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BIOLOGICAL ASPECTS OF LITTLE TUNNY *EUTHYNNUS ALLETTERATUS* FROM SPANISH AND PORTUGUESE WATERS

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SUMMARY

This study provides information on some biological aspects of Euthynnus alletteratus *from the western Mediterranean (Spanish coast) and in the Atlantic Ocean (south of Iberian Peninsula). A total of 1266 individuals were measured between 2003 and 2017. The L-W relationship was calculated with W equal to 0.01242 $FL^{3.058}$. Histological analysis of the ovaries and the monthly variation of the gonadosomatic index for both sexes suggested that the spawning season for* Euthynnus alletteratus *in the western Mediterranean Sea takes place from June to August. The lengths at first maturity (L_{50}) were estimated to be 50.1 cm and 43.4 cm FL for female and male, respectively. Age at first maturity (A_{50}) was calculated.*

RÉSUMÉ

*Cette étude fournit des informations sur quelques aspects biologiques de la thonine commune (*Euthynnus alletteratus*) de l'ouest de la Méditerranée (côte espagnole) et de l'océan Atlantique (sud de la péninsule ibérique). Un total de 1.266 spécimens a été mesuré de 2003 à 2017. La relation taille-poids a été calculée selon W = 0,01242 $FL^{3,058}$. Des analyses histologiques des ovaires et la variation mensuelle de l'indice gonado-somatique des deux sexes suggéraient que la saison de frai de la thonine commune (*Euthynnus alletteratus*) dans l'ouest de la Méditerranée avait lieu de juin à août. Il a été estimé que les tailles à la première maturité (L50) s'élevaient à 50,1 cm FL pour les femelles et 43,4 cm FL pour les mâles. L'âge à la première maturité (A_{50}) a été calculé.*

RESUMEN

Este estudio proporciona información sobre algunos aspectos biológicos del Euthynnus alletteratus *del Mediterráneo occidental (costa española) y el océano Atlántico (al sur de la península Ibérica). Desde 2003 hasta 2017 se midieron en total 1.266 ejemplares. La relación L-W se calculó con W igual a 0,01242 $FL^{3,058}$. Los análisis histológicos de los ovarios y la variación mensual del índice gonadosomático para ambos sexos sugería que la temporada de desove para el* Euthynnus alletteratus *en el mar Mediterráneo occidental se produce de junio a agosto. Se estimó que las tallas de primera madurez (L_{50}) eran 50,1 cm FL para las hembras y 43,4 cm para los machos. Se calculó la edad de primera madurez (A_{50}).*

KEYWORDS

Euthynnus alletteratus, *L-W relationship,
sex ratio, sexual maturity, spawning season, L_{50}, A_{50}*

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1. Introduction

Little tunny *Euthynnus alletteratus* is widely distributed in Atlantic tropical and subtropical waters and Mediterranean Sea. This study examines various aspects of the biology of *Euthynnus alletteratus* sampled during 15 years in the western Mediterranean (Mediterranean Spanish coast) and in the Atlantic Ocean (south of Portugal and Spain). The study aims were to examine the length-weight relationship, sex ratio, macroscopic and microscopic maturity of gonads, spawning season, and length and age at 50% maturity.

2. Material and Methods

2.1. Sampling collection

Fresh individuals of *Euthynnus alletteratus* (Little tunny, LTA) were collected from 30 localities (corresponding to 20 squares of a 1x1 degrees grid) throughout the Atlantic Ocean and Mediterranean Sea between 2003 and 2017 (**Figure 1**).

Individuals were caught in the Mediterranean Sea (Spanish coast) and the Atlantic Ocean (south of the Iberian Peninsula) in a variety of fishing gears (**Table 1**). In decreasing order according to the number of samples collected: trap fisheries (TRAP); recreational fisheries (TROL); longline fisheries (mainly targeting *Thunnus alalunga* (LLALB)), purse seine (PS) (targeting coastal species, mainly sardine and anchovy); longline home-based (LLHB); trammel net (TN) targeting small coastal species, and bottom or deep longliners (BLL). On the Portuguese coast all individuals sampled were caught by TRAP.

Fork length (FL) was measured either to the nearest 0.1 cm or to the nearest 0.5 cm (fish measured on longline vessels). Total body weight (W) also referred as Raw Weight (RW) was measured to the nearest gram. A total of 1266 individuals were both measured and weighted. The length-weight relationship was calculated using a two-parameter power function with a multiplicative error term (Froese, 2006):

$$W_i = \alpha L_i^{\beta} e^{\epsilon_i}$$

The model is linearised by means of a logarithmic transformation, which has the added benefit of making the errors additive and stabilizing the variances about the model. Simple linear regression methods are used to fit the relationship between $\log_e(W)$ and $\log_e(L)$.

$$log_e(W_i) = log_e(\alpha) + \beta \log(L_i) + \epsilon_i$$

A total of 1261 measured fish were sampled to identify their sex: male (n = 414), female (n = 461), undetermined individuals (n = 384), and intersex (n = 2). Undetermined individuals are those with small gonads, which sex cannot be identified macroscopically (visually), and intersex fish are those with gonads that presented both testicular and ovarian tissue (for more information see Macías et al., 2014). Sex ratio was calculated as the proportion of females by length class.

Maturity stages of gonads (visual stating method) were assigned using similar criteria to those developed for *Sarda sarda*, *Euthynnus alletteratus* and *Scomberomorus tritor* Diouf (1981). The five macroscopic stages are: (1) Immature (virgin); (2) Developing; (3) Spawning capable; (4) Spawning; (5) Regressing and Regenerating (see description of stages in **Table 2**). Some doubts came up when staging and so intermediate stages were assigned. These intermediate stages: immature or developing (1−2) and immature or regressing/regenerating (1−5) were not included in the analysis of the length at 50% maturity (L_{50}).

Weights of the fresh gonads (GW) were recorded to the nearest 0.01 gram. A 2−3 cm cross-section from the central part of the right or left lobe was cut and fixed in Bouin’s fluid for four hours, and then preserved in 70% ethanol. For small gonads, the whole gonad was preserved. Gonadosomatic index (GSI) was calculated for males and females to identify the spawning periods according to the equation (Gibson and Ezzi, 1980):

$$GSI = GW / (W - GW) \times 100$$

That is, the GSI was calculated from the ratio of gonad weight (GW) to fish gonad-free weight (W - GW) times 10^2; GW and W, both in grams.

2.2. Histological processing and microscopic maturity staging of gonads

A representative portion of the preserved gonad tissue was dehydrated in ascending concentrations of ethanol, cleared with n-butanol, and embedded in paraffin. Sections were cut at 10 µm and stained with Mallory's trichrome stain. Microscopic maturity stating of gonads was based on a modification of the criteria of Schaefer (1998) and Farley *et al*. (2013).

Females: the most advanced group of oocytes (MAGO) was determined for each ovary; primary growth, cortical alveolar (lipid-stage), early vitellogenic, mid vitellogenic, advanced vitellogenic, migratory nucleus and hydrated oocyte stages (see description of oocyte stages in **Table 3**). Ovarian stages were assigned based on the developmental stage of the MAGO, the presence/absence of postovulatory follicles (POFs), the level of alpha and beta atresia of vitellogenic oocytes, the presence/absence of late stages of atresia (gamma/delta) and, the observation of the thickness of the *tunica albuginea* (gonad wall). Six ovarian stages were defined: (1) immature (virgin); (2) Developing which included two substages (2a) LP- Developing, if cortical alveolar oocytes as the MAGO are present and (2b) Developing, when early vitellogenic oocytes as the MAGO are present; (3) Spawning capable; (4) Spawning; (5) Regressing and regenerating (postspawning), with two substages, (5a) Regressing and (5b) regenerating and finally, the stage (6) Abnormal (**Table 4**).

Males: the four cellular stages (spermatogonia, spermatocytes, spermatids, and spermatozoa) were differentiated and recorded. Testes stages were assigned based on: the relative abundance of cysts containing the several cellular stages, the presence or absence of spermatozoa within seminiferous tubules, and the amount of sperm (when present) within the central longitudinal sperm duct (vas deferens) (**Table 5**).

2.3. Length and age at 50% maturity

Length and age at maturity are important life history parameters that are often used when assessing stock status and estimating spawning stock biomass or reproductive potential. They are typically obtained by examining gonads using macroscopic or histological techniques to determine maturity status of individuals and then applying statistical models to determine the proportion mature as a function of length or age (Chen and Paloheimo, 1994).

Macroscopic maturity data of both female and male and by sex were used to estimate the length at 50% maturity (L_{50}) (i.e. the length at which 50% of individuals are mature). Fish were classified as either immature (stage 1) or mature (stages 2-5).

Additionally, L_{50} was estimated using the microscopic maturity data of female. Fish were classified as either immature or mature depending on the criteria considered. In tuna studies immature females are those with previtellogenic (primary growth and cortical alveolar oocytes) or early vitellogenic oocytes (Schaefer, 1998; Farley et al., 2013), that is, stages 1, 2a and 2b. In contrast, in other fish studies, females whose ovaries contained cortical alveolar oocytes as the MAGO, are considered to be mature (Brown-Peterson et al., 2011; ICES, 2014), i.e only ovaries at stage 1 are considered immature. Therefore in order to assess possible implications in the future assessment, in the present report three calculation of L_{50}, based on microscopic examination of ovaries, were estimated on the basis of the criteria given in **Table 6**.

L_{50} was calculated by fitting the proportion of mature females by 2-cm size classes to a logistic equation, assuming a binomial error distribution, to model the probability of maturity (*p*) by length. Since the relationship is not linear (primarily due to the constraint that the probability is between 0 and 1), the logistic regression approach transforms *p* to obtain a linear equation. The required transformation is the *logit* transformation. From this transformation a linear model is obtained:

$$log_e\left(\frac{p}{1-p}\right) = \alpha + \beta\ FL$$

Confidence intervals for the parameters of the logistic regression were estimated via bootstrapping. A thousand bootstrap samples (with replacement) were used to derive the sampling distribution of the statistic of interest by means of the bootstrap percentile interval (the empirical quantiles are used to form a confidence interval for the parameter of interest). The 95% bootstrap confidence interval for a parameter was based on the values of the parameter estimate that had ±2.5% of bootstrap sample values (i.e., the 2.5% and 97.5% quantiles of the parameter estimates).

The growth parameters to estimate the age at 50% maturity (A_{50}), i.e. the age at which 50% of individuals are mature, were obtained from the literature review (Kahraman and Oray, 2001; Hattour, 2009). The values of the growth parameters are shown in (**Table 7**).

3. Results and Discussion

3.1 Length-Weight relationship

Length–weight relationship ($W = aL^b$) was developed for *Euthynnus alletteratus* (LTA). The sample size, the range of fork length and weight, on which the L-W relationships is based, the estimated parameters (a and b) and R^2 value are presented in **Table 8** and **Figure 2**. The relationship between the two variables was significant ($p < 0.001$). The slope (allometry coefficient) was higher than 3, indicating a positive allometric growth, that is, fish became more rotund as length increases.

3.2. Sex ratio

The size of males ranged between 30.6 and 101.0 cm FL (n = 414) and females ranged between 28.5 and 99.0 cm FL (n = 461). Significant differences in the proportion of females respect to males were found between length classes (p <0.001). The analysis showed that males were more abundant in the larger length classes (> 96 cm FL). Predicted sex-ratio (% females) by length (FL, cm) is shown in **Figure 3**.

3.3 Maturity classification of gonads

A total of 1232 fish measured (FL, cm) were macroscopically classified. A total of 580 fish (47.1%) were immature (stage 1); 628 fish (51.0%) were mature (stages 2 – 5); and 24 fish (1.9%) were classified as immature or mature (i.e. immature or developing (1−2) and immature or regressing/regenerating (1−5)), which were not included in the analysis of the length at 50% maturity (L_{50}). A total of 20 gonads out of the 24 fish were further histologically processed in order to determine the maturity status.

3.4 Microscopic examination of the gonads

A total of 282 gonads were microscopically examined. The number of individuals by microscopic maturity stages is given in **Table 9**. A total of 27 gonads of undetermined individuals were histologically processed in order to determinate their sex, 22 of them were successfully identified as male or female. After microscopic examination of the 20 gonads macroscopically classified as immature or mature (1−2 and 1−5) it was determined that 5 of them were immature, 14 were mature and 1 remained uncertain.

3.5 Spawning season

The gonadosomatic index (GSI) values were calculated monthly for both mature males and females by stock (Atlantic and Mediterranean).

The monthly variation in mean GSI values of mature males and females in the Mediterranean Sea suggest that the spawning season for LTA is between June and August, with the highest values in July (9.75 for female and 8.20 for male). Mean GSI values decreased sharply from September to December (**Figure 4**). Histological analysis of ovaries confirmed that spawning activity occurred from June to August (**Figure 5**). Our results are in line with other studies conducted in the Mediterranean Sea in (Hajjej et al., 2010; Kahraman et al., 2008; Mohamed et al., 2014).

Unfortunately, few samples of LTA in the Atlantic were collected; therefore spawning season for this species cannot be assessed (**Figures 4 and 5**).

3.6 Length at 50% maturity

A total of 1208 LTA were macroscopically classified as immature (48%) or mature (52%). The estimated length at which 50% of LTA is mature was 51.13 cm FL (*50.07 cm - 52.20 cm*, 95% confidence interval). The estimated length at which 90% of LTA is mature was 65.23 cm FL (*63.11 cm - 67.26 cm*, 95% confidence interval). Logistic fit for the proportion of mature LTA is shown in **Figure 6**.

Significant differences in the logistic regressions between sexes were found ($p = 0.002$). The estimated L_{50} were 50.07 cm FL (*48.31 cm - 51.64* cm, 95% confidence interval) for females and 43.44 cm FL (*36.89 cm - 47.34* cm, 95% confidence interval) for males. The estimated L_{90} were 65.24 cm FL (*62.16 cm - 68.65 cm*, 95% confidence interval) and 69.70 cm FL (*65.30 cm - 75.34* cm, 95% confidence interval) for female and male, respectively. These results show that LTA males reached the size at first maturity at smaller sizes than females; however the estimated L_{90} is smaller for females. Logistic fit for the proportion of mature LTA by sex is shown in **Figure 7**.

The histological analysis of LTA ovaries showed that 69 (36%) were at stage 1 (immature), 53 (28%) at stage 2a (LP-developing), 30 (8%) at stage 2b (developing) and, 39 (20%) at stages 3-5b (mature individuals in all of the three criteria considered). Logistic regression models show different slopes ($p = 0.020$) with equal intercepts ($p = 0.395$), which indicates that there is significant difference in the logistic regressions among microscopic maturity scenarios. L_{50} was estimated to be 53.19 cm FL when females with ovaries at stage 2a (ovaries containing cortical alveolar as MAGO) and onward were considered mature. This estimate noticeable increased to 68.74 when the second, and 83.33 cm FL when the third, criteria were applied (i.e., when the maturity threshold was defined as at stages 2b and 3, respectively) (**Figure 8**).

The estimated L_{50} according to macroscopic and microscopic criteria is shown in **Table 10**.

The present study showed that 50% of LTA attained first maturity at 51.13 cm FL. This value is higher than those reported in other Mediterranean areas, namely the Tunisian (44.8 and 42.8 cm FL for female and male, respectively) or Egyptian (42 cm FL) waters (Hajjej et al., 2010; Mohamed et al., 2014). In contrast, other studies reported that LTA reached maturity in larger size classes, between 57 and 65 cm FL (Rodríguez-Roda, 1966; Kahraman et al., 2008).

3.7 Age at 50% maturity

The age at first maturity (A_{50}) was estimated using size-age relationships from the literature review (**Table 7**). Resultant ages at 50% maturity are shown in **Table 11**. From our point of view, the growth parameters obtained by Hattour (2009) are in line with the knowledge about the reproductive biology of the species, that is, LTA would reach maturity at 2 year.

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Table 1. Number of *Euthynnus alletteratus* collected between 2003 and 2017 in the Atlantic Ocean (ATL) and Mediterranean Sea (MED) by gears (sorted in decreasing order). Trap fisheries (TRAP), recreational fisheries (TROL), longline targeting *Thunnus alalunga* (LLALB), purse seine (PS) targeting coast species mainly sardine and anchovy, longline home-based (LLHB), trammel net (TN) targeting small coast species, bottom or deep longliners (BLL).

Gear	ATL	MED	Total	(%)
TRAP	53	684	737	(57.1)
TROL	3	335	338	(26.2)
LLALB		93	93	(7.2)
PS		84	84	(6.5)
LLHB		26	26	(2.0)
TN		11	11	(0.9)
BLL		2	2	(0.2)
Total	56	1235	1291	

Table 2. Maturity stages of gonads (visual stating method). Based on the criteria of Diouf (1981) (Collect. Vol. Sci. Pap. ICCAT, 15 (2): 327–336).

Sex / Reproductive stage	Macroscopic criteria
Undetermined: Immature (virgin)	Gonads are very small (thread-like). Sex not distinguished by naked eye.
Females	
I. Immature (virgin)	Gonads are small and cylindrical in shape; more or less translucent-pinkish.
II. Developing (early developing)	Gonads are increasing in size; orange, pink or reddish colour. External blood vessels start to develop around the gonads (vascularisation).
III. Spawning capable (late developing)	Gonads are well developed; yellow -orange colour. Opaque oocytes are visible.
IV. Spawning	Gonads are greatly enlarged; orange –reddish colour, with conspicuous superficial blood vessels. Large translucent hydrated oocytes visible if those are presented.
V. Regressing and regenerating (postspawning)	Gonads are bloody and flaccid, show a wrinkled wall; reddish colour.
Males	
1. Immature (virgin)	Gonads are small, thin and flattened; more or less translucent – lightly pink.
2. Developing (early developing)	Gonads are increasing in size and triangular in cross section; orange, whitish - pinkish colour.
3. spawning capable (late developing)	Gonads are well developed; whitish - pinkish colour. Accumulation of sperm in the spermatic ducts, under pressure sperm is expelled.
4. Spawning	Gonads are greatly enlarged with conspicuous superficial blood vessels; pinkish colour. Large amount of sperm, under very lightly pressure sperm is expelled.
5. Regressing and regenerating	Gonads are bloody and flaccid, show a wrinkled wall; reddish colour.

NOTE: in Diouf (1981) stage II refers to Developing or Regenerating stages. Sometimes, at the early beginning of the spawning season, these mentioned stages are difficult to distinguish in individuals that spawned in the previous spawning season.

Table 3. Description of the oocyte stages in ovaries according to their histological characteristics (Saber et al., 2015).

Stage oocytes	MAGO. Histological characteristics
*Primary growth oocytes (PG)	In an early phase: homogeneous basophilic cytoplasm with no cytoplasmic inclusions.
*Cortical alveolar (CA) or lipid-stage oocyte (LP)	Small lipid droplets in the cytoplasm but still no yolk granules.
Primary or early vitellogenic oocytes (VT1)	Yolk granules in the periphery of the cytoplasm and lipid droplets occupy more of the cytoplasmic area than the yolk granules;
Secondary or mid vitellogenic (VT2)	Yolk granules and lipid droplets are spread throughout the cytoplasm.
Tertiary or advanced vitellogenic (VT3)	Larger yolk granules than that of VT2 stage oocytes. Lipid droplets fuse and are distributed around the nucleus.
Migratory nucleus (MG)	Lipid droplets fuse into 1−3 large droplets. Migration of the nucleus toward the animal pole. In a later phase, yolk granules fuse progressively.
Hydrated (HY)	The nucleus has disintegrated. All yolk granules fuse into a homogeneous yolk mass and the oocyte increases in size due to hydration. The oocyte is still surrounded by the follicle layer, i.e. ovulation has yet not taken place.

* Commonly PG and CA oocyte stages in tuna species are reported as one oocyte stage called ‘unyolked oocytes’ or ‘previtellogenic oocytes’.

* According to oogenesis studies in bluefin tuna (*Thunnus thynnus*) cortical alveoli appears after the lipid droplets (Abascal and Medina, 2005; Sarasquete et al., 2002). For this reason, the term lipid-stage oocyte is used instead of cortical alveolar stage in studies of tuna species (Corriero et al., 2003; Figueiredo et al., 2008; Aragón et al., 2010).

Table 4. Microscopic classification criteria for females based on a modification of the criteria of Schaefer (1998) and Farley *et al*. (2013).

Maturity stage. Females	Microscopic characteristics
1. Immature (virgin)	Only oogonia and primary growth oocytes present. No atresia. Absence of POFs. Thin ovarian wall and little space between oocytes.
2. Developing	
2a. LP-Developing	Lipid-stage oocytes present as MAGO. Some atresia may be present. Absence of POFs.
2b. Developing	Early vitellogenic oocytes present as MAGO. Some AT may be present. Absence of POFs.
3. Spawning capable	Mid or advanced vitellogenic oocytes present as the MAGO. Atresia (<50%) can be present. Absence of POFs.
4. Spawning	POFs present and /or migratory nucleus or hydrated oocytes present as the MAGO. Atresia, when present at all, only in limited amounts.
5. Regressing and regenerating	
5a. Regressing	In an early phase: cortical alveolus or early vitellogenic oocytes as the MAGO. Abundant alpha and/or beta atresia. Absence of POFs. In a latter phase: cortical alveolus oocytes as the MAGO. Abundant beta atresia. Absence of POFs. Disorganization of ovary structures, with some spaces. Thick and/or wrinkled gonad wall is observed (in some ovaries). Only primary growth oocytes as the MAGO present, with some spaces. Absence of POFs. Late stages of atresia. Thick and/or wrinkled gonad wall is observed (in some ovaries).
5b. Regenerating	At the early beginning of the spawning season. Difficulties are found to distinguish between this stage and LP-developing (2a), that is, individuals that spawned in the previous spawning season and immature individuals developing for the first time.
6. *Abnormal	Intersex, both oocytes and sperm cells are present at the same time; sclerosis, the ovary is dominated by atretic oocytes and large amounts of connective tissue; infections; necrosis (atrophy).

*Abnormal stage was defined according to the International Council for the Exploration of the Sea (ICES) (ICES, 2014).

Table 5. Microscopic classification criteria for males based on a modification of the criteria of Schaefer (1998).

Maturity stage. Males	Microscopic characteristics
6. Immature (virgin)	Only spermatogonia present. No sperm in the sperm duct. Small space of lobule lumen.
7. Developing (early developing)	Spermatocytes, spermatids, and spermatozoa. Small space of lobule lumen.
8. spawning capable (late developing)	Abundant spermatids and some spermatozoa within seminiferous tubules. Sperm duct relatively full of sperm.
9. Spawning	Some spermatids and abundant spermatozoa. Greatly enlarged tubules, sperm duct full of sperm.
10. Regressing and regenerating	In regressing, residual spermatozoa. In regenerating stage only spermatogonia present.
11. *Abnormal	Intersex, both oocytes and sperm cells are present at the same time; sclerosis; infections; necrosis (atrophy).

*Abnormal stage was defined according to the International Council for the Exploration of the Sea (ICES) (ICES, 2014).

Table 6. Immature or mature females according to three criteria considered (microscopic maturity scenerios) to estimate L_{50}.

	Immature. Stages:	Mature. Stages:
Criterion mat_a	(1) Immature	(2a) LP-Developing (2b) Developing (3) Spawning capable (4) Spawning (5a) Regressing (5b) Regenerating
Criterion mat_b	(1) Immature (2a) LP-Developing	(2b) Developing (3) Spawning capable (4) Spawning (5a) Regressing (5b) Regenerating
Criterion mat_c	(1) Immature (2a) LP-Developing (2b) Developing	(3) Spawning capable (4) Spawning (5a) Regressing (5b) Regenerating

Table 7. The growth parameters (sexes combined) for *Euthynnus alletteratus* (LTA).

L_∞	k	t_0	area	Reference / (Reference number)	
117	0.19	-1.13	Med Tunisia	Hattour, 2009	(1)
123	0.13	-3.84	Med Turkey	Kahraman and Oray, 2001	(2)
128	0.11	-4.18	Aegean Turkey	Kahraman and Oray, 2001	(2)

Table 8. Number of fish measured and weighted; range of both fork length and weight; and estimated parameters of length–weight relationships for *Euthynnus alletteratus* (LTA).

N	FL (cm)	W (g)	Parameters L-W relationship ($W = a\ FL^b$)			
	Range	Range	a	b	SE (b)	R^2
1266	10.5 – 101.0	12 – 15000	0.01242	3.058	0.006	0.995

Table 9. Number of females and males by microscopic maturity stages for *Euthynnus alletteratus*.

Gonad stage	Undetermined	Intersex	Females	Males
Immature	5		69	9
LP-Developing			53	
Developing			30	45
Spawning capable			15	18
Spawning			19	1
Regressing				10
Regenerating			5	1
Abnormal		2		
Total	5	2	191	84

Table 10. Estimated length at first maturity (length at 50% maturity, L_{50}) using macroscopic (MACRO) and microscopic (MICRO) criteria for *Euthynnus alletteratus*. The estimated L_{50} based on microscopic examination of ovaries were calculated on the basis of three criteria: Mat_a, Mat_b and Mat_c (see **Table 6** for descriptions).

MACRO			MICRO (only femles)		
Sex combined	Females	Males	Mat_a	Mat_b	Mat_c
51.13	50.07	43.44	53.19	68.74	83.33

Table 11. Age at first maturity (A_{50}) estimated using size-age relationships for *Euthynnus alletteratus* from the literature review. Reference number: (1), Hattour, 2009; (2), Kahraman and Oray, 2001 (MT, refers to Mediterranean Turkey and AT, to Aegean Turkey). The values of length were estimated in the present study.

Species	Length	Sex	Length Value	Ref. # (1)	Ref. # (2) MT	Ref. # (2) AT
LTA	L50	both	51.13	1.89	0.29	0.46
LTA	L50 lower	both	50.07	1.81	0.18	0.33
LTA	L50 upper	both	52.20	1.98	0.41	0.58
LTA	L90	both	65.23	3.16	1.97	2.30
LTA	L90 lower	both	63.11	2.95	1.70	2.00
LTA	L90 upper	both	67.26	3.37	2.25	2.60

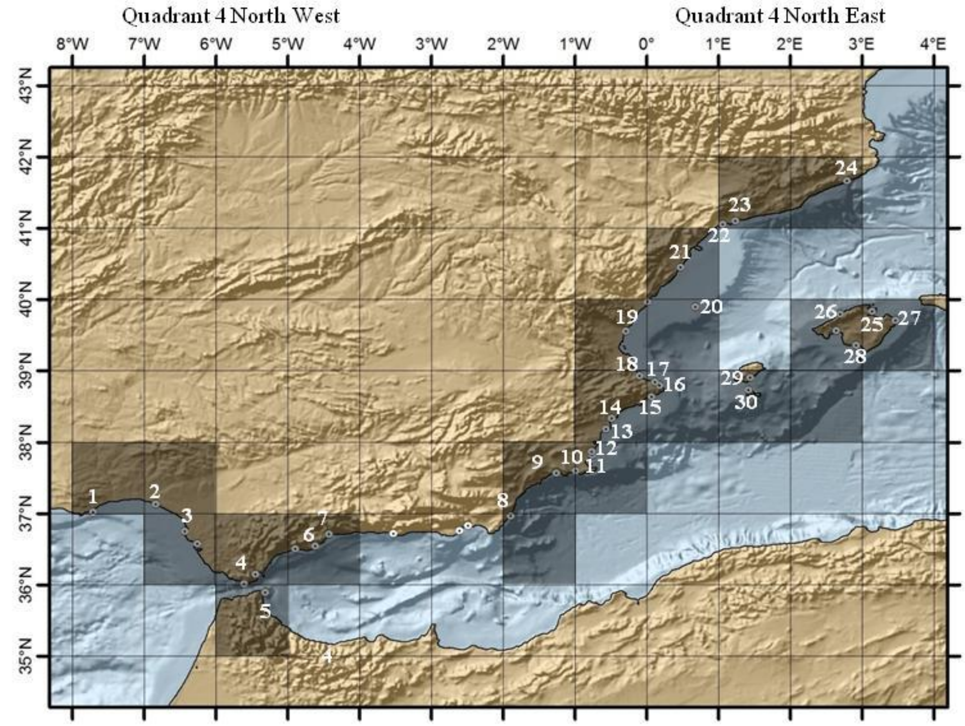

Figure 1. Colored grids 1x1 (squares of 1 by 1 degrees) indicating where BLT, BON and LTA were sample. Sampling period: 2003-2017. Ports: 1, Olhão; 2, Mazagón; 3, Chipiona; 4, Tarifa; 5, Ceuta; 6, Fuengirola; 7, Málaga; 8, Carboneras; 9, Mazarrón; 10, Cartagena; 11, San Pedro del Pinatar; 12, Torre de la Horadada; 13, Santa Pola; 14, Alicante; 15, Calpe; 16, Jávea; 17, Dénia; 18, Oliva; 19, Pobla de Farnals; 20, Islas Columbretes; 21, Vinaròs; 22, Cambrils; 23, Tarragona; 24, Blanes; 25, Alcúdia; 26, Sóller, 27, Cala Ratjada; 28, S'Estanyol; 29, Ibiza; 30, Formentera.
Geographical areas: Atlantic, 1 – 3; Gibraltar Strait, 4 – 5; Alboran Sea, 6 – 7; Mediterranean Sea, 8 – 30.

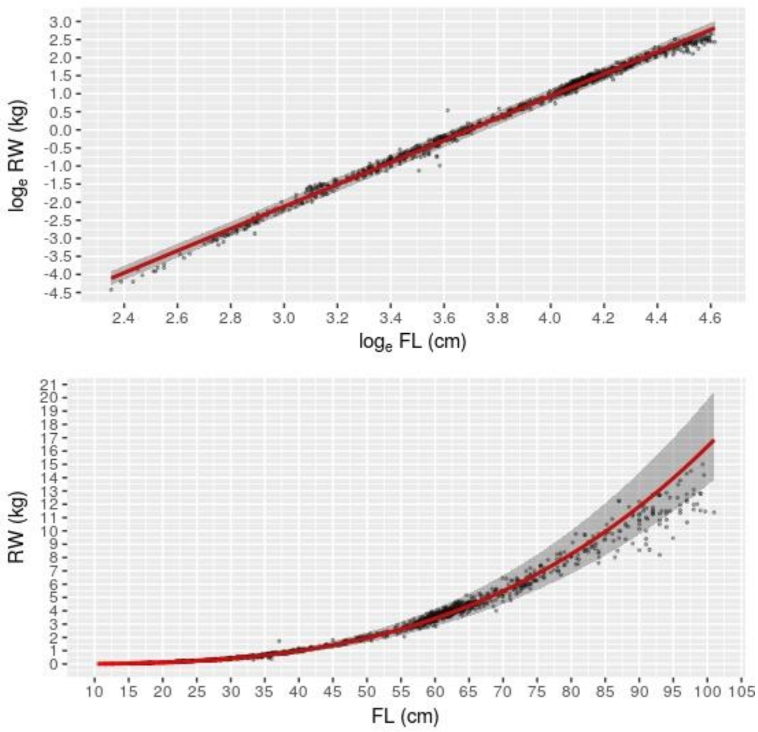

Figure 2: Estimated length-weight relationship for *Euthynnus alletteratus* (LTA) (upper panel, logarithmic scale; lower panel, linear scale). RW is Raw Weight and FL is Fork Length.

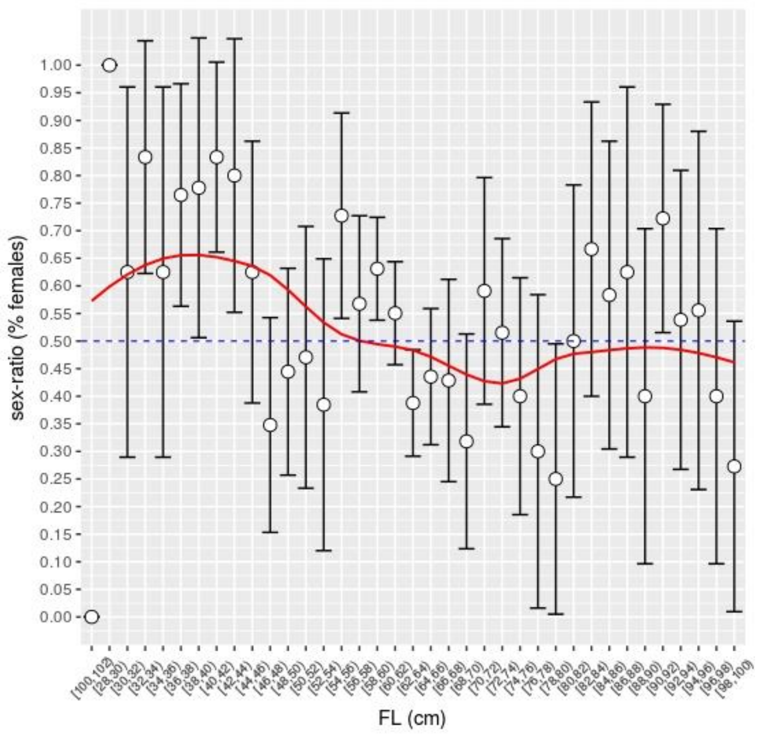

Figure 3. Predicted sex-ratio (% females) by length (FL, cm) for *Euthynnus alletteratus* (LTA) with 95% confidence interval (based on normal approximation).

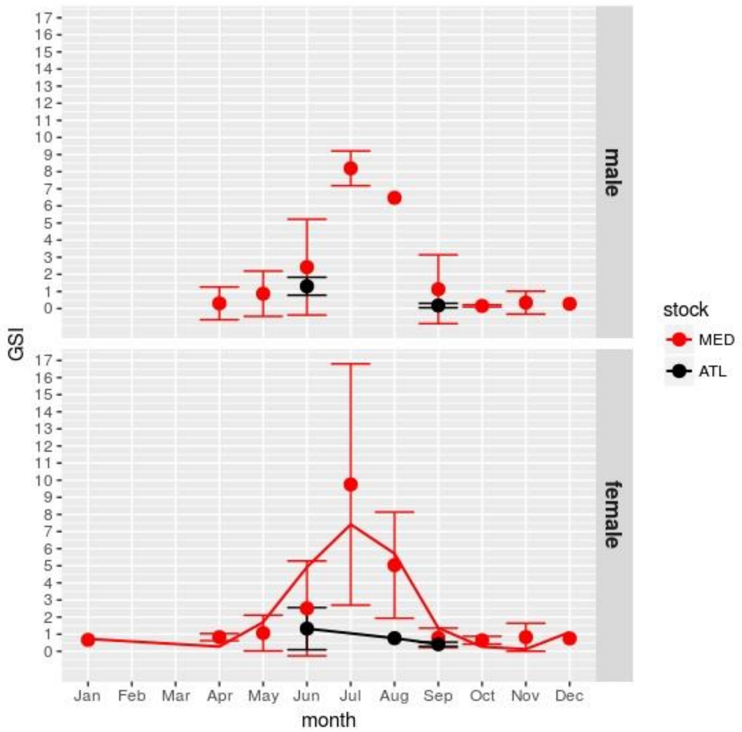

Figure 4. Monthly variation of the gonadosomatic index (GSI) for Mediterranean and Atlantic mature female and male *Euthynnus alletteratus* (LTA).

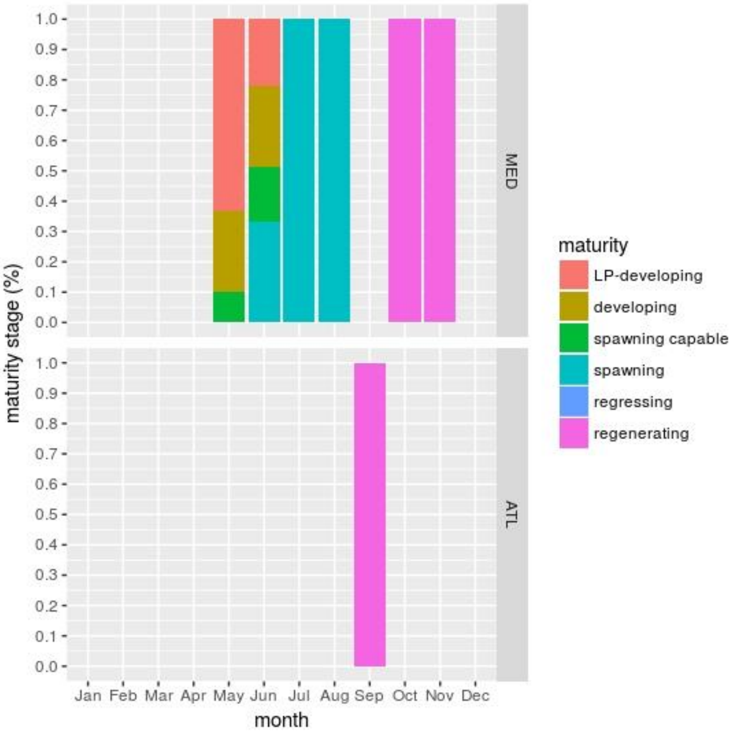

Figure 5. Monthly relative frequency of gonad stages (by microscopic examination) for Mediterranean and Atlantic mature female *Euthynnus alletteratus* (LTA).

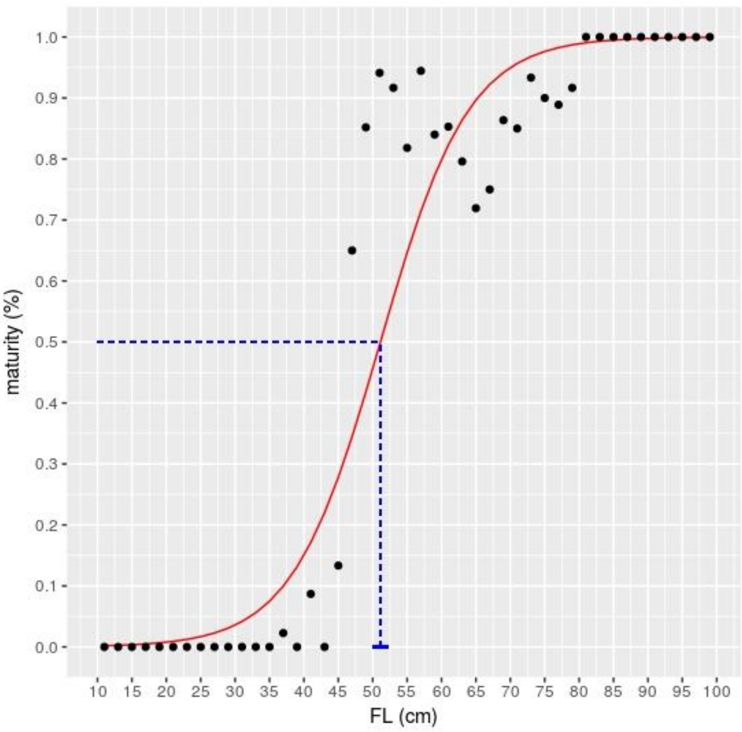

Figure 6. Logistic fit for the proportion of mature *Euthynnus alletteratus* (LTA). Macroscopic maturity staging (n = 1208).

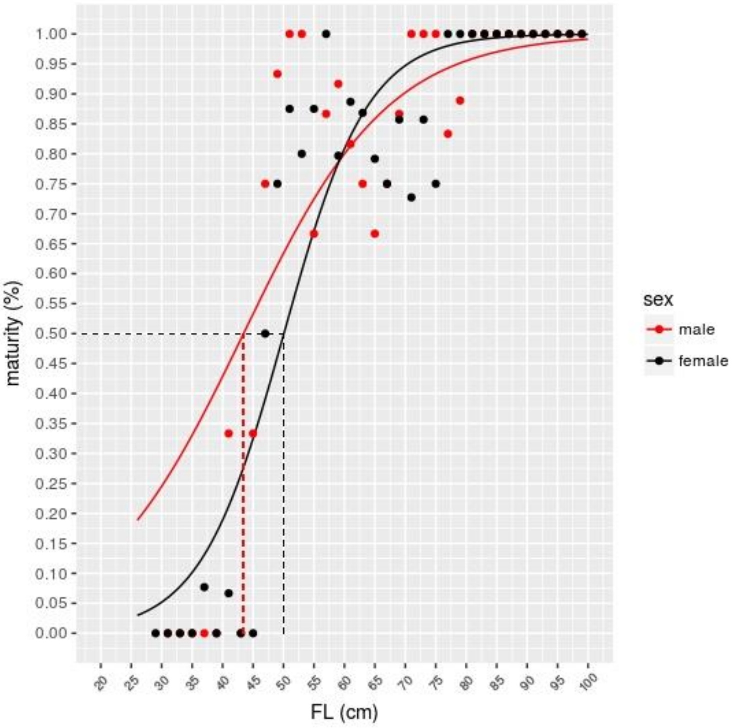

Figure 7. Logistic fit for the proportion of mature *Euthynnus alletteratus* (LTA) by sex. Macroscopic maturity staging.

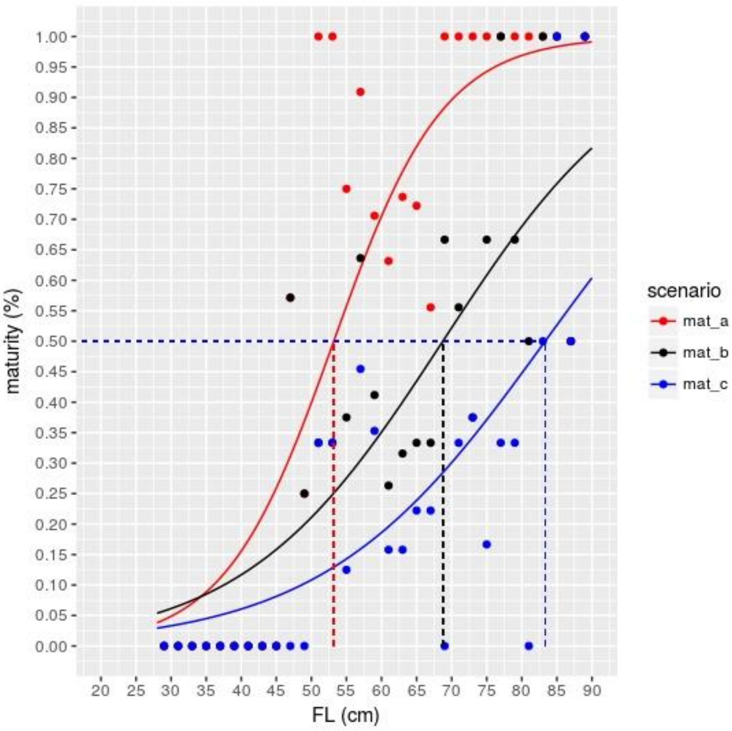

Figure 8. Logistic fit for the proportion of mature female *Euthynnus alletteratus* (LTA) based on microscopic examination of ovaries were calculated on the basis of three criteria: Mat_a, Mat_b and Mat_c (see **Table 6** for descriptions).