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REPRODUCTIVE PATTERN AND GROWTH IN *LEPIDOPUS CAUDATUS* (OSTEICHTHYES, TRICHIURIDAE) FROM THE CANARY ISLANDS (EASTERN-CENTRAL ATLANTIC)

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Abstract: The silver scabbardfish *Lepidopus caudatus* (Euphrasen 1788) is a bathypelagic fish living off the Canary Islands. The sexual pattern, reproductive cycle and growth were studied from samples collected from October 1996 and December 1997. The macroscopic observations and the histological analysis of the gonads reveal that this species is a gonochoristic species, showing the ovary a group-synchronous development. A new macroscopic and histological maturity scale of six stages was elaborated according to multiple spawning pattern observed in females. The development of the testis is continuous or unrestricted spermatogonial type being acinar the type of spermatogenesis. Range of length of fish was between 141 and 189 cm FL predominating the females in the catches (89.6% of the total). The length-weight and length-length relationships studied did not point out differences between sexes. Age was determined in whole otoliths ranging from 1 to 7 years in females and between 1 and 5 years in males. The von Bertalanffy growth parameters estimated for females were L_∞= 186.90 cm FL, k= 0.23 year^{-1} and t_o= -1.23 years.

Key words: Reproduction Pattern, Growth, *Lepidopus caudatus*, Fishes, Trichiuridae, Canary Islands

Introduction

The silver scabbardfish, *Lepidopus caudatus* (Euphrasen 1788), although absent from the North Pacific, is a cosmopolitan temperate species (Mikhaylin 1978; Demestre et al. 1993) found in outer-shelf waters, over off-shore pinnacles and sea mounts. It is occasionally beachcast (Robertson 1980). In the Atlantic Ocean it is found from France to Senegal, including the Azores, Madeira, the Canaries and offshore seamounts, off South Africa from Cape Frio to Agulhas Bank, and it is occasionally found in Iceland, Norway, Scotland and England. It is also found in the Western Mediterranean. In the Pacific, it is distributed off Australia from New South Wales to Southern West Australia, and New Zealand, with a doubtful record from Cape San Lucas, Mexico. In the Indian Ocean, it is recorded at offshore seamounts along 30º to 35º S (Parin 1984; Nakamura and Parin 1993). In the Canary Islands, it is a benthopelagic species inhabiting near the bottom, between depths of 250 and 750 m during daytime and moving to shallower waters (up to 50 m) at night (Franquet and Brito 1995; Brito et al. 2003).

This species is included in the FAO catalogue of species of interest to fisheries (Fischer et al. 1981). In the last years (1991-2001) its catches had varied between 21,697 and 8,989 tons (Froese and Pauly 2004). Longline and trawling are the fishery methods used to capture this species between 150 and 350 m, although both present differences in the sampling recollected. Longline only collects large individuals while trawling catches small and immature specimens (D'Onghia et al. 2000). In spite of commercial interest, its biological knowledge is scarce and it has been never studied very thorough. In general, the studies are focused for establishing the age and growth (Moli et al., 1990; Demestre et al., 1993; D'Onghia et al., 2000) and for determining the spawning season (Karlovac and Karlovac 1976; Orsi Relini et al. 1989; Demestre et al. 1993; D'Onghia et al. 2000). Nevertheless, due to the complexity of the population structure of this species seems very complicated an approach in detail.

In the Canary Islands, silver scabbardfish is not target of fishery appearing occasionally in the longlines. The objective of this paper is to provide preliminary information on the sexuality, reproductive cycle, length-weight relationship, age and growth of the Canary Island silver scabbardfish population.

Material and methods

A total of 336 individuals of silver scabbardfish were collected at depth from 127 m to 755 m between October 1996 and December 1997. The samples were taken from commercial catches of the local and artisanal fisheries in the Gran Canaria Island (Canary Islands, Eastern-central Atlantic Ocean), although due to characteristics of the local fishery was impossible to obtain samples from July to September. For each fish, total length (TL, cm), fork length (FL, cm), total weight (TW, g) and gutted weight (GW, g) were measured (Table 1). Relationships between FL-TL, FL-TW and FL-GW were calculated for the whole sample using a power function to fit the data (TL, TW or GW=a(FL)^b). A *t*-test was used to determine if the slope of both relationships was significantly different between sex.

Sexuality and reproduction

Gonads were removed, weighted (GNW, g) and determined macroscopically the sex (males, females or undetermined) and maturity stages (MS) according to Demestre et al. (1993) and D'Onghia et al. (2000). All gonads were fixed in buffered formaldehyde 4%, before being dehydrated and embedded in paraffin wax. Longitudinal or cross-sections, 4 to 5 µm thick, were stained with Harris'hematoxylin-Puttis'eosin. Histological analysis of the gonads enabled the re-identification of sexual type. Oocytes were classified according to Selman et al. (1993) in: primary growth (nucleolar and perinucleolar stages), yolk-vesicle formation, vitellogenesis, oocyte maturation and mature egg; while the spermatogenic cells according to Grier (1981) in: spermatogonia, primary and secondary spermatocytes, spermatid and spermatozoon. The diameters of 100 oocytes randomly chosen were measured using an ocular micrometer. Measurements were taken only on oocytes sectioned through the nucleus. The histological analysis also allowed re-assign the maturity stages in relation to the development of the ovary and testis and also by the presence/absence of different types of oocytes (i.e. whether organized by ovarian lamellae or not) and spermatocytes (García-Díaz et al. 1997, 2002).

Age and growth

The sagittal otoliths were extracted, cleaned and stored dry in plastic vials for all individuals. To find the best method for age determination, a random subsample of 20 individuals were studied interpreting growth rings from whole otoliths and sections of them for several experimental readers in a workshop.

Table 1. Macroscopical scale of females used in silver scabbarfish on date and the scale proposal in the present study with its macroscopic and histological description.

Demestre el al. (1993)	D'Onghia et al. (2000)	Scale proposal (present study)	Description	
			Macroscopic	**Histological**
I. Immature	I. Virgin gonad	I. Virgin gonad (immature)		
II. Resting	II. Resting (immature)	I. Developing virgin or recovering-spent	Ovary appears white or yellowish. Gonad wall is thin and transparent in virgin individuals and gross and opaque in individuals that have spawned at least once. Internally it is a yellowish mass with a granular aspect, although oocytes are indistinguishable by the naked eye. Ovary occupied 25% of the body cavity.	Ovarian lamellae show a compact organization and contained oogonia and oocytes in nucleolar and perinucleolar chromatin stage (primary growth phases), radially distributed from the join part of both lobules to the exterior. Oogonia are organized in cysts within the germinal epitelium with large nucleus, a single nucleolus and a small band of light cytoplasm. Oocytes in the nucleolar chromatin stage appear similar to the oogonia, but have a larger diameter (diameter size = 15-80 μm) and a large nucleus with scatteredd chromatin surrounded by a small band of cytoplam. Oocytes increase in size before starting the perinucleolar stage, which is characterised by the presence of nucleolus near the nuclear membrane and by a strongly basophilic cytoplasm (diameter size = 80-160 μm).
III. Onset of maturity IV. Maturity	III. Maturing	III. Maturing	Ovary appeared white or light pink in colour. Gonad wall becomes thicker and the ovary occupies 50% of the body cavity at the end of this stage.	Small oocytes in the early yolk-vesicle formation. Oocytes distinguish by the presence of some inclusions in the cytoplasm called yolk vesicles, and because of the observation of the layer follicle wrapping the oocyte (diameter size = 160-250 μm). The oocytes then begin the vitellogenic stage, in which lipidic inclusions appear around the nucleus in addition to the presence of yolk granules in the cytoplasm and to the formation of the zona radiata. The more advanced oocytes show abundant vitellogenic corpuscles in the cytoplasm, uniformly distributed with the inclusions (diameter size = 250- 820 μm).
V. Ripe	IV. Mature V. Running ripe	IV. Ripe	Ovary appeared orange in colour. Gonad wall is very thick and the ovary occupies 75% of the body cavity. Mature oocytes begin to hydrate when spawning is imminent, the wall becoms thinner and the ovary occupies 100% of the body cavity.	Ovary contained all the developmental stages of oocytes, a small number of yolkless oocytes, various sized vitellogenic oocytes and some oocytes maturing or fully mature. Mature vitellogenic oocytes (diameter size = 820-980 μm) are characterized by the migration of the nucleus towards the animal pole and by the presence of a large oil drop formed by fusion of the lipid inclusions. Yolk granules are fused in a homogeneous mass creating the hydrated oocyte (diameter size = 1500-1800 μm).
		V. Re-developing	Ovaric wall is very thick and the oocytes cannot be observed by the naked eye. Inside, some transparent and large oocytes remaining from the last spawning event can be observed. The ovary occupies 50% of the body cavity.	Two different groups of oocytes are observed; primary growth oocytes and the remains of larger maturing vitellogenic oocytes. Some remnants of hydrated eggs from the inner spawn cycle can also be found. Oocytes are located in the ovarian lamellae where primary growth oocytes predominate near the join part of both lobes, while the ovulated eggs are more abundant in the opposite zone.
VI. Recently spent	VI. Spent	VI. Spent	Ovary appeared garnet in colour, flaccid and sometimes completely empty. Gonad wall is thick and the main vessel appears well-developed. Remains of oocytes can still be observed in the ovary occupying about 25% of body cavity.	Ovary empty and the ovigerous lamellae, which was almost disrupted, contains remaining vitellogenic oocytes, atretic bodies and postovulatory follicles in different stages of reabsortion. Tunica albuginea is swelled because of the retraccion of the ovarian parenquima after spawning. Ovaries start the reabsorption stage, at the end of which the ovarian parenquima is formed only by reserve primary growth oocytes, although some hydrated eggs can be found in the lumen.

The results did not indicate differences between both reading methods, so age was estimated from whole otoliths. Otoliths were placed in a black disk with alcohol (70º) and examined under a compound microscope with reflected light. Two readers independently counted opaque zones in each otolith, and only coincident readings were accepted. The opaque and translucent zone deposition pattern was considered as an annual event according to literature (Moli et al. 1990; Demestre et al. 1993, D'Onghia et al. 2000).

Nonlinear regression was used to estimate parameters of the von Bertalanffy growth equation:

$$L = L_{\infty}[1 - e^{-K(t-t_0)}]$$

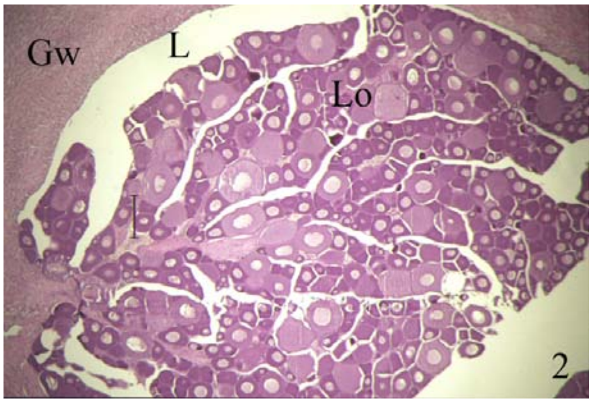
where L is total length (cm), L_{∞} asymptotic length, k growth coefficient, t age (years) and t_0 hypothetical age at which length is zero. Growth performance (Φ) was calculated to compare with the growth parameters obtained for other authors (Pauly and Munro 1983).

Results

Gonad anatomy and sexuality

The macroscopic and histological analyses of the gonads reveal that silver scabbardfish is a gonochoristic species. The gonads are paired structures joined to the body cavity by peritoneal membranes in a posterior-dorsal position, close to the swimbladder. They consist of two elongate tubular lobes, of which the right is always longer than the left, covered by a gross membrane of connective tissue or tunica albuginea. The blood vessels are distributed over the whole surface of the gonad and the main vessel irrigates both lobes and extends to the anterior part of the gonad. A short common duct opens externally via a genital pore located posterior to the anus. During the gonad development, the sexual glands formed by the two small lobes, enlarge until they completely fill the abdominal cavity, and the lobes, which in the early stages of maturation were two completely separate structures join together until, at the end of the maturing stage, they form a single structure that is difficult to remove (Figure 1).

The histological analysis revealed that the ovary is composed of numerous longitudinal folds or ovigerous lamellae, radially oriented towards the lumen located in a lateral position (Figure 2).



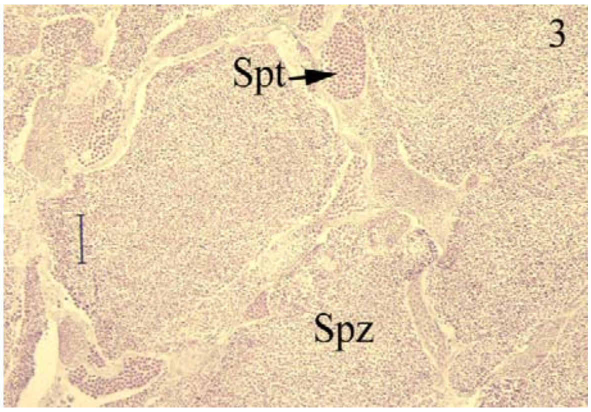


Figure 1. Macroscopical view of gonad.

Figure 2. Histological section of an ovary in pre-reproductive stage. x50. Gw, gonal wall; L, lumen; Ol, ovigerous lamellae.

Figure 3. Histological section of a testis in reproductive stage. x50. Spz, spermatozoa Spt, spermatocytes.

Each lamella is covered by an epitellium within the oogonia, which are becoming in maturing and mature oocytes, are present. The testis are formed by numerous seminiferous ducts that contain the spermatogonia which, as they divide form secondary spermatogonia, pass though a number of sperm-atocytes stages, spermatids and finally, spermatozoa which are situated in the lumen of the tubule (Figure 3). In both cases (ovary and testis) did not detect morphological differences among different zones of the gonad.

In total of 84.8% of the gonads were identified correctly the sex, being the assignation percentage in males (57.1%) lower than in females (89.7%). This error in the classification of the sex in males was due mainly to small size of the gonad when it is in later recovering stages. Then, the posterior studies were carried out using the sex determined by histological assignation. Of the 336 individuals histologically sexed, 35 (10.4%) were males and 301 (89.6%) females. The overall sex-ratio was clearly unbalanced in favour to females (1:9.6) being the smallest mature of 85.0 cm FL in both sexes.

Reproductive cycle

The histological analysis revealed that the ovary of *L. caudatus* is classified as group-synchronous, according to Wallace and Selman (1981). The macroscopic and histological study of female gonad revealed two different morphologies after the ripe stage: i) ones with remaining of hydrated oocytes in the dorsal of the gonadal lobule, slightly flaccid, without blood-stained vessels, with a very thin ovaric wall and the gonad occupying more of 50% of the abdominal cavity. Histologically, this observations corresponding with gonads containing two different groups of oocytes: previtellogenics and the remains of larger maturing vitellogenics. The oocytes are located in the ovarian lamellae where the previte-llogenic oocytes predominate near the join part of both lobes, while the ovulated eggs are more abundant in the opposite zone; ii) others with aspect very flaccid, of garnet colour, the main vessel appears well-developed, the gonad wall is thick, sometimes completely empty or with remains of oocytes can still be observed in the ovary occupying less of 25% of the abdominal cavity. The histological observation of these gonads showed an empty ovary and the ovige-rous lamellae, which was almost disrupted, contained remaining vitellogenic oocytes, atretic bodies and postovulatory follicles in different stages of reabsortion. The tunica albuginea was swelled beca-use of the retraction of the ovarian parenquima after spawning. At this moment the ovaries start the reabsorption stage, at the end of which the ovarian parenquima is formed only by reserve previtellogenic oocytes, although some hydrated eggs can be found in the lumen.

Consequently, the reproductive cycle of females revealed three phases of the seasonal cycle: pre-reproductive, reproductive and post-reproductive. The reproductive phase is represented by an inner cycle, consisting of ovulation, spawning and redevelopment, during the spawning season where a small portion of the oocytes are hydrated and ovulated in several batch (Figure 4). Furthermore, a new macroscopic scale based in our macroscopic and histological observa-tions of maturity stages was newly made for females (Table 1, Figures 5-9.). Females in reproductive phase (stages III, IV or V) were observed in all months sampled (Table 2).

In accordance with the description of Grier (1981) for teleosts, the spermatogenesis found in *L. caudatus* is of the acinar type; the sperm develops in small cysts, all the cells of one cyst being in the same stage of meiosis within the wall of the seminiferous ducts. The macroscopical scale was in four categories: immature, developing, mature and spent (Figures 10 and 11). Males in reproductive phase (stage III) were detected mainly in the first quarter (Table 2).

Allometric relationships

The data indicated that the females reaching a maximum size (189.0 cm FL) higher than males (141.1 cm FL) (Table 3). The results of the all regressions between fork length and total length, total weight and gutted weight for males, females and all individuals are summarized in Table 4. The slope analyses of all regressions were not significantly different between sexes (Table 4).

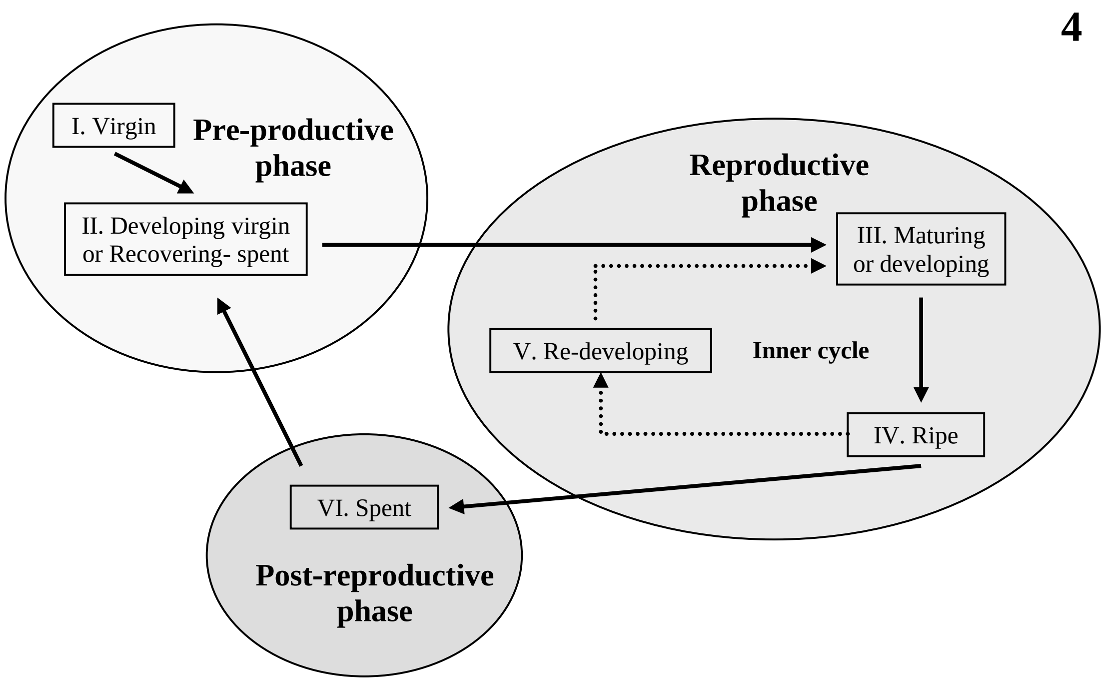


Figure 4. Phases of sexual cycle in females of silver scabbarfish.

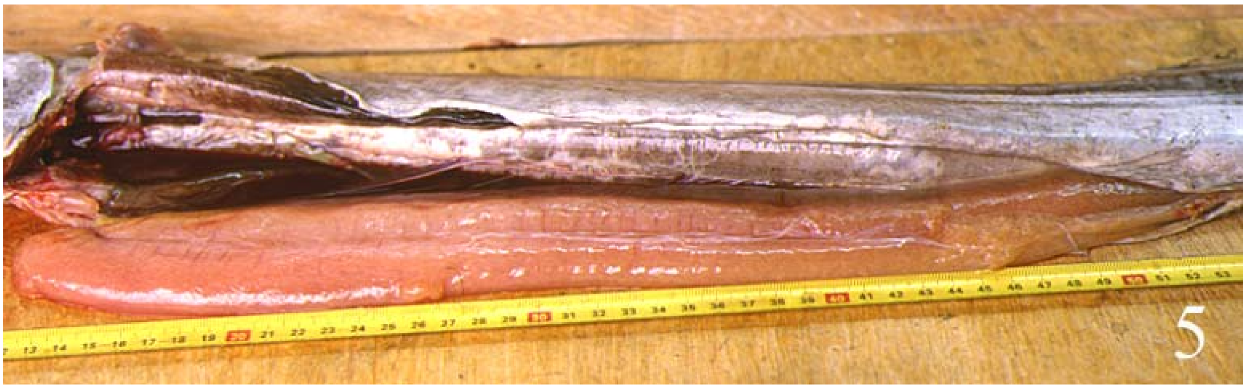
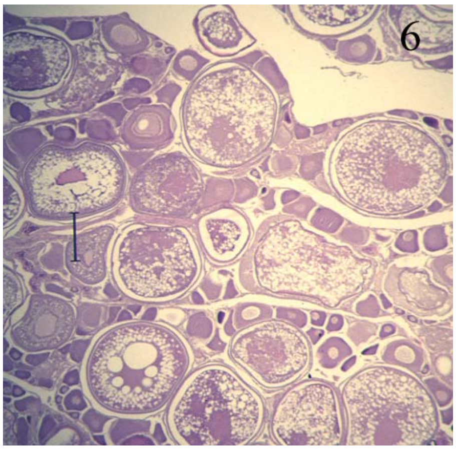
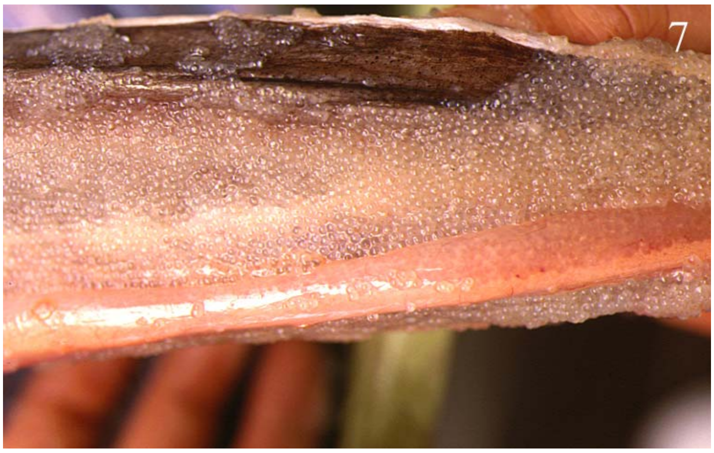
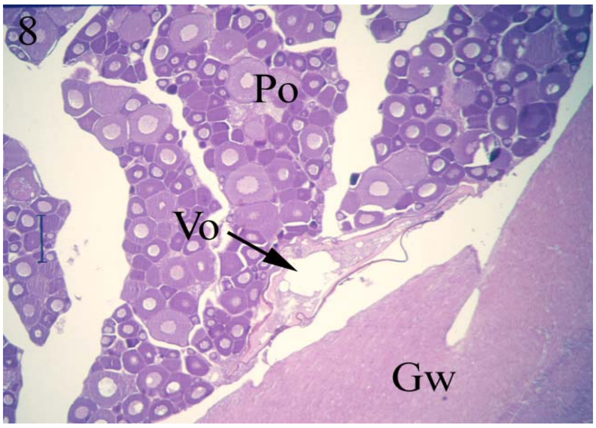
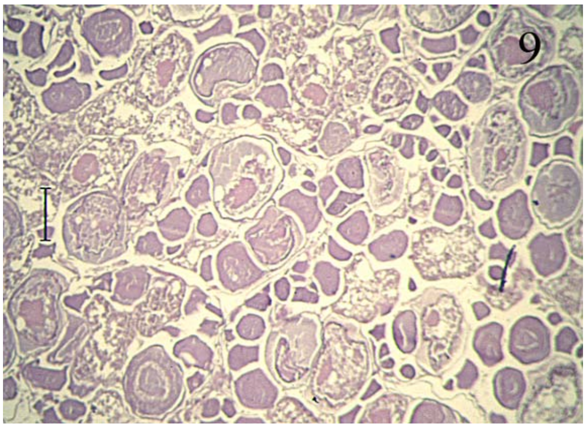


Figure 5. Macroscopical view of ovary in ripe stage.









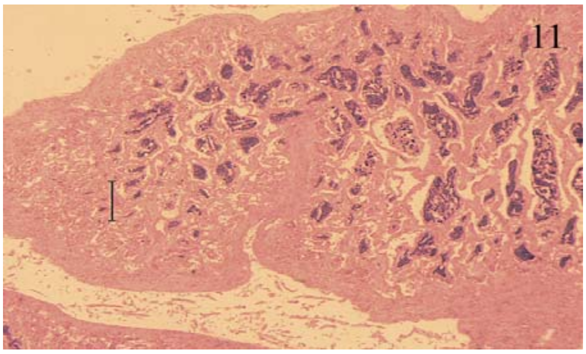


Figure 6. Histological section of an ovary in ripe stage. x50.
Figure 7. Ovary with hydrated eggs filling the body cavity.
Figure 8. Histological section of an ovary in redeveloping stage. x50. Gw, gonad wall; Po, pre-vitellogenic oocytes; Vo, vitellogenic oocytes.
Figure 9. Histological section of an ovary in post-reproductive stage. x50.
Figure 10. Macroscopical view of testis in post-reproductive stage.
Figure 11. Histological section of a testis in post-reproductive stage. x50.

Table 2. Percentage of the reproductive stages observed in females and males in the samples studied.

Maturity stage	1st Quarter		2nd Quarter		4th Quarter	
	Females	Males	Females	Males	Females	Males
II	39.8	20.0	18.0	25.0	5.9	-
III	15.7	66.7	12.0	5.0	35.3	-
IV	17.6	13.3	32.0	70.0	23.5	-
V	11.6		14.0		2.9	
VI	15.3		24.0		32.4	

Table 3. Summary statistics on sizes and weight for each sex.

Variable	Males		Females	
	n	range	n	Range
Total length (cm)	35	84.5-145.0	301	87.8-195.8
Fork length (cm)	35	82.4-141.1	301	84.9-189.0
Total weight (g)	35	368-1969	301	440-5149
Gutted weight (g)	35	355-1846	301	421-5090

Table 4. Estimated parameters of the relationship between fork length (FL, cm) and total length (TL, cm), total weight (TW, g) and gutted weight (GW, g) for males, females and all individuals and, *t*-test (P>0.05) to analyze the isometry and to compare of slopes between sex.

Variable	a	b	SE (b)	r^2	n	Comparison
FL-TL equation						
Males	1.045	0.999	0.022	0.985	35	*t*= 0.000 df= 334
Females	1.039	0.999	0.004	0.996	301	
Total	1.039	0.999	0.003	0.996	336	
FL-TW equation						
Males	0.0003	3.167	0.108	0.966	35	*t*= 1.003 df= 334
Females	0.0007	3.001	0.055	0.916	301	
Total	0.0006	3.035	0.051	0.921	336	
FL-GW equation						
Males	0.0003	3.159	0.113	0.964	35	*t*= 1.120 df= 334
Females	0.0007	3.005	0.045	0.944	301	
Total	0.0007	2.983	0.036	0.957	336	

Table 5. Age-length key for females of silver scabbarfish from Canaries. SD, standar deviation.

TL (cm)	Age								
	0	1	2	3	4	5	6	7	Total
84			1	1					2
86			2						2
88			1	1					2
90			1	1					2
92			1						1
94			1						1
96									
98									
100				3					3
102									
104									
106									
108				3	1				4
110				4					4
112				1	1				2
114									
116				3	1				4
118				2	1				3
120				2	4				6
122				3	1				4
124				4	4	1			9
126				3	2	3			8
128				1	4				5
130				2	4	2			8
132				6	7	6			19
134				3	4	6	1		15
136					7	9	2		18
138					3	8			11
140					8	14	2		24
142					4	6	2		12
144					1	12	1		14
146						1	2		3
148						3	1		4
150						3	1	1	5
152							3	1	4
154							2	2	4
156						2			2
158							3		3
160							2	1	3
162								1	2
164									
166									
168							1		1
170									
172							1		1
174								1	1
176									
178							1		1
180									
182									
184								1	1
186									
188								1	1
Mean			88.6	118.2	131.5	139.2	151.2	164.2	
SD			3.6	13.0	8.2	6.3	11.3	14.3	
Number			7	43	57	76	25	9	218

Age and growth

Otoliths of silver scabbarfish were thin and show clearly the growth rings. Of the 336 otoliths removed only 317 otoliths could be examined, 245 (77.2%) were readable and used for the age and growth study. Fish from age 1 to 7 years were found for females (Table 5) and from 2 to 5 years for males. The von Bertalanffy growth parameters estimated for females were (n= 218) L_∞= 186.90 cm FL, k= 0.23 $year^{-1}$ and t_o= -1.23; and for whole population were (n= 245) L_∞= 191.23 cm FL, k= 0.23 $year^{-1}$ and t_o= -1.04 year.

Discussion

The macroscopic and histological study of the gonad in silver sccabarfish population from the Canary Islands allowed to conclude that it is a gonochoristic and indetermined species (spawns may take place many times during a protracted spawning season) where the females expelled out the oocyte by clutches. These acts of spawning in time is reflected in the gonad morphology where remaining of hydrated eggs were observed in gonads in the reproduction stage. This is the reason because the macroscopic scales used on date for females had not correctly described the gonad morphology. Orsi Relini et al. (1989) noted this characteristic in samples from the Liguria Sea, but they did not give a biological interpretation in their macroscopic scale. In general, it seems that the reproductive strategy is similar in all trichurids. Figueiredo et al. (2003) had described in Aphanopus carbo that the ovary development is also by group-synchronous.

The studies have suggested that this species spawns almost all the year round with peaks more prominent between spring and autumn (Karlovac and Karlovac 1976; Orsi Relini et al. 1989; Demestre et al. 1993; D'Onghia et al. 2000). Although it was not possible to define the reproductive peak, data on the maturity stages of the gonads also indicated that the reproductive activity in females is prolonged during the year. The number scarce of males sampled did not allow the knowledge of its reproductive strategy. This result has been described in others geographical areas of the Mediterranean (Orsi Relini et al. 1989; D'Onghia et al. 2000). Wirtz and Morato (2001) argued several options to explain this kind of sexual phenomenon: variation in spatial distribution, different response to the given hook size or bait size or differing in their feeding behaviour. D'Onghia et al. (2000) demonstrated that the fishing method (longlines and trawling) did not influence in the maximum size captured, being always the females bigger. It could be more probably that this difference is related with the species growth. Moli et al. (1990) and Demestre et al. (1993) found in slightly variations in growth rate between sexes growing slower and longer the females while D'Onghia et al. (2000) provided values of growth rate similar between sexes reaching an asymptotic length higher the females (Table 6). Moreover, data revealed that the growth performance index did not vary among geographical areas. Furthermore, it seems logical to conclude that differences in growth rates are the responsible of this sex-ratio a favour to females in all cases and presenting in all regions.

Acknowledgments

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Table 6. Comparison of growth parameters cited in the literature.

Growth parameters	Moli et al. (1990) Northwestern Mediterranean		Demestre et al. (1993) Northwestern Mediterranean		D'Onghia et al. (2000) Eastern-central Mediterranean		Present study
	Females	Males	Females	Males	Females	Males	Females
L_∞ (TL in cm)	195.4	185.2	243.9	201.8	182.5	174.3	195.1
k ($year^{-1}$)	0.207	0.333	0.142	0.226	0.30	0.31	0.23
t_o (year)	0.317	0.344	1.632	0.919	-0.50	-0.53	-1.23
Φ	3.90	4.06	3.93	3.96	4.00	3.98	3.94

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