Spawning activity and batch fecundity of skipjack, *Katsuwonus pelamis*, in the Western Indian Ocean

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ABSTRACT

This study is focused on the reproductive biology of the skipjack, *Katsuwonus pelamis*, of the Western Indian Ocean. The reproductive strategy of the skipjack is defined, by histological analysis of the ovaries and oocyte size frequency distribution. Moreover, the reproductive capacity of individuals was assessed by estimating the size at first maturity and the fecundity parameters, such as the batch fecundity and relative batch fecundity.

A total of 1269 skipjack tuna, ranging from 32 to 68 cm in fork length, were sampled from commercial catches on-board of a purse-seiner in three different areas of the Western Indian Ocean: SE and NW Seychelles, Somalia and the Mozambique Channel in three different periods (from January 2009 to May 2010).

The histological analysis was carried out in 501 skipjack females. The skipjack was defined as indeterminate fish with an asynchronous ovarian development organization and asynchronous spawning. The size at first maturity was estimated in 37.8 cm. The batch fecundity ranged from 100,828 to 627,325 oocytes, and the mean relative batch fecundity was estimated in 150 oocytes/g. From the results it seems that batch fecundity and relative batch fecundity were related to the condition of fish. From the monthly variation of the proportion of different stages (determined by histological analysis) and GSI data, it is concluded that the spawning is observed all year around in the three sampling areas, with increasing periods of the activity that could be related to monsoon events: North monsoon (November to March) and South monsoon (June to August).

INTRODUCTION

The skipjack tuna, *Katsuwonus pelamis,* is a cosmopolitan species widely distributed throughout the world's tropical and subtropical oceans. Its geographical limits are 55º-60ºN and 45º-50ºS, being more abundant in the region of the equator all year around (Collete & Nauen, 1983; Matsumoto et al., 1984). Its distribution is limited by the surface water temperature of 17º to 30º. Inhabits predominantly the mixed layer forming large schools and in association with other tunas (Wild and Hampton, 1994).

Skipjack is the primary target specie of large-scale purse-seiner (PS) fisheries, responsible for around 35 % of the total tropical tuna catches (4 million tons in 2007 worldwide, FAO (2009)). In the Indian Ocean, the catch of tropical tunas was around 0.85 million tons in 2008 of which around 0.41 million were skipjack (*Katsuwonus pelamis*) (IOTC, 2009a). These figures show the importance of skipjack to the fishing sector in the Indian Ocean. Although no quantitative assessment is currently available for skipjack in the Indian Ocean, the specie is resilient and not prone to overfishing due to high productivity (IOTC, 2009).

The understanding of the reproductive biology (i.e. reproductive potential) of fish, quantifying size or age-specific parameters (i.e. size at first maturity, sex ratio and fecundity) and its variation in the temporal and spatial extent, are essential for the determination of the reproductive success of the specie (Murua et al., 2003; Lambert, 2008). The knowledge of the reproductive biology of a species is essential to understand the effect of fishing on the reproductive potential of the stock, which, in turn, will contribute to a better assessment and management of the stock (Schaefer, 2001b. 2001c; Morgan et al., 2009; Kjesbu, 2009).

Little is known about the reproductive biology of tunas in general and for skipjack in particular. The information of the reproductive biology of skipjack available in the literature is not extensive. In the Atlantic and Pacific Ocean several authors studied the reproduction of this specie: Schaefer et al. (1956), Yoshida (1966), Batts (1972), Matsumoto (1982), Cayre (1981); Cayre & Ferrugio (1986), Batalyants, (1989), Schaefer (2001a). In the case of the Indian Ocean, although there are few documents dealing with

skipjack reproduction, the available information is to large extent not updated (Raju, 1964; Stequert, 1976; Golberg & Au, 1986; Stequert & Ramcharrun, 1995; Stequert & Ramcharrun, 1996; Timohina & Romanov, 1996)

Previous studies classify the skipjack (*Katsuwonus pelamis*) as a multiple batch spawner fish with asynchronous oocyte development in which oocytes of all stages are present in the ovary with no dominant population present (Cayre & Ferrugio, 1986; Golberg & Au, 1986; Stequert & Ramcharrun, 1996, Schaefer (2001a, 2001b, 2001c). Little is known, however, of the reproductive strategy of this species in relation to the mode of fecundity regulation (i.e. indeterminate or determinate fecundity regulation) (Murua et al., 2003; Kjesbu 2009). The specie has an opportunistic mode of reproduction and spawning occurs in offshore waters, wherever the hydrologic conditions are favourable (Schaefer, 2000). The latitudinal range of the spawning is delineated on the north and south by the 24ºC isotherm (Matsumoto et al., 1984; Cayre & Farrugio, 1986; Schaefer, 2000). The spawning season take place all year around with periods of more intensive sexual activity during the north monsoon (i.e. November to March) and south monsoon (i.e. June to July) (Stequert & Ramcharrun 1996; Timohina & Romanov, 1996). Regarding to different reproductive parameters, the latest studies of the skipjack in the Indian Ocean estimated a size at first maturity of 41 and 43 cm, corresponding with 1.5 years (Stequert & Ramcharrun, 1996; Timohina & Au, 1996). The relative batch fecundity is estimated to vary from 40 to 130 eggs/g body weight, which varies with the season and size of the fish (Stequert & Ramcharrun, 1995). Laboratory experiments suggest a mean spawning interval of 1.18 days for skipjack in the Pacific region (Hunter et al., 1986).

The objectives of this study are to define the reproductive strategy of the skipjack in the Western Indian Ocean basin, by histological analysis of the ovaries, and to quantify the reproductive capacity of skipjack by estimating the length at first maturity as well as the batch fecundity. Moreover, the relation of the reproductive parameters with several morphometrics characteristics of the individuals, season and area will be analysed.

MATERIAL AND METHODS

A total of 1269 individuals were collected onboard a purse-seiner during three cruises carried out in the Western Indian Ocean (Fig 1.). During 2009, two surveys were carried out covering the area of the East South Seychelles, North West Seychelles and Chagos (i.e. area 1, from 0ºN to 10ºS) and the area of Somalia.(i.e. area 2, from 0ºN-10ºN) In 2010, the third cruise was carried out in the Mozambique Channel (i.e. area 3, from10ºS to 20ºS (Table 1).

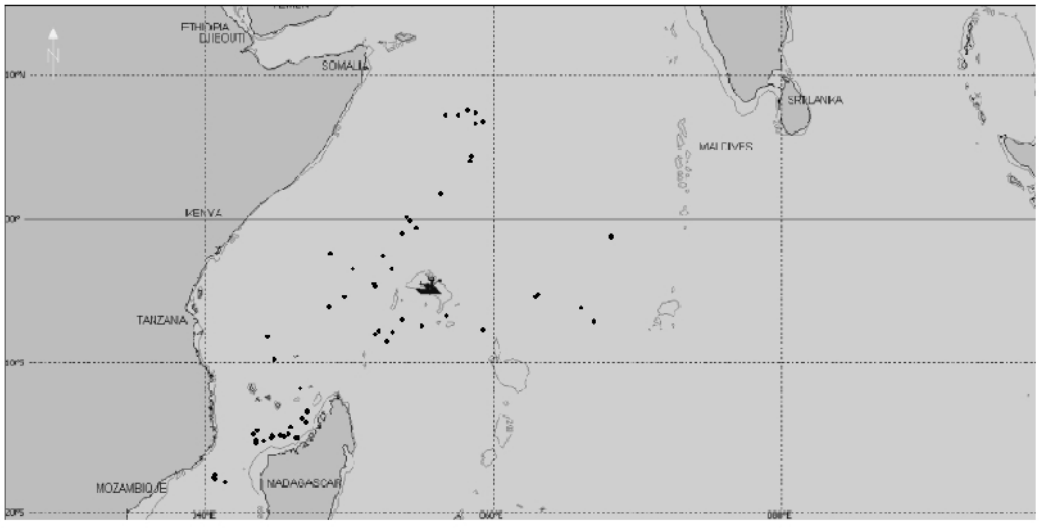


Fig 1. Sampling locations in the study area.

Table 1.- Information of the sampling carried out during the three surveys.

Survey	Data	Area	Nº of fish	FL (cm)
1	22/01/2009 - 13/03/2009	Area 1 and 2	569	33 - 65
2	11/06/2009 - 17/07/2009	Area 1 and 2	214	33 - 65
3	03/04/2010 - 24/05/2010	Area 1 and 3	486	32 - 68

The fishes were measured (i.e. fork length) with callipers to the nearest 0.5 cm and weighted (i.e. total weight) with marine balances to the nearest gram. The sampled fish were between 33-68 cm. Sex was determined in the sea, whenever possible, by the examination of the gonad. Ovaries were weighted on board and a sub-sample of the posterior right lobule was collected and preserved in a solution of 4 % buffered

formaldehyde (Hunter, 1985) for histological analysis, oocyte size-frequency distribution and estimation of batch fecundity**.**

The Gonadosomatic Index (GSI) was calculated as the gonad weight (Wg) divided by the gonad free weight expressed as a percentage.

$$GSI = \left(\frac{Wg}{TW - OW} \right) x10^2$$

For histological analysis, cross sections of about 5 mm of 501 female ovaries were dehydrated by moving them through increasing concentrations of alcohol (i.e. from 70% to 96%) and embedded in ascending solutions of resin. Then, the samples were polymerised into resin blocks. The histological sections of 3 to 5 μm were cut using a microtome and were stained with the Harri´s Hematoxylin and Eosin.

Using the microscopic slides the ovaries were classified in different stage according to the most advanced oocyte present in the gonad (West, 1990; Murua & Motos, 2000; Murua & Motos 2006). Oocyte development stages were defined following the classification proposed by Wallace & Sellman, 1981 and West, 1990; and applied for other species by Murua et al (1998); Murua & Motos (2000) and Mackie & Lewis (2001).

For the assessment of the temporal extent of the spawning and to separate the immature females from the ones in postspawning the atresia was determined (Hunter & Macewicz, 1985a). The oocyte resorption seems to follow similar development process in all the teleost (Hunter et al., 1985) and, therefore, the atretic oocytes were staged based on the classification proposed by Hunter & Macewicz (1985b) for the northern anchovy. It was proved to be the most useful classification and was used for the skipjack in previous works (Hunter et al., 1986). In the study only α and β stages were identified. The prevalence of atresia, defined as the percentage of females showing some sign of α -atresia, together with the relative intensity of atresia, i.e. the percentage of α-atretic yolked oocytes in relation to total number of yolked oocytes, were recorded from histological sections. Subsequently, the atretic condition of the ovary of each individual was established following the criteria defined by Hunter & Macewizc (1985a). In this sense, postspawning or inactive mature

females, i.e. adult females not capable of spawning within the near future, were identified when >50% of yolked oocytes were atretic (atretic stages 3 and 4 of Table 2). In contrast, mature active females, defined as those capable of spawning at the time of sampling or in the near future, were categorized as atretic stages 0, 1 and 2 (Table 2).

Table 2. Atretic status according to Hunter & Macewiwicz (1985a) and applied to skipjack.

Atretic state	Atretic condition
0	α-atresia does not appear in cortical alveoli oocytes nor in vitelogenic oocytes. β-atresia may be present but is not considered.
1	Incidence of α-atresia in <50% of the yolked oocytes.
2	Incidence of α-atresia in >50% of the yolked oocytes
3	α or β atresia appear in the 100% of the cortical alveoli or yolked oocytes.

According to the histological ovarian classification the fishes were classified into mature (i.e fish that certainly will spawn or have spawned showing cortical alveoli stage or vitellogenic stage as most advanced oocyte classes, post ovulatory follicles or atresia condition 2/3) or immature (i.e. have all the oocytes in the primary growth stage) (Murua & Motos, 2000; Murua et al., 2003).

The samples preserved in formaldehyde were also used to analyse the frequency distribution of the oocyte diameters in 37 mature individuals. The samples (i.e 0.04 gr. to the nearest 0.0001gr), after being sieved were covered with Rose Bengal stain. After one day exposure the oocytes were stained and ready for image analysis**.** The sample was placed in a Petri dish and with the help of a needle the oocytes were separated. A photograph of the sample was taken and processed using the Image J software. The oocytes with a diameter bigger than 150 μm present in the samples were counted and the diameter measured.

The size at first maturity (i.e. size in which the 50% of the population is estimated to be mature) was estimated by calculating the proportion of histological mature females by 1cm length classes (Cayre & Ferrugio, 1986; Stequert & Ramcharrun, 1996). The data were fitted to a logistic equation (Ashton, 1972).

$$\bar{P} = \frac{e^{\alpha+\beta FL}}{1+e^{\alpha+\beta FL}}$$

Where P is the predicted mature proportion, α and β are the coefficients of the logistic equation and FL is the fork length in cm. A nonlinear regression (the Marquardt method without restrictions; Marquardt, 1963) was used to fit the logistic equation to the data.

The batch fecundity was determined using the gravimetric method (Hunter et al., 1985) by counting the oocytes in the most advanced stage (i.e. advance migratory nucleus stage and hydration) (Hunter & Macewicz, 1985a; Schaefer, 2001b, 2001c) in a given sub-sample. 3 sub-samples of around 0.1 g (to the nearest 0.01) (Schaefer, 1987) of each ovary were immerse in glycerine and the count of the oocytes was done under a stereomicroscope. Batch fecundity was calculated as the weighted mean density of the three subsamples multiplied by the total weight of the ovaries. Relative batch fecundity was calculated as the batch fecundity divided by the ovary-free female weight.

RESULTS

Size distribution and Sex ratio

A total of 1269 fish were sampled from which 704 (55.5 %) were females, 488 (38.2 %) males and 77 (6.1 %) indeterminate. The fork length (FL) of sampled fish varied from 32 to 68 cm. FL of females varied from 32 to 68 cm, and from 37 to 67 in the case of males (Fig. 2).

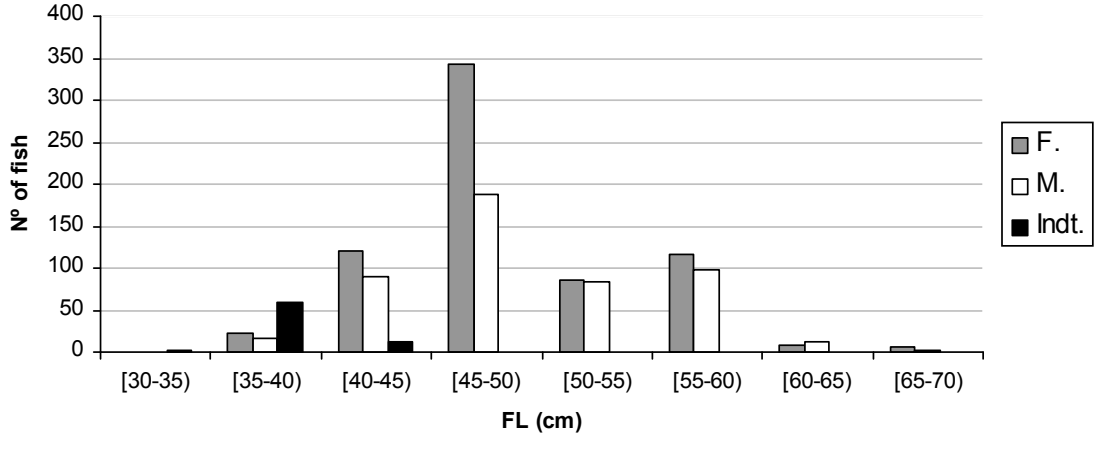


Figure 2. Fork-length frequency distribution of the sampled fish. (F.: Females; M.: Males; Indt.: Indeterminate)

During the first two fishing trips the fish where randomly sampled. The third sampling, due to the needs of the study, was focused on females. The sex was identified by gonad in-situ observations and, thus, the sex of some not developed ovaries or testis could not be identified, classifying those as indeterminate. Therefore, for sex-ratio analysis, the individuals of the third cruise and those classified as indeterminate were not included in the analysis.

The overall sex ratio for the pooled data do not differed significantly from the expected 1:1 (Table 3)**.** When doing the estimation by 5 cm classes, the sex ratio significantly departs for the established 1:1 in larger fishes with a dominance of males (i.e. P-value < 0.05 (Table 3). Although it is not seen a statistically significant dominance of females by size class, the number of females exceed the number of males in almost all the groups, except in the larger fish.

Table 3.- Sex ratio of the *Katsuwonus pelamis* sampled during the first survey (January to March, 2009) and second survey.(June to July, 2009).

FM (cm)	F	M	Total	M:F	Chi-Squared	P-value
[35-40)	17	12	29	1:0.71	0.86	0.99
[40-45)	53	48	101	1:0.91	0.25	1
[45-50)	136	121	257	1:0.89	0.87	0.99
[50-55)	79	78	157	1:0.99	0.006	1
[55-60)	109	95	204	1:0.87	0.96	0.99
[60-65)	10	12	22	1:1.2	0.18	$12*10^{-7}$*
Total Nº	404	366	770	1:0.90	1.87	0.06

* P-value < 0.05

Stage of oocyte development

By analyzing the histological sections the ovary appears to be a random mixture of oocytes in all stages of development with no dominant population present. Therefore, the skipjack´s oocyte development seems to be asynchronous (Wallace & Selman, 1981; Murua & Saborido-Rey, 2003). The oocytes develop from previtellogenic the standing stock of advanced vitellogenic ocytes, passing through different developing stages. After the hydratyon process a portion of the standing stock of yolked oocytes is spawned in several batches. This development process is described in the Table 4 and Fig 3. Following the classification proposed by Wallace & Sellman (1981) and West (1990), we defined 9 stages.

Table 3. Summary of the oocyte development stage defined for skipjact. A morphological description of each stage and the range size of the oocytes in μm are given.

Oocyte development stage	Description	Diameter (μm)
Prymary growth		
Chromatin nucleolar	The initial oocyte is surrounded by squamous follicle cells and contains a central nucleus with a single nucleolus in it. Scarce layer of cytoplasm surround the germinal vesicle	35-91
Perinucleiolar	The nucleus increase in size and nucleolies appear in the periphery. The "Balbiani bodies" migrate from the nucleus to the perihery. Surrounding the oocyte appear the definitive follicle: the scamous follicle cells, the theca cells and epithelial cells in the most external layer.	90-140
Cortical alveoli	This stage is the primary indicator of the onset of the oocyte development for the breeding season. It is characterized by the appearance of small spherical vesicles in the periphery of the cytoplasm which increase in size and number. Those lead to the cortical alveoli as the oocyte develops. Oil vacuoles begin to accumulate in the cytoplasm. The chorion (i.e. zona radiata) also appears in this stage.	140-250
Vitellogenic stage	This stage last from the appearance of yolk vesicles in the cytoplasm to the fusion of them during the final maturation. It is subdivided in 3 sub stages, taking into account the accumulation of the granules containing the yolk proteins (i.e. vitelogenin) within the oocyte´s cytoplasmic area.	
VIT 1	Oil droplets occupy more cytoplasmic area than yolk granules .	250-350
VIT 2	Oil droplets occupy similar cytoplasmic area than yolk granules.	350-450
VIT 3	Oil droplets occupy less cytoplasmic area than yolk granules .	450-500
Maturation		
Initial migration	The geminal vesicle start to migrate towards the animal pole where the micropyle is situated, while the lipoid vesicles coalescence in a unique oil globule.	500-550
Advanced migration	Nuclear migration continues while the yolk granules start to fuse.	550-650
Hydratation	It is characterized by a size increase of the oocyte due to the expansion of the nucleous content within the cytoplasm, followed by the fusion of the yolk granules, and the uptake of fluids. At the end of the process the oocyte has a hialine appearance and could have and irregular shape in the histological slides, due to the loose of fluid during the preparation (Schefer, 1987).	

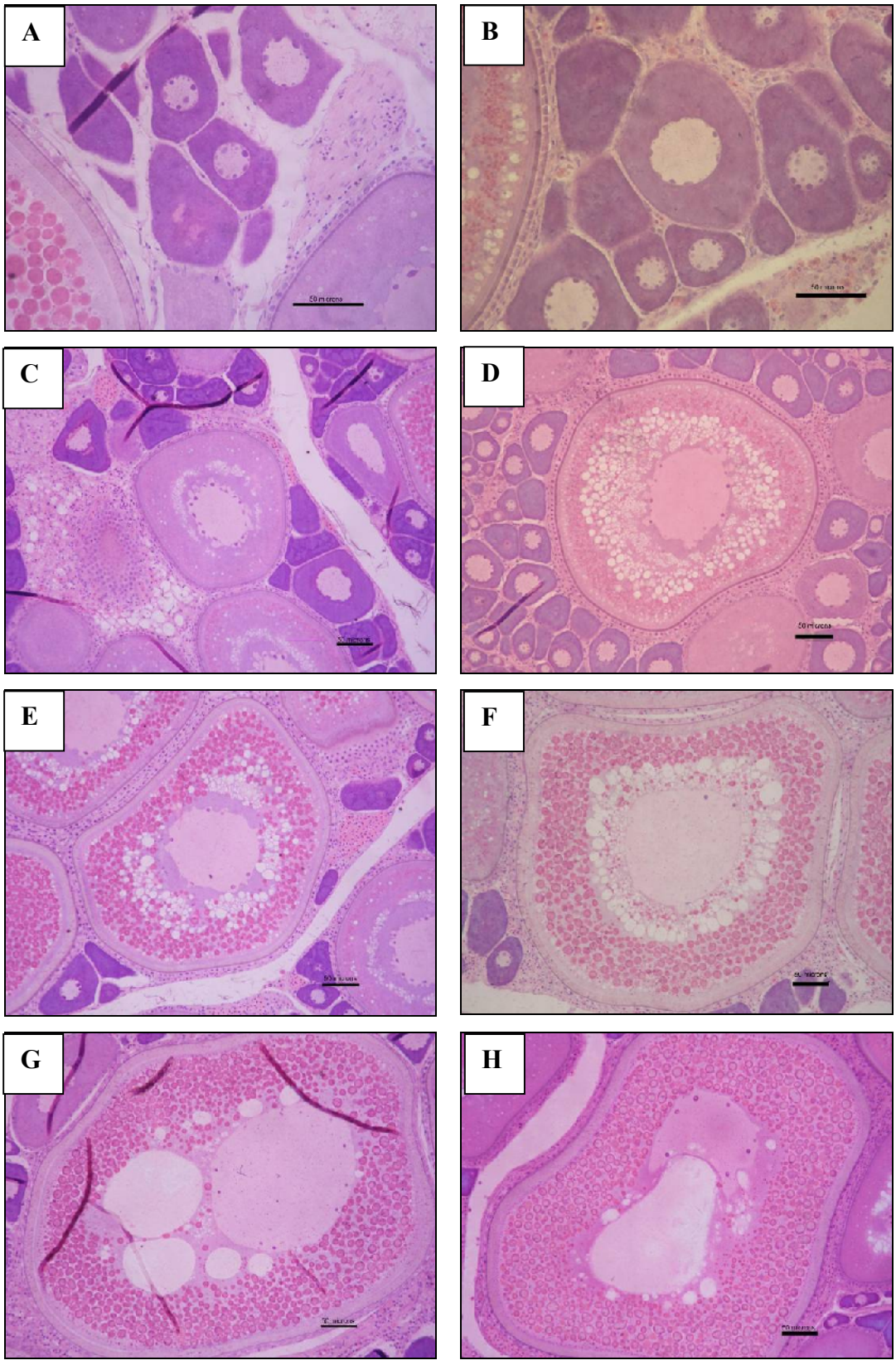


Figure 3.- Stages of oocyte development in skipjack. (A) Cromatin nucleolar oocyte; 40x. (B) perinucleolar oocyte; 40x. (C) Cortical alveoli oocyte; 20x. (D) VIT 1 oocyte; 20x. (E) VIT 2 oocyte; 20x. (F) VIT 3 oocyte; 20x. (G) IM oocyte, 20x. (H) AM oocyte; 20x. Bar on the image is equivalent to 50 µm.

After the hydration process the oocytes are released from their follicle (i.e. monolayer of granulose cells and an outer layer of theca cells) into the lumen. The follicle remains in the ovary as a postovulatory follicle (POF) (Hunter & Macewicz, 1985). Those are transitory structures which are reabsorbed in the ovary. In the case of the skipjack the POF are short-lived structures which cannot be accurately identified in 24 h after ovulation (Hunter et al., 1987; Schaefer, 2001b, 2001c). Upon the histological sections, POF in an advance stage of degeneration were identified (Fig. 4). The thin granulose layer appears surrounded by the thick theca layer. Cells in both layers are in degeneration process which pycnotic nuclei and in irregular aligment (Schaefer, 1987). The POF identified in the ovaries correspond to previous spawning event, occurred more than 12h before sampling. Not new POF were present.

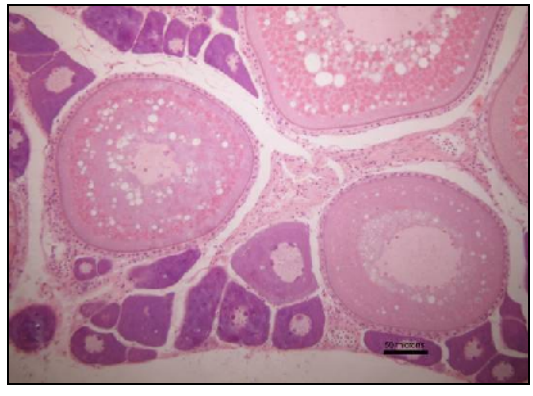


Figure 4.-.A Postovulatory follicle of more than 12 hours; 20x. The bar in the image is equivalent to 50 μm.

The oocytes that do not go through the ovulation suffer a resorption process, atresia, which is subdivided in different stages (Hunter & Macewicz, 1985b). In the initial stage of the atresia (i.e. alpha (α)) the entire oocyte including the yolk (if present) is reabsorbed by the hypertrophying granulosa cells of the follicle (Fig 5). Subsequently, mayor degeneration of the granulosa and theca cells occurs, reducing the size of the follicle (i.e. beta (β)).

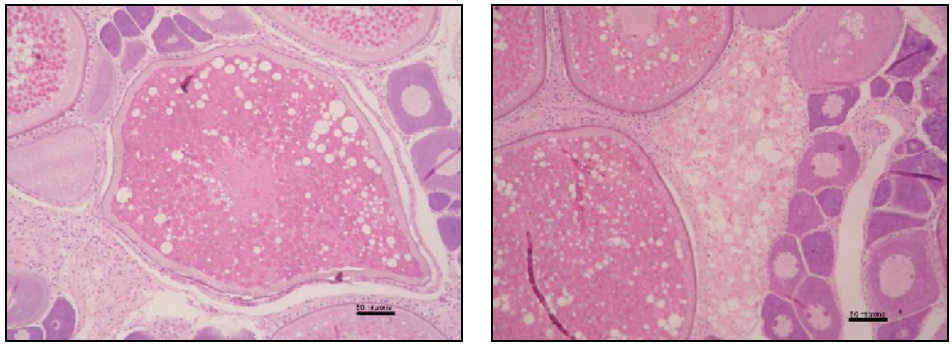


Figure 5.- Left: Alpha atresia; 20x. Right: Beta atresia; 20x. The bar of the image is equivalent to 50 μm

Oocyte Diameter distribution

Skipjack ovaries show a continuous distribution of oocyte diameters throughout the stages of mature gonad development. No hiatus can be observed between previtellogenic oocytes (< 150 μm) and vitellogenic oocytes, which can be considered as an evidence for indeterminate fecundity strategy. In the ovary of active mature females the oocytes gradually increase in size along with the ovarian development (Fig. 6). At the cortical alveoli stage, the ovary contains small oocytes in diameter (140-250 μm) distributed uniformly in number. During the vitellogenesis process the size of the oocytes increases from around 250 μm in VIT 1 to a maximum of 450 μm in VIT 2. At this stage a group of oocytes starts to separate from the adjacent group of smaller oocytes. At the tertiary yolk stage (VIT. 3) the group of oocytes is almost completely isolated. The hiatus starts to form at around 400-450μm which will be developed to form the next batch. At the final stage of maturation (i.e. migratory stage and hydration) the oocytes in the most advanced batch, increase in size while the remainder group remains in the vitellogenic phase. This group of oocytes will be spawned in the subsequent batch, while the remaining vitlellogenic oocytes form the oocyte reservoir for successive batches.

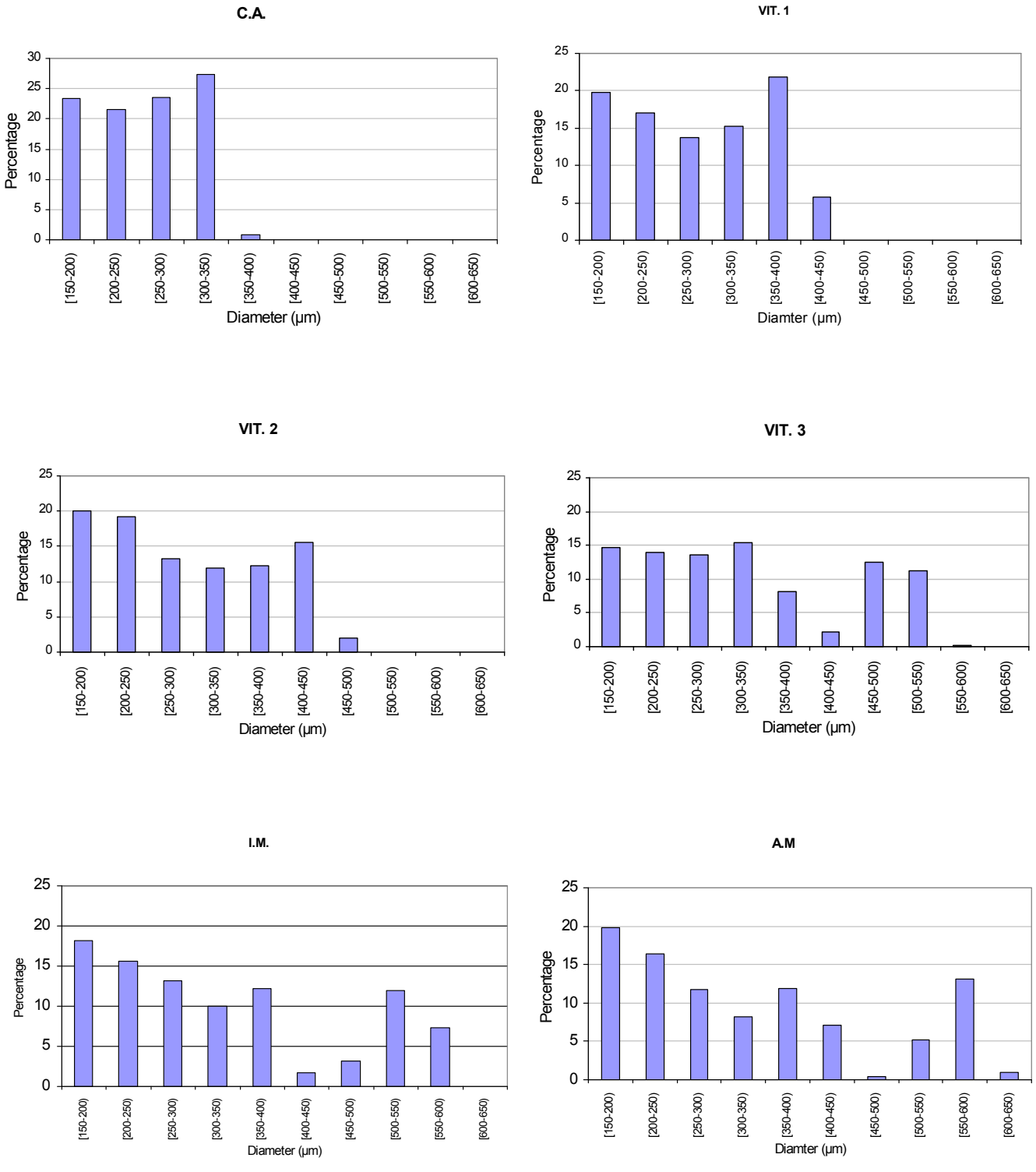


Figure 6. The evolution of the percentage of abundante of oocyte by 50 µm size class through different oocytegrowth stage.

Seasonal variation in occurrence of various oocyte stages

4.2% of the sampled individuals were classified as primary growth or immature, 5.4% as C.A, 81.2% in vitellogenic stage and 9.2% in maturation stage (Table 4.). In the histological sections only atretic hydrated oocytes were present; which correspond to previous spawning batch. Therefore none of the fish was classified as hydrated. In this species hydration of the oocytesand the subsequent spawning occurs rapidly and therefore is difficult to find running-ripe skipjacks.

Table 4. The number of fish classified as immature and those classified as mature (i.e. cortical alveoli, vitelogenesis, initial migration and advanced migration), by 1 cm size (i.e. fork length) classes. The total number of fish by FL class is also given.

FL (cm)	Inmature	Mature						Total number
		C.A..	VIT 1	VIT 2	VIT 3	I.M	A.M	
33-34				1				1
36-37	1							1
37-38	1		1	1				3
38-39	2	3						5
39-40	4	1	2	2		1		10
40-41	3	2	1	1				7
41-42	4	3			2	1		10
42-43	2	2	1	1	4	1		11
43-44	2	3	2	4	3			14
44-45	1	4	2	9	13	2		31
45-46			3	7	26	8	1	45
46-47	1	2	3	12	36	9	2	65
47-48		2	2	12	34	4	2	56
48-49			1	6	22	4	1	34
49-50		1		11	5			17
50-51		2			8	3		13
51-52			1	5	8	2		16
52-53		1	1	6	3	1		12
53-54			2	7	5	2		16
54-55				6	14			20
55-56			1	6	19	1		27
56-57			2	7	15	1		25
57-58				9	16			25
58-59		1		4	12			17
59-60			2	3	6			11
60-61				3				3
61-62					1			1
62-63				1				1
63-64				1				1
64-65					3			3
Total number	21	27	27	125	255	40	6	501

The number of fish in the immature stage is low. This can be attributed to bias in the sampling related to the fishing activity. The purse-seiner´s target fish are mainly the ones over 35 cm fork length, close to the size at first maturity.

When analyzing how the percentage of fishes in each stage of oocyte development varies with the fishing area and season (Fig. 7), it is observed that around 70 % of the individuals is active and close to spawning from January to June in the Western Indian Ocean. There are not significant differences between areas. On the other hand, a major proportion of active mature females is observed in January; where more than the 95 % of the individuals are active and close to spawning. During May the number of PG individuals seems to increase.

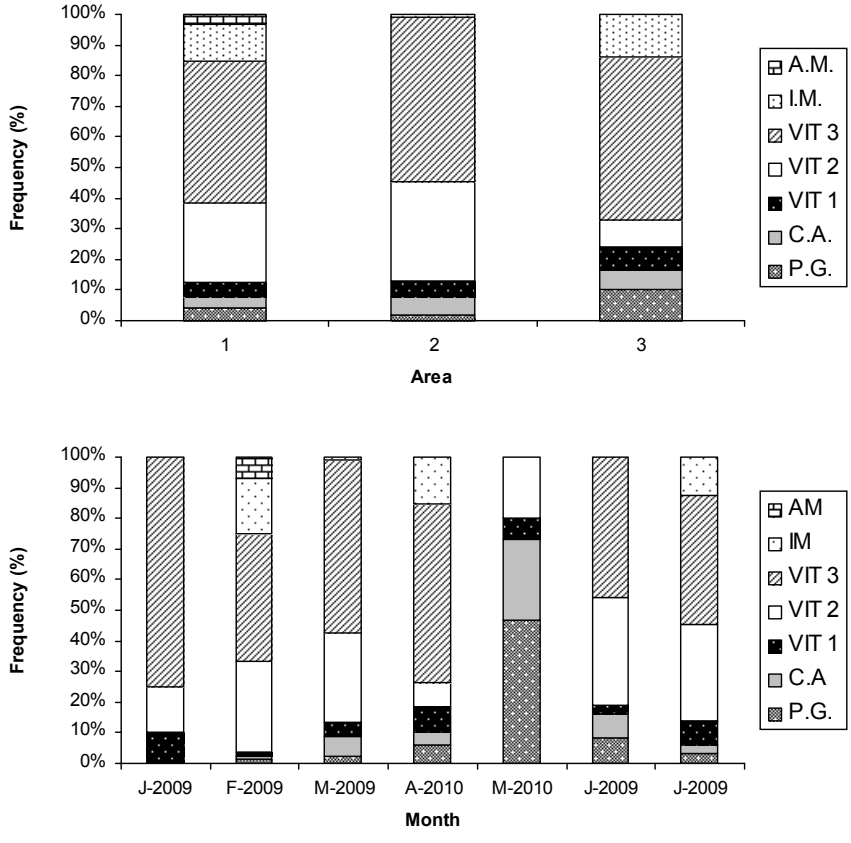


Figure 7.- Upper panel: The percentage of the individual in each of the reproduction stage (primary growth, cortical alveoli, vitellogenesis, and maturation) by sampling area: NE and SW Seychelles (1), Somalia (2) and Mozambique channel (3). Lower panel: Percentage of individual in each reproduction stage by month.

In Seychelles´ sampling area (Fig. 8), which is the area covered by the three trips, and thus, were sampled over a longer period of time, the same pattern is observed: Maximum in the activity during January and an increase of the immature fish (i.e. primary growth) in May.

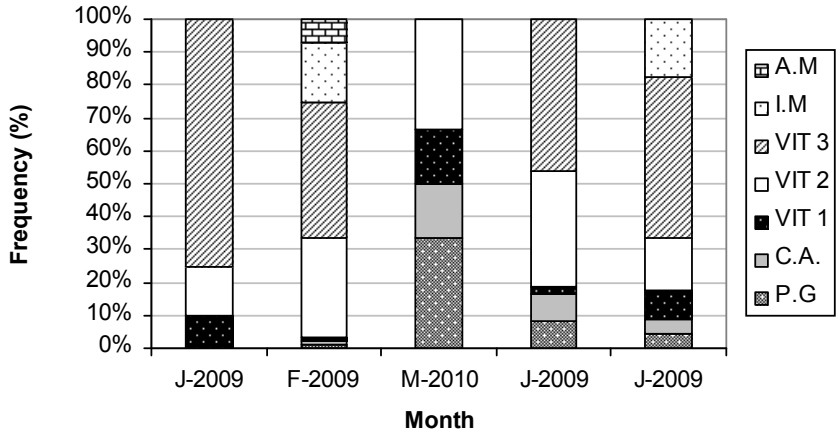


Figure 8.- The percentage of the individual in each of the reproduction stage (primary growth, cortical alveoli, vitellogenesis, and maturation) by month in the sampling area of Seychelles.

With regard on the incidence of atresia, we observe that the mean prevalence of atresia during the sampling period do not exceed 30 %; however, during February, March and April the frequency of individual showing signs of atresia was higher (Fig. 9). Maximum values were reached in March (35.33%), from which the 13.59% were in atretic state 2 (i.e. >50 % of the yolk oocytes show signs of atresia) and state 3 (i.e. the 100% of the oocytes are in atretic the α or β atretic stage)

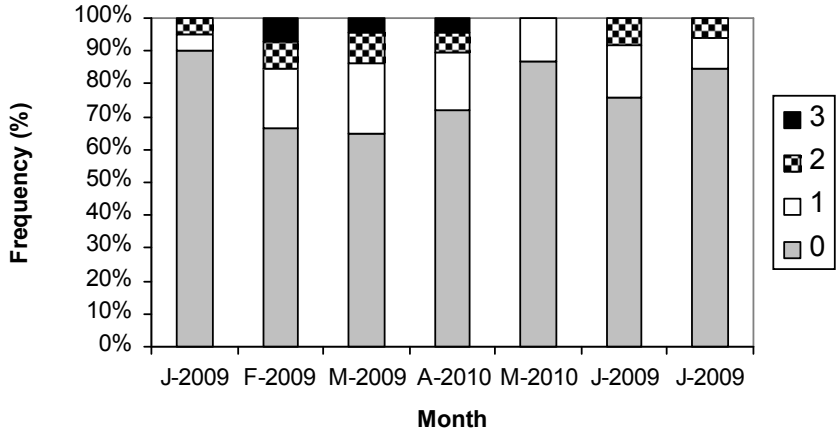


Figure 9.- Monthly variation of the percentage of individuals in each of the atretic condition stages defined following Hunter & Macewicz (1985).

Gonadosomatic index:

The relation of the females´ GSI value and fork length could not be explain with a linear regression model, as previous studies did in the skipjack (Golberg & Au, 1986) and in other tuna species (Schaefer, 1987). The GSI value tents to increase with the fork length, however, the maximum values appear at intermediate length classes (i.e. 45 to 50) (Fig. 10). When the reproduction stage of individual is introduced in the analysis is observed that GSI values are higher for individuals in VIT 3 stage ranging from 45 to 50 than for those bigger than 50 cm.

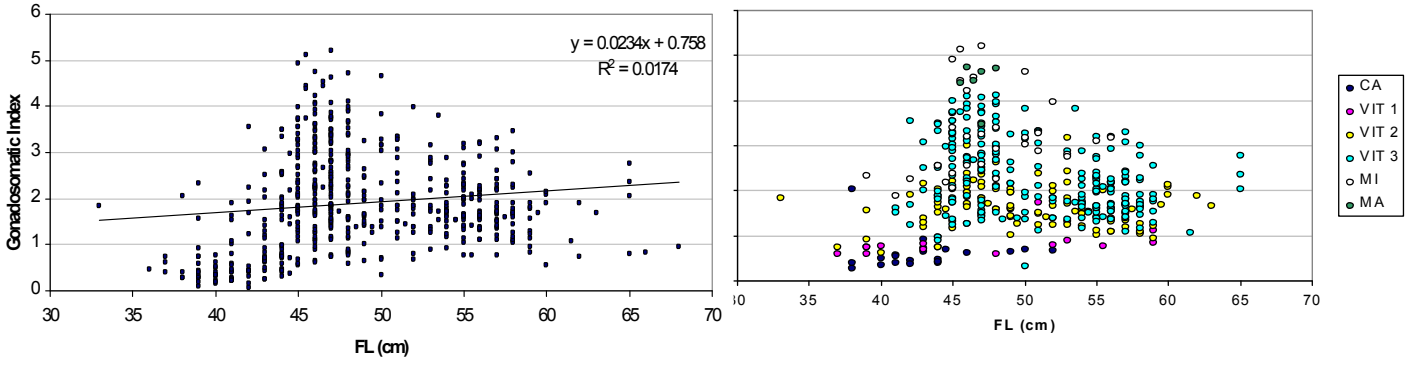


Figure 10.- Left graph: The variation of the GSI with FL (cm) of female skipjack. Right graph: The variation of GSI with the fork length (cm) of those females whose ovaries were histologicalLy analyzed and classified in different oocyte development stage (cortical alveoli, vitellogenesis or maturation stages).

The Gonadosomatic index was calibrated with the histological information (Table 5); which, in turn, can be used to asses the spatio-temporal patterns of the spawning activity of the specie (Schaefer, 1987; West 1990; Schaefer, 2001b).

Table 5. Gonadosomatic index values for different maturity stages of skipjack.

Number	Stage of maturity	Range of variability	Average
21	P.G	0.15-0.65	0.4
27	C.A	0.27-1.5	0.885
27	VIT 1	0.59-1.79	1.19
125	VIT 2	0.63-3.17	1.9
255	VIT 3	0.89-4.12	2.505
46	M	1.89-5.22	3.555

During the period of sampling the mean GSI value by month was over 1.5 (with the exception of May), which is related at least with the VIT 1 stage (Fig. 11). It suggests that a high proportion of females were active matures and that the population spawning occurred during all the sampling period. There is one marked peak during January and

February (north monsoon season) reaching values of 2.88**.** Then, GSI decreased to the lowest levels at 1.44 in May (inter-monsoon season). It starts increasing again from June on (south monsoon season). Focusing in the area of Seychelles (Fig 11), the GSI values follow the same pattern as in all the Western Indian Ocean, with maximum values in February and minimum in May.

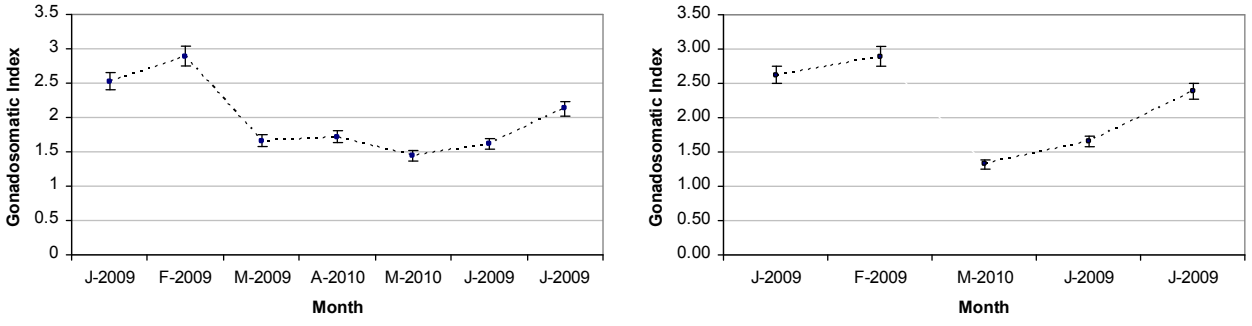
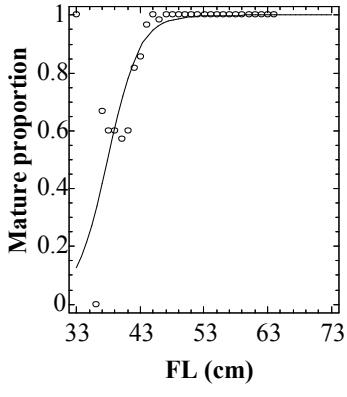


Figure 11.- Left graphs: Variation of the mean GSI by month. Right graph: Variation of the mean GSI by month in Seychelles sampling area.

Length at first maturity ($L_{50\%}$)

The results of the estimation of the proportion of mature fish by 1 cm fork length size class are showed in the figure 12. The figure 12 also shows the parameters used in the prediction of the proportion of mature females by 1 cm fork length class. The logistic model as fitted, explains 71.8 % of the variability of the proportion of mature females by FL. The length at which the 50% of the female population reach maturity was estimated at 37.8 cm.



Parameter	Estimate	Standard Error
α	-15.235	10.867
β	0.403	0.287

Nº of females	501
$L_{50}(-\alpha/\beta)$	37.81
R^2	71.84

Figure 12.- The proportion of mature skipjack females by fork length (cm) with the logit regression fitting curve. Also, the logit parameters (α and β) and the estimated L_{50} (size at first maturity) are shown.

Batch Fecundity

Females at the most advanced stage of maturation were selected for the batch fecundity analysis. All of them were in the initial and advanced migratory nucleus stage, with the hiatus well developed. None of them had recent POFs, since their presence indicated part of the batch had ovulated and the batch fecundity thus would be underestimated. The batch fecundity was estimated in 37 ovaries and batch fecundity ranged from 100,828 oocytes to 627,325 oocytes. It is observed that the B.F. increases linearly with the ovary weight (Fig. 13).

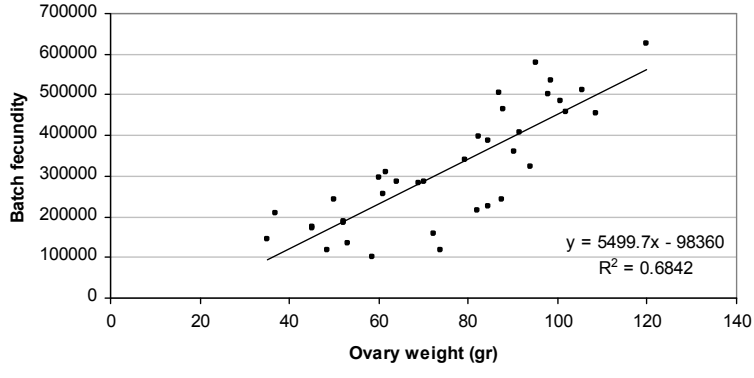


Figure 13.- Batch fecundity in relation to ovary weight (g.).

Figure 14 shows that there is a high variability when plotting the batch fecundity against the fork length and the gonad free weight of the individuals. It seems that the number of advanced oocytes in the ovary tends to increase with fork length and gonad free weight, however, there is not statistically significant relatioinship between BF and somatic variables ($p > 0.1$). There is a peak of the batch fecundity in individuals ranging from about 45 to 47 cm with around 2 kg in gonad free weight (Fig. 14.).

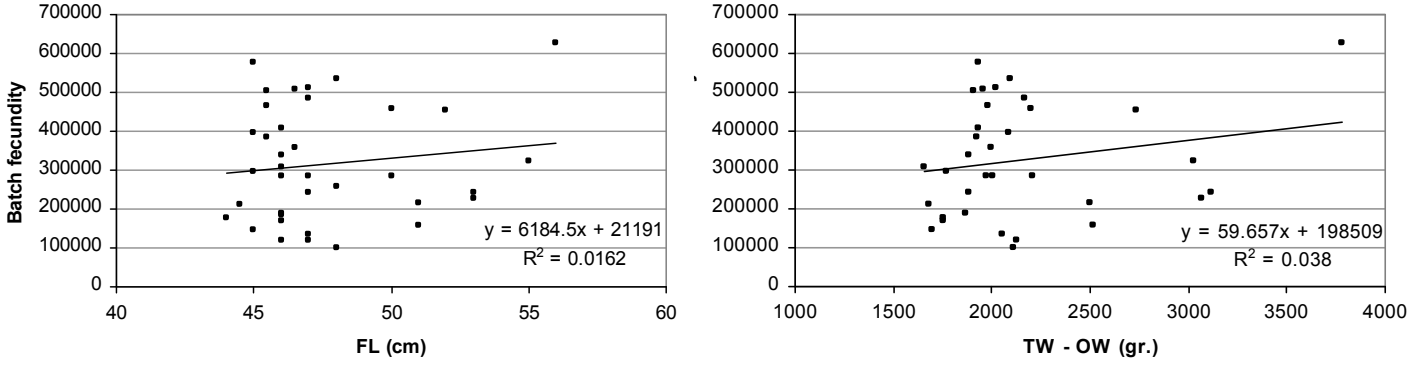


Figure 14.- The variation of batch fecundity with fork length and gonad free wigth.

The relative batch fecundity (RBF) of the individuals ranged from 48 to 299 eggs per gram of female, with a mean of 150 eggs per gram. Fishes ranging from 45 to 47 cm have the highest RBF values. There is not a significant relationship between the fork length and the ovary free weight with the RBF (Fig. 15), however, a slight negative trend of RBF is seen as the value of the independent variables increase.

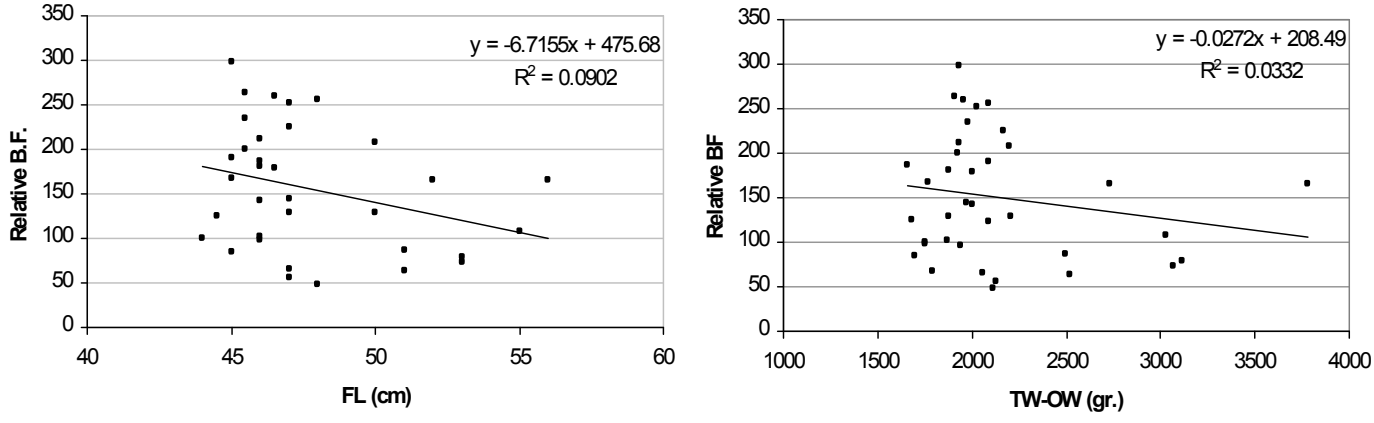


Figure 15.- The variation of relative batch fecundity with fork length and gonad free female weight.

DISCUSSION

The results obtained from this analysis showed that the sex-ratio in skipjack of the Western Indian Ocean without classifying the fish by size groups is 1:1 which is in accordance with data obtained by Cayre & Ferrugio (1986) for the skipjack of the Atlantic Ocean. However, when studying by 5 cm size classes the data show that the sex ratio significantly departs from the established 1:1 in larger fishes, in which there is a predominance of males. Other works in the same area (Timohina & Romanov, 1996; Stequert & Ramcharrun, 1996) observed the same pattern and it is thought to be a common characteristic of all the species of tropical tuna worldwide (Timohina & Romanov, 1996). The causes of the differences in number between sexes as the fish grow could be diverse: differences in the mortality rate by sex, different growth rates, different behaviour or being one of them more vulnerable to fishing (Stequert & Ramcharrun, 1996; Murua et. al., 2003). On the other hand, we found that in almost all the size classes the number of female (although not statistically significant) seems to be higher. We attribute that to sampling biases, because priority was given to females in order to study the reproductive biology.

The size (i.e. fork length) at first maturity (L_{50}) found for the skipjack females in the study area was 37.8 cm. Other studies in the same area (Stequert & Ramcharrun, 1996; Timohina and Romanov, 1996) reported higher values. For example, Stequert and Ramcharrun (1996) estimated the length at first maturity at 42 cm for females and 43.5 cm for males. The deviance of our estimations from the ones given by other studies could lead in the selection of the stage at which the individual can be considered as mature. We classified individuals as mature when oocytes in cortical alveoli stage appear in the ovary. Stequert and Ramcharrun (1996), however, classified the mature individuals as IVa and IVb, which correspond to the VIT 3 or migration stage in the classification applied in this study. If we applied the classification used by Stequert and Ramcharrun (1996) in our data the length at first maturity would be 42.0 cm for skipjack females; which is similar to the one reported by Stequert and Ramcharrum (1996). However, taking into account the reproductive biology of this species and the length range of oocytes in CA and vitellogenesis, it seems more adequate to use CA stage as the threshold of maturation (Brown-Peterson et al., in press).

Based on the histological sections and the oocyte size frequency distribution, we describe the ovarian development organization of the skipjack as asynchronous and we consider the fecundity of skipjack indeterminate (Murua & Saborido-Rey, 2003). Regarding to the spawning pattern it is confirmed that the spawning occurs in batches (i.e. asynchronous ovulators) over a protracted period. When the oocytes starts to enter the final maturation stage and reach a size of around 450-500μm a batch of oocytes gets isolated to be ovulated. Other studies (Stequert & Ramcharrun, 1995; Timohina & Romanov, 1996; Stequert & Ramcharrun, 1996) show the formation of the hiatus at the same interval size.

In species with indeterminate fecundity type, the annual fecundity should be calculated by counting the number of oocytes per batch, the percentage of females spawning per day (i.e. spawning fraction) and the duration of the spawning season (Hunter et al., 1985; Murua & Saborido-Rey, 2003). However, in our case the spawning fraction could not be determined due to the lack of POFs in the samples. This seems to be because a rapid resorption process of POF occurs in the skipjack inhabiting tropical waters (Schaefer, 2001a; 2001b; 2001c).. The batch fecundity values ranged from 100,828 to 627,325 oocytes, and the mean relative batch fecundity was estimated in 150 oocytes per gram of fish. If compared with previous studies in the Indian Ocean (Raju, 1964; Stequert, 1976; Amarasiri & Joseph, 1987; Stequert & Ramcharrun, 1995) and in others (Batts, 1972; Yoshida 1966; Matsumoto et al., 1984; Cayre & Ferrugio, 1986; Golberg & Au, 1986), the batch fecundity values fit in the data range of the previous estimations (i.e. 100,000 and 1 million of eggs). Regarding to the relative batch fecundity, the estimate value is close but slightly higher than the one estimated by Stequert & Ramcharrum (1995) for the Indian Ocean (110eggs/g).

The batch fecundity was directly related with gonad weigth. Contrary to the results showed by other authors (Cayre & Ferrugio, 1986; Stequert & Ramcharrun, 1995; Timohina & Romanov, 1996), and although the relationships between the variation in BF and length/weight were positive; they explained little of the variability in BF. Upon the analyzed fish, the batch fecundity tent to increase with the size, but high values also appear in intermediate sizes (ranging from 45 to 47 cm in fork length). This relationship is also observed when analyzing the variation of GSI with length, which tends to increase with the increasing length, but showing maximum values in intermediate sizes. This could be due to batch fecundity being highly variable amongst the same length/ gutted weight classes; which will be surely dependent on the season in which the fish is captured, i.e. individual

at different stages in the sequence of egg batch production. As the spawning season progresses the BF diminishes (Murua et al., 2006); therefore, the batch fecundity may greatly vary for a fish with the same length/gutted weight depending on the capture date during the spawning season, i.e. at the beginning or at the end of the individual spawning season (Korta et al., 2010). This also could be evidence of different spawning timing between fishes with different classes, as larger fishes are reported to start the individual spawning season earlier in the season (Kjesbu et al., 1998).

On the other hand, the relative batch fecundity is not constant with size and tent to decrease, presenting maximum values at mentioned intermediate sizes. This relationship was also seen by Cayre & Ferrugio (1986) in the Atlantic Ocean, who could not confirm the hypothesis which state that the relative batch fecundity is constant with the age of the fish.

Fishes in intermediate sizes have the ovary proportionally bigger in relation to body size (or weight) than older fishes in the same reproductive stage; which is observed analysing the GSI with fish size. Thus, if high gonad weight and GSI values are observed in the middle length range it means that relative batch fecundity would be maximum in the middle length range; which can be interpreted as batch fecundity and relative batch fecundity being independent of the size (i.e. fork length or weight) of the fish and being related to the condition of the individuals. Therefore, with those assumptions, we state that the gonad weight is the best factor explaining the batch fecundity and the relative batch fecundity. In this sense the GSI can be considered as an index of gonad condition and, hence, batch fecundity. Thus, the size of the fish and the fecundity parameters are independent and conditioned by the fish condition, which are to large extend affected by sampling period (Stequert & Ramcgarrun, 1995; Kjesbu et al., 1998; Korta et al., 2010).

More than the 70% of the female's population was active during the duration of the surveys and in different sampling areas. Also, from the mean GSI value by month we observed that the majority of mature females were at least in the VIT 1 stage. Moreover, the prevalence of atresia did not exceed the 30%, with the exception of May (i.e. 35%). Therefore, as Stequert & Ramcharrun (1995 and 1996) and Stequert et al. (2001), we suggest that the skipjack in the Indian Ocean may spawn all year around. The GSI data show that at least one peak on the spawning is identified from January to February (north

monsoon season) and a minor spawning during April and May (inter-monsoon season). Also, an increase in the activity is detected during June and July (south monsoon season). The same pattern is corroborated when looking separately the Seychelles area. Therefore, the reproduction of the skipjack in the study area could be modulated by climatic events, as suggested by Stequert & Ramcharrun (1995 and 1996) and Stequert et al. (2001) and fish condition as suggested by this study.

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