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AGE DETERMINATION OF CAPE HORSE MACKEREL (*TRACHURUS CAPENSIS*) USING OTOLITHS

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ABSTRACT

The Cape horse mackerel (*Trachurus capensis*) is one of the important commercial fish species of Namibia. The fishery is assessed using an age-structured production model (ASPM), which requires catch at age data that can be derived from the otolith microstructure. For this study, a total of 1604 otolith were randomly selected from annual acoustic surveys conducted in February or March of 2011, 2012, 2014 and 2015, covering the coast of Namibia. Whole otoliths were examined under a binocular microscope. Age was determined by identifying and counting annuli on otoliths. Annuli consisted of opaque and translucent zones, each of which were interpreted as one year of growth. To estimate the age of the fish, translucent zones were counted. Eight age classes were determined, ranging from 0 to 7 year olds. Most fish belonged to the age 3 year class and fewest fish belonged to the age 7 year class. In young fish (0 to 4 year olds), annuli were relatively clear as the zones were wide and distinctive, however in older fish the growth zones were closely packed and often indistinct. Corresponding length data were combined with derived ages in order to construct an age-length key for Cape horse mackerel. The age-length key showed a wide variation in length at age data.

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1 INTRODUCTION

Fisheries contribute significantly to national economies worldwide. Fish provide high quality protein and vital nutrients to many. In many developing states, fish is the most important protein source. In addition to supplying food, marine capture and aquaculture production provides for livelihoods for fishermen and fish processors, and fish sales provides foreign currency from the export of fishery products, contributes to gross domestic product (GDP) and boosts government revenues through fishing agreements and taxes (FAO, 2014).

Namibia has one of the most productive fishing grounds in the world, resulting from the Benguela Current system, one of the four eastern boundary upwelling systems in the world. Other upwelling systems are off North-West Africa, and off the coasts of California and Peru. The Benguela current is characterised by strong seasonal variations with higher temperatures in during summer and autumn (December- March) and lower temperatures during winter and spring (August- November) (Kreiner, 2015). Namibian waters are characterised by a high level of production and abundant resources that promote the production of plankton, driven by the intense upwelling in the region, supporting many commercially valuable fish (Boyer and Hampton, 2001).

The marine fishery sector in Namibia is industrial. The demersal fishery mainly targets hake, monkfish, sole, snoek and kingklip and the pelagic fishery comprises of the midwater trawlers that target horse mackerel and purse seiners that target pilchard, juvenile horse mackerel and anchovy. Other industries include tuna, rock lobster and deep-sea crab fishing. The industry is dominated by the cape hake (*Merluccius capensis*), deep water hake (Merluccius paradoxus), cape horse mackerel (*Trachurus capensis*) and pilchard (*Sardinops sagax*) (MFMR, 2013).

In terms of value, hake is the most important commercial species in Namibia which is mainly exported to European markets, with the main markets in Spain. Cape horse mackerel is the most abundant commercially exploited fish species in Namibia with annual catches exceeding 300,000 tonnes (t) but due to its low market value, horse mackerel is the second highest economic contributor to the fishing industry, after the Cape hake (Kirchner *et al.*, 2010). Horse mackerel is mainly exported to Africa, with the main export market in the Democratic Republic of Congo (DRC) (MFMR, 2013). Commercial fishing and fish processing sector is rapidly growing in terms of employment, export earnings, and the contribution to gross domestic product (GDP). In 2012, the fishing industry contributed 3.9% to GDP, this was attributed to an increase in the fishing processing (MFMR, 2013).

Horse mackerel is one of the eight species regulated by total allowable catch (TAC) based on recommendations by scientist employed at the Ministry of Marine Fisheries and Marine Resources (MFMR). One of the main aims of the MFMR is to collect and provide reliable biological data, such as length, weight, sex, and age structure data for stock assessment and management for the commercial fish regulated by TAC.

Age estimation plays a vital role in the age-based assessments, such as the age-structured production model (ASPM) used for Namibian stocks. Age data forms the basis for calculations of growth rate, mortality rates and productivity, age is the most influential amongst all biological variables (Campana, 2001).

Knowledge of the age at maturity and age of the fish when caught is an important aspect of fishery assessment and management. Size limits and gear restrictions can be put in place to

protect fish until they have spawned at least once (Wallace and Fletcher, 2000). Errors in age estimation can adversely affect assessments and management decisions, which can result in overfishing or over exploitation of stocks (O'Sullivan, 2007).

Fish age can be estimated by counting and interpreting growth patterns that occur on calcified structures (otoliths, scales, fin rays, vertebrae). Otoliths are preferred for age estimation, because of the precision of age estimates based on annuli and the relative ease of otolith preparation and counting annuli (Campana and Thorrold, 2001).

The sagittal otoliths are used to determine the age of horse mackerel in Namibia. The otoliths are collected from fish caught during the acoustic surveys and samples from commercial catches. Otoliths have been collected over the years dating back from pre-independence (1990). Once analysed, Otolith are used to construct age-length keys (ALKs) which are subsequently used to estimate catch-at-age data and age structured indices for the stock assessment model. ALKs for horse mackerel were last updated in 2004, and the 2004 ALKs are still used for the present assessments.

The lack of ALKs for the Namibian horse mackerel, attributed to the lack of technical expertise required to age horse mackerel has been identified as one of the main shortcomings with the current stock assessment models. There is therefore a need for training on horse mackerel ageing and otolith interpretation to develop a reliable ageing method for cape horse mackerel.

1.1 Aims and Objectives

This project aims to estimate the ages of the Namibian horse mackerel using otoliths. In order to achieve this, the following specific objectives were pursued in this study:

- To be trained in horse mackerel otolith reading and age determination
- To develop criteria for horse mackerel otolith reading and annuli interpretation
- To build a reference collection of horse mackerel otoliths images for future readings
- To analyse otolith-derived age estimates and growth parameters by year

2 THE HORSE MACKEREL FISHERY AND MANAGEMENT

Horse mackerel is targeted by midwater fishery for whole rounded production, in the past purse seine fishery targeted juvenile horse mackerel for fish meal production and canned fish. Horse mackerel is also caught as by-catch in the demersal trawl for hake. The purse seine industry stopped in 2014, now only adult fish are harvested for whole round production by the midwater, and a portion of the midwater catch is used for value addition in factories owned by the purse seine operators (MFMR, 2013).

The horse mackerel fishery started in the early 1960s with an average catch of 50,000t which gradually increased to around 500,000t between 1978 and 1987. During this time, the fishery suffered from overfishing as a result of open access exploitation of the fishery. At that time, mainly foreign vessels from Eastern Europe and Cuba exploited the horse mackerel fishery. Increased catches in 1975 was attributed to the change in target species from hake and sardine to horse mackerel (Boyer and Hampton, 2001). In 1990, after Namibia gained independence, commercial stocks were depleted and average catches for horse mackerel decreased to around

325,000t. Average catches for 1990 to 2013 has been between 300,000t - 350,000t, with a very low catch of 125,000t in 2006. In 2015, the TAC for horse mackerel was set to 350,000t (MFMR, 2015).

In terms of TAC and catch, the horse mackerel fishery is the largest. In 2013, the subsector had 67 right holders, with total Namibian ownership of 85.82% and 14.17% was foreign owned; there are 14 vessels licenced to catch adult horse mackerel and there is a total of 1,866 employees both onshore and offshore (MFMR, 2013).

Since 1990, the stock has been monitored and managed by the Ministry of Fisheries and Marine Resources. Assessment of the stock is based on a combination of catch statistics from commercial vessels, providing fishery dependent information and acoustic surveys providing the fishery independent data. The information from the surveys is mainly used for the assessment of horse mackerel.

Horse mackerel is managed through total allowable catch (TAC) which is based on the calculation of maximum sustainable yield (MSY), and control of fishing effort: minimum mesh size limits of 60 mm in the midwater fishery and fishing is not allowed at depth shallower than 200m (BCLME, 2012). TAC for the horse mackerel fishery is determined each year by adjusting the TAC from the previous year depending on whether the size of the resources have increased or decreased. The rate of change of the TAC depends on two indices, the commercial catch per unit of effort (CPUE) and the abundance index from scientific surveys (Paterson and Kainge, 2014). These indices are then entered into an age-structured production model that estimates the size of the resource, by using these data along with proportions of fish caught per age class from both surveys and commercial catches (Paterson and Kainge, 2014). The assessment of the stock in 2015 shows that the biomass of the stock is declining, however the biomass is still above the MSY level (MFMR, 2015).

3 GENERAL BIOLOGY OF THE CAPE HORSE MACKEREL

Cape horse mackerel (*Trachurus capensis*) (Figure 1), a species in the family Carangidae is a long-lived species, reaching a maximum age of above 30 years (Abaunza *et al.*, 2003). Horse mackerel grows to about 60 cm length, with a common length range of 15 - 40 cm. They grow rapidly during the first years of life and much more slowly after age 3, reaching their length at first maturity at a length range of 20-33 cm (FishBase, 2016).



Figure 1. Horse mackerel (Trachurus capensis) (FishBase, 2016)

Cape Horse Mackerel has a continuous distribution from Port Alfred on the south-eastern coast of South Africa to the Northern border of the Benguela, Tombwa in Southern Angola (Axelsen *et al.*, 2004). In the past, it was assumed that the west and south coast horse mackerel

populations were separate stocks, however, these populations are genetically identical, and hence they are believed to be a single stock (Axelsen *et al.*, 2004). The South African and Namibian stock is separated by the strong Lüderitz upwelling cell, the two stocks are assessed and managed separately. The distribution and migration patterns of the Cape horse mackerel in Namibia is illustrated in Figure 2, with the highest concentration of horse mackerel found in the northern part of the country, between 17°00 S-20°00 S.

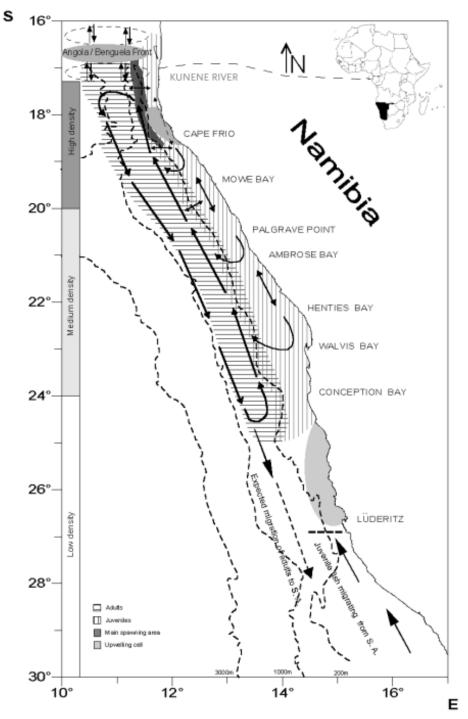


Figure 2. Generalized distribution and migration of Cape Horse Mackerel (Axelsen *et al.*, 2000).

Horse mackerel are highly migratory species and often the distribution of stocks overlap, they have distinct areas for spawning, feeding and over-wintering. Their migration is driven by water

temperatures and availability of food. In winter the horse mackerel form dense schools in deep waters, while in winter they become dispersed and migrate northwards with increasing temperatures (Abaunza *et al.*, 2003). In the Namibian waters, horse mackerel spawn continuously from October to March/April, with spawning in spring being isolated and patchy, whereas spawning in summer and early autumn is intense and widespread (Kreiner *et al.*, 2015). The Namibian Cape horse mackerel is mainly pelagic, but as fish become older they are seen in demersal waters. Adults form monospecific schools, but juveniles can be found in mixed schools with the sardine (*Sardinops sagax*), anchovy (*Engraulis capensis*) and round herring (*Etrumeus whiteheadii*) (Axelsen *et al.*, 2004). Adult and juvenile horse mackerel mainly feed in the pelagic zone with their diets varying according to their size. The juvenile diet consists mainly of small zooplankton such as copepods and adult diet consist of euphasids, copepods, lantern fish, gobies and polychaetes. The most active feeding period of horse mackerel is daytime, mainly at dusk (Axelsen *et al.*, 2004).

4 AGE DETERMINATION OF FISH AND ITS APPLICATION

Fishing affects stock size as it reduces abundance and changes the stock composition. When fishing mortality rates are high, there will be a fewer older fish in the stock and the age of the fish will be shifted towards younger fish and the length structure will be shifted to smaller fish. Catch composition data is required to estimate the relative abundance of different age classes or cohorts (Hoggarth, 2006). Age and length composition data from commercial fisheries or surveys are used in most assessments of major fish stocks. Age based stock assessment models require the number of fish caught for each age group as input (Maunder and Piner, 2014).

Annual recruitment fluctuations, cohort strengths, growth rates, natural mortality rates and age specific selectivity for fishing gear can be established for stock assessment models that need catch at age data (Wilhelm *et al.*, 2015). Ageing fish accurately is essential to the understanding of the dynamics of stocks. Ageing errors adversely affect the estimates of these parameters, and subsequently have detrimental consequence in fishery management (Wilhelm *et al.*, 2015). Life-history traits of fish provide a better understanding of the vulnerability of fish populations to exploitation and stocks can be assessed on how they respond to exploitation and management changes (Stocks *et al.*, 2011).

Fish age can be estimated directly by analysing the length- frequency distribution, where mean length of each group can be obtained or directly by counting annual growth rings on calcified structure (Pilling *et al.*, 2003). Assessment of individual ages and growth from calcified structures is more precise and gives more information than length frequency data (Panfilli *et al.*, 2002). The study of calcified structures not only allows for the estimation of age, but also the times and duration of the life history events from the periodic growth increments produced in these structures (Panfilli *et al.*, 2002).

The response of the fish to environmental conditions causes variations in growth rates of calcified structures over a period of time ranging from days to years. The determination of the age of fish occurs at both daily and annual scales. Daily ageing is usually used for recruitment studies and for studies based on younger fish, whereas annual ageing is done to support harvest calculations and population studies (Campana, 2001).

Estimating the age of fish is more difficult and costly than measuring length, hence ALKs that require a smaller sample, are used to estimate age composition of fish populations. To estimate

the age, a large random length sample is taken to obtain a length frequency of the landed fish. Age is derived from a stratified subsample taken from the length samples so that sampling is evenly allocated, covering both small and large fish (Gerritsen *et al.*, 2006). The aged sample is then raised to the total length frequency using an ALK, which consists of proportions at each length class. Age length classes can be specific to stations, strata or can be broadly applied to the entire survey areas (Jennings *et al.*, 2001). ALKs are usually resampled every year, as the sum of fish in each age class vary depending on annual recruitment strengths, and growth and mortality rates can also change over time (Hoggarth, 2006).

Otoliths are the most used structure for ageing bony fish, as they consistently record daily events in the early life stages and annular events throughout the fish lifespan. Otolith growth is continual and the annuli in older fish otoliths are visible. Otolith growth occurs in isolation from the physiological environment of the fish, as the otoliths are isolated within a semi permeable inner ear membrane bathed in a more regulated fluid. The crystalline structure of the otolith is aragonite, which is a useful indicator of age in fish. Otoliths are not reabsorbed under stressful conditions (Campana and Thorrold, 2001; Mendoza 2006).

Otoliths are small calcified structures that are located in the inner ears of teleost fish. Otoliths play an important role in the balance, detecting sounds and spatial orientation of the fish (Popper *et al.*, 2005). All teleost fish have three pairs of otoliths: sagitta, asteriscus, and lapilla, as shown in Figure 3. In most species sagitta is the largest amongst the three sets of otoliths, and the one mostly used for age determination studies (Wright *et al.*, 2001; Abaunza 2003).

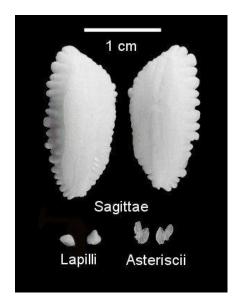


Figure 3. Images of three sets of otoliths of Cod, the saggitae, lapilli and asteriscii (Pétursdóttir, 2004)

Otoliths are three dimensional but do not grow at the same rate equally in all dimensions. The size and shape of the otoliths vary considerably among species, as shown in Figure 4 (Popper *et al.*, 2005; Mendoza, 2006).

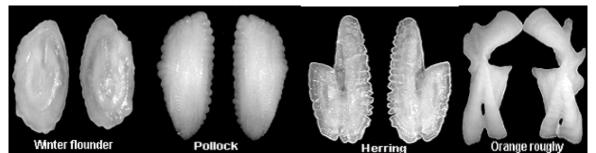


Figure 4. Examples of the diversity of otolith shapes amongst teleost, (Campana, 2001B).

Age estimation of the fish depends on visible changes in the otolith growth, which occur at four levels of resolution: daily increments; seasonal increments, annual increments and discontinuities (or growth checks) in the otolith's microstructure, that form under stressful conditions (Wright *et al.*, 2002). Otoliths are considered to give precise age estimates, due to the ease of preparation of material for age determination as well as the ease of counting annuli (Campana and Thorrold, 2001) Age estimation from otoliths in most larval and juvenile fish is possible, due to the presence of distinctive daily increments on the otolith (Mendoza, 2006).

4.1 Morphological Characteristics of the Horse Mackerel Sagitta Otolith

Abaunza (2003) described the shape of the horse mackerel Sagitta otolith as inconsistent, ranging from an oval to elliptical shape. The anterior end is more pointed than the posterior; the dorsal margin is irregular with a few cuts or lobes, the ventral margin sinuated. The posterior end is highly variable too. Rostrum is big and bluntly pointed, whereas the dorsal margin is smooth, and the ventral margin of the rostrum is lobed. Anti-rostrum is small; rounded or pointed; with a strongly convex surface smooth inside. Sulcus is moderately deep, with a very shallow area present dorsal of the sulcus with a slightly concave outside. Details are illustrated in Figure 5 below.

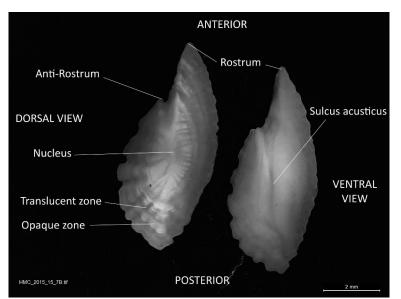


Figure 5. Morphological characteristic of a pair of horse mackerel sagittal otoliths (Shivute, 2016).

An annual increment of otoliths consists of an opaque and a translucent zone, interpreted as one-year growth (Wright *et al.*, 2002). To estimate the age of the fish, the opaque zones or translucent zones are counted. The opaque zone is denser than the translucent zone, as it

contains more calcium carbonate (Jennings *et al.*, 2001). The opaque zone restricts light, when compared to the translucent zone. Under transmitted light, the opaque zone appears dark and translucent zones appears bright, whereas under reflected light the opaque zones appear bright and the translucent zone appears dark. The translucent zone allows the passage of greater quantities of light than the opaque zone (Secor *et al.*, 1995).

The timing of zone formation varies among species, age classes and according to geographical location of the fish (Jennings *et al.*, 2001). Opaque zones are associated periods of rapid growth of the fish in summer, due to high temperatures and increased food supply. Translucent zones are associated with periods of slower growth, in adults this period is also associated with spawning (Jennings *et al.*, 2001). The formation of opaque and hyaline zones on the otoliths are also attributed to environmental variations. Therefore, otoliths can only be used for age estimation if growth is synchronised to periodic events (Moralis-Nin, 2000).

4.2 Age Validation

Before otoliths are used to age a fish, it is important to validate whether the opaque and translucent zones are deposited annually (Jennings *et al.*, 2001). Validation entails estimating the accuracy of the ageing method. Quantifying the error that is associated with the ageing method and it also indicates that the ageing method is sound and based on facts (Campana, 2001). Validation of the age of fish should be mandatory, especially for species where no age estimation have been done before. Panfili *et al.* (2002) identified four categories of validating methodologies:

- Indirect validations which considers a precise temporal marking on a calcified structure like otoliths to other growth marks; this method involves making markings and rearing.
- Semi-indirect validations, that entails observing time series of growth marks on a population.
- Indirect validation that entails comparing individual age estimates with statistical age estimates from length frequency distributions and,
- Verification, that involve multiple interpretations, which are obtained from readings of calcified structures.

It is important to distinguish between accuracy and precision of age estimates. Precision refers to the degree of reproducibility and thus relates to the variability between readers or between readings. Accuracy relates to the degree of closeness to the true value and thus relates to the departure from the true age (Morrison *et al.*, 2005). Investigations that involve determining the age of fish usually deal with the problem of precision, but comparatively little effort has been done on the problems of accuracy. Standard statistical techniques can be used to estimate the precision of age determination. More commonly is the use of a "percent agreement" comparison, whereby one set of readings is in agreement with another by a certain percent and ± 1 year by another percent, as illustrated in this study.

4.3 Interpretation Criteria for Cape Horse Mackerel

The criteria used to estimate the ages for this study was established by ICSEAF (1986), ICES (1999 and 2015) and the validation methods by Waldron and Kerstan (2001).

Horse mackerel ages are estimated by counting the number of paired growth zones. The annual growth zone (AGZ) of horse mackerel is described by ICES (1999) as one opaque and one

translucent. One pair of these alternating zones is called annulus (plural annuli). For horse mackerel an annulus is defined as the brightest contrast between the preceding translucent and subsequent opaque zone deposited in the following year. On whole otolith analysis, the annulus should be traceable on the whole otolith, with the exception of the dorsal- medial surface of the rostrum (ICES, 2015).

Waldron and Kerstan (2001) validated the ages of horse mackerel up to four years of age using two methods: daily increment counts and measurements of marginal increment widths. In the first method, whole otoliths were examined, and age was estimated by identifying and counting annuli and marginal increment widths were also measured and were used to identify ages of fish in the range of 0.4-4.3 years. In the second validation method, a scanning electron microscope was used to examine otoliths and estimated ages of the fish using daily increment counts. This study also confirmed that estimated ages that were read from the whole otoliths were generally correct up to age 4. They also confirmed the presence of false rings on the otolith structure which resembles the appearance of annuli, however most annuli could be distinguished from the false rings by their sharp images and high contrast.

4.4 Interpretation Difficulties

Cape horse mackerel otoliths have complicated ring structures making them difficult to age. Annual growth zones on the otolith do not always consist of only one opaque and one translucent zones, but double or multiple translucent zones with false rings, which are often inaccurately interpreted as true annual rings (Waldron and Kerstan, 2001).

Horse mackerel usually have a distinct pre-annual ring surrounding the first year's ring, called the juvenile ring, which is separated from the first translucent ring or joined with it, therefore forming a broad translucent zone (Karlou-Riga and Sinis, 1997). The presence of the juvenile ring can exacerbate the difficulties of age estimation, since juvenile rings can be misinterpreted as the first annulus (Waldron and Kerstan, 2001).

Frequently fluctuating environmental conditions as a result of upwelling in the pelagic zone where horse mackerel mostly inhabit, and fluctuating food availability are the cause of multiple ring formation on the otoliths of Cape horse mackerel (ICSEAF, 1986; ICES 1999). Multiple ring formation is most prevalent in juvenile fish as they experience drastic change in their life in terms of adopting to their environment until they reach age 1 and shortly after they reach maturity and start reproducing. Fat and energy capacity of horse mackerel drops significantly after spawning, therefore irregularities in the formation of increments during spawning causes false rings (Altigan and Bascanar, 2015).

5 MATERIALS AND METHODS

5.1 Sampling

The otoliths and data used for this study were collected during annual acoustic horse mackerel surveys that take place in summer (February or March). The survey is conducted by the Ministry of Fisheries and Marine Resources in Namibia. Samples were randomly selected from the 2011, 2012, 2014 and 2015 otolith samples. The aim of the annual horse mackerel survey is to determine abundance, spatial distribution, and demographic structure of the horse mackerel fish greater than 10 cm and small pelagic fish using acoustics combined with targeted trawling.

The survey covers the area from $17^{\circ}15$ to $25^{\circ}00$ ' S and a depth distribution from the coast (approximately 20m) to the offshore limit of the stock. The survey area is divided into two regions: inshore and offshore, separated by the 200m depth contour. The two regions are divided into three discrete strata and these strata signify areas of low, medium and high densities as previously observed, in total 6 strata, shown in Figure 6. The total number of stations in the survey is around 50.

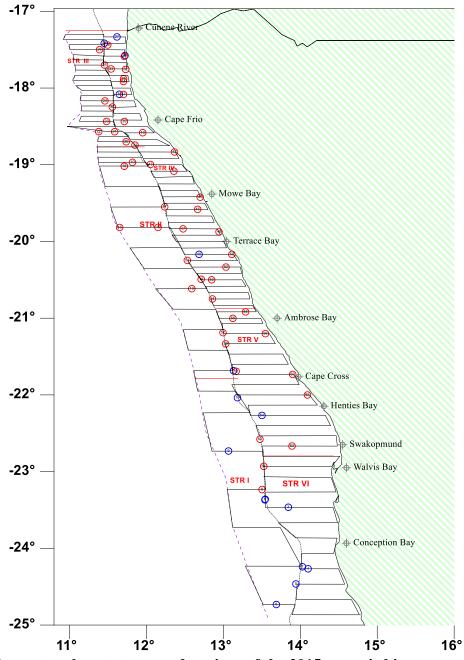


Figure 6. Survey tracks, stratums and stations of the 2015 acoustic biomass survey. Red circles show stations with horse mackerel and blue circles are the stations with no horse mackerel (Uanivi and Van Der Plas, 2015).

The sampling frequency in each stratum is determined based on the proportion of biomass in each of the strata during previous surveys, the recent catch distribution of commercial vessels and the current state of the stock are also considered when allocating transects to the strata. Bottom and mid-water trawls are conducted during the surveys.

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For each station, 50 otolith pairs were collected from a random sample, or ten otoliths representing each length class (1cm) of which 5 are male and 5 are female. Both sagittal otoliths were extracted from each fish using a tweezer.

After otoliths were removed they were stored in a vial containing distilled water that was clearly labelled with a permanent maker. All vials belonging to a station was stored together in a plastic Ziploc bag labelled with the year the survey was conducted and the station number. The vial was labelled with the station number, fish number and fish length. The otoliths were only cleaned after the survey ended. Otoliths were removed from the vials, rinsed with distilled water and adhering organic tissue was removed using tweezers. After cleaning, otoliths were returned to clean, labelled vials and allowed to air dry, then stored.

5.2 Data Available

The available biological data collected from the fish were:

- total length (0.1 mm accuracy)
- total weight (0.1 g accuracy)
- gutted weight (0.1 g accuracy)
- sex (M = 1/F = 2/J = 0) gonad maturity stage (1-7)
- gonad weight (0.1 g accuracy)
- stomach fullness (0-5) according to fullness scale on the scale of 0-5, where 0 corresponds to absolutely empty, 1: 1-25 %, 2: 25-50 %, 3: 50-75 %
 4: 75 -99%, and 5: 100 % (absolutely full or overfilled/ bulky)
- stomach weight (0.1 g accuracy)

5.3 Otoliths Used for Ageing

In total, 1,899 otoliths were examined for age estimation, however only 1,604 otoliths were aged. The remaining 295 otoliths were excluded for various reasons: broken otoliths (especially when both posterior and anterior tips were not attached); crystallised otoliths; majority of the otoliths were rejected because of sampling faults, such as no fish number on the vials or information written on vials that did not match the biological data. All otolith reading was done by the same person. The sample was randomly selected covering all six stratums of survey area. The number of otoliths aged is shown in Table 1.

		Total			
Stratum	2011	2011 2012 2014 2015		Total	
1	46	0	0	0	46
2	136	114	99	80	429
3	118	111	192	84	505
4	122	79	47	82	332
5	39	0	61	32	132
6	29	83	50	0	162
Total	490	387	449	278	1604

5.4 Age Estimation Method

- Observation: Both sagittal otoliths were observed in a binocular microscope inside a black plate and immersed in a clarifying solution (96% ethanol) prior to analysis. Refractory period was not needed for this study.
- Illumination: Reflected light was used. Translucent zones appeared dark and opaque zones bright.
- Magnification: The otoliths were observed with an initial magnification of 10x or reduced to 6.4x. Higher magnification was avoided as it resulted in over interpretation of the ages as the false rings can be misinterpreted as annuli.
- Reading axes: Otoliths were positioned with their distal surface turned up facing the light source and its proximal surface (*Sulculus acusticus*) turned down. In this position, the opaque and translucent zones were exposed to the light, the alternating opaque and translucent zones are visible, especially towards the anterior and posterior edges. During the analysis the otolith was moved around with a pair of tweezers to view it from different angles.

5.5 Age Estimation Criteria

The criterion used for determining the ages of the fish for this study was established by ICES (1999 and 2015); the ICSEAF otolith interpretation guide (1986) and the age validation studies by Waldron and Kerstan (2001). The date of birth is assumed to be 1 July (ICSEAF, 1986). One opaque and one translucent ring constituted an annulus which is characterised by the brightest gap between the preceding translucent and the subsequent opaque zone deposited in the following year. Annuli that were observed at the post-rostrum and the post-rostrum of the otolith was counted. Accurate age estimations of fish using otoliths requires a lot of experience, therefore annuli were identified and aged with the guidance of an ageing specialist at MRI, during the image analysis. The results of the estimated ages were entered into a database for further analysis.

5.6 Reference collections of otoliths

A reference collection of 300 otoliths was created. Age readings for the reference collection was carried out by two readers and disagreements were evaluated by a third reader. Images of aged otoliths was captured with a Leica IC80 HD camera and stored for the reference collection. Observation, illumination, magnification and reading axes were the same as in microscopic analysis, using reflected light, a black background, and immersed otoliths in clarification solution (96% ethanol). Magnification used was 1.0x, but for bigger otoliths the magnification was reduced to 0.8x. The images were calibrated, by adding a scale bar of 2 mm for all otoliths. The images were saved using a newly assigned sample identity number (e.g. HMC_2015_10_21, where:

- HMC represents the fish species code.
- 2015 represents the year the fish was captured.
- 10 represents the station number.
- 21 represents the fish identity number.

The colour of the images was changed to grey, enhancing the visibility of the growth zones. The translucent zones of the otoliths were marked using ImageJ software. Marked images will be used as a reference set for the age reader.

5.7 Quality Control

The precision of the age reader was tested with a blind test using 100 otoliths that were randomly selected and aged again by the same reader. Average percent error (APE) was used for comparisons of the two age readings and percent agreements, which is an index of ageing agreement precision (Campana, 2001), was calculated.

5.8 Growth

Growth is one of the most important measurable life history parameters for individual fish or species as growth relates with a number of life history traits including natural mortality (Vasconcelos *et al.*, 2006). Parameters of the von Bertalanffy growth equations describes fish life histories. Using the length and age data a combined age-length key was developed using the data of 2011, 2012, 2014 and 2015. The lengths of the fish were rounded to whole numbers and the total length distribution by age was fitted to the von Bertanlanffy growth function (VBGF) to estimate growth parameters.

The VBGF describes the relationship between age and length as follows:

 $Lt = L\infty [1 - e - k (t - t0)].$

where: Lt = mean is the fish length at age t (cm)

- $L\infty$, K and t0 are the parameters that that determine the shape of the growth curve
- $L\infty$ = asymptotic mean length (cm)
- k = body growth coefficient
- t0 = age at length 0

t = fish age (years from birth)

The body growth coefficient (k) describes the curvature of a growth curve while t0 acts as an adjustment factor moving the curve to the left or right (Vasconcelos *et al.*, 2006). A high k is associated with low age, size at maturity, high reproductive output, short life spans and low asymptotic length. On the contrary, species with a low k, have greater age and size at maturity, lower reproductive output, lower lifespans and greater asymptotic length (Jennings *et al.*, 2001).

5.9 Analysis of Age Data

The age data were combined with biological data collected during surveys. The data were analysed with R statistical software. Differences in age composition between the years and stratum was analysed. Growth parameters K, $L\infty$, and t were estimated using R statistical software.

6 **RESULTS**

6.1 Age Composition

Eight year classes including: 0, 1, 2, 3, 4, 5, 6, 7 age classes were determined during this study, as shown in Figures 7 and 8. For this study, the age 3 is the predominant age, which was concentrated in stratum 3 in 2014.

In the 2011 sample, all ages were represented, and it is worth mentioning that age 0 was only represented in the 2011 sample. Age 2 is most prevalent followed by age 1 and 3 that is equally represented in the sample.

In the 2012 sample, age 2 is most prevalent, followed by age 3. A relative strong year class of 3-year old fish appeared in stratum 3 representing 42% of the 2014 sample. Following the prevalent occurrence of 3 year olds in 2014, one would expect 2015 to be dominated by 4 year old fish, but this was not the case, age 3 fish dominated then sample.

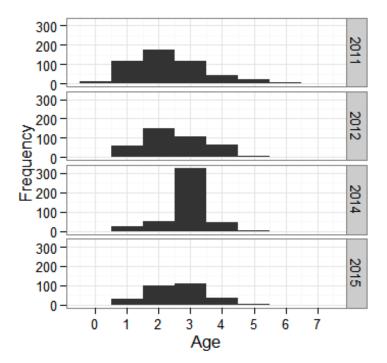


Figure 7. Age Composition by year

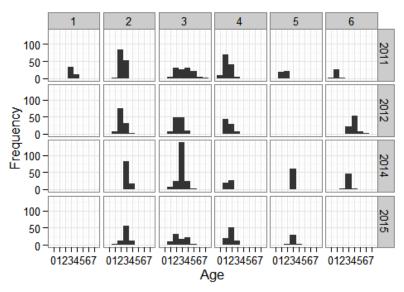


Figure 8. Age Composition by stratum

Growth zones consisting of opaque and translucent zones on the otoliths were fairly clear, with opaque zones appearing bright and translucent zones appearing dark, as illustrated in Figures 9 and 10.

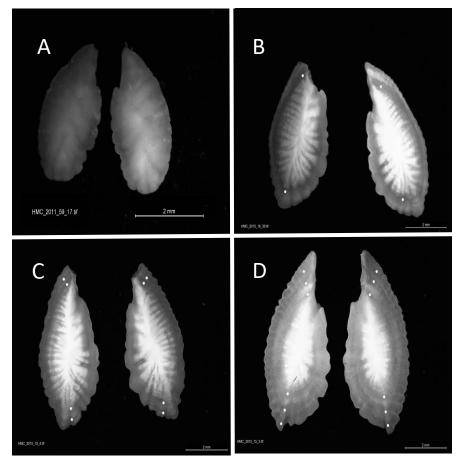


Figure 9. Age 0 year class; B. Age 1 year class; C. Age 2 year class; D. Age 3 year class

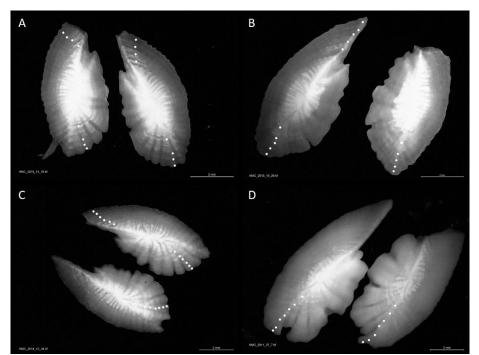


Figure 10. A. Age 4 year class; B. Age 5 year class; C. Age 6 year class, D. Age 7 year class

6.2 Length Composition

Length frequency distribution among the 1,604 fish was determined as shown in Figure 11. The total length ranged between 9.70 cm - 35.1 cm. According to the length frequency distribution, majority of the fish length were between 20cm-24 cm.

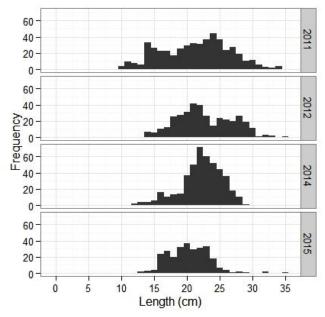


Figure 11. Length Distribution for Horse Mackerel

6.3 Age and Growth

The results of the age readings were combined with length data which was rounded to the nearest whole number data to establish an age-length key (Table 2). From the 1,604 age

readings, only 1,585 were used for the age-length key as length data were not available for 19 otoliths. Eight length classes ranging from 0-7 years were defined. Modal age was age 3 (N=658, which made up 42 % of the samples).

Length	Age (Years)						Total		
(cm)	0	1	2	3	4	5	6	7	10141
10	5								5
11	7	1							8
12		11							11
13		11							11
14		48							48
15		37	4						41
16		64	21	1					86
17		19	39	1					59
18		19	61	7					87
19		2	73	11					86
20		1	92	54	3				150
21		1	66	61	7	1			136
22			69	116	15				200
23			28	90	12				130
24			19	115	8	3			145
25			5	72	17	1			95
26				69	25	1			95
27				31	24	2			57
28				23	31	6			60
29				5	19	4			28
30				2	15	4	2		23
31					3	3			6
32					6		2		8
33					1	3			4
34						2	1	1	4
35						1	1		2
Total	12	214	477	658	186	31	6	1	1585
%	1%	14%	30%	42%	12%	2%	0%	0%	100

 Table 2. Age-Length Key for Horse Mackerel in Namibia based on otolith reading

 Length
 Age (Years)

The growth parameters were estimated by applying the von Bertalanffy equation to the observed lengths at age reading from otoliths of 1,585 fish (Table 3). The horse mackerel von Bertalanffy growth curve in (Figure 12), has been drawn after applying the length at age data to the relative equation. Each point represents an estimate of mean length at age in the sample. Growth parameters estimated were $L\infty = 42$, K = 0.2, t0 = -1.56.

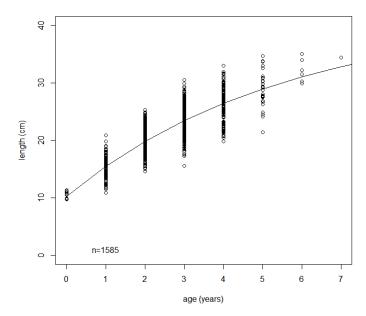


Figure 12. Von Bertalanffy growth curve for horse mackerel

6.4 Agreement Percent Readings

The results of the precision test on a total 100 otoliths, showed an agreement percent of 95%, with 5 age disagreements of which ages of 2 fish were underestimated by 1 year, and ages of 3 fish were overestimated by 1 year, as shown in Table 3. There were no observed biases.

1 00	Cape Horse Mackerel						
Age	-2(+)	-1	Agreed	+1	+2(+)		
0	0	0	2	0	0		
1	0	0	17	0	0		
2	0	0	25	0	0		
3	0	2	35	3	0		
4	0	0	16	0	0		
5	0	0	0	0	0		
Totals	0	2	95	3	0		
% Agreed	0%	2%	95%	3%	0%		

Table 3. Results from precision test.

QC Target %: 95 QC Result %: 9.50 APE: 1.750

7 DISCUSSION

The morphological structure of the horse mackerel varied according to the age, the rostrum was the most noticeable difference. As the fish grew older the rostrum elongated. The anterior and posterior of the otolith were the most important parts, as the annuli were most visible on both ends of the otolith. The first annuli usually border the rostrum on the anterior of the otolith, but this was not always the case for all otoliths, making it difficult to decide the first ring structure. During this study the same challenges highlighted by ICSEAF (1986) guide and Waldron and Kerstan (2001) were experienced, these were locating the first annulus; differentiating checks

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and annuli and distinguishing annuli near the edges of the otoliths of older fish as annuli are closely packed. Some otoliths displayed double or more annuli that resembled prominent, narrow translucent zones that were closely packed, hence they were only counted as one year. This study identified 8 age classes for Horse mackerel, with the minimum age being 0 and maximum age 7. Growth patterns were relatively clear on young fish (0-4 years old) and ages were assigned with little difficulty as the growth patterns were wide and distinctive, which is similar to the observation of the ICSEAF (1986) guide. The presence of juvenile rings and false rings in younger fish, was the main source of bias when estimating the ages.

The ICSEAF (1986) guide found that the otoliths of juvenile horse mackerel up to 3 or 4 years old consisted of one growth zone, with each year consisting of multiple rings in the opaque and translucent zones, attributed to fluctuations of environmental conditions. In this study, multiple rings were commonly found in younger fish. The checks were differentiated from the annuli by their weak appearance compared to the more noticeable annuli.

Reference collections available from the ICSEAF (1986) and ICES (1999 and 2015) were helpful in distinguishing the checks from the prominent annuli. With the experience of age reading it became easier to distinguish the checks from annuli.

In older fish (5-7 years old), the ages were relatively difficult to estimate, as the growth slowed, and the annuli were frequently closely spaced and indistinct, making the annuli difficult to distinguish. Older fish otoliths were observed at lower magnification and when images were taken, reducing the size of the image made the annuli more visible.

The modal age estimated in this study is age 3, predominately found in stratum 3 of 2014. In the acoustic survey of 2014, horse mackerel was most abundant in stratum 3, this could explain the 42% occurrence in the 2014 sample.

From the age-length key constructed one can conclude that there is a wide variation in horse mackerel length at age data, therefore assigning of ages from age-lengths keys might be challenging.

Growth parameters need to be checked for the quality and validity. The growth rate of horse mackerel is high during its first years of the lifespan. The von Bertallanfy parameters for this study was $L\infty = 42$, K = 0.2, t0 = -1.56.

Length-at-age data for this study was compared to other studies as shown in Table 4. With the exception of the Wengrzyn (1976), there was wide variations in the length-at-age obtained from the different studies. Length-at-age of this study resembled that of Wengrzyn (1976).

Naish *et al.* (1991) observed bigger fish than this study, while Sosa (1981) and Terre (1976) observed smaller fish.

	Length at age (mm)							
Age	Wengrzyn (1976)	Sosa (1981)	Terre (1976)	Babayan and Bulgakova (1983)	Naish <i>et al</i> (1991)	This study		
1	153	113	80	167	170	153		
2	194	163	138	208	306	199		
3	231	207	182	248	383	234		
4	264	246	222	287	428	264		
5	294	280	258	335	454	289		
6	321	309	290	363	468	322		
7	344	330	318	393	477	344		
8	366	359	344	417	482			
9	385	379	367	441	484			

 Table 4. Comparisons of calculated lengths-at-age data for Cape horse mackerel off

 Namibia in ICSEAF Divisions and values obtained for this study.

The results of the precision test revealed percent agreements of 95%, which indicates that the age reader is consistent with age estimates and the precision of the ageing for this study was high.

8 STUDY LIMITATIONS

Otoliths were omitted from age estimation due to sampling errors, 1,899 otoliths were examined for age estimation, however only 1,604 otoliths were aged.

Vials are currently marked with permanent markers with labels that include fish number and length of fish, and all fish belonging to one station are stored together in a Ziploc plastic bag that is also marked with a permanent marker with labels of the date of sampling, and station number. These labels fade with time especially when exposed to alcohol during otolith analysis. There is no sampling form where the information regarding the otoliths collected and fish identity is recorded.

Horse mackerel otoliths are fragile, especially those of older fish. There were many broken otoliths in this sample. Not all broken otoliths were excluded from the analysis, only the otoliths that had both posterior and anterior tips detached were excluded, since the annuli is most visible at the tips of the otolith.

The visibility of annuli was reduced on stained otoliths. The stained otoliths can be attributed to organic matter depositing on the otolith because of not washing the otoliths immediately after extraction. Otoliths are washed and cleaned only after the survey ends.

Data entry was poor as numerous discrepancies between the information on the vials and the database.

9 **RECOMMENDATIONS**

Sampling protocols should be updated to incorporate changes that will improve the quality of the otoliths and supporting data. The current labelling method should be replaced, to one that is more resistant to alcohol and water. Tracing paper is an alternative inexpensive option, to use as labels that are inserted in the vial. A separate data collection form for the otoliths should be used, where survey name, date, station, fish species and number are recorded. Otoliths should be washed immediately after sampling, to prevent build-up of organic tissue on the otolith. To improve the quality of age readings, more age readers need to be trained for reading comparisons.

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