Genetic stock structure of white steenbras *Lithognathus lithognathus* (Cuvier, 1829), an overexploited fishery species in South African waters

RH Bennett[1,2]*, K Reid[3,4], G Gouws[1], P Bloomer[3], PD Cowley[1,2]

[1]South African Institute for Aquatic Biodiversity, Private Bag 1015, Grahamstown, 6140, South Africa

[2]Department of Ichthyology and Fisheries Science, Rhodes University, P.O. Box 94, Grahamstown, 6140, South Africa

[3]Molecular Ecology and Evolution Programme, Department of Genetics, University of Pretoria, Pretoria, 0002, South Africa

[4]Present Address: Ecology and Evolutionary Biology Department, University of California, Santa Cruz, 95060, USA

*Corresponding author, email: r.bennett@saiab.ac.za

Abstract

White steenbras (*Lithognathus lithognathus*, Sparidae) is an overexploited fishery species endemic to South Africa. Overexploitation in recreational, subsistence and commercial fisheries has resulted in stock collapse, and the need for improved management of the species. Adults are thought to undertake large scale annual spawning migrations, yet movement studies indicate low levels of connectivity among coastal regions. To address this, mitochondrial DNA sequencing and genotyping of microsatellite loci in the nuclear genome were conducted to determine the genetic stock structure and level of gene flow within this species. Genetic diversity was high throughout the species' core distribution, with no evidence of isolation by distance or localised spawning. Low, non-significant pairwise fixation indices (F_{ST}, R_{ST} and Jost's D_{est}) indicated low genetic differentiation and high levels of gene flow. The observed results, and agreement between mitochondrial and microsatellite DNA, confirm that white steenbras exists as a single genetic stock with high levels of gene flow throughout its distribution.

Keywords: fisheries, microsatellites, mitochondrial control region, population genetics, Sparidae

Introduction

Understanding genetic stock structure and movement patterns is fundamental to understanding a species' ecology and essential for its effective management (Meyer et al. 2000, Balloux and Lugon-Moulin 2002). Larval dispersal, post-settlement movements and spawning migrations can play a major role in defining the levels of gene flow within a species (Turchin 1998). Genetic analyses can provide valuable information on reproductive isolation and the level and direction of gene flow throughout the species' distribution range (Shaklee and Bentzen 1998). This information is essential for understanding and predicting responses to natural and anthropogenic environmental changes (e.g. global climate change) and for identifying the most appropriate measures for successful management and conservation (Gold and Turner 2002, Nicastro et al. 2008, Zardi et al. 2011)

In South African waters, numerous marine fish species have evolved to share a common life-history pattern, with adults migrating north-eastwards along the South African east coast to spawn in warmer waters, where after eggs and larvae are transported south-westwards in shelf waters inshore of the south-west flowing Agulhas Current (Beckley 1993, Hutchings et al. 2002). Owing to such dispersal, many teleost species (including important fishery species) show no genetic structuring throughout their South African distributions (Teske et al. 2010, Henriques et al. 2012, 2014, Mirimin et al. 2015, Reid et al. 2016). However, phylogeographic breaks have been identified in the genetic structures of several marine taxa, at Cape Point, Cape Agulhas, and in the vicinities of Mossel Bay, Algoa Bay and Transkei (Figure 1) (Teske et al. 2011, Murray et al. 2014). While the movement patterns of most marine species in South Africa have been well documented, movement studies cannot provide information on the consequences of dispersal at the molecular level (Waples 1998), and information on the genetic stock structure of many South African marine species is lacking (Mann 2000, Teske et al. 2011). Genetic analyses should thus complement movement studies, to determine the spatial delineation of genetic stock structure within a species (von der Heyden 2009) and, concomitantly, the

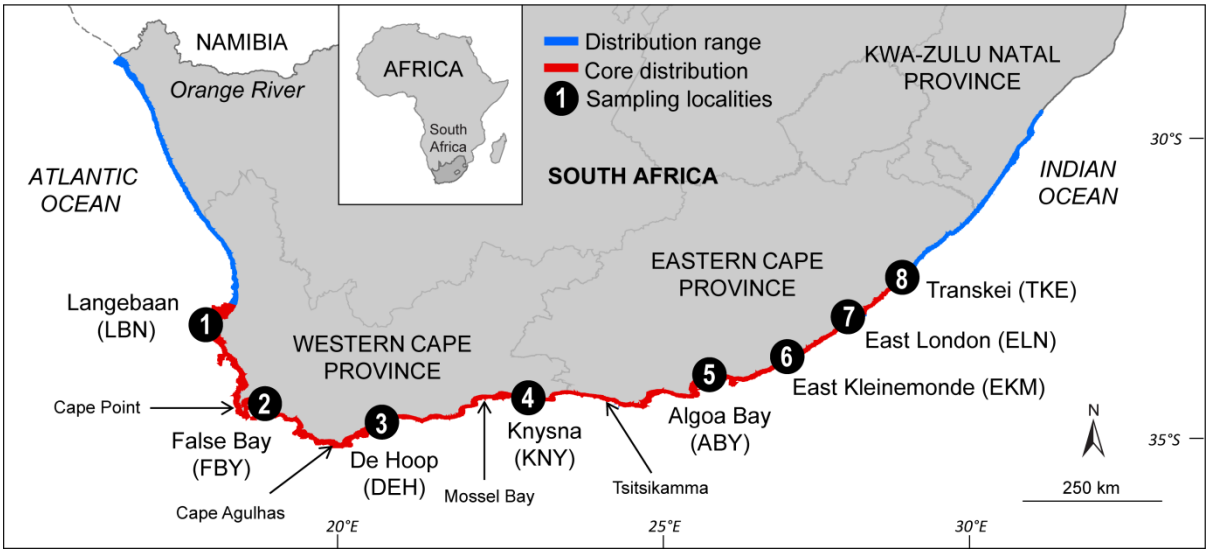

Figure 1: Map of the study region, showing the white steenbras distribution range and core distribution, the eight sampling localities (Langebaan and Transkei = adults only, East Kleinemonde = juveniles only) and locations referred to in text.

magnitude and spatial extent of gene flow and connectivity among populations or geographic localities (Waples 1998).

White steenbras *Lithognathus lithognathus* (Teleostei: Sparidae) is a coastal fishery species, endemic to South African waters (Lamberth and Mann 2000). It is heavily targeted in the recreational and subsistence estuarine, shore- and spear-fisheries in South Africa (Brouwer et al. 1997, Pradervand and Baird 2002, Cowley et al. 2013) and historically constituted a significant proportion of the catches in the commercial beach-seine and purse seine fisheries in South Africa's Western Cape Province (Penney 1991, Bennett 1993b, Lamberth et al. 1994). However, overexploitation of white steenbras in all sectors has resulted in rapid population decline and reductions in catch-per-unit-effort (CPUE) over the past five decades (Lamberth et al. 1994, Pradervand and Baird 2002, Bennett and Lamberth 2013). This is despite a series of increasingly stringent catch restrictions being imposed for this species in the recreational sector (including minimum legal size and maximum daily bag limits), and eventually a complete ban on the commercial harvesting and sale of the species (Bennett 2012). With a spawner biomass per recruit (SB/R) ratio of just 6% (Bennett 1993a), the white steenbras stock is considered collapsed, according to the South African Linefish Management Protocol (Griffiths et al. 1999). The decreasing population trend has led to the species' conservation status recently being elevated to "Endangered A2bcd; B2ab(ii,v)" (Mann et al. 2014) on the IUCN Red List of Threatened Species, based on a decline of more than 50% in the number of mature individuals in the species over the past three generations (IUCN 2001).

White steenbras has historically been managed as a single stock; however, prior to the current study, no attempt had been made, using genetic or other techniques, to assess the stock structure of the species. Juvenile white steenbras are resident in estuaries for up to three years, after which they move to the marine environment where they remain resident in the nearshore zone (Bennett 1993b, Bennett 2012, Bennett et al. 2015). According to Bennett (1993b) adults are thought to undertake large-scale

annual spawning migrations between an aggregation area in False Bay and the species' spawning grounds along the Transkei coast. Based on this perceived spawning migration (Bennett 1993b) and the dispersal of eggs and larvae by means of coastal oceanographic features (Hutchings et al. 2002), it was expected that white steenbras would be represented by a single genetic stock, with low levels of spatial genetic differentiation among coastal regions. However, more recently, dart tagging studies have indicated low levels of coastal connectivity in this species, and little evidence of large-scale annual migrations (Cowley 1999, Bennett 2012). Therefore, this study analysed mitochondrial (control region) DNA (mtDNA) and 11 microsatellites in the nuclear genome, to assess the demographic history and genetic stock structure of white steenbras throughout its distribution.

Material and methods

Sampling protocol and DNA extraction

Pectoral fin clippings were taken from 343 white steenbras from eight discrete localities, representing the species' core distribution (Figure 1). Juveniles (< 350 mm FL) were sampled in estuaries and adults (> 600 mm FL) predominantly in the marine environment. Samples were stored in absolute ethanol, and genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, USA).

Mitochondrial control region sequencing and microsatellite genotyping

A 720-bp fragment of the white steenbras mitochondrial control region was amplified using primers PT (forward) and PU (reverse) (Jean et al. 1995). Polymerase chain reactions (PCR) were conducted in 50-μL solutions, containing 1X buffer, 3 mM $MgCl_2$, 0.2 μM of each primer, 0.8 mM dNTPs, 0.5 U of DNA Taq Polymerase (Southern Cross Biotechnology, South Africa), and 5 μL of extracted DNA template, made up to final volume with ultrapure water. The PCR cycling profile comprised initial denaturation (94°C) for 3 minutes; 35 cycles of denaturation (94°C for 50 s), annealing (56°C for 40 s) and extension (72°C for 90 s); and final extension (72°C) for 7 minutes. PCR products were

purified and sequenced at a commercial sequencing facility (Macrogen Inc., Korea). Sequences were edited in Chromas Lite 2.01 (Technelysium Pty Ltd), and aligned manually in Seqman Pro™ (DNASTAR®). A final sequence alignment was produced in Clustal X (Larkin et al. 2007), which agreed with the manual alignment.

Fifteen microsatellite markers were used in this study; *LLt002, LLt005, LLt006, LLt007, LLt011, LLt014, LLt020, LLt024* and *LLtr004* were isolated in white steenbras (from Reid et al. 2012), while *PBt003, PBt007, PBt013, PBt018, PB106* (from Reid et al. 2012) and *Clat11* (from Teske et al. 2009) were isolated from related species. These loci were designed into multiplexes and amplified using the Quantitect® Multiplex PCR Kit (QIAGEN®). Reactions were conducted in 10-μL solutions, each containing 5 μL of Quantitect Multiplex PCR Master Mix®, 10-100 ng of DNA template, and 0.2 pmol of each forward and reverse primer in that multiplex, made up to final volume with Quantitect Multiplex deionised water. The PCR cycling profile comprised an initial polymerase activation step (95°C) for 15 minutes, then 50 cycles of annealing (94°C for 60 s) and extension (60°C for 90 s) (after Reid et al. 2012). Microsatellite profiles were examined using GeneMarker 1.95 (SoftGenetics® LLC) and peaks were scored manually. To ensure consistent scoring among runs, all runs contained four control samples. For the complete microsatellite dataset, Micro-Checker 2.2.3 (Van Oosterhout et al. 2004) was used prior to any analysis to detect possible amplification or genotyping errors (e.g. the presence of null alleles, large allele dropout and stuttering).

Data analysis

A priori delineation of populations was defined by geographic locality and by age class, with certain analyses conducted using two different groupings of samples. The first comprised eight localities (Figure 1) (five of which contained both juvenile and adult samples), and the second comprised thirteen populations (six juvenile and seven adult populations from the eight localities).

Genetic diversity

Genetic diversity in the mtDNA control region was calculated for each locality and population, using the number of polymorphic sites (*S*), haplotype diversity (*h*) and nucleotide diversity (π), in DnaSP 5 (Librado and Rozas 2009). A minimum spanning haplotype network was created to illustrate schematically the relationships among mtDNA haplotypes, using Network 4.6.0.0 (Fluxus Technology Ltd).

Genetic diversity in the microsatellite DNA was calculated as the mean number of alleles at each locality (averaged across all loci), and mean allelic richness for each locus across all localities, and for each locality across all loci, using FSTAT 2.9.3 (Goudet 1995, 2001). Mean observed and expected heterozygosities were calculated for each locus within each locality and overall, using Arlequin 3.5 (Excoffier and Lischer 2009). An exact test (Guo and Thompson 1992) was conducted to test for departure of the observed allele frequencies from Hardy-Weinberg Equilibrium (HWE), using Arlequin (with 100 000 steps in the Markov chain and 10 000 dememorisation steps). Linkage disequilibrium tests were conducted between pairs of loci within each locality and across all localities to test for significant non-random association of alleles at different loci, using an Expectation-Maximisation (EM) algorithm in Arlequin (based on 16 000 permutations of alleles at each locus, and five initial conditions from which the EM was started). Standard Bonferroni corrections were applied for multiple statistical tests (Rice 1989).

Genetic differentiation and population structure

Prior to the investigation of population differentiation, POWSIM 4.1 (Ryman and Palm 2006) was used to investigate the power of the data and the suitability of the present sample sizes to detect low [F_{ST} = 0.001; with the 1 000 simulations set using N_e = 10 000 and t (generations of drift) = 20] and moderate (F_{ST} = 0.05; N_e = 10 000, t = 1 026) levels of differentiation between two populations. Sample sizes for both the mitochondrial DNA and the microsatellite datasets were guided by the

second sample grouping, i.e. thirteen (six juvenile and seven adult) populations from eight localities, as this arrangement presented the lowest sample sizes per population.

Exact probability tests of population differentiation (Raymond and Rousset 1995) were used to assess pairwise genetic differentiation among sampling localities (n = 8) and among populations (n = 13). Tests were run in Arlequin with 100 000 steps in the Markov Chain and 10 000 dememorisation steps. Genetic differentiation among localities and among populations was further assessed by pairwise comparisons of haplotype frequencies in the mtDNA, using F_{ST} values (Wright 1951), and allele frequencies in the microsatellite data, using Slatkin's (1995) R_{ST}. The significance of each observed F_{ST} and R_{ST} value was calculated by permutation (10 000 permutations in each case). Genetic differentiation was also assessed in the microsatellites with Jost's D_{est}, in SMOGD version 1.2.5 (Crawford, 2010).

Analysis of Molecular Variance (AMOVA, Excoffier et al. 1992) was used to assess genetic structure at different hierarchical levels, for mtDNA and microsatellite genotypes. The different levels of assessment were defined as *among geographic localities* (n = 8 localities, Figure 1), *among populations within localities* (5 of 8 geographic localities had juvenile and adult populations), and *within individual populations* (n = 13 populations). For the microsatellite DNA, a fourth level of variability, i.e. *within individuals*, was included. AMOVA was also used to test for genetic differences between juvenile and adult samples; hierarchical levels included *among groups* (juvenile and adult), *among populations within groups* (n = 7 adult populations, n = 6 juvenile populations) and *within populations* (n = 13 populations), as well as *within individuals* for the microsatellite DNA. AMOVA analyses were conducted in Arlequin, with 10 000 permutations to determine significance at each level.

To further investigate population structure from the microsatellite genotypes, a Bayesian model-based clustering analysis was run in Structure 2.3.3 (Pritchard et al. 2000). The analysis probabilistically assigned individuals on the basis of their multilocus genotypes to one of a number of discrete putative populations (*K*). The term "population" in this analysis does not refer to the 13 sample populations described, but rather to hypothetical stocks. Analyses were run for $K = 1$ to $K = 8$ (the number of sampling localities). Mean log probability that the observed set of genotypes in the global sample was drawn from the *K* populations was estimated from 20 iterations for each value of *K*. Each iteration consisted of 100 000 burnin steps, and an additional 100 000 steps in the Markov chain. The most probable number of real populations present was taken as the value of *K* that maximised the log probability (Falush et al. 2003). The analysis was based on the *admixture ancestry model* and the *correlated allele frequencies model* in the Structure software.

Isolation by distance (Slatkin 1993) in the mtDNA and microsatellite DNA was assessed using the Mantel (1967) test in Mantel for Windows 1.16 (Cavalcanti 2005). This was assessed for the six juvenile populations, the seven adult populations and for the eight sampling localities (juveniles and adults combined). Matrices of pairwise F_{ST} values (for the mtDNA) and Slatkin's (1995) linearised genetic distance [$F_{ST}/(1 - F_{ST})$] values (for the microsatellite dataset) between localities were created and correlated with a similarly constructed matrix of geographic distances (km). Significance was determined by 10 000 permutations of one matrix, while the other remained constant.

Demographic history

Demographic history was assessed using the mismatch distribution (frequency distribution of numbers of nucleotide differences between all pairs of haplotypes) (Rogers and Harpending 1992), and a Bayesian Skyline Plot (BSP) approach that reconstructed the effective population size through time using coalescent-based genealogies (Heled and Drummond 2008). Significance of the mismatch test was based on the sum of squared deviations (SSD) between parameters of the observed and

expected distributions, and the probability determined from 1 000 simulated parameter sets (Excoffier and Lischer 2009). The mismatch was run in Arlequin under the assumptions of a demographic expansion model. Harpending's (1994) raggedness index (*r*) was calculated as a measure of the goodness-of-fit of the mismatch distribution to the demographic expansion model. The BSP was implemented in BEAST (Heled and Drummond 2008) using 20 million iterations, with a strict molecular clock, with the HKY substitution model. Model selection in MEGA 5 (Tamura et al. 2011) identified HKY as the most suitable substitution model, of those available in BEAST. A mutation rate of 9.4% per million years with a standard deviation of 2.45% was used to calibrate the BSP (Hoareau 2016). The outputs were assessed in TRACER version 1.5 (Rambaut and Drummond 2007).

Results

Genetic diversity

Mitochondrial control region sequences were successfully generated for 307 white steenbras, which produced 174 haplotypes (169 excluding gaps), and 143 polymorphic sites. Haplotype and nucleotide diversities were high, and consistent among localities and among populations. Numbers of polymorphic sites and mean numbers of nucleotide differences between sequences were high, producing high numbers of private haplotypes (Table 1).

The haplotype network (Figure 2) identified two common haplotypes, separated by six mutations, with many rare haplotypes. Haplotype 1 (found in 28 individuals from seven localities) formed the centre of the generally star-like topology. Haplotype 2 (15 individuals) formed the centre of a small, second cluster (indicated by "A" in Figure 2), in which all eight sampling localities were represented. A long branch (indicated by "B" in Figure 2) extended from the main clade and included haplotypes differing by up to 22 mutations from haplotype 1; within this clade all eight geographic localities were again represented. The network indicated no association between haplotype genealogy and geographic location.

Table 1: Genetic diversity indices calculated from 720-base pair mtDNA control region sequences for 307 white steenbras (n = 8 localities/13 populations), showing numbers of sequences (*N*), polymorphic sites (*S*), haplotypes (*H*), private haplotypes (*P*), mean number of nucleotide differences between sequences (*k*), haplotype diversity (*h*) and nucleotide diversity (π) (J = juvenile, A = adult)

Sample grouping		*N*	*S*	*H*	*P*	*k*	*h*	π
Grouped by locality								
LBN	Langebaan (A)	21	48	18	10	9.152	0.990	0.013
FBY	False Bay (A + J)	55	71	47	27	6.692	0.985	0.009
DEH	De Hoop (A + J)	44	64	35	21	7.734	0.979	0.011
KNY	Knysna (A + J)	47	61	40	18	7.067	0.988	0.010
ABY	Algoa Bay (A + J)	50	68	37	17	8.543	0.980	0.012
EKM	East Kleinemonde (J)	25	53	24	11	9.090	0.997	0.013
ELN	East London (A + J)	40	73	29	20	8.831	0.979	0.013
TKE	Transkei (A)	25	51	23	8	9.193	0.993	0.013
Grouped by population								
LBN A	Langebaan (A)	21	48	18	10	9.152	0.990	0.013
FBY J	False Bay (J)	25	51	25	8	7.397	1.000	0.010
FBY A	False Bay (A)	30	50	25	18	6.143	0.996	0.009
DEH J	De Hoop (J)	21	42	18	9	7.476	0.986	0.011
DEH A	De Hoop (A)	23	52	19	11	8.150	0.960	0.011
KNY J	Knysna (J)	22	45	21	8	7.351	0.996	0.010
KNY A	Knysna (A)	25	47	22	9	6.937	0.987	0.010
ABY J	Algoa Bay (J)	25	47	21	10	8.240	0.987	0.012
ABY A	Algoa Bay (A)	25	53	20	6	9.010	0.980	0.013
EKM J	East Kleinemonde (J)	25	53	24	11	9.090	0.997	0.013
ELN J	East London (J)	25	63	22	13	9.363	0.987	0.013
ELN A	East London (A)	15	36	12	5	8.114	0.971	0.012
TKE A	Transkei (A)	25	51	23	8	9.193	0.993	0.013
	Overall	*307*	*143*	*169*	-	*8.036*	*0.985*	*0.011*

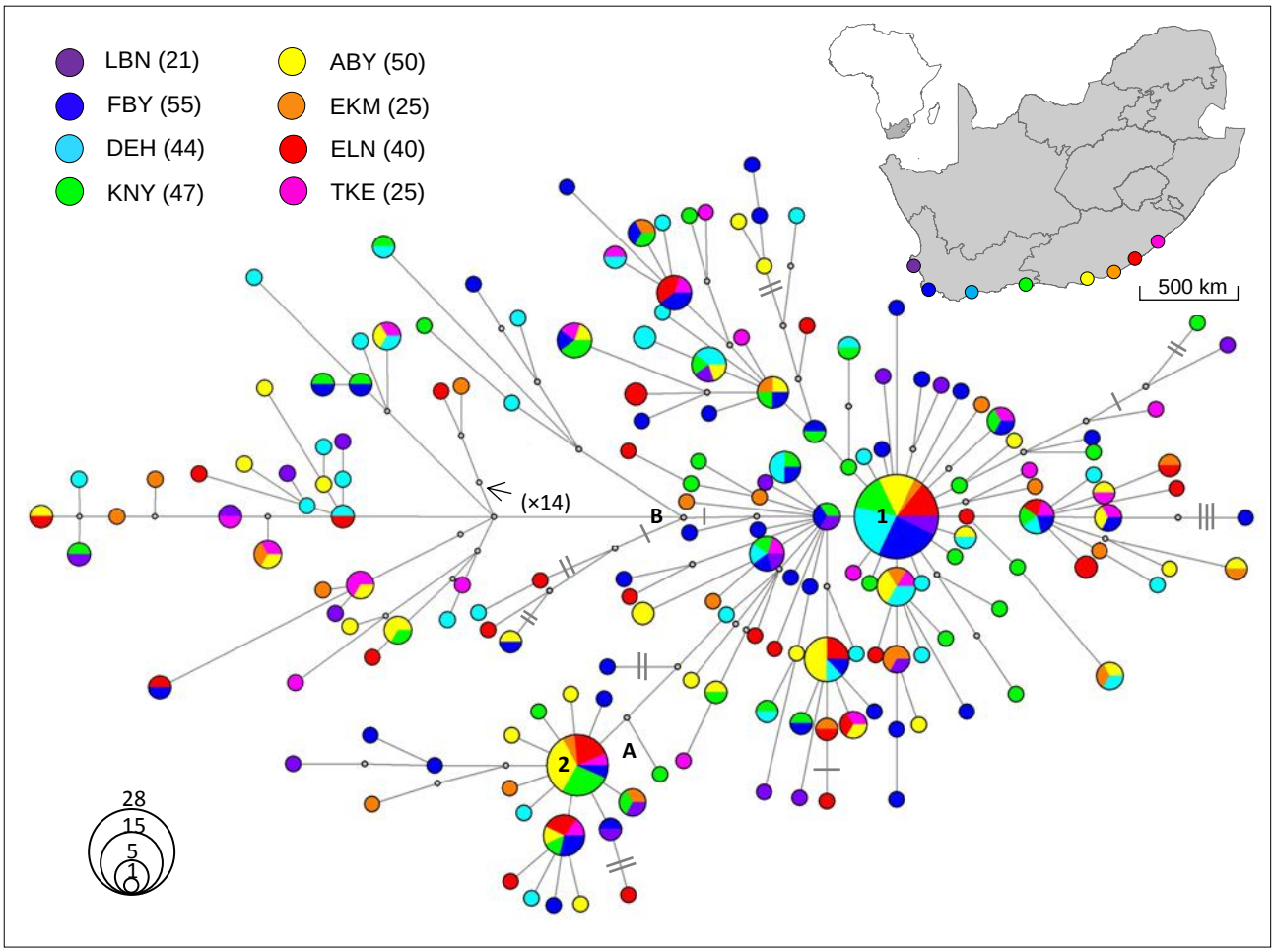

Figure 2: Minimum spanning haplotype network showing genealogical relationships among 174 haplotypes identified from the mitochondrial control region sequences of 307 white steenbras from the eight sampling localities (sample sizes in parentheses). Common haplotypes 1 and 2 are labelled. Sizes of circles are proportional to haplotype frequencies and colours identify localities. Branches indicate one mutational step, with additional steps indicated by transverse bars (×14 indicates 14 mutational steps). Intermediate nodes represent unsampled extant haplotypes or ancestral haplotypes.

Fifteen microsatellite markers were initially evaluated, of which three (*PBt007*, *LLt024* and *PBt013*) were immediately excluded due to inconsistent amplification. Micro-Checker detected no significant amplification or genotyping errors at any of the remaining loci, except for *Clat11* for which there was a significant probability ($p < 0.001$) of null alleles, as well as evidence of stuttering. This locus was therefore excluded from all further analyses. Considering all cases of polymorphism, involving all loci and localities, only two deviations from HWE were observed after standard Bonferroni correction. Linkage disequilibrium was observed in only 12 of the 440 pairwise locus comparisons (8 localities × 55 locus pairs), after Bonferroni correction. In the global sample, four of the 55 pairwise comparisons showed significant linkage disequilibrium (Appendix Table A).

Summary statistics were calculated for the eleven remaining loci (Appendix Table B). The mean number of alleles recovered at each locus ranged from 4.00 (*PBt018*) to 15.75 (*PBt003*), mean allelic richness ranged from 3.32 (*PBt018*) to 12.65 (*PBt003*), and observed heterozygosities were close to those expected under HWE for all eleven loci. Summary statistics were calculated across the eight sampling localities and 13 sampling populations (Table 2). Mean numbers of alleles, mean allelic richness and inbreeding coefficients averaged across all loci showed no trends among localities.

Genetic differentiation and population structure

Given the high number of haplotypes, the POWSIM analyses of the control region dataset excluded singletons and were restricted to those 48 haplotypes occurring more than once in the base population (i.e. the total sample). For both the control region and the microsatellite datasets, the simulations indicated that the data had sufficient power to detect moderate differentiation (at $F_{ST} = 0.05$) between two populations representing the smallest sample sizes in each of the data sets (i.e. 15 for East London adults and 21 for Langebaan adults/De Hoop juveniles for the control region data; 16 for East London adults and 23 for De Hoop adults for microsatellites). For these datasets, there was, respectively, a 98.3% and a 100% chance of detecting a significant F_{ST} of 0.05. There was much less power in the

Table 2: Summary statistics for white steenbras (n = 8 localities/13 populations), averaged (± SD) across 11 microsatellite loci (n = sample size, N_A, A_R, H_O and H_E refer to the mean number of alleles, mean allelic richness (based on 14 individuals per population) and observed and expected heterozygosities, F_{IS} = inbreeding coefficient estimated across all 11 loci) (J = juvenile, A = adult)

Sample grouping		n	N_A		A_R		H_O		H_E		F_{IS}
Grouped by locality											
LBN	Langebaan (A)	25	8.33	(± 3.11)	7.80	(± 2.93)	0.72	(± 0.18)	0.78	(± 0.14)	0.08
FBY	False Bay (A + J)	55	9.75	(± 4.16)	7.87	(± 2.98)	0.77	(± 0.13)	0.79	(± 0.14)	0.02
DEH	De Hoop (A + J)	47	9.67	(± 4.56)	7.98	(± 3.25)	0.77	(± 0.18)	0.79	(± 0.13)	0.02
KNY	Knysna (A + J)	48	9.50	(± 3.87)	7.86	(± 2.78)	0.75	(± 0.18)	0.79	(± 0.16)	0.04
ABY	Algoa Bay (A + J)	48	10.00	(± 4.07)	8.15	(± 2.91)	0.76	(± 0.21)	0.79	(± 0.15)	0.05
EKM	East Kleinemonde (J)	41	9.08	(± 3.40)	7.81	(± 2.76)	0.76	(± 0.17)	0.79	(± 0.14)	0.05
ELN	East London (A + J)	41	9.42	(± 3.78)	7.96	(± 2.91)	0.79	(± 0.17)	0.80	(± 0.12)	0.01
TKE	Transkei (A)	25	8.17	(± 2.89)	7.62	(± 2.66)	0.72	(± 0.20)	0.78	(± 0.17)	0.08
Grouped by population											
LBN A	Langebaan (A)	25	8.73	(± 2.94)	7.79	(± 2.58)	0.75	(± 0.04)	0.78	(± 0.04)	0.06
FBY J	False Bay (J)	25	8.36	(± 2.77)	7.42	(± 2.38)	0.77	(± 0.05)	0.76	(± 0.04)	0.01
FBY A	False Bay (A)	30	9.00	(± 4.05)	7.69	(± 2.99)	0.79	(± 0.04)	0.79	(± 0.04)	0.01
DEH J	De Hoop (J)	24	8.91	(± 3.27)	7.92	(± 2.59)	0.82	(± 0.04)	0.79	(± 0.03)	-0.01
DEH A	De Hoop (A)	23	8.82	(± 3.71)	7.90	(± 3.04)	0.80	(± 0.05)	0.78	(± 0.04)	0.00
KNY J	Knysna (J)	24	8.73	(± 3.50)	7.83	(± 2.80)	0.78	(± 0.06)	0.78	(± 0.05)	0.03
KNY A	Knysna (A)	24	8.36	(± 2.73)	7.65	(± 2.34)	0.77	(± 0.05)	0.79	(± 0.05)	0.04
ABY J	Algoa Bay (J)	24	8.91	(± 3.08)	7.95	(± 2.49)	0.76	(± 0.06)	0.79	(± 0.04)	0.07
ABY A	Algoa Bay (A)	24	9.55	(± 3.75)	8.21	(± 2.93)	0.82	(± 0.06)	0.79	(± 0.05)	-0.02
EKM J	East Kleinemonde (J)	41	9.45	(± 3.30)	7.74	(± 2.49)	0.78	(± 0.05)	0.79	(± 0.04)	0.03
ELN J	East London (J)	25	9.00	(± 3.58)	7.96	(± 2.81)	0.81	(± 0.04)	0.79	(± 0.04)	0.00
ELN A	East London (A)	16	7.82	(± 2.09)	7.76	(± 2.10)	0.86	(± 0.04)	0.79	(± 0.03)	-0.06
TKE A	Transkei (A)	25	8.55	(± 2.70)	7.62	(± 2.31)	0.73	(± 0.06)	0.77	(± 0.05)	0.07
	Overall	330	13.09	(± 5.74)	7.84	(± 2.60)	0.79	(± 0.01)	0.78	(± 0.01)	0.02

data for detecting low levels of differentiation between two populations with the above sample sizes, with a 6.7% chance of detecting F_{ST} = 0.001 for the control region data and 7.8% for the microsatellites. This was not improved much considering the mean sample sizes for each dataset, with the power increasing to 7.6% for the control region (considering two populations of 24 individuals) and to 9% for the microsatellites (25 individuals per population). This is probably a consequence of the high diversity in the base (total) population (see above), but caution is needed in the interpretation of the lack of differentiation based on the pairwise F_{ST} values and AMOVA results alone.

Exact tests revealed no significant differentiation between juvenile and adult samples within each of the five localities represented by both, allowing juvenile and adult populations to be pooled within each locality. Exact tests based on mtDNA identified significant differentiation in just three of the 28 pairwise comparisons between localities (n = 8); between East London and De Hoop (p = 0.041), East London and Langebaan (p = 0.043), and Langebaan and Algoa Bay (p = 0.040). There was no significant differentiation in the microsatellite data between localities (Appendix Table C). At the population level, only one of 78 mtDNA pairwise comparisons showed significant differentiation, that between False Bay adults and Transkei adults (p = 0.035), and there were again no significant pairwise differences in the microsatellite data (Appendix Table D). Pairwise genetic comparisons based on the mtDNA (F_{ST}) and microsatellite DNA (R_{ST}, Jost's D_{est}) also showed low genetic differentiation and no significant differences between localities (Table 3) or between populations (Table 4).

The AMOVA analyses identified low levels of genetic variation at all hierarchical levels in the mtDNA and microsatellite dataset (Table 5). The source of the greatest percent variation in the mtDNA was identified as that *among individuals within populations*, and in the microsatellite DNA as that *within individuals*. The analyses revealed no significant variation at any level, except *among*

Table 3: Pairwise genetic comparisons between localities (n = 8) for white steenbras, showing F_{ST} values based on 720-base pair mtDNA sequences (above diagonal), and pairwise R_{ST} (and Jost's D_{est}) values based on 11 microsatellite loci (below diagonal)

	LBN	FBY	DEH	KNY	ABY	EKM	ELN	TKE
LBN	-	0.021	0.002	0.000	-0.015	-0.015	-0.007	-0.013
FBY	0.005 (0.000)	-	0.007	-0.007	0.015	0.009	0.000	0.015
DEH	-0.005 (0.001)	-0.005 (0.000)	-	-0.001	0.002	0.000	-0.001	-0.002
KNY	-0.002 (0.007)	-0.005 (0.000)	-0.007 (0.000)	-	0.003	0.002	-0.002	0.006
ABY	-0.008 (0.000)	0.002 (0.000)	-0.003 (0.000)	-0.005 (0.000)	-	-0.010	-0.012	-0.008
EKM	0.010 (0.000)	-0.005 (0.003)	-0.001 (0.003)	0.003 (0.002)	0.005 (0.000)	-	-0.015	-0.012
ELN	-0.008 (0.000)	-0.004 (0.000)	-0.001 (0.000)	-0.001 (0.000)	-0.007 (0.000)	-0.004 (0.000)	-	-0.007
TKE	0.007 (0.005)	-0.007 (0.000)	-0.013 (0.000)	-0.015 (0.000)	-0.006 (0.000)	0.007 (0.000)	-0.002 (0.000)	-

Table 4: Pairwise genetic comparisons between populations (n = 13) for white steenbras, showing F_{ST} values based on 720-base pair mtDNA sequences (above diagonal), and pairwise R_{ST} (and Jost's D_{est}) values based on 11 microsatellite loci (below diagonal)

	LBN A	FBY J	FBY A	DEH A	DEH J	KNY J	KNY A	ABY J	ABY A	EKM J	ELN J	ELN A	TKE A
LBN A	-	0.006	0.025	-0.003	-0.009	-0.013	0.000	-0.018	-0.023	-0.015	-0.004	-0.023	-0.013
FBY J	0.003 (0.009)	-	-0.006	-0.004	0.000	-0.014	-0.023	-0.004	0.000	0.002	-0.018	-0.009	-0.002
FBY A	0.001 (0.000)	-0.015 (0.000)	-	0.002	0.000	-0.013	-0.003	0.010	0.024	0.008	0.006	0.004	0.021
DEH A	-0.005 (0.005)	0.005 (0.000)	0.004 (0.000)	-	-0.024	-0.020	-0.005	-0.005	-0.010	-0.006	-0.007	-0.015	-0.013
DEH J	0.000 (0.000)	-0.014 (0.000)	-0.020 (0.000)	0.005 (0.005)	-	-0.019	-0.003	-0.006	-0.013	-0.010	-0.009	-0.017	-0.006
KNY J	0.015 (0.015)	-0.011 (0.000)	-0.010 (0.000)	0.010 (0.004)	-0.015 (0.000)	-	-0.018	-0.020	-0.014	-0.013	-0.011	-0.026	-0.008
KNY A	-0.014 (0.000)	0.001 (0.000)	-0.006 (0.000)	-0.016 (0.000)	0.004 (0.000)	0.006 (0.000)	-	-0.001	0.006	0.002	-0.002	-0.012	0.006
ABY J	-0.002 (0.000)	0.002 (0.001)	-0.006 (0.000)	-0.003 (0.000)	-0.014 (0.001)	-0.004 (0.000)	-0.013 (0.000)	-	-0.019	-0.015	-0.013	-0.034	-0.007
ABY A	-0.024 (0.000)	-0.006 (0.000)	-0.019 (0.000)	-0.009 (0.000)	-0.005 (0.000)	-0.005 (0.000)	-0.016 (0.000)	-0.025 (0.000)	-	-0.017	-0.015	-0.030	-0.018
EKM J	0.010 (0.000)	-0.010 (0.014)	-0.008 (0.000)	0.013 (0.020)	-0.009 (0.001)	0.008 (0.006)	0.004 (0.000)	0.009 (0.000)	-0.010 (0.000)	-	-0.012	-0.032	-0.012
ELN J	-0.017 (0.000)	0.003 (0.000)	-0.016 (0.000)	0.004 (0.006)	0.000 (0.000)	0.003 (0.000)	-0.004 (0.000)	-0.018 (0.000)	-0.015 (0.000)	-0.005 (0.000)	-	-0.017	-0.005
ELN A	0.006 (0.000)	-0.017 (0.000)	-0.012 (0.000)	0.012 (0.000)	-0.021 (0.000)	-0.007 (0.000)	-0.001 (0.000)	-0.007 (0.000)	-0.019 (0.000)	-0.007 (0.000)	-0.008 (0.000)	-	-0.021
TKE A	0.007 (0.005)	-0.012 (0.000)	-0.011 (0.000)	-0.004 (0.008)	-0.016 (0.000)	-0.018 (0.000)	-0.006 (0.000)	-0.009 (0.000)	-0.015 (0.000)	0.007 (0.000)	-0.005 (0.000)	-0.001 (0.000)	-

Table 5: Results of the AMOVAs to determine genetic variation in 1) mtDNA (full dataset); 2) mtDNA (between juvenile and adult groups); 3) microsatellite dataset (full dataset); 4) microsatellite dataset (between juvenile and adult groups) (d.f. = degrees of freedom)

Dataset	Source of variation	d.f.	Sum of squares	Variance component	Percent variation	Fixation index	p
1. mtDNA	Among localities	7	28.12	0.0417	1.02	F_{CT} 0.010	0.007
(among localities	Among populations	5	12.38	-0.0698	-1.71	F_{SC} -0.017	0.999
and populations	Within populations	294	1205.36	4.0999	100.69	F_{ST} -0.007	0.946
within localities)	*Total*	*306*	*1245.86*	*4.0718*	*100.00*		
2. mtDNA	Between groups	1	2.32	-0.0074	-0.18	F_{CT} -0.002	0.881
(juveniles vs. adults)	Among populations	11	38.17	-0.0266	-0.66	F_{SC} -0.007	0.908
	Within populations	294	1205.36	4.0999	100.84	F_{ST} -0.008	0.946
	Total	*306*	*1245.86*	*4.0657*	*100.00*		
3. microsatellites	Among localities	7	20.487	-0.005	-0.160	F_{CT} -0.0016	0.852
	Among populations	5	16.837	0.001	0.020	F_{SC} 0.0002	0.413
(among localities	Among individuals	317	1058.142	-0.017	-0.050	F_{IS} -0.0050	0.716
and populations	Within individuals	330	1112.500	3.371	100.640	F_{IT} -0.0064	0.763
within localities)	*Total*	*659*	*2410.391*	*3.350*			
4. microsatellites	Between groups	1	3.262	0.001	0.020	F_{CT} 0.0002	0.396
(juveniles vs. adults)	Among populations	11	34.061	-0.005	-0.140	F_{SC} -0.0014	0.843
	Among individuals	317	1058.142	-0.017	-0.500	F_{IS} -0.0046	0.724
	Within individuals	330	1112.500	3.371	100.620	F_{IT} -0.0062	0.765
	Total	*659*	*2410.391*	*3.350*			

localities in the mtDNA (p = 0.007), although the magnitude of this variation (F_{CT} = 0.010) and the percent contribution to the observed variation (1.02%) were low. The AMOVA analyses to test for differences in mtDNA and microsatellite DNA among juvenile and adult populations showed no significant differences at any level, with low (negative) fixation indices (Table 5).

The Structure analysis estimated the greatest probability (negative log likelihood) was that the global sample, given the observed genotypes, was drawn from a single population (i.e. K = 1) (Appendix Figure A). The isolation-by-distance analyses showed no association between pairwise genetic differentiation and geographic distance between localities, in the mtDNA (F_{ST}) for juveniles (R^2 = 0.123, p = 0.904), adults (R^2 < 0.001, p = 0.535), or juveniles and adults combined (R^2 = 0.001, p = 0.393), or in the microsatellite data (Slatkin's linearised F_{ST}) for juveniles (R^2 = 0.018, p = 0.307), adults (R^2 = 0.125, p = 0.943), or juveniles and adults combined (R^2 = 0.147, p = 0.227).

Demographic history

The mismatch distribution (SSD = 0.004, p = 0.506) (Figure 3a) did not differ significantly from that expected under a population expansion, and the low, non-significant Harpending's (1994) raggedness index r (r = 0.005, p = 0.574) indicated a good fit of the observed data to the model. The slightly bimodal nature of the distribution reflects the mutational differences between the main clade and the long branch of the haplotype network, although this did not affect the significance of the model fit to the data. The BSP (Figure 3b) supported an expansion, occurring within the last 20 000 years.

Discussion

Population structure

Analyses of the mitochondrial control region and eleven polymorphic microsatellite loci in the nuclear genome revealed high levels of gene flow and little evidence of spatial genetic variability in white steenbras, throughout its core distribution. The low estimates of genetic divergence along the

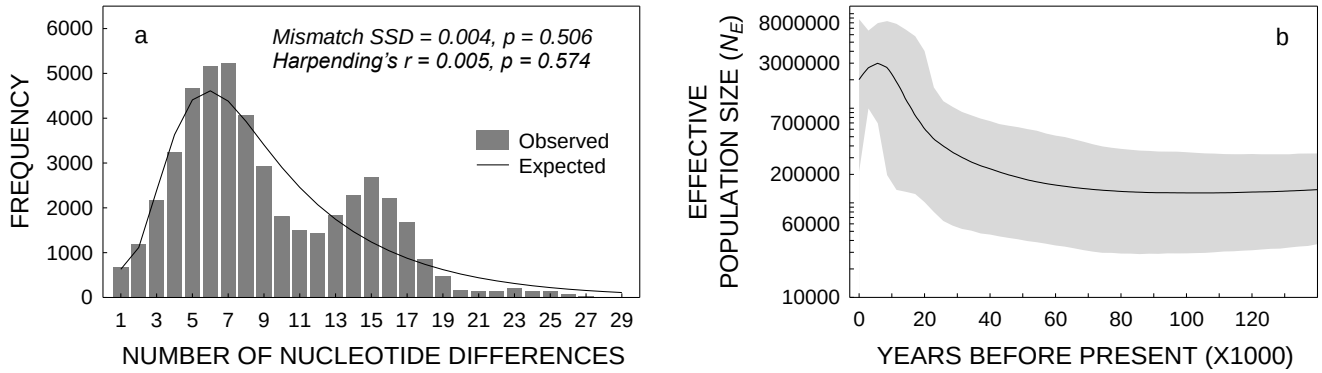

Figure 3: Demography of white steenbras based on the mitochondrial control region, showing: a) mismatch distribution graph: Observed (bars) and expected (line) mismatch distributions (under the demographic expansion model) of the frequency distribution of all pairwise nucleotide differences between haplotypes (mean = 8.15, variance = 24.05, n = 174 haplotypes); b) Bayesian skyline plot of white steenbras effective population size over time. The black line in (b) indicates the median effective population size through time and the grey band the 95 % highest posterior density (HPD).

coastline and the lack of association between haplotype genealogy and geographic location dispel the possibility of abrupt phylogeographic breaks or genetically distinct stocks in this species. The lack of isolation by distance confirms the absence of gradual genetic differentiation across the species' distribution range, and that average dispersal distances per generation are not less than this range (Slatkin 1993, Dupanloup et al. 2002). Furthermore, the Structure analysis indicated the most likely number of actual populations from which the 330 sample individuals were drawn was one, indicating that white steenbras sampled in different coastal regions were drawn from the same gene pool. The presence of a single, genetically homogenous stock and mechanisms such as larval dispersal and adult migration can improve the resilience of a species and prevent localised extinction that may occur due to unfavourable environmental conditions or through anthropogenic impacts (Bernhardt and Leslie 2013).

Similarly low genetic differentiation was exhibited by several other teleosts along the South African coastline, for example Cape stumpnose *Rhabdosargus holubi*, an endemic Sparid (Oosthuizen 2006), spotted grunter *Pomadasys commersonnii* (Klopper 2005) and dusky kob *Argyrosomus japonicus* (Mirimin et al. 2015), estuary-dependent coastal migrants, and shallow-water hake *Merluccius capensis* (von der Heyden et al. 2007). In South Africa, numerous species spawn along the East Coast, using the inshore waters of the southward flowing Agulhas Current to transport and disperse eggs and larvae to nursery grounds further south-westwards along the coast (Beckley 1993, Hutchings et al. 2002). This suggests that movement during the adult life stage (i.e. spawning migration) is an important mechanism for maintaining gene flow in these species, as many of these migratory species show a lack of population structure along the South African coastline, for example geelbek *Atractoscion aequidens* (Henriques et al. 2014) and shad *Pomatomus saltatrix* (Reid et al. 2016).

In contrast, many marine invertebrates (Evans et al. 2004, Teske et al. 2006, Nicastro et al. 2008, Zardi et al. 2011, Qhaji et al. 2015) and small, non-migratory fish species (e.g. Knysna seahorse *Hippocampus capensis* (Teske et al. 2003), bluntnose klipfish *Clinus cottoides* (von der Heyden et al.

2008) and sandgoby *Psammogobius knysnaensis* (Drost et al. 2016)) exhibit genetic divergence along the South African coastline associated with environmental transitions (Teske et al. 2011). Therefore, taxa with low levels of adult dispersal generally exhibit multiple genetically distinct populations, whereas species capable of undertaking large-scale adult movements generally exhibit higher levels of gene flow (as predicted by Avise et al. 1987).

The AMOVA analyses conducted on the mtDNA and microsatellite loci were generally in agreement, and revealed low spatial genetic variation and identified the source of greatest genetic variation as that among and within individuals, reflecting the high genetic diversity. The significant, albeit low, variability observed *among localities* in the mtDNA but not in the microsatellite loci is likely a result of the high number of unique and rare haplotypes, and may reflect historical divergence. With 174 haplotypes and sample sizes ranging from 21 to 55, it was not possible for a single locality to be entirely representative of the haplotypes identified. In a global sample exhibiting high haplotype diversity and a high proportion of unique or rare haplotypes, minor differences among localities might be expected (Excoffier et al. 1992).

Coastal connectivity and movement patterns in white steenbras

While the genetic analyses revealed high levels of gene flow in white steenbras, a study on the coastal movements of this species, based on more than 350 tag-recapture records along the South African coastline, revealed low levels of connectivity among coastal regions, and little movement between the Eastern Cape and Western Cape provinces (Bennett 2012). The author thus posed the question of whether the species is represented by separate stocks. In South Africa, white stumpnose *Rhabdosargus globiceps* is morphologically represented by separate stocks along the south and south east coasts (Griffiths et al. 2002), and carpenter *Argyrozona argyrozona* exhibit low levels of exchange between Tsitsikamma and Mossel Bay along the south coast (Figure 1) (Griffiths and Wilke 2002). Similarly, Murray et al. (2014) found evidence of limited gene flow in black musselcracker

Cymatoceps nasutus across this region. The similar locations of phylogeographic breaks in these species suggest separation by a common oceanographic feature, such as the cold-water ridge that extends offshore from the coast between Tsitsikamma and Mossel Bay (Hutchings et al. 2002, Murray et al. 2014). A similar phenomenon could present a barrier to the movement of white steenbras between the Eastern and Western Cape provinces. Numerous other phylogeographic breaks have also been observed in marine species within the white steenbras distribution, for example at Cape Point (Teske et al. 2011) and Cape Agulhas (Evans et al. 2004, Teske et al. 2006, von der Heyden et al. 2008) (Figure 1). However, the absence of isolation by distance and the lack of association between genealogy and geographic locality in the haplotype network indicate no regional clustering of haplotypes throughout the white steenbras core distribution. Thus, gene flow in white steenbras appears not to be affected by geographic or oceanic features identified as barriers to gene flow in other South African marine taxa.

Banded goby *Caffrogobius caffer*, a cryptic gobiid, and red roman, a territorial reef-dwelling sparid, are southern African endemics with similar distribution ranges to white steenbras, and also exhibit low levels of genetic differentiation among coastal regions (Neethling et al. 2008, Teske et al. 2010). The similarly high gene flow throughout their ranges, despite low levels of adult movement and different life history strategies, is likely a result of dispersal during long larval phases (Neethling et al. 2008, Teske et al. 2010). Considering the generally limited coastal movements of white steenbras (Bennett 2012), the high levels of gene flow observed in this species throughout its distribution are possibly also facilitated by larval dispersal. This highlights the importance of larval dispersal by ocean currents as a mechanism for maintaining gene flow along the South African coastline (Beckley 1993, Hutchings et al. 2002).

Demographic history

The star-like topology of the white steenbras haplotype network, with few common and many rare haplotypes, has been observed in the mitochondrial control regions of numerous marine fish species, for example red snapper *Lutjanus campechanus* (Camper et al. 1993), red drum *Sciaenops ocellatus* (Gold et al. 1993) and white seabream *Diplodus sargus* (Bargelloni et al. 2005), and indicates a population expansion (Slatkin and Hudson 1991, Fu and Li 1993). This is congruent with the population expansion revealed by the white steenbras mismatch distribution and the BSP.

Population expansions and bottlenecks are likely a result of climatic oscillations and major glaciations during the Pleistocene period (Grant and Bowen 1998), which would have shaped the contemporary population structures of most extant species (Excoffier 2004). The BSP indicated that the white steenbras population expansion occurred within the last approximately 20 000 years. Population expansions during this period have been identified in the demographic histories of several other South African marine taxa, such as geelbek (c 24 000 years ago, Henriques et al. 2014), shallow-water hake (17 100 to 7 500 years ago, von der Heyden et al. 2007), spiny lobster *Palinurus delagoae* (13 000 to 9 000 years ago, Gopal et al. 2006), black musselcracker *Cymatoceps nasutus* (10 000 to 4 800 years ago, Murray et al. 2014) and deep-water hake *Merluccius paradoxus* (6 000 years ago, von der Heyden et al. 2010). While estimates of time since expansion are characterised by broad confidence intervals, and should thus be treated with caution (Henriques et al. 2014), the concordance among South African taxa, from crustaceans to teleosts, provides evidence that the population structures of marine organisms within this region have been shaped by similar historical environmental changes and biogeographical factors. Population expansions occurring within the last 20 000 years likely resulted from the glacial Holocene temperature increase of about 5.5°C and the increased habitat associated with the increase in sea-level (Sachs et al. 2001, von der Heyden et al. 2010), since the end of the last glaciation period approximately 19 000 years ago (Sachs et al. 2001).

The long branch of the white steenbras haplotype network could represent the more recent part of the genealogy (Fu and Li 1993), with the high number of rare haplotypes representing new mutational derivatives of the common haplotypes (Billington and Hebert 1991), reflecting the demographic expansion (Harpending and Rogers 2000). A similar haplotype network, with a long external branch and high numbers of mutations, was observed for red roman, which the authors proposed may have been an artefact of a separate genetic lineage (Teske et al. 2010). The long branch of the haplotype network could therefore be interpreted as evidence of a separate genetic lineage in white steenbras. However, there was no association between genealogy and geographic location within this long branch of the network, therefore negating the possibility of extant allopatric lineages in white steenbras. This may thus be indicative of secondary contact between, or a relic of, previously genetically differentiated allopatric lineages in this species.

Extreme levels of haplotype and, to a lesser extent, nucleotide diversity for the mitochondrial control region are common features in many marine fishes (Von der Heyden et al. 2010), including sparids off the South African coast (Teske et al. 2010; Murray et al. 2014; Duncan et al. 2015), possibly as a consequence of large effective population sizes and the high mutation rate of the marker. Despite concerns around the potential impact of this variability on analyses, the marker has proved effective in providing evidence for panmixia (Teske et al. 2010; Duncan et al., 2015; Agiulli et al. 2016) and varying degrees of structure among populations (González-Wangüemert et al. 2011; Murray et al. 2014) in sparids.

Considerations for management and future research

In the absence of morphological, meristic or biological differences to delineate discrete stocks, white steenbras has historically been managed as a single stock. The current study confirms that white steenbras is characterised by high levels of gene flow and low, non-significant levels of genetic

differentiation throughout its distribution. Therefore, the species should continue to be managed as a single stock.

Differential fishery regulations are often applied in different areas, for example commercial harvesting of white steenbras was historically permitted in certain areas of the Western Cape Province only (Penney 1991, Bennett 1993a). For species that are genetically homogenous throughout their distribution ranges, excessive local harvest in one area, particularly in spawning or aggregation areas, may affect the persistence of the stock and concomitantly predator/prey relationships and resource users dependent on the resource in other areas. Furthermore, the number of migrants per generation required to mask inherent stock structure is low, and the actual number per generation from one area may be insufficient to rebuild a depleted population in another (Hauser and Carvalho 2008). This could allow independent responses of populations in different areas to different demographic perturbations (Hastings 1993), such as localised overexploitation. Therefore, management of a fishery species should consider the ecological and fishery implications of localised overexploitation, even if the species is represented by a single genetic unit.

While the results of the current study have confirmed high levels of gene flow in white steenbras throughout its core distribution, genetic analyses alone cannot quantify the level of adult movement within a species, nor empirically confirm whether adults undertake annual spawning migrations, and movement studies based on dart tagging have indicated low levels of longshore movement in this species (Bennett 2012). Comprehensive management of a species thus requires both information on genetic stock structure and an understanding of the movement behaviour at different life history stages, which will need to be determined for white steenbras by dedicated, long-term assessments of movement and migration.

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Appendix I

Appendix tables

Table A: Tests for pairwise linkage disequilibrium in the global sample, for all pairs of loci, across all eight localities (underlined values indicate significant differences, after Bonferroni correction)

Locus	Llt005	LLt006	PB106	LLt014	Llt011	LLt007	PBt018	LLtr004	LLt020	LLt002
LLt006	0.429									
PB106	0.000	0.649								
LLt014	0.224	0.194	0.051							
Llt011	0.000	0.183	0.000	0.734						
LLt007	0.756	0.481	0.390	0.062	0.761					
PBt018	0.877	0.281	0.633	0.976	0.222	0.591				
LLtr004	0.306	0.766	0.343	0.845	0.045	0.106	0.222			
LLt020	0.348	0.265	0.355	0.111	0.962	0.134	0.149	0.914		
LLt002	0.323	0.207	0.447	0.884	0.745	0.164	0.318	0.650	0.001	
PBt003	0.301	0.887	0.567	0.585	0.077	0.927	0.765	0.370	0.000	0.001

Table B: Summary statistics for 11 microsatellite loci, showing total numbers of alleles per locus (*N*), mean (± SD) number of alleles (N_A) and mean (± SD) allelic richness (A_R) averaged across all eight localities, observed and expected heterozygosities (H_O and H_E), inbreeding coefficients (F_{IS}) and Hardy-Weinberg exact test p-values (*HWE*)

Locus	*N*	N_A		A_R		H_O	H_E	F_{IS}	*HWE*
LLt005	6	5.00	(± 0.00)	4.82	(± 0.12)	0.66	0.70	0.07	0.55
LLt006	14	9.88	(± 0.35)	8.72	(± 0.43)	0.82	0.87	0.06	0.08
PB106	13	10.38	(± 0.92)	8.94	(± 0.59)	0.83	0.87	0.04	0.71
LLt014	11	8.50	(± 0.93)	7.58	(± 0.37)	0.83	0.85	0.03	0.35
LLt011	14	11.25	(± 1.49)	9.27	(± 0.74)	0.82	0.83	0.01	0.92
LLt007	15	10.00	(± 1.51)	8.23	(± 0.59)	0.83	0.83	0.00	0.23
PBt018	5	4.00	(± 0.76)	3.32	(± 0.42)	0.40	0.42	0.03	0.12
LLtr004	8	6.25	(± 0.46)	5.61	(± 0.26)	0.74	0.76	0.03	0.56
LLt020	19	13.38	(± 1.69)	11.13	(± 0.86)	0.91	0.90	-0.01	0.27
LLt002	14	11.50	(± 0.93)	9.95	(± 0.48)	0.89	0.88	-0.01	0.44
PBt003	25	15.75	(± 2.76)	12.65	(± 0.84)	0.91	0.91	0.00	0.18

Table C: P-values for pairwise exact tests for genetic differentiation between localities (n = 8), for 720-base pair mtDNA sequences (above diagonal), and for 11 microsatellite loci (below diagonal); (underlined values indicate significant differences)

	LBN	FBY	DEH	KNY	ABY	EKM	ELN	TKE
LBN	-	0.653	0.530	0.767	<u>0.040</u>	1.000	<u>0.043</u>	0.674
FBY	0.569	-	0.683	0.996	0.303	0.370	0.801	0.474
DEH	1.000	0.512	-	0.579	0.213	0.248	<u>0.041</u>	0.116
KNY	1.000	0.483	1.000	-	0.652	0.715	0.292	0.547
ABY	1.000	0.465	1.000	1.000	-	0.245	0.434	0.479
EKM	0.503	0.242	0.508	0.488	0.481	-	0.147	0.874
ELN	1.000	0.485	1.000	1.000	1.000	0.529	-	0.121
TKE	0.471	0.331	0.568	0.567	0.543	0.264	0.497	-

Table D: P-values for pairwise exact tests for genetic differentiation between populations (n = 13), for 720-base pair mtDNA sequences (above diagonal), and for 11 microsatellite loci (below diagonal); (underlined values indicate significant differences)

	LBN A	FBY J	FBY A	DEH A	DEH J	KNY J	KNY A	ABY J	ABY A	EKM J	ELN J	ELN A	TKE A
LBN A	-	1.000	0.338	0.864	0.576	1.000	0.675	0.206	0.246	1.000	0.558	0.371	0.675
FBY J	1.000	-	0.169	0.178	0.799	1.000	0.991	0.643	0.842	0.970	0.964	0.944	0.996
FBY A	0.500	0.503	-	1.000	0.063	0.693	0.225	0.194	0.224	0.073	0.758	0.563	<u>0.035</u>
DEH A	1.000	1.000	0.492	-	0.111	0.908	0.323	0.205	0.108	0.267	0.261	0.198	0.069
DEH J	1.000	1.000	0.523	1.000	-	0.363	0.538	0.606	0.435	0.584	0.677	0.128	0.596
KNY J	1.000	1.000	0.505	1.000	1.000	-	0.932	0.865	0.680	0.950	0.882	0.842	0.962
KNY A	1.000	1.000	0.504	1.000	1.000	1.000	-	0.671	0.816	0.785	0.507	0.782	0.406
ABY J	1.000	1.000	0.507	1.000	1.000	1.000	1.000	-	0.767	0.724	0.776	0.745	0.756
ABY A	1.000	1.000	0.503	1.000	1.000	1.000	1.000	1.000	-	0.655	0.813	0.598	0.734
EKM J	0.502	0.547	0.243	0.492	0.522	0.533	0.537	0.516	0.513	-	0.828	0.518	0.878
ELN J	1.000	1.000	0.468	1.000	1.000	1.000	1.000	1.000	1.000	0.535	-	0.901	0.633
ELN A	1.000	1.000	0.570	1.000	1.000	1.000	1.000	1.000	1.000	0.586	1.000	-	0.761
TKE A	0.485	0.503	0.231	0.467	0.482	0.490	0.449	0.490	0.475	0.261	0.496	0.541	-

Appendix figure legends

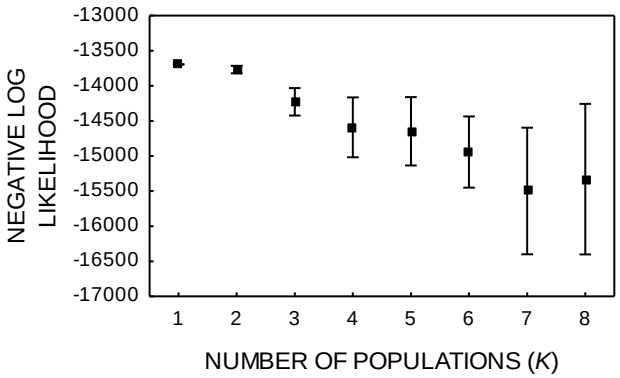

Figure A: Inference of white steenbras population structure in the microsatellite DNA, based on mean (± SD) probabilities (negative log likelihood), given the observed genotypes, of the global sample having being drawn from different numbers of putative populations (*K*).