

AGE DETERMINATION AND GROWTH RATE OF THE NORTHERN BENGUELA
SARDINE (*Sardinops sagax*)

THESIS
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Abstract

The main objective of this study was to determine sardine age by counting rings on otoliths and to analyse the length, age and growth through regression and Von Bertalanffy Growth models. Otoliths amounting to 826 pairs were collected from the annual sardine research survey of the Ministry of Fisheries and Marine Resources in October 2013. A total of 713 sardine otoliths were mounted on black Perspex plates and aged by means of counting rings using a Zeiss microscope at a magnification of 22x. A micrometer (4 μ m = 1 mm) was used to measure the radius and distance of rings from the nucleus on each otolith. In total, six age groups were represented in the sample, including, zero, one year, two years, three years, four years and five year age groups. The one year old age class was the dominant age group. Mean lengths at age were determined for each age group and were as follows: Zero age group had a mean length of 13.8 cm, one year olds were 16.8 cm, two year olds were 19.6 cm, three year olds were 21.4 cm, four year olds 22.4 cm and mean length for the five year age group was 23.4 cm. All these lengths were significantly different from each other, except the four and five year age groups. Fish length and otolith radius relationship was strong ($r = 0.75$, $p < 0.05$). The estimated parameters for the Von Bertalanffy Growth model were: $L_{\infty} = 26.6$ cm, $K = 0.28$, and $t_0 = 2.69$. Growth rate of females ($K = 0.2256$) was lower than males ($K = 0.3188$).

Keywords: Sardine, Von Bertalanffy Growth models, Benguela, otoliths, growth rate, length, age

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List of Abbreviations

TACs – Total Allowable Catches

BCLME – Benguela Current Large Marine Ecosystem

MFMR – Ministry of Fisheries and Marine Resources

BC – Benguela Current

FAO – Food and Agricultural Organization

LFA - Length Frequency Analysis

LMEs – Large Marine Ecosystems

FiSAT - FAO-ICLARM stock assessment tool

VBG – Von Bertalanffy Growth

MEKP - Methyl Ethyl Ketone Peroxide

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Dedication

Miss Taimi Nashilongo, my mother, for always believing in me and encouraging me to study. If it wasn't for you, I would not have thought of even studying for a masters. My little brother Jacob Shiimi, for making me feel like the smartest person ever. I know one day you will get to go further than I did. Tulimo Uushona, your drive for excellence has motivated me.

Declaration

I, Maria Malakia, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education

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Date.....

Maria Malakia

Chapter 1

Introduction

The Namibian fishing industry is based on the Benguela Current upwelling system and supports one of the richest fisheries in the world (Lange, 2003). The fishing industry is the second biggest export earner of foreign currency in Namibia after mining (INFOSA, n.d.) and it remains the third largest economic sector with a contribution of 3.2% to the Gross Domestic Product (GDP) (NPC, 2013). Namibian Fisheries consists of more than 20 commercially harvested species within its 200 nautical mile exclusive economic zone (EEZ). Economically the three most important fish species are: hake, horse mackerel and sardine (Sumaila & Vasconcellos, 2000; Namibian Brief, 1998). However, the sardine stock contributes little to the country's economy, and the non-recovery of the sardine biomass observed thus far could act as a hindrance to the success of the fishing industry as the fisheries relies on higher yields to cover their costs and make optimal profits (MFMR report, 2013b). Canned fish make up more than 90% of the export earnings from the pelagic fishery (Boyer & Hampton, 2001). Canning is thus the most profitable activity of the industry and companies require large amounts of fish in order to maximise their returns.

Since independence in 1990 the Namibian government initiated measures to ensure increasing ownership as well as economic and social benefits for Namibians from the fishing industry. Two such measures aimed specifically to increase economic gains and employment creation were: (a) the requirement and incentives for onshore processing as

well as (b) incentives for registering vessels as Namibian when 80% of the crew consists of Namibian Nationals (Namibian Brief, 1998). During 2011 the fishing sector employed approximately 14 000 people (MFMR annual report, 2011).

The sardine fisheries had a humble start in 1948 when only 1000 tonnes were caught (Sumaila & Steinshamn, 2004). Since then there was a steady increase in catch, where after the industry collapsed in the early 1970s (Table 1). According to a report of the National Planning Commission (NPC) of Namibia (2013), the Namibian fishing industry landed a total of 467 004 tonnes of fish in 2012. The sardine stock contributed less than 40 000 tonnes of the total amount of fish landed (BCLME, 2012).

Table 1: Historical catch data of sardine

Year	Catch (tonnes)	Reference
1948	1 000	(Sumaila & Steinshamn, 2004)
1953	262 000	(Sumaila & Steinshamn, 2004)
1968	1.4 – 2 million	(Sumaila & Steinshamn, 2004)
1978	100 000	(Sumaila & Steinshamn, 2004)
1992	80 784	(Namibia Brief, 1998)
1993	114 812	(Namibia Brief, 1998)
1994	116 429	(Namibia Brief, 1998)
1995	42 797	(Namibia Brief, 1998)
1996	1 171	(Namibia Brief, 1998)
2003	20 000	(MFMR annual report, 2013)
2005	25 000	(MFMR annual report, 2013)
2010/11	25 000	(MFMR annual report, 2013)
2011/12	25 000	(MFMR annual report, 2013)
2012/13	25 000	(MFMR annual report, 2013)

The continuous decline of the sardine stock poses a major concern to both management and the scientific community. Even though the decline in stock size has been explained by over-fishing that critically reduced the population in the late 1960s and early 1970s, the question on why the stock has not been able to recover to at least half the level it was in the 1960s remains unanswered. Fishing is selective, and has a tendency of removing bigger fish in the population and reduces the age classes of fish populations (Bond, 1996). Hence, recovery of a depleted stock such as Sardine will depend on an increase in the number of age classes, and favourable environmental conditions (Boyer et al., 2001). A reduction in fishing pressure could also be a viable factor, but this is supposed to be controlled by the current management practices such as Total Allowable Catches (TACs), closed seasons and protected areas. However, variation in catches of sardine in the northern Benguela Current are more directly related to changes in oceanographic conditions and not only related to fishing mortality (O'Tool, 1997). The annual report of the MFMR (2013a) reported that, total length and length at 50% maturity of Sardine has been decreasing over the years and this is usually an indication of a declining fish stock often caused by overfishing. The persistence of a low biomass of sardine leads to degradation of the ecosystem through the loss of biodiversity and it results in under-performance of the pelagic and demersal fisheries (MFMR, 2013b).

The reduction in age classes of sardine only leaves behind a much younger population which is of smaller size and not as valuable as the adults. A population made up of several age classes is not only advantageous for the survival of the stock, it is equally important for the survival of the industry and the economy of the country. The survival

of the fishing industry is highly dependent on the allocation of TACs which are obtained through the evaluation of the stock on an annual basis. An age structured stock assessment model is used to assess the stock, and it has been developed to estimate the absolute biomass and predict the effect of harvesting (MFMR, 2013b)

1.1 Stock Assessment and management

The sardine stock is annually monitored through acoustic surveys. The surveys are used as an index of the stock assessment model. The model used is the age structured model which requires an accurate age structure of the population to be known in order to estimate the growth of the stock, this in turn leads to effective stock assessment analysis and viable sustainable initiatives.

Currently the number of age classes within the sardine stock is not really known, because there is no validated age determination method and, according to Thomas and Agnalt (as cited in Boyer et al., 2001, p.70), it is due to the absence of seasonality in the northern Benguela marine ecosystem, which causes irregular formation of rings on otoliths, that are not related to a time scale.

The lack of age data has necessitated the use of length frequencies to split the stock into separate cohorts (De Oliveira, Boyer, & Kirchner, 2007). However, separation of cohorts based on length frequencies is not the best method for determining age structure of a stock as it proves difficult if not impossible for the application to adult fish (Fossen et al., 2001). In contrast, the use of otoliths is known to be a much more precise method of determining age of fish (Campana, 2013).

1.2 Fish Ageing

The northern Benguela sardine is a tropical species and there is a general consensus that otoliths of tropical fish are harder to read due to the lack of a strong seasonality (FAO, 1998). Pannella (1973) states, “age determination by means of otolith growth rings can be accurate if one is able to separate the patterns due to seasonal variations from those due to other causes that do not have annual periodicity.” Otoliths of sardine are also tiny and are prone to break easily if not handled with care; the size also makes extraction difficult, especially in smaller fish.

The use of otoliths and other ageing structures for age determination purposes is one of the most useful tools available in fish biology and fisheries science (Bagenal, 1973). There is a lack of literature on ageing of the Northern Benguela sardine. Most of the studies were done decades ago. The most prominent studies were done by Thomas in the 1980s. He described the method of reading sardine otoliths and he also concluded that mean lengths at age of sardine approximately agree with those calculated from independent age-reading methods. Boyer et al. (2001), states that, age-length keys for sardine are not available. Therefore, catch-at-length data were split into three size categories, with sardine of 16.5 cm and less representing the 0 year age group, sardine of 16.6 – 22.4 cm representing the 1 year age group, and Sardine of more than or equal to 22.5 cm representing the 2+ years age fish (Boyer et al., 2001). All the ages are based on length based estimates of Von Bertalanffy growth parameters.

Ageing of fish is also important in estimating the overall growth rate or part of the life history of an individual fish by using ages derived from ring counts and sizes back calculated from the relationship between fish length and otolith size (Rice, 1990).

The main objective of this study was to determine the age composition of sardine by means of the growth rings on otoliths and in the process the results were compared with those obtained by the LFA method. In addition, an evaluation of the growth rate and biological parameters such as length and weight were conducted.

The specific objectives of this study were to:

- i. Determine the different age classes of the current sardine population
- ii. Compare age distribution with length and weight of sardine.
- iii. Compare age estimates from otolith zonation with previous age estimates
- iv. Determine the growth rate of males and females
- v. Determine the dominant age group in the population

The following hypotheses were investigated:

- i. The sardine population consists of more than two year age classes, since the LFA which is the current age determination method, is unreliable in estimating age of older fish.
- ii. There is a positive linear relationship between age and the length and weight of the fish, because younger fish are expected to be smaller in size and lighter in weight compared to adult fish.
- iii. Based on observations from previous literature, there is no significant difference between otolith age estimates and previous LFA estimates.

- iv. There is a significant difference in mean length at age between males and females
- v. The dominant age group is expected to be the recruits as the adult population of a depleted stock is usually overfished, resulting in higher catches of younger fish.

1.3 Significance of study

Although thousands of otoliths were collected over many years of research at the Ministry of Fisheries and Marine Resources (MFMR), the otolith based method is not being applied for ageing of sardine. The LFA however can only be used confidently once it is cross validated with an aging method. According to Rochet (as cited in Fossen et al., 2001, p. 111), the adult population of an extremely exploited species such as sardine are often the ones to be removed from the stock first, leaving younger fish that are forced to mature earlier. However, there is a need for constant monitoring to record any changes and to ensure that outdated information is not used which could give unreliable results. A precise and accurate method of ageing is needed to confidently estimate the age composition of a stock. Currently only two year age classes are known to exist within the sardine stock, yet there is no clear age composition data since the LFA which is used for age determination currently is an unreliable estimate of older fish (Fossen et al., 2001).

The use of otoliths for age determination of sardine is critical since there is no current age validation of the species. There is a gap in knowledge of the precise sardine age composition due to a lack of literature and research on ageing of sardine by means of otolith zonation. Unlike other fish species, sardine otoliths are tiny and difficult to read,

requiring a special method to be able to read them clearly. The annuli on the otoliths are also known to be unclear due to the lack of seasonality and variability of the environment in the northern Benguela marine system. These have been the main reasons why ageing with the use of otoliths was never fully implemented at MFMR.

This study established whether there has been a change in sardine age classes and growth rate. Accurate age determination is crucial for stock assessment analysis because age is a prerequisite for the estimation of growth within a stock and for the determination of cohorts. Information generated from stock assessment analysis is used in decision making regarding the sustainable utilization of the stock, with the main aim of improving management practices. Catches can only be controlled sustainably if the cohorts within the fish population are known and this can be achieved through precise ageing methods such as otolith analysis.

If otolith age estimates are found to be comparable to those of LFA, it will be possible to compile an age length key, thus avoiding the necessity to read otoliths on a regular basis.

Chapter 2

Literature Review

Growth rates of fish stocks around the World are determined mainly through the application of ageing techniques. These techniques are fundamental in the understanding of life cycles of species and are useful tools in the sustainable management of fisheries resources (Campana, 2001). The main focus of this study is on age determination of the northern Benguela sardine (*Sardinops sagax*) using age determination from otoliths and comparison of the results to historical age related data obtained through Length Frequency Analysis (LFA). Several studies were conducted on sardine species in other upwelling marine ecosystems, for example, in California (Yaremko, 1996), Chile (Araya et al., 2003) and on the west coast of South Africa (Van der Lingen & Durholtz, n.d.). However, limited age related studies on the northern Benguela sardine exists and the most prominent studies were done by Thomas in the 1980s, henceforth validating the otolith method and results obtained in those studies are still applicable today.

2.1 The Benguela Large Marine Ecosystem

There are 49 Large Marine Ecosystems (LMEs) in the world, four of which are synonymous with current upwelling systems (Sherman, 1994). The Benguela current is one of the four upwelling LMEs, and is highly productive. Boyer and Hampton (2001) refer to the Benguela as being the second most productive in fish among all the eastern boundary upwelling systems, only falling short behind the Humboldt system.

The Benguela LME covers the coast of Angola, Namibia and the western coast of South Africa. It extends from the continental shelf between the Angola-Benguela frontal zone off northern Namibia to the Agulhas retroflection area, typically between 36 and 37°S (Cochrane et al, 2009; Shannon & O'Toole, 1998). The Benguela current covers the entire coast of Namibia between 17°S and 29°S (Boyer & Hampton, 2001). Currently, harvesting of sardine in Namibia is mainly focused between 25°S to 17°S. The Lüderitz upwelling cell separates the northern Benguela from the southern Benguela which is at present is considered very different from the southern Benguela and is unique among all the world's upwelling systems, since it is the only upwelling system without a large biomass of wasp-waist species. Wasp-waist species is a species which is a regulator of the abundance of both predator and prey species (MFMR, 2013b, Atkinson, et al., 2012). Although the northern Benguela is an upwelling system, it does not function ecologically as one due to the absence of a large sardine biomass (MFMR, 2013b). Low biomass of wasp-waist species such as sardine, can lead to drastic changes in the functional diversity of an ecosystem, altering not just the food webs, but other biological processes such as changes in biomass of functional groups (Griffiths, et al., 2012). This will result in an overall loss of biodiversity in the ecosystem. Furthermore, Griffiths et al. (2012) states that, changes in the structure of marine ecosystems can have adverse effects on recreational and commercial fisheries. However, recently the wasp-waist theory has been challenged through a number of studies, for example stable isotope analysis studies conducted by Madigan et al. (2012). They indicated that there is a higher degree of trophic connectivity compared to what was demonstrated by the wasp-waist theory.

The Benguela ecosystem is also characterised by highly variable environmental conditions which had so far resulted in phenomena such as the ‘Benguela Ninos’ of 1984 and 1995, eutrophication in 1993-1994, sulphur eruptions and red tides (O’Toole, 1997). These conditions have a direct impact on plankton production, temperature and nutrient availability, leading to changes in fish distribution, spawning and growth (BCLME, 2012) and thus causing further degradation of an already highly depleted stock such as sardine (Boyer, et al., 2001).

2.2 Ageing of fish

Age plays an important role in determining the growth and size of a population. Human beings use age in demographic studies whereby it is incorporated in policy frameworks for the purpose of economic and development strategies (Silby et al., 2003). For environmental and conservation organizations, it is vital to determine and monitor the age groups of wildlife populations, in order to protect organisms against overexploitation and possible extinction (Gosselin et al., 2014). To maintain a sustainable population of fish stocks, the age distribution of a population should be taken into account. It is imperative for countries such as Namibia to practice accuracy in age determining of fish, thus ensuring the sustainable utilization of the resource. Different methods are used to determine the age of organisms and the methods used depend on the type of organism under study.

Otoliths are composed of calcium carbonate and they are embedded in the head of fish with a specific location in the labyrinths of fish ears (Simkiss, 1973). Age is determined

by means of rings which are also known as annuli, produced as a result of daily growth zones formed from daily growth increments (Beamish & McFarlane, 1987; Sparre & Venema, 1998). The growth zones are most commonly referred to as opaque and translucent bands. Already in 1973, Williams Bedford observed that the opaque band is laid down during the time of fast growth and is usually much wider than the hyaline band which is laid down during the time of year when there is minimal or no growth. According to Yaremko (1996), opaque zones are significantly wider than translucent zones in juvenile fish, forming because of rapid growth. The difference in width of opaque and translucent rings becomes smaller as fish get older (Yaremko, 1996). The bands can clearly be viewed and distinguished by light microscopy, appearing as either transparent or dark bands, respectively. However, according to Pannella (1973), there is conflicting use in the distinction between the bands as one translucent band of one writer could be the opaque band of another. The type of incident light used to view the otolith usually brings about the inconsistency. When an otolith is viewed by reflected light, the opaque band will appear white or transparent and the translucent band will appear dark. In contrast when viewed with transmitted light opaque bands are shown as dark and translucent bands as white (Williams, & Bedford, 1973; Pannella, 1973; Thomas, 1985; McFarlane et.al., 2010). This mainly causes confusion for those who describe the bands based on their dark and light appearance. Thomas (1983) who referred to Sardine otolith hyaline bands as appearing dark and opaque bands as white probably used reflected light when observing the rings on the otoliths.

In most temperate species, the opaque ring is formed during summer and the translucent ring is formed during winter (Thomas1985), however the same cannot be said for tropical species. It is difficult to distinguish annual rings in tropical species due to a low variability in seasonal changes (Dorval et al., 2013). However, in a study done on South African Sardine, Waldron (1998) indicated that on average a translucent band was deposited a year after hatching thus signifying an annual ring. Pannella (1979) has cautioned that the translucent and opaque rings should not necessarily be used as indicators of annual periodicity for tropical species and those patterns should be observed by means of daily or monthly rings. Daily and monthly rings also validate the ageing method of otoliths, as was done in a study by Thomas (1986) on the northern Benguela sardine. Thomas (1983) concluded that translucent rings on sardine otoliths were formed in late winter. Similar results were found for the California sardine (Yaremko, 1996), South African sardine (Waldron, 1998), and Canadian sardine (McFarlane et al., 2010).

It is imperative to note that not all bands are considered to be seasonal bands. Some bands are laid down during the spawning period and must therefore not to be mistaken for an annual or seasonal ring. There are also some translucent bands which form within opaque bands and this may result in the band being considered as an annual ring, leading to inaccurate results. Such bands are referred to as 'false rings' and the bands which are considered as annual rings, are referred to as 'true rings' (Williams & Bedford, 1973, McFarlane et al., 2010). Being able to recognize a 'true' ring from a 'false' ring is one of the most valuable skills in determining the age of fish from an otolith. Yaremko

(1996), has described some methods for distinguishing true rings from false rings. Some of these methods include:

- observing spacing between rings as it tends to get narrower as fish age
- using otolith radius to distinguish fish of different ages, but this is only applicable for differentiating younger ages from older ones.
- observing check marks of otoliths from the same year class, However it is stressed that the best way to learn aging is through repetition and experience, at least reading 1000 pairs before meeting the qualifying criteria.

It is also important to note and keep in mind that, as fish become older, otoliths become thicker, resulting in some of the rings becoming faint (Thomas, 1985).

Otoliths of bigger fish such as hake can be viewed under a microscope without any special treatment; however some otoliths have to be cross-sectioned to be able to view the rings clearly on a microscope. It is difficult to apply this method to small otoliths of fish such as sardine, since cutting might shatter the otolith into several pieces. Alternatively, sectioning for smaller otoliths could be achieved by embedding otoliths in a medium for grinding and polishing purposes (FAO, n.d.). Another method involves mounting small otoliths on glass slides using resin and viewing them under a black background. This method is referred to as otolith surface reading and it remains the main form of age determination used among all ageing laboratories of the northern Pacific sardine (Dorval et al., 2013). It involves mounting or fixing whole otoliths (sulcus side down) on glass slides using resin and viewing them under a black background (Dorval et al., 2013). McFarlane et al. (2010) also used the surface method by immersing otoliths in

a film of water in a shallow container and observing them under a dissecting microscope. Subsequently he found the polishing method of using fine sand paper -to make otoliths more transparent - superior to the surface method as rings which were not visible during surface reading became clearly visible after applying the polishing method. Nonetheless, Dorval et al. (2013), questions McFarlane's polishing method by stating that it was not validated. Findings from McFarlane's study affirmed that ages determined using annulus measurements in conjunction with the polished otolith, were generally 1 to 3 years older than the estimates obtained from the surface reading method. This implies that surface reading without polishing underestimates the age of fish by quite some margin. Methods of storage and viewing otoliths have also been described in detail by Williams & Bedford (1973). Chemical marking on otoliths is also another method that could be applied to cultured fish. This method however results in high mortality rates for the larvae under study (MacFarlane et al., 2010).

2.3 Length Frequency Analysis

Growth parameters are used to predict the body size of a fish when it reaches a certain age (FAO, 1998). The length frequency analysis (LFA) method is best applied for fish younger than two year old, and fish with irregular or unclear markings on ageing structures such as otoliths, and scales. Furthermore, LFA is also used as a supplement to other methods of age determination (Fagade, 1973; FAO, 1998), thus serving as a tool in the validation process. Although the LFA might be best suited for smaller fish and fast growing species, it is of less value in determining the age of older and slower growing

fish. It creates overlaps between age groups to such an extent that length modes cannot be identified (Linfield, 1973; FAO, 1998). However, it could be the most inexpensive ageing method and less time consuming.

Estimation of age from length frequencies is commonly done with the Bhattacharya method and modal progression analysis (Sparre & Venema, 1998), and calculations can be carried out in computer programmes such as FiSAT (Sparre & Venema, 1998). The modal progression method has however been found to generate questionable results (Pauly & David, 1981). This was attributed to the observation that, age groups cannot be allocated to some length frequency samples due to batch spawning which might be a cause factor in the occurrence of certain peaks in those samples (Pauly & David, 1981).

Length at age data can be analysed with several methods. Amongst these methods are, the Bhattacharya method (1967), Gulland and Holt plot(1959), the Ford-Walford plot (1933), Chapman's method for the derivation of growth parameters (Sparre & Venema, 1998) and the Electronic Length Frequency Analysis (ELEFEN) (Pauly & David, 1981). All these methods can be used to estimate K (the rate at which the size of a fish approaches L_{∞}), and asymptotic length (L_{∞}), while the Von Bertalanffy plot can be used to estimate K and t_0 (the theoretical time at which a fish is at zero length), but it requires an estimated value of L_{∞} as an input (Sparre & Venema, 1998).

The Gulland and Holt plot (1959) (Sparre & Venema, 1998), can be used to obtain an estimate of L_{∞} . The equation is: $\frac{L}{t} = (K \times L) - (K \times L_{(t)})$ (Equation 1)

where L is the length; L(t) is the length range from age zero (t_0) to $L_{(t+t)}$ at age $t+t$; K is the growth coefficient (FAO, 1998; Hart & Reynolds, 2002).

Von Bertalanffy introduced the Von Bertalanffy Growth (VBG) method in 1934 (Sparre & Venema, 1998). While other methods are simpler to use such as Ford-Walford plot, VBG is considered a stronger method as it gives a reasonable K estimate (Sparre & Venema, 1998). The VBG model requires input data of length and age. Another method is the least squares method which is assumed to be more superior to all the other methods (Sparre & Venema, 1998). The ELEFEN program uses an approach which is considered to provide results with logic and objectivity found lacking in methods such as modal progression (Pauly & David, 1981).

One good example of the error that could result from the LFA methods is the case where the age of Lamprey was determined as described in Beamish and McFarlane, (1987), where it was believed from an analysis that lamprey had a life cycle of 5 to 9 years and that, subsequent research by Purvis (1980) using known age of fish indicated that the life cycle could be about 20 years.

Namibian Sardine has been aged with LFA for some time, probably for lack of better alternatives. However, according to Beamish and McFarlane (1987), using only one method for age determination of a species will result in bigger errors being obtained than when more than one method is employed.

Thomas (1985) and Armstrong et al. (1987) quoted in Armstrong et al. (1989, p.99) stated that sardine of the Benguela ecosystem grow to an asymptotic length of about 23 cm, with sardine of 14 -15 cm allocated to the one year age class and those of 18 -20 cm to the two year old age class. Additionally, Boyer et al. (2001) classified sardine of the northern Benguela with 16.5 cm and less as the 0 age group, those of 16.6 -22.4 cm

represented the one year olds, and sardine with a length of more or equal to 22.5 cm represented the two year olds and older.

The estimates obtained by Thomas (1983) from the Von Bertalanffy model according to the equation $(L(t) = L_{\infty}*[1-\exp(-K*(t- t_0))])$ (Equation 2) were as follows: $L_{\infty} = 21.2$ cm, $K = 1.19$, and $t_0 = - 0.38$ years. From the independent methods the parameters were: $L_{\infty} = 22.6$ cm, $K = 1.09$, and $t_0 = - 0.003$ years (Thomas, 1984). He also obtained an estimate of the radius and length relationship of sardine, with a strong correlation of ($r = 0.999$) (Thomas, 1985).

2.4 Ecological significance and Biology of the northern Benguela sardine

Apart from the benefits to human beings, sardine also plays a vital role in the marine ecosystem. The species is considered as a regulator of the abundance of both predator and prey species (Curry et al., 2000), and therefore performs a critical role in the marine food web.

“Clupeoids (Sardine and Anchovy) in upwelling system food webs are an essential link between secondary producers and upper trophic levels providing high quality prey for valuable predatory fish stocks”(MFMR report, 2013). A decline in biomass of forage fish such as sardine and anchovy directly affects the breeding capacity of birds such as gannets and penguins by reducing their success for producing offspring (Attwood, Hamukwaya, Willemse, 2012). As a consequence, the populations of African penguins and Cape gannets in Namibian fell by 85% and 95% respectively between the years 1956

and 2005 (BCLME, 2007). According to the 2013 MFMR report, there has been a significant drop in dietary quality of predators since the collapse of the Sardine stock.

Sardine is distributed all over the world, especially in upwelling ecosystems. This fish has been overexploited almost in all regions of the world (Kreiner et al., 2001), and is found in Australia, Japan, Morocco, Spain, South Africa, Chile, United States of America, New Zealand, and Canada. It is a short-lived species and the stock has been known to be composed of more than six age classes, however currently, it is hard to find sardine of three years and older (Fossen et al., 2001). Hence, the modal length has decreased from 25.5 cm in the 1950s to the current mode of 22 cm (MFMR report, 2014). Sardine in the Benguela ecosystem attains a length of 28 cm (Smith & Heemstra, 1988) and it is distributed along the south Atlantic coast from southern Angola to Kwazulu Natal, northeast of South Africa (De Goede, 2004; Kreiner et al., 2001), the species is found very close to shore, extending along the entire continental shelf just beyond the surf zone (Boyer & Hampton, 2001; BCLME, 2013).

Most spawning takes place in spring and summer (De Goede, 2004). They are batch spawners and spawn during September-October and February-March (MFMR report 2014). They reach sexual maturity during the second year of life and will spawn at an age of approximately 2 years (MFMR report, 2014; BCLME, 2013). Northern Benguela sardine spawning areas were in the vicinity of Walvisbay and Palgrave point, however spawning in Walvis bay grounds is hypothesised to have declined in importance or possibly ceased (van der Lingen & Durholtz, n.d.)

Sardine otoliths could be used as a reliable method for age determination as it was demonstrated by Walford and Mosher, (1943) (as cited in Yaremko, 1996) who revealed that age estimates from scales highly correlated with age estimates from otoliths. According to Yaremko, (1996), Otolith analysis is the preferred method for age determining of California sardine.

Van der Lingen & Durholtz, (n.d) stated that, continuous and consistent time series growth rates for Benguela sardine are not available and growth rate data is relatively limited.

Benguela sardine usually shoals with anchovy coexisting as separate stocks (Boyer, & Hampton, 2001). The fish has a series of dark spots on the lateral side with a silver and greenish bluish colour.



Figure 2: Northern Benguela sardine

It is a round and robust fish which swims in shoals making it an easy target for purse seiners and fishermen. *Sardinops sagax* of South Africa is responsible for the famous sardine run (Smith & Heemstra, 1988), along the south east coast of South Africa. Sardine is both a filter and particulate feeder consuming phytoplankton and zooplankton (De Goede (2004)).

Chapter 3

Materials and Methods

3.1 Collection of Samples

Random samples of fish were collected from trawls caught from the lowest depth possible for a vessel to trawl, to the 500 m isobar along the Namibian coast, covering the area between 25°S to 17°S (Figure 3). Samples were collected with the research vessel *Welwitchia*. The vessel searches for fish strictly in accordance with a pre-determined search grid (MFMR, 2013c) which is based on a randomized stratified design. The survey is an adaptive two-stage survey with pre-determined transect spacing and Purse seine nets with 12 mm cod ends were used for trawling (MFMR, 2013).

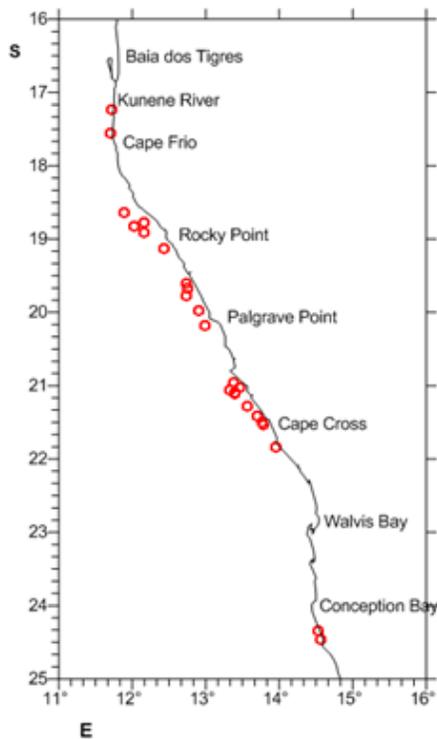


Figure 3: Sampling sites of sardine during the sardine survey.

Otoliths were collected from fish during the annual small pelagic survey in October 2013. A sample of a maximum number of 50 fish or less, depending on the size of the trawl, was collected for biological sampling (Table 2).

Table 2: October 2013 catch positions of sardine biological samples. Source: Ministry of Fisheries and Marine Resources (NatMIRC)

survey	station no	samples	date	Longitude (°S)	Latitude (°E)
201310	10	18	10/21/2013	13.56667	21.27517
201310	11	50	10/23/2013	13.32133	21.0685
201310	12	9	10/23/2013	13.38383	20.962
201310	13	4	10/23/2013	13.40083	21.106
201310	14	50	10/23/2013	13.40517	21.09917
201310	16	50	10/23/2013	13.463	21.03283
201310	18	50	10/25/2013	12.74267	19.77767
201310	2	17	10/14/2013	14.5365	24.33967
201310	20	10	10/25/2013	12.72867	19.60783
201310	21	50	10/25/2013	12.75183	19.67367
201310	22	50	10/26/2013	12.90667	19.98233
201310	23	50	10/26/2013	12.99467	20.17567
201310	24	50	10/27/2013	12.43667	19.13133
201310	26	50	10/28/2013	12.0255	18.8355
201310	27	24	10/28/2013	12.16267	18.9185
201310	28	50	10/28/2013	12.15583	18.78317
201310	29	50	10/28/2013	11.89167	18.64867
201310	3	50	10/14/2013	14.56883	24.472
201310	30	50	11/29/2013	11.71583	17.2395
201310	31	50	10/29/2013	11.66317	17.11467
201310	32	50	10/29/2013	11.7055	17.56633
201310	33	50	10/30/2013	14.70233	16.84083
201310	34	50	10/30/2013	11.7015	16.93367
201310	35	50	10/30/2013	11.721	16.8325
201310	4	3	10/20/2013	13.96483	21.8505
201310	5	41	10/20/2013	13.9495	21.84917
201310	7	2	10/20/2013	13.794	21.543
201310	8	50	10/21/2013	13.70833	21.4115
201310	9	50	10/21/2013	13.77333	21.50833

Length of fish was measured with a measuring board and sex of fish was recorded. The reproductive maturity of fish was determined with the aid of a maturity stage key

(MFMR, 2013) and the fish were weighed. Otoliths were extracted with the use of forceps. After extraction, a pair of otoliths from each fish was placed in separate 15 ml plastic vials filled with distilled water.

3.2 Drying of Otoliths

The otoliths were stored in vials filled with distilled water until time of analysis. Otoliths were removed from vials with forceps and dried on jumbo roll paper. Immediately after drying, the otoliths were transferred to a new vial and the information on the previous vial was copied onto the new vial (Figure 4).



Figure 4: Vials with otoliths labelled with fish length, sex, and station

All the otoliths from fish sampled or caught from the same trawl were placed in the same vinyl plastic bags. Each trawl is referred to as a station and it is allocated a station number which corresponds to the trawl number. In total, 826 vials were recorded which contained 826 otoliths, some appearing in pairs and some appearing single due to misplacement or breakage of otoliths during extraction.

3.3 Preparation of Resin and Catalyst Mixture

A drop of Methyl Ethyl Ketone Peroxide (MEKP), a catalyst is added to 10 ml of resin (Figure 5). If there is a pair of otoliths in a vial, only one otolith was taken and placed in one of the moulds on the black Perspex plates. The otolith was turned onto its lateral side, with the sulcus invisible and the rostrum visible. Drops of the resin and MEKP mixture were added onto each mould on the black Perspex plate. One drop of the mixture was added into each mould with a syringe.



Figure 5: Crystic resin and catalyst

Each black Perspex plate consists of 16 moulds and in total 27 (Figure 6) plates were mounted with 800 otoliths. In total, 826 otoliths should have been mounted; however, some of the otoliths were destroyed during the collection process.



Figure 6: Black perspex plates mounted with otoliths

A card cut from cardboard paper was placed behind each plate (Figure 7). The card was divided into the number of moulds on the plate and the fish number for each respective otolith was written on the block on the card which corresponds to the mould on the plate in which the otolith in question is placed. That allowed for the identification of otoliths in order to trace the fish from which each otolith originated. It also allowed for matching of otoliths with biological data of a particular fish. The otoliths were placed on the resin mixture with the aid of a forceps and it was positioned in the mixture with a tooth pick. After all the otoliths were mounted in the moulds, the plates were air dried for 4 to 5 days for the resin to get completely dry. The capacity of each plate was 36 otoliths, representing 36 fish samples (Figures 6 and 7) (Ministry of Agriculture, Forestry and Fisheries (South Africa)).

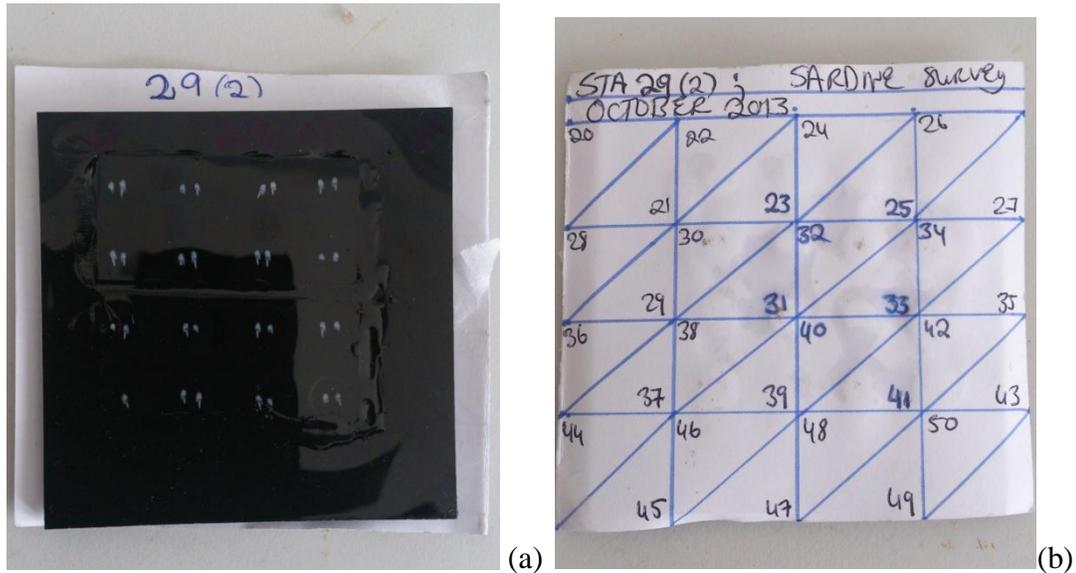


Figure 7: (a) Black Perspex plates with embedded otoliths, (b) paper with written numbers corresponding to the fish number of otolith mounted on a plate

Resin enough to fill a 30 ml vial was mixed with a drop of acetone and 2 drops of MEKP. The mixture was then poured over the plate containing the embedded otoliths. This was done slowly and evenly to avoid excessive air bubbles. Existing bubbles were removed by popping them with a paper clip dipped in acetone. It was also essential to ensure that all moulds were sufficiently covered in resin. Microscope cover slips were used to cover the plates by placing them from the left hand side and then dropping them slowly toward the other end of the plate. This was done cautiously in order to avoid creation of air bubbles. It took 5 to 7 days for the plates to get dry (Ministry of Agriculture, Forestry and Fisheries (South Africa)).

3.4 Reading of otoliths

All the otoliths were viewed under a Zeiss microscope at 25X magnification with reflected light. Caution was always taken to make sure that magnification always remained at 25X in order to get consistent results.

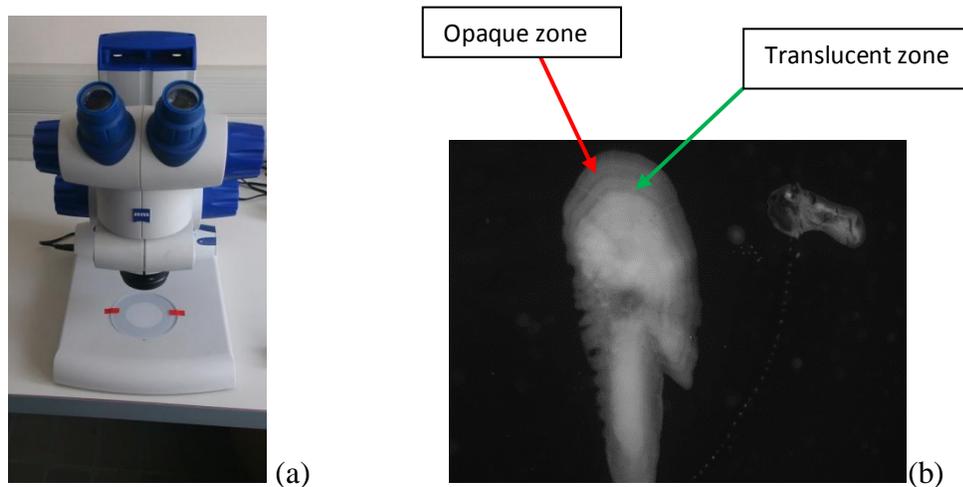


Figure 8: (a) Zeiss microscope, (b) sardine otolith: dark zones are hyaline rings and white zones are opaque rings

The translucent zones appeared dark and the opaque zones appeared white (Figure 8 b). Otolith radii were measured with an eyepiece micrometer (4 epu = 1mm). The radius was measured from the centre or nucleus of otolith to the posterior edge on the ventral side of the otolith. Each visible ring was also measured from the nucleus to the end of the ring, with distance from the nucleus increasing as rings get closer to the edge of the otolith. If a ring occurred within the otolith and continued to the sides of the otolith, it was considered a ‘true ring’, whereas if the line of the ring disappeared along the sides or edges of the otolith, it was considered a ‘false ring’, the same principle was applied to

a ring that was too close to the first ring or nucleus. Some translucent rings also faded into other rings as they approached the edges of the otolith.

The first visible ring was always read, sometimes it occurred faint and at times it occurred dark, appearing quite close to the nucleus. This ring is also known as the secondary ring (Thomas, 1985). The distance between the second ring and the ring first observed to be further away from the nucleus should always be greater than the distance between the third and fourth ring. If distance between the third and subsequent rings appears to be greater than the distance between the second and third ring, then either the second ring or the third ring was a false ring and should be read again. Distance between rings gets narrower as they approach the edge of the otolith and should be read even if they appear too close.

The length of the fish from which each otolith originated was not known to the reader, in order to avoid bias. Reading of otoliths was done by only one person and should ideally be validated by two other readers. However, since the second and third readers were unavailable during the study period, validation will only occur as a follow-up from this study. The first annual ring was considered to be the second ring in most otoliths and most of the time it occurred distinct, dark and wider than most rings. Rings closer to the nucleus were not considered as annual rings and an otolith with annuli of no greater than 0.95 mm distance from the nucleus were considered as the 0 age group. The first annual ring was considered as the annulus with a distance of between 0.95 mm and 1.1 mm from the nucleus. That ring would be allocated to the 1 year old age group. The ring counted as the second annual ring should at least be 0.25 mm from the first annual ring.

However, if a clear 3 year old age group ring is found at a longer distance from a 2 year old ring, reading of the 2 year old ring should be repeated or alternatively, repeating the reading of the first annual ring. Most of the rings which follow the 3 year old age group are easier to read except when they appear too faint and care should be taken not to count the rings which fade into other rings. As the rings get closer to the edge, they clump together with a distance of less than 0.05 mm between them. A ring which is very close to the edge of the otolith is not counted as an annual ring. Most otoliths of one year olds have the first annual ring, but if the distance between that ring and the second ring is not big enough, it is not allocated to the 2 year old age group. Some of them do not have the occurrence of any other ring except the annual ring, which occurs very close to the edge of the otolith.

When an otolith has a bigger radius, and it appears not to have any rings, it is not aged at that time, but it will be compared to other otoliths of the same radius. Some otoliths of smaller radius also have multiple clear rings, but they are close together and occur at a distance which is not considered as an annual ring.

Age of some otoliths could not be read due to bubbles, unclear nuclei, some were broken, discoloured rings (making the rings invisible), resulting in only 713 otoliths being clearly read and allocated an age. Images of sardine otoliths were taken with the Leica microscope at a magnification of 2.5X ($1\mu = 20.3\ \mu\text{m}$).



Figure 9: Leica light microscope

3.5 Data Analysis

The R statistical package (version 3.0.2) with R studio package was used for Pearson's regression analysis to determine relationship between radius and length of fish. Shapiro Wilkison test and QQ plots were used to test the length at age data for normality. One way ANOVA was used to test for significance of mean length at age between different age groups and males and females in conjunction with the Tukey test for comparison of mean length at age between different age groups and mean length at age between males and females. The Fligner-killeen test was used to test for homogeneity of variances. A two sample t-test was also used for groups with smaller sample number.

Length and weight of fish was analysed with linear regression in Microsoft Excel (2007), linear regression lines were fitted to mean length at age for age groups using the

Bhattacharya modal regression method in FISAT (FAO-ICLARM stock assessment tool) software.

growth parameters were estimated with the Gulland and Holt plot using linear regression and these parameters were used to fit the Von Bertalanffy growth model to length at age data. Analysis was carried out in Microsoft Excel (2007), equations were obtained from Sparre & Venema (1998).

Chapter 4

Results

4.1 Length distribution

The length distribution of *Sardinops sagax* was bimodal with two peaks at 17 cm and 22 cm (Figure 10). Maximum lengths measured were 25 cm and the minimum length was 11 cm.

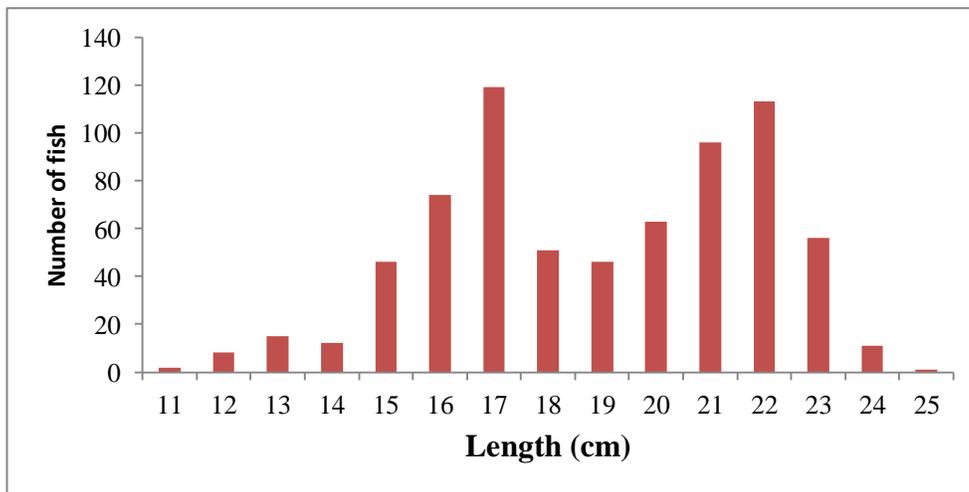


Figure 10: Length frequency distribution of sardine.

There is a significant positive relationship between fish length and the radius of the otolith and 75% of variation in otolith radius is explained by the independent variable length ($R = 0.75$, $p < 0.05$) (Figure 11). The observed values' distribution along the predicted points has outliers to the left and a few on the right. The model's fit to the data

is significant ($p = 2e-16$). The mean for the length frequency distribution obtained from FISat was 17 cm.

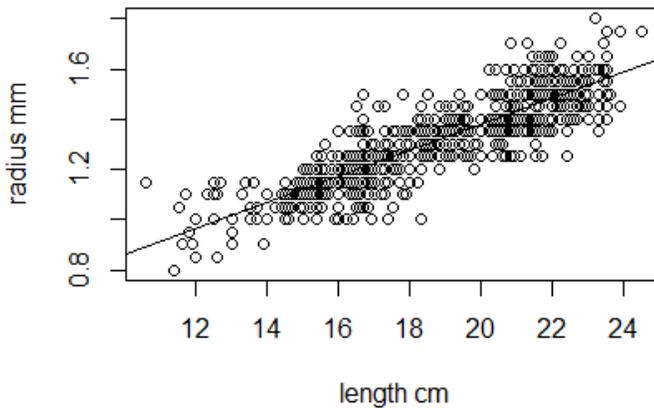


Figure 11: Relationship between otolith radius and fish length

4.2. Different age classes of the current sardine population

Most of the otoliths of younger age groups had shorter rostrums and narrower width compared to otoliths of older age groups such as four and five. Younger age group otoliths also had very faint rings or no visible rings. Most of the otoliths from the two year and three year age group had clearer rings and a well-defined shape, while the four and five year age groups otoliths had faint rings, especially the first rings, some of which had faded completely. On the otoliths of most of the older age group otoliths, only the last rings closer to the edge could be identified. The edge of these otoliths also became more transparent, making it difficult to identify rings at the very edge of otoliths. A total of six age groups were observed from the sample and are shown in Figures 12-16.

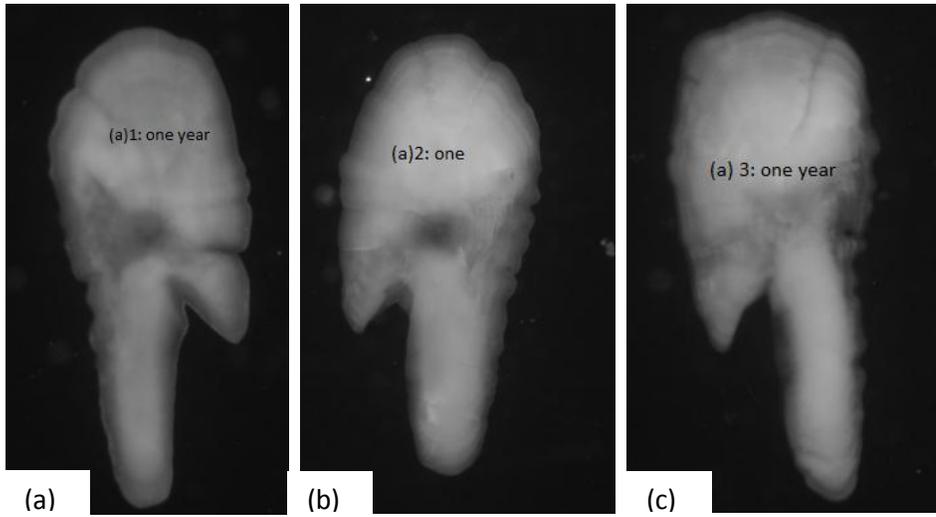


Figure 12: One year old age group: (a) radius = 1.25 mm, 17.2 cm (b) radius = 1.1 mm, 14.9 cm (c) radius = 1.05 mm, 15.4 cm

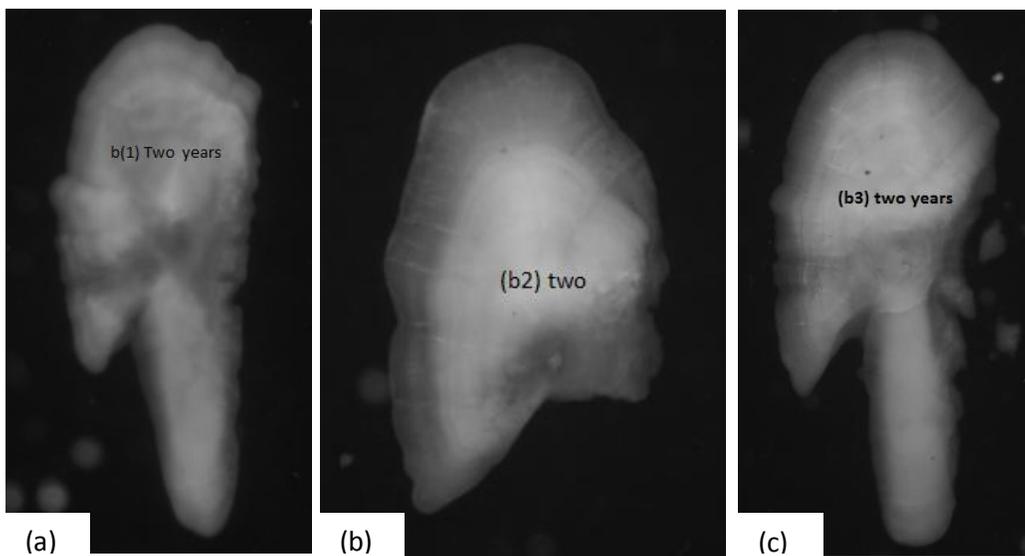


Figure 13: Two year old age group: (a) radius = 1.3 mm, 19.2 cm (b) radius = 1.2 mm, 19.8 cm (c) radius = 1.35 mm, 21 cm

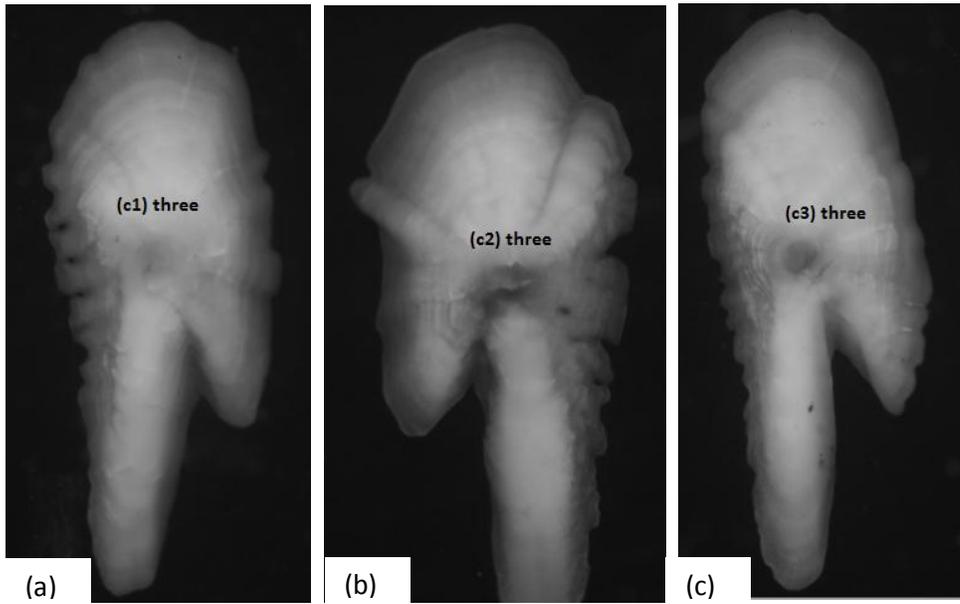


Figure 14: Three year old age group: (a) radius = 1.5 mm, 21.7 cm (b) radius = 1.5 mm, 22.4 cm (c) radius = 1.4 mm, 21.3 cm

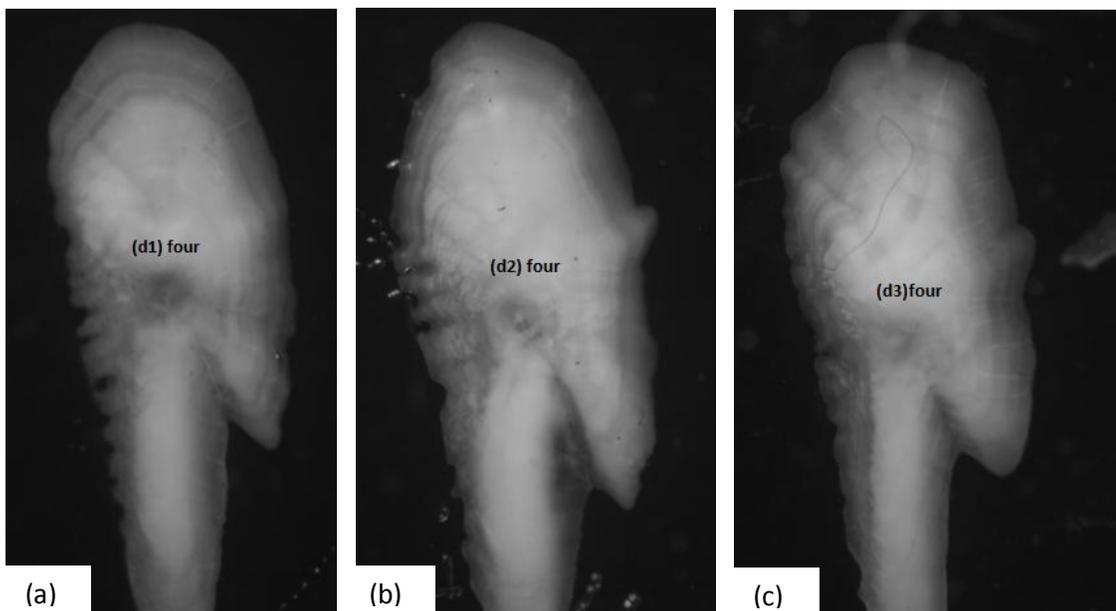


Figure 15: Four year old age group: (a) radius = 1.55 mm, 22 cm (b) radius = 1.6 mm, 21 cm (c) radius = 1.65 mm, 22 cm

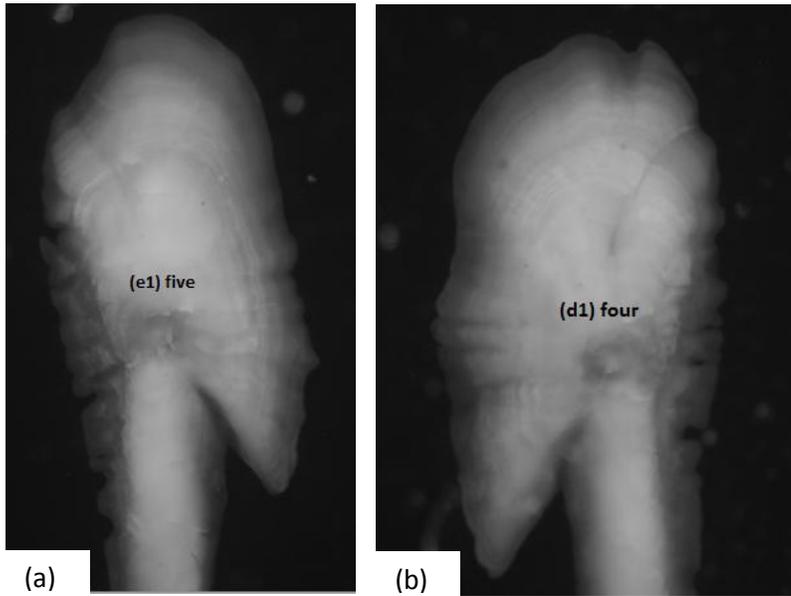


Figure 16: (a) Five year old age group: radius = 1.8 mm, 23.2 cm (b) Four year old age group: radius = 1.55 mm, 23.4 cm

In some cases it was difficult to distinguish individual rings (Figure 17) and these otoliths were rejected.

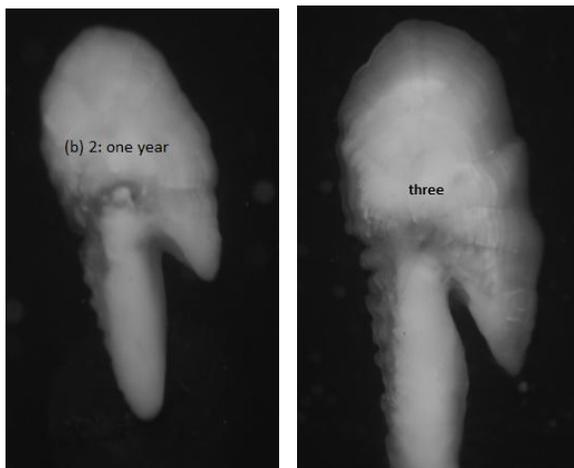


Figure 17: Otoliths with unclear rings and very faint outer rings.

A total of six age groups were observed from the sample (Table 3). Length class intervals used was 1 cm. The highest number of fish (260) was found in the 1 year old age group, accounting for 36% of the total sample. The lowest number of fish (14) was observed for the 5 year old age group, accounting for only 2% of the fish sampled. The minimum length was 10.5-11.5 cm and maximum length was 24.5-25.5 cm.

Table 3: Age classes obtained from otolith readings and their corresponding length classes

Age count	Age						
Length(cm)	0	1	2	3	4	5	Total
10.5-11.5	2						2
11.5-12.5	6	2					8
12.5-13.5	12	3					15
13.5-14.5	9	3					12
14.5-15.5	15	30	1				46
15.5-16.5	7	62	4	1			74
16.5-17.5		102	16	1			119
17.5-18.5		30	19	2			51
18.5-19.5		13	31	2			46
19.5-20.5		6	44	13			63
20.5-21.5		6	46	37	7		96
21.5-22.5		3	15	66	29		113
22.5-23.5			2	13	32	9	56
23.5-24.5				2	5	4	11
24.5-25.5						1	1
Total	51	260	178	137	73	14	713

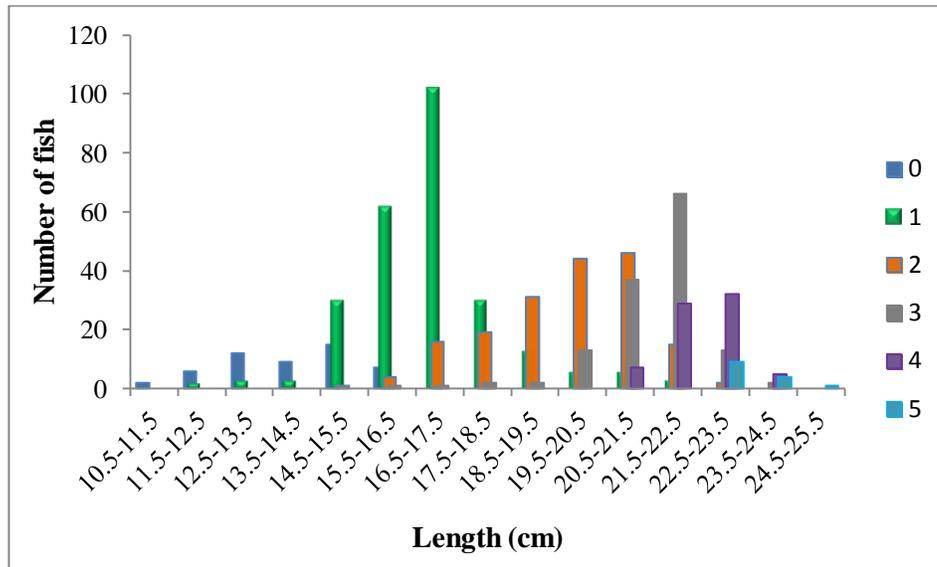


Figure 18: Length distribution of age groups.

4.3. Relationship between length and weight of fish

A strong positive correlation between length and weight of fish was found ($R = 0.98$) (Figure 19). Length and weight relationship for sardine was transformed into a linear equation and converted into the following parabolic equation: $W = aL^b$, where W is weight in grams, L is total length in centimetres, and a and b are constants (FAO, 1998). The following parameters were estimated, $b = 3.32$; $a = -5.84$, the exponential of which was 0.003. The length weight relationship derived was $W = 0.003 * L^{3.32}$. The 95% confidence limits about the equation were 3.317; 3.32. ($t = 1.96$). Further investigations revealed that the weight of females have a wider distribution, ranging from 20 g to 120 g compared to males which only reached about 100 g (Figure 20). The median weight of females was 65.75 g, while that of males was 58.8 g (Figure 21).

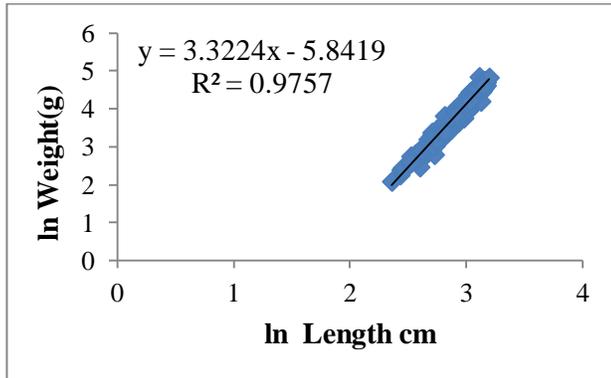


Figure 19: Relationship between length and weight (n = 713).

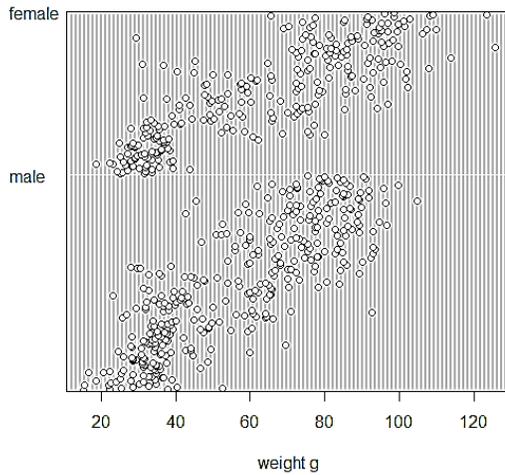


Figure 20: Weight of females and males.

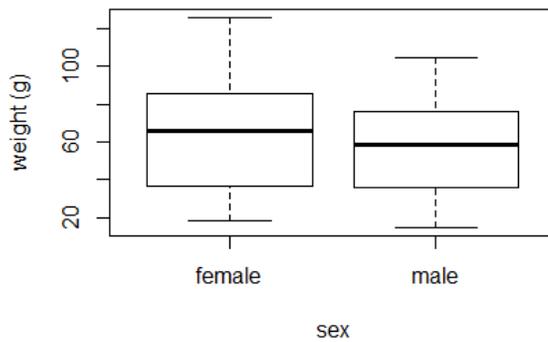


Figure 21: Box and whisker plots of the weight of males and females.

Females attained larger lengths than males (Figure 22). Length distribution of females is in the range of 14 cm to 25 cm, while that of males is between 13 cm and 23 cm. The median length of females is higher than that of males and skewed towards smaller length classes (skewed to the left) (Figure 23).

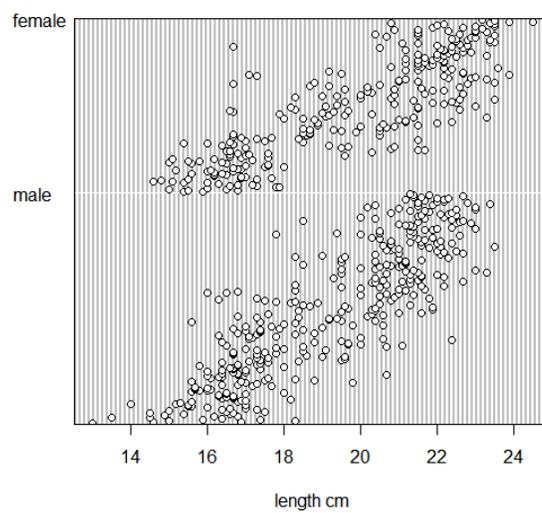


Figure 22: Scatter plot for length distribution of males and females.

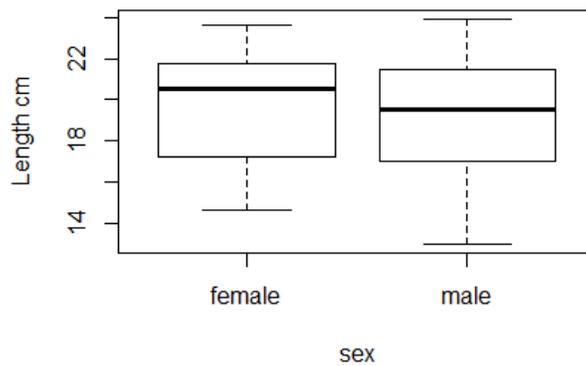


Figure 23: Box plot of lengths of males and females.

There was a higher contribution of males in the sample accounting for almost 60% of the sample (Table 4). The most represented age groups for both sexes were age groups one and two, and the least represented were age groups zero and five. Length distribution between age groups for males is not very different from that of females, except for the four and five year age groups. Length distribution for ages of both sexes gets narrower with an increase in age, with the exception of the zero age group.

Table 4: Length at age for males and females

Length (cm)	Male						Male Total	Female						Female Total	Total
	0	1	2	3	4	5		0	1	2	3	4	5		
12.5-13.5	1						1								1
13.5-14.5	1	1					2								2
14.5-15.5	5	3	1				9	3	7					10	19
15.5-16.5	4	28	3	1			36	2	17	1				20	56
16.5-17.5		54	10	1			65		39	1				40	105
17.5-18.5		20	12	2			34		10	7				17	51
18.5-19.5		8	13				21		5	18	2			25	46
19.5-20.5		3	27	12			42		3	16	1			20	62
20.5-21.5		5	28	27	5		65		1	18	10	2		31	96
21.5-22.5		1	6	36	14		57		2	9	28	15		54	111
22.5-23.5				4	11	3	18			2	7	21	6	36	54
23.5-24.5					1		1				2	4	4	10	11
24.5-25.5													1	1	1
Grand Total	11	123	100	83	31	3	351	5	84	72	50	42	11	264	615

The length distribution of all age groups is between 11 cm and 24 cm (Figure 24).

Median lengths for the different age groups were: zero age group = 13.95 cm; 1 year old age group = 16.7 cm; two year olds = 19.7 cm; three year olds = 21.5 cm; four year olds

= 22.5 cm and five year olds = 23.35 cm (Figure 25). The two year old age group had the widest range of length distribution, followed by the three year old age group. The narrowest range in distribution of lengths is that of the five year old age group.

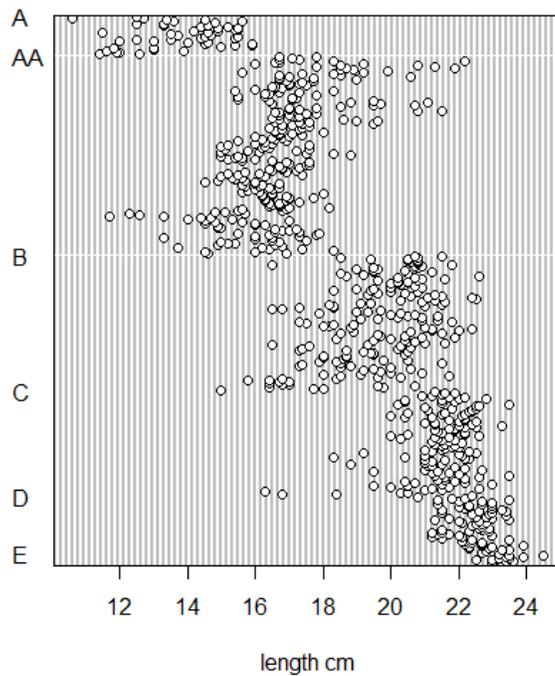


Figure 24: Scatter diagram of length distribution of age groups (A=Zero; AA=one; B=two; C=three; D=four; E=five)

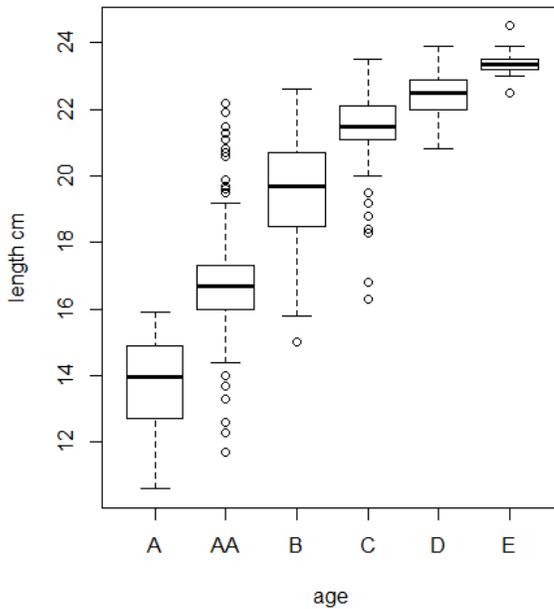


Figure 25: Box plots indicating median lengths for age groups (A=zero; AA=one; B=two; C=three; D=four; E=five)

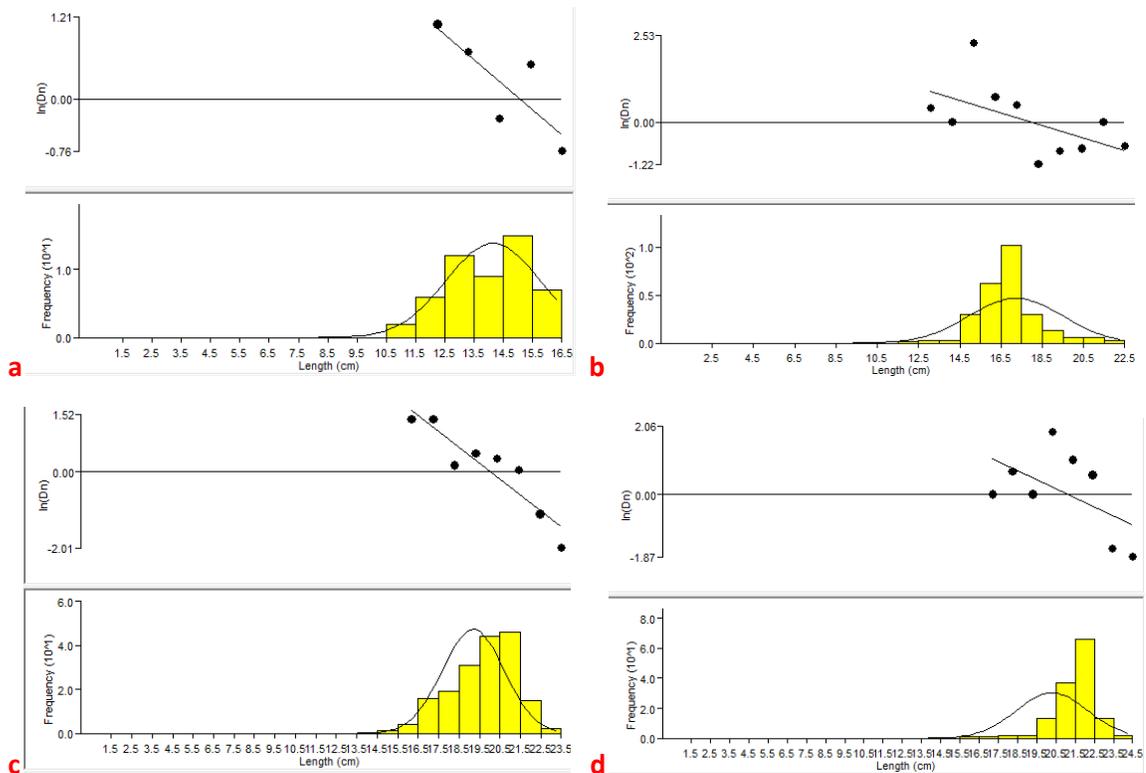
4.4 Relationship between age and length

The relationship between age and length was established for each age group separately (Figure 28) (Table 5). Mean length for zero age group is 14.4 cm (Figure 28a). A positive relationship exists between this age group and length with a correlation of $R = 0.663$. Mean length of the one year old age group was $17.21 \text{ cm} \pm 1.5$. Correlation between age and length was weak ($R = 0.320$) for the one year old age group (Figure 28b). The mean length of age group two was $19.20 \text{ cm} \pm 1.51$, and the relationship with length was very strong ($R = 0.852$) (Figure 28c) Mean length of age group 3 was $20.31 \text{ cm} \pm 1.88$ and correlation with length was very strong ($R = 0.8$) (Figure 28d). The mean length for age group 4 was $22.43 \text{ cm} \pm 0.78$. Age-length relationship was very strong (R

= 0.98) (Figure 28 e). Growth parameters of the five year old class could not be estimated by the model (Figure 28f). The mean length was $23.43 \text{ cm} \pm 2.78$.

Table 5: Age-length relationship estimates from Bhattacharya regression

Age	mean length (cm)	S.D	R
0	13.5	1.5	0.713
1	16.86	2.39	0.5
2	19.82	1.51	0.725
3	21.34	2.9	0.09
4	21.94	1.23	0.762
5	Could not be determined (number of fish in sample too few)		



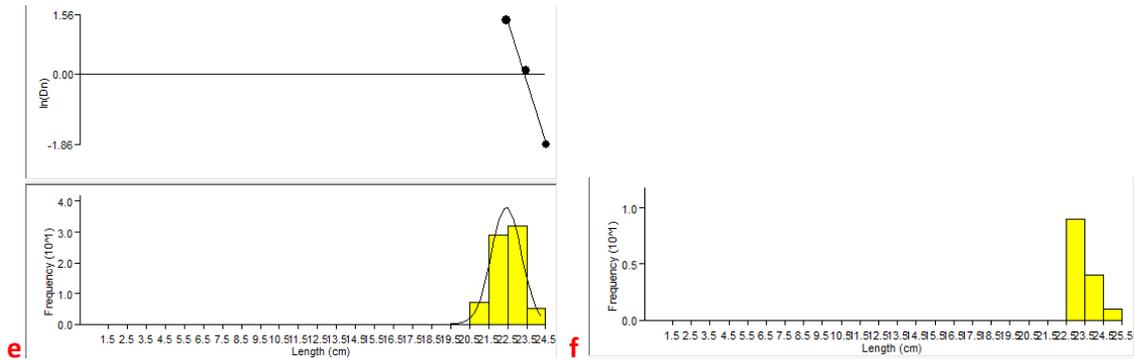


Figure 28: Bhattacharya modal regression for length at age for all age groups

4.4.5. Mean length at age for males and females

4.4.5.1 Zero age group

Length distribution for males and females of age group zero was between 13 cm and 16 cm (Figure 29). Males had a wide distribution which is skewed to smaller length classes, while females had a very narrow distribution with some outliers (Figure 30).

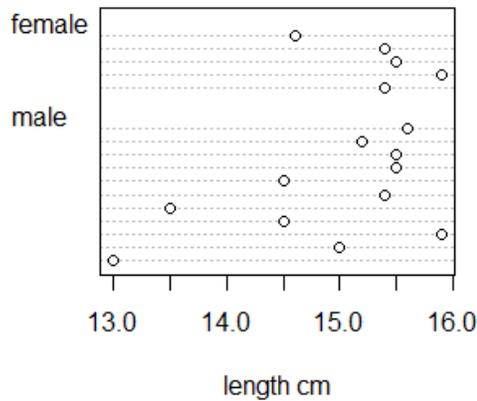


Figure 29: Zero age group length distribution for males and females.

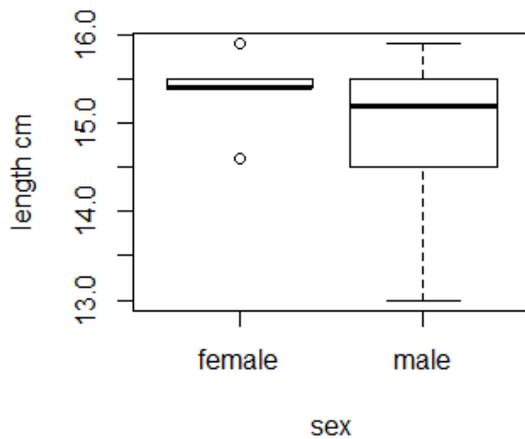


Figure 30: Box and Whisker plot for lengths of males and females in the age group zero.

4.4.5.2 One year old age group

Length distribution of males and females in the one year old age group was between 14 cm and 22 cm (Figure 31). Both variables show outliers to the right of the distribution, and the medians are almost identical (Figure 32). Males had a median of 17.21 cm, and females of 16.46 cm.

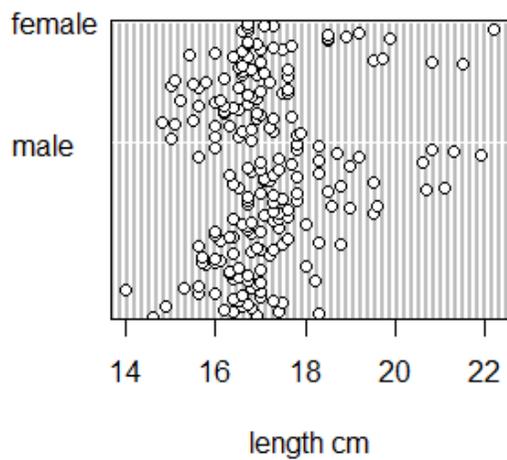


Figure 31: Length distribution of males and females in the one year old age group

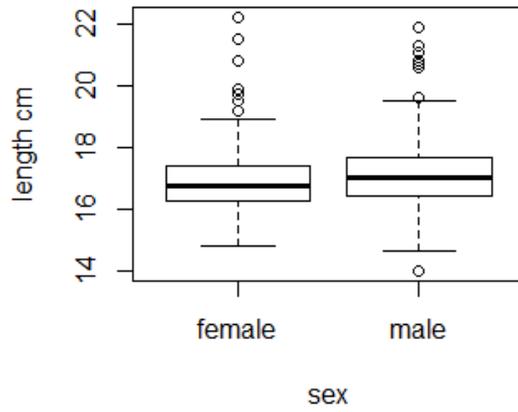


Figure 32: Box plot of lengths of males and females in the one year old age group

4.4.5.3 Two year old age group

The length range was between 15cm and 22cm (Figure 33). The medians of lengths of females and males are almost the same. Males have a wider length distribution than the females. Median length for females was 19.93 cm) and for males 19.47 cm (Figure 34).

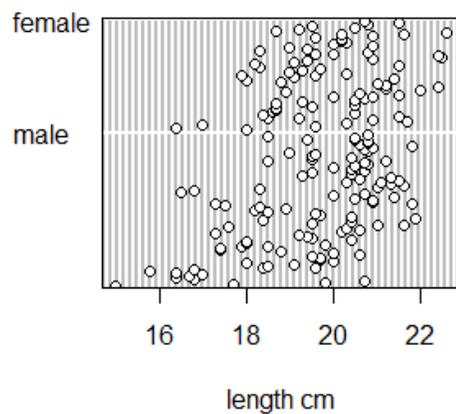


Figure 33: Length distribution of males and females in the two year old age group.

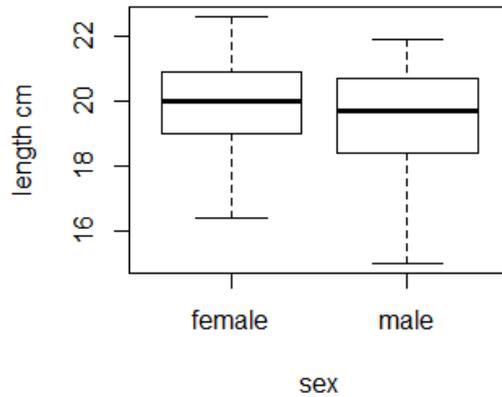


Figure 34: Box plot of males and females in the two year old age group.

4.4.5.4 Three year old age group

Both groups are centred on the same length classes showing an inclination towards bigger length classes. Length distribution is between 17 cm and 23 cm (Figure 35). There were more outliers for males than females. Females had an equal distribution on both sides of the distribution. Median length for males was 21.22 cm, which was less than that of females (22 cm). Both groups demonstrated very narrow length distributions (Figure 36).

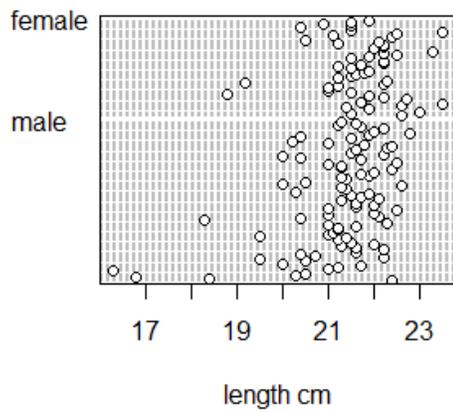


Figure 35: Length distributions of males and females in the three year old age group.

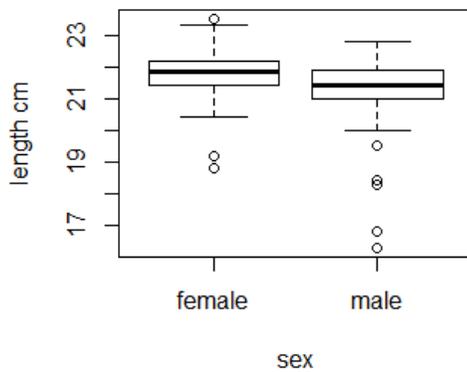


Figure 36: Box plot of females and males in the three year old age group.

4.4.5.5 Four year old age group

The length distribution of males and females was in the range of 21 cm and 24 cm (Figure 37). Males have a wider length distribution with a skewed distribution to smaller length classes. Median length for males was 22.15 cm, which is lower than that of females (22.58 cm) (Figure 38).

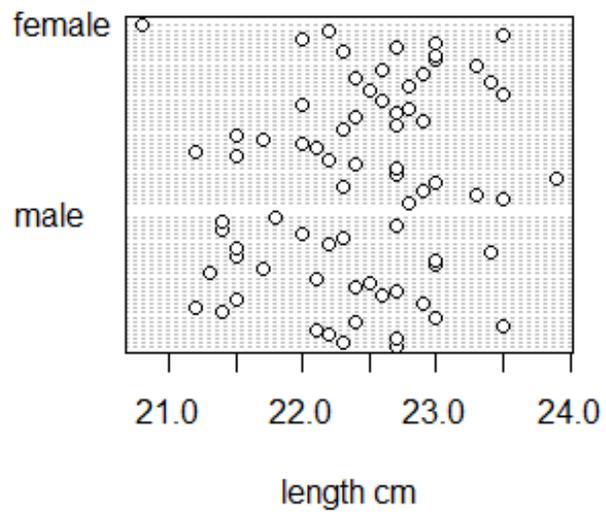


Figure 37: Length distribution of males and females in the four year old age group.

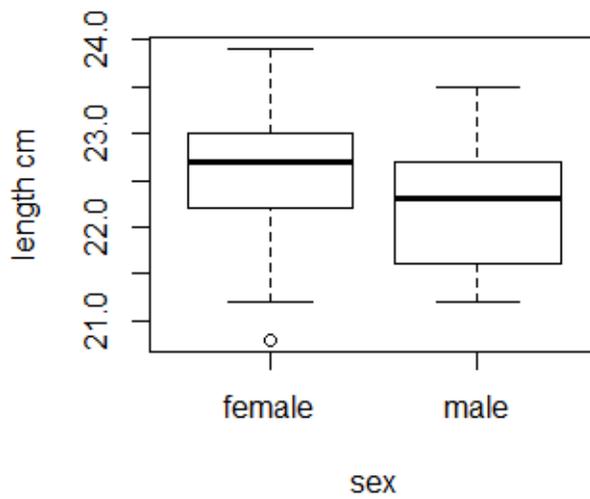


Figure 38: Box plot of length distribution of males and females in the four year old age group.

4.4.5.6 Length distribution of males and females in the five year old age group

Length distribution of males and females was between 22.5 cm and 24.5 cm (Figure 39). Females had an equal distribution on both sides of the median. Median length for females was 23.7 cm, which is higher than for males (22.7 cm) (Figure 40).

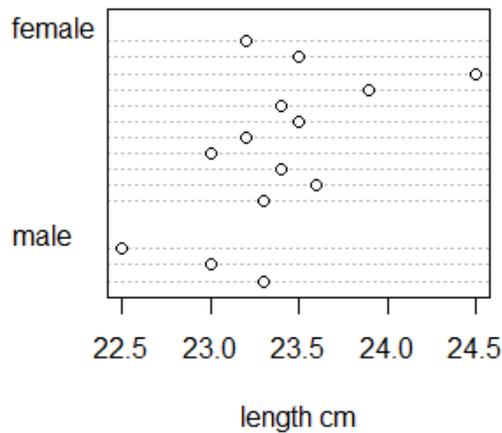


Figure 39: Length distribution of males and females in the five year old age group.

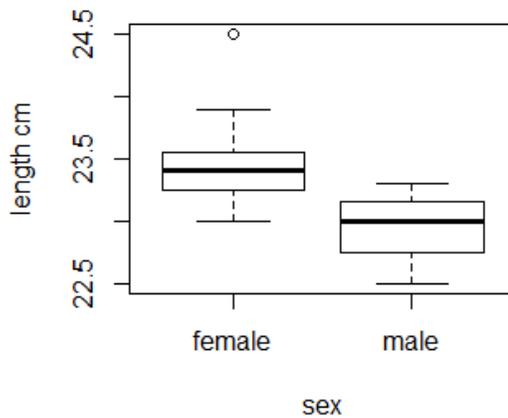


Figure 40: Box and Whisker plot of length distribution of males and females in the five year old age group.

A summary of results from all age groups for length at age for males and females is provided in Table 6.

Table 6: mean length at age for females and males of different age groups

Age	Mean Length		P value
	Females	Males	
0	15.78	15.68	0.793
1	16.5	17.21	0.266
2	19.93	19.47	0.03
3	21.2	21.8	0.003
4	22.57	22.16	0.03
5	23.68	22.66	0.054

4.5 Growth rate of Sardine

Growth coefficient (K) from the Gulland and Holt plot obtained was -0.3732 and L_{∞} was 26.606 cm, $t_0 = -3.45$. A strong relationship exists between growth rate and mean length at age ($R = 0.84$) (Figure 41). The Von Bertalanffy growth equation for sardine was, $L(t) = 26.6068*[1-\exp(-0.3901*(t-(-3.4524))]$ (Figure 42). Growth parameters obtained from the Gulland and Holt plot for males were: $a = 7.0205$, $b = -0.2732$, $K = 0.3189$, $L = 25.7143$ cm, (Figure 41), and the estimated $t_0 = -2.4079$. The VBG growth equation for males was, $L(t) = 25.7143*[1-\exp(-0.3189*(t-(-2.4079))]$ (Figure 44). Parameters obtained from the Gulland and Holt model for females were: $a = 5.593$, $b = -0.2017$, $K = 0.2256$, $L_{\infty} = 27.6733$, $t_0 = -3.45$ (Figure 45). VBG growth equation for females was, $L(t) = 27.6733*[1-\exp(-0.2256*(t-(-2.3737))]$ (Figure 45).

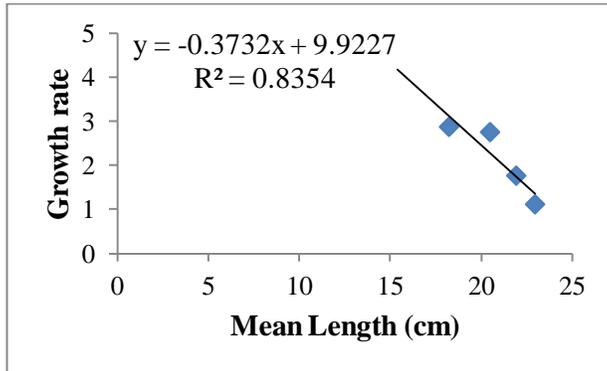


Figure 41: Gulland and Holt growth plot for sardine.

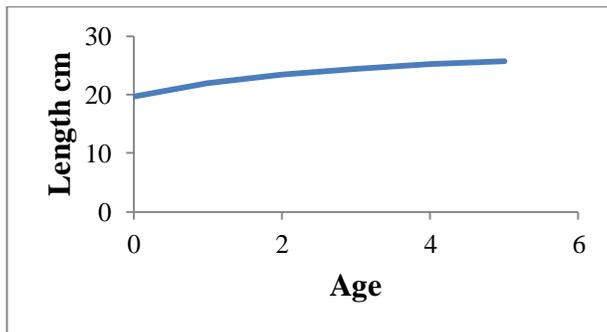


Figure 42: Von Bertalanffy growth plot for sardine with Length plotted against age.

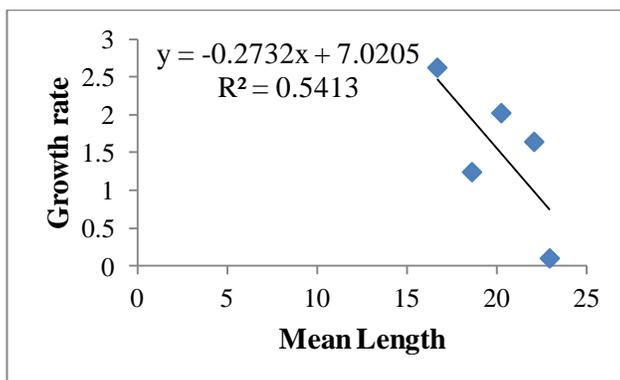


Figure 43: Gulland and Holt growth plot for males.

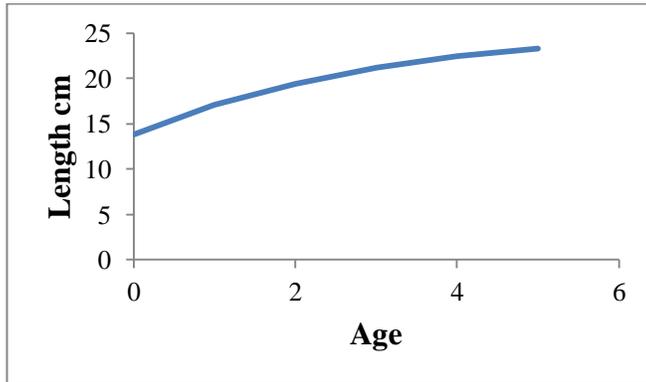


Figure 44: VBG growth curve for males.

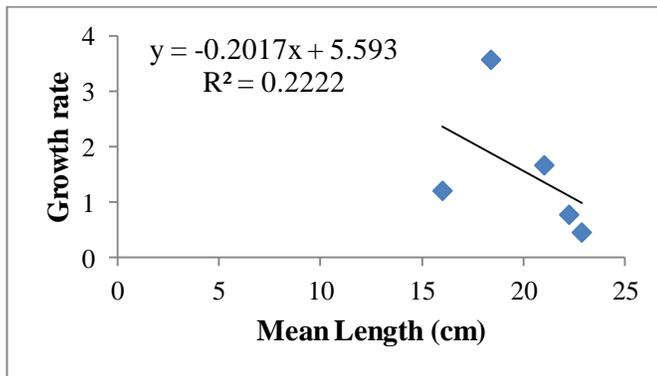


Figure 45: Gulland and Holt plot for females.

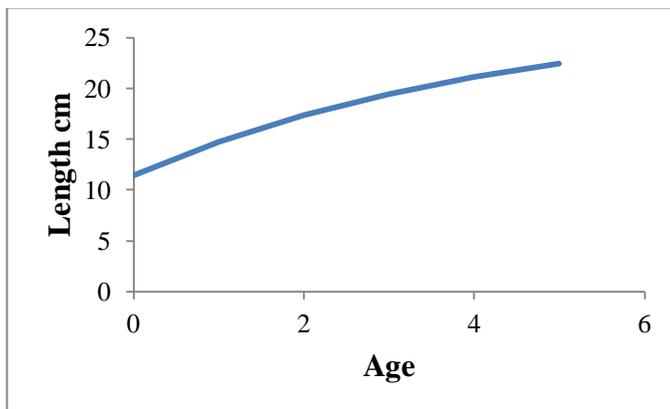


Figure 46: VBG growth curve for females.

4.6 Determination of the dominant age group in the population

The dominant age group observed was the one year old age group, comprising 36% of the sample, with 260 observations (Figure 47)

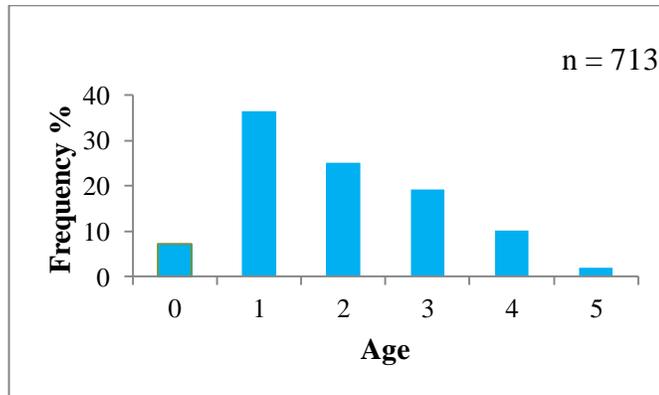


Figure 47: Age frequency graph.

Chapter 5

Discussion

5.1 Length distribution of sardine

The minimum and maximum lengths of sardine are 11 cm and 25 cm, respectively. These lengths were also composed of the lowest number of fish sampled. This is probably due to the fact that smaller fish could easily escape from the net and recruits are always caught first, reducing the numbers which could reach large sizes. Length frequency distributions indicated that fish of intermediate size (17 cm and 22 cm) made up most of the sample. This length range represents the recruits, when fish reach the length at which they are first caught or enter the fishery (Boyer et al., 2001). Length frequency distribution is bimodal, with mode at 17 cm and at 22 cm. Research survey data often showed length frequency distributions with more than one mode, indicating spatial variability in Length frequency distribution which results in separation of cohorts (Fossen et al., 2001). These are the mean lengths for the one year old age group and the three year old age group obtained from modal regression analysis. Lorenzen (2008) stated that when fishing mortality is high recruits dominate as a regulatory mechanism from the population in response to fishing pressure. This is further support for the fact that the sardine population is still experiencing high fishing mortality. However, it should be noted that recruitment is a complex process regulated by multiple mechanisms in fish populations (Lorenzen, 2008).

The relationship between otolith radius and fish length is highly significant ($p < 0.05$) with a strong correlation ($R = 0.73$). Although the correlation is not as strong as that of Thomas (1983) who found $R = 0.991$, it gives an indication of proportionality in growth of otoliths and fish length.

The length distribution of age groups indicated overlaps between all ages. Overlapping becomes more extreme at older age classes (Yaremko, 1996), and this was demonstrated in the results where the older (3-5 year) classes shared a similar, narrow length distribution. This is due to a slower growth rate as fish grow older (FAO, 1998).

5.2 Age classes of the current sardine population

Fossen et al. (2001), stated that splitting length frequencies of northern Benguela sardine in the 1990s showed a few fish which were two years and older. For this study, it was observed that a total of six age groups exist within the sardine population as opposed to what was found with LFA, which never estimated beyond three years. Estimates obtained from length frequencies were probably the only reliable estimates which could be used due to the depleted state of the stock, even though the results were obtained without validation. The difference in age estimates of this study and previous LFA estimates strengthens the general assumption that LFA is an unreliable method in estimating the age of older fish. According to Fossen et al. (2001), there was a higher proportion of the zero year age group in the 1990s and 80s and age groups beyond two year olds were very few. Results from the current study indicated that the sardine fish population on the Namibian coast consists of older fish and less juveniles compared to

the findings of Fossen et al. (2001). However, the minimum length is a function of the mesh size used, since smaller fish could escape capture. The two year old age group contributed to 25% of all the fish caught in the sample and had a wide length distribution from 16.5 cm to 22.5 cm. Fish of this age are most commonly caught or harvested as they have attained the length at which fish enter the fisheries (Boyer et al., 2001).

The first ring with the lowest distance from the nucleus observed was (1.6 epu = 0.4 mm), also known as the secondary ring and the maximum was (6.8 epu=1.7 mm). A ring at this distance was almost always visible and it was used as the basis for allocating a first annual ring. The mean distance for the first secondary ring was difficult to allocate, as some otoliths had a very clear secondary ring and some had none at all. The appearance was inconsistent. Secondary rings also tend to disappear as fish get older (Thomas 1983), which was the case with most of the otoliths of bigger sized fish. Thomas (1985) validated that the first ring formed on fish of 13-15 cm, which were mainly juvenile fish. Otoliths from the one year old age group usually had a clear translucent zone near the edge of the otolith and most of those otoliths were in the 15-17cm length classes, only a few were found in 14 cm length classes and smaller. Annuli measurements from the nucleus also resulted in the first annual ring being allocated a distance ranging between 0.95 mm and 1.1 mm and the second annual ring allocated a distance of at least 1.20 mm. These observations concur with results obtained from McFarlane et.al. (2010), whereby the mean first annulus diameter was estimated at 0.92+ mm and the second annulus were 1.21+ mm. Most of the otoliths aged one (mostly occurring in the 15 cm – 17 cm length classes) were only allocated to the one year age

group and beyond, when those specific otoliths had a translucent zone which was at least ≥ 0.95 mm from the nucleus.

In older age groups, differences in otolith size become more apparent. These otoliths were much bigger than those of the one year old age group, clearly indicating that otoliths increase in size as fish grow older. Rings on most of the older aged otoliths were very faint, which is also a common occurrence for sardine species in the northern American pacific region as stated by Dorval et al. (2013) and McFarlane et al. (2010). It is very easy to consider the first visible ring on these otoliths as a secondary ring, because of the size of the otolith most first rings appear to be closer to the nucleus, when actually the distance is much bigger than anticipated. In this case, the measurement of annuli from the nucleus becomes indispensable. McFarlane et al. (2010) employed the mean annulus measurement method to determine location of the first and second annuli when they were not visible. Annuli distance from the nucleus could be used to allocate a translucent zone to a reasonable annual time. The method is subjective, but it is better than allocating an otolith to an age group simply based on the length of fish or size of the otolith.

5.3 Comparison of age distribution with length and weight

The relationship between length and weight of fish was very strong ($r = 0.98$) and the $b(3.32)$ constant is comparable with what is considered as the average constant for length weight relationships, which is 3.0, a case where weight maintains a constant proportion to length, Weatherly and Gill (as cited in Benedito-Cecilio et al., 1997,

pp.57). Males made up a bigger contribution to the fish caught than females, but they had a narrower length distribution with a mean length significantly lower than that of females ($p > 0.05$). The bigger size and lower abundance of females could be attributed to several factors such spawning, whereby according to Parker (2006), females maintain a higher weight so as to conserve energy during spawning. However environmental factors such as food availability and temperature in different areas could also play a role. The significant difference may indicate sexual dimorphism. According to Parker (2006), sardine females show a higher growth rate than their male counterparts. The higher significant mean length at age for females indicates females to be bigger than males for sardines in this sample. There was a significant difference in variances; therefore log transformation of length was applied. But variances were still found to be heterogenous ($p > 0.05$).

The relationship between age and length was strong, since all age groups had a correlation coefficient bigger than 0.7, except for the one year old age group ($r = 0.320$) and the five year old age group for which the model could not predict mean length at age and correlation. The weak correlation of one age group could be due to the fact that this group had a wide length distribution creating high variability in observed data from estimated points on the regression line. Number of observations in five year age group was probably too low for the model to form any trend. The four year old age group had the strongest relationship between age and length ($r = 0.988$), probably due to the narrow length distribution.

No significant differences in mean lengths between females and males of zero age group existed. Biologically, the zero age group is not expected to be composed of any matured specimens such as males and females, only juveniles. It is assumed that northern Benguela sardine only attain maturity at two years (MFMR, 2013b), and the zero age is not included in that estimate.

Mean length at age for females and males of one and five year age groups were the same. Within the age groups two, three and four, the mean length at age between females and males were significantly different, with females always attaining a higher length than males. However males always had a wider length distribution in all age groups indicating that males had a higher representation than females.

5.4 Comparison of age estimates from otolith zonation with previous age estimates

The mean length at age for age groups were as follows: zero age group (13.8 cm), one year old age group (16.75 cm), two year old age group (19.6 cm), three year old age group (21.43 cm), four year old age group (22.43 cm), five year old age group (23.38 cm).

The previous estimated length at age derived from catch at length data for the northern Benguela sardine were as follows: Zero age group (<16.5 cm), one age group (16.6 – 22.4 cm) 2+ age groups (22.5 cm) (Boyer et al., 2001). The previous estimated length at age for zero age groups and one year old age groups and estimates from this study fall in the same range, except for age group two and beyond, for which the estimated mean length at age starts from (19.6 cm) for this research as opposed to the previous estimate

of 22.5 cm . In the current study the four and five year age groups had a mean length of 22.5 cm. Mean length estimates from this study could also be compared to average length at age of sardine for countries such as Morocco and Spain obtained in the 1970s (FAO, n.d.). The estimated length at age for two year age class for all the countries represented in the FAO (n.d.) paper, never went beyond 19 cm, and 22 cm - 23 cm was allocated to four and five year classes. Higher length estimates from Boyer et al. (2001) and lower length estimates from this study could mean that younger fish are reaching sexual maturity earlier than before, probably due to environmental factors or overfishing resulting in a continual decline in fish stock. Rochet (as cited in Fossen et al., 2001, p. 111) has stated that decline in fish biomass puts pressure on younger fish to mature early. However the differences could be due to the limitations of LFA in estimating older ages. This is further strengthened by results from a study conducted by McFarlane et al. (2010), indicating that a 22.6 cm sardine male was aged 4+ when using the otolith surface reading method. Thomas (1985) also stated that otolith age estimates were comparable to LFA estimates for younger age classes, but not for older age groups.

5.5 Determination of growth rates of males and females

The results obtained from Gulland and Holt showed a strong inverse regression relationship ($r = -0.83$) between growth rate and mean length of sardine, illustrating a decrease in growth rate as fish grow older. A strong negative correlation ($r = -0.754$) between fish length and growth rate was also obtained by Thomas (1985). According to Sparre & Venema (1998), length of fish may increase with age, however, growth

decreases with age, gradually getting to zero as fish reach asymptotic length as is shown by the VBG growth curve of sardine.

Growth parameters obtained as input data for VBG model for sardine: ($L_{\infty} = 26.6$ cm, $K = 0.39$, and $t_0 = -3.45$) were all different from those estimated by Thomas (1984) ($L_{\infty} = 21.2$ cm, $K = 1.19$, and $t_0 = -0.38$). The growth coefficient parameter (K) was estimated to be lower than the previous K (1.19), alluding a decrease in growth rate of sardine. In such a case, sardine is expected to have a higher lifespan since it will take a longer time for the fish to reach maximum length.

The estimated asymptotic length (26.59 cm) is larger than the maximum length (24.6 cm) of the observations. The model assumes that the fish will reach 26.59 cm if it were to grow indefinitely. Smith states that Benguela sardine grows to a maximum length of 28 cm, another estimate is 23 cm stated in Armstrong (1989).

The predictive capacity of Gulland and Holt model was not very strong for males ($r = 0.54$). Females showed an even less predictive capacity ($r = 0.22$). The higher K value estimate for males (0.3188) and a lower K estimate for females (0.2256) however, indicates a higher growth rate for males than females. This could have been derived from the differences in composition between females and males in different age groups. The ratio of males and females in different age groups is not the same, as Younger age groups such as zero, one and two are comprised of more males than females, while the older age groups such as four and five have more females than males. According to Yaremko (1996), younger fish experience faster growth rates compared to adults. This

also implies that a younger population of sardine is composed of more males than females.

5.6 Determination of the dominant age group in the population

The age group which was most dominant was the one year old age group followed by age group two. Age group one had a very weak relationship with length, meaning the observations do not have a good fit to the regression line. This could be due to the wide length distribution of the one year age group. Mean length at age for age group one was about 17 cm and males and females in this group have a length distribution between 14 cm and 22 cm, placing this group around 19 cm which is regarded as recruits as stated by Boyer et al. (2001). Jensen (1990) stated that when fishing pressure and fishing mortality is high fish populations compensate through increased survival of young and a decrease in age at maturity.

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

The relationship between otolith radius and length of fish is strong, although weaker compared to the estimate from Thomas (1985).

The number of age classes estimated from this study was six in total. The zero age class and the five year old age class were not as abundant in the samples compared to the other age groups. Allocation of age groups is very subjective and it depends on the reader and the type of method applied. Measurements of radii make things easier as a pattern is formed and it helps to differentiate 'false' rings from 'true' rings. As a rule of thumb, closer rings at the edge of the otolith are most often not 'false' rings, but they could be false rings when they are closer to the nucleus.

Opaque zones are much wider in younger age groups and get narrower as age increases, since fish are assumed to grow faster at a younger age and slower as they reach maturity (Yaremko, 1996).

Length distribution of sardine ranged between 11 cm and 24 cm. The mean length for age groups were as follows: zero year old age group(13.8 cm), one year old age group(16.75 cm), two year old age group(19.6 cm), three year old age group(21.43 cm), four year old age group(22.43 cm), and five year old age group(23.38 cm). All these mean lengths were significantly different from each other.

Estimates of otolith zonation length at age obtained from this study and those obtained by Thomas (1985) were more comparable or precise than length at age estimated from otoliths and those obtained through LFA.

Correlation of age groups with length was very strong except for the five year old age group and the one year old age group which had a very weak correlation between length and age ($r = 0.32$)

Guland and Holt estimates produced a higher growth rate for males, indicative of younger fish composed of more males than females as shown in the results of length at age between males and females. In contrast, mean length at age and mean length at weight for females was always higher than that for males with the exception of the zero and one year olds for which there was no significant difference in mean length at age between females and males.

Growth rate estimates of VBG models were not similar to those obtained by Thomas (1984), however mean length at age was almost similar to all estimates from Boyer et al. (2001) and Thomas & Armstrong (1987).

The dominant age group for this study was the one year old age group, and although there was high variability within the observations, this year class had a mean length which was comparable to previous mean length estimates of one year age group for sardine.

It was discovered during this study that age determination through otolith ageing method could be considered as a reliable method for ageing of the northern Benguela sardine. Although otoliths contain false rings and some rings appear too faint, there are ways of

finding patterns which could aid in allocating an otolith to an age group. The technique of measurement of the distance of annuli from the nucleus is very useful and could possibly be a reliable method if the mean distance of the secondary ring could be determined.

Overall, length at age of the northern Benguela sardine could have been underestimated in the past, even for the younger age groups which are considered to be accurately estimated with the LFA method. Ageing method techniques such as polishing of otoliths might just lead to a whole new dimension for age estimates for the Benguela sardine, possibly refuting all previous age estimates and creating new and improved methods for management of the species.

Input data for the stock assessment model is based on age derived data, which is based on length of sardine as a determining factor. Inaccurate length at age data could lead to underestimates or overestimates of cohorts within the sardine population with specific reference to the recruits and spawning stock. This will lead to poor management practices as predictions of future populations are based on estimates derived from the present population, and as was stated by Tyler et al. (1989), underestimation of ages would overestimate mortality rates, leading to overestimation of recruitment and possibly higher allocation of catches resulting in overfishing in the long run.

6.2 Recommendations

Data should be collected over different seasons so as to assess temporal variation in age distribution and to establish any differences in biological parameters. Precision is crucial

in reading otoliths, at least more than two readers should each read the same otoliths at least twice, because it becomes easier to read when it is done consistently, patterns begin to form.

Validation of rings was done according to Thomas (1983), but only for small fish, perhaps bigger fish could also be used for otolith age validation through the counting of daily rings. The distance of the first annual ring should be known so there can be consistency in the allocation of age, and it is especially important when the rings are faint or when there are too many multiple rings. Another method of storing otoliths right after extraction should be employed, because water damages otoliths over time, or else mounting should be done right after surveys.

Data should be collected on a time series basis, so that environmental data can be collected at each sampling area in order to assess if there is any cause and effect between environmental conditions and biological parameters of fish such as length and weight. Fisheries managers should make sure that more research on ageing of the northern Benguela sardine by means of otoliths is conducted in order to assess changes in length at age for each age group and to derive reliable and accurate length at age data. Any changes in age structure should be incorporated into stock assessment models. Other methods of ageing such as polishing should be adopted to test for the most reliable otolith ageing method.

Chapter 7

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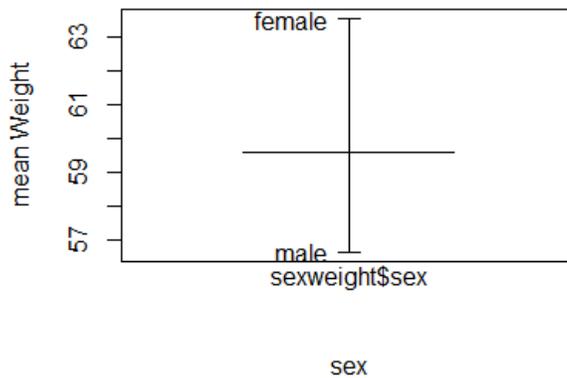
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Chapter 8

Appendices

Appendix 1: Comparison of mean weight of males and females



Appendix 2: Model output for mean weight

```

Model1<-aov(log(sexweight$weight)~sexweight$sex)
> summary.aov(Model1)

```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sexweight\$sex	1	1.56	1.5635	8.174	0.00439 **
Residuals	613	117.26	0.1913		

Appendix 3: Weight estimates

	Min	1 st Qu.	Median	Mean	3 rd Qu	Max
Females	17.41	49.83	66.70	66.03	83.40	138.30
males	47.49	54.61	56.62	56.62	58.92	65.38

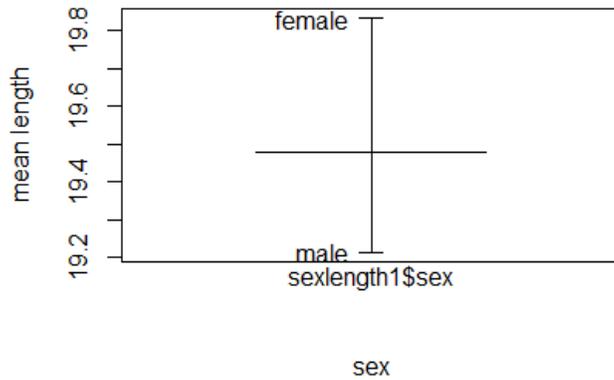
Appendix 4: Shapiro-Wilk normality test for weight of males and females

Shapiro test	W	P-value
Females	0.9959	0.7208
Males	0.9969	0.6541

Appendix 5: Homogeneity of variances test for length

Fligner-Killeen test of homogeneity of variances
data: log(sexlength1\$length) by sexlength1\$sex
Fligner-Killeen:med chi-squared = 1.5206, df = 1, p-value = 0.2175

Appendix 6: Comparison of mean length for males and females



Appendix 7: Model output for mean length

```
Model1<-aov(log(sexlength1$length)~sexlength1$sex)
> summary.aov(Model1)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sexlength1\$sex	1	0.141	0.14120	8.466	0.00375 **
Residuals	613	10.224	0.01668		

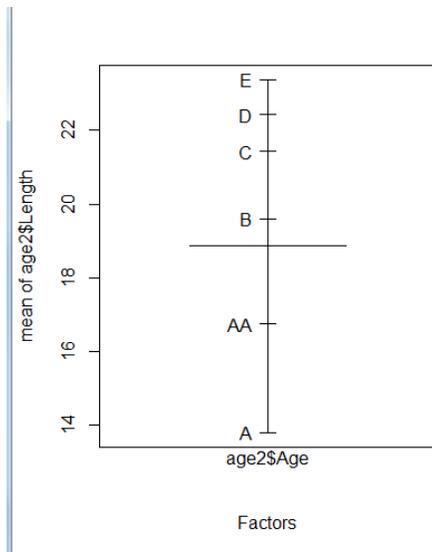
Appendix 8: Length estimates of males and females

	Min	1 st Qu.	Median	Mean	3 rd Qu	Max
Females	11.73	18.22	19.80	19.94	21.64	26.27
males	11.38	17.64	19.05	18.97	20.32	24.41

Appendix 9: Shapiro-Wilk normality test for length of males and females

Shapiro test	W	P-value
Females	0.9969	0.8887
Males	0.9961	0.5405

Appendix 10: Comparison of means between age groups



Appendix 11: Model output for different age groups

summary.aov(Model1)						
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
age2\$Age	5	4660	932.0	519.6	<2e-16	***
Residuals	707	1268	1.8			

Appendix 12: Tukey test

Tukey multiple comparisons of means Age groups				
95% family-wise confidence level				
Fit: (Length ~ age)				
	diff	lwr	upr	p adj
AA-A	2.9581073	2.3672695	3.548945	0.0000000
B-A	5.8031910	5.1906069	6.415775	0.0000000
C-A	7.6351971	7.0028306	8.267564	0.0000000
D-A	8.6361370	7.9335511	9.338723	0.0000000
E-A	9.5845714	8.4273025	10.741840	0.0000000
B-AA	2.8450837	2.4730389	3.217129	0.0000000
C-AA	4.6770898	4.2733010	5.080879	0.0000000
D-AA	5.6780297	5.1712901	6.184769	0.0000000
E-AA	6.6264641	5.5764978	7.676431	0.0000000
C-B	1.8320061	1.3970170	2.266995	0.0000000
D-B	2.8329460	2.3010108	3.364881	0.0000000
E-B	3.7813804	2.7190248	4.843736	0.0000000
D-C	1.0009399	0.4463381	1.555542	0.0000048
E-C	1.9493743	0.8754901	3.023259	0.0000041
E-D	0.9484344	-0.1682423	2.065111	0.1483195

Appendix 13: Length estimates for males and females in the zero age group

	Min	1 st Qu.	Median	Mean	3 rd Qu	Max
Females	15.11	15.79	15.84	15.78	15.86	16.31
males	13.80	15.05	15.61	15.65	16.24	17.29

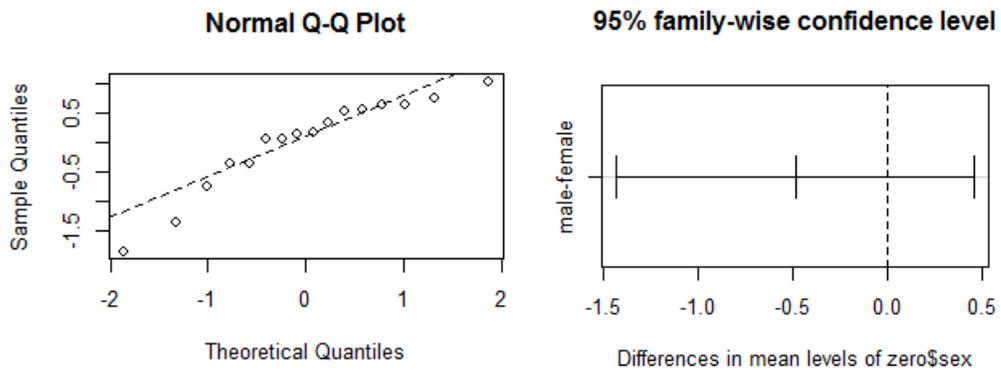
Appendix 14: Shapiro-Wilk normality test for length of males and females in the zero age group. No significant difference females ($p = 0.98$), males ($p = 0.2145$).

Shapiro test	W	P-value
Females	0.8561	0.2145
Males	0.9823	0.9775

Appendix 15: Homogeneity of variances test for zero age group

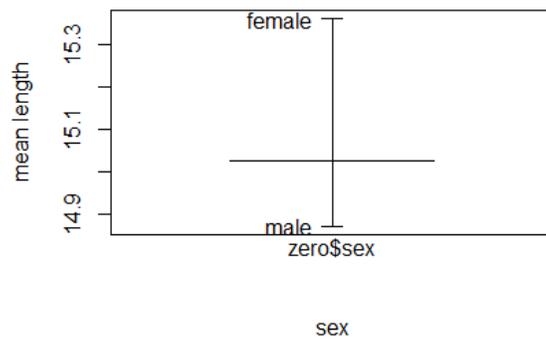
Fligner-Killeen test of homogeneity of variances	
data: zero\$length by zero\$sex	
Fligner-Killeen:med chi-squared = 1.1735, df = 1, p-value = 0.2787	

Appendix 16: Model output for males and females in the zero age group



Appendix 17: Comparison of means for males and females in the age group zero

summary(Model1)					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sex	1	0.816	0.8162	1.224	0.287
Residuals	14	9.334	0.6667		



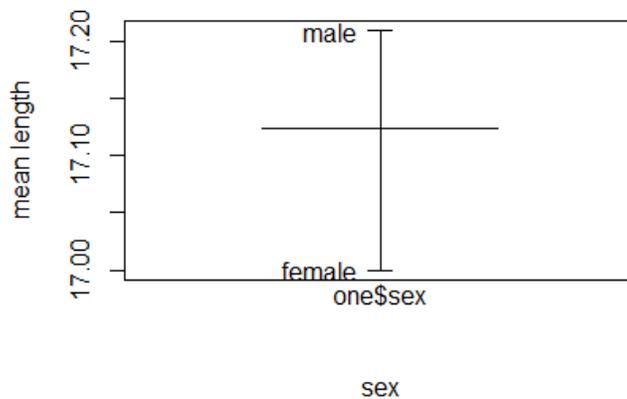
Appendix 18: t-test for males and females of zero age group

Two Sample t-test	
data: females and males	
t = 0.2698, df = 14, p-value = 0.7913	
alternative hypothesis: true difference in means is not equal to 0	
95 percent confidence interval:	
-0.9265347	1.1931473
sample estimates:	
mean of females	: mean of males
15.78111	15.64781

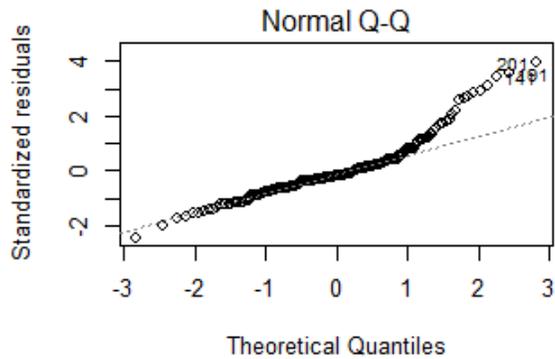
Appendix 19: Homogeneity of variances test for males and females in the one year old age group

Fligner-Killeen test of homogeneity of variances	
data: one\$length by one\$sex	
Fligner-Killeen:med chi-squared = 0.3343, df = 1, p-value = 0.5632	

Appendix 20: Comparison of means for males and females in the one year old age group



Appendix 21: Model output for males and females in one year old age group



summary.aov(Model1)					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
one\$sex	1	2.2	2.196	1.239	0.267
Residuals	205	363.3	1.772		

Appendix 22: Tukey test for age group one

TukeyHSD(Model1)					
Tukey multiple comparisons of means					
95% family-wise confidence level					
Fit: aov(formula = length ~ sex)					
sex					
	diff	lwr	upr	p adj	
male-female	0.2097561	-0.1617665	0.5812787	0.2669528	

Appendix 23: Length estimates of males and females in age group one.

	Min	1 st Qu.	Median	Mean	3 rd Qu	Max
Females	14.45	15.72	16.46	16.50	17.12	19.60
males	15.05	16.56	17.21	17.24	17.94	19.85

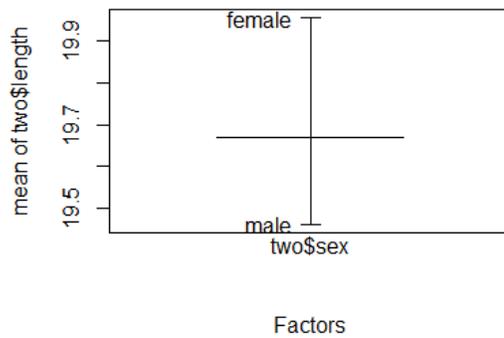
Appendix 24: Shapiro-Wilk normality test for males and females length of age group one. No significant difference females ($p=0.9318$), males (0.9874).

Shapiro test	W	P-value
Females	0.9929	0.9318
Males	0.9874	0.3164

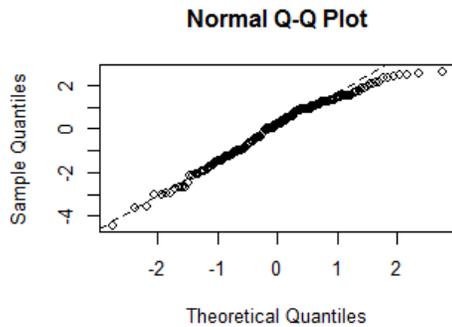
Appendix 25: Homogeneity of variances test for males and females in age group two

Fligner-Killeen test of homogeneity of variances
data: two\$length by two\$sex
Fligner-Killeen:med chi-squared = 1.3668, df = 1, p-value = 0.2424

Appendix 26: Comparison of means for males and females in age group two



Appendix 27: Model output for males and females in age group two



```
plot (Model1)
> summary(Model1)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
two\$sex	1	10.2	10.156	4.773	0.0303 *
Residuals	170	361.7	2.128		

Appendix 28: Tukey test for age group two

Tukey multiple comparisons of means				
95% family-wise confidence level				
	diff	lwr	upr	p adj
male-female	0.2097561	-0.1617665	0.5812787	0.2669528

Appendix 29: Length estimates of males and females in age group two

	Min	1 st Qu.	Median	Mean	3 rd Qu	Max
Females	16.68	19.04	19.93	19.90	20.70	22.95
males	15.34	18.66	19.47	19.56	20.50	23.35

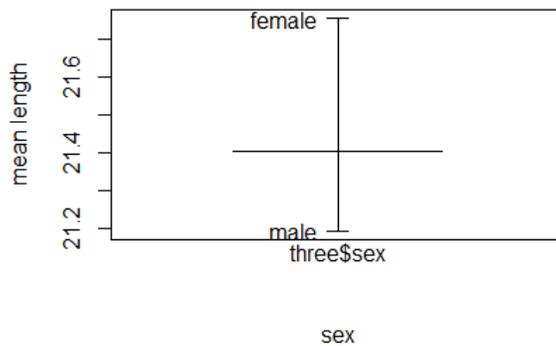
Appendix 30: Shapiro-Wilk normality test for lengths of males and females in age group two. No significant difference females ($p = 0.9318$), males ($p = 0.9874$).

Shapiro test	W	P-value
Females	0.9869	0.6631
Males	0.9874	0.03

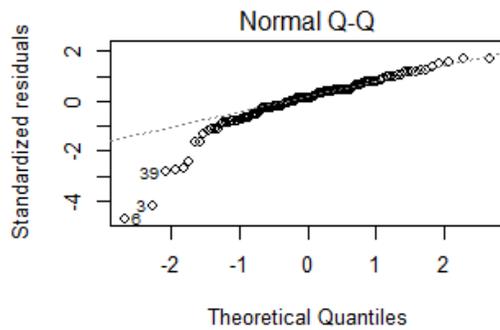
Appendix 31: Appendix 31: Homogeneity of variances test for males and females in age group three.

Fligner-Killeen test of homogeneity of variances
data: three\$length by three\$sex
Fligner-Killeen:med chi-squared = 0.3369, df = 1, p-value = 0.5616

Appendix 32: Comparison of means test for males and females in age group three



Appendix 33: Model output for males and females in age group three



summary(Model1)						
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
three\$sex	1	9.81	9.814	8.938	0.00334	**
Residuals	131	143.84	1.098			

Appendix 34: Tukey test for age group three

TukeyHSD(Model1)				
Tukey multiple comparisons of means				
95% family-wise confidence level				
Fit: aov(formula = length ~ sex)				
sex				
	diff	lwr	upr	p adj
male-female	-0.5608193	-0.9319155	-0.189723	0.0033371

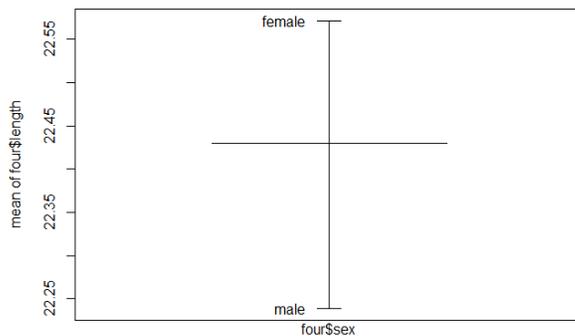
Appendix 35: Length estimates of males and females in age group three

	Min	1 st Qu.	Median	Mean	3 rd Qu	Max
Females	20.26	21.14	21.76	21.80	22.6	24.4
males	19.22	20.57	21.22	21.22	21.97	23.3

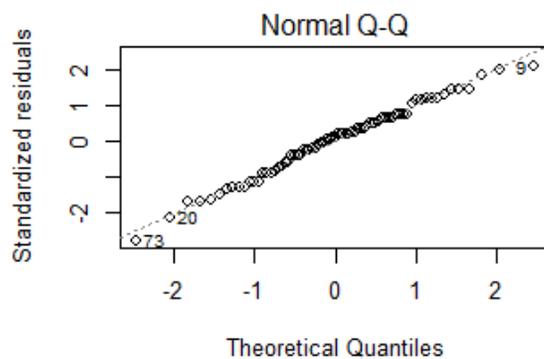
Appendix 36: Shapiro-Wilk test for normality for lengths of males and females in age group three. No significant difference females ($p = 0.46$), males ($p = 0.72$).

Shapiro test	W	P-value
Females	0.9837	0.715
Males	0.9852	0.46

Appendix 37: Comparison of means for males and females in age group four.



Appendix 38: Model output for males and females in age group four



Appendix 39: Tukey test for age group four

summary.aov(Model1)						
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
four\$sex	1	1.974	1.9744	4.707	0.0334	*
Residuals	71	29.779	0.4194			

TukeyHSD(Model1)				
Tukey multiple comparisons of means				
95% family-wise confidence level				
Fit: aov(formula =length ~ sex)				
sex				
	diff	lwr	upr	p adj
male-female	-0.3327189	-0.6384901	-0.02694771	0.0333802

Appendix 40: Length estimates of males and females in age group four.

	Min	1 st Qu.	Median	Mean	3 rd Qu	Max
Females	20.89	22.25	22.58	22.57	22.90	24.16
males	20.42	21.87	22.15	22.16	22.66	23.56

Appendix 41: Shapiro-Wilk test for normality for lengths of males and females in age group four. No significant difference females ($p = 0.77$), males ($p = 0.24$).

Shapiro test	W	P-value
Females	0.9785	0.7694
Males	0.9659	0.2399

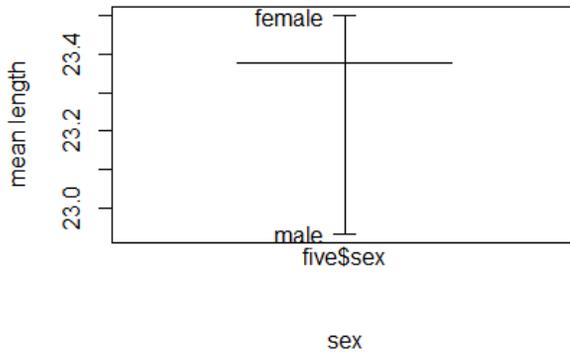
Appendix 42: Homogeneity of variances test for males and females in age group five.

Fligner-killeen test of homogeneity of variances
data: five\$length by five\$sex
Fligner-killeen:med chi-squared = 0.0851, df = 1, p-value = 0.7706

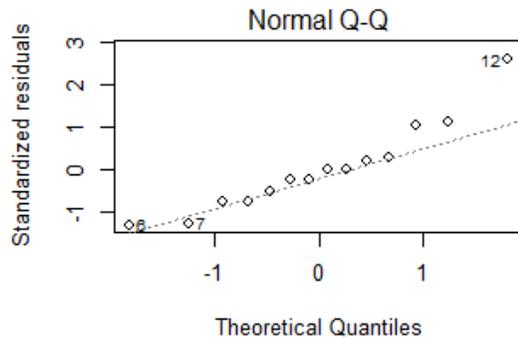
Appendix 43: Tukey test for age group five

TukeyHSD(Model1)				
Tukey multiple comparisons of means				
95% family-wise confidence level				
Fit: aov(formula =length ~ sex)				
sex				
	diff	lwr	upr	p adj
male-female	-0.5666667	-1.144096	0.01076267	0.0537632

Appendix 44: Comparison of means for males and females in age group five



Appendix 45: Model output for males and females in age group five



summary.aov(Model1)						
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
five\$sex	1	0.7569	0.7569	4.572	0.0538	.
Residuals	12	1.9867	0.1656			

Appendix 46: Length estimates of males and females in age group five

	Min	1 st Qu.	Median	Mean	3 rd Qu.	Max
Females	23.23	23.40	23.68	23.73	24.10	24.29
males	22.13	22.39	22.66	22.62	22.86	23.06

Appendix 47: Shapiro-Wilk test for normality for lengths of males and females in age group five. No significant difference females ($p = 0.16$), males ($p = 0.84$).

Shapiro test	W	P-value
Females	0.8947	0.1593
Males	0.9933	0.8431

Appendix 48: t-test for age group five

Two Sample t-test	
data: females and males	
t = 1.0005, df = 12, p-value = 0.3368	
alternative hypothesis: true difference in means is not equal to 0	
95 percent confidence interval:	
-0.8288226	2.2363497
sample estimates:	
mean of females :	mean of males
23.50823	22.80447

Appendix 49: FAO (n.d.) average length by age group, in catches by country

Country	Fishing zone	Years of observation	Age					
			I	II	III	IV	V	VI
Morocco	A	1973-1978	16.2	17.8	19.2	20.0	20.8	
Spain	B	1975-1978	14.8	18.8	20.4	21.2	21.7	22.0
USSR ¹	C	1971-1977	12.1	11.8	20.7	22.1		
Poland	C	1973-1976 (2nd quarter)	14.2	17.4	19.7	21.2	22.3	23.0

Appendix 50: Gulland and Holt linear regression data for sardine

Age	Length	growth rate	mean length
years		y	x
0	13.98		
1	16.85	2.87	15.415
2	19.60112	2.751124	18.22556
3	21.36496	1.76384	20.48304
4	22.47945	1.114489	21.92221
5	23.42857	0.949119	22.95401

Appendix 51: Gulland and Holt linear regression data for males and females

males				
	Age	Length	growth rate	mean length
	years		y	x
	0	15.38		
	1	18	2.62	16.69
	2	19.24	1.24	18.62
	3	21.26	2.02	20.25
	4	22.9	1.64	22.08
	5	23	0.1	22.95

Appendix 52: Gulland and Holt growth estimates for females

FEMALES				
	Age	Length	growth rate	mean length
	years		y	x
	0	15.4		
	1	16.61	1.21	16.005
	2	20.18	3.57	18.395
	3	21.85	1.67	21.015
	4	22.63	0.78	22.24
	5	23.09	0.46	22.86