

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

Iker Zudaire<sup>1</sup>, Iraide Artetxe-Arrate<sup>1</sup>, Jessica Farley<sup>2</sup>, Hilario Murua<sup>3</sup>, Deniz Kukul<sup>1</sup>, Annie Vidot<sup>4</sup>, Shoaib Abdul Razzaque<sup>5</sup>, Mohamed Ahusan<sup>6</sup>, Evgeny Romanov<sup>7</sup>, Paige Eveson<sup>2</sup>, Naomi Clear<sup>2</sup>, Patricia L. Luque<sup>1</sup>, Igaratza Fraile<sup>1</sup>, Nathalie Bodin<sup>8,9</sup>, Emmanuel Chassot<sup>8,10</sup>, Rodney Govinden<sup>4</sup>, Ameer Ebrahim<sup>4</sup>, Umair Shahid<sup>6</sup>, Theotime Fily<sup>8</sup>, Francis Marsac<sup>8</sup>, Gorka Merino<sup>1</sup>

<sup>1</sup> AZTI, Marine Research, Basque Research and Technology Alliance (BRTA), Herrera Kaia, Portualdea z/g, 20110 Pasaia – Gipuzkoa, Spain.

<sup>2</sup> CSIRO Oceans and Atmosphere, Hobart, Australia.

<sup>3</sup> International Seafood Sustainability Foundation, Washington DC, USA.

<sup>4</sup> Seychelles Fishing Authority, Mahe, Seychelles.

<sup>5</sup> WWF Pakistan, Karachi, Pakistan.

<sup>6</sup> Maldives Marine Research Institute, Moonlight Hingun, Male, Maldives.<sup>7</sup> CAP RUN - CITEB (Centre technique de recherche et de valorisation des milieux aquatiques), Île de la Réunion.

<sup>8</sup> Research Institute for Sustainable Development (IRD), Victoria, Seychelles

<sup>9</sup> Sustainable Ocean Seychelles (SOS), Beau Belle, Seychelles

<sup>10</sup> IOTC Secretariat, Victoria, Seychelles

\*Corresponding author: izudaire@azti.es

### **Executive Summary**

*NOTE: A preliminary document of this paper was submitted as INFO paper to the SC24 with the reference IOTC-2021-SC24\_INF01.*

This paper describes preliminary work to estimate reproductive parameters for bigeye tuna (*Thunnus obesus*) in the Indian Ocean as part of the 'GERUNDIO' project<sup>1</sup>. The 2019 stock assessment for bigeye 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 2021). The assessment model used a maturity ogive equivalent to that used by Shono et al. (2009), for which length at 50% maturity was 110.9 cm fork length (FL) and full maturity was around 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 information 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

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<sup>1</sup> 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.

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 between 2009-2019 and were also predominantly from the western Indian Ocean.

Here, preliminary estimates of sex ratio, spawning periodicity, batch fecundity and length at maturity are provided for bigeye tuna, mainly from the west region of the Indian Ocean. Further work is required to finalize these 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.

The shape of the preliminary maturity ogive (i.e., the proportion of mature individuals at age or length) 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 (ISSF 2022). In the Indian Ocean (IO), average catch between 2016-2020 was 86,880 tonnes (IOTC 2021). Bigeye tuna is a valuable species 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 2022). However, high piracy activity in the region during the mid-2000s resulted in a decline in LL activity and in the

catches of bigeye tuna (Fu 2019). Longline 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 for which 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 (Fu et al. 2019; 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 (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 (Stéguert and Marsac 1989; Stobberup et al. 1998; Nootmorn 2004; Ariz et al. 2006; Zhu et al. 2011, Zudaire et al., 2016), 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 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”.

However, due to the limited number of bigeye individuals histologically analysed in this study, results for some analyses were only shown for ALL projects dataset.

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-a). 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 FL, and from 9.5 to 34.5 kg total weight (Table 1 and Fig 2).

Gonadosomatic index (GSI) was calculated as  $\text{gonad weight}/(\text{total weight} - \text{gonad weight}) \times 100$ . 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 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 GERUNDIO project. The individuals were collected from 2009-2019 predominantly from the western Indian Ocean (Fig 1-b). 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).

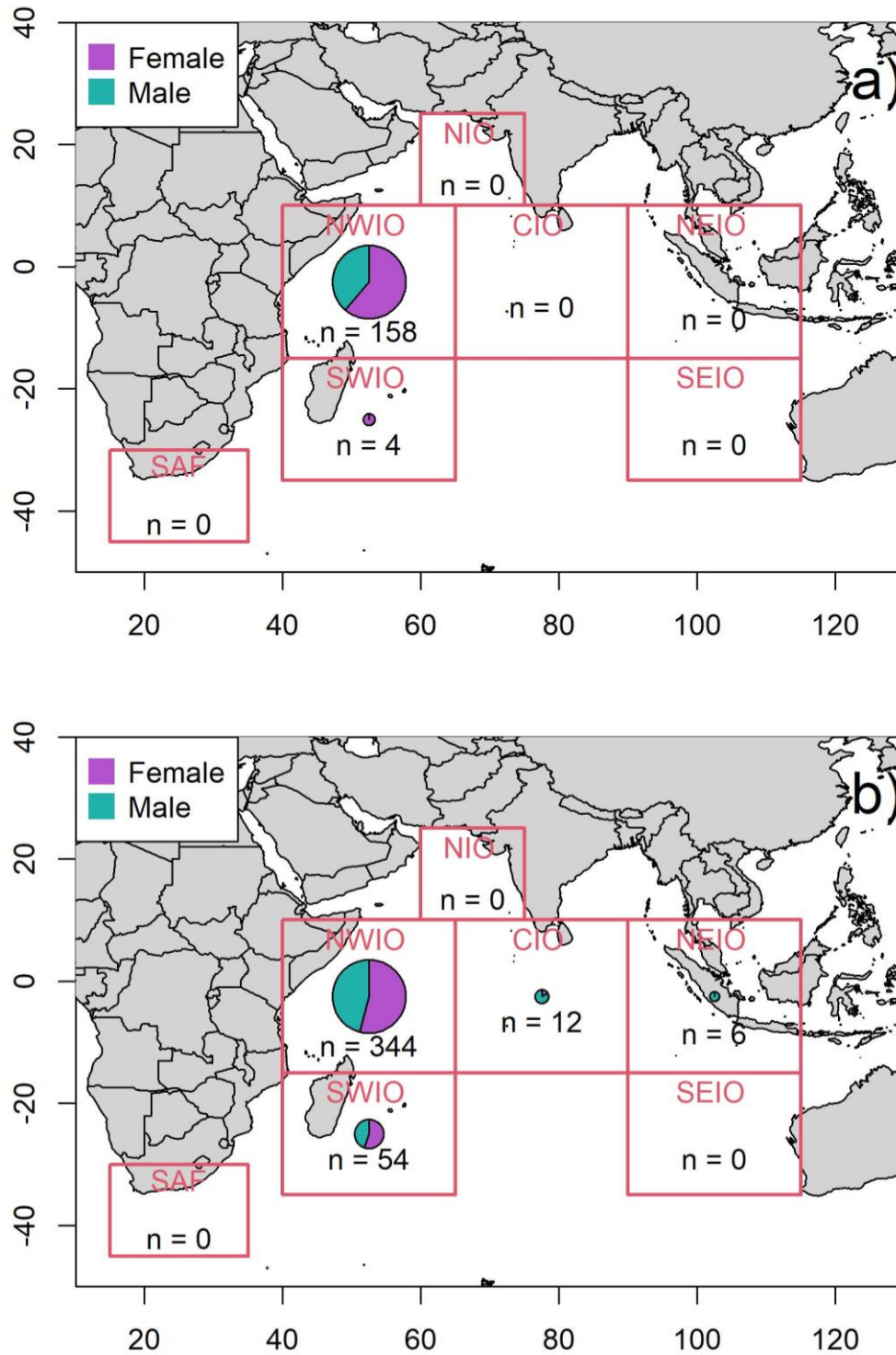


Figure 1 Map showing sampling locations and number individual bigeye tuna (*Thunnus obesus*) with sex data used in the analysis for a) GERUNDIO project and b) ALL project data sets in the Indian Ocean. Sampling regions across the Indian Ocean were defined as South Africa (SAF), Southwest Indian Ocean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

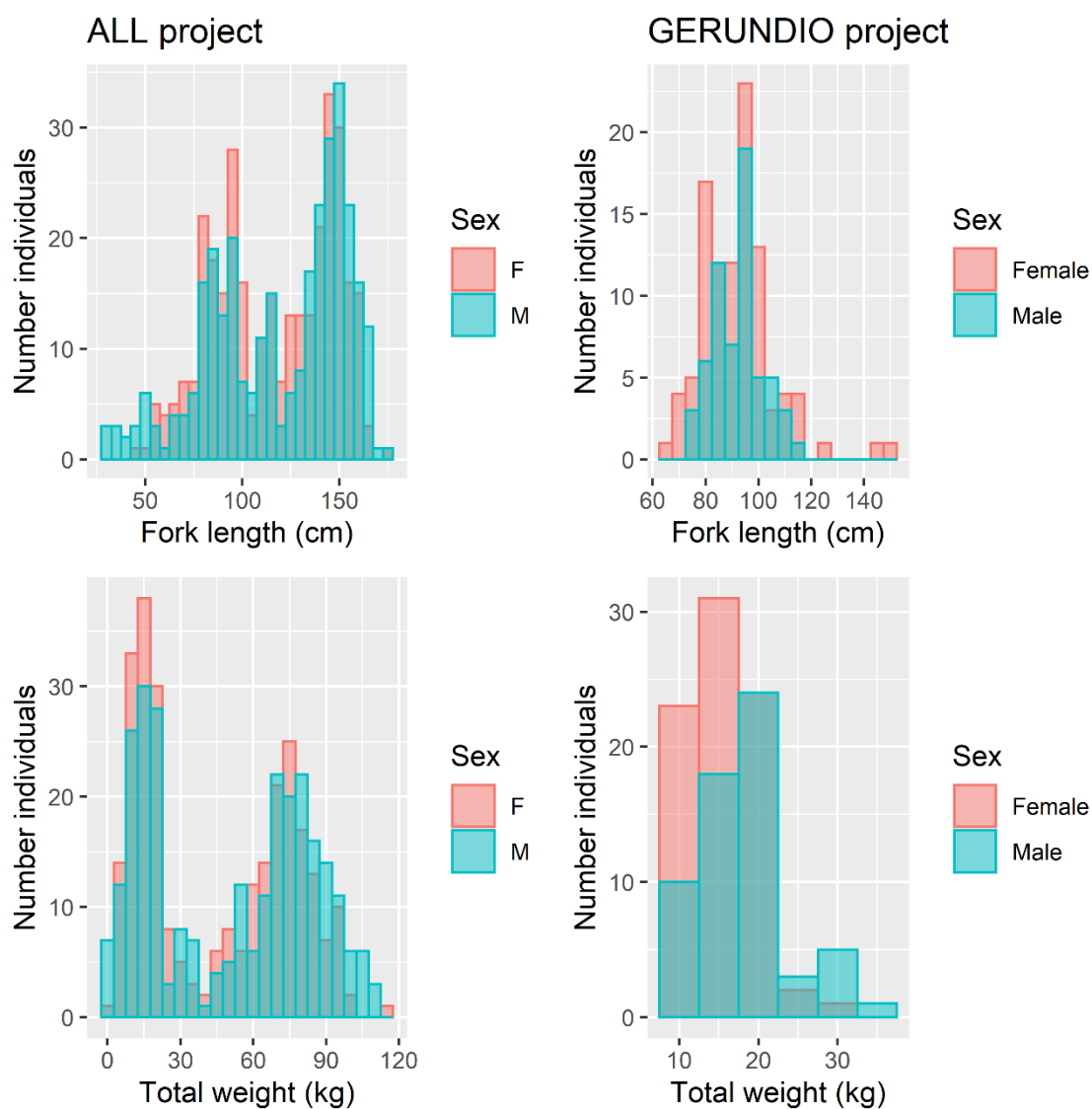


Figure 2 Fork length (in cm) and total weight (in kg) class frequency of bigeye tuna (*Thunnus obesus*) by sex sampled within the ALL project and GERUNDIO project.

Table 1 Number of bigeye tuna (*Thunnus obesus*) included in the analysis from the GERUNDIO project and from previous projects (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	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	-	-
PSTBS-IO (Davies et al., 2020)	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  $\mu\text{m}$  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 (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 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 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  $\alpha$  and  $\beta$  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 25 ovaries, at which the hydration stage oocyte appeared as the most advanced stage of development (0 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 (Stéquent and Ramcharrun, 1996). For BF analyses, three subsamples of 0.1 g ( $\pm 0.01$  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 (FL 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 FL class in the sample, and Chi-square tests ( $\chi^2$ ) 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**

### **Length-weight relationships and sex-ratio**

Sex did not significantly affect the relationship between FL and weight in bigeye tuna ( $p$ -value = 0.112). The FL to weight relationship of both sexes combined is comparable to that currently used within IOTC (2021) for all size ranges (Fig 3).



### ALL Project

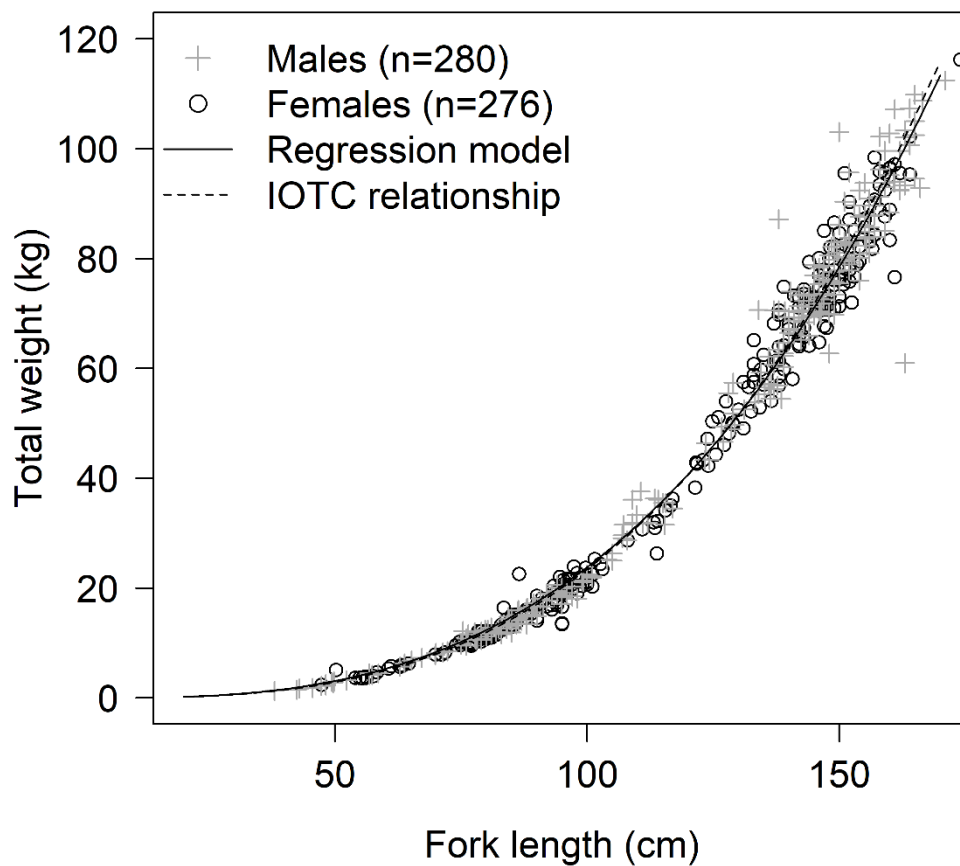


Figure 3 Relationship between fork length (cm) and total weight (kg) for male (cross) and female (open circle) bigeye tuna (*Thunnus obesus*) sampled in the Indian Ocean. Solid line indicates the regression model fitted to all individuals; Dashed line indicates the IOTC relationship currently used.

## 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 ( $\chi^2$  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	$\chi^2$	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

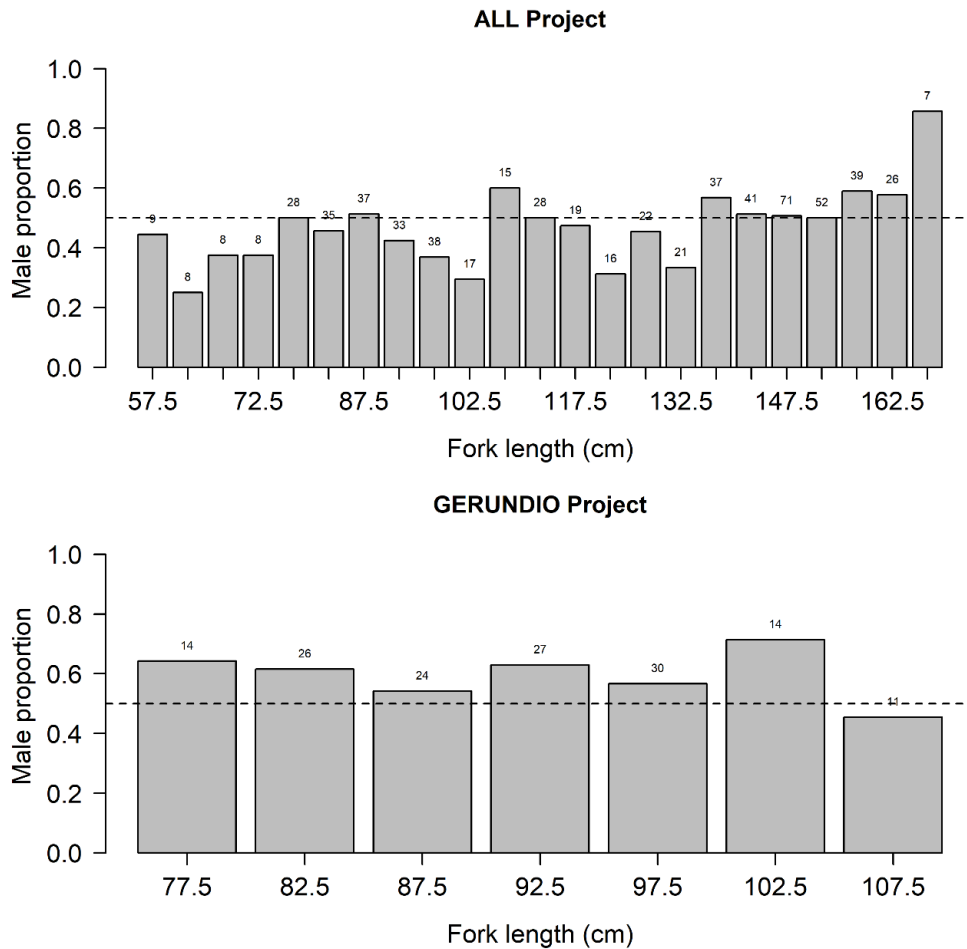


Figure 4. Variations of sex-ratio with fork length (cm) for bigeye tuna (*Thunnus obesus*) in the Indian Ocean. The horizontal dotted line indicates 50% of male proportion. Numbers above bars indicate the number of individuals included in each size class.

### Histological classification of ovaries and spawning season

Table 3 and figure 5 show 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 early vitellogenic stage (including Vit 1 and Vit 2) and onward, 77% of the analysed females were mature, from which 18% 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

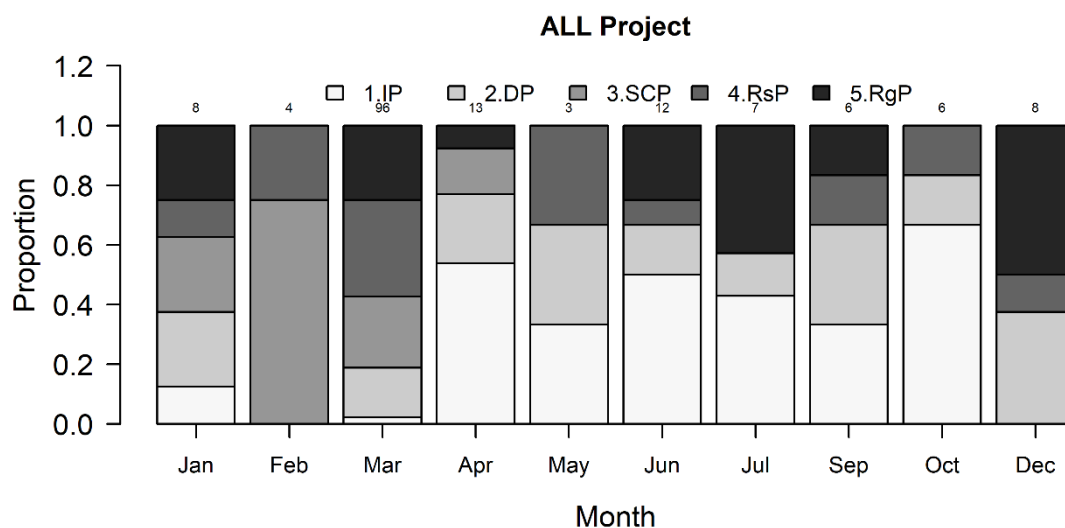


Figure 5 Monthly variations of the proportions of ovary development phases for female bigeye tuna (*Thunnus obesus*) selected from ALL project dataset in the Indian Ocean. IP = Immature phase; DP = Developing phase; SCP = Spawning capable phase; RsP = Regressing phase; RgP = Regenerating phase. Numbers above bars indicate the number of individuals included in each month.

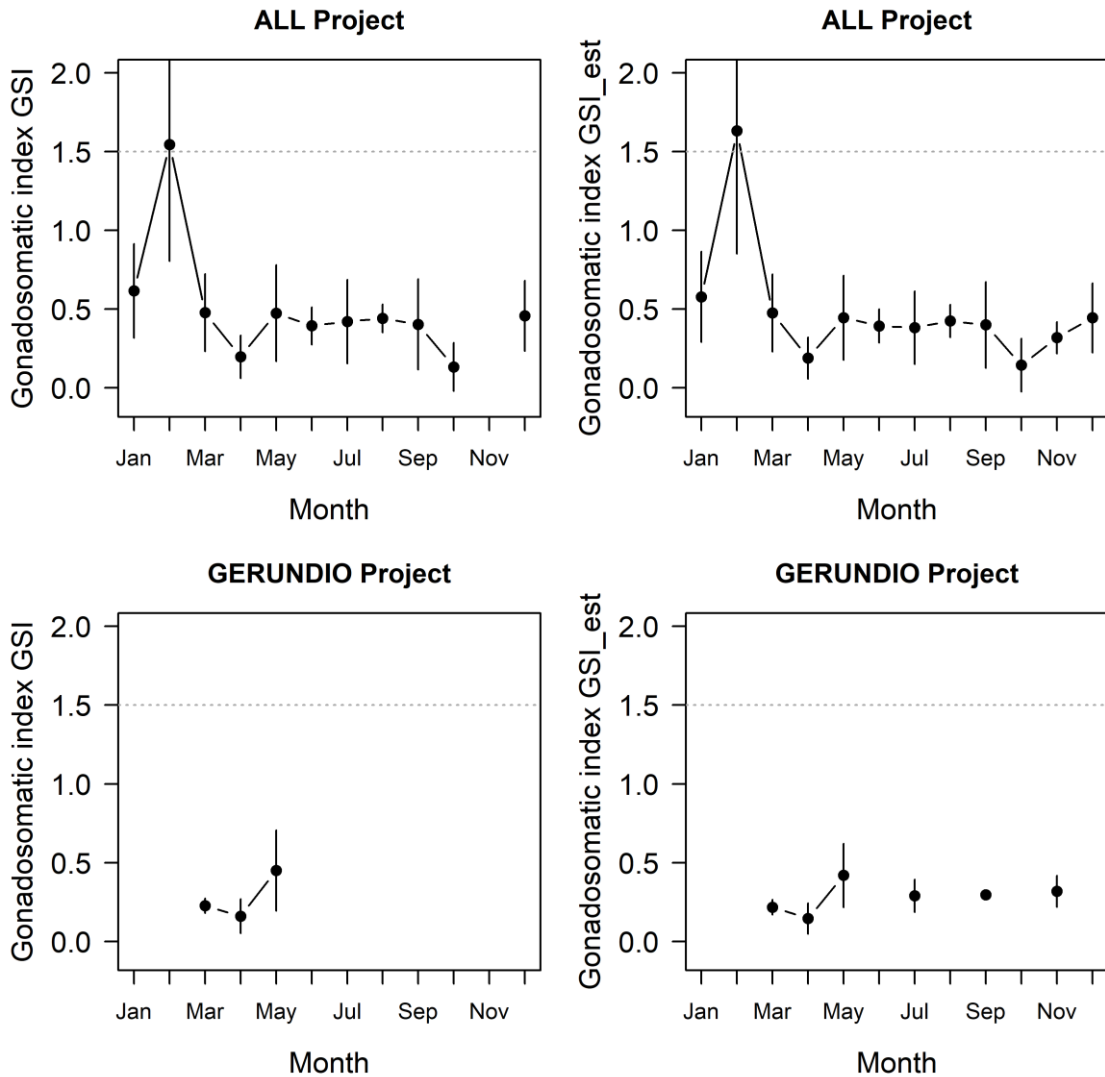


Figure 6 Monthly variations of the gonadosomatic index (GSI) and estimated gonadosomatic index (GSI\_est) values for female bigeye tuna (*Thunnus obesus*) selected from ALL project and GERUNDIO project datasets in the Indian Ocean.

### Length at maturity

$L_{50}$  was estimated at  $100.3 \pm 1.02$  cm FL 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). Further work is required to finalize the analyses as it is currently based on data from a subset of the ovaries collected in the GERUNDIO project. It was also not possible to undertake the required cross-checking (re-reading) of all histological sections within the project timeframe.

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 ( $L_{50}$ , cm). Ovaries in stages iii to iv were considered mature.  $\alpha$  and  $\beta$  are the coefficients of the equation and  $L_{50}$  was computed as  $-\alpha / \beta$  for the maturity threshold used: Vit 3 = tertiary vitellogenesis for data from ALL Project.

ALL project			
Parameters	$\alpha$	$\beta$	$L_{50}$
Estimates	-5.46	0.054	100.3
Standard error	1.02	0.008	
Significance	$p$ -value < 0.001		

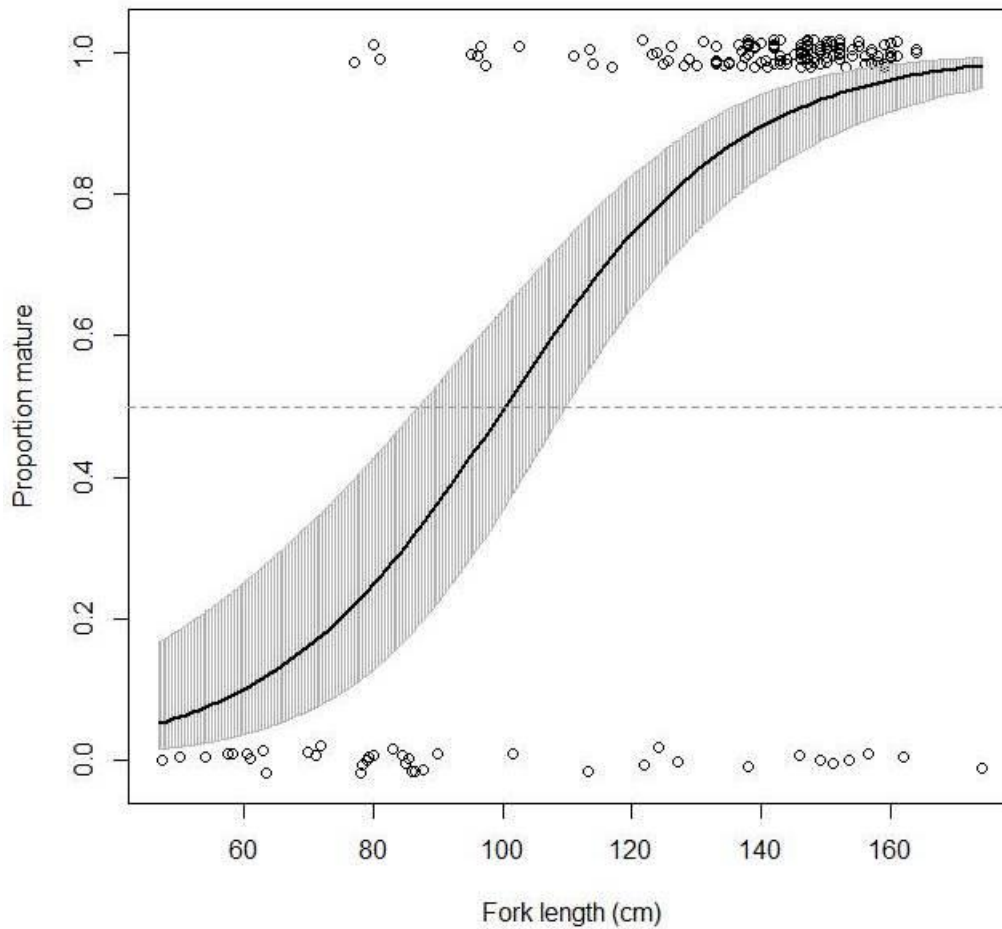


Figure 7 Estimated proportion of mature female bigeye tuna (*Thunnus obesus*) in the Indian Ocean by fork length. Open circles show the maturity status (0 = immature; 1 = mature) estimated for each fish in the ALL Project dataset (note that the points have been jittered to reduce overlap). The solid 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  $0.61 \pm 0.46$  million oocytes and varied from 0.1 million to 1.88 million oocytes. The mean relative batch fecundity relBF was estimated at  $8.29 \pm 5.86$  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 FL ( $p=0.094$ ). However, the 10% quantile regression line fitted to BF did significantly increase with FL ( $p=0.022$ ) (Fig 8). The maximum levels of batch fecundity were observed in the largest females. No relationship was found between relBF and FL (Fig 8). Both BF and relBF increased significantly with gonad weight ( $p$ -value  $< 0.01$ ), showing a positive pattern in all three quantile regressions (Fig 8).

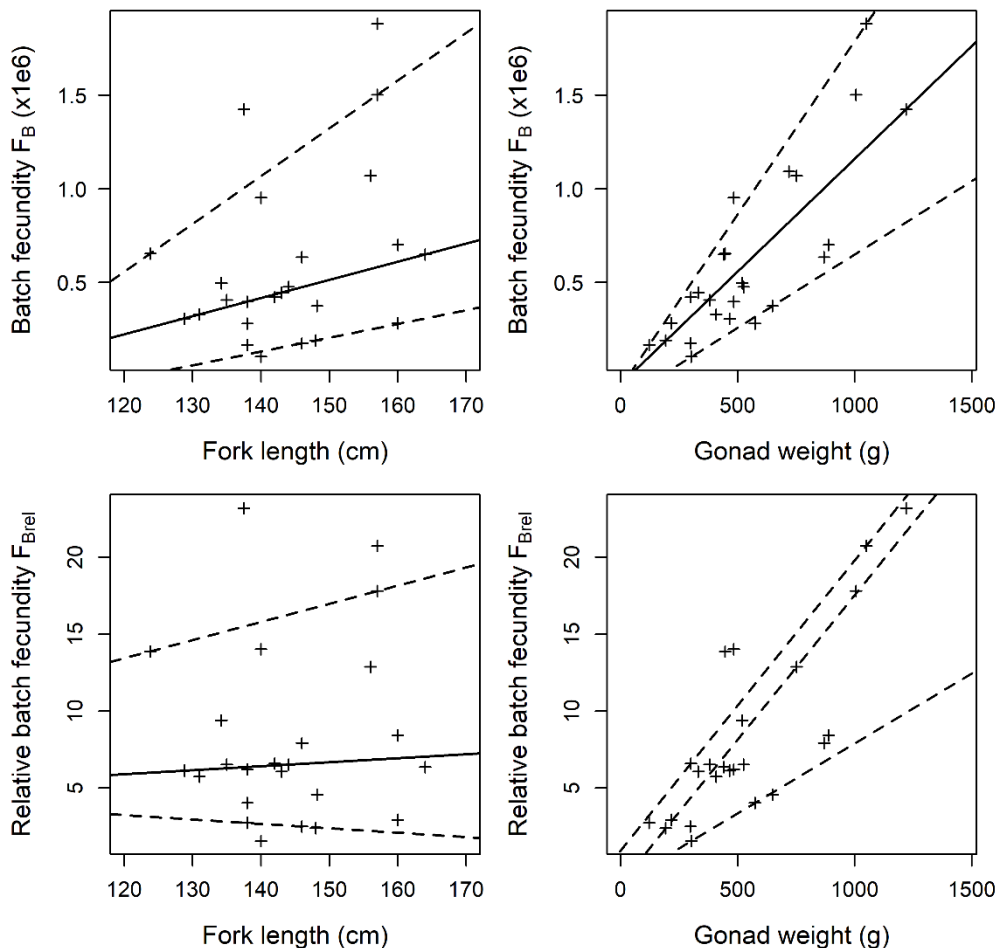


Figure 8. Relationships between batch fecundity (BF, millions of oocytes) (top) and relative batch fecundity (relBF, oocytes per gram of fish body weight) (bottom) with fork length (cm) and ovary weight (g) for female bigeye tuna (*Thunnus obesus*) using the ALL project data dataset in the Indian Ocean.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

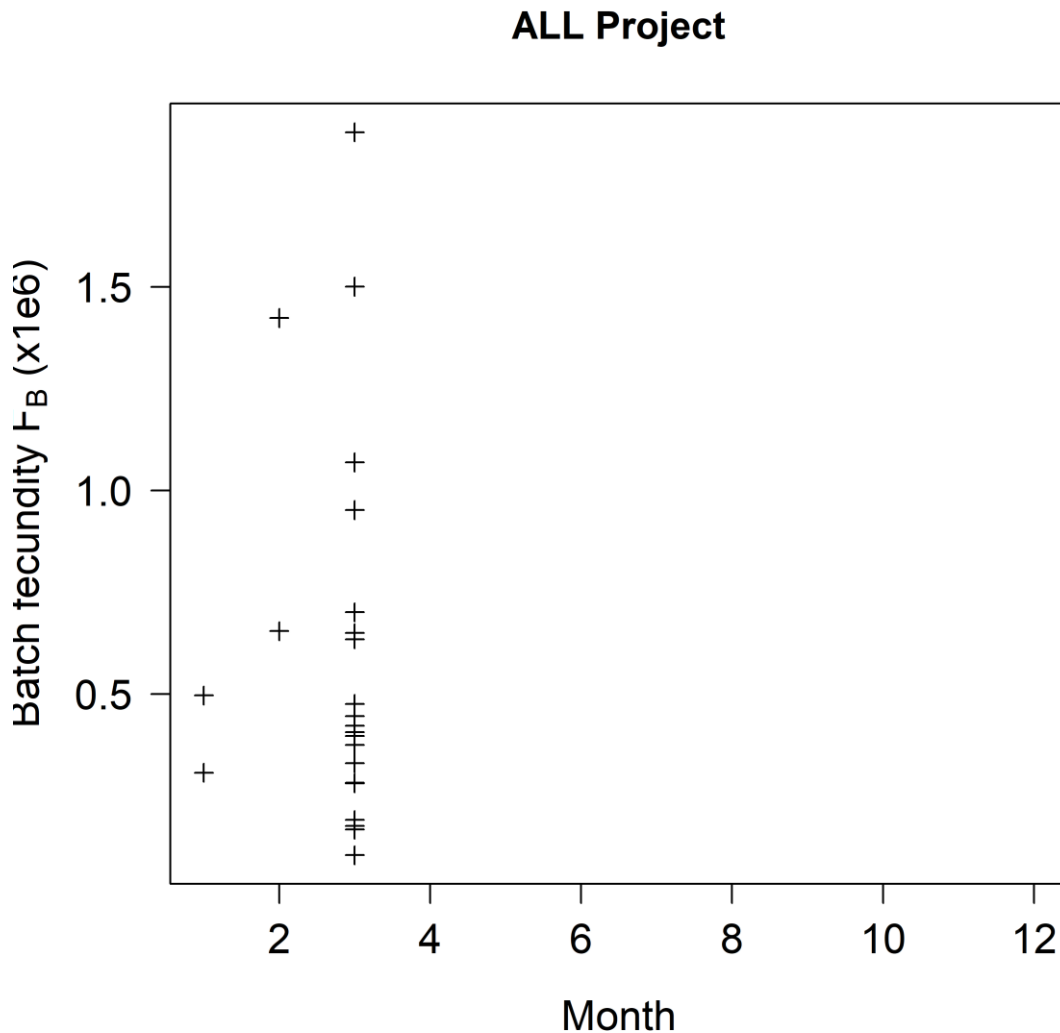


Figure 9. Batch fecundity estimates by month from ALL project datasets for bigeye tuna (*Thunnus obesus*) in the Indian Ocean.

#### 4. Conclusion 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 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 region of the Indian Ocean. We recommend that additional gonad samples are collected and analysed from other 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 and to analyse whether differences could exist between areas. Fish >60 cm FL (~minimum size at maturity) are particularly important to increase the sample size available for maturity, fecundity and spawning fraction analyses. Sampling performed on monthly basis is important to obtain reproductive data throughout the year. We also recommend that additional gonad samples from different fishing gears (e.g., longline) are collected to improve both the size and population spatial range coverage. Continuing to collect and analyse gonads over time will be particularly important for assessing inter-annual variation in reproductive parameters, which in turn, will help to reduce the uncertainty in stock assessment models used for bigeye tuna management in the Indian Ocean.

## **Acknowledgements**

We are grateful to the many vessel skippers and crew involved in the project. We gratefully acknowledge all the observers, port samplers and coordinators for collecting, storing and transporting gonads across the Indian Ocean. We want to thank the EMOTION, PEVASA, CANAL, MADE, PSTBS-IO, SAUMTEST, IOTTP, projects for permitting the use of available biological data collected during these projects. We want to thank the PROBIO project for providing an excellent sampling platform to obtain samples required for this project. The GERUNDIO project is supported by financial assistance of the European Union (contract no. 2020/SEY/FIDTD/IOTC - CPA 345335). The views expressed herein can in no way be taken to reflect the official opinion of the European Union.

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