THE POPULATION STRUCTURE OF TWO ESTUARINE FISH SPECIES, *Atherina breviceps* (Pisces: Atherinidae) AND *Gilchristella aestuaria* (Pisces: Clupeidae), ALONG THE SOUTHERN AFRICAN COASTLINE

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OLIVIA BRIDGET NORTON

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Abstract

Phylogeographic patterns of coastal organisms with different life histories and breeding strategies may reveal patterns not consistent with the current delineation of the biogeographic provinces around South Africa. The subdivision of the South African coastline into these three main climatological or biogeographic regions: namely the cool temperate west coast, the warm temperate south coast and the subtropical east coast, is based on average seawater temperatures and hydrological conditions.

Genealogies of two estuarine fish species *Atherina breviceps*, a marine breeder, and *Gilchristella aestuaria*, an estuarine spawner, were reconstructed using mitochondrial DNA (mtDNA) control region sequences. The study comprised two components, an assessment of a small dataset of both fish species to compare their population structure along the South African coastline and a more comprehensive investigation of the phylogeography of *G. aestuaria* collected from 21 estuaries around the coast.

The comparative study of *A. breviceps* and *G. aestuaria* indicate different population distribution patterns along the South African coastline. Results of the *A. breviceps* analysis demonstrate substantial gene flow due to the random mixing of alleles, while the comparative *G. aestuaria* dataset indicates a more structured population and considerably less gene flow. The *G. aestuaria* population demonstrates geographic separation into four groups, namely the west coast (Great Berg), Bot (south coast), Seekoei (south coast) and east coast (Bushmans, Kasouga and Cefane).

Results from the larger *G. aestuaria* dataset indicate that the phylogeographic patterns observed during this study do not conform to existing biogeographic boundaries along the southern African coastline. The delineation identified during this study between the warm temperate and subtropical regions is further south than originally perceived and this southward extension can be ascribed to the prevailing hydrology.

The life history patterns and ecology of these two estuarine fish species appears key to understanding their population structure. These factors interact with environmental characteristics such as physical oceanography and the distribution of estuaries (along the coastline) to explain the observed distribution patterns and population structure of *A. breviceps* and *G. aestuaria*.

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Chapter 1

Introduction

1.1 General Introduction

The structure of natural populations can be viewed from two standpoints: demographic structure, and genetic structure (Chenoweth *et al.*, 1998). Variation in population genetic structure is connected to fundamental evolutionary processes such as mutation, drift, selection, speciation, breeding strategies, local extinction and gene flow (Lambert *et al.*, 2003), whereas demographic structure is affected by birth, death and dispersal (Chenoweth *et al.*, 1998). From these two standpoints, dispersal (and migration) and gene flow are tightly linked and refer to the movement of individuals and gametes among populations (Chenoweth *et al.*, 1998). Whereas gene flow and dispersal have homogenising effects, genetic differences within and among populations arise through the processes of genetic drift or localised selection (Arndt and Smith, 1998; Lambert *et al.*, 2003). These genetic differences give rise to a set of unique mutations in finite populations that are separated for sufficient time (Bernardi *et al.*, 2001). The successful participation in reproduction (gene flow) will oppose this differentiation through the random mixing of alleles from differing local populations. The balance between these forces determines the scale and pattern of divergence among populations (Arndt and Smith, 1998).

Genetic studies provide opportunities to study the variation induced by stochastic (genetic drift) and deterministic forces (gene flow and localised natural selection), and enable identification of hierarchical levels of heterozygosity (Wimmer *et al.*, 2002). These studies are significant if progress is to be made beyond the description of genetic patterns, and inferences are to be drawn about the processes that shape the distribution of genetic variation within and among populations (Lambert *et al.*, 2003).

Levels of gene flow between natural populations may vary greatly between species. Some show such high rates of gene flow that they become almost panmictic, but for other species, levels of gene flow may be so low that natural selection and genetic

drift may occur almost independently in each local population (Mariani *et al.*, 2002). In terrestrial environments, habitat characteristics can change over short distances as mountains, deserts and water gaps create effective barriers. In marine systems, it is difficult to imagine how boundaries become locally concentrated, as a single, continuous dispersal medium connects all habitats, creating gradients which are not distinctive (Gaylord and Gaines, 2000). Marine fishes often have high vagility, large effective population sizes and extensive geographic ranges, leading to higher gene flow among populations (Borsa, 2003; Collin, 2001; Jones and Quattro, 1999). Many studies confirm this expected pattern of species with high dispersal capabilities having populations that are genetically similar to one another. There are, however, exceptions to this generalisation that suggest a complex paradigm of marine population structure (Palumbi, 1995). Schizas *et al.* (1999) suggest that population subdivision in the marine environment occurs in species independent of high dispersal rates. This indicates that high dispersal ability does not necessarily mean geographic homogeneity (Borsa, 2003) and subdivision of populations is attributable to factors such as geographic isolation (by large expanses of ocean), barriers (features that influence patterns of ocean circulation), behavioural limits to dispersal, natural selection and recent history, thus leading to the cessation of gene flow (Collin, 2001; Schizas *et al.*, 1999).

For example, populations of the non-dispersing gastropod, *Nucella lapillus*, separated by less than 10km showed genetic differences at protein coding allozyme loci (Grosberg and Cunningham, 2001). Palumbi (1995), found large genetic differences between populations of tide-pool copepods separated by only a few kilometres, despite the three to six week larval stage of the species and the ability of the adults to drift between the rocky outcrops on which they live. Waples (1987) found a correlation between estimated dispersal potential and genetic differentiation among ten species of shore fish, however, in a separate study, no relationship was found between life history parameters and phylogeographical structure in seven species of Caribbean coral reef fishes (Chenoweth *et al.*, 1998).

In terrestrial ecosystems, the ranges of species often track variation in habitat quality, whereas in marine environments, the association between range borders and nearshore current features is assumed to be a consequence of the gradients in water properties

that arise at major current interfaces (Gaylord and Gaines, 2000). Jones and Quattro (1999) investigated the effect of the zoogeographic barrier, Cape Hatteras, on the genetic divergence among samples of summer flounder (*Paralichthys dentatus,* Linnaeus). The divergence of the Gulf Stream current from the coast, as it collides with the lower leg of the Labrador Current at this well-known barrier in the western Atlantic Ocean, is an example of a potential barrier to gene flow in a marine system. Large-scale ocean currents that originate from different depths and latitudes result in water masses with different characteristics such as water temperature. The convergence of currents causes steep water temperature changes, which impose physiological challenges creating range limits and biogeographic boundaries (Gaylord and Gaines, 2000). The environmental differences north and south of Cape Hatteras are dramatic, and form a boundary to fish. Thus, while life history characteristics appear to promote genetic homogeneity, summer flounder populations might be structured due to the effects of philopatry in combination with restricted dispersal due to currents. If adults return to the same spawning grounds, and their larvae are subject to currents that differentially influence dispersal, population subdivision will result (Jones and Quattro, 1999).

The affect of currents on population subdivision, however, ignores the impact of flow fields on population distributions (Gaylord and Gaines, 2000). Species with dispersing larvae have an increased capacity for ocean flows to affect abundance patterns through their influence on recruitment processes. This questions whether certain ocean circulation patterns have the potential to influence species abundance and the geographical distribution of taxa with pelagic young, even when species' demographic parameters are insensitive to water property gradients (Gaylord and Gaines, 2000).

Organisms with lengthy pelagic larval stages have a greater dispersal capacity and show greater variability in the degree of genetic differentiation than species with direct development, as they remain suspended in the water column where they are at the mercy of ocean currents (Arndt and Smith, 1998; Collin, 2001). Collin (2001) found species with direct development have more population structure than species with planktonic development as haplotypes formed genetically distinct monophyletic clades. Based on molecular data, differences in population structure and estimated

levels of gene flow of two species of sea cucumber were attributed to length of pelagic larval duration (Arndt and Smith, 1998), whereas dissimilarity in levels of population structure between two direct-developing species of *Littorina* were attributed to diversity in generation time (Collin, 2001). However, as the length of the pelagic larval development increases, the degree of local genetic differentiation decreases (Arndt and Smith, 1998). Thus, modes of development and resultant dispersal ability affect a species' geographic range (Gaylord and Gaines, 2000). Lambert *et al.* (2003) conducted studies on the genetic structure of populations of two intertidal nudibranchs, *Goniodoris nodosa* and *Adalaria proxima*, using polymorphic allozymes. A relationship between larval strategies and spatial differences in allele frequencies showed that the planktotrophic species (*G. nodosa*) lacked spatial heterogeneity in population structure over distances of >1000km, indicating, as expected, considerable levels of gene flow (i.e. larval dispersal). In contrast, populations of the lecithotrophic species (*A. proxima*) showed significant spatial heterogeneity and marked disjunctures in allele frequencies among populations over distances of as little as 100 – 1000m, even in locations with highly dispersive tidal currents. Temporal studies support the spatial studies, illustrating that the population structure for both species is closely related to their realised larval dispersal (Lambert *et al.*, 2003).

Gaylord and Gaines (2000) explored the possible theoretical role of ocean currents in the geographical distribution of larval settlement using a modified version of Possingham and Roughgarden's (1990) original advection-diffusion approach. As suggested, advective ocean currents may have a strong potential to influence adult shoreline abundance and distribution in species that have planktonic larvae. The model developed by Possingham and Roughgarden (1990) "explores the population dynamics of a marine species with a dispersing larval phase by explicitly linking temporal changes in an adult shoreline distribution to offshore concentrations of larvae produced by those adults". The pattern of larval concentration is explained with a two-dimensional advection-diffusion equation, which assumes that larvae are well mixed in water of a constant depth, or remain within a single layer of the water column. Results suggest that circulation patterns can play a strong role in setting species geographical distributions (Gaylord and Gaines, 2000).

As an example, in harpacticoid copepod species the contribution of transportation by clinging to floating marine algal mats or by ballast of sailing vessels is unknown, but previous studies report differing degrees of genetic differentiation on a scale between a kilometre, and hundreds of kilometres (Schizas *et al.*, 1999). Over short distances (<1000m) between rock pools, salt marshes or offshore habitats, distinct populations can be maintained. However, at larger scales, latitudinally separated *Coullana canadensis* populations show differences in growth, reproduction and energy budgets. These differences suggest that the geographically separated populations of *C. canadensis* could be genetically distinct (Schizas *et al.*, 1999).

A review by Sweijd *et al.* (2000), illustrates the wide variety of biochemical and molecular techniques applied to identify species, for conservation and management purposes. The genetic identification techniques of species from cryptic life-cycle stages or of morphologically indistinct species are an indispensable tool for marine scientists, conservators and managers. The requirements for methods that identify samples of processed marine products to species level has become a conservation priority. For example, in a case where South African abalone were poached and marked in cans as “Australian”, the poachers were released as the defence contended that South African regulations did not have jurisdiction over the case. The contents were subsequently proved to be of South African origin using a DNA-based species identity kit, and as a result of this case the SA police have a molecular tool to help protect this abalone species from exploitation. This illustrates the importance of species identity in conservation, and how molecular markers such as those described below play a role in conservation and management of marine species

1.2 A comparison of molecular markers

In recent years, molecular techniques have been used as a tool for population structure studies by analysing dispersal, colonisation patterns and gene flow between populations over a variety of geographic scales (Duran *et al.*, 2004). There is, however, a need for expanding the array of molecular tools available in the context of population genetics. Inferences made from datasets may be influenced by the use of different molecular techniques, as, for example, allozymes evolve at a slower rate than mitochondrial DNA (which is maternally inherited) and nuclear DNA such as microsatellites (Duran *et al.*, 2004).

1.2.1 Mitochondrial DNA

Animal mitochondrial DNA (mtDNA) is a closed circular molecule, approximately 16 – 20 kilobases (kb) in size and consists of 37 genes encoding thirteen proteins, two ribosomal RNAs (small 12S and large 16S rRNA), 22 transfer RNAs (tRNAs) and one major non-coding region (control region) that contains the initiation sites for mtDNA replication and RNA transcription (Figure 1.1) (Inoue *et al.*, 2000; Yamauchi *et al.*, 2003; Yamauchi *et al.*, 2002; Meyer, 1993). The control region contains areas that are constrained in primary sequence or secondary structure to regulate replication and transcription and is characterised by the displacement loop (D-loop). A stretch of DNA that is complementary to the light L-strand, the D-loop strand replaces the H-strand where the origin of the H-strand replications and the initiation site of D-loop synthesis are identical (Meyer, 1993).

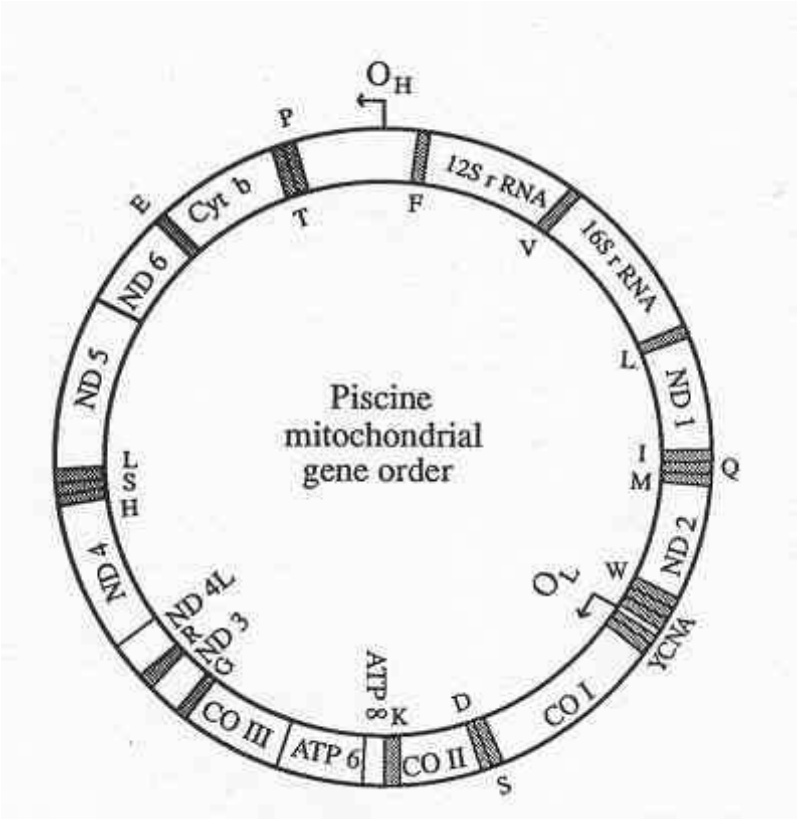

Figure 1.1: Piscine mitochondrial gene order, illustrating the control region in fish that contains the initiation sites for mtDNA replication and RNA transcription (after Meyer, 1993).

Animal mitochondrial sequence data is a powerful tool for tracing founder events, population bottlenecks and population range fluctuations, and has become the method of choice for intraspecific phylogeographic studies (Neigel, 1997). Animal mitochondrial DNA (mtDNA) was the first DNA-based genetic marker system that could be applied to surveys of genetic variation in natural populations.

The introduction of the polymerase chain reaction (PCR) and improvements in DNA sequencing methods have made it possible to determine the exact nucleotide sequence of amplified regions of mtDNA for large numbers of individuals (Neigel, 1997). Mitochondrial DNA is compact and is inherited maternally therefore it represents only a partial view of species history without recombination as a single linkage unit of about 15kb (Inoue *et al.*, 2000). In comparison to nuclear DNA, mitochondrial DNA has a fast evolutionary rate, higher mutational rate, shorter coalescence time and a greater sensitivity in reflecting the genetic impact of population subdivision over large geographic scales, which makes mtDNA a useful marker for population genetics studies (Duran *et al.*, 2004; Inoue *et al.*, 2000). There are also more detectable polymorphisms than there are for single allozyme loci, making the inheritance of mtDNA similar to a single haploid locus. More significantly, characterisation of sequence differences can be used to infer genealogical relationships and estimate divergence times (Neigel, 1997).

For more adequate resolution of higher-level relationships in organisms and more effective uses of mtDNA, it appears that longer DNA sequences or the whole mitochondrial genome (mitogenome) is required from many taxa (Miya *et al.*, 2003). Although it is technically difficult to obtain a number of such sequences for determination of the complete mitochondrial genome, and the process is constrained by time and resources, the development of a long Polymerase Chain Reaction (PCR) technique has been employed by Miya and Nishida (1999). In this approach, the entire mitochondrial genome is amplified from long PCR, and then the product is subsequently used as a template for PCR with fish-versatile primers in various combinations that amplify overlapping regions of the individual genes (Miya *et al.*, 2003). Contiguous PCR products are then sequenced, producing the complete mtDNA sequence in a single or two reactions. Sequencing mitogenomes reduces the possibility of amplification of mitochondrial pseudogenes in the nuclear genome, and

also allows an accurate determination of the complete mtDNA sequence (Inoue *et al.*, 2000).

As indicated by Mindell *et al.* (1999), features consistent with mitochondrial origin are a) presence of a conserved reading frame in protein-coding genes among all taxa, with decreasing rates of variability at third, first and second codon positions respectively; b) absence of extra stop codons, frameshifts, or unusual amino acid substitutions; and c) no sequence changes indicating a loss of known secondary structure in tRNA (transcription RNA) and rRNA (ribosomal RNA) genes that would indicate translocation to the nucleus. Nuclear copies of DNA have distinguishing features such as double peaks as a result of coamplification of mtDNA and nuclear DNA sequences, frameshifts or stop codons, uncalculated insertions or deletions, and mismatches in overlapping sequences for a given taxon from different amplification products when examining on electropherograms (Mindell *et al.*, 1999).

There are, however, some general problems associated with the use of mtDNA. Analysis of genetic variation in short segments of mtDNA, have, in some cases, illustrated ambiguous geographic structures of local populations, mainly because the sequence amplified was either too short to contain significant genetic variations or the evolutionary rate of the segment was not suitable for the specific purpose of the study (Inoue *et al.*, 2000).

1.2.2 Nuclear DNA

A few studies have estimated gene flow using nuclear DNA (nDNA) by using two forms of nuclear sequence variation as genetic markers: variable numbers of tandem repeats (VNTRs) and base substitutions (Neigel, 1997). The latter are more difficult to survey in populations but provide the potential of inferring genealogical relationships among sequences (Neigel, 1997). VNTR sequences are classified by size as microsatellites or minisatellites. Microsatellites consist of up to 50 copies of tandemly repeated sequences of 1 – 10 base pairs (bp) in length. Length variation can be analysed by direct size measurements of PCR-amplified sequences on electrophoretic gels as their total length is usually less than a few hundred base pairs. Too large to be amplified by PCR, minisatellite sequences contain up to several hundred copies of

repeated units 10 – 200bp in length, with total lengths up to 50kb (Neigel, 1997). Length polymorphisms are usually detected as Restriction Fragment Length Polymorphisms (RFLPs) with Southern hybridization.

Recombination (largely responsible for variation in minisatelllite sequences) and replication slippage (responsible for distribution of length variation in microsatellite sequences) are two mechanisms expected to alter the number of tandem repeats (Neigel, 1997). Recombination may generate length changes at rates as high as $5x10^{-2}$, whereas replication slippage that occurs during DNA replication favours small stepwise changes in the number of tandem repeats at rates of $1x10^{-4}$ and $1x10^{-3}$. The variation generated by replication slippage expects a correlation between the accumulated length differences and the number of generating events, providing the number of events is not too large (Neigel, 1997).

From the onset of DNA studies, it was recognised that any DNA segment can be useful over a limited divergence range. Outside that range, the historical signal may either be too undeveloped or too attenuated to be reliable (Naylor and Brown, 1998). Perhaps, however, it is a matter of poor understanding and failure to incorporate knowledge of molecular evolutionary processes that leads to inaccurate phylogenetic estimates based on molecular sequences, rather than a lack of historical information (Mindell *et al.*, 1999). Reductions in potential biases in analyses could be through the use of weighted maximum parsimony (MP) analyses of nucleotide and amino acid sequences, and the use of maximum likelihood (ML) analyses with model parameters accommodating base composition heterogeneity as well as among-sites and among-taxa rate heterogeneity (Mindell *et al.*, 1999). Also, improvements in understanding sequence evolution and the addition of taxa and further data sets improve the hypothesis being tested.

1.3 Study Area

1.3.1 Biogeographic Regions

Covering a wide range of climatic and oceanic conditions, the South African coastline stretches for 3400km from the Orange River mouth on the west coast (Atlantic Ocean) to Kosi Bay on the east coast (Indian Ocean) (Harrison, 2004). Based on average seawater temperatures, rainfall and river flow, Allanson and Baird (1999), Day (1981)

and Whitfield (1994) subdivided the coastline into three broad climatological/biogeographic regions (Figure 1.2). The subtropical region extending from the northern border of KwaZulu-Natal to the Mbashe River, and the warm temperate region from the Mbashe River to Cape Point in the South (Maree *et al.*, 2000). The third zone, the cool temperate region, incorporates the west coast of the Western and Northern provinces, and is under the influence of the cold Benguela system of upwelled inshore waters (Allanson and Baird, 1999; Harrison, 2002).

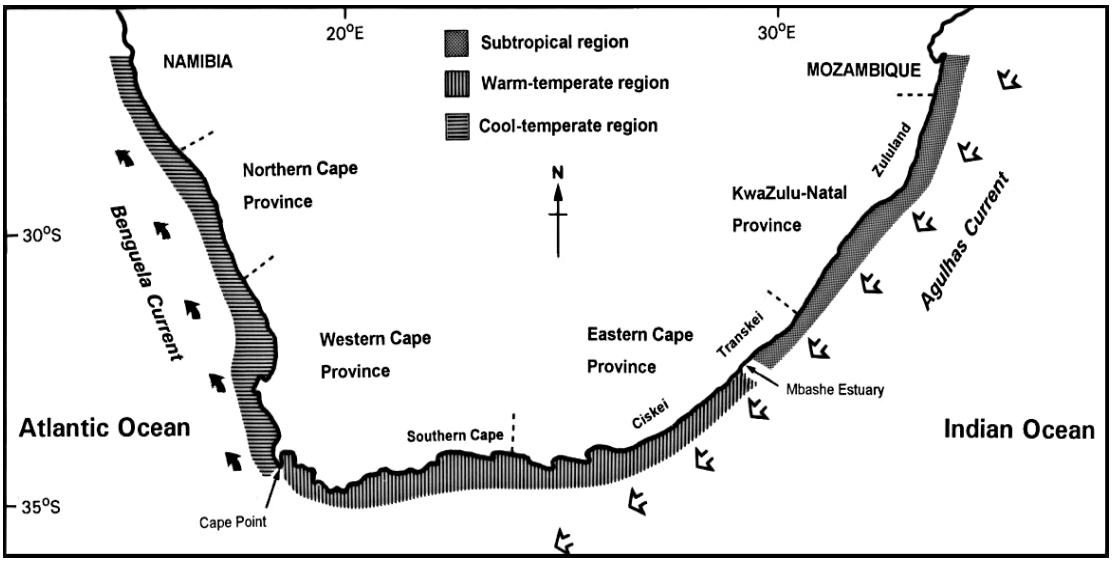

Figure 1.2: Map of South Africa illustrating the three biogeographic regions along the coastline (after Whitfield, 2000). Arrows at Cape Point and Mbashe Estuary indicate the breaks between the different biogeographic zones. Also shown are the two main current systems (warm Agulhas and cool Benguela) that influence the South African coastline.

The boundaries of these three faunistic provinces are not precise (Maree *et al.*, 2000), as estuaries are subjected to terrestrial, marine and seasonal influences and previous studies have relied on historical data, limited field collections and existing distribution records (Harrison, 2002). On the basis of fish communities however, Harrison (2002), suggests that the break between the subtropical and warm temperate zones lies further north, just south of Port St Johns, in the region of the Mdumbi estuary. In this region of overlap between the warm temperate and subtropical regions, a change from tropical to temperate communities including rock pool fishes, rocky shore biota, beach macrofauna, marine molluscs, estuarine vegetation, shelf-associated fishes and

estuarine and freshwater fishes have been observed (Harrison, 2002). Harrison (2002) further suggests that the break between the warm temperate and cool temperate zones occurs at Cape Agulhas, east of Cape Point as suggested by Allanson and Baird (1999), Day (1981) and Whitfield (1994).

Thus, borders between biogeographic regions are not clearly defined and to produce a comprehensive picture, a variety of systems for the delineation of regions needs to be used and tested with different organisms and different criteria (both presence/absence and abundance) using both modern and available historical data (Bolton *et al.*, 2004).

1.3.2 Physical Oceanography

The marine environment around southern Africa, for a region of its size, is one of the most varied and complex in the world. Surrounded by three oceans, the Indian to the east, Atlantic to the west and Southern Ocean to the south, two main oceanic currents dominate these waters (Payne and Crawford, 1989). The Agulhas Current flowing along the east coast (red in Figure 1.3), is the major western boundary current of the southern hemisphere, and the cold Benguela Current (blue in Figure 1.3) on the western side of the subcontinent, have different physical, chemical and biological properties (Lutjeharms and Ballegooyen, 1988).

The Benguela Current is difficult to define, as it is an extremely complex system of flows (Payne and Crawford, 1989). However, there is a general drift of surface water northwards and north westwards, originating from the South Atlantic gyre. The surface water temperatures of the Benguela system average between 13°C and 15°C, with an upwelling season during summer (September – March) (Allanson and Baird, 1999; Harrison, 2002). The subtropical and warm temperate regions border the Indian Ocean, and this coastal environment off south eastern Africa is dominated by the Agulhas Current. This current system is complex and diverse, including the waters of the east coast of Madagascar and extending from the tropics to a region adjacent to the Subantarctic (Lutjeharms, 2005).

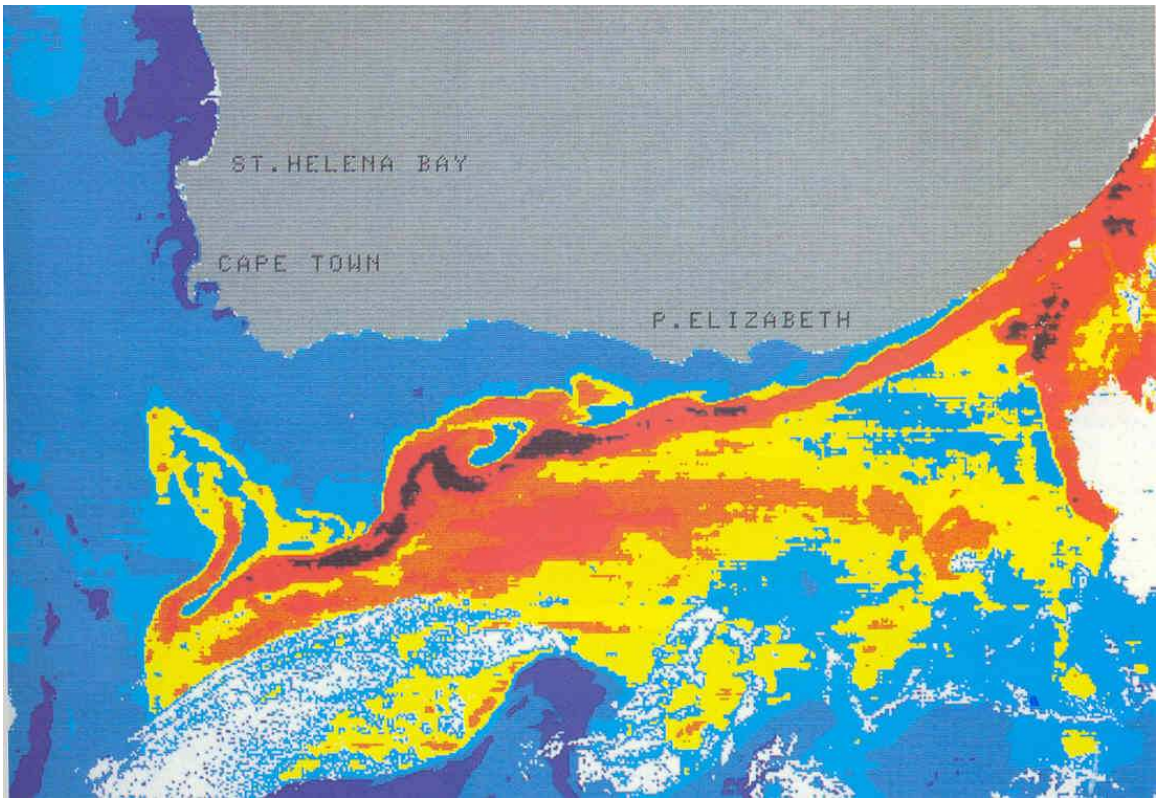

Figure 1.3: Satellite image of sea surface temperatures around South Africa on 16 July 1979. Note the warm Agulhas Current (red) on the south coast and the cold upwelling water of the Benguela Current (dark blue) on the west coast (after Payne and Crawford, 1989).

The Agulhas Current is composed of water from two different sources; the South Equatorial Current and recirculation in the South West Indian Ocean subgyre. How the South Equatorial Current acts as a source for the Agulhas Current has not been determined, although the classic portrayal is that as the current reaches the east coast of Madagascar it bifurcates into the southern and northern branches of the East Madagascar Current (Figure 1.4). As the northern current passes the northern tip of Madagascar and joins with the remainder of the South Equatorial Current, it was thought to move towards the east coast of Africa, splitting again. Some water then passes northwards into the Somali Current and the remainder moves southward into the Mozambique Channel forming the warm, low salinity Tropical Surface Water of the Mozambique Current. The Mozambique Current and the southern limb of the East Madagascar Current then converge somewhere off South Africa and contribute to the Agulhas Current (Lutjeharms, 2005; Payne and Crawford, 1989).

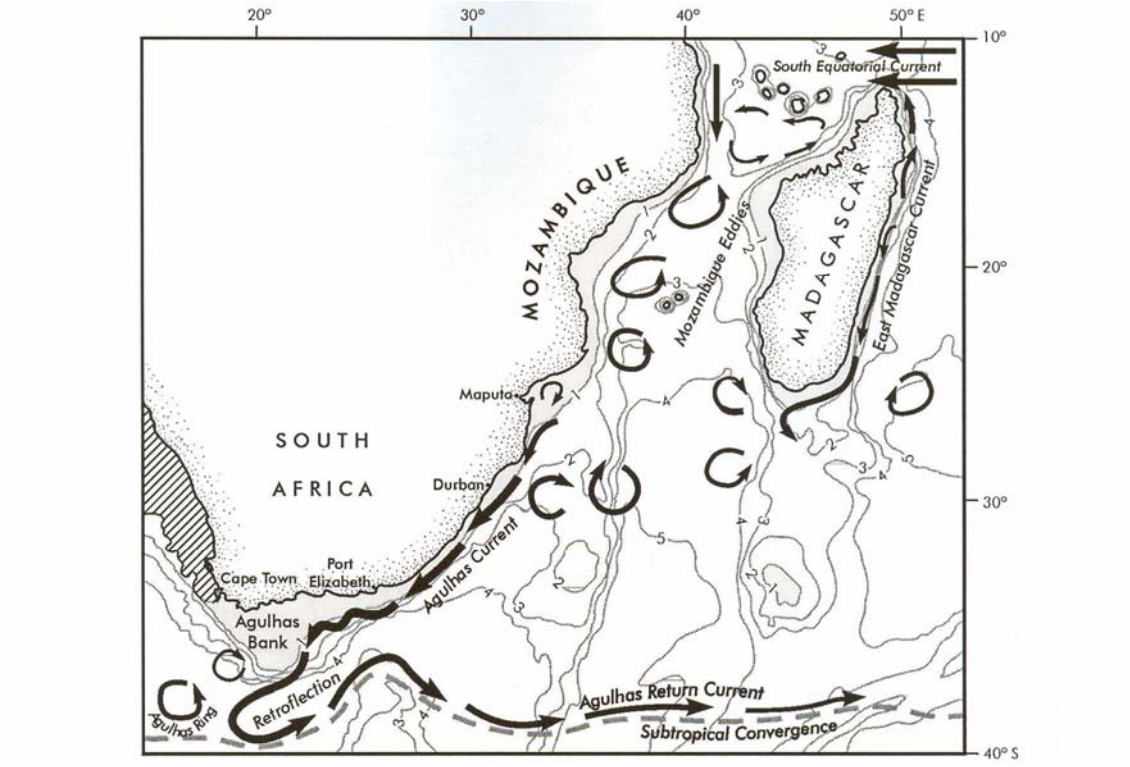

Figure 1.4: The major circulation features of the South West Indian Ocean. Shelf regions shallower than 1km are indicated by the first bathymetry line and upwelling is indicated by hatching (after Lutjeharms, 2005).

Recent observations show that no continuous, unbroken western boundary current exists in the Mozambique Channel. Instead, mesoscale eddies form at the narrows of the channel and shift southwards along the shelf edge at speeds of about 5cm.s^{-1} forming the major elements of circulation of the Mozambique Current (Figure 1.4). As eddies may ultimately combine with the Agulhas Current, these features are seen as a minor and intermittent source of water for the Agulhas Current. Although these Mozambique eddies and the East Madagascar Currents do not form a continuum with the Agulhas Current itself, they do influence its behaviour and can be considered as part of the Agulhas system (Lutjeharms, 2005).

As illustrated in Figure 1.4, the Agulhas Current proper is established at approximately 28ºS, along the east coast of South Africa between Maputo and Durban (Lutjeharms, 2005). Remote sensing enables understanding of movement of surface waters by measuring the temperature of the ocean. A narrow ribbon of warm water shows the position of the northern Agulhas Current (Figure 1.3), which flows close inshore where the continental shelf is narrow and the continental slope is steep off the

coast of northern KwaZulu-Natal. Here the course of the current is very stable, and the core of the northern Agulhas Current meanders less than 15km to either side, following the shelf edge closely (Lutjeharms, 2005). This has important consequences for the circulation on the adjacent shelf as the surface waters of the current are not as stable and penetrate into the shelf waters at irregular intervals (Lutjeharms, 2005). More importantly, short term current reversals have been observed at the edge of this current, possibly due to shear edge eddies or the effect of the wind (Lutjeharms, 2005).

Towards northern KwaZulu-Natal, just upstream of Durban, the Agulhas Current flows closer inshore due to the wider shelf and more gentle continental slope, forming an elongated system of eddies called the Natal Bight circulation (Figure 1.5) (Payne and Crawford, 1989). Here inshore water is transported in the opposite direction to that of the main stream of the Agulhas Current and the Agulhas Current on the shelf edge forms a formidable barrier to the free exchange of water and biota with the open ocean (Lutjeharms, 2005). A mechanism is thus provided for retaining water and associated fauna and flora on the KwaZulu-Natal shelf. According to Whitfield (1990), the inshore currents along the KwaZulu-Natal coast retain eggs, embryos and fish larvae in the region. If, however, spawning takes place in offshore waters along this coastline, eggs, embryos and larvae are rapidly transported southward in the Agulhas Current (Whitfield, 1990). This is the case with the strepie, *Sarpa salpa*, and leervis, *Lichia amia*, whose juveniles frequent south east and southern Cape estuaries but are absent from KwaZulu-Natal estuaries and inshore reefs (Whitfield, 1990).

The Agulhas Current moves offshore and flows more slowly further south, where the shelf is wider and the slope much broader with a gentler gradient (visible in Figure 1.3 and Figure 1.4) (Payne and Crawford, 1989). Near Port Elizabeth, as off central KwaZulu-Natal, eddies form and filaments sheared off these eddies move parcels of Agulhas Current water onto the shelf (Payne and Crawford, 1989). When the eastern section of the broad shelf region off South Africa (the Agulhas Bank), is reached, the nature of the current changes considerably. The ‘jet’ can no longer be controlled, and the main body of the current turns back on itself or ‘retroflects’ in an anticlockwise direction forming the Agulhas Return Current (Figure 1.4) (Lutjeharms and Ansorge, 2001; Lutjeharms and Ballegooyen, 1988; Payne and Crawford, 1989).

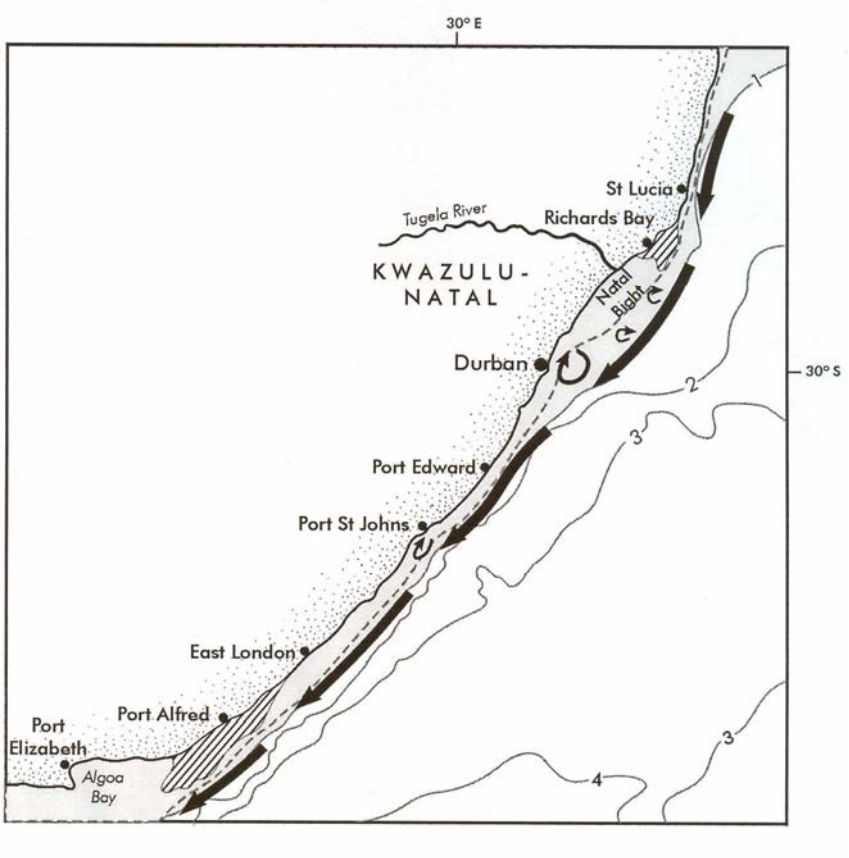

Figure 1.5: The continental shelf along the northern Agulhas Current illustrating the Natal Bight region. The 200m isobath is shown by a broken line and the hatched area denotes upwelling (after Lutjeharms, 2005).

The retroflection loop caused by this mechanism is an unstable configuration, exhibiting some of the highest levels of mesoscale variability in the worlds oceans (Garzoli *et al.*, 1996). This is due to the generation of large Agulhas rings or warm and cold core-eddies that subsequently drift into the south Atlantic Ocean as a function of the volume flux of the Agulhas Current (Lutjeharms and Ballegooyen, 1988; Lutjeharms and Gordon, 1987). This leads to a substantial transfer of water from the Indian to the Atlantic Ocean systems (Lutjeharms and Ballegooyen, 1988) and may play a role in carrying surface water directly from the Agulhas Current northwards past the western edge of the Agulhas Bank (Lutjeharms, 2005). For many marine planktonic or nekton species, the Agulhas Retroflection area acts as a barrier to gene flow, dividing the South Atlantic and South Indian Oceans (Payne and Crawford, 1989). For others, it forms a conduit or bridge between masses of water in the two oceans (Barker *et al.*, 2002; Payne and Crawford, 1989).

Roy *et al.* (2001) encountered warm water originating from the Agulhas in the main upwelling cells of Cape Point, Cape Columbine and north of Hondeklip Bay during a study of the oceanographic events recorded in the Southern Benguela during the 1999 – 2000 summer season (Figure 1.6). This anomaly caused a collapse in the upwelling conditions, covering the entire continental shelf north of the Cape Peninsula with surface water warmer than 19°C. Two weeks later, the Cape Point and Cape Columbine upwelling cells were once again fully developed and cold water covered the entire shelf.

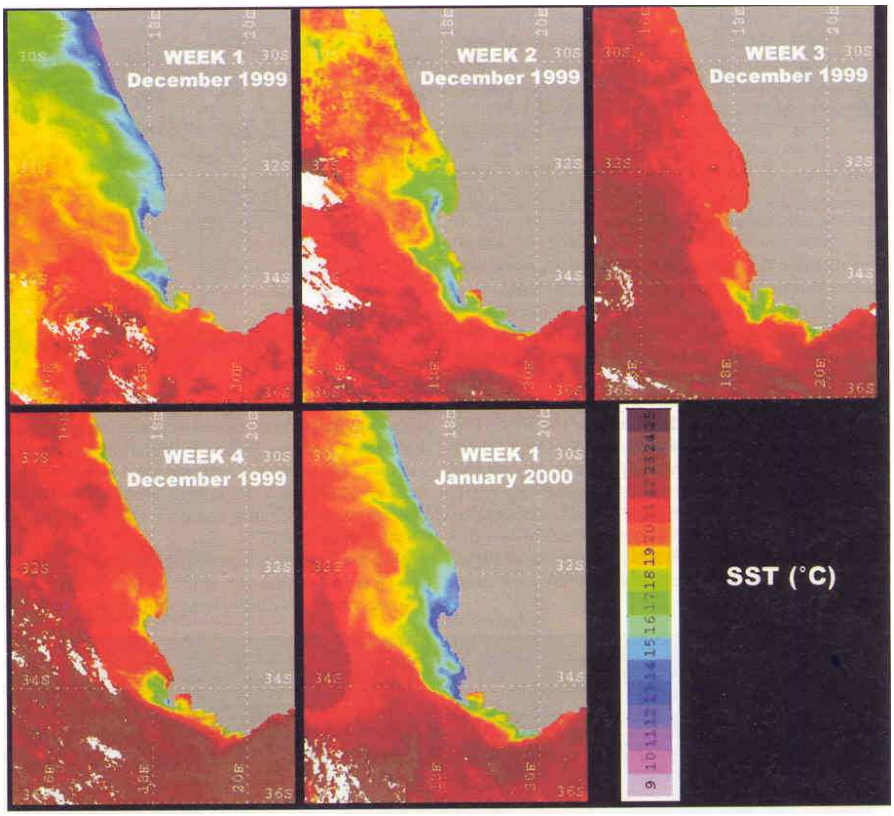

Figure 1.6: Mean sea-surface temperatures (°C) illustrating the movement of warm water (red) from the Agulhas region to the area north of the Cape Peninsula from the first week of December 1999 to the first week of January 2000 (after Roy *et al.*, 2001).

In addition to eddies generated by instabilities of the Agulhas Retroflection, intense wind-driven surface Ekman transport has been shown to facilitate the free exchange of water around the southern end of the African continent (Figure 1.7) (Sherman *et*

al., 1993). This transport may provide an additional mechanism that would allow the transportation of larvae from the south east coast to the west coast (Sherman *et al.*, 1993). For example, the spawning habitat of the southern Benguela anchovy and sardine populations is over the Agulhas Bank. Ekman transport serves to either retain drifting larvae within the neritic habitat over the Agulhas Bank, or due to the geostrophic current pattern, westwards around the Cape Peninsula and northwards into the rich Benguela upwelling system (Sherman *et al.*, 1993).

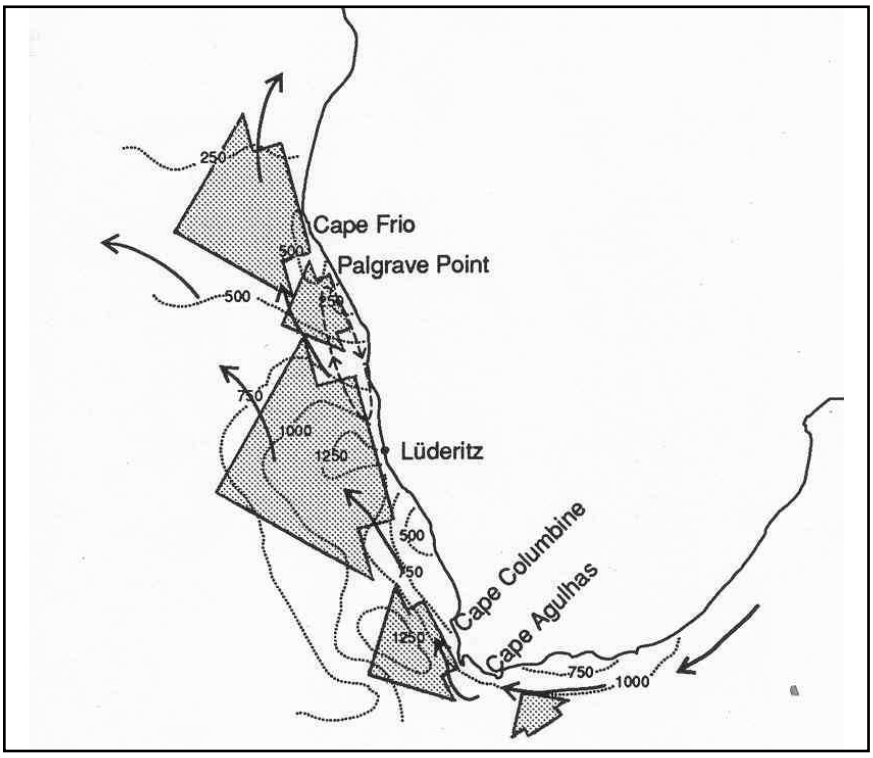

Figure 1.7: Diagram of characteristic flow features and wind mixing index for the Benguela Current region. Broad shaded arrows indicate surface Ekman transport and solid arrows indicate the general trend of underlying geostrophic current flow (after Sherman *et al.*, 1993).

1.4 Study Species

1.4.1 *Atherina breviceps*

Silverside fishes (families Atherinidae and Atherinopsidae) can be found in freshwater, estuarine and marine environments of temperate and tropical regions around the world (Beheregaray and Sunnucks, 2001). *Atherina breviceps* (Cape Silverside) are numerically abundant along the south east and west coasts of South Africa, from northern KwaZulu-Natal to southern Namibia (Figure 1.8) (van der Elst,

1988; Whitfield, 1998). A small, translucent, elongated fish with a small head and silver lateral stripe down each flank (Figure 1.9), this common endemic species of estuarine and coastal waters moves about in large shoals in the nearshore marine environment, particularly in sheltered bays in the Eastern Cape (van der Elst, 1988; Whitfield, 1998). Whitfield (1998) describes this fish species as a Class 1b breeder, leading a life-style whereby they inhabit shallow estuarine waters, but undergo annual spawning migrations into the marine environment, suggesting a high dispersal capacity.

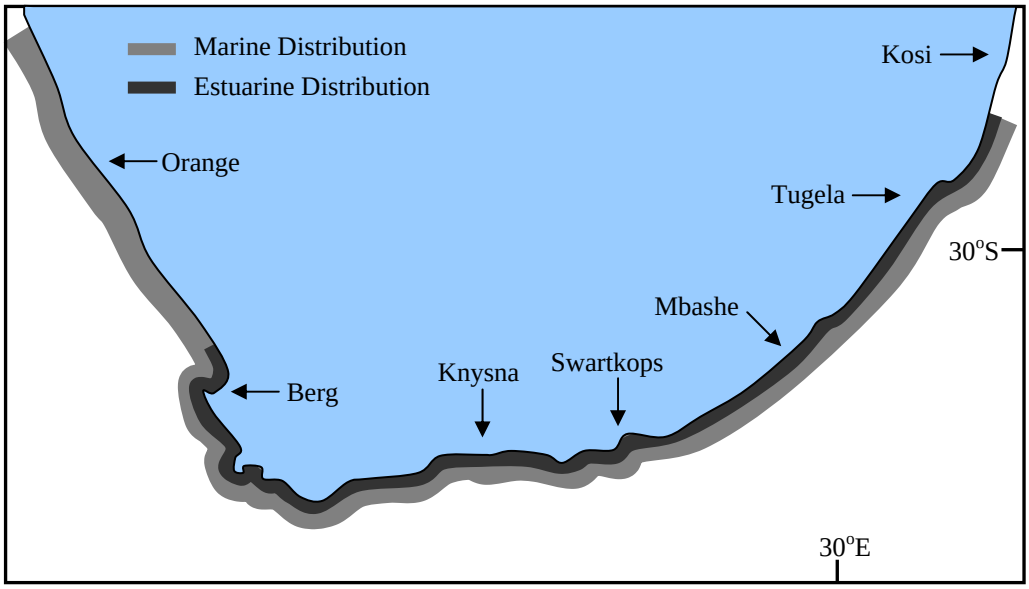

Figure 1.8: Distribution of *Atherina breviceps* along the South African coastline (after Whitfield, 1998).

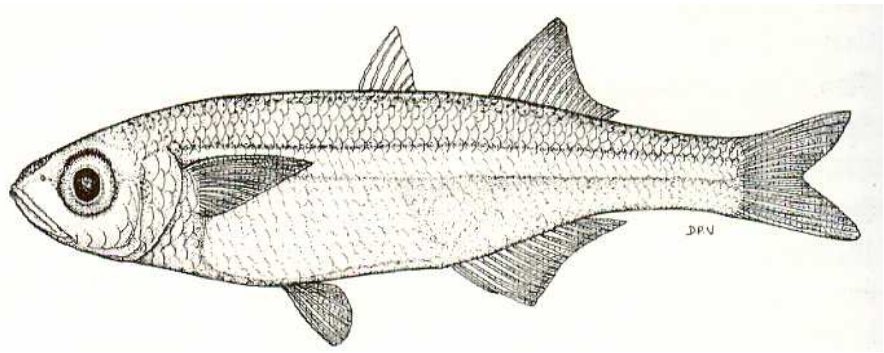

Figure 1.9: Schematic diagram of *Atherina breviceps* Valenciennes, 1835 (Family: Atherinidae, Common name: Cape Silverside, Smiths' Sea Fishes number: 111.1). Illustrated specimen length: 64mm SL (after Whitfield, 1998).

Foraging during nocturnal hours, *A. breviceps* feeds mainly on planktonic organisms such as copepods, amphipods, isopods, gastropods, ostracods, decapods, crab and insect larvae and the fry of other fishes (van der Elst, 1988; Whitfield, 1998). *Atherina breviceps* thus forms an important component of estuarine ecosystems as a link in the foodweb between primary producers and consumers, and a wide variety of gamefish and piscivorous birds, which feed upon it. Sexual maturity of *A. breviceps* is reached within eight months; at a standard length (SL) of 40mm.

Breeding takes place during spring and summer, resulting in an abundance of pelagic larvae in surface waters between September and March (Tweddle, 2004; Whitfield, 1998). Eggs are approximately 1.5mm in diameter, somewhat larger by comparison to most other fish, and are equipped to attach to submerged plants and other objects with well-developed chorionic or adhesive filaments (van der Elst, 1988).

1.4.2 *Gilchristella aestuaria*

Discernible from *A. breviceps* by the absence of a lateral line and second dorsal fin (Figure 1.10), the estuarine roundherring (*Gilchristella aestuaria*) is a shoaling fish abundant in all types of estuaries, bays and vleis, and also certain freshwater coastal lakes along the South African coastline (Figure 1.11) (van der Elst, 1981; Whitfield, 1998).

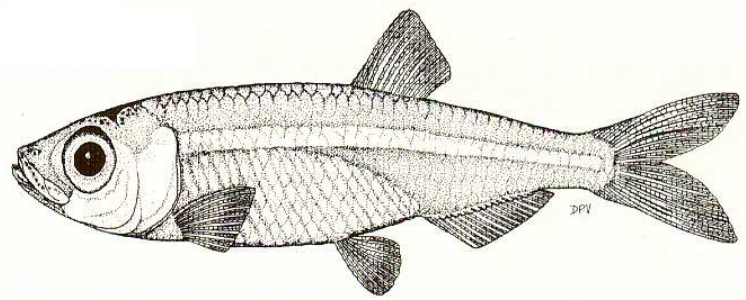

Figure 1.10: Schematic diagram of *Gilchristella aestuaria* Gilchrist, 1914 (Family: Clupeidae, Common name: Estuarine Roundherring, Smiths' Sea Fishes number 54.3). Illustrated specimen length: 59mm SL (after Whitfield, 1998).

G. aestuaria lives for a maximum of six years, but few individuals reach this age as they are preyed upon by a wide variety of piscivorous birds and fishes (especially the ladyfish, *Elops machnata*, dusky kob, *Argyrosomus japonicus*, and leervis, *Lichia amia*). In the Swartkops estuary, 99% of *G. aestuaria* were less than two years old, indicating that <1% of the population reaches three years of age (Whitfield, 1990). To compensate for this short life span, a sex ratio of 0.69 males: 1.00 females (n=1345) and that both sexes have a gonadosomatic index (GSI) which exceeds 17, enhances the reproductive potential of this species (Whitfield, 1990).

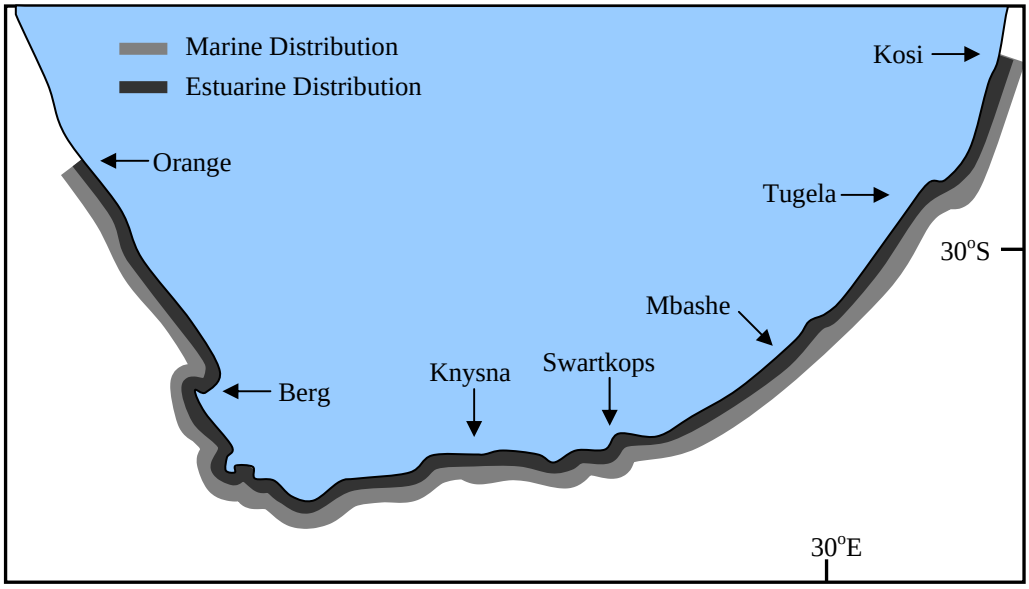

Figure 1.11: Distribution of *Gilchristella aestuaria* illustrating marine and estuarine occurrences (after Whitfield, 1998).

This small filter-feeding planktivorous clupeid forages mainly during daylight hours, with feeding rates peaking in the afternoon (Coetzee, 1982; Whitfield, 1998). Mature within seven months (approx 28mm SL), spawning peaks occur in estuaries during spring and summer although breeding has been noted throughout the year in an attempt to counter unfavourable environmental conditions which may occur during the larval and juvenile periods (Strydom *et al.*, 2002). This species has a comparatively high fecundity, produces lots of small eggs and exhibits no parental care (Whitfield, 1990). Classified by Whitfield (1998) as a class 1a species, *G. aestuaria* is one of the very few euryhaline fishes that spawns and breeds within estuaries, and has not been recorded spawning in marine or freshwater environments (van der Elst, 1981; Whitfield, 1998).

Although these species have different breeding strategies as *G. aestuaria* appears to be restricted to estuaries and *A. breviceps* has a marine phase, both utilise estuaries as physical protection for juveniles, or as nursery habitats. As two of the most numerically abundant species in South African estuaries (reaching densities in excess of 80 fish per m^3), both *A. breviceps* and *G. aestuaria* represent a very important link in the food web (Whitfield, 1998). Thus the degradation of these finely balanced estuarine environments could well limit, or greatly reduce, the abundances of both species, placing stress on the overall food web as an intermediate between zooplankton and the gamefish and piscivorous birds which prey upon them.

Determining the spatial scale and dispersal potential of *Atherina breviceps* and *Gilchristella aestuaria* is also important in understanding population biology, conservation, management and the evolution of species (Schizas *et al.*, 1999). The link between dispersal ability and gene flow needs further investigation to validate the power of population genetic models in predicting demographically informative dispersal patterns. The high dispersal capacity of *A. breviceps* as it migrates into the marine environment to spawn, suggests that extensive transport could result in high levels of within-population gene flow (greater than that of estuarine dependant species *G. aestuaria*) and low levels of among-population gene flow. This phylogeographic study of *A. breviceps* and *G. aestuaria* is the first of its kind to take place on these species around the South African coastline, and is important as the ecological significance of estuaries to ichthyofauna needs to be understood to make decisions about management and conservation.

1.5 Aims and Objectives

From information provided by this mitochondrial DNA study, an evaluation of the degree of genetic divergence within and between *Atherina breviceps* and *Gilchristella aestuaria* populations has been undertaken using control region sequences. Both these species are found in estuaries spanning large spatial scales and across well-known biogeographic boundaries.

Chapter 2

Materials and Methods

2.1 Sampling

Although the same materials, methods and data analysis techniques have been used throughout this study, the results obtained have been divided into two separate chapters: results from a comparison between *Atherina breviceps* and *Gilchristella aestuaria* (Chapter 3), and an analysis of *Gilchristella aestuaria* only (Chapter 4).

For Chapter 3, a total of 60 *Atherina breviceps* and 60 *Gilchristella aestuaria* individuals were collected from six sites (locations in Table A1.1 and A1.2, Appendix One) along the South African coastline from June 2004 to January 2005 (Figure 2.1). This enabled a comparison between the two species in terms of their genetic structure. In cases where one species and not the other were found within an estuary, sites in very close proximity were treated as the same site (e.g. Kasouga / Kariega and Qolora / Cefane in the warm temperate region).

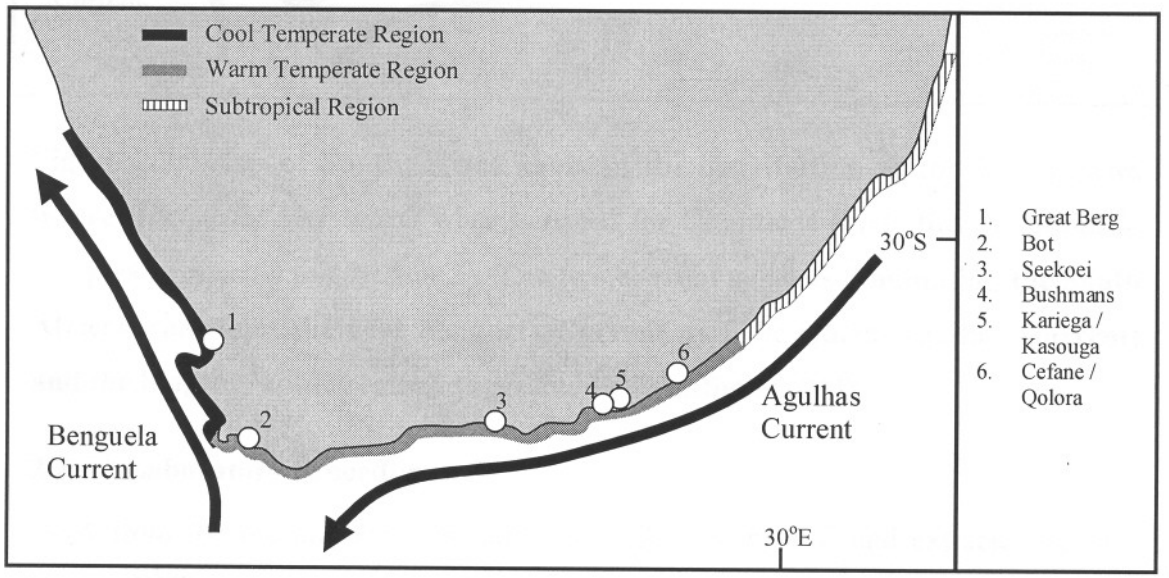

Figure 2.1: Map of South Africa showing the six estuaries sampled for Chapter 3, the two dominating current systems (cold Benguela Current and warm Agulhas Current) and the biogeographic regions suggested by Whitfield (1994).

In addition, 138 *Gilchristella aestuaria* individuals were collected from a further 15 sites (21 sites in total) (locations in Table A1.3, Appendix One) within the three biogeographic regions along the southern African coastline (Figure 2.2) for inclusion in Chapter 4. All samples were collected with a 5m (500μm mesh size) or 15m (1cm stretch mesh) seine net, stored directly in 90% ethanol at ambient temperature in the field and transported to the laboratory and stored at 4°C. Due to time constraints relatively small sample sizes have been used in both studies and it is acknowledged that to detect signal of genetic differentiation, especially within species with marine dispersal phases, one would typically standardise sampling to a particular age class and time of year and analyse large sample sizes (50 – 100) per site from fewer sites.

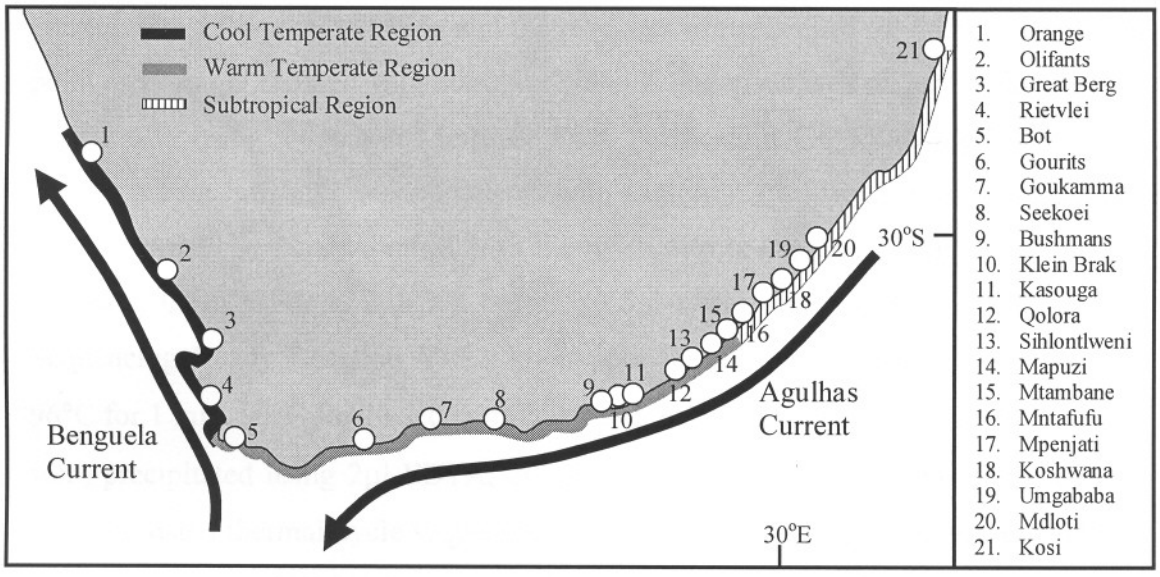

Figure 2.2: Map of South Africa showing the distribution of the 21 estuaries where *Gilchristella aestuaria* were sampled for Chapter 4 (including *G. aestuaria* samples presented in Chapter 3). The two current systems dominating the South African coastline (the cool Benguela Current and the warm Agulhas Current) and the biogeographic regions suggested by Whitfield (1994).

2.2 Laboratory Procedures

DNA from the muscle tissue of individual fish was isolated and extracted using a Qiagen DNEasy tissue kit following manufacturer's animal tissue protocol, with an overnight homogenisation at 55°C in extraction buffer. Using aliquots of 5μl as a template for Polymerase Chain Reaction (PCR) (Saiki *et al.*, 1988), a 608 base pair fragment of the control region of mitochondrial DNA was

amplified for *Atherina breviceps* (F: 5'-CATCTTAGCATCTTCAGTG-3' and R: 5'-TATTCCTGGCATTTGGTTCC-3') and a 636 base pair fragment of the control region was amplified for *Gilchristella aestuaria* (F: 5'-ACATGAATTGGAGGAATACCAGT-3' and R: 5'-GCCCTGAAATAGGAACCAGA-3') in a Thermo Hybaid Px2 Thermal Cycler. To amplify 596 base pairs of the mitochondrial DNA control region of the 138 *G. aestuaria* individuals, the same forward and reverse primes were used as for the smaller *G. aestuaria* dataset. PCR cycling conditions involved an initial denaturation step of 3 mins at 94°C, followed by 35 cycles of 30 seconds at 94°C, annealing for 1 min at 48 – 50°C, extension for 1 min at 72°C, and a final elongation step for 10 mins at 72°C. Amplification was always carried out with a negative control to test for contamination and the reactions were checked on a 1% low-melting point agarose gel, stained with ethidium bromide and visualised under UV light. PCR purification using a Qiagen MinElute PCR Purification Kit following instructions using a microcentrifuge, was completed with elution in 10µl water. Cycle-sequencing was performed in 20µl volumes with the reaction mix containing 2µl purified PCR template, 0.5µl primers, 2µl 5 x buffer, 11.5µl water and 4µl BigDye Terminator Sequencing Ready Reaction V 3.1 kit (Applied Biosystems) under the conditions: 96°C for 1 min, 96°C for 10 sec, 50°C for 5 sec, 60°C for 4 min at 25 cycles. Samples were precipitated using 2µl EDTA, 2µl 3M Sodium Acetate and 50µl 100% ETOH and automated thermal-cycle sequencing was utilised according to the manufacturer's instructions on an ABI 3100 sequencer. Sequences were obtained from both strands of DNA and were checked in GENESTUDIO (GeneStudio Inc, 2004) and aligned using CLUSTAL X within the program GENESTUDIO.

2.3 Substitution model and Phylogenetic analysis

Ambiguous regions with missing data were removed from both the 5' and 3' ends of each dataset. The Akaike Information Criterion (AIC) was employed to facilitate comparisons between 56 alternative models of evolution within the program MODELTEST version 3.06 (Posada and Crandall, 1998) to determine the best-fit nucleotide substitution model for each set of aligned sequences. The Ti:Tv ratio, base frequencies, proportion of invariable sites (I) and the α value of the gamma distribution (rate of variation among sites) were determined from the chosen model.

To reconstruct phylogenetic relationships from the alleles of the different species and datasets, the neighbour-joining (NJ) method of Saitou and Nei (1987) was performed in PAUP* version 4.0b10 (Swofford, 2002). The model of sequence evolution that best described the data sets, as determined by the Akaike Information Criterion (AIC) within MODELTEST version 3.06 (Posada and Crandall, 1998), was incorporated into the reconstructions. Midpoint rooting was applied, as no closely related outgroups were available in Genebank, and lineage support was estimated using 1000 bootstrap replicates.

2.4 Diversity Indices

Estimates of genetic variation were obtained using ARLEQUIN version 2.000 (Schneider *et al.*, 2000). Gene diversity δ (Nei, 1987), which is defined as the probability that two randomly chosen alleles are different in the sample, and nucleotide diversity π (Nei and Jin, 1989), which is the average number of nucleotide differences per site between two sequences, were calculated with standard errors for each population within each species.

2.5 Structure Analysis (Exact tests and AMOVA)

Using ARLEQUIN, exact tests of population differentiation were performed among all the populations. To examine population structure, ARLEQUIN was used to perform an analysis of molecular variance on the control region alleles (Excoffier *et al.*, 1992). This is a hierarchical analysis of population differentiation which estimates the proportion of total genetic variation attributable to different hierarchical levels based on geographical distribution of alleles and pairwise distances between them (Milot *et al.*, 2000). An analogue of Wright's (1965) F_{ST}–statistics which incorporates both haplotype frequencies and the number of nucleotide differences between each pair of haplotypes, Φ_{ST} values were generated to determine how genetic variance is partitioned into the hierarchical categories.

This approach requires that an *a priori* definition of group structure is used to group sets of populations together to form different hierarchical levels in the analysis. Three data partitions were defined and analysed separately for *A. breviceps* in Chapter 3: (a) three groups with divisions based on the biogeographic regions suggested by Whitfield (1994), namely the cool temperate (Great Berg), western warm temperate

(Bot and Seekoei) and eastern warm temperate regions (Bushmans, Kariega and Cefane); (b) four groups based on the results of the neighbour-joining tree; the west coast (Great Berg), Bot, Seekoei and east coast (Kariega, Bushmans and Cefane); (c) five groups, namely individual Great Berg, Bot, Seekoei and Cefane populations, with Bushmans and Kariega grouped as one group as they are the closest two estuaries. Based on the same criteria, three similar hierarchical structures were defined for the comparative *G. aestuaria* dataset in Chapter 3. The first structure divides estuaries into (a) three groups: the cool temperate (Great Berg), western warm temperate (Bot and Seekoei) and eastern warm temperate (Bushmans, Kasouga and Qolora); (b) four groups based on results of the neighbour-joining tree, namely the west coast (Great Berg), Bot, Seekoei and east coast (Bushmans, Kasouga and Cefane); and (c) five groups consisting of individual populations with the two closest estuaries, Kasouga and Bushmans, grouped as one group. Using the gamma corrections found in MODELTEST 3.06, the Tamura-Nei model of substitution was used to calculate genetic distances. Based on these distances, Φ_{ST} values were calculated with 10 000 replicate analysis to test for significance.

Three hierarchical structures were also defined for the large *Gilchristella aestuaria* dataset (Chapter 4). The first structure divides estuaries into three groups according to biogeographic regions as suggested by Whitfield (1994): (a) cool temperate region (Orange, Olifants, Great Berg, Rietvlei), warm temperate region (Bot, Gourits, Goukamma, Seekoei, Bushmans, Klein Brak, Kasouga, Qolora, Sihlontlweni) and subtropical region (Mapuzi, Mtambane, Mntafufu, Mpenjati, Koshwana, Umgababa, Mdloti, Kosi). The second structure (b), forming four groups, includes the cool temperate region (Orange, Olifants, Great Berg, Rietvlei), the subtropical region (Mapuzi, Mtambane, Mntafufu, Mpenjati, Koshwana, Umgababa, Mdloti, Kosi) and divides the warm temperate region into the south coast (Bot, Gourits, Goukamma, Seekoei), and south east coast (Bushmans, Klein Brak, Kasouga, Qolora, Sihlontlweni). This break is based on the potential barrier formed by the Alexandria Coastal Dunefields between the Sundays and Boknes estuaries where Teske *et al.* (In press), found effects of this biogeographic boundary on the phylogeographic patterns of estuarine crustaceans. The third structure (c) made up of five distinct groups; the cool temperate region (Orange, Olifants, Great Berg, Rietvlei), warm temperate region (Bot, Gourits, Goukamma, Seekoei, Bushmans, Klein Brak, Kasouga, Qolora,

Sihlontlweni) and the subtropical region which was divided into two groups. The division was made between the Kosi estuary and the remaining subtropical estuaries (Mapuzi, Mtambane, Mntafufu, Mpenjati, Koshwana, Umgababa, Mdloti) due to the large geographical distance between the furthest estuary sampled northwards on the east coast (Mdloti, north of Durban) and the Kosi system (on the Mozambique border).

Again the Tamura-Nei model with gamma corrections found in MODELTEST 3.06 was used when estimating Φ_{ST} and significance levels were obtained using a permutation approach with 10 000 iterations. Significance testing was conducted by permuting the individuals among the various hierarchical levels and recalculating the null distribution to determine significance (Dyer and Sork, 2001).

2.6 Estimates of gene flow

To asses the estimation of parameters such as the direction of past gene flow between populations, the coalescent based approach in the program MIGRATE v. 2.0.6 (Beerli and Felsenstein, 1999; Beerli and Felsenstein, 2001) was employed to validate inferences made by other phylogeographic methods. This analytical method calculates directional maximum-likelihood estimates of gene flow among populations, allowing for different subpopulation sizes and unequal migration rates (Beerli and Felsenstein, 2001; Gysels *et al.*, 2004). Recently, coalescent based theory approaches have overcome the biologically unrealistic limitations of equal population sizes and symmetrical rates of gene flow imposed by traditional population genetic models (Beerli and Felsenstein, 1999). More powerful than cladistic approaches and superior to traditional pairwise estimators such as those using F_{ST} based methods, this method provides more robust estimates of gene flow (Beerli and Felsenstein, 1999; Nielsen and Wakely, 2001).

Three initial short runs and one longer run were conducted with individual populations for *A. breviceps* and *G. aestuaria* populations in Chapter 3. This was done to test the consistency of results of each run for both species. Parameters for the longer run were (short run settings in parenthesis) ten short chains, each with 5000 (2000) generations and a sampling increment of 250 (100) steps, and three long chains of 50 000 (20 000) generations and 2500 (1000) steps. For both long and short runs,

the first 10 000 generations were discarded (burnin). Default values were implemented for all other parameters.

To avoid computational difficulties and to increase sample sizes within the larger *G. aestuaria* dataset for Chapter 4, individuals were pooled into larger regional populations, similar to Bowie *et al.* (In press). In this case, estuaries were pooled into three regional populations based on the three biogeographic regions along the South African coastline (Whitfield, 1994), namely, the cool temperate region consisting of estuaries on the west coast, the warm temperate region with estuaries from the south and south east coasts, and the subtropical region containing estuaries from the east coast of South Africa. It is possible that these groupings highlight potential problems regarding the current delineation of biogeographic regions, and will be used to test biogeographic boundaries as an explanation for genetic structure.

For the migration analysis of this larger *G. aestuaria* dataset, again three initial short runs were conducted, followed by one longer run. Parameters for the long run (short run values in brackets) were ten short chains, each with 100 000 (2000) generations and a sampling increment of 1000 (100) steps, and three long chains of 1 000 000 (20 000) generations and 10 000 (1000) steps. For both long and short runs, the first 10 000 generations were discarded (burnin). For all other settings, default values were implemented.

Chapter 3

Population structure of two estuarine fish species: *Atherina breviceps* and *Gilchristella aestuaria*

3.1 Sampling

Atherina breviceps samples were found in only six estuaries, although sampling efforts in estuaries around the South African coastline from the Orange to the Kosi systems were conducted. The successful collection of *Gilchristella aestuaria* samples from the same regions as *A. breviceps* enabled a comparison between the two species, which is discussed in this Chapter.

3.2 Results

3.2.1 Substitution model and Phylogenetic analysis

A 636 base pair (bp) fragment of the control region was obtained from 60 *G. aestuaria* individuals, and a 608bp fragment was obtained from 60 *A. breviceps* samples. The *A. breviceps* dataset yielded 46 alleles (Table 3.1) and 92 polymorphic sites. Allele A13 was most common, shared among Seekoei (two individuals), Bushmans (two individuals) and Kariega (one individual). Each individual from the Great Berg population had a unique allele, and in addition, none of the alleles sampled from this estuary were shared with any other population. The *G. aestuaria* dataset yielded 54 unique alleles (Table 3.2), 111 polymorphic sites, and 68 parsimony informative sites. Allele G12 was shared between two sites, the nearby Bushmans (one individual) and Kasouga (one individual) populations. All of the remaining alleles were unique to a single population.

Tamura-Nei (Tamura and Nei, 1993) was determined as the substitution model for *A. breviceps* under the Akaike Information Criterion that best fits the data, with a Ti:Tv ratio of 2.078, I = 0.7869 and $\alpha = 0$. Estimates of base frequencies under this model were A) 25%, C) 23.5%, G) 17.5% and T) 34%. The most suitable model for *G. aestuaria* was the Kimura-2-parameter model, with a Ti:Tv ratio of 1.90, I =

0.6337 and α = 0.7558. Estimates of base frequencies were (A) 41%, (C) 17.8%, (G) 12.7% and (T) 28.5%.

Table 3.1: Frequency of *Atherina breviceps* mtDNA control region alleles for the six sampling sites along the South African coastline.

Allele Number	*N*	Great Berg	Bot	Seekoei	Bushmans	Kariega	Cefane
A1	1	-	1	-	-	-	-
A2	2	-	2	-	-	-	-
A3	2	-	2	-	-	-	-
A4	1	-	1	-	-	-	-
A5	1	-	1	-	-	-	-
A6	1	-	1	-	-	-	-
A7	1	-	1	-	-	-	-
A8	1	-	1	-	-	-	-
A9	3	-	-	-	2	1	-
A10	3	-	-	1	1	-	1
A11	1	-	-	-	1	-	-
A12	2	-	-	1	1	-	-
A13	5	-	-	2	2	1	-
A14	1	-	-	-	1	-	-
A15	1	-	-	-	1	-	-
A16	1	-	-	-	1	-	-
A17	1	-	-	-	-	-	1
A18	2	-	-	-	-	-	2
A19	1	-	-	-	-	-	1
A20	1	-	-	-	-	-	1
A21	1	-	-	-	-	-	1
A22	1	-	-	-	-	-	1
A23	1	-	-	-	-	-	1
A24	1	-	-	-	-	-	1
A25	1	1	-	-	-	-	-
A26	1	1	-	-	-	-	-
A27	1	1	-	-	-	-	-
A28	1	1	-	-	-	-	-
A29	1	1	-	-	-	-	-
A30	1	1	-	-	-	-	-
A31	1	1	-	-	-	-	-
A32	1	1	-	-	-	-	-
A33	1	1	-	-	-	-	-
A34	1	1	-	-	-	-	-
A35	1	-	-	-	-	1	-
A36	1	-	-	-	-	1	-
A37	1	-	-	-	-	1	-
A38	2	-	-	1	-	1	-
A39	1	-	-	-	-	1	-
A40	1	-	-	-	-	1	-
A41	1	-	-	-	-	1	-
A42	1	-	-	-	-	1	-
A43	1	-	-	1	-	-	-
A44	2	-	-	2	-	-	-
A45	1	-	-	1	-	-	-
A46	1	-	-	1	-	-	-
Total	**60**	**10**	**10**	**10**	**10**	**10**	**10**

Table 3.2: Frequency of *Gilchristella aestuaria* mtDNA control region alleles from the small dataset.

Allele Number	*N*	Great Berg	Bot	Seekoei	Bushmans	Kasouga	Qolora
G1	1	-	1	-	-	-	-
G2	1	-	1	-	-	-	-
G3	1	-	1	-	-	-	-
G4	1	-	1	-	-	-	-
G5	1	-	1	-	-	-	-
G6	1	-	1	-	-	-	-
G7	1	-	1	-	-	-	-
G8	1	-	1	-	-	-	-
G9	1	-	1	-	-	-	-
G10	1	-	1	-	-	-	-
G11	1	-	-	-	1	-	-
G12	2	-	-	-	1	1	-
G13	1	-	-	-	1	-	-
G14	1	-	-	-	1	-	-
G15	1	-	-	-	1	-	-
G16	1	-	-	-	1	-	-
G17	1	-	-	-	1	-	-
G18	1	-	-	-	1	-	-
G19	1	-	-	-	1	-	-
G20	1	-	-	-	1	-	-
G21	1	1	-	-	-	-	-
G22	4	4	-	-	-	-	-
G23	1	1	-	-	-	-	-
G24	1	1	-	-	-	-	-
G25	1	1	-	-	-	-	-
G26	1	1	-	-	-	-	-
G27	1	1	-	-	-	-	-
G28	1	-	-	-	-	1	-
G29	1	-	-	-	-	1	-
G30	2	-	-	-	-	2	-
G31	1	-	-	-	-	1	-
G32	1	-	-	-	-	1	-
G33	1	-	-	-	-	1	-
G34	1	-	-	-	-	1	-
G35	1	-	-	-	-	1	-
G36	1	-	-	-	-	-	1
G37	1	-	-	-	-	-	1
G38	2	-	-	-	-	-	2
G39	1	-	-	-	-	-	1
G40	1	-	-	-	-	-	1
G41	1	-	-	-	-	-	1
G42	1	-	-	-	-	-	1
G43	1	-	-	-	-	-	1
G44	1	-	-	-	-	-	1
G45	1	-	-	1	-	-	-
G46	1	-	-	1	-	-	-
G47	1	-	-	1	-	-	-
G48	1	-	-	1	-	-	-
G49	1	-	-	1	-	-	-
G50	1	-	-	1	-	-	-
G51	1	-	-	1	-	-	-
G52	1	-	-	1	-	-	-
G53	1	-	-	1	-	-	-
G54	1	-	-	1	-	-	-
Total	**60**	**10**	**10**	**10**	**10**	**10**	**10**

The substitution model that best described each dataset was incorporated into the neighbour-joining analysis in PAUP* version 4.0b10 (Swofford, 2002). Figure 3.1 and 3.2 show the distance trees for *A. breviceps* and *G. aestuaria* respectively.

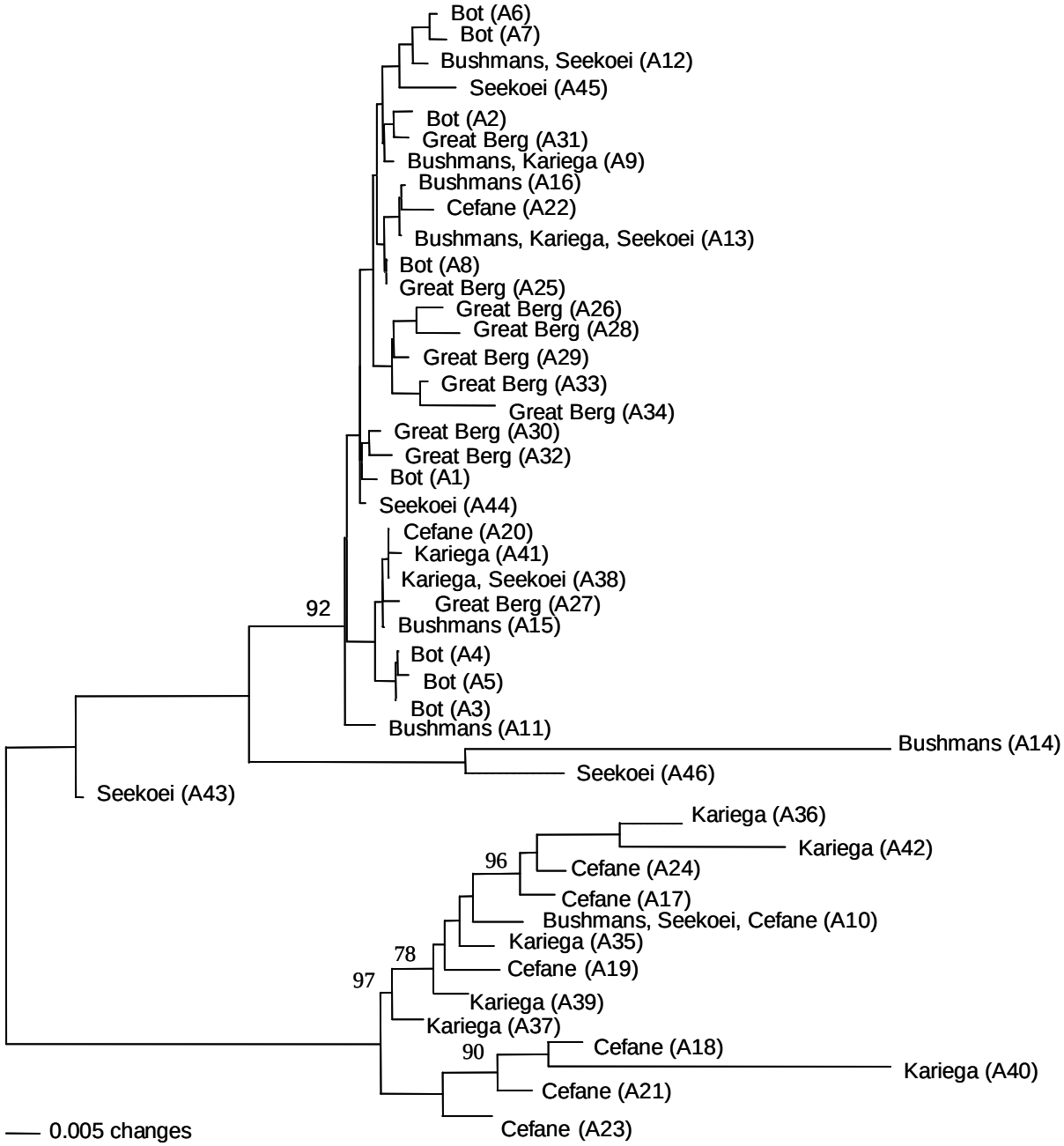

Figure 3.1: The Neighbour-joining phylogram of *Atherina breviceps* mtDNA sequence data. The Tamura-Nei model (Tamura and Nei, 1993) was selected with I = 0.7869 and α = 0. Mid-point rooting was used and numbers at nodes show statistical support obtained from 1000 bootstrap replicates.

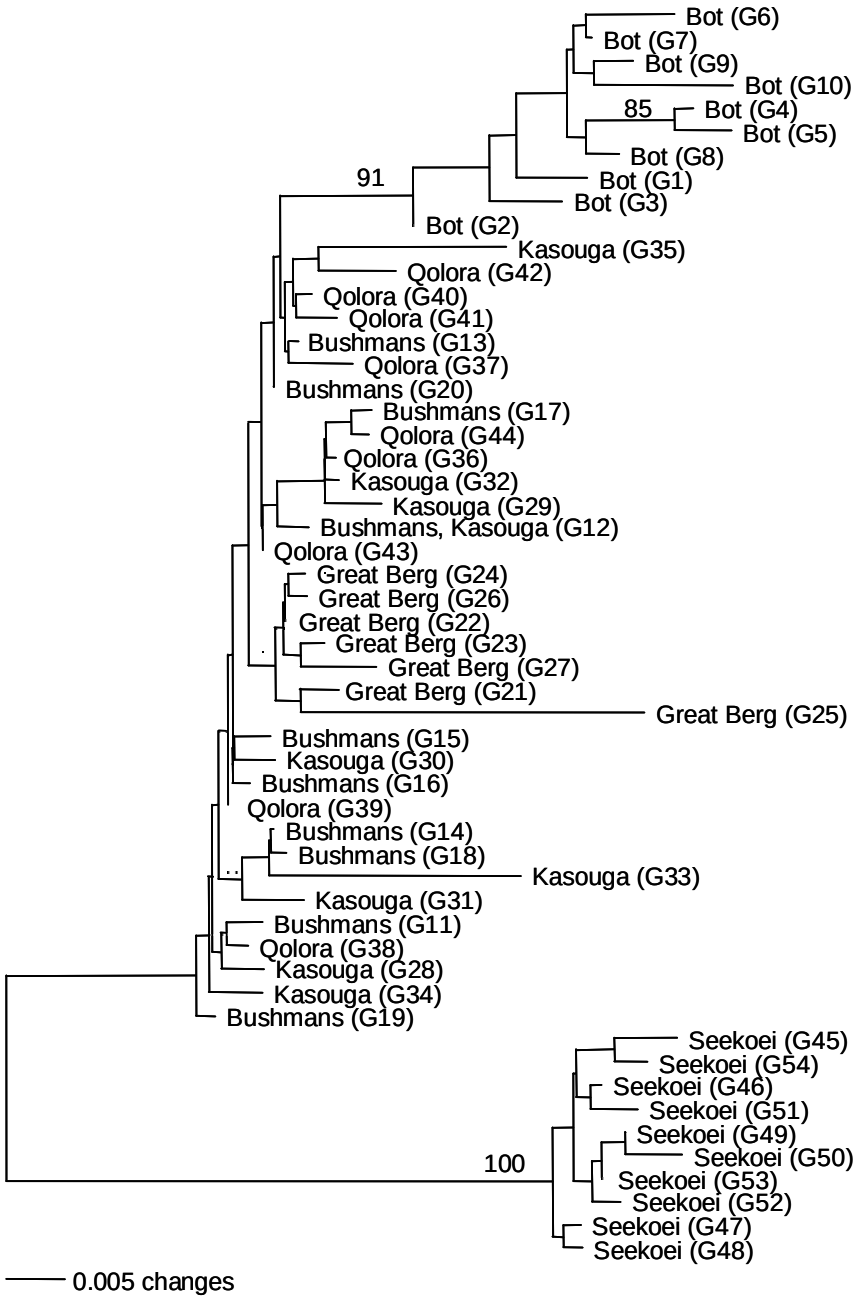

Figure 3.2: Neighbour-joining tree built from the *Gilchristella aestuaria* mtDNA sequence data. The transversional model was selected with I = 0.6337 and α = 0.7558. Mid-point rooting was used and numbers at nodes show statistical support obtained from 1000 bootstrap replicates.

Nested clade analysis (NCA) was not conducted on the *A. breviceps* and *G. aestuaria* datasets. NCA requires confidence in the cladograms as constructed with the program TCS (Templeton *et al.*, 1992), using the rules in Templeton *et al.* (1987) and Templeton and Sing (1993). This confidence was not attained with these datasets, as too many ambiguous branches occurred in the cladograms.

3.2.2 Diversity Indices

Atherina breviceps nucleotide diversity per site ($\pi \pm SE$) is highest for the Kariega sample (0.0478 ± 0.026), which is significantly different from the Great Berg and the Bot estuaries (Bot = 0.007 ± 0.005 and Great Berg = 0.011 ± 0.007) (Figure 3.3; Table A1.1, Appendix One). *Gilchristella aestuaria* nucleotide diversity values (Table A1.2, Appendix One), however, are not significantly different from each other and are generally lower than that for *A. breviceps*. The nucleotide diversity is highest for the Bot sample (0.017 ± 0.009) and lowest for the Bushmans estuary (0.008 ± 0.005).

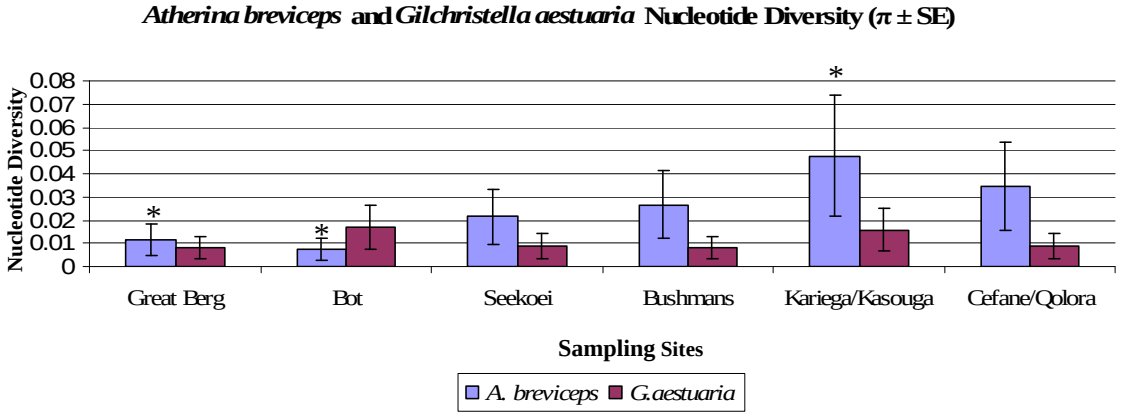

Figure 3.3: Nucleotide Diversity ($\pi \pm SE$) of *Atherina breviceps* and *Gilchristella aestuaria* populations (asterisks indicate significant difference, $P < 0.05$).

The gene diversity for both *Atherina breviceps* and *Gilchristella aestuaria* did not differ significantly from each other, ranging from 0.867 ± 0.107 (*G. aestuaria* from Great Berg) to 1 ± 0.045 (Figure 3.4; Table A1.1 and A1.2, Appendix One).

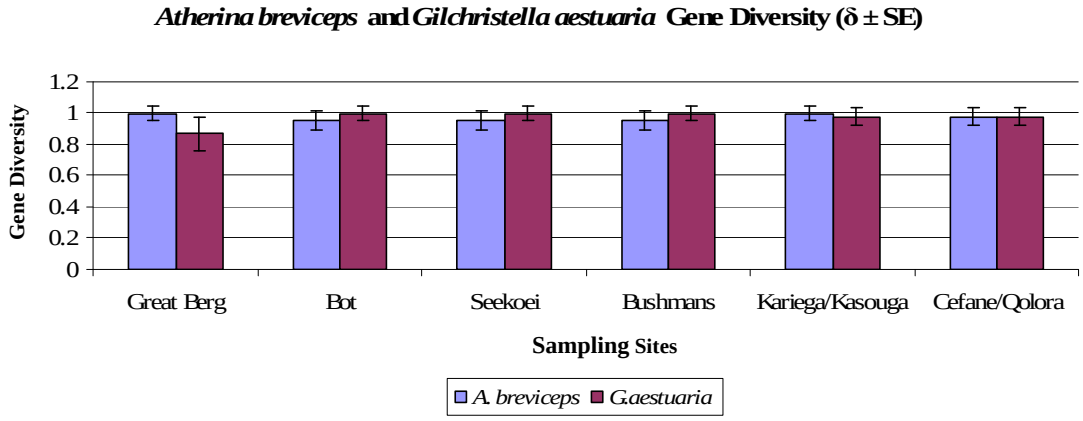

Figure 3.4: Gene Diversity ($\delta \pm SE$) of *Atherina breviceps* and *Gilchristella aestuaria* populations.

3.2.3 Structure Analysis (Exact tests and AMOVA)

The hypothesis of random distribution of alleles between populations, conducted with exact tests in ARLEQUIN, was not rejected for comparisons within the *Atherina breviceps* dataset (P > 0.05) (Table 3.3). In the pairwise population comparisons of *Gilchristella aestuaria* (Table 3.4), the null hypothesis of random distribution of alleles was rejected between the Great Berg and Kasouga and the Great Berg and Qolora populations (P < 0.05) due to differences in allele frequencies.

Table 3.3: Exact test results for individual populations of *A. breviceps* (P > 0.05).

	Bot	Bushmans	Cefane	Great Berg	Kariega	Seekoei
Bushmans	0.052±0.001					
Cefane	0.110±0.002	0.275±0.002				
Great Berg	0.226±0.002	0.224±0.002	0.477±0.003			
Kariega	0.222±0.002	1.000±0.00	0.471±0.003	1.000±0.000		
Seekoei	0.051±0.001	0.676±0.002	0.279±0.002	0.228±0.002	0.777±0.002	

Table 3.4: Exact test results for individual *G. aestuaria* populations (values in bold are statistically significant, P < 0.05).

	Bot	Bushmans	Great Berg	Kasouga	Qolora	Seekoei
Bushmans	1.000±0.000					
Great Berg	0.083±0.002	0.084±0.002				
Kasouga	0.472±0.003	0.721±0.002	**0.042±0.001**			
Qolora	0.474±0.003	0.472±0.003	**0.044±0.001**	0.226±0.002		
Seekoei	1.000±0.000	1.000±0.000	0.087±0.002	0.467±0.003	0.476±0.003	

AMOVA results for *Atherina breviceps* indicates that the majority of variation is explained within populations (a) three groups = 65.94%, (b) four groups = 69.69% and (c) five groups = 69.07% (Table 3.5). Less differentiation occurs between populations within groups, and even less among groups for all *a priori* structures.

Most of the variation between the *Gilchristella aestuaria* samples, as measured with AMOVA, was explained by differentiation among groups in two of the three specified *a priori* structures (b) four groups = 72.60% and (c) five groups = 69. 36% (Table 3.2). Variation among populations within groups accounted for very little variation when (b) four groups and (c) five groups were specified (0.14% and 0.22% respectively), and variation within populations also explained less of the diversity compared to the differentiation among groups. The *a priori* structure where estuaries in which *G. aestuaria* were sampled were divided up into only three groups, based on the west, south and east coasts, showed a different trend. The most differentiation was observed between populations within groups (62.34%), the within population differentiation was similar to the values obtained by the four and five group *a priori* structures (31.36%), and the among group variation was the lowest (6.30%). These results suggest that separation of estuaries into three groups was a weak explanation of genetic structuring compared to the *a priori* structures where more groups were defined. From AMOVA results of the comparative *G. aestuaria* dataset, it appears that the grouping of estuaries into four structures; west coast (Great Berg), Bot (south coast), Seekoei (south coast) and east coast (Bushmans, Kasouga and Cefane) provides a better explanation of genetic structuring.

The overall Φ_{ST} values for *G. aestuaria* are all large (0.686, 0.727 and 0.696) and significant ($P > 0.05$), whereas *A. breviceps* overall Φ_{ST} values are considerably lower (0.341, 0.303 and 0.309). Φ_{ST} Values for all *A. breviceps a priori* structures are also significant.

Table 3.5: *Gilchristella aestuaria* and *Atherina breviceps* results for the *a priori* population structures defined in AMOVA, using the program ARLEQUIN v 2.000. Asterisks indicate significant results (P < 0.05).

	Variance Components					
Source of variation	**(a) 3 Groups**		**(b) 4 Groups**		**(c) 5 Groups**	
	G. aestuaria	***A. breviceps***	***G. aestuaria***	***A. breviceps***	***G. aestuaria***	***A. breviceps***
Among Groups	0.712 (6.30%)	1.402 (11.39%)	9.427 (72.60%)*	-0.446 (-4.11%)	8.072 (69.36%)	0.127 (1.16%)
Populations within groups	7.038 (62.34%)*	2.791 (22.67%)*	0.018 (0.14%)*	3.734 (34.42%)*	0.025 (0.22%)	3.260 (29.77%)*
Within populations	3.540 (31.36%)*	8.119 (65.94%)*	3.540 (27.26%)*	7.561 (69.69%)*	3.540 (30.42%)*	7.561 (69.07%)*
Overall Φ_{ST}	0.686*	0.341*	0.727*	0.303*	0.696*	0.309*

3.2.4 Estimates of gene flow

MIGRATE v 2.0.6 results from the *Atherina breviceps* dataset (Table 3.6) shows *m* values that are consistently between 0 and 4.782 for all systems, and that do not fluctuate dramatically between estuaries as seen in the *Gilchristella aestuaria* results (Table 3.7). Although values for migration between some estuaries within the *A. breviceps* dataset did not converge, which leaves gaps in the data; very few values were zero, which indicates a lack of migration. Migration from the Kariega to the Great Berg and from the Great Berg to the Bot estuaries is higher than the other population comparisons, but lack duplicate results from the three other runs to validate this migration.

MIGRATE results from the *Gilchristella aestuaria* dataset (Table 3.7) shows a separation of the Seekoei estuary individuals from all other systems as no detectable migration occurs between the Seekoei and the Great Berg estuary on the west coast, the Bot estuary on the south coast and the Bushmans estuary on the east coast. The results for migration in the opposite direction from the Great Berg, Bot and Bushmans to the Seekoei, support this lack of migration between these systems. Little migration was detected between the Seekoei and the Kasouga and Qolora estuaries; with results from all four MIGRATE runs ranging between 0 and 0.667.

The largest volume of *G. aestuaria* migration occurs between the Kasouga and Bushmans estuaries, the two closest systems situated approximately 8km's apart. In the four runs conducted with this dataset, the values recorded were for migration from the Kasouga to the Bushmans (15.930 to 191.707), or from the Bushmans to the Kasouga (19.100 to 167.337). There are also high levels of migration in both directions between the Bushmans, Kasouga and Qolora systems, which is consistent with the subdivision of these three systems from the other estuaries in the neighbour-joining tree. From Bushmans to Qolora, three runs showed values of 18.850, 21.330 and 24.922 and from Qolora to Bushmans, values ranged from 2.715 to 44.194, and from Qolora to Kasouga from 14.935 to 61.381

Table 3.6: Results from four runs of the *Atherina breviceps* data on the computer software program MIGRATE. Values presented are the effective number of migrants per generation (*m*).

		RECEIVING POPULATION					
		Bot	**Bushmans**	**Cefane**	**Great Berg**	**Kariega**	**Seekoei**
DONATING POPULATION	**Bot**		0.3076 0.4146 0.6219 0.4918	0 0 0 1.2439	1.2303 3.7317 0.3109 5.4094	0.3076 0 0 0	2.7681 2.7956 0 1.697
	Bushmans	0.1658 4.525		1.1721 19.9956	0.0003 1.746	0.2921 3.7269	
	Cefane	0.481 1.1905 0.2025 0	3.377 0.5953 0 1.6527		0.5953 0.1012 0.4832 0.6611	2.9764 0.2025 0.9656 0.9916	4.7622 0.8098 2.1701 1.3222
	Great Berg	31.0858	4.7824	4.7824		2.3912 0.0002	4.7824
	Kariega		1.9488		58.1478		0.0007
	Seekoei	0.8057 0 0.1261 0.1367	0.1404 0.9407 0.3782 1.9137	1.2634 2.0156 0.1261 0.5468	1.5442 1.6115 0.2521 1	0.5615 0.4031 1.8908 0	

The *G. aestuaria* results for movement from the Bot estuary to all other systems are consistently low for all runs and show an equal amount of migration from the Bot River into the Berg, Kasouga and Qolora estuaries. Most migration from the Bot estuary was towards the Great Berg system, around the Cape Peninsula. The most anomalous results are the high migration occurring from the Bushmans and Kasouga to the Great Berg estuary. All values showing migration from the Great Berg to other systems in the opposite direction to the flow of the main oceanic current is very low, between 0 and 0.539.

Table 3.7: MIGRATE results from four separate runs conducted with the small *Gilchristella aestuaria* dataset. Values presented are the effective number of migrants per generation (*m*).

		RECEIVING POPULATION					
		Bot	**Bushmans**	**Great Berg**	**Kasouga**	**Qolora**	**Seekoei**
DONATING POPULATION	**Bot**		0 0 0 0.4935	0 0.6902 0.5912 0.6062	0 0 0 0.6062	0 0 0 0.6902	0 0 0 0
	Bushmans	0 0 0 0.6037		0 6.8234 9.844 86.1755	167.3368 25.2259 40.3948 19.1004	21.3309 18.8509 0.001 24.9225	0 0 0 0
	Great Berg	0 0 0 0	0.5386 0.2936 0.069 0.1146		0 0.207 0.1146 0.482	0 0 0.2936 0.069	0 0 0 0
	Kasouga	0 0 0 0.0002	191.7074 15.9309 42.6535 47.9861	106.5086 13.853 21.8736 20.8633		4.1559 6.5621 4.1727 9.0803	0 0 2.0864 4.4583
	Qolora	0 0 0.0061 1.2096	2.7154 44.1938	0.0399 1.3577 58.9257	14.9345 61.3812		0 0 0.0023 0.1726
	Seekoei	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0.436	0 0.6671 0.4209 0.436	

3.3 Discussion

The results of the comparative study between *Atherina breviceps* and *Gilchristella aestuaria*, have demonstrated two different population structure patterns for these two estuarine fish species. The *A. breviceps* results indicate a mixed, population along the South African coastline with high levels of gene flow between estuaries. Conversely, the results from the data analysis of *G. aestuaria* indicate a more structured genetic pattern along the coastline, with differentiation of the population into four groups.

Grouping of *G. aestuaria* individuals is evident in the neighbour-joining tree constructed in PAUP (Figure 3.2), where samples from the Seekoei and the Bot estuaries form two separate lineages and Great Berg individuals remain together in the third lineage. The fourth lineage consists of individuals from the east coast. The separation of the *G. aestuaria* population into these four lineages is supported by the AMOVA results (Table 3.5), where genetic structuring among groups was highest when the structures were defined as; Great Berg, Bot, Seekoei and east coast estuaries. The high overall Φ_{ST} values in AMOVA indicate high levels of structuring in these *a priori* groupings and similarly, the subdivision of populations is supported by MIGRATE results (Table 3.7). Only one allele was shared between two estuaries and all other alleles were unique to individual estuaries indicating limited gene flow of this species. In the pairwise population comparisons of *G. aestuaria* conducted with exact tests, the null hypothesis of random distribution of alleles was rejected between the Great Berg and Kasouga and the Great Berg and Qolora populations (Table 3.4) and therefore structured populations along the coastline are expected.

The *Atherina breviceps* dataset yielded fewer alleles than the *G. aestuaria* dataset due to sharing of alleles by more than one individual within estuaries. In addition, one allele was shared between estuaries from the east and south coasts. The hypothesis of random distribution of alleles between populations, conducted with exact tests (Table 3.3), was not rejected, indicating a lack of population structure. This is supported by the Φ_{ST} values from the AMOVA analysis (Table 3.3), which are lower than for *G. aestuaria*, indicating less population differentiation. The neighbour-joining analysis in PAUP (Figure 3.1) also suggests gene flow, as there is mixing of individuals in both lineages. MIGRATE results (Table 3.4) indicate gene flow between all estuaries, in both directions along the east, south and west coasts of South Africa.

The abovementioned differences in population structure of *A. breviceps* and *G. aestuaria* along the South African coastline can to some extent be related to the life history patterns and ecology of these two fish species. According to Jones and Quattro (1999) and Gold *et al*. (1999), differences in population structure may be attributed to differences in biology of marine animals, environmental influence or behaviour. The fact that *A. breviceps* does not complete its entire life cycle within the estuarine

environment and breeds at sea (Whitfield, 1990)**,** spawning and releasing offspring in bays in the marine environment or near estuaries in the open ocean, may contribute to the high gene flow observed. This breeding strategy, coupled with the fact that this species employs shoaling as an anti-predation behaviour, results in a large well-mixed larval population where ocean currents, eddies and other transport mechanisms are able to disperse them over a wide region.

Conversely, *G. aestuaria* has a completely estuarine life cycle, spawning in the upper reaches of estuaries and avoiding the open ocean. As they only extend their distribution towards the mouth with an increase in size and when they are strong enough to maintain their position, *G. aestuaria* also avoids being swept out to sea by selecting areas with lower current velocities (Whitfield, 1989). In a study by Wooldridge and Bailey (1982), very few *G. aestuaria* eggs were recorded in the lower half of the Sundays estuary in comparison to the upper half. In another study on the Swartvlei estuary by Whitfield (1989), *G. aestuaria* larvae were not recorded in three of four sampling sessions; despite the fact that early life stages of this species were abundant in the lake-like upper reaches of the system. This different breeding strategy results in comparatively few individuals dispersing in ocean water bodies, and results in more structured populations.

As *G. aestuaria* has not been recorded shoaling or breeding in the marine environment, the only opportunity for individuals to be exported to the open ocean is through flushing of estuaries during flood events or breaching. In a study by Strydom *et al.*, (2002), *G. aestuaria* larvae and juveniles were flushed out of the Great Fish system as a result of excessive river flow received from an interbasin water transfer scheme. Larvae spawned in the upper-reaches of a high flow estuary, like the Great Fish, run a risk of being washed out to sea as flushing influences the early developmental stages of fish larvae (Harvey, 1987), particularly when larvae hatch in the underdeveloped state characteristic of *G. aestuaria*. Although larvae of this species are known to use tidal currents in stratified water columns to keep position in an estuary, the effectiveness in the mouth region is reduced where current stratification is decreased by turbulence of fast flowing water. The abovementioned study by Strydom *et al.* (2002), have demonstrated that movement of *G. aestuaria* does occur from estuaries into the marine environment.

Once the individuals of these two species are within coastal water bodies and available for dispersal, the physical oceanography along the South African coastline contributes to the explanation of the different population structures identified. The Agulhas Current, up until the region of Port Alfred, flows within a narrow band in close proximity to the continental shelf, playing a role in the transportation of species down the east coast of South Africa. Surface waters of the current are able to penetrate the shelf waters at regular intervals (e.g. Natal Bight) due to short term current reversals as a result of shear edge eddies or wind activity (Lutjeharms, 2005). These factors combined, allow for specimens entrained within the surface waters of the current to recruit into nearby estuaries and maintain their position on the east coast.

Both *A. breviceps* and *G. aestuaria* datasets demonstrate the abovementioned effects on their population structure, as, although individuals from all six sampling sites are randomly distributed in the *A. breviceps* neighbour-joining tree, the bottom lineage is comprised mainly of individuals from the Cefane, Kariega and Bushmans estuaries (east coast) and MIGRATE results indicate migration in both directions between these three systems. The equivalent east coast estuaries in the *G. aestuaria* dataset, namely the Qolora, Kasouga and Bushmans systems, formed the fourth lineage in the neighbour-joining tree, and were identified by the AMOVA results. The MIGRATE results also support migration of this species between these three systems. Both the *A. breviceps* and *G. aestuaria* datasets reflect the interaction between these biota and the inshore Agulhas Current.

South of Port Elizabeth, the Agulhas Current begins to move offshore where the continental shelf becomes wider (Lutjeharms, 2005). This dramatically reduces the probability of an organism trapped within a current being able to recruit back into estuaries. At this southern tip of the African continent, where the shelf is wider, the Agulhas sheds large rings of warm water due to baroclinic instabilities at the Agulhas Retroflection, leading to a substantial transfer of water from the Indian to the Atlantic Ocean systems (Lutjeharms and Ballegooyen, 1988).

The abovementioned currents along the coastline assist in explaining the structure of the Bot and Seekoei estuary lineages in the *G. aestuaria* dataset, and the presence of a

more contiguous *A. breviceps* population. As *A. breviceps* is able to move in large shoals within bays around the coastline, this species avoids possible transfer offshore when the shelf becomes wider, resulting in recruitment of *A. breviceps*, into estuaries on the south coast of South Africa. This is evident in the neighbour-joining tree, where samples from the Seekoei region (south coast), appear in the bottom lineage with Cefane, Kariega and Bushmans samples (east coast). Conversely, *G. aestuaria* samples collected from the Seekoei estuary form their own lineage with 100% bootstrap support, contributing to the population structure evident in this species. Similar patterns occur regarding the Bot system, as *G. aestuaria* individuals form a third lineage in the neighbour-joining tree, comprised of Bot estuary samples only. As *G. aestuaria* have no marine-phase characteristics such as shoaling and use of bays, and the current no longer flows close inshore, it appears more difficult for this species to recruit from one south coast estuary into another and isolation by distance plays a role in their population structure.

For the same reasons, migration of *G. aestuaria* around the Cape Peninsula and into west coast systems seems unlikely, and the Great Berg individuals form a fourth lineage on the neighbour-joining tree. Although all Great Berg samples remain together on their own arm within this lineage, this fourth group falls within the lineage comprised of east coast individuals. In addition, MIGRATE results indicate a small amount of migration from the Bot to the Great Berg, and migration from the east coast estuaries to the Great Berg system. This migration from the east coast to the west coast can be explained by eddies that shear off from the Agulhas Retroflection zone and contribute to the Benguela Current running up the west coast. This would provide an organism with a mechanism of rounding Cape Point from the east coast, but around the south coast estuaries, there are less influential inshore currents, contributing to the lack of movement of *G. aestuaria*. *Atherina breviceps* samples from the Bot and Great Berg estuaries are dispersed between individuals from the four other systems in the neighbour-joining tree, once again indicating a more contiguous population.

Although *A. breviceps* is adapted for a marine phase and utilises bays along the coastline, there are additional mechanisms which facilitate movement onto the west coast, enabling this species to round Cape Point. Such an event has been discussed by Roy *et al.* (2001) (Figure 1.6), who encountered water originating from the Agulhas in

the main upwelling cells of Cape Point, Cape Columbine and north of Hondeklip Bay. This anomaly caused a collapse in the upwelling conditions, covering the entire continental shelf north of Cape Peninsula with warm Agulhas surface water. In addition, intense wind-driven surface Ekman transport has been shown to facilitate the free exchange of water around the southern end of the African continent (Figure 1.7) (Sherman *et al.*, 1993). This transport provides an additional mechanism allowing the transportation of species from the south east coast to the west coast.

This comparative study of the population structure of *A. breviceps* and *G. aestuaria* has demonstrated two different patterns of distribution. *Atherina breviceps* exhibits gene flow between the six estuaries sampled, whereas *G. aestuaria* demonstrates structuring of the population into four groups. This grouping separates estuaries into the west coast (Great Berg), east coast (Qolora, Kasouga and Bushmans), Bot (south coast) and the Seekoei (south coast). The different phylogeographic patterns observed for these two small estuarine fish species can be explained by a combination of biology, behaviour and the complex physical environment along the southern African coastline.

Chapter 4

Population structure of *Gilchristella aestuaria*

4.1 Sampling

In the previous chapter detailing the comparison of *Atherina breviceps* and *Gilchristella aestuaria*, sampling was restricted to six estuaries as *A. breviceps* could not be located in some systems. Sampling was, however, conducted in estuaries spanning the South African coastline from the Orange to the Kosi systems, and an additional 138 *Gilchristella aestuaria* individuals were sequenced from a further 15 sites within the cool temperate, warm temperate and subtropical biogeographic regions. This enabled a broader analysis of the population structure of *G. aestuaria*, and is discussed in this chapter.

4.2 Results

4.2.1 Substitution model and Phylogenetic analysis

The General Time Reversible model was determined as the substitution model that best fits the dataset, with a Ti:Tv ratio of 2.421, invariable sites (I) = 0.6144 and gamma distribution (α) = 0.7203. The base frequencies under this model were A) 43%, C) 16%, G) 12% and T) 29%. The 596 base pair fragment of mtDNA for the 138 *Gilchristella aestuaria* individuals analysed, yielded 116 alleles (Table 4.1) with 135 polymorphic sites and 73 parsimony informative sites. Alleles 22, 57 and 73 were each shared between three estuaries. Allele 22 was shared between Bushmans, Mntafufu and Umgababa, allele 57 between Kosi Bay, Qolora and Mntafufu and allele 73 between Qolora, Sihlontlweni and Mapuzi. The majority of this sharing thus occurred between populations in the warm temperate and subtropical regions. Allele 30 was shared between the Great Berg population and the Olifants population on the West Coast.

Table 4.1: Frequency of *Gilchristella aestuaria* mtDNA control region alleles from the large dataset.

Allele No.	*N*	Orange	Olifants	Great Berg	Rietvlei	Bot	Gourits	Goukamma	Seekoei	Bushmans	Klein Brak	Kasouga	Qolora	Sihlontlweni	Mapuzi	Mtambane	Mntafufu	Mpenjati	Koshwana	Umgababa	Mdloti	Kosi
1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
6	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	2	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
17	2	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
18	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
19	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
20	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
21	2	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-
22	3	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	1	-	-
23	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
24	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
25	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
26	2	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
27	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
28	2	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
29	2	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	5	-	1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4.1 continued…

Allele No.	*N*	Orange	Olifants	Great Berg	Rietvlei	Bot	Gourits	Goukamma	Seekoei	Bushmans	Klein Brak	Kasouga	Qolora	Sihlontlweni	Mapuzi	Mtambane	Mntafufu	Mpenjati	Koshwana	Umgababa	Mdloti	Kosi
32	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	2	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
51	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
52	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
53	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
54	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
55	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
56	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
57	3	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	1
58	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
59	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
60	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
61	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
62	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
63	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

Table 4.1 continued…

Allele No.	*N*	Orange	Olifants	Great Berg	Rietvlei	Bot	Gourits	Goukamma	Seekoei	Bushmans	Klein Brak	Kasouga	Qolora	Sihlontlweni	Mapuzi	Mtambane	Mntafufu	Mpenjati	Koshwana	Umgababa	Mdloti	Kosi
64	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
65	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
66	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
67	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
68	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
69	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
70	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
71	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
72	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
73	3	-	-	-	-	-	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-
74	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
75	2	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-
76	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
77	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
78	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
79	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
80	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
81	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
82	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
83	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
84	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
85	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
86	2	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
87	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
88	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
89	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
90	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
91	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
92	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
93	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
94	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
95	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4.1 continued…

Allele No.	*N*	Orange	Olifants	Great Berg	Rietvlei	Bot	Gourits	Goukamma	Seekoei	Bushmans	Klein Brak	Kasouga	Qolora	Sihlontlweni	Mapuzi	Mtambane	Mntafufu	Mpenjati	Koshwana	Umgababa	Mdloti	Kosi
96	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
97	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
98	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
99	2	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-
100	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
101	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
102	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
103	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
104	2	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
105	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
106	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
107	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
108	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
109	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
110	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
111	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
112	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
113	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
114	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
115	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
116	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Total	**138**	**9**	**6**	**10**	**3**	**10**	**10**	**5**	**10**	**10**	**6**	**8**	**10**	**2**	**5**	**5**	**4**	**3**	**5**	**4**	**3**	**10**

In Figure 4.1, the phylogenetic relationship between all *Gilchristella aestuaria* alleles based on the neighbour-joining method with mid-point rooting is shown. A lineage is formed by alleles from estuaries within the warm temperate region (Bot, Gourits and Seekoei), and a second lineage comprised of alleles from the cool temperate region is also evident. Exceptions to these groupings consists of the cluster of alleles; 87, 94, 86, 88 and 39 (indicated on the phylogram with asterisks). Alleles from the subtropical region on the east coast and the warm temperate region on the south east coast are mixed.

To explore phylogeographic structure, network approaches have been developed as they take into account the unique characteristics of intraspecific datasets and avoid the violation of the assumptions of maximum likelihood (ML) and maximum parsimony (MP) methods (Posada and Crandall, 2001). Nested clade analysis has power to detect geographical associations and allows a wider range of gene-flow parameters to be estimated, however, this analysis requires confidence in the cladograms derived by TCS (Templeton *et al.*, 1992), which was not possible in this dataset as there were too many ambiguous branches.

4.2.2 Diversity Indices

Haplotype Diversity is similar throughout all samples, with the lowest value for Rietvlei (0.6667 ± 0.3143) and the highest value at 1 ± 0.5 for Sihlontlweni (Table A1.3, Appendix One; Figure 4.2). Nucleotide diversity per site (π ± SE) is highest for the Mpenjati sample (0.021436 ± 0.01672) and the lowest for the Sihlontlweni sample (0.003403 ± 0.00416) (Table A1.3, Appendix One; Figure 4.3). The Sihlontlweni sample is significantly different from the Koshwana, Kasouga and Gourits samples.

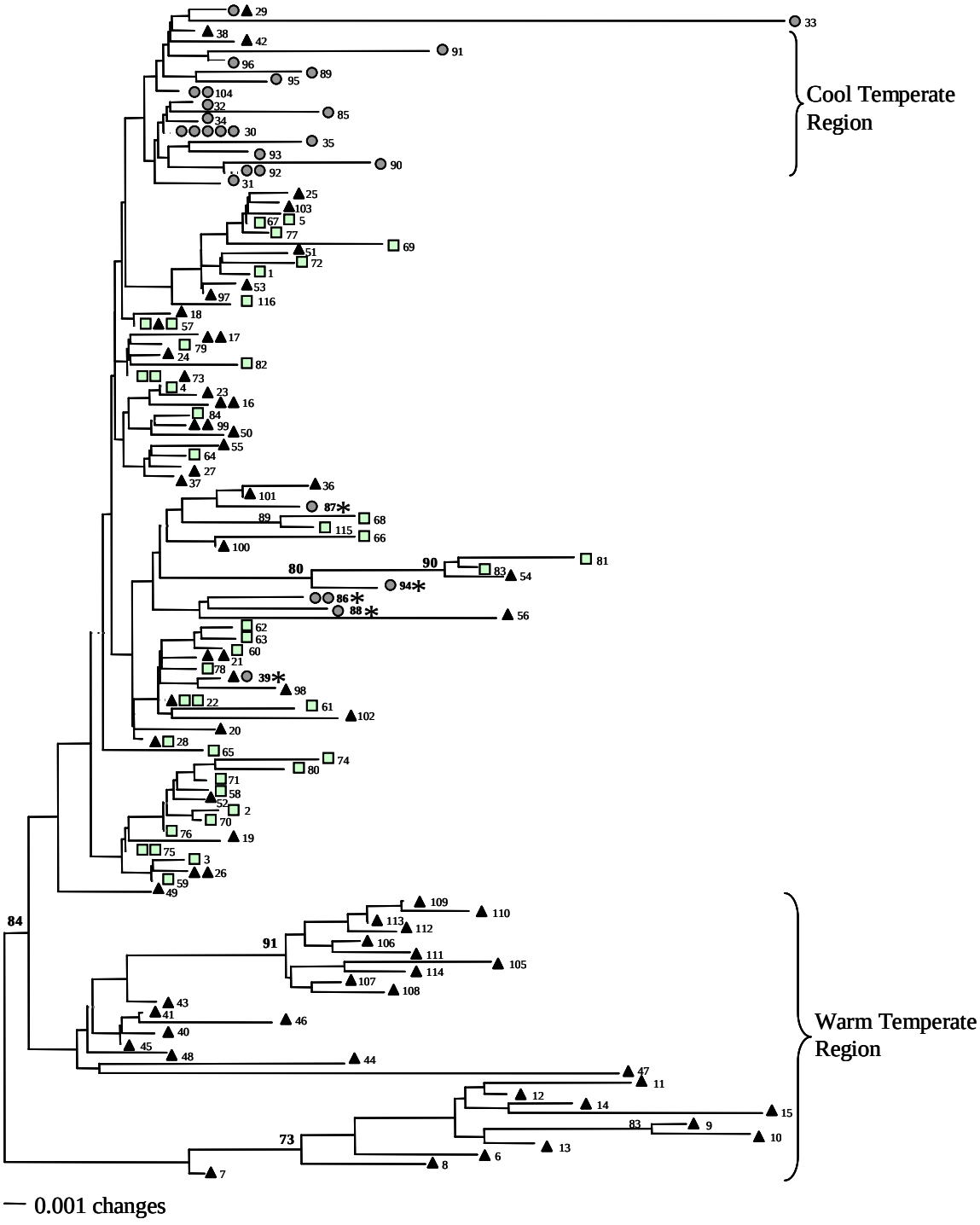

Figure 4.1: Neighbour-joining phylogram built from *Gilchristella aestuaria* mtDNA sequence data, with mid-point rooting. Numbers at nodes indicate the statistical support obtained from 1000 bootstrap replicates. The General Time Reversible model was selected with invariable sites (I) = 0.6144 and gamma distribution (α) = 0.7203. The biogeographic region where each allele is found is indicated by shapes at the tips of branches; ▲ = Warm Temperate Region, ○ = Cool Temperate Region and □ = Subtropical Region.

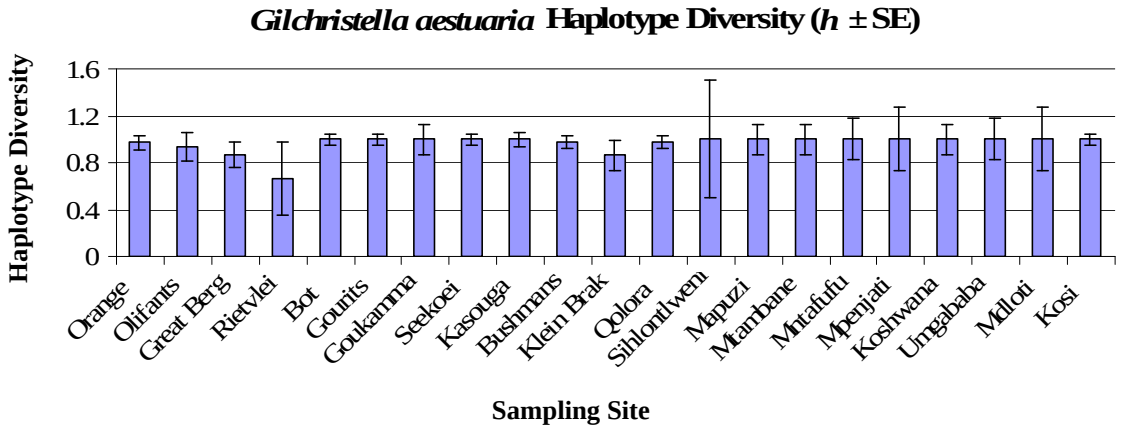

Figure 4.2: Haplotype Diversity (*h* ± SE) of *Gilchristella aestuaria* populations.

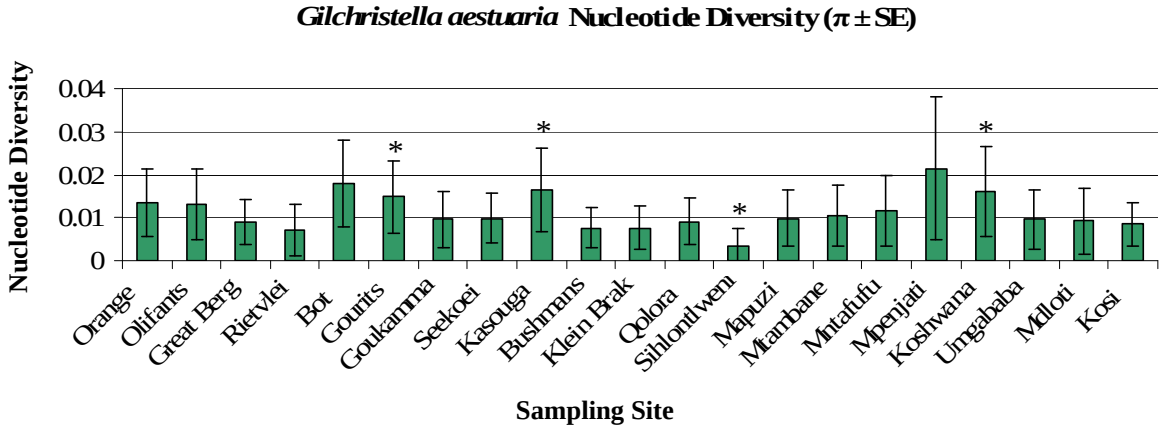

Figure 4.3: Nucleotide Diversity (π ± SE) of *Gilchristella aestuaria* populations. Asterisks indicate statistical significance (P < 0.05).

4.2.3 Structure Analysis (Exact tests and AMOVA)

When exact tests were performed in ARLEQUIN, the null hypothesis of random distribution of alleles was rejected in some of the pairwise population comparisons of *Gilchristella aestuaria*. The Great Berg in comparison to the Klein Brak, Bushmans, Qolora and Orange systems respectively showed significant differences in allele frequencies.

Most of the variation between the *Gilchristella aestuaria* samples, as measured with AMOVA (Table 4.2), was explained by differentiation within populations in all of the three specified *a priori* structures. Variation among groups accounted for very little

variation in all three instances (three groups = 3.35%, four groups = 10.99% and five groups = 8.24%), and variation of populations within groups also explained less of the diversity (three groups = 31.08%, four groups = 24.02% and five groups = 26.21%) compared to the differentiation within populations. All three *a priori* groupings give very similar results, and neither can be preferentially selected over the other. From these AMOVA results, it appears that the grouping of estuaries into the three biogeographic regions defined by Whitfield (1994); namely the cool temperate, warm temperate and subtropical regions, does not form a better structure than the other *a priori* structures.

Table 4.2: *Gilchristella aestuaria* population structure based on analysis of molecular variance (AMOVA). Asterisks indicate statistically significant values (P < 0.05).

	Variance Components		
Source of variation	**(a)** **3 Groups**	**(b)** **4 Groups**	**(c)** **5 Groups**
Among Groups	0.175 (3.35%)	0.565 (10.99%)*	0.420 (8.24%)
Populations within groups	1.623 (31.08%)*	1.236 (24.02%)*	1.337 (26.21%)*
Within populations	3.424 (65.56%)*	3.344 (64.99%)*	3.344 (65.55%)*
Overall Φ_{ST}	0.344*	0.350*	0.344*

4.2.4 Estimates of gene flow

The four MIGRATE runs produced similar results of migration between the three regions, and the different runs were used to verify one another (Table 4.3). Most gene flow occurred from the warm temperate to the subtropical region, and a notable amount occurred from the warm temperate to the cool temperate region. The values from the warm temperate region show a trend as being the highest throughout all four runs. It appears that no discernable gene flow was detected from the subtropical east coast to the cool temperate region on the west coast; however a small amount can be detected from the cool temperate to the warm temperate regions.

Table 4.3: MIGRATE results for *G. aestuaria* with populations separated into the cool temperate, warm temperate and subtropical regions. Values presented are the effective number of migrants per generation (*m*).

		RECEIVING POPULATION		
		Cool Temperate	**Warm Temperate**	**Subtropical**
DONATING POPULATION	**Cool Temperate**		4.69 0 2.179 0.715	0 1.702 1.112 2.629
	Warm Temperate	10.815 9.167 7.875 9.152		111.755 136.783 168.892 180.099
	Subtropical	0 0 0 0.28	0.588 0 0 0	

4.3 Discussion

Results of the comparative study between *Atherina breviceps* and *Gilchristella aestuaria* (Chapter 3) demonstrated more population differentiation for the estuarine roundherring, *G. aestuaria*, than *A. breviceps*. The larger *G. aestuaria* dataset discussed in this chapter provides a more comprehensive overview with the inclusion of samples from an additional 15 sites. The results of this study indicate a structured population along the South African coastline, with potential differentiation of populations into three regions.

The majority of the 116 alleles from this dataset (Table 4.1) are represented by a single individual and are found at only one location, however, some geographical patterns in allele relationship distribution are evident. Sharing of alleles only occurs between populations in the warm temperate and subtropical regions, and this structuring is supported by MIGRATE results (Table 4.3) which indicate substantial migration of *G. aestuaria* between the warm temperate and subtropical regions, in the direction of the subtropical region. The neighbour-joining tree constructed in PAUP (Figure 4.1) demonstrates the subdivision of this population into three lineages, which

is supported by the AMOVA analysis where all the *a priori* groupings show structuring within populations. Thus, the grouping of estuaries into the three biogeographic regions as suggested by Whitfield (1994); is not necessarily the most reasonable explanation of genetic structuring. Similarly, exact tests reject the null hypothesis of random distribution of alleles in some of the pairwise population comparisons of *G. aestuaria,* suggesting population differentiation.

The abovementioned population structure of *G. aestuaria* along the South African coastline can to some extent be related to the life history patterns and breeding strategy of this estuarine species. As discussed in Chapter 3, *G. aestuaria* breeds in the upper reaches of estuaries and completes its entire life cycle in the estuarine environment. This results in relatively few individuals available for dispersal and migration through the open ocean. However, as discussed by Strydom *et al.* (2002), flushing events result in the movement of *G. aestuaria* from the estuarine to the marine environment where physical oceanography and the nature of the South African coastline contributes to the migration of *G. aestuaria* between different regions.

The proximity of estuaries on the east and south east coasts of South Africa (Table A2.1, Appendix Two) may explain the observed gene flow of *G. aestuaria*. Approximately 70% of the 600km^2 estuarine region of South Africa lies in the subtropical areas of KwaZulu-Natal and the Eastern Cape (Whitfield, 1998). The average distance between the 159 estuaries (estuarine count and map distances calculated from Harrison *et al.*, 2000) within the subtropical zone is ≈ 5.3km, whereas the average distance between the 34 estuaries located in the cool temperate region on the west coast is ≈ 21km. In the warm temperate zone, the average distance between the 151 estuaries is ≈ 8.6km. The close proximity of estuaries in the subtropical zone suggests that if *G. aestuaria* is flushed into the marine environment, the probability of it recruiting into a neighbouring estuary is greater.

This migration is assisted by the flow of the Agulhas Current, which follows the continental shelf closely up until the region of Port Alfred (Lutjeharms and Ansorge, 2001). Shelf waters mix with surface waters of the Agulhas Current (Lutjeharms, 2005), which assists species to recruit into nearby estuaries on the east and south east coasts. This mixing of water bodies explains some of the overlap and migration

between the subtropical and northern extent of the warm temperate biogeographic regions (approximately 300km south of the suggested boundary).

South of Port Elizabeth, the Agulhas Current moves offshore where the shelf becomes wider. This offshore movement reduces the probability of *G. aestuaria* trapped within the current being able to recruit back into estuaries. Eddies in the Port Elizabeth region, however, allow water onto the shelf and therefore the possible migration of species back up the coast against the flow of the main Agulhas Current (Lutjeharms, 2005). The Agulhas Current at this point changes significantly, where the shelf is wider, and it sheds large rings of warm water at the Agulhas Retroflection leading to a substantial transfer of water from the Indian to the Atlantic Ocean systems (Lutjeharms and Ballegooyen, 1988).

Along the south coast of southern Africa, the absence of inshore currents coupled with isolation-by-distance between estuaries, make the recruitment of *G. aestuaria* from the marine environment into estuaries less likely. Consequently, individuals in the warm temperate lineage on the neighbour-joining tree comprised of Seekoei, Bot and Gourits samples (western section of south coast only), form a distinct group from the cool temperate and subtropical groups.

Migration of *G. aestuaria* around the Cape Peninsula and into the west coast systems seems unlikely for this estuarine species, and distances between estuaries on this coastline are, on average ≈ 21km apart (greater than in the warm temperate and subtropical regions). This grouping is reflected in the neighbour-joining tree by a distinct cool temperate region. It should be noted that MIGRATE results indicate a noticeable amount of migration from the warm temperate to the cool temperate region. The migration of individuals from the warm temperate region to the cool temperate region is likely to be mediated by the transport of water around the Cape Peninsula to the west coast by intense wind-driven surface Ekman transport (Figure 1.7) (Sherman *et al.*, 1993) or the Retroflection loop which generates large Agulhas rings that drift into the south Atlantic Ocean and into the Benguela system (Lutjeharms and Ballegooyen, 1988).

The inclusion of five cool temperate alleles in the subtropical lineage on the neighbour-joining tree (Figure 4.1) is possibly due to these alleles originating in the subtropical region and being transported around the Cape Peninsula. Conversely, the migrate table demonstrates minimal migration of cool temperate individuals into the subtropical region which may be explained by close inshore currents and eddies that move in the opposite direction to the main current systems (Lutjeharms, 2005), thus transporting species back round the Cape Peninsula and up the south east coast.

Whitfield (1994) subdivided the South African coastline into three broad biogeographic regions based on average seawater temperatures. These were the subtropical region extending from the northern border of KwaZulu-Natal to the Mbashe River, the warm temperate region from the Mbashe River to Cape Point in the south, and the third zone, the cool temperate region, incorporating the west coast of the Western and Northern provinces. Results from Chapters 3 and 4 appear to demonstrate that the structuring of *G. aestuaria* populations is based on oceanic water bodies, rather than hydrological conditions. The location of the phylogeographic break does not correspond to Whitfield's (1994) biogeographic boundary at the Mbashe river. In the case of *G. aestuaria* the new boundary occurs further south, including the entire region where the Agulhas Current flows close inshore along South Africa's east coast as the subtropical region. The new break is suggested as being between the Seekoei and Bushmans estuaries. It is worth noting that Teske *et al.* (In press) found evidence of a boundary in a similar area separating the warm temperate and subtropical provinces for the cumacean, *Iphinoe truncata*. This was attributed to the Alexandria Coastal Dunefield located between the Sundays and Boknes estuaries where the large distance between the estuaries, the effect of the strong perpendicular southwesterly winds that prevent drifting and the lack of suitable habitat along the dunefield form a dispersal barrier to the cumacean. Teske *et al.* (In press) also suggested that the region where the Agulhas Current moves offshore functions as a mechanical barrier to other invertebrate species, such as the mudprawn (*Upogebia africana*) and the isopod (*Exosphaeroma hylecoetes*), as, where the continental shelf widens, southward-flowing Agulhas water is deflected into the open ocean. In addition, previous studies by Stephenson and Stephenson (1972) and Wallace and van der Elst (1975) attributed changes in species composition in this region to temperature changes.

The phylogeographic patterns of *G. aestuaria* suggest that the subtropical biogeographic region extends further south of the Mbashe River to Port Elizabeth. This result is in agreement with a recent study by Teske *et al*. (In press) on the phylogeography of three estuarine invertebrates. The phylogeographic break at Cape Point observed within *G. aestuaria* and estuarine invertebrates (Teske *et al.,* in press) coincides with a previously suggested biogeographic break between the cool temperate and warm temperate regions (Whitfield (1994). Further studies should investigate this potential phylogeographic break as it may form a transition zone stretching between Cape Point and Cape Agulhas.

Chapter 5

General Discussion and Conclusions

This study compared the population structure of two common estuarine fish species along the South African coastline. Two different phylogeographic patterns emerged, with the marine spawning species, *A. breviceps,* exhibiting high dispersal, gene flow and a homogenous population. Conversely, the estuarine resident species, *G. aestuaria,* demonstrated less gene flow and more population structure.

Cape Point has been widely accepted as the location of the boundary between the cool temperate and warm temperate biogeographical regions (Maree *et al.,* 2000). Results from the current study of *G. aestuaria* confirm the importance of this region as a biogeographic boundary. The position of the warm temperate and subtropical boundary, however, is not as clear (Maree *et al.*, 2000) and has traditionally been based on average water temperatures (Whitfield, 1994). Results of the current study of *G. aestuaria* suggests that hydrology along the South African coastline plays an important role in the delineation of the biogeographic regions, with the division between the subtropical and warm temperate regions occurring further south than the Mbashe estuary, between the Bushmans and the Seekoei estuaries where the Agulhas Current moves offshore (Figure 5.1).

The characteristics of the current regime in the subtropical KwaZulu-Natal province and the northern section of the Eastern Cape region (counter current systems and eddies) is conducive to the retention of progeny in this region (Maree *et al.*, 2000). Thus in instances where *G. aestuaria* are flushed out to sea, they have a greater probability of recruiting back into a neighbouring estuary on the east and south east coasts. This probability of recruitment of fish into estuaries is assisted by the close proximity of systems to one another in this region. These factors lead to the mixing of *G. aestuaria* populations on the east coast with population subdivision occurring south of the Bushmans estuary. On the other hand, marine adaptations (shoaling in the nearshore environment and bays) demonstrated by *A. breviceps*, and the exchange

of water between the Indian and Atlantic Oceans mediated by Agulhas rings and Ekman transport, allows *A. breviceps* to expand its distribution range throughout the three biogeographic zones identified along the southern African coastline.

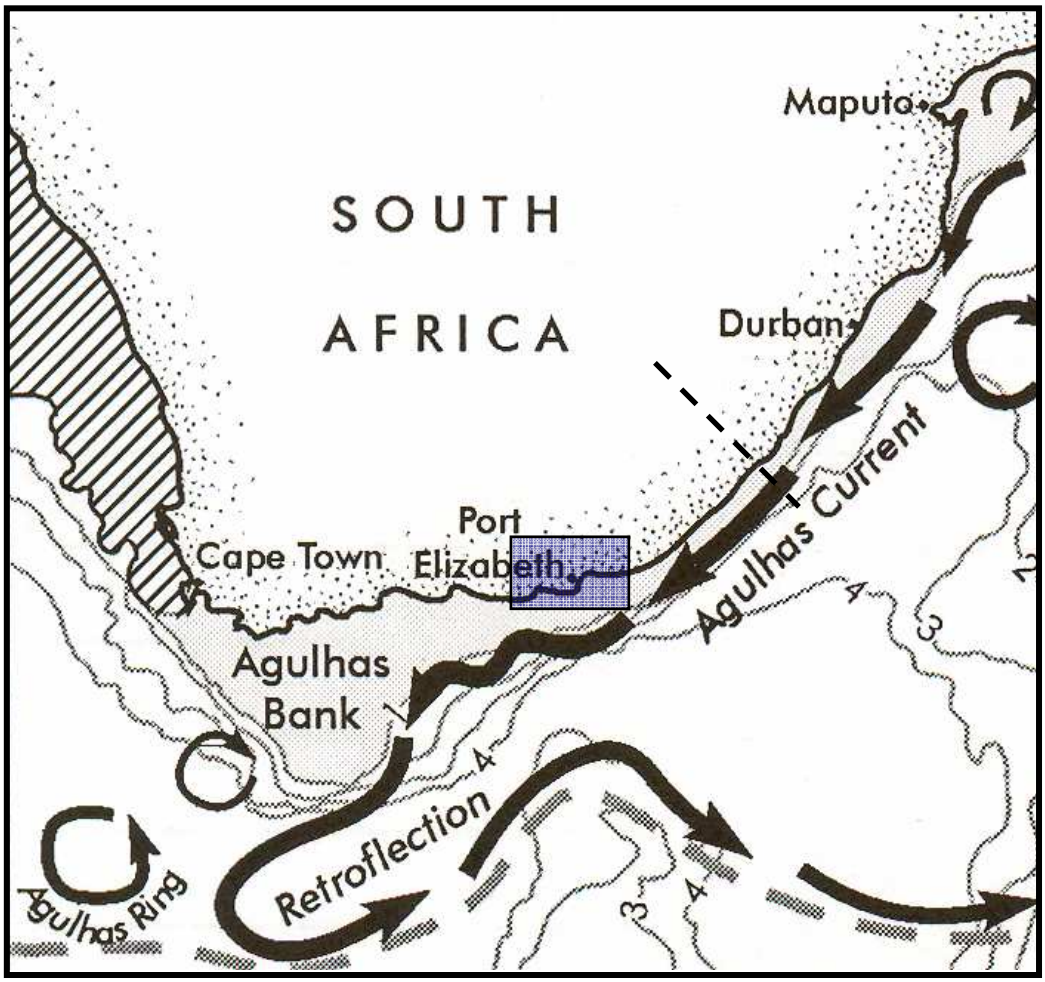

Figure 5.1: Map of southern Africa, showing the Agulhas Current and the region along the coastline were it moves offshore, the separation between the warm temperate and subtropical biogeographic regions according to Whitfield (1994) (dotted line) and the zone identified in this study according to *Gilchristella aestuaria* where the new biogeographic separation should be (shaded box). Map modified from Lutjeharms (2005).

Approximately 70% of the estuaries along the South African coast are temporarily open/closed (TOC) estuaries and are characterised by a sandbar across their mouth that acts as a barrier between the estuarine and marine environment for varying periods of time (Kemp and Froneman, 2004). These TOC estuaries may restrict gene flow along the coastline as this mouth closure for extended periods restricts access

opportunities for fish from the marine environment into the estuarine environment and vice versa (Ayvazian *et al.*, 1994). However, opportunities do arise in the form of breaching or overtopping events, which have significant impacts on the community structure within these systems (Kemp and Froneman, 2004). When these events occur, marine water washes over the sandbar due to high tides or large swell (overtopping), or there is a response to precipitation in the catchment, and a link between the estuary and marine environment is established.

Whitfield (1992) describes a situation in the temporarily closed Haga Haga estuary where two month old marine spawning species were present in the estuary despite the system being closed for a period of six months prior to the survey. A similar situation was described by Vivier and Cyrus (2001) in the Nhlabane estuary in KwaZulu-Natal where recruitment was recorded during closed mouth conditions. Both authors identified overtopping events as the means by which recruitment occurred. In a study by Kemp and Froneman (2004) in the West Kleinemond estuary, results from overtopping events revealed that seven fish species, including *A. breviceps* and *G. aestuaria*, utilised these events to recruit into the estuary. This suggests that overtopping events provide a strategy for these two species to gain access and recruit into TOC estuaries, contributing to gene flow.

Anthropogenic influences may affect an estuary's ability to contribute to gene flow by altering the frequency of marine – estuarine interactions (Whitfield, 1990). Whitfield (1990) describes how historical evidence of the Bot River estuary indicates that it has undergone topographical changes during the past century as it was closed to the sea, but is now opened artificially every three to five years. The *G. aestuaria* results from the current study suggest that the Bot estuary population is genetically divergent from neighbouring systems, whereas *A. breviceps* populations do not demonstrate this isolation. The observed pattern may be due to the fact that *A. breviceps* mainly occupies the lower reaches of estuaries and artificial opening may allow this species to migrate to the open ocean. Conversely, *G. aestuaria* would not be influenced, as it occupies the upper reaches of estuaries and the volume of water flowing through the Bot upon artificial breaching would not flush individuals into the sea, resulting in the isolation of this *G. aestuaria* population over time.

In addition to the obvious barrier that a closed river mouth represents to gene flow, several authors have identified other natural barriers such as changes in water temperature, physical oceanography and water bodies. In a study by Ridgway *et al.* (1998), results showed that despite morphological similarities between populations of the limpet *Patella granularis* along the coast of southern Africa, individuals from the northern sites on the east coast represented a gene pool distinct from the west, south and south east coast populations. Ridgway *et al.* (1998) attributes the lack of gene flow between the south east coast populations and the north east coast populations to physical oceanography and the marked discontinuity of the inshore water characteristics in the Mbashe area. The population subdivision is further north than that identified in the current study for *G. aestuaria*, and was attributed to the pelagic environment in this region being influenced by the upwelling of cool Indian Ocean central water onto the shelf.

Ridgway *et al.* (1999) also examined patterns of genetic and morphological variation among eight populations of the bearded limpet *Patella barbara* along the coast of South Africa. He found little geographic structuring and no genetic differentiation between populations, except for the Dwesa population (near the extreme end of the geographic range) which contained a second allele for two loci not present in the other seven populations. This result is similar to the observed patterns for *A. breviceps* in this study, with both this limpet species and *A. breviceps* having a pelagic larval phase.

As discussed in previous chapters, the work by Teske *et al.* (In press) reveals the impacts of biogeographic boundaries on the phylogeographic patterns of three estuarine crustaceans, each with a different mode of dispersal. A population division between the cool temperate and warm temperate regions near Cape Agulhas affected all three species, with the strongest influence on the cumacean, *Iphinoe truncata,* which was not found west of this boundary. The mudprawn, *Upogebia africana,* revealed a monophyletic lineage comprising specimens collected west of Cape Agulhas that was not strongly differentiated from specimens collected east of the boundary and similarly, the isopod, *Exosphaeroma hylecoetes,* showed considerable genetic differentiation across Cape Agulhas. This corresponds with work conducted by Evans *et al.* (2004) who concluded that populations of *Haliotis midae,* on either

side of Cape Agulhas, represented two independent reproductive stocks and that the area of transition between the stocks coincided with oceanographic features of the region. Teske *et al.* (In press) also found evidence of an eastern boundary separating the warm temperate and subtropical regions in the mudprawn and isopod, which he attributed to a mechanical barrier formed by the region where the Agulhas Current diverges from the coast as the continental shelf break moves further from the coastline. No evidence of genetic discontinuity was found for the cumacean, but a boundary was detected south of this region. This was attributed to the Alexandria Coastal Dunefield where distances between the estuaries, the effect of the strong perpendicular winds and the lack of suitable habitat along the dunefield form a dispersal barrier to the cumacean. The position of this barrier is similar to that observed for *G. aestuaria* during the current study.

In a study by Collin (2001) three species of *Crepidula* (Gastropoda: Calyptraedidae) were found ranging across the barrier created by the Cape Hatteras biogeographic break. This suggests a tolerance of these species to the geographically abrupt changes in water temperature in this region. Conversely, studies by Stephenson and Stephenson (1972) and Wallace and van der Elst (1975) attribute varying species compositions along the South African coastline to water temperature changes. However, there is no population differentiation of *A. breviceps* and *G. aestuaria* from this study based on water temperatures and both species appear to tolerate temperature changes in a similar manner to the species studied by Collin (2001).

An additional study by Gold *et al.* (1999) identified genetic differences among red drum adults (*Sciaenops ocellatus*) across localities but believed this to be minimal due to the dispersal of eggs and larvae on oceanic currents. This life history is similar to the result for *A. breviceps* in this study, with the marine phase of this species allowing for extensive dispersal and gene flow.

5.1 Future directions

According to Whitfield (1998), *Atherina breviceps* and *Gilchristella aestuaria,* can be regarded as the most numerically abundant fish in the cool temperate and warm temperate biogeographic regions. During this study, both species were available for collection and analysis from these two biogeographic regions. However, within the

subtropical region, *A. breviceps* numbers decline (Whitfield, 1998). The low abundances within this zone account for the absence of specimens within this region during this study. Future research should increase sampling effort to ensure that samples are collected from the subtropical zone so that a comprehensive study on the phylogeography of *A. breviceps* along the southern African coastline can be undertaken.

Alternative strategies for the estimation of gene flow using different genetic markers, different models of population genetics and demography, and different methods of parameter estimation could be employed to further investigate the boundaries of the biogeographic zones (Neigel, 1997). In the late 1960's, surveys of allozyme variation in populations set a new direction for gene flow studies. However, a shift to DNA sequences, which presents a more diverse set of genetic markers, has changed the way population genetic variation is described (Neigel, 1997). Each technique has advantages and disadvantages and alternative strategies have progressed sufficiently. The addition of a nuclear gene (nDNA), in conjunction with mitochondrial DNA may provide a more comprehensive picture of the population structure of *A. breviceps* and *G. aestuaria*. The feasibility of nDNA sequence surveys has increased since the advent of PCR and two forms of nuclear sequence variation can be used as genetic markers: variable numbers of tandem repeats (VNTRs) and base substitutions (Neigel, 1997). Alternatively, it appears that adequate resolution of higher-level relationships in any organism will require longer DNA sequences or the sequencing of the complete mitochondrial genome (Miya and Nishida, 2000). For example, in mammals, the transition from an unsolvable to solvable problem occurred from the availability of complete mtDNA sequences, and preliminary studies have indicated that sequencing the complete mitochondrial genome is applicable to a wide variety of teleosts (Miya and Nishida, 2000). Although it remains unclear as to whether nuclear or mitochondrial genes are generally more efficacious for such purposes, these alternatives or combinations of markers could provide a more conclusive overall perspective of the population structure and gene flow between these two species.

An additional direction could be to assess the comparative phylogeographic patterns and impacts of marine biogeographic boundaries using other estuarine or sandy beach species. Teske *et al.* (In press) used three estuarine crustaceans: the mudprawn

(*Upogebia Africana*) the isopod (*Exosphaeroma hylecoetes*) and the cumacean (*Iphinoe truncata*), each characterised by a different mode of passive dispersal, to assess the impacts of biogeographic boundaries on their comparative phylogeographic patterns. Results of the study suggest evidence of a boundary in a similar area to that found in the current study, separating the warm temperate and subtropical provinces, which was attributed to the Alexandria Coastal Dunefield. Teske *et al.* (In press) also identified the region where the Agulhas Current moves offshore as a mechanical barrier to other invertebrate species. Estuarine species seem suitable model organisms to investigate the impacts of marine biogeographic boundaries on genetic structure, as most have wide distribution ranges, and are not limited by salinity fluctuations (Teske *et al.*, In press).

"Occurrences in this domain are beyond the reach of
exact prediction because of the variety of factors in
operation, not because of any lack of order in nature."

Albert Einstein (1879 – 1955)
World Renown Physicist

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Appendix One

Population locations, sample sizes, number of haplotypes and nucleotide and haplotype diversities

Table A1.1: Population locations, sample sizes, number of alleles and nucleotide and gene diversities for *Atherina breviceps* mtDNA control region variation from six sites around the South African coastline.

Atherina breviceps Populations	Latitude, Longitude	Sample Size	Total number of alleles	Nucleotide Diversity (π ± SD)	Gene Diversity (δ ± SD)
Great Berg, CT	32º46´S;18º09´E	10	10	0.01141 ± 0.006634	1 ± 0.0447
Bot, WT	34º21´S;19º04´E	10	8	0.007393 ± 0.0004499	0.9556 ± 0.0594
Seekoei, WT	34º05´S;24º55´E	10	8	0.021389 ± 0.0011918	0.9556 ± 0.0594
Bushmans, WT	33º42´S;26º40´E	10	8	0.026678 ± 0.0014715	0.9556 ± 0.0594
Kariega, WT	33º41´S;26º44´E	10	10	0.047795 ± 0.025874	1 ± 0.0447
Cefane, WT	32º49´S;28º08´E	10	9	0.034562 ± 0.018882	0.9778 ± 0.054

Table A1.2: Population locations, sample sizes, number of alleles and nucleotide and gene diversities for *Gilchristella aestuaria* mtDNA control region variation from six sites around the South African coastline.

Gilchristella aestuaria Populations	Latitude, Longitude	Sample Size	Total number of alleles	Nucleotide Diversity (π ± SD)	Gene Diversity (δ ± SD)
Great Berg, CT	32º46´S;18º09´E	10	7	0.008249 ± 0.004929	0.8667 ± 0.1072
Bot, WT	34º21´S;19º04´E	10	10	0.016764 ± 0.009444	1 ± 0.0447
Seekoei, WT	34º05´S;24º55´E	10	10	0.008956 ± 0.005303	1 ± 0.0447
Bushmans, WT	33º42´S;26º40´E	10	10	0.007887 ± 0.004736	1 ± 0.0447
Kasouga, WT	33º39´S;26º44´E	10	9	0.015893 ± 0.008984	0.9778 ± 0.054
Qolora, WT	32º38´S;28º25´E	10	9	0.009046 ± 0.005353	0.9778 ± 0.054

Table A1.3: Population locations, sample sizes, number of haplotypes and nucleotide and haplotype diversities for *Gilchristella aestuaria* mtDNA control region variation from all twenty one sites sampled.

Population	Latitude, Longitude	Sample Size	Total number of haplotypes	Nucleotide Diversity (π ± SD)	Haplotype Diversity (h ± SD)
Orange, CT	28º38´S;16º27´E	9	8	0.013441 ± 0.00783	0.9722 ± 0.064
Olifants, CT	31º42´S;18º11´E	6	5	0.013198 ± 0.00827	0.9333 ± 0.1217
Great Berg, CT	32º46´S;18º09´E	10	7	0.008844 ± 0.00528	0.8667 ± 0.1072
Rietvlei, CT	33º53´S;18º28´E	3	2	0.006996 ± 0.00592	0.6667 ± 0.3143
Bot, WT	34º21´S;19º04´E	10	10	0.01796 ± 0.01012	1 ± 0.0447
Gourits, WT	34º21´S;21º53´E	10	10	0.014816 ± 0.00845	1 ± 0.0447
Goukamma, WT	34º05´S;22º57´E	5	5	0.009701 ± 0.00653	1 ± 0.1265
Seekoei, WT	34º05´S;24º55´E	10	10	0.009897 ± 0.00584	1 ± 0.0447
Bushmans, WT	33º42´S;26º40´E	10	9	0.007609 ± 0.00462	0.9778 ± 0.054
Klein Brak, WT	33º37´S;26º56´E	6	4	0.007625 ± 0.00504	0.8667 ± 0.1291
Kasouga, WT	33º39´S;26º44´E	8	8	0.016625 ± 0.00971	1 ± 0.0625
Qolora, WT	32º38´S;28º25´E	10	9	0.00905 ± 0.00539	0.9778 ± 0.054
Sihlontlweni, WT	32º29´S;28º39´E	2	2	0.003403 ± 0.00416	1 ± 0.5
Mapuzi, ST	31º58´S;29º10´E	5	5	0.009755 ± 0.00657	1 ± 0.1265
Mtambane, ST	31º39´S;29º30´E	5	5	0.010437 ± 0.00698	1 ± 0.1265
Mntafufu, ST	31º34´S;29º38´E	4	4	0.011495 ± 0.00819	1 ± 0.1768
Mpenjati, ST	30º58´S;30º17´E	3	3	0.021436 ± 0.01672	1 ± 0.2722
Koshwana, ST	30º39´S;30º31´E	5	5	0.016194 ± 0.01048	1 ± 0.1265
Umgababa, ST	30º09´S;30º50´E	4	4	0.009589 ± 0.00694	1 ± 0.1768
Mdloti, ST	29º38´S;31º08´E	3	3	0.009313 ± 0.00766	1 ± 0.2722
Kosi, ST	26º54´S;32º48´E	10	10	0.008472 ± 0.00508	1 ± 0.0447

Appendix Two

Distances between Estuaries

Table A2.1: Distances (in km) between estuaries sampled for *Gilchristella aestuaria* in Chapter 4.

Estuary	Distance to next sampled estuary (km)
Orange, CT	354.29
Olifants, CT	113.78
Great Berg, CT	256.53
Rietvlei, CT	141.64
Bot, WT	366.17
Gourits, WT	127.86
Goukamma, WT	226.94
Seekoei, WT	217.37
Bushmans, WT	2.63
Klein Brak, WT	4.74
Kasouga, WT	205.8
Qolora, WT	33.71
Sihlontlweni, ST	83.59
Mapuzi, ST	56.73
Mtambane, ST	17.22
Mntafufu, ST	98.62
Mpenjati, ST	42.68
Koshwana, ST	67.76
Umgababa, ST	66.75
Mdloti, ST	433.67
Kosi, ST	

Table A2.2: Total estuary counts and average distances between estuaries in the subtropical, warm temperate and cool temperate regions (after Harrison, 2000).

Subtropical Region	
Average Distance between estuaries	**5.319**
Std. Deviation	13.168
Total Estuary Count	**159**

Warm Temperate Region	
Average Distance between estuaries	**8.604**
Std. Deviation	11.871
Total Estuary Count	**151**

Cool Temperate Region	
Average Distance between estuaries	**21.050**
Std. Deviation	16.230
Total Estuary Count	**34**