Chapter 3

Mitochondrial DNA variation in the spotted grunter, *Pomadasys commersonnii* (Lacepède, 1801), from South African estuaries

Abstract

Pomadasys commersonnii is a western Indian Ocean species that inhabits the subtropical and warm temperate waters off South Africa's eastern and southern seaboard. Estuaries play a vital role in the species' survival, as juveniles are totally dependent on estuaries as nurseries for their first year of development. Sexual maturity is reached at about three years of age. Tagging data suggest that adults are resident around estuaries and post-spawning individuals frequently utilize estuaries as rich feeding grounds. However, adults from the warm temperate waters off the south coast undergo spawning migrations to KwaZulu-Natal during late winter and spring. Larvae are carried southwestwards by currents inshore of the Agulhas system to estuaries as far west as Swartvlei on the south coast. Analysis of the mitochondrial DNA control region shows that genetic diversity ($h = 0.98$, $\pi = 2.19\%$) is comparable with reports for other marine species. Seventy-four haplotypes were detected from 139 control region sequences analysed from four localities. No pattern between haplotype genealogy and geographic locality was evident. Analysis of Molecular Variance allocated 100% of the variance to within localities, resulting in an F_{ST} value of zero. A finding of a lack of genetic differentiation and high maternal gene flow along the South African coast is in concordance with biological data indicating a single South African intermixing population.

Introduction

Spotted grunter, *Pomadasys commersonnii* (Lacepède, 1801) (Haemulidae), occurs in coastal waters of the western Indian Ocean, from India to False Bay in South Africa (Heemstra & Heemstra, 2004). In the latter region they are mainly found in estuaries and shallow water at depths of less than 30 meters (Branch *et al.*, 1994; Smith & McKay, 1986). Along the South African coast, estuaries are important for this species' survival because of the role these systems play as nursery areas (Whitfield, 1994). Juveniles inhabit estuaries from the southern Cape eastwards up the coast into southern Mozambique (Fig 3.1). While their marine distribution can occasionally reach False Bay during the summer, they are more abundant off subtropical KwaZulu-Natal (KZN) and the eastern portion of the Eastern Cape Province (Wild Coast), becoming less common west of Algoa Bay (Smith & McKay, 1986; Wallace *et al.*, 1984; Whitfield, 1988; Whitfield, 1989). Estuaries also serve as rich feeding grounds for adults, mainly in the spring and summer months, when they are in a post-spawning condition and need to build up depleted energy reserves (Wallace, 1975b; Whitfield, 1998).

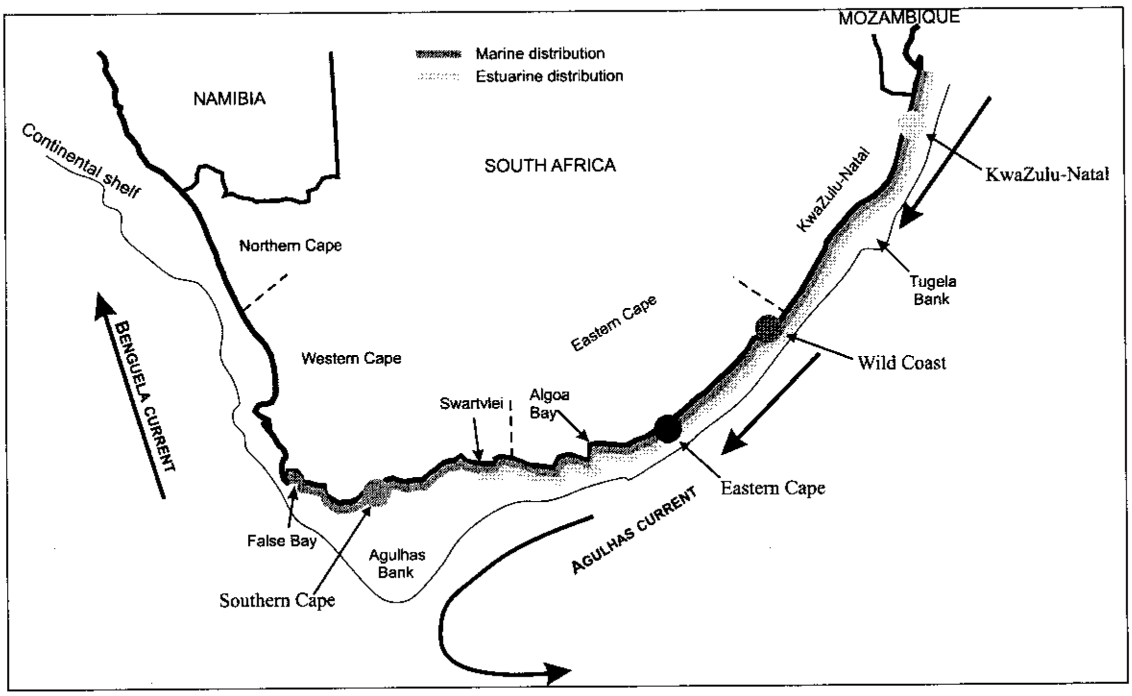

Fig. 3.1 Distribution and sampling localities of *P. commersonnii* along the South African coast (adapted from Whitfield, 1998).

Spawning occurs at sea in the shallow inshore zone from August to December, although 'ripe running' specimens have occasionally been found in the lower reaches of certain estuaries (Wallace, 1975b). Although spawning has only been recorded off the KZN coast, indications are that it might occur throughout the species' distribution (Wallace, 1975b; Wallace &

Schleyer, 1979; Wallace & van der Elst, 1975). This has recently been questioned by Webb (2002) who found that, in contrast to the observations from KZN estuaries, 'ripe' and recently 'spent' adults are absent from southern and Eastern Cape estuaries, thus suggesting that spawning did not occur within these regions. This evidence indicates that reproductively mature fish from the Cape regions undergo a spawning migration to KZN during spring and summer, followed by a return migration to the southern and Eastern Cape estuaries (Webb, 2002).

Larval development occurs in the marine environment, but eggs have been found in estuary mouths (Connell, 1996; Wallace, 1975b). Juveniles are recruited into estuarine nursery areas at approximately 20–30 mm total length (TL), and they are most abundant in the upper reaches, even entering freshwater (Wallace & van der Elst, 1975; Whitfield, 1998). Early juveniles in estuaries feed mainly on pelagic copepods and mysids (Wooldridge & Bailey, 1982), while subadults and adults prey predominantly on benthic anomurans and bivalves (Hecht & van der Lingen, 1992; Webb, 2002). Although *P. commersonnii* feeds during the day, they are predominantly crepuscular and nocturnal (Marais, 1984; Whitfield, 1998).

Juveniles tend to return to the sea after one year (approximately 15 cm TL) where they stay until maturity (Wallace, 1975a). Males are mature by 30 cm and females by 36 cm standard length (SL), about three years of age. The maximum recorded size is 87 cm TL, approximately 15 years old (Wallace & Schleyer, 1979). Tagging data suggest that adults are often resident within a particular region and tend to congregate in the vicinity of estuary mouths, although a few individuals were recorded migrating more than 50 km (Fennessy & Radebe, 2000, Bullen & Mann, 2004, P. Cowley, pers. comm.)

Estuarine and shore anglers along the species' distribution target *P. commersonnii* for its excellent eating and sporting qualities (van der Elst, 1998), making it one of South Africa's most targeted linefish species (Lamberth & Turpie, 2003). In estuaries alone, *P. commersonnii* catches make up nearly 20% of the total fish catch weight. Along the south coast, despite the fact that it is less abundant there than off KZN, they comprise 45% of catches, with a 31% catch contribution along the east coast and 11% in KZN. In total this amounts to over 400 tons of a total ±2100 tons of fish caught in South African estuaries. This clearly shows that *P. commersonnii* is an important species for the recreational angling industry, where the total value of estuarine and estuary-associated fisheries was estimated to be worth R1.2 billion in 2000 (Lamberth & Turpie, 2003). Currently *P. commersonnii* is maximally or optimally

PCR Amplification

The setting up of Polymerase Chain Reaction (PCR) amplifications were implemented as for *A. japonicus* (Chapter 2) but with the following variations. Only the 5' end of the mtDNA control region was successfully amplified using oligonucleotide primers Pc L16565 (AAC GCC GGT CTT GTA AGC CG) forward and H404 reverse (Fig. 3.2). Primer Pc L16565 was designed specifically for this study in the same way as Per L16565 in Chapter 2. The reverse primer H404 (AGG AAC CAR ATG CCA GKA ATA) was developed for previous studies in our laboratory based on published salmonid sequences (P. Bloomer, pers. comm.) and anneals in the central conserved block of the mtDNA control region (Fig. 3.2).

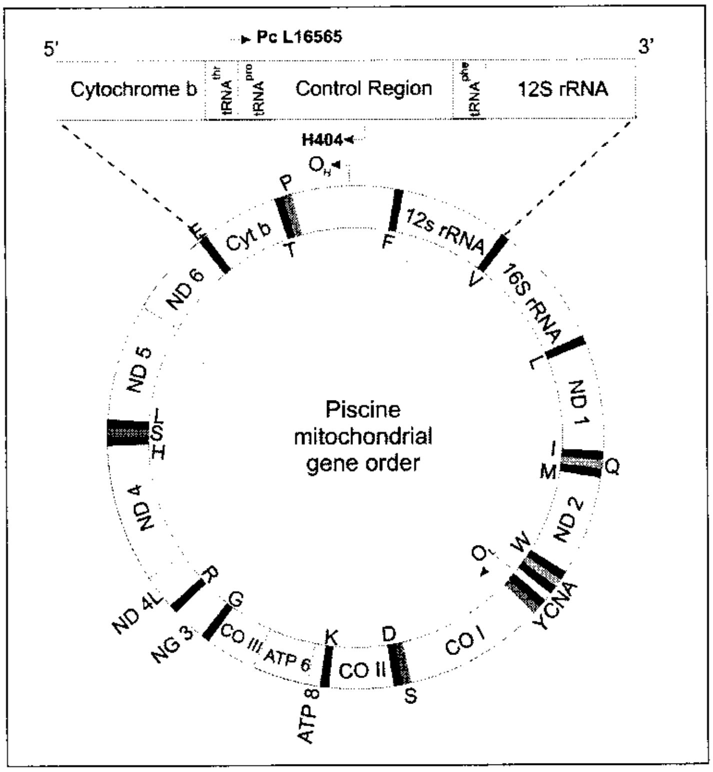

Fig. 3.2 Map of the piscine mitochondrial DNA showing annealing positions of primers used for amplification of *P. commersonnii* 5' end of the mtDNA control region (modified from Meyer, 1993).

Basic PCR set-up, DNA visualization and precipitation were as described in Chapter 2 with the exception of the amplification steps. Amplifications were conducted in a Geneamp® PCR System 9700 (Applied Biosystems), commencing with a five minute 94°C denaturing step. This was followed by 35 cycles of denaturing at 94°C (20 s), primer annealing at 64°C (30 s) and extension at 72°C (30 s). The final extension step was undertaken at 72°C for seven minutes.

Sequencing

Approximately 80–100 ng purified PCR template, 3.2 *pmol* of the specific primer and 1 µl BigDye® was mixed and made up with Sabax® water to a final volume of 5 µl and cycled in a Geneamp® PCR System 9700 running the BigDye® program. Sequencing and verification of successful sequences were undertaken as described in Chapter 2.

Population Genetic Analysis

A summary of the population genetic analysis is given below; for a more comprehensive outline refer to Chapter 2. Genetic diversity within each sampling locality was estimated by calculating haplotype diversity (h) (Nei, 1987) and nucleotide diversity (π) (Tajima, 1983, 1993), while between locality diversity was calculated as net nucleotide sequence divergence (Reynolds *et al.*, 1983; Slatkin, 1995). Based on pairwise differences between the haplotypes (ARLEQUIN 2.0, Schneider *et al.*, 2000), a Minimum Spanning Network (Excoffier *et al.*, 1992) was constructed. Frequency and locality information was plotted onto this analysis to visually examine the genealogical relationships among *P. commersonnii* lineages. An endemism index was calculated for each locality by dividing the number of 'private' haplotypes at a locality by the total number of haplotypes found at that locality, thus expressing the proportion of haplotypes at a locality that were found nowhere else. To test if the marker is evolving according to neutral expectations, Tajima's D, Fu's F_s and the R_2 test were used (Fu, 1997; Ramos-Onsins & Rozas, 2002; Tajima, 1989).

Mismatch distributions for the localities and the South African population were calculated and compared to those expected under a population growth and decline model (Harpending, 1994; Rogers, 1995). Homogeneity of mtDNA haplotype frequencies was tested via a randomisation procedure (Monte Carlo) (Roff & Bentzen, 1989). To determine if any genetic differentiation between localities exists, and to estimate patterns of spatial genetic structuring, Analysis of Molecular Variance (AMOVA) (Excoffier *et al.*, 1992) was executed in ARLEQUIN 2.0. However, to determine the effect that a large number of homoplasy sites may have on the analyses, all calculations were done on the fragment before and after removal of the homoplasy sites and the results compared.

exploited, as the spawner biomass is estimated at 40% of pristine biomass (Lamberth & Turpie, 2003).

Based on life-history information it is suggested that the South African population of *P. commersonnii* comprises a single stock made up of several geographically related sub-populations differentiated by their growth patterns (Webb, 2002). The aim of this study was to determine the genetic diversity and spatial differentiation among *P. commersonnii* within South African waters and to determine whether fish from the various estuaries are connected via gene flow. As discussed in Chapter 2, the use of the mtDNA control region in detecting population structure within marine fish species can be informative. The inferred mtDNA haplotype genealogy was combined with the geographical distribution of the sampled populations then analysed to establish whether the genetic variation is associated with particular geographic localities within the overall South African population. Tests were also conducted to assess whether isolation-by-distance exists, with discrete spawning events potentially taking place along the coast, or if spawning only occurs off the KZN coast leading to little or no differentiation within the South African *P. commersonnii* population.

Materials and Methods

Samples

Fin clips or gill filaments were collected from fish in estuaries or the adjacent surf zone by seine netting, gillnet or hook and line. Material was placed in 80% ethanol and later stored at –20°C. Length measurements were recorded, as well as locality and collection date. Sampling was conducted at four sites across the species' distribution in South African waters (Fig 3.1). Samples from the southern Cape were collected from the Breede River, Gouritz and Heuningnes estuaries. Although these estuaries are some distance apart (96 km), fish found in these systems are primarily summer migrants and were therefore treated as a single locality. Eastern Cape samples were collected from the Great Fish River, East Kleinemonde and Kowie River estuaries (25 km apart), while the Wild Coast samples were obtained from two adjacent estuaries, Mngazi and Mngazana (4 km apart). All KZN samples were collected from the St. Lucia estuary. Total distance along the coast between the southern Cape and KZN localities is 1 494 km. A list of all samples analysed can be found in Appendix IV.

DNA Extraction

DNA extraction was conducted using the procedure outlined in Chapter 2.

Results

Control region

A ± 450 base pair (bp) fragment was sequenced, including 350 bp from the 5' end of the control region as well as some bases from of the flanking tRNAs, threonine ($tRNA^{thr}$) and proline ($tRNA^{pro}$), where the 5' primer annealed. No evidence of heteroplasmy was found and no length polymorphisms were detected in any of the samples analysed. The first 41 bp segment and the last 24 bp segment of the mtDNA control region fragment were conserved between all samples (Fig. 3.3). Nucleotide composition of the 5' end of the mtDNA control region in *P. commersonnii* was C 21.67%, T 31.44%, A 34.36% and G 12.53%. The fragment contained a total of 66 polymorphic sites, with 76 substitutions that consisted of 65 transitions at 64 sites and 11 transversions at 11 sites. An alignment of all the variable positions is shown in Table 3.1 (a complete alignment is available on request). By mapping the mutations onto the initial Minimum Spanning Network, a total of 26 sites with homoplasy characters were identified. All analyses were undertaken by comparing the original 350 bp fragment to the 324 bp fragment obtained after the homoplasy characters were removed (Table 3.2). Homoplasy characters induce ambiguities in the relationship between haplotypes, potentially creating noise that might obscure any population signal (Avise & Lansman, 1983). Comparing results from the various analyses revealed that removal of homoplasy sites did not yield significantly different answers; therefore only results on the full 350 bp fragment is reported here in detail.

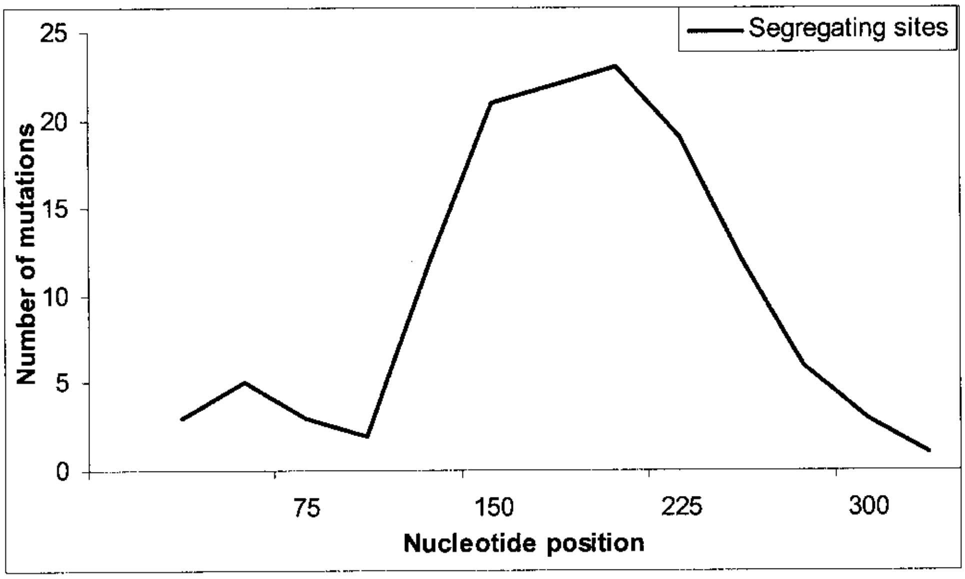

Fig. 3.3 Sliding window of substitutions in the 5' end of mtDNA control region from *P. commersonnii* (window length 50, step size 25 bp).

Table 3.1 Sixty-six variable sites defining 74 *P. commersonnii* haplotypes in the South African population. The number of individuals that shared a specific haplotype is indicated under the "frequency" column. Base one corresponds to base number one in the reference sequence (*Salmo salar* GenBank NC 001960)

Haplotype nr	Frequency	
		1111111111111111111111111111111111222222222222222222222223
		444558122223333444555666777788888999999000011111123333333345667
		236899967890789036267467034592345901347925784567895013568929165
1	8	ACTCTTCTCTACCTATAAAAGCACAAAAAATTTAATAATTTCTCATCATTACGTATATACATT
2	1	....C.
3	2	.....................T...
4	1	.....C...............T................C........................
5	1	....C..............
6	1	....A..C..............
7	1	...............................C...............................
8	3	.................G.............C...............................
9	6	.................G.............C.....................C.........
10	4	.................G..A..........C.....................C.........
11	1	.................G..A..........C.....................C...C.....
12	1	.................G..A..........C.....................C.........
13	1	.................G..AT.........C.....................C.........
14	1	....
15	1	.........C...
16	1	........AC.......G...............G.....................C.......
17	2	...............C................................C..............
18	1	....C..G...........
19	2	.............C...G...............................C.............
20	1	........T..
21	1	........T.G......G...
22	1	........T........G.................CGG.........................
23	1	....C......................
24	11	.............C...G......................C.................G.G..
25	1	.............C...G......................C.....G...........G.G..
26	2	..C..........C...G......................C.................G.G..
27	1	....C........C...G......................C.................G.G..
28	1	.............C...G......................C......G..........G.G..
29	1	.............C...G.............C........C.................G.G..
30	1	.............C...G......................C...G.............G.G..
31	1	.............C...G......................C...G.............G....
32	1	.............C...G.....G..............C.C.................G.G..
33	1	.................G..................................A..........
34	5	.........C.......G.........G........................A.......G..
35	4	.......C.C.......G.........G........................A.......G..
36	6	T.......TC.T.....G.........C........................A..........
37	1	........TC.T.....G.......G.C........................A..........
38	1	................GG...........................C.................
39	7	................GG.................C.........C.................
40	1	................GG....T............C.........C.................
41	3	.................G.................C.........C.................
42	1	....C...........GG.................C.........C.................
43	1	................GG.................C.........C.....G...........
44	1	................GG..............C..C.........C.................
45	1	................GG.................C........GC.................
46	3	................GG.................C..........T................
47	1	................GG.................C.........CG.......G..C.....
48	3	.......C......G..GG..........................C.G...............
49	1	...T........T....G..A........................C................C
50	2	....................A...G..........C................A..........
51	2	....................A...G..........C..C.............A..........
52	1	....................A...G.....C....C........G.......A..........
53	1	.T...........C...G................................G............
54	1	.T.T.............G................................G............
55	1	.T...............G........G.......................G............
56	1	.................G.....T................................GC.....
57	1	.................G..A..T................................C......GC.....
58	1	.................G.....T.................................C.....
59	1	.................G..A..T.................................C.....
60	1	...................TA.............G..G.............G...........
61	1	........A........G...........G........C.........C.......G.G.G..
62	2	........A........G...........G........C....T....C.......G.G.G..
63	6	......T.A........G...........G........C....T....C.......G.G.G..
64	1	......T.A....C...G...........G........C....T....C.......G.G.G..
65	1	.....CT.A........G...........G........C....T....C.......G.G.G..
66	1	......TCA........G...........G........C....T....C.......G.G.G..
67	1	......T.A........G...........G........C..T.T....C.......G.G.G..
68	2	......T.A........G...........G........C....T....C.......G.G....
69	1	......T.A....C...G....G...............C....T....CC.GC...G.G....
70	1	...A....A........G....................C....T....C.........GTG..
71	1	...A..T.A........G..........G.........C....T....C..T......GTG..
72	1	...A..T.AC.......G..A.C...............C....T....C..T....G.GTG..
73	1	...A....A..T.C...G....................C....T.C..C.........GTG..
74	2	........A........G.......G............CC..CT....C.........G.G..

Table 3.2 Comparison of variability indices calculated for 139 *P. commersonnii* based on the 5' end of the mtDNA control region (350 bp fragment) and the fragment obtained after removal of 26 homoplasy sites (324 bp fragment)

	350 bp fragment	**324 bp fragment**
Nr of polymorphic sites	66	40
Substitutions	76	45
Transitions	65	11
Transversions	11	6
Nr of haplotypes	74	40
Mean haplotype divergence	2.19%	1.03%
Nr of haplotypes represented by single samples	52 (70.3%)	23 (57.7%)
Haplotypes at single locality	54 (73.0%)	27 (67.5%)
Spatial homogeneity test	$\chi^2 = 235.6773$, $P = 0.0644$	$\chi^2 = 119.2160$, $P = 0.4130$
F_{ST}	-0.00999 (P = 0.9365)	-0.0118 (P = 0.9462)

Haplotype variation and distribution

For the 139 individuals analysed from four South African regions, 74 haplotypes were defined from 66 polymorphic sites. Genetic distances ranged from 0.29% (one bp difference), to 5.34% (19 bp difference), with a mean divergence of 2.19% (Appendix V). The most common haplotype (nr 24) was shared by 11 samples (15.29%), followed by haplotype nr 1 with eight samples (11.12%). Fifty-two (72.28%) of the haplotypes were represented by a single sample (Table 3.1).

From the Minimum Spanning Network (Fig. 3.4) it is clear that there is no relationship between haplotype genealogy and geographic locality. In several instances star-like radiations are visible and this may be an indication of recent population growth. The latter is supported to some extent by the mismatch distributions for the four localities (Appendix VI) and for the South African population overall (Fig 3.5) which were unimodal. Observed differences between the localities all have similar distributions although not nearly as smooth as that obtained for the overall mismatch distribution. A relative good fit to a model of sudden expansion was observed in all cases, as reflected in the S.S.D. values (Appendix VI), although this is not reflected in the significance of the P-values. The overall ruggedness index was low (r = 0.0058) indicating a good fit, but it was also not significant (P = 0.7599).

At all localities, as well as the population overall, the Tajima D test for neutrality and Fu's F_s test indicated that the mtDNA control region of *P. commersonnii* is evolving according to neutral expectations (Table 3.3). Although R_2 values were significant at all localities, as well as for the South African population, the values are not small enough to indicate that the populations have undergone historical growth or that the marker is under selection (Table 3.3).

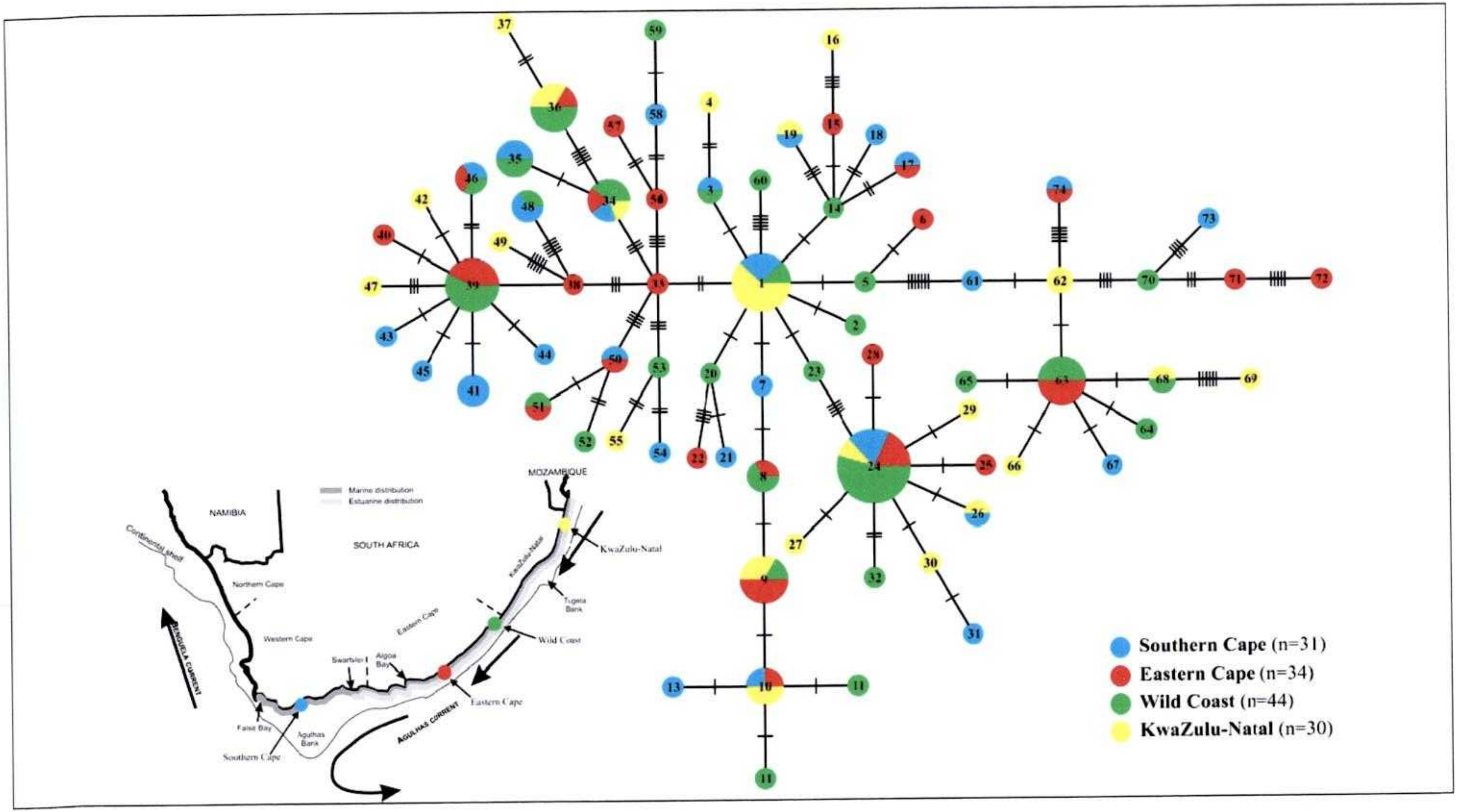

Fig. 3.4 Minimum Spanning Network showing the relationships among the 74 South African *P. commersonnii* mtDNA control region haplotypes. Circle diameter is proportional to haplotype frequency. Pie charts display the localities where the haplotype has been found and numbers indicate haplotype numbers as in Table 3.2. All haplotypes are separated by at least one mutational step, with hatch marks across the connecting lines indicating the number of mutations.

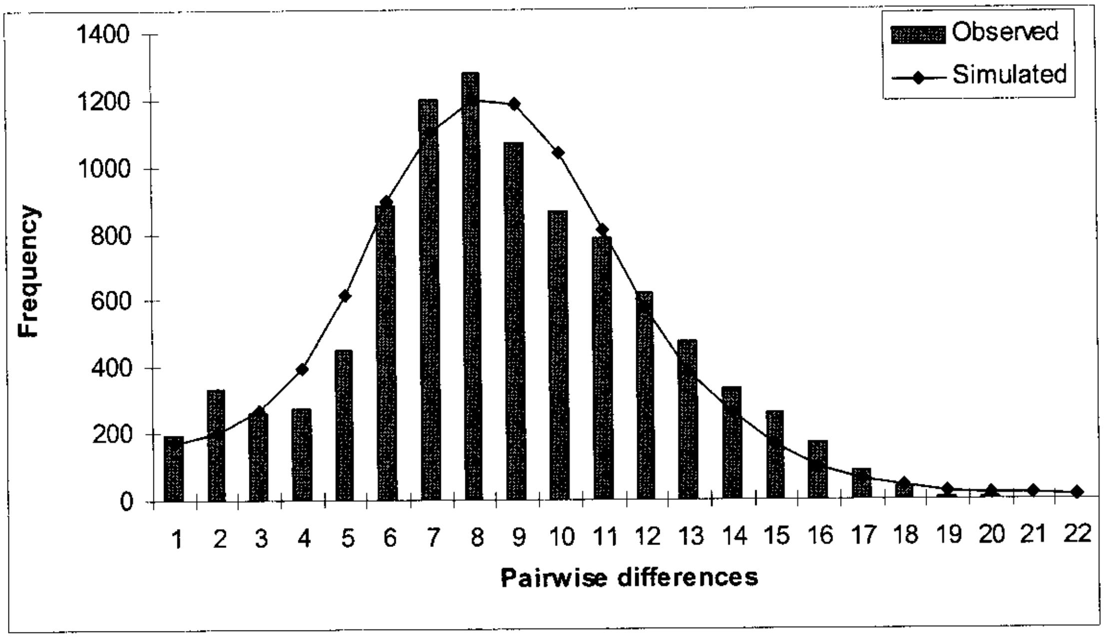

Fig. 3.5 Mismatch distribution of pairwise differences between 139 *P. commersonnii* individuals based on the 5' end of the mtDNA control region. The expected frequency is based on a population growth-decline model (S.S.D. = 0.0014 $P = 0.500$, r = 0.0058 $P = 0.7599$ $\theta_0 = 1.481$ and $\theta_1 = 55.190$) determined using ARLEQUIN.

Table 3.3 Comparison of several neutrality tests for the South African *P. commersonnii* mtDNA control region (350 bp fragment) analysed per locality and the South African population overall

Locality	**Tajima D**	***P***	**Fu's F_s**	***P***	**R_2**	***P***
Southern Cape	-0.0721	0.5417	0.3400	0.4433	0.1617	0.0000
Eastern Cape	-0.0802	0.5397	0.1074	0.4722	0.1671	0.0000
Wild Coast	-0.0752	0.5385	0.0955	0.4791	0.1673	0.0000
KwaZulu-Natal	-0.0757	0.5375	0.0837	0.4757	0.1663	0.0000
SOUTH AFRICA	*-0.0814*	*0.5475*	0.3325	0.4509	0.1621	0.0000

Genetic diversity within and among sample localities

Fifty-four of the 74 haplotypes (72.97%) were found at only a single locality. KZN had the most number of private haplotypes, with 59% of haplotypes from KZN found nowhere else (Endemism index, Table 3.4). Haplotype and nucleotide diversity observed at the four localities was similar to that for the overall population (Table 3.4). Evaluation of nucleotide divergence between localities revealed similar values, whereas corrected pairwise divergence showed that, in all instances, within locality diversity was greater that between locality diversity (negative values) (Table 3.5).

Table 3.4 Genetic diversity estimates for *P. commersonnii* mtDNA control region variability within sampling localities

Locality	n	No of haplotypes	Endemism index	*h* (±SD)	π %	Mean pairwise differences (bp)
Southern Cape	31	26	0.54	0.9871 ± 0.0122	2.066	7.2323
Eastern Cape	34	26	0.46	0.9804 ± 0.0125	2.301	8.0517
Wild Coast	44	29	0.52	0.9683 ± 0.0139	2.199	7.6966
KwaZulu-Natal	30	22	0.59	0.9678 ± 0.0208	2.282	7.9885
SOUTH AFRICA	***139***	***74***		***0.9800* ± 0.0041**	***2.194***	***7.6783***

Table 3.5 Nucleotide divergences between localities. Above diagonal: average percentage pairwise difference between localities. Below diagonal: corrected average pairwise difference

	Southern Cape	**Eastern Cape**	**Wild Coast**	**KwaZulu-Natal**
Southern Cape		2.1724	2.1299	2.1693
Eastern Cape	-0.0386		2.2126	2.2641
Wild Coast	-0.0099	-0.1302		2.2002
KwaZulu-Natal	-0.0179	-0.0956	-0.1418	

Geographic variation among localities

Testing for spatial homogeneity of mtDNA haplotype frequencies between the four localities as non-significant, with the *P*-value ($\chi^2 = 235.6773$, $P = 0.0644$) indicating that significant structure does not exist between the four localities. However, after removal of homoplasy characters, the result obtained ($\chi^2 = 119.2160$, $P = 0.4130$) differs noticeably from that above. Results from AMOVA revealed that 100% of the variation was found among samples within localities and little thereof could be attributed to variation among localities. This resulted in an overall low F_{ST} value ($F_{ST} = -0.00999$, $P = 0.9365$) suggesting homogeneity among the localities. All pairwise F_{ST} values were negative and non significant (Table 3.6).

Table 3.6 *Pomadasys commersonnii* pairwise F_{ST} and associated probability (*P*) values between four South African localities. F_{ST} values are below the diagonal and corresponding *P* values above the diagonal

	Southern Cape	**Eastern Cape**	**Wild Coast**	**KwaZulu-Natal**
Southern Cape		0.6099	0.4869	0.4698
Eastern Cape	-0.0052		0.9825	0.8205
Wild Coast	-0.0016	-0.0167		0.9911
KwaZulu-Natal	-0.0023	-0.0121	-0.0183	

Discussion

This is the first study to determine the genetic variation in any of the species in the genus, *Pomadasys*. The 5' end of the mtDNA control region is comparable to that reported for other fish species, with most of the variation found in the middle of the fragment. No evidence of tandem repeats, resulting in size variation typically found in many species, were observed (Lee *et al.*, 1995). The 5' end of the mtDNA control region has been shown to be selectively neutral, and is thus a suitable molecular marker for population studies.

High levels of mtDNA genetic diversity ($h = 0.98$, $\pi = 2.19\%$) found in South African *P. commersonnii* compare well with that typically observed in other marine fish species (Beheregaray & Sunnucks, 2001; Carvalho & Hauser, 1998; Gold & Richardson, 1991; Grant & Bowen, 1998; Graves, 1998; Hauser & Ward, 1998; Rocka-Olivares *et al.*, 2000; Zardoya *et al.*, 2004). In general a marine fish species typically exhibits both high h and π diversity due to either secondary contact of previously differentiated allopatric lineages or as a result of a long evolutionary history in a large stable population (Grant & Bowen, 1998). This is also to be expected of a large population such as *P. commersonnii*, since the loss of genetic diversity due to drift is lower and therefore mutations accumulate over time (Hauser & Ward, 1998; Kimura, 1983). The large mutational difference observed in the Minimum Spanning Network between haplotype numbers 5 and 61, could point to integration of two differentiated maternal lineages. A few star-like radiations are apparent, possibly indicating that *P. commersonnii* off South Africa's coast experienced recent population growth, but in general it appears that this species comprises a large stable population and that mutations have accumulated over time. The latter is evident in the large number of homoplasy characters observed. The mismatch distribution fits well to the model of population expansion and shows no apparent evidence of the integration of differentiated maternal lineages.

Genetic variation within the four South African localities was very similar. Haplotype and nucleotide diversity from the individual localities were comparable to the values recorded for the overall South African population. The near identical percentage nucleotide divergence values within and among *P. commersonnii* sampling localities indicate that, on average, any two individuals drawn at random from a given locality will be about as different as any two individuals drawn from different localities, as for as their mtDNA haplotypes. In all cases, within locality diversity were greater than among locality diversity, resulting in a negative value in the corrected pairwise differences.

Analysis of the 139 *P. commersonnii* samples revealed no evidence of genetic differentiation between the four South African localities. Although the spatial homogeneity test gave a near significant value ($P = 0.064$) when analysing the full fragment, thus indicating some structure, it changed six fold after the removal of homoplasy characters ($P = 0.413$). This was the only result where there was a large discrepancy between the two comparisons. By examining the haplotypes, and their distribution, it is evident that the large number of haplotypes represented by only a single sample (70.27%) heavily influences the spatial homogeneity test. As the number of haplotypes were reduced by removal of the homoplasy characters, the test gave a better reflection of the lack of genetic differentiation and is in agreement with the F_{ST} value indicating homogeneity among localities. The high number of haplotypes that were only found at a single locality resulted in high the endemism index values. These were spread evenly between the four localities, indicating similar diversity along the coast.

Effectively an F_{ST} of zero was recorded between the various localities, indicating extensive female gene flow along the coast. This seems to be in contrast with tagging information that indicate that adults are resident around estuaries (Bullen & Mann, 2004b; Fennessy & Radebe, 2000). Recently reported biological information shows that no spawning occurs in a large portion of the species' distribution and all indications are that mature adults migrate up the coast to the warmer waters of KZN for spawning (Webb, 2002). A combination of the above genetic and ecological evidence (Fig 3.6) suggests that adults spawn off the KZN coast, the inshore currents randomly disperse eggs and larvae, and the early juveniles enter shallow coastal waters when they are ready to recruit into estuaries. Similarly, *Pomatomus saltatrix* migrate to the warmer waters of KZN to spawn, with ocean currents transporting larvae from the KZN spawning ground to the south coast in 3-4 weeks (Beckley & Connell, 1996). However, *P. saltatrix* spawn further offshore (50–100 m) (Beckley & Connell, 1996; van der Elst, 1976) than *P. commersonnii* (30 m maximum recorded depth) (Smith & McKay, 1986) and are therefore more likely to be influenced by the strong southward flowing Agulhas Current. Consequently, it might be expected that *P. commersonnii* larvae will not to be dispersed as rapidly, or as far to the southwest, as those of *P. saltatrix*. Evidence to support this view is provided by the lack of juvenile *P. saltatrix* in KZN estuaries whereas *P. commersonnii* juveniles are common in the estuaries of this region (Wallace & van der Elst, 1975). Conversely, *P. saltatrix* juveniles are regularly found in the lower reaches of south coast estuaries but *P. commersonnii* juveniles are rare in these systems (Whitfield & Kok, 1992).

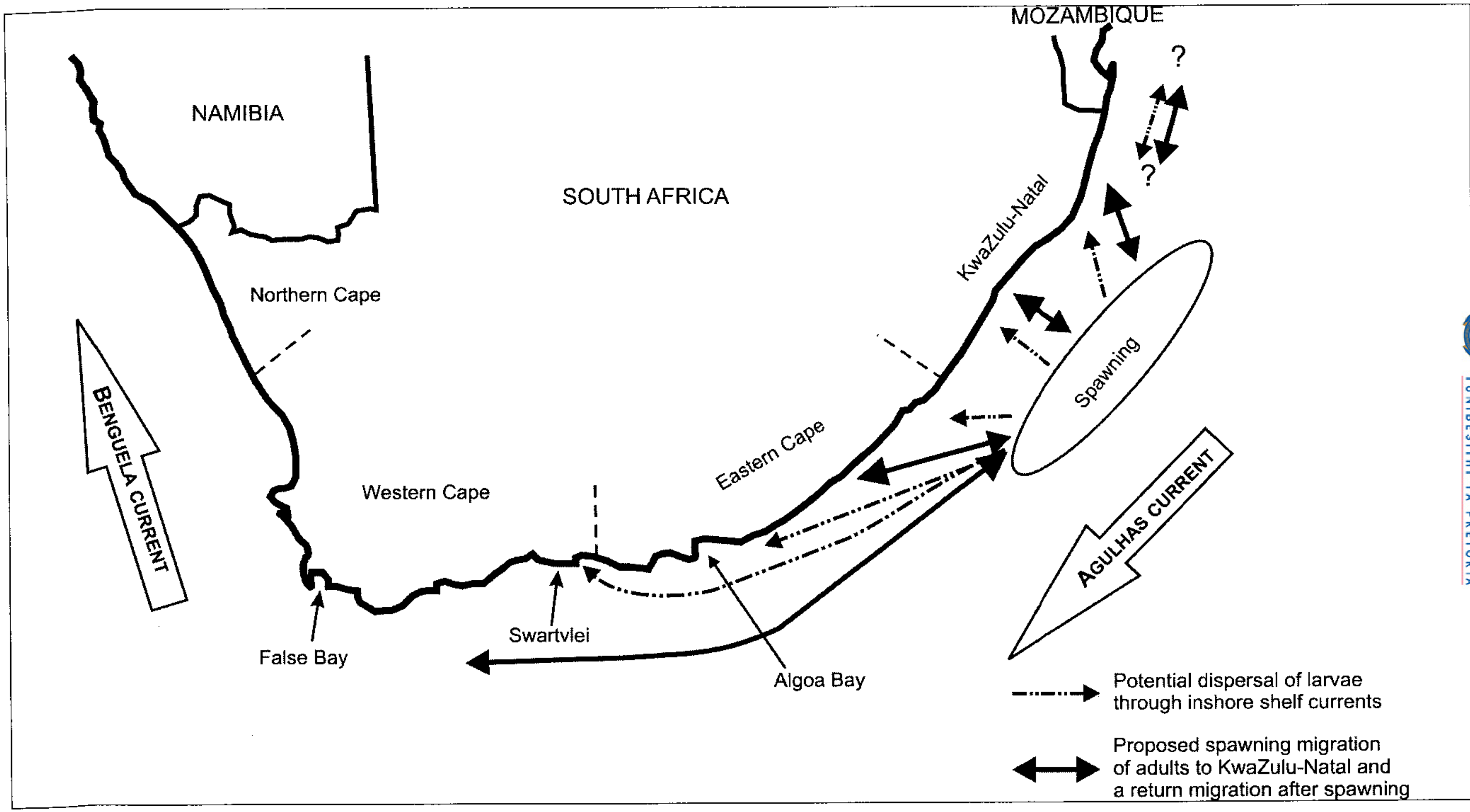

Fig.3.6 Suggested adult and larval movements along the coast that may influence genetic structuring. Based on life history data adult *P. commersonnii* undergoes a spawning migration to KZN, larvae get dispersed by inshore currents influenced by the Agulhas Current, resulting in recruitment of juveniles into estuaries as far southwest as Swartvlei. Gene flow between South African and Mozambican *P. commersonnii* is currently unknown.

Determining larval distribution accurately is difficult and is usually done through a variety of indirect techniques. Although fish population origins can be inferred from genetic studies, this can however still not determine the exact extent of larval dispersal (Thresher, 1999). Circumstantial evidence, based on juvenile abundance in estuaries, suggests that *P. commersonnii* larvae undergo a major reduction in abundance west of Algoa Bay (A.K. Whitfield, pers. comm.). Juveniles grow up and stay close to their natal estuaries with adults only migrating to spawn. Thus larval dispersal southwards and movement back up the coast by reproductively active adults ensures that the South African *P. commersonnii* population remains undifferentiated (Fig 3.6). A possible explanation for the discrepancy between tagging and life-history data might be that adult fish catchability in the marine environment and during their spawning migration is very low (P. Cowley, pers. comm.). Tagging data does show that *P. commersonnii* are capable of migrating long distances, with eight individuals having been recaptured between 105 km and 832 km from their tagging locality (Bullen & Mann, 2004b).

Current sample size and the mixture of age classes analysed from the four localities is not ideal for the detection of structure. Furthermore, it runs the risk of failing to identify any such structure that may well exist since small sample sizes might not have the power to detect differentiation (Hauser & Ward, 1998; Ward, 2000). The marker is not without limitations and high levels of homoplasy can occur in the mtDNA control region which introduces ambiguity into the relationship of the haplotypes (Avise & Lansman, 1983). However, comparison of analyses (Table 3.2) in *P. commersonnii* indicated that the removal of the homoplasy sites did not have a marked effect on the results. The high mutation rate and small effective population size of mtDNA makes it an ideal marker for population studies (Hauser & Ward, 1998).

The current analysis does give a good representation of the *P. commersonnii* genetic diversity that occurs within the South African population as a whole. High levels of gene flow observed are in concordance with life-history information, indicating that despite the apparent residency of adults, a highly interconnected population occurs along the coast. In South Africa, the population of *P. commersonnii* is at the southern extremity of its western Indian Ocean distribution. Along this coast the species inhabits subtropical and warm temperate waters, compared to tropical waters throughout most of its western Indian Ocean habitat. In order to fully comprehend the dynamics of the South African *P. commersonnii* population it will be necessary to compare populations throughout their range. This will assist towards the development of regional and global plans for the long-term sustainable utilization of this valuable species.

Chapter 4

Conclusion

Argyrosomus japonicus and *Pomadasys commersonnii* have a similar distribution and to a large extent utilize the same inshore and estuarine habitats in the subtropical and warm temperate waters off South Africa's eastern and southern coast. Both are classified as estuarine dependent IIa species, with estuaries acting as nursery areas for their juveniles. Estuaries are therefore integral to their survival along the South African coast. In both species a spawning migration up the east coast to the warmer waters of KwaZulu-Natal (KZN) takes place during late winter to early summer. However, for *A. japonicus* spawning has been recorded as far southwest as Cape Infanta on the south coast. Inshore currents along the species' distribution are strongly influenced by the fast-flowing Agulhas Current which moves in a southwesterly direction. These inshore currents, together with the Agulhas Current, have the potential to transport larvae from the spawning grounds off the KZN coast to the southern Cape coast within three to four weeks. This passive movement of larvae from the subtropical waters to the warm temperate waters off the south coast could potentially result in high gene flow in only one direction. No such evidence was observed in either species. The potential one-directional gene flow via larval dispersal is most likely countered by the migration of adult fish up the coast to spawn. In *P. commersonnii*, where spawning has only been recorded off the KZN coast, the South African population may therefore be completely interconnected. However, in the case of *A. japonicus*, spawning in both KZN and Cape waters has the potential to result in population differentiation between the coastal regions.

Analyses of the mitochondrial DNA control region revealed that both species have high levels of diversity (haplotype and nucleotide diversity), certainly comparable to that found in many other marine fish species. There is, however, a marked difference in the phylogenetic relationships among the haplotypes within the two species; *P. commersonnii* shows signs of star-like radiations, an indication of recent population growth, whereas the genealogy of *A. japonicus* haplotypes shows the possible mixing of several isolated lineages in the regional populations. A potential explanation is that *A. japonicus* was once more widely distributed, given the species' current global distribution, and as the species' range contracted, several lineages that were previously isolated, came into contact with each other to form the South African population. An alternative explanation is that 'vagrant' individuals from other parts of the world have, from time to time, entered South African waters and contributed to the local gene pool. One possible way to understand the observed local pattern of mtDNA variation, would be to conduct future genetic analyses using multiple markers at a broader (worldwide) as well as at a finer (multiple cohorts from a many estuaries and large numbers of marine adults) scale. Preliminary analyses between the South African and Australian populations indicated that these two populations have been isolated of a long time. Indications are that the Australian population also contains several divergent maternal lineages similar to that observed for the South African population and similar population processes may thus be operating throughout the species' range.

To obtain a better understanding of the processes shaping genetic diversity in the South African *P. commersonnii* population, the local population would have to be compared to those found along the Mozambican coast and beyond. The latter is especially important given the need for the identification of transboundary stocks that have to be equitably managed by neighbouring countries to ensure sustainable utilization.

No significant population differentiation results were obtained in any of the tests that were conducted, probably due to insufficient numbers of samples analysed. However, the results obtained are supported by the previously described spawning patterns observed for the two species. In the case of *A. japonicus*, where spawning has been recorded off the KZN and southern Cape coasts, possible isolation-by-distance was detected. Whereas for *P. commersonnii*, which is only known to spawn off KZN, no population differentiation was evident. *P. commersonnii* larvae are dispersed from the spawning grounds as far to the southwest as Swartvlei, although abundantly only as far as Algoa Bay. This might also give an indication of the potential dispersal of *A. japonicus* larvae, with juveniles found along the southern Cape coast being a result of the spawning observed within that region. This would explain the differentiation observed between this region and especially KZN. However, the isolation-by-distance observed in *A. japonicus* sampled from the Eastern Cape and KZN is more difficult to explain but may be due to the larvae of this species being closer inshore and therefore less susceptible to longshore currents that would transport them away from the spawning areas.

In conclusion, although no definitive results regarding the species' population structure were obtained, the present study has provided a valuable baseline for future research. More intensive sampling, including multiple larval cohorts from spawning and nursery areas as well as marine adults sampled during breeding and non-breeding seasons, could potentially reduce noise in mtDNA data and result in better estimates of female gene flow patterns. In

A. japonicus, where some differentiation potentially exists within the South African population, the use of microsatellite markers will enable the detection of fine-scale patterns of genetic differentiation and allow the indirect estimation of overall (male and female mediated) gene flow. Analyses at various spatial and temporal scales will enhance our understanding of intraspecific variation in marine fish species which is not only of relevance to stock management but also more broadly to the identification and protection of marine biodiversity.