

SOME REPRODUCTIVE ASPECTS OF BULLET TUNA (*AUXIS ROCHEI*) FROM THE SOUTH WESTERN SPANISH MEDITERRANEAN

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SUMMARY

The bullet tuna (*Auxis rochei*) is one of the most abundant small tuna species in the Mediterranean Sea. This species is commercially exploited in the Spanish coast by traditional fisheries. Nevertheless, the biological and reproductive information about this species is currently scarce in the Western Mediterranean. Gonads of Bullet tuna from a Spanish Mediterranean trap (la Azohia, Murcia) were collected along June (2003) in order to determine the reproductive characteristics (Sex-ratio, maturity, oocyte developmental features, etc) of the population. The fish collected ranging from 334 to 470 mm in fork length. The sex-ratio was 1:1.7 F/M. All these fishes were mature and their reproductive characteristics indicate a spawning area near to the trap location. These and other biological aspects (size distribution, size/weight relations, etc) will be discussed in this paper.

RÉSUMÉ

Le bonitou (*Auxis rochei*) est l'une des plus abondantes espèces de petits thonidés en Méditerranée. Cette espèce est commercialement exploitée sur la côte espagnole par les pêcheries traditionnelles. On dispose néanmoins de peu d'informations sur la biologie et la reproduction de cette espèce à l'ouest de la Méditerranée. Les gonades de bonitous provenant d'une madrague méditerranéenne espagnole (la Azohia, Murcie) ont été prélevées au mois de juin (2003) afin de déterminer les caractéristiques reproductives (sex-ratio, maturité, caractéristiques du développement des ovocytes, etc.) de la population. Les poissons recueillis mesuraient entre 334 et 470 mm de longueur à la fourche. Le sex-ratio était de 1 :1.7 F/M. Tous ces poissons avaient atteint le stade de maturité et leurs caractéristiques reproductives indiquent une zone de frai proche de l'emplacement de la madrague. Ces aspects biologiques et d'autres (distribution des tailles, rapports taille/poids, etc.) seront examinés dans le présent document.

RESUMEN

La melva (*Auxis rochei*) es una de las especies de pequeños túnidos más abundantes en el mar Mediterráneo. Las pesquerías tradicionales explotan comercialmente esta especie en las costas españolas. Sin embargo, actualmente existe poca información sobre la biología y reproducción de esta especie en el Mediterráneo occidental. Se recogieron gónadas de melva (*Auxis rochei*) de una almadraba española del Mediterráneo (La Azohía, Murcia) durante junio de 2003, con el fin de determinar las características reproductivas (ratio de sexos, madurez, rasgos del desarrollo de los oocitos, etc.) de la población. La talla de los peces recogidos osciló entre 334 y 470 mm de longitud a horquilla. La ratio de sexos fue 1:1,7 H/M. Todos los peces habían alcanzado la madurez y sus características reproductivas apuntaban hacia la existencia de una zona reproductiva situada cerca de la almadraba. En este documento se discuten estas y otras cuestiones biológicas (distribución por tallas, relaciones talla-peso, etc.).

KEYWORDS

Auxis rochei, bullet tuna, spawning, sexual maturity, South Western Mediterranean.

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1. Introduction

The genus *Auxis* distributes world-wide in tropical and subtropical waters. Bullet tuna (*Auxis rochei*) is an epi and meso-pelagic fish that chose a seasonal coastal distribution in temperate and tropical areas including the Mediterranean (Uchida 1981; Collete 1986). This species is abundant in the Strait of Gibraltar, North coast of Africa and Spanish Mediterranean coast (Postel 1973). The bullet tuna is one of the most abundant small tuna species in the Spanish Mediterranean Sea where has been commercially exploited by seasonal artisanal fisheries (Sabatés and Recasens 2001). In Spain, the species have been caught traditionally by seasonal coastal fisheries. Several fishing gears have been used to catch it: Traps and other minor fixed gears, purse-seine and hand-line (Uchida 1981). Little is currently known about the biology of this small tuna in the western Mediterranean Sea.

The first maturity size has been stated in 35 cm (FL) when the fish is two years old (Rodríguez-Roda 1983). Certain areas in the Mediterranean have been suggested as possible spawning sites of *Auxis* spp: Greece and Gulf of Catania (Bellot 1954), Balearic Islands (Duclerc *et al.* 1974), and Tunisian and Algerian waters (Postel 1964). The spawning period in the Mediterranean has been reported to occur from June to September (Ehrenbaum 1924; Piccinetti *et al.* 1996; Alemany 1997). The species is a multiple spawner with asynchronous oocyte development that carried out several spawning step by reproductive season (Niiya 2001).

Information concerning to migration patterns is scarce and fragmented (Rey and Cort 1981; Sabatés and Recasens 2001). Several authors have suggested a genetic migration from the Atlantic Ocean to the spawning areas in the Mediterranean trough the Gibraltar Strait (Sabatés and Recasens 2001).

The aim of this paper is to describe and discuss the results obtained about the reproductive characteristics of the Bullet tuna caught in the southwestern Mediterranean.

2. Material and methods

2.1 Bullet tuna samples

2.1.1 Specimen collection

A total of 177 bullet tuna were measured and weighed during a scientist survey developed during 2003 in the “la Azohía” trap (Murcia) in the South Western Mediterranean. 46 specimens caught from June 2 to 10 were dissected to obtain gonad, liver and muscle tissue for reproductive studies and hard structures for age determination like spines, vertebrae and otoliths. For each specimen fork length (FL) to the nearest millimeter, round and dressed weight and sex were determined. In addition the gonad weight to the nearest g was obtained with the purpose of knowing the Gonad-somatic index (Kume and Joseph, 1969) indicating the levels of sexual maturity of the bullet tuna specimens. Gonads were collected from each fish. GLM analysis was used to compare the length-weight relationships obtained with those reported previously.

Each couple of gonads (ovaries, testis) were removed from the gut cavity and identified as male or female, then a section of 2 cm width from the central part of only one single gonad was removed and preserve in 10 % buffered formalin and stored. A portion of each section preserved was washing in buffer solution, dehydrated in ethanol and n-butanol series and embedded in paraffin. Then samples were sectioned at approximately 10 μ m with a microtome and then mounted on slides and stained with Mallory’s Trichrome Stain for a general assessment of the histology of the gonad. Finally, the slides were examined and photographed in a Leyca photo-microscope.

2.1.2 Staging and measurement of oocytes

Five well-defined developmental stages of oocytes (perinucleolar, previtelogenic, partially yolked, totally yolked and Hidrated oocytes) were defined using histology following several authors (Yamamoto 1956, Forberg 1982 & Hunter *et al.* 1992). Developmental stage and measurement of whole oocytes were determined from 5 females in different developmental stages. Oocyte sizes were obtained by measuring of two diameters of 100 oocytes of each stage using an analysing system of only those oocytes that had been sectioned through the nucleus. Finally, the histological analysis and oocyte diameter distribution in each female was used to assess the spawning sequence (West 1990).

2.1.3 Histological classification

To estimate reproductive condition of bullet tuna, two different histological classification systems were used: one for estimating the sexual maturity and the other for assessing the activity stage of mature females. Each ovary was histologically classified according to both systems (Hunter and Goldberg 1980; Hunter and Macewicz 1980, 1985a, b; Hunter, Macewicz and Sibert 1986).

Sexual maturity

It is considered that a female is a mature one when has the capability to reproduce in a determinate spawning season. Histological signs of maturity are the presence in the ovary of yolked oocytes, hydrated oocytes or postovulatory follicles. The immature females have not reached the sexual maturity and are unable to reproduce in a determinate season.

Sexual activity

Five different stages of activity have been taken into consideration in the bullet tuna female:

Inactive females: the histological analysis indicates that the ovary contains no yolked oocytes and no atresic structures.

Active females: females were classified as active when the ovary contained yolked oocytes and there was no atresia or only minor considered. Active females were further classified into other stages according to additional criteria:

Ripening females (Maturing): Those females showing signs of sexual maturity (yolked oocytes) but not signs of imminent spawn or signs of past spawns steps.

Pre-spawning females (Ripe): Those females showing signs of an imminent spawning like hydrated or nuclear migration phase oocytes but not postovulatory follicles or extended atresia. High density of oocytes in the ovary can be seen.

Spawning females: spawning females were considered that whose ovaries present postovulatory follicles or imminent spawning signs like hydrated or migratory-nucleus oocytes. The histological analysis shows signs of past spawning (postovulatory follicles) and enough vitellogenic oocytes to complete more spawning.

Post-spawning females: Those females showing signs of past spawning (postovulatory follicles) but have not enough vitellogenic oocytes to complete more spawning. Extended atresia in vitellogenic oocytes. Low oocytes density in the ovary.

The gonadosomatic index was calculated according to Kume and Joseph (1969).

3. Results

3.1 Histological analysis

Ovary structure and oocyte development

The ovary consists of a muscle wall and a lot of follicles in different stages of development inside connective tissue. The follicles are made of an oocyte and a single layer of follicular cells. The type and number of follicles vary with the maturity and activity stages.

The histologic analysis let us characterise six stages of oocyte development:

Perinucleolar stage (\varnothing 22-146 μm) (**Figura 1 A**). Oocytes displayed polygonal shape with numerous small nucleoli near nuclear envelope. The average diameter was 61 μm , the median 60 μm and the modal value was 60 μm .

Previtellogenic stage (Lipid cortical alveoli) (\varnothing 68-264 μm) (**Figure 1 B**). The previtellogenic oocytes exhibited small lipid droplets and cortical alveoli in the peripheral ooplasm. No yolk granules can be found. The average diameter of this stage was 165 μm , the median 163 μm and the modal value was 174 μm .

Partially yolked stage (\varnothing 147-413 μm) (**Figure 1 C**). These oocytes present small spherical yolk granules in the peripheral ooplasm. The ooplasm are not completely filled of yolk granules. The average value of the diameter was 265 μm , the median 260 μm and the modal value was 250 μm .

Totally yolked stage (\varnothing 211-583 μm) (**Figure 1 D**). Totally yolked oocytes display the ooplasm entire filled of yolk granules. The zona radiata increase it thickness but the nucleus remains in the central location. The average diameter was 380 μm , the median 359 μm and the modal value was 310 μm .

Nuclear migration stage (\varnothing 352-564 μm) Nuclear migration oocytes display the ooplasm entire filled of yolk granules and the nucleus located in the animal pole. The average diameter was 484 μm , the median 490 μm and the modal value was 488 μm .

Hidrated stage (\varnothing 419-723 μm) (**Figure 1 E**). The final of the oocyte maturation is characterised by the coalescence of lipid and yolk globules and ooplasm hydration. The average diameter of these oocytes was 594 μm , the median 605 μm and the modal value was 641 μm .

Atretic follicles can be found in a different frequency in several ovaries. The atretic structures had an average size of 223 μm and their size vary from 96 μm to 421 μm . These broad range indicate that atresia can be found in all stages of oocytes development.

3.2 Maturity

Activity:

Immature female. Only one female 362mm in FL was found immature in the study sample (5.8%). The ovary of this female only displayed previtellogenic oocytes and no showed signs of recent spawning steps.

Ripening females. Three females (17.6%) were classified like maturing. These females displayed ovary profile characterised by the presence of perinucleolar, Previtellogenic and yolked oocytes in the ovary. No atretic follicles and post-ovulatory follicles can be found in the ovary.

Ripe females. Six females (35.3%) were classified like totally mature. These females displayed ovary profile characterised by the presence of perinucleolar, Previtellogenic, partially yolked and post-vitellogenic oocytes (nuclear migration or hydrated oocytes) in the ovary (**Figure 2 A**). No atretic and post-ovulatory follicles can be found in the ovary (**Figure 2 B**). No signs of imminent spawning activities can be found in the ovary (**Figure 2 C**).

Spawning females. Six spawning females (35.3%) were caught in the sample. These females showed some differential characteristics. All these females showed perinucleolar, previtellogenic, yolked oocytes, atretic structures and postovulatory follicles. But only some of these females presented hydrated oocytes. The location of postovulatory follicles in all females indicate us recent spawning steps but not all of these females are in the same stage of maturing for the next spawning step. The ovary profile shown in **Figure 3**.

Post-spawning females. Only one female was post-spawning one (5.8%). This female showed perinuclear and previtellogenic oocytes and large extended atresia of yolked and hydrated oocytes (**Figure 4**).

First maturity size

Only one female caught in this study were immature. The size of this female was 362 mm.

3.3 Sex ratio and Gonadosomatic index (GSI)

The fishes collected ranged from 334 mm to 470 mm in fork length (FL). The average length was 38cm, the median 37 and the modal value was 37.5 cm of FL. Size distribution has been shown in **Figure 5 A**. Detailed information on date, fork length (cm.), maturity, GSI and sex are given in **Table 1**. The sex-ratio was 1:1.7 F/M.

Males were more abundant than females in the studied sample. Cannot be saw clear trend in the proportion of females when the length increased (see **Figure 5B**).

Mean GSI by size class are represented in the **Figure 6 A**. In general, GSI grew up when the length increase, but this increment of GSI is clearer in males than females.

Once females were classified using histology, mean GSI was calculated by activity stage. The results have been summarised in the **Figure 6 B**. Mean GSI shows an increasing trend along maturing progress. The minimum GSI values were shown by the immature female, after the maturing ones, and the highest values are reached by the totally mature pre-spawning females. Finally, mean IGS fall to values only a few higher than the immature females in post-spawning females.

3.4 Spawning pattern

The microscopic appearance of the oocytes and the oocyte size frequency distribution had been used to define the spawning pattern shown by these species. The maturation of the bullet tuna follicles began in oocytes ranging from 68 μm (Pre-vitellogenic oocytes) to 148 μm (early vitellogenesis). Vitellogenesis initiates at 148 μm but the average size of partially yolked oocytes was 265 μm . Usually, fully yolked oocytes can be found at 211 μm but the average size of this stage was 381 μm . The nuclear migration entailed a qualitative change in the morphologic characteristics of the follicles but not involved a size increment. The hydration started when the oocyte reached at least 419 μm in diameter. Maximal size of hydrated oocytes in this study was 723 μm . The oocyte size frequency distributions broadly overlapped each of the different stages of development. This characteristic indicated us that perinucleolar stage oocytes were constantly recruited to the maturing stages. Postovulatory follicles appear once the hydrated oocytes have been spawned. Atretic oocytes can be seen in all the developmental stages.

3.5 Length-weight relationships

Pairs of observations on length and weight (round – **Figure 7A**- and dressed- **Figure 7 B**) were used to fit the following relationships:

$$\begin{aligned} \text{Total weight} &= 1.60943\text{E}^{-5} * \text{FL}^{3.00293229} \\ \text{Dressed weight} &= 4.56376\text{E}^{-06} * \text{FL}^{3.31734402} \end{aligned}$$

The comparison (Using GLM analysis) of Length-weight relationships with those reported previously (Megalophonou *et al.* 2000; de la Serna *et al.* 2004) showed that there no were significant differences at 95% of confidence level.

4. Discussion

Oocyte and gonad development

Developmental characteristics of oocyte maturation of bullet tuna are similar to those described for other species with asynchronous development. Oogonia and perinuclear oocytes are found in all ovaries all year and are considered to be part of the general stock of the ovary. Later, when cortical lipid alveoli develop pre-vitellogenic oocytes emerge. Yolk accumulation occurs in vitellogenic phase and, after migration of the nucleus to the animal pole, hydration and ovulation occurs (Forber 1982). In Bullet tuna hydrated oocytes were used to define the mature pre-spawning stage according to Arocha's (2002) criteria for swordfish. After spawning step numerous post-ovulatory follicles are found in the ovary. Since post-ovulatory follicles become soon indistinguishable from the connective tissue (Matsuyama *et al.*, 1988), they have been commonly interpreted as a sing of recent spawning. The mature spawning ovary never was empty of vitellogenic oocytes, only in post-spawning (recovering ovaries) females we can observed empty ovaries.

The modal value of the diameter of the three main clusters of maturing oocytes showed by the Hydrated females were 250, 310 and 641 μm . These results agree basically with those found for this species in the Pacific Ocean (Niiya, 2001). Nevertheless, our result indicates that the cluster of yolked oocytes usually presented several modal values. Also the proportion of yolked oocytes is higher than the hydrated ones. Both facts could suggest that the cluster of yolked oocytes really represent several maturing clusters.

Spawning pattern

The results of this study indicate that bullet tuna is a multiple spawner with asynchronous oocyte development. These results agree with those of previous studies (Megalophonou *et al.* 2000; Niiya 2001). Our result indicates that there are no gaps between distributions of dominating stages of oocyte development. We also found several modal values in yolked oocytes. These characteristics suggest that bullet tuna are a species with indeterminate fecundity. This fecundity pattern depends on estimates of batch fecundity and spawning frequency to determinate potential annual fecundity (Hunter and Macewitz 1985; Hunter *et al.* 1992).

Spawning season and areas

From the histology analysis of bullet tuna ovaries we have deduced a reproductive activities pattern in the studied area. During June the females are spawning near the trap location. The spawning season occurs mainly in later spring and early summer, with a peak in June. These results agree with the observations of larvae of this species in the Catalanian coast along July (Sabatés and Recasens 2001).

Spawning areas for Bullet tuna has been stated all over the Mediterranean Sea using the presence of larvae in the plankton (Duclerc *et al.* 1974; Piccinetti *et al.* 1996; Alemany *et al.* 1997). The catches of pre-spawning, spawning and recent post-spawning mature female in the study area during June highly support the hypothesis of a reproductive area in the South-Western Spanish Mediterranean. These results agree with those of Sabatés and Recasens (2001).

In conclusion our results indicate that the bullet tuna has a reproductive and spawning area near to “la Azohía” in the Western Mediterranean.

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Table 1. Bullet tuna sample characteristics.

CODE	Length (cm)	Weight (g)	Dressed W	GONAD W	GSI	SEX	Maturity
FRI1	44	1510	1284	78,5	9,2	F	Ripe
FRI10	37	922	780	61,5	12,1	F	Ripe
FRI13	38,7	1002	856	60	10,4	F	Ripe
FRI14	37,2	828	742	32,5	6,3	F	Ripe
FRI17	38,6	970	828	62,5	10,9	F	Ripe
FRI19	37	810	692	51,5	10,2	F	Ripe
FRI23	38,7	984	863	42,5	7,3	F	Spawning
FRI24	38	960	818	43	7,8	F	Spawning
FRI28	35,8	708	658	38	8,3	F	Ripening
FRI30	35,9	802	694	39,5	8,5	F	Ripening
FRI31	35,8	758	666	22,5	4,9	F	Spawning
FRI35	36	802	692	24,5	5,3	F	Spawning
FRI37	37,7	918	802	49	9,1	F	Ripening
FRI39	38,5	924	782	41,5	7,3	F	Spawning
FRI42	37,7	842	766	17	3,2	F	Post-spawning
FRI43	36,5	750	680	33,5	6,9	F	Spawning
FRI6	36,2	730	658	11,5	2,4	F	Inmature
FRI11	39	1056	898	86,5	14,6	M	Mature
FRI12	36,6	764	676	33	6,7	M	Mature
FRI15	35,9	710	648	15	3,2	M	Mature
FRI16	37,7	940	826	48,5	9,1	M	Mature
FRI18	35,2	702	638	21	4,8	M	Mature
FRI2	44,8	1638	1400	138	15,3	M	Mature
FRI21	36,4	756	696	10,5	2,2	M	Mature
FRI22	33,5	560	508	11	2,9	M	Mature
FRI25	37	910	796	50	9,9	M	Mature
FRI26	34	636	568	21	5,3	M	Mature
FRI27	37,7	856	768	16,5	3,1	M	Mature
FRI29	37,1	866	774	33	6,5	M	Mature
FRI3	44,6	1678	1426	142,5	16,1	M	Mature
FRI32	36,3	770	674	34	7,1	M	Mature
FRI33	36,7	836	744	36,5	7,4	M	Mature
FRI34	36,8	680	618	9	1,8	M	Mature
FRI36	36,2	710	640	30,5	6,4	M	Mature
FRI38	36,8	722	662	22,5	4,5	M	Mature
FRI4	42,3	1254	1142	47	6,2	M	Mature
FRI40	34,9	656	600	7,5	1,8	M	Mature
FRI41	39,5	986	840	81,5	13,2	M	Mature
FRI44	38,6	906	776	69,5	12,1	M	Mature
FRI45	35	720	638	14,5	3,4	M	Mature
FRI46	36,3	702	642	11,5	2,4	M	Mature
FRI5	43	1348	1270	123	15,5	M	Mature
FRI7	39,5	898	794	24	3,9	M	Mature
FRI8	38	902	828	19	3,5	M	Mature
FRI9	37	882	778	41,5	8,2	M	Mature
FRI20	40,4	1012	896	38	5,8	M	Mature

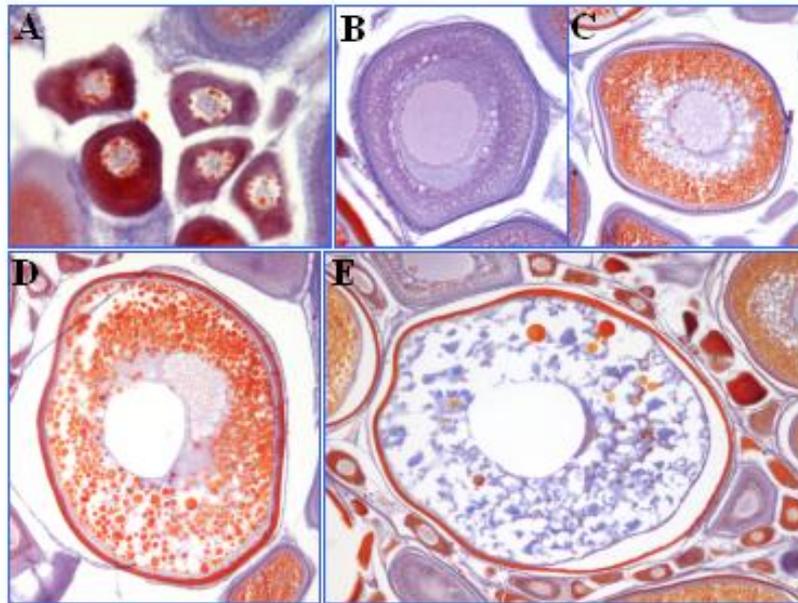


Figure 1. Oocyte development of *Aedes vexans*. **A.** Perinuclear stage oocytes. **B.** previtellogenic oocyte. **C.** Partially yolked oocyte. **D.** Totally yolked oocyte. **E.** Hydrated oocyte.

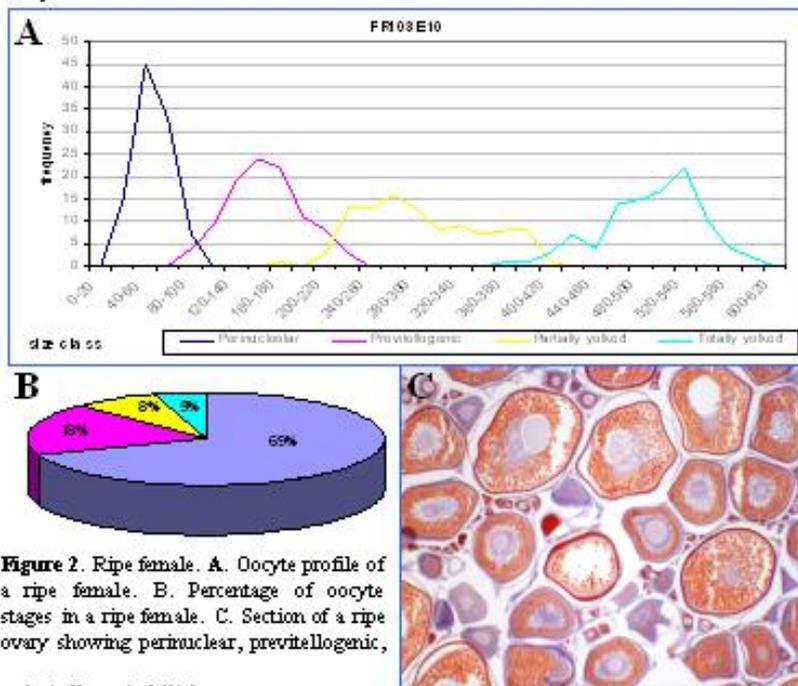


Figure 2. Ripe female. **A.** Oocyte profile of a ripe female. **B.** Percentage of oocyte stages in a ripe female. **C.** Section of a ripe ovary showing perinuclear, previtellogenic, and vitellogenic follicles.

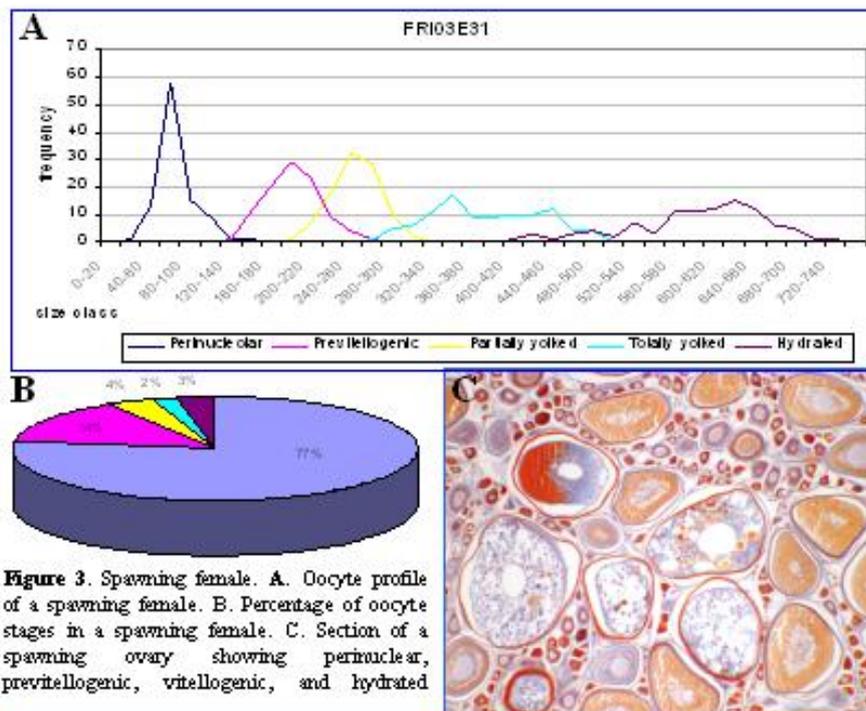


Figure 3. Spawning female. **A.** Oocyte profile of a spawning female. **B.** Percentage of oocyte stages in a spawning female. **C.** Section of a spawning ovary showing perinuclear, previtellogenic, vitellogenic, and hydrated

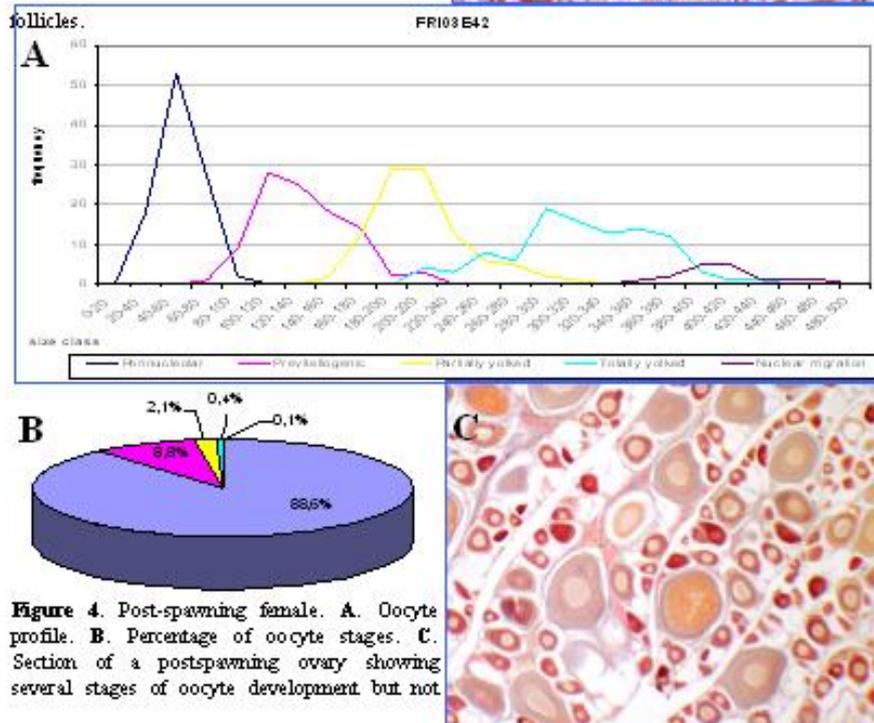


Figure 4. Post-spawning female. **A.** Oocyte profile. **B.** Percentage of oocyte stages. **C.** Section of a postspawning ovary showing several stages of oocyte development but not enough amount for a complete spawning step.

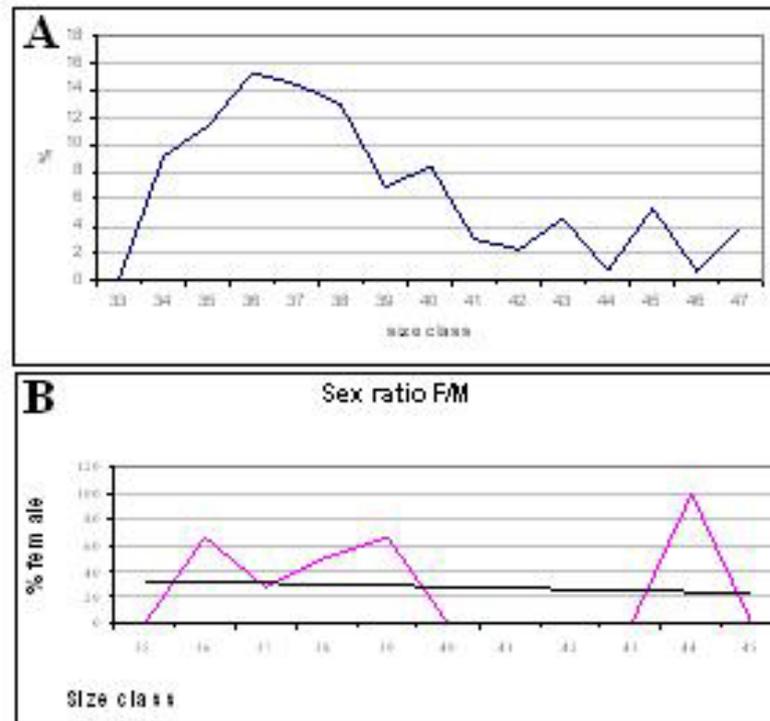


Figure 5. A. Size distribution of the *A. rochei* sample. B. Sex-ratio of *A. rochei* caught in the "la Azohia" trap.

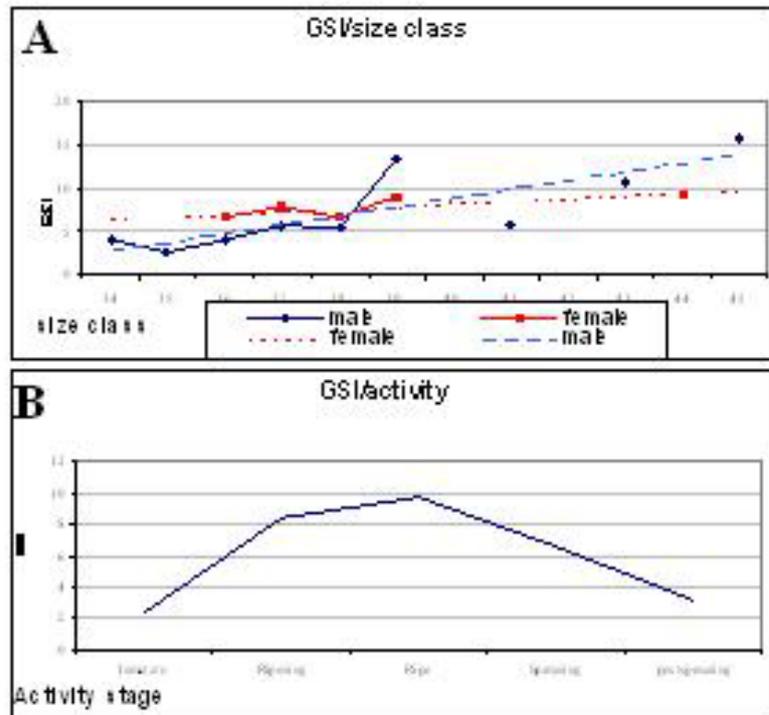


Figure 6. A. Gonadosomatic index variation by size class. B. Gonadosomatic index variation with the different sexual activity stages.

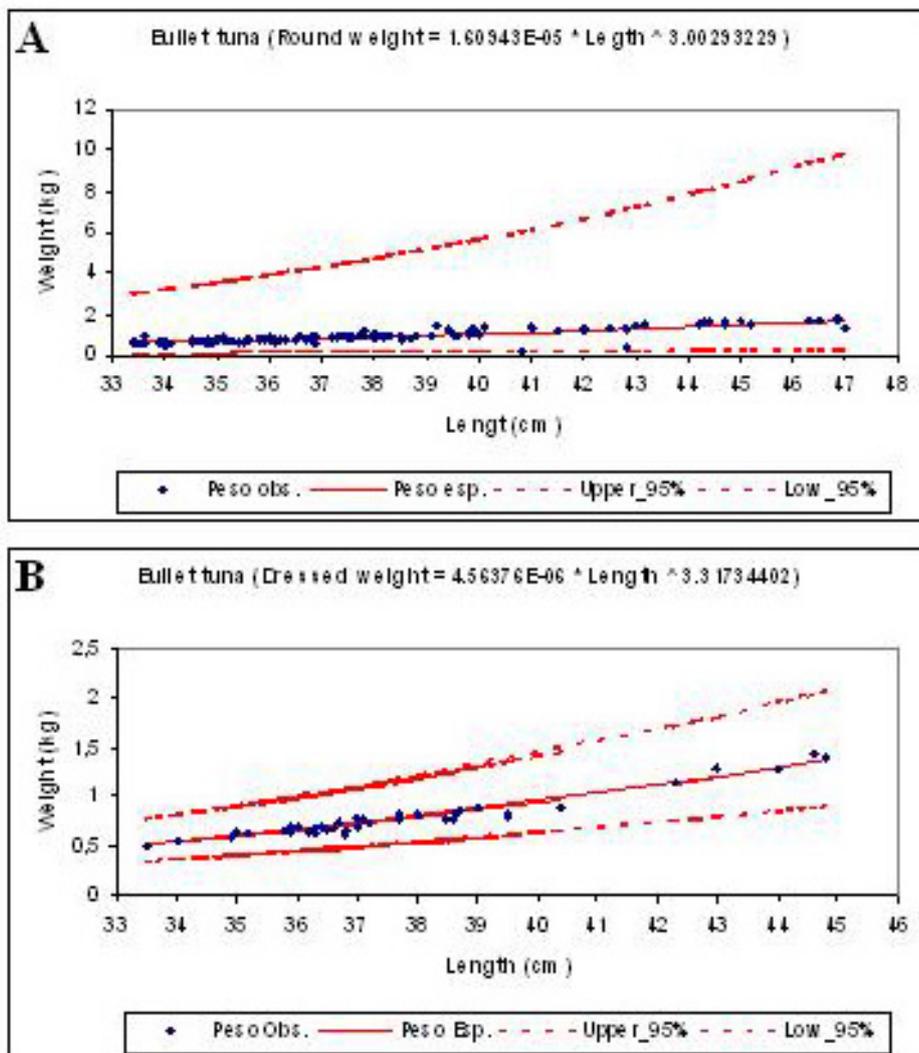


Figure 7. Length-weight relationships of *Auxis rochei*. A. Round weight-Length relationship. B. Dressed weight-Length relationship.