Szent István University

OVARIAN CYCLE OF FISH SPECIES OF NATURAL WATERS

Thesis of Ph.D. dissertation

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1. BACKGROUND, OBJECTIVES

1.1. Background of the work

Ecological rationale of the work

A major part of Europe's rivers has changed as a result of the regulation works of the 19th century. The 20th century saw the construction of dams and hydroelectric facilities on rivers. One-sided interventions resulted in negative ecological consequences. Typical riverine habitats lost their previous character, spawning grounds that were of primary importance for the reproduction of riverine fish became isolated. Human interventions fnally resulted in the loss of diversity in fish communities of the rivers. Water quality problems associated with industrial production that increased towards the second half of the century further contributed to this loss (SZABÓ 2001).

The majority of endangered fish species in Hungary lives in rivers. This is indicated by the fact, that 61% of Hungarian riverine species were enlisted as protected in the near past in contrast with the 12% of those inhabiting stillwaters (GYÖRE et al. 2000). During the past century and a half human activities have significantly altered the natural status of river systems which had an undesired effect of fish stocks, as well (KIRCHHOFER és HEFTI 1996). Regulation of rivers, growing industrial activities and associated pollution resulted in a decrease of water quality and a loss of fish populations in flowing waters.

The objective of the project entitled „Fisheries management, fishing, angling" of the National Biodiversity Monitoring Program is the conservation of the biological diversity of rivers. This is partly possible through the protection of areas still considered natural and reconstruction

of habitats already altered by human activities. Hatchery spawning of fish, fry rearing in protected ponds and subsequent stocking into natural waters can also contribute to the conservation of rheophylic fish species (LUSK 1995).

Economic justification of the topic

Riverine species belong to the group of fish of economic interest due to their importance in commercial and recreational fisheries. Demands of the Hungarian angling society and the Western European market justify the hatchery propagation and subsequent stocking of these fish species into natural waters. Knowledge of the gonadal cycle and gametogenesis of these species is a basic prerequisite of the production of fry for stocking.

Scientific justification of the topic

Interest toward the biology of reproduction of teleost fish has increased considerably over the past few years due to the environmental and economic importance of several species. Induced spawning of fish species involved in Hungarian pond aquaculture is carried out in the hatcheries from the hormonal treatment of the broodstock to the production of fry. One of the key factors for their successful propagation was knowledge of their biology of reproduction. First, the sexual cycle and gametogenesis of the common carp (*Cyprinus carpio*) was described (HORVÁTH 1980). This was later used as a background for similar studies in other species, i.e. Chinese carps, the tench (*Tinca tinca*), the pike (*Esox lucius*) and the wels catfish (*Silurus glanis*).

These processes have also been explored in species involved in foreign aquaculture, such as the rainbow trout (*Oncorhynchus mykiss*), tilapia species farmed in tropical countries and the African catfish (*Clarias gariepinus*) (RICHTER et al. 1982), as well as sturgeons (*Acipenseridae*) cultured for the production of caviar (DOROSHOV 1985). In addition, the gametogenesis of the zebra fish (*Danio rerio*) which is important molecular biology and biotechnology research and is used as a model animal for the study of other vertebrate animals has also been explored (SELMAN et al. 1993).

1.2. Objectives

The objective of my studies was the determination of several reproductive indices in different stages of the ovarian cycle such as:

gonado-somatic index,

per cent proportion of cells in different stages of development present in the ovary,

follicle diameter,

and the concentration of three sexual steroid hormones (testosterone, progesterone, 17β-estradiol)

in the asp (*Aspius aspius*, Linnaeus, 1758), the white-eye bream (*Abramis sapa*, Pallas, 1814), the ide (*Leuciscus idus*, Linnaeus, 1758), the barbel (*Barbus barbus*, Linnaeus, 1758), the roach (*Rutilus rutilus*, Linnaeus, 1758), the white bream (*Blicca bjoerkna*, Linnaeus, 1758) and the nase (*Chondrostoma nasus*, Linnaeus, 1758).

A further objective of the work was the analysis of the ovarian cycle of white bream and roach in the lake Balaton in order to compare

the effects of lake and river habitats on the gonadal cycles in both species.

2. MATERIALS AND METHODS

2.1. Location and conditions of investigations

Analyses were carried out at the Department of Aquaculture, Institute of Environmental and Landscape Management, Faculty of Agriculture and Environmental Science, Szent István University (Gödöllő) and the Department of Pathology and Forensic Veterinary Medicine, Faculty of Veterinary Science, Szent István University (Budapest).

Experimental fish stocks were collected between 2003-2007 on the Szigetköz, Ercsi and Paks sections of the river Danube. Samples from the lake Balaton were collected between 2006-2007.

2.2. Experimental fish species

The gonadal cycle and oogenesis of the asp, white-eye bream, ide, barbel, roach, white bream and nase were investigated.

2.3. Investigated reproductive indices

2.3.1. Determination of the gonado-somatic index (GSI)

Proportion of the ovary weight to the body weight gives a good indication of the gonadal cycle. The gonado-somatic index was used for statistical analyses which was calculated according to the formula:

$$\text{GSI (\%)} = \text{ovary weight (g)} \times 100 / \text{body weight (g)}$$

2.3.2. Analysis of the proportions of gametes in different stages of development to each other

Oocytes in the stages of primary growth, cortical alveoli and vitellogenesis were counted in histology sections made of individual

ovaries at different sampling times. Stages of oogenesis were determined according to the classification by BROMAGE and CUMARANATUNGA (1988). An additional stage, that of annular cortical alveoli was rendered by me which allowed me to compare these to those in the complete stage of cortical alveoli.

2.3.3. Determination of the follicle diameters in the ovaries

One gram of fish ovary was dissected and follicles were measured individually using an ocular micrometer. Calculated data were extrapolated to the complete ovary and later these results were used for evaluation.

2.3.4. Determination of sex steroids in the blood plasma

Analyses were completed by blood sampling in two experimental years (2006 and 2007) as observation of the changes in sex steroid levels in the blood plasma were possible at this time.

Blood samples were taken from the caudal vein (*vena caudalis*) from experimental animals within 1-2 hours following capture.

The volumes of blood plasma required for the analyses was assessed experimentally prior to the investigations which were the following: testosterone (T) 50µl, 17β-estradiol (E_2) 100µl, progesterone (P_4) 200µl.

Steroids were extracted by diethyl-ether, the ether phase was evaporated and stored at -20°C in absolute ethanol until use.

Quantitative determination of sex steroid concentrations was carried out by a radioimmunoassay (RIA). Determination of the levels of testosterone was conducted according to the RIA by JALLAGEAS

(1975), that of progesterone by ABRAHAM et al. (1971), and that of 17-β-estradiol by MIKHAIL et al. (1970).

2.4. Statistical analysis

Softwares Microsoft Excel 97 and GraphPad Prism 4.0 for Windows were used for the statistical analyses. Results obtained in different sampling months during the determination of the GSI values were compared by a one-was analysis of variance (ANOVA) (using Tukey's Multiple Comparison test as a post-hoc test), and a two-sample t-test at a significance level of $P \leq 0.05$.

3. RESULTS

3.1. Ovarian cycle of the species collected in the Danube

3.1.1. Ovarian cycle of the asp

The GSI value during the Early-Spring pre-spawning period in the asp is very high. Only oocytes in the stages of cortical alveoli and vitellogenesis were found in the ovary which clearly indicates that oocytes ready for spawning are present. The diameter of mature follicles is the highest at this time when it reaches 2000 µm. Concentrations of 17-β-estradiol are considered intermediate which is explained by the pre-spawning termination of oogenesis. Following the spawning in April GSI value falls below 1%, the major part of the ovary is occupied by oocytes in the stage of primary growth, those in the stage of vitellogenesis empty from the ovary. Mitotically dividing oogonia, non-ovulated oocytes and non-spawned eggs were also present in the ovaries. The latter two groups of eggs were going through a process of reabsorption. Early spring spawning is followed by a long summer period of regeneration. Testosterone concentration reaches its maximum at this period. According to the reproductive parameters development of oocytes takes a new momentum in October. The weight of the ovary grows eight-fold due to the start of yolk accumulation. The largest part of the ovary weight is occupied by oocytes in vitellogenesis whose diameter reaches 1800 µm. As oocyte development and yolk accumulation is regulated by 17-β-estradiol its concentration in the blood plasma reaches its maximum in this period. A further growth of GSI during the winter months indicates that intensive cell formation processes are present in the females in the 3-4 months prior to spawning. These, however, are not “qualitative”

changes (shift from one developmental stage to another) but quantitative growth of already formed cells in the stage of vitellogenesis. This is supported by the growth of follicle diameters which is very low between October and March.

3.1.2. Ovarian cycle of the white-eye bream

In the ovaries of the white-eye bream qualitative changes are not typical in the Early Spring. Oocytes in the stage of vitellogenesis fill the ovaries ready for ovulation. Apart from these, only cells in the stage of primary growth are present in the ovaries. GSI value is high characteristically for this species which indicates the size and amount of cells mature for spawning in vitellogenesis. Size of follicles reaches 2400 µm. Sex steroid concentrations in the blood plasma are high. Progesterone regulates ovulation processes as a steroid stimulating final oocyte maturation (SCOTT és CANARIO, 1987). Its generally low concentrations are characteristically higher in this period. In April, the relative weight of ovaries grows further, presumably this is when spawning takes place, then GSI values suddenly decrease. During mid-summer only oocytes in the stages of primary growth and cortical alveoli are present in the ovaries. The relative weight as well as the level of steroid hormones responsible for ovarian development gradually decrease. The diameter of follicles does not exceed 1400 µm. In August, the GSI value falls below 2% and a very short summer regeneration period starts which lasts only a few weeks. The concentration of 17-β-estradiol regulating cell development is almost undetectable and the size of cells shows a homogeneous distribution between 800-1200 µm. On the other hand, testosterone concentration shows an increasing tendency, it

transforms into 17-β-estradiol which stimulates the processes following the short Summer rest. In the white-eye bream, yolk deposition starts in September, by October the majority of cells are in the stage of vitellogenesis and during the Winter and early Spring months qualitative changes are not typical in the oocytes. By this time, they grow to 60-70% of their spawning GSI value and oocyte diameter reaches 1800µm.

3.1.3. Ovarian cycle of the ide

According to the data concerning the ide, the relative weight of the ovary is high already in the pre-spawning season and GSI increases all the way up to spawning. It reaches its extremely high maximum value (19.02 %) in March. At this time, cells in the stage of vitellogenesis increase in size, however, their numerical growth cannot be excluded, either. Eighty per cent of yolk-rich oocytes fall into the category between 1300-1600 µm. Ide spawning continues in April, accompanied by follicle growth when their diameter can reach 2100 µm. Following spawning, the amount of cells in vitellogenesis falls below 20% and empty ovulated follicles are detectable in histology sections. Due to prolonged spawning the relative weight of the ovary significantly decreases and in parallel blood plasma progesterone concentration also reduces. However, gonad development continues due to the increase of 17-β-estradiol and testosterone concentrations. By July the GSI value of 1% indicates the short regeneration period that lasts only a few days in the ide, the size of follicles in this period hardly exceeds 1000µm. Following its minimum value in June, the concentration of 17-β-estradiol increases and it stimulates the oocytes in the stage of primary growth to enter that of cortical alveoli. Vitellogenesis starts early, in August, significantly increasing the relative weight of the ovaries and the size of follicles. The

process of cortical alveoli formation is very intensive in the second half o of the Summer but it finishes by September-October. The size of oocytes in vitellogenesis is very homogeneous in September-October and by the end of this period they approach the follicle size characteristic of the spawning period.

3.1.4. Ovarian cycle of the barbel

During the Spring, the proportion of cells in different stages of development does not change significantly in the ovary of the barbel. However, according to the GSI values, cell growth is continuously present from January onwards. This is supported by the extremely high blood plasma testosterone concentration and the diameters of follicles which can reach 1800 µm. The first spawning presumably starts in March as GSI reaches its maximum at this time. Progesterone also reaches its maximum which facilitates the final maturation of oocytes. Follicle diameter increases to 2200 µm and this growth continues in April. In May GSI values gradually decrease together with the 17-β-estradiol concentration of blood plasma. Oocytes in the stage of vitellogenesis are found in the ovaries until the beginning of summer months together with cells in the stage of primary growth which indicates continuous cell supply. 17-β-estradiol and testosterone responsible for cell development fluctuates until July following the tendency of gamete development. GSI does not reach its minimum in July as the retention of mature non-ovulated and ovulated non-spawned eggs is in process. The very short regeneration period which is similar to that of the white bream ends in August. In September, cells in the ovaries of the barbel and the white bream show a similar pattern from several aspects. Their ovaries contain

oocytes in different stages of development in similar proportions, progesterone concentration of the blood plasma is characterized by a slow growth while testosterone levels show a decrease. In October, the GSI of the barbel is half of that in the white bream. The index however, continues to grow in the late Autumn and Winter moths. The qualitative transformation of one stage into another in the cells follows a slower pace than in the white bream. Vitellogenesis presumably is not halted in either species during the Winter as according to my observations, the number of oocytes in vitellogenesis in samples collected in the early Spring months exceeded that in October. The number of oocytes in the stages of cortical alveoli and vitellogenesis significantly increased between October and January.

3.1.5. Ovarian cycle in the roach

In Hungary, roach spawning takes place in the early Spring, in March-April at a temperature of 8.1-13.4°C. In March, only oocytes in the stages of primary growth and vitellogenesis were found in the ovaries of Danubian roaches. The number of yolk-rich oocytes exceeded that of cells in the stage of primary growth which is a clear indicator of approaching ovulation. GSI is also high (13.77%), very close to that measured in the asp and the white-eye bream. Diameter of cells in the stage of primary growth does not exceed 400 µm while yolk-rich oocytes show a homogeneous distribution between 1100-1700 µm. Roach spawning season is short, mature oocytes empty from the ovaries in April and most of the ovary (83%) is occupied by cells in the stage preceeding primary growth. GSI value decreases and by the end of the Summer it falls by a further 0.74%. 17-β-estradiol and testosterone concentrations

responsible for oogenesis decrease to the lowest levels while progesterone concentrations increases compared to the value in April. By October, the proportion of cells in vitellogenesis reaches 40%. GSI increases drastically and reaches 63% of the spawning value. Concentrations of sex steroids show a growing tendency similarly to those in the ide. During the Winter months (between October and March) GSI growth, change in the per cent proportion of different development stages and growth of follicles in size shows that intensive cellular growth processes are present in the 3-4 months preceding spawning. The growth of already formed cells in the stage of vitellogenesis is not quantitative, but rather an increase of sizes.

3.1.6. Ovarian cycle in the white bream

The GSI value is relatively high (7.88%) in the month (March) before spawning and oocytes representing all three developmental stages are present in the ovary. However, follicle diameters change only to a small extent up to the following month. Sex steroid concentrations in the blood plasma are intermediate in this period. The concentrations of both hormones responsible for undisturbed oogenesis (17-β-estradiol, testosterone) grow and individuals are prepared for reproduction. Oogenesis gains a new momentum in April which is explicable by the increasing daytime light, temperature and food (GLENN és WILLIAMS 1976). GSI values almost double within a short period of time (12.6 %). In parallel, the number of oocytes in vitellogenesis also increases and it reaches that of cells in vitellogenesis. Diameter of cells in vitellogenesis reaches 1500 µm. The concentration of testosterone detectable in the blood plasma increases drastically, the highest value was reported during

final oocyte maturation (RINCHARD et al., 1997). In parallel, the concentration of 17-β-estradiol also increases. Spawning probably starts in the end of April, beginning of May at a water temperature of 13.4-17.8°C. A possible explanation of the low concentration of 17-β-estradiol during spawning is that a characteristic peak does not appear due to the continuous spawning as the white bream is a multiple spawner species, thus, several volumes of eggs mature in its ovary during one spawning season. Investigations of BRYLIŃSKA and ŹBIKOWSKA (1997) confirmed the previously described observation that the white bream can spawn once, twice and even three times within a reproductive season depending on the habitat. The most characteristic frequency (78.3%) is two spawnings a season. The second spawning takes place in July at the temperature of 18-29°C. In my studies no single-spawner individuals were found, sampled fish were all characterized as multiple spawners according to the investigated reproductive parameters.

In this species, the prolonged spawning season of April-May and June is followed by a rather short regeneration period of only a few weeks in July. Oogenesis gains its momentum in August-September. Increase of GSI values (3.81 %) starts in August. Oocytes entering the stage of cortical alveoli as well as those in vitellogenesis contribute primarily to the increase of relative ovary weight. The diameter of oocytes in the stage of primary growth is 300-550 µm, while that of cells in vitellogenesis is between 600-1000 µm. A gradual growth of progesterone concentration starts in this period, although the level of this steroid stays low during the entire sexual cycle in other species, as well. The number of oocytes in the stage of vitellogenesis in this period is negligible compared to that of cells in the stage of cortical alveoli. In October however, the number of cells in

the stage of cortical alveoli increases drastically and thus, the number of oocytes filled with yolk also grows. The proportion of cells in the stage of cortical alveoli is almost double of those in vitellogenesis. The qualitative transformation from the stage of cortical alveoli to that of vitellogenesis is accompanied by a significant growth of relative ovary weight. GSI value grows two-fold compared to the value in September. Between October and March the ovary enters a Winter period of rest. No significant changes occur in either relative ovary weight nor in the proportion of oocytes in different stages of development. Progesterone levels decrease while the concentration of 17-β-estradiol responsible for final maturation and ovulation increases. The blood plasma concentration of testosterone as a precursor of 17-β-estradiol also increases.

3.1.7. Ovarian cycle in the nase

The relative weight of the ovary in the nase is very high in the period preceding spawning and GSI keeps increasing up to spawning. It reaches the extremely high GSI value (15.7%) of other single-spawner species in March already. In this period, only oocytes in the process of vitellogenesis and those in the stage of primary development are present in the ovary, apart from the few per cent of those in the stage of “annular” cortical alveoli. GSI keeps increasing in April. Cell diameter reaches its maximum value and shows a homogeneous distribution of 2000-3000 µm. Spawning starts in the end of March and contiunues through April at water temperatures of 9.6-13.4°C. In April, the concentration of steroids responsible for the regulation of oogenesis (17-β-estradiol, testosterone) decreases and the level of progesterone necessary for ovulation increases compared to the value in March. Following spawning the relative weight

of the ovary decreases to 2.14%, mature yolk-rich oocytes are emptied from the organ and the concentration of 17-β-estradiol which stimulates development also decreases. A slight increase of progesterone concentration is detected which is probably due to the progesterone secretion of follicles emptied following ovulation as it was observed by KAGAWA et al. (1983) in the goldfish. The short Summer regeneration period of a few weeks starts in June. In July, the diameter of follicles remaining in the ovaries does not exceed 1400 µm, however, the transformation of cells in the stage of cortical alveoli continues with greater intensity, thus, the relative weight of the ovary also grows by a few per cent. Similarly to the ide, the process of vitellogenesis starts early, in August, thus increasing the relative weight of the ovary and the size of follicles. The concentration of 17-β-estradiol shows a continuously increasing tendency reflecting intensive gamete development. The continuous increase of cells transforming into the stage of vitellogenesis results in a significant growth of GSI values. Relative weight of the overy reaches 50% of the spawning value before Winter. The homogeneous distribution of follicle diameters reaches 2400 µm by October.

3.2. Evaluation of the results of individuals collected from Lake Balaton

3.2.1. Ovarian cycle in the roach

The spawning of roach starts in the early Spring (March) at very low water temperatures (4.0-14.2°C). In this period, only oocytes in the stage of primary growth and vitellogenesis are present in the ovary. The number of yolk-rich oocytes exceeds that of cells in the stage of primary growth by far which is clear indicator of the approaching spawning. GSI

value is high (15%) and it exceeds that of roach from the Danube (13.77%), although only by a few per cent. Both testosterone and 17-β-estradiol are characterized by very high concentrations. Following spawning the concentrations of testosterone and 17-β-estradiol rapidly decrease along with the relative weight of the ovary to less than 1.5%. While only a few per cent (1.52%) of yolk rich oocytes and empty follicles remain in the individuals from the Danube, in those from the Lake Balaton, this percentage (2%) is occupied by empty follicles and atretic cells. Cell diameter in this period does not exceed 1700µm. Ovaries are emptied by July and only (100%) cells in the stage of primary growth are present. The concentration of 17-β-estradiol in the blood decreases to minimal but testosterone levels reduce as well. However, progesterone concentrations start of increase following the low levels in late Spring. By the end of the Summer, a qualitative change of cells begins and oocytes in the stage of „annular" cortical alveoli appear to the ratio of 31%. These cells, however, contribute to ovary weight increase only to a small degree which is reflected by the moderate increase of GSI. By November, the relative weight of the ovary reaches 11% and most of the ovary is filled with cells in the stage of vitellogenesis, whose weight significantly exceeds that of oocytes in the stage of primary growth. Cells are large, their size is almost equal to that of the follicles analyzed in March. The concentration of 17-β-estradiol increases drastically, and in parallel, the levels of the two other sex steroids shows a growing trend, as well which indicates intensive oocyte development.

In the winter months (between October and March), the 3-4 months prior to spawning intensive cell growth is present in the females.

3.2.2. Ovarian cycle in the white bream

In this species, intensive cell growth processes are present in the ovary even in the period prior to spawning which is indicated by the high number of oocytes in the stage of cortical alveoli. Follicle diameter in this period does not exceed 900 µm and the concentration of progesterone that regulates final oocyte maturation is also relatively high. Development reinitiates following the Winter period of rest in parallel with increasing water temperature. In April, oogenesis gains a new momentum and GSI values increase four-fold within a short period of time. Spawning takes place mostly in April, however, the post-spawning fluctuation of GSI indicates that spawning is a similarly prolonged process in Lake Balaton as it is in the Danube. In this month, the concentrations of 17-β-estradiol and testosterone responsible for oogenesis increases drastically, the size of cells reaches 1400µm and the heterogeneous distribution of follicle sizes indicates the partial ovulation that follows spawning in April. Although ovary weight decreases in May, oocytes awaiting final maturation remain in the ovaries which will ovulate only in June and this will be followed by spawning. By the end of Spring, ovaries contain cells in the stage of cortical alveoli only in a low percentage, testosterone concentration also decreases, however, 17-β-estradiol levels remain the same which indicates further gamete production. In June, GSI increases again, new cells reach the stage of final maturation, whose diameter reaches 1600µm. The prolonged spawning season is followed by a short regeneration period of a few weeks in July (RINCHARD and KESTEMONT 1996). In this period, cells in the stage of vitellogenesis are emptied from the ovary, and oocytes in the stage of primary growth occupy most of the organ. Hormone concentrations decrease and GSI

does not exceed 1%. Energy spent on reproduction decreases to minimum. Oogenesis restarts in August, similarly to Danubian individuals. In August, oocytes entering the stage of cortical alveoli and those in the stage of vitellogenesis (present in a few per cent, 1.42%) contribute to the increasing relative weight of the ovary. In parallel, the concentration of 17-β-estradiol also increases in the blood plasma. In the fall, the formation of cortical alveoli starts in the cells. Follicle diameters show a relatively homogeneous distribution between 200-800 µm. Intensive yolk deposition starts in November which is accompanied by a significant increase in the relative weight of the ovary. The concentration of steroid hormones regulating oogenesis increases in parallel to gamete development. GSI increases to double of that measured in September. This is a clear difference between the white bream stocks living in the Danube and Lake Balaton as these changes in Danubian individuals start already in October. This is probaby due to the more balanced conditions in Lake Balaton. Food is more abundant in Balaton due to its stagnant waters, there are less competitor species, the vast amount of stagnant water cools later during the Fall due to its shallow levels and more balanced temperature, thus, its white bream stocks begin to prepare for the Winter period of rest later.

No significant changes occur in the proportion of oocytes in the stages of cortical alveoli and vitellogenesis in the Winter period of rest.

3.3. New scientific results

1. Changes of the gonado-somatic index (GSI) during the annual gonadal cycle have been determined in the asp, white-eye bream, ide, barbel, roach, white bream and nase.

2. The dynamics of cell growth in the ovary have been described through the appearance of different development stages and by the determination of the diameters of developing oocytes.

3. Changes of the concentrations of most important sex steroids regulating gametogenesis during the sexual cycle have been described.

4. The ovarian morphology and hormonal background of spawning frequency (single and multiple spawner) in the investigated species has partly been described.

5. A previously published information (HALAČKA et al., 1997) stating that vitellogenesis in the nase ends in October has been refuted as according to my experiments these processes continue through the Spring months.

6. A comparison has been made between the reproductive characteristics of identical species (roach and white bream) inhabiting different natural waters (stagnant waters and rivers) and differences have been described.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusions

The period of the formation of cortical alveoli occurs in the Fall in the asp, white-eye bream, roach (including its stocks in the Lake Balaton), ide and nase. In the 3-4 months preceding spawning, already developed cells in the stage of vitellogenesis develop further. This is not an increase in their numbers but rather in size.

Results on the changes in GSI and oocyte diameters confirm earlier observations that the asp, white-eye bream, roach, ide and nase are single-spawner species.

Not only quantitative changes are present in the ovary of the barbel in the late Fall and Winter months as shown by the significant increase of GSI values. The number of cells in the stage of cortical alveoli has signficantly decreased between October and April while the number of oocytes in the stage of vitellogenesis has increased. This qualitative change means that the formation of vesicles finishes in the cells in the stage of cortical alveoli and the process of vitellogenesis starts.

In the barbel and the white bream (including those in the Lake Balaton) formation of cortical alveoli continues in the Spring moths. This period is characterized by quantitative as well as qualitative changes, meaning a transition from one stage to another.

Results on GSI and oocyte diameter confirm earlier observations that the barbel and the white bream are multiple spawner species.

A better knowledge of reproductive processes of individual species allows the precise timing of induced spawning of riverine species and ensures the period of broodstock capture and maintenance. This is

considered an important trait at a fish farm. Precise timing allows the reduction of stress on the broodstock to be spawned.

4.2. Recommendations

If the species-wise prohibition period recommended by SALLAI (2008) would be considered for introduction, I would recommend that results of this work are taken into account when calculating the protection period for the species studied here. The currently valid specific prohibition period for the barbel is May 2nd – June 15th. Due to the reproductive characteristics of the species (its spawning starts in the middle of April) its specific protection period should be modified accordingly.

According to the joint regulation 88/2009 (VII. 17.) FVM-KvVM, as of 2010, ide, nase and vimba bream cannot be captured between May 2nd and June 15th. This regulation offers limited protection to the vimba bream, which is the latest spawning species of the three in Hungary. My experiments, however, prove that the spawning periods of both the ide (**March**-April) and the nase (March-**April**) fall beyond the period mentioned in the regulation. Thus, a revision of the regulation is recommended.

I also recommend the further and in-depth study of the ovarian cycle of these species, a more detailed investigation of changes in sex steroid concentrations which would provide a more sophisticated view of the reproductive characteristics of the fish species in our habitats.

5. PUBLICATIONS RELATED TO THE TOPIC OF THE DISSERTATION

5.1. Publications in scientific journals

- **LEFLER K.**, GÁL J., DEMETER GY. (2006): A vízhőmérséklet hatása a szivárványos pisztráng (*Oncorhynchus mykiss*) embrionális fejlődésére és a kelési eredményekre. Effect of water temperature on the embryonic development and hatching results of rainbow trout (*Oncorhynchus mykiss*). *Magyar Állatorvosok Lapja*, 128 (9) 565-569.
- **K. K. LEFLER**, Á. HEGYI, F. BASKA, J. GÁL, Á. HORVÁTH, B. URBÁNYI, T. SZABÓ (2008): Comparison of ovarian cycles of Hungarian riverine fish species representing different spawning strategie. *Czech Journal of Animal Science*, 53 10: 441-452.
- KOTRIK L., HETYEY CS., HEGYI Á., GÁL J., URBÁNYI B., **LEFLER K. K.** (2008): Az ultrahang vizsgálat alkalmazása az afrikai harcsa ivari működésének jellemzésében *Magyar Állatorvosok Lapja*, 130 (8), 475.

5.2. Book chapters

- SZABÓ T., **LEFLER K. K.,** HORVÁTH L., (2004): A fenékjáró küllő (*Gobio gobio*) szaporodásbiológiájának áttekintése irodalmi adatok és saját vizsgálatok alapján. In: Kálmán E., Csanády A-né: A Tisza és környezete a 2000. évi rendkívüli vízszennyezések után. Bay Zoltán Alapítvány Anyagtudományi és Technológiai Intézete. 293-295.
- SZABÓ T., **LEFLER K. K.,** URBÁNYI B., HORVÁTH L., BASKA F., (2004): Folyóvízi keszegfélék szaporodásbiológiájának vizsgálata és indukált szaporításuk fejlesztése. In: Kálmán E., Csanády A-né: A Tisza és környezete a

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5.2. Conference proceedings

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