SCI. MAR., 69 (2): 275-283 SCIENTIA MARINA 2005

Reproductive aspects of *Microchirus azevia* (Risso, 1810) (Pisces: Soleidae) from the south coast of Portugal*

ISABEL AFONSO-DIAS [1,2], CATARINA REIS [2] and J. PEDRO ANDRADE [2]

[2] Universidade do Algarve, FCMA/CCMar, Campus de Gambelas, 8005-139 Faro, Portugal. E-mail: idias@ualg.pt
[1] CCMar, FCMA - Universidade do Algarve.

SUMMARY: Fresh fish obtained from commercial landings in the harbours of Olhão and Quarteira (south Portugal) in 1998 and 1999, were examined in order to study different aspects of the reproductive biology of *Microchirus azevia* (Risso, 1810): spawning season, ovary maturation, length/age at first maturity and sex ratio. A five-stage maturity scale, based on external appearance was used to classify the ovaries. *M. azevia* is a winter-spring batch spawner with a protracted spawning season. Females outnumbered males in length classes greater than 19 cm and in all age groups. The estimated mean size at first maturity ($L_{50\%}$) for females was 23 cm total length at 3 years of age ($t_{50\%}$).

Keywords: reproduction, *Microchirus azevia*, maturity.

RESUMEN: ASPECTOS DE LA REPRODUCCIÓN DE *MICROCHIRUS AZEVIA* ((RISSO, 1810) (PISCES: SOLEIDAE) DE COSTA SUR DE PORTUGAL. – Ejemplares de *Microchirus azevia* (Risso, 1810) capturados en la pesca comercial en los puertos portugueses de Olhão y Quarteira en 1998 y 1999, fueron examinados para el posterior estudio de varios aspectos de su biología reproductiva: estación de freza, maduración del ovario, talla/edad de la primera madurez y proporción sexual. Una escala de madurez de cinco estados, basada en el aspecto externo, fue utilizada para clasificar los ovarios. *M. azevia* es un reproductor secuencial con una estación de freza prolongada entre invierno y primavera. El número de hembras entre los ejemplares capturados fue siempre superior al número de machos en clases de talla mayores de 19 centímetros y en todas las categorías de edad. La talla de primera madurez ($L_{50\%}$) para las hembras fue 23 centímetros y la edad ($t_{50\%}$) 3 años.

Palabras clave: reproducción, *Microchirus azevia*, madurez.

INTRODUCTION

The sole, *Microchirus azevia* (Risso, 1810), is a flatfish that can be found at depths up to 250 m (Queró and Vayne, 1997; Whitehead *et al*., 1986; Fischer *et al*., 1987). Its northern limits are in the eastern Atlantic (Portugal) but it is found throughout the southern range of the Iberian Peninsula, in the western part of the Mediterranean Sea (Spain and Algeria) and off the African coast, southward to Senegal (Whitehead *et al*., 1986; Fischer *et al*., 1987).

This species is mainly caught by the artisanal fleet (with trammel and gill nets). It is also caught by bottom trawl as a by-catch with other commercially important species. According to official data (DGPA - Direcção Geral das Pescas e Aquicultura, Portugal) the *M. azevia* landed by the bottom trawl fleet, from 1993 to 2001, accounted for only 24% of the total Portuguese landings of this species. Among the Soleidae species landed in Portugal, this sole is one of the most important, because it represents about

*Received January 13, 2004. Accepted November 4, 2004.

26% of the total landings of Soleidae. However, this figure may be well underestimate since around 45% of flatfish are landed under the name of Soleidae. From 1993 to 2001, about 365 tonnes/year (not including "Soleidae") of *M. azevia* were landed in Portugal, 50% of this on the south coast (Algarve). Although the weight of the landings is not high, the same cannot be said about their value, considering that this species can fetch up to 20 EUR/kg (depending on size) in the fish market.

Despite the economic importance of this species, there is little information published on its biology. A number of authors have studied the growth of sole on the south coast of Portugal (Andrade, 1998), and off the Moroccan coast (Marfin and Hajji, 1988). There are other studies on particular aspects of reproduction (Belaid and Marinaro, 1983), and egg and larvae development (Marinaro, 1991), of *M. azevia* in the Mediterranean Sea. This paper presents original information on the reproductive biology of *M. azevia* on the south coast of Portugal.

MATERIAL AND METHODS

Sampling procedures

Randomly selected samples of fresh fish were collected monthly in two fishing harbours in the south of Portugal, Olhão and Quarteira (Fig. 1), between June 1998 and July 1999. The total length was measured to the nearest mm below. Total weight, gutted weight and gonad weight were recorded to the nearest 0.1 g. Since there is no external difference between sexes in *M. azevia,* sex determination was possible only by examining the gonads.

A five-stage maturity scale, based on the external appearance, was used to classify the ovaries. The characteristics used to classify each ovary were size, shape, volume, degree of vascularization, degree of opacity, size and appearance of the oocytes (Table 1). It was not possible to establish the stage of the male gonads with the naked eye.

Gonads for histological investigation were dissected in small portions and fixed in 10% buffered formalin. After dehydration in different grades of ethanol the samples were embedded in resin (Technovit 7100 Kit - TAAB), sectioned at 2-3 μm and stained with 1% toluidine blue in 1% borax.

The maximum diameter of the oocytes, whose nucleus was clearly visible in cross-section, was measured from histological sections using image analysis software (Image Pro Plus 3.0).

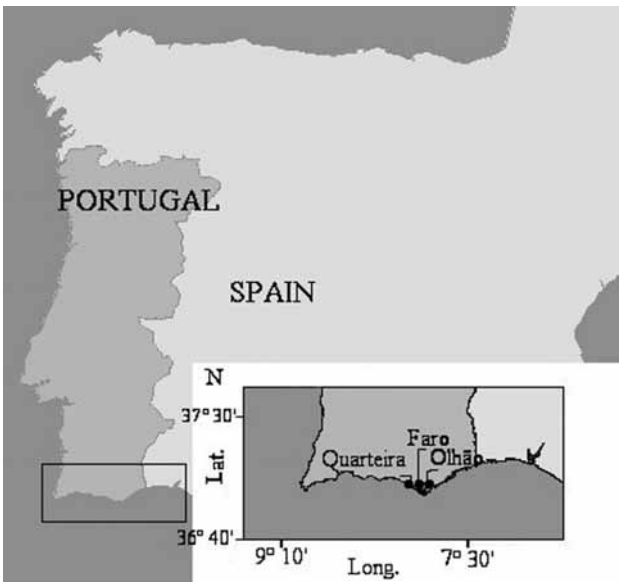

FIG. 1. – Location of the two fishing harbours where sampling took place (mod. from http://www.geog.uni-hannover.de/phygeo/geodaten.html).

Sagittal otoliths were used to age *M. azevia*. The otoliths of each fish were removed, washed with tap water, labelled and stored dry. The whole otolith was removed from the container and placed in a mixture of ethanol and 50% glycerol (1:1) in a black porcelain dish and viewed with reflected light under a low power binocular microscope with constant magnification.

The age-reader read each pair of otoliths twice (with one month interval between readings) to assess the consistency of the age readings. Whenev-

TABLE 1. – Macroscopic maturity scale for *M. azevia* ovaries.

I- Immature or Resting
Very thin ovaries not extending more than 4 cm down the side of the body. Pinkish ovaries with very thin walls and easily broken. No visible oocytes.

II- Developing
The ovaries increase in length and in width; they become thicker and opaque. Small opaque specks are visible.

III- Maturing
The ovaries increase considerably in volume and usually distend the body. Many opaque and a few hydrated oocytes are visible.

IV- Ripe
Full ovary with hydrated oocytes visible. In the running stage the oocytes are extruded copiously under light pressure of the ovary.

V- Spent and recovering
Ovaries are empty or partially empty, flaccid, with mainly opaque oocytes in a state of reabsorption with a lot of slime. Gonads are highly vascularized with some ruptured capillaries, bloodshot in appearance.

TABLE 2. – Summary of data collected monthly. In June only two 7 years old fish, one male and one female, were sampled.

Sampling Period	Numbers		Size Range (cm)		Age Range (year)	
	Female	Male	Female	Male	Female	Male
June	112	54	15.2-33.2	14.2-24.3	1-7	1-7
July	51	14	18.1-31.2	17.5-24.0	1-5	1-3
August	32	7	15.1-28.6	12.6-19.2	1-3	1-2
September	40	36	17.7-26.9	17.0-20.4	1-3	2
October	83	37	16.7-26.1	16.4-24.4	2-4	2-3
November	35	18	17.7-31.9	18.3-24.9	2-5	2
December	70	17	18.6-32.2	15.8-26.7	2-5	2-3
January	21	29	17.1-31.9	16.2-23.5	2-6	2-3
February	40	28	14.8-37.6	13.7-36.0	1-5	1-5
March	44	8	13.2-33.3	12.9-26.3	1-4	1-3
April	43	24	14.7-30.8	14.8-25.0	1-4	2
May	52	5	19.8-36.2	19.7-27.3	2-5	2
Total	623	277	13.2-37.6	12.6-36.0	1-7	

er, the first reading was not the same as the second, a third reading was performed. The otoliths were discarded if two out of three age readings were not the same.

Sample description

A total of 900 *M. azevia*, 277 males and 623 females, was examined. Total lengths ranged from 13.2 cm to 37.6 cm for females and from 12.6 to 36.0 cm for males. Age groups ranged from 1 to 7 years for both sexes (Table 2).

Data analysis

The sex ratio was expressed as the proportion of females in the sample, and was calculated for 5-cm length classes and 1-year age groups, according to the month the fish was caught in.

Females sampled throughout the sampling period were pooled to increase the sample size. A total of 617 females was used to determine the length/age at which 25% ($L_{25\%}$, $t_{25\%}$), 50% ($L_{50\%}$, $t_{50\%}$) and 75% ($L_{75\%}$, $t_{75\%}$) were sexually mature (stages III, IV and V – see Table 1).

Inspection of the relative frequency distributions for length/age classes of mature females suggests that the relationship between maturity and length/age is adequately described by a logistic curve,

$$p_L = \frac{e^{(a+b*L)}}{1-e^{(a+b*L)}}$$

This analysis was performed only for females since male gonads were impossible to stage macroscopically. Parameter estimates were produced as suggested by King (1995), and Jennings *et al.* (2001).

The timing and duration of the spawning season were deduced from the monthly data on the incidence of mature individuals and changes in the gonadosomatic index (GSI). The GSI was estimated as the quotient between the gonad weight and gutted weight. As the samples contained many small specimens, the analysis of the maturity stages and the estimation of the GSI were restricted to females at or above $L_{25\%}$.

RESULTS

Morphology and development of the gonads

The gonads of *M. azevia* are quite distinct between sexes and easy to identify. They show a well-marked size asymmetry between the eyed and blind side. Male gonads are bean shaped and extremely small, occupying less than 1% of the abdominal cavity. The ovaries exhibit a flattened tubular structure much wider in the anterior region that runs along the ventral part of the animal's body. In a transverse section the ovaries exhibit an external ovigerous membrane. From this very thin layer many ovigerous lamellae project into the lumen.

The different macroscopic stages used to classify the ovaries were examined microscopically (Table 3). The cellular development of the ovary during maturation can be summarized as follows: In the early stages (I and early II) of development each ovary contained many pre-vitellogenic oocytes in two to three maturation stages: chromatin nucleo-

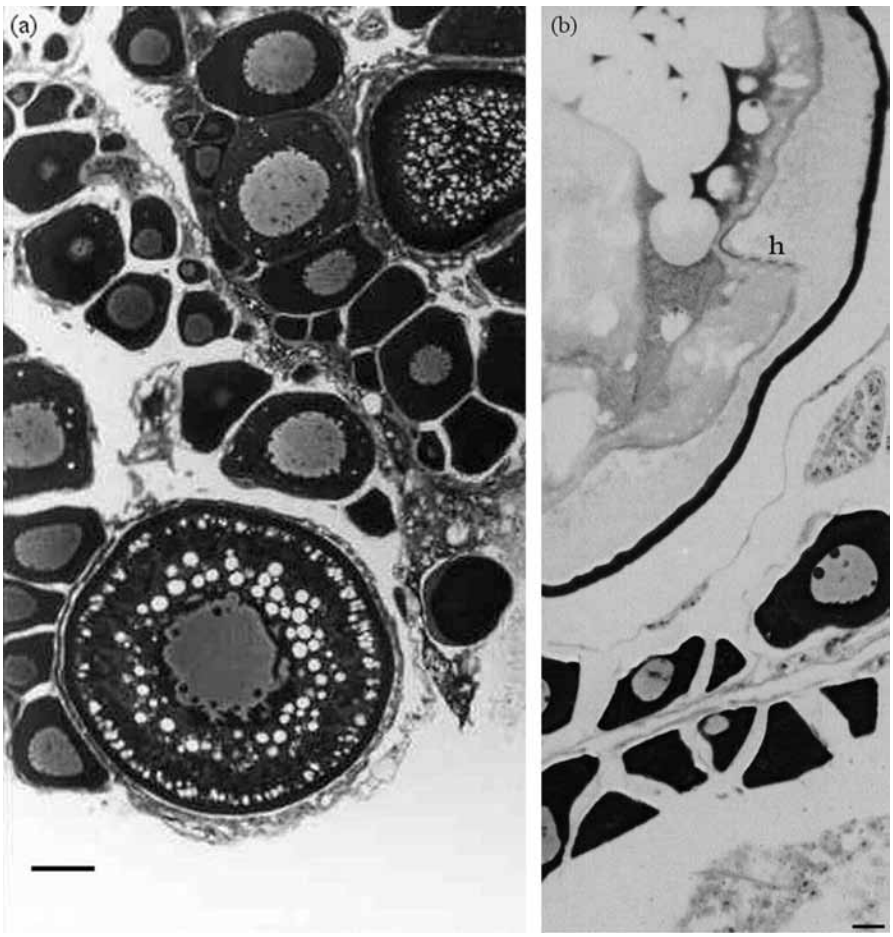

FIG. 2. – Section of two *M. azevia* ovaries showing (a) pre-vitellogenic oocytes (scale bar=0.06 mm) and (b) hydrated oocytes (h) with pre-vitellogenic oocytes (scale bar=0.03 mm).

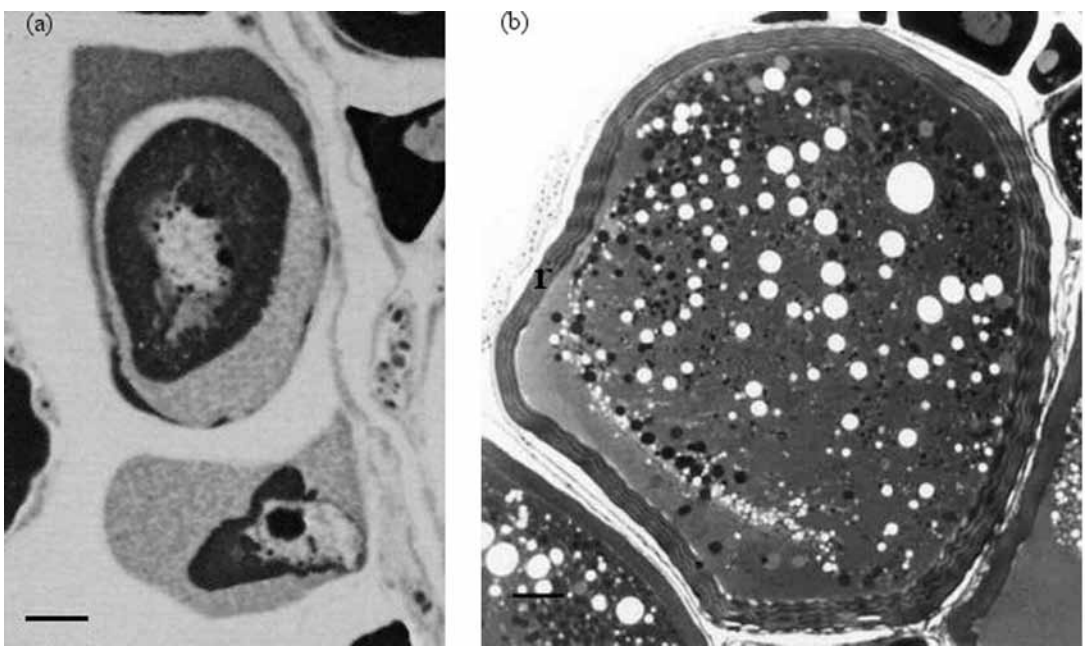

FIG. 3. – Atretic oocytes of *M. azevia* in different development stages showing (a) bizarre markings in the cytoplasm and (b) the thickening of the zona pellucida (r) in vitellogenic oocytes.

TABLE 3. – Microscopic description of oocyte development for the 5 stage maturity scale used to classify *M. azevia* ovaries.

Stage I- Immature or Resting
Oogonias and small oocytes in chromatin nucleolus stage (CNS); no oocytes beyond the perinucleolus stage (PNS) were found. Resting ovaries have vitellogenic oocytes in atresia visible by the advanced stage of reabsorption of the cytoplasm and by the undulations of the zona pellucida. Diameter range: 0.004-0.144 mm

Stage II- Developing
Some oocytes in CNS but most of the small oocytes found are in PNS with one or more nucleoli in the periphery of the nucleus. In some oocytes the Balbiani's body (BB) is visible in the cytoplasm adjacent to the nucleus. As the oocytes develop, the BB migrates to the periphery of the oocyte. The larger oocytes are in the cortical alveoli stage (CAS). Diameter range: 0.008-0.552 mm

Stage III- Maturing
Oocytes continue to increase in size, most in CAS. Vitellogenic (Vit) and small numbers of mature (Mat) oocytes are present. Small PNS oocytes are still present. Diameter range: 0.009-1.039 mm

Stage IV- Ripe
Many mature and hydrated oocytes are present. Numerous oocytes in CAS and in an advanced stage of vitellogenesis; pre-vitellogenic oocytes are still present. Diameter range: 0.021-1.110 mm

Stage V- Spent
Many oocytes in different stages of maturation being reabsorbed. Many empty spaces in the ovaries. Mature oocytes that were not released in different stages of atresia; numerous post-ovulatory follicles are present. Diameter range: 0.071-0.2511 mm

lus, perinucleolus and cortical alveoli stages (Fig. 2a). As the ovarian maturation proceeded the number of oocyte stages increased, but the numbers of oocytes in pre-vitellogenic stages decreased. At the end of maturation, the ovary was filled with hydrated oocytes, but there were still oocytes in earlier development stages (Fig. 2b). Atretic oocytes were common in females of all maturity stages other than stage I. In the early stages of maturity (perinucleolus, cortical alveoli and early vitellogenesis) the first sign of oocytes becoming atretic was the formation of bizarre marks in the cytoplasm (Fig. 3a). The first visible sign of atresia, in vitellogenic oocytes, was the thickening of the zona pellucida (Fig. 3b).

Oocyte diameter frequency distributions

Oocytes in different stages of development (Table 3) were found in each macroscopic maturity stage (Table 1). The diameter of the oocytes measured varied between 0.004 to 1.110 mm (Table 4). Small pre-vitellogenic oocytes were present in all stages of maturity; even ovaries classified in stage IV, with hydrated oocytes had pre-vitellogenic oocytes (Figs. 2 and 4).

In Fig. 4 it is possible to observe a threshold in the oocyte diameter at about 0.20 mm. Oocytes smaller than 0.02 mm were considered to belong to a group of 'resident oocytes', i.e. a group of oocytes that are present in the ovary throughout the spawning season. Oocytes larger than 0.20 mm seem to develop and contribute to the oocyte spawning stock of the season. The data also suggests that oocytes larger than 0.80 mm constitute the batch stock of oocytes.

Sex ratio

The monthly proportion of females varied considerably (Table 2). Although no seasonal pattern in sex ratio was observed, samples with larger fish tended to include greater numbers of females (Fig. 5). Females outnumbered males in almost all length classes (Fig. 5a) and in all age groups (Fig. 5b).

Length/age at first maturity

The percentage of mature females was plotted against length and age (Fig. 6) and was adequately described by a logistic curve. The estimated mean length ($L_{50\%}$) and age ($t_{50\%}$) at first maturity was 22.8

TABLE 4. – Summary of the measurements (Diameter) taken in ovary sections according to the development stages of *M. azevia*. Mean ± 95% Confidence limits (C.l.)

Stage of development	n.	Diameter (mm) Minimum	Maximum	Mean ± C.l.
Oogonia	37	0.004	0.017	0.010±0.001
Chromatin Nucleolus	60	0.012	0.060	0.027±0.004
Perinucleolus	30	0.051	0.092	0.070±0.004
Cortical Alveoli	32	0.108	0.319	0.166±0.025
Vitellogenic	50	0.180	0.651	0.398±0.041
Mature	30	0.068	0.994	0.667±0.086
Hydrated	13	0.896	1.110	0.988±0.034
Total	252	0.004	1.110	

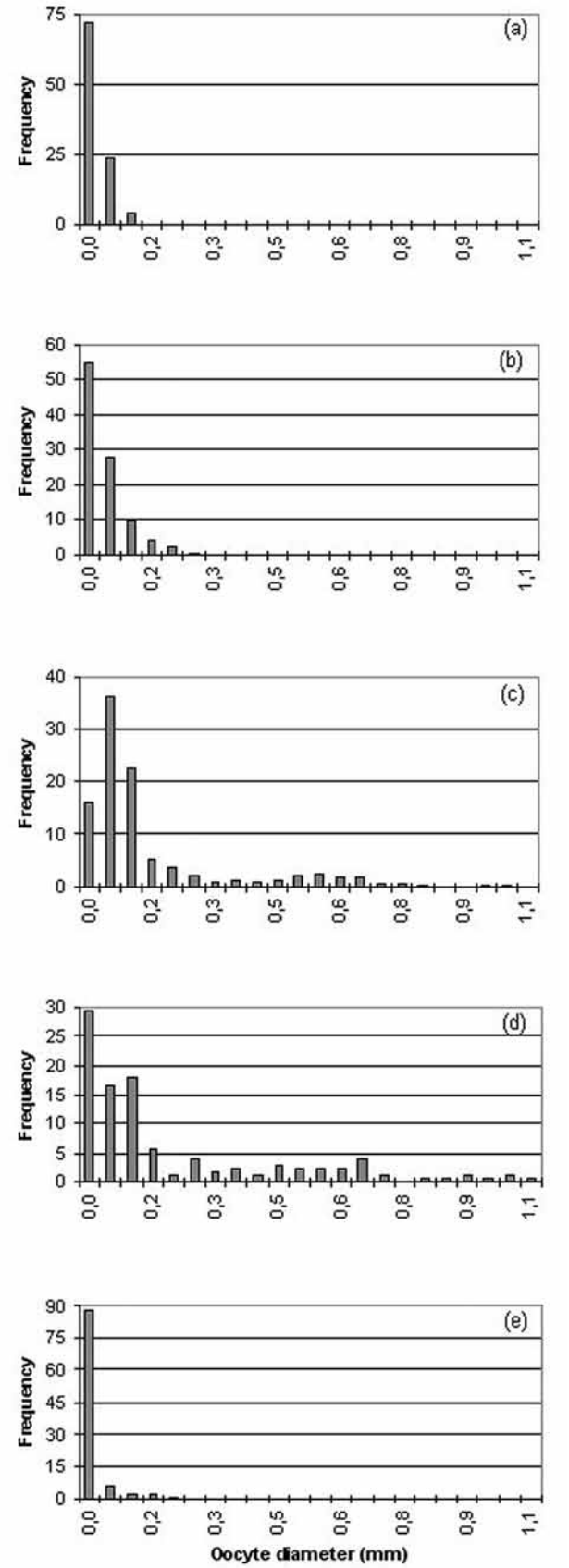

FIG. 4. – Distribution of oocyte diameter according to the macroscopic maturity stage: (a) Stage I, n=544; (b) Stage II, n=449; (c) Stage III, n=983; (d) Stage IV, n=173; (e) Stage V, n=270.

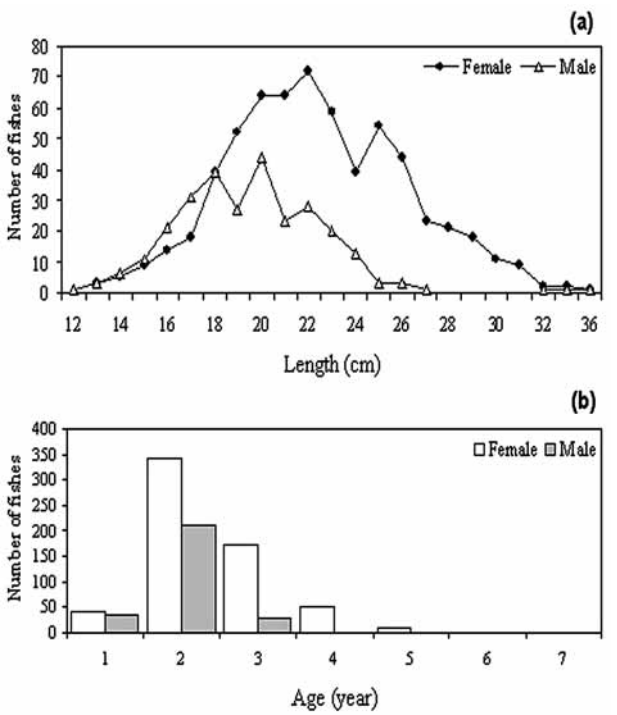

FIG. 5. – Numbers of *M. azevia* sampled according to length class (a) and age group (b). Few specimens were sampled in age group 6 (n=1) and 7 (n=2).

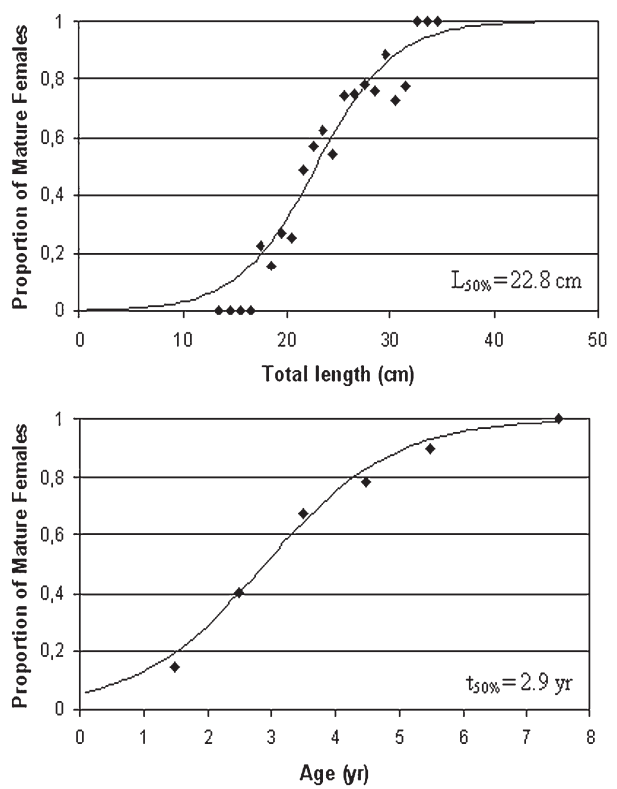

FIG. 6. – Percentage of mature females according to length (left) and age (right) class.

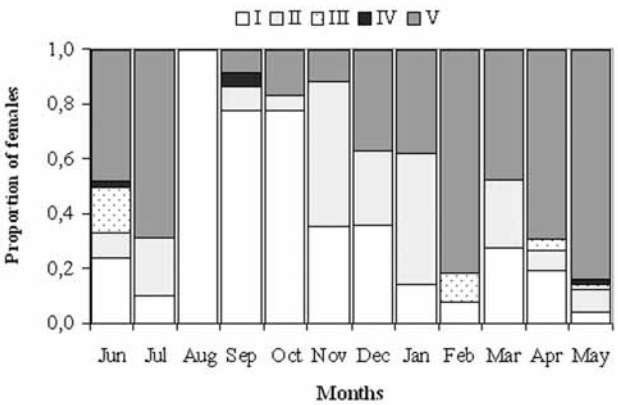

Fig. 7. - Percentage of *M. azevia* females sampled throughout the year, according to macroscopic maturity stage.

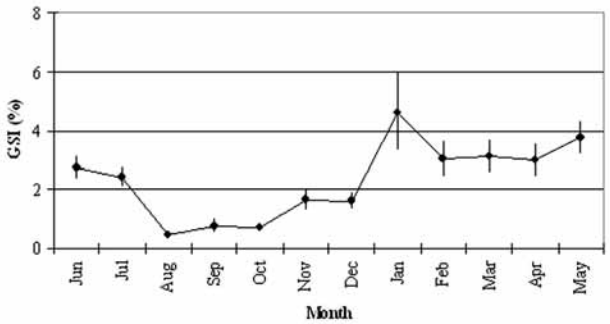

FIG. 8. – Monthly variation of the GSI for *M. azevia* females. The bars represent the mean ± 95% confidence limits.

cm ($L_{25\%=}$18.8 cm and $L_{75\%}$=26.9 cm) and 2.9 years ($t_{25\%}$= 1.8 years and $t_{75\%}$=4.0 years), respectively.

Spawning season

Ripe females (stage IV) were only found in May (n=1), June (n=2) and September (n=2) (Fig. 7). Maturing females were found mainly in June and February. Spent females were caught more frequently from December to July and were absent in August. Although the largest numbers of immature fish were found from August to December, immature females were found throughout the year (Fig. 7).

The GSI values were higher between January and May (Fig. 8) and clearly decreased between June and August. There seems to be a resting period from August to September; after this period the gonad seems to increase in weight continuously in relation to body weight, with some acceleration around January.

DISCUSSION

Our data suggest that a high percentage of *M. azevia* landed by commercial fleets are sexually immature. Although there is a minimum landing size for this species (18 cm total length), which corresponds to $L_{25\%}$, about 13% of the fish measured were below this size. However, little to no attention is paid to this species that is a part of traditional artisanal fisheries and a valuable species.

Sexual maturity of *M. azevia* ($L_{50\%}$) seems to be constant on the south coast of Portugal: $L_{50\%}$=23 cm and $t_{50\%}$=3 yr, in females since 1986 (see Andrade (1990, 1998)). However, in Algerian waters Belaid and Marinaro (1983), reported that sexual maturation starts at the same age, but at slightly smaller sizes, at about 20 cm total length.

This study shows that *M. azevia* is a winter/spring spawner with asynchronous ovaries. This sole releases its oocytes in groups over a protracted season, which seems to be a common feature amongst other species of flatfish like plaice (*Pleuronectes platessa*) and Dover sole (*Solea solea*) (Urban, 1991).

The size frequency distributions of oocyte diameters suggest that *M. azevia* is a determinate spawner, so the annual fecundity can be estimated before the spawning season, because there seems to be a gap between the pre-vitellogenic "resident oocytes" or "reserve fund" (Foucher and Beamish, 1977), and the maturing oocytes. However, this gap is not clear enough to completely rule out the possibility of an indeterminate number of pre-vitellogenic oocytes maturing after the onset of spawning. Although the fate of these intermediate sized oocytes is somewhat controversial (see West, 1990), and the use of oocyte size-frequency distributions limiting (De Vlaming, 1983), it seems that the intermediate sized oocytes of *M. azevia* are not spawned. Although no intermediate sized oocytes were measured in stage V ovaries, many degenerating vitellogenic oocytes were found in maturing (stage III) fish along with perfectly healthy oocytes.

The two spawning strategies, determinate and indeterminate, are common among different species of flatfish (Urban and Alheit, 1988), and it has been suggested that the two strategies are a consequence of different feeding habits. Even the same species may adopt different strategies depending on the environmental conditions. Witthames and Walker (1995), found that *Solea solea* can exhibit the two spawning strategies depending on the spawning area. Although these authors discuss that it is unlikely that *S. solea* has two different spawning strategies

in adjacent waters, the results show this to be so.

A second, and probably the most likely explanation for the occurrence of intermediate sized oocytes, is the variability in the oocyte measurements depending on the sample site. Although the work published by Emerson *et al*. (1990), showed no significant differences in oocyte density between the two ovaries and between sample sites of Sole (*S. Solea*), Witthames and Walker (1995), showed that while producing a batch of oocytes the hydrated oocytes were concentrated in the lumen and at the anterior end of the ovary close to the cloaca. Therefore, a section in that area would not be representative of the whole ovary. Moreover, a section in the opposite site would also not represent the ovary development completely. The site sampled in this study was taken randomly based on the study by Emerson *et al*. (1990). This might have influenced the oocyte measurements, considering that *M. azevia* has the same ovary development as *S. solea*, and there are strong indications that it does.

The ovaries classified as partially spent (Stage V) had many vitellogenic oocytes in atresia. Although oocyte atresia has been reported in many different species, and it is well documented throughout the literature, the reason why so many cells degenerate during the normal maturation process is still not clearly understood. It seems that atresia is a highly regulated process within the vertebrate ovary, and it is believed to be essential for maintaining ovarian homeostasis (Foghi *et al*., 1998). In oviparous species, such as *M. azevia*, a substantial quantity of energy is invested into yolk granules within the oocytes. Mechanisms have evolved to recycle this energy in the event of atresia (Wood and Van Der Kraak, 2001). Although apoptosis (cell death) is a fundamental mechanism in follicular atresia and postovulatory regression in mammals, its role in teleost ovarian function is currently unknown (Wood and Van Der Kraak, 2001).

Individual GSI values from our study were in the range of 0.08-10.96%. They were smaller than the values obtained during previous studies of this species in 1986, 1987 and 1988 (Andrade, unpublished data). The mean GSI obtained in the former study increased from a minimum in July to October (1.5% - 2.5%) until spawning commenced in February (12.5%). This data set indicates that the GSI for this sole increases with age and that during spawning it reaches a level of about 12-17%. Since we took samples during one calendar year and did not find indices so high, it is possible that the females that we sampled had the maturity process in progress and that in the ripe females sampled spawning had already began. This statement is in accordance with the fact that large numbers of spent and partially spent females (54.5% at or above $L_{25\%}$) were found in the samples. The small numbers of maturing (Stage III) and ready to spawn females (Stage IV) in the samples may be due to sampling bias. These gonads usually distend the body and are quite visible just by visual inspection. These specimens are very valuable on their own on the black market (direct sale to restaurants and fish mongers), therefore they probably do not reach the fish market where the fish samples were obtained. In addition, stage IV may develop quite fast, making the odds of finding females in this development stage quite small.

The sex ratio varied with length, possibly because there are differences in the growth rate between sexes, with females attaining larger sizes and living longer than males (Andrade, 1998). This difference in growth between the two sexes, with females attaining larger sizes than males, coupled with the fact that they live longer, may be caused by a distinct difference in metabolism between the two genders (Pauly, 1994).

Future studies on the maturity of this species should include the calibration of the macroscopic scale by microscopic examination of the ovaries, including atresia quantification, to try to establish what is the effect of cell death on the reproduction of this species.

ACKNOWLEDGEMENTS

We would like to acknowledge the European Commission for financing our project (DG XIV 97/0083), allowing us to collect very diverse and important biological data on five species of Soleidae. We wish to thank the Direcção Geral das Pescas e Aquicultura (Portugal) for kindly giving us commercial landing data on *M. azevia*. To Dr. John R.G. Hislop we would like to express our deepest thanks for the important contribution given to this manuscript.

REFERENCES

Andrade, J.P. – 1990. *A importância da Ria Formosa no ciclo biológico de Solea senegalensis Kaup 1858, Solea vulgaris Quensel 1806, Solea lascaris (Risso, 1810) e Michrochirus aze-*

via (Capello, 1868). Ph. D. Thesis, Univ. Algarve.
Andrade, J.P. – 1998. Age and growth of the bastard sole, *Microchirus azevia* (Capello, 1868) (Pisces, Soleidae) from the south coast of Portugal. *Fish. Res*., 34: 205-208.
Belaid, B. and J.Y. Marinaro. – 1983. Biologie de la reproduction de *Microchirus azevia*_(Capello) (Téléostéen soléidé). *Rapp. Comm. Int. Mer Médit*., 28(5): 59-60.
De Vlaming, V. – 1983. Oocyte development patterns and hormonal involvements among Teleosts. In: J.C. Rankin, T.J. Pitcher and R.T.N. Duggan (eds.), *Control Process in Fish Physiology,* pp. 176-199, Croom. Helm, London.
Emerson, L.S., M. Greer Walker and P.R. Witthames. – 1990. A stereological method for estimating fish fecundity. *J. Fish. Biol*., 36: 721-730.
Fischer, W., M. Schneider and M.-L Bauchot. – 1987. *Fisches. FAO d'identification des espèces pour les besoins de la pêche. Méditerranée et Mer Noire* (Zone de Peche 37).Vol. 11-Vertébrés: 1325-1342.
Foghi, A., K.J. Teerds, H. van der Donk, N.C. Moore and J. Dorrington. – 1998. Induction of apoptosis in thecal/interstitial cells: action of transforming growth factor (TGF) plus TGFß on bcl-2 and interleukin-1ß-converting enzyme. *J Endocrinol*, 157: 489-494.
Foucher, R.P. and R. J. Beamish. – 1977. A review of oocyte development in fishes with special reference to Pacific hake (*Merluccius productus*). *Fish. Mar. Serv. Tech. Rep*. 755: 1-16.
Jennings, S., M.J. Kaiser and J.D. Reynolds. – 2001. *Marine Fisheries Ecology*. Blackwell Science, Oxford.
King, M. – 1995. *Fisheries Biology, Assessment and Management*. Blackwell Science, Oxford.
Marfin, J. P. and N. Hajji. – 1988. Croissance de *Microchirus azevia* (Capello, 1867) du Sud Marocain. *Cybium*. 12(2): 123-128.
Marinaro, J.Y. – 1991. Eggs of the Michrochirus species (Soleidae) from the Bay of Algiers. *J. mar. biol. Ass. U.K*., 71:117-125.
Pauly, D. – 1994. *On the Sex of Fish and the Gender of Scientists: Essays in Fisheries Science*. Fish and Fisheries Series. Chapman & Hall, London.
Quéro, J.C. and J.J. Vayne. – 1997. *Les poissons de mer des pêches françaises, en collaboration avec l'IFREMER, coll. Les encyclopédies du naturaliste, éd*. Delachaux et Niestlé, Paris.
Urban, H.J. – 1991. Reproduction strategies of North Sea plaice *Pleuronectes platessa*; and North Sea Sole, *Solea solea*. Batch spawning cycle and batch fecundity. *Meeresforschung-Rep. Mar. Res*, 33(4): 330-339.
Urban, J. and J. Alheit – 1988. Oocyte development cycle of plaice, *Pleuronectes platessa*, and North Sea sole, *Solea solea*. *ICES Doc. C.M. 1988/G: 52*, 8 (mineo).
West, G. – 1990. Methods of Assessing Ovarian Development in Fishes: a Review. *Aust. J. Mar. Freshwater Res*., 41: 199-222.
Whitehead, P.J.P., M.L. Bauchot, J.C. Hureau, J. Nielsen, and E. Tortonese (eds). – 1986. *Fishes of the North-eastern Atlantic and the Mediterranean*. Unesco, Paris.
Witthames, P.R. and M. G. Walker. – 1995. Determinacy of fecundity and oocyte atresia in sole (*Solea solea*) from the Chanel, the North Sea and the Irish Sea. *Aquat. Living Resour*., 8: 91-109.
Wood, A.W. and G.J. Van Der Kraak. – 2001. Apoptosis and Ovarian Function: Novel Perspectives from the Teleosts. *Biology of Reproduction*, 64: 264-271.

Scient. ed.: A. Sabatés