Marine biodiversity assessment through baited underwater camera monitoring and maturity assessment of selected demersal fish species

Report produced by The University of Exeter for Africa's Eden

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Forever Príncipe! conservation alliance

Contents

Report on activities conducted from 01st February 2018 to 31st March 2019

A. INTRODUCTION

This report has been produced by the University of Exeter (UoE) as part of the collaboration between UoE and Africa's Eden (AE) for the implementation of the Marine Project entitled "Marine biodiversity assessment through baited underwater camera monitoring and maturity assessment of selected demersal fish species", under the collaborative agreement between both institutions ("The Agreement"). This collaboration falls within the "Forever Principe" programme, a Conservation Tourism Programme initiated by AE which aims to create a close link between high end nature tourism, meaningful conservation research and national park management and financing activities. The research outcomes of the Marine Project will provide managers, conservation organisations and the government with information that can be used to design management measures based on solid scientific foundations; namely 1) better understanding of the spatial distribution of predatory fish around Principe Island, which can be used to design a potential network of Marine Protected Areas; and 2) better understanding of the reproduction of a key, predatory demersal fish species, which can be used by managers to protect critical moments of the species' life cycle.

This final project document reports on the Marine Project activities conducted from February 1st 2018 to March 31st 2019 as well as the achievements towards project deliverables and outputs, as per established on The Agreement.

The report on activities has been separated in the following sections: **B)** Fish Maturity Study; **C)** Baited Remote Underwater Video Surveys. Each section has been structured as follows: 1) Objectives; 2) Project deliverables; 3) Outputs. These are followed by: **D)** List of materials to be returned to Belo Monte; **E)** Financial report. Following the report on activities, the research reports on the fish maturity study (pages 10 to 19) and the BRUV surveys (pages 20 to 28) are presented.

B. FISH MATURITY STUDY

B.1. Objectives and research questions

This study component aims to better understand the life cycle of the Golden African Snapper (*Lutjanus fulgens*), a predatory demersal fish species. This information will be used to inform government, managers and fishers for a more sustainable management of the fishery. Specifically, this research aims to understand the general aspects of reproductive biology of this species, including maturation, and meet the following objectives:

- Collecting crucial information for fisheries management, including: *length-at-maturity*, *determination of the spawning seasons* and *length-weight relations*.

- Provide recommendations based on fish size and identify times of the year that are critical for the species reproduction.

B.2. Project deliverables

Expected project deliverables, as defined in The Agreement	Comments
"*Description of the reproductive biology and –if possible- life cycle of five demersal fish species*"	Study of five species was deemed unfeasible within the time and budget available (see Output B.1.2). Paragraph modified as follows: "Description of the reproductive biology and life cycle of one demersal species (*L. fulgens*)" **Achieved**, see *RESULTS*, page 15
"*Identified of the size corresponding to reproductive age of the five demersal fish species.*"	Otoliths for age estimations were collected for future potential studies but their analysis has been deemed unfeasible within the time of the project. Paragraph modified as follows: "*Identify the size-at-50%-maturity of the demersal species* Lutjanus fulgens*, as well as length and weight distributions of the different maturity stages*" **Achieved**, see *RESULTS*, page 15
"Comparison of average catch size in relation to reproductive size per demersal fish species"	**Achieved**, see *DISCUSSION*, page 19
"Written recommendations for fishing regulations (size quota, seasonal closures to avoid reproductive season or area or other)"	**Achieved**, see *DISCUSSION*, page 19
"Guided visit to the laboratory and demonstration of the reproductive biology research in action for tourists and hotel visitors."	**Achieved**, see Output B.4

B.3. Outputs

Output B.1 Design and preparation

Output B.1.1 Development of field-sampling methods: including: **1)** meetings with experts from the University of Exeter in the UK; **2)** literature research; **3)** defining and developing protocols, including procedure for extraction, fixation and transport of the gonads that is feasible within the resources available in São Tomé and Principe; **4)** Selection of sampling sites, which involved analysis of landing data. Initially, only Burras was going to be involved in the project (as described in the Agreement); but four communities were finally selected instead (Abade, Hospital Velho, Sto António, Campanha), as none of the monthly landings of any community alone reached the minimum sample size.

Output B.1.2 Selection of the study species: including **1)** developing criteria for selecting the species; **2)** analysis of Omali Vida Nón's landing data to understand which species are caught more often; **3)** final selection of species after first phase of testing the methods, in which the costs and feasibility of studying

the selected species were assessed. Conducting a maturity study on five species was considered unfeasible as explained below. Initially, three species of Lutjanid snappers were selected for the study: *Lutjanus fulgens*, *L. dentatus* and *L. goreensis*. The study was finally restricted to *Lutjanus fulgens*, which is captured in higher number and its small size makes it affordable within the budget available. The study of the other two species was deemed unfeasible due to: **A)** not captured in enough numbers; **B)** not enough budget for purchasing enough sample size for three different species; **C)** not enough time to process samples necessary to provide robust results for three species.

Output B.1.3 Purchase of materials: including: **1)** laboratory materials (UK); **2)** fixative solutions (formaldehyde, ethanol and distilled water, in UK and STP) and **3)** cooling boxes for transporting the fish. This was done continuously over the whole project duration.

Output B.2 Training

Output B.2.1 Training focal points: including **1)** meetings in the five fishing communities to explain and present the research activity; **2)** selection of focal points (fish traders or *palaiês*) that would purchase the fish, conduct landing survey and send it to Belo Monte; **3)** training of focal points. Training included: **A)** conducting landing surveys; **B)** maximum number of fish to be purchased and how to conduct random sampling; **C)** storing the fish for transport to ensure fish from different canoes are kept separated. At least a monthly visit to the focal points in the five communities was conducted to ensure their engagement in the project (15 visits to the communities were conducted). From August 2018 onwards, fish was mainly purchased by the lead researcher in Hospital Velho, so as to allow for a more intensive sampling (focal points' availability for purchasing fish was limited).

Output B.2.2 Training in histological processing: In June 2018, the lead researcher Guillermo Prieto Porriños was trained on histological techniques (mounting and staining the samples and observation in the microscope). Training was delivered for eight days (80 hours) by Dr Anke Lange in Prof Charles Tyler's laboratory at the University of Exeter.

Output B.3 Data collection and processing

Output B.3.1 Sampling and dissection of fish: From April 2018 to March 2019, 657 individuals were sampled, and 367.3 kg of fish purchased. In comparison with other published maturity studies on snappers, sample size is up to 6 times higher than many published studies on snapper maturity (e.g. Nanami *et al.*, 2010; Shimose and Nanami, 2015). For each fish sampled, whole gonads, otoliths, fin clips and stomach contents were collected, labelled and preserved. In addition, its weight, length, gonad weight and length and maturity stage have been recorded. On average, 40 minutes were required to process each individual fish, including time required for: **1)** dissection, extraction and labelling of the samples; **2)** preparation of the fixative solutions; **3)** changing solutions and sealing samples for travelling (from 10% formaldehyde to 10% ethanol). Approximately 600h (50h per month) have been invested in field sampling.

Output B.3.2 Processing tissue samples: In order to assess the accuracy of the macroscopic assessment of maturity done in the field, a subsample of the collected gonads was analysed using histological techniques, which are more accurate for assessing fish maturity. This includes: **1)** cutting 3mm sections the anterior, posterior and middle portions of each gonads; **2)** twelve-hour dehydration process of the 3mm sections; **3)** embedding each 3mm section in separated blocks of paraffine (wax); **4)** cutting with the microtome 5μm sections of the blocks with the samples embedded and mounting them on a mounting plate; **5)** staining the mounted sections using the haematoxylin-eosin method. In total, 312 samples have

been processed up to stage 3 (embedding in wax), involving 192 hours of laboratory work. Of those, 106 samples have been processed up to stage 5, involving 150 hours of laboratory work. The remaining samples will be analysed in the future, in order to provide a more comprehensive analysis of gonad development.

Output B.3.3 Analysis of sectioned gonads at the microscope: In total, 106 gonads were analysed microscopically, so as to assess the accuracy of the macroscopic assessment done in the field. Analysis was done over 5 days (40 hours).

Output B.3.4 Results: Summarised in the Research report, pages 10 to 19.

Output B.4 Outreach

Output B.4.1 Social media and presentations: This includes **1)** Social media and regular updates in social media and project website showing the progress done in the field (project's website, twitter, Facebook) and in the lab (project's website, Facebook); **2)** Fifteen presentations were given at Belo Monte hotel. From July to December, a presentation and overnight visit to Belo Monte was provided weekly every Monday by the lead researcher, Guillermo Porriños, when available, besides the overnight stays during fish maturity sampling. The standard presentation is available here, which was accompanied by the screening of videos and other resources. In addition, summary findings were presented by Dr Ana Nuno at 5 fishing communities in March 2019 and a regional event on the 1st April 2019 attended by regional government, relevant departments, project partners and fishing communities. A summary report with recommendations was produced and distributed to all relevant stakeholders in English and Portuguese, available here.

Output B.4.2 Visits to the lab: Visits to the laboratory were organised for journalists and photographers that visited the hotel. Although visits by tourists were not organised by the hotel, the lead researcher, Guillermo Porriños, showed his availability.

C. BRUV SURVEYS

C.1. Objectives

Baited Remote Underwater Video stations (BRUVS) were used to better understand spatial variation in presence/absence of species of commercial importance and conservation concern in the island of Principe and to compare their relative abundances between sampling sites. The main objective is to provide baseline information to inform Marine Spatial Planning, which can be used to create a potential network of Marine Protected Areas around the island. On the other hand, this information contributes to understanding how spatial patterns of fish occurrences and diversity correlate with variables such as marine habitat, and whether these patterns may be influenced by fishing pressure.

C.2. Project deliverables

Expected project deliverables, as defined in The Agreement	Comments
"Assessment of key fish species presence, diversity and relative abundance on 10 sites around Principe across 4 seasons (one-year duration baseline study)"	Sampling 4 seasons per year was deemed unfeasible within the time and resources available. In order to increase sample size per site, the island was divided in 6 bigger sectors, instead of 10 sampling sites. Likewise, the sampling effort was increased from 50 sampling points (5 points per site) to 60 (10 points per site). Project deliverable modified to:

	"*Assessment of key fish species presence, diversity and relative abundance on 6 sectors (60 sampling points) around Principe across 2 seasons (one-year duration baseline study)*" **Partially achieved**, video analysis will not be finished until the end of June, although findings have been produced with the data available, see research report, pages 20 to 24.
"Production of distribution maps for selected fish species across Principe, based on interest (threatened species, commercial relevance, unique species, endemic species)"	A map representing the likelihood of finding different types of species will be produced using habitat information from other studies in the island, although this was unfeasible within the study timeframe. Project deliverable modified to: "*Explore differences in distribution of selected fish species, families and functional groups across habitats and sectors"* **Partially achieved**: ANOVA was used to detect differences in CPUE between habitat types. As the video analysis has not concluded yet and the six sectors (NE, E, SE, SW, W, NW) were not equally represented in the videos analysed to date, differences between sector have not been analysed yet and results will be provided by the end of June 2016. In addition, once the video analysis is complete, extra analysis will be done to detect potential differences in the fish species depending at the different deployment times. See *RESULTS* page 21 and *DISCUSSION*, page 24. A distribution map will be produced in the next months.
"Written recommendations on priority marine management areas around Principe based on the collected data to be shared and discussed with relevant stakeholders"	**Achieved**: see *DISCUSSION*, page 24
"Public presentation and at least one environmental awareness event or multiple if spread out over the 6 fishing communities using audio-visual material obtained from BRUVS"	**Achieved**, see Output C.4
"A short video compilation for display at the Belo Monte Museum"	**Achieved**, video material produced and available to Belo Monte when required, see *Output C.4*

C.3. Outputs

Output C.1 Design and preparation

Output C.1.1 Purchasing and building materials and transporting them to the country: this comprised: **1)** purchasing material for BRUV frames, diving weights, bait cages, ropes, amongst others (UK); **2)** building BRUV frames (UK); **3)** purchase and construction of other materials for the frame in Principe; **4)** modifications of the design of BRUV frame to better suit sampling needs.

Output C.1.2 Mapping Principe's marine environment: necessary for designing sampling strategy. This included **1)** digitizing a nautical chart with accurate depth data and transforming it into useful digital files; **2)** visiting fishing grounds and marking their GPS locations and mapping fishing pressure with data from Omali Vida Nón; **3)** participatory mapping of habitat types using fishers' knowledge.
Output C.1.3 Designing final sampling strategy: This involved: **1)** literature review and meetings with experts (UK and STP); **2)** Rapid habitat assessment on 25 random points, done in four days between 28th of June and the 2nd of July, involving 32 hours of field-work; **3)** Using Geographical Information Systems to process the data produced in the Output 2, define the sampling sites and locate sampling points in the map.
Output C.2 Training
Output C.2.1 Testing protocol for deploying BRUVs: During April and May 2018, the protocol for deploying the BRUVs was developed and tested in the field, visiting twenty-nine sampling points and recording 43.5 hours of video during 8 sampling days (72 hours of field work). Sampling sites were specifically chosen by the researcher to evaluate the performance of the method, targeting specific fishing grounds and habitats, using fishers' knowledge and the researcher's criteria.
Output C.2.2 Video analysis: Six videos of different habitat types, collected during the testing phase (Output C.2.1), were analysed to develop the video analysis protocol, recording all the species present and developing a data collection sheet. Approximately 4 hours per video were required for the analysis.
Output C.2.3 Training observers: two observers were trained for video analysis during 20 hours and 5 hours each, using 5 BRUV videos to that end: Atenizia Camblé, a BSc student (University of São Tomé) (Output C.3.1); and Dr. Liliana Colman, a post-doctoral researcher (University of Exeter) (Output C.3.2).
Output C.3 Data collection and processing
Output C.3.1 Completion of first sampling round: 60 sampling points -selected randomly around the island- were visited during July and August 2018, recording 90 hours of underwater videos. This involved the following tasks: **1)** Twelve sampling days over 4 weeks and 100 hours of field work at the sea (approximately 8-10 hours per sampling day); **2)** Purchase of bait in the fish market or directly at the landing sites; **3)** Daily maintenance of materials and downloading videos into hard drives; **4)** Rapid viewing of the videos to ensure the sampling point is valid; **5)** Periodic maintenance of materials.
Output C.3.2 Completion of the second sampling round: 60 random sampling points were visited during December 2018 and January 2019. Same tasks and time as Output C.3.1.
Output C.3.3 Video analysis: Twenty videos of the first phase were analysed by the lead researcher from October 2018 to January 2019 (80 hours) and 30 videos were analysed by Atenizia Camblé (120 hours). Thirty videos from the second phase have been analysed to date by Dr Liliana Colman and analysis will carry on in May and June 2019.
Output C.4 Outreach
Output C.4.1 Social media and presentation: This included: **1)** A short film showcasing the work done during the testing phase (output 4) was produced and posted online in YouTube, the project's website, Facebook, and twitter; **2)** Footage showing emblematic species, including *barracudas* (YouTube, Facebook, twitter); *octopus* (YouTube, Facebook, twitter); *moray eel* (YouTube, Facebook, twitter); *nurse shark* (YouTube, Facebook, twitter) and *snapper* (YouTube). **3)** Periodic updates on social media on research

progress (twitter, project's website, twitter, project's website, project's website); **4)** Fifteen presentations given at Belo Monte hotel to date. From July to December, a presentation and overnight visit to Belo Monte was provided weekly every Monday by the lead researcher, Guillermo Porriños. The standard presentation is available here, which is accompanied by the screening of videos and other resources; **5)** Summary findings were presented by Dr Ana Nuno at 5 fishing communities in March 2019 and a regional event on the 1st April 2019 attended by regional government, relevant departments, project partners and fishing communities; **6)** Some footage was included in Omali Vida Nón's documentary (March 2019) directed by Dário Pequeno Paraíso (available in YouTube).

Output C.4.2 **Follow up studies**: This methodology will be applied over the next five years and used as a monitoring tool at national level.

D. LIST OF MATERIAL TO BE RETURNED TO BELO MONTE

Equipment to be returned to Africa's Eden - Belo Monte at Guillermo Porriños' arrival at Principe Island (June 2019):

- 2 hard drives with BRUV videos, one no longer functional.
- 2 digital scales
- 5x GoPro Hero, one no longer functional, and 6 diving cases, 2 leaking.

E. FINANCIAL REPORT

Exchange rate: 1 EUR = £0.8533	**Staff costs**	**Travel and subsistence**	**Consumables and equipment**	**TOTAL**
Actual expenditure (GBP)	£13,996.93	£7,273.79	£6,822.21	**£28,092.93**
Actual expenditure (EUR)	€16,403.29	€8,524.31	€7,995.09	**€32,922.69**

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).

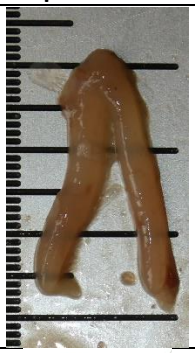
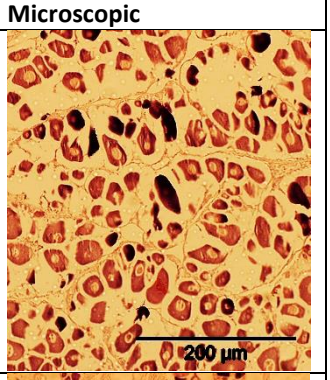
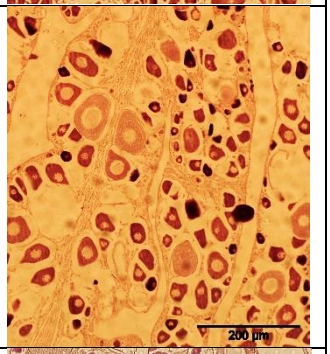
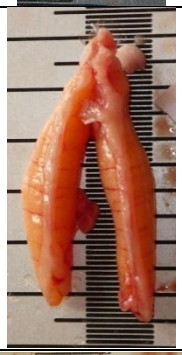
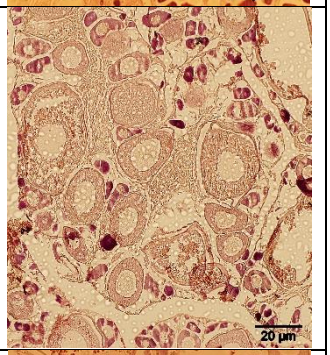
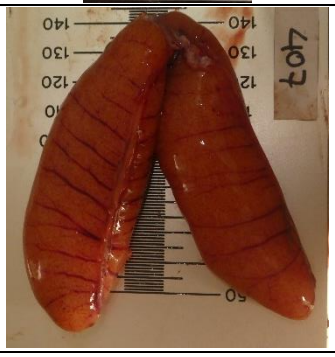
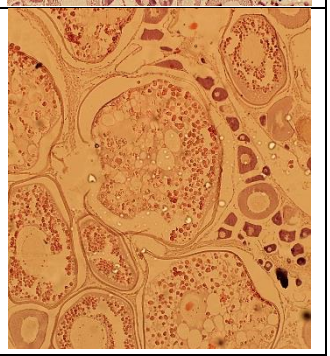
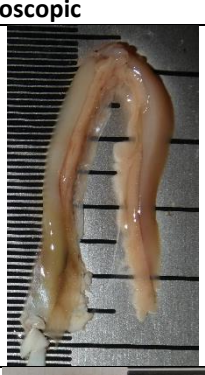
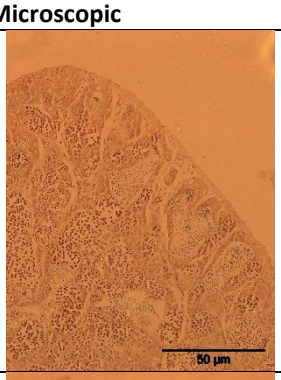
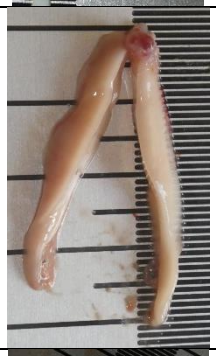
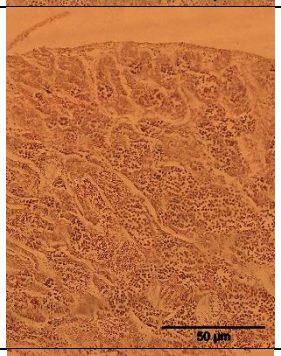
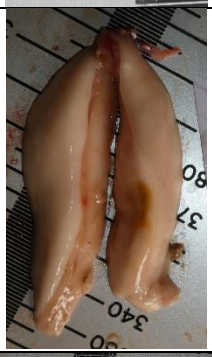
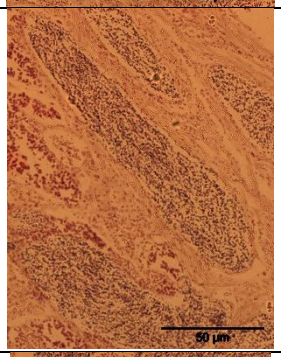
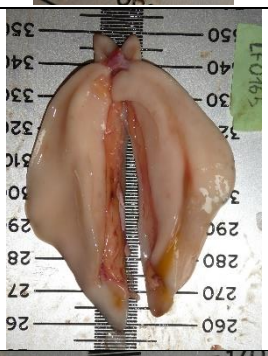
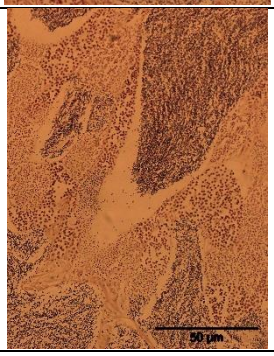
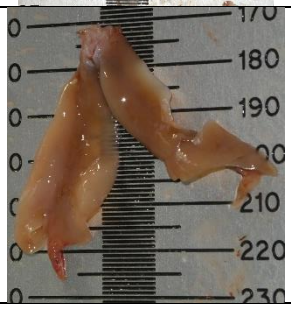
Brown-Peterson Maturity scale (2011)			Macroscopic	Microscopic
F I	**Immature**	**Small ovaries, clear no blood vessels present** Only oogonia and PG oocytes present, thin ovarian wall and little space between oocytes		
F II	**Early Maturing**	**Small ovaries, clear, blood vessels reduced but present** 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.

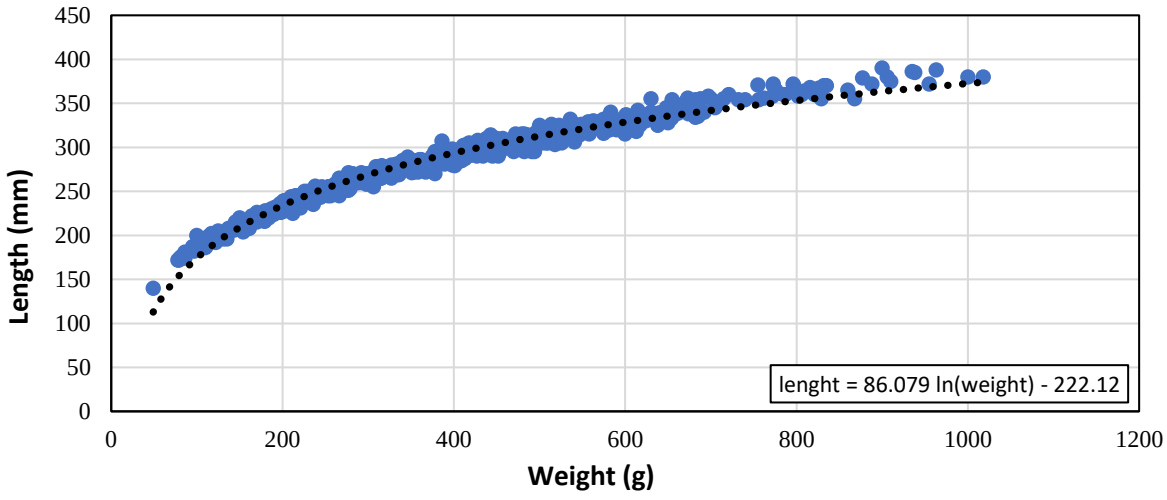


Figure 2: Length (L) vs. Weight (W) of *L. fulgens*. Estimated fitted distribution: *L = 86.079 ln(W) - 222.12*; equivalent to: $W = 14.484e^{0.0113 * L}$

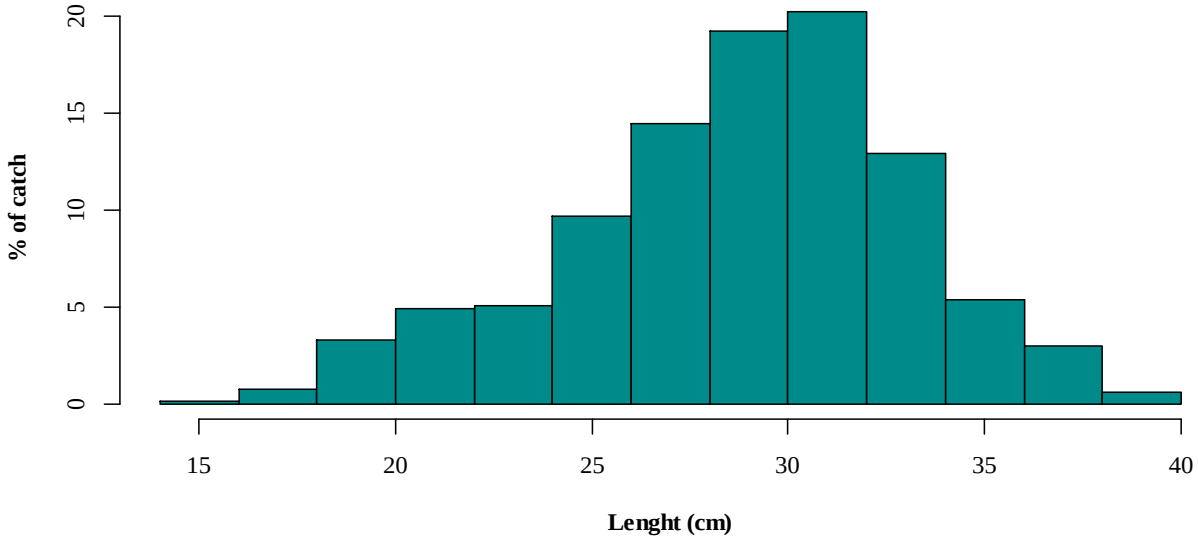


Figure 3: Distribution of length in 628 fish individuals used in this study. Average length of fish caught was 28.8 cm (S.D. 4.4). Fifty-eight percent of the sampled fish were below 30 cm.

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).

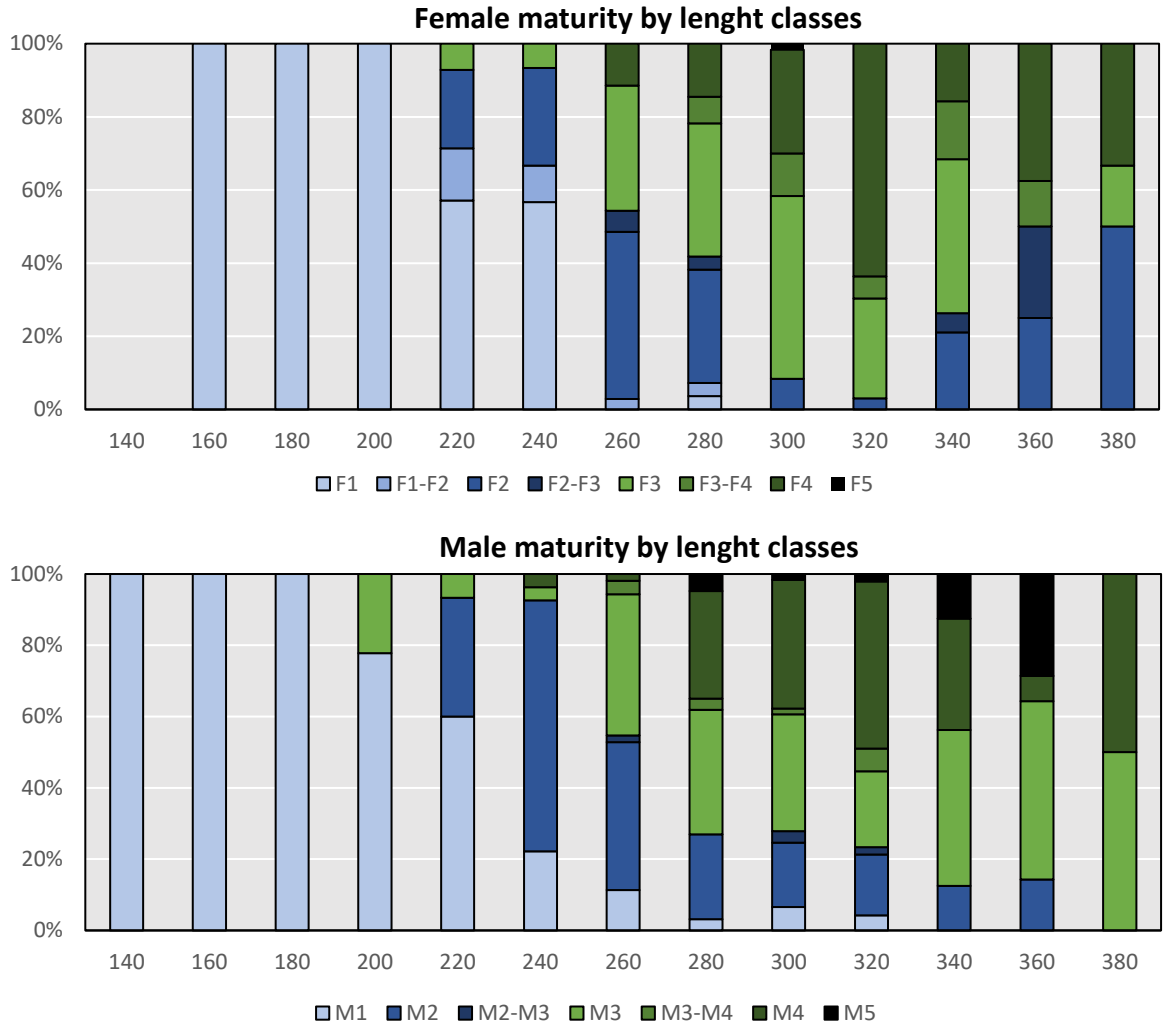


Figure 4: Proportion of each maturity stage at different length classes (N = 628)

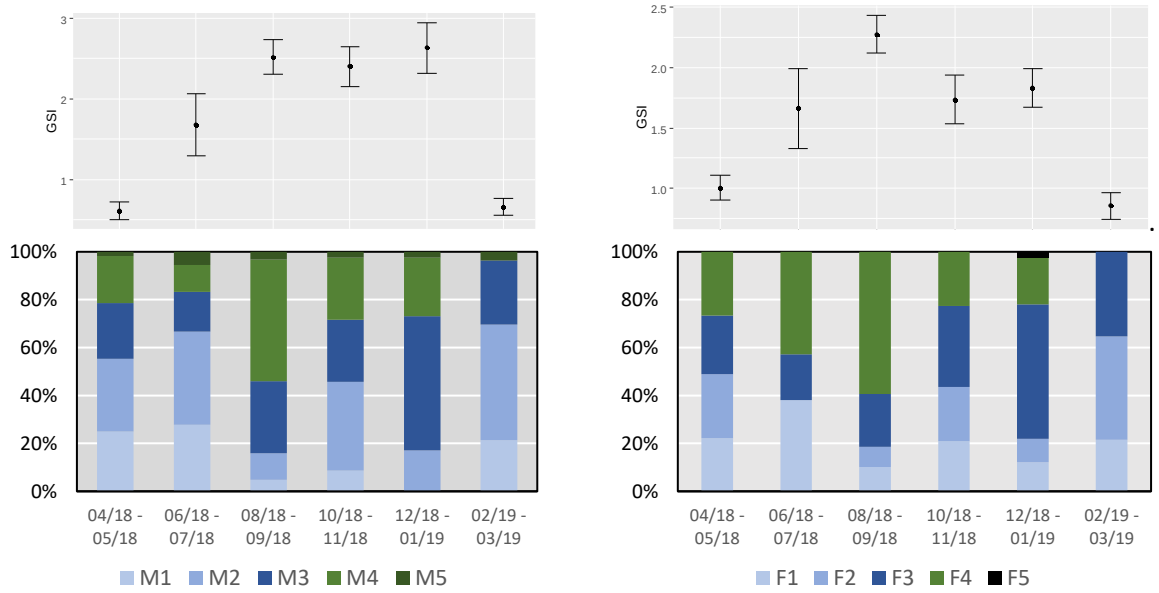


Figure 5: Bimonthly variations of Gonado-Somatic Index and maturity stages of male (left) and female (right) *L. fulgens* (n=628)

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 using histology	Classification in the field: F1	F2	F3	F4	F5	TOTAL
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 using histology	Classification in the field: M1	M2	M3	M4	M5	TOTAL
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.

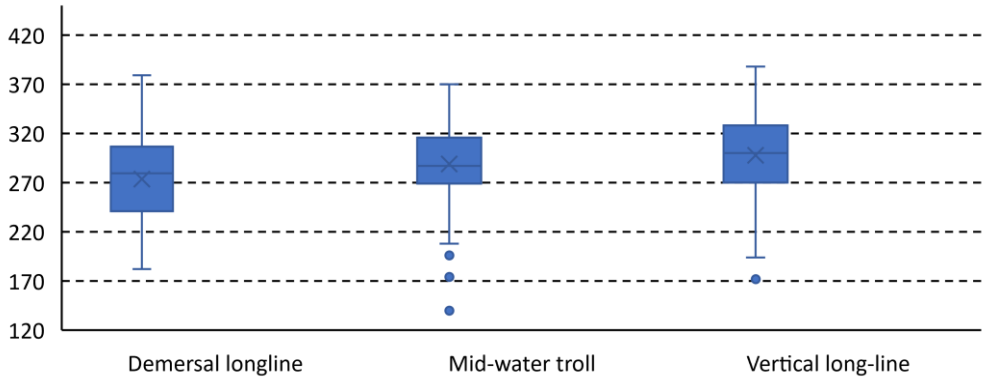


Figure 6: Distribution of length by fishing gear (n=439, no information was available for the remaining samples)

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.

Mapping Principe's fish biodiversity using Baited Remote Underwater Video

A. INTRODUCTION

Baited Remote Underwater Video (BRUV) is a non-invasive technique for studying fish fauna (for example, their presence, relative abundance and behaviour), consisting on attracting fish species towards an underwater camera using a bait (Kelaher *et al.*, 2014). In addition, BRUV systems can also be used for estimation of biomass (using stereo-BRUVs) or used in different environments (both demersal and pelagic, Whitmarsh *et al.* 2017).

When compared to other methods, benefits include being a non-invasive technique (for example, scientific fishing requires harvesting) and fieldwork and data collection that does not require intensive training or previous fish identification skills (for example, underwater visual census require experienced SCUBA divers and accurate identification of fish species underwater) (Brooks *et al.*, 2011). In addition, it creates a permanent record of the sampling and the video material from BRUVs can be used for training students, technicians and researchers on fish identification, as well as being useful for outreach and environmental awareness activities.

The method was used to understand differences in fish composition associated to the different habitats, and to create baseline information on Principe's marine environment for selected key species.

B. METHODS

B.1. Data collection

Five BRUV devices were used for this study. Each device consists on a weighed PVC frame holding a front-facing camera 35 cm over the sea floor, with a bait cage located 120 cm in front of the camera. For each deployment, 600 g of chopped "Fulu fulu" (*Euthynnus alletteratus*) were used, a small species of tuna caught in high numbers by Principe's artisanal fishers during the whole year. Fish was kept frozen in a cooling box until taken to the sea, and only unfrozen immediately deploying the camera. Each BRUV device was deployed for 90 minutes at each sampling point (due to battery life restrictions), tied to a buoy in the surface with a long rope marking the position of the camera. An 8-10 kg weight was tied to the middle of the rope, separated 10 metres to the camera, to prevent waves or very strong surface currents from moving the device.

The study was limited to a maximum depth of 28 metres, due to low visibility below that. The area between 2 and 25-metre deep around the island was divided in six sectors (NE, E, SE, SW, W and NW) of equal size. Ten sampling points were randomly allocated in each of them, setting a minimum distance between them of 400 metres, totalling 60 sampling points (10 per sector) (Hill *et al.*, 2014).

This sampling was conducted twice: one period in July-August 2018 and another one in December 2018-January 2019. For July and August 2018, only five points were sampled per day, deploying 5 BRUV devices simultaneously from 9AM to 11AM. For December 2018 and January 2019, 15 sampling points were deployed per sampling day due to time constrains, deploying 5 BRUV devices in the morning (around 09:00), noon (around 12:00) and afternoon (around 15:00).

B.2. Video analysis

BRUV videos were analysed by three observers, one of them being the lead researcher. All observers were trained for at least 20 hours using previously collected video material from Principe (previous scoping study, September 2017). Comparability of the video analysis will be assessed by June 2019 by the lead researcher, analysing 15 videos analysed by the other two observers and comparing the results. Maximum number of individuals of each species per frame (MaxN) were recorded (Whitmarsh *et al.* 2017), identifying species to the lowest taxonomical level possible. All species were recorded, including sea turtles and invertebrates such as octopuses and other molluscs, crabs and polichaetes, although only finfish was considered for statistical analysis. For each species, MaxN was recorded, alongside the time in the video. The species was only registered again in a new entry if the number of individuals was higher than in the last data entry. Time and position of the species not identified was recorded and will be re-analysed using input of experts. To date, 78 of the videos have been analysed, remaining 32 to be analysed. Twelve videos were lost due to failure of the hard-drives and back-ups.

B.3. Data analysis

Habitat was classified after Abreu et al (2016), distinguishing between three main habitats: *rocky reefs*, *sandy grounds* and *maerl beds* (see Figure 9). Species recorded in the videos were classified by family, trophic group and maximum size of the species (*small*, <30 cm; *medium*, between 30 and 90 cm; and *large*, over 90 cm; average maximum species size 45 cm) using information from FishBase.org. Catch Per Unit Effort (CPUE) was defined as MaxN per hour. Occurrence was defined as presence of a species or functional group per sampling site. MaxN of the small-sized, schooling species *Prionurus biafraensis* and *Paranthias furcifer*, was divided by 10 to make it comparable to other less numerous species (1 school unit = 10 fish).

ANOVA was used to detect differences in CPUE between habitat types. As the video analysis has not concluded yet and the six sectors (NE, E, SE, SW, W, NW) were not equally represented in the videos analysed to date, differences between sector have not been analysed yet and results will be provided by the end of June 2016. In addition, once the video analysis is complete, extra analysis will be done to detect potential differences in the fish species depending at the different deployment times.

C. RESULTS

To date, 78 videos (112 hours) have been analysed, resulting in the identification of 92 different species (Table 7); we were not able to assign 12.5% of the data entries to any taxa for now. The most common family in terms of occurrence (number of species per sampling site) was Carangidae (pelagic; jacks and pompanos), comprising 16% of all the observed species, with a total CPUE of 3.16 fish per hour. CPUE of carangids did not show significant differences between habitats ($p>0.1$).

Snappers were most common in rocky habitats, showing significantly higher CPUE and occurrence than sandy and maerl habitats ($p<0.001$). However, they were also occasionally found in sandy and maerl habitats and strongly interacting with the bait cage (Figure 9).

CPUE for elasmobranchs was 0.04 sharks per hour and 0.06 rays per hour. Three different species of sharks were identified: nurse shark (*Ginglymostoma cirratum*), lemon shark (*Negaprion brevirostris*) and an unidentified hammerhead shark. Two different species of rays were identified, both belonging to Myliobatiformes: *Taeniura grabata* and *Daysatis pastinaca*. All sharks recorded were found in December/January and 3 out of 4 of them from 15:00 onwards.

CPUE was largely dominated by predatory fish for all the habitat types (Figure 8). Herbivore fish are almost absent from sandy grounds and maerl beds, with small, medium and large tertiary consumers comprising 57% of the total MaxN in maerl and 61% in sand. CPUE of rocky habitats is significatively higher than sandy grounds and maerl beds, for all the trophic and size categories ($p<0.001$). Total MaxN for rocky habitats is comprised by medium and small herbivores (10%); by small, shoal-forming, medium and large secondary consumers (50%) and small, medium and large tertiary consumers (40%).

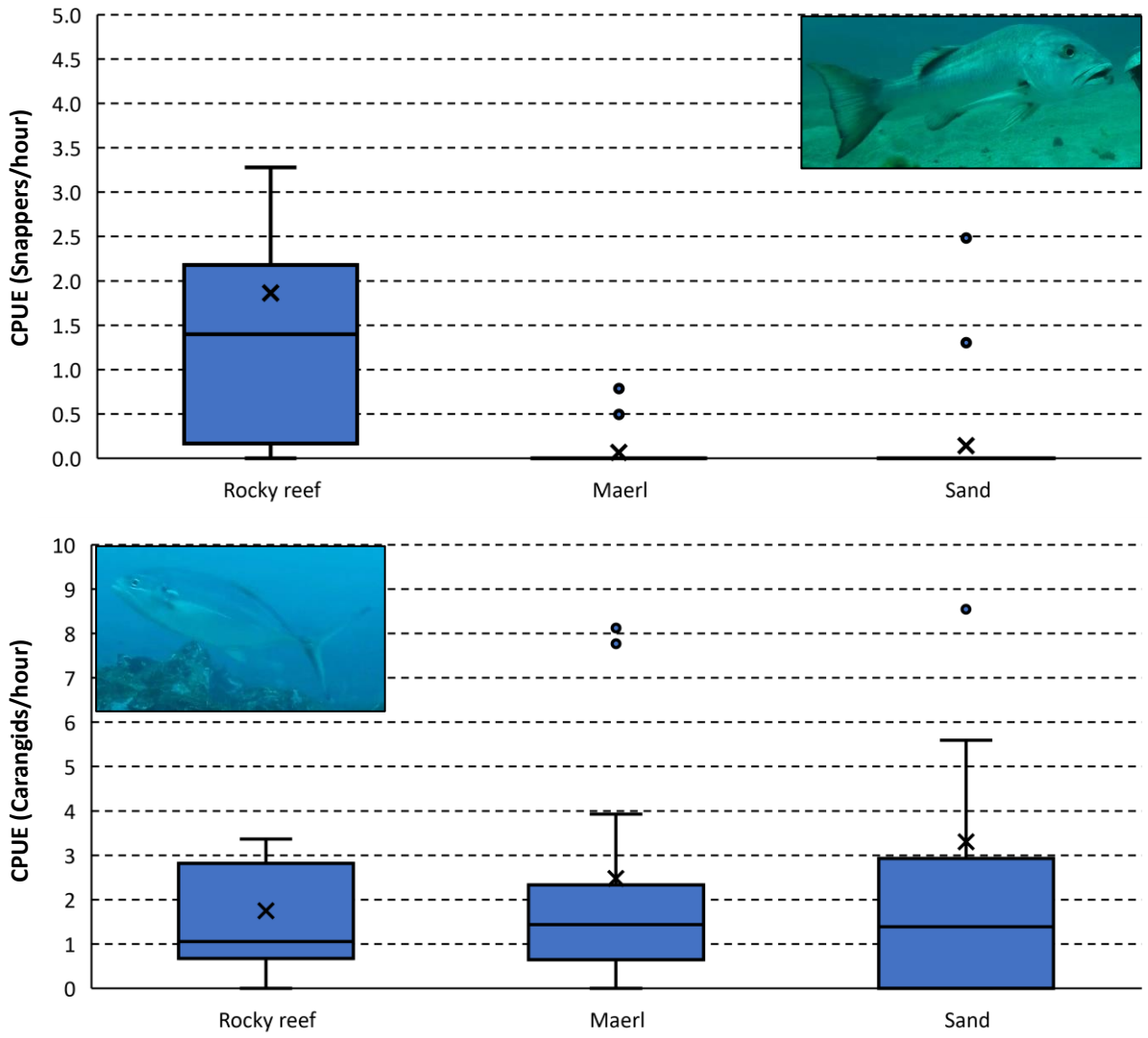


Figure 7: CPUE by habitat of lutjanid snappers and carangids.

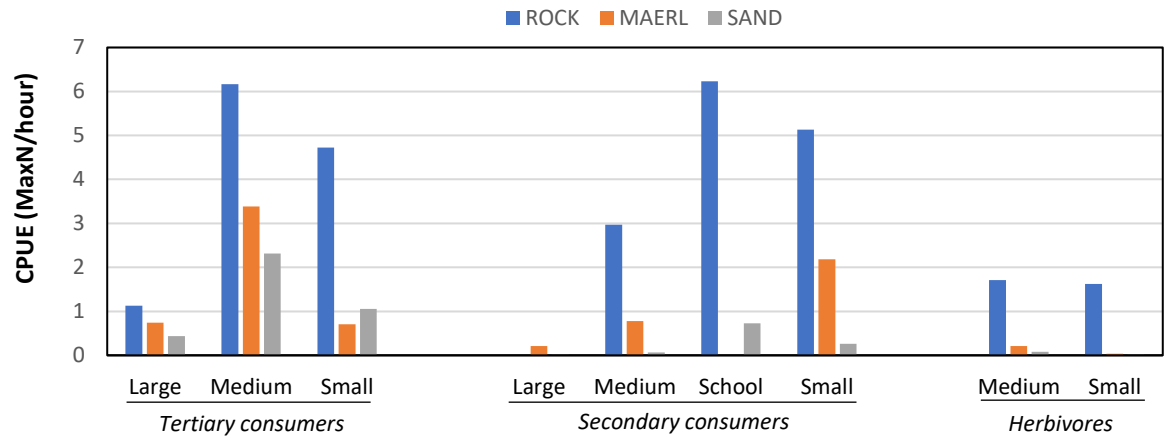


Figure 8: CPUE of the different trophic groups of finfish registered in the BRUVs: Large (max length =<90cm); Medium (max length between 30 and 89 cm); Small (max length <30cm); School (schooling species *Prionurus biafraensis* and *Paranthias furcifer*, below 30cm). For all the categories, CPUE was expressed in MaxN per hour, except for "School" (the unit for schooling species was considered to be 10 fish, so as to reduce the contrast).

Figure 9: Three main habitat types. **A)** Rocky reef, with corals (marked with an arrow); **B)** Sandy ground, with a Brown African Snapper (*Lutjanus dentatus*) attacking the bait cage; **C)** Maerl bed with a nurse shark (*Ginglymostoma cirratum*) approaching the BRUV device.

D. DISCUSSION

The results obtained for carangids suggest that carangids are highly motile species without a preferential habitat. This means that any spatial management measure -such as no-take zones- designed to protect these species would probably not have a strong positive impact on its populations, given that the geographical range of these species would probably surpass the boundaries of the managed areas. However, the ubiquity of these species and especially *Caranx crysos* and *Carangoides bartholomaei* makes these two species a robust indicator species to detect long-term changes in the fishery using BRUVs.

The significantly higher presence and MaxN of snappers in rocky habitats indicate that snappers are probably found in these grounds for most of the time. However, the presence of snappers in sandy grounds and maerl beds, and especially the fact that some snappers attacked the bait cage, indicates that snappers might leave the rocky reefs and go to sandy habitats to hunt. This means that management or protection of rocky habitats is a priority in order to protect snappers, but it would also be necessary to create a buffer zone around the rocky reefs, in order to protect them when they adventure out to hunt. To estimate the appropriate size of the buffer zone, information from published studies should be collected to estimate the geographical range for these species or close relatives.

CPUE of elasmobranchs was markedly lower when compared to other parts of the world (Jabado *et al.*, 2018), and this value could be amongst the lowest CPUE recorded in the world. Given that sharks are more active at dawn, this low abundance of sharks might be related to the sampling strategy, and the fact that 3 out of 4 of the sharks recorded were found from 15:00 onward might be a consequence of this. The strong dominance of predators over herbivorous fish does not necessarily reflects the actual trophic structure of the system, but it might be due to the lack of other taxa different than finfish and the fact that herbivorous fish might not be attracted to the bait at all.

Future improvements of the study include increasing sampling the same points in the morning (09:00) and in the evening (15:00), to account for potential differences in fish behaviour at different times of the day.

E. SPECIES LIST

Table 7: Preliminary species list based on XX videos analysed so far. Information on family, trophic level, trophic group and size (*Large*, max length =<90cm; *Medium*, max length between 30 and 89 cm; *Small*: max length <30cm) from FishBase.org

species	Occurrences (nº points)	Family	Trophic level	Size	Trophic group
Abudefduf hoefleri	3	Pomacentridae (damselfishes)	2.7	Small	Herbivores
Abudefduf saxatilis	2	Pomacentridae (damselfishes)	2.7	Small	Herbivores
Abudefduf taurus	1	Pomacentridae (damselfishes)	2.1	Small	Herbivores
Acanthocybium solandri	1	Scombridae (wahoo)	4.3	Large	Tertiary consumers
Acanthostracion guineensis	2	Ostraciidae (cowfishes)	2.4	Medium	Herbivores
Acanthostracion notacanthus	3	Ostraciidae (cowfishes)	2.4	Medium	Herbivores

Acanthurus monroviae	15	Acanthuridae (doctorfishes)	2.5	Small	Herbivores
Aluterus scriptus	6	Monacanthidae (filefishes)	2.91	Large	Herbivores
Aulostomus strigosus	1	Syngnathiformes (trumpetfishes)	4.2	Medium	Tertiary consumers
Balistes carolinensis	7	Balistidae (triggerfishes)	4.1	Medium	Tertiary consumers
Balistes punctatus	7	Balistidae (triggerfishes)	3.4	Medium	Secondary consumers
Bodianus pulcellus	3	Labridae (wrasses)	3.6	Small	Secondary consumers
Bodianus speciosus	13	Labridae (wrasses)	3.6	Medium	Secondary consumers
Bothus guibei	16	Pleuronectiformes (flatfishes)	3.7	Small	Secondary consumers
Cantherhines pullus	10	Monacanthidae (filefishes)	2.6	Small	Herbivores
Canthidermes sufflamen	1	Balistidae (triggerfishes)	3.5	Medium	Secondary consumers
Canthigaster supramacula	7	Tetraodontidae (puffers)	3	Small	Secondary consumers
Carangoides bartholomaei	36	Carangidae (jacks and pompanos)	4.5	Medium	Tertiary consumers
Caranx crysos	37	Carangidae (jacks and pompanos)	4.1	Medium	Tertiary consumers
Caranx hippos	2	Carangidae (jacks and pompanos)	4.1	Medium	Tertiary consumers
Caranx latus	1	Carangidae (jacks and pompanos)	4.2	Medium	Tertiary consumers
Caranx lugubris	2	Carangidae (jacks and pompanos)	4.5	Large	Tertiary consumers
Cephalopholis nigri	11	Serranidae, epinephelinae (groupers)	4.1	Small	Tertiary consumers
Cephalopholis taeniops	9	Serranidae, epinephelinae (groupers)	4.1	Small	Tertiary consumers
Chaetodon hoefleri	1	Chaetodontidae (butterflyfishes)	3.5	Small	Secondary consumers
Chaetodon robustus	1	Chaetodontidae (butterflyfishes)	3.3	Small	Secondary consumers
Chilomycterus reticulatus	1	Diodontidae (porcupinefishes)	3.5	Medium	Secondary consumers
Cirrhitus atlanticus	6	Cirrhitidae (hawkfishes)	3.6	Small	Secondary consumers
Clepticus africanus	2	Labridae (wrasses)	3.5	Small	Secondary consumers
Coris atlantica	13	Labridae (wrasses)	3.5	Small	Secondary consumers
Dactylopterus volitans	15	Dactylopteridae (flying gurnard)	3.65	Medium	Secondary consumers
Dasyatis pastinaca	3	Myliobatiformes (stingrays)	4.1	Rays	Tertiary consumers

Decapterus punctatus	1	Carangidae (jacks and pompanos)	4.4	Small	Tertiary consumers
Diodon sp	3	Diodontidae (porcupinefishes)	3.9	Medium	Secondary consumers
Echeneis naucrates	3	Echenidae (remoras)	3.7	Medium	Secondary consumers
Elagatis bipinnulata	7	Carangidae (jacks and pompanos)	4.27	Large	Tertiary consumers
Enchelycore nigricans	11	Anguiliformes (eels and morays)	4.5	Medium	Tertiary consumers
Epinephelinae	1	Serranidae, epinephelinae (groupers)	4.1	Medium	Tertiary consumers
Epinephelus adscensionis	3	Serranidae, epinephelinae (groupers)	4.1	Medium	Tertiary consumers
Epinephelus aeneus	1	Serranidae, epinephelinae (groupers)	4.02	Large	Tertiary consumers
Fistularia tabacaria	1	Syngnathiformes (trumpetfishes)	3.7	Medium	Secondary consumers
Ginglymostoma cirratum	2	Sharks	4.15	Large	Tertiary consumers
Grammonus longhursti	1	Bythitidae (viviparous brotulas)	3.4	Small	Secondary consumers
Hemiramphus balao	1	Beloniformes (needlefishes)	3.9	Small	Secondary consumers
Holocantus africanus	9	Pomacanthidae (angelfishes)	2.86	Small	Herbivores
Holocentrus adscensionis	10	Holocentridae (squirrelfishes, soldierfishes)	3.11	Medium	Secondary consumers
Kyphosus incisor	5	Kyphosidae (sea chubs)	2	Medium	Herbivores
Labrisomus nuchipinnis	1	Labrisomidae (labrisomids)	3.6	Small	Secondary consumers
Lagocephalus laevigatus	2	Tetraodontidae (puffers)	4	Medium	Tertiary consumers
Lethrinus atlanticus	15	Lethrinids (emperor)	3.54	Medium	Secondary consumers
Lutjanus agennes	8	Lutjanidae (snappers)	4	Large	Tertiary consumers
Lutjanus dentatus	10	Lutjanidae (snappers)	4	Large	Tertiary consumers
Lutjanus fulgens	2	Lutjanidae (snappers)	4	Medium	Tertiary consumers
Lutjanus goreensis	4	Lutjanidae (snappers)	4	Medium	Tertiary consumers
Melichthys niger	1	Balistidae (triggerfishes)	2.4	Medium	Herbivores
Microphis aculeatus	1	Syngnathiformes (trumpetfishes)	3.4	Small	Secondary consumers
Microspathodon frontatus	2	Pomacentridae (damselfishes)	2.3	Small	Herbivores

Mulloidichthys martinicus	10	Mullidae (goatfishes)	3.2	Medium	Secondary consumers
Muraena melanotis	3	Anguiliformes (eels and morays)	3.5	Medium	Secondary consumers
Myrichthys pardalis	1	Anguiliformes (eels and morays)	3.5	Medium	Secondary consumers
Myripristis jacobus	5	Holocentridae (squirrelfishes, soldierfishes)	3.39	Small	Secondary consumers
Negaprion brevirostris	1	Sharks	4.3	Large	Tertiary consumers
Ophichthus ophis	2	Anguiliformes (eels and morays)	4.5	Medium	Tertiary consumers
Pagrus caeruleostictus	1	Sparidae (porgies)	3.7	Medium	Secondary consumers
Pagrus pagrus	1	Sparidae (porgies)	3.9	Large	Secondary consumers
Paranthias furcifer	15	Serranidae (seabasses)	3.2	Small	Secondary consumers
Pomadasys rogeri	5	Haemulidae (grunts)	3.6	Medium	Secondary consumers
Prionurus biafraensis	7	Acanthuridae (doctorfishes)	2.5	Small	Herbivores
Pseudupeneus prayensis	1	Mullidae (goatfishes)	3.2	Medium	Secondary consumers
Rypticus saponaceus	12	Serranidae (seabasses)	4.1	Medium	Tertiary consumers
Scarus hoefleri	4	Scaridae (parrotfishes)	2	Medium	Herbivores
Selar crumenopthalmus	1	Carangidae (jacks and pompanos)	3.8	Medium	Secondary consumers
Seriola rivoliana	3	Carangidae (jacks and pompanos)	4.45	Large	Tertiary consumers
Serranus cabrilla	2	Serranidae (seabasses)	3.4	Medium	Secondary consumers
Serranus pulcher	14	Serranidae (seabasses)	3.4	Small	Secondary consumers
Sparisoma choati	13	Scaridae (parrotfishes)	2	Small	Herbivores
Sparisoma rubripinne	6	Scaridae (parrotfishes)	2	Medium	Herbivores
Sphoeroides marmoratus	16	Tetraodontidae (puffers)	3.4	Small	Secondary consumers
Sphyraena barracuda	15	Sphyraenidae (barracudas)	4.49	Large	Tertiary consumers
Sphyrnidae	1	Sharks	4.2	Large	Tertiary consumers
Stephanolepis hispidus	1	Monacanthidae (filefishes)	2.6	Small	Herbivores
Synodus synodus	2	Synodontidae (lizardfishes)	4.2	Small	Tertiary consumers
Taeniura grabata	3	Myliobatiformes (stingrays)	4	Rays	Tertiary consumers

Thalassoma newtoni	8	Labridae (wrasses)	3.5	Small	Secondary consumers
Trachinotus ovatus	2	Carangidae (jacks and pompanos)	3.7	Medium	Secondary consumers
Xyrichthys novacula	13	Labridae (wrasses)	3.51	Small	Secondary consumers
NA	72	NA	NA	NA	NA

References

Abreu, A. D. *et al.* (2016) 'Final Report Scientific Expedition Bio-Principe 2016', *IU-ECOAQUA, Universidad de las Palmas de Gran Canaria, Spain*.

Allen, G.-R. (1985) 'Snappers of the world', *FAO SPECIES CATALOGUE*, 6(125).

Brooks, E. J. *et al.* (2011) 'Validating the use of baited remote underwater video surveys for assessing the diversity , distribution and abundance of sharks in the Bahamas', 13(Iucn 2010), pp. 231–243. doi: 10.3354/esr00331.

Brown-Peterson, N. J. *et al.* (2011) 'A Standardized Terminology for Describing Reproductive Development in Fishes A Standardized Terminology for Describing Reproductive', *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science*, 3, pp. 52–70. doi: 10.1080/19425120.2011.555724.

Cadrin, S. X. (2000) 'Advances in morphometric identification of fishery stocks', *Reviews in Fish Biology and Fisheries*, 10(1), pp. 91–112. doi: 10.1023/A:1008939104413.

Hill, N. A. *et al.* (2014) 'Quantifying Fish Assemblages in Large , Offshore Marine Protected Areas: An Australian Case Study', *PLoS ONE*, 9(10). doi: 10.1371/journal.pone.0110831.

Jabado, R. W. *et al.* (2018) 'Low abundance of sharks and rays in baited remote underwater video surveys in the Arabian Gulf', *Nature Scientific Reports*. Springer US, 15597(8), pp. 1–11. doi: 10.1038/s41598-018-33611-8.

Johnson, R., Wolf, J. and Braunbeck, T. (2009) 'Guidance Document for the Diagnosis of Endocrine-Related Histopathology of Fish Gonads', *OECD report*, pp. 1–96.

Kelaher, B. P. *et al.* (2014) 'Changes in Fish Assemblages following the Establishment of a Network of No-Take Marine Reserves and Partially-Protected Areas', 9(1). doi: 10.1371/journal.pone.0085825.

Kilongo, K., Barros, P. and Diehdiou, M. (2007) 'Diet of large-eye dentex Dentex macrophthalmus (Pisces: Sparidae) off Angola and Namibia', *African Journal of Marine Science*, 29(1), pp. 49–54. doi: 10.2989/AJMS.2007.29.1.4.69.

Morais, L. *et al.* (2015) *Lutjanus fulgens*, *The IUCN Red List of Threatened Species 2015*. Available at: http://www.iucnredlist.org/details/194389/0.

Nanami, A. *et al.* (2010) 'Age, growth and reproduction of the humpback red snapper *Lutjanus gibbus* off Ishigaki Island, Okinawa', *Ichthyological Research*, 57(3), pp. 240–244. doi: 10.1007/s10228-010-0160-8.

Shimose, B. T. and Nanami, A. (2015) 'Age , growth , and reproduction of blackspot snapper *Lutjanus fulviflammus* (Forsskal 1775) around Yaeyama Islands , southern Japan , between 2010 and 2014', *Applied Ichtyology*, 31, pp. 1056–1063. doi: 10.1111/jai.12894.

Tous, P. (2015) 'Consulta para a avaliação dos recursos de peixes demersais', *Report to the Fisheries Department, Ministry of Economy and International Co-operation, São Tomé and Príncipe*.

Whitmarsh, S. K., Fairweather, P. G. and Huveneers, C. (2017) 'What is Big BRUVver up to? Methods and uses of baited underwater video', *Reviews in Fish Biology and Fisheries*. Springer International Publishing, 27(1), pp. 53–73. doi: 10.1007/s11160-016-9450-1.

Woods, M. K. *et al.* (2003) 'Size and age-at-maturity of female red snapper *Lutjanus campechanus* in the Northern Gulf of Mexico', *Gulf and Caribbean Fisheries Institute*, 54, pp. 526–537