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ANNUAL REPRODUCTIVE CYCLE AND FECUNDITY OF *ASPITRIGLA OBSCURA* (TELEOSTEI, TRIGLIDAE)

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TRIGLIDAE
ASPITRIGLA OBSCURA
REPRODUCTION
CYCLE ANNUEL
FÉCONDITÉ

RÉSUMÉ. – Dans ce travail nous analysons les changements histologiques saisonniers des gonades, différents indices relatifs à la reproduction et à la fécondité de *Aspitrigla obscura* (Teleostei : Triglidae). Les femelles sont plus grosses que les mâles et elles sont matures lorsqu'elles atteignent la longueur standard de 130 mm, tandis que les mâles deviennent sexuellement matures à 120 mm. Il existe de grandes réserves de graisse mésentérique et hépatique qui sont essentiellement utilisées pour la synthèse de la vitellogénine. La ponte multiple a lieu entre janvier et avril et produit de 7 000 à 22 500 œufs d'un diamètre de plus de 1000 µm. La fécondité de l'espèce est déterminée par la taille et le poids des individus.

TRIGLIDAE
ASPITRIGLA OBSCURA
REPRODUCTION
ANNUAL CYCLE
FECUNDITY

ABSTRACT. – In this study about *Aspitrigla obscura* (Teleostei: Triglidae) we analyse the seasonal histological changes in the gonads, various indices related to reproduction, and fecundity. The females of this species are larger than the males and mature when they reach a standard length of 130 mm, while the males become sexually mature at 120 mm. There are large hepatic and mesenteric fat reserves which are basically used in the synthesis of vitellogenin. Multiple spawning takes place between January and April, consisting of between 7000 and 22500 eggs, each more than 1000 µm in diameter. The fecundity of the species is determined by the size and weight of the individuals.

INTRODUCTION

The family Triglidae is to be found all over the world, and enjoys a certain economic importance, since most of the species are fished in abundance and are often found in fish markets (Fischer *et al.* 1987).

More specifically, the longfin gurnard (*Aspitrigla obscura* Linneus, 1764) is a benthonic species commonly encountered in the Mediterranean and the East Atlantic, although Papaconstantinou (1983) and Tsimenides *et al.* (1992) suggested that it does not inhabit Greek seas. It lives in soft and rocky substrates off the coast up to depths of 170 m (Whitehead *et al.* 1986). While some works refer to its morphology (Bini 1969) and feeding (Moreno-Amich 1996), until recently most aspects related to its reproduction were unknown.

Our previous study has already examined the gonadal structure and process of gametogenesis of this species (Muñoz *et al.* 2001), and now we aim to provide an in-depth analysis of its annual cycle studying the seasonal histological changes of the gonads and of various indices related to its reproduction. Finally, we also estimate and analyse the fecundity of this species.

MATERIALS AND METHODS

For the description of the different stages of maturity of the gonads, the ovaries and the testes were embedded in Histosec 56-58 pF (Merck) or in hydroxyethyl methacrylate, and were sectioned at between 4-10 µm, depending on the sex and the stage of maturity. Transverse and longitudinal sections were obtained for both sexes. The following stains were used for the samples kept in Histosec: haematoxylin-eosin for general histology; Mallory as a trichrome; PAS reaction (periodic acid-Schiff) for the demonstration of neutral mucopolysaccharides; and Alcian blue for acid mucopolysaccharides. The samples kept in methacrylate were stained with methylene blue-basic fuchsin, toluidine blue, and PAS as well.

The stages of development in the oocytes were determined by following the criteria established by Wallace & Selman (1981) and West (1990). The ovaries were classified according to the more developed type of oocyte. The stage of development of the testicles was determined following the criteria laid down by Grier (1981).

In order to study the indices related to reproduction, we used 335 examples of *Aspitrigla obscura* which were caught during a year-long period at various ports along the Costa Brava (Northwest Mediterranean). The fish were fixed immediately after capture in 10% formaldehyde and preserved in 4% formaldehyde. The following parameters were analysed:

– Sexual dimorphism. The total standard lengths and weights of the males and females were compared by means of an analysis of variance (ANOVA).

– The size at sexual maturity. This is achieved macroscopically, by observing the gonads during the spawning season of the species.

– Sex Ratio (SR = Number of males / Number of females) of the total population and of the specimens classified by size, as the monthly variations of this index. We looked to see if the result was significantly different from 1 by means of the χ^2 test.

– The gonadosomatic index (GSI = weight of gonad × 100 / eviscerated weight), the hepatosomatic index (HSI = weight of liver × 100 / eviscerated weight), and the condition factor (K = eviscerated weight × 100 / standard length^3). We also calculated the mesenteric fat index (MFI = weight of fat × 100 / eviscerated weight), which complements the information offered by the previous indices. It was only measured in those individuals that had visible accumulations of fat between the viscera and the mesentery, with the assumption that the lipids stored in other parts of the body change in a similar way (Delahunty & de Vlaming 1980).

All the indices were calculated as a function of the eviscerated weight, in order to avoid possible variations arising from differences in the digestive tract contents or energy reserves of the specimens. We calculated the indices, separating the fish by sex and month of capture. Later, we observed any significant differences in the monthly variations by means of an analysis of variance (ANOVA). All the statistical analyses in this section have been carried out in line with the criteria set out by Sokal and Rohlf (1995) with the group of statistical programmes of the SPSS for Windows 7.5. The significance levels are expressed as follows:

n.s. = not significant differences ($\geqslant 0.05$)

* = significant differences (<0.05)

** = highly significant differences ($\leqslant 0.001$)

Fecundity was estimated in 31 females by means of the gravimetric method (Burd & Howlett 1974, Hunter *et al.* 1985). The separation of the oocytes was achieved by introducing samples of completely mature ovaries in Gilson's solution, as modified by Simpson (1951). The eggs were then filtered and, once sorted into different diameters, they were counted. We repeated the process twice for each ovary. The individual, or absolute, fecundity refers to the number of eggs produced per female per year (Wootton 1979), and can be defined as the number of mature oocytes present in the ovary immediately before spawning (Bagenal 1973). Where species utilise multiple spawning, it is the number of oocytes destined for spawning, i.e., the ones that will mature during the current reproductive cycle, which are usually taken into account (Aboussouan & Lahaye 1979). And so we only counted those with an equal or greater diameter than the oocytes at the cortical alveoli stage, since it is considered that these are the ones due to be released for spawning in the current reproductive cycle. This absolute fecundity tends to increase in line with the size and age of the fish, and therefore, in order to facilitate the comparison, we also calculated the relative fecundity, i.e. the number of eggs per unit eviscerated weight (Bagenal 1978). In order to study the relationship between the fecundity and the size or the total weight of the individual, we used the linear regression analysis by means of the logarithm $\log Y = \log a + b \cdot \log X$. This is calculated using the least squares method and corresponds to an exponential function of the type: $Y = a \cdot X^b$. The significance levels are the same as we described above. Finally, we also determined the frequency distribution of the egg diameters.

RESULTS

Seasonal histological changes in the gonads

Testicles

The *spermatogonial proliferation period* occurs between April and May. The lobular lumens only contain a few spermatogonial cysts here and there on the periphery, these always being enclosed in the Sertoli cells which cover the inside of the seminiferous lobule. Some residual spermatozoa can be detected. From June the testicles enter to the *early recrudescence period*: the first spermatocytes appear, although they are still more scarce than spermatogonia. Between August and December, the testicle is in the *mid recrudescence period* and contains germinal cells in all the stages of development: spermatogonia, spermatocytes and spermatids. Already little groups of free spermatozoa can be seen in the lobular lumen. From October onwards, the lobules of some specimens still show all the cited stages, but also spermatids in various stages of development and spermatozoa: the testicle is in the *late recrudescence period.* During the *functional maturity period,* which occurs from January to April, the lobules and all the ducts are full of sperm.

Ovaries

Throughout the period from May to October the ovary is in the *period of previtellogenesis*: the presence of oogonia and oocytes in various stages of development can be detected, without, however, any vitellogenic oocytes present. The first oocytes in the phase of formation of cortical alveoli appear in August. The appearance of the yolk granules and their progressive growth can be observed between November and December: when the ovary is in the *vitellogenic period.* During the *period of maturation*, between January and April, a lot of mature oocytes which are full of yolk granules, as well as oocytes with migrated germinal vesicle, and hydrated oocytes are detected. The ovary also contains postovulatory follicles, so it can therefore be

assumed that spawning takes place within this period. At the end of this period some ovaries show a lot of atretic oocytes.

Reproductive indices

Table I shows the mean values obtained for the standard lengths and total weights of the males and females of *Aspitrigla obscura.* There are highly significant differences between the values obtained for the two sexes: as we have said, females are bigger than males. On the other hand, the males of the longfin gurnard are sexually mature when they reach a standard length of 120 mm, while the females mature at a larger size, beginning at a standard length of 130 mm.

The annual and monthly sex ratio values are shown in Table II: 61.2% of the 335 individuals are female and the remaining 38.8% male, so the sex ratio is 0.6 – a highly significant difference from 1 (χ^2=16.791, g.d.l.=1, p=0.000), although there are no significant differences in most of the sex ratios. The analysis of the sex ratios in relation to size is shown in Table II. Significant differences between the values obtained for the two sexes start from the standard length of 14 cm.

Table I. – Standard length (mm) and total weight (g) of *Aspitrigla obscura.*

	n	mean ± SE	minimum	maximum	SD	significance
SL						
males	130	138,2 ± 1,6	84,0	191,0	16,68	
females	205	154,5 ± 1,5	103,0	204,0	22,32	**
total	335	148,1 ± 1,2	84,0	207,0	22,42	
TW						
males	130	41,1 ± 1,5	7,1	85,8	16,67	
females	205	61,4 ± 1,9	15,8	152,8	27,40	**
total	335	53,5 ± 1,4	7,1	152,8	25,76	

Table II. – Top, monthly sex ratio values in *Aspitrigla obscura*. Bottom, sex ratio values by size in *Aspitrigla obscura.*

	males		females			
	n	%	n	%	χ2	significance
January	20	43,5	26	56,5	0,783	n.s.
February	8	23,5	26	76,5	9,529	*
March	10	47,6	11	52,4	0,048	n.s.
April	6	35,3	11	64,7	1,471	n.s.
May	7	33,3	14	66,7	2,333	n.s.
June	8	28,6	20	71,4	5,143	*
July	18	40,9	26	59,1	1,455	n.s.
August	10	58,8	7	41,2	0,529	n.s.
September	11	44,0	14	56,0	0,360	n.s.
October	6	28,6	15	71,4	3,857	n.s.
November	15	45,5	18	54,5	0,273	n.s.
December	11	39,3	17	60,7	1,286	n.s.
annual	130	38,8	205	61,2	16,791	**

Size	males		females			
(cm)	n	%	n	%	χ2	significance
≤ 9	1	100,0	0	0,0	-	-
9,1 - 10	2	100,0	0	0,0	-	-
10,1 - 11	8	72,7	3	27,3	2,273	n.s.
11,1 - 12	12	46,1	14	53,9	0,154	n.s.
12,1 - 13	21	63,6	12	36,4	2,455	n.s.
13,1 - 14	28	52,8	25	47,2	0,170	n.s.
14,1 - 15	23	36,5	40	63,5	4,587	*
15,1 - 16	22	43,1	29	56,9	0,961	n.s.
16,1 - 17	9	22,5	31	77,5	12,100	**
17,1 - 18	2	8,7	21	91,3	15,696	**
18,1 - 19	1	5,5	17	94,5	14,222	**
≥ 19,1	1	7,1	13	92,9	10,286	**

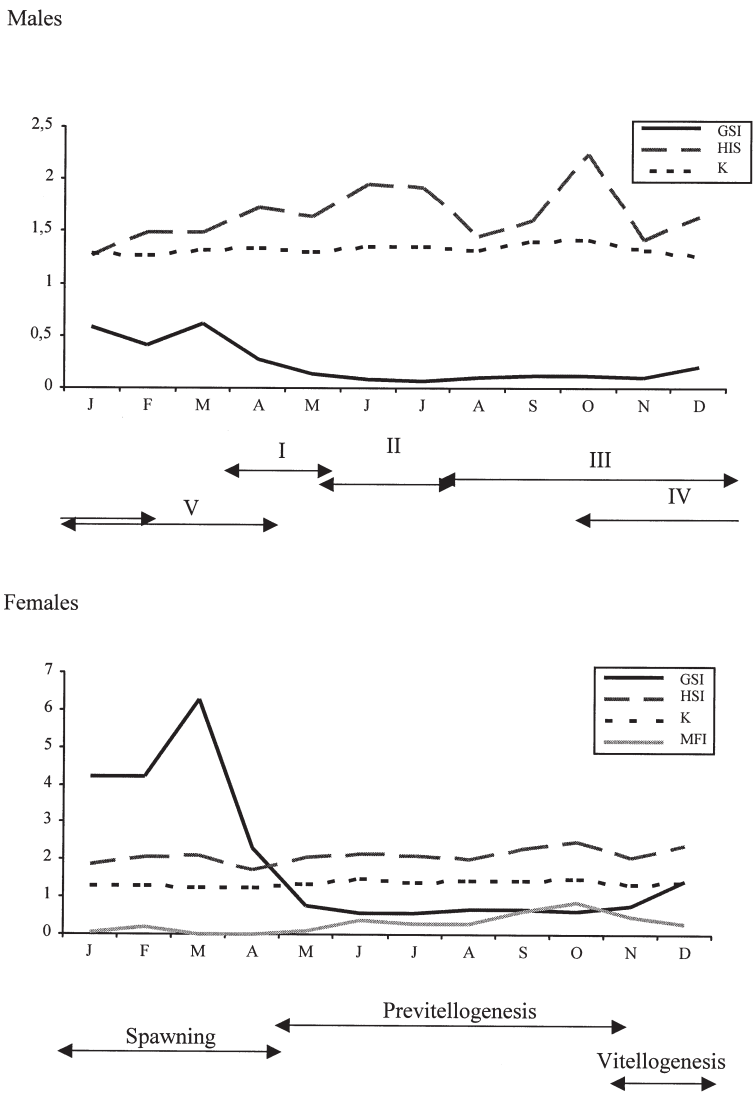

Fig. 1. – Annual cycle of *Aspitrigla obscura*. The graphs show the annual development of the indices with statistical significant monthly variations related to reproduction. Below graphs are details of the stage which the gonads are going through. In the case of the testicles, there are the following stages: (I) spermatogonial proliferation, (II) early recrudescence, (III) mid recrudescence, (IV) late recrudescence, and (V) functional maturity.

Figure 1 shows the annual development of the various indices we analysed, in relation to which phase the gonad is in. The gonadosomatic index (GSI) shows highly significant differences between different phases for both sexes (ANOVA, p=0.000), and the maximum values for males and females coincide – appearing between January and March. For the hepatosomatic index (HSI), which also shows significant monthly changes, the highest values appear in October in males (ANOVA, p=0.003), and from September to December in females (ANOVA, p=0.044). The condition (K) of the individuals we studied showed highly significant differences among the females and significant differences among the males (ANOVA, p=0.000 and p=0.032, respectively). In both sexes, the maximum values appear during the summer and the beginning of autumn, and the minimum values are detected after spawning, between March and April. Finally, although the monthly variations of the mesenteric fat index (MFI) cannot be considered statistically significant for males, they are significant for females (ANOVA, p=0.012). Mesenteric reserves begin to be accumulated in May reaching their peak in October, and then drop sharply during spawning.

Fecundity

The distribution of egg diameter frequency and fecundity are shown in the Table III. The relationship between the absolute and relative fecundity with the size and total weight of the specimens was significant in all the cases.

Table III. – Frequency distribution of eggs by diameter (mm) and fecundity of *Aspitrigla obscura*. M=month; SL=standard length (mm); TW=total weight (g); EW=eviscerated weight (g); GW=gonadal weight (g).

	M	SL	TW	EW	GW	D. 0,25	D.0,3	D. 0,4	D. 0,5	D. 0,6	D. 0,7	D. 0,8	D. 0,9	D. 1	F abs.	F rel.
1	12	169	76,9	67,7	1,0477	3579	3021	505	147	53	47	0	0	0	7352	108,6
2	12	174	86,0	75,6	1,2489	4436	4388	4914	213	93	0	0	0	0	14044	185,8
3	12	147	55,4	47,8	1,1612	2937	3237	2921	2947	210	100	0	0	0	12352	258,4
4	1	169	85,1	70,3	2,6330	2414	6534	3897	1091	398	131	63	0	0	14528	206,6
5	1	176	87,2	72,8	4,3586	1621	4189	2323	1603	591	286	234	170	0	11017	151,3
6	1	191	108,0	95,0	5,2421	1360	4949	4348	2538	1633	373	102	152	0	15455	162,7
7	1	164	71,9	60,0	3,1320	1116	2232	4128	2552	773	209	0	0	0	11010	183,5
8	1	174	76,8	66,1	3,7118	2321	2654	2672	6768	702	113	0	0	0	15230	230,4
9	1	162	67,4	56,4	4,8417	3287	2675	2737	4137	2731	75	0	0	0	15642	277,3
10	1	188	97,1	85,6	5,1146	1936	2702	3943	4519	2234	202	119	0	0	15655	182,9
11	1	172	81,3	67,9	5,5398	493	1705	2205	2198	3487	282	320	513	57	11260	165,8
12	2	131	32,3	27,7	2,1449	3861	3122	3050	2261	1094	155	0	0	0	13543	488,9
13	2	153	58,5	47,3	2,7560	1142	2289	4426	1636	773	108	159	136	57	10726	226,7
14	2	156	51,9	43,9	2,8667	1897	2227	2630	1806	1250	113	56	147	113	10239	233,2
15	2	186	105,0	88,4	6,6336	700	1660	1966	1360	1140	107	53	93	0	7079	80,1
16	2	169	72,4	58,3	3,5432	1576	2747	3017	1717	1153	141	141	247	117	10856	186,2
17	2	198	133,0	113,7	8,1595	2267	4316	6788	3563	1373	634	464	781	21	20207	177,7
18	2	170	76,2	62,1	4,9000	1225	1775	2156	1837	312	56	25	137	193	7716	124,2
19	2	171	66,3	55,5	4,7368	1808	1685	3080	1154	2061	197	37	234	308	10564	190,3
20	3	147	40,0	34,4	1,2185	2984	2149	1835	1010	362	186	37	0	0	8563	248,9
21	3	131	32,1	27,7	1,0511	3721	2095	1505	926	363	62	31	105	0	8808	317,9
22	3	140	40,3	34,0	1,8283	3695	2473	2418	1766	576	255	59	108	0	11350	333,8
23	3	162	63,6	47,3	5,5686	3294	2904	2615	1730	1132	89	102	423	192	12481	263,8
24	3	137	37,9	32,4	2,1318	2922	2078	2894	1587	733	250	116	50	0	10630	328,1
25	3	138	40,5	33,5	1,6607	2972	2706	3538	2701	804	385	181	97	0	13384	399,5
26	3	207	138,0	114,3	9,8866	4895	4582	5768	4132	2574	515	37	22	0	22525	197,1
27	3	143	44,6	37,1	2,1007	2731	2084	2626	2963	1784	110	51	0	0	12349	332,8
28	3	178	76,4	63,1	4,5375	2792	2359	2122	2823	1798	152	0	24	0	12070	191,2
29	4	150	45,0	40,0	1,1081	2368	1884	1400	652	173	79	263	0	0	6819	170,5
30	4	146	38,1	34,3	1,1656	3242	2384	1558	1879	300	147	0	0	0	9510	277,2
31	4	153	50,2	43,3	2,0102	3120	1219	1797	2895	835	159	31	0	0	10056	232,2

DISCUSSION

In the *Aspitrigla obscura* population studied, the number of females is much greater than that of the males, especially in those of larger sizes. In general, the variations in the sex ratio at different sizes are related to unequal rates of growth and mortality (Turner *et al.* 1983), although a supplementary analysis of more samples of each size would be advisable in order to corroborate the obtained results. The morphometric analysis carried out shows that the females of *A. obscura* are larger and have a larger size at sexual maturity than the males; this data also suggests that growth is greater in individuals of the female sex. The triglids are characterized by fast growth and long life (Booth 1997), and Baron (1985a) states that a species from the same genus, *A. cuculus*, can reach the 21 years of life and that the maximum size of the females is greater than that of the males. In general, it is considered that a greater number of females is advantageous since, as pointed out by Wootton (1982), populations with a sex ratio biased towards the females have a greater rate of reproduction.

The energy used up in reproduction is clearly seen in the poor condition in which the females are found at the end of the reproductive period: the energy that is normally used for maintenance and growth of the individual is diverted towards the production of eggs. Even so, both the number of prey eaten by these fish and the mean weights of this prey are at maximum levels in the spring (Moreno-Amich 1996), in such a way that these good feeding conditions allow for the recovery and the storage of fat reserves detected in the examples captured from May onwards.

In August, and coinciding with the appearance of the first cortical alveolar oocytes in the ovaries and the formation of the first spermatozoids in the testicles, the activity of the gonads begins to increase progressively. From autumn onwards, the fish are in very good condition and both the hepatic and mesenteric reserves reach their highest levels. The HSI levels are higher in the females, but this is a common feature of many species (Bruslé & González 1996). The transitory increase in the weight of the liver before the rapid increase in the weight of the ovary is also detected in *A. cuculus* (Mouneimné 1971). The explanation lies in the fact that the liver usually has an important role in the

processing of fatty acids, mobilised from the muscles, before being transferred to the ovary. The beginning of vitellogenesis in the females and the beginning of the late recrudescence phase in the testicle in the males will bring with it a subsequent decrease in these reserves.

In December, the gonadosomatic index increases sharply reaching maximum levels that are prolonged from January until April, coinciding with the spawning period. This progressive development with its various peaks is typical of multiple spawning species (Rinchard & Kestemont 1996). The only data known so far with reference to the spawning of the longfin gurnard is in full agreement with the results we obtained: i.e. the capture of males and females fully mature in the month of March (Marinaro 1968). The development of the condition factor shows that, while the females do not begin their recovery until after the spawning, the condition of the males begins to improve progressively from January onwards. Thus, the greatest energy use in the males takes place when the high production of spermatozoids coincides with the development of cysts, that is to say, when the testicle has entered the phase of late recrudescence and not when functional maturity occurs.

The number of eggs produced by *Aspitrigla obscura* that mature in each reproductive cycle ranges from approximately 7,000 to 22,500. The distribution of eggs per frequency of diameter indicates that the release of the most advanced oocytes is followed by the development and release of the second group of oocytes, confirming, therefore, that the longfin gurnard is a multiple spawning species. In fact, the hydrated oocytes increase in volume so much that the ovary can hardly contain all of them in this state and they have to be released successively, although this occurs at short intervals of time. The absolute and relative fecundity of the species studied here increased with the increase in size and weight of the individuals.

Comparing two examples of the same size, one captured at the beginning and the other at the end of the reproductive period (individuals nº3, captured in December and nº30, captured in April), it can be seen that the total number of eggs is smaller in the latter, a characteristic feature of species with determinate fecundity (Greer Walker *et al.* 1994). At the same time, the greater relative fecundity is clearly determined by the worse condition of the second individual, since its eviscerated weight is very much lower than that of the individual of the same size captured at the beginning of spawning. In fact, the annual development of the condition factor already shows that at the end of the reproductive period the fish is in its worst condition and, according to Rothschild (1986), the nutritional state of the individual is the principle factor influencing fecundity.

Some of the females we analysed contained eggs of a diameter even greater than that detected in histological slices (Muñoz *et al.* 2001). This is probably because the more mature oocytes get separated from the ovarian tissues and float free in the lumen, hence they do not appear in the histological slices. Even so, the eggs of the longfin gurnard analysed by Marinaro (1968) have an even larger diameter, between 1200 and 1280 μm. Although it is known that Gilson fluid can reduce the diameter of oocytes (Lowerre-Barbieri & Barbieri 1993), it has been demonstrated that for various species of Triglidae, the differences in size caused by the Bouin and Gilson fluids are negligible (Baron 1985b). It should be noted that the light of the largest filter used is 1000 μm, so it is not known whether these larger eggs were retained by the largest filter or whether the maximum diameter measured really was smaller than that which appears in the natural environment. The second option appears to be unlikely, since according again to Baron 1985b, the eggs of Triglidae swell inside the female genital tract before being laid, rather than doing so in contact with the marine environment as occurs in most of the teleost species.

The large number of oocytes that, in April, still remain in the initial state of vitellogenesis suggests that either the process of maturation is extremely fast or that there is a generalised atresia at the end of the period of reproduction. Whereas there is no data about the rate of maturation in this species, some of the post-spawning ovaries analysed in this study showed a considerable amount of atretic oocytes. We probably did not detect atresia in more ovaries because it seems to be a quick process. Not many attempts have been made to estimate the time needed for a follicle to disappear by atresia in teleosts, but the knew rates are always high: eight days for the alpha atresia in *Engraulis mordax* (Hunter & Macewicz 1985), ten days for *Gadus morhua* (Kjesbu *et al.* 1991) or eleven days for *Poecilia reticulata* (Lambert 1970), for instance. In spite of generalised atresia is a phenomenon usually associated with fish that have an indeterminate fecundity (Hunter & Macewicz 1985), in *A. obscura* it seems that the excess of vitellogenic oocytes which are produced during spawning can also become atretic towards the end of spawning and finally be absorbed.

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