SEYCHELLES CONSERVATION AND CLIMATE ADAPTATION TRUST
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"Enabling the Seychelles Marine Spatial Plan"

Determining Biological Parameters of Brownspotted grouper (*E. Chlorostigma) and* White-blotched grouper (*E. Multinotatus*): A Contribution to Sustainable Fishery Management in Seychelles

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Abstract

The Brownspotted grouper (*Epinephelus chlorostigma*) and White-blotched grouper (*Epinephelus multinotatus*) are two commercially targeted species in the Seychelles demersal fishery, yet information regarding their populations remains limited. This study explored the biological parameters of Brownspotted and White Blotched grouper samples focusing on sex ratio, gonad staging, the Length-Weight (LW) relationship, Gonadosomatic Index (GSI) trends, and Size at Maturity. The study aimed to address the same objectives for both species; however, differences in sample sizes led to some analyses being omitted for the White-blotched grouper, resulting in different outcomes. A total of 307 Brownspotted groupers were sampled, with the heaviest female caught weighing 1.20 kg and measuring 43.5 cm (TL). In contrast, the sample of White-blotched groupers consisted of 48 fish, with the heaviest female weighing 7.14 kg and measuring 74 cm in TL. The LW relationship of Brownspotted groupers indicated positive allometric growth, meaning that weight increases slightly faster than length as the fish grows. Seasonal variations in GSI values were observed, with peaks in March, August, and November, and observed decrease in April and September. The estimated size at maturity (L50) for Brownspotted groupers was approximately 23 cm, although caution should be taken in interpretating the result due to sample composition variability. Overall, this research underscores the complexities and variations in biological parameters among grouper species.

1. Background

The artisanal fishery in Seychelles predominantly operates in the inshore coastal waters of the main granitic inner islands (Glass et al., 2022) and focuses on capturing demersal and semi-pelagic species (Robinson, 2021) such as barracuda *(Sphyraenidae)*, snappers *(Lutjanidae),* groupers *(Serranidae)*, and trevallies *(Carangidae)*, among others (Glass et al., 2022). Artisanal fishers in Seychelles use a range of fishing gears, most commonly handlines and nets (Robinson, 2021). Each gear type differs in terms of the species targeted, selectivity, and the sizes of the catches.

Over time, the artisanal fishery in Seychelles has undergone significant growth, marked by an increase in fleet size and fishing activities. This expansion has contributed to declines in stocks of some targeted species, compounded by the effects of climate change (Robinson, 2021). However, species such as the Emperor Red Snapper *(L. sebae)*, Green Jobfish *(A. virescens)*, and trevallies, which experienced reduced catches since 2010, have shown signs of recovery, though they still require ongoing attention (Robinson, 2021).

Evidently, the artisanal fishery plays a crucial role in ensuring food security and supporting economic growth in Seychelles (SFA, 2020; Robinson & Shroff, 2020). Given the significant socio-economic value of fish resources to the Seychellois people, management strategies under the Mahé Plateau Trap and Line Fishery Co-Management Plan have been developed to promote the sustainable utilization of commercially targeted species.

The co-management plan was developed in collaboration with stakeholders and aims to implement management interventions in phases (Phase 1 and 2) to maintain and restore healthy demersal fish populations. To bolster the development and execution of the plan, the Seychelles Fisheries Authority (SFA), partnered with the Seychelles Conservation and Climate Adaptation Trust (SeyCCAT), to undertake the OCEANS5 project, aimed at determining the mean size at maturity (L50) for three key demersal species: Brownspotted grouper (*Epinephelus chlorostigma*), White-blotched grouper (*Epinephelus multinotatus*) and Twospot red snapper *(Lutjanus Bohar)*, for the implementation of minimum landing size as one of the management measures (SFA, 2019).

Understanding the specific growth progression of a species is a fundamental process that influences productivity and population dynamics. Therefore, gathering biological information on life history traits is crucial for conducting fish stock assessments and in providing scientific advice to inform management decision-making (Kadir et al., 2023). The species under investigation in this report include the Brownspotted grouper (*Epinephelus chlorostigma*) and White blotched grouper (*Epinephelus multinotatus*). The report begins with an introduction to the sampled species, followed by a description of the sampling methodology and data analysis. It then presents the results on the biological parameters of both species, ultimately leading to the conclusion and recommendations for the study. The overarching goal of the project was to determine fundamental baseline data on the growth and reproductive parameters of the Brownspotted grouper *(E. chlorostigma)* and White-blotched grouper (*E. multinotatus).* This information will support the implementation of minimum catch sizes as a management strategy of the Mahé plateau co- management plan.

The specific objectives of the study were to:

1. Determine sex-specific growth parameters.
2. Determine the size at sexual maturity.
3. Determine reproductive periodicity.

1.1. Detailed Characteristics of the Brownspotted and White-blotched grouper.

The **Brownspotted grouper** *(E. chlorostigma)*, known locally as "Vyey Makonde," and the **White-blotched grouper** *(E. multinotatus)*, known as "Vyey Plat," are both members of the Serranidae family.

Both species inhabit coral reefs, rocky areas, and seagrass beds in tropical and subtropical waters throughout the Indo-Pacific, including the Maldives, Seychelles, Great Barrier Reef, and the Philippines (Allen & Erdmann, 2012; Froese & Pauly, 2024), though at different depths. The Brownspotted grouper is usually found on the Mahé Plateau at depths between 40 and 60 meters, while the White-blotched grouper is more commonly found at depths ranging from 1 to 110 meters (Nevill, 2023).

The Brownspotted grouper (Fig. 1) has a brownish body with dark spots, distinct markings on its head and fins, and a white line on the back edge of its caudal fin (Allen & Erdmann, 2012; Froese & Pauly, 2022; Nevill, 2023). It is a small to medium-sized fish with a slightly curved space between its eyes and can grow up to 80 cm TL. It has a protruding jaw with 2-4 rows of teeth, 11 dorsal spines (with the 3rd or 4th spine longest), and slightly longer pectoral fins than pelvic fins. The caudal fin is either straight or slightly indented (Froese & Pauly, 2022; Nevill, 2023).

It is often confused with the Areolate grouper *(Epinephelus areolatus)* due to similar spots and tail fins (Nevill, 2023). However, the Brownspotted grouper typically has an extra fin ray on the dorsal and pectoral fins, and its spots are smaller, more abundant, and darker. It is also often mistaken for the multi-spotted grouper *(Epinephelus polylepis)* and the Small-scale grouper *(Epinephelus gabriellae)* (Chatla et al., 2020; Nevill, 2023).

The Brownspotted grouper is a protogynous hermaphrodite, starting life as a female and later transitioning to a male, though not all females undergo this change (Allen & Erdmann, 2012; Froese & Pauly, 2024). During the breeding season, it forms spawning aggregations (Froese & Pauly, 2024). Females reach sexual maturity at 23-29 cm TL and change sex around 34 cm TL, while males become sexually active at about 37 cm TL (Fennessy et al., 2018; Moussac, 1996; Heemstra & Randall, 1993). Regional variations exist, females in the Seychelles mature at up to 31 cm TL (Fennessy et al., 2018; Moussac, 1996), while in Kenya, they mature at about 25 cm SL (Fennessy et al., 2018; Sanders et al., 1988).

Figure 1: A photograph of a Brownspotted grouper *(E. Chlorostigma)*. Source: Nevill (2023).

The **White-blotched grouper** can grow up to 100 cm in length and is known for its irregular white blotches and spots on its body. Its color ranges from grey, dark grey, brown, to purplish grey. The grouper has a slightly curved head, and its upper jawbone reaches near the back of its eye (Nevill, 2023). It also has two rows of teeth in the middle of its lower jaw and sub-angular preopercle. The caudal fin is either straight or slightly indented, like the Brownspotted grouper (Froese & Pauly, 2022; Nevill, 2023). In Seychelles, the White-blotched grouper shares the same Creole name as the Blue-and-yellow grouper (*Epinephelus flavocaeruleus)* (which is deep blue) and the Wavy-lined grouper *(Epinephelus undulosus)* (which can be misidentified as its color fades after capture) (Nevill, 2023).

The White-blotched grouper is also a protogynous hermaphrodite and forms spawning aggregations during its breeding season. It reaches sexual maturity between 32 and 34 cm in length (Mahe et al., 2022). Groupers have varying growth rates depending on their location, which is important to consider when studying their development (Schemmel & Dahl, 2023; Mun et al., 2019).

Figure 2: A photograph of a White- blotched grouper *(E. multinotatus).* Source: Nevill (2023).

2. Materials and Methods

2.1. Study Area.

The biological sampling of Brownspotted and White-blotched groupers was conducted over 22 months, from March 2022 to December 2023. Sampling occurred on Mahé Island, situated on the Seychelles Bank (also known as the Mahé Plateau) in the Southwest Indian Ocean (Fig 3). Fish were sourced from both the inner and outer Seychelles islands, as fishers typically operate in these areas. These regions, with their extensive shallow waters, coral reefs, and diverse underwater formations, offer optimal habitats for various fish species (Lea et al., 2016; Collier & Humber, 2012).

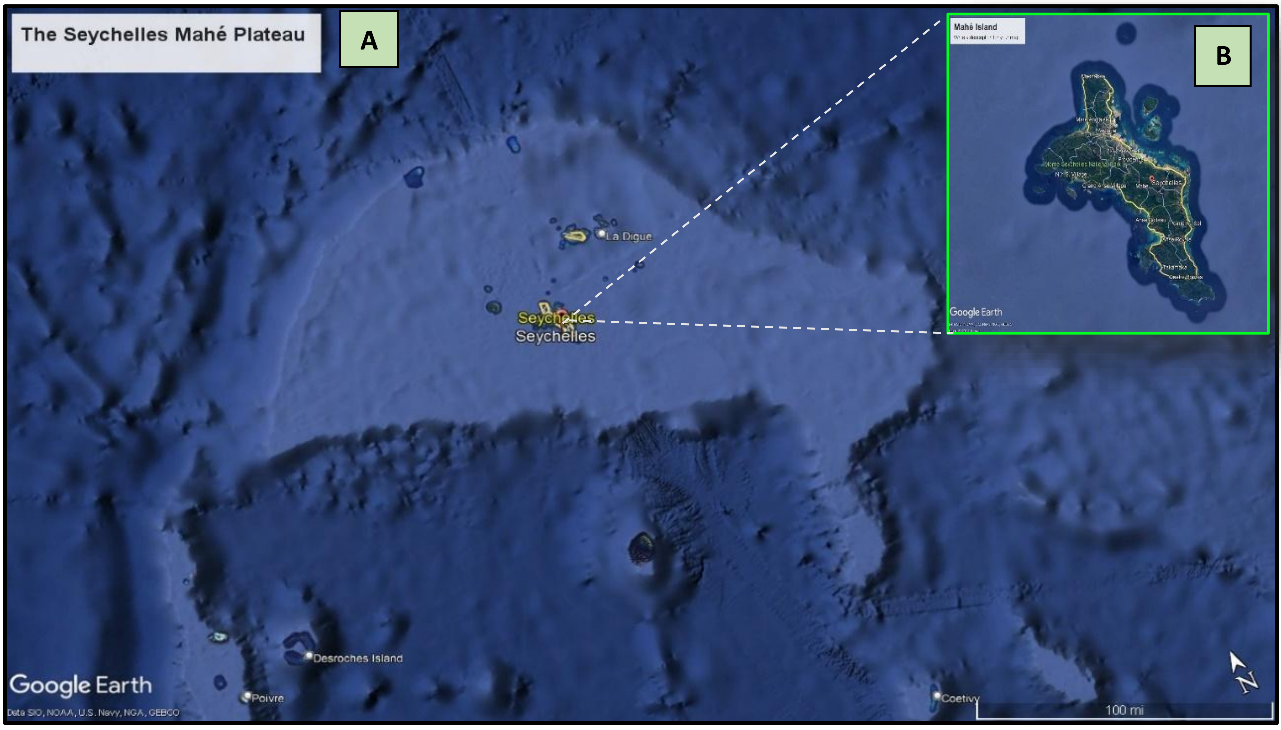

Figure 3: (A) Map of the Seychelles bank; Mahé plateau. (B) The main island of Mahé. Source: google LLC (2024). Google earth.

2.2 Data Collection

Specimens of Brownspotted groupers and White-blotched groupers were collected from artisanal fishers at multiple landing sites on Mahé Island. These sites included the central area, such as the Victoria market and the Victoria fishing port, as well as the northeastern coast, particularly La Retraite.

Biological measurements included total body length (nearest cm) (DeMartini et al., 2011), as well as both gross weight and eviscerated weight in kilograms (kg). Fish collected during the survey were measured within a size range, with the minimum length recorded at 20 cm and the maximum at 70 cm. Gonads were also weighed to the nearest gram, dissected, and then macroscopically examined to ascertain the sex (female, male, intersex or unknown) using a developmental stages guide (Nikolsky, 1976) (Appendix I).

A cross-section of the gonad or the entire gonad for smaller specimens were preserved in 10% formalin for subsequent histological analysis. This analysis included gonad segmenting, tissue dehydration, paraffin wax embedding, staining, and slide preparation (DeMartini et al., 2011). Then on, the microscopic stage of each gonad sample was determined using the ' SFA Histology Identification guide (2018)' (Appendix II). The slides were internally verified in two rounds within the organization, then sent to CSIRO in Australia for further verification by international partners.

3. Data Analysis

3.1. Sex ratio

The sex ratio, expressed as the ratio of males to females in a population, is a crucial metric in understanding population dynamics reproductive potential and for assessing the balance between the sexes within a population and can provide insights into reproductive strategies and behaviors (Kiørboe, 2006). To calculate the sex ratio, the count of males is divided by the count of females (Donald, 2007). Data from both males and females were used to identify patterns in the sex ratio, expressed as (M: F) and calculated using equation:

$$\text{Sex Ratio} = Count\,\frac{Male}{Female}$$

The **Chi-square analysis** was employed to evaluate whether the proportion of sexes adhered to an expected ratio, such as 1:1, or if there are significant deviations (Franke et al., 2011). This statistical method allows researchers to determine if observed differences in sex ratios are statistically significant and not merely due to chance.

3.2. Size Frequency distribution

The length and weight frequency distributions of Brownspotted and White-blotched groupers were examined using total length and gross weight measurements. However, due to the small amount of White-blotched grouper samples, histograms were created exclusively for Brownspotted groupers, with size groups classified into 2-bin intervals
Additionally, box plots were produced to visualize the dataset. The study also utilized chi-squared tests to investigate any differences in sizes between males, females, and intersex individuals. This comprehensive approach allowed for a detailed examination of the length and weight frequency distribution and size variations within the samples.

3.3. Length- weight relationship

The length-weight relationship (LWR), a crucial growth parameter essential for fishery management was determined. The relationship is expressed as $W = aL^b$, where W is weight, L is length, and parameters ***a*** and ***b*** are constants. To simplify the computations, this equation can be linearized by taking the natural logarithm of both sides: $\ln(W) = \ln(a) + b*\ln(L)$. The parameters ***a*** and ***b*** can then be estimated using simple linear regression, where ln(a) corresponds to the y-intercept (β0) and b represents the slope (β1). The regression model was applied to the dataset with the equation:

$$\log(W) = \beta 0 + \beta 1 \cdot \log(L)$$

The log-transformed (log(W)) represented the gross weight of the fish in kilograms, while log-transformed (log (L)) denoted the logarithm of the ratio of the total length of the fish in centimetres. The coefficient of determination, R-squared (r^2), obtained from the regression analysis indicated the strength of correlation between these two variables: total length and gross body weight. The goodness of fit of the regression model was evaluated using the F-test statistic (Zlateva, 2017).

3.3.1. Growth pattern

Allometric and isometric growth are essential concepts in understanding the relationship between length and weight in fish species. Allometric growth refers to a situation where the body proportions of a fish changes as it grows, with the weight increasing at a different rate compared to the length. This can be further classified into positive allometric growth (b > 3), where the fish becomes more roundish or heavier relative to its length, and negative allometric growth (b < 3), where the fish becomes slimmer or lighter as it grows (Hamuna, 2023). On the other hand, isometric growth occurs when the weight and length of a fish increase proportionally at the same rate (b = 3), maintaining consistent body proportions as the fish grows (Garcia-Ayala et al., 2023).

These growth patterns, determined by the value of 'b' in the length-weight relationship equation, provide valuable insights into the morphological changes that fish undergoes as they develop. Understanding whether a fish species exhibits allometric or isometric growth is crucial for assessing its biology, ecology, and overall development.

3.4. Gonadosomatic Index (GSI):

The Gonadosomatic Index (GSI) was calculated for both mature and immature females in the sample of Brownspotted groupers. Immature specimens can provide insights into the overall reproductive potential of the population and indicate the proportion of fish that have not yet reached reproductive maturity.

The GSI plays a vital role in fish biology, offering valuable perspectives on the reproductive status and spawning behavior of fish (Priyanka, 2024). GSI was calculated as the ratio between the gonad weight and gutted weight of the individual fish:

$$GSI = \frac{Gonad\ weight\ (grams)}{Gutted\ weight\ (grams)}\ X\ 100$$

3.5. Size at maturity

The size at which 50% of all specimens were sexually mature (L50) was determined for Brownspotted females using a logistic function, as follows:

$$Pmature = \frac{1}{1 + \exp(-a - bxL)} x\ 100$$

where Pmature is the proportion of mature specimens, L is the total length (TL), and the parameters ***a*** and ***b*** were to be estimated. The parameter ***c*** is the slope of the curve and describes the rate of change in the proportion of mature females, and b = L50.

4. Results

White- Blotched Grouper

4.1. Sample size and Sex ratio

A total of 48 White- blotched groupers, including 2 males, 45 females, and 1 unknown specimen was sampled. The observed sex ratio (males to females) was 0.044. It can be expressed as approximately 1:23, indicating that there are about 23 females for every 1 male. A chi-squared test revealed a highly significant difference (X-squared = 135.71, p-value < 2.2e-16) between the observed sex ratio and the expected 1:1 ratio.

4.2. Size and Weight Distribution per sex.

Females had a greater size and weight range, measuring from 43.5 - 76.5 cm TL and 1.38 - 7.14 kg in weight. Only two male specimens were sampled, with total lengths of 36 cm and 37 cm, both weighing 0.78 kg. The smallest female measured 43.5 cm in TL and weighed 1.41

kg, while the smallest male was 36 cm TL and weighed 0.78 kg. The white-blotched sample had a median length of 66.5 cm.

Brownspotted grouper

4.3. Sample Size and Sex ratio

A total of 307 Brownspotted groupers were sampled, including 176 females, 15 males, 96 intersex individuals, and 20 unknowns. The observed sex ratio was 0.085, which translates to a ratio of approximately 1:12, indicating that there are about 12 females for every 1 male. The chi-squared test result showed a highly significant deviation (X-squared = 135.17, p-value < 2.2e-16), from the expected sex ratio.

4.4. Sex determination

To validate sex and gonad staging, the sexes identified macroscopically during sampling were cross-checked with microscopic examination. While both methods are used to determine maturity stages, macroscopic assessment can be prone to errors due to difficulties in distinguishing between immature and spent fish based solely on gonad appearance. The results from both techniques showed considerable variation in sex identification, underscoring the value of employing multiple methods to ensure reliable data and acknowledges the possibility of human error in scientific research. The differences between the two gonad staging methods are highlighted in Figure 4.

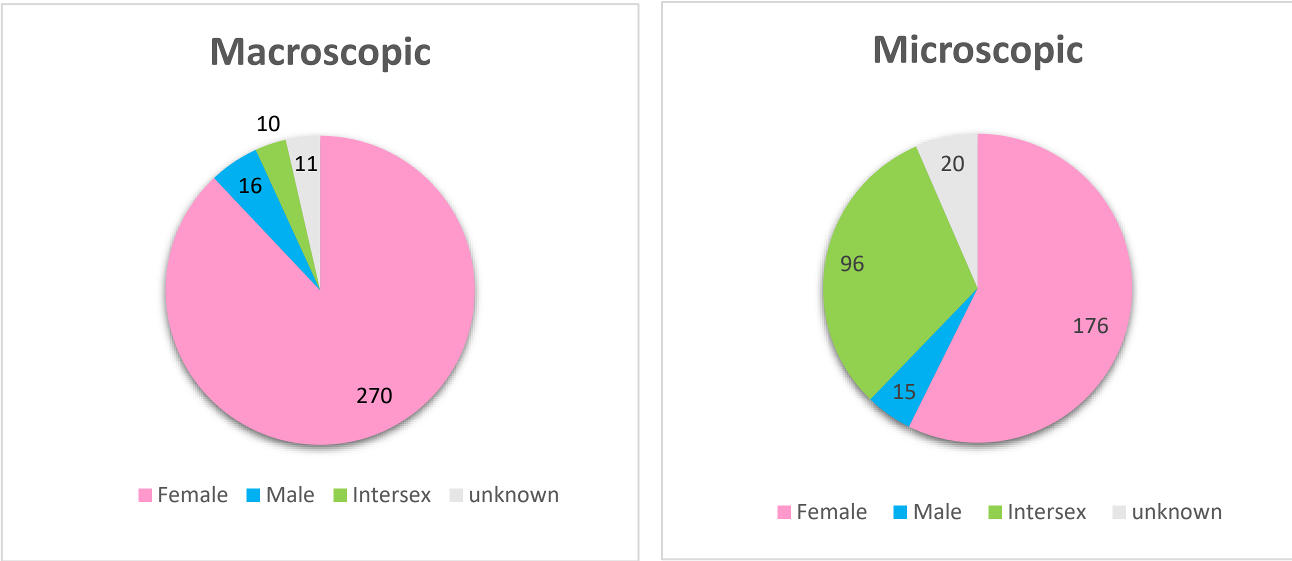

Figure 4: Comparison in sex counts between visual (macroscopic) and using microscope (microscopic) gonad staging in Brownspotted groupers.

4.5. Size and Weight distribution per sex.

The size and weight of female Brownspotted groupers ranged from 23.5 – 43 cm TL and 0.17 – 1.14 kg in weight, respectively. Males varied from 35 – 40.5 cm TL and 0.66 – 1.01 kg in weight. Intersex individuals ranged from 31.5 – 46 cm TL and 0.54 – 1.06 kg in weight. The smallest female was 23.5 cm TL and weighed 0.22 kg. The smallest male was 35 cm TL and 0.66 kg, and the smallest intersex fish was 35.5 cm TL and weighed 0.54 kg (Figure 5 & 6).

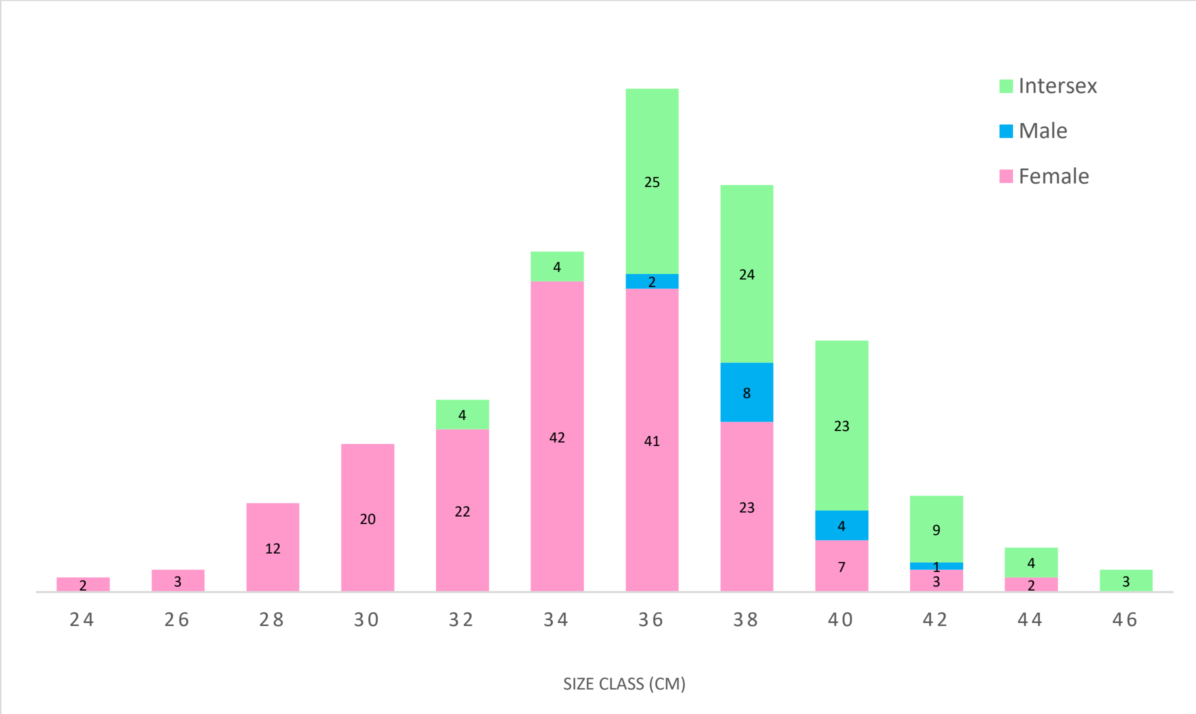

Figure 5: Length frequency histogram of Brownspotted groupers per sex categories (female n= 176, males n=15, intersex n=96)

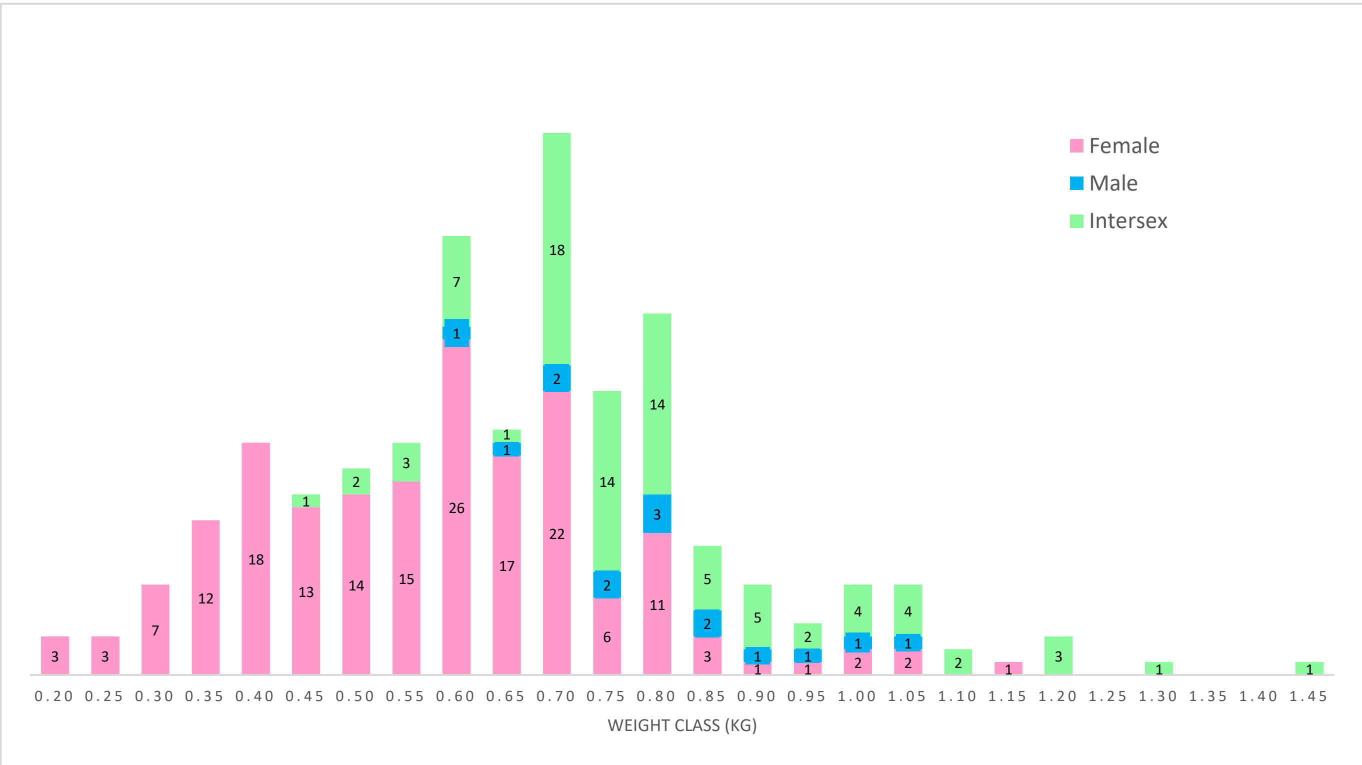

Figure 6: Weight frequency histogram of Brownspotted groupers per sex categories (female n=176, male n=15, intersex n=96).

Box plots to summarize the length distribution of Brownspotted groupers were produced to show data variability and central tendency (Fig 7). The median length of all sampled Brownspotted groupers was 35 cm, with lengths varying by approximately 4.02 cm around this value. Most fish measured between 32 cm and 38 cm, with some outliers. For males and intersex groupers, the median length was around 37.5 cm, while for females, was 34 cm. Males lengths ranged from 37 cm to 38.8 cm, females from 30.3 cm to 35.2 cm, and intersex from 35.2 cm to 38.8 cm.

The chi-square test revealed a significant difference in size distribution among male, female, and intersex fish. With a chi-square statistic of 84.375, 10 degrees of freedom, and a very small p-value of 6.93e-14, the results strongly indicated that the sizes of males, females, and intersex fish differ significantly. In other words, the size distribution is not the same across these three groups.

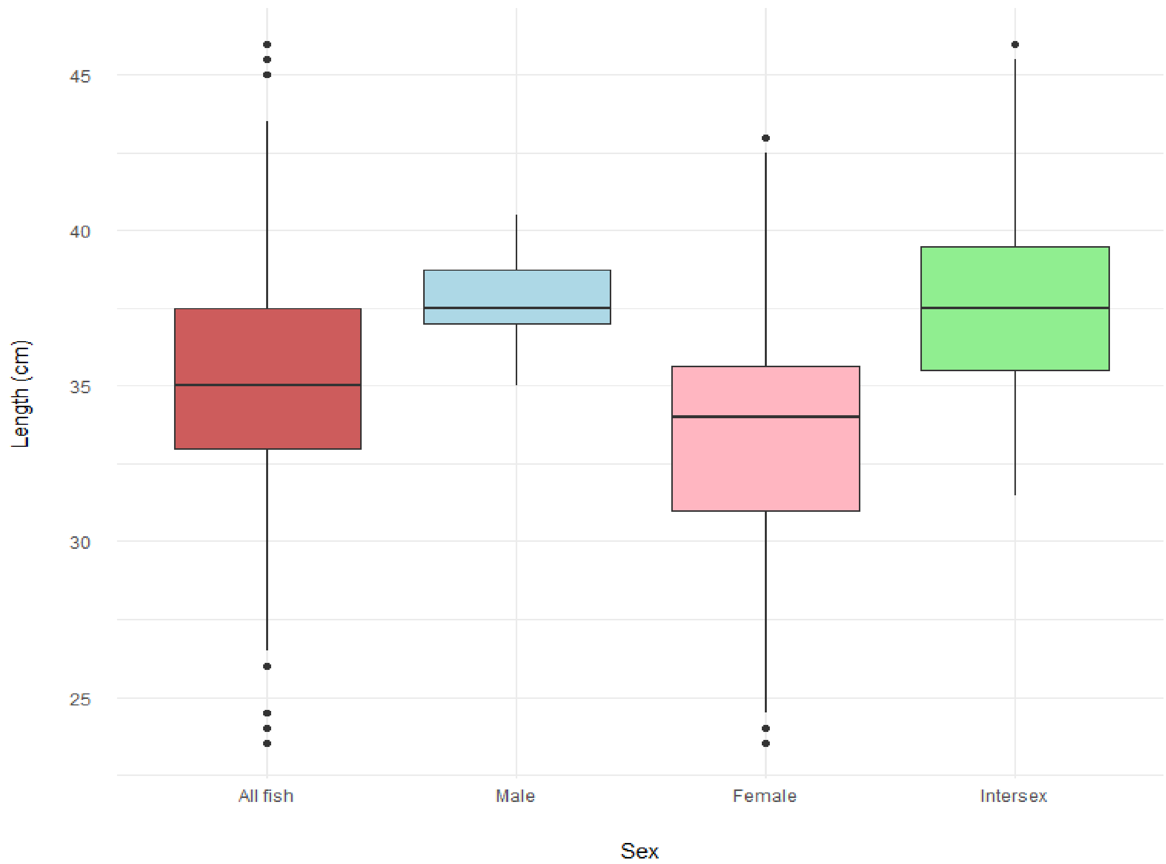

Figure 7: Boxplots illustrating the range of lengths observed in the Brownspotted grouper sample (female n=176, male n=15, intersex n=96).

Box plots summarizing the weight distribution of Brownspotted groupers, showed the median weight for all groupers was about 0.63 kg, with weights fluctuating by approximately 0.21 kg from this average. Most fish weighed between 0.5 kg and 0.67 kg. For males, the median weight was around 0.79 kg, with weights ranging from 0.71 kg to 0.86 kg. In comparison, females weighed slightly lower than the males, with a median weight of 0.56 kg. Weights for females ranged between 0.4 kg and 0.65 kg. The median weight for Intersex groupers was 0.72 kg, and their weights ranged from 0.63 kg to 0.82 kg. A few individuals weighed more than the rest.

Due to the lack of samples in several weight classes, it was not possible to undertake the chi-squared test.

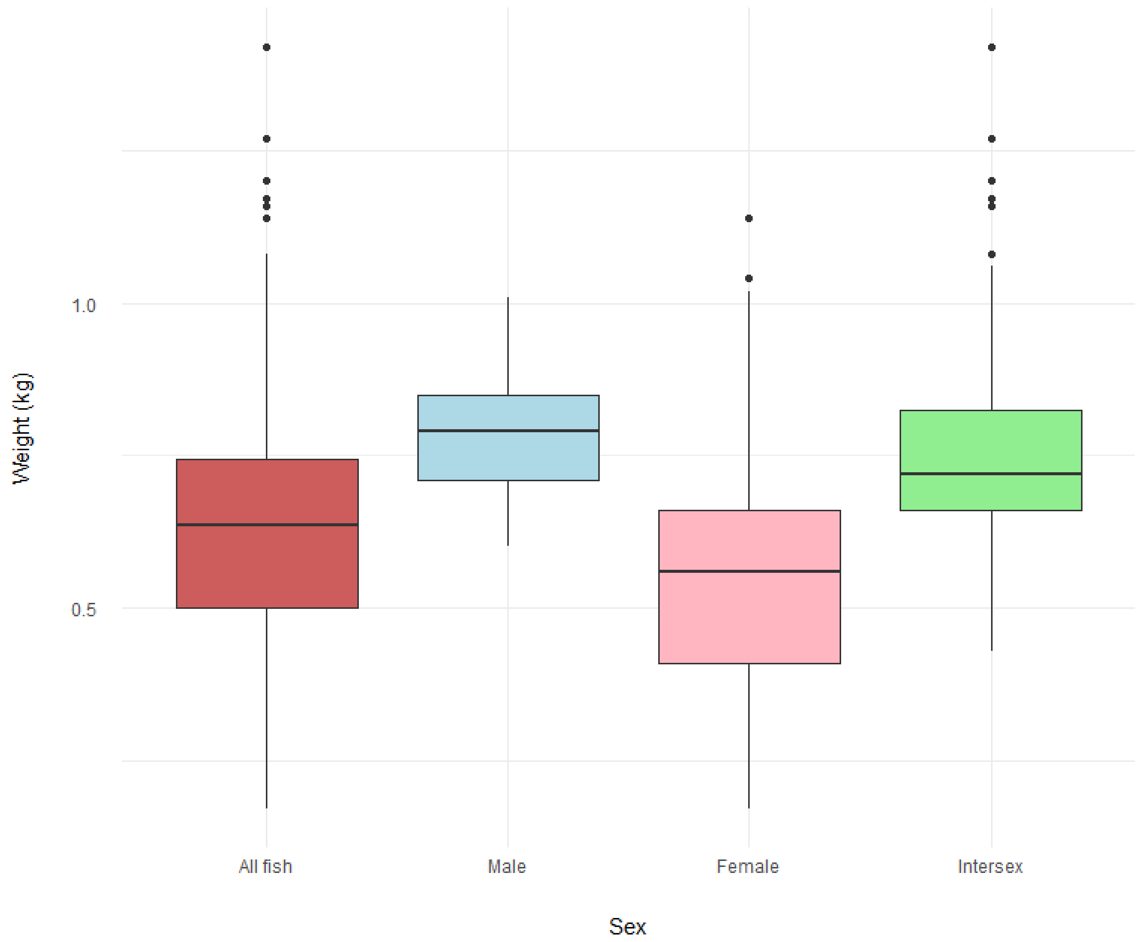

Figure 8: Boxplots illustrating the range of weights observed in the Brownspotted grouper sample for combined and sex categories (female n=176, male n=15, intersex n=96.)

4.6. Length – Weight Relationship of Brownspotted groupers.

The linear regression analysis conducted to examine the relationship between the logarithm of fish length (`logL`) and the logarithm of fish weight (`logW`) revealed a strong and statistically significant relationship. The model indicated that for each unit increase in the logW, the logL increased by approximately 0.31 units. An intercept of 3.71 represented the estimated logL when the logW was zero. The residuals, which measured the differences between observed and predicted values, ranged from -0.14850 to 0.45156, reflecting the model's accuracy and showing that most predictions were close to the actual measurements. An R-squared value of 0.8575 indicated that about 85.8% of the variability in fish length could be explained by fish weight, suggesting a strong correlation. The adjusted R-squared value of 0.857 supported the model's robustness. Both the intercept and slope were highly significant (p-values < 2e-16), confirming that the relationship was statistically reliable. The high F-statistic of 1836 further indicated the model's effectiveness in predicting fish length based on weight.

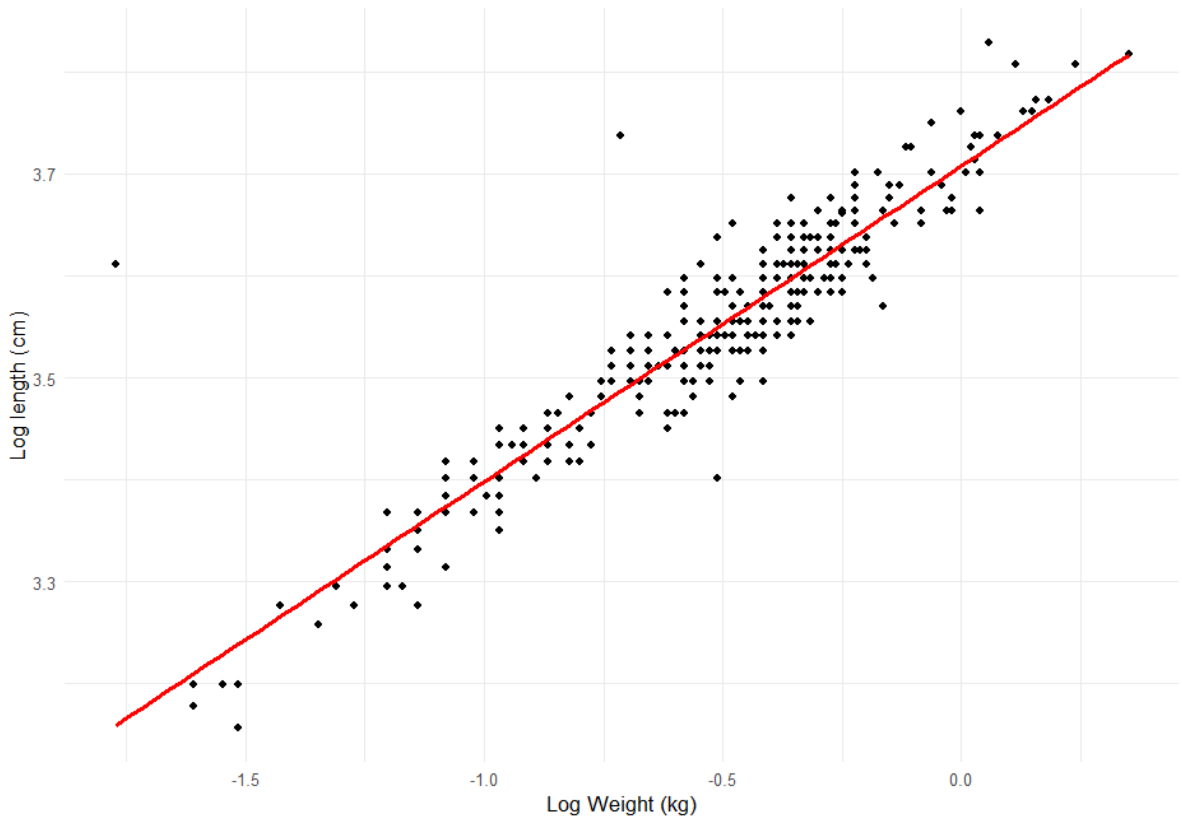

Figure 9: Length-Weight relationship plot for Brownspotted grouper.

4.7. Gonadosomatic Index (GSI)

For this study, GSI data were only analyzed for the months that had consistent data across both years. Due to the variability in data collection, only months with available data for both years were included in the analysis. Although consistent sample collection during the same months each year is recommended, this study lacks data for January and June, resulting in their exclusion from the graph (Fig. 10). Changes in GSI values over time can reveal the onset of spawning periods in different fish species, hence making this analysis essential. It is important to highlight that the focus was specifically on female Brownspotted groupers.

Analyzing GSI values across different months (Fig 10) revealed notable variations in the reproductive condition throughout the year. The highest average GSI was observed in August (Mean = 2.15), with a substantial range (0.17 to 9.82) and high standard deviation (SD = 3.22), indicating significant variability and a peak in reproductive activity during this period. Conversely, April exhibited the lowest mean GSI (0.668), with a narrower range (0.05 to 2.35) and lower variability (SD = 0.640), suggesting minimal reproductive activity at the beginning of the year. Elevated GSI values were also observed in March and November, both with a mean of 1.69, indicating periods of increased reproductive effort. The moderate GSI values in February (Mean = 0.854) and May (Mean = 1.33) indicate transitional phases in the reproductive cycle.

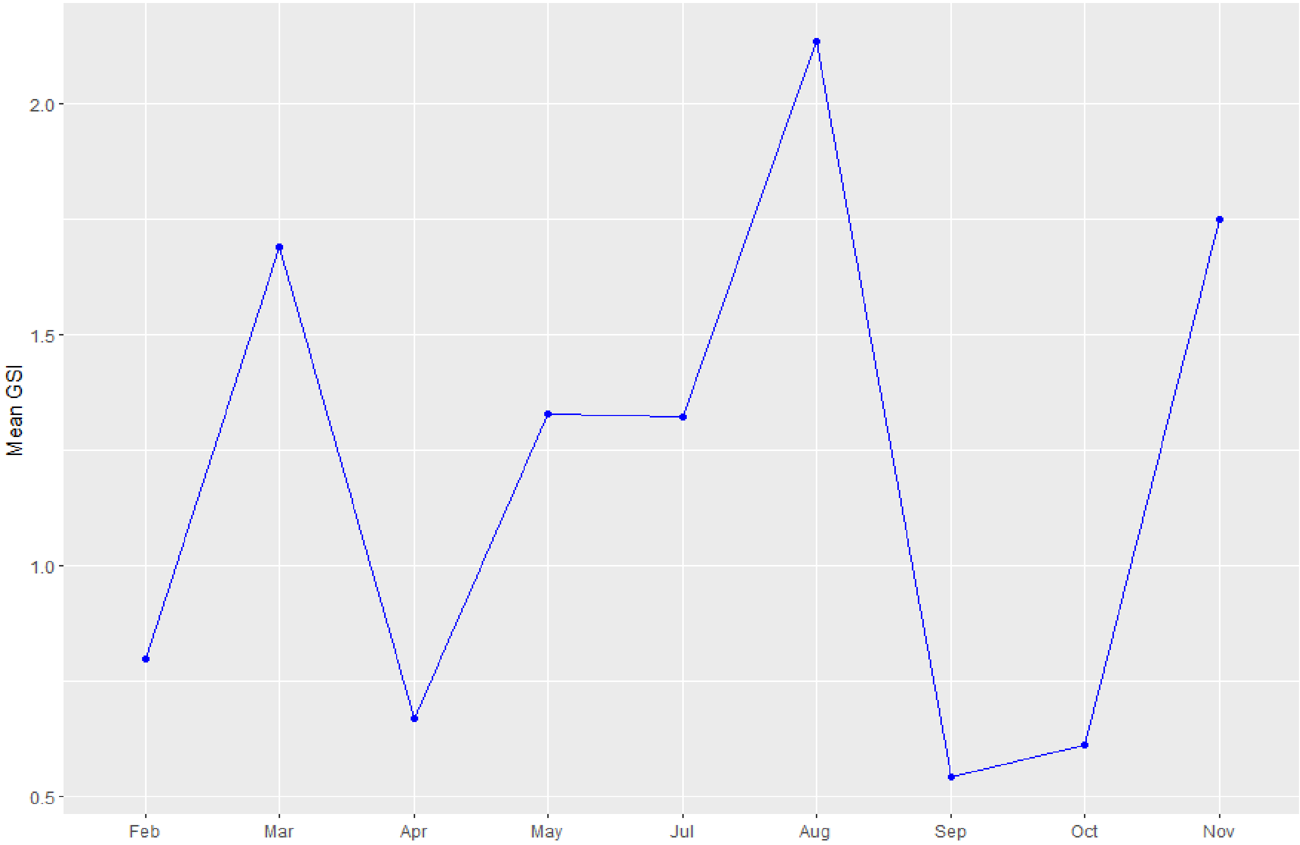

Figure 10: Mean GSI values across months (mar 2022 - oct 2023. NB: Data for January and June are excluded from the graph due to lack of data.

4.8. Size at Maturity

In the analysis of size at maturity in Brownspotted groupers, the study examined the relationship between total length and the proportion of mature individuals. However, in this instance, the investigation included 169 females (5 immature: 164 mature), 76 intersex fish, and 15 males, all of which were classified as mature.

The summary statistics for the Brownspotted grouper indicate that size plays a key role in determining maturity. The logistic regression formula used shows that the **A coefficient** (intercept) is negative (-9.9014 original, -10.0075 bootstrap median), suggesting that as the fish grow, the likelihood of reaching maturity increases. The **B coefficient** (slope) is positive (0.4405 original, 0.4432 bootstrap median), meaning that larger fish are more likely to be mature, with the probability increasing at a moderate rate as their size grows. The **L50** value, which represents the size at which 50% of the fish are mature, is around 22.6 cm. The **R^2 value**, which shows how well the model fits the data, is 0.2999 (original) and 0.4405 (bootstrap median), suggesting that while size is a crucial factor in maturity, other variables may also influence this process, as the model doesn't explain all the variation.

In most of the literature, size at maturity is often studied exclusively in female fish. However, to ensure comprehensive analysis and validate data reliability, a separate examination focusing solely on females was also conducted. This analysis found that the **L50** value was 22.2 cm, with an R^2 of 0.24.

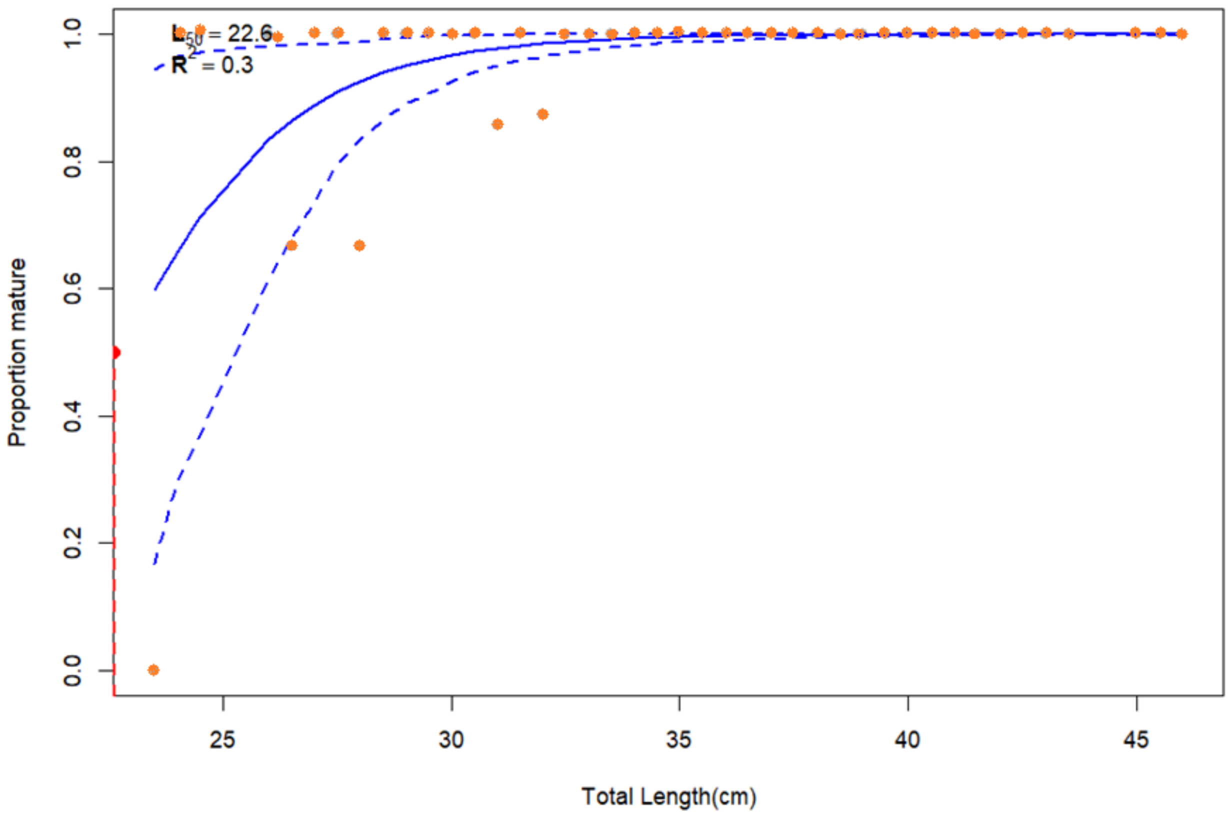

Figure 11: The size at maturity (L50) among female (n=169), males (n=15) and intersex (n=76) Brownspotted groupers. Lm50 was approx. 23 cm).

5. Discussion

5.1. Sex Ratio

The skewed sex ratio favoring females was evident in both the Brownspotted and White-blotched groupers. This skew may be due to insufficient sampling, especially for the White-blotched grouper, leading to a lack of representation from certain size classes which could have further affected the observed sex ratio.

The sampling was unevenly distributed, with fish collected from multiple fishermen, and it is possible that, on certain fishing days, these fishermen targeted aggregations primarily composed of females. Since hermaphroditic groupers, such as the Brownspotted grouper, exhibit a predominance of females in their populations (Achmad et al., 2021; Caballero-Arango et al., 2010; Murata et al., 2021; Li et al., 2023), females were likely caught more often, especially since fishermen frequently returned to the same fishing grounds. Additionally, as male groupers transition to larger sizes later in life, these larger males may have been missed due to gear selectivity (McClanahan & Mangi, 2004). This could skew the representation of the population, with females being overrepresented and males underrepresented, ultimately reducing the overall diversity of the samples.

5.2. Length - Weight relationship

Figure 9 illustrates the relationship between fish weight and length for Brownspotted groupers. It reveals that as weight increases, length also tends to increase. The red trend line, based on regression analysis, highlights a positive correlation, which follows a typical logarithmic growth pattern seen in biology. This visualization supported the statistical findings, where the model explained approximately 86% of the variability in fish length is based on weight. The close fit of the predicted values to the observed measurements suggested that the model effectively captures the relationship between these variables.

It is also noteworthy that the positive coefficient and the intercept estimate of 3.708 suggested positive allometric growth in this species (Froese et al., 2011). In this case indicating that as the length of the Brownspotted grouper increases, its weight also increases at a faster rate. Available literature states that groupers may exhibit both isometric and allometric growth patterns but these specific growth pattern can vary among different grouper species and life stages (Josh et al.,2022). Overall, the results highlighted a robust and meaningful correlation between fish length and weight, reflecting significant biological insights into the growth patterns of Brownspotted groupers. However, it is important to note that the observed correlation from the model does not imply a direct causal relationship between weight and length, as the analysis is based on the logarithm of weight rather than actual weight values. Practical applications should account for the model's limitations and assumptions, including the theoretical baseline at zero weight.

5.3. Gonadosomatic Index (GSI)

The GSI data for female Brownspotted groupers highlighted notable seasonal patterns in reproductive activity. The pronounced peak in GSI values observed in August, with a mean of 2.15 and a high standard deviation, suggests a significant reproductive event during this month. This peak indicates a period of intense gonadal development and spawning activity, aligning with the observed increase in GSI values. Similarly, elevated GSI values in March and November (Mean = 1.69) further support the notion of heightened reproductive effort during these months (Joshi et al., 2018; Farooq et al., 2019; Fontoura et al., 2009). Elevated GSI was also observed in November.

In contrast, the lower mean GSI in April (0.668), coupled with a narrower range and lower variability, indicates a period of reduced reproductive activity. This might represent a recovery phase following the peak spawning season or a transitional period leading up to increased reproductive efforts (Denusta et al., 2019). Moderate GSI values in February (Mean = 0.854) and May (Mean = 1.33) suggest transitional phases within the reproductive cycle, potentially reflecting periods of preparation or recovery.

These results illustrate a clear seasonal pattern in reproductive activity, with higher GSI values indicating peak reproductive periods and lower values corresponding to quieter phases.

While the tropical climate of the Seychelles may influence these patterns, the data primarily highlight intrinsic reproductive cycles and strategies of the Brownspotted groupers. The variability in GSI across different months provides valuable insights into the timing and intensity of spawning, offering guidance for future studies and conservation efforts focused on understanding and managing the reproductive dynamics of this species.

Nevertheless, it is important to recognize that these patterns can vary across varied species (Hughes et al., 2020). Numerous factors influence these variations, including seasonal changes, water temperature, time of day (day or night), depth, and habitat characteristics such as seafloor structure or benthic composition (Desiderà et al., 2019). Additionally, geographic location, population structure, and dynamics also play significant roles in shaping reproductive patterns.

5.4. Size at Maturity

The results of the size-at-maturity analysis for Brownspotted grouper show that size plays a significant role in determining the likelihood of sexual maturity. The **L50** value of approximately 23 cm suggests that 50% of the population reaches maturity at this size. This provides valuable information for understanding the reproductive cycle of this species, which is essential for effective fisheries management, especially when considering size-based regulations.

The $\mathbf{R^2}$ values of 0.2999 (original) and 0.4405 (bootstrap median) indicate that while size is a key factor in maturity, it does not explain all the variability in the data. This suggests that other factors, such as age or environmental conditions, may also influence the timing of maturity. The relatively low $\mathbf{R^2}$ values highlight the complexity of maturity in Brownspotted groupers and suggest that a more comprehensive understanding of their reproductive biology will require examining additional variables beyond size.

The consistency between the original and bootstrap results strengthens the reliability of the estimates. Bootstrapping provides a more robust estimate of the coefficients, helping to account for uncertainty and variability in the data, particularly in biological studies where natural variability is common.

Overall, these findings contribute to our understanding of the reproductive biology of Brownspotted groupers. The **L50** value offers a useful benchmark for maturity, which can help inform size limits in fisheries management. The **L50** value of 22.6 cm found in this study aligns closely with the maturity sizes reported in the literature for Brownspotted groupers, which typically range between 23 cm and 31 cm (Moussac, 1996; Heemstra & Randall, 1993). This consistency supports the reliability of the findings and suggests that the observed size at maturity is within the expected range for this species.

However, further research is needed to explore other factors influencing maturity, such as age, environmental conditions, and potential social dynamics, to develop a more complete picture of the species' reproductive strategies.

6. Conclusion

The findings revealed several key insights about the Brownspotted grouper. First, the L-W relationship showed a positive allometric growth pattern, with weight increasing slightly faster than length as the fish grew. Regarding reproductive trends, although data collection was incomplete for some months, higher GSI values were observed in March, and November, peaking in August before decreasing in September. This pattern may vary depending on the species, season, or location. Finally, the size at maturity (L50) for Brownspotted groupers was found to be 23 cm, based on data from females, males, and intersex individuals.

Caution should be exercised when interpreting these results, as the limited variation in size classes may have influenced the findings. Unfortunately, the sample size for the White-blotched grouper was too small to conduct comprehensive reproductive analyses.

Several factors contributed to these challenges such as securing funds for purchasing fish. A considerable amount of time to establish an efficient distribution system, resulted in missed sampling opportunities. Obtaining samples was also impacted during periods of the Southeast Monsoon due of rough sea conditions.

It is evident that this study encountered several challenges in data collection. However, the research conducted as part of the SFA project successfully provided some valuable insights into the biological parameters of the Brownspotted grouper *(E. chlorostigma)* and White-blotched grouper *(E. multinotatus).* Despite the challenges, these findings contribute to achieving some of the study's key objectives.

7. Recommendations

To improve the accuracy and reliability of this research, it is recommended to expand the sample size, focusing on individuals closer to the size at maturity as well as larger fish. Conducting longitudinal studies would provide valuable insights into growth patterns and reproductive behaviors over time, helping to understand how factors such as temperature, habitat quality, and food availability influence these parameters (Perkin, 2023). Additionally, age determination techniques, such as otolith analysis, could be used to better assess growth rates and reproductive timing. Genetic studies, including those on size dimorphism and sex determination, would offer alternative and complementary methods for evaluating maturity. Investigating the potential impacts of human-induced stressors, such as habitat degradation, pollution, and climate change, on fish growth in changing environments would also be valuable. Gathering data on spawning aggregations and comparing fish stocks between the Mahe Plateau and outer islands would further contribute to a deeper understanding of the species' dynamics.

Moreover, incorporating some of these approaches into a separate study could strengthen the findings of this one, and help establish more precise recommendations for size at maturity for both to undertake genetic studies (size dimorphism and sex determination) as an alternative and complementary metrics to assessing maturity.

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9. Appendices

9.1. Appendix I

TABLE 1: AN SIX-POINT MATURITY SCALE FOR TELEOST FISH, ADOPTED FROM NIKOLSY (1976).

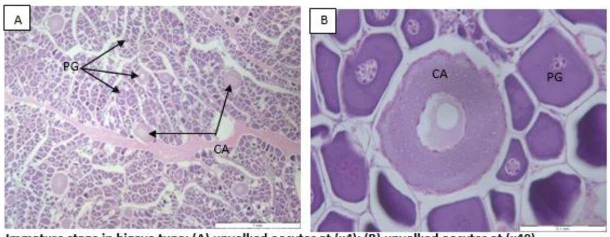
Maturity stage		Females - Ovary	Males - Testis
0	Undetermined	Sex not distinguished with the naked eye	
1	Immature	Rounded translucent up to 2 mm broad; less than a quarter of length of body cavity; no oocytes are visible with the naked eye. Sex may be difficult to determine	Flattened, 1-2 mm broad, translucent; less than a quarter of length of body cavity
2	Resting	Rounded translucent, yellow to orange; about a third of length of body cavity; oocytes are visible only with the use of microscope	Flattened, pink; about a third of length of body cavity
3	Developing	Rounded yellow or orange; about a half of length of body cavity; oocytes are visible with naked eye	Becoming fatter; off white; about a half of length of body cavity
4	Maturing	Firm and yellow; half to whole of length of body cavity; hydrated oocytes may be visible as grey spots, which may run on big pressure	Firm becoming whiter; half to whole of length of body cavity
5	Mature	Fill the whole length of body cavity; hydrated grey oocytes are visible as grey spots on the ovary surface, which run from vent on slight pressure	Becoming soft; fill the whole length of body cavity; milt runs from vent on slight pressure
6	Spent	Flaccid dark red; ; less than half of length of body cavity; a few large residual oocytes may be visible	Flaccid off yellow; less than half of length of body cavity

9.2. Appendix II: SFA Histological Identification guide.

Maturity Phase -IMMATURE

1. Immature stage

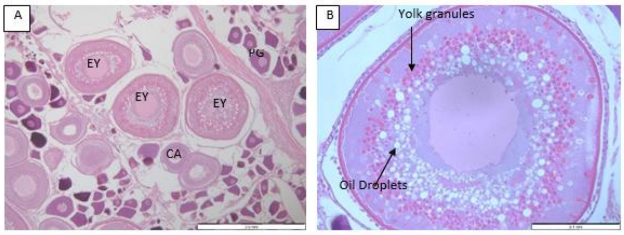
The Most Advanced Group Oocytes (MAGO) in this stage are unyolked – which consists of primary growth (PG) and cortical alveoli (CA) oocytes. PG oocytes stain dark purple while CA oocytes are paler and have a granular perinuclear zone. No POFs, atresia or maturity markers are present in the ovary (see below).



Immature stage in bigeye tuna: (A) unyolked oocytes at (x4); (B) unyolked oocytes at (x40)

2. Immature developing stage

MAGO are Early Yolked (EY) – these oocytes are in the early stages of yolk deposition (vitellegenis) (Vtg1 or Vtg2). The yolk granules are sharply stained and occupy up to 2/3 of the perinuclear zone. No POFs, atresia or maturity markers present are present in the ovary.

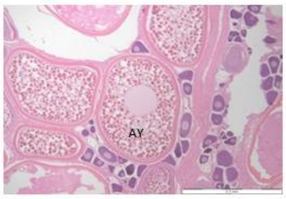


Early Yolked oocytes (EY) in bigeye tuna at: (A) x10; (B) x40. PG and CA oocytes also indicated.

Maturity Phase = MATURE

3. Non-Spawning - Spawning capable stage

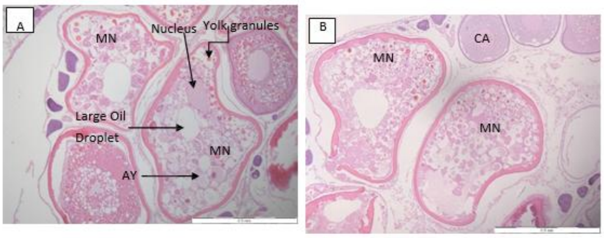
MAGO is Advanced Yolked oocytes (AY) - the yolk granules gets bigger, they are spread throughout the oocyte, and the nucleus is still in the middle of the oocyte. No POFs present, but atresia and/or maturity markers may be present in the ovary.



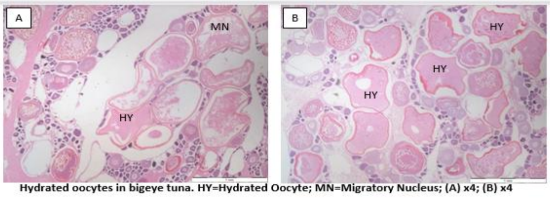
Advanced oocyte in bigeye tuna at; (A) x10

4. Actively Spawning- Spawning capable stage

MAGO is Migratory Nucleus oocytes (MN) or hydrated (H) – the oocyte gets bigger and oil droplets starts to fuse together to form larger oil droplets, while the nucleus moves towards the edge of the oocyte. The large oil droplet is an indicator that the oocyte is at Migratory Nuclear stage. The yolk granules then join together to form Yolk Plates (YP).Hydration (HY) then occurs (uptake of fluid). The yolk plates fuse completely, forming a uniform pink stain. The nucleus disappears (nuclear membrane disintegrates). The oocyte significantly increases in size and becomes irregular in shape. The granulosa and thecal cells stretch. POFs, < 50% α atresia, β atresia and/or maturity markers may be present in the ovary.



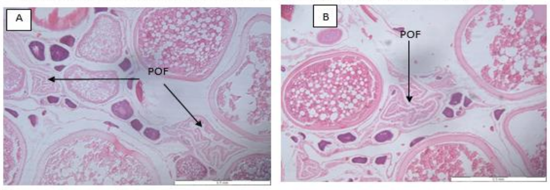
Migratory Nucleus Phase in Yellow Fin Tuna: (A) Oil droplet fusing and nucleus moving towards the edge of oocyte at (x10); (B) Large oil droplet and visible yolk plates at (x10).



Hydrated oocytes in bigeye tuna. HY=Hydrated Oocyte; MN=Migratory Nucleus; (A) x4; (B) x4

Post Ovulatory Follicles (POFs)

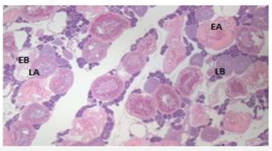
Post ovulatory follicles (POFs) may be present in Active - Spawning stage fish. A POF is what is left behind when an egg is ovulated (released from its follicle). This follicle is made up of the thecal and granulosa cells which stretch during hydration; it will remain in the ovary and will collapse into a distinctive folded structure called POFs. POFs are reabsorbed over time and become smaller and less well defines. POFs are generally classified into New (0 hr), young (0-12 hrs) or old (12-24 hrs).



New POFs in Swordfish at (x10)

5. Inactive - Regressing Stage

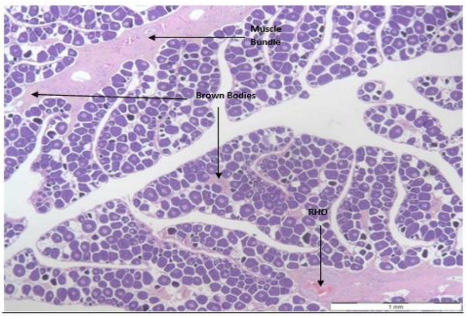
As the reproductive cycle ends, the fish moves into the regressing phase, which is recognize by the presence of atretic oocyte, a reduced number of vitellogenic oocytes and the presence of Post Ovulatory Follicles (POFs).



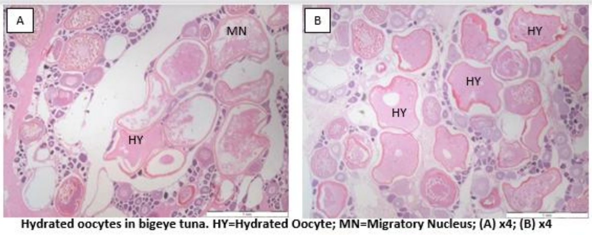
The different stages of atresia in yellow fin tuna at (x4): EA=Early Alpha; LA= Late Alpha; EB= Early Beta; LB= Late Beta

6. Regenerating Stage

The regenerating stage is characterized by ovaries containing only PG oocytes, similar to immature stage. It can be differentiated from the immature stage by a thicker ovarian wall, the presence of muscle bundles, brown bodies and residual hydrated oocytes. Regenerating stage is equivalent to mature-resting.



9.2.1. Appendix II. continued.



Hydrated oocytes in bigeye tuna. HY=Hydrated Oocyte; MN=Migratory Nucleus; (A) x4; (B) x4

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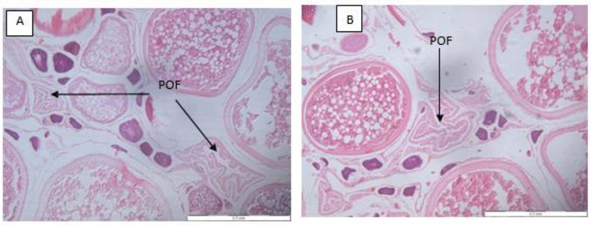

New POFs in Swordfish at (x10)

5. **Inactive - Regressing Stage**

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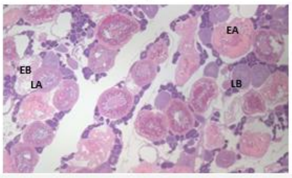

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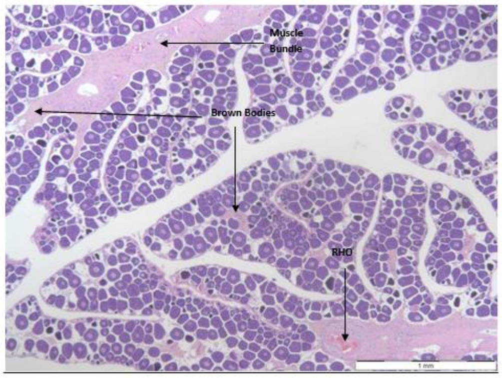
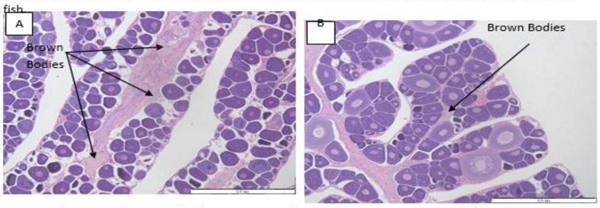


Brown Bodies

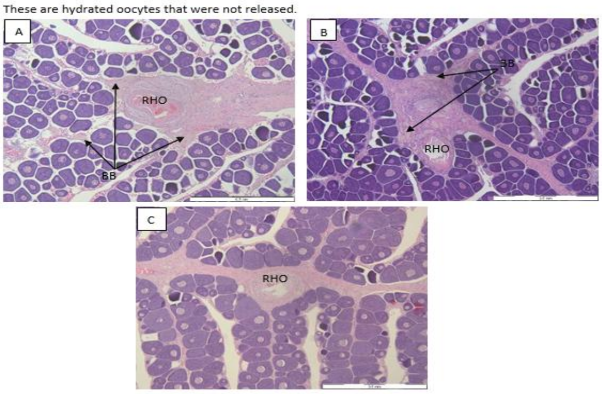
They are atresia that has been reabsorbed. If brown bodies are present it means that this is a mature fish.



Brown Bodies in Southern Pacific Blue Fin Tuna at (x10); (A) Large Brown Bodies; (B) Small Brown Bodies

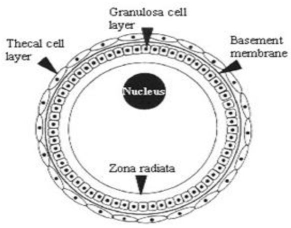
Residual Hydrated Oocytes (RHO)

These are hydrated oocytes that were not released.



RHO and BB in Southern Bluefin Tuna at (x10); RHO=Residual Hydrated Oocytes; BB= Brown Bodies

Oocyte Structure



Generalised diagram of an egg (oocyte) surrounded by the granulosa cells, basement membrane and thecal cells. Nucleus is migrating to the margin of the oocyte. (Adapted from Takashima and Hibiya, 1995).

Summary Table

MATURITY STATUS	PHASE	SUB-PHASE	MAGO AND POF STAGE	ATRESIA OF ADVANCED YOLKED OOCTYTES	MATURITY MARKERS[2]
Immature	Immature		Unyolked, no POFs	Absent	Absent
Immature	Developing		Early yolked, no POFs	Absent	Absent
Mature	Spawning Capable	Non-spawning	Advanced yolked, no POFs	α and β atresia may be present	Possible
Mature	Spawning Capable	Actively spawning	Migratory nucleus or hydrated and/or POFs	α and β atresia may be present	Possible
Mature	Regressing		Unyolked or early yolked, no POFs	All yolked Oocytes are in the α and β stages of atresia	Possible
Mature	Regenerating[1]		Unyolked or early yolked, no POFs	Absent	Present

[1] Regenerating is equivalent to mature-resting.

[2] Thickness of ovary wall, or the presence of brown bodies, muscle bundle or residual hydrated Oocytes.

9.2.2. Appendix II. continued - Histological ID guide for protogynous hermaphrodites.

Histology guide on protogynous hermaphrodites e.g., Vielle Makonde and vielle plat

Gonadal histology revealed that mature oocytes of brown-spotted and white blotched grouper comprise of primary growth, Pre vitellogenic, vitellogenic, spawning and regressing gonads (Figure 1) or intersex gonad (Figure 2). Intersex gonads include early intersex, mid intersex, and late intersex phases, in which both ovarian and testicular tissue coexist (Figure 2).

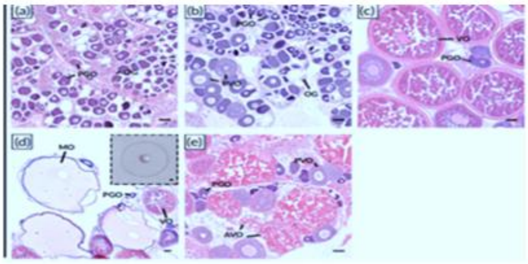
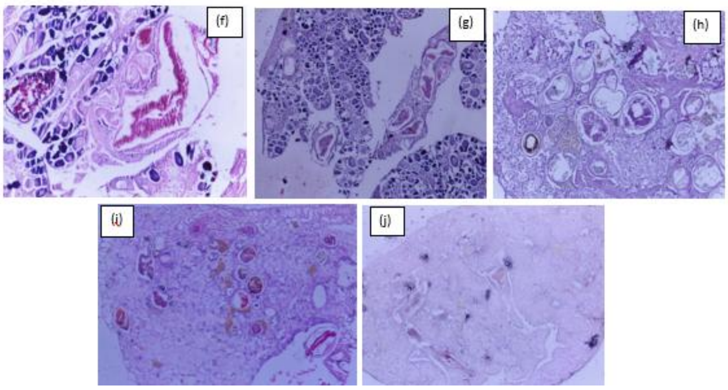


Figure 1: Histology of different gonadal stages in the Epinephelus Sp. Ovary Stage: (a) primary growth ovary (PGO) stage; (b) pre-vitellogenic ovary (PVO) stage; (c) vitellogenic ovary (VO) stage; (d) Spawning ovary (SO) stage; (e) regressing ovary (RO) stage.



Identification guide

Ovary (female gonad)	Maturity markers present in a normal female gonad: - Primary growth oocytes (PG) - Pre vitellogenic oocytes (CA) - Vitellogenic or Yolk granules (AY) - Migratory Nucleus or Hydration (M) (H) - Regressing ovary (Resting phase)
Intersex gonad	When both ovarian and testicular tissue are present. - Early intersex (the ovary is in the earliest phase of transition)– appearance of scattered nests of gonial cells. - Mid intersex (mid transition phase) – Atretic oocytes are present. There is an increase of male cells (spermatocytes) Brown bodies can also be observed. - Late intersex (last phase of transition) – significant increase of sperm cells and spermatocytes at a more development stage.
Male Gonad	Only male cells present. After sex change (full transition), no ovarian tissues are observed in testes.