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PRESENT AND FUTURE OF REPRODUCTIVE STUDIES OF YELLOWFIN TUNA (*THUNNUS ALBACARES*) IN THE EASTERN ATLANTIC OCEAN

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SUMMARY

*The reproductive biology of yellowfin tuna (*Thunnus albacares; YFT*) was studied through the estimation of some important traits, such as sex-ratio, spawning seasonality, fish condition and stomach content analysis, in the Eastern Atlantic Ocean from February 2014 to August 2014. In total 1016 YFT individuals (475 females and 541 males) were collected in a weekly sampling at the cannery in the fishing port of Abidjan, Ivory Coast. Based on the macroscopic identification of the gonads, a high reproductive activity of female (76.4-166 cm FL) and male (72.8-170.9 cm FL) YFT was observed during boreal winter, being significantly active in December and January. The preliminary results of the condition indices by sex showed a possible pattern in the energy allocation dynamic during the spawning season but with differences among both sexes. This study establishes the bases for future biochemical analysis on the species and accurate histological analysis will be performed to further study the energy allocation strategy during reproduction of this socio-economically important tuna species.*

RÉSUMÉ

*La biologie reproductive de l'albacore (*Thunnus albacares, YFT*) a été étudiée au moyen de l'estimation de quelques facteurs importants, tels que le ratio des sexes, le caractère saisonnier de la reproduction, le facteur de condition et l'analyse des contenus stomacaux, dans l'océan Atlantique Est de février 2014 à août 2014. Au total, 1.016 spécimens d'albacore (475 femelles et 541 mâles) ont été recueillis lors d'un échantillonnage hebdomadaire dans la conserverie du port de pêche d'Abidjan (Côte d'Ivoire). Sur la base de l'identification macroscopique des gonades, une activité reproductive intense des femelles (76,4-166 cm FL) et des mâles (72,8 - 170,9 cm FL) a été observée pendant l'hiver boréal, plus particulièrement en décembre et janvier. Les résultats préliminaires des indices de condition par sexe montraient un schéma possible de dynamique de distribution de l'énergie pendant la saison de frai, présentant toutefois des différences entre les deux sexes. Cette étude jette les bases des futures analyses biochimiques consacrées à cette espèce et des analyses histologiques précises seront réalisées afin d'étudier plus en profondeur la stratégie de distribution de l'énergie pendant la reproduction de cette espèce de thon socio-économiquement importante.*

RESUMEN

Se estudió la biología reproductiva del rabil (Thunnus albacares, YFT) *mediante la estimación de algunos rasgos importantes, como la ratio de sexos, la estacionalidad del desove, la condición de los peces y el análisis del contenido estomacal, en el océano Atlántico oriental desde febrero de 2014 hasta agosto de 2014. En total, se recopilaron 1016 ejemplares de rabil (475 hembras y 541 machos) en un muestreo semanal en una conservera en el puerto pesquero de Abiyán, Côte d'Ivoire. Basándose en la identificación macroscópica de las gónadas, se observó una elevada actividad reproductiva de las hembras (76,4-166 cm FL) y los machos (72,8-170,9 cm FL) durante el invierno boreal, siendo especialmente activos en diciembre y enero. Los resultados preliminares de los índices de condición por sexo mostraban un posible patrón en la dinámica de asignación de energía durante la temporada de reproducción, pero con diferencias entre ambos sexos. Este estudio establece las bases para futuros análisis bioquímicos de esta especie y se llevarán a cabo análisis histológicos precisos para estudiar más en profundidad la estrategia de asignación de energía durante la reproducción de esta socio-económicamente importante especie de túnido.*

KEYWORDS
Reproduction, Sex ratio, Condition index, Yellowfin Tuna, Atlantic Ocean

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1. Introduction

Yellowfin tuna (*Thunnus albacares*; YFT), is a large epipelagic species widely distributed in tropical and subtropical regions (Collette and Nauen, 1983). It is an important commercial value highly harvested by different fishing methods worldwide. Tuna purse-seine (PS) fishery is the main fishing gear corresponding 82.3% of the total annual catches of this species in the Eastern Atlantic Ocean (ICCAT, 2013). Two different fishing types with different size-composition at catches are used by PS fleet (Joseph, 2003): Fish Aggregating Devices (FADs) and, Free-Swimming Schools (FSC) of tuna. YFT total annual catches represented 101,866 tons (t) in 2012 from which around 45-50% were caught in FADs (ICCAT, 2013).

While fisheries science in the last decades has focused on the development of more realistic population dynamic models, the collection of biological data has been widely neglected. In this context, the assessment of important reproductive traits such as sex-ratio, timing in terms of maturation and spawning, and fish condition, has been described to be important for the assessment of the reproductive potential of the populations as well as to well understand the productivity of fish populations and their resilience to fisheries and environmental changes. Reproductive studies on yellowfin tuna were already carried out previously in the Eastern (Albaret, 1977; Bard, 1991; Orange, 1963), and Western Atlantic Ocean (Arocha, 2001), as well as in the Pacific Ocean (McPherson, 1991; Schaefer, 1996; 1998) and more recently in the Indian Ocean (Zhu *et al*., 2008; Zudaire *et al*., 2013a; 2013b). However, some important parameters such as fecundity remains not well studied and it is necessary an update of them for the quality of stock assessment.

Thus, the objective of this work is to evaluate these reproductive traits, as well as the stomach content and length-weight relation estimation of the yellowfin tuna caught in the Eastern Atlantic Ocean in an all year around research project. The monitoring of these reproductive traits will contribute to the knowledge of those basic parameters for the study of yellowfin tuna population dynamics.

2. Material and methods

2.1 Field sampling

Yellowfin tuna caught by the purse-seine fleet operating in the Eastern Atlantic Ocean (**Figure 1**), were processed in a weekly (every two days) sampling at the cannery of the fishing port of Abidjan from February 2014 to August 2014. Species identification was performed based on species external characters (Collette and Nauen, 1983). For each fish sex was determine and fork length (cm), first dorsal length (cm), thorax length (cm) and total weight (Kg) were recorded. From sampled females ovaries, liver and stomach were removed and total weight recorded to the nearest gram. A cross section of ovaries of 4–5 cm was cut between the middle and end part of the right or left lobe and preserved in 4% buffered formaldehyde for reproductive analysis. Samples of gonad, liver and white muscle, approximately 15 g, were collected and preserved frozen for biochemical analysis. The fish traceability regarding, vessel name, fishing date and area, sea surface temperature, and fishing type (FADs and FSC) were obtained from the vessels logbooks.

2.2 Sex Ratio

Sex was reported based on visual examination of the gonads. Sex ratio (SR) was calculated as the proportion of females by 5 cm length classes. In the present study was assumed that the sex-ratio of males and females was equal to 1(Schaefer 1987).

$$SR = \frac{Nf}{Nt} \times 10^2$$

Where Nf is the number of females and Nt is the total number of sampled fish.

2.3 Condition indices

Fish condition was evaluated by applying three conditions indices: gonadosomatic index (GSI), hepatosomatic index (HSI) and condition factor (K)

$$GSI = \frac{Wg}{W} \times 10^2$$

$$HSI = \frac{W_l}{W} \times 10^2$$

$$K = \frac{W}{LF^3} \times 10^2$$

where Wg is fish gonad-free weight (g), W is total fish weight (g),and W_l.is the liver weight (g) The variation of the condition indices was analyzed by month and by maturity development phases.

2.4 Reproductive analysis

The classification of the maturity stage of yellowfin tuna ovaries and testis was based on macroscopic identification (ICCAT, 2014) shown in **Table 1**.

2.5 Stomach content analysis

For the stomach content analysis each stomach was rinsed, and weighted full. After removing all the content the empty stomach was weighted again. The classification of preys was based on global characteristic of species and five different groups were identified (Fish, Crustaceans, Cephalopods, Mix of previous prey groups and others).

3. Results

3.1 Length-weight relationships and Sex ratio

A total of 1016 yellowfin (475 females and 541 males) were collected from February 2014 to July 2014. From the total 650 specimens of yellowfin tuna, 238 males (72.8 to 170.9 cm FL) and 321 females (76.4 to166.2 cm FL) contributing 42.57% and 57.42% respectively, were used for the length-weight relationship studies The results for combined values of both sexes (**Figure 2**) indicated the same evolution with ICCAT officially representation. In this analysis data corresponding to large size individuals was not included due to the lack of a suitable scale to weight them during the sampling.

Sex ratio for female and male yellowfin tuna was analyzed by 5cm size classes. It was not a clear predominance of both sexes at small and intermediate size. In contrast, males' proportion was predominant at large sizes, from 148 to 172 cm (**Figure 3**).

3.2 Reproductive analysis

3.2.1 Macroscopic observation

The macroscopic identification of yellowfin tuna ovaries and testis described 5 development stages in females (**Figure 4**) and 4 development stages in males with the absence of stage **Figure 5**.

3.2.2 Spawning seasonality

According to the classification summarized in the **Table 1**, 56.63 % of the female individuals were at the stages 1, 12.63 % were at stage 2, 12.63 % were at stage 3, 17.89 % at stage 4 and 0.2 % of the fishes showed ovaries at stage 5 (**Table 2**). The 30.74 % of sampled females were mature. In the case of males 59.15 % of male individuals were at the stages 1, 4.44 % were at stage 2, 10.72 % were at stage 3, and 25.69 % of male population were at stage 4.

Regarding the seasonal development of ovaries and testis, females with developed ovaries at stage 3 and 4 appeared in a high percentage from December to April. In contrast, from May to August a high percentage of females with less developed ovaries, stage 1 and 2 as the most advanced stage of development, were found in the sampled population (Fig.6). Similar trend was observed for the males in which testis appeared more developed from December to April with testis at stages 3 and 4. In contrast, ovaries were less developed from May to August, with almost 100% of the ovaries at stage 1 (**Figure 7**).

3.3 Condition Indices

The analysis of the seasonal development of condition indices (Fig.8) in yellowfin tuna females showed a period of high mean GSI values; from December (2.47±0.76) to April (2.22±1.58) with values higher than 1.5. This result correspond with previously described pattern on the seasonal development of ovaries. During the following months, from May (0.31±0.12) to August (0.38±0.09), the GSI values decreased sharply and kept low (around 0.31 GSI). Similar pattern in GSI values was observed in males with same period of high values from December (1.32±0.42) to April (1.01±0.52) and low values from May to August.

Similarly, the values of HSI and K were analyzed by month. K values remained almost constant through the spawning period in both sex, around 1.02±0.05 for females and 1.03±0.07 for males. However, a different development pattern of HSI values was observed between sexes during the spawning season. In females, the mean HSI values followed the GSI pattern, being HSI high from December (1.05±0.17) to April (0.90±0.27) and afterwards performing a decrease from May (0.61±0.19) to August (0.68±0.11). In contrast, males did not show much variance in HSI values being July the month of highest values (0.79±0.15) (**Figure 9**).

The variability of GSI values at each ovary and testis development stage are shown in figure 10 and figure 11 respectively. The variability of GSI values at most developed ovaries is wider and there is an increase of GSI values as the ovary develops. Stage 3 and 4 at both sexes showed the highest mean GSI values (1.79±0.60 and 2.80±1.07 in females and 1.02±0.19 and 1.33±0.33 in males). In the case of female GSI values for these stages were found over 1.5. In contrast, HSI values had a different pattern by sex. Female HSI followed the GSI trend increasing as ovary develops describing the highest values at stage 3 (0.95±0.16) and 4 (1.05±0.20). In contrast, HSI values in males did not show variation as testis develops keeping constant from stage 1 (0.66±0.33) to stage 4 (0.56±0.11). Regarding the K values it was not observed a clear pattern by sex and the values kept constant through the development of the ovary and testis.

3.4 Stomach content analysis

The preliminary results of the stomach content analysis showed that female yellowfin tuna fed principally on cephalopods with more than 50% of the stomachs containing this prey group in females at immature and developing stages (**Figure12**). Females with ovaries at stage 1 fed also in a large extend on fish prey (41% of the stomachs), however it was observed an increase of fish prey, mainly small pelagic, from stage 2 (10%) to stage 4 (47%) females. In males the main group of prey was the cephalopods with more than 50% in individuals with testis at stage 1, 2 and 3 of development. Only in males with testis at stage 4, the percentage of fish prey increased over the 50% (**Figure13**).

4. Discussion and future perspective

This document contains the preliminary results of the research on reproduction of tropical tunas in the Eastern Atlantic Ocean carried out in the CRO with the collaboration of IRD. At present, funding limitations for research and the lack of laboratory material do not allow (1) a precise biologic manipulation and (2) a projection on the future research activities in CRO. However, the acquired knowledge by the CRO researchers in the Seychelles Fisheries Authority laboratory will allow the implementation of different methodologies in the near future. Therefore, works on yellowfin tuna, bigeye tuna (*Thunnus obesus*) and skipjack (*Katsuwonus pelamis*) will carry on for an accurate histological and biochemical analysis.

Similar to previous studies in the region (Capisano, 1991), in our preliminary results was described a significant dominancy of males for specimens larger than 150 cm. This predominance of male yellowfin tuna at large sizes were already reported in the main three oceans (Capisano, 1991; Schaefer, 1998; Timochina and Romanov, 1996). However, the female dominant pattern described for the Atlantic Ocean (125-140 cm) was not observed in the present study. These differences in the sex-ratio by size are likely to be the consequence of a sexually dimorphic growth patterns in yellowfin tuna (Marsac et al., 2006; Timochina and Romanov, 1996), and/or the natural and fishing mortalities by sex. Hence, its intensive sampling and comparative analysis with other ocean estimations has been recommended (Fonteneau, 2002). In this context, the all year around sampling at the cannery of Abidjan’s fishing port will provide valuable information to update possible trend in the sex-ratio of this species.

The assessment of the seasonal development of gonads in both sex, described a main spawning period from December to April, and this high reproductive activity period was also supported by the description of GSI values. The observed pattern is similar to the results previously described in the Eastern Atlantic Ocean (ICCAT, 2011). However, has to be noted that these results are based on the macroscopic identification of gonads and thus a more precise analysis applying microscopic identification of ovaries and testis are required:(a) to perform a comparison of both methods (macroscopic vs. microscopic) in order to calibrate macroscopic identification of gonads in cannery and (b) to accurately identify the ovary and testis development phases as it has been recommended for reproductive analysis (Brown-Peterson et al., 2011). Therefore, yellowfin tuna individuals presented in this document will be analyzed histologically and fecundity will be estimated applying the gravimetric and image analysis methods. This will allow us to perform a comparison of reproductive parameters obtained in the Atlantic and Indian Ocean for this species.

The protracted spawning season and population asynchronicity in spawning activity shown by yellowfin tuna could mask temporal variations on energy allocation and mobilization analyzed by the study of condition indices throughout the spawning season (Zudaire *et al*., 2013). Therefore, the assessment of energy reserves variation by female and male maturity phases is required in order to study the energetic dynamic cycle in individuals undergoing gonad development and reproductive activity. The preliminary results showed that in female yellowfin tuna the GSI and HSI followed a similar increasing trend as ovary develops. In contrast the HSI in males kept constant during testis development while GSI increased. These differences in the pattern of both sexes could be related to the accumulation and depletion of important compounds (i.e., lipids and proteins) involved in the reproduction. The future biochemical analysis of liver, muscle and gonad will allow us to better understand how these compounds are mobilized for the reproduction in both sexes. Besides, further feeding analysis will be performed to study the energy acquisition during reproduction and to observe if there is any difference in the energy acquisition strategy by sex.

CRO in collaboration with IRD project to study in short time the reproduction of small tuna species in the Eastern Atlantic Ocean applying microscopic analysis of ovaries and image analysis method to study the fecundity regulation strategy as well as to perform fecundity estimations. In other words, the work carrying out in large tunas will be reproduced for small tuna species catch widely in the region. However, these histological works are jeopardized due to the lack of necessary equipments: 1) Tissue embedding processor used to pass histological sampling, 2) microtome used to cut processed tissues and 3) tissue tek used to make inclusion. If we get this material, the research works on large tuna will carry on and collection of biological data for the small tuna would begin at the end of the year 2014.

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Table 1. Maturity stage classification for visual examination of gonads for large pelagic (ICCAT, 2014).

Stage	*Criteria*	
	Males	*Females*
I	Gonads small ribbon-like, not possible todetermine sex by gross examination	Gonads small ribbon-like, not possible todetermine sex by gross examination
1	Immature; testes extremely thin, flattened and ribbon-like, but sex determinable by gross examination	Immature; gonads elongated, slender, but sex determinable by gross examination
2	Enlarged testes, triangular in cross section, no milt in central canal	Early maturing; gonads enlarged but individual ova not visible to the naked eye
3	Maturing; milt flows freely if testes pinched or pressed	Late maturing; gonads enlarged, individual ova visible to the naked eye
4	Ripe; testes large, milt flows freely fromtestes	Ripe; ovary greatly enlarged, ova translucent, easily dislodged from follicles or loose in lumen of ovary
5	Spent; testes flabby, bloodshot, surface dull red, little or no milt in central canal	Spawned; includes recently spawned and postspawningfish, mature ova remnants in various stages of resorption, and mature ova remnantsabout 1.0mm in diameter

Table 2. Summary of the sampled individuals by 5 cm size classes and maturity development for male and female individuals.

Size classes	***Maturity Females***						***Maturity Males***				
	1	***2***	***3***	***4***	***5***	***Total***	***1***	***2***	***3***	***4***	***total***
<60										1	1
60-65							1				1
70-75							1				1
75-80	5					5	3				3
80-85	8					8	5				5
85-90	5	1				6	14				14
90-95	9					9	14				14
95-100	13					13	11				11
100-105	50	4				54	39				39
105-110	78	14				92	107	1			108
110-115	54	13				67	75	3			78
115-120	8	7				15	14				14
120-125	9	4	1			14	19				19
125-130	28	4	5	3		40	14	3	5	1	23
130-135	1	4	5	6		16	3	7	3	5	18
135-140	1	2	13	22	1	39		1	8	10	19
140-145		2	13	21		36		1	14	21	36
145-150		2	13	22		37		4	12	31	47
150-155		2	8	7		17		3	6	32	41
155-160		1	1	3		5			6	24	30
160-165			1			1		1	3	9	13
165-170				1		1			1	2	3
170-175										3	3
Total	**269**	**60**	**60**	**85**	**1**	**475**	**320**	**24**	**58**	**139**	**541**

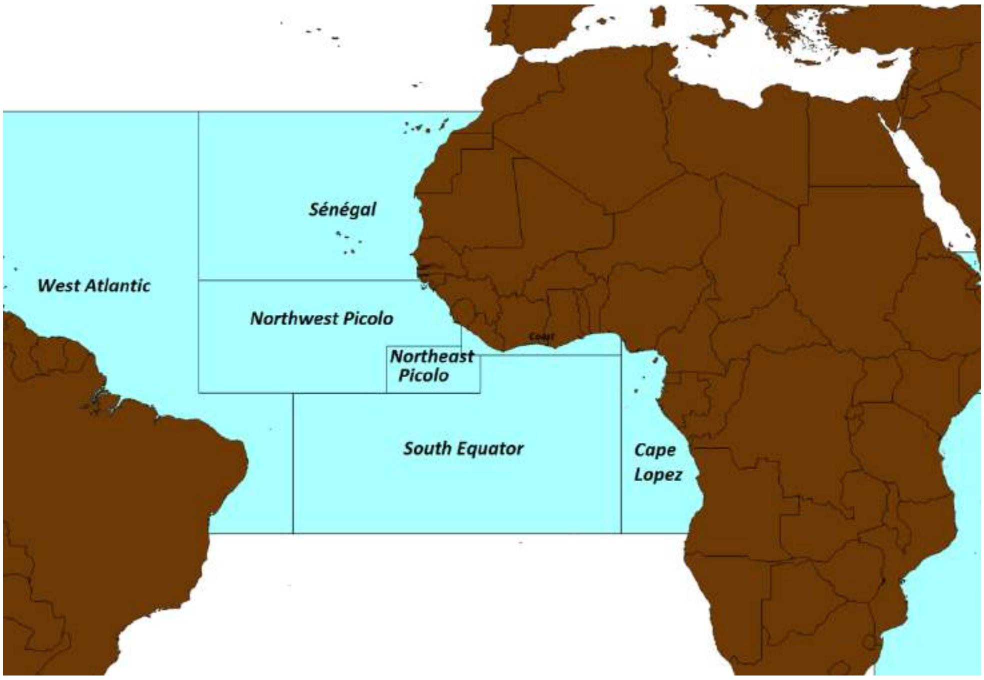


Figure 1. Fishing areas of the yellowfin tuna caught by purse seiners in this study.

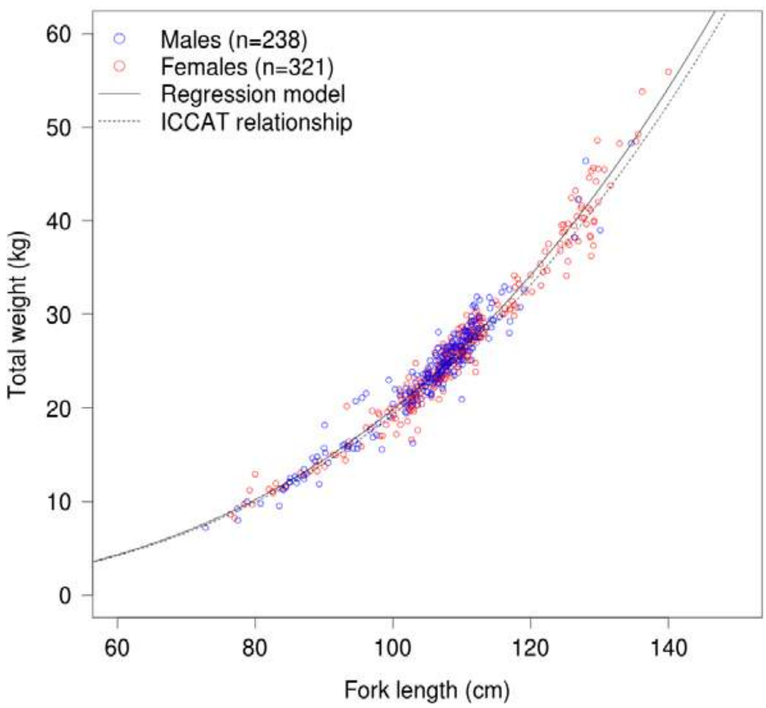


Figure 2. Relationship between fork length (cm) and body weight (kg) for male and female yellowfin tuna sampled in the cannery of the fishing port of Abidjan from January to August 2014.

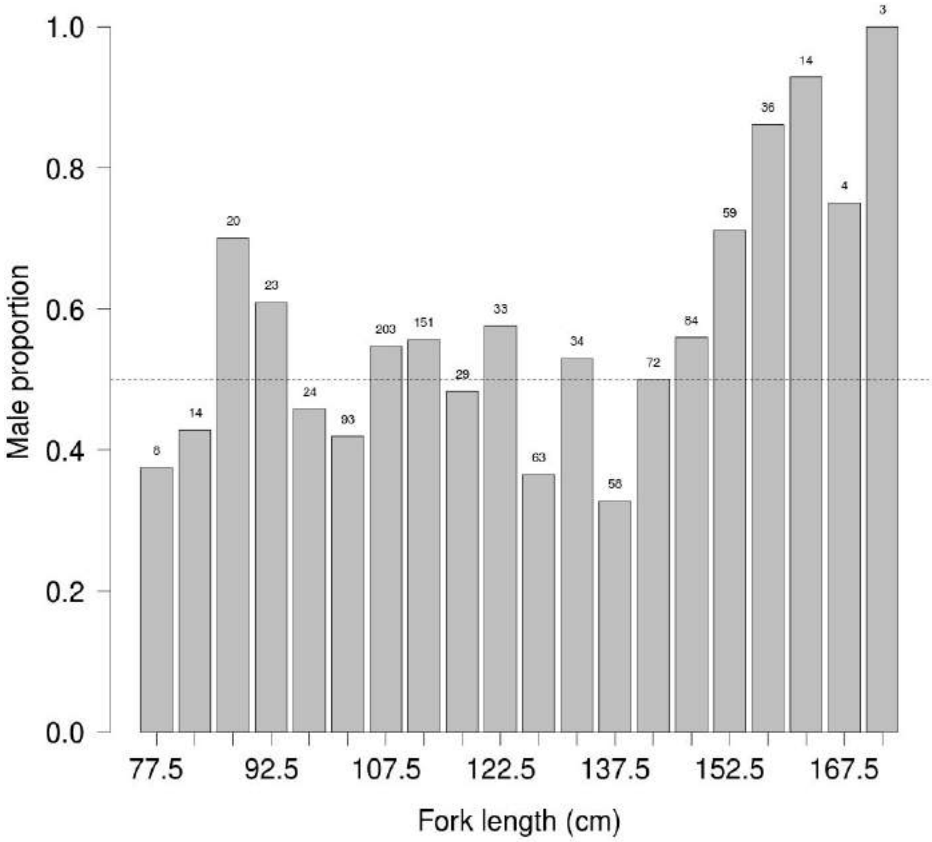
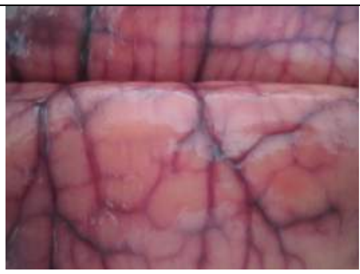
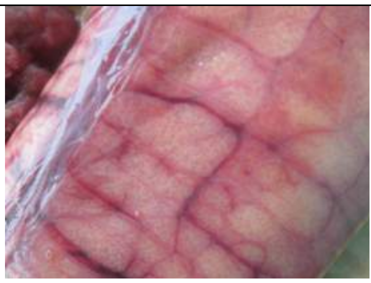


Figure 3. Monthly variations in sex-ratio of yellowfin tuna in the Eastern Atlantic Ocean.

<table>
<tr><th>General aspect</th><th>External characteristics</th><th>Internal characteristics</th></tr>
<tr><td></td><td>
</td><td></td></tr>
<tr><td colspan="3">Stage 1 : Immatures : Gonads elongated, slender, but sex determinable by gross examination</td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td colspan="3">Stage 2 : Early maturing: Gonads enlarged but individual ova not visible to the naked eye</td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td colspan="3">Stage 3 : Late maturing; gonads enlarged, individual ova visible to the naked eye</td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td colspan="3">Stage 4 : Ripe: ovary greatly enlarged, ova translucent, easily dislodged from follicles or loose in lumen of ovary</td></tr>
</table>

		
Stage 5 : Spawned; includes recently spawned and post spawning fish, mature ova remnants in various stages of resorption, and mature ova remnantsabout 1.0mm in diameter		

Figure 4. Macroscopic identification of maturity stages for female yellowfin tuna ovaries in Eastern Atlantic Ocean.

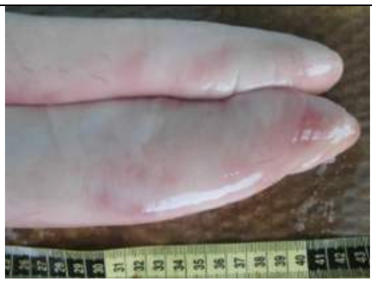
General aspect	External characteristics	Internal characteristics
		
Stage 1 : Immature : testes extremely thin, flattened and ribbon-like, but sex determinable by gross examination		
		
Stage 2 : Enlarged testes, triangular in cross section, no milt in central canal		
		
Stage 3 : Maturing; milt flows freely if testes pinched or pressed		
		
Stage 4 : Ripe; testes large, milt flows freely from testes		

Figure 5. Macroscopic identification of maturity stages for male yellowfin tuna testis in Eastern Atlantic Ocean.

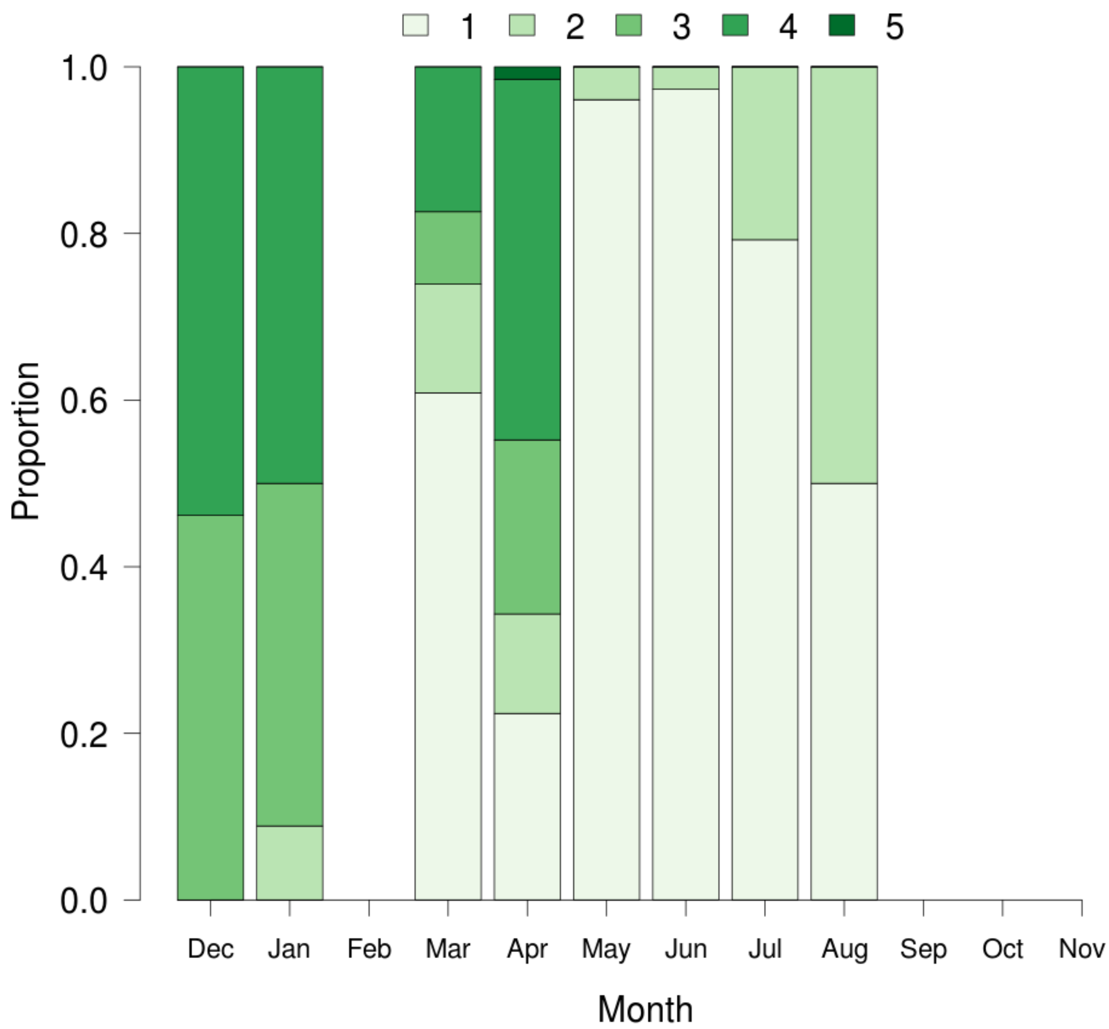


Figure 6. Monthly proportion of ovary development stages for female yellowfin tuna.

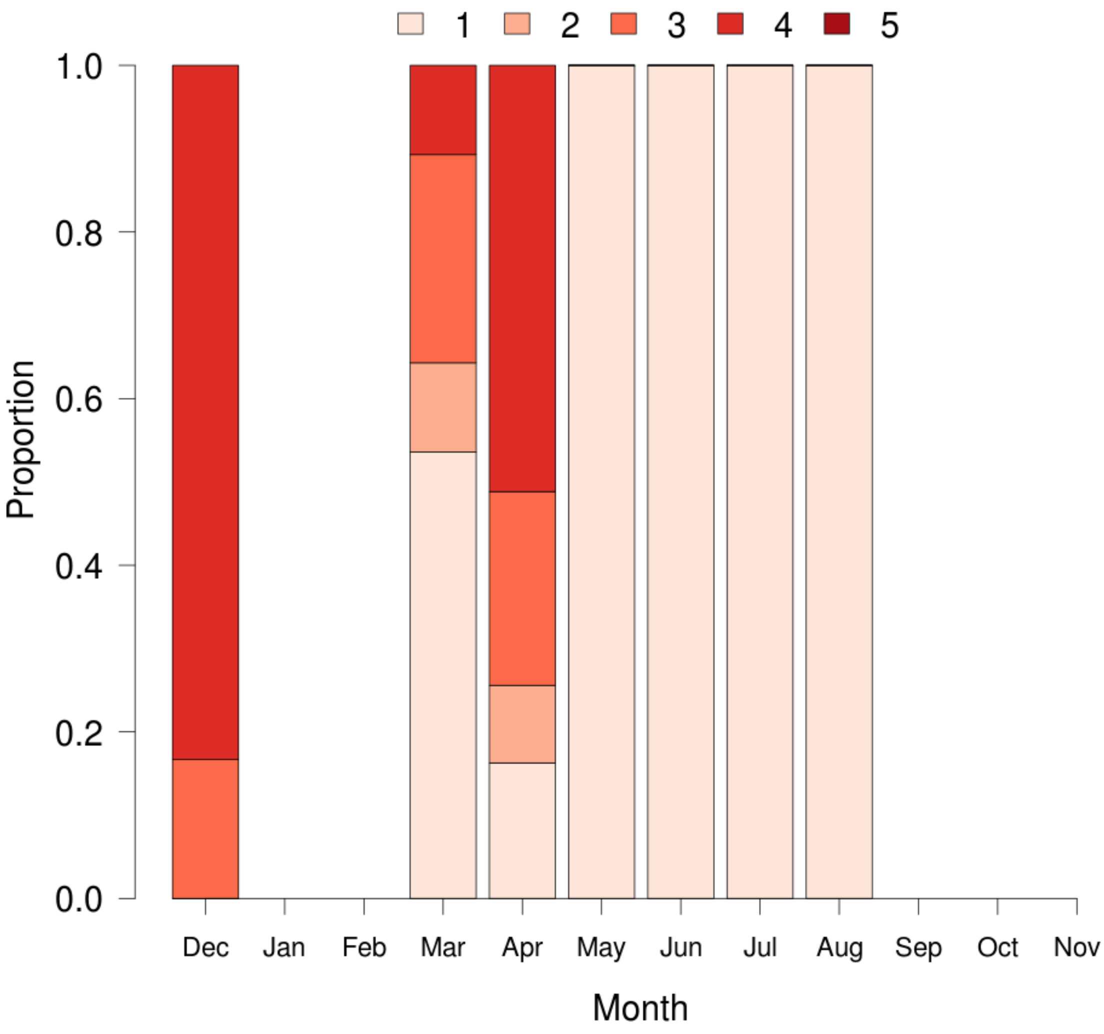


Figure 7. Monthly proportion of testis development stages for male yellowfin tuna.

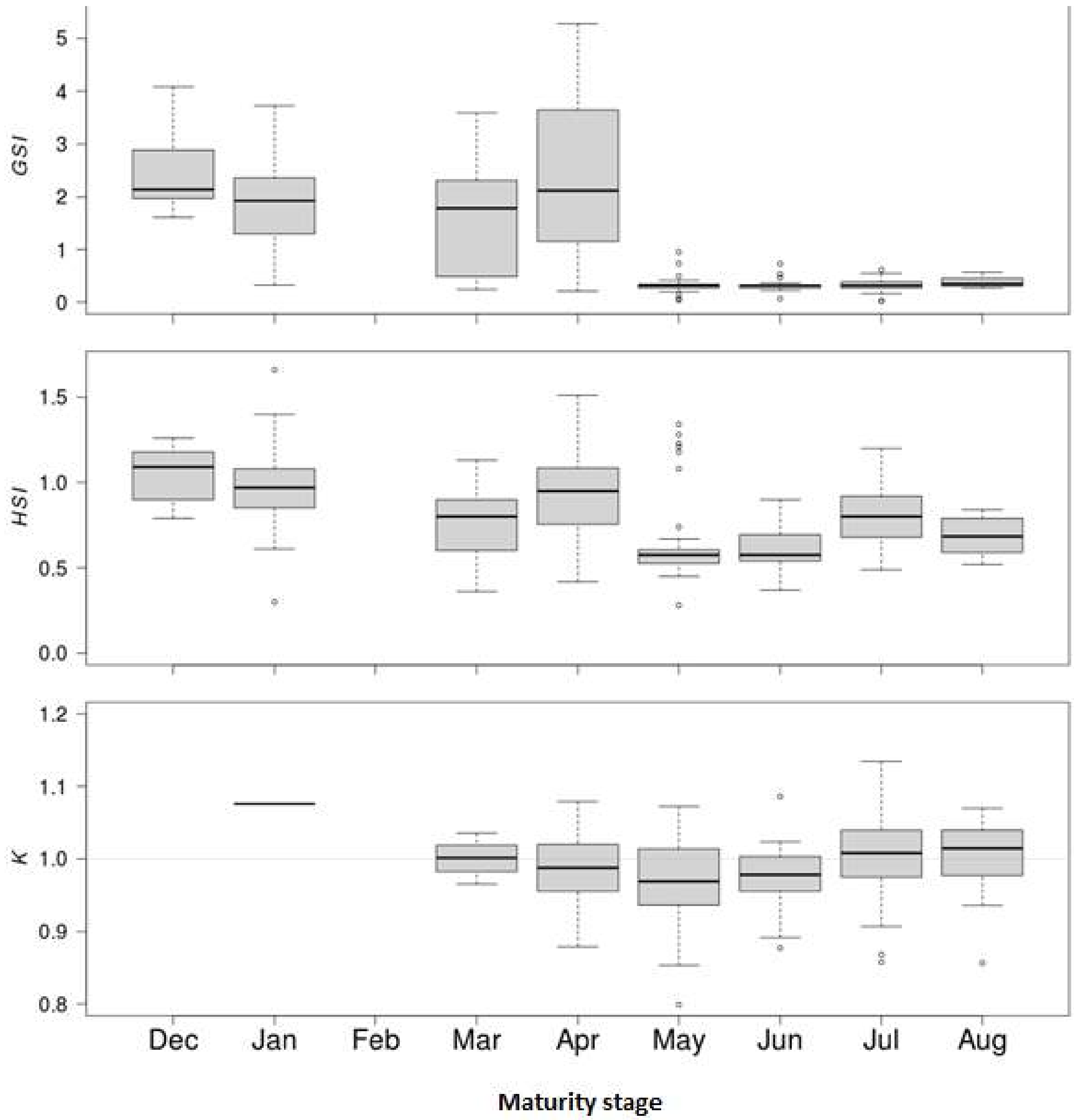


Figure 8. Seasonal variation of GSI, HSI and K for female yellowfin tuna caught by purse seiner in the Eastern Atlantic Ocean. Vertical bars indicate the standard error of the mean.

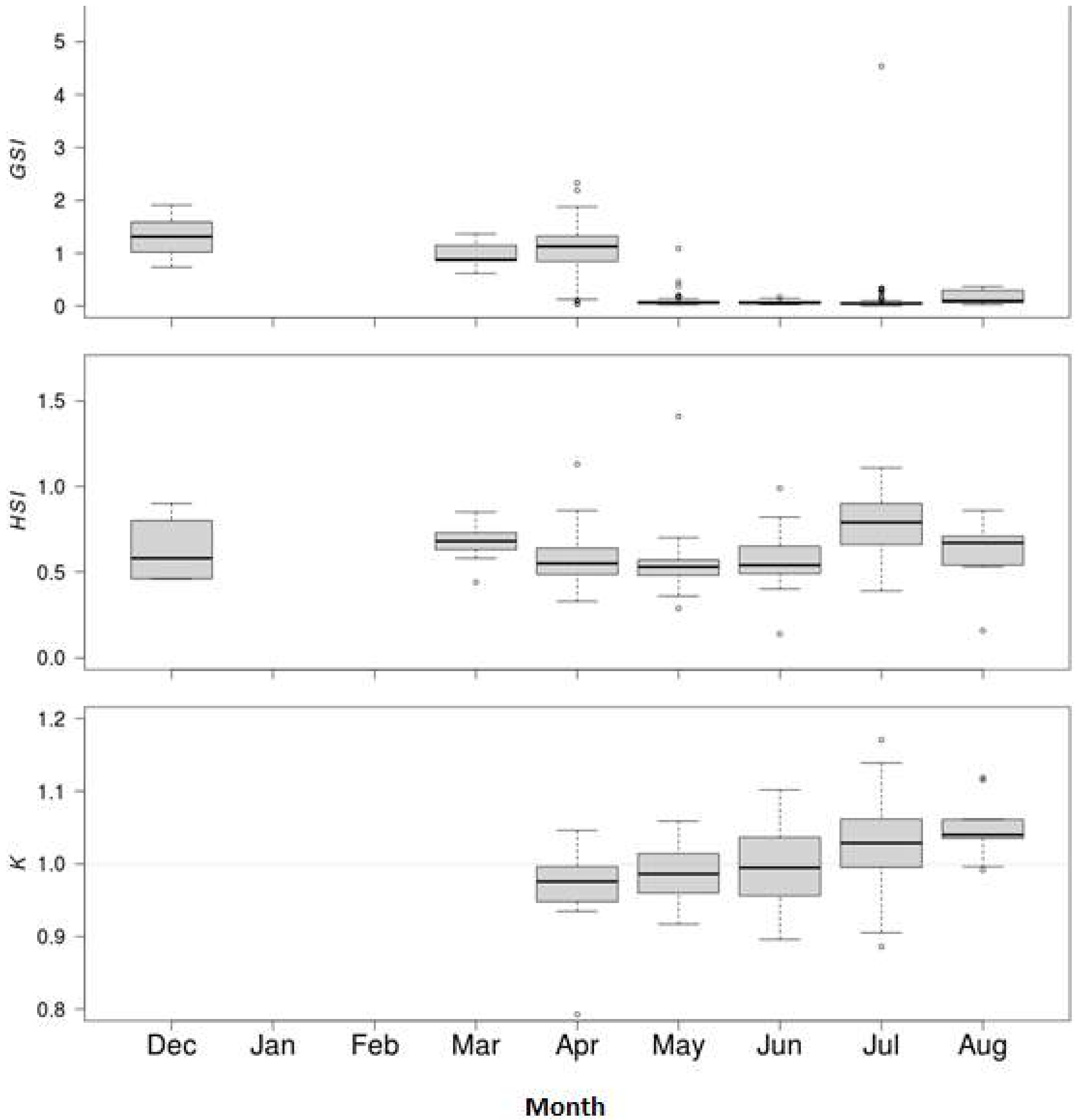


Figure 9. Seasonal variation of GSI, HSI and K for male yellowfin tuna caught by purse seiner in the Eastern Atlantic Ocean. Vertical bars indicate the standard error of the mean.

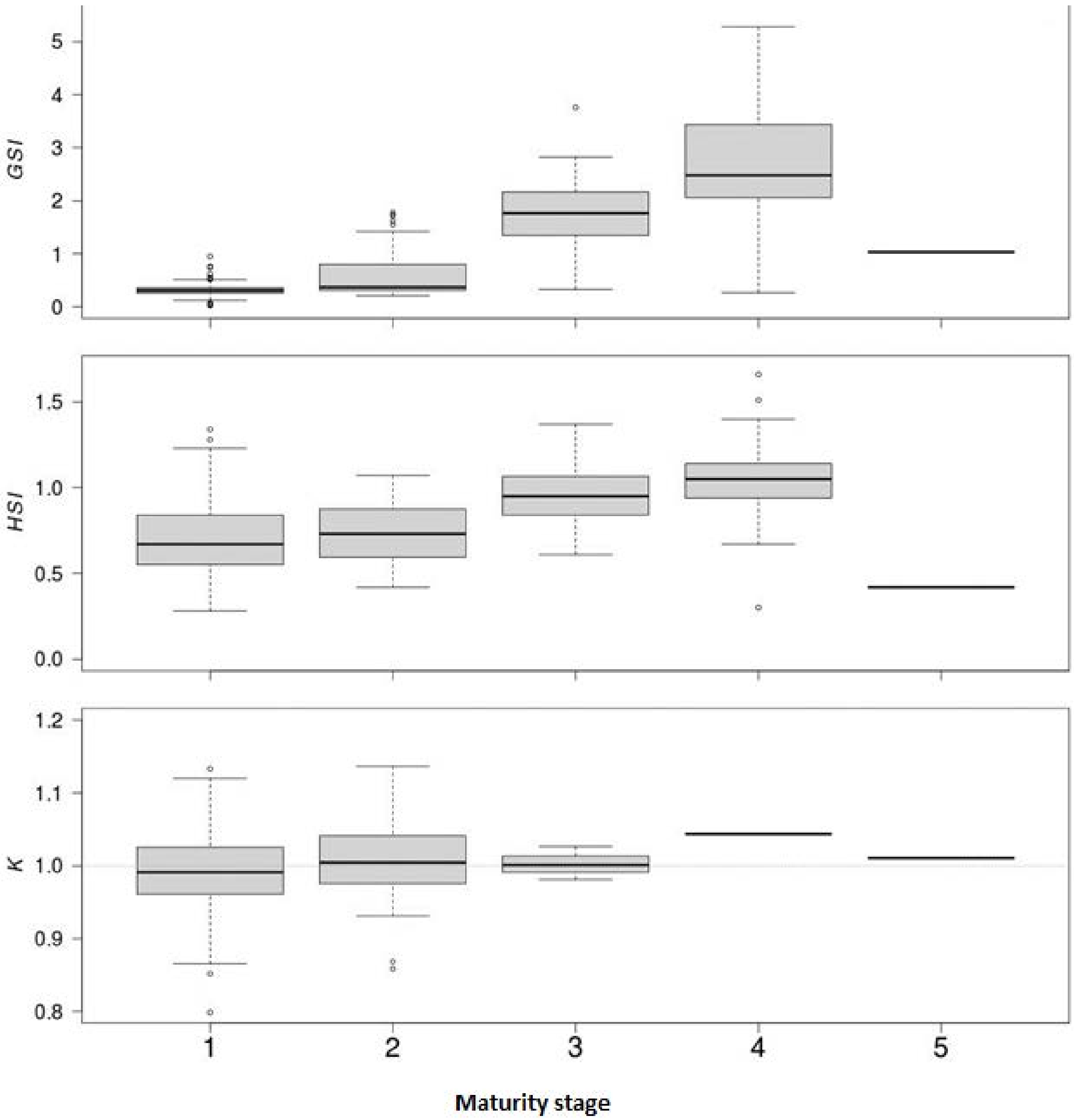


Figure 10. Variation of GSI, HSI and K by different ovarian development stages for female yellowfin tuna caught by purse seiner in the Eastern Atlantic Ocean. Vertical bars indicate the standard error of the mean.

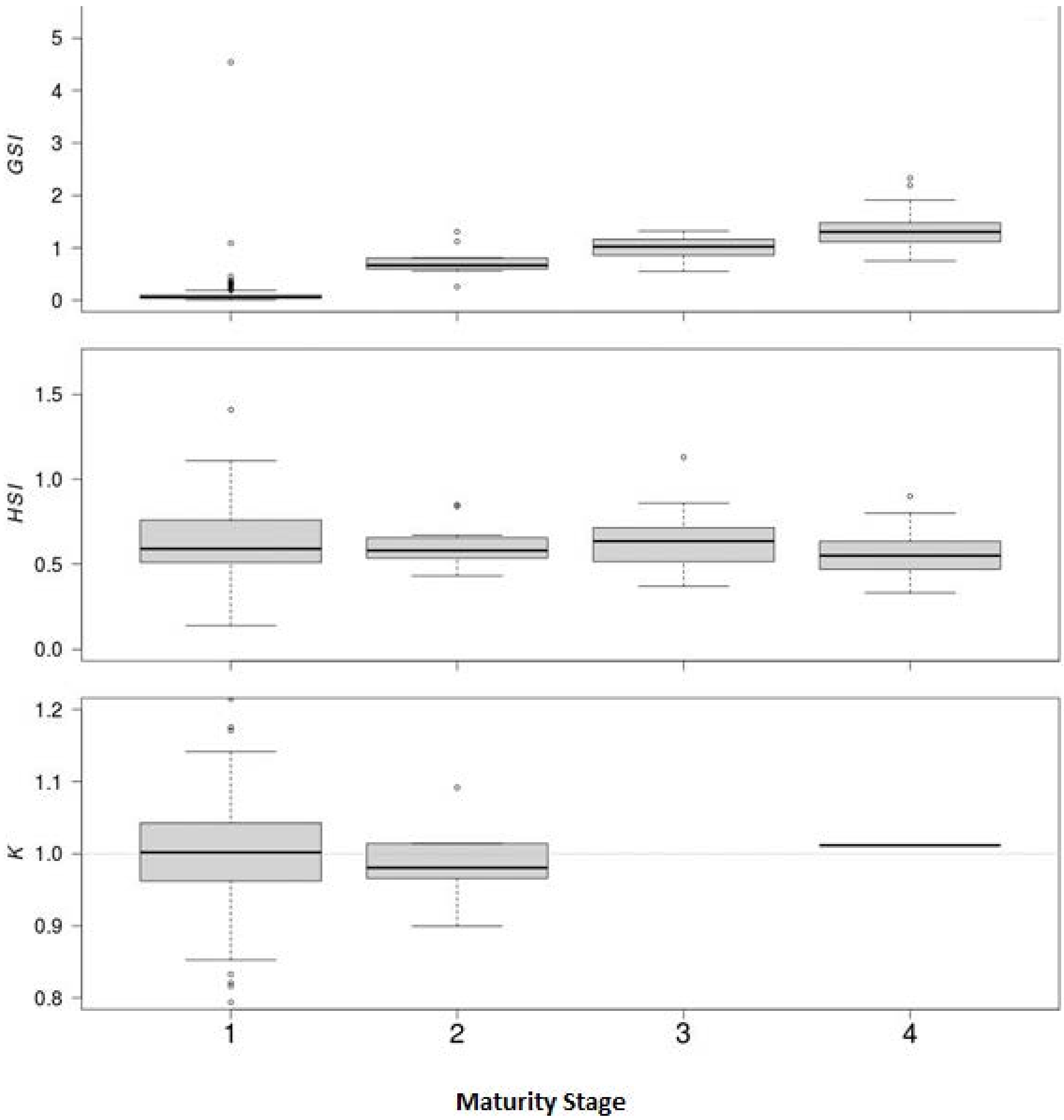


Figure 11. Variation of GSI, HSI and K by different testis development stages for male yellowfin tuna caught by purse seiner in the Eastern Atlantic Ocean. Vertical bars indicate the standard error of the mean.

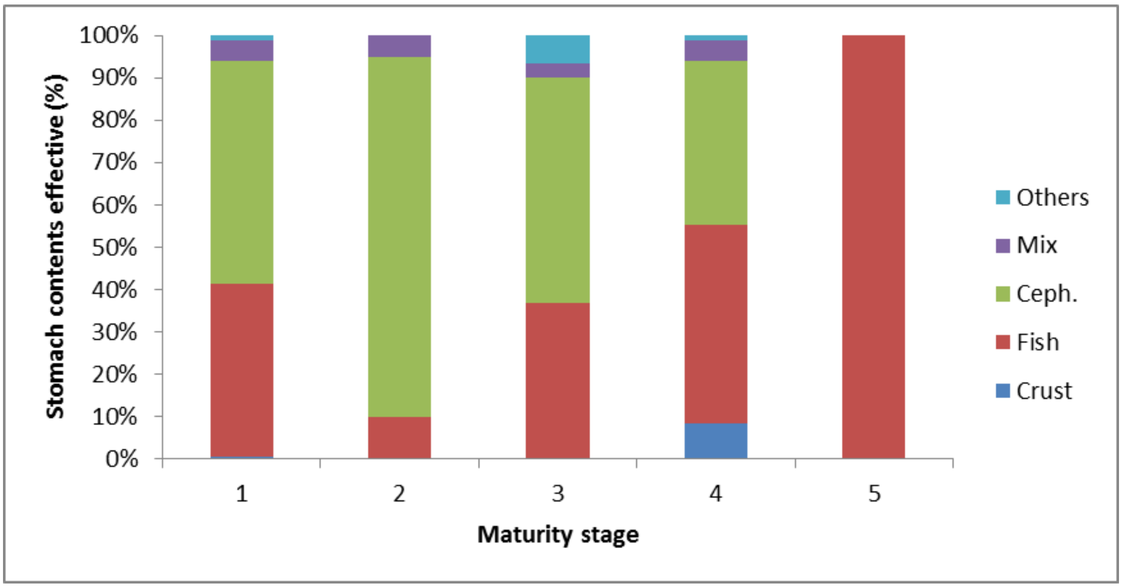


Figure 12. Percentage of different prey groups identified in the stomachs of female yellowfin tuna by ovary maturity stages in the Eastern Atlantic Ocean.

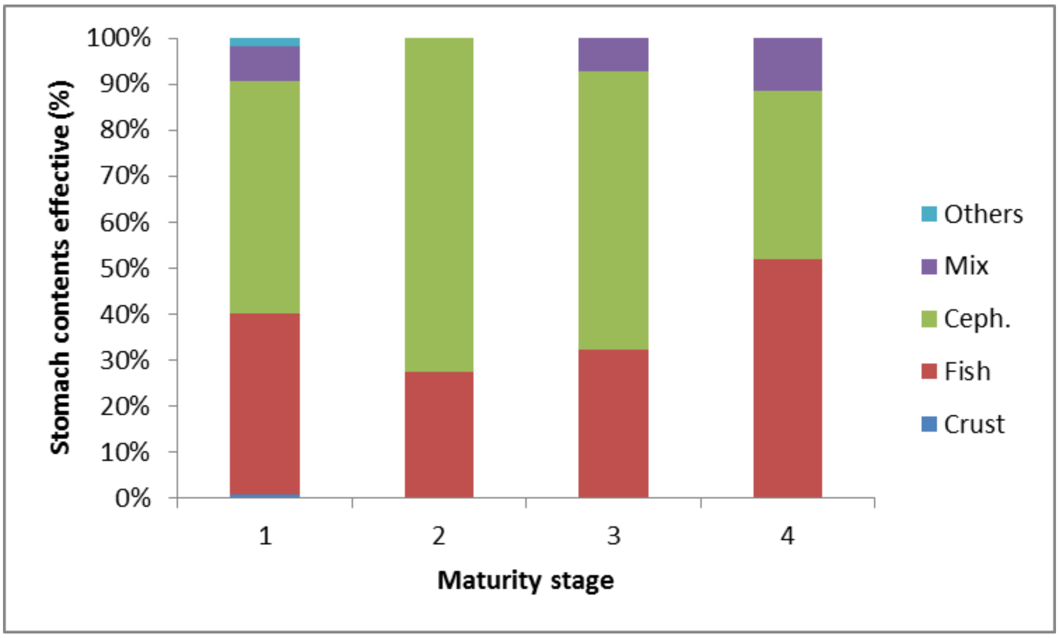


Figure 13. Percentage of different prey groups identified in the stomachs of male yellowfin tuna by testis maturity stages in the Eastern Atlantic Ocean.