

Reproduction and gonad histology of *Aidablennius sphyinx* (Pisces: Blenniidae) of the Catalan Sea (northwestern Mediterranean)*

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SUMMARY: The reproductive biology of the Mediterranean blenny fish *Aidablennius sphyinx* was studied on the basis of microscopic and macroscopic analysis. *A. sphyinx* is a resident species of the shallow waters of this region, lays demersal eggs and provides parental care to the developing embryos. Mature male gonads occur between March and July, whereas mature females appear between April and August. Male gonads are composed of the testis and the testicular gland. Spermatozoa are released into the testicular gland. The testicular gland is relatively more prominent than the testis after the reproductive season. Six stages of oocyte development (including atretic ones) are considered. Seasonal variations of gonads of both males and females are illustrated. Low fecundity was recorded, as the ovaries may contain 432 to 1682 oocytes according to female size. Equations describing the relationship between gonad weight and length and weight of males and females are presented. Equations describing the relationship between absolute individual fecundity and length and weight of females are also presented.

Key words: *Aidablennius sphyinx*, reproduction, gonad histology, fecundity, Catalan Sea.

RESUMEN: REPRODUCCIÓN E HISTOLOGÍA GONADAL DE *AIDABLENNIUS SPHYNX* (PISCES: BLENNIIDAE) EN EL MAR CATALÁN (MEDITERRÁNEO NOROCCIDENTAL). – La biología reproductiva del blénido mediterráneo *Aidablennius sphyinx* ha sido estudiada en base a un análisis macroscópico y microscópico. *A. sphyinx* es una especie residente de las aguas someras de esta región, pone huevos demersales y proporciona cuidado parental a los embriones en desarrollo. En machos se encuentran gónadas maduras entre marzo y julio, mientras que en hembras aparecen entre abril y agosto. Las gónadas masculinas están compuestas por el testículo y la glándula testicular. Después de la estación reproductiva, la glándula testicular es relativamente más prominente que el testículo. Se han considerado seis estadios de desarrollo ovocitario (incluyendo el estado de atresia). Se han ilustrado las variaciones estacionales de las gónadas de machos y hembras. Se ha registrado una baja fecundidad, ya que los ovarios pueden contener de 432 a 1682 ovocitos, dependiendo del tamaño de la hembra. Se han presentado las ecuaciones que describen la relación entre el peso de las gónadas y la longitud y el peso de los machos y hembras, respectivamente. Asimismo, se han descrito las ecuaciones que relacionan la fecundidad absoluta con la longitud y peso de las hembras.

Palabras clave: *Aidablennius sphyinx*, reproducción, histología gonadal, fecundidad, mar Catalán.

INTRODUCTION

Biologists have long been familiar with the maturation or so-called ripening of the gonads of teleost fishes and the subsequent onset of the spawning

period. The reproductive biology of several species of blennies and their close relatives of the northeastern Atlantic and the Mediterranean and adjacent seas have been studied from a variety of viewpoints. Many studies have been published that describe the reproductive behaviour of blenny species in their natural habitat or in captivity (Almada and Santos,

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1995; Almada *et al.*, 1990; Fishelson, 1975; Gibson, 1969; Guitel, 1893; Phillips, 1977; Qasim, 1956; Stephens *et al.*, 1966). Blennid fishes lay demersal eggs and provide parental care to the developing embryos (Gibson, 1969). Other studies have provided a description of gonad development macroscopically (Ruchon *et al.*, 1993) or microscopically (Lahnsteiner and Patzner, 1990a, 1990b; Lahnsteiner *et al.*, 1990; Santos, 1995).

Aidablennius sphynx (Valenciennes, 1836) is an endemic species of the Mediterranean Sea that lives on rocky shores from 0 to 1.5 metres depth with a distribution extending to the northern Atlantic coast of Morocco (Zander, 1986) (Illich and Kotschal, 1990). The species is sexually dimorphic. Adult males reach 78 mm in size (maxim total length), whereas adult females reach 55 mm (Kraak, 1996a). In particular, *A. sphynx* is a common benthic littoral species of blenny from the northwestern Mediterranean Sea (Macpherson, 1994). Studies on reproductive behaviour in its natural habitat and in the laboratory (Guitel, 1893; Gibson, 1969; Kraak and Videler, 1991; Kraak and Van Den Berghe, 1992; Kraak and Weissing, 1996; Kraak and Groothuis, 1994; Kraak 1996a, 1996b), on larval development (Sabatés, 1994), and on the structure of the testicular gland, testis and spermatic duct (Lahnsteiner and Patzner, 1990 a, 1990b) have been carried out. Histological studies, while time-consuming, yield the most reliable, objective information on spawning cycles (West, 1990). Reproductive studies of fishes, such as assessment of size at maturity, duration of the spawning season and fecundity, require knowledge of the state of gonad development in individual fish.

In this paper we provide a description of gonad development of *A. sphinx* in the Catalan Sea (northwestern Mediterranean). The histology of the gonads is examined in relation to the process of maturation and reproductive effort. Oocyte development stages are identified to describe the previtellogenesis and vitellogenesis phases. The reproductive style and annual cycle are also evaluated using the gonadosomatic index (GSI) and fecundity.

MATERIAL AND METHODS

A total of 124 fishes were sampled between March 1993 and December 1995 (Table 1) along the coast of north-east Spain (Girona). Sampling was carried out by snorkelling. The animals were killed with an overdose of the anaesthetic MS222 and

TABLE 1. – Sampling date and number of specimens collected for *Aidablennius sphynx* in the present study. Unid. = unidentified.

| Month | Unid. | Males | Females | Total |
|-----------|-------|-------|---------|-------|
| February | | 1 | 2 | 3 |
| March | | 4 | 4 | 8 |
| April | | 8 | 5 | 13 |
| June | | | 3 | 3 |
| July | 2 | 2 | 5 | 9 |
| August | 7 | 12 | 15 | 34 |
| September | 5 | 24 | 15 | 44 |
| October | | | 1 | 1 |
| December | 1 | 6 | 2 | 9 |
| | 15 | 57 | 52 | 124 |

fixed in 10% buffered formalin immediately after capture (after opening the abdominal cavity to facilitate fixation). Once in the laboratory, they were measured (total length: TL, to the nearest mm) and dissected. The gonads and liver were obtained from recently dead fishes. Their weight was recorded to the nearest 0.1 mg. The eviscerated body weight (W) was also taken to the nearest mg.

In the females, no differences in the left and right ovaries size were found. Indistinctly, one ovary was assigned for histological study and the other for fecundity analysis. In the males a gonad was used for histological study.

One gonad fixed in formalin was dehydrated in alcohol and xylol, and infiltrated with paraffin. Sections between 6 and 10 μ m were stained with the eosine haematoxiline dye. Development of the gonads was described from histological preparations of the gonads of a total of 48 males and 48 females.

The ovaries of 15 females (36 to 53 mm TL) fixed in formalin, caught from April to August, were used to estimate fecundity. To estimate the absolute fecundity, all of one ovary of each female was used. It was damp dried on filter paper and weighed, and the number of the oocytes in the vitellogenic and ripe stage contained counted. An estimate of the total number of vitelline oocytes in both ovaries could then be made (the proportional number relative to the weight of two ovaries). Relative fecundity was also calculated (absolute fecundity / total length). Relationships between fecundity, total length, eviscerated weight and gonad weight were derived by regression analysis. According to the methodology of Childress *et al.* (1980) and Davis (1982) the diameter of the 10 largest oocytes of each ovary in all females was measured to obtain the average maximum diameter. The diameter of eggs was corrected by the shrinkage of eggs in formalde-

hyde solution (2%) (West, 1990). The average maximum diameter provides useful descriptive information on the ovarian maturation and on the duration of the spawning season.

The gonadosomatic index (gonad weight x 100 / eviscerated weight) was computed to quantify changes in gonad size related to the month. Temporal variations in gonad maturity and liver weight of males and females were investigated for data pooled by months by means of analysis of variance (one

way ANOVA). When the original data did not fit the assumptions of ANOVA (normality and homogeneity of variances) (IGS in females, liver weight in males), we transformed the data. If it has been found empirically that the standard deviation (σ) of the untransformed variable (IGS in females, liver weight in males) is a particularly potential function of the mean value (μ) ($\sigma = a \cdot \mu^b$), we can obtain an appropriate transformation, to stabilize variance, by using the transformed variable:

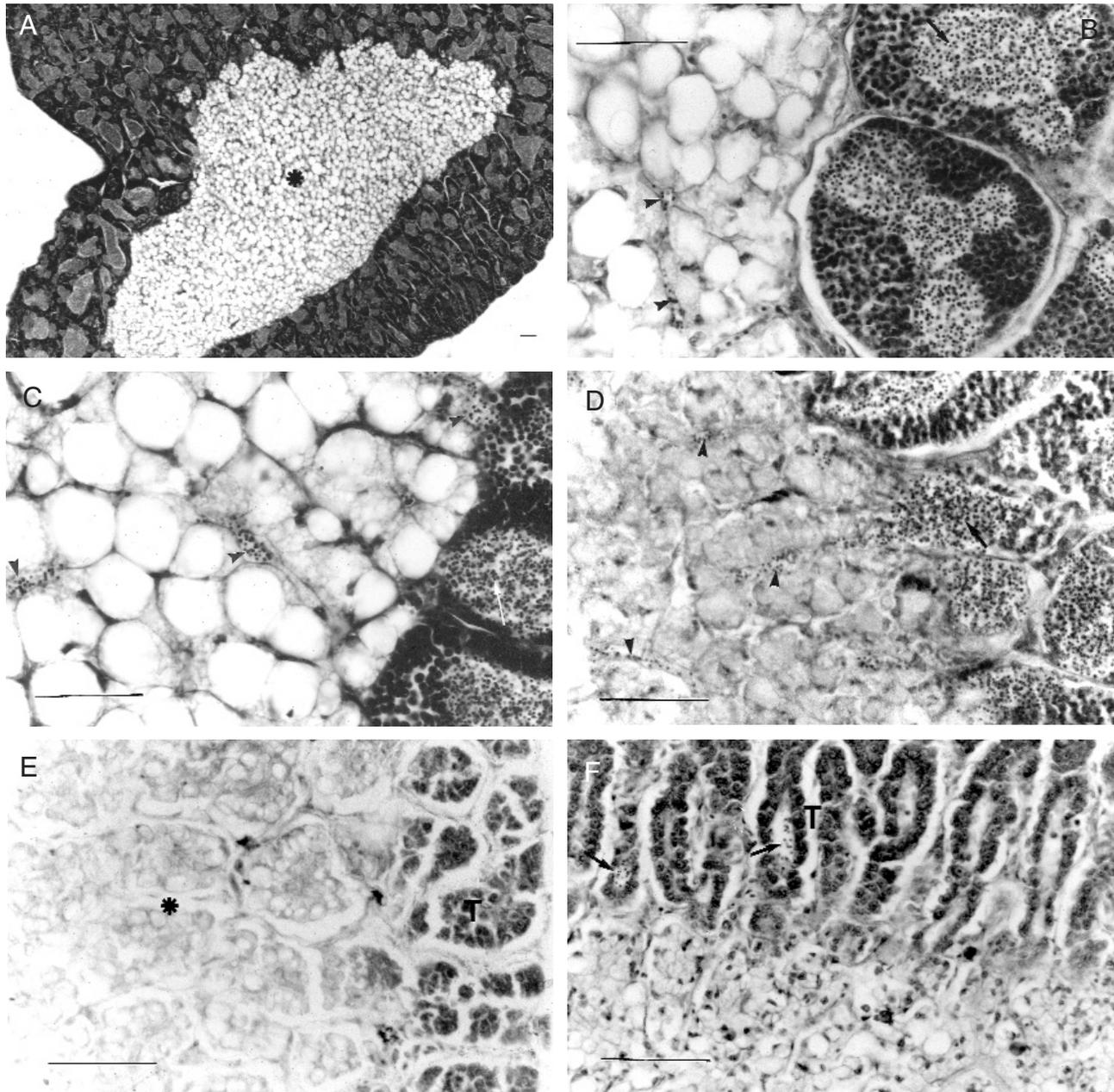


FIG. 1. – Histology of male gonads: (a) Testis and testicular gland (*) of a male from April. (b) Male from March, showing an abundance of spermatids in the testis (→) and some in the seminiferous tubules into the ducts of the testicular gland (▶). (c) Male from April, showing numerous spermatids in the seminiferous tubules (▶). (d) Male from July, still showing spermatids in the testicular gland. (e) Male from August, testis (T) looks like a fine band over the gland (*). (f) Male from December, gonad recovery is initiated. (Scale: 50 μ m).

If $b \neq 1$, $g(x) = x^{1-b}$

If $b = 1$, $g(x) = \ln x$ (Draper and Smith, 1981).

The mean values of the transformed data were then compared by analysis of variance, followed by a Scheffé multiple-comparison test.

Furthermore, mean values of the transformed diameter of the larger eggs ($\bar{O}^{1/2}$) of maturity females were investigated (one-way ANOVA and a posteriori Scheffé test) for data pooled by months.

RESULTS

Males

Male gonads are in different stage of maturity throughout the year. The gonad weight (GW) range is between 0.0001 and 0.0388 g.

Male gonads are composed of two main and very distinctive components, the testis and the testicular gland. The testicular gland is situated in the middle of the testis, surrounded by it (Fig. 1a). It is composed of tubuli, which are separated by cell membranes. We observe well-developed gonads during the months March to July, and testes are full of cells in different stages of spermatogenesis.

In March (maturing) the testis has spermatocites and spermatids. The testicular gland has numerous large lipid vacuoles in the luminal cell regions (Fig. 1b). The spermatids are released from the seminiferous tubules into the ducts of the testicular gland. In April (ripe and spawning) the gonad is at its maximum stage of development, and in the testis and in the ducts of the testicular gland the spermatid number is greater than in March (Fig. 1c). In July, spermatids can still be seen (Fig. 1d), and the lipid vacuoles have a smaller diameter. In

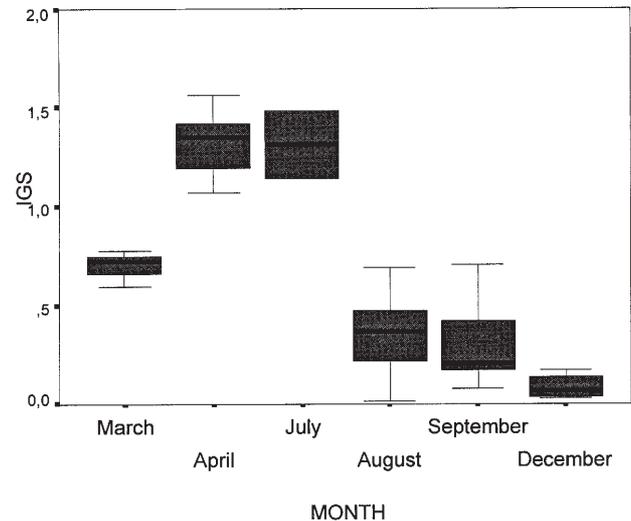


FIG. 2. – Evolution of male gonad GSI during the sampling period. Lines show full range, bars show 25-75% quartile range.

August and September (post-spawning) the testis and testicular gland shrink substantially. The proportional relationship between the testicular gland and the testis is clearly distinct after the reproductive season, the testicular gland being relatively more prominent than the testis in August and September. The testis looks like a fine band over the gland and mainly spermatogonium can be observed in the testis. In this period, the lipid vacuoles in the testicular gland are not visible (Fig. 1e). In December (recovering), though a significant increase in gonad weight cannot be observed, recovery of the gonad is initiated histologically and expediting of spermatogenesis can be observed again. In the seminiferous tubules spermatocytes begin to accumulate and in the luminal cell regions of the testicular gland the lipid vacuoles can be seen again (Fig. 1f).

TABLE 2. – Relationship between total length (TL) and gonad weight (GW), eviscerated weight (EW) and gonad weight, total length and fecundity (F), eviscerated weight and fecundity, and gonad weight and fecundity. Indep = independent variable; Dep = dependent variable.

| | Variables Indep-Dep | N | r ² | p | Equation regression |
|----------------|------------------------|----|----------------|-------|---|
| ♂ annual | TL-GW | 51 | 0.56 | 0.000 | $GW = 1.8 \times 10^{-10} \times TL^{4.2948}$ |
| ♂ annual | EW-GW | 50 | 0.82 | 0.000 | $GW = -0.0041 + 0.0126 \text{ EW}$ |
| ♂ adults April | TL-GW | 8 | 0.98 | 0.000 | $GW = 7.0 \times 10^{-8} \times TL^{3.0787}$ |
| ♂ adults April | EW-GW | 8 | 0.93 | 0.000 | $GW = 0.0027 + 0.0115 \text{ EW}$ |
| ♀ annual | TL-GW | 46 | 0.59 | 0.000 | $GW = 1.4 \times 10^{-12} \times TL^{6.1068}$ |
| ♀ annual | EW-GW | 45 | 0.53 | 0.000 | $GW = -0.0150 + 0.0764 \text{ EW}$ |
| ♀ adults April | TL-GW | 5 | 0.94 | 0.007 | $GW = 5.5 \times 10^{-10} \times TL^{4.8835}$ |
| ♀ adults April | EW-GW | 5 | 0.91 | 0.012 | $GW = -0.0317 + 0.1330 \text{ EW}$ |
| ♀ adults April | TL-F | 5 | 0.97 | 0.003 | $F = -2146.4 + 71.0700 \text{ TL}$ |
| ♀ adults April | EW-F | 5 | 0.94 | 0.007 | $F = -28.458 + 1533.03 \text{ EW}$ |
| ♀ adults April | GW-F | 5 | 0.99 | 0.000 | $F = 302.871 + 9809.36 \text{ GW}$ |

The highest gonadosomatic index (IGS) occurs from March to July (Fig. 2). Significant differences in IGS between the months analysed were found (one-way ANOVA, $F = 56.181$, $P < 0.001$). An a posteriori Scheffé multiple rank test revealed significant differences between IGS from March to July and other months analysed. No significant differences in liver weight (transformed by $LW^{1/4}$) were found (One-way ANOVA, $p > 0.05$). Based on the presence of spermatids in the gonad of the smallest individual male present in this sample, we estimated the minimum size for first maturity to be 36 mm TL. The length for first maturation at which 50% of the fish had become mature could not be determined due to the lack of sufficient data in the relevant size range.

We plotted gonad weight against fish length and fish weight for two stages (April, when gonad development is maximum, and annual) and found that they showed quite different regressions (Table 2). The gonad weight in April was best correlated with total length rather than with eviscerated weight. At all times, the determination coefficient is greater in adult males in April.

Females

The female gonad is composed of two duct-shaped ovaries that extend along the body cavity in a dorsal position above the intestines. In a single ovary there were oocytes in several stages of development. The maximum diameter of mature oocytes was 0.7 to 0.9 mm.

Due to the methodology used (sections in paraffin between 6 and 10 μm), oogonies cannot be observed. In *A. sphynx* during the reproductive season an ovary contains oocytes in all phases of oogenesis (Fig. 3b, 3c, 3d). Only a small proportion of the oocytes mature at one time. Oocytes can be divided into six stages, based on major morphological characteristics of developing oocytes and follicles. The description of the different stages of oocyte growth is given below.

1. Chromatin nucleolar stage. The small oocyte has a large nucleus surrounded by a thin layer cytoplasm. Primary oocytes exhibited a strongly basophilic cytoplasm and a prominent nucleus that contains a single, large nucleolus (Fig. 3a, 3e).

2. Perinucleolar stage. In the early perinucleolar stage (a) the oocyte is slightly enlarged and the nucleolus has split into several smaller nucleoli, which spread towards the periphery of the nucleus (Fig. 3). The cytoplasm shows uniform dark staining

(Fig. 3a, 3e). In the late perinucleolar stage (b) the oocyte is on average almost double the size of the former stage and now many small nucleoli can be seen spread around the periphery of the nucleus. Also, there are some vacuoles in the cytoplasm, whose presence usually characterises the cortical alveoli stage (Fig. 3a, 3c). Towards the end of this stage the chorion begins to form.

3. Cortical alveoli stage (yolk vesicle). This stage is characterised by the appearance of cortical alveoli in the cytoplasm; these spherical structures appear empty. The cortical alveoli increase in number to form peripheral rows. The chorion is fully formed. (It is not yolk in a strict sense since it does not serve as a nutrient source for the embryo (West, 1990). The oocyte has enlarged by 50-100% on average (Fig. 3a, 3c).

4. Vitellogenic (yolk) stage. The oocyte is once more enlarged and reaches its maximum size at the end of this stage. The cytoplasm begins to fill with yolk spheres, granules or globules, shown by wine staining with the haematoxylin and eosin method (H&E). Translucent areas at the periphery of the vitelline area that would correspond to the partial coalescence of yolk granules were not seen. The yolk spheres maintain their integrity throughout the oocyte growth, without merging into a continuous mass of fluid yolk (Fig. 3b).

5. Ripe (mature) stage. The start of this stage is indicated by the peripheral migration of the nucleus and the dissolution of its membrane. The final stage of oocyte maturation is difficult to follow because of the shrinkage and distortion of these cells during normal processing (Fig. 3c, 3d).

6. Atretic stage. This occurs when oocytes have either failed to mature or be released with their original batch and are now being reabsorbed (Fig. 3c). The atretic is usually a vitellogenic stage as observed by the acidophyllic staining. This state is a good indicator of recent spawning (within the previous two to three months) and also that the fish have reached maturity. This state is an additional evidence for multiple spawning.

Stages 1 and 2 are observed in the ovarians throughout the year (Fig. 3). Stage 3 is observed in one female in March, and in all the mature females from April to August. Stages 4 and 5 are observed in the females from April to August. In September and December the ovarians of juvenile and adult females appear to be similar (Fig. 3e). In January and February the ovarians will be similar to those of December, and immature as in March.

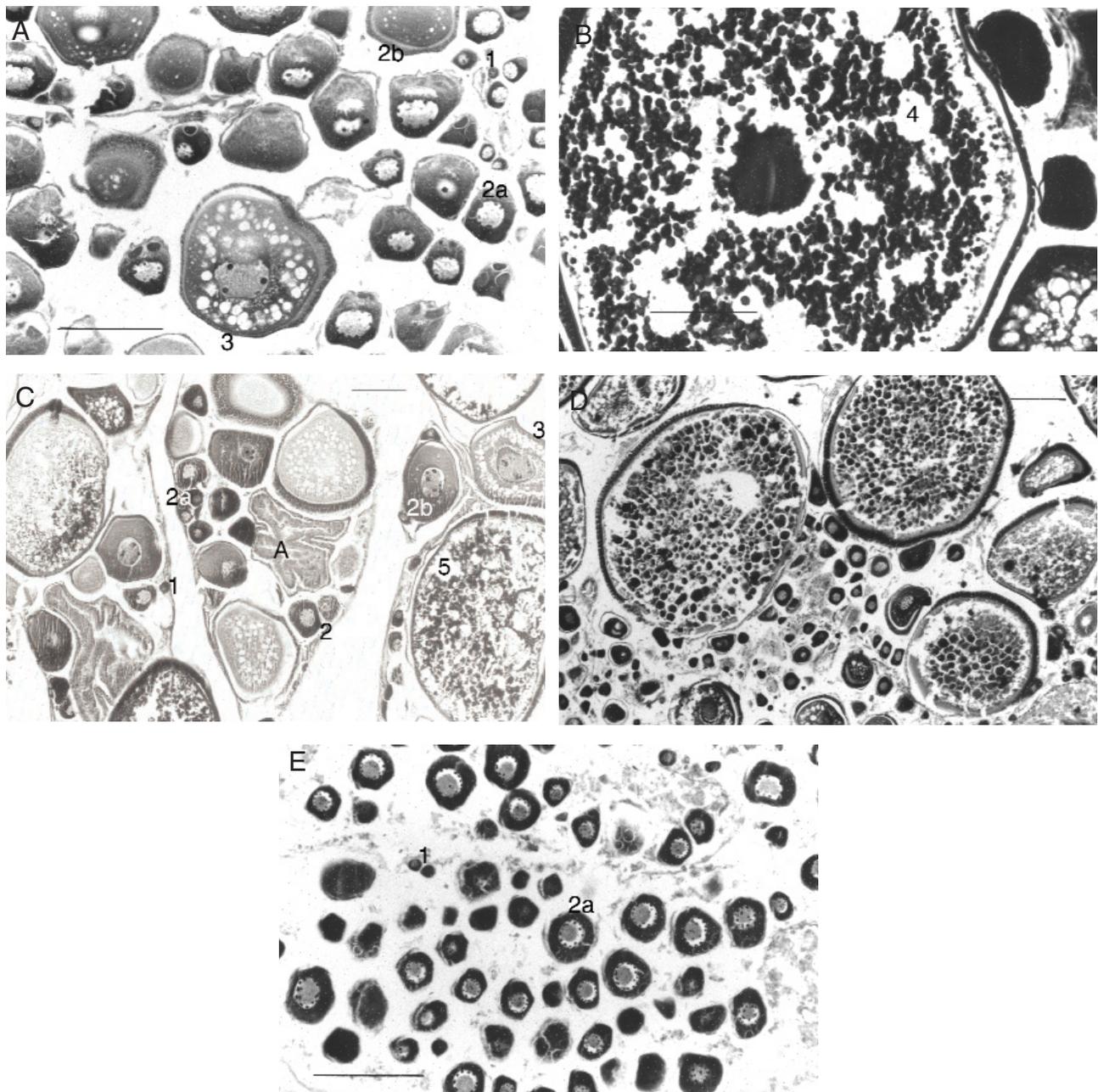


FIG. 3. – Histology of female gonads. (A) Ovary from March; only oocytes in stages 1 to 3 can be seen. (B) Ovary from April. (C) Ovary from July, ripe (5) and atretic (A) oocytes can be seen. (D) Ovary from August of a female with 37 cm TL; ripe oocytes (5) can be seen. (E) Female with 46 cm TL, from September; only oocytes in stages 1 and 2 can be seen. Stages of oocyte development: 1 = chromatin nucleolar stage; 2a = early perinucleolar stage; 2b = late perinucleolar stage; 3 = cortical alveoly stage; 4 = vitellogenic stage; 5 = ripe stage; A = atretic stage. (Scale: 100 μ m).

The gonad weight ranges from 0.0001 to 0.0546 and there was one gonad with the exceptional weight of 0.1424 g. The gonadosomatic index is greater from April to August than in the remaining months (Fig. 4), and significant differences were obtained in transformed IGS ($IGS^{1/3}$) of females larger than 36 mm TL (One way ANOVA, $F = 49.157$, $p < 0.001$). In particular, significant differences were obtained between the months April to August and March and September

(a posteriori Scheffé rank test, with $p < 0.05$). No significant differences in liver weight were met (One way ANOVA, $F = 2.435$, $p > 0.05$).

In females we also plotted gonad weight against fish length and fish weight for two stages (April, when spawning initially, and annual) and the results were similar to those of males (Table 2). The best regression is with fish length. The determination coefficient (r^2) is greater in adults of April.

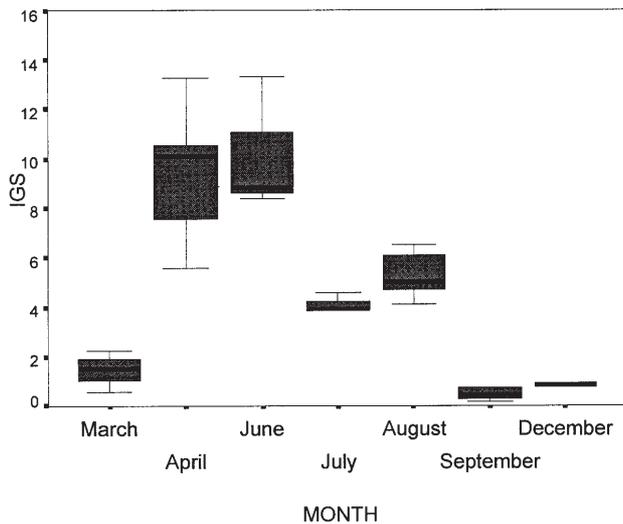


FIG. 4. – Evolution of female GSI during the sampling period. Lines show full range, bars show 25-75% quartile range.

The vitellogenesis (stage 4) starts from an oocyte diameter of 0.25 mm. Macroscopically oocytes in the vitellogenic stage are recognised to show a light yellow coloration. Based on the presence of vitellogenic oocytes in the ovary of the smallest adult female present in this sample, we estimated the minimum size for first maturity for females to be 36 mm TL.

Histological examination showed that all stages of sexual maturity were represented in the ovarians of the females from April to August, indicating that the spawning in *A. sphynx* is asynchronous, occurring over an extended period from April to August. As it is a multiple spawner, it is likely that only a small proportion of the oocytes in the ovary are released at any one time. Therefore, in multiple spawning for species such as this, absolute fecundity in terms of eggs released is difficult to estimate. Since the egg-laying starts at the end of April (Kraak, 1996a, b), the fecundity in April will be

TABLE 3. – Oocyte maximum diameter for juveniles (TL < 36 mm) and adults (TL ≥ 36 mm) of *Aidablennius sphynx* and oocyte stages in different months of year.

| | Oocyte maximum diameter | | Oocyte stages |
|-----------|-------------------------|--------|---------------|
| | Juveniles | Adults | |
| February | 0.096 | - | 1 to 2 |
| March | 0.159 | 0.230 | 1 to 3 |
| April | - | 0.920 | 1 to 4 |
| June | - | 0.740 | 1 to 5 |
| July | - | 0.700 | 1 to 5 |
| August | 0.185 | 0.701 | 1 to 5 |
| September | 0.099 | 0.099 | 1 to 2 |
| October | 0.073 | - | 1 to 2 |
| December | - | 0.112 | 1 to 2 |

greater than in the rest of the spawning season. In fact, marginally significant differences were found between relative fecundity of April, June, July and August (One way ANOVA, $F = 3.185$, $p < 0.10$). The number of oocytes in the ovary varied from just under 432 in a female of 45 mm total length to over 1682 in a female of 53 mm TL (for females of April). It was found that the number of eggs (F) was best correlated with total length (TL) rather than with eviscerated weight (EW) (Table 2). The relative fecundity (absolute fecundity / total length) varied from 12.00 to 31.74 oocytes per mm of length.

Temporal changes in the mean size of the larger eggs also provide information on the seasonality of the breeding cycle. The oocyte stages in different months analysed also indicate that the breeding season starts in April (Table 3). For females larger than 36 mm TL, significant differences in transformed oocyte diameter ($\phi^{1/2}$) between months were found (One way ANOVA, $F = 111.027$, $p < 0.001$). In particular, significant differences were obtained between March and September and April, June, July and August (A posteriori Scheffé multiple rank test, with $p < 0.05$).

DISCUSSION

Male gonads of blennioids are curious among teleosts in possessing a testicular gland. It stores lipids and spermatids (Lahnsteiner and Patzner, 1990a; Lahnsteiner *et al.*, 1990). The size of the testicular gland in relation to the testis varies in blennioid fish (Lahnsteiner *et al.*, 1990). In *Aidablennius sphynx*, which has a voluminous testicular gland and very small testis, those spermatids that are released into the testicular gland are at an early stage of maturation. The reduction of the testis in favour of the testicular gland, which then takes over the testicular functions in the process of spermiogenesis, which is observed in *A. sphynx*, is also reported in *Lipophrys adriaticus*, *Lipophrys canevae*, *Lipophrys dalmatinus* and *Parablennius incognitus* (Lahnsteiner *et al.*, 1990). Testicular glands that are proportionally bigger than the testis in the post-breeding season compared with the breeding season, as is observed in *A. sphynx*, also appear in *Parablennius sanguinolentus* (Santos, 1995). The maturation of the testis in *A. sphynx* of the northwestern Mediterranean starts in March, with a production of spermatids until July, in contrast to the shorter spawning season described by Blüm (1972) for the Adriatic Sea, and by Lahnsteiner

er and Patzner (1990 a) for other Mediterranean blennies. Males have mature testes before female gonads mature. Spawning in blennies may occur on successive days over a long period of time (Gibson, 1969; Lahnsteiner and Patzner, 1990 a; Kraak, 1996a). Under these circumstances, the ability to accelerate the initial rate of spermatogenesis, associated with the possibility of spermatozoa storage in the testicular gland, is understood to be an advantage (Santos, 1995). The further differentiation of spermatids in the testicular gland of blennioid fish may be interpreted in favour of the production of high numbers of germ cells in the testes in a short interval. The storage of germ cells ensures sufficient numbers for fertilisation (Lahnsteiner and Patzner, 1990b).

The development of the oocytes is similar to that of other species of blennies. Stage 2a y 2b correspond to stage II and III in *P. sanguinolentus* (Santos, 1995). The vitellogenic phase, stage 4, agrees with the stages V, VI and VII of *P. sanguinolentus*. In *A. sphyinx*, as in *P. sanguinolentus* (Santos, 1995), lipid globules are first seen in the cortical cytoplasm and then migrate to the juxtannuclear region. The ring circling the nucleus without vacuoles that is observed in the early stage 4 also is observed in *P. sanguinolentus*. On the other hand, in most teleosts (Wiegand, 1982; Nagahama, 1983; Selman and Wallace, 1989), lipid globules are first found in the vicinity of the nucleus and then move to the periphery of the oocyte. The non-coalescence of yolk granules, which is also recognised in *P. sanguinolentus*, is frequent among teleosts that spawn demersal eggs.

De Vlaming (1983) emphasizes that ovulation and spawning are separate events under different control mechanisms and that it is difficult to predict the number of spawnings and the number of eggs spawned. Direct observations of Kraak (1996a) on spawning females of *A. sphyinx* show the oocytes distributed by several spawning acts as late as 24 oviposition days in 63 days monitored. Histology shows that only a small proportion of the oocytes mature at a time and this proportion occupies the majority of the ovary volume. In species such as *A. sphyinx*, in which the yolk granules do not coalesce at the ripe stage or hydrate, ripe oocytes are difficult to distinguish from yolked oocytes on the basis of their external appearance, as happens in other marine teleosts (Forberg, 1983). Notwithstanding, ripe oocytes of *A. sphyinx* are observed in April, according with the start of the breeding season described by Kraak (1996a). Kraak (1996a), in

Stareso (Corsica, France), observed that egg laying in the nests still continued when the fieldwork ended (8 August). In our study it was confirmed that the breeding season concludes at the end of August. Females captured on 20 August still carried ripe oocytes, but in September all were immature.

The maximum fecundity observed in this study was 1682 eggs in the largest female of April analysed (LT = 53 mm). This value does not reach the 75% of the mean numbers of eggs that 15 females laid in aquaria that Kraak (1996a) computed. Kraak (1996a) considered that the egg production of females determined in his study might be overestimated, because the females in the aquaria were fed abundantly with high quality food. Our results confirm that natural egg production is lower.

Most species with asynchronous oocyte development have protracted spawning seasons with multiple spawnings (De Vlaming, 1983). The production of multiple batches of eggs, reaching maturation and being spawned at different times, may present some advantages. A high reproductive effort is achieved by summation of repeat spawning in which successive batches of eggs are produced during a lengthy period of reproduction (Santos, 1995). *A. sphyinx* distributes the eggs among several males (Kraak, 1996a), like other blennies (Santos, 1995; Robertson, 1990), and reduces the risks of absolute loss of progeny due to wrong choice of the male. Burt *et al.* (1988) and Santos (1995) associate multiple spawning within years with less-seasonal environments (for example, in warm temperate and subtropical areas without marked seasonality of primary production), smaller body sizes and smaller relative ovary sizes. The Mediterranean Sea is a seasonal environment, so only smaller body and ovary sizes are applicable. Distributing eggs for several spawnings during several months also assures that larvae will occur in the plankton at different times, and reduces the risks of total loss of progeny due to extremely unfavourable conditions during planktonic life.

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