# FEASIBILITY STUDY INTO THE APPLICATION OF GENETIC TECHNIQUES FOR DETERMINING FISH STOCK IDENTITY OF TRANSBOUNDARY POPULATIONS IN THE BCLME REGION

Project LMR/CF/03/04

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## EXECUTIVE SUMMARY

The BCLME programme is a multi-sectoral regional initiative by Angola, Namibia and South Africa whose objective is to facilitate the integrated management, sustainable development and protection of its unique eastern boundary upwelling ecosystem. The BCLME Strategic Action Plan calls for harmonising management of shared marine resource stocks. Most commercially harvested resources are shared between two or three BCLME countries. Thus overexploitation in one country can cause depletion in another country or in the region as a whole. The identity of transboundary stocks is in most cases unknown and this has important implications for management.

This report reviews the potential application of genetic techniques for identification of stocks. From the literature consulted, interviews with fisheries scientists (MCM, NatMIRC and IIM) and responses from local and international geneticists, it is clear that genetic analyses can make a contribution towards the identification of transboundary populations in the BCLME region. Future genetic work should however be carefully prioritised and planned. The latter is especially relevant to ensure adequate sampling that would be crucial for obtaining conclusive results. It is also important that fisheries scientists, managers and geneticists work towards the same goal, i.e. understand and identify stocks using only one working definition of a stock.

Several species with potential transboundary populations or with potential separate stocks have been identified as priorities for genetic study, although the list as provided in this report may not be exhaustive. It is recommended that a pilot investigation into a number of species representative of different life histories should be conducted to guide a larger research initiative in the region. In particular, such a pilot investigation should address the effects of current boundaries and strong upwelling zones on genetic differentiation within living marine resources. The pilot study should also address the feasibility of sampling at spawning grounds as well as sampling of different cohorts to allow determination of the temporal stability of patterns of genetic variation. The pilot study should additionally focus on utilising a range of statistical methods that have recently been developed for analysis of genetic data, specifically with reference to defining boundaries between stocks and to estimate gene flow rates.

## TERMS OF REFERENCE

**Objectives:**

The specific objectives of project LMR/CF/03/04 are to critically review the work on stock identity / separation which has been attempted in the BCLME region **(section 2)**, to assess the applicability of the previous techniques used (and possible new techniques) as tool for management of stocks which straddle or migrate across national boundaries between countries **(section 1.2)**, and to assess human resources and training needs for the application of genetic techniques in the region **(sections 4 & 5)**.

**Scope:**

The contractee will be expected *inter alia* to undertake the following:

- Review results of previous genetic studies conducted to identify transboundary stocks of exploited marine resources in the BCLME region (and elsewhere in southern Africa) **(section 2)**
- Identify other exploited and non-exploited resources in the BCLME mandate that might require application of genetic stock level analysis **(section 2, Appendix I)**
- Identify suitable capacity (or lack thereof) in the national (marine) research institutions (government and academic) and facilities to carry out stock level genetic analyses necessary in BCLME region and potential international partners **(section 4, Appendix II)**
- Identify training needs / opportunities for genetics in the BCLME countries **(section 5)**

**Outputs:**

A comprehensive report to include the following:

- Review of previous genetic studies conducted to identify transboundary stocks of exploited marine resources in the BCLME region (and elsewhere in southern Africa) **(section 2, see section 2.x.2 for each species "Genetics done")**
- List other transboundary exploited and non-exploited resources in the BCLME mandate that might require application of genetic stock level analysis **(section 2, Appendix I, section 3)**
- Capacity (or lack thereof) in the national (marine) research institutions (government and academic) and facilities to carry out stock level genetic analyses necessary in BCLME region and potential international partners **(section 4, 6)**
- Training needs / opportunities for genetics in marine science **(section 5)**
- Recommendations on potential applications of genetic analyses on (sub-) populations of the following exploited resources: hake, sardine, linefish, red crab, dentex, rock lobster, snoek, sardinella (transboundary with Guinea Current LME) **(section 1.4.2, 2, 6, Appendix III)**
- Recommendations regarding the appropriate genetic markers that would need to be developed or could be acquired for the analyses **(sections 1.2, 2 & 6, Appendix III)**
- Draft terms of reference / specification for future work (project) in respect of genetic techniques to address stock identity issues in the BCLME **(section 7)**

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**LIST OF ACRONYMS AND SYMBOLS**

AFLP – Amplified Fragment Length Polymorphism

BCLME – Benguela Current Large Marine Ecosystem

ICSEAF – International Commission for the Southeast Atlantic Fisheries

IIM - Instituto de Investigação Marinha

Ma – Million years ago

MCM – Marine and Coastal Management

MtDNA – mitochondrial DNA

NatMIRC – National Marine Information and Research Centre

RAPD – Random Amplified Polymorphic DNA

RFLP – Restriction Fragment Length Polymorphism

SSCP – Single Strand Conformation Polymorphism

VNTR – Variable Number of Tandem Repeat

**GLOSSARY**

Genetic distance: A quantitative estimate of how genetically divergent two entities (individuals, populations or taxa) are. This measure can be applied to allozyme data (Nei's D; the net number of codon substitutions per locus that have accumulated since two populations separated) or DNA restriction site or sequence data (the number of base substitutions per nucleotide).

Heterozygosity (H): The mean percentage of loci heterozygous per individual (for most allozyme surveys H-values are in the range 0.0-0.2).

$F_{ST}$: Statistical description of population structure; the variance of allele frequencies among populations. Generally there is a negative relationship between $F_{ST}$ and dispersal ability. Values range between 0 and 1; the closer to 0 the less the genetic structuring.

Haplotype: Although this term has alternative meanings, in this report it is used to refer to unique maternally inherited mtDNA alleles.

Haplotype diversity (h): The number and frequency of alleles at a locus. The value ranges from 0 to 1 and indicates the probability that two individuals drawn from a population at random will have different alleles. Values closer to 0 indicate greater similarity.

Nucleotide diversity ($\pi$): A weighted sequence divergence between individuals in a population, regardless of the number of haplotypes. Within populations, values are typically between 0.0005 and 0.020 (i.e. less than 2%).

VNTR: A class of nuclear DNA dispersed tandem repeats. Originally loci with a repeated core sequence of 10-15 bp were used in multilocus DNA fingerprinting. Microsatellites are shorter VNTR's with repeat units usually consisting of 2-6 bases; these markers are common in most genomes and are highly variable.

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# 1. BACKGROUND REVIEW

## 1.1 What is a stock?

It was not the aim of the present report to review the stock concept. For recent comprehensive reviews refer to Carvalho and Hauser (1998), Hauser and Ward (1998), Begg and Waldman (1999), Begg *et al.* (1999), Booke (1999), Secor (1999), Waldman (1999), Ward (2000) and references therein. For the purposes of this report it is however very important to realise the potential conflict if managers and geneticists define stocks differently. A widely applied definition of a biological stock was formulated by Ihssen *et al.* (1981): " a stock is an intraspecific group of randomly mating individuals with temporal and spatial integrity". By contrast genetic stocks are defined on the basis of reproductive isolation (Ovenden 1990). While the latter is useful in identifying historically isolated lineages, such "pure" genetic definitions are not of value from a short-term management perspective where some degree of genetic exchange may not prevent the maintenance of spatial and temporal integrity of biological stocks. In addition, many of the above authors agreed that a combination of approaches to the identification of stocks would probably always lead to more informed management decisions. Therefore, while the focus of this report is on the potential application of genetic methods for stock identification, a combination of genetic data with phenotypic (morphometrics, parasites, otolith microchemistry, population parameters) as well as ecological (demography, migration, oceanographic features) information is **recommended** (6.1).

## 1.2 Genetic markers

Many different genetic markers are available for the study of genetic differentiation within and between populations of living marine resources. Each method typically has its advantages and disadvantages and for any particular question, genetic markers should be chosen with care so as to maximise scarce resources. For recent reviews see Hillis and Moritz (1990), Avise (1994), Carvalho and Hauser (1998), Hauser and Ward (1998), Sweijd *et al.* (1999), amongst others. In summary however it should be noted that allozymes represents one of the most extensive comparative data bases and this technique can be widely applied to questions related to genetic identity, parentage, relationships among conspecific populations (the focus of this report) as well as relationships at shallow to intermediate taxonomic levels (Table 1). One of the potential disadvantages of allozymes for understanding the history of connectedness among populations is that loci may be under selection. In such cases, this could however act as another marker to understand processes

involved in population structuring. DNA sequencing has become far more accessible than in the not too distant past and can also give insight at all the levels as indicated for allozymes (Table 1). Furthermore, neutral loci can specifically be chosen for study thereby excluding the confounding effects of selection. An additional advantage is the potential for analysis of archived material such as scales and otoliths (see for example Hutchinson *et al.* 1999). Mitochondrial DNA (mtDNA) analysis provides useful data in cases where sex biases can affect population structuring and due to the rapid rate of evolution recorded in this cytoplasmic genome, it can be used to retrace recent connections over a variety of geographic scales. One of the current markers of choice in stock level genetic analysis is the analysis of Variable Number of Tandem Repeat (VNTR) loci such as microsatellites (Table 1, Appendix III). These short, tandem repeat DNA sequences are highly variable and can provide better resolution than allozymes and mtDNA for questions relevant to short term management and can also be used for archived material (see for example Hauser *et al.* 2002).

**TABLE 1 Summary of the genetic tools available and their potential applications ('+++' indicates the highest potential resolution; '-' indicates that the marker is not appropriate at a particular level)**

| Technique | Allozyme | Microsatellite | mtDNA |
|---|---|---|---|
| **Individual identity** | ++ | +++ | + |
| **Relatedness** | ++ | +++ | + |
| **Population genetics** | +++ | +++ | ++ |
| **Stocks** | ++ | +++ | +++ |
| **Between closely related species** | ++ | + | +++ |
| **Species identity** | ++ | + | +++ |
| **Deep phylogenetic relationships** | + | - | +++ |

## 1.3 International trends in the application of genetic techniques in stock identification

Two recent reviews summarise the most important considerations for the application of genetic techniques in stock identification (Hauser and Ward 1998, Ward 2000). A complex interaction between physical factors in their environment, life history characteristics and population genetic processes affect genetic variability and differentiation in marine taxa (Figure 1, Hauser and Ward 1998) and all of these should be taken into account when addressing stock level questions using genetic techniques.

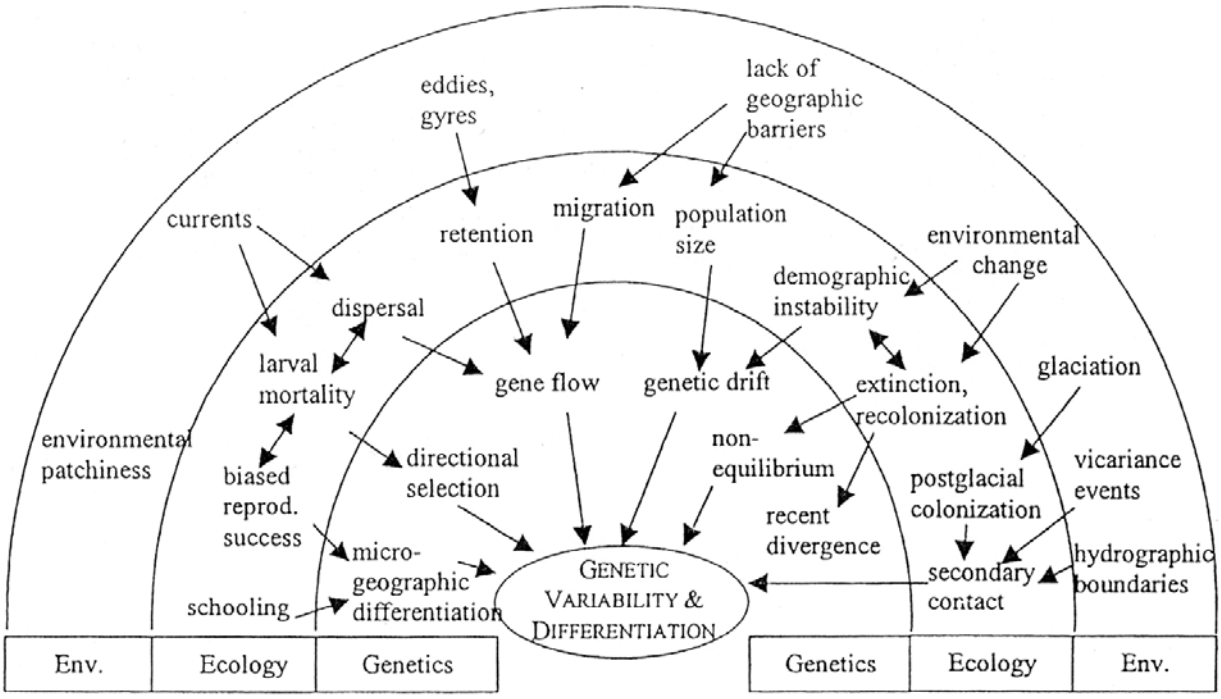



**Figure 1** Genetic differentiation within pelagic fish (and other marine organisms) are influenced by a complex interaction between physical characteristics of the marine environment, ecological factors unique to particular life histories and the degree to which these processes increase or decrease genetic variability and differentiation (from Hauser and Ward 1998).

One of the best examples of the contributions of many researchers to understanding the population structuring within a single species over various temporal and spatial scales is research relating to the Atlantic cod *Gadus morhua*. One study will be singled out to illustrate the scale at which structuring within this species has been addressed. Cod is widespread, abundant and migratory and part of its range includes an area of 3000 km in the northwest Atlantic. Ruzzante *et al.* (2000) sampled over the period 1992 to 1995 and used five microsatellite loci to analyse 1300 cod from 14 populations representing offshore and inshore areas as well as different age classes. They found differences on continental shelf scale where regions are separated by trenches and channels but also at spawning bank scale in relation to oceanographic features and spatiotemporal distribution of spawning.

## 1.4 Sampling strategies

### *1.4.1 International guidelines for sampling design*

Due to large population sizes and high levels of gene flow, differentiation in many species will be low and the challenge to molecular geneticists is therefore to differentiate between signal and noise. Adequate sampling is consequently of the utmost importance in addressing these issues (Ward 2000). Carvalho and Hauser (1998) highlighted important considerations for sampling design. The first consideration is the particular question asked, i.e. whether the study pertains to relatedness, population structuring or species identification. The latter will determine the best choice of genetic marker or combination of markers (Table 1) as well as the sample sizes required for adequate statistical evaluation of results. Additional considerations include environmental features such as hydrography, bathymetry and biotic factors such as food availability and predation. As much biological information as possible should be used to ensure adequate sampling: Important information include the geographic distribution of the species, social structure (schooling or lekking for example), dispersal ability, fidelity to natal or spawning sites, life history stage (larval/feeding/spawning) most likely to show differentiation or most likely to represent a stage where mixing will take place. If consideration is not given to biological data, patterns that are observed may be misinterpreted. Another important consideration that was previously often ignored is the stability of any patterns that may be observed and therefore sampling over several years or spawning events would be required. This is especially relevant due to biased reproductive success. Very few studies conducted on BCLME resources in the past had adequate temporal and spatial sampling to yield conclusive results regarding stock identity.

### *1.4.2 Recommendations for sampling*

With the above background in mind, several unique features of the Benguela system (Figure 2) in addition to specific life history characteristics of the resources, of which some aspects are outlined in section 2, should be taken into consideration for future application of genetic stock identification of exploited resources.

1. It is **recommended** that for each species, available biological data, catch trends and specific management issues should be plotted against the background of the dominant physical features of the Benguela (Fig. 2). Such visualizations of the relevant data will guide sampling strategies, will identify overlap in life history traits between taxonomically unrelated taxa and focus short term research efforts to determine the feasibility of obtaining conclusive results regarding the genetic status of transboundary populations. Such a review for each species will also identify gaps in our understanding of life history traits and guide research in that regard.

2. Sampling is **recommended** across current boundaries (such as the Benguela/Agulhas and Benguela/Angola boundaries), on either side of the area of strongest upwelling at Luderitz and at spawning sites (Figure 2).

3. The value of archived biological material has already become apparent in many studies. It is therefore **recommended** that all fisheries surveys should be utilized for the collection of specimens that can be deposited in either a dedicated fisheries tissue bank facility or the existing fish collection of the South African Institute for Aquatic Biodiversity (SAIAB) in Grahamstown. For each specimen collected the following should be recorded: Accurate location, weight, length, sex, information on samples taken (otolith, scales, muscle), etc. A database including all this information should be maintained by the tissue bank and genetic data should also be linked to each sample record. Collection of voucher specimens for morphological analysis should also be done.

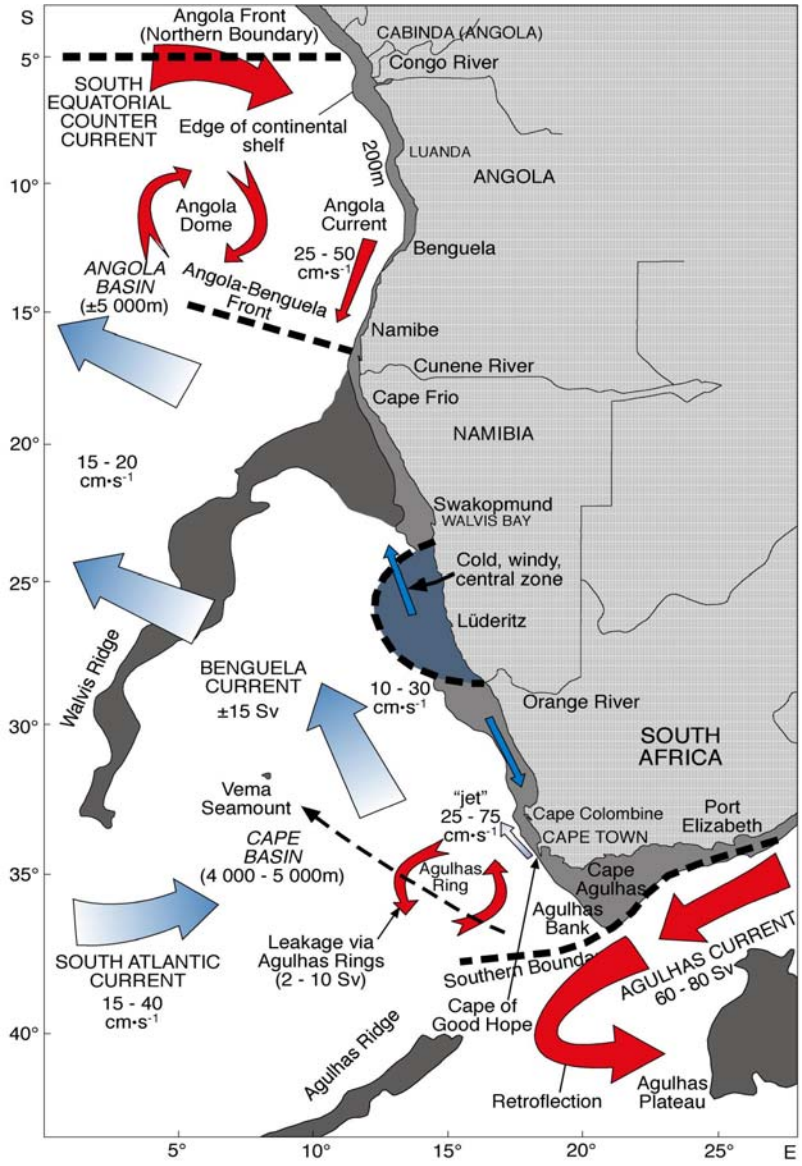



**Figure 2** Major physical features of the Benguela Current (from, BCLME Transboundary Diagnostic Analysis Report) that have a major impact on spawning, recruitment and therefore gene flow patterns in marine species. These features should specifically be taken into consideration when planning sampling strategies for identification of transboundary populations based on genetic techniques.

## 2. THE PAST APPLICATION OF GENETIC TECHNIQUES IN SOUTHERN AFRICA AND IDENTIFICATION OF EXPLOITED RESOURCES IN THE BCLME THAT REQUIRE APPLICATION OF GENETIC STOCK LEVEL ANALYSIS

***This section addresses the following TOR's:***

- ***Review of previous genetic studies conducted to identify transboundary stocks of exploited marine resources in the BCLME region (and elsewhere in southern Africa)***
- ***List other transboundary exploited and non-exploited resources in the BCLME mandate that might require application of genetic stock level analysis***
- ***Recommendations on potential applications of genetic analyses on (sub-) populations of the following exploited resources: hake, sardine, linefish, red crab, dentex, rock lobster, snoek, sardinella (transboundary with Guinea Current LME)***

Over the past 20 years genetic studies have been conducted using a variety of techniques on a number of southern African species in relation to taxonomic questions, species identity and forensics, as well as to stock identification. With respect to the latter, however, attention to southern African resources lags far behind international research efforts.

This section lists most of the exploited transboundary species that were identified by MCM, NatMIRC and IIM staff as species in need of genetic stock level analysis (List summarised in Appendix I). The section is organised in such a way that for each species the following aspects are dealt with:

***Biology/Life History***: For several species very little biological information appears to be available, however, the aim of this section is to reflect some of the biological information that is needed to assist in (1) planning of sampling for genetic stock identification, (2) interpretation of genetic results and (3) integration of genetic results into a holistic stock identification procedure. In cases where other methods of stock identification have been applied to a particular resource, the information has also been incorporated here. Some information was retrieved from FishBase.

***Genetics done***: An attempt was made to find as many previously conducted genetic studies on BCLME resources (or related species) as possible. These studies are summarised with respect to the genetic markers used and the results obtained.

***Questions that should be addressed***: Some of the issues that could potentially be resolved through the incorporation of genetic methods are listed.

Resources for which most biological and genetic data are available are outlined first, not surprisingly these include among the most important demersal (hakes) and pelagic resources (horse mackerel and Clupeiformes) in the region. The remaining resources were organised according to the fisheries as outlined by Booth and Hecht (2000), namely demersal fisheries, linefisheries, rock lobster fishery, beach seine and gillnet fisheries and recreational molluscs and crustaceans.

## 2.1 *Merluccius capensis* (shallow-water Cape hake), *M. paradoxus* (deep-water Cape hake), *M. polli* (Benguela hake)

Hake species represent the most important commercial resource for Namibia (90% of the demersal fishery; Van der Westhuizen 2001) and makes up 70% of the South African demersal fishery. *Merluccius capensis* occurs from 12°S-38°S, *M. paradoxus* from 18°S -39°S, while *M. polli* occurs from 20°N to 22°S.

### *2.1.1 Biology/Life history*

Morphological distinction between *M. paradoxus* and *M. capensis* is based on the number and pigmentation of the gill rakers, pectoral fin length, otolith shape and the number of vertebrae. There is ecological separation between the deep-water hake species (*M. paradoxus* and *M. polli*) that occur at depths of 300-1000 and 50-600 m respectively (temperatures of 8-3° C) versus shallow water Cape hake that occur at 50-350 m and 12-4 °C. Shallow water Cape hake are top predators and cannibalism forms a major part of their diet (Kainge 2002 and references therein). *Merluccius capensis* and *M. paradoxus* have low resilience with an estimated population doubling time of 4.5-14 years whereas *M. polli* has medium resilience with a doubling time of 1.4-4.4 years. Hake can apparently live for up to 9-11 years and the age at first breeding is four years (Iilende pers. comm.). Cape hake are serial spawners with batch fecundity of 160 and 306 eggs/g in *M. capensis* and *M. paradoxus* respectively (Osborne *et al.* 1999). Their eggs are pelagic and larval duration is relatively short at approximately two months (Fahay 1974 as cited in Quinteiro *et al.* 2000).

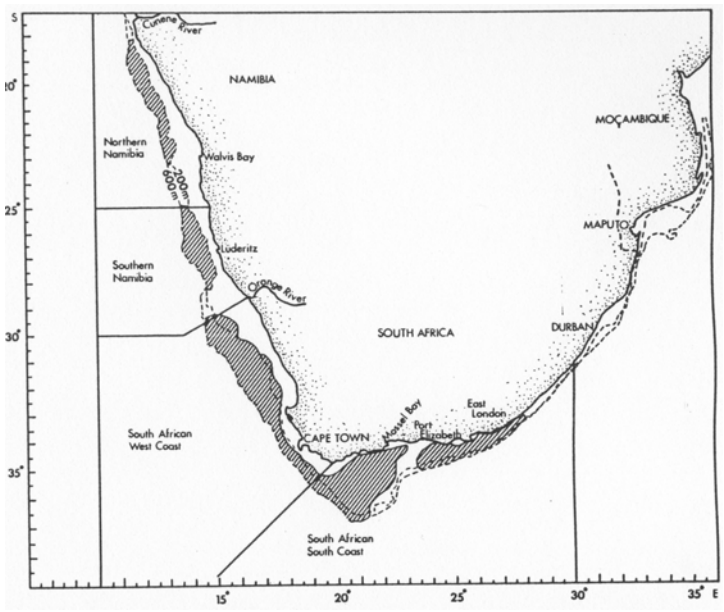
Of particular importance for the management of hake off Namibia and South Africa is the fact that in the past the two countries managed their "stocks" independently of each other but no distinction in either fishery was made between the two species (Burmeister 2000, see Fig. 3). Off Namibia approximately 60% of catches are however attributed to *M. paradoxus* (Kainge 2002). Despite setting a single total

allowable catch for South African hakes, the west and south coast populations are believed to be closed and separate TAC recommendations are therefore made (Punt 1994); the same applies to northern and southern Namibian "stocks" (Punt 1992, see Fig. 3a).

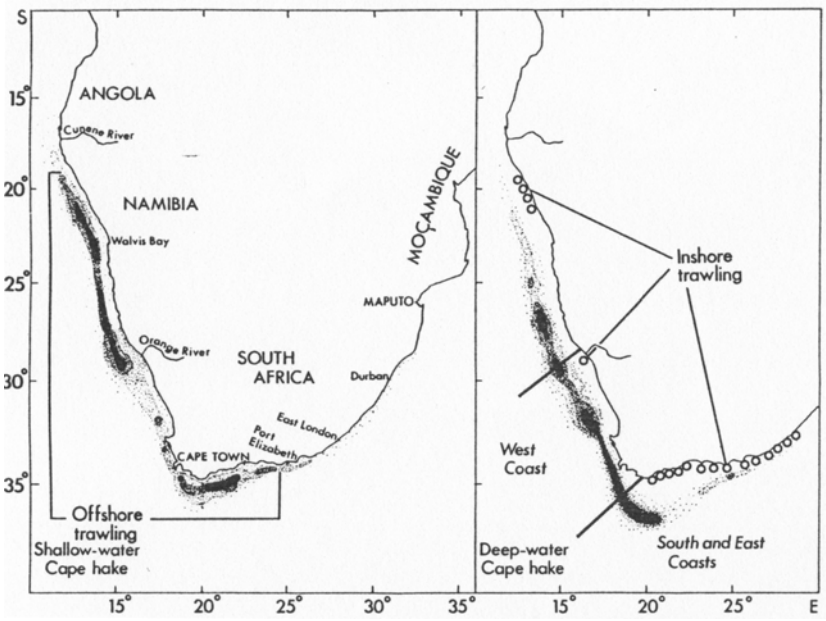
Burmeister (2000) recorded a lack of young year classes off Namibia and Kainge (2002) found no evidence of *M. paradoxus* spawning off the Namibian coast whereas known spawning sites exist between St Helena Bay and Cape Town and on the Agulhas Bank off the South African coast. Based on these observations and the lack of allele frequency differences reported by Grant *et al.* (1987), Burmeister (2000) suggested recognition of a single stock of *M. paradoxus* off South Africa and Namibia, with spawning and nursery grounds off South Africa and recruitment to Namibia. Another interesting observation of Kainge (2002) was the potentially skewed sex ratio in adult *M. paradoxus* off southern Namibia and northern South Africa indicating that adult males and females may form separate aggregations in preparation for spawning off South Africa.

Kainge (2002) reported spawning sites of *M. capensis* off Namibia at 22-24° S and the most intensive spawning was in the area between Walvis Bay and Conception Bay. The latter areas correspond with regions of mesoscale gyres. Spawning probably continues throughout the year but with a peak in Sept-November.

Other important factors to consider when studying genetic stock identification of hake species are their ability to undergo long migrations and their vertical mobility. They are reported to migrate inshore to spawn and show daily offshore and vertical feeding migrations (Quinteiro *et al.* 2000). In addition the effect of the environment on spawning (Hamukuaya *et al.* 2001; Sundby *et al.* 2001; Van der Westhuizen 2001) needs consideration.




(a)




(b)

**Figure 3** (a) Fishing grounds of Cape hakes (northern Namibia, southern Namibia, west coast of South Africa and south coast of South Africa). These four areas are presumed to represent separate stocks (from Punt 1992); (b) The distribution of shallow and deep water hake; for deep water hake the boundary between south and west coast stocks are indicated (from Punt *et al.* 1995).

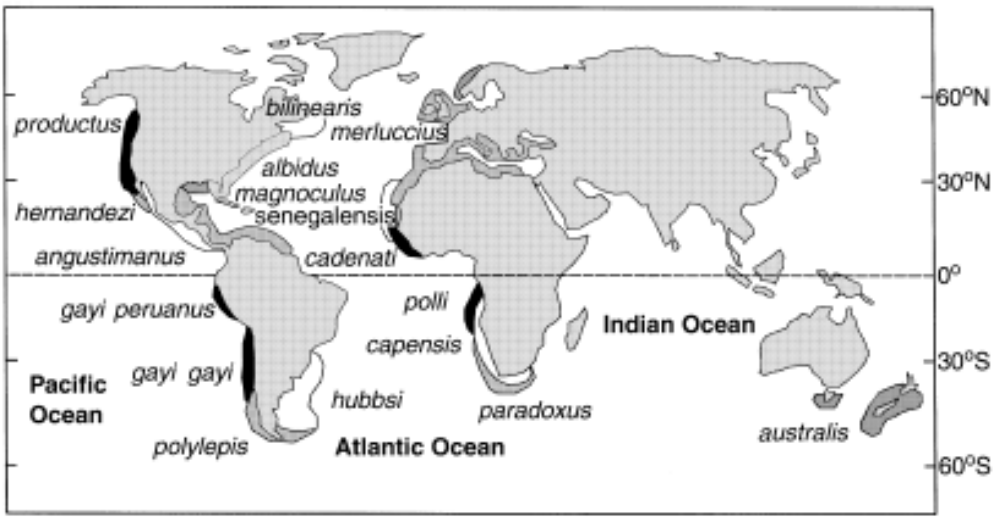
### *2.1.2 Genetics done*

#### *2.1.2 (a) Phylogenetic relationships*

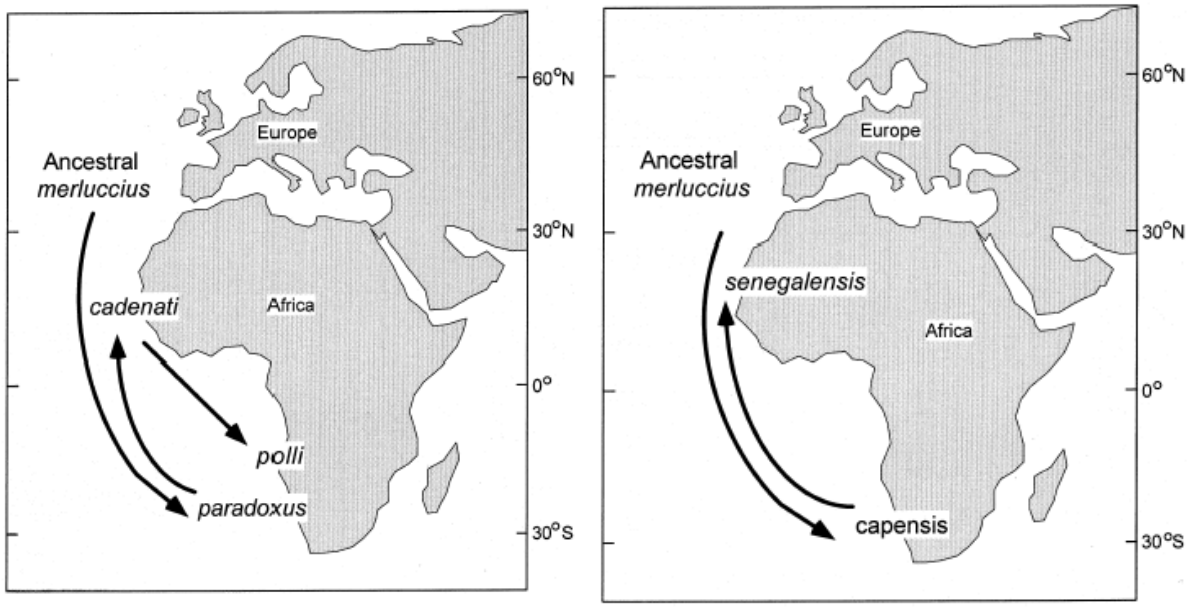
Southern African hake species have received a reasonable amount of attention. Becker *et al.* (1988) used both allozyme electrophoresis as well as mtDNA RFLPs to study the relationship between the sympatric *M. capensis* and *M. paradoxus*. Their data showed that the two species were genetically quite distinct. Roldan *et al.* (1999) reported allozyme distinction between hake species including between *M. capensis* and *M. paradoxus* sampled off Namibia. In this study only two sites of each species were analysed and no significant differences were reported within species.

Quinteiro *et al.* (2000) analysed variation in the variable mtDNA control region to study the phylogeny and biogeographic history of hake. They found two well-supported groups comprising west Atlantic/east Pacific and east Atlantic species respectively. Although the number of sites and samples were low they did report some variation within all the southern African species. Their results agreed with those of Grant and Leslie (2001) who investigated 13 hake species worldwide and based on a combination of nuclear (allozyme) and mtDNA markers found that Old World hake species (Figure 4) first moved into southern Africa approximately 10-15 Ma. This colonization occurred independently on two separate occasions; the first species that reached these waters was deep-water hake *M. paradoxus*. The latter species later re-crossed warm equatorial waters where *M. cadenati* arose in West Africa. It is believed that *M. polli* recently speciated from the latter. A second wave of colonization of southern African waters from the ancestral European *M. merluccius* resulted in *M. capensis* being restricted to southern Africa while *M. senegalensis* speciated from it and became isolated in West Africa.

Based on these results it is therefore clear that the three southern African hake species are taxonomically distinct and it would be possible to develop species specific markers for compliance enforcement and for species identification of eggs and larvae (see section 2.1.3). The latter is necessary for accurate recording of population trends (abundance, recruitment patterns, growth, etc.).




(a)




(b)

**Figure 4** (a) Global distribution of 13 hake species and (b) a proposed scenario for the colonization of the southeast Atlantic (Grant and Leslie 2001).

*2.1.2 (b)* *Stock structure*:

Grant *et al.* (1988) reported weak allozyme differentiation between *M. capensis* from northern Namibia and South Africa and no differentiation between *M. paradoxus* sampled from the two regions. The study of Becker *et al.* (1988) found no evidence of stock separation in South Africa for both species but their sample sizes for mtDNA analysis was very low. These earlier studies should be followed up by analyses with highly variable microsatellite markers as applied to European hake (refer to 2.1.2c and Appendix III).

*2.1.2 (c)* *Lessons to be learnt from more detailed studies on the related European hake* M. merluccius

Based on allozyme analysis of 10 sites in the North Atlantic and the Mediterranean, Roldan *et al.* (1998) found restricted migration between the two oceans potentially due to the Strait of Gibraltar. It is believed that fish from the Atlantic have only recently been able to move into the Mediterranean and despite low levels of gene flow it was recommended that the two populations should be managed as separate stocks. It was also proposed that the unidirectional gene flow into the Mediterranean likely occurred during the extended pelagic larval stage. Lundy *et al.* (1999, 2000) studied European hake using highly variable mtDNA and microsatellite markers. Their later study specifically aimed to test the temporal stability of population structuring and they included adult samples collected during autumn as well as two spring seasons at spawning sites (total sample size = 600 hake). They found no fixed differences in mtDNA alleles between presumed northern and southern Atlantic stocks (separated at the Bay of Biscay, a 4000 meter deep trench). Based on the microsatellite markers they found most variation within rather than between populations and found that temporal instability was more important than spatial differences. Temporal differences within sites could either be explained due to large variance in reproductive success or to the presence of two or more breeding stocks spawning at different times but recruiting to the same areas. The Breton Canyon was therefore found not to be an effective barrier to gene flow yet the stock structure within northeast Atlantic hake was certainly more complicated than previously anticipated.

*2.1.3 Questions that should be addressed*

- Can biochemical or molecular based methods be developed for accurate identification of early life stages of the different species? These methods should be fast and applicable on survey vessels rather than laboratory based. Laboratory based methods using PCR with species specific primers would be easy to implement and could even be done on ship, however the PCR result would not be available within minutes and the best equipment for such an application (quantitative PCR) is currently still very expensive. The best methods would therefore be biochemical incorporating some form of antigen-antibody recognition system.
- What is the temporal and spatial distribution of *M. paradoxus* stocks? What is the link between *M. paradoxus* spawning adults from South Africa and recruits in Namibia? Does the increased abundance of *M. paradoxus* in Namibian waters represent a recent range expansion? – The latter questions all relate to the need to establish whether *M. paradoxus* represents a transboundary population. It would be crucial to sample both adults at spawning areas as well as recruits over several years. Both mtDNA and microsatellite markers should be utilized.
- Are there separate stocks of *M. capensis* off the three countries based on microsatellite markers?
- Is there spatial genetic variation within *M. polli* and what is the relationship of *M. polli* to its sister species *M. paradoxus* and *M. cadenati*? Do the latter three species represent isolated gene pools?
- Development of a species- specific diagnostic test for compliance enforcement using existing sequences generated by previous studies.

## 2.2 *Trachurus trachurus* (Atlantic horse mackerel), *T. capensis* (Cape horse mackerel), *T. trecae* (Cunene horse mackerel)

*2.2.1 Biology/Life history*

Horse mackerel are of considerable commercial importance within the BCLME region having been exploited since the 1940's. They are pelagic shoaling species occurring at depths of up to 600 m. Sexual maturity is reached at two to four years and spawning occurs from approximately September to April. The three species have medium resilience (minimum population doubling time 1.4-4 years); longevity ranges from seven to 11 years. The Atlantic horse mackerel occurs up to 600 m from 66°N

to 28°S, *T. capensis* occurs at depths of 0-400 m from 14°N to 28°S and *T. trecae* occurs at 20–100 m from Morocco to Angola (36°N to 18°S). The relationship between *T. trachurus* and *T. capensis* remains unclear while several stocks have been identified in the Cape horse mackerel (Draganik and Sacks 1991), one off Namibia and southern Angola and another off the south coast of South Africa. These apparently separate spawning stocks may be demographically isolated by the Luderitz upwelling cell (Babayan *et al.* 1983 as cited by Boyer and Hampton 2001). Off South Africa, Cape horse mackerel shows both temporal and age class related shifts in distribution with recruits generally found between the Orange River and Mossel Bay and adults found along the shelf edge east of Mossel Bay. Juveniles appear to be concentrated inshore (Barange *et al.* 1998, see Fig. 5). Boyer and Hampton (2001) also made reference to along shore and cross shelf migrations by juveniles and adults off Namibia. Recent observations from Namibia have shown a lack of older age classes (only ages up to three years old recorded; Kanendjembo pers. comm.). It would be important to establish whether the latter trend relates to declines due to exploitation or to environmental anomalies.

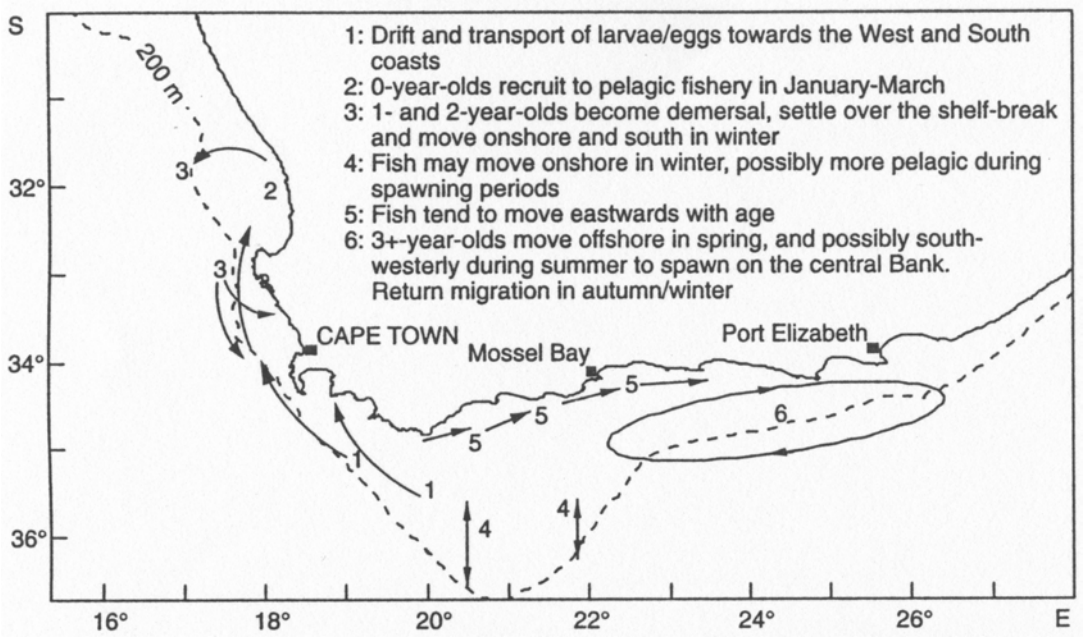



**Figure 5** Movements of Cape horse mackerel based on research surveys off the south coast of South Africa. The survey data were used to document horse mackerel distribution and to postulate their migratory and spawning strategies (from Barange *et al.* 1998).

### *2.2.2 Genetics done*

Two studies on horse mackerel stand out amongst all the investigations on local species in terms of temporal sampling as well as excellent sample sizes used. Zenkin and Komarov (1981) analysed two allozyme loci (muscle esterases) in Cape horse mackerel *T. capensis* (N=9550) from 40 sites off the Namibian coast over a five-year period (1975-1979). They reported allele frequency differences and recognised two stocks although the exact geographic location of these stocks are not immediately apparent from their article. Their results appear to separate the Namibian (ICSEAF divisions 1.3-1.4) and South African stocks (ICSEAF division 2.1; for ICSEAF divisions see Draganik and Sacks 1991). Sardinha and Naevdal (2002) studied differentiation within and between *T. trecae* (N=1037 from 11 sites) and *T. capensis* (N=192 from two sites) off Angola. Among six polymorphic allozyme loci they found fixed allelic differences at one locus and allele frequency differences at three loci between the two species. They further reported significant differentiation within the Cunene horse mackerel, with the Benguela region distinct from the Cabinda and Luanda areas.

### *2.2.3 Questions that should be addressed*

- Do highly variable genetic markers (microsatellites) support previously suggested Cape horse mackerel and Cunene horse mackerel stocks?
- Do these stocks show temporal stability?
- What is the extent of exchange between the different Cape horse mackerel in the different regions of the Benguela system?
- What are the phylogenetic relationships between *Trachurus* species, specifically the relationship between the Atlantic and Cape horse mackerel?
- Can the allozyme markers developed by Sardinha and Naevdal (2002) be used for species identification for compliance enforcement?

**Small pegalics**

***Etrumeus whiteheadi* (round herring), *Sardinops sagax* (pilchard or sardine), *Sardinella maderensis* (Madeiren sardinella), *S. aurita* (round sardinella), *Engraulis encrasicolus* (anchovy)**

The Clupeidae is represented by 13 species off southern Africa and these species contribute significantly to the pelagic fishery. Based on total mass, Clupeidae globally contributes approximately 25% of the world's catch. The literature related to the biology and genetic studies on Clupeiformes are extensive and the following only represents a brief summary. More genetic papers will also soon be published (see Grant, Appendix II). One important general conclusion drawn by Grant and Bowen (1998) is that despite the extreme abundance of these pelagic resources, these species are vulnerable to overexploitation and environmental stochasticity over ecological and evolutionary time frames.

## 2.3. *Sardinops sagax* (pilchard or sardine)

### *2.3.1 Biology/Life history*

Beckley and Van der Lingen (1999) provides a comprehensive review of the biology, ecology and management of sardines in southern Africa. Sardines form dense shoals and are important prey species for other fish, birds and marine mammals. This epipelagic cool water species is generally associated with anti-tropical upwelling areas and an important feature of the life history of the species is long-term fluctuations mediated by global climate changes; in southern Africa biomass peaked in the 1960's. Sardines are fast growing, short-lived and highly fecund; larval duration is short. The species is widespread from southern Angola to the KwaZulu-Natal coast of South Africa. Despite extensive migrations, two stocks are recognised: central Namibia/Angola (27-15°S) and southern Namibia/South Africa (27°S and southeastwards). The Luderitz upwelling cell apparently prevents mixing between these stocks. There may be substocks between central and northern Namibia/southern Angola but the latter division is not supported unequivocally.

The complex distribution patterns of sardines appear to be linked to seasonal hydrological features and age- and size specific behavioural differences. Off South Africa most spawning occurs in spring and summer on the Agulhas bank while the contribution of east coast spawning to the South African stock is not well understood

(Fig. 6). Off Namibia the species spawns north of Walvis Bay with peaks in August/September and February/March.

Kreiner *et al.* (2001) compared sardine from the northern and southern Benguela. Northern biomass was found to be much lower than in the south. They found a lack of agreement in condition factor time-series between the two regions suggesting that they are independent. Tagging data showed no movement from the Western Cape to Namibia and minimal movement in the opposite direction.

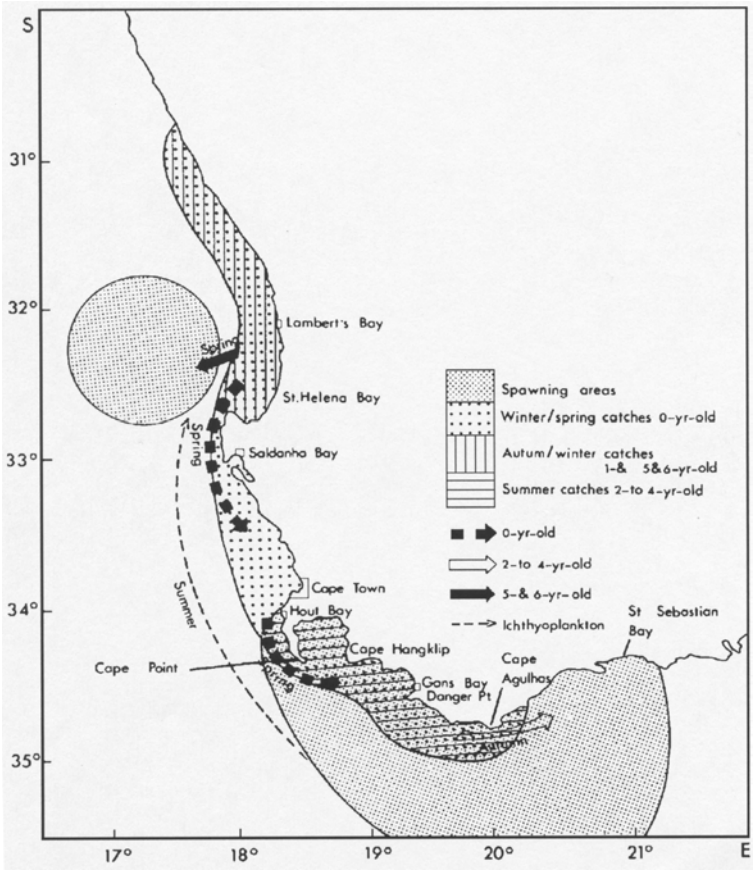



**Figure 6** Spawning areas and catch data for sardine on the southwest coast of South Africa (from Beckley and Van der Lingen 1999).

### *2.3.2 Genetics done*

Bowen and Grant (1997) studied mtDNA control region sequences of global *Sardinops* populations. They found strong structuring but shallow divergence within and between regions (South Africa, southern Australia, southern California and Japan). They proposed a stepping stone model, hypothesizing that the current patterns of genetic relationships suggest a series of Pleistocene dispersal events

around the Indo-Pacific continental margin. They further suggested that regional populations are recent, unstable and ephemeral; these observations support other suggestions of short and long term fluctuations in the abundance of the species. Their data revealed very high levels of haplotype diversity (h = 0.998) but low levels of nucleotide diversity (average $\pi$ = 0.038). Low levels of allozyme diversity were also reported for populations within the species (H = 0.01-0.045) indicating rapid population growth from small ancestral populations. The deepest division was recorded between Japan and South Africa and using an approximate molecular clock the diversification within all sardine populations was dated to within the past 200 000 years. Low diversity is therefore the result of metapopulation processes of extinction and recolonization due to climate-driven population turnover. Scales in sediments off California showed fluctuations in *Sardinops* abundance over the past 1800 years; these fluctuations are therefore natural and not due to exploitation levels. Overexploitation could however have a severe impact by limiting recolonization.

### *2.3.3 Questions that should be addressed*

- Using highly variable microsatellite markers, what is the extent of exchange between central Namibia/Angola and southern Namibia/South Africa? Do coalescent based modelling of genetic data indicate unidirectional movement (and perhaps gene flow) as suggested by tagging data?
- Do southern and northern Benguela populations represent separate stocks? Is there any genetic evidence of substocks within the southern and northern Benguela respectively?
- How should long term cycles of extinction and recolonization be managed within the BCLME?

## 2.4 *Etrumeus whiteheadi* (Whitehead's round herring) [the South African East coast *E. teres* should also be considered]

### *2.4.1 Biology/Life history*

Round herring are pelagic with a depth range of 0-200 m and is subtropical in distribution from 22-36°S. The species appears to be particularly abundant over the central and eastern Agulhas Bank (Geldenhuys 1978 as cited by Boyer and Hampton 2001). The species is of high commercial importance but relative to other clupeids it

is probably underexploited. It has high resilience with a minimum population doubling time of less than 15 months. Roel and Armstrong (1991, see Fig. 7) found adults to be widespread off the west and south coast of South Africa at depths of 100-400 m and a temperature range of 10-20°C. They move closer inshore during late summer and early autumn. Spawning occurs in deep water in July-November and was found to be most intense on the west coast. Juveniles and young adults occur in water shallower than 100 m; mostly juveniles are caught off Namibia. Seasonal movements affect catches. Boyer and Hampton (2001) suggested limited exchange between the southern and northern Benguela stocks.

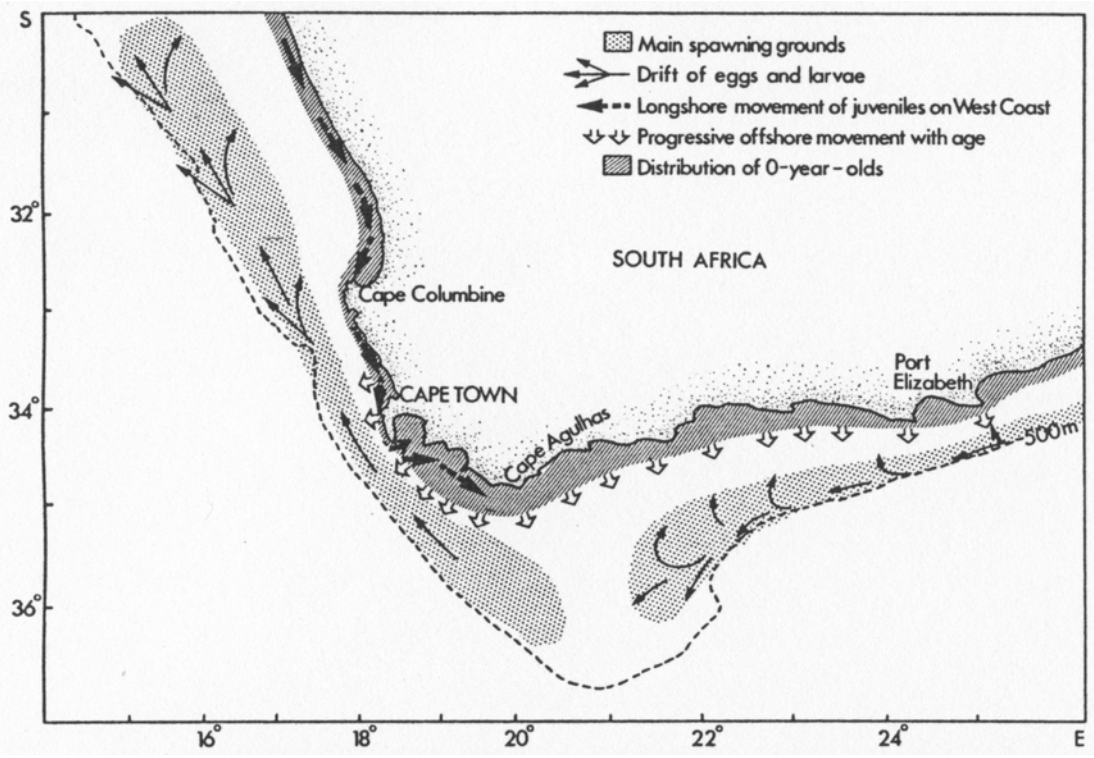



**Figure 7** Main spawning grounds and seasonal movement patterns of round herring off the southwest coast of South Africa (from Roel and Armstrong 1991).

### *2.4.2 Genetics done*

No information is available.

### *2.4.3 Questions that should be addressed*

- What are the phylogenetic relationships of *E. whiteheadi* based on mtDNA and nuclear genes? This is an important first step to determine the geographic scale over which stock level questions should be addressed.
- If *E. whiteheadi* can be investigated further (independent of other *Etrumeus* species), is there evidence of two stocks in the northern and southern Benguela based on mtDNA and microsatellite markers?

## *2.5 Sardinella aurita, S. maderensis* (Round and Madeiren sardinella)

### *2.5.1 Biology/Life history*

*Sardinella aurita* is reef-associated at depths of 0-350 m and is subtropical in distribution from 46°N to 36°S. It has high resilience reaching sexual maturity at one year and with a maximum age of seven years. Juveniles tend to concentrate on nursery grounds. The species is strongly migratory. The species can breed throughout the year but in some areas there are discrete spawning times. The Madeiren sardinella is pelagic (0-80 m) and ranges from 40°N to 25°S. It has medium resilience with recruitment at three years and a maximum age of six years. It breeds from July to September and both juveniles and adults show north-south migrations from Gabon to Angola in response to seasonal upwelling.

### *2.5.2 Genetics done*

Chikhi *et al.* (1998) studied allozyme variability in *S. aurita* from the Congo, Ghana, Ivory Coast and Venezuela (sample sizes ranged from 21-97 per region). They recorded three polymorphic loci but extremely low levels of diversity (H = 0.01) and low differentiation between populations ($F_{ST}$ = 0 - 0.0055). As in many other pelagic marine resources, they attributed the low levels of diversity to large variance in reproductive success, demographic instability, evolutionary population size reductions or to the potential effects of selection.

### *2.5.3 Questions that should be addressed*

- What are the phylogenetic relationships of *Sardinella* species based on mtDNA and nuclear genes? This is an important first step to determine the geographic scale over which stock level questions should be addressed.
- Do variable mtDNA and microsatellite markers provide evidence of differentiation? In light of the previous allozyme study, the influence of selection could be addressed through analysis of adaptively significant loci (such as some allozyme loci). Larger sample sizes collected over multiple cohorts would make it possible to account for the effects of large variance in reproductive success and demographic instability. Coalescent based analyses of mtDNA could test for changes in effective population size.

## 2.6 Anchovy (*Engraulis capensis* has been synonymised with *E. encrasicolus*)

### *2.6.1 Biology/Life history*

Anchovy are pelagic (0-400 m) and subtropical in distribution from 62°N to the east coast of South Africa. The species is of extremely high commercial value and is characterised by high resilience. Anchovy has probably been the most intensively studied marine fish species off South Africa over the past 20-30 years. Hutchings *et al.* (1998) provides a good summary of anchovy life history in the region (Fig. 8). The South African anchovy spawn on the western Agulhas bank over the summer period (October – February) at temperatures of 16-19°C. Eggs spawned on the Agulhas Bank are subject to both cannibalism and advective loss. Nursery areas are found along the west coast. Environmental impact on spawning, transport and nursery areas have impacted on the recruitment to the fishery and the spawning stock over the past 13 years. The species is managed as two separate stocks in the northern and southern Benguela although the extent of exchange between the regions is unknown. During most years the Luderitz cell may act as a barrier to recruitment of pelagic larvae from the Cape to the northern Benguela (Boyer and Hampton 2001 and references therein).

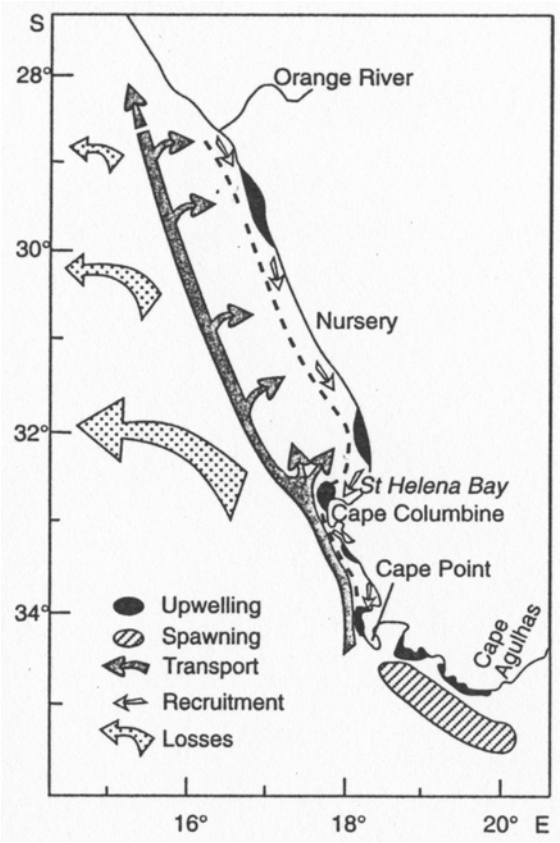



**Figure 8**. Current hypothesis of the life history of anchovy in the southern Benguela (from Hutchings *et al.* 1998).

### *2.6.2 Genetics done*

Grant and Bowen (1998) found deep lineages within anchovy separated by 6-10 million years of divergence but reported shallow divergences among Old World species with dispersal events dating to within the last 100 000 years. In contrast to low levels of mtDNA diversity within South African anchovy (h = 0.21 and $\pi$ = 0.004) they found high levels of allozyme diversity (H = 0.091-0.115). The difference between the two marker systems may be due to sex specific dispersal patterns or to skewed sex ratios. Low mtDNA diversity may relate to a low effective population size in the recent past. They found that in contrast to *Sardinops*, the Cape anchovy population did not show a link with Australia but in fact with Europe. They predicted that this was due to an extinction event and recent recolonization from Europe.

*2.6.3 Questions that should be addressed*

- Do the northern and southern Benguela represent separate anchovy stocks based on highly variable microsatellite markers?
- Grant (Appendix II) recommended coastwide analysis of mtDNA variation.
- How should long term cycles of extinction and recolonization of this species be managed within the BCLME?

*General questions that should be addressed for small pelagic resources:*

- What is the relationship of pelagic fish off the Orange River region to the northern and southern Benguela (see Kanandjembo *et al.* 2001)?
- What is the influence of the complex ecological interactions between Clupeiforms on comparative genetic structuring within the different species?

**OTHER EXPLOITED RESOURCES:**

**<u>Demersal fisheries</u>**

**2.7 *Dentex angolensis* (Angola dentex), *D. macrophthalmus* (Large-eye dentex)**

*2.7.1 Biology/Life history*

Two species of dentex occur off the Angolan coast. These deep water species occur at depths of 15-500 m on the continental shelf and slope. The Angolan dentex occurs from 33°N to 13°S while the large-eye dentex occurs from 42°N to 22°S. Both species have medium resilience and reach maximum ages of seven and 13 years respectively. Big-eye dentex undergo seasonal migrations and has a single clear spawning peak (April-May).

*2.7.2 Genetics done*

No information is available.

2.7.3 *Questions that should be addressed*

- What are important life history aspects that should first be studied before genetic analysis is planned?

- What are the phylogenetic relationships between dentex species based on mtDNA and nuclear genes?
- Is there a shared large-eye dentex stock between Angola and Namibia?

## 2.8 *Lophius vomerinus* (Cape monk/anglerfish)

### *2.8.1 Biology/Life history*

The Cape monk is bathy-demersal (150-400 m) occurring in deep water off Namibia and South Africa between 21°-28°S. North of Walvis Bay, the Cape monk is replaced by *L. vaillanti.* Cape monk has been exploited since the 1970's (initially as bycatch of the hake fishery) and during the late 1990's the species represented one of the five most important resources in terms of landed mass and income in Namibia but was found to be fully or overexploited (Maartens and Booth 2001). The species has low resilience with maturity reached at four years. The species is slow growing and long lived (11 years or even up to 20-30 years). Indications are that these predatory fish may be quite sedentary (Smith and Heemstra 1986). Monk spawn throughout the year. There are two recruitment areas of Cape monk off Namibia: Walvis Bay and Orange River. The relationships between these areas and the extent of adult longshore movements are unknown (Boyer and Hampton 2001).

### *2.8.2 Genetics done*

Grant and Leslie (1993) used allozymes to study the phylogenetic relationships between the seven *Lophius* species. They suggested that *L. vomerinus* dispersed along West Africa and that it is a sister species of *L. budegassa* from the Mediterranean. Allozyme analysis did not indicate any evidence of Cape monk stocks (Leslie pers. comm.).

### 2.8.3 *Questions that should be addressed*

- Do *L. vomerinus* from the northern and southern Benguela represent separate stocks based on mtDNA and microsatellite markers? Primary areas for sampling should be the two recruitment areas off Walvis Bay and the Orange River.
- Does genetic evidence support the sedentary behaviour of adults?

## 2.9 *Beryx decadactylus* (Alfonsino or beryx), *B. splendens* (Splendid alfonsino or slender beryx)

### *2.9.1 Biology/Life history*

Berycids occur worldwide (with the exception of the eastern Pacific) in tropical and temperate latitudes at depths of 200-800m (Alfonsino) and 25-1300 m (splendid alfonsino). The adults are demersal and the juveniles pelagic. Splendid alfonsino are often found over seamounts. These species have low resilience with sexual maturity reached at 2-8 years and longevity of up to 23 years. Off Namibia, Alfonsino is more widely distributed than orange roughy and given its earlier maturity and shorter life span, it is probably more productive. Very little is known about its life history including spawning and breeding areas. The extent to which the species extend into South Africa and Angola is unknown (Boyer and Hampton 2001).

### *2.9.2 Genetics done*

No information is available.

### 2.9.3 *Questions that should be addressed*

- What are important life history aspects that should first be studied before genetic analysis is planned?
- What are the phylogenetic relationships between *Beryx* species based on mtDNA and nuclear genes?
- Is there a shared stock within the BCLME region?

## 2.10 *Hoplostethus atlanticus* (Orange roughy)

### *2.10.1 Biology/Life history*

The slimeheads are medium sized fish found at great depths (600-1400 m) in cold waters (3-9°C) off steep continental slopes, ocean ridges and sea mounts (see Fig. 1 of Branch 2001). The orange roughy is circumglobal in distribution; in the southern hemisphere there are major stocks off New Zealand and Australia, along the mid-Atlantic ridge off Namibia and along the Madagascar ridge in the southern Indian

Ocean (Boyer *et al.* 2001; Branch 2001). Important life history characteristics include extreme longevity (> 100 years), late and irregular recruitment (at 22-40 years), low fecundity (large but small numbers of eggs), low natural mortality and slow growth rates (Mace *et al.* 1990, Boyer *et al.* 2001, Branch 2001). The species has a short spawning period of less than one month duration in late July during which time they form dense aggregations. These aggregations persist throughout the year but at lower densities assumed to be feeding aggregations (Boyer and Hampton 2001; Boyer *et al.* 2001). Observations indicated a very wide range of year classes in samples suggesting that not all adults spawn each year. The planktonic phase of the eggs is extremely short (roughly 10 days) and the larvae are epibenthic (Smith *et al.* 2002). The species is adapted to low productivity environments where chances of regular recruitment is low (Smith and Benson 1997). Given its life history characteristics the species is very susceptible to over-exploitation (Boyer *et al.* 2001, Branch 2001).

### *2.10.2 Genetics done*

A variety of stock discrimination methods have been applied to orange roughy especially off New Zealand and Australia where the fisheries already developed in the 1980's. These methods include morphometrics, otolith shape and microchemistry, parasites, length at maturity, fecundity and spawning time and genetic methods (allozymes, mtDNA restriction enzyme patterns or sequencing, RAPDs). While parasite distribution, morphometrics and otolith trace elements suggested differentiation in Australasia, initial genetic studies showed moderate to high levels of genetic diversity but little regional differentiation (Smith and Benson 1997, Branch 2001, Smith *et al.* 2002). Smolenski *et al.* (1993) did however find temporal changes in genetic composition.

Smith and Benson (1997) used 11 allozyme loci to study differentiation within and between geographically separated spawning areas off the east coast of New Zealand and off the Chatham Rise. They reported significant heterogeneity at three loci indicating that the sites did not represent a single panmictic population. They did not find any heterogeneity among the east coast populations but found significant differentiation between the east coast and Chatham Rise . Among the Chatham Rise sites they found evidence for isolation-by-distance and both temporal and spatial heterogeneity. The temporal changes are important to consider in management as it is a reflection that not all adults spawn every year.

Smith *et al.* (2002) used a holistic approach to study stock differentiation among four fisheries in the eastern Tasman Sea. They investigated life history traits (age and length at maturity), otolith morphology, mtDNA RFLP's and microsatellites, size frequency and timing of spawning. Based on size differences there appeared to be little short-term movement between fisheries but there could still be slow age-related movement. Their study highlighted several important considerations in stock differentiation in roughy: it is important to collect spawning and non-spawning samples, small spatial scales are important (e.g. 200 km) and there could be temporal changes in spawning time. Some of the latter issues are reiterated by Branch (2001) who reviewed stock studies to date; there are strong indications that orange roughy are sedentary with potentially discrete stocks over distances of less than 500 km.

Off Namibia there are four management areas assumed to be separate stocks (Boyer *et al.* 2001). Flint *et al.* (1998/99) reported on a preliminary allozyme analysis of two of the management units showing low but significant differentiation (Nei's D = 0.004, $F_{ST}$ = 0.019).

### *2.10.3 Questions that should be addressed*

- Non-genetic issues that should be addressed include gathering of accurate distribution information off Namibia, South Africa and Madagascar. Methods for ageing and tagging roughy need to be developed.
- A long-term sampling strategy should be put in place to be representative of the major aggregations and to ensure monitoring of temporal changes.
- Do the separate aggregations represent demographically isolated stocks?
- As the Namibian fishery is much younger than in Australia and New Zealand it offers opportunities to study the link between natural changes, fishing pressure and levels of genetic diversity over time.

## 2.11 *Genypterus capensis* (Kingklip)

### *2.11.1 Biology/Life history*

Kingklip are bathydemersal occurring in deep water (50-500m) from 23°S - 35°S (endemic to southern Africa from Walvis Bay to Algoa Bay). The species is commercially exploited in South Africa (trawling and more recently demersal

longlining) and an experimental fishery is being developed in Namibia. The species has low resilience with maturity reached at 4-5 years, they are slow growing and can reach 24 years of age. Spawning occurs from August to October (with spawning aggregations reported from the south coast on the eastern Agulhas bank) and juveniles are found in shallower water. Fisheries scientists disagree in terms of the number of stocks recognised (see Punt and Japp 1994 and references therein): Some advocate a single stock, others recognise a Namibian (north of Luderitz) and South African stock, while others also consider separation between the west and south coast of South Africa. Evidence used for these considerations included catch statistics, morphometrics, meristics and otolith morphology. Due to the inconclusive nature of the data, a precautionary principle has been applied and the South African resource managed as two stocks (west and south coasts). In their assessment of the South African stocks Punt and Japp (1994) found that the stocks were severely depleted (potentially to less that 50% of the pristine levels) and the west coast appeared to be in a poorer state.

*2.11.2 Genetics done*

No information is available. Smith and Paulin (2003) used genetic markers and morphology to study the relationships of *G. blacodes* and *G.microstomus* in New Zealand and Australian waters. They found evidence of a single species *G. blacodes* but with two apparent groups in New Zealand waters (northern and southern corresponding to major water masses) confirming their earlier allozyme data. They reported significant genetic divergence between the New Zealand and Tasmanian stocks. Microsatellite markers have recently been developed for *G. blacodes* (Appendix III).

*2.11.3 Questions that should be addressed*

- Do spawning only occur on the south coast?
- How many stocks are represented off Namibia and South Africa based on allozyme, mtDNA and microsatellite markers?
- What is the phylogenetic relationship between *G. capensis* and *G. blacodes* from Australia and New Zealand?

## 2.12 *Chaceon maritae* (Deep sea red crab)

### *2.12.1 Biology/Life history*

The benthic deep-sea red crab is distributed from 27°S off Namibia and northwards to the Ivory Coast. The species occurs at depths of 300-900 m and forms part of a northern slope decapod crustacean assemblage (Macpherson 1991). Beyers (1994) estimated population size using tag-recapture off Namibia and reported the highest density from 18-20°S. Sex ratio differ markedly between different parts of the coast; off Namibia female densities were highest in the south 19-20°C (Beyers 1994). While males tend to remain in a particular fishing ground, females move to shallower water and emigrate from Namibia to Angola (Le Roux 2001). Egg production and larval release are reported from shallower parts of the continental slope (Boyer and Hampton 2001). Spawning appears to occur throughout the year (Le Roux 1994 as cited by Boyer and Hampton 2001). Pollock and Melville-Smith (1993) indicated that fecundity is low and growth rate slow. They predicted that females likely only spawn twice in their lifetime ($0.225 \times 10^6$ eggs per lifetime). Larval duration is up to 18 weeks.

The deep sea red crab has been exploited off Namibia since the 1970's; catches showed a sharp decline at the end of the 80's and remained low. Since 1993 a depth restriction has been implemented to protect the female stock (Le Roux 2001).

### *2.12.2 Genetics done*

No information is available. A study has been initiated at the Dept of Genetics, Stellenbosch University in partnership with IIM, NatMIRC and BENEFIT.

### *2.12.3 Questions that should be addressed*

- Do deep sea red crab off Namibia and Angola represent a single stock as suggested by reports of female crab emigration from Namibia to Angola?

**Linefisheries**

## 2.13 *Carcharhinus brachyurus* (copper shark or bronze whaler)

### *2.13.1 Biology/Life history*

Bronze whalers are important reef-associated (at depths of 0-100 m) gamefish and are subtropical to temperate in distribution. The species is frequently caught by anglers, commercial lineboats, as by-catch in trawlers and in KwaZulu-Natal shark nets (Compagno *et al.* 1989). Between 1978 and 1990, 1800 copper sharks were caught in these nets representing 10% of the total shark catch; interestingly most of these catches were associated with the sardine run (Cliff and Dudley 1992). They have low resilience reaching sexual maturity at 13-20 years and attain a maximum age of 25-30 years. They are internal live bearers with a long gestation period (of up to 12 months) and produce 7-20 pups. The low fecundity and the advanced age at maturity, combined with a slow growth rate makes the species especially vulnerable to overfishing (Walter and Ebert 1991). Based on data gathered from specimens caught in shark nets, mating appears to occur after the winter period; pupping peaks in spring to early summer (Cliff and Dudley 1992). No new borne sharks have been caught off southern KwaZulu-Natal and a possible nursery area may be off the Eastern Cape (Smale 1991 as cited in Cliff and Dudley 1992). From limited tagging returns, these sharks appear to range over considerable distances; up to 1320 km. Field observations further indicate that copper sharks may live in packs (Cliff and Dudley 1992). Walter and Ebert (1991) suggested the existence of two separate populations, one off Namibia from south of Walvis Bay northwards and one off South Africa from the Western Cape eastwards. This suggestion was supported by a lack of catch data of this species from the Luderitz area and differences in breeding seasonality (along the Namibian coast near term embryos were observed in December to March and off South Africa during October to December (Walter and Ebert 1991).

### *2.13.2 Genetics done*

No information is available. See Appendix III.

*2.13.3 Questions that should be addressed*

- What is the extent of exploitation and the status of copper sharks in the three countries?
- Do copper sharks represent one, two or three separate stocks based on mtDNA and microsatellite markers?

## 2.14 *Lichia amia* (leerfish or garrick)

*2.14.1 Biology/Life History*

*Lichia amia* is an aggressive predatory species that prefer the surf zone and water to a depth of 50 meters. The aggressive behaviour makes it a popular game fish. It is subtropical in distribution and ranges along the entire African west coast around the Cape to southern Mozambique. Sexual maturity is attained at a fork length of 60 cm (approximately two years) and a total length of more than one meter can be reached. The species has medium resilience (population doubling time of 1.4 – 4.4 years) and undergoes seasonal migrations. During winter those found off the south coast of South Africa migrate into the warmer waters off KwaZulu-Natal, coinciding with the annual sardine migration. Spawning takes place in these warmer waters from September to November. The occurrence of juveniles at the Orange River indicates that spawning may also occur off the west coast. Juveniles are mainly associated with estuaries (Smith and Heemstra 1986, Van der Elst 1998, Whitfield 1998).

*2.14.2 Genetics done*

No information is available. Genetic markers will need to be developed.

*2.14.3 Questions that should be addressed*

- Do leervis within the BCLME represent a transboundary population based on mtDNA and microsatellite markers?

## 2.15 *Dichistius capensis* (Galjoen)

### *2.15.1 Biology/Life History*

The Coracinidae only occur off southern Africa and Madagascar (Smith and Heemstra 1986). Galjoen is one of southern Africa's sought after angling species and is endemic to the region occurring from southern Angola to Sodwana Bay (Smith and Heemstra 1986, Van der Elst 1998). It is commonly caught by rock-and-surf anglers along the Namibian coast and the coast of the southwestern Cape (Van der Lingen 1994). It used to be much more abundant throughout its range living in small groups in the shallow turbulent waters of the close inshore environment. Migrations between Namibia and South Africa have been documented but they also appear to be semi-resident for part of the year. Sexual maturity is at 34 cm and spawning is in deeper waters during summer. Large numbers of pelagic eggs are produced but very little is known regarding the biology and distribution of eggs and larvae (Van der Lingen 1994). Based on a laboratory study of temperature tolerance of eggs and embryos, Van der Lingen (1994) suggested that West Coast galjoen may be adapted to a lower temperature range than South Coast galjoen. The species has low resilience (minimum population doubling time of 4.5-14 years) and its decline is in part attributed to overfishing.

### *2.15.2 Genetics done*

No information is available. Genetic markers will need to be developed.

### *2.15.3 Questions that should be addressed*

- What are the phylogenetic relationships based on mtDNA and nuclear genes between the three galjoen species, in particular, what is the relationship between *C. capensis* and *C. multifasciatus*?
- Do galjoen from Namibia and South Africa represent a transboundary population based on microsatellite markers?

## 2.16 *Thyrsites atun (Snoek)*

### *2.16.1 Biology/Life History*

The Gempylidae are large fast predators found in all oceans at depths of 200-500 m where they form schools near the bottom or midwater over continental shelves but sometimes moving to the surface at night. Snoek are mesopelagic in cold waters (13-18°C; in the BCLME from southern Angola to Cape Agulhas) of the southern hemisphere. The species feeds on pelagic fishes, euphausiids and cephalopods. It is an important commercial species in southern Australia, New Zealand, southern South America and especially South Africa (Smith and Heemstra 1986). Snoek are pelagic nomads able to cover large distances over short time periods and cannot be found predictably. Snoek off the western and southwestern Cape are regarded as a single stock (Griffiths 2000). Catch rates throughout the 20$^{th}$ century have fluctuated and it appears as though the species has not declined to the same extent as other linefish species (Griffiths 2000). The species has medium resilience reaching maturity at two to three years and with a maximum age of about 10 years. Spawning patterns are complex with different stocks spawning at different times of the year; off Tasmania spawning occurs from October to March. Snoek spawn off the western edge of the Agulhas Bank (150-400 m depth) during late winter and early spring (August to November); eggs and larvae are transported by the Benguela current to nursery areas north of Cape Columbine (Griffiths 2000). Spawning has also been recorded from southern Namibia and the west coast of South Africa (Boyer and Hampton 2001). The presence of snoek eggs and larvae throughout the Benguela during winter and spring have led some researchers to believe that a single migratory stock exists. Griffiths has however suggested that there are two stocks separated by the Luderitz upwelling zone but with medium term exchange between them.

### *2.16.2 Genetics done*

No information is available. Genetic markers will need to be developed.

### *2.16.3 Questions that should be addressed*

- What are the relationships between snoek stocks throughout their southern hemisphere distribution based on mtDNA and nuclear gene sequences?

- Does snoek represent a single transboundary population off the southwest coast of South Africa and Namibia based on microsatellite markers?

## 2.17 *Pomatomus saltatrix* (bluefish, elf)

### *2.17.1 Biology/Life History*

Elf is found in almost all oceans (except the eastern Pacific) between the 50° latitudes and a single species is recognised (Smith and Heemstra 1986). It appears in all coastal waters of the BCLME region, however most of the available data is for the stock found of the South African southern and eastern seaboard. There the indications are that a spawning migration takes place with fish from the Cape region migrating to KwaZulu-Natal during winter to spawn (Van der Elst 1998). The Tugela Bank has been identified as one of the spawning areas (Whitfield 1998). *Pomatomus saltatrix* has a prolonged asynchronous spawning season (Govender 1999); off KwaZulu-Natal the peak is October to January. The pelagic larvae are transported back to the Cape waters by the Agulhas current (Van der Elst 1998). Graves (1998) reported larval duration times of one month. Some juveniles appear to utilise estuaries as nursery grounds. Growth is rapid and sexual maturity is reached after one to two years; the maximum reported age is nine years. The species has medium resilience (minimum population doubling time of 1.4-4.4 years) and high fecundity. Graves (1998) also reported on seasonal migrations and tagging data indicated substantial dispersal potential (in excess of 1300 km). Wilber *et al.* (2003) reported considerable variation in year-class strength off the coast of northern New Jersey.

### *2.17.2 Genetics done*

Graves (1998) studied the relationships among the six geographically distinct populations recognised worldwide and reported higher levels of diversity than found within other cosmopolitan marine fishes (h = 0.92; $\pi$ = 1.09% based on mtDNA RFLP analysis). He found that the South African population was most closely related to the western (d = 0.38%) and eastern (d = 0.35%) North Atlantic. Graves *et al.* (1992) reported high levels of mtDNA haplotype (h = 0.7) and nucleotide diversity ($\pi$ = 1.23%) among bluefish from the northwest Atlantic. Grant and Bowen (1998) suggested that these high levels of diversity were an indication of large stable populations with a long evolutionary history. Australian bluefish showed low levels of diversity (h = 0.11, $\pi$ = 0.07%).

*2.17.3 Questions that should be addressed*

- What is the extent of population structuring based on mtDNA and microsatellites in the Benguela system?
- How do these levels compare with those reported for other populations?
- Does high dispersal potential translate into effective gene flow?
- Is there genetic evidence (based on allozymes) of selection for faster growth rates and increased survival among cohorts and if so, what is the long term impact of this?

## 2.18 *Atractoscion aequidens* (Geelbek croaker)

*2.18.1 Biology/Life History*

Geelbek is a medium-sized sciaenid and is one of the six most important linefish species in South Africa (Hutton *et al.* 2001). It is distributed along South Africa's southern and eastern seaboard, abundant in Angolan waters and also occurs off northwest Africa and eastern Australia (Smith and Heemstra 1986). The species is a shoaling predatory fish that inhabits coastal waters between 30m and 100m in depth. Sexual maturity is at 90 cm fork length that corresponds to six years of age. Geelbek is considered to be a coastal migrant and inshore waters of the Agulhas reportedly transport eggs and larvae from KwaZulu-Natal to southern Cape nursery areas (Griffiths 2000). Stock data is available for the population found of South Africa's southern and eastern seaboard indicating a single migratory stock (Griffiths and Hecht 1995, Hutton *et al.* 2001). There are three age/size related subpopulations: Juveniles are abundant in the southeastern Cape, sub-adults in the southwestern Cape while KwaZulu-Natal has an annual increase in spawning adults during winter (Hutton *et al*. 2001). The current size limit is set at 60 cm, far less than the 50% maturity that has been set for other linefish species. The latter is due to the fact that a greater size limit would have excluded most of the catches made in the SW Cape. The stock is estimated to be below 3% of pristine level (Hutton *et al.* 2001). The species has low resilience (minimum population doubling time of 4.5-14 years).

*2.18.2 Genetics done*

No information is available.

*2.18.3 Questions that should be addressed*

- What is the phylogenetic relationship between geelbek (from the Indo-Pacific) to other *Atractoscion* species from the eastern Pacific and the western Atlantic based on mtDNA and nuclear DNA sequences?
- What is the relationship between the southern and northern Benguela populations based on mtDNA and microsatellite markers?

## *2.19 Argyrosomus inodorus*, *A. coronus* (Kob species)

*2.19.1 Biology/Life History*

*Argyrosomus inodorus* (silver kob) is a medium-sized slow-growing and long-lived sciaenid. The species has been exploited for more than 150 years and is the regions' most valuable linefish species (commercially and recreationally) (Griffiths 1997a, Holtzhausen *et al.* 2001, Kirchner 2001), although it was only recently correctly classified (Griffiths and Heemstra 1995). The species' distribution ranges from the Kei River on South Africa's east coast to as far north as Cape Frio in northern Namibia although it is uncommon between Cape Point and central Namibia (Griffiths 1997a, Kirchner 2001). Along South Africa's south coast it is co-distributed with *A. japonicus*, a species that usually occurs in or just beyond the surf zone while *A. inodorus* is mostly found in deeper water (1-100 m). In Namibian waters it is mostly found in the surf zone (1-20 m). In the north of their distribution another sciaenid *A. coronus* (the West Coast dusky kob) gradually replaces *A. inodorus* (Van der Bank and Kirchner 1997). *Argyrosomus coronus* is only recorded from the southeastern Atlantic off Namibia and Angola; the species is found in estuaries, the surf zone and offshore (typically at 20-40 m).

Griffiths (1997b) described at least three separate stocks in South Africa based on growth rate, fish-length and otolith-dimension relationships, appearance of growth zones, size at maturity, sex ratios and separate spawning grounds; south-western Cape, southern Cape and south-eastern Cape. Although differences in growth rate were found between northern and southern populations in Namibia (Kirchner and Voges 1999) they are treated as one stock since tagging data suggest mixing in the central region (Kirchner 1999 as cited by Kirchner and Voges 1999). Tagging data in Namibia support the single stock concept (Holtzhausen *et al.* 2001). Tagging data from South Africa indicates that *A. inodorus* have the ability to migrate over vast distances, however most fish do not migrate more than 50km from their tagging localities. Most of the migrations that did occur still supported the three stock

concept. An offshore migration during winter occurs in all three stocks (Griffiths 1997a).

There is no indication of silver kob migration between South Africa and Namibia. From 13 000 kob tagged in Namibian waters, only two out of 171 recaptures were recorded from the west coast of South Africa (Kirchner 1999 as cited by Kirchner and Voges 1999). To date, no silver kob tagged off South Africa has been recaptured off Namibia (Holtzhausen *et al*. 2001). Adult *A. coronus* is rarely found off Namibia but abundant off southern Angola, potentially indicating a single shared stock.

Indications are that some spawning occurs throughout the year for the South African silver kob stocks but there is a clearly defined breeding season with the peak spawning during spring (September-November). Inshore distributions of adults in spring as well as the absence of larvae in the Agulhas Current suggest that spawning occurs in shallow water (<50 m depth). Shoreward currents are probably responsible for the movement of eggs and larvae towards the nursery areas that is just seaward of the surf zone. As the juveniles grow they move further offshore (Griffiths 1997a).

A spawning migration occurs in Namibian waters during summer as fish from the northern end of the distribution migrates south to Sandwich Harbour and Meob Bay, the southern end of the Namibian distribution. Larvae are transported northward by currents into the central region of their distribution that act as the nursing grounds. As the juveniles reach the age of about two years they start moving northward. Post spawning adults also migrate back to the northern area (Holtzhausen *et al* 2001). Kirchner (2001) concluded that *A. inodorus* is a migratory species with distinct areas for each part of their lifetime.

Across their distribution, catches of kob have declined (Griffiths 1997a, Kirchner and Voges 1999, Kirchner 2001). Predictions are that for the Namibian stock the current level of depletion is 39%. The total line fish recourses in Namibia is worth an estimated N$35 million and *A. inodorus* is the most important species of this industry (Kirchner 1999). The South African stocks are even worse off; Griffiths (1997c) showed that the stocks are at between 3% and 12% of pristine condition, indicating that it is not the resilient species it is believed to be, and that the current protection on size and bag limit does not provide sufficient protection. The age at first capture is three years and this can therefore lead to severe recruitment overfishing. The high number of recruits and low numbers of older adults (> seven years) in catches combined with large aggregations at predictable localities, indicate that this reasonably long-lived species ($t_{max}$ = 25 years) is vulnerable to overexploitation (Griffiths 1997a).

*2.19.2 Genetics done*

Van der Bank and Kirchner (1997) reported fixed differences between *A. coronus* and *A. inodorus* at seven allozyme loci and the genetic distance of D = 0.272 and $F_{ST}$ of 0.559 supported their status as congeneric species. Their study only reported on variation between two populations of silver kob, Terrace Bay (n=5) and Swakopmund (n=8). They found limited variation between the two populations (D = 0.013) but this result based on small sample sizes is inconclusive. Given differentiation reported between West coast steenbras populations between Meob Bay and Rocky Point (see 2.21.2), the allozyme study of silver kob should be extended to a wider area and include larger sample sizes off Namibia.

A preliminary study (n=40 from four apparent stocks) using mtDNA and microsatellites indicated a distinction between South African samples and those from Namibia; the Namibian samples were the most divergent ($F_{ST}$ between Namibia and South Africa = 0.04-0.05 based on microsatellites). Significant $F_{ST}$ values were also obtained between South African localities ($F_{ST}$ = 0.03 between Cape Infanta and Algoa Bay) suggesting reduced gene flow between the different stocks. Overall mtDNA haplotype diversity was high (h=0.988) while nucleotide diversity was low ($\pi$ = 0.008) (Morabe 2003). During 2004 the latter preliminary study will be extended to include larger sample sizes of all stocks.

Klopper *et al.* (unpublished data) are investigating the genetic relationship between juvenile *A. japonicus* from estuaries along different parts of the South African coastline using mtDNA sequences. Although mtDNA alleles appear to be shared between different parts of the coast, the results may indicate the presence of mixed stocks. Spawning areas off KwaZulu-Natal (winter/spring) and the eastern Cape (spring/summer) where previously suggested by Griffiths (1996) and indications were that juveniles recruited to estuaries at an age of four weeks.

*2.19.3 Questions that should be addressed*

- What are the phylogenetic relationships among *Argyrosomus* species, with specific emphasis on *A. coronus*, *A inodorus*, *A. japonicus* and *A. thorpei* based on genetic markers (allozymes and mtDNA sequences; perhaps also including nuclear gene sequences)?
- Do larger sample sizes indicate a genetic basis for growth differences between silver kob from northern and southern Namibian fishing areas and for morphological

differences between South African silver kob stocks? Genetic markers that can be included are allozymes (see Van der Bank and Kirchner 1997), mtDNA and microsatellites (see Morabe 2003).

- Do genetic markers (as above) support the lack of exchange between Namibian and South African silver kob stocks based on coalescent modeling of gene flow?
- Do genetic markers (as above) support the suggestion of a transboundary population of West coast dusky kob between northern Namibia and southern Angola?

## 2.20 *Diplodus sargus* (Cape white seabream/black tail/dassie)

### *2.20.1 Biology/Life History*

*Diplodus sargus* is a tropical reef associated species. The Cape white seabream (*D. s. capensis*) ranges from Angola to Mozambique and southern Madagascar. It is primarily an inshore species (< 30m) preferring rocky shores, but can also occur on deeper reefs. Sexual maturity is attained at 22 cm FL or four years of age and the maximum recorded age is 21 years. The species has medium resilience (minimum population doubling time of 1.4-4.4 years). Only a part of the male population undergoes sex change. Spawning occurs almost all year round (May – December) but with a peak period of July-September in KwaZulu-Natal and October-December in the southern Cape. Juveniles inhabit tidal pools and inshore reefs and estuaries as nursing grounds (Smith and Heemstra 1986, Van der Elst 1998, Whitfield 1998).

### *2.20.2 Genetics done*

No information is available.

### *2.20.3 Questions that should be addressed*

- What are the phylogenetic relationships between *Diplodus* species based on mtDNA and nuclear gene sequences?
- Does *D. sargus capensis* represent a single transboundary population between BCLME countries based on mtDNA and microsatellite markers?

## 2.21 *Lithognathus aureti* (West coast steenbras)

### *2.21.1 Biology/Life History*

*Lithognathus aureti* is one of three *Lithognathus* species inhabiting southern African waters. It ranges from Cape Point to Angola (13°S to 28°S; Smith and Heemstra 1986) but is more abundant from Meob Bay and especially from Walvis Bay northwards. Big shoals of mature fish form in shallow water during spring and summer (September-December), the spawning season, a time when the species is most vulnerable to exploitation. They occur over sandy seabed areas in the shallow surf zone as well as in deeper waters (Van der Elst 1998). The species is a protandrous hermaphrodite with some individuals developing male and female gonads simultaneously (Smith and Heemstra 1986, Holtzhausen *et al.* 2001). It is a long-lived species that can reach an age of 50 years and a size of 100 cm (Holtzhausen and Kirchner 2001). Sexual maturity is reached at 5-10 years and the species has low resilience.

The species is a sought after target mostly for recreational angles but also for commercial skiboats and lineboats (Van der Bank and Holtzhausen 1998/99). It is the second most (only after *A. inodorus*) important species caught by shore-anglers and the third most landed species by commercial fisherman off Namibia (Holtzhausen and Kirchner 2001). Best estimates are that *L. aureti* is at 42% of pristine levels. Tag-recapture data show two isolated populations, a small closed population at Meob Bay and a wider distributed northern population from Sandwich harbour northwards. The results showed that individuals from the northern region moved considerable distances (mostly males) while those from the southern population were mostly recaptured at the same locality (Beyer *et al.* 1999). Distinct differences exist between the Meob Bay population and the northern population including differences in growth rates, otolith morphology, size at maturity, sex ratios and length-at-age (Holtzhausen *et al.* 2001). Sea temperatures and food availability are said to account for some of the differences observed in growth and age between the two populations (Holtzhausen and Kirchner 2001).

*2.21.2 Genetics done*

Van der Bank and Holtzhausen (1998/99) reported on a preliminary study of allozyme differentiation between two *L. aureti* populations off Namibia, Meob Bay (n=55) and Rocky Point (18°59'S, n=83). Although no fixed allelic differences were found, two loci showed significant genotypic frequency differences between these areas. The $F_{ST}$ of 0.014 indicated reduced gene flow between the two populations. The reduction in gene flow may be related to changes in circulation and turbulence off the Meob Bay region. This region is believed to represent a biological discontinuity for several species (Agenbag and Shannon 1988 as cited by Van der Bank and Holtzhausen 1998/99). The reported genetic distance value (D = 0.002) was however very low and should be reevaluated within the context of variation across the entire distributional range of the species as well as by taking into consideration the potentially recent diversification between *L. aureti* and *L. lithognathus*.

*2.21.3 Questions that should be addressed*

- What are the phylogenetic relationships between *Lithognathus* species with special emphasis on the closely related southern African endemics, *L. aureti* and *L. lithognathus* based on allozymes, mtDNA and nuclear gene sequences?
- What is the extent of genetic differentiation among West Coast steenbras across the distribution range of the species (Angola to South Africa) based on allozyme, mtDNA and microsatellite markers? How does this compare with differentiation in the white steenbras and differentiation between the two species?
- How do homing and migratory behaviour impact on the population structuring within *Lithognathus* species?
- What is the influence of hermaphroditism on genetic structuring?

## 2.22 *Brachydeuterus auritus* (Bigeye grunt)

*2.22.1 Biology/Life History*

Very little information is available. Bigeye grunt are benthopelagic inhabiting coastal waters up to 100 m in depth. The species is tropical in distribution (27°N - 13°S) in

the Eastern Atlantic from Morocco to Angola. It is of high commercial value and medium in resilience.

*2.22.2 Genetics done*

Mitochondrial DNA data for French grunt from the Caribbean showed high levels of haplotype diversity (h = 0.78) but relatively low levels of nucleotide diversity ($\pi$ = 0.62) potentially indicating population growth after a time of low effective population size (Shulman and Bermingham 1995 as cited in Grant and Bowen 1998).

*2.22.3 Questions that should be addressed*

- What are important life history aspects that should first be studied before genetic analysis is planned?
- What are the phylogenetic relationships between grunt species based on mtDNA and nuclear genes?
- Is there a shared stock within the BCLME region?

**2.23 *Thunnus alalunga* (Albacore), *T. albacares* (yellowfin tuna)**

*2.23.1 Biology/Life History*

Scombrids are fast epipelagic predators, many species form large schools and they are very important food fishes. Albacore tuna are cosmopolitan between 45°-50°N and 30°-40°S. In the BCLME region, the species is mostly caught by longline fishers off the western Cape (Smith and Heemstra 1986). In the South Atlantic albacore has been exploited since the 1950's where it is managed separately from the North Atlantic stock. Punt *et al.* (1995) estimated that the stock was significantly depleted, to 20% of pre-exploitation levels. Tagging data found no evidence of movement between the North and South Atlantic albacore stocks whereas continuous catches off southern Africa may indicate exchange between the South Atlantic and the Indian Ocean stock (Punt *et al.* 1995).

Yellowfin tuna occurs worldwide in tropical and subtropical seas (rarely in areas with temperatures below 18°C). The species is abundant off Angola and also occurs around the South African coastline where it is caught by longlining off the Cape and by anglers in KwaZulu-Natal (Smith and Heemstra 1986). Spawning occurs

throughout the year. The species exhibits rapid growth, reaches maturity at two years and may live up to eight years. Tagging data show that the species can move over vast distances including trans-Atlantic migrations but most fish only move a few hundred kilometers (Graves 1998). Most migrations tend to be within rather than between regions (Appleyard *at al*. 2001 and references therein).

*2.23.2 Genetics done*

Mitochondrial DNA did not reveal significant differentiation between albacore tuna from the Bay of Biscay and Brazil (ICCAT 1995 as cited in Punt *et al.* 1995). Graves (1998) analysed yellowfin tuna from five Pacific and one Atlantic sites using mtDNA RFLPs and found high levels of haplotype diversity (h = 0.82-0.87) but low levels of nucleotide diversity ($\pi$ = 0.28-0.39%). Some haplotypes were shared among oceans showing a lack of within and between population structure. Chow *et al.* (2000) used RFLP analysis of three mtDNA regions to compare Atlantic and Indo-Pacific stocks of bigeye tuna (*T. obesus*). They reported haplotype frequency differences between these stocks indicating that migration between the Atlantic and Indian Ocean is restricted; their results confirmed earlier suggestions that the distinct stocks mix off the coast of South Africa. The limited presence of Indo-Pacific haplotypes in the south Atlantic and the lack of Atlantic haplotypes in the Indo-Pacific suggest that mixing off South Africa is either influenced by die Agulhas current or by philopatric behaviour of the tuna. Chow and Ushiama (1995) used a similar but slightly less sensitive RFLP test and reported a similar result for albacore. Most studies did not report local scale differentiation within ocean basins (e.g. Pujolar *et al.* 2003 failed to detect heterogeneity in allozyme allele frequencies among five albacore populations in the Mediterranean and East Atlantic). Takeyama *et al.* (2001) developed a specific RFLP test for species identification of *Thunnus* species. Several independent research groups recently developed microsatellite loci for tuna (mostly for *T. thunnus* but demonstrating the cross amplification of these loci in yellowfin, albacore and bigeye tuna; see Clark *et al.* 2004 and references therein). Appleyard *et al*. (2002) reported mtDNA and microsatellite variation and found west versus east Indo-Pacific differentiation based on mtDNA but they could not reject the null hypothesis of panmixia. Appleyard *et al*. (2001) documented microsatellite variation of yellowfin tuna in the western and eastern Pacific. They found no correlation between genetic variation and geographic distance. The overall $F_{ST}$ (0.002) was very low but significant.

*2.23.3 Questions that should be addressed*

- What are the mixing rates between Indo-Pacific and Atlantic stocks based on representative spatial and temporal sampling within the BCLME region and from the Agulhas current (especially including the retroflection zone)? A combination of the diagnostic mtDNA RFLP previously developed and microsatellites should be used.
- Can mixing rates off South Africa and unidirectional gene flow from the Indo-Pacific to the Atlantic be quantified using microsatellite markers?

## 2.24 *Loligo vulgaris reynaudii* (Squid)

*2.24.1 Biology/Life History*

*Loligo vulgaris reynaudii* occurs from southern Namibia to East London on the south coast of South Africa. The species is closely related to the European and West African *L. vulgaris vulgaris* (Augustyn *et al*. 1992).

On average *Loligo* lives for one year while large males may live up to 18 months (Lipinski 1992). The species is characterised by a long larval duration (up to five months of their life span) of which a major part is a passive planktonic phase (Augustyn *et al*. 1992). As predator avoidance, the species tend to be more dispersed on their feeding grounds but are more aggregated on their spawning grounds (Sauer and Smale 1991). Cephalopods are dynamic predators and are highly adapted to unfavorable conditions (Lipinski 1992).

A tag recapture study by Sauer *et al.* (2000) showed that squid move significant distances during the protracted spawning season during which eggs are deposited at a number of spawning sites. A mean distance of 43 km was covered over a period of 14 days. A distance of up to 203 km was recorded. Adults tend to move eastwards along the South African coast during their spawning migration (Augustyn 1990) although a more random pattern mediated by food availability and environmental conditions was reported for squid from the central parts of the south-east coast of South Africa (Sauer *et al*. 2000).

Spawning occurs in inshore areas (< 50 m deep) and is most intense during spring and summer (October-December) between Plettenberg Bay and Algoa Bay. Larvae are transported westwards by the Agulhas (Augustyn 1990). The is no evidence of spawning on the West Coast of South Africa north of Cape Town (Augustyn *et al*. 1992).

Two life history aspects that impact significantly on the management of the species are its short life cycle and the stochastic nature of spawning and recruitment (Augustyn *et al*. 1992). Catches of *L. v. reynaudii* in the southern Benguela appear to have remained relatively stable over a 10 year period (1980-1991; Lipinski 1992, Augustyn *et al*. 1992). The jigging industry has become the dominant fishery for squid since 1985.

### *2.24.2 Genetics done*

Augustyn and Grant (1988 as cited by Augustyn *et al*. 1992) found minimal genetic differentiation between *L. vulgaris* subspecies with gene pools potentially isolated by a barrier off Namibia. Their results however suggested a single transboundary population. No additional genetic studies of squid within the BCLME have thus far been published although research by Shaw and Sauer is in press.

Allozyme studies on squid have generally revealed low levels of differentiation ($H_O$ = 0.000-0.069) (see Shaw *et al*. 1999, Reichow and Smith 2001 and references therein). Microsatellite markers have recently been applied to a number of *Loligo* species. Shaw *et al*. (1999) studied population structuring in the veined squid *L. forbesi* and reported higher levels of genetic variation based on microsatellite loci compared with previous studies using allozymes and mtDNA markers. In agreement with these markers they found divergence between the Azores and populations off the European shelf of the NE Atlantic ($F_{ST}$ = 0.245). But in contrast to previous studies, they also reported subtle structuring among NE Atlantic offshore banks and the shelf population ($F_{ST}$ = 0.002-0.006). Water depth and currents appear to be effective barriers to migration. Reichow and Smith (2001) used microsatellite markers to assess differentiation among California squid *L. opalescens* from different spawning grounds and over several spawning seasons. Their data suggested that extensive gene flow prevented genetic differentiation in the species (overall $F_{ST}$ = 0.0028). The authors however recommended continued genetic monitoring of the population to allow more detailed estimates of migration, genetic changes over time and effective population size. Emery *et al*. (2001) used microsatellites to study paternity of veined squid hatchlings.

*2.24.3 Questions that should be addressed*

- Are squid continuously distributed from southern Namibia to the east coast of South Africa and does the species display any variation in terms of morphology, growth rates and seasonality?
- What are levels of within and between population genetic diversity based on microsatellite markers?
- Do data collected over multiple years indicate any fine-scale changes in allele frequencies?
- What is the relationship of *L. v. reynaudii* to *L. v. vulgaris*?

**Rock lobster fisheries**

## 2.25 *Jasus lalandii* (Spiny lobster/ West coast rock lobster)

*2.25.1 Biology/Life History*

*Jasus lalandii* occurs in the littoral zone off the southern African west coast (from 23°S to 28°S; highest densities are recorded from 25°S to east of Cape Point) while a closely related species, *J. tristani* occurs along the Tristan da Cunha Island, Gough Island and the Vema seamount. All spiny lobsters have phyllosoma larvae that can disperse over wide distances over lengthy periods (including 11-25 larval moult stages). The final larval stage is triggered by specific environmental factors and the resulting fast swimming small spiny lobster shows directional swimming "homing" to specific populations across the continental shelf (Pollock and Melville-Smith 1993). Each year West coast rock lobster larvae hatch in spring and most are dispersed offshore where they enter the gyral system of the South Atlantic. The specific environmental triggers that ensure that *J. lalandii* settle on the west coast and *J. tristani* at the oceanic islands, have not yet been identified. The average number of eggs produced by a female was estimated to be 1 x $10^6$ for the West Coast rock lobster and 0.3-0.5 x $10^6$ for the Tristan rock lobster. These species have a long lifespan (30-40 years of egg production) and the high fecundity estimates probably counter the high mortality due to the extended pelagic larval phase as well as lack of recruitment of the postlarval stage (Pollock and Melville-Smith 1993).

Utilization of the species date back to the Holocene while commercial catches commenced in the 1950's. The resource has been very stable until 1989 when a

major environmental anomaly triggered reduced growth rates (Cockcroft and Payne 1999). Past tagging studies suggested the recognition of three discrete stocks: Namibia, west coast of South Africa and south coast of South Africa although the stocks may not be isolated due to larval recruitment (Pollock 1986).

*2.25.2 Genetics done*

Ovenden *et al*. (1991) used mtDNA RFLP analysis to study genetic diversity in *J. edwardsii* and despite high levels of diversity could not detect any genetic subdivision across 4600 km of southern ocean habitat. It was proposed that genetic homogeneity is maintained through larval dispersal (the species has a larval duration of 6-23 months). Endemann *et al*. (unpublished data) are currently working with MCM and NatMIRC staff to study genetic stock structure of West Coast rock lobster. Thus far samples from eight sites have been analysed using allozymes and mtDNA 16S rRNA sequences. Allozyme analysis showed low levels of differentiation between sites ($F_{ST}$ = 0.007) suggesting the presence of a single stock. MtDNA revealed high haplotype diversity (16 unique alleles among 24 individuals) but the levels of within population variation (96.47% of the overall variance) was much higher than between populations (3.53%).

*2.25.3 Questions that should be addressed*

- Do microsatellite markers confirm the lack of differentiation between rock lobster populations as suggested by allozyme and mtDNA markers?
- What is the extent of gene flow based on modeling of genetic data?
- What is the significance of divergent mtDNA alleles as detected by Endemann *et al*.?

**Beach seine and gillnet fisheries**

**2.26 *Liza richardsonii* (South African mullet)**

*2.26.1 Biology/Life History*

Southern mullet is an estuarine dependant endemic species that ranges from northern Namibia to KwaZulu-Natal, although not abundant in the warmer waters east of East London. Juveniles and adults are frequently found in estuaries but are

more abundant in the nearshore environment. Dense shoals can be found in the cooler waters of the southwest Cape coast (Smith and Heemstra 1986, Van der Elst 1998). Sexual maturity is at 18 cm and the species can attain a maximum-recorded length of 32 cm. Spawning occurs close inshore during September to March (Whitfield, 1998). Shore-based fishers (small scale commercial and subsistence) from Namibia and South Africa exploit mullet (some 10 000 tons annually). Morphological variability has been observed between estuarine and marine sub-populations and between coasts (Lamberth pers. comm.).

*2.26.2 Genetics done*

No information is available. Rossi et al. (1998) reported allozyme variation among global populations of the striped mullet *Mugil cephalus*. Several populations showed fixed allelic differences. Average genetic distance was D = 0.117. High rates of gene flow were estimated among Mediterranean populations but extremely low levels among disjunct populations in the Indian, Pacific and Atlantic oceans.

*2.26.3 Questions that should be addressed*

- Do estuarine and marine subpopulations represent separate stocks?
- Are different stocks present on the west and south coast?
- Do morphological variation in the species reflect species or stock level separation?

<u>Recreational molluscs and crustaceans</u>

Recreational fisherman utilise Octopus species, mussels, oysters and other mollusks and prawns (Booth and Hecht 2000). Many of these species are also exploited through mariculture and although outside the scope of the present report, it is recommended that the relationships between natural and mariculture stocks should be monitored genetically. Little biological information is available for many of the taxa but genetic data ( not reported here) are available for mussels, oysters, prawns and shrimps.

## 2.27 *Octopus vulgaris* (common octopus)

### *2.27.1 Biology/Life History*

The common octopus is probably the most widespread cephalopod species, found worldwide in tropical and subtropical waters in shallow water up to depths of 200 m. The species has a fast growth rate and reaches sexual maturity within a few months. As in squid, the lifespan appears to be short (12-18 months). The species apparently spawns throughout the year but with peaks in spring and summer. Fecundity is high; there is no larval stage but the small octopus are planktonic for 2-3 months after hatching. Following this period they become benthic and mostly active nocturnally. The species may migrate offshore during winter (information from CephBase and Smith and Griffiths 2002).

Morphometric analysis of octopus from False Bay showed similar trends to studies conducted on the northwest coast of South Africa, the east coast of South Africa and in the Mediterranean, suggesting that all these areas represent the same species (Smith and Griffiths 2002).

### *2.27.2 Genetics done*

No information is available. Greatorex *et al*. (2000) isolated microsatellite markers for the species to allow investigation of population structuring.

### *2.27.3 Questions that should be addressed*

- Which aspects of the life history of *O. vulgaris* are priorities for research in terms of an expansion of utilization of the species in the BCLME?
- What are the phylogenetic relationships between *Octopus* species and within *O. vulgaris* based on mtDNA and nuclear gene sequences?
- What are the relationships between *O. vulgaris* populations within the BCLME region based on microsatellite markers?

## 3. NON-EXPLOITED RESOURCES OF THE BCLME REGION

The principal focus of this report has been on currently exploited resources. There are however several species where fisheries may develop, for example, *Sepia australis*. For such resources it is **recommended** (6.4) that relevant biological and life history information should be gathered. Assessment of pre-exploitation levels of genetic variation and stock structuring would contribute to future sustainable utilization and to set baselines for future monitoring.

More generally, genetic studies can make a significant contribution to our understanding and conservation of marine biodiversity. Gibbons (2000) reviewed the state of local taxonomic knowledge and expertise for metazoan taxa. From the review it is clear that many marine groups have never been studied in South Africa and there is a clear need to develop local expertise and to implement a holistic approach (incorporating morphology, behaviour, ecology and genetics) in taxonomic and systematic studies.

The BCLME region is also of particular interest as it includes at least four biogeographic provinces: the Tropical West Coast off southern Angola, the Cool Temperate North-West Coast north of Luderitz, the Cool Temperate South-West Coast south of Luderitz and north of Cape Point, the Warm Temperate South Coast from Cape Point to East London (see Emanuel *et al.* 1992, Turpie *et al.* 2000 and references therein). Genetic studies can contribute to the evaluation of biogeographic hypotheses. Furthermore, phylogeographic boundaries (i.e. genetic boundaries within species) may correspond with biogeographic boundaries (see for example Avise 1992, Bernardi 2000). Future protection of representative areas (such as marine reserves) within these unique provinces could therefore ensure that not only species diversity, but also genetic diversity is preserved; both are fundamental components of biodiversity (see Convention on Biological Diversity). Phylogeographic and landscape genetic approaches also enable testing of hypotheses regarding processes that have generated these levels of biodiversity, enabling management decisions that will preserve future potential for ecological and evolutionary adaptation (Manel *et al.* 2003, Knowles 2004).

## 4. CAPACITY IN THE NATIONAL MARINE RESEARCH INSTITUTES AND FACILITIES TO CARRY OUT GENETIC STOCK LEVEL ANALYSIS

***TOR:***

- ***Capacity (or lack thereof) in the national (marine) research institutions (government and academic) and facilities to carry out stock level genetic analyses necessary in BCLME region and potential international partners***

Several local research groups are already actively involved in marine genetic projects:
Prof. Herman van der Bank, RAU (various linefish and marine invertebrate taxa)
Prof. Mark Gibbons, UWC (demersal fish and plankton, marine invertebrate taxonomy in general)
Dr. Eugenia D'Amato, Stellenbosch University Genetics (Abalone, rock lobster, pelagic fish)
Dr. Conrad Matthee and Dr. Raurie Bowie, Stellenbosch University Zoology (Rock lobster, marine mollusks)
Dr. Colleen O'Ryan, UCT (Harmful algal blooms)
Prof. Warwick Sauer, RU (Cephalopods)
Proffs. Christopher McQuaid and Nigel Barker, RU (Mussels and estuarine herring)
Prof. Paulette Bloomer, UP (Linefish and marine invertebrates)

Facilities at these universities include capacity for allozyme electrophoresis as well as DNA based technologies (PCR, RFLP, DNA sequencing, SSCP, AFLP). The Centro nacional de Recursos Fitogeneticos, University Agostinho Neto, Luanda has the capacity to conduct allozyme work and will soon be able to do DNA based PCR analyses and collaborations can be fostered with IIM. NatMIRC has allozyme electrophoresis equipment. MCM conducts all genetic work in collaboration with academic institutions.

Southern Africa has the potential to conduct stock level analyses as required within the BCLME region and as outlined in section 2. This potential include the laboratories and the theoretical, technical and analytical expertise (although the latter is still in need of considerable development). Several of the Universities have world class automated DNA sequencing facilities necessary for high throughout sequencing and microsatellite analysis; these include facilities at UCT, Stellenbosch, Rhodes and Pretoria. Through partnerships, these facilities will also be available to other participating institutions. It will be possible for fisheries scientists to interact directly with research groups at universities, for example, Arved Staby indicated his interest in getting involved in the genetic analysis of orange roughy, Heidi Skrypzeck has an interest in bronze whaler genetics and Craig Smith is

planning a PhD study on swordfish in collaboration with Marc Griffiths and Paulette Bloomer. Several potential international partners have been identified: Gary Carvalho, Bob Ward and Nick Elliott (CSIRO), Peter Smith, Stewart Grant (Appendix II, see also section 6).

It is **recommended** (6.1) that a meeting should be convened as soon as possible to pull together all the BCLME expertise necessary to address genetic stock identification for a selection of species to serve as a pilot investigation. This meeting should include not only geneticists but also fisheries scientists, managers and oceanographers. Several international scientists have indicated their willingness to participate in such a meeting. The major objective of the meeting should be the development of a short-term strategy for genetic stock identification in the region. A short term funding proposal should be drafted to fund a pilot investigation. The choice of species to include in the pilot study will be crucial.

In terms of the other resources it is **recommended** (6.3) that a small task team for each species/group of related resources should be formed to collate all relevant information on each species. Each team should formulate recommendations for long-term sampling needs as well as for life history and biological research.

## 5. TRAINING NEEDS AND OPPORTUNITIES FOR GENETICS IN THE BCLME COUNTRIES

**TOR:**

- ***Training needs / opportunities for genetics in marine science***

Marine science training is offered at many institutions in BCLME countries and several courses emphasize living marine resource management. Generally courses are offered at both undergraduate and postgraduate levels:

University of Namibia, Dept of Natural Resources and Conservation (jpmsangi@unam.na)
Dept of Zoology (Biodiversity and Conservation Biology Programme), UWC (www.uwc.ac.za/zoology/index.htm)
Marine Biology Research Institute, Dept of Zoology, UCT (www.zoology.uct.ac.za/docs/courses.html)
Dept of Zoology, UPE (www.zoo.upe.ac.za)
Rhodes University, Dept of Zoology and Dept of Ichthyology and Fisheries Science (www.ru.ac.za/academic/calender2003/zoology.html)

(www.ru.ac.za/academic/departments/difs)
School of Life and Environmental Sciences, University of Natal
(www.nu.ac.za/sles/honours/default.htm)
Dept of Zoology, RAU (general.rau.ac.za/zoology)
Staff from SAIAB and ORI also participate in training at Rhodes University and the University of Natal respectively.

Opportunities for genetic training exist at several of the above institutions (UWC, RU, NU Pietermaritzburg) as well as some other Universities:
Centro nacional de Recursos Fitogeneticos, University Agostinho Neto, Luanda (fitogen@ebonet.net)
Dept of Molecular and Cell Biology, UCT (www.mcb.uct.ac.za)
Dept of Genetics (www.sun.ac.za/genetics) and Zoology Dept, Stellenbosch University (www.sun.ac.za/zoology)
Dept of Zoology & Entomology (www.up.ac.za/academic/zoology/top.htm) and Dept of Genetics, University of Pretoria (www.up.ac.za/academic/genetics)

There is however a need to integrate genetic training into marine science education at both undergraduate and postgraduate levels (see 6.1). Also, more linkages should be encouraged between the three BCLME partner countries. Both Namibia and Angola have strong relationships with European based institutions, but more students and researchers could be encouraged to collaborate with South African institutions. Interaction between the above institutions should also generally be encouraged.

## 6. RECOMMENDATIONS (General recommendations follow; also refer to section 1.4.2: Recommendations for sampling)

1. A pilot study should be developed through consultation among managers, fisheries scientists, marine biologists, oceanographers and geneticists. Three to four species should be selected to represent different life history patterns. Special consideration should be given to larval duration, age at maturity, longevity, migratory behaviour and morphological variability. Sampling should in particular focus on current boundaries, upwelling zones and spawning grounds. The feasibility of sampling different cohorts should be considered. Stocks should be defined on a holistic basis (incorporating genetic, morphological, behavioural and ecological data).
2. Depending on the outcome of the pilot investigation, a comprehensive proposal should be developed to address genetic identification of transboundary populations within the other priority resources.
3. A conference should be held to bring together managers, fisheries scientists, marine biologists, oceanographers and geneticists to discuss planning and priorities. In preparation participants should be tasked to compile comprehensive summaries of all available data for each species.
4. Funding should be sought to address other general issues in the region: Compliance enforcement, species identification kits, biogeographic zones and the distribution of genetic diversity (a focus on marine biodiversity rather than just a focus on exploited resources), the location and efficacy of marine protected areas, the use of archived material to address the impact of exploitation on genetic diversity and long-term viability of marine resources, the application of molecular techniques to pressing ecological questions.
5. Exchange of researchers between BCLME countries as well as among local institutions and overseas laboratories should be considered.
6. Training in genetics should be integrated as part of marine science education.

**Summary of recommendations by overseas experts (see also Appendix II):**

**Peter Smith**: Larval duration or pelagic phase duration are important factors in determining genetic structure. Sampling should be conducted at oceanic/current boundaries. Several species with different life histories should be sampled across the same range including current boundaries as a pilot study.

**Gary Carvalho**: From the outset there should be careful coordination and consultation. Genetics should be given a high priority as part of long term fisheries

related research. In the past poor sampling design, inappropriate genetic markers and genetics done in isolation of other methods have limited the contribution it could have made to fisheries management. Large sample sizes (around 100 fish per site) should be sampled preferably from spawning individuals. Temporal stability of spatial patterns should be tested. Statistical methods should include mixed stock analysis and assignment tests. Factors determining variance in reproductive success should be examined among cohorts.

**Bob Ward**: A combination of genetic approaches should be used (including mtDNA, microsatellites and allozymes). Sample sizes should have a minimum of 50 but preferably 100 fish per site. Fish near the point of spawning should be used (preferably 0 class individuals or at least fish from a single cohort). Temporal sampling from sites should be included. A lack of genetic differentiation should be tested to distinguish no mixing from some mixing.

**Stewart Grant**: Based on previous results a coast wide survey of mtDNA variation in anchovy should be undertaken. The preferred technique for management purposes should be microsatellites. When dealing with phylogeography, sampling should extend outside southern African waters.

## 7. TERMS OF REFERENCE FOR FUTURE GENETIC STOCK LEVEL ANALYSIS

**Objective**

The specific objectives of a pilot study applying genetic techniques to determine fish stock identity of transboundary populations in the BCLME are to:

1. Through consultation between managers, fisheries scientists, statisticians and geneticists, select several species representative of different life histories for study;
2. To sample these species at a spatially and temporally appropriate scale including important features of the Benguela such as current boundaries and strong upwelling areas;
3. To analyze samples using appropriate markers for the questions posed;
4. To statistically determine genetic boundaries and quantify the extent of gene flow;
5. To incorporate genetic data with other stock level and biological data to formulate recommendations for management of these resources.

**Scope**

The research team should consist of all the relevant BCLME managers and fisheries scientists, statisticians and geneticists and the team should undertake to do the following:

1. Organise a planning meeting at the earliest convenience to address the choice of species for the pilot study and to address sampling and funding needs for these species and in general for BCLME resources. In preparation for such a meeting all the relevant information on each species should be collated.
2. Conduct sampling and at the same time initiate laboratory optimization of the relevant genetic markers and test existing genetic data modeling approaches.
3. Conduct genetic analyses within a reasonable time frame.
4. Continually liaise as a team during the data analysis phase.
5. Meet again to bring together all the data and formulate recommendations.

**Outputs**

1. Regular interaction between managers, fisheries scientists, statisticians and geneticists within the BCLME region.
2. A comprehensive proposal for the pilot study and following from that a comprehensive proposal for the integration of genetics in stock identification in the BCLME region.
3. Reports and scientific papers for each species.

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**APPENDIX I**

**MEETINGS WITH LOCAL FISHERIES AUTHORITIES AND IDENTIFICATION OF PRIORITY SPECIES FOR FUTURE APPLICATION OF GENETIC METHODS FOR STOCK IDENTIFICATION OF TRANSBOUNDARY POPULATIONS**

One of the most important components of the project was the personal visits and interviews with managers from all three fisheries departments. The following summarizes the visits:

30-31 July MCM [Short discussions with: Johann Augustyn, Andy Cockcroft, Renee Osborne, Steve Lamberth, Carl van der Lingen, Craig Smith, Marek Lipinski and Larry Hutchings]; UCT [Colleen O'Ryan]; subsequent correspondence with Rob Leslie and Doug Butterworth.

1 August University of Stellenbosch [Dept of Genetics, Eugenia D'Amato; Dept of Zoology, Conrad Matthee]

2 August-7 August BENEFIT [Neville Sweijd, Filipe Vianda], NatMIRC [We first had a general meeting between Neville Sweijd, Ben van Zyl, Angie Kanandjembo and Titus Iilende. This was followed by a short general presentation and discussion with a large number of NatMIRC staff. Then personal meetings with: Helen Boyer, Anja Kreiner and Beau Tjizoo; Hannes Holtzhausen and Heidi Skrypzeck; Titus Iilende; Fabian Haufiko; Angie Kanandjembo; Peter Schneider; Arved Staby], MFMR [Brief discussion with Moses Maurihungirire; meeting with Burger Oelofsen], University of Namibia [Dept of Natural Resources, Prof Msangi, Lineekela Kandjengo], Meeting with Phoebe Barnard; subsequent correspondence with Jean-Paul Roux and Kolette Grobler.

2-4 October IIM and BCLME [short personal meeting with Victoria de Barros Neto; informal discussion with Filomena Vaz Velho and Milu Sardinha; presentation and discussion attended by eight representatives of IIM, one from BCLME and four from the University], University Agostinho Neto [meeting at Herbario de Luanda attended by Filomena, Milu and Elizabeth Matos (CNRF), Fernanda Lages (Faculty of Science), Francisca Delgado (IIM), Julia Ferreira (IIM)]

Based on the visits to MCM, NatMIRC, BENEFIT and IIM, the exploited species in need of investigation of genetic stock structure were identified (Table 1).

**Table 1 BCLME exploited marine resources identified for application of genetic stock identification (√√ - highest priorities, √ - listed as deserving attention, √ - suggested but not strongly)**

| Species | MCM | NatMIRC | IIM |
|---|---|---|---|
| Hake species | √√ | √√ | √ |
| Horse mackerel spp. | √ | √ | √√ |
| Anchovy | √ | √ | √ |
| Pilchard | √ | √√ | √√ |
| Sardinella | | | √ |
| Roundherring | | | √ |
| Linefish: Silver kob | √ | √ | √ |
| West coast steenbras | √ | √ | |
| Geelbek | √ | | √ |
| Snoek | √ | √ | |
| Elf/Shad | √ | | |
| Harder | √ | | |
| Galjoen | √ | √ | |
| Leervis | √ | | |
| Dassie | √ | | |
| Bronze whalers | | √√ | √ |
| Other: | | | |
| Kingklip | √ | √ | |
| Tuna | | | √ |
| Bigeye grunt | | | √ |
| Dentex | | | √ |
| Orange roughy | | √ | |
| Alphonsino | | √ | |
| Monk | | √ | |
| Invertebrates: | | | |
| Deep sea red crab | | √√ | √√ |
| Shrimp | | | √ |
| Cephalopods | √ | | √ |
| Rock lobster | √ | √ | √ |
| Brown mussel | √ | √ | |

## APPENDIX II
## LETTERS TO AND FROM INTERNATIONAL EXPERTS

Subject: Application of genetic techniques for determining fish stock identity
Date: Wed, 08 Oct 2003 12:10:59 +0200
From: Dr P Bloomer <pbloomer@postino.up.ac.za>
To: Bob.Ward@csiro.au, g.r.carvalho@hull.ac.uk, spalumbi@stanford.edu, gold.sh@tamu.edu, robin.waples@noaa.gov, lhauser@u.washington.edu, hutton@zoology.ubc.ca, c.lundy@uea.ac.uk, p.smith@niwa.cri.nz, footec@mala.bc.ca, hermit@tokyo-u-fih.ac.jp, john@hudsonriver.org

Dear Colleagues, I have been contracted by the Benguela Current Large Marine Ecosystem (BCLME) programme to do a short feasibility report on the application of genetic techniques for determining fish stock identity of transboundary populations in the BCLME region. I have consulted recent reviews authored by many of you in this regard and the reason for me writing to you now is that we want to learn from your experience and in some instances you may be interested to become partners in future investigations that may be undertaken.

For your background, the specific objectives and scope:
The specific objectives of project LMR/CF/03/04 are to critically review the work on stock identity / separation which has been attempted in the BCLME region, to assess the applicability of the previous techniques used (and possible new techniques) as tool for management of stocks which straddle or migrate across national boundaries between countries, and to assess human resources and training needs for the application of genetic techniques in the region. " "Scope:
· Review results of previous genetic studies conducted to identify transboundary stocks of exploited marine resources in the BCLME region (and elsewhere in southern Africa)
· Identify other exploited and non-exploited resources in the BCLME mandate that might require application of genetic stock level analysis
· Identify suitable capacity (or lack thereof) in the national (marine) research institutions (government and academic) and facilities to carry out stock level genetic analyses necessary in BCLME region and potential international partners
· Identify training needs / opportunities for genetics in the BCLME countries"

There hasn't been many studies of this nature in southern Africa and some studies that have been published have suffered from very limited geographic sampling or too small sample

sizes. There is a clear need from management's side for the use of genetic techniques to add to other tools that they are already employing extensively in stock identification. The identification of stocks within transboundary populations are relevant to a long list of species, amongst others: Hake; horse mackerel; Clupeids and Engraulidae, members of the Sciaenidae, Carangidae, Coracinidae, Gempylidae, Lutjanidae, Mugilidae, Pomatomidae, Scombridae, Ophiidae and Sparidae; Orange roughy; deep sea red crab, various cephalopods, lobsters, shrimps and mussels.

Following meetings with the marine managers in all three countries (South Africa, Namibia and Angola), I have a strong feeling that we will need to undertake in-depth planning in terms of appropriate sampling strategies for all the species. There may also be some instances where genetic techniques may not answer the questions posed. Fortunately the region has been studied in depth from a physical oceanographic point of view and we can be guided by that.

If you could take the time to consider the background and to send me any advice you could offer us at this stage, I would really appreciate it. If there are any of the species/families on the list that appeals to your personal interests, please indicate that. The general consensus here is to rather plan a comprehensive collaborative project rather than to split off the separate projects for each species. Also, it may not be financially feasible to start with too many species, but again the sampling can start for all resources. We may be able to plan a mini-symposium to get together fisheries biologists, fisheries managers, geneticists, oceanographers. Would any of you consider contributing to such a planning meeting?

If your publication list does not appear on your websites, would you mind to please send me a copy of that so that I can include your work in my literature review.

I know you are all very busy but as you are already contributing extensively to our understanding of the marine environment and marine resources, your contributions are highly valued.

Kind regards
Paulette

Subject: Re: Application of genetic techniques for determining fish stock identity
Date: Thu, 9 Oct 2003 08:58:44 1200
From: "Peter Smith" <p.smith@niwa.co.nz>
Organization: NIWA
To: Dr P Bloomer <pbloomer@postino.up.ac.za>

Dear Paulette

Many thanks for your long email re your very interesting genetics project. I am about to leave the lab for ten days, so this will be a very quick reply.

Some of our recent genetics work on New Zealand marine fishes suggests two important (and fairly obvious!) factors to consider in genetics projects: 1. species with high dispersal ability, either as pelagic larvae/juveniles or adults, show little genetic differentiation. If we contrast orange roughy and oreos, both seamount species but with very different dispersal capabilities (orange roughy pelagic <one month, oreo pelagic 4 years) then orange roughy shows genetic differentiation and oreos do not. 2. Genetic differentiation occurs at oceanic/current boundaries. For example we have found genetic uniformity over 1000+ kms in snapper off NE New Zealand, but there is an abrupt change in allele frequencies across a a current boundary.

My suggestion, off the top of my head, would be to choose two or more species with different dispersal capabilities and test them over a similar range that included current boundaries. This approach would allow you to evaluate the usefulness of genetic markers for your project.

Finally I am interested in samples of orange and silver roughy from your region. In particular silver roughy (Hoplostethus mediterraneus). We are currently working on global populations of this species complex.

Best wishes
peter

Attached recent publication list
**********

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Subject: RE: Application of genetic techniques for determining fish stock identity
Date: Thu, 9 Oct 2003 11:08:00 -0400
From: "John Waldman" <john@hudsonriver.org>
To: "'Dr P Bloomer'" <pbloomer@postino.up.ac.za>

Dear Paulette,

Thank you for querying me about this initiative. I am interested in large endeavors of this kind that address major questions and which might allow for comparisons among taxa. It is to South Africa's credit that they are looking at these questions comprehensively.

Most of my work has involved anadromous, estuarine, and inshore species, although I remain interested in "blue water" questions. I do not have my own lab--all of my genetic stock structure work has been in collaboration with an excellent "hands on" colleague, Dr. Isaac Wirgin.

It is good that you recognize that all questions may not be responsive to genetic analysis; sometimes phenotypic approaches are preferable.

You may be interested to know that the first book devoted to fish stock identification techniques will be published by Elsevier next year. I am one of the editors. The work originates through ICES.

I've attached my curriculum vitae, which contains my publications. If I can be of use, please let me know.

John Waldman
Senior Scientist

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Date: Sun, 19 Oct 2003 14:20:59 +0100
To: Dr P Bloomer <pbloomer@postino.up.ac.za>
From: "G.R.Carvalho" <g.r.carvalho@hull.ac.uk>
Subject: Re: Application of genetic techniques for determining fish stock identity

Dear Dr Bloomer,

Many thanks for your message. Please excuse the delay in my reply, but I have been away on several trips.

I was naturally most interested to hear of the BCLME programme, and agree at the outset that careful coordination and consultation is likely to be the most efficient and effective way to proceed. I am currently involved with several fisheries genetics projects, including some instigated by our national body responsible for marine fisheries, DEFRA. I am pleased to say that at long last the UK authorities are beginning to appreciate that recent advances in genetic techniques have increased significantly the scope of what we can do, and now genetics appears to be a high priority in their 10 year forward look research programme.

With this background, it is clear that I would be most enthusiastic about participating in your proposed study, including any opportunities to contribute to a meeting. Across the years it has become apparent that marine fishes may well exhibit much higher levels of population structuring than originally anticipated, and that many previous projects suffered either from poor sampling design or the use of inappropriate molecular tools, sometimes in complete isolation to non-molecular approaches. Such issues as appropriate spatial scales, a sufficient sample size (e.g. around 100 for microsatellite studies, though this will depend on number and level of polymorphism at loci), the targeting of spawning individuals where possible, the testing of temporal stability of spatial patterns, are among the critical aspects that would need to be considered. In addition to these, modifications of established statistical procedures can be useful in identifying the source of recruits/adults by using such things as mixed stock analysis (MSA) (commonly employed in salmonids) and assignment tests. For example, we currently have an EU-funded project testing the feasibility of an MSA-based approach to managing the North Sea herring fishery.

In addition to the question of stock boundaries, such issues as effective population size are very relevant, especially since recent studies have indicated that marine fishes may be vulnerable to genetic drift and loss of genetic diversity: see attached paper, and our recent work on cod (Proc. Roy. Soc. London. B: 270, 2125-2132- abstract can be found at http://www.pubs.royalsoc.ac.uk/. Understanding the dynamics and determinants of variance in reproductive output of families and cohorts may also greatly increase the accuracy of such things as spawning stock-recruitment relationships. Worthwhile things can now be done using comparisons of genetic diversity among specific cohorts for example.

Suffice is to say, that I would be keen to embark on such a venture if you feel that I could play a useful role. In terms of species, I have no great preferences since we work on a variet of fin and shell fish at Hull, though much of my work has included snappers, clupeids and gadoids.

I would be interested in hearing from you concerning future prospects.

with best wishes,

Gary

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Subject: Re: [Fwd: Application of genetic techniques for determining fish stock identity]
Date: Thu, 20 Nov 2003 10:46:26 -0600
From: "John Gold" <goldfish@tamu.edu>
To: <pbloomer@postino.up.ac.za>

Hi Paulette,

As I don't maintain a website (laziness, among other things), I've attached a list of our pubs on marine fish genetics. Most deal with stock structure in one way or another (more-or-less). If you want any specific papers, let me know. Some are in Word files and some are in PDF files; I have reprints of all (I think).

Previously (re your list), we have worked on sciaenids, carangids, lutjanids, and scombrids - sparids would be quite interesting (because of the possible hermaphroditism).
I'd be interested in a planning meeting but (as is probably true for many others), funds for travel not tied to a specific project are almost impossible to obtain.
Keep in touch if you have any questions, et cetera.

Regards,

John

15 December 2003

Dr. Paulette Bloomer
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0002 Pretoria
SOUTH AFRICA

Dear Paulette,

I'm sorry for the delay in responding to you – to be honest, I put your request aside and then proceeded to forget about it until reminded by Nick Elliott.

Anyway, in response to your questions:

As far as sampling strategy goes, it is less than desirable to use only one genetics approach in stock structure studies. Wherever possible, it would be better to include both mitochondrial DNA and nuclear DNA approaches. The latter might be either of allozymes or microsatellites (preferably both!). Such multi-faceted genetics approaches are unfortunately expensive and can be logistically very demanding.

Sample sizes should be at least 50 per site, preferably 100. It is beneficial to use 0-class fish for this, near the point of their spawning, but this is much easier to write than to do! Often it will be impossible. If impossible, then it is good to use fish from (as much as possible) a single cohort, so that any possible inter-cohort differences don't confound results. Again, this might be very difficult (and for some fish, e.g. orange roughy, impossible).

Ideally, temporally separated samples should be collected from each site. Again, this gets very expensive. Maybe it would be more practical to re-sample any collection areas which look genetically different, to be assured that the heterogeneity is real and not artefactual.

Very few if any stock structure studies meet these desirable attributes. A suboptimal but reasonable study of a single species is still expensive – studying multiple species as proposed in your case becomes very expensive.

You should always bear in mind that where gene frequency differences are detected (and confirmed), then that is a very strong indicator of stock differentiation and the need for separate management; however, the alternative result, a lack of significant genetic differentiation, may not be very helpful. The latter can result from anything from full to very limited mixing, and very limited mixing might benefit from separate management.

So, genetic approaches must also be complemented by non-genetic approaches in order to reasonably discriminate between the full mixing and limited mixing scenarios.

Obviously these are very simple thoughts and comments put very briefly.

Some species we are specially interested in at the moment are toothfish and tunas and, to a lesser extent, shrimps and oysters. Nick Elliott is interested in abalone.

I might be able to contribute to any planned mini-symposium, subject to timing and finance.

My publication list follows.

I hope some of the above is useful,

Yours,
Bob Ward

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Subject: Re: Benguela current
Date: Sat, 13 Dec 2003 15:02:33 -0800 (PST)
From: Stewart Grant <phylogeo@yahoo.com>
To: Dr P Bloomer <pbloomer@postino.up.ac.za>

Hi Paulette, thanks for you kind email. Oh, yes I remember you, but you must have married in the meantime because I don't remember your last name. Didn't you also work in Cape Town for a while?

Yes, I'd like to become involved, but since I am not associated with a lab, all I can offer is my experience and writing skills. And yes there are some very good questions needing answers for SA marine fishes.

So I'll send along the few PDF files I have of pertinent papers and my publication list. With this email, I'm sending a draft of a manuscript on Old World anchovies and SA anchovies are spotlighted as you will read. Since we have not submitted this yet please restrict distribution. This paper points out the need for a coast-wide survey of mtDNA variability in SA anchovies. We have cytochrome b data for only 20 fish or so and base our

story on this sample. We found a very close connection with European anchovies and a very low haplotype diversity, but need to know the generality of these conclusions. I'm involved with another study of anchovies along the California coast (which because of length will send along in another email) that illustrates some expectations in a high gene flow species.

May I offer some insights into possible future work on SA's marine fishes. You might consider different methods for different kinds of questions. Usually funding is easier to get for 'fishery management' questions. But, since there are very high levels of gene flow and since the Benguela Current System has been very unstable throughout the Pleistocene, there are unlikely to be any regional mtDNA partitions. There were virtually no allozyme differences up and down the coast for the species we looked at. So if the question is management the technique should be microsatellites.

You however mentioned phylogeography, which implies the analysis of mtDNA. If this is your interest then sampling must extend outside SA waters. A while back Schulman and Bermingham (Evolution) did a study of mtDNA in Caribbean reef fishes and their glorius conclusion was that they had to sample beyond the Caribbean to understand the evolutionary dynamics of these fishes. The same is especially true for SA marine fishes.

If you do get a project going on marine fishes, you might consider including Rob Leslie, a very good naturalist, knows the importance of good sampling, is excellent with computer data analyses, and in fact knows a good deal about population genetics.

Another person also worth contacting is Brian Bowen who is now in Hawaii. At the moment he's busy with a new born child and getting set up in a new DNA lab, but he's great for helping to see the right story for a set of data. His current research focus is on tropical marine fishes on a global scale. In fact some of his past works include samples of fish and sea turtles from SA waters.(bbowen@hawaii.edu)

So anyway, good to hear from you.

From the frozen north,
Stew

## RECENT PUBLICATIONS OF SOME OF THE RESPONDENTS

**PJ Smith, NIWA, Private Bag 14901, Wellington, New Zealand**
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**Recent publications (2000-03)**

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Smith, P.J., Benson, P.G. 2001. Biochemical identification of shark fins and fillets from the coastal fisheries off New Zealand. *Fishery Bulletin 99* (2): 351-355.

Smith, P.J., Griggs, L., Chow, S. 2001. DNA identification of Pacific bluefin tuna (*Thunnus orientalis*) in the New Zealand fishery. *New Zealand Journal of Marine & Freshwater Research 35*: 843-850.

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Ryan, A.W., Smith, P.J.*, Mork, J. 2002. Genetic differentiation between the New Zealand and Falkland Island populations of southern blue whiting *Micromesistius australis*. *New Zealand Journal of Marine & Freshwater Research 36*: 621-627. *corresponding author

Smith, P.J., Paulin C. D. 2003. Genetic and morphometric evidence for a single species of pink ling *Genypterus blacodes* (Forster) in New Zealand and Australian waters. *New Zealand Journal of Marine & Freshwater Research* 37: 183-194.

Bernal-Ramírez, J.H., Adcock, G.H., Hauser, L., Carvalho, G.R., Smith P.J.* 2003. Temporal stability of genetic population structure in the New Zealand snapper, *Pagrus auratus*, and relationship to coastal currents. *Marine Biology 142*: 567-574. *corresponding author

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Smith, P.J. 2004: Genetic analyses: Random Amplified Polymorphic DNA (RAPD). In Stock Identification Methods. Ed. by Cadrin, S.X., Friedland, K.D., and Waldman, J. Academic Press Database book. In press

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**Bob Ward**

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**Appendix III**

**CURRENTLY AVAILABLE MICROSATELLITE LOCI WITH RELEVANCE TO BCLME RESOURCES**

**(the order of species in the table follow the order as presented in the report)**

| **Resource** | **Reference**[1] | **Note on microsatellites** |
|---|---|---|
| Hake | D'Amato *et al.* 1999 | 10 loci for *Macruronus magellanicus*; di-, tri- & tetranucleotide repeats; amplified and polymorphic in other gadoid genera |
| | Moran *et al.* 1999 | 6 loci for *Merluccius merluccius*; dinucleotide repeats; not tested for cross amplification |
| Horse mackerel | Ohara *et al.* 2003 | 5 loci in *Seriola quinqueradiata*; dinucleotide repeats; amplify and polymorphic in *S. dumerili* |
| Clupeids | Miller *et al.* 2001 | 15 loci for *Clupea pallasi*; di-, tri- & tetranucleotide repeats; not tested for cross amplification |
| | Olsen *et al.* 2002 | 14 loci for *C. pallasi*; tetranucleotide repeats; not tested for cross amplification |
| | O'Connell *et al.* unpubl. | 8 loci for *C. pallasi*; tetranucleotide repeats |
| | McPherson *et al.* 2001 | 9 loci for *C. harengus*; tetranucleotide repeats |
| | Brown *et al.* unpubl. | 6 loci for *Alosa sapidissima*; tri- & tetranucleotide repeats |
| Anchovy | Chiu *et al.* 2002 | 6 loci for *Engraulis japonica*; di- & pentanucleotide repeats; not tested for cross amplification |
| | Zampicinini & Magoulas unpubl. | 16 loci for *E. encrasicolus*; dinucleotide repeats |
| Dentex | - | |
| Monk | Garoia *et al.* 2003 | 6 loci for *Lophius budegassa*; dinucleotide repeats; also amplify in *L. piscatorius* |
| Alphonsino | - | No information available but loci developed for orange roughy may cross amplify in this family |
| Orange roughy | Oke *et al.* 1999 | 10 loci for *Hoplostethus atlanticus*; amplified in three other *Hoplostethus* species and in two other genera, *Gephyroberyx* and *Paratachichthys*. |
| Kingklip | Ward & Reilly 2001 | 15 loci for *Genypterus blacodes*; di-, tri- & tetranucleotide repeats; not tested for cross amplification |
| Deep sea red crab | Seeb *et al.* 2002 | 6 loci for *Paralithodes camtschaticus*; tri- & tetranucleotide repeats; cross amplified and polymorphic in *P. platypus* and *Lithodes aequispinus*<br>NOTE: Markers are being developed by Eugenia D'Amato |

| | | |
|---|---|---|
| Bronze whalers | Keeney & Heist 2003<br><br>Feldheim unpubl., Feldheim *et al.* 2001, Pardini *et al.* 2000, Heist & Gold unpubl. | 16 loci for *Carcharhinus limbatus*; dinucleotide repeats; 9 loci amplified in *C. brachyurus*, 5 appeared polymorphic<br>Other appropriate loci include ones developed in the lemon shark (*Negaprion brevirostris*), the great white (*Carcharodon carcharias*) and the sandbar shark (*C. plumbeus*) |
| Leervis | Ohara *et al.* 2003 | See horse mackerel above. |
| Galjoen | - | |
| Snoek | - | |
| Elf | - | |
| Geelbek | Turner *et al.* 1998<br><br><br>O'Malley *et al.* 2003<br>Maccatrozzo *et al.* 2002 | 30 loci for *Sciaenops ocellatus*; di-, tri- & tetranucleotide repeats; nine loci cross amplify in red spotted sea trout *Cynoscion nebulosus*, black drum *Pogonias cromis* and Atlantic croaker *Micropogonias undulatus*<br>38 loci for *Sciaenops ocellatus*; dinucleotide repeats including a set of linked loci<br>2 loci linked to a specific gene for *Umbrina cirrosa* |
| Silver kob | Turner *et al.* 1998<br>O'Malley *et al.* 2003<br>Maccatrozzo *et al.* 2002 | See as for geelbek above. Three loci of Turner et al and 12 loci of O'Malley *et al.* have been amplified in silver kob; four polymorphic loci were analysed (Morabe 2003) |
| Dassie/blacktail | Batargias *et al.* 1999<br>Stockley *et al.* 2000<br><br>Yap *et al.* 2000<br>Published and unpublished loci on GenBank | 6 loci for *Sparus aurata*; dinucleotide repeats; not tested for cross amplification<br>10 loci for *Pagellus bogaraveo*; di-, tri- & tetranucleotide repeats; not tested for cross amplification<br>5 loci for *Acanthopagrus butcheri*; dinucleotide repeats; not tested for cross amplification<br>Other appropriate loci include *Pagellus erythrinus* (8 loci), *Sparus aurata* (12 and 6 loci), *Acanthopagrus schlegelii* (8 loci), *A. latus* (2 loci), *Pagrus auratus* (6 loci), *Chrysophrys major* (5 loci) |
| West coast steenbras | See as for dassie. | See as for dassie above. |

| | | |
|---|---|---|
| Grunt | - | |
| Tuna | McDowall *et al.* 2002<br><br>Clark *et al.* 2004 | 7 loci for *Thunnus thynnus thynnus*; tetranucleotide repeats; 5-6 loci cross amplify in *T. macoyii, T. albacares, T. alalunga* and *T. obesus*<br>25 loci for *Thunnus thynnus thynnus*; di- & tetranucleotide repeats; all loci amplified and were polymorphic in *T. albacares, T. alalunga* and *T. obesus* |
| Squid[2] | Guarniero *et al.* 2003<br>Shaw 1997<br>Emery *et al.* 2000 | 5 loci for *Loligo vulgaris*; dinucleotide repeats; not tested for cross amplification<br>Loci are also available for *L. forbesi*, *L. opalescens*, *L. gahi*, *L. pealeii* and *L. bleekeri*. Seven *L. forbesi* loci cross amplified in *L. vulgaris* but levels of polymorphism were lower than in *L. forbesi*. |
| Rock lobster[2] | Jones *et al.* 2003<br><br>Streiff *et al.* 2001 | 13 loci for *Homarus americanus*; di-, tri- & tetranucleotide repeats; cross-amplification tested within the genus<br>5 loci for *Nephrops norvegicus*; di- & trinucleotide repeats<br>NOTE: Eugenia D'Amato is developing species specific loci for *Jasus lalandii* |
| Mullet | - | |
| Brown mussel[2] | Holland 2001<br>Teh & Tan unpubl. | 2 loci for *Perna perna*; dinucleotide repeats<br>4 loci for *P. viridus*; trinucleotide repeats |
| Octopus[2] | Greatorex *et al.* 2000 | 6 loci for *Octopus vulgaris*; di-, tri & tetranucleotide repeats; not tested for cross amplification |
| Prawns[2] | Xu *et al.* 1999 | 10 loci for *Penaeus monodon*; di-, tri & tetranucleotide repeats; cross amplified in another *Penaeus* species |

[1] Dashes indicate species for which no relevant information could be retrieved on GenBank (www.ncbi.nlm.nih.gov/nucleotide).

[2] For invertebrates in general it is recommended that species specific microsatellite loci should be developed. Attempted cross amplification even within genera have shown that either flanking sequences have undergone mutations so that no amplification result or the microsatellite repeats themselves had changed over the time of divergence. Often loci developed in one species tended to be monomorphic in other species.

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