

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

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Executive Summary

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This paper describes preliminary work to estimate reproductive parameters for skipjack tuna (*Katsuwonus pelamis*) in the Indian Ocean as part of the 'GERUNDIO' project. The most recent stock assessment of skipjack tuna in the Indian Ocean shows that the stock is not overfished and is not subject to overfishing (Fu 2020, IOTC 2020). The assessment model used a maturity ogive (i.e., the proportion of mature individuals at age or length) for the western Indian Ocean from Grande et al. (2010), with length at 50% maturity (L₅₀) at 37.8 cm fork length (FL) and full maturity at around 42 cm FL. A slightly updated ogive was presented in Grande et al. (2014) with L₅₀ estimated at 39.9 cm FL. Grande et al. (2010; 2014), used the 'cortical alveolar' oocyte development stage and above as the threshold indicating a fish was mature. One of the aims of the current project was to produce updated estimates of key biological reproductive parameters for skipjack tuna in the Indian Ocean. This included updating information on length-at-maturity using the (older) 'early vitellogenic' oocyte stage as the maturity threshold, which is considered a more reliable threshold for determining a female is mature and contributing to egg production (Schaefer 2001).

A total of 635 skipjack tuna were sampled in the GERUNDIO project (296 females and 339 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 subset of 84 ovary samples, which were read using an agreed classification system. Additional ovaries collected in the project will be processed soon to update the current analysis.

Data from an additional 1151 skipjack tuna (862 females and 649 males) were obtained from previous projects (EMOTION database, see Bodin et al. 2018), which included histological data from 756 females classified using a similar classification scheme to that agreed in the GERUNDIO 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 skipjack 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 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.

The estimate of L_{50} (41.3 cm FL) obtained in this study is slightly higher than that obtained by Grande et al. (2010; 2014), probably affected by different maturity thresholds used in both studies. Our L_{50} estimate was slightly lower than estimated by Stequert and Rammcharrun (1996) and Timohino and Romanov (1996) (42 and 43 cm FL respectively) using the same maturity threshold.

There was insufficient data to examine region-specific reproductive parameters in this project since most of the gonads were samples 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 >30 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. Continuing to collect and analyse gonads over time will be particularly important for assessing inter-annual variation in reproductive parameters.

1. Introduction

Skipjack tuna (*Katsuwonus pelamis*) is a cosmopolitan species inhabiting tropical and subtropical waters of the Indian, Pacific and Atlantic Oceans (Collette & Nauen 1983). It is one of the most commercially valuable fish; it accounts for more than the half of global tuna production, and is the third most harvested marine species worldwide (FAO 2020, McKinney et al. 2020). Skipjack tuna have specific life history attributes, such as relatively fast growth rates and early maturation, that render the species more resilient

and less susceptible to overfishing than other commercial tuna species (Schaefer 2001, Murua et al. 2017). Currently, skipjack tuna stocks in all oceans are considered to be in a healthy status, both in terms of stock abundance and fishing mortality (ISSF 2021). Based on the results of the stock assessment of skipjack tuna in 2020, the Indian Ocean skipjack was considered not overfished and that overfishing was not occurring (Fu 2020). However, due to the high levels of exploitation of this species, sound management is necessary to ensure that this remains the case. The Indian Ocean Tuna Commission (IOTC) adopted an annual catch limit of 513,572 tonnes for skipjack for the period 2021-2023 following the application of the Harvest Control Rule in Resolution 16/02 (IOTC 2020).

Skipjack, like other tuna species, show asynchronous oocyte development and have an indeterminate fecundity type. Skipjack are capable of spawning daily and can spawn year-round in tropical waters, and during summer and early autumn in subtropical waters. The spawning period seems to shorten as the distance from equator increases (Matsumoto et al. 1984). Available studies on the reproductive potential of skipjack tuna in the Indian Ocean are scarce and limited to the western Indian Ocean region. The mean relative batch fecundity was estimated at 140 ± 64 eggs g^{-1} of fish (Grande et al. 2012, 2014), while individual batch fecundity ranged from 80,000 to 1.25 million oocytes, for 44 to 75 cm FL females, respectively (Stéquent & Ramcharrun 1995). In the western Indian Ocean between 10-20°S, skipjack tuna can spawn throughout the year, with peaks of intensity between November-March and June-July, coinciding with the two monsoonal seasons of the region (Stéquent et al. 2001, Grande et al. 2014). Year-round spawning has also been reported for skipjack tuna off India, however peak in activity occurs between December-March, with a secondary peak from June to August (Koya et al. 2012).

An essential part of the skipjack stock assessment is knowing the size of the spawning stock biomass (SSB); for which maturity data-at-size/age is necessary. In addition, reliable estimates of a population's resilience to fisheries required the fluctuations on stock productivity to be monitored over time, and the underlying mechanisms of these fluctuations to be understood (Wright & Trippel 2009). The 2020 stock assessment model used a maturity ogive for the western Indian Ocean from Grande et al. (2010), with L_{50} at 37.8 cm FL and full maturity at around 42 cm FL. A slightly updated ogive was estimated by Grande et al. (2014) with L_{50} at 39.9 cm FL. Grande et al. (2010; 2014) studies used the 'cortical alveolar' oocyte development stage and above as the threshold indicating a fish was mature. Grande et al. (2014) also estimated a maturity ogive using the (older) 'advanced vitellogenic' oocyte stage as the maturity threshold, which is considered a more reliable threshold for determining a female is mature and contributing to egg production (Schaefer 2001). The estimate of L_{50} using this alternative maturity ogive was 43.5 cm FL. Two earlier studies estimated L_{50} to be slightly higher at 42-43 cm FL (Stéquent and Rammcharrun 1996, Timohino and Romanov 1996) using the same 'advanced vitellogenic' oocyte maturity threshold.

The lack of skipjack tuna reproductive studies at an oceanic scale make it difficult to determine the productivity of this species in the Indian Ocean. More comprehensive studies are needed to better understand the potential fluctuations in population

dynamics, which in turn, will allow to better assessment of skipjack tuna resilience to both fishing activities and environmental changes in this 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 skipjack tuna sex ratio, spawning periodicity, 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 skipjack 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 information of samples 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”.

From the GERUNDIO project a total of 635 skipjack tuna (296 females and 339 males) were used for analyses. These individuals were collected between 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 36 to 72 cm FL, and from 0.88 to 7.6 kg total weight (Table 1 and Figure 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 skipjack 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 1511 skipjack tuna were obtained from the EMOTION project (862 females and 649 males), which included histological data from 756 females classified using a similar classification scheme to that agreed in this project. The individuals were collected from 2009-2019 predominantly from the western Indian Ocean

(Figure 1-b). Fish ranged from 30 to 72.3 cm FL and from 0.5 to 8.5 kg total weight (Table 1 and Figure 2). The fish sampled in the “GERUNDIO project” and the combined “ALL project” were geographically determined by sampling region (Figure 1).

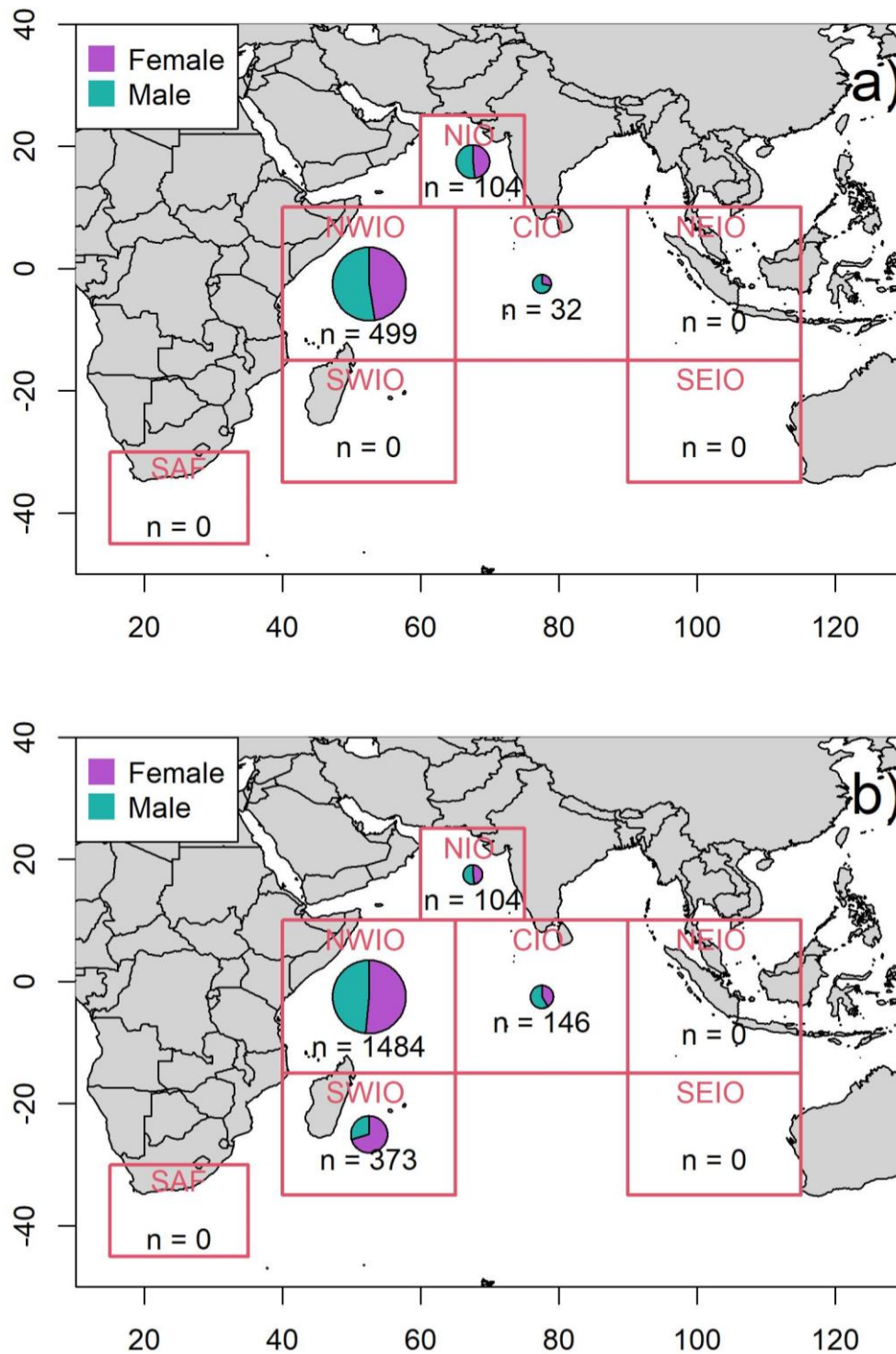


Figure 1 Map showing sampling locations and number individual skipjack tuna (*Katsuwonus pelamis*) with sex data used in the analysis for a) GERUNDIO project and b) ALL project data sets in the Indian Ocean. Sampling regions across the Indian Ocean were defined as South Africa (SAF), Southwest Indian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

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

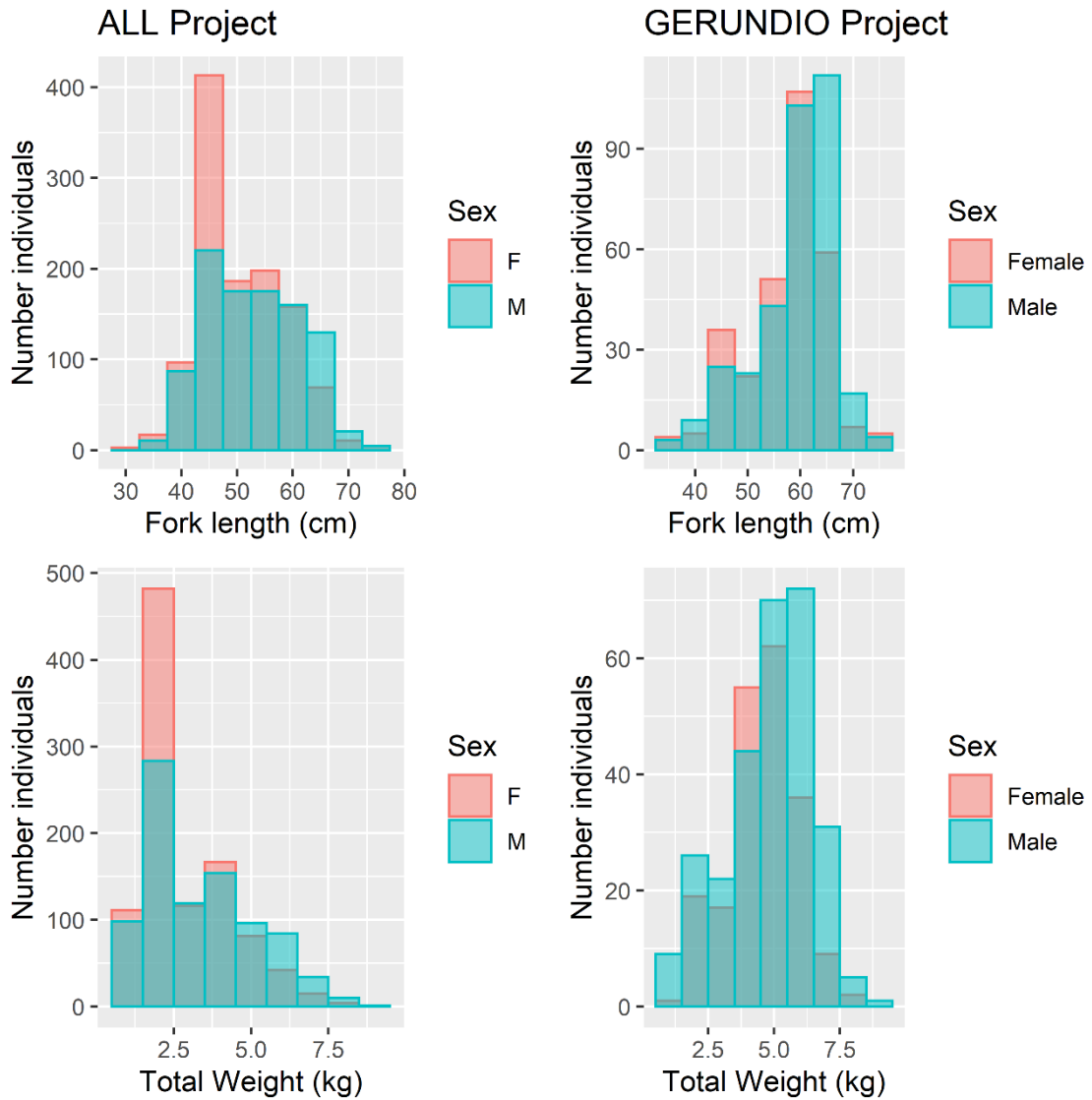


Figure 2 Fork length (in cm) and total weight (in kg) class frequency of skipjack tuna (*Katsuwonus pelamis*) by sex sampled within the ALL project (top panel) and GERUNDIO project (bottom panel).

Table 1 Number of skipjack tuna (*Katsuwonus pelamis*) samples 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).

projects	Female	Male	Total	Length range (cmFL - Female)	Length range (cmFL - Male)	Weight range (Kg - Female)	Weight range (Kg - Male)	
ASCLME-POP	7	5	12	47.4 - 58.9	51.2 - 57.9	2.3 - 4.1	2.8 - 4.6	
CANAL	81	92	173	30 - 72.3	41 - 71.6	0.5 - 8.1	1.4 - 8.5	
EMOTION	2	0	2	39.4 - 40.6	-	1.3 - 1.4	-	
(Bodin et al., 2018)	IOT_quality	5	4	9	45.4 - 58.5	51.3 - 60.4	1.8 - 4.7	2.7 - 5.4
Liver test	0	1	1	-	66.4 - 66.4	-	7.2 - 7.2	
MADE	20	26	46	44 - 57	40 - 74	-	-	
PEVASA	733	499	1232	32 - 68	36 - 67	0.75 - 7.4	0.83 - 7.2	
SAUMTEST	1	4	5	54.9 - 54.9	38.4 - 56.7	3.7 - 3.7	1.1 - 4	
PSTBS-IO								
(Davies et al., 2020)	PSTBS-IO	13	18	31	45 - 62	45 - 67	-	-
GERUNDIO	GERUNDIO	296	339	635	36 - 70	36 - 72	1.08 - 7.6	0.88 - 7.08
ALL Project		1158	988	2146	30 - 72.3	36 - 74	0.5 - 8.1	0.8 - 8.5

Histological classification of ovaries

A subset of 84 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 756 ovary sections prepared from previous projects (see Bodin et al. 2018) were obtained for a combined total of 840 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. All ovaries included in the "ALL project" dataset 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 (PsP) 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 skipjack 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 will be 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 (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 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 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), or the total number of oocytes released per batch, was estimated for 55 ovaries (of which none were from the GERUNDIO project) by gravimetric method (Hunter et al., 1985), , 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 (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 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 skipjack 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 and discussion

Length-weight relationships

There were not significant differences (p -value = 0.339) by sex in the FL- weight relationship of skipjack tuna when data from the ALL project dataset was analysed. 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 and large size fish (Figure 3).

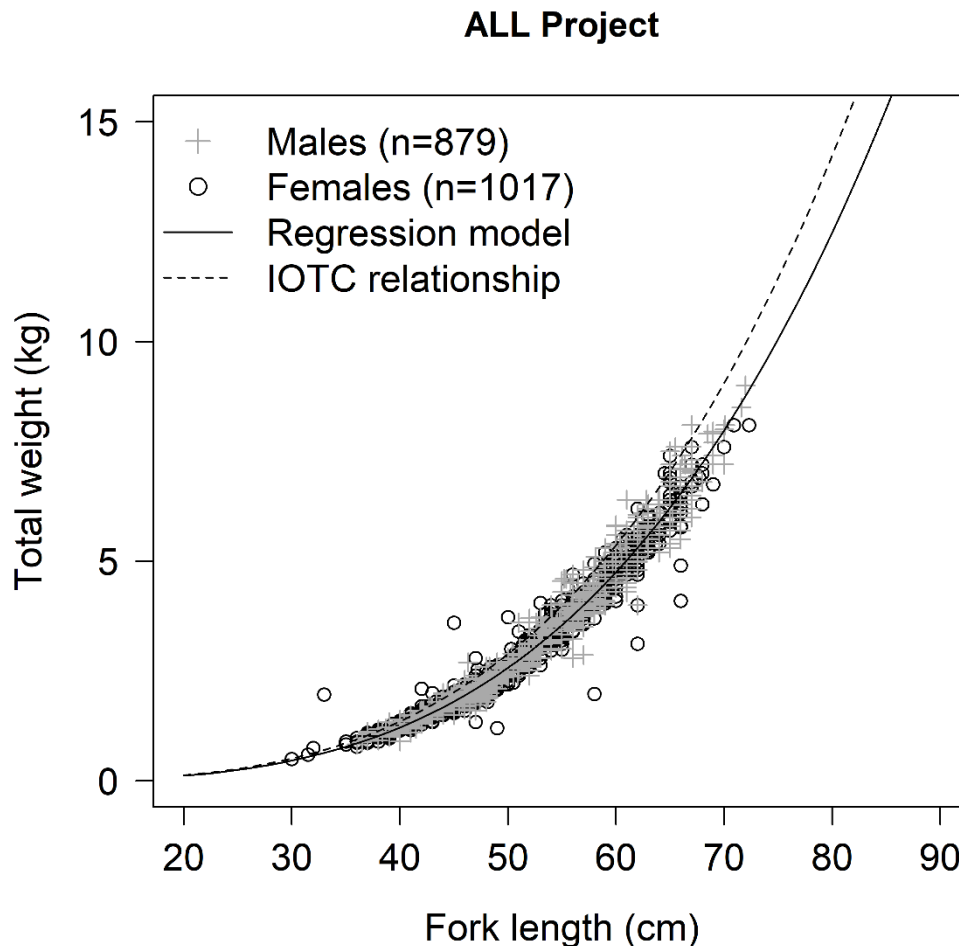


Figure 3 Relationship between fork length (cm) and body weight (kg) for male (cross) and female (open circle) skipjack tuna (*Katsuwonus pelamis*) sampled in the Indian Ocean. Solid line indicates the regression model fitted to all individuals; dashed line indicates the IOTC relationship currently used.

Sex ratio

The sex-ratio differed significantly from 1:1 in the smallest size classes (<50 cm FL) with a higher proportion of males, and in the larger length classes (60-70 cm FL) with a higher proportion of females (Table 2 and Figure 4). The largest size class (70-74 m FL) was also dominated by females, but the difference was not significant due to the small sample size available (Table 2 and Figure 4). A similar pattern was observed when considering only the GERUNDIO project dataset (Figure 4).

Table 2 Summary of the number of female and male skipjack tuna (*Katsuwonus pelamis*) 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
30 - 34	4	0	4	0.0455	*	4
35 - 39	50	32	3.9512	0.04684	*	82
40 - 44	164	119	7.1555	0.007474	*	283
45 - 49	419	253	41.006	1.52E-10	**	672
50 - 54	145	149	0.054422	0.8155		294
55 - 59	219	193	1.6408	0.2002		412
60 - 64	98	133	5.303	0.02129	*	231
65 - 69	50	91	11.922	0.0005548	**	141
70 - 74	5	11	2.25	0.1336		16
75 - 79	3	3	0	1		6

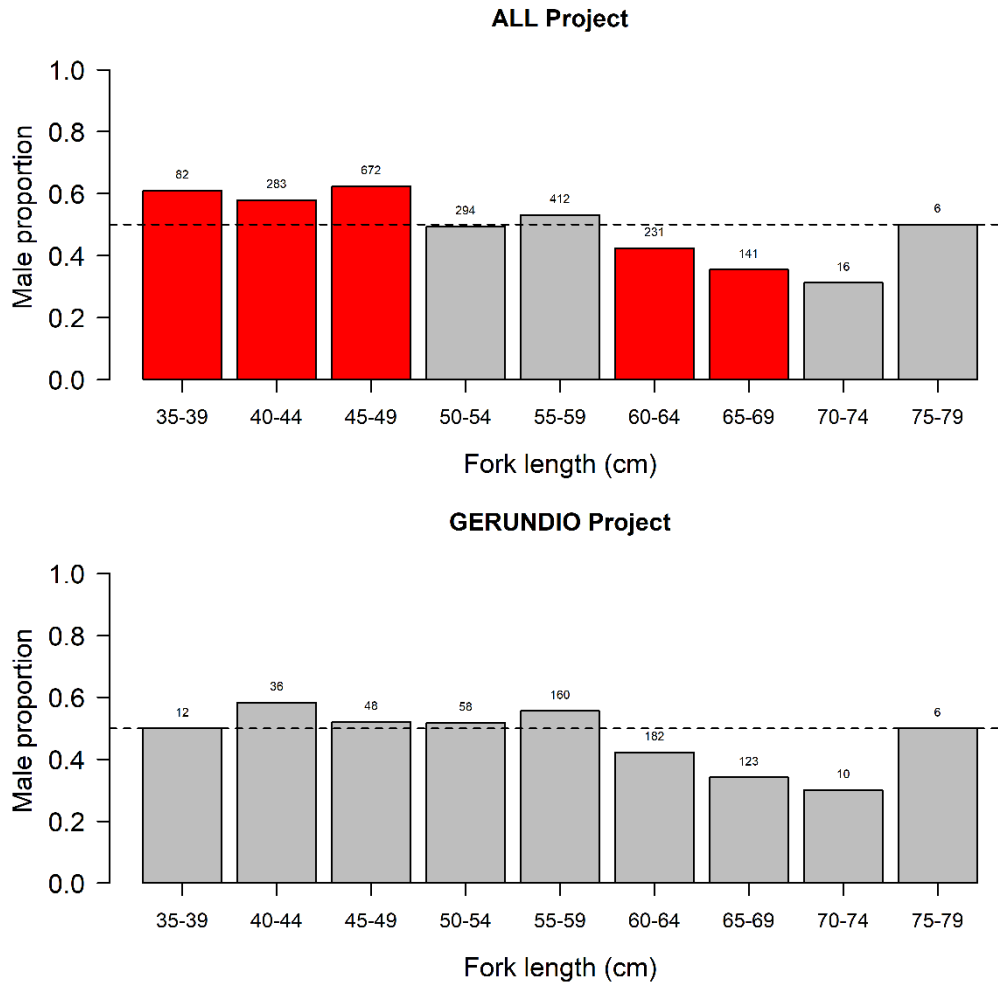


Figure 4. Variations of sex-ratio with fork length (cm) for skipjack tuna (*Katsuwonus pelamis*) in the Indian Ocean. The horizontal dotted line indicates 50% of male proportion. Numbers above bars indicate the number of individuals included in each size class. Bars in red identifies the significant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

Histological classification of ovaries and spawning season

Table 3 and figure 5 show the selection of ovaries used for the histological classification. According to this classification, 9% of females were at IP, 26% were at DP, 54% were at SCP, 12 % at RsP, and 0% were at RgP (Table 3). Applying the maturity threshold at early vitellogenic stage (including Vit 1 and Vit 2) and onward, 83% of the analysed females were mature, from which 12% were undergoing oocyte maturation (ovaries contained GVM, GVBD or Hyd oocytes) indicating spawning was imminent. A high proportion of ovaries at SCP was recorded all year round with values above 30%. The highest proportions of reproductively active females were present from January (82%) to March (57%), although high proportions of SCP ovaries were also found from July to September (>50%). In contrast, females with less developed ovaries (i.e., IP and DP) were dominant from September to December (around 50% of individuals at IP and DP) (Figure 5). A similar pattern of population ovary maturation process was also observed in the monthly evolution of the GSI (Figure 6). The GERUNDIO project dataset alone did not have sufficient samples to provide a representative assessment of spawning

periodicity for the population as all individuals were classified as mature and were from a limited fish size and temporal range.

Table 3 Summary of the number of female skipjack tuna (*Katsuwonus pelamis*) sampled by 5-cm class of fork length (FL) and maturity development. Immature phase (IP), developing phase (DP), spawning capable phase (SCP), regressing phase (RsP) and regenerating phase (RgP).

Size range	IP	DP	SCP	RsP	RgP	Total
30 - 34	3	1	0	0	0	4
35 - 39	29	8	1	1	0	39
40 - 44	27	58	37	9	0	131
45 - 49	10	82	190	61	2	345
50 - 54	2	28	56	16	0	102
55 - 59	2	29	104	10	0	145
60 - 64	0	9	42	0	0	51
65 - 69	0	2	19	0	0	21
70 - 74	0	0	2	0	0	2
TOTAL	73	217	451	97	2	840

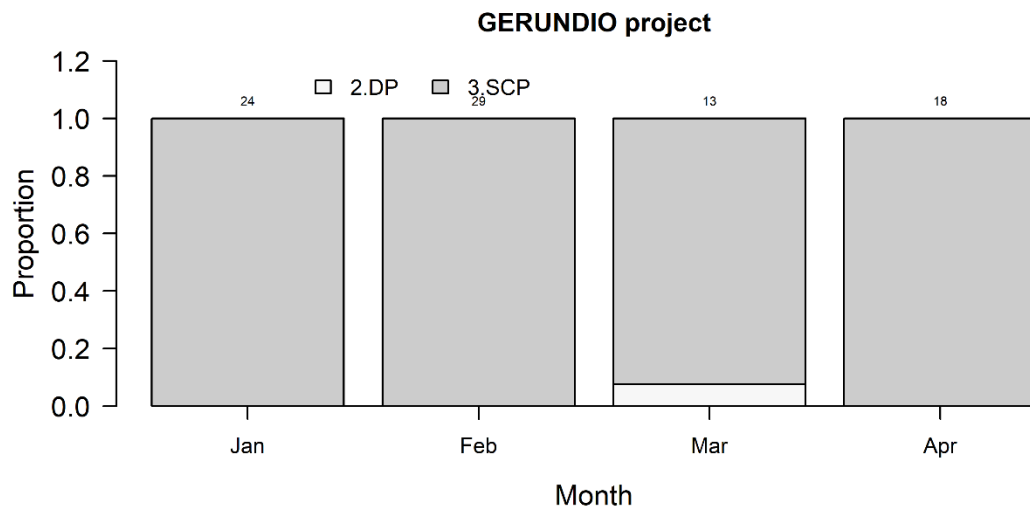
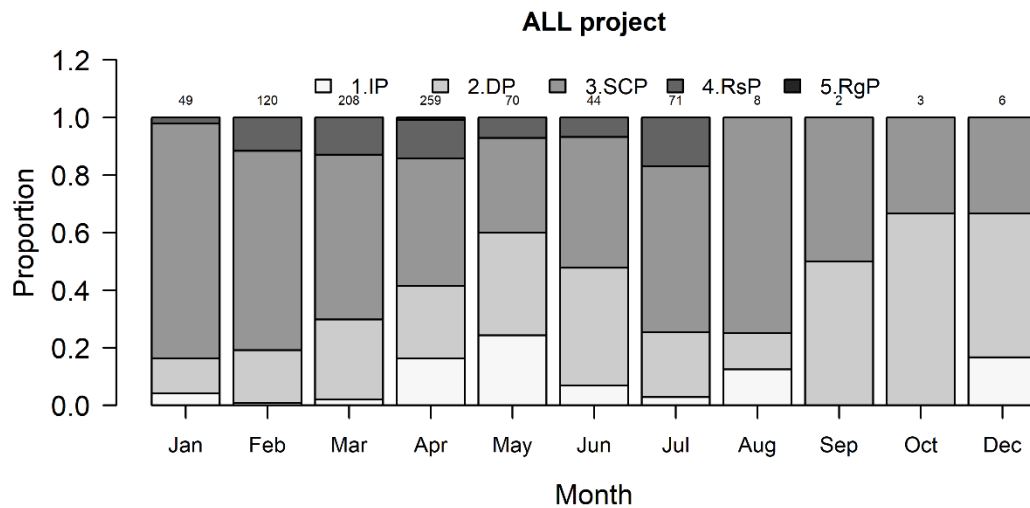


Figure 5 Monthly variations of the proportions of ovary development phases for female skipjack tuna (*Katsuwonus pelamis*) selected from ALL project (top) and GERUNDIO project (bottom) datasets in the

Indian Ocean. IP = Immature phase; DP = Developing phase; SCP = Spawning capable phase; RsP = Regressing phase; RgP = Regenerating phase.

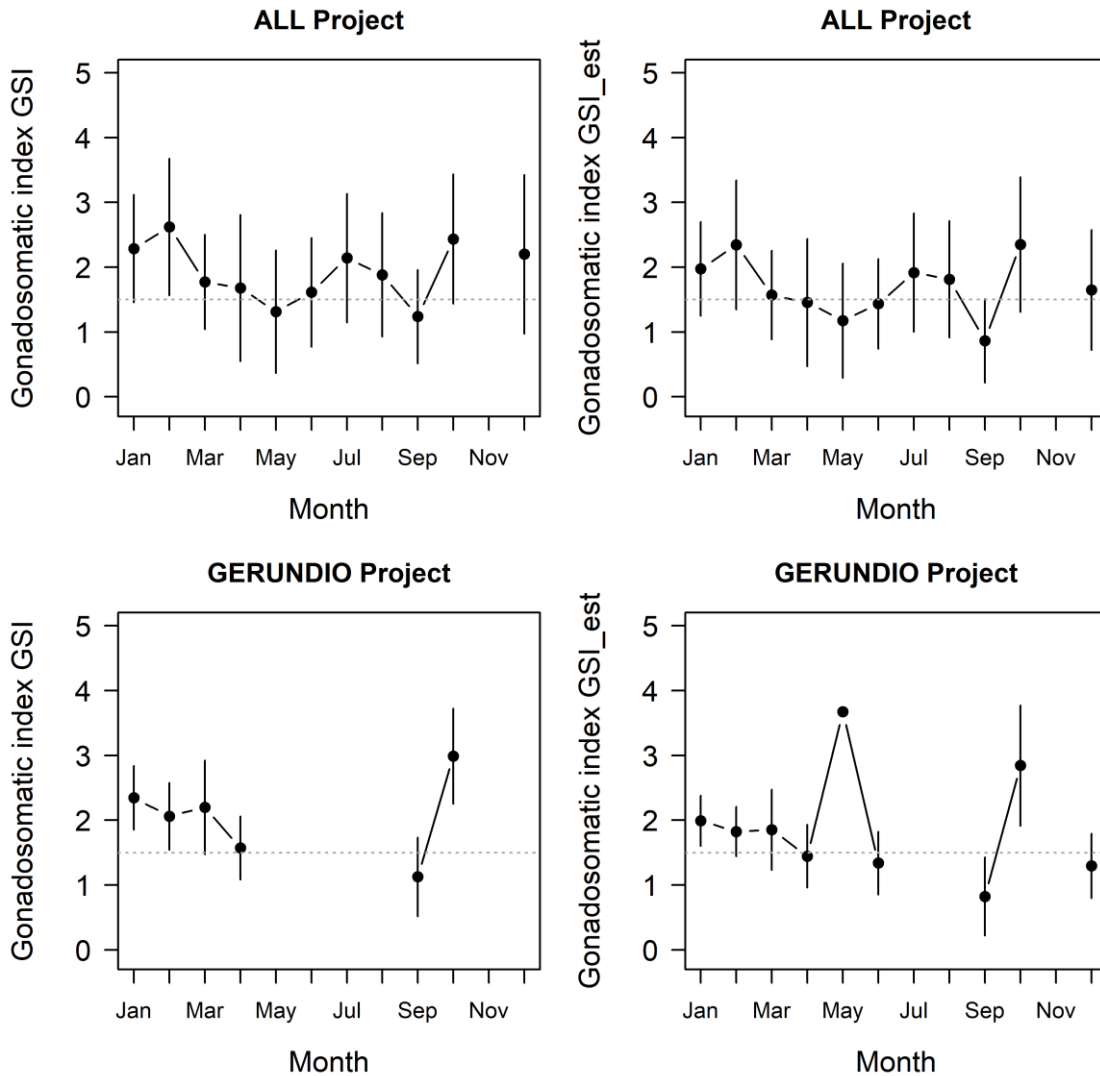


Figure 6 Monthly variations of the gonadosomatic index (GSI) and estimated gonadosomatic index (GSI_est) values for female skipjack tuna (*Katsuwonus pelamis*) selected from ALL project and GERUNDIO project datasets in the Indian Ocean.

Length at maturity

L_{50} was estimated at 41.3 ± 0.5 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. It was not possible to estimate L_{50} for individuals from the GERUNDIO project as all individuals were classified as mature (Table 4 and Figure 7).

Table 4 Parameters for the logistic regression model for estimating the fork length of female skipjack tuna (*Katsuwonus pelamis*) in the Indian Ocean at which 50% of the population is mature (L_{50} , cm). α and β are the coefficients of the equation and L_{50} was computed as $-\alpha/\beta$ for the maturity threshold used: Vit 3 = tertiary vitellogenesis for ALL project data.

	ALL project		
Parameters	α	β	L_{50}
Estimates	-13.78	0.333	41.3
Standard error	1.41	0.032	
Significance	p -value < 0.001		

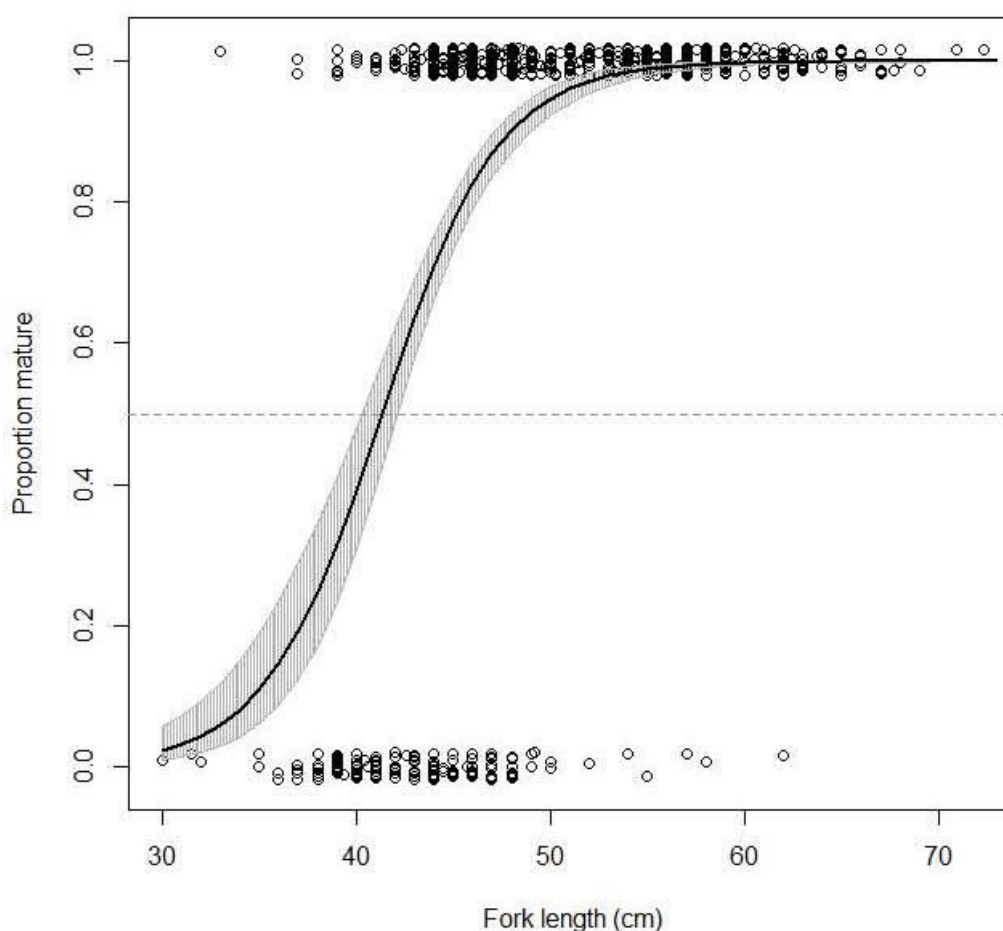


Figure 7 Estimated proportion of mature female skipjack tuna (*Katsuwonus pelamis*) in the Indian Ocean by fork length. Open circles show the maturity status (0 = immature; 1 = mature) estimated for each fish in the ALL Project dataset (note that the points have been jittered to reduce overlap). The solid black line indicates the logistic regression curve fitted to the data, and the grey shaded area indicates the 95% confidence interval. An estimate of L_{50} , i.e. the length at which 50% of the female population is mature, is given by the length at which the dashed horizontal line intersects the maturity curve.

Fecundity estimation

The estimated mean batch fecundity (BF) was 0.28 ± 0.14 million oocytes and ranged from 0.05 million to 0.62 million oocytes. The mean relative batch fecundity (relBF) was estimated at 131.4 ± 62.9 oocytes per gram of gonad-free body weight and fluctuated between 284.9 and 29.7 oocytes per gram.

The maximum levels of BF were not observed in the largest females, but in the intermediate sizes. BF increased significantly with FL ($p < 0.05$). However, 50% and 90% quantile regression lines fitted to BF did not significantly increase with FL ($p = 0.098$ and $p = 0.438$, respectively) (Figure 8). Only the 10% quantile regression line significantly increased with FL ($p < 0.05$) (Figure 8). No relationship was found between relBF and FL (Figure 8). Both BF and relBF increased significantly with gonad weight (p -value < 0.01) (Figure 8). Also, the variability in fecundity estimates increased with gonad weight.

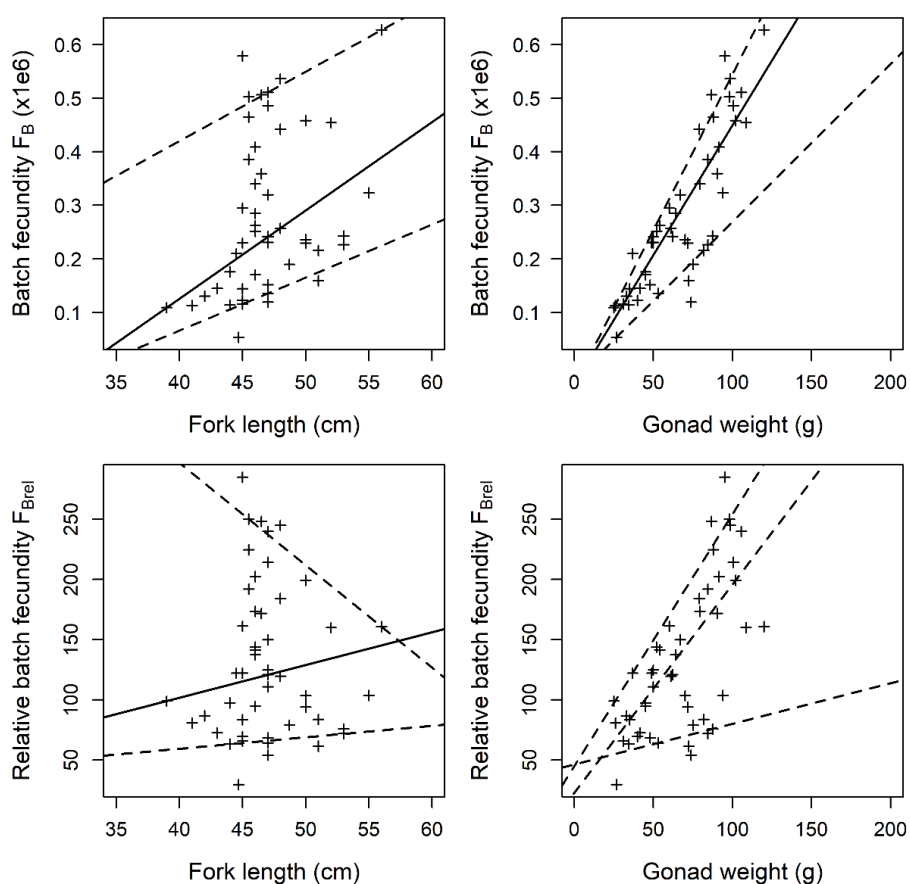


Figure 8. Relationships between batch fecundity (BF, millions of oocytes) (top) and relative batch fecundity (relBF, oocytes per gram of fish body weight) (bottom) with fork length (cm) and ovary weight (g) for female skipjack tuna (*Katsuwonus pelamis*) using data from ALL project datasets in the Indian 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 and reIBF varied significantly by month at a 95% confidence level ($F_{(5,42)}=3.558$, p -value <0.01 ; $F_{(5,42)}=2.987$, p -value <0.05 , respectively). BF estimates appeared highly variable within months (Figure 9).

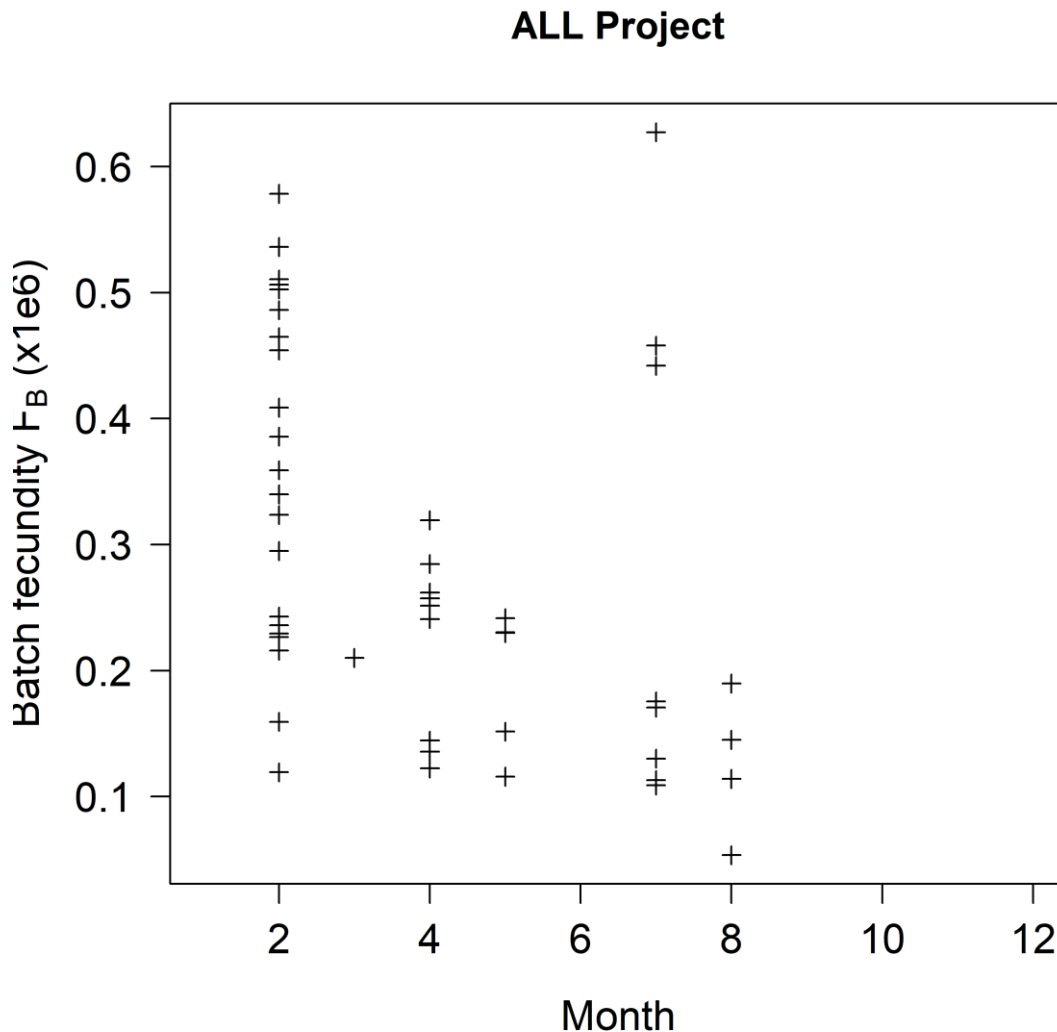


Figure 9. Batch fecundity estimates by month from the ALL project dataset for skipjack tuna (*Katsuwonus pelamis*) in the Indian Ocean.

4. Conclusion and Recommendations

This paper provides preliminary estimates of sex ratio, spawning periodicity, batch fecundity and length at maturity for skipjack 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 within 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 before completing the analysis 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.

The estimate of L_{50} (41.3 cm FL) obtained in this study is slightly higher than that obtained by Grande et al. (2010; 2014), probably affected by different maturity thresholds used in both studies. Our L_{50} estimate was slightly lower than estimated by Stequert and Rammcharrun (1996) and Timohino and Romanov (1996) (42 and 43 cm FL respectively) using the same maturity threshold.

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 >30 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. Continuing to collect and analyse gonads over time will be particularly important for assessing inter-annual variation in reproductive parameters.

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