INTRODUCTION

Mugilidae is a widely distributed family and its memberspecies occur in coastal marine and brackish waters in all tropical and temperate seas. They are euryhaline fish and are more commonly found in coastal waters and lagoons than in fresh waters. They reproduce at sea; subsequently the fish fry approach the shore in dense schools and enter lagoons, rivers, or even lakes, where they continue to grow. When mugilids (known also as grey mullets) approach sexual maturity, they move again towards the sea, where their maturation is completed and their spawning takes place. This migration is well known for Mugilidae (see Brusle 1981), but no solid evidence exists that spawning occurs in a specific location based on known sea characteristics (currents, depth, temperature, etc.). As actual spawning has never been observed, information about reproductive activities can be gathered only from studies inside lagoons and/or during the migration of fish into or out of the lagoon (Hotos et al. 2000).

Brusle (1981), in his review of mugilid reproduction, mentioned that even though mugilid fish are so widely distributed, limited information is available on their reproduction (spawning period, spawning grounds, fecundity, etc.) usually because it is very difficult to collect fully mature specimens. This is why information on the spawning period of grey mullets is usually concluded from the appearance of juveniles to coastal lagoons or estuarine systems (Cambrony 1984, Koutrakis et al. 1994, Katselis et al. 1994, Koutrakis 2004). In the Mediterranean, this is even more evident, since very limited information is published on their spawning (Tsikliras et al. 2010) and mainly concerns Liza ramada (Tunisia: Farrugio and Quignard 1973; Egypt: El Maghraby 1974, Turkey: Ergene 2000) and Liza aurata (Ionian Sea: Hotos et al. 2000). Nothing has been published for Liza saliens, apart a study on the eggs and early life stages in Israel (Yashouv and Berner-Samsonov 1970) and a reference on the spawning period based on maturity stages in Egypt (El Zarka and El-Sedfy 1970). Moreover, in the Aegean Sea no relevant information on the grey mullet reproduction is still available. In view of the increasing interest for extensive culture (“valicoltura”) of these species all over the

REPRODUCTIVE BIOLOGY OF TWO GREY MULLET SPECIES (ACTINOPTERYGII: MUGILIFORMES: MUGILIDAE) IN A NORTHERN AEGEAN SEA ESTUARINE SYSTEM

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Background. Limited information is available on the reproductive biology of grey mullets mainly because it is very difficult to collect fully mature specimens. In the presently reported study the reproductive biology of two mugilid species, namely Liza saliens and Liza ramada, was studied in the Vistonis and Porto Lagos estuarine systems. Materials and methods. Gonadosomatic index (GSI) and standard length at first maturity (SL50) were calculated. In order to estimate which oocytes are going to mature, oocyte size-frequency profiles were constructed for each fish and only the most advanced yolked oocytes were considered. The fecundity estimates were obtained using the volumetric method.

Results. The results indicated that the sex ratio was in favour of females for both species. The gonadosomatic index indicated that the spawning season of L. saliens extends from June through October and that of L. ramada—from September through January. L. ramada sexually matures at a greater size and older age than L. saliens. For a correct estimate of the fecundity, the smallest group of eggs in both species (<0.20 mm) was rejected. For L. saliens the absolute fecundity varied from 245 000 to 555 000 eggs, while for L. ramada from 150 000 to 685 000 eggs. Fecundity is probably more correlated with the weight of the fish for both species.

Conclusion. For the estimation of fecundity the total number of the oocytes is often used. However, this can be misleading as shown from the frequency distribution of the oocytes in the two grey mullet species, where a large proportion of the oocytes will never mature and therefore they do not contribute to the reproduction process. The percentage of these oocytes can be high, as shown in the current study (3.9%–28.1%).

Keywords: Mugilidae, Liza saliens, Liza ramada, reproductive biology, sex ratio, GSI, fecundity, Aegean Sea
Mediterranean lagoons and estuarine systems, all information on their reproductive biology is valuable.

The aim of this research project was to study the reproductive biology of two mugilid species, namely the leaping grey mullet, *Liza saliens* (Risso, 1810), and the thin lipped grey mullet, *Liza ramada* (Risso, 1827), of the estuarine system of Lake Vistonis and Porto Lagos Lagoon (Thracian Sea, North Aegean Sea). These were the most abundant mugilid species in this system and thus it was possible to find a meaningful number of samples mature or close to maturity. More specifically the sex ratio, the gonadosomatic index, the length and age at first maturity, the oocyte growth and development, the absolute and relative fecundity, and the relation of absolute fecundity with length, weight and age were studied.

**MATERIALS AND METHODS**

**Study area.** Lake Vistonis (40 km²) is a shallow lake (average depth of 2 m), connected to the Porto Lagos Lagoon (3.6 km²) by two narrow channels and to the Vistonikos Gulf in the Thracian Sea (northern Aegean Sea), by a channel 60 m wide and 600 m long (Fig. 1). The lake is also connected directly to the sea by a narrow (5 m), artificial channel 1.5 km long. A horizontal salinity gradient divides the lake in two parts: the northern, upstream part is affected by the freshwater inflow of three rivers (annual discharge of $515.3 \times 10^6$ m³) into the lake (Babajimopoulos and Antonopoulos 1992), whereas the southern part is more saline due to the inflow of the seawater in the lake through Porto Lagos Lagoon. The latter situation is more intense during summer due to the low freshwater input, leading to negative water balance. At the northern end of the channels connecting the Porto Lagos Lagoon and Vistonis Lake (Fig. 1), grilled barriers (10 mm width between grills) prevent adult fish from moving between the two systems, but allow the entrance of juveniles and small sized adult fish in the lake. These screens open between January and April each year allowing adult fish to move between systems. Market-size individuals of commercially important fish species are harvested during their reproductive migration to the sea at a specially designed entrapment device, constructed by grilled screens, similar to the French ‘bordigue’ described by Pauly and Yáñez-Arancibia (1994), which are situated at the southern part of the lagoon. The rest of the fish caught are returned either in the lagoon or in artificial deep channels (wintering channels) to spend the cold season and to grow until the next harvest period. No artificial feeding is provided (Koutrakis et al. 2005).

**Sampling and sample elaboration procedure.** The study was carried out from December 1988 through November 1990. One more sample was also collected on August 1991 in order to collect more mature specimens. Monthly fish samplings were carried out in the Porto Lagos Lagoon, Lake Vistonis and the Vistonikos Gulf for two consecutive days each month. Three different sampling techniques were used in order to collect all size classes of the grey mullets:

- Five nylon twine gillnets of 50 m length and 1.5 m height (bar mesh size of 8, 14, 22, 28, and 34 mm, positioned randomly) were deployed in gangs, tied end to end perpendicular to the shore for 12-h periods (from dusk till dawn);

**Fig. 1.** Map of the study area. Arrows indicate connection with the Porto Lagos Lagoon and the Vistonicos Gulf
A trammel net of 850 m length, height 4.5 m, mesh size 26 mm, was used randomly in order to catch migrating mature specimens; samples were taken from a stationary entrapment device, used by local fishermen.

The fish collected were kept in ice and then frozen in the laboratory. Standard length (SL) was measured to the nearest 0.1 mm and total weight (TW) was recorded to the nearest 0.01 g. Fish were classified into maturity stages visually, following the Kesteven scale (Bagenal and Braum 1971). Ovaries of all female fish were separated, weighted and placed in 8% formalin. All ovaries were subsequently transferred into a Gilson solution for three months in order to facilitate egg separation (Bagenal and Braum 1971). The fecundity estimates were obtained using the volumetric method (Alvarez-Lajonchere 1982, Snyder 1985).

Gonadosomatic index (GSI) was calculated for all fish with gonad weight over 0.01 g, separately for each fish and all values were averaged monthly; then GSI was calculated for males and females of each species, according to the formula: GSI = (GW / TW) \times 100, where GW = gonad weight [g].

The predicted standard length at first maturity was estimated as (Echeverría 1987):

\[ SL_{50} = \frac{-a}{b} \]

All specimens assigned to the III or higher stage of maturity (according to the Kesteven scale) were considered mature. The estimation of age at first maturity was based on the growth parameters previously determined for the same species (Koutrakis and Sinis 1994).

The absolute fecundity was defined as the mean number of oocytes found in the ovaries of the female specimens of each species that are probable to be released during spawning. Wootton (1979) defined the absolute fecundity as the number of oocytes released during spawning. Because, in the presently reported study, it would be impossible to follow the above-mentioned definition the number of oocytes found in the gonads was estimated, even if this estimation could lead to biased results, since not all of these oocytes would eventually mature or would be released. In order to estimate which oocytes are going to mature, oocyte size-frequency profiles were constructed for each fish and only the most advanced yolked oocytes, representing the larger diameter were considered for the absolute fecundity count for each species (Greeley et al. 1987, Hotos et al. 2000). Absolute fecundity and relative fecundity (number of eggs per unit of length or weight) were calculated for both standard length and net weight for each specimen. The age used was the one that corresponds to the length of the specimens, as it was determined by Koutrakis and Sinis (1994). The relation between absolute fecundity \( F \) and the growth parameters was described (Blaxter 1969) by the equation:

\[ F = a \times x^b \]

where \( x \) = length, weight, or age, \( a \) and \( b \) = constants.

Chi square \( \chi^2 \) test was used to identify statistical differences from the theoretical ratio 1 : 1 of the sex ratio for the two mugilid species for each length class (Zar 1984).

**RESULTS**

**Sex ratio.** Sex was determined in 227 \( L. saliens \) specimens from which 81 (35.7%) were males longer than 118 cm and 146 (64.3%) were females longer than 93 cm. The resulting sex ratio was 1 : 1.8, statistically different from unity \( \chi^2 = 5.44, P < 0.05 \) (Fig. 2). Females dominated the length classes 90–110 mm and in size classes greater than 210 mm, with ratio statistically different from unity. Males dominated the length classes from 110 mm through 210 mm. Their dominance was gradually decreased with increasing size.

Sex identification was possible for 118 \( L. ramada \) specimens longer than 190 mm (TL). From these, 55 were males (46.6%) and 63 (53.4%) females. Sex ratio was 1 : 1.14, not statistically different from unity \( \chi^2 = 0.54, P < 0.05 \).

**Gonadosomatic index (GSI).** The GSI was calculated for 209 specimens of \( L. saliens \) (74 males and 135 females) and 127 specimens of \( L. ramada \) (60 males and 67 females), with gonad weight over 0.001 g (Fig. 3). The mean monthly GSI values for \( L. saliens \) were low (< 0.5) during the first two months of spring (March–April) and the first winter month (November). During May, there was an increase that peaked in July for females (1.35 ± 0.62) and in August for males (1.03 ± 0.3). No mature sample was found in September and only one in October, and since there was a remarkable decrease in November for both sexes, it can be concluded that the spawning period ended in November. However, in January of the first year of samplings and in February of the second year, there were 4 female specimens of maturity stage III with GSI >1 (1.0–1.4). These specimens were considered delayed spawners, which could be the result of their age, being between the oldest specimens caught (> 250 mm SL, thus of age >6+). Late spawning of old specimens has also been reported by Hotos et al. (2000). Due to the individual variability in maturation of the mugilid species, which could influence the average monthly values, another approach was used in order to verify the reproduction period. This is to record the temporal distribution of the most mature specimens (GSI > 2) (Fig. 4). According to this, 5 specimens (2 males and 3 females) were caught in May, 6 in June and July, and 3 in August. The highest individual GSI value (10.8) was recorded in June; it was a female thin lipped grey mullet in stage V of maturity. The maximum GSI values were recorded for stages IV and V and they were 2.78–4.71 for males and 4.04–10.87 for females.

The mean monthly GSI values for \( L. ramada \) were low (< 0.5) during the end of winter (February) till the end of spring (May) and no mature samples were found in June and July (Fig. 3). In August, there was an increase in GSI values...
Fig. 2. Sex ratio (%) of mature males (M) and females (F) of *Liza saliens* and *Liza ramada* by size class (SL, mm)

Fig. 3. Monthly variation of the gonadosomatic index (GSI) mean values of males and females of *Liza saliens* and *Liza ramada* caught during the sampling period (samples from different years were averaged monthly)
that peaked in September both for females (1.47 ± 0.49) and for males (2.09 ± 0.61). In October, there was a decrease in the GSI of both sexes, which diminished in November for males (0.24 ± 0.09), and in December for females. However, in January, there was again a high monthly GSI, for females (1.19 ± 0.53), due to one old female specimen with GSI 5.2 (age: 7+ and 344 mm SL). According to the temporal distribution of the most mature specimens (>2), 3 specimens (1 male and 2 females) were found in August, 8 specimens (4 males and 4 females) in September, 1 male in October, and 1 female in January (Fig. 4). The highest GSI (6.2) was recorded in August from a female specimen. The maximum GSI values occurred for stages IV and V and they were 2.1–3.47 for males and 3.08–5.31 for females.

**Length and age at first maturity.** The first mature males of *L. saliens* appeared in the length class of 160 mm (Fig. 5). The percentage of the mature individuals increased gradually with body length reaching 50% in the class of 190 mm. All males greater than 210 mm were mature. The SL_{50} (±SE) of males was 195.9 ± 1.62 mm (a = −15.19, b = 0.077, r^2 = 0.94, n = 21). The first mature females appeared in the
length class of 170 mm and all female individuals were mature at the length class of 220 mm. The female SL50 (±SE) was greater than the male one reaching 206.5 ± 1.29 mm ($a = -23.08$, $b = 0.115$, $r^2 = 0.97$, $n = 21$). The SL50 of both sexes corresponded to the age of 3+.

The first mature male of *L. ramada* appeared in the 240 mm length class (Fig. 5). Males are all mature after the size class of 290 mm. The SL50 (±SE) of males was 277.9 ± 1.42 mm ($a = -19.26$, $b = 0.069$, $r^2 = 0.85$, $n = 25$). The first mature female specimens appeared in the 250 mm size class and after the 320 mm class all females were mature. The SL50 (±SE) of females was 267.6 ± 1.34 mm ($a = -21.91$, $b = 0.081$, $r^2 = 0.88$, $n = 25$). These lengths corresponded to individuals aged 3+.

**Fecundity.** The counting of oocytes and the calculation of the average diameter was possible only for nine specimens of *L. ramada* and for six specimens of *L. saliens*. For a better understanding of the procedure of the oocytes’ increase, which leads to a grouping of mature eggs, a representative frequency distribution of the oocytes of the two species is presented for every gonadal maturity stage, according to the scale developed by Greeley et al. (1987). Stages I, V, and VI are not presented as there were no individuals having oocytes of such maturity stage. Two categories of oocytes were recognised in the ovaries of maturity stage greater than IIIa of both species examined. The first group of oocytes was small and had a diameter range of 0.1–0.2 mm for all maturity stages examined for both species. A second group with larger oocytes of diameter attaining 0.22–0.47 mm for stage IIIa, 0.32–0.47 mm for stage IIIb, and finally 0.40–0.65 mm for stage IV was found for *L. ramada* (Fig. 6). The second group of larger oocytes of *L. saliens* measured 0.22–0.45 mm in diameter for stage IIIa, 0.35–0.55 mm for stage IIIb, and finally 0.45–0.57 mm for stage IV (Fig. 6). The average diameter of the oocytes of stage IV was 0.517 ± 0.065 mm for *L. ramada* and 0.498 ± 0.030 mm for *L. saliens*.

The proportion of the immature oocytes varied between 6.3% and 18.4% for *L. saliens* and between 3.9% and 28.1% for *L. ramada*. The remaining number of oocytes was considered as the absolute fecundity of the species. For *L. saliens*, absolute fecundity varied from about 245 000 to 555 000 eggs, for specimens of 210–253 mm SL and ages

**Fig. 6.** Size-frequency profile corresponding to stages of oocyte maturity, used in order to estimate fecundity of *Liza saliens* and *Liza ramada* during the spawning period.
from 3+ through 6+ years (Table 1). For *L. ramada*, absolute fecundity varied from about 150 000 to 685 000 eggs for specimens of 293–395 mm SL of ages from 4+ through 9+ years (Table 1).

The relation between absolute fecundity (*F*) and standard length (SL), net weight (NW), and age (*A*) was best described as follows: For *L. ramada* $F = 0.0000063 \times SL^{4.276}$ ($r = 0.796$), $F = 279.2 \times NW^{1.109}$ ($r = 0.779$), $F = 18749 \times A^{1.67}$ ($r = 0.867$). For *L. saliens* $F = 0.0076 \times SL^{3.219}$ ($r = 0.699$), $F = 1217 \times NW^{1.073}$ ($r = 0.661$), $F = 94842 \times A^{0.782}$ ($r = 0.558$). From the above relations, the estimated fecundity of the two species can be calculated for specific lengths but only within the range of lengths from which the equations were worked out. Absolute fecundity increases, in both species with length, weight and age increase.

Considering the low number of samples and the limited range of the parameters used, especially for *L. saliens*, the correlation coefficients can be considered acceptable.

The relative fecundity to the net weight found for both species and for the range of age and length that was also reported for absolute fecundity, varied between 1507–2501 eggs per 1 g, with a mean value of 1822 ± 220 (SE) for *L. saliens* and 269 to 871 eggs per 1 g, with a mean value of 553 ± 55 SE) for *L. ramada* (Table 1). No correlation was found between relative fecundity and the growth parameters.

**DISCUSSION**

Difficulties in sex identification in small-size mugilids have been reported by several authors who studied fishes of this family. According to Brusle (1981), the gonads of *L. ramada* and *L. saliens* can be recognised only after age 2. In the presently reported study, sex was identified in smaller specimens for *L. saliens* (age 1+, 193 mm). Data on sex ratio are rather complicated, but in general, the sex ratio especially in high length classes favours the females (Brusle 1981). This is in accordance with the results of this study, where the females *L. saliens* and *L. ramada* dominated the populations of both species. Female domination is rather common for members of the family Mugilidae. Several theories have been put forward to explain the female domination:

- Segregation of the sexes through different periods of the year, including discrimination arising from differences in age and size of maturity;
- Different natural and fishing mortality of the two sexes;
- Environmental conditions favouring one sex over the other;
- Differences of the two sexes in length and age (growth rate and longevity, life span);
- Reproductive migrations (mobility, different behaviour of the two sexes) (El Zarka and El Sedfy 1970, Brusle 1981).

The domination of females in the larger length classes could be attributed to differential growth in favour of the females along with increased natural mortality of the males in smaller size/age (Albaret and Legendre 1985). Nikolaevsky (1969) supported the above statement referring in general to sex ratios among the fish species. For certain fish species, male individuals mature earlier but have a shorter life span allowing for the female domination in larger age classes. The populations of grey mullets studied here fall within the above trend. Therefore, males dominated the small length classes since the females are still immature and impossible to be identified and females dominated the larger length groups.

Several methods have been used in the past for the determination of the reproductive period of grey mullets, since the capture of a significant number of sexually mature individuals is not always possible. To determine the reproduction period, some workers observed the changes of the gonadosomatic index (Farrugio and
Quignard 1973, Bruhlet 1975, El Maghraby et al. 1974, Ergene 2000), however, the most widely used method, especially in the Mediterranean Sea, is the determination of the time the young individuals migrate towards inland waters and their size distribution. With this method, the determination of the reproductive period is possible by counting, according to the length of individuals, the time after hatching (El Maghraby et al. 1974, Albertini-Berhaut 1975, Cambry 1984, Rossi 1986, Katselis et al. 1994, Koutrakis et al. 1994). For the estuarine system of Lake Vistonis and Porto Lagos Lagoon, Koutrakis et al. (1994) have used the seasonal distribution of grey mullet fry in order to estimate the reproduction period of each species. Based on the results of the present study, the spawning season of *L. saliens* is confirmed (June to October), even though November can also be considered as month of the reproduction period. El Zarka and El-Sedfy (1970) in Egypt, based only on the temporal distribution of maturity stages, suggest two spawning periods in Egypt (March–June and October–December). Concerning *L. ramada* however, based on the results of the present study, it can be concluded that the reproduction starts two months earlier, thus from early autumn to mid winter (September to January). Ergene (2000), in the Levantine Sea (Eastern Mediterranean) based on the GSI temporal variation, suggests a shorter spawning period (November to December), probably because temperature drops later than the northern Aegean, since the spawning period is influenced by sea temperature (El Zarka and El Sedfy 1970).

As far as length and age at first sexual maturity are concerned, *L. ramada* sexually matures in a greater size and older age than *L. saliens* (277.9 mm SL for males and 267.6 mm SL for females, aged 3 years old). *L. saliens* reaches maturity at 195.9 mm SL for males and at 206.5 mm SL for females and age over 2 years old. It seems that there is also differentiation in maturity between the two sexes. Most males reach maturity smaller and younger than females. This explains the greater duration of life of the females that mature later (Nikolsky 1969, Albaret and Legendre 1985). The previous statement could also explain the high female : male ratio observed in the high length classes. These results are rather similar in areas with similar environmental conditions. Ergene (2000) gives average fork length 267–271 mm and age III for the first sexual maturity of *L. ramada*. Brusle (1981) observed that there is a relation between first sexual maturity and temperature in every region. Hence, younger ages and smaller sizes at maturity are observed in warm waters, while, in cooler waters, both age and size at maturity are higher.

For the estimation of fecundity the total number of the oocytes is often used. However, this can be misleading as shown from the frequency distribution of the oocytes in the two species, where a large proportion of the oocytes will never mature and therefore they do not contribute to the reproduction process. The percentage of these oocytes can be high, as shown in the current study (3.9%–28.1%). Greeley et al. (1987) report almost 30% of oocytes in *Mugil cephalus* gonads are immature, while Naama et al. (1986) report that the immature oocytes in *Liza abu* (Mugilidae) in Iraq were always more numerous than the mature ones.

Concerning the oocyte size, it has been reported that the egg diameter from mature gonads of *L. ramada* varies from 0.55 to 1.03 mm (Yashouv and Berner-Samsonov 1970, Brusle 1981, Ergene 2000) and for *L. saliens* from 0.60 to 0.88 mm (Yashouv and Berner-Samsonov 1970, Brusle 1981). According to Brusle (1981), the differences in the mean oocyte diameter and the number and the size of the oil drops they contain, are attributed to geographical differences or even to the collection technique of eggs from the gonad. In the present study, the average (±SE) size of the eggs (stage IV) is smaller (0.517 ± 0.065 mm) both for *L. ramada* and for *L. saliens* (0.498 ± 0.030 mm), probably because the specimens collected had not reached final maturity. Hotos et al. (2000) has also reported two groups of eggs for *L. aurata* in western Greece, but that the small group of oocytes ranged from 0.1 to 0.4 mm for stages IV and V. This could be attributed either to the different species or to environmental parameters. All the remaining oocytes increased at the same time for both species, hence during the reproduction period oocytes reached maturity at the same time and thus the oocyte deposition occurred once for each female. It can be concluded that these two species are non-intermittent spawners and that their spawning occurs only once during their reproduction period. Thus, absolute fecundity is the annual fecundity, similar to spawning strategies observed for other mugilids (Brusle 1981, Wijeyarratne and Costa 1986, Hotos et al. 2000).

The fecundity reported by other workers for the two species is similar with the results of this study. Thus, for *L. ramada*, Farrugio and Quignard (1973) in Tunis reported an absolute fecundity of 82 202 to 434 787 eggs and relative fecundity of 604 to 1454 eggs per 1 g for lengths ranging from 255 to 345 mm (SL). In Egypt, El Maghraby et al. (1974) reported 45 568 to 316 828 eggs absolute fecundity and 728 to 992 eggs per 1 g relative fecundity. Hickling (1970), in southern England, found only two ripening females of *L. ramada* with 581 000 and 1 243 000 eggs with lengths 490 to 530 mm, respectively, and Ergene (2000) found 234 720 to 435 265 eggs for ages III and IV respectively. Brusle (1981) mentioned (citing Popescu) that *L. saliens*, in the Danube’s Delta contained 1 175 000–2 347 000 eggs. From the above it can be concluded that the differences can be attributed either to the high spatial variation of the studies, thus to different environmental conditions or to the methods used that produce variable results (e.g., counting or not the oocytes that will not mature), but mainly to the differences in length, weight or age in the samples of the different authors, since absolute fecundity increases as those parameters increase (Hotos et al. 2000). Bagenal and Braum (1971) reported that usually, in the relation between absolute fecundity (*F*) and standard length (SL), net weight (NW) and age (*A*), when the exponent is around 3, the fecundity is correlated with length, while when fecundity is correlated with weight or age the exponent is around 1. In the relations found in the current study, the exponent is
around 1 only in the weight equations ($P < 0.05$), hence fecundity is probably more correlated with the weight of the fish and it is also affected by various other factors such as the environment (Hotos et al. 2000).

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