

Artificial reproduction of pikeperch

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Preface

Further development of European freshwater aquaculture needs diversification of its production with reliable culture methods of new fish species. From the last two decades, more research efforts were done in order to develop culture of Eurasian perch (*Perca fluviatilis* L.) and pikeperch (*Sander lucioperca* L.). Both species are highly valuable commercial fish and have an acceptable growth rate to market size under intensive culture. Pikeperch is also a valuable recreational species. Up to now, most market size pikeperch come from open waters (lakes, rivers, ponds or lagoons) and relatively few are produced under intensive and/or indoor conditions. In consequence, their availability on the market is fluctuating strongly, as well as their market price and the quality of their flesh.

For these reasons different investigations were conducted in various aspects of pikeperch culture, but the availability of high quality of eggs, fry and weaned juveniles was rapidly identified as one of the main bottlenecks of pikeperch culture. Research focusing on the improvement of broodstock management and its effects on egg and larval quality has been considered among the priorities from both the scientific and technical perspectives.

In several countries producing pikeperch for restocking or food markets, e.g. in Poland, Hungary, Finland, The Netherlands and Germany, breeders are caught from open waters a short time before spawning or reared in ponds during winter and early spring. Some weeks before the reproductive period, males and females are harvested and kept separated in tanks or cages. When the females are ready to spawn, mature males and females are stocked in spawning tanks or cages in which nests have been installed in order to stimulate the females laying the eggs. Fertilization occurs naturally. The results of production of fry based on this method are highly variable. Reliable data about fully artificial spawning are still needed.

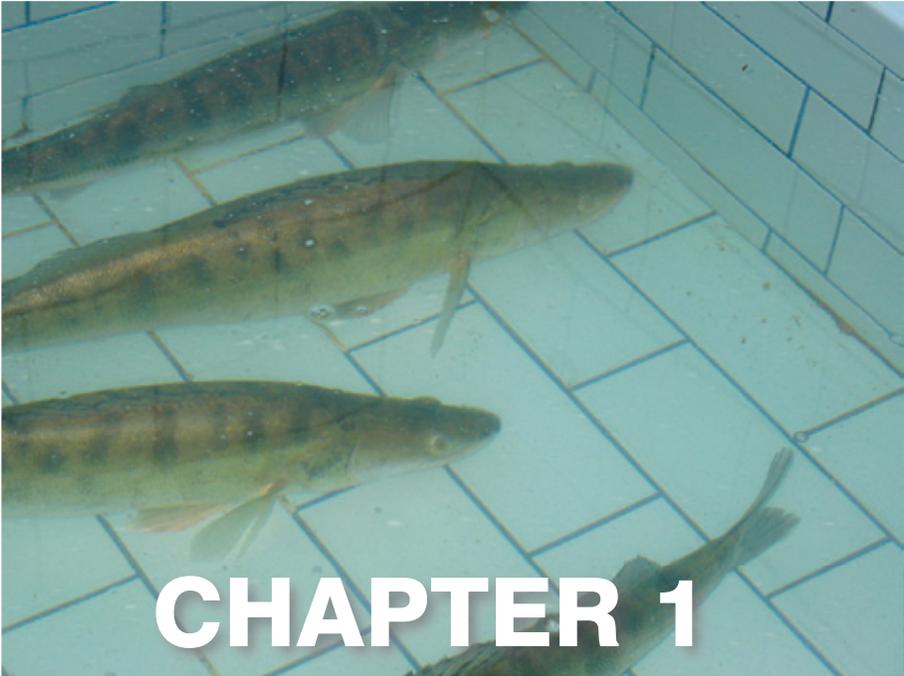
In November 2005, a EC funded a project (Luciopercimprove, COOP-CT 2005-17646) which was initiated by consortium composed of 11 partners (6 SME's and 5 RTD partners) from four European countries (Belgium, France, Poland, The Netherlands) in order to improve the egg and larval quality of pikeperch by improving the management, husbandry and nutrition of breeders. The control of the reproductive cycle, including out-of-season spawning and artificial fertilization, was proposed among some other specific objectives of the project. The University of Warmia and Mazury, in Olsztyn (Poland) has gained a wide expertise in

the control of maturation and spawning on many freshwater fish species belonging to different families (cyprinids, percids, esocids, coregonids) and offered, within the framework of Luciopercimprove, to prepare a manual on artificial reproduction of pikeperch. This manual has been revised by the different partners of the project in order to produce a practical tool which can be used by all aquaculturists interested to artificially reproduce pikeperch in captivity. The editors and the technical assistant are very grateful to the members of the Luciopercimprove consortium (P. Fontaine, H. Jansen, R. Mandiki, C. Mélard, E. Philipsen, E. Rezzouk, A. Roem, C. Rougeot, L. Tamazouzt, F. Téléchéa, J. Van Dooren, J. Van Mechelen, N. Wang) for their valuable comments on a former version of this manual.

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Photo. *Pikeperch eggs in Weiss jars shortly before hatching*



CHAPTER 1

Artificial reproduction of pikeperch – organization of the manual

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Chapter 1. Artificial reproduction of pikeperch – organization of the manual

This manual is divided into 9 chapters and provides the most important practical information about the artificial spawning of pikeperch under controlled conditions.

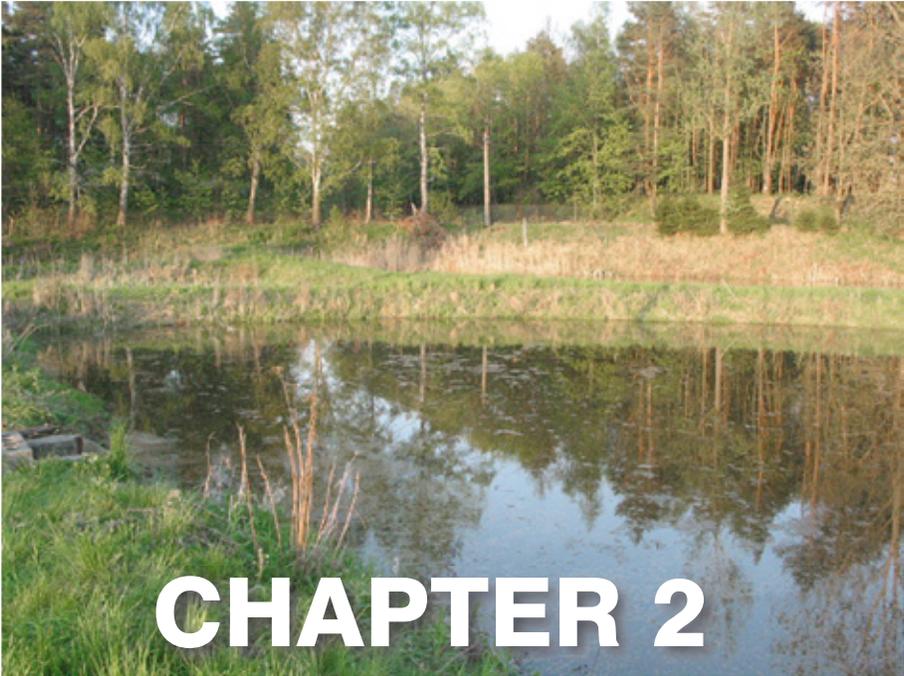
Chapter 2 includes a short review of the state of art in pikeperch reproduction, comparing natural spawning, semi-controlled reproduction and controlled reproduction in captivity. Chapter 3 describes the conditions of breeder stocking and handling. An important aspect of artificial spawning is checking female maturation, in order to induce mature females to ovulate successfully by hormonal treatment (chapter 4). The spawning agents and their application are compared in chapter 5, and the collec-



Fig.1.1. *Pikeperch eggs in Weiss jars shortly after fertilization.*

tion of gametes, both ovules and sperm, is described in chapter 6. Egg fertilization and incubation (Fig.1.1) are detailed in chapter 7, including the issue how to solve the problem of eggs adhesiveness. Chapter 8 briefly describes the hatching of larvae as well as the assessment of their viability. This chapter is complemented by a short annex explaining how to sample eggs or larvae. Chapter 9 concerns an important aspect in pikeperch broodstock management which is the veterinary issue as pikeperch are extremely sensitive to handling and might die from many different causes before, during or after the reproduction phase. The last chapter (chapter 10) presents the short summary and conclusions as well as some information about further possibility of development pikeperch aquaculture.

The manual is extensively illustrated by pictures clearly describing the different steps of the whole artificial reproduction procedure.



CHAPTER 2

State of art of pikeperch reproduction – a short review

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Chapter 2. State of art of pikeperch reproduction – a short review

Pikeperch is one of the most important species in freshwater aquaculture in many European countries. In some of them, pikeperch are cultured up to commercial size fish, i.e. in Holland (1.0-2.0 kg), in other (i.e. in Poland) mainly as a stocking material: fry, summer or autumn fingerlings (Wojda et al. 1995). The fish are produced using different systems and methods, i.e. in pond (in mono and polyculture), cages and tanks. In this last one mainly in a closed water system.

Generally, three methods of pikeperch reproduction are propagated: controlled reproduction (artificial insemination and incubation of eggs in controlled conditions); semi-controlled reproduction in net cages or tanks; natural spawning in ponds (Kausch 1975, Antalffy 1979, Steffens et al. 1996).

Natural spawning

Natural spawning, carried out in ponds, is the oldest method of pikeperch propagation, where spawners are kept in ponds until the catch of summer fry (on average from five to six weeks) (Korycki, 1976), or fingerling pikeperch (7.7-20.0 cm, depending on the initial stocking density) are harvested in autumn (Raat 1991, Steffens et al 1996) (Fig.2.1).

In this method, the pikeperch spawners are usually put into a pond without hormonal stimulation (Wojda et al. 1994a, 1994b). Depending on the pond area one or a few pairs of breeders are put into each spawning pond. Sometimes spawners used the pond ground as spawning substrate, or, more often, artificial nest should be applied. The nests are usually introduced into the pond at the same time as breeders are. There are many different types of nests, depending on the country and region, but nowadays the nests made of artificial grass are usually applied (Fig.2.2). The size of the nest is very important. For smaller females (1.0 – 2.0 kg BW) the minimum size of the nest is 50 cm X 50 cm or 40 cm X 60 cm. From our knowledge, bigger nests should be used, because pikeperch females are very fecund. Usually they produce more than 100,000 eggs per kg of body weight.



Fig.2.1. Pond used for pikeperch propagation.



Fig.2.2. Nest (artificial grass) applied for pikeperch reproduction.

Semi-controlled reproduction

Semi-controlled reproduction in cages requires preparation of spawning nests, made of either natural materials (pine branches, tiny willow roots, and sedge roots) or synthetic materials (Szkudlarek & Zakes 1995, Skrzypczak et al. 1998, Demska-Zakes & Zakes 2002). The broodstock

spawn in cages (Fig.2.3) placed into ponds or lakes usually within few days after stocking.

Semi-artificial reproduction can be also carried out by keeping spawners in pairs in plastic tanks (usually 1-4 m³) (Fig.2.4) also equipped with spawning nests. Fish might be stimulated hormonally or not.

Depending on the number of fish, one or two spawning pairs may be placed in a cage. For each spawning pair one or two nests should be placed on the bottom of the cage (at least 0.5m x 0.5m when female is up to 2 kg of body weight). The nests should be checked twice a day, in the morning and in the evening. Spawning begins when the water temperature is 10 – 14°C. After spawning, breeders should be removed from the cages or tanks. The nests with eggs (Fig.2.5) may be transported to the hatchery and placed in special incubation tanks, or farmers might leave them in the cage. It is also possible to move them to ponds.

Using this method it is also possible to apply spawning agents. The injections are usually made before fish are transferred to cages or tanks.

Controlled reproduction in captivity

Controlled reproduction is the most reliable method for obtaining a high number of pikeperch larvae. Spawners, kept in tanks, are hormonally



Fig.2.3. Cages for pikeperch reproduction. FFP Poland.



Fig.2.4. Tanks for pikeperch reproduction. W, The Netherlands.

stimulated (Ronyai 2007). Gametes are collected by manual stripping, and fertilized eggs are incubated in Zug or Weiss jars (Berka 1979). In recent years, significant progress has been achieved in the development of techniques for the controlled reproduction of many fish species with application of different spawning agents (Fig.2. 6).

The knowledge of physiological processes in fish has facilitated the use of substances that stimulate maturation and reproduction (Peter et al. 1991). Obtaining the maturity can be stimulated in percids (perch, pikeperch) using carp pituitary extract (CPE), human chorionic gonadotropin (hCG) and luteinizing hormone-releasing hormone (LH-RH) or super-active analogs (LH-RHa), sometimes with dopamine antagonists (Kucharczyk et al. 1996, 1998, 2001, Demska-Zakes & Zakes 2002, Kaszubowski 2005, Ronyai 2007, Sosiński 2007).

Some experiments showed that gonadotropin (GtH) and human chorionic gonadotropin (hCG) can be applied in the artificial reproduction of pikeperch, and they might be also used to induce spawning in net cages



Fig.2.5. Nest (artificial grass) with pikeperch eggs (FFP, Poland).



Fig.2.6. Application spawning agent in artificial reproduction of pikeperch.

or even to induce out-of-season spawning (Steffens et al. 1996, Demska-Zakes & Zakes 2002, Zakes & Szczepkowski 2004, Kaszubowski 2005). A commercial product which contains mammalian GnRH analogue with the dopamine inhibitor metoclopramide (Ovopel) (Horvath et al. 1997) was considered as ineffective in stimulation of pikeperch reproduc-

tion (Zakes & Demska-Zakes 2005). On the other hand, the research conducted by Kaszubowski (2005), Ronyai (2007) and Sosiński (2007) showed that it was possible to obtain gametes of good quality using this spawning agent, but applied doses should not be too high.

References

Antalfi A. (1979) Propagation and rearing of pike perch in pond culture. *EIFAC Technical Paper*, 35: 120-125.

Berka, R. (1979) Incubation of pikeperch eggs (a review). *Bull. VURH Vodnany*, 15-42-48 (In Czech with English summary).

Demaska-Zakes, K., Zakes. Z. (2002) Controlled spawning of pikeperch, *Stizostedion lucioperca* (L.), in lake cages. *Czech J. Anim. Sci.*, 47(6): 230-238.

Kaszubowski, R. (2005) Artificial reproduction of pikeperch under controlled conditions. *MSc thesis, UWM Olsztyn*, 30 pp (In Polish with English summary)

Kausch H. (1975) Breeding habits of the major cultivated fishes of EIFAC region and problems of sexual maturation in captivity. *EIFAC Technical Paper*, 25: 43-52.

Korycki, A. (1976) Pikeperch (in Polish). PWRiL, Warszawa, 164 pp.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Skrzypczak, A., Wyszomirska, E. (1996) Induced spawning in perch, *Perca fluviatilis* L. using carp pituitary extract and HCG. *Aquacult. Res.* 27: 847-852.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Skrzypczak, A., Wyszomirska, E. (1998) Induced spawning in perch, *Perca fluviatilis* L., using FSH + LH with pimozide or metoclopramide. *Aquacult. Res.* 29: 131-136.

Kucharczyk D., Szczerbowski A., Luczynski M.J., Kujawa R., Mamcarz A., Wyszomirska E., Szabo T., Ratajski S. (2001) Artificial spawning of Eurasian perch, *Perca fluviatilis* L., using ovopel. *Arch. Ryb. Pol.* 9: 39-49.

Peter R.E., Trudeau V.L., Sloley B.D. (1991) Brain regulation of reproduction in teleosts. *Bull. Inst. Zool., Academia Sinica, Monograph*, 16: 89-118.

Raat J.P. (1991) Production, growth, condition and angling vulnerability of zander, *Stizostedion lucioperca* (L.), in relation to the availability of prey fish in ponds. *Aquaculture and Fisheries Management*, 22: 93-104.

Ronyai, A. (2007). Induced out-of-season and seasonal tank spawning and stripping of pike perch (*Sander lucioperca* L.). *Aquacult. Res.* 1-8.

Steffens, W., Geldhauser F., Gerstner P., Hilge V. (1996) German experiences in the propagation and rearing of fingerling pikeperch (*Stizostedion lucioperca*). *Ann. Zool. Fennici.*, 33: 627 - 634.

Skrzypczak, A., Kucharczyk, D., Mamcarz, A., Kujawa, R., Furgala-Selezniow, G. (1998): A new breeding technique of pikeperch (*Stizostedion lucioperca*) in net cages. *Czech J. Anim. Sci.*, 43: 398-398.

Sosiński, M. (2007). The application of Ovaprim in artificial reproduction of pikeperch. *MSc thesis, UWM Olsztyn*, 30 pp (In Polish with English summary)

Szkudlarek M., Zakes Z. (1995) Pikeperch spawning in lake cages – a little known method. *Komunikaty Rybackie*, 5: 8-10. (In Polish)

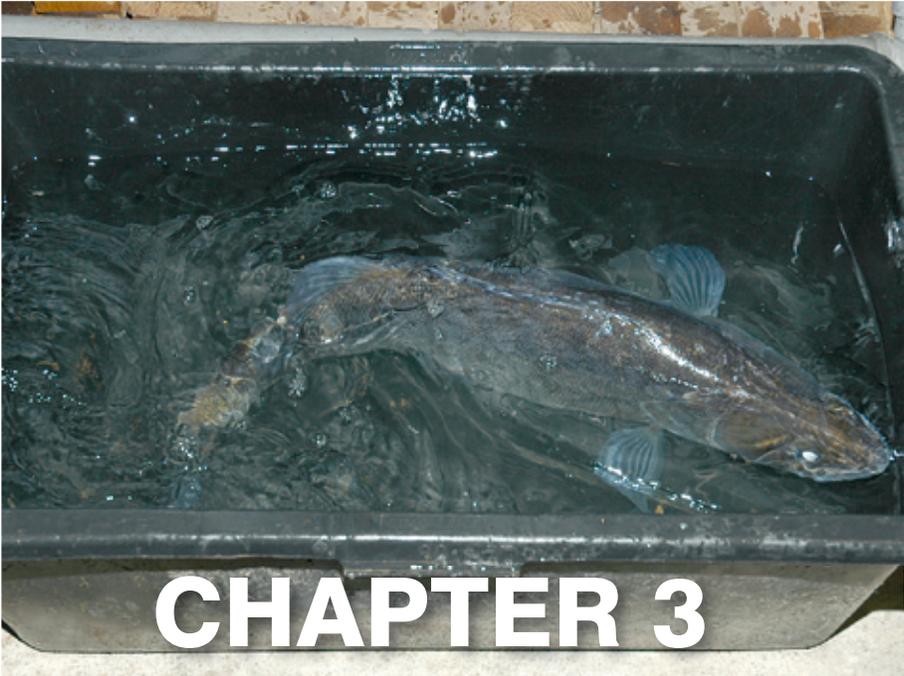
Wojda R., Sliwinski J., Ciesla M. (1994a) Natural spawning of pikeperch in carp ponds. *Komunikaty Rybackie*, 2: 13-16. (In Polish).

Wojda R., Sliwinski J., Ciesla M. (1994b) Results of studies on natural spawning in pikeperch, *Stizostedion lucioperca* (L.). *Roczniki Naukowe PZW*, 7: 71-80. (In Polish with English summary).

Wojda R., Ciesla M., Sliwinski J. (1995) Rearing of pikeperch, *Stizostedion lucioperca* (L.), fingerlings in carp ponds. *Roczniki Naukowe PZW*, 8: 75-93 (In Polish with English summary).

Zakes Z., Demska-Zakes K. (2005) Artificial spawning of pikeperch (*Sander lucioperca* (L.)) stimulated with human chorionic gonadotropin (hCG) and mammalian GnRH analogue with a dopamine inhibitor. *Arch. Pol. Fish.*, 13: 63-75.

Zakes Z., Szczepkowski M. (2004) Induction of out-of-season spawning of pikeperch, *Sander lucioperca* (L.). *Aquacult. Int.*, 12: 11-18.



Spawners and handling

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Chapter 3. Spawners and handling

Obtaining good quality gametes, especially in such species as pikeperch, needs the use of high quality breeders. In pikeperch aquaculture spawners are collected from different environments. Wild fish are often collected from natural environments: lakes, rivers, lagoons. Another way is to obtain fish from pond culture. Such fish are much less sensitive than wild ones. The third possibility is obtaining breeders from intensive aquaculture, reared under controlled conditions.

Wild pikeperch spawners are often obtained from lakes - caught at natural spawning grounds - using trap gear (fyke nets) in the pre-spawning season (March - April) (Peter et al. 1991; Steffens et al. 1996; Demska-Zakes & Zakes 2002; Kaszubowski 2005, Zakes & Demska-Zakes 2005, Sosiński 2007).

The main disadvantage of this method is that wild spawners are very sensitive to stress and manipulation and improper handling usually results in high losses, caused mainly by fungi diseases (see chapter 9) (Kaszubowski 2005, Sosiński 2007).

The pikeperch breeders should be transported separately in plastic bags with oxygen (20 L of water and 20 L oxygen), one fish per bag (Zakes & Demska-Zakes 2005). From our own experience we obtained very good results transporting fish in bags made of opaque, blue plastic



Fig.3.1. *Pikeperch breeders in anaesthesia.*



Fig.3.2. Pikeperch breeder in tank with controlled water temperature and aeration.



Fig.3.3. Pikeperch breeders in pond.

(FFP, Poland). Fish are not stressed and the only disadvantage is that breeders cannot be observed during transport. The anti-stress agent, i.e. Propiscin (0.2% etomidate, IFI Olsztyn, Poland, Kazun & Siwicki 2001) can be added to each bag at concentration of $0.02 \text{ cm}^3/\text{dm}^3$ (Zakes & Demska-Zakes 2005).



Fig.3.4. Tanks for intensive pikeperch culture.

Following transport, the spawners are moved to tanks (Fig.3.2) at water temperature close to that during transportation. The fish should be sorted by sex (males are identified by the appearance of semen when a gentle pressure is applied to the abdomen, as well as the colour of the belly – they are darker than females) and body weight is determined.

All manipulations are conducted after the fish are anaesthetized (i.e. with Propiscin at a concentration of 2.0 mL/L) (Zakes & Demska-Zakes, 2005) (Fig.3.1). There are different chemicals allowed to be used as anaesthetics in different countries, so we can not propose any of them as “universal” anaesthetic. We also applied other anesthetics, but always with doses similar to those suggested for salmonid fish. If the 2-phenoxy-ethanol is used, fish can not be at full anaesthesia, because it causes high mortality.

The spawners might be also kept in semi-natural conditions, e.g. in ponds or cages (Fig.3.3), but it often results in quite high mortality, especially if no medical treatment is applied.

Spawners obtained from pond or culture tanks are less sensitive than wild ones. They might be kept in ponds or tanks, without serious influence on fish health, survival and gamete quality (Fig.3.4.)

During succeeding other manipulations, i.e. checking maturity stage of oocytes, applying injection, obtaining gametes, etc. fish should be kept in full anaesthesia. One of the methods, which also improves fish



Fig.3.5. Pikeperch female in anaesthetic bath.



Fig.3.6. *Checking maturity stage of pikeperch male.*

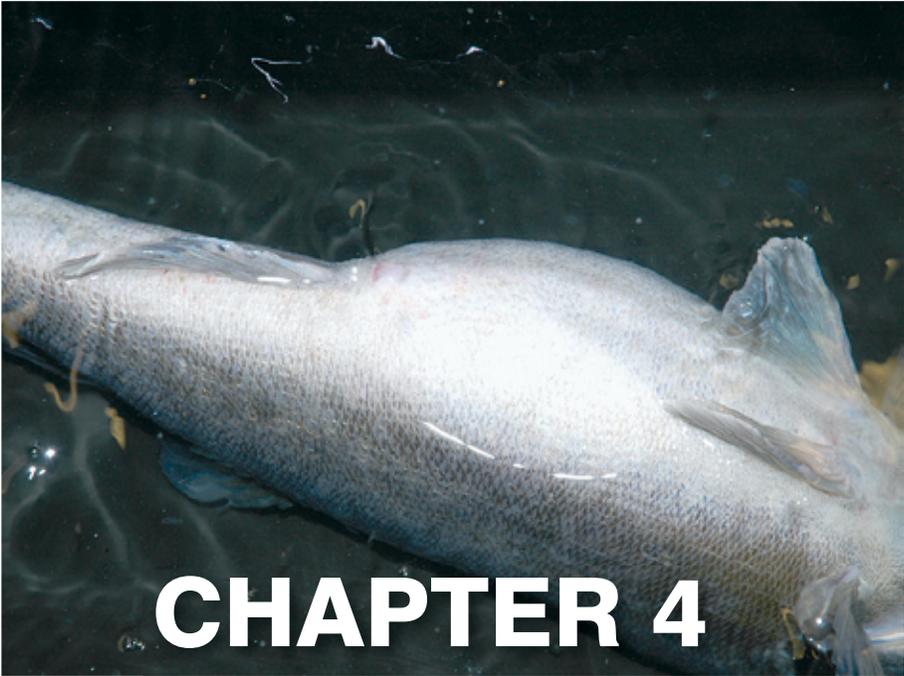
survival and reduces health problems, is adding 1% salt (NaCl) to the bath (Fig.3.5.)

The best method for pikeperch breeders is keeping them in anaesthetic solution separately. It is very important because sometimes spawners are very nervous during such bath and can hurt other ones.

Also, any other manipulations with fish should be carried out very gently. During all manipulations fish should be kept on a wet towel (Fig.3.6.).

References

- Demska-Zakes, K., Zakes. Z. (2002) Controlled spawning of pikeperch, *Stizostedion lucioperca* (L.), in lake cages. *Czech J. Anim. Sci.*, 47(6): 230-238.
- Kaszubowski, R. (2005) Artificial reproduction of pikeperch under controlled conditions. *MSc thesis, UWM Olsztyn*, 30 pp (In Polish with English summary)
- Kazun, K., Siwicki A. (2001) Propiscin – a new safe anaesthetic for fish. *Arch. Pol. Fish.*, 9: 183-190.
- Peter R.E., Trudeau V.L., Sloley B.D. (1991) Brain regulation of reproduction in teleosts. *Bull. Inst. Zool., Academia Sinica, Monograph*, 16: 89-118.
- Sosiński, M. (2007). The application of Ovaprim in artificial reproduction of pikeperch. *MSc thesis, UWM Olsztyn*, 30 pp (In Polish with English summary)
- Steffens, W., Geldhauser F., Gerstner P., Hilge V. (1996) German experiences in the propagation and rearing of fingerling pikeperch (*Stizostedion lucioperca*). *Ann. Zool. Fennici.*, 33: 627 - 634.
- Zakes Z., Demska-Zakes K. (2005) Artificial spawning of pikeperch (*Sander lucioperca* (L.)) stimulated with human chorionic gonadotropin (hCG) and mammalian GnRH analogue with a dopamine inhibitor. *Arch. Pol. Fish.*, 13 (1): 63-75.



CHAPTER 4

Checking maturation stage of females

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Chapter 4. Checking maturation stage of females

During the selection of suitable females for reproduction their health status (see Chapter 9) and the maturation stage of oocytes (eggs still in the ovary) should be taken into account.

Maturation of the oocytes is a long process that involves complex physiological and biochemical changes. One important step, vitellogenesis, is a process in which yolk proteins are produced in the liver, transported to the ovary and stored in the egg, resulting in tremendous egg enlargement. Also critical are germinal vesicle (GV) migration and germinal vesicle breakdown (GVBD).

The maturity of oocytes can be determined by using biopsy techniques. In this technique eggs (oocytes) are taken from the ovary, cleared



Fig.4.1. *The female of pikeperch before checking maturity stage of oocytes.*

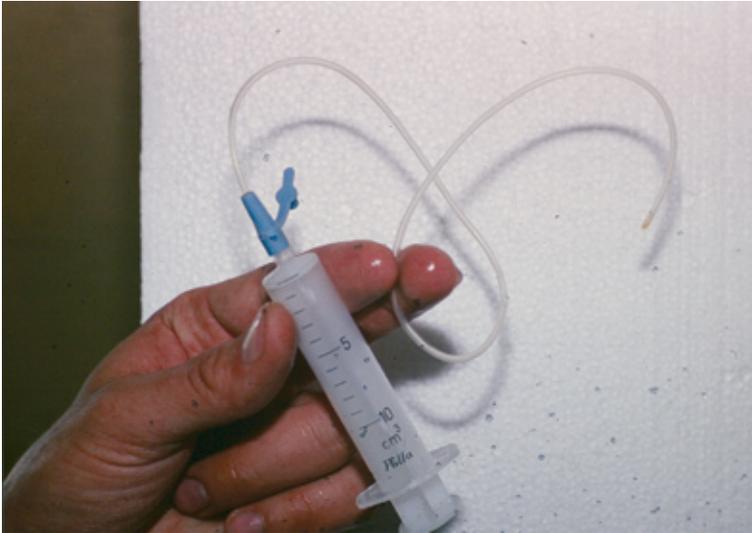


Fig.4.2. A catheter for sampling pikeperch oocytes.



Fig.4.3. Pikeperch in anaesthesia bath before taking oocytes sample.

with a prepared solution (e.g. Serra's solution), and viewed under a microscope.

There are many different methods of sampling oocytes from fish ovary. One of them is taking sample using a catheter (Kujawa & Kucharczyk 1996) (Fig.4. 2). This method was tested many times in perch (Kucha-

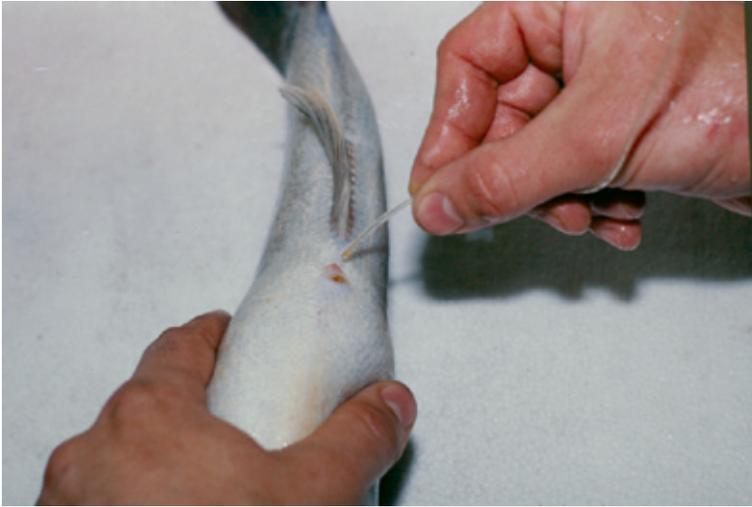


Fig.4.4. *Shortly before bringing in a catheter to pikeperch female.*

rczyk et al. 1996, 1998) pikeperch (Wojnowska 1998, Demska-Zakes & Zakes 2002, Zakes & Szczepkowski 2004, Zakes & Demska-Zakes 2005, Ronyai 2007), as well as in many other fish species, especially in cyprinids (Kucharczyk et al. 1997, 2005).

Before taking an egg sample, fish should be treated with an anaesthetic solution. It is very important, because pikeperch are extremely sensitive fish. On the other hand, taking samples sometimes is time consuming and requires a few minutes. During this time the fish should not move. Another problem is: when should the catheter be applied? Into which pore?

In pikeperch females there are three pores on the belly and the catheter (Fig.4.4) should be brought into the middle one. The genital pore is very easily visible, especially during the spawning season, when it is the biggest one, and usually pink or reddish.

The main evaluation criteria of pikeperch oocytes maturity stages like other *Teleostei* fishes are the location of germinal vesicle (GV) and additionally coalescence of the oil droplets.

Oocytes classified according the above-mentioned criteria are divided into four stages:

- I. Oocytes in I (first) maturity stage have GV in the central position and many small oil droplets (Fig.4.7.). In this stage freshly sampled oocytes from the ovary have a yellow-white colouration and they are



Fig.4.5. *Sampling oocytes from pikeperch female.*



Fig.4.6. *Oocytes in the catheter.*

relatively opaque. The clearing time in Serra's fluid to obtain full transparency varies from two to five minutes.

- II. Oocytes classified as II (second) maturity stage have shifted GV less than a half radius. In addition, the oil droplets are less numerous and have bigger dimensions (Fig.4.8). In this stage oocytes freshly

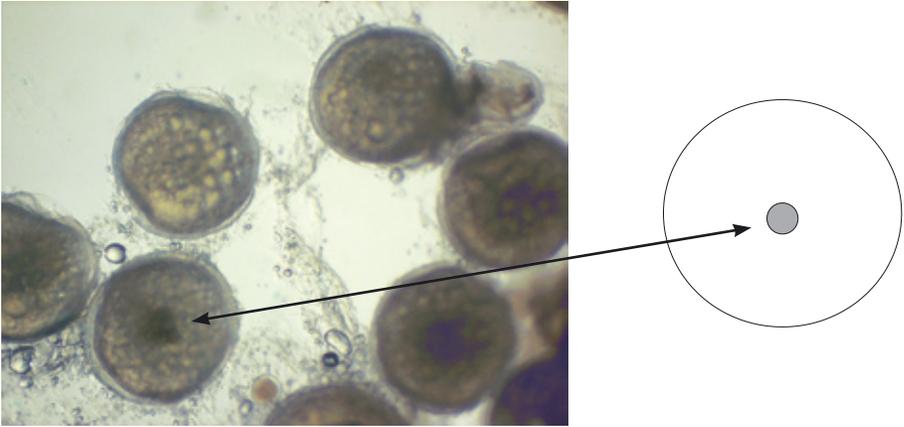


Fig.4.7. *The oocyte in stage I.*

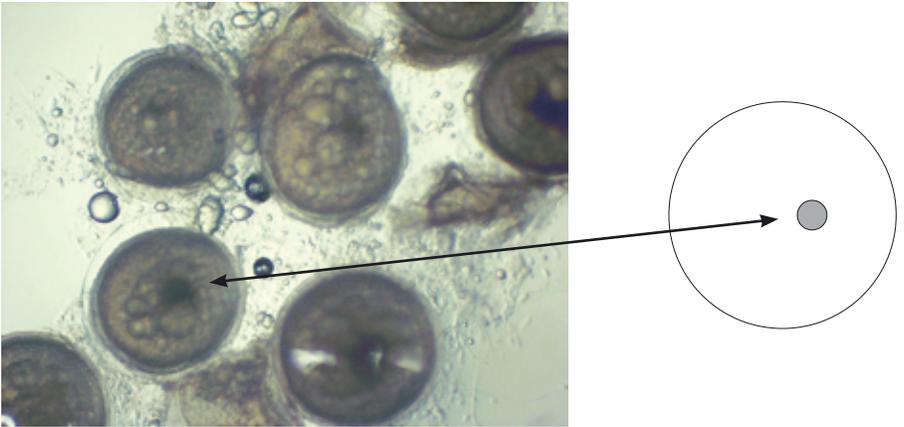


Fig.4.8. *Oocyte in stage II.*

sampled from the ovary are straw-coloured and less opaque than in the previous stage. The clearing time in Serra's fluid to obtain full transparency is usually shorter than in the previous stage of maturity and takes about 2 -3 minutes.

III. Oocytes classified as maturity stage III have positioned GV on the periphery, near the oocyte membrane, and oil coalesced in one big droplet (Fig 4.9). In this stage oocytes freshly sampled from the ovary are more transparent than in the previous stage. The clearing time in Serra's fluid to obtain full transparency is shorter than in the previous stage and takes one to three minutes.



Fig.4.9. Oocyte in stage III.

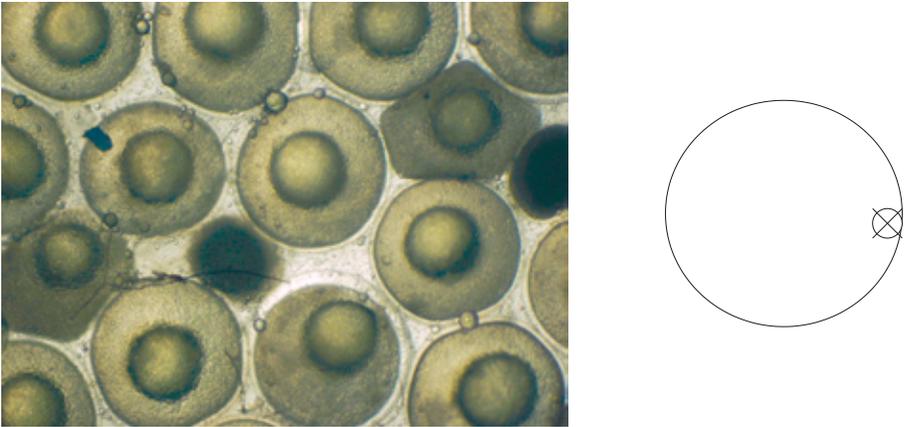


Fig.4.10. Oocyte in stage IV.

IV. Oocytes without visible GV, i.e. in which the process of GV breakdown (GVBD) has begun or GV is present near the zone, should be classified as maturity stage IV (Fig.4.10). In this stage oocytes freshly sampled from the ovary have light yellow colouring and clear transparency. The clearing time in Serra's fluid to obtain full transparency is shorter than in the previous stage and equals half to two minutes.

If the oocytes placed in Serra's solution are damaged it might mean that oocytes are already in the ovulation or resorption process. When the oocytes are in stages I – IV, the shape of oocytes in Serra's solution is still

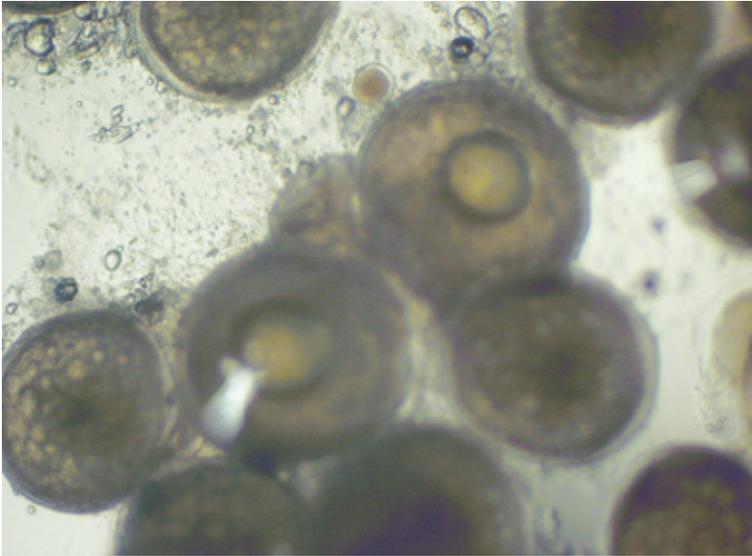


Fig.4.11. *Asynchronous oocyte maturation.*

constant – they are round. If they are in different developmental stages (Fig.4.11) the quality of eggs should be very low and during this spawning season such females should be omitted from broodstock.

Annex: for an ovarian biopsy, proceed as follows:

1. All equipment has to be ready, cleaned and sterilized in advance.
2. Add anaesthetic in sufficient quantity to obtain sedation (for type of drug and dosage see Chapter 3.). On a tray prepare one Petri dishe per female, a couple of flexible sterile catheters (1.5 – 2.0 mm internal diameter), a glass or plastic tubes, clearing fluid (Serra's fixative - solution of ethyl alcohol 96%: formalin: glacial acetic acid, 6:3:1, v/v).
3. Take one female at a time and move into the container with solution of anaesthetic. After sedation introduce the sterile catheter into the genital papilla and the oviduct, up to the ovary for a few cm, then suck carefully a small sample of oocytes up into the catheter and place the sample into a tube.
4. After sampling, release the female into the tank, where recovery from sedation will take place.
5. Fill the tube with Serra's fluid (1-2 cm above oocytes level), cork it and shake energetically for few minutes, then move the sample of oocytes

- (c. 30) to a Petri dish and examine maturity stages under the microscope at 5 and 10 magnifications (for details see above).
6. Place the catheter in alcohol, and sample the next female in the same way with another catheter. Repeat sampling changing the catheter each time. Handle fish with care (use cotton gloves) and do not damage the genital papilla area when introducing the catheter. The use of cotton gloves or towel to handle fish is recommended.

Females, depending on their oocytes maturity stage, should be divided into four groups, from I to IV, and located into separate tanks (or cages) respectively.

References

- Demska-Zakes, K., Zakes, Z. (2002) Controlled spawning of pikeperch, (*Stizostedion lucioperca* L.), in lake cages. *Czech J. Anim. Sci*, 47: 230-238.
- Kucharczyk, D., Kujawa, R., Mamcarz, A., Skrzypczak, A., Wyszomirska, E. (1996) Induced spawning in perch, *Perca fluviatilis* L. using carp pituitary extract and HCG. *Aquacult. Res.* 27: 847-852.
- Kucharczyk, D., Kujawa, R., Mamcarz, A., and Wyszomirska, E. (1997) Artificial spawning in bream (*Abramis brama* L.). *Pol. Arch. Hydrobiol.*, 44: 203-207.
- Kucharczyk, D., Kujawa, R., Mamcarz, A., Skrzypczak, A., Wyszomirska, E. (1998) Induced spawning in perch, *Perca fluviatilis* L., using FSH + LH with pimozide or metoclopramide. *Aquacult. Res.* 29: 131-136.
- Kucharczyk D., Kujawa R., Mamcarz A., Targońska-Dietrich K., Wyszomirska E., Glogowski J., Babiak I., Szabó T. (2005) Induced spawning in bream (*Abramis brama* L.) using GnRH-containing pellets. *Czech J. Anim. Sci*, 50(3): 89-95.
- Kujawa, R., Kucharczyk, D. (1996) The collection of eggs using catheter from cyprinids and percids. *Komunikaty Rybackie* 4: 20-21 (In Polish).
- Ronyai, A. (2007) Induced out-of-season and seasonal tank spawning and stripping of pike perch (*Sander lucioperca* L.). *Aquacult. Res.* 1-8.
- Wojnowska, E., (1998) New methods of rearing pikeperch (*Stizostedion lucioperca* L.) larvae. *MSc Thesis. Olsztyn University of Agriculture & Technology* (In Polish)
- Zakes Z., Demska-Zakes K. (2005) Artificial spawning of pikeperch (*Sander lucioperca* (L.)) stimulated with human chorionic gonadotropin (hCG) and mammalian GnRH analogue with a dopamine inhibitor. *Arch. Pol. Fish.*, 13: 63-75.
- Zakes Z., Szczepkowski M. (2004) Induction of out-of-season spawning of pikeperch, *Sander lucioperca* (L.). *Aquacult. Int.*, 12: 11-18.



CHAPTER 5

Spawning agents and their application

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Chapter V. Spawning agents and their application

One of the most important problems in modern aquaculture is obtaining good quality gametes. For this reason many hormonal treatments were used for stimulation gamete maturation in commercial fish culture, e.g. mainly freshwater like cyprinids, percids, etc. One of the most commonly applied spawning agents is carp pituitary extract (CPE), especially in cyprinids, in some cases with addition of human chorionic gonadotropin (hCG). Good results in induced ovulation in freshwater fish were also obtained after a hormonal stimulation with a synthetic analogue of gonadotropin releasing hormone (GnRH), frequently with strong dopamine antagonists.

In last years many kinds of spawning agents, as well as doses were tested in pikeperch reproduction (Ronyai 2007). Also, a lot of new spawning agents present at the aquaculture market involve new research (Brzuska 2000, 2001, 2004, 2005). Some of them are now tested also in the case of pikeperch (Sosiński 2007).



Fig.5.1. Pikeperch spawners in anaesthesia bath, before application of spawning agents.

Spawning agents

It is possible to apply many kinds of hormones. The most frequently used are:

- Extract from pituitary glands, i.e. carp (CPE) (Wojnowska 1998, Muller et al. 2004, Kaszubowski 2005, Ronyai 2007)
- Chorionic gonadotropins (i.e.: human, horse) (Wojnowska 1998, Demska-Zakes & Zakes 2002, Zakes & Szczepkowski 2004, Demska-Zakes & Zakes 2005, Kaszubowski 2005, Ronyai 2007, Sosiński 2007)
- GnRH or its analogues sometimes combined with dopamine antagonists, i.e. Ovopel (Kaszubowski 2005, Sosiński 2007, and Ronyai 2007). One Ovopel pellet (average weight about 25 mg) contains a mammalian GnRH analogue (D-Ala⁶, Pro⁹Net-mGnRH at dose 18-20 μ g) and dopamine antagonist: metoclopramide (dose 8-10 mg) (Horvath et al. 1997).

Preparing of injection solution

All spawning agents are usually prepared with 0.9% NaCl. Carp pituitary extract is usually homogenised in mortar or homogenizer, hCG, FSH, LH dissolved and Ovopel pellets are pulverised in a mortar and then dis-



Fig.5.2. Application of spawning agents in pikeperch female.

solved. Some other agents were produced ready to use as liquid, i.e. Ovaprim. Spawning agent solutions should be prepared shortly before application. The temperature of hormone solution should be the same as the temperature of water in which the breeders are kept.

Application of injections

There is a possibility to apply the injection in a few different places. Injections of hCG in many cases are intramuscular in the dorsal area of the body (Thalathiah et al., 1988). Injections of pituitary (Kucharczyk et al., 1997) and Ovopel (Horvath et al., 1997) extracts are usually intraperitoneal at the base of the pelvic fin. But, in many cases, the places in fish body where injections are applied really depend on the farmer's experience. In our opinion, if only one spawning agent is applied, the best place for application is **the base of the pelvic fin**.

Are injections really necessary?

In intensive aquaculture the application of spawning agents is really necessary and, in many cases, it is basically impossible to obtain good qual-



Fig.5.3. Possible places of application of spawning agents. The arrows indicate possible places for preparing injections.

ity gametes without hormonal stimulation in captivity. The data from many research showed that **non-stimulated females in captivity usually do not ovulate** (Zakes & Szczepkowski 2004, Kaszubowski 1995, Sosiński 2007). There are many different reasons for such a situation: i.e. stress, different photo-thermal regimes, lack of spawning grounds, etc. Results of different spawning agents in pikeperch reproduction under controlled conditions are presented in Table 5.1.

Table. 5.1. Efficiency of different spawning agents for artificial reproduction of pikeperch.

	Control (0.9% NaCl)	CPE	hCG	Ovopel
No. of females	15	15	15	15
Ovulation (%)	20	47	93	73
Embryo survival (%)*	49.1 ± 4.2 B	25.9 ± 4.5 C	81.4 ± 2.5 A	78.9 ± 4.2 A
Females' survival (%)	60	40	87	80

* - data (± SD) marked with the same letter did not differ statistically (Duncan's multiple range test, $\alpha = 0.05$)

The application of spawning agents usually influences not only the spawning synchronization, but the percentage of fish which are ready to spawn and the quality and quantity of gametes, as well.

How many injections: one, two, or more?

In case of pikeperch two different ways might be recommended. One of them is preparing a single injection (Muller et al. 2004, Kaszubowski 2005, Sosiński 2007). In this case fish receive the whole spawning agent dose at a time. This method is specially recommended when hCG is applied. In case of other hormones, especially when CPE or GnRH α with dopamine antagonist is used, spawning agents might be applied in two doses. More injections than two are not recommended (except out-of-season spawning: Zakes & Szczepkowski 2004, Ronyai 2007), because pikeperch are very sensitive to stress.

In our opinion fish of both sexes should receive the same doses of spawning agents, especially in the case of out-of-season spawning or when fish sex is not recognized.

Doses

The doses of spawning agents depend on many factors:

- Time of spawning (season, out-of-season)
- Maturation stage of fish
- Kind of hormones (i.e. human or horse gonadotropins)

The range of total doses of chosen spawning agents should be as follows:

- Chorionic gonadotropins (200 – 1000 IU/kg BW) (Demska-Zakes & Zakes 2002, Demska-Zakes et al. 2005, Kaszubowski 2005, Ronyai 2007, Sosiński 2007)
- CPE (3 – 15 mg / kg BW) (Wojnowska 1998, Muller et al. 2004, Kaszubowski 2005)
- Ovopel (1,2 – 2,0 pellets / kg BW) (Kaszubowski 2005, Zakes & Demska-Zakes 2005, Sosiński 2007)

If two injections are planned, the initial dose should be 20 – 50 % in the case of chorionic gonadotropins and 10 – 20 % in the case of other spawning agents.

Photo-thermal manipulations

After injection spawners should be kept in good conditions. It is better if fish of both sexes are kept separately. In other case, the male starts to fight for the best place for reproduction. The temperature should be between 14 – 16 °C, the photo-period 12 h Light : 12 h Dark, dissolved oxygen level at minimum 6 ppm. This last factor is very important. If its level decreased to 3 – 4 ppm, usually the fish (females) spawning in this season was not possible.

Effects

If all the processes are made using the described instruction, results as shown in Fig.5.4. are obtained. Because the fecundity of pikeperch is very high, obtaining good quality gametes of a few females is enough for commercial hatchery to take a constant production.



Fig.5.4. Mixed gametes of artificially stripped pikeperch. Effects of well performed artificial reproduction.

References

Brzuska E. (2000) Artificial spawning of carp *Cyprinus carpio* L.: differences between the effects on reproduction in females of Polish and Hungarian provenance treated with carp pituitary and (D-Ala⁶) GnRH ProNHET (Kobarelin). *Aquaculture Research* 31, 457-465.

Brzuska E. (2001) Artificial spawning of carp *Cyprinus carpio* L.: the use of Aquaspawn and carp pituitary to induce ovulation in females of Lithuanian line B. *Aquaculture Research* 32, 357-364.

Brzuska E. (2004) Artificial spawning of carp (*Cyprinus carpio* L.); differences between the effects of reproduction in females of Hungarian, Polish and French origin treated with carp pituitary homogenate or [D-Tle⁶,ProNHET⁹]GnRH (Lecirelin). *Aquaculture Research* 35, 1318-1327.

Brzuska E. (2005) Artificial spawning of carp (*Cyprinus carpio* L.): differences between females of Polish strain 6 and Hungarian strain W treated with carp pituitary homogenate, Ovopel or Dagin. *Aquaculture Research* 36, 1015-1025.

Demska-Zakes, K., Zakes, Z. (2002) Controlled spawning of pikeperch, (*Stizostedion lucioperca* L.), in lake cages. *Czech J. Anim. Sci.*, 47: 230-238.

Demska-Zakes, K., Zakes, Z., Roszuk, J. (2005) The use of tannic acid to remove adhesiveness from pikeperch, *Sander lucioperca*, eggs. *Aquacult. Res.*, 36: 1458-1464.

Horvath, L., Szabo, T., Burke, J. (1997) Hatchery testing of GnRH analogue-containing pellets on ovulation in four cyprinid species. *Pol. Arch. Hydrobiol.*, 44, 221-226.

Kaszubowski, R. (2005) Artificial reproduction of pikeperch under controlled conditions. *MSc thesis, UWM Olsztyn*, 30 pp (In Polish with English summary)

Muller, T., Taller, J., Nyitrai, G., Kucska, B., Cernak, I., and Bercesenyi, M. (2004) Hybrid of pikeperch, *Sander lucioperca* L. nad Volga perch, *S. volgense* (Gmelin). *Aquacult. Res.*, 35: 915-916.

Ronyai, A. (2007) Induced out-of-season and seasonal tank spawning and stripping of pike perch (*Sander lucioperca* L.). *Aquacult. Res.* 1-8.

Sosiński, M. (2007). The application of Ovaprim in artificial reproduction of pikeperch. *MSc thesis, UWM Olsztyn*, 30 pp (In Polish with English summary)

Wojnowska, E., (1998) New methods of rearing pikeperch (*Stizostedion lucioperca* L.) larvae. *MSc Thesis. Olsztyn University of Agriculture & Technology* (In Polish)

Zakes Z., Demska-Zakes K. (2005) Artificial spawning of pikeperch (*Sander lucioperca* (L.)) stimulated with human chorionic gonadotropin (hCG) and mammalian GnRH analogue with a dopamine inhibitor. *Arch. Pol. Fish.*, 13: 63-75.

Zakes Z., Szczepkowski M. (2004) Induction of out-of-season spawning of pikeperch, *Sander lucioperca* (L.). *Aquacult. Int.*, 12: 11-18.

The protocol for spawning activity after checking maturity stages of oocytes

- **Stage I.**

Females at this stage should be kept longer in water at temperature of about 13-15 °C or should obtain the initial injection from hCG at dose 150 – 300 IU kg⁻¹. Fish should be checked after 4 -5 days for evaluating oocytes maturation.

- **Stage II**

Fish in which oocytes are in this stage should be treated in the same way as in case of stage I. The next controlling of fish should take place earlier, after 2 – 3 days.

- **Stage II/III**

Fish at this stage should receive the normal doses of spawning agents. The spawning time in the temperature of 15 °C should be after 3.5 to 4.5 days.

- **Stage III**

Fish at this stage should receive the normal doses of spawning agents. The spawning time in the temperature of 15 °C should occur after 2.5 to 3.5 days.

- **Stage IV**

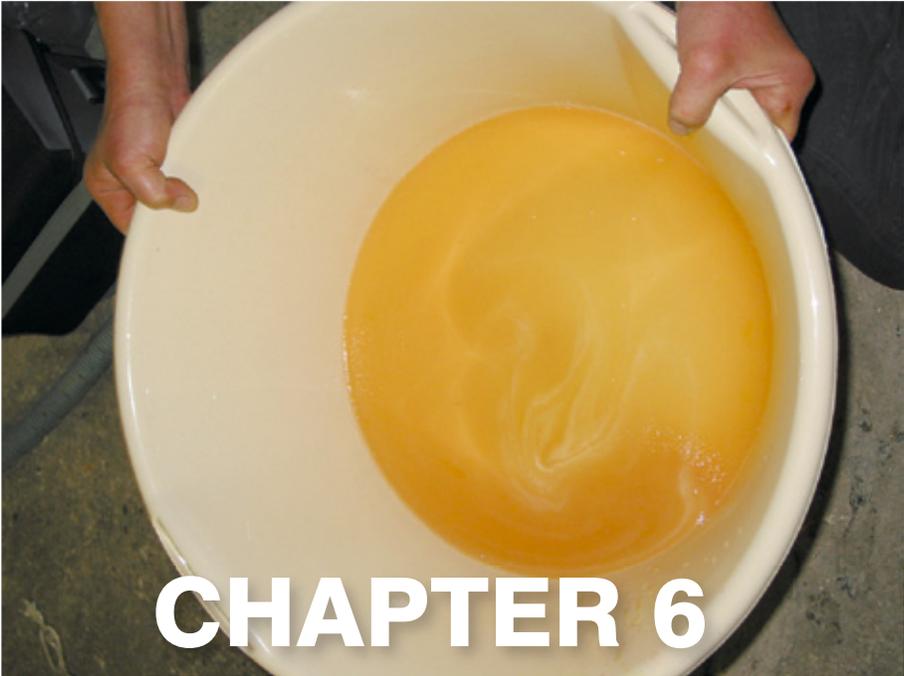
If the GV is visible and is near the external zone (membrane, III/IV stage) it is possible to apply spawning agents but in a smaller dose than usual, i.e. 200 – 300 IU kg⁻¹. If the GV is broken down and can not be seen, it is not necessary to apply hormones. Fish will usually spawn spontaneously after 10 – 20 hours.

- **Oocytes during ovulation**

When the oocytes are destroyed in Serra's solution, it usually means that they are ovulating. In this case, fish should be checked after 2 – 3 hours, and later (if necessary) also after every 2 hours.

- **Oocyte in different maturity stages**

If the oocytes taken from one female are in different stages, i.e. some of them in stage I, some of them in stage III, or GV migration is not synchronous with aggregation of oil droplets, such spawners should be omitted in this season. The biological quality of eggs obtained from such a female is usually very low.



CHAPTER 6

Obtaining gametes and short storage

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Chapter VI. Obtaining gametes and short storage

Collection of high quality gametes is one of the most important problems in modern aquaculture. Their quality might be influenced by many factors, but their sampling and short storage using a well worked out protocol is really important. It may be really responsible for high/low fertilization rate.

Collection of gametes

Pikeperch breeders must be handled very gently. They are very sensitive to shocks or temperature changes and may die during handling (Steffens et al. 1996, Kaszubowski 2005, Sosiński 2007). In this situation fish before handling should be anaesthetized with “propiscin” at a concentration of $2.0 \text{ cm}^3 \cdot \text{dm}^{-3}$ (or other solution applied in aquaculture as anaesthetics). While handling the fish, gentle firmness should be the rule. Covering the fish with a wet towel or cloth, will help to keep them calm (Fig.6.1.)

When the pikeperch female is ready to spawn, the eggs usually start to drop spontaneously from the genital pore. It is very well visible, because the colour of eggs is yellow (Fig.6.2).

Before stripping both the male and female should be cleaned and dried using a soft towel. Water should not be allowed to mix with the gametes as they are stripped. To strip the fish, the female should be held around the caudal fin with one hand, and a slight pressure to the abdomen should be done with the other hand. If ovulation has occurred, a stream of eggs will emerge. If there is a stream of eggs, the abdomen should be massaged from the front to the back to strip out all the eggs (Fig.6.3 and 6. 4).

After collecting the eggs the skin in female belly goes inside (Fig.6.5.) and looks empty. Eggs are usually collected in a small plastic basin (Fig.6.2, 6.3 and 6.4). The artificially stripped eggs usually wait for adding milt (Fig.6.6.).

The best way to obtain milt is collecting it using plastic syringes (Fig.6.7) or with a pipette (Steffens et al. 1996). Milt from individual males should be collected separately and later added to the eggs. Milt should be white without any pollution, especially blood and urine. It is also possible to strip milt directly onto the eggs. (Fig.6.8).

Ovulation time

The time of pikeperch females ovulation strictly depends on the oocytes maturity stage. The relationship between the oocytes maturity stage and ovulation time is presented in Fig.6.9. Fish are injected with a single dose of hCG (400 – 500 IU / kg). The water temperature is constant at 15 °C.

Short storage

The short-time storage of milt is not easy in practice, but this is a very useful tool in many cases, i.e. shortage of males when females are ripe. Freshly collected milt kept in plastic syringes, at temperature 0.0 – 4.0 °C is able to inseminate eggs even after three or four hours. But a very important thing is to keep contact with air or oxygen. Milt kept without air or oxygen will usually die very soon.



Fig.6.1. *Handling pikeperch female before obtaining the eggs.*

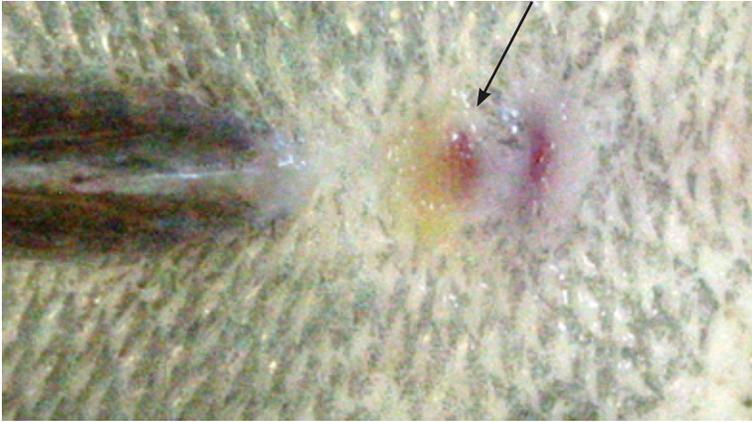


Fig.6.2. Eggs dropping spontaneously from pikeperch females. The arrow shows the eggs.



Fig.6.3. Stream of pikeperch eggs during stripping.



Fig.6.4. *Slight pressure of the pikeperch female belly during stripping of the eggs.*



Fig.6.5. „Empty” females after stripping eggs.



Fig.6.6. *Collected eggs in a plastic basin.*

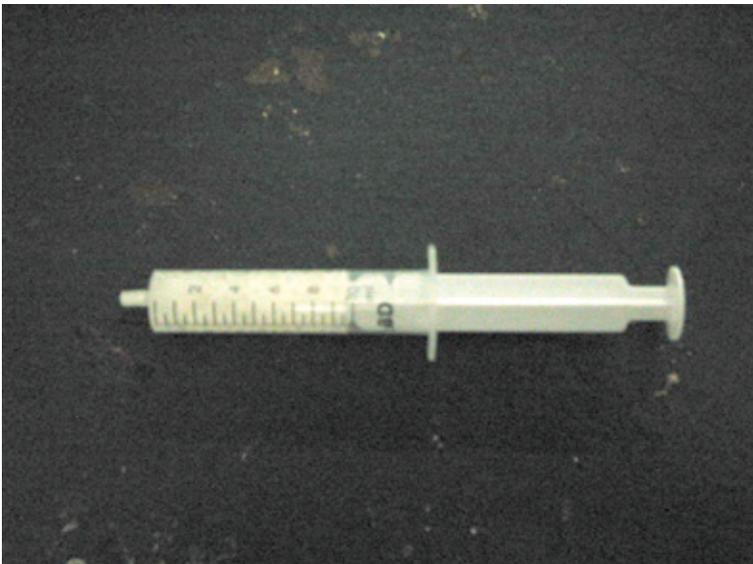


Fig.6.7. *Pikeperch milt in a syringe. It is possible to take a short storage of such collected semen for about one hour.*



Fig.6.8. Adding sperm to the pikeperch eggs.

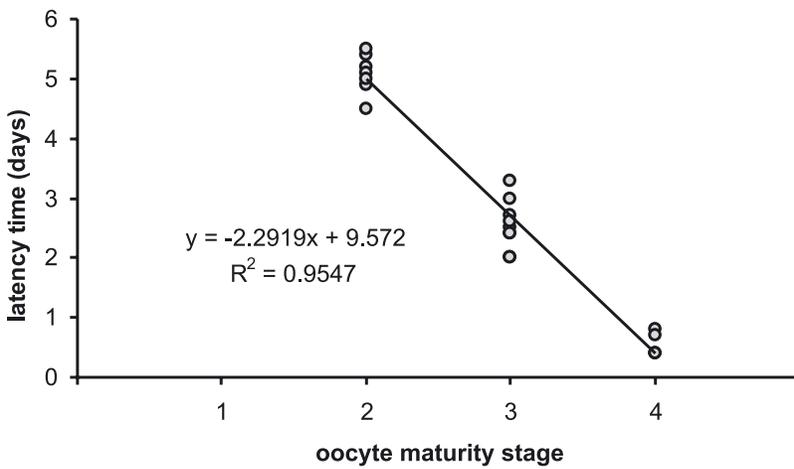


Fig.6.9. Relationship between the oocyte maturity stage and ovulation time.

If a farmer needs more time than one or two hours, the following guidelines are proposed for a short-term storage of pikeperch semen:

- preserved semen containers should be stored in a fridge at temperature 2-3 °C and should be oxygenated once a day.
- the best effects of storage will be obtained when milt is not diluted. In such conditions spermatozoa can be activated even after 10 days of storage. The level of depth of milt should not exceed 3 mm.

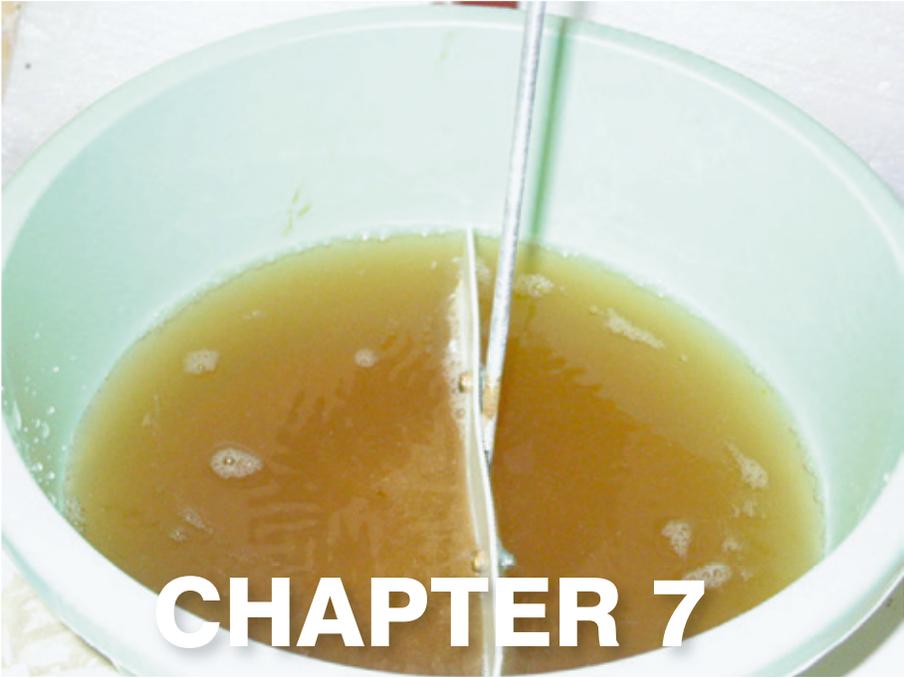
A lot of problems appear if the farmer needs to store the eggs. It is not possible to preserve eggs in the fridge. They should be kept at the same temperature conditions as during egg stripping. They also should be protected against drying. The maximum time for short storage, without the influence on egg survival, is usually about 30 min.

References

Kaszubowski, R. (2005) Artificial reproduction of pikeperch under controlled conditions. *MSc thesis, UWM Olsztyn*, 30 pp (In Polish with English summary)

Sosiński, M. (2007) The application of Ovaprim in artificial reproduction of pikeperch. *MSc thesis, UWM Olsztyn*, 30 pp (In Polish with English summary)

Steffens, W., Geldhauser F., Gerstner P., Hilge V. (1996) German experiences in the propagation and rearing of fingerling pikeperch (*Stizostedion lucioperca*). *Ann. Zool. Fennici.*, 33: 627 - 634.



CHAPTER 7

Egg fertilization and incubation

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Chapter VII. Egg fertilization and incubation

Obtaining eggs and milt of good quality is among the most important step in finfish artificial reproduction. Another important step is the egg fertilization and incubation. In many freshwater fish, the egg stickiness which occurs during egg fertilization, becomes a big problem. This is easily seen in the case of many cyprinids, i.e. common carp, common tench, goldfish, ide, as well as many other species: European catfish or northern pike. The problem is very well known in pikeperch aquaculture (Wojnowska 1998, Demska-Zakes et al. 2005, Kaszubowski 2005, Sosiński 2007). Sticky eggs are aggregated and their incubation in hatchery conditions, i.e. in Weiss jars, is not possible.

The most frequently, eggs of pikeperch are incubated on nests (Fig.7.1): in tanks, ponds or cages. Moving the nests with eggs to the hatchery usually influences egg survival, in a positive way in comparison to natural conditions, but these methods have to be further developed.

Fertilization of artificially stripped eggs

Pikeperch eggs, which are incubated in special hatchery units, must be fertilized artificially and then kept in un-sticking solution. Fertilization is a



Fig.7.1. Pikeperch eggs on the nest during incubation.



Fig.7.2. Fertilization of pikeperch eggs.

very simple method. Gametes, sampled using “dry method”, are mixed together, and a small amount of water from the hatchery unit is added (Fig.7.2). Then they should be mixed gently using a plastic spoon.

The time of mixing eggs, milt and water is short and depends on the type of un-stick solution. In Polish hatcheries it is usually about 5 min and 20 min in case of using tannin acid and talk with salt solution, respectively.

Un-sticking solution bathing

There are a few different kinds of un-sticking bath solutions, which might be used in pikeperch aquaculture (doses are presented in Table 7.1):

- Solution of talk and salt (NaCl). In this method the grey talk and salt is used.
- Tannin acid solution
- Enzyme bath

Application of un-sticking solution

After fertilization, the un-sticking solution should be added to the eggs (Fig.7.3). At this time eggs should be mixed manually or automatically (Fig.7.4, 7.5).

Table. 7.1. The tested doses (for 10 L of solution) of chosen bath solutions using in pikeperch aquaculture.

Un-stick solution	Bath composition	Time (min)	Survival of 3-days-old embryos (%)
Talk (Tk) and salt (S)	Tk – 40g S – 10g	20; 30; 40	6.4 ± 1.1; 4.7 ± 1.0; 0.0
Talk (Tk) and salt (S)	Tk – 80g S – 20g	20; 30; 40	3.7 ± 0.8; 1.6 ± 0.5; 0.0
Tannin acid	5 g	2; 5	56.1 ± 3.4; 62.2 ± 4.4
Tannin acid	10 g	2; 5	53.0 ± 4.3; 52.2 ± 5.3

For commercial aquaculture the application of tannin solution is recommended in dose 5 – 10 g /10 L (500 – 1000 ppm) for time 2 – 5 min, starting at 5 min after egg fertilization.

Within a few minutes, when the eggs are kept in a bath solution, they are changing colour (Fig.7.4 and 7.5) from yellow to light brown (in tannin) or white-grey (in talk). After the bath, the eggs should be rinsed with water from the hatchery, but pure water should be added slowly and mixed. Before moving the eggs to incubating chambers, the aquaculturist should be sure that the eggs stopped to be sticky. The best method for it is the “glass test”. Samples of eggs should be moved to the glass with water from hatchery. If the eggs are not sticky, they might be moved to the hatchery. In other case, the un-stick bath should be repeated, but for a shorter time.

Incubation of eggs

Fertilized eggs after un-sticking bath should be moved to incubation chambers. One of best for it are Weiss jars, routinely used in Polish hatcheries. The amount of eggs should not be too high: maximum 2-3 dm³ of hydrated eggs per 8 l jars (Fig.7.7).

The temperature of water should be kept between 15 – 18 °C and the water flows between 1 – 4 l / min in each chamber. The water flow depends on the type of chambers, amount of eggs and developmental stage of embryos. At the beginning, the water flow is usually lower than just before hatching. Dead eggs move to the top, so it is very easy to remove them from the incubation chambers.



Fig.7.3. Adding un-sticking solution to fertilized eggs of pikeperch.



Fig.7.4. Eggs in un-sticking solution. The beginning of the process.



Fig.7.5. Eggs in un-sticking solution. The end of the process.



Fig.7.6. Pikeperch eggs after bath in un-sticking solution.



Fig.7.7. Eggs incubated in Weiss jars in one of commercial hatcheries.

Incubation of eggs on nests

Eggs might be also incubated at the hatchery on nests. For this reason the nests should be placed in the tanks with slow water flow and aeration (Korolev & Tereshenkov 1985). Incubation on the nest usually induces more problems with egg health (see chapter 9).

References

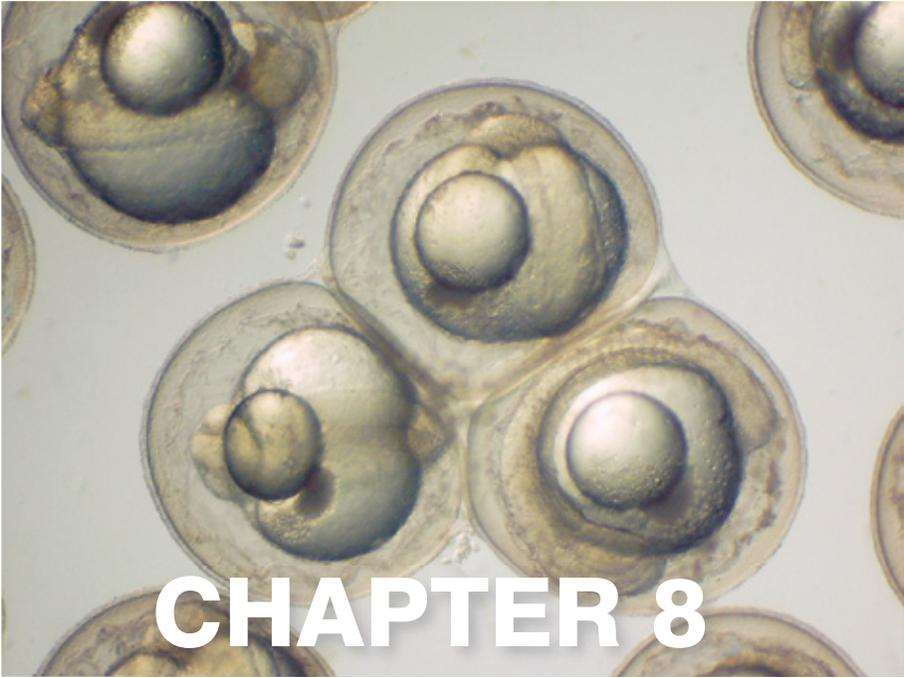
Demska-Zakes, K., Zakes, Z., Roszuk, J. (2005) The use of tannic acid to remove adhesiveness from pikeperch, *Sander lucioperca*, eggs. *Aquacult. Res.*, 36: 1458-1464.

Kaszubowski, R. (2005) Artificial reproduction of pikeperch under controlled conditions. *MSc thesis, UWM Olsztyn*, 30 pp (In Polish with English summary).

Korolev A.Y and Tereshenkov I.I. (1985) Incubation of pikeperch eggs in tanks. *Rybnoe Khozyaystvo*, 7: 31-32 (In Russian).

Sosiński, M. (2007) The application of Ovaprim in artificial reproduction of pikeperch. *MSc thesis, UWM Olsztyn*, 30 pp (In Polish with English summary).

Wojnowska, E., (1998) New methods of rearing pikeperch (*Stizostedion lucioperca* L.) larvae. *MSc Thesis. Olsztyn University of Agriculture & Technology* (In Polish).



CHAPTER 8

Hatching

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Chapter VIII. Hatching

In natural conditions spawning of pikeperch takes place at water temperature of 8-16 °C, depending on the geographical region or climatic conditions. This range seems to fit closely the temperatures in which the normal development of eggs until hatching is possible (Lappalainen et al. 2003). Data on the subject of optimal incubation temperature are slightly divergent and indicate that they range from 11.5 to 20 °C (Kokurewicz 1969, Kudrinskaya 1970, Muntian 1977). Within this temperature, the longest (4-5 mm) and most developed larvae hatched at 12-16 °C (Kokurewicz 1969). In practice the suitable temperature for hatching is between 15-17 °C. The incubation time until hatching can be estimated with the equations enclosed below, according to data from almost the whole range of pikeperch occurrence (Lappalainen et al. 2003):

$$DD = 1255 \times T^{1.07}$$

and

$$I = 30124 \times T^{2.07}$$

where **DD** is the incubation time of eggs needed for the onset of hatching in degree-days; **I** is the incubation time of eggs needed for the onset of hatching in hours, and **T** is the temperature in °C.

The hatching period is often extended in time (2-3 days) and can be accelerated by a short-term increase of temperature. According to Geldhauser (1992), it is advisable to reduce the incubation temperature by approximately 1°C per day shortly before hatching, to ensure a constant and full development before hatching. Thereafter the increase in temperature of 1 to 3 °C per day results in fast and complete hatching. Hatching success rate using this method is about 90% (Steffens et al. 1996). An alternative method to accelerate hatching is to reduce the oxygen supply by stopping the inflow of the incubators for a short time (15-25 min) a few hours before the expected hatching.

The hatching of fry obtained after artificial reproduction and incubated in Weiss jar is a little different than in case when eggs are incubated in the nest. It depends on the used un-stick bath solution. If the enzyme "protease" was used, the embryos hatch rapidly, without any problems. Sometimes they start to hatch too early. On the contrary, if the tannin acid bath was used, embryos face hatching problems. The egg zone is

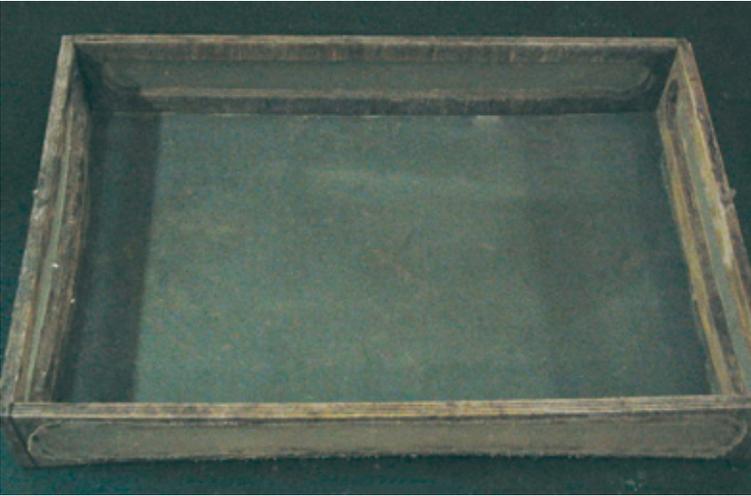


Fig.8.1. A wooden frame with net for hatching pikeperch.



Fig.8. 2. The frame from the other side.

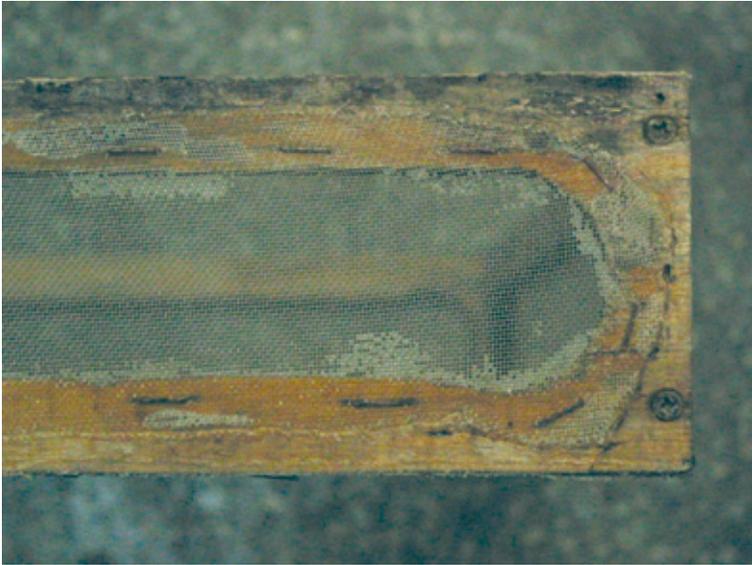


Fig.8.3. *The view of the wooden frame yet another side.*

very strong. If the method described above does not work, eggs should be moved from the incubation chambers and put on floating wooden frames, with the bottom made of net (mesh screen about 1 mm) (Fig.8.1, 8.2 and 8.3). The hatching of embryos should be accelerated by increasing the temperature by 2 – 3 °C. Sometimes, when tannin acid is used to avoid egg adhesiveness, embryo eyes are pigmented. This is unusual situation, because normally pikeperch larvae hatched before eyes pigmented stage.

The eggs are bigger than mesh size, so before hatching eggs are placed on the net. Hatched fry are smaller than mesh size and larvae can swim out from the frame. Usually two times per day, the non hatched eggs are moved out from the frame, slightly shake and put back on the frame.

As soon as the hatching begins (Fig.8.4), environmental parameters should be reset according to the indications presented in Annex 8.1. During the hatching newly hatched larvae should be siphoned from the incubators into holding tanks having high oxygen concentrations. Stocking density in holding tanks may be up to 15 000 larvae/L. (Steffens et al. 1996). It should be noted that newly hatched larvae of pikeperch have very positive phototropism resulting in crowding at high densities (Proteau et al. 1993). This can be neutralised by appropriate colouration of the walls of holding tanks or moderate illumination (about 100 lx). In darkness larvae do not swim, but



Fig.8.4. *The beginning of hatching.*

often rest on the bottom, vertical movement could only be initiated when a light source is provided. Larvae should remain in the holding tanks c. 3-4 days (depending on temperature), then moved to the rearing units about 1 day prior to disappearance of the yolk. During this period the holding tanks should be siphoned in order to remove empty egg shells and dead larvae once or twice a day. The survival rate from hatching to the end of yolk resorption depends mainly on viability of larvae and/or environmental conditions. It varies on average from 80 to 90%. Positive phototaxis of larval pikeperch has been also utilized in methods of rearing in illuminated cages (Gensch 1979, Schlumpberger and Zieborh 1981, Jancke 1989).

Viability of newly hatched larvae

One day after hatching the viability of larvae (Fig.8.5) should be assessed. From each batch at least 10-20 specimens are sampled (the procedure is described in detail in Annex 8.1), and are then placed on a watch glass in water and observed under the stereomicroscope at low magnification (5 and 10x).

They are checked for normality in respect of:

- overall shape and dimensions;
- disposition of internal organs;

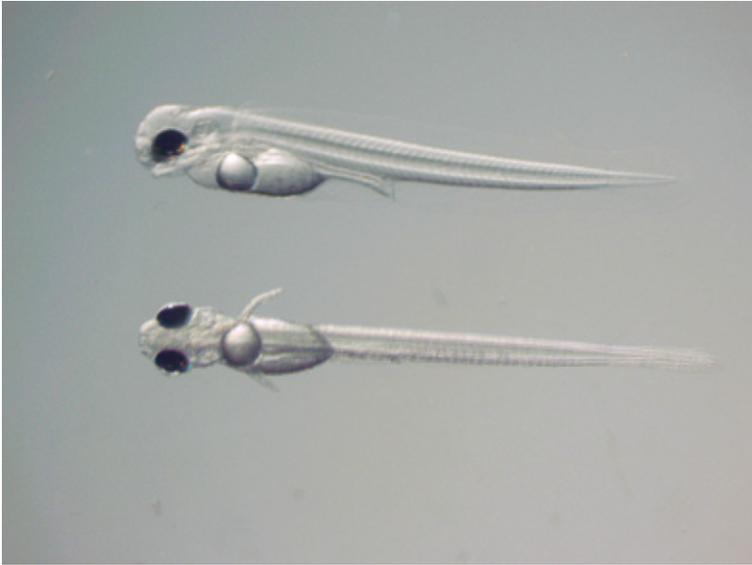


Fig.8.5. Pikeperch larvae shortly after hatching.

- integrity of larval (primordial) fin, that should not present malformations and/or erosions;
- absence of external parasites.

On the first day after hatching pikeperch larvae show a typical behaviour and their observation contributes to evaluate their viability and condition. Examination of this behaviour can take place directly in the holding tank, as well as on a sample of at least 50 larvae taken in a transparent container. The larval motion occurs in a peculiar way, which is called 'candle-swimming' – they swim upwards in a spiral for a few seconds, and then sink slowly head downwards. A few days later (c. 2-3) they start to swim horizontally. Larvae showing a different pattern (being totally passive or hyperactive), reveal poor viability and should be eliminated.

References

Geldhauser F. (1992) Practical aspects of the embryonic and larval development of pike-perch (*Stizostedion lucioperca* L.). In: *Proceedings of the Scientific Conference on Fish Reproduction, Vodnany, 2–4 March, 1992*, pp. 65–69.

Gensch R. (1979) Ergebnisse und Erfahrungen beim Vorstrecken von Fischbrut in beleuchteten Gazekäfigen in VEB Binnenfischerei Frankfurt/Oder. *Zeitschrift für die Binnenfischerei der DDR*, 26: 38-41.

Jancke G. (1989) Erfahrungen beim Erbrüten und Vorstrecken von Hecht, Zander und Maräne. *Zeitschrift für die Binnenfischerei der DDR*, 36: 27-33.

Kokurewicz B. (1969) The influence of temperature on the embryonic development of the perches: *Perca fluviatilis* L. and *Lucioperca lucioperca* (L.). *Zoologica Poloniae* 19: 47-67.

Kudrinskaya O. (1970) The effect of feeding and temperature factors on growth, development and survival of larval pikeperch and perch. *Voprosy Ikhtologii*, 10: 1035-1046. (In Russian with English summary).

Lappolainen J., Dörner H., Wysujack K. (2003) Reproduction biology of pikeperch (*Sander lucioperca* (L.)) – a review. *Ecology of Freshwater Fish* 12: 95-106.

Muntian S.P. (1977) Effect of constant incubation temperatures on hatching and the morphological characteristics of pike perch embryos. In: Karzinkin G. S., (ed.) *Metabolism and biochemistry of fishes*. New Delhi: Indian National Scientific Documentation Centre, pp. 214-221.

Salminen M., Ruuhijärvi J. 1988. Kuhan mädinhankinnan ja -haudonnan uudet niksit. *Suomen Kalastuslehti* 4: 176-180 (In Finnish).

Schlumpberger W., Zieborth G. (1981) Produktion von vorstrecken Zandern in beleuchteten Gazekäfigen. *Zeitschrift für die Binnenfischerei der DDR*, 5: 143-144.

Schlumberger O., Proteau, J.P. (1996) Reproduction of pike-perch (*Stizostedion lucioperca*) in captivity. *Journal of Applied Ichthyology* 12: 149-152. pp. 42-47.

Steffens W., Geldhauser F., Gerstner P., Hilge V. (1996) German experiences in the propagation and rearing of fingerling pikeperch (*Stizostedion lucioperca*). *Ann. Zool. Fenici* 33: 627-634.

Virbickas J., Gerulaitis A., Misiūnienė D., Sinevicienė D. (1974) Biology and Fishery of the Pike-Perch in the Water Bodies of Lithuania. *Vilnius: State publishing house 'Mintis'*, 277 pp. (in Russian with English summary).

Woynarovich, E. (1961) Die künstliche Erbrütung des Zanders. *Zeitschrift für Fischerei X (N.F.)*: 677-680 (in German).

Wundsch, H.H. (1973) Barsch und Zander. *Die Neue Brehm-Bücherei*. Wittenberg: A. Ziemsen Verlag, 76 pp (in German).

8.1. Annex: sampling eggs or larvae

1. Prepare on a tray four sterilized Petri dishes and one 100 ml beaker, note-pad and pencil.
2. Take four samples of 100 ml water from any point of the larval tank at least 10 cm away from the centre and from the walls.

3. Transfer larvae of each sample to a Petri dish; record normal/abnormal larvae on a specific form.
4. Empty the Petri dishes and repeat steps (2) and (3) at least five times, in order to sample at least two liters of tank water.
5. Calculate average and multiply by 10 to obtain average density of eggs/larvae per liter.

Alternative method

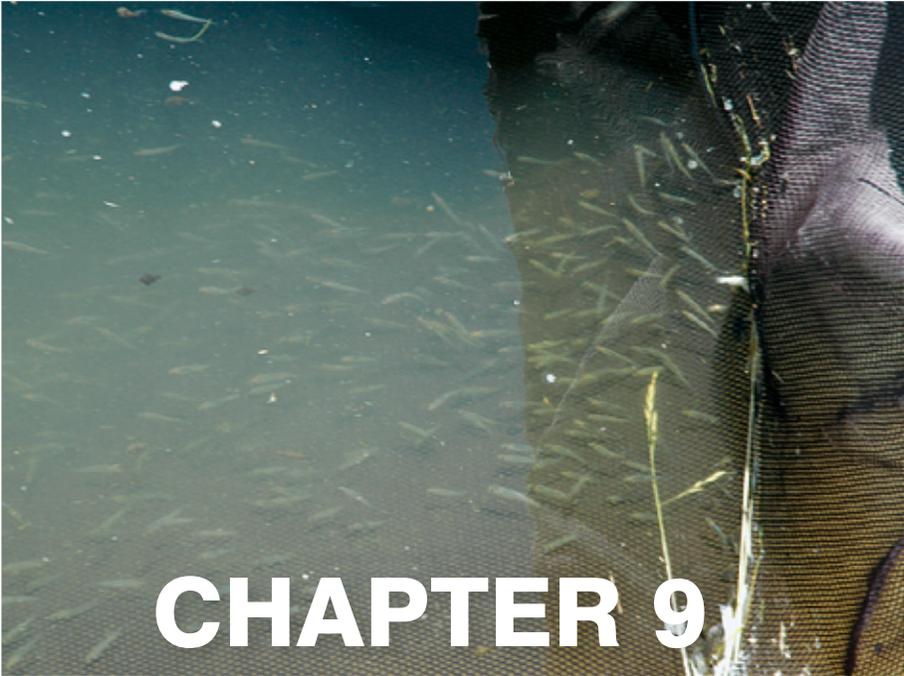
1. Prepare one 1l glass beaker (clean and sterilized), one 100 ml glass beaker and three Petri dishes.
2. Take a 1-l sample; stir it carefully before quickly taking one 100-ml sub-sample; repeat such sub-sampling procedure twice.
3. Transfer the three sub-samples to Petri dishes and allow time to settle.
4. Count larvae or eggs.
5. Repeat (2), (3) and (4) at least five times, sampling in different tank points.
6. Obtain the average density as above.

NOTE

When sampling newly hatched larvae:

- prior to sampling adjust aeration so as to evenly distribute the larvae in the whole tank water volume;
- examine larvae visually in the Petri dishes when the samples have settled, over a black surface;
- to check more carefully for abnormalities prepare a sample on a slide, avoiding as much as possible physical shocks (such as filtering or repeated pipetting) in order to preserve body integrity, then observe under the microscope;
- a more practical way consists of preparing a slide directly from each 100 ml sub-sample.

Examine at least 20 larvae (an optimal number would be 50) and in any case consider the whole sample to avoid any form of selection.



CHAPTER 9

Artificial pikeperch propagation – veterinary purposes

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Chapter 9. Artificial pikeperch propagation – veterinary purposes

The problem with fish health is one of the most important in modern aquaculture. This is especially true in pikeperch culture, because these fish are very sensitive and might die from many different causes.

The role of hatcheries in pathogen transmission

Intensive fish production creates conditions which make the transmission of pathogens between fish easier. Weak resistance induced by stress and high density of fish stock allows pathogens to grow rapidly and to



Fig.9.1. *Pikeperch male under the nest.*

settle new fish easily. Decreased food utilization, growth impairment and fish mortality caused by pathogens belong to one of the main sources of economical losses of fish farmers across the world.

Hatchery is a very specific place regarding fish pathogen transmission:

1. using of spawners or eggs of outside origin (from the wild or from other farms) increases the possibility of entrance of new pathogens inside the hatchery;
2. fish larvae and young fish are highly susceptible to any transmissible disease due to not fully developed immune system;
3. hatchery product – young fish or eggs – can be a very important source of fish pathogens for numerous fish farms. It is important to be aware that hatcheries can play a key role in dispersing some important (dangerous) fish diseases.

It means, looking from the other side, that elimination of health threats in hatcheries can be an important mean of successful controlling of fish diseases. In pikeperch aquaculture only fish without any health problems might be used for reproduction (Fig.9.1.).

Having one's own brood stock is the best solution for fish hatcheries from the veterinary point of view. It enables regular examinations of spawners' health status, prophylaxis (diet supplementation before spawning, vaccinations, biostimulation etc.) and decreases the stress impact on fish. The immune system of spawners reared in the hatchery is ready to fight against the autochthonic micro organisms and parasites. This immunocompetency can be passed to young larvae *via* eggs and protect them till their own immune system matures.

However, in case of pikeperch reproduction, wild spawners are used very commonly. The choice of the best available source of fish for spawn is of crucial importance. First of all, the hatchery owner should ensure that the future spawners' area of origin is "clear" regarding to infectious diseases. The veterinary "certificate of origin" is, to some extent, a guaranty that no dangerous infectious fish disease was noticed on the area of their living.

The second important point to consider is the experience of fishermen we are going to obtain fish from. As the collection of the fish is a source of stress and potential damage of body surface, the method and equipment giving the lowest impact on fish should be applied. Stress reaction during catching and stocking of spawners leads to strong depletion of fish resistance. The damaged skin is a gate of pathogen entrance. The coexistence of these two factors always results in protozoan, bacterial or fungal infection (Fig. 9.2.)



Fig.9.2. *The typical fungal infection in pikeperch.*

The transport of the spawners to the hatchery is another source of stress and possible damage to fish. The duration of transport is very important as the longer transport usually causes more injuries and stronger stress reaction in fish. However, regarding to above deliberations, sometimes it is better to choose a more distant location to obtain fish of better quality.

To prevent body damage, the sedative concentrations of anaesthetics can be used during wild pikeperch transportation. The best results were achieved with etomidate (Hypnomidate, Propiscin) in concentration 0.02 – 0.2 mg/l of active ingredient. In contrast to other anaesthetics, etomidate inhibits the production of cortisol (one of the stress hormones) in fish body and so it can, to some extent, decrease the stress reaction.

Another method to minimize losses caused by transport stress is the addition of 0.05 – 0.2% NaCl (table salt) (0.5 – 2.0 g/l) to the transport water. Some practitioners reported good results in higher concentrations, up to 0.5% (5 g/l) or even 1.0% (10 g/l). Pikeperch is quite resistant to increased water salinity (some populations live in brackish waters, i.e. in the Baltic Sea). However, one should be very careful using the higher salt concentrations due to a possible harmful effect on fish gills and osmoregulatory processes.

Decrease of Cl⁻ ions concentration in blood plasma is one of the effects of stress reaction in fish (Schreck, 1981). Addition of salt during the transport acts by enabling fish to restore physiological concentration of chlorine in plasma and thus to decrease energy loss, which results in



Fig.9.3. *Pikeperch female during anaesthetic bath with salt addition.*

a lower mortality rate. So, for this reason, salt should be added during many manipulations with fish, i.e. before applying hormonal stimulation (Fig.9.3.).

Every fish introduced into the hatchery should be regarded as a potential source of pathogens. The hatchery owner should provide conditions preventing direct or indirect (by water, equipment or by staff) contact of this fish with other hatchery stock fish. As the wild pikeperch spawners usually stay in the hatchery only through the spawn period, they should be kept in separate tanks or ponds. Effluents shouldn't contact other fish or should be disinfected (by ultraviolet radiation or ozone). Equipment used for the spawners (nets, buckets, rubber tubes etc.) can not be used for other fish and should be regularly disinfected.

There are a lot of preparations (chemicals) for disinfection on the European market. In most countries, substances like sodium hypochlorite, hydrogen peroxide and peracetic acid, chloramin T, potassium permanganate, formalin, quaternary ammonium compounds, or iodophores are allowed for aquaculture purposes (see Tab. 9.1.). One should remember to rinse the disinfected equipment with clean tap water very carefully.



Fig.9.4. *Dead pikeperch male over the nest with developing eggs.*

As dead fish can be a very serious source of pathogenic bacteria, viruses or other pathogens, it should be removed from the water as quickly as it is possible. Dead fish should be destroyed according to sanitary regulations relevant for a given country. During pikeperch reproduction, especially in ponds or cages, dead breeders are sometimes kept for a few days with developing eggs. In some cases, died males are located directly on the nest (Fig.9.4.).

Manipulations, necessary in the artificial pikeperch propagation, like injections of spawning agents, checking eggs maturity or eggs stripping, induce strong stress reaction and cause skin destruction. Proper performing of these procedures is the best preventive measure and usually results in very low or none mortality (for details see earlier chapters).

The narcotic concentrations of anaesthetics are usually used to facilitate all the above procedures and to decrease damages. None anaesthetic is registered for fish in EU. However, preparations containing etomidate are registered for use in other animals and in human. These substances can be used in “out of label” manner for veterinarians prescription. Etomidate is used in 2.0 – 4.0 mg/l concentration and produces enough and safe anaesthesia for fish manipulation (Kazun and Siwicki 2001). Note; etomidate does not fully eliminate the sense

of pain, all procedures should be done very cautiously (Brown 1988). Note; the recovery time after etomidate anaesthesia can be quite long. One can shorten it by managing gentle flow of clear water (without anaesthetic) through the oral cavity for a few minutes before releasing fish back to the tank.

Another promising anaesthetic for fish is clove oil (Keene et al. 1998, Hamáčková et al. 2001). It is approved for use as food supplementary substance so it should be safe for people. In concentration 30 - 60 mg/l, clove oil produces deep anaesthesia but its therapeutic index is very low (1.27) – it means that clove oil isn't safe drug for fish and its concentration in anaesthetic bath should be measured very carefully (Velišek 2005). Note; the strong excitation can occur just after dipping pikeperch into the anaesthetic solution.

After eggs or sperm stripping, local fungal or bacterial infection occurs on the abdominal area of the body very often. Local infection on small, limited body surface can be treated with 1% water solution of gentian, administered directly onto the changed place with cotton gauze and tweezers. Mould infections of high percentage of body surface are usually mortal for pikeperch.

To prevent generalized bacterial infection after spawning, oxytetracycline treatment is used in some hatcheries. The drug is administered via a catheter (4 – 5 mm in external diameter) directly to the fish stomach immediately after egg or sperm stripping. The drug, in powdered form, is added to loose paste (prepared earlier by mixing clean tap water and wheat flour) to reach the concentration 100 mg of oxytetracycline in 1 cm³.

Above mixture is administered to fish in dose of 1 cm³ per 1 kg of body weight. Fish should be still anaesthetized. The catheter is introduced gently through the oral cavity to the gullet and next to the stomach. The needed volume of the mixture is slowly administered by a syringe. The catheter is removed after several seconds. The treatment should be repeated for 5 days. Fish should be anaesthetized every time before drug administration.

Oxytetracycline is an antibiotic substance effective against a broad spectrum of bacteria species. However, many strains of bacteria are resistant to oxytetracycline. Other antibiotics can be used according to a veterinarian prescription.

References

Brown L.A. (1988) Tropical fish medicine. Anesthesia in fish. *Veterinari Clinic North American Small Animal Practice*, 18, 317-330.

Hamáčková J., Sedova J.M., Pjanova S.V., Lepičová A. (2001) The effect 2-phenoxyethanol, clove oil and Propiscin anaesthetics on perch (*Perca fluviatilis*) in relation to water temperature. *Czech J Anim Sci* 46: 469-473

Kazuň K., Siwicki K. (2001) Propiscin – a safe new anaesthetic for fish. *Arch. Pol. Fish.* Vol. 9 (2); 183-190.

Keene, JL, Noakes, DLG, Moccia, RD, Soto, CG. (1998) The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquacult. Res* 29: 89-101.

Schreck C.B. (1981) Stress and compensation in teleostean fish: response to social and physical factors., pp 295. *In Pickering A. D. (Ed.), Stress and fish. Academic Press, London.*

Velišek J., Svobodova Z., Piačkova V. (2005) Effects of Clove Oil Anaesthesia on Rainbow Trout (*Oncorhynchus mykiss*). *Acta Vet. Brno* 74: 139-146.

Table 9.1. *Disinfectants used in aquaculture*

compound	concentration	time	application
chloramin T	30 g /l	5 min	foot bath, plastic and glass equipment
sodium hypochlorite	100 ppm	10 min	hand nets, cages, boats, tanks, plastic and glass equipment
formaline	5 %	10 -20 min	hand nets, glass equipment
iodophor	100 – 300 mg /l	10 min	nets, foot bath, glass equipment
peracetic acid and hydrogen peroxide mix.	0,4%	5 min	tanks, boats, glass equipment
potassium permanganate	5 g/l	10 – 20 min	tanks, glass equipment
quarternary ammonium compounds	125 ppm	5 min	plastic and glass equipment



CHAPTER 10

Summary and conclusions

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Chapter 10. Summary and conclusions

The manual “Artificial reproduction of pikeperch” was prepared as a part of EC funded project: **“Improving pikeperch larval quality and production by broodstock management and nutrition, husbandry and sex control”**, No. COOP-CT-2005-017646. It is a kind of compilation the original data obtained during the project, as well as, published knowledge. For now, the most of marked-size pikeperch come from open waters: lakes, rivers, ponds or lagoons. Such collection of fish influenced to high fluctuations of their availability on the market, meat quality, as well as, their market price. The application of the knowledge of this manual gives the possibility to preparing artificial reproduction of pikeperch on industrial scale. But reproduction is only one of the aspects of pikeperch intensive culture.

Many different researches were done in many aspects of pikeperch culture (Mamcarz et al. 1997, 1998, Skrzypczak et al. 1998a, 1998b, Demska-Zakes & Zakes 2002, Demska-Zakes et al. 2005). In many countries, i.e. in Poland, the natural propagation method of this species is usually applied. The results of production fry using this method are highly fluctuated. For this reason some modification of pikeperch reproduction are



Fig.10.1. *Before application of hormones the fish should be weighted.*



Fig.10.2. *Adding the sperm to pikeperch eggs.*

earlier done (Demska-Zakes & Zakes, 2002), but reliable data about fully artificial spawning was still needed. This lack of complete information was changed by this manual.

As the results of these and many other researches, mainly in collaboration with fish farms, the new facilities for percids culture are built in France, Netherlands, Ireland or Denmark. Generally, these new fish farms used closed water system. But successful culture requires developing detailed biotechnology of reproduction. One of the main problems in pikeperch culture is obtaining high quality gametes and later larvae. These are determining the culture success.

The artificial propagation of pikeperch is not easy. There are many bottlenecks in artificial reproduction of pikeperch, i.e. hormonal stimulation, obtaining gametes or removing eggs adhesiveness or high mortality of spawners. This create to develop new methods of dissolved these problems (Demska-Zakes et al. 2005). In the present manual, the most of information are detailed described but for successful reproduction of pikeperch also the own expertise of farmers are very important. On the other hand, also the genomic manipulation was applied to improving pikeperch culture. The intensive research on sex differentiation and sex reversal was carried out in case of pikeperch (Demska-Zakes & Zakes 1997). This last one was concerning the masculinization (Demska-Zakes & Zakes 1999) and feminization (Targońska-Dietrich 2001). One of other



Fig.10.3. The hatchery used for pikeperch reproduction. Fish Farm Pasłek, Poland.

method is crossbreeding between pikeperch and Volga perch (Muller et al. 2004). The hybrids were growth slower than pikeperch, but were less sensitive to some environmental factor, i.e. dissolved oxygen or stress and handling.

The data described at this manual present whole process of pikeperch artificial reproduction under controlled conditions. Obtaining good



Fig.10.4. The view of the pikeperch farm. Excel Fish, The Netherlands.



Fig.10.5. The pikeperch summer fry.

quality gametes and larvae is the first step of production this species on mass scale. But still is needed the complete data about intensive rearing of pikeperch larvae and juveniles in captivity.

References

Demska-Zakes, K., Zakes, Z. (1997): Effects of 17 α -methyltestosterone on gonadal differentiation in pikeperch, *Stizostedion lucioperca* L. *Aquacult. Res.*, 28: 59-63.

Demska-Zakes, K., Zakes, Z. (1999): Effect of 11 β -hydroxyandrostendione on pikeperch *Stizostedion lucioperca* (L.). *Aquacult. Res.*, 30: 731-735.

Demska-Zakes, K., Zakes, Z. (2002): Controlled spawning of pikeperch, (*Stizostedion lucioperca* L.), in lake cages. *Czech J. Anim. Sci.*, 47: 230-238.

Demska-Zakes, K., Zakes, Z., Roszuk, J. (2005): The use of tannic acid to remove adhesiveness from pikeperch, *Sander lucioperca*, eggs. *Aquacult. Res.*, 36: 1458-1464.

Mamcarz, A., Kucharczyk, D., Kujawa, R., Skrzypczak, A. (1997): Influence of fish density on survival, growth and cannibalism development of pikeperch (*Stizostedion lucioperca* L.) larvae. In: *Martinique 97. Island Aquaculture and Tropical Aquaculture. Short communications and abstracts. Les Trois-Ilets, Martinique, French West Indies, May 4-9, 1997.*, edited by L.Creswell, et al, pp. 343-346. *European Aquaculture Society, Oostende.*

Mamcarz, A., Kucharczyk, D., Skrzypczak, A., Kujawa, R. (1998): A new rearing technique of pikeperch (*Stizostedion lucioperca* L.) larvae using *Paramecium aurelia* as live food. *Europ. Aquacult. Soc., Spec. Publ.*, 26: 191-192.

Muller, T., Taller, J., Nyitrai, G., Kucska, B., Cernak, I., Bercesenyi, M. (2004): Hybrid of pikeperch, Sander lucioperca L. and Volga perch, *S. volgensis* (Gmelin). *Aquacult. Res.*, 35: 915-916.

Skrzypczak, A., Kucharczyk, D., Mamcarz, A., Kujawa, R., Furgala-Selezniow, G. (1998a): A new breeding technique of pikeperch (*Stizostedion lucioperca*) in net cages. *Czech J. Anim. Sci.*, 43: 398-398.

Skrzypczak, A., Mamcarz, A., Kucharczyk, D., Kujawa, R. (1998b): Use of floating pump to collect and transfer live zooplankton as food for percid larvae reared in net cages. *Progve Fish.-Cult.*, 60: 239-241.

Targońska-Dietrich, K. (2001): Pikeperch (*Stizostedion lucioperca*) feminization by diethylstilbestrol (DES). *MSc Thesis, Warmia and Mazury University in Olsztyn (in Polish)*.