Reproductive biology of the Golden African Snapper *Lutjanus fulgens* in Principe Island (São Tomé and Príncipe)

RESEARCH REPORT

A. INTRODUCTION

Understanding reproductive biology of demersal fish is essential for fisheries management, and it was highlighted as priority information by the Santomean fisheries department (Tous, 2015). Although demersal fish comprises only around 10% of the total catch in Principe (Omali Vida Nón's landing surveys, Dec 2016 to Dec 2018), its populations are resident to the specific grounds and therefore much more sensitive to fishing pressure, and the health of its populations determines the health of the whole marine ecosystem around the island (Tous, 2015).

Lutjanus fulgens (Vermelho terra, in Santomean Portuguese) is one of the four species of lutjanid snappers captured by fishers in Príncipe Island. Lutjanid snappers are highly valued by the local population for trade and consumption, and they comprise almost 40% of the biomass of demersal fish landed in Principe (data from Omali Vida Nón's landing surveys, Dec 2016 to Dec 2018). In particular, *L. fulgens* comprises 5% of the demersal catch in terms of biomass, and 15% in number of individuals. This species has a wide distribution along the West African coast, from Senegal and Cape Verde to Angola and in the islands of the Gulf of Guinea, and it is found to depths down to 150 m - deeper than the depth most of the trawls operate in the region (Morais *et al.*, 2015). Given its wide depth and geographical ranges, it is classified as a species of *Least conservation concern* by the IUCN Red List, although due the lack of population information and catch statistics for this species, the potential long-term impacts of fisheries on this species are uncertain (*ibid.*).

B. METHODS

B.1. Sampling

Fish was bought directly from the fishers at the landing sites in four communities in Principe Island: Hospital Velho, Abade, Campanha and Sto António (sampling size of 655 fish purchased from 125 fishing trips/landings). Initially, it had been defined that a maximum of 15 fish would be purchased per canoe, deliberately aiming to collect a representative sample of all the sizes classes present in the canoe (roughly dividing the catch in 7-8 size classes, from the smallest to the largest, and picking two of each class). However, in 96% of the 125 landings, the number of *L. fulgens* in the canoe was below 14 so all the individuals in the canoe were purchased. When purchasing fish, a short survey was conducted to record name of the fisher, gear, location, weather, fishing time and landing time. Sampling was conducted monthly, from April 2018 to March 2019, with a monthly target of at least 30 fish of each sex per month (Woods *et al.*, 2003). The target was sometimes not reached due to logistic reasons, so data was grouped bi-monthly (average sample size per month, 50 fish per month for maturity data).

B.2. Fish processing

Fish was processed at the field laboratory or directly at the beach and kept in proper cooling facilities (cooling boxes with ice or a fridge) until processing, to preserve the tissues for histology and avoid wastage of fish. A labelled and standardised picture of the fish for morphometric studies was taken before measuring length and weight (Cadrin, 2000). Fish was then dissected, using dissection scissors to open a shallow cut from the anus to gills to expose the body cavity (Johnson *et al.* 2009). Gills and innards were extracted whole, by cutting the lateral and dorsal attachments of the gills to the body

first and then cutting the edges of the swimming bladder, which is attached to the innards. Gonad length and weight were recorded, and a labelled and scaled picture of the gonads was taken. Gonads' maturity stage was assessed macroscopically using the Brown-Peterson (2011) maturity scale, which uses a standardised, comparable terminology to describe macroscopic and histological reproductive development in all species of fish. Whole gonads were fixed and kept in 10% formaldehyde for at least two days, using at least 4 times volume of fixative as the volume of the gonad. Samples were transferred to ethanol 70% immediately before its transport by plane (higher concentration of ethanol must not be used, as it causes shrinkage of the tissues; Johnson *et al.* 2009). Stomach was opened to extract its content, if any, and kept in ethanol 90% (Kilongo *et al.* 2007). Finally, otoliths were removed, and a fin clip was taken, keeping them in ethanol 97% (Nanami *et al.*, 2010). All samples were labelled using small pieces of paper written in pencil and kept inside the tubes for transport of the samples. Presence of parasites and damage in the fish due to decompression were also recorded in side notes.

Figure 1: **A)** Standardised picture of the fish being processed directly at the landing site. **B)** and **C)** Dissection of fish and extraction of gonads. **D)** Otoliths. **E)** Otic capsule was exposed after cleaning and removing the tissue remainings of the gills, and opened to extract the otoliths.

Table 1: Female maturity scale, following Brown-Peterson (2011). Pictures correspond to gonad of snappers at each maturity stage sampled in the field and processed at UoE's histology laboratory (pictures by G. Porriños).

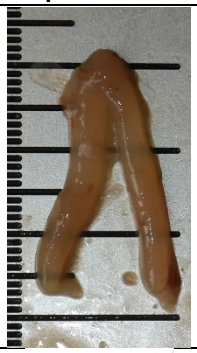
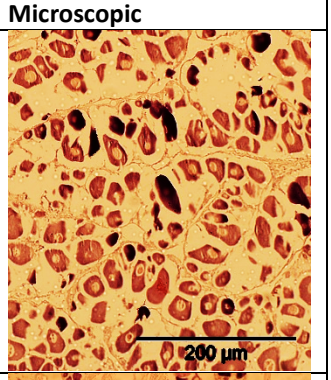
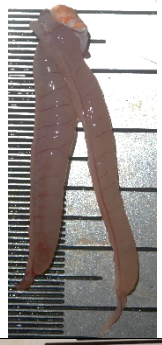
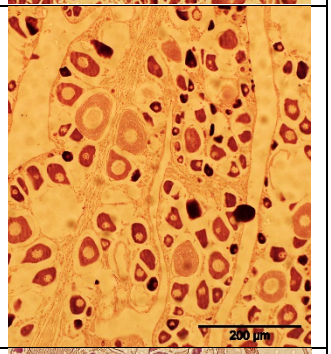
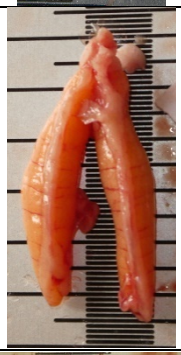
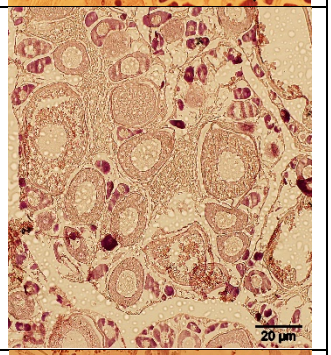
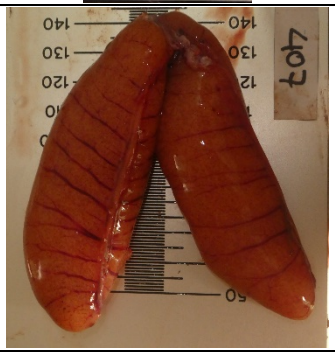
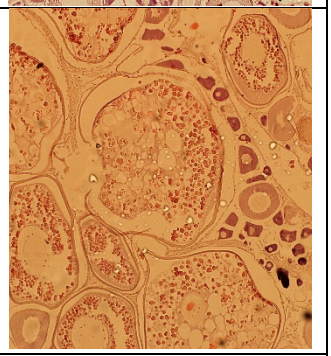
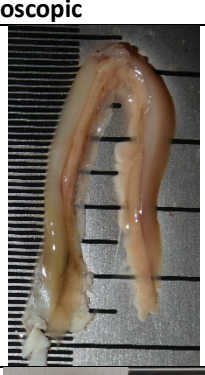
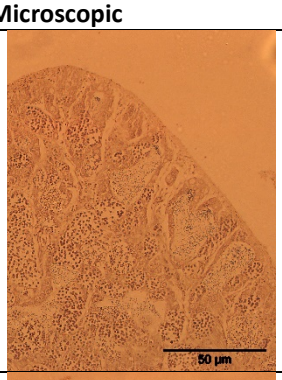
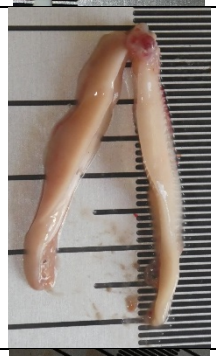
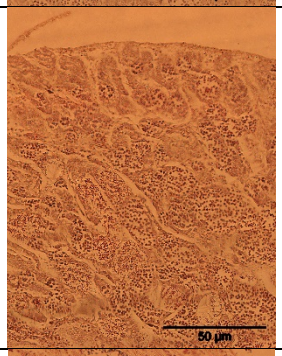
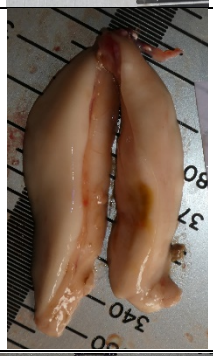
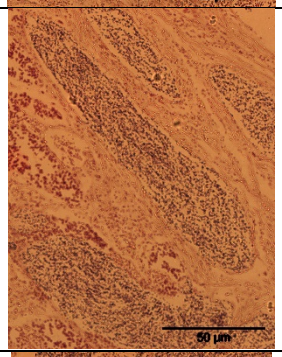
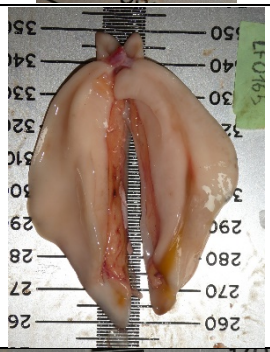
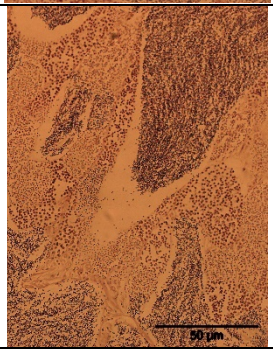
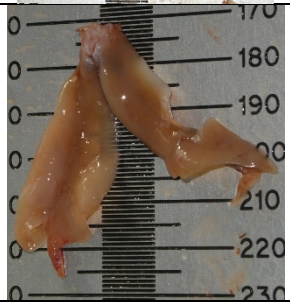
Brown-Peterson Maturity scale (2011)			Macroscopic	Microscopic
F I	**Immature**	**Small ovaries, clear no blood vessels present** Only oogonia and PG oocytes present, thin ovarian wall and little space between oocytes		
F II	**Early Maturing**	**Small ovaries, clear, blood vessels reduced but present** Only PG and CA oocyte present		
F III	**Late Maturing**	**Enlarging ovaries, blood vessels more distinct. Ovaries orange in colour due to the accumulation of yolk in vitellogenic oocytes** PG, CA, vtg1, vtg2 oocytes present.		
F IV	**Ripe**	**Large ovaries, blood vessels prominent. Individual oocytes visible macroscopically. Ovaries orange in colour** Vtg3 oocytes present or POFs present in batch spawners. Atresia of vtg and/or hydrated oocytes may also be present		
F V	Spent	**Flaccid ovaries yet still large, blood vessels reduced but present** Atresia and POFs present. Some CA and/or vtg1/vtg2 oocytes present	**NO PICTURE AVAILABLE**	**NO PICTURE AVAILABLE**

Table 2: Male maturity scale, following Brown-Peterson (2011). Pictures correspond to gonad of snappers at each maturity stage sampled in the field and processed at UoE's histology laboratory (pictures by G. Porriños).

Brown-Peterson Maturity scale (2011)			Macroscopic	Microscopic
F I	**Immature**	**Small testes, clear and threadlike (difficult to assign sex)** Primary germ cell stage. Spermatogonia (Sg) present. Lobules packed very close together so no lumens are present (or they are barely visible).		
F II	**Early Maturing**	**Small, threadlike testes** Lobule lumens are now visible and are narrow and long. The lobule wall is thin and there are large numbers of spermatogonia (Sg). May be a few spermatocytes present but no spermatids.		
F III	**Late Maturing**	**Small testes but easily identified.** Lobule with thicker wall and larger lumen, although may not be visible as they are full of spermatids (St). Spermatocytes (Sc) are present. Spermatozoa (Sz) may also be present but difficult to see.		
F IV	**Ripe**	**Large and firm testes** Lobule completely packed with Spermatozoa (Sz). All the flagella are pointing towards the centre of the lobule. Large numbers of spermatids (St) also present.		
F V	**Spent**	**Small, flaccid testes.** Lobule walls are thick and strongly eosinphillic. Thick layers of spermatogonia cells inside the lobule wall, some residual spermatozoa remaining but mostly large empty lumens		**NO PICTURE AVAILABLE**

B.3. Processing of gonad samples for histology

A subsample of the collected gonads was processed using histological techniques in order to assess the accuracy of the macroscopic assessment done in the field. Processing of 312 gonad samples for histology was done at Prof Charles Tyler's laboratory (University of Exeter), under the supervision of Dr. Anke Lange. For each gonad, anterior, middle and posterior 4mm sections were cut. Sections were processed using a tissue processor, which automatically uses increasing concentrations of ethanol to dehydrate the gonads and finally embeds them in wax as described in Table 3 (Johnson *et al.* 2009). Samples were embedded in wax blocks and sectioned to 5μm thick using a microtome, and sections were attached to glass slides. Of the 312 samples processed up to this stage, a subsample of 106 gonads representing all maturity stages were stained using Hematoxilin-Eosin method (Table 4). Sections were observed on microscope and classified according to Table 1 and Table 2.

Table 3: Embedding of samples in wax (steps automated using a tissue processor, modified from Johnson *et al* . 2009) .

Step	Reagent	Pressure/Vacuum Cycle	Heat (°C)	GONAD program (min.)
1	70 % Ethanol	On	Ambient	40
2	80 % Ethanol	On	Ambient	40
3	95 % Ethanol	On	Ambient	40
4	95 % Ethanol	On	Ambient	40
5	100 % Ethanol	On	Ambient	40
6	100 % Ethanol	On	Ambient	40
7	100 % Ethanol	On	Ambient	40
8	Clear Rite 3	On	Ambient	60
9	Clear Rite 3	On	Ambient	60
10	Paraffin	On	60	60
11	Paraffin	On	60	60
12	Paraffin	On	60	60
13	Paraffin	On	60	45

Table 4: Hematoxiline and eosing staining (Johnson *et al.* 2009)

Step	Reagent	Minutes in Reagent
1	Xylene	4
2	Absolute Alcohol	2
3	80% Alcohol	1
4	Water	1
5	Hematoxylin	3
6	Water	2
7	Clarifier	1
8	Water	1
9	Bluing	1
10	Water	2
11	95% Alcohol	1
12	Eosin	1
13	Absolute Alcohol	4
14	Xylene	3

B.4. Statistical analysis

Length-at-maturity (length at which 50% of the fish population have reached maturity for the first time) was determined by fitting a generalised linear model for binary data (Binomial Likelihood with a Logit-link function); given binary nature of variable, this required reclassifying the five maturity stages into "Immature" and "Mature". Stages 1 and 2 were reclassified into immature and stages 3 to 5 into mature, both for male and female.

Gonado-Somatic Index (GSI, *Gonad weight / body weight*) indicates how developed are the gonads in comparison to the size of the body, and it is used as a proxy for maturity. To explore variations in GSI and maturity throughout the year, months were pooled by pairs to increase sample size per unit of time being compared and improve robustness of the statistical analysis. One-way ANOVA and Post-hoc comparisons using the Tukey correction were used to explore bimonthly variations in GSI; Levene and Shappiro-Wilk tests were used to test the null hypotheses of normality and homoscedasticity. Chi-squared tests and post-hoc comparisons using the Bonferroni correction were used to explore variations in the proportion of mature and immature fish bimonthly. ANOVA was used to detect differences in the average size of fish caught by the different fishing gears. All analyses were conducted using the statistical software R.

B.5. Processing of otoliths, fin clips, stomach contents and pictures

Otoliths, fin clips, stomach contents and pictures of the fish will be used to study growth, population genetics, diet and morphological variation of the species, respectively. Remaining gonad samples will be analysed using histological techniques in order to collect in-depth histological data on maturity development of *Lutjanus fulgens*. These studies fall out the scope of the study and will be conducted at the University of Exeter in the future. AE's support will be acknowledged for any potential future study resulting from this work.

C. RESULTS

For this study, 655 fish were analysed: 628 *L. fulgens*, 7 *Lutjanus dentatus* and 20 *Lutjanus goreensis*. The study of *L. dentatus* and *L. goreensis* was deemed unfeasible and abandoned after the first month of sampling. The following results refer only to *L. fulgens*.

Mean length and weight of *L. fulgens* was 28.8 cm (S.D.=4.4) and 421 g (S.D.=183). Maximum and minimum length and weight respectively were 140 and 390 mm and 49 and 1018 g. The length-weight relationship for the species is illustrated in Figure 2.

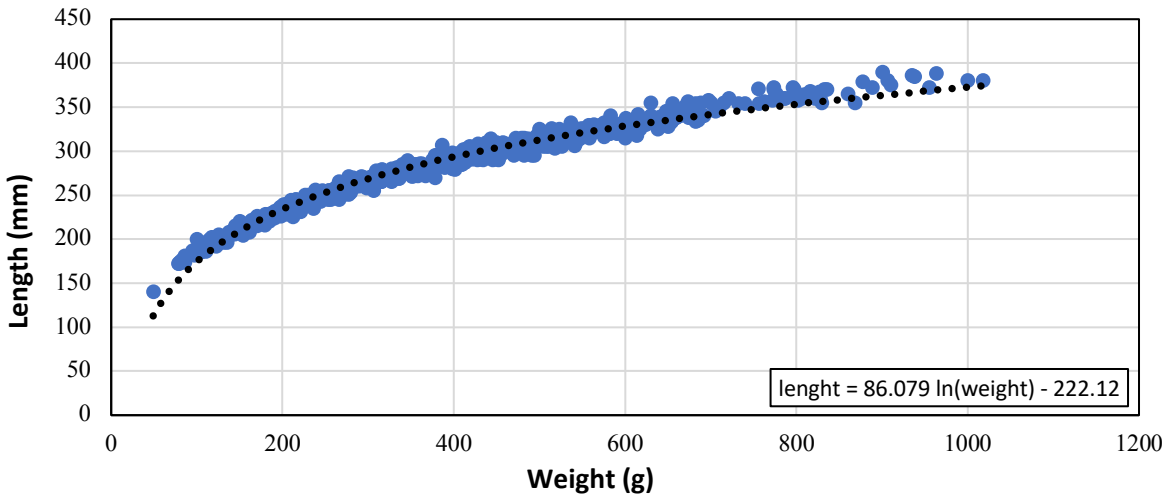


Figure 2: Length (L) vs. Weight (W) of *L. fulgens*. Estimated fitted distribution: *L = 86.079 ln(W) - 222.12*; equivalent to: $W = 14.484e^{0.0113 * L}$

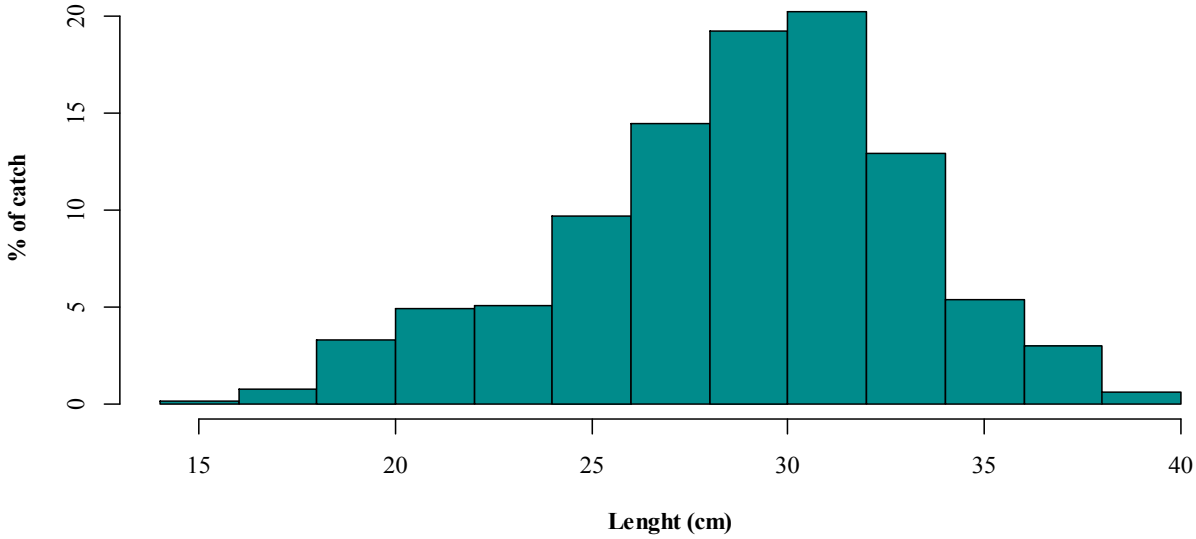


Figure 3: Distribution of length in 628 fish individuals used in this study. Average length of fish caught was 28.8 cm (S.D. 4.4). Fifty-eight percent of the sampled fish were below 30 cm.

Table 5: Body length and gonad weight at each reproductive stage (N = 628).

	Fish length (mm)			Gonad weight (g)		
	Average	Min	Max	Average	min	Max
F1	22.7	17.2	29.0	0.16	<1 g	3
F2	28.4	22.0	38.6	3.05	<1 g	10
F3	30.4	23.5	38.5	8.42	<1 g	15
F4	31.9	27.0	38.8	16.58	3	88
F5	31.6	31.6	31.6	14.00	14	14
M1	24.3	14.0	32.8	0.12	<1 g	2
M2	28.1	22.0	37.2	1.80	<1 g	8
M3	30.4	23.0	37.5	8.55	<1 g	28
M4	31.4	26.1	38.0	21.37	1	72
M5	33.7	29.0	37.9	13.36	3	36

Length at reproductive maturity -length at which 50% of the population is mature- was calculated to be 27.8 ± 0.1 cm for both female and male fish. However, this analysis might slightly over-estimate this value, as a macroscopic assessment of the gonads does not allow to distinguish between fish that have never reproduced and big-sized fish that might appear immature at the beginning of their reproductive cycle. As shown in Figure 4, late maturing females start appearing at 22.0 cm; and 20.0 cm for male and almost 50% of the fish sampled (male and female) are in phase III or IV (late mature or ripe) in the size class 26-28 cm.

GSI of the period of June-July for both male and female fish did not show any significant difference compared to the rest of the time periods. Male and female GSI for the periods of August 2018 to December 2018 was significantly higher than GSI for the periods of April-May 2018 and February-March 2019. The proportion of mature fish was significantly higher than the rest of the months in August-September and significantly lower in February-March for both male and female (Bonferroni corrected post-hoc comparisons, $P<0.01$), with the exception of June-July, that did not show any significant differences (see Figure 5).

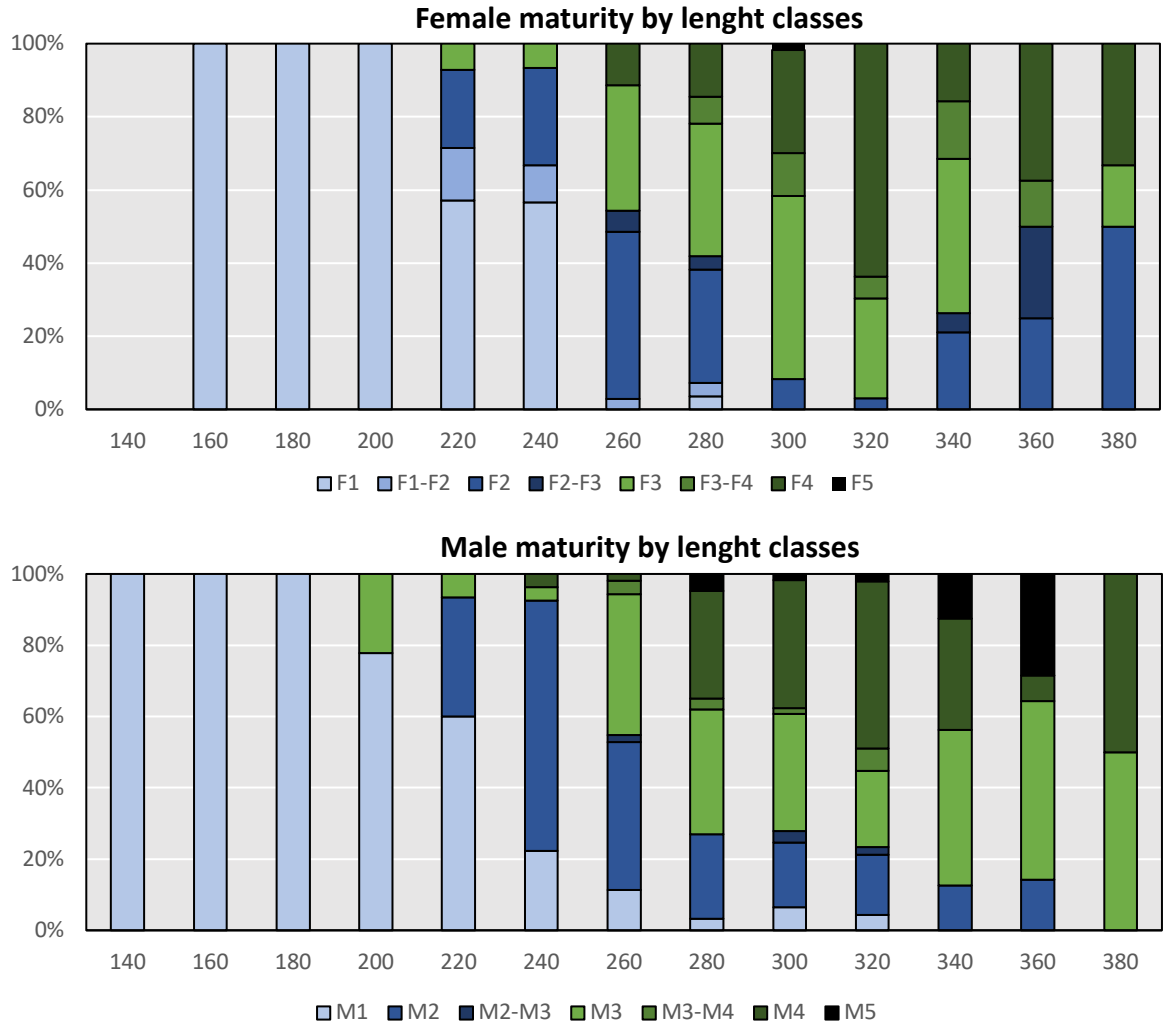


Figure 4: Proportion of each maturity stage at different length classes (N = 628)

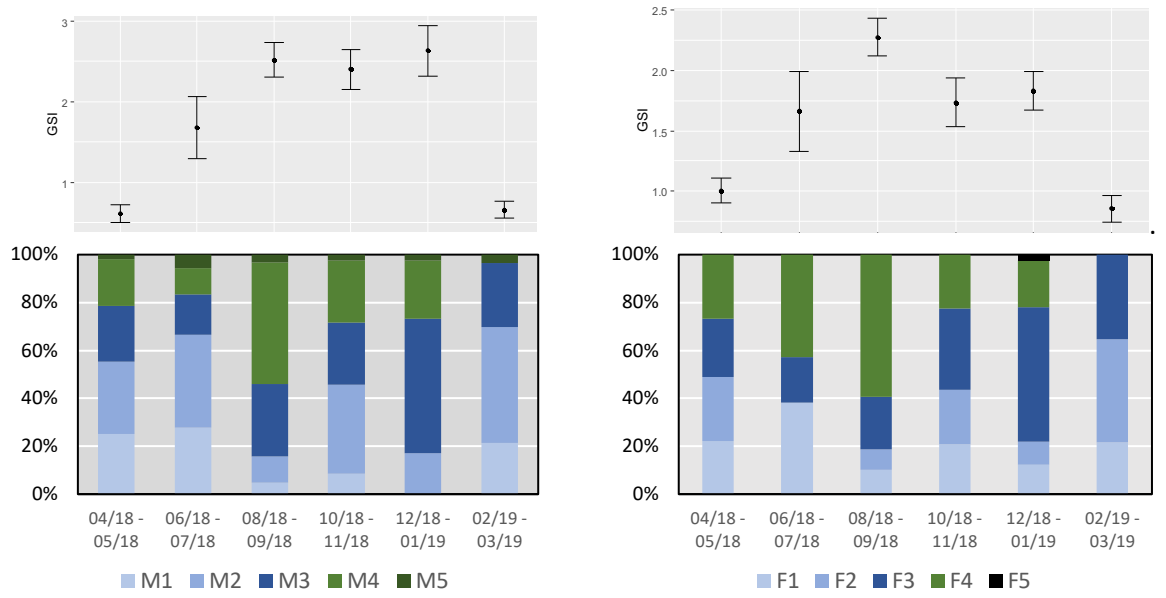


Figure 5: Bimonthly variations of Gonado-Somatic Index and maturity stages of male (left) and female (right) *L. fulgens* (n=628)

Additional laboratory assessments showed that in-field assessment of maturity was between 85.7-100% accurate for female and 76.9-100% accurate for male, depending on the maturity stage; with an overall accuracy of 91.4% for female and 79.4% for male.

Table 6: Comparison of the accuracy of assessment of maturity stage in field and in the laboratory, in female snappers (top) and male snappers (bottom). Cells in green indicate the samples for which the macroscopic and histological assessment of maturity matched. Cells in blue indicate the cells for which the maturity assessment in the field underestimated the actual maturity level, and cells in yellow those which maturity was overestimated.

		Classification in the field					
		F1	**F2**	**F3**	**F4**	**F5**	**TOTAL**
Classification using histology	**F1**	12					12
	F2	2	15				17
	F3			14			14
	F4			2	12	1	15
	TOTAL	14	15	16	12	1	58
	Accuracy	85.7%	100.0%	87.5%	100.0%	0.0%	91.4%

		Classification in the field					
		M1	**M2**	**M3**	**M4**	**M5**	**TOTAL**
Classification using histology	**M1**	10					10
	M2	3	7				10
	M3		1	11	2		14
	M4			1	10	3	14
	TOTAL	13	8	12	12	3	48
	Accuracy	76.9%	87.5%	91.7%	83.3%	0.0%	79.2%

This study suggests that adult *L. fulgens* in Principe Island is a component of the catch of three main gears: *mid-water troll* (n=92; line with 150 to 200 non-baited hooks, with small stripes of coloured plastic tied to the hook, dragged in mid-water, but close to the sea floor, with a weight in front of the line); *demersal longline* (n=124; 200-300 baited hooks, anchored to the seafloor for an hour and a half with a rock at each end signalled with a buoy); *vertical longline* (n=223; 7-8 hooks, baited, boat stopped and "agitated" up and down by the fisher). In addition, it is also caught by *purse seine nets* (personal observation). Although average length shows significant differences on the average length of fish caught by each gear ($p<0.001$), these differences are very small to consider meaningful.

Figure 6: Distribution of length by fishing gear (n=439, no information was available for the remaining samples)

Most of the *L. fulgens* analysed (397 out of 628, 63%) presented severe damage due to decompression, with the stomach partially or completely turned inside-out and the swimming bladder notably distended and slightly pressing some of the innards through the anus. At least 36 out of 623 sampled fish (5%) presented a parasitic isopod attached to the gills and tongue (for some of the fish, the presence of the parasite was not recorded); it mostly seems to appear in pairs or threes, with a bigger one (approx. 2.5-3.0 cm length) attached to the tongue and one or, more rarely, two smaller ones (approx. 1 cm length) attached to the gills. Some of the bigger isopods appeared gravid, with larvae developing in a ventral bag.

D. DISCUSSION

Fisheries statistics for *L. fulgens*, including average sizes and maximum length, do not exist (Morais *et al.*, 2015). However, the average and maximum lengths obtained in this study (28.8 and 39.0 mm, respectively) are markedly below those described by Allen (1985, location and source not provided), who reports a maximum size of 60 cm for this species, with individuals up to 50 cm long commonly caught. Thirty-five percent of the caught fish recorded in our study was below 27.8 cm, the estimated length-at-first-maturity, and 60% of the caught fish were below 30 cm, which means that a considerable proportion of the fish harvested in Principe has never reproduced or has probably reproduced for few seasons only.

These results suggest that catching Golden African Snapper below 28 cm is not recommended, as most of them would not have had the chance to reproduce even once. However, targeting fish bigger than a certain size might be challenging. Release does not seem to be an option, as most of the fish analysed showed evidence of severe, permanent damage due to decompression and were probably dead by the time they reached surface. The restriction of specific fishing gears does not seem to be a suitable option in this case, as differences in fish length according to fishing gear used were minimal. Further analyses would be required to understand whether the size of the fish correlates with depth or any specific fishing grounds, and more detailed information should be collected on fishing gears to understand whether specific hook sizes or bait target fish of different sizes.

The variations in GSI and maturity ratio throughout the year indicate that August to December/January might be a period of higher reproductive activity, with a spawning peak in August-September, revealed by the higher number of spawning-capable (phase 4) male and females. February to May seems to be a period of low or non-existent reproductive activity. No significant differences were found for June and July, which might relate to the low sample size during this period.

Although spawning might be happening continuously from August to December / January, reducing the fishing effort on this species during the months of August and September -when the number of mature fish peaks- is recommended. This can be done by restricting the use of gears targeting this species (mid-water troll, demersal long-line, vertical longline) in the fishing grounds where it is caught more frequently. However, *Lutjanus fulgens* is only one of the many species targeted by Principe's artisanal fishers using the three described gears. Full consideration of potential socioeconomic implications is needed for identifying robust management interventions and more information should be collected on fishing grounds where the fish is caught more often and techniques used for targeting this species.

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