The biological basis for hypothesizing multiple stocks in South African sardine *Sardinops sagax*

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Introduction - A multidisciplinary approach to assessing stock structure in South African sardine is being applied, including studies on distribution patterns, spawning areas and environmental characterizations thereof, meristics (vertebral counts, gill raker number) and morphometrics (body shape, otolith shape, gill raker length and spacing), parasites, life history characteristics (length-at-maturity and length-at-age), otolith microchemistry, and genetics. Many of these are on-going but final results from some studies and initial data from others will be described here.

Distribution patterns – Sardine distribution patterns were described by Coetzee *et al*. (2008) who reported a consistent separation between sardine found to the west and east of Cape Agulhas at low and medium biomass levels (Fig. 1). Sardine to the west occupied the Western Agulhas Bank (WAB) at all biomass levels and their distribution extended northwards over the shelf and shelf-edge up the West Coast with increasing biomass and also eastwards onto the inner shelf of the Central Agulhas Bank (CAB) . Sardine to the east primarily occupied the Eastern Agulhas Bank (EAB) at low biomass levels and extended westwards along the outer shelf of the CAB at moderate and high biomass. Overlap between the western and eastern parts of the population only observed at high biomass levels. Those authors suggested that their data provided support for the hypothesis of separate spawning units, one on either side of Cape Agulhas.

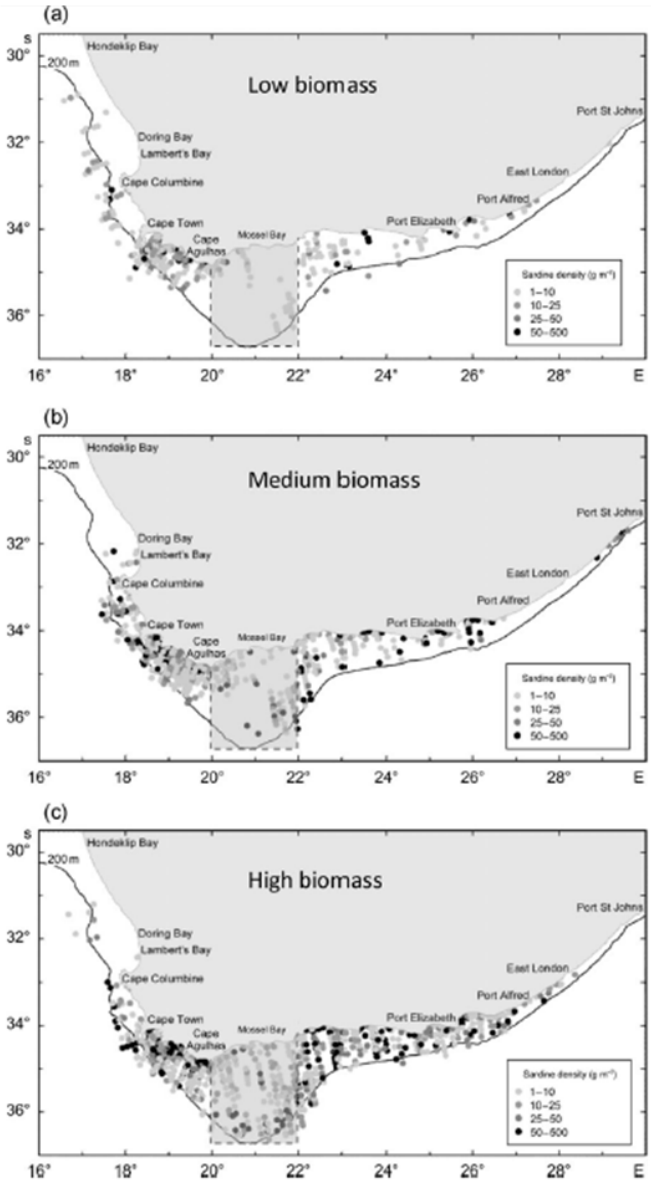

Figure 1: Composite maps of sardine density derived from hydroacoustic data collected during November surveys 1984-2007 for periods of (a) low, (b) medium, and (c) high sardine biomass (from Coetzee *et al.* 2008).

Spawning areas – Sardine spawn around South Africa's coast, and their eggs have been collected between Hondeklip Bay and Durban

(van der Lingen and Huggett 2003). A composite map of sardine egg distributions from CalVET net samples collected during pelagic spawner biomass surveys conducted in spring (Fig. 2a) clearly shows two discrete spawning areas, separated by the CAB. The western spawning area extends across the shelf and shelf edge from Cape Agulhas to Cape Columbine, and along the shelf edge and offshore between Cape Columbine and Hondeklip Bay. The eastern spawning area extends along the shelf edge from the eastern part of the CAB to Port Alfred on the EAB. Environmental characteristics of the western and eastern spawning areas differ, with eggs off the West Coast being found primarily in waters of 14-17° C compared to 19-22° C for eggs off the South Coast (Fig. 2b). Sardine eggs have also been found off the South Coast during autumn surveys, but not off the West Coast.

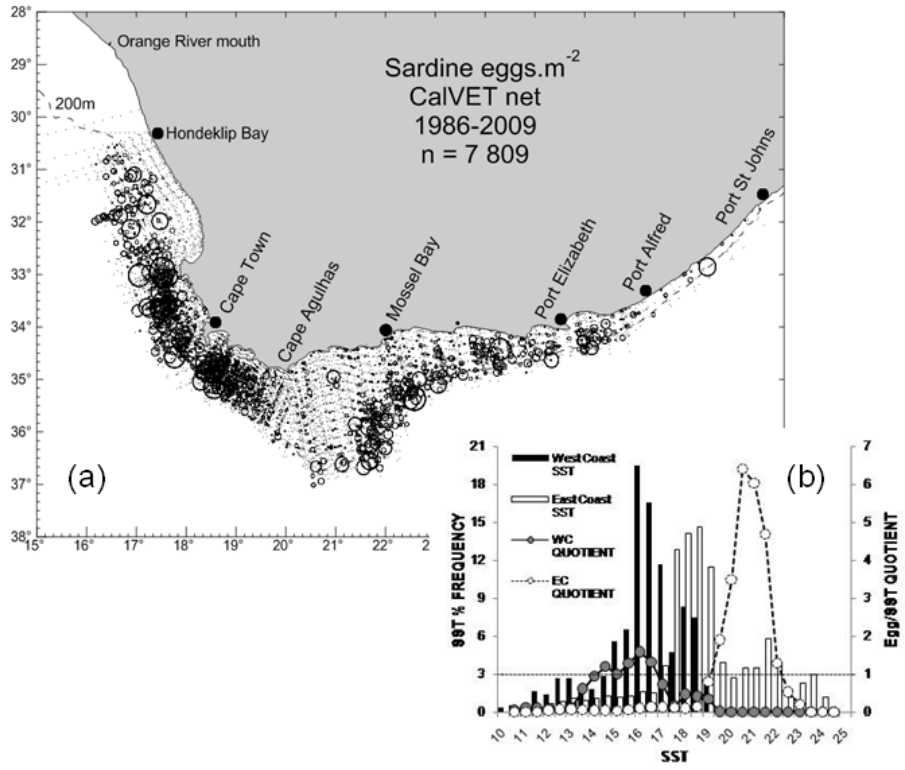

Figure 2: (a) Composite sardine egg density map derived from CalVET net samples (7 809 stations) collected during November surveys 1986-2009 (C. van der Lingen, DAFF, unpub.); and (b) characterization of sardine spawning habitat using SPQ analysis for SST and CUFES egg data from the west and east of Cape Agulhas from the 2008 PSB survey. SST %FO distributions are histograms, and the quotients (dots and lines) are 3-point running means (C. van der Lingen, DAFF, unpub.).

Miller *et al*. (2006) used an individual-based model coupled with a 3-D hydrodynamic model to explore how spatial variability in sardine spawning and nursery grounds impacted on the transport and retention of their eggs and larvae, and concluded that sardine life history strategy could be divided, at Cape Agulhas, into two main systems. Fish in the "West Coast system" spawn on the western Agulhas Bank and the West Coast, and recruit to the West Coast nursery grounds, whilst those in the "Agulhas Bank system" spawn on the central and eastern Agulhas Bank, and mostly recruit to the South Coast nursery grounds, although some transport from east of Cape Agulhas to the West Coast does occur (Fig. 3). Whilst the bulk of sardine recruitment occurs of the West Coast juvenile fish are frequently found in bays off the South Coast during hydroacoustic surveys conducted in winter and spring. Sardine also have a third spawning area off the East Coast, with eggs found between Port Alfred and Durban during the annual winter sardine run off KwaZulu-Natal. The sardine run is considered most likely to be a seasonal reproductive migration of a genetically distinct

subpopulation (Fréon *et al.* 2010). Sardine eggs are found off Park Rynie (60 km south of Durban) from June to December but not during the remainder of the year (Connell 2010).

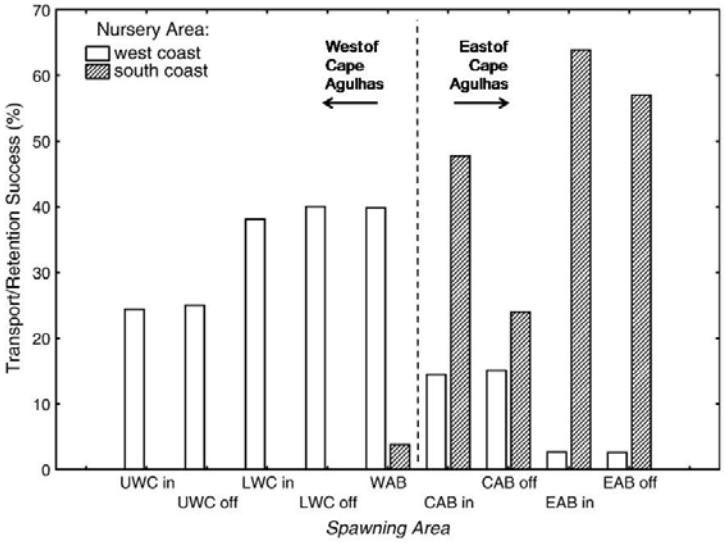

Figure 3: Modeled transport to, or retention in, West (between Cape Columbine and the Orange River) and South (between Cape Infanta and Plettenberg Bay) coast nursery areas for sardine eggs released in nine different spawning areas (UWC = Upper West Coast; LWC = Lower West Coast; WAB = Western Agulhas Bank; CAB = Central Agulhas Bank; EAB = Eastern Agulhas Bank; in = inshore and off = offshore). The dashed line indicates the position of Cape Agulhas (from Miller *et al*. 2006).

Meristics and morphometrics – Spatial variability in sardine meristic characters and morphometric variables has been assessed by comparing these traits between fish collected from the West (west of Cape Agulhas), South (east of Cape Agulhas) and East (KZN) coasts. Sardine from Namibia have also been examined in order to act as a partial control, in terms of the ability of a given trait to reflect stock differentiation, given that sardine from the Northern (Namibia) and Southern (South Africa) Benguela systems are considered to be discrete stocks separated by the upwelling cell off Lüderitz that are separately managed.

Sardine from Namibia and the South African West and South coasts showed a significant (KW; $p < 0.001$) difference in vertebral number, and *post hoc* tests indicated that the difference was due to Namibian fish having more vertebrae than sardine from either the West or South coast of South Africa. Sardine from the latter two regions showed no difference in vertebral count (Figure 4a). Analyses of vertebral count data of fish from the KZN sardine run from a small sample collected in 2009 and a larger combined sample from 1978, 1979 and 1980, and comparison of these counts with those from the West and South South African coasts, again showed a significant spatial difference (KW; $p < 0.001$). *Post hoc* tests showed that historical (1978-1980) and contemporary (2009) KZN data were significantly different and that each of these was also significantly different to data for West coast and South coast fish (Figure 4b). Vertebral counts again showed no difference between West and South coast fish. These results show a continuum in sardine vertebral count number around the southern African coast, with Namibian fish having the most and KwaZulu-Natal sardine the fewest vertebrae. This fits with the pattern of a higher vertebral number in fish that underwent larval development in cooler temperatures and fewer vertebrae for those that developed in warmer temperatures (Jordan's rule; Jordan 1892 in Swain and Foote, 1999). Whereas geographic variation in vertebral number is generally ascribed to environmental influences such as temperature during development, controlled-rearing experiments have shown a shown genetic component of such variability in some species, suggesting that geographic variation in this parameter may reflect genetic

divergence in response to changes in the phenotypes favoured by selection (Swain and Foote, 1999).

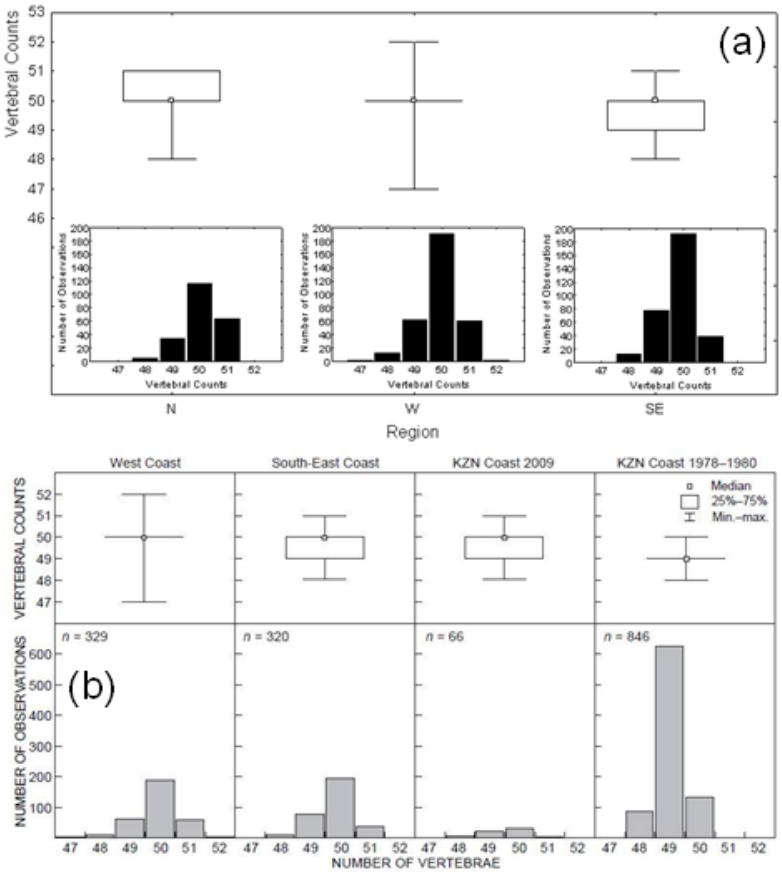

Figure 4: (a) Box and whisker plots (upper panel) showing the median, quartile (25-27%) and range (min-max), and frequency distributions (lower panel) for vertebral counts of sardine from Namibia (N), and the South African West (W) and South (SE) coasts (from Wessels *et al*. submitted); and (b) Box and whisker plots, and frequency distributions for vertebral counts of sardine from the South African West, South and KZN coasts (2009 and the 1978-1980 sardine runs) (from van der Lingen *et al.* 2010).

A box truss network comprising 10 landmarks and the resultant 21 morphometric measurements (Fig. 5a) were used to compare the body shape of sardine from Namibia, and the South African West and the South coasts. Morphometric measurements were transformed to size-independent shape variables, and PCA was used to examine morphological variation and stepwise DFA subsequently used to identify which morphometric measurements differed between sardine from the three regions. PCA indicated differences in body shape among fish from the three regions, and DFA centroids (Fig. 5b) for the three regions were significantly ($p<0.001$) different (Wessels *et al*. submitted). The main differences were thicker bodies, bigger heads and smaller tails for Namibian sardine, and thinner bodies and smaller heads for South Coast sardine. Body shape analysis was also conducted on a small sample collected during the 2009 KZN sardine run, and data were compared to those for fish collected from the South African West and South coasts. Overall discrimination between sardine from the three regions was highly significant ($p < 0.001$) and whereas the 95% confidence interval ellipses of the DFs showed a good deal of overlap (Fig. 5c), significant ($p < 0.001$) differences were found between the means of all regions. Condition factor was not an important covariate for KZN sardine (van der Lingen *et al*. 2010).

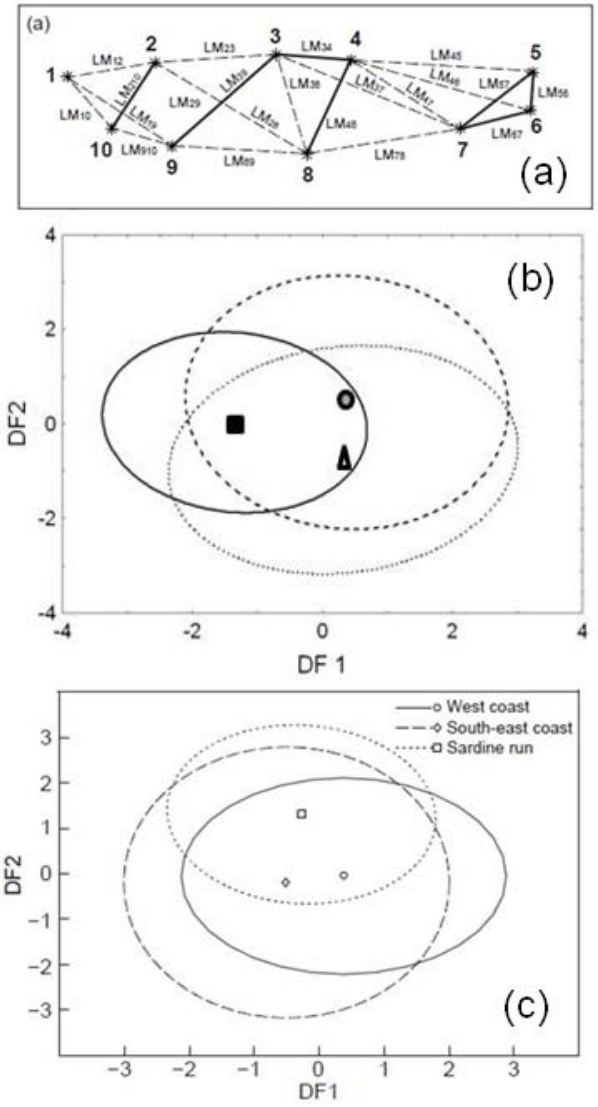

Figure 5: (a) The box-truss network linking landmarks and showing morphometric measurements used in the DF model (solid black lines show those used in the analysis of van der Lingen *et al.* 2010); (b) group centroids and 95% confidence interval ellipses for DFA scores for sardine from Namibia (black square and solid line), and the South African West (grey circle and dashed line) and South (white triangle and dotted line) coasts (from Wessels *et al*. submitted); and (c) group centroids and 95% confidence interval ellipses for DFA scores for sardine from the South African West (circle and solid line), South (diamond and dashed line) and KZN (square and dotted line) coasts (from van der Lingen *et al.* 2010).

Results from the morphological analyses conducted to date indicate that there is significant variation in sardine body shape between the four regions around the coast of southern Africa. Whereas morphometric characters typically show ontogenetic changes associated with allometric growth and may be liable to environmental influences throughout life, it is not uncommon for population structure to be indicated by variation in this parameter even in the absence of direct measures of genotypic variation (Swain and Foote, 1999). Spatial variability in meristic and morphological characters of the branchial baskets of sardine from Namibia and the South African West, South and East coasts was examined by Idris *et al.* (submitted). Statistical analyses (ANCOVA and *post hoc* Tukey tests; CL used as covariate) indicated significant differences in some branchial basket characteristics of sardine from the four regions, although not always consistently across all fish size classes. Gill arch length differed between large fish from all regions with East Coast sardine having the shortest and West Coast sardine the longest gill arches. No significant difference in gill raker number was observed between regions for large fish, but analysis of small fish showed a difference (as for gill arch length) between West and South Coast sardine, with the latter having significantly fewer gill rakers than the former. The relationship between gill raker spacing and CL was consistent across all four regions and showed significant spatial variability, with West Coast sardine having the smallest predicted gill raker spacing, followed by South then East Coast fish, and Namibian sardine the largest spacing, across all size classes (Fig. 6).

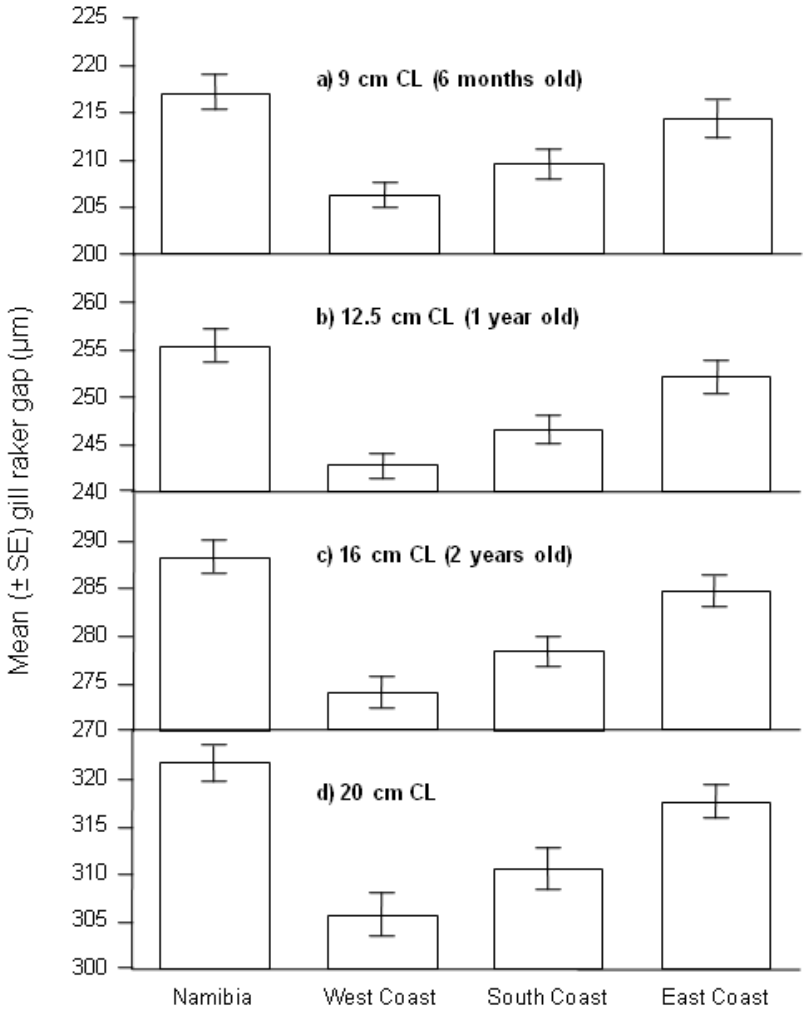

Figure 6: Results of the ANCOVA model predicting mean gill raker spacing (±SE) for sardine of different sizes (approximate ages) in the four regions (Namibia, and the SA West, South and East [KZN] coasts). a) 9 cm CL, b) 12.5 cm CL, c) 16 cm CL and d) 20 cm CL (from Idris *et al.* submitted).

Parasitological studies - The use of parasites as biological tags for stock identification of marine fish has been successfully implemented elsewhere, and the potential for this method in answering questions concerning stock structure in SA sardine was assessed by Reed *et al.* (submitted). Those authors examined 120 fish from 7 localities and recorded seven parasite taxa, and suggested that a digenean tetracotyle metacercariae of the genus *Cardiocephaloides* that infected sardine's eyes and showed a distcontinuous distribution had the greatest potential as a biological tag. Examination of more fish (484) from a larger area (Hondeklip Bay to Durban) confirmed the discontinuous distribution of this infection, with fish from the West Coast having substantially higher prevalence rates (% of sample infected; Fig. 7) and higher infection intensities (mean number of parasites per fish; not shown) than sardine from the South and East coasts. A comparison of prevalence as a function of size for the three regions shows that sardine off the West Coast can be infected at a small (<10cm CL) size and that prevalence increases rapidly to >50% at sizes >14 cm CL (Fig. 7). Sardine from the South Coast appear to become infected at a larger size, and prevalence levels are lower at a given CL, than for fish off the West Coast, although sample sizes for small and large fish from the South Coast are low. Larger (17-19cm CL) sardine from the KZN sardine run had substantially lower prevalence levels compared to West Coast fish of the same size.

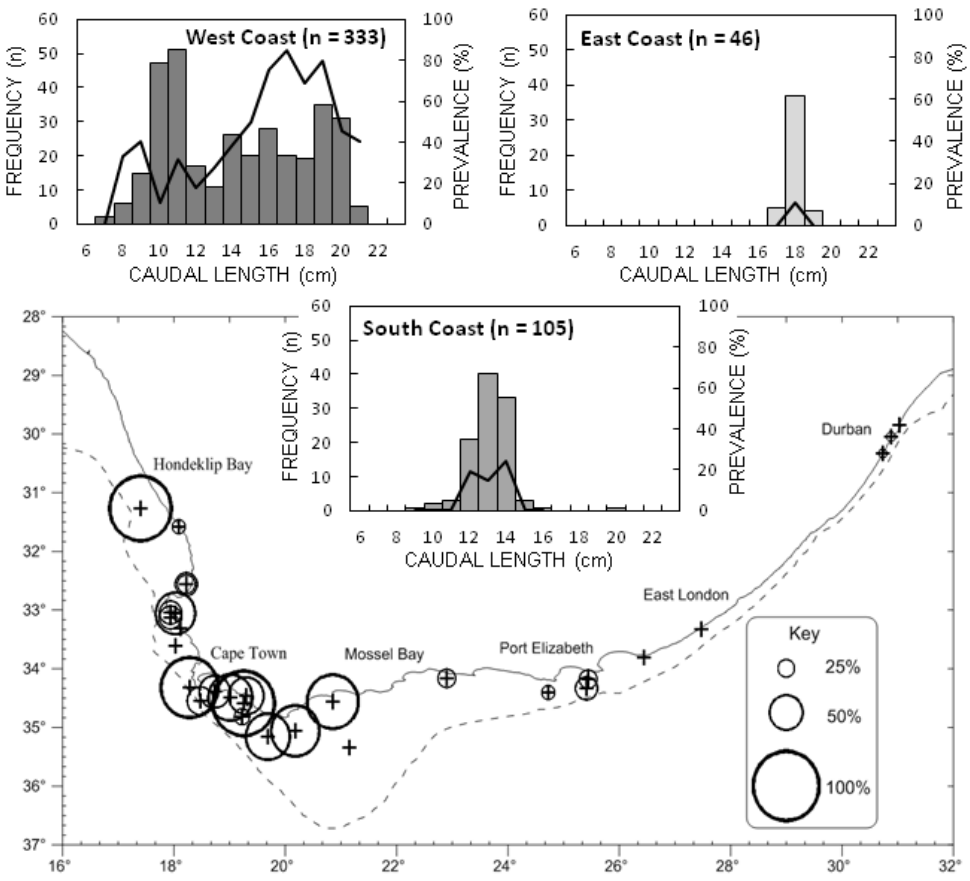

Figure 7: Sample locations (crosses) and prevalence (%) of infection by the parasite *Cardiocephaloides* sp of sardine (map); and length frequency distribution (n; histograms) and prevalence as a function of length (%; line) for sardine from the West (<21°E), South (21-28°E) and East (>28°E) coasts respectively (C. Reed *et al*, UCT, unpub.).

The use of parasites as biological tags depends on several factors, amongst them that the area of infection by the parasite (known as the endemic area) is not continuous within the host's distribution range; that the host should show different levels of infection in different parts of the study area; that the parasite infection is site specific and preferably internal (ectoparasites can sometimes be easily detached); and that the parasite lives sufficiently long in the host (suggested minimum is 2 years) and its life cycle is understood. Several of these apply to the tetracotyle parasite described here, and this genus of parasite has been successfully used to indicate discrete stocks in other species elsewhere, including Argentian anchovy (*Engraulis anchoita*; Timi 2003). The results described here suggest that it may also discriminate between western and southern sardine stocks off South Africa. Some (50) sardine from northern Namibia and Angola have also been examined but showed no infection. Fish are intermediate hosts of *Cardiocephaloides*, which first infects a gastropod and subsequently a fish-eating bird (Niewiadomska, 2002). The initial and final host species of this particular digenean parasite in South African waters are still unknown, but *Cardiocephaloides physalis* was reported from jackass penguins in South Africa by Randall and Bray (1983) and more recently by Horne *et al.* (2011), so the metacercariae from the sardine may also be of this species. Further work is underway to increase the numbers, size range (particularly for South Coast sardine) and seasonal sampling of sardine collected for parasite analysis, and work on elucidating the life cycle of *Cardiocephaloides* will also be conducted. A second parasite, the coccidian *Eimeria sardinea* that infects the testes of male sardine, also shows a discontinuous

distribution with larger fish from the West Coast tending show infection whereas those from the South Coast tend not to (not shown).

Life-history characteristics – Annual maturity ogives for sardine from commercial catches taken to the west and east of Cape Agulhas over the period 1984-2005 have been generated under the assumption that fish with a standardized gonad mass (SGMn) >0.25 were mature (Fairweather *et al.* 2006). The time-series of annual mean L_{50} shows a consistent pattern of sardine off the South Coast maturing at a larger size than those off the West Coast (Fig. 8a), with this difference being larger in recent years. Annual L_{50} values off the West and South coasts vary synchronously and are significantly correlated.

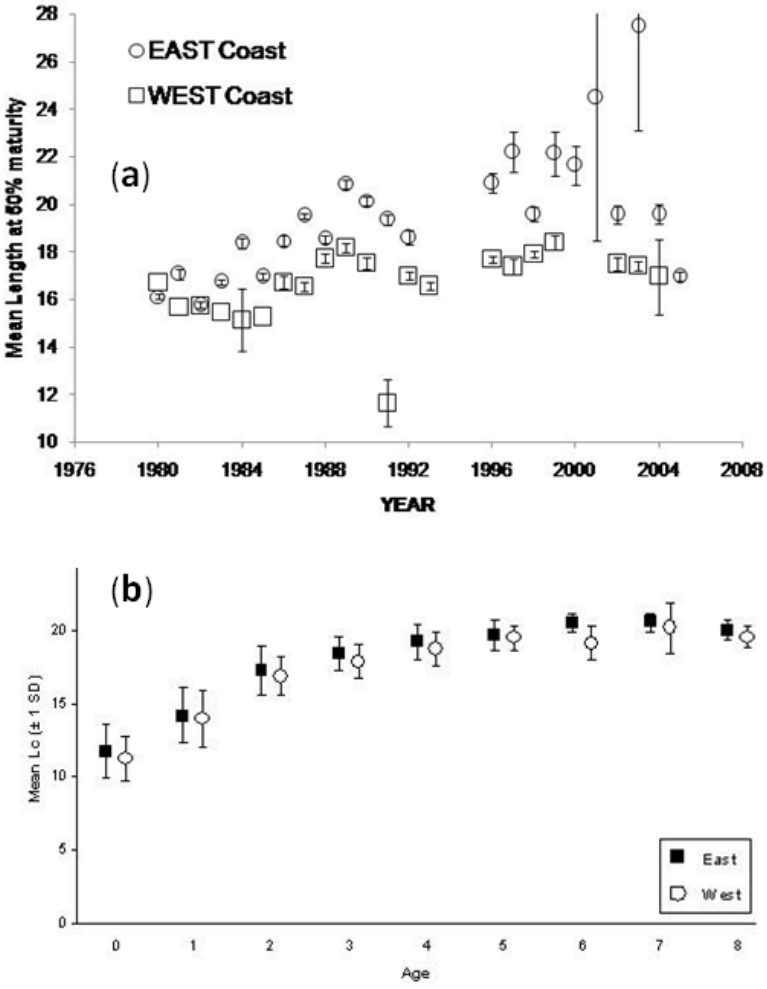

Figure 8: (a) Annual mean (±1 SD) length-at-50% maturity (SGM > 0.25) of female sardine collected from commercial catches taken to the west and east of Cape Agulhas, 1980-2005 (T. Fairweather, DAFF, unpub.); and (b) mean (±1 SD) length-at-age of sardine from research survey samples collected from east and west of Cape Agulhas during 1993, 1994, 1996, 2001-04, and 2006-07 (D. Durholtz, DAFF, unub.).

Mean length-at-age values for sardine from the west and east of Cape Agulhas calculated from otolith samples collected during routine spawner biomass surveys are shown in Figure 8b. No evidence for spatial differences in length-at-age is seen, although there does appear to be a slight tendency for fish from the west to be slightly smaller than those from the same age group in the east. A similar trend, although again slight, is apparent in otolith size-at-age, with sardine from the West Coast having slightly larger otoliths than those from the South Coast (not shown).

Otolith elemental composition – The elemental composition of otoliths of juvenile sardine collected from around the South African coast has been investigated as a first step to assess whether such analyses could be useful in determining stock structure. Spatial heterogeneity in otolith composition should be observed for this to be the case. Concentrations of boron (B), magnesium (Mg), strontium (Sr) and barium (Ba) from the otolith margin of 5-10 juvenile

sardine collected from 4 stations during the 2008 recruit survey and 5 stations during the 2009 recruit survey were measured. Elemental concentrations were transformed (normalized and logged) and a PCA was performed on those data. PCA results indicate that otolith elemental composition does do show spatial heterogeneity (Fig. 9), which may be sufficient for assessing sardine population structure and discriminating between putative stocks. Otolith elemental composition provides "apparently robust indications of regional structuring of some marine fish populations" (Thresher 1999), and initial results thus look promising for application to South African sardine.

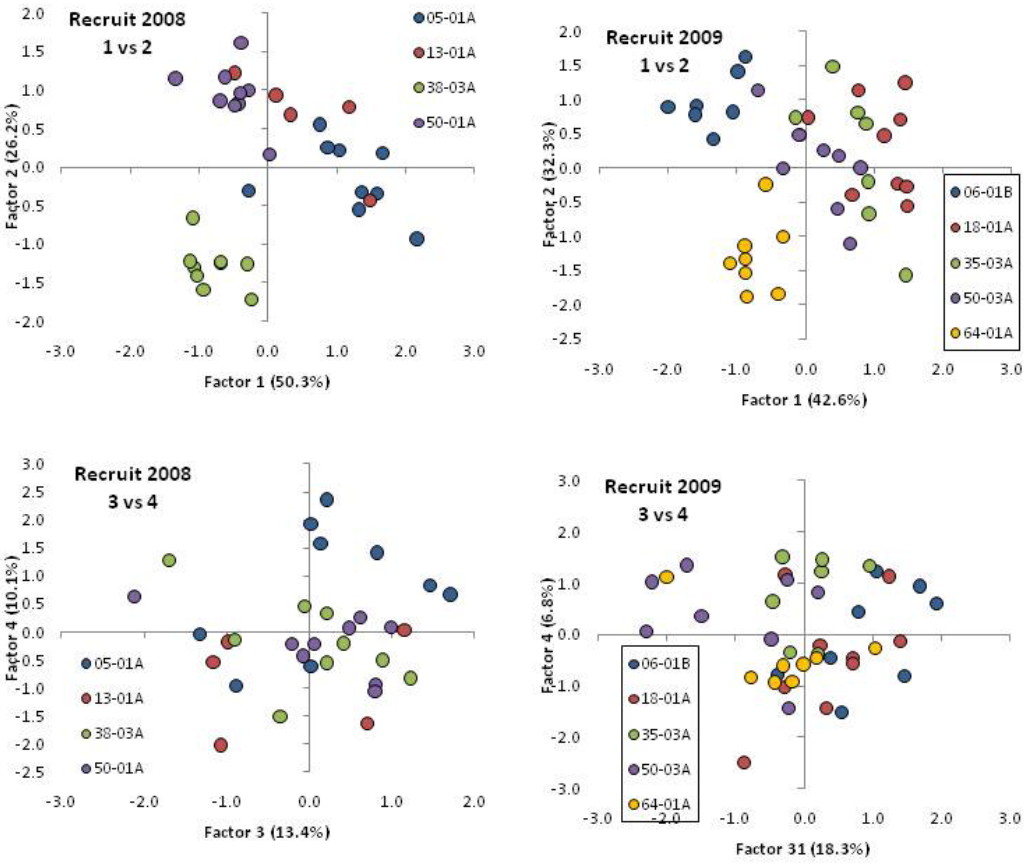

Figure 9: Results from a PCA of concentrations of boron, magnesium, strontium and barium in the otoliths of juvenile sardine from four sites sampled during the 2008 recruit survey (left plots), and five sites sampled during the 2009 recruit survey (right plots). PCA loadings for the first 4 factors are shown, Factors 1 versus 2 in the upper plots and Factors 3 versus 4 in the lower plots (M. Laybonne *et al.*, IRD, unpub. data).

Genetics and otolith shape – Investigations into the genetic structure of southern African sardine using analysis of mitochondrial and nuclear (microsatellite) DNA are currently underway. Preliminary results from the mitochondrial analysis have shown a large number of haplotypes for South African sardine that do not show spatial structuring (Fig. 10). Results of analyses of microsatellite data are, as yet, inconclusive. PCA analysis of otolith shape variability has indicated the presence of three, well separated otolith groups that are not spatially defined and are not related to fish size. Reasons for the otolith group shaping remain unclear and work on this and the genetic analysis is continuing.

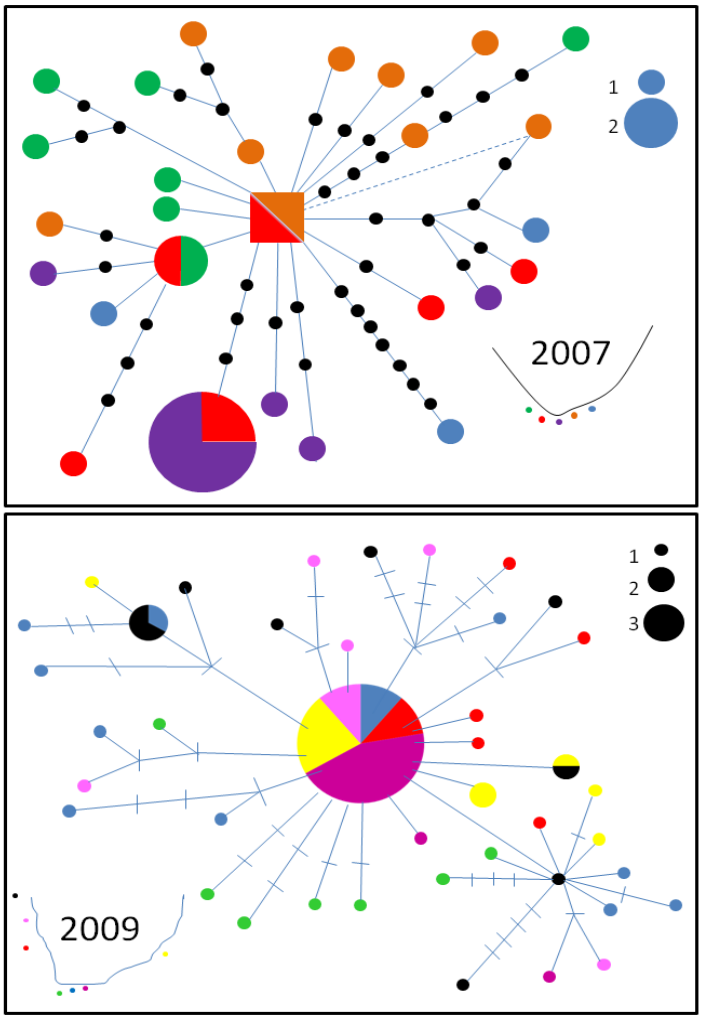

Figure 10: Haplotype maps for sardine collected off South Africa during 2007 (upper plot), and off southern Africa (including from Namibia and the KZN coast) during 2009 (lower plot). Sample locations are shown schematically, and symbol colours in the haplotype map correspond to sample location colours (S. Hampton, UCT, unpub.).

Conclusions – Consistent differences in most but not all (age-at-length) biological characteristics of sardine from the South African West, South and East coasts have been observed. The separation (except at high biomass levels) between West and South coast distributions and spawning areas, and hypothesized different life history strategies of western and southern sardine, support the hypothesis of ecologically, or functionally discrete stocks or sub-populations. Similarly, sardine from the East Coast may comprise a third sub-population. Additionally, meristic and morphological data also indicate the existence of three sardine morphs or phenotypes off South Africa. Differences in distribution, spawning areas and phenotypic characteristics also exist between Namibian and South African sardine, considered to be separate populations. Phenotypic differences may be environmentally induced and could result from differences in life history strategies, exposure to different environmental cues during important developmental periods, and trophic differences (Cadrin *et al.*, 2005).

Whilst studies on biological characteristics of Benguela sardine are on-going the results to date provide strong support for the incorporation of the multi-stock nature of South African sardine into assessment models and management procedures. Preliminary results from genetic analysis do not suggest the existence of genotypic stocks, but could indicate that there is sufficient mixing between phenotypic stocks to prevent genetic difference, and this seems likely. Some studies (*e.g.* of parasites and otolith microchemistry) may potentially be used to estimate mixing. The Eastern (KZN) stock can be ignored for present purposes of management of the sardine fishery, since the amount of sardine caught during the annual KZN sardine run is low (<1 000 t per annum) compared to that landed off the West and South coasts.

References – Available on request.