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REVIEW PAPER

# Controlled reproduction of pikeperch *Sander lucioperca* (L.): a review

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**Abstract.** Mastering techniques for conducting the controlled reproduction of a given fish species, the procedures for performing hormonal stimulation, and the choice of the appropriate hormonal preparation and dosage all have a significant impact on the effectiveness of spawning, and, in turn, on the quantity of stocking material produced. This paper presents reproduction techniques for pikeperch, one of the most economically valuable species of freshwater European fish. The methods discussed include cage spawning, the artificial reproduction of wild fish (obtained from the natural environment), and out-of-season spawning. The principles of stimulating spawning with either photothermal or hormonal methods are also described. It is demonstrated that using *in vivo* methods for determining the maturation stage of oocytes and hormonal stimulation (especially in less mature females) permits increasing cage and artificial spawning of wild fish. Among the hormones and hormonal preparations tested to date in the artificial spawning of pikeperch (gonadotropins (GtH), gonadoliberins (GnRH), and synthetic analogues (GnRHa)), the most effective have been carp pituitary extract (CPE) and human chorionic gonadotropin (hCG). This paper also presents the most recent techniques used in the production of pikeperch stocking material.

**Keywords:** pikeperch, cage spawning, artificial reproduction, out-of-season spawning, hormonal stimulation

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## Introduction

Pikeperch, *Sander lucioperca* (L.), is a highly valued fish in Europe thanks to the organoleptic qualities of its meat (Dil 2008). The supply of pikeperch, however, has decreased drastically, and in the past fifty years catches of it from natural waters has declined four-fold (FAO 2007). This species is also very popular among recreational fishers (Bninska and Wolos 2001). Consequently, the demand for pikeperch stocking material for both stocking programs in natural waters and for intensive culture is continually increasing. Pikeperch is currently being cultivated in several European fish farms in recirculation systems (RAS) using commercial feed (Philipsen 2008). The increasing demand for stocking material and the highly variable effectiveness of methods used to date for the artificial reproduction of pikeperch have prompted several groups of scientists to study and then develop more effective methods for reproducing this species, and, as a consequence, increase the production of stocking material.

The challenges presented by the controlled reproduction of pikeperch have long inspired ichthyologists. The first reports of attempts to obtain the sex products of this species date to the nineteenth century (Sakowicz 1928). However, to date the methods usually used to accomplish this are either natural spawning or semi-natural spawning conducted in earthen ponds (Wojda et al. 1994, Steffens et al. 1996, Wojda 2006). Fertilized pikeperch eggs are

also obtained by deploying various types of artificial nests in the natural spawning grounds of this species (Korycki 1976, Horváth et al. 1984). Another way to obtain eggs is to conduct pikeperch spawning in earthen ponds equipped with spawning nests made of various materials (Horváth et al. 1984, Wojda et al. 1994, Cieśla et al. 1996, Wojda 2006). Spawning nests are also deployed in a rare method of pikeperch reproduction known as lake cages (e.g., Kiełczewski 1939, Terlecki 1955, Korycki 1976, Antila et al. 1988, Salminen et al. 1992, Craig 2000, Demska-Zakęś and Zakęś 2002). Prior to stocking the ponds or cages with fish they are stimulated with hormonal preparations; this procedure is usually performed on females (Horváth et al. 1984).

The history of fish reproduction aided by hormonal stimulation dates to the early twentieth century. This is when fresh (and slightly later – preserved) fish pituitary extract was used to stimulate reproduction (Houssay 1930). Despite the passage of time, this pituitary extract homogenate (mainly from carp, *Cyprinus carpio* L.) remains one of the most commonly applied hormonal preparations in aquaculture (Zohar and Mylonas 2001). The main active ingredient in these is gonadotropins (GtH), which plays a key role in the stimulation of the synthesis of the sex steroids, gamete maturation, and ovulation and spermiation. In addition to carp pituitary extract (CPE), human chorionic gonadotropin (hCG) is also used successfully in fish reproduction. Other gonadotropins, such as pregnant mare serum gonadotropin (PMSG) or composite preparations containing pituitary and placental GtH have not found wider applications. It should be mentioned that PMSG is commonly used in the reproduction of domesticated animals such as cattle, sheep, and rabbits. This preparation has a similar influence as do the pituitary gonadotropins of FSH (follicle stimulating hormone) and LH (luteinizing hormone).

The development of studies in the field of veterinary endocrinology and reproductive techniques has spurred interest in the gonadoliberins (GnRH, formerly LHRH). This hypothalamus hormone (decapeptide) controls the pituitary gland and stimulates it to secrete GtH. In aquaculture, applications have also been found for natural gonadoliberins (mammalian – mGnRH or salmon – sGnRH), as well as their synthetic analogues (GnRHa/LHRHa) (Peter et al. 1993, Crim and Bettles 1997). The latter often differ from the natural GnRH amino acid sequence (especially in position 6), the length of the peptide chain (they can be nonapeptides) as well as in activity. The most frequently used synthetic GnRH analogues include the following: D-Ala$^{6}$Pro$^{9}$NEt-mGnRH; D-Ala$^{6}$desGly$^{10}$-mGnRH; D-Phe$^{6}$-mGnRH; D-Trp$^{6}$-mGnRH; D-Arg$^{6}$Pro$^{9}$NEt-sGnRH; D-Lys$^{6}$-sGnRH. In some fish species, the introduction of exogenous GnRH causes increased dopamine secretion that inhibits the effects of gonadoliberins (Peter et al. 1993, Zohar and Mylonas 2001). The negative influence of dopamine can be counteracted by applying dopamine receptor inhibitors (e.g., metoclopramide, domperidone, reserpine, pimozide). These types of substances, besides GnRH/GnRHa, are also included in mixed preparations. One of the most widely applied mixed preparations is Ovopel (D-Ala$^{6}$Pro$^{9}$NEt-mGnRH and metoclopramide; Horváth et al. 1997).

The aim of this paper is to summarize the most important achievements in controlled pikeperch reproduction techniques, the principles of stimulating spawning, the application of hormonal preparations, and wide-ranging hatchery techniques for this economically and ecologically important species.

## Spawning in cages

Pikeperch is an atypical species among European freshwater fish because it spawns in nests, and this peculiarity was exploited in techniques of controlled reproduction in cages. The development of this reproduction method also exploited the plasticity of this species with regard to the substrate on which it releases its eggs; pikeperch uses either mineral (sand, gravel) or vegetation substrates (Schlumberger and Proteau 1996, Lappalainen et al. 2003). Vegetation substrate is mainly used to build spawning nests for lake cages reproduction (Steffens et al. 1996, Zakęś and Demska-Zakęś 2001, Zakęś

2009). Pikeperch spawners are caught during the pre-spawning season or at spawning grounds. Depending on latitude, the natural spawning period ranges from April to June (Korycki 1976, Ruuhijärvi and Hyvärinen 1996, Lappalainen et al. 2003). It is recommended to use trap gear (fyke-nets) to catch spawners (Antila et al. 1988, Ruuhijärvi and Hyvärinen 1996, Demska-Zakęś and Zakęś 2002).

Spawning cages are constructed with frames built of wooden slats, plastic pipes, metal rods, and the walls are usually made of netting material with a mesh bar length of about 10 mm. The shape of the cages is either rectangular (Korycki 1976, Demska-Zakęś and Zakęś 2002) or tubular (Antila et al. 1988, Salminen et al. 1992, Ruuhijärvi and Hyvärinen 1996). The cages used in Poland in the 1950-1970 period were known as "Swedish cages" with dimensions of 1.20 (L) × 0.60 (W) × 0.80 (H) m. They were comprised of a holding cage for the spawners, and a lower part comprised of the spawning mattress (1.20 (L) × 0.60 (W) × 0.10 (H) m) made of natural substrate (e.g., juniper branches and roots from willows, alders). The cages were secured to piers in lakes (Korycki 1976), and were stocked with one set of spawners each (1 ♀ + 1-2 ♂). Unfortunately, there is a no documented data on the effectiveness achieved with this method. It is known, however, that because of the small size of Swedish cages (volume – 0.6 $m^3$), eggs were often deposited in thick layers on the substrate. This often led to high mortality rates during further development because of insufficient gas exchange and the development of *Saprolegnia* sp.

Steffens et al. (1996) recommend using larger cages (volume – 30-50 $m^3$) for pikeperch reproduction. It is also recommended to use larger spawning nests (1.8 (L) × 0.7 (W) m; surface area 1.26 $m^2$) (Schlumpberger and Schmidt 1980). The spawners are usually stocked into such cages at a density of 2-5 ♀ and 4-10 ♂, and spawning usually occurs after two to three days. Hormonal stimulation is used very rarely, but if it is, it generally contains GtH. Usually, the nests and the fertilized eggs are removed to the hatchery, where the larvae hatch (recommended water temperature 16-20°C; Steffens et al. 1996).

Salminen et al. (1992) used tube-shaped floating cages with a diameter of 1.5 to 2.5 m and a height of 2.0 to 2.5 m (made of netting material with a mesh bar length range of 6.0 to 10.0 mm) to reproduce pikeperch. The cages were fitted with spawning nests made of 2.0 mm-thick perforated aluminum sheets with rice hay as the substrate. The size of the nest should correspond to that of the female, although the nests used were usually square in shape with a surface area of 0.16 or 0.42 $m^2$ (Salminen and Ruuhijärvi 1991). Erm (1981, cited by Lappalainen et al. 2003) reported that the mean diameter of natural pikeperch nests is approximately 0.5 m, thus, using square spawning nests of the size reported by Salminen and Ruuhijärvi (1991) of 0.42 $m^2$ (0.65 (L) × 0.65 (W) m) should be allow pikeperch females to deposit their eggs. It was confirmed, however, that even nests with a surface area of 0.4 $m^2$ were too small for the larger females to spawn in (body weight > 2.5 kg). Salminen and Ruuhijärvi (1991) suggest that each cage should contain one nest and be stocked a maximum of 3 ♀ and 4 ♂. The period during which the fish are held in the cages for the purpose of spawning is usually within the range of 6 to 13 days, but females can begin to spawn after as many as 40 days (Salminen et al. 1992). The nests with fertilized eggs are removed to empty incubation cage. One day before hatching, they were transported to the hatchery or ponds. This method usually produced an average of 80-90 thousand larvae 1 $kg^{-1}$ of female body weight (Salminen et al. 1992).

The results of the study by Salminen et al. (1992) indicates that pikeperch spawning in lake cages extended over time. This might have been due to the varied degree of maturity of the fish used for reproduction. It was confirmed that even individuals caught in the same basin could have gonads in distinctly different stages of maturity (Antila et al. 1988). No hormonal stimulation was applied in the studies by Salminen et al. (1992), and this could have influenced the time period during which the fish were held in the cages. Greater spawning synchronization could be obtained by using hormonal injections (Demska-Zakęś and Zakęś 2002). The effects of hormonal stimulation with GnRHa (5 μg D-$Ala^6$ $Pro^9$

NEt-mGnRH $kg^{-1}$ b.w.) exclusively or in combination with hCG (500-2500 IU hCG $kg^{-1}$ b.w.) was studied by Antila et al. (1988). The fish were administered the hormones by intramuscular injection in either one or two doses (time interval between injections was 48 h). What is significant is that the studies were conducted under similar climate (southern Finland) and technical (cylindrical lake cages) conditions as those by Salminen et al. (1992). Each cage (1.8 m diameter, 1.8 m deep) was fitted with from 1-3 square nests with a surface area of 0.20 $m^2$ (0.45 × 0.45 m; rice hay substrate) and from 1-3 ♀ and 2-6 ♂. It was confirmed that the percentage of ovulating females in the group that was stimulated hormonally was higher than that in the control group (25.0-37.5% versus 0.0-11.1%). However, hormonal stimulation was not noted to have had a significant influence on the timing of spawning (the length of time the pikeperch females were held in cages until spawning). In the group that were stimulated hormonally, the latency time in subsequent years ranged from 2.7 to 3.8 days, while in the control group the average time period was 3.5 days. Higher spawners mortality was noted in these studies. Female mortality was higher in the group that was stimulated hormonally (counted to the fifth day of holding the fish) than in the control group (31.3-36.4% versus 10.5-11.1%). Antila et al. (1988) confirmed that the effect of hormonal injections depends on the maturation stage of the female pikeperch, and that hormonal stimulation has a positive effect on females whose oocytes are in the final stage of maturation. These authors also recommend a minimum of two injections of GnRHa or GnRHa and hCG.

The application of methods for determining the maturity stage of females can improve the effectiveness of controlled pikeperch reproduction. The basic condition for performing this is that the method is *in vivo* and that it allows for determining the maturity of the eggs quickly. A relatively non-invasive method for doing this to use a catheter passed into the ovary through the genital opening and suctioning out a sample of oocytes (Rothbard and Yaron 1995). This method was developed initially for carp, but it can be used successfully to determine the maturity stage of females of other species, including pikeperch (Steffens et al. 1996, Demska-Zakęś and Zakęś 2002, Zakęś and Szczepkowski 2004, Müller-Belecke and Zienert 2008). The *in vivo* method for collecting oocytes is presented in Photo 1. The oocyte sample is placed in a test tube with the preservative and fixing Serra solution (ethyl alcohol

Photo 1. Collecting samples of oocytes from female pikeperch with a catheter.

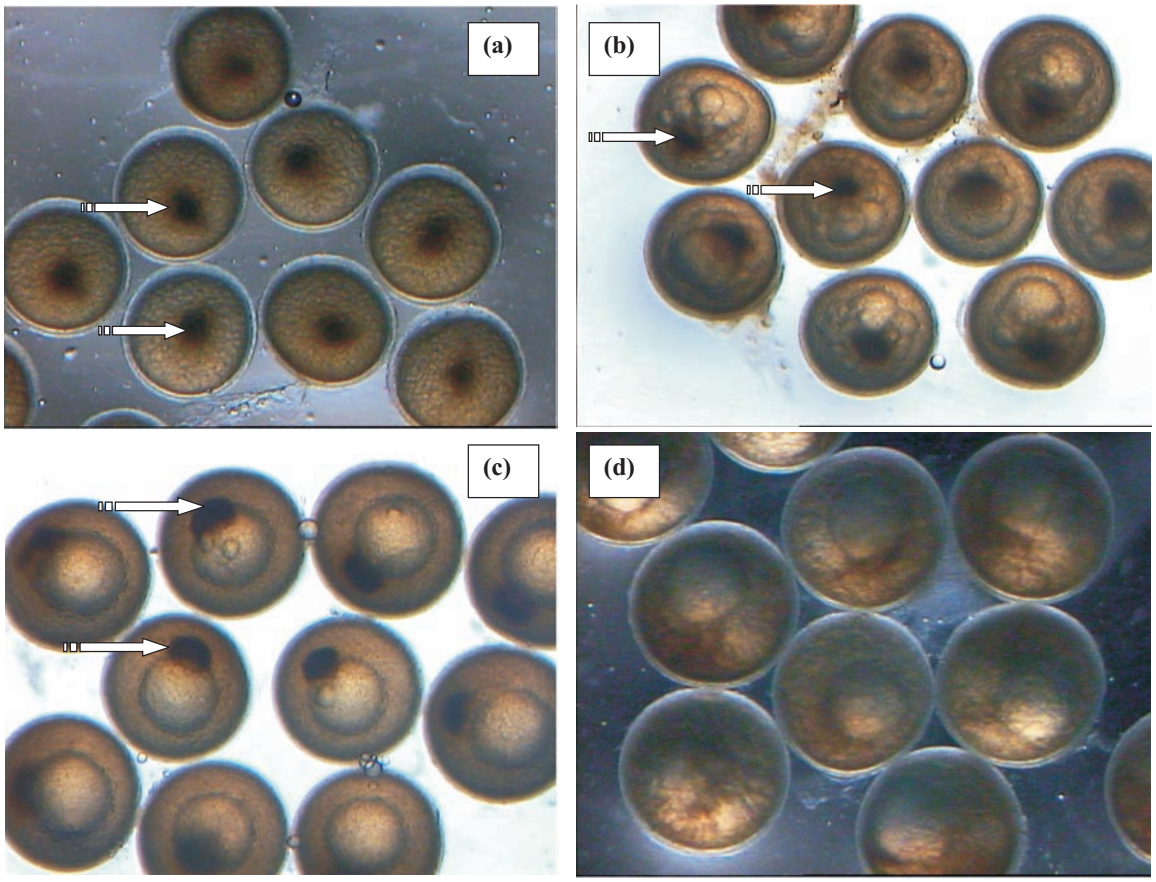


Photo 2. Maturity stage of pikeperch oocytes: (a) – stage I (nucleus in the center of the cell, dissipated lipid drops); (b) – stage II ( nucleus migration, lipid occurs in several larger drops); (c) – stage III (nucleus shifted beyond radiant field of egg cell, lipid drop in one ball); (d) – stage IV (disintegration of the morphological nuclear structure (GVBD)) (arrows indicate nucleus position in chosen oocytes).

96%:formalin:glacial acetic acid, 6:3:1, v/v) (Brzuska and Bieniarz 1977). With pikeperch, the fixing time lasts from 20 to 170 s depending on the maturity stage of the sex cells (Zakęś and Demska-Zakęś 2001). Following this, the oocytes can be examined under a microscope to determine their maturity. The main evaluation criteria are the placement of the cell nucleus and the degree of lipid drop dissipation. Oocytes in the first stage of maturation have a centrally-located nucleus, and the lipid drops are dissipated throughout the cytoplasm (Photo 2a). Oocytes with nuclei that have shifted past the center of the cell are classified as stage II. The lipid drops are less numerous, but slightly larger (Photo 2b). The nuclei of stage III oocytes are located peripherally (near the cell membrane), and there are single, large lipid drops (Photo 2c). Cells with no nuclei are classified as stage IV, and in these oocytes germinal vesicle breakdown (GVBD) has begun (Photo 2d; Zakęś and Szczepkowski 2004). While the sex cells are being sampled, the fish should be anesthetized. Pikeperch can be anesthetized using etomidate (Propiscin, IFI, Olsztyn; Kazuń and Siwicki 2001) in doses of 1.0-2.0 $cm^3$ $dm^{-3}$ (Demska-Zakęś and Zakęś 2002).

The application of *in vivo* methods for determining the maturity stage of pikeperch oocytes allows making an initial estimation of the degree to which the fish are ready to spawn (i.e., before applying hormonal stimulation and stocking spawners into cages), observing the progress of maturation in individual females, and predicting the time of spawning (Demska-Zakęś and Zakęś 2002). It also permits achieving significant improvement in the effectiveness of reproduction conducted in cages (Zakęś and

Demska-Zakęś 2001). In this study discussed, cubic cages with a volume of 8 $m^3$ fitted with spawning nests recommended by Salminen et al. (1992) (surface area 0.25 or 0.36 $m^2$). It was noted that hormonal stimulation with hCG (400-600 IU $kg^{-1}$ b.w.) significantly increased the percentage of females that spawned. The number or dose of the hormone was not noted to have had an effect on spawning (percentage of ovulating females or latency time). Tests were performed on the effects of a single (400 IU hCG $kg^{-1}$ b.w.) or a double (first dose 200 IU hCG $kg^{-1}$ b.w., second dose, administered after 24 h, 300-400 IU hCG $kg^{-1}$ b.w.) dose of hormone on the effects of reproduction (Zakęś and Demska-Zakęś 2001, Zakęś et al. 2001). Hormonal stimulation increased the percentage of ovulating females most significantly in the group of fish that were the least mature (oocytes in stage I). In this group, 83.4% of the females spawned, while none of the females from the control group did so (placebo of 0.9% NaCl solution administered). Among the females with oocytes in stage II that were stimulated with hCG, the percentage that ovulated was 96.2% (57.4% more than in the control group). Hormonal stimulation did not have such a significant impact on females with oocytes in stage III; however, in comparison to the females from the control group, 20% more females ovulated (80.0 vs. 59.6%). Hormonal stimulation was counter indicated for the most mature females (stage IV oocytes) since all the fish from this group spawned spontaneously (Demska-Zakęś and Zakęś 2002). During two spawning events conducted in the Mazurian Lakeland (northern Poland), females with oocytes in stages I, II, III, and IV comprised 36.5, 38.1, 22.9, and 2.5%, respectively, of all the females examined. After hormonal stimulation, as many as 87% of the females with oocytes in stages I, II, or III spawned, while only 33% of the spawners from the control group were spent (Demska-Zakęś and Zakęś 2002). When expressed as the percentage of ovulating females, the results obtained were better than those reported by Antila et al. (1988). The reason for this discrepancy could have been different prevailing thermal conditions during the tests as well as the particular reaction of the spawners to the hormones applied.

Demska-Zakęś and Zakęś (2002) not only noted a greater percentage of ovulating females, but they also noted a shortened latency time and synchronization in spawning, which is contrary to the findings reported by Antila et al. (1988). The latency time among females with oocytes in stages II or III that were subjected to hormonal stimulation was 3.6 and 1.7 days, respectively, while in the control group it was 5.4 and 2.8 days, respectively. The lack of progress in maturation among the females with the least mature oocytes which were not stimulated hormonally is puzzling. After 6 to 9 days of holding the fish in the cages, only slight progress was noted in the maturation of the oocytes. In the samples collected, the majority of eggs were still in stage I (50-70%), while in 30-50% of the oocytes the beginning of nuclear migration was noted (stage II). It is possible that this resulted from the stress stemming from capture, transport, and manipulation. The processes of oocyte maturation and ovulation can be halted as a consequence of physiological reactions that occur in females subjected to stress (Schreck et al. 2001). Heightened levels of cortisol, a hormone released as a reaction to stress, might have an impact on the functioning of the hypothalamus-pituitary-gonadal axis (HPGA), which leads to disturbances in the maturation of oocytes and ovulation (Carragher et al. 1989). The application of hCG hormonal stimulation had a clearly therapeutic effect that led to increased systemic levels of gonadotropin and the release of regulatory mechanisms.

Spawners death are often noted within the first five days that the fish are held in cages during this type of reproduction. It was also confirmed that losses among the fish that are stimulated with hormones are higher than in the control group (Antila et al. 1988). Zakęś and Demska-Zakęś (2001) and Demska-Zakęś and Zakęś (2002) did not note this negative phenomenon in either the control groups or those stimulated hormonally. Losses throughout spawning (8-13 days) reached 1-2% of all the fish. Symptoms of fungal infection were observed especially in the less mature females. It must be noted that the fish were manipulated after having been anesthetized, which could have had a advantageous impact on their condition.

Variable atmospheric conditions (especially lower temperatures that occurred, known as "May cooling"), that are often observed during the natural pikeperch spawning period (review in Lappalainen et al. 2003) can influence the course of spawning migrations and natural spawning, as well as the effectiveness and timing of cage spawning. If there are drastic decreases in water temperature, pikeperch can discontinue spawning, and if these conditions hold, the females can even reabsorb their oocytes (Golovanenko et al. 1970). Salminen et al. (1992) confirmed that when pikeperch cage spawning was conducted during a year when average spring weather conditions prevailed, the females deposited their eggs within a period of 5.9 to 13.1 days. During colder spring weather, the pikeperch needed as many as 40 days to spawn. Demska-Zakęś and Zakęś (2002) conducted cage spawning in two years with significantly different thermal conditions. During the first year, the mean water temperature was 10.0°C (range of 8.1-15.6°C), and in the second year the mean was 16.2°C (range 13.8-18.1°C). The lower water temperature resulted in a spawning period that was five days longer (eggs obtained for 13 days). The effectiveness of spawning at the two water temperatures expressed as the percentage of females that deposited eggs, their working fecundity, and the percentage of eggs survival to the eyed-egg stage was similar. It should be emphasized that in the colder year, the females deposited eggs even at temperatures lower than 10.0°C (i.e., 8.0°C). Thus, it would appear that females which had already attained the appropriate maturation stage spawned despite the significantly lowered water temperature (Zakęś 2009). In less mature individuals, it is necessary to apply hormonal stimulation (Demska-Zakęś and Zakęś 2002). Holding fish in cages for long periods when lower temperatures prevail can have a negative impact on the condition and health of the fish thus lowering the effectiveness of this method.

## Artificial spawning of wild pikeperch

Most of the pikeperch spawners used in artificial reproduction are wild specimens obtained from natural water bodies, although cultivated spawners held in earthen ponds are sometimes used (Horváth et al. 1984, Schlumberger and Proteau 1996, Steffens et al. 1996, Zakęś and Demska-Zakęś 2005, Zakęś 2009). Spawners from natural waters are caught in fall (October-November) or in spring (March-May), during spawning migrations (Zakęś 2009). The fish caught in fall are held for the winter in earthen ponds. Pikeperch feed rather intensively during the winter, so it is important to ensure that an appropriate food base is available. The minimum monthly feed fish biomass should be 20% of the pikeperch spawner biomass (Horváth et al. 1984, Wojda 2006). This species prefers feeding on small-sized fish, thus the body weight of the prey fish should not exceed 8-10% of the spawner body weight (Steffens et al. 1996, Szczepkowski and Zakęś 2003).

It is very important to ensure that the spawners are transported under appropriate conditions. It is also recommended to use some sort of anti-stress agent such as sodium chloride (3-5 g NaCl $dm^{-3}$) or etomidate (0.02-0.05 $cm^3$ $dm^{-3}$; Schlumberger and Proteau 1996, Zakęś and Demska-Zakęś 2005, Zakęś 2009).

Applying thermal and hormonal stimulation is possible if reproduction is conducted in a hatchery equipped with a RAS. As a rule, males do not require hormonal stimulation (Zakęś and Demska-Zakęś 2005, Rónyai 2007). However, if they are not ripe when they are delivered to the hatchery, they should be stimulated hormonally with half the dose given to the females (Steffens et al. 1996). Mainly gonadotropins are used in the hormonal stimulation of pikeperch (CPE and hCG; Antalfi 1979, Steffens et al. 1996, Zakęś and Demska-Zakęś 2005, 2006, Wang et al. 2009). Effective dosages of these preparations range from 2.4 to 5.0 mg CPE $kg^{-1}$ b.w. and 100-700 IU hCG $kg^{-1}$ b.w. (Table 1). The hormonal preparations are administered in either one or two doses. The period between injections is usually 24 hours, although Steffens et al. (1996)

recommend a 12-hour interval between injections of CPE. These authors also recommend administering the total hCG dose in three to five injections. Zakęś and Demska-Zakęś (2005), however, did not note that the number of injections or the sizes of the hCG doses (400 or 700 IU $kg^{-1}$ b.w.; administered in two portions) had any effect on the effects of reproduction (Table 1). The size of the doses and the number of injections should be determined based on the maturity of the females (Rónyai 2007, Zakęś 2009). Females with oocytes in stage I can be stimulated twice, but for those with oocytes in stages II or III (Photo 2) it is sufficient to use one injection (i.e., 200 IU hCG $kg^{-1}$ b.w.; Zakęś and Demska-Zakęś 2005, 2006). Either etomidate (1.0-2.0 $cm^3$ $dm^{-3}$, Zakęś and Demska-Zakęś 2005) or phenoxyethanol (0.1-0.3 $cm^3$ $dm^{-3}$; Schlumberger and Proteau 1996, Wang et al. 2009) can be used to anesthetize the fish prior to manipulation (collecting oocyte samples, injections).

Studies have been conducted of the effects of other gonadotropins on the maturation of oocytes and ovulation in pikeperch including pregnant mare serum gonadotropin (PMSG) (Zakęś and Demska-Zakęś 2006). It should be emphasized that studies of stimulating fish reproduction with PMSG are not numerous (Brzuska and Ryszka 1990, Bieniarz and Epler 1991). The dose of PMSG applied was the same as that of hCG (i.e., 200 IU $kg^{-1}$ b.w.). While stimulation with hCG produced significant progress in oocyte maturation 48 h following injection, progress in the group stimulated with PMSG was only slight (Zakęś and Demska-Zakęś 2006). Not until the second injection of PMSG (200 IU $kg^{-1}$ b.w.) was there a significant stimulatory effect. The application of PMSG resulted in a lengthened latency period and a lowered degree of spawning synchronization (Table 1), but eggs was obtained from all females, and, more importantly, the weight of the eggs expressed as a percentage of female body weight was significantly higher in the PMSG group than in the hCG group (15.9 and 10.4% b.w., respectively). The gonadotropins administered were not observed to effect the quality of the eggs (Zakęś and Demska-Zakęś 2006). It is possible that a higher dose of PMSG would likely lead to synchronized spawning. Brzuska and Ryszka (1990) reported, however, that with carp even doses of PMSG as high as 2000 IU $kg^{-1}$ b.w. did not have a significant effect on the oocyte maturation process or on ovulation. These authors also noted that fish stimulated with PMSG first and then CPE, matured earlier than those injected exclusively with CPE. It was also confirmed that the carp stimulated with both PMSG and CPE produced a greater amount of eggs.

Currently, domestic animals are stimulated to reproduce using a hormonal preparation comprising both hCG and PMSG. Initial studies indicate that using this preparation in pikeperch production is not fully justified because of the higher cost and the fact that it does not produce a greater percentage of ovulating females. Additionally, as was the case with using PMSG exclusively, the latency time is relatively long and the degree of spawning synchronization not high (Zakęś et al., unpublished data).

Gonadoliberins are used less frequently to stimulate the artificial spawning of wild pikeperch, and the available literature indicates that it is more common to use GnRHa (D-$Ala^6$ des$Gly^{10}$-mGnRH) and Ovopel (Schlumberger and Proteau 1996, Zakęś and Demska-Zakęś 2005; Table 1). The effects of stimulating pikeperch with GnRHa are satisfactory (Schlumberger and Proteau 1996), but the results achieved with Ovopel are not conclusive. Zakęś and Demska-Zakęś (2005) noted that this preparation had a negative impact on pikeperch reproduction. In comparison to the females stimulated with hCG, the percentage of those who deposited eggs was lower when Ovopel was the stimulant. Additionally, spawner mortality increased and the quality of the eggs was poorer (Table 1). Similarly, high mortality was noted in wild ide, *Leuciscus idus* (L.), that had been stimulated with Ovopel (Kucharczyk et al. 1999). It must be emphasized that Ovopel is used successfully in the reproduction of cyprinids and catfishes (i.e., Horváth et al. 1997, Brzuska and Grzywaczewski 1999, Brzuska 2003, 2004). It should be noted that the fish material used in the cited studies were highly domesticated (in ponds) making them more resistant to all kinds of manipulation and the stress associated with it.

**Table 1**
Hormones and hormonal preparations and the effects of the artificial reproduction of wild pikeperch (CPE – carp pituitary extract. hCG – human chorionic gonadotropin, PMSG – pregnant mare serum gonadotropin, GnRHa – superactive GnRH analogue, Ovopel – mammalian GnRH analogue with dopamine inhibitor (Horváth et al. 1997)

| Hormone/ preparation | Dose (per kg body weight (b.w.)) | Administration of doses and time interval between injections | Water temperature (°C) | Latency time (h) [1] | Ovulation (%) | Survival (%) | Author |
|---|---|---|---|---|---|---|---|
| CPE | 3.0-5.0 mg | 2 doses (10 and 90%), 24 h | 14.0 | 28 | 80 | 80-95 | Antalfi (1979) |
| | 2.4 mg | 2 doses (0.8 and 1.6 mg $kg^{-1}$ b.w.), 24 h | 12.0-15.5 | 28-35 | 75 | 75[2] | Zakęś et al., unpublished data |
| | 4.0 mg | 2 doses (1.0 and 3.0 mg $kg^{-1}$ b.w.), 24 h | 15.5-16.5 | 24-38 | 90 | > 95 | Rónyai (2007) |
| hCG | 100-200 IU | n.d. | 19.0-20.0 | 10 | n.d. | n.d. | Steffens et al. (1996) |
| | 700 IU | 2 doses (200 and 500 IU $kg^{-1}$ b.w.), 24 h | 12.0-15.5 | 20-28 | 70 | 79[2] | Zakęś et al., unpublished data |
| | 200 IU | 1 dose | 10.0-15.8 | 24-70 | 100 | 73[2] | Zakęś and Demska-Zakęś (2006) |
| | 400 IU | 2 doses (200 and 200 IU $kg^{-1}$ b.w.), 24 h | 10.2-15.5 | 22-32 | 83 | 72[2] | Zakęś and Demska-Zakęś (2005) |
| | 700 IU | 2 doses (200 and 500 IU $kg^{-1}$ b.w.), 24 h | 10.2-15.5 | 25-36 | 100 | 68[2] | |
| PMSG | 400 IU | 2 doses (200 and 200 IU $kg^{-1}$ b.w.), 48 h | 10.0-15.8 | 48-96 | 100 | 76[2] | Zakęś and Demska-Zakęś (2006) |
| GnRHa | 100 µg + pimozide 10 mg | n.d. | 14.0-20.0 | 25-30 | n.d. | > 80 | Schlumberger and Proteau (1996) |
| Ovopel[3] | 0.75 pellet | 2 doses (0.25 and 0.50 pellet $kg^{-1}$ b.w.), 24 h | 10.2-15.5 | - | 0 | - | Zakęś and Demska-Zakęś (2005) |
| | 1.25 pellet | 2 doses (0.25 and 1.00 pellet $kg^{-1}$ b.w.), 24 h | 10.2-12.5 | 42-62 | 50 | 3[2] | |
| No stimulation | 0.5 ml 0.7% NaCl | 2 doses (0.2 and 0.3 ml $kg^{-1}$ b.w.), 24 h | 12.0-15.5 | 35-72 | 25 | 82[2] | Zakęś et al., unpublished data |
| | 0.7 ml 0.7% NaCl | 2 doses (0.2 and 0.5 ml $kg^{-1}$ b.w.), 24 h | 10.2-15.0 | 52-99 | 50 | 70[2] | Zakęś and Demska-Zakęś (2005) |

[1] time measured from last hormone dose; [2] survival determined at the eyed-egg stage; [3] 1 pellet of an average weight of 25 mg contains 18-20 µg D-Ala$^6$ Pro$^9$NEt-mGnRH and 8-10 mg metoclopramide); n.d. – no data

Hodson and Sullivan (1993) recommend using gonadotropin instead of gonadoliberin when reproducing wild fish. They conformed in a study on wild bass, *Morone saxatilis* (Walb.), that stimulating with hCG, a hormone that acts directly on the gonads, had a more positive impact on the maturation of oocytes, ovulation, and spermiation than did GnRH. The high effectiveness of this hormone for stimulating and synchronizing reproduction is explained by the relatively long half-life of hCG in the bloodstream (Ohta and Tanaka 1997). Gonadoliberins act at the higher level of the HPG axis, which is why the latency period is usually longer than after the application of gonadotropin. It cannot be ruled out that the longer latency period and accompanying stress might be the cause of the high mortality among wild spawners (Zohar and Mylonas 2001).

Currently, two methods for reproducing pikeperch are used at hatcheries: the tank method and artificial spawning. In the first, after hormonal stimulation, a set of spawners (1 ♀ + 1-2 ♂) is placed in a tank on the bottom of which is a spawning nest (these fish spawn on a substrate; Horváth et al. 1984, Schlumberger and Proteau 1996, Steffens et al. 1996, Rónyai 2007, Wang et al. 2009, Zakęś 2009). After injecting the fish with the hormonal stimulant, it is recommended to conduct thermal stimulation. Steffens et al. (1996) recommend increasing water temperature to 19-20°C, while other authors recommend temperatures from 10-12 and 14-16°C (Antalfi 1979, Zakęś 2009). This method reduces the number of manipulations the spawners are subjected to during the process of obtaining sex products, but quite frequently a portion of the eggs are deposited outside of the spawning nests. The eggs can also be deposited in a thick layer, which promotes the development of *Saprolegnia* sp., and consequently reduces the effects of spawning (Zakęś 2009). It would appear that the optimal solution is to conduct artificial reproduction. Pikeperch eggs obtained artificially are fertilized with milt collected from males with syringes (Steffens et al. 1996, Zakęś and Demska-Zakęś 2005). Pikeperch have thick semen with sperm concentrations that range from 16 to 20 million $cm^{-3}$ of semen (mean 19.8 million $cm^{-3}$; Zakęś and Demska-Zakęś 2005). To fertilize 100 g of eggs it is recommended to use 1.0-2.0 $cm^3$ semen (Steffens et al. 1996, Zakęś and Demska-Zakęś 2005). Sperm motility usually ranges from 50 to 90%, which is why it is recommended to fertilize any given portion of eggs with the milt taken from 2 to 3 males (Zakęś and Demska-Zakęś 2005).

Adhesiveness can be removed from the eggs by bathing it in a talc-sodium chloride solution (100 g salt + 25 g talc + 10 $dm^3$ water) for 45 to 60 min (Schlumpberger and Schmidt 1980). The disadvantage of this method is that it is time and labor intensive. Removing adhesiveness with a tannin solution is much faster and can be done at a solution concentration of 0.5-1.0 g $dm^{-3}$ of water for a period of 5 min (Demska-Zakęś et al. 2005). Pikeperch eggs can also be cleared of adhesiveness with an aquatic solution of protease (recommended concentration of 0.5% (5 $cm^3$ $dm^{-3}$ water; length of procedure – 2 min; Zakęś et al. 2006). The eggs are incubated in standard Weiss jars at a recommended water temperature of 16.0 to 17.0°C (Steffens et al. 1996). Kokurewicz (1969) reported, however, that the best larval development and longest body length is obtained when the eggs are incubated at a water temperature of 12.0-16.0°C. Jars of a volume of 7 $dm^3$ can accommodate from 0.5 to 5.0 $dm^3$ of eggs. The recommended water flow rate is 0.5 $dm^3$ $min^{-1}$ at the beginning of egg incubation and 4.0-5.0 $dm^3$ $min^{-1}$ in later periods (Steffens et al. 1996). During incubation, prophylactic baths to prevent the development of fungi can be used (i.e., 100 ppm of formalin for 5 min; Rónyai 2007). Based on data available in the literature, Lappalainen et al. (2003) that the pikeperch egg incubation period (from fertilization to hatching larvae) can be calculated with the following formulae: $DD = 1255 \times T^{-1.07}$ or $I = 30124 \times T^{-2.07}$, where: DD – incubation time (°D), I – incubation time (h), T – water temperature (°C).

When catching spawners prior to the spawning season, it should be borne in mind that male pikeperch generally mature earlier and reach the spawning grounds earlier (Salminen et al. 1992, Lappalainen et al. 2003). In the initial stages of catching spawners, it is usually only possible to

obtain males, which then have to be held for several days to even two to three weeks until mature female spawners are caught. An alternative solution, that is less costly than holding the males, is to strip them of milt and store it under the appropriate conditions. To date, studies of short-term storage of semen have been conducted mainly on salmonids (Stoss 1983, McNiven et al. 1993) and cyprinids (Ravinder et al. 1997, Glogowski et al. 2008). Moore (1987) developed a method for the short-term storage of the semen of walleye, *Sander vitreus* (Mitch.). Demska-Zakęś and Zakęś (2003) confirmed that undiluted pikeperch semen held under refrigeration (+4°C) in an oxygen atmosphere remains viable for up to 12 days. Using diluting agents, such as those used for the storage of semen from other fish species (i.e., Cortland and Moore fluids; Brown and Moore 1996) did not improve the biological quality of the semen. What determines how long pikeperch sperm remains viable is the maintenance of appropriate oxygen conditions (daily oxygen exchange) in the container in which the semen is stored. Pikeperch milt stored in open containers at a temperature of + 4°C was viable for just a day (Demska-Zakęś and Zakęś 2003). The positive effects of oxygen on semen quality was also observed in salmonid fish (Stoss and Holtz 1983). It is noteworthy, however, that not all fish species exhibit this same dependency (Glogowski et al. 2008). A high percentage of fertilized eggs (80-90%) can be obtained when sperm motility does not fall below 50% (Brown and Moore 1996). Such viability was confirmed in pikeperch sperm up to 7 days after semen collection (Demska-Zakęś and Zakęś 2003). Bokor et al. (2007) reported promising results using cryopreserved sperm to fertilize the eggs of this species. They also confirmed that good effects are guaranteed by using methanol (MeOH) or dimethyl sulfoxide (DMSO) as cryoprotectants. When cryopreserved pikeperch milt was used, in excess of 40% of the eggs were fertilized. These same authors suggested that contamination with urine appears to be the key obstacle to overcome when developing more effective methods for the cryopreservation of the semen of this species.

## Out-of-season pikeperch reproduction

Some of the most recent studies of pikeperch are focused on out-of-season spawning of this species. The first documented reproduction of pikeperch prior to the natural spawning season were performed by Zakęś and Szczepkowski (2004). Currently, this method is also being used to reproduce wild pikeperch (Zakęś and Szczepkowski 2004, Zakęś et al. 2005, Rónyai 2007), as well as cultivated pikeperch reared in RAS (Zakęś 2007). The process of intense yolk accumulation (exogenic vitellogenesis) begins as early as in late October and early November (ovary maturation stage IV; Sakun and Bucka 1968). During the subsequent 5-6 months the oocytes achieve their full size and following a relatively short maturation period they are ready to be deposited during spawning (ovulation). It was confirmed that subjecting pikeperch to photothermal stimulation shortens the duration of vitellogenesis and reproduction can be achieved several months prior to the natural spawning season (Zakęś and Szczepkowski 2004, Rónyai 2007). The full thermal cycle of stimulation for spring spawning fish comprises three phases: cooling phase (CP), chilling phase (CHP), and warming phase (WP) (Migaud et al. 2002). Zakęś and Szczepkowski (2004) applied a 16-week thermal stimulation cycle (CP – 6 weeks; CHP – 6 weeks; WP – 4 weeks). This resulted in obtaining eggs 3-4 months prior to the natural spawning season of this species. The length of the full thermal stimulation cycle for pikeperch is significantly shorter than that for perch, *Perca fluviatilis* L. Migaud et al. (2002) noted that with this species the duration of CHP, which should last about 5 months, is significant. Rónyai (2007) suggests that with pikeperch a cooling period of 3 months is sufficient. In turn, Zakęś (2007) concluded that the duration of the CHP might be shortened to 6 weeks (temperature ≤ 8.0°C) or 8-9 weeks (temperature ≤ 10.0°C). Similar solutions are also recommended by Müller-Belecke and Zienert (2008).

There are several ways of performing thermal stimulation. In variant I, the fish undergo CP under natural conditions (ponds or lakes). Spawners are

caught in fall and placed in a hatchery under fully controlled conditions, where they are subjected to the two final phases of stimulation (CHP + WP). In variant II, the fish are stocked into ponds and then removed during winter. This allows conducting the first two phases (CP + CHP) under natural conditions. Only WP is conducted under controlled conditions. In variant III, all three phases of thermal stimulation are conducted under fully controlled conditions (Zakęś et al. 2005). Rónyai (2007) applied the second method successfully, while Zakęś and Szczepkowski (2004) and Zakęś (2007) obtained good results with variant III. Rónyai (2007) caught subsequent batches of spawners in the ponds from January to May, and concluded that the fish that had been held in ponds during the fall and winter for phases CP and CHP required just a 7-day period of increased temperature (WP) after being moved to the hatchery. The extension of this phase (14 days) did not improve the results of spawning (Rónyai 2007). During WP, the water temperature is generally increased to 15-16°C (Rónyai 2007, Zakęś 2007; Table 2), at a maximum daily temperature increase of 2°C (Rónyai 2007, Müller-Belecke and Zienert 2008). Rónyai (2007) observed that applying higher temperatures (18°C) shortened the latency time, but it did not affect the percentages of ovulating females or fertilized eggs. The latency time was also affected by the period when the fish were caught in the ponds and moved to the hatchery. From January to April, this period was shortened significantly by 120 to 60 h, respectively (Rónyai 2007). One of the advantages of variants I and II is that the thermal stimulation is accompanied by the natural photoperiod. One solution that permits exploiting the natural photoperiod is to place the fish in recirculating systems (controlled temperature) located in glasshouses (Zakęś and Szczepkowski 2004). In the studies cited above, a shifted photoperiod was only applied during WP. Over a month, along with increasing water temperature, the photoperiod was also changed gradually from 8:16 to 14:10 L:D (Zakęś and Szczepkowski 2004, Zakęś 2007). The stimulatory effect of photoperiod on percid fish is not unequivocal (Malison et al. 1998, Migaud et al. 2002, Rónyai 2007). Kayes and Calbert (1979) noted that yellow perch, *Perca flavescens* (Mitch.), held under various photoperiod regimes (L:D; 13.5:10.5; 10.5:13.5; 6:18; 18:6) spawned at similar times. Rónyai (2007) concluded that even the application of the photoperiods of L:D; 12:12 and 0:24 did not influence the out-of-season spawning of pikeperch. Dabrowski et al. (1994) and Migaud et al. (2002) suggest that with percids that reproduce in spring, such as yellow perch and European perch, photoperiod does not have as significant an effect on the process of gametogenesis, as does water temperature.

In addition to photothermal stimulation, one of the conditions for achieving full oocyte maturation, spermiation, and ovulation in out-of-season pikeperch reproduction was to apply hormonal injections (Zakęś and Szczepkowski 2004). In the fish from the control group, which were injected with a placebo and subjected only to environmental stimulation, no progress was noted in the maturation of oocytes (Zakęś and Szczepkowski 2004, Rónyai 2007, Zakęś 2007). Malison et al. (1998) reached similar conclusions in studies of out-of-season spawning of walleye. Good pikeperch reproduction results can be anticipated when hCG and CPE are administered either separately or in combination (Table 2). In out-of-season reproduction males are not as mature as wild individuals caught in the pre-spawning period, which is why it is necessary to conduct hormonal stimulation (i.e., 200 IU hCG $kg^{-1}$ b.w.; Zakęś 2007). The number of doses of this hormone (1-3 doses) administered was not noted to have affected either the latency time or the biological quality of the sex products (Zakęś and Szczepkowski 2004). Likewise, the dose of hormone (200 and 400 IU hCG $kg^{-1}$ b.w.) or the age of cultivated pikeperch females (2+ and 3+) were not noted to have influenced the latency period or the working fecundity (Zakęś 2007). When CPE or a combined injection of CPE and hCG are applied, it is recommended to use two doses (Rónyai 2007). Three injections of CPE do not significantly improve the effects of spawning in comparison with females that were injected twice with CPE. However, with fish that do not spawn within 5-6 days, the application of a third injection might be effective (i.e., 3 mg CPE $kg^{-1}$ b.w.; Rónyai, 2007). The shortest latency time is guaranteed with two injections (first dose of

**Table 2**
Impact of various hormonal preparations on the effects of out-of-season pikeperch reproduction (hCG – human chorionic gonadotropin; CPE – carp pituitary extract; Ovurelin – GnRH analogue (Rónyai 2007); mtc – metoclopramide; Ovopel – mammalian GnRH analogue with a dopamine inhibitor (see Table 1) (range or mean values ± SD)

| Hormone/ preparation | Dose (per kg body weight (b.w.)) | Administration of doses, time interval between injections, characteristic of fish | Water temperature (°C) | Latency time (h)[1] | Ovulation (%) | Survival (%) | Author |
|---|---|---|---|---|---|---|---|
| hCG | 200 IU | 1 dose | 12.0-15.5 | 66 ± 7 | 100 | 77.5 ± 5.6[4] | Zakęś and Szczepkowski (2004) |
| | 400 IU | 2 doses (200 and 200 IU$kg^{-1}$ b.w.), 24 h | 12.0-15.5 | 71 ± 9 | 100 | 73.0 ± 8.9[4] | |
| | 600 IU | 3 doses (200, 200, 200 IU $kg^{-1}$ b.w.), 24 h | 12.0-15.5 | 70 ± 5 | 100 | 71.5 ± 9.1[4] | |
| | 200 IU | 1 dose, cultivated spawners, age 2+ | 12.0-16.0 | 101 ± 13 | 80 | 61.0 ± 14.8[4] | Zakęś (2007) |
| | 200 IU | 1 dose, cultivated spawners, age 3+ | 12.0-16.0 | 88 ± 14 | 93 | 70.7 ± 10.2[4] | |
| | 400 IU | 1 dose, cultivated spawners, age 2+ | 12.0-16.0 | 98 ± 13 | 100 | 64.0 ± 20.7[4] | |
| | 400 IU | 1 dose, cultivated spawners, age 3+ | 12.0-16.0 | 99 ± 7 | 100 | 73.3 ± 11.3[4] | |
| | 400 IU | 2 doses (200 and 200 IU $kg^{-1}$ b.w.), 24 h[2] | 15.5-16.5 | 76 ± 6 | 100 | 87 ± 8[5] | Rónyai (2007) |
| hCG + CPE | 200 IU + 3 mg | first dose hCG, second dose CPE, 24 h[2] | 15.5-16.5 | 108 ± 23 | 75 | 85 ± 10[5] | |
| | 200 IU + 3 mg | first dose hCG, second dose CPE, 24 h[3] | 15.5-16.5 | 120 ± 4 | 50 | > 95[5] | |
| hCG + CPE | 200 IU + 3 mg | first dose hCG, second dose CPE, 24 h[2] | 15.5-16.5 | 67 ± 3 | 100 | 87 ± 6[5] | |
| CPE | 6 mg | 2 doses (3 and 3 mg $kg^{-1}$ b.w.), 24 h[2] | 15.5-16.5 | 119 ± 23 | 75 | 75 ± 23 [5] | |
| | 6 mg | 2 doses (3 and 3 mg $kg^{-1}$ b.w.), 24 h[3] | 15.5-16.5 | 115 | 25 | > 95 [5] | |
| | 6 mg | 2 doses (3 and 3 mg $kg^{-1}$ b.w.), 24 h[2] | 15.5-16.5 | 71 ± 5 | 100 | 91 ± 6[5] | |
| | 6 mg | 2 doses (3 and 3 mg $kg^{-1}$ b.w.), 24 h[3] | 15.5-16.5 | 74 ± 6 | 100 | 88 ±13[5] | |
| CPE + hCG | 3 mg + 200 IU | first dose 1.5 mg CPE $kg^{-1}$ b.w. + 100 IU hCG $kg^{-1}$ b.w., second dose 1.5 mg CPE $kg^{-1}$ b.w. + 100 IU hCG $kg^{-1}$ b.w., 24 h[2] | 15.5-16.5 | 78 ± 8 | 100 | 90 ± 12[5] | |
| Ovurelin | 20 µg | first dose 5 µg $kg^{-1}$ b.w., second dose 15 µg $kg^{-1}$ b.w., 24 h | 15.5-16.5 | 86 ± 9 | 100 | 43 ± 34[5] | |
| Ovurelin + mtc | 20 µg + 10 mg mtc | first dose 5 µg $kg^{-1}$ b.w. + 10 mg mtc. second dose 15 µg $kg^{-1}$ b.w., 24 h | 15.5-16.5 | 93 ± 38 | 50 | 56 ± 22[5] | |
| Ovopel | 0.2 pellet | 2 doses (0.1 and 0.1pellet $kg^{-1}$ b.w.), 24 h | 15.5-16.5 | 78 ± 1 | 100 | 54 ± 43[5] | |

[1] time measured from administration of first hormone dose; [2] fish caught from ponds and held at spawning temperature for 7 days prior to injection; [3] fish caught from ponds and held at spawning temperature for 14 days before injection; [4] survival determined at the eyed-egg stage; [5] survival determined 16-18 h after fertilization

hCG, second dose of CPE; Rónyai, 2007). Results to date also indicate that in out-of-season reproduction, stimulation with GnRH preparations (i.e., Ovopel and Ovurelin (D-Phe$^6$-mGnRH); Rónyai 2007) produce worse results than GtH (longer latency period and/or lower percentage of fertilized eggs; Table 2). Gonadoliberins, and in particular their super active analogues (GnRHa), potentially have properties that could render them more advantageous than GtH for the maturation of gametes, for example, by influencing the synthesis and excretion of somatropins, tyreotropins, and prolactin. However, the excretion of GtH after the application of GnRH might be inhibited by dopamine (Zohar and Mylonas 2001). This is why GnRH is administered along with dopamine blockers (Zohar and Mylonas 2001). Rónyai (2007) did not note more positive effects when metoclopramide was administered along with GnRH (Ovurelin) to stimulate pikeperch spawning. Kouril et al. (1997) also failed to note a more advantageous effect on the spawning of European perch when GnRH was administered along with a dopamine blocker.

Hormonal stimulation procedures should be preceded by determining the stage of maturity of the females. It is recommended to use the same *in vivo* method for doing this as was used in cage reproduction and in artificial reproduction in the natural spawning period for this species (Zakęś and Szczepkowski 2004, Müller-Belecke and Zienert 2008). Additionally, it was confirmed that changes in body weight observed in hormonally stimulated cultivated female pikeperch corresponded to gonadal maturity degree. This might be a valuable aid in determining their stage of maturity, as well as predicting the period in which eggs will be obtained (Zakęś 2007).

One of the problems encountered in out-of-season reproduction is the high incidence of post-spawning mortality of spawners. Rónyai (2007) confirmed that all of the females that had been stripped artificially died within five days of obtaining eggs. Mortality among fish that spawned in tanks (eggs deposited on substrate) was also high at about 60%. The cause of such high losses might have manipulation stress. The origin of the fish is also significant. The material used in Rónyai's (2007) reproduction study were wild fish, which are decidedly more susceptible to stress than are cultivated fish. When conducting artificial reproduction of pikeperch that have been reared from larvae in RAS on commercial feed, post-spawning losses are not high and do not exceed 10% (Zakęś 2007). The same females held in RAS can reproduce for the subsequent three or four seasons without any negative impact on the quality of the eggs obtained (Zakęś et al., unpublished data).

Doubtlessly, catching, transporting, and manipulating fish during the spawning season leads to higher losses. This is especially true when inappropriate methods are applied (Zakęś 2009). One solution that would minimize fish manipulation would be to subject them only to photothermal stimulation. The photothermal stimulation procedures presented above do not prepare pikeperch for spawning without the application of hormonal stimulation (Zakęś and Szczepkowski 2004, Rónyai 2007, Zakęś 2007). However, Müller-Belecke and Zienert (2008) applied photothermal stimulation, following which the majority of fish (91% of the females) held in the tanks spawned (eggs were deposited in nests). After initial, year-long rearing in RAS (temperature 22-24°C), the pikeperch were moved to lake cages, where their rearing continued. It was confirmed that the application of a chilling phase of 43 to 60 days (CHP was conducted in lake cages; temperature $\leq$10.0°C), followed by a warming phase of 44 to 68 days (water temperature approximately 15°C; photoperiod L:D 16:8; fish held in RAS) allowed obtaining fertilized eggs two months prior to the natural spawning period (Müller-Belecke and Zienert 2008). Using this procedure decreased post-spawning losses, which were 12.5% for the females. The mean weight of the eggs obtained in various experimental groups ranged from 9.8 to 24% b.w., and in most instances, these were higher than that obtained during artificial, out-of-season, hormonally-stimulated reproduction of this species (Rónyai 2007, Zakęś 2007). It was symptomatic that photothermal stimulation was more effective among females than among males (Müller-Belecke and Zienert 2008). These authors

put forth the hypothesis that perhaps holding females and males in the same tanks (especially during WP) would have a more advantageous impact on their maturation (potential effect of pheromones and visual stimulation).

## Final comments, research priorities

Knowledge pertaining to pikeperch reproduction has been increased substantially, especially in recent years. Methods for pikeperch reproduction in lake cages have been improved, and the application of *in vivo* methods for determining the oocyte maturity stage and hormonal stimulation (especially in less mature females) have permitted increasing the effectiveness of cage spawning and the artificial reproduction of wild fish. Preparations containing GtH (CPE and hCG) have proved to be particularly effective. The application of CPE carries the risk of transmitting diseases borne by the fish from which the pituitary extract is obtained. Another disadvantage of CPE is that is is often a non-standard product, and other hormones contained in the pituitary can have side effects. After the application of GtH, which are proteins, and particularly after the administration of hCG, it is possible for the immune system to produce anti-bodies (Zohar and Mylonas 2001). Consequently, when using hCG in subsequent reproductive seasons, it might be necessary to use increasingly higher doses of the preparation. No such reaction was noted among the pikeperch spawners that were stimulated with hCG in three to four subsequent spawning seasons (Zakęś et al., unpublished data). It cannot be ruled out, however, that this type of problem will arise in the subsequent stages of the domestication of this species. Bearing in mind the effectiveness of this preparation, its standardization, the ease with which it can be prepared, the small dose required, its low cost, as well as the repeatability of the results achieved, hCG can be recommended for use in pikeperch reproduction. Because of the potentially positive factors of GnRH, it is highly recommended that further studies be conducted to develop optimal methods for stimulating pikeperch reproduction with this type of preparation (Rónyai 2007). As the importance of so-called "organic aquaculture" increases, undoubtedly so too will the role of methods for reproducing pikeperch that are based on only on photothermal stimulation (Müller-Belecke and Zienert 2008).

The negative influence of intense rearing conditions and domestication on fish reproduction is noted relatively frequently (Brooks et al. 1997). The growth of intense pikeperch cultivation will depend on the development of effective reproduction techniques for cultivated pikeperch spawners held in RAS, and fed diets of commercial feed. Initial studies have confirmed that this type of reproducer is suitable for artificial spawning (Zakęś 2007, Müller-Belecke and Zienert 2008). However, the priority has to be developing recipes for commercial feeds that meet the nutritional needs of the selects and spawners of this species which, in turn, ensures obtaining high quality sex products and progeny. Studies of fatty acid profiles have indicated that the eggs of cultivated pikeperch differs from that of wild pikeperch in the contents, for example, of polyunsaturated fatty acids (PUFA) (Kowalska et al. 2006). Such differences were noted with regard to fatty acids that determine the quality of eggs and progeny, including, for example, arachidonic (C 20:4 n-6), eicosapentaenoic (C 20:5 n-3), and docosahexaenoic (C 22:6 n-3) (Izquierdo et al. 2001). Wang et al. (2009) confirmed that feeding pikeperch commercial feed resulted in lowered artificial reproductive effectiveness in comparison to that in the group of spawners fed a diet of fish. However, the diet of pikeperch spawners was not noted to have had an impact on the biological quality of the larvae (Wang et al. 2009).

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## Streszczenie

### Kontrolowany rozród sandacza *Sander lucioperca* (L.) – artykuł przeglądowy

W pracy podsumowano najważniejsze osiągnięcia dotyczące kontrolowanego rozrodu sandacza, jednego z najcenniejszych gospodarczo i ekologicznie europejskich gatunków ryb słodkowodnych. Zawarto informacje na temat tarła sadzowego, sztucznego tarła ryb dzikich (pozyskanych ze środowiska naturalnego) i tzw. tarła pozasezonowego tego gatunku. Opisano metody i zasady przeprowadzania stymulacji tarła – fototermiczej i/lub hormonalnej. Wykazano, że zastosowanie metody przyżyciowego określania stadiów dojrzałości oocytów (fot. 1 i 2) i stymulacji hormonalnej, szczególnie w przypadku mniej dojrzałych samic, pozwala na istotne zwiększenie efektywności (odsetek owulujących samic) tarła sadzowego i sztucznego oraz zsynchronizowanie akcji tarłowej. Spośród testowanych do tej pory hormonów i preparatów hormonalnych (gonadotropiny (GtH), gonadoliberyny (GnRH) i ich syntetyczne analogi (GnRHa)), we wszystkich ww. metodach rozrodu sandacza, najbardziej skuteczne okazały się GtH – homogenat przysadki mózgowej karpia (CPE) i ludzka gonadotropina kosmówkowa (hCG) (tabela 1 i 2). Praca zawiera również najnowsze informacje na temat krótkoterminowego przechowywania nasienia, odklejania i inkubacji ikry sandacza.