MEASUREMENTS OF THE BRANCHIAL SIEVE OF SARDINE (*Sardinops sagax ocellatus*) FROM THE WEST AND SOUTH COASTS OF SOUTHERN AFRICA

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DECLARATION

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Thank you, shukran, baie dankie, trimakassie, abregado, terima kasih…

ABSTRACT

Branchial sieves of southern African sardine (*Sardinops sagax ocellatus*) were collected from three geographical regions along the southern African coast: Namibia, the south coast and the west coast of South Africa. Sardine from Namibia represented the northern Benguela upwelling ecosystem while sardine from the west and south coasts of South Africa represented the southern Benguela upwelling ecosystem. Morphometric measurements (gill arch length and gill raker spacing) and counts (number of gill rakers) of branchial sieves on the left side of the first gill arch were taken to determine whether the sardine from these regions have different branchial sieve structures. A total of 221 samples of branchial sieves was measured, representing 35 samples from Namibia, 96 samples from the west coast and 90 samples from the south coast of South Africa. Results from a General Linear Model (GLM) analysis indicated that fish size (caudal length) has a significant effect on the measured variables (gill arch length: $F_{(1, 216)} = 4887.047$, $p < 0.05$; number of gill rakers: $F_{(1, 216)} = 2579.356$, $p < 0.05$; gill raker spacings: $F_{(1, 216)} = 2170.765$, $p < 0.05$). Further GLM analysis revealed that there were significant differences among regions in gill arch length ($F_{(2,216)} = 4.079$, $p < 0.05$), number of gill rakers ($F_{(2,216)} = 6.287$, $p < 0.05$) and gill raker spacings ($F_{(2,216)} = 7.020$, $p < 0.05$). Post hoc analyses (Tukey test) revealed that statistical differences occurred on all measured variables except gill arch length between sardine from Namibia and either one or both South African regions (west and south coasts). Sardine from the west and south coasts of South Africa showed significant differences in gill arch length and the number of gill rakers, but not in gill raker spacings. Differences in branchial sieve morphology could be related to differences in the size and type of prey consumed by sardine in these regions. The outcomes of this study support the hypothesis that South African and Namibian sardine are independent stocks, and the possibility of subpopulations of sardine from the west and south coasts of South Africa.

Chapter 1. Introduction. Biology and feeding ecology of southern African sardine *Sardinops sagax ocellatus*.

Sardine (*Sardinops sagax ocellatus*) (Grant *et al.* 1998) is one of the mid-water pelagic fish species found in southern Africa (King and Macleod 1976, Fairweather *et al.* 2006b, Coetzee *et al.* 2008, Crawford *et al.* 2008). This species, together with anchovy (*Engraulis encrasicolus*), has formed the main catches in purse seine fisheries since the early 20th century (Fairweather *et al.* 2006a, van der Lingen *et al.* 2006b). Globally, distributions of sardines (the genera *Sardinops* and *Sardinella*) can be found in areas where tropical and subpolar currents mix (Lluch-Belda *et al.* 1989), between the latitudes of 60^{0}N and 50^{0}S (Culley 1971) and also in areas where coastal upwelling carries cold and nutrient rich deep water to the surface in all three oceans (Bailey 1992). In total, intensive fisheries on sardines are conducted in five current regions of the world, namely the Kuroshio and Oyashio Currents (Japan), California Current (Canada, USA and Mexico), Humboldt Current (Peru and Chile), Canary Current (Morocco, Western Sahara, Mauritania, and Senegal) and Benguela Current (South Africa and Namibia) (Lluch-Belda *et al.* 1989, Rodriguez-Sanchez and Villalobos 2002, van der Lingen *et al.* 2006b, Checkey *et al.* 2009) (Figure 1.1).

Commercial, industrial scale sardine fisheries in South Africa and Namibia (previously known as South West Africa) started in 1943 and 1951 respectively (Culley 1971, Crawford *et al.* 2008). The earliest fishing grounds for sardine were Walvis Bay in Namibia and St. Helena Bay in South Africa (Culley 1971). Although sharing the same Benguela upwelling ecosystem, the sardine from Namibia and South Africa are separated (Newman 1970) geographically by an intense perennial upwelling cell off Lüderitz, which forms a thermal and circulation barrier restricting north and south movements of sardine (Lett *et al.* 2007).

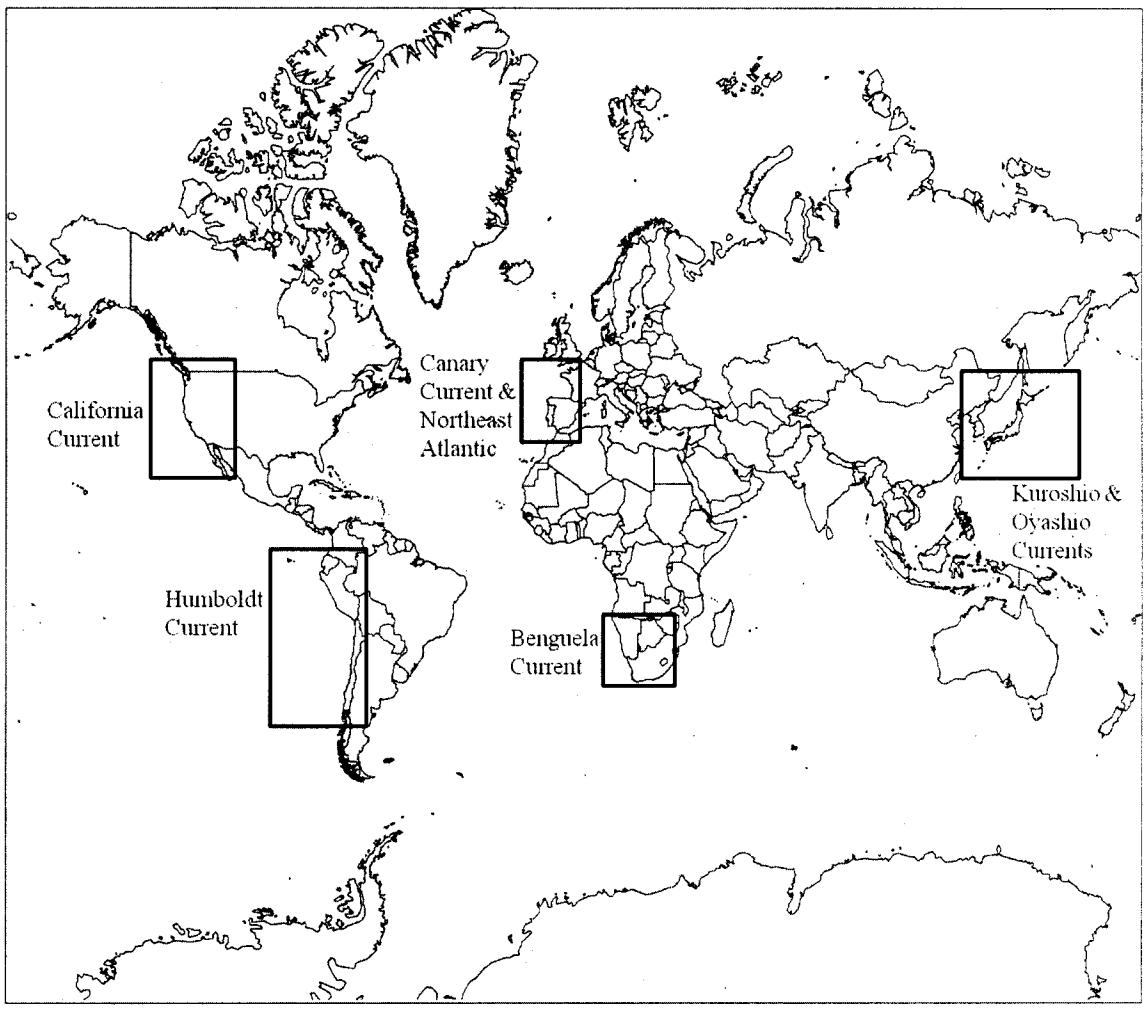

Figure 1.1: Main sardine fishery areas (after Lluch-Belda *et al.* 1989, Checkley *et al.* 2009).

In South Africa, sardine catches reached their peak in 1962 before a massive decrease by the late 1960s. Since then, the magnitude of catches has fluctuated with a continuous decrease during the 1980s. However, introduction of a total allowable catch (TAC) during the mid 1980s allowed slow recovery of the population. Exceptional recruitment at the end of the 1990s led to high catches, averaging around 200 000 tons between 2001 and 2005. Poor recruitment since 2004 has led to a rapid decrease in sardine biomass and subsequently catches since 2005 (Coetzee *et al.* 2008). In Namibia, the catches reached their peak later than those in South Africa, in the late 1960s, with the highest catch recorded in 1968 (1.4 million tons) (Boyer and Hampton 2001). Subsequently, the catches declined (Schwartzlose

et al. 1999) resulting in a temporary ban of all sardine-directed fishing in 1981 (Cram 1981, as stated in Boyer *et al.* 2001). However, fishing continued again in 1982 and by 2000 the catch was a mere 25000 tons (Boyer and Hampton 2001) and continued low biomass levels led to a zero TAC being allocated for the 2002/2003 fishing season (Sumaila and Stephanus 2006).

From 1997 to 2005, the distribution of sardine in South Africa shifted about 400km to the south and east (van der Lingen *et al.* 2005, Fairweather *et al.* 2006a, Coetzee *et al.* 2008, Crawford *et al.* 2008). Several hypotheses have been suggested to explain this phenomenon, including 1) local depletion of fish stocks on the west coast and Western Agulhas Bank due to high exploitation, 2) environmentally induced changes in the distribution of sardine spawners and 3) successful spawning and recruit survivorship on the south coast compared with the west coast, contributing to a distribution imbalance (van der Lingen *et al.* 2005). Recent findings, however, indicated that for the past two years (2008 and 2009), sardine originated from the west coast appear to be moving back to their original distribution (Hutchings, MCM, pers. comm.).

Biology of *Sardinops sagax ocellatus*

Southern African sardine belongs to the family Clupeidae (Whitehead 1985), which also includes other commercial species such as Atlantic menhaden (*Brevoortia tyrannus*), Atlantic herring (*Clupea harengus*), Baltic herring (*Clupea harengus membras*) and Pacific herring (*Clupea pallasii*) (Bailey 1992). These species forage in neritic areas in large schools, which serve to protect them from predation and also increase feeding efficiency in the presence of predators (Gerking 1994, Bone *et al.* 1995) such as penguins, seals, dolphins (Crawford *et al.* 2008) and other large pelagic fish (Shannon *et al.* 2000).

Categorized as an oily fish similar to southern African anchovy (*Engraulis encrasicolus*) and west coast redeye (*Etrumeus whiteheadi*), sardine is not only important for the economy of Namibia and South Africa as part of pelagic fisheries production (Boyer *et al.* 2001, van der Lingen 2002, Fairweather *et al.* 2006b), but also in the trophic structure of the Benguela upwelling ecosystem (Cury *et al.* 2000, van der Lingen 1998). Sardine is positioned at a crucial intermediate trophic level which is believed to control not only the population size of its prey (plankton) but also its predators (Cury *et al.* 2000). Any large changes in abundance of these so-called wasp-waist populations (Cury *et al.* 2000, Bakun 2006), caused either by natural (e.g. climate change, predation) or anthropogenic (e.g. fishing) (van der Lingen *et al.* 2005) causes can destabilize populations of their predators as well as their prey.

The population of sardine in southern Africa is believed to be divided into two main populations, namely off Namibia and South Africa (Newman 1970, Kreiner *et al.* 2001, Wessels 2009). An early study by Newman (1970) suggested that sardine from the Western Cape (South Africa) should be treated as an independent population from sardine from Walvis Bay-Lüderitz (Namibia), based on migration movements. Kreiner *et al.* (2001) found that there was a lack of coherence in condition factor (CF) between sardine from the northern and southern Benguela ecosystems, which led to the suggestion of two separate and independent stocks between Namibia and South Africa. Recent studies by Wessels (2009) from her studies on morphometric variation among sardine populations in southern Africa found that sardine from Namibia have different head measurements than sardines from the west and south coasts of South Africa. At the same time, she also suggested that the positions of the pectoral fin and head measurements of sardine from the west coast are different from those from the south coast of South Africa, which could possibly indicate the existence of subpopulations of sardine within the southern Benguela upwelling ecosystem.

Feeding ecology of *Sardinops sagax ocellatus*

Like most other clupeoids, sardine is an omnivorous plankton feeder, feeding on both zooplankton and phytoplankton (King and Macleod 1976, James 1988, van der Lingen 1998, van der Lingen 2002, Emmett *et al.* 2005, van der Lingen *et al.* 2006a). Previous studies, particularly in the northern Benguela upwelling region, concluded that sardine feed largely on zooplankton in their juvenile stage and change to phytoplanktivory when adult (King and Macleod 1976). These conclusions were based on quantitative studies that assessed diet in terms of relative numerical abundance of prey in sardine gut contents and also measurements of gill raker morphology. However, it was subsequently revealed that numerical abundance is not an appropriate way to address the issue of dietary preference and relative dietary importance; analysis of dietary components (nitrogen and carbon) is more reliable (James 1988). Based on his review of previous studies, James (1988) concluded that zooplankton forms between 60 and 89% of the diet of sardine in the Benguela. Further studies done by van der Lingen (2002) and Mketsu (2008) on sardine in the southern Benguela upwelling region and on the east coast of southern Africa, respectively, have confirmed James's (1988) conclusion that zooplankton dominates sardine diet, with micro-zooplankton such as small copepods and fish eggs being the main dietary items for sardine. Although King and Macleod (1976) erroneously concluded that sardine preferred phytoplankton in their studies in Namibia (northern Benguela upwelling system), their studies pioneered further feeding studies of sardines in southern Africa subsequent to that of Davies (1957).

Sardine have the ability to alternate between filter feeding and particulate feeding behaviour depending on the size and concentration of available prey (van der Lingen 1994). This provides sardine with a wide range of possible food items and allows the species to avoid competition with other small pelagic fish such as anchovy (Louw *et al.* 1998, van der Lingen 1994) which feed on larger (>1.5mm) zooplankton through size-selective particulate

feeding and filter feed on smaller zooplankton and phytoplankton (van der Lingen *et al.* 2009a). Diet partitioning as the result of distinctive feeding patterns also has been observed in a few species such as herring (*Clupea harengus*), which is a particulate feeder and feeds only on zooplankton (Casini *et al.* 2004), Atlantic menhaden (*Brevoortia tyrannus*), which is a filter feeder on both phytoplankton and zooplankton (Friedland *et al.* 2006), and dogtooth herring (*Chirocentrodon bleekerianus*) which is a predaceous fish-eater (Sazima *et al.* 2004).

An important factor in determining successful feeding (feeding efficiency) and the type of food consumed is the adaptation of the branchial basket for filter feeding activity (King and Macleod 1976, Drenner *et al.* 1984, MacNeill and Brandt 1990, Castillo-Rivera *et al.* 1996, Molina *et al.* 1996, Friedland *et al.* 2006). The branchial basket holds the gills, gill arches and gill rakers, with the latter two structures being directly involved in filter feeding activity (Bone *et al.* 1995, Gerking 1994) (Figure 1.2).

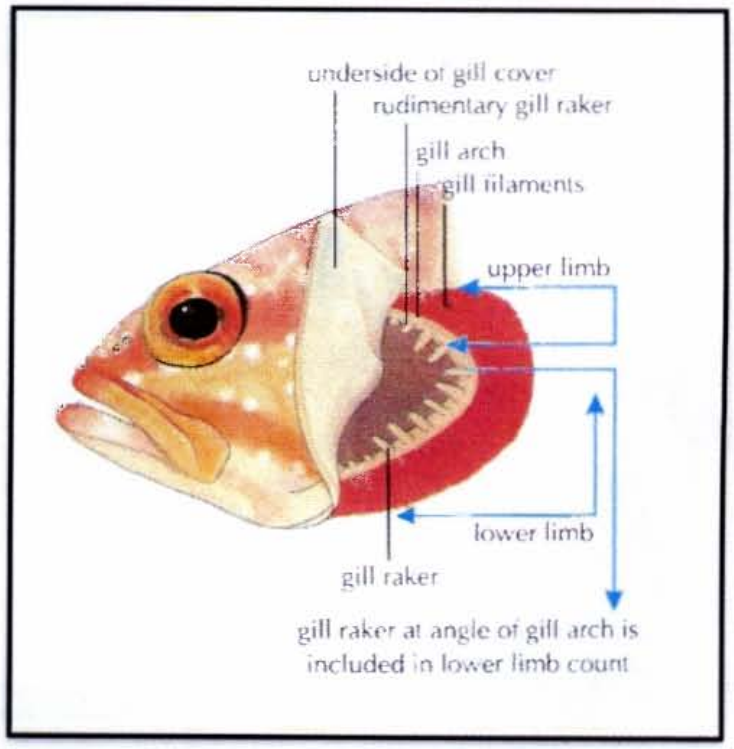

Figure 1.2: Morphology of the branchial basket in a typical fish (from Heemstra and Heemstra 2004).

The gill arch works in two important aspects: 1) as a fundamental structure to hold gill filaments and the main capillaries (afferent and efferent), preventing collapse and ensuring respiration efficiency and 2) in filter feeding fish, the gill arch works as a firm base for the attachment of gill rakers (Gerking 1994, Bone *et al.* 1995, Jobling 1995, Heemstra and Heemstra 2004). The gill rakers' form and function work differently based on the feeding behaviour and dietary preferences of fish. Carnivorous fish like barracuda (*Sphyraena* spp.) and Shakalin trout (*Parahucho perri*; Pichugin and Sidorov 2006) do not have any gill rakers at all or they are 'reduced' (small, rough tubercles), whereas filter feeding fish such as alewife (*Alosa pseudoharengus*) (MacNeill and Brandt 1990), sardine (*Sardinops sagax ocellatus*) and anchovy (*Engraulis encrasicolus*) (King and Macleod 1976) have numerous, elongated and thin gill rakers lying very close together (Drenner *et al.* 1984, Hammann 1985, Gerking 1994, Bone *et al.* 1995, Friedland *et al.* 2006) (Figure 1.3).

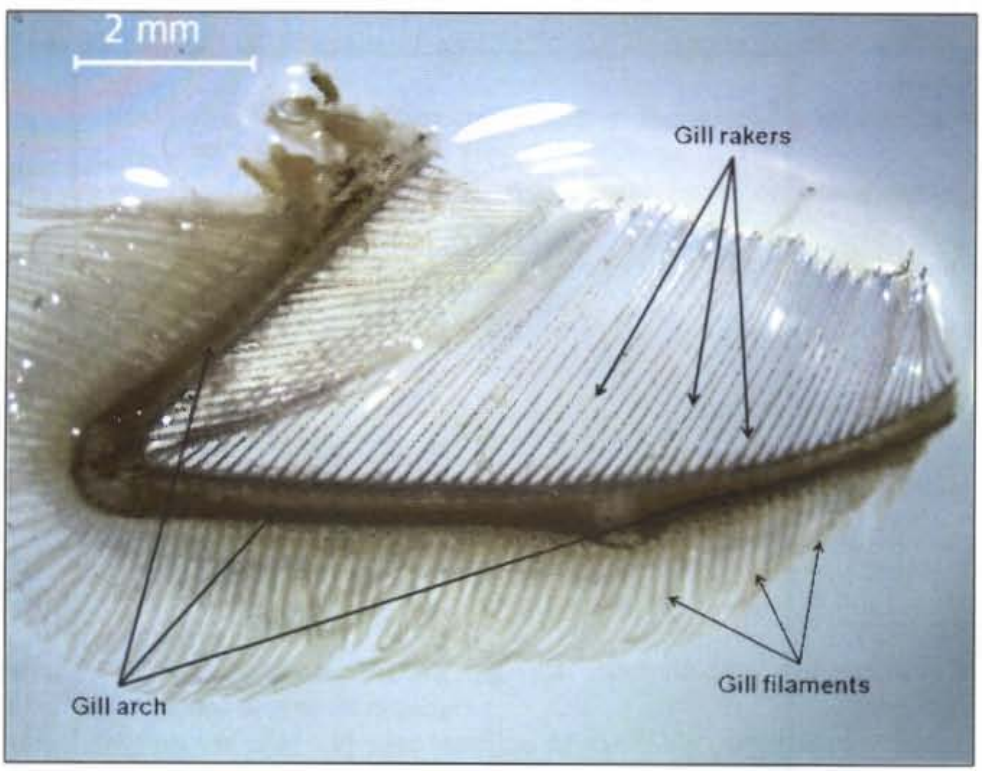

Figure 1.3: Gill arch with elongated gill rakers from sardine (*Sardinops sagax ocellatus*).

In filter feeding fish, plankton (phytoplankton and zooplankton) and suspended organic particles of a sufficiently large size in the water column become trapped in the meshes of the gill rakers while smaller particles pass through and are expelled through the opercular opening (Gerking 1994, Bone *et al.* 1995). This happens either when fish swim forward with their mouths agape and opercules flaring (ram filtration strategy) or by a series of rapid, non-directed suction actions while the fish is stationary (pump filtration strategy) (Gerking 1994). The mesh of the gill rakers can function as a sieve, according to the mechanical sieve model (Gerking 1994), previously described for *S. s. ocellatus* as interlocking of the gill rakers (King and Macleod 1976), with the interraker gap being assumed to be the 'pore' size of the sieve. The overlap of gill rakers from one gill arch with those from another gill arch next to it creates a finer mesh size (Magnuson and Heitz 1971).

The concept of a mechanical sieve in filter feeding forage fish has been challenged with another two concepts: 'mucous entrapment' (Gerking 1994, Sanderson *et al.* 1996) and crossflow filtration (Sanderson *et al.* 2001). Fish that employ mucus entrapment possess mucus-secreting cells located on the anterior part of the gill raker blade where food particles come in contact with it. The mucus-food complex then travels down to the buccal cavity before proceeding to the oesophagus (Friedland 1985). In the crossflow filtration technique, the high velocity crossflow retains the food particle inside the oral cavity, and not on the gill rakers. At the same time, the filtrate (water and smaller particles) exits between the gill rakers. The crossflow velocity then carries trapped food particles to the oesophagus (Sanderson *et al.* 2001).

Gill raker morphology has been studied widely for various filter feeding species such as Pacific mackerel (*Scomber japonicus*) (Magnuson and Heitz 1971, Molina *et al.* 1996), alewife (*Alosa pseudoharengus*) (MacNeill and Brandt 1990), and Atlantic menhaden (*Brevoortia tyrannus*) (Friedland *et al.* 2006). The majority of these studies compared prey

size in the stomach with the gill raker morphology, especially the gill raker spacing (*e.g.* Tanaka *et al.* 2006). In addition, gill raker morphology has been used to identify subpopulations (Mais 1972) and to describe characteristics of exploited stocks (Ramirez-Granados 1958, as cited by Rodriguez-Sanchez and Villalobos 2002).

Gill raker morphology studies on sardine

Since sardine is an important small pelagic fish for both economic and ecological reasons (van der Lingen 2002), understanding the feeding ecology of sardine is important for better management of this resource, which is positioned at an intermediate trophic level (Cury *et al.* 2000). One of the ways to better understand feeding ecology is by looking at gill raker morphology, which is one of the main factors determining the feeding efficiency and food partitioning between marine forage species at similar trophic levels (King and Macleod 1976, Drenner *et al.* 1984, James 1988, van der Lingen 1994, van der Lingen *et al.* 2009a).

Gill raker morphology of sardine from other regions has been studied widely for *S. s. sagax* (previously known as *S. caeruleus*) in California (Rodriguez-Sanchez and Villalobos 2002, Rykaczewski 2009) and *S. melanostictus* in Japan (Nakai 1938, Matsuoka 1997). The only study on gill raker morphology of sardine in the Benguela upwelling ecosystem was done by King and Macleod (1976) off Namibia who described the feeding apparatus of sardine as consisting of five gill arches with gill rakers situated on top of each arch. The first three arches have gill rakers in anterior positions, while the fourth gill arch has gill rakers situated anteriorly and posteriorly, and the last gill arch has gill rakers in a posterior direction. King and Macleod (1976) also reported that the sardine gill raker number, gill arch length, gill raker gap and gill raker length increase almost linearly with fish size. They used a formula to determine gill raker gap using data on gill arch length, number of gill rakers and mean gill raker width. Apart from branchial sieve measurements, King and Macleod (1976)

also looked at the food of juvenile and adult sardine in Namibia and reached the conclusion that juveniles are zooplanktophagus while adults are phytoplanktophagus. This conclusion however was challenged for southern Benguela sardine when James (1988), van der Lingen (1999) and Mketsu (2008) revealed that sardine is primarily zooplanktophagus throughout its life stages.

Since King and Macleod's (1976) paper, there have been numerous feeding behaviour and preference studies on sardine (James 1988, van der Lingen 1994, Louw *et al.* 1998, van der Lingen 1998, van der Lingen 1999, van der Lingen 2002, Mketsu 2008), but no additional studies on the morphology of sardine's gill rakers. This lack of knowledge of branchial morphology of sardine in the southern Benguela upwelling ecosystem means there is a gap in our understanding of the trophic ecology of sardine in the Benguela upwelling ecosystem.

Objectives of this study

The objectives of this project consist of two aspects. The first objective is to understand the characteristics of the branchial sieve of southern African sardine from the northern and southern Benguela upwelling ecosystems. The second objective of this study is to determine if there are differences in branchial sieve structures of sardine between the northern and southern Benguela upwelling ecosystems and also between the west and south coasts of South Africa. These objectives have been chosen to determine whether the phenotypic variation reported by Wessels (2009) for sardine populations in the northern and southern Benguela upwelling ecosystems also occur in the branchial sieves. Both objectives are achieved by applying the methods of King and Macleod (1976) to new data sources.

Chapter 2. Measurements of the branchial sieve of sardine (*Sardinops sagax ocellatus*) from the west and south coasts of southern Africa

Many marine filter feeding fish come from the family Clupeidae which includes American shad (*Alosa sapidissimaa*) (Hammann 1985), herring (*Clupea harengus*) (Batty *et al.* 1986, Casini *et al.* 2004), Atlantic menhaden (*Brevoortia tyrannus*) (Friedland *et al.* 2006), and sardine (*Sardinops sagax ocellatus*) (van der Lingen 2002). As filter feeders, these planktivores consume either only phytoplankton (*Dorosoma cepedianum*; Drenner *et al.* 1984), or zooplankton (*Alosa pseudoharengus*; MacNeill and Brandt 1990) or are able to feed on both (*Brevoortia tyrannus*; Friedland *et al.* 2006).

Feeding, among other factors, plays a very important role in the growth and development of fish (Gerking 1994). Success in feeding is not only determined by the availability of preferred food for positive growth, but also by how the feeding apparatus (Magnuson and Heitz 1971) and strategy are able to capture sufficient amounts of food (Holanov and Tash 1978).

The distinct feature of filter feeding fish is the presence of substantial numbers of long and thin gill rakers positioned mostly on the anterior part of gill arches (Gerking 1994, Bone *et al.* 1995, Heemstra and Heemstra 2004). Regardless of whether the fish employs ram or pump filtration strategies (Gerking 1994), gill rakers are believed to trap plankton and suspended organic matter from the water column in their meshes (Nelson 1967, Sanderson *et al.* 2001, Rodriguez-Sanchez and Villalobos 2002, Tanaka *et al.* 2006). The meshes work as sieves which are able to retain food sources according to the size and types preferred by the fish. They are also able to discard small particles through the opercular opening (Gerking 1994).

Apart from its use in feeding, the branchial sieve is believed to indicate the existence of different stock populations of a genetically similar species (Mais 1972), since the feeding morphology of individual fish within the same species can vary as a result of polymorphism (Amundsen *et al.* 2004). The causes of this variability range from habitat characteristics (Malmquist 1992) to availability of preferred and suitable food (Day *et al.* 1994) and developmental stage (Kinsey *et al.* 1994). In some cases, phenotypic variation has nothing to do with genetic variability since external factors may play a large 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 conditions can display quite distinct characteristics (Stearns 1989), sometimes resulting in individuals or populations which display different morphology or behaviour. Among fish species, examples of phenotypic variability are phenotypic plasticity (environmentally induced phenotypic variation) causing morphometric variation among sardine (*Sardine pilchardus*) from the northeastern Atlantic and the western Mediterranean (Silva 2003), the presence of different stocks of horse mackerel in the Mediterranean Sea (Turan 2004), and diet induced phenotypic plasticity of stickleback (Day *et al.* 1994).

Phenotypic variability of branchial sieves has been detected in Mediterranean horse mackerel (*Trachurus mediterraneus*) by Turan (2004). Morphometric and meristic work on *T. mediterraneus* (including branchial sieve studies) revealed that this species forms a number of independent populations, especially in the Marmara Sea, whereas some intermingling is believed to occur between stocks from the Black, Aegean and Eastern Mediterranean Seas. Similar conclusions were made by Haddon and Willis (1995) for orange roughy (*Hoplostethus atlanticus*) in New Zealand. Morphometric measurements and meristic counts on this species, including for the gill rakers, revealed that orange roughies from Puysegur Bank and those caught on the Lord Howe Rise are separate stocks. Intermingling

between these populations is believed to cause genetic homogeneity but is not strong enough to prevent phenotypic variability from occurring.

In the Benguela Current ecosystem, southern African sardine (*Sardinops sagax ocellatus*) and anchovy (*Engraulis encrasicolus*) are the important species in the small pelagic fishery (Fairweather *et al.* 2006a, Fairweather *et al.* 2006b, Coetzee *et al.* 2008, MacCall 2009). Sardine species can be found from the boundary of the warm Angola Current north of Namibia to the KwaZulu Natal province in South Africa (Beckley and van der Lingen 1999). The presence of an intense perennial upwelling cell off Lüderitz, however, is believed to isolate sardine between Namibia and South Africa (Lett *et al.* 2007). In addition, a comparison of sardine's condition factor (CF) from each subsystem shows a disparity in CF trends between the two that suggests that the northern and southern Benguela sardine stocks are independent (Kreiner *et al.* 2001)

Although they are environmentally separated, sardine from Namibia and South Africa are believed to be genetically similar (Beckley and van der Lingen 1999), although more extensive genetic studies are currently being done (Hampton, UCT, pers. comm.). However, Namibian sardine is believed to be different in body form from South African sardine (Wessels 2009).

The only branchial sieve measurements of sardine in the Benguela Current system were done by King and Macleod (1976). Their work, which concentrated on sardines (previously known as *S. ocellata*) and anchovy (previously known as *E. capensis*) from the northern Benguela Current system off Namibia, concluded that both species are non-selective filter feeders that change their preferred diet from zooplankton to phytoplankton once they reach 100mm and 80mm in body length respectively. Those authors also found that the number of gill rakers for anchovy does not increase when reaching the size of 80mm in

contrast to sardine. No work has yet been done on branchial sieves of sardine from the southern Benguela upwelling ecosystem, which includes the west and south coasts of South Africa. The study by King and Macleod (1976) also concentrated on the branchial sieve morphometry but not on the variability of branchial sieves among different regions.

The aims of this study are to determine the morphologies of the branchial sieve (gill arch length, number of gill rakers, and gill raker spacings) of sardine from the west and south coasts of South Africa and to update the data from Namibia by applying some of the methods of King and Macleod (1976) to further samples. Sardine from Namibia are assumed to represent the northern Benguela upwelling ecosystem whereas samples from the west and south coasts of South Africa represent the southern Benguela upwelling ecosystem. This study also aims to determine whether there are differences in branchial sieve measurements of sardine from different areas, which might indicate different subpopulations, particularly in the southern Benguela region where two sardine subpopulations are thought to exist (Fairweather *et al.* 2006b).

Materials and Methods

Samples of branchial sieves of adult and juvenile *S. s. ocellatus* were obtained from four research cruises and one commercial trawler in 2008 and 2009. The research cruises were: 1) Recruitment Biomass Survey (RBS 2008), 2) August Acoustic Survey (AAS 2008), 3) Pelagic Spawner Biomass Survey (PSB 2008), and 4) Namibian Pelagic Survey, 2009. The first three of these cruises were conducted by Marine and Coastal Management of South Africa, and the last one by the Namibian Government. Sardines from commercial trawling came from the FV Borderer off Mossel Bay, South Africa in 2009. These samples covered the west and south coasts of southern Africa (Figure 2.1).

Samples were divided into three main groups representing geographical location: Namibia, the west coast of South Africa, and the south coast of South Africa. The west and the south coasts are divided at Cape Agulhas as this location is viewed as the easternmost boundary of the southern Benguela upwelling ecosystem (Miller *et al.* 2006). The caudal length (mm) of each fish was measured to the nearest mm using a measuring board, and then the branchial sieve was extracted from the fish cavity, fixed in 10% formaldehyde and preserved in 70% alcohol prior to measurement (Magnuson and Heitz 1971, King and Macleod 1976, Malborough 1981).

Branchial sieve measurements and counting of gill rakers were done using a Leica L2 stereo microscope with 25X and 60X magnifications, which gave measurement resolutions of 40μm and 17μm respectively. An ocular micrometer with 12 grid lines (each gridline divided into 10 smaller grid lines), embedded in the eye-piece, was used for measurement. The micrometer was calibrated prior to measurement. The left side of the first gill arch was removed from the branchial sieve under the stereo microscope, following the methods of King and Macleod (1976). Measurements were taken of the gill arch length (lower and upper limb) and gill raker spacings, and the number of gill rakers on both lower and upper limbs

was counted (Figure 2.2 and 2.3). The central (ceratobranchial) and lower (hypobranchial) limb data were combined as lower limb measurements while the epibranchial section represented the upper limb. Measurements were made of five gill raker spacings closest to the middle of the gill arch, based on work done by Tanaka *et al.* (2006). Gill raker spacing measured in this study ranges from the mid-point of one gill raker to the next. (Figure 2.3). This measurement is equated to the "gill raker gap" measurements of King and Macleod (1976), assuming that all gill rakers were of equal thickness.

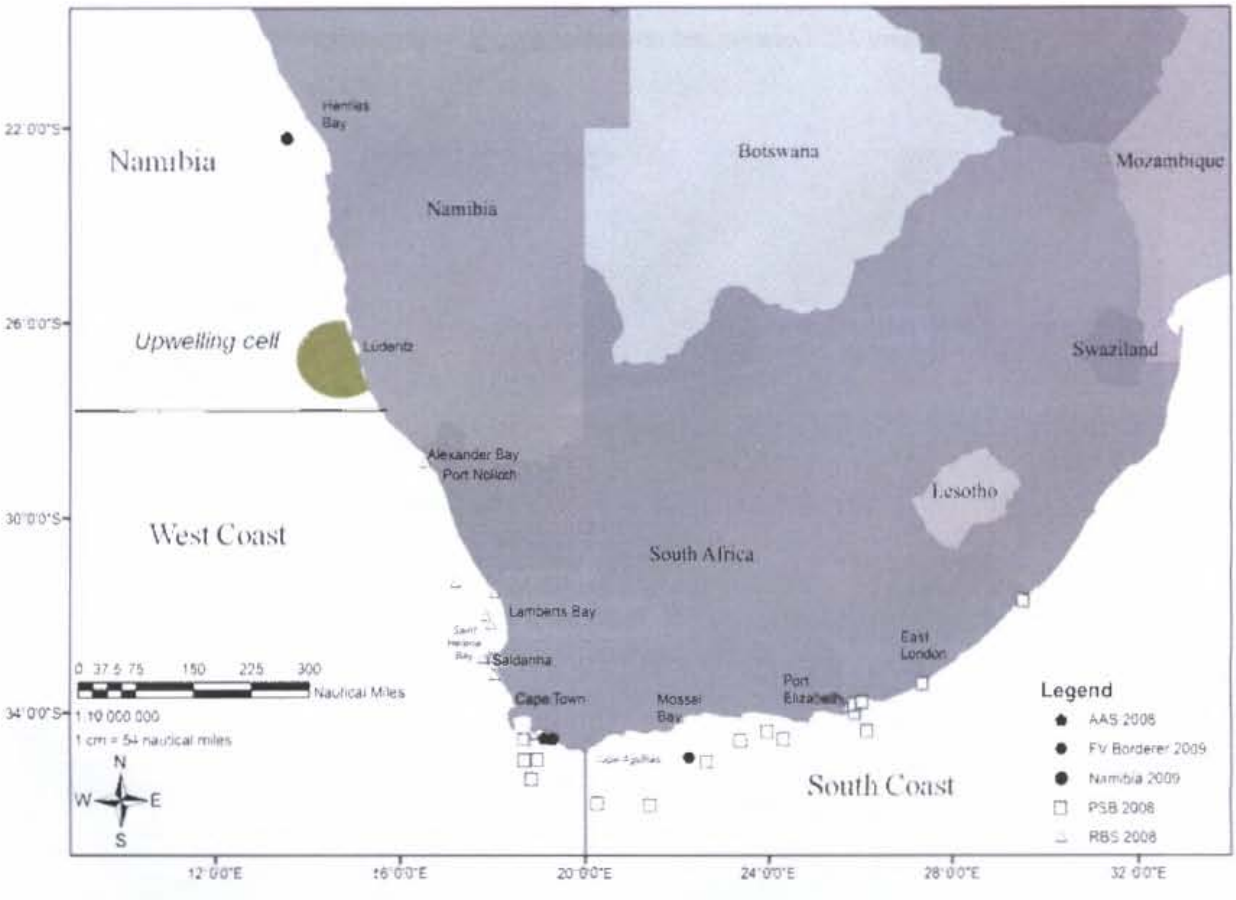

Figure 2.1: Locations of survey and commercial trawls from which samples of branchial sieves of sardine were taken for this study. The upwelling cell off Lüderitz is shown as a dark semi circle. Straight lines indicate biological and/or environmental borders for the regions mentioned in the text.

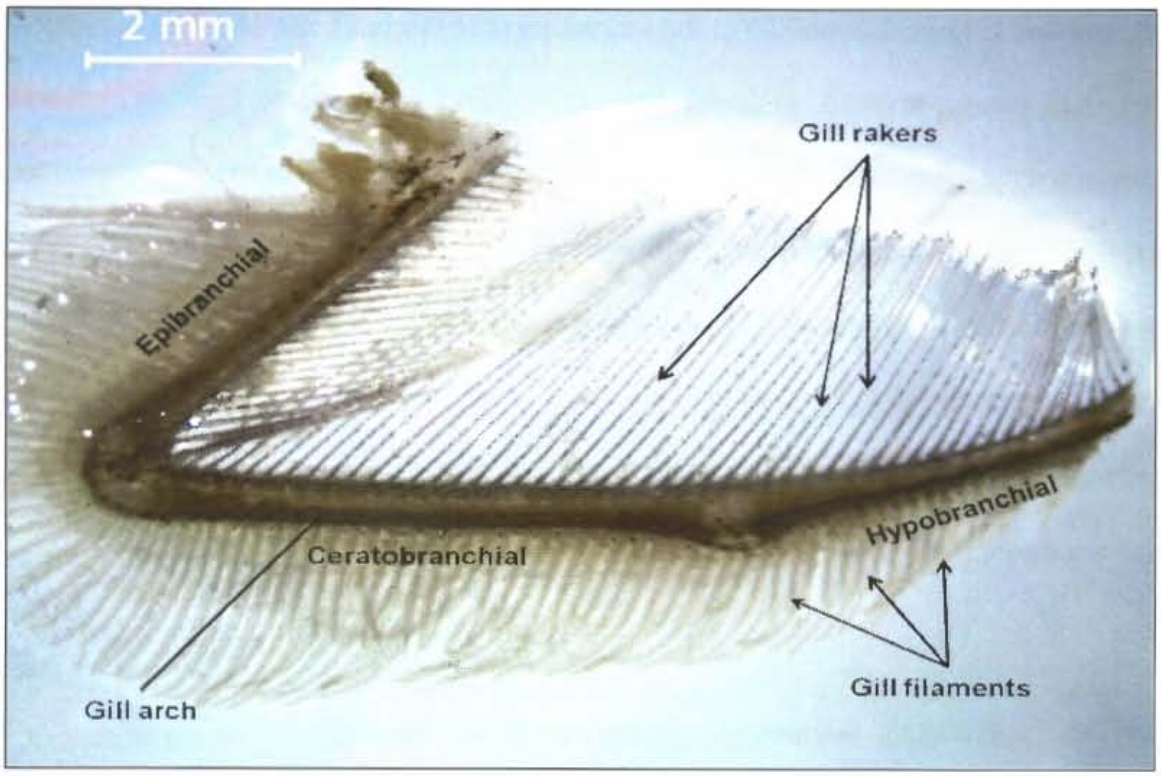

Figure 2.2: Location and orientation for gill raker measurements

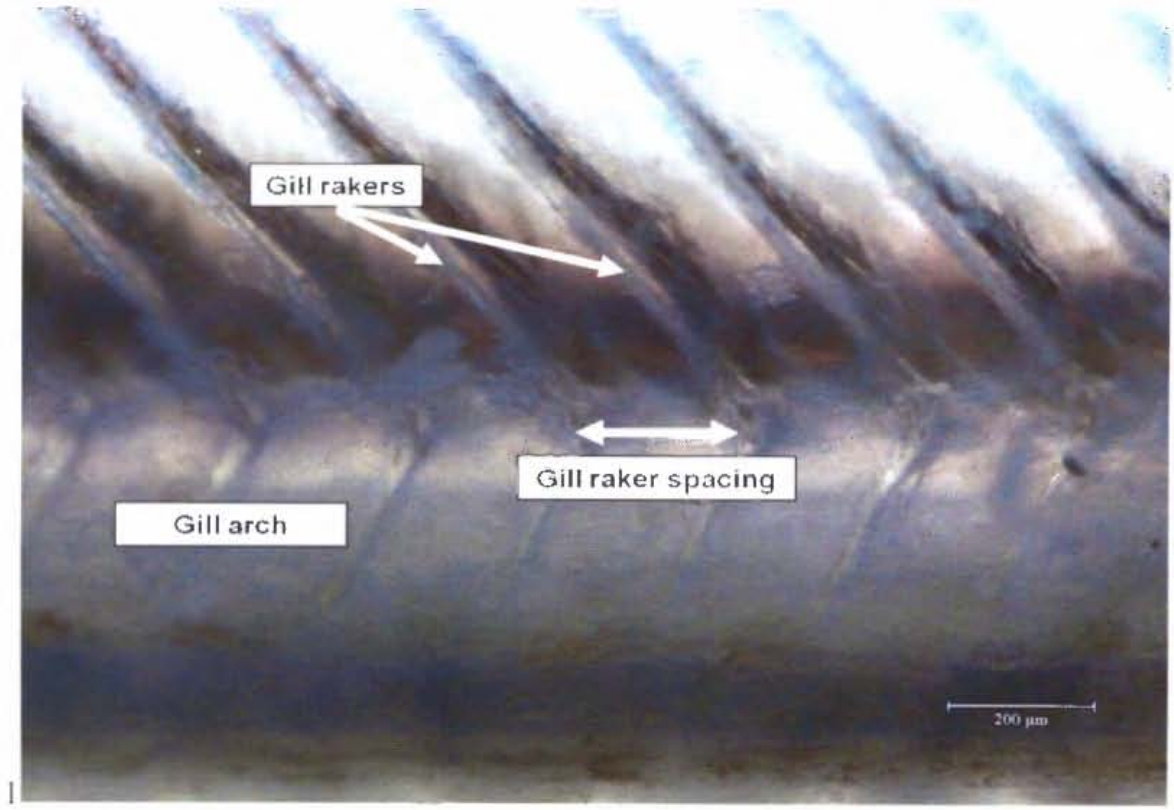

Figure 2.3: Location and orientation for gill raker spacing measurements

To correct for measurement errors incurred by an inexperienced person measuring the gill raker spacings, the first set of gill raker spacing measurements for sardine from Namibia were repeated at the end of all measurements (Figure 2.4). The results indicate a consistent bias in the first set of measurements which underestimated the values. As a result, analyses of data of gill raker spacings from Namibia were based on the second measurements (repeated).

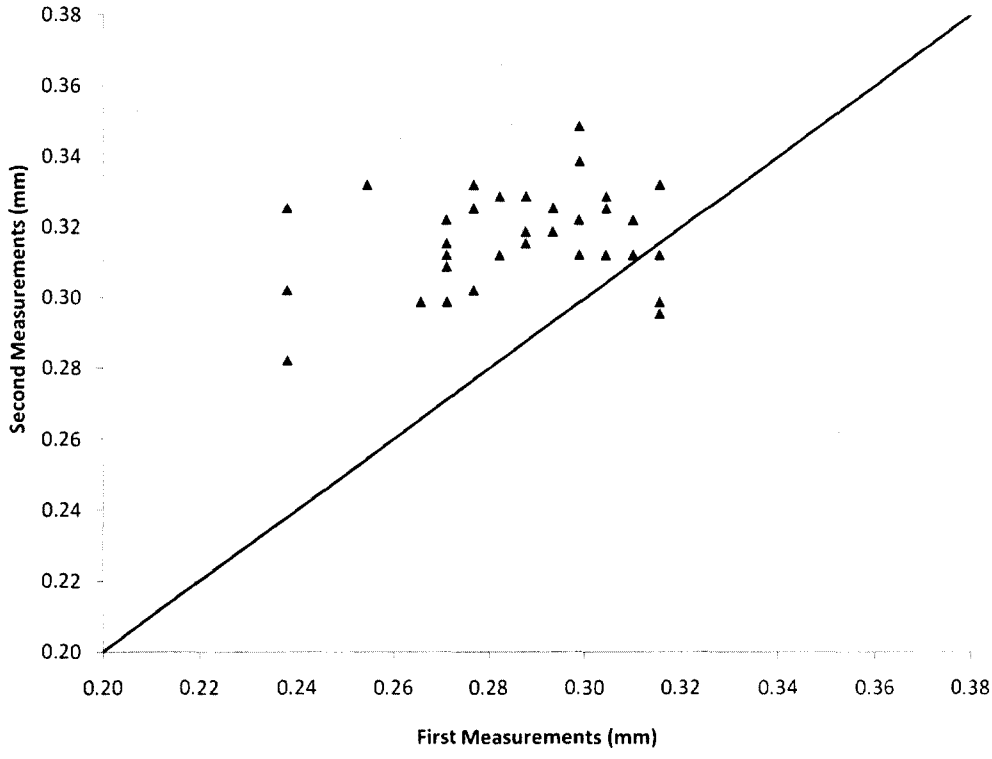

Figure 2.4: Comparison of first and second (repeated) measurements of gill raker spacings of sardine from Namibia. The 1:1 line is shown.

All measurements and counts were analysed using statistical software STATISTICA Ver. 8 (StatSoft Inc., 2008), using a significance level of 95% ($\alpha = 0.05$). All data were log transformed before any statistical analysis was conducted because they are allometric measurements and transformation was required to normalize the residuals. Relationship plots between measured (gill arch length and gill raker spacing) and counted variables (number of gill rakers) and caudal length from the three regions were plotted together with the predicted

values which were obtained from the General Linear Model (GLM) analysis (see below). Prior to GLM analyses, the slopes of these log-log relationships were tested for homogeneity using ANCOVA.

The effects of size (caudal length) and variability of measured and counted variables in different regions were tested using a GLM analysis. A GLM was used because there was a range of fish sizes (caudal length) and size needed to be included as a covariate. Results from the GLM analysis comparing the three regions were based on the mean covariate value of caudal length of sardines from these regions. The model that was used in this analysis is:

$$\text{Log (measured/counted variables of branchial sieve)} = \beta 0 + \beta_1 * CL + \text{Region} + \text{Error}$$

Where:

β_0 : intercept value

β_1 : parameter describing the influence of $\log_{10}$ caudal length on the dependent variable

CL : $\log_{10}$ caudal length

Region : parameter describing the influence of each of the three regions on the dependent variable (Namibia, South Africa south coast and South African west coast)

Error : the difference between predicted and observed values of the dependent variable

Post hoc analysis (Tukey test) was conducted to determine which of the regions were significantly different. This analysis used the least squares means (LS means) obtained from the GLM analysis and compared pairs of mean values of measured variables between two regions. Hence, for each measured variable, three comparison tests were done (S vs W, S vs N, W vs N) (S: south coast, W: west coast, N: Namibia) with the comparison sequence starting with the biggest and the smallest values for each variable. The confidence limit for this test is 95% (α: 0.05)

Results

A total of 221 branchial sieves of sardine with caudal lengths ranging from 60 – 220mm was measured in this study, with 35, 96, and 90 fish from Namibia, the west coast and the south coast of South Africa, respectively. The reduced number of sardines from Namibia is due to small catches obtained from trawling. One sample from the west coast of South Africa was discarded from statistical analysis due to error during preparation for measurement, resulting in 95 samples (Table 2.1).

Table 2.1: Data (mean ± SD) from measurements of branchial sieves of sardine from Namibia and the west and south coasts of South Africa. Least square means are based on caudal lengths of 140.3mm from the GLM analysis.

Region	No. of Samples	Caudal Length Range	Mean Gill Arch Length		Mean No. Gill Rakers		Mean Gill Raker Spacings	
			Actual	LS Means	Actual	LS Means	Actual	LS Means
Namibia	35	190-220mm	44.28 ± 3.20mm	30.70 ± 1.63mm	154 ± 6	124 ± 5	0.32 ± 0.01mm	0.268 ± 0.02mm
West Coast of South Africa	95	60-200mm	29.81 ± 10.82mm	31.69 ± 0.97mm	121 ± 26	128 ± 3	0.25 ± 0.05mm	0.257 ± 0.05mm
South Coast of South Africa	90	60-210mm	32.83 ± 10.33mm	30.77 ± 0.94mm	127 ± 28	124 ± 3	0.27 ± 0.04mm	0.261 ± 0.01mm

Sardine obtained from Namibia had a narrow caudal length range and were positioned at the large end of the overall caudal length range compared to sardine from the west and south coasts of South Africa. As a result, the mean values of measured variables from Namibia were much higher compared to the other two regions.

Gill arch length ($F_{(1, 216)} = 4887.047$, $p < 0.05$), the number of gill rakers ($F_{(1, 216)} = 2579.356$, $p < 0.05$) and gill raker spacing ($F_{(1, 216)} = 2170.765$, $p < 0.05$) of sardine from Namibia, the west and south coasts of South Africa all increased with caudal length (Figure 2.4a,b,c), indicating that fish size (caudal length) has a significant effect on the measured

variables. Homogeneity tests of the slopes of these relationships indicated that they were all equal ($p > 0.05$), allowing the GLM analyses to be conducted.

There were significant differences among the regions in gill arch length ($F_{(2,216)} = 4.079$, $p < 0.05$), number of gill rakers ($F_{(2,216)} = 6.287$, $p < 0.05$) and gill raker spacings ($F_{(2,216)} = 7.020$, $p < 0.05$) after accounting for size differences. Post hoc analyses (Tukey test) indicated that for gill arch length, although a significant difference occurred between the south and west coasts of South Africa, both had similar lengths to Namibia, making the results inclusive. The numbers of gill rakers in sardine from the south coast of South Africa were statistically similar to Namibian sardine but significantly different from sardine from the west coast, and west coast sardine were also different to fish from Namibia. Significant differences in gill raker spacings occurred between sardine from Namibia and South Africa (from both the south and west coasts) but no difference was seen for South African sardine. Details of the post hoc tests are shown in Table 2.2.

These results are illustrated in Figure 2.5 using predicted values and in Figure 2.6, where caudal length has been standardized to a mean value of 14.03mm to remove the body size effect. The predicted values for gill arch length (Figure 2.5a) and number of gill rakers (Figure 2.5b) of sardine from the west coast appear to be higher than those from both Namibia and the south coast of South Africa although the large variability of the Namibian sample did not allow conclusive results (Table 2.1). Similarly, for the LS mean results in Figure 2.6a (gill arch length: south coast = 30.77 ± 0.94mm, west coast = 31.69 ± 0.97mm, and Namibia = 30.70 ± 1.63mm) and Figure 2.6b (number of gill rakers: south coast = 124 ± 3, west coast = 128 ± 3 and Namibia = 124 ± 5). Namibian sardines have predicted mean values for gill arch length (Figure 2.6a) and number of gill rakers (Figure 2.6b) very similar to those of the south coast of South Africa. For gill raker spacings, Namibian sardine has the

largest (0.268 ± 0.010mm) and is statistically different compared to the South African sardine (south coast = 0.261 ± 0.005mm, west coast = 0.257 ± 0.005mm) (Figure 2.6c).

Table 2.2: Results from post hoc analyses (Tukey test) (α = 0.05) on measured variables (gill arch length, number of gill rakers and gill raker spacings) of sardine from Namibia, the south and west coasts of South Africa. Tests were done using data (mean) from Least Square means of GLM. Ho: Null hypothesis, Difference: difference between mean values of compared regions, q_{stat}: q value from calculation, q_{crit}: q value from Tukey table.

Post hoc analyses (Tukey Test) (significance level = 95%)							
Measured Variable	Comparison	Ho	Difference	Standard Error	q_{stat}	q crit (Tukey)	Conclusion
Gill Arch Length	W vs N	μW=μN	0.0138	0.0045	3.0929	3.356	**Do not reject Ho**
	S vs W	μS=μW	0.0128	0.0033	3.8636	3.356	**Reject Ho**
	S vs N	μS=μN	0.0010	0.0045	0.2170	3.356	**Do not reject Ho**
Number of Gill Rakers	W vs N	μW=μN	0.0133	0.0037	3.5567	3.356	**Reject Ho**
	S vs W	μS=μW	0.0136	0.0028	4.9029	3.356	**Reject Ho**
	S vs N	μS=μN	0.0003	0.0038	0.0900	3.356	**Do not reject Ho**
Gill Raker Spacings	N vs W	μW=μN	0.0180	0.0031	5.8690	3.356	**Reject Ho**
	N vs S	μS=μN	0.0119	0.0031	3.8359	3.356	**Reject Ho**
	S vs W	μS=μW	0.0061	0.0023	2.6946	3.356	**Do not reject Ho**

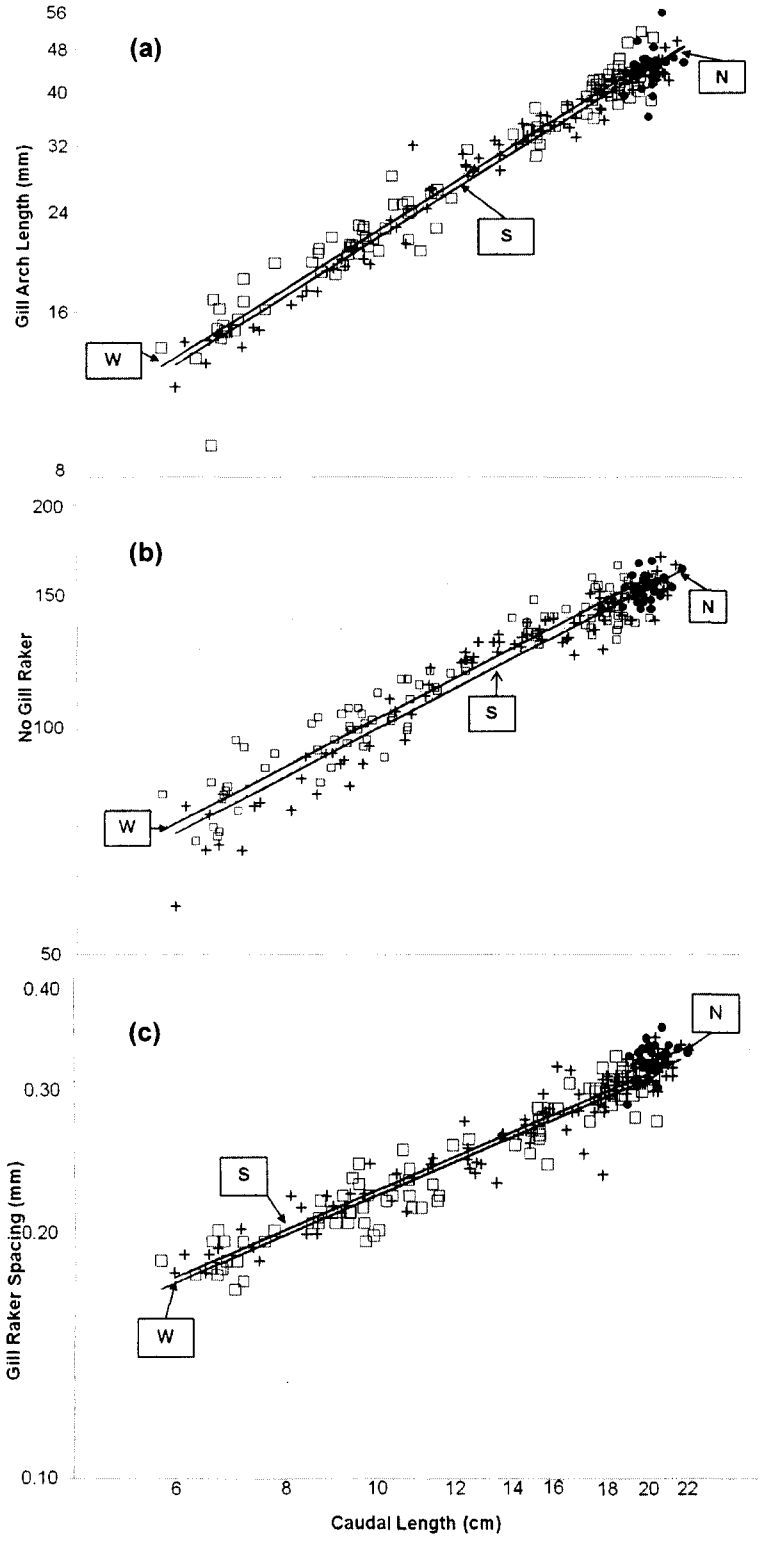

Figure 2.5: The relationship between caudal length and gill arch length (a), number of gill rakers (b) and gill raker spacing (c) of sardines from Namibia (●), and the south (+) and west coasts () of South Africa. The lines (N), (S) and (W) represent the predicted values of regions for these variables. The predicted lines for Namibia for gill arch length and number of gill rakers are hidden in the cloud of data points. Note that logarithmic axes are used.

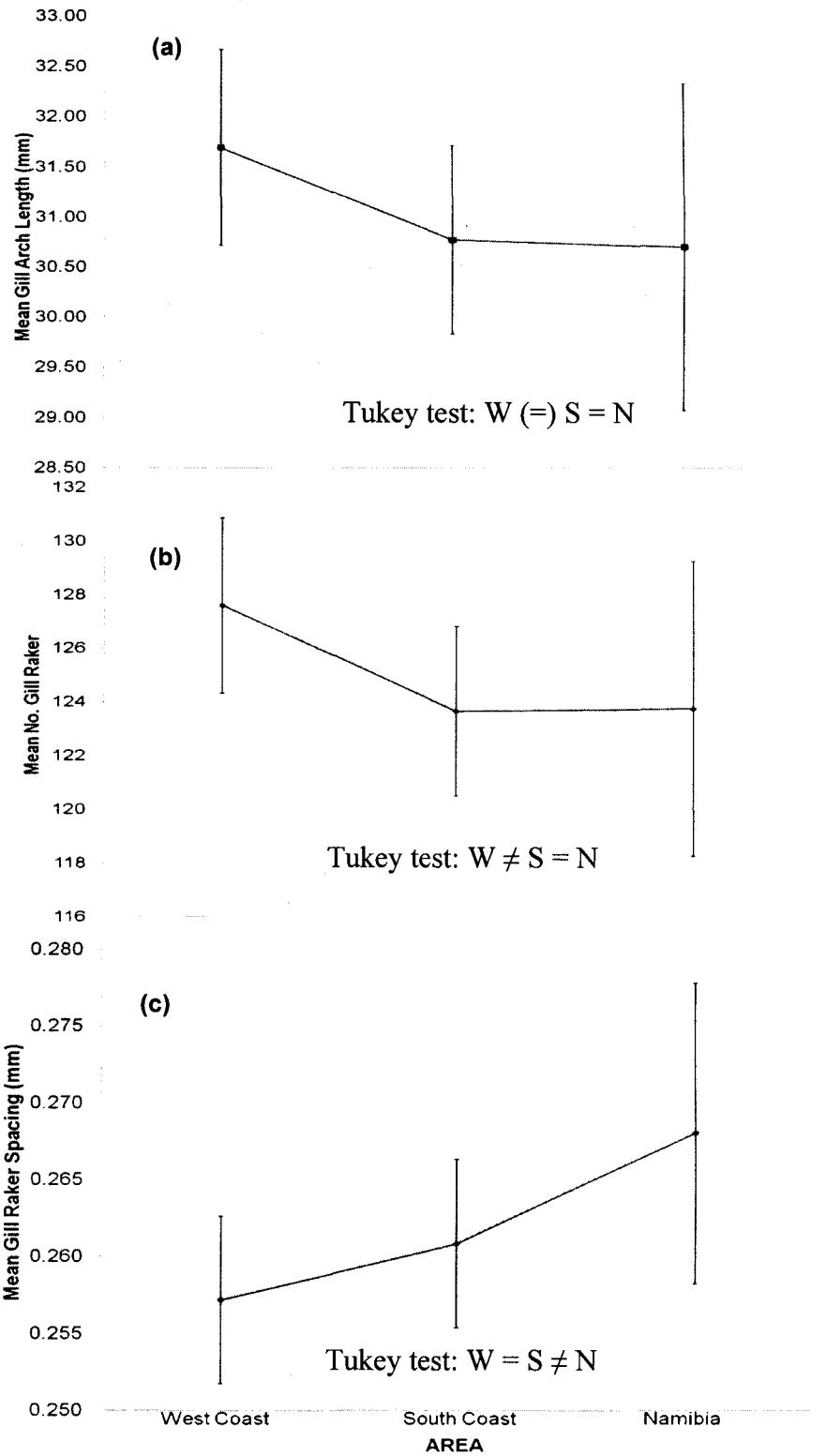

Figure 2.6: Least square mean values (± SE) of a) gill arch length (mm), b) number of gill rakers and c) gill raker spacings (mm) of sardines from the west and south coasts of South Africa and from Namibia. All values are calculated for a mean caudal length of 140.3mm. The results of the Tukey test (Table 2.2) are shown, with (=) indicating that although the west and south coasts were different, both were similar to Namibia, making the results inclusive. The lines have been drawn between the points to highlight the trends.

Discussion

Sardine from Namibia and the south and west coasts of South Africa have been shown to have similar growth trends with regard to branchial sieve morphology, with gill arch length, number of gill rakers and gill raker spacings increasing with an increase in caudal length. These relationships are similar to those shown by King and Macleod in (1976) for sardine from Namibia which followed a power curve (displayed as a straight line on logarithmic axes in Figure 2.4). *S. s. sagax* (Pacific sardine) from the California Current ecosystem also shows similar relationships of branchial sieve measurements with fish size (Rykaczewski 2009).

The increase in the number of gill rakers and gill arch length with fish size for sardine from the west and south coasts of South Africa is not unexpected because increases in body length will increase sizes of other body parts including the branchial sieve (Bone *et al.* 1995). These trends were also observed in other filter feeding species such as Atlantic menhaden (*Brevoortia tyrannus*) (Friedland *et al.* 2006), herring (*Clupea harengus*) (Gibson 1988), Pacific mackerel (*Scomber japonicus*) (Molina *et al.* 1996), and alewife (*Alosa pseudoharengus*) (MacNeill and Brandt 1990).

The gill raker spacing or gill raker gap in sardine from the three areas also shows an increase with body size. Similar trends were reported for other filter feeding fish like herring (Gibson 1988) and anchovy (*E. encrasicolus*) (King and Macleod 1976). The gill raker gap data from King and Macleod's (1976) study cannot be merged with the current data from Namibia because different methods were used. King and Macleod (1976) calculated gap size using a formula based on measurements of other variables (total length of gill arch, total number of gill rakers and gill raker thickness) whereas this study employed direct measurement.

Development trends in the feeding apparatus of fish, particularly among small pelagic fish, can vary for the variables measured in this study. King and Macleod (1976) found that the branchial sieve measurements of *E. encrasicolus* (previously known as *E. capensis*) had different development trends from those found for sardine in this study. The number of gill rakers for anchovy appear to remain constant beyond a caudal length of 80mm. This pattern of development affects the mean gill raker gap which for anchovy are almost double the size of sardine's when passing the 80mm caudal length mark (King and Macleod 1976). In a more recent study of branchial morphology of small pelagic fish off Japan, Tanaka *et al.* (2006) concluded that the numbers of gill rakers of Japanese anchovy *E. japonicus*, Pacific jack mackerel *Trachurus japonicus* and Pacific round herring *Etrumeus teres*, were almost constant at fish lengths greater than 100mm, 80mm, and 90mm, respectively. Those authors also concluded that the gill raker spacing for these three species showed linear relationships with fish length.

Gill raker spacings increase as fish increase in size (MacNeill and Brandt 1990, Gerking 1994, Friedland *et al.* 2006). This increase was believed to cause a switching of feeding preferences of juvenile and adult filter feeders, with feeding strategies changing from filter feeding to particulate feeding in the case of anchovy (King and Macleod 1976). This might be true for certain species such as Atlantic menhaden (Friedland *et al.* 2006). However, more recent research (James 1988, Louw *et al.* 1998, van der Lingen 2002, Mketsu 2008) on sardine has concluded that microzooplankton is the dominant dietary component of juveniles and adults of this species, although phytoplankton is occasionally important. In addition, laboratory experiments have demonstrated that filter feeding is the main feeding strategy for sardine (van der Lingen 1994).

The increase in gill raker spacing with fish size is not believed to result in negative effects on the ability of sardine to feed on small zooplankton. Gill rakers not only occur on

the first gill arch but are found on all five gill arches in the branchial basket of sardine. Gill rakers on gill arches other than the first were also observed in other filter feeding species such as *E. encrasicolus* (King and Macleod 1976), *Clupea harengus* (Gibson 1988) and Nile tilapia (*Oreochromis niloticus*) (Northcott *et al.* 1991). The occurrence of gill rakers on more than one gill arch is believed to increase the efficiency of filter feeding by creating a series of meshes in the branchial basket (the mechanical sieve model, Gerking 1994). The pore size in this sieve is determined by the combination of gill raker spacing or gill raker gap and the number of gill rakers on arches in sequence (Drenner *et al.* 1984, Hammann 1985). Although not measured in this study, the presence and role of denticles for filter feeding in sardines has been noted in various other studies, increasing the efficiency of the sieve in retaining food particles (Castillo-Rivera *et al.* 1996, Rykaczewski 2009).

Some filter feeding fish show a decrease in gill raker gap with increasing body length, for example Pacific mackerel (*S. japonicus*) (Molina *et al.* 1996). A smaller gill raker gap means that a larger size range of food can be trapped by the gill rakers, which is particularly important for this species because gill rakers are found only on the first gill arch.

Variability of branchial sieve measurements of Sardinops sagax ocellatus *between regions*

Sardine from Namibia are believed to be isolated from those off South Africa (Lett *et al.* 2007) whereas sardine from South Africa's west coast are believed to spawn on the western Agulhas Bank (van der Lingen and Hugget 2003) with some spawning activity recorded off St Helena Bay (Beckley and van der Lingen 1999), and sardine from the south coast of South Africa are believed to spawn over the shelf-edge region of the central Agulhas Bank (van der Lingen and Huggett 2003, Coetzee *et al.* 2008). Hence, most of the adult biomass of South African sardine is found on the southwest coast and east of the Agulhas Bank (Coetzee *et al.* 2008).

The northern Benguela ecosystem is sandwiched between two large upwelling cells at Lüderitz (which is the largest cell in the world) and the northern Namibian cell (Shillington 2003). The Lüderitz upwelling cell is so intense and permanent that it acts as an impermeable barrier for sardine from Namibia from going to the southern Benguela region (Lett *et al.* 2007). In the southern Benguela upwelling ecosystem however, there are periodic intrusions of warm water from the Agulhas Current via Agulhas Rings from the Indian Ocean (Lutjeharms and Gordon 1987). Occasional upwelling occurs as far south and east as Cape Agulhas (Shillington 2003) and the bathymetry of the continental shelf results in cold water being detected as a coastal counter current along the south and east coast of South Africa (Lutjeharms 2006). Although the oceanographic characteristics are different starting from Cape Agulhas towards the east (Miller *et al.* 2006), there is no oceanographic condition that restricts any movements of sardine from the west coast to the east to avoid unfavourable conditions on the west coast (van der Lingen *et al.* 2005) and vice versa. These environmental conditions allow South African sardine to have a wider and larger distribution than sardine from Namibia.

Statistical tests (*post hoc* analysis - Tukey test) (Table 2.2) indicated that the sardine from Namibia and South Africa are significantly different in terms of gill raker spacing. This result is in line with the results of the study by Wessels (2009), who found that Namibian sardine have different head measurements than South African sardines. For the gill arch length, the *post hoc* analysis gave inconclusive results, but for the number of gill rakers, the west coast was significantly different from the south coast and Namibian fish. The estimated difference in gill raker spacings for Namibian and South African sardine at 140.3mm caudal length is approximately 7-11μm for mean gaps of 257-268μm. This is a relatively small difference and it is not clear how it might affect feeding in the field.

Variability in the morphology of the branchial sieve within a single species has been reported for Spanish sardine (*Sardinella aurita*) (Kinsey *et al.* 1994), *Sardina pilchardus* (Andreu 1969), orange roughy (*Hoplostethus atlanticus*) (Haddon and Willis 1995), and Arctic char (*Salvelinus alpinus*) (Malmquist 1992). Genetic homogeneity for these species probably is caused by intermingling among individuals from different subpopulations, but with sufficient separation for phenotypic variance to occur (Haddon and Willis 1995). The phenotypic variability detected in sardine branchial morphology may be environmentally-mediated and an adaptive response to different trophic environments. It is possible that sardine from these three regions vary in their branchial sieve morphometry because of different diets. Day *et al.* (1994) conducted experiments on larvae of stickleback fish (*Gasterosteus* spp.) and found that the morphology of their branchial sieves changed when given different types of diets. Van der Lingen (2002), in his study of the diet of sardine from the areas off Cape Columbine to east of Port Elizabeth, concluded that the percentage of calanoid copepods consumed by sardines increased to 96% as the sampling stations moved towards the east. Calanoid copepods were also reported to be the greatest contributor to sardine diet (after fish eggs) in terms of percentage carbon on the east coast of South Africa (Mketsu 2008). Sardine from the west coast of South Africa feed mainly on cyclopoid copepods (van der Lingen 2002). Louw *et al.* (1998) found that juvenile sardines on the west coast tended to eat prey of 300-500μm prosome length, which is within the size range of cyclopoid copepods (Stuart and Verheye 1991). This difference in the trophic environment could explain why the gill raker spacing for sardine from the west coast of South Africa is generally smaller (although not significantly so) than that from the south coast for similar sized fish.

Off Namibia, King and Macleod (1976) found that the calanoid copepod, *Calanoides carinatus* has the greatest representation among zooplankton species found in the stomach of

this species. This finding seemed to match with the measurement data in this study which showed that sardine from Namibia have the widest gill raker spacing compared to South African sardine. However, high quantities of phytoplankton also were found in the stomach of sardine in Namibia (King and Macleod 1976). A recent study using stable isotope analyses of Namibian sardine indicated that this species feeds at a lower trophic level than South African sardine (van der Lingen, MCM. pers.comm.), which supported King and Macleod's (1976) findings on phytoplankton consumption. The trophic characteristic of Namibian sardine might be influenced by the presence of upwelling cells (Lüderitz, northern and central Namibian cells), which provide ample nutrients triggering high primary productivity in the northern Benguela ecosystem (Estrada and Marrasé 1987, Timonin *et al.* 1992, Hewitson and Cruickshank 1993, Hansen *et al.* 2005).

Sardine in South Africa employ filter feeding on food particles up to 1230μm, and for food larger than this may filter or particulate feed, depending on the density of the food particles (van der Lingen 1994). The ability of sardines to change their feeding behaviour from filter feeding to particulate feeding (van der Lingen 1994) means that changing the gill raker spacing drastically is not necessary to cater for different sizes and densities of prey. Sardine is able to move from its spawning ground on the Agulhas Bank (Beckley and van der Lingen 1999) to the south, west and east coasts of South Africa and vice versa (Barange *et al.* 1999). Since the structure of the branchial sieve cannot be changed (Berg *et al.* 1992) to suit the sizes of available foods, the phenotypic plasticity of the feeding apparatus of sardine must be able to cope with different sizes of available prey in different areas.

Nonetheless, caution should be applied to the outcomes from this study. The statistical results obtained in this study may be caused by different sample sizes obtained from each region. This can be seen clearly from the mean values of measured variables (Table 2.1), specifically for sardine from Namibia. Apart from different sample sizes,

statistically different results can also arise from bias in measurements as a result of error during sample preparation. For example, the number of gill rakers and the gill arch length on the edge of the hypobranchial and epibranchial limbs are not easily measured because they need to be separated from the branchial basket. Measurements of gill raker spacing are also subject to error, caused either by the human factor (viewing error), the orientation of denticles (secondary rakers on each gill raker), or the poor condition of the gill raker itself as a result of the preparation (fixation and preservation) process.

Chapter 3. Conclusions and Future Work.

The branchial sieves of sardine from Namibia, the west and the south coasts of South Africa show some differences in their gill arch length, number of gill rakers and gill raker spacings. Sardine from the west coast have the longest gill arches and most gill rakers, whereas sardine from Namibia have the widest gill raker spacing. For all measured variables except the gill arch length, sardine from Namibia were statistically different to either one or both of the regions in South Africa. This supports the work done by Wessels (2009), who concluded that Namibian sardine is from a different stock than the South African sardines. However, data and results from Namibian sardine should be treated with caution because of the small sample size and narrow size range compared to sardine from the west and south coasts of South Africa. This small number of data points made it difficult to find differences as the power of the statistical tests is reduced.

The results suggest that sardine from these regions might be from separate populations, especially between the northern and southern Benguela upwelling ecosystem as proposed by Newman (1970), Kreiner *et al.* (2001), and Wessels (2009). A strong environmental barrier arising from the intense upwelling cell off Lüderitz separates the two and supports the concept of Namibian sardines being an independent stock. Hence, separate management regimes are appropriate for sardine in Namibia and South Africa.

Although *post hoc* analyses gave mixed results, some branchial sieve measurements of sardine from the west and south coasts of South Africa showed differences (Figure 2.4, Figure 2.5), suggesting that the west coast and south coast sardine could comprise separate subpopulations, similar to suggestions made by de Moor and Butterworth (2009), de Moor (2009), van der Lingen *et al.* (2009b) and Wessels (2009). One of the proposed reasons for this variability may be the difference in prey consumed by sardine in these regions. Sardine from the west coast is believed to feed mainly on cyclopoid copepods whereas calanoid

copepods are mostly consumed by sardine from the south coast. Sardine from the west coast also consumed greater concentrations of phytoplankton (Davies 1957) compared to the south coast (van der Lingen 2002, van der Lingen *et al.* 2009a).

This study indicates the possibility of using branchial sieve morphology as part of the process to determine the population structure of sardine in southern Africa. This is similar to a number of studies that used data from branchial sieves to establish different stock populations of fish (Malmquist 1992, Kinsey *et al.* 1994, Haddon and Willis 1995, Amundsen *et al.* 2004, Turan 2004). However, results from branchial sieve morphology should be used together with other analyses such as genetics and other morphological measurements before final conclusions on stock identification can be made.

Future work

Use of branchial sieves as part of a stock identification analysis is relevant for sardine in the Benguela upwelling ecosystem. In addition, it would be useful to carry out laboratory feeding experiments similar to the work of Day et al. (1994) to determine whether different types of food and environmental variables can change the morphology of branchial sieves and whole fish. This current study should also be repeated using greater ranges of caudal length with adequate sample sizes, especially for samples from Namibia. Adequate sample size and greater ranges will increase the power of the analysis to detect any differences and avoids the use of extrapolated values. Although the results from a single measurement exercise might indicate accidental rather than actual differences (Haddon and Willis 1995), samples from South Africa were collected at different locations and dates, suggesting that the differences are not affected by sample collection. Detailed measurements on gill rakers such as measurements of the actual gill raker thickness to determine the gill raker gap as was done by King and Macleod (1976), could also be done. However, a microscope with higher resolution than used here is needed for this measurement. Gill raker spacing could also be measured throughout the left gill arch giving a possibility for the gill raker spacings to form a

cumulative size frequency distribution because of different development stages and positions (Drenner *et al.* 1984).

Small pelagic fish species in the Benguela upwelling ecosystem also include anchovy (*E. encrasicolus*) and west coast redeye (*Etrumeus whiteheadi*). Comparative studies between these species should be conducted to determine whether variability in branchial sieve structures occurs. Knowledge from these studies can be used to identify the population structure of small pelagic fish and may help in understanding the cause of alternating species abundance in the same trophic level.

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