Ovarian atretic rates and sexual maturity of European horse mackerel, *Trachurus trachurus* (L.), in the Saronikos Gulf (Greece)

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Abstract.—Histological sections of European horse mackerel, *Trachurus trachurus* (L.) ovaries were used to follow oogenesis and to describe the atretic states. Atretic states were used to examine the reproductive cycle during two successive reproductive seasons. During the first season, spawning lasted from December to May, with peak spawning occurring between the end of March and the beginning of April. The duration of the spawning season for the average female was estimated to be about 3 months. Atretic state 1 (yolked oocytes where <50% were in alpha (α) stage atresia) was the most common atretic condition during the spawning season. Atretic states 2 (yolked oocytes where ≥50% were in α-stage atresia) and 3 (no yolked oocytes present but β atresia or later atresia stages) increased near the end of the season, and at the end of the season, females in atretic state 3 were predominant. The mean number of potential and past-spawning stages per female increased throughout the spawning season, indicating an increase in spawning frequency. During the second season, spawning occurred from January to May. High numbers of females in atretic states 2 and 3 were noted during the period considered to be the middle of the season, followed by an increase of females classified in atretic state 0 (yolked oocytes with no α-atresia stage), indicating a species flexibility to different conditions. The use of traditional macroscopic methods, such as gonadosomatic and hepatosomatic indices, revealed a conformity with the histological data concerning the identification of the spawning season. Total body lengths at female maturity (ML_{50}), estimated histologically and macroscopically, were found to be 211 and 220 mm, respectively.

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In the Greek seas, the genus *Trachurus* is represented by three species: European horse mackerel, *T. trachurus* (L.); Mediterranean horse mackerel, *T. mediterraneus* (S.); and blue jack mackerel, *T. picturatus* (B.). The first two species are of commercial value and are very abundant, whereas the third is very scarce. Their average catch over the years 1987–90 constitutes about 10% of the total annual yield of the country and 15% of the total Saronikos Gulf catch (NSSG, 1989–93). Although these species are commercially important fish for the entire Mediterranean region, little is known about their biology, especially their reproduction. The genus *Trachurus* comprises multiple spawners with a prolonged spawning season (Polonsky and Tormosova, 1969; Kaiser, 1973; Macer, 1974; Andrianov, 1985; Eaton, 1989; Lisovenko and Andrianov, 1991; Macewicz and Hunter, 1993; Komarov[1]).

The most convenient method of determining the reproductive cycle in a female is to observe the seasonal developmental changes of the gonads. Until recently these changes for species of *Trachurus* were examined by studying the seasonal variations of maturity stages or the gonadosomatic index, or both (Kaiser, 1973; Macer, 1974; Nazarov, 1977; Arruda, 1984; Andrianov, 1985; Kerstan, 1985; Komarov[1]). However, for multispawners the use of gross anatomical criteria provides a less accurate assessment of fish maturity and reproduction in comparison with that of histological methods (Hunter and Macewicz, 1985a; Dickerson et al., 1992; Eltink[2]). Histological examination is essential for detecting maturing females, partly spawned fish, the postovulatory follicles, and atretic oocytes.

In the present work we used the ovarian atretic states defined by Hunter and Macewicz (1985b) in order to follow the reproductive cycle of European horse mackerel. Specifically, we estimated the rate at which females spawn and pass from the active (when fish are capable of spawning in the current season) to the inactive state (when fish have completed their spawning cycle), the peak period of spawning, and the duration of the spawning

[1] Komarov, J. A. 1964. On the reproduction of horse mackerel (*Trachurus trachurus*) off the southwestern coast of Africa. ICES Council Meeting 94, 5 p. (Mimeo.)

[2] Eltink, A. 1991. Batch fecundity and fraction spawning of horse mackerel (*Trachurus trachurus* L.). European Community Study Contract BO-1990-207, 71 p.

season for the average female. In addition, anatomical maturity stages and the gonadosomatic index were compared to histological results in order to show the extent that these data can provide accurate information concerning the reproductive cycle. Finally, we estimated the length at which 50% of females are mature, using both histological and macroscopic criteria.

Materials and methods

Sampling and macroscopic classification

Samples of European horse mackerel were collected in the Saronikos Gulf (Fig. 1) by a commercial trawler operating from October through May. In the study area, the mean annual temperature and salinity at a depth range of about 100–200 meters were 14°C and 38‰, respectively. At this depth range, which characterizes European horse mackerel distribution (Fischer et al., 1987), there was little monthly temperature variation during the sampling period (Georgopoulos[3]). The samples were taken almost monthly, from October 1989 until May 1991, but they were taken more often during the peak spawning period until the end of the sampling period. Supplementary samples were obtained with a purse seiner during the period closed to trawling (i.e. June–September) (Table 1).

European horse mackerels were randomly selected from the catch, and the total length of the fish was measured to the nearest centimeter. Ten fish from each 1-cm length class (throughout the available range) were retained. These fish were measured (total length) to the nearest millimeter, sexed, and weighed (total weight) to the nearest gram. After gross examination of the gonads, the fish were classified into maturity stages according to Macer (1974). Because some of the stages used by Macer were difficult to distinguish macroscopically, (such as developing virgin (maturing/immature) versus resting, ripe versus running, and spent versus recovering), three pairs of Macer's stages were grouped into single stages. Thus, the maturity scale used consisted of seven stages: I = virgin, II = resting, III = early developing, IV = later developing, V = ripe, VI = partly spent, and VII = spent. Gonads and livers were removed and weighed to the nearest 0.1 gram.

Ovaries classified into maturity stages greater than II (about a third of the length of the body cavity) were preserved in 7% neutral, buffered formalin for histological examination. A number of randomly selected ovaries in maturity stages I and II were also preserved in 7% buffered formalin.

Histological method and classification

A small piece (about 0.5–1 cm long) of each preserved ovary was removed, dehydrated, and embedded in paraffin. Subsequently, 3-μm sections were cut and stained with Harris hematoxylin followed by eosin counterstain (H+E). In each sectioned ovary we recorded the following characters (Wallace and Selman, 1981; Mayer et al., 1988; Macewicz and Hunter, 1993): (a) oocytes that had not begun vitellogenesis; (b) oocytes in early vitellogenic stages; (c) advanced yolked oocytes; (d) migratory-nucleus-stage oocytes; (e) hydrated oocytes; and (f) postovulatory follicles. Postovulatory follicles were aged following the criteria used for *T. symmetricus* (Macewicz and Hunter, 1993; Macewicz[4]). The ovarian sections were also

[3] Georgopoulos, D. 1993–95. National Centre for Marine Research, Aghios Kosmas, 16 604 Hellinikon, Greece. Personal commun.

[4] Macewicz, B. J. 1993. Southwest Fisheries Science Center, Natl. Mar. Fish. Serv., NOAA, P. O. Box 271, La Jolla, CA 92038. Personal commun.

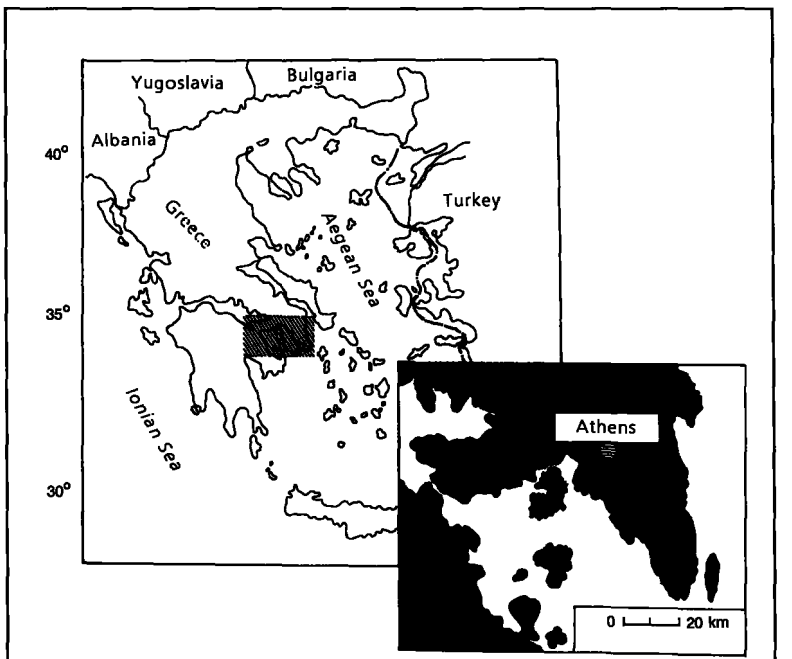

Figure 1
Map of the Saronikos Gulf (Greece).

Table 1

Sampling data and numbers of horse mackerel, *T. trachurus*, females in various histological subclasses during the sampling period in the Saronikos Gulf (Greece).

Sampling date	Fishing gear[1]	Immature[2]	Mature			Females sectioned	Total no. females sampled
			Active		Inactive		
			Spawning[3]	Nonspawning[4]	Postspawning[5]		
24 Oct 1989	TR	34	0	1	0	35	68
20 Nov 1989	TR	4	0	9	0	13	32
28 Dec 1989	TR	0	1	12	0	13	27
24 Jan 1990	TR	0	2	14	1	17	53
15 Feb 1990	TR	0	13	12	1	26	30
12 Mar 1990	TR	0	32	8	1	41	79
28 Mar 1990	TR	0	17	0	3	20	48
18 Apr 1990	TR	0	14	0	5	19	67
09 May 1990	TR	0	11	0	5	16	57
29 May 1990	TR	0	6	0	9	15	39
20 Jun 1990	PS						24[6]
05 Jul 1990	PS	0	1	0	14	15	29
12 Sep 1990	PS	25	0	0	0	25	51
19 Oct 1990	TR	8	0	0	0	8	83
20 Nov 1990	TR	7	0	0	0	7	34
18 Dec 1990	TR	7	0	20	0	27	70
24 Jan 1991	TR	0	9	8	0	17	53
13 Mar 1991	TR	0	4	1	19	24	30
31 Mar 1991	TR	0	15	2	4	21	90
14 Apr 1991	TR	0	13	0	15	28	42
07 May 1991	TR	0	5	0	0	5	24
24 May 1991	TR						50[6]
30 May 1991	TR						72[6]
Total		85	143	87	77	392	1,152

[1] TR = Trawler, PS = Purse seiner.
[2] No yolked oocytes and no postovulatory or atretic follicles were present.
[3] Migratory-nucleus-stage oocytes and/or hydrated oocytes were present, or yolked oocytes and postovulatory follicles.
[4] Yolked oocytes were present and no migratory-nucleus-stage or hydrated oocytes, or postovulatory follicles.
[5] Postovulatory or atretic follicles could be detected, but either no yolked oocytes were present or if present, ≥50% were undergoing α-atresia.
[6] Samples of females with were macroscopically classified as immature (virgin or resting) and were not sectioned.

examined for the presence of alpha (α), beta (β), gamma (γ) or delta (δ) stage atresia (Bretschneider and Duyvene de Wit, 1947). Our definition of an atretic follicle, which characterizes an oocyte undergoing atresia in β or subsequent atretic stages, was adopted from Hunter and Macewicz (1985b).

Because females tend to reabsorb their oocytes once the spawning season comes to an end, the criteria of 50% or more of yolked oocytes (not those in early vitellogenic stages) with α-stage atresia, or the absence of yolked oocytes but presence of postovulatory follicles or atretic follicles, or both, were used to indicate that these females had finished their spawning season (Hunter and Macewicz, 1985, a and b; Hunter et al., 1986; Dickerson et al., 1992; Macewicz and Hunter, 1993). It should be mentioned that the occurrence of α-stage atresia in yolked oocytes is the best characteristic to backcalculate the time of past reproductive activity because the type of oocyte undergoing atresia is still discernible, and this atretic oocyte can be easily distinguished from postovulatory follicles (Hunter and Macewicz, 1985b). Hence, all the yolked oocytes (not those in early vitellogenic stages) in each section were counted under the mi-

croscope, and the percentage (< or ≥50%) of oocytes in α-stage atresia was noted.

Females with ovaries containing yolked oocytes were classified as active, i.e. capable of spawning in the current spawning season. Active females were separated into spawning and nonspawning groups. Spawning females contained ovaries with histological characteristics of past spawning (postovulatory follicles) or imminent spawning (hydrated oocytes or migratory-nucleus-stage oocytes). The nonspawning females that displayed no such characteristics were assumed to be capable of spawning in the near future. Females with yolked oocytes where 50% or more were in α-stage atresia, or had no yolked oocytes but only postovulatory or atretic follicles, or both, were classified as postspawning or inactive in the current season. The active and the postspawning females were both considered mature. Females that showed no histological evidence of past, imminent, or future reproductive activity were classified as immature.

Reproductive cycle

Atretic states Description of atresia stages (α, β, γ, and δ) follows Bretschneider and Duyvene de Wit (1947), Hunter and Macewicz (1985, a and b) and Macewicz and Hunter (1993). These stages are common among several species studied including the jack mackerel, *Trachurus symmetricus* (Macewicz and Hunter, 1993).

Classification of ovaries based on oocyte and atresia stages follows Hunter and Macewicz (1985b) and is briefly summarized as follows:

Atretic state 0 No α atresia of yolked oocytes.
Atretic state 1 <50% (1 oocyte to 49%) of yolked oocytes were in α atresia.
Atretic state 2 ≥50% of yolked oocytes were in α atresia.
Atretic state 3 No remaining yolked oocytes, but β atresia or later atresia stages were present.

Atretic state 3 was used to separate immature females from postspawners, because advanced atresia stages indicate the possibility of past spawning. The late postspawning ovary was also characterized by disorganized septa, a condition which indicated that this particular female might have spawned during the latest reproductive season.

We defined the reproductive season for European horse mackerel as the period from the date of first active females with yolked oocytes, not necessarily spawning, until the last active females sampled. The fraction of active females classified as spawning was used to estimate the spawning rate index, while the fraction of the postspawning females was used as an index of the rate at which females passed from the active to the inactive state. These data were fitted to logistic curves ($y=1/(1+e^{a-bx})$, where y is the fraction of the females used and x is the number of days elapsed since the initiation of spawning; Hunter et al., 1992). We calculated the approximate spawning season for individual females by subtracting the days from the 50% spawning point to the 50% postspawning point.

In some females, multiple spawning stages (migratory-nucleus oocytes, hydrated oocytes, 1-d-old postovulatory follicles, 2-d-old postovulatory follicles) occurred within the same ovary indicating different potential or past spawnings. Under the assumption that the stages of advanced yolked oocytes and partially yolked oocytes could represent two potential spawnings, as many as six different spawning stages could be distinguished histologically in the same ovary. Hence, the mean number of stages per sampling date was estimated, providing information about seasonal changes of spawning frequency (Hunter et al., 1992).

Gonadosomatic and hepatosomatic index We also followed the seasonal changes in ovarian development by calculating the mean monthly gonadosomatic (GSI) and hepatosomatic (HSI) indices. The formulae used were the following: GSI= 100 (ovary weight)/(body weight–ovary weight) and HSI= 100 (liver weight)/(body weight–liver weight).

Estimation of length at 50% maturity

Total lengths of all histologically identified females were used to estimate the length at 50% maturity (ML_{50}) of European horse mackerel during the spawning season. We assumed that all females with vitellogenic ovaries would reach sexual maturity, and therefore such females were included in the proportions of mature females. The proportions were estimated at length classes of 10 mm, and the data fitted to a logistic curve. Lengths of females with ovaries classified macroscopically were similarly fitted to a logistic function for comparison of the ML_{50} value. Macroscopically mature females possessed ovaries that were classified to maturity stages above stage II.

Results

Oogenesis

In the ovaries of European horse mackerel, oocyte development is divided into a primary growth phase

and a secondary growth phase as in most teleosts (Wallace and Selman, 1981; Mayer et al., 1988). The secondary growth phase is characterized by yolk deposition, nucleus migration to the animal pole, and hydration. In particular, oocytes in early vitellogenic stages (partially yolked), and advanced yolked oocytes that were included in the mature horse mackerel females had the following characteristics:

Partially yolked oocytes Yolk vesicle deposition following by the appearance of yolk granules and the formation of the zona radiata.

Advanced oocytes Increase of yolk granules resulting in compact dense yolk spherules, which filled the cytoplasm. The nucleus was eventually surrounded by the lipid droplets.

Reproductive cycle

Atresia stages and atretic states During the whole sampling period, 392 ovaries out of 1,152 females sampled were sectioned, and 85 were identified as immature, 143 as spawning, 87 as nonspawning, and 77 as postspawning (Table 1). Ninety-six ovaries were found having α-atresia in advanced yolked oocytes (Table 2).

We observed atresia throughout the spawning period in sequential stages—α(alpha), β(beta), and δ (delta), (Fig. 2, A–C). The α- and δ-stages of atresia showed similar characteristics to those described for northern anchovy (Hunter and Macewicz, 1985b). The γ (gamma) stage atresia was not observed; this may have been due to its short duration or to the fact that the follicle passes directly from the β to the δ stage without passing through the intervening γ stage (Hunter and Macewicz, 1985, a and b). In addition, the low incidence of δ-stage atresia observed in regressing ovaries of European horse mackerel indicates that some follicles might be completely resorbed during the β stage. In particular, the β-stage atretic follicle of European horse mackerel was characterized by the presence of many vacuoles, possible remnants of the lipid droplets that take longer than yolk to be absorbed (Macewicz and Hunter, 1993).

The spawning potential of females with atretic ovaries is generally thought to be low (Hunter and Macewicz, 1985b). To verify this expectation in the regressing ovaries of European horse mackerel, we estimated the percentages of females with the histological criteria of imminent spawning, past spawning, and nonspawning, which were classified into atretic states 1 and 2. Females in atretic state 1 showed evidence of past or imminent spawning (82.9%), whereas the percentage of spawning females in atretic state 2 was very low (7.7%) (Table 2). By the time that ovaries reached atretic state 2, more than half of the advanced yolked oocytes were not viable. In particular, about 80% of the ovaries in atretic state 2 had 90–100% atretic advanced yolked oocytes.

During this study, we identified two successive reproductive seasons, the first one from October 1989 to early July 1990, and the second one from December 1990 to May 1991. Onset of spawning was observed in December and January, respectively, and continued until July and May of the first and second seasons, respectively. In June 1990, no ovaries were sectioned because females were macroscopically classified as immature, whereas in July 1990 all but one (spawning) of the females were late postspawning (atretic state 3).

On the basis of histological classification of ovaries, 192 and 115 mature females were examined in the first and second reproductive seasons, respectively. The occurrence of α atresia was seen from the onset of spawning. During the first spawning season we observed that atretic state 1 was the most common atretic condition (Fig. 3A). Females in atretic

Table 2

Percentage of European horse mackerel, *T. trachurus*, females classified into two atretic states which occurred in each of four reproductive classes.

Atretic state	% totally yolked oocytes with α-atresia stage	Immenent spawning[1]	Past spawning[2]	Spawning past or imminent	Active nonspawning[3]	Total no. females
1	<50	15.3	67.6	82.9	17.1	73
2	≥50	0.0	7.7	7.7	92.3	23
					Total	96

[1] Migratory-nucleus-stage oocytes or hydrated oocytes present. Postovulatory follicles may be present.
[2] No migratory-nucleus-stage oocytes or hydrated oocytes present. Postovulatory follicles present.
[3] Yolked oocytes present and no histological characteristics as mentioned above.

states 2 and 3 appeared gradually in increasing numbers. The high percentage of atretic-state 2 females was evident near the end of the season, whereas at the end, females with yolked oocytes in the ovaries were not present, and atretic-state 3 females were predominant.

In the ovaries sectioned from September to November 1990, we observed only primary growth oocytes, and the onset of vitellogenesis was not detected until December 1990. During this second season, atretic patterns did not follow the trends observed in the first season (Fig. 3B). During March 1991 (mid-season), high numbers of postspawning females (atretic states 2 and 3) were noted, followed by an increase in the females classified in atretic state 0, which suggests a hiatus in spawning during a period considered to be the middle of the spawning season.

Spawning and postspawning females during the season In the first reproductive season, the sample of 28 December was the first to contain spawning females with evidence of past spawning (Table 1). Since the previous sample taken (20 November) had no spawning females but rather females with advanced yolked oocytes, 9 December (the middle of these dates) was taken as an approximate start of the spawning period. The fractions of spawning and postspawning females were plotted as a function of time elapsed since 9 December (Fig. 4).

A sharp increase occurred in spawning rate between January and February, indicating a change in the spawning frequency, and by the end of March all active females were spawning (Fig. 4). The rate at which females passed from the active to the inactive stage (postspawning rate) accelerated in April. Thus, the period from the end of March to the beginning of April was considered to be the peak of spawning in 1990 on the basis of histological samples.

During the spawning season, the sex ratio (females/total) from all fish sampled remained constant (mean=0.57, SD=0.035), indicating that no sex-based migration probably occurred in and out of the sampled area. The number of sections were insufficient to detect whether the incidence of atresia was age specific. However, it is known that all individuals of European horse mackerel do not have the same spawning season (Arruda, 1984), as is the case for northern anchovy (Hunter and Macewicz, 1985b). For this reason, we estimated the approximate spawning season for an average female by sub-

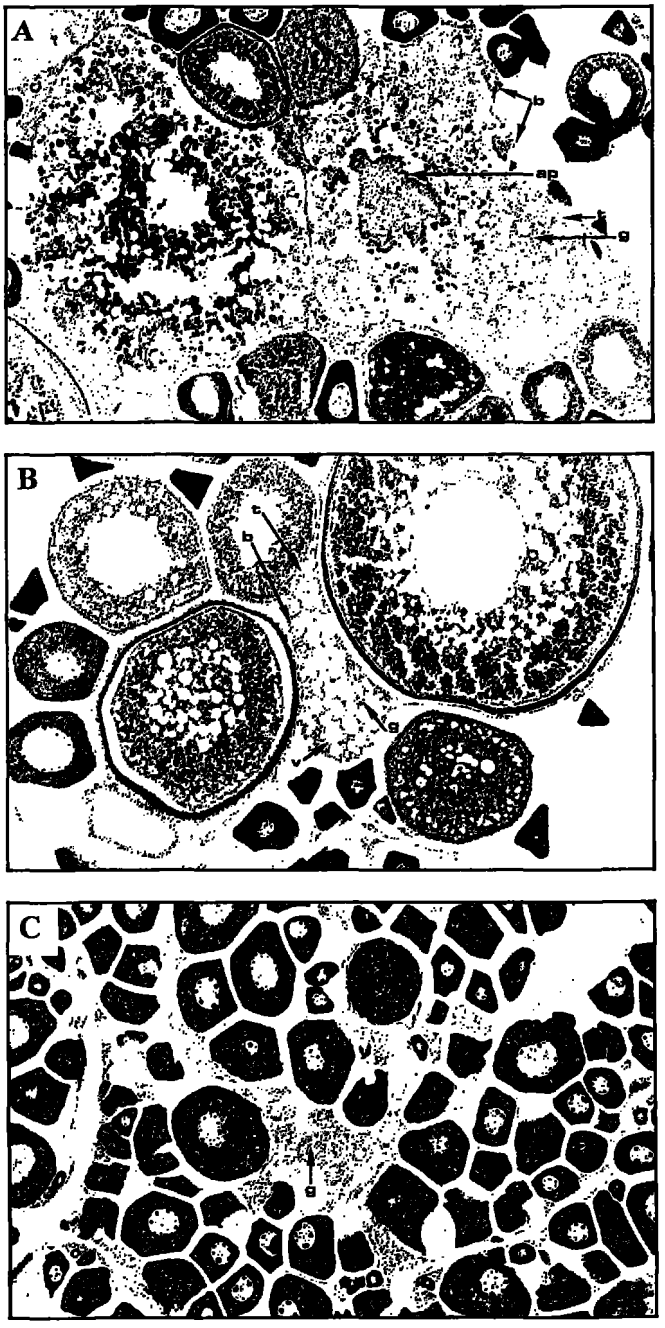

Figure 2

Atresia stages of European horse mackerel, *Trachurus trachurus*, oocytes. (**A**) α-stage atresia of two yolked oocytes. The zona radiata has been dissolved. The hypertrophied granulosa cells (g) have invaded the yolk. The thecal cell layer (t) with blood capillaries (b) is seen. The amorphous particle (ap) may be the remnant of the zona radiata or yolk. (**B**) β-stage atresia. The yolk spherules show degeneration. Granulosa cells (g) and many vacuoles (v) are present. The thecal cell layer (t) with blood capillaries (b) is seen. (**C**) δ-stage atresia. Few granulosa cells (g) in the ovarian tissue stroma. The thecal cell layer and the blood capillaries no longer encompass the follicle. Harris haematoxylin and eosin stains were used. Magnification: 137×.

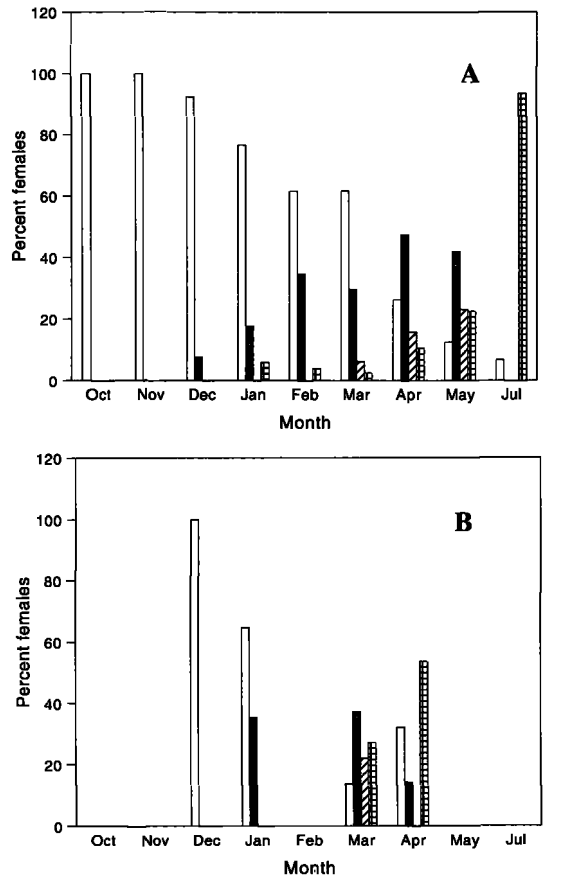

Figure 3

Percentage of mature European horse mackerel, *Trachurus trachurus*, females classified into atretic states (**A**) during the first reproductive season and (**B**) during the second reproductive season. No ovaries were sectioned in June (A). Five ovaries sectioned in May (B) were not classified into atretic states because of their low number. Open solid bar = atretic state 0; filled solid bar = atretic state 1; diagonally hatched bar = atretic state 2; cross-hatched bar = atretic state 3.

tracting the number of days to the 50% spawning point (67 days) from the number of days to the 50% postspawning point (161 days) which resulted in a spawning duration estimate of 94 days.

An increase in the mean number of potential and past-spawning stages per female occurred until early May, indicating a possible increase in spawning frequency (Fig. 5). By the end of May, when atretic states 2 and 3 were estimated to be very high, the mean number of these spawning stages decreased sharply. This shows that no other oocytes were recruited into vitellogenesis as the spawning period was ending.

Gonadosomatic and hepatosomatic index From the histological study we observed that no females of age less than 2 years spawned (Karlou-Riga[5]). Therefore, GSI and HSI were both calculated for females older than 1 year of age. The peak GSI value occurred in February 1989, when the histological data indicated that half of the mature females (52%) were spawning (Fig. 6). GSI decreased in the following months, whereas the peak HSI value was observed near the end of the first reproductive period (around May). During the summer and autumn of 1990, the two indices remained low, and an increase occurred in December 1990 denoting the onset of the second reproductive season (Fig. 6). During the second season, the peak GSI value was about one third of the respective value of the same index observed in the previous period (Fig. 6).

The correlation of the two indices was tested by using Spearman's rank correlation procedure (Zar, 1984). Disagreement was found during the sampling period (Oct. 1989–Nov. 1990: r_s=–0.2829, P<0.001, n=551; Dec. 1990–May 1991: r_s=–0.1955, P<0.001, n=429). The negative correlation between GSI and HSI indicates that GSI and HSI did not follow the same course, indicating most probably that ovarian growth proceeds at the expense of liver content as is the case for other teleost species (Wallace and Selman, 1981), including *Trachurus murphyi* (Kaiser, 1973).

Estimation of length at 50% mature

The sex ratio was examined by applying the paired-sample t-test in both reproductive periods, and no difference was found at 5% level of significance (t=1.92, P=0.127). Length-frequency distributions of females for the two periods were tested by applying the Kolmogorov-Smirnov two-sample procedure. No significant length differences were found between females that were macroscopically staged (P=0.96 [>0.05]) and those that were histologically staged (P=0.37 [>0.05]) for maturity status. Therefore, the data from the two periods were combined, and the ML_{50} values were estimated from the logistic curves separately for histological and macroscopical data (Fig. 7).

Fish with lengths close to ML_{50} were two to three years old (Karlou-Riga[5]). The macroscopically estimated ML_{50} (220 mm) was a little higher than the histologically estimated ML_{50} (211 mm), probably owing to the macroscopical misclassification of some mature females as immature. However, analysis of

[5] Karlou-Riga, C. 1995. Age and growth of *Trachurus trachurus* (L.) in the Saronikos Gulf (Greece). Unpubl. manuscr., Fisheries Laboratory, Ministry of Agriculture, 15 Karaoli and Dimitriou St., GR-18531 Piraeus, Greece.

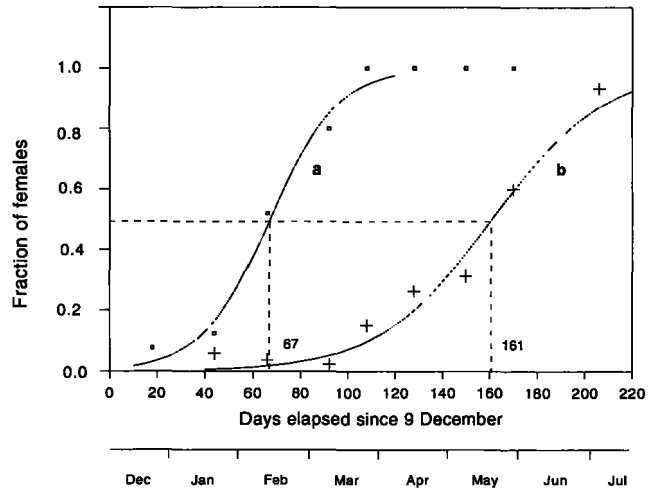

Figure 4

Fraction of European horse mackerel, *Trachurus trachurus*, spawning and postspawning females during the period from 9 December to 5 July (1989–90). (**a**) Females with active ovaries, which had one or more spawning stages (open squares); trend line is a logistic curve, where $a = 4.699$, $b = 0.070$, $r^2 = 0.98$. (**b**) Females in atretic states 2 or 3, which have finished their spawning season (crosses); trend line is a logistic curve, where $a = 6.757$, $b = 0.042$, $r^2 = 0.97$. The approximate spawning season for the individual female (94 days) was estimated by subtracting the 50% spawning point (67 days) from the 50% postspawning point (161 days).

covariance of the $\log_e$-transformation of percent mature females on the length and maturity-staging method indicated that the difference between the two ML_{50} estimates was not significant (F=0.85, P=0.373).

Discussion

Reproductive cycle

In the present work, we found that histological atretic states were useful as an index of the population's reproductive capability for European horse mackerel. Atretic-state 1 females (<50% of advanced yolked oocytes in α-stage atresia) were apparently capable of spawning, whereas atretic-state 2 females (50% or more of advanced yolked oocytes in α-stage atresia) had a lower spawning probability. It seems most likely that once females are in atretic state 2 they do not spawn or spawn infrequently (Hunter and Macewicz, 1985b). Therefore, this state was the best absolute measure of ovary resorption. Atretic-state 3 females (showing no yolked oocytes but late atresia stages) were those in late postspawning condition. These females could not be distinguished from immature females by gross anatomical criteria. Consequently, this histological state was very useful in classifying mature females and estimating length at first maturity.

The high percentages of atretic oocytes observed in European horse mackerel throughout the spawning season are a normal occurrence in multispawners (Hunter and Macewicz, 1985b; Dickerson et al., 1992), an attribute which probably characterizes most species of *Trachurus* (Macewicz and Hunter, 1993). High numbers of atretic oocytes are also observed in Atlantic mackerel (*Scomber scombrus* L.) ovaries around peak spawning (Walsh et al.[6]). Therefore, atretic state 1 is expected to be the most common condition during the main spawning season.

During the first spawning season, we found that atretic state 2 increased near the end of the season, and that it was completely replaced by atretic state 3 at the end. Thus, it is useful to study both of these atretic states during the entire spawning season. However, it is also important to estimate atretic states near and after the end of the season. We were not able to assess the atretic state in June because of a lack of histological samples. However, because atretic state 3 lasts for one month or more in the ovary (Hunter and Macewicz, 1985, a and b), it is very likely that ovary resorption observed in high levels in late May continued through July, because all the mature females were in late postspawning condition by July. Low GSI values in June and July supported this conclusion. Thus, high levels of atretic state 2 actually forecasted the end of the European horse mackerel spawning season as has been noted for other fishes (Hunter and Macewicz, 1985b). Because GSI remained low during the summer and no atresia stages were observed in early September 1990, vitellogenesis probably did not occur in the time interval between July and September.

Females were inactive until December 1990, when the next reproductive season began. By the middle of this second season (March 1991), high levels of atretic states 2 and 3 were noted, and subsequently percentages of females in atretic state 0 (yolked but no atretic oocytes) increased, resulting in an unexpected pattern. It was not possible to explain either the "delay" of onset for this reproductive season or the mid-season increase in atretic rates. Variation

[6] Walsh, M., P. Hopkins, P. Witthames, M. Greer-Walker, and J. Watson. 1990. Estimation of total potential fecundity and atresia in the western mackerel stock, 1989. ICES Council Meeting H:31, 22 p.

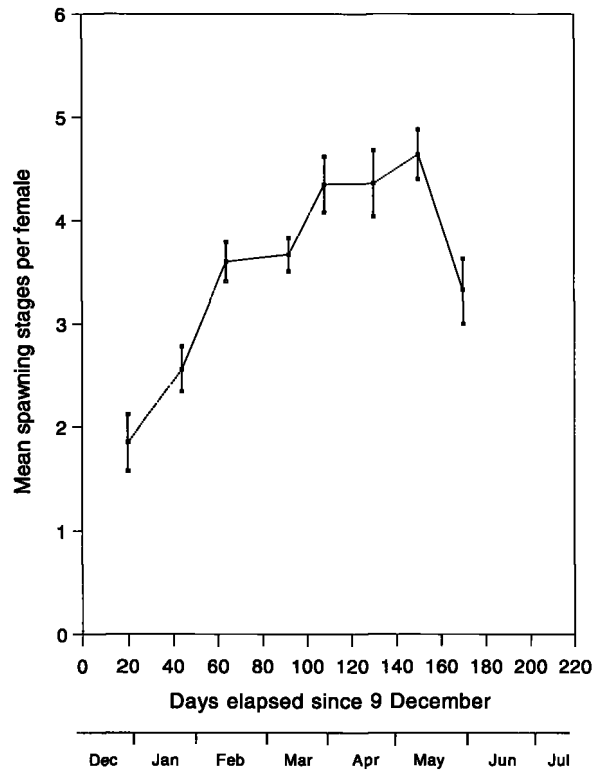

Figure 5

Mean number of potential and past-spawning stages per European horse mackerel, *Trachurus trachurus*, female during the period from 9 December to 5 July (1989–90); bars are two standard errors of the mean.

in temperature, one of the main factors influencing maturation (Billard et al., 1981), could not have affected these second season changes because temperature remained almost constant in the study area. Another factor known to stimulate ovary resorption is an imbalance in the sex ratio (Trippel and Harvey, 1990). However, in the present work this ratio did not differ between the two reproductive seasons and remained constant. Although a reactivation of regressing ovaries has been postulated as a possible event (Hunter and Macewicz, 1985b), it is not known whether postspawning European horse mackerel females can be reactivated during a spawning season. The high levels of atretic states 2 and 3 for European horse mackerel in the middle of the season may not necessarily indicate cessation of spawning but merely the end of a spawning cycle within the spawning season. This implies that European horse mackerel seems to be very flexible in adjusting its spawning time to different conditions and that its spawning cycle might be more variable than previously assumed. Such a flexibility may be very important for the survival of the species in highly oligotrophic waters, such as the Greek and northeast Mediterranean waters in general (Stergiou, 1993).

The index of spawning rate provided information about the spawning behavior of active European horse mackerel females during the first reproductive season. The increase of this rate, beginning very early in the season, indicated an increase in spawning frequency in the same period (Hunter et al., 1992). This increase was supported by an increase in the mean number of potential or past-spawning stages. The decrease in this number toward the end of the season likely indicated a decrease of spawning frequency, although all the remaining active females were spawning.

We noticed in using both histological and macroscopic data that the spawning season of European horse mackerel lasts about six months. This season ranges from 3 to 6 months for northern to southern areas of the eastern Atlantic, respectively (Arruda, 1984; Eaton, 1989; Eltink[7]). However, we believe the duration of the season, during which spawning was observed, differs from the spawning duration for the average female, which may be very representative of the population. Smaller northern anchovy females have a shorter spawning season because of high levels of atresia (Hunter and Macewicz, 1985b). The same may be true for European horse mackerel because all females did not start and finish spawning on the same day. The estimated spawning season for the average European horse mackerel female (94 days) is higher than that for the same species in the eastern Atlantic which is assumed to be 65 days (Eltink[2]).

The GSI, as an index of ovarian changes during the spawning season, is of lesser importance than histological data for multiple spawners. For instance, this index does not distinguish partly spent (active females with postovulatory follicles) and spent (inactive females with regressing ovaries) females. The peak GSI value may not indicate the peak spawning time but rather a later developing stage for most females (Arruda, 1984). However, high values of this index can be used to determine the period when hydrated oocytes can be identified (Hunter and Macewicz, 1985a). This is very useful for batch fecundity estimates, where hydrated oocytes need to be counted. GSI calculated over the entire year indicated the approximate reproductive period, and the low GSI values near the end of the season constituted a helpful guide to validate the end of the sea-

[7] Eltink, A. 1991. Horse mackerel egg production and spawning stock size in the North Sea in 1990. ICES Council Meeting H:27, 14 p. (Mimeo.)

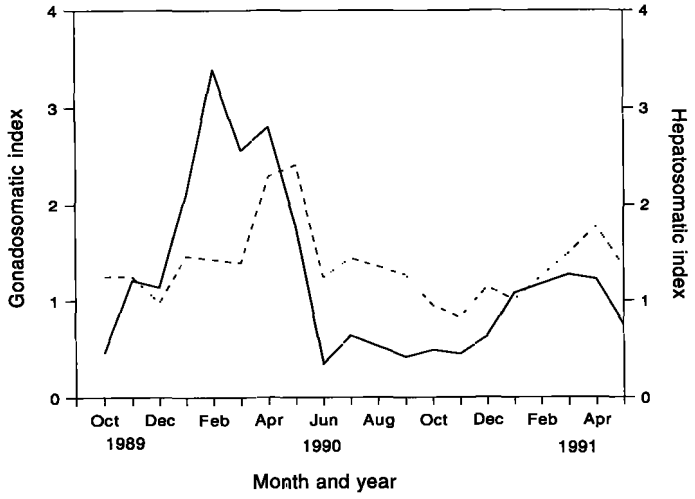

Figure 6

Gonadosomatic (mean GSI) and hepatosomatic (mean HSI) index of European horse mackerel, *Trachurus trachurus*, females per sampling month during the period from October 1989 to May 1991. Number of females examined = 980. Solid line = GSI; dashed line = HSI.

son as was indicated by high atretic states. During the second reproductive period, we noticed that GSI attained about one third of the value recorded in the previous period. The lower GSI values during the second period also support the high numbers of postspawning females that were observed histologically during mid-season, resulting in a possible drop of annual fecundity.

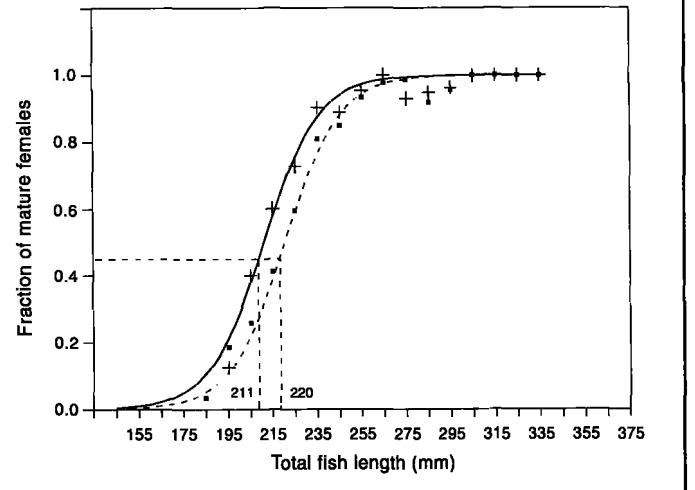

Figure 7

Fraction of European horse mackerel, *Trachurus trachurus*, females that were sexually mature on the basis of histological criteria (crosses and solid line) and macroscopic criteria (filled squares and dashed line) during two successive reproductive periods. The ML_{50}, based on 307 mature females, was estimated histologically to be 211 mm (logistic curve parameters: a=17.104, b=0.081, r^2=0.98); the ML_{50}, based on 595 mature females, was estimated macroscopically to be 220 mm (logistic curve parameters: a=17.863, b=0.081, r^2=0.99).

Sexual maturity

We found that the values of ML_{50} estimated histologically (211 mm) and macroscopically (220 mm) were very similar. This indicates that the separation between mature and immature females by gross examination of the gonads was relatively unbiased. However, a number of females with early developing or regressing ovaries were grossly misclassified as immature. These could be correctly identified only by histological examination, resulting in lower proportions of mature females with the macroscopic method. On the other hand, it is possible that ML_{50}, determined histologically, may be overestimated when measurements are made during the spawning season (Hunter et al., 1992). This did not happen in the present work because immature females were identified only at the beginning of the reproductive period, before spawning had started. Consequently, we believe the ML_{50} determined from histological sections was the least-biased estimate. Although the age at first maturity (i.e. 2 years) is in general agreement with Arruda's (1984) estimation, our ML_{50} values were not strictly comparable to those of Arruda, because the latter refers to standard length units and no conversion equation is given. However, our value was probably lower because Arruda considered only macroscopically "ripe" individuals as mature. A smaller number of stages used to define maturity was also the reason for a higher value of ML_{50} (254 mm) estimated by Kerstan (1985). Finally, the differences in sexual maturity referred to by various investigators should be compared carefully owing to possible different sampling times or, more importantly, to a strict use of anatomical criteria to identify the mature individuals resulting in a significant bias, especially for multiple spawners.

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