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

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Executive Summary

This paper describes preliminary work to estimate reproductive parameters for bigeye tuna (*Thunnus obesus*) in the Indian Ocean as part of the 'GERUNDIO' project[1]. The 2019 stock assessment for yellowfin tuna in the Indian Ocean (IOTC) indicated that the stock is not considered to be overfished but is subject to overfishing (Fu et al. 2019; IOTC 2020). The assessment model used a maturity ogive equivalent to that used by Shono et al (2009) where length at 50% maturity was 110.9 cm FL and full maturity was around

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[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.

125 cm FL. The source of this maturity ogive is unclear. The aim of the current project was to produce updated estimates of key biological reproductive parameters for bigeye tuna in the Indian Ocean, including informaion on length at maturity.

A total of 162 bigeye tuna were sampled in the project (101 females and 61 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 from a small number of ovary samples (i.e., females only), which were read using an agreed classification system. Additional ovaries collected in the current project will be processed soon to update the current analysis.

Data from an additional 485 bigeye tuna (230 females and 255 males) were obtained from previous projects (EMOTION database, see Bodin et al. 2018), which included histological data from 158 females classified using a similar classification scheme to that agreed in the project. The individuals were collected from 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 bigeye tuna predominantly from in 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 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.

The shape of the preliminary maturity ogive obtained in this study is very different to the ogive currently used in the stock assessment, although the estimates of length at 50% maturity are similar (112.7 cm versus 110.9 cm FL). The proportion mature at length does not reach 100% as expected in the larger length classes and requires further investigation.

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. 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.

1. Introduction

Bigeye tuna (*Thunnus obesus*) is a large cosmopolitan pelagic tuna species inhabiting tropical and subtropical waters (Collette and Nauen 1983). Catches worldwide represent

~9% of total catch of all tuna and correspond to a global catch of about 440,000 tonnes over the last decade. In the Indian Ocean (IO), annual bigeye catches have been about 100,000 tonnes annually in recent years. Bigeye tuna is a valuable meat for the sashimi market with its commercial value increasing over recent decades. Indonesian and Taiwanese longline (LL) fisheries are the principal fisheries harvesting bigeye tuna in western IO taking over 50% of total annual catches (ISSF, 2016). However, high piracy activity in the region during the mid-2000s resulted in a decline in LL activity and catches of bigeye tuna (Fu 2019).Catches have fluctuated but have continued to decline to ~86,000 tonnes in 2018 (Fu 2019). The purse seine (PS) fleet, with 28% of bigeye total annual catches, is the second largest fishery in the area and fishing activity has been relatively stable since 2000. The last bigeye tuna stock assessment conducted in 2019 by the Indian Ocean Tuna Commission (IOTC) reported that the stock is considered not to be overfished but is subject to overfishing.

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 (Holden & Raitt 1974). In the Indian Ocean, the analysis of important reproductive traits for bigeye tuna has remained preliminary and little information is available regarding sex-ratio, size at maturity and fecundity (Stéquert and Marsac 1989; Stobberup et al. 1998; Nootmorn 2004; Ariz et al. 2006; Zhu et al. 2011), and most of the information on bigeye tuna reproduction is available for the Pacific Ocean bigeye tuna (Farley et al. 2003; Schaefer et al. 2005; Farley et al. 2006; Zhu et al. 2010; Sun et al. 2013). The lack of important reproductive data makes it difficult to incorporate life-history parameters at the appropriate scale into the stock assessment model (Artetxe-Arrate et al. 2021). This, in turn, reduces the reliability of the stock assessment model and can undermine the sustainable management of bigeye 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 very preliminary results of bigeye 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 the reproductive parameters of bigeye 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 162 bigeye tuna were sampled (101 females and 61 males). The individuals were collected in 2020-2021 predominantly from purse seine vessels operating in the western Indian Ocean and were sampled at canneries (Figure 1-b). All fish were sexed, measured to the nearest 0.1 cm fork length (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 66 to 150 cm fork length, and from 9.5 to 34.5 kg total weight (Table 1 and Fig 2).

Gonadosomatic index (GSI) was calculated as gonad weight/(total weight - gonad weight) × 100. 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 bigeye 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 485 bigeye tuna were obtained from the EMOTION project (230 females and 255 males), which included histological data from 158 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-a). Fish ranged from 31 to 174 cm FL and from 1.3 to 116.2 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).

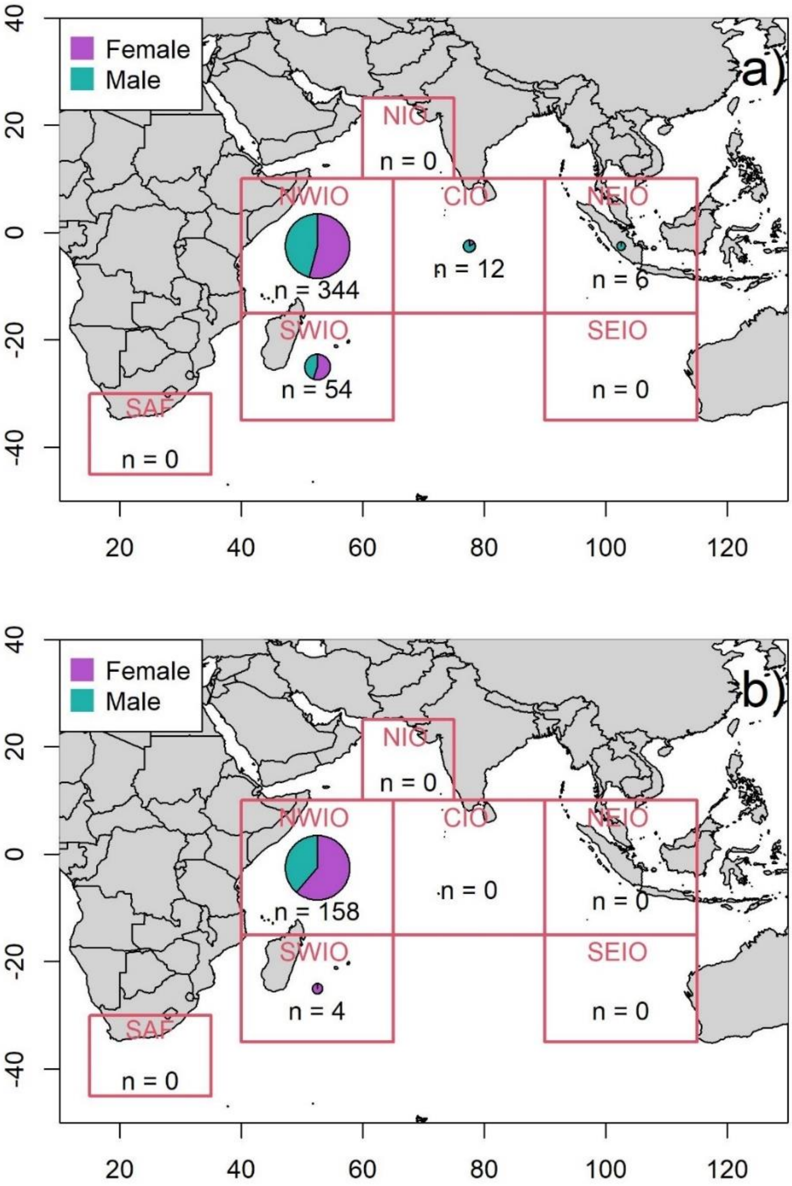

Figure 1 Map showing sampling locations and number individual bigeye tuna (Thunnus obesus) with sex data used in the analysis for a) ALL project and b) GERUNDIO project data sets in the Indian Ocean. Sampling regions across the Indian Ocean were defined as South Africa (SAF), Southwest Indian Ocean (SWIO), Northwest Indian Ocean (NWIO), North Indian Ocean (NIO), Central Indian Ocean (NIO), Northeast Indian Ocean (NEIO) and Southeast Indian Ocean (SEIO).

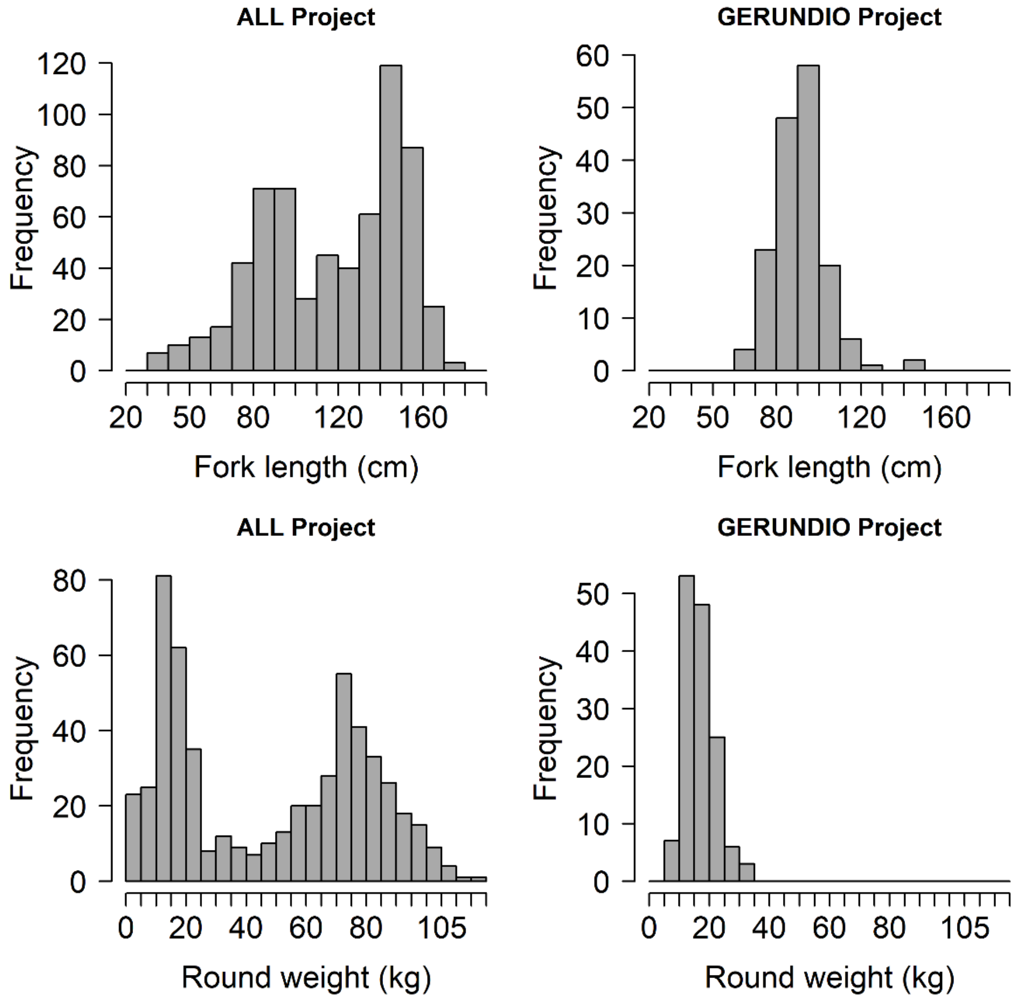

Figure 2 Fork length (in cm) and weight (in kg) class frequency of bigeye tuna (Thunnus obesus) sampled within the ALL project and GERUNDIO project.

Table 1 Number of bigeye tuna (Thunnus obesus) included in the analysis from the GERUNDIO project and from previous projects included in the EMOTION database published by 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	71	83	154	47.3 - 174	38 - 166.5	2.4 - 116.2	1.3 - 108.7
	EMOTION	130	137	267	74.7 - 164	76 - 171	10.1 - 102.25	10.8 - 112.42
	IOTTP	2	3	5	126 - 130	118.3 - 133	-	-
	Liver test	1	2	3	56 - 56	92 - 94	3.9 - 3.9	17.7 - 19.5
	PSTBS-IO	26	30	56	109 - 167	31 - 174	-	-
GERUNDIO	GERUNDIO	101	61	162	66 - 150	75 - 117	9.5 - 28.7	9.5 - 34.5
ALL Project		331	316	647	47.3 - 174	31 - 174	2.4 - 116.2	1.3 - 112.42

Histological classification of ovaries

A subset of 5 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 158 ovary sections from previous projects (see Bodin et al. 2018) were obtained for a combined total of 163 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 [22]: (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 [27] and on the classification for atresia stages described in [14]. Postovulatory follicles were not recorded for these samples to estimate spawning fraction.

The bigeye tuna from the EMOTION project were classified into the same development phases based on the histological data available. 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 female reach maturity (L_{50}), was calculated by fitting a logistic model to the proportion of females mature [28] 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 the fork length 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 data aggregated by 5 cm class of fork length. 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 [29]. The maturity curve was fitted to the data on the basis of the assumptions regarding female maturity threshold: ovaries in stages iii to v [16,24] were considered mature.

Batch fecundity estimation

Batch fecundity (BF), i.e., the total number of oocytes released per batch, was estimated for 25 ovaries (of which 0 were from the GERUNDIO Project) by gravimetric method [30], where the number of hydrated oocytes present in spawning capable ovaries were counted. Homogeneity in oocyte density among whole ovary was assumed on the basis of previous works on tuna [31]. 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 fish gonad-free body weight computed as weight - gonad weight.

Statistical analyses

Multiple linear regression modelling was applied on the subset of sampled bigeye tuna with available morphometric measurements (fork length and 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 fork length 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 fork length as well as gonad weight [32]. 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 fork length and gonad weight.

3. Results and discussion

Length-weight relationships and sex-ratio

Sex did not significantly affect the relationship between fork length and weight in bigeye tuna (p-value = 0.112). The fork length to weight relationship of both sexes combined is comparable to that currently used within IOTC [33] for all size ranges (Fig 3).

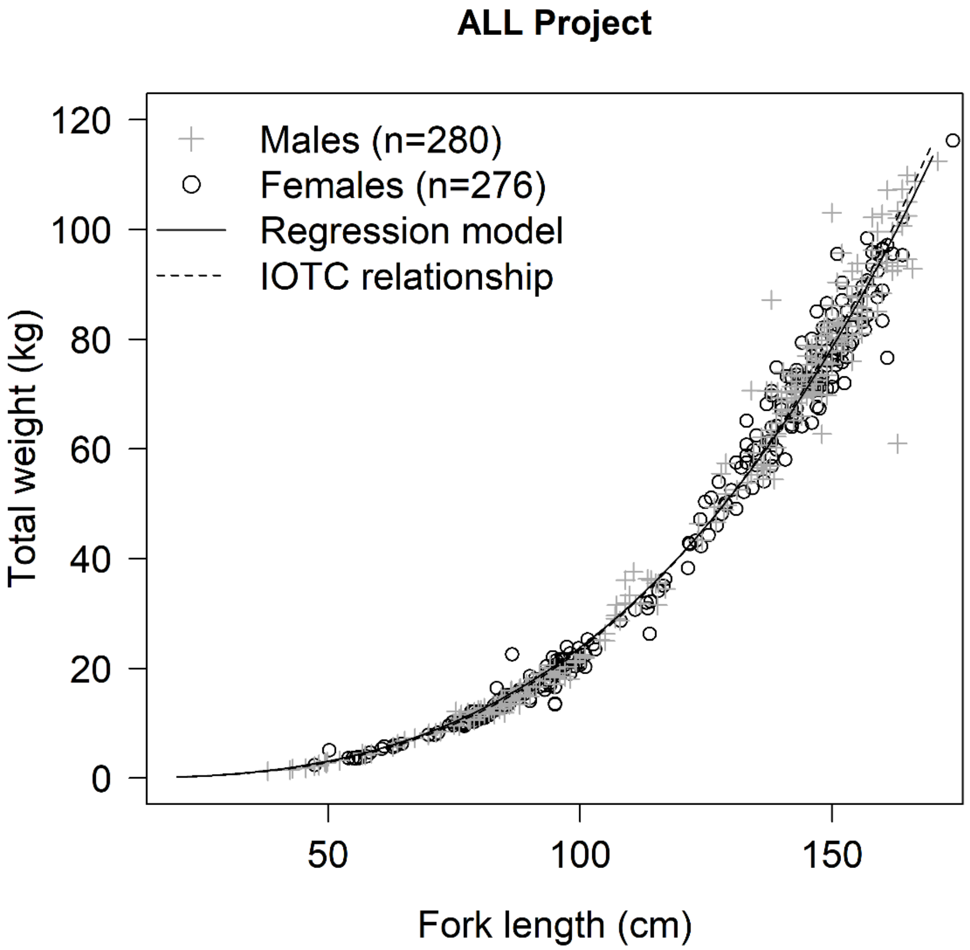

Figure 3 Relationship between fork length (cm) and body weight (kg) for male (cross) and female (open circle) bigeye tuna (Thunnus obesus) sampled in the Indian Ocean. Solid line indicates the regression model fitted to all individuals; Dashed line indicates the IOTC currently used relationship.

Sex-ratio

The sex-ratio did not differ significantly from 1:1 in any of the size classes analysed. Only the largest size classes generally had slightly more males than females, although this difference was not statistically significant (Table 2 and Fig 4).

*Table 2 Summary of the number of female and male bigeye tuna (Thunnus obesus) 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	Male	Female	χ^2	p-value	Total
55 - 59	4	5	0.111	0.739	9
60 - 64	2	6	2.000	0.157	8
65 - 69	3	5	0.500	0.480	8
70 - 74	3	5	0.500	0.480	8
75 - 79	14	14	0.000	1.000	28
80 - 84	16	19	0.257	0.612	35
85 - 89	19	18	0.027	0.869	37
90 - 94	14	19	0.758	0.384	33
95 - 99	14	24	2.632	0.105	38
100 - 104	5	12	2.882	0.090	17
105 - 109	9	6	0.600	0.439	15
110 - 114	14	14	0.000	1.000	28
115 - 119	9	10	0.053	0.819	19
120 - 124	5	11	2.250	0.134	16
125 - 129	10	12	0.182	0.670	22
130 - 134	7	14	2.333	0.127	21
135 - 139	21	16	0.676	0.411	37
140 - 144	21	20	0.024	0.876	41
145 - 149	36	35	0.014	0.906	71
150 - 154	26	26	0.000	1.000	52
155 - 159	23	16	1.256	0.262	39
160 - 164	15	11	0.615	0.433	26
165 - 169	6	1	3.571	0.059	7
170 - 174	2	1	0.333	0.564	3

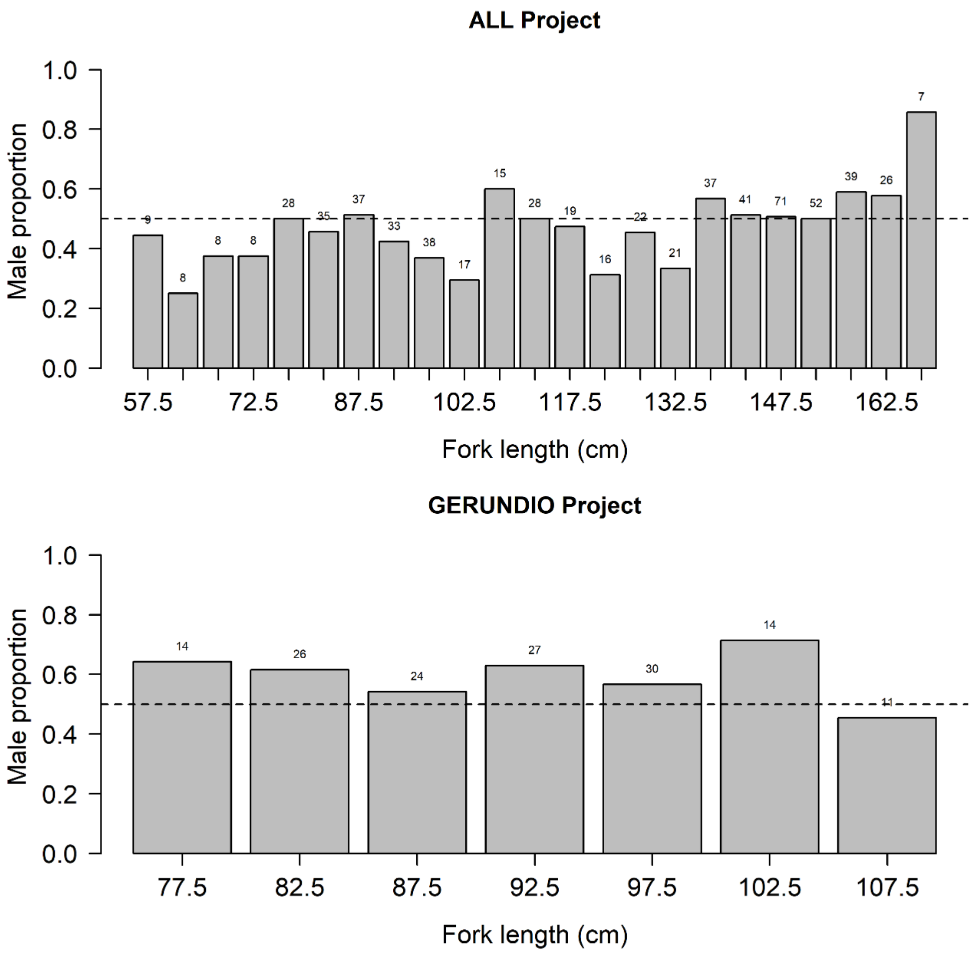

Figure 4. Variations of sex-ratio with fork length (cm) for bigeye tuna (Thunnus obesus) in the Indian Ocean. The horizontal dotted line indicates 50% of male proportion. Numbers above bars indicate the number of males included in each size class.

Histological classification of ovaries and spawning season

Table 3 shows the selection of ovaries used for the histological classification. According to this classification, 16% of females were at IP, 19% were at DP, 18% were at SCP, 23% at RsP, and 23% at RgP (Table 3). Applying the maturity threshold at spawning capable stage and onward, 65% of the analysed females were mature, from which 25% 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 January to April, with the highest proportions of reproductively active females present in January (25%), February (75%) and March (24%). In contrast, females with less developed ovaries (i.e., IP and DP) were dominant from April to October (around 47% of individuals at IP and around 23% at DP) (Fig 5). Females at RsP and RgP phases were observed throughout the year. In general, both datasets (ALL project and GERUNDIO project) are limited by sample size. However, similar patterns of population ovary maturation process were observed in the monthly evolution of the GSI (Fig 6).

Table 3 Summary of the number of female bigeye tuna (Thunnus obesus) sampled by 5-cm class of fork length (LF) and maturity development. IP = immature phase, DP = developing phase, SCP = spawning capable phase, RsP = regressing phase, RgP = regenerating phase.

Size range	1.IP	2.DP	3.SCP	4.RsP	5.RgP	TOTAL
45 - 49	1	0	0	0	0	1
50 - 54	2	0	0	0	0	2
55 - 59	2	0	0	0	0	2
60 - 64	4	0	0	0	0	4
65 - 69	1	0	0	0	0	1
70 - 74	2	0	0	0	0	2
75 - 79	4	0	0	0	1	5
80 - 84	1	2	2	0	0	5
85 - 89	3	2	0	0	0	5
90 - 94	1	0	0	0	0	1
95 - 99	0	2	0	1	1	4
100 - 104	0	1	0	0	1	2
110 - 114	0	2	0	0	2	4
115 - 119	0	0	0	0	1	1
120 - 124	0	2	2	0	2	6
125 - 129	0	2	1	0	2	5
130 - 134	0	2	2	2	1	7
135 - 139	1	2	5	2	5	15
140 - 144	0	0	5	8	4	17
145 - 149	2	4	4	12	7	29
150 - 154	1	3	3	7	7	21
155 - 159	1	3	3	4	2	13
160 - 164	0	3	3	2	2	10
170 - 174	0	1	0	0	0	1
TOTAL	26	31	30	38	38	163

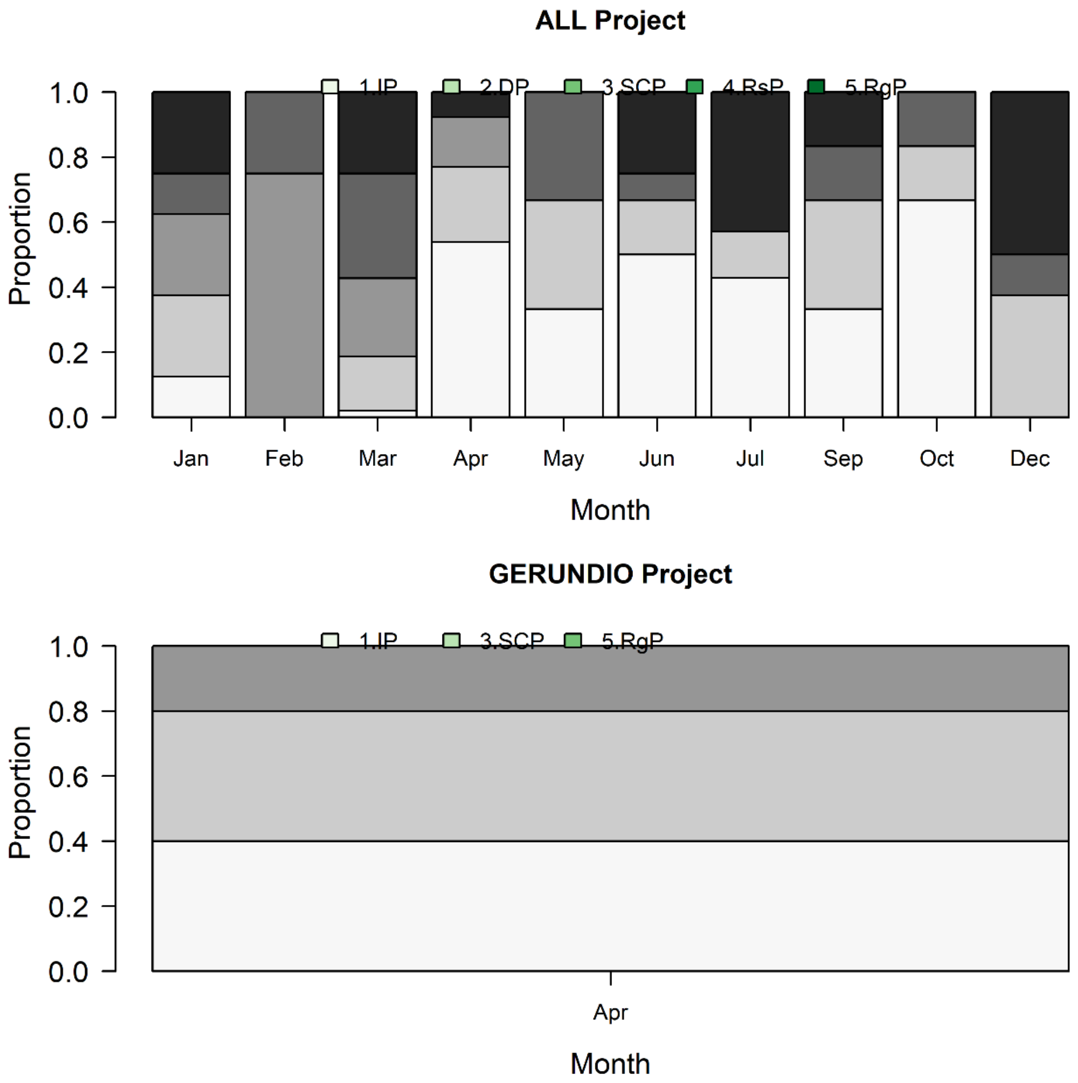

Figure 5 Monthly variations of the proportions of ovary development phases for female bigeye tuna (Thunnus obesus) selected from ALL project (top) and GERUNDIO project (bottom) datasets in the Indian Ocean. IP = Immature phase; DP = Developing phase; SCP = Spawning capable phase; RsP = Regressing phase; RgP = Regenerating phase.

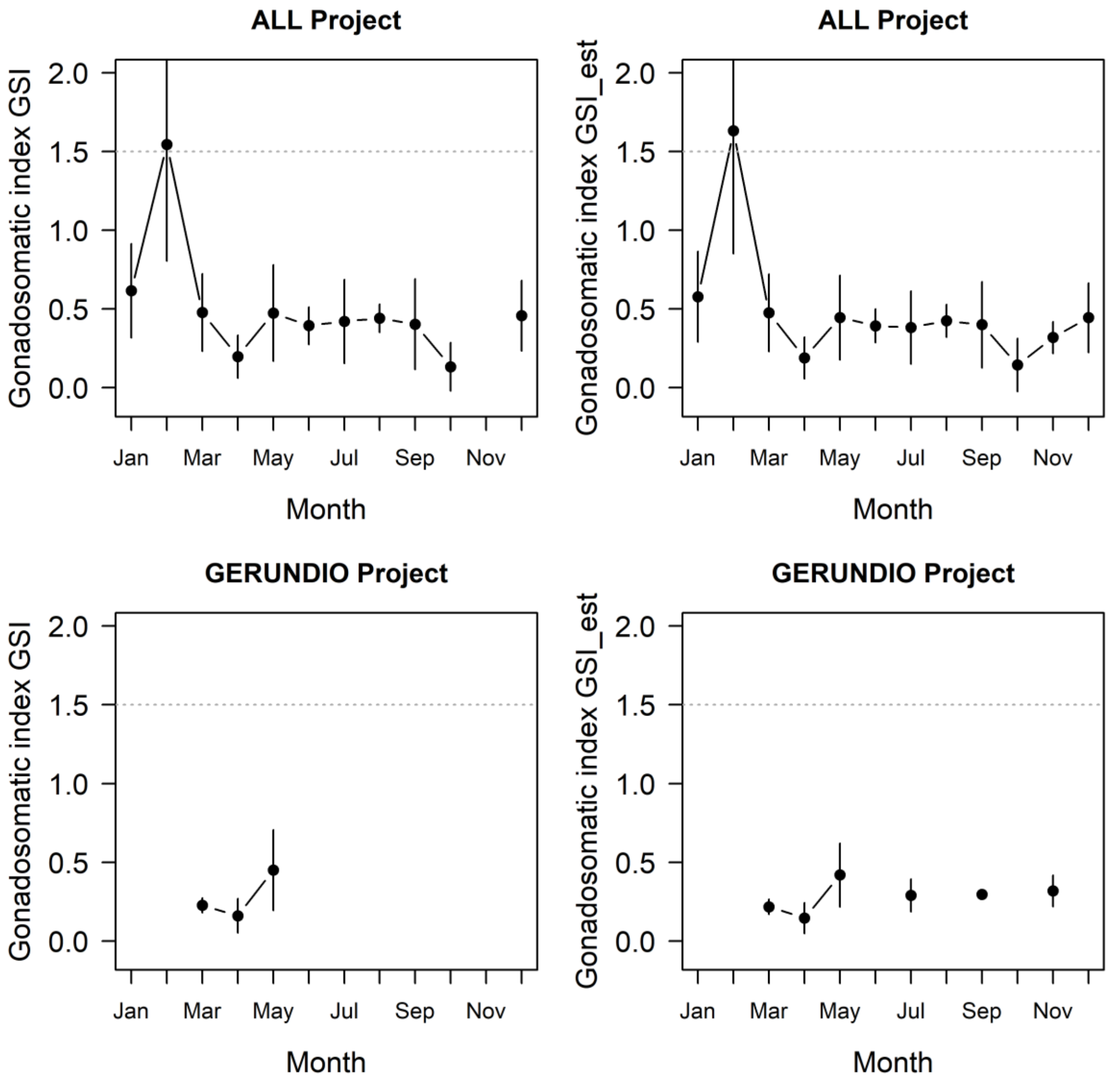

Figure 6 Monthly variations of the gonadosomatic index (GSI) and estimated gonadosomatic index (GSI_est) values for female bigeye tuna (Thunnus obesus) selected from ALL project and GERUNDIO project datasets in the Indian Ocean.

Length at maturity

L_{50} was estimated at 112.7±5.8 cm fork length for the ALL project dataset when females with ovaries in stages iii to iv were considered mature. As only data from 5 females were available from the GERUNDIO project, an accurate maturity ogive could not be estimated (Table 4 and Fig 7).

Table 4 Parameters for the logistic regression model for estimating the fork length of female bigeye tuna (Thunnus obesus) in the Indian Ocean at which 50% of the population is mature (L50, 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	-4.362	0.038	112.7			
Standard error	0.934	0.007				
Significance	*p-value* < 0.001			*p-value*		

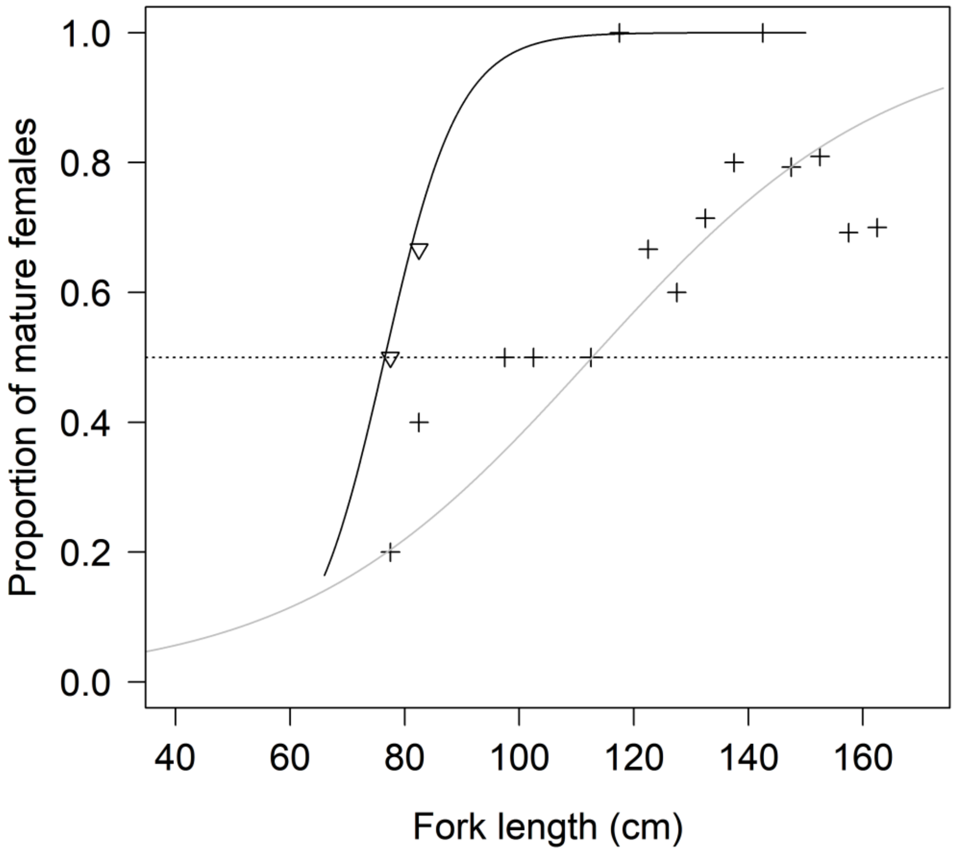

Figure 5 Proportion of mature female bigeye tuna (Thunnus obesus) in the Indian Ocean at 5 cm fork length intervals. Crosses represent the proportions of females considered mature estimated from the ALL project dataset and the grey solid line indicates the logistic regression curve fitted to the data. Reversed triangles represent the proportions of females estimated using the limited GERUNDIO project dataset (n=5); the black solid line indicates the logistic regression curve fitted to these data. The horizontal dotted line indicates L_{50}, i.e. the length at which 50% of the female population was mature. However, as only data from 5 females were available, the ogive is not accurate.

Batch fecundity estimation

The estimated mean batch fecundity (BF) was 0.61±0.46 million oocytes and varied from 0.1 million to 1.88 million oocytes. The mean relative batch fecundity relBF was estimated at 8.29±5.86 oocytes per gram of gonad-free body weight and fluctuated between 1.55 and 23.19 oocytes per gram.

The BF did not increase significantly with fork length (p=0.094). However, the 10% quantile regression line fitted to BF did significantly increase with fork length (p=0.022) (Fig 8). The maximum levels of batch fecundity were observed in the largest females. No relationship was found between relBF and fork length (Fig 8). Both BF and relBF increased significantly with gonad weight (*p*-value <0.01), showing a positive pattern in both estimates and for minimum (10% quantile) and maximum (90% quantile) levels (Fig 8).

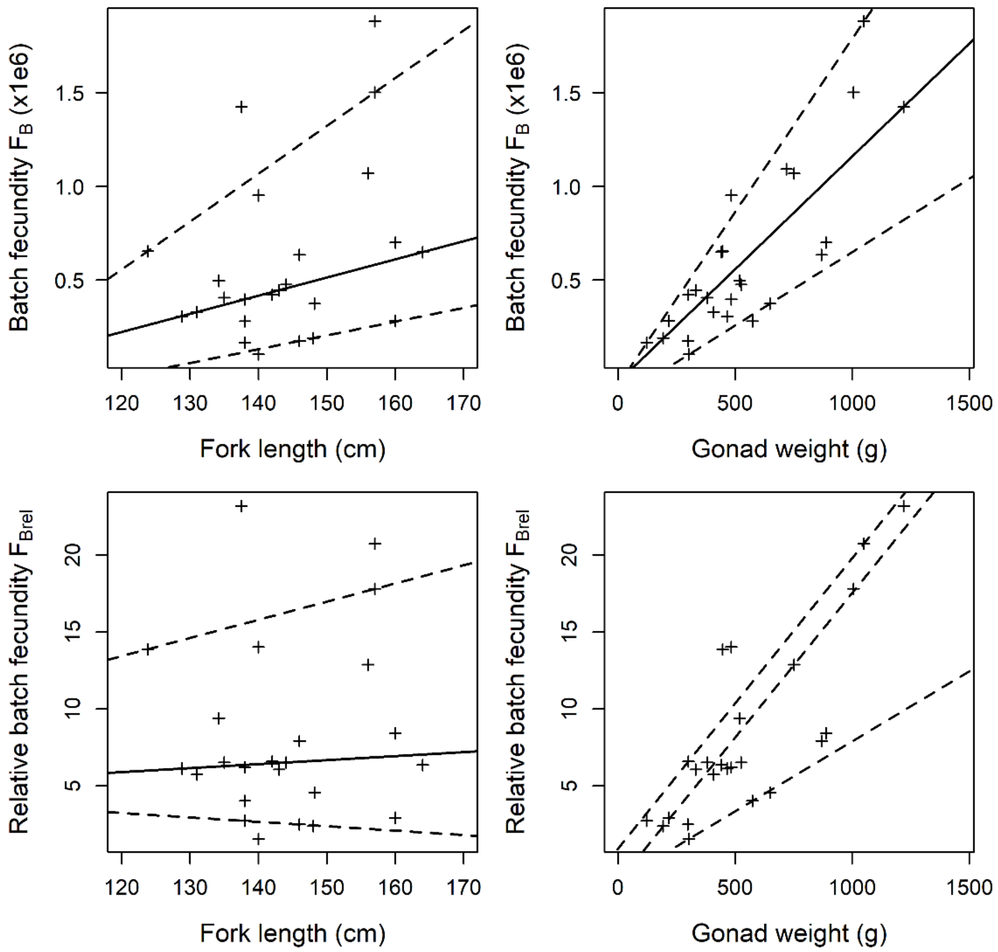

Figure 8. Relationships between batch fecundity (BF, millions of oocytes) (top) and relative batch fecundity (relBF, oocytes per gram of fish body weight) (bottom) with fork length (cm) and ovary weight (g) for female bigeye tuna (Thunnus obesus) using the ALL project data dataset in the Indian Ocean.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

The analysis of variance revealed BF did not vary significantly by month at a 95% confidence level ($F_{(2,21)}$=1.155, *p*-value =0.334), while relBF varied significantly ($F_{(2,21)}$=4.273, *p*-value <0.05). BF estimates appeared highly variable within months (Fig 9).

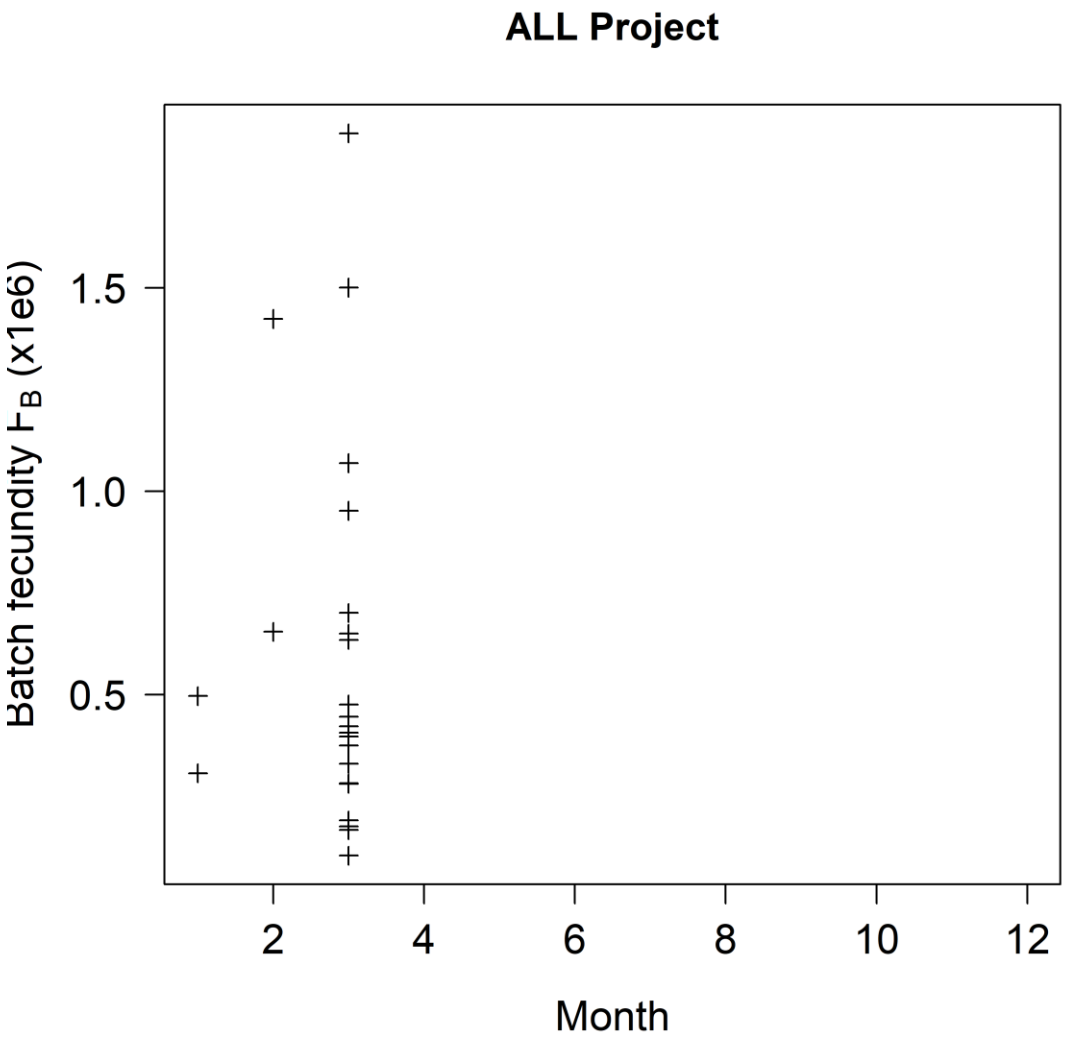

Figure 9. Batch fecundity estimates by month from ALL project datasets for bigeye tuna (Thunnus obesus) in the Indian Ocean.

4. Discussion and Recommendations

This paper provides preliminary estimates of sex ratio, spawning periodicity, batch fecundity and length at maturity for bigeye tuna sampled predominantly in the western Indian Ocean. Further work is required to finalize the analyses, particularly the spawning periodicity and maturity results. 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 project timeframe. In addition, it was not possible to cross-check the histological data obtained from the EMOTION database.

The shape of the preliminary maturity ogive obtained in this study is very different to the ogive currently used in the stock assessment, although the estimates of length at 50% maturity are similar (112.7 cm versus 110.9 cm). The proportion mature at length does not reach 100% as expected in the larger length classes and requires further investigation.

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. 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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