

## Comparison of ovarian cycles of Hungarian riverine fish species representing different spawning strategies

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**ABSTRACT:** Investigations on the ovarian cycle of fish species that inhabit Hungarian rivers are necessitated by both environmental and economic reasons. The objective of our research was to explore new fundamental knowledge concerning the ovarian cycle of the white bream (*Blicca bjoerkna*, Linnaeus, 1758), barbel (*Barbus barbus*, Linnaeus, 1758), orfe (*Leuciscus idus*, Linnaeus, 1758) and nase (*Chondrostoma nasus*, Linnaeus, 1758). Histological investigation of ovaries and determination of proportions of oocytes in different stages of development is an appropriate method for the description of spawning characteristics of these species. Our results show that the GSI value for all four investigated species starts to increase at the end of summer and reaches its maximum before spawning. In the barbel and white bream, the presence of oocytes in the stage of cortical alveoli and the heterogeneous size of oocytes in the stage of vitellogenesis in the pre-spawning period indicate that barbel and white bream are multiple spawners. In contrast, in the orfe and nase, the absence of oocytes in the stage of cortical alveoli and the homogeneous size of cells in the stage of vitellogenesis indicate that orfe and nase are single spawners.

**Keywords:** ovogenesis; cyprinids; single spawners; multiple spawners

Most of the endangered fish species in Hungary inhabit rivers. Recently, 61% of riverine species and only 12% of stillwater species were placed under protection (Györe et al., 2000). The appearance of our rivers has undergone a significant change as a consequence of regulation works that began in the 19<sup>th</sup> century. River habitats lost their character, thus the conditions necessary for the reproduction and life of riverine fish species have changed or disappeared. Human intervention resulted in a long-term loss of diversity in riverine fish species.

Traditional fishing on Hungarian rivers has lost some of its earlier importance, however, angling has become an important recreational activity. Fish re-

stocking is an important work on intensive angling waters, the area of which has considerably increased recently. Demands of the Hungarian angling community and the Western European market economically justify the induced spawning of fish species and systematic restocking of natural waters.

Therefore, investigations in the field of biology of reproduction in fish species that inhabit Hungarian rivers are necessitated by both environmental and economic reasons.

The characteristics of gonadal cycle and gametogenesis have been described for several fish species of economic importance such as the common carp (*Cyprinus carpio*, Linnaeus, 1758) (Horváth,

1985; Hassanin et al., 2002; Sivakumaran et al., 2003; Snyder et al., 2004; Carballo et al., 2005), rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) (Bromage and Cumaranatunga, 1988; Springate et al., 1985), brown trout (*Salmo trutta morpha fario*, Linnaeus, 1758) (Billard, 1987), grass carp (*Ctenopharyngodon idella*, Valenciennes, 1844) (Horváth, 1985; Glasser et al., 2003) and African catfish (*Clarias gariepinus*, Burchell, 1822) (Van den Hurk, 1984), the wels catfish (*Silurus glanis* L., 1758) (Hochman, 1967), and of course, sturgeons (*Acipenseridae*) necessary for the production of caviar (Doroshov, 1985).

Similar investigations were conducted on Cyprinids by Rinchard and Kestemont (1996, 2003), who described the spawning strategies and ovarian cycles of single spawner and multiple spawner species such as the white bream (*Blicca bjoerkna* L., 1758), the bleak (*Alburnus alburnus* L., 1758) and the roach (*Rutilus rutilus* L., 1758).

These types of investigations have not been carried out on Hungarian riverine fish species yet, thus the description of their ovarian cycle and spawning strategy gives a good opportunity for the investigation of interactions in the fields of gamete biology and ecology that have new scientific importance.

In our study, the ovarian cycle of fish species and the reproductive biological status before spawning were examined. Investigations were carried out on the white bream, the barbel (*Barbus barbus* L., 1758), the orfe (*Leuciscus idus* L., 1758) and the nase (*Chondrostoma nasus* L., 1758).

## MATERIAL AND METHODS

Investigations were carried out in three consecutive years from 2003 to 2005. Examined female individuals were collected at different times and different sections of the Danube, in Paks, Sződliget and Dunakiliti (Hungary). Electrofishing apparatus, gill nets or other fishing equipment suitable for the given fish species were used for the collection of experimental fish samples. Attempts were made to collect the experimental fish samples on a monthly basis, however, it was impossible to catch individuals of all species even with systematic fishing due to actual weather conditions.

Monitoring the relation of ovary weight to body weight gives a detailed description of the ovarian cycle. Gonado-somatic index is determined at different stages of the ovarian cycle.

Ovarian samples were fixed in Bouin's solution (75 ml saturated picric acid, 25 ml 35% formaldehyde and 5 ml glacial acetic acid) and after 10 to 16 hours (depending on the size of the sample) they were placed into 70% alcohol. Before being embedded in paraffin, the tissues were dehydrated in increasing concentrations of alcohol (70%, 80%, 96% and absolute ethanol) and sections of 4–5  $\mu$ m in thickness were prepared with a microtome. Samples were stained with haematoxylin-eosin so that the nucleus was stained blue and the cytoplasm pink to red (Blazer, 2002).

According to our preliminary studies as well as those found in the literature (Rinchard and Kestemont, 1996; Blazer, 2002), no difference was found among samples taken from the anterior, central or posterior section of the ovary, thus, samples were dissected randomly from ovaries of studied individuals. Denominations described by Bromage and Cumaranatunga (1988) were used for different stages of oogenesis. Histological sections showed a distinctive pattern for all species in the given periods of the ovarian cycle.

Oocytes in the stages of primary growth, cortical alveoli and vitellogenesis were counted on histological sections using a light microscope (Nikon Eclipse E 600). Ovarian samples collected at different times were compared for each species. The proportions of oocytes in the three stages of development were analyzed as well as the change in this proportion with the advance in the ovarian cycle. Samples collected at the same time were compared for different species.

Samples of 1 g were dissected from the ovaries of each fish to determine oocyte diameter. Ovarian samples were stored in 10% formalin until use. The ovarian tissue was removed from the samples and oocyte diameter was determined using an ocular micrometer inserted in one ocular of a Zeiss Technival (Germany) stereo microscope. Results were extrapolated for the entire ovary.

Quantitative results were expressed as mean  $\pm$  SD. Statistical analyses were conducted using the softwares Microsoft Excel '97, GraphPad Prism 4.0 and SPSS 13.0 for Windows. Changes in GSI values and oocyte diameters of fish collected in different periods of the cycle were analyzed. Data were tested for normality and evaluated using one-way analysis of variance (ANOVA) with Tukey's Multiple Comparison test as post-test at a significance level of  $P \leq 0.05$ . Levene's test was used to verify the homogeneity of variances.

**RESULTS**

**Changes in GSI**

Significant differences were found among the pre-spawning GSI values for each species. The pre-spawning increase in GSI values (Figures 1, 2, 3 and 4) suggests that the intensive cell growth is

in process during spring months in all four investigated fish species.

It has been determined that the pre-spawning GSI of multiple spawner species such as the white bream and the barbel has a medium value (6–15%). It is fully visible in the white bream (Figure 1) that ovary weight gradually increases during the spring months and peaks at the beginning of May (15.56%).

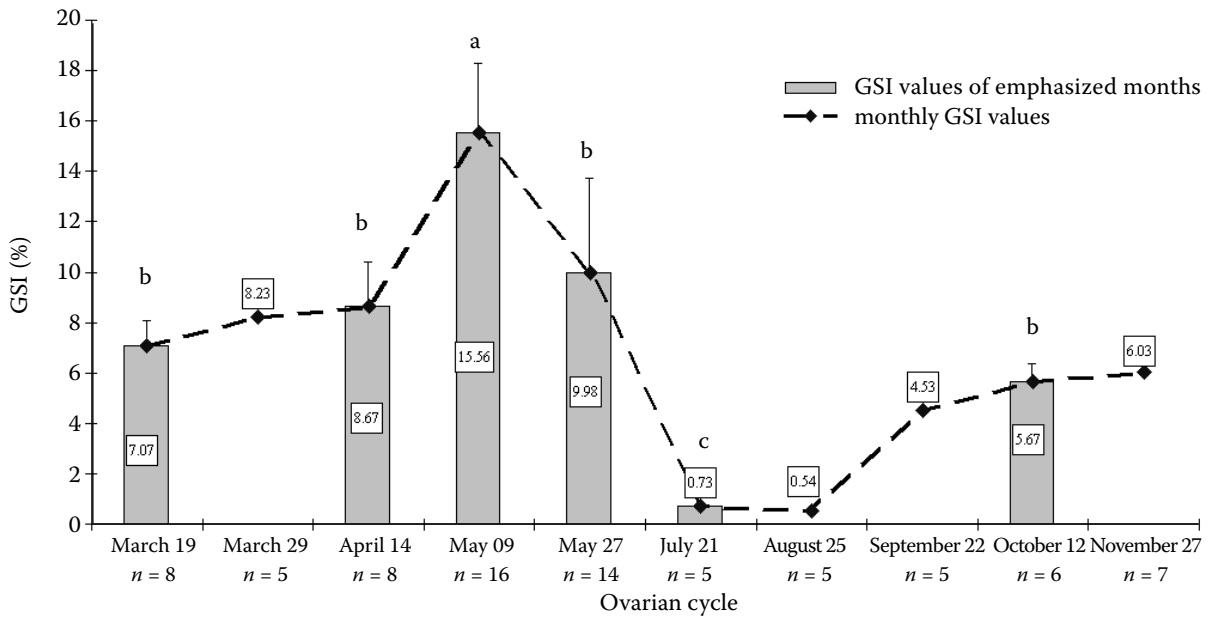


Figure 1. Changes in GSI of white bream during the ovarian cycle (mean ± SD) (Columns marked with the same letter are not significantly different at  $P < 0.05$ )

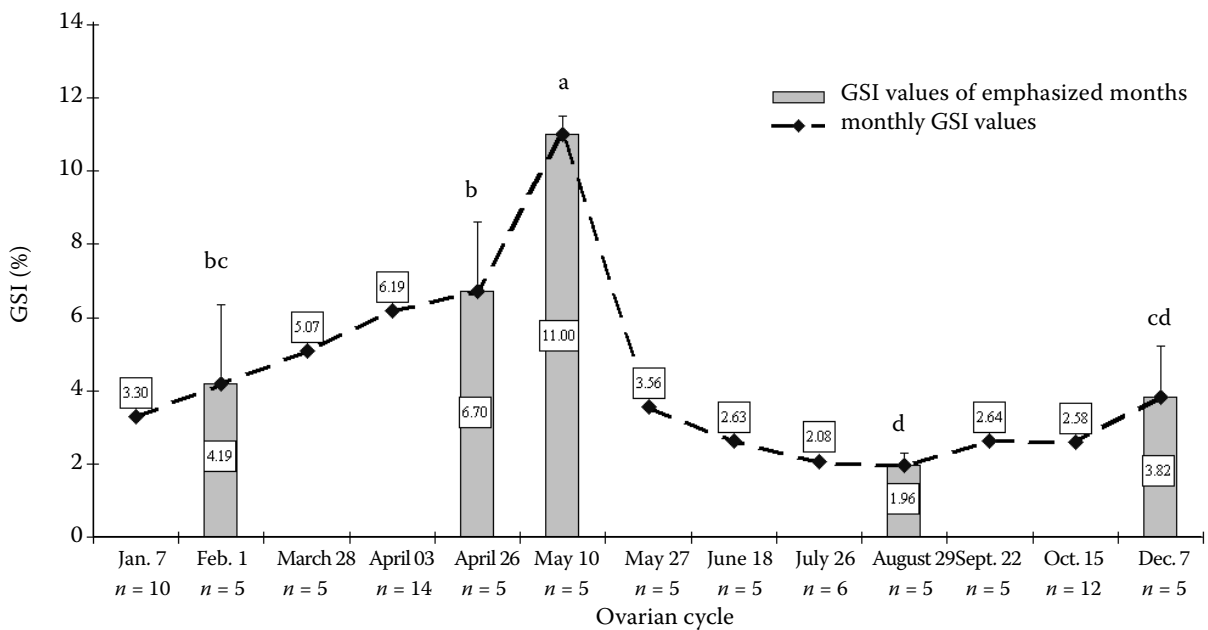


Figure 2. Changes in GSI of barbel during the ovarian cycle (mean ± SD) (Columns marked with the same letter are not significantly different at  $P < 0.05$ )

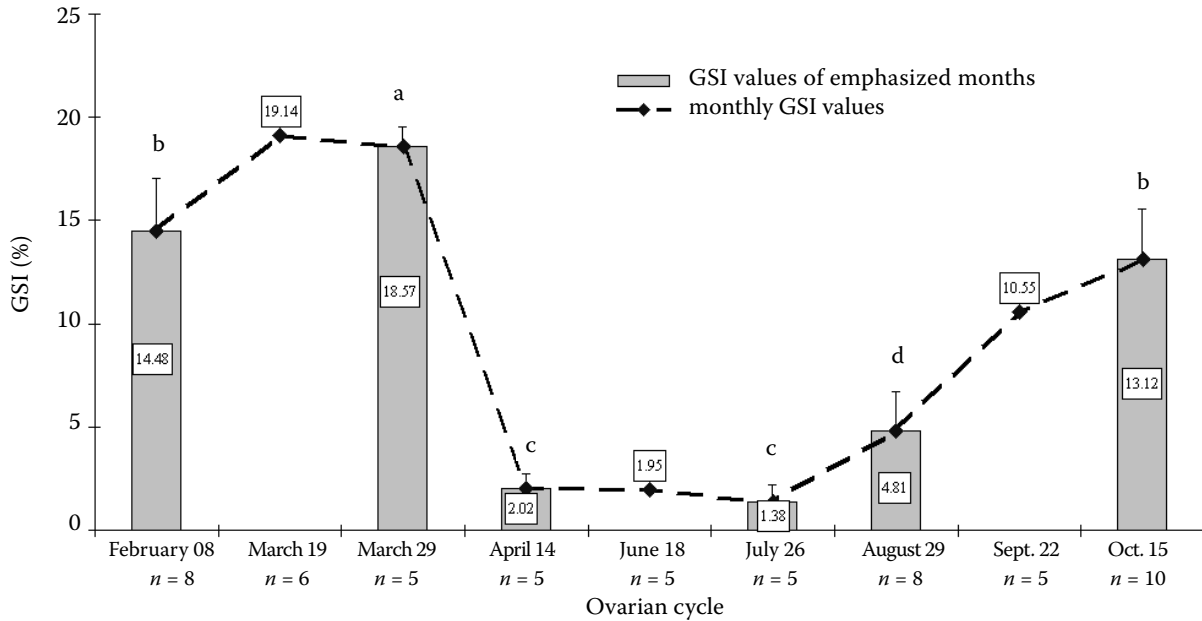


Figure 3. Changes in GSI of orfe during the ovarian cycle (mean ± SD) (Columns marked with the same letter are not significantly different at  $P < 0.05$ )

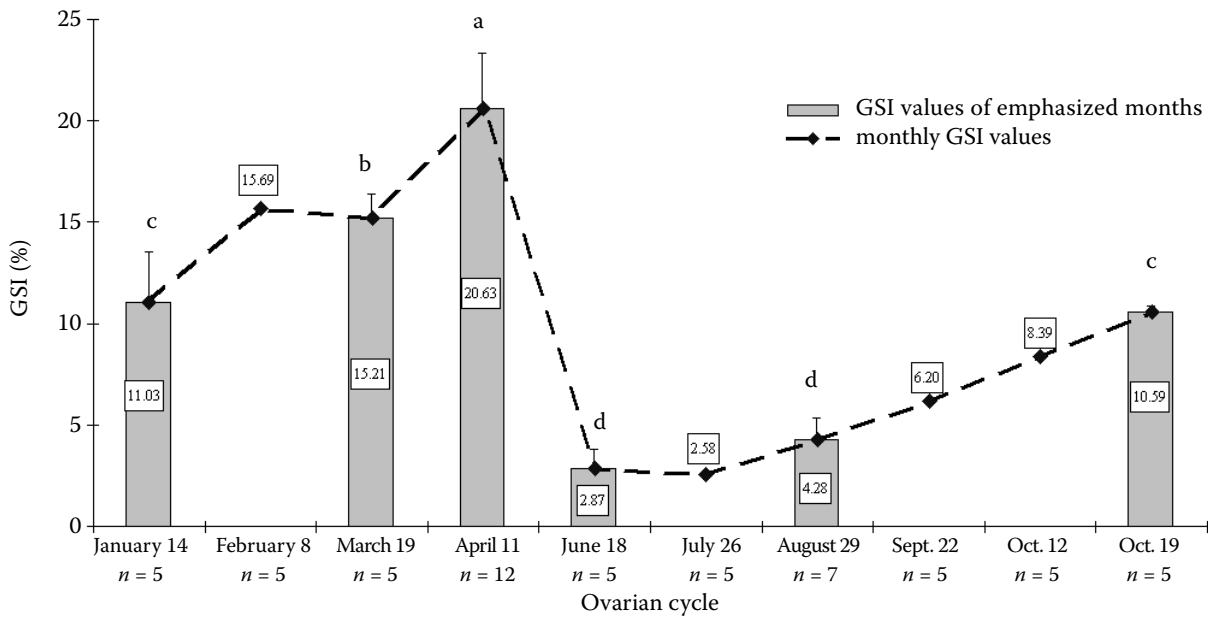


Figure 4. Changes in GSI of nase during the ovarian cycle (mean ± SD) (Columns marked with the same letter are not significantly different at  $P < 0.05$ )

Following this period, the GSI value gradually decreases to a minimum value of 0.54% as a sign of continuous release of eggs. Similarly, in the barbel, a gradual and continuous increase of GSI value is obvious with the approach of the spawning season (Figure 2). The increase is not very strong but continuous and even. GSI reaches its maximum value at the beginning of May (11.00%). The subsequent

sampling in May (3.56%) presumably took place after the first spawning.

According to the ANOVA, the advance of time had a significant main effect on GSI values of both species ( $P < 0.0001$  and  $P < 0.0033$  for the white bream and the barbel, respectively).

It has also been observed that pre-spawning GSI values of fish species that spawn only once during a

spawning season are relatively high (19–20%) such as the orfe and the nase (Figures 3 and 4). GSI values show clearly that the relative weight of ovaries in both species is higher, already in October (8–13%), than in the barbel and white bream. The GSI value keeps increasing, although at a slower rate, during the winter months.

According to the ANOVA, the advance of time had a significant main effect on GSI values of both species ( $P < 0.0001$  and  $P < 0.0001$  for the orfe and the nase, respectively).

**Changes in oocyte diameter**

The most vivid results can be presented for the white bream where we were able to monitor the changes in oocyte diameter for several months. In September, oocyte diameter did not exceed 600  $\mu\text{m}$  (Figure 5).

Oocytes of 500  $\mu\text{m}$  in diameter were in majority (47%) but 400  $\mu\text{m}$  diameter oocytes were also found in great numbers (36%). In March, the rela-

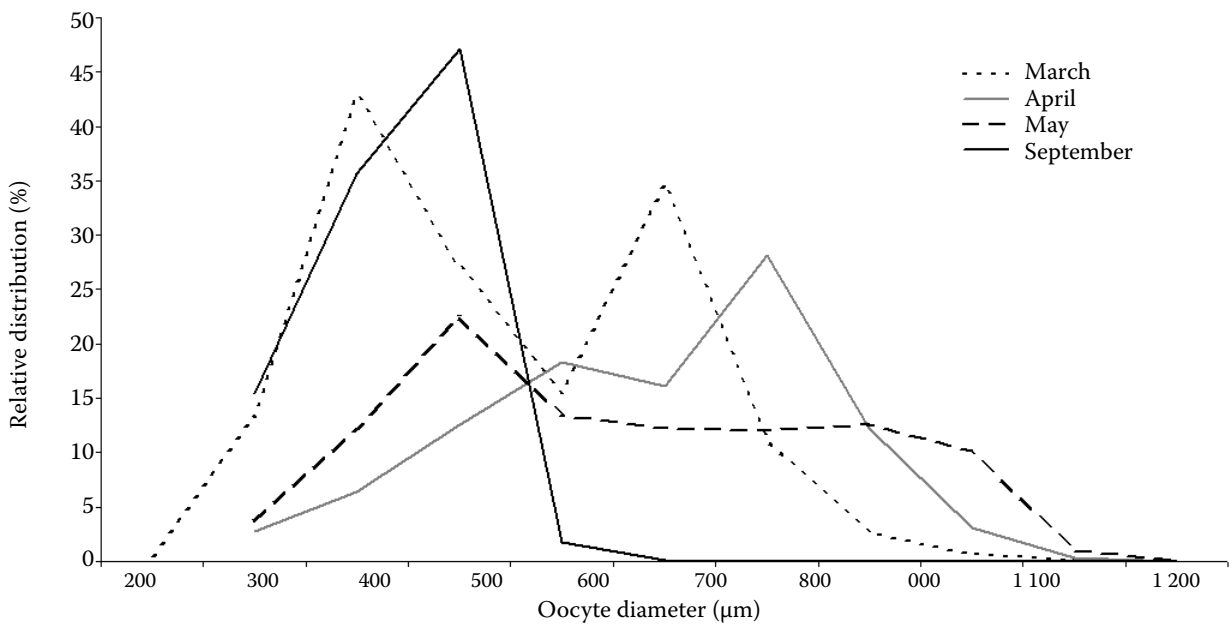


Figure 5. Relative distribution of (mean) diameters of white bream oocytes sampled at different times during the ovarian cycle

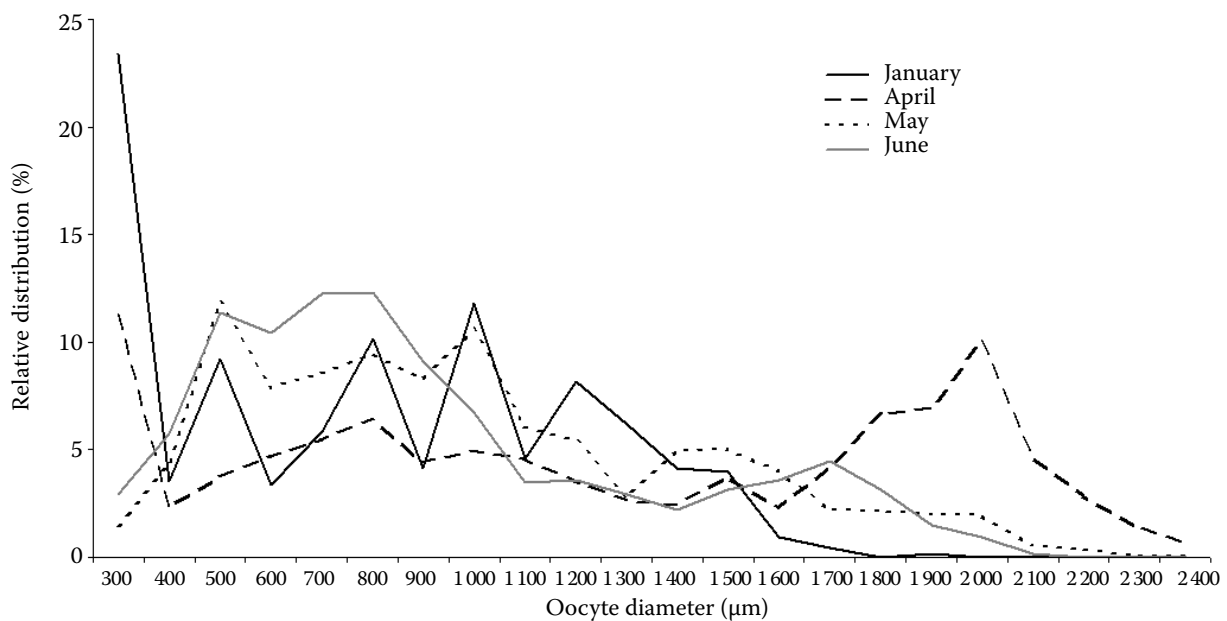


Figure 6. Relative distribution of (mean) diameters of barbel oocytes sampled at different times during the ovarian cycle

tive distribution of oocyte diameters has two peaks at 400  $\mu\text{m}$  (42.78%) and at 700  $\mu\text{m}$  (34.63%), and then the number of larger cells gradually decreases. In April, oocytes of 800  $\mu\text{m}$  in diameter were in majority (28.17%) while samples in May contained oocytes of different sizes in a roughly equal distribution. Only oocytes of 500  $\mu\text{m}$  were present in greater numbers. The number of oocytes representing the largest and smallest diameter was very low. The last sampling of white bream ovaries took place

in September when the sizes of cells in the ovaries visibly decreased, only oocytes of 300 to 600  $\mu\text{m}$  in diameter were found in greater numbers.

Relative distribution of oocyte diameters of the barbel (Figure 6) is in a much wider range than that of the white bream.

Smaller, 300  $\mu\text{m}$  oocytes can be found in greater numbers in January, however cell diameters continue to grow. In April, the distribution of cells peaks at 2 000  $\mu\text{m}$  (10.05%) and at 300  $\mu\text{m}$  (11.25%). Oocyte

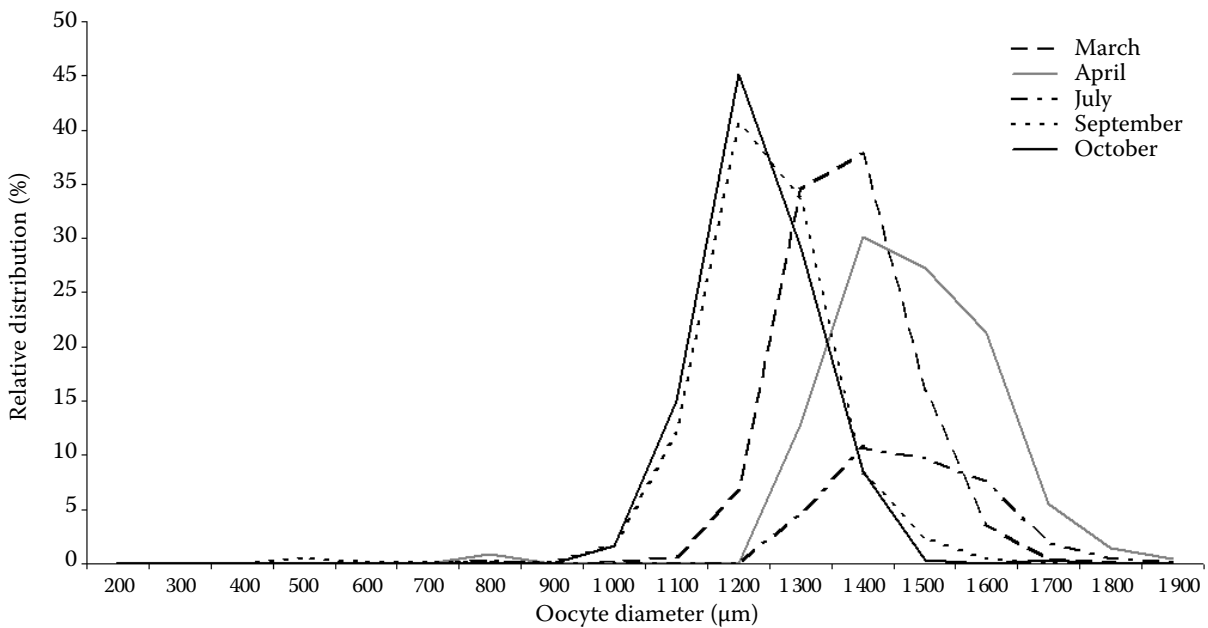


Figure 7. Relative distribution of (mean) diameters of orfe oocytes sampled at different times during the ovarian cycle

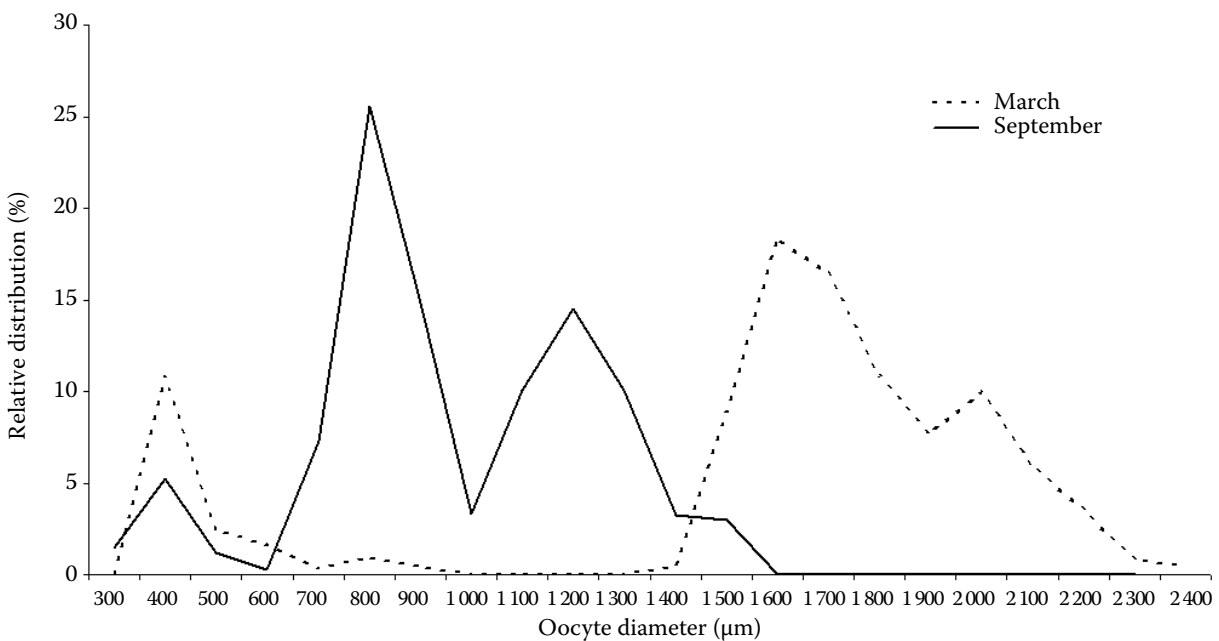


Figure 8. Relative distribution of (mean) diameters of nase oocytes sampled at different times during the ovarian cycle

diameters peak at 500 µm in May (11.89%) and after this period oocyte sizes continuously decrease as a sign of spawning. By June, the percentage of larger oocytes gradually decreases and the number of smaller ones (400–1 000 µm) increases.

Relative distribution of oocyte diameters in ovaries of the orfe was determined at 5 sampling dates throughout the year (Figure 7).

Oocyte sizes taken at the two autumn sampling times show a similar distribution and range between 1 000 and 1 600 µm. Oocyte sizes vary within

very narrow limits in March and April, they show a distinctive peak at 1 400 µm. The diameter of oocytes did not change in July (1 200–1 800 µm) but their distribution decreased to 10–11%.

Samples for determination of oocyte diameters in the nase in the months of March and September could be collected. In September, oocyte diameters had two distinctive peaks at 1 000 µm and 1 400 µm (Figure 8), whereas oocyte diameter significantly increased with the approach of the spawning season in March with peaks at 1 700 µm and 2 100 µm.

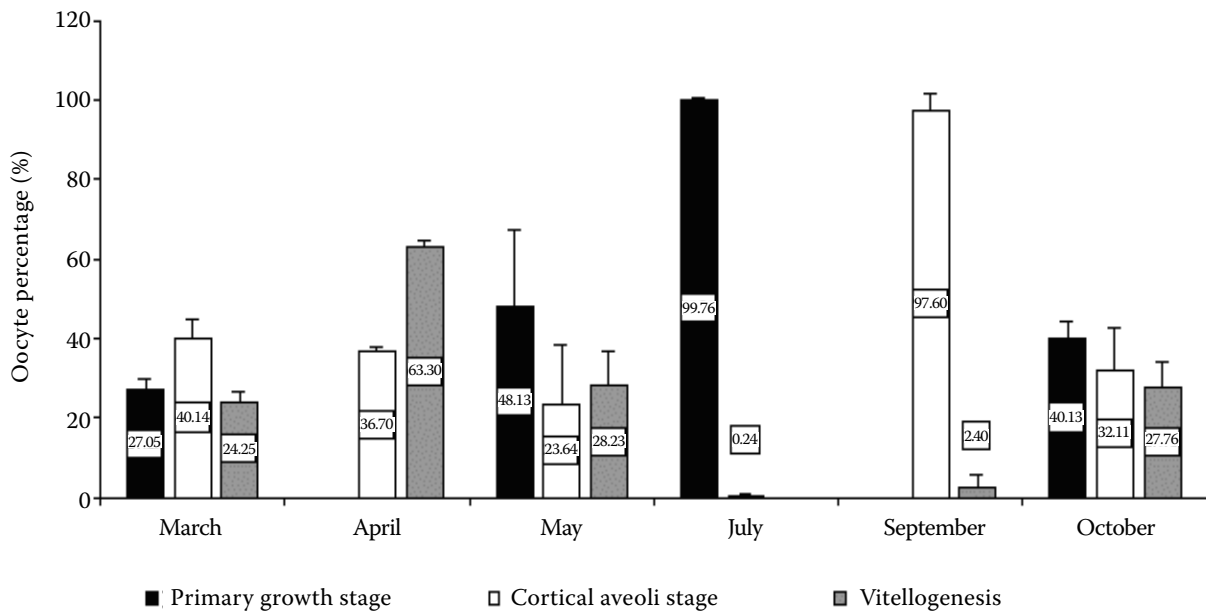


Figure 9. Proportion of oocytes in the stages of primary growth, cortical alveoli and vitellogenesis in samples of white bream taken in different periods of the ovarian cycle

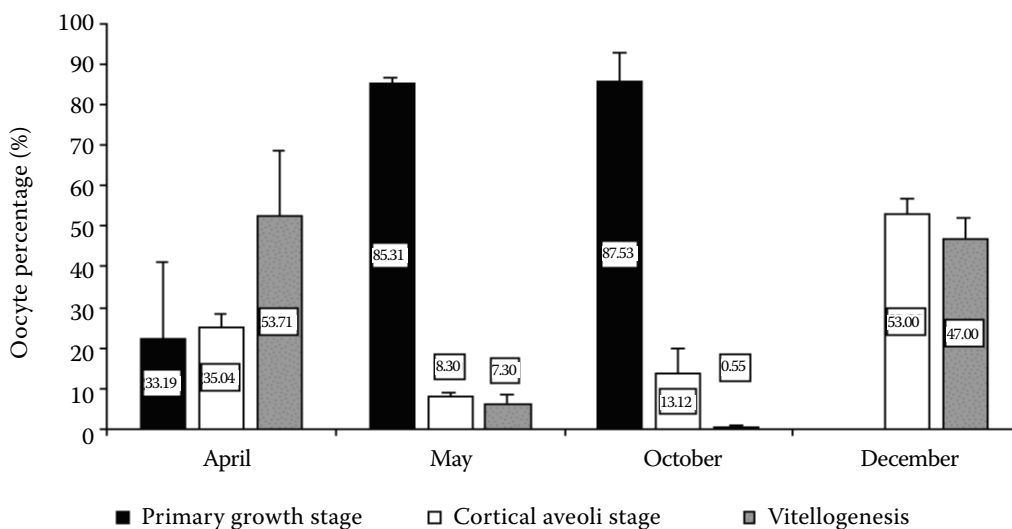


Figure 10. Proportion of oocytes in the stages of primary growth, cortical alveoli and vitellogenesis in samples of barbel taken in different periods of the ovarian cycle

## Histological studies

Further information for the better knowledge of the ovarian cycle and gonadal development was acquired following the processing and evaluation of histological samples. Percentages of oocytes in different stages of development were compared in different periods of the ovarian cycle.

In the white bream, the number of oocytes in the stage of cortical alveoli was higher than that of vitellogenic oocytes in the samples taken in March (Figure 9).

In April, before spawning no cells in the stage of primary growth were found in the ovaries whereas the number of oocytes in the stage of vitellogenesis doubled compared to that of cells in the stage of cortical alveoli. The subsequent sampling in May probably took place already after spawning as the number of oocytes in the stage of vitellogenesis was reduced and the number of those in the stage of cortical alveoli drastically increased. In July, only the number of oocytes in the stages of primary growth and cortical alveoli could be determined but the latter ones were present in small quantities. In September, the number of cells in the stage of cortical alveoli increased and oocytes in the stage of vitellogenesis appeared as well. In October, the number of oocytes in the stage of cortical alve-

oli exceeded that of cells in the stage of vitellogenesis.

In the ovary of the barbel oocytes in the stage of vitellogenesis were in majority in the month of April. The first spawning probably took place in this month because the number of oocytes in the stage of primary growth in May became much higher than that of oocytes in the stage of cortical alveoli or vitellogenesis. In October, oocytes in the stage of primary growth were still in majority in the ovaries of examined individuals (Figure 10). This proportion changed between October and December, the number of oocytes in the stage of vitellogenesis almost reached that of cells in the stage of cortical alveoli.

In the orfe (Figure 11), only oocytes in the stage of vitellogenesis were present in the ovaries in March. In the samples collected in July, the number of oocytes in the stage of primary growth was much higher than that of oocytes in the stage of cortical alveoli and no vitellogenic oocytes were found. In September, the number of oocytes in the stage of primary growth and vitellogenesis was almost equal while cells in the stage of cortical alveoli were present only in small numbers. In October, the oocytes in the stage of vitellogenesis were in majority and the number of cells in the stage of cortical alveoli was still low.

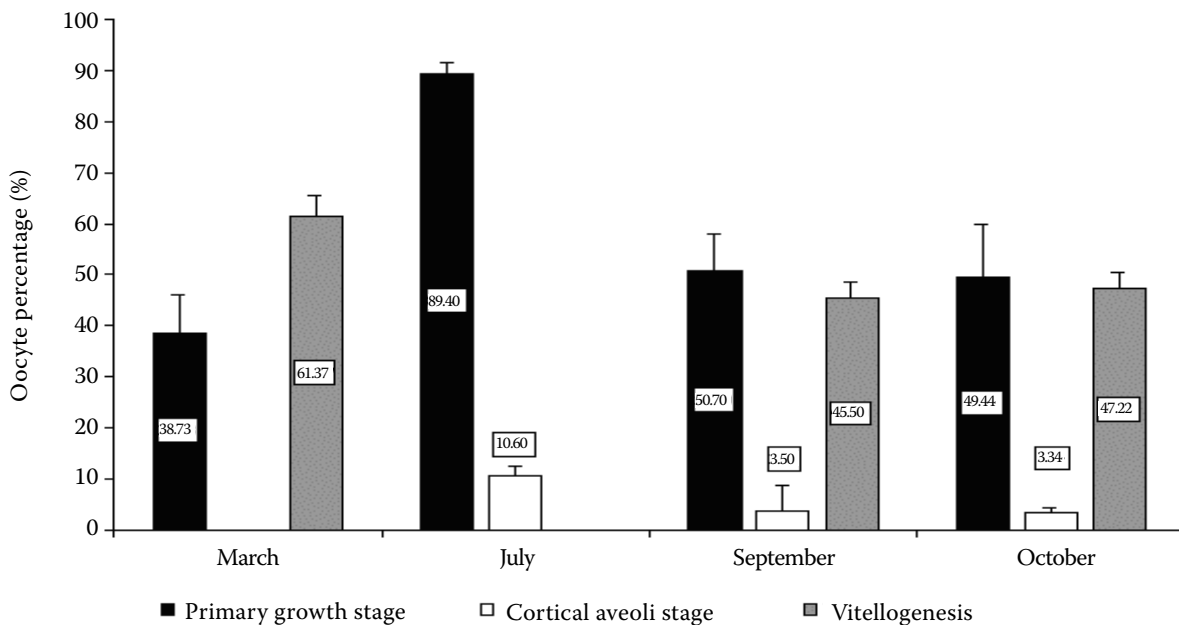


Figure 11. Proportion of oocytes in the stages of primary growth, cortical alveoli and vitellogenesis in samples of orfe taken in different periods of the ovarian cycle



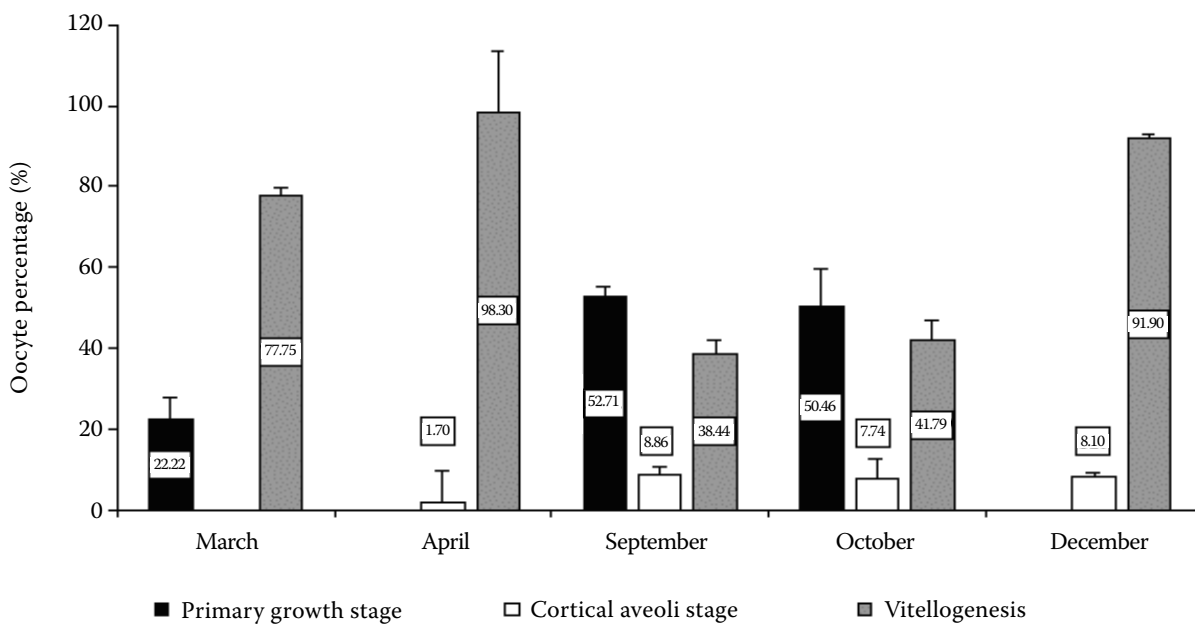


Figure 12. Proportion of oocytes in the stages of primary growth, cortical alveoli and vitellogenesis in samples of nase taken in different periods of the ovarian cycle

In the nase, samples from five months were available (Figure 12) for the observation of the proportion of oocytes in different stages of development. In March, only oocytes in the stages of primary growth and vitellogenesis were found. The number of oocytes in the stage of vitellogenesis was three times higher than that of oocytes in the stage of primary growth.

The next sampling took place in April, when almost only oocytes of the highest stage of development were present in the ovaries (98.30%). In September, oocytes in the stages of primary growth and vitellogenesis were present in the ovaries of examined individuals. In October, this rate was similar, however, the number of cells in the stage of vitellogenesis increased by 1.12% only. The majority of oocytes was in the stages of primary growth and vitellogenesis while cells in the stage of cortical alveoli were found only in the rate of 7–8%. In December, a different proportion of oocytes reflected the early cellular growth characteristic of the species as the majority of cells in the ovary was in the stage of vitellogenesis.

## DISCUSSION

The intensity of ovogenesis is not the same during the ovarian cycle of the white bream as shown by the GSI values, investigations of histological

samples and the proportion of oocytes of different diameters.

GSI is less than 1% following spawning and only oocytes in the stage of primary growth are found in the ovary, 300  $\mu\text{m}$  or less in diameter. The intensity of ovogenesis began to increase in August and September. A similar observation was reported by Rinchar and Kestemont (1996), who found that oocytes in the stage of cortical alveoli were present in the ovaries of females already in September. Based on our investigations the GSI value is about 4.5% at the end of September, which can clearly be explained by oocytes entering the stage of cortical alveoli. Thus, an increase in the relative weight of the ovary in September is a result of a qualitative change, namely the onset of the stage of cortical alveoli. The number of oocytes in the stage of vitellogenesis is negligible compared to that of cells in the stage of cortical alveoli.

Oocytes of more than 500  $\mu\text{m}$  in diameter in the stage of vitellogenesis appear in the ovary at the end of September. Their number continues to increase in October and the number of cells in the stage of cortical alveoli decreases, although their quantity is still twice higher than that of vitellogenic oocytes. The qualitative transition from the stage of cortical alveoli into the stage of vitellogenesis does not yet coincide with a significant increase in the relative ovary weight. The ovary of the white bream en-

ters a period of tranquillity between November and March. No significant changes appear in the relative ovary weight or in the proportion of oocytes in the stages of cortical alveoli and vitellogenesis.

The regeneration period of a couple of weeks develops as a result of prolonged spawning period or low water temperature in autumn and becomes a distinct characteristic of this species (1996). According to previous studies (Bromage and Cumaranatunga, 1988) the regeneration period serves for the absorption of ripe but not ovulated follicles, ovulated but not released eggs and blood clots occurring during spawning. No important quantitative or qualitative processes are active in the ovaries during this period. Fish are engaged in intensive feeding and consumed food is utilized for the energy stores of the body.

The long period of tranquillity is followed in early spring by a rapid gain in ovary weight which is a result of increasing day length, temperature and improving food supply (Glenn and Williams, 1976). By April, the intensity of ovogenesis in the ovaries increased again. The GSI value increased one and a half times in a short period of time (from 5.67% to 8.67%). Simultaneously, the number of oocytes in the stage of vitellogenesis increased and exceeded that of cells in the stage of cortical alveoli. The increase in the number of oocytes in the stage of vitellogenesis contributes to the growth of the relative ovary weight on the one hand, and on the other accumulation of yolk in the cells increases the size of individual oocytes and thus the GSI value. Changes in cell sizes are clearly shown by the growth of oocyte diameters in the months prior to spawning. Thus, in the one – one and a half months prior to spawning very intensive qualitative and quantitative processes can be observed in the ovary. This is the most intensive period of the ovarian cycle which is shown by quantifiable reproductive indicators.

The ovary of the barbel in the late autumn – winter months is not only characterized by quantitative changes as shown by a significant increase in GSI values. After October, in December the number of cells in the stage of cortical alveoli and in the stage of vitellogenesis increases significantly. This qualitative change means that the formation of cortical alveoli begins in October in the numerically dominating oocytes in the stage of primary growth and that in a fraction of oocytes in the stage of cortical alveoli the formation of vesicles comes to an end and they enter the stage of vitellogenesis which is the accumulation of yolk.

The process of vitellogenesis in the ovary of the barbel begins somewhat later than in that of the white bream. This is confirmed by the proportion of cells in the stage of vitellogenesis in October which is much higher than in the white bream. As vitellogenesis in the barbel does not stop in winter months, the number of oocytes in the stage of vitellogenesis in samples collected in December almost reaches that of cells in the stage of cortical alveoli.

In samples collected in April, the number of oocytes in the stage of vitellogenesis was almost twice higher than that in the stage of cortical alveoli. In contrast, in the white bream the number of oocytes in the stage of vitellogenesis was only one and a half times higher than that of cells in the stage of cortical alveoli.

Comparing the reproductive traits of the two species, we can assume that the proportion of oocytes in the stages of cortical alveoli and vitellogenesis can be species specific in the same sampling time. Thus, a conclusion can be made that the spawning season of the white bream is longer than that of the barbel and white bream releases its eggs in several portions. Another possible explanation is that the spawning of the white bream starts a few weeks later than that of the barbel. Oocytes in the stage of cortical alveoli can develop further into the stage of vitellogenesis during this period. The diameter of an oocyte in the same stage of development in the barbel is almost the double of that in the white bream.

In the orfe, the October value of GSI was 13.12% and in the nase it was 8.39% in the same month. In spite of the fact that the relative weight of the ovary is significant already in October, the GSI value continues to increase during winter months, although at a slow rate. In both species, oocytes in the stage of vitellogenesis dominate in the ovaries already in autumn and early winter months and the number of cells in the stage of cortical alveoli is relatively low. An increase in the GSI value during winter months indicates that the intensive cell growth is in process 3–4 months before spawning. This process is not a transition from one stage to another but the growth in size of already developed cells in the stage of vitellogenesis.

Following early spring spawning oocytes in the stage of primary growth are in majority in the ovary. Mitotically dividing oogonia, non-ovulated oocytes and eggs that were not released during spawning are also present in the ovaries. The latter two groups of

cells undergo the process of resorption. Continuous atresia is also in process in the ovary (Springate et al., 1985; Witthames and Greer-Walker, 1991) that affects primarily the least developed functioning oocytes during the post-spawning period, i.e. those in the stage of primary growth. In all probability their number will further decrease immediately after the spawning season.

Data collected in the orfe and nase show that the transition of oocytes in the stage of primary growth into the stage of cortical alveoli takes place in July–August and vitellogenesis already starts in August–September. Thus the formation of cortical alveoli is intensive in the second half of summer and is terminated at the end of October. Vitellogenesis starts at the end of summer and lasts until the beginning of spawning season. The size of oocytes in the stage of vitellogenesis is already very homogeneous in October. Almost 80% of oocytes fall into the range of 1 300–1 400 µm in diameter.

It can be concluded that the fish species investigated in the present study and teleosts that live in waters with a temperature cycle also have an annual ovarian cycle. Although studies were earlier carried out on the ovarian cycle of several fish species in other countries (Völlestad and L'Abée-Lund, 1987; Kestemont, 1991; Rinchar and Kestemont, 2003), we conclude that the white bream and the barbel are multiple spawners while the orfe and the nase are single spawner species within one spawning season.

## Acknowledgements

The authors also thank the Paks Fishing Cooperative for their help.

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Received: 2008–01–29

Accepted after corrections: 2008–07–17

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