Intraspecific genetic variation in the percoid teleosts *Argyrosomus japonicus* (Temminck & Schlegel, 1843) and *Pomadasys commersonnii* (Lacepède, 1801), as inferred from the mitochondrial control region

by

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Thesis Abstract

Dusky kob, *Argyrosomus japonicus* and spotted grunter, *Pomadasys commersonnii* occur off South Africa's southern and eastern seaboard. They are the preferred target species for both shore and estuarine anglers. In order to sustain the billion rand recreational angling industry, healthy fish populations are of the utmost importance. However, *A. japonicus* is currently overexploited, with the species' spawner biomass estimated at 1–4.5% of pristine levels. *Pomadasys commersonnii* spawner biomass is estimated at 40% of pristine levels, thus indicating that the species is not overexploited. For effective management of our marine resources, information about the population size, structure, dynamics and population history of individual species is needed. Genetic data can make a valuable contribution to a holistic stock determination approach, as powerful tools in unravelling population history. Genetic variation gives a reflection of the evolutionary differences within and between populations and allows for indirect assessment of population connectivity and gene flow levels.

The mitochondrial DNA (mtDNA) control region is a useful marker in population studies, due to its high substitution rate. The haploid nature of the mtDNA, maternal inheritance and the absence of recombination, means that the signal obtained from genetic drift is stronger than that for nuclear loci. For this reason the mtDNA control region was analysed for 133 juvenile

A. japonicus and 139 *P. commersonnii* samples from four localities along their South African distribution, to determine the genetic diversity and differentiation among the coastal regions. Juvenile *A. japonicus* are resident around their natal estuaries until they reach sexual maturity. This residency of juveniles makes them ideal candidates to give a reflection of *A. japonicus* population dynamics, especially to determine if any isolation-by-distance exists along the coast. In the case of *P. commersonnii*, both juveniles and adults were analysed, since adults are resident around their natal estuaries when not undertaking spawning migrations.

High levels of genetic diversity were found in both *A. japonicus* and *P. commersonnii*, comparable to that observed in other marine fish species. No significant population differentiation results were obtained, possibly due to the small sample sizes collected or to lack of resolution in the marker. In *A. japonicus*, where spawning is known to occur off KwaZulu-Natal and off the southern Cape coast, possible isolation-by-distance was detected. This indicates that the adult *A. japonicus* population probably does not consist of one freely intermixing unit, but rather of geographically separated spawning units. For *P. commersonnii*, where spawning has only been recorded off the KwaZulu-Natal coast, no population differentiation was observed, indicating that the different regions along the South African coast are highly connected.

In conclusion, through future analysis of larger sample sizes, preferably from single cohorts, some of the noise will be reduced and more conclusive answers with respect to female gene flow could be provided. In the case of *A. japonicus* the use of microsatellite markers which are better at detecting fine-scale differentiation and provide estimates of total gene flow, will be informative. At a broader geographic scale, it will be important to assess differentiation among Dusky kob populations from throughout the Western and Eastern Indian Ocean. As far as *P. commersonnii* is concerned, it is recommended that a comparison be undertaken to determine the relationship of the South African population to that found along the Mozambican coast.

DECLARATION

I declare that the dissertation, which I hereby submit for the degree Magister Scientiae at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature : ..... Date : 4/08/2005.....

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Chapter 1

General introduction

Estuaries, although limited in surface area when compared to South Africa's marine environment, represent one of the most valuable habitats along the coast. Usually calm, sheltered and shallow, estuaries are highly productive systems (Correll, 1978). It is a specialised environment that serves as an important nursery area for juvenile fish (Blaber, 1974b) and a rich feeding ground for the adults of certain species (Tilney & Hecht, 1993). Dusky kob, *Argyrosomus japonicus* (Temminck & Schlegel, 1843) and spotted grunter, *Pomadasys commersonnii* (Lacepède, 1801) are two of South Africa's most targeted angling species, and both are dependent on estuaries for their survival.

Two oceans flank South Africa's coastline, the warm Indian Ocean and the cool Atlantic Ocean (Fig. 1.1). The warm Agulhas Current in the southwestern Indian Ocean flows along the eastern and southeastern coast of Africa, before meandering offshore in the vicinity of the Agulhas Bank (Branch *et al.*, 1994). The cool Benguela Current in the southeastern Atlantic Ocean flows northwards along the west coast of South Africa and Namibia, this marine environment influences physico-chemical conditions in the entire coastal zone, including the adjacent estuaries (Schumann, 2000). Depending on their distribution along the coast, South African estuaries can be divided into three groups: subtropical, warm-temperate and cool temperate (Fig. 1.1). The Agulhas current interacts with 117 subtropical and 123 warm-temperate estuaries, while the Benguela interacts with 10 cool-temperate estuaries (Whitfield, 1998).

There are few sheltered shores along South Africa's approximately 3100 km coastline, most of which is almost constantly subjected to turbulent wave action. Estuaries, on the other hand, offer sheltered environments but have varying turbidities, temperature and salinity fluctuations (Whitfield, 1998). Day (1980) defined an estuary in a South African context as follows: "An estuary is a partially enclosed coastal body of water, which is either permanently or periodically open to the sea, and within which there is a measurable variation of salinity due to the mixture of sea water with fresh water derived from land drainage". Estuaries act as nursery areas for juvenile fishes and feeding grounds for adults of certain species and therefore play an important role in the life histories, maintenance of diversity and productivity of coastal fish communities (Blaber, 1981; Braird *et al.*, 1996; Whitfield, 1998). However, estuaries represent

a highly specialised habitat and less than 10% of the approximately 1500 fish species along the South African coast makes use of this environment. Estuarine dependent fishes are defined as those taxa whose southern African populations would be adversely affected by the loss of estuaries from the subcontinent (Whitfield, 1994). Based on the level of dependence on estuaries, fish species can be divided into five categories (Table 1).

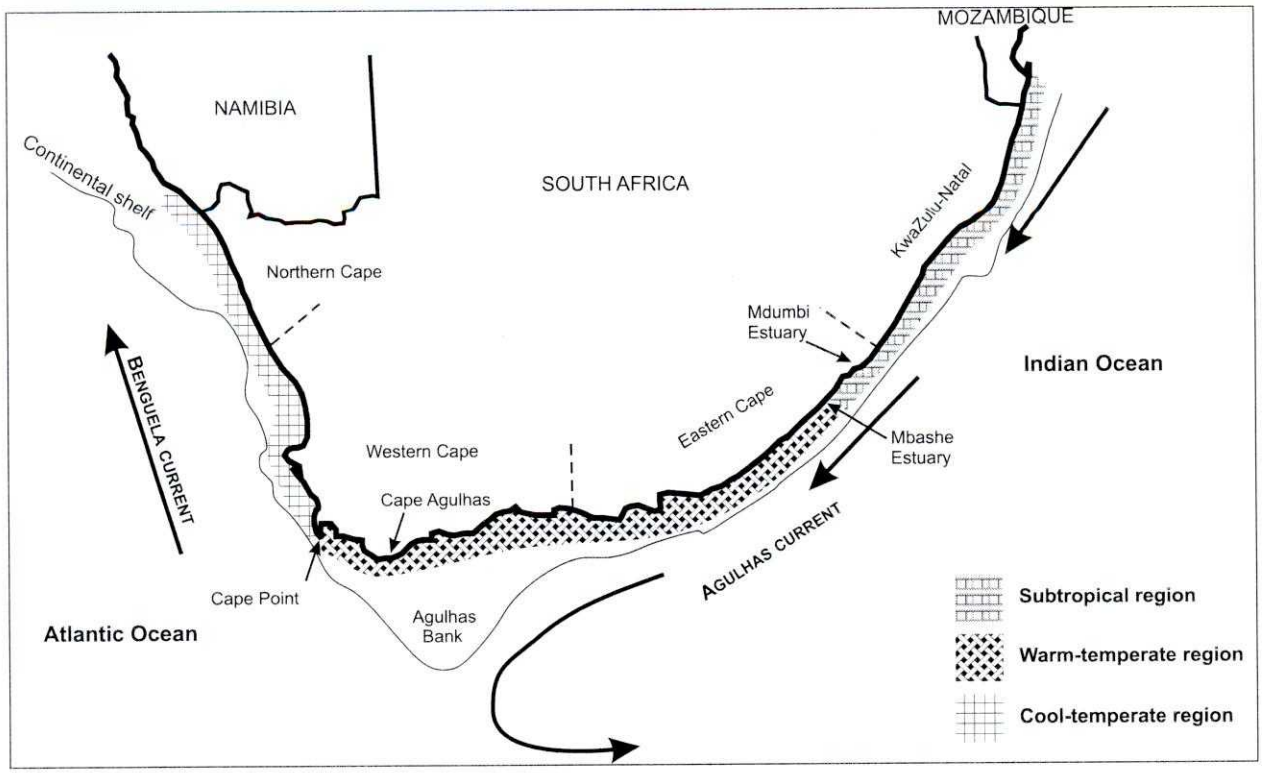

Fig. 1.1 Map of the southern African coastline. Arrows indicate major ocean currents that influence the adjacent estuarine environment. Cape Point and the Mbashe Estuary were regarded as the boundaries of the warm-temperate estuarine region (Maree *et al.*, 2000) although more recent research (Harrison, 2002) have placed these boundaries at Cape Agulhas and the Mdumbi Estuary respectively.

Larval and juvenile fish enter the estuaries mainly from late winter to early summer, and grow rapidly in these systems due to elevated temperatures, abundant food supplies and protection from predators (Blaber, 1974a). Fish generally migrate back to sea before they become sexually mature, while adult fish numbers can increase seasonally as they enter estuaries to feed (Wallace *et al.*, 1984).

Two of South Africa's fish species for which the estuarine environment is a vital habitat are *A. japonicus* and *P. commersonnii*. Whitfield (1998) lists *A. japonicus* and *P. commersonnii* as species that are dependent on estuaries and both species have been placed in category IIa. Apart from dependence at the juvenile stage, both *P. commersonnii* and *A. japonicus* adults also frequently enter estuaries to feed.

Table 1.1: The five major categories of fish utilizing southern African estuaries (Table 11 from Whitfield, 1998)

Category	Description
I	Estuarine species that breed in southern African estuaries. Further subdivided into:
Ia	Resident species which have not been recorded spawning in the marine environment.
Ib	Resident species which also have marine or freshwater breeding populations.
II	Euryhaline marine species which usually breed at sea with the juveniles showing varying degrees of dependence on southern African estuaries. Further subdivided into:
IIa	Juveniles dependent on estuaries as nursery areas.
IIb	Juveniles occur mainly in estuaries, but are also found at sea.
IIc	Juveniles occur in estuaries, but are usually more abundant at sea.
III	Marine species which occur in estuaries in small numbers, but are not dependent on these systems.
IV	Euryhaline freshwater species, whose penetration into estuaries is determined primarily by salinity tolerance. Includes some species which may breed in both freshwater and estuarine systems.
V	Obligate catadromous species which use estuaries as transit routes between marine and freshwater environments.

Estuarine dependent fish taxa include some of South Africa's most important angling species, both for recreational anglers and as a food source for subsistence fishermen and to a lesser extent the commercial fishery. Dusky kob comprises approximately 18% of the catch composition by weight in estuaries and spotted grunter 20%. Only the mugilid, *Liza richardsonii,* is a more important fishery target (32%) but utilization of this species is mainly on the west coast where large numbers are netted (Lamberth & Turpie, 2003).

Argyrosomus japonicus

Argyrosomus japonicus (Fig. 1.2) is distributed from the Cape of Good Hope to southern Mozambique, although only abundant from Cape Agulhas to northern KwaZulu-Natal. Although most abundant in the warm-temperate and subtropical waters of south-east Africa, they also occur along Australia's southern seaboard and from Hong Kong northwards along the Chinese coast to southern Korea and Japan. It also occurs off Oman, Pakistan and India in the northern Indian Ocean. In South African waters, *A. japonicus* is a continental coastal species that can be found in estuaries, the surf and nearshore zone with a maximum recorded depth of 100 meters (Griffiths, 1996b; Griffiths & Heemstra, 1995; Heemstra & Heemstra, 2004). They utilise estuaries throughout their range, especially the larger systems such as Lake St Lucia (Whitfield, 1994). In estuaries they prefer turbid water where they hunt by combining

olfactory and lateral line senses and are therefore well equipped to forage in these waters (Van der Elst, 1988).

Spawning takes place at night in the nearshore marine environment (Griffiths, 1996b) and usually coincides with the summer rainfall season along the East Coast. Elevated river flow into the coastal zone increases water turbidities and boosts planktonic productivity (Heydorn *et al.*, 1978) upon which *A. japonicus* larvae depend for food. Recruitment of the postflexion larvae and 0+juveniles into estuaries occur mostly at 20–30 mm total length (TL), which corresponds to an age of approximately one month (Griffiths, 1996b). According to Whitfield (1994) the olfactory cues from estuaries in the adjacent marine environment are probably the most important factor influencing the recruitment of estuarine dependent species such as *A. japonicus*.

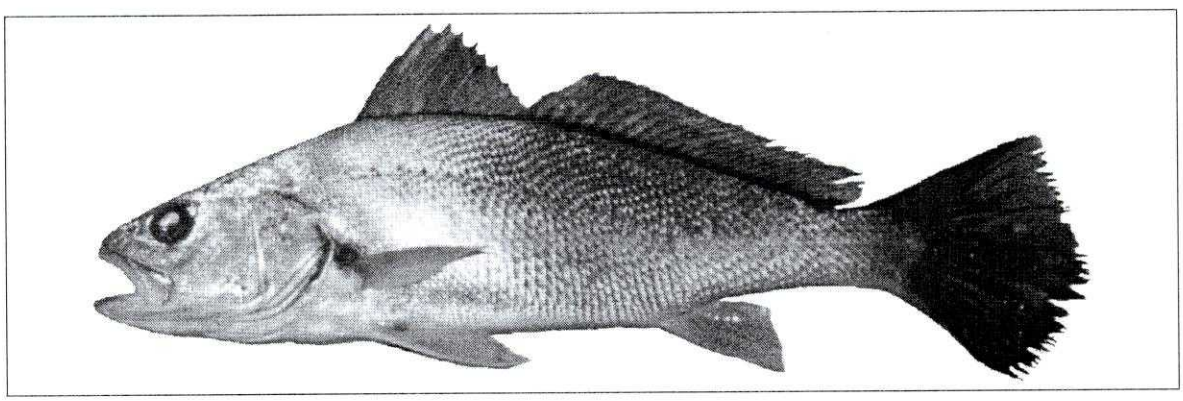

Fig. 1.2 Juvenile *Argyrosomus japonicus* (128 mm standard length).

Recruitment of *A. japonicus* into estuaries occurs mainly between October and February (Wallace & van der Elst, 1975). Early juveniles, 20–30 mm TL, appear to remain in the upper reaches of estuaries where they concentrate in the more turbid, oligohaline areas (<5% salinity). At approximately 15 cm TL they move into the middle and lower reaches, with some entering the adjacent marine surf zone (Griffiths, 1996b). Juveniles grow rapidly and can attain 35 cm TL in the first year. Individuals older than a year still tolerate a wide range of salinities (3–66‰) but subadults cannot survive prolonged exposure to very low salinities (<3%), especially in combination with low temperatures (Blaber & Whitfield, 1976). Juveniles (<50 mm TL) prey mainly on calanoid copepods and mysids (Griffiths, 1996b). Larger juveniles (>17 cm TL) feed mainly on fish, shrimps and penaeid prawns, with small fish species such as *Gilchristella aestuaria* dominating the diet of these size classes. Subadult *A. japonicus* also target these prey species, as well as the juveniles of marine migrant species (Griffiths, 1997a, b; Whitfield, 1998).

Tagging data show that juvenile *A. japonicus* do not travel far and are likely to remain close to their natal estuaries, forming localised populations until they reach sexual maturity (Bullen & Mann, 2004a; Griffiths, 1996b). Fifty percent of females reaches sexual maturity at 100 cm TL and males at 95 cm TL. These sizes translate to six years of age in females and five years in males, while all males are mature at the of age seven years and females at the age of eight years (Griffiths & Hecht, 1995). Most *A. japonicus* caught inshore (estuaries and surf zone) are less than seven years old, whereas those from the offshore linefishery are generally older than six years. Although an age of 42 years has been recorded, individuals older than 27 years are rare. Adult *A. japonicus* with a body mass in excess of 45 kg are often caught, with some individuals attaining 75 kg (Griffiths & Heemstra, 1995).

The increase of mature individuals ready to breed, which are caught by lineboats in KwaZulu-Natal between August and November, suggests that adult fish from the southeastern and southern Cape migrate there to spawn. Spawning also occurs in the Cape region, where spawning as far west as Cape Infanta has been recorded from October to January. This indicates that breeding might commence in KwaZulu-Natal and continues as a portion of the spawning population returns to southern waters. A general absence of individuals with spent gonads in KwaZulu-Natal and their presence in southeastern and southern Cape waters also implies a return migration once spawning is completed (Griffiths, 1996b; Griffiths & Heemstra, 1995).

Because of its life history characteristics, age at sexual maturity, and having evolved with relatively low rates of natural mortality, *A. japonicus* is unlikely to be able to sustain high levels of exploitation. Estuarine and shore anglers have been targeting this species for more than four decades and it is estimated that the species' biomass is between 1.0 and 4.5% of its pristine levels (Griffiths, 1997d; Lamberth & Turpie, 2003). There are at least three phases where they are particularly vulnerable to human activities. Recreational and commercial lineboat fishermen target the dense spawning aggregations off the KwaZulu-Natal coast. Recreational anglers target juveniles that inhabit the surf zone and estuaries. Although early juveniles are not exploited directly, these size classes are estuarine dependent and most of South Africa's estuaries have already been degraded in various degrees, mostly by impacts in the catchments (Griffiths, 1996b).

Pomadasys commersonnii

Pomadasys commersonnii (Fig. 1.3) is a western Indian Ocean coastal species, that occurs from India to South Africa (Heemstra & Heemstra, 2004). It has an estuarine distribution in South African waters from the southern Cape eastwards up the coast into southern Mozambique, while their marine distribution can occasionally reach False Bay during the summer (Whitfield, 1998). *Pomadasys commersonnii* is mainly found in shallow water with depths of less than 30 meters (Smith & McKay, 1986).

Spawning occurs at sea in the shallow inshore zone from August to December, although ripe and running specimens have occasionally been found in estuaries (Wallace, 1975b). Larval development occurs in the marine environment. Juveniles are recruited into estuarine nursery areas at approximately 20–30 mm TL, and they are most abundant in the upper reaches, even entering fresh water (Wallace & van der Elst, 1975; Whitfield, 1998). Size classes between 4 and 16 cm TL predominate in estuaries but post-spawning individuals also enter estuaries to feed. Juveniles 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. Tagging data suggest that adults are fairly resident and tend to congregate in the vicinity of estuary mouths (Bullen & Mann, 2004b; Fennessy & Radebe, 2000). Currently *P. commersonnii* is maximally or optimally exploited, with the spawner biomass estimated at 40% of pristine levels (Lamberth & Turpie, 2003).

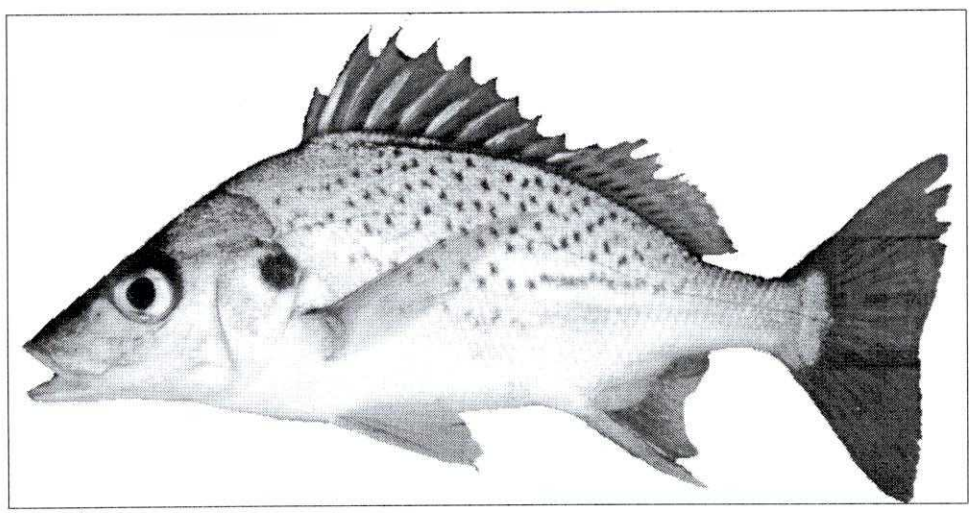

Fig. 1.3 Juvenile *Pomadasys commersonnii* (123 mm standard length).

Pomadasys commersonnii can survive in salinities from 0–90‰, but mass mortalities have been recorded when low salinities (<4%) coincide with water temperatures below 13°C (Blaber & Whitfield, 1976). Sampling and experiments have shown that *P. commersonnii* is indifferent to water turbidity (Cyrus & Blaber, 1987). 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). Although *P. commersonnii* feeds during daylight hours they are mainly crepuscular and nocturnal (Marais, 1984). The maximum recorded size is 87 cm TL, approximately 15 years old (Whitfield, 1998).

Species importance

Both *A. japonicus* and *P. commersonnii* are preferred target species of shore and estuarine anglers. Estuaries are favoured by recreational anglers and subsistence fishermen due to the high abundance of fish and the accessibility and protected waters of these systems (Baird *et al.*, 1996). In the warm-temperate and subtropical estuaries, *A. japonicus* and *P. commersonnii* are the two most important angling species. On the Cape south coast *P. commersonnii* makes up 45% of catches and *A. japonicus* 6% of catch weight. Catches on the Cape east coast are dominated by *A. japonicus* (48%) and *P. commersonnii* (31%), whereas in KwaZulu-Natal *A. japonicus* constitutes 35% and *P. commersonnii* 11% of catch weight. Altogether this amounts to an annual ±377 tons of *A. japonicus* and ±416 tons of *P. commersonnii* of a total ±2100 tons of fish caught in estuaries. Recreational angling in estuaries is worth nearly R429 million per annum. Furthermore, it is estimated that the contribution of estuarine dependent fish species to inshore fisheries is worth R519 million per annum. In total, the value of estuarine and estuarine dependent fisheries in 1997 was estimated to be ±R952 million with a projected value of about R1.162 billion in 2000 (Lamberth & Turpie, 2003).

From the above, it is clear that the economic value of *A. japonicus* and *P. commersonnii* cannot be underestimated, even at the individual estuary level. In order to sustain this source of income, healthy fish populations are of utmost importance. The increase in fishing pressures to unsustainable levels has caused severe depletion of fish stocks in South African and global waters. In the 1950s fishing efforts intensified and over exploitation of marine resources commenced. Since the late 1980s, world fish landings have started to decline and future prospects are uncertain unless drastic measures are put in place to reduce the rates of exploitation (Pauly *et al.*, 2003; Pauly & Watson, 2003). Overfishing not only depletes fish

populations but also has the effect of altering the entire marine ecosystem (Jackson *et al.*, 2001). To maintain a profitable fishery, information on the species' population history and composition is needed to formulate effective recovery and management strategies. Fish is the last major food source on this planet that is harvested from natural populations (Ryman *et al.*, 1995). The fishing industry is worth over a billion Rand to South Africa each year (Lamberth & Turpie, 2003), yet little detailed information is available about the population sizes, structures and dynamics of most of our fish species (Mann, 2000); information that is of utmost importance for the sustainable utilisation of these resources.

Stock determination

The oceans cover more than 70% of the earth's surface and most of them are interlinked. When compared to continental land masses there are few natural barriers in the oceans, with many marine species having a high dispersal potential, resulting in widespread areas that are genetically connected (Avise, 1998; Palumbi, 1994; Waples, 1998). Several studies have shown that marine species, especially those along continental margins typically show high levels of gene flow. This might be due to dispersal of pelagic larvae, through migrating adults or large effective population sizes. Consequently this translates into low levels of population genetic differentiation (Carvalho & Hauser, 1998; Exadactylos *et al.*, 1998; Gold *et al.*, 1999; Hilbish, 1996; Waples, 1998; Ward, 2000; Ward *et al.*, 1994). However, increasingly studies are showing that a lack of barriers and high dispersal potential do not necessarily translate into high gene flow or low levels of population differentiation (Avise, 1998; Hilbish, 1996; Hutchinson *et al.*, 2001; Lundy *et al.*, 2000; Luttikhuizen *et al.*, 2003; Smith *et al.*, 2002; Stephenson, 1999; Waples, 1998). Behavioural philopatry during some stage in the life cycle of a species might be responsible for low gene flow and differences in population characterisitics (Avise, 1998; Carvalho & Hauser, 1998).

Most organisms show some form of discontinuous aggregation of individuals in space and/or time. Each aggregation has a specific character or genetic make-up that is influenced by gene flow from other aggregations, genetic drift and natural selection. In exploited marine species these aggregations are referred to as "stocks" (Shaklee & Bentzen, 1998). Correct identification of stocks are important for the proper management of exploited species and the preservation of their genetic diversity (Booke, 1999). Various definitions of a stock exist, including that of Ihssen, *et al.* (1981): "a stock is an intraspecific group of randomly mating individuals with temporal and spatial integrity". Most fisheries consist of more than one

fishing ground and the question then arises: do these different fishing grounds exploit one or more stocks of the same species? The high diversity that exists at population and species levels, and the risk due to overexploitation, drives the quest to describe and monitor fish population structure (Carvalho & Hauser, 1998). The answer is of critical importance to management because, for each stock, a separate assessment of sustainable fishing should be determined (Ward, 2000). It is the genetic variation that exists at not only the species level but within and among populations that is essential in the management programme of species. Genetic variation is what allows species to keep on adapting to changing environments and to respond to pressures (O'Connell & Wright, 1997).

Considering the above, it is surprising how few fishery assessments implement stock identification requirements. For effective management and stock rebuilding programmes it is not only stock structure that is important, but also the distribution of fishing effort and mortality among stocks. Correct stock identification and estimation of exchange between them is a major challenge for scientists and managers. It assists in the recognition and protection of spawning and nursery areas, the development of an optimal harvesting and monitoring strategy, and in catch allocations between competing fisheries (Begg *et al.*, 1999).

Determining stock structure is a critical biological and management issue. Various environmental factors, biological information, phenotypic and genotypic variation have been studied in order to infer stock structure (Begg *et al.*, 1999; Waldman, 1999). Phenotypic characters usually reflect short-term environmentally induced differences, such as prolonged separation of postlarval fish in different environments, thus providing an indirect measure of genetic isolation (Begg *et al.*, 1999; Waples, 1998). Results obtained from phenotypic markers should only be interpreted with the understanding of their inheritance (Booke, 1999). Information obtained from mark/recapture surveys on later life stages (juveniles and adults) and collection of early life stages (eggs and larvae) from different regions gives some indication of movement. How far and in which direction larvae actually disperse cannot, however, be accurately mapped (Thresher, 1999). The overall recapture rate for the South African Sedgewick's/ORI/WWF Tagging Programme is 5.2% (Bullen & Mann, 2004b). In addition, movement or migration does not necessarily translate into gene flow (Ferris & Berg, 1987; Waples, 1998). However, baseline information provided by life history parameters usually assists in the initial recognition and delineation of geographically isolated stocks (Begg *et al.*, 1999).

Genetic variation on the other hand reflects evolutionary differences between stocks (Begg *et al.*, 1999; Begg & Waldman, 1999). Failure to detect differences indicates that (a) it might be one stock, (b) that the specific marker could not resolve the question, (c) recent separation has occurred or (d) that the sampling was inadequate. Even low amounts of gene flow can result in genetic homogeneity among samples, yet the actual amount of gene flow is important in assessing stock models. In large populations neutral markers will diverge relatively slowly through genetic drift and it may take millions of generations before they reach equilibrium and only then will the marker fully reflect the reproductive isolation among existing populations (Carvalho & Hauser, 1998; Dizon *et al.*, 1992; Waldman, 1999; Ward, 2000). Although very little gene flow is necessary to obscure any evidence of separate stock structure, limited gene flow cannot be relied on for the rebuilding of a depleted stock (Waples, 1998). Failure in recognising stock structures can lead to depletion of spawning components that result in a loss of genetic diversity and unknown ecological consequences (Begg *et al.*, 1999).In species that are not amenable to direct study, gene frequencies can be used to draw inferences about species biology (Beaumont & Nichols, 1996).

Genetics is a valuable tool in stock identification, it is however not without problems and limitations (Ward, 2000). Specific genetic markers, protein or DNA, all have shortcomings and limitations. For a marker to be useful, its functional behaviour needs to be understood and monitored (Booke, 1999). Genetic data, combined with information on aspects of species biology, biogeography, behaviour, migration, reproduction and oceanographic data will contribute to the best possible management plan. Genetic data can provide a direct basis for stock structuring and for the interpretation of other observed patterns, e.g. phenotypic patterns (Ihssen *et al.*, 1981). Although a multi-disciplinary approach is likely to be very costly for most species, the integration of all available information before decision-making is of the utmost importance for an optimal management strategy (Waldman, 1999).

It is difficult to determine stock structure in species with a high gene flow (= low F_{ST} values). Mean F_{ST} for marine species are estimated at 0.062, while 60% of marine species have a F_{ST} value of less than 0.03 (Ward *et al.*, 1994). Estuarine species especially those that spawn within estuaries generally show lower levels of gene flow (= higher F_{ST} values) because of retention mechanisms (Bilton *et al.*, 2002; Chenoweth & Hughes, 2003; Chenoweth *et al.*, 1998; Stefanni & Thorley, 2003). Estuarine dependant marine species breeds at sea and particularly those that undergo spawning migrations shows gene flow comparable to that found in typical marine species. Low levels of F_{ST} observed in marine species reflect the high

dispersal capabilities and lack of barriers. The low levels of differentiation observed in many marine species necessitate the use of large sample sizes, as well as multiple markers to discriminate the genetic signal from genetic noise. The estimation of population genetic parameters can be affected by the noise. The common principle in all stock identification studies is that the signal from the among-stock variation must exceed the noise of within-stock variation. Apart from large sample sizes, temporally separated samples should also be analysed, since patterns of genetic relatedness or differentiation that is consistent over time are unlikely to be due to sampling problems (Gold *et al.*, 1999; Waldman, 1999; Waples, 1998; Ward, 2000). Taking all the problems that might arise into consideration, it is important that one understands the ecology and life history of the species under investigation. This will help facilitate effective strategies to minimize sampling errors and to adjust for possible bias that might occur (Waples, 1998).

Genetic markers

Stock identification based on genetic methods allows for indirect testing of reproductive isolation and gene flow between stocks (Shaklee & Bentzen, 1998). The initial method employed to determine genetic differences was based on indirect expression of DNA loci, in the form of blood group and protein variation. This method was followed with the advent of allozyme analysis (protein electrophoresis) in the 1970s, and allozymes have become the primary marker that most studies have relied upon. Allozymes is a cost-effective application that is easy to apply and large sample sizes can be analysed (Begg & Waldman, 1999; Carvalho & Hauser, 1998; Park & Moran, 1994; Shaklee & Bentzen, 1998; Ward, 2000). Allozymes have been used for 30 years, and is far from obsolete and can still yield valuable information. The speed and simplicity of allozyme analysis is one of its major advantages. Disadvantages are that it requires fresh or frozen material, most species show low levels of variability at allozyme loci and some allozyme loci may be under selection. Methods were then developed that more directly examined DNA variability and initially employed the use of restriction enzymes. However the development of polymerase chain reaction (PCR) technologies and DNA sequencing provided the best method to determine genetic differences (Carvalho & Hauser, 1998; Ward, 2000). The most rapidly expanding marker used in population and fisheries biology studies is microsatellites. Microsatellites are co-dominant nuclear markers that are inherited in a Mendelian way and is ideal for fine scale stock structure investigations (Carvalho & Hauser, 1998; DeWoody & Avise, 2000; O'Connell & Wright, 1997; Shaklee & Bentzen, 1998; Ward, 2000).

The advantage of PCR-based genetic markers over protein electrophoresis is the ease of sample collecting and storage. Non-lethal sampling can be done since only a small piece of material is required, eggs and larvae can be used and archived material such as scales and otoliths can be analysed (Hutchinson *et al.*, 1999; Ward, 2000). Analysing archive material such as scales have been used with success to determine the loss in genetic diversity that coincided with a decline in population size (Hauser *et al.*, 2002). Access to several markers, ranging from low to high variability, and the use of non-coding regions make the data less liable to the influence of natural selection and more likely to reflect stochastic processes. The limitation of the method is that it is more expensive and that it takes more time to develop (Ward, 2000).

Initially most studies were limited to mitochondrial DNA (mtDNA), a small (±16 000 base pairs in length) circular molecule in the cytoplasm. In most organisms, including most fish, it is maternally inherited and thus haploid. Therefore, studying mtDNA allows one to characterise maternal lineages within and among species. The mtDNA contains 13 protein-coding genes, two ribosomal-coding genes, 22 genes that code for transfer RNAs and one non-coding segment, the control region. Furthermore the mitochondrial genome does not undergo recombination and the genes have different mutation rates (Avise, 1998; Faith & Pollock, 2003; Ferris & Berg, 1987; Meyer, 1993; Moritz *et al.*, 1987; Park & Moran, 1994). However, it must be noted that several studies have indicated that recombination in the mtDNA genome do occur in animals including vertebrates such as humans and fish (Awadalla *et al.*, 1999; Hoarau *et al.*, 2002; Ladoukakis & Zouros, 2001; Lunt & Hyman, 1997; Rokas *et al.*, 2003)

The section with the highest substitution rate in vertebrates (two to five times higher than mitochondrial protein coding genes) is the control region, making it useful for population studies. The length of this segment in fish varies from 800–1500 base pairs (bp), mostly due to tandem repeat sequences and large insertions, but it also contains conserved sequence blocks similar to those found in mammals. The hypervariable region I (5' end) has considerable size variation among species due to the presence of tandem repeat sequences. It also shows high levels of nucleotide substitution, which is most useful in intraspecific studies. The hypervariable region II (3' end) in some species have long repetitive sequences making it less suitable for population studies (Lee *et al.*, 1995).

Due to the haploid nature and absence of recombination in mtDNA, the signal obtained from genetic drift is stronger than for nuclear loci. If there is equilibrium between drift and

migrations, and drift and mutation, together with equal dispersal of males and females between stocks, mtDNA effective population size is expected to be one quarter that of nuclear genes. This means that genetic drift and loss of genetic variation within reproductively isolated stocks occurs at a faster rate in mtDNA than in nuclear genes. Thus, on average, mtDNA variation is expected to be greater between stocks and less within stocks. In most species, mtDNA has a higher mutation rate than single copy nuclear DNA, providing high-resolution analyses of recent evolutionary events (Birky *et al.*, 1989; Ferris & Berg, 1987; Meyer, 1993; Shaklee & Bentzen, 1998; Waples, 1998; Wilson *et al.*, 1985).

In order to fully understand the dynamic processes that shape species and their populations, more than one type of molecular marker needs to be investigated. Only then can the genetic data in combination with biological and life history data lead to an optimal management strategy for the sustainable utilisation of the species.

Thesis preview

The following chapters deal with the intraspecific variation of *Argyrosomus japonicus* (chapter 2) and *Pomadasys commersonnii* (chapter 3) based on analysis of their mtDNA control region sequences.

The overall aim of the study was to determine the genetic diversity and population structure of the species based on the genetic marker, and to link the genetic data with existing survey and other ecological and life history data.

Chapter 4 gives an integrated summary for the two species.

Chapter 2

Mitochondrial DNA variation in South African dusky kob, Argyrosomus japonicus (Temminck & Schlegel, 1843)

Abstract

Argyrosomus japonicus is abundant from Cape Agulhas to northern KwaZulu-Natal in South African coastal waters. The species is also found along Australia's southern seaboard, from Hong Kong northwards in the South China Sea to southern Korea and Japan and in the northern Indian Ocean off Oman, Pakistan and India. In South Africa *A. japonicus* juveniles are estuarine, while subadults and adults inhabit inshore waters from the surf zone to a depth of 100 meters. Juveniles remain close to their natal estuaries until they reach sexual maturity. Indications are that spawning commences in KwaZulu-Natal from August to November and continues south to the Cape region. Based on otolith dimensions and fish length relationships, it has been suggested that the South African population of *A. japonicus* consists of several allopatric juvenile subpopulations and a single offshore migratory adult population. Analysis of mitochondrial DNA shows that the genetic diversity (mean $h = 0.95$, $\pi = 0.89\%$) compares well with other marine fish species, with 35 haplotypes detected from the 133 control region sequences analysed. Haplotype relationships showed no clear geographic pattern. Analysis of Molecular Variance allocated more than 99% of the variance to within localities and no significant F_{ST} values were found. Lack of genetic differentiation and high maternal gene flow between localities would be in concordance with morphometric and otolith data, which indicate a single South African intermixing adult stock. On the other hand, the isolation-by-distance observed between fish from the southern and Eastern Cape localities and those of KwaZulu-Natal cannot be ignored and deserves further investigation.

Introduction

The dusky kob *Argyrosomus japonicus* (Temminck & Schlegel, 1843) is a large sciaenid with a fragmented global distribution. Along South Africa's coast it is found from Cape Point to southern Mozambique, although only abundant from Cape Agulhas to northern KwaZulu-Natal (KZN) (Fig. 2.1). The species also occurs off the Australian west and south coast and along the South China Sea from Hong Kong to southern Korea and Japan (the type locality). It is also known from Oman, Pakistan and India (Heemstra & Heemstra, 2004). In South African waters, *A. japonicus* is a coastal species that can be found in estuaries, the surf and nearshore zone, with a maximum recorded depth of 100 meters (Griffiths, 1996b; Griffiths & Heemstra, 1995). This species utilise estuaries throughout their range, especially the larger systems such as Lake St. Lucia (Whitfield, 1994). Estuaries with turbid waters are the preferred habitat and, as an ambush predator, the species can effectively forage in murky waters using olfactory and lateral line senses (Van der Elst, 1988). Juveniles are dependent on estuaries as nursery grounds and recruitment of juveniles into estuaries occurs at 20–30 mm TL (approximately one month old). Tag-recapture data suggest that juveniles do not undertake substantial coastal movements and are likely to remain close to their natal estuaries, forming localised populations until sexual maturity is reached (Bullen & Mann, 2004a; Griffiths, 1996b; Whitfield, 1998). Interpreting tagging data for the species has been complicated by misidentification prior to the mid-1990s and, because of similarities in the morphology of *A. japonicus* and *A. inodorus*, misidentification still occurs (Bullen & Mann, 2004a).

In South African waters, prior to the mid-1990s the species was misidentified as *A. hololepidotus* (Lacepède, 1801), and was also not distinguished from the sympatric congeneric, *A. inodorus* (Griffiths & Heemstra, 1995), that co-occurs with *A. japonicus* from Algoa Bay in the Eastern Cape Province westwards to False Bay. Correct identification has proved crucial for management purposes, since the two species have very different life histories (Griffiths, 1996b, 1997c). *Argyrosomus japonicus* is a long-lived species, that can reach an age of 42 years, although individuals older than 27 years are uncommon (Griffiths & Hecht, 1995). Sexual maturity is attained at seven years of age for males and eight years for females, with both sexes having a total length of more than a meter at maturity. In contrast, *A. inodorus* reaches maturity between one and two years of age (310–375 mm TL) and rarely exceeds an age of 13 years (Griffiths, 1996a; Griffiths & Hecht, 1995).

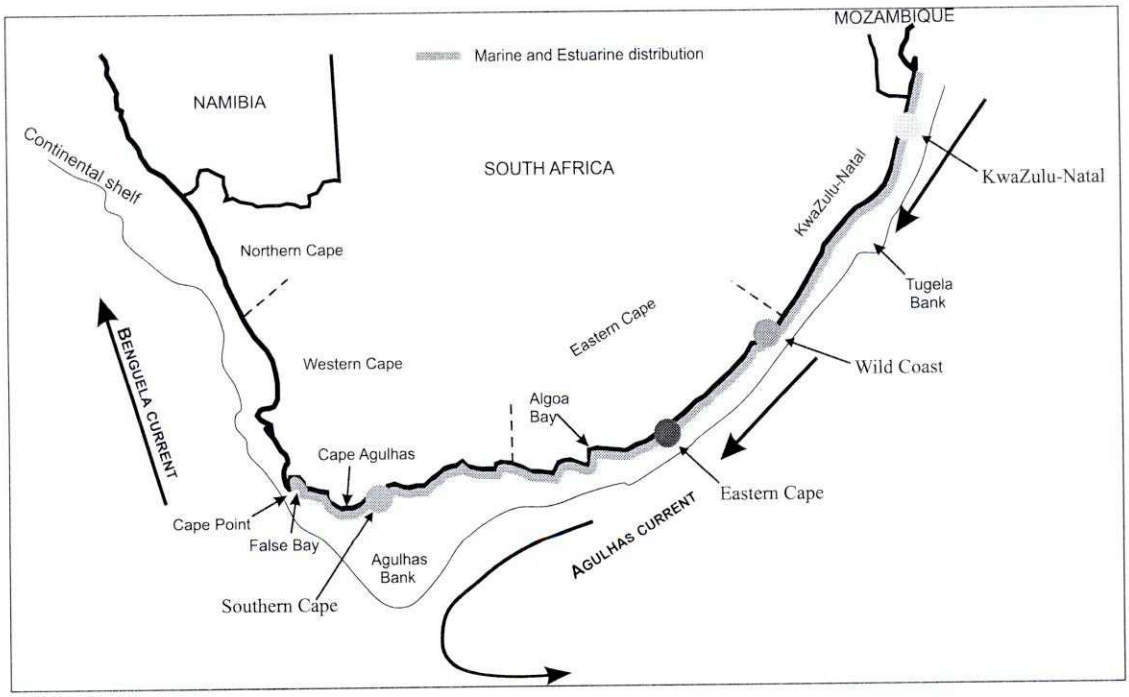

Fig. 2.1 Distribution of *A. japonicus* along South Africa's eastern and southern coast. The four sampling localities where fish were collected are indicated (adapted from Whitfield, 1998).

Increasing numbers of reproductively active *A. japonicus* in linefish catches off the KZN coast during August to November suggests that adult fish from the southeastern and southern Cape migrate to KZN waters to spawn. Spawning from October to January also occurs in the Cape region as far west as Cape Infanta (Griffiths, 1996b). This suggests that breeding commences in KZN and continues as a portion of the spawning population returns to southern waters. A general absence of individuals with spent gonads in KZN, and their presence in southeastern and southern Cape waters, also implies a return migration once spawning has been completed (Griffiths, 1996b; Griffiths & Heemstra, 1995).

Argyrosomus japonicus is one of South Africa's most important angling species and constitutes approximately 18% of the total catch (Lamberth & Turpie, 2003). Per recruit stock assessment models estimate that the South African population has been overexploited and that the spawner biomass is between 1.0 and 4.5% of pristine levels (Griffiths, 1997d; Lamberth & Turpie, 2003). Because of its life history characteristics, namely, age at sexual maturity, longevity and having evolved with relatively low rates of natural mortality, *A. japonicus* is unlikely to sustain high levels of exploitation (Griffiths 1997). There are at least three phases where it is particularly vulnerable to human activities. First, recreational and commercial lineboat fishermen target the dense spawning aggregation off the KZN coast. Second, recreational anglers have targeted juveniles that inhabit the surf zone and estuaries since the 1960s. On the Eastern Cape coast, catches are dominated by *A. japonicus* and they constitute 48% of catch

weight, 35% in KZN and 6% along the southern Cape coast. Third, although early juveniles are not exploited directly, they are estuarine dependent and most of South Africa's estuaries are degraded to various degrees (Griffiths, 1996b, 1997d; Mander *et al.*, 2001).

Based on otolith dimensions and fish length relationships, Griffiths and Hecht (1995) suggested that three or more South African *A. japonicus* stocks might exist for at least part of the species' life cycle. They concluded that the South African population of *A. japonicus* consists of a single offshore migratory adult population with several allopatric juvenile subpopulations in their natal estuaries and adjoining surf zone. Because of the migratory adults, differences among juveniles would reflect environmental conditions rather than genetic differences and it was recommended the species should be managed on a national rather than regional level (Griffiths, 1997d).

The use of the mitochondrial DNA (mtDNA) control region in detecting population structure in marine fish has been successfully applied to many species (Alvarado Bremer *et al.*, 2005; Bernardi *et al.*, 2003; Bernardi & Talley, 2000; Bernardi & Vagelli, 2004; Chenoweth & Hughes, 2003; Fauvelot *et al.*, 2003; Gold & Richardson, 1991; Graves, 1998; Ovenden *et al.*, 2002; Ovenden *et al.*, 2004; Ravago-Gotanco & Juinio-Menez, 2004; Smith *et al.*, 2002; Stefanni & Thorley, 2003). In some cases it has been of limited use due to large intra-oceanic differences and high levels of genetic diversity, e.g. in *Mugil cephalus* (Rocka-Olivares *et al.*, 2000). In the case of *Merluccius merluccius*, analysis of several mtDNA regions was not sensitive enough to reveal structure, whereas microsatellite markers were successful (Lundy *et al.*, 2000). The control region is the section of the mitochondrial genome with the highest substitution rate in most vertebrates, making it useful for population studies. Because of its haploid nature and absence of recombination, the signal obtained from genetic drift is strong and, on average, mtDNA variation is expected to be greater between populations and less within populations (Birky *et al.*, 1989; Ferris & Berg, 1987; Gold & Richardson, 1991; Meyer, 1993; Shaklee & Bentzen, 1998; Waples, 1998; Wilson *et al.*, 1985).

In this study mtDNA control region sequences were analysed to examine the levels of genetic diversity and spatial differentiation among juvenile *A. japonicus* sampled from South African waters. Sampling of estuarine dependent juveniles was important to investigate the possibility that isolated spawning events occur in a specific region or regions, to test if an isolation-by-distance effect exists or whether the South African *A. japonicus* population consist of a single breeding population.

Materials and Methods

Samples

Juvenile *A. japonicus* were collected in estuaries or the adjacent surf zones by seine netting, gillnet or hook and line. Fin clips or gill filaments were taken, placed in 80% ethanol and later stored at -20°C. Length measurements were recorded, as well as locality and date of collecting. Sampling was done at four sites across the species' distribution in South African waters (Fig. 2.1). Samples from the southern Cape were collected in the surf zone at De Hoop Nature Reserve. Eastern Cape samples were collected from the Great Fish River estuary, while the Wild Coast samples were obtained from two adjacent estuaries (Mngazi and Mngazana) with mouths four kilometres apart (samples from these two estuaries were subsequently combined for analysis). All KZN samples were collected from the St. Lucia estuary or the adjacent surf zone. The total distance along the coast between De Hoop in the southern Cape and St Lucia in KZN is 1 542 km. In addition, samples from specimens off Australia's west coast were obtained for comparative purposes. A list of all samples analysed is given in Appendix I.

DNA Extraction

Three different methods for total genomic DNA extraction were used during the course of this study.

1. Phenol-chloroform extraction: A small piece of material (0.01–0.02 g) was placed in 500 µl amniocyte buffer (50 mM Tris, pH 7.6, 100 mM NaCl, 1M EDTA, pH 8.0, 0.5%SDS) with 0.5 mg proteinase K (Roche Diagnostics). The mixture was incubated at 55°C for a minimum of 2.5 hours or until the material was digested, 1.25 µg RNAse A (Roche Diagnostics) was added and incubated at 37°C for 30 min. Next the samples were extracted twice with phenol and then twice with a 24:1 chloroform:isoamyl alcohol solution. Total DNA precipitation was done overnight at -20°C with 0.1 volumes 3M NaAc and two volumes 96% ethanol. The DNA was pelleted through centrifugation at 21 000 *g* for 30 min. The pellet was washed with 2 volumes 70% ethanol, collected through another 30 min at 21 000 *g* centrifugation step and dried. The dried DNA was resuspended in 100 µl Sabax® (Adcock Ingram) water and stored at -20°C (Sambrook *et al.*, 1989).
2. DNA was extracted with the Qiagen DNeasy® tissue kit (Qiagen Inc). A volume of 180 µl ATL buffer and 0.5 mg proteinase K was added to a small piece of material

(0.01–0.02 g) and incubated at 55°C for three hours. This was followed by a RNA digestion with 0.1 mg RNAse A for one hour at 37°C. Next 200 µl AL buffer was added to the solution and incubated at 70°C for 30 min. An equal volume of 100% ethanol was added to this and the solution pipetted into the DNeasy column. The column was centrifuged at 8 000 *g* for one minute. Five hundred microlitres of AW1 buffer was added and the column centrifuged at 8 000 *g* for one minute. Then 500 µl AW2 buffer was added and centrifuged for three minutes at 24 000 *g*. A volume of 50 µl Sabax® water was pipetted onto the membrane and incubated at room temperature for 30 min before being centrifuged for one minute at 8 000 *g*. This procedure was then repeated and the DNA collected in a new centrifuge tube. The DNA solutions were stored at -20°C.

3. The last method of DNA extraction followed Estoup *et al.* (1996). Five hundred microlitres of 10% Chelex resin (Sigma-Aldrich) solution at 60°C was added to a small piece of material (0.01–0.02 g). This was incubated at 100°C for 15 min. After adding 0.5 mg proteinase K, the solution was incubated at 55°C for one hour with constant shaking. The same solution was once again incubated for 15 min at 100°C and then left to cool down before storage at 4°C. Before the Chelex resin extractions were used in a reaction, the tubes were vortexed and centrifuged at 24 000 *g* for 20 s (Estoup *et al.*, 1996).

PCR Amplification

In each Polymerase Chain Reaction (PCR) (Saiki *et al.*, 1988), 80–100 ng of the total genomic DNA was used as template. The full length of the mtDNA control region was amplified using oligonucleotide primers Per L16565 (5' AGC GCC GGT CTT GTA AAC CG 3') and H1033 (5' CAT CTT AAC AGC TTC AGT G 3') (Fig. 2.2).

Primer Per L16565 was designed specifically for this study. Amplification and sequencing of a fragment that included the 3' end of cyt *b*, the two tRNAs, threonine ($tRNA^{thr}$) and proline ($tRNA^{pro}$), as well as the 5' end of the control region was done to achieve this. The obtained sequence was then aligned with sciaenid sequences obtained from GenBank. A conserved sequence in the $tRNA^{thr}$ was identified and selected as template for primer Per L16565. The reverse primer H1033 was developed in our laboratory based on published salmonid sequences (P. Bloomer, pers. comm.). Both primers were numbered according to corresponding nucleotide positions on reference sequence *Salmo salar* (GenBank sequence number NC 001960).

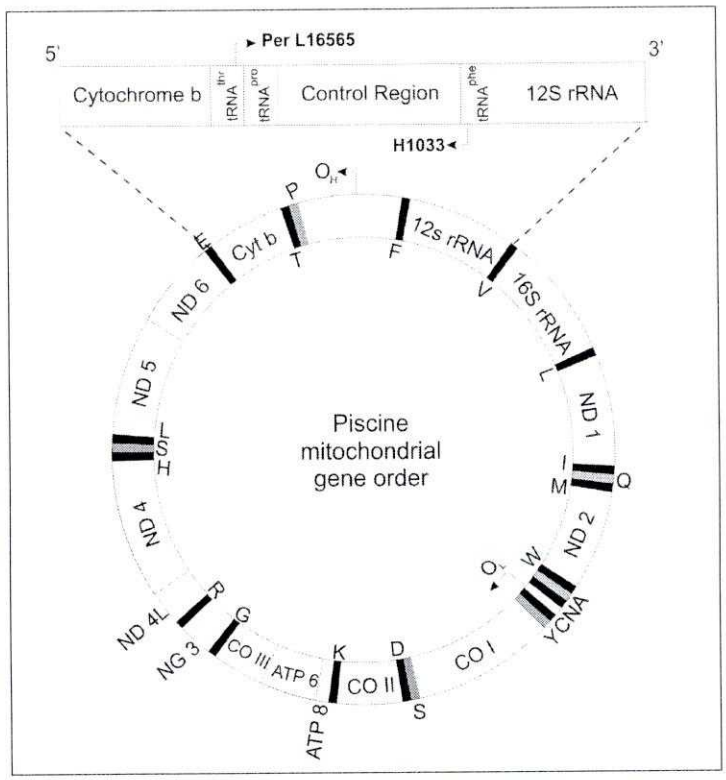

Fig. 2.2 Map of the piscine mitochondrial DNA showing the annealing positions of primers for the amplification of *A. japonicus* complete mtDNA control region (modified from Meyer, 1993).

Each double-stranded amplification reaction contained the following: 1x reaction buffer, 2.0 mM $MgCl_2$, 2 mM of each of the four nucleotides (dNTP's; Promega), 1.5 U Super-therm® DNA Polymerase (Southern Cross Biotechnology) and 10 pmols of each primer. Each reaction was made up with Sabax® water to a final volume of 50 µl. Negative controls were included each time a PCR was set up to screen for possible DNA contamination of solutions. Amplifications were done in a Geneamp® PCR System 9700 (Applied Biosystems) and started with a five minute 94°C denaturing step. This was followed by 35 cycles of denaturing at 94°C (30 s), primer annealing at 58°C (30 s) and extension at 72°C (40 s). The final extension step was done at 72°C for seven minutes. Four microlitres of the PCR product was run through a 1% agarose gel (Invitrogen), stained with ethidium bromide and visualised under UV light to verify successful amplification.

Successfully amplified PCR products were purified directly using a modified sodium acetate precipitation method (Applied Biosystems). This was done by adding 10 µl Sabax® water, 2 µl NaAc (3M) and 90 µl 100% sequencing grade ethanol to the PCR product. The mixture was left to stand at room temperature for 15 min before being centrifuged at 24 000 *g* for 20 min. The ethanol supernatant was removed and the pellet washed by adding 90 µl 70% ethanol (sequencing grade). The DNA was again pelleted by a 10 min centrifugation at 24 000 *g*. All

ethanol was removed and the DNA pellet dried before being resuspended in 15–30 µl Sabax® water. A quality check was done by running some of the resuspended DNA through a 1% agarose gel, stained with ethidium bromide and visualised under UV light.

Sequencing

For all the samples two sequencing reactions were performed, one with Per L16565 and the other with the H1033 primer. Sequencing reactions were done using the BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems). Approximately 100–120 ng purified PCR template, 3.2 pmol of the specific primer and 2 µl BigDye® were mixed and made up with Sabax® water to a final volume of 10 µl and cycled in a Geneamp® PCR System 9700 using the BigDye® program. Purification of cycle sequencing products was done with the same protocol as for PCR products (see PCR amplification above). The one exception was that the pellet was not resuspended in water, but with Hi-Di™ formamide (Applied Biosystems) before loading on the ABI 3100 automated sequencer (Applied Biosystems). Electropherograms of both sequencing reactions per sample were imported into Sequence Navigator™ version 1.0.1 (Applied Biosystems, 1994), aligned and checked for ambiguous bases. The resulting consensus sequence obtained from the alignment was used in multiple sequence alignments performed in Clustal X version 1.8 (Thompson *et al.*, 1997). All substitutions and inferred insertion/deletion (indels) events were double-checked on the original electropherograms to ensure that all bases were scored accurately.

Population Genetic Analysis

Juveniles analysed in this study varied in size and were collected over an extended period from the various sampling localities. Most of the samples were obtained from fish tagged and released as part of other studies. Due to the reported high variability in length-at-age, *A. japonicus* age cannot be estimated accurately from size and should be based on otolith analysis (Griffiths & Hecht, 1995). Since otoliths were not collected, the fish could not be accurately aged. The result was that temporal stability of observed patterns of genetic variability between cohorts at the different localities could not be tested with any certainty and was thus not addressed in the present study. Juvenile fish, irrespective of size class or date collected from a given locality, were grouped to test for geographic variation among localities.

DNASP version 4.0 (Rozas *et al.*, 2003) was used to compute a sliding window of the polymorphisms observed in the analysed fragment. Aligned sequences of all individuals were used to construct an input file for ARLEQUIN 2.0 (Schneider *et al.*, 2000). Only sequences

with less than 5% missing data were used for analysis. Several genetic diversity indices were calculated. First, within sampling locality diversity was estimated by calculation of haplotype diversity (h) (Nei, 1987) and nucleotide diversity (π) (Tajima, 1983, 1993). Second, between locality diversity was calculated as net nucleotide sequence divergence (Reynolds *et al.*, 1983; Slatkin, 1995). Haplotypes and their frequencies at localities were used to construct a Minimum Spanning Network (Excoffier *et al.*, 1992) based on pairwise differences obtained from ARLEQUIN 2.0 to visually examine their genealogical relationship. The number of haplotypes that were only found at a single locality, regardless of frequency, is expressed by the endemism index. This is calculated by dividing the number of 'private' haplotypes at a locality by the total number of haplotypes at that locality.

Before any conclusions on population structure can be drawn from the data, it is important to show that the marker is evolving according to neutral expectations and thus not affected by selection. Population differentiation is assumed to be a result of reproductive isolation, but in the presence of selection this assumption becomes invalid. A mixture of test of neutrality can serve as indicators of demographic parameters like population growth/decline in the evolutionary history of the taxa (Rand, 1996). To test for neutrality of the locus, Tajima's D, Fu's F_s and the R_2 test were used. Tajima (1989) test statistic D is the difference between the average number of nucleotide differences (π) between sequences drawn from random samples and the number of segregating sites (θ). Under the neutral model, in a population at equilibrium, $\pi = \theta$. A negative value for D may either indicate a selective sweep, a population expansion after a bottleneck, or a small founder event. Positive D-values are consistent with models of positive and balancing selection, or an admixture of distinct isolated populations (Rand, 1996). Fu's F_s is a test that works well in detecting population growth for larger sample sizes (Fu, 1997; Ramos-Onsins & Rozas, 2002). F_s tends to be negative when there is an excess of recent mutations (thus an excess of rare alleles). A large negative value is taken as evidence against neutrality of mutations (Fu, 1997). Lastly, the R_2 neutrality test works well for smaller sample sizes. This test evaluates the number of singletons where lower values of R_2 are expected after a recent population expansion (Ramos-Onsins & Rozas, 2002).

Mismatch distributions for all pairwise number of differences observed were calculated and compared to those expected under a population growth and decline model for all localities and for the overall South African population (Harpending, 1994; Rogers, 1995). Homogeneity of mtDNA haplotype frequency was tested via a randomisation procedure (Monte Carlo) (Roff & Bentzen, 1989), with 10 000 permutations.

Analysis of Molecular Variance, AMOVA (Excoffier *et al.*, 1992) was executed in ARLEQUIN 2.0 (Schneider *et al.*, 2000), to determine which part of the variance can be attributed to variance between regions, and variance among individuals within regions. F_{ST} (Wright, 1951) values were used to determine the extent of genetic differentiation between localities and to estimate patterns of spatial genetic structuring. Population pairwise F_{ST} values were calculated and significance at the 5% level determined.

To determine the relationship between genetic differentiation and geography, pairwise F_{ST} values were plotted against geographic distance. A Mantel test (10 000 random permutations) (Smouse *et al.*, 1986) was used to determine the correlation between genetic differentiation (pairwise F_{ST}) and geographic distance between sampling localities, using ARLEQUIN 2.0.

Results

Control region

A fragment of 950 base pair (bp) was obtained through sequencing, including 819 bp from the control region as well as some bases from the flanking tRNA's where the primers annealed. No evidence of heteroplasmy was found since no double peaks were observed in the sequences and a consistent pattern of variability was found across all sequences. From the sliding window it is clear that the distribution of variation in the mtDNA control region of *A. japonicus* is comparable to observations from other vertebrates. Most of the variation was found within the hypervariable region I followed by hypervariable region II, with very conserved 5' (first 72 bp) and 3' (last 84 bp) terminal ends and a relatively conserved central region (Fig. 2.3).

At the 5' end of the control region, one individual had three tandem repeats of a ten bp segment, while all other individuals had only a single copy. Tandem repeats in the 5' end is commonly observed in fish species (Lee *et al.*, 1995). The repeat occurred 18 bp from the 5'end of the control region, and as this falls within the first 72 bp conserved segment, the insert and the first 18 bp of all samples were excluded from all analyses.

By mapping the mutations onto the initial Minimum Spanning Network containing 56 haplotypes, a total of 22 homoplasy characters were identified and removed before analysis to exclude any ambiguity in the relationship among the haplotypes (Avise & Lansman, 1983).

Six sites with insertion/deletions (indels) were also removed. The final fragment analysed was 735 bp in length. Nucleotide composition of the fragment was C 22%, T 30%, A 32% and G 16%, with a total of 58 polymorphic sites. The polymorphic sites contained 61 substitutions, 56 transitions at 55 sites and five transversions at five sites. An alignment of all the variable positions is shown in Table 2.1 (a complete alignment is available on request).

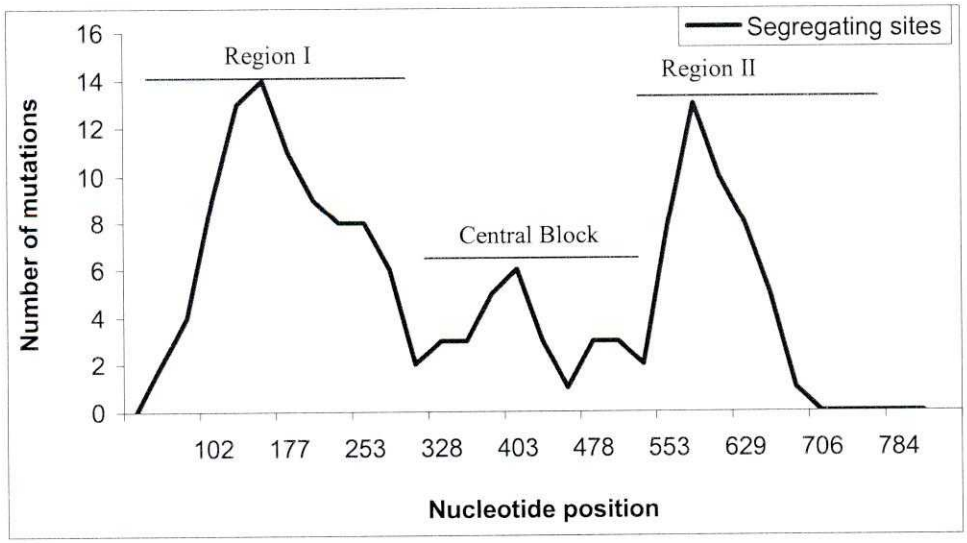

Fig. 2.3 Sliding window of substitutions in the complete mtDNA control region of South African *A. japonicus* indicating the distribution of variable sites; window length 50, step size 25 bp.

Haplotype variation and distribution

The 58 polymorphic sites defined 35 unique haplotypes among 133 individuals analysed from the four South African localities (Table 2.1). Genetic distances among haplotypes ranged from 0.14% (one bp difference) to 2.04% (15 bp difference) with a mean divergence of 1.1% (Appendix II). The most common haplotype (#11) was shared by 14 samples, followed by haplotype numbers three and eight with eleven samples each. Fourteen haplotypes (40%) were represented by a single sample (Table 2.1).

Samples analysed from Australia did not share any haplotypes with those from South Africa. The percentage divergence between the two groups varied between 7.46 and 9.03%. Because of this, the Australian samples were excluded from all the analyses performed on the South African samples.

From the Minimum Spanning Network (Fig. 2.4) it is clear that there is no relationship between haplotype genealogy and geographic location. There are a number of links that contain five or six mutational steps in the network. These large steps are found throughout the network and split the network into several smaller clusters. There is also no apparent

geographic pattern within the smaller haplotype groups created by the large mutational differences.

Table 2.1 Variable sites defining 35 South African *A. japonicus* haplotypes. 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	1111111111111111122222222233333333445555555555566666 5578800111233444555679000122459024788890733444567778901334 6761801014046157478295156036204805624868247156122369167355
1	5	AAACATATTCCTCTCTGATTTGCAACATCGCGTTCATACCTAGTATATTAAAAAGAGG
2	1	....G.....
3	11	.....C.....
4	1	.....G.....C.....
5	9	.....A....C.....
6	4	.....T.....A....C.....
7	5	.....C.....
8	11	.....C.....C.....
9	3	.....C..A.....C.....
10	1	.G....C..A.....C.....
11	14	.....C.....A.....A...
12	4	.....C.....A.....C.....A...
13	1	.....C.....A.....G.....A...
14	1	.....C.....A.....G...A...
15	1	.....C.....A.....A.....A...
16	4	.....C....C.....A.....T.....
17	1	.....A....C.....A.....T.....C.....
18	1	.....C..C....C.....A.....T.....G....
19	5	.....C....C..A....AT..T.....T.....C.....
20	1	.....C.C.....A.....C.....C.....
21	1	.....G.....C.....T.....G.....GA.
22	1	.....C.....T.....G.....G...GA.
23	4	....T.....C.....CA.....G.....G.....
24	2	....T.....CTC.....CA.....G.....G.....
25	7	....T.....T..C.....CA.....TCG.....
26	9	...TT.....C.....CA.....A.....
27	1	...TT.....C...G..CA.....A.....
28	2	...TT.....C.....CA.....A..C.....
29	1	G..TT.....C....C.CA.....A.....
30	3	..GTT.....C.....CA..G.....
31	1	...TT.....C.....CCA.....G.....GG.....
32	8	....T....T...C.....CA.....C.....G.C.....
33	2	....T....T...CA.....CA.....C.....G.C.....
34	3	....T....T...C.....CA.....C.....G.C.....A
35	4	....T....T...C.....CA....G.....C.T.....G.C.....

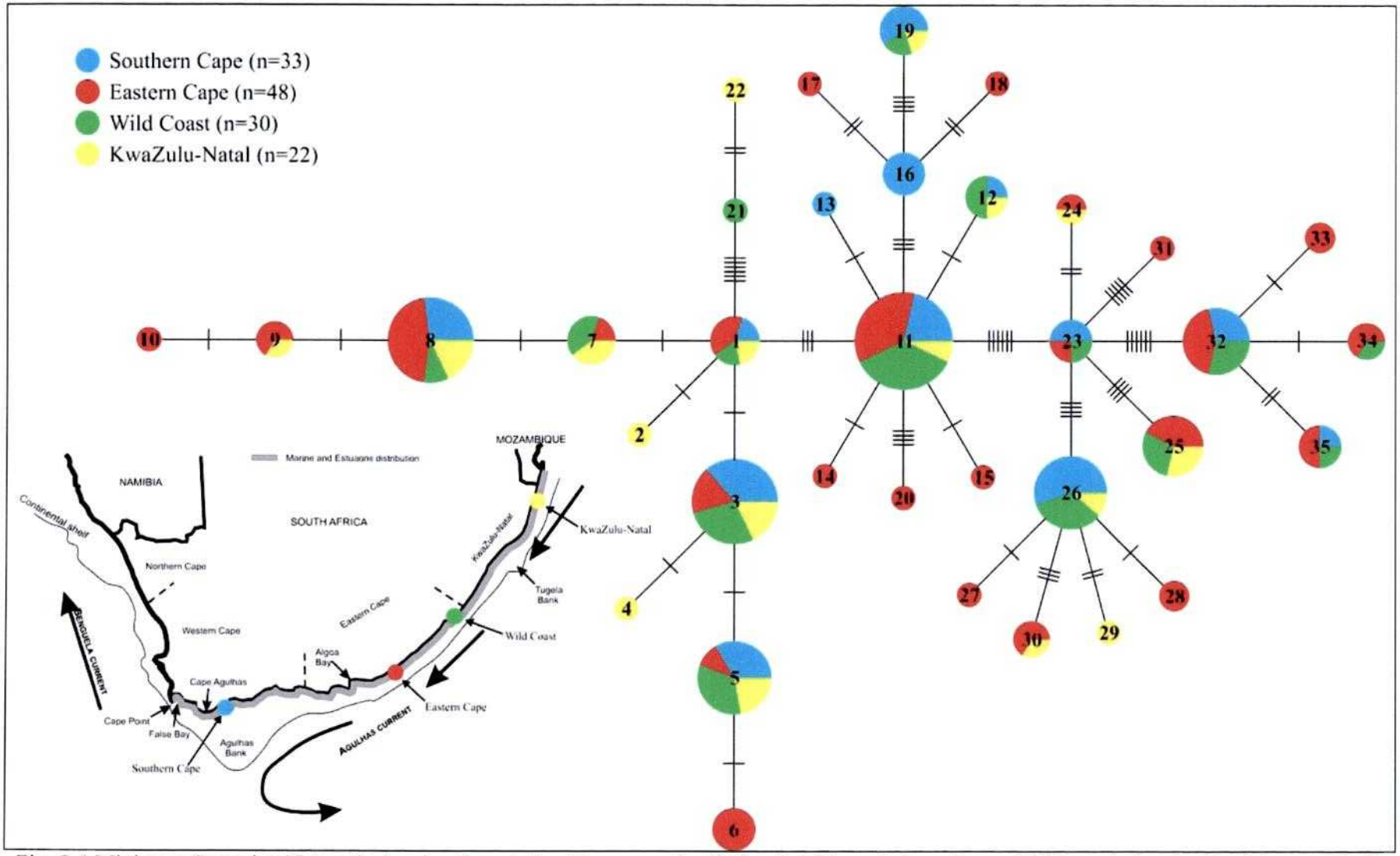

Fig. 2.4 Minimum Spanning Network showing the relationship among the 35 South African *A. japonicus* mtDNA control region haplotypes. Circle diameter is proportional to haplotype frequency. Pie charts display the localities where each haplotype was found and numbers indicate haplotype numbers as in Table 2.1. All haplotypes are separated by at least one mutational step, with hatch marks across the connecting lines indicating the number of mutations.

The mismatch distributions for the four localities (Appendix III) and for the South African population overall (Fig. 2.5) were unimodal. Mismatch distributions from the four localities all have similar distributions that are comparable with the overall mismatch distribution, with that obtained for the southern Cape and KZN being the most different. A relative good fit to a model of sudden expansion was observed in all cases as reflected in the S.S.D. values (Appendix III), although this is not reflected in the *P*-value. The overall ruggedness index was low (r = 0.001) indicating a good fit, but it was not significant (*P* = 0.910).

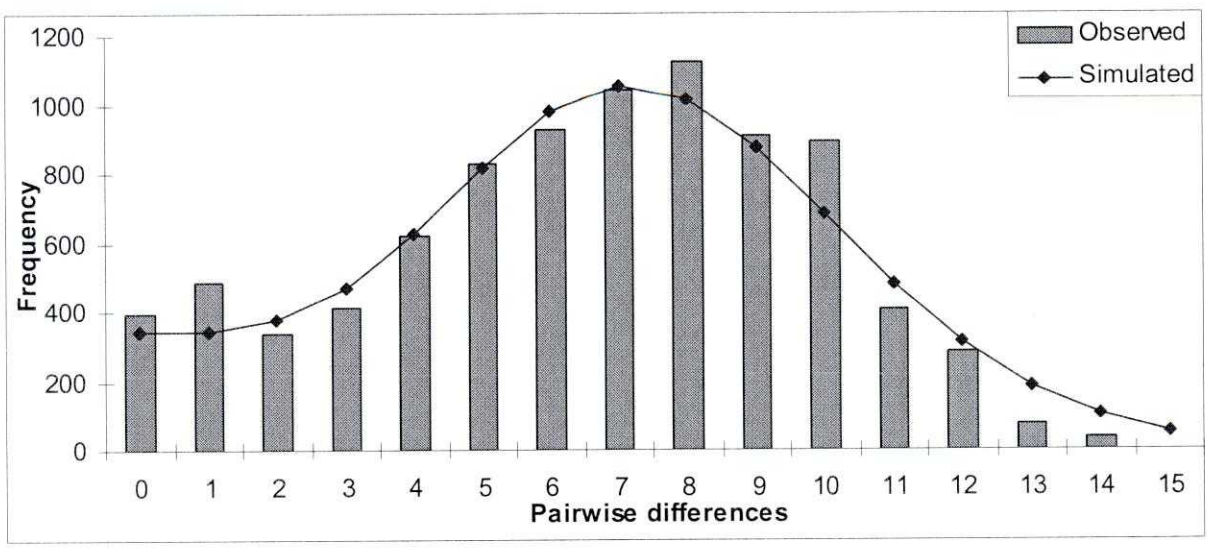

Fig. 2.5 Mismatch distribution of pairwise differences between 133 *A. japonicus* individuals based on 739 bp of the mtDNA control region. The expected frequency is based on a population growth-decline model (S.S.D. = 0.003 *P* = 0.627, r = 0.001 *P* = 0.910, θ_0 = 0.001 and θ_1 = 25.000) determined using ARLEQUIN 2.0.

Tajima D test for neutrality and Fu's F_s test indicated that the mtDNA control region of *A. japonicus* is evolving according to neutral expectations at all the localities, as well as for the South African population overall (Table 2.2). Although R_2 values were significant, both within the 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 2.2).

Table 2.2 Comparison of several statistical tests of neutrality for the South African *A. japonicus* mtDNA control region, analysed per locality and the overall population

Locality	**Tajima D**	***P***	**Fu's F_s**	***P***	**R_2**	***P***
Southern Cape	-0.0699	0.5373	0.3278	0.4554	0.1624	0.0000
Eastern Cape	-0.0650	0.5302	0.3417	0.4465	0.1619	0.0000
Wild Coast	-0.0813	0.5402	0.3296	0.4470	0.1622	0.0000
KwaZulu-Natal	-0.0794	0.5381	0.3518	0.4403	0.1618	0.0000
SOUTH AFRICA	-0.0862	0.5465	0.3458	0.4382	0.1617	0.0000

Genetic diversity within and among sample localities

Eighteen of the 35 haplotypes were only found at a single locality. The Eastern Cape had by far the highest number of private haplotypes, with 44% of the haplotypes from the Eastern Cape not found at other localities (endemism index, Table 2.3). Haplotype diversity at localities were similar to that of the population overall (Table 2.3) and comparable with other marine species. Variation in nucleotide diversity within localities was more variable, with that from the Eastern Cape being the highest (Table 2.3). Comparison of nucleotide divergence between localities indicated that the Eastern Cape and KZN were the most divergent. Corrected pairwise divergence values less than zero indicate that within locality diversity is greater than between locality diversity (Table 2.4).

Table 2.3 Genetic diversity estimates for *A. japonicus* from four South African localities and the South African population overall

Locality	n	No of haplotypes	Endemism index	h (±SD)	π (%)	Mean pairwise differences (bp)
Southern Cape	33	13	0.15	0.9318 ± 0.0178	0.8122	5.9697
Eastern Cape	48	25	0.44	0.9645 ± 0.0111	0.9700	7.1294
Wild Coast	30	15	0.07	0.9425 ± 0.0207	0.8523	6.2644
KwaZulu-Natal	22	17	0.24	0.9784 ± 0.0189	0.8623	6.3377
SOUTH AFRICA	***133***	***35***		***0.9551 ± 0.0057***	***0.8883***	***6.5287***

Table 2.4 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		0.9002	0.8261	0.8467
Eastern Cape	0.0672		0.8984	0.9294
Wild Coast	-0.0453	-0.0934		0.8644
KwaZulu-Natal	0.0695	0.0979	0.0520	

Geographic variation among localities

Testing for spatial homogeneity of mtDNA haplotype frequencies among the four localities was non-significant ($\chi^2 = 98.72$, $P = 0.6515$). This indicates that no significant structure exists between the four sampling localities.

Results from AMOVA revealed that most of the variation (99.79%) was found among samples within localities and that little could be attributed to variation among localities (0.12%). The overall low F_{ST} value ($F_{ST} = 0.0021$, $P = 0.3537$) also suggested homogeneity among the localities. Similarly pairwise F_{ST} analysis indicated very low differentiation between localities (Table 2.5). Plotting pairwise F_{ST} values against geographic distance indicate that there might

be some isolation-by-distance since a positive correlation (Mantel r = 0.357, P = 0.291) was found between the two variables, this was however not significant (Fig. 2.6).

Table 2.5 *Argyrosomus japonicus* pairwise F_{ST} and associated probability (P) values between the 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		0.17871	0.58984	0.24316
Eastern Cape	0.00917		0.89746	0.19238
Wild Coast	-0.00740	-0.01476		0.26660
KwaZulu-Natal	0.01168	0.01262	0.00827	

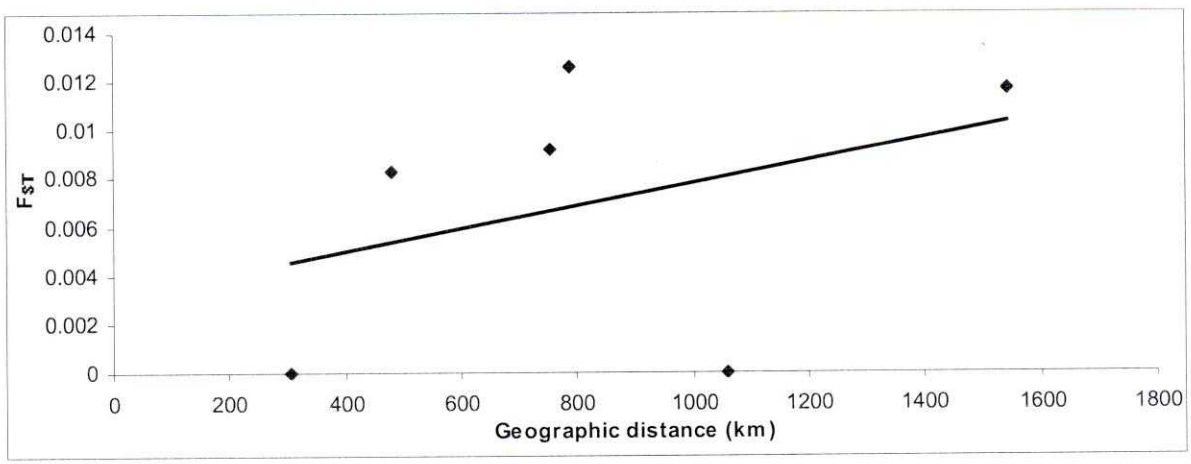

Fig. 2.6 Pairwise F_{ST} values plotted against geographical distance between four South African localities of *A. japonicus*. Mantel correlation value r = 0.413, P = 0.295.

Discussion

This is the first in depth study to determine the genetic variation within any of the *Argyrosomus* species. Allozyme data have been used to distinguish between two sympatric species, *A. inodorus* and *A. coronus*, found off the Namibian coast. The allozymes was also use to determine if any differentiation exits within the Namibian *A. inodorus* population (van der Bank & Kirchner, 1997). In the present study the complete mtDNA control region was shown to be selectively neutral, and thus a suitable molecular marker for a population study. High levels of mtDNA genetic diversity (h = 0.95, π = 0.89%) were found in *A. japonicus* from South Africa. These high levels compare well with that 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). According to Grant and Bowen (1998) species typically exhibit 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. From the *A. japonicus* Minimum Spanning Network it seems that the high h and π could be due to previously differentiated allopatric lineages as this is evident by a number of steps in the network containing five or six mutations. These divergent lineages could be the result of previous isolated groups that have come into secondary contact and are now integrated in the South African population. The unimodal mismatch distribution (Fig. 2.5) shows a high frequency of large mutational differences (at ±8 steps), which may be a signature left by these divergent lineages. The mismatch distribution obtained from the original 819 bp fragment (Appendix II, Fig II.2) was multimodal, potentially a clearer indication of the possible integration of divergent lineages in the population although population subdivision has a very small effect on the mismatch distribution or the raggedness index (Harpending, 1994; Rogers, 1995).

High h and π diversity is more likely to be expected of a large population since the loss of genetic diversity due to drift is slower and therefore mutations accumulate over time (Hauser & Ward, 1998; Kimura, 1983). Large populations tend to lose more alleles than smaller populations when population size is reduced dramatically and this can result in a considerable change in genetic makeup of the population. But a population do need to reach a new equilibrium after a "crash" before the impact can be determined and in populations with large sizes, such as in many marine fish species, this can take many generations to obtain (if ever). Analysing a small number of individuals might not detect the genetic loss and change in genetic makeup that occurred in the population (Hauser & Ward, 1998; Ryman *et al.*, 1995). It is important to note that species with large population sizes (e.g. *A. japonicus*) are likely to have slow genetic drift at neutral loci, thus taking a long time to reflect differentiation (Hauser & Ward, 1998). *Argyrosomus japonicus* is long-lived, only reaching sexual maturity at an age of 7–8 years, has overlapping generations and a large population size. Consequently, a neutral locus such as the mtDNA control region will be slow in sorting lineages and slow to reach equilibrium.

Very little difference in genetic variation within the four South African localities was observed. Haplotype and nucleotide diversity from the localities is comparable with the overall values for the South African population. The exception is the high nucleotide diversity observed in the Eastern Cape, as well as the high endemism index compared to the other localities. This does not appear to be a result of the larger than average number of samples analysed for this locality, π drops to comparable levels when the samples size is randomly reduced to that of the other localities but the endemism index remains higher. The near identical percentage nucleotide

divergence values within and among *A. japonicus* sampling localities indicates that, on average, any two individuals drawn at random from a given locality will be as different for their mtDNA haplotypes as any two individuals drawn from different localities. Gold and Richardson (1991) also observed this in *Sciaenops ocellatus*, an estuarine dependent sciaenid with a similar life history, from the Gulf of Mexico and the southern United States Atlantic coast. Despite this, they have shown that isolation-by-distance does exist in the Gulf, with gene flow more likely between adjacent estuaries. This also means that conservation should not be focused on individual estuary level but on a wider geographic scale (Gold *et al.*, 2001).

Homogeneity of haplotype frequencies (spatial homogeneity test P=0.65) across the localities, coupled with a low non significant overall F_{ST} value (F_{ST}=0.002, P=0.35), also indicates that no population structure exists among juveniles found at the four South African localities. However, F_{ST} values between the southern Cape and KZN as well as between the Eastern Cape and KZN, although not significant, were five to six times greater than the overall F_{ST} value. This, and the positive correlation found between geographic distances and pairwise F_{ST} values, indicates that some isolation-by-distance does exist.

Sampling of estuarine dependent juveniles is essential to determine if localised spawning occurs within a region, or to detect an isolation-by-distance effect (Gold *et al.*, 1999). This will only give a true reflection if juveniles stay close to their natal estuaries and this appears to be the case for juvenile *A. japonicus* (Bullen & Mann, 2004a; Griffiths, 1996b). Most juveniles found in an estuary is likely to be the result of the proximate spawning events and would thus be expected to reflect the genetic composition of adults from the region. Based on the genetic evidence obtained and life history data, it seems that spawning off KNZ and that observed later in the season off Cape Infanta do not involve the same adult aggregation. The similar differentiation between the two warm temperate localities and KZN show that the juveniles recruiting into estuaries off the Eastern Cape are as isolated from KZN as those off the southern Cape. This indicates that recruitment of juveniles in the Eastern Cape is not merely a result of spawning adults off KZN returning south but that isolated spawning probably occurs within the region (Fig. 2.7). It has been suggested that larvae spawned off South Africa's east coast can be transported by shelf currents southwestwards and that they can reach nursery areas along the south coast in three to four weeks (Beckley & Connell, 1996). However, it would be reasonable to assume that estuaries further from a particular spawning site would receive fewer recruits than estuaries closer to the breeding event. With spawning reported as far south as Cape Infanta, juveniles found in southern Cape estuaries are more likely to be recruited from a

spawning event in this region than from similar events off KZN (Fig. 2.7). Passive larval dispersal would however result in gene flow predominantly in a southwestward direction. This would result in samples from the southern Cape having the highest diversity whereas this study revealed that sample from the Eastern Cape where the most divergent. It might be that the southwestward dispersal of larvae is counter balanced by the migration of adults up the coast to spawn. Total lack of isolation-by-distance between the southern Cape and the Wild Coast as well as between the Eastern Cape and the Wild Coast cannot be explained, specially given that some differentiation is observed between the southern and Eastern Cape. Current life history data indicate that adult *A. japonicus* along the South African coast form a single stock. This cannot be verified due to the lack of tagging information for adult *A. japonicus* (Bullen & Mann, 2004a). The potential isolation-by-distance observed in the current investigation could indicate that not all adult *A. japonicus* travel to spawn off KZN but that most of them tend to spawn close to their natal estuaries.

The general absence of definitive trends in the various tests conducted in this study might be due to the lack of structure in the South African *A. japonicus* population. Another possible reason could be that recent separation occurred therefore not allowing enough time for the accumulation of differences at the neutral locus due to genetic drift. In addition, the ability of tests to detect low levels of differentiation from small sample sizes, like that of the current study, is very limited and therefore run the risk of failing to identify any structure that might exist (Hauser & Ward, 1998; Ward, 2000). Further, individuals analysed in this study were a mixture of juveniles from various cohorts collected over an extended period. This may potentially add a lot of 'noise' to the analysis that could obscure any signal that does exist. Gene flow of only a few migrants per generation among spawning groups would make the isolated populations genetically indistinguishable yet this would not be enough to help overexploited populations to recover (Carvalho & Hauser, 1994; Hauser & Ward, 1998). Thus, due to the lack of power in all the population analysis tests conducted, it would be premature to make any definitive conclusions regarding *A. japonicus* population structure. The marker is not without its limitations. It only gives a reflection on maternal patterns and female dispersal and it reflects historical patterns rather than that of the current conditions (Ferris & Berg, 1987). An increase in sample sizes, as well as better structuring thereof, will be needed to detect if any genetic differentiation does exist within the South African *A. japonicus* population. The issue of temporal stability at localities, although important, is secondary to spatial structure. If spatial differentiation had been detected, then an analysis of temporal

stability would have been important to indicate the stability of breeding populations within a region.

Final conclusions should not be based on only one marker and it would be advisable to make use of a different marker (e.g. microsatellite markers) that is more appropriate at detecting fine-scale genetic population differentiation (Ward, 2000). Given appropriate sampling, analysis of microsatellites will also reflect on the current population gene flow rather than historical genetic information as is the case with mtDNA (Hauser & Ward, 1998). Analyses of spawning adults along the coast might give us a better understanding of the processes operating in the adult *A. japonicus* population along the South African coast. If genetic differentiation is detected between the spawning aggregations the data could be used as baseline information to detect the possible mixing of these stocks as juveniles at the various estuaries.

The mtDNA diversity observed between samples of *A. japonicus* from South Africa and Australia was more than seven times greater than the observed differences within the South African population (data not shown). Studies on other marine taxa with fragmented global distributions have revealed that populations found off South Africa and Australia are either closely related as is the case for *Sardinops* (Bowen & Grant, 1997) but, in general it seems that the South African and Australian populations are not closely related as observed in *Engraulis* (Grant & Bowen, 1998) and *Pomatomus* (Graves, 1998). Similar to the divergence observed in *A. japonicus* from South Africa and Australia was reported by Alvarado *et al.* (2005) that found 8.7% divergence in the variable region I of the mtDNA control region of Atlantic bluefin tuna (*Thunnus thynnus*). This high divergence was attributed to the introgression of maternal lineages from two sister species, *T. orientalis* and *T. alalunga*. The relationship between the two isolated populations (South Africa and Australia) of *A. japonicus* was beyond the scope of this study, but it deserves further investigation. Samples from the South China Sea off Japan, the source locality for the type specimen of this species, will have to be included in such an investigation to unravel the fragmented global distribution of *A. japonicus*.

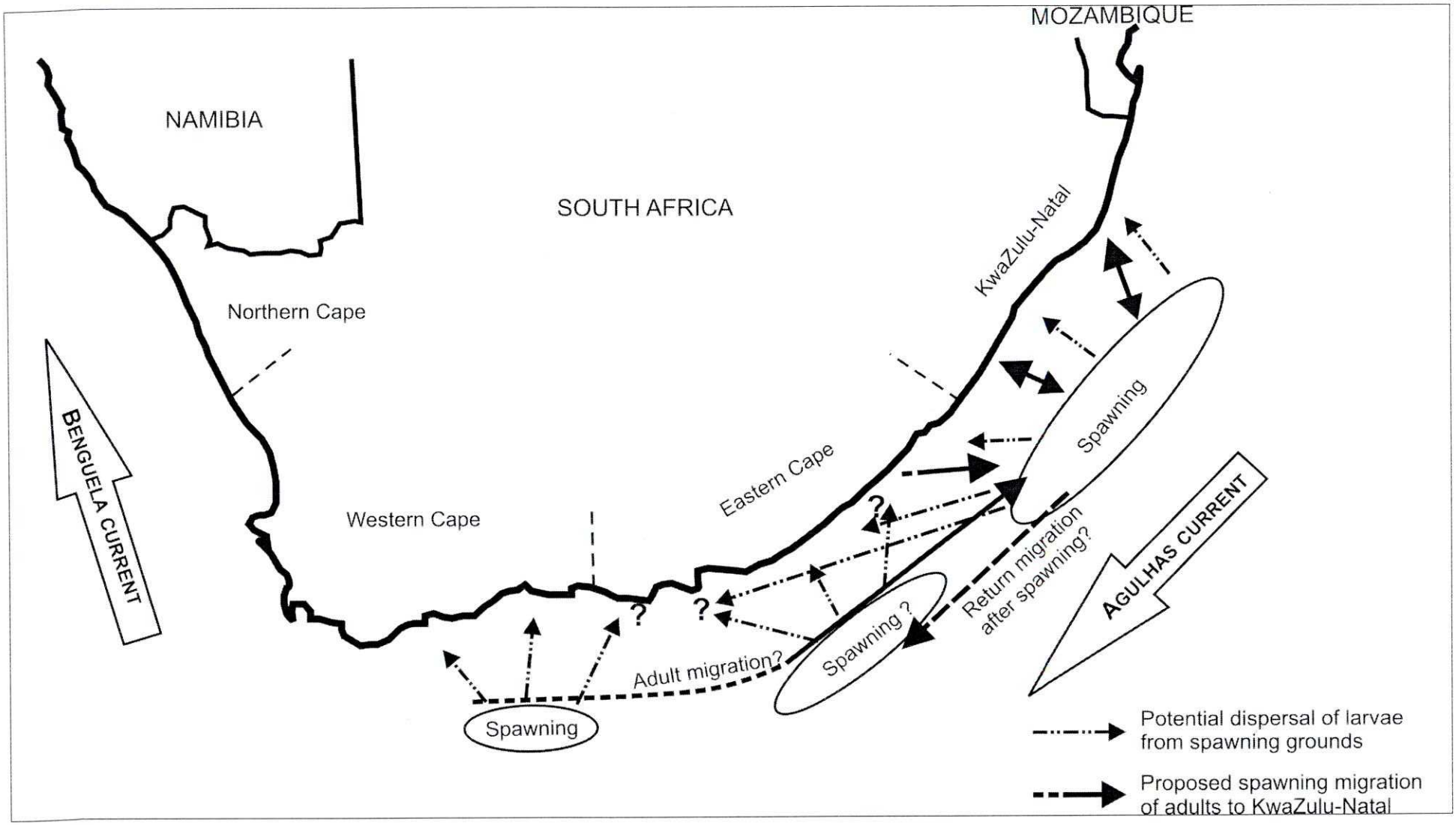

Fig.2.7 Hypotheses regarding maternal gene flow in South African *A. japonicus*. Based on life history data, adult *A. japonicus* undergoes a spawning migration to KZN, but spawning has also been recorded along the south coast later in the season. Influenced by the Agulhas Current, dispersal of larvae would predominantly be in a southwesterly direction whereas adults migrate up the coast to spawn.

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).

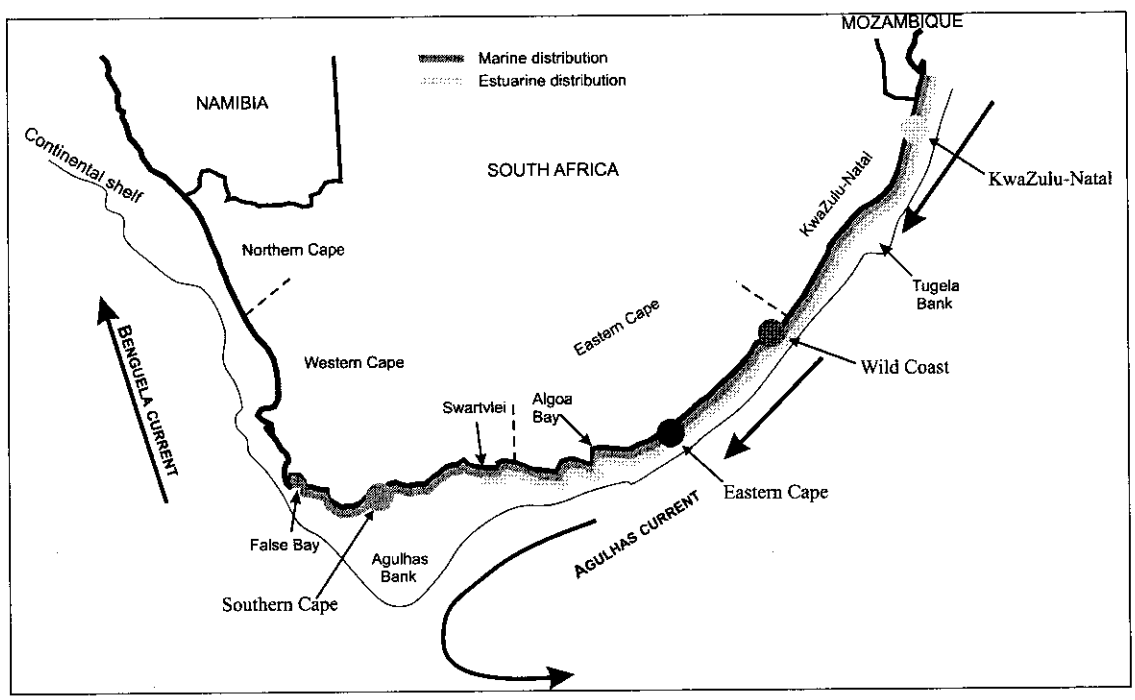

Fig. 3.1 Distribution and sampling localities of *P. commersonnii* along the South African coast (adapted from Whitfield, 1998).

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).

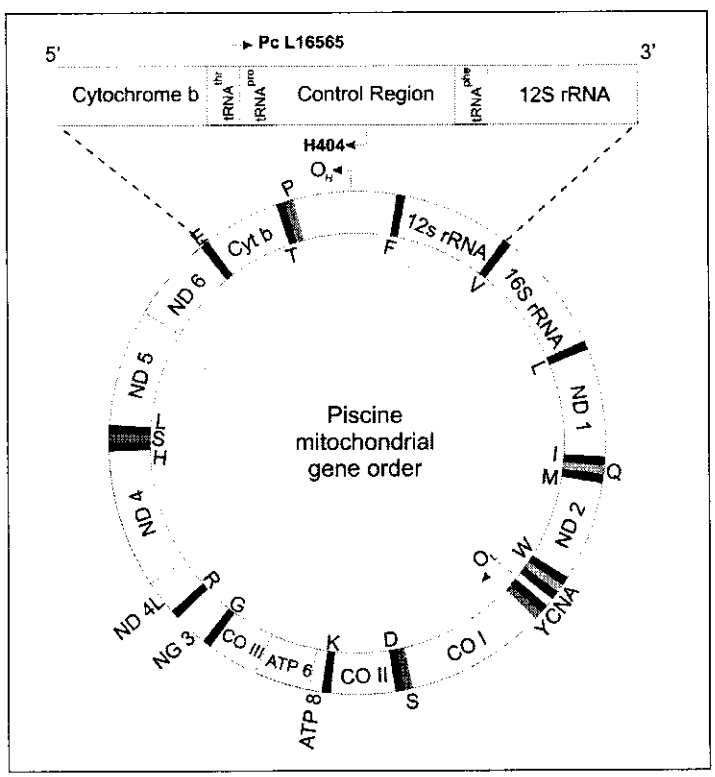

Fig. 3.2 Map of the piscine mitochondrial DNA showing annealing positions of primers used for amplification of *P. commersonnii* 5' end of the mtDNA control region (modified from Meyer, 1993).

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.

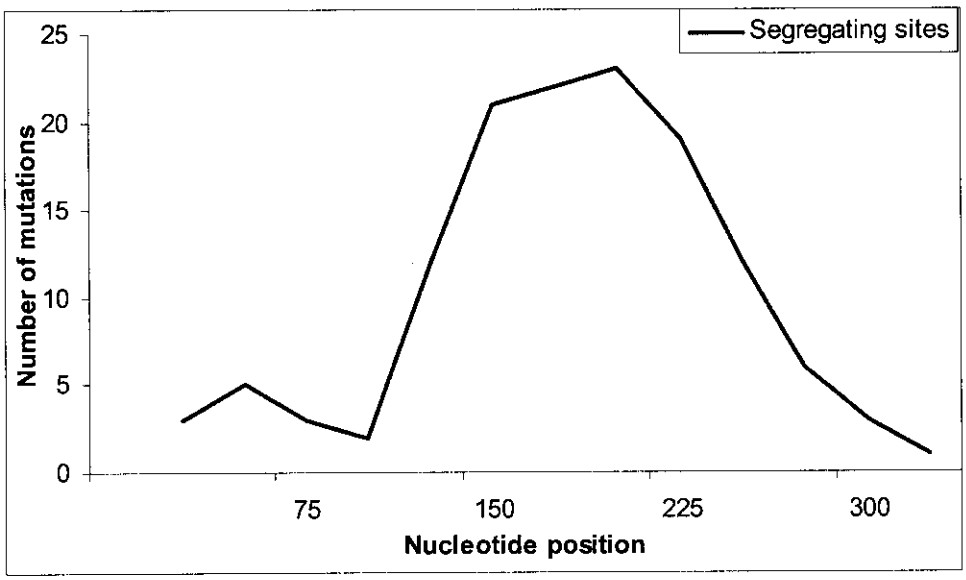

Fig. 3.3 Sliding window of substitutions in the 5' end of mtDNA control region from *P. commersonnii* (window length 50, step size 25 bp).

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	1111111111111111111111111111111111112222222222222222222222222223 4445581222233334445556667777788888999999000011111123333333456 67 2368999678907890362674670345923459013479257845678950135689291 65
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.....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).

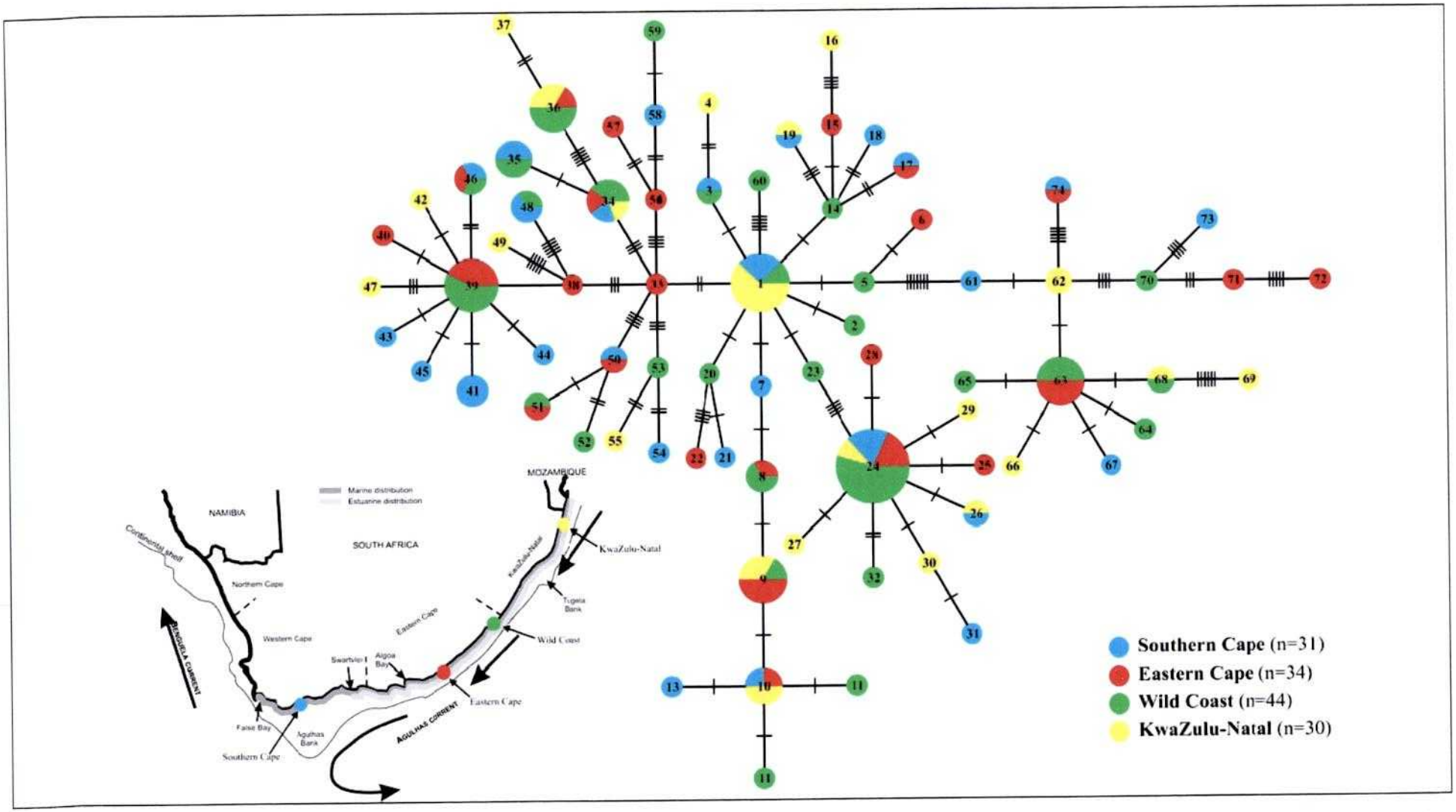

Fig. 3.4 Minimum Spanning Network showing the relationships among the 74 South African *P. commersonnii* mtDNA control region haplotypes. Circle diameter is proportional to haplotype frequency. Pie charts display the localities where the haplotype has been found and numbers indicate haplotype numbers 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.

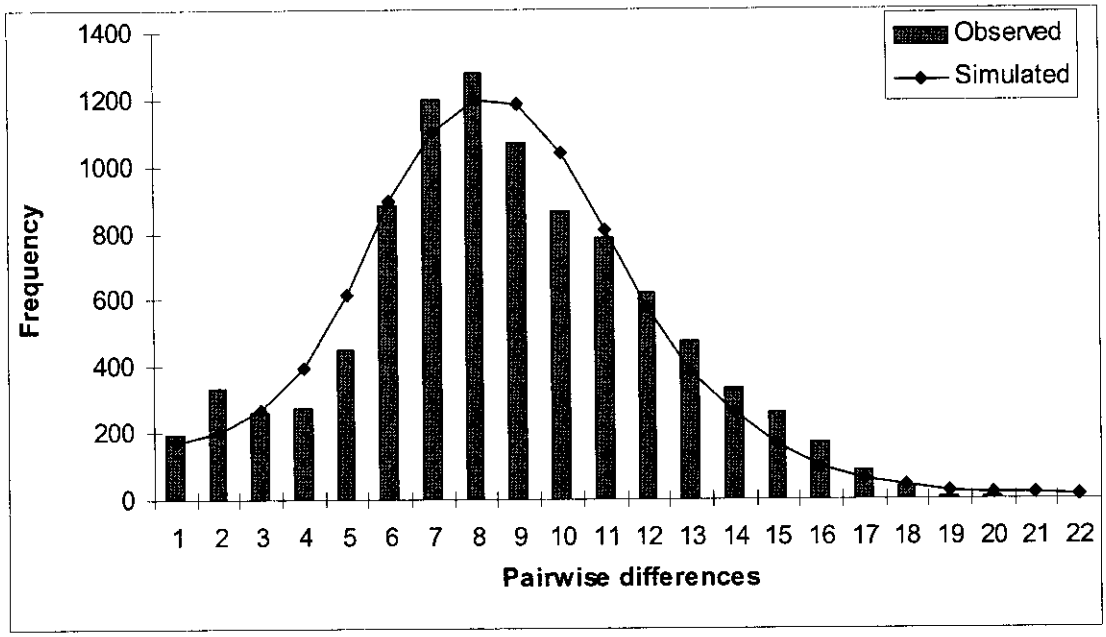

Fig. 3.5 Mismatch distribution of pairwise differences between 139 *P. commersonnii* individuals based on the 5' end of the mtDNA control region. The expected frequency is based on a population growth-decline model (S.S.D. = 0.0014 $P = 0.500$, r = 0.0058 $P = 0.7599$ $\theta_0 = 1.481$ and $\theta_1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

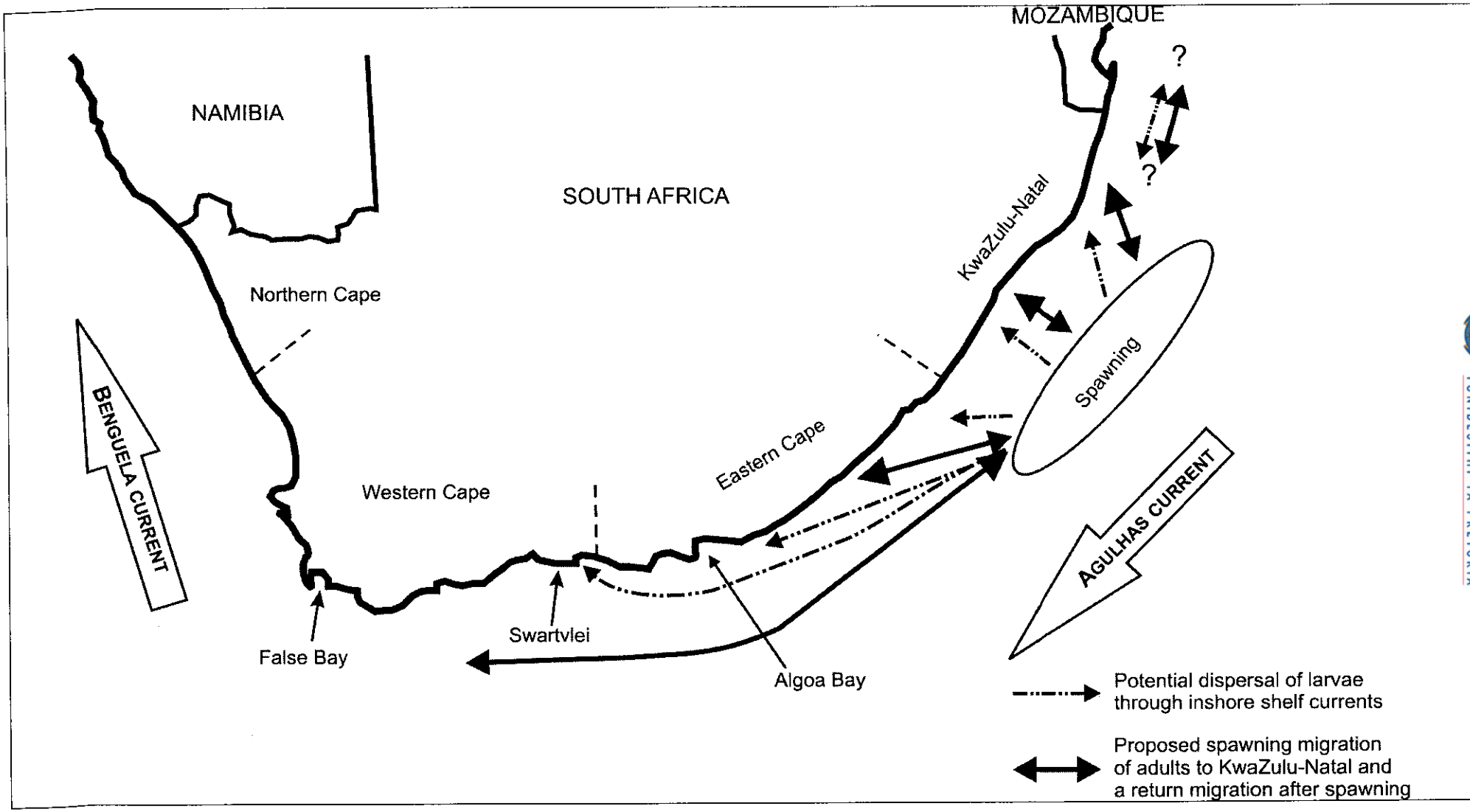

Fig.3.6 Suggested adult and larval movements along the coast that may influence genetic structuring. Based on life history data adult *P. commersonnii* undergoes a spawning migration to KZN, larvae get dispersed by inshore currents influenced by the Agulhas Current, resulting in recruitment of juveniles 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.

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Appendix I

Argyrosomus japonicus samples analysed in the present sudy. Samples are listed by locality, and haplotype nr, date collected and fish size are shown.

Southern Cape

Sample number	*h* nr	Date collected	Size TL (mm)
Aj0172DeH	3	23/11/00	702
Aj0173DeH	19	23/11/00	752
Aj0174DeH	16	23/11/00	940
Aj0293KAl	5	22/02/01	795
Aj0294KAl	13	22/02/01	696
Aj0295KAl	26	22/02/01	744
Aj0296KAl	3	22/02/01	798
Aj0297KAl	26	22/02/01	705
Aj0298KAl	3	22/02/01	645
Aj0299KAl	12	24/02/01	654
Aj0301KAl	23	22/02/01	695
Aj0492DeH	23	12/07/01	788
Aj0493DeH	26	12/07/01	745
Aj0494DeH	16	12/07/01	585
Aj0495DeH	19	12/07/01	558
Aj0496DeH	16	12/07/01	475
Aj0497DeH	32	14/07/01	800
Aj0498DeH	16	24/08/01	553
Aj0499DeH	3	24/08/01	460
Aj0500DeH	5	24/08/01	562
Aj0501DeH	19	24/08/01	570
Aj0502DeH	11	25/08/01	775
Aj0503DeH	8	25/08/01	492
Aj0504DeH	35	24/08/01	760
Aj0538KAl	1	01/11/01	540
Aj0539DeH	32	01/11/01	690
Aj0540DeH	5	01/11/01	850
Aj0542DeH	26	01/11/01	512
Aj0543DeH	11	01/11/01	470
Aj0544DeH	8	01/11/01	483
Aj0545DeH	8	01/11/01	482
Aj0550DeH	26	01/11/01	518
Aj0551DeH	11	01/11/01	790

Eastern Cape

Sample number	*h* nr	Date collected	Size TL (mm)
Aj0303Fis	7	14/04/01	400
Aj0304Fis	6	14/04/01	272
Aj0305Fis	34	14/04/01	350
Aj0306Fis	1	14/04/01	720
Aj0307Fis	25	14/04/01	240
Aj0308Fis	35	14/04/01	370
Aj0309Fis	28	14/04/01	280
Aj0310Fis	11	14/04/01	320
Aj0311Fis	11	14/04/01	290
Aj0312Fis	32	14/04/01	650
Aj0313Fis	32	14/04/01	300
Aj0314Fis	27	14/04/01	260
Aj0315Fis	17	14/04/01	450
Aj0316Fis	18	14/04/01	430
Aj0317Fis	26	14/04/01	450
Aj0318Fis	16	14/04/01	510
Aj0319Fis	8	14/04/01	460
Aj0320Fis	16	14/04/01	730
Aj0321Fis	14	14/04/01	570
Aj0322Fis	28	14/04/01	450
Aj0323Fis	10	14/04/01	970
Aj0324Fis	26	14/04/01	430
Aj0325Fis	35	14/04/01	290
Aj0326Fis	11	14/04/01	420
Aj0327Fis	16	14/04/01	460
Aj0328Fis	1	14/04/01	500
Aj0329Fis	34	14/04/01	480
Aj0330Fis	33	14/04/01	460
Aj0331Fis	33	14/04/01	480
Aj0332Fis	9	14/04/01	320
Aj0333Fis	30	14/04/01	290
Aj1125Fis	24	20/09/01	430
Aj1126Fis	3	20/09/01	500
Aj1127Fis	8	20/09/01	260
Aj1128Fis	8	20/09/01	400
Aj1129Fis	11	20/09/01	600
Aj1130Fis	8	10/10/01	330
Aj1131Fis	9	10/10/01	250
Aj1132Fis	15	10/10/01	320
Aj1133Fis	20	10/10/01	350
Aj1134Fis	30	10/10/01	800
Aj1137Fis	31	10/10/01	400
Aj1138Fis	8	20/10/01	440
Aj1139Fis	5	20/10/01	410
Aj1140Fis	3	20/10/01	550
Aj1141Fis	23	14/11/01	300
Aj1142Fis	11	14/11/01	280
Aj1144Fis	32	14/11/01	350

Wild Coast

Sample number	*h* nr	Date collected	Size TL (mm)
Aj0017Mng	32	23/01/01	juv
Aj0020Mng	5	24/01/01	juv
Aj0021Mng	1	24/01/01	juv
Aj0022Mng	11	24/01/01	juv
Aj0023Mng	8	24/01/01	juv
Aj0024Mng	3	24/01/01	juv
Aj0046Mng	7	24/01/01	juv
Aj0047Mng	5	24/01/01	juv
Aj0048Mng	12	24/01/01	juv
Aj0051Mng	11	24/01/01	juv
Aj0054Mng	3	24/01/01	juv
Aj0055Mng	32	24/01/01	juv
Aj0073Mng	35	25/01/01	juv
Aj0074Mng	3	25/01/01	juv
Aj0079Mng	5	25/01/01	juv
Aj0103Mng	11	26/01/01	juv
Aj0104Mng	11	26/01/01	juv
Aj0105Mng	23	26/01/01	juv
Aj0106Mng	26	26/01/01	juv
Aj0160Mna	25	29/01/01	juv
Aj0162Mna	26	29/01/01	juv
Aj0163Mna	32	29/01/01	juv
Aj0389Mna	11	07/06/01	244
Aj0411Mna	26	07/06/01	380
Aj0425Mna	34	08/06/01	145
Aj0452Mng	21	10/06/01	46
Aj0469Mng	7	11/06/01	470
Aj0475Mng	26	11/06/01	405
Aj0477Mng	19	11/06/01	310
Aj1153Mng	12	23/01/02	280

KwaZulu-Natal

Sample number	*h* nr	Date collected	Size TL (mm)
Aj0619StL	19	15/07/02	
Aj0620StL	1	23/08/02	345
Aj1038StL	5	1/05/03	juv
Aj1039StL	26	1/05/03	juv
Aj1040StL	26	1/05/03	juv
Aj1041StL	8	1/05/03	juv
Aj1042StL	5	1/05/03	950
Aj1043StL	7	1/05/03	juv
Aj1044StL	3	1/05/03	juv
Aj1045StL	7	1/05/03	juv
Aj1046StL	3	1/05/03	juv
Aj1047StL	12	1/05/03	820
Aj1048StL	11	1/05/03	102
Aj1049StL	8	1/05/03	800
Aj1050StL	30	1/05/03	107
Aj1051StL	29	1/05/03	850
Aj1052StL	2	1/05/03	100
Aj1054StL	4	1/05/03	juv
Aj2550StL	22	11/08/03	850
Aj2551StL	26	11/08/03	870
Aj2552StL	9	11/08/03	870
Aj2553StL	22	11/08/03	730

Appendix II

Absolute pairwise differences between *Argyrosomus japonicus* control region haplotypes from four regions along the South African coast.

h nr	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1	0																																		
2	1	0																																	
3	1	2	0																																
4	2	3	1	0																															
5	2	3	1	2	0																														
6	3	4	2	3	1	0																													
7	1	2	2	3	3	4	0																												
8	2	3	3	4	4	5	1	0																											
9	3	4	4	5	5	6	2	1	0																										
10	4	5	5	6	6	7	3	2	1	0																									
11	3	4	4	5	5	6	4	5	6	7	0																								
12	4	5	5	6	6	7	5	6	7	8	1	0																							
13	4	5	5	6	6	7	5	6	7	8	1	2	0																						
14	4	5	5	6	6	7	5	6	7	8	1	2	2	0																					
15	4	5	5	6	6	7	5	6	7	8	1	2	2	2	0																				
16	4	5	5	6	6	7	5	6	7	8	3	4	4	4	4	0																			
17	5	6	6	7	7	8	6	7	8	9	4	5	5	5	5	2	0																		
18	6	7	7	8	8	9	7	8	9	10	5	6	6	6	6	2	4	0																	
19	8	9	9	10	10	11	9	10	11	12	7	8	8	8	8	4	4	6	0																
20	5	6	6	7	7	8	6	7	8	9	4	5	5	5	5	5	6	7	9	0															
21	6	7	7	8	8	9	7	8	9	10	7	8	8	8	8	8	9	10	12	9	0														
22	6	7	7	8	8	9	7	8	9	10	7	8	8	8	8	8	9	10	12	9	2	0													
23	6	6	7	8	8	9	7	8	9	10	5	6	6	6	6	6	7	8	10	7	10	10	0												
24	8	8	9	10	10	11	9	10	11	12	7	8	8	8	8	8	9	10	12	9	12	12	2	0											
25	8	8	9	10	10	11	9	10	10	11	7	8	8	8	8	8	9	10	12	9	12	12	4	6	0										
26	6	6	7	8	8	9	7	8	9	10	5	6	6	6	6	6	7	8	10	7	10	10	4	6	6	0									
27	7	7	8	9	9	10	8	9	10	11	6	7	7	7	7	7	8	9	11	8	11	11	5	7	7	1	0								
28	7	7	8	9	9	10	8	9	10	11	6	7	7	7	7	7	8	9	11	8	11	11	5	7	7	1	2	0							
29	8	8	9	10	10	11	9	10	11	12	7	8	8	8	8	8	9	10	12	9	12	12	6	8	8	2	3	3	0						
30	7	7	8	9	9	10	8	9	10	11	6	7	7	7	7	7	8	9	11	8	11	11	5	7	7	3	4	4	5	0					
31	9	9	10	11	11	12	10	11	12	13	8	9	9	9	9	9	10	11	13	10	13	11	5	7	9	5	6	6	7	6	0				
32	8	8	9	10	10	11	9	10	11	12	7	8	8	8	8	8	9	10	12	9	12	12	6	8	8	6	7	7	8	7	9	0			
33	9	9	10	11	11	12	10	11	12	13	8	9	9	9	9	9	10	11	13	10	13	13	7	9	9	7	8	8	9	8	10	1	0		
34	9	9	10	11	11	12	10	11	12	13	8	9	9	9	9	9	10	11	13	10	13	13	7	9	9	7	8	8	9	8	10	1	2	0	
35	10	10	11	12	12	13	11	12	13	14	9	10	10	10	10	10	11	12	14	11	14	14	8	10	10	8	9	9	10	9	11	2	3	3	0

Appendix III

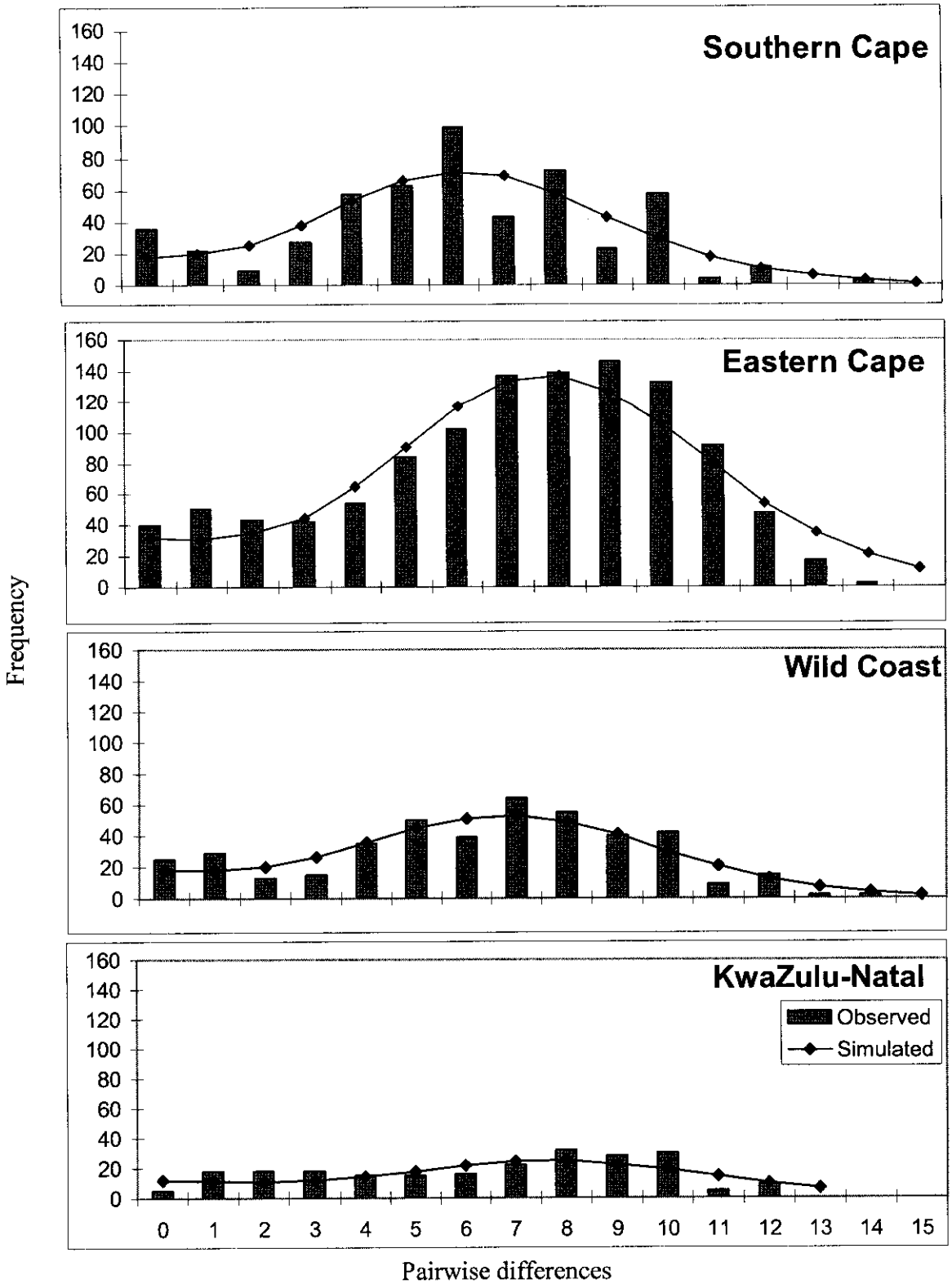

Fig. III.1 Mismatch distributions of *A. japonicus* at the four South African localities.

Table III.1 Mismatch distribution results from four populations of *A. japonicus*. Parameters of the model of sudden expansion are presented as well as goodness–of–fit to the model. Harpending's index of raggedness and its *P* value are given (Harpending, 1994; Rogers, Harpending, 1992).

	Southern Cape	Eastern Cape	Wild Coast	KwaZulu-Natal	***SOUTH AFRICA***
Parameters					
Θ_0	0.003	0.000	0.000	0.000	0.003
Θ_1	28.690	35.336	21.448	23.452	18.814
τ	6.912	8.624	8.542	7.738	8.770
Test of Goodness–of–fit					
S.S.D.	0.014	0.002	0.003	0.005	0.010
P	0.149	0.691	0.867	0.648	0.548
Raggedness index	0.049	0.006	0.011	0.017	0.020
P	0.046	0.924	0.833	0.565	0.718

n = number of haplotypes involved in the computations, Θ_0 = pre–expansion, Θ_1 = post-expansion, τ = time in generations since expansion.

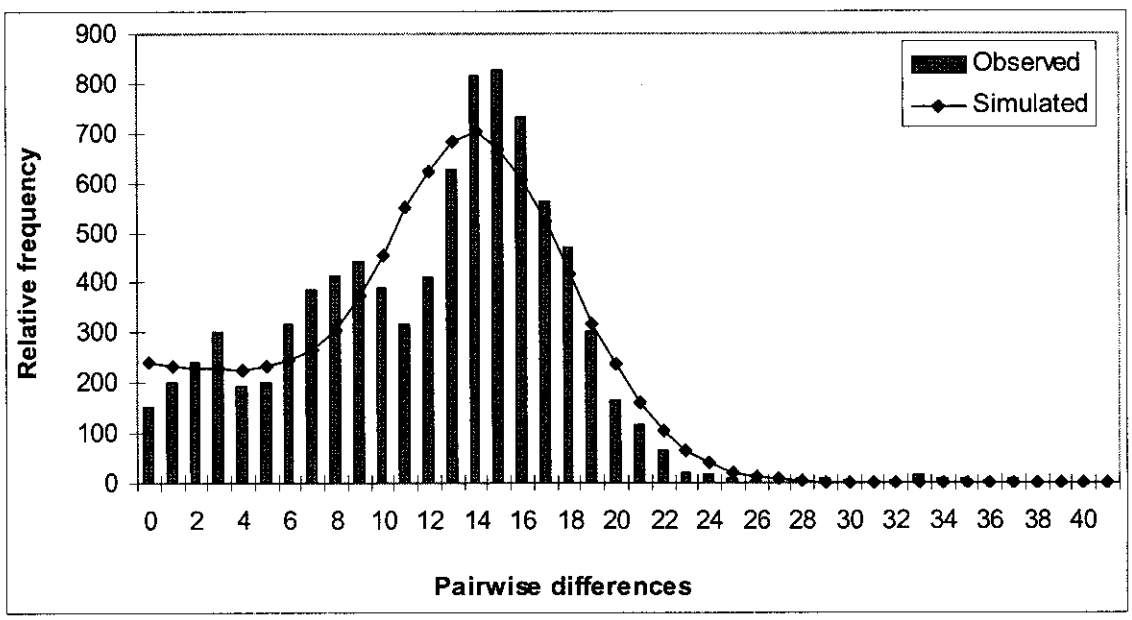

Fig III.2 Mismatch distribution based on the complete mtDNA control region (819bp) between 133 *A. japonicus* individuals. The expected frequency is based on a population growth-decline model (S.S.D. = 0.003 *P* = 0.667, r = 0.003 *P* = 0.907, θ_0 = 0.003 and θ_1 = 35.285, τ = 15.131) determined using ARLEQUIN 2.0.

Appendix IV

Pomadasys commersonnii samples analysed in the present study. Samples are listed by locality, and haplotype nr, date collected and fish size are shown.

Southern Cape

Sample number	*h* nr	Date collected	Size TL (mm)
Pc2198Grt	17	16/09/02	542
Pc2199Grt	41	16/09/02	245
Pc2200Grt	10	16/09/02	553
Pc2201Grt	13	16/09/02	575
Pc2202Grt	61	16/09/02	685
Pc2203Grt	41	16/09/02	552
Pc2204Grt	73	16/09/02	555
Pc2205Grt	34	16/09/02	571
Pc2206Grt	1	16/09/02	538
Pc2215Brd	54	19/09/02	598
Pc2216Brd	43	20/09/02	555
Pc2226Brd	50	20/09/02	609
Pc2227Brd	24	20/09/02	588
Pc2228Brd	45	20/09/02	542
Pc2229Brd	48	20/09/02	602
Pc2230Brd	46	20/09/02	665
Pc2231Brd	3	20/09/02	620
Pc2232Brd	1	20/09/02	440
Pc2233Brd	31	20/09/02	655
Pc2275Hnn	18	05/10/02	534
Pc2276Hnn	41	05/10/02	500
Pc2277Hnn	19	30/10/02	620
Pc2278Hnn	26	05/10/02	522
Pc2279Hnn	24	18/11/02	675
Pc2280Hnn	58	20/11/02	485
Pc2281Hnn	44	28/10/02	520
Pc2282Hnn	7	18/11/02	635
Pc2283Hnn	48	26/10/02	655
Pc2284Hnn	21	19/10/02	503
Pc2285Hnn	67	26/10/02	500
Pc2286Hnn	74	20/10/02	500

Eastern Cape

Sample number	*h* nr	Date collecte	Size TL (mm)
Pc0247EKM	9	8/02/01	420 SL
Pc0248EKM	74	8/02/01	291 SL
Pc0249EKM	24	8/02/01	229 SL
Pc0250EKM	57	8/02/01	275 SL
Pc0251EKM	39	8/02/01	295 SL
Pc0334Fis	51	14/04/01	360
Pc0335Fis	25	14/04/01	450
Pc0338Fis	9	14/04/01	430
Pc0339Fis	36	14/04/01	500
Pc0341Fis	63	14/04/01	740
Pc0342Fis	22	14/04/01	310
Pc0343Fis	46	14/04/01	470
Pc0344Fis	10	14/04/01	530
Pc0345Fis	39	14/04/01	400
Pc0346Fis	63	14/04/01	420
Pc0348Fis	6	14/04/01	640
Pc0349Fis	34	14/04/01	400
Pc0350Fis	40	14/04/01	260
Pc0351Fis	72	14/04/01	390
Pc0352Fis	17	14/04/01	211
Pc0353Fis	35	14/04/01	530
Pc0354Fis	24	14/04/01	510
Pc0356Fis	39	14/04/01	320
Pc0358Fis	8	14/04/01	341
Pc0360Fis	63	14/04/01	390
Pc0361Fis	38	14/04/01	340
Pc0362Fis	71	14/04/01	480
Pc0365Fis	28	14/04/01	196
Pc0366Fis	50	14/04/01	222
Pc0367Fis	33	14/04/01	215
Pc0368Fis	15	14/04/01	300
Pc0369Fis	56	14/04/01	680
Pc0385Kow	9	01/05/01	205 SL
Pc0386Kow	35	01/05/01	152 SL

Wild Coast

Sample number	*h* nr	Date collecte	Size TL (mm)
Pc0008Mng	34	23/01/01	juv
Pc0014Mng	24	23/01/01	juv
Pc0015Mng	60	23/01/01	juv
Pc0016Mng	51	23/01/01	juv
Pc0018Mng	3	24/01/01	juv
Pc0019Mng	35	24/01/01	juv
Pc0028Mng	1	24/01/01	juv
Pc0029Mng	63	24/01/01	juv
Pc0030Mng	53	24/01/01	juv
Pc0042Mng	46	24/01/01	juv
Pc0043Mng	63	24/01/01	juv
Pc0044Mng	24	24/01/01	juv
Pc0045Mng	2	24/01/01	juv
Pc0050Mng	8	24/01/01	juv
Pc0052Mng	34	24/01/01	juv
Pc0053Mng	35	24/01/01	juv
Pc0056Mng	8	25/01/01	juv
Pc0062Mng	70	25/01/01	juv
Pc0063Mng	39	25/01/01	juv
Pc0064Mng	24	25/01/01	juv
Pc0065Mng	24	25/01/01	juv
Pc0066Mng	39	25/01/01	juv
Pc0067Mng	52	25/01/01	juv
Pc0068Mng	65	25/01/01	juv
Pc0071Mng	32	25/01/01	sub adult
Pc0072Mng	63	25/01/01	juv
Pc0075Mng	11	25/01/01	juv
Pc0076Mng	59	25/01/01	juv
Pc0077Mng	24	25/01/01	juv
Pc0078Mng	68	25/01/01	juv
Pc0102Mng	14	25/01/01	juv
Pc0123Mng	5	26/01/01	juv
Pc0124Mng	64	26/01/01	juv
Pc0125Mng	20	26/01/01	juv
Pc0158Mna	23	28/01/01	juv
Pc0161Mna	36	29/01/01	juv
Pc0165Mna	39	29/01/01	juv
Pc0410Mna	48	07/06/01	450
Pc0414Mna	24	08/06/01	382
Pc0416Mna	9	08/06/01	403
Pc0426Mna	36	08/06/01	79
Pc0437Mng	39	10/06/01	234
Pc0470Mng	36	11/06/01	387
Pc1152Mng	12	23/01/02	159

KwaZulu-Natal

Sample number	*h* nr	Date collecte	Size TL (mm)
Pc0482StL	4	01/06/01	310
Pc0483StL	1	12/06/01	420
Pc0484StL	47	12/06/01	540
Pc0485StL	30	12/06/01	500
Pc0486StL	69	12/06/01	510
Pc0487StL	37	12/06/01	550
Pc0488StL	36	12/06/01	565
Pc0489StL	27	12/06/01	540
Pc0591StL	66	21/05/02	402
PC0603StL	26	21/05/02	258
Pc0604StL	1	22/05/02	325
Pc2455StL	1	6/06/03	530
Pc2456StL	1	6/06/03	525
Pc2457StL	9	6/06/03	522
Pc2458StL	49	6/06/03	535
Pc2459StL	36	6/06/03	581
Pc2460StL	62	6/06/03	492
Pc2467StL	10	6/06/03	535
Pc2468StL	9	6/06/03	560
Pc2469StL	68	6/06/03	555
Pc2471StL	19	6/06/03	530
Pc2472StL	29	7/06/03	570
Pc2473StL	55	7/06/03	582
Pc2474StL	10	7/06/03	550
Pc2475StL	24	7/06/03	524
Pc2476StL	1	7/06/03	478
Pc2477StL	34	7/06/03	532
Pc2478StL	62	7/06/03	534
Pc2479StL	16	7/06/03	504
Pc2480StL	42	7/06/03	580

Appendix V Absolute pairwise differer ontrol region haplotypes from four regoins along the South African coast.

h nr	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	0																								
2	1	0																							
3	1	2	0																						
4	3	4	2	0																					
5	1	2	2	4	0																				
6	2	3	3	5	1	0																			
7	1	2	2	4	2	3	0																		
8	2	3	3	5	3	4	1	0																	
9	3	4	4	6	4	5	2	1	0																
10	4	5	5	7	5	6	3	2	1	0															
11	5	6	6	8	6	7	4	3	2	1	0														
12	5	6	6	8	6	7	4	3	2	1	2	0													
13	5	6	4	6	6	7	4	3	2	1	2	2	0												
14	1	2	2	4	2	3	2	3	4	5	6	6	6	0											
15	2	3	3	5	3	4	3	4	5	6	7	7	7	1	0										
16	6	7	7	9	7	8	7	6	7	8	9	9	9	5	4	0									
17	3	4	4	6	2	3	4	5	6	7	8	8	8	2	3	7	0								
18	3	4	4	6	2	3	4	5	6	7	8	8	8	2	3	7	2	0							
19	4	5	5	7	5	6	5	4	5	6	7	7	7	3	4	6	5	5	0						
20	1	2	2	4	2	3	2	3	4	5	6	6	6	2	3	6	4	4	5	0					
21	3	4	4	6	4	5	4	3	4	5	6	6	6	4	5	6	6	6	5	2	0				
22	5	6	6	8	6	7	6	5	6	7	8	8	8	6	7	8	8	8	7	4	4	0			
23	1	2	2	4	2	3	2	3	4	5	6	6	6	2	3	7	4	4	5	2	4	6	0		
24	5	6	6	8	6	7	6	5	6	7	8	8	8	6	7	9	8	8	5	6	6	8	4	0	
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30	6	7	7	9	7	8	7	6	7	8	9	9	9	7	8	10	9	9	6	7	7	9	5	1	2
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73	13	14	14	14	12	12	14	13	14	15	16	16	16	12	13	13	12	12	11	13	13	15	14	10	11
74	10	11	11	11	9	10	11	10	11	12	13	13	13	11	12	12	11	11	12	10	10	12	11	9	10

h nr	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
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28	2	0																							
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34	7	7	7	7	8	8	3	0																	
35	8	8	8	8	9	9	4	1	0																
36	11	11	11	11	10	12	5	5	6	0															
37	11	11	11	11	10	12	5	5	6	2	0														
38	7	7	7	7	6	8	3	6	7	8	8	0													
39	8	8	8	8	7	9	4	7	8	9	9	1	0												
40	9	9	9	9	8	10	5	8	9	10	10	2	1	0											
41	7	7	7	7	6	8	3	6	7	8	8	2	1	2	0										
42	7	9	9	9	8	10	5	8	9	10	10	2	1	2	2	0									
43	9	9	9	9	8	10	5	8	9	10	10	2	1	2	2	2	0								
44	9	9	9	9	8	10	5	8	9	10	10	2	1	2	2	2	2	0							
45	9	9	9	7	6	10	5	8	9	10	10	2	1	2	2	2	2	2	0						
46	8	8	8	8	7	9	4	7	8	9	9	3	2	3	3	3	3	3	3	0					
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53	6	6	6	6	5	7	4	7	8	9	9	5	6	7	5	7	7	7	7	6	9	8	8	8	9
54	8	8	8	8	7	9	4	7	8	9	9	5	6	7	5	7	7	7	7	6	9	8	6	8	9
55	9	9	9	9	8	10	5	8	9	10	10	6	7	8	6	8	8	8	8	7	10	9	9	9	10
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61	8	8	8	8	9	7	8	9	10	12	12	9	10	11	9	11	11	11	11	10	13	12	12	12	11
62	9	9	9	9	10	8	9	10	11	13	13	10	11	12	10	12	12	12	12	11	14	13	13	13	12
63	10	10	10	10	11	9	10	11	12	14	14	11	12	13	11	13	13	13	13	12	15	14	14	14	13
64	9	9	9	9	10	8	11	12	13	15	15	12	13	14	12	14	14	14	14	13	16	15	15	15	14
65	11	11	11	11	12	10	11	12	13	15	15	12	13	14	12	14	14	14	14	13	16	15	15	15	14
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67	11	11	11	11	12	10	11	12	13	15	15	12	13	14	12	14	14	14	14	13	16	15	15	15	14
68	11	11	11	11	10	10	9	12	13	13	13	10	11	12	10	12	12	12	12	11	14	13	13	13	12
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70	9	9	9	9	10	8	9	10	11	13	13	10	11	12	10	12	12	12	12	11	14	13	12	13	12
71	12	12	12	12	13	11	12	13	14	16	16	13	14	15	13	15	14	15	15	14	17	16	15	16	15
72	15	15	15	15	16	14	15	14	15	17	17	16	17	17	16	18	17	18	18	17	20	19	16	17	16
73	11	11	11	11	12	10	13	14	15	15	15	12	13	14	12	14	14	14	14	15	16	15	14	17	16
74	10	10	10	10	11	9	10	11	12	14	12	11	12	13	11	13	13	13	13	12	15	14	14	14	13

h nr	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74
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56	10	6	6	7	0																		
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58	10	6	6	7	2	4	0																
59	9	7	7	8	3	3	1	0															
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67	17	13	13	14	11	11	13	14	16	3	2	1	2	2	2	0							
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70	15	11	10	12	11	11	11	12	14	5	4	5	6	6	6	6	6	10	0				
71	18	14	13	15	14	14	14	15	16	8	7	6	7	7	7	7	7	10	3	0			
72	19	17	16	18	15	13	17	16	17	9	8	7	8	8	8	8	8	10	6	5	0		
73	19	13	14	14	15	15	15	16	18	9	8	9	8	10	10	10	10	12	4	7	10	0	
74	16	12	12	13	12	12	12	13	15	6	5	6	7	7	7	7	7	11	5	8	11	9	0

Appendix VI

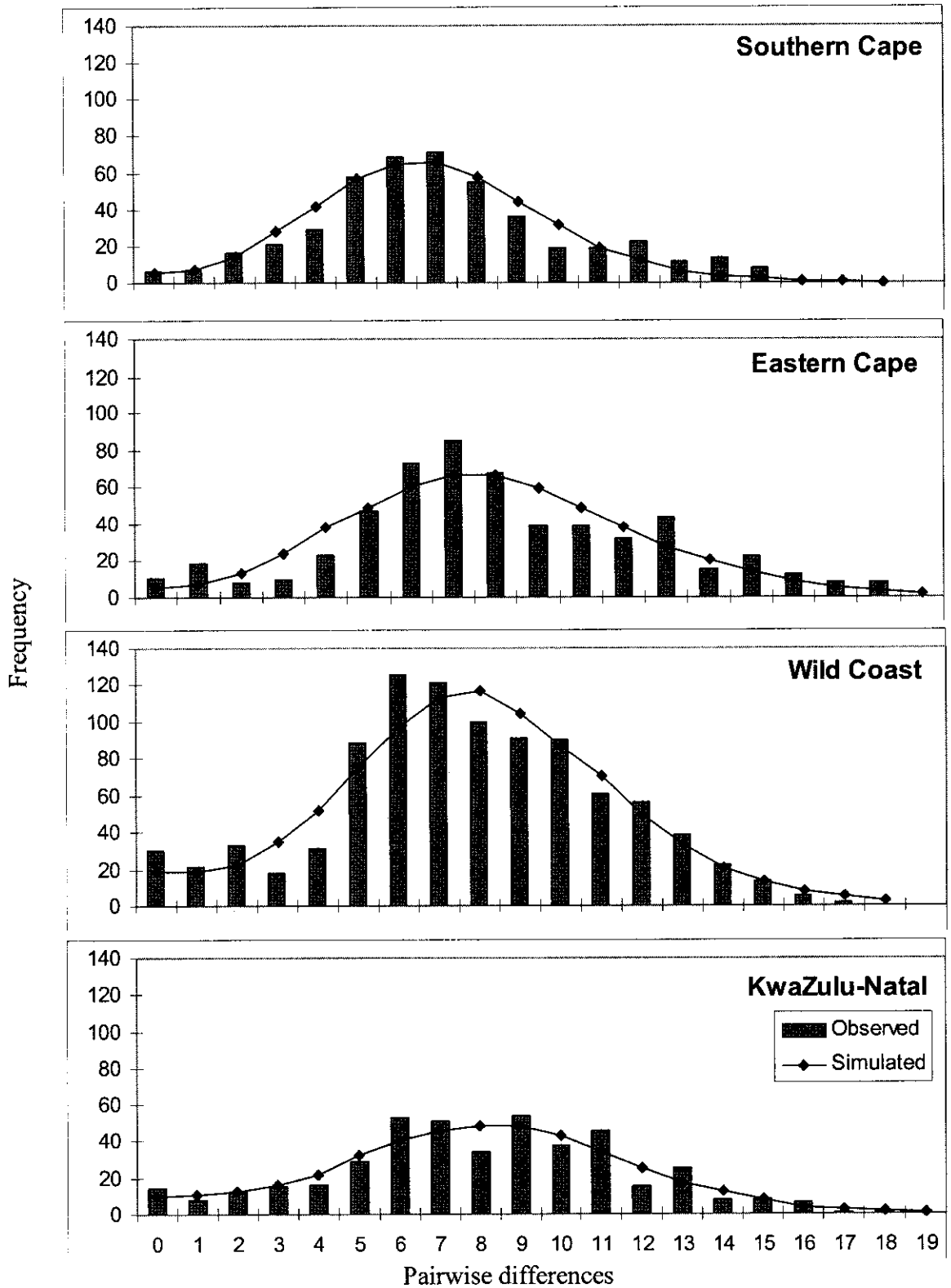

Fig VI.1 Mismatch distributions of *P. commersonnii* control region haplotypes within the four South African localities.

Table VI.1 Mismatch distribution results from four populations of *P. commersonnii*. Parameters of the model of sudden expansion are presented as well as goodness of fit to model. Harpending's index of raggedness and its *P* value given (Harpending, 1994; Rogers, Harpending, 1992).

	Southern Cape	Eastern Cape	Wild Coast	KwaZulu-Natal	***SOUTH AFRICA***
Parameters					
Θ_0	1.223	2.068	1.153	1.064	1.481
Θ_1	122.5456	106.055	53.990	43.047	55.190
τ	6.041	6.404	7.256	7.872	6.815
Test of Goodness of fit					
S.S.D.	0.004	0.006	0.003	0.005	0.001
P	0.400	0.260	0.550	0.490	0.504
Raggedness index	0.010	0.013	0.008	0.017	0.006
P	0.680	0.320	0.630	0.290	0.685

n = number of haplotypes involved in the computations, Θ_0 = pre expansion, Θ_1 = post-expansion, τ = time in generations since expansion.