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Fecundity regulation strategy of the yellowfin tuna (*Thunnus albacares*) in the Western Indian Ocean

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ABSTRACT

The oocyte development process and fecundity regulation (i.e. whether yellowfin tuna shows determinate or indeterminate fecundity) were analyzed to investigate the reproductive strategy of yellowfin tuna, *Thunnus albacares*. A total of 819 yellowfin ovaries were sampled at sea and at the Seychelles cannery during 2009 and 2010 from purse-seiners operating in the Western Indian Ocean. Histological analysis and automated computer-controlled image analysis software were used to study four main criteria applied for fecundity style determination: (a) oocyte size–frequency distribution, (b) number of cortical alveoli and total vitellogenic oocytes in different ovary maturation phases, (c) differences in mean diameter of tertiary vitellogenic oocytes, and (d) seasonal development of atresia.

The results revealed an asynchronous oocyte development and a continuous oocyte size–frequency distribution throughout all ovarian developmental phases over the spawning season. The percentage and number of cortical alveoli and total vitellogenic oocytes remained constant through the spawning season. The mean diameter of tertiary vitellogenic oocytes decreased as spawning progressed. Also, the incidence of atresia was higher at the end of the spawning season as a consequence of over-recruitment of oocytes during this period. These findings revealed that yellowfin tuna exhibit indeterminate fecundity.

1. Introduction

Yellowfin tuna (*Thunnus albacares*) is a large epipelagic species widely distributed in the tropical and subtropical waters of the major oceans (Collette and Nauen, 1983). It is an important component of tuna fisheries worldwide and is one of the major target species for the tuna fishery in the Indian Ocean (Somvanshi, 2002). Due to its high demand, yellowfin is harvested widely, and many types of fishing gear are used. Contrary to the situation in other oceans, the artisanal fishery component in the Indian Ocean (mainly using pole and line, driftnet and hand line) is substantial, taking around 35% of the total yellowfin tuna catch during recent years (2000–2008). The Indian Ocean Tuna Commission (IOTC) estimated that spawning stock biomass has decreased markedly over the last decade (IOTC, 2010). The IOTC recommended that catches of yellowfin tuna should not exceed 300,000 tonnes per year in order to increase the biomass to levels that will sustain catches at levels of Maximum Sustainable Yield (MSY) in the long term (IOTC, 2010). During the last decade the IOTC has recommended and encouraged research on the reproductive biology of yellowfin in order to acquire updated information to be used in the assessment and management process of the population (Somvanshi, 2002; Zhu et al., 2008). A proper estimation of the reproductive potential of the stock would contribute to better and sound management advice for this species in the area.

In the Indian Ocean, yellowfin tuna spawning seems to occur mainly in the equatorial area (0–10°S), with the main spawning ground west of 75°E (IOTC, 2003). Different spawning periods have been described for yellowfin tuna in the Indian Ocean, extending from December to March (IOTC, 2003), and from January to June (Zhu et al., 2008). Also Stéquert et al. (2001) described two reproductive seasons that are related to the north monsoon (main spawning period) and the south monsoon (less reproductive activity). Yellowfin tuna is considered a batch spawner (Joseph, 1963; McPherson, 1991; Orange, 1961; Schaefer, 1998), with a protracted spawning season (Itano, 2000; Schaefer, 1998, 2001b). The pattern of oocyte development is defined as asynchronous (Schaefer, 1998, 2001a), the ovary presenting different oocyte development stages with no clear dominant oocyte stage (Wallace and Selman,

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1981), and fecundity was assumed to be indeterminate (Schaefer, 1996, 1998). However, these findings were only based on indirect evidence such as the absence of the gap between the diameter of oocytes in primary and secondary growth stages (i.e. asynchronous oocyte development) and/or based on the spawning pattern (i.e. batch fecundity). Nevertheless, evidence of these two reproductive characteristics does not necessarily confirm an indeterminate oocyte recruitment type as noted in other studies (Greer Walker et al., 1994; Alonso-Fernández et al., 2008). Therefore, in addition to the spawning pattern (i.e. batch fecundity) and the oocyte development pattern (i.e. asynchronous), other key characteristics need to be applied to test the oocyte recruitment type of a species (Murua and Saborido-Rey, 2003; Kjesbu, 2009). For example, knowledge of the seasonal variation in the percentage and number of different oocyte stages, seasonal variation in the mean diameter of the tertiary vitellogenic oocytes, and the incidence of atresia through the spawning season, are necessary (Murua and Saborido-Rey, 2003) to ascertain accurately the oocyte recruitment type of a species.

Fecundity regulation is related to the manner in which individuals of a species recruit and develop oocytes until they are ovulated (Murua and Motos, 2006). Fecundity pattern can be classified according to the time lag between oocyte recruitment and spawning, and generally two types are defined: determinate and indeterminate (Hunter et al., 1992; Murua and Saborido-Rey, 2003). In fishes with determinate fecundity, oocyte recruitment is completed before onset of spawning and hence the number of advanced oocytes in the ovary corresponds to the potential annual fecundity. In contrast, in fishes displaying indeterminate fecundity, oocyte recruitment and spawning period overlaps, i.e. potential fecundity is not fixed before commencement of spawning. Thus, annual fecundity in fishes with indeterminate fecundity should be estimated multiplying the number of oocytes spawned per batch, the percentage of females spawning per day (i.e. spawning fraction), and the duration of the spawning season (Hunter and Macewicz, 1985a). Therefore, knowledge of oocyte recruitment and development pattern is essential for appropriate fecundity estimation, as the selected method to estimate fecundity is based on the reproductive strategy (Armstrong and Witthames, 2012; Hunter et al., 1992; Kjesbu, 2009; Korta, 2010; Murua and Saborido-Rey, 2003). Moreover, appraisal and acquiring knowledge of the fecundity type is necessary before applying any Egg Production Methods (EPM) (Armstrong and Witthames, 2012; Bernal et al., 2012). For example, the daily egg production method (DEPM) (Lasker, 1985; Parker, 1980) in principle is suitable to be applied to all batch spawner species with indeterminate and determinate fecundity, while the annual egg production method (AEPM) and daily fecundity reduction method (DFRM) are suitable for species with determinate fecundity (Armstrong and Witthames, 2012). Any possible successful application of the egg production survey method would help in the estimation of spawning potential of this specie providing a fishery independent alternative to catch based assessments (Stratoudakis et al., 2006).

The main objective of this research is to determine whether yellowfin tuna exhibits indeterminate or determine fecundity. For that purpose and based on previous studies on fish reproductive strategies (Greer Walker et al., 1994; Hunter et al., 1989; Murua and Saborido-Rey, 2003), we investigated, for the first time, four principal factors related to oocyte development and recruitment in yellowfin tuna. These are: (a) phase-specific and month-specific variation of oocyte size–frequency distribution; (b) seasonal variation in the percentage and number of different oocyte stages during spawning season, (c) seasonal variation in the mean diameter of the tertiary vitellogenic oocytes; and (d) seasonal incidence of atresia through the spawning season.

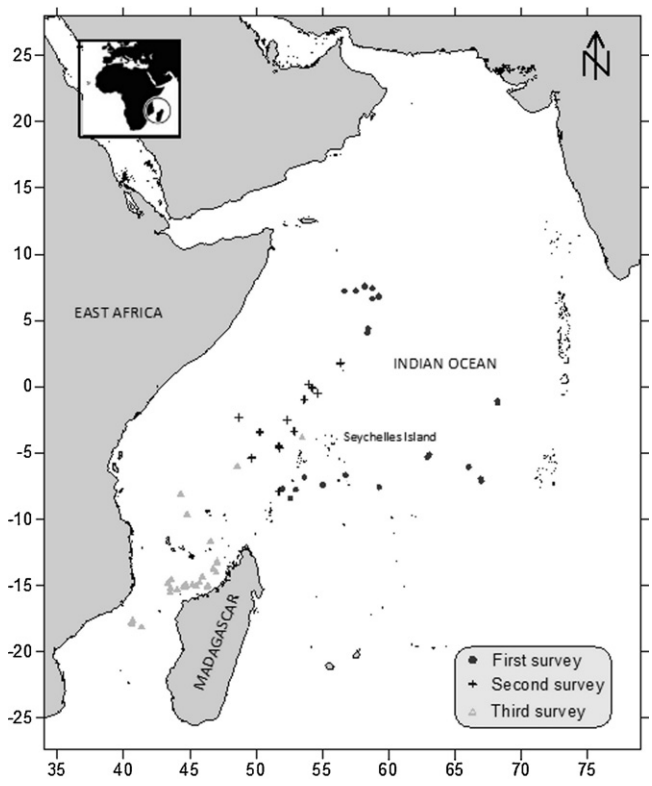

Fig. 1. Location of yellowfin tuna sample collection in the Western Indian Ocean during 2009 and 2010.

2. Material and methods

2.1. Field sampling

Sampling was carried out by scientific observers during three surveys onboard a commercial purse seiner in the Western Indian Ocean during 2009 (2 surveys) and 2010 (1 survey). The first survey was performed from 21st January to 23rd March 2009, the second survey was conducted between 5th June and 25th July 2009, and the third survey from 3rd April to 21st May 2010. The sampling sets were distributed throughout the areas of Somalia, South East Seychelles, North West Seychelles, Chagos and the Mozambique Channel (Fig. 1). In addition to sampling at sea, ovaries were collected at Seychelles cannery from the purse seiner fleet operating in the Western Indian Ocean during the same period.

A total of 319 female yellowfin tuna were sampled onboard a purse seiner, ranging in size from 48 cm to 153 cm fork length, and 500 female yellowfin tuna were collected from the cannery, ranging between 61 and 147 cm fork length (Table 1). Each fish was measured (fork length) to the nearest cm and weighed to the nearest 0.1 kg. Gutted weight, maturity and sex were also recorded. Ovaries were removed from all specimens and weighed to the nearest g, and a 4–5 cm cross-section of the gonad was taken from the middle to end part of the right or left lobe and preserved in 4% buffered formaldehyde immediately after the sampling until they were processed in the laboratory.

2.2. Description of oocyte development stages and atresia

A cross-section (approximately 1 cm) from the preserved portion of the ovary was embedded in resin sectioned at 3–5 μm and stained with H&E. The yellowfin tuna ovaries were classified histologically following the criteria of Wallace and Selman (1981) and modified by Schaefer (1996, 1998). In the present work, the

Table 1
Summary of the number of female yellowfin tuna sampled onboard purse seiner (by surveys) and at the cannery by 20 cm size-group.

Fork length	30–50	50–70	70–90	90–110	110–130	130–150	150–170	Total
1st Survey	4	48	28	19	6	4	1	110
2nd Survey	–	37	5	14	17	22	–	95
3rd Survey	–	43	4	54	12	1	–	114
Cannery	–	19	251	115	45	70	–	500

Table 2
Summary of oocyte developmental stages in yellowfin tuna. The morphological characteristics and size ranges are given for each oocyte stage. Measurements are made using histological material.

Oocyte development stage	Characteristic	Oocyte diameter (μm)
Primary growth		
Chromatin nuclear	Oocyte is surrounded by a few squamous follicle cells. The nucleus is large and centrally located, surrounded by a thin layer of cytoplasm and containing a large and single nucleolus	45–75
Perinucleolar	Nucleus increase in size and multiple nucleoli appear at its periphery. The "Balbiani bodies" migrated from the nucleus to the cytoplasm. At the end, some vacuoles appear in the cytoplasm and the chorion precursors material start to accumulate in patches	75–120
Cortical alveoli formation (CA)	Spherical vesicles start to appear at the periphery of the cytoplasm. They increase in size and number forming rows and giving rise to cortical alveoli. Oil drops begin to accumulate in the cytoplasm. At this stage chorion and follicle layers are apparent	120–200
Vitellogenic (Vtg)	This stage is characterized by the appearance of yolk vesicles in the cytoplasm. Besides, the separation of the chorion in two different layers occurred: inner and outer zona radiata. This stage is subdivided in 3 different stages	
Vtg1	Oil droplets occupy more cytoplasmic area than yolk granules	200–310
Vtg2	Oil droplets occupy similar cytoplasmic area than yolk granules	310–390
Vtg3	Oil droplets occupy less cytoplasmic area than yolk granules	390–470
Oocyte maturation (OM)		
Germinal Vesicle Migration (GVM)	The nucleus (germinal vesicle) starts to migrate to the animal pole and the oil droplets fuse to coalescence into a unique oil globule	470–510
Germinal Vesicle Breakdown (GVBD)	The nucleus completes its migration to the animal pole and the unique oil droplet is clearly evident at central part of the oocyte	510–600
Hydration	Yolk granules fuse in yolk plates, and eventually form a homogeneous mass. The nucleus has disintegrated and the cortical alveoli and cytoplasm are restricted to a thin peripheral layer. The oocyte significantly increases in size due to the uptake of fluids. Hydrated oocyte has a translucent appearance	600–780

terminology used for describing the different oocyte stages and ovarian phases is based on Brown-Peterson et al. (2011). Each ovary was classified according to the most advanced oocyte stage present in the ovary (Murua and Motos, 2000) (Table 2). The atretic condition to appraise the regressing phase was based upon the criteria of Hunter and Macewicz (1985b) modified for yellowfin tuna as shown in Table 3. The prevalence of atresia, defined as the percentage of females presenting any sign of alpha-atresia (Murua and Motos, 2006) was recorded at vitellogenic or further stages of oocyte development. The incidence of atresia defined as the number of atretic oocytes in relation to total number of oocytes was also estimated. The prevalence and incidence of atresia were estimated only for ovaries collected using sea sampling. The ovaries collected at the cannery were excluded from these analyses due to their exposure to the brine conservation process used in the purse seiners. This conservation method produces breakages in the follicle wall and chorion and, hence, precise quantification of alpha-atresia was not possible.

Table 3
Atresia categories classification following the description of Hunter and Macewicz (1985b) and Murua and Motos (2006).

Atretic state	Atretic condition	Spawning condition
0	α-Atresia does not appear in cortical alveoli oocytes or in vitellogenic oocytes	Prespawning active
1	Incidence of α-atresia is 10–50% in cortical alveoli oocytes.	Prespawning active
2	Incidence of α-atresia is > 50% in cortical alveoli oocytes.	Prespawning inactive
3	Incidence of α-atresia is < 50% in yolked oocytes	Spawning
4	Incidence of α-atresia is > 50% in yolked oocytes	Regressing
5	Incidence of α-atresia in cortical alveoli or yolked oocytes is 100% or β-atresia is present in all oocytes	Regressing

The size range for each oocyte development stage (Table 2) was estimated measuring the diameter of 75 oocytes for each stage estimated on histological slides using ImageJ software. Then, the upper and lower limits of the size range for each oocyte stage were estimated applying a 99% confidence interval. When the limits between two consecutive stages overlapped, the threshold between both stages was estimated as the average between the maximum value of one range and the minimum value of the other.

2.3. Oocyte size–frequency distribution

The gonads selected for oocyte size–frequency distribution analysis ($n = 83$) covered all ovarian development phases (Table 4). From each preserved ovary a 0.04 g (±0.0001 g) sample was removed and stained with Rose Bengel for at least 24 h. The dyed sample was placed into a 125-μm sieve and sprayed with high pressure water in order to separate the oocytes from connective tissue. Then, oocytes were collected in a Petri dish in order to be photographed with a digital camera. Subsequently, each image was analyzed by ImageJ analysis software, which automatically counted and measured all the oocytes in the image.

2.4. Occurrence of various oocyte stages and pattern in oocyte diameter during the spawning season

The percentage and the number of cortical alveoli oocytes and total vitellogenic oocytes (Vtg1, Vtg2 and Vtg3) were estimated by

Table 4
Summary of the number of yellowfin tuna ovaries (classified by the most advanced oocyte class) analyzed by image analysis by month.

	January	February	March	April	May	June	July	August	September	October	November	December	Total
CA	–	–	1	–	–	1	–	–	–	–	–	–	2
Vtg1	2	4	1	–	2	1	1	–	–	–	–	–	11
Vtg2	2	4	–	–	2	4	3	–	–	–	–	–	15
Vtg3	4	5	1	–	–	10	1	–	–	–	7	5	33
GVM	11	1	–	–	–	3	–	–	–	–	–	–	15
HYDR.	1	5	–	–	1	–	–	–	–	–	–	–	7
Total	20	19	3	0	5	19	5	0	0	0	7	5	83

ovary maturation phases (Brown-Peterson et al., 2011), i.e. developing and spawning capable phase in order to find patterns in oocyte development and oocyte recruitment. The same analysis was performed by month with those ovaries at the spawning capable phase with Vtg3 as the most advanced oocytes. On the other hand, variation in the mean diameter of the Vtg3 oocytes was analyzed during the spawning season by estimating the mean diameter of the largest 100 oocytes from ovaries at the spawning capable phase. For these analyses, and based on previous reproductive studies carried out in the study area, two reproductive periods were assumed: a main one from November to March and a second period in June (Stéquert et al., 2001). Assumed reproductive periods are supported by the result described in (Zudaire et al., submitted for publication) in which yellowfin tuna reproductive season was analyzed.

2.5. Statistical analysis

Analysis of variance (ANOVA) was applied to investigate the differences between the number of cortical alveoli and total vitellogenic oocytes in ovaries at the spawning capable phase by month. ANOVA was also used to assess the monthly variation in the ratio between the total vitellogenic oocytes and the total oocytes for ovaries at the spawning capable phase. The trend in the mean diameter of the largest 100 oocytes from ovaries at the spawning capable phase by month was fitted to a linear regression and additionally the variance at mean diameter among months was analyzed by applying ANOVA.

3. Results

3.1. Stages of oocyte development

Histological analysis of yellowfin tuna ovaries revealed an asynchronous ovarian development in which all oocyte stages were observed, without a dominant population present. Fig. 2 summarizes the different oocyte developmental stages according to the classification described in Table 2.

3.2. Oocyte size–frequency distribution

The oocyte size–frequency distribution at different ovarian developmental phases from the developing (CA–Vtg1–Vtg2 oocytes) to the spawning capable phase (Vtg3 and OM) was continuous without any gap in diameter between primary and secondary growth oocytes in all months studied (Fig. 3). A gap in diameter at around 440 μm in the tertiary vitellogenic stage (Vtg3) separated the less developed oocytes from the largest modal group of oocytes forming a batch which was clearly separated during the early germinal vesicle migration (GVM) stage at around 490 μm. At the hydration stage, the oocytes increased in size to above 700 μm. Although developing and spawning capable phases were investigated for the oocyte diameter distribution during the whole studied period, not all the different oocyte stages, as the most advanced oocyte, were represented in the ovaries analyzed (Fig. 3). Yet the oocyte development and oocyte recruitment pattern is clearly observed along the studied period.

3.3. Occurrence of various oocyte stages along the spawning season

Table 5(A) shows that the percentage of cortical alveoli oocytes by ovary maturation phases decreased from ovaries at developing phase (with Vtg1 as the most advanced oocytes) to ovaries at spawning capable phase (with oocytes in GVM stages) and it remained stable in ovaries with hydrated oocytes. On the other hand, the percentage of total vitellogenic (Vtg1–3) oocytes increased significantly during the developing phase ovaries (with oocytes in the Vtg1 and Vtg2 stages), and then it was at a similar level in the spawning capable phase ovaries (Vtg3, GVM and hydration). Table 5(B) shows that the proportion of cortical alveoli over total vitellogenic oocytes by month (November, December, January, February, March, June and July) was relatively constant during the spawning season in the case of ovaries showing a spawning capable phase with Vtg3 as the most advance stage. Moreover, no significant differences were found at the 95% confidence level (ANOVA, $n = 31$, d.f. = 4; F-ratio = 1.02; $P > 0.05$) between the number of cortical alveoli oocytes in ovaries at the spawning capable phase (Vtg3) by month. The percentage of total vitellogenic oocytes increased through the spawning season from November (56.6%) to June (59.7%). Except for July, when values decreased to 47.5%, the other months did not show a decreasing trend as the spawning season progressed. There were no significant differences in the number of total vitellogenic oocytes by month (ANOVA, $n = 31$, d.f. = 4; F-ratio = 2.66; $P > 0.05$). The ratio between the number of total vitellogenic oocytes and the number of total oocytes in ovaries at the spawning capable phase (Vtg3) did not differ by month (ANOVA, $n = 31$, d.f. = 4; F-ratio = 2.41; $P > 0.05$).

3.4. Mean diameter of tertiary vitellogenic oocytes during the spawning season

The estimated mean diameter of tertiary vitellogenic oocytes from November to March, ranged from 511.2 μm to 469.4 μm. Subsequently, this value increased in June to 491.8 μm and then decreased again in July to 481.1 μm. The regression line between mean diameter of tertiary vitellogenic oocytes for the period from November to March, described a significant decreasing trend ($r^2 = 0.351$, $n = 22$, $P < 0.1$). In contrast, no significant relationship between mean diameter and month was observed from November to July ($r^2 = 0.01933$, $n = 33$, $P > 0.1$) (Fig. 4). For a comparative analysis among mean diameters by month, only those months with four or more measurements were selected. There was no significant difference among mean diameter by month at the 95% confidence level for values corresponding to the period from November to March (ANOVA, $n = 21$, d.f. = 3, $P > 0.05$), or for the period from November to July (ANOVA, $n = 31$, d.f. = 4, $P > 0.05$).

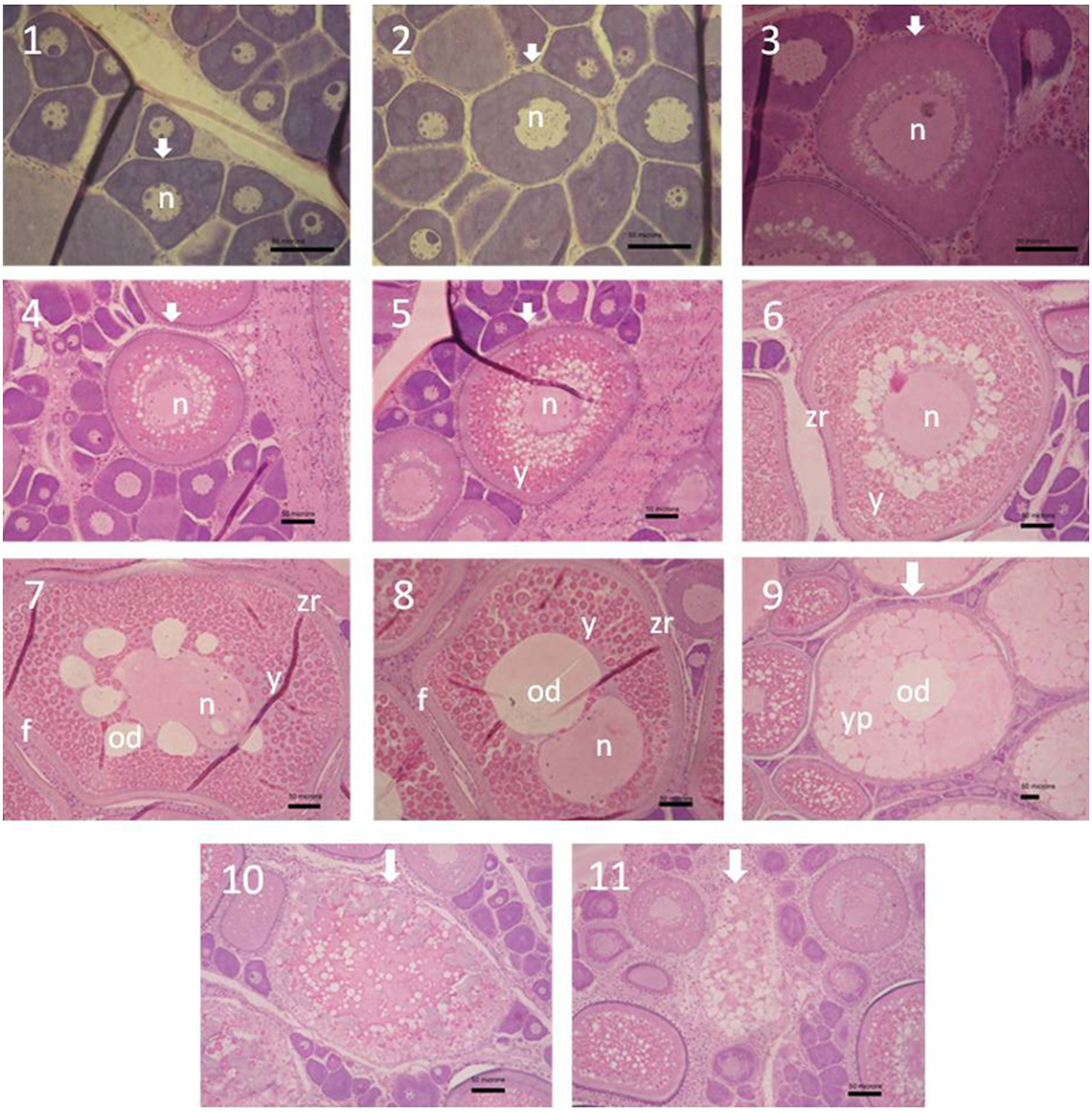

Fig. 2. Different ovarian development phases showing different oocyte stages. (1) and (2) Immature phase with oocytes in the primary growth stage (chromatin nuclear and perinuclear stage respectively); (3) early developing sub-phase with the most advanced oocyte stage in CA stage; (4) and (5) developing phase with the most advanced oocyte stage in Vtg1 stage and Vtg2 stage respectively; (6) spawning capable phase with the most advanced oocyte stage in Vtg3 stage; (7)–(9) actively spawning sub-phase with the most advanced oocyte stage in the GVM, GVBD and hydration stages respectively; (10) alfa and (11) beta atresia. n = nucleus; zr = zona radiate; f = follicle (granulose and theca layers); y = yolk; yp = yolk plates; od = oil droplets. Bar on images is equivalent to 50 μm.

Table 5
(A) Percentage of cortical alveoli and total vitellogenic oocytes by ovary developmental phases. (B) Percentage of cortical alveoli and total vitellogenic oocytes in spawning capable phase ovaries (Vtg3) by month.

(A)

	Developing phase ovaries		Spawning capable phase ovaries		
	Vtg1	Vtg2	Vtg3	GVM	HID
CA	65.6	41.7	32.6	25.9	27.4
Total Vtg (Vtg1, Vtg2, Vtg3)	34.4	56.6	56.0	54.4	57.8

(B)

	November	December	January	February	March	June	July	Total
CA	29.6	34.1	29.8	34.3	36.6	32.1	45.5	32.6
Total Vtg (Vtg1, Vtg2, Vtg3)	51.0	52.3	56.6	59.7	58.9	58.1	47.5	56.0

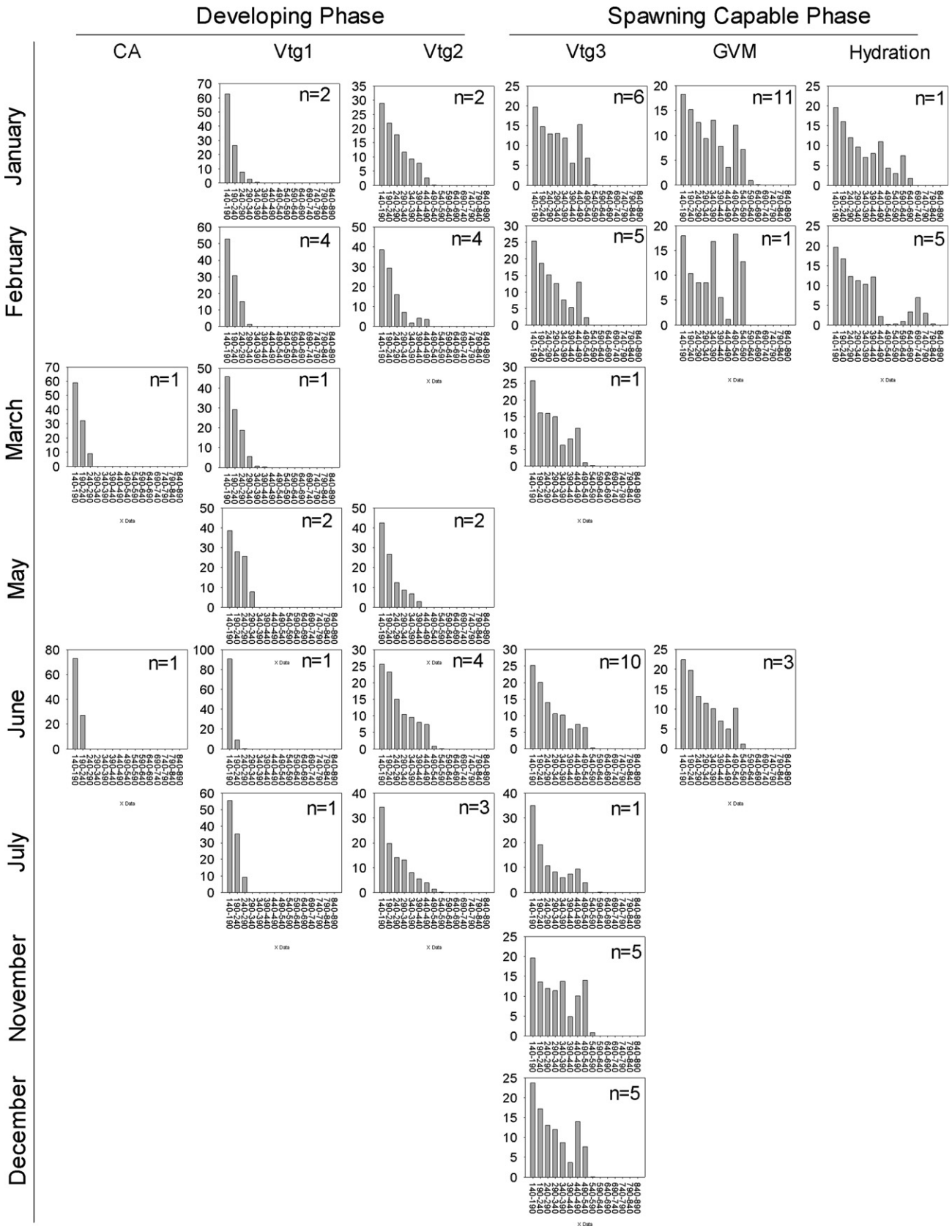

Fig. 3. Monthly changes in the oocyte size–frequency distribution for each ovarian development phase. The number of individuals included in each ovarian development phase is given by month.

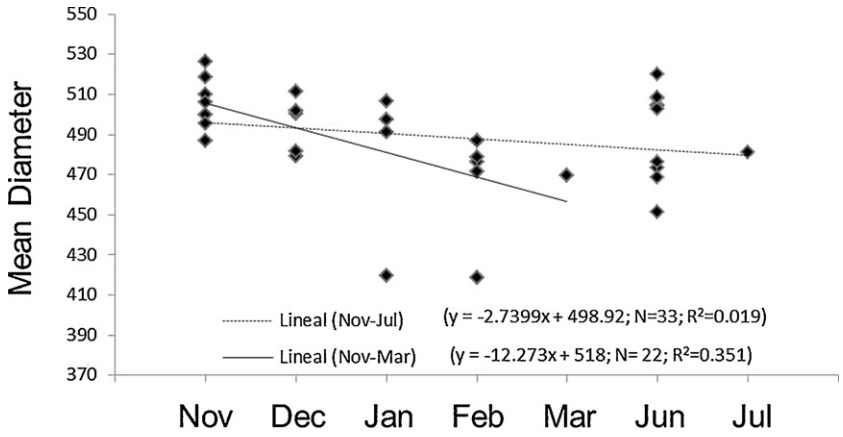

Fig. 4. Monthly mean diameters (μm) of the largest 100 Vtg3 oocytes in ovaries in the spawning capable phase. Linear regression lines for period from November to March (solid line) and for the period from November to July (dahsed line) are also shown.

3.5. Atresia

The prevalence of alpha-atresia increased throughout the spawning season (Table 6). The prevalence of alpha-atresia was relatively low between January and March (6.2, 9.4 and 3.2% respectively) and afterwards started to increase until June, when the highest values were observed (40.5%). The prevalence of alpha-atresia in each oocyte developmental stage was variable. The percentages of females with presence of atresia in early developing sub-phase ovaries (CA) was 24.8%, 80% in developing phase (Vtg1 and Vtg2) ovaries, and there was no alpha-atresia in actively spawning sub-phase ovaries. An ovary was considered to be at the regressing phase when more than 50% of vitellogenic oocytes were in alpha-atresia. The proportion of regressing females increased from January to May but decreased to 9.5 and 11.3% in June and July (Table 6).

4. Discussion

4.1. Oocyte size–frequency distribution

In the present study, continuous oocyte size–frequency was observed during all ovarian maturation phases from the early developing sub-phase until the actively spawning sub-phase (CA, Vtg1–3, GVM, GVBD, hydration) and this spanned the entire study period; which is in agreement with a previous report in the Pacific (Schaefer, 1998). The continuous oocyte size–frequency without any gap in the diameter between primary and secondary growth oocytes in different spawning phases as well as in different months has been considered as a sign of indeterminate fecundity (West, 1990), as it may indicate the existence of a continuous recruitment of primary growth oocytes during the spawning season (Murua and Saborido-Rey, 2003). However, some species with determinate fecundity lack also this gap in the oocyte size diameter (Andersen et al., 2000; Alonso-Fernández et al., 2008; Greer Walker et al., 1994; Hislop and Hall, 1974; Tyler et al., 1994). Therefore, this evidence is not an unequivocal sign of indeterminate fecundity for all fish species.

4.2. Seasonal variation in occurrence of various oocyte stages

The number and percentage of cortical alveoli, along with the analysis of total vitellogenic (Vtg1–3) oocytes in different ovarian maturation phases indicated that yellowfin tuna may exhibit indeterminate fecundity. The percentage of cortical alveoli oocytes decreased as the ovary developed until the initiation of the spawning capable phase (Vtg3). Afterwards, at the spawning capable phase (Vtg3) and the actively spawning sub-phase (GVM and hydration), the percentage of cortical alveoli oocytes remained constant, showing that the recruitment of newly vitellogenic oocytes continued. The higher percentage of cortical alveoli oocytes observed in the developing phase (Vtg1) ovaries could be related to a high pulse of oocyte recruitment at the beginning of the reproductive season in order to produce the main stock of vitellogenic oocytes. Similar results on oocyte development patterns were observed in European hake with a "matching pulse" of cortical alveoli oocytes in the developing phase (Vtg1) ovaries (Korta et al., 2010). In the case of determinate fecundity species, a decrease

Table 6
Summary of monthly incidence of each atretic state for female yellowfin tuna. Prevalence of atresia for ovaries at spawning capable phase (Vtg3 as most advanced vitellogenic oocyte) takes into account the atretic state 3–5. Also, the percentage of ovaries at regressing phase is shown.

State of atresia	January	February	March	April	May	June	July	Total general
0	11	27	39	43	38	21	31	210
1	4	2	11		4	1	9	31
2	–	–	10	4	7	3	6	30
3	1	2	–	–	1	13	1	18
4	–	–	1	–	–	3	1	5
5	–	1	1	5	12	1	5	25
No. of fish	16	32	62	52	62	42	53	319

	Percent occurrence						
	January	February	March	April	May	June	July
Prevalence of atresia	6.2	9.4	3.2	9.6	21.0	40.5	13.2
Regressing	0.00	3.1	3.2	9.6	19.3	9.5	11.3

in cortical alveoli oocytes would be expected as a consequence of the fact that no additional oocytes are recruited once the spawning season commenced (Hunter et al., 1992). On the other hand, the results of the percentages of cortical alveoli oocytes and total vitellogenic oocytes (Vtg1–3) by month for ovaries at the spawning capable phase (Vtg3) were similar. There was no evidence of a decreasing tendency in the percentage of cortical alveoli and total vitellogenic oocytes as the spawning season progressed, which was indicative of recruitment of new oocytes. Apart from July (47%), all months exhibited a percentage of total vitellogenic oocytes higher than 50%, which represented a replenishment of newly formed primary vitellogenic oocytes (Vtg1) into the standing stock of secondary and tertiary vitellogenic stages, i.e. indeterminate fecundity. In contrast, in determinate fecundity fishes, a decrease in the standing stock of vitellogenic oocytes is expected as there is no replacement of oocytes after each spawning event (Murua and Saborido-Rey, 2003). Therefore, it can be concluded that continuous oocyte replenishment occurred from primary growth oocytes into the secondary growth stages (i.e. the so-called de novo vitellogenesis) in order to replace those oocytes shed during the spawning season (Gordo et al., 2008; Korta, 2010).

4.3. Mean diameter of tertiary vitellogenic oocytes

Replenishment by newly formed vitellogenic oocytes to the standing stock of advanced vitellogenic oocytes causes a decrease in the mean diameter of tertiary vitellogenic (Vtg3) oocytes (Hunter et al., 1992). This effect on the diameter is evidence of indeterminate fecundity (Murua and Saborido-Rey, 2003). In our study, the mean diameter of Vtg3 oocytes decreased significantly from November to March. However the mean diameter of Vtg3 oocytes did not show a temporal significant variation from November to June, although the reduced number of spawning capable phase (Vtg3) ovaries reduces the reliability of this analysis. The pattern found in relation to mean diameter could be explained by the temporal extension of the spawning seasons described in previous studies, where a main reproductive season from November to March and a second peak with less spawning intensity from June to August have been postulated (Koido and Suzuki, 1989; Nootmorn et al., 2005; Stéquert and Marsac, 1989; Stéquert et al., 2001). In that sense, the significant decrease in the mean diameter from November to March could be explained by the completion of the main spawning season, and the higher values in November and June could correspond to the beginning of the reproductive period; i.e. the main spawning period in November and a second period with a lower spawning activity in June.

4.4. Atresia

The seasonal development of atresia throughout the reproductive cycle differs between species with indeterminate and determinate fecundity (Murua and Saborido-Rey, 2003). Fishes with indeterminate fecundity show an increase in the intensity and prevalence of atresia at the end of the spawning season (Korta, 2010; Murua and Motos, 2006). The continuous recruitment of newly formed oocytes into the standing stock of vitellogenic oocytes generates a surplus production of oocytes that become atretic when individuals approach the end of their spawning season and need to be resorbed (Murua and Motos, 2006). In the present study, a higher incidence of atresia was found in the actively spawning sub-phase and regressing phase. The prevalence of alpha-atresia increased throughout the spawning season, as well as the number of regressing individuals showing high incidence of atretic vitellogenic oocytes (>50%). Higher occurrences of alpha-atresia in vitellogenic oocytes were observed at the end of the spawning season, a result that concurred with those of several published studies (Koido and Suzuki, 1989; Nootmorn et al., 2005; Stéquert and Marsac, 1989; Stéquert et al., 2001), but not during earlier months (Ndjaula et al., 2009), which is also consistent with an indeterminate fecundity species.

5. Conclusion

The intention of this study was to describe the type of fecundity of yellowfin tuna in the Western Indian Ocean by a comprehensive assessment of the recruitment process of oocytes based on modern histological and image analyses protocols. Our research, which was based on the acceptable set of principles to be examined to ascertain fecundity type of a species (Kjesbu, 2009; Murua and Saborido-Rey, 2003), revealed asynchronous oocyte development and a continuous oocyte size–frequency distribution throughout all ovarian developmental phases over the spawning season. Moreover, the percentage and number of cortical alveoli and total vitellogenic oocytes remained constant through the spawning season. The mean diameter of tertiary vitellogenic oocytes decreased as spawning progressed, while the incidence of atresia was higher at the end of the spawning season as a consequence of over-recruitment of oocytes during the spawning period. Thus, we concluded that Indian Ocean yellowfin tuna exhibit indeterminate fecundity.

Resolving the type of fecundity in yellowfin tuna would contribute to a better understanding of reproductive potential of this species; which, in turn, will help to investigate the processes of fish recruitment in this very important commercial fish species. Moreover, the work carried out here would be necessary for any possible application of an egg production method (EPM); which would provide a fishery-independent estimation of the spawning stock biomass.

Conflict of interest

The authors declare that they have no conflicts of interest.

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References

Alonso-Fernández, A., Domínguez-Petit, R., Bao, M., Rivas, C., Saborido-Rey, F., 2008. Spawning pattern and reproductive strategy of female pouting *Trisopterus lucus* (Gadidae) on the Galician shelf of north-western spain. Aquat. Living. Resour. 21, 383–393.

Andersen, T.E., Witthames, P.R., Kjesbu, O.S., Bromley, P., Tyler, C.R., 2000. Development of a Surgery Protocol for Unilateral Ovariectomy (ULO) on Cod (*Gadus morhua*). Dept. Fish. Mar. Biol., Bergen, Norway, p. 304 http://www.ifm.uib.no/repro/.

Armstrong, M.J., Witthames, P.R., 2012. Development in understanding of fecundity of fish stocks in relation to egg production methods for estimating spawning stock biomass. Fish. Res. 117–118, 35–47.

Bernal, M., Somarakis, S., Witthames, P.R., van Damme, C.J.G., Uriarte, A., Lo, N.C.H., Dickey-Collas, M., 2012. Egg production methods in marine fisheries: an introduction. Fish. Res. 117–118, 1–5.

Brown-Peterson, N.J., Wyanski, D.M., Saborido-Rey, F., Macewicz, B.J., Lowerre-Barbieri, S.K., 2011. A standardized terminology for describing reproductive development in fishes. Mar. Coast. Fish.: Dyn. Manage. Ecosyst. Sci. 3 (1), 52–70.

Collette, B.B., Nauen, C.E., 1983. FAO Species Catalogue. Vol. 2 Scombrids of the World: An Annotated and Illustrated Catalogue of Tunas, Mackerels, Bonitos, and Related Species Known to Date. FAO, Rome, 137 pp. (Fish. Synop., 125).

Gordo, L.S., Costa, A., Abaunza, P., Lucio, P., Elnk, A.T.G.W., Figueiredo, I., 2008. Determinate versus indeterminate fecundity in horse mackerel. Fish. Res. 89, 181–185.

Greer Walker, M., Witthames, P.R., Bautista de los Santos, I., 1994. Is the fecundity of the Atlantic mackerel (*Scomber scombrus*: Scombridae) determinate? Sarsia 79, 13–26.

Hislop, J.R.G., Hall, W.B., 1974. The fecundity of whiting *Merlangus merlangus* (L), in the North Sea, the Munch and Iceland. ICES. J. Conseil. 36, 162–165.

Hunter, J.R., Macewicz, B.J., 1985a. Measurement of spawning frequency in multiple spawning fishes. In: Lasker, R., (Ed.) An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy, *Engraulis mordax*. NOAA Tech. Rep. NMFS 36, pp. 79–94.

Hunter, J.R., Macewicz, B.J., 1985b. Rates of atresia in the ovary of captive and wild northern anchovy, *Engraulis mordax*. Fish Bull. 83 (1), 19–136.

Hunter, J.R., Macewicz, B.J., Kimbrell, C.A., 1989. Fecundity and other aspects of the reproduction of sablefish, Anaplopoma fimbria, in Central California waters, California Cooperative Oceanic Fish. Investig. Rep. 30, pp. 61–72.

Hunter, J.R., Macewicz, B.J., Lo, N.C.H., Kimbrell, C.A., 1992. Fecundity, spawning, and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. Fish. Bull. 90, 101–128.

IOTC (Indian Ocean Tuna Commission), 2003. Report of the Fifth Session of the IOTC WPTT, Victoria, Seychelles.

IOTC (Indian Ocean Tuna Commission), 2010. Report of the Twelve Session of the IOTC WPTT, Victoria, Seychelles.

Itano, D.G., 2000. The reproductive biology of yellowfin tuna (*Thunnus albacares*) in Hawaiian water and the western tropical Pacific Ocean: project summary. PFRP, JIMAR, UH, HI, p. 69 (JIMAR Contribution 00-328).

Joseph, J., 1963. Fecundity of yellowfin tuna (*Thunnus albacares*) and skipjack (*Katsuwonus pelamis*) from the eastern Pacific Ocean. Inter-Am. Trop. Tuna Commun. Bull. 7(4), 255-92.

Koido, T., Suzuki, Z., 1989. Main spawning season of yellowfin tuna (*Thunnus albacares*) in the western tropical Pacific Ocean. Bull. Far. Seas Fish. Res. Lab. 26, 153–163.

Kjesbu, O., 2009. Applied fish reproductive biology: contribution of individual reproductive potential to recruitment and fisheries management. In: Jokobsen, T., Fogarty, J., Megrey, B., Moksness, E. (Eds.), Fish Reproductive Biology: Implications for Assessment and Management. Wiley-Blackwell, United Kingdom, pp. 293–332.

Korta, M., 2010. New methodologies applied to quantify the dynamics of the ovary in indeterminate fecundity species. Ph.D Thesis. Dep. Zool. Anim. Cell Biol., Univ. Basque Country, Spain.

Korta, M., Murua, H., Kurita, Y., Kjesbu, O., 2010. How are the oocytes recruited in an indeterminate fish? Applications of stereological techniques along with advanced packing density theory on European hake (*Merluccisu merluccius* L.). Fish. Res. 104, 56–63.

Lasker, R. (Ed.), 1985. An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy, *Engraulis mordax*. U.S. Dep. Commer. NOAA Tech. Rep., NMFS 36, 99 pp.

McPherson, G.R., 1991. Reproductive biology of yellowfin tuna in the eastern Australian fishing zone with special reference to the North-western Coral Sea. Aust. J. Mar. Freshw. Res. 42, 465–477.

Murua, H., Motos, L., 2000. Reproductive biology of roughhead grenadier. Sarsia 85, 393–402.

Murua, H., Motos, L., 2006. Reproductive strategy and spawning activity of the European hake *Merluccius merluccius* (L.) in the Bay of Biscay. J. Fish Biol. 69, 1288–1303.

Murua, H., Saborido-Rey, F., 2003. Female reproductive strategies of commercially important fish species in the North Atlantic. J. Northw. Atl. Fish Sci. 33, 23–32.

Ndjaula, H.O.N., Hansen, T., Krüger-Johnsen, M., Kjesbu, O.S., 2009. Oocyte development in captive Atlantic horse mackerel *Trachurus trachurus*. ICES J. Mar. Sci. 66 (4), 623–630.

Nootmorn, P., Yakoh, A., Kawises, K., 2005. Reproductive Biology of Yellowfin Tuna in the Eastern Indian Ocean. IOTC, Victoria.

Orange, C.J., 1961. Spawning of yellowfin tuna and skipjack in the eastern tropical Pacific, as inferred from studies of gonad development. Inter-Am. Trop. Tuna Commun. Bull. 5 (6), 457–526.

Parker, k., 1980. A direct method for estimating northern anchovy *Engraulis mordax* spawning biomass. Fish Bull. 78, 541–544.

Schaefer, K.M., 1996. Spawning time, frequency, and batch fecundity of yellowfin tuna *Thunnus albacares*, near Clipperton Atoll in the eastern Pacific Ocean. Fish. Bull. 94, 98–112.

Schaefer, K.M., 1998. Reproductive biology of yellowfin tuna (*Thunnus albacares*) in the eastern Pacific Ocean. Inter-Am. Trop. Tuna Commun. Bull. 21 (5), 205–221.

Schaefer, K.M., 2001a. Reproductive biology of tunas. In: Block, B.A., Stevens, E.D. (Eds.), Tuna: Physiology Ecology, and Evolution. Acad. Press, San Diego, California, US, pp. 225–270.

Schaefer, K.M., 2001b. Estimation of the maturity and fecundity of tunas. Report on the Working Group on Modern Approaches to Assess Maturity and Fecundity of Warm and Cold Water Fish and Squids. John Grieg Grafisk AS, Bergen, Norway, pp. 117–124.

Somvanshi, V., 2002. Review of biological aspects of yellowfin tuna (*Thunnus albacares*) from the Indian Ocean. IOTC Proc. 5, 420–426.

Stéquert, B., Marsac, F., 1989. Tropical tuna–surface fisheries in the Indian Ocean. FAO Fish. Tech. Pap. No. 282, Rome, Italy, 238 pp.

Stéquert, B., Nuñez, J., Cuisset, B., Le Menn, F., 2001. Gonadosomatic index and seasonal variations of plasma sex steroids in skipjack tuna (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) from the western Indian ocean. Aquat. Living. Resour. 14, 313–318.

Stratoudakis, Y., Bernal, M., Ganias, K., Uriarte, A., 2006. The daily egg production method: recent advances current applications and future challenges. Fish Fish. 7, 35–57.

Tyler, C.R., Nagler, J.J., Pottinger, T.G., Turner, M.A., 1994. Effects of unilateral ovariectomy on recruitment ang growth of follicles in the rainbow trout, *Oncorhynchus mykiss*. Fish. Physiol. Biochem. 13 (4), 309–316.

Wallace, R.A., Selman, K., 1981. Cellular and dynamic aspects of oocyte growth in teleosts. Am. Zool. 21, 325–343.

West, G., 1990. Methods of assessing ovarian development in fishes: a review. Aust. J. Mar. Freshw. Res. 41, 199–222.

Zhu, G., Xu, L., Zhou, Y., Song, L., 2008. Reproductive biology of yellowfin tuna (*T. albacares*) in the west-central Indian Ocean. J. Ocean Univ. China (Engl. Ed.) 7, 327–332.

Zudaire, I., Murua, H., Grande, M., Submitted Reproductive potential of yellowfin tuna (*Thunnus albacares*) in the Western Indian Ocean. Fish. Bull., submitted for publication.