Preliminary estimates of sex ratio, spawning season, batch fecundity and length at maturity for Indian Ocean yellowfin tuna

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Executive Summary

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This paper describes preliminary work to estimate reproductive parameters for yellowfin tuna (*Thunnus albacares*) in the Indian Ocean as part of the 'GERUNDIO' project[1]. The 2018 stock assessment for yellowfin tuna in the Indian Ocean (IOTC) indicated that the stock is overfished and subject to overfishing (Fu et al. 2018; IOTC 2020). The assessment base case model used a maturity ogive for the western Indian Ocean from Zudaire et al. (2013), which used the 'cortical alveolar' oocyte development stage and above as the threshold indicating a fish was mature. Zudaire et al. (2013) also estimated a maturity ogive using the (older) 'advanced vitellogenic' oocyte stage as the maturity threshold, which is a more reliable threshold for determining a female is mature and contributing to egg production (Schaefer 2001). The two length based ogives from Zudaire et al. (2013) were converted to age based ogives in Langley (2015) and age at 50% maturity was estimated to be ~9 quarters and ~12 quarters, respectively. Sensitivity

[1] Collection and analysis of biological samples of tropical tunas, swordfish, and blue shark to improve age, growth and reproduction data for the Indian Ocean Tuna Commission (IOTC), FAO Contract No. 2020/SEY/FIDTD/IOTC - CPA 345335.

analyses conducted in Langley (2015) using the alternate maturity ogive gave a slightly more pessimistic outlook for the status of the stock relative to the base case model.

The aim of the current study was to: (i) identify and compile gonad samples, histological sections or histological data from previous studies in the Indian Ocean; (ii) design a gonad sampling plan to collect representative samples across the Indian Ocean, particularly where gaps currently exist; (iii) develop and apply a standardized histological based classification method to all ovary samples; (iv) provide training on sample collection and reproductive analysis; and (iv) produce updated estimates of key biological reproductive parameters.

A total of 1145 yellowfin tuna were sampled in the project (476 females and 669 males). The individuals were collected in 2020-2021 predominantly from purse seine fisheries unloading at canneries in the western Indian Ocean. Histological sections were prepared for 212 ovary samples (i.e., females only), which were read by project partners using an agreed classification system after receiving training at an online workshop in July 2021. Additional ovaries collected in the current project will be processed soon to update the current analysis.

Data from an additional 921 yellowfin tuna (476 females and 445 males) were obtained from previous projects (EMOTION database, see Bodin et al. 2018), which included histological data from 388 females classified using a similar classification scheme to that agreed in the project. The individuals were collected between 2009-2019 and were also predominantly from the western Indian Ocean.

Preliminary estimates of sex ratio, spawning periodicity, batch fecundity and length at maturity are provided for yellowfin tuna predominantly from the western Indian Ocean. Further work is required to finalize the analyses, particularly the spawning periodicity and maturity results. The analysis is currently based on data from a subset of the ovaries collected in the GERUNDIO project as it was not possible to process all the ovaries collected or to undertake the required cross-checking (re-reading) of histological sections within the project timeframe. In addition, it was not possible to cross-check the histological data obtained from the EMOTION database. Postovulatory follicles were not recorded in this study to estimate spawning fraction (the proportion of females spawning per day), but it may be possible to estimate when the data are available. There was insufficient data to examine region-specific reproductive parameters in this project since most of the gonads were sampled in the western Indian Ocean. Genetic studies indicate that the population structure of yellowfin tuna within the Indian Ocean is complex (Kunal et al. 2013; Barth et al. 2017), and a recent study found evidence for genetic structure north and south of the equator (Grewe et al. 2020).

We recommend that additional gonad samples are collected and analysed from all regions of the Indian Ocean, but particularly from the northern and eastern areas (from all size classes and months) to improve the reproductive parameters obtained in this project. Fish >60 cm fork length (~minimum size at maturity) are particularly important to increase the sample size available for maturity, fecundity and spawning fraction analyses. Monthly sampling is important in reproductive studies to obtain reproductive data throughout the year. We also recommend collecting additional gonad samples from different fishing gears (e.g., longline) to improve the size coverage and have better representation of the population spatial range. Continuing to collect and analyse gonads

over time will be particularly important for assessing inter-annual variation in reproductive parameters.

1. Introduction

Yellowfin tuna (*Thunnus albacares*) is a highly mobile pelagic species inhabiting the tropical and subtropical waters around the globe. It supports the second largest tuna fishery worldwide, being an important source of nourishment and livelihood for numerous nations (Guillotreau et al. 2017, FAO 2020). Over the last three decades, yellowfin tuna has been subject to high fishing pressure, with global catches reaching about 1.45 million tonnes in 2018 (FAO 2020). These high exploitation rates have raised concerns about the urgent need of effective management measures for the conservation of the species. This is particularly the case for yellowfin tuna in the Indian Ocean, where the most recent stock assessment by the Indian Ocean Tuna Commission (IOTC) indicated that the yellowfin tuna stock in the IOTC area is overfished and subject to overfishing (Fu et al. 2018; IOTC 2021).

Fishery stock assessment models are demographic analyses designed to determine the effects of fishing on fish populations, where reproductive information, together with age and growth estimates, provide the fundamental basis for assessing the condition and resilience of a fish stock (Methot & Wetzel 2013). For example, knowledge of size/age at maturity (i.e., the length or age at which individuals are reproductively active) is critical as it influences future recruitment (Mangel et al. 2010). Another important element when defining reproductive potential includes fecundity, or the numbers of eggs that may be spawned in each season (Morgan et al 2009; Murua et al 2003).

In the case of yellowfin tuna from the Indian Ocean, mean size at maturity, i.e., length at which 50% individuals are classed as mature (L_{50}), has been estimated at 75.0 cm to 120.0 cm fork length (FL). Reported differences are mainly due to: (1) the threshold oocyte development stage considered to indicate maturity (i.e., cortical alveolar vs tertiary vitellogenic), (2) the sex of the fish analysed (i.e., potential sexual dimorphism in growth) and (3) the region where the fish were captured. For example L_{50} for female yellowfin tuna captured from the west Indian Ocean was estimated at 102.0 cm FL (Zudaire et al. 2013), while in the eastern and west-central Indian Ocean it was estimated at 109.6 cm and 114.0 cm respectively (Nootmorn et al. 2005, Zhu et al. 2008). Regarding fecundity, yellowfin tuna exhibit an indeterminate fecundity (i.e., oocyte maturation is continuous during their extended spawning periods) and mean batch fecundity was estimated at 3.1 million oocytes for females from the western Indian Ocean (Zudaire et al. 2013), and between 0.3 and 5.3 million oocytes for females from the eastern Indian Ocean (Nootmorn et al. 2005). Spawning frequency has not been reported for yellowfin tuna in the Indian Ocean, but in the Pacific Ocean mean spawning interval was estimated as 1.53 days (Schaefer 2001).

The most recent stock assessment for yellowfin tuna in the Indian Ocean (Fu et al. 2018) used a maturity ogive for the western Indian Ocean from Zudaire et al. (2013) in the base case model. The ogive used the ‘cortical alveolar’ oocyte development stage as the threshold indicating a fish was mature. Zudaire et al. (2013) also estimated a maturity ogive using the (older) ‘advanced vitellogenic’ oocyte stage as the maturity threshold,

which is a more reliable threshold for determining a female is mature and contributing to egg production (Schaefer 2001). The two length based ogives from Zudaire et al. (2013) were converted to age based ogives in Langley (2015) and age at 50% maturity was estimated to be ~9 quarters and ~12 quarters, respectively. Sensitivity analyses conducted in Langley (2015) using the alternate maturity ogive gave a slightly more pessimistic outlook for the status of the stock relative to the base case model.

The lack of oceanic scale studies, together with the fact that available studies of yellowfin tuna reproductive biology in the Indian Ocean are based on fish from different size ranges and using different analytical methods, makes it difficult to incorporate accurate life-history parameters at the appropriate scale into the stock assessment model (Artetxe-Arrate et al. 2021). This reduces the reliability of the stock assessment model and affects the management advice of yellowfin tuna in the Indian Ocean.

In this context, the European Union and the IOTC supported the “GERUNDIO” project for the “*collection and analysis of biological samples of tropical tunas, swordfish, and blue sharks to improve age, growth and reproduction data for the IOTC*”. The aim of the project is to produce updated estimates of age, growth, and reproduction parameters for the stock assessments of Indian Ocean tropical tunas (bigeye, skipjack, and yellowfin), swordfish and blue shark. This paper provides preliminary results of yellowfin tuna sex ratio, spawning seasonality, length at maturity and batch fecundity in the Indian Ocean, undertaken within this project.

2. Material and methods

Sample collection and data available from previous projects

Two sources of data were used to estimate reproductive parameters of yellowfin tuna in this study: i) data collected as part of the ‘GERUNDIO’ project and ii) data available in the database developed during the EMOTION project (Bodin et al., 2018), which contains data from previous projects related to biological studies of tropical tuna in the Indian Ocean (Table 1). The availability of sex data has been the minimum condition for the selection of individuals for the analysis. For clarity, the analyses are shown for the “GERUNDIO project” data alone and for the combined dataset labelled "ALL project".

For the GERUNDIO project, a total of 1145 yellowfin tuna were sampled (476 females and 669 males). The individuals were collected in 2020-2021 predominantly from purse seine vessels operating in the western Indian Ocean and were mainly sampled at canneries (Fig 1-a). All fish were sexed, measured to the nearest 0.1 cm FL and weighed to the nearest 0.1 kg (total weight). Whenever possible, the ovary was removed and weighed to the nearest 0.1 g. Fish ranged from 43.0 to 184.0 cm FL and from 4.45 to 91.1 kg total weight (Table 1 and Fig 2).

Gonadosomatic index (GSI) was calculated as gonad weight/(total weight - gonad weight) × 10^2. Additionally, estimated GSI (GSI_est) was calculated using estimated fish total weight by applying FL and weight relationship for those females without weight measurement. For histological analysis, an ovary sub-sample was removed from the middle part of the right or left lobe from each fish and fixed in 4% buffered formaldehyde.

Whenever possible, the date and location of capture were obtained from the record of the brine-freezing well and vessels logbooks through close collaboration with fishing companies and the cannery. Some uncertainty arose when yellowfin tuna came from a well containing multiple fishing sets. In such cases, the median date of fishing was calculated for defining the month of capture.

Data from an additional 921 yellowfin tuna were obtained from the EMOTION project (476 females and 445 males), which included histological data from 388 females classified using a similar classification scheme to that agreed in the project. The individuals were collected from 2009-2019 predominantly from the western Indian Ocean (Fig 1-b). Fish ranged from 46.0 to 170.5 cm FL and from 1.6 to 91.0 kg total weight (Table 1 and Fig 2). The fish sampled in the “GERUNDIO project” and the combined “ALL project” were geographically determined by sampling region (Fig 1).

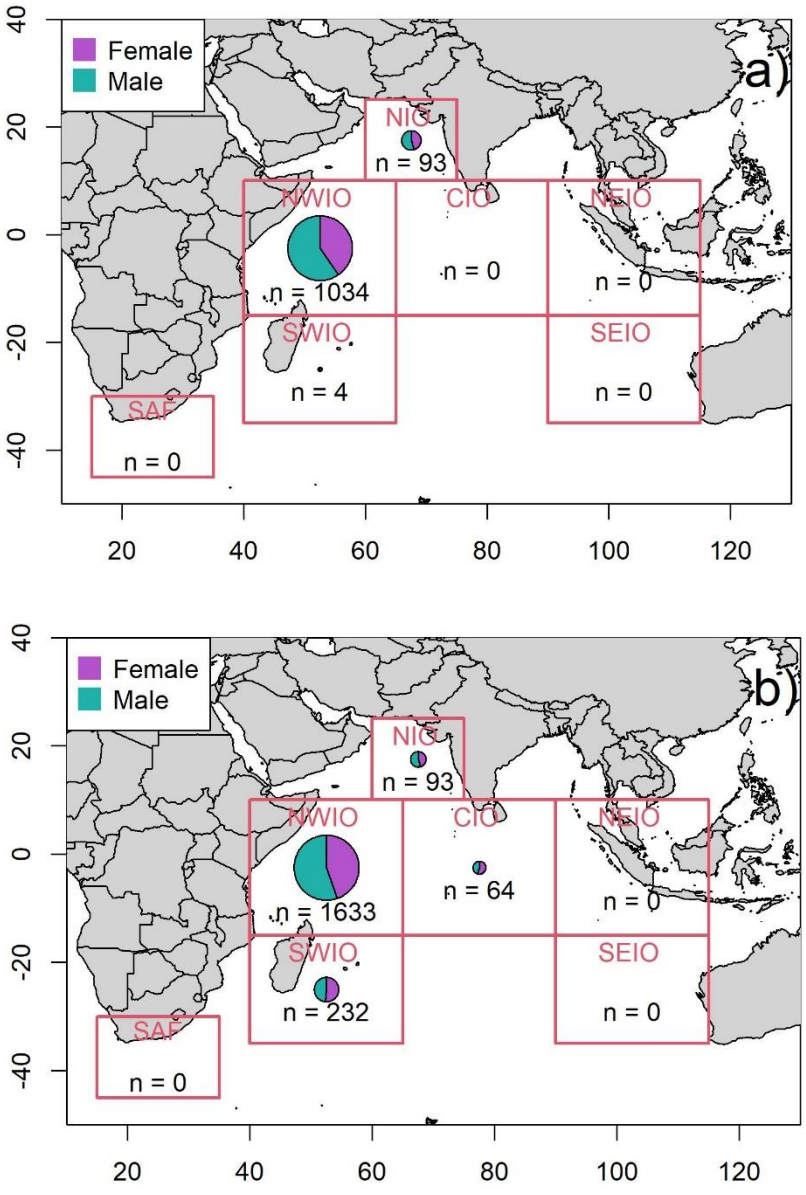

Figure 1 Map showing sampling locations and number individual yellowfin tuna (Thunnus albacares) with sex data used in the analysis for a) GERUNDIO project and b) ALL project data sets in the Indian Ocean. Sampling regions across the Indian Ocean were defined as South Africa (SAF), Southwest Indian Ocean

(SWIO), Northwest Indian Ocean (NWIO), North Indian Ocean (NIO), Central Indian Ocean (NIO), Northeast Indian Ocean (NEIO) and Southeast Indian Ocean (SEIO).

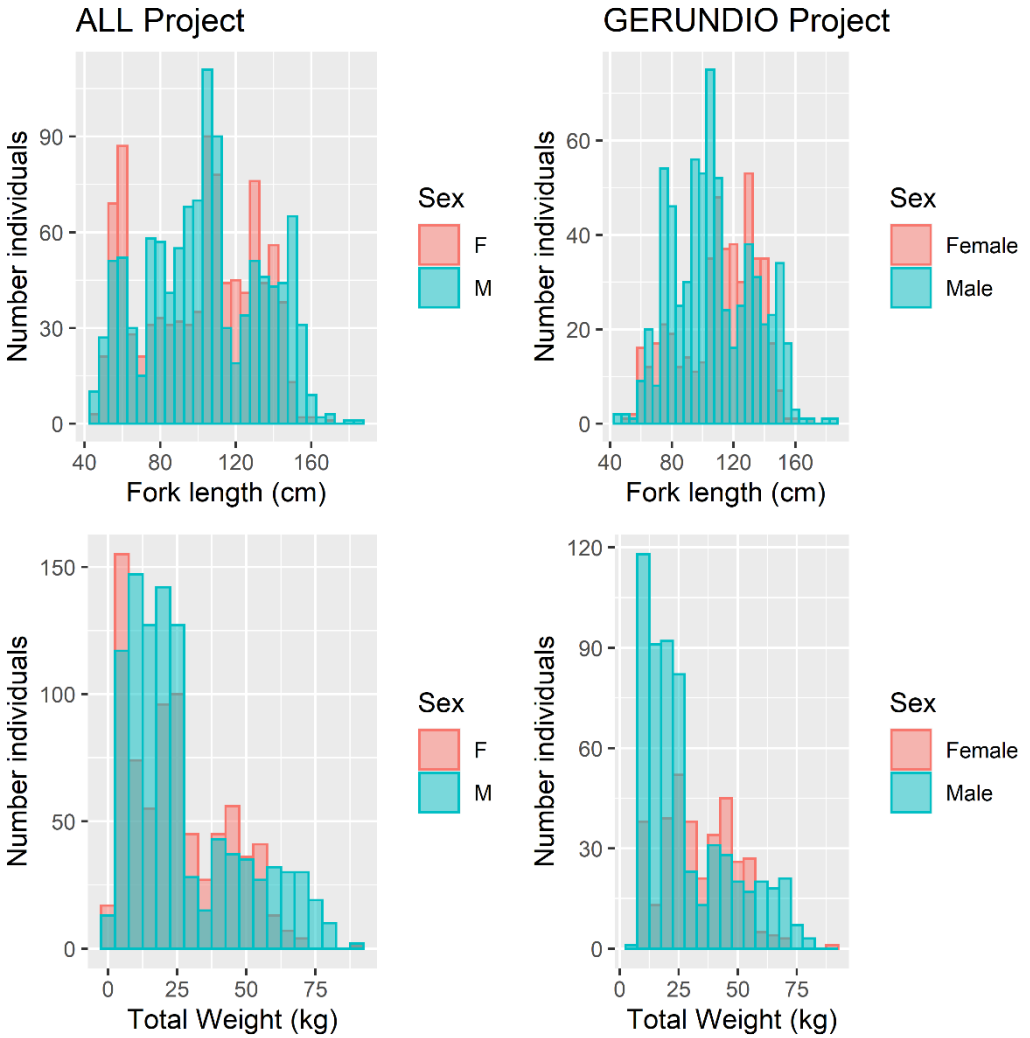

Figure 2 Fork length (in cm) and total weight (in kg) frequency of yellowfin tuna (Thunnus albacares) by sex sampled within the ALL Project and GERUNDIO project.

Table 1 Number of yellowfin tuna (Thunnus albacares) included in the analysis from the GERUNDIO project and from previous projects (i.e., included in PSTBS-IO project (Davies et al., 2020) and EMOTION project (Bodin et al., 2018)). Data is described by sex, including range of fork length (cm) and total weight (kg).

Database	projects	Female	Male	Total	Length range (cmFL - Female)	Length range (cmFL - Male)	Weight range (Kg - Female)	Weight range (Kg - Male)
EMOTION (Bodin et al., 2018)	CANAL	81	93	174	46 - 149.5	43.8 - 165.2	1.9 - 67.1	1.6 - 90.1
	EMOTION	11	25	36	128 - 152	50.3 - 157.7	43.7 - 70.5	2.6 - 91
	IOT_quality	2	1	3	57.4 - 58.2	53.8 - 53.8	--	--
	IOTTP	4	5	9	110.5 - 125.3	137.9 - 148.2	36.5 - 36.5	--
	Liver test	1	0	1	77.5 - 77.5	--	9.9 - 9.9	--
	MADE	11	5	16	58 - 106	57 - 101	--	--
	PEVASA	343	281	624	48 - 153	49 - 161	2.05 - 63	2.24 - 79
	SAUMTEST	0	2	2	--	48.5 - 50.4	--	2.3 - 2.6
PSTBS-IO (Davies et al., 2020)	PSTBS-IO	23	33	56	55 - 158	47.3 - 170.5	--	--
GERUNDIO	GERUNDIO	476	669	1145	51 - 171	43 - 184	7.9 - 91.1	4.45 - 78.5
ALL Project		952	1114	2066	46 - 171	43 - 170.5	1.9 - 91.1	1.6 - 91

Histological classification of ovaries

A subset of 212 ovaries collected in the GERUNDIO project were initially chosen for histological classification. A cross-section of around 1 cm from the preserved portion of each ovary was embedded in paraffin or resin, sectioned at 5-7 µm and stained with haematoxylin and eosin. An additional 388 ovary sections prepared in the previous projects (see Bodin et al. 2018) were obtained for a combined total of 600 ovary histological sections for the ALL project dataset. More ovaries collected in GERUNDIO will be processed soon to update the current analysis.

A standardized ovary classification method was agreed by Project partners. Ovaries were classified according to the most advanced oocyte stage present, atresia of Vtg2 or Vtg3 oocytes, and maturity markers following (Brown-Peterson et al., 2011): (i) immature phase (IP) which includes oocytes in the primary growth stage; (ii) developing phase (DP) which includes oocytes in the stages of cortical alveoli (CA), primary (Vtg1) and secondary vitellogenesis (Vtg2); (iii) spawning-capable phase (SCP) which includes oocytes in the stages of tertiary vitellogenesis (Vtg3), germinal vesicle migration (GVM), germinal vesicle breakdown (GVBD) and hydration (Hyd); (iv) regressing phase (RsP) characterized by the presence of atretic oocytes (any stage), and few healthy Vtg2 and Vtg3 oocytes; and (v) regenerating phase (RgP) characterized by the presence of maturity markers, late-stage atresia and a thicker ovarian wall than seen in immature fish. The atretic condition to appraise the RsP and RgP was based on (Hunter and Macewicz, 1985) and on the classification for atresia stages described in Brown-Peterson et al. (2011). Postovulatory follicles were not recorded in these samples to estimate spawning fraction.

The yellowfin tuna from the EMOTION project were classified into the same development phases based on the histological data available. However, future cross-checking (re-reading) of a subset of the histological slides is required to confirm consistent classification.

Length at maturity

The size at which 50% of the females reach maturity (L_{50}), was calculated by fitting a logistic model to the proportion of females mature (Saborido-Rey et al., 1998) following:

$$P_l = \frac{\exp(\alpha + \beta \times l)}{1 + \exp(\alpha + \beta \times l)}$$

where P_l is the proportion of mature females identified through histological analysis in FL class l, and α and β are coefficients of the logistic equation. A binomial distribution with logit link function was used to fit the above equation to the raw fork length data. L_{50} was estimated as the ratio of the coefficients ($-\alpha\beta^{-1}$). The variance of the estimate of L_{50} was derived from the delta method using a first-order Taylor approximation (Xu et al., 2005). The maturity curve was fitted to the data on the basis of the assumptions regarding female maturity threshold: ovaries in early vitellogenic stage including primary (Vtg1) and secondary vitellogenesis (Vtg2) stages (Schaefer, 1998; Zhu et al., 2008) were considered mature.

Batch fecundity estimation

Batch fecundity (BF), i.e., the total number of oocytes released per batch, was estimated for 38 ovaries, at which the hydration stage oocyte appeared as the most advanced stage of development (21 of which were from the GERUNDIO project). BF was estimated by gravimetric method (Hunter et al., 1985), where the number of hydrated oocytes present were counted. Homogeneity in oocyte density among whole ovary was assumed on the basis of previous works on tuna (Stéquert and Ramcharrun, 1996) . For BF analyses, three subsamples of 0.1 g (±0.01 g) were collected from each ovary. Each subsample was saturated with glycerin and the hydrated oocytes were counted under a stereomicroscope. BF was calculated as the weighted mean density of the three subsamples multiplied by the total weight of the ovary. A threshold of 10% for the coefficient of variance was applied for the three subsamples, and when this threshold was surpassed, more subsamples were counted until this value was reached. Relative batch fecundity (relBF) was estimated as the ratio between BF and gonad-free body weight (computed as total weight - gonad weight).

Statistical analyses

Multiple linear regression modelling was applied on the subset of sampled yellowfin tuna with available morphometric measurements (FL and total weight) to assess the variability in weight as a function of length and sex. Model parameters were estimated using the 'lm' function in R (R Core Team, 2016). Gaussian error distribution and homoscedasticity hypotheses were checked using the residuals. Sex-ratio was calculated as the proportion of females to males by 5 cm FL class in the sample, and Chi-square tests (χ^2) were used to examine differences from an expected 1:1 by size class. Monthly reproductive activity of females was assessed applying a non-parametric Kruskal-Wallis (KW) test to estimate variability in GSI. Quantile linear regression models were used to describe the relationship between fecundity (BF and relBF) and fish FL as well as gonad weight (Koenker, 2013). 10%, 50% and 90% quantiles were used to respectively describe the minimum, median and maximum levels of fecundity (BF and relBF) as a function of FL and gonad weight.

3. Results & discussion

Length-weight relationships

There were no differences in the body length-weight relationship when analysed by sex for yellowfin tuna (*p*-value = 0.0979). Besides, the FL to weight relationship of both sexes combined is comparable to that currently used within IOTC (IOTC, 2020) for small sizes, but is slightly different for medium to large size fish (Fig 3).

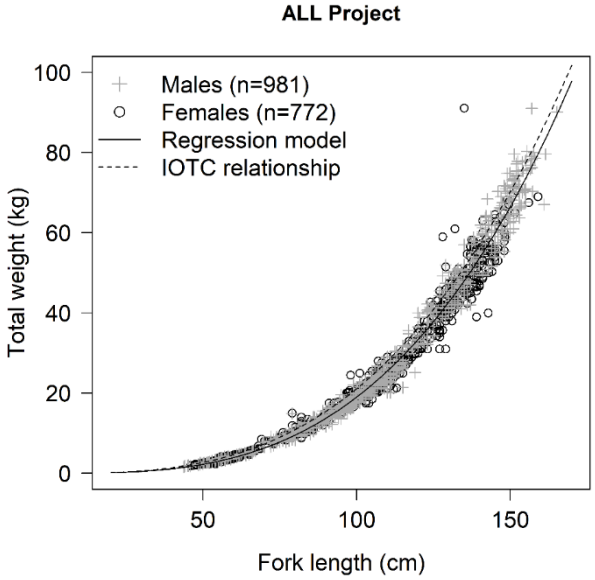

Figure 3 Relationship between fork length (cm) and body weight (kg) for male (cross) and female (open circle) yellowfin tuna (Thunnus albacares) sampled in the Indian Ocean. Solid line indicates the regression model fitted to all individuals; dashed line indicates the IOTC relationship currently used.

Sex-ratio

The sex-ratio did not differ significantly from 1:1 for most the small and intermediate size classes (<115 cm FL), with only the 55-59 cm class having significantly more females (χ^2= 4.119, *p*-value < 0.05) and the 75-79, 90-94 and 95-99 cm classes having significantly more males. A clear male predominance was observed at larger sizes (>150 cm FL; *p*-values < 0.05; Table 2 and Fig 4). A dominance of males in larger length classes has been found in many tunas and has been linked to sexual dimorphism in growth, mortality and/or availability (Schaefer 2001). Farley et al. (2021) found some evidence that male yellowfin tuna in the Indian Ocean growth faster and reach larger sizes than females, which may have contributed to these sex ratio results. The dominance of females in the length classes before the male dominance (i.e., between ~115 and 144 FL) adds further evidence for differential growth between sexes in yellowfin tuna.

*Table 2 Summary of the number of female and male yellowfin tuna (Thunnus albacares) sampled by 5 cm fork length class and by sex. Chi-square test results (χ^2 and p-value) are provided for size classes with more than 5 individuals. *: p-value<0.05; **: p-value<0.001. NA indicates Not Available*

Size classes	M	F	χ^2	p-value		Total
45 - 49	19	10	2.793	0.095		29
50 - 54	23	31	1.185	0.276		54
55 - 59	75	102	4.119	0.042	*	177
60 - 64	35	52	3.322	0.068		87
65 - 69	22	20	0.095	0.758		42
70 - 74	24	24	0.000	1.000		48
75 - 79	68	33	12.129	0.000	**	101
80 - 84	47	36	1.458	0.227		83
85 - 89	45	32	2.195	0.139		77
90 - 94	59	25	13.762	0.000	**	84
95 - 99	75	35	14.545	0.000	**	110
100 - 104	76	54	3.723	0.054		130
105 - 109	114	91	2.581	0.108		205
110 - 114	63	62	0.008	0.929		125
115 - 119	23	43	6.061	0.014	*	66
120 - 124	30	41	1.704	0.192		71
125 - 129	34	62	8.167	0.004	*	96
130 - 134	57	59	0.034	0.853		116
135 - 139	39	51	1.600	0.206		90
140 - 144	42	49	0.538	0.463		91
145 - 149	51	32	4.349	0.037		83
150 - 154	60	4	49.000	0.000	**	64
155 - 159	18	3	10.714	0.001	*	21
160 - 164	5	0	5.000	0.025	*	5
>164	7	1	4.500	0.034	*	8

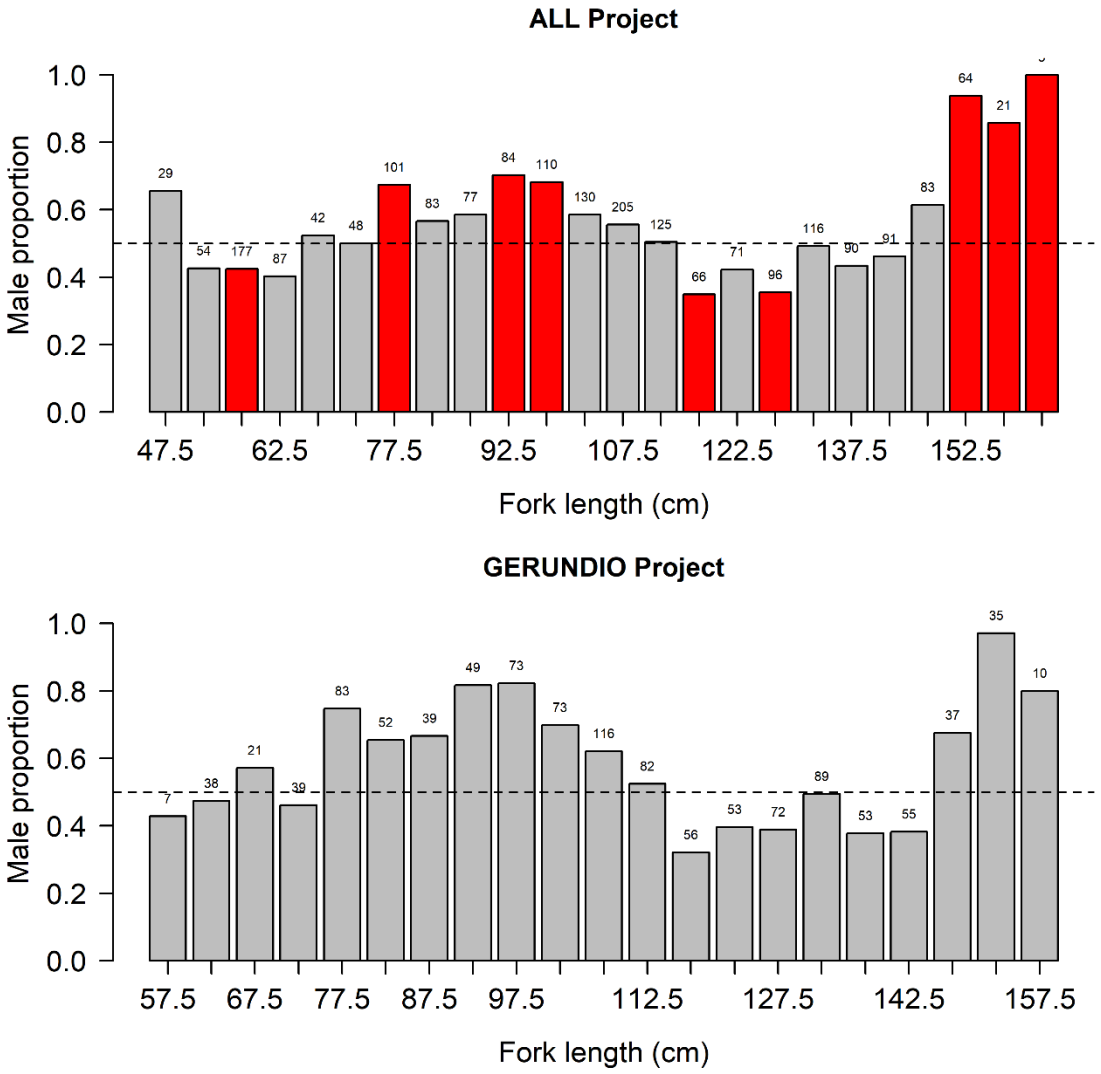

Figure 4. Variations of sex-ratio with fork length (cm) for yellowfin tuna (Thunnus albacares) in the Indian Ocean. The horizontal dotted line indicates 50% of male proportion. Numbers above bars indicate the number of individuals included in each size class. Bars in red identifies the significant size-ranges.

Histological classification of ovaries and spawning season

Tables 3 and 4 show the selection of ovaries used for the histological classification. According to this classification, 34% of females were at IP, 27% were at DP, 30% were at SCP, 4% at RsP, and 6% were at RgP (Table 3). Applying the maturity threshold at early vitellogenic stage (including Vit 1 and Vit 2) and onward, 46.5% of the analysed females were mature, of which 22.2% were undergoing oocyte maturation (ovaries contained GVM, GVBD or Hyd oocytes) indicating spawning was imminent. A high proportion of females at SCP was recorded from December to April with the highest proportions present from December to February (>50%) (Fig 5). As expected, the proportion of females at RsP and RgP (post-spawning) increased between February and May as spawning declined. An increase in SCP females was also present in June followed by an increase of RsP and RgP females in July and also September. In contrast,

females with less developed ovaries (i.e., IP and DP) were dominant from April to October (around >40% of individuals at IP and around 25% at DP) (Fig 5). A similar pattern of population ovary maturation process was also observed in the monthly evolution of the GSI (Fig 6). The GERUNDIO project dataset, although it is more limited in sample size and temporal coverage, showed a pattern on ovary maturation similar to that described with the ALL project dataset.

Table 3 Summary of the number of female yellowfin tuna sampled by maturity development and dataset origin. IP = immature phase, DP = developing phase, SCP = spawning capable phase, RsP = regressing phase, RgP = regenerating phase.

Database	Project	IP	DP	SCP	RsP	RgP	Total
EMOTION (Bodin et al., 2018)	CANAL	33	16	9		1	59
	EMOTION		1	9			10
	PEVASA	157	110	29	1	22	319
GERUNDIO	GERUNDIO	15	35	132	20	10	212
Total		205	162	179	21	33	600

Table 4 Summary of the number of female yellowfin tuna (Thunnus albacares) sampled by 5-cm class of fork length (FL) and maturity development. IP = immature phase, DP = developing phase, SCP = spawning capable phase, RsP = regressing phase, RgP = regenerating phase.

Size range	IP	DP	SCP	RsP	RgP	total
45 - 49	7	0	0	0	0	7
50 - 54	25	3	0	0	0	28
55 - 59	73	6	0	0	0	79
60 - 64	23	4	0	0	0	27
65 - 69	8	2	0	0	0	10
70 - 74	3	1	0	1	1	6
75 - 79	5	9	0	1	4	19
80 - 84	9	20	0	3	2	34
85 - 89	7	15	1	1	1	25
90 - 94	5	10	1	1	0	17
95 - 99	10	10	3	1	1	25
100 - 104	14	18	5	2	3	42
105 - 109	14	22	20	4	13	73
110 - 114	2	21	19	2	3	47
115 - 119	0	5	16	1	0	22
120 - 124	0	0	12	0	1	13
125 - 129	0	4	23	0	0	27
130 - 134	0	5	22	1	0	28
135 - 139	0	4	26	0	0	30
140 - 144	0	2	21	2	2	27
145 - 149	0	0	9	1	2	12
150 - 154	0	1	1	0	0	2
TOTAL	205	162	179	21	33	600

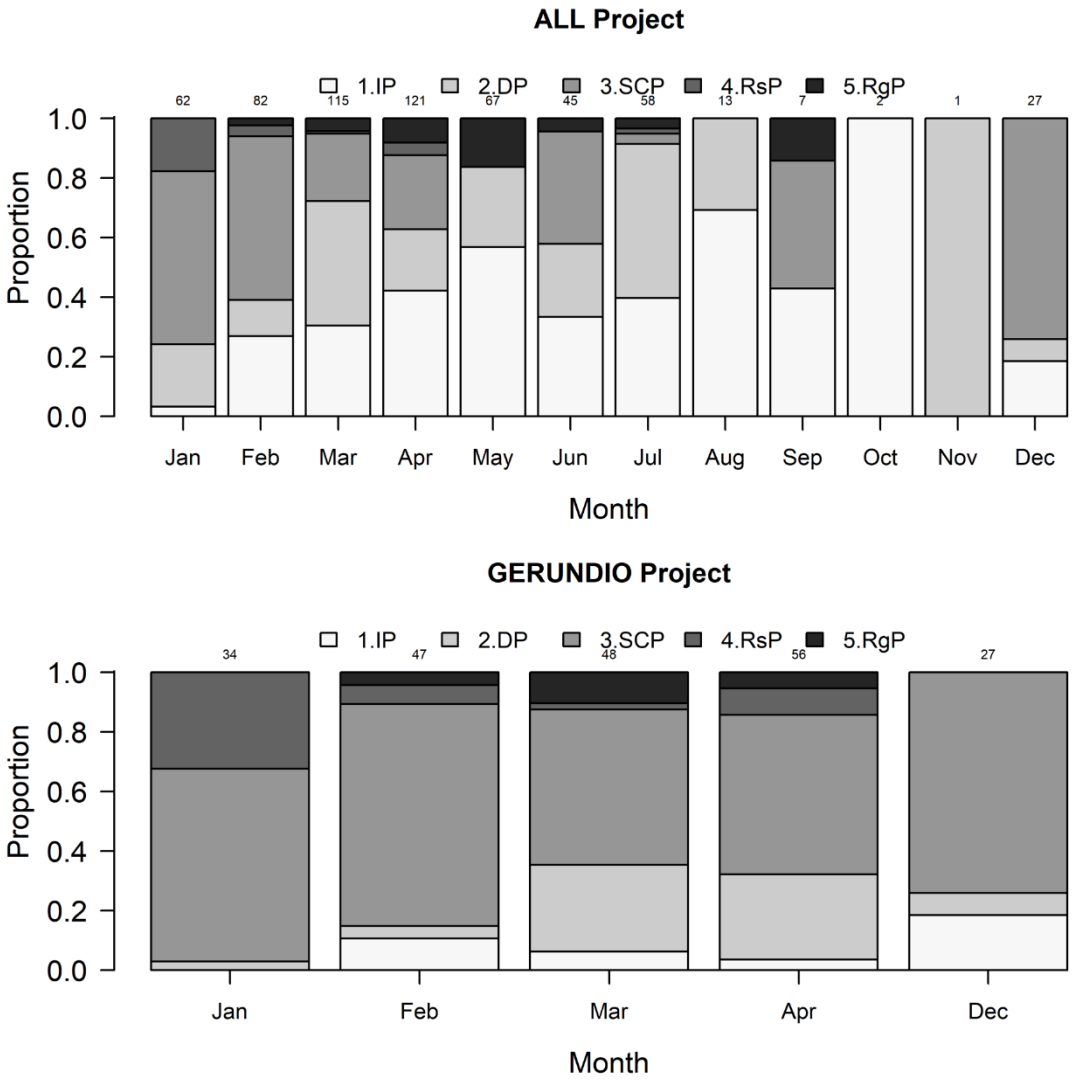

Figure 5 Monthly variations of the proportions of ovary development phases for female yellowfin tuna (Thunnus albacares) selected from ALL Project (top) and GERUNDIO project (botton) datasets in the Indian Ocean. IP = Immature phase; dP = Developing phase; SCP = Spawning capable phase; RsP = Regressing phase; RgP = Regenerating phase. Numbers above bars indicate the number of individuals included in each month.

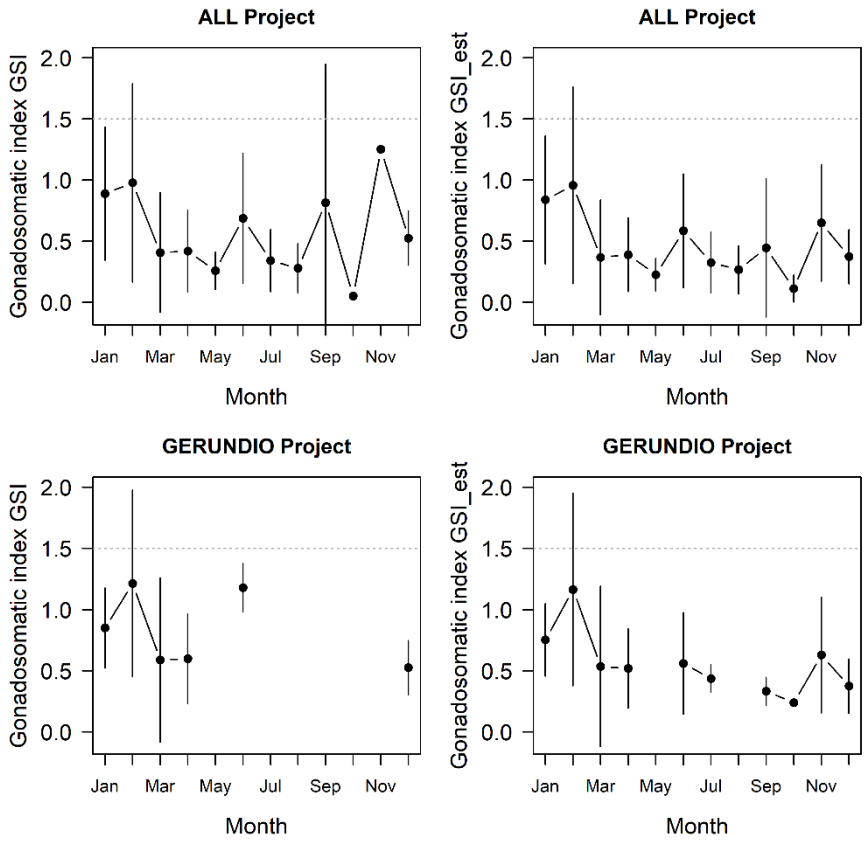

Figure 6 Monthly variations of the gonadosomatic index (GSI) and estimated gonadosomatic index (GSI_est) values for female yellowfin tuna (Thunnus albacares) selected from ALL project and GERUNDIO project datasets in the Indian Ocean.

Length at maturity

L_{50} was estimated at 101.7±1.3 cm FL for the ALL project dataset when females with ovaries in early vitellogenic stage including primary (Vtg1) and secondary vitellogenesis (Vtg2) stages were considered mature (Table 5 and Figure 7). This estimate decreased to 84.2±1.3 cm FL when only individuals from the GERUNDIO project were analysed (Table 5). The difference between estimates is likely due to the limited sample size in the GERUNDIO dataset as the full size range of females was not covered, and the number of individuals was lower in comparison to the ALL project dataset. However, further investigation is also required to determine the reason for the high proportion of mature individuals observed in the smaller size classes as it was also not possible to undertake the required cross-checking (re-reading) of all histological sections within the project timeframe.

Table 5 Parameters for the logistic regression model for estimating the fork length of female yellowfin tuna (Thunnus albacares) in the Indian Ocean at which 50% of the population is mature (L_{50}, cm). Ovaries in stages iii to iv were considered mature. α and β are the coefficients of the equation and L_{50} was computed

as - α / β for the maturity threshold used: Vit 3 = tertiary vitellogenesis for both data from ALL Project and GERUNDIO datasets.

	ALL Project			GERUNDIO project		
Parameters	α	β	L_{50}	α	β	L_{50}
Estimates	-9.25	0.091	101.7	-9.692	0.115	84.2
Standard error	0.78	0.007		1.764	0.012	

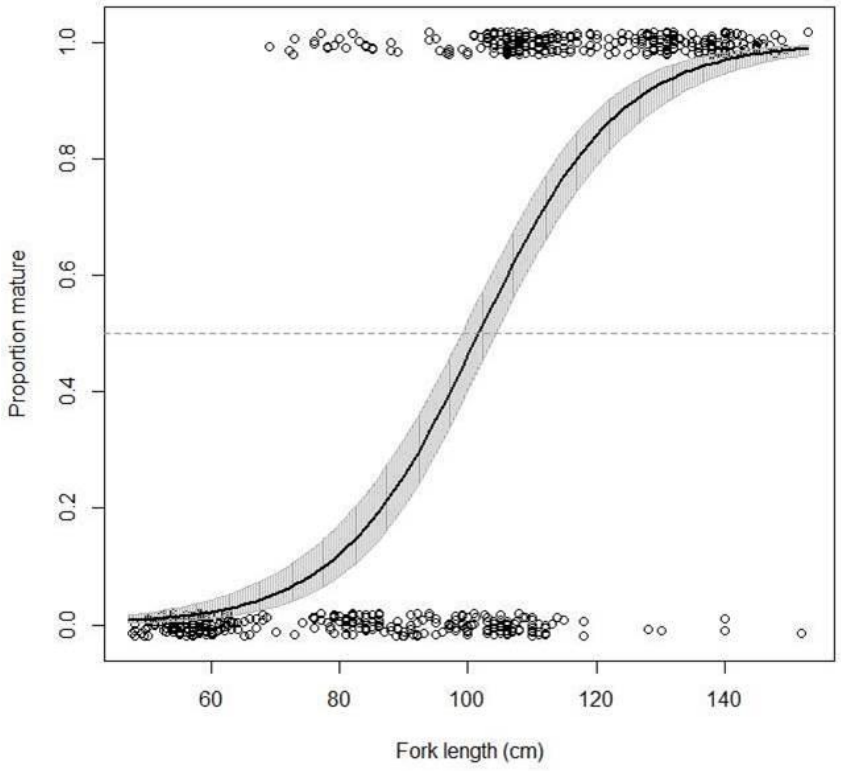

Figure 5 Estimated proportion of mature female yellowfin tuna (Thunnus albacares) in the Indian Ocean by fork length. Open circles show the maturity status (0 = immature; 1 = mature) estimated for each fish in the ALL Project dataset (note that the points have been jittered to reduce overlap). The solid black line indicates the logistic regression curve fitted to the data, and the grey shaded area indicates the 95% confidence interval. L_{50}, i.e. the length at which 50% of the female population is mature, is the length at which the dashed horizontal line intersects the maturity curve.

Batch fecundity estimation

The estimated mean batch fecundity (BF) was 2.3±1.2 million oocytes and ranged from 0.3 million to 4.82 million oocytes. The mean relative batch fecundity (relBF) was estimated at 48.6±25.6 oocytes per gram of gonad-free body weight and fluctuated between 6.0 and 106.6 oocytes per gram. Fecundity varied greatly with yellowfin tuna size. The maximum levels of batch fecundity were observed in the largest females. The mean BF increased significantly with FL ($p<0.05$). However, a 50% quantile regression line fitted to BF did not significantly increase with FL ($p=0.368$) (Fig 8). Similarly, no relationship was found between relBF and length (Fig 8). Both BF and relBF increased significantly with gonad weight (*p*-value <0.05), showing a positive pattern in all three quantile regressions (Fig 8).

The analysis of variance revealed BF and relBF did not vary significantly by month at a 95% confidence level ($F_{(4,33)}$=0.493, p-value=0.493; $F_{(4,27)}$=0.929, p-value=0.462, respectively). BF estimates appeared highly variable within each month (Fig 9). The maximum monthly mean BF value was found in September (2.7± 0.05), however, only to measurements were available in that month. The next larger value was found in February (2.4±1.2 million oocytes) with 25 measurements. The minimum estimate was observed in January (2.0±1.0 million oocytes).

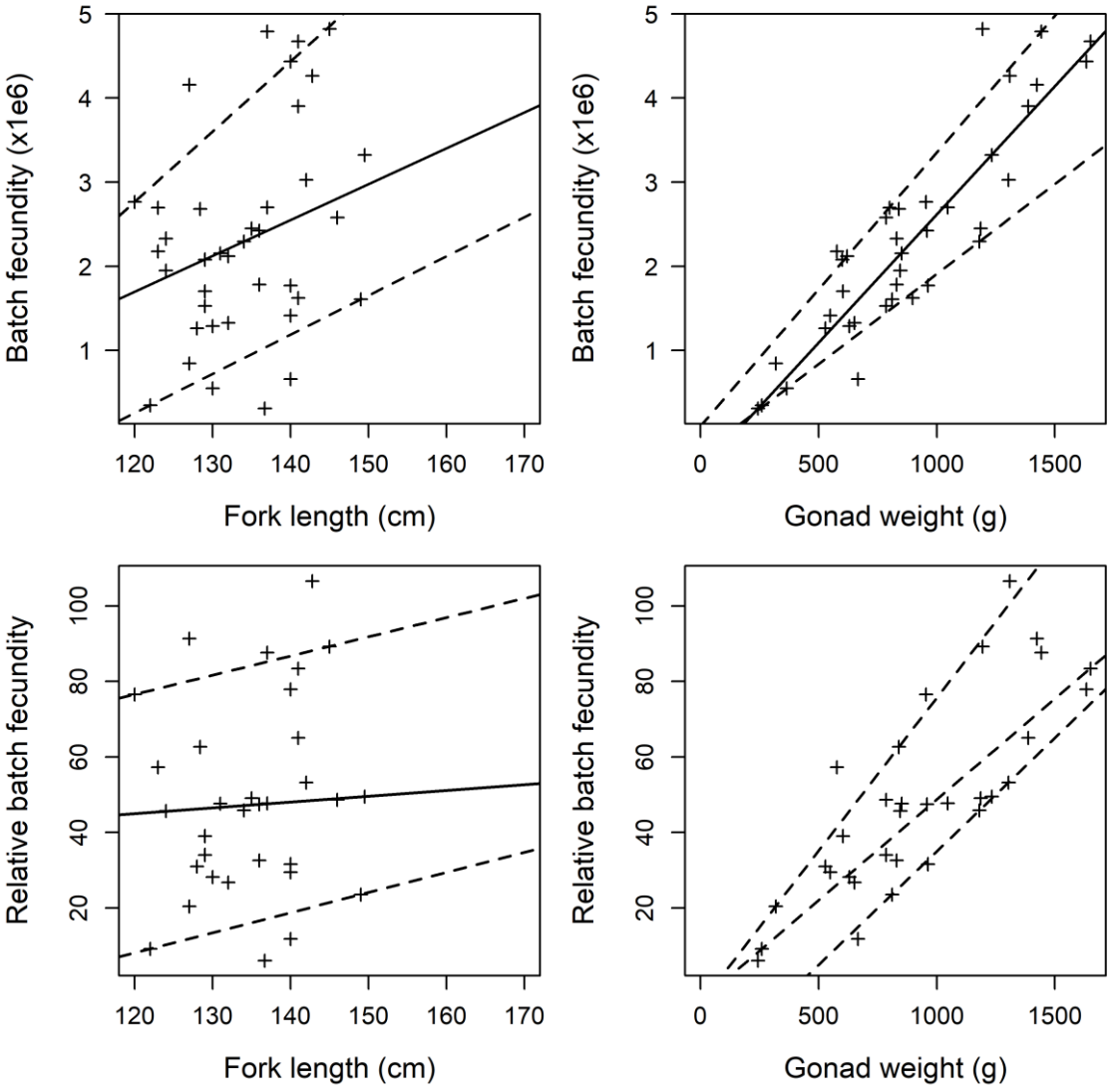

Figure 8. Relationships between batch fecundity (BF, millions of oocytes) (top) and relative batch fecundity (relBF, oocytes per gram of fish body weight) (bottom) with fork length (cm) and ovary weight (g) for female yellowfin tuna (Thunnus albacares) using the ALL Project dataset in the Indian Ocean. Dotted lines represent 10% and 90% quantiles, while solid line represents the median regression line (50% quantile).

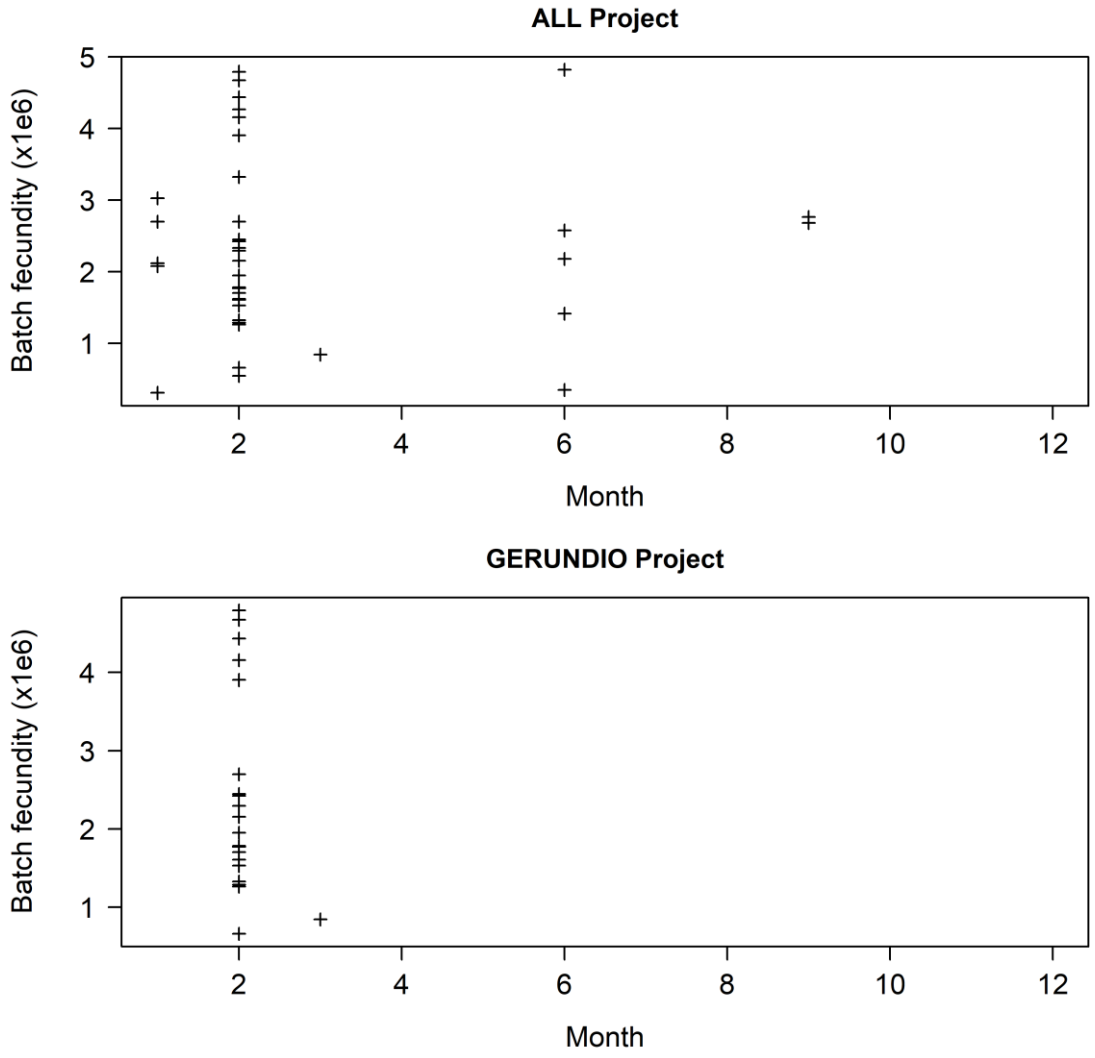

Figure 9. Batch fecundity estimates by month from ALL Project (top) and GERUNDIO project (botton) datasets for yellowfin tuna (Thunnus albacares) in the Indian Ocean.

4. Conclusion & Recommendations

This paper provides preliminary estimates of sex ratio, spawning periodicity, batch fecundity and length at maturity for yellowfin tuna sampled predominantly in the western Indian Ocean. Further work is required to finalize the analyses, particularly the spawning periodicity and maturity results. Spawning fraction (the proportion of females spawning per day) will also be estimated when the necessary data are available. Only a subset of the ovaries collected in the GERUNDIO project were included in the analyses as it was not possible to process all the ovaries collected or to undertake the required cross-checking (re-reading) of histological sections within the timeframe of the project. In addition, it was not possible to cross-check the histological data obtained from the EMOTION database. Further investigation is also required to understand the differences detected in maturity ogives estimated using data from the current project and the combined dataset, as well as determine the reason for the high proportion of mature individuals observed in the smaller size classes in both datasets. There was insufficient

data to examine region-specific reproductive parameters in this project since most of the gonads were obtained from the western Indian Ocean.

We recommend that additional gonad samples are collected and analysed from all regions of the Indian Ocean, but particularly from the northern and eastern areas (from all size classes and months) to improve the reproductive parameters obtained in this project. Fish >60 cm FL (~minimum size at maturity) are particularly important to increase the sample size available for maturity, fecundity and spawning fraction analyses. Monthly sampling is important in reproductive studies to obtain reproductive data throughout the year. We also recommend collecting additional gonad samples from different fishing gears (e.g., longline) to improve the size coverage and have better representation of the population spatial range. Continuing to collect and analyse gonads over time will be particularly important for assessing inter-annual variation in reproductive parameters.

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