Genetic connectivity of the slinger *Chrysoblephus puniceus* among Marine Protected Areas and unprotected areas along the east coast of South Africa

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SAMANTHA ANGELIQUE NATASHA OCKHUIS

ORCID ID: https://orcid.org/0000-0002-5955-6279

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ABSTRACT

Marine Protected Areas (MPAs) are advocated worldwide for the conservation of biodiversity and fisheries management, as well as for adaptation and resilience in the face of climate change. Their effectiveness has been noted through increases in abundances, biomass, sizes and diversity of species, as well as the recovery of overexploited fisheries. For MPAs to realise their full potential, these benefits need to expand beyond their boundaries through larval dispersal and/or adult spillover. Connectivity between MPAs is critical for planning and placement of MPAs, as this promotes the persistence of metapopulations, their recovery from disturbance, as well as productivity in marine ecosystems. The effectiveness of MPAs in conserving biodiversity along the east coast of South Africa has been established; however, limited evidence is available on whether they offer benefits beyond their boundaries, enhancing biodiversity and fisheries in adjacent areas.

The slinger *Chrysoblephus puniceus*, an endemic southern African sparid, is a major component of the commercial linefishery in KwaZulu-Natal (KZN), South Africa and southern Mozambique, and is also caught in the recreational ski-boat fishery in these regions. The spawning of slinger occurs in shoals on offshore reefs from southern Mozambique to the north of KZN, and larvae are likely transported southwards, assisted by the southward-flowing Agulhas Current and promoted by a relatively long larval duration. Slinger are relatively resident, but occasional northward migrations have been observed. These life history characteristics suggested that slinger would be a suitable biological model to test connectivity along the east coast, and particularly connectivity between MPAs. The aim of this study was therefore to investigate connectivity of slinger between MPAs and the surrounding areas along the east coast of South Africa, using restriction-site-associated DNA (RAD) sequencing. This

formed part of the larger CAPTOR project investigating east coast MPA connectivity across a range of taxa and habitat types.

A temporal comparison of genetic diversity in slinger spanning two sampling events (2012 and 2018) revealed no substantial changes in genetic diversity, as well as a lack of genetic structure along the KZN coast. Similarly, no substantial changes in effective population size (N_e) were found between 2012 and 2018 for microsatellites and Single Nucleotide Polymorphisms (SNPs). However, subtle, albeit non-significant, differences were observed both spatially and over time in the microsatellite data, indicating some instability along the KZN coast.

A lack of genetic structure based on RAD sequencing was observed between east coast MPAs and the surrounding areas. This implies high connectivity along the coast - not only are east coast MPAs connected, the surrounding areas are also connected, and there is replenishment between all areas via larval dispersal and/or adult spillover. Analyses of multidirectional gene flow between MPAs, together with known spatial spawning patterns and prior particle dispersal modelling, identified the Richards Bay area as a key source of recruits.

However, parentage analysis – a direct method to estimate connectivity – failed to match any parent-offspring pairs in this study; successful parentage assignments are highly dependent on intensive sampling of potential parents and are seldom feasible in marine systems, as most populations have large effective population sizes. Estimates of intergenerational effective population size for slinger in this study are considered large - >2 000 and infinite based on adults and juveniles, respectively. Population genetics revealed close genetic similarities between slinger adult and juvenile slinger sites, emphasizing genetic connectivity and the lack of structure between sites observed before.

This was the first study on South African sparids to use RAD sequencing to assess genetic connectivity between MPAs along the east coast of South Africa. Overall, results provide

evidence that MPAs along the east coast of South Africa are connected, and can readily replenish or be replenished by other sites along the east coast of South Africa, whether they are within MPAs or not. So too are the areas surrounding MPAs interconnected, and slinger forms a homogenous single population on the east coast of South Africa. This notwithstanding, it is important that east coast MPAs, particularly those that host spawning shoals of slinger, are well managed in order that they continue to provide recruits to sustain the population and support sustainable fisheries.

DECLARATION

I, Samantha Angelique Natasha Ockhuis, hereby declare that this thesis submitted to the Department of Ichthyology and Fisheries Science, Rhodes University, is my original work and has not been previously submitted in any form to another university. I have not included ideas, phrases, passages or illustrations from another person without acknowledging their authorship.

ETHICAL CLEARANCE

Some slinger fin clips used in Chapter 3 were collected during previous projects, approved by the South African Institute for Aquatic Biodiversity's Animal Ethics Committee under the reference numbers: 2012/08 1 ('Molecular systematics of marine fishes') and 2012/08 2 ('Modelling the effects of climate change on the distribution of shared fishery species in the Western Indian Ocean'). For Chapters 3, 4 and 5, fin clips were collected by the Oceanographic Research Institute, ethics approval was granted under its legal entity (South African Association for Marine Biological Research) in terms of its Annual Ethics Policy (SAAMBR POLICY 013, first issued on 22 May 2014) and its Standard Operating Procedures for ethical handling, conventional tagging, fin clip sampling of, and surgical implantation of transmitters in fishes.

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Chapter 1: General introduction

Marine Protected Areas

The first documented Marine Protected Area (MPA) in the world is the Royal National Park, in Australia, located near Sydney, which was promulgated in 1879 (Laffoley et al. 2018). This MPA was proclaimed to protect a large tidal inlet. Initially, MPAs were declared for the protection of threatened species or scenic locations. However, this focus shifted to include a wide range of marine and coastal habitats, and the first MPA taking an ecosystem-based approach was the Fort Jefferson National Monument in Florida, United States, proclaimed in 1935 (Laffoley et al. 2018). According to the International Union for Conservation of Nature (IUCN), an MPA is defined as "an area of intertidal or subtidal terrain, together with its overlaying waters, and associated flora, fauna, historical and cultural features, which has been reserved by law or other effective means to protect part or all of the enclosed environment" (Kelleher and Kenchington 1992: 7).

Globally, MPAs have been created to restore and sustain fisheries, conserve biodiversity (Mora et al. 2006, Wood et al. 2008), which includes threatened species, and support the recovery of depleted species (Edgar et al. 2007). MPAs sustain fisheries (through spillover) and support conservation, but they also preserve areas of cultural significance, and generate aesthetic and education benefits (Edgar et al. 2014). The benefits of MPAs as a fisheries management tool have been demonstrated globally by increased abundances, mean sizes and ages, and per-capita fecundity of exploited species within protected areas (Lester et al. 2009, Graham et al. 2011, Edgar et al. 2014, Baskett and Barnett 2015).

Initially, coastal MPAs in South Africa were implemented to protect intertidal resources for purposes of biodiversity conservation (Sink et al. 2019). The establishment of MPAs in South

Africa came with the rapid increase in coastal populations and the mounting demands made on the marine environment (Mann et al. 1998). These demands were seen in the deterioration of coastal fisheries to the point where stock estimates were below commonly-accepted threshold values, and shifts from targeted species to less desirable species, with more valuable species disappearing in the commercial linefishery (Buxton 1992, Bennett 1993, Griffiths 1997a). Catches by shore-anglers declined consistently, where ordinary catches in the past became rarities (Bennett 1991a). Even shellfish resources showed signs of overexploitation. Much later, the protection of offshore resources was considered and the potential role of MPAs in fisheries was taken into account (Sink et al. 2019).

In South Africa, the first MPA was declared in 1964 at Tsitsikamma on the south coast (Attwood et al. 1997b). This MPA was incorporated as a conservation measure to provide refuge for highly-threatened linefish and the recovery of adjacent fisheries (Lombard et al. 2019). By 2011, 25 MPAs had been declared in South African waters, covering 23% of the length of the coastline. In 2019, an expanded network of 20 new MPAs (mainly offshore) was declared (RSA 2019), which included the expansion of some existing MPAs. There is now a total of 42 MPAs along the South African coast, including a very large MPA surrounding the Prince Edward Islands (Sink et al. 2019). Kirkman et al. (2023) provide the most recent review of the history of MPAs in South Africa.

MPAs were also introduced as a fisheries management tool for the South African linefishery when other restrictive measures, such as closed seasons, daily bag limits, and minimum size limits, were not practical to enforce or proved ineffectual (Bennett 1991b). The effectiveness of MPAs in this respect was first shown by Bennett and Attwood (1991), and MPAs have been specifically chosen as one of the management tools for a variety of linefish species (Bennett and Attwood 1991, Cowley et al. 2002, Kerwath et al. 2008, Currie et al. 2012, Maggs et al. 2013a). South African MPAs, as in many other parts of the world, have also been shown to

enhance reef fish abundance, size and diversity (Buxton and Smale 1989, Cowley et al. 2002, Götz et al. 2008, Kerwath et al. 2008, 2013, Currie et al. 2012, Floros et al. 2013, Mann et al. 2016). This has proven an effective form of management for resident, long-lived species susceptible to overfishing (Attwood et al. 1997a, 1997b). MPAs also provide additional fisheries benefits by (i) providing refugia for resident stocks within MPAs (Bennett and Attwood 1991), (ii) providing spillover (the export of adult fishes beyond MPA boundaries) to adjacent fishing areas (Kerwath et al. 2013), (iii) enhancing the recovery of depleted stocks (Bennett and Attwood 1991), and (iv) aiding the recovery of exploited populations (Buxton 1987, Buxton and Smale 1989, Mann et al. 2016).

MPAs and connectivity

MPAs replenish their adjacent areas through larval dispersal and adult spillover (Rowley 1994, Cowen et al. 2000, Russ 2002, Jones et al. 2007, Halpern et al. 2010), but can be more effective in replenishing areas beyond their boundaries when they are connected to other MPAs, promoting persistence in marine ecosystems (Planes et al. 2009). Furthermore, knowledge of connectivity of marine fauna between MPAs and adjacent areas is crucial for marine conservation (Almany et al. 2009, McCook et al. 2009, Beger et al. 2010, Kininmonth et al. 2011, Olds et al. 2012a) and fisheries management (Leis et al. 2011), and is key in developing spatial management tools for designing MPAs (Goñi et al. 2010).

Spillover is variable and depends on several factors, such as the mobility of the species, social tendencies, spawning habits of a given species and local oceanographic conditions (Lester et al. 2009, Claudet et al. 2010). Empirical evidence of spillover is still sparse, with a few exceptions (Goñi et al. 2010, Halpern et al. 2010, Kerwath et al. 2013). In South Africa, a few studies have demonstrated adult spillover based on tag-and-release or acoustic telemetry, and

provided evidence of movement of adult fish from MPAs to exploited adjacent areas (Attwood and Bennett 1994, 1995a, Brouwer 2002, Brouwer et al. 2003, Attwood and Cowley 2005, Maggs et al. 2013b, Mann et al. 2015, Maggs and Cowley 2016). Information on larval dispersal from MPAs is scarce due to difficulties involved in tracking small larvae, and the large spatial and temporal variation in larval production and survivorship (Pelc et al. 2010). However, biophysical models, which use high-resolution oceanographic data and biological information, are now able to predict larval dispersal (Pelc et al. 2010). A few studies in South Africa have provided information on larval dispersal, but mainly focussed on the Tsitsikamma MPA on the south coast (Sauer 1995, Tilney et al. 1996, Attwood et al. 2002, Brouwer et al. 2003, Roberts and van den Berg 2005).

Knowledge of biological life history characteristics, as well as animal movements, is vital for the spatial design of MPAs, and implementation of corrective fisheries management strategies (Attwood and Cowley 2005). Residency (when a species remains within a circumscribed area) is a common behaviour/movement pattern found in both temperate and tropical reef fish species (Roberts and Polunin 1991, Brouwer et al. 2003, Zeller et al. 2003, Attwood and Cowley 2005, Maggs et al. 2013b). Several studies have demonstrated residency in sparids throughout their life history. (Attwood and Bennett 1994, Cowley et al. 2002, Griffiths and Wilke 2002, Brouwer et al. 2003, Attwood and Cowley 2005, Kerwath et al. 2007, 2013, Götz et al. 2008, Maggs et al. 2013b). However, other sparids are resident as juveniles, but migrate as sub-adults or adults. These include the white steenbras *Lithognathus lithognathus*, considered to be partial migrators where some migrate but most remain resident (Bennett et al. 2017a), the white musselcracker *Sparodon durbanensis* (Bennett and Attwood 1991, Booth 2000), and red steenbras *Petrus rupestris* (Brouwer 2002). Despite residency being the predominant behaviour, some species also display nomadic behaviour, which is mainly foraging-related (Mann et al. 2015). In other studies on non-sparid coastal species, movement behaviour was

related to reproduction, with adults migrating up to the South African east coast for spawning to ensure their progeny recruited to nursery areas in the south via dispersal assisted by inshore processes of the Agulhas Current (AC). These included the shad *Pomatomus saltatrix* (van der Elst 1976), geelbek *Atractoscion aequidens* (Griffiths and Hecht 1995), leervis *Lichia amia* (Dunlop et al. 2015) and galjoen *Dichistius capensis* (Attwood and Cowley 2005).

However, in order to gain a better understanding of movement within MPAs, spillover, and connectivity between MPAs and adjacent open areas, genetic data are crucial. Recent advances in molecular techniques, as well as biophysical models, they have enabled the quantification of population connectivity and larval export. For example, microsatellites have been successful in resolving population genetic structure, genetic diversity, average kinship and effective population size (Lemopoulos et al. 2019). More recently, Single Nucleotide Polymorphisms (SNPs) have become the preferred marker in population genetic studies (Davey and Blaxter 2011, Andrews et al. 2016). Restriction-site-associated DNA sequencing (RADseq) is one of several techniques to obtain SNPs, producing thousands of loci, and, therefore, these markers can provide more reliable inferences of population structure compared to microsatellites (Bruneaux et al. 2013). Certainly, SNPs provide more detailed information on genetic structure (Sunde et al. 2020), and more accurate and informative parentage/relatedness analyses (Lemopoulos et al. 2019) relative to microsatellites. Although several studies using microsatellites determined genetic connectivity between MPAs and unprotected/surrounding areas in South Africa (Teske et al. 2010) and in other temperate/tropical areas (van der Meer et al. 2015, Calò et al. 2016, Eastwood et al. 2016, Sahyoun et al. 2016, Le Port et al. 2017, Guzmán-Méndez et al. 2020). Only a few studies used SNPs for assessing connectivity among MPAs and surrounding areas in tropical areas (Stockwell et al. 2016, Beltrán et al. 2017, Mzingirwa et al. 2019).

Linefisheries in South Africa

The South African linefishery is described as the harvesting of fish with hook and line, but excludes the use of longlines (Mann 2013). The linefishery dates back to the indigenous Khoi people and the European seafarers in the 1500s (Thompson 1913), originally developing in the Cape region on the southern tip of Africa, and eventually spreading to the KwaZulu-Natal (KZN) region on the east coast. Despite the high abundance of fish, the fishery was slow to develop due to various restrictions when the Dutch colonised the Cape in 1652 (Thompson 1913). These fishing restrictions were removed in 1795 when the British captured the Cape Colony, and in the 1800s a boat-based linefishery became a thriving industry (Thompson 1913).

The South African linefishery is divided into three sectors; the commercial, subsistence and recreational (also referred to as angling) sectors (Mann 2013). The commercial linefishery is exclusively boat-based and provides livelihoods for a variety of people along the entire South African coast, albeit that it is a low-earning, labour-intensive industry (Mann 2013). The subsistence sector consists mainly of fishers who are poor, and for whom fishing provides a source of food or a commodity to sell to meet the basic needs of food security (Sowman 2006). These participants mainly live in close proximity to the resources and use low-technology gear (Sowman 2006). The marine recreational fishery sector is by far the largest in terms of the number of participants, and has considerable economic value, although participants are not allowed to sell their catch (DAFF 2016). This sector is divided into four distinct facets: shore angling, ski-boat angling, estuarine angling and spearfishing (van der Elst 1989).

The commercial linefishery off KZN only developed towards the end of the 19th century, after some of the larger steam-powered vessels, which were initially introduced to exploit the rich demersal fish resources of the southern Cape (Lees 1969), moved up the east coast. At present, commercial linefishing in KZN is undertaken from much smaller, surf-launched vessels, which

undertake daily trips. In the late 1990s, the boat-based linefishery off KZN accounted for approximately 35% of the capital value of all the KZN fisheries and landed an estimated 40% of the total annual weight of marine fish caught in the province (McGrath et al. 1997, Penney et al. 1999).

The South African linefishery has changed tremendously over the years, particularly in the latter half of the 20th century, with an increase in fishing effort, increase in operational range through the introduction of motorised, surf-launched ski-boats, and the improvement of fishing technology (DAFF 2016). The substantial increase in fishing effort led to overfishing of most linefish resources around the coast during the last quarter of the 20th century (Griffiths 2000). As a result, there were notable changes in the catch composition along the entire coast of South Africa (Hecht and Tilney 1989, Bennett 1991b, Penney et al. 1999, Brouwer and Buxton 2002) with a decrease in mean length of fishes (Hecht and Tilney 1989, Yemane et al. 2004). Seabreams (sparids) are the most commonly caught fish in the commercial linefishery, and are also important to the recreational sector (Penney et al. 1989). They are generally endemic, slow-growing and late-maturing fish species with complicated life histories, including sex change, which makes them susceptible to overexploitation (Griffiths 2000, Comeros-Raynal et al. 2016).

There are regulations in place to manage the linefish resources (Marine Living Resources Act No. 18 of 1998), but the fishery is not easily managed due to the multi-species, multi-sector, and multi-area nature of the fishery, and the life histories of the target species (Winker et al. 2014). The commercial linefishery is managed by a Total Allowable Effort (TAE) allocation (number of licenses), and bag and minimum size limits for some species (Winker et al. 2014). The recreational sector is managed by size and bag limit restrictions for all species. No-take areas and closed seasons for certain species apply to both sectors (Winker et al. 2014).

Regulation of the subsistence sector is in its implementation phase and will be managed by a combination of the abovementioned (DAFF 2016).

Concerns around the overfishing of some linefish species were first raised in the 1940s (Griffiths 2000), but the first comprehensive management framework for the linefishery was only introduced in 1985 (Penney et al. 1989). Data from research surveys conducted during the 1990s still indicated continuing declines in the linefish resources (Punt 1993, Griffiths 1997a, 1997b, 2000).

Following this, an emergency in the linefishery was declared in 2000 by the then Minister of Environmental Affairs and Tourism, and species-specific management plans were implemented during the mid-2000s (Mann 2013). This led to a reduction in TAE of approximately 70% in the commercial linefishery sector in 2000, which continued at this level during the 2003 and 2005 rights allocation process (Winker et al. 2012). A reduction in recreational fishing pressure was also implemented, with more stringent species-specific daily bag and size limits since 2005 (DAFF 2016).

Study species

Chrysoblephus puniceus, commonly known as the slinger, is a major component of the commercial linefishery in KZN (Dunlop and Mann 2013) and southern Mozambique (Garratt 1985a, Lichucha 1999, 2001), and is also important in the recreational skiboat fishery in these regions (Dunlop and Mann 2013). This species is endemic to the south-eastern coast of southern Africa from Vilanculos in southern Mozambique to Algoa Bay in the Eastern Cape, South Africa, with the core distribution between Ponta Zavora, Mozambique and Coffee Bay in the Eastern Cape (Garratt 1985a; Figure 1.1). Adults inhabit offshore reefs from 20 –130 m (Heemstra and Heemstra 2004), while very small juveniles [<5 cm fork length (FL)] have been

recorded on shallow reefs off Pondoland MPA at 10 – 30 m depth (Mann et al. 2006); juveniles >5 cm FL are widely spread along the KZN coast at 20 – 60 m depth (Garratt 1984). Spawning is restricted to offshore reefs from southern Mozambique to northern KZN, peaking from August to October (Garratt 1985b).

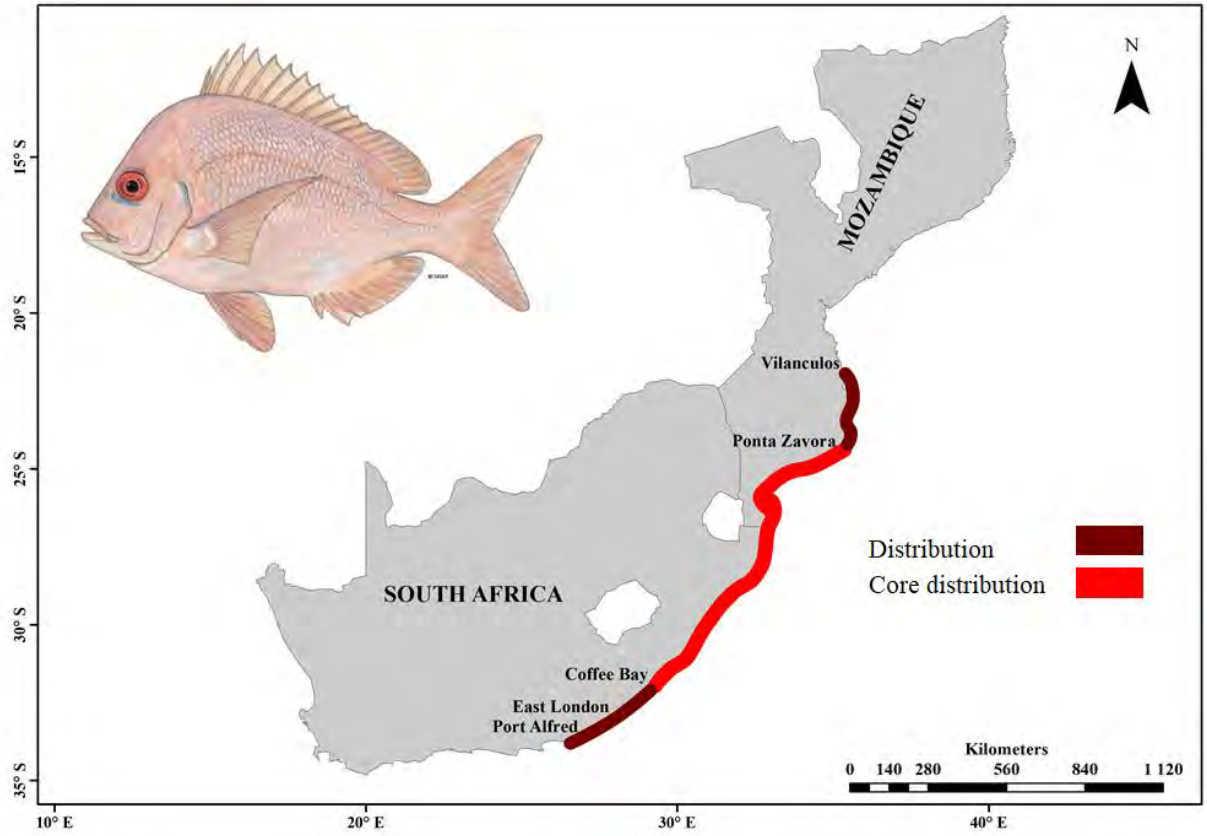

Figure 1.1: The distribution (maroon) and core distribution (red) of the slinger *Chrysoblephus puniceus* (pictured) along the east coast of southern Africa. Illustration from Heemstra and Heemstra (2004)

There was long-held speculation that slinger larvae were transported southward by the powerful southward-flowing Agulhas Current (AC) (Heydorn et al. 1978, Garratt 1988, van der Elst and de Freitas 1988). However, there were limited empirical fish larval studies on the east coast of South Africa to support this contention, and Beckley (1993) subsequently found that processes at the shoreward edge of the AC, not the AC itself, were instrumental in linefish larval dispersal. Essentially, linefish larvae were not retained in the main current, but on the

shelf around 100 – 150 m depth. Hutchings et al. (2002) further elaborated on the spatial spawning strategies of several fish species on the east coast, involving the northward migration of mature fishes from Western and Eastern Cape waters, and southward dispersal of their larvae. This strategy was also suggested for slinger by Duncan et al. (2015), who used evidence of genetic panmixia to infer a high likelihood of southward dispersal by the prevailing oceanography acting on slinger larvae.

The complex life history of the slinger makes this species vulnerable to over-exploitation. It is a protogynous hermaphrodite (changing sex from female to male) (Garratt 1985b), and is slow-growing, reaching maturity at the age of three years and changing sex at the age of five (Garratt et al. 1993). The slinger is also fairly resident, but tagging studies have revealed occasional long-distance northward movements by adults (Maggs et al. 2013b). This species is found in several no-take MPAs, which have been shown to provide protection for slinger (Mann et al. 2006, Maggs et al. 2013a).

Rationale, aims and objectives

Five relatively large MPAs (iSimangaliso, uThukela, Aliwal Shoal, Protea Banks and Pondoland) are present along the east coast of South Africa, from the northern KZN coast to the northern Eastern Cape (Figure 1.2). The largest, iSimangaliso, was established to conserve vulnerable coral habitats and turtle nesting sites, and fortuitously also included large populations of slinger (Mann et al. 1998, 2006). A strong motivation for the establishment of the Aliwal Shoal and Pondoland MPAs was to conserve endemic fishes, including slinger. In 2019, the uThukela and Protea Banks MPAs were established, and the iSimangaliso and Aliwal Shoal MPAs were expanded to achieve marine habitat conservation targets (Sink et al. 2019), providing further protection for endemic fishes. While these MPAs undoubtedly conserve their

inhabitants and associated habitats, there is limited evidence on whether they offer benefits beyond their boundaries, promoting persistence in marine ecosystems.

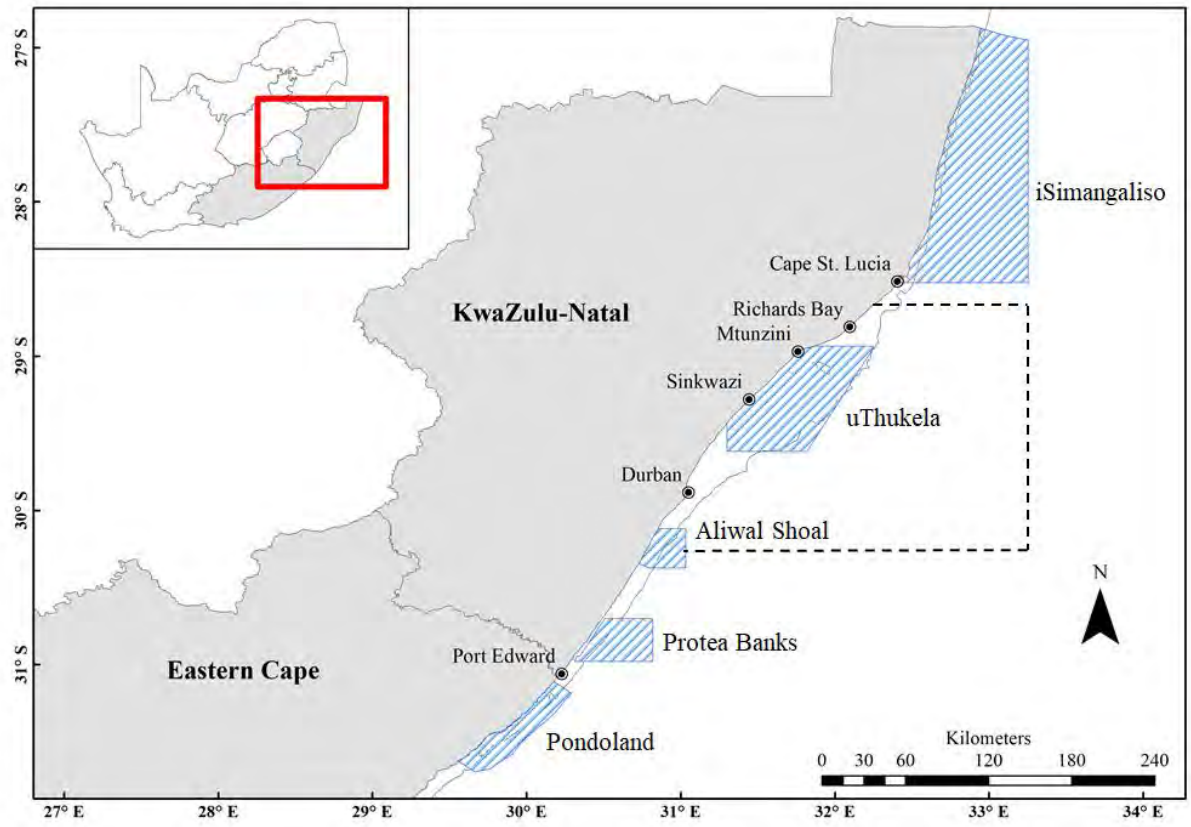

Figure 1.2: Study region along the east coast of South Africa including five Marine Protected Areas (iSimangaliso, uThukela, Aliwal Shoal, Protea Banks and Pondoland) and unprotected areas Richards Bay and Port Edward. Dashed line polygon denoted the KwaZulu-Natal Bight

The CAPTOR project

The CAPTOR (Connectivity And disPersal beTween prOtected aReas) MPA connectivity project was funded by the National Research Foundation (DSI-NRF) and African Coelacanth Ecosystem Programme (ACEP), and sought to determine whether the network of new and existing MPAs mentioned above are connected, and if they offer benefits beyond their boundaries. The main objective of CAPTOR was to examine connectivity and dispersal of neritic fauna between South African east coast MPAs, and surrounding habitats, by integrating

information on physical oceanographic processes (currents, eddies and turbulence) and biological processes (reproduction, migration and recruitment) to understand the mechanisms and extent of connectivity. The work in this thesis was conducted in parallel with other studies that form part of the CAPTOR project, investigating connectivity in hard and soft corals, as well as in marine benthic infauna. The slinger is used as a model species (for many co-distributed sparid species with similar life histories and of commercial importance) and these results will be integrated with the other studies on taxa with life histories contrasting that of the slinger, which is more mobile, to determine the drivers of connectivity.

The overall aim of this thesis was to use the slinger as a biological model to determine whether east coast MPAs are connected and whether they replenish their surrounding areas. Two molecular markers, microsatellites and SNPs, were used to test genetic connectivity among MPAs and the surrounding areas. This study also re-examined the conclusions drawn from previous published genetic data based only on microsatellites (Duncan et al. 2015) to see if these authors' conclusions of a single population are still applicable, here using a genome-scale SNP dataset which is generally regarded to have greater power in resolving fine-scale population structure or lack thereof (Lemopoulos et al. 2019, Sunde et al. 2020), thus, providing more robust and reliable measures of connectivity. In detail:

The first objective aimed to provide an assessment of temporal genetic diversity in slinger over a period of six years (2012 and 2018) using both microsatellites and SNPs. Slinger is heavily exploited and is a protogynous hermaphrodite. Sex-changing fish can present a highly skewed sex ratio, which decreases the effective population size and in turn leads to higher variations in allele frequencies, which increases genetic differentiation (Hartl and Clark 1997). If temporal genetic stability is demonstrated, there would be greater confidence in inferring spatial population genetic structure (or the lack thereof) or source-sink dynamics in subsequent chapters (4 and 5, respectively) being independent of when sampling occurred. Alternatively,

should there be any temporal structure; this should be considered in the interpretation of spatial population genetic structure or source-sink dynamics. This component of the study aimed to provide more insight into this.

The second objective aimed to investigate genetic structure and connectivity in slinger among MPAs and the surrounding areas using SNPs. Knowledge of genetic structure and connectivity of slinger would help to better inform fisheries management and marine spatial planning for this important fishery species.

The third objective aimed to determine the sources (where they are spawned) and sinks (where they recruit to) of slinger recruits. Information on source-sink dynamics of slinger could be included in marine spatial planning, identifying areas where continued or increased exploitation could place the resource at risk.

This thesis is divided into six chapters, including the present general introduction (Chapter 1), and a study area description (Chapter 2). Chapter 3 investigates temporal and spatial genetic diversity in slinger using both microsatellites and SNPs, over a period of six years. Chapter 4 investigates connectivity between east coast MPAs and the surrounding areas. The sources and sinks of slinger are determined in Chapter 5. Chapter 6 discusses the main findings, shortcomings and recommendations, as well as the management implications of this study. There is, thus, some degree of repetition in the chapters; abstracts are included in Chapters 3 to 5 in preparation for their intended submission for publication.

Chapter 2: Study area

Oceanography of study area

The study sites were situated on the east coast of South Africa. This region falls into the known distribution of slinger, where this species inhabits offshore reefs from Vilanculos in southern Mozambique to Algoa Bay in the Eastern Cape, South Africa. The oceanographic regime on the east coast of South Africa is dominated by the Agulhas Current (AC), a fast, warm, southward/poleward-flowing, western boundary current (Roberts et al. 2010). The AC is highly variable and this variability is derived from the recirculation of the Southwest Indian Ocean sub-gyre, the flow through the Mozambique Channel, and the South East Madagascan Current (Stramma and Lutjeharms 1997, Lutjeharms 2006) (Figure 2.1). The AC closely follows the continental slope, and becomes detached as a free-flowing jet south of the Delgoa Bight of Mozambique (Figure 2.1). The continental shelf on the east coast is generally narrow, but the shelf widens between Cape St Lucia and Durban, an area known as the KwaZulu-Natal (KZN) Bight (Figure 1.2). The KZN Bight is responsible for generating complex circulation patterns. For example, three types of upwelling occur: (1) topographically-induced upwelling off St Lucia/Richards Bay in the north; (2) shelf-edge upwelling; and (3) cyclonic, lee-trapped, eddy–induced upwelling to the south of Durban (Guastella and Roberts 2016, Roberts and Nieuwenhuys 2016) (Figure 2.1). Together, these features with the riverine inputs, make this region nutrient-rich compared to the nutrient-poor AC (Bustamante et al. 1995). The KZN Bight is thought to offer refuge from the offshore AC, thus providing a suitable spawning ground and nursery for fish larvae (Hutchings et al. 2002).

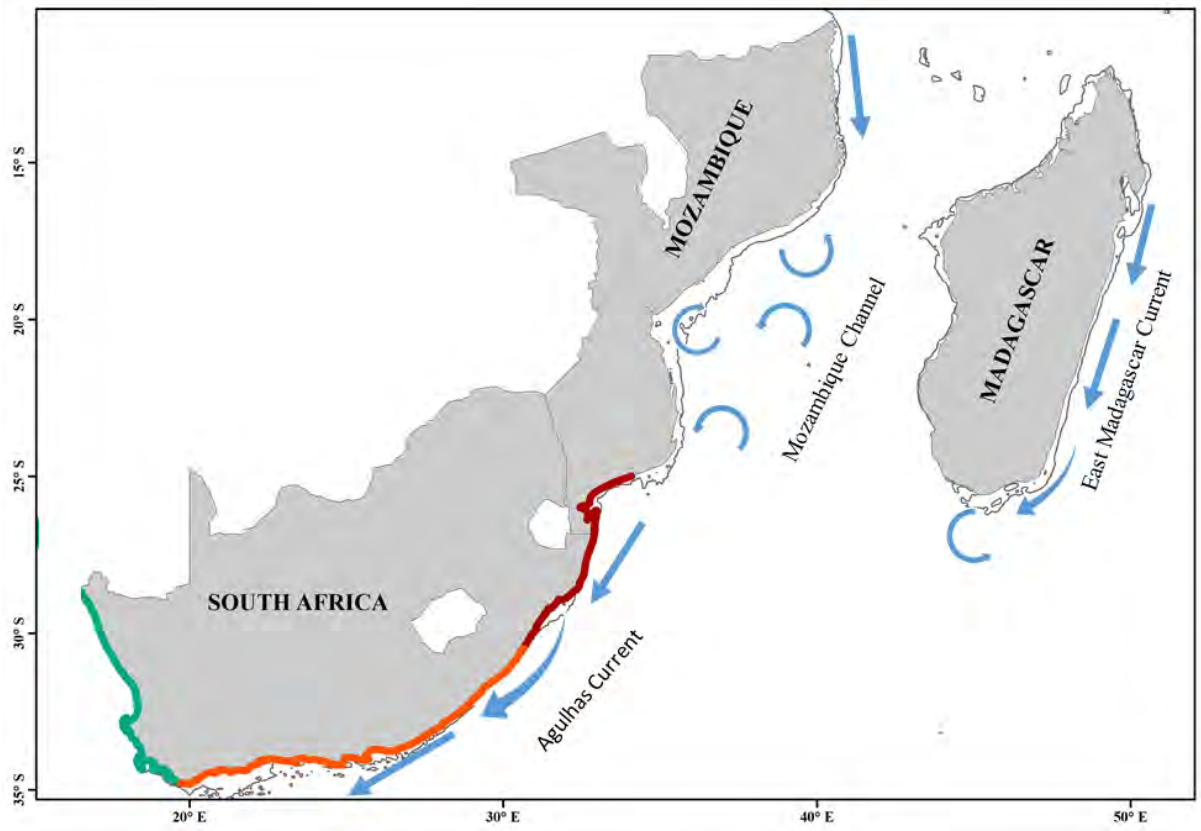

Figure 2.1: Circulation features of the greater Agulhas Current. The Agulhas Current is sourced through the anti-cyclonic circulation northwest of Madagascar and mesoscale eddy activity from both the Mozambique Channel and south of Madagascar (adapted from (Lutjeharms 2006). Biogeographic regions indicated, subtropical (red), warm-temperate (orange) and cool-temperate (green)

Biogeography of study area

Three biogeographic provinces were initially recognised along the South African coastline, the cool-temperate West Coast, the warm-temperate South Coast and the subtropical East Coast (Stephenson 1939, 1944, 1948) (Figure 2.1). The National Biodiversity Assessment identified four biogeographic provinces (Sink et al. 2019), confirmed in a recent revised ecosystem classification scheme that incorporated biogeographical zonation and introduced new types (van Niekerk et al. 2020). These included four coastal ecoregions, the cool-temperate southern Benguela, the warm-temperate Agulhas, the subtropical Natal and tropical Delagoa ecoregions. The southern Benguela ecoregion is further divided into two sub-regions, the Namaqua and Cape, while the Natal ecoregion is divided into the southern KZN and Wild Coast sub-region,

and the KZN Bight sub-region. Further offshore, two deep ocean ecoregions are recognised – the Southeast Atlantic Deep Ocean ecoregion and the Southwest Indian Deep Ocean ecoregion. The eastern ecoregions support a high diversity of marine species, as well as a large number of range-restricted endemics (Turpie et al. 2000, Awad et al. 2002, Bolton and Stegenga 2002). The warm-temperate Agulhas ecoregion is an important linefishing ground, and supports the greatest number of South African endemics, including sparid reef fishes, octocorals and algae, and provides spawning and nursery grounds for many species (Thandar 1989, Griffiths and Robinson 2016, Atkinson and Sink 2018). The subtropical Natal ecoregion is characterised by high turbidity and riverine influences (De Lecea et al. 2013, Porter et al. 2014, 2017, Scharler et al. 2016), supporting a high diversity of flora and fauna, as well as a high percentage of endemic species (Emanuel et al. 1992, Turpie et al. 2000, Mann et al. 2006). The Delgoa ecoregion is considered transitional between subtropical and tropical waters (Schleyer et al. 2018) and is dominated by algae, zooxanthellate corals and soft corals, in particular, found on shallow reefs (Porter et al. 2013, Schleyer and Porter 2018).

As of 2020, South Africa has 42 marine protected areas (MPAs) along its coast, , including the very large MPA surrounding the Prince Edward Islands (Whitehead et al. 2019). The declaration of 20 MPAs in 2019 increased the protection of the marine environment around mainland South Africa from less than 0.5% in 2018 to 5.4%. Twenty-six of these 42 MPAs are considered coastal, while 15 are offshore (Sink et al. 2019). These MPAs also have varying levels of protection, which include sanctuary zones (no access and/or no resource use), restricted zones (no resource use but access for tourism, including diving and fishing, is encouraged), and controlled zones (certain types of fishing, often with limitations on gear or species, are permitted). Although few, there are some completely no-take MPAs, as well as some seasonal ones (Sink et al. 2019).

This study incorporated three MPAs, iSimangaliso, uThukela and Aliwal Shoal, and unprotected (open to fishing) areas off Richards Bay and Port Edward, all located on the east coast of South Africa (Figure 1.2). This region is home to large populations of slinger, occurring on the reefs along the coast.

Marine Protected Areas in the study area

iSimangaliso MPA, the largest of the three MPAs in KZN, covers an area of 10 700 km^2 and is the main shallow coral ecosystem in South Africa (Floros 2010, Schleyer et al. 2018), supporting unique biodiversity and important fisheries resources (Schleyer and Tomalin 2000, Schleyer and Celliers 2003, 2005). This MPA consists of two contiguous MPAs, St. Lucia and Maputaland, proclaimed in 1979 and 1986, respectively, to protect coral reef ecosystems, turtle nesting sites and fortuitously protecting populations of slinger (Mann et al. 1998, 2006). This MPA was declared a World Heritage Site in 2000 (Tunley 2009).

The uThukela MPA is situated offshore of the uThukela River and was proclaimed as recently as 2019 (Sink et al. 2019). The uThukela MPA covers an area of 5 666 km^2 and is a nursery area for numerous fish species, and protects a variety of unique faunal communities and habitats, including unconsolidated seabed types (Sink et al. 2019).

The Aliwal Shoal MPA is a reef system with the southernmost shallow-water corals in Africa, situated approximately 50 km south of Durban (Du Preez et al. 2012). This MPA is located within a transition zone between the tropical/subtropical Maputaland and warm-temperate Pondoland reefs (Olbers et al. 2009) (Figure 2.2). This is also evident in the rich biological diversity of flora and fauna found within this MPA (Schleyer 2000, Bolton et al. 2004). First proclaimed in 2004, Aliwal Shoal was extended in 2019 to be approximately 670 km^2 in size, to protect key deep reefs and the historical spawning grounds of the overexploited seabream, seventyfour *Polysteganus undulosus* (Sink et al. 2019).

Chapter 3: A temporal comparison of genetic diversity in the slinger *Chrysoblephus puniceus* along the east coast of South Africa between 2012 and 2018

Abstract

The slinger *Chrysoblephus puniceus* is endemic to the South West Indian Ocean around South Africa and southern Mozambique. This species is important in both the commercial linefishery and recreational fishery in KwaZulu-Natal (KZN), South Africa and southern Mozambique. While currently optimally-exploited, slinger was overexploited in the early 1990s, with overexploitation likely being exacerbated due to the complex life history of slinger, including hermaphroditism, late maturation and resident behaviour, which makes it vulnerable to fishing pressure. The aim of this study was to do a temporal comparison of genetic variation of slinger between two sampling events (2012 and 2018) at two sites (Richards Bay and southern KZN). Two markers were considered: microsatellites and genome-wide Single Nucleotide Polymorphisms (SNPs). Levels of genetic diversity were consistent between sampling years, suggesting no change of genetic diversity between the two sampling periods, spanning a period of six years. Similarly, there was no differences in effective population sizes between 2012 and 2018 for both markers. All pairwise F_{ST} values were non-significant, indicating a lack of temporal or spatial structure. However, subtle differences were reflected in the microsatellite Principal Coordinate Analysis (PCoA) plot indicating genetic instability along the KZN coast over time. Bayesian clustering indicated one and two genetic clusters for SNP and microsatellite data, respectively. However, the probabilities of assignment of each individual identified clusters were similar, indicating no temporal or spatial genetic structure. This study demonstrates genetic stability in slinger along the east coast of South Africa, considering

optimal exploitation over the study period, which can aid and inform fisheries management of this commercially important species.

Introduction

Chrysoblephus puniceus, commonly known as the slinger, is a sparid seabream endemic to the South West Indian Ocean, around South Africa and southern Mozambique (Garratt 1985a). The slinger is the main targeted species in the commercial line-fishery off KwaZulu-Natal (KZN) on the east coast of South Africa (Dunlop and Mann 2013), and southern Mozambique (Garratt 1985a, Lichucha 1999, 2001), and accounts for > 60% of landings from KZN skiboats (Dunlop and Mann 2013). It is also important to the recreational skiboat fishery in these regions (Dunlop and Mann 2013).

The complex life history of the slinger makes this species vulnerable to fishing pressure and slow to recover from exploitation (Garratt 1985a, 1993). In particular, slinger is a protogynous hermaphrodite, reaching sexual maturity at the age of three years and then changing sex from female to male from the age of five years (Garratt 1985b). This species is relatively slow-growing and attains a weight of 3 kg after 10-12 years (Garratt 1993). Slinger are highly resident as adults, but occasional long-distance northward movements of adults have been revealed in tagging studies (Punt et al. 1993, Maggs et al. 2013a).

The slinger became the most commonly caught species in KZN with the decline of the larger sparid, seventy-four *Polysteganus undulosus* (Penney et al. 1999). Increased fishing pressure on the slinger in the early 1990s led this species to become overexploited, along with several other sparids (Punt et al. 1993). Stock status indicators, such as strongly skewed male: female sex ratio (1:19) indicated this, particularly in KZN. The removal of larger (male) fish possibly also reduced the reproductive capacity of the slinger (Garratt 1985a, Punt et al. 1993). Of concern are reports that the size at sex-change appears to have decreased by 50 mm (around

10% of slinger maximum body size) over the last three decades (Mariani et al. 2013). Skewed sex ratios in the slinger are expected to reduce effective population size and, in turn, reduce genetic diversity and increase genetic drift. The slinger has recovered from being heavily exploited in the early 1990s, and the stock is assumed to be optimally exploited (as of 2015) based on biological and fisheries data (Winker et al. 2013, Maggs et al. 2017).

Genetic diversity is described as the variety of alleles and genotypes retained by a group of organisms, either by population or species (Ovenden et al. 2015). Genetic diversity is an invaluable resource for the sustainability of a marine fish species (Antoro et al. 2006), and is a key measure of resilience and abundance (Ovenden et al. 2015). The loss of genetic diversity is caused by genetic drift, with population bottlenecks from overexploitation accentuating drift, as well as when the flow of genes between populations is changed by, for example, habitat loss or alterations to patterns of connectivity (Ovenden et al. 2015). These changes may severely decrease the evolutionary potential of the species and sustainability of populations, particularly if abundance remains low and diversity continues to decay (Pinsky and Palumbi 2014).

Previous genetic studies on the slinger found high levels of genetic diversity and a lack of population structure (Duncan et al. 2015, Coscia et al. 2016). However, these were based on a single sampling occasion across the species' distribution, which could only depict spatial structure and genetic diversity at that time. Temporal genetic studies, which assess the stability of genetic composition and trajectories over time, give a better understanding of the stability of genetic diversity over several snapshots of time rather than one.

Overexploitation, resulting in population bottlenecks and accelerating drift, can cause the loss of genetic variation in fish, which consequently reduces the evolutionary potential and adaptive ability (Allendorf et al. 2008). Sex-changing fish are particularly vulnerable to heavy exploitation, as experienced by slinger, because severely skewed sex ratios are known to further reduce the effective population size (N_e) in natural populations (Wright 1931). Consequently,

sex-changing fish might present a higher potential for exhibiting more spatially structured populations, because of lower N_e and increased genetic drift (Hartl and Clark 1997). Slinger was overexploited in the early 1990s, but recovered by 2015 following a reduction in fishing effort (Winker et al. 2012, Maggs et al. 2017) and has been assumed to be optimally exploited since then as there has been no change in fishing effort (ORI, unpublished. data). During the period of heavy exploitation, genetic diversity in slinger could have been lost. A temporal comparison, allows the estimation of effective population size (N_e) considered one of the most important parameters for genetic monitoring for management and conservation (Waples and Do 2010, Hare et al. 2011). Furthermore, estimates of N_e may provide information on the level of inbreeding and the amount of genetic variation lost due to random genetic drift particular in commercially exploited populations, such as slinger. A temporal comparison of N_e could provide information on the potential continued impact of past overexploitation, the effects of current optimal exploitation or evidence of recovery, and particularly aid in understanding the population dynamics of slinger. In slinger, a substantial change in genetic diversity over a period of six years is hypothesized, as slinger is a protogynous hermaphrodite, which has implications for sex ratios and effective population size as discussed above, and also because it is heavily exploited.

The aim of the current study was therefore to provide a temporal comparison of genetic diversity of slinger across two sampling events: 2012, with sampling conducted for the previous study by Duncan et al. (2015); and 2018, with sampling conducted for the current study, involving populations from two different areas (north vs south) of KZN. This study used both microsatellites and Single Nucleotide Polymorphisms (SNPs) and compared these two marker sets for diversity and resolution. Microsatellites have been used successfully for decades to resolve genetic structure, and provide information on population genetic diversity, average kinship and effective population size (Lemopoulos et al. 2019). However, over the

years, restriction associated DNA sequencing (RADseq) has gained more popularity (Davey and Blaxter 2011, Andrews et al. 2016), and can identify and produce, unlike typical microsatellite studies, thousands of polymorphic markers across the genome (Bruneaux et al. 2013, Bradbury et al. 2015, Jeffries et al. 2016). The use of two differing marker sets could be critical for improved understanding of genetic diversity in slinger and its implication for sustainability, considering the importance of this species as the main target of the commercial and recreational linefishery sectors in KZN (Dunlop and Mann 2013) and southern Mozambique. This information would help to better inform fisheries management of the slinger.

Materials and methods

Sampling

Fin clips from adult slinger used in an earlier microsatellite study by Duncan et al. (2015) were sourced from the National Research Foundation – South African Institute for Aquatic Biodiversity's (NRF-SAIAB) Biomaterials Collection. These samples were collected in 2012 from two sites along the KZN coast – Richards Bay and Shelley Beach (Figure 3.1), once commercial vessels returned after a days' fishing; no further sampling details were available. Fin clips were collected from adult slinger in 2018 from Richards Bay and Port Edward (Figure 3.1), as part of the multidisciplinary African Coelacanth Ecosystem Programme CAPTOR (Connectivity And disPersal beTween prOtected aReas) project. Samples from 2018 were collected from commercial fishing vessels upon their return to port or launch site with the day's catch of slinger (Table 3.1). The four combinations of sampling events (2012 and 2018) and sampling sites (north – Richards Bay; south – Shelley Beach and Port Edward) are hereafter

defined as samples. Sampling sites are referred to as North 2012 and North 2018 (Richards Bay) and South 2012 (Shelley Beach) and South 2018 (Port Edward).

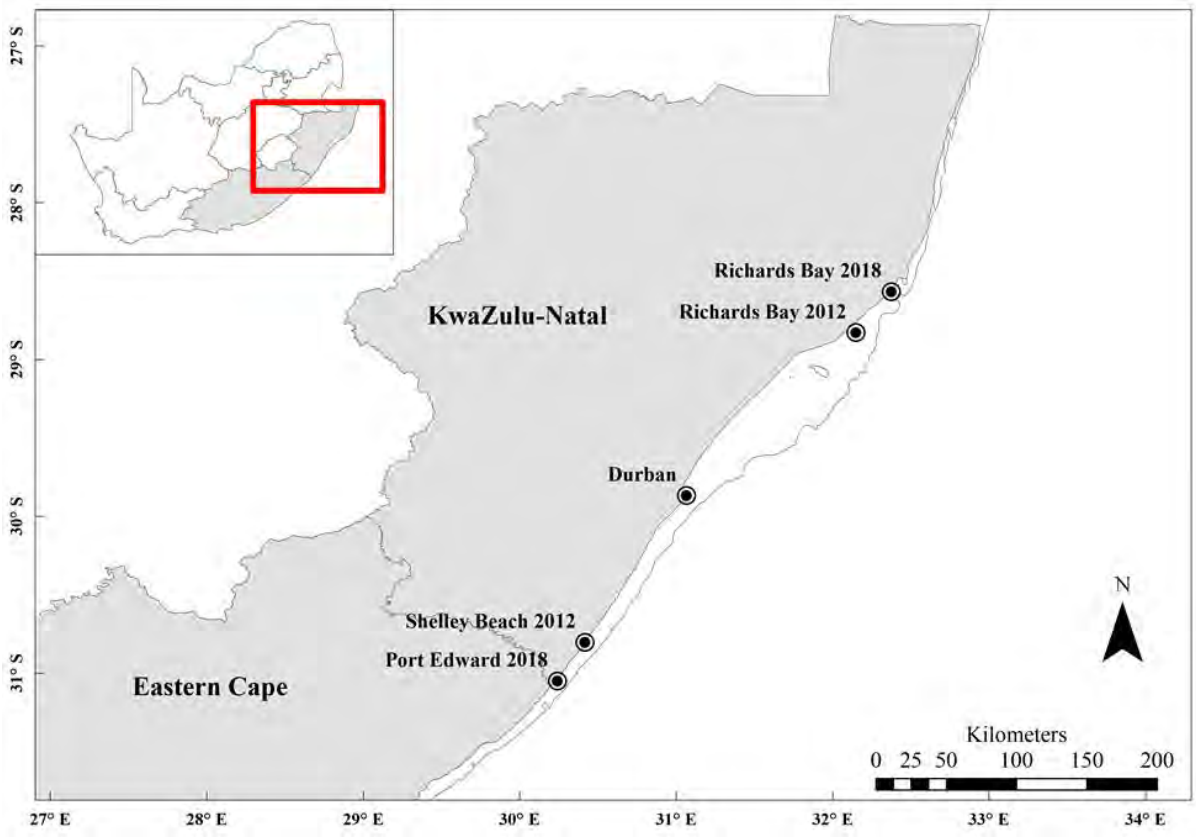

Figure 3.1: Sampling sites from which slinger individuals were sampled along the KwaZulu-Natal coast, temporally (2012 and 2018) and spatially north (Richards Bay) and south (Port Edward and Shelley Beach)

Table 3.1: Sampling sites for slinger collected along the east coast, South Africa, temporally (2012 and 2018) and spatially north (Richards Bay), south (Shelley Beach and Port Edward)

Sampling site	Region	Microsatellites sample size	SNPs sample size	Sampling details
North 2012	Richards Bay	48	4	Fin clips were collected through various approaches, including commercial vessels on their return to port. From small-scale fishermen on their return to the launch site, and active sampling aboard fishing vessels.
South 2012	Shelley Beach	48	31	
North 2018	Richards Bay	48	9	Fin clips were collected randomly from part (ca. 50%) of a single day's catch of slinger (medium-sized category fish only), made by a commercial boat on 23 May 2018, once the vessel had returned to harbour. Fishing was between Nhlabane and Mapelane, likely from several reefs.
South 2018	Port Edward	48	13	Fin clips were collected randomly from part (ca. 60%) of the day's catch of slinger made by a commercial boat on 15 June 2018, once the vessel had returned to the launch site (Margate). The catch was made from fishing on several reefs in the Port Edward area.

DNA extraction

Genomic DNA was extracted from fin clips using the 'salting out' method (Sunnucks and Hales 1996). DNA concentration was initially measured using a Nanodrop 2000 (Thermoscientific, ThermoFisher Scientific, Waltham, Massachusetts), and final DNA concentrations prior to digestion-ligation reactions (see below) for the SNP analysis were more accurately quantified by Qubit 3.0 (Invitrogen, Carlsbad, California) Broad Range fluorescence assay.

Microsatellite genotyping

For the microsatellite genotyping, eight microsatellite loci (SL1, SL7, SL25, SL26, SL29, SL33 SL34 and SL35), developed specifically for slinger (Chopelet et al. 2009a) and sourced from the study by Duncan et al. (2015), were used.

Amplifications were carried out in 27 µL volumes, containing 1 x Multiplex Polymerase Chain Reaction (PCR) Master Mix Proline MyTaq HS, 0.2 µM of each primer and 100 ng of DNA. PCRs were performed as two multiplexes, according to Chopelet et al. (2009a). The

thermocycling profile included an initial 15 min at 95 °C, followed by 30 cycles of 45 s at 94 °C, 45 s at 60 °C and 45 s at 72 °C, followed by 45 min at 72 °C. PCR products were then combined with ABI (Applied Biosystems, Austin, Texas) Hi-Di Formamide and an internal lane standard (GS600LIZ), and fragment analysis was undertaken on an ABI-Hitachi Genetic Analyser 3500 at the Aquatic Genomics Research Platform (AGRP), NRF-SAIAB. GeneMapper Software 5.0 (Applied Biosystems) was used to size fragments, call alleles and genotype individuals.

Prior to analysis, identification of null alleles, large allele dropout, PCR slippage and genotypic errors with a 95% confidence interval by Monte Carlo simulation, was conducted in Micro-checker version 2.2.3 software (Van Oosterhout et al. 2004).

Single Nucleotide Polymorphism (SNP) data generation

Population genetic data in the form of multilocus SNP genotypes were generated using a "reduced representation" genome sequencing approach – RADseq (Peterson et al. 2012). For this study, a more recent variation of this method, quaddRAD sequencing (Franchini et al. 2017), was considered. This approach is more efficient in that the digestion and ligation steps are done in one step and the adapters have a stretch of degenerate bases, enabling the identification and later removal of PCR duplicates.

Digestions and ligations were performed in a single 40 µL reaction, following the Franchini et al. (2017) protocol. This was achieved combining genomic DNA (200 ng), 1 x Tango Buffer (ThermoFisher), 15 units of restriction enzyme *MspI* (ThermoFisher), double the amount of restriction enzyme *PstI* (ThermoFisher) to allow the double digestion, 0.5 mM ATP to facilitate the simultaneous digestion and ligation using 400 CEU ThermoFisher T4 DNA ligase, 0.188 µM of each quaddRAD-i5 and quaddRAD-i7 adapter, and double-distilled water to reach the final volume. Samples were incubated at 30 °C for four hours in a PCR machine, after which

the reaction was stopped with EDTA to 20 mM. Samples, individually indexed by the twelve unique combinations of the quaddRAD-i5 and quaddRAD-i7 adapters, were pooled. Pooled samples were then purified and size selected using Ampure XP (Beckman Coulter, Brea, USA) beads (at 0.5 x and 0.8 x) and eluted in 40 µL elution buffer. DNA concentrations were determined using a Qubit High Sensitivity (HS) assay. A second pair of Illumina indexes were added to each pool through an indexing PCR step. The PCR reaction followed Franchini *et al.* (2017), with 1 x buffer (ammonium buffer, 15 Mm $MgCl_2$) (Amplicon A/S, Odense, Denmark), 0.8 U AccuPOL DNA Polymerase, 20 µL digestion-ligation template, 0.2 mM of each dNTP and double-distilled water to reach a final volume of 100 µL. The PCR conditions were as follows: 95 °C for 2 min, 10 x [95 °C for 10 s, 65 °C for 30 s and 72 °C for 30 s], and 72 °C for 5 min. Amplified products were then purified using Ampure XP beads (0.8 x) and eluted in 40 µL elution buffer. The DNA concentration was determined using a Qubit Broad Range assay. The size distribution and concentration of indexed pooled samples were then determined on an Agilent BioAnalyser 2100 (Agilent Technologies, Santa Clara, California), with a High Sensitivity kit. The desired size range was selected and the indexed pooled samples combined equimolarly, according to the concentration of DNA for this range. Size selection of the desired size range was done by Pippin Prep (Sage Science, Beverly, Massachusetts) by the Central Analytical Facility at the University of Stellenbosch, South Africa. The concentrations of the size-selected products were then determined using a High Sensitivity BioAnalyzer analysis. Two sequencing runs were conducted on an Illumina Miseq (Illumina Inc., San Diego, California) using v3 150-cycle kits at the AGRP.

Trimming and demultiplexing of sequencing reads, and *de novo* assembly of SNP loci were performed using STACKS version 2.41 (Catchen et al. 2011, 2013) on a computer cluster at the Centre for High Performance Computing, Council for Scientific and Industrial Research, South Africa (http://www.chpc.ac.za). PCR duplicate reads were identified from raw fastq

files, through the ambiguous base stretch in the adapters and removed using the STACKS module *clone-filter*. Reads were then demultiplexed, cleaned, removing erroneous and low-quality reads, and truncated to a length of 64 bp using the STACKS module *process-radtags*. This was determined to be 64 bp from the distribution of read lengths from the first read sets for each of the sample pools, allowing for the inline barcodes. Since there is no reference genome available for slinger specifically, the *de novo* pipeline of STACKS was applied to assemble loci. First, parameters for the *ustacks* and *cstacks* components of the STACKS were optimized on a subset of the 20 samples with the greatest number of reads, following Paris et al. (2017) and Rochette and Catchen (2017). The options chosen by optimization, including the maximum of nucleotide mismatches among primary reads (*M*) was run at various iterations, *M* was tested: $M = 0$ to 8. The minimum stacks depth (*m*) was kept at three and the maximum nucleotide differences (*n*) allowed during elaboration of the stacks catalogue in *cstacks*, were kept equal to *M*. The final parameters revealed $M = n = 2$ to be optimal.

The '*populations*' component in STACKS was used to call genotypes. The data set retained only those loci that were present in 50% (*-r* 0.5) of individuals in a population, loci present in a minimum of two populations (*-p* 2), and alleles with frequency >0.02 (*-min_maf* 0.02). Mean coverage was evaluated and the data set reduced to include only individuals with ≥7.5x coverage. The STACKS program '*populations*' was also used to generate output files for input into downstream programs. From this data set, GENEPOP 4.2 (Rousset 2008) and STRUCTURE 2.3.4 (Pritchard et al. 2000) input files were produced, implementing the 'write-single-snp' option, to prevent linked SNPs from being processed by STRUCTURE.

Data analysis

For the microsatellite data, Genepop version 4.2 (Rousset 2008) was used to test for deviations from Hardy-Weinberg equilibrium (HWE) at all loci for all samples, and linkage disequilibrium to determine the extent of distortion from the independent segregation of loci. Exact tests for conformity to HWE were performed by the Markov Chain method with 10 000 dememorization steps, 100 batches, and 5 000 iterations. The nominal statistical significance of these tests (5%) was adjusted for multiple comparisons using a B-Y False Discovery Rate correction (FDR) (Narum 2006). Null allele frequencies were estimated for each microsatellite locus at all samples with FreeNA (Chapuis and Estoup 2007), according to the expectation maximum (EM) algorithm of Dempster et al. (1977). For the SNP data, deviations from HWE were determined in the '*populations*' module of STACKS.

Genetic diversity

Standard genetic diversity indices, such as the mean number of alleles (*Na*), mean number of effective alleles (*Ne*), observed heterozygosity (*Ho*), expected heterozygosity (*He*) and fixation index (*Fis*), were calculated for each sample from the microsatellite data, using GenAlEx version 6.503 (Peakall and Smouse 2012). Allelic richness (*An*), a standardized measure of the number of alleles per locus independent of the sample size, was calculated using FSTAT version 1.2 (Goudet 1995).

For the SNP data, diversity estimates were calculated in the '*populations*' module of STACKS for each sample. These include the mean number of individuals (*N*), number of private alleles (*Pa*), number of polymorphic sites (*Ps*), proportion of polymorphic SNPs across all nucleotides sequenced (*P%*), nucleotide diversity (π), observed heterozygosity (*Ho*), expected heterozygosity (*He*) and inbreeding/fixation index (*Fis*).

Population differentiation

Prior to the investigation of population differentiation, POWSIM v 4.1 (Ryman and Palm 2006) was used to evaluate the statistical power of the data for both markers, given the present sample sizes, for detecting low (F_{ST} = 0.001) or moderate (F_{ST} = 0.05) levels of differentiation. For the microsatellites, 1 000 simulations were run using an effective population size (N_e) of 10 000 and 20 and 1026 generations of drift (t) for the respective scenarios of F_{ST}. For the SNP dataset, 200 simulations were run using N_e of 1 000, and t = 2 and t = 103. For both markers, combinations of N_e and t were used to model specific values of F_{ST} as detailed in the software documentation. For the microsatellite data set, the executable Powsim_c was used, whereas for the SNPs, the executable Powsim_b was used to accommodate > 1000 loci.

For the microsatellite data, genetic differentiation among samples were investigated using pairwise F_{ST} (Weir and Cockerham 1984) comparisons in GenAlEx. Significance was tested using 999 permutations. To prevent overestimation of F_{ST} values due to the presence of null alleles, pairwise F_{ST} values were determined using the excluding null alleles (ENA) correction in FreeNA. The 95% confidence intervals for corrected pairwise F_{ST} values were obtained by bootstrapping 1 000 times over loci. In addition, a Principal Coordinate Analysis (PCoA), using Nei's (1972) unbiased genetic distance, was performed in GenAlEx to graphically show the genetic differentiation or similarity between the samples. Pairwise F_{ST} values based on SNP data were estimated in Arlequin version 3.5.2.2 (Excoffier and Lischer 2010). Significance of values for F_{ST} were based on 10 000 permutations. A Principal Component Analysis (PCA) was conducted in ADEGENET 2.1.3 (Jombart 2008) to visualise the genetic relationships among samples.

Hierarchical Analyses of Molecular Variance (AMOVA) (Excoffier et al. 1992) were carried out in Arlequin version 3.5.2.2 for microsatellite and SNP data to examine genetic differentiation among samples, testing for temporal variation between two sampling events

(2012 and 2018) and for spatial structure (integrated over time) between the northern and southern KZN coast (sampling sites).

Population structure

Clustering analyses, based on a Bayesian assignment approach, using STRUCTURE version 2.3.2 (Pritchard et al. 2000), were performed using the microsatellite and the SNP data sets to infer the optimal number of homogenous genetic units (*K*) within the total sample. This method attempts to partition samples into *K* group(s), independent of sampling region or event, such that the loci in those groups are in HWE and linkage equilibrium. The software was run considering *K* values from one to four. For each *K*, 20 replicates were tested for the microsatellite data, using 100 000 Markov Chain Monte Carlo repetitions (after a burn-in period 100 000). Ten replicates were used for the SNP data due to computational and time constraints. This analysis was run under an admixture model and considered allele frequencies as being independent. The most likely *K* was assessed using the four statistics (MEDMED, MEDMEAN, MAXMED and MAXMEAN as implemented in StructureSelector (Li and Liu 2018) and by plotting Ln(PD) and implementing the ΔK method, as in Evanno et al. (2005). The former has been described as more accurate than previous methods to determine the best fit number of clusters, for both even and uneven sampling (Puechmaille 2016). Results of all the replicates for the optimal *K* (20 for the microsatellite data and 10 for the SNPs) were averaged with CLUMPP (Jakobsson and Rosenberg 2007) and visualised with DISTRUCT (Rosenberg 2004).

Effective population size

Finally, estimates of effective population size (N_e) were obtained temporally (2012 and 2018) for both markers, using the Linkage Disequilibrium method (Hill 1981, Waples 2006, Waples and Do 2010) in NeEstimator v2 (Do et al. 2014). Samples from 2012 (both north and south

sample sites) were pooled together and 2018 (both north and south samples sites) were combined to get an estimate of N_e over time in the slinger. A random mating model was assumed, and parametric and jackknife methods were used for estimating confidence intervals for microsatellites and SNPs, respectively. The jackknife method is recommended when the number of loci is large (>100) (Jones et al. 2016). Critical values 0.05, 0.02 and 0.01 were selected to represent the minimum allele frequency cut-off for the microsatellites; alleles at a frequency < 0.05 were already excluded through the earlier filtering of the SNP data. A negative N_e-value was interpreted as being infinite N_e.

Results

Microsatellites

For this study, 192 samples were genotyped at eight polymorphic microsatellite loci. Forty-eight samples were included from each of the northern sampling sites 2012 and 2018 (Richards Bay) and from southern sampling sites in 2012 (Shelley Beach) and 2018 (Port Edward). Exact probability tests of HWE were performed across eight loci and for all samples. Loci SL1 and SL26 conformed to HWE. Significant departures from HWE after B-Y FDR correction (P = 0.012) were observed at loci SL7, SL25, SL29, SL33, SL34 and SL35 across all samples. Further analysis with Microchecker indicated that the deviations at these six loci could be due to the presence of null alleles. Significant positive values of *Fis* indicated a deficit of heterozygotes at these loci. There was no consistent pattern for the presence of null alleles and these were not observed in more than two samples. The mean frequency of null alleles for loci that deviated from HWE ranged from 0 to 0.091 across all samples (data not shown). The highest null allele frequencies were recorded in two samples, South 2012 and North 2012. At South 2012, null allele estimates were recorded for SL7 (0.112), SL25 (0.122), SL34 (0.116), SL35 (0.153), and for North 2012 at SL7 (0.115) and SL29 (0.206). After FDR correction (P

= 0.013), significant linkage disequilibrium was detected at three pairs of loci (SL7–SL25, SL7–SL34 and SL25–SL34) out of 28 comparisons among pairs of loci involving all samples. There was no consistent pattern of linkage disequilibrium observed, with different pairs of loci identified as linked in different samples. Hence, the full set of loci was included for further analysis.

Genetic diversity

Genetic diversity indices are presented in Table 3.2. *Na* across sampled sites and sampling events ranged from 11.250 ± 1.688 to 12.125 ± 1.894; it was slightly higher for North 2018 but similar amongst the other samples. *Ne* ranged from 6.473 ± 1.148 to 7.516 ± 1.326, with the highest recorded at South 2012. Overall, *Ho* was similar among samples and was consistently lower than *He*, indicating a deficiency of heterozygotes. This was also seen in the mostly positive fixation index values. *Ho* ranged from 0.720 ± 0.037 to 0.804 ± 0.036, and *He* ranged from 0.807 ± 0.041 to 0.828 ± 0.034. The *He* values were similar among samples, with the highest *He* was recorded at South 2012 (0.828 ± 0.034) and the lowest at South 2018 (0.807 ± 0.041). Allelic richness (*An*) was similar across all samples and ranged from 10.018 to 10.776.

Table 3.2: Genetic diversity estimates for eight microsatellite loci for slinger sampled temporally over two sampling events (2012 and 2018) and sampling sites, along the north (Richards Bay) and south (Port Edward and Shelley Beach) east coast of South Africa. *N* = mean sample size, *Na* = number of mean alleles per locus, *Ne* = mean number of effective alleles, *Ar* = allelic richness, *Ho* = observed heterozygosity, *He* = expected heterozygosity, *Fis* = fixation index

Samples				
Estimates	**North 2012**	**South 2012**	**North 2018**	**South 2018**
N	24.250 ± 1.830	21.750 ± 0.366	21.875 ± 0.915	22.000 ± 2.928
Na	11.250 ± 1.688	11.750 ± 1.666	12.125 ± 1.894	11.378 ± 1.841
Ne	6.473 ± 1.148	7.516 ± 1.326	6.625 ± 1.237	6.901 ± 1.256
Ar	10.018 ± 4.342	10.633 ± 3.997	10.776 ± 4.307	10.259 ± 4.392
Ho	0.724 ± 0.062	0.720 ± 0.037	0.804 ± 0.036	0.773 ± 0.037
He	0.809 ± 0.031	0.828 ± 0.034	0.808 ± 0.033	0.807 ± 0.041
Fis	0.096 ± 0.095	0.115 ± 0.069	-0.005 ± 0.057	0.025 ± 0.069

Population differentiation

The statistical power analysis performed in POWSIM with the present sample sizes indicated that microsatellites had sufficient power to detect moderate differentiation (F_{ST} = 0.05) in 100% of simulations. There was less power in the data for detecting low levels of differentiation, with a 17.6 % chance of detecting F_{ST} = 0.001. This is probably a consequence of the high diversity found in this dataset (see Table 3.2 above), and there may be limitations to accurately detect low levels of diversity. Pairwise estimates of F_{ST} (Table 3.3) ranged between 0 (North 2012 and North 2018, and South 2012 and South 2018) and 0.019 (North 2018 and South 2018), indicating low levels of differentiation overall. No significant differentiation was recorded after B-Y FDR correction (P = 0.02). ENA-corrected F_{ST} values ranged between 0 (South 2012 and North 2012) and 0.019 (North 2018 and North 2012) and, like the F_{ST} values, no significant differentiation was recorded after B-Y FDR correction (Table 3.3).

Table 3.3: The pairwise F_{ST} (below diagonal) and excluding null alleles (ENA) F_{ST} values (above diagonal), based on eight microsatellite loci, between slinger sampled temporally over two sampling events (2012 and 2018) and spatially, along the north (Richards Bay) and south (Port Edward and Shelley Beach) east coast, South Africa

	North 2012	South 2012	North 2018	North 2018
North 2012	-	0.000	0.011	0.019
South 2012	0.014	-	0.015	0.015
North 2018	0.000	0.001	-	0.005
South 2018	0.001	0.000	0.019	-

The clustering from the PCoA indicated four distinct genetic clusters. The samples in the north (North 2012 and North 2018) clustered more closely together and were genetically more similar, reflecting some temporal stability between 2012 and 2018. Despite the similarity, they could still be considered to be differentiated from each other, and from all other distinct populations (Figure 3.3). The samples in the south (South 2012 and South 2018) were clearly distinct, indicating temporal structure.

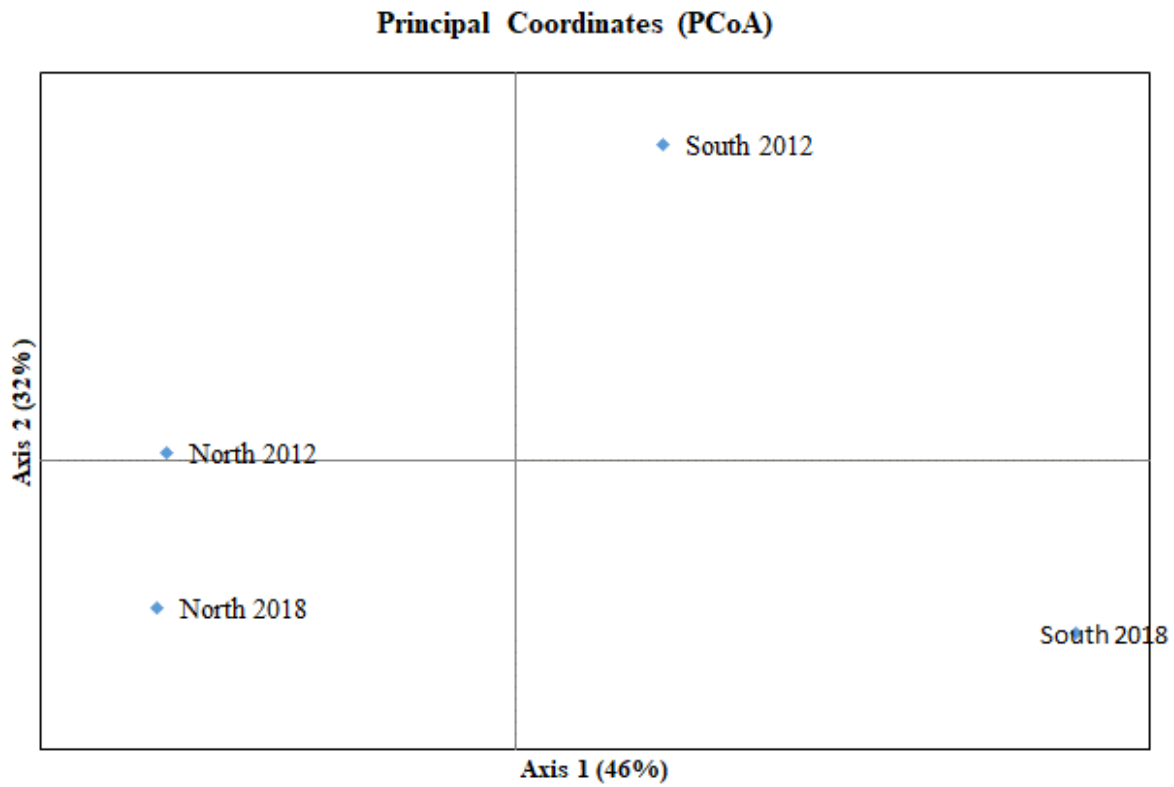

Figure 3.2: Principal Component Analysis based on eight microsatellite loci depicting the relationship of four slinger populations, sampled temporally over two sampling events (2012 and 2018) and spatially along the north (Richards Bay) and south (Port Edward and Shelley Beach) east coast, South Africa.

The hierarchical AMOVA, testing temporal differentiation (Table 3.4), revealed that 106% of genetic variance was distributed among individuals (F_{ST} = -0.059; P = 0.996). Those components of genetic variation explained by differences between sampling events and among populations within each sampling event were -4.86% and -1.07%, respectively. For both these groups, P values were not significant ($P > 0.05$), indicating no significant temporal structure. For the spatial investigation, 104% of genetic variance was again found among individuals (F_{ST} = -0.040; P = 0.999). Genetic variation explained by differences between the northern (North 2012 and 2018) and southern (South 2012 and 2018) sampling sites and among populations within the north and south sampling sites was 0.64% and -4.66%, respectively. Again, no significant differences were recorded to indicate differentiation between northern and southern sampling sites (Table 3.4).

Table 3.4: Hierarchical Analysis of Molecular Variance (AMOVA) based on eight microsatellite loci, considering the arrangement of slinger sampled from the South African east coast into two groups: temporal (two sampling events 2012 and 2018) and spatial [north (Richards Bay) and south (Port Edward and Shelley Beach)]. Source of variation, sum of squares, variance components, percentage (%) of variation, fixation index and p value (* $P < 0.05$) are indicated

Source of variation	d.f	Sum of squares	Variance components	% Variance	F statistics	*p value*
Temporal (2012 – 2018)						
Among groups	1	-9.485	-0.079	-4.86	F_{CT} = -0.048	1.000
Among populations within groups	2	1.216	-0.017	-1.07	F_{SC} = -0.010	0.793
Among individuals	252	434.456	1.724	105.94	F_{ST} = -0.059	0.996
Total	255	426.188	1.627			
Spatial (North and South)						
Among groups	1	-1.885	0.011	0.64	F_{CT} = 0.006	0.676
Among populations within groups	2	-6.383	-0.077	-4.66	F_{SC} = -0.047	0.991
Among individuals	252	434.456	1.724	104.02	F_{ST} = -0.040	0.099
Total	255	426.188	1.657			

Population structure

StructureSelector identified two genetic clusters, while the Evanno et al. (2005) method revealed three genetic clusters (K = 3) among the microsatellite genotypes (Table 3.5). The probabilities of assignment of each individual to each of the identified populations were similar, indicating a lack of temporal and spatial structure (Figure 3.4).

Table 3.5: Analyses of STRUCTURE outputs following StructureSelector and Evanno et al. (2005) method of eight microsatellite loci for slinger sampled from two regions, north (Richards Bay) and south (Shelley Beach and Port Edward) along the east coast, South Africa, 2012 and 2018

	StructureSelector				Evanno method				
K	MedMedK	MedMeanK	MaxMedK	MaxMeanK	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'P(*K*)	|Ln"P(*K*)|	Delta *K*
2	2	2	2	2	-3679.59	351.61	N/A	N/A	N/A
3	0	0	0	0	-3492.43	60.95	187.16	302.03	4.96
4	0	0	0	0	-3607.30	201.61	-114.87	N/A	N/A

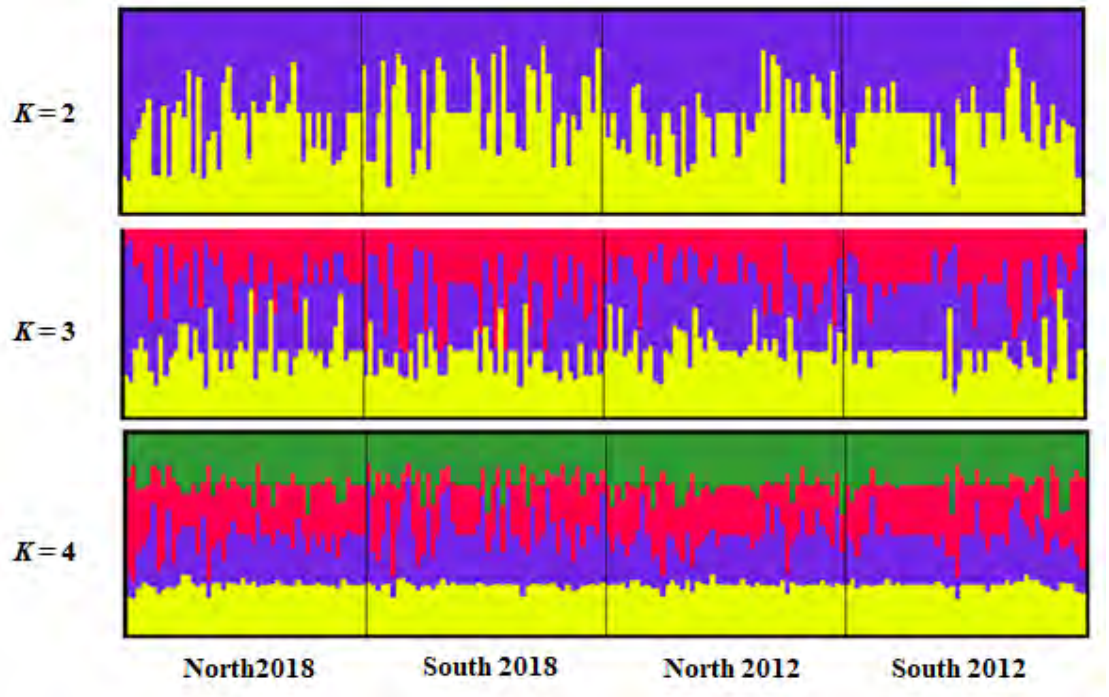

Figure 3.3: Assignment/admixture plots for K = 2 to 4 from the STRUCTURE analysis of eight microsatellite loci from slinger sampled from two region, north (Richards Bay) and south (Port Edward and Shelley Beach) along the KwaZulu-Natal coast, South Africa, 2012 and 2018. Geographic regions are shown at the distinct populations. Each colour represents a unique genetic cluster. Each vertical line represents an individual with the proportional representation of admixture of each of these genetic populations within the genotype indicated

Effective population size

Estimates of contemporary effective population size (N_e) slinger based on the microsatellites for 2012 were 199.5, 338.5 and 349.4 for critical values 0.05, 0.02 and 0.01, respectively,

whereas the 2018 N_e estimates were 67.1, 207.3 and 195.0 for critical values 0.05, 0.02 and 0.01, respectively (Table 3.6).

Table 3.6: Estimates of effective population size (N_e) in slinger based on eight microsatellite loci sampled temporally over two sampling events (2012 and 2018) and spatially along the north (Richards Bay) and south (Port Edward and Shelley Beach) east coast, South Africa. Three allele frequencies are used and the corresponding parametric 95% confidence interval is reported for each

Lowest allele frequency			
	0.05	0.02	0.01
2012			
N_e (Parametric 95% CIs)	199.5 (73.9-infinite)	338.5 (128.5-infinite)	349.4 (150.6-infinite)
2018			
N_e (Parametric 95% CIs	67.1 (36.9-218.2)	207.3 (97.4-infinite)	195.0 (108.2-746.3)

SNPs

In total, 31 399 144 pairs of sequencing reads were obtained, with 1 064 681 to 5 380 852 pairs for each sample pool. PCR duplicates, constituting between 1.29 and 18.21% of reads in each pool, were identified and removed, retaining 1 016 083 to 5 311 641 read pairs per pool, and 29 172 144 read pairs in total (Appendix 1). After filtering for length and quality, between 20 983 and 1 764 399 reads were assigned to individual samples, with a mean of 330 311 (± 292 068.8 SD) (Appendix 2).

Parameters were optimized using the 20 samples with the greatest number of reads. This procedure revealed $M = n = 4$ to be optimal and further analysis proceeded using these parameters.

The final dataset was reduced to only include samples with the mean coverage >7.5 and loci that were present in 50% of individuals in a population. In total, 57 samples (from 4 to 31 samples per population) were genotyped. A total of 80 292 loci could be assembled from the paired reads and were genotyped, with a mean sample coverage of 11.4x (SD = 5.1x), a

minimum coverage of 6.7x, a maximum of 26.6x, and a mean number of sites per locus of 103.4. Genotype filtering was then applied to account for missing data, retaining only loci genotyped in more than 50% of individuals in a population and present in at least two populations. A total of 23 777 loci was genotyped, composed of 2 688 201 sites, with a mean number of sites per locus of 111.26 ± 0.08. The total number of variant SNPs was 17 171.

A number of variable SNPs were found to be significantly out of HWE ($P < 0.01$) in most samples (Table 3.7), with the highest recorded at South 2012 (533). No variable SNPs were identified as deviating from HWE in the North 2012 sample.

Table 3.7: Summary genetic diversity estimates for 17 171 SNP loci for slinger sampled temporally over two sampling events (2012 and 2018) and spatially, along the north (Richards Bay) and south (Shelley Beach and Port Edward) east coast, South Africa. *n* = sample size, *N* = mean number of samples genotyped across all loci, *Pa* = number of private alleles, *Ps* = number of polymorphic sites across all nucleotide sequences, *P%* = proportion of polymorphic SNPs across all nucleotides sequenced, HWE = number of SNPs out of Hardy Weinberg Equilibrium, π = nucleotide diversity, *Ho* = observed heterozygosity, *He* = expected heterozygosity, *Fis* = inbreeding index

	Samples			
Estimates	**North 2012**	**South 2012**	**North 2018**	**South 2018**
n	4	31	9	13
N	3.198 (± 0.007)	23.428 (± 0.037)	7.892 (± 0.013)	10.098 (± 0.017)
Pa	209	2 595	866	440
Ps	6 025	14 664	10 519	9 645
P%	0.260	0.605	0.149	0.452
HWE	0	533	43	85
π	0.001 (± 0.166)	0.171 (± 0.001)	0.171 (± 0.001)	0.001 (± 0.168)
Ho	0.002 (± 0.158)	0.154 (± 0.001)	0.165 (± 0.002)	0.002 (± 0.172)
He	0.001 (± 0.138)	0.167 (± 0.001)	0.160 (± 0.001)	0.001 (± 0.160)
Fis	0.017 (± 0.015)	0.093 (± 0.037)	0.023 (± 0.013)	0.013 (± 0.000)

Standard diversity indices

Overall, there was not much difference in genetic diversity among samples (Table 3.7). The numbers of polymorphic SNPs (*Ps*) were highest in South 2012 (14 664) and North 2018

(10 519). Nucleotide diversity was also highest in South 2012 and North 2018 (both 0.171 ± 0.001). Observed heterozygosity was highest in North 2018 (0.165 ± 0.002), and *He* was highest in South 2012 (0.167 ± 0.001). *Ho* was generally higher than expected heterozygosity in most samples, reflecting an excess of heterozygotes. The exception is South 2012, where *He* (0.167 ± 0.001) was higher than observed heterozygosity (0.154 ± 0.001). The *Fis* was also higher (0.093 ± 0.037), indicating a deficiency of heterozygosity at South 2012; this is also in line with the highest number of loci out of HWE at South 2012.

Population differentiation

The simulations performed in POWSIM highlighted the high-resolution power of the SNP dataset and revealed that this dataset has enough statistical power to detect moderate F_{ST} values (0.05) in 100% of tests, and a high probability (> 60%) of detecting F_{ST} values as low as 0.001, with sample sizes as low as four individuals. All pairwise F_{ST} values were very low (< 0.000) and non-significant, indicating a lack of temporal and spatial genetic differentiation (Table 3.8). In the PCA, the first and second principal components accounted for 3.21% and 2.82% of the total variation, respectively, and a lack of clustering and separation of individuals according to temporal or spatial sampling was observed (Figure 3.5).

Table 3.8: The matrix of pairwise F_{ST} values, based on 17 171 SNP loci, among samples of slinger sampled temporally over two sampling events (2012 and 2018) and spatially, along the north (Richards Bay) and south (Shelley Beach and Port Edward) east coast, South Africa

	North 2012	South 2012	North 2018	South 2018
North 2012	-			
South 2012	-0.030	-		
North 2018	-0.032	-0.006	-	
South 2018	-0.032	-0.010	-0.012	-

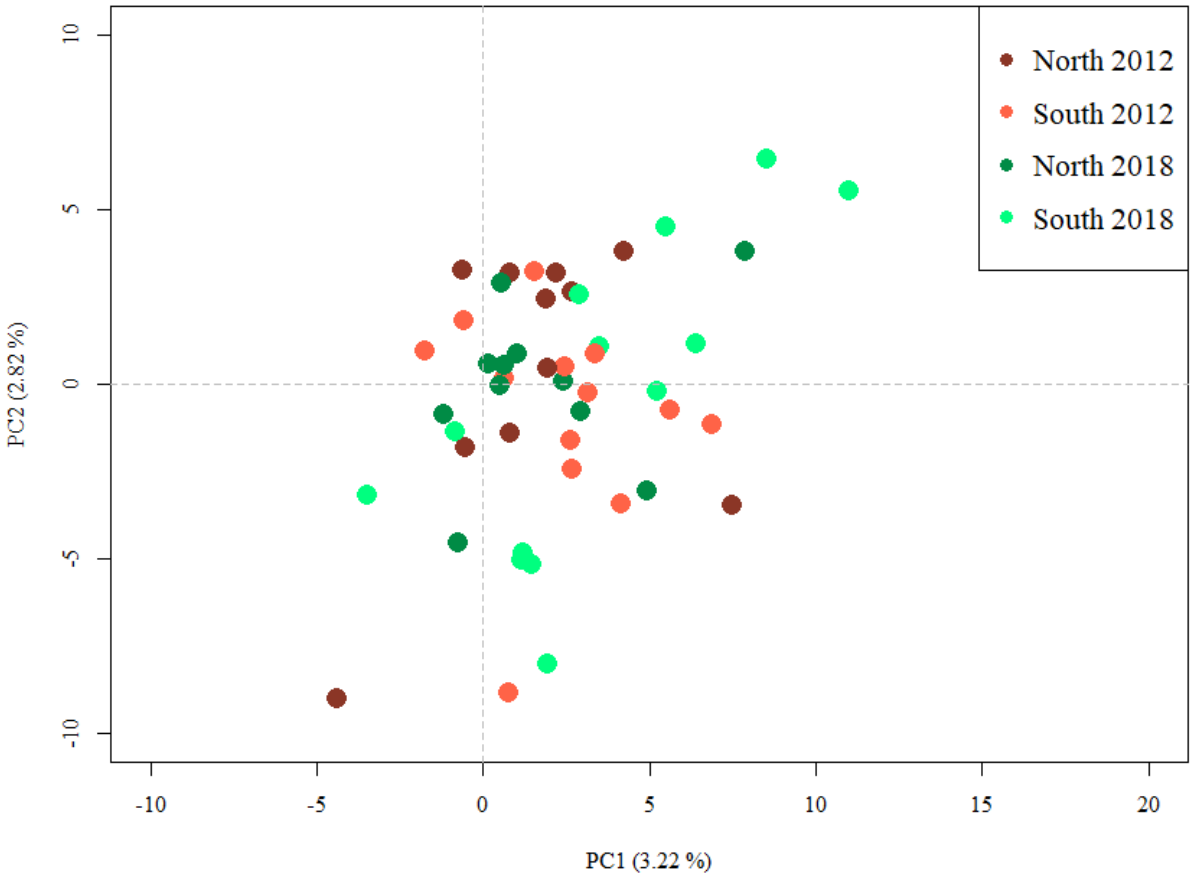

Figure 3.4: Principal component analysis of 17 171 SNP loci for slinger individuals from four populations, sampled temporally over two sampling events (2012 and 2018) and spatially along the north (Richards Bay) and south (Port Edward and Shelley Beach) east coast, South Africa. Percentages of total variation explained by each axis are shown

For the SNP data (Table 3.9), the hierarchical AMOVA testing for temporal differences found the highest variation (101%) among individuals (F_{ST} = -0.012 P = 0.947). The components of genetic variation explained by differences between sampling years and among populations within sampling years were 0.75% and -1.92%, respectively. No significant temporal structure was recorded among sampling years (P > 0.05). On examining differentiation between the north (North 2012 and 2018) and south (South 2012 and 2018) sampling sites, the highest variation was 101% among individuals (F_{ST} = -0.012; P = 0.945). The components of genetic variation explained between the north and south sampling sites and among temporally-separated populations at these sampling sites were 0.31% and -1.54%, respectively. No significant spatial structure was recorded among sampling sites (P > 0.05).

Table 3.9: Hierarchical Analysis of Molecular Variance (AMOVA) based on 17 171 SNP loci, considering the arrangement of slinger sampled from South African east coast into two groups: temporal (two sampling events 2012 and 2018) and spatial north (Richards Bay) and south (Shelley Beach and Port Edward). Source of variation, degrees of freedom, sum of squares, variance components, percentage (%) of variation, fixation index and *P* value indicated

Source of variation	d.f	Sum of squares	Variance components	% Variation	F statistics	*P* value
Temporal (2012-2018)						
Among groups	1	493.511	5.013	0.75	F_{CT} = 0.007	0.342
Among populations within groups	2	882.876	-12.868	-1.92	F_{SC} = -0.019	0.905
Among individuals	110	74551.596	677.742	101.17	F_{ST} = -0.012	0.947
Total	113	75927.982	669.886			
Spatial (North and south)						
Among groups	1	511.276	2.073	0.31	F_{CT} = 0.003	0.663
Among populations within groups	2	865.110	-10.279	-1.54	F_{SC} = -0.015	0.970
Among individuals	110	74551.596	677.742	101.23	F_{ST} = -0.012	0.945
Total	113	75927.982	669.536			

Population structure

One genetic cluster (*K*) was identified with StructureSelector (Li and Liu 2018) (Table 3.10), indicating a lack of temporal or spatial structure, and a single slinger population.

Table 3.10: Analyses of STRUCTURE outputs following StructureSelector of 17 171 SNP loci for slinger sampled during two years, 2012 and 2018, from two regions, north (Richards Bay) and south (Shelley Beach and Port Edward) along the east coast, South Africa

StructureSelector				
K	MedMedK	MedMeanK	MaxMedK	MaxMeanK
1	1	1	1	1
2	1	1	1	1
3	1	1	1	1

Effective population size

The estimates of effective population size in slinger for SNPs were 254.0 based on the 2012 samples and negative/infinite for the 2018 samples.

Table 3. 11: Estimates of effective population size (N_e) based on 17 171 SNP loci from slinger sampled temporally over two sampling events (2012 and 2018) and spatially along the north (Richards Bay) and south (Port Edward and Shelley Beach) east coast, South Africa.

N_e (Jackknife 95% CIs	
2012	
N_e (Jackknife 95% CIs)	254.0 (40.3-infinite)
2018	
N_e (Jackknife 95% CIs)	Infinite (67.2-infinite)

Discussion

This study used both microsatellites and SNPs to examine genetic diversity in the slinger, across two sampling events 2012 and 2018 from two areas off KZN. Results provided no evidence of substantial changes in genetic diversity over a period of six years, but there was subtle differentiation both spatially and over time along the KZN coast.

No genetic differentiation was observed among sampling events and sampling sites from the pairwise F_{ST} values for both the microsatellite and SNP data sets, indicating high levels of temporal and spatial genetic connectivity and gene flow among sampling sites.

Similarly, there was neither significant temporal nor spatial structure recorded with AMOVA for both the microsatellite and SNP data. This was also evident in the PCA plot based on the SNP data.

For the Bayesian clustering analysis, both microsatellite and SNP data revealed neither temporal nor spatial structure, but rather a single population. This confirms findings of previous

studies based on microsatellites alone, which revealed the slinger to form one population (Duncan et al. 2015, Coscia et al. 2016)

The lack of spatial genetic differentiation in this area is consistent with the life history and behaviour of the slinger. Adults are abundant on offshore rocky reefs from 20 m to 130 m depth (Garratt 1984, Heemstra and Heemstra 2004). In KZN, very small juveniles occur on shallow rocky reefs (10 to 30 m depth) in the Pondoland MPA south of Port Edward (Mann et al. 2006), and larger juveniles (> 0.5 cm FL) are widespread on reefs off KZN from 20 m to 60 m depth (Garratt 1984). Adults show fairly resident behaviour (Punt et al. 1993), and there is no evidence of spawning south of Durban (Figure 3.1). At least some adults migrate northwards from southern Eastern Cape/southern KZN province to northern KZN/southern Mozambique (Buxton 1992, Maggs et al. 2013b). There is an extended spawning period, with peak spawning occurring between August and October in KZN; spawning occurs in southern Mozambique and northern KZN on offshore reefs (Garratt 1985b). The species receives protection from fishing in several no-take MPAs situated along this stretch of coastline. The largest, the iSimangaliso MPA to the north of Richards Bay, is the site of several spawning aggregations, and has long been thought to provide recruits to support the wider KZN population, notwithstanding considerable fishing pressure outside of MPAs (Punt et al. 1993). The Pondoland MPA is the only region where very small juveniles are known to occur, which is highly suggestive of north-south dispersal of spawning products, assisted by inshore processes of the south-flowing Agulhas Current (Beckley 1993). The KZN coast is relatively linear, with few substantial bays or other refugia from a dynamic, turbulent oceanography (Schumann 1988), although recent particle dispersal modelling suggests the feasibility of greater retention of fish larvae between Richards Bay and Durban (Heye et al. 2022) . Thus, genetic homogeneity might be expected (Duncan et al. 2015), with southward larval dispersal followed by northward

adult migration resulting in the constant redistribution of alleles across the area, establishing population cohesion.

The PCoA plot based on the microsatellite data revealed all sampling sites to be distinct, but with the Richards Bay 2012 and 2018 samples clustering closer together, indicating greater similarity compared to the southern sampling sites. The southern sampling sites (Shelley Beach and Port Edward) were more distant in the plot and genetically differentiated, reflecting greater differentiation between the north and south KZN coasts, but these themselves are also quite differentiated, indicating greater temporal instability along the southern KZN coast between 2012 and 2018. This indicates that the genetic structure of the slinger may not be stable over time and differences may occur temporally. The greater genetic similarity between the two Richards Bay (northern) sampling events may reflect a sampling artefact, with the potential sampling of overlapping generations. It cannot entirely be excluded that samples collected in 2012 and 2018 were from fishes in the same cohort; but the nature of fishing involves the visiting of dispersed multiple reefs and hence catching (sampling) of different shoals of fish of a range of sizes, which are unlikely to have all been from one cohort. The greater genetic divergence seen between the two southern samples (Port Edward and Shelley Beach) could be because there is greater dispersion of diversity in the south as one moves from a northern source, and more cohesion closer to the source. Heye et al. (2022) shows greater particle retention in the north, which supports the suggestion of greater cohesion in the north closer to the source of eggs/larvae. Alternatively, although unlikely (see Chapters 4 and 5), the genetic divergence in the south could also reflect spatial sampling, with two sites representing the 2012 and 2018 southern samples opposed to the same area being sampled in the north in 2012 and 2018. The observed temporal differences could emanate from differential reproductive success via the “sweepstake hypothesis”, whereby only few adults contribute towards the next generation of offspring (Hedgecock 1994). This is explored further in Chapter 5, which

employs a design that allows disentangling true intergenerational variation from stochastic variation through the targeted sampling of juvenile cohorts.

Diversity indices indicated no changes in genetic diversity in the slinger between 2012 and 2018 for both microsatellites and SNP data. Slinger was heavily overexploited in KZN by the 1990s, indicated by skewed sex ratios (female-dominated) and a decline in mean size, and confirmed by stock assessment (Punt et al. 1993). In southern Mozambique, the status is less clear, but indications are that the stock was heavily exploited from the mid-1990s (Lichucha 2001), and catch rates have been very low in recent years (Fennessy et al. 2012). Catch rates in KZN have improved with a decline in commercial fishing effort, and the stock is recovering, evidenced by an approximately 30% increase in biomass (Winker et al. 2014), as a result of the reduction in commercial fishing effort since 2003. The lack of change in genetic diversity observed in this study suggests that the earlier high levels of fishing effort leading to overexploitation may not have had a detrimental effect on slinger genetic diversity. More recent stock assessments suggest that the slinger stock should continue rebuilding if the current fishing effort is not increased and could possibly sustain a 5% increase of fishing mortality without the risk of overexploitation (Maggs et al. 2017).

Other studies have indicated stable genetic diversity over time in several species of marine fishes despite intensive exploitation. Based on analysis of DNA extracted from archived and contemporary samples, Therkildsen et al. (2010) found genetic diversity in the Atlantic cod *Gadus morhua* population from the southern Gulf of St. Lawrence, Canada, to be stable over 80 years, even with intensive fishing. Future evolution in the cod population is unlikely to be constrained by reduced genetic diversity through fisheries-induced bottlenecks. Ruzzante et al. (2001) showed the temporal stability of the genetic differentiation between inshore and offshore populations of Atlantic cod off Newfoundland, Canada, at a scale of 2–3 years. Poulsen et al. (2006) found temporal stability in Atlantic cod from Moray Firth (North Sea)

and Bornholm Basin (Baltic Sea) over 37 and 69 years, respectively, while Jakobsdóttir et al. (2011) found temporal stability of Atlantic cod over 54 years in Icelandic waters. The Atlantic herring *Clupea harengus* off the Baltic Sea and Skagerrak waters showed no changes in genetic diversity, even with this species being subjected to intense exploitation over a period of 24 years (Larsson et al. 2010). However, in the latter study, samples did not pre-date major exploitation, so genetic diversity could have been lost before the sampled period. Similarly, sampling in the present study occurred after several decades of heavy fishing pressure experienced by slinger, and genetic diversity may already have been lost before the sampled period.

Furthermore, most of these studies indicated large effective population sizes in the focal species, which could explain why no genetic diversity was lost despite intense exploitation. For this study, no differences in estimates of effective population sizes were reported between 2012 and 2018 for both microsatellites and SNPs, and estimates were in the order of 10^2. Previous studies on slinger reported effective population sizes in the order of 10^4 (Duncan et al. 2015, Coscia et al. 2016). Although a lower N_e was reported in this study compared to previous studies, there was no evidence of loss of genetic diversity between 2012 and 2018. These high effective population sizes could explain why no genetic diversity was lost during the period slinger was heavily exploited. However, sampling for this study, as for those above, did not pre-date exploitation and genetic diversity could have been lost before sampling occurred.

Two molecular markers were used in this study, microsatellites and SNPs. The latter gave lower estimates for heterozygosity compared to microsatellites. This is consistent with previous studies comparing microsatellites and RAD sequencing. These differences are suggested to be influenced by technical and methodological differences rather than biological differences (Sunde et al. 2020). For example, this is likely reflected in the high mutational rate of

microsatellites compared to SNPs (Sunde et al. 2020). Moreover, SNPs are mostly biallelic, providing less information per locus and lower allele diversity compared to microsatellites (Coates et al. 2009). Despite these differences, mostly similar patterns were detected for both markers, i.e., no significant pairwise population differentiation. Based on the PCoA plot of the microsatellite dataset, slight/subtle spatial and temporal differences were recorded. However, no clear patterns were observed from the PCA plot using the SNPs. The most likely number of genetic clusters for the SNP dataset was one and two for microsatellites. Similarly, other studies found similar trends in diversity estimates when comparing microsatellites and SNPs (Bradbury et al. 2015, Jeffries et al. 2016, Hodel et al. 2017, Guzinski et al. 2018, Morgan et al. 2018, Lemopoulos et al. 2019, Sunde et al. 2020). However, in some of these studies, SNPs outperformed microsatellites in detecting fine-scale structuring. This is likely explained by the fact that RADseq produces a higher number of loci compared to microsatellites (Sunde et al. 2020). This would always be the case, as increasing the number of the markers, regardless of the marker type, increases power and allows for clearer detection and higher resolution of genetic structure (Sunde et al. 2020).

There were differences in statistical power between the two markers. SNPs highlighted the power of high-resolution, next-generation sequencing markers, with significant power for detecting genetic differentiation with both low and moderate F_{ST} values in comparison to microsatellites, and considering small sample sizes as low as four individuals for the SNP dataset.

This study was conducted to understand temporal genetic structure in the slinger and genetic stability over time. Results from some analyses indicated subtle differences both spatially and over time, while all showed a lack of substantial changes in genetic diversity over a period of six years. Studies of both temporal and spatial genetic variation serve as important tools for the genetic conservation and management of marine resources (Ward 2000). Temporal monitoring

helps to understand genetic compositional stability and trajectory over time. This could be vital information for the accurate forecasting of changes in the genetic diversity of marine populations (Francisco and Robalo 2020). This study cannot indicate whether the KZN slinger population is genetically robust or not, since genetic diversity could have been lost before the sampled periods. Notwithstanding that slinger in KZN belongs to one population unit, there may be limited genetic instability along the KZN coast over time. This study demonstrates genetic stability in slinger along the east coast of South Africa, considering optimal exploitation over recent time and the study period, which can aid to better inform fisheries management of this commercially important species.

Chapter 4: Connectivity of slinger *Chrysoblephus puniceus* between Marine Protected Areas and surrounding areas along the east coast of South Africa

Abstract

Marine connectivity refers to the demographic linking of individuals between local populations, occurring through larval dispersal and adult migration. Estimates of connectivity are key factors to consider when designing protected areas, as this process promotes the persistence of metapopulations, their recovery from disturbance (e.g. overfishing), as well as productivity in marine ecosystems. This study investigated genetic structure and gene flow of the slinger *Chrysoblephus puniceus* between three Marine Protected Areas (MPAs) (iSimangaliso, uThukela and Aliwal Shoal) and unprotected areas on the east coast of South Africa. Restriction Associated DNA sequencing was used to genotype 6 850 SNP loci. Results showed similar genetic diversity indices for both MPAs and unprotected areas. Pairwise F_{ST} values across all sites were low and not significant. STRUCTURE, fineRADstructure and Principal Component Analysis failed to detect any population structure in slinger within or between MPAs and unprotected areas. Multidirectional migration of slinger was observed between MPAs and unprotected areas, with net migration from Richards Bay (an unprotected area) to all sites, suggesting that Richards Bay is a key source of recruits. An overall lack of genetic structure across all sites implies high connectivity of all slinger sites, and confirms that MPAs along the east coast form a connected network and contribute to the biological replenishment of surrounding areas. Further, the surrounding areas are also connected, and there is replenishment between all areas via larval dispersal and/or adult spillover. This study reinforces the importance of maintaining MPAs along the east coast which serve as spawning grounds for this important fisheries species.

Introduction

Connectivity refers to the demographic linking of individuals between local populations and occurs through larval dispersal and adult migration, with larval dispersal being the primary mechanism (Sale et al. 2005, Planes et al. 2009, Green et al. 2014). Most non-pelagic marine fishes are relatively sedentary as adults and produce large numbers of eggs and larvae, which are dispersed over large distances by ocean currents (Leis and McCormick 2002). Therefore, the export of larvae has greater potential for wider dispersal compared to adult spillover (Sale et al. 2005). Connectivity is a key ecological factor to consider when designing protected areas, as it promotes the persistence of metapopulations, their recovery from disturbance, as well as productivity in marine ecosystems (Botsford et al. 2003, Roberts et al. 2006, Salm et al. 2006, Almany et al. 2009, McCook et al. 2009, McLeod et al. 2009). However, connectivity was rarely considered in the past when designing Marine Protected Areas (MPAs), as the focus was more on conservation (Almany et al. 2009). With better knowledge of the focal species, more informed decisions can be made to maximise benefits for biodiversity, conservation, fisheries management and improving resilience against climate change (Micheli et al. 2012, Olds et al. 2012b, Magris et al. 2014, Mann et al. 2016, Duncan et al. 2019). Furthermore, connectivity could also enhance increased genetic diversity via intermixing with other populations. High genetic diversity is vital for maintaining the adaptability of natural fish populations and sustainable yields in fisheries (Kenchington et al. 2003).

MPAs are advocated worldwide for the protection of biodiversity, preventing fisheries stocks from being overfished and for the recovery of overexploited species (Roberts et al. 2005, Mora et al. 2006, Edgar et al. 2007, Levin and Lubchenco 2008, Wood et al. 2008, Gaines et al. 2010, Green et al. 2014). MPAs have been a particularly effective tool for fisheries management, and have contributed to the restoration of overfished populations in multiple countries (Attwood et al. 1997a, Kleczkowski et al. 2008, Russ et al. 2008, Barrett et al. 2009, Lester et al. 2009).

While the benefits of MPAs conserving their inhabitants have been established, the full potential of MPAs are realised when these benefits are exported beyond their boundaries, either through larval dispersal or spillover of adult fishes (Sale et al. 2005).

Dispersal through spillover of adult fishes has been demonstrated in several studies (e.g. McClanahan and Mangi 2000b, Russ et al. 2004, Abesamis and Russ 2005, Goñi et al. 2010, Kerwath et al. 2013). However, measuring larval dispersal still presents many challenges. These are due to difficulties involved with tracking small larvae, and the large spatial and temporal variation in larval production and survivorship (Pelc et al. 2010). Therefore, initial inferences of larval dispersal come from biophysical models (Pelc et al. 2010), otolith chemical tags (Elsdon et al. 2008), genetic assessments of genetic flow and connectivity (Palumbi 2003, Levin 2006) and, more recently, parentage analysis (Jones and Ardren 2003, Jones et al. 2010).

It has been established that MPAs along the east coast of South Africa conserve their inhabitants (Mann et al. 1998, 2006), but whether they are connected either via larval dispersal or adult spillover, the extent of their connectivity, and whether they enhance biodiversity and fisheries in adjacent areas, is yet to be understood.

This study investigated the patterns of genetic connectivity between MPAs and unprotected areas off the KwaZulu-Natal (KZN) coast using the slinger seabream *Chrysoblephus puniceus* as a model species. Three MPAs (iSimangaliso Wetland Park, uThukela and Aliwal Shoal), where this species is protected (Sink et al. 2019), were considered. This seabream is endemic to the south-eastern coast of Africa from southern Mozambique to Algoa Bay in the Eastern Cape (Garratt 1985a) (Figure 4.1), and is the most commonly caught fish in the commercial linefishery in KZN and southern Mozambique (Garratt 1985a, Lichucha 1999, 2001, Dunlop and Mann 2013). It is also important in the recreational skiboat fishery in these regions (Attwood et al. 1997b, Penney et al. 1999, Fennessy et al. 2012, Dunlop and Mann 2013).

Previous research on slinger using microsatellites indicated a panmictic stock with high levels of connectivity throughout its distributional range (Duncan et al. 2015). Results from that study also suggested that the high connectivity in slinger is a result of current-driven larval dispersal. The spawning of slinger occurs in shoals on offshore reefs of southern Mozambique and northern KZN (Garratt 1985b), which likely explains how slinger larvae are transported southwards with the prevalent southward-flowing Agulhas Current (AC) assisting. Furthermore, this also explains why very small juvenile slinger (<0.5 cm fork length) are only found in the Pondoland MPA in the Eastern Cape (Mann et al. 2006). This species is believed to be mostly resident; although occasional northward movements have been recorded in adults (Punt et al. 1993, Maggs et al. 2013b).

These characteristics, i.e. the occasional long-distance migrations of adults, adult residency and suspected wide larval dispersal of this species, potentially make the slinger a good biological model for connectivity studies in the considered region. This chapter aims to establish whether MPAs along the KZN coast form a connected network and contribute to the biological replenishment of surrounding areas at multiple scales, by determining the spatial genetic structure of the species along the KZN coast, and examining gene flow between MPAs and exploited areas. This information will be beneficial for marine spatial planning and fisheries management of the slinger, and other species with similar life histories.

Methods

Sampling

Sampling was conducted at five sites along the KZN coast – in MPAs [iSimangaliso Wetland Park (Red Sands), uThukela (Sinkwazi) and Aliwal Shoal (Scottburgh)] and unprotected adjacent areas (Richards Bay and Port Edward) (Table 4.1). Sampling locations were roughly 100 to 150 km apart (Figure 4.1). Fin clips of ca. 100 slinger adults were collected at each of these five sampling sites in 2018 and, in the case of Scottburgh, in 2019. Non-sacrificial sampling was undertaken by boat at protected sites (Scottburgh and Red Sands) by researchers. These entailed repeated drifts over the reefs until 100 fish were caught using conventional rod and line methods. Fin clips were taken from the pectoral fins and the fish were released back into the water. Samples from unprotected areas Richards Bay and Port Edward, as well as the protected site Sinkwazi were obtained from commercial catches. Slinger from Richards Bay and Port Edward were collected from single day catches upon the boats' return to harbour (Richards Bay) or launch site (Margate), respectively. Sinkwazi slinger were collected over three days from a commercial boat upon its return to the launch site (Sinkwazi) or Durban Harbour. At the time of sampling, the areas from which the Sinkwazi and Aliwal Shoal samples were collected were not protected, as these areas were promulgated for protection in August 2019.

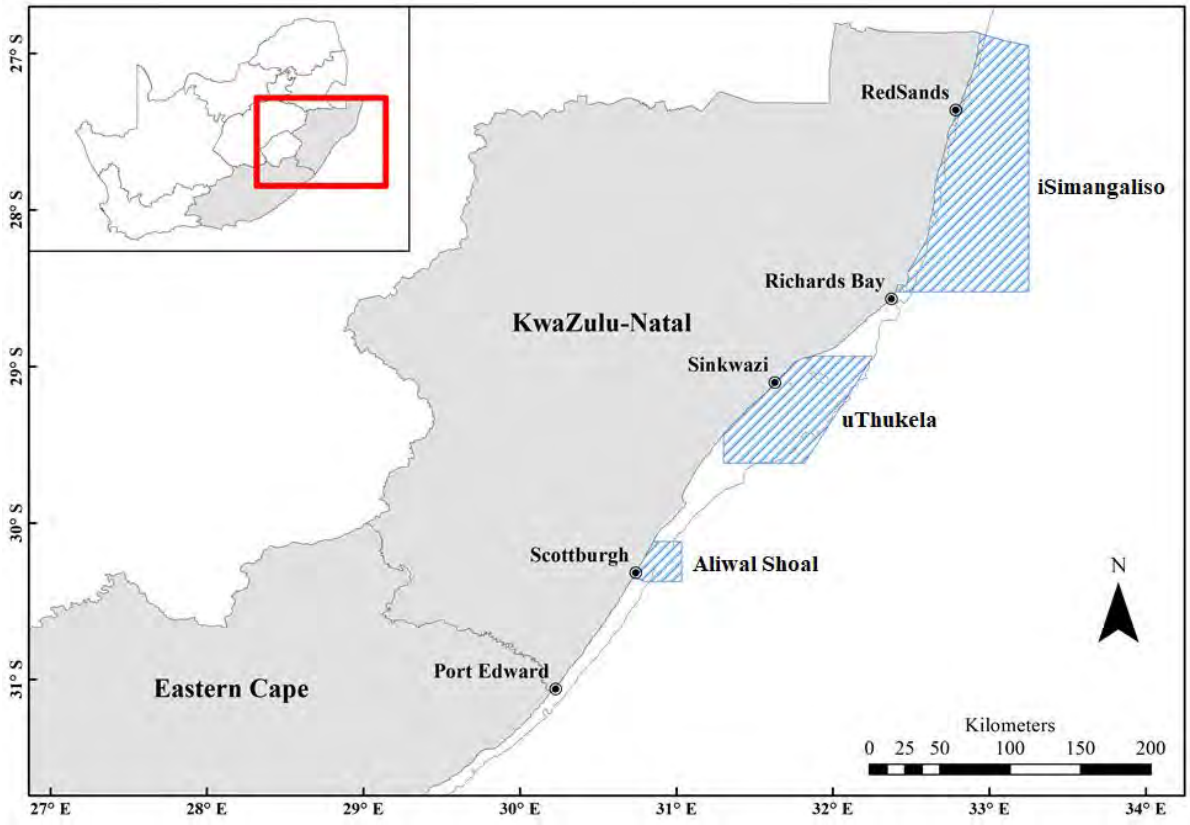

Figure 4.1: Sampling sites for slinger, within protected areas iSimangaliso (Red Sands), uThukela (Sinkwazi) and Aliwal Shoal (Scottburgh) and unprotected areas Richards Bay and Port Edward along the east coast, South Africa

Table 4.1: Sampling sites along the east, South Africa, coast, where slinger were collected in the present study

Sampling site	Region	Sample size	Sampling year	Sampling details
Red Sands	iSimangaliso MPA	102	2018	Slinger were sought by research fishing on the reefs where slinger were caught during the 1980s (Garratt 1993). Fishing was undertaken off SAIAB's RV Phakisa, entailing repeated drifts over the reefs until 100 fish were caught. Fin clips were taken and the fish were returned to the water immediately
Richards Bay	Unprotected area	100	2018	Fin clips were collected randomly from part (ca. 50%) of a single day's catch of slinger (medium-sized category of fish only), made by a commercial boat on 23 May 2018, once the vessel had returned to harbour. Fishing occurred between Nhlabane and Mapelane, likely on several reefs
Sinkwazi	uThukela MPA	99	2018	Fin clips were collected from catches by a commercial boat, on three days, once the vessel had returned to the launch site (Sinkwazi/Matikulu, 22 May 2018, whole slinger catch (n=31) sampled; 6 June 2018, whole slinger catch (n=48); sampled; 14 June 2018, whole slinger catch (n=20) sampled. Note that the fished area only became part of uThukela MPA in 2019
Scottburgh	Aliwal Shoal MPA	60	2018	Fin clips were collected from the catch by a commercial boat, once the vessel had returned to the launch site (Park Rynie). Fish were caught in the vicinity of Scottburgh (26 October 2018; whole catch), likely from several reefs. Note that the fished area was not in the extended Aliwal Shoal MPA at the time of sampling
Scottburgh	Aliwal Shoal MPA	40	2019	Fins were collected from part of the catch by a commercial boat, once the vessel had returned to the launch site (Park Rynie) Fish were caught in the vicinity of Scottburgh (7 July 2019). Note that the fished area was not in the extended Aliwal Shoal MPA at the time of sampling
Port Edward	Unprotected area	100	2018	Fin clips were collected randomly from part (ca. 60%) of the day's catch slinger made by a commercial boat on 15 June 2018, once the vessel had returned to the launch site (Margate). The catch was made from a fishing vessel on several reefs in the Port Edward area

DNA extraction, SNP library construction and genotyping

Genomic DNA was extracted from fin clips using the 'salting' out method (Sunnucks and Hales 1996). DNA concentrations were quantified by a Qubit 3.0 (Invitrogen, Carlsbad, California) broad-range fluorescence assay.

A detailed description of library preparation, single nucleotide polymorphism (SNP) data generation and genotyping is provided in Chapter 3. Briefly, the quaddRAD protocol of Franchini et al. (2017) was followed by preparing double-digest libraries for sequencing, using the restriction enzymes *PstI* and *MspI.* Sequencing of prepared, size-selected libraries was undertaken on an Illumina Hiseq platform using 150 cycle kits by a commercial sequencing facility (Admera Health, South Plainfield, New Jersey). More details on the data cleaning, demultiplexing, SNP and genotype calling can be found in Chapter 3. Raw reads were first screened for duplicate reads and these were discarded using the STACKS *clone-filter* module. Following this, the data set was reduced by removing two sample pools (S132 and S133) with very low reads (749 533 and 323 506, respectively) (Appendix 3). The *process_radtags* module was then used to clean data, removing erroneous and low quality reads. Another five pools (S134, S135, S137, S137 and S148) with very low reads (4 455 860, 4 978 704, 4 098 184, 9 368 928 and 3 111 935 reads per sample, respectively) were discarded (Appendix 3). Prior to running the *denovo_map* pipeline to build a catalogue of loci, optimization of parameters (Paris et al. 2017, Rochette and Catchen 2017) was conducted on a subset comprising the 12 samples spanning the mean number of reads as calculated across all samples. This was to avoid bias, as considering samples with larger numbers of reads would result in the optimisation of a large number of loci which are not found in the samples with fewer reads.

Prior to genotype filtering, samples with < 100 000 reads incorporated by the module *gstacks* were excluded from the dataset due to the high percentages of missing data (>80%) found in

these samples. These included 50 individuals; 17 from Port Edward, 14 from Richards Bay, 17 from Red Sands and two from Sinkwazi.

Furthermore, the dataset was filtered to include only those loci shared by a minimum overall percentage (*R*) of 70% of all the retained individuals across all populations, using the STACKS '*populations'* module. Lastly, output files were produced for downstream programs, including GENEPOP 4.2 (Rousset 2008), STRUCTURE 2.3.4 (Pritchard et al. 2000) and fineRADstructure (Malinsky et al. 2018). The 'write-single-snp' option was implemented, which prevents obviously linked data from being processed by STRUCTURE.

Data analysis

Deviations from Hardy Weinberg Equilibrium (HWE) at each sampling site were determined in the *'populations'* module of STACKS.

Population diversity

Standard diversity measures were calculated for each site in the '*populations*' module of STACKS. These included the mean number of samples (*N*), number of private alleles (*Pa*), number of polymorphic sites (*Ps*), proportion of polymorphic SNPs across all nucleotides sequenced (*P%*), nucleotide diversity (π), observed heterozygosity (*Ho*), expected heterozygosity (*He*) and the inbreeding /fixation index (*Fis*).

Population differentiation

POWSIM v4.1 was used to evaluate theoretical statistical power of the dataset to detect genetic differentiation (Ryman and Palm 2006). Simulations were carried out for low (F_{ST} = 0.001) or moderate (F_{ST} = 0.05) levels of genetic differentiation. A total of 200 replicates was run using an effective population size (N_e) of 1000 and 2 generations of drift (*t*) and *t* = 106 for low and

moderate levels of genetic differentiation, respectively, using the combinations of N_e and t detailed in the software documentation. The power of the analysis was indicated by the proportion of tests that were significant at $P < 0.05$ based on the chi-square test.

To detect spatial genetic structure, pairwise F_{ST}-estimates (Weir and Cockerham 1984) were then calculated among all sampling sites. Statistical significance was determined based on 1 000 permutations of the dataset using Arlequin version 3.5.2.2 (Excoffier and Lischer 2010).

Population structure

A Principal Component Analysis (PCA) was performed to examine patterns of genetic differentiation/similarity among sampling sites using the R package 'Adegenet' version 2.1.1 (Jombart 2008). The software package fineRADstructure (Malinsky et al. 2018) was used to infer population structure using a non-parametric Bayesian clustering approach that groups together individuals with high levels of shared co-ancestry. A "co-ancestry matrix", defined as a summary of nearest-neighbour haplotype relationships, is required as input and was generated using the "Radpainter" module of fineRADstructure. Default parameters were used, which included 100 000 Markov Chain Monte Carlo (MCMC) iterations with a burn-in of 100 000 iterations, and sampling occurred every 1 000 iterations.

A tree was then constructed with 10 000 hill-climbing iterations and the results were visualised using the scripts FINERADSTRUCTUREPLOT.R and FINESTRUCTURELIBRARY.R, which are available via http://cichlid.gurdon.cam.ac.uk/fineRADstructure.html. Lastly, to infer genetic structure among sampling sites a Bayesian approach using STRUCTURE version 2.3.4 (Pritchard et al. 2000) was conducted. STRUCTURE analyses were run with the number of genetic clusters (K) set from 1 to 5, with 10 replicate runs of 100 000 MCMC iterations, after an initial burn-in period of 500 000 iterations. The best fit for K was determined by plotting Ln(PD) and implementing the ΔK method of Evanno et al. (2005), and using the four statistics

MEDMED, MEDMEAN, MAXMED and MAXMEAN, as implemented in StructureSelector (Li and Liu 2018). These four estimates have been found to be more accurate than previous methods to determine the best fit number of clusters, for both evenly and unevenly sampled datasets (Puechmaille 2016).

Gene flow

According to Hedgecock et al. (2007), Bayesian approaches for inferring migration rates are more applicable for structured populations and less so for weakly structured populations. Bayesian methods can produce accurate estimations on condition that genetic differentiation is not too low ($0.1 \geq F_{ST} \geq 0.05$) and migration rats are high ($m = 0.1$) (Faubet et al. 2007). However, when these assumptions are violated (mean $F_{ST} = 0.041$; this study), accurate estimations may be difficult to obtain. However, numerous published studies (Rossi et al. 2019, Machado et al. 2020, Moreira et al. 2020, Loera-Padilla et al. 2022, Velasco-Montoya et al. 2022, Rumisha et al. 2023) have included migration rates calculated under similar conditions, such as a lack in genetic structure observed in slinger, and provided biologically-plausible insights into gene flow and connectivity when considered alongside other results. Contemporary gene flow between sites, particularly between MPAs and unprotected areas, was estimated using BayesAss version 3.0.4 (Wilson and Rannala 2003). This programme estimates the fraction of immigrants in a population using Bayesian inference. The mixing parameters for migration rates (m), allele frequencies (a) and inbreeding coefficients (f) were optimized to ensure that the acceptance rates for each of the parameters were between 0.2 and 0.6 (Wilson and Rannala 2003). However, this procedure is impractical when it comes to examining input files with a large number of loci. Therefore, an automated process was used which uses a binary search algorithm in an additional program – BA3-SNPS-autotune (Mussmann et al. 2019). Firstly, BA3-SNPS-autotune was used to find optimal mixing parameters for each run, with exploratory analyses employing 10 000 MCMC generations. A maximum of 10 exploratory

analyses were conducted for each data file. The number of repetitions required to find optimal mixing parameters was recorded for each, and mixing parameters verified to produce adequate MCMC acceptance rates (i.e., 0.2 < acceptance rate < 0.6). The program required only five rounds of optimization to obtain suitable parameters. Final mixing parameters for migration rates, allele frequencies and inbreeding coefficients were 0.156, 0.550 and 0.025, respectively. The final analysis included all individuals and was completed using 10 million MCMC generations with 1 million iterations discarded as burn-in. Results were visualized using R (R Core Team 2022) with a custom R-script and the R-packages 'circlize' (Gu et al. 2014), 'migest' (Abel 2012) and 'dplyr' (Wickham and Francois 2015).

Results

In total, 211 samples were genotyped across all sites after the various stages of data cleaning and culling – Scottburgh (n = 12), Sinkwazi (n = 24), Port Edward (n = 42), Richards Bay (n = 79) and Red Sands (n = 54). Sequencing produced 743 081 562 pairs of sequences, with a minimum of 329 229 and a maximum of 100 716 505 pairs for each sample pool. The identified PCR duplicates ranged from 1.11 to 20.87% of reads in each pool, and were removed. Retained reads ranged between 323 506 and 95 299 208 read pairs per pool and totalled 683 365 175 read pairs. After filtering for quality, between 39 939 and 46 596 509 reads were assigned to individual samples, with a mean of 4 059 154 (± 5 156 483.8 SD). Optimization with the *denovo_map* pipeline revealed optimal parameters of m = 3, M = 4, and n = 4 (Appendix 5). Overall, 702 312 loci were assembled from paired reads, with a mean sample coverage of 11.1x (SD = 9.7x), a minimum coverage of 4.1x, a maximum coverage of 54.9x and the mean number of sites per locus of 160.4.

The final dataset was then subjected to genotype filtering to account for missing data, and included only loci that were present in 70% of individuals across all populations. In total, 114

individuals were retained for this final dataset, with 11 to 32 samples per population (Table 4.2). A total of 6 850 loci were shared by at least 70% of these individuals and were composed of 1 099 226 sites, with a mean number of nucleotides per locus of 160.47 ± 0.27. The total number of variant SNPs was 6 592.

A number of loci were found to be significantly ($P < 0.05$) out of HWE at all sampling sites (Table 4.2). The highest of these was recorded at Scottburgh (2 459) and the lowest at Richards Bay (1 518).

Population diversity

Overall, there were no clear differences or patterns found in genetic diversity among the sampling sites (Table 4.2). The number of private alleles was highest at Richards Bay (*Pa* = 4 796) and Red Sands (*Pa* = 4 127), and these sites had the highest number of genotyped samples (32 and 27, respectively). The diversity in terms of polymorphic sites (*Ps*) was highest at Richards Bay (*Ps* = 24 797), Red Sands (*Ps* = 22 824) and Sinkwazi (*Ps* = 20 596). Nucleotide diversity was very similar among all sites, but slightly higher at Sinkwazi (0.088). The *Ho* across sampling sites ranged from 0.069 to 0.077, whereas *He* ranged from 0.072 to 0.085. *Ho* was similar among sites and consistently lower than expected heterozygosity (*He*), indicating a deficiency in heterozygosity, and inbreeding. The inbreeding coefficient (*Fis*) values were rather low but positive, suggesting heterozygote deficiency, with the highest value recorded at Red Sands (0.040) and the lowest at Port Edward (0.023). Furthermore, *Fis* values do not follow the same trends as HWE.

Table 4.2: Summary of genetic diversity based on 6 850 SNP loci in slinger sampled within Marine Protected Areas and unprotected areas along the east coast, South Africa. *n* = sample size, *N* = mean number of samples genotyped across all loci, *Pa* = number of private alleles, *Ps* = number of polymorphic sites, *P%* = proportion of polymorphic SNPs across all nucleotides sequences, π = nucleotide diversity, *Ho* = observed heterozygosity, *He* = expected heterozygosity, *Fis* = inbreeding index, *HWE* = number of loci out of Hardy Weinberg Equilibrium

Estimates	Red Sands	Richards Bay	Sinkwazi	Scottburgh	Port Edward
n	27	32	22	11	22
N	21.597	27.487	15.822	8.341	14.128
Pa	4 127	4 796	2 954	1 300	2 331
Ps	22 824	24 797	20 596	13 937	17 558
P%	2.076	2.256	1.874	1.268	1.597
$\boldsymbol{\pi}$	0.080 ± 0.001	0.080 ± 0.001	0.088 ± 0.001	0.080 ± 0.001	0.075 ± 0.001
Ho	0.071 ± 0.001	0.072 ± 0.001	0.077 ± 0.001	0.069 ± 0.001	0.069 ± 0.001
He	0.078 ± 0.001	0.078 ± 0.001	0.085 ± 0.001	0.075 ± 0.001	0.072 ± 0.001
Fis	0.040 ± 0.014	0.032 ± 0.012	0.038 ± 0.014	0.030 ± 0.009	0.023 ± 0.017
HWE	1 567	1 518	1 888	2 459	1 988

Population differentiation

According to the power estimations, implemented in POWSIM, the dataset had sufficient power to detect moderate genetic differentiation (F_{ST} = 0.05) in 100% of tests, and also high probabilities (> 60%) for detecting low genetic differentiation (F_{ST} = 0.001).

Genetic differentiation (based on overall F_{ST} values) among the five sampling sites was low and non-significant ($P > 0.05$), ranging from 0 to 0.065 (Port Edward and Richards Bay) (Table 4.3).

Table 4.3: Pairwise genetic differentiation based on 6 850 SNP loci among slinger sampled within Marine Protected Areas and unprotected areas. Pairwise F_{ST} values are shown below the diagonal and *P* values based on 1 000 permutations above the diagonal

	Red Sands	Richards Bay	Sinkwazi	Scottburgh	Port Edward
Red Sands	-	0.464	0.999	0.999	0.570
Richards Bay	0.003	-	0.999	0.234	0.165
Sinkwazi	0.000	0.000	-	0.327	0.487
Scottburgh	0.000	0.057	0.034	-	0.855
Port Edward	0.000	0.065	0.047	0.000	-

Population structure

A principal component analysis (PCA) was performed to evaluate population structure at the individual level. The first and second principal components accounted for 1.45% and 1.35% of the total variation, respectively (Figure 4.2). The PCA results revealed no clustering based on the geographic origin of the samples. Next, a non-parametric Bayesian clustering approach was implemented in fineRADstructure to infer population structure via shared ancestry using 6 850 loci across 114 slinger samples. The resulting cladogram and co-ancestry matrix are shown in Figure 4.3 and confirm the results of the PCA, i.e. no geographical structure among sampling sites as there is no pattern of shared co-ancestry based on geographic origin of each sample. However, some individuals are clearly more genetically related to each other than to others. StructureSelector found one genetic cluster (Table 4.4), indicating a single slinger population.

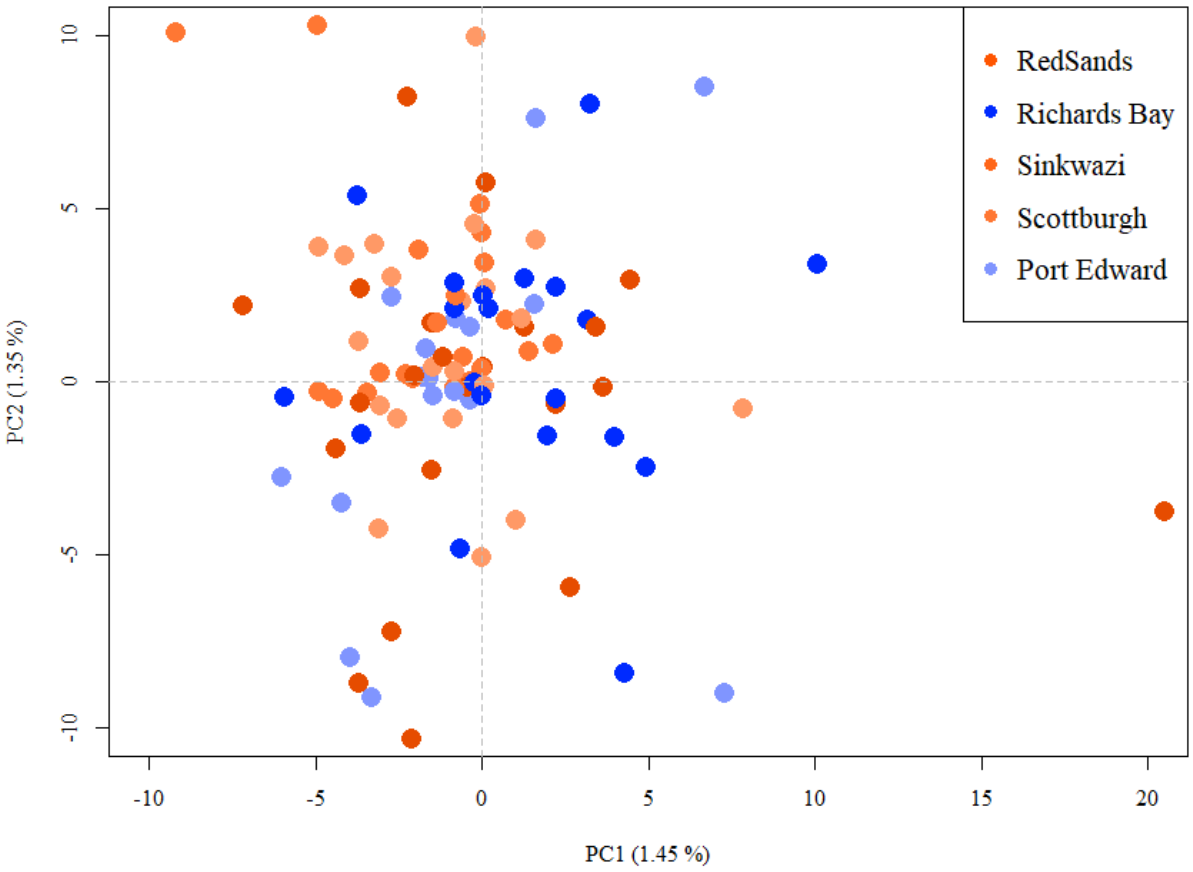

Figure 4.2: Individual-based principal component analysis (PCA) plot based on 6 850 SNP loci of slinger samples collected within protected sites (orange shades) and unprotected sites (blue shades) along the east coast, South Africa

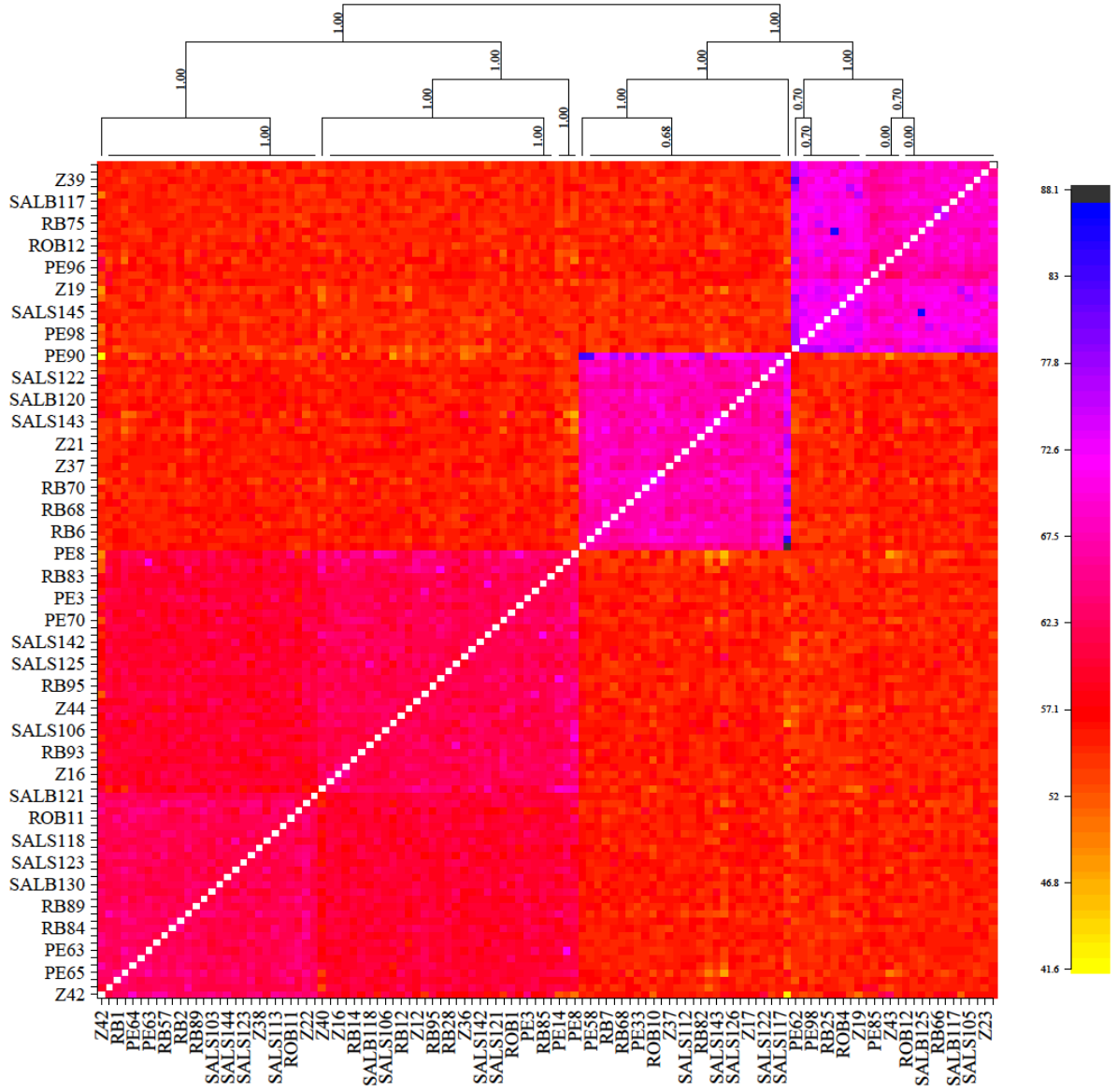

Figure 4.3: Co-ancestry matrix of slinger individuals based on 6 850 SNP loci sampled among three Marine Protected Areas (Red Sands, Sinkwazi and Scottburgh) and unprotected areas (Richards Bay and Port Edward). Colours indicate scale of relatedness between individuals, with yellow being low relatedness and blue/black indicating high relatedness

Table 4.4: Analyses of STRUCTURE following StructureSelector of 6 850 SNP loci sampled among three Marine Protected Areas (Red Sands, Sinkwazi and Scottburgh) and unprotected areas (Richards Bay and Port Edward)

StructureSelector				
K	MedMedK	MedMeanK	MaxMedK	MaxMeanK
1	1	1	1	1
2	1	1	1	1
3	1	1	1	1
4	1	1	1	1
5	1	1	1	1

Gene flow

Contemporary gene flow between MPAs and unprotected areas was estimated using the Bayesian approach implemented in BA3-SNPS (Wilson and Rannala 2003, Mussmann et al. 2019). Results indicated multidirectional migration between sites. Migration rates for individuals being retained in the source populations varied between 0.708 (Scottburgh) to 0.928 (Richards Bay). These migration rates were the highest values recorded and were much higher than migration recorded between different populations. The highest migration rates were recorded from Richards Bay to all other sites; for example, to Red Sands (0.250), Scottburgh (0.187), Sinkwazi (0.161) and Port Edward (0.161) (Table 4.4; Figure 4.5). There appears to be a source-sink relationship between Richards Bay and the other sites, with very low migration rates (ranging from 0.009 to 0.027) in the opposite direction, suggesting the Richards Bay area as a key source of recruits. Furthermore, low migration rates were recorded from Sinkwazi to Scottburgh (0.063) and from Port Edward to Sinkwazi (0.049). Overall, multidirectional migration rates between sites indicated connectivity among MPAs themselves and the surrounding areas.

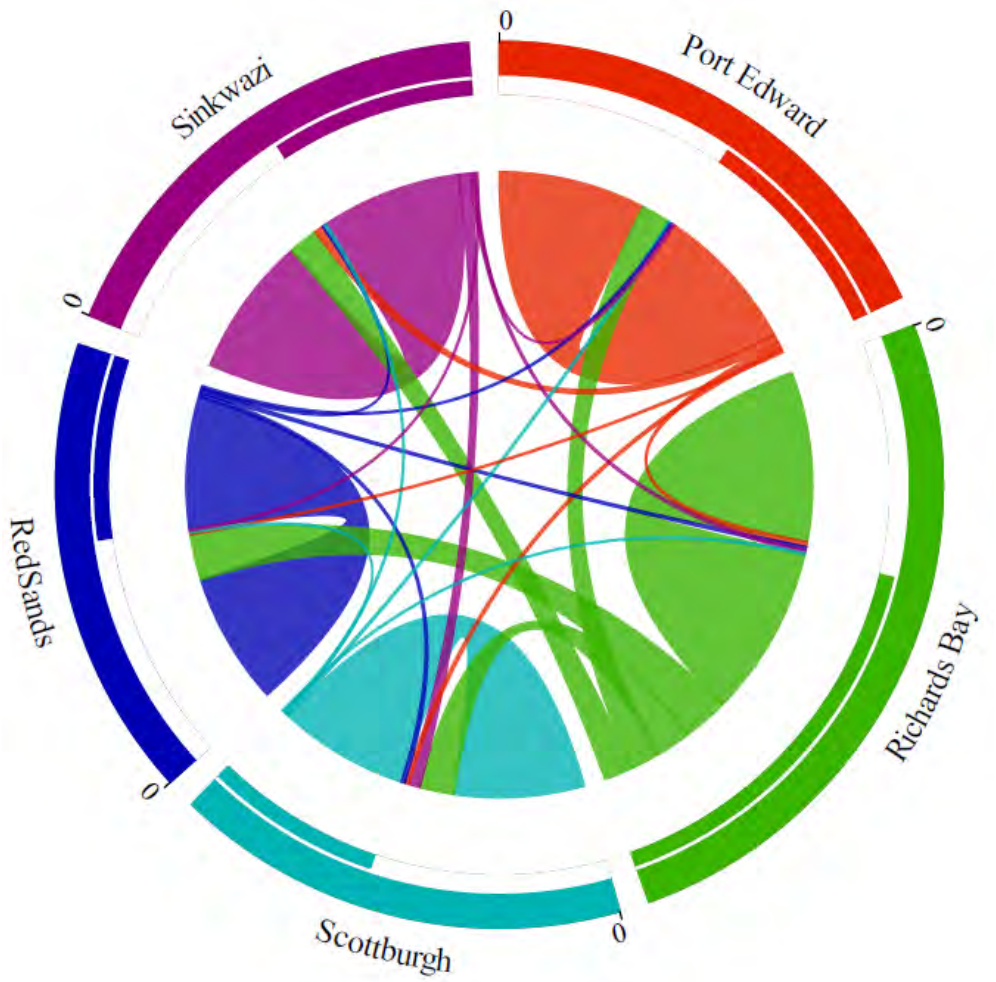

Figure 4.4: Plot of contemporary gene flow using the program BayesAss for slinger based on 6 850 SNP loci. Colours correspond to sites: Richards Bay (green), Scottburgh (turquoise), Red Sands (blue), Sinkwazi (purple) and Port Edward (red)

Table 4.5: Posterior mean migration rates (*m*) and the standard deviation of the marginal posterior distribution for each estimate among sampling sites of slinger in protected and unprotected sites along the east coast, South Africa, as calculated using BayesAss based on 6 850 SNP loci. The populations into which individuals are migrating are listed in the rows, while the origins of the migrants are listed in the columns. Migration rates ≥ 0.10 are in bold

		Source				
		Red Sands	**Richards Bay**	**Sinkwazi**	**Scottburgh**	**Port Edward**
Sink	Red Sands	**0.719** (± 0.021)	**0.250** (± 0.025)	0.010 (± 0.010)	0.011 (± 0.010)	0.011 (± 0.010)
	Richards Bay	0.018 (± 0.012)	**0.928** (± 0.022)	0.018 (± 0.012)	0.009 (± 0.009)	0.027 (± 0.015)
	Sinkwazi	0.012 (± 0.012)	**0.161** (± 0.032)	**0.765** (± 0.029)	0.012 (± 0.012)	**0.049** (± 0.022)
	Scottburgh	0.021 (± 0.020)	**0.187** (± 0.040)	**0.063** (± 0.032)	**0.708** (± 0.027)	0.021 (± 0.020)
	Port Edward	0.012 (± 0.012)	**0.161** (± 0.032)	0.012 (± 0.010)	0.012 (± 0.012)	**0.803** (± 0.031)

Discussion

This study aimed to investigate whether existing MPAs along the east coast are connected, form a network and contribute to the biological replenishment of surrounding areas; quaddRAD sequencing was used to examine genetic structure and gene flow in slinger between MPAs and unprotected areas along the east coast of South Africa. This is the first large-scale generation of novel SNPs for any South African sparid which has been used to examine genetic connectivity between these MPAs and unprotected areas. Results provided conclusive evidence of a lack of genetic structure among MPAs and the adjacent exploited areas, with high levels of genetic connectivity among MPAs themselves, and between MPAs and unprotected areas along the east coast. These results confirm the contention by Duncan et al. (2015), in a less detailed study based on 10 microsatellite loci, that there is a single population of slinger with high levels of connectivity recorded throughout its distributional range.

Genetic diversity indices in this study indicated no differences among sampling sites along the KZN coast. Genetic similarity indicates that, at the scale of this study, gene flow is sufficient to maintain homogeneity in allele frequencies between MPAs and unprotected areas (i.e. 100–150 km apart). Very low genetic differentiation between sites indicates high genetic gene flow between MPAs and surrounding areas. Similarly, population structure analysis from PCA, fineRADstructure and STRUCTURE failed to detect genetic structure among sites, emphasizing the extent of gene flow in slinger, spanning MPAs and unprotected areas along the east coast of South Africa. Although fineRADstructure showed a lack of structure, clusters of individuals with higher levels of co-ancestry are apparent. These individuals (from MPAs and adjacent exploited areas) were more closely related, indicative of connectivity/spillover of cohorts of larvae or adults.

Similar patterns of a lack of genetic structure have been observed in South African coastal fishes co-occurring in this study area and at larger scales, particularly from False Bay (southern coast of South Africa) to KZN (Teske et al. 2010, Henriques et al. 2012, 2014, 2016, Murray et al. 2014, Reid et al. 2016, Bennett et al. 2017b). This lack of genetic structure among sites may be expected, with the coastline on the east coast being relatively linear, with no geographical barriers, thus allowing easy dispersal or movement for fishes. Moreover, high connectivity among MPAs and the surrounding areas has also been reported in other parts of the world. For example, the saddled bream *Oblada melanura*, showed no differentiation among protected areas and unprotected areas in the Western Mediterranean Sea at spatial scales of 500– 1000 km (Calò et al. 2016). Similarly, Mzingirwa et al. (2019) found a lack of genetic structure among protected and exploited sites in the sky emperor *Lethrinus mahsena* along the coast of Kenya. Sahyoun et al. (2016) found a lack of genetic structure between an MPA and surrounding exploited areas in the two-banded seabream *Diplodus vulgaris* in the Mediterranean Sea. Restriction site-associated DNA sequencing indicated a lack of population

structure in the dusky parrotfish *Scarus niger* spanning >400 km across the central the central Philippines and revealed that MPAs established within the central Philippines likely supply varying levels of larvae to overfished reefs (Stockwell et al. 2016).

Bayesian analysis of contemporary gene flow among MPAs and unprotected areas in the present study revealed multidirectional gene flow, with the highest migration rates observed from Richards Bay (unprotected area) towards other sites. This suggested the Richards Bay area as a key source of recruits. The highest migration rates were predominantly southwards and were likely influenced by the prevalent southward-flowing Agulhas Current (AC). Initial studies suggested that this current is responsible for southward distribution of recruits of many species on the east coast (Heydorn et al. 1978, Garratt 1988). Subsequent research indicated that processes at the shoreward edge of the AC were responsible for dispersal and not the current itself (Beckley 1993). Although studies on larval development and the associated potential for dispersal of slinger are currently lacking, these are believed to be similar to those of the santer *Cheimerius nufar*, a co-occurring sparid in the region (Connell et al. 1999). *Cheimerius nufar* has a flexion stage of larval development, which is complete after 21 days, giving ample time for widespread current-driven dispersal.

Spillover or movement by either juvenile or adult fish could be another aspect promoting the connectivity demonstrated in this study. Although predominantly southward, gene flow was also recorded in a northward direction, from both Richards Bay to Red Sands, and from Port Edward to Sinkwazi. Migration from Richards Bay to Red Sands could be effected via adult dispersal, or larval dispersal with occasional northward flow inshore of the AC entraining larvae, while migration from Port Edward to Sinkwazi is possibly through juveniles or adults moving northwards, since no spawning has been recorded this far south. Slinger are highly resident, but tagging studies have revealed occasional northward movements by adults (Punt et al. 1993, Maggs et al. 2013b). For example, a total of 4 613 slinger have been tagged with

standard spaghetti tags, and 157 (3.4%) have been recaptured, with a mean distance moved of only 8 km (Maggs et al. 2013b). Migrations were only recorded in older slinger (>4 years old) individuals, of which only a few individuals have been observed to undertake extensive migrations (Punt et al. 1993). These included slinger tagged within the Pondoland MPA. One individual nearly covered its distribution range and was recaptured off Quissico, Mozambique, covering a distance of 1 059 km within 582 days (Maggs et al. 2013b). Another slinger was recaptured on the KZN south coast, covering a distance of 163 km within 581 days (Maggs et al. 2013b).

The resident behaviour and occasional movement of slinger is also supported by more recent tagging and recapture data from the Oceanographic Research Institute's Cooperative Fish Tagging Project (ORI-CFTP) from 1985-2023 (Jordaan and Mann 2023). Between 1984 and 2023, a total of 5 253 slinger were tagged, with 223 (4%) recaptures reported, including 14 multiple recaptures (recaptured two or more times). The majority of tag recaptures was reported from two localities, iSimangaliso and Pondoland, where most tagging occurred. In terms of movement, 187 (87%) individuals were recaptured at their tagging locality or very close to it (< 6 km); 19 (8.5%) moved between 6 and 1 000 km, and only one (0.5%) individual moved > 1 000 km from the original tag-release site. This individual, tagged at the offshore Mtentu Reef in the Pondoland MPA, moved 1 100 km and was recaptured 1 350 days (3.7 years) later in Ponta Zavora/Inharrime, Mozambique.

This northward migration behaviour is likely to be spawning-related and has been recorded in other South African east coast sparids. These include the black musselcracker *Cymatoceps nasutus* (Murray et al. 2019), red steenbras *Petrus rupestris* (Brouwer 2002), white steenbras *Lithognathus lithognathus* (Bennett et al. 2017a), white musselcracker *Sparodon durbanensis* (Buxton and Clarke 1991, Watt-Pringle et al. 2013) and scotsman *Polysteganus praeorbitalis* (Maggs et al. 2013b, Mann et al. 2023). The spawning of slinger is restricted to the north of

Durban, on the central KZN coast, with no reproduction recorded south of Durban (Garratt 1985b); thus fish would have to migrate up the east coast to spawn.

In conclusion, this study found a lack of genetic structure in slinger among sites, which indicates a single population with high levels of genetic connectivity between MPAs and the surrounding areas; confirming MPAs are connected and can readily replenish or be replenished by other sites along the east coast of South Africa, whether they are within protected areas or not. For MPAs to be beneficial, they should be connected to other MPAs, either through larval dispersal or spillover of adult fishes (Sale et al. 2005, Planes et al. 2009). Given the lack of structure and high levels of connectivity observed, and the relatively high mobility of at least some slinger individuals, this species may not be particularly informative for exploring marine spatial planning and management of MPAs off the South African east coast, when considered in isolation. Other studies, which were done in parallel with this work and form part of the larger CAPTOR project, investigating connectivity in more sessile organisms, such as corals or benthic meiofauna, should also be considered.

Whilst the current network of MPAs along the east coast may provide adequate protection for South African slinger, the lack of protection in the Richards Bay area, which is indicated as being a key source of recruits, is potentially concerning, should patterns of fishing effort change. While the slinger population has recovered, based on stock assessment data up to 2015 (Maggs et al. 2017), it is conceivable that fishing pressure in Richards Bay may increase. This is a possibility because launching there is via a sheltered port, and with the implementation of the small-scale fisheries policy, there are likely to be new entrants to the fishery who would choose to be based there – thereby increasing local fishing pressure (Oceanographic Research Institute, unpublished. data). The Richards Bay reefs are known to host spawning slinger shoals (Garratt 1985b), and their importance as a source of recruits has been demonstrated in the current study. Only the Red Sands area in the iSimangaliso MPA was sampled in the current

study and more research on the relative importance of this MPA as a source of recruits is needed, as there are several reefs there, which host spawning slinger shoals (Garratt 1993). While the protected slinger shoals in iSimangaliso MPA can biologically replenish other protected or non-protected sites, they require continued protection in the face of recent increasing pressures (Anon 2021), because of the recruits and gene flow from spawning shoals of slinger there.

Chapter 5: Source-sink dynamics of the slinger *Chrysoblephus puniceus* along the east coast of South Africa

Abstract

Many non-pelagic marine species' life cycles include a dispersal larval phase, which can be transported great distances with prevailing currents. Understanding the processes behind larval dispersal is important for determining the persistence and productivity in marine populations, which, in turn, can be useful to establish the extent of connectivity between populations. This is also critical for planning and placement of Marine Protected Areas (MPAs) in support of fisheries. It is assumed that slinger *Chrysoblephus puniceus* larvae are transported via inshore processes associated with the prevailing south-flowing Agulhas Current. This is supported by observations that slinger's spawning is restricted to reefs from southern Mozambique to northern KwaZulu-Natal (KZN) (Garratt 1985b). Spawning is suggested to occur on the down-current edges of reefs, producing small pelagic eggs containing an oil droplet which provides the eggs with extra buoyancy and increased dispersal potential. The aim of this study was to determine likely sources and sinks of slinger recruits along the east coast of South Africa. Parentage analysis and population genetics were used to assign juveniles back to their natal populations, to unravel larval dispersal patterns and connectivity in the slinger along the east coast of South Africa coast. Parentage analysis identified no parent-offspring pairs in this study, but population genetic analyses revealed genetic similarities between slinger adult and juvenile sites, emphasizing genetic connectivity and a lack of genetic structure between these sites. Additionally, BayesAss results suggested mainly southward larval dispersal, influenced by the prevailing southward flowing inshore of the AC. Contemporary effective population size (N_e), depicting intergenerational variation between slinger adult and juvenile sample sites was >2 000 and infinite for adults and juveniles, respectively. This study, therefore, provides

valuable information to support the role of the network of KZN MPAs for fisheries management as well as for marine spatial planning.

Introduction

Many non-pelagic marine species' life cycles consist of a dispersal larval phase and a relatively sedentary adult phase (Planes et al. 2009, D'Aloia et al. 2015). During the larval phase, larvae can be transported great distances with prevailing currents (see Roberts 1997, Jenkins et al. 1999, Cowen et al. 2000, 2006, Mora and Sale 2002, Olsen et al. 2002, Treml et al. 2008, Simpson et al. 2014). This dispersive larval phase drives the exchange of individuals and alleles, leading to connectivity among populations within many marine metapopulations (Kritzer and Sale 2006, Liggins et al. 2019).

Understanding the processes behind larval dispersal is important for determining persistence and productivity in marine populations (Dubé et al. 2020), which, in turn, can be useful to establish the extent of connectivity between populations (Almany et al. 2009). Moreover, larval dispersal patterns characterize patterns of connectivity, which can shape metapopulation dynamics and gene flow (Shima and Swearer 2016). This is also critical for the planning and placement of Marine Protected Areas (MPAs) in support of fisheries (Andrello et al. 2017).

Measuring larval dispersal is challenging, with the earliest attempts being predictive models of passive larval dispersal (Roberts 1997). Other methods used to determine larval dispersal in the marine environment include coupled biophysical models (Cowen et al. 2006, Paris et al. 2007, Cowen and Sponaugle 2009), otolith chemistry (Elsdon et al. 2008), and population genetics (Palumbi 2003, Levin 2006).

Parentage analysis, which assigns individuals to their parents or birth populations, is one method that can be used to overcome the challenge of directly measuring larval dispersal (Hedgecock et al. 2007, Planes et al. 2009). This method is a useful and robust tool for

quantifying connectivity, and has resolved fine-scale recruitment dynamics in marine systems (von der Heyden et al. 2014), as well as complementing efficacy assessments and the design of MPAs (Berumen et al. 2012). Although this method requires extensive sampling to match potential parents with recruits (Christie et al. 2017) and performs best in structured populations (Almany et al. 2017, Harrison et al. 2020, Catalano et al. 2021), it has proven successful in several homogenous metapopulations (Saenz-Agudelo et al. 2009, Christie et al. 2010a, Berumen et al. 2012, Pusack et al. 2014, D'Aloia et al. 2015, Salles et al. 2015). High levels of gene flow and the associated lack of population structure in many marine species is another drawback to assign recruits to their natal populations (Underwood et al. 2007), specifically with challenges in sampling a sufficiently large proportion of the parental population required for parentage analysis (Saenz-Agudelo et al. 2009). However, new sequencing approaches, producing more loci, can overcome this challenge (Christie et al. 2017).

Larval dispersal studies have been applied mainly to tropical reef systems (Jones et al. 2005, Planes et al. 2009, Saenz-Agudelo et al. 2009, Christie et al. 2010a, Berumen et al. 2012, Herrera et al. 2016, Almany et al. 2017), while studies in temperate regions are rare (Schunter et al. 2014, Le Port et al. 2017, Baetscher et al. 2019). Tropical reef species frequently have restricted home ranges (Saenz-Agudelo et al. 2011, Almany et al. 2013) and/or form reproductive aggregations (Almany et al. 2013), which aid sampling sufficiently large proportions of parental populations for successful detection of parent-offspring pairs (Underwood et al. 2007). Contrastingly, temperate regions pose more challenges in sampling a sufficient number of Individuals, as temperate species are suggested to have broader dispersal than tropical species, and larval dispersal is highly influenced by dominant alongshore currents (Baetscher et al. 2019).

Slinger *Chrysoblephus puniceus* is a protogynous hermaphrodite endemic to the south-eastern coast of Africa (Garratt 1985a), and is characterized by high levels of gene flow (Chapters 3

and 4), which Duncan et al. (2015) suggested was maintained primarily by a highly dispersive larval phase. It is assumed that slinger larvae are transported along the south east coast of southern Africa via inshore processes associated with the prevailing southward-flowing Agulhas Current (AC) (Beckley 1993, Punt et al. 1993, Hutchings et al. 2002). This is supported by observations that slinger spawning is restricted to reefs from southern Mozambique to northern KwaZulu-Natal (KZN) (Garratt 1985b). Spawning is suggested to occur on the down-current edges of reefs, producing small pelagic eggs, containing an oil droplet, which provides the eggs with extra buoyancy and increased dispersal potential. While details of slinger larval development are not known, they are inferred to be similar to the co-occurring confamilial santer *Cheimerius nufar* (Connell et al. 1999), whose dispersal is likely similar. Previous work on reef-associated sparids and other fish species also suggested that the inshore currents on the east coast of South Africa are responsible for larval dispersal (Beckley 1993, Hutchings et al. 2002). This may also explain why juveniles < 50 mm of slinger have only been found off the Pondoland MPA in the Eastern Cape (Mann et al. 2006) and not further northward where spawning occurs.

The life history characteristics of slinger, notably a pelagic larval phase and wide larval dispersal, make this species potentially suitable to determine the sources (where they are spawned) and sinks (where they recruit to), as well as to assess larval dispersal and connectivity along the KZN coast. This study used parentage analysis and population genetic analyses of single nucleotide polymorphisms (SNPs) to assign slinger recruits to adults, and to decipher patterns of dispersal and connectivity in the slinger among a network of MPAs and surrounding areas along the KZN coast.

Methods

Sampling

For the genetic tracing of offspring, and the identification of sources and sinks of recruits, slinger adults and juveniles were sampled along the east coast of South Africa. Adults were from sites sampled in 2018, as in Chapter 4, specifically Red Sands, Richards Bay, Sinkwazi, Scottburgh and Port Edward. Approximately one-year old juveniles (identified on the basis of size) were sampled in the Aliwal Shoal MPA early in 2019 (Figure 5.1; Table 5.1), with the rationale that the adults sampled previously in 2018 could have spawned these juveniles. Fishing for juvenile samples entailed repeated drifts over the reef until 50 fish were caught using conventional rod and line methods. Fin clips were taken from pectoral fins and the fish were released back into the water immediately.

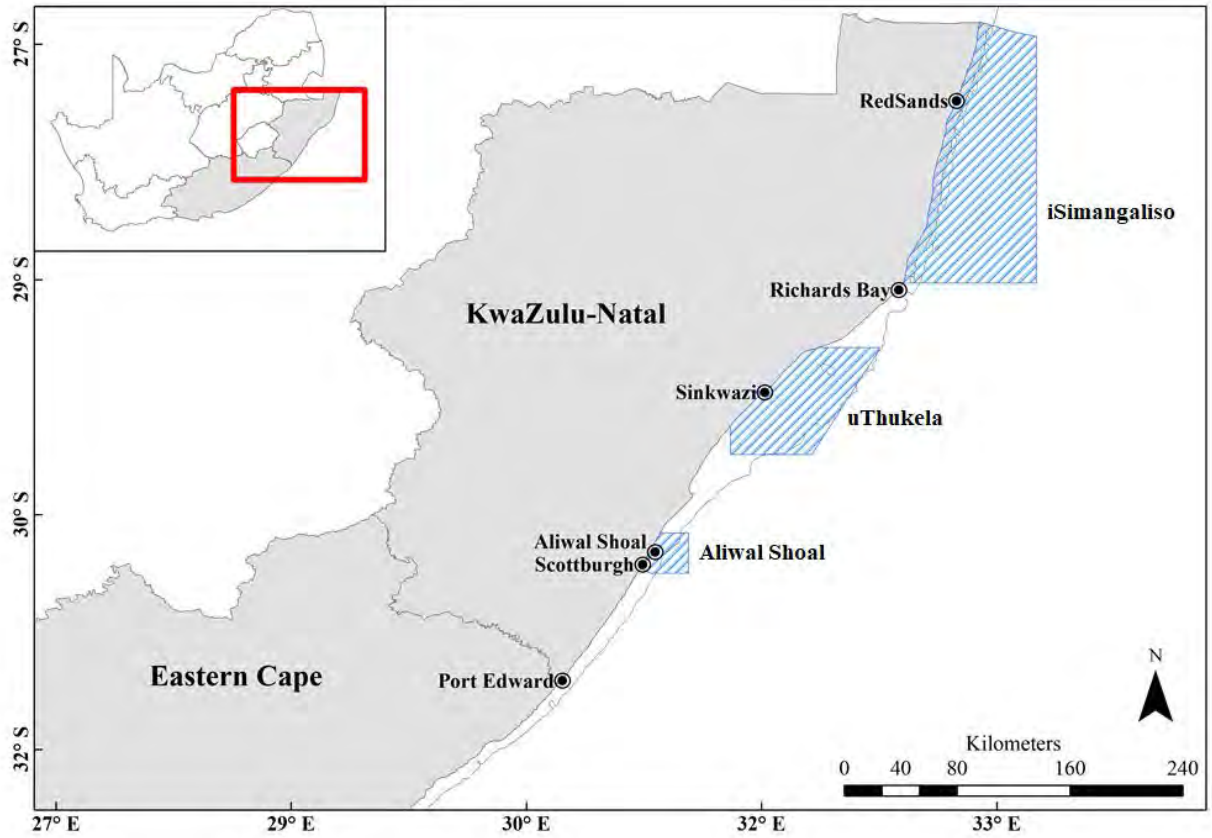

Figure 5.1: Sampling sites of slinger adults (Red Sands, Richards Bay, Sinkwazi, Scottburgh and Port Edward sampled in 2018) and juveniles (Aliwal Shoal sampled in 2019) along the east coast, South Africa.

Table 5.1: Sampling sites of slinger adults (Red Sands, Richards Bay, Sinkwazi, Scottburgh and Port Edward) and juveniles (Aliwal Shoal) collected along the east coast, South Africa in 2018 and 2019.

Sampling site	Sample size	Sampling year	Life stage	Sampling details
Red Sands (iSimangaliso) **MPA**	102	2018	Adults	Slinger were sought by research fishing on the reefs where slinger were caught during the 1980s (Garratt 1993). Fishing was undertaken off SAIAB's RV Phakisa, entailing repeated drifts over the reefs until 100 fish were caught. Fin clips were taken and the fish were returned to the water immediately
Richards Bay **Unprotected area**	100	2018	Adults	Fin clips were collected randomly from part (ca. 50%) of a single day's catch of slinger (medium-sized category of fish only), made by a commercial boat on 23 May 2018, once the vessel had returned to harbour. Fishing occurred between Nhlabane and Mapelane, likely on several reefs
Sinkwazi (uThukela) **MPA**	99	2018	Adults	Fin clips were collected from catches by a commercial boat, on three days, once the vessel had returned to the launch site (Sinkwazi/Matikulu, 22 May 2018, whole slinger catch (n=31) sampled; 6 June 2018, whole slinger catch (n=48); sampled; 14 June 2018, whole slinger catch (n=20) sampled. Note that the fished area only became part of uThukela MPA in 2019
Scottburgh (Aliwal Shoal) **MPA**	60	2018	Adults	Fin clips were collected from the catch by a commercial boat, once the vessel had returned to the launch site (Park Rynie). Fish were caught in the vicinity of Scottburgh (26 October 2018; whole catch), likely from several reefs. Note that the fished area was not in the extended Aliwal Shoal MPA at the time of sampling
Scottburgh (Aliwal Shoal) **MPA**	40	2019	Adults	Fins were collected from part of the catch by a commercial boat, once the vessel had returned to the launch site (Park Rynie) Fish were caught in the vicinity of Scottburgh (7 July 2019). Note that the fished area was not in the extended Aliwal Shoal MPA at the time of sampling
Port Edward **Unprotected area**	100	2018	Adults	Fin clips were collected randomly from part (ca. 60%) of the day's catch slinger made by a commercial boat on 15 June 2018, once the vessel had returned to the launch site (Margate). The catch was made from a fishing

				vessel on several reefs in the Port Edward area
Aliwal Shoal **MPA**	50	2019	Juveniles	Slinger were sought by research fishing. Fishing was undertaken off SAIAB's Phakisa, entailing repeated drifts over the reef until 50 fish were caught. Fin clips were taken and fish were returned to the water immediately

The DNA extraction protocol and procedure details are provided in Chapter 3. The SNP library construction and genotyping are described in Chapter 4. The SNPs were generated using quaddRAD sequencing (Franchini et al. 2017). Detailed information on the data cleaning, demultiplexing and SNP genotype calling processes performed in this chapter are available in Chapter 4. For this chapter, juvenile samples from Aliwal Shoal and adults from Red Sands, Richards Bay, Sinkwazi, Scottburgh and Port Edward (used in Chapter 4) were included for downstream analysis.

Data filtering was conducted in STACKS version 2.60 (Catchen et al. 2011, 2013), using the '*populations*' module, and included only those loci shared by a minimum overall percentage of 70% of all retained individuals across all populations. Output files were produced for further downstream analysis in GENEPOP 4.2 (Rousset 2008), STRUCTURE 2.3.4 (Pritchard et al. 2000) and fineRADstructure (Malinsky et al. 2018), and a 'populations.haplotypes.tsv' file was generated for analysis in 'apparent' (Melo and Hale 2019). To avoid linked data being processed by STRUCTURE, the 'write-single-snp' option was implemented.

Data analysis

Parentage assignments

To assign juveniles from Aliwal Shoal MPA to potential parents from other sites, the R package 'apparent' (Melo and Hale 2019) was used. Unlike most progeny-parent assignment tests,

'apparent' does not require *a priori* knowledge of family structure, for example, sex, or pedigree, to enable a robust parentage test. This software uses homozygous parental loci for discriminating true- and false-offspring assignments. It performs parentage analysis based on a test of genetic identity between expected progeny (EP_{ij}), using homozygous loci from all pairs of loci from all possible parents (i and j), and all potential offspring (POk). The Gower Dissimilarity (GD) metric (Gower 1971), the genetic identity between EPij and POk is taken as evidence that individuals i and j are the true parents of offspring k. Evaluation of triad (two parents + offspring) significance is based on the distribution of all GD (EP_{ij}|POk) values. The significance is assessed using a Dixon test (Dixon 1950, 1951), which identifies a gap-based threshold that separates true triads from spurious associations. The GD ranges from 0 to 1, where a value of 0 indicates perfect identity at all loci which is expected for 'true' parent-offspring triads. For parentage analysis in this study, a subset of the dataset was used to accommodate the limit on loci used for this software. Genotype filtering was applied to include only those loci shared by a minimum overall percentage of 88% of all retained individuals across all populations, resulting in 114 SNP loci.

Diversity estimates

The 'populations' module in STACKS was used to examine deviations from Hardy Weinberg Equilibrium (HWE), as well as to calculate diversity indices at each site. Diversity estimates included the mean number of samples (*N*), number of private alleles (*Pa*), number of polymorphic sites (*Ps*), proportion of polymorphic SNPs across all nucleotides sequenced (*P%*), nucleotide diversity (π), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and the inbreeding/fixation index (*Fis*).

Population differentiation

Pairwise genetic differentiation values (F_{ST}: Weir and Cockerham 1984) between juvenile and adults sample sites and their significance were determined in Arlequin version 3.5.2.2 (Excoffier and Lischer 2010). Significance for pairwise F_{ST} tests was based on 10 000 permutations.

Population structure

A Principal Component Analysis (PCA) was performed to assess and visualise genetic distance and relatedness between juvenile and adult sample sites using the R package 'adegenet' version 2.1.1 (Jombart 2008).

Additionally, genetic structure was investigated to test for genetic similarity or divergence between juvenile and adult sample sites using a Bayesian approach in STRUCTURE. More details on this can be found in Chapter 4. For this study, STRUCTURE was run with the number of genetic clusters *(K)* set from 1 to 6. The analyses were conducted with 100 000 generations for burn-in and 500 000 recorded generations for each *K*. Runs for each *K* were repeated 10 times (Pritchard et al. 2000). Details on extracting the results, determining the most likely *K*, summarising the results and visualization are found in Chapter 4. Furthermore, population structure between juveniles and adult sample sites was assessed using a Monte Carlo Markov Chain (MCMC) method in fineRADstructure (Malinsky et al. 2018) – see Chapter 4. The MCMC chain ran with a burn-in of 100 000, 100 000 iterations and sampling occurred every 1 000 iterations.

Migration rates

Recent migration rates were determined in BayesAss (Wilson and Rannala 2003), as in Chapter 4. The final mixing parameters for migration rates, allele frequencies and inbreeding coefficients were 0.156, 0.550 and 0.250, respectively. These parameters were then applied to

the dataset in BA3-SNPS, and run for 10 000 000 MCMC generations, with 1 000 000 iterations discarded as burn-in. Finally, a custom R-script employing the R packages 'circlize' (Gu et al. 2014), 'migest' (Abel 2012) and 'dplyr' (Wickham and Francois 2015) were used to visualize the results in R (R Core Team 2022).

Effective population size

Contemporary effective population size (N_e) was estimated for the juveniles and adult samples, respectively, to depict intergenerational variation, using the Linkage Disequilibrium (LD) method (Waples and Do 2008) with a random mating model and the Jackknife method for estimates of confidence intervals, as implemented in NeEstimator v2.01 (Do et al. 2014). Negative N_e was interpreted as being infinite N_e.

Results

After genotype and sample filtering, a final dataset of 8 234 SNP loci was obtained for 137 individuals: 114 adults from five sample sites, Red Sands (27), Richards Bay (32), Sinkwazi (22), Scottburgh (11) and Port Edward (22), and 23 juveniles from Aliwal Shoal.

Parentage assignments

The Triad gap analysis was not significant at the declared alpha level. Hence, no triads (pairs of parents + offspring) were found in this study.

Diversity indices

A number of loci were found to be significantly out of HWE at all sampling sites (Table 5.2), with the highest recorded at Scottburgh (5 940) and lowest at Richards Bay (4 061). Diversity estimates indicated no clear patterns between adult and juvenile sample sites (Table 5.2). *Pa* was highest at Richards Bay (796), Red Sands (774) and Aliwal Shoal (729), and these values were correlated to the number of individuals genotyped at each site. The proportion of

polymorphic SNPs across all nucleotide sites varied between 0.163 (Scottburgh) and 0.321 (Richards Bay). *Ho* varied between 0.058 and 0.067, and *He* ranged from 0.056 to 0.067. *Ho* and *He* values at each site were similar. Fixation index values were similar across sites with the highest recorded at Red Sands (0.015 ± 0.030) and lowest at Port Edward (0.004 ± 0.043).

Table 5.2: Diversity estimates of 8 234 SNP loci for slinger juveniles and adults sampled along the east coast, South Africa, between 2018 and 2019. *n* = sample size, *N* = mean sample size, *Pa* = number of private alleles, *Ps* = number of polymorphic sites, *P%* = proportion of polymorphic SNPs across all nucleotides sequenced, HWE = number of SNP loci out of Hardy Weinberg Equilibrium, π = nucleotide diversity, *Ho* = observed heterozygosity, *He* = expected heterozygosity, *Fis* = inbreeding index

	Juveniles	Adults				
Estimates	Aliwal Shoal	Port Edward	Scottburgh	Sinkwazi	Richards Bay	Red Sands
n	23	22	11	22	32	27
N	20.178	13.250	27.132	15.340	27.132	21.585
Pa	729	392	218	509	796	774
Ps	3 888	2 830	2 184	3 524	4 299	3 978
P%	0.291	0.212	0.163	0.263	0.321	0.297
$\boldsymbol{\pi}$	0.063 (± 0.001)	0.058 (± 0.001)	0.060 (± 0.001)	0.069 (± 0.001)	0.062 (± 0.001)	0.063 (± 0.001)
Ho	0.061 (± 0.001)	0.059 (± 0.001)	0.058 (± 0.001)	0.067 (± 0.001)	0.063 (± 0.001)	0.060 (± 0.001)
He	0.062 (± 0.001)	0.056 (± 0.001)	0.056 (± 0.001)	0.067 (± 0.001)	0.061 (± 0.001)	0.061 (± 0.001)
Fis	0.013 (± 0.023)	0.004 (± 0.043)	0.007 (± 0.023)	0.011 (± 0.031)	0.006 (± 0.029)	0.015 (± 0.030)
HWE	4 357	5 384	5 940	4 759	4 061	4 278

Population differentiation

Pairwise F_{ST} values between juvenile and adult sample sites were low to moderate, ranging from 0 to 0.111 for Aliwal Shoal and Richards Bay (Table 5.3). The largest estimates of

differentiation ($F_{ST} \geq 0.086$) were seen between two adult sample sites (Richards Bay and Sinkwazi) and the juvenile site (Aliwal Shoal), suggesting that juveniles possibly did not emanate from these adult sample sites. However, no significant *(P* > 0.05) genetic differentiation was detected between juvenile (Aliwal Shoal) and adult sample sites.

Table 5.3: Pairwise F_{ST} values based on 8 234 SNP loci for slinger juveniles and adults sampled along the east coast, South Africa, between 2018 and 2019. Population pairwise F_{ST} values are shown below the diagonal and P values based on 1 000 permutations above the diagonal

	Juveniles	Adults				
Sites	Aliwal Shoal	Port Edward	Scottburgh	Sinkwazi	Richards Bay	Red Sands
Aliwal Shoal	--	0.789	0.059	0.946	0.448	0.246
Port Edward	0.000	--	0.172	0.849	0.585	0.485
Scottburgh	0.000	0.000	--	0.248	0.448	0.999
Sinkwazi	0.086	0.047	0.034	--	0.999	0.325
Richards Bay	0.111	0.065	0.057	0.000	--	0.999
Red Sands	0.000	0.000	0.000	0.000	0.000	--

Population structure

The PCA also revealed that the majority of samples clustered together, indicating no genetic differentiation between juvenile and adult sampling sites (Figure 5.2). The variation in each of the principal components was very low, with components one and two accounting for 1.21% and 1.09% of total genetic variation, respectively.

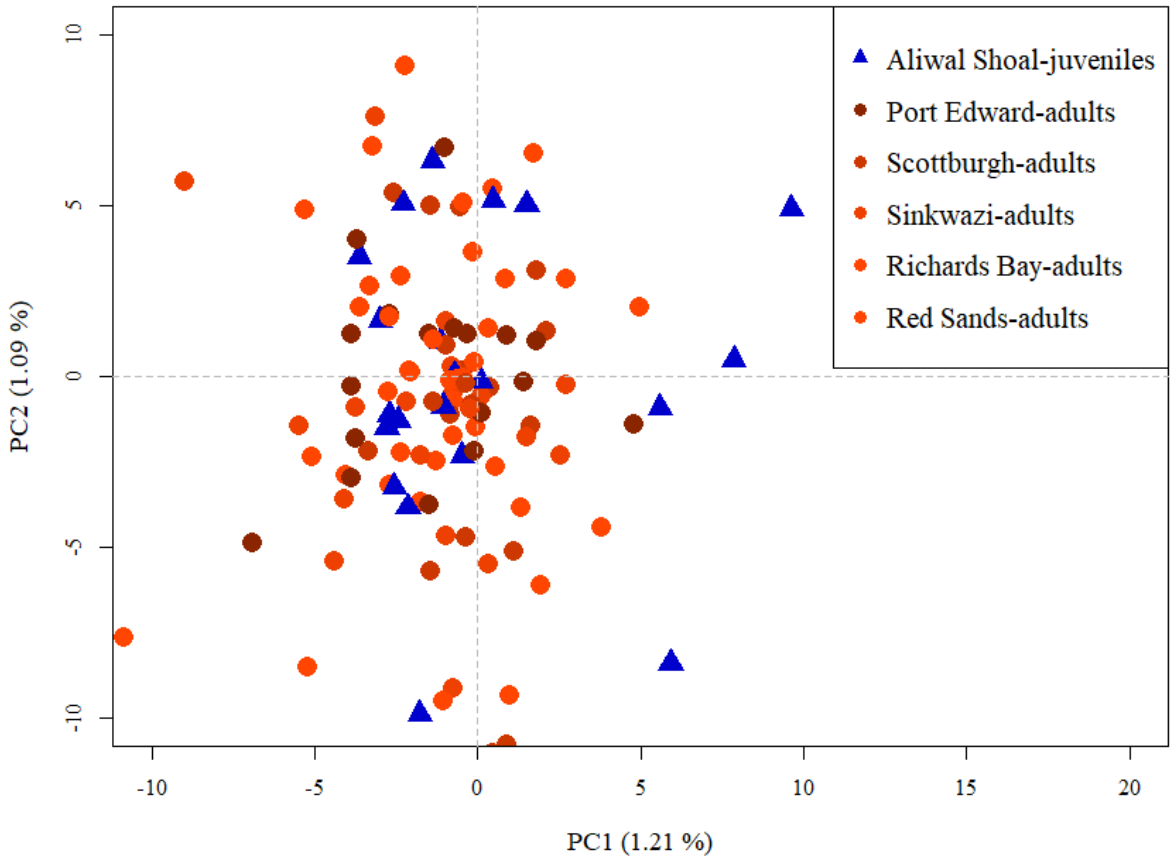

Figure 5.2: Principal Component Analysis (PCA) based on 8 234 SNP loci of slinger juveniles (Aliwal Shoal; blue triangles) and adults (Port Edward, Scottburgh, Sinkwazi, Richards Bay and Red Sands; orange-red shade circles) along the east coast, South Africa

StructureSelector revealed one genetic cluster for the adult and juvenile sampling sites (Table 5.4). Similarly, fineRADstructure analysis corroborated both the PCA and STRUCTURE results, with no geographical genetic structure, but high relatedness between adult and juvenile sample sites. (Figure 5.4). Specifically, in the lower left of Figure 5.4, individuals from both juvenile and adult sample sites clustered together with a high degree of relatedness. This comprised adults from Red Sands, Richards Bay, Sinkwazi and Port Edward, and excluded adults from Scottburgh.

Table 5.4: Analyses of STRUCTURE outputs following StructureSelector of 8 234 SNP loci for slinger juveniles (Aliwal Shoal) and adults (Red Sand, Richards Bay, Sinkwazi, Scottburgh and Port Edward) sampled along the east coast, South Africa

StructureSelector				
K	MedMedK	MedMeanK	MaxMedK	MaxMeanK
1	1	1	1	1
2	1	1	1	1
3	1	1	1	1
4	1	1	1	1
5	1	1	1	1
6	1	1	1	1

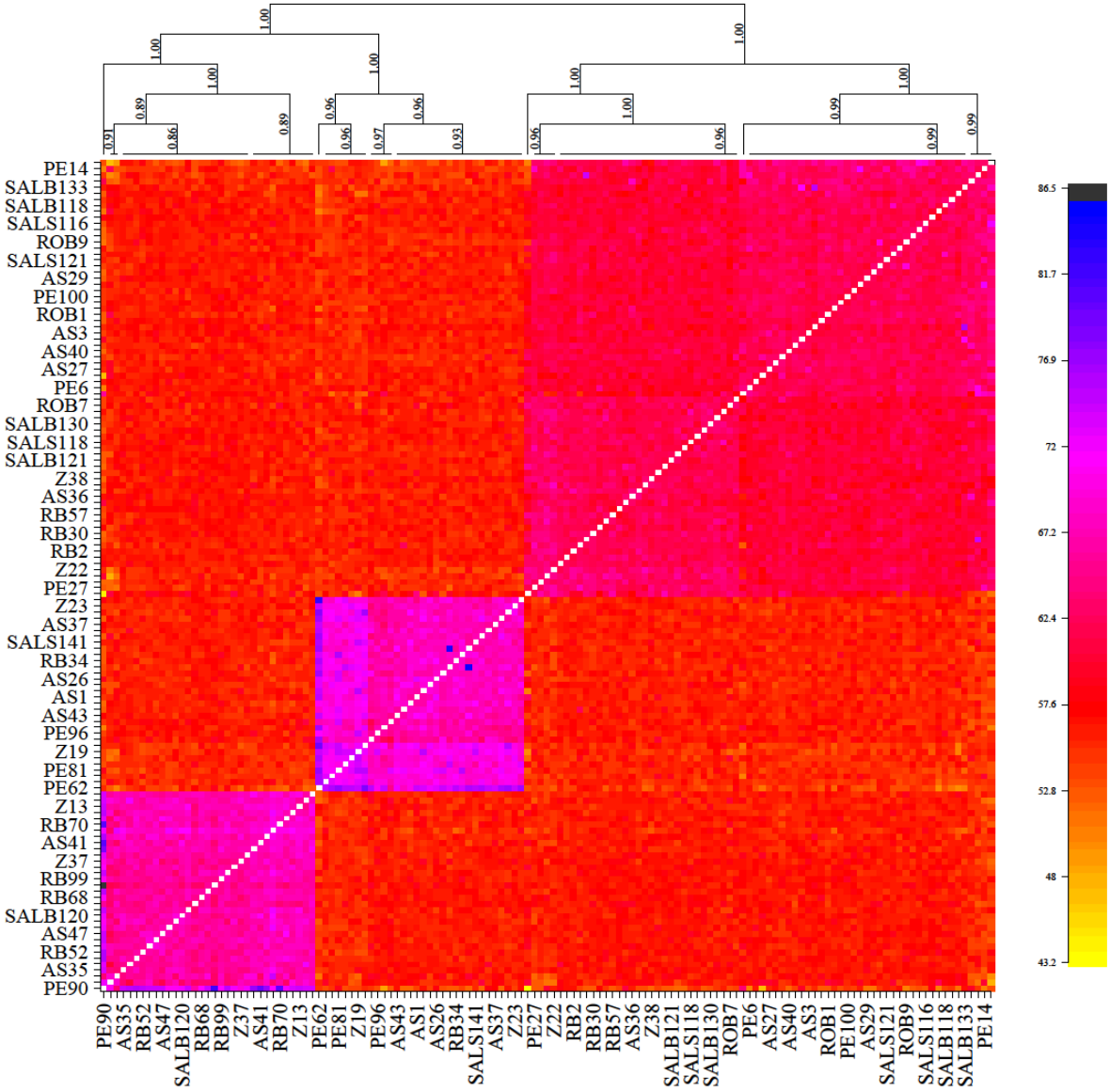

Figure 5.3: FineRADstructure coancestry matrix based on 8 234 SNP loci for slinger juvenile (Aliwal Shoal) and adults (Red Sands, Richards Bay Sinkwazi, Scottburgh and Port Edward) sampled along the east coast, South Africa. Colours indicate scale of relatedness between individuals, with yellow being low relatedness and blue/black high relatedness

Migration rates

The mean posterior migration rates between juvenile and adult sample sites of slinger from BayesAss revealed asymmetrical migration (Table 5.5.) Migration rates for individuals being retained in the source population varied between 0.694 and 0.914. The highest migration rate reported into the juvenile sample site at Aliwal Shoal was 0.260 from Richards Bay, which

suggests southward movement of slinger larvae from spawning events in northern KZN. Furthermore, migration was predominantly in a north to south direction, and predominantly from Richards Bay southwards to the other sites and northwards to Red Sands (0.229).

Effective population size

Estimates of contemporary effective population size (N_e) derived from slinger juveniles and adults are reported in Table 5.6. Based on the juveniles, estimates of N_e were infinite and > 2 000 for slinger adults.

Table 5.5: Migration rates (*m*) and standard deviation among slinger juvenile and adult sample sites using BayesAss based on 8 234 loci. The populations into which individuals migrate are listed in rows, while the columns represent origins of migrants. Migration rates ≥ 0.10 are in bold

		Source					
		Adults					**Juveniles**
		Red Sands	Richards Bay	Sinkwazi	Scottburgh	Port Edward	Aliwal Shoal
Sink	Red Sands	**0.718** (± 0.021)	**0.229** (± 0.046)	0.010 (± 0.010)	0.010 (± 0.010)	0.010 (± 0.010)	0.023 (± 0.038)
	Richards Bay	0.018 (± 0.012)	**0.914** (± 0.030)	0.009 (± 0.009)	0.009 (± 0.007)	0.035 (± 0.016)	0.016 (± 0.021)
	Sinkwazi	0.012 (± 0.012)	**0.166** (± 0.031)	**0.750** (± 0.027)	0.012 (± 0.012)	**0.048** (± 0.022)	0.012 (± 0.012)
	Scottburgh	0.020 (± 0.019)	**0.129** (± 0.042)	**0.059** (± 0.030)	**0.734** (± 0.035)	0.039 (± 0.025)	0.020 (± 0.018)
	Port Edward	0.012 (± 0.012)	**0.152** (± 0.032)	0.012 (± 0.012)	0.012 (± 0.011)	**0.798** (± 0.030)	0.015 (± 0.015)
	Aliwal Shoal	0.012 (± 0.011)	**0.260** (± 0.056)	0.012 (± 0.011)	0.012 (± 0.011)	0.011 (±0.011)	**0.694** (± 0.053)

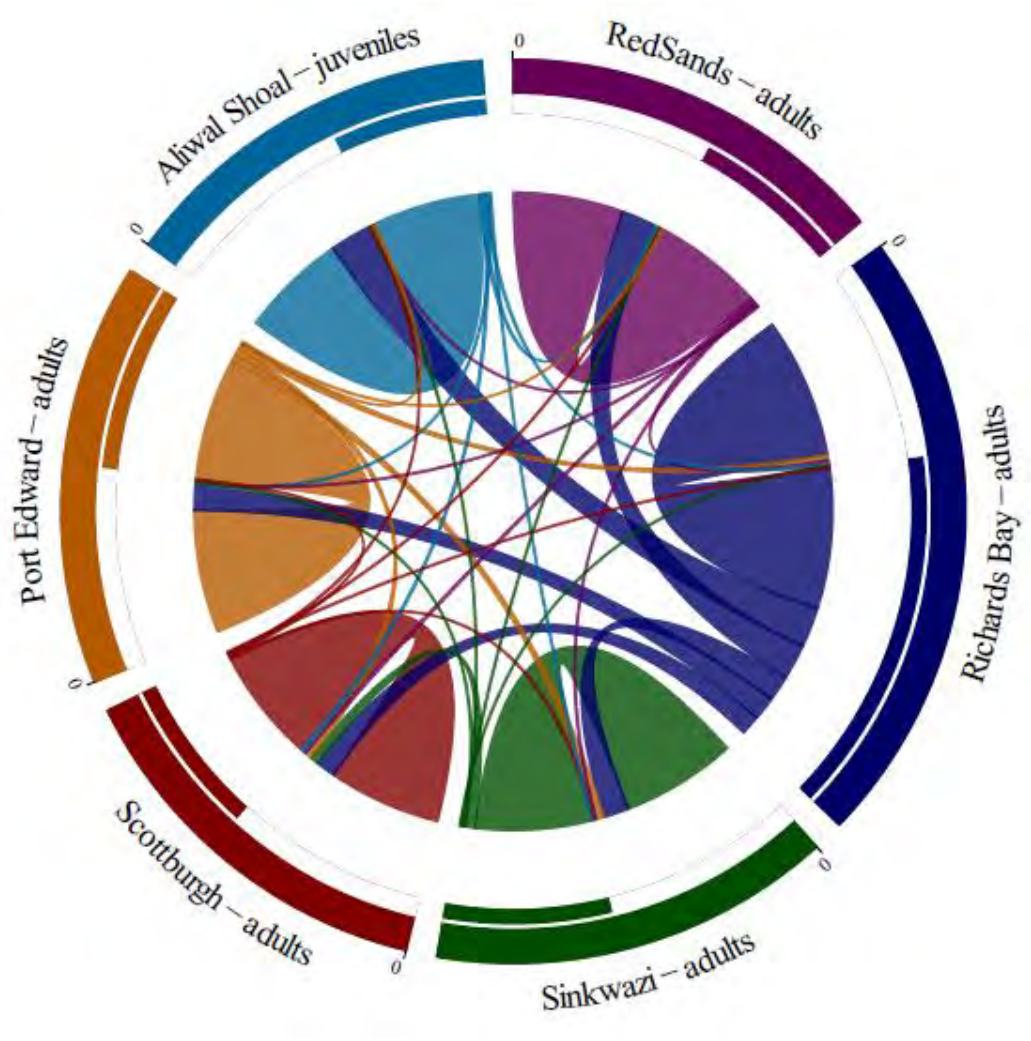

Figure 5.4: Schematic of contemporary gene flow among juvenile and adult slinger sample sites using BayesAss based on 8 234 SNP loci

Table 5.6: Effective population size (N_e) estimates based on the Linkage Disequilibrium method by NeEstimator between adults and juvenile sample sites along the east coast, South Africa using 6 850 and 8 234 SNP loci respectively.

N_e (Jackknife 95% CIs)	
Adults	**Juveniles**
2049.0 (278.3-infinite)	Infinite (122.4-infinite)

Discussion

This study used parentage analysis and population genetics to attempt to assign slinger recruits to adults, and to elucidate larval dispersal patterns and connectivity in the slinger along the east coast of South Africa. Understanding larval dispersal patterns of slinger could be important for marine spatial planning by revealing whether and how populations within MPAs and those in the surrounding areas are connected (Harrison et al. 2012, Christie et al. 2017).

Parentage analysis, a direct method to estimate connectivity, identified no parent-offspring pairs in this study. Although this approach performs well in structured marine populations (Almany et al. 2017, Harrison et al. 2020, Catalano et al. 2021), there has also been success in determining parentage in populations with low genetic structure, provided that enough loci are sampled (Christie et al. 2017). Slinger showed a lack of genetic structure among MPAs and unprotected areas (Chapter 4), but a large number (8 234) of SNP loci were used in the parentage analysis, so, in principle, juveniles should have been assignable to adult parents successfully; however, this was not the case. This could be due to several reasons. Firstly, a small sample size of potential parents was collected in this study, followed by a further reduction of sample numbers by genotype filtering. Successful parentage assignments are highly dependent on intensive sampling of potential parents (Underwood et al. 2007, Saenz-Agudelo et al. 2009). Moreover, intense sampling of potential parents is normally not feasible in marine systems, as most populations have large effective population sizes (Marandel et al. 2019). The estimated effective population size of slinger in this study (> 2000 based on the adult samples and infinite when using the juvenile samples) is considered to be large. These estimates of N_e are often higher than true census estimates (Do et al. 2014). It should also be noted that slinger is a protogynous hermaphrodite and the sex ratio of sex-changing species is biased towards the ‘first sex’, which is known to reduce effective population size (Chopelet et

al. 2009b). NeEstimator (Do et al. 2014), using the Linkage Disequilibrium method (Waples and Do 2008), does not consider sex ratios or reproductive strategies.

Although parentage analysis has hitherto been applied in tropical reef systems at relatively small spatial scales (Jones et al. 2005, Planes et al. 2009, Saenz-Agudelo et al. 2009, Christie et al. 2010a, Berumen et al. 2012, Herrera et al. 2016, Almany et al. 2017), successful parentage analysis has been undertaken in large-scale studies. For example, patterns of larval dispersal were studied using parentage analysis in two exploited coral reef groupers, the bar-cheek coral trout *Plectropomus maculatus* and leopard coral trout *P. leopardus,* within and among three clusters of reefs separated by distances of 60– 220 km in the Great Barrier Reef Marine Park, Australia (Williamson et al. 2016). Both short and long distance dispersal, with a maximum of 250 km larval dispersal among study regions were found, which implies larval exchange among reefs, and demonstrates that established reefs function as a connected network, contributing larvae to replenish fished areas. Another parentage analysis study on the yellow tang *Zebrasoma flavescens* among reefs around the island of Hawai'i documented larval dispersal distances ranging from 15 to 184 km (Christie et al. 2010b), providing evidence of MPAs successfully seeding exploited areas through larval dispersal.

Overall, genetic diversity estimates were similar among adult and juvenile sample sites in this study, indicating consistent exchange of alleles among sites, resulting in a panmictic population. F_{ST} values were low to moderate, with moderate values recorded between the juvenile site (Aliwal Shoal) and the adult sites of Richards Bay and Sinkwazi. The moderate genetic differentiation possibly indicates alternative parental sources for these sites not sampled in the current study e.g. further north in the iSimangaliso MPA or in Mozambique. However, low genetic differentiation was recorded between Aliwal Shoal (juvenile site) and Red Sands (adult site), indicating a close relationship between these sites and another potential northern source for slinger.

Population structure analyses conducted with PCA, Structure and fineRADstructure indicated a lack of genetic structure among adult and juvenile sample sites. Overall, juvenile and adult sample sites of slinger were genetically similar, consequently indicating high connectivity along the entire KZN coast.

Contemporary migration rates indicated asymmetrical migration in this study. Overall, the juvenile sample site (Aliwal Shoal) received the highest proportion of migrants from Richards Bay, suggested as a key source of recruits in Chapter 4. This implies a southward movement of juveniles from spawning sites in northern KZN, most likely influenced by the prevalent southward-flowing AC.

Similarly, gene flow in this study occurred mainly in a southward direction, with the highest migration rates predominantly from Richards Bay towards the other sites. However, relatively strong migration rates were observed northwards from Port Edward to Sinkwazi, as well as from Richards Bay to Red Sands, which could be accounted for by migration of adults for spawning. This is supported by tagging studies on slinger which indicated occasional northward migration by adults from southern populations (Mann et al. 2006, Maggs et al. 2013b). Other research, using virtual particles to model MPA connectivity along the KZN coast revealed overall strong southward MPA connectivity, driven by the Agulhas Current (Heye 2021). However, this author also observed modelled occurrences of a less frequent northward connection. This latter pattern was mainly influenced by the northward flow of the Natal Bight Coastal Counter Current, which is derived from the semi-permanent Durban Eddy in the southern KZN Bight. Notably, the Natal Bight Coastal Counter Current increased water retention within the uThukela MPA, but did not extend into the iSimangaliso MPA to create a northward MPA connection. Furthermore, a previous study on penaeid prawns within the uThukela MPA indicated northward flow of larvae from there towards the iSimangaliso MPA

(Forbes et al. 1999). The potential for northward flow from the uThukela MPA to northern KZN was also demonstrated by Guastella and Roberts (2016) using current drifters.

The high connectivity of slinger observed in this study is promoted by a pelagic larval stage, which results in the larvae being widely transported by inshore currents along the KZN coast (Hutchings et al. 2002). During spawning, slinger produces small pelagic eggs with oil droplets that provide extra buoyancy and therefore increased dispersal potential (Connell et al. 1999). Other studies have indicated that larval dispersal maintains connectivity in sparid species. These species all share similar life history traits such as residency and a pelagic larval phase. These include the slinger (Duncan et al. 2015), roman *Chrysoblephus laticeps* (Teske et al. 2010), the black musselcracker *Cymatoceps nasutus* (Murray et al. 2014) and white steenbras *Lithognathus lithognathus* (Bennett et al. 2017b).

The oceanographic regime on the east coast of South Africa is dominated by the strong southward-flowing AC (Lutjeharms 2006). This predominant southerly flow is occasionally disturbed by short northerly flow events, which can last for a few days, e.g., off Sodwana Bay in the iSimangaliso MPA (Morris 2009). This northerly flow is primarily driven by southerly winds, as well as the influence of occasional cyclonic mesoscale eddies. Spawning of slinger is suggested to occur during these northerly flow events with spawning activity taking place in resident shoals on the down-current outer edge of reefs in the iSimangaliso MPA (Garratt 1993). This spawning has not yet been observed visually, but changes in the behaviour and social structure and interactions of fish during the spawning season suggested that such spawning occurred (Garratt 1993). This timing of spawning is suggested to reduce predation of eggs/larvae by maximising dispersal and assisting in survival of pelagic larvae. These northerly flows may be entraining slinger larvae and preventing larvae from getting lost further offshore with the AC. Moreover, Roberts et al. (2016) investigated circulation of shelf waters in the KZN Bight and found a northward flow, derived from the semi-permanent Durban Eddy

in the southern KZN Bight (Guastella and Roberts 2016). This region could offer refuge from the strong southward flowing AC. In support of this, the central KZN region has been suggested to provide a suitable spawning and nursery area for other important KZN fish species (Hutchings et al. 2002).

The levels of connectivity observed in this study and Chapter 4 suggest that there is sufficient scope for supply of slinger larvae to sustain populations further south under current oceanographic conditions. Previous research suggested that slinger resources off KZN are sustained by 0-year olds from the north, especially from the St Lucia MPA, which makes up the southern portion of the iSimangaliso MPA, and from Mozambique (Punt et al. 1993). However, results from BayesAss in this study indicated that Richards Bay is an important key source of recruits. Given that Richards Bay is a heavily exploited area, with the highest number of commercial skiboats in KZN, as well as a large number of recreational skiboats operating from this area (Oceanographic Research Institute, unpublished. data), this is an important region to monitor. Chapter 3 indicated no changes in genetic diversity over a period of six years of exploitation, supporting the contention that slinger is optimally exploited, and the stock will continue building if the current level of harvesting is maintained (Maggs et al. 2017). Notably, though, these authors determined stock status as at 2015, and a follow-up assessment is required to determine current status. Increased fishing pressure on slinger, particularly in the Richards Bay region, could have detrimental effects for future sustainability of slinger. The imminent roll-out of plans for the small-scale fisheries sector, whereby new rights holders will enter the fishery, may well lead to greater boat-based fishing effort in the Richards Bay area because of the relative ease of launching in the sheltered port there compared to elsewhere in KZN.

In conclusion, this study could not assign slinger recruits to adults along the east coast, but population genetic analyses provided an idea of possible sources and larval dispersal patterns

in slinger. Results indicated mainly southward larval dispersal, which is a consequence of the prevailing southward flow inshore of the AC, with occasional northward flow of the AC entraining larvae allowing for northward dispersal. The Richards Bay area was suggested to be a key source of recruits in Chapter 4, contributing to sustaining populations further south, although other northern areas also contribute. This study, therefore, provides valuable information to elucidate the role of east coast MPAs and adjacent areas in slinger recruitment, which will support fisheries management as well as marine spatial planning.

Chapter 6: General Discussion

Marine Protected Areas (MPAs) are widely implemented for the conservation of biodiversity and fisheries management (Roberts et al. 2003, Gaines et al. 2010), as well as to support adaptation and resilience in the face of climate change (McLeod et al. 2009, Micheli et al. 2012, Carr et al. 2017). The benefits of MPAs have been observed in the higher abundances, biomass, larger sizes and more diverse target species, as well as the recovery of overexploited fisheries (Halpern 2003, Olds et al. 2012a, 2012b). MPAs also provide benefits beyond their boundaries to other MPAs and exploited areas, through larval dispersal or adult spillover (Halpern et al. 2010, Harrison et al. 2012). Connectivity between MPAs and exploited areas through the dispersal of individuals as larvae, juveniles or adults (Sale et al. 2005) is important as it promotes the persistence of populations and the recovery from disturbance (Botsford et al. 2003, Almany et al. 2009, McCook et al. 2009).

The benefits of MPAs on biodiversity in South Africa have been demonstrated by increases in the population density, biomass, size and diversity of their fauna and flora, e.g. in the De Hoop (Bennett and Attwood 1991, Attwood and Bennett 1995b); Tsitsikamma (Cowley et al. 2002); Pondoland (Maggs et al. 2013a, Mann et al. 2022) and iSimangaliso (Mann et al. 2016) MPAs. There are five large MPAs on the east coast of South Africa, comprising (from north to south) iSimangaliso, uThukela, Aliwal Shoal, Protea Banks and Pondoland (Figure 6.1). While these benefits have been shown within the boundaries of these MPAs [e.g. Pondoland (Maggs et al. 2013) and iSimangaliso (Mann et al. 2016)], less is known regarding whether these MPAs replenish adjacent areas through larval dispersal or adult spillover.

Therefore, in order to determine whether MPAs along the east coast are capable of this replenishment, the genetic connectivity of slinger sample sites collected within MPAs and from surrounding unprotected areas along the KZN coast was investigated. This would establish

whether these MPAs are connected, function as a network, and replenish themselves and adjacent exploited areas through larval dispersal and adult spillover.

This study used two genetic markers, microsatellites and high density single nucleotide polymorphisms (SNPs) to, firstly, determine variation in genetic diversity in slinger, a species of high commercial and recreational importance distributed along the east coast over a six-year exploitive period and secondly, to estimate genetic structure and gene flow in slinger among MPAs and adjacent unprotected areas along the east coast using SNPs; and, lastly, to attempt to assign slinger juveniles to potential parents in order to determine sources and where juveniles recruit to (sinks), using SNPs.

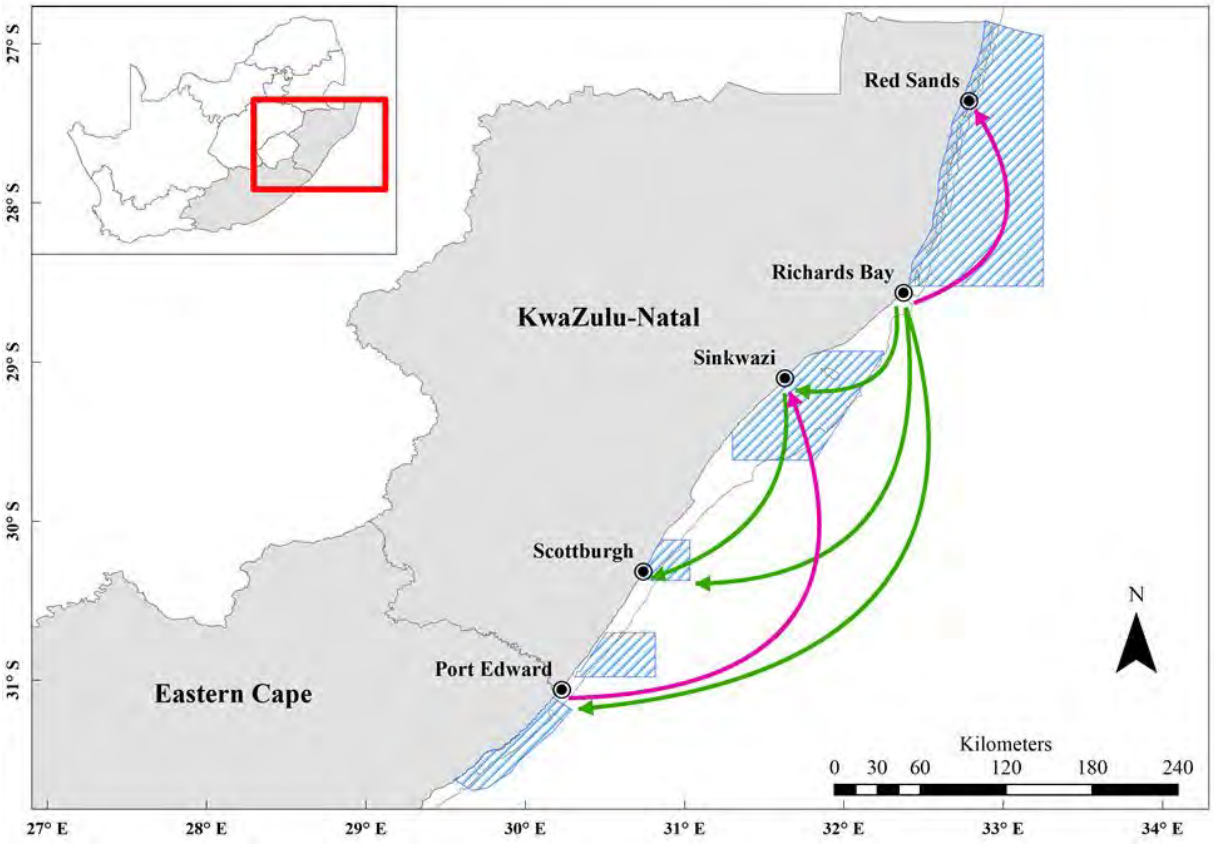

Figure 6.1: Map showing the main findings of gene flow of slinger populations among Marine Protected Areas (MPAs; iSimangaliso, uThukela, Aliwal Shoal, Protea Banks and Pondoland, denoted by blue striped blocks) and exploited areas (Richards Bay and Port Edward) along the east coast of South Africa. Green arrows indicate south-ward gene flow, with the pink arrow indicating north-ward gene flow

MPA connectivity

The main findings of this thesis indicated a lack of genetic structure among slinger sample sites along the east coast, implying high levels of gene flow among all locations, including among MPAs and adjacent unprotected areas (Chapter 4). As such, MPAs along the east coast are connected and function as a network, and can readily replenish or be replenished by other sites, whether these sites are within protected areas or not, through larval dispersal and adult spillover. Furthermore, multidirectional migration rates were observed between sites (Figure 6.1) along the coast, with most net migration rates being southward, probably influenced by the prevailing southward-flowing Agulhas Current (Chapter 4).

This lack of genetic structure has also been documented in several other marine teleosts found off the South African coast. These include the Cape stumpnose *Rhabdosargus holubi* (Oosthuizen 2006), spotted grunter *Pomadasys commersonnii* (Klopper 2005), dusky kob *Argyrosomus japonicus* (Mirimin et al. 2016), white steenbras *Lithognathus lithognathus* (Bennett et al. 2017b) and shallow water hake *Merluccuis capensis* (von der Heyden et al. 2007). Furthermore, Teske et al. (2010) found high connectivity of the roman *Chrysoblephus laticeps* among MPAs (Tsitsikamma and Bird Island) and exploited areas along the south coast of South Africa.

Similarly, studies of networks of MPAs worldwide have found high levels of genetic connectivity among MPAs and surrounding areas using various genetic markers. For example, Mzingirwa et al. (2019) found a lack of genetic differentiation in populations of the commercially-important sky emperor *Lethrinus mahsena* across MPAs and surrounding areas off coastal Kenya, indicating high levels of gene flow among sites. Using SNPs, a lack of genetic structure in the dusky parrotfish *Scarus niger* was observed among MPAs and unprotected areas (>500 km apart) in the Philippines (Stockwell et al. 2016). The saddled sea bream *Oblada melanura* showed patterns of connectivity among protected areas and unprotected areas in the Western Mediterranean Sea (Calò et al. 2016). A lack of genetic structure indicated high levels of connectivity in the two-banded sea bream *Diplodus vulgaris* within an MPA and the surrounding fished areas along 200 km of the Apulian Adriatic coast in the Adriatic Sea, and (Sahyoun et al. (2016) concluded that this high connectivity is maintaining local populations.

The marine environment can be remarkably genetically homogenous, indicated by numerous population genetic studies (Riginos et al. 2016, Selkoe et al. 2016), and evolving next generation sequencing permitting detection of subtle genetic differentiation has not substantially changed this picture (Liggins et al. 2019). However, genetic structure has been

observed in several fish studies, mainly due to restricted gene flow observed at small spatial scales caused by geographical or ecological barriers to dispersal or gene flow. Although Sahyoun et al. (2016) observed a lack of genetic structure of the two-banded seabream across a scale of at least 200 km (see above), with genetic divergences observed locally at two sites. These divergences were suggested to be either due to different source populations or from chaotic genetic patchiness occurring under temporal variation in reproductive success. Similarly, the saddled bream revealed subtle local genetic heterogeneity (Calò et al. 2016), which reflected the geography and the main oceanographic features (currents and barriers) of the study area. Furthermore, Murray et al. (2014) found no genetic structure of the black musselcracker *Cymatoceps nasutus* throughout most of its distribution in South Africa. However, moderate to substantial differentiation was found in individuals sampled furthest to the west, with differentiation attributed to a near-permanent thermal barrier (a cold-water ridge) along the south coast of the country.

There is, thus, a considerable body of evidence demonstrating that MPAs have the capacity to sustain populations in adjacent areas. Similarly, high connectivity found between sites in this study is probably maintaining the local supply of recruits to the slinger population. This also explains the lack of reduction in genetic diversity despite intense exploitation (see Chapter 3 and above). The constant mixing of alleles among sample sites is another means whereby genetic diversity remains constant, even during intense fishing pressure.

Larval dispersal

Most of the abovementioned studies showed high connectivity promoted by a pelagic larval phase and oceanographic currents within the region. Similarly, a pelagic larval phase and the southward-flowing Agulhas Current could promote the high connectivity found among slinger

sites in this study. While there is limited information available on the larval life history of slinger, it is believed to be similar to that of the confamilial santer *Cheimerius nufar* (Connell 2012). The lack of genetic structure and population patterns of slinger in this study are likely promoted by larvae being dispersed southwards with inshore currents along the east coast of South Africa. This also explains why very small juveniles are found in the Pondoland MPA further south (Mann et al. 2006), and conforms to the suspected life history of this species. Santer has a flexion stage of larval development which lasts for 21 days (Connell 2012); if larval slinger have a similar life history, then they could be transported widely assisted by the prevailing Agulhas Current. Slinger have an extended spawning period, peaking between August to October in KZN (Garratt 1985b). Spawning occurs in shoals on reefs from southern Mozambique to the northern KZN coast, producing small pelagic eggs containing oil droplets which provide extra buoyancy and increased dispersal potential (Connell 2012). It was initially suggested that larvae of slinger may be transported southwards with the Agulhas Current (Heydorn et al. 1978, Garratt 1993, Punt et al. 1993). Subsequent work on slinger and other reef-associated sparid species suggested that inshore currents were the driving force of dispersal of these fishes (Beckley 1993, Hutchings et al. 2002). These results are consistent with other studies on sparids for which larval dispersal was assisted inshore of the Agulhas Current, maintaining connectivity (Teske et al. 2010, Murray et al. 2014, Duncan et al. 2015). Connectivity through larval dispersal processes is, thus, highly promoted by oceanographic features within the region.

Adult spillover

The export of individuals through the movement of juveniles, sub-adults and adults is referred to as spillover (Gell and Roberts 2003), and is another mechanism that might explain the high

connectivity observed in this study. Gene flow was also inferred north-eastwards along the east coast, particularly from Port Edward and Richards Bay towards Red Sands (iSimangaliso MPA) (Chapter 4). This observation suggests adult movement and/or larval dispersal of slinger up the coast. The adult migration patterns are suggested to be spawning-related in this species, as maximum reproductive activity has only been observed off the northern KZN coast with virtually no spawning observed south of Durban (Garratt 1985b); the most recent available biological sampling of slinger (in 2010-2011) does not indicate that the situation has changed (ORI, unpublished. data). Slinger are mostly resident, but occasional long-distance northward movements from sites south of Durban have been observed in tagging studies (Maggs et al. 2013b). These authors described the greatest distance covered by a slinger as 1 059 km (582 days), from the Pondoland MPA to Quissico, Mozambique, nearly covering its entire distribution range. Another slinger covered a northward distance of 163 km in approximately 581 days from the Pondoland MPA to the KZN south coast. This movement pattern of subadult/adult slinger likely contributes towards the high genetic connectivity observed in this study. Similar migration patterns have also been observed in other sparids along the east coast of South Africa, for example, the black musselcracker (Murray et al. 2019), red steenbras *Petrus rupestris* (Brouwer 2002), white steenbras (Bennett et al. 2017a) and white musselcracker *Sparodon durbanensis* (Buxton and Clarke 1991). Similarly, genetic studies of highly-migratory fish species geelbek *Atractoscion aequidens* (Henriques et al. 2014) and shad *Pomatomus saltatrix* (Reid et al. 2016) on the east coast found lack of genetic structure, suggesting that the movement of fish contributes to the high connectivity found in these species.

Northward larval dispersal of slinger larvae could occur during reversals of inshore processes associated with the AC at times in central/northern KZN. This was evidenced by the northward movements of drifters found in a study by Roberts et al. (2016) which investigated the

circulation patterns in the central KZN Bight. These northerly flow events could also account for the northward dispersal suggested by the BayesAss results found in this study.

Shortcomings and future research

This was the first study to use SNPs to determine MPA connectivity in South Africa. Restriction Associated DNA sequencing identifies and genotypes hundreds to thousands of SNPs across the genome (Davey and Blaxter 2011, Andrews et al. 2016). As genome-wide markers, large numbers of SNPs can resolve more subtle patterns of connectivity, allowing for finer-scale insights into population structure and connectivity. Hence, this molecular marker has become the more preferred marker in both population genetics (Andrews et al. 2016) and parentage analysis (Flanagan and Jones 2019). Furthermore, accurate estimates of diversity and differentiation have been observed with this marker (Helyar et al. 2011, Fischer et al. 2017, Hodel et al. 2017). However, RADseq data are still prone to sources of sequencing and genotyping errors (Shendure and Hanlee 2008). These include allele dropout and null alleles, PCR duplicates and genotyping errors and variance in depth coverage among loci (details discussed in Andrews et al. (2016)). However, the RADseq technique used in this study (quaddRAD sequencing), removes all PCR duplicates during the filtering stage. A substantial reduction in the number of sample pools were lost during data filtering which could be the result of sequencing and genotyping errors. This is still a challenge in high-throughput sequencing technologies. These challenges are dealt with during the genotyping filtering stage, which produces reliable data sets for further downstream analysis. Furthermore, this study incorporated fairly temporally confined sampling, which strengthens this approach, allowing it to be focussed on that given time and space. Future studies should incorporate larger sampling sizes; this study sampled 100 individuals per sample site, to account for sample dropout during

sequencing and genotyping filtering. Furthermore, the differences observed in effective population size estimates between Chapters 3 and 5 are probably due to the two different data sets used in this study. Two markers were used in Chapter 3, microsatellites and SNPs, while different SNP filtering approaches were applied in these two chapters.

This study failed to assign juveniles to potential parents from adult sites. The primary reason for this was insufficient sampling relative to the effective population size, and again samples lost during sequencing and genotyping filtering. Future research should employ a much larger sampling of potential parents e.g. 200 individuals per sample site, to overcome loss of samples during genotype filtering, to increase the likelihood of reliably identifying parent-offspring pairs. Another drawback of parentage analysis is that it provides a snapshot of dispersal capabilities over just one generation, and may not capture temporal and spatial variability in dispersal patterns. Furthermore, coupling genetic approaches with biophysical models could help define larval dispersal patterns and provide additional insights to source-sink dynamics in the slinger population. Reconstruction of sibling groups (sibships) is another genetic approach which could be used to infer larval dispersal and connectivity patterns (Blouin 2003). This approach has been used in studies of family structure and reproductive output in natural populations. The combination of both parentage analysis and sibship reconstruction are recommended for future studies to provide a complete picture of dispersal dynamics, variability in reproductive contributions/sweepstakes and population connectivity of slinger sites.

Management implications

Excessive fishing led slinger to be overexploited by the early 1990s, indicated by both skewed sex ratios in favour of females and a decline in the mean size (Garratt 1985a, Winker et al. 2014, Maggs et al. 2017). More recent stock assessments indicate that the stock has recovered

with the application of reduced fishing effort (Maggs et al. 2017) and is thought to still be optimally exploited, as there has been no increase in commercial fishing effort since then. The high connectivity between MPAs (particularly iSimangaliso with its high abundance of spawning slinger), and the adjacent exploited areas, observed in this study, has likely supported the recovery of slinger after intense exploitation, with high connectivity known to promote persistence of populations and recovery from disturbance (Botsford et al. 2003, Almany et al. 2009, McCook et al. 2009).

Early research suggested that slinger sites further south are sustained by input of 0-year olds from the iSimangaliso MPA and southern Mozambique (Punt *et al.* 1993). The current study suggested the Richards Bay area as a key source of recruits, but it is likely that the iSimangaliso MPA also provides 0-year olds, which help sustain slinger populations further south. In the long term, the effects of high fishing effort in the Richards Bay area may manifest, particularly if effort there increases, and could have detrimental effects on the slinger population, as excessive exploitation in this area could affect the whole population. MPAs in this context do provide a reservoir for the slinger population, and the recent (2019) promulgation of new MPAs (uThukela and Protea Banks) and expansion of existing MPAs (Aliwal, iSimangaliso) will assist in mitigation - provided these MPAs are well managed. A recent review of effective governance and management of MPAs in South Africa, spanning a period from 1964-2023, revealed improvement in legislation and policies, MPA establishment, planning and design, and staff training and skills; however, there is still great need for improvement of enforcement and compliance in MPAs in South Africa (Kirkman et al. 2023). Some of these new east coast MPAs are yet to have management plans produced and implemented, and there are indications of poaching in established MPAs (Anon 2021), which is concerning for the ongoing sustainability of slinger.

In conclusion, this was the first study to provide an overview and understanding of genetic connectivity of slinger sample sites within MPAs and the surrounding areas along the east coast of South Africa. A lack of genetic structure in slinger sites between MPAs, inferring a single slinger population with high levels of genetic connectivity among MPAs themselves as well as among and between adjacent exploited areas along the east coast, reinforces the conclusion that the MPAs along the east coast are connected, form a network, and contribute to the local persistence of the slinger population.

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APPENDICES

Appendix 1: Demultiplexed library information on slinger pools, number of individuals, number of reads, percentage of PCR duplicates and reads retained are shown

Indexed pool identifier	Number of individuals	Number of reads	Percentage PCR duplicates	Reads retained
G1	12	1 733 881	7.31	1 607 121
G2	12	1 472 664	16.74	1 226 109
G3	12	3 396 234	5.79	3 199 590
G4	12	1 574 224	3.70	1 516 042
G5	12	1 069 923	8.54	978 534
G6	12	1 064 681	4.56	1 016 083
G7	12	3 577 581	18.21	2 926 208
G8	12	2 462 476	7.64	2 274 380
G9	12	1 721 305	10.10	1 547 446
G10	12	3 304 835	2.70	3 215 490
G11	12	5 380 852	1.29	5 311 641
G12	12	4 660 488	2.68	4 535 500

Appendix 2: For each slinger individual sample, locality of sample, barcode combination used, total number of raw filtered reads, number RAD-loci identified using the *denovo* pipeline of STACKS and their mean depth of coverage is shown

Pool	MiSeq	Sample	Locality	Inner adapter barcode	Truseq indexes	Reads	Retained reads	Depth of coverage
1	1	18RB72	Richards Bay2018	AACCCG-AGTCAT	D501 – D701	52 936	44 912	4.10
1	1	18RB82	Richards Bay2018	AACCCG-GATCGT	D501 – D701	309 000	272 053	6.14
1	1	18RB1	Richards Bay2018	AACCCG-GCATTG	D501 – D701	193 876	174 985	5.43
1	1	18RB75	Richards Bay2018	AACCCG-TTAATG	D501 – D701	233 266	205 331	5.82
1	1	18RB88	Richards Bay2018	AAGGGA-AGTCAT	D501 – D701	371 040	321 800	6.62
1	1	18RB52	Richards Bay2018	AAGGGA-GATCGT	D501 – D701	621 052	545 878	8.53
1	1	18RB97	Richards Bay2018	AAGGGA-GCATTG	D501 – D701	517 834	449 796	7.53
1	1	18RB94	Richards Bay2018	AAGGGA-TTAATG	D501 – D701	234 970	196 183	5.52
1	1	18RB12	Richards Bay2018	CAACTA-AGTCAT	D501 – D701	288 646	258 749	6.29
1	1	18RB98	Richards Bay2018	CAACTA-GATCGT	D501 – D701	68 770	58 346	4.20
1	1	18RB29	Richards Bay2018	CAACTA-GCATTG	D501 – D701	131 832	115 881	4.88
1	1	18RB16	Richards Bay2018	CAACTA-TTAATG	D501 – D701	121 998	105 070	4.39
2	2	18RB28	Richards Bay2018	AACCCG-AGTCAT	D501 – D702	193 236	153 238	5.03
2	2	18RB71	Richards Bay2018	AACCCG-GATCGT	D501 – D702	113700	77 139	4.82
2	2	18RB57	Richards Bay2018	AACCCG-GCATTG	D501 – D702	259700	217 001	5.77
2	2	18RB25	Richards Bay2018	AACCCG-TTAATG	D501 – D702	193720	154 713	5.55
2	2	18RB33	Richards Bay2018	AAGGGA-AGTCAT	D501 – D702	532274	419 885	6.83
2	2	18RB70	Richards Bay2018	AAGGGA-GATCGT	D501 – D702	260940	206 378	5.70
2	2	18RB89	Richards Bay2018	AAGGGA-GCATTG	D501 – D702	474268	371 313	6.78
2	2	18RB62	Richards Bay2018	AAGGGA-TTAATG	D501 – D702	81 472	65 848	4.22
2	2	18RB6	Richards Bay2018	CAACTA-AGTCAT	D501 – D702	88 796	73 153	4.10
2	2	18RB30	Richards Bay2018	CAACTA-GATCGT	D501 – D702	75 958	63 524	4.04
2	2	18RB34	Richards Bay2018	CAACTA-GCATTG	D501 – D702	84 536	72 523	4.00
2	2	18RB99	Richards Bay2018	CAACTA-TTAATG	D501 – D702	25 374	20 983	4.23
3	1	18RB54	Richards Bay 2018	AACCCG-AGTCAT	D501 – D703	489 602	454 810	7.54
3	1	18RB93	Richards Bay 2018	AACCCG-GATCGT	D501 – D703	225 234	209 688	5.92
3	1	18RB7	Richards Bay 2018	AACCCG-GCATTG	D501 – D703	397 352	369 930	4.10

3	1	18RB2	Richards Bay 2018	AACCCG-TTAATG	D501 – D703	247 352	232 537	5.65
3	1	18RB66	Richards Bay 2018	AAGGGA-AGTCAT	D501 – D703	879 940	806 540	10.63
3	1	18RB78	Richards Bay 2018	AAGGGA-GATCGT	D501 – D703	1 872 578	1 764 399	17.99
3	1	18RB53	Richards Bay 2018	AAGGGA-GCATTG	D501 – D703	113 913	106 713	4.36
3	1	18RB83	Richards Bay 2018	AAGGGA-TTAATG	D501 – D703	1 109 484	1 029 534	13.04
3	1	18RB40	Richards Bay 2018	CAACTA-AGTCAT	D501 – D703	156 448	139 773	4.89
3	1	18RB61	Richards Bay 2018	CAACTA-GATCGT	D501 – D703	207 788	193 155	4.97
3	1	18RB63	Richards Bay 2018	CAACTA-GCATTG	D501 – D703	235 158	220 483	5.33
3	1	18RB68	Richards Bay 2018	CAACTA-TTAATG	D501 – D703	313 008	300 098	5.84
4	2	12RB2	Richards Bay 2012	AACCCG-AGTCAT	D501 – D704	232 454	211 931	5.89
4	2	12RB8	Richards Bay 2012	AACCCG-GATCGT	D501 – D704	92 102	83 303	4.12
4	2	12RB9	Richards Bay 2012	AACCCG-GCATTG	D501 – D704	202 378	183 877	5.57
4	2	12RB10	Richards Bay 2012	AACCCG-TTAATG	D501 – D704	69 542	62 687	6.14
4	2	12RB11	Richards Bay 2012	AAGGGA-AGTCAT	D501 – D704	475 208	433 773	6.66
4	2	12RB16	Richards Bay 2012	AAGGGA-GATCGT	D501 – D704	420 338	370 358	6.39
4	2	12RB17	Richards Bay 2012	AAGGGA-GCATTG	D501 – D704	529 208	479 333	7.48
4	2	12RB33	Richards Bay 2012	AAGGGA-TTAATG	D501 – D704	379 950	336 896	6.36
4	2	12RB40	Richards Bay 2012	CAACTA-AGTCAT	D501 – D704	124 278	107 098	4.87
4	2	12RB41	Richards Bay 2012	CAACTA-GATCGT	D501 – D704	188 362	168 170	5.04
4	2	12RB51	Richards Bay 2012	CAACTA-GCATTG	D501 – D704	163 900	145 091	4.97
4	2	12RB55	Richards Bay 2012	CAACTA-TTAATG	D501 – D704	86 026	78 282	4.19
5	1	12RB58	Richards Bay 2012	AACCCG-AGTCAT	D502 – D701	169 492	145 877	4.60
5	1	12RB59	Richards Bay 2012	AACCCG-GATCGT	D502 – D701	104 640	86 051	4.18
5	1	12RB60	Richards Bay 2012	AACCCG-GCATTG	D502 – D701	68 638	56 152	3.98
5	1	12RB66	Richards Bay 2012	AACCCG-TTAATG	D502 – D701	134 204	112 452	4.45
5	1	12RB67	Richards Bay 2012	AAGGGA-AGTCAT	D502 – D701	241 294	205 526	4.85
5	1	12RB69	Richards Bay 2012	AAGGGA-GATCGT	D502 – D701	170 890	144 577	4.19
5	1	12RB70	Richards Bay 2012	AAGGGA-GCATTG	D502 – D701	273 894	231 926	5.18
5	1	12RB71	Richards Bay 2012	AAGGGA-TTAATG	D502 – D701	217 204	184 315	4.59
5	1	12RB72	Richards Bay 2012	CAACTA-AGTCAT	D502 – D701	113 056	96 878	4.33
5	1	12RB73	Richards Bay 2012	CAACTA-GATCGT	D502 – D701	137 374	117 053	4.40
5	1	12RB75	Richards Bay 2012	CAACTA-GCATTG	D502 – D701	148 746	125 898	4.57

5	1	12RB76	Richards Bay 2012	CAACTA-TTAATG	D502 – D701	137 118	116 697	4.44
6	2	12RB80	Richards Bay 2012	AACCCG-AGTCAT	D502 – D702	100 828	88 112	4.19
6	2	12RB82	Richards Bay 2012	AACCCG-GATCGT	D502 – D702	174 670	169 794	5.11
6	2	12RB83	Richards Bay 2012	AACCCG-GCATTG	D502 – D702	93 726	82 468	4.40
6	2	12RB84	Richards Bay 2012	AACCCG-TTAATG	D502 – D702	150 452	135 225	4.86
6	2	12RB85	Richards Bay 2012	AAGGGA-AGTCAT	D502 – D702	155 202	129 180	4.73
6	2	12RB86	Richards Bay 2012	AAGGGA-GATCGT	D502 – D702	257 672	235 624	5.61
6	2	12RB87	Richards Bay 2012	AAGGGA-GCATTG	D502 – D702	287 870	264 575	5.83
6	2	12RB63	Richards Bay 2012	AAGGGA-TTAATG	D502 – D702	221 854	205 052	5.21
6	2	12RB7	Richards Bay 2012	CAACTA-AGTCAT	D502 – D702	183 422	168 731	5.30
6	2	12RB35	Richards Bay 2012	CAACTA-GATCGT	D502 – D702	77 182	64 937	4.15
6	2	12RB47	Richards Bay 2012	CAACTA-GCATTG	D502 – D702	171 888	153 395	4.95
6	2	12RB56	Richards Bay 2012	CAACTA-TTAATG	D502 – D702	103 388	89 371	4.25
7	1	18PE63	Port Edward 2018	AACCCG-AGTCAT	D502 – D703	787 120	672 538	8.89
7	1	18PE62	Port Edward 2018	AACCCG-GATCGT	D502 – D703	331 742	232 914	5.69
7	1	18PE6	Port Edward 2018	AACCCG-GCATTG	D502 – D703	916 970	737 774	9.53
7	1	18PE58	Port Edward 2018	AACCCG-TTAATG	D502 – D703	355 384	279 528	6.06
7	1	18PE66	Port Edward 2018	AAGGGA-AGTCAT	D502 – D703	383 892	263 674	5.12
7	1	18PE81	Port Edward 2018	AAGGGA-GATCGT	D502 – D703	1 085 546	811 364	9.08
7	1	18PE3	Port Edward 2018	AAGGGA-GCATTG	D502 – D703	902 754	698 173	9.16
7	1	18PE37	Port Edward 2018	AAGGGA-TTAATG	D502 – D703	213 050	153 084	5.17
7	1	18PE41	Port Edward 2018	CAACTA-AGTCAT	D502 – D703	256 294	200 424	5.57
7	1	18PE68	Port Edward 2018	CAACTA-GATCGT	D502 – D703	184 638	143 484	4.66
7	1	18PE13	Port Edward 2018	CAACTA-GCATTG	D502 – D703	89 878	65 633	4.40
7	1	18PE77	Port Edward 2018	CAACTA-TTAATG	D502 – D703	155 112	119 511	4.49
8	2	18PE74	Port Edward 2018	AACCCG-AGTCAT	D502 – D704	627 608	578 821	9.35
8	2	18PE46	Port Edward 2018	AACCCG-GATCGT	D502 – D704	388 100	350 135	7.33
8	2	18PE88	Port Edward 2018	AACCCG-GCATTG	D502 – D704	456 640	406 189	7.71
8	2	18PE71	Port Edward 2018	AACCCG-TTAATG	D502 – D704	220 686	196 044	6.17
8	2	18PE42	Port Edward 2018	AAGGGA-AGTCAT	D502 – D704	443 192	396 432	7.73
8	2	18PE56	Port Edward 2018	AAGGGA-GATCGT	D502 – D704	728 850	632 453	8.90

8	2	18PE14	Port Edward 2018	AAGGGA-GCATTG	D502 – D704	851 438	765 463	9.86
8	2	18PE90	Port Edward 2018	AAGGGA-TTAATG	D502 – D704	129 844	110 512	5.20
8	2	18PE65	Port Edward 2018	CAACTA-AGTCAT	D502 – D704	199 402	178 950	5.56
8	2	18PE7	Port Edward 2018	CAACTA-GATCGT	D502 – D704	40 154	35 124	4.38
8	2	18PE8	Port Edward 2018	CAACTA-GCATTG	D502 – D704	226 164	205 517	6.09
8	2	18PE99	Port Edward 2018	CAACTA-TTAATG	D502 – D704	125 810	114 367	4.54
9	1	18PE94	Port Edward 2018	AACCCG-AGTCAT	D503 – D701	182 108	150 129	5.40
9	1	18PE27	Port Edward 2018	AACCCG-GATCGT	D503 – D701	391 902	350 814	7.21
9	1	18PE5	Port Edward 2018	AACCCG-GCATTG	D503 – D701	163 144	141 788	5.76
9	1	18PE100	Port Edward 2018	AACCCG-TTAATG	D503 – D701	388 360	345 642	7.40
9	1	18PE36	Port Edward 2018	AAGGGA-AGTCAT	D503 – D701	292 264	257 124	6.31
9	1	18PE26	Port Edward 2018	AAGGGA-GATCGT	D503 – D701	164 110	132 172	4.95
9	1	18PE45	Port Edward 2018	AAGGGA-GCATTG	D503 – D701	320 782	269 704	7.06
9	1	18PE83	Port Edward 2018	AAGGGA-TTAATG	D503 – D701	266 058	224 955	5.72
9	1	18PE64	Port Edward 2018	CAACTA-AGTCAT	D503 – D701	154 256	131 787	4.89
9	1	18PE98	Port Edward 2018	CAACTA-GATCGT	D503 – D701	183 850	156 279	5.47
9	1	18PE70	Port Edward 2018	CAACTA-GCATTG	D503 – D701	221 224	192 850	5.62
9	1	18PE33	Port Edward 2018	CAACTA-TTAATG	D503 – D701	285 964	251 210	6.13
10	2	12SB7	Shelley Beach 2012	AACCCG-AGTCAT	D503 – D702	525 998	477 684	7.98
10	2	12SB19	Shelley Beach 2012	AACCCG-GATCGT	D503 – D702	439 872	393 697	7.22
10	2	12SB28	Shelley Beach 2012	AACCCG-GCATTG	D503 – D702	533 320	484 259	8.07
10	2	12SB35	Shelley Beach 2012	AACCCG-TTAATG	D503 – D702	428 250	385 595	7.05
10	2	12SB38	Shelley Beach 2012	AAGGGA-AGTCAT	D503 – D702	817 182	732 136	9.30
10	2	12SB39	Shelley Beach 2012	AAGGGA-GATCGT	D503 – D702	750 366	677 833	9.45
10	2	12SB49	Shelley Beach 2012	AAGGGA-GCATTG	D503 – D702	863 066	782 320	9.92
10	2	12SB72	Shelley Beach 2012	AAGGGA-TTAATG	D503 – D702	734 508	611 041	9.22
10	2	12SB73	Shelley Beach 2012	CAACTA-AGTCAT	D503 – D702	252 348	226 889	5.73
10	2	12SB74	Shelley Beach 2012	CAACTA-GATCGT	D503 – D702	370 202	336 867	6.74
10	2	12SB76	Shelley Beach 2012	CAACTA-GCATTG	D503 – D702	310 934	280 988	6.11
10	2	12SB80	Shelley Beach 2012	CAACTA-TTAATG	D503 – D702	258 310	238 894	5.72
11	1	12SB83	Shelley Beach 2012	AACCCG-AGTCAT	D503 – D703	612 710	576 495	9.23

11	1	12SB85	Shelley Beach 2012	AACCCG-GATCGT	D503 – D703	43 152	41 010	3.81
11	1	12SB90	Shelley Beach 2012	AACCCG-GCATTG	D503 – D703	398 038	358 651	5.98
11	1	12SB91	Shelley Beach 2012	AACCCG-TTAATG	D503 – D703	578 136	535 845	8.27
11	1	12SB107	Shelley Beach 2012	AAGGGA-AGTCAT	D503 – D703	1 891 944	1 809 797	19.00
11	1	12SB108	Shelley Beach 2012	AAGGGA-GATCGT	D503 – D703	1 413 056	1 352 248	15.41
11	1	12SB109	Shelley Beach 2012	AAGGGA-GCATTG	D503 – D703	1 626 366	1 558 079	17.07
11	1	12SB110	Shelley Beach 2012	AAGGGA-TTAATG	D503 – D703	1 421 562	1 365 834	15.48
11	1	12SB114	Shelley Beach 2012	CAACTA-AGTCAT	D503 – D703	250 044	240 315	5.88
11	1	12SB117	Shelley Beach 2012	CAACTA-GATCGT	D503 – D703	850 752	816 894	11.61
11	1	12SB118	Shelley Beach 2012	CAACTA-GCATTG	D503 – D703	739 828	710 028	10.83
11	1	12SB119	Shelley Beach 2012	CAACTA-TTAATG	D503 – D703	567 568	542 811	9.46
12	2	12SB121	Shelley Beach 2012	AACCCG-AGTCAT	D503 – D704	1 643 896	1 548 166	17.66
12	2	12SB122	Shelley Beach 2012	AACCCG-GATCGT	D503 – D704	1 751 978	1 642 770	17.92
12	2	12SB123	Shelley Beach 2012	AACCCG-GCATTG	D503 – D704	510 390	460 806	8.73
12	2	12SB124	Shelley Beach 2012	AACCCG-TTAATG	D503 – D704	441 138	409 723	6.83
12	2	12SB125	Shelley Beach 2012	AAGGGA-AGTCAT	D503 – D704	338 240	294 696	7.02
12	2	12SB126	Shelley Beach 2012	AAGGGA-GATCGT	D503 – D704	381 478	321 967	6.73
12	2	12SB127	Shelley Beach 2012	AAGGGA-GCATTG	D503 – D704	817 686	751 885	10.48
12	2	12SB137	Shelley Beach 2012	AAGGGA-TTAATG	D503 – D704	697 394	601 304	9.14
12	2	12SB139	Shelley Beach 2012	CAACTA-AGTCAT	D503 – D704	407 476	369 476	7.49
12	2	12SB142	Shelley Beach 2012	CAACTA-GATCGT	D503 – D704	683 380	638 055	9.77
12	2	12SB143	Shelley Beach 2012	CAACTA-GCATTG	D503 – D704	836 690	781 644	11.25
12	2	12SB144	Shelley Beach 2012	CAACTA-TTAATG	D503 – D704	382 230	352 442	7.32

Appendix 3: Demultiplexed library information on slinger pools, number of individuals, number of reads, percentage of PCR duplicates and reads retained are shown. Highlighted in grey indicate pools with lowest number of reads

Indexed pool identifier	Pools	Number of individuals	Number of reads	Percentage PCR duplicates	Reads retained
S131	1	12	52 758 172	4.64	50 307 650
S132	2	12	771 261	2.82	749 533
S133	3	12	329 229	1.74	323 506
S134	4	12	4 537 401	1.80	4 455 860
S135	5	12	5 036 672	1.15	4 978 704
S136	6	12	30 963 875	3.78	29 792 352
S137	7	12	4 281 555	4.28	4 098 184
S138	8	12	100 716 505	5.38	95 299 208
S139	9	12	9 641 136	2.82	9 368 928
S140	10	12	32 207 150	3.23	31 167 037
S141	11	12	17 365 535	3.60	16 739 729
S142	12	12	28 019 937	3.62	27 005 609
S143	13	12	38 105 043	4.30	36 468 214
S144	14	12	42 786 409	4.19	40 993 653
S145	15	12	89 667 184	6.88	83 495 467
S146	16	12	17 405 132	1.11	17 212 781
S147	17	12	18 284 390	16.16	15 329 359
S148	18	12	3 738 375	16.76	3 111 935
S149	19	12	30 462 002	20.82	24 120 740
S150	20	12	27 810 846	17.51	22 942 267
S151	21	12	30 859 055	12.74	26 928 753
S152	22	12	34 193 224	4.39	36 692 702
S153	23	12	36 734 734	20.59	29 169 549
S154	24	12	17 666 141	16.84	14 691 814
S159	25	12	28 462 296	13.58	24 595 915
S161	26	12	13 038 586	20.87	10 317 791
S162	26	12	27 239 717	15.54	23 007 935

Appendix 4: For each individual slinger sample, locality of sample, barcode combination used, total number of raw and filtered reads, number of RAD-loci identified using the *denovo* pipeline of STACKS and their mean depth of coverage are shown

Pool	HiSeq	Sample	Locality	Inner adapter barcode	Truseq indexes	Reads	Retained reads	Depth of coverage
	B	ROB1	Scottburgh	AACCCG-AGTCAT	D501 – D701	10 154 520	7 759 868	22.92
1	B	ROB2	Scottburgh	AACCCG-GATCGT	D501 – D701	8 965 342	7 427 807	24.26
1	B	ROB3	Scottburgh	AACCCG-GCATTG	D501 – D701	3 490 658	2 800 466	
1	B	ROB4	Scottburgh	AACCCG-TTAATG	D501 – D701	6 390 090	5 680 945	22.81
1	B	ROB5	Scottburgh	AAGGGA-AGTCAT	D501 – D701	5 698 962	5 393 266	23.19
1	B	ROB6	Scottburgh	AAGGGA-GATCGT	D501 – D701	19 077 078	18 823 750	30.85
1	B	ROB7	Scottburgh	AAGGGA-GCATTG	D501 – D701	19 969 076	19 764 166	30.65
1	B	ROB8	Scottburgh	AAGGGA-TTAATG	D501 – D701	6 391 866	6 328 406	25.19
1	B	ROB9	Scottburgh	CAACTA-AGTCAT	D501 – D701	5 350 788	5 068 805	22.13
1	B	ROB10	Scottburgh	CAACTA-GATCGT	D501 – D701	3 221 688	3 187 736	14.63
1	B	ROB11	Scottburgh	CAACTA-GCATTG	D501 – D701	4 071 762	3 996 569	19.85
1	B	ROB12	Scottburgh	CAACTA-TTAATG	D501 – D701	5 071 230	5 031 701	23.09
4	B	ROB38	Scottburgh	AACCCG-AGTCAT	D501 – D704	1 858 456	1 344 397	
4	B	ROB39	Scottburgh	AACCCG-GATCGT	D501 – D704	790 664	610 210	
4	B	ROB40	Scottburgh	AACCCG-GCATTG	D501 – D704	610 930	564 149	
4	B	ROB42	Scottburgh	AACCCG-TTAATG	D501 – D704	937 522	853 365	
4	B	ROB43	Scottburgh	AAGGGA-AGTCAT	D501 – D704	906 514	854 990	
4	B	ROB44	Scottburgh	AAGGGA-GATCGT	D501 – D704	41 323	39 939	
4	B	ROB45	Scottburgh	AAGGGA-GCATTG	D501 – D704	660 174	647 560	
4	B	ROB46	Scottburgh	AAGGGA-TTAATG	D501 – D704	261 046	257 138	
4	B	ROB47	Scottburgh	CAACTA-AGTCAT	D501 – D704	894 318	859 451	
4	B	ROB48	Scottburgh	CAACTA-GATCGT	D501 – D704	273 944	270 436	
4	B	ROB49	Scottburgh	CAACTA-GCATTG	D501 – D704	881 882	869 504	
4	B	ROB50	Scottburgh	CAACTA-TTAATG	D501 – D704	502 318	495 468	
5	B	ROB51	Scottburgh	AACCCG-AGTCAT	D502 – D701	2 245 088	1 346 349	
5	B	ROB52	Scottburgh	AACCCG-GATCGT	D502 – D701	1 610 442	1 057 930	
5	B	Z1	Sinkwazi	AACCCG-GCATTG	D502 – D701	527 976	379 550	

5	B	Z2	Sinkwazi	AACCCG-TTAATG	D502 – D701	771 626	638 559	
5	B	Z3	Sinkwazi	AAGGGA-AGTCAT	D502 – D701	898 892	785 581	
5	B	Z5	Sinkwazi	AAGGGA-GATCGT	D502 – D701	1 039 318	1 006 203	
5	B	Z6	Sinkwazi	AAGGGA-GCATTG	D502 – D701	1 070 992	1 049 749	
5	B	Z7	Sinkwazi	AAGGGA-TTAATG	D502 – D701	557 320	550 949	
5	B	Z8	Sinkwazi	CAACTA-AGTCAT	D502 – D701	244 818	210 771	
5	B	Z9	Sinkwazi	CAACTA-GATCGT	D502 – D701	146 284	141 179	
5	B	Z10	Sinkwazi	CAACTA-GCATTG	D502 – D701	182 206	177 548	
5	B	Z11	Sinkwazi	CAACTA-TTAATG	D502 – D701	181 786	179 455	
6	B	Z12	Sinkwazi	AACCCG-AGTCAT	D502 – D702	6 641 598	5 067 382	18.59
6	B	Z13	Sinkwazi	AACCCG-GATCGT	D502 – D702	6 755 434	5 547 078	20.78
6	B	Z14	Sinkwazi	AACCCG-GCATTG	D502 – D702	5 781 114	5 382 447	22.51
6	B	Z15	Sinkwazi	AACCCG-TTAATG	D502 – D702	2 637 610	2 453 309	15.27
6	B	Z16	Sinkwazi	AAGGGA-AGTCAT	D502 – D702	8 731 436	8 515 955	27.05
6	B	Z17	Sinkwazi	AAGGGA-GATCGT	D502 – D702	6 881 218	6 753 397	25.40
6	B	Z18	Sinkwazi	AAGGGA-GCATTG	D502 – D702	1 975 882	1 954 433	12.80
6	B	Z19	Sinkwazi	AAGGGA-TTAATG	D502 – D702	2 761 226	2 729 746	16.59
6	B	Z20	Sinkwazi	CAACTA-AGTCAT	D502 – D702	1 139 334	1 121 098	9.55
6	B	Z21	Sinkwazi	CAACTA-GATCGT	D502 – D702	5 884 946	5 807 193	23.33
6	B	Z22	Sinkwazi	CAACTA-GCATTG	D502 – D702	2 810 100	2 772 828	18.06
6	B	Z23	Sinkwazi	CAACTA-TTAATG	D502 – D702	6 021 454	5 977 883	23.93
7	B	Z24	Sinkwazi	AACCCG-AGTCAT	D502 – D703	932 560	748 092	
7	B	Z25	Sinkwazi	AACCCG-GATCGT	D502 – D703	609 970	504 847	
7	B	Z26	Sinkwazi	AACCCG-GCATTG	D502 – D703	413 200	392 322	
7	B	Z27	Sinkwazi	AACCCG-TTAATG	D502 – D703	666 974	640 954	
7	B	Z28	Sinkwazi	AAGGGA-AGTCAT	D502 – D703	878 830	859 513	
7	B	Z29	Sinkwazi	AAGGGA-GATCGT	D502 – D703	992 554	974 132	
7	B	Z30	Sinkwazi	AAGGGA-GCATTG	D502 – D703	723 090	716 665	
7	B	Z31	Sinkwazi	AAGGGA-TTAATG	D502 – D703	832 022	823 523	
7	B	Z32	Sinkwazi	CAACTA-AGTCAT	D502 – D703	429 456	411 694	
7	B	Z33	Sinkwazi	CAACTA-GATCGT	D502 – D703	362 344	357 075	
7	B	Z34	Sinkwazi	CAACTA-GCATTG	D502 – D703	630 332	624 058	

7	B	Z35	Sinkwazi	CAACTA-TTAATG	D502 – D703	524 568	521 074	
			Sinkwazi					
8	B	Z36	Sinkwazi	AACCCG-AGTCAT	D502 – D704	13 069 850	11 480 479	27.56
8	B	Z37	Sinkwazi	AACCCG-GATCGT	D502 – D704	13 522 538	12 586 512	28.58
8	B	Z38	Sinkwazi	AACCCG-GCATTG	D502 – D704	9 485 814	9 273 479	25.74
8	B	Z39	Sinkwazi	AACCCG-TTAATG	D502 – D704	8 924 810	8 680 816	25.73
8	B	Z40	Sinkwazi	AAGGGA-AGTCAT	D502 – D704	24 102 966	23 716 787	32.76
8	B	Z41	Sinkwazi	AAGGGA-GATCGT	D502 – D704	25 324 928	25 073 865	32.67
8	B	Z42	Sinkwazi	AAGGGA-GCATTG	D502 – D704	23 553 688	23 359 662	31.55
8	B	Z43	Sinkwazi	AAGGGA-TTAATG	D502 – D704	15 771 446	15 626 596	30.24
8	B	Z44	Sinkwazi	CAACTA-AGTCAT	D502 – D704	13 024 522	12 835 031	28.29
8	B	Z45	Sinkwazi	CAACTA-GATCGT	D502 – D704	17 572 278	17 389 308	30.13
8	B	Z46	Sinkwazi	CAACTA-GCATTG	D502 – D704	9 642 730	9 558 891	26.49
8	B	Z47	Sinkwazi	CAACTA-TTAATG	D502 – D704	12 801 516	12 714 939	29.04
			Sinkwazi					
9	B	Z48	Sinkwazi	AACCCG-AGTCAT	D503 – D701	2 303 434	1 762 831	
9	B	Z49	Sinkwazi	AACCCG-GATCGT	D503 – D701	1 828 738	1 355 569	
9	B	Z50	Sinkwazi	AACCCG-GCATTG	D503 – D701	1 466 820	1 303 673	
9	B	Z51	Sinkwazi	AACCCG-TTAATG	D503 – D701	2 004 422	1 916 279	
9	B	PE61	Port Edward	AAGGGA-AGTCAT	D503 – D701	1 811 242	1 739 765	
9	B	PE74	Port Edward	AAGGGA-GATCGT	D503 – D701	2 225 814	2 184 915	
9	B	PE46	Port Edward	AAGGGA-GCATTG	D503 – D701	1 662 734	1 637 650	
9	B	PE88	Port Edward	AAGGGA-TTAATG	D503 – D701	1 945 986	1 901 068	
9	B	PE71	Port Edward	CAACTA-AGTCAT	D503 – D701	864 192	825 336	
9	B	PE42	Port Edward	CAACTA-GATCGT	D503 – D701	651 820	636 446	
9	B	PE56	Port Edward	CAACTA-GCATTG	D503 – D701	1 200 214	1 154 910	
9	B	PE93	Port Edward	CAACTA-TTAATG	D503 – D701	211 232	208 710	
10	B	PE14	Port Edward	AACCCG-AGTCAT	D503 – D702	8 838 388	6 337 226	20.03
10	B	PE60	Port Edward	AACCCG-GATCGT	D503 – D702	4 245 356	3 439 738	
10	B	PE63	Port Edward	AACCCG-GCATTG	D503 – D702	7 806 892	7 174 757	25.53
10	B	PE62	Port Edward	AACCCG-TTAATG	D503 – D702	3 043 404	2 749 309	17.89
10	B	PE6	Port Edward	AAGGGA-AGTCAT	D503 – D702	12 948 230	12 549 581	31.16
10	B	PE58	Port Edward	AAGGGA-GATCGT	D503 – D702	7 651 582	7 454 940	27.55

10	B	PE66	Port Edward	AAGGGA-GCATTG	D503 – D702	2 696 058	2 642 387	
10	B	PE81	Port Edward	AAGGGA-TTAATG	D503 – D702	6 656 210	6 547 685	24.57
10	B	PE3	Port Edward	CAACTA-AGTCAT	D503 – D702	2 784 186	2 683 792	14.17
10	B	PE37	Port Edward	CAACTA-GATCGT	D503 – D702	1 087 696	1 068 581	10.31
10	B	PE41	Port Edward	CAACTA-GCATTG	D503 – D702	1 738 724	1 717 401	12.72
10	B	PE68	Port Edward	CAACTA-TTAATG	D503 – D702	899 272	893 690	7.7
11	B	PE13	Port Edward	AACCCG-AGTCAT	D503 – D703	1 721 910	1 414 553	13.17
11	B	PE22	Port Edward	AACCCG-GATCGT	D503 – D703	1 525 412	1 282 792	11.18
11	B	PE25	Port Edward	AACCCG-GCATTG	D503 – D703	1 372 612	1 210 903	10.93
11	B	PE94	Port Edward	AACCCG-TTAATG	D503 – D703	1 521 610	1 437 477	15.99
11	B	PE77	Port Edward	AAGGGA-AGTCAT	D503 – D703	4 651 628	4 503 447	22.18
11	B	PE90	Port Edward	AAGGGA-GATCGT	D503 – D703	3 176 368	3 095 979	23.65
11	B	PE65	Port Edward	AAGGGA-GCATTG	D503 – D703	6 955 116	6 841 876	29.44
11	B	PE7	Port Edward	AAGGGA-TTAATG	D503 – D703	687 892	621 504	12.75
11	B	PE8	Port Edward	CAACTA-AGTCAT	D503 – D703	2 634 510	2 545 107	18.96
11	B	PE99	Port Edward	CAACTA-GATCGT	D503 – D703	1 875 620	1 834 526	12.67
11	B	PE27	Port Edward	CAACTA-GCATTG	D503 – D703	4 452 566	4 377 715	22.36
11	B	PE5	Port Edward	CAACTA-TTAATG	D503 – D703	2 072 380	2 060 671	18.09
12	B	PE72	Port Edward	AACCCG-AGTCAT	D503 – D704	2 855 320	2 149 141	12.91
12	B	PE12	Port Edward	AACCCG-GATCGT	D503 – D704	4 052 450	2 714 387	15.84
12	B	PE55	Port Edward	AACCCG-GCATTG	D503 – D704	2 868 652	2 072 058	8.62
12	B	PE51	Port Edward	AACCCG-TTAATG	D503 – D704	2 307 800	1 955 927	12.11
12	B	PE100	Port Edward	AAGGGA-AGTCAT	D503 – D704	10 686 936	10 477 624	27.30
12	B	PE26	Port Edward	AAGGGA-GATCGT	D503 – D704	1 607 390	1 558 366	12.72
12	B	PE64	Port Edward	AAGGGA-GCATTG	D503 – D704	4 988 832	4 892 259	19.49
12	B	PE98	Port Edward	AAGGGA-TTAATG	D503 – D704	5 906 276	5 851 354	24.35
12	B	PE70	Port Edward	CAACTA-AGTCAT	D503 – D704	2 492 54	2 376 335	12.42
12	B	PE33	Port Edward	CAACTA-GATCGT	D503 – D704	4 283 962	4 182 235	19.33
12	B	PE28	Port Edward	CAACTA-GCATTG	D503 – D704	1 652 318	1 587 823	8.22
12	B	PE50	Port Edward	CAACTA-TTAATG	D503 – D704	8 777 006	8 695 752	25.83
13	B	RB72	Richards Bay	AACCCG-AGTCAT	D504 – D701	2 398 060	1 964 904	8.71

13	B	RB82	Richards Bay	AACCCG-GATCGT	D504 – D701	10 211 769	8 474 877	22.18
13	B	RB1	Richards Bay	AACCCG-GCATTG	D504 – D701	5 965 166	5 515 833	19.44
13	B	RB75	Richards Bay	AACCCG-TTAATG	D504 – D701	7 144 126	6 761 048	21.42
13	B	RB88	Richards Bay	AAGGGA-AGTCAT	D504 – D701	7 228 210	6 991 058	22.36
13	B	RB52	Richards Bay	AAGGGA-GATCGT	D504 – D701	11 811 784	11 644 234	27.22
13	B	RB97	Richards Bay	AAGGGA-GCATTG	D504 – D701	10 807 256	10 679 834	26.39
13	B	RB94	Richards Bay	AAGGGA-TTAATG	D504 – D701	6 142 624	6 088 296	22.16
13	B	RB12	Richards Bay	CAACTA-AGTCAT	D504 – D701	3 468 918	3 347 774	15.01
13	B	RB98	Richards Bay	CAACTA-GATCGT	D504 – D702	1 565 826	1 592 214	8.64
13	B	RB29	Richards Bay	CAACTA-GCATTG	D504 – D701	2 258 844	2 216 789	11.18
13	B	RB16	Richards Bay	CAACTA-TTAATG	D504 – D701	2 086 518	2 061 366	11.57
14	B	RB28	Richards Bay	AACCCG-AGTCAT	D504 – D702	9 805 170	7 116 794	19.39
14	B	RB71	Richards Bay	AACCCG-GATCGT	D504 – D702	6 151 218	4 666 924	
14	B	RB57	Richards Bay	AACCCG-GCATTG	D504 – D702	11 682 498	10 325 251	26.01
14	B	RB25	Richards Bay	AACCCG-TTAATG	D504 – D702	6 444 978	5 668 282	21.56
14	B	RB33	Richards Bay	AAGGGA-AGTCAT	D504 – D702	18 310 580	17 578 175	30.24
14	B	RB70	Richards Bay	AAGGGA-GATCGT	D504 – D702	9 325 436	9 098 222	26.03
14	B	RB89	Richards Bay	AAGGGA-GCATTG	D504 – D702	12 177 374	11 997 972	27.54
14	B	RB5	Richards Bay	AAGGGA-TTAATG	D504 – D702	304 548	297 737	5.8
14	B	RB74	Richards Bay	CAACTA-AGTCAT	D504 – D702	906 862	803 739	7.33
14	B	RB26	Richards Bay	CAACTA-GATCGT	D504 – D702	544 174	524 188	7.07
14	B	RB84	Richards Bay	CAACTA-GCATTG	D504 – D702	2 788 808	2 770 785	11.51
14	B	RB62	Richards Bay	CAACTA-TTAATG	D504 – D702	1 130 874	1 122 065	7.3
15	B	RB95	Richards Bay	AACCCG-AGTCAT	D504 – D703	18 975 430	12 204 549	14.58
15	B	RB6	Richards Bay	AACCCG-GATCGT	D504 – D703	19 342 512	11 728 629	11.76
15	B	RB30	Richards Bay	AACCCG-GCATTG	D504 – D703	14 805 798	13 125 506	23.82
15	B	RB34	Richards Bay	AACCCG-TTAATG	D504 – D703	10 761 410	9 219 876	19.49
15	B	RB14	Richards Bay	AAGGGA-AGTCAT	D504 – D703	14 659 296	13 572 362	25.3
15	B	RB99	Richards Bay	AAGGGA-GATCGT	D504 – D703	3 834 504	3 638 513	12.94
15	B	RB9	Richards Bay	AAGGGA-GCATTG	D504 – D703	1 169 258	1 052 461	7.17
15	B	RB54	Richards Bay	AAGGGA-TTAATG	D504 – D703	47 244 400	46 596 509	
15	B	RB93	Richards Bay	CAACTA-AGTCAT	D504 – D703	5 346 724	4 699 824	13.61

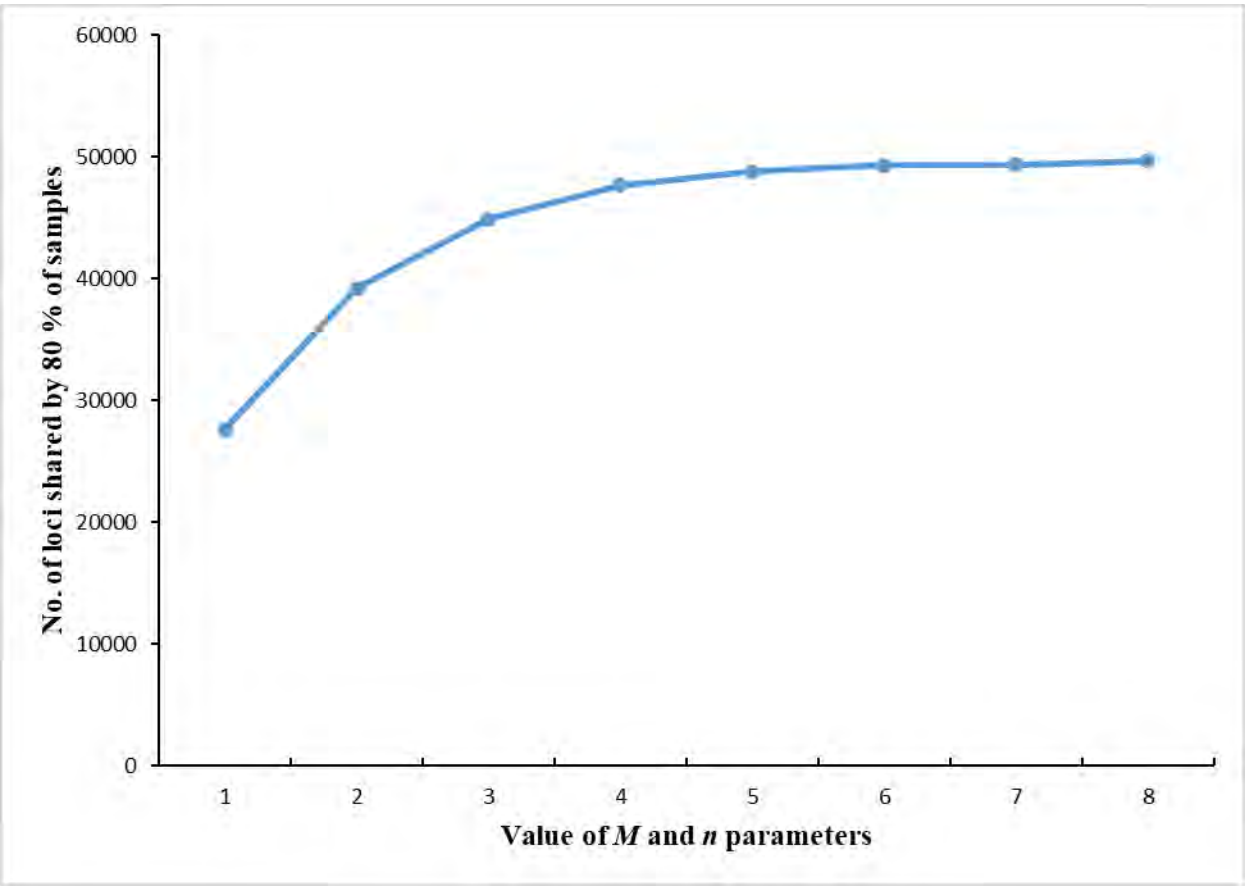
15	B	RB7	Richards Bay	CAACTA-GATCGT	D504 – D703	17 304 778	16 948 594	28.02
15	B	RB2	Richards Bay	CAACTA-GCATTG	D504 – D703	2 262 894	2 234 450	8.25
15	B	RB66	Richards Bay	CAACTA-TTAATG	D504 – D703	4 504 242	4 427 735	12.67
16	B	RB96	Richards Bay	AACCCG-AGTCAT	D504 – D704	3 294 960	1 735 171	4.84
16	B	RB78	Richards Bay	AACCCG-GATCGT	D504 – D704	15 892 708	8 710 164	7.24
16	B	RB77	Richards Bay	AACCCG-GCATTG	D504 – D704	968 636	549 188	4.9
16	B	RB53	Richards Bay	AACCCG-TTAATG	D504 – D704	1 288 944	912 503	4.64
16	B	RB83	Richards Bay	AAGGGA-AGTCAT	D504 – D704	4 092 550	3 141 167	8.88
16	B	RB40	Richards Bay	AAGGGA-GATCGT	D504 – D704	1 076 106	943 853	5.63
16	B	RB48	Richards Bay	AAGGGA-GCATTG	D504 – D704	369 826	295 620	5.45
16	B	RB61	Richards Bay	AAGGGA-TTAATG	D504 – D704	845 438	811 306	5.40
16	B	RB63	Richards Bay	CAACTA-AGTCAT	D504 – D704	972 562	716 705	4.91
16	B	RB68	Richards Bay	CAACTA-GATCGT	D504 – D704	1 682 600	1 604 312	6.66
16	B	RB42	Richards Bay	CAACTA-GCATTG	D504 – D704	666 972	628 099	4.60
16	B	RB32	Richards Bay	CAACTA-TTAATG	D504 – D704	971 502	940 441	5.76
17	C	RB85	Richards Bay	AACCCG-AGTCAT	D501 – D701	2 916 592	2 474 795	8.83
17	C	RB45	Richards Bay	AACCCG-GATCGT	D501 – D701	2 526 300	2 211 318	8.27
17	C	RB79	Richards Bay	AACCCG-GCATTG	D501 – D701	2 927 436	2 812 745	
17	C	RB76	Richards Bay	AACCCG-TTAATG	D501 – D701	2 416 490	2 369 244	
17	C	RB49	Richards Bay	AAGGGA-AGTCAT	D501 – D701	4 352 170	4 310 737	
17	C	RB43	Richards Bay	AAGGGA-GATCGT	D501 – D701	3 643 938	3 608 469	
17	C	RB24	Richards Bay	AAGGGA-GCATTG	D501 – D701	3 554 512	3 530 061	
17	C	RB58	Richards Bay	AAGGGA-TTAATG	D501 – D701	2 925 698	2 912 333	
17	C	RB8	Richards Bay	CAACTA-AGTCAT	D501 – D701	1 086 486	1 066 071	
17	C	RB47	Richards Bay	CAACTA-GATCGT	D501 – D701	855 012	844 277	
17	C	RB18	Richards Bay	CAACTA-GCATTG	D501 – D701	937 726	930 598	
17	C	RB27	Richards Bay	CAACTA-TTAATG	D501 – D701	1 250 250	1 245 611	
18	C	RB44	Richards Bay	AACCCG-AGTCAT	D501 – D702	695 156	530 079	
18	C	RB15	Richards Bay	AACCCG-GATCGT	D501 – D702	446 724	404 022	
18	C	RB23	Richards Bay	AACCCG-GCATTG	D501 – D702	404 352	384 349	
18	C	RB59	Richards Bay	AACCCG-TTAATG	D501 – D702	242 696	228 736	

18	C	RB64	Richards Bay	AAGGGA-AGTCAT	D501 – D702	419 886	409 964	
18	C	RB80	Richards Bay	AAGGGA-GATCGT	D501 – D702	536 644	530 990	
18	C	RB81	Richards Bay	AAGGGA-GCATTG	D501 – D702	383 996	379 400	
18	C	RB37	Richards Bay	AAGGGA-TTAATG	D501 – D702	1 281 558	1 277 872	
18	C	RB91	Richards Bay	CAACTA-AGTCAT	D501 – D702	399 592	394 873	
18	C	RB90	Richards Bay	CAACTA-GATCGT	D501 – D702	410 658	407 910	
18	C	RB11	Richards Bay	CAACTA-GCATTG	D501 – D702	499 008	496 556	
18	C	RB50	Richards Bay	CAACTA-TTAATG	D501 – D702	269 658	268 437	
19	C	RB36	Richards Bay	AACCCG-AGTCAT	D501 – D703	4 023 008	3 895 442	
19	C	RB55	Richards Bay	AACCCG-GATCGT	D501 – D703	2 722 098	2 559 541	
19	C	RB92	Richards Bay	AACCCG-GCATTG	D501 – D703	2 938 054	2 888 539	
19	C	RB21	Richards Bay	AACCCG-TTAATG	D501 – D703	2 411 526	2 366 989	
19	C	RB3	Richards Bay	AAGGGA-AGTCAT	D501 – D703	5 374 192	5 335 532	
19	C	RB56	Richards Bay	AAGGGA-GATCGT	D501 – D703	6 109 342	6 071 509	
19	C	RB10	Richards Bay	AAGGGA-GCATTG	D501 – D703	10 049 572	10 018 280	
19	C	RB65	Richards Bay	AAGGGA-TTAATG	D501 – D703	5 291 378	5 274 175	
19	C	RB17	Richards Bay	CAACTA-AGTCAT	D501 – D703	1 472 536	1 465 005	
19	C	RB86	Richards Bay	CAACTA-GATCGT	D501 – D703	1 885 764	1 874 119	
19	C	RB51	Richards Bay	CAACTA-GCATTG	D501 – D703	1 866 260	1 860 732	
19	C	RB31	Richards Bay	CAACTA-TTAATG	D501 – D703	2 318 888	2 312 783	
20	C	RB87	Richards Bay	AACCCG-AGTCAT	D501 – D704	3 994 868	3 469 443	
20	C	RB35	Richards Bay	AACCCG-GATCGT	D501 – D704	3 300 548	2 901 771	
20	C	RB69	Richards Bay	AACCCG-GCATTG	D501 – D704	3 274 538	3 186 816	
20	C	RB67	Richards Bay	AACCCG-TTAATG	D501 – D704	3 571 784	3 504 495	
20	C	RB41	Richards Bay	AAGGGA-AGTCAT	D501 – D704	13 868 698	13 779 174	
20	C	RB38	Richards Bay	AAGGGA-GATCGT	D501 – D704	5 950 818	5 919 775	
20	C	RB22	Richards Bay	AAGGGA-GCATTG	D501 – D704	7 334 724	7 300 883	
20	C	PE96	Port Edward	AAGGGA-TTAATG	D501 – D704	1 043 036	1 037 214	6.62
20	C	PE29	Port Edward	CAACTA-AGTCAT	D501 – D704	403 780	393 101	4.70
20	C	PE57	Port Edward	CAACTA-GATCGT	D501 – D704	335 070	323 017	4.59
20	C	PE43	Port Edward	CAACTA-GCATTG	D501 – D704	209 786	206 377	4.30
20	C	PE85	Port Edward	CAACTA-TTAATG	D501 – D704	690 782	687 715	5.60

21	C	PE21	Port Edward	AACCCG-AGTCAT	D502 – D701	13 268 098	10 298 919	14.00
21	C	SALS102	Red Sands	AACCCG-GATCGT	D502 – D701	9 231 470	4 978 344	5.68
21	C	SALS103	Red Sands	AACCCG-GCATTG	D502 – D701	3 924 080	2 946 027	8.49
21	C	SALS105	Red Sands	AACCCG-TTAATG	D502 – D701	3 739 984	2 962 205	9.30
21	C	SALS106	Red Sands	AAGGGA-AGTCAT	D502 – D701	4 934 180	4 635 394	14.3
21	C	SALS112	Red Sands	AAGGGA-GATCGT	D502 – D701	5 336 654	5 132 952	14.3
21	C	SALS113	Red Sands	AAGGGA-GCATTG	D502 – D701	2 698 624	2 622 639	9.99
21	C	SALS115	Red Sands	AAGGGA-TTAATG	D502 – D701	798 512	777 755	6.68
21	C	SALS116	Red Sands	CAACTA-AGTCAT	D502 – D701	2 664 658	2 502 485	8.73
21	C	SALS117	Red Sands	CAACTA-GATCGT	D502 – D701	2 134 576	2 055 269	7.50
21	C	SALS118	Red Sands	CAACTA-GCATTG	D502 – D701	1 672 650	1 642 345	7.14
21	C	SALS120	Red Sands	CAACTA-TTAATG	D502 – D701	299 504	295 335	5.18
22	C	SALS121	Red Sands	AACCCG-AGTCAT	D502 – D702	32 812 690	17 332 423	7.90
22	C	SALS122	Red Sands	AACCCG-GATCGT	D502 – D702	9 965 916	6 303 417	8.65
22	C	SALS123	Red Sands	AACCCG-GCATTG	D502 – D702	6 337 168	4 494 322	9.37
22	C	SALS124	Red Sands	AACCCG-TTAATG	D502 – D702	2 413 004	1 350 641	4.82
22	C	SALS125	Red Sands	AAGGGA-AGTCAT	D502 – D702	2 475 392	1 840 979	8.48
22	C	SALS126	Red Sands	AAGGGA-GATCGT	D502 – D702	2 360 508	2 084 276	8.84
22	C	SALS127	Red Sands	AAGGGA-GCATTG	D502 – D702	1 083 864	912 400	6.67
22	C	SALS128	Red Sands	AAGGGA-TTAATG	D502 – D702	634 382	565 843	5.66
22	C	SALS129	Red Sands	CAACTA-AGTCAT	D502 – D702	754 818	367 749	5.29
22	C	SALS131	Red Sands	CAACTA-GATCGT	D502 – D702	1 037 400	912 400	5.88
22	C	SALS132	Red Sands	CAACTA-GCATTG	D502 – D702	151 484	129 392	4.46
22	C	SALS137	Red Sands	CAACTA-TTAATG	D502 – D702	194 136	187 941	6.02
23	C	SALS138	Red Sands	AACCCG-AGTCAT	D502 – D703	976 474	613 873	5.26
23	C	SALS139	Red Sands	AACCCG-GATCGT	D502 – D703	1 548 952	1 136 168	6.57
23	C	SALS140	Red Sands	AACCCG-GCATTG	D502 – D703	1 289 896	1 131 628	6.85
23	C	SALS141	Red Sands	AACCCG-TTAATG	D502 – D703	2 058 610	1 899 394	7.96
23	C	SALS142	Red Sands	AAGGGA-AGTCAT	D502 – D703	5 688 204	5 502 477	13.66
23	C	SALS143	Red Sands	AAGGGA-GATCGT	D502 – D703	14 563 580	14 353 844	21.92
23	C	SALS144	Red Sands	AAGGGA-GCATTG	D502 – D703	3 923 794	3 890 637	12.13

23	C	SALS145	Red Sands	AAGGGA-TTAATG	D502 – D703	16 383 064	16 324 409	25.10
23	C	SALS146	Red Sands	CAACTA-AGTCAT	D502 – D703	2 463 360	2 428 251	9.63
23	C	SALS148	Red Sands	CAACTA-GATCGT	D502 – D703	1 644 286	1 622 977	
23	C	SALS149	Red Sands	CAACTA-GCATTG	D502 – D703	4 347 250	4 328 665	15.45
23	C	SALS150	Red Sands	CAACTA-TTAATG	D502 – D703	1 192 392	1 188 522	9.51
24	C	SALB104	Red Sands	AACCCG-AGTCAT	D502 – D704	1 211 164	770 958	4.54
24	C	SALB113	Red Sands	AACCCG-GATCGT	D502 – D704	1 054 760	760 573	4.81
24	C	SALB115	Red Sands	AACCCG-GCATTG	D502 – D704	267 896	215 304	4.23
24	C	SALB117	Red Sands	AACCCG-TTAATG	D502 – D704	2 961 468	2 823 742	10.53
24	C	SALB118	Red Sands	AAGGGA-AGTCAT	D502 – D704	8 791 816	8 657 547	25.97
24	C	SALB120	Red Sands	AAGGGA-GATCGT	D502 – D704	5 073 112	5 010 263	15.67
24	C	SALB121	Red Sands	AAGGGA-GCATTG	D502 – D704	3 341 394	3 305 411	10.20
24	C	SALB125	Red Sands	AAGGGA-TTAATG	D502 – D704	1 925 212	1 911 813	7.42
24	C	SALB126	Red Sands	CAACTA-AGTCAT	D502 – D704	656 470	601 443	5.48
24	C	SALB129	Red Sands	CAACTA-GATCGT	D502 – D704	452 058	430 051	5.72
24	C	SALB130	Red Sands	CAACTA-GCATTG	D502 – D704	1 574 968	1 561 311	7.80
24	C	SALB131	Red Sands	CAACTA-TTAATG	D502 – D704	859 394	855 073	6.05
25	C	SALB133	Red Sands	AACCCG-AGTCAT	D504 – D701	26 589 828	20 978 719	36.61
25	C	SALB136	Red Sands	AACCCG-GATCGT	D504 – D701	2 894 224	1 755 130	10.65
25	C	SALB137	Red Sands	AACCCG-GCATTG	D504 – D701	2 396 116	1 677 604	
25	C	SALB138	Red Sands	AACCCG-TTAATG	D504 – D701	1 085 574	732 689	
25	C	SALB140	Red Sands	AAGGGA-AGTCAT	D504 – D701	1 230 898	925 966	
25	C	SALB141	Red Sands	AAGGGA-GATCGT	D504 – D701	341 062	316 562	
25	C	SALB147	Red Sands	AAGGGA-GCATTG	D504 – D701	174 366	170 984	
25	C	AS1	Aliwal Shoal	AAGGGA-TTAATG	D504 – D701	2 603 606	2 593 836	10.62
25	C	AS3	Aliwal Shoal	CAACTA-AGTCAT	D504 – D701	3 067 154	3 034 780	10.13
25	C	AS4	Aliwal Shoal	CAACTA-GATCGT	D504 – D701	2 634 462	2 603 700	9.43
25	C	AS5	Aliwal Shoal	CAACTA-GCATTG	D504 – D701	1 749 932	1 717 553	7.73
25	C	AS6	Aliwal Shoal	CAACTA-TTAATG	D504 – D701	1 812 010	1 799 937	8.42
26	C	AS8	Aliwal Shoal	AACCCG-AGTCAT	D504 – D703	1 595 230	1 196 761	5.98
26	C	AS10	Aliwal Shoal	AACCCG-GATCGT	D504 – D703	711 306	585 185	4.86

26	C	AS12	Aliwal Shoal	AACCCG-GCATTG	D504 – D703	617 702	574 616	5.02
26	C	AS14	Aliwal Shoal	AACCCG-TTAATG	D504 – D703	351 148	342 814	4.73
26	C	AS18	Aliwal Shoal	AAGGGA-AGTCAT	D504 – D703	594 372	577 243	5.33
26	C	AS19	Aliwal Shoal	AAGGGA-GATCGT	D504 – D703	1 433 164	1 426 067	7.58
26	C	AS22	Aliwal Shoal	AAGGGA-GCATTG	D504 – D703	869 526	866 564	6.50
26	C	AS26	Aliwal Shoal	AAGGGA-TTAATG	D504 – D703	7 437 636	7 414 677	23.57
26	C	AS29	Aliwal Shoal	CAACTA-AGTCAT	D504 – D703	2 002 002	1 989 277	10.20
26	C	AS30	Aliwal Shoal	CAACTA-GATCGT	D504 – D703	3 297 516	3 284 169	14.12
26	C	AS33	Aliwal Shoal	CAACTA-GCATTG	D504 – D703	345 820	343 843	4.75
26	C	AS34	Aliwal Shoal	CAACTA-TTAATG	D504 – D703	619 074	617 413	5.08
27	C	AS25	Aliwal Shoal	AACCCG-AGTCAT	D504 – D704	10 005 984	6 768 182	13.34
27	C	AS35	Aliwal Shoal	AACCCG-GATCGT	D504 – D704	4 291 702	3 257 348	10.35
27	C	AS36	Aliwal Shoal	AACCCG-GCATTG	D504 – D704	1 963 384	1 466 635	6.23
27	C	AS37	Aliwal Shoal	AACCCG-TTAATG	D504 – D704	1 913 656	1 729 301	7.80
27	C	AS40	Aliwal Shoal	AAGGGA-AGTCAT	D504 – D704	5 233 580	5 154 472	16.17
27	C	AS41	Aliwal Shoal	AAGGGA-GATCGT	D504 – D704	3 267 448	3 210 404	11.8
27	C	AS42	Aliwal Shoal	AAGGGA-GCATTG	D504 – D704	2 821 042	2 785 511	10.94
27	C	AS43	Aliwal Shoal	AAGGGA-TTAATG	D504 – D704	1 541 368	1 525 186	7.41
27	C	AS27	Aliwal Shoal	CAACTA-AGTCAT	D504 – D704	6 376 188	6 266 087	21.75
27	C	AS47	Aliwal Shoal	CAACTA-GATCGT	D504 – D704	2 715 426	2 699 836	9.81
27	C	AS48	Aliwal Shoal	CAACTA-GCATTG	D504 – D704	1 335 774	1 322 374	6.71
27	C	AS50	Aliwal Shoal	CAACTA-TTAATG	D504 – D704	2 427 336	2 420 102	9.36



Appendix 5: Selection of assembly parameters in a *de novo* analysis. Increasing the *M* and *n* assembly parameters affects the number of loci shared by 80 % of samples. Both *M* and *n* parameters are kept equal, and the *m* parameter is fixed to 3. The optimal assembly parameter value for the slinger dataset for Chapters 4 and 5 dataset is when *M* equals 4