# An update on the reproductive biology of the *Merluccius capensis* (Shallow water hake) in the south coast of South Africa

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By

LUNGELWA NOMXEGO

ORCID ID

http://orcid.org/0009-0001-9647-6901

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Supervisors: Prof. W. Sauer, Dr M. Lipinski and Dr O.S. Kjesbu

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## Preface

This thesis consists of the general introduction with background information of *M. capensis* and illustration of the time series of biomass of the species (Chapter 1); Chapters 2-5 are the primary research chapters that due to their qualitative and quantitative nature are in prep for publications as stand-alone publications. The conclusion of this research is enclosed in the implications for fisheries management (Chapter 6). The repetition in the work was avoided as different methods were used to process and analyse different samples. Repetition was also avoided by independent figures and tables that refer to particular investigations and by having the accurate reference list for each chapter.

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**Conference and other presentations:**

- Oral presentation in the SAMSS in Port Elizabeth (2017)
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- Oral presentation of the preliminary results at Rhodes University (October 2023), Titled: An update on the reproductive biology of the *Merluccius capensis* (Shallow water hake) in the south coast of South Africa
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- Nomxego L.C., O.S. Kjesbu, W. Sauer and M. R. Lipinski (*Manuscript in preparation*). Determination of *Merluccius capensis* fecundity through the volume based equations.
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## Declaration

I, Lungelwa Nomxego declare that the work described in this thesis was carried out in the Department of Ichthyology and Fisheries Science, Rhodes University, under the supervision of Prof. Warwick Sauer, co-supervisors Dr Marek Lipinski and Dr Olav Kjesbu. The components of this thesis comprise original work by the author and have not been submitted to any other university.

Signed: _____

Date:_____

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## Abstract

The reproductive biology of the *Merluccius capensis* was investigated in the south coast of South Africa for the period between 2014- 2016 and 2019. In the present study the whole-mount (WM) technique is introduced for field assessment, applying the digital auto-diametric method for maturity staging of female Cape hake (*M. capensis*). The updated maturity-scale-designed oocyte diameter (OD) thresholds for six maturity stages were given as ≤ 250 μm for the immature, 251 - 350 μm for the developing, > 351 μm for the spawning (which includes spawning capable, actively spawning and the regressing stages) and 100 - 300 μm for the regenerating stages. There was 100% correspondence between WM and histological assessments for the developing and actively spawning stages, with an overall agreement across all other stages reaching >85%. The mean OD were significantly different ($P < 0.001$) for all stages when combined stages were tested, however separate testing of testing of stages showed no significant difference between the developing and the regenerating stages ($P > 0.05$). Furthermore, the test of the mean OD of the leading cohort (the most advanced oocytes) from all maturity stages indicated that the actively spawning stage was clearly the largest, ($P < 0.05$). Features including the existence of *de novo* vitellogenesis, i.e., the continuous recruitment of previtellogenic oocytes to the developing pool of oocytes, reconfirmed *M. capensis* as an indeterminate spawner. Furthermore, the presence of many oocyte cohorts at one time, as observed in the present study, supports the observation that this species is also a batch spawner. Original outputs from the random forest model pointed at all tested features as best in testing compatibility between histology and the WM technique, though oocyte diameter, area, and axis length yielded the same shape in oocyte categories. Thus, this simple method can not only be effectively applied in the laboratory but also in the field for more basic research to improve maturity staging quality and the understanding of reproductive patterns.

The fecundity of *M. capensis* for the three - year period (2014-2016) was also investigated through a dedicated quantitative analysis on the various stages of oocyte developmental phases which are inclusive of the previtellogenic (PVO), cortical alveoli (CAO), vitellogenic (VTO) and hydrated oocytes

(HO). The established algorithms– rooted in principal physics–made it possible to successively estimate the single oocyte volume, the total occupied volume by phase and thereby the fecundity of these different oocytes present at a given point in the reproductive cycle. The HO were significantly highest in volume, while the PVO were significantly highest in abundance (*Fec,i*). The ratio *Fec,i* for CAO and VTO were the same or partly differ during the season. A minimum VTO threshold where the relative fecundity (*RF*) and the subsample volume intersect is determined as a point from which batch fecundity (*BF)* can be calculated using the volumetric formulae. Our calculation exercise suggested a mean *RF* of 283 (SE = 30) per gram ovary-free (somatic) body weight and a (*BF*) of 566 (SE = 98) thousands during the peak season of austral autumn. More generally, over the three years of study the grand mean *RF* was 259 ± 20 per gram somatic body weight, while the grand mean BF was 522 ± 64 thousands. At least at the population level *M. capensis* spawns all year round, with the two highest peaks seen respectively in austral autumn (March and May) and austral spring (August and September). Naturally, the gonadosomatic index (GSI) increased before spawning activity and decreased during the high peak of spawning. The biggest gonads, found in the biggest females, produced the greatest BF values, which indicates that this parameter (BF) is both gonad-size and body-size dependent. Essentially, this study advocates for vigorous investigations on earlier oocyte developmental stages as opposed to the current status of focusing primarily on the advanced stages in fecundity estimations.

This study also presents the exploration of the life history parameters of female *Merluccius capensis* off South Africa (N = 1817) which are inclusive of the gonadosomatic index (GSI), length-at-maturity, length-weight relationships and condition indices (relative condition (*k*) and Fulton's condition factor (*K*)). GSI was slightly higher in spring and autumn (≥7%) but could not be confirmed during winter- and summertime due to less access to data in those periods. The opposite was true for the actively spawning stage as GSI values were higher than 7 % when compared to other maturity stages. The length (*L*) at 50% maturity was around 38 cm ($L_{50}$), though differences occurred between the two applied staging methods, histology and visual (macroscopic) classification, when *L* approached infinity. The latter method presented underestimated length at maturity values at the 75 and 95 percentiles (48 and 60 cm) compared to the corresponding percentiles given from histology (50 and 65 cm). There

were trivial across-method differences in $L_{50}$. However, we found a clear reduction in $L_{50}$ in view of published information in prior years when this estimate was 48 (1985), 42 (2008), 53 (2011) and 24.8 (2015) cm. Overall, *L* explained 90% of the variation in whole body weight (*W*). As the bootstrapped, grand mean growth coefficient was *b* = 2.98, indicating a slight allometric growth function, there were no significant variations between years, though an isometric growth existed for 2016 with *b* = 3.0, whereas for 2014 and 2015 this *b* was 2.98 and 2.93, respectively. In terms of demography, females < 60 cm generally showed isometric growth (*b* = 3) with less eggs as opposed to allometric growth (*b* = 2.95) at > 60 cm which laid more eggs. The relative condition index (*k* = 1) exhibited higher values than Fulton's *K* which was 0.80. Taken together, the maternal stock of *M. capensis* along the south coast seems to be in good condition, likely spawn throughout the year but we found that the macroscopic data tend to give biased maturity ogives.

Spatial and temporal distribution revealed that the body size increased with water depth and was significant at $p<0.05$, though this relationship between the body size (cm) and water depth (m) was rather weak ($r^2 = 0.22$). The vertical adjustment of the position in the water column between the immature to regressing stage appeared trivial, however, those in the regenerating stage stayed significantly deeper ($P<0.05$), specifically at about 225 m mean depth compared to 160-200 m for the other stages. *M. capensis,* as a serial spawner, tended to move upwards by about 25 m to release egg batches. Spawning females occupied the latter mentioned mean depths in April to May and in September, evidenced by the improved incidence of actively spawning females. A latitudinal shift was also apparent as the females moved northwards before spawning (35.4-35.3ºS), followed by spawning (34.9ºS), and subsequently returning southwards (35.2-35.4ºS). These latitudinal and longitudinal movements came together with relocations to the east at pre-spawning (21.7-21.8ºE), then even further east when spawning (22.8ºE) and then going back to the west (21.2ºE) after spawning. Thus, the various phases of the reproductive cycle of this species are characterized by highly specific spatiotemporal dynamics.

# Chapter 1: General Introduction

## 1.1 Introduction

Cape hakes *(Merluccius capensis* and *M. paradoxus*) also known as the shallow water hake and the deep-water hake, are found in the vast coastal areas of South Africa which include both the Atlantic and the Indian Ocean (Jansen et al. 2016). This vast existence allows the species not to be bound in South Africa only, but to the Benguela region as well as other neighbouring coastal countries, like Namibia. *Merluccius capensis* co-exist with the sister species *M. paradoxus* and their difference is not only by genetics and the taxonomy, but also by distribution in the water column (Jansen et al. 2016). The adult *M. capensis* is mostly found with the juveniles of the *M. paradoxus* and due to their mixing, both species (*M. paradoxus* and *M. capensis*) were earlier caught as one stock. The sizes of both species (*Merluccius capensis and M. paradoxus*) increase with depth, which concurs with the recent investigations (Nomxego et al. 2024; Jansen et al. 2016; Durholtz et al. 2015).

The distribution of *M. paradoxus* is greatest at 330m depth and their coexistence is between 220 and 440m depth, however, it has a maximum depth beyond 640m, which is different to that of *M. capensis* (Botha, 1980; 1986). *Merluccius capensis* occurs in great abundance in depths of about 150m and becomes lesser abundant in around 300m and merely exist beyond 440m depth (Botha, 1985; 1986) and its greatest abundance moved from 4500 tons to 1800 tons and less than 500 tons for the said depths respectively (Botha, 1985; 1986). The distribution of *M. capensis* was not only observed along the water depth, but also along water oxygen content and the seas surface temperature (SST), wherein it was observed to be distributed in hypoxic (low water oxygen content) conditions off Namibia and in the Benguela region (Mc Pherson et al. 1991). Furthermore, it was reported that the relationship between its distribution off northern South Africa and southern Namibia and the sea surface temperature (SST), indicated that there was negative correlation between ages 1 and 0 as opposed to positive correlation of

ages 4 and older, implying that immature stages prefer deeper, cold and dark waters as opposed to spawning maturity stages that survive in opposing conditions (Mc Pherson et al. 1991).

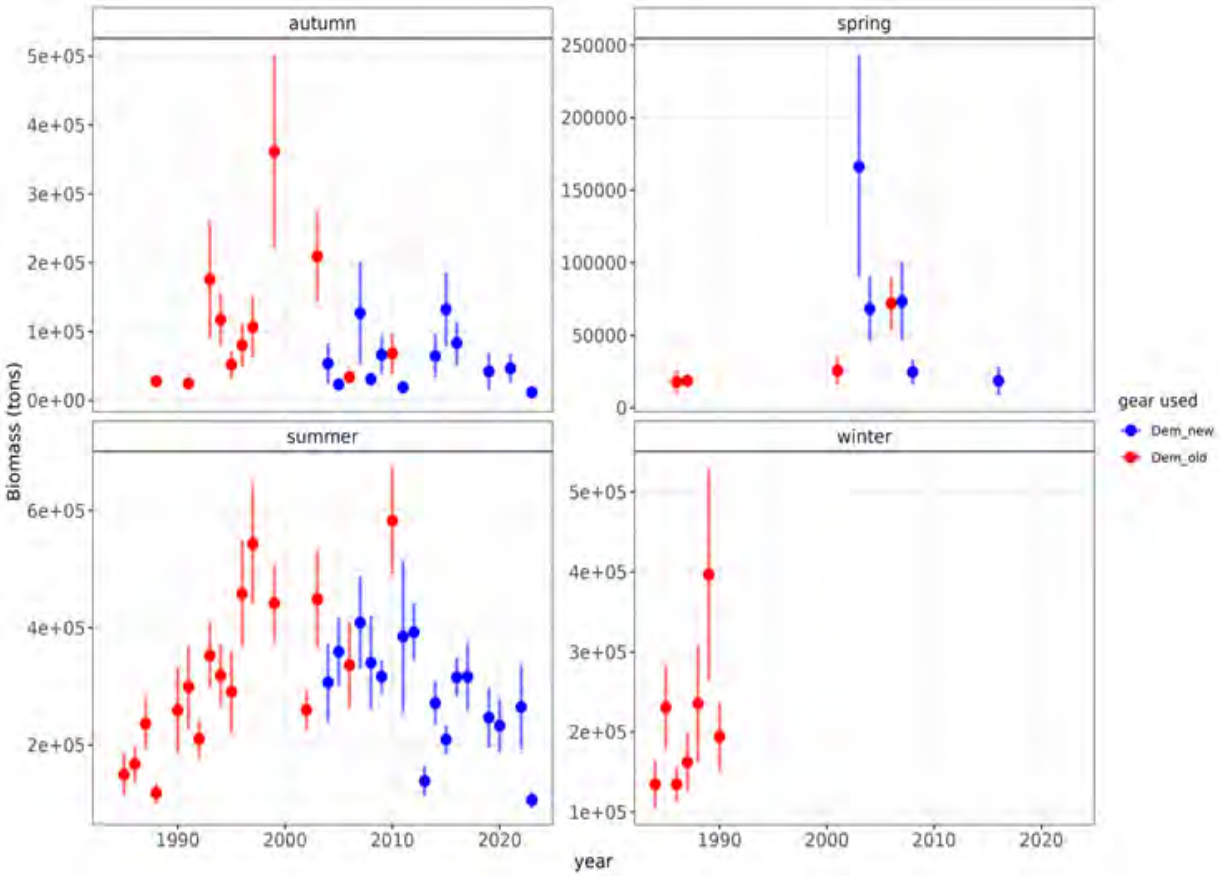
### 1.2 Rationale

South African hakes *(Merluccius capensis* and *M. paradoxus) are* the mostly exploited fish stocks in South Africa and they form part of the general contribution to the country's gross domestic production (GDP) of 4% as per statistics South Africa. They also form part of the demersal fishery species that are not only directly exploited at local level but are also exploited and exported to other neighbouring countries and to the international community (Durholtz et al. 2015; DFFE, 2020). Since 2006, the both of these demersal species have been managed by the operational management procedures (OMP), which is a set of rules that specify exactly how the total allowable catch (TAC - which is a fisheries management tool that sets a limit on the amount of specific fish stock that can be harvested in a given period, usually a year) should be calculated using stock specific monitoring data (commercial and fishery - independent indices of abundance), and is revised every four years to account for possible revised datasets and improved understanding of the resource and fishery dynamics (DAFF, 2012). Subsequent to the use of OMP, the South African trawl fishery had to meet the new Marine Stewardship Council (MSC) standards for trade and marketing value but also ensuring biological and ecological sustainability. The new MSC Fisheries Standards feature better protections for marine life, as well as stronger fisheries management and compliance requirements. The South African hake trawl fishery received Marine Stewardship Council (MSC) certification and eco- label from 2006, and recertification in the following years (2010, 2015 and 2021). The MSC certification and eco- labelling of the South African trawl fisheries provides the constant increase in demand for the MSC certified seafood products, which results to provision of socio economic benefits to the country and end- users, especially through the international community (DFFE, 2023).

It was difficult to separate *M. capensis* from *M. paradoxus* and were generally targeted, processed and marketed as single commodities for four different fisheries (hake handline, hake longline, deep sea trawl and Inshore trawl: DAFF, 2012). The separation into different stocks occurred later (DFFE, 2023), hence the time series present them as separate fish stocks (Figure 1.1 and Figure 1.2). Fish stock biomass estimates started to be a necessary feature from the start of fishery administration, thus when the rights were allocated for fishing and is expressed as the total weight of all matured fish in a stock which could be determined by multiplying the number of mature fish by age, by their average weight at that age.

Most catches of *M. paradoxus* were prominent in autumn and summer, though a reduction with the use of a different gear occurred in both most prominent seasons (Figure 1.1). The biomass estimates for *M. capensis* are illustrated before 1990 till current (Figure 1.2**)**. It is noted though that the old gear was more efficient in terms of collection and harvesting of samples as compared to the new gear that is less catching. The new gear has not been operational in the winter season after 1990. After 2020 the biomass estimates were just above 200 000 tons in summer in the south coast and were below 100 000 tons in autumn in the west coast.

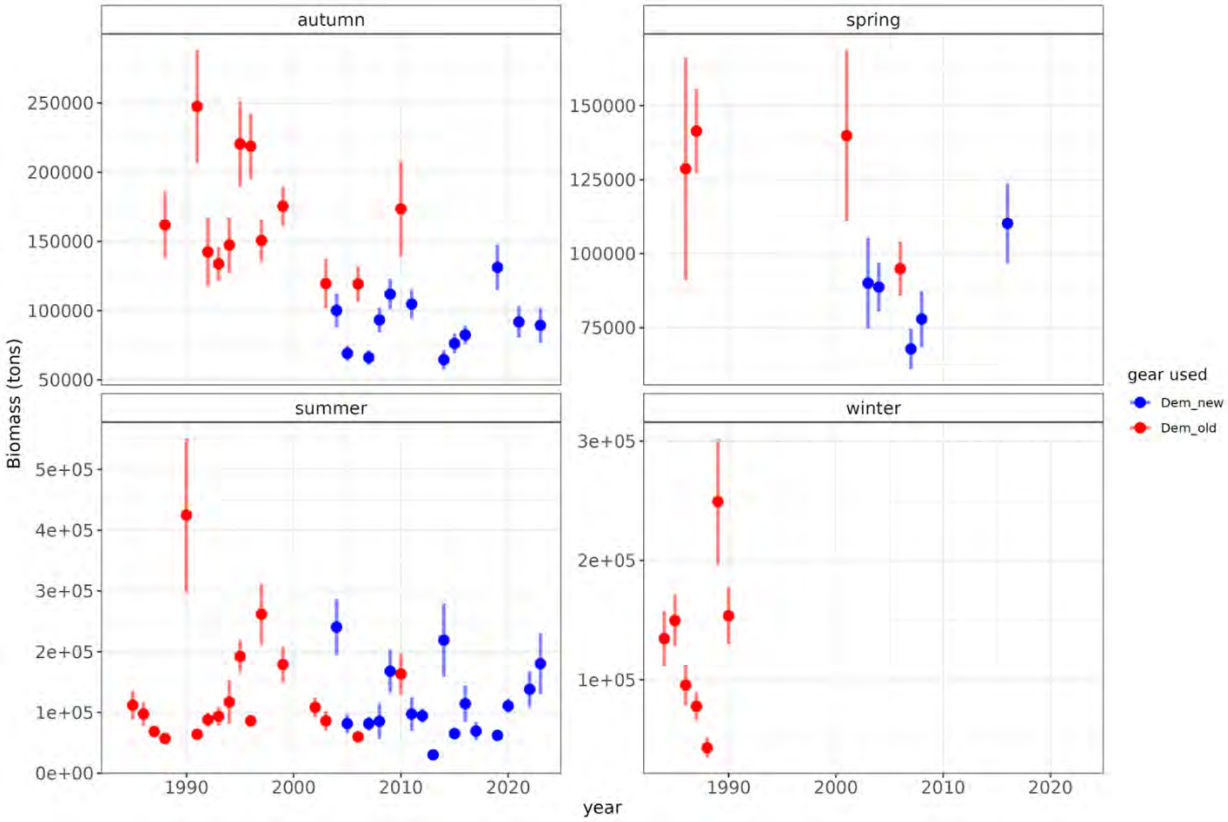
According to Botha 1986, the stock status of the shallow water hake in South African waters was generally considered to be in a healthy state with a least concern by the International Union for Conservation of Nature (IUCN) red list. The biomass estimates of *M. capensis* in South Africa show fluctuations over time, with both increases and decreases in exploitable biomass observed and their stock status (allocated in tonnes for right holders) is considered healthy at the moment (Durholtz et al. 2015).




143

144 **Figure 1.1:** Distribution of biomass estimates and the years for *M. paradoxus*. The series runs before the
145 1990 to current. The data was collected in both the south and the west coast of South Africa and was
146 partitions in seasons (autumn, spring summer and winter). The gear use for the collection of samples is
147 denoted by the red (for the old gear) and the blue (for the new gear) colours.

148




149

150 **Figure 1.2:** Distribution of biomass estimates and the years for *M. capensis*. The series runs before the
151 1990 to current. The data was collected in both the south and the west coast of South Africa and was
152 partitions in seasons (autumn, spring summer and winter). The gear use for the collection of samples is
153 denoted by the red (for the old gear) and the blue (for the new gear) colours.

154 The total biomass of *M. capensis* in South Africa increased by 6.1% between 2021 and 2022 surveys,
155 reaching 736 000 tonnes and the non-fishable biomass increased by 33.3 % to 536 000 tonnes, while
156 fishable biomass decreased by 9.5 % to 200 000 tonnes (DFFE, 2023). Therefore, the overall biomass
157 of *M. capensis* showed an increase while the fishable biomass showed a decrease.

158 However, more studies on reproductive indices of the stock would improve our information and
159 understanding of the stocks. Efforts are encouraged to improve stock reproductive potential (SRP) indices
160 for potential application in assessment and management in order to establish reference points, which are
161 basic to a precautionary approach to fisheries management and sustainable fisheries (Tomkiewicz et al.

2003). Furthermore, ignorance of variability in SRP adds uncertainty to stock-recruitment relationships, which affect the reliability of associated reference points (Tomkiewicz et al. 2003).

Much as it is important to exploit cape hakes (*M. capensis* and *M. paradoxus*) for food security and socio- economic reasons, their harvest is directly linked to the fishing pressure, the fishing mortality and the reduction of food for the next trophic level in the food chain. These species experience significant amount of fishing pressure, due to their popularity and commercial importance which is more evident in the West coast fisheries, where they are directly exploited with other commercial fisheries like, anchovy, sardine and are significant in the offshore fishing grounds mostly on the larger size (*M. capensis*), which occupy more deeper depths. However, while total allowable catch (TAC) is currently high, there are some concerns about the potential decrease in abundance, particularly in recent years. Fish mortality is taken care of by sustainable fishing, which is attained when fishery resources are exploited such that the values of the ecosystem approach to fisheries (EAF) are upheld. It is common knowledge that, it is required that when harvesting fish, there should be minimal or no environmental degradation. For instance, the destruction of the sea bottom is not allowed as the bottom species also need to be sustained. The destruction of the other species that co-exist that are found at similar depth and environments should also be sustained (Murawski et al. 2001). Sustainable resource utilisation should be such that the resources are healthy and resilient for the benefit of future generations. The examination of bycatch species and its reduction thereof should be closely monitored to ensure maximum exploitation of the lucrative species while ensuring sustainability and co-existence of other species and healthy environment (Murawski et al. 2001). Ecosystem approach to fisheries puts emphasis on scientific and socio - economic recommendations for control and sustainable management of fisheries resources, which reflects as the permit conditions on the licence for the fishery right holders. The permit conditions are enforced by the compliance (division of management), inclusive of the fishery police officers, who ensure apprehension of illegal, unreported and unregulated (IUU) fishing. The enforcement of the permit conditions may include the presence of the fishery observers to ensure that operations like high - grading

are avoided and the catches submitted to the research body are a true reflection of what has happened in the wild. The submission of the wrong catch data to the research body leads to spurious biomass estimates that are far from precision.

Although the Shallow water hake *M. capensis* is one of the most economic valuable resources for the South African demersal fisheries, there are a paucity of information of the most critical parameters of the SRP, such as its maturity scale, fecundity pattern, the maternal impacts and the species spatial and temporal distributions. Consequently, it is the purpose of this research to contribute in filling the knowledge gap on the reproductive dynamics of *M. capensis*, and so to provide more information for assessment and management of this species. The inadequate information on life history parameters of the *M. capensis* leads to inaccurate resource status information and estimations. Improvement of resource information prompted the current investigation by collection of data from the south coast of South Africa by bottom trawls from research and commercial surveys from 2014- 2016 and 2019.

## 1.3 Aims and Objectives

### *1.3.1 Introduction of the Whole mount technique*

The aim of the present investigation is to update the gonad maturity scale of *M. capensis* as the original one was published by authors (Botha 1985) only consist of the macroscopic assessment. There is a need for an updated histological scale that is scientifically reviewed. The maturity scale provides a set of keys to be used when staging the fish based on their gonads and the current maturity scale used for *M. capensis* is only macroscopic as that of Botha 1985, but is extended with the inclusion of the sixth stage. The definition of those microscopic stages can be realised through the process of histological validation, which allows the assessment of the gonads under microscope whereby the cell organelles are read and analysed (Brown - Peterson et al. 2011). All the six stages are readable under the microscope, as they are identified by the presence of cell organelles (including the alpha and beta atresia, different stages of post ovulatory follicles (POF), the movement of the nucleus, thick and thin ovarian wall, etc.) and the

most advanced oocyte stages ((primary growth oocytes (PGO) which are oogonia and perinucleolar); cortical alveoli oocytes (CAO); primary, secondary and tertiary vitellogenic oocytes (PVTO, SVTO, TVTO); hydrated oocytes (HO)). The study also intended to apply the whole mount innovative methods which are currently used by the global scientific community. Whole mount technique is two- fold. It can be used onboard the vessel to identify the matured from immature (Kjesbu 1991, Kjesbu *et al*. 2003), which is a primary parameter for stock assessment, but remains a grey area for *M. capensis* in South Africa. Whole mount can also be used as an auto-diametric method that creates qualitative and quantitative data from the oocytes' measurements (Thorsen and Kjesbu, 2001). The latter further allows easy assessment of oocyte development, maturity staging, fecundity estimates, etc. Comparison and examination of compatibility between whole mount and histological assessment methods is also crucial so that they may be used as a proxy when conditions are stringent. The present study also assesses whether the *M. capensis* is an indeterminate and or batch spawner which allows better choice of methods for further estimates for resource management (Ganias et al. 2015).

*1.3.2 Spawning activities*

The number of eggs that are spawned in a spawning event (Batch fecundity) (Hunter and Macewicz, 1985; Ganias, 2013) needs to be updated for the *M. capensis* in the south coast of South Africa. Batch fecundity was last recorded by authors (Osborne *et al.* 1999, Osborne 2004) as 417± 64 thousand. The latter was based on the average of the range of fish weight and length at the time. There may be variation in BF if different samples are to be collected, as it is gonad size dependant and takes into account on whether the fish are repeat spawners or not (Murua and Motos, 2006). Batch fecundity also differs by seasons as there may be separate peaks, depending on the species, while the relative BF gives a better estimate of the number of eggs spawned by individuals of a certain species per spawning event (Macchi (2005), which may not be the same for the population estimate. Furthermore, the latter should also consider the size range, especially of the gonad and the body size inclusive of length and weight (Macchi, 2005)). Some innovative methods are used to calculate BF and can be used as proxies when accurately

examined. This investigation also introduces the use of the oocyte volumetric formulae (Thorsen and Kjesbu 2001; Kjesbu et al. 2011, Korta et al. 2010) and the volumetric fecundity (VF) (dos Santos Schmidt et al. 2021; Bagenal, 1978) to assess the number of eggs spawned per ovary per event. It was also imperative to assess the months of the year that were closest to spawning or the spawning season. Gonadosomatic index (GSI) is a popular method that could be used to assess the spawning period or season of *M. capensis.*

*1.3.3 Life history parameters*

It is a current day – to - day activity to use the macroscopic method on board the vessel to assess the maturity stage of the caught fish, which is the primary data that is used for the development of the maturity ogives. Through the maturity ogives, it is possible to determine the percentage of the population that is immature versus the matured and to declare the age at 50 % maturity ($A_{50}$) or the length at 50 % maturity ($L_{50}$). However, understanding the percentage of the matured fish versus the immature is another procedure that is not easily established, but requires expert knowledge of the species in question, otherwise, subjective methods may spoil the SSB estimations. The current reading of the immature maturity stages *of M. capensis* in South Africa indicates that stages 1 and 2 are immature stages while the other stages (stage 3 and above) are categorised as matured.

Maturity ogives are the primary information that is used for population studies and various stock assessments. The macroscopic method is widely known to be subjective (Honji et al. 1993; Vitale et al. 2006), and if used, it cannot be used alone but need histology or other assessment method for validation. In the absence of validation, the use of the macroscopic results is nothing but spurious information for further estimations. The present study presents the maturity ogives validated by histology and whole mount methods. The length -weight relationships were examined for the body condition with the understanding that if the slope (b) and intercept (a) of the species under study is below, above 3 or is equal to 3 (Jisr et al. 2018), then the species is considered to follow the negative, positive allometric

growth or isometric growth pattern, respectively (Schneider et al. 2000). The latter gives an estimate of whether the fish stock is healthy or not. When either of the two (length or weight) is known and are compatible, it is easy to use the other as the proxy for the other and for further extrapolations. Fulton's condition factor and the relative condition factors we also used as the body condition parameters to check whether the stock in the south coast is either fast or slow growing.

### *1.3.4 Distribution*

As much as *M. capensis* is pronounced to be a healthy resource (Rademeyer et al. 2008; Durholtz et al. 2015; DFFE, 2020), there is no updated information about its spatial and temporal distribution. There is no updated information as to the specific depth that is preferred by the species. It is also the objective of the present to assess the specific depth preferences by the maturity stages of *M. capensis*. It is also interesting to know at what time of the year those depth and spaces will be preferred by different maturity stages. The study of movements along the lines of latitude and longitude assigned by the present investigation could also enlighten us whether the timing for this species has shifted or not and whether or not, the spawning grounds have also shifted (Jansen et al. 2016).

## **1.4 Conclusion**

The data processed and analysed to be the relevant information about the *M. capensis* in the south coast of South Africa will enhance improvement in methods for data collection and enlighten the scientific community about the importance of raw biological data usage and the consequences should that be ignored. The biological estimates made on resource status can only be close to the precision when biological data is available and is put to good use.

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# Chapter 2: Understanding the whole mount technique as applied to fish maturation process of *Merluccius capensis* (Merlucciidae) and a new maturity scale

## 2.1 Introduction

Cape hakes (*Merluccius capensis and M. paradoxus*) are the most commercially important species in the South African fishery with annual contribution of R5.2 billion in the Gross Domestic Production (GDP) of the country (DFFE, 2023). Their abundance in depth tend to vary but larger individuals of both species are found deeper than the smaller ones (Botha, 1985). *Merluccius capensis* being the sister species is also found shallower than the *M. paradoxus*, though its larger sizes are found to share the similar depth with juveniles of *M. paradoxus* as they are cannibalistic in nature (Botha, 1986; Trout, 1996). They are distributed on the continental shelf and upper slope around the coast of southern Africa and are targeted by four fishery sectors, namely, hake deep-sea trawl, hake inshore trawl, hake longline and hake handline (DFFE, 2023). Due to their commercial importance, the biological information that serves as the support for stock assessment models ought to be accurate and precise for the betterment of the population estimates (Lambert, 2008). The current use of the macroscopic method as the only method for maturity staging of the gonads of the cape hakes compromises the output, as the results need to be verified and validated by the more precise method, namely histology or the whole mount technique (Nomxego et al. 2024). The macroscopic method used at sea on board the vessel to stage the gonads of the *M. capensis* is subjective and cannot be used independently (Honji et al. 1993; Osborne et al. 1999; Kainge et al. 2007). Better and innovative techniques for collection, processing and analysis of data serve as an integral part in improving fisheries research and fisheries management, for example, other authors have practiced the use of whole mount technique, although in different species and regions (Kjesbu, 1991; Thorsen and Kjesbu, 2001; Serrat et al. 2019; Anderson et al. 2020).

Employment of alternative methods for classifying gonads maturity stages is ideal as opposed to the subjective macroscopic staging. The whole-mount (WM) technique offers an alternative way of classifying

ovarian stages by the external appearance and size of the intact oocytes (Sivertsen, 1935; West, 1990; Kjesbu et al. 1991). Although it is known as a laboratory routine, it is also possible to directly apply these staging principles in the field (using a stereomicroscope) to assess the immature versus mature stages (Kjesbu 1991). The WM measurements are also the key elements of the more sophisticated auto-diametric method, which produces numerous quality data points while automatically digitizing the oocytes (Thorsen and Kjesbu 2001). In the latter process, the WM information can be linked to Image J to characterize oocytes based on the set of macros set up in the software.

Many maturity scales have been devised and described in the literature to determine the degree of ripeness of the ovaries of various teleosts (e.g. West 1990; Honji et al. 1993; Arocha 2002; Tomkiewicz et al. 2003; Vitale et al. 2006; Costa 2009). The latter mentioned studies cover the differences and variability of oocyte development and maturity within a single species. It has been widely accepted that a maturity scale should be arrived at using two procedures: by the macroscopic scale in the field, and following subsequent validation by histological analysis in the laboratory and the of both procedures is important as it ensures that maturity stage identification is an accurate estimate of reproductive status and maturation process (Honji et al. 1993; Vitale et al. 2006; Costa, 2009).

The macroscopic scale in use for maturity staging of the Cape hake *Merluccius capensis* is taken from Botha (1986). The Botha scale consists of five macroscopic maturity stages namely, inactive, active, ripe, ripe and running and spent. Although the use of a sixth macroscopic stage has been practised by the Fisheries Department in South Africa for more than two decades (Osborne 2004). Botha's scale defines the external, gonadal morphological characteristics used to classify maturity stages for *M. capensis* for both sexes but lacks the histological validation. Meanwhile, that of Osborne (2004) has extended the description of the stages to histological assessment, including the sixth maturity stage, though the latter authors' investigation does not bridge the gap between the previous scale (with 5 stages) and theirs (with six stages), the investigation also passed through to maturity scale as opposed to paying attention to a scientifically reviewed maturity scale. The six maturity stages stated by Osborne (1999) are from 1-6 as

419 virginal, immature, maturing, mature/ripe, partially spent and regressing/ resting stages, respectively.
420 Furthermore, the latter stages were sourced from Herrera et al. (1988) and the authors (Osborne et al.
421 1999) changed the partially spent stage, which was stage 3a (partially spent) from the latter author and
422 adopted by Osborne (1999), to be stage 5 (partially spent) without changing the name. Further research
423 of other authors (Osborne 2004; Osborne and Mullins 2005; Kainge et al. 2007; Nomxego et al. 2024)
424 highlighted numerous inadequacies of the macroscopic maturity scale for Southern African hakes, but
425 despite this, there has been continuous use of those maturity stages in the field-based population
426 dynamics studies and none of the latter mentioned studies have applied the WM technique. There are
427 no investigations to our knowledge that have used WM technique and the auto- diametric method, neither
428 on any other fish stocks in this region for the specific purpose of maturity scale and improvement of the
429 maturity ogives.
430 The aim of the present study is to re- analyse the tissue content of the ovaries through the histological
431 procedure so that there can be scientifically reviewed maturity scale that clearly justifies the distinct
432 microscopic and morphological characteristics that makes each maturity stage different from the other. It
433 is also an objective of this investigation to introduce the WM technique onboard the vessel at sea with
434 the intention to obtain immediate and accurate results that clearly identify and separate the immature
435 versus the maturity of the individuals, so that we can determine the length or age at which the *M. capensis*
436 population is matured. Such identification will be followed by the statistical handling, but with the
437 consequence of the unbiased maturity ogives of *M. capensis*. The histological assessment that validates
438 macroscopic method, does not allow the counting of the oocytes from the slides under microscope as
439 some stages are not clearly measurable (Kjesbu et al. 2011; Nomxego et al. 2024). However, the WM
440 auto-diametric method provides both the quality and the quantity data, so that numerous cell organelles
441 including oocytes could be measured (Nomxego et al. 2024). This investigation will also assess how the
442 WM auto-diametric method becomes an alternative for the provision of the calculation and measurements
443 of the oocytes, as it is not possible under the microscope during histological assessment. Hydrated

444 oocytes are the main oocytes that rupture during the formation of the slides and therefore do not allow
445 the quantification under microscope. It is equally important to investigate compatibility between the WM
446 and histological assessments and also to devise a maturity scale for application to *M. capensis* as an
447 alternative to the macroscopic method. The present study also aims to further assess the indeterminate
448 reproductive style of this species off South Africa using WM information as a complement to histologically
449 based insights given from samples collected off Namibia, i.e. towards the northern part of this species'
450 distribution area (Kainge et al. 2007). Consequently, it is expected that the expertise of reading whole-
451 mount oocytes under the stereomicroscope onboard, originally recommended by Kjesbu (1991), and
452 subsequent automation in the laboratory, may improve accuracy in determining reproductive status, and
453 enhance understanding of the maturation processes (Thorsen and Kjesbu 2001).
454 The best oocyte feature determined by the WM auto-diametric method could also enhance accuracy
455 on further oocyte development studies. To follow up on this issue, we took advantage of the fast
456 progresses in machine learning techniques to characterise the maturity stage by WM oocyte features,
457 confer Flores et al. (2024) who have just recently used random forest to classify the maturity stage of
458 *Merluccius gayi gayi* by gonadosomatic index, body descriptors and geographical location (>71%
459 agreement). Hence, the results of the present study may be used to appraise the true fecundity type and
460 spawning pattern of this species under investigation or any other wherein the methods are deemed
461 suitable.

462
463
464
465

## 2.2 Methods

### *2.2.1 Field collection, and macroscopic staging*

The study material was composed of female samples of *M. capensis* collected by bottom trawl on the upper slope and shelf of the south coast of South Africa during the *R/V Africana* surveys in April 2014 and March 2015, as well as the *EAF-Nansen* research survey of *R/V Dr. Fridtjof Nansen* in March 2019. Random samples were taken from each trawl and the following variables were recorded: total length (TL) in cm below and total weight (TW), gutted weight, gonad weight and liver weight, all to the nearest gram. The ovaries were classified according to the five -stage macroscopic maturity scale as published by Botha (1986), with addition of the sixth stage (Osborne et al. 1999), however, the current investigation did not take the pattern of Herrera et al. (1988) as the latter authors. According to Osborne et al 1999, stage 3a- partially spent from Herrera 1988, was fitted in order to come up with six maturity stages. The current study added the sixth stage on the already existing Botha scale, (Botha,1986), between stage 4 - (ripe and running) and stage 5 (spent). The order of the maturity stages became, stage1 - inactive, stage 2 - active, stage 3 - ripe, stage 4 - ripe and running, stage 5 - regressing and stage 6 – spent and inactive as guided by the other authors (Costa, 2009 and Brown - Peterson et al. 2011; Table 2.1). It is clear in the current investigation that only two stages are inactive, thus (stage1 and stage 6), and both stages contain primary growth oocytes (PGO) only and further growth in the ovary only starts at stage 2, hence it is called the active stage. Small subsamples were collected from the nearest to the vent part of one ovarian lobe, weighed, fixed, and stored in 4% buffered formaldehyde. All the remaining pieces of ovary were also fixed separately to provide extra ovarian material, if required.

**Table 2.1.** Indication of the source of the maturity stages of *M. capensis* as they initially did not include stage 6. Maturity stages included the sixth one in Osborne et al. (1999), though they are explained differently from the current investigation.

| Maturity stages | Botha, (1986) | Herrera, (1988) | Osborne et al. (1999) | Nomxego et al. (2024) - current |
|---|---|---|---|---|
| 1 | Inactive | Virginal | Virginal | Inactive |
| 2 | Active | Immature | Immature | Active |
| 3 | Ripe | Maturing<br>3a - Partially spent | Maturing | Ripe |
| 4 | Ripe and running | Mature/ Ripe | Mature/ Ripe | Ripe and running |
| 5 | Spent | Regressing/ Resting | Partially spent | Regressing |
| 6 | ---------- | ---------- | Regressing/ Resting | Spent and inactive |

*2.2.2 Laboratory protocol, and microscopic staging*

Standardized histological procedures were adopted (Hunter and Macewicz 1985). Cross sections of one of the lobes of the ovary nearest to the vent were dehydrated through a series of ethanol immersions, embedded in paraffin, sectioned at 6-10 µm, transferred onto slides, cleared with xylene, rehydrated, and stained using haematoxylin and eosin. Slides were analysed under the microscope at 40× magnification to confirm the stage of maturity of the ovary. The histological maturity stage was classified based on the most advanced type of oocytes but also considering the presence of any post-ovulatory follicles (POFs) or atretic oocytes (Hunter and Macewicz 1985; Brown - Peterson et al. 2011). In more detail, the indicative features during histological assessment of *M. capensis* maturity status were oogonia and perinucleolar

499 also known as primary growth oocytes (PGO), cortical alveoli oocytes (CAO), vitellogenic oocytes (VTO;
500 with protein and yolk granules and lipid vacuoles), alpha and beta atretic sub – stages, newer and older
501 (disintegrating) POFs, migrating nucleus and the hydrated oocytes (HO). Moreover, PGO and (CAO)
502 together form part of the previtellogenic oocytes (PVO) as they are the oocyte developmental stages with
503 no vitellogenic characteristics. The microscopic stages were identified based on the criteria derived from
504 the current study after the authors (Costa, 2009; Brown - Peterson et al. 2011; Table 2.2).

505 *2.2.3 Laboratory protocol and the whole mount staging*

506 The whole-mount (WM) method was followed as in Kjesbu (1991) and Kjesbu et al. (2003), using
507 image analysis (Thorsen and Kjesbu, 2001). The ultrametric method, which employs ultrasonication to
508 separate and enumerate the tiny PVOs but also all other oocytes (Anderson et al. 2020) was routinely
509 used as a supplement. The samples were transferred to Petri dishes and images of the tissue were taken
510 with a Carl Zeiss Camera (Axiocam ERc5s) connected to the dissecting microscope and a computer with
511 Zen-lite 2.3 software. The camera was set to take 8 - bit RGB images and transmitted light was used so
512 that the oocytes appeared as dark objects on a bright background. The resolution of the images was 0.88
513 pixels $\mu m^{-1}$. Macros were developed using ImageJ software to measure roundness, ellipsoid shape,
514 diameter, area, volume, minor and major lengths and counts of the oocytes, following scripts developed
515 by Thorsen and Kjesbu (2001).

516 To keep the microscopic staging (WM and histology) separate from the macroscopic staging, we
517 employed a letter system instead of a numerical system. Thus stage 1 (immature) = A; stage 2
518 (developing) = B; stage 3 (spawning capable) = C; stage 4 (actively spawning) = D; stage 5 (regressing)
519 = E, and stage 6 (regenerating) = F. During the WM analysis, stage C, D and E were subsequently
520 merged, i.e., labelled as C+D+E, and named “spawning”, as a multiple batch spawner like *M. capensis*
521 may go quickly from D to C and there- after from C to D, and possibly also from E to F as some oocytes

may undergo atresia (Kainge et al. 2007; Kjesbu, 2009). After the histological validation and WM analysis, the available oocyte developmental stages were recorded.

Obtaining accurate measurements and calculations from the fixed ovarian tissue requires conversion and extrapolations from the fixed state to fresh material and the fresh water correction from formaldehyde fixed ovarian samples of oocyte diameter was calculated using the following equation (Kjesbu et al. 2011):

$$OD_{fresh} = 0.947x\ OD_{formalin.wm+19} \quad \text{Equation 1}$$

where OD formalin is the oocyte diameter of the formalin fixed samples calculated by the WM method. The number of oocytes per fresh sample weight, thus fresh oocyte density ($g^{-1}$) was calculated as per authors (Ganias, 2013) using the following equation:

$$D_{oocyte} = N_{oocytes/}W_{sub-sample} \quad \text{Equation 2}$$

where $D_{oocyte}$ is the oocyte density, $N_{oocytes}$ is the total number of oocytes calculated by the whole mount method and $W_{sub\text{-}sample}$ is the total weight of the sub-sample. All the samples with already identified maturity stages by the histologically assessment were taken through the whole mount assessment method. The statistical mean of means was obtained from the formula:

*2.2.4 Maturity validation*

The comparison of the maturity stages between the macroscopic method and the histology was done in the laboratory. Also, the stages that were identified and validated by histology were further compared with the features identified by whole mount auto-diametric method. The whole mount presented the oocyte diameters for each maturity stage and those were fitted to the histological stages.

*2.2.5 Data analysis*

Macroscopic and histological maturity assessment were compared statistically to assess the level of agreement between these two staging methods and later also to compare the histological and WM assessment. In the latter case, typically close to 1200 oocytes were digitalized per female ($N_{females}$ = 92). Due to this large data set of oocyte recordings ($N_{oocytes}$ ≈110400), the distribution of the leading oocyte cohort (LC) was firstly analysed to assess the pattern of the most advanced oocytes in each maturity stage, complemented with presentations of complete oocyte frequency distributions from a random selection of individuals with the largest oocyte diameters (OD) within each maturity stage. The simplest account was to develop the histograms which indicated the distribution of oocytes in different size categories, while also showing the biggest LC for each ovary and, later, for maturity stages.

Further testing for the level of agreement between the histological and WM results, reassessing the large data sets of thousands of whole-mount observations (see above),five machine-learning models were considered: i) random forest, ii) neural network, iii) boosted trees, iv) support-vector machine with polynomial kernel, and v) support-vector machine with radial basis of kernel function (Alathea, 2015; Allaire et al. 2020; Henry and Wickham, 2019; Robinson and Hayes, 2020; Wickham et al. 2020a, 2020b; and Xie, 2020; respectively). Different oocyte features were assessed to determine those most suitable for maturity staging based on optimal values following tuning procedures. All the analyses, visualisation and report generation were done in R (R Core Team, 2020). Multiple R packages were used for data processing, visualization, analysis, and summary of results (Alathea, 2015; Allaire et al. 2020; Henry and Wickham, 2019; Robinson and Hayes, 2020; Wickham et al. 2020a, 2020b; Xie, 2020). There was a consideration of four sets of models of increasing complexity:

$$\begin{array}{c|c|c|lcr}\text{Model} & \text{formula} \\\hlineModel1;

histology \sim diameter\\Model2;

histology \sim diameter+roundness\\Model3;

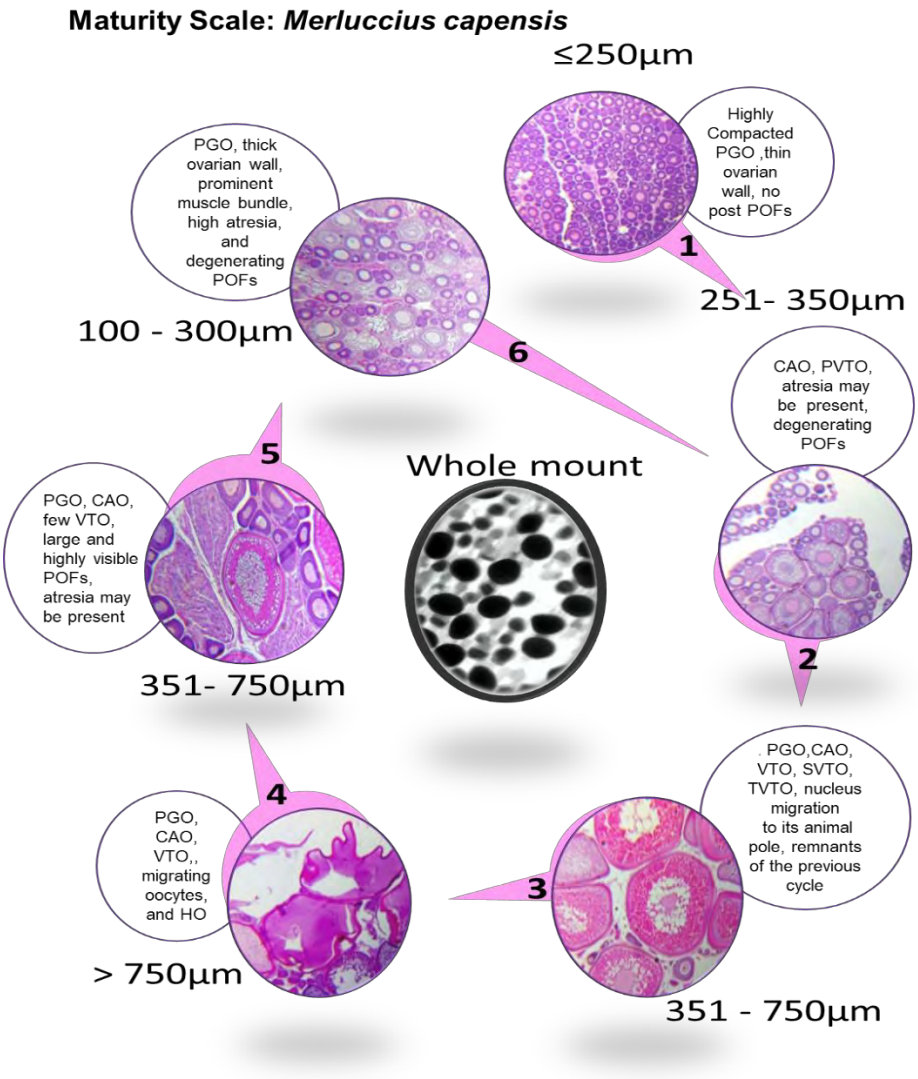
histology \sim diameter+roundness+ellipcitiy\\\end{array}$$

One-Way ANOVA was used to test the difference of mean OD between the maturity stages (immature, developing, spawning and resting) and also the difference between the mean LC diameter of the six different maturity stages. The contingency tables were used to test correlations between the WM and histological stages. The two means from different samples were compared by a Student's t-test and the corresponding variation by F-test, when applicable. The post-hoc Tukey HSD test was used to test the difference between pairs of group means.

## 2.3 Results

### *2.3.1 Maturity scale development*

The OD threshold of the histologically- assessed immature stage (stage 1) was ≤ 250 µm. The oocytes of the developing stage (stage 2) were compatible with mean OD between 251 and 350 µm, the spawning-capable (stage 3) and regressing (stage 5) stages with 351-750 µm, the actively spawning stage (stage 4) with > 750 µm and the regenerating (stage 6) stages with 100- 300 µm (Figure 2.1). In terms of the most advanced oocyte stage presence, the immature (1) stage showed only PVOs, while different cellular classification features were present in the other stages. For instance, the developing (2) stage had additionally cortical alveoli oocytes (CAOs) but also with some primary vitellogenic oocytes (VTOs), the spawning capable (3) stage had well-developed VTOs (secondary and tertiary VTO), and the actively spawning (4) stage had, in addition, hydrated oocytes (HOs) (Figure 2.1). The OD size maturity stages assessed by WM are immature (≤250 µm), developing (251- 350 µm), spawning (> 351 µm) and regenerating (100- 300 µm). The spawning stage is the combination of all the stages with spawning activities (spawning capable, actively spawning and regressing stages). New post-ovulatory follicles (POFs) were observed in the regressing (5) stage, while old and degenerating POFs, atresia and connective tissue were features observed in the regenerating (6) stage, meanwhile some old and degenerating POFs were sometimes present in the developing stage (Figure 2.1). The process leading up to spawning included the migration of the nucleus to the animal pole (spawning capable), then ovulation (release of HOs to the lumen) followed by regressing (Figure 2.1). The current investigation adopted the definitions of the ovarian characteristics and maturity stages of the *Merlucciidae* by the other authors (Brown - Peterson et al. 2011; Figure 2.1).




593

594 **Figure 2.1:** Maturity scale and observed features of the indeterminate reproductive style of *M. capensis*
595 as oocyte development advances throughout the major stages ( 1- 6) as immature (≤ 250 µm), maturing/
596 developing (251 - 350 µm), spawning potential (351 - 750 µm), actively spawning (>750 µm), regressing
597 (351 - 750 µm).spawning (> 350 µm) and regenerating stage (251 - 350 µm), respectively, as per authors
598 (Costa, 2009; Brown - Peterson et al. 2011). Maturity stages 3 - 5 are condensed to one spawning stage
599 as they are inclusive of the spawning activities.

600 *2.3.2 Introduction of the WM method*

601 Maturity stages determined by histological method were fitted to the oocyte diameters (OD) that were
602 generated by the WM method (Table 2.2). A contingency table revealed that 44% of the samples collected
603 in 2019 were immature as opposed to 56% that was matured (Table 2.4).

604 **Table 2. 2:** Classification of microscopic maturity stages and the oocyte diameters (OD) derived through
605 the whole mount (WM) auto - diametric method for the *M.capensis* females. The table represent the
606 results of the current study and the names of the stages on the left have been adopted from Brown -
607 Peterson et al. 2011.

| Maturity stages | Histological (Microscopic criteria) | Stage of maturity assigned by WM (OD) |
|---|---|---|
| Immature (1) | Highly compacted primary growth oocytes (PGO; oogonia and perinucleolar), thin ovarian wall, no POFs | ≤ 250 μm |
| Developing (2) | PGO, CAO, old atresia may be present, disintegrating POFs | 251- 350 μm |
| Spawning capable (3) | PGO, CAO, PVTO, SVTO, TVTO, nucleus migrating to its animal pole, remnants of the previous cycle. | 351 - 750 μm |
| Actively spawning (4) | PGO, VTO, migrating nucleus, and the HO. | >750 μm |
| Regressing (5) | PGO, CAO, few VTO, highly visible POFs, atresia may be present. | 351 - 750 μm |
| Regenerating (6) | PGO, thick ovarian wall, prominent muscle bundle, high atresia and degenerating POFs. | 100 - 300 μm |

608

609 The combined spawning activities i.e., labelled as “spawning” (stage 3+ 4+ 5) are the spawning
610 capable, actively spawning and the regressing stages and they all contain the vitellogenic oocytes, though
611 hydrated oocytes may only be common in the middle stage of spawning (actively spawning stage) and
612 ovulation only occurs in actively spawning stage (Figure 2.2). The distribution indicates that all stages
613 have advanced oocytes that are beyond > 350 μm, which is the threshold of spawning stage.

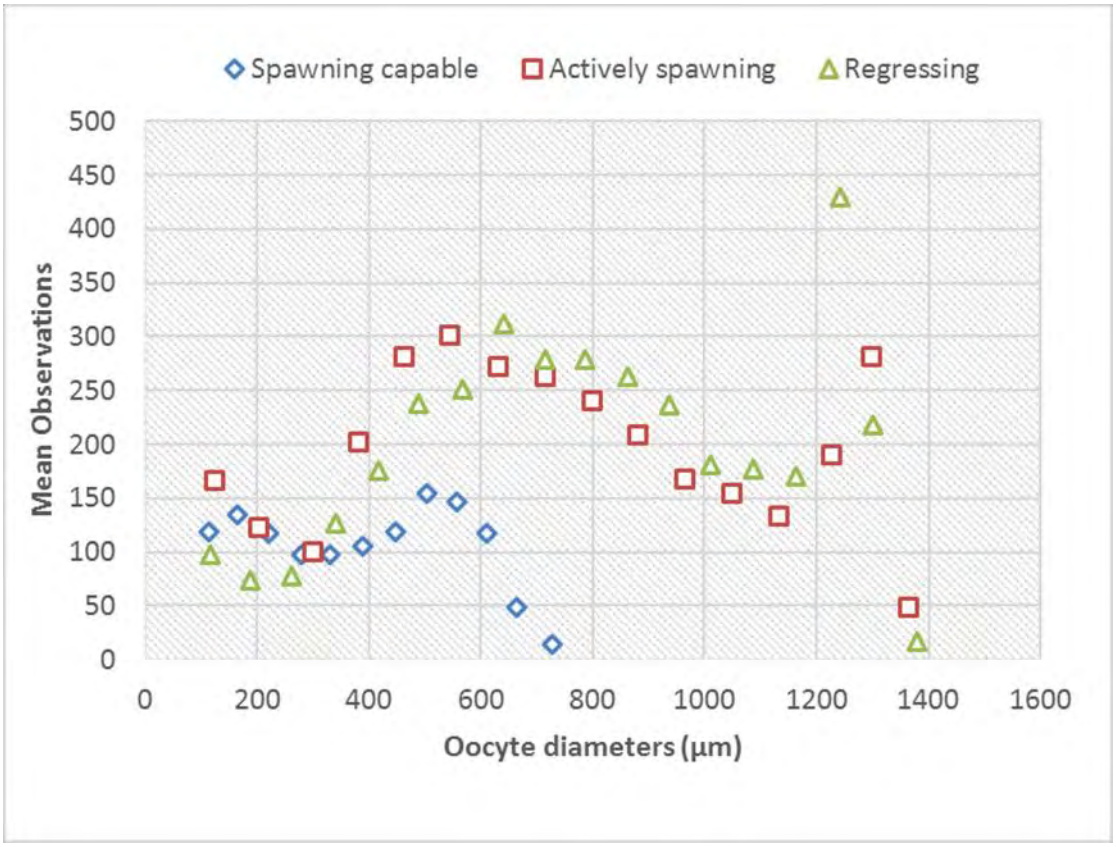



**Figure 2.2:** Distribution of the mean oocyte diameters during spawning stages (spawning capable, actively spawning and regressing stages - which are maturity stages 3, 4 and 5). The number of oocytes in a particular stage is (n = 2171, 3132 and 2818) from an individual female, respectively.

Regarding the WM auto-diametric method, separation of the leading cohorts from the rest of the oocytes helped to identify the maturity stage defined by their size (LC diameter) (Table 2.3). Considering the WM data quality-assessed by histology, the grand mean LC diameter was lowest (167 µm) for the immature stage (stage 1), followed by the developing stage (312 µm) (stage 2), > 350 µm of the spawning stage (stage 3 + 4 + 5) and the regenerating stage (265 µm) (stage 6). The mean OD of all the maturity stages, separated and combined was significantly different ($P < 0.05$). Meanwhile the distribution of the mean values for the LC, including the distribution of the rest of the maturity stage values, indicated some overlap in maturity stage immature, developing and the regenerating stages and there was no significant difference ($P > 0.05$) in the OD of such maturity stages (Figure 2.3).

**Table 2.3:** Distribution of leading cohort (LC) oocyte diameters per maturity stage. Measurements of LC diameter are from the 50 samples of 2019. N refers to the advanced LC oocytes found when females of a particular maturity stage (1- immature, 2- developing, 3- spawning capable, 4- actively spawning, 5- regressing and 6- regenerating), were combined.

| Maturity stage | Leading cohort oocyte diameter (µm) | | | N |
|---|---|---|---|---|
| | Mean | -95% | 95% | |
| 1 | 167 | 164 | 170 | 850 |
| 2 | 312 | 308 | 316 | 564 |
| 3 | 377 | 372 | 381 | 747 |
| 4 | 886 | 875 | 896 | 88 |
| 5 | 558 | 552 | 563 | 293 |
| 6 | 265 | 252 | 278 | 51 |

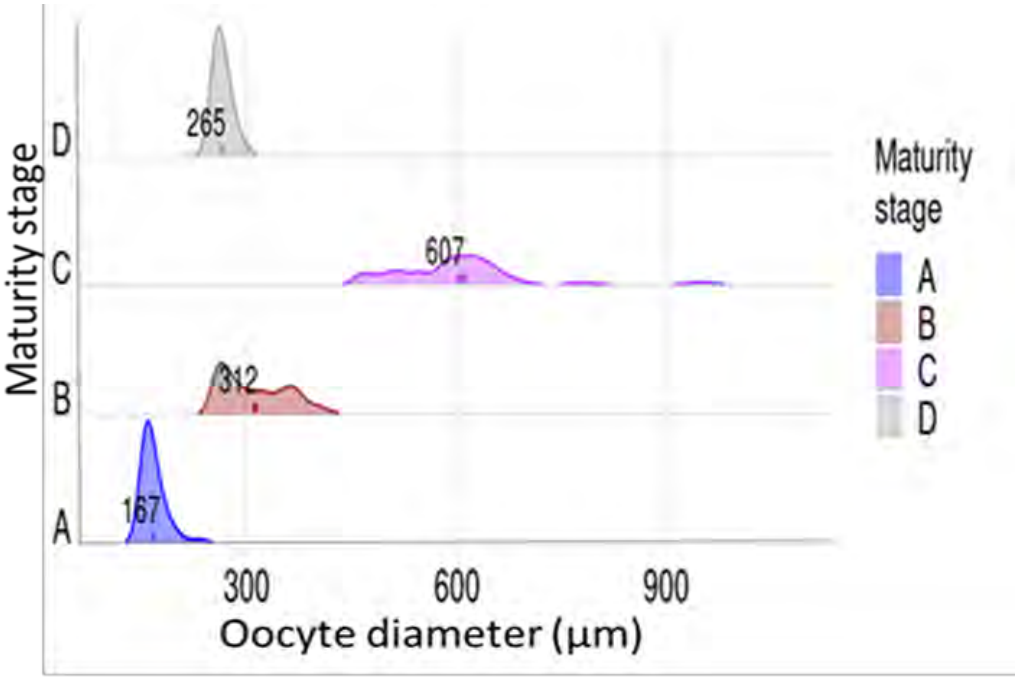



**Figure 2.3:** Distribution of oocyte diameters (µm) of the leading cohorts (LC) and the 95% intervals in different maturity stages (A = 1 - immature, B = 2 - developing, C = 3 - spawning (combining stages 3 - 5 in Table 2.3 as spawning capable, actively spawning and the regressing stages) and D = 4 - regenerating stages). The graph is a representation of the samples of 2019 survey data.

*2.3.3 Compatibility of assessment methods*

The correlation between the two variables is represented in the diagonal bold percentage values from maturity stage 1- 6 (Table 2.4). Other values not highlighted are the misplaced values in percentage. The overall, across-stage compatibility between the WM and histological assessments resulted in 86% agreement, when all stages were combined (Table 2.4). The developing and the actively spawning stages were 100% compatible, whereas the regenerating stage was the least compatible, with 33% disagreement.

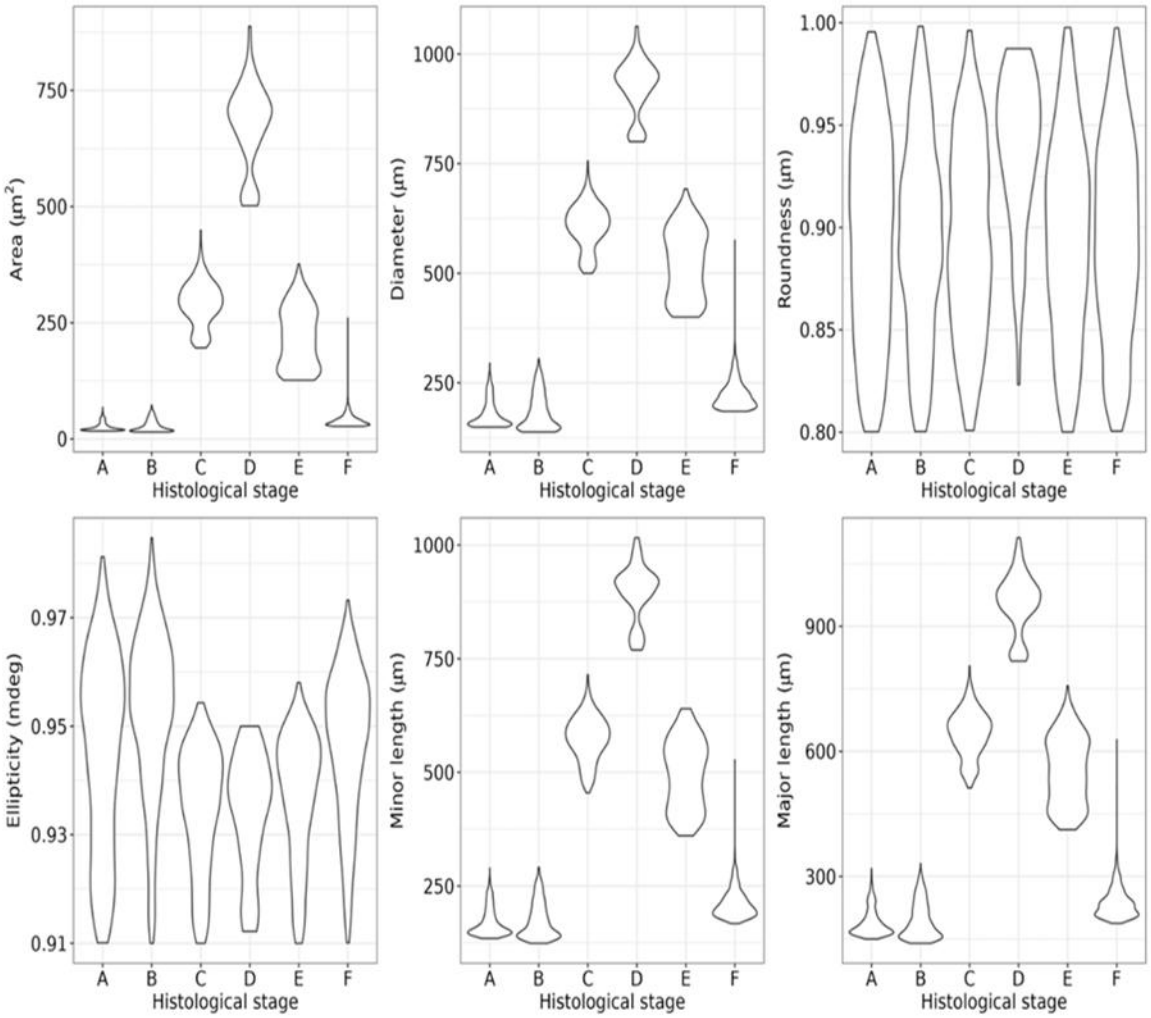
**Table 2.4:** Contingency table showing the distribution of histology in rows and the whole mount values in columns. Letters represent the maturity stages (1 = Immature, 2 = developing, 3 = spawning capable, 4 = actively spawning, 5 = regressing and 6 = regenerating maturity stages). N = 50 samples collected in 2019 survey.

| Maturity stages | 100-250µm | 251-350µm | 351-750µm | >750µm | 351-750µm | 100-300µm | Histology (N) |
|---|---|---|---|---|---|---|---|
| 1 | **91** | 9 | 0 | 0 | 0 | 0 | 22 |
| 2 | 0 | **100** | 0 | 0 | 0 | 0 | 2 |
| 3 | 0 | 18 | **73** | 9 | 0 | 0 | 11 |
| 4 | 0 | 0 | 0 | **100** | 0 | 0 | 4 |
| 5 | 0 | 0 | 0 | 0 | **80** | 20 | 5 |
| 6 | 33 | 0 | 0 | 0 | 0 | **67** | 6 |
| Whole mount | 22 | 6 | 8 | 5 | 4 | 5 | 50 (**86.2%**) |

*2.3.4 WM oocyte features*

Machine learning models indicated six important WM oocyte features (the area, diameter, roundness, ellipticity, minor and the major length) to assess the microscopic maturity stage validated by histology. A violin plot indicates the distribution of the density data at different values (Figure 2.4). There was symmetrical shape with even distribution for all features for all maturity stages, though, there was significant difference for area, OD, minor and major length of oocytes for all maturity stages as the actively

655 spawning stage (stage 4) was greatest in all mentioned features ($P < 0.05$). The median for area was
656 >500 μm (mean = 700 μm), stages 1, 2 and 6 were not significantly different with each other and stages
657 3, 5 and 6 were also not significantly different with each other ($P > 0.05$). The OD and minor length
658 performed in the similar way when tested, with their median >750 μm (mean = 900 μm), meanwhile the
659 major length was slightly higher than the latter with median >800 μm (mean = 1000 μm), though they
660 were not significantly different ($P > 0.05$). Roundness for all maturity stages as the violins ranged from
661 80- 100% for all stages combined and separated (P > 0.05). Distribution of ellipticity for all maturity stages
662 indicated no significant difference with values ranging from 91- 99 mdeg ($P > 0.05$). Overall, four WM
663 features showed similar patterns (area, diameter, major and minor length) across histological stage,
664 whereas ellipticity and roundness indicated different shapes. (Figure 2.4). The said, machine- learning
665 techniques illustrated that the roundness increases for large oocytes (cf. stage D) and that a high degree
666 of ellipticity is characteristic for relatively small oocytes (cf. stages A, B and F).




667

668 **Figure 2.4:** Violin plot summarising distribution of oocyte morphological attributes (oocyte area, oocyte
669 diameter, ellipticity, major length, minor length and roundness) from the whole mount data for the A- F
670 maturity stages (A - immature, B - developing, C - spawning and D - regenerating stages).

671 Classification of the WM data was performed by five models and only random forest could classify all
672 data points of high magnitude (> 111 000 data points). Random forest model appear to perform well in
673 classifying oocytes to the relevant histologically validated stage as the overall classification was 87% that
674 also attributes to the compatibility of histology and whole mount auto-diametric methods. Due to high
675 magnitude of data, the information was reduced to LC of 10%, to measure how well a model based whole-
676 mount attributes of oocytes will perform in classification of the histologically generated maturity stages
677 (Figure 2.5). The models indicated the confusion matrix as shown with bolded levels of agreement

between the two methods (WM and histology). All models (Boosted tree = 0.81, nnet,= 0.82, random forest = 0.82, svm_polynomial = 0.84 and svm_radial - basis = 0.83) accredited for different values and the svm_polynomial accredited for 0.84, which is slightly higher than others, though not significant ($P > 0.05$).

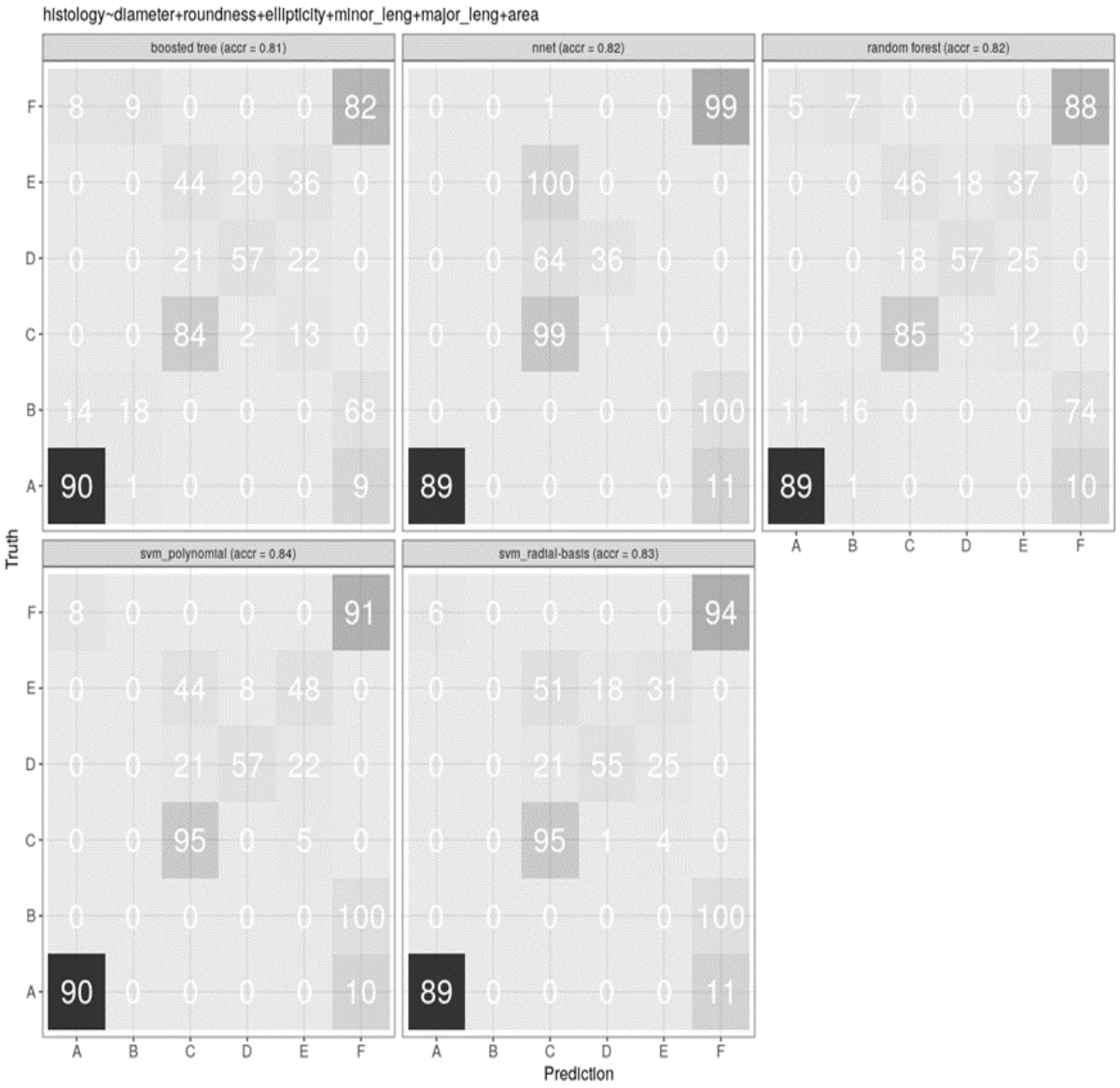



**Figure 2.5:** Confusion matrix: showing five different model classification performance based on the selected sets of oocyte attributes: diameter, roundness, and ellipticity. Rows are classification from histological work whereas the columns are predicted class from the model. The source of data is the

686 histology and the whole mount auto-diametric processed samples and A - F and maturity stages from 1-
687 6.

688 *2.3.5 Fecundity pattern*

689 As maturity advanced, there was further development in the WM - measured size of oocytes as
690 vitellogenesis progressed, more protein granules becoming apparent (when assessed histologically) –
691 resulting in increased darkness (contrast) under the stereomicroscope – and other oocytes developing
692 from the PVOs to constitute the most advanced stage (Table 2.5). The number of oocytes per weight of
693 ovarian subsample was always greatest for PVO oocytes, followed by VTO, HO and CAO oocytes and
694 was significant at P<0.05 when a single factor Anova was run (Table 2.5).

695 **Table 2.5:** Distribution of the WM oocyte categories, average, and the standard error between the groups.
696 N is the total number of fish in assessment, from where oocyte categories (primary growth oocytes (PGO),
697 cortical alveoli (CAO), vitellogenic oocytes (VTO) and the hydrated oocytes (HO)) were calculated.

| Summary of Anova: Single Factor Analysis | | | | | | |
|---|---|---|---|---|---|---|
| *Groups* | *Count (N)* | *Sum* | *Average* | *Variance* | | |
| PGO | 91 | 139261 | 1530.3 | 1866536.9 | | |
| CAO | 71 | 21775 | 306.7 | 197909.7 | | |
| VTO | 59 | 32018 | 542.7 | 266506.3 | | |
| HO | 44 | 40253 | 914.8 | 481762.2 | | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 68591139 | 3 | 22863713 | 27.371627 | 2.01E-15 | 2.639188 |
| Within Groups | 2.18E+08 | 261 | 835307 | | | |
| Total | 2.87E+08 | 264 | | | | |

698

699 The oocyte density was different when all oocyte categories were compared ($P < 0.05$), with PGO
700 oocytes showing the greatest values, than all other oocyte categories. However, when the Turkey HSD
701 test was done between groups, the oocyte density of HO and PGOs was not significantly different, and
702 so was that of CAO and VTO ($P > 0.05$). It was also apparent that the oocyte diameter size (≤ 250 µm)
703 of the PGO was constantly present with every maturity stage as the first oocytes that develop to be the

secondary and tertiary oocytes (Figure 2.6). There is always above 50% frequency of the PGO in the immature, the developing and the regenerating stages, though the content of PGO has shown some reduction (> 10 %) in the spawning stages. All stages have highest frequency of the PGO, meanwhile other oocyte stages (CAO, VTO and HO), reflected to be < 10 % beyond 300 oocyte density.

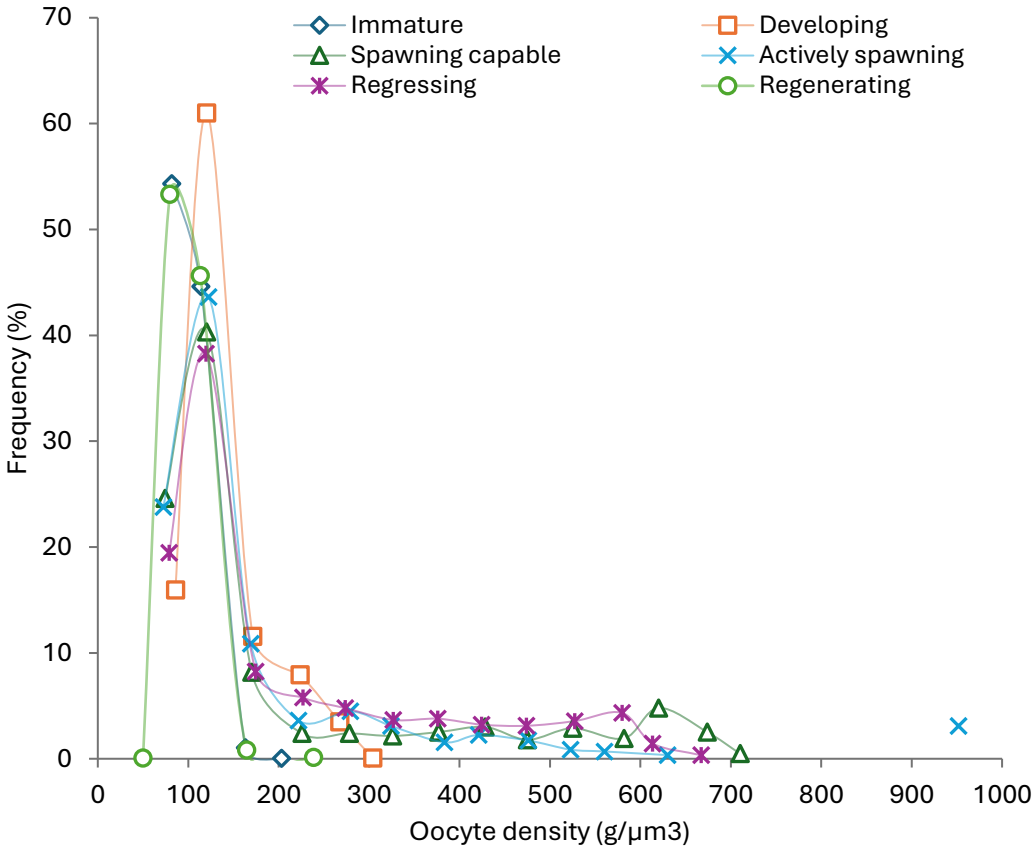



**Figure 2.6:** Distribution of mean oocyte density on the x- axis and the frequency on the y- axis for the six maturity stages. Different maturity stages are indicated by different marks and colours. Analysis is from 50 samples collected in 2019.

## 2.4 Discussion

### *2.4.1 Maturity scale*

A new maturity scale that uses both the whole-mount method and histological features for *M. capensis* was developed in the present study with four major maturity stages depicted based on OD categories and six primary microscopic stages from histology (Figure 2.1). The four-stage maturity scale superficially resembles that proposed by Osborne and Mullins (2005). However, the latter does not provide a correct estimate of physiological maturity based on field-friendly staging through the use of histology. The suggested maturity scale in this study is two-fold, composed of oocyte diameter categories from the whole-mount auto-diametric method (Serrat et al. 2019) and oocyte characteristics from histologically assessed stages (Brown- Peterson et al. 2011).

The presented maturity scale addresses four size oocyte feature related whole mount categories and six histological feature related categories. The first size category (≤250 OD) corresponds to ovaries that contain mainly PVOs (the immature stage), which provides a good estimate for future but not current recruitment to the fishery, avoiding erroneous counts which possibly lead to spurious population biomass estimates (Murawski et al. 2001). The threshold for mean OD, which is less than 250 µm for the immature stage and above 350 µm for spawning-capable to regressing stages, agrees with other investigations (Kainge et al. 2007; Jansen et al. 2015). The second category (251- 350 µm) contains PVOs and the larger, different and more advanced oocytes, the cortical alveoli advanced oocytes (CAO; the developing stage). The third oocyte size category (>350 µm OD) is the largest in the whole-mount auto-metric method in the present study and reflects sequence of all spawning activities. The beginning of spawning is indicated by the presence of tertiary vitellogenic oocytes (TVTO), the migration of the nucleus to its animal pole, the enzymatic disintegration of yolk granules resulting in more water being accumulated in the hydrated oocyte, and imminent ovulation (Brown- Peterson et al. 2011; Lowerre- Barbieri et al. 2011). For the histologically assessed stages, this oocyte size category (>350 µm OD) includes the spawning-capable, actively spawning and regressing stages. It is necessary to be able to separate these stages as

737 they differ in oocyte types and represent different physiological solutions in the process of reproduction.
738 The most advanced oocytes for the spawning-capable stage are the TVTOs and the migration of nucleus
739 to its animal pole, while HOs are the most important feature for the actively- spawning stage and the new
740 POFs are a feature of the regressing stage. Although there is only one spawning category combining
741 stages (spawning capable, actively spawning and the regressing stages) with OD >350 μm stages from
742 the auto-diametric method (Figure 2.1), different features and purpose necessitate histological
743 separation. For instance, only ovaries that show the presence of HOs and the absence of POFs in the
744 histological analysis are normally used for batch fecundity analysis (Hunter et al. 1985, 1992; Brown –
745 Peterson et al. 2011; Lowerre- Barbieri et al. 2011). The OD peaked during actively stage, though it had
746 similar range of OD (spawning capable stage C: 351-750 μm) and the (regressing stage E: 351-750 μm)
747 which is the stage after egg release. New POFs are a prominent feature of the regressing stage, hence
748 ovaries containing POFs are necessary for calculation of the spawning fraction, in the process of
749 calculating annual fecundity (Hunter and Goldberg, 1980; Hunter and Macewicz, 1985).
750 The fourth category is the regenerating stage (100 - 300 μm) which contained only the PVO and the
751 remnants of the previous cycle as atresia and the old POFs. The last stage of the maturity process of a
752 serial spawner, which spawns in batches at intervals during a spawning season, can only return to the
753 developing stage after a resting period (Tomkiewicz et al. 2003, Brown-Peterson et al. 2011). Hence, the
754 remnants of the previous cycle include the old POFs, atresia and epithelial bodies (West, 1990; Tyler and
755 Sumpter, 1996; Tuene et al. 2002). The developing and the regenerating stages were not significantly
756 different when their mean ODs were tested as both stages contain PVO as the primary oocytes and their
757 OD size overlaps. Both stages (developing and regenerating) displayed the presence of remnants of the
758 previous cycle as atretic POFs, though the developing stage may not have sometimes. Old and
759 degenerating POFs and atresia are common remnants of both developing and regenerating stages,
760 mostly in the developing stage of a fish that has spawned before (Honji et al. 1993; Rideout et al. 2011).

*Merluccius capensis* is a serial spawner and the remnants of the previous cycle are a prominent feature of the developing stage (Osborne et al. 1999; Osborne, 2004).

*2.4.2 Introduction of the WM method*

This study reiterated the importance of a combination of a morphological analysis, histological and whole-mount analysis to eliminate errors and provide necessary resolution to estimate reproductive status from data collected in the field. The maturity stage of the gonads is an important biological parameter to be used in the determination of maturity ogives which are later used for estimation of the spawning stock biomass (SSB) and if errors in estimating maturity ogives occur, they may lead to spurious SSB estimates (Vitale *et al*., 2006). Maturity staging is also critical factor for fecundity estimates and spawning investigations, which when erroneous may lead to wrong spawning estimates (Murawski et al. 2001; Arocha, 2002; Murua and Saborido - Rey, 2003). The use of a quantitative whole-mount method at sea could produce better estimates of maturity ogives and could replace the erroneous macroscopic method (Kjesbu, 1991). Furthermore, the whole-mount auto- diametric method may be a beneficial method as it reduces the time, cost and labour incurred by histology when applied accurately (Kjesbu, 1991; Kjesbu et al. 2003; Nomxego et al. 2024). The current study has recorded that; HO of the actively spawning stage cannot be measured by histology (due to rapture during slide production) and are therefore dependant on a whole-mount auto-diametric method for measurements and further assessment. Likewise, stages sharing similar OD (spawning capable and regressing stages) from the whole-mount data create a histological dependency for identification and validation as internal organelles (presence of POFs) can only be determined by microscopic analysis, thereby leaving histology as a validating tool for both the macroscopic and whole-mount techniques. The inclusion of the WM auto-diametric method with the histological assessment has improved our understanding of the oocyte development of the investigated species.

784 *2.4.3 Compatibility of the assessment methods*

785 Earlier histological assessments indicate the existence of a high degree of error in the macroscopic,
786 morphological staging of the maturation process, particularly between immature and post-spawning hake
787 (Osborne, 2004; Kainge et al. 2007). The cited authors reported overall percentage errors of 40% and
788 30% respectively for the subjective macroscopic assessment, when validated through histology for both
789 *M. paradoxus* and *M. capensis.* Other studies have highlighted the errors of the macroscopic method
790 (Honji et al. 1993; Tomkiewicz et al. 2003; Vitale et al. 2006; Costa, 2009; Ferreri et al. 2009; Brown -
791 Peterson et al. 2011). All indicated a need for an improved maturity scale and better methods for maturity
792 staging. Other than the traditionally used histological method, which validates the macroscopic errors of
793 the maturity scale for *M. capensis*, the present study has introduced the whole-mount technique for
794 application for maturation stage determination for this species in the field. The present study suggests a
795 relatively good agreement (86 %) between histological assessment and the whole-mount auto-diametric
796 method for all stages combined and an accurate assessment of both the developing and the actively
797 spawning stage. Compatibility of maturity stage assessment method increases the credibility of data,
798 thereby improving precision of estimated results (Honji et al. 1993; Kjesbu et al. 2003; Ferreri et al. 2009).
799 The high compatibility of these methods can allow the use of each method independently with similar
800 results (Kjesbu, 1991; Nomxego et al. 20024).

801 *2.4.4 WM oocyte features*

802 In the present study, there are three major stages that explained the spawning activity (spawning capable,
803 actively spawning and regressing stages) of an indeterminate spawner and they showed increased
804 oocytes in ovulating stages (actively spawning and the regressing stages). The highest OD was on the
805 oocytes ready for ovulation and there was always a leading cohort of the biggest oocytes that are ready
806 to be spawned, which is an indication of batch spawning (Murua and Motos 2006). The findings of the
807 present study agree with the observed presence of the LC especially in the actively spawning or hydrated

808 stages, which carried the largest oocytes ready for ovulation (Murua and Motos, 2006). The presence of
809 many cohorts at various stages of development is an indication of a batch spawner, and this result
810 concurs with those of other authors (Osborne, 2004; Brown- Peterson et al. 2011; Lowerre-Barbieri et al.
811 2011). Continuous development of cohorts is an indication that *M. capensis* is a group-synchronous
812 spawner, wherein oocytes of all stages are present in the ovary without dominant populations, so the
813 ovary appears to be a random mixture of oocytes at every conceivable stage and such a species is
814 capable of ovulating on a regular basis, sometimes every day over a prolonged period (Murua and
815 Saborido - Rey, 2003).
816 The random forest model was the best model among the selected candidates as it is not limited by
817 dimensional dataset and functions well with subsets of data (Robinson and Hayes, 2020; Wickham *et al.*,
818 2020a, 2020b; Xie, 2020). In the present study, oocyte area, oocyte diameter, and the major and minor
819 length of the oocyte were the best parameters when the histological stage and the whole-mount features
820 of the oocytes were tested. The order of the maturity stages (stages A - F) that was assessed by histology
821 was also compatible with features (area, diameter and length) measured by the whole-mount technique.
822 There was a clear distinction of stages with the actively spawning/ hydrated stage characterized by the
823 largest OD, area, major and minor length. The model tested the compatibility of the two methods and
824 whether or not the aforementioned features are viable for maturity staging assessment. Results from the
825 random forest model suggest that the whole-mount method is compatible with histological assessment
826 regarding the oocyte features.

827 *2.4.5 Fecundity type*
828 The continuous presence of PVOs observed in the present study is a prominent feature of indeterminate
829 fecundity, as supported by other studies (Osborne, 2004; Kainge et al. 2007; Gordo et al. 2008; Brown -
830 Peterson et al. 2011; Ganias et al. 2015; Serrat et al. 2019). Species exhibit an indeterminate fecundity
831 type when the oocyte recruitment of the unyolked PVOs continues on to secondary growth throughout

the spawning season (Kjesbu, 2009; Lowerre - Barbieri et al. 2011; Ganias, 2013). Indeterminate batch spawners exhibit continuous oocyte recruitment (Serrat et al. 2019) and repeatedly recruiting oocytes from primary to secondary growth, increasing their fecundity and their ability to spawn over an extended time (Lowerre-Barbieri et al. 2011). New batches of developing oocytes were also clear in the present study, with the OD size increasing in the actively spawning stage, which was indicative of serial spawning. In serial spawners, the presence of a new batch of developing oocytes can be observed on a size-frequency histogram as an isolated group of oocytes that are larger than the remaining VTOs (Murua and Saborido - Rey, 2003; Dominguez - Petit et al. 2017).

## 2.5 Conclusion

The whole-mount technique produces quantitative information and is a simple field-friendly method, which is applicable on-board the research vessel with adequate facilities, while its automation is applicable for staging in the laboratory (Forberg et al. 1983; Kjesbu, 1991; Dominguez - Petit et al. 2017). Both latter methods are applied in a new way in this paper and may bring innovation in the region. The results of the present investigation, specifically the analysis of OD, lays the foundation for a new approach in batch fecundity studies of *M. capensis*.

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# Chapter 3: Determination of batch fecundity of Merluccius capensis by principal volume based equations

## 3.1 Introduction

Estimation of fecundity is a key parameter in Egg Production Methods (EPM) but is also a very relevant trait in fundamental life history and evolutionary framework studies (Murua et al. 2006). According to other authors (Hunter and Macewicz, 1985; Ganias 2013; Saber et al. 2015), investigation of batch fecundity (BF) primarily seeks to get useful measure of the number of eggs released in a single spawning event. Such information permits evaluation of the reproductive potential of species, helps provide proxies for spawning stock biomass and improves information regarding the relationship between spawning stock biomass and recruitment (Murua et al. 1998). Reporting of individual fecundity can be done directly by manual counting of developing oocytes (Bagenal, 1978), however, new advancements and innovative methods have surfaced over time. Among other aspects, these advancements, as the major implementation of digital techniques, have clarified that *de facto* quantification of different oocyte developmental phases, including the early ones, is the best practice to get an in-depth understanding of the spawning style (fecundity regulation) *per se*, and thereby the realised formation of annual, seasonal or batch fecundity (Thorsen and Kjesbu, 2001; Kurita and Kjesbu, 2009; Kjesbu et al. 2011; Saber et al. 2015; ; Serrat et al. 2019; Anderson et al. 2020).

Batch fecundity relates to the gonadosomatic index (*GSI*) which reflects the spawning period (Coker et al. 2008). Once more energy is invested in gonad development, the gonads increase in size as the maturity stage develops from inactive immature to actively spawning, however, this process typically results in reduction in body weight, implying that the gonad size increased towards the actively spawning stage, while the body weight decreased (Coker et al. 2008; Lowerre- Barbieri et al. 2011). Thus, the relationship of the gonad size and the body weight becomes important, hence, *GSI* is generally used as an indicator for gonad size relative to body size and the larger is the *GSI*, the higher the chances of spawning or the closer is the spawning period (Coker et al. 2008).

1014 Demographic variation in e.g. total length composition influences the number of eggs spawned
1015 (Marshall et al. 1999) in a single spawning event (batch fecundity; Saber et al. 2015; Ganias et al. 2018,),
1016 which varies during the spawning season (Macchi et al. 2005; Korta et al. 2010). Batch fecundity is also
1017 known to be size and age dependent with large and older fish producing more eggs and releasing more
1018 batches and thereby spawning over a longer time period in the season (Marteinsdottir and Begg, 2002;
1019 Macchi et al. 2005). Other authors relate to the release of more eggs by bigger and older fish as the
1020 BOFFFF effect (Field et al. 2008). In this sense, fecundity estimates per length class could give
1021 information on the number of potential offspring that could be produced (Field et al. 2008; Hixon et al.
1022 2014).
1023 Though *M. capensis* is one of the most lucrative species in Benguela Upwelling System, and
1024 pronounced to be a biologically and ecologically healthy resource (Rademeyer et al. 2008; Durholtz et al.
1025 2015; DFFE, 2020, Nomxego et al. 2024), several scientific research deficiencies regarding its biology
1026 (life history) are obviously in place. It is therefore imperative to improve the knowledge base to which
1027 degree the reproductive investment and phenology may vary seasonally, such as for BF *vs*. spawning
1028 period and body size. In parallel, we aimed at contributing to launching more effective, general ways to
1029 work up ovaries from active spawner to get better insight in the underlying oocyte recruitment pattern as
1030 such.
1031 Batch fecundity of the shallow water hake *M. capensis* has been investigated by Osborne et al. (2004)
1032 off south-west South Africa, reporting a mean relative fecundity of 160 (SE = 12) per gram ovary - free
1033 (somatic body weight ) or 143 (SE = 10) per gram whole body weight). These authors used 'Hydrated
1034 oocyte method' (Osborne et al. 2004), which is a variant of 'the Gravimetric method', that is targeting
1035 hydrated oocytes rather than vitellogenic oocytes. Other than the above-mentioned fecundity estimation
1036 of Osborne et al. (1999) more than two decades ago using classical, non-digital methodology, no other
1037 published fecundity information apparently exists for *M. capensis* in the area. The current study aims to
1038 explore volumetric methods to investigate the BF of *M. capensis* in the south coast of South Africa and

1039 also to investigate whether there are other oocyte developmental stages that can be useful in determining
1040 estimates of fecundity other than HO. The study also intends to investigate the link between BF, GSI and
1041 the spawning time. The relationship between BF and the fish size will also be explored to assess whether
1042 there is dependency on the said parameters. It is expected that there may be changes in the BF of the
1043 *M. capensis* due to the changes in the environment, in distribution, in the methods used and the species
1044 life history parameters (Hunter et al. 1985; Macchi et al. 2005; Nomxego et al. 2024). Such changes
1045 became the rationale and encouraged the current investigation, thereby updating the methods and the
1046 literature regarding the species in the region.

1047 Methodologically, it should be noted that the frequently applied auto-diametric method was designed
1048 for homogenous ovaries (cf. pre-spawners) only, that is, it functions rather poorly on heterogeneous
1049 ovaries (cf. spawners) (Thorsen and Kjesbu, 2001). Rightly so, the closely related ultra-diametric method
1050 works well on ovarian samples from spawners too but then limited to presenting oocyte size frequency
1051 distribution (OSFD), albeit even for thinnest previtellogenic oocytes (PVOs). Here, we firstly developed
1052 principal equations to be able to quantify fecundity by oocyte developmental phase based on these
1053 automated, digital measurements, thus, not by consulting, supplementary parameterisations from
1054 stereological techniques in this calculation exercise (Kurita and Kjesbu, 2009; Kjesbu et al. 2011; Saber
1055 et al. 2015; Serrat et al. 2019). The current study used histology to characterize and detail the maturity
1056 stage for each of the examined individuals. Thereafter, we used this new toolbox of algorithms on *M.*
1057 *capensis* to provide up-dated fecundity figures by oocyte phase for this species. The alongside
1058 investigation on *GSI* dynamics throughout the sampling period was used to trace the months closest to
1059 spawning activity (with the highest *GSI*).

## 3.2 Materials and methods

### *3.2.1. Field samples*

Samples of spawning females of *M. capensis* were collected by the bottom trawl gear on the shelf and upper slope of the south coast of South Africa during both commercial and research surveys for three years, 2014 (N = 64), 2015 (N = 212), and 2016 (N = 119). Variables collected were: total length (TL) in centimetres, and total weight (TW), gutted weight and gonad weight in grams. Gonads were extracted and fixed in 4% buffered formaldehyde for further processing in the laboratory.

### *3.2.2. Tissue preparation*

#### *3.2.2.1. Histological protocol*

The collected ovarian samples were processed in the laboratory following standardized histological procedures (Hunter and Macewicz, 1985). Cross sections of one of the lobes of the ovary nearest to the vent were removed for histological analysis, dehydrated through a series of ethanol immersions, embedded in paraffin, sectioned at 6-10 µm, put on slides, and cleared with xylene. The slides were thereafter rehydrated, stained by haematoxylin and eosin, and, finally, analysed under the microscope at 40× magnification to establish the degree of maturity of the ovary. A total of six maturity stages (1 - 6) were identified as immature, developing, spawning capable, actively spawning, regressing and the regenerating, respectively, based on the presence of the most advanced oocytes and the cell organelles in the ovary (Nomxego et al. 2024).

#### *3.2.2.2. Whole-mount protocol*

A separate piece of tissue was cut from the centre of the ovarian lobe, weighed to the nearest gram and placed in a vial filled with 4% buffered formaldehyde  An ultrasonic pen was used for a maximum period of 20 seconds, depending of the softness of the tissue, to separate the oocytes (Anderson *et al.,* 2020). The samples were transferred to a petri dish, and pictures of the tissue taken using the computer with

Zen-lite 2.3 software and with the Carl Zeiss Camera (Axiocam ERc5s) connected to the dissecting microscope and the light source. The camera was set to take 8-bit converted to RGB pictures and transmitted light was used so that the oocytes appeared as dark objects on a bright background. The resolution of the pictures was 0.88 pixels/µm. Oocyte features were automatically measured using the open source image analysis program image-J (v.1.52, https://imagej.nih.gov/ij/) with the plug in object-J (https://silsfnwi.uva.nl/bcb/objectj/) and an adapted variant of the elliptical oocytes project (https://silsfnwi.uva.nl/bcb/objectj/examples/oocytes/Oocytes.htm). Scale was propagated at 0.13px/µm and the threshold ranges for oocytes size was 100-1600 µm. The macros in the Image-J software measured diameters, roundness, ellipticity, area, volume and counts of the oocytes from each picture.

*3.2.3 Oocyte developmental phases*

The different oocyte developmental stages were categorized based on their oocyte diameter size (and corresponding appearance), with previtellogenic oocytes (PVO), cortical alveoli oocytes (CAO), vitellogenic oocytes (VTO) and the hydrated oocytes (HO) being ≤ 250, 251- 350, 351- 750 and >750 µm, respectively (Nomxego et al., 2024). The WM maturity stages were based on oocyte diameter size also 1- 6 as immature (≤ 250 µm), developing (251- 350 µm), spawning capable (351- 750 µm, actively spawning (> 750 µm), regressing (351- 750 µm) and the regenerating stage (100- 300 µm; Nomxego et al. 2024).

*3.2.4 Batch fecundity estimation by principal, volume-based equations*

Basically, the study developed equations inspired by 'the volumetric method', an alternative to 'the Gravimetric method', both classical fecundity methods (Bagenal, 1978). However, instead of counting the number of oocytes in a given sub-sample volume (SV) multiplied by the rasing factor (RF), that is ovary volume (OV) divided by SV (RF = OV)/SV), we mathematically estimated SV, and 'transferred' the ovary weight into OV by accounting for the ovarian specific gravity. This algorithms required that *all* oocytes in the petri dish could be presented by their undamaged size, and oocyte shrinkage could be accounted for

(as OW was weighed fresh). Likewise, ovarian stroma (debris) contributes to SV and thereby needs to be added in, handled as for shrinkage by a correction factor. All these calculation steps could today be performed due to 1) access to specific literature sources, when needed (cf. correction factors), 2) presentation of detailed, accurate size spreadsheet data on individual oocytes, even the tiny ones, as part of 'the Ultrametric method' (Anderson et al. 2020), and 3) the reliable measurement of OW in the field. Altogether seven equations were put together, ending with *Fec, i,* that the oocyte-phase specific fecundity, including for hydrated oocytes, that is *BF* (Table 3.1).

**Table 3.1:** Illustration of the calculation for batch fecundity using volumetric fecundity method. EQ. represent the equations and they are from 1 - 7.

| EQ. | PARAMETERS | EXPRESSION | Reference |
|---|---|---|---|
| Raising factor (RF) using principles from the classical volumetric fecundity method | | | |
| 1 | Estimating ovary volume (OV, in $cm^3$) as a function of ovary weight (OW, in g) and ovary specific gravity (ρ, in g $cm^{-3}$) | OV = OW/ρ; OV = OW/1.047 | Volumetric method: Bagenal, T. B. (1978). Chapter 4: Aspects of fish fecundity. In S. D. Gerking (Ed.), Ecology of Freshwater Fish Production (pp. 75-101). Blackwell Scientific Publications. ρ: dos Santos Schmidt, T. C., Thorsen, A., Slotte, A., Nøttestad, L., & Kjesbu, O. S. (2021). First thorough assessment of de novo oocyte recruitment in a teleost serial spawner, the Northeast Atlantic mackerel (Scomber scombrus) case. Scientific Reports, 11, Article 21795. https://doi.org/10.1038/s41598-021-01234-1 |
| 2 | Accounting for oocyte swelling in fixative (3.6% buffered formaldehyde) to get fresh oocyte diameter, $OD_{fresh,\ 1}$ (in µm) from $OD_{fixed}$ (in µm) | $OD_{fresh,\ 1} = 19 + 0.947 \times OD_{fixed}$ | Fixation effect: Thorsen, A., & Kjesbu, O. S. (2001). A rapid method for estimation of oocyte size and potential fecundity in Atlantic cod using a computer-aided particle analysis system. Journal of Sea Research, 46, 295-308. https://doi.org/10.1016/s1385-1101(01)00090-9 |
| 3 | Changing the unit for $OD_{fresh,\ 1}$ (Eq. 2) to cm; $OD_{fresh,\ 2}$ | $OD_{fresh,\ 2} = OD_{fresh,\ 1}/10^4$ | |
| 4 | Estimating the oocyte volume of a given class (phase) of oocyte i (OoVi) represented by fresh oocyte diameter ($OD_{fresh,\ 2}$ i) (Eq. 3), using the standard formula for the volume of a sphere. PS: no need to first estimate volume-based diameter when working on phases of oocytes (narrow size range per phase) | $OoVi = 3.14 \times OD_{fresh,\ 2}i^3/6$ | Phase-specific oocyte diameter: Korta, M., Murua, H., Kurita, Y., & Kjesbu, O. S. (2010). How are the oocytes recruited in an indeterminate fish? Applications of stereological techniques along with advanced packing density theory on European hake (Merluccius merluccius L.). Fish. Res., 104, 56-63. |
| 5 | Getting the analysed whole-mount, ovarian sub-sample volume (SV, in $cm^3$), which equals the volume of all oocytes (Eq. 4) recorded (N) by the image analyser times a correction factor for unaccounted debris (CFd) | $SV = CFd \times \sum N \times OoVi$; $SV = 1.03 \times \sum N \times OoVi$ | Debris: Buzeta, M. I., & Waiwood, K. G. (1982, 1982). Fecundity of Atlantic cod (Gadus morhua) in the southwestern Gulf of St. Lawrence. Canadian Technical Report of Fisheries and Aquatic Sciences, 1110, iii + 6p. Fixation: |
| 6 | Finding the resulting RF (raising factor) (no unit) (Eq. 1 and Eq. 5) | OV/SV | |
| Fecundity per oocyte phase | | | |
| 7 | Presenting successively the fecundity of different phases of oocytes (PVO, CA, EVO, VO and HO), where Fec,i is given from the general RF (Eq. 6) | $Fec, i = RF \times Ni$ | |

| | | | |
|---|---|---|---|
| | times the phase-specific number of recorder oocytes in whole mount analysis, $N_I$ | | |

*3.2.5 Spawning period*

Gonadosomatic index (*GSI*) was estimated using the formula:

$$GSI = 100\ x(W_{gonad}\ /W_{FISH}\ ) \qquad \text{Equation 8}$$

*3.2.6 Spawning size*

Measurements of the total length (cm) together with the amount of the eggs spawned in an event were used to assess whether there is a relationship between the two parameters. The mean values of the *Fec,i* of VTO were tested for correlations with the size of the fish and sometime the gonad size. Regression analysis were used to test the relationship.

*3.2.7 Data analysis*

For data analysis, both non- parametric and parametric tests were used, pending on which one was most suitable. All relationships were tested using different regressions. The relationship between the BF and the length and weight was tested by power regressions. T-tests were used to analyse and test the relationship between the two means. F- Test from two sample means were also used for testing variation. When more than three variables to be analysed, more complicated tests were used e.g. factorial ANOVA, etc.

## 3. 3 Results

### *3.3.1 Oocyte developmental phase*

The size of the oocytes is investigated by plotting the distribution of the subsample volume (SV, in $cm^3$), which is the total volume recorded in the image analyser is presented in Figure 3.1. There was different oocyte volume presented by different oocyte developmental phases as the mean volume of the HO was greatest at (84.2 %) followed by that of the VTO (13.3 %), CAO (2.0 %) and the PVO (0.5 %) with actual averages of 1.65, 0.26, 0.04 and 0.01 $cm^3$, respectively and they were all significantly different at $P <$ 0.01 (F - test).

The volume of the subsample per ovary indicated that when the SV of VTO increases, the volume of the HO decreases, thus they had an inverse pattern (Figure 3.1). The mean SV of the HO indicated to be the greatest than other oocyte categories as illustrated in Figure 3.1. The combined content of the PVO and the CAO was < 10 % of the ovarian content, while the VTO was between 5 and 25 % and the HO was between 70 and 95 % (Figure 3.1).

The distribution of abundance of oocytes (oocyte counts) in the oocyte developmental phases ($Fec_i$) is indicated in Figure 3.1. The four identified oocyte development phases indicated different mean counts in the ovaries and were altogether significantly different at $P < 0.05$, as the PVO were the most abundant at averages: 89.2% followed by the 5% for each of the CAO and VTO and the 0.8% of the HO, with actual values for means as 15506269; 934877; 654136.2 and 76680.97, respectively. Furthermore, CAO and VTO were not significantly different from one another, when the test of two means was run ($P > 0.05$) and the HO were extremely low < 1% (Table 3.2).

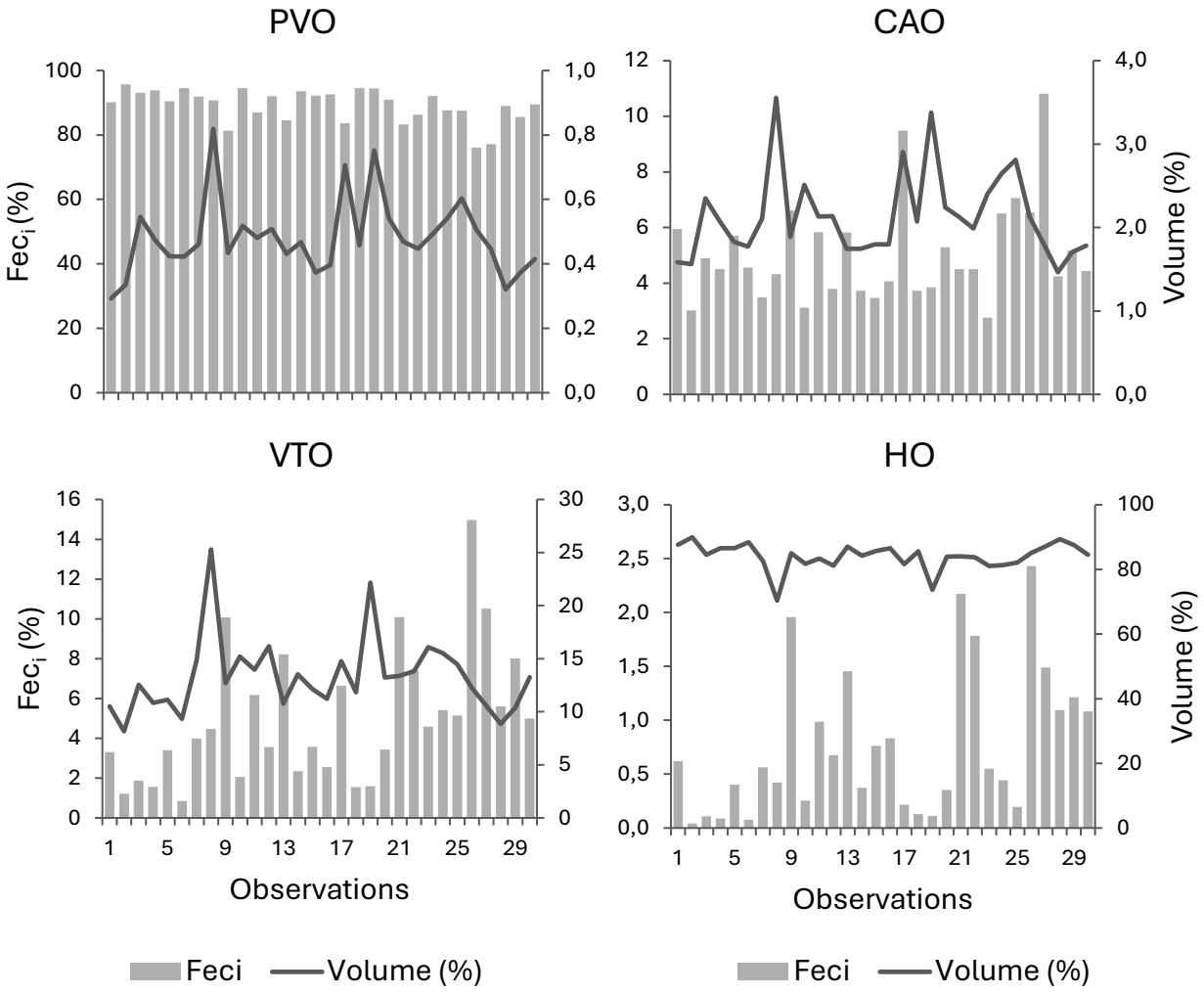


**Figure 3.1:** The distribution of the oocyte counts (Fec$_i$) on the primary y- axis and the volume for the different oocyte developmental stages in the secondary y-axis. Observations (N = 30) are indicated on the x-axis. Indicating the amount (%) of individual different oocyte developmental phases (namely, PVO, CAO, VTO and HO) in the ovaries of *M. capensis*.

The abundance of the oocyte developmental phases showed no significant difference between the mean abundance of the CAO and the VTO ($P$>0.05), while the HO were lowest and the PVO greatest in numbers and were significantly different to other oocytes at (P< 0.05) as illustrated in Table 3.2 and 3.3.

Furthermore, there was difference when all groups were tested and they were significant at P<0.05 (F-test) as in Table 3.3.

**Table 3.2:** Mean *Fec,i* - value for the PVO was greatest followed by the CAO, the VTO and the HO. There was no significant difference between the *Fec,i* - values of CAO and VTO, while HO values were significantly smaller than all oocyte phases.

| *OOCYTE PHASE ($Fec_i$)* | *N* | *Sum* | *Average* | *Variance* | *-95%* | *95%* |
|---|---|---|---|---|---|---|
| PVO | 49 | 7.6E+08 | 15506269 | 5.16E+14 | 9016739 | 21995798 |
| CAO | 49 | 45808971 | 934877 | 1.71E+12 | 561152.3 | 1308602 |
| VTO | 49 | 32052676 | 654136.2 | 3.92E+11 | 475346.9 | 832925.6 |
| HO | 49 | 3757367 | 76680.97 | 4.05E+09 | 58501.66 | 94860.28 |

**Table 3.3:** Single Factor ANOVA showing analysis of the difference between the mean number of oocytes ($Fec_i$) in each oocyte developmental phase (PVO, CAO, VTO and HO). The values were significantly different at $P < 0.05$.

| *ANOVA: Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 8.23E+15 | 3 | 2.74E+15 | 21.19324 | 6.67E-12 | 2.65164 |
| Within Groups | 2.49E+16 | 192 | 1.3E+14 | | | |
| Total | 3.31E+16 | 195 | | | | |

Oocyte developmental phases were quantified in oocyte counts (Feci) and the difference in oocyte phases were calculated and tested. The PVO phase was the most abundant phase ranging in million, so its difference with other phases was already known. However, the assessment between CAO and VTO

phases indicated that DOP changes in months of spawning and the mean difference oocyte phase (DOP) percentage in oocyte development phases was presented in Figure 3.2. The abundance of CAO was higher than the VTO (February and April) and that of the VTO was more than the CAO (March, May and September) were significant at $P < 0.05$. The mean DOP of the CAO exceeded the VTO in the ovaries of February and April by 59 and 30% respectively, while the opposite was true for March, May and September as the CAO were 35, 31 and 33% less than VTO. Furthermore, the DOP between the VTO and the HO was significantly different as VTO phase was always more than HO phase for all months combined ($P < 0.05$; Figure 3.2). There was no difference in DOP for the month of April and May and between March and September ($P > 0.05$). February (mean = 9 %) and September (mean = 19 %), were significantly different ($P < 0.05$). The months that had the highest DOP for VTO and HO phases was March and September with means of 17 and 19 % as opposed to February, April and May with means 9, 13 and 12 %, respectively and were significantly different ($P < 0.05$).

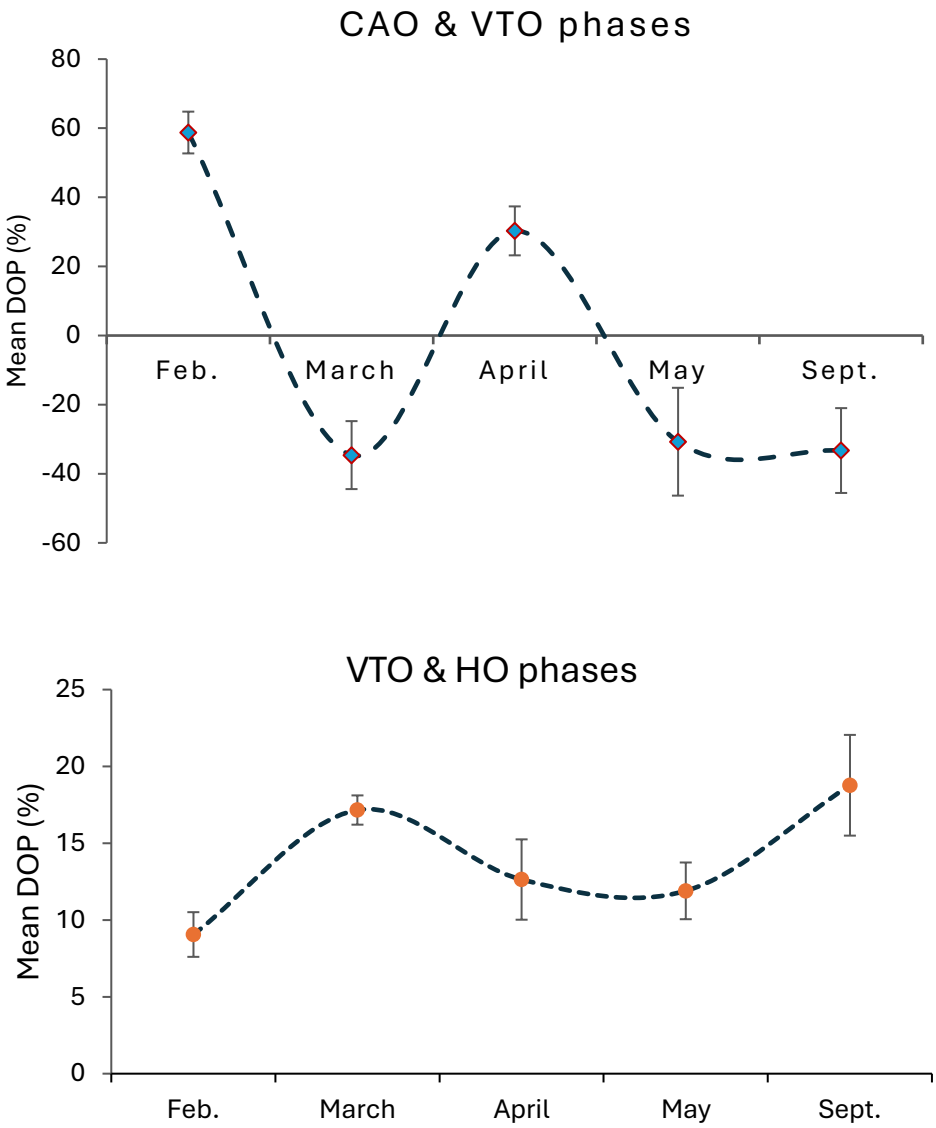


**Figure 3.2.** Distribution of the difference oocyte phase (DOP) on the y- axis and the period in months on the x- axis. The DOP of CAO and VTO is indicated by the diamond shape mark while that of VTO and HO is indicated by circular mark.

### *3.3.2 Batch Fecundity*

The exponential curves display the relationship between relative fecundity (RF) and the subsample volume (SV). The RF shows the exponential regress/decay while oocyte volume (V) shows exponential growth (Figure 3.3). The asymptotic relationship between the RF (red line) and the SV (blue line) shows a point where they intercept, which is the mean point where the minimum size of the VTO for the spawning stage can be calculated. Graphing all the oocyte developmental phases assist in identification of the convergence point, hence the right side of the graph contains only the VTO and the HO. Both y- values (primary and secondary) decrease towards zero reciprocal functions. The point where they cross marks the true minimum VTO threshold from which fecundity of the actively spawning females of *M. capensis* can be calculated (Table 3.4). The regression relationship is highly significant at $P < 0.05$ (Table 3.5).

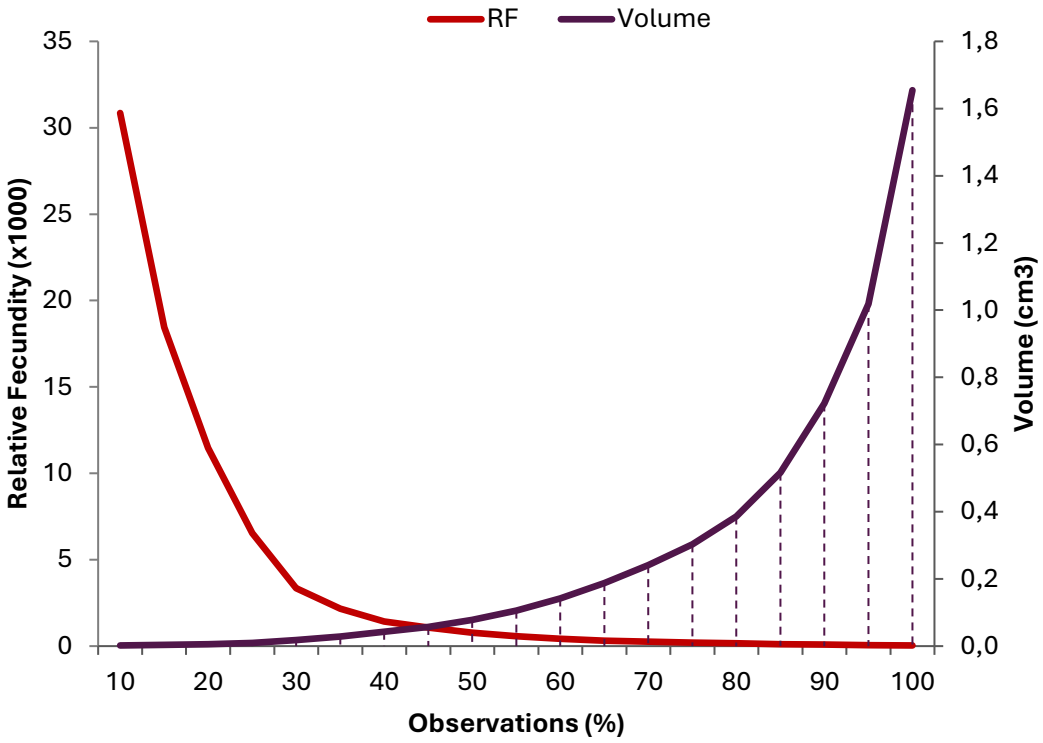


**Figure 3.3:** Distribution of the mean relative fecundity (RF) and the mean subsample volume from an ovary with n=1895 oocytes. The asymptotic relationship between the RF (red line) and the volume (blue line) shows a point where they meet. Both y- values (primary and secondary) decrease towards zero reciprocal functions.

**Table 3.4:** Showing the mean VTO thresholds from which the fecundity can be calculated. The VTO threshold is the minimum value of the VTO oocyte diameter (µm).

| Period | Mean VTO (µm) Thresholds | SE | -95 | 95 | N |
|---|---|---|---|---|---|
| February | 430.8 | 9.2 | 412.4 | 449.2 | 6 |
| March | 402.9 | 4.7 | 393.5 | 412.2 | 14 |
| April | 398.1 | 10.2 | 377.7 | 418.5 | 10 |
| May | 403.7 | 3.2 | 397.2 | 410.2 | 13 |
| September | 432.7 | 8.6 | 415.5 | 449.8 | 3 |

**Table 3.5:** Illustration of the regression results between the relative fecundity (RF) and the GSI (%). Correlation was 86% while $r^2$ was 75% and the observations were 49.

| *ANOVA* | *df* | *SS* | *MS* | *F* | *P-value* |
|---|---|---|---|---|---|
| Regression | 1 | 311.9703 | 311.9703 | 139.6188 | 1.1233E-15 |
| Residual | 47 | 105.0188 | 2.234443 | | |
| Total | 48 | 416.9892 | | | |

Relative batch fecundity for the austral autumn was calculated and illustrated in Figure 3.4 from the ovary free weight with a mean of 283 (SE=30) g-1 and the range between eggs per g female and mean BF of 566076 and SE= 98421. All results were significant at $P < 0.05$.

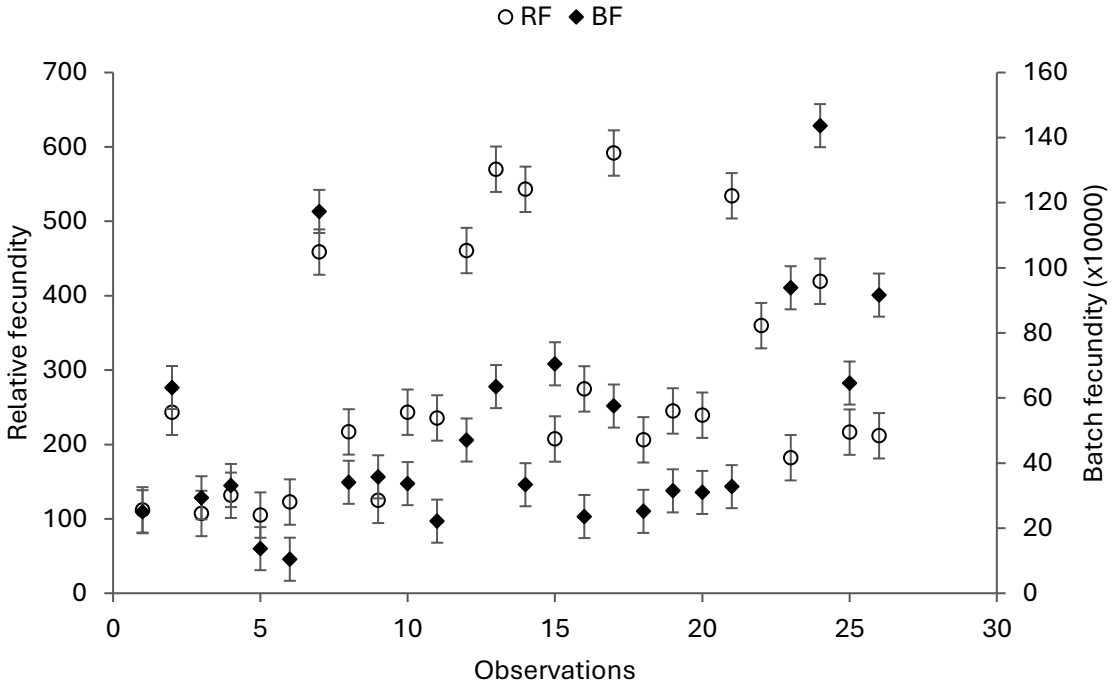


**Figure 3.4:** Distribution of the relative fecundity (RF) in the primary y- axis and the batch fecundity (BF) in the secondary y- axis in Austral Autumn in 2024. N=27.

*3.3.3 Spawning period*

Table 3.6. The mean SV ranged between 0.37- 0.53% for PVO; 1.65- 2.30% for CAO; 10.42- 15.57% for VTO and 81.61- 87.30% for HO in all sampled months in 2015 (Feb., March, April, May and Sept.) respectively. The HO volume in all the oocytes was always beyond 80%.

**Table 3.6:** Illustrating the mean values of the subsample volume of each oocyte developmental stage per month in 2015. PVO, CAO, VTO and HO represent the previtellogenic oocytes, cortical alveoli oocytes, vitellogenic oocytes and the hydrated oocytes, respectively. N=30. The volume of the hydrated oocytes is always highest followed by VTO, CAO and the PVO and they were all significantly different at $P< 0.01$.

| Months | PVO ($cm^3$) | CAO ($cm^3$) | VTO ($cm^3$) | HO ($cm^3$) |
|---|---|---|---|---|
| February | 0.01 | 0.03 | 0.14 | 1.17 |
| March | 0.01 | 0.06 | 0.44 | 1.97 |
| April | 0.01 | 0.03 | 0.19 | 1.24 |
| May | 0.01 | 0.05 | 0.30 | 1.95 |
| September | 0.01 | 0.04 | 0.29 | 2.06 |

**Table 3.7:** Indication of the mean relative oocyte counts ($Fec_{,i}$ ) as per oocyte developmental phases (ODP). The phases are represented by PVO- previtellogenic oocytes, CAO-cortical alveoli oocytes, VTO-vitellogenic oocytes and the HO- hydrated oocytes. N=30.

| Months | Mean Relative $Fec_{,i}$ counts of oocytes per ODP | | | |
|---|---|---|---|---|
| | PVO | CAO | VTO | HO |
| February | 23735 | 1143 | 435 | 36 |
| March | 6543 | 284 | 276 | 41 |
| April | 8667 | 534 | 323 | 34 |
| May | 4563 | 324 | 344 | 42 |
| September | 3123 | 236 | 247 | 32 |

An individual ovarian analysis on oocyte phases has shown that some ovaries contain more counts of VTO than the CAO and this relates to the time or some months of the year (Figure 3.5).

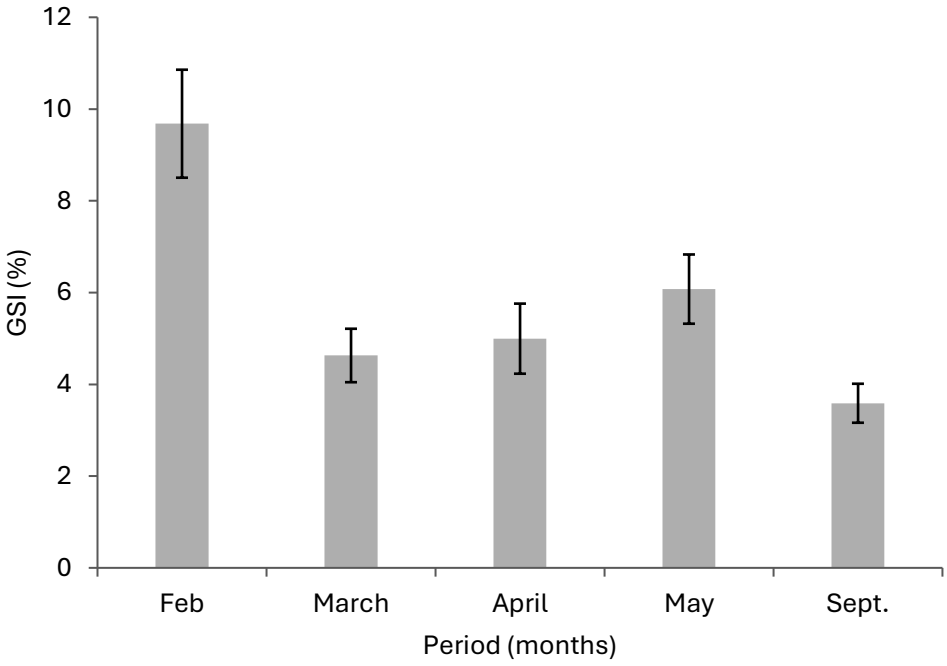


**Figure 3.5:** Representation of the *GSI* in percentage of the oocyte developmental phase (VTO) on the y- axis and the period in months of the occurrence of spawning.

Analysis of the combined data for the three-year period resulted to the RBF grand mean 258(SE=20) and BF with grand mean of 522772 (SE=64085) and all is presented in Figure 3.6. All the spawning females were tested to assess the month with the most spawning for the rest of the study period ($P < 0.05$). The austral autumn and the austral spring indicated to be the most spawning time.

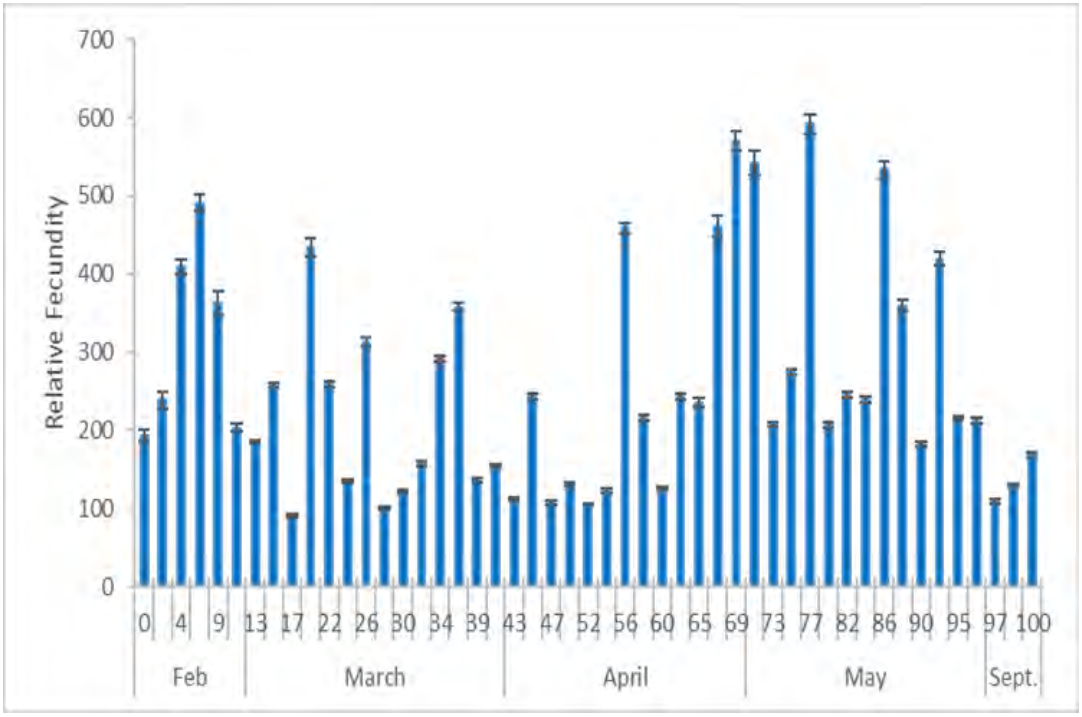


**Figure 3.6:** Indication of the relative batch fecundity calculated from oocyte phase of VTO. The relative fecundity is on the y- axis, while the cumulative % of the observations is on the primary x- axis and the months of spawning on the secondary x- axis. N= 50, Grand mean RF =258.45, SE= 19.95

**Table 3.8:** Illustration of the correlation and the strength between the BF calculated from the vitellogenic oocytes (VTO) through the volumetric formulae and from the hydrated oocytes (HO) through the auto-diametric method. The overall correspondence is 78% though low samples of September yielded <50% correlation.

| Period | BF- Vol.Mean | BF-HO.Mean | Pearson Correlation | t- Stat | P-value | N |
|---|---|---|---|---|---|---|
| Feb. | 732685 | 163223 | 0,88 | -2,96 | 0,0157 | 6 |
| March | 380098 | 106107 | 0,82 | -4,81 | 0,0002 | 14 |
| April | 431663 | 195215 | 0,84 | -4,99 | 0,0002 | 12 |
| May | 725660 | 241802 | 0,66 | -3,50 | 0,0022 | 13 |
| Sept. | 393457 | 159082 | 0,34 | -3,77 | 0,0319 | 3 |

Testing for variance for all the months was done by F- test and the results indicated significant difference as the BF calculated by volumetric formulae was three times more than that of hydrated oocyte method (means 175585 and 531487, respectively, $P < 0.05$, F = 0.2167 and F-critical = 0.61). Single factor analysis of the BF and relative BF calculated by each formulae indicated no significant differences between months ($P > 0.05$).

The power regression relationship between *GSI* and the relative fecundity was strong and correlated at 86% with $r^2$ of 75% ($P<0.05$) and illustrated in Figure 3.7.

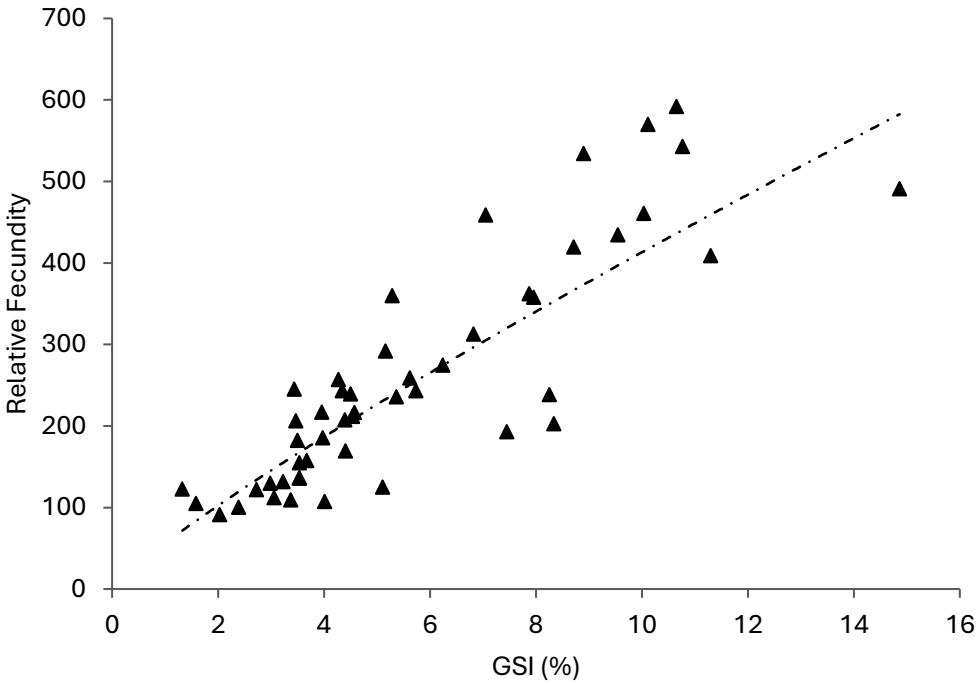


**Figure 3.7:** Illustration of power regression relationship between the Relative fecundity (RF) on the y-axis and the Gonadosomatic index (GSI) on the x- axis. N=50.

All the maturity stages were assessed to ascertain the stage that has the most GSI and maturity stage 4 or the actively spawning stage had the most *GSI* as compared to other five maturity stages (Immature, Developing, spawning capable, regressing and regenerating stages). The difference in *GSI* of the maturity stages was significant at $P< 0.05$. Spawning in *M.capensis* occurred in all months that were sampled, however, there was a difference in the incidence of those female spawners and the time in

which spawning occurred and the distribution of incidence of female spawners in mean numbers with the GSI over a period of three years (2014- 2016) are illustrated in (Figure 3.8). There is peak in GSI in May for 2014, in April for 2015 and in May and September for 2016. The peak around September, which is in Austral Spring is not as high as that of March - May in Austral Autumn.

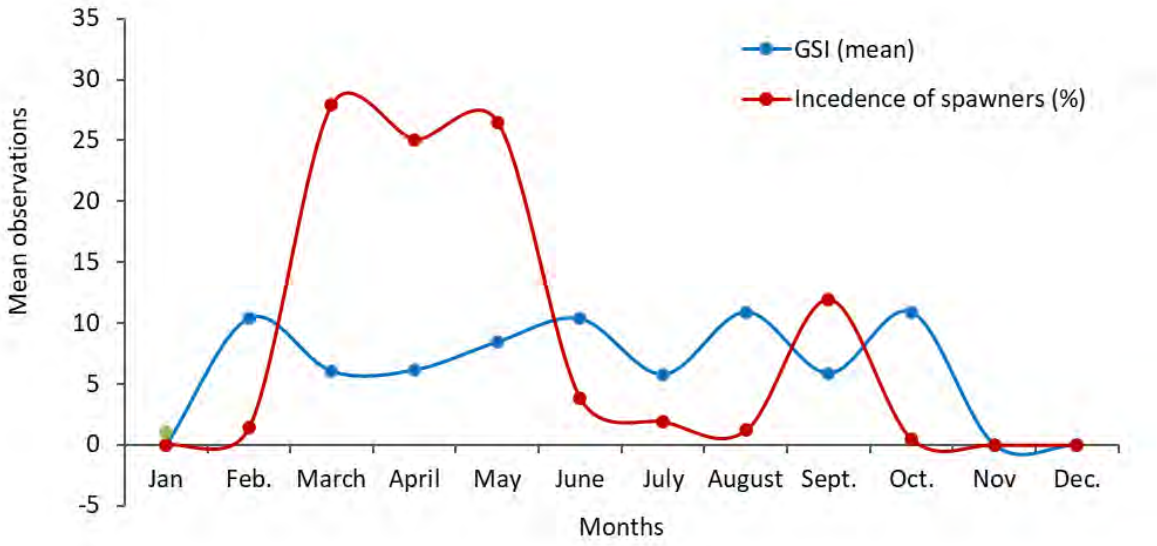


**Figure 3.8:** Distribution of the mean amounts of gonadosomatic index (*GSI*) and the amount of mean incidence of spawners (%) in months that were observed from the year 2014 - 2016. N=50.

The distribution of spawners and the GSI has suggested that spawning season has two peaks in a year. It is the peak that is around May (Austral Autumn) and the other in September (Austral Spring), however, the earlier one in the year has indicated almost double the spawner abundance as compared to the later one.

### *3.3.4 Spawning size*

The mean length for spawners along the south coast was 61cm, with the minimum and the maximum length at 34 and 97 cm respectively and all the fish smaller than 65 cm laid eggs below 600 000 BF, and those beyond > 65cm in length could lay up to 1.6 million eggs. These differences were significant at $P < 0.05$. The volumetric BF ranged from 150 - 1400 (thousands) for all the length classes (Figure 3.9). The different length size laid different batch sizes, BF was significantly different at $P < 0.05$, and all fish beyond 65cm laid more eggs than those < 65 cm. The number of eggs laid by fish smaller than 65cm were not significantly different ($P > 0.05$) and a similar pattern occurred with those fish beyond 70 cm length class, except that the leading cohort of 90cm laid the greatest number of eggs than all other length classes ($P < 0.05$).

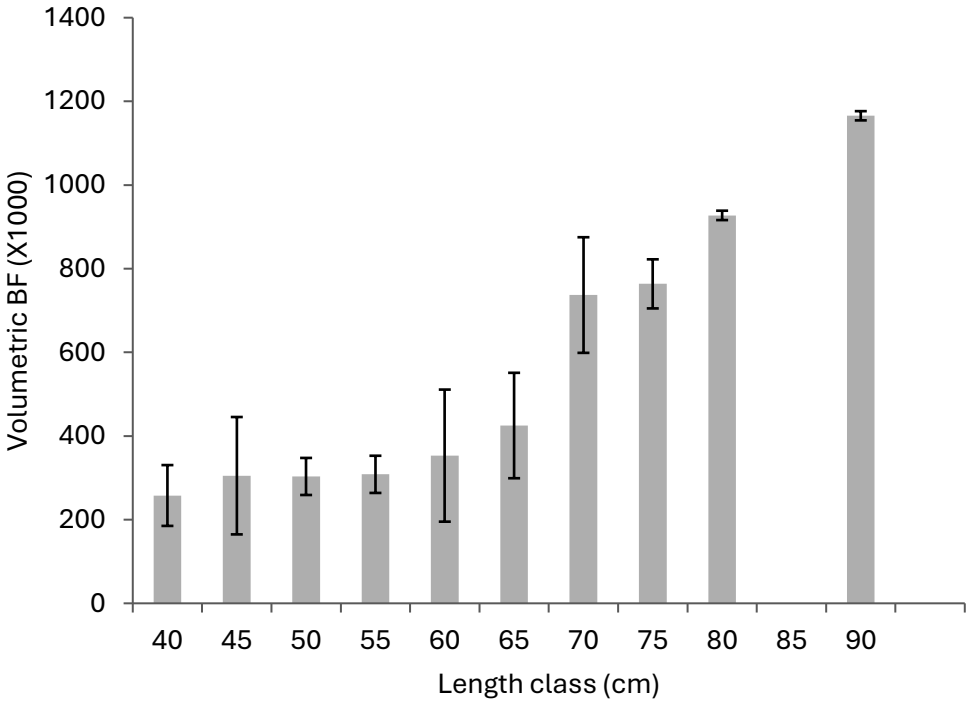


**Figure 3.9:** Distribution of the length class on the y- axis Illustration of length distribution versus the batch fecundity calculated from the volumetric formulae on the y-axis. The grey columns represent the mean length and the error bars represent the standard errors.

## 3. 4 Discussion

Distribution of spawners of *M. capensis* in the south coast of South Africa, was in the western part of the Agulhas Bank, within the central Agulhas Bank and the east of Agulhas Bank (Garavelli *et al.* 2012). The current study concurs with the latter and it could account for all the months of the year, except for December wherein there were no spawners found, and the months that were not sampled (November and January).

### *3.4.1 Oocyte developmental phases*

Different types of oocytes were assessed in all the actively spawning oocytes and the VTO were the matured size that preceded the spawning phase. The common mean oocyte diameter size of the combined spawning stage (spawning capable, actively spawning and regressing maturity stages) is beyond 350µm (Nomxego *et al.* 2024), therefore the estimation of spawning and batch fecundity concurred with the latter authors. Some authors investigated the oocyte phases and have used the oocyte packing density (OPD) to calculate fecundity and suggested the calculation of fecundity from the primary oocyte phases as opposed to the advanced oocyte stages (Kurita et al. 2009; do Santos et al. 2021). Other authors (Kjesbu *et al.* 2011; Saber *et al.* 2015; Anderson *et al.* 2020) who studied different species have demonstrated that BF could be calculated from the VTO phase as opposed to the use of the HO phase that has been commonly considered. In the current study, the intersection from the graphical presentation of the oocyte volume (V) and the RF also presented a true minimum VTO phase threshold that provides further guidance from which the BF can be calculated when oocyte developmental phase is used. In the current study, the volume of the oocyte development phases increased with the development of the oocytes from PVO, CAO, VTO and HO phases, thus greatest volume was when spawning becomes active (HO phase), while the oocyte counts decreased with the growth of the phases. Similar to oocyte ratio category (ORC), the current study uses the oocyte development phase, which as ORC increased and when fish approached the end of spawning, there was a transition towards a lower

number of oocyte cohorts in the 250- 1200 um range which is reflective of vitellogenic oocytes (VO) depletion, (Anderson *et al.* 2020), which concurs with the current study.

*3.4.2 Batch Fecundity*

The most advanced oocytes (hydrated oocytes) are known as a good measure for the calculation of batch fecundity (BF) (Hunter *et al*. 1985; Osborne *et al.* 1999; Osborne *et al*. 2004, Kainge *et al*. 2007; Loweri-Barbieri *et al.* 2011), however, the use of whole mount auto- diametric method **(**Thorsen and Kjesbu, 2001). produces readily available quantitative data to determine thresholds for developmental stages from the oocyte diameter (OD). More often than not, actively spawning maturity stage, the stage with the HO, is difficult to find as opposed to other maturity stages (e.g spawning capable), therefore, finding other stage options for fecundity estimation alleviate the challenge of calculation of BF from minimal samples (Osborne et al. 2009) or not pursuing the investigation at all due to data unavailability. Developmental stages of oocytes have been identified to have different thresholds by some authors (Kainge *et al*. 2007; Osborne *et al.* 1999; Osborne *et al*. 2004; Nomxego *et al.* 2024). Kainge *et al.* (2007) identified the threshold for the hydrated oocytes to be beyond 450µm, while Osborne et al., 1999 used >700µm. Other authors (Van Damme 2014; Ganias 2013; El Habouz *et al.* 2011) who studied hake and other indeterminate spawners, used hydrated oocytes >750µm threshold, which was also adopted in the current study (also Murua *et al.* 1998 and Alheit 1986). Refined maturity stages of *M. capensis* were determined to be <250 µm for immature stage, 251- 350 µm for the developing stage, >351 µm for spawning stage and 100- 300 µm for regenerating stage (Nomxego *et al*. 2024). In the current study, relative fecundity was calculated as oocyte counts ($Fec_i$) wherein a direct calculation of the oocytes beyond 351µm oocyte diameter gave a mean of 283 (SE=30) $g^{-1}$ and mean BF of 566076 (SE= 98421) $g^{-1}$. The current study results is almost double (as the previous is 56% of the current study RF) the outcome of Osborne et al. 1999, with the relative mean of 160 eggs per female, however the BF of the current study falls within the range of the said study which was 147000- 723 658 eggs per female. Batch fecundity was found to be within the range of what was found earlier by Osborne *et al.* 1999 and Osborne,

2004, though the relative fecundity was lower in the previous study (Osborne *et al*. 1999) by almost 45%. The reason for the increase is not known but may be due to the different size of fish sampled as the fish lay eggs depending on size of both the gonad weight and the body length (Nomxego *et al.* 2024). It may also be due to the time of collection as the latter sample collection was in June as opposed to the present study which was in April- May, which is the peak of the spawning season and also collected all year round with minimal or fewer samples in June. It may also be due to the different methods used as the manual count was used in the previous study as opposed to automation in the current study and the volumetric method which is used for the first time in this species.

The calculation of fecundity in the current study was from the lesser advanced stage (vitellogenesis) than the hydrated oocytes (dos Santos et al. 2021) but has yielded a reasonable outcome as it can be compared with other studies (Osborne et al. 1999, Kainge et al. 2007). Therefore, investigation of the earlier oocytes than the most advanced hydrated oocytes can give a good estimate of the Batch fecundity. Volumetric BF from the other developmental stages (hydrated oocytes only or primary growth oocytes (PVO and CAO)) was either too much (in millions) or too little (ten hundreds) to compare with other values already in existence in literature.

The current study has also revealed that another OD threshold for calculation of BF, which is over 99% compatible with > 351μm, can be calculated from the simple plot from the two parameters (the relative fecundity and the oocyte or subsample volume). Together these parameters create an asymptotic relationship and on the right-hand side of the graph, lies the size of the OD and the number of oocytes to be calculated for BF from the point where they intercept. The thresholds that presented in Table give the mean values for the minimum VTO in particular months and they are all slightly greater than the 351μm, supporting the calculation of BF from the volumetric formulae as they are precisely within the range of spawning females of *M. capensis* (Nomxego et al. 2024).

### *3.4.3 Spawning period*

The timing of spawning was assessed by *GSI* as it is a metric that represents the relative weight of a gonad to the fish weight and is also widely used to evaluate spawning season or the reproductive timing (Lowerre- Barbieri *et al.* 2011). Regarding the *GSI*, the significantly highest peaks were found in the spawning phase for three years for *M. merluccius* (Candelma *et al.* 2021) and they were in austral autumn and in austral spring, hence the increased development of VTO during those periods. The results of the current study indicated that at the high values of *GSI*, follows the spawning which reduces gonad size as quoted in other investigations (Papaconstantinou *et al.* 1986; Anderson *et al.* 2020; Candelma *et al.* 2021; El Habouz *et al*. 2011; Serrat *et al.* 2019). The latter implies that gonad size relative to body weight is at highest peak until spawning deflates it with ovulation, hence the peak patterns follow each other, the spawners frequency always follow *GSI* peak. The size of gonads increases towards the spawning season (Marshall et al. 1999), meaning *GSI* also increased when approaching spawning season. The current investigation also suggests that the difference in oocyte phase plays a role in timing of spawning as the DOP of CAO phase was greater than that of the following phase (VTO) in February and April, but VTO phase was greater than CAO in March, May and September, which is both the austral autumn and austral spring. The pattern continues where the DOP of VTO phase is always greater than that of HO phase but high in September and March, though not significant with one another, but with all other months. In a study by Anderson *et al.* 2020, significant somatic energetic resources were used during the earlier stages of spawning and the condition decreased with increased spawning, oocyte ratio category (ORC) and the number of pre- vitellogenic oocytes (PVO). Furthermore, GSI decreased markedly as oocyte ratio category (ORC) increased which is reflective of serial egg release over a spawning season (Anderson *et al.* 2020). Other authors explored the lipid energy that stored in gonads for spawning and suggests its assessment as a measure of fecundity, which leads to increase in gonad size and reduction in body weight.

### *3.4.4 Spawning size*

Batch fecundity increased proportionally with fish length and with the ovary free weight, similar to when it increased with female gutted weight (Murua *et al.* 1998). In a study of *M. capensis* and *M. paradoxus,* fecundity increased exponentially with fish length, weight and age*,* though correlation was better with weight and length than with age (Osborne *et al.* 1999). The similar pattern occurred in the current study that BF increased with length and with gonad weight and the correlation was better with both parameters. The current study also suggests that smaller fish have a potential to lay fewer eggs than the large fish and concurs with the big old fat fecund female fish (BOFFFF) effect. The length assessment has shown that below 65cm length, BF was always below 600000 as opposed to doubling and more of that number just 5cm beyond that length. Therefore, it is a fact that large fish spawn more eggs than smaller fish (Hixon *et al.* 2014). It is also known that BF is size and age dependent with large and older fish producing more eggs and spawning more frequently (Field *et al*. 2008; Hixon *et al.* 2014; Macchi *et al.* 2005; Marteinsdottir and Begg, 2002). According to Serrat et al., 2019, egg production is mostly dependent on female size, and female energetics, including their feeding capacity. Furthermore, spawning dynamics do not only differ by female size, but also among seasons (Serrat *et al.* 2019). Hence some authors suggest that the (BOFFFF) effect is underappreciated as batch spawning species tend to have earlier and longer spawning seasons and may spawn in more locations than the smaller females (Field *et al.* 2008; Hixon *et al.* 2014). According to Field et al. 2008, conservation of the BOFFFF should be seriously considered as they have the larger contribution to fish production and therefore population stability and the current study support such narrative.

## 3.5 Conclusion

In the current study, volumetric formulae were used to calculate oocyte counts (Feci) and earlier oocyte developmental phase like VTO was used to estimate BF, The mean oocyte per gram per ovary from VTO for *M. capensis* gives the best estimate and proxy for BF, which concurs with Kjesbu *et al*. 2011. The

volume of oocyte developmental phase was found to be greatest at HO phase, while the Feci was greatest at PVO phase. The DOP assessed the peak of the spawning period and austral autumn and austral spring were found to be the peak spawning times. The relationship between the proxies and fecundity needs to be strong, well described and based on time series before the use of proxies for management advice (Murua and Saborido - Rey 2003, Van Damme *et al*. 2014).

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# Chapter 4: Life history parameters of adult females of *Merluccius capensis* (Gadidae) in the south coast of South Africa

## 4.1 Introduction

*Merluccius capensis* caught in spawning condition in the area off Cape of Good hope (33°- 34.5° S and 17°- 18.5° E), suggests that most spawning took place in November and December, with reduced spawning, again in February, March and August and only a few spawning fish were found in other months (Botha, 1986). Other studies also located the spawning stock in the south coast of South Africa (Osborne et al. 2004; Garavelli and Gruss, 2012; Durholtz et al. 2015). It was shown in Botha 1985 that mature hake leave the sea-bed and spawn in mid water and the level of 50 % sexual maturity was reached at the age of 1.9 years calculated by von Bertalanffy growth model, while the length at 50 % maturity ($L_{50}$) was 48 cm by the females of *M. capensis* (Botha, 1986). Fish body cavity limits the reproductive allocation of each spawning event resulting in a correlation between reproductive investment and body size (Lambert, 2008). The reproductive investment includes the storing of energy in gonads towards spawning activities; the gonads increase in size as they develop from inactive immature to actively spawning. Therefore, gonadosomatic index (GSI) is generally used as a reproductive indicator, and the larger the GSI is, the higher the chances of spawning or the closer is the spawning period (Coker et al. 2008; Lowerre- Barbieri et al. 2011). Because body cavity relates to fish total length, potential fecundity in many fishes increases strongly with the latter (Wootton, 1992; Ali and Wootton, 1999).

A related aspect is that the length at maturity reflects the proportion of mature fish at a certain length, with the established maturity ogive typically being a logistic curve that describes the proportion of the fish at first maturity, often specified as the size (length) or age at which 50% of the population is considered sexually mature ($L_{50}$) (Flores et al. 2015), or, $A_{50}$. $A_{50}$ is more frequently used in stock assessments, though $L_{50}$ becomes an alternative when the age reading of the species in question becomes unreliable

(Trippel, 1995). Hence, along with detecting fluctuations in stock size, one of the most important management measures to be undertaken include updates of the estimation of maturity schedules, since this class of parameter can change in exploited stocks (da Costa et al. 2015).

Addressing growth and condition issues are also key parts of reproductive studies. The length-weight relationship provides information about the growth pattern, as well as morphological characteristics of the fish, as emphasised by other authors (Schneider *et al*., 2000; Froese, 2006). Length-weight relationship may indicate growth pattern, for instance, according to a study on skipjack tuna (*Katsuwonus pelamis*) in the western and central Pacific Ocean, those beyond 60 cm were more elongated in body shape (Jin et al. 2015). Statistically, the growth pattern is often characterized by the exponent *b* in the power function equation ($W_p = aL^b$), with body weight as response variable and length as effector: negative allometric: $b < 3$, isometric: $b = 3$, and positive allometric: $b > 3$ (Costa et al. 2013).

Condition index, such as Fulton's condition factor (*K*) (Lambert et al. 2000) is often suitable to indicate the nutritional status of fish, since this proxy indirectly reflects changes in energy content and proximate composition in the different tissues studied (Lambert and Dutil, 1997a; Marshall et al. 1999; Ballón et al. 2008). According to Costa et al., 2013, the condition factor gives a general idea of the weight gain and loss – standardized by length (*L*) – along the year (Bolger and Connolly, 1989; Nash et al. 2006). Furthermore, the condition index may be used to evaluate the sensitivity of a given fish to ambient factors or "health conditions" during old age (Ouellet et al. 2001). So, the expression of condition index varies due to the reproductive state (with links to *GSI*) and the general conditions of nutrition brought about by seasonality or other circumstances in the environment (Bolger and Connolly, 1989). Hence, not only female attributes like size or age but also the nutritional condition shape the resulting realized fecundity (Hislop, 1998), with the repeat spawners typically producing bigger and more eggs (Lambert et al. 2000; Lambert, 2008).

Based on the association of the length at maturity with the fish body structure in the form of length-weight relationship; the *GSI* and the condition of the parental stock may improve the understanding of

1609 the recruitment variations observed in natural populations (Hislop, 1998; Marteinsdottir and Thorarisson,
1610 1998; Marshall et al. 1999). The current study is aimed at revising and complementing such information
1611 for the commercially and ecologically valuable *Merluccius capensis* off South Africa. As for the congeneric
1612 *M. paradoxus* in the Benguela region, *M. capensis* display two peaks of spawning, one in austral autumn
1613 and one in austral spring (Kainge et al. 2007). This investigation attempts to validate the length at 50 %
1614 maturity, explore any allometry in the length-weight relationship, and track seasonal patterns in GSI and
1615 the condition factor of *M. capensis*. To do so, we complemented the already existing on-board biometric
1616 information, when relevant, by in-depth histological studies in the laboratory. Finally, we aimed at
1617 updating the values of these biometric parameters and report systematic changes that may have occurred
1618 over time by comparing them with other recorded or published values where we can.

## 4.2 Materials and Methods

### *4.2.1 Ethical statement*

The care and use of study animals complied with Marine Living Resources Act (Act 18 of 1998) and the guidelines and policies as provided by the Department of Forestry, Fisheries and Environment (DFFE), South Africa, as the permitting authority with the following reference numbers: RES2014/81A, RES2015/92 (A) and RES2016/90-RR.

### *4.2.2 Research material*

The collection of samples of *M. capensis* was conducted during the routine research and commercial surveys of the hake resource by bottom trawl gear on the shelf and upper slope of the south coast of South Africa. A total of 1819 female samples were collected for the years 2014, 2015 and 2016 (N = 352, 932 and 535, respectively) on the study site at 34º- 37º S and 18º- 27º E (Figure 4.1). The following variables were collected: total length (*L*) in centimeters below and total weight (*W*), and gonad weight ($W_{gonad}$), all in grams.

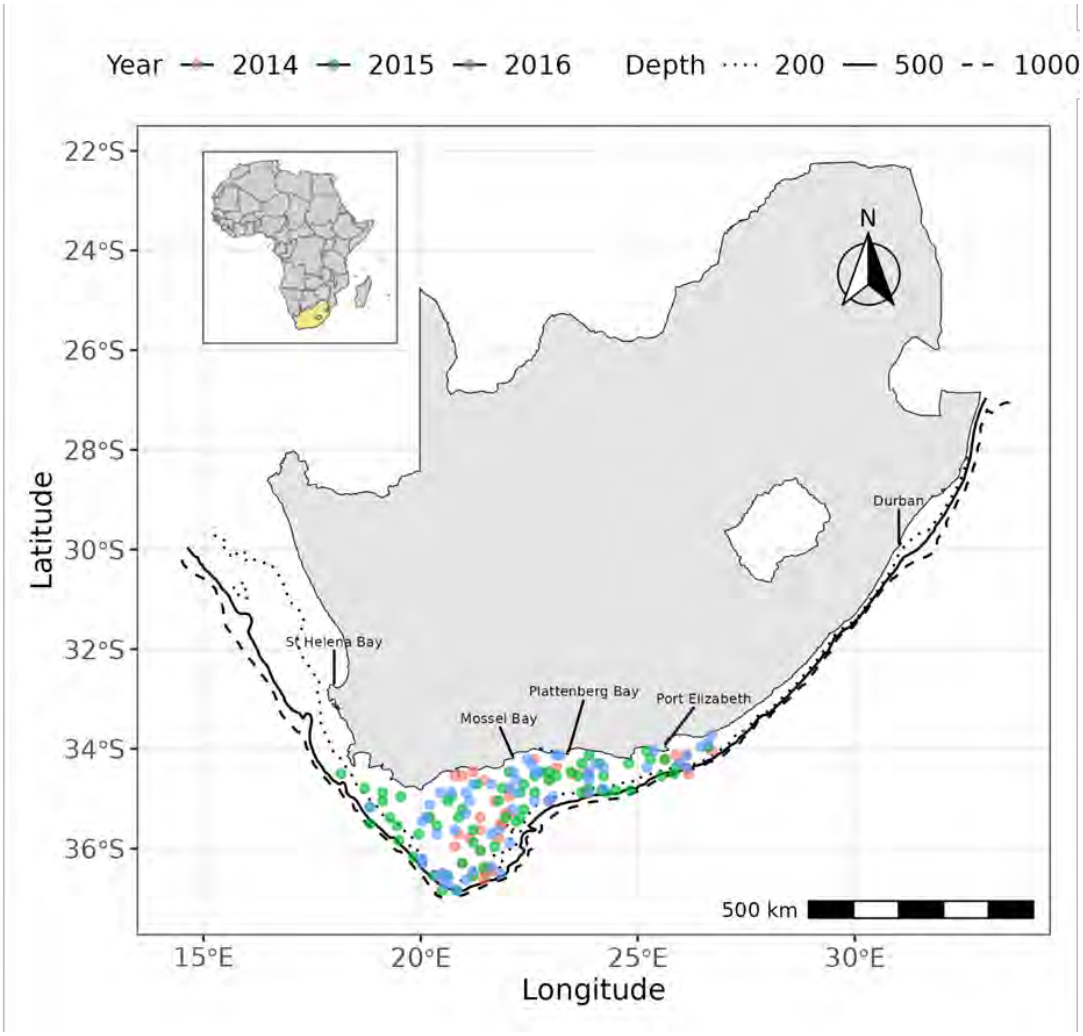


**Figure 4.1:** Map representation of the latitudinal and longitudinal positions of the data collected from surveys from the southern coast of South Africa. Different colours denote different years (2014-2016), pink, green and red, respectively.

*4.2.2.1 Maturity stage classification*

While on board the vessel, the ovaries were classified by the six-degree macroscopic maturity scale (the sixth stage was added by DFFE technicians to Botha's scale (Botha, 1986)) and preserved in 10 % buffered formalin as per (Osborne et al. 1999). Inactive and immature females were set as maturity stage 1, while sexually mature females were from the developing (stage 2) to the regenerating stage (stage 6) (see a fuller description below). The regenerating stage was also considered as inactive as the ovaries were shrunk and almost empty.

Further processing of samples in the laboratory followed standard histological procedures for paraffin embedding, with the following adopted protocol for *M. capensis*. A cross section of one of the lobes of the ovary nearest to the vent was removed for histological analysis. This ovarian tissue was dehydrated through a series of ethanol immersions, embedded, sectioned at 6 -10 μm, transferred onto slides, cleared with xylene, rehydrated and stained using a standard haematoxylin and eosin counterstain. Analysis of slides was under the microscope at 40× magnification to establish the degree of ripeness of the ovary. The classification of histological maturity stage of each ovary was based on the most advanced type of oocytes, atretic oocytes and the occurrence of post-ovulatory follicles (POFs) (Hunter and Macewicz, 1985, Brown-Peterson et al. 2011). The maturity stage 1 was the only sexually immature stage, while stages 2-6 were considered as sexually matured stages. The presence of only oogonia and perinucleolar forming the previtellogenic oocytes (PVO) signified immature stage, while that of the cortical alveoli oocytes (CAO) as the most advanced oocytes represented maturity stage 2 (developing stage). Maturity stage 3 (spawning capable) was noticed by the presence of vitellogenic oocytes (VTO) with protein (yolk) granules and lipid vacuoles, while the migrating nuclei and hydrated oocytes (HO) were indicative features of the actively spawning stage (stage 4). Maturity stage 5 (regressing stage) was marked by the VTO and the presence of new POFs. Primary vitellogenic oocytes marked maturity stage 6 (regenerating stage) but differed from stage 1 by the presence of atretic oocytes (alpha and beta atretic sub-stages) and old disintegrating POFs. Sometimes the developing stage would consist of degenerating POFs and atresia (especially when the fish has just completed a spawning cycle; Brown- Peterson et al. 2011), but the presence of the CAO as the advanced oocytes marked the maturity stage to be the developing stage.

*4.2.2.2 Maturity assessment methods*

Fish maturity stage was determined by macroscopic method which was later verified by the microscopic (histological) method. There are gonad morphological characteristics that are widely used for stage identification under macroscopic assessment, but they always vary with the person and are subjective (Honji et al. 2006). On the other hand, histological assessment uses cellular characteristics (see above section) detected under the microscope and can therefore be repeated by different persons yielding the same outcome (Vitale *et al.,* 2006). Binary classification and or confusion matrix was used in order to measure accuracy and precision of assessment of the macroscopic and the microscopic methods. Macroscopic values were treated as predicted, while the microscopic values were treated as the actual values. The accuracy rate determined the misclassification, i.e, the true positive and the true negative.

Further, a whole-mount maturity scale was introduced and applied in this investigation to stage the gonads, by *in vitro* examination of un-sectioned oocytes, a process that was taken from Kjesbu et al. (1991), used onboard the vessel to assess sexaully immatures versus sexually matures. In the current study it was further used as an auto-diametric method (Thorsen and Kjesbu, 2001), that assess oocyte development through investigation of oocyte type and dimensions. Different mean oocyte diameter dimensions were allocated for each maturity stage based on the advancement of oocyte development, which rose to six stages that ranged from ≤ 250 μm (immature), 251 - 350 μm (developing), 351 - 750 μm (spawning capable), > 750 μm (actively spawning), 351 - 750 μm (regressing), and 100-300 μm (regenerating). The > 350 μm relates to spawning activities, hence the combination of spawning capable, actively spawning and the regressing stages. In effect, two reliable microscopic assessment methods were used in this study, namely, the one that focuses on histological oocyte developement structures and the presence of other tissue markers, and the one that focused on whole oocyte diameter and frequency distribution.

*4.2.2.3 Gonadosomatic index*

The gonad weight ($W_{gonad}$) and body total weight of fish (W) were used to calculate the gonadosomatic index (*GSI)*. The computation of the *GSI* (in percentage) was done as per (Coker et al. 2008):

$$GSI = 100\ x\ W_{gonad}\ /\ W - W_{gonad}). \qquad \text{Equation 1}$$

*4.2.2.4 Maturity ogives*

Measurements of fish length together with the maturity staging were used to identify the sexually mature versus sexually immature fraction of the population, including the length at which 50, 75 and 95 % of the population gets matured. Microscopic followed macroscopic reading in order to correct the possible misclassification of stages on maturity data. The maturity data (inclusive of the validated macroscopic data of all stages) from females of *M. capensis* was used to model the standard sigmoid-shaped (logistic) curve used within fisheries sciences:

$$p_l = \frac{1}{1+e^{-(l-l_{50})/\delta}} \qquad \text{Equation 2}$$

where $p_l$ is the proportion of the fish sexually mature at length *L*(fish total length from snout to the fin tail end) , $L_{50}$ the length at which 50 % of the animals are sexually mature and δ is the inverse of the rate at which animals sexually mature, i.e., a small value denotes a high rate. The model parameters were minimised in Excel Solver using a negative binomial log likelihood of the form:

$$\ln L = \sum(y_l \ln p_l + (n_l - y_l) \ln(1 - p_l)) \qquad \text{Equation 3}$$

where ln *L* is length, at which half of the fish are sexually matured, $n_l$ is the number of fish sampled in length class *L* assessed for maturity, and $y_l$ is the number of fish in that sample that were sexually mature.

*4.2.2.5 Length-weight relationship*

Fish length and weight measurements were used to estimate length- weight relationship (L/W) . Fitting the standard length-weight (power function) model was done as follows:

$$W_p = aL^b \quad \text{Equation 4}$$

where $W_p$ is the predicted weight in grams at observed length (L), *a* and *b* are parameters of the model to be estimated, with the confidence interval given by bootstrapping with 1000 replicates as per (Robinson and Hayes, 2020).

*4.2.2.6 Body condition*

Fish body condition was represented by Fulton's *K* and relative condition index *k* (Lambert *et al*., 2000; Nash *et al*., 2006):

$$K = 100 * \frac{W}{L^3} \quad \text{Equation 5}$$

$$k = \frac{W}{W_p} \quad \text{Equation 6}$$

where $W_p$) is based on Equation 5.

*4.2.2.7 Data analysis*

Excel Solver and Statistica v.13.6 were applied to test for any differences and agreements within and among groups, with *P* = 0.05 set as significance level. In addition, multiple R packages (R-studio, R Core Team, 2020) were utilised for data processing, visualization, analysis and summary of results (Alathea,

1725 2015; Henry and Wickham, 2019; Allaire *et al.* 2020; Robinson and Hayes, 2020; Wickham *et al.* 2020a,

1726 2020b; Xie, 2020).

## 4.3 Results

### *4.3.1 Maturity staging*

The characteristics of the classification of females by macroscopic assessment was taken from Botha's scale, with present modifications (Table 4.1).

**Table 4.1:** Classification of the macroscopic maturity stage of *M. capensis* females (Botha et al. 1986). The current study modified stage 5 and added stage 6. The staging system to the left is adopted from Brown-Peterson et al. 2011.

| MATURITY STAGES | WHOLE MOUNT | VISUAL APPEARANCE OF OVARIES *IN SITU* | STAGE OF MATURITY ASSIGNED |
|---|---|---|---|
| Immature (1) | <250 µm | Ovaries small, slender, transparent, no visible signs of eggs | Inactive |
| Developing (2) | 251- 350 µm | Ovaries larger and filling with small, pink - orange, opaque visible eggs | Active |
| Spawning capable (3) | 351- 750 µm | Ovaries are large in relation to fish size, distended and clearly fill with visible opaque eggs. Some eggs already translucent. Colour of ovaries is bright orange to deep pink | Ripe |
| Actively spawning (4) | >750 µm | Translucent eggs can be extruded through the cloaca with slight abdominal pressure | Ripe and running |
| Regressing (5) | 351- 750 µm | Flaccid ovaries, blood vessels present, some translucent eggs present | Regressing |
| Regenerating (6) | 100- 300 µm | Ovaries visually completed empty, but large flappy, prominently veined and often bloodshot | Spent and inactive |

Further validation by histological and whole-mount auto-diametric methods resulted in the development of a most detailed microscopic maturity scale for *M. capensis* (Figure 4.2).

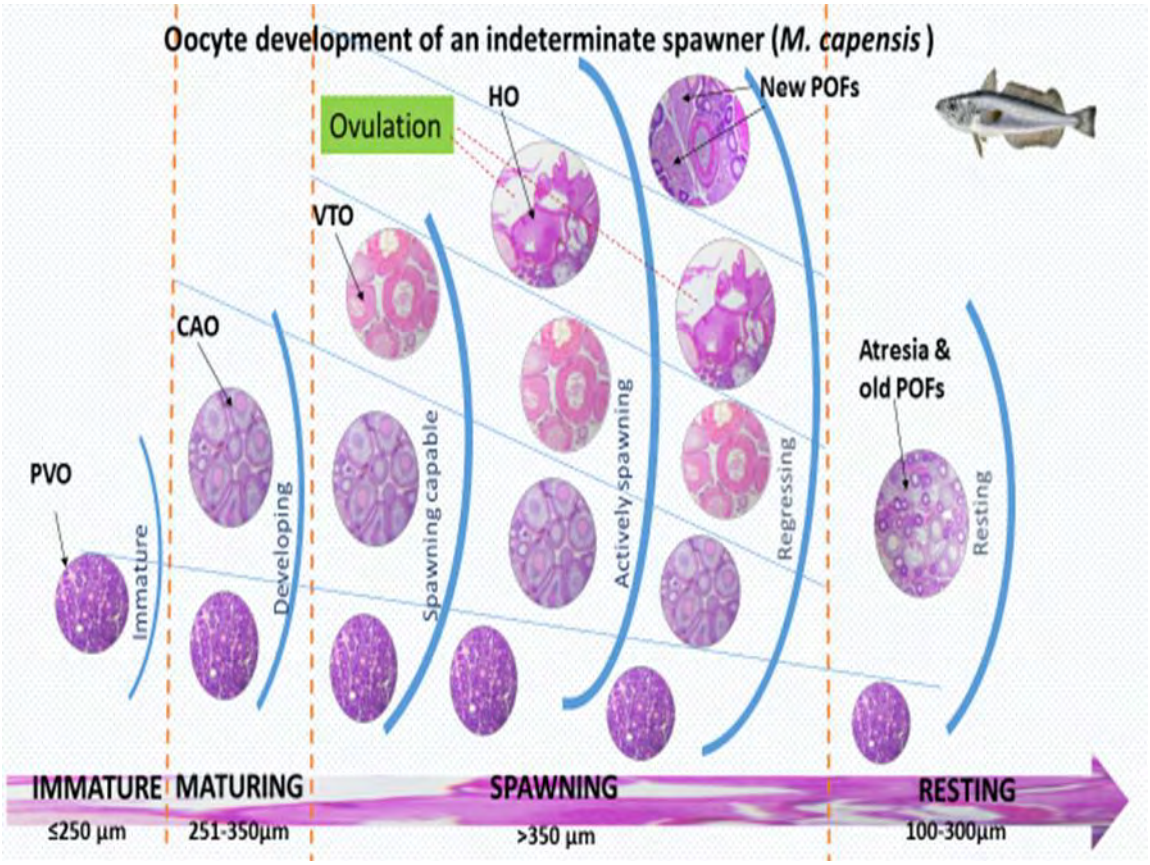


**Figure 4.2:** Microscopic maturity scale and observed features of the indeterminate reproductive style of *M. capensis* as oocyte development advances throughout the major stages (immature (≤ 250 µm), maturing (251 - 350 µm), spawning (> 350 µm) and resting (100 – 300 µm). The spawning stage includes all the spawning activity stages (spawning capable, actively spawning and regressing).

*4.3.2 Maturity assessment methods*

The undertaken macroscopic *vs.* microscopic maturity assessment of *M. capensis* – with the percentage of agreements placed into a so-called confusion matrix (Figure 4.3) – indicated an overall agreement of 35 %, i.e., when all the years were combined, and with the following agreement values when separated: 38, 30 and 39 % for 2014, 2015 and 2016 respectively. The highest agreement was for stage 1, ending at 76, 54 and 85 % for 2014 - 2016, respectively (Figure 4.3). The other stages were poorly identified by macroscopic staging.

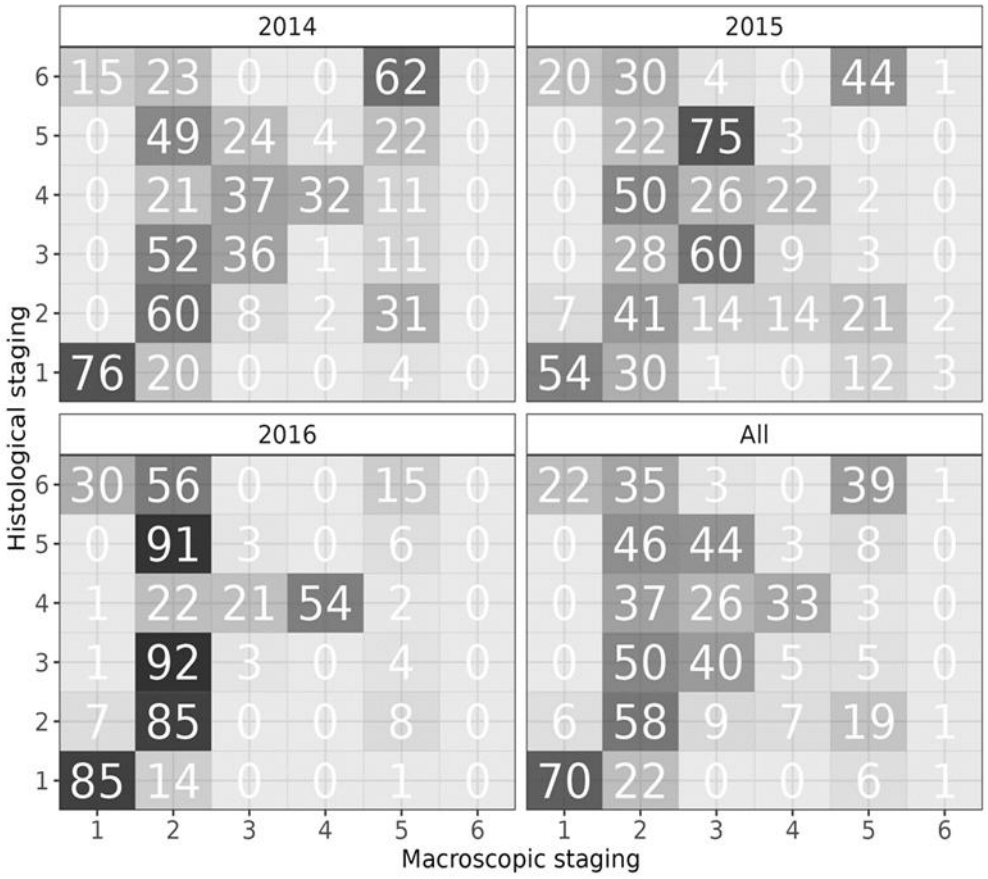


**Figure 4.3:** Confusion matrix for the percentages of agreement for histologically and macroscopically staged *M. capensis* individuals for the different sampling years and for all years combined. The macroscopic staging gives the predicted values while the histological staging gives the actual ones. Maturity stages are represented by 1 - 6 (1: immature stage, 2: developing stage, 3: spawning potential stage, 4: actively spawning stage, 5: regressing stage and 6: regenerating stage). The agreement is between the diagonal corresponding stages for a particular year. For instance, macroscopic stage 1 corresponds with the histological stage 1 in 2014 at 76 %.

*4.3.3 Gonadosomatic index*

There was relatively little variation in *GSI* across months but seeing weak indications of higher levels around March/April (2014 - 2016) and in August (2016) (Table 4.2). For 2015 and 2016 in total 5 months were represented whereas in 2014 only by 3 months (Table 4.2).

**Table 4.2**. Distribution of the mean values and the standard errors of the gonadosomatic index (GSI) for the three years (2014 - 2016) of investigation. All the maturity stages were considered for each month.

| Year | Month | Mean *GSI* (%) | -0,95% | 0,95% | N |
|---|---|---|---|---|---|
| 2014 | April | 3.0 | 2.4 | 3.6 | 98 |
| | May | 4.8 | 4.2 | 5.4 | 134 |
| | Sept. | 2.5 | 2.2 | 2.7 | 120 |
| 2015 | Feb. | 3.8 | 3.1 | 4.4 | 133 |
| | March | 5.2 | 4.6 | 5.8 | 116 |
| | April | 3.5 | 3.2 | 3.9 | 270 |
| | May | 4.5 | 3.8 | 5.2 | 86 |
| | Dec. | 3.4 | 2.8 | 4.0 | 186 |
| 2016 | April | 4.9 | 3.6 | 6.2 | 45 |
| | May | 4.4 | 3.8 | 5.0 | 172 |
| | Aug | 6.5 | 5.7 | 7.3 | 71 |
| | Sept. | 2.3 | 1.7 | 2.9 | 148 |
| | Oct. | 2.9 | 2.2 | 3.5 | 86 |

Statistically speaking, GSI was significantly different between the three months analysed in 2014 when all months were combined ; highest in May and lowest in April and in September ($P < 0.05$) (Figure 4.4, Table 4.2), However, there was no significant difference between April and September. Significant differences were also seen for the five months in 2015 at $P < 0.05$, when combined, with the highest value in March and the lowest in February. GSI across February, April, May and December 2015 were not different from each other. Significant difference was also noted in 2016 for all months when combined ($P < 0.05$), highest in August and lowest in September. Individual months (April and August; September and October) were not significantly different from each other ($P > 0.05$).

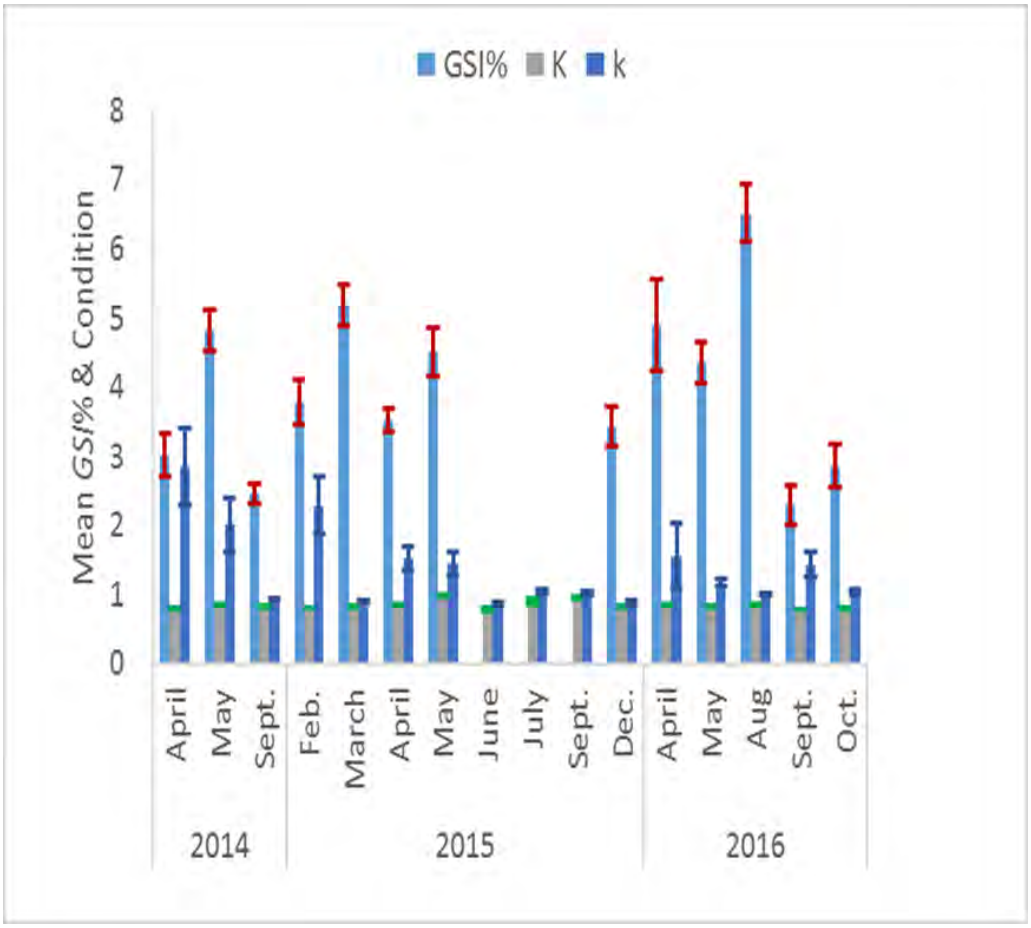


**Figure 4.4:** Distribution of the mean values (markers) and standard errors (caps) of the Gonadosomatic index (GSI*I %)* with light blue markers and red caps and the Fulton's condition index (*K*) in grey markers and green caps and the Relative condition index (*k*) in blue markers and dark blue caps over a three-year period (2014 - 2016). The total number of *GSI* values (1534) varied from that of the *k* and *K* (N = 1815) due to absent months (June, July and September) in 2015.

The highest GSI noticed in August 2016 was clearly different ($p < 0.01$) for all other months studied in this investigation, reaching a mean value of 6.5 %, while about one month later detecting the lowest figure (2.3 %) among all (Table 4.2). Note that the statistical anlyses were sensitive to the number of observations per analysed month ($60 < N < 270$) (Table 4.2). The comparison by year also resulted in low GSI means of 3.4; 4.1 and 4.2% for 2014, 2015 and 2016 respectively, with ($p > 0,05$). A comparison

of the GSI of the actively spawning stage showed that February in 2015 was highest, but not significant (Figure 4.5).

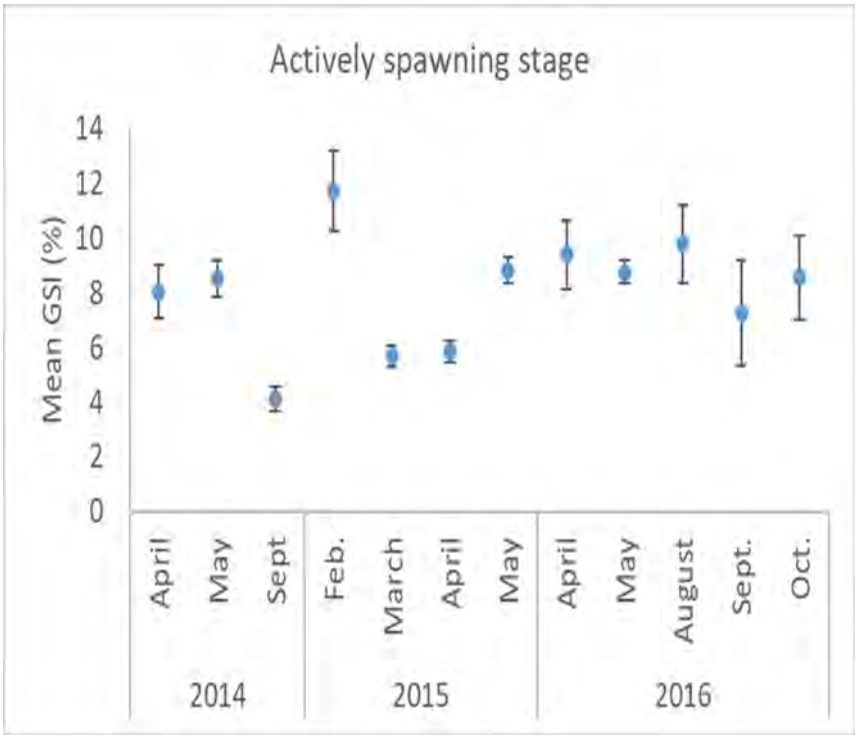


**Figure 4.5:** The distribution of the gonadosomatic index (GSI) of the actively spawning stage (stage 4) on the y-axis split by sampling months on the x-axis for the years 2014 to 2016. The blue centre dots represent the mean GSI with the error bars (in black) in both ends. Sampling was opportunistic, hence not efficiently collected in all the months in years of investigation.

The mean values for the actively spawning stage were much higher (6.9; 8.0 and 8.7%) than those of all maturity stages presented above (Figure 4.5), for the same years (2014 - 2016), though not significantly different. Furthermore, the within year comparisons indicated months differences as, September was significantly lowest than April and May in 2014 ($P < 0.05$). March and April were significantly lower (5 – 7 %) than February and May (8 – 15 %) in 2015 ($P < 0.05$). In 2016 there was no difference in months.

*4.3.4 Maturity ogives*

Using histology, the grand mean $L_{50}$ for *M. capensis* was 38 cm (2014 - 2016), seeing an underlying difference of a few centimeters across years: 38, 35 and 40 cm for 2014, 2015 and 2016, respectively (Figure 4.6).

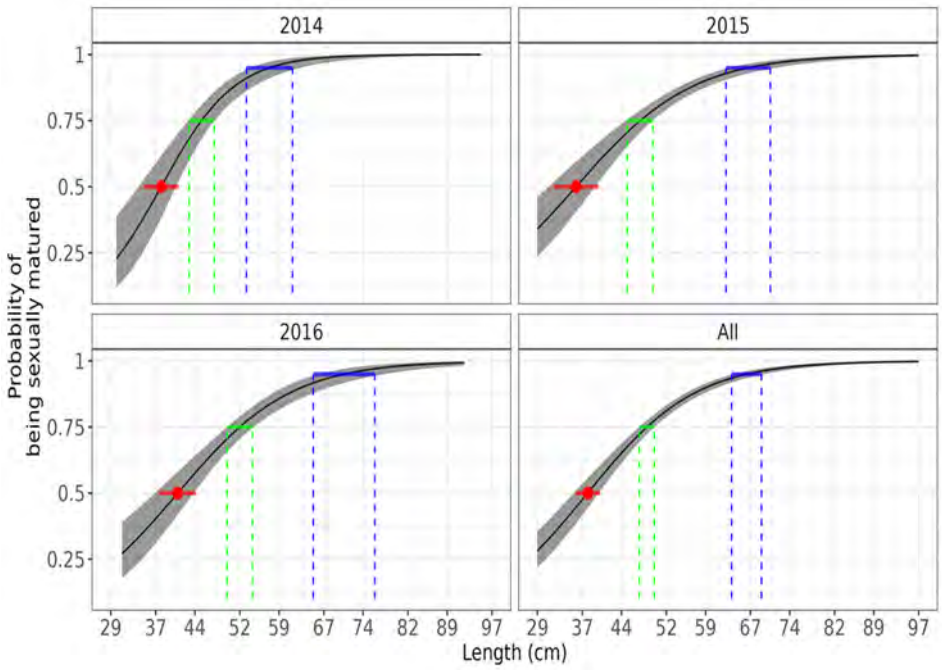


**Figure 4.6:** Established histology-based maturity ogives for the different sampling years and data sources with the corresponding 95% confidence interval (CI). $L_{50}$ is shown with the filled dot (and 95% CI). The 95% CI of for $L_{75}$ and $L_{95}$ are denoted by coloured line segments (green and the blue, respectively), with vertical dashed lines denoting the location of the corresponding length.

The resulting comparison with the corresponding macroscopic maturity ogive, focusing on $L_{75}$ and $L_{95}$, this visual staging resulted in lower figures than those seen under the microscope; macroscopically: 48 and 60cm, histologically: 50 and 65 cm, respectively. $L_{50}$ of the microscopic method was 39 cm (Figure 4.7), thus, a trivial change compared to the one from macroscopic method.

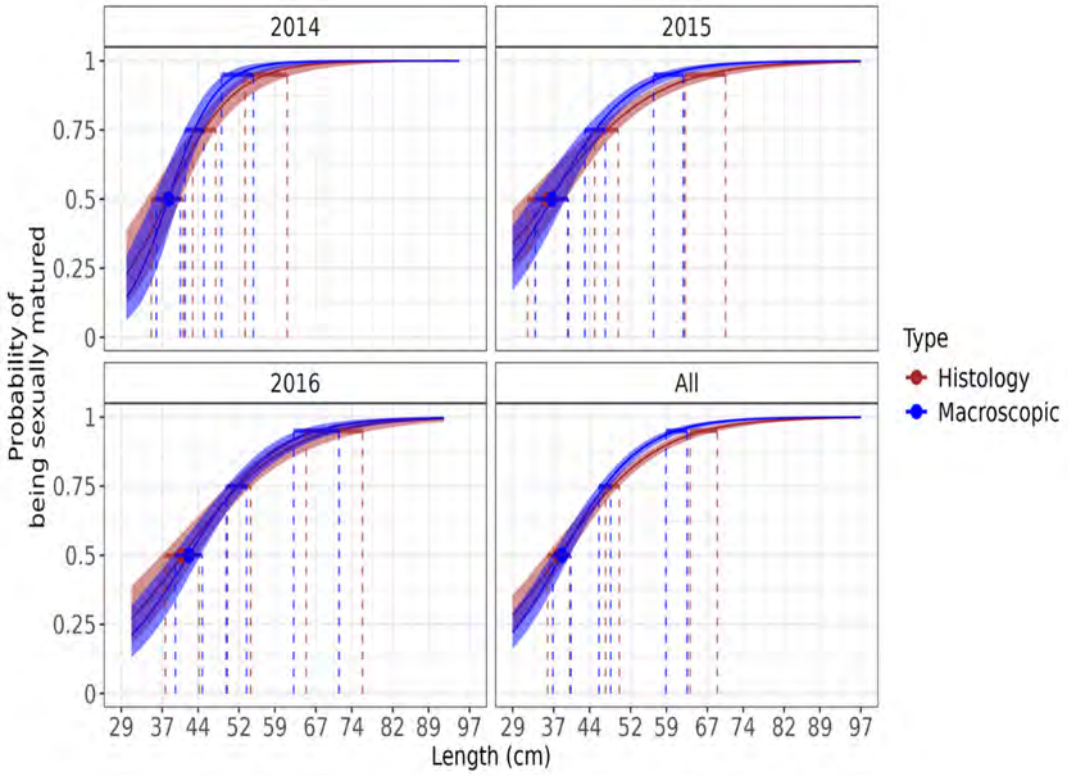


**Figure 4.7:** Illustration of the histological (pink) and macroscopic (blue) maturity ogives, and $L_{50}$ for the different sampling years (2014 - 2016), separated and combined. There is always slight overestimation by the macroscopic assessment for $L_{50}$, but more visible underestimation for $L_{75}$ and $L_{95}$ for both the separated and combined years. The 95% confidence interval of length for $L_{75}$ and $L_{95}$ are denoted by corresponding coloured line segments. $L_{25}$ was not considered in this analysis.

The delta (δ) and variation (sum of squares) were higher in histological ogives as compared to macroscopic ogives (Table 4.3), which implies that there was more variation around the mean for the histological data as opposed to the macroscopic data

**Table 4.3:** Illustrating the indicative values (δ (delta) and the sum of squares) from Excel Solver when the analysis was made for the length at which 50% of the population gets sexually matured ($L_{50}$).

| Maturity parameters | Macroscopic | Histology |
|---|---|---|
| $L_{50}$ | 38.54 | 37.88 |
| Delta | 8.39 | 5.10 |
| Sum of squares | 212.58 | 133.00 |

*4.3.5 Length-weight relationships*

There were signs of isometric pattern when length- weight in years (2014, 2015 and 2016) was compared separately, as they were not different from three. However, the growth pattern was evidently allometric when all years were combined (Figure 4.8).

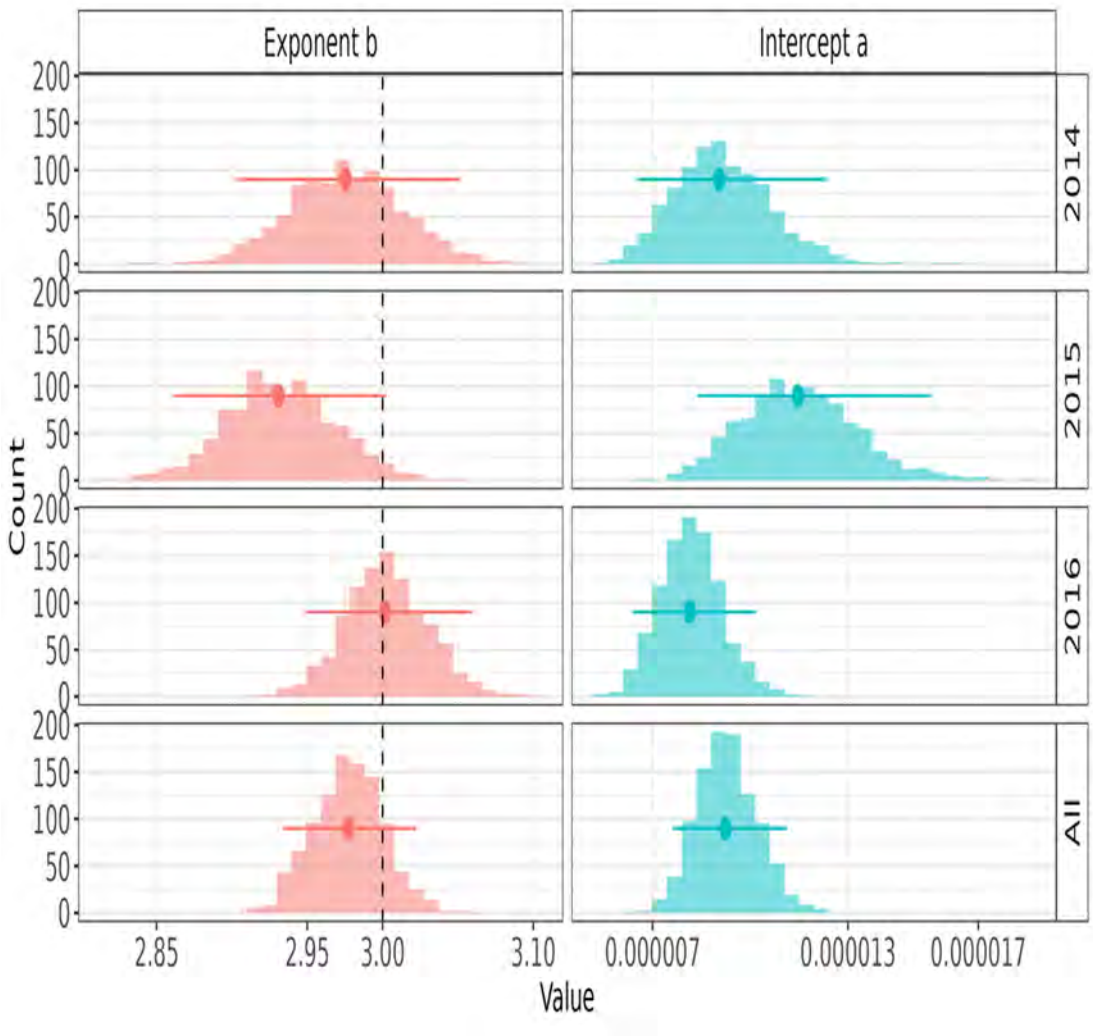


**Figure 4.8:** Histograms of the bootstrapped distribution estimated parameters of the length - weight relationship showing the intercept (*a*) and the exponent (*b*) calculated for 2014 - 2016 separated and combined years. Horizontal line with filled circle shows mean ± 95 % confidence interval.

The exponent *b* in the growth power function was respectively 2.98, 2.93 and 3.0 for 2014, 2015 and 2016, and 2.98 for all years combined (Table 4.4). The relationship was very strong for all the years from 2014 to 2016 combined and separated, though 2015 was slightly lower in growth with *b* = 2.9. Both the slope and the intercept were highly significant within groups with $P < 0.001$ for each year.

**Table 4.4:** Regression statistics for the length-weight relations for the years 2014-2016. The strength of the relationship between the two parameters is represented by $r^2$; the variation from the mean is indicated by the SE (standard errors), while N gives the total number of samples.

| Bootstrapping Results (Year) | Coefficients | Means | SE | $r^2$ | N | Growth pattern |
|---|---|---|---|---|---|---|
| 2014 | a | 8.96E-06 | 1.40E-06 | 0.95 | 350 | Allometric |
| | b | 2.98E+00 | 3.70E-02 | | 350 | |
| 2015 | a | 1.13E-05 | 1.80E-06 | 0.89 | 931 | Allometric |
| | b | 2.93E+00 | 3.70E-02 | | 931 | |
| 2016 | a | 8.09E-06 | 9.50E-07 | 0.96 | 535 | Isometric |
| | b | 3.00E+00 | 2.80E-02 | | 535 | |
| All | a | 9.24E-06 | 9.00E-07 | 0.92 | 1816 | Allometric |
| | b | 2.98E+00 | 2.30E-02 | | 1816 | |

The corresponding coefficient of determination ($r^2$) between *W* and *L* was high ($P < 0.001$): overall 0.92 for all the years combined, whereas 0.95, 0.89 and 0.96 for 2014, 2015 and 2016, respectively (Table 4.4). The mean slope values for the below and above 60 cm length when years are combined was 2.98 and 2.91, respectively, and suggests negative allometric growth for both categories.

**Table 4.5:** The distribution of the growth patterns derived from the slope and the intercept for length frequency below and above 60 cm from 2014 - 2016. The samples numbers are displayed as N.

| Year | Length class | Mean Length (cm) | Slope (b) | Intercept | N | Growth pattern |
|---|---|---|---|---|---|---|
| 2014 | <60cm | 48.24 | 2.87 | -1.80 | 233 | Allometric |
| | >60cm | 71.09 | 2.98 | -2.00 | 119 | Allometric |
| 2015 | <60cm | 49.94 | 3.12 | -2.20 | 498 | Isometric |
| | >60cm | 71.78 | 2.95 | -2.00 | 428 | Allometric |
| 2016 | <60cm | 49.49 | 2.96 | -2.00 | 346 | Allometric |
| | >60cm | 68.59 | 2.81 | -1.70 | 176 | Allometric |

Overall, those < 60 cm showed isometric growth ($b$ = 3), while those > 60 cm indicated allometric growth being in place ($b$ = 2.9) (Figure 4.9). Furthermore, when still $L$ separated, those > 60 cm in 2016 followed allometric growth, though still not that very strongly ($b$ = 2.8).

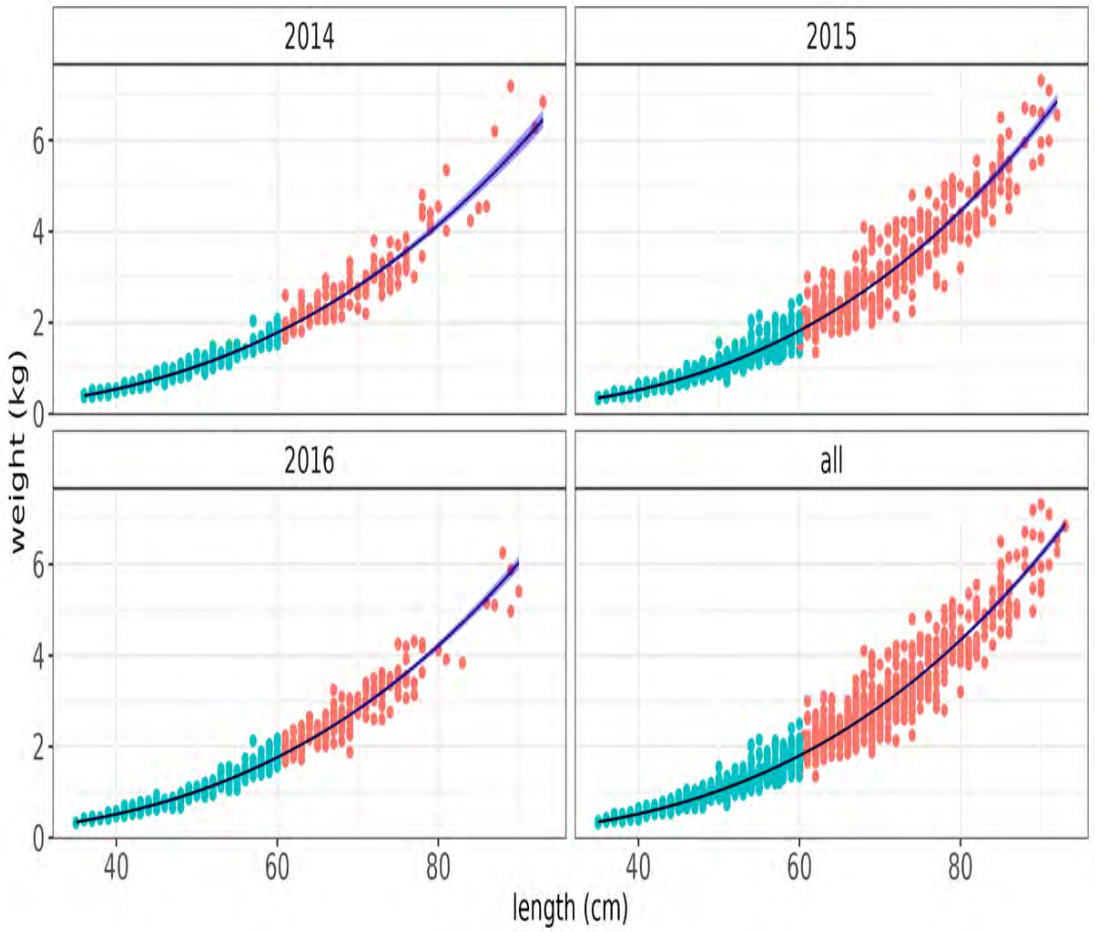


**Figure 4.9:** Observed length *vs*. weight together with power regression indicating the strength of the relationship, separately for each sampling year and all data combined. The two length classes were created: < 60 cm (blue colour) and > 60 cm (pink colour). Total number of samples is 1816 combined, collected from 2014 - 2016.

*4.3.6 Body condition*

The alternative use of either relative body condition (*k*) or Fulton's condition factor (*K*) resulted in very much the same insights, overall and across years (Figure 4.10).

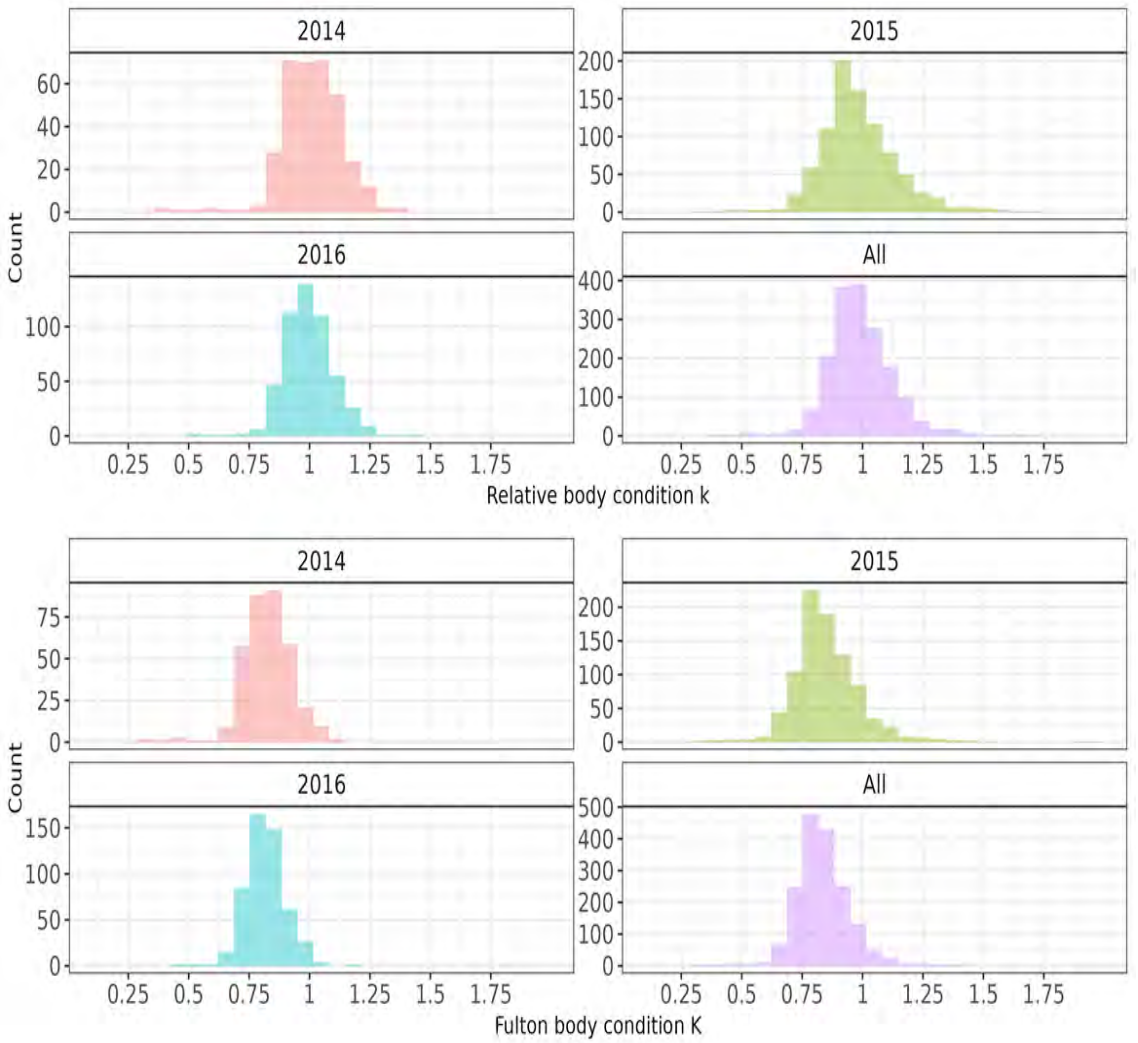


**Figure 4.10:** Histograms showing distribution of the counts or observations versus the relative body condition index (*k*) and Fulton's body condition (*K*) for data from each sampling year and years combined (2014 - 2016). The calculations of the condition factors are from the length and weight parameters for each year and thereafter for all fish combined

The k and *K* indices were consistently centred at 0.79 - 1.68 and 0.80 - 0.86, respectively (Table 4.6), though seeing a broader range in 2014 followed by 2016 in comparison with 2015, which showed a tight distribution.

**Table 4.6:** Distribution of the mean values and the standard errors (*SE*) of the body condition calculated from the relative condition index (*k*) and the Fulton's condition factor (*K*) from 2014 - 2016, separated and combined.

| **Relative condition index (k)** | | | | | |
|---|---|---|---|---|---|
| **Years** | **Mean Condition** | **SE** | **-0.95** | **0.95** | **N** |
| 2014 | 1.93 | 0.32 | 1.28 | 2.57 | 351 |
| 2015 | 1.25 | 0.11 | 1.03 | 1.47 | 931 |
| 2016 | 1.24 | 0.15 | 0.94 | 1.54 | 533 |
| All | 1.37 | 0.16 | 1.05 | 1.70 | 1815 |
| **Fulton's condition index (K)** | | | | | |
| 2014 | 0.82 | 0.01 | 0.81 | 0.84 | 351 |
| 2015 | 0.86 | 0.02 | 0.83 | 0.90 | 931 |
| 2016 | 0.82 | 0.01 | 0.81 | 0.84 | 533 |
| All | 0.84 | 0.01 | 0.82 | 0.87 | 1815 |

The latter year exhibited a significantly higher *k* than *K* ($p < 0.05$). The *k* was slightly higher in 2014, specifically in April ($k = 2.31$) and May $k = 1.82$) (Table 4.7.), than all other months in 2015 and 2016 ($p < 0.05$), however, this measure of relative body condition did not show any significant difference when combined data for years were compared.

1878 **Table 4.7:** Distribution of the relative condition index for the months in the years 2014 - 2016.

| Year | Month | Mean k | SE | -0.95 | 0.95 | N |
|---|---|---|---|---|---|---|
| 2014 | April | 2.8 | 0.6 | 1.7 | 4.0 | 98 |
| | May | 2.0 | 0.4 | 1.2 | 2.8 | 134 |
| | Sept. | 0.9 | 0.0 | 0.9 | 1.0 | 120 |
| 2015 | Feb. | 2.3 | 0.4 | 1.5 | 3.1 | 133 |
| | March | 0.9 | 0.0 | 0.9 | 0.9 | 116 |
| | April | 1.5 | 0.2 | 1.2 | 1.8 | 290 |
| | May | 1.4 | 0.2 | 1.1 | 1.8 | 151 |
| | June | 0.9 | 0.0 | 0.8 | 0.9 | 53 |
| | July | 1.1 | 0.0 | 1.0 | 1.1 | 29 |
| | Sept. | 1.0 | 0.0 | 1.0 | 1.1 | 61 |
| | Dec. | 0.9 | 0.0 | 0.9 | 0.9 | 89 |
| 2016 | April | 1.6 | 0.5 | 0.6 | 2.5 | 45 |
| | May | 1.2 | 0.1 | 1.1 | 1.3 | 172 |
| | Aug. | 1.0 | 0.0 | 1.0 | 1.0 | 71 |
| | Sept. | 1.4 | 0.2 | 1.1 | 1.8 | 148 |
| | Oct. | 1.0 | 0.0 | 1.0 | 1.1 | 86 |

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## 4.4 Discussion

Maturity information is used as an input to stock assessment models to make inferences and estimations about the fisheries stock status (Hunter and Macewicz 1985; Colman 1998; Lambert 2008; Brown-Peterson et al. 2011; ). Macroscopic maturity staging is regularly used at sea for *M. capensis* to estimate the relative portions of sexually immature and mature fish, though it is known for its subjectivity (Vitale et al. 2006). When histological validation is absent, the macroscopic data are used as the baseline information for maturity ogives. The present investigation found the overall length at 50 % maturity assessed by macroscopic method to be 39 cm whereas 38 cm by histological means. Thus, a non-significant difference, although seeing a reduction in length at maturity at the 75 and 95 percentiles to 48 cm instead of 50 cm, and 60 cm instead of 65 cm, respectively, suggesting that histological validation should be a regular practice within this stock assessment, as commonly recommended.

The current study suggests the use of the maturity stage 2 (developing stage) and up as the active matured stages versus the inactive immature maturity stage one for the calculation of the length at 50 % maturity and that is in agreement with other authors (Osborne et al. 2004: Wilhelm et al. 2015). The use of stage 2 and up for maturity active stages is due to two basic reasons. Firstly, the microscopic assessment of the immature stage indicates that only the primary growth oocytes (oogonia and the perinucleolar) and or previtellogenic oocytes (PVO) are available in that stage, which then emphasises the inactivity and the immaturity of the stage (Brown- Peterson et al. 2011). The immature stage does not contain any matured oocytes other than the mentioned ones, hence mean oocyte diameter size of 250 µm should be treated as a threshold or a cut-off point for immaturity in the present study. Secondly, once PVO grow bigger in size, they develop into cortical alveoli oocytes (CAO) which is the main identifier of stage 2 and they are the most advanced oocytes at that stage, though sometimes there may be primary vitellogenic oocytes. (Brown- Peterson et al. 2011). The developing stage comes in two forms, as some are a direct development from the immature stage, while others are from resting stage of the previous

cycle. Due to the nature of *M. capensis* being a serial spawner and an inderteminate spawner (Osborne et al. 2004), after the ovary has finished a spawning cycle, it rests (stage 6/ regenerating stage) and allows the development of the new oocytes (PVO), which when grown enough, they become CAO and the ovary can be categorised as stage 2/ developing stage again. The latter stage two is slightly different from the basic one as it contains the remnants of the previous cycle such as the post ovulatory follicles (POFs), atretic follicles in different forms and other degenerating structures (Brown- Peterson et al. 2011; Lowerre- Barbiere et al. 2011). Therefore the two cited reasons emphasise the calculation of maturity ogives between the immature (stage 1) and the matured females (stage 2 and up) to improve the precision of the spawning stock biomass estimates.

It is clear that the use of different methods has resulted in different length at 50 % maturity in the four studies conducted at different times, in the similar area (the south coast of South Africa with the warm Agulhas current mixing with the waters from the Indian Ocean) as the authors (Botha 1985; Fairweather and Leslie 2008; Singh et al. 2011) used macroscopic methods and their results (48, 42 and 53cm) were different as opposed to when microscopic analysis was applied in the current study with the result of 38cm. There are various authors who have demonstrated the erroneous macroscopic method (Honji et al. 2006, Vitalle et al. 2006, Osborne et al. 1999, Kainge et al. 2007) with emphasis that it cannot be used for the development of the maturity ogives. The major problem with macroscopic information is that, it does not give insight as to the contents of the ovaries which then limits its usage, as opposed to microscopic analysis. Microscopic information of *M. capensis* directs the threshold for maturity ogives in the current study to be stage 2 and up and the reasons (including calculation of ogives from stage 3 and up, reliance on macroscopic data, unfavourable conditions and stopping of maturity process from stage 2 to stage 6, mentioned in other studies (Singh et al. 2011; Fairweather and Leslie, 2007)) need scientific backing and thorough investigation.

Furthermore, the current study also noted that, the investigation of the $L_{50}$ in the adjacent Namibian region on the same species (Kainge et al. 2007; Wilhelm et al. 2015) wherein similar methods as the

current study were used, yielded different results as $L_{50}$ = 25cm while $L_{50}$ = 38cm in the present study. The difference in the mentioned studies can be attributed to the difference in the regions as the Namibian area represents the South Eastern Atlantic upwelling cold Benguela Current region as opposed to the south coast of South Africa with warm coastal Agulhas Current region.

*Merluccius australis* off New Zealand in the study by Colman (1998) appears to become sexually mature at a smaller size than reported by previous studies. The latter author argues that the difference may not be as great as that observed from the data as the samples were restricted to the spawning season, implying that the time of sample collection has an effect. However, the data for the current study were collected across the South coast area, where spawning occurs all-year-round with apparent two spawning peaks.

Over and above the different methods used for determination of the maturity ogives, the different region and the different times for sample collection, the change in life history strategies over time cannot be ruled out as marine fish evolve life- history strategies that maximise lifetime reproductive success within environmental and genetic constraints (Roff, 1984). Their optimal size at maturity is dependent on trade-offs between the interrelated traits fecundity, body growth rate and maturity (Roff, 1984) and any factor (be it environmental, biological or anthropogenic) that alters one of the above parameters, could shift the optimal size at maturity (Griffiths, 2002). In terms of trade-offs scenario, the results by Punt and Leslie (1991) could not justifiably support the hypothesis that age at 50% maturity of hakes (*M. capensis* included), with the length data fitted into von Bertalanffy growth equation, has increased in response to a decrease in fishing mortality. This calls for further research of the responsible factors behind the change of $L_{50}$.

Gonadosomatic index of all maturity stages of *M. capensis* turned out to be generally low (< 4.2 % per annum) and displaying relatively little variation across the study months and years. It is expected to be around 7 % for all the maturity stages in the region as suggested by other authors (Kainge *et al*., 2007), while it was between 2-10% by other authors (Jansen et al. 2015). However, in the current study, the GSI

means of the actively spawning stage was on average above (2014 = 6.9 %, 2015 = 8.0 % and 2016 = 8.9 %) the suggested threshold of 7%. This species (*M. capensis)* seems to be able to spawn all-year-round (Durholtz et al. 2015) as GSI values fluctuated and actively spawning stages was found all year round in the current study. GSI is inexpensive and easy to compute; given as the relative weight of the gonad to the fish weight. Not only so, GSI is regarded a good indicator of reproductive activity, with, for instance, the spawning season being determined by an association between GSI and frequency distribution of maturity stages (Flores et al. 2015). Consequently, it is widely used to evaluate reproduction timing (Coker etal. 2008; Lowerre-Barbieri et al. 2011). Thus, our work in these regards followed a long tradition.

Spawning seasons of *M. capensis* were found to be in winter months and in late spring (October – December), for the northern and central Namibia (Olivar et al. 1988). However, all these conclusions were based on macroscopic maturity assessments and was later supported by studies of ichthyoplankton that observed spawning of *M. capensis* to peak between austral spring (October and December) and winter months in 1979 – 1980 (Olivar et al. 1988). A study using gonad weights data concluded that peak spawning of *M. capensis* occurred from July to October (winter to spring) between 22° S and 24° S (off Namibia) (Kainge et al. 2007). Nevertheless, the present study suggests that there are two spawning peaks, which are in austral autumn around March to May and in austral spring around August in the south coast of South Africa (34°- 36.5° S and 18°- 27° E), agreeing with suggestions made by other authors (Osborne et al. 2004; Jansen et al. 2015; Durholtz et al. 2015).

Fish length-weight relations are used to convert length observations into weight estimates in order to obtain stock biomass (Froese, 1998), An underlying reason may be that it is sometimes difficult to get reliable weight measurements on board due to weighing scale imbalances (Sinovcic, 2004). The length of the *M. capensis* in the south coast of South Africa can trustworthy be used to give the corresponding weight as 92 % of the variance was explained (cf. $r^2$). In the present study, the exponent *b* for 2014 and

2015 reflected signs of allometric growth, while 2016 showed isometric growth, therefore the females of 2014 and 2015 were relatively more elongated and thinner. Thus, the smaller individuals in those two years were possibly in a (slightly) better nutritional condition at the time of sampling (Zaher et al. 2015). Contrary to what was experienced in the current study in years 2014 and 2015, exposure to more food resource and favourable conditions may have led to fish getting fatter with the improved positive allometric growth (b > 3).

There was a slight difference in growth pattern indicated for different length size as 60 % and 40 % observations were below and above 60 cm with an average of $b$ = 3 and 2.90 respectively, for all years. Therefore, different growth patterns were possibly in place across the studied length range. However, the samples of the current study were collected in different seasons, with the most collection in autumn: samples constituted of 12, 58, 8 and 22 % of observations in summer, autumn, winter and spring, respectively. Therefore, it remains a possibility that a seasonal effect was in play, which could not be properly tested due to this unequal monthly representation. So, changes in growth observed may well be attributed to the period of collection (Jisr et al. 2018); the value of b may change during different time periods, illustrating, for instance, the fullness of stomach and gonad stages (Zaher et al. 2018). Furthermore, growth process can differ in the same species dwelling in diverse locations, being influenced by different biotic and abiotic factors (Jisr et al. 2018).

In the present study, relative fish condition ($k$) was at or near 1 in all years separated and combined, however, the Fulton's condition ($K$) was consistently below 1, though slightly higher in 2015 as compared to 2014 and 2016. The latter result suggests that $k$ indicated a better condition as opposed to $K$, which concurs with the investigation by Bolger and Connolly (1989). Although $k$ is calculated using a pooled estimate of $b$, it may be the best index when length vary significantly amongst the groups to be compared, i.e. $k$ more properly accounts for length dependency than $K$ (Bolger and Connolly, 1989). More in detail, $K$ assumes that the growth is always isometric, which is not the case (Bolger and Connolly, 1989). For $k$, the 'fitness' is assumed when $k$ = 1 or close to 1 (Mensah, 2015), whereas for $K$ above 1 are relatively

2003 better and below 1 are worse than the standard fish (Lambert *et al.,* 2000). Many factors affect the body
2004 condition of fish including reproductive cycles, availability of food as well as habitat and environmental
2005 factors (Morato et al. 2001). Fish in poor condition and first-time spawners may have reduced fecundity
2006 or reproductive success or may fail to spawn at all, affecting future recruitment (Marteinsdottir and Begg
2007 2002; Saborido-Rey et al. 2004; Dominguez et al. 2010). According to Macchi et al. (2016), *M. hubbsi*
2008 may skip spawning due to poor and low maternal condition. Low maternal condition has also been linked
2009 to a high incidence of atresia in anchovy *Engraulis capensis* (Melo, 1994) and herring *Clupea harengus*
2010 (Óskarsson et al. 2002). Conversely, good somatic conditions of the maternal stock of e.g. larger
2011 individuals of *M. hubbsi* are linked with better egg conditions with the hydrated oocytes containing larger
2012 oil droplets (Macchi et al. 2013).

2013 ## 4.5 Conclusion

2014 This life-history study on adolescents and adults of *M. capensis* is based on a relatively large field
2015 sampling program, studying totally of 1819 females between 2014 - 2016. Comprehensive work efforts
2016 were also made in the laboratory to validate the macroscopic data by histology and whole-mount analysis,
2017 with varying outcomes depending on the maturity stage in question. The update of the biometric
2018 parameters was fulfilled as we learned that $L_{50}$ from the macroscopic staging closely reflected the one
2019 from histology, though also seeing that there were deviating patterns at $L_{75}$ and $L_{95}$ indicting that the
2020 practice of naked-eye staging runs in short in cases, depending upon the material examined and personal
2021 expertise. This investigation also presents a series of biometric formulae of relevance in the hake
2022 assessment, using bootstrapping techniques when required to emphasize uncertainty (cf. *a* and *b* values)
2023 in growth functions. The current study also serves as a good report on life history parameters inclusive
2024 of length- weight relations and condition of *M. capensis*, though improved precision could have been
2025 reached with the use of gutted weight as opposed to total weight. Further investigations on energy

reserves, via for instance the hepatosomatic index, but also on how variation in body condition impacts on fecundity seem logical next steps following up studies on *M. capensis*.

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## 4.6 Author Contributions

This work is the contribution to the PhD thesis in the Department of Ichthyology and Fisheries Science at Rhodes University, South Africa. The work was conceived by L.N. and M.L. L.N proceeded with the survey design, sample collection and laboratory analysis. O.S.K acquired funding from Annenberg Foundation and trained L.N. on processing samples, analytical and digital methods at IMR. Data analysis and write up was done by L.N. under the supervision of O.S.K; M.L; and W.S. All authors commented on manuscript drafts and approved the final version of the manuscript.

ORCID

Lungelwa Nomxego: https://orcid.org/0009-0001-9647-6091

Olav Sigurd Kjesbu: https://orcid.org/0000-0002-8651-6838

Warwick Sauer: https://orcid.org/0000-0002-9756-1757

Marek Lipinski: https://orcid.org/0000-0001-9362-4610

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# Chapter 5: Spatial and temporal distribution of *Merluccius capensis* off south coast of South Africa

## 5.1 Introduction

The fishery for South African hakes *Merluccius capensis and M. paradoxus* is the second largest in the country (DFFE 2023), though knowledge of their life history contains important gaps (Durholtz et al. 2015). *Merluccius capensis* (shallow-water hake) is distributed from Southern Angola to KwaZulu Natal in South Africa and is mostly abundant in waters shallower than 400 m depth where as adults of *M. paradoxus* (deep-water hake) are distributed from northern Namibia to southern Mozambique, in depths of 110m to above 1000 m (Botha 1986, DFFE 2023). The horizontal and vertical distribution of eggs and larvae of hakes in the southern Benguela have been investigated by Stenevik et al. (2008), but research on the spawning grounds, nursery grounds, and feeding grounds in the southern region of South Africa makes limited reference to timing of spawning and its stages (Botha 1985; Payne and Punt, 1995). This is particularly so over the past decade as new research has not been reported.

It is not known whether ready-to-spawn *M. capensis, the present objective of the study,* show spatial and temporal shifts, therefore it remains a possibility that their spawning activity is associated with movement to areas that are related to favourable environmental conditions and/or ecologically suitable, where food is available both for the spawning fish and the larvae. Saunders and McFarlane (1997) discovered that female *Merluccius productus* (North Pacific hake) reside in deeper waters until they are ready to spawn, then move to shallow waters where spawning takes place, and then return to deeper water once all batches of eggs have been released and spawning is completed. Whereas *M. capensis* are still juveniles at <38cm total length (Nomxego et al. 2024) they appear in bottom trawls with the fine - mesh codend (Singh et al. 2011). According to Jansen et al. (2016), this size class appears to occupy depths of between 100 and 200 m, after which the fish gradually migrate to the deeper waters. Burmeister (2001) suggested that > 75 % of the *M. capensis* resource is in waters shallower than 300 m, and that

this species does not occur deeper than 500 m. Spawning females of of *Merluccius hubbsi* (Argentine hake) in Patagonian waters were observed to shift southwards as the spawning progressed (Pajaro et al. 2005). This geographical displacement was inferred from the observation of monthly spatial and temporal distribution patterns of individuals with hydrated oocytes. The spawning peak of *M. hubbsi* occurred between January and February, with 10 % of the females having either hydrated oocytes or new post-ovulatory follicles (POFs), whereas in March the prevalence of such females dropped to 6 %, indicating that the spawning of *M. hubbsi* varies with time (Pajaro et al. 2005). These authors also showed that mature males typically remain in the spawning region, and when the adult females leave the area many immature males replace them. Towards the end of spawning, a shift of post- reproductive *M. hubbsi* to generally deeper waters and a decrease in fish density in shallow waters is observed (Macchi, 2005). In the southeast Atlantic region (northern Benguela), spawning areas of *M. capensis* were located at around 20° - 21° 30' S in the 1970's and early 1980s (Assorov and Berenbeim, 1983), which then shifted to 22° - 24° S in the 1990's (Kainge et al. 2007) and father south to 26° - 28° S after 2010 (Wilhelm et al. 2015a). Whether this southward shift in spawning area off northern Namibia (Kainge et al. 2007; Wilhelm et al. 2015), has been replicated off the south coast of South Africa is unknown and would require a similarly long time series of data to beinvestigated.

The eggs and larvae of *M. capensis* that spawn on the south coast of South Africa are transported to the west coast by the Benguela Current (Hutchings et al. 2002; Jansen et al. 2016). Also , there is evidence of an inshore-offshore spawning migration off the south coast, revealed by large catches of spawning hake by handline between Port Elizabeth and St Francis Bay (Durholtz et al. 2015). A similar but eastward movement of spawning *M. capensis* along the south coast of South Africa has also been observed, i.e. counter to the Agulhas Current (Durholtz et al. 2015). However, there is a paucity of recent literature dedicated to spawning activities of the female of *M. capensis* about andtheir possible temporal shifts and variations in density off the south coast. In this area, where the continental shelf (including the Agulhas Bank) is broad, the *M. capensis* stock contributes as much as 70% of the South African

commercial hake fishery, which is carried out by the inshore trawl, deep-sea trawl and handline sectors (DFFE 2023). *Merluccius capensis* is also an important component of several southern African ecosystems (Payne et al. 1987). This study provides both updated and new information on the distribution of spawning activity of *M. capensis* in space and time, including the apparent occupation of different locations by different maturity and reproductive stages.

## 5.2 Materials and Methods

### *5.2.1 Sample collection and processing*

A total of 1807 *M. capensis* females were collected with bottom trawl on the shelf and upper slope off the south coast of South Africa during both commercial and research surveys between 2014 and 2016. The intensity of sampling and abundance of samples differed between years and were dependent on cruise availability and therefore the number of bottom trawls. To enable the analysis of the movement of the spawning stock, the time and geographic position and the water depth of each sample was recorded. It was assumed that all fish in the trawl were collected on the bottom and not in the water column. It was also assumed that the sexual maturity of the fish sampled was approximately representative around the given station and of the season in which the fish were caught. Each trawl was subsampled by randomly collecting 30 *M. capensis* females with no body size restriction, if they were present in sufficient numbers. The number of fish caught was standardized to the number per 10 $m^2$ of sea surface area based on the depth and duration of each tow as described in Smith and Richardson (1977).

For each fish, the following variables were collected: total length (TL, cm), and total weight (TW), gutted weight, gonad weight (GW) and liver weight, all in grams. The ovaries were classified according to a five-degree macroscopic maturity scale following Botha (1986), with the addition of stage 6 (Osborne et al. 1999), supported in all instances by photographic records for further evaluation. Detailed morphological descriptions of the 1807 *M. capensis* ovaries were recorded on board before fixation in 3.6 % buffered formaldehyde. In the laboratory, the macroscopically assessed ovary stages were validated using histology and corrected, if necessary. The histological validation identified maturity stages based on the most advanced oocyte development and on the presence of certain cellular organelles (following the microscopic criteria given below). Females in stages 1 and 6 were classified as inactive, whereas those in stages 2-5 were classified as active (Nomxego et al. 2024). Standard microscopic criteria applied in teleost reproductive biology (Hunter and Macewicz 1985; Osborne 2004; Kjesbu 1991; Brown - Peterson et al. 2011; Nomxego et al. 2024) were adopted when establishing the following staging system.

Stage 1 is the ‘immature stage’ and contains previtellogenic oocytes (PVOs; oogonia and perinucleolar). Stage 2 is the ‘developing stage’ and contains PVO, plus cortical alveoli oocytes (CAO), and usually, primary vitellogenic oocytes (PVTO) with protein (yolk) granules and lipid vacuoles. Stage 3 is the ‘spawning capable stage’ that has PVO, CAO, PVTO, as we ll as secondary (SVTO) and tertiary vitellogenic oocytes (TVTO) (including eventual migration of the nucleus to its animal pole). Stage 4 is the ‘actively spawning stage’ with PVO, CAO, all VTOs, nucleus migration and hydrated oocytes (HO). Stage 5 is the ‘regressing stage’ with all the features of Stage 3 but can be identified by the new post ovulatory follicles (POFs). Stage 6 is the ‘regenerating stage’ and has the oocyte content of Stage 1, but also the degenerating POFs and atretic oocytes (alpha and beta atretic sub - stages), the remnants of the previous cycle, as the identifying features. The gonadosomatic index (GSI, expressed as a percentage) was used to assess the relationship between TW and GW by month: GSI = 100 × GW / TW.

*5.2.2 Data analysis*

Standard parametric tests were conducted. One-way ANOVA (including F - test) were applied when comparing variance between three or more sets of observations of the same type of variable. A Student’s t - test was run instead when there were two sets of observations. Regression (or correlation) analysis was used to check relationships between the parameters. Significance levels are indicated by the *p*-values.

## 5.3 Results

### *5.3.1 Body-size and bottom-depth related patterns in maturity development*

Differences in total length associated with each maturity stage were investigated. The mean TL of females in the immature stage (stage 1) was smaller (48 cm) than the mean TL of those in other stages ($P < 0.05$) (Table 5.1, Figure 5.1).

**Table 5.1:** Total length distribution of female *M. capensis* in different maturity stages off the South coast of South Africa. The effect of the relationship between maturity stage and total length: $F (5, 1792) = 70.986$, $P < 0.05$ ($N = 1807$).

| Maturity stage | Mean length(cm) | -0.95 | 95% CI | Frequency % |
|---|---|---|---|---|
| Immature (1) | 47.9 | 46.8 | 48.9 | 17 |
| Developing (2) | 55.6 | 54.4 | 56.8 | 15 |
| Spawning capable (3) | 61.3 | 60.3 | 62.4 | 30 |
| Actively spawning (4) | 62.8 | 61.4 | 64.2 | 21 |
| Regressing (5) | 60.1 | 58.2 | 62.0 | 9 |
| Regenerating (6) | 56.6 | 55.0 | 58.1 | 8 |

Females in the developing (stage 2) and the regenerating stage (stage 6) had the same mean TL (56 cm) and they were significantly different in size from those being spawning capable (stage 3), actively spawning (stage 4) and regressing (stage 5) ($P > 0.05$) (60 - 63 cm) (Table 5.1, Figure 5.1). Thus, the relationship between mean TL and maturity stage represented a convex curve (Figure 5.1).

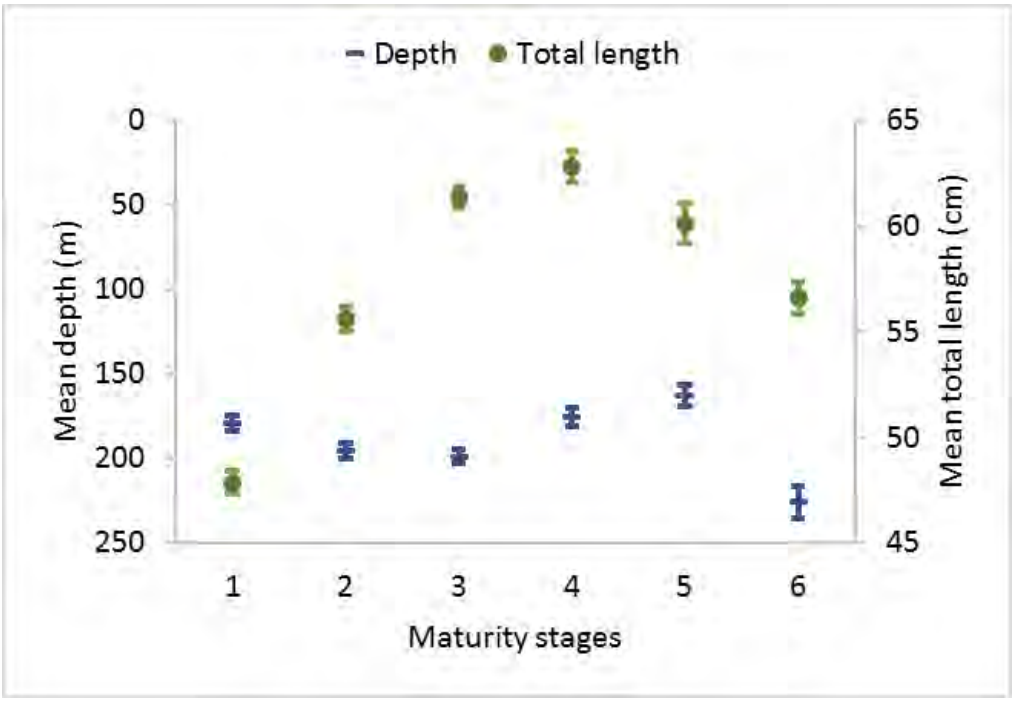



**Figure 5.1:** Study overview of the total length of female *Merluccius capensis* and the water depth plotted against maturity stage. Maturity stage 1 = immature; 2 = developing; 3 = spawning capable; 4 = actively spawning; 5 = regressing; 6 = regenerating. Error bars represent SE. The data were combined for 2014 – 2016.

Fish of stages 3 and 4 contributed 51 % of the total number of samples collected across the three years, whereas those of stages 5 and 6 were lower in abundance (9 and 8 %), respectively, as compared to all other stages.

The body size and water depth of females increased with maturity stage for the first three stages (immature, developing and spawning capable); mean size increased from 48 cm to 61 cm TL and mean water depth increased from 179 to 199 m (Tables 5.1 and 5.2). In comparison with other stages, females in maturity stage 6 (regenerating stage) were found significantly deeper ($p < 0.05$) (on average at 226 m).

**Table 5.2:** Maturity stage of female *M. capensis* by mean depth off the south coast of South Africa. The effect of the relationship between maturity stage and depth: F (5, 1757) = 12.737, P < 0.05 (N = 1807).

| Maturity stage | Mean depth(m) | -0.95 | 0.95 | Frequency % |
|---|---|---|---|---|
| Immature (1) | 179.0 | 169.8 | 188.1 | 17 |
| Developing (2) | 195.6 | 185.8 | 205.4 | 16 |
| Spawning capable (3) | 199.0 | 190.8 | 207.2 | 30 |
| Actively spawning (4) | 175.5 | 165.0 | 185.9 | 22 |
| Regressing (5) | 163.1 | 150.5 | 175.7 | 8 |
| Regenerating (6) | 225.9 | 207.4 | 244.4 | 7 |

Maturity stages 1-5 (immature to regressing) all tended to be found at depths shallower than 200 m ($p$ > 0.05). Combining reproductive stages, the greatest number (43 %) of *M. capensis* occur at about 140 m depth, when smaller in size (TL = 55 cm), whereas larger specimens (TL = 62 cm) were found at about at about 240 m, and those larger (TL > 67 cm) at depths greater than 300 m (Table 5.3). About 60 % of *M. capensis* females examined were found shallower than a mean depth of 141 m, and 40 % were deeper (mean depth 244- 421 m) (Table 5.3). Mean TL increased steadily with mean depth (Table 5.3).

**Table 5.3:** Overall depth occupation by different female sizes of *M. capensis* (N = 1807) in the south coast of South Africa over the three-year study period (2014-2016).

| Stratified depth (m) | Mean depth (m) | Mean length (cm) | Frequency (%) |
|---|---|---|---|
| 0-100 | 81.2 | 51.3 | 16.9 |
| 101-200 | 140.7 | 54.9 | 42.5 |
| 201-300 | 243.5 | 62.0 | 24.9 |
| 301-400 | 349.2 | 67.8 | 15.1 |
| 401-500 | 420.8 | 73.1 | 0.6 |

All maturity stages, except the regenerating stage (stage 6), were concentrated at depths shallower than 200 m (93 %), whereas individuals in the regenerating stage (7 %) were located some 20 m deeper (Table 5.2). Spawning stages 3-5 (spawning capable, actively spawning and regressing stages) were

found at mean depth between 163 and 199 m (Table 5.2, Figure 5.1). An *F* - test indicated that all stages occupied significantly different depths ($p<0.05$).

*5.3.2 Temporal patterns in maturity development*

In terms of longitudinal component, there were no significant differences in spatial distribution across years and stage 1 (immature), 2 (developing), and 6 (regenerating) with their annual distributions overlapping east of 20º E (regression; $p>0.05$). However, stages 3 (spawning capable) and 4 (actively spawning) showed slight spatial changes across years, though again not significant ($p > 0.05$) (Figure 5.2). Stage 6 (regenerating) was commonly found at 22º E. In terms of latitudinal changes, stage 4 (actively spawning) had one major centre of distribution for all three years at about 34.5° S.

Generally, *M. capensis* females in the other stages were either concentrated at 36.5° or 34.5° S, though with indications of a change in the distribution pattern towards 35.5° S for stage 1(immature), 5 (regressing) and 6 (regenerating) in 2015, 2014 and 2016, respectively (Figure 5.2). Collectively, all six maturity stages of *M. capensis* were distributed between 34 and 37º S and 18 and 27º E (Figures 5.2).

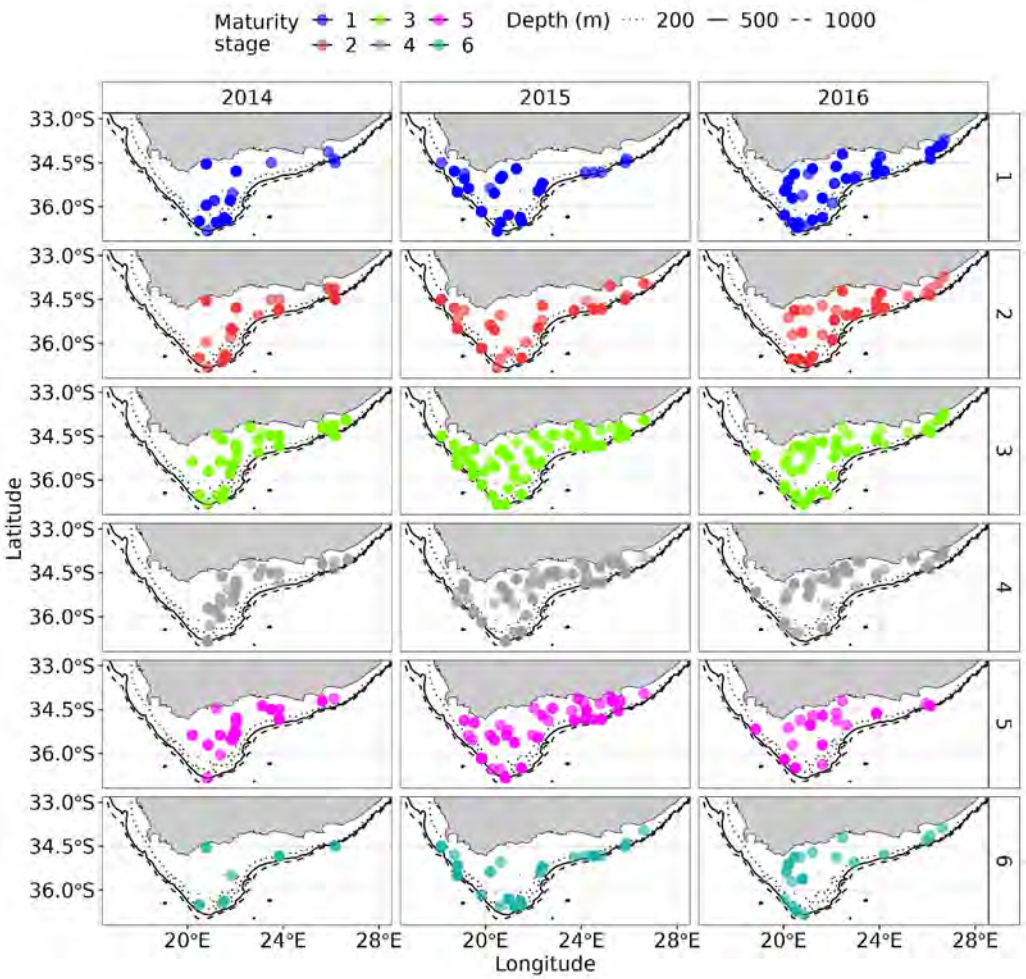



**Figure 5.2:** Spatial distribution of each maturity stage (vertical columns) of female *M. capensis* by sampling year (2014 - 2016). Maturity stage 1 = immature; 2 = developing; 3 = spawning capable; 4 = actively spawning; 5 = regressing; 6 = regenerating. A colourful dot represent a position of at least one specimen of a given maturity stage.

Monthly gonadosomatic index (GSI) varied, though no significant difference in the mean values ($p > 0.05$) across the combined years (Figures 5.3 and 5.4).

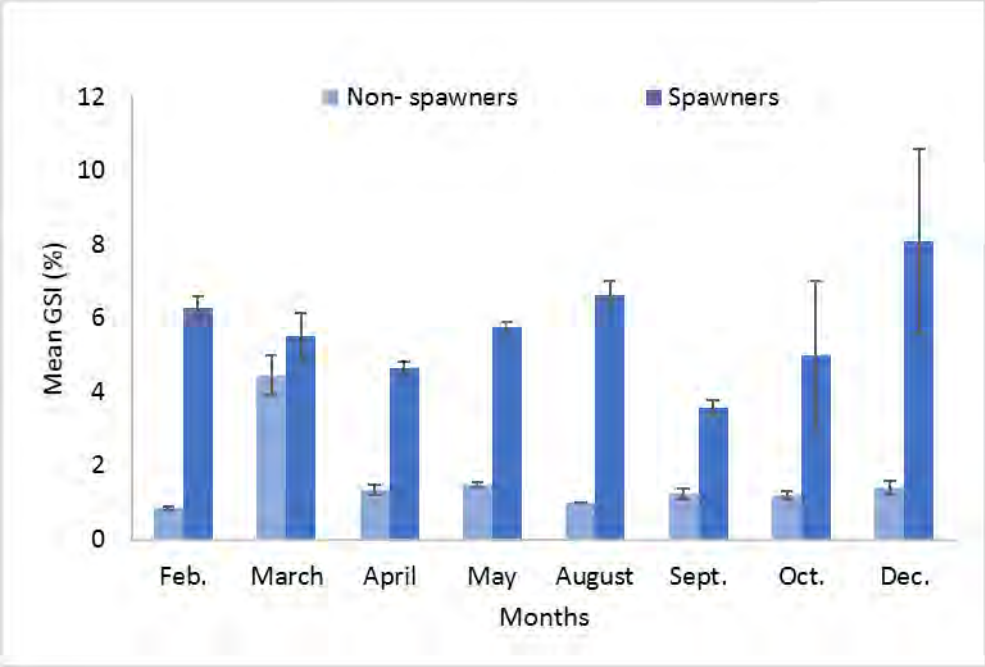



**Figure 5.3:** Study overview of gonadosomatic index (GSI) of female *Merluccius capensis* for each month of sample collection for spawners (maturity stages 3, 4, and 5) in dark blue and non-spawners (maturity stages 1, 2, and 6) in light blue. The error bars represent standard errors (SE). The data were combined for 2014 - 2016.

The GSI of spawners (stages 3, 4, and 5) was significantly lower in September as compared with all other months ($p < 0.05$), with the exception of October, with which it did not differ ($p > 0.05$), whereas for non- spawners (stages 1, 2 and 6), only the March value differed with those of other months, with higher GSI (Figure 5.3). For spawners, GSI in the month of February, August and December was high, though variation with individual months could be considerable, e.g. in December and October (Figures 5.3 and 5.4). The percentage of spawners was highest around April - May, also with high variation, though year round variation was apparent (Figure 5.4).

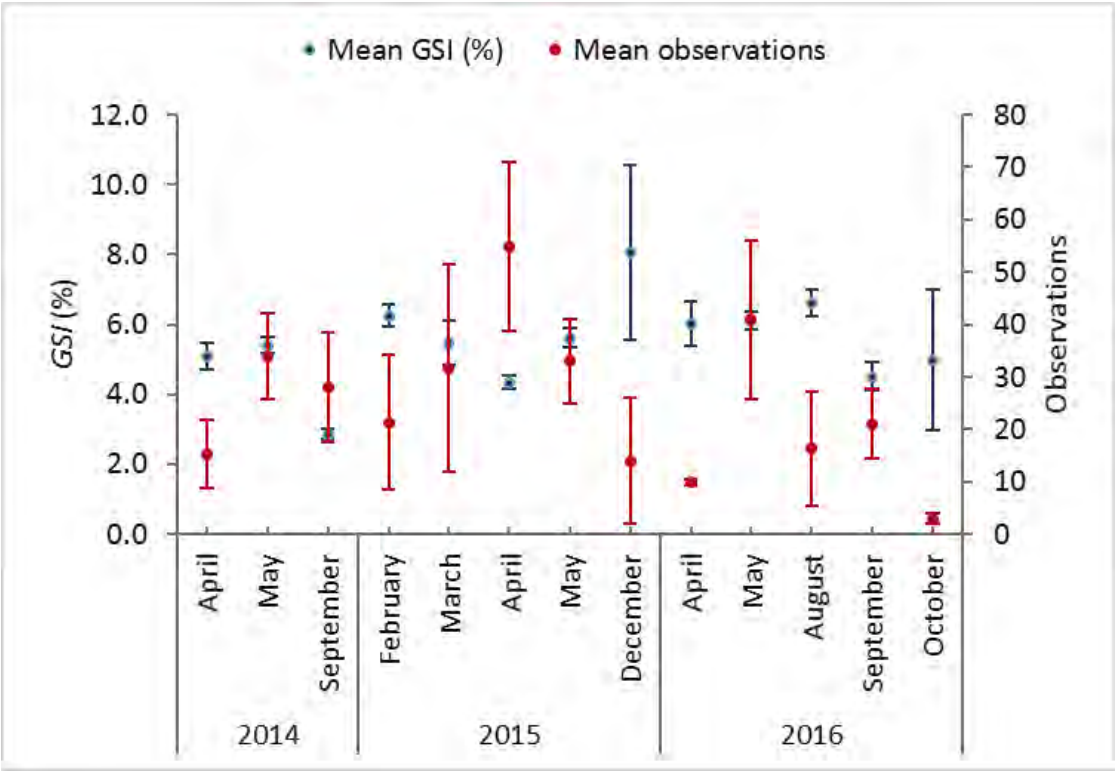
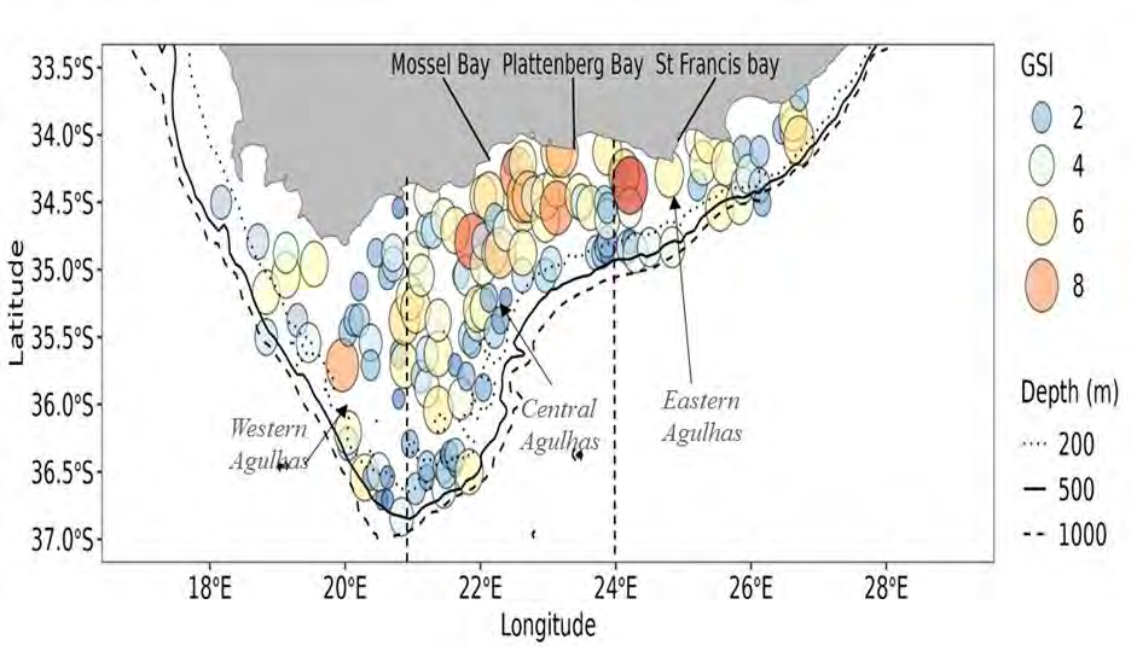



**Figure 5.4:** Temporal distribution of gonadosomatic index (GSI) of spawning female *Merluccius capensis* and the frequency of observations by study month and year (2014 - 2016)). Error bars represent the standard errors (SE).

*5.3.3 Spatial patterns in maturity development*

GSI values were highest in the central Agulhas Bank, marking that as area where most spawning occurred (*F* - test; $p < 0.05$) (Figure 5.5No samples were collected in January and November, and the gonad weights reported by the industry for June and July could currently not be verified using fishery-independent data.




2383

2384 **Figure 5.5:** Geographic distribution of gonadosomatic indices (GSI) of female *Merluccius capensis* along
2385 latitudinal and longitudinal co-ordinates of the Agulhas Bank (inclusive of the Western, Central and
2386 Eastern Agulhas Bank) of the southern coast of South Africa. GSI values are grouped into four categories:
2387 2, 4, 6 and 8 % as > 2 - 4, > 4 - 6, > 6 - 8 and > 8 %, respectively.

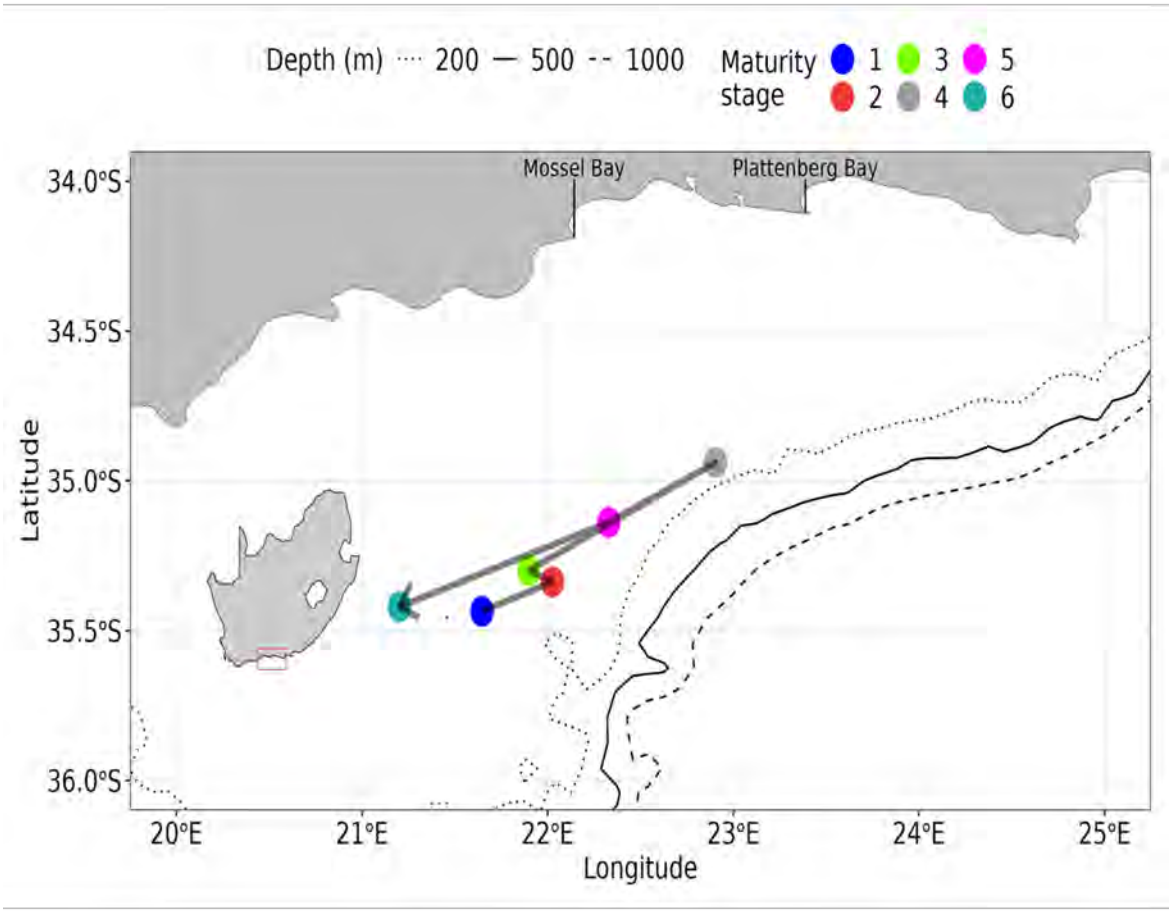
2388 *Merluccius capensis* samples could be grouped roughly as those sampled shallower and deeper than
2389 200 m (Table 5.4). The mean depths of individual fish sampled in 2014 and 2016 were shallower than
2390 200 m, except for in August 2016 (225 m), whereas the fish sampled in 2015 were all found deeper than
2391 200 m. Overall, the cumulative frequency of those located deeper than 200 m was 54 % versus 46 % for
2392 those located shallower than 200 m. Generally, *M. capensis* occupied different depths at different months
2393 (85-330 m mean depth); the *F* - test was significant at $p < 0.01$ when all the data were combined for each
2394 of the 12 calendar months.

2395 **Table 5.4:** Monthly-resolved depth occupancy of female *M. capensis* from 2014 to 2016 off the south
2396 coast of South Africa, with the number of fish observations expressed as percentage (%) of the total

sample. No data were collected in November and January. The effect of the relationship between depth occupancy and month: F (9, 1793) = 63.097, $p < 0.01$.

| Year | Month | Mean depth (m) | -0.95% | 0.95% | Fish samples at mean depth (%) |
|---|---|---|---|---|---|
| 2014 | April | 163 | 140 | 186 | 5 |
| | May | 122 | 117 | 128 | 7 |
| | September | 171 | 161 | 181 | 7 |
| 2015 | February | 208 | 199.2 | 215.4 | 7 |
| | March | 286 | 274.3 | 295.5 | 7 |
| | April | 214 | 198.4 | 229.0 | 14 |
| | May | 208 | 188.5 | 226.4 | 9 |
| | June | 313 | 303.5 | 321.1 | 3 |
| | July | 330 | 313.2 | 342.9 | 2 |
| | September | 248 | 244.6 | 250.3 | 3 |
| | December | 207 | 207.0 | 207.0 | 5 |
| 2016 | March | 169 | 142.8 | 203.5 | 2 |
| | April | 85 | 83.2 | 86.2 | 3 |
| | May | 131 | 123.8 | 138.1 | 9 |
| | August | 225 | 207.0 | 238.6 | 4 |
| | September | 136 | 123.7 | 148.8 | 8 |
| | October | 138 | 129.2 | 146.4 | 5 |

The geographic position occupied by the various maturity stages was significantly different (regression; $p<0.05$). The immature (stage 1) and regenerating stages (stage 6) were the most south - westerly and the actively spawning stage (stage 4) the most north - easterly, i.e., closest to the mainland, as illustrated for April 2014 (Figure 5.6). In addition, the regenerating stage was farthest from the ovulating stages (maturity stages 4 and 5) and also the farthest offshore (Figure 5.6). The developing (stage 2) and the spawning capable stages (stage 3) were in close proximity (Figure 5.6).




2406

2407 **Figure 5.6:** Distribution (mean position) of the different maturity stages of female *Merluccius capensis* on
2408 the central Agulhas Bank in April 2014. Maturity stage 1= immature; 2 = developing; 3 = spawning
2409 capable; 4 = actively spawning; 5 = regressing; 6 = regenerating.

2410 Consequently, based on this dataset, *M. capensis* evidently moved closer to the shore and the actively
2411 spawning stage was more northerly (mean 34.96° S) than all other stages ($p$< 0,05;Figure 5.7). The
2412 regressing stage was relatively remote from the immature, actively spawning and the regenerating stages
2413 ($p$ < 0,05; Figure 5.7), though the mean positions of other stages (immature, developing, spawning
2414 capable and the regenerating) were not significantly different from each other ($p$ > 0.05; Figures 5.7).
2415 Overall, the geographic position of the actively spawning stage differed statistically from all the others ($p$
2416 < 0,05).

2417

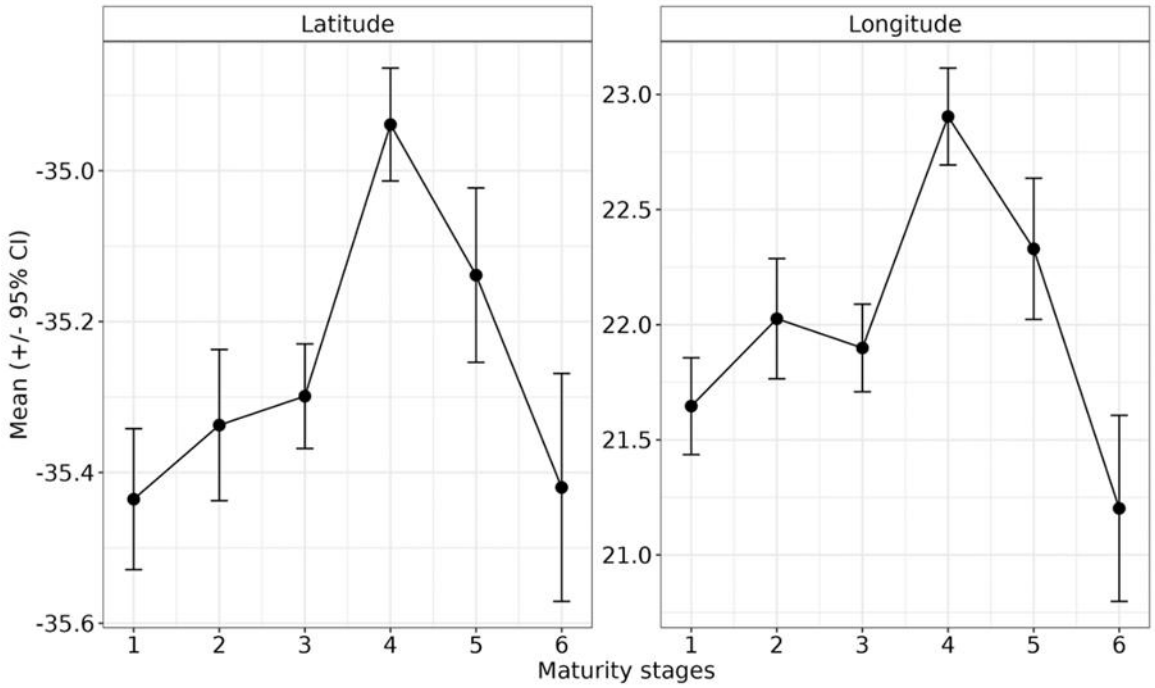



**Figure 5.7:** Mean latitudinal and longitudinal positions (with 95 % CI) of female *Merluccius capensis* of each maturity stage for the entire period of three years (2014 - 2016). For details of calculation and interpretation, refer to Materials and methods.

## 5.4 Discussion

The Agulhas Bank is an important nursery area for species that spawn on the narrow shelf further northeast (Hutchings et al. 2002), and it is also a centre of abundance of many warm-temperate species (Sink et al. 2012). The area is also essential for numerous life-cycle processes that occur on the bank (including spawning, oceanographic larval retention, recruitment, connectivity and provision of nursery and foraging areas) and is moderately productive but has locations of relatively higher productivity (Sink et al. 2012). There is a cold ridge of water that is a prominent subsurface feature during most summers on the central Agulhas Bank (Swart and Largier, 1987).

### *5.4.1 Body-size and bottom-depth related patterns in maturity development*

*Merluccius capensis* is more abundant inshore, in shallower habitats than the congeneric *M. paradoxus* (Botha 1980; Assorov and Berenbeim 1983; Gordoa et al. 1995, Wilhelm et al. 2015). In particular, *M. capensis* is primarily concentrated at depths shallower than 200 m (Botha 1985; Durholtz et al. 2015), which concurs with the results from the present study.

Some individuals with the mean size of > 67 cm were found deeper than 300 m and constituted 15 % of entire sample collection. Those fish were also at regenerating stage (Table 3) meaning that they were resting from the previous spawning cycle. The regenerating stage of *M. capensis* showed old post ovulatory follicles (POFs) - which are an indication of a completed spawning cycle, as well as atresia and the oocyte in primary growth, the latter an indication of iteroparity, i.e. an oocytic reservoir for the coming season(s) (Brown- Peterson et al. 2011; Nomxego *et al.* 2024). The large body - size category of fish that remained in the deeper water may do so due to foraging behaviour, as Botha (1985) reported that small numbers of large *M. capensis* coexisted with large numbers of small *M. paradoxus* at depths around 330 m. Such *M. capensis* actively target *M. paradoxus* as prey (Inada 1981; Payne *et al*. 1987; Traut 1996);

feeding studies on Cape hakes off South Africa have demonstrated a large proportion of *M. paradoxus* in the diet of *M. capensis* (Pillar and Wilkinson 1995; Pillar and Barange 1997).

We observed that the immature and developing stages were located shallower than 200 m depth (as also reported by Singh *et al*. 2011). The novel finding of the current study, however, was that the other maturity stages – spawning capable, actively spawning and the regressing stages– were also found at mean depth shallower than 200 m, more specifically at ≈200, 176 and 163 m depth, respectively. This observation implies that the actively spawning and the regressing stages were located significantly shallower than the other maturity stages, though close to the depth recordings given for immatures, which had a mean depth of 179 m. Collectively, the maturity stages that appeared shallower than 200 m depth constituted 93 % of the entire dataset, while the regenerating stage, at a mean depth of ≈225 m, constituted 7 %. Overall, the key result was that a convex relationship was found between maturity stage and body size (mean TL), whereas the relationship with water depth as the response variable was flatter, albeit with a decline to greater depths for those individuals in the regenerating stage.

Our results confirm the finding that *M. capensis* remain in deeper water until their gonads are mature and move to shallower depths for spawning (Garavelli et al. 2012; Jansen et al. 2016). Females apparently return to deeper waters immediately after spawning (Botha 1985), which may explan the distribution of spawning stages in the shallow waters. In this study, females in the regressing stage moved slightly shallower – in the order of 10 m – than those actively spawning. This is similar to *M. productus,* where the females remain in deeper waters until they are ready to spawn, move to the shallow water where spawning takes place, and then return to deeper water once all batches of eggs have been released and spawning is completed (Saunders and McFarlane 1997). While moving inshore to spawn (Olivar et al. 1988; Jansen et al. 2015), *M. capensis* also move upwards in the water column, above the low oxygen layer, to mate and then extrude eggs (Olivar 1990; Olivar and Shelton 1993; Mbatha et al. 2019). Agulhas Bank waters occurring north of the subtropical convergence to the east of the 18° E have a low oxygen content (<5.0 ml $l^{-1}$; Chapman 1988) and are avoided by *M. capensis* as confirmed by the

low number of samples (15 %) presently collected in this subregion. Low concentrations of oxygen on the Agulhas Bank are due to stability of the summer thermocline and stagnant water masses on the bank leading to the oxygen depletion (Chapman 1988).

*5.4.2 Temporal patterns in maturity development*

According to Botha (1985), the peak of the spawning season for *M. capensis* is in March and in September. Our data suggested the spawning season to be between April and May and in September. High concentrations of *M. capensis* were found in samples collected in shallow depths in April-May and September - October in 2014 and 2016 (though data were lacking for November and January), which supports to the existence of two main spawning seasons (Botha 1985; Jansen et al. 2015), with some annual variance. Furthermore, as is the case for *M. capensis* off Namibia, seasonal tracking of GSI indicates that the main spawning season as such is in austral spring (Kainge et al. 2007; Jansen et al. 2015). However, as is typical for indeterminate spawners, egg release may happen throughout the year (Rijnsdorp et al. 2015), as the GSI in the current study indicated year-round spawning. Numbers of the spawning females were relatively high in April, May and in September, which have been shown previously to be periods with spawning peaks (Garavelli et al. 2012; Durholtz et al. 2015).

*5.4.3 Spatial patterns in maturity development*

In the current study, *M. capensis* females remained on the southern part of the shelf prior to spawning,then moved northwards and inshore during the spawning season, and shifted south again after spawning, a distribution pattern which fits well into the earlier findings (Grote et al. 2007; Stenevik et al. 2008). Within the Central Agulhas Bank (CAB), the ready-to-spawn females showed a shift from west to east, to the area associated with the cool, productive and oceanic feature known as the cold ridge (CR) (Jacobs et al. 2022). The CR is the central feature of the CAB that occurs between November and April (Jacobs et al. 2022). Currents on the Agulhas Bank transport nutrient-rich waters westwards, leading to

large phytoplankton blooms on the CAB, with the highest productivity in the vicinity of the CR (Jacobs et al. 2022). The CR is in the area perpendicular to Mossel Bay, Plettenberg Bay and St. Francis Bay (Jacobs et al. 2022), that is, where highest GSI values for *M. capensis* were recorded in this study, indicating spawning activity. Generally, the higher the GSI, the greater the content of hydrated oocytes and the more imminent the spawning event (Jansen et al. 2015). Our study suggested that the spawning ground of *M. capensis* on the south coast is found in the CAB between 34 - 35°S and 22 - 24° E. There is evidence of an inshore-offshore spawning migration on the south coast, revealed by the large catches of hake by handline between Port Elizabeth and St Francis Bay (Durholtz et al. 2015).

Our analysis fits in with this description, where females in developing and spawning stages moved towards north - eastwards to spawn (offshore of Plattenberg Bay), then south - westwards to release the remaining egg batches (offshore of Mossel Bay), in agreement with Garavelli et al. (2012), and finally farther south-westwards to rest (regenerating stage). The spawning area according to the current study is inshore on the Agulhas Bank, in an area between Mossel Bay and Plettenberg Bay at mean depths below 200 m. Continuous batch production is evident from the presence of the hydrated oocytes, indicating that ovulation is imminent, whereas the presence of new POFs suggests that eggs have just been released (Grote et al. 2007; Stenevik et al. 2008).

## 5.5 Conclusion

This study confirms that *M. capensis* are widely but not randomly distributed off the south coast of South Africa, specifically between 34 - 35° S and 18 - 25° E. They are found in depths ranging between 50 and 400 m but mostly occur shallower than 300 m. Spawning happens year-round, but with two peaks, one around April and the other around September. The immature and developing stages occur at greater depth than the spawning stage. The spawning-capable female moves north - eastwards to shallow water to spawn and then return to south-westwards when in the regenerating stage to rest. Thus, the study provides fine scale details of the spatiotemporal distribution of M. capensis off the south coast, with statistically significant movement between depths. The findings are consistent with those of previous studies (Pillar and Wilkinson, 1995; Grote et al. 2007; Stenevik et al. 2008; Garavelli et al. 2012; Jansen et al. 2016). However, further studies are needed to investigate these movements on a larger geographical and temporal scale, including the role of biophysical factors that may be contribute towards the spatial and temporal distribution of this species.

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# Chapter 6: Conclusion- Implications for fisheries management

## 6.1 Introduction

Errors and biases as a result of poor input maturity data have already been highlighted and discussed in depth in other studies (Burton 1999; Tomkiewicz et al. 2003; Vitale et al. 2006; Honji et al. 1993; Costa 2009; Fererri 2009). Developing a more precise and accurate maturity staging tool ensures more qualitative input maturity data in stock assessment models, better input data in the Operational Management Plan (OMP), thereby improving fisheries management strategies of the *M. capensis*. In quest to complement the primary maturity staging information for the *M. capensis*, the current study has developed the maturity scale, which has six maturity stages that are validated by microscopic or histological method and the whole - mount method. The whole mount method that is operative onboard the research vessel should replace the use of the subjective macroscopic method that is currently widely used in research expeditions in the region, though highly erroneous. The maturity scale for *M. capensis* was built with the oocyte diameter thresholds for the maturity stages (immature stage (< 250 μm); developing stage (251 - 350 μm); spawning (> 351 μm, which includes spawning potential, actively spawning and regressing); regenerating (100 - 300 μm)). The maturity scale fuses together the histology and the whole mount technique so that the oocyte characteristics read through the microscope can also be quantified through the whole mount automation process. The compatibility between the microscopic or histological method and the whole mount technique was found to be above 88 %, which means either of the methods can produce rational results. *M. capensis* was re- emphasised as an inderteminate and the batch spawner as it continuously develop primary growth oocytes throughout its spawning cycle and lays its eggs in batches (Brown- Peterson et al. 2011; Nomxego et al. 2024). The current study also notes that *M. capensis* follows iteroparity strategy as it conserves its eggs for future reproductive cycle (Brown-Peterson et al. 2011). Volumetric formulae were designed in the current study as an alternative to calculate batch fecundity (BF) and the results suggested that the mean relative fecundity (RF) was 283

eggs per gram per ovary and the mean BF found was comparable with other studies. Calculation of BF was found to be reasonable and compatible with other formulae used (Osborne et al. 1999; Kainge et al. 2007) when the vitellogenic oocytes (VTO) were used as opposed to the hydrated oocytes (HO) that are commonly used, though VTO fall on oocyte developmental stage prior to actively spawning stage. The latter resulted to the current study concluding that focusing on less advanced oocyte developmental stage than the most advanced stages like the hydrated oocytes, could also help determine the number of eggs shed in an event, which concurs with other authours (Serrat et al. 2019; Anderson et al. 2020).

The current study notes that the total fish weight used for this investigation was ungutted as opposed to gutted weight. The use of the gutted weight eliminates the biasness in results that may be caused by the stomach weight and content (Marteinsdottir and Begg, 2002). However, the latter is noted as a point of improvement for further studies. The power regression results for the length and weight (L/W) relationship indicated above 92 % correlation ($r^2$) as a result one variable could be used to estimate the other. The length - weight relationships indicated a negative allometric growth pattern for other periods as the overall slope was $b < 3$, meaning that the fish were more elongated and more slender, though for the year 2016, fish with total length beyond 60 cm, had a positive allometric growth pattern ($b > 3$), meaning that they were larger and more stout and round. However, the growth pattern results were not significantly different between the years (2014 - 2016). Condition of the fish was estimated with two parameters, the relative condition (k) and the Fulton's condition (K) and the stock in the south coast was deemed to be healthy and normal as the latter was $k = 1$ while the other less than 1 ($K = 0.8$). The study also noted that the data was not enough to draw further inferences about the stock in the south coast as the samples only covered three years (2014 - 2016).

*Merluccius capensis* spawns all year round in the south coast with two peaks, one in austral autumn and the other in austral spring. The gonadosomatic index (GSI) of the actively spawning stage was above 10 % while that of the combined spawning stages (3, 4 & 5) was > 7 %. This species is widely distributed off the south coast and is found between 34 -35° S and 18 - 25° E. They occupy the mean depths between

50 and 400m, though they are mainly below 300m mean depth. The current study recorded that > 75 % female samples were found in less than 200 m mean depth. In terms of the spatial and temporal distribution, the study suggests that immature and the developing maturity stages occur at greater depths as compared to the spawning stages. Furthermore, the spawning potential stage moves to north - eastwards to the shallow water to spawn (as the actively spawning stage), slightly moves to shed the last batches of eggs (as the regression stage) and return south - westwards to rest as the regenerating stage. This study updates the biological information of *M. capensis* off the south coast of South Africa, which should serve as the benefit to fisheries management in obtaining biological reference points that are more useful for future inferences.

### 6.2 Constructive summary

In the current study biological data through research expeditions had been gathered, processed in the laboratories, analysed and interpreted, thereby producing information which can be trusted towards making inferences about the shallow water hake estimations. Certainly, the level of accuracy diminishes when the estimations, instead of the raw biological information, are used in numerous models, one advancing the other, to create further inferences about the population (Murawski et al. 2001). Maturity scale inferences are known to be inaccurate when only the macroscopic method is used, and to be specific, the studies (Osborne et al. 1999; Kainge, 2002; Osborne 2004; Kainge et al. 2007) of *M. capensis* have made it clear that errors with macroscopic method were beyond 40 %. The current study has also shown this discrepancy by 57 % inaccuracy. Furthermore, there was 100% macroscopic maturity staging error for the developing stage (stage two) in 2019 data and the same for the regenerating stage (stage six) in data collected between 2014 - 2016. It is therefore not in line with the principles and methods of the established scientific knowledge to include the on- board the vessel erroneous macroscopic maturity scale readings, together with the invalidated maturity stages that are based on the latter macroscopic readings in fish stocks management models (e.g. OMP). Upon inaccurate reading of the maturity stages by macroscopic method, determination of the mature versus immature (maturity

2728 ogives) follows, which cannot help, but to be erroneous being affected by the first step. The percentage
2729 of the immature versus matured population (maturity ogives) is determined by the size (length was used
2730 in this study) in *M.capensis* and $L_{50}$ was overestimated by macroscopic method by the tune of 1 % (38
2731 versus 39 cm), however, $L_{75}$ and $L_{95}$ were way underestimated by 60%. The lack of scientific research
2732 skill regarding the microscopic reading and interpretation of the histologically produced slides, leads to
2733 lack of understanding of the contents of the ovaries and the type and quality of different organelles that
2734 marks or the determining structures and characteristics identified for that particular maturity stage. For
2735 instance, microscopic features that determine the immature stage are different from those of the
2736 developing stage (Costa et al. 2009; Brown – Peterson et al. 2011), hence they are named as such. The
2737 same happens for the developing and the spawning potential stages, they are both matured stages, but
2738 the developing stage marks the beginning of the matured stages. Therefore, it is not scientifically sound
2739 to input data in the OMP that marks the developing stage as the immature stage, wherein the spawning
2740 potential stage marks the beginning of the matured stages. Spurious information regarding $L_{50}$ results to
2741 further erroneous estimations, which cannot be trusted. In the current study, errors are minimised by the
2742 microscopic or histological assessment, which validated macroscopic results, however, the method is
2743 highly laborious and time consuming. The agreement between the macroscopic and microscopic
2744 methods was about 40 %, while it was above 60 % for the microscopic and the whole mount technique,
2745 which then allows the replacement of the macroscopic method with the whole mount technique. The
2746 current study comes with an option of determining the primary information regarding the reproductive
2747 potential of the species through the whole mount reading of the oocytes while on-board (after Kjesbu et
2748 al. 2001), so as to at least determine the immature versus mature more accurately.

## 6.3 Implications to fisheries management

One of the main purposes of stock assessment is to evaluate the spawning stock size in order to conserve sufficient reproductive potential to allow for sustainable exploitation (Marshall et al. 1998; Morgan, 2009). Spawning stock size in Cape Hake populations is indicated by the spawning stock biomass (SSB). However, evidence is growing that the SSB is a poor measure for the reproductive potential of exploited marine fish stock (Trippel et al. 1997; Marshall et al. 1998; Marshall and Frank, 1999). SSB is a short term indicator of spawning stock size, which estimates the magnitude of spawning in the next spawning season, but it does not give the exact estimate as it has been observed that some mature fish do not spawn annually or their fecundity is extremely low due to unfavourable environmental and physiological conditions (Burton, 1999; Kraus, 2002; Saborido - Rey et al. 2004). On the other hand, factors such as spawning experience, sex –ratio, growth, or offspring survival also determine reproductive success and future recruitment (Marteinsdottir and Begg, 2002; O'Brian, 2003; Morgan and Brattey, 2005).

Because SSB cannot account for the long-term reproductive ability of the stock, fisheries management, Trippel (1999) emphasized the importance of integrating basic reproductive biology such as spawners' ages, size, maturation, condition and reproductive history into stock assessment, and introduced the new term of Stock Reproductive Potential (SRP) that "represents the annual variation in a stock's availability to produce viable eggs and larvae that may eventually recruit to adult population of fishery". SRP is beyond the SSB, as there is evidence indicating that SSB may not be directly proportional to reproductive potential (Marteinsdottir and Begg, 2002; Marshall et al. 2003). Besides, not only it is necessary to take into account spawners' reproductive characteristics, but also their spatial –temporal variation (Morgan and Brattey, 2005; O'Brian, 2006).

According to (Lambert 2008), one possible outcome of measuring the reproductive potential is the calculation of the intrinsic rate of population increase (r), which is an essential parameter in population dynamics and evolutionary ecology, which can be used in determining harvesting, resilience, potential

rates of population recovery. Meanwhile, Murua et al. 2003, writes that fecundity is one of the most important determinants of a stock reproductive potential (SRP). Findings of this study enhance and improve our understanding of the spawning dynamics of *M. capensis* at species level, but also provide updated information for fishery stock assessment and resource management for facilitation of harvest conditions. This study presents improved pragmatic methods, which provide information of better quality and thereby offering potential solutions to stock assessment and fisheries management of *M. capensis* in South African coastal water.

In consequence, stock-recruitment relationship normally improved when the SRP index is used instead of SSB (Wigley, 1999; Marshall et al. 2003; O'Brian, 2003; Tomkiewicz et al. 2003). Moreover, the reproductive potential can be used to evaluate the long- term reproductive ability of the standing stock, considering the biological parameters and the age composition. The current study has solved finer problems that were not necessarily spoken about and or indicated due to them being primary information in nature and due to lack of scientific research and skill to support the novel truth.

The main focus of the current study was to appraise reproductive information of the *M. capensis* and the innovative techniques of whole mount used to assess the maturity ogives and automation to develop the maturity scale, are of international standard though they are also used by other authors (Kjesbu, 1991; Thorsen and Kjesbu 2001; Kjesbu et al. 2003; Anderson et al. 2020, Nomxego et al. 2024). The use of the whole mount technique, especially onboard the research vessel can somehow eliminate the use of the erroneous macroscopic assessment that when continuously used, it negatively impacts on the further models that use such primary data for stock estimations. The whole mount automation provides an alternative to the use of the laborious histological methods in identifying maturity stages. The estimation of batch fecundity using volumetric formulae (BF-VF) calculated from the volume of the VTO, instead of the HO is also an innovative and modern method that is internationally used (Thorsen and Kjesbu 2001; Korta et al. 2010; Kjesbu et al. 2011; Saber et al. 2015; ; Serrat et al. 2019; Anderson et al. 2020; dos Santos Schmidt et al. 2021). However, further collection of matured female gonads within the 48 – 72

hour - period, could assist in investigations of the spawning frequency, thereby estimations of annual fecundity for *M. capensis*.

### 6.4 Concluding remarks

The use of biological information in setting reference points for any biological estimations result in better precision and thereby improving fisheries management. Gathering biological data from the field, process, analyse and interpret it towards making inferences about the entire population should be more encouraged than the development of models that use numbers to estimate the wild population, especially when the methods are proven to be flawed by other scientific studies (Burton, 1999; Morgan, 2009). It is imperative to note that the field of fisheries research has two very different approaches with two very different conclusions: biological research on the ground (mainly research surveys) which point out to a moderately good state of the resource (*M. capensis*); and modelling, strictly stock assessment approach with OMP, which points out to unhealthy state of the same resource. It is envisaged that the OMP could use more biological information so as to improve precision, while more improved models and biological research is necessary to enhance reconciliation of the two popular views.

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