

Spatial variability in branchial basket meristics and morphology of southern African sardine *Sardinops sagax*

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We examined spatial variability in meristic and morphological characteristics of the branchial basket of sardine *Sardinops sagax* collected from four geographical regions around the southern African coast, namely Namibia and the South African west, south and east coasts. Our analysis tested the hypothesis of three putative sardine stocks off South Africa, one in each of the three geographical regions. We therefore collected fish data from Namibia to compare with South Africa, because sardine from the two countries are considered to be separate stocks. Morphometric measurements (gill arch length and gill raker spacing) and meristic data (number of gill rakers) were collected from the left side of the first gill arch from a total of 377 sardine, approximately equally divided between the regions. A multivariate general linear model with caudal length as covariate was used to assess differences among fish from the four regions and significant differences were observed, although not always consistently across all fish size classes. Small South Coast sardine had shorter gill arches than small West Coast sardine, but adults had gill arches of similar length, longer than those from Namibia and the East Coast. Small sardine from the South Coast had fewer gill rakers than small sardine from the West Coast, but larger fish had similar numbers of gill rakers, significantly more than sardine from Namibia and the East Coast. Sardine from the West and South coasts had similar gill raker spacings, which were smaller than those of fish from the East Coast and Namibia. Despite spatially and particularly temporally unbalanced sampling, we consider that these differences provide evidence of spatial variation in Benguela sardine phenotype and that it would support the hypothesis of discrete sardine stocks off Namibia and South Africa. The results are consistent with the hypothesis of three sardine stocks within the southern Benguela.

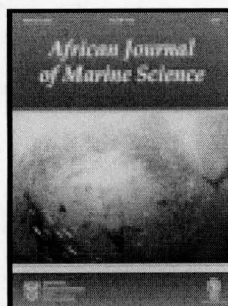
Keywords: gill arch, gill raker, population structure

Introduction

Many fish belonging to the order Clupeiformes are omnivorous, filter-feeding planktivores (Blaxter and Hunter 1982; James 1988). A characteristic of these filter-feeding microphagous fish is the presence of substantial numbers of gill rakers, positioned on the anterior side of the gill arches (Gerking 1994; Bone et al. 1995) that collectively form the branchial basket, which traps plankton and suspended organic matter from the water column (Nelson 1967; Sanderson et al. 2001; Villalobos and Rodriguez-Sanchez 2002; Tanaka et al. 2006). Regardless of whether a fish employs ram or pump filtration strategies (Gerking 1994), the branchial basket acts as a sieve that determines the retention of food particles, depending on their size and shape. Retained particles are concentrated by the epibranchial organs (paired dorsal diverticula at the posterior end of the pharynx) and then swallowed, whereas particles not retained exit through the opercular opening (Blaxter and Hunter 1982; Gerking 1994). Clupeiform branchial baskets range from coarsely to finely meshed and many species show a change in their branchial basket morphology as they grow, which results in changing prey compositions and size spectra with age (Blaxter and

Hunter 1982). Interspecific differences in branchial basket morphology are considered to contribute to resource partitioning between co-occurring Clupeiformes, as observed for anchovy *Engraulis* spp. and sardine *Sardinops sagax* and *Sardina pilchardus* in several marine ecosystems (van der Lingen et al. 2009).

In addition to such ecological considerations, spatial variability in branchial basket meristics (gill raker number) and morphology has been used to infer the existence either of different stocks within single, genetically homogeneous populations, or of ecophenotypic morphological variation, for some clupeiform species. These include northern anchovy *Engraulis mordax mordax* (McHugh 1951), Iberian sardine *Sardina pilchardus* (Andreu 1969), Pacific sardine *Sardinops caeruleus* (Mais 1972), Spanish sardine *Sardinella aurita* (Kinsey et al. 1994) and European anchovy *Engraulis encrasicolus* (Tudela 1999). Population structure in several other species has also been assessed using gill raker number, including New Zealand orange roughy *Hoplostethus atlanticus* (Haddon and Willis 1995), European whitefish *Coregonus lavaretus* (Amundsen et al. 2004) and Mediterranean horse mackerel *Trachurus*



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existence of three sardine morphs within the southern Benguela, with fish from the West, South and East coasts having significantly different body shapes (van der Lingen et al. 2010; Chiazzari 2014). The body shape of sardine from the northern Benguela is also significantly different from those from either the South African west or south coasts (Wessels 2009) and sardine from the South African east coast can also be differentiated from fish elsewhere off South Africa or Namibia by their otolith morphology (Hampton 2014). A meristic characteristic, vertebral number, also differs among sardine from these regions; sardine from the West and South coasts do not differ in vertebral number, but have significantly fewer vertebrae than Namibian fish and significantly more vertebrae than East Coast fish (Wessels 2009; van der Lingen et al. 2010). Those results support the hypothesis of three phenotypic sardine stocks in the southern Benguela, with phenotypic stocks being defined as groups that maintain 'characteristics that are expressed in one or more ways depending on the type of environment' (Booke 1981). Hence phenotypic variability likely reflects local adaptation to different environments, and recognition of intraspecific groups with persistent phenotypic differences in life-history traits, even if these differences do not reflect genetic differentiation, is important for appropriate fisheries management (Cadrian and Friedland 1999).

Genetic differentiation of the three putative stocks has yet to be shown unequivocally. Hampton (2014) examined seven microsatellite loci and the mitochondrial DNA marker ND2 and found no evidence for the occurrence of genotypically differentiated sardine stocks. In contrast, examination of nine exon-primed, intron-crossing (EPIC) DNA markers and the mitochondrially encoded cytochrome oxidase I (mtCOI) region of DNA from sardine from around the South African coast showed moderately low, but significant, genetic structuring and indicated that KZN (eastern) sardine are genetically different from the rest of the population (Chiazzari 2014), supporting the hypothesis of Fréon et al. (2010). A genomic analysis of the sardine population using next-generation-sequencing is presently under way (P Teske, University of Johannesburg, pers. comm.).

Research using parasites as bio-tags for population-structure studies has provided further support for the hypothesis of multiple sardine stocks within the southern Benguela. Sardine from the putative western stock off the South African west coast show significantly higher infection levels of a digenean 'tetracotyle' type metacercarian parasite, likely of the genus *Cardiocephaloides*, than do fish from either the putative southern (Weston et al. 2015) or eastern stocks (van der Lingen et al. 2015) and fish from the East Coast do not appear to be infected by the coccidian parasite *Eimeria sardinae*, whereas those from the West and South coasts are (van der Lingen et al. 2015).

The sardine population off South Africa consequently appears to comprise three phenotypically differentiated stocks and stock-specific assessment models for the putative western and southern sardine stocks have now been developed (de Moor and Butterworth 2015) for consideration and possible inclusion in management of the purse-seine fishery for this species. The aim of this study was to examine meristic (gill raker number) and morphological (gill

arch length and gill raker spacing) characteristics of the branchial basket of sardine from the South African west, south and east coasts in order to test further the hypothesis of three putative phenotypic sardine stocks in the southern Benguela. Additionally, we collected data on fish from Namibia in order to compare branchial morphology of sardine from the two subsystems of the Benguela, which we assume to be separate stocks. Previous work on the number and structure of gill rakers of sardine from the northern Benguela was conducted by King and Macleod (1976), who examined ontogenetic changes in these variables and compared them with similar data for anchovy *Engraulis encrasicolus*.

Material and methods

Samples of branchial baskets of juvenile and adult *Sardinops sagax* from around the southern African coast were obtained in 2008 and 2009, primarily from midwater trawl catches made during four research cruises, but also from catches made by a gillnet, a purse-seine net and a beach-seine net (Table 1; Figure 1). The research cruises were: (1) the pelagic recruit survey in May/June 2008 (PRS 2008), during which samples were collected off the South African west coast; (2) an acoustic experimental survey conducted in August 2008 (AES 2008), during which samples were collected off the South African west coast; (3) the pelagic spawner survey in October/December 2008 (PSS 2008), when samples were collected off the South African west and south coasts; and (4) a pelagic survey in February 2009 conducted off Namibia (NPS 2009). The first three of these cruises were conducted by the South African Government through what is now Branch: Fisheries Management of the Department of Agriculture, Forestry and Fisheries and the fourth by the Namibian Government. Sardine were also collected from a gillnet catch taken in Lüderitz Lagoon, Namibia, in November 2009 (LL 2009); from a purse-seine catch made by the FV *Borderer* off Mossel Bay, South Africa, in March 2009 (MBCC 2009); and from a beach-seine catch taken at Amanzimtoti in KZN in July 2009 (SRCC 2009) during the sardine run (see van der Lingen et al. 2010).

Samples were frozen after collection until subsequent analysis and were divided into four main groups representing geographical location: (i) the west-, (ii) south- and (iii) east coasts of South Africa and (iv) Namibia. The West and the South coasts are separated at Cape Agulhas (20° E; Figure 1), because this location divides the coastal upwelling system to the west from the temperate shallow shelf system to the east (Hutchings et al. 2009). The east coast of South Africa is defined here to extend north from the provincial border between the Eastern Cape and KZN, ending at the international border with Mozambique.

In the laboratory, the fish were thawed and their caudal length (CL) measured to the nearest 0.1 cm. The branchial basket was extracted, fixed in 10% formaldehyde and preserved in 70% alcohol prior to measurement (Magnuson and Heitz 1971; King and Macleod 1976; Malborough 1981). Branchial basket measurements and counting of gill rakers was done using a Leica L2 stereo microscope fitted with a calibrated ocular micrometer with 12 gridlines (each

mediterraneus (Turan 2004). Whether such differences reflect genetic stock structure is arguable, but if these indicate some degree of isolation then this would be important for fisheries management (Jemaa et al. 2015).

Such intraspecific differences arise because the morphology of individual fish within a single species can vary as a result of polymorphism (Amundsen et al. 2004), the causes of such variability including habitat characteristics (Malmquist 1992), developmental stage effects (Kinsey et al. 1994) and the availability of preferred and suitable food (Day et al. 1994). In these cases, phenotypic variation may not necessarily be linked to genetic variability, because external factors can play a role in suppressing a suitable gene rather than changing the gene's sequence (Swain and Foote 1999). Organisms that are genetically identical, but reared under different environmental conditions, can display quite distinct characteristics (Stearns 1989), sometimes resulting in individuals or subpopulations that display different morphologies or behaviour. In the examples listed above where gill raker number has been used to infer population structure, intermingling between different stocks or morphs within a single population is believed to cause genetic homogeneity, but this degree of mixing may not be enough to prevent the occurrence of phenotypic variability. However, gill raker number has been shown to be heritable in some fish, including threespine stickleback *Gasterous aculeatus* (Hagen 1973), rainbow trout *Oncorhynchus mykiss* (Leary et al. 1985) and sockeye salmon *Oncorhynchus nerka* (Foote et al. 1999).

In the Benguela Current ecosystem, southern African sardine *Sardinops sagax* is an ecologically and economically important species that is distributed from the Angola–Benguela Front in southern Angola, through Namibia and around the southern tip of the African continent to the KwaZulu-Natal (KZN) province on South Africa's east coast (Beckley and van der Lingen 1999). The intense, perennial upwelling cell off Lüderitz (26°–27.5° S) divides the Benguela into northern (Namibian) and southern (South African) subsystems and is thought to act as a barrier to the exchange of pelagic fauna (including sardine) between the two (Lett et al. 2007). Tagging studies showed no movement of sardine from the southern to the northern Benguela and virtually no movement in the opposite direction (Newman 1970). Despite a lack of genetic difference between sardine in the northern and southern Benguela, as observed from protein electrophoresis (Grant 1985) and analyses of mitochondrial and microsatellite DNA (Hampton 2014), fish from the two subsystems are considered to be independent stocks with little interaction (Boyer and Hampton 2001). That hypothesis is supported by disparities in the population dynamics of the two stocks and in other biological characteristics (Kreiner et al. 2001).

Changes in the distribution patterns of southern Benguela sardine off the West and South coasts at differing abundance levels have been observed over the past three decades, with the species occupying two distinct areas, consistently separated by the region between Cape Agulhas (considered to be the division between the West and South coasts; see Figure 1) and Mossel Bay at low and moderate biomass levels (Coetzee et al. 2008). Together with data on sardine egg distribution

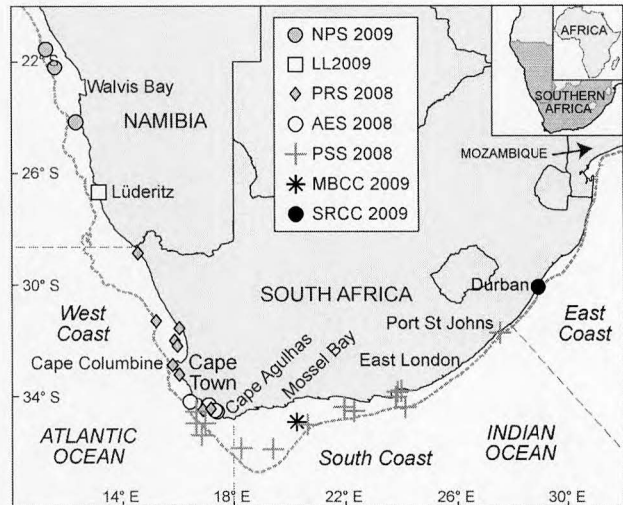


Figure 1: Map of southern Africa showing the locations from which sardine were collected for this study: NPS – Namibian pelagic survey; LL – Lüderitz Lagoon; PRS – pelagic recruit survey; AES – acoustic experimental survey; PSS – pelagic spawner survey; MBCC – Mossel Bay commercial catch; and SRCC – sardine run commercial catch. The divisions between the four regions (Namibia, West, South and East coasts) are shown as dashed lines. Places mentioned in the text are indicated

patterns, which also show two distinct spawning grounds (van der Lingen and Huggett 2003; van der Lingen et al. 2015), this suggests the possible existence of at least two separate, adult spawning assemblages. An individual-based model (IBM) coupled to a 3D-hydrodynamic model of the southern Benguela indicated that eggs spawned in these two regions had different fates, with those spawned to the west of Cape Agulhas being transported to a West Coast nursery area and those spawned to the east mostly being retained in a South Coast nursery area (Miller et al. 2006). Those authors concluded that sardine life history could be divided between two main systems separated at Cape Agulhas and, should these two systems represent largely self-sustaining components of the southern Benguela sardine population, then they each meet a current definition of a stock (Cadrin et al. 2005).

Sardine that undertake the annual 'sardine run' that occurs off South Africa's east coast in austral winter may represent a third stock, with consistent spawning observed there during winter and spring (Connell 2010). Whereas previous publications considered the sardine run to arise from an eastward extension of suitable habitat in winter, a recent and detailed analysis rejected that hypothesis and considered the sardine run to be a 'reproductive migration of a genetically distinct subpopulation' (Fréon et al. 2010).

Given these observations of three discrete sardine spawning areas off South Africa that may be indicative of western, southern and eastern stocks, a variety of complementary research approaches (as recommended by Begg and Waldman 1999) has since been used to examine the population structure of southern Benguela sardine. Examination of morphological variability has shown the

regions, with caudal length as a covariate. The model was of the form:

$$Y_{ijk} = e^{\beta_0 + \beta_1 \times \log_e(CL_{jk}) + \text{region}_k + \log_e(CL_{jk}) \times \text{region}_k + \varepsilon_{ijk}} \quad (1)$$

where Y_{ijk} is one of the three response variables i for each fish j from each region k ; β_0 is a constant, β_1 is a parameter describing the influence of the covariate $\log_e(CL)$ on the dependent variables, region_k is a vector of parameters describing the influence of each of the four regions on the dependent variable(s), $\log_e(CL_{jk}) \times \text{region}_k$ is an interaction term and ε_{ijk} is the difference for each fish between the predicted and observed values of each dependent variable. A sigma-restricted parameterisation with type VI sums of squares was used and four common multivariate statistics (Wilks' lambda, Hotelling's trace, Pillai's trace and Roy's maximum root) were examined to identify significant results, in addition to F -statistics for univariate results. Normality and homogeneity of variances were checked through examination of residuals.

To allow for the possibility that there is more mixing of cohorts from putative stocks among large than small fish, the results were presented for fish of four sizes: 9 cm CL (which corresponds to an age of approximately six months, Fréon et al. 2010), 12.5 cm CL (one year old), 16 cm CL (two years old) and 20 cm CL. Results for smaller fish from the East Coast and Namibia are presented, but not emphasised, because there were no fish from Namibia <10 cm and only five fish from the East Coast <16 cm. Fish of ≥ 20 cm CL were only obtained from Namibia and the South African south coast.

Results

In total, 377 branchial baskets of sardine with caudal lengths ranging from 6.1 to 23.6 cm were examined in this study, with 90, 95, 90 and 102 fish, respectively, from Namibia and the west, south and east coasts of South Africa (Figure 4). Samples obtained from the East Coast contained only large fish (Figure 4d) and those from Namibia had medium to large fish (Figure 4a), whereas samples from the West and South coasts also included smaller fish (Figure 4b and 4c). Gill arch length, the number of gill rakers and gill raker spacing all increased with fish length (Figure 5), indicating that the effect of fish size needed to be considered when analysing differences among the four regions.

The results of the GLM indicated significant influences of the explanatory variables on the three response variables, with the model explaining 89.4–93.8% of the variability in the data (Table 2). The results confirmed the significant effects of caudal length and indicated differences among regions (Table 2). Univariate results showed a significant interaction between fish caudal length and region for gill arch length ($F_{3,369} = 13.052$, $p = 4 \times 10^{-6}$) and number of gill rakers ($F_{3,369} = 8.894$, $p = 1 \times 10^{-5}$), but not for gill raker spacing ($F_{3,369} = 2.040$, $p = 0.108$). This result indicates that regional differences are not consistent across the fish size range, possibly because of migrations and mixing of older fish. Predicted values for smaller fish from the East Coast and Namibia should be viewed with caution, because they require extrapolations beyond the range of the data.

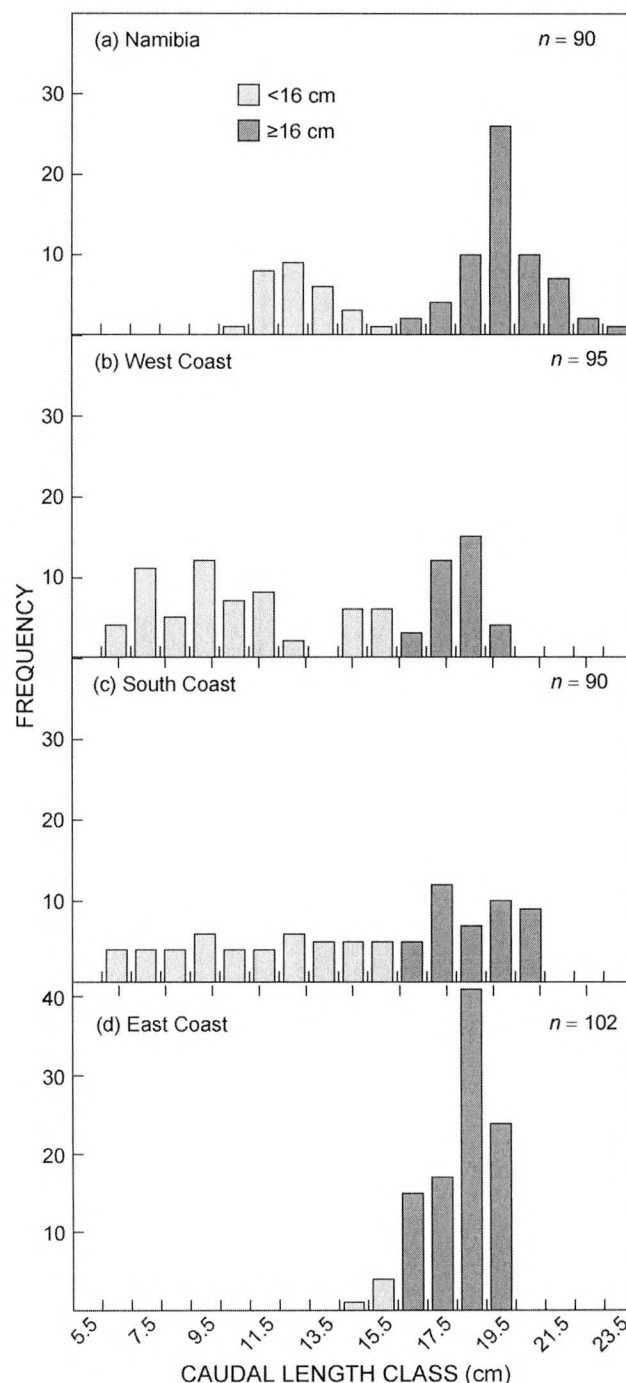
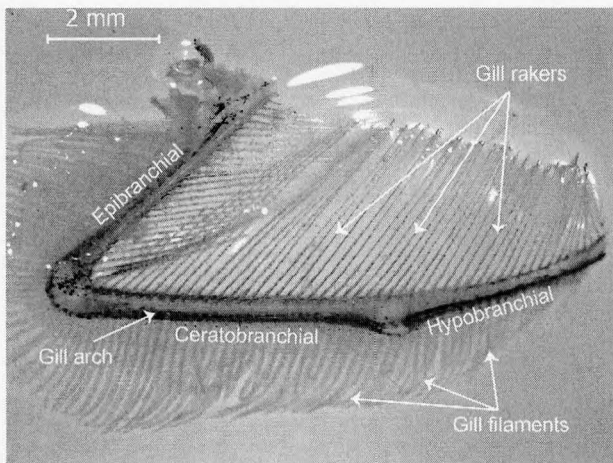
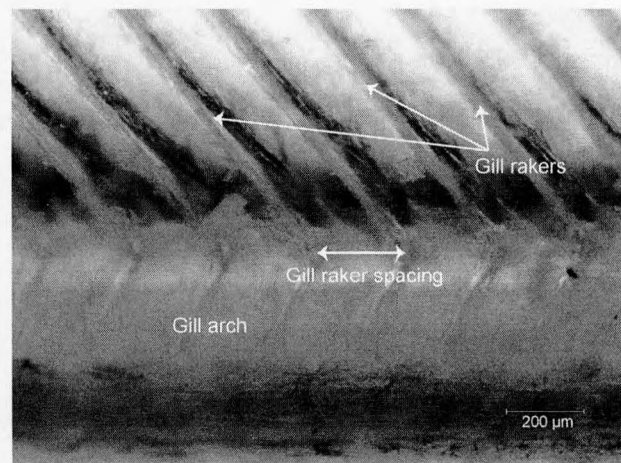


Figure 4: Frequency distributions of sardine size classes in the samples collected from (a) Namibia, (b) West Coast, (c) South Coast and (d) East Coast. Fish of <16 cm CL are approximately <2 years old

Predicted mean values of the three response variables for four different sizes of fish were compared among regions. Large sardine on the West and South coasts have longer gill arches than those from Namibia or the East Coast (Figure 6j), whereas smaller sardine from the South

Table 1: Summary of collection details of sardine used in the analyses (for locations see Figure 1)

Source	Region	Dates	Gear	Number of stations; number of fish	Caudal length (cm) (mean and SD)
Namibian pelagic survey (NPS)	Namibia	February 2009	Midwater trawl	3; 83	17.1 (3.3)
Lüderitz Lagoon (LL)	Namibia	November 2009	Gillnet	1; 7	21.9 (1.0)
Pelagic recruit survey (PRS)	South Africa: West Coast	May/June 2008	Midwater trawl	9; 73	13.0 (4.6)
Acoustic experimental survey (AES)	South Africa: West Coast	August 2008	Midwater trawl	3; 5	9.1 (1.1)
Pelagic spawner survey (PSS)	South Africa: West and South coasts	October–December 2008	Midwater trawl	14; 81	13.4 (3.5)
Mossel Bay commercial catch (MBCC)	South Africa: South Coast	March 2009	Purse-seine	1; 26	19.5 (0.9)
Sardine run commercial catch (SRCC)	South Africa: East Coast	July 2009	Beach-seine	1; 102	18.1 (1.1)

**Figure 2:** Photograph of the left side of the first gill arch excised from a Benguela sardine showing the three elements (epibranchial, ceratobranchial and hypobranchial) that make the gill arch and the location and orientation of gill rakers and gill filaments**Figure 3:** Photograph of a region of the ceratobranchial showing the gill rakers and gill raker spacing

divided into 10 smaller gridlines). The left side of the first gill arch was removed from the branchial basket following the methods of King and Macleod (1976). Measurements were taken of gill arch length (lower and upper limb) at 25 \times magnification (measurement resolution of 40 μ m) and the number of gill rakers was counted and gill raker spacing measured on both lower and upper limbs at 60 \times magnification (measurement resolution of 17 μ m; Figures 2 and 3). Measurements from the central (ceratobranchial) and lower (hypobranchial) limbs were combined as lower limb measurements, whereas the epibranchial section represented the upper limb. Measurements were made of five gill raker spacings closest to the middle of the gill arch, following Tanaka et al. (2006). Gill raker spacing was measured from the mid-point of one gill raker to that of the next (Figure 3). This measurement is analogous to the 'gill raker gap' estimates of King and Macleod (1976), assuming that all gill rakers were of equal thickness. Unfortunately, however, the gill raker gap data from King and Macleod's

(1976) study cannot be merged with gill raker spacing data for Namibian sardine collected during this study, because different methods were used. King and Macleod (1976) calculated gap size using a formula based on measurements of total length of the gill arch, total number of gill rakers and gill raker thickness, whereas this study employed direct measurement.

All measurements and counts were analysed using statistical software STATISTICA 12 (StatSoft Inc., 1984–2013), using a significance level of 0.05. All data were log₁₀ transformed to reduce heteroscedasticity after examination of diagnostic plots of residuals with and without data transformations. This also linearised the relationship between number of gill rakers and caudal length, which was curvilinear. Because the three dependent variables were significantly correlated ($r > 0.85$ for all combinations), a multivariate general linear model (GLM with three dependent variables) was used to assess the differences in measurements (gill arch length and gill raker spacing) and counts (number of gill rakers) for individual fish among the four

Table 2: Results from the multivariate GLM (Equation 1) showing statistics of the model for each of the response and explanatory variables for Benguela sardine from each of the four regions; the results of four multivariate tests of significance are shown for each of the explanatory variables. All statistics are significant at $p < 0.000001$

Model variable	Test statistic	Value	Effect df	Error df	Adjusted R^2
<i>Response variables</i>					
$\text{Log}_e(\text{gill arch length})$	F	1 418	4	372	0.938
$\text{Log}_e(\text{number of gill rakers})$	F	790	4	372	0.894
$\text{Log}_e(\text{gill raker spacing})$	F	804	4	372	0.895
<i>Explanatory variables</i>					
Constant (β_0)	Wilks	0.101	3	367	
	Pillai	0.899	3	367	
	Hotelling	8.933	3	367	
	Roy	8.933	3	367	
Region	Wilks	0.857	9	893	
	Pillai	0.148	9	1 107	
	Hotelling	0.161	9	1 097	
	Roy	0.111	3	369	
$\log_e(\text{CL})$	Wilks	0.252	3	367	
	Pillai	0.748	3	367	
	Hotelling	2.970	3	367	
	Roy	2.970	3	367	
Region \times $\log_e(\text{CL})$	Wilks	0.862	9	893	
	Pillai	0.142	9	1 107	
	Hotelling	0.155	9	1 097	
	Roy	0.112	3	369	

Discussion

Sardinops sagax from Namibia and the three South African subregions show similar trends in the development of their branchial basket, with gill arch length, gill raker number and gill raker spacing increasing with increasing caudal length. Despite these similarities, there are significant differences in some branchial basket meristic and morphometric characteristics of fish from the four regions (summarised in Table 3). These results provide further evidence of spatial variation in Benguela sardine phenotype and support previous hypotheses of discrete sardine stocks in the northern and southern Benguela and at least three morphs (or stocks, if phenotypic variability is indicative of distinct stocks) within the southern Benguela itself (van der Lingen et al. 2010, 2015).

A limitation of the current study is the fact that data were collected from fish that had been frozen prior to examination. Freezing has been shown to result in a small, but significant, decrease in both caudal length and wet body weight of sardine (Wessels et al. 2010), as has preservation in both formaldehyde and ethanol in other species (e.g. Kristoffersen and Salvenes 1998). Hence it is likely that a small degree of shrinkage and possibly a change in shape of the branchial basket may have occurred during preservation of the fish examined. The effects of preservation on branchial basket morphology, and whether such effects are consistent across the size range of fish examined, were not assessed in this study; hence measurements reported here should be considered relative and not absolute. Another possible limitation is the temporal mismatch in sampling, with fish from the different regions collected in different seasons over two years. This may confound

interpretation of our results, because of possible temporal variation in oceanic influences on sardine development and distributions, which were not explicitly accounted for. The climatology of the period 2008–2009 (when samples were collected) and earlier (1–4 years, when sampled fish were spawned), could indeed have played a role in the observed differences, but whereas Blamey et al. (2012) report some environmental variation during the late 2000s off the South-West Coast, Hutchings et al. (2012) document little change in the West Coast environment at that time. We recommend that future studies be designed to sample the same locations in different seasons and years in order to evaluate temporal variability and test whether differences among sardine from different regions observed in this study are repeatable.

The relationships between fish size and gill arch length, gill raker number and gill raker spacing are similar to those previously shown by King and Macleod (1976) for sardine from Namibia and for *Sardinops* species elsewhere, including the California Current ecosystem (Villalobos and Rodriguez-Sanchez 2002; Rykaczewski 2009) and the Kuroshio Current ecosystem (Tanaka 2006; van der Lingen et al. 2009). The increase in gill arch length and gill raker number with increasing size is not unexpected, because increases in body length will increase the size of other body parts, including the branchial basket (Bone et al. 1995). Size-related increases in branchial basket characteristics have also been observed in other filter-feeding marine fish, such as Namibian anchovy *Engraulis encrasicolus* (King and Macleod 1976), Atlantic herring *Clupea harengus* (Gibson 1988), alewife *Alosa pseudoharengus* (MacNeill and Brandt 1990), Pacific mackerel *Scomber japonicus* (Molina et al. 1996), Atlantic menhaden *Brevoortia tyrannus* (Friedland et

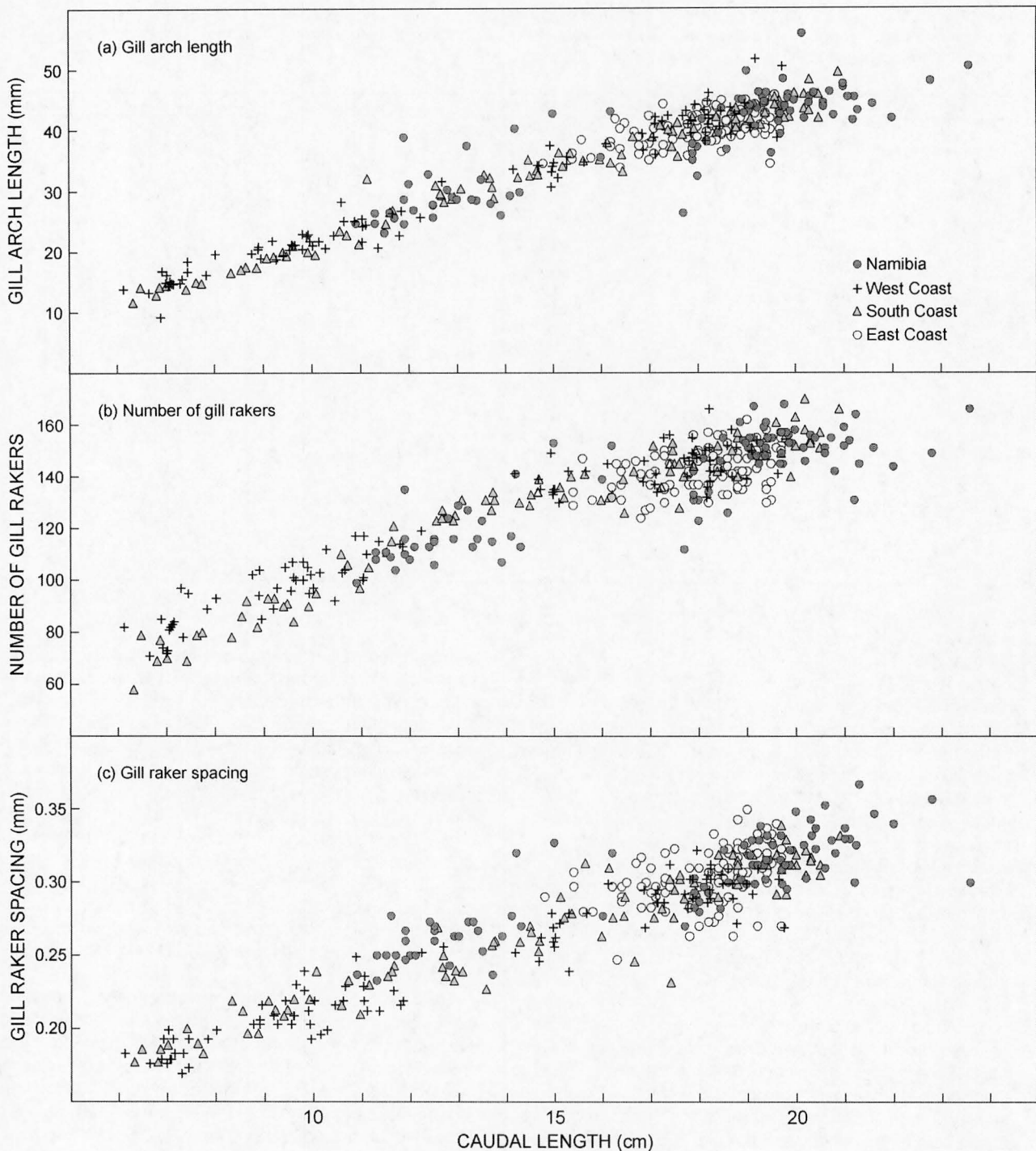


Figure 5: Relationships between (a) gill arch length, (b) number of gill rakers and (c) gill raker spacing and sardine caudal length for samples collected from the four regions (note that the data were not transformed in this figure, but were transformed for the statistical analyses)

Coast have significantly shorter gill arch lengths than fish from elsewhere (Figure 6d). Small sardine from the South Coast had fewer gill rakers than similar-sized sardine from the West Coast (Figure 6b and 6e), likely related to the shorter gill arch length of those fish. This pattern was not apparent in adult fish, with fish from the South and West

coasts having similar numbers of gill rakers, but significantly more than sardine from Namibia and the East Coast (Figure 6h and 6k). Sardine from the West and South coasts had similar gill raker spacings for all fish sizes (Figure 6c, 6f, 6i and 6l), but these were significantly smaller than those of fish from the East Coast and Namibia.

by creating a series of meshes in the branchial basket (the mechanical sieve model; Gerking 1994). The porosity of this sieve is determined by the combination of the number of gill rakers on arches in sequence and gill raker spacing (Drenner et al. 1984; Hammann 1985), but also by the presence of denticles that are thought to increase the efficiency of the sieve in retaining small food particles (Castillo-Rivera et al. 1996; Rykaczewski 2009). Laboratory experiments have demonstrated that filter-feeding is the dominant feeding mode of adult southern Benguela sardine (van der Lingen 1994).

The difference in gill raker number between large fish from some of the four regions is similar to the findings of studies listed in the Introduction, which generally reported differences in this meristic characteristic in other Clupeiformes from elsewhere. The apparent loss of differences from small to large sardine from the West and South coasts could indicate a high degree of mixing between adults of these stocks and recently developed stock-specific assessment models for the putative western and southern stocks have suggested that mixing between the two via the movement of recruits is appreciable (de Moor and Butterworth 2015). Future research on gill raker number should focus on the examination of smaller fish. We are aware of only a single paper describing intra-specific differences in gill raker spacing of a clupeiform fish, namely that by Andreu (1969) for European sardine *Sardina pilchardus*, which showed larger spatial variation in this characteristic (ranging from a gill raker spacing of c. 285 μm for a 20 cm TL fish from Vigo to c. 430 μm for a 20 cm TL fish from Madeira) than observed in this study. Similarly, a latitudinal gradient in branchiospinule (small denticles projecting from each gill raker) spacing for Atlantic menhaden *Brevoortia tyrannus* was reported by Friedland et al. (2006), who found that this decreased from north to south.

Differences in biological traits, such as those reported here, cannot themselves provide direct evidence of stock structure (Begg et al. 1999) and may instead represent phenotypic variability in a single stock. Such phenotypic variability can have both genetic and environmental causes (Swain and Foote 1999), but differentiating between the two requires controlled rearing experiments of fish from suspected stocks under the same environmental conditions, which has not been done for Benguela sardine. If such variability is mediated environmentally and not genetically, this does not necessarily reduce the likelihood of discrete stocks, because phenotypic variability could reflect local adaptation to different environments.

The phenotypic variability in sardine branchial basket morphometry reported here could be environmentally mediated and an adaptive response to different trophic environments, with sardine from the four regions showing differences because of different diets. The diet of sardine between Cape Columbine (West Coast) and Port Elizabeth (South Coast) was examined by van der Lingen (2002), who reported that whereas sardine is omnivorous and ingests both phytoplankton and zooplankton, the relative importance of these two food types varied both spatially and temporally. A clear difference in the relative contribution to dietary carbon by prey type and size between sardine from the west and east of Cape Agulhas was

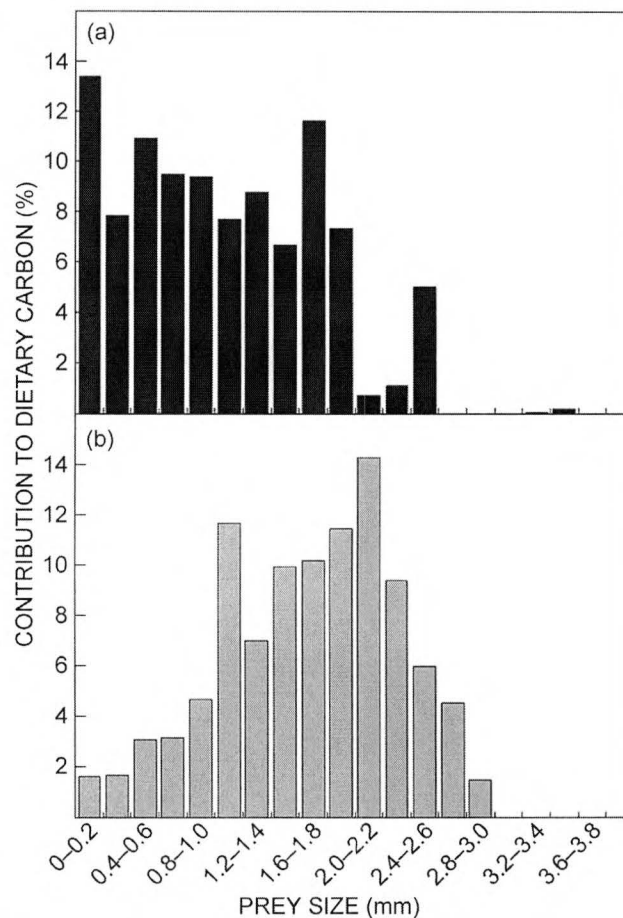


Figure 7: Size frequency distribution of the diet (expressed as percentage contribution to dietary carbon) of sardine sampled from (a) the west and (b) the east of Cape Agulhas (based on data in van der Lingen 2002)

observed, with the diet of sardine to the west comprising more phytoplankton, cyclopoid copepods and crustacean eggs and nauplii than the diet of sardine to the east, which consumed more calanoid copepods and fish eggs. When expressed in terms of prey size, sardine to the west derived 51% of their dietary carbon from prey <1 mm TL, whereas those to the east obtained only 14% of dietary carbon from prey of this size (Figure 7). Farther east, between East London and Port St Johns, fish eggs and calanoid copepods were again the greatest contributors to sardine dietary carbon (Mketsu 2008). Hence the cline in sardine gill raker spacing (which increases from west to east) along the South African coastline may reflect differences in their trophic environment and hence diet, as hypothesised for three-spine sticklebacks (*Gasterosteus* spp.) by Day et al. (1994). Should this be the case it could have significant implications for an ecosystem-based approach to fisheries management, given that sardine are important in pelagic food webs of the region and a key link between plankton and upper trophic levels. However, the match between diet and gill raker spacing observed for South African sardine does not appear to fit for Namibian sardine,

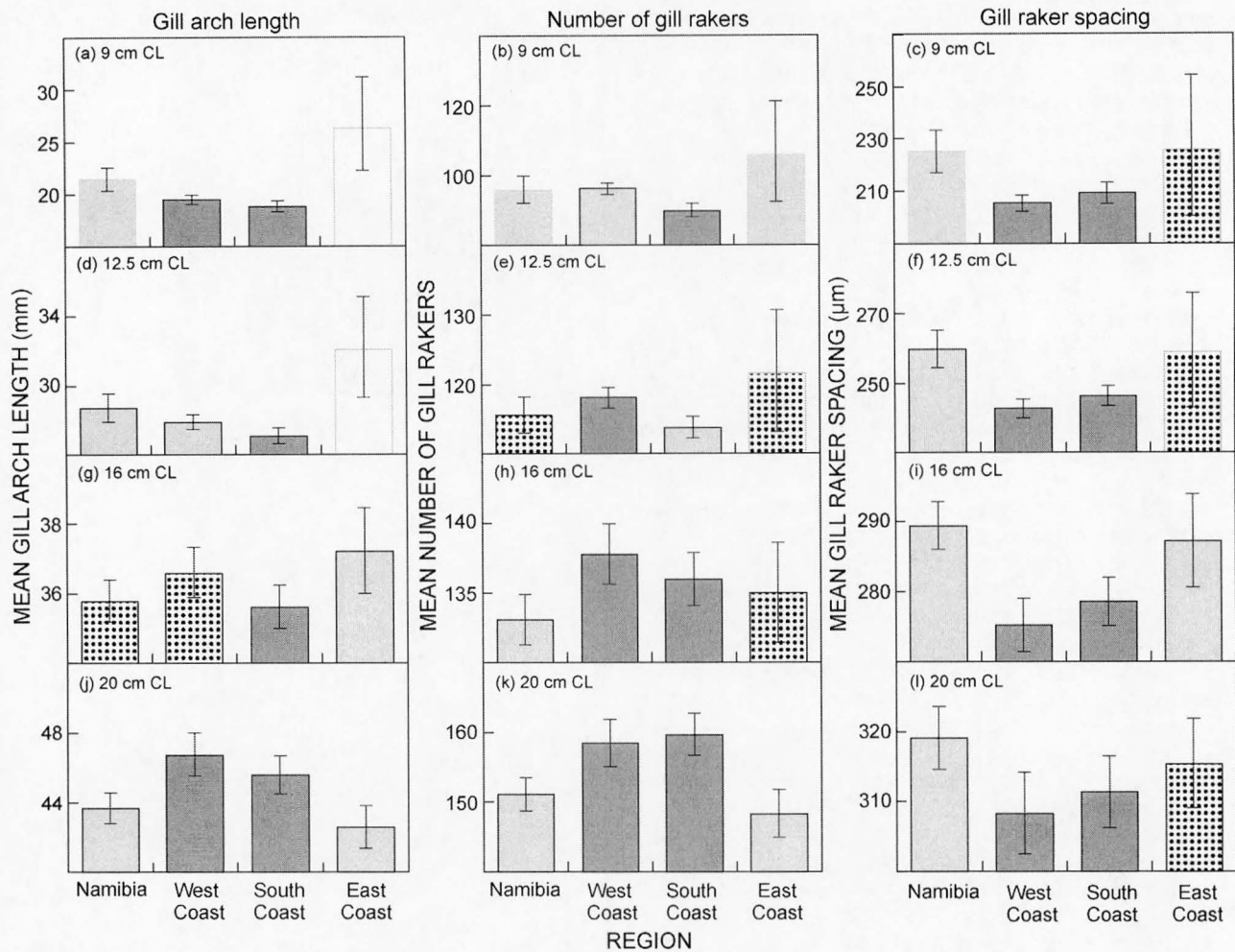


Figure 6: Results of the multivariate GLM showing predicted mean gill arch lengths, numbers of gill rakers and gill raker spacings for sardine of different sizes (approximate ages) in the four regions; (a, b, c) 9 cm CL (6 months old), (d, e, f) 12.5 cm CL (1 year old), (g, h, i) 16 cm CL (2 years old) and (j, k, l) 20 cm CL. Error bars denote $\pm 95\%$ CI. Different solid shadings (none, light or dark) indicate statistically significant differences between groups, patterned bars indicate the region could be assigned to more than one group and histograms with a pale outline for smaller (9 and 12 cm CL) fish represent model extrapolations beyond the range of the data

Table 3: Summary of results showing size effects and spatial patterns in branchial basket characteristics of Benguela sardine. EC = East Coast, SC = South Coast, WC = West Coast, Nam = Namibia

Characteristic	Fish size effect	Fish size	Pattern
Gill arch length	Varies among regions	Large (≥ 16 cm CL) Small (< 16 cm CL)	EC = Nam < SC = WC SC < WC
Gill raker number	Varies among regions	Large (≥ 16 cm CL) Small (< 13.9 cm CL)	EC = Nam < SC = WC SC < WC
Gill raker spacing	Consistent among regions	All sizes	WC = SC < EC = Nam

al. 2006), Japanese anchovy *Engraulis japonicus*, Pacific round herring *Etrumeus teres* and Japanese jack mackerel *Trachurus japonicus* (Tanaka et al. 2006).

The above authors report that gill raker spacing generally increases as fish increase in size and this increase has been implicated in a switch in feeding mode from filter- to particulate-feeding (e.g. Namibian anchovy; King and

Macleod 1976). However, an increase in gill raker spacing with increasing fish size may not necessarily reduce the ability of filter-feeders to entrap small prey. The reason is that in many species (including sardine) gill rakers occur not only on the first gill arch, but are found on all five gill arches and the occurrence of gill rakers on more than one gill arch is believed to increase the efficiency of filter-feeding

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which have the largest gill raker spacing, but were considered by King and Macleod (1976) to be primarily phytophagous, with a diet dominated by the diatoms *Fragilaria* and *Chaetoceros*. However, those authors assessed relative dietary importance using frequency-of-occurrence data and estimation of prey volume, both of which are inappropriate because they overestimate the importance of small prey, such as phytoplankton (van der Lingen et al. 2009). Consequently, re-evaluation of Namibian sardine diet using an objective method, such as calculation of the contribution to dietary carbon, might change perceptions of the diet of this species from being primarily phytophagous to essentially zoophagous (see, for example, Espinoza and Bertrand 2008).

Differences in gill raker number and spacing have been related to differences in diet both within and between species. Andreu (1969) identified two 'populations' (stocks) of *S. pilchardus* based on gill raker number and gill raker spacing, with fish from the Mediterranean and from Atlantic islands (Canary Islands, Azores and Madeira) having larger gill raker spacings compared to those from the Atlantic coast. Significant differences in gill raker number between *S. pilchardus* from the north-western Mediterranean (fewer gill rakers) and the Atlantic Iberian coast (more gill rakers) were related to differences in their prey species and size compositions (Costalago et al. 2015), with sardine from the Mediterranean feeding mainly on prey of 750–1 500 µm and 3 000–4 000 µm, corresponding to cladocerans, decapods and copepods, whereas those from the Atlantic fed primarily on smaller prey of 50–500 µm and 1 000–1 500 µm, which included phytoplankton, copepods and fish eggs. This marked difference in trophic ecology was considered to have arisen from local adaptation to contrasting feeding environments (Costalago et al. 2015). Bodaly (1979) found that two forms of lake whitefish *Coregonus clupeaformis* were characterised by differences in gill raker counts and spacings and that fish with few gill rakers and wide spacings were benthic feeders, whereas those with more gill rakers and narrower spacings fed pelagically. Similarly, Gillespie and Fox (2003) reported that two morphs of the freshwater pumpkinseed *Lepomis gibbosus*, which could be differentiated by internal morphometric measures including gill raker spacing, had different diets, with the pelagic morph feeding on a higher proportion of zooplankton prey than the littoral morph. Interspecifically, Castillo-Rivera et al. (1996) compared the trophic morphology and diet of finescale menhaden *Brevoortia gunteri* with that of the sympatric gulf menhaden *B. patronus* and found that the latter had a finer-meshed branchial basket and consumed significantly more phytoplankton and less zooplankton than did the former. Tanaka et al. (2006) attributed trophic partitioning between *E. japonicus*, *E. teres* and *T. japonicus* to differences in branchial basket morphology, as well as differences in feeding behaviour, and van der Lingen et al. (2009) describe a consistent pattern of wider gill raker spacing and a diet dominated by larger zooplankton for anchovy compared to a narrower spacing and smaller zooplankton-dominated diet for sardine, in several systems where the two genera co-occur. Vorsatz et al. (2015) also describe interspecific differences in gill raker spacing of four southern African Clupeiformes (*S. sagax*, *E. encrasicolus*, *Etrumeus*

whiteheadi and *E. wongratanae*), indicative of resource partitioning through differential feeding on zooplankton of different sizes.

Conclusions

Significant differences in some branchial basket characteristics were observed between sardine from Namibia and the west, south and east coasts of South Africa, supporting previous hypotheses of discrete stocks between the northern and southern Benguela and the possibility of three phenotypically differentiated sardine stocks off the South African coast. For South African fish, this phenotypic variability may be an adaptation to the local trophic environment.

The fisheries for Namibian and South African sardine are managed separately, and although a single homogeneous stock was previously assumed for South African sardine, consideration is being given to a multiple-stock hypothesis (van der Lingen et al. 2015), including the development of stock-specific assessment models for the putative western and southern stocks that are targeted by purse-seiners (de Moor and Butterworth 2015). Many marine fish species exhibit complex stock structure, comprising metapopulations with discrete spawning components (or stocks) rather than a single discrete population (Stephenson 1999). Failure to consider this structure in management could lead to the erosion of spawning components and we would argue that management strategies should aim to preserve stock complexity.

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