Investigating the biological parameters of Two-spot red snapper (*Lutjanus bohar*) on Mahé Plateau, Seychelles

photograph by Gianemilio Rusconi (2013), from Fishbase

Prepared by
Fisheries Research Department
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Table of content

Abstract 4

Acknowledgements 5

Introduction..... 6 - 8

- Background..... 6
- Species profile ..... 7 - 8
- Aim and objectives..... 8

Methodology..... 9 - 11

- Study area..... 9
- Sampling methodology..... 10
- Data analysis ..... 10 – 12

Results..... 13 - 19

- Length and Weight statistics of *L. bohar* (overall & by sex) ..... 13 - 14
- Length frequency distribution (overall & by sex) ..... 14 -15
- Weight frequency distribution (overall & by sex) ..... 15 -16
- Length - weight relationship (overall & by sex) ..... 16 - 17
- Size at sexual maturity (female only) ..... 18 -19
- Gonadosomatic Index (female only) 19

Discussion ..... 19 – 23

Conclusion..... 23

References..... 24 - 28

Appendices..... 29 - 36

List of Figures

Figure 1: Two-spot red snapper (*Lutjanus bohar)*..... 8
Figure 2: The Seychelles Bank/Mahé plateau..... 9
Figure 3: Sex distribution of *L. bohar*(n=170) 13
Figure 4: Overall size frequency distribution of *L. bohar (*n=170) 14
Figure 5: Size frequency distribution of Male *L. bohar* (n=75) 15
Figure 6: Size frequency distribution of Female *L. bohar* (n=84).....15
Figure 7: Overall weight frequency distribution of *L. bohar* (n=170) 15
Figure 8: Weight frequency distribution of Male *L. bohar* (n=75) 16
Figure 9: Weight frequency distribution of Female *L. bohar* (n=84) 16
Figure 10: Overall Length-weight linear regression of *L. bohar* (n=170) 16
Figure 11. Length-weight linear regression of Female *L. bohar* (n=84) 17
Figure 12: Length-weight linear regression of Male *L. bohar* (n=75) 17
Figure 13: Female *L. bohar* length at maturity (Lm50) logistic curve (n=84) 18
Figure 14: Gonadosomatic indices Female *L. bohar* (n=84) 19

List of Tables

Table 1: Summary of descriptive statistics of overall length and weight of *L. bohar.*..... 13
Table 2: Summary of descriptive statistics of length and weight of *L. bohar* by sex..... 14

List of Appendices

Appendix 1: Macroscopic differences between Male and Female gonads..... 30
Appendix 2: Paraffin Histology processes..... 31
Appendix 3: Female and Male gonad transection macroscopic identification..... 32
Appendix 4: Female teleost gonad development stages..... 33
Appendix 5: Summary of length-weight relationship -overall..... 34
Appendix 6: Summary of Length-Weight relationship of Females *L. bohar* only..... 34
Appendix 7: Summary of Length-Weight relationship of Males *L. bohar* only..... 34
Appendix 8: Comparison with other studies on *L. bohar* size at maturity..... 35

Abstract

Lutjanus bohar (locally known as Var vara) is a popular species caught as part of the demersal fishery in the Seychelles. The aim of the study was to determine the biological parameters of *Lutjanus bohar* (Two-spot red snapper) caught on the Mahé plateau to support the implementation of phase two of the co-management plan. In essence, it aimed at giving a better understanding of the size at which the fish is considered as matured and hence has the potential to contribute towards recruitment of the population. A total of 170 fish was sampled between July 2022 and December 2023, with their gonads macroscopically and microscopically identified, of which there were 84 females, 75 males and 11 of unknown sex. The sex ratio was 0.89:1 (M: F). The females ranged between 30 - 78 cm in total length and in fork length between 28 - 75 cm, while the male ranged between 30 - 82.5 cm total length and in fork length 28-78 cm. The overall length-weight relationship gave a r-squared value (r2) of 0.98 indicating a strong correlation between the length and the weight. An overall b-value (interception) of 3.63 was obtained, as the same was obtained for the sex specific relationship (Male with a b value= 3.620, r^2 =0.99 and female with b value of 3.917, r^2 =0.94) showing a positive allometric growth which means that the fish have relatively deeper bodies as it increases in length. This indicates that the fish is in good health and has access to enough food in its environment. The size at maturity was modelled and it was found to be 43.4cm (FL) for females. The GSI described spawning peaks throughout February to May, August, September and November, though some uncertainties in the data due to lack of consistently, available monthly samples.

Key words: Seychelles • size at maturity • reproduction • growth • length-weight relationship • Lutjanus bohar

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Introduction

Background

The artisanal fishery in the Seychelles is crucial to the food security and economic development of the nation, particularly through employment and export (Williams et al., 2017 & Christ et al., 2020). The main fishing area supporting this fishery is the Mahé plateau, which sustains an array of fish species from various families ranging from snappers, emperors, groupers, rabbitfish and many others (SFA, 2019). However, with technological development and modernization in this sector, there has been increasing fishing pressure in this area (Friedlander & DeMartini ,2002), resulting in a reduction in the catch and with smaller individuals of certain target species (Hilborn et al., 2020). Another factor challenging the fisheries is varying degrees of exploitation which can alter the size at maturity of fish species (Jørgensen et al., 2008 & Sharpe & Hendry, 2009).

Hence, there has been a need for management intervention to ensure sustainability of this resource (SFA, 2019). All this led to the initiation and development of the Mahé Plateau trap and line fishery co-management plan for the demersal resources for this particular area, a process spearheaded by stakeholders in 2019 and was regulated early in 2020. This plan provides a pathway for the phased introduction of a series of management measures to rebuild and maintain healthy fish stocks (SFA, 2019). The co-management plan is integrated as part of an even bigger initiative, the Seychelles Marine Spatial Plan (SMSP). With the Marine Spatial Planning process, 30% of the Seychelles' territorial waters and Exclusive Economic Zone (EEZ) has been designated as marine protected areas, with these areas further divided into 3 main zones. The focus of the co-management plan is on Zone 2 (sustainable use zone) and Zone 3 (multiple use zone) which will enable certain level of economic activity and resource extraction within the EEZ. In support and to initiate the implementation of phase 2 of the co-management plan and with the aim of moving from zoning to implementation of the Marine Spatial plan, this study under the Ocean 5 project aim to provide critical, baseline information about the biological parameters, most specifically the size of maturity of Two-spot red snapper for the introduction of minimum landing size as one of the management measures.

Species profile

Two-spot red snapper (*Lutjanus bohar*) commonly known as Var vara in Seychelles (Figure 1) is a commercially important demersal fish species of the Seychelles' artisanal fishery. Two-spot red snapper is one of the 125 species in the Lutjanidae family (Nelson et al., 2016). It is characterized as a long-lived reef fish reaching around 60 cm in fork length, with a maximum estimated aged of more than 55 years old (Marriott et al., 2007). This species is slow-growing, with late maturity making them have a K-selected life history (Marriott et al., 2007), which makes them vulnerable to overfishing (Fortaleza & Nañola, 2017). Snappers in general are popular commercial fish in the tropical and subtropical seas and are large components of the landings (Grandcourt et al., 2008). They are commonly found on coral reef habitat in depth range from 10 – 70 m but they can be found up to 180m (Fishbase, n.d. -a). *Lutjanus bohar* is a large predatory reef fish (Miller & Cribb, 2007) and their diet consists of small fish, crustaceans, cephalopods and molluscs (Fishes of australia, nd), amphipods, stomapods, gastropods and urochordates (Fishbase,n.d.-a).

In the Seychelles, *L. bohar* comprises a major proportion of the total catch of the snapper family and is one of the main snappers caught by local fishers for consumption (Marriott et al., 2007). However, in certain region such as on the Great Barrier Reef, Australia, historically this species has been avoided due to its toxicity in causing ciguatera poisoning (Marriott et al., 2007). Research about the life history (e.g. maturity and age) of Two- spot red snapper is well known and more detailed studies on population biology has been undertaken in other tropical countries (Marriott & Mapstone, 2006, Marriott et al., 2007, Fortaleza & Nañola, 2017 & Ong et al., 2017).

Being one of the prevalent species of demersal fishery, there was a need to determine its biological parameters, more specifically the size at maturity to provide information necessary to improve stock assessments (Grandcourt, 2005) and management measures.

Figure 1: Two-spot red snapper (*Lutjanus bohar*). *Photograph by Pascal Irz(2009), from Fishbase*

Aim and Objectives

The aim of this project was to determine the biological parameters of Two-spot red snapper (*Lutjanus bohar*) caught on the Mahé plateau to support the implementation of phase two of the co-management plan, with the introduction of minimum landing size for certain demersal species.

The specific objectives were to:

i. Determine sex-specific growth parameter (length and weight frequency)
ii. Determine sex-specific size at sexual maturity
iii. Determine reproductive periodicity

Methodology

Study area

Two-spot red snapper was collected from the Seychelles Bank, also known as the Mahé Plateau (Figure 2) which is located in the South-west Indian Ocean. The plateau has a surface area of 41, 338 km^2 and surrounded by an incomplete shallow rim of 0 – 20 meters. Granite and coral outcrops form small banks with a maximum depth reaching 65m (MRAG, 1996).

Figure 2: The Seychelles Bank/ Mahé Plateau. *Photo credit of FBOA: Seychelles Fishing Boat Owners Association*

Sampling Methodology

Samples were collected from the artisanal commercial fishery by purchasing whole, ungutted fish from catch landings with local fishers around Mahé island. Mahe, is the main island and the largest granite island on the Mahe plateau (Figure 2). The landing sites included Aux Cap,

Victoria old fishing port and Victoria market. Additionally, fish was also sought according to a size class distribution. The sampling took place from July 2022 to December 2023 and this included biometric measurements, fork length, total length and weight (whole and eviscerated) and the measurements were recorded to the nearest centimetre and nearest kilogramme respectively. The fish was also sexed (male and female) macroscopically (using visual analysis) (Appendix 1) and the gonads were weighed (to the nearest gramme). A cross section of the gonads was collected or the whole gonads for some individuals and these were preserved in 10% formalin for further paraffin embedded histological processes (Appendix 2) at SFA laboratory. A transection of the gonads was fixed on microscope slides for further reading under the light microscope, support by an identification guide. This facilitated confirmation of the sexes (Appendix 3) and the stages of development of the female gonad could also be deduced (Abdel-Baki et al., 2015) (Appendix 4). The slides were then sent to external experts at CSIRO, Australia for validation.

Data analysis

The data collected was analysed using Microsoft excel (Windows 10) and R programme software 4.4.0. A significant threshold of $p > 0.05$ with 95% confidence interval was applied.

A. Sex ratio

The sex ratio is an important measure in the relevance of the sex composition in population dynamics, specifically regarding reproductive potential of a given species. Sex ratios represent the number of males to females in a sample. In sexually reproducing biological populations, the sex ratio tends to be 1:1 with equivalent numbers of males and females. The ratio is given as (M: F) and calculated using the following equation: The males count divided by the female count.

$$\boldsymbol{Sex\ ratio} = \frac{Total\ number\ of\ males}{Total\ number\ of\ females}$$

The chi-square (χ^2) was used to verify the existence of significant differences between the sex ratio of the study species and commonly expected 1:1 sex ratio.

B. Length-weight relationship

Length-weight relationship is an important aspect of the biology of any fish, from which the physical wellbeing of a fish can be ascertained for a given body at a given time. The length and weight vary depending on reproductive cycles and environmental factors, availability of foods, the age and the physiological state of the fish (Asres et al., 2023; Islam et al., 2002).

The formula for the relationship is given as: $W = aL^b$

W = total weight (g),
L = total length of fish(cm)
a= intercept (initial rate of growth or condition factor; rate of change of weight)
b= exponent weight at unit length (slope; coefficient of growth or relative growth rate)

(*b = 3, isometric growth; when b < 3, negative allometric growth and when b > 3, positive allometric growth*) (Fafioye & Ayodele, 2018)

C. Size frequency distribution

A statistical representation of the distribution of sizes within a sample. It illustrates the range of sizes amongst individuals sampled from the population.

D. Weight frequency distribution

A statistical representation of distribution of weight within a sample. Weight frequency is used to understand the variability of distribution amongst the individuals in a dataset.

E. Length at maturity (Lm_{50})

The proportion of matured gonad of stage III to stage V of fish sampled was used to deduce the length at which 50% of the sample is maturate. The principle of length at 50% of sexual maturity for fish population is mostly used in fish stock management. The 50% threshold in Lm50 signifies a pivotal point in the life history of the population (Asres et al., 2023). It is an

important parameter of life history where there are significant implications for population dynamics, reproductive ecology, and ecosystem functioning (Reed et al.,2023).

The formula is as follows:

$Pm = 1 \times 100 / 1 + e^{(-aL+b)}$

Pm= Proportion of mature (%)

L=length in cm

a=fitted constants

b=fitted constants

(Asres et al., 2023)

F. Gonadosomatic index (GSI)

Gonadosomatic Index (GSI) is the ratio between the gonad weight and the total weight of mature individuals, and it is used to describe the reproductive periodicity. The formula is given as:

$$GSI = \frac{Gonad\ weight, g}{Total\ weight, g}\ x\ 100$$

Results

At the end of the sampling programme, a total of 170 individuals were sampled. This comprised of 84 females (49.4%), 75 males (44.1%) and 11 categorised as unknown (6.5%), (Figure 3).

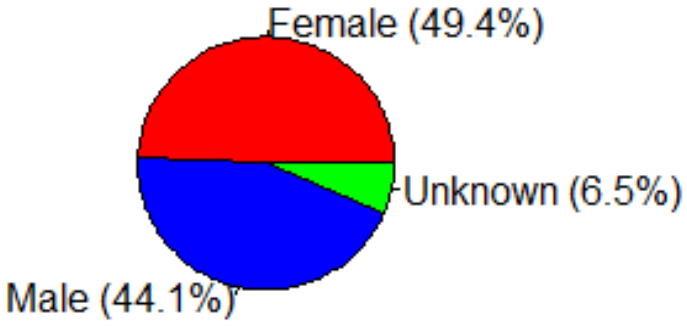


Figure 3: Sex distribution of *L. bohar* sampled (n=170)

The sex ratio was 0.89:1 (M: F). The outcome of the chi-squared (χ^2) test revealed a squared value of 0.403 and p value of 0.526 (df = 1). Therefore, there was no statistically significant difference between female and male among the samples.

Length and Weight statistics of *L. bohar* (overall & by sex)

In terms of length, on average the *Lutjanus bohar* sampled were 52.5cm in TL and 49.7cm in FL. There was slightly difference in the range, between 30 cm to 82.5 cm for TL and 28-78 cm for the FL. As for the weights, the mean weight of the individuals sampled were 3.11 kg, with a range of 0.42 kg to 10.51 kg (Table 1).

Table 1: Summary of descriptive statistics of overall length and weight of *L. bohar*

Statistical analysis	**Total length overall (cm)**	**Fork length overall (cm)**	**Weight overall (kg)**
Mean	52.5	49.7	3.117
Median	51.2	47.4	2.095
Standard deviation	15.1	14.3	2.496
Female length range	30 - 78	28-75	0.43 - 8.33
Male length range	30 - 82.5	28-78	0.42 - 10.51

The comparison of total length, fork length, and weight between male and female is presented in Table 2. The males tend to be slightly longer and heavier than the females, in both length and weight. The males measured 54.57 cm in TL and 51.4 cm in FL, compared to the females which were 53.46 cm in TL and 50.6 cm in FL. In terms of weight, on average males weighed 3.48 kg and the females weighed 3.11kg (Table 2).

Table 2: Summary of descriptive statistics for length and weight of *L. bohar* by sex

	Total Length (cm)	Fork Length (cm)	Weight (Kg)
Male			
Mean	54.57	51.4	3.48
Median	51.5	48.5	2.36
Standard deviation	15.21	14.4	2.71
Male length range	30 – 82.5	28 - 78	0.42 – 10.51
Female			
Mean	53.46	50.6	3.11
Median	53.5	50.25	2.39
Standard deviation	14.12	13.7	2.28
Female length range	30 -78	28 - 75	0.43 – 8.33

Length frequency distribution (overall & by sex)

The length frequency histograms were plotted for all the Two-spot red snapper sampled, as well as by sex using the fork length data only. Overall, the highest proportion of the samples were in the 35-40 cm range (Figure 4).

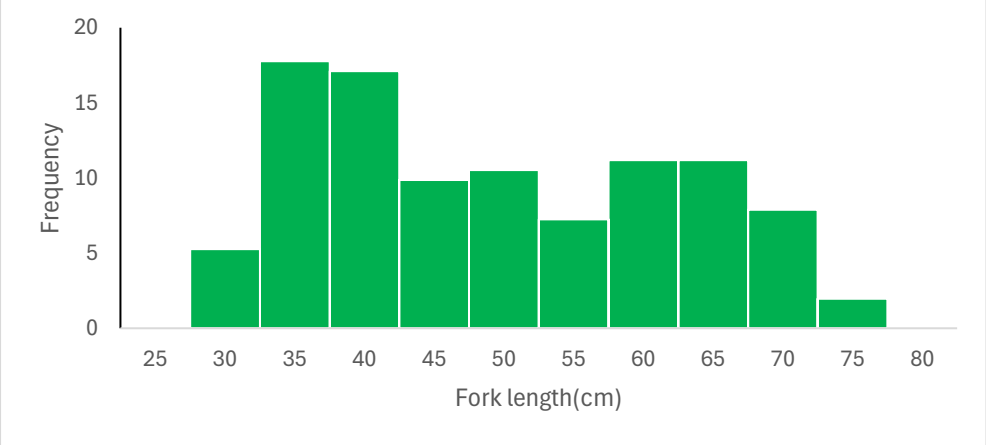


Figure 4: Overall size frequency distribution of *L. bohar* (n=170)

The male length distribution showed that the highest proportion of males were 40 cm, representing 18% of the total males (Figure 5). As for the females, they had a slightly different distribution, with most of the females measuring 35cm, representing 17% of the total females (Figure 6). In comparing males and females, a more even distribution was observed for females compared to males. The Wilcoxon test was carried out and there were no significant differences.

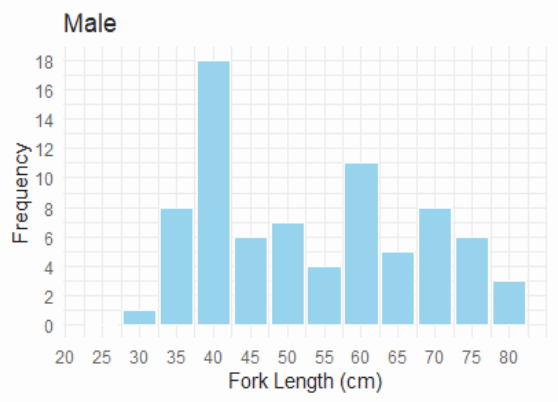


Figure 5: Size frequency distribution of Male *L. bohar* (n=75)

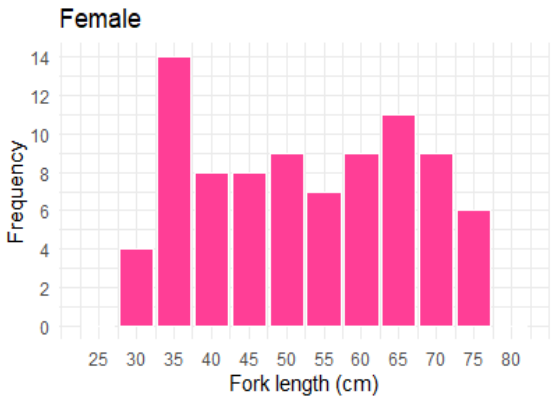


Figure 6: Size frequency distribution of Female *L. bohar* (n=84)

Weight frequency distribution (overall & by sex)

Weight frequencies were plotted for all individuals and by the sexes. Overall, the weight of the samples ranged between 1 to 9 kg, with most of the samples in 1.5- 2 kg extent. Fewer fish weighed more than 7kg (Figure 7).

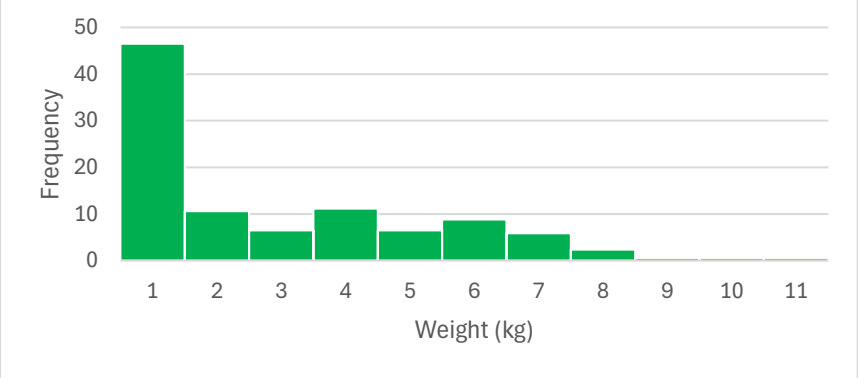


Figure 7: Overall weight frequency distribution of *L. bohar* (n= 170)

In terms of the sex specific weights, the highest proportion of males were in 1 and 2 kg range, representing 16% of the total number of males (Figure 8). As for the females, the majority were smaller than 5 kg, with most in the 1 kg class, which accounted for 20% of the total number of females (Figure 9). The variation in the weights between the sexes was not significant (Wilcoxon test; p>0.05).

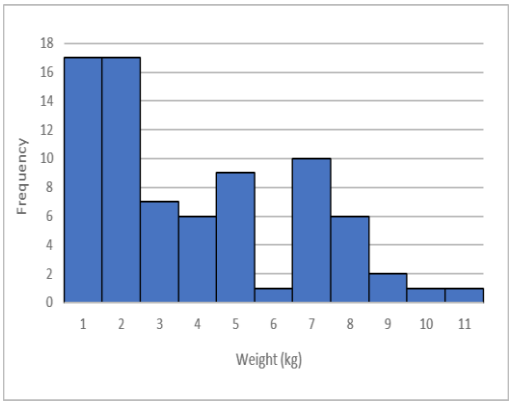


Figure 8. Weight frequency distribution of Male *L. bohar* (n= 75)

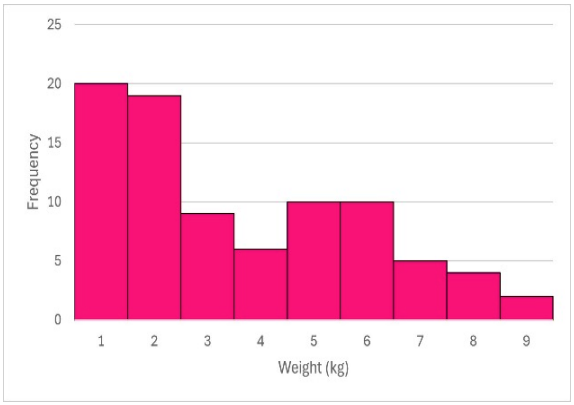


Figure 9. Weight frequency distribution of Female *L. bohar* (n=84)

Length-weight relationship (overall & by sex)

The length-weight regression showed positive correlation between the length and the weight. The strength of the correlation is deduced from the R-squared, and a value of $r^2 = 0.98$ was obtained, hence indicating a strong correlation. A b-value (intercept) of 3.63 was attained (Appendix 5), which indicated a positive allometric growth. This means that the weight increases more with increase in length (Figure 10).

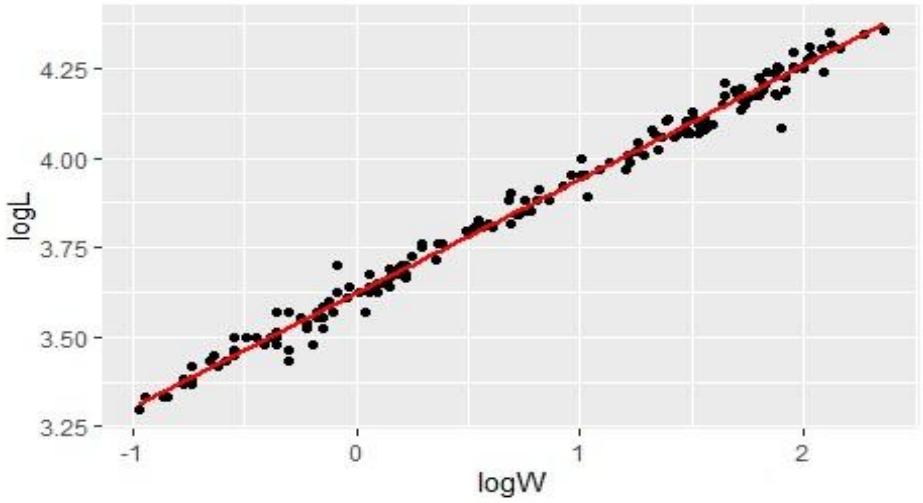


Figure 10: Overall length-weight regression of *L. bohar* (n= 170)

A slightly stronger correlation was observed in the female length-weight relationship compared to the males. A R-squared (r^2) value of 0.99 and a b-value (intercept) of 3.620 was obtained (Appendix 6; Figure 11) for the females and a R-squared (r^2) value of 0.94 and the b-value of 3.617(Appendix 7; Figure 12) for the males. Both results revealing a positive allometric growth.

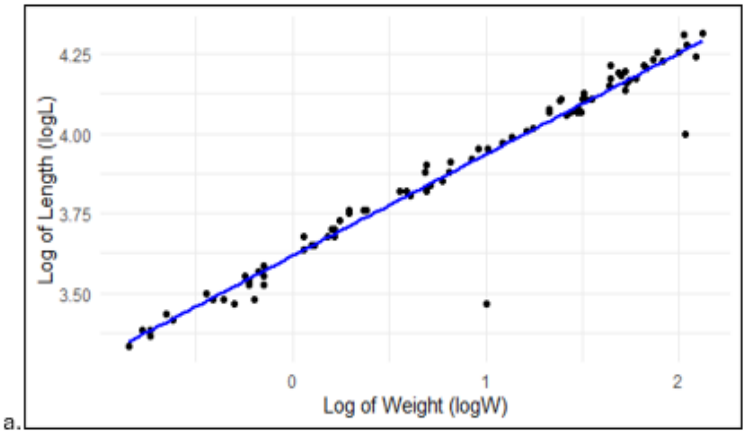


Figure 11: Length-weight regression of Female *L. bohar* (n= 84)

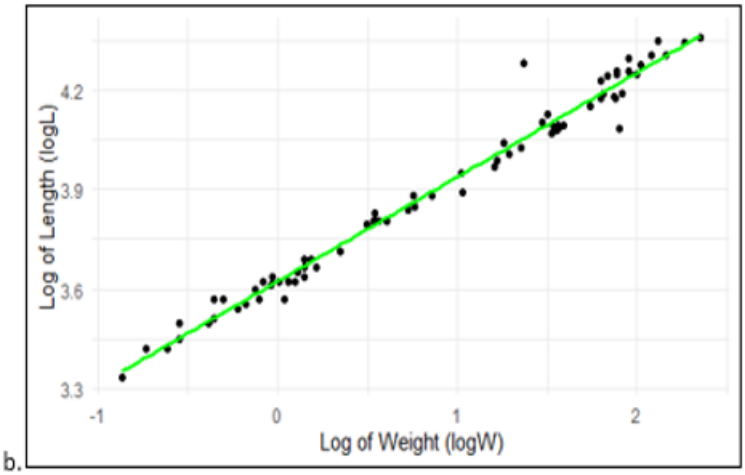
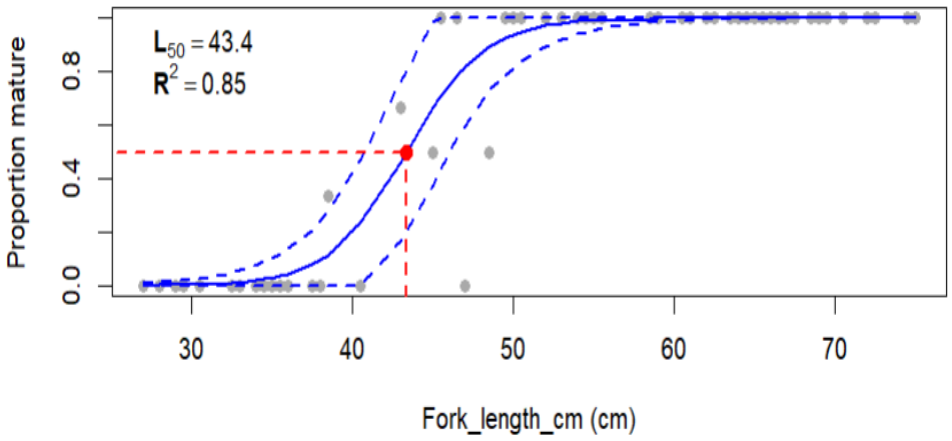


Figure 12: Length-weight regression of Male *L. bohar* (n= 75)

Size at sexual maturity (female)

The logistic curve showed the relative observed and estimated curve of proportion of mature females, in fork length in cm. Size at maturity of 50% of the female within the sample of *L. bohar* was investigated using the fork length and it was found to be 43.4 cm (Figure 13). The female length at maturity had an r^2 value 0.85, in such case the model provides an overall good fit to the data.



Size at maturity	43.4 cm
Confidence intervals	40.8 - 46
R-square	0.85

Figure 13: Female *L. bohar* length at maturity (Lm 50) logistic curve

Gonadosomatic index (female only)

The Gonadosomatic index (GSI) showed differences in the reproductive pattern during the sampling periods of 2022 to 2023. A higher GSI value indicated pre-spawning and spawning phase where the gonad of the female fish enters in the mature phase therefore increasing in size. As per Figure 14, the GSI graph showed peaks in February to May, September and November. The highest GSI recorded was 3.7 in May. The lowest GSI recorded was in December with a value of 0.065. However, it should be noted that for certain months over the two-year sampling, no samples were recorded (such as January) or the number of samples was low (e.g. in June and October and December). Hence with a lack of consistent monthly sample, the result should be taken with caution.

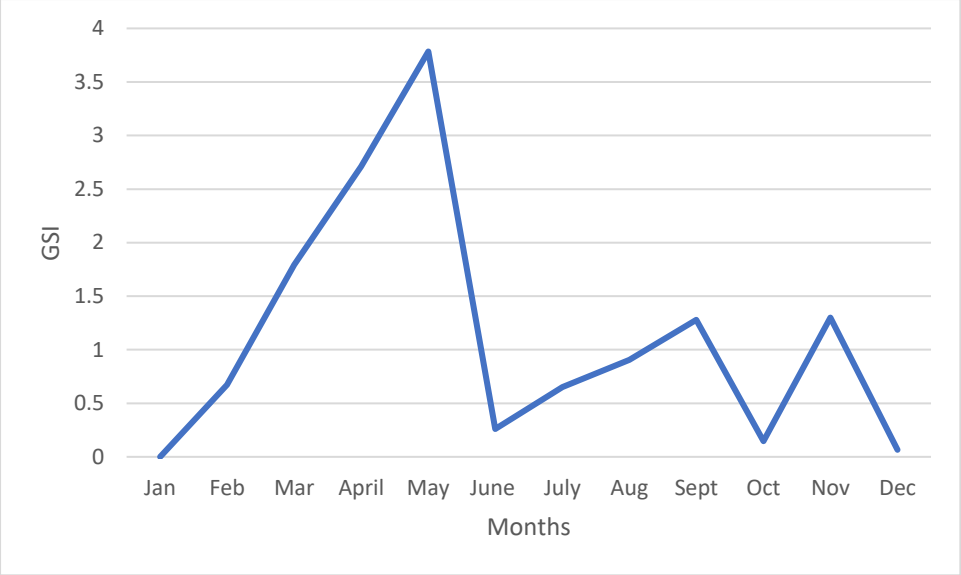


Figure 14: Gonadosomatic indices female *L. bohar* (GSI) (n=84)

Discussion

Overall, the study provided insights of some key parameters of *Lutjanus bohar* in terms of growth and reproductive biology, specifically through the size at maturity, length-weight relationship and spawning periodicity. The biometric analysis of the *Lutjanus bohar* gives information on the growth characteristics of the fish, the general health, food availability, seasonality, sex, habitat condition etc., as it would establish a benchmark for future pattern to be observed in the species stock. (Jisr et al, 2018).

Lutjanus bohar is a K-strategist species implying that they are long-lived, with delayed maturity, having a complex life history and reproductive strategies, longevity of survival and they tend to compete in stable environments (Marriott et al., 2006). K-strategist are vulnerable to excess of fishing pressure (Parent and Schriml 1995, Jennings et al., 1998; Musick, 1999). *Lutjanus bohar* have deep bodies, with slow growth rate and a long-life span, hence they can accommodate larger organs or reserves in support of reproductive strategies. The positive allometric growth indicates that this species is healthy, which may be due to stable environments where resources are used effectively.

Research on Lutjanus family have previously been undertaken in the Seychelles; Wheeler & Ommanney (1953) investigated age and growth of some Lutjanus species to determine population trend and size at maturity. This study deduced that the Lm_{50} of *Lutjanus bohar* was 51 cm. Marriott et al., (2007) investigated the length-at-age of *Lutjanus bohar* in Seychelles and a comparison was made to that from the Great barrier reef Australia. This kind of studies provides evidence-based information required to support the formulation of management strategies of these resources, to ensure its sustainability (Marriott et al., 2007).

A determined sex ratio of 0.89:1 (0.89 male for every female), conformed to the expected 1:1 sex ratio in the population. This implies that the population is in a state which can uphold the required reproduction capability. In terms of size range, it was found that the range of length for both sexes were comparable to other past studies undertaken in the Indo-Pacific region. For instance, study on *Lutjanus bohar* by Talbot (1960) recorded a size range of 42.5 cm – 66cm, as for Wright, Dalzell, & Richards (1986), it was noted that the length ranged from 20.3 cm – 61 cm, in Marriott (2002), the samples were between 30 cm – 70 cm, Marriott et al., (2007) recorded length between 10 cm to 70 cm, in Fortaleza & Nañola (2017) reported a range of 13.4 – 30-4 cm, Elamin, Elamin & Ali (2020) recorded a range of 29 cm -70cm and lastly, the samples from the Bailey (2021) study was between 14.5 cm - 70 cm.

In terms of weight, a similar observation could be made with other studies, with a recorded range of 1-11kg, with most being less than 7 kg. In other past studies., Marriott (2005) recorded a weight range of 0.2 Kg – 6 kg, as for Fortaleza & Nañola (2017), a range of 0.42kg-4.60 kg was obtained, Elamin, Elamin & Ali (2020) noted samples between 2 kg to 18kg range and lastly Bailey (2021) recorded a range of 0.3 kg- 7kg.

There are various factors which may influence the size of a fish and this include food availability (Bailey, 2021.), habitat, sea conditions, maturity phase ,behaviour (MRAG 1995; Marriott & Mapstone 2006; Piñón, Amezcua & Duncan, 2009),as well as environmental factors (Elamin, Elamin & Ali ,2020) and anthropogenic factors such as fishing pressure (Fortaleza & Nañola,2017).

Growth parameters are mainly based on age and length and are important to fish stock assessments and therefore management of fisheries (Gwinn et al., 2010). Length-weight relationship analysis provides a growth model from which the growth pattern can be

determined (Huxley, 1924). The outcome of the analysis from this study found that *L. bohar* had a positive allometric growth pattern, indicating that the weight of the fish increases more with increase in length. A positive allometric growth pattern indicates good conditions for growth (Luklhurst 2000; Newman & Dunk 2003). In addition, a strong coefficient of determination (r^2) of 0.9896 was attained, indicating a strong relationship and a good fit to the model. However, though the model has a practical use in fisheries biology, it does have some limitations such as factors that cannot be incorporated such homogeneity whereby all individuals adhere to this relationship regardless of age, sex, condition (Jisr et al., 2018). With better sample size, a wider range of lengths could be integrated and hence strengthening the accuracy of the model.

Another key component to fisheries reproductive biology is the size at sexual maturity. It plays a crucial role in understanding the life history of fish species and health of the stock, particularly those with commercial importance. The length at maturity (Lm50) is a vital metric in determining suitable fishing sizes, serving as a critical indicator for effective fish stock management (Sadovy, 1996). Changes in the length-at-maturity (Lm50) can signal the effects of fishing pressure (Reed et al.,2023).

Specifically, Lm50 refers to the length at which 50% of individuals are considered to have reached sexual maturity, a commonly used estimate that reflects the mean value within the population (Sadovy, 1996). For the sex-specific size at maturity, female was recorded a Lm50 of 43.4 cm, with a r^2 of 0.85, whilst for the males it was 60.1, with a r2 of 0.33. The females had a better fit to the model than the males.

In comparison with previous studies undertaken on the *L. bohar* species, it was determined that the length at maturity in the Indo-Pacific region varied across locations. Research conducted by Wheeler and Ommaney (1953) in Seychelles found that the Lm50 for females was 51 cm (length measurement unspecified). Talbot (1960) reported an Lm50 of 44.5 cm (SL) in East Africa, while Wright et al., 1986 found it to be 45.0 cm (FL) in Papua New Guinea. In Australia, Marriott et al (2007) reported an Lm50 of 42.8 cm (FL) of the species (Appendix 8).

However, there were certain limitations to this analysis such as the total monthly sample which influenced the rigorosity of the model. Also, fish populations can exhibit temporal variability in growth rates and maturation schedules due to differed environmental factors

and changes, varying degree of fishing pressures and so on. The models might not account for these changes effectively, hence leading to less applicable results (Soares et al., 2020). Therefore, the model should be used along with other information for decision making.

Sexual maturity can also be determined using the reproductive periodicity (Soares et al., 2020) as it is the seasonal trend in the gonad development over the year (Marriot et al., 2007). From the study, elevated GSI peaks were noted for May, September and November and this aligns with the conclusion of other studies regarding multiple spawning patterns exhibited by Lutjanid species (Freire et al., 2002). In comparison with other studies conducted in Seychelles by Grandcourt et al., 2008, it was found that the spawning season of *Lutjanus sebae* spanned from February to April and September to October. As in Marriott et al. (2007) a study of *Lutjanus bohar* of Australia, it was observed that spawning took place from August to April. However, the result from our study should be interpreted with caution as samples were not available or adequate throughout all the months of the sampling period.

From this study, several limitations were encountered and this included:

- Issues with attaining samples from the various sizes range
- The availability of fish within the sampling period, particularly during the Southeast Monsoon.
- The disbursement of funds was also a challenge, particularly with the amount of funds which was made available at one given time. It should be noted that this amount was shared between three other fish species, hence there was a major limitation on the number of fish that could be purchased at one given time.
- Delays were also encountered with the disbursement and acquiring the funds on time.
- Logistical setback in terms of transportation to the various landing sites. Additionally, with our work schedule, most fish were purchased within the vicinity of Victoria and opportunistically on other sites.

Recommendations

In terms of lessons learnt from the implementation of this project and its associated outcomes, it is recommended that there is:

- Better sensitization and awareness raising to fishers prior to undertaking such research to encourage their engagement and participation in the sampling programme.
- More collaboration and better communication with fishers' association which may lead to more proactiveness for different fishing communities
- Capacity building in microscopic gonad staging, to build confidence in interpreting the data.
- Sampling period to be extended beyond one year to ensure samples are obtained for all months of the year.
- To focus on sampling at the two ends of the distribution (smaller and larger samples)
- Incorporate non-reproductive factors relative to stock. These can include diet, environmental factors and habitat.

Conclusion

This study provided baseline information on the biological parameters of the locally commercial Two-spot red snapper, which will help support the implementation of phase two of the Mahé Plateau co-management plan. The study was undertaken over the 2022 and 2023 period and the results included length at maturity (Lm50) of 43.4 cm, spawning periodicity to be mainly during May, September and December. Lastly, in terms of the fitness of the fish samples, they demonstrated a positive allometric state (b= 3.62).

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Appendices

Appendix 1: Macroscopic differences between Male and Female gonads

Macroscopic gonad stage	Female Description	Male Visual observation
Immature	Ovaries pinkish, translucent. Eggs (oocytes) not visible to naked eye	Testis whitish. Thin thread-like
Developing	Wider Gonad. Pinkish to yellow-green colour; Blood vessel more apparent. Eggs not visible to naked eye	Testis whitish, symmetrical. Enlargement of testis becoming rounded and whitish-grey. Generally thin and smooth testicular wall
Spawning capable/Ripe	Larger oocyte, ovary pinkish to yellow green color with granular appearance. Not transparent eggs and blood vessel are visible	Testis larger and sperm often visible in sinuses. Exudes milt with light pressure on the abdomen
Active spawning/ Spent	Flaccid occupying less than 1/3 of body cavity. Slight brown and orange with heavy discharge of blood from the blood vessels	Testis bloodshot and flabby. Testis flaccid and thick walled, generally bloody red in colour
Regression	Flaccid oocyte. Small and broad ovaries, slightly brown colour, blood vessels less prominent	
Regenerating	Small ovaries blood vessels reduced but present	

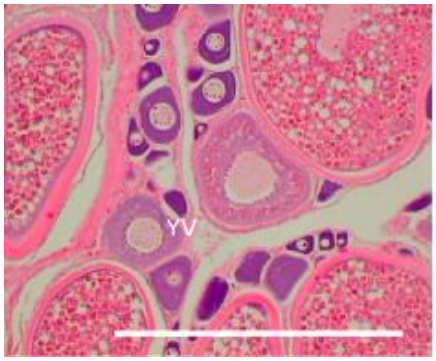
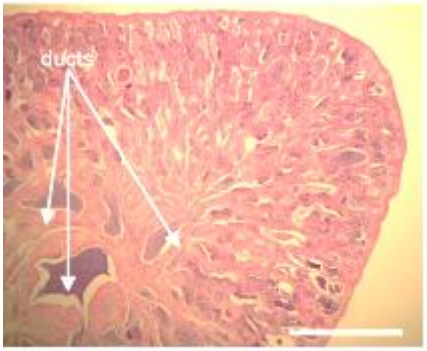
(Robinson et al., 2007) (modified from de Souza 1988; Ntiba and Jaccarini 1990; Samoilys and Roelofs 2000; West 1990)

Appendix 2: Paraffin Histology processes

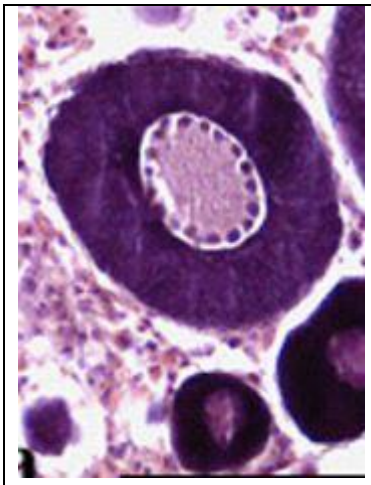
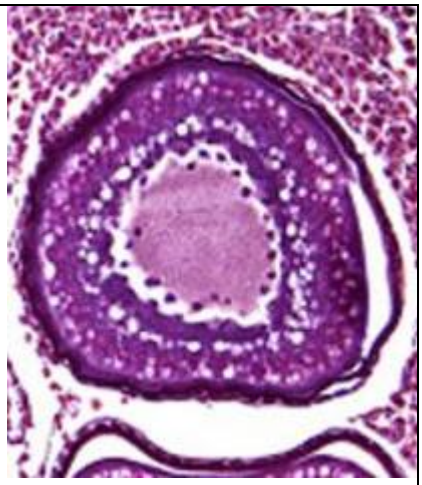
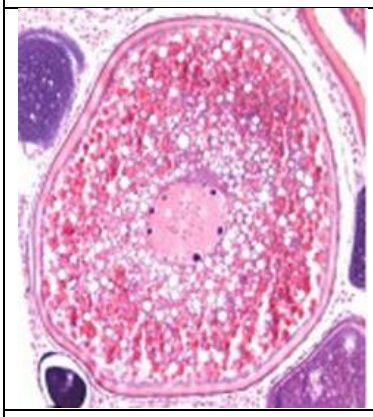
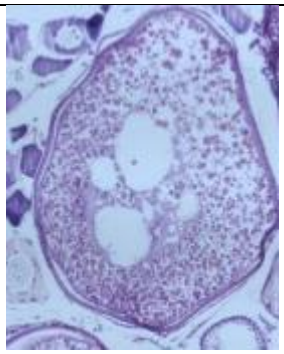
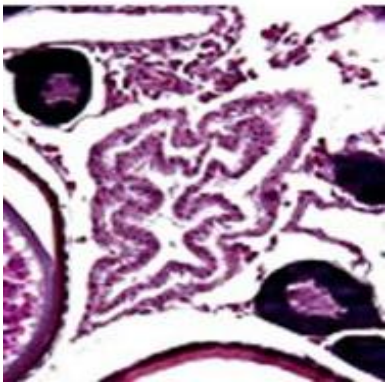
Tissue Fixation – Gonad was placed to set/fix in a 10 % formalin solution. Fixation allows for preservation of the tissue structures
Tissue processing - Dehydration – This process carried out by immersing specimens in a series of ethanol solutions of increasing concentrations, then in a xylene solvent then liquid paraffin. This process allows the tissue to be fixed to maintain its structure for the rest of the process. The cassettes are then removed after 16 hours (overnight).
Embedding – Wax is placed in a wax embedding machine a day prior turned on and left to melt. For the embedding, the cassette is opened and the cross section is placed in a mould. The mould is filled with liquefied paraffin, and the cassette is placed into it. The paraffin in filled to topped then let the paraffin solidify on the cold plate or on the side of the wax machine. The mould with the cross section is then removed and cleaned reading for sectioning.
Tissue sectioning - The blocks are mounted on a microtome. The paraffin block containing the gonad is first trim 10 µm thickness to remove some paraffin and better access the embedded gonad, then cut at a 5 µm thick. The resulting ribbons is placed in waterbath (40° c). A microscope slide is used to scoop the ribbon and adjusted to slide. The slides are left dry overnight. The slides are then collected in a slide rack.
Tissue staining – The slide rack was taken to the staining station. The gonad slides go through a cycle of submersion in various chemical 1. Ethanol 2. Ethanol 3. Tap or Distilled water 4.Hematoxylin 5. Tap or Distilled water 6. Acid alcohol (0.5 %) 7. Distilled water 8. Eosin 9.Ethanol 10. Ethanol 11. Xylene 12. Xylene
Slide coverslip mounting – The slides in the slide rack are kept in xylene taken to the fume hood. Using a pipette place Eukitt glue was placed on the cover slip. Add a few drops of xylene on the cover slip then slowly placed in a horizontal manner on the slide. Carefully adjust the cover slip and the slide using a tweezer, press the cover slip to stick without bubbles and uneven glue (this is to prevent distorting the gonad cross section). Allow slides to set for 1-2 days after which reading can be done on the slides.

(Balette, 2023 & Wijayanti et al., 2017)

Appendix 3: Female and Male gonad transection microscopic identification

1  	1 
Microscopic view of a Female gonad (© Marriott et al., 2007)	Microscopic view of Male gonad (© Marriott et al., 2007)

Appendix 4: Female teleost fish gonad development stages

		
1)Immature Oocytes are small, irregular shaped but circular nucleus and prominent pale circular nucleus containing multiple nucleoli. Nucleus occupies less 30% of cell space. The zona radiata is absent	1) Immature Thicker gonad wall than immature females, no evidence of prior spawning (see below). Tightly packed previtellogenic oocytes throughout lamellae	2) Maturing (Developing) Cell outline/oocytes are smooth. Moderately-dark-staining cytoplasm contain many pale lipid vesicles. Nucleus become irregularly shaped.
		
3) Ripe (non-spawning) Cell with uniform vitellin globules which stain moderately with toluidine blue/purple. Globules initially appear at the oocyte periphery then distribute evenly throughout cytoplasm. Cytoplasm lighter than previous stages. Zona radiata and follicular layer prominent.	4) Spent (spawning) Cells double-in-size when compared to previous stage. Nucleus indistinct or absent. Vitellin coalesces into larger globules of different sizes or forming uniformly staining "sheets" within cytoplasm. Lipid vesicles(non-staining) also coalesce into larger droplets. Cells shape becoming irregular. In late maturation zona radiata thin and pull away from follicle.	5) Regressing Only oogonia and PG oocytes present, muscle bundle, enlarged blood vessels, thick ovary wall, gamma/ delta atresia and post-ovulatory follicles present in ovary indicate recent spawning

(Brown-Peterson et al., 2011, Longenecker et al., 2020 and Yamaguchi et al., 2006)

Appendix 5: Summary of Length-Weight relationship overall

Residuals	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-0.142033	-0.01606	-0.00078	0.018913	0.105347
Coefficients:	Est. Std Error	t-value	t-value	Pr (>|t|)	
(Intercept)	3.622539	0.003003	1206.5	<2e-16 ***	
LBLW_fishdata$logW	0.318817	0.002518	126.6	<2e-16 ***	
Significant codes	0	0.001	0.01	0.05	0.1
Residual standard error	0.03023	168 df			
Multiple R-squared	0.9896	Adjusted R-squared		0.9896	
F-statistic	1.60E+04	on 1 & 168 DF		p-value :<2.2e-16	

Appendix 6: Summary of Length-Weight relationship of Females

Residuals	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-0.075118	-0.015478	0.001025	0.016588	0.05535
Coefficients: (Intercept)	Est. Std Error 3.620965	t-value 0.003782	t-value 957.3	Pr (>|t|) <2e-16 ***	
LBLW_fishdata$logW	0.325552	0.003195	101.9	<2e-16 ***	
Significant codes	0	0.001	0.01	0.05	0.1
Residual standard error	0.02527	82 df			
Multiple R-squared	0.9922	Adjusted R-squared		0.9921	
F-statistic	10380	on 1 & 82 DF		p-value :<2.2e-16	

Appendix 7: Summary of Length-Weight relationship of Males

Residuals	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-0.46988	-0.00723	0.00786	0.02521	0.07021
Coefficients: (Intercept)	Est. Std Error 3.617067	t-value 0.009796	t-value 369.25	Pr (>|t|) <2e-16 ***	
LBLW_fishdata$logW	0.318349	0.008274	38.48	<2e-16 ***	
Significant codes	0	0.001	0.01	0.05	0.1
Residual standard error	0.06544	82 df			
Multiple R-squared	0.9475	Adjusted R-squared		0.9469	
F-statistic	1480	on 1 & 82 DF		p-value :<2.2e-16	

Appendix 8: Comparison of other studies on Lutjanus bohar size at maturity

Author	Year	Country	Region	Species	Sample size	Size at maturity
Wright et al.	1986	Island Papua New Guinea	Indo-pacific	*Lutjanus bohar*	283 (159 (F)/123 (M)	45 cm
Marriott	2005	Great Barrier reef	Indo-pacific	*Lutjanus bohar*	308	42.8 cm (F) 30.0 cm (M)
Fortaleza & Mañola	2017	Davao Gulf, Philippines	Indo-pacific	*Lutjanus bohar*	50 (21 F/ 29 M)	20.8 cm
Talbot	1960	Lamu, Kenya and South west	Indo-pacific	*Lutjanus bohar*	854	44.5cm (F)27.0 cm (M)
Fishbase (n.d, -b)	1995 - 2003	Australia	Indo-pacific	*Lutjanus bohar*		42.9 cm (F)