Report on Hake Genetics

Stock assessment workshop
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Current state of knowledge:

In an attempt to define genetic stock structure in *M. paradoxus* and *M. capensis*, two research projects were initiated in two separate laboratories (at Stellenbosch and Pretoria Universities). The first involved mitochondrial DNA analyses performed at Stellenbosch; this study aimed to provide a first indication of population substructure in both species. Simultaneously, DNA of the same specimens used in the mtDNA analyses, were provided to the research group in Pretoria to perform microsatellite analyses on both species using species specific microsatellite primers designed by the laboratory in Pretoria. The latter approach is generally accepted to provide a more accurate/sensitive method to define differences in the distribution of genetic alleles. Both these research projects provided valuable information that can be used as a baseline for future investigations and a short summary of the outcome of each of these studies are provided below.

Mitochondrial DNA stock structure results: The initial study utilized 644 hake sequences derived from the variable 5' region of the mtDNA control (311 *M. capensis* and 333 *M. paradoxus*). Samples were obtained between Lüderitz (southern Namibia) to south of Cape Point (South Africa). *Merluccius capensis* showed a high level of haplotypic diversity (107 haplotypes) while only 8 haplotypes were recovered for *M. paradoxus*. No evidence of mtDNA population structure could be found for *M. capensis*, however significant genetic differentiation was present in adult *M. paradoxus* (>3 years old). No significant population genetic structuring for detected for fish <3 years (~30cm).Two populations were identified which corresponded to Namibian and South African 'populations' (to the north and south of the Orange River). There was also significant structure along the South African coast.

To establish the temporal stability of the *M. paradoxus* pattern a total of 1013 adult *M. paradoxus* (longer than 30cm), sampled over a period of three years were analysed (in addition to 134 adult fish taken from the 2005 study, 267 additional fish were included for the same period, 412 specimens were sampled in 2006 and 334 in 2007). For 2005, we sampled fish from southern Namibia to the western Agulhas Bank, in 2006 we included the most northern distribution on the border with Angola, but none from southern Namibia. For 2007, samples ranged from southern Namibia to the western Agulhas Bank. Analyses of population structure revealed a similar northern (Namibian) southern (South African) differentiation but the boundaries were not as exact as in the first study. Notably, only two common haplotypes represent the majority of the sampling effort (Fig 1).

In 2005, Namibia was significantly differentiated to SA1, SA2 and SA3 (F_{st} = 0.04-0.08, P < 0.05), but not SA4 (Fig 1). In 2006, the Cunene samples (no samples from southern Namibia were available), showed significant differentiation between SA3 and SA4 (F_{st} = 0.13-0.14, P < 0.03), but none of the other regions. In 2007, SA1 and SA3 were genetically differentiated. Combining the three single year data sets recovered significant structure between the Cunene River samples and all other sampling areas, except for southern Namibia (F_{st} = 0.13-0.19, P < 0.04), between southern Namibian samples and SA1, SA2 and SA4 (F_{st} = 0.02-0.04, P < 0.05) and between SA1 and SA3 (F_{st} = 0.009, P < 0.05). No significant inter-annual differentiation was present.

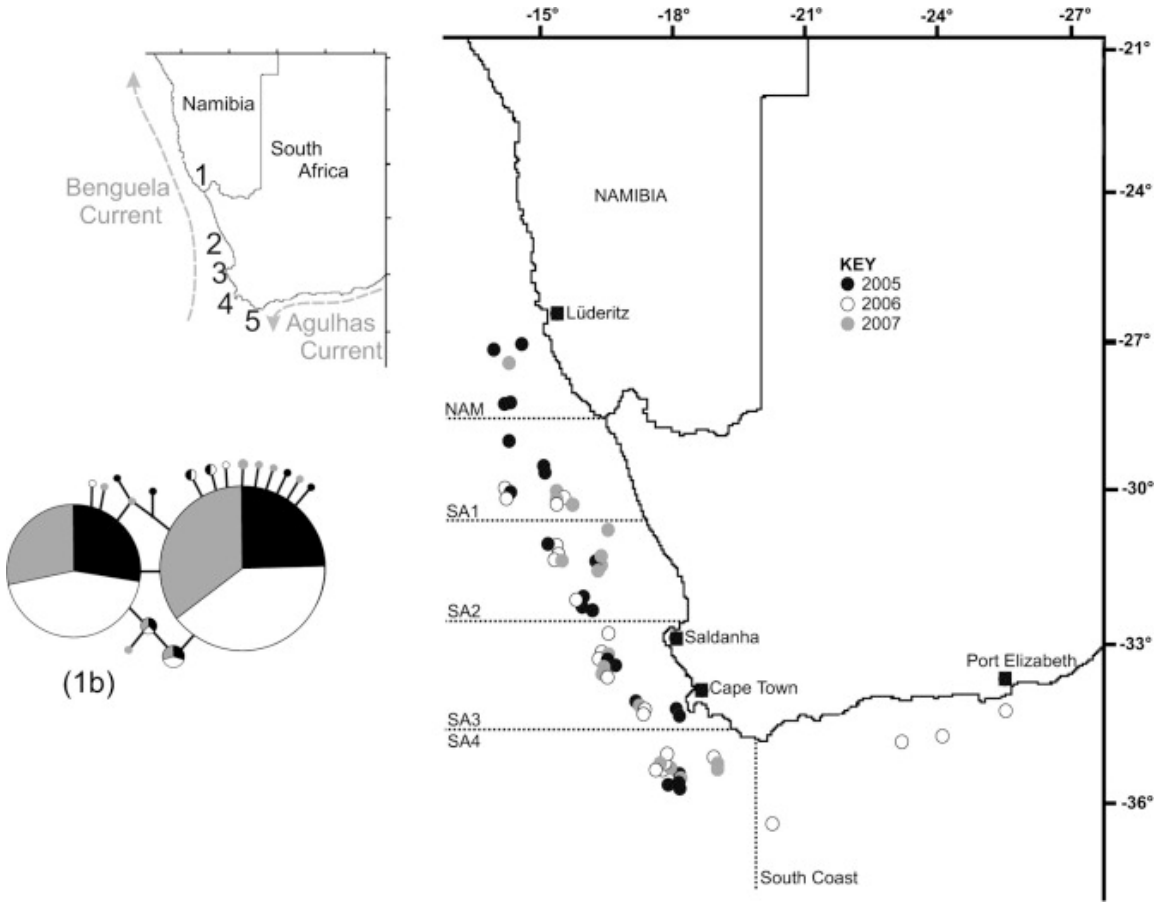

Fig. 1. Figure taken directly from von der Heyden et al. 2010 showing sampling areas of *M. paradoxus.* The inset shows the two main southern African current systems. 1 = Orange River, the border between Namibia and South Africa, 2 = Hondeklip Bay, 3 = St. Helena Bay, 4 = Cape Point, 5 = Cape Agulhas. Main map shows areas sampled for the period 2005–2007 (see key). Statistical parsimony haplotype network showing the two dominant haplotypes of 1013 *M. paradoxus* sampled. The size of the circles represents the relative frequency of individuals defining each haplotype; haplotypes are shaded according to year sampled.

Microsatellite stock structure results: Since *M. paradoxus* showed mtDNA structure, and this species appears to be the older species within the southern African system, microsatellites were developed for *M. paradoxus.* Eight loci (six newly developed tetranucleotide loci and two European hake dinucleotide

loci) were genotyped in 209 *M. paradoxus* (57 trawls) and 222 *M. capensis* (24 trawls) adults collected during 2005 and 2006. Based on Bayesian STRUCTURE analyses the preliminary report indicated three likely stocks in *M. capensis* and one in *M. paradoxus*. The finding is in sharp contrast to the mtDNA results. Small sample sizes per sampling trawl precluded a definitive outcome. Nevertheless, to determine regions where genetic patterns change abruptly, the software BARRIER was used. The geographic position of the significant genetic barriers in *M. capensis* was inferred to be (1) south of the Orange River on the west coast and (2) in the vicinity of Hermanus on the South African south coast (Fig 2). In general *M. paradoxus* showed higher levels of genetic variability than *M. capensis*, (14.75 alleles for *M. paradoxus* versus 11.13 *M. capensis*) and higher levels of heterozygosity (average expected heterozygosity 0.72 versus 0.61). Deviations from Hardy-Weinberg expectations could be due to null-alleles or to recent demographic changes (as suggested by the mtDNA data).

Figure 2: Taken directly from Bloomer et al. 2009: Two barriers inferred across the sampling of *M. capensis* genotypes off the coasts of South Africa and Namibia; the locations of the two barriers are shown.

Future Research direction: The present information given above offer a first step in providing genetic data useful for stock assessment in two hake species along the South African coastline. The data, however, suffer from several deficiencies and most of this relate to appropriate sampling and or the insufficient resolution of the genetic markers used. To rectify the problem we aim to sample 20-40 stations (20 adult fish from each species covering the entire range of the species) over three consecutive seasons (March/April each

year for three years). A mtDNA control region data set will be generated to test previous hypotheses that were inconclusive due to insufficient temporal/spatial sampling. For the microsatellites, it is recommended that at least 10 to 20 polymorphic loci is needed for the detection of stock differentiation. In addition to the loci already developed, we will utilize new generation sequencing techniques to generate an additional 50+ polymorphic microsatellites useful for screening both hake species. It is anticipated that sampling will commence early in 2012 and final results will be available by the end of 2014. The team involved includes: M Lipinski, R Leslie, P Bloomer, C Matthee and S von der Heyden.

Data Sources:

1) von der Heyden S., Lipinski M.R. and Matthee C.A. 2007. Mitochondrial DNA analyses of cape hakes reveal an expanding, pan-mictic population for *Merluccius capensis* and population structuring for mature fish in *Merluccius paradoxus.* Molecular Phylogenetics and Evolution 42:517-527.
2) von der Heyden S., Lipinski M.R., Matthee C.A. 2007. Species-specific genetic markers for identification of early life history stages of Cape hakes, *Merluccius capensis* and *M. paradoxus* in the southern Benguela Current. Journal of Fish Biology 70 (Supplement B):262-256.
3) Bloomer P, Dos Santos SMR, Oosthuizen CJ, Hoareau TB & Klopper AW 2009. “Development of microsatellite markers and screening of microsatellite locus variation in Cape hakes, *Merluccius paradoxus* and *M. capensis*, from the Namibian and South African coasts” Benefit Report
4) von der Heyden, S., Lipinski, M., Matthee C.A. 2010. Remarkably low mtDNA control region diversity in an abundant demersal fish. Molecular Phylogenetics and Evolution 55: 1183-1188.