STOCK SEPARATION OF THE SHALLOW-WATER HAKE *MERLUCCIUS CAPENSIS* IN THE BENGUELA USING OTOLITH SHAPE ANALYSIS AND PARASITE INFESTATION

A RESEARCH THESIS SUBMITTED IN FULFILMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE (FISHERIES AND AQUATIC SCIENCES)
OF

THE UNIVERSITY OF NAMIBIA

BY

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201303830

APRIL 2020

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Abstract

The fishing industry is an important sector in Namibia with hake contributing about one third of the total commercial catch. *Merluccius capensis,* the shallow water hake, forms the bulk of this resource. Studies on the distribution of spawners and juveniles, spawning areas and genetics have proposed three stock structure hypotheses of *M. capensis* in the Benguela: (1) one stock throughout, (2) one in the northern and one in the southern Benguela or (3) three stocks: one in the northern, one in southern Namibia and the Orange River (border between Namibia and South Africa), and one in the southern Benguela. This study aimed to investigate these hypotheses of *M. capensis* stock structure within the Benguela using otolith shape analysis and parasite infestation, and to identify parasite species as potential biological tags. *M. capensis* otolith samples were collected during demersal trawl surveys along the Namibian coast in 1992, 2004 and 2005 and along the South African West coast in 2005. The Benguela ecosystem was divided into three areas, northern (17.00-25.29 S), central (25.30 - 29.05 S) and southern Benguela (South African waters, 28.86 - 35.81 S), for this study. A total of 1628 otolith images were analyzed using the ShapeR package in R. Otolith outlines from Wavelet transformations and ANOVA permutation tests indicated no significant differences between the northern and central Benguela for the years 1992, 2004 and 2005 ($p > 0.05$ for all) but showed significant differences between the northern and southern Benguela in 2005 ($F = 14.81$, $p < 0.001$). Comparing otolith size descriptors and otolith shape indices showed that otoliths from the southern Benguela were significantly more elongated (longer, narrower and less round) than those from the northern and central Benguela. 80 *M. capensis* were collected off Namibia and examined for parasites and compared with a study done on *M. capensis* parasites in South Africa in the previous year. A total of 10 parasite species were found on Namibian *M. capensis*. Prevalence (number of infected fish/number of fish examined)*100 and abundance (total number of parasites/total number of fish examined) were compared between areas. The following species have the potential to be used as biological tags (1) *Chondracanthus merlucii* indicating significant differences in abundance between the northern and central Benguela, and northern and southern Benguela; (2) *Parabrachiella australis*, only present in the northern and central Benguela, and (3) *Stephanostomum multispinosum*, only present in the southern Benguela. Otolith shape analysis and parasite infestation levels therefore revealed one *M. capensis* stock in the northern Benguela and another in the southern Benguela, with a possible separation further north than the Orange River, as shown by parasites, supporting stock structure hypothesis number (2). Further studies should include sampling for parasites conducted seasonally and adding stock identity methods such as otolith microchemistry along the Benguela.

List of Publication(s) / Conferences proceedings

1. South African Marine Science Symposium (SAMSS), PE, South Africa

   Title: Stock separation of the shallow-water hake *M. capensis* in the Benguela using otolith shape analysis

   Authors: E.N.G. Shoopala, M.R. Wilhelm

   Date: 4-7 July 2017

   Format: Poster presentation

2. 2nd Annual Research Conference of the Sam Nujoma Marine and Coastal Resources Research Centre (SANUMARC), Henties Bay, Namibia

   Title: Stock separation of the shallow-water hake *M. capensis* in the Benguela using otolith shape analysis and parasite infestation

   Authors: E.N.G. Shoopala, M.R. Wilhelm

   Date: 28-29 September 2017

   Format: Oral and poster presentation

3. The 7th Annual Science & Governance Forum of the Benguela Current Convention (BCC), Windhoek, Namibia

   Title: Stock separation of the shallow-water hake *M. capensis* in the Benguela using otolith shape analysis and parasite infestation

   Authors: E.N.G. Shoopala, M.R. Wilhelm

   Date: 25-26 October 2017

   Format: Oral presentation

4. 3rd Annual Research Conference of the Sam Nujoma Marine and Coastal Resources Research Centre (SANUMARC), Henties Bay, Namibia

   Title: Stock separation of the shallow-water hake *M. capensis* in the Benguela using otolith shape analysis and parasite infestation

   Authors: E.N.G. Shoopala, M.R. Wilhelm

   Date: 26-27 September 2018

   Format: Oral and poster presentation

5. 2nd International Conference on Agriculture and Natural Resources Research and Innovation (2018), Windhoek, Namibia

   Title: Stock separation of the shallow-water hake *M. capensis* in the Benguela using otolith shape analysis and parasite infestation

   Authors: E.N.G. Shoopala, M.R. Wilhelm

   Date: 15-16 October 2018

   Format: Oral presentation

Table of contents

List of Figures

List of tables

Acknowledgements

I would like to thank the Department of Fisheries and Aquatic Sciences for the financial support and student bursary. Am grateful for the financial assistance from the BCC EcoFish project (EuropeAid grant 2010/222387) and DTU Aqua, Denmark for travelling and equipment financial assistance.

Am extremely grateful to my supervisor Dr. Margit Wilhelm for seeing me through this project, getting me funds where needed, getting me the necessary articles, arranging for me to attend all the appropriate conferences and most importantly for the statistical analysis assistance and for patiently seeing me through to producing a good quality report. I would like to thank Ms. Sarah Paulus for the otolith collections from the Ministry of Fisheries and Marine Resources, Namibia.

Am also very grateful to the Department of Agriculture Forestry and Fisheries (DAFF), South Africa (Dr. Deon Durholtz), for the 2005 otolith collection. I would also like to thank Ms. Justine Cruickshank and Dr. Cecile Reed for the parasite data, part of this thesis would not have been possible without your contribution. Irfan Nunkoo, thank you very much for seeing me through the identification of parasites. Thank you Dr. Hilkka Ndjaula for making all the necessary arrangements with Tunacor fishing company for fish collections.

Thank you very much Annette Amakali for assisting me with the parasite identification and for the long hours in the laboratory. Greg Mbaimbai, am grateful for helping me draw my maps!!

Last but not least, am very grateful for all the support from my parents, family (Ultimate cousins) and friends. Your ultimate support played a major role in completing this thesis.

Dedication

To my parents who taught me that anything is possible and today’s dream can become tomorrow’s reality. Your prayers and motivation have kept me going.

Declaration

I, [**Ester Ndapewa Gift Shoopala**], hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof has not been submitted for a degree at any other institution.

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Research ethics

This research is primarily based on secondary data that was lawfully retrieved from the Ministry of Fisheries and Marine Resources (MFMR) database and otolith collections (S. Paulus, MFMR, 2017). Each fish was killed by decompression during the trawling operation. Honest reporting of data, results, methods and procedures was maintained during the entire study. The study did not fabricate, falsify or misrepresent data or copy text from authors.

CHAPTER 1: INTRODUCTION

1.1. General description of the study area

One of the unique eastern upwelling systems in the world is the Benguela current system (Hutchings et al. 2009). Upwelling caused by strong southwesterly winds brings nutrient-rich waters up from the depths. This brings about the growth of microscopic phytoplankton and zooplankton. The ecosystem is in turn enriched with a vast biomass of fish species including hake (BCC 2012).

The Benguela current system is divided into the southern and northern Benguela (Hutchings et al. 2009). A seasonal inter-annual Angola-Benguela front functions as the border of the northern Benguela to the north. The Angola-Benguela front is influenced by moderate upwelling-favourable winds, with well-defined seasons and the moderate productive Angolan current system. The southern Benguela is enclosed by seasonal stratification and mixing, and the well-oxygenated temperate Agulhas current system. The northern Benguela is a typical coastal upwelling system with equator winds, cool water and high plankton biomass (Duncombe Rae 2005). The Luderitz upwelling cell (at about 26°S) serves as the border between the northern and southern Benguela. This cell is driven by strong seasonal winds and experiences sea surface temperatures of about 11°C from June to October (Bartholomae and van der Plas 2007).

The surface layer along the narrow shelf region at Luderitz seems to be the main barrier mechanism (Lett et al. 2007) for the pelagic fish populations, which divides them into northern and southern components (Hutching et al. 2009). However, this barrier does not apply to demersal populations and mobile predators (Lett et al. 2007).

The southern Benguela is characterized by seasonal endurable hypoxic water in the inshore regions and upwelling winds (Hutchings et al. 2009). Several teleost fish spawn on the Agulhas Bank in the southern Benguela and are transported to nursery areas on the South African west coast (Hutchings et al. 2009).

1.2. The hakes- Genus *Merluccius*

The hake genus *Merluccius* means sea pike (Flick et al. 1990), belonging to a demersal finfish (Pitcher and Alheit 1995) and comprising of twelve identified species (Flick et al. 1990; Silva-Segundo et al. 2011). These species include *M. merluccius*, *M. capensis*, *M. senegalensis*, *M. paradoxus*, *M. polli*, *M. bilinearis*, *M. albidus*, *M. hubbsi*, *M. gayi gayi*, *M. productus*, *M. angustimanus* and *M. australis*. Species within the genus *Merluccius* have analogous external features, a complicated taxonomy and usually coexist, hence making it difficult to distinguish the species from one another (Silva-Segundo et al. 2011). Two hake species overlap in their geographical ranges in most parts of the world, these include the two Cape hakes (*M. capensis* and *M. paradoxus*) (Durholtz et al. 2015; Wilhelm et al. 2015a) in southern Africa; the eastern North America have the silver and offshore hake (*M. bilinearis* and *M. albidus*) (Lorenzo and Defeo 2015; Vaz dos Santos and Schwingel 2015) co-existing, Patagonia water have the Austral and Argentine hakes (*M. hubbsi* and *M. australis*) (Arkhipkin et al. 2015), the Senegalese and Benguela are both present in West Africa and the Austral and Chilean hakes (*M. australis* and *M. gayi gayi*) (Horn 2015; Arkhipkin et al. 2015; Gatica et al. 2015) are both existing in southern and central Chile (Pitcher and Alheit 1995).

Hake are piscivores, with strong needle-like teeth, which are adapted to snapping and biting (Pitcher and Alheit 1995). The ecological role of *M. capensis* includes being a predator, frequently feeding on fish and cephalopods (Pillar and Wilkinson 1995), and also serving as prey for fur seals, sea birds and demersal fish species (Roux and Shannon 2004). They have a good vision at low light levels because of their large eyes. This characteristic particularly makes them good ambush predators (Pitcher and Alheit 1995). *M. capensis* reach their maximum length at around 110 cm (Wilhelm et al. 2015a).

1.3. The Namibian hake fishery

Hake is a dominant, commercially important demersal fish species in Namibia (Wilhelm et al. 2015a) and it is commercially caught by trawlers and longliners. The hake industry encompasses two main exploited stocks *M. capensis* and *M. paradoxus*. *M. capensis* was the first species to be commercially targeted since they live in shallower waters. The hake industry alone serves as the major source of employment in the fisheries sector in Namibia; in 2013 it had an employment of 9508 people (Musariri 2015).

The fishing sector is Namibia’s second largest earner of foreign currency after the mining sector. One third of the total fisheries catch is contributed by the hake fishery (Kathena et al. 2016). The majority of the total hake survey biomass consists of *M.*

capensis, but most commercial catches consist of *M. paradoxus* (Kirchner et al. 2012; Wilhelm et al. 2015b). Fishing of two hake species takes place along the entire Namibian coast, between 19˚S and 25˚S at 200-400 m bottom depth and between 25˚S and 29˚S at 300-500 m bottom depth (Johnsen and Kathena 2012). The mid-water trawl fishery (targeting horse mackerel), the monk and sole trawl fishery and large pelagic hook and line fishery catch hakes as bycatch (Wilhelm et al. 2015a).

Exploitation of hakes in Namibia started with about 100 trawlers in 1964 with open access fishing on hake by fleets mainly from Cuba, Israel, Italy, Japan, Poland, Portugal, South Africa, Spain and USSR (Wilhelm et al. 2015a). Between 1968 and 1972, Soviet and Spanish fleets caught about 90% of the hake of all foreign fleets off the Namibian coast (Paterson et al. 2013). The fishery was managed from 1976 following advice by the International Commission for Southeast Atlantic Fisheries (ICSEAF), implementing a minimum mesh size of 110 mm and member country quotas or total allowable catch (TAC) limits (1977-1989). Namibia’s waters remained an open access area until 1990 when the Exclusive Economic Zone (EEZ) was proclaimed by the independent Namibia (Paterson et al. 2013).

The Ministry of Fisheries and Marine Resources (MFMR) took over management of the hake fishery in 1990 after independence. Rebuilding the hake stocks and to ‘Namibianise’ the fishing industry were two of the main aims (MFMR 1992). Certain regulations were implemented such as immediate reduction of the TAC, limitation of entry licenses, enforcement of minimum mesh size of 110 mm, enforced catch and discard monitoring and establishment of an observer programme (van der Westhuizen 2001). A 200-m depth restriction was also implemented in 1990, which was further extended to 300-m from 25°S to the Orange River in 2006 (Wilhelm et al. 2015a). Closure of the hake-directed fishery in October was also implemented in 2006 (Wilhelm et al. 2015a).

The Namibian market does not experience a high demand for hake (Musariri 2015). Hence the Ministry of Fisheries and Marine Resources instructed the fishing companies to locally supply 30% of the allocated quota. On the other hand, hake is highly in demand in the international market and is regularly exported to Spain, USA, Australia, Norway, Germany, Holland, and Russia (Musariri 2015). Hake is processed in different varieties such as frozen skin-on, skinless fillets, headed and gutted into either baby hake, cutlets, tails, minced, blocks, sausages glazed hake steaks, roe and prime quality fresh chilled products (Musariri 2015).

1.4. The South African hake fishery

Hake was initially caught as a bycatch in South Africa, while targeting Agulhas sole (*Austroglossus pectoralis*) and West coast sole (*A. microlepis*) (Durholtz et al. 2015). The hake fishery came into place towards the end of the First World War and developed excessively after the Second World War. During the expansion, recently discovered fishing grounds on the south coast were heavily targeted by larger modern vessels now equipped with on-board freezing facilities and echo-sounders. In 1962, foreign fleets were targeting hake in South African waters and further into Namibian waters from countries like Japan, Spain, Soviet Union, Poland, Romania and the German Democratic Republic. About 300 000 t of hake were caught in 1972, but catch rates decreased after an increase in effort. This led to the overexploitation of hake which initiated the formation of the ICSEAF with the same measures that were applied on the Namibian hake fishery.

South Africa declared a 200-mile Exclusive Fishing Zone (EFZ) in 1977, allowing only few permitted foreign vessels operating in their waters (Durholtz et al. 2015). Several management measures were introduced in 1979, aiming to reduce fishing effort. This included a total allowable catch (TAC) measure, vessel restrictions, capacity management measures, gear restrictions, closed areas, ring-fencing, mitigation of seabird mortalities and bycatch limitation measures. Currently, hake is targeted by the deep-sea and inshore demersal trawl fisheries, the hake longline and hake handline sectors. The horse mackerel midwater trawl, the demersal shark longline and linefish sectors catch hake as a bycatch.

The hake industry plays a major role in the South African economy. Cape hake (*M. capensis* and *M. paradoxus*) generates a 53% output value alone compared to other fishery sectors, creating 8400 jobs. Equally important, exports of 8000 t of hake are made to European retailers once a year (Durholtz et al. 2015).

1.5. *M. capensis* distribution

M. capensis occurs at 100–450 m bottom depths (Burmeister 2001) and inhabit the continental shelf and upper slope from around 16°S in Angola to about 31°E in South Africa (Payne 1989). In South Africa, *M. capensis* is mainly caught on the south coast, strictly east of 20˚E, in water shallower than 150 m (Durholtz et al. 2015).

M. capensis is observed to have size distribution and depth-preferences due to several factors such as food availability and intra species competition (Gordoa et al. 1995; Wilhelm et al. 2015b). Hypoxic conditions affect the depth distributions of juvenile *M. capensis* (Hamukuaya et al. 1998). Salvanes et al. (2015) observed that *M. capensis* in Namibia are adapted to hypoxic conditions and often migrate offshore during severe low oxygen conditions but no similar migration has been observed for the southern Benguela *M. capensis*.

Historically, *M. capensis* spawning centres in the northern Benguela were located at 20-21°S (Olivar et al. 1988) and around Walvis Bay (23-24°S) in austral spring (October-December) and Cape Frio / Torra Bay (19-20°S) in winter (mid-July) (Assorov and Berenbeim 1983). Recently, two spawning centres have been described for the northern Benguela / Namibia, one at Walvis Bay at 22-25°S and another south of Luderitz at about 27°S, identified using the Namibian biomass survey length-distribution data (1990-2007) (Wilhelm et al. 2015b). Spawning in Namibia has the highest intensities in July-August (winter). *M. capensis* on the South African West coast mainly spawn around January in summer (Jansen et al. 2015), thus proposing the existence of separate stocks in the northern and southern Benguela by looking at their different spawning grounds and reproduction periods based on the stock definition of "intraspecific semi-discreet group of randomly mating individuals" (Begg and Waldman 1999; Gauldie 1988).

From Namibian summer survey length distributions, the youngest (<10 cm) *M. capensis* were found on the mid-shelf, whilst the 15-24 cm fish were found on the inner-shelf (Wilhelm et al. 2015b). This might be a consequence of their diet shift from crustaceans such as euphausiids to a cannibalistic diet (Wilhelm et al. 2015b). After 24 cm they move offshore again as they grow in size. Medium sized fish (30-45 cm) increase their energy for maturation and spawning via migrations from central to northern Namibia (Wilhelm et al. 2015b). Medium-sized *M. capensis* occur at the Angola-Benguela front, which serves as a feeding and spawning area for pelagic and mesopelagic fish (Crawford at al. 1987). A southward migration and migration from the mid-shelf to the outer-shelf of fish follows (>45 cm fish), showing that one separate stock of *M. capensis* (from juvenile to spawning adult fish) exists in central to northern Namibia (Wilhelm et al. 2015b).

Wind, temperature and oxygen level are some of the environmental factors reported responsible for the movement of Cape hake off South Africa (Durholtz et al. 2015). Alongshore migration patterns have been reported off South Africa (Jansen et al. 2016). *M. capensis* were observed moving eastward along the south coast of South Africa towards the Agulhas Bank and their distributions varied with age (Jansen et al. 2016). Hutchings et al. (2009) observed that *M. capensis* usually spawn on the Agulhas Bank, while the eggs and larvae are transported to their nursery areas on the South African west coast. This observation was based on the numerous juveniles commonly found on the west coast off South Africa during demersal research surveys. In contrast, the Agulhas Bank usually has few juveniles present. Large-sized *M. capensis* are usually caught on the Agulhas Bank in shallow waters between 50 and 60 m during commercial fishing by the hake handline fishery. These catches indicated seasonal inshore-offshore spawning migrations of *M. capensis* off South Africa (Durholtz et al. 2015).

1.6. Stock assessment methods used on *Merluccius* sp. in Namibia

From 1990, the Namibian hake fishery has used total allowable catch (TAC) to manage the hake fishery through a stock assessment based on an age-structured production models (Wilhelm 2012). Since 1998, Namibia has used statistical catch at age analysis for stock assessment. Catch at age data has been provided by the International Commission for South East Atlantic Fisheries (ICSEAF) which was formed in 1969 (Kirchner et al. 2012). This is done by reading otoliths assuming that a zone pair that consists of the opaque and translucent forms annually (Wilhelm 2012). Annual catch at age data is used to estimate the current biomass and the state of the stock (Kirchner et al. 2012). The model requires several input data such as catch data, commercial catch-per-unit effort (CPUE) indices, a survey biomass index, and proportions of catch-at-age from survey and commercial catches, and mean weight-at-age calculated from surveys (Wilhelm 2012). Kirchner et al. (2012) reported that the stock assessment of hake using catch at age data indicate that the hake resource is still below the maximum sustainable yield level (MSY). *M. capensis* and *M. paradoxus* are both assessed as one stock due to their similarity and not being separated into commercial catches until 1997 (Kirchner et al. 2012). Kathena et al. (2016) carried out a single species assessment study on *M. capensis* and *M. paradoxus* separately, and results indicated an increase in stock recruitment biomass of *M. capensis*. This was however not observed in a combined-species stock assessment.

Data from fur seal scat analysis indicate that *M. capensis* between the ages of 6 to 12 months grow at a fast rate with females maturing faster than males (Wilhelm et al. 2012). Data from length-frequency distributions also indicate that older fish grow at a fast rate mostly at the rate of 1.4 and 0.8 per month in fish aged between 1 and 5 years

(Wilhelm et al. 2017). *M. capensis* have a high natural mortality but with a greater stock productivity (Wilhelm 2012).

1.7. Importance of identifying fish stock structure

The definition of the stock unit is important as a stock is often used as a fisheries management unit. A stock is commonly defined as an intraspecific group of randomly mating individuals with temporal or spatial integrity (Waldman 2005; Tracey et al. 2006). The fish stock is the basic unit to which population dynamics models are applied both to know its status and to adopt appropriate management measures to ensure its sustainability. Stock structure is therefore a fundamental concept to the management of fisheries on fish resources (Hare 2005).

Different stocks could show different responses to exploitation because of differences in life history characteristics, for instance growth, mortality and reproduction (Gauldie 1988; Begg and Waldman 1999; Gauldie and Jones 2000) and replacement yield (Jónsdóttir et al. 2006). As a result, it is essential to identify stock units including their distribution, migration, spawning area and temporal and spatial degree of overlap with other stocks (Leguá et al. 2013). Studies indicate that sometimes when stock structure studies present results contradicting past studies (Gauldie and Jones 2000; Ukomadu 2017), these are ignored. Ignorance comes with negative events such as overexploitation of fish stocks resulting in the poor management of the fishery. Stock assessments conducted under the assumption of homogenous life history characteristics and closed populations could produce inaccurate results (Cadrin and Friedland 1999). The reliability of stock assessments and therefore the effectiveness of fishery management are limited for many principal fishery resources because stock delineation is uncertain (Waldman 2007).

1.8. Methods used in fish stock identification

The three main types of techniques used to obtain stock structure are: 1) natural methods, including morphometric and meristic analyses, use of parasites as biological tags and genetic studies 2) the use of externally and internally attached artificial tags and 3) studies of biological parameters in relation to life cycles (Templeman 1983; Smith and Jamieson 1986). This study will focus on 1) natural methods, namely the use of otolith shape analysis (e.g. Campana and Casselman 1993; Stransky et al. 2008) and parasites as biological tags (MacKenzie and Abaunza 1998) for the stock identification of *M. capensis* in the Benguela.

Methods based on phenotype provide perceptions into stock structure as opposed to genetic methods that may not be sufficient for defining the geographical limits of stocks (Grant et al. 1999).

1.8.1. Otolith shape analysis as a tool for stock identification

Otoliths are considered in stock discrimination studies because they grow throughout the life of the fish and are metabolically inert (Campana and Neilson 1985; Gauldie and Jones 2000). Otoliths are calcium carbonate structures in the fish “ears” and play an important role in the fish’ ability to detect sounds (Popper and Lu 2000; Popper et al. 2005) and maintain equilibrium (Payan et al. 2004). Otolith shapes regulate the sensitivity of acoustical sound it can take and their adaptation to different frequencies (Gauldie and Jones 2000; Gauldie and Crampton 2002). Sagittal otoliths are easy to prepare, observe and measure, thus they are frequently used for stock discrimination studies by using image techniques to obtain otolith measurements (Campana and Casselman 1993; Mapp et al. 2017).

Otolith shapes were shown to be species specific (Stransky and MacLellan 2005). Otolith shape analysis is based on the principle that the shape of the otolith varies geographically, even within a species (Campana and Casselman 1993). Otolith shape may also vary according to genetics and other environmental factors such as food availability, salinity, water temperature and depth (e.g. De Vries et al. 2002; Cardinale et al. 2004).

The otoliths of fish from different areas are usually compared using linear measurements of definable structures (Smith 1992; Leguá et al. 2013). This method’s importance to fisheries management has been recognized and has been successfully used on spatial and temporal discrimination of stocks (e.g. Griffiths 1996; Bolles and Begg 2000; Begg et al. 2001; Jónsdóttir et al. 2006; Stransky et al. 2008; Leguá et al. 2013; Zhang et al. 2017). These studies were based on otolith morphometry to determine the stock structure based on applications of basic size descriptors (area, perimeter, width and otolith size), shape indices (circularity, squareness, shape factor, roundness, rectangularity and ellipticity). Otolith shape analysis produces accurate measurements (Bolles and Begg 2000), and has the advantage as a monitoring tool because of its lower cost and easier preparation procedures compared to other methods such as genetic studies (Cadrin and Friedland 1999; Lord et al. 2012; Leguá et al. 2013). Despite this method’s advantages, using otoliths comes with a risk of having broken or damaged otoliths reducing the number of samples (Bolles and Begg 2000).

The appearance of growth zones within otoliths has also been used to differentiate between fish stocks (Rätz 1994).

Castonguay et al. (1991) questioned the successful use of otolith shape to discriminate between stocks. They highlighted the need to carefully draw conclusions about stock structure from Fourier analysis because the discrimination might have been caused by age and year class effects and would therefore highlight sample differences rather than stock discrimination. Begg and Brown (2000) therefore suggested that otolith shape characteristics for stock discrimination have to be recalculated for each major age group.

Otolith shape analysis has been used for stock identification in many studies, for example three stocks of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean (Stransky et al. 2008). Two stocks of Icelandic cod (*Gadus morhua* L.) were identified, one stock in the north and another in the south with a further distinction south of Iceland, one stock spawning below and another above the depths of 125 m (Jónsdóttir et al. 2006). Leguá et al. (2013) distinguished between southern blue whiting *Micromesistius australis* populations in the Pacific and Atlantic using otolith shape analysis. The two stocks of South African sardine (*Sardinops sagax*), the western stock from the Orange River to Cape Agulhas and the southern stock, east of Cape Agulhas, were separated based on otolith shape indices together with other complementary methods such as meristic and parasite markers (Ukomadu 2017). A study conducted on angler fish *Lophius piscatorius* in the Northeast Atlantic to observe a separation between the northern and southern stocks by using otolith shape (Cañás et al. 2012) observed minimal differences in their otolith shapes, hence concluding that there was only one stock in the Northeast Atlantic. Bolles and Begg (2000) also used otolith morphometrics to identify two stocks (a northern stock from the Gulf of Maine to northern Georges Bank and a southern stock from southern Georges Bank to the middle Atlantic) of silver hake (*Merluccius bilinearis*) in the USA waters of the Northwest Atlantic. Identification of five *Pampus* species (*Pampus chinensis*, *P. cinereus*, *P. chinensis*, *P. punctatissmus* and *P.* sp.) of the Chinese coast was conducted using sagittal otolith morphology (Zhang et al. 2017). Multiple otolith techniques including otolith shape, elemental chemistry of otolith cores and stable isotopes of otoliths were used to discriminate the stock structure of Australian blue grenadier *Macruronus novaezelandie* in three fishery regions; west Tasmania, east Bass Strait and Great Australian Bight (Hamer et al. 2012). Sadighzadeh et al. (2014) identified two stocks of John's snapper *Lutjanus johnii* using otolith shape; one stock was present in the Persian Gulf and another stock was found in the Oman Sea. Another study applied the otolith shape analysis method on *Coilia ectenes* in the Yangtze Basin in China, which found different ecotypes. The *C. ectenes* anadromous and land-locked ecotypes were found similar to each other, specifically in the rostrum and antirostrum whilst the freshwater ecoctype was different from them (Radhakrishnan et al. 2012).

1.8.2. The use of parasites as biological tags

Parasites have been extensively used as biological tags to provide information on the stock structure of their fish hosts (Lester 1990; MacKenzie and Abaunza 1998). The biological tag method is centered on the geographical distribution of parasites. This method is based on the assumption that hosts can become infected only when they are within the endemic area of a parasite (the geographical region in which the conditions are suitable for transmission). If infected hosts are found outside of this area then one can assume that these hosts had been within the endemic area at some time in their life cycle (MacKenzie and Abaunza 1998). Scientists can estimate the maximum time since infection and since the hosts left the endemic area provided that there is information on the life span of the parasite (MacKenzie and Abaunza 1998). Environmental conditions suitable for a host determine the endemic area for parasites with direct life cycles, whereas parasites with indirect life cycles need two or more hosts for all developmental stages (MacKenzie and Abaunza 1998).

MacKenzie (1999) highlighted certain advantages of the use of parasites as biological tags over the use of artificial tags. Firstly, biological tags are more appropriate than artificial tags for studies of deep water fish, which are unlikely to survive capture and handling. Secondly, they are less expensive than artificial tags, since samples can be obtained from sampling surveys. Lastly, they eliminate doubts concerning abnormal behavior of artificially tagged hosts. However, there are some limitations to the use of parasites as tags (Sindermann 1983). These include lack of information on the ecology and biology of aquatic parasites and disagreement among taxonomists because of the uncertainty of the identification of many parasites. It is also desirable to know the age of the host individuals, for which in some species the age determination methods have not been validated.

Two methods can be used when using parasites as biological tags (MacKenzie 1999). The first is to select a few parasite species and to examine a large number of hosts only for these parasites. This method only makes use of simple univariate statistical analyses. The second method looks at the entire parasite community and analyzes the results with more sophisticated multivariate statistical systems. If the first method is used, only certain parasites can be used. MacKenzie (1999) identified some criteria to help asses if a parasite is useful as a biological tag: different levels of infection should exist in the subject in different parts of the study, parasites should persist in the host for a long time, the level of infection should remain constant from year to year, parasites should be easily detected and identified and lethal pathogenic parasites should not be used. Having a complete understanding of the parasite community infecting *M. capensis* in the Benguela would help to identify potential biological tags, to help define the *M. capensis* population structure.

The use of parasites as biological tags for stock identification has been successfully applied in the Benguela on sardine (Reed et al. 2012; van der Lingen et al. 2015; Weston et al. 2015; Ukomadu 2017) and horse mackerel (Le Roux 2013).

1.8.3. Parasitology studies on hakes (Genus *Merluccius*)

Parasites of some hake species have been well documented in several studies. A study on *Merluccius gayi gayi* using a metazoan parasite as a tool for stock discrimination was conducted resulting in the separation of two stocks (southern and central Chile) (Oliva and Ballón 2002). Identification of these stocks was based on significant differences in mean intensity of adult *Clestobothrium crassiceps* and larval *Hepatoxylon trichiuri* (Eucestoda), the monogenean *Anthocotyle merluccii* and the copepod *Neobrachiella insidiosa f. pacifica* (Oliva and Ballón 2002).

A parasite study on Argentine hake *Merluccius hubbsi* resulted in the identification of three stocks: 1) one stock present in the Argentine-Uruguayan Common fishing zone, 2) one stock consisting of hake from San Matias Gulf and 3) one stock including hake from both San Jorge Gulf and Patagonian shelf (Sardella and Timi 2004). The first stock, the Argentine-Uruguayan stock was characterized by low prevalence of most parasites. The second stock, the San Matias Gulf stock was characterized by *Kudoa rosenbushi*, *Grillotia* sp. 1 and *Contracaecum* sp. The third stock, the San Jorge Gulf and the Patagonian Shelf was classified based on the shared parasites, *Grillotia* sp., *Pseuophyllidea* sp.1 *plerocerci* and *Hysterothylacium aduncum* (Sardella and Timi 2004).

An assessment on the parasites of *M. australis* and *M. hubbsi* around the Falkland Island waters resulted in the identification of potential parasite biological tags characterized on their presence in specific areas such as the unidentified fungus; the protozoans *Alatospora merluccii kalavati*, *Goussia* sp., *Kudoa rosenbuschi*, *Microsporidium ovoideum*, *Myxidium baueri*, and *Myxoproteus meridianalis*; the metacestodes *Grillotia* sp. and *Hepatoxylon trichiuri* (Holten 1802); and the parasitic copepod *Trifur tortuosus* and the adult digenean *Elytrophalloides oatesi* (MacKenzie and Longshaw 1995).

Parasitology studies specifically for stock identity on *M. capensis* have not been conducted in Namibia or South Africa. However, a few studies documented the parasite communities found on the Cape hakes. Two ectoparasitic copepods (from the gills and buccal cavity) have been observed on South African *M. capensis* (Barnard

1955), namely *Chondracanthus merluccii* and *Parabrachiella australis*. Szidat (1955) also noted the occurrence of *C. merluccii* on *Merluccius merluccius* (Eastern Atlantic), *M. hubbsi* and *M. bilinearis* (Western Atlantic). However, Botha (1986) did not observe *C. merluccii* on the South African *M. capensis*. Immense infestations of *Anisakis* larvae (a cestode endoparasite infecting the gonads and liver) have been observed in many marine teleosts (Botha 1986). *Anisakis* is perceived as one of the most important parasites dominating parasite communities of both species of *M. capensis* and *M. paradoxus,* because of its high intensity of infestation, predominantly in >80 cm (Botha 1986). Botha (1986) noted that euphausiids, in the diet of the Cape hakes, are intermediate hosts of *Anisakis*. Another endoparasitic helminth observed in Cape hakes is the cestode *H. trichiuri*, which is large and cream-coloured and is often found in the coelomic cavity (Botha 1986).

A parasitology study conducted on *M. capensis* off Namibia (Krzeptowski 1980) also observed the occurrence of the larval nematode *Anisakis simplex*, *H. trichiuri* and the parasitic copepod *P. australis*. *A. simplex* larvae were observed in the body cavity and mostly on the liver of the fish. They were present in all hake age groups, but a clear increase of the degree of infestation was observed with increase in age. There were age-related differences in both sexes for *A. simplex,* being that the number of parasites in females increased with age, while 3-year-old males showed the highest level of infestation with a decrease in infestation level in the 4- and 5- year-old males. *P. australis* was the second most frequently occurring parasite on *M. capensis* after *A. simplex* and was found on gill lamellae of fish examined. *H. trichiuri* showed the lowest infestation level on *M. capensis,* which was irregular with fish age (Krzeptowski 1980).

Reimer (1993) studied parasites of *M. capensis* and *M. paradoxus* from the coast of Namibia. Parasitation by *Leptotheca* sp., *Anthoctyle merluccii* and *Anisakis* sp. was observed to be increasing with an increasing age in *M. capensis.* Reimer (1993) stated that the northern and southern parts of the investigation area (between 22˚03'S and 27˚01' S) were clearly divided with a boundary at about 25˚30'S. South of this border *Brachiella merluccii* was absent and the prevalence of *Scolex pleuronectis* and *Leptotheca* species was higher, which Reimer (1993) suggested to be because of the existence of different stocks of *M. capensis* north and south 25˚30' S. This is further investigated in this study.

Canning (2001) also noted the presence of the species *Myosporidium* in several fish such as Artic blue trout and sockeye salmon and stressed that these infections were noted to have detrimental effect on the production, quality and safety of human food sources. *Myosporidium merluccius* was observed infecting muscles of commercial hake (*M. capensis*) from fisheries near Namibia (Baquero et al. 2005).

Parasite assemblages of *M. capensis* and *M. paradoxus* were recently studied in Southern Africa off the southern Benguela and East coast (Cruickshank 2017). Seven parasite species were recorded on *M. capensis* hosts from both the southern Benguela and South African East coast stations. *Anisakis* sp., *Stephanostomum multispinosum*, *C. merlucci*, *Corynosoma* sp., an unidentified cestode and a digenean were found on *M. capensis*. Cruickshank (2017) proposed that *A. merlucci*, *Bolbosoma* sp. (present in *M. paradoxus*) and *H. trichiuri* could be potential biological tags that could be used to discriminate between *M. capensis* and *M. paradoxus* because of the noticeable differences in prevalence, abundance and intensity of infection of these parasites between *M. capensis* and *M. paradoxus*. Results of Cruickshank (2017) were compared with data collected in this study.

1.9. Stock structure of *M. capensis* in the Benguela

A number of genetic studies have been documented on the population structure of *M. capensis* in Namibia and South Africa in the past. A study by Grant et al. (1988) by means of allozymes advocated the presence of two populations in the Benguela, separated by the Orange River for *M. capensis* whilst Mitochondrial DNA (mtDNA) data revealed the presence of one single panmictic population for *M. capensis* in the Benguela (von der Heyden et al. 2007). The most recent studies have observed two *M. capensis* populations in the Benguela, one in Namibia and one in South Africa along the west coast. A third, South coast *M. capensis* stock exists between 20˚E - 26˚E along the South African South coast (Henriques et al. 2016). Mixed origins were also observed in the area between the Orange River in Namibia to the West coast of South Africa at about 29-30˚S (Henriques et al. 2016), hence perceiving this area as where the barrier to gene flow occurs. A study on seasonal migration of *M. capensis* and *M. paradoxus* (using eight nuclear microsatellite markers) concluded that southern Benguela *M. capensis* migrate to the northern Benguela in winter (June to August) and the northern Benguela *M. capensis* migrate to the southern Benguela in summer (February to March) (Kapula 2017).

Jansen et al. (2016) identified three spawning aggregations along the entire Benguela (Walvis Bay around 25˚S, Orange River around 29˚S and Agulhas around 34˚S) using a geostatistical population model fitted to survey length-distributions (corresponding with the Walvis Bay and Orange River aggregation of Wilhelm et al. (2015b)). A north-south return migration was observed for the Walvis Bay and Agulhas Bank aggregations, hence making these the nursery areas (Jansen et al. 2016).

The spatial stock structure, and especially the degree of mixing and the exact border between the northern and southern Benguela *M. capensis* stocks remains uncertain. This led to three stock structure hypotheses for the Benguela (West coast): (1) One stock of *M. capensis* occurs throughout Namibia and the South African West coast (von der Heyden et al. 2007), (2) two stocks of *M. capensis* occur, one in the northern Benguela and one in the southern Benguela (South African West coast) (Grant et al. 1988; Henriques et al. 2016) or (3) three stocks of *M. capensis* occur from three spawning aggregations, one in the northern Benguela, one on the Orange River and one in the southern Benguela / Agulhas Bank (Wilhelm et al. 2015b; Jansen et al. 2016).

Because of these reasons, further information and understanding of stock structure and mixing that could be incorporated into the stock assessments of *M. capensis* in Namibia and South Africa, is needed for optimal fisheries management at regional level.

1.10. Project aims and hypotheses

Given the importance of hake and the uncertainty regarding population structure and especially the mixing area of *M. capensis* in the Benguela, this study aimed to test if there are three stocks in the Benguela with two stocks of *M. capensis* in Namibian waters, one north and one south of 25˚30'S as previously shown by spawning aggregations (Wilhelm et al. 2015b; Jansen et al. 2016) and parasite communities (Reimer 1993).

The specific aims of this study were to investigate the stock structure of *M. capensis* within the Benguela using (1) otolith shape analysis (Chapter 3.1) and (2) parasite infestation (Chapter 3.2) and to identify parasite species as potential biological tags for *M. capensis* (Chapter 3.2).

The null hypothesis tested in Chapter 3.1 was that there is no significant difference between the *M. capensis* otolith shape found in the northern and central Benguela (northern and southern Namibia) and southern Benguela (South African West coast). Chapter 3.2 tested the null hypothesis of no significant differences in infection level of parasites between the northern, central and southern Benguela *M. capensis* samples.

1.11. Limitations of the study

M. capensis otoliths were only collected in specific years in certain areas. Future studies should analyse otoliths of *M. capensis* collected along the whole Benguela covering the northern, central and southern area in the same year and season, along with complementary methods such as parasites, otolith microchemistry and genetics, hence using multiple stock identification methods. Robust results are achieved through a combination of different methodologies when discriciminating fish stocks (Ukomadu 2017). In addition, a univariate study on parasites of *M. capensis* as potential biological tags is required with more samples including the mixing area, with comparisons of parasite prevalences and abundances between seasons. This is also essential to explore the proposed mixing of the northern and southern Benguela *M. capensis* stocks in the Orange River area.

Using parasites as biological tags in stock identification has one main restriction, specifically lack of knowledge about the biology and ecology of parasites. Therefore, further research should study the biology and ecology of *P. australis* and *S. multispinosum* (such as preferred temperatures, specific diet studies of hosts by size) in order to understand why the northern and southern Benguela *M. capensis* had specific localized parasite species of *P. australis* and *S. multispinosum* present.

CHAPTER TWO: MATERIAL AND METHODS

2.1. OTOLITH SHAPE ANALYSIS

2.1.1. Sample collection

Otolith samples were collected during the Ministry of Fisheries and Marine Resources (MFMR), Namibia, demersal trawl surveys on board different research vessels along the Namibian coast, in 1992 on board R.V. *Dr. Fridjof Nansen*, and in 2004 and 2005 on board *F/V Blue* Sea 1 and along the South African West coast during the Department of Agriculture Forestry and Fisheries (DAFF), South Africa, demersal trawl survey by *R/V Africana* in 2005 (Table 1). The different years were selected to investigate if the otolith shape (and stock structure of *M. capensis*) changed over time. The Namibian coastline was divided into two areas, namely, northern Benguela (northern Namibia) (17.31˚S-25.29˚S) and central Benguela (southern Namibia) (25.30˚S-29.05 ˚S) (data available for 1992, 2004 and 2005), and the South African west coast made up the southern Benguela (28.86.0˚S- 35.81˚S) (data only available for 2005) for this study (Figure 1).

Biological information such as fish total length, fish weight, sex, and maturity stage was recorded for each fish. Station number, latitude, depth and date for each station were also recorded.

Table 1: *M. capensis* otolith samples collected from different areas in the Benguela, northern (N) (17.31˚S-25.29˚S, in northern Namibia), central (C) (25.30˚S-29.05˚S, in southern Namibia) and southern (S) (28.86˚S-35.81˚S, in South Africa) Benguela (Figure 1). Year of collection, collection months, fish length ranges of samples (cm) and number of samples collected per area (N) are indicated.

Year	**Month**	**Area**	**Fish total length range (cm)**	**N**
1992	October- December	N	19 – 51	174
		C	19 – 51	46
2004	January-February	N	19 – 59	500
		C	19 – 58	73
2005	January –February	N	19 – 58	146
		C	19 – 62	168
		S	21 – 57	371

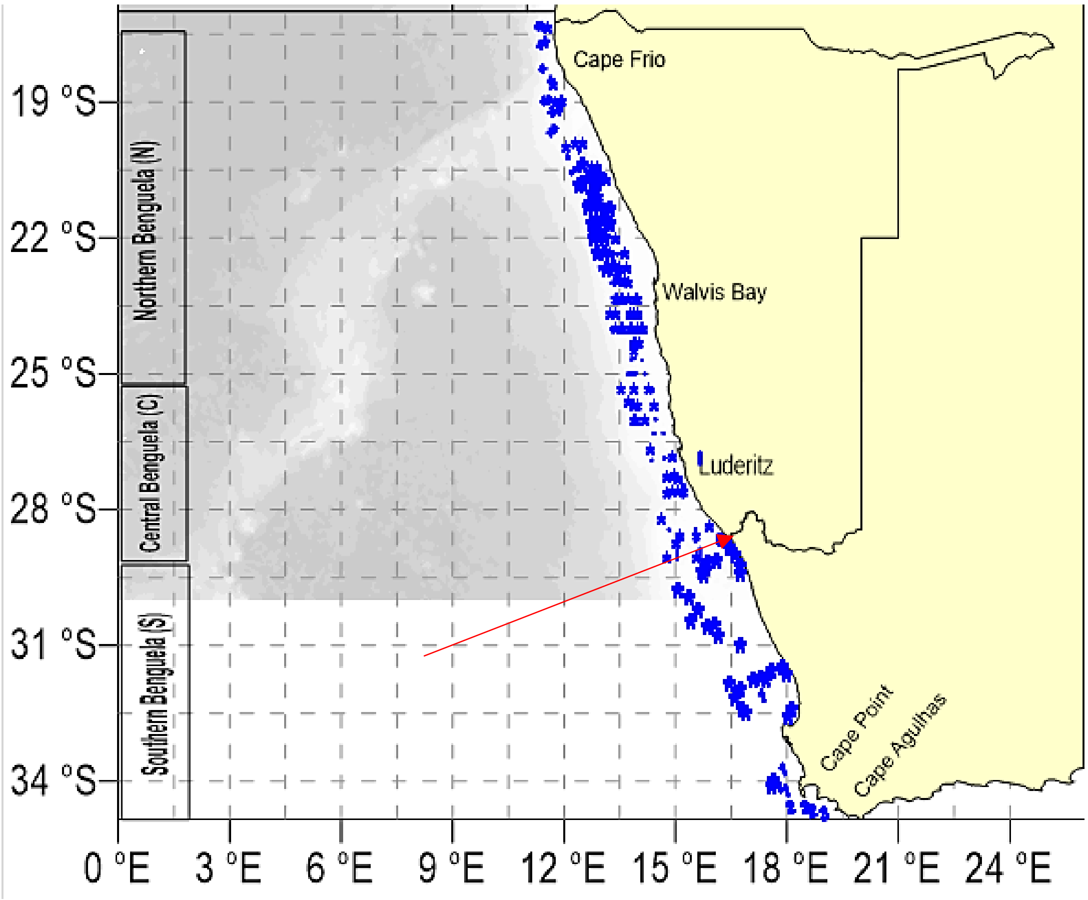


Figure 1: Sampling stations of *M. capensis* otoliths in the Benguela. Latitude ranges of areas are indicated. (Drawn using Surfer ® 9 Golden software LLC). The red line indicates the national border (Orange River).

2.1.2. Otolith Image analysis

For photographing, otoliths were positioned onto a black background with the sulcus down and the rostrum to the right. Otolith images were taken under reflected light with the Zeiss Stemi DV4 Stereotype microscope and a Zeiss Axio-camera using Zeiss AxioVision 4.0 software (Carl Zeiss, Thornwood, NY) (Eliceiri et al. 2012) for adjusting the camera and photographing (Appendix 1, Figure A1). An external light source was used to produce clear images. All images were taken at the lowest magnification (0.63x). Broken or damaged otoliths were excluded from the analysis.

Since the hake otoliths were between 4 and 24 mm in diameter, many otoliths were too large for the field of view of the microscope at 0.63x magnification. In this case, two images were taken per otolith stitched together using “Pairwise stitching” plugin in ImageJ with the Fiji (Fiji Is Just ImageJ) (https://imagej.net/Fiji) platform for ImageJ (Preibisch et al. 2009).

ShapeR (Libungan and Pálsson 2015) an R package, used with R (version 3.3.1) (R Core Team 2016) and the R studio (version 3.3.1) platform (R Studio Team 2016), was used to study otolith shape variation in *M. capensis*. The package is used for otolith shape analysis by permitting the user to automatically extract closed contour outlines from a large number of images, transform outlines via Fourier or Wavelet transformations and visualize the average shape (Libungan and Pálsson 2015).

Fourier coefficients and Wavelet transformation methods have been frequently used to determine shape differences in morphological studies (Jónsdóttir et al. 2006; Cañás et al. 2012; Leguá et al. 2013; Libungan and Pálsson 2015; Lee et al. 2017). Fourier coefficients produce an average otolith shape with fewer variations due to its high number of harmonics (Figure 2) (Parisi-Baradad et al. 2005; Libungan and Pálsson 2015). The large number of Fourier coefficients makes it difficult to provide shape differences at specific regions. In contrast, Wavelet transformations required a low wavelet number (Figure 2), and were therefore used for *M. capensis* otolith shape transformations in this study (Parisi-Baradad et al. 2005; Libungan and Pálsson 2015).

Wavelet transformation is one of the latest well-organized and adequate methods to find similar features or differences in otolith images (Parisi-Baradad et al. 2005). Using the wavelet transformation technique comes with many advantages. Firstly, this method can be carried out by a qualified individual that can obtain the desired otolith morphological features manually. Secondly, resolution of wavelet transformations are adapted to size structures. Lastly, irregularities of otolith contours can be quantified and precision position determined (Parisi-Baradad et al. 2005).

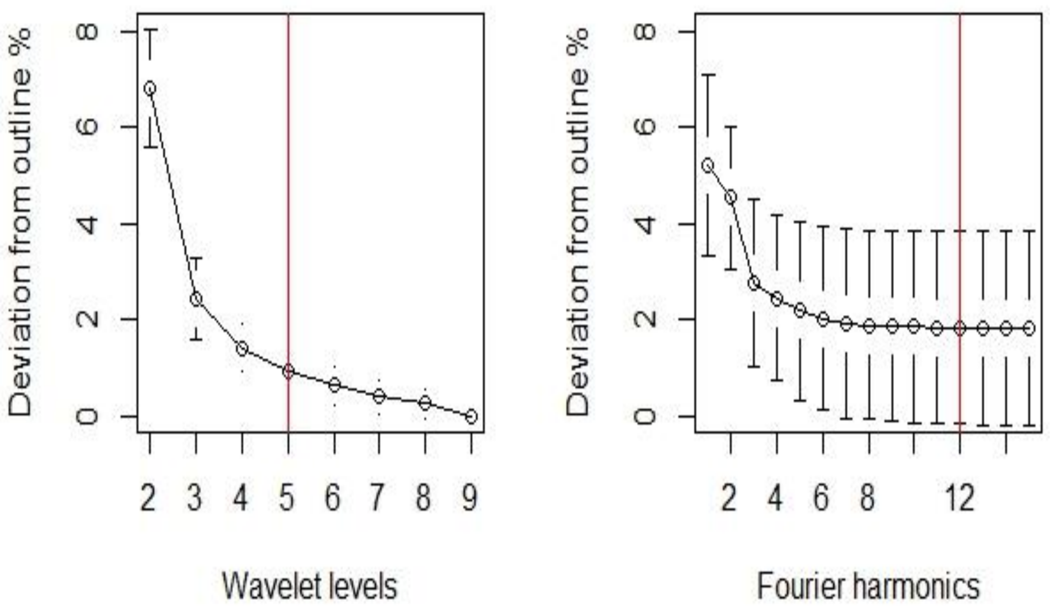


Figure 2: Quality of the Wavelet and Fourier reconstruction of otolith outline. The vertical line serves to show the number of Wavelet levels and number of Fourier harmonics required for a 98.5% accuracy.

The ShapeR package produced otolith outlines (Figure 3) mostly from a threshold pixel value of 0.1 or lower. All shape coefficients used for extracting the otolith outline were standardised by fish length to remove size effects (Libungan and Pálsson 2015; Lee et al. 2017). Otolith outlines were standardized by fish length to remove the allometric effect, in order remove all the information related to size.

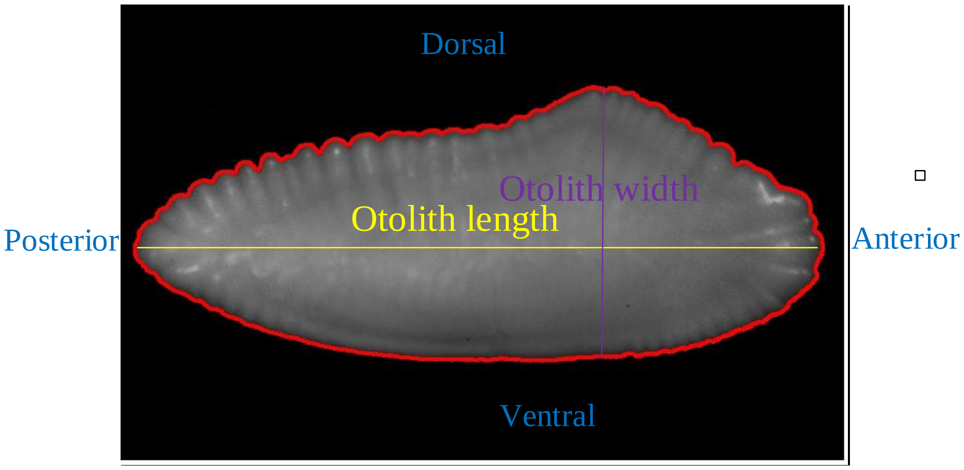


Figure 3: *M. capensis* otolith produced in the ShapeR package (Libungan and Pálsson 2015) using Wavelet coefficients. The red line indicates the perimeter. The yellow line indicates the otolith length. The purple line indicates the otolith width.

2.1.3. Statistical analyses

All analyses were done in R version 3.3.1 (R Core Team 2016).

Shape measurements (basic descriptors) such as otolith length, otolith width, otolith area and otolith perimeter were obtained from the outlines (Figure 3, Table 2) and shape indices (form factor, roundness, circularity, rectangularity, ellipticity, perimeter/area and aspect ratio) were calculated from the basic descriptors (Table 2).

Table 2: Otolith shape parameters measured and shape indices calculated for each *M. capensis* otolith.

Shape parameter	**Equation**	**Description**	**References**
Otolith length (mm) (OL)		The longest distance between any two points along the selection boundary otherwise known as the diameter (Figure 3).	
Otolith width (mm) (OW)		Shortest distance between any two points on the otolith (Figure 3)	
Otolith area (mm^2) (A)		Total surface area of the otolith	
Otolith perimeter (mm) (P)			
Perimeter / Area	$\frac{P}{A}$	Describes otolith contour complexity.	Cañás et al. (2012); Almeland (2015)
Form factor	$\frac{4\pi * A}{(P)^2}$	The similarity of the otolith outline to a perfect circle is presented by this value. Ranges from almost 0 to 1, 1 being a flawless circle.	Leguá et al. (2013); Ukomadu (2017)
Roundness	$\frac{4A}{\pi OL^2}$		Lord et al. (2012); Leguá et al. (2013)
Circularity	$\frac{P^2}{A}$		Bolles and Begg (2000); Jònsdòttir et al. (2006); Lord et al. (2012); Leguá et al. (2013); Ukomadu (2017)
Rectangularity	$\frac{A}{OL * OW}$	The otolith area divided by the area of its minimum enclosing rectangle.	Bolles and Begg (2000); Jónsdóttir et al. (2006)
Ellipticity	$\frac{OL - OW}{OL + OW}$		Lord et al. (2012); Leguá et al. (2013)
Aspect ratio	$\frac{OL}{OW}$		Almeland et al. (2015)

Otolith size descriptors and shape indices were standardized by otolith length (OL) according to the equation calculated from a linear regression of the particular descriptor against OL (Bolles and Begg 2000; Jónsdóttir et al. 2006; Stransky et al. 2008; Leguá et al. 2013) (Table 3). Rectangularity was standardized by 1/x. Otolith size descriptors and shape indices were standardized by otolith length because otolith length was used in certain equations for the otolith size descriptors and shape indices.

Table 3: Slope and intercept for standardizing *M. capensis* otolith size descriptors and shape indices by otolith length (OL), where standardized Y = mx + c, where m = slope and c = intercept. T values (T), P values (P) are shown of the regression and significance levels indicated by ***< 0.0001. For all regressions, total n = 1136.

Variables	Transformation	Slope	Intercept	T	P
Size descriptors					
Otolith width	None	0.322	0.618	88.46	< 0.0001***
Otolith area	Log-transformed	1.814	0.743	194.99	< 0.0001***
Otolith perimeter	None	2.318	1.094	158.64	< 0.0001***
Shape indices					< 0.0001***
Form factor	None	-0.005	0.714	-11.30	< 0.0001***
Roundness	None	-0.005	0.449	19.85	< 0.0001***
Circularity	None	0.163	17.398	11.06	< 0.0001***
Ellipticity	None	0.004	0.405	14.15	< 0.0001***
Perimeter/area	Log-transformed	-0.856	1.729	-116.10	< 0.0001***
Aspect ratio	None	0.028	2.330	14.11	< 0.0001***

Fish were assigned to three “age groups” based on the fish total length (Wilhelm et al. 2017; 2018), using the lengths of < 19 cm for 0-year-old, 19 to 34 cm for 1-year-old, 35 to 48 cm for 2-year old and 49 to 59 cm for 3-year-old individuals (Wilhelm et al. 2017; 2018; 2019 (Figure 4).

For each of three age groups, 1 to 3 years, standardized basic size descriptors (otolith area, otolith perimeter and otolith width) and shape indices were averaged. (Age group 0 had too few individuals present). An ANOVA test was performed to test for significant differences of means of each standardized size descriptor and shape index for each age group between areas.

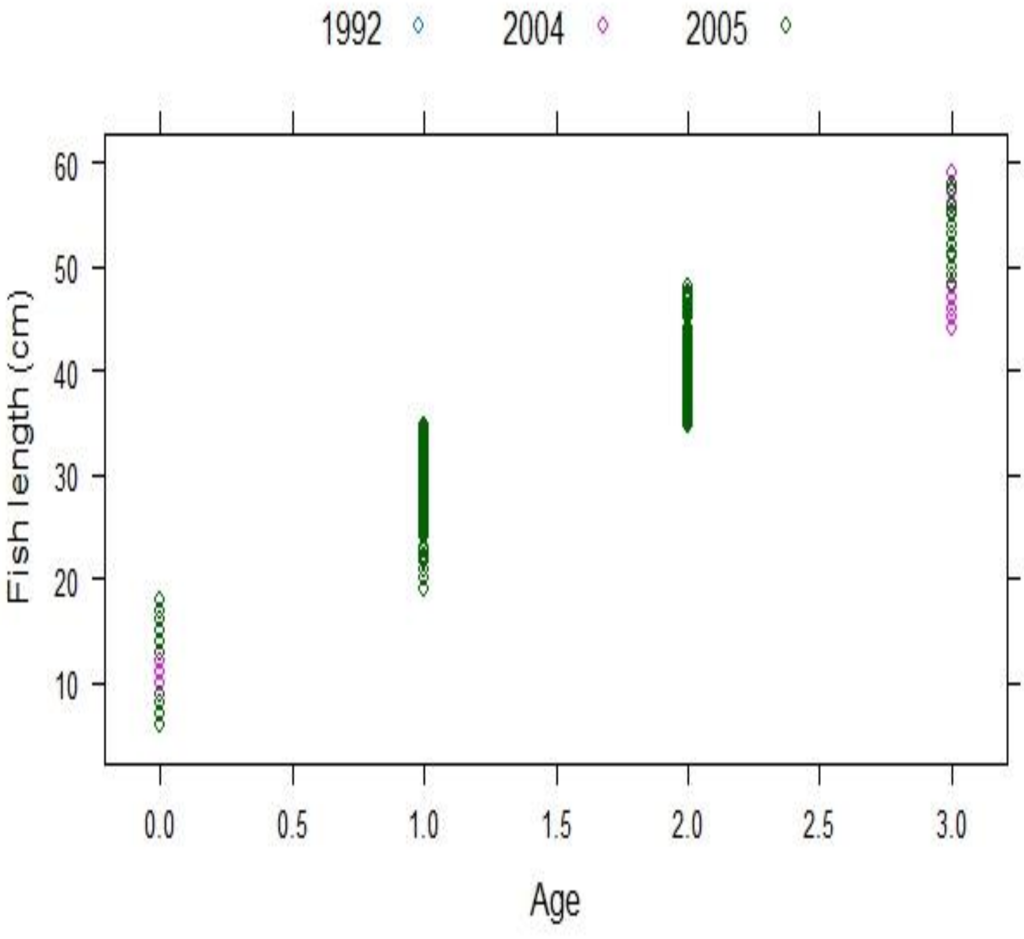


Figure 4: The relationship between fish length (cm) and assigned *M. capensis* age for the years 1992, 2004 and 2005.

To remove the influence of age and year class effects, a Canonical analysis of Principle Coordinates (CAP) (Anderson and Willis 2003) using standardized otolith size descriptors and shape indices between the areas was performed on three age groups between three populations northern, central and southern Benguela and also between the three years 1992, 2004 and 2005 to test for possible changes in structuring over time. This was done by showing groupings or mixture of samples for all areas using the “vegan” package in R (Oksanen et al. 2008). A Linear Discriminant Analysis (LDA) was applied to confirm the observed grouping structure using the “MASS” package in R (Venables and Ripley 2002). An ANOVA test (Legendre et al. 2011) using 1000 permutations was used to test for significant differences in otolith shape between the areas for each year and between years.

The means and standard deviations of wavelet coefficients were plotted against the otolith angle to investigate how the variation of the wavelet coefficients is dependent on the position along the otolith outline. Interclass correlation (ICC) was then calculated to compute the proportion of variation among the northern (N), central (C) and southern (S) groups for each year 1992, 2004 and 2005.

2.2. PARASITE INFESTATION

2.2.1. Sample collection

A total of 80 fish were collected for parasite analysis from commercial catches, 40 from 24.4˚S, 13.4˚E (northern Benguela) on 13 September 2017 and 40 from 26.4˚S, 14.2˚E (central Benguela) on 26 March 2018 (Table 4, Figure 5).

Forty *M. capensis* samples collected off South Africa coast 31.0˚S-34.0˚S between January and October 2017, and studied for parasites (Cruickshank2017), were used as comparisons with this study. A total of 22 *M. capensis* samples were collected from the South African west coast (southern Benguela) during January and February 2017, while 17 *M. capensis* samples were collected from the East coast of South Africa during September and October (Table 4, Figure 5).

Table 4: *M. capensis* samples examined for parasites collected from different areas in the Benguela, northern Benguela (24.4 ˚S, in northern Namibia) (N), central Benguela (26.4 ˚S, in southern Namibia) (C) and southern Benguela (31.0 ˚S -34.0 ˚S, in South Africa) (S) and East coast (25.1˚S E-26.7 ˚E, in South Africa) (E) (Figure 5).

Year	Month	Area	Fish length range (cm)	N	Reference
2017	September	N	34 – 64	40	This study
2018	March	C	27 – 72	40	This study
2017	January and February	S	26 – 81	23	Cruickshank (2017)
2017	September and October	E	34 – 69	17	Cruickshank (2017)

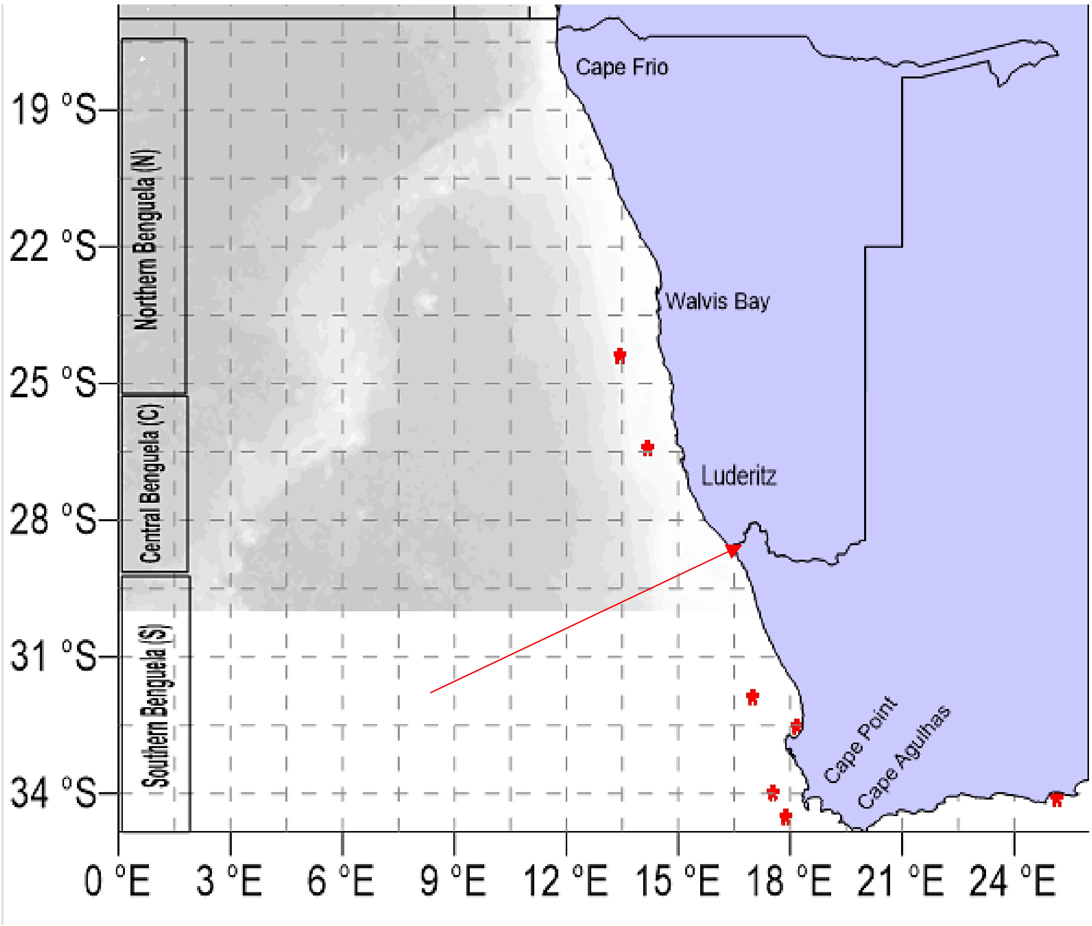


Figure 5: Sampling stations of *M. capensis* samples examined for parasites. Grey shading shows the depth contours off Namibia, starting at 100m (Drawn using Surfer ® 9 Golden software LLC). The red line indicates the national border (Orange River).

M. capensis from each fishing station were labelled, bagged and frozen immediately after capture. In the laboratory, fish were thawed and examined for parasites. Each fish was individually measured in total length to the nearest cm and weighed to the nearest gram using a Mettler Toledo SB 8001 scale (Appendix 2, Table A2). Each fish organ was individually examined for parasites using a Motic SFC 11 stereo microscope. The liver, gall bladder and skin smear tissues were examined on a microscope slide using a Motic B series compound microscope (Appendix 1, Figure A2).

All parasites had the following information recorded: family, genus or species level as far as possible, location on host and the number of individuals found.

Levels of infection were calculated for each parasite species in each area (and at each host length class) using the equations described by Bush et al. (1997):

$$\text{Prevalence (P\%)} = \frac{\text{Number of infected fish}}{\text{Total number of fish examined}} \text{ x } 100\% \qquad (1)$$

$$\text{Mean infection intensity (MI)} = \frac{\text{Total number of parasites}}{\text{Number of infected fish}} \qquad (2)$$

$$\text{Mean parasite abundance (MA)} = \frac{\text{Total number of parasites}}{\text{Total number of fish examined}} \qquad (3)$$

Comparisons of MA of each parasite species between areas was done using a Wilcoxon signed-rank test ($p < 0.05$). A Wilcoxon signed-rank test was used to compare different parasite species between the different areas in the Benguela (northern, central and southern) and East coast because the test could indicate which areas differs in terms of parasite levels the test could indicate which areas differs in terms of parasite levels.

For the most abundant seven parasite species, fish were grouped into 10 cm total length classes, and mean and standard deviation of abundance were calculated for each species at each length class for each area.

CHAPTER 3: RESULTS

3.1. OTOLITH SHAPE ANALYSIS

3.1.1. Otolith morphometrics

A positive linear relationship was observed between fish length and otolith length for *M. capensis*, but did not differ between areas (Figure 6).

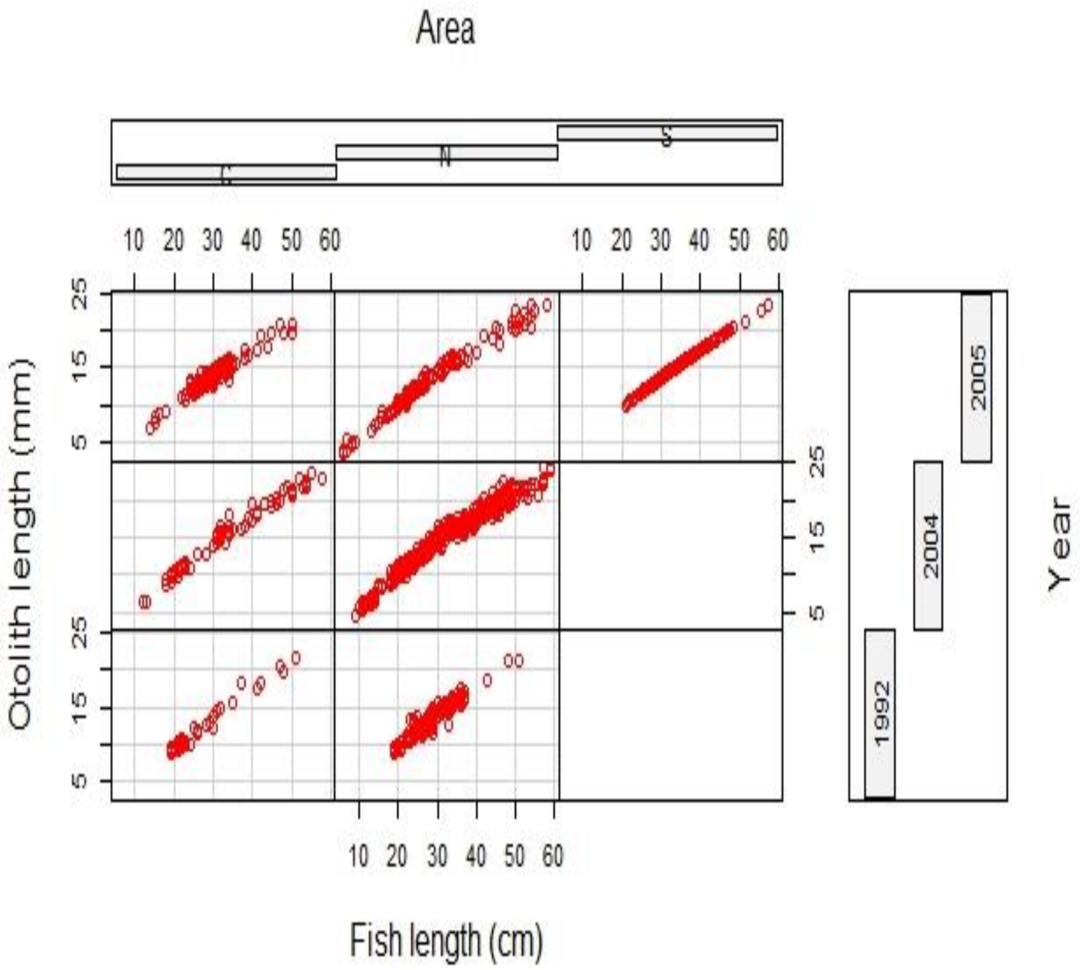


Figure 6: The relationship between otolith length (mm) and fish length (cm) of *M. capensis* for the years 1992, 2004 and 2005 in the northern (N) and central (C) and southern (S) Benguela.

Southern Benguela *M. capensis* of all three age groups had otoliths with a lower otolith width, area and perimeter compared to the northern and central Benguela *M. capensis* of the same age group, which decreased (differences increased) with an increase in age groups (Figures 7, 8 and 9). All these differences were highly significant (Table 5). Central Benguela 2-year-old *M. capensis* had a significantly higher otolith perimeter compared to the northern Benguela *M. capensis* of the same age group in 1992 (Figure 9, Table 5). No other differences were observed in all otolith size descriptors between the northern and central Benguela *M. capensis* (Figures 7, 8 and 9, Table 5).

Form factor was significantly lower in central Benguela 2-year-old *M. capensis* otoliths than in northern Benguela *M. capensis* of the same age group in 1992 and significantly higher in southern Benguela 1- and 2-year-old *M. capensis* than in the northern and central Benguela *M. capensis* of the same age group in 2005 (Figure 10, Table 5). Southern Benguela *M. capensis* otoliths had a significantly lower roundness than the northern and central Benguela *M. capensis* in all three age groups, and the differences increased with an increase in age (Figure 11, Table 5). Circularity indicated significant differences between areas in 1- and 2-year-old *M. capensis* in 2005 (Figure 12, Table 5). Rectangularity indicated significant differences between areas in 1-year-old *M. capensis* in 1992 and 2005 (Figure 13, Table 5).

M. capensis from the southern Benguela had significantly higher otolith ellipticity than the northern and central Benguela *M. capensis* in 2005 for all three age groups (Figure 14, Table 5). Southern Benguela *M. capensis* otolith ellipticity increased (differences in ellipticity between areas increased) with an increase in age. In 2005, a significantly higher otolith perimeter/area was observed in 2- and 3-year-old southern Benguela *M.*

capensis otoliths compared to the northern and central Benguela *M. capensis* of the same age (Figure 15, Table 5). Southern Benguela *M. capensis* collected in 2005 also had a significantly higher otolith aspect ratio than the nothern and central Benguela *M. capensis* in all age groups (Figure 16, Table 5).

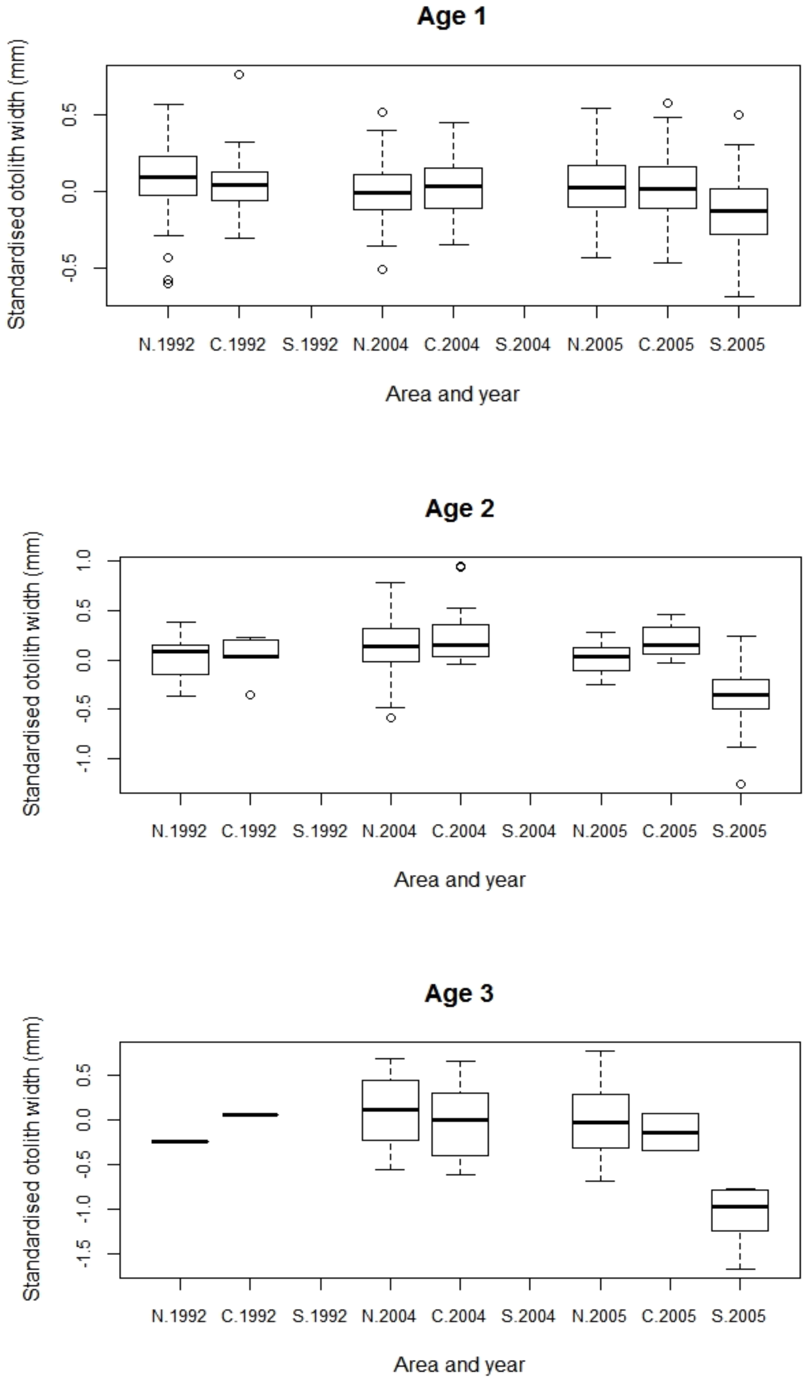


Figure 7: Box plots of standardized otolith width for three age groups of *M. capensis* collected from the northern (N), central (C) and southern Benguela (S) in the years 1992, 2004 and 2005.

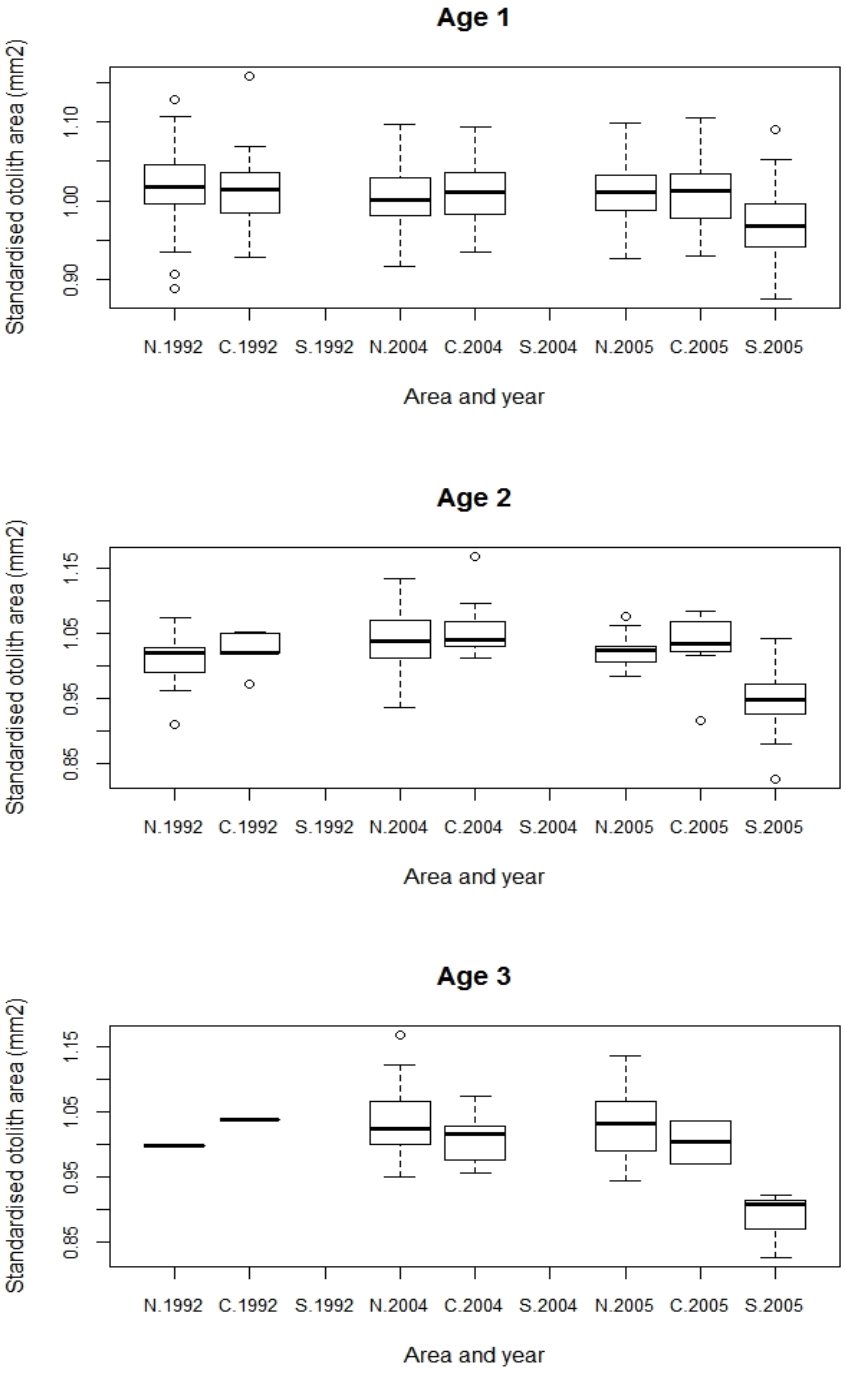


Figure 8: Box plots of standardized otolith area for three age groups of *M. capensis* collected from the northern (N), central (C) and southern Benguela (S) in the years 1992, 2004 and 2005.

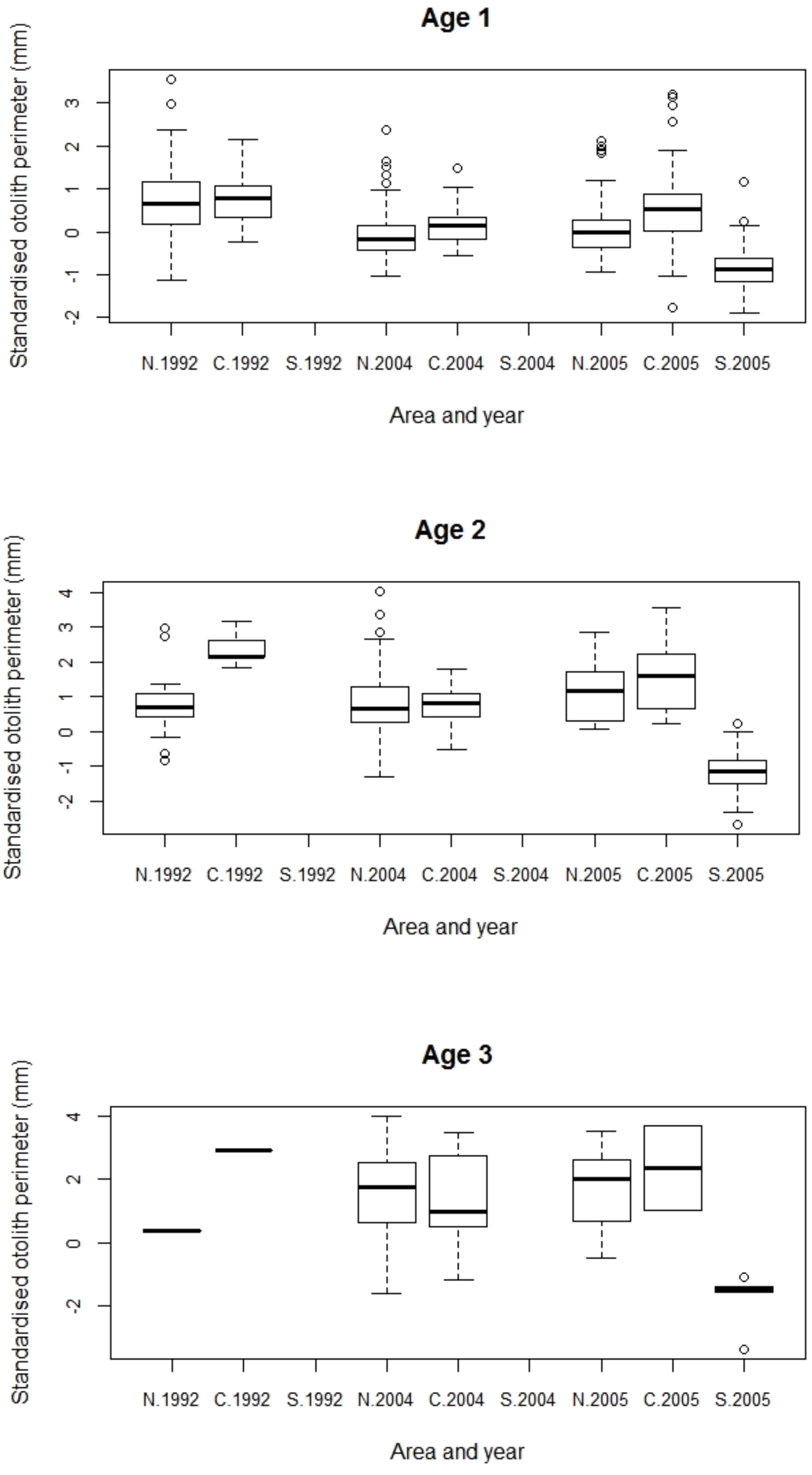


Figure 9: Box plots of standardized otolith perimeter for three age groups of *M. capensis* collected from the northern (N), central (C) and southern Benguela (S) in the years 1992, 2004 and 2005.

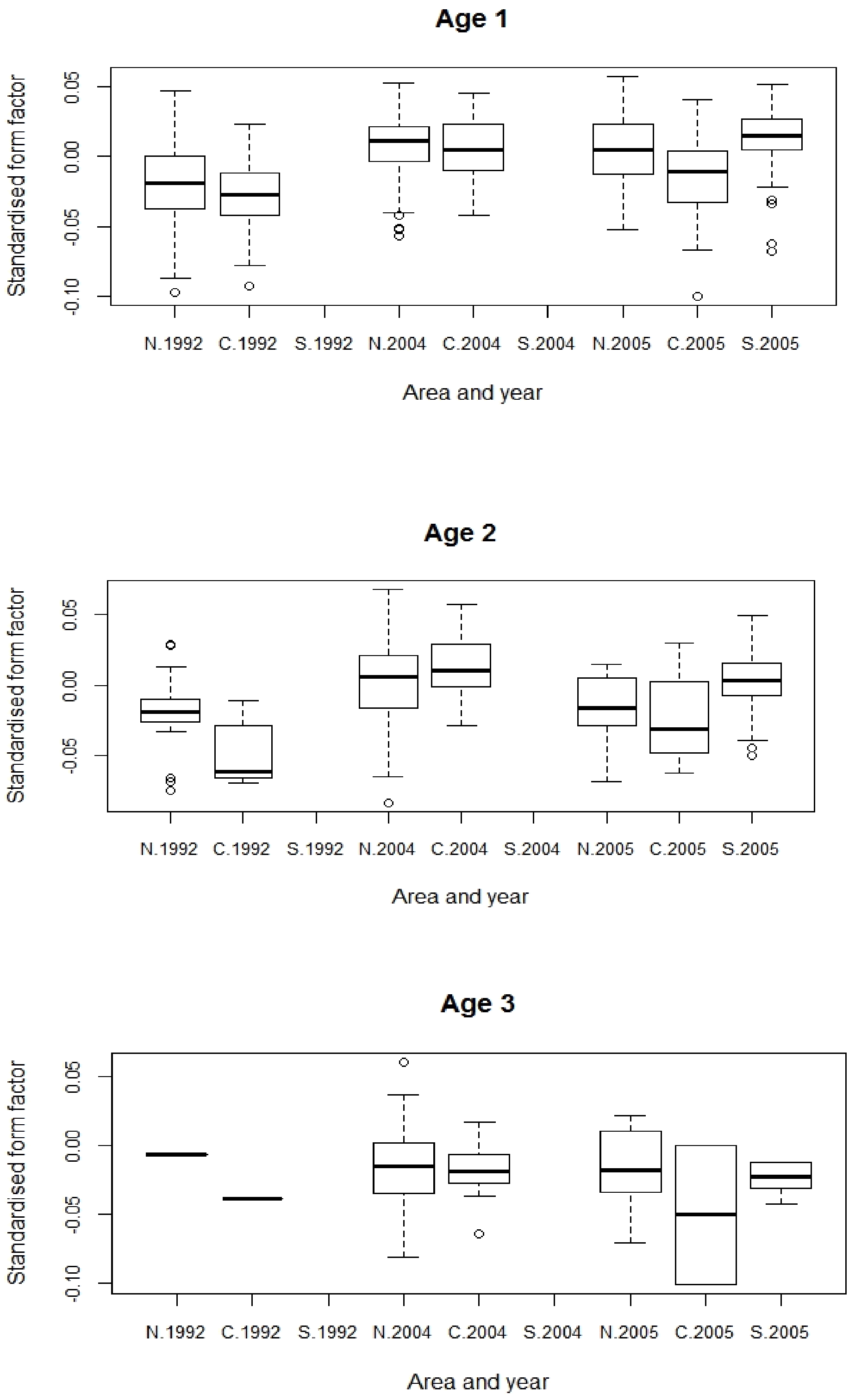


Figure 10: Box plots of standardized otolith form factor for three age groups of *M. capensis* collected from the northern (N), central (C) and southern Benguela (S) in the years 1992, 2004 and 2005.

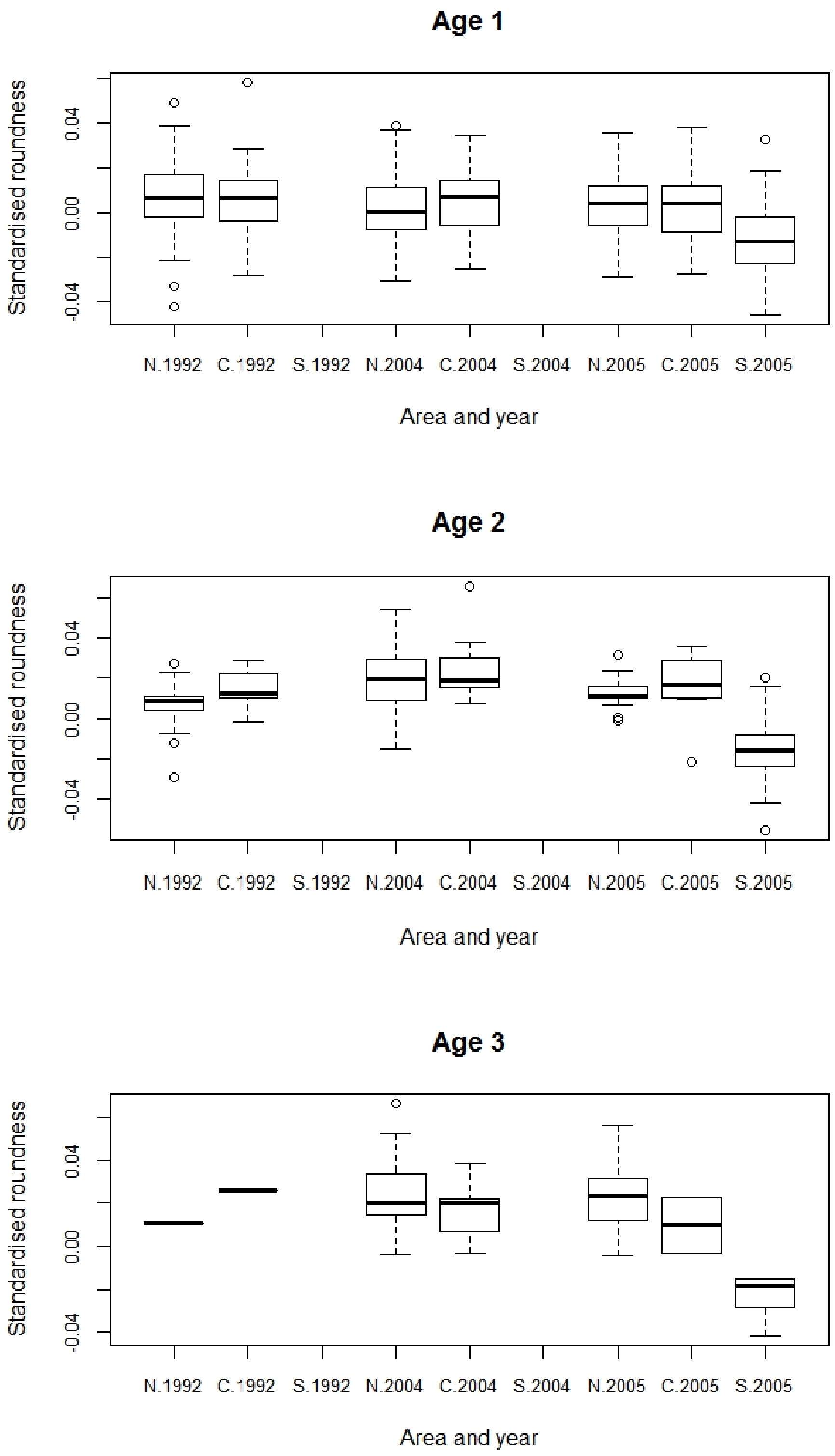


Figure 11: Box plots of standardized otolith roundness for three age groups of *M. capensis* collected from the northern (N), central (C) and southern Benguela (S) in the years 1992, 2004 and 2005.

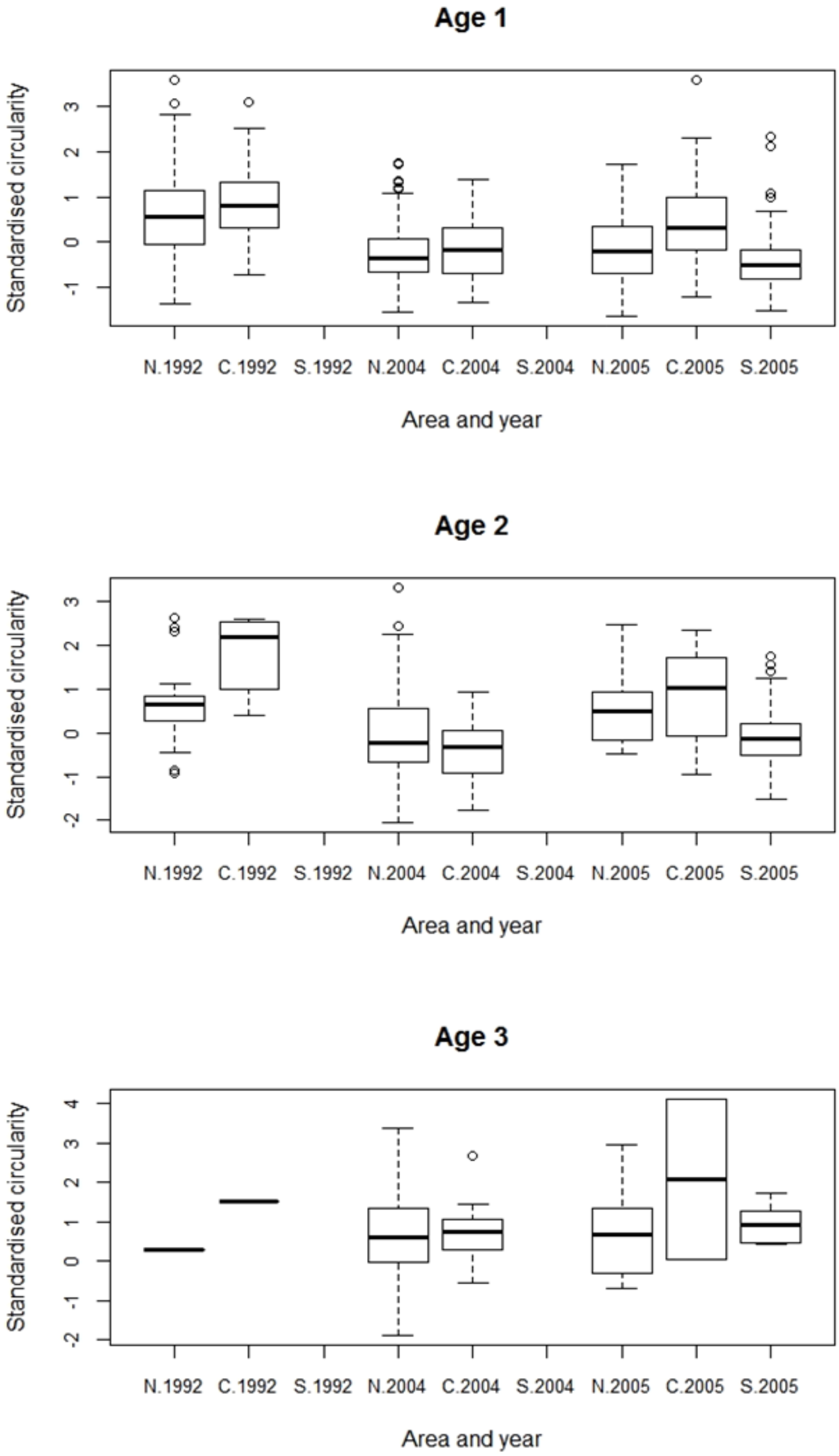


Figure 12: Box plots of standardized otolith circularity for three age groups of *M. capensis* collected from the northern (N), central (C) and southern Benguela (S) in the years 1992, 2004 and 2005.

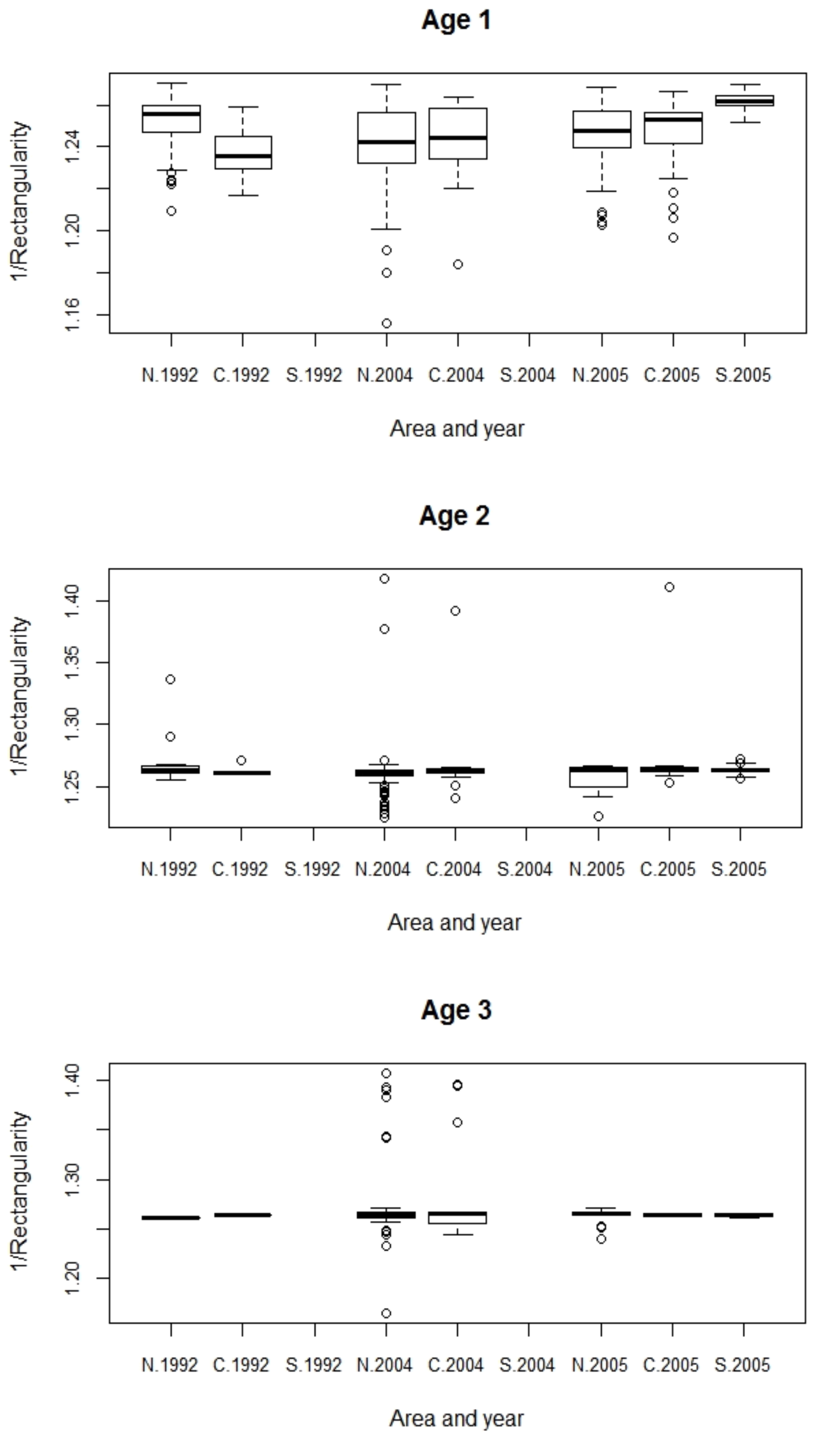


Figure 13: Box plots of standardized otolith rectangularity for three age groups of *M. capensis* collected from the northern (N), central (C) and southern Benguela (S) in the years 1992, 2004 and 2005.

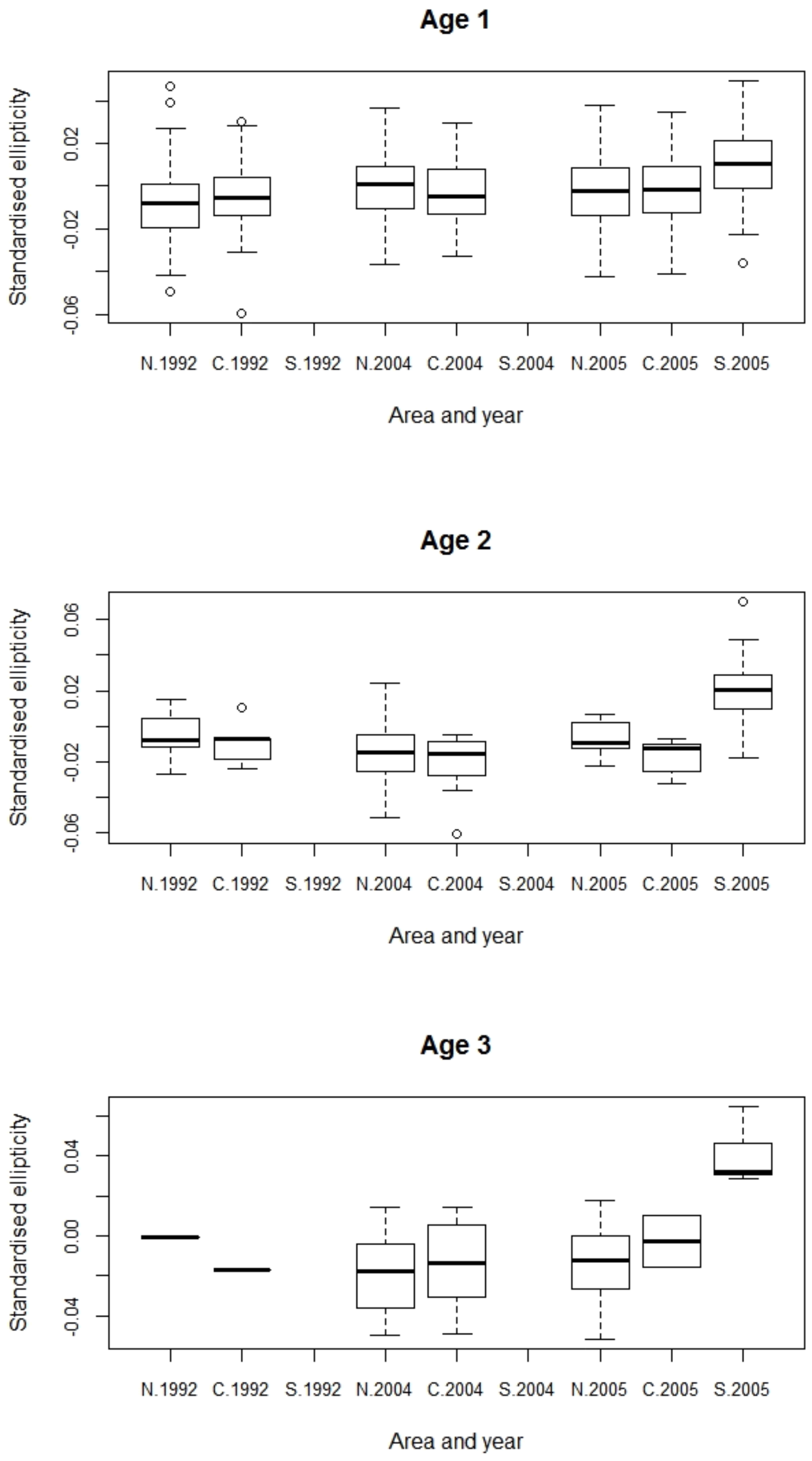


Figure 14: Box plots of standardized otolith ellipticity for three age groups of *M. capensis* collected from the northern (N), central (C) and southern Benguela (S) in the years 1992, 2004 and 2005.

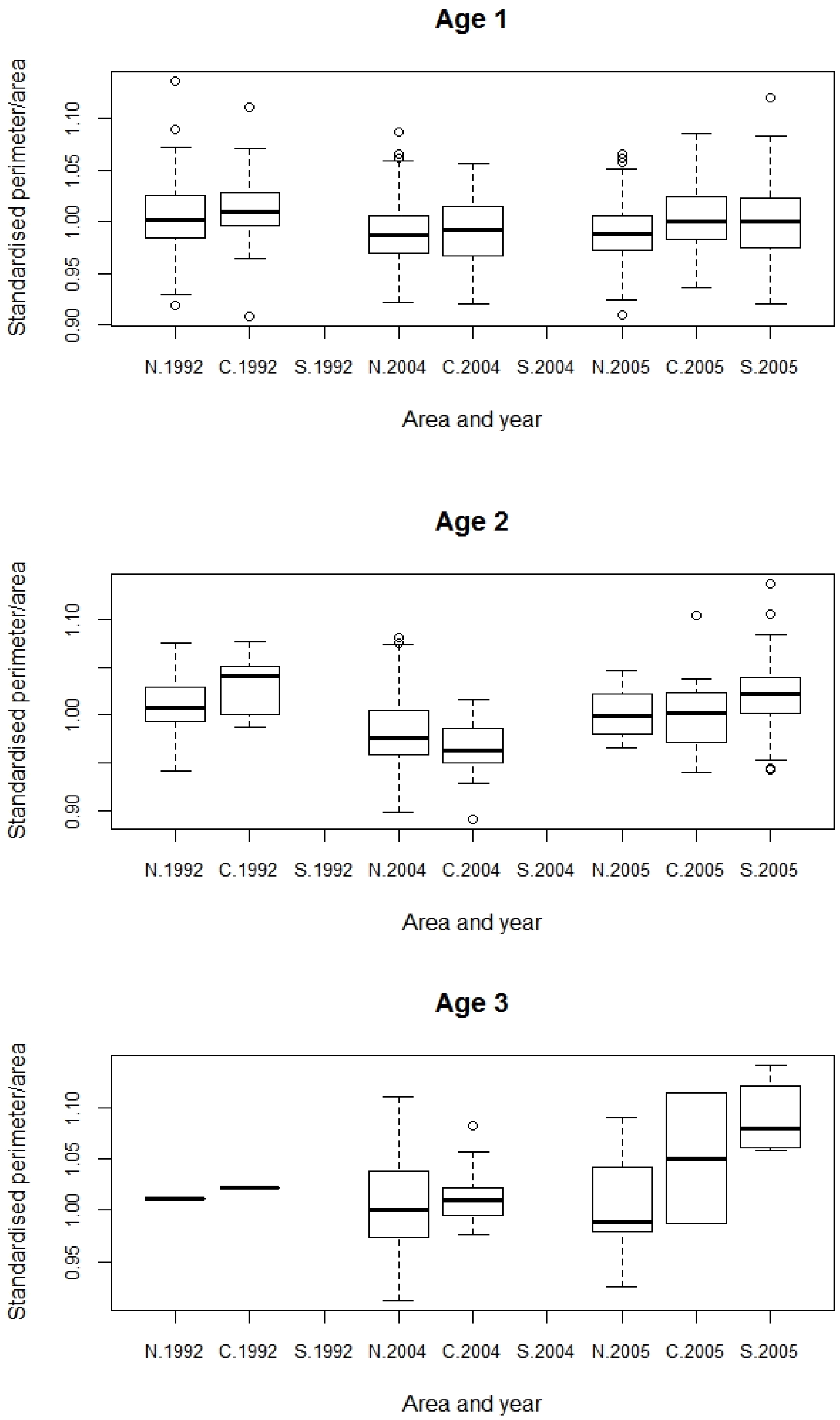


Figure 15: Box plots of standardized otolith perimeter/area for three age groups of *M. capensis* collected from the northern (N), central (C) and southern Benguela (S) in the years 1992, 2004 and 2005.

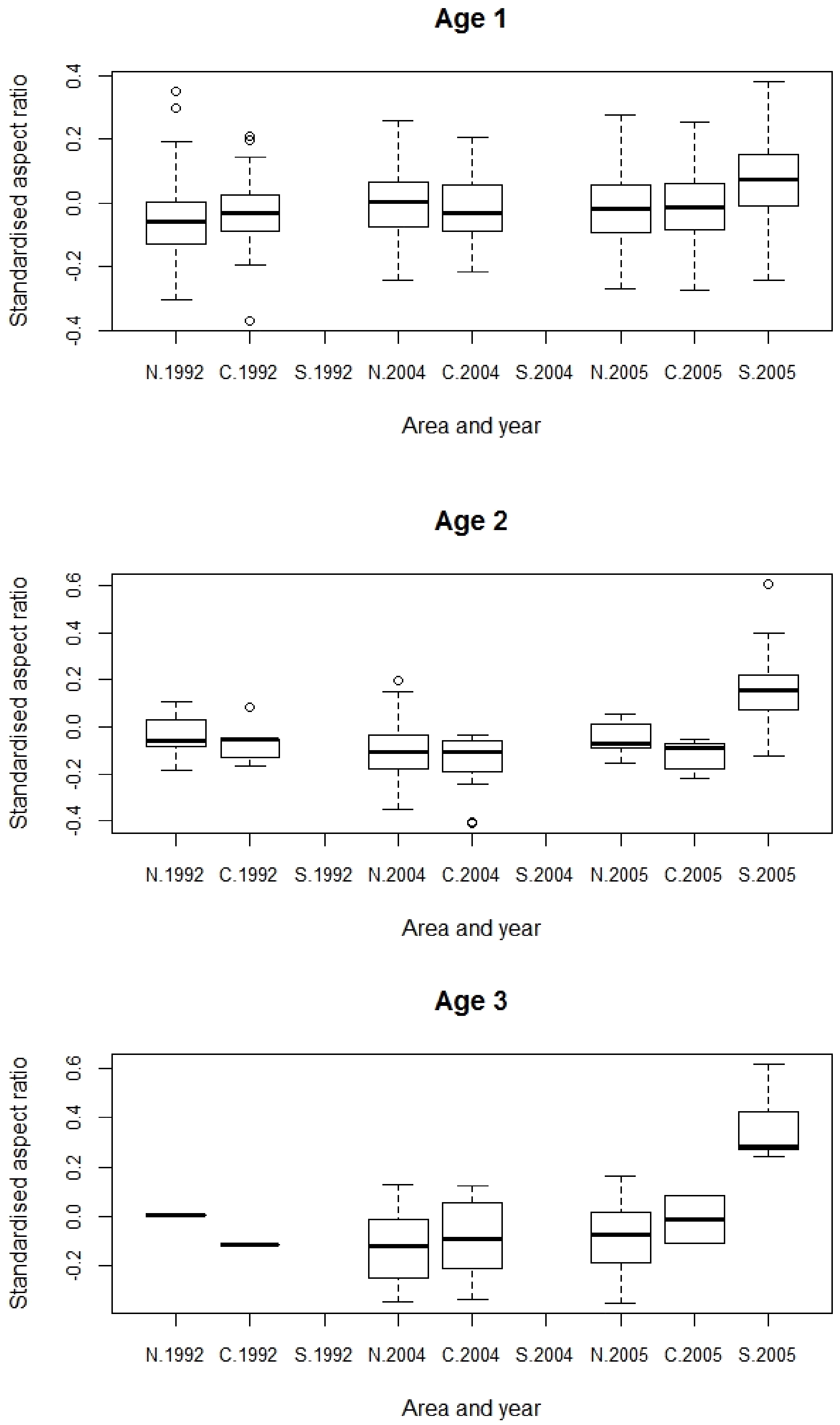


Figure 16: Box plots of standardized otolith aspect ratio for three age groups of *M. capensis* collected from the northern (N), central (C) and southern Benguela (S) in the years 1992, 2004 and 2005.

Table 5: ANOVA test results, F-ratio (F), P value (P) and significance levels indicated by * < 0.05, ** < 0.01, *** < 0.0001, of *M. capensis* standardized otolith descriptors: width, area and perimeter and standardized otolith shape indices: form factor, roundness, circularity, rectangularity, ellipticity, perimeter/area and aspect ratio compared between northern, central and southern Benguela for each age group in each year 1992, 2004 and 2005. (ANOVA could not be performed for 3-year old *M. capensis* collected in 1992 because only two samples were available).

Age 1									
Shape descriptors	**1992**			**2004**			**2005**		
	F	P	N	F	P	N	F	P	N
Width	1.42	0.23	188	1.73	0.18	275	53.12	< 0.0001***	232
Area	0.35	0.55	188	1.58	0.21	275	87.42	< 0.0001***	232
Perimeter	0.2	0.65	188	6.37	0.01*	275	252.4	< 0.0001***	232
Form factor	4.32	0.03*	188	0.72	0.39	275	54.27	< 0.0001***	232
Roundness	0.03	0.86	188	1.85	0.17	275	64.81	< 0.0001***	232
Circularity	3.43	0.06	188	0.85	0.35	275	53.30	< 0.0001***	232
Rectangularity	60.76	<0.001***	188	0.11	0.73	275	123.12	< 0.0001***	232
Ellipticity	0.48	0.48	188	1.88	0.17	275	25.84	< 0.0001***	232
Perimeter/area	2.68	0.10	188	0.02	0.87	275	3.82	< 0.0001***	232
Aspect ratio	0.64	0.42	188	1.79	0.18	275	25.84	< 0.0001***	232
Age 2									
Width	0.04	0.83	54	3.76	0.05	158	164.40	< 0.0001***	135
Area	0.89	0.35	54	1.65	0.20	158	216.00	< 0.0001***	135
Perimeter	10.12	<0.01*	54	0.55	0.45	158	277.90	< 0.0001***	135
Form factor	3.22	0.08	54	2.92	0.08	158	9.93	< 0.0001***	135
Roundness	2.41	0.13	54	1.92	0.16	158	292.67	< 0.0001***	135
Circularity	3.64	0.06	54	3.06	0.08	158	10.95	< 0.0001***	135
Rectangularity	0.46	0.50	54	2.22	0.13	158	3.01	0.05	135
Ellipticity	0.69	0.41	54	3.71	0.05	158	281.83	< 0.0001***	135
Perimeter/area	1.05	0.31	54	2.87	0.09	158	57.56	< 0.0001***	135
Aspect ratio	0.61	0.44	54	3.70	0.05	158	282.01	< 0.0001***	135
Age 3									
Width	-	-	2	0.47	0.49	67	23.26	< 0.0001***	25
Area	-	-	2	1.38	0.24	67	23.70	< 0.0001***	25
Perimeter	-	-	2	0.35	0.55	67	17.80	< 0.0001***	25
Form factor	-	-	2	0.06	0.79	67	0.57	0.56	25
Roundness	-	-	2	0.92	0.34	67	24.37	<0.0001***	25
Circularity	-	-	2	0.03	0.84	67	0.60	0.55	25
Rectangularity	-	-	2	0.51	0.47	67	0.77	0.46	25
Ellipticity	-	-	2	0.19	0.66	67	20.96	< 0.0001***	25
Perimeter/area	-	-	2	0.63	0.43	67	11.12	< 0.0001***	25
Aspect ratio	-	-	2	0.23	0.63	67	26.74	< 0.0001***	25

3.1.2. Multivariate analyses and Wavelet coefficients

Mean shape of 1992 of all age groups combined did not differ significantly between the northern (N) and central (C) Benguela *M. capensis* otoliths reproduced from wavelet coefficients (Figure 17a, Table 6). Minor varitations between areas were observed on the otolith outline at 0-20˚ and 170-300˚ (Figure 18a). No significant differences (Table 6) were observed in the average otolith shape between N and C for the year 2004 of all ages combined (Figure 17b). Otolith outline at regions 90-120˚ and 180-240˚ revealed a slight variation in the Wavelet coefficients between areas (Figure 18b). Significant differences were observed in the average otolith shape for the year 2005 (of all age groups combined), with the southern Benguela otolith average shape looking different compared to the northern and central Benguela otolith average shape (Figure 17c, Table 6). The highest variations were seen on the ventral (upper) part of the otolith at 90-180˚ and dorsal/posterior part at 270-0˚ (Figure 18c). Northern Benguela otoliths and central Benguela otoliths were shorter than the southern Benguela otoliths, and the southern Benguela otoliths were longer and narrower than the otoliths collected in the other two areas in the Benguela (Figure 17c).

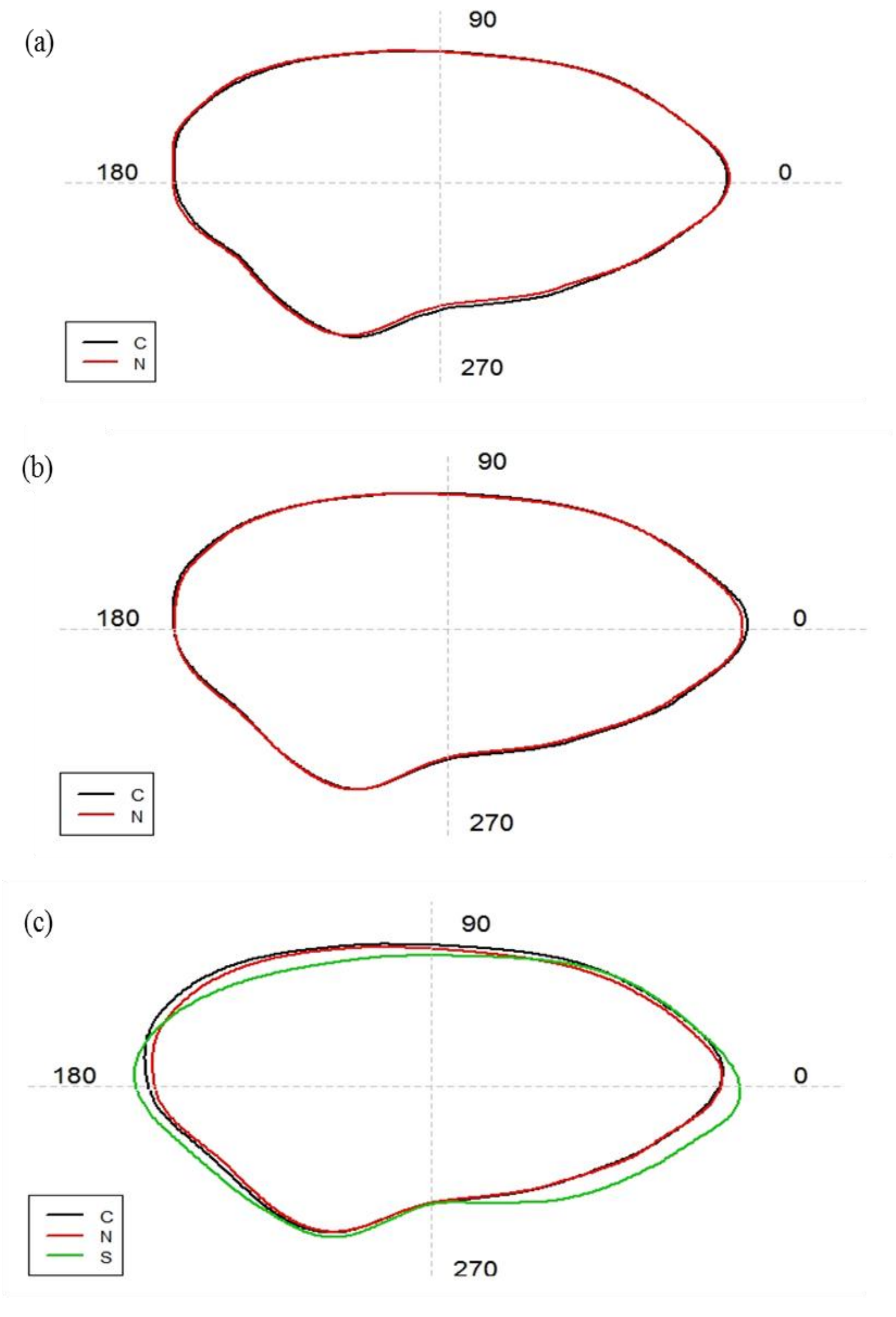


Figure 17: Average otolith shapes produced from outlines of mean Wavelet coefficients for *M. capensis* otoliths (all age groups combined) from northern Benguela (N), central Benguela (C) and southern Benguela (S) sampled in (a) 1992: (N: n=174 and C: n= 46) (b) 2004: (N: n=500 and C: n=73) (c) 2005 (N: n=146 and C: n=168, S: n=371).

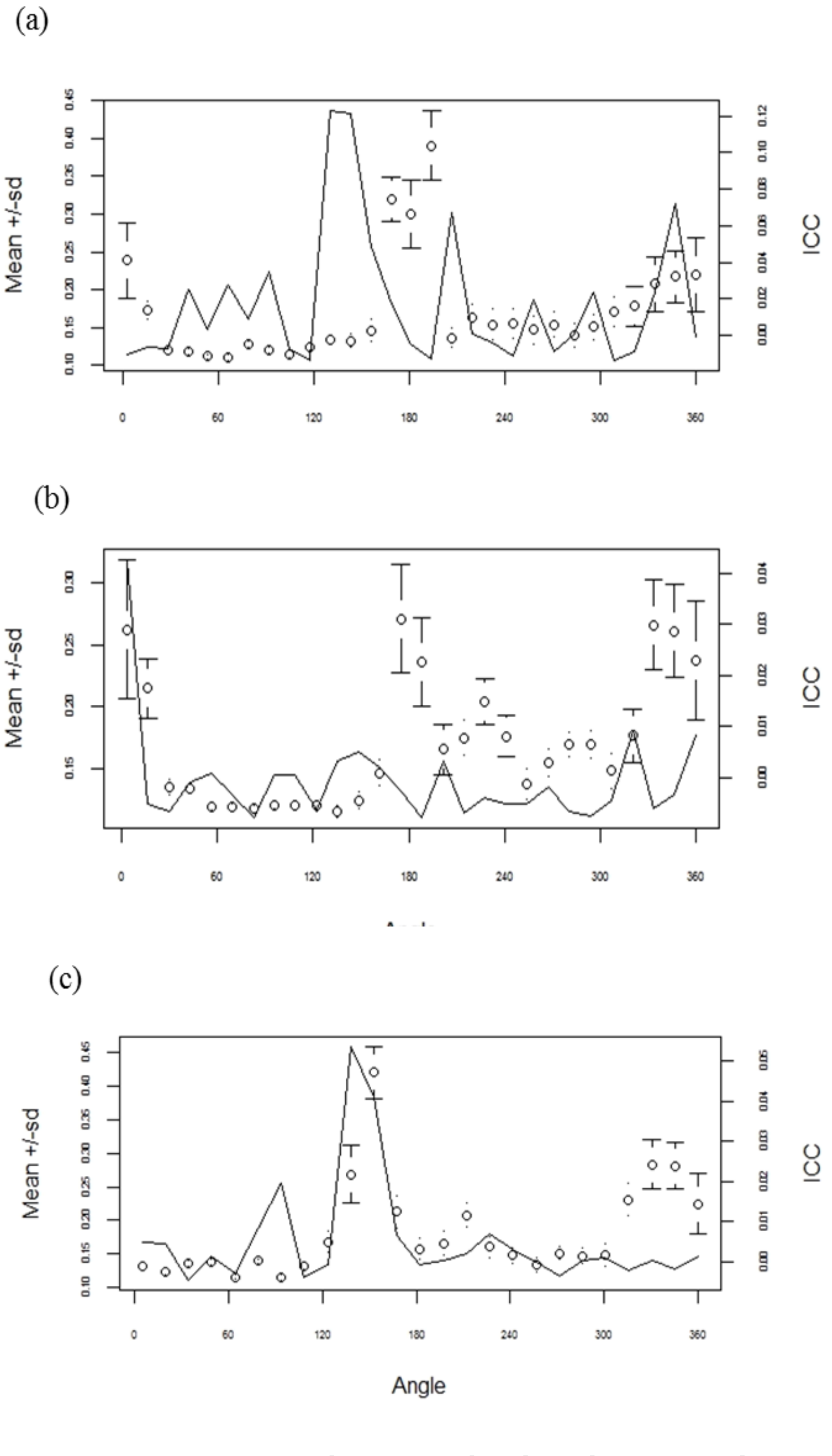


Figure 18: Variance among northern, central and southern Benguela or interclass correlation (ICC) (black solid line) investigated by mean and standard deviation (error bars) of Wavelet coefficients for *M. capensis* sampled in (a) 1992 (b) 2004 (c) 2005.

Table 6: ANOVA permutation test with 1000 iterations: degrees of freedom (DF), variances (Var), F- ratios (F), P values (P) are indicated for each year of sampling and significant difference levels indicated by * < 0.05, ** < 0.01 on otolith shapes of *M. capensis* caught from the northern (N) and central (C) Benguela using Wavelet coefficients for shape reconstruction for the years 1992, 2004 and 2005 and northern (N), central (C) and southern (S) Benguela for the year 2005.

Years and Sample area	**DF**	**Var**	**F**	**P**
1992 N and C				
Population	1	0.29	1.36	0.190
Residual	219	46.70		
2004 N and C				
Population	1	0.00	1.38	0.210
Residual	572	0.93		
2005 N and C				
Population	1	0.00	0.45	0.640
Residual	313	2.45		
2005 N, C and S				
Population	2	0.01	14.81	0.001**
Residual	683	0.22		

Figure 19 shows that the highest classification of *M. capensis* individuals to the different areas was best observed in *M. capensis* of age groups 2 and 3 (35-48 cm and 49-59 cm) with a grouping of southern Benguela otoliths from the northern and central Benguela samples in the first discriminant axis. Classification of individuals by years indicated a single grouping of *M. capensis* in all age groups examined (Figure 20).

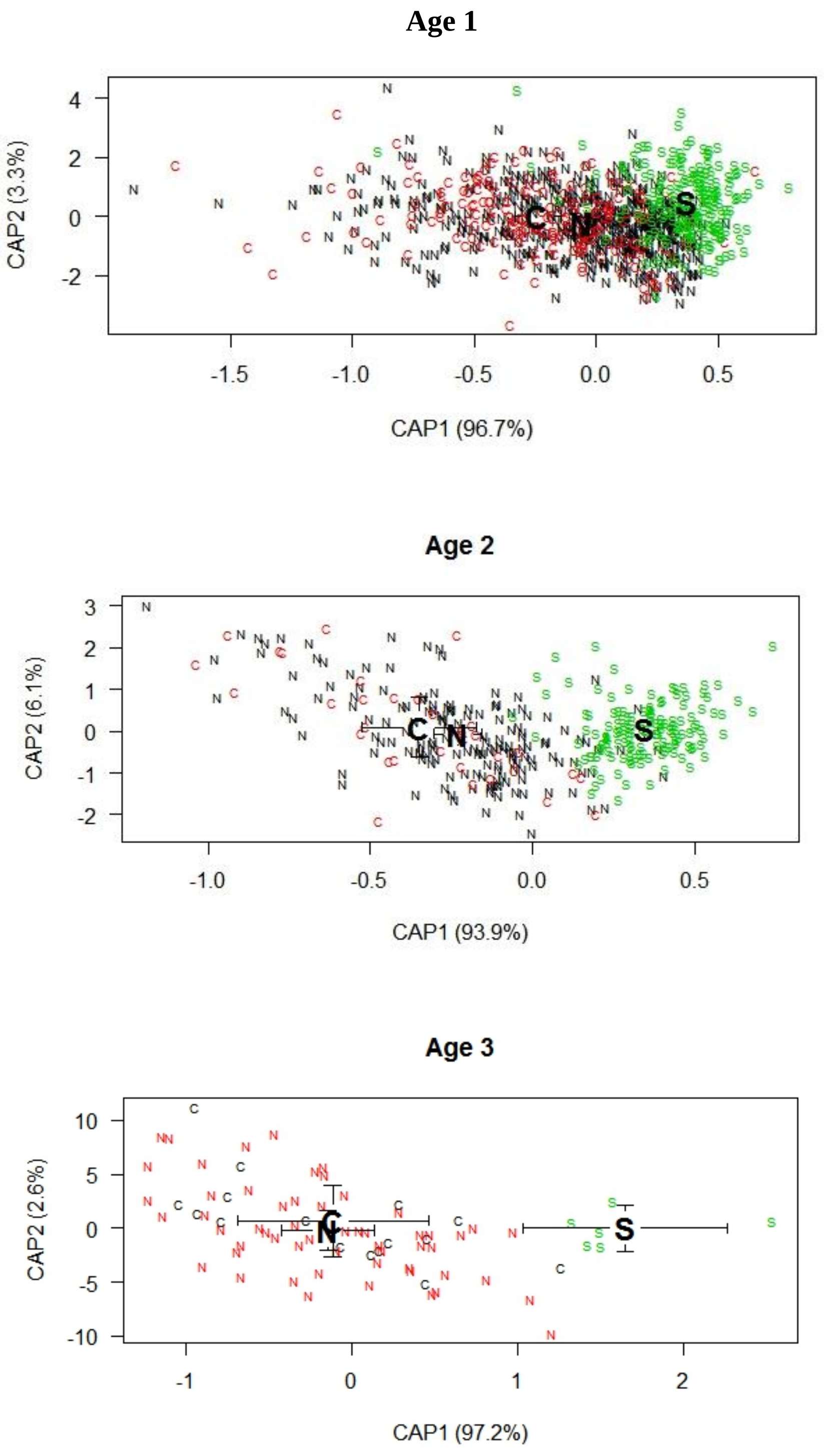


Figure 19: Canonical analysis of Principal Coordinates (CAP) of northern (N), central (C) and southern (S) Benguela *M. capensis* otolith shapes in each of the three age groups for all years combined using wavelet coefficients. The mean canonical value for each population surrounded by the interval standard error is shown.

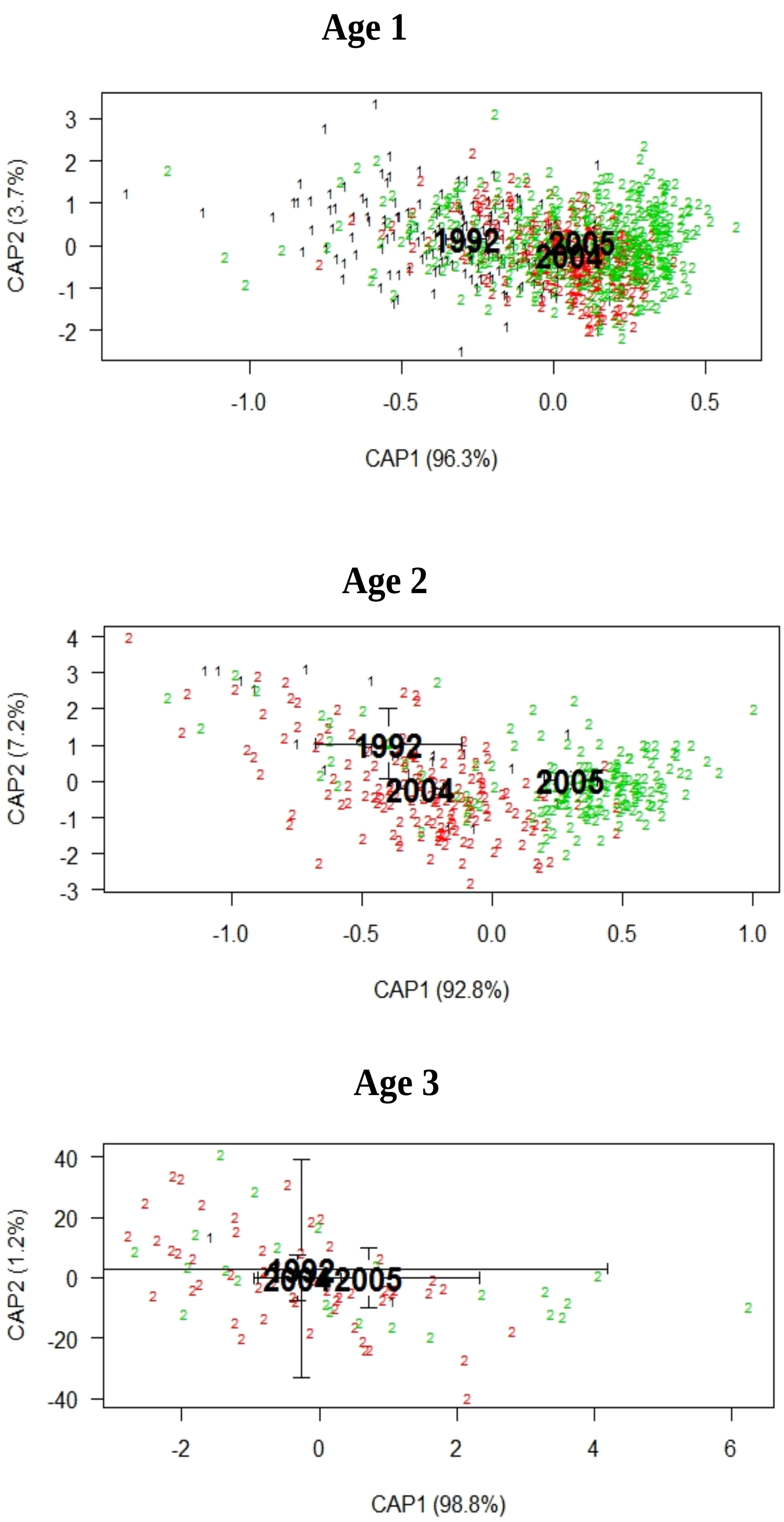


Figure 20: Canonical analysis of Principal Coordinates (CAP) of 1992, 2004 and 2005 *M. capensis* otolith shapes for all areas combined, for each of three age groups using wavelet coefficients. The mean canonical value for each year surrounded by the interval standard error is shown.

3.2. PARASITE INFESTATION

3.2.1. Parasite assemblages on *M. capensis* off the Namibian coast (northern and central Benguela)

A total of 10 parasite species, seven identified to genus level, three identified to class/subclass level, and one unidentified, were found on *M. capensis* off Namibia at two stations (N=80) (Table 7).

Two gill copepods were observed, *Chondracanthus merluccii* (Appendix 1, Figure A3) and *Parabrachiella australis* (Appendix 1, Figure A4). *P. australis* were also found in the fish mouth. *Anisakis* sp. (Appendix 1, Figure A5) larvae, brown or clear nematodes, were observed infesting most fish. They were commonly found on the liver, in the body cavity, on the gonads, in the intestines and along the stomach lining. Monogeneans *Anthocotyle merluccii* (Appendix 1, Figure A6) were observed in the gill filaments. *Hepatoxylon trichiuri* (cestodes) (Appendix A1, Figure A7) were found in the body cavity (Table 7).

Anisakis sp. was the most prevalent parasite species in both the northern and central Benguela (Table 7) with a slightly higher prevalence in northern Benguela *M. capensis* (85%) than in central Benguela *M. capensis* (80%) and the most abundant species in the northern Benguela (8.4 per host) and the second-most abundant (7.3 per host) in the central Benguela after the endoparasite Unknown 2 (36.1 per host). *A. merluccii* had the second highest prevalence and abundace of all parasite species. *A. merluccii* had a higher prevalence in central Benguela *M. capensis* (73%) than in northern

Benguela *M. capensis* (65%). *C. merluccci* showed the highest differences in prevalence between the northern (30%) and central Benguela (5%) *M. capensis*. The mean parasite abundance of *C. merluccii* was also higher in northern Benguela *M. capensis* (0.5 per host) than in central Benguela *M. capensis* (0.1 per host). The cestode Unknown 0 was only present in central Benguela *M. capensis* with a 5% prevalence and 0.1 per host abundance. The trematode Digenea (metacercaria) was only present in northern Benguela *M. capensis* with a 8% prevalence and 0.8 per host abundance. *Corynosoma* sp. was also only found in northern Benguela *M. capensis* with a 3% prevalence and 0.1 per host abundance level. The Acanthocephala Unknown 1 was only present in the central Benguela *M. capensi*s with 18% prevalence and 1.7 per host abundance. The endoparasite Unknown 2 was only present in central Benguela *M. capensis* with a 55% prevalence and 36.1 per host abundance (Table 7).

P. australis was more prevalent in central Benguela *M. capensis* (15%) compared to northern Benguela *M. capensis* (10%), but with similar abundances. Northern *M. capensis* had a higher *H. trichiuri* prevalence (18%) than the central Benguela (10%) fish and about the same abundance (0.3 and 0.2 per host respectively) (Table 7).

Table 7: Parasite species found infecting *M. capensis* collected off Namibia at 24.4˚S (n=40) and at 26.4˚S (n=40).

Parasite class/subclass	**Parasite species / family**	**Reference**	**Site**	**24.4˚S**			**26.4˚S**		
				Prevalence (%)	**Mean infection intensity**	**Mean abundance**	**Prevalence (%)**	**Mean infection intensity**	**Mean abundance**
Ectoparasites									
Copepoda	*Chodrachanthus merluccii*	Holten (1802)	Mouth	30	1.8	0.5	5	1.5	0.1
Copepoda	*Parabrachiella australis*	Wilson (1923)	Mouth, Gills	10	4.5	0.5	15	3.5	0.5
Monogenea	*Anthocotyle merluccii*	Van Beneden and Hesse (1863)	Gills	65	6.1	4.0	73	4.0	2.9
Trematoda	Digenea (Metacercaria)		Gills	8	11.0	0.8	-	-	-
Endoparasites									
Nematoda	*Anisakis* sp.	Botha (1986)	Body cavity, intestines, stomach, liver, gonads	85	9.8	8.4	80	9.1	7.3
Cestoda	*Hepatoxylon trichiuri*	Holten (1802)	Body cavity	18	1.7	0.3	10	1.8	0.2
Cestoda	Unknown 0		Intestines	-	-	-	5	1.5	0.1
Acanthocephala	*Corynosoma* sp.	Lühe 1904	Body Cavity	3	0.1	0.1	-	-	-
Acanthocephala	Unknown 1		Intestines	-	-	-	18	9.7	1.7
Unknown	Unknown 2		Intestines and stomach	-	-	-	55	65.7	36.1

3.2.2. Comparison of northern, central and southern Benguela *M. capensis* parasite structure

Three parasite species, *Chondracanthus merluccii*, *Anthocotyle merluccii* and *Anisakis* sp., were present in all areas in the Benguela and South African East coast and *Hepatoxylon trichiuri* was present in northern, central and southern Benguela, but not on the East coast (Table 8).

The northern Benguela had the highest prevalence of *C. merluccii* (30%) compared to the central (5%) and southern (5%) Benguela. Northern Benguela *M. capensis* had the highest abundance of *C. merlucci* (0.5 per host) compared to the central and southern Benguela *M. capensis* (0.1 per host) (Table 8), with significant differences between the northern and central Benguela, northern and southern Benguela, and northern Benguela and East coast (Table 9).

P. australis was only found infesting *M. capensis* collected in the northern and central Benguela, but it was not found infesting *M. capensis* collected in the southern Benguela (Table 8). There was no significant difference in mean abundance of *P. australis* between the northern and central Benguela *M. capensis* (Table 9).

The southern Benguela *M. capensis* had the highest mean abundance of *Anisakis* sp. (37.3 per host) compared to the northern (8.4 per host) and central (7.3 per host) Benguela (Table 8), but *Anisakis* sp. indicated no significant differences in mean abundance between the northern, central and southern Benguela hosts (Table 9). However, significant differences in mean abundance of *Anisakis* sp. were observed between the southern Benguela *M. capensis* and the East coast *M. capensis* (16.4 per host) (Table 9).

A. merluccii had the second highest prevalence of all parasites, after *Anisakis* sp. in all areas with the central Benguela at 73%, the northern Benguela at 65% and the southern Benguela at 59%. Southern Benguela (7.3 per host), northern Benguela (4.0 per host) and central Benguela (2.9 per host) *M. capensis*, had similar abundance levels of *A. merluccii* (Table 8), which all were significantly higher than *A. merluccii* abundance levels in East coast *M. capensis* (1.6 per host) (Table 9).

Hepatoxylon trichiuri was only observed infesting *M. capensis* from the northern Benguela, central Benguela and southern Benguela (Table 8). The southern Benguela *M. capensis* (36%) were more prevalent with *H. trichiuri* and the central Benguela *M. capensis* were least prevalent (10%), but with no significant differences in mean abundance of *H. trichiuri* between all areas (Table 9).

Stephanostomum multispinosum was only found on *M. capensis* in the southern Benguela and East coast but not on *M. capensis* in the northern and central Benguela (Table 8). No significant difference in mean abundance of *S. multispinosum* was observed between these areas (Table 9).

Corynosoma sp. was only present in *M. capensis* from the northern Benguela, southern Benguela and East coast but absent in the central Benguela *M. capensis* (Table 8), with no significant differences in abundance observed between the northern Benguela, southern Benguela and East coast (Table 9). The unidentified Acanthocephala (Unknown 1), the unknown cestode (Unknown 0) and Unknown 2 were only recorded on central Benguela *M. capensis* but absent in the other areas (Table 8).

Table 8: Summary of prevalence and abundance of parasites found in the Benguela: northern (N), central (C) southern (S) Benguela and East coast *M. capensis*. Data from S and East coast: Cruickshank (2017), used with permission. Prevalence is the (number of fish infected / total number of fish examined)*100 and abundance is the number of parasites / total number of fish examined.

Parasite class/subclass	**Parasite species**	**Prevalence (%)**				**Abundance (per host individual)**			
		N	**C**	**S**	**East coast**	**N**	**C**	**S**	**East coast**
Ectoparasites									
Copepoda	*Chondracanthus merluccii*	30	5	5	6	0.5	0.1	0.1	0.2
Copepoda	*Parabrachiella australis*	10	15	-	-	0.5	0.5	-	-
Monogenea	*Anthocotyle merluccii*	65	73	59	61	4.0	2.9	7.3	1.6
Digenea (Metacercaria)		8	-	-	5.5	0.8	-	-	0.1
Endoparasites									
Nematoda	*Anisakis sp.*	85	80	90	89	8.4	7.3	37.3	16.4
Cestoda	*Hepatoxylon trichiuri*	18	10	36	-	0.3	0.2	1.0	-
Cestoda	Unknown 0	-	5	-	-	-	0.1	-	-
Trematoda	*Stephanostomum multispinosum*	-	-	45	33	-	-	18.5	3.7
Acanthocephala	*Corynosoma* sp.	3	-	18	6	0.1	-	0.7	0.2
Acanthocephala	Unknown 1	-	18	-	-	-	1.7	-	-
Unknown	Unknown 2	-	55	-	-	-	36.1	-	-
Unknown	Unknown 3	-	-	0.27	6	-	-	2.1	0.1

Table 9: Wilcoxon signed-rank test p-values of comparing seven parasite species' abundances between *M. capensis* from the northern Benguela (N), central Benguela (C), southern Benguela (S) and East coast. Significance levels indicated by: * < 0.05, ** < 0.01, *** < 0.0001.

Parasite species	**N-C**	**N-S**	**C-S**	**N-East**	**C-East**	**S-East**
Chondracanthus merluccii	0.033*	0.016*	0.750	0.024*	1.000	1.000
Parabrachiella australis	0.156	-	-	-	-	-
Anisakis sp.	0.683	0.353	0.174	0.278	0.583	0.035*
Anthocotyle merluccii	0.316	0.665	0.829	< 0.001***	< 0.001***	0.006***
Hepatoxylon trichiuri	0.520	0.804	0.322	-	-	-
Corynosoma sp.	-	0.313	-	1.000	-	0.313
Stephanostomum multispinosum	-	-	-	-	-	0.376

3.2.3. Parasite prevalence-fish length and abundance-fish length relationships in the Benguela

Both prevalence of infection and abundance of *Chondracanthus merluccii* appeared to increase with host total length (Figure 21).

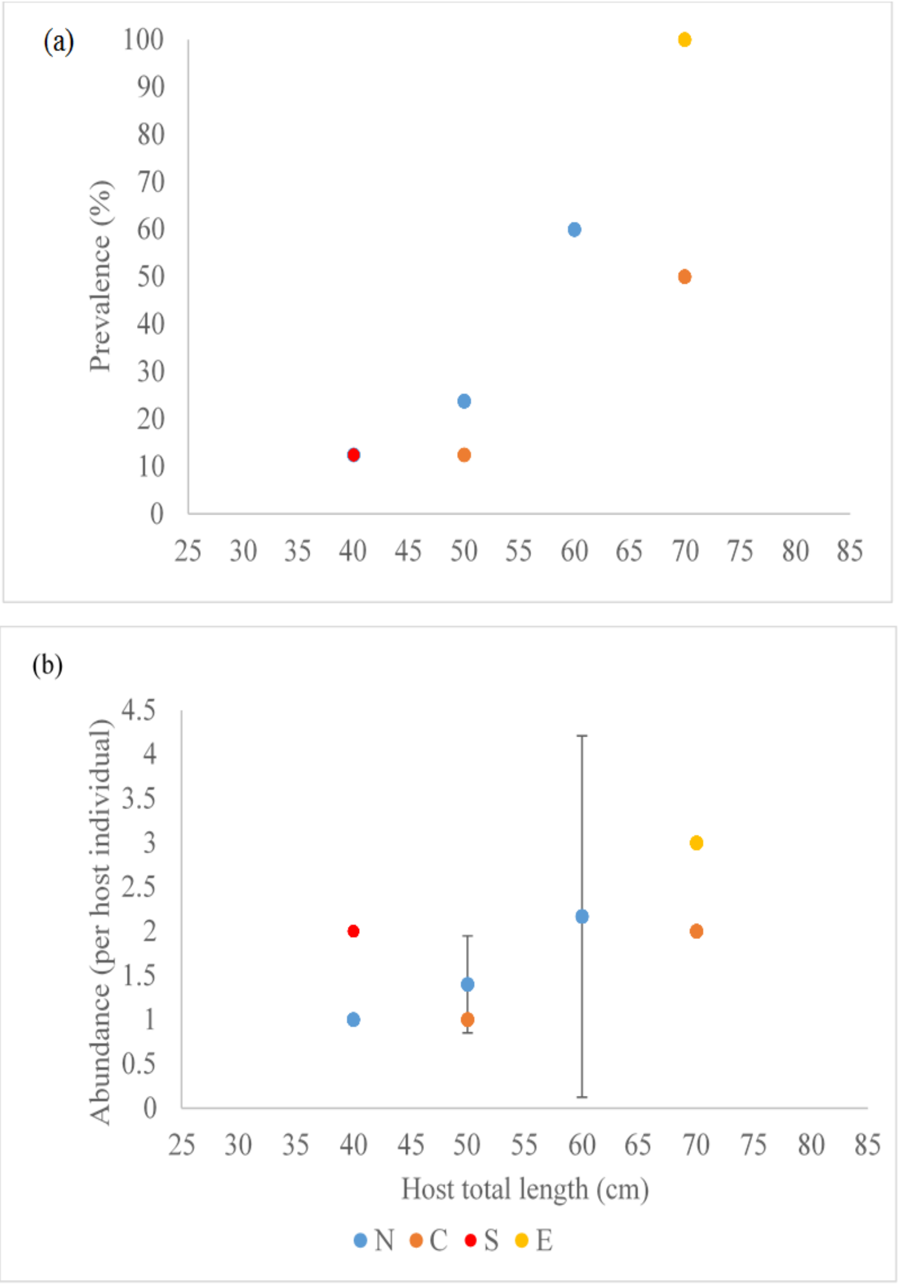


Figure 21: (a) Prevalence (%) and (b) mean abundance (± one standard deviation) of *Chondracanthus merluccii* at each 10 cm total length class of *M. capensis* caught in northern Namibia (N) at 24.4˚S, central Namibia (C) at 26.4˚S, southern Benguela (S) from the west coast of South Africa between 31 and 34˚S and South African East coast (E) between 25.1 and 26.7˚E. (Standard deviations could not be calculated for length classes that had only one fish present, hence no error bars for these points).

Figure 22 indicates an increase in prevalence of infection and mean abundance of *Parabrachiella australis* with host total length in central Benguela *M. capensis*.

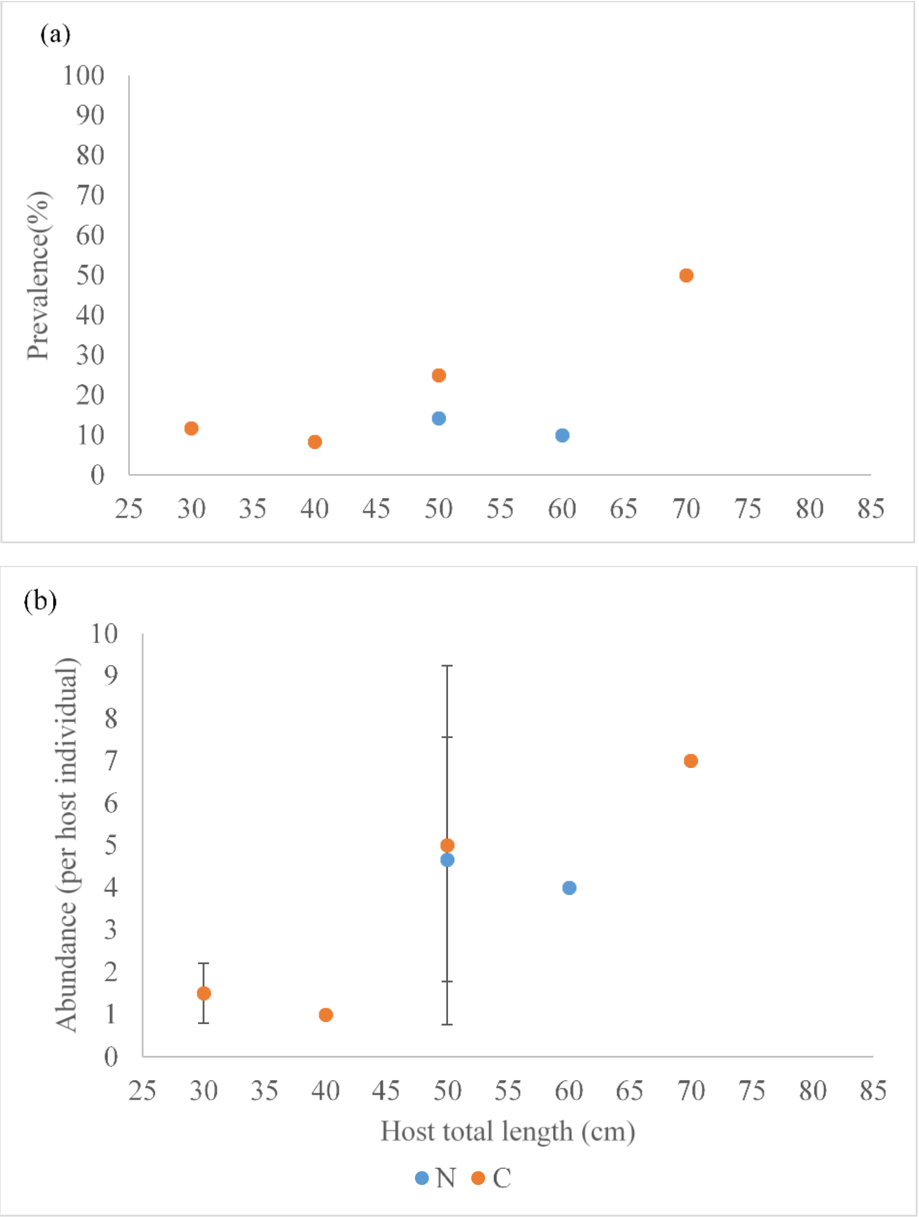


Figure 22: (a) Prevalence (%) and (b) mean abundance of (± one standard deviation) of *Parabrachiella australis* at each 10 cm total length class of *M. capensis* caught in northern Namibia (N) at 24.4˚S and central Namibia (C) at 26.4˚S. (No *P. australis* were found on *M. capensis* from the southern Benguela and South African East coast. Standard deviations could not be calculated for length classes that had only one fish present, hence no error bars for these points).

Infestation prevalence of *Anisakis* sp. showed a 100% prevalence in fish ≥30 cm total host length in the northern, central and southern Benguela (Figure 23a). *Anisakis* sp. mean abundance increased expotentially with host total length (Figure 23b).

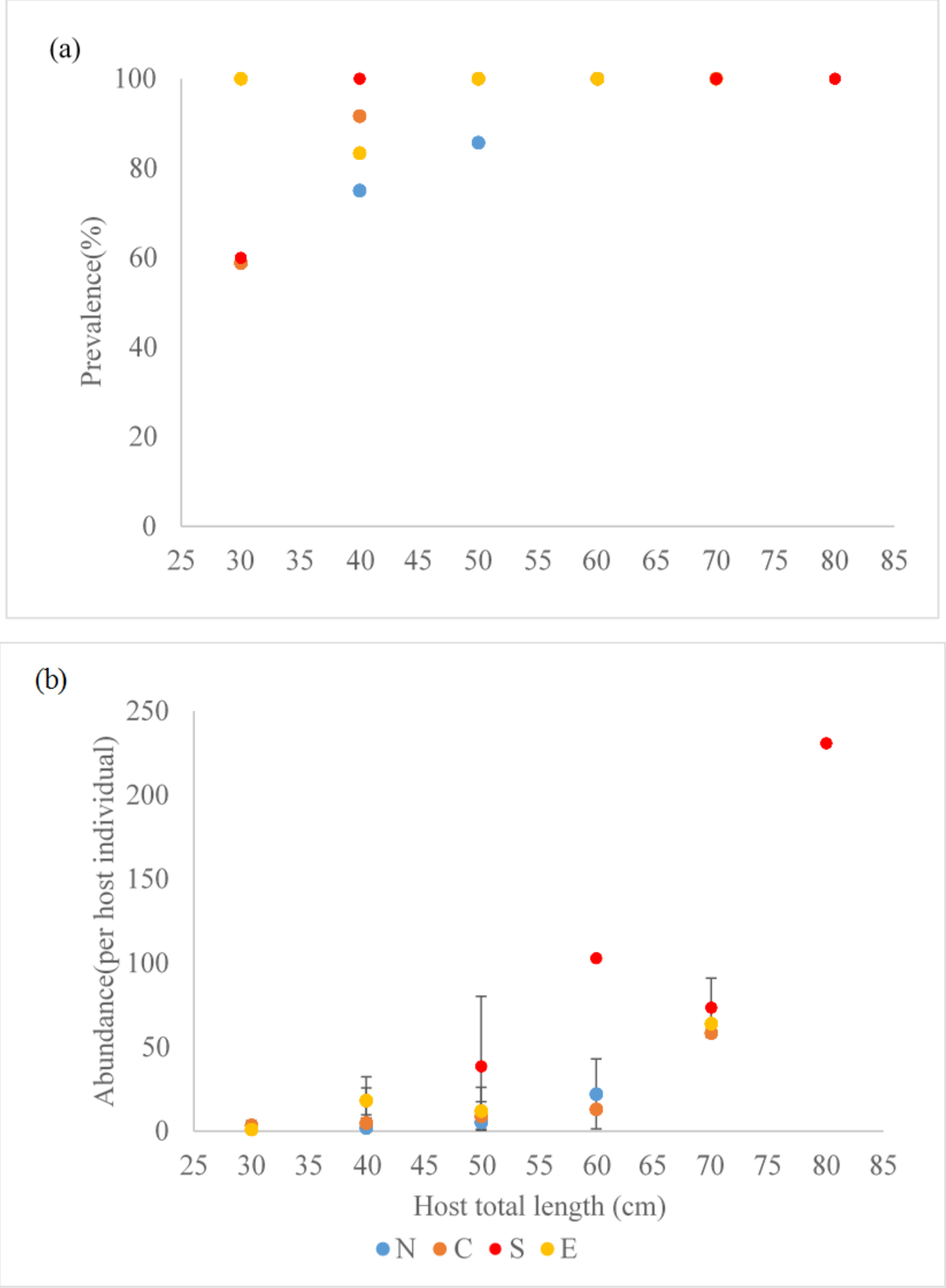


Figure 23: (a) Prevalence (%) and (b) mean abundance (± one standard deviation) of *Anisakis* sp. at each 10 cm total length class of *M. capensis* caught in northern Namibia (N) at 24.4˚S, central Namibia (C) at 26.4˚S, southern Benguela (S) from west coast of South Africa between 31 and 34˚S and South African East coast (E) between 25.1 and 26.7˚E. (Standard deviations could not be calculated for length classes that had only one fish present, hence no error bars for these points).

Anthocotyle merluccii prevalence and abundance showed no clear relationship with host total length.

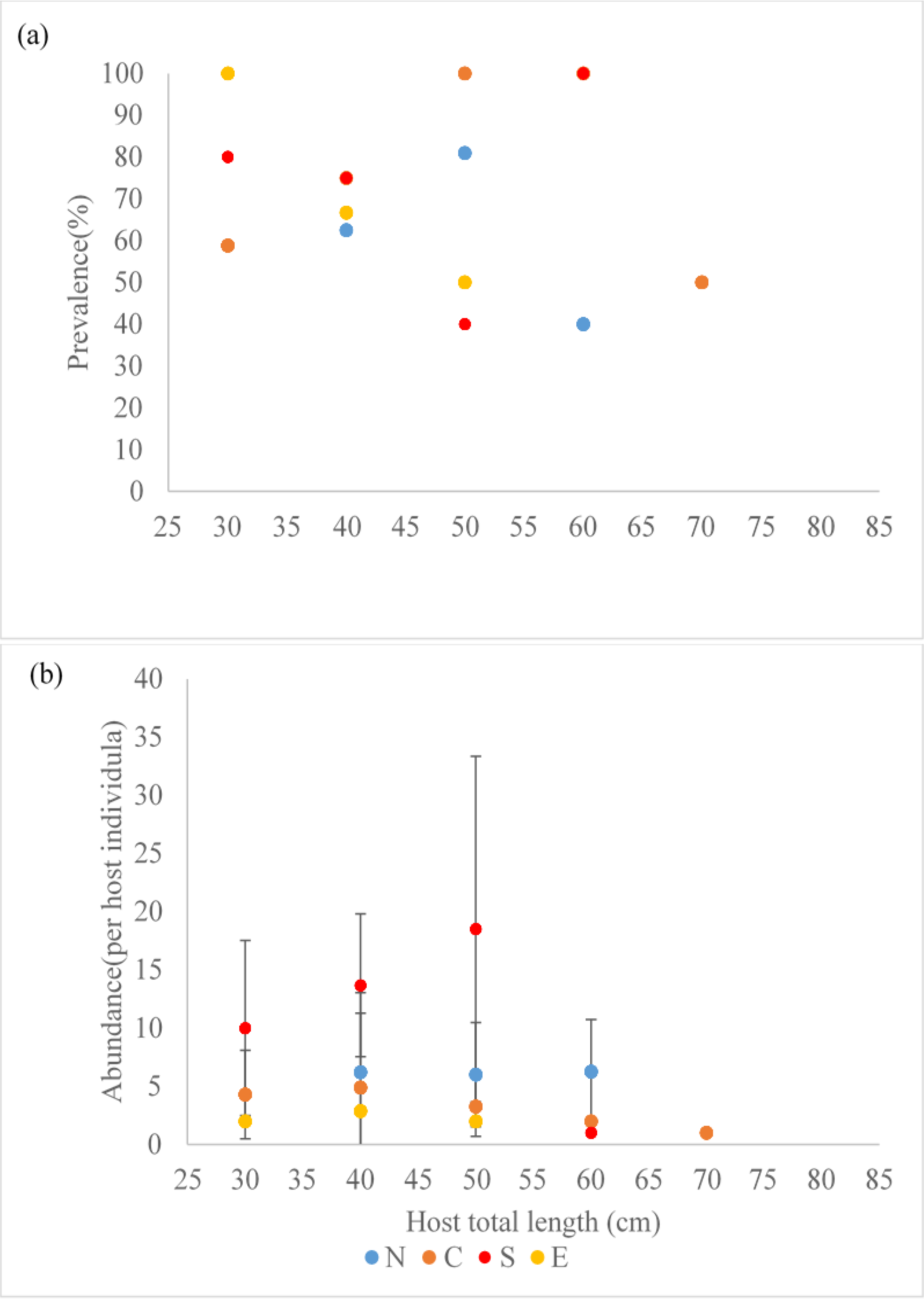


Figure 24: a) Prevalence (%) and (b) mean abundance (± one standard deviation) of *Anthocotyle merluccii* at each 10 cm total length class of *M. capensis* caught in northern Namibia (N) at 24.4˚S, central Namibia (C) at 26.4˚S, southern Benguela (S) from the west coast of South Africa between 31 and 34˚S and South African East coast (E) between 25.1 and 26.7˚E. (Standard deviations could not be calculated for length classes that had only one fish present, hence no error bars for these points.)

Hepatoxylon trichiuri prevalence of infection and abundance showed a positive relationship with host total length (Figure 25).

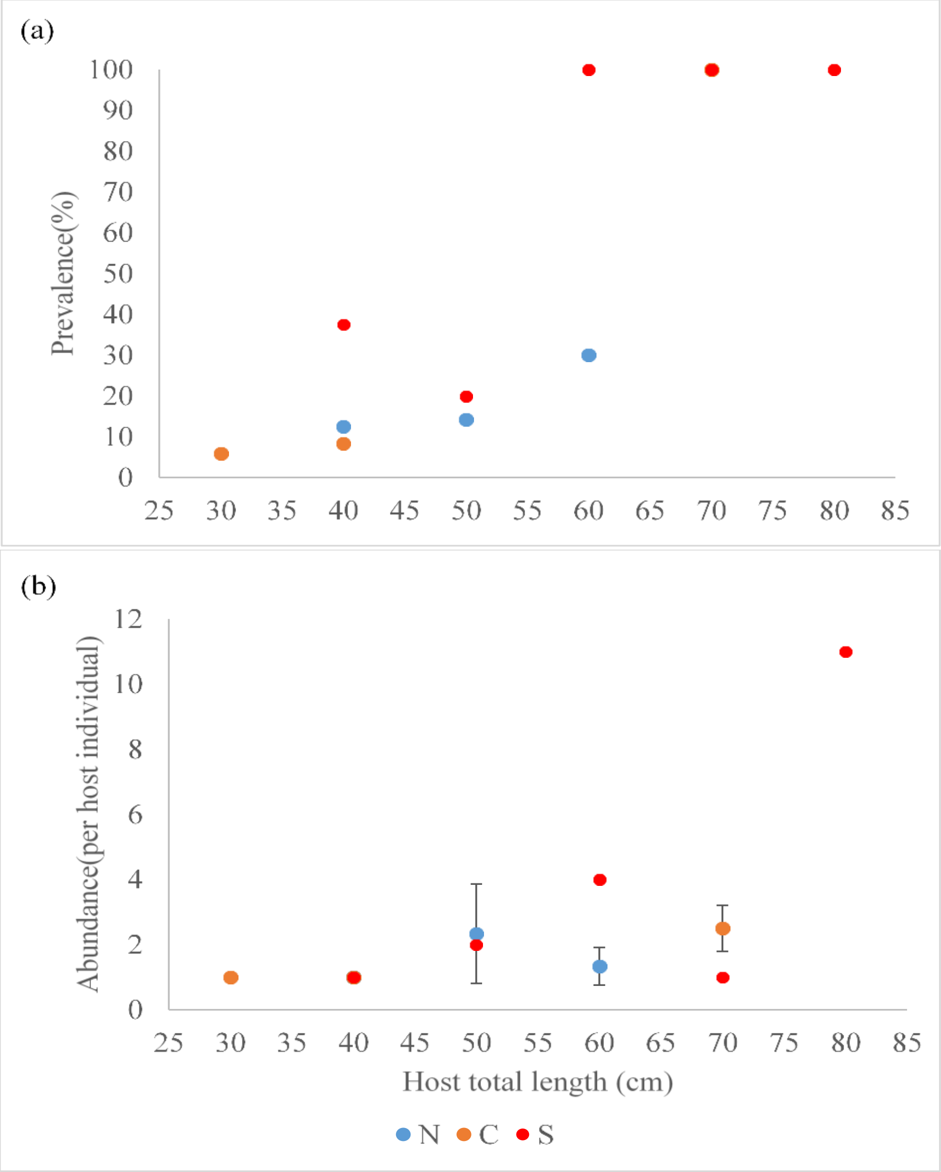


Figure 25: (a) Prevalence (%) and (b) mean abundance (± one standard deviation) of *Hepatoxylon trichiuri* at each 10 cm total length class of *M. capensis* caught in northern Namibia (N) at 24.4˚S, central Namibia (C) at 26.4˚S and the southern Benguela (S) from the west coast of South Africa between 31 and 34˚S. (No *H. trichiuri* were found on *M. capensis* from the South Africa East coast. Standard deviations could not be calculated for length classes that had only one fish present, hence no error bars for these points.)

Stephanostomum multispinosum indicated no relationship between prevalence of infection or abundance and host total length (Figure 26). *S. multispinosum* was most abundant in medium-sized hosts between 40 and 60 cm in the southern Benguela (Figure 26b).

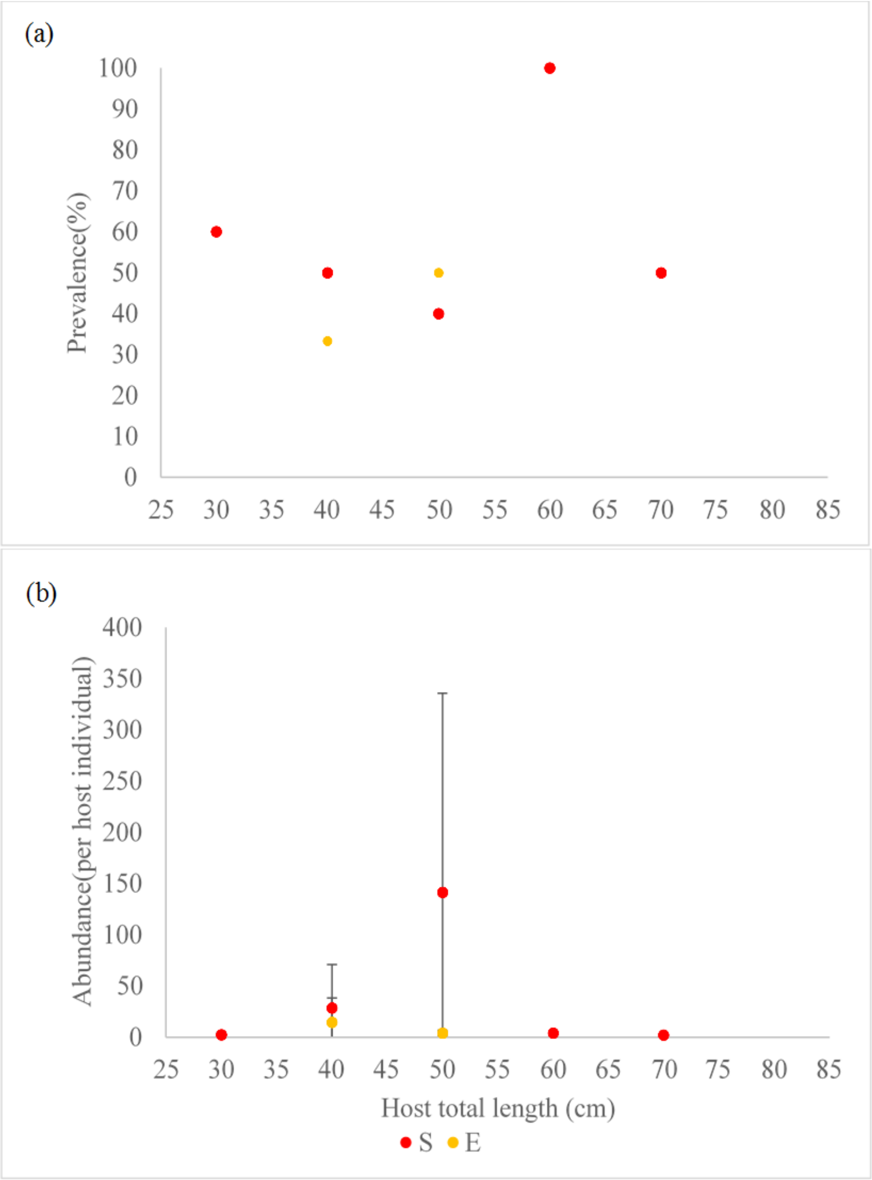


Figure 26: (a) Prevalence (%) and (b) mean abundance (± one standard deviation) of *Stephanostomum multispinosum* at each 10 cm total length class of *M. capensis* caught in the southern Benguela (S) between 31 and 34˚S and South African East coast (E) between 25.1 and 26.7˚E. (No *S. multispinosum* were found on *M. capensis* from the northern and central Benguela. Standard deviations could not be calculated for length classes that had only one fish present, hence no error bars for these points)

Corynosoma sp. indicated no relationship between prevalence of infection or abundance and host total length (Figure 27).

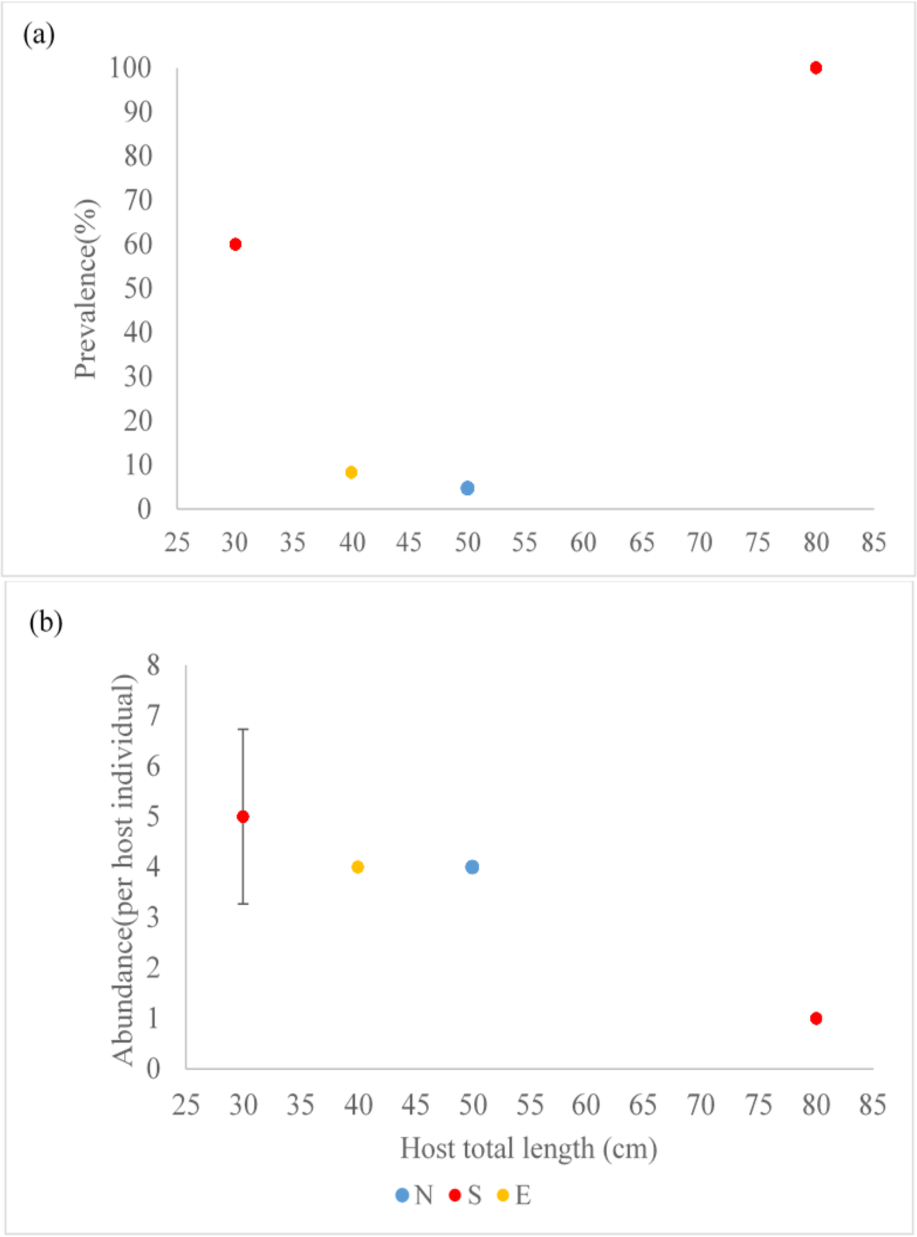


Figure 27: (a) Prevalence (%) and (b) mean abundance (± one standard deviation) of *Corynosoma* sp. at each 10 cm total length class of *M. capensis* caught in the northern Benguela (N) at 24.4, southern Benguela between 31 and 34˚S and South African East coast (E) between 25.1 and 26.7˚E. (No *Corynosoma* sp. were found on *M. capensis* from the central Benguela. Standard deviations could not be calculated for length classes that had only one fish present, hence no error bars for these points).

CHAPTER 4: DISCUSSION

4.1. Otolith shape analysis

Otolith morphometrics and otolith mean shape results for the years 1992, 2004 and 2005 indicated a single stock in the northern and central Benguela, with similar otolith shapes between the northern and central Benguela, but with significantly different shapes to the southern Benguela otoliths. This therefore indicated two stocks in the Benguela: one stock in the northern Benguela up to the Orange River, and another stock in the southern Benguela, separated at around 28˚S to 29˚S. Otoliths from the northern and central Benguela area were wider and shorter (with significantly wider otoliths, bigger area and longer perimeter in relation to otolith length) compared to the southern Benguela otoliths. Southern Benguela otoliths were longer and narrower with a significantly lower roundness, higher ellipticity, perimeter/area and aspect ratio in relation to otolith length. Differences in shapes (width, area, perimeter, roundness, ellipticity, perimeter/area and aspect ratio) between the southern Benguela and the rest increased with age.

There are two factors that account for regional similarity in otolith shapes. Firstly, it could be due to a high population mixing (genetic/phenotypic effects) and secondly, homogenous factors (environmental effects) might have affected the shapes of otoliths with no mixing involved (Hamer et al. 2012).

These results are complementary to the migration, distribution and population stock structure study (Jansen et al. 2016) that observed regional differences in distributions of *M. capensis* older than 2-years-old. The identification of two stocks of *M. capensis* in this study also confirmed results of genetic analysis of *M. capensis* by Henriques et al. (2016), who also found a single stock of *M. capensis* in the northern and central Benguela and two genetically different stocks in the Benguela. Samples of *M. capensis* used by Henriques et al. (2016) were collected in Namibia from northern Namibia 17˚S to 26˚S (northern Benguela in this study) and in southern Namibia 26˚S to 29˚S (central Benguela) and in South Africa on the West coast from 29˚S to 34˚S (southern Benguela), Southwest coast 18˚E to 20˚E and South coast 20˚E to 26˚E (East coast). The Orange River area around 28˚S to 29˚S, was observed as the mixing zone and border for Namibia and South African *M. capensis* (Henriques et al. 2016), complementary to Jansen et al. (2016) who also found adult aggregations in this area. It is possible that *M. capensis* from the northern and southern Benguela mix at age 1 (and younger) at the Orange River area, since the regional differences of otolith shapes increased with age from 2-year-old *M. capensis*.

Southern Benguela 3-year-old *M. capensis* had lower otolith width, area and perimeter, and because of this southern otoliths tended to be less round and narrower, with a higher ellipticity, perimeter/area and aspect ratio compared to the northern and central Benguela fish. Ellipticity is an indication of how narrow an otolith is, perimeter divided by area indicates how lobed the otolith edge can be (Almeland 2014) and aspect ratio shows how elongated the otolith shape is. These otolith morphometric differences between the northern and southern stock were similar to the differences observed between the northern and southern stocks of silver hake *Merluccius bilinearis* (Bolles and Begg 2000). Northern silver hake had slower growth rates, hence their otoliths were longer (similar widths), greater in area and perimeter (age 1 and 2 years) compared to the southern stock otoliths, and southern stock otoliths were rounder. In this study, the northern Benguela *M. capensis* had a higher otolith area and perimeter, but were wider and rounder compared to their length than the southern Benguela *M. capensis*. The northern *M. capensis* probably grow at a faster rate (larger fish length at the same age) than the southern ones (Wilhelm 2012; Jansen et al. 2016; Wilhelm et al. 2017) and therefore overall have larger otoliths compared to the southern Benguela *M. capensis*. Differences in otolith growth rates and therefore otolith shapes between the northern and southern Benguela are probably a result of combination of genetically different stocks (Henriques et al. 2016) and long-term environmental differences (Hutchings et al. 2009) observed between the northern and southern Benguela. The northern Benguela is a typical cold temperate wind-driven upwelling system and the southern Benguela is a temperate shelf system (Hutchings et al. 2009).

Henriques et al. (2016) stressed that different upwelling events in the northern and southern Benguela could be responsible for the high abundances of northern *M. capensis* reported off the southern Benguela (west coast) during its upwelling season (summer-autumn), which is pronounced by low oxygen water. This is perceived to be in favour of the northern *M. capensis* because they are more tolerant to low oxygen water. Northern Benguela *M. capensis* seasonally migrate to the southern Benguela during summer, and southern Benguela *M. capensis* migrate to the northern Benguela during winter (Kapula 2017).

Different life history characteristics have been outlined by several studies between *M. capensis* from the northern and southern Benguela (Gordoa et al. 1995; Jansen et al. 2015; Salvanes et al. 2015; Henriques et al. 2016), which could account for the differences in the otolith shapes between the two areas. A study on gonadosomatic indices revealed different spawning periods between the northern and southern Benguela (Jansen et al. 2015). *M. capensis* in the northern Benguela spawn mainly in winter and southern Benguela *M. capensis* are regarded as summer spawners. Otolith shape variations witnessed in five species (*Hoplostethus atlanticus, Hoplostethus mediterraneus, Paratrichichthys trailli, Pagrus major* and *Trachurus murphyi*) indicated that otolith complexity and life history are related (Gauldie and Crampton 2002). Gauldie and Jones (2000) stated that variations in otolith shapes within a species could be a consequence of different growth rates, therefore illustrating biological variability. Argentine hake *Merluccius hubbsi* was distinguished into three stocks based on their life history parameters. The *M. hubbsi* stock in the San Jorge Gulf was distinguished based on morphometric measurements and growth attributes (Perez 1990) and parasite infestation (Sardella and Timi 2004). The *M. hubbsi* stock in the San Matias Gulf was identified based on its spawning aggregation and complete life cycle in that area (Di Giácomo et al. 1993) and parasite infestation (Sardella and Timi 2004).

The differences in otolith shape between the northern and southern Benguela also correspond with Gordoa et al. (1995), who noted morphological differences between *M. capensis* north and south of the Orange River. North of the Orange River *M. capensis* have a white anal fin, whereas those south of the Orange River have grey anal fins with black edges. Namibian and South African *M. capensis* also have different iris colours (Durholtz et al. 2015). There is a permanent cold water body at the Orange River, therefore this area has been declared as the mixing zone and gene barrier between the northern and southern *M. capensis* (Henriques et al. 2016). Depth is another environmental factor that is responsible for otolith differences (Cardinale et al. 2004; Marengo et al. 2017) and Namibian *M. capensis* older than 2.5 years were caught at deeper depths compared to the South African *M. capensis* of the same age (Jansen et al. 2016).

Different environments with different sounds cause different otolith morphometrics and otolith shape variations, which are adopted to enhance fish survival (Gauldie and Crampton 2002). *M. capensis* from the southern Benguela are most abundant at the Agulhas Bank (von der Heyden et al. 2007; Henriques et al. 2016). This area is characterized with seasonal upwelling and low oxygen areas (Monteiro et al. 2008). Different seasonal peaks of phytoplankton blooms have been detected in the northern Benguela (winter-spring) and southern Benguela (summer-autumn) (Hutchings et al. 2009). These environmental differences between the northern and southern Benguela could also account for the differences in otolith morphometrics. Equally, environmental differences between the Patagonian Shelf and South Sandwich Islands were also responsible for otolith shape variability between the Pantagonian toothfish (*Dissostichus eliginoides*) and Antractic toothfish (*D. mawsoni*) (Lee et al. 2017). Otolith differences between *M. gayi* from Chile and Peru, and between *M. merluccius* from the Atlantic and Mediterranean were also a result of environmental differences (Torres et al. 2000). Spawning at different latitudes were discovered to have a prominent effect on the genetic differences on *M. capensis* from Namibia and South Africa (Henriques et al. 2016). Genetic differences also probably accounted for significant differences between otolith shapes from northern Benguela and southern Benguela. Classification of individuals between years displayed a single grouping, thus indicating temporal stability of otolith shape among areas in the three years examined.

Stocks that are not homogenous and entirely isolated from each other react to environmental conditions and exploitation differently (Begg and Brown 2000; Marengo et al. 2017). Otolith shape analysis results from this study indicate that two different hake stock strutures exist in the Benguela, hence these different stock units should be managed differently.

Otolith morphology can be used to observe interactions among groups of fishes (Gauldie and Crampton 2002). In this present study, otolith shape analysis did not show any differences between the northern and central Benguela stocks although a spawning and nursery study on *M. capensis* in the northern Benguela concluded that there are two spawning aggregations in the northern Benguela, one aggregation in northern Namibia and another in southern Namibia ("central Benguela" in the present study) (Wilhelm et al. 2015b). This present study confirms that both aggregations are entirely contained in the northern Benguela with a closed life cycle from adults to nursery areas (Wilhelm et al. 2015b; Jansen et al. 2016), and are morphometrically similar to each other. According to Wilhelm et al. (2015b) and Jansen et al. (2016), northern Benguela 1-year-old *M. capensis* (juveniles) were mostly located around Walvis Bay and the Orange River, their place of origin, corresponding to the presence of adult *M. capensis* that usually aggregate in these areas from 5- to 9-years-old *M. capensis*. *M. capensis* that were 2- and 3-years-old mostly move away from their place of origin (Jansen et al. 2016) in central Namibia (Walvis Bay) to northern Namibia, whilst the southern Namibia *M. capensis* migrate to central Namibia (Wilhelm et al. 2015b). Otolith shapes of these fish are probably influenced by the environments from the age of 2 years. Both the northern and central 2- and 3-years-old *M. capensis* were influenced by the northern Benguela environment, influenced by the Luderitz cell (at about 26˚S), which could explain their similar otolith shapes (Wilhelm et al. 2015b).

Begg and Brown (2000) suggested that otolith shape characteristics for stock discrimination have to be recalculated for each major age group. Classification of *M. capensis* individuals between age groups indicated two groupings, a southern Benguela stock different from the northern and central Benguela stock. Accurate classification of otolith morphometrics increased with age group in this study. Variations between age groups observed in otolith shape development appear to be influenced by genetics (Hüssy 2008). Bolles and Begg (2000) reported that accurate classification decreased with age group in silver hake (*M. bilinearis*) and Hamer et al. (2012) gave a similar report about blue grenadier *Macruronus novaezelandiae*, further stating that these is because younger fish are more sensitive to the environment.

4.2. Parasite species of *M. capensis* in the northern and southern Benguela

Results presented in the present study rejected the null hypothesis of no significant differences in infection level of parasites between the northern and central Benguela and between the northern and southern Benguela *M. capensis* and concluded that there are significant differences in *Chondracanthus merluccii* abundances between the northern and central Benguela, and northern and southern Benguela with a possible border at 25˚30 S based on the high prevalence and abundance of *C. merluccii* in the northern Benguela as compared to the central and southern Benguela. The following parasites have the potential to be used as biological tags (1) *C. merluccii*, which indicated significant differences in abundance between the northern and central Benguela, and the northern and southern Benguela hosts, (2) *Parabrachiella australis,* which was only present in northern and central Benguela hosts, (3) *Stephanostomum multispinosum*, which was only present in southern Benguela hosts.

Reimer (1993) suggested the presence of two *M. capensis* stocks in the Namibian waters separated by a border at 25˚30 S based on the absence of *Brachiella merluccii* and also the significantly higher prevalences of *Scolex pleuronectis* and *Leptotheca* sp. south of this border. *B. merluccii* is also called *Parabrachiella merluccii* (WoRMS 2008). *S. pleuronectis* and *Leptotheca* sp., were, however, not observed in the present study or by Cruickshank (2017).

The prime cause of parasite differences is dissimilar oceanographic conditions (Marengo et al. 2017). The northern Benguela is influenced by the upwelling cell off Luderitz and the Angola-Benguela front, whilst, the southern Benguela is influenced by the upwelling cell off Cape Point and the East coast is influenced by the Agulhas Bank (Hutchings et al. 2009). Sardella and Timi (2004) found three distinctive Argentine hake (*Merluccius hubbsi*) stocks based on parasite infestation levels and proposed that the varrying environmental conditions might have played a role in the different hake stocks observed since Argentine hake are influenced by the Malvis current, the Brazil current and shelf water.

This study revealed the presence of *C. merluccii* Holten (1802) in the northern and central Benguela, with a mean infection intensity of ≤ 2 in both areas and low mean parasite abundance ≤ 0.5 in the northern, central and southern Benguela. Barnard (1955) also identified *C. merluccii* on South African *M. capensis*. *C. merluccii* have not been identified by Botha (1986), Krzeptowski (1980) and Reimer (1993) on South African and

Namibian *M. capensis*. Grabda and Soliman (1975) also found *C. merlucci* in other *Merluccius* species such as *M. bilinearis* from Atlantic coast, *M. senegalensis* from Mauretania and *M. mediterraneus* from the Meditarrenean sea.

Copepods *C. merluccii* that are parasitic on fish have a single host life-cycle (Paterson and Poulin 1999). Females produce egg sacs, which hatch into nauplii. These nauplii then develop into copepods that infect hosts after contact (Paterson and Poulin 1999). *C. merluccii* revealed a positive relationship between both the prevalence of infestation and mean abundance and *M. capensis* total length in the present study. *C. merluccii* is an ectoparasite that was usually found in the mouth of *M. capensis*. Infection of *M. capensis* with *C. merluccii* probably takes place when the hosts open their mouths during feeding. Large-sized hosts have bigger mouths with a larger surface area, thus take in more water and come into contact with free-living ectoparasites, hence accumulating more of these parasites during this process than smaller-sized hosts. Significant differences in *C. merlucii* abundances were observed between the northern and central Benguela, and northern and southern Benguela and, northern Benguela and East coast. These results suggest that the northern Benguela *M. capensis* only are exposed to the endemic area of *C. merluccii,* different from the central Benguela and also different from the southern Benguela *M. capensis*. The sampling period in the central and southern Benguela both took place in summer (March and January), during the period that northern Benguela *M. capensis* usually migrate seasonaly to the southern Benguela (Kapula 2017). This means that *M. capensis* caught in the central and southern Benguela area probably never migrated into the far northern Benguela to get infected by *C. merluccii*, which confirms the presence of two distinct geographically separated stocks. *C. merluccii* are about 10 mm in total length and can be identified with a naked eye. MacKenzie and Abaunza

(1998) stressed that parasites that are suitable as biological tags (1) indicate significantly different levels in different areas, (2) with a single-host life cycle (3) can be easily identified and (4) their presence does not lead to host mortality. This indicates that *C. merluccii* could serve as a biological tag of *M. capensis* because of fulfilling all the above mentioned criteria.

P. australis Wilson (1923), a parasitic copepod, was only observed in *M. capensis* from the northern and central Benguela. *P. australis* probably gets attached on *M. capensis* gills and mouth through gaseous exchange because they are free-roaming ectoparasites. *P. australis* revealed an increase in prevalence of infection and abundance with host total length in northern and central Benguela *M. capensis*, which is in agreement with Krzeptowski's (1980) findings. Large-sized hosts have bigger gills with a larger surface area, which provides enough space for *P. australis* to inhabit.

P. australis have one direct life cycle and their presence does not lead to host mortality and can be easily identified, hence these characteristics make them good biological tags (MacKenzie and Abaunza 1998). MacKenzie and Abaunza (1998) stated that parasites with one direct life cycle like *P. australis* require suitable environmental conditions. Hutchings et al. (2009) portrayed the differing environmental conditions in the northern, central and southern Benguela. In this case the northern and central Benguela might have favourable environmental conditions suitable for *P. australis* to thrive in, unlike the southern Benguela. Reimer (1993) also found *Brachiella merluccii* (also known as *Parabrachiella merluccii*) only present in the northern Namibia (northern Benguela) *M. capensis*, similar to *P. australis* found in the present study only present in the northern and central Benguela.

Botha (1986) reported very low prevalence of infection of *P. australis* off South Africa, (generally less than 3%), while their infection intensity was ≤ 0.002. The very low infection intensity of *P. australis* observed off South Africa could indicate that these few *M. capensis* only got infected with *P. australis* after they had been in the endemic area of *P. australis*. Henriques et al. (2016) observed the northern Benguela *M. capensis* genotype off the South African coast, while Kapula (2017) observed the seasonal migration of the northern Benguela *M. capensis* genotype to the southern Benguela during summer (February to March), whereas, the southern Benguela *M. capensis* genotype migrated to the northern Benguela during winter (June to August). It is through these occasional migrations that southern Benguela *M. capensis* could have acquired the low levels of infection of *P. australis* as reported by Botha (1986).

The trematode *Stephanostomum multispinosum* Manter (1940) was only present in *M. capensis* caught in the southern Benguela and East coast in the present study. *S. multispinosum* indicated no relationship between prevalence of infection or abundance with host total length. The first intermediate hosts of *S. multispinosum* are gastropods and their second intermediate hosts are other fish species (Bray et al. 2005). Piscivorous fish like *M. capensis* are the definite hosts who acquire them after ingesting the second intermediate hosts. Crustaceans feed on small gastropods and species such as horse mackerel and young *M. capensis* feed on crustaceans. Large *M. capensis* prey on horse mackerel and young *M. capensis* (Durholtz et al. 2015) which is how *M. capensis* would get infected with *S. multispinosum. S. multispinosum* was only present in the southern Benguela *M. capensis* but absent from the northern and central Benguela *M. capensis*, which indicate that it is endemic to these areas, and could serve as a potential biological tag.

However, Reimer (1993) revealed the presence of infection of *S. multispinosum* on *M. capensis* and *M. paradoxus* off the Namibian coast with prevalence of infection of 14.5% and their infection of intensity of 0.17. Reimer's (1993) study did not provide the sampling period so comparisons of seasonality could not be made with the present study.

P. australis and *S. multispinosum* could be seasonal species because *P. australis* only occurred in the northern and central Benguela (summer and spring), whereas *S. multispinosum* only occurred in the southern Benguela (winter and spring) in this study and should be re-sampled seasonally for confirmation.

Anisakis sp. is a commonly found nematode, mostly found infesting the liver, body cavity, the inside of the stomach, along the stomach lining and intestines. It was previously reported to be one of the dominating species in *M. capensis* and *M. paradoxus* (Krzeptowski 1980; Botha 1986; Reimer 1993), *Merluccius gayi gayi* (Oliva and Ballon 2002) as well as in more than 80 fish and mammalian hosts worldwide (Colón-Llavina et al. 2009). *Anisakis* sp. prefer cold waters (Smith and Wootten 1978), which explains why this study revealed a high prevalence of *Anisakis* sp. in the cold Benguela current system (Hutchings et al. 2009). *Anisakis* sp. were the most abundant parasite species in *M. capensis* observed in the northern and southern Benguela and were found present in almost all host total length classes. The abundance of *Anisakis* sp. increased with host total length, which is a consequence of the feeding habits of their hosts. Young *M. capensis*' diet is composed of euphausiid crustaceans, which are intermediate hosts of *Anisakis* sp., whilst older hosts have a primarily cannibalistic diet (Wilhelm et al. 2015a), thus accumulating more *Anisakis* sp. (Krzeptowski 1980; Botha 1986). As a result, older *M. capensis* are associated with a high abundance of *Anisakis* sp. (Krzeptowski 1980;

Reimer 1993). Sardella and Timi (2004) reported that parasite populations of the Argentine hake were determined by the diet composition. *Merluccius hubbsi* hosts from Patagonian waters had the highest parasite infestation levels because their main prey was anchovy *Engraulis anchoita* as compared to *Merluccius hubbsi* from Argentine-Uruguayan waters that fed more on crustaceans (Sardella and Timi 2004). Hence parasite abundance for some fish species is greatly determined by the type of prey in the host's diet (Sardella and Timi 2004; Gauldie and Jones 2008; Marengo et al. 2017), which is also the case with *M. capensis*. Similarly, *Anisakis* sp. indicated a positive and significant relationship between infection of prevalence and host total length in Chilean hake *Merluccius gayi gayi* (Oliva and Ballon 2002).

A genetic study of seven *Anisakis* sp. was done on European hake from Atlantic and Mediterranean waters, suggesting the existence of different stocks of *Merluccius merluccius* (Mattiucci et al. 2004). *Anisakis* sp. have specific characteristics of an ideal parasite that can be used as a biological tag as described by McKenzie and Abaunza (1998): they inhabit the host for a long period, their infection level remains constant from year to year, they can be easily identified and their presence does not lead to host mortality. Cantabrian Sea and Galalcian waters, two areas that were considered to be part of the south stock were found with significant levels of *Anisakis* sp. infestation (Abaunza et al. 2008). Le Roux (2013) acknowledged separate stocks of *Trachurus capensis* in the northern and southern Benguela based on significant differences between *Anisakis* sp. Despite the outstanding features of *Anisakis* sp. that makes it a potential biological tag, this study indicated similar abundance levels of *Anisakis* sp. in the Benguela. Hence this study does not identify *Anisakis* sp. as a potential biological tag in the Benguela. *Anisakis* sp. can be used as biological tags in the case of the South African East coast stock, however, as the abundance of *Anisakis* sp. indicated significant differences between the southern Benguela *M. capensis* and East coast *M. capensis*. This feature is adequate to assume different *M. capensis* stocks between the southern Benguela and East coast.

Reimer (1993) and Oliva and Ballon (2002) also recorded *A. merluccii* Van Beneden and Hesse (1863) in *M. capensis* off Namibia, a monogenean that thrives in fish gills. *A. merluccii* was recorded in the northern, central and southern Benguela in this study. Northern, central and southern Benguela *M. capensis* showed similar abundance patterns of *A. merluccii* in young fish with a decrease in abundance in fish >60 cm. On the contrary, Reimer (1993) shows that *A. merluccii* increased with age in *M. capensis*. However, Reimer (1993) showed that *A. merluccii* decreased in infestation level with age in *M. paradoxus* because as this parasite seems to be less abundant at lower depths. *M. capensis* mostly move deeper as their size increases due to food availability, light, salinity and inter / intra species competition (Wilhelm et al. 2015a). This may help explain why the *A. merluccii* prevalence of infection decreased with fish length in the present study as *M. capensis* migrate deeper with increased length. Cantatore et al. (2016) observed that *K. rosenbuschi* was more prevalent and abundant in *Merluccius hubbsi* juveniles compared to adults and that the decrease in prevalence and abundance with host age could be because older fish are more resistant to certain parasites because of their fully developed immune responses as compared to the younger ones (Cantatore et al. 2016). This could also be the case with *A. merluccii* in *M. capensis*. Another possible explanation of decreased levels of *A. merluccii* in larger *M. capensis* could be the selective (fishing) mortality of heavily infected hosts, in this case the older fish.

There were no significant differences in abundance levels of *A. merluccii* between the areas in the Benguela. On the other hand, significant differences in *A. merluccii* were observed between *M. capensis* from the northern Benguela and East coast, and central Benguela and East coast and southern Benguela and East coast. The life history information of *A. merluccii* is lacking (Oliva and Ballon 2002). However, *A. merluccii* have previously been used to discriminate the Chilean hake *Merluccius gayi gayi* (Oliva and Ballon 2002). A parasite study on *Merluccius australis* and *Merluccius hubbsi* in the waters of Falkland Islands, southern Chile and Argentina also suggested that *A. merluccii* could be a potential biological tag for *M. autralis*. Therefore, *A. merluccii* could potentially be used to discriminate between the Benguela *M. capensis* and East coast *M. capensis*.

The low infection intensity of *H. trichiuri* in *M. capensis* in the present study was confirmed by Krzeptowski (1980), Botha (1986) and Reimer (1993). This cestode could easily be confused as a fish muscle due to its white colour. *H. trichiuri* can stretch to about 60 to 90 mm (Reimer 1993). The life cycle of *H. trichiuri* is such that, the egg hatches in the water and the ciliated coracidium is ingested by a copepod crustacean, which then develops into a procercoid larva. Ingestion of the crustacean by a teleost leads to the larva being passed in the coelomic cavity, where it then develops into a post-larval stage (Botha 1986). In this case, small *M. capensis* would be infected as they feed on copepods and accumulate infections over their lifetime as, medium sized hosts between 50 cm and 60 cm had higher abundances compared to the younger hosts. *H. trichiuri* were present in all ages and their abundances increased with host total length in this study. However, Krzeptowski (1980) and Botha (1986) both reported no relationship between the abundace of *H. trichiuri* and host total length. Krzeptowski (1980) observed *H. trichiuri* absent in 1 and 5 year old hosts.

H. trichiuri was only observed off the southern Benguela but not off the East coast. This could mean that *M. capensis* off the southern Benguela and East coast are separate stocks, or at least phenotypically, as is the case with South African sardine (*Sardinops sagax*) (Ukomadu 2017), although Henriques et al. (2016) only found one genetic stock of *M. capensis* off South Africa. Future studies with more *M. capensis* samples could consider *H. trichiuri* useful in stock structure discrimination studies for several reasons such that: they are long lived and can provide useful information about the fish diet (Nunkoo 2015). *H. trichiuri* have also been identified as a useful biological tag in the Chilean hake *Merluccius gayi gayi* (Oliva and Ballón 2002) and also in hakes in the Falkland Islands *M. australis* and *M. hubbsi* (MacKenzie and Longshaw 1995). *H. trichiuri* could also be potentially used as a potential biological tag to distinguish between the Benguela *M. capensis* and East coast *M. capensis*.

Corynosoma sp. larvae, belonging to the phylum Acanthocephala were recorded on the walls of the stomach, liver and other organs of the abdominal cavity of *M. capensis* by Reimer (1993). Acanthocephala have an indirect life cycle, where the fish serves as the final host and the crustacean as the first host (Nunkoo 2015). *M. capensis* juveniles usually feed on euphausiids (Wilhelm et al. 2015b) which could explain the presence of *Corynosoma* sp. in *M. capensis* hosts.

4.3. Conclusions and Recommendations

Southern Benguela otoliths were significantly less round, longer, narrower and more elongated with a higher ellipticity, perimeter/area and aspect ratio compared to the northern Benguela otoliths, confirming previous genetic results (Henriques et al. 2016). The wavelet method proved to be an effective method that can be succesfully used in discriminating stock structures in this study (Libungan and Pálsson 2015). Using otolith shape provides a more efficient way of stock discimination than genetic analyses and can be applied in the future to establish the degree of mixing between two stocks.

Most of the parasite species found on *M. capensis* in the present study have previously been reported by other studies (Krzeptowski 1980; Botha 1986; Reimer 1993). *C. merluccii*, *A. merluccii*, *Anisakis* sp. and *H. trichiuri* were all found in the northern, central and southern Benguela. *C. merlucii* indicated significant differences in abundance between the northern and central Benguela suggesting two stocks of *M. capensis* in the Namibian waters, and two stocks in the Benguela; one in the northern and one in the central and southern Benguela separated by a border at 25˚30 S. *P. australis*, was only present in the northern and central Benguela while *S. multispinosum*, was only present in the southern Benguela.

Otolith shape analysis and parasite structure confirmed the presence of one *M. capensis* stock in the northern Benguela (including the Orange River area) and another stock in the southern Benguela. The two complementary methods prove to be uselful in the investigation of stock structure of *M. capensis* in the Benguela. Ideally, sampling for these methods should be combined, together with genetic sampling for future studies for *M. capensis* as well as *M. paradoxus* in the Benguela.

Previous genetic results (Henriques et al. 2016) and spawning aggregation studies (Jansen et al. 2016) and the present otolith morphometrics analysis and parasite analysis all suggest two stocks of *M. capensis* in the Benguela, which are always moving and mixing to some degree, thus probably reacting to environmental conditions and exploitation differently. This means that the northern Benguela (Namibia) and southern Benguela (mainly South Africa) *M. capensis* stocks comprise of sufficiently different phenotypic (morphometry) and life history (growth rates) to be modelled as separate units for stock assesment and management purposes.

This research shows that otolith shape analysis and parasite infestation could be used to determine the degree of mixing of the two *M. capensis* stocks in the Benguela at the Orange River/border area. These two methods are less expensive and require less time and effort than genetic analyses, and would therefore be useful alternatives for genetic analyses. Otolith shape analysis and parasite infestation analysis also have the potential for describing stock structure of other species to ensure the sustainable management of fisheries in the Benguela. Successful discrimination of stocks using otolith shape analysis and parasite infestation could also reveal different life histories, which could be used in the formulation of conservation management measures of overexploited species.

REFERENCES

Abaunza P, Murta AG, Campbell N, Cimmaruta R, Comesaña AS, Dahle G, Magoulas A. 2008. Stock identity of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean Sea: Integrating the results from different stock identification approaches. *Fisheries Research* 89: 196-209.

Almeland OW. 2015. Phenotypic plasticity in Atlantic herring (*Clupea harengus*) juveniles reared at two different salinities. Masters thesis, The University of Bergen, Norway.

Anderson MJ, Willis TJ. 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology* 84: 511-525.

Assorov VV, Berenbeim DY. 1983. Spawning grounds and cycles of Cape hakes in the Southeast Atlantic. *Collection of Scientific Papers of the International Commission for Southeast Atlantic Fisheries* 10: 27-30.

Arkhipkin AI, Laptikhovsky VV, Barton AJ. 2015. Biology and fishery of common hake (*Merluccius hubbsi*) and southern hake (*Merluccius australis*) around the Falkland/Malvinas Islands on the Patagonian Shelf of the Southwest Atlantic Ocean. In Arancibia H (ed), *Hakes: Biology and Exploitation*, Oxford, UK: Wiley Blackwell. pp 154-184.

Baquero E, Rubio M, MOURA IN, Pieniazek NJ, Jordana R. 2005. Myosporidium merluccius ng, n. sp. infecting muscle of commercial hake (*Merluccius* sp.) from fisheries near Namibia. *Journal of Eukaryotic Microbiology* 52: 476-483.

Barnard H. 1955. *South African parasitic Copepoda*. South African Museum.

Bartholomae CH, van der Plas AK. 2007. Towards the development of environmental indices for the Namibian shelf, with particular reference to fisheries management. *African Journal of Marine science* 29: 25-35.

BCC. 2012. Status of the Fishery Resources in the Benguela Current Large Marine Ecosystem Report No. 3. Swakopmund, Namibia: Benguela Current Commission.

Begg GA, Brown RW. 2000. Stock identification of haddock *Melanogrammus aeglefinus* on Georges Bank based on otolith shape analysis. *Transactions of the American Fisheries Society* 129: 935-945.

Begg GA, Overholtz WJ, Munroe NJ. 2001. The use of internal otolith morphometrics for identification of haddock (*Melanogrammus aeglefinus*) stocks on Georges Bank. Fishery Bulletin 99: 1.

Begg GA, Waldman JR. 1999. An holistic approach to fish stock identification. *Fisheries research* 43: 35-44.

Bolles KL, Begg GA. 2000. Distinction between silver hake (*Merluccius bilinearis*) stocks in US waters of the northwest Atlantic based on whole otolith morphometrics. *Fishery Bulletin* 98: 451-451.

Botha L. 1986. Major endoparasites of the Cape hakes *Merluccius capensis* and *M. paradoxus,* with brief notes on some conspicuous ectoparasites. *South African Journal of Marine Science* 4: 45-49.

Bray RA, Webster BL, Bartoli P, Littlewood DTJ. 2005. Relationships within the Acanthocolpidae Lühe, 1906 and their place among the Digenea. *Acta Parasitologica*: 281-291.

Burmeister LM. 2001. Depth-stratified density estimates and distribution of the Cape hake *Merluccius capensis* and *M. paradoxus* off Namibia deduced from survey data, 1990–1999. *South African Journal of Marine Science* 23: 347-356.

Bush AO, Lafferty KD, Lotz JM, Shostak AW. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of Parasitology* 83: 575-583.

Cadrin SX, Friedland KD. 1999. The utility of image processing techniques for morphometric analysis and stock identification. *Fisheries Research* 43: 129-139.

Campana SE, Casselman JM. 1993. Stock discrimination using otolith shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 1062-1083.

Campana SE, Neilson JD. 1985. Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* 42: 1014-1032.

Cañás L, Stransky C, Schlickeisen J, Sampedro MP, Fariña AC. 2012. Use of the otolith shape analysis in stock identification of anglerfish (*Lophius piscatorius*) in the Northeast Atlantic. *ICES Journal of Marine Science* 69: 250-256.

Canning EU. 2001. Microsporidia. In: Gillespie S, Pearson RD (eds). *Principles and Practice of Clinical Parasitology*. John Wiley & Son Ltd, Chichester, UK. pp 171–195.

Cantatore DMP, Irigoitia MM, Holzer AS, Timi JT. 2016. Myxozoans as biological tags for stock identification of the Argentine hake, *Merluccius hubbsi* (Gadiformes: Merlucciidae). *Parasitology* 143: 732-740.

Cardinale M, Doering-Arjes P, Kastowsky M, Mosegaard H. 2004. Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* 61: 158-167.

Castonguay M, Simard P, Gagnon P. 1991. Usefulness of Fourier analysis of otolith shape for Atlantic mackerel (*Scomber scombrus*) stock discrimination. *Canadian Journal of Fisheries and Aquatic* Sciences 48: 296-302.

Colón-Llavina MM, Mignucci-Giannoni AA, Mattiucci S, Paoletti M, Nascetti G, Williams EH. 2009. Additional records of metazoan parasites from Caribbean marine mammals, including genetically identified anisakid nematodes. *Parasitology Research* 105: 1239.

Crawford RJM, Shannon LV, Pollock DE. 1987. The Benguela ecosystem. Part IV. The major fish and invertebrate resources. *Oceanography Marine Biology* 25: 252–505.

Cruickshank ML. 2017. Assessing the parasite assemblages of the South African Cape hakes, *Merluccius capensis* and *Merluccius paradoxus*. Honors thesis, University of Cape Town, South Africa.

De Vries DA, Grimes CB, Prager MH. 2002. Using otolith shape analysis to distinguish eastern Gulf of Mexico and Atlantic Ocean stocks of king mackerel. *Fisheries Research* 57: 51-62.

Di Giácomo EE, Calvo J, Perier MR, Morriconi E. 1993. Spawning aggregations of *Merluccius hubbsi*, in Patagonian waters: evidence for a single stock? *Fisheries research* 16: 9-16.

Duncombe Rae CD. 2005. A demonstration of the hydrographic partition of the Benguela upwelling ecosystem at 26° 40'S. *African Journal of Marine Science* 27:617-628.

Durholtz MD, Singh L, Fairweather TP, Leslie RW, van der Lingen CD, Bross CAR, Hutchings L, Rademeyer RA, Butterworth DS, Payne AIL. 2015. In: Arancibia H (ed), *Hakes: Biology and Exploitation*, Oxford, UK: Wiley Blackwell. pp 38-69.

Eliceiri KW, Berthold MR, Goldberg IG, Ibáñez L, Manjunath BS, Martone ME, Stuurman N. 2012. Biological imaging software tools. *Nature Methods* 9: 697.

Flick GJ, Hong GP, Hwang JW, Arganosa GC. 1990. Groundfish. In *The Seafood Industry*. Springer, Boston, MA. pp 32-66.

Gatica C, Neira S, Arancibia H, Vásquez S. 2015. The biology, fishery and market of Chilean hake (*Merluccius gayi gayi*) in the Southeastern Pacific Ocean. In

Arancibia H (ed), *Hakes: Biology and exploitation*, Oxford, UK: Wiley Blackwell. pp 126-153.

Gauldie RW. 1988. Tagging and genetically isolated stocks of fish: a test of one stock hypothesis and the development of another. *Journal of applied ichthyology* 4: 168-173.

Gauldie RW, Crampton JS. 2002. An eco-morphological explanation of individual variability in the shape of the fish otolith: comparison of the otolith of *Hoplostethus atlanticus* with other species by depth. *Journal of Fish Biology* 60: 1204-1221.

Gauldie RW, Jones JB. 2000. Stocks, or geographically separated populations of the New Zealand orange roughy, *Hoplostethus atlanticus*, in relation to parasite infestation, growth rate, and otolith shape. *Bulletin of marine science* 67: 949-971.

Gordoa A, Macpherson E, Olivar MP, Scharm W. 1995. Biology and fisheries of Namibian hakes (*M. paradoxus and M. capensis*). *In* Hake, Springer, Dordrecht. pp 49-88.

Grabda J, Soliman IAFM. 1975. Copepods-parasites of Genus *Merluccius* from the Atlantic Ocean and Mediterranean sea. *Acta Ichthyologica et piscatorial* 1: 31-40.

Grant WS, Becker II, Leslie RW. 1988. Evolutionary divergence between sympatric species of Southern African hakes, *Merluccius capensis* and *Merluccius paradoxus*.1. Electrophoretic analysis of proteins. *Heredity* 61: 13–20.

Grant WS, Garcia-Marin JL, Utter FM. 1999. Defining population boundaries for fishery management. *Genetics in Sustainable Fisheries Management* 1: 27-72.

Griffiths MH. 1996. Age and growth of South African Silver kob *Argyrosomus inodorus* (Sciaenidae), with evidence for separate stocks. *South African Journal of Marine Science* 17: 37-48.

Hamer PA, Kemp J, Robertson S, Hindell JS. 2012. Multiple otolith techniques aid stock discrimination of a broadly distributed deepwater fishery species, blue grenadier, *Macruronus novaezelandiae*. *Fisheries Research* 113: 21-34.

Hamukuaya H, O'Toole MJ, Woodhead PMJ. 1998. Observations of severe hypoxia and offshore displacement of Cape Hake over the Namibian shelf. *South African Journal of Marine Science* 19: 57–59.

Hare JA. 2005. The use of early life stages in stock identification studies. In: Cadrin SX, Friedland KD, Waldman JR (eds). *Stock Identification Methods. Applications in Fishery Science*. Elsevier, Amsterdam. pp 89–117.

Helser TE, Alade L. 2012. A retrospective of the hake stocks off the Atlantic and Pacific coasts of the United States: uncertainties and challenges facing assessment and management in a complex environment. *Fisheries Research* 114: 2-18.

Henriques R, von der Heyden S, Lipinski MR, Toit N, Kainge P, Bloomer P, Matthee CA. 2016. Spatio-temporal genetic structure and the effects of long-term fishing in two partially sympatric offshore demersal fishes. *Molecular Ecology* 25: 5843-5861.

Horn PL. 2015. Southern hake (*Merluccius australis*) in New Zealand: biology, fisheries and stock assessment. In: Arancibia H (ed), *Hakes: Biology and Exploitation*, Oxford, UK: Wiley. pp 101-125.

Hüssy K. 2008. Otolith shape in juvenile cod (*Gadus morhua*): Ontogenetic and environmental effects. *Journal of Experimental Marine Biology and Ecology* 364: 35-41.

Hutchings L, Van der Lingen CD, Shannon LJ, Crawford RJM, Verheye HMS, Bartholomae CH, Fidel Q. 2009. The Benguela Current: An ecosystem of four components. *Progress in Oceanography* 83: 15-32.

Jansen T, Kainge P, Singh L, Wilhelm M, Durholtz D, Strømme T, Erasmus V. 2015. Spawning patterns of shallow-water hake (*Merluccius capensis*) and deep-water hake (*M. paradoxus*) in the Benguela Current Large Marine Ecosystem inferred from gonadosomatic indices. *Fisheries Research* 172: 168-180.

Jansen T, Kristensen K, Kainge P, Durholtz D, Strømme T, Thygesen UH, Degel H, Wilhelm M, Fairwather TP, Paulus S, Lipinski MR, Beyer JE. 2016. Migration, distribution and population (stock) structure of shallow-water hake (*Merluccius capensis*) in the Benguela Current Large Marine Ecosystem inferred using as geostatistical population model. *Fisheries Research* 179: 156-167.

Johnsen E, Kathena JN. 2012. A robust method to separate Namibian commercial hake catches by species. A necessary step towards a biologically realistic hake stock assessment. *African Journal of Marine Science* 43: 43-53

Jónsdóttir IG, Campana SE, Marteinsdottir G. 2006. Otolith shape and temporal stability of spawning groups of Icelandic cod (*Gadus morhua* L.). *ICES Journal of Marine Science* 63: 1501-1512.

Kapula V. 2017. Seasonal transboundary movement of cape hake (*Merluccius capensis*) across the western coast of southern Africa. Masters thesis, University of Namibia, Namibia.

Kathena JN, Nielsen A, Thygesen UH, Berg CW. 2016. Hake species (*Merluccius capensis and M. paradoxus*) assessment in the Benguela Current Large Marine Ecosystem. *Environmental Development* 17: 193-201.

Kirchner CH, Kainge P, Kathena J. 2012. Evaluation of the status of the Namibian hake resource (*Merluccius* spp.) using statistical catch-at-age analysis. *Environment for Development Discussion Paper Series* 12: 1-52.

Krzeptowski M. 1980. Occurrence of larval nematode *Anisakis simplex*, larval cestode *Hepatoxylon trichiuri*, and parasitic copepod *Parabrachiella australis* in juvenile *Merluccius capensis* off Namibia. *Acta ichthyologica et piscatoria* 10: 35-44.

Le Roux JL. 2013. Parasite assemblages of Cape horse mackerel (*Trachurus capensis* Castelnau, 1861) from the northern and southern Benguela. Masters thesis, University of Cape Town, South Africa.

Lee B, Brewin PE, Brickle P, Randhawa H. 2017. Use of otolith shape to inform stock structure in Patagonian toothfish (*Dissostichus eleginoides*) in the south-western Atlantic. *Marine and Freshwater Research* 69: 1238-1247.

Legendre P, Oksanen J, ter Braak CJ. 2011. Testing the significance of canonical axes in redundancy analysis. *Methods in Ecology and Evolution* 2: 269-277.

Leguá J, Plaza G, Pérez D, Arkhipkin A. 2013. Otolith shape analysis as a tool for stock identification of the southern blue whiting, *Micromesistius australis*. *Latin American Journal of Aquatic Research* 41: 479-489.

Lester RJG. 1990. Reappraisal of the use of parasites for fish stock identification. *Marine and Freshwater Research* 41: 855-864.

Lett C, Veitch J, van der Lingen, CD, Hutchings L. 2007. Assessment of an environmental barrier to transport of ichthyoplankton from the southern to the northern Benguela ecosystems. *Marine Ecology Progress Series* 347: 247-259.

Libungan L A, Pálsson S. 2015. ShapeR: an R package to study otolith shape variation among fish populations. *PloS One* 10: e0121102.

Libungan LA, Slotte A, Husebø Å, Godiksen JA, Pálsson S. 2015. Latitudinal gradient in otolith shape among local populations of Atlantic herring (*Clupea harengus* L.) in Norway. *PloS One* 10: e0130847.

Lord C, Morat F, Lecomte-Finiger R, Keith P. 2012. Otolith shape analysis for three *Sicyopterus* (Teleostei: Gobioidei: Sicydiinae) species from New Caledonia and Vanuatu. *Environmental Biology of Fishes* 93: 209-222.

Lorenzo MI, Defeo O. 2015. The biology and fishery hake (*Merluccius hubbsi*) in the Argentinean-Uruguayan Common Fishing Zone of the Southwest Atlantic Ocean. *Hakes: Biology and Exploitation.* Oxford: Wiley Blackwell. pp 185-210.

MacKenzie K. 1999. Parasites as biological tags in population studies of marine fish. *Parasitology International* 47: 43.

MacKenzie K, Abaunza P. 1998. Parasites as biological tags for stock discrimination of marine fish: a guide to procedures and methods. *Fisheries Research* 3: 45–56.

MacKenzie K, Longshaw M. 1995. Parasites of the hakes *Merluccius australis* and *Merluccius hubbsi* in the waters around the Falkland Islands, southern Chile, and Argentina, with an assessment of their potential value as biological tags. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 213-224.

Mapp J, Hunter E, Van Der Kooij J, Songer S, Fisher M. 2017. Otolith shape and size: the importance of age when determining indices for fish-stock separation. *Fisheries Research* 190: 43-52.

Marengo M, Baudouin M, Viret A, Laporte M, Berrebi P, Vignon M, Marchard M, Durieux EDH. 2017. Combining microsatellite, otolith shape and parasites community analyses as a holistic approach to assess population structure of *Dentex dentex*. *Journal of Sea Research* 128: 1-14.

Mattiucci S, Abaunza P, Ramadori L, Nascetti G. 2004. Genetic identification of *Anisakis* larvae in European hake from Atlantic and Mediterranean waters for stock recognition. *Journal of Fish Biology* 65: 495-510.

MFMR. 1992. *Sea Fisheries Act* (*Act no.27 of 2000*). Government Gazette of the Republic of Namibia: 493.

Monteiro PMS, Van Der Plas AK, Melice JL, Florenchie P. 2008. Interannual hypoxia variability in a coastal upwelling system: Ocean–shelf exchange, climate and ecosystem-state implications. *Deep Sea Research Part I: Oceanographic Research Papers* 55: 435-450.

Musariri C. 2015. *Guide to Namibias Fisheries.* Windhoek, Namibia: John Meinert Printing.

Nunkoo MAI. 2015. A survey of the metazoan parasite assemblage of snoek*, Thyrsites atun* (Euphrasen, 1791*)*, off South Africa with an assessment of host-parasite relationships and potential biological tags. Masters thesis, University of Cape Town, South Africa.

Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson GL, Solymos P, Wagner H. 2008. The vegan package. *Community Ecology Package*. http://r-forge. rproject.org/projects/vegan/.

Oliva ME, Ballón I. 2002. Metazoan parasites of the Chilean hake *Merluccius gayi gayi* as a tool for stock discrimination. *Fisheries Research* 56: 313-320.

Olivar MP, Rubiés P, Salat J. 1988. Early life history and spawning of *Merluccius capensis* Castelnau in the northern Benguela current. *South African Journal of Marine Science* 6: 245-254.

Parisi-Baradad V, Lombarte A, García-Ladona E, Cabestany J, Piera J, Chic O. 2005. Otolith shape contour analysis using affine transformation invariant wavelet transforms and curvature scale space representation. *Marine and Freshwater Research* 56: 795-804.

Paterson AM, Poulin R. 1999. Have chondracanthid copepods co-speciated with their teleost hosts? *Systematic Parasitology* 44: 79-85.

Paterson B, Kirchner C, Ommer RE. 2013. A short history of the Namibian hake fishery a social ecological analysis. *Ecology and Society* 18: 66-75.

Payan P, De Pontual H, Bœuf G, Mayer-Gostan N. 2004. Endolymph chemistry and otolith growth in fish. *Comptes Rendus Palevol* 3: 535-547.

Payne AIL. 1989. Cape hakes. In: Payne AIL, Crawford RJM (eds). *Oceans of life off southern Africa*. Cape Town: Vlaeberg Publishers.

Perez CJA. 1990. Biology and distribution of the Argentine hake (*Merluccius hubbsi*) considerations on its stocks structure, migrations and dynamics of its nursery ground at San Jorge Gulf (Argentina). Masters thesis, University of Washington, United States.

Pillar SC, Wilkinson IS. 1995. The diet of Cape hake *Merluccius capensis* on the south coast of South Africa. *South African Journal of Marine Science* 15: 225-239.

Pitcher TJ, Alheit J (eds). 1995. What makes a hake? A review of the critical biological features that sustain global hake fisheries. Springer, Dordrecht. pp 1-14.

Popper AN, Lu Z. 2000. Structure–function relationships in fish otolith organs. *Fisheries Research* 46: 15-25.

Popper AN, Ramcharitar J, Campana SE. 2005. Why otoliths? Insights from inner ear physiology and fisheries biology. *Marine and Freshwater Research* 56: 497-504.

Preibisch S, Saalfeld S, Tomancak P. 2009. Globally optimal stitching of tiled 3D microscopic image acquisitions. *Bioinformatics* 25: 1463-1465.

Radhakrishnan KV, Li Y, Jayalakshmy KV, Liu M, Murphy BR, Xie S. 2012. Application of otolith shape analysis in identifying different ecotypes of Coilia ectenes in the Yangtze Basin, China. *Fisheries Research* 125: 156-160.

Rätz HJ. 1994. Assessment of the migration of Atlantic cod (*Gadus morhua* L.) between the stocks off West and East Greenland in 1984-86 by means of otolith typing. *Journal Northwest Atlantic Fisheries Sci*ence 16: 7-18.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/.

R Studio Team. 2016. RStudio: Integrated Development for R. [Online] RStudio, Inc., Boston, MA. http://www.rstudio.com/.

Reed C, MacKenzie K, van der Lingen CD. 2012. Parasites of South African sardines, *Sardinops sagax*, and an assessment of their potential as biological tags. *Bulletin of the European Association of Fish Pathologists* 32: 41-48.

Reimer LW. 1993. Parasites of *Merluccius capensis* and *M. paradoxus* from the coast of Namibia. *Applied Parasitology* 34: 143-150.

Roux JP, Shannon LJ. 2004. Ecosystem approach to fisheries management in the northern Benguela: the Namibian experience. *African Journal of Marine Science* 26: 79-93.

Sadighzadeh Z, Valinassab T, Vosugi G, Motallebi AA, Fatemi MR, Lombarte A, Tuset VM. 2014. Use of otolith shape for stock identification of John's snapper, *Lutjanus johnii* (Pisces: Lutjanidae), from the Persian Gulf and the Oman Sea. *Fisheries Research* 155: 59-63.

Salvanes AGV, Bartholomae C, Yemane D, Gibbons MJ, Kainge P, Krakstad JO, Sundby S. 2015. Spatial dynamics of the bearded goby and its key fish predators off Namibia vary with climate and oxygen availability. *Fisheries Oceanography* 24: 88-101.

Sardella NH, Timi JT. 2004. Parasites of Argentine hake in the Argentine Sea: population and infracommunity structure as evidence for host stock discrimination. *Journal of Fish Biology* 65: 1472-1488.

Silva-Segundo CA, Brito-Chavarria M, Balart EF, Barriga-Sosa IDLA, Rojas-Esquivel R, Roldán MI, García-De León FJ. 2011. Clarifying the taxonomic status of *Merluccius* spp. in the northeastern Pacific: a combined morphological and molecular approach. *Reviews in Fish Biology and Fisheries* 21: 259-282.

Sindermann CJ. 1983. Parasites as natural tags for marine fish: a review. *NAFO Science Council Studies* 6: 63-71.

Smith JW, Wootten R. 1978. *Anisakis* and *anisakiasis*. In *Advances in parasitology*. Academic Press. pp 93-163.

Smith MK. 1992. Regional differences in otolith morphology of the deep slope red snapper Etelis carbunculus. *Canadian Journal of Fisheries and Aquatic Sciences* 49: 795-804.

Smith PJ, Jamieson A. 1986. Stock discreteness in herrings: a conceptual revolution. *Fisheries Research* 4: 223-234.

Stransky C, MacLellan SE. 2005. Species separation and zoogeography of redfish and rockfish (genus *Sebastes*) by otolith shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 2265-2276.

Stransky C, Murta AG, Schlickeisen J, Zimmermann C. 2008. Otolith shape analysis as a tool for stock separation of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean. *Fisheries Research* 89: 159-166.

Szidat L. 1955. La fauna de parásitos de"*Merluccius hubbsi*" como caracter auxiliar para la solución de problemas sistemáticos y zoogeográficos del genero"Merluccius" L. *Comunicaciones del Museo Argentino de Ciencias Naturales"Bernardinosz Rivadavia", Ciencias Zoológicas* 3: 1-54.

Templeman W. 1983. Stock discrimination in marine fishes. *NAFO Science Council* 6: 57-62.

Torres GJ, Lombarte A, Morales-Nin B. 2000. Sagittal otolith size and shape variability to identify geographical intraspecific differences in three species of the genus *Merluccius*. *Journal of the Marine Biological Association of the United Kingdom* 80: 333-342.

Tracey SR, Lyle JM, Duhamel G. 2006. Application of elliptical Fourier analysis of otolith form as a tool for stock identification. *Fisheries Research* 77: 138-147.

Ukomadu NMI. 2017. Assessment of the population structure of the South African sardine *Sardinops sagax* using a multi-method approach and the morphological and molecular characterization of a stock-discriminating digenean parasite biotag of the genus *Cardiocephaloides*. PhD thesis, University of Cape Town, Cape Town, South Africa.

van der Lingen CD, Weston LF, Ssempa NN, Reed CC. 2015. Incorporating parasite data in population structure studies of South African sardine *Sardinops sagax. Parasitology* 142: 156-167.

Van der Westhuizen A. 2001. A decade of exploitation and management of the Namibian hake stocks. *South African Journal of Marine Science* 23: 307-315.

Vaz-dos-Santos AM, Schwingel PR. 2015. Biology and fisheries of hake (*Meluccius hubbsi*) in Brazilian waters, Southwest Atlantic Ocean. In Arancibia H (ed), *Hakes: Biology and Exploitation. Oxford: Wiley Blackwell*: pp 211-233.

Venables WN, Ripley BD. 2002. Tree-based methods. *Modern Applied Statistics with S*. Springer, New York, NY. pp 251-269.

von der Heyden S, Lipinski MR, Matthee CA. 2007. Mitochondrial DNA analyses of the Cape hakes reveal an expanding, panmictic population for *Merluccius capensis* and population structuring for mature fish in *Merluccius paradoxus*. *Molecular Phylogenetics and Evolution* 42: 517-527.

Waldman JR. 2005. Definition of stocks: an evolving concept. In: Cadrin SX, Friedland KD, Waldman JR (eds), *Stock Identification Methods. Applications in Fishery Science*. Amsterdam: Elsevier Academic Press. pp 7–16.s

Waldman JR. 2007. Definition of stock: an evolving concept. In: S.X. Cadrin, K.D. Friedland J.R (eds). Stock identification methods. Elsevier, Amsterdam. pp 719.

Weston LF, Reed CC, Hendricks M, Winker H, van der Lingen CD. 2015. Stock discrimination of South African sardine (*Sardinops sagax*) using a digenean parasite biological tag. *Fisheries Research* 164: 120-129.

Wilhelm MR. 2012. *Growth and otolith zone formation of Namibian hake, Merluccius capensis*. PhD thesis, University of Cape Town, South Africa.

Wilhelm MR, Kirchner CH, Roux JP, Jarre A, Iitembu JA, Kathena JN, Kainge P. 2015a. Biology and fisheries of the shallow-water hake (*Merluccius capensis*) and the deep-water hake (*Merluccius paradoxus*) in Namibia. In: Arancibia H (ed), *Hakes: Biology and Exploitation,* Oxford, UK: Wiley Blackwell. pp 70-100.

Wilhelm MR, Jarre A, Moloney CL. 2015b. Spawning and nursery areas, longitudinal and cross-shelf migrations of the *Merluccius capensis* stock in the northern Benguela. *Fisheries Oceanography* 24: 31-45.

Wilhelm MR, Moloney CL, Paulus S, Roux JP. 2017. Fast growth inferred for northern Benguela shallow-water hake *Merluccius capensis* using annual survey-and monthly commercial length-frequency distributions. *Fisheries Research* 193: 7-14.

Wilhelm MR, Moloney CL, Paulus SC, Kashava S, Brinkman FR, van der Plas AK, Roux JP. 2018. Biannual otolith zonation of Cape hake (*Merluccius capensis*) in response to fish physiology and environment in the northern Benguela. *Journal of Marine Systems* 188: 98-108.

Wilhelm MR, Paulus SC, Kashava S. 2019. New age-determination method based on fast growth rate of Cape hake *Merluccius capensis* in Namibia. *African Journal of Marine Science* 41(2): 119-124.

WoRMS. 2008. Ostend, Belgium. marinespecies.org.

Zhang C, Fan Y, Ye Z, Li Z, Yu H. 2017. Identification of five *Pampus* species from the coast of China based on sagittal otolith morphology analysis. *Acta Oceanologica Sinica* 36: 51-56.

APPENDIX

Figure A1: Laboratory set up for taking *M. capensis* otolith images using a Zeiss Stemi DV4 Stereotype microscope embedded with a Zeiss Axio-camera.

Figure A2: Laboratory set up for a stereo and compound microscope for examining parasites.

Figure A3: Copepod *Chondracanthus merluccii* found in the mouth of *M. capensis* hosts.

Figure A4: Copepoda *Parabrachiella australis* found in the mouth and gills of *M. capensis* host.

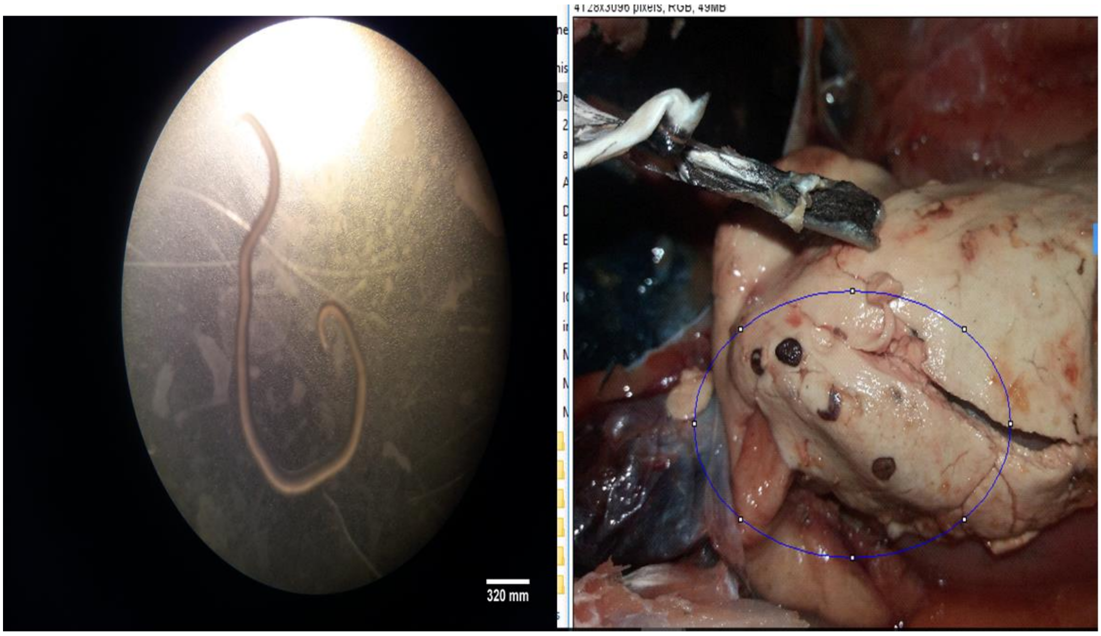


Figure A5: Nematode *Anisakis* sp. found on *M.capensis* liver, intestines and body cavity.

Figure A6: Monogenea *Anthocotyle merluccii* found on *M. capensis* gill fillaments.

Figure A7: Monogenea *Hepatoxylon trichiuri* found on *M. capensis* body cavity.

Appendix 2

Table A2: Sampling form for parasite raw data

Fish dissection and parasitological data sheet

Date: ____________________ Species: ______________ Locality: _______________

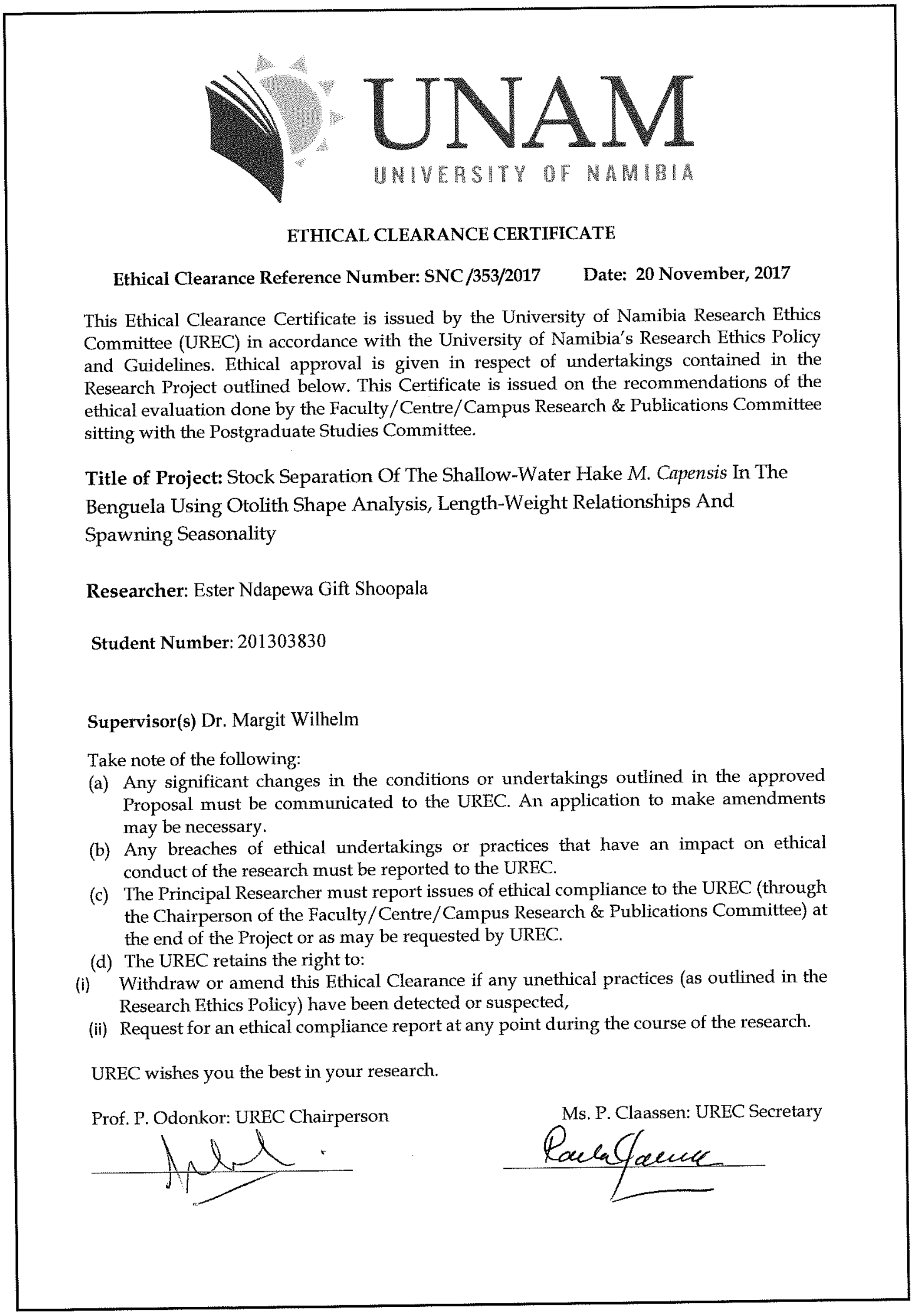
Landed on: ________________ Fish no: _______________ Captured by: ________________

TL (mm)	
FL (mm)	
Mass (board only) (g)	
Mass (board + fish) (g)	
Mass (fish) (g)	
Sex (F/M/H)	
Gonad Stage (1-7)	
Gonad Mass (g)	
Fat score (1-3)	
Stomach mass (full) (g)	
Stomach mass (empty)(g)	
Bait mass (g)	
Prey mass (g)	
Prey %	

Parasite species	SOI	No

Notes:

Appendix 3



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ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: SNC /353/2017 **Date:** 20 November, 2017

This Ethical Clearance Certificate is issued by the University of Namibia Research Ethics Committee (UREC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the Faculty/Centre/Campus Research & Publications Committee sitting with the Postgraduate Studies Committee.

Title of Project: Stock Separation Of The Shallow-Water Hake *M. Capensis* In The Benguela Using Otolith Shape Analysis, Length-Weight Relationships And Spawning Seasonality

Researcher: Ester Ndapewa Gift Shoopala

Student Number: 201303830

Supervisor(s) Dr. Margit Wilhelm

Take note of the following:

(a) Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the UREC. An application to make amendments may be necessary.
(b) Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the UREC.
(c) The Principal Researcher must report issues of ethical compliance to the UREC (through the Chairperson of the Faculty/Centre/Campus Research & Publications Committee) at the end of the Project or as may be requested by UREC.
(d) The UREC retains the right to:
  (i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,
  (ii) Request for an ethical compliance report at any point during the course of the research.

UREC wishes you the best in your research.

Prof. P. Odonkor: UREC Chairperson

Ms. P. Claassen: UREC Secretary