabeth
Limnoria quadripunctata	Unknown	Table Bay to Port Elizabeth
Limnoria tripunctata	Unknown	Table Bay
Paracerceis sculpta	NE Pacific	Port Elizabeth
Sphaeroma serratum	Europe	Durban Bay
Sphaeroma walkeri	N Indian Ocean	KZN coastline
Amphipoda		
Apocorophium acutum	N Atlantic	Durban
Chelura terebrans	Pacific N America	Saldanha Bay to Port Elizabeth
Cerapus tubularis	Atlantic N America	Saldanha Bay to Kosi Bay
Erichthonius brasiliensis	N Atlantic	Widespread coastal
Ischyrocerus anguipes	N Atlantic	Entire coast
Jassa marmorata	N Atlantic	Table Bay to KZN
Jassa morinoi	N Atlantic	False Bay to KZN
Jassa slatteryi	Pacific N America	Saldanha Bay to Knysna
Monocorophium ascherusicum	N Atlantic	Alexander Bay to Durban
Orchestia gammarella	Europe, Meditteranean	Cape Peninsula
Platorchestia platensis	Origin unknown	Gansbaai
Decapoda		
*Carcinus maenas**	Europe, Meditteranean	Cape Peninsula
Xantho incicus	Europe, Meditteranean	Kleinsee
PYCNOGONIDA		
Ammothella appendiculata	Pacific	Durban Bay
MOLLUSCA		
Gastropoda		
Catriona columbiana	N Pacific	Cape Town
Tarebia granifera	SE Asia	KZN coastal strip
Thais blanfordi	Tropical Indo-Pacific	Durban
Thais tissoti	Tropical Indo-Pacific	Durban
Bivalvia		
Crassostrea gigas	Japan, NW Pacific	S Cape estuaries
Lyrodus pedicellatus	Uknown	Simon's Town

*Mytilus galloprovincialis**	Meditteranean, NE Atlantic	Alexander Bay to East London
Ostrea edulis	Europe, Meditteranean	Alexander Bay
Perna viridis	SE Asia	East London harbour
Semimytilus algosus	Pacific S America	Alexander Bay to Cape Town
Teredo navalis		
BRACHIOPODA		
*Discinisca tenuis**	Namibian coast	Saldanha Bay, Port Elizabeth (aquaculture facility)
BRYOZOA		
Bugula dentata	Indo-Pacific	Table Bay to Sodwana Bay
Bugula flabellata	Unknown	Port Nolloth to Plettenberg Bay
Bugula neritina	Europe?	Port Nolloth to Durban
Conopeum seurati	Europe	Saldanha Bay, False Bay
Cryptosula pallasiana	Europe	Saldanha Bay to False Bay
Watersipora subtorquata	Caribbean	Saldanha Bay to Knysna
CHORDATA		
Ascidiacea		
Ascidia sydneiensis	Asia	False Bay to Bushman's River
Ascidiella aspersa	North Sea	Saldanha Bay to Table Bay
Botryllus schlosseri	Northeast Atlantic	Durban to Alexander Bay
*Ciona intestinalis**	Europe	Durban to Alexander Bay
*Clavellina lepadiformis**	Europe	Durban to Saldanha Bay, harbours
Cnemidocarpa humilis	Unknown	Alexander Bay to Cape Agulhas
Diplosoma listerianum	Europe	Durban to Alexander Bay
Microcosmus squamiger	Australia	Mossel Bay to Richards Bay
*Styela plicata **	W Pacific	Saldanha Bay to Richards Bay

* Species marked with an asterisk are those species used in the target list for the visual (1x1m) samples.