

## **CONTROLLED REPRODUCTION OF PIKEPERCH, SANDER LUCIOPERCA (L.) – ACHIEVEMENTS AND PROSPECTS**

DANIEL ŻARSKI, SŁAWOMIR KREJSZEFF, KATARZYNA  
PALIŃSKA-ŻARSKA, DARIUSZ KUCHARCZYK

*Department of Lake and River Fisheries, University of Warmia and Mazury,  
ul. Oczapowskiego 5 (pok. 327), 10-719 Olsztyn, Poland*

### **KONTROLISANA REPRODUKCIJA SMUĐA, SANDER LUCIOPERCA (L.) – DOSTIGNUĆA I IZGLEDI ZA BUDUĆNOST**

#### *Apstrakt*

Smuđ, *Sander lucioperca*, je vrsta ribe koja ima visoku komercijalnu vrednost na Evropskom tržištu i jedan je od najboljih kandidata za intenzivnu proizvodnju u slatkim vodama. Do sada su se mnogobrojne studije bavile različitim aspektima razmnožavanja smuđa. Ipak, još uvek postoje mnogi aspekti koji nisu proučeni, koji su nejasni i veoma često nepredvidivi. To je ono što čini ovaj deo akvakulture veoma teškim za mnoge odgajivače riba i naučnike. Stoga je i cilj ovog članka da da precizan osvrt na trenutna dostignuća u kontrolisanoj reprodukciji smuđa kao i da ukaže na nove načine istraživanja u ovom polju. Da bi se dao opis trenutnog statusa veštačke reprodukcije smuđa opisani su: trenutno stanje hormonske stimulacije ovulacije i spermijacije, nova klasifikacija stupnjeva sazrevanja, metod za procenu kvaliteta jaja kao i protokoli za tretman jaja.

Još uvek jako malo znamo o nekim aspektima kontrolisane reprodukcije smuđa uprkos veoma intenzivnim istraživanjima. Rezultati objavljeni u poslednje tri godine pokazuju da još mnogo treba da se radi na razmatranju ove faze proizvodnje smuđa u akvakulturi.

Jedan od glavnih problema je, još uvek, optimizacija hormonalne stimulacije i mužjaka i ženki. Razvoj protokola za hormonsku indukciju ovulacije može da prouzrokuje ne samo usklađivanje i predviđanje trenutka ovulacije već i kontrolu nad kvalitetom jaja. Prema najnovijim nalazima efekta hormonske stimulacije i temperature na period latencije može se zaključiti da kombinovana stimulacija temperaturom i hormonskim agentima može da postane dobra metoda u kontroli ovulacije ove vrste. Ipak, potrebno je detaljnije istražiti finalno sazrevanje jajnih ćelija (process koji između ostalog uključuje migraciju vezikula gaminativnih ćelija i njihovu razgradnju [GVBD]) i efekte različitih doza i različitih preparata na datoj temperaturi i uslovima gajenja. Ovaj

aspektat je još važniji u gajenim nasadima koji su u potpunosti zavisni od uslova koje obezbeđuju farmeri.

Što se tiče daljih istraživanja gajenih nasada potrebno je razviti posebne protokole reprodukcije za sve uslove gajenja. Pored stimulacije ovulacije, pažnju treba posvetiti i stimulaciji spermijacije koja predstavlja veliki problem kod ove vrste (lični podatak).

Opšti rezultat reprodukcije smuđa se takođe može poboljšati primenom različitih metoda za in vitro oplodjenje. Protokoli za tretman jaja posle oplodjenja takođe treba da se unaprede. Iskustvo nekih naučnika ukazuje na to da su protokoli razvijeni u laboratorijama nedovoljno precizni i manje efikasni u određenim komercijalnim uslovima. Zbog toga je potrebno posebno ispitati transfer metoda razvijениh u laboratorijama na uslove komercijalne proizvodnje.

*Кljučne reči: Sander lucioperca, reprodukcija, hormonalni stimulacija*

*Keywords: Sander lucioperca, reproduction, hormonal stimulation*

## INTRODUCTION

The pikeperch, *Sander lucioperca*, is a species with a very high commercial value on the European market and it is one of the most promising candidates for intensive freshwater aquaculture production. It resulted in recent years in increased scientific activity aiming at the artificial reproduction, egg treatment, larvae and juveniles intensive rearing (e.g. Kestemont and Mélard 2000, Kestemont et al. 2007, Źarski et al. 2012a, 2012b, 2013). Among the production steps, one of the main bottleneck in this species is the control over the reproduction which directly affect effectiveness of further culture procedures (e.g., Kucharczyk et al. 2007, Zakeš and Demska-Zakeš 2009, Źarski et al. 2012a).

To this date numerous studies aimed at different aspects of reproduction of pikeperch. However, as considering controlled reproduction there is still many aspects which were not studied or which remains unclear and very often unpredictable what makes this step of aquaculture quite difficult for many fish farmers and scientists. Therefore, the aim of this article is to concisely review the current achievements in controlled reproduction of pikeperch as well as to point out the probable ways of further research in this field.

## CURRENT ACHIEVEMENTS IN CONTROLLED REPRODUCTION OF PIKEPERCH

### *Hormonal stimulation*

One of the biggest problem in controlled reproduction of pikeperch is to obtain high quality 'dry' eggs which are necessary for further procedures of *in vitro* fertilization. To this end, initially various kinds and doses of hormonal preparations in stimulation of ovulation in wild or pond reared females were tested (e.g., Kucharczyk et al. 2007, Zakeš and Demska-Zakeš 2009). This resulted in designation of the most suitable hormonal preparations for induction of ovulation in this species. According to the literature review provided by Zakeš and Demska-Zakeš (2009) the most suitable for ovulation induction was the hCG (human chorionic gonadotropin) and the gonadoliberine analogues (GnRH<sub>a</sub>), which are commonly used in aquaculture of cyprinids (e.g. Kucharczyk et al. 2008), were less efficient. However, in a recent study Źarski et al. (2012c) suggested that effectiveness of hormonal preparation (mostly GnRH<sub>a</sub>) was dependent on the ma-

turity stage of the fish. The fish more advanced in maturation responded for hormonal treatment (with GnRH $\alpha$ ) more efficiently. Nevertheless, the hormonal injection with hCG seems to be still the most suitable for controlled reproduction in pikeperch while the application of GnRH $\alpha$  was characterized by a lower efficiency in stimulation of ovulation as comparing to hCG (Kristan et al. 2012a, Źarski et al. 2012c).

One of the less studied aspect as considering hormonal treatment in aquaculture is the stimulation of spermiation with application the hormonal agents. In recent years, few studies have been made to improve the sperm quantity and quality in freshwater fishes by hormonal therapy (e.g. Cejko et al. 2010). In the case of pikeperch it was reported that males usually spermiate without the need of hormonal treatment (Kucharczyk et al. 2007). However, Źarski et al. (2012c) reported that hormonal stimulation generally improved the sperm motility regardless the hormonal preparation used (hCG or GnRH $\alpha$ ).

#### *Synchronization of ovulation*

The other obstacle in controlled reproduction of pikeperch is prediction of moment of ovulation (Źarski et al. 2012a). Especially, when pikeperch females are able to release the eggs in the tank even without the presence of the males (e.g., Kucharczyk et al. 2007, Źarski et al. 2012a). It was obvious that the moment of ovulation was strictly related with the maturation stage of the females, which was always determined before the hormonal injection, analogously to the protocols widely applied for cyprinids (see e.g. Brzuska 1979, Kucharczyk et al. 2007, Zakęś and Demska-Zakęś 2009). However, it still did not allow to predict moment of ovulation accurately. Therefore, Źarski et al. (2012a), after the extensive studies on Eurasian perch, *Perca fluviatilis* L., which allowed the development of new classification of maturation stages for freshwater percids (Źarski et al. 2011), implicated (with minor changes) the new system of maturational stages for pikeperch (see Źarski et al. 2012a). This 'new system' allowed considerable better synchronization of ovulation and much more precise prediction of ovulation in pikeperch. Also, the results obtained by those authors suggested that even effectiveness of hCG was strictly dependent on the maturation stage of females, where hCG treatment of less advanced fish (stage I and II of classification given by Źarski et al. 2012a) affected lower survival rate of embryos. Such observation explained in some extent variable embryonic survival of eggs obtained from hormonally stimulated pikeperch. However, the variable egg quality still widely observed during controlled reproduction of this species indicates that there is much more work needed to develop efficient hormonal treatment protocols.

#### *Egg quality evaluation*

Possibility of evaluation of the egg quality in aquaculture is of obvious importance (e.g. Bobe and Labbe 2010). It allows for evaluation of the effectiveness of culture (e.g. photothermal regime, feeding) and reproductive (e.g. hormonal treatment) protocols. On the other hand, fast, easy and objective egg quality indicator applicable before the *in vitro* fertilization may allow to choose only the highest quality eggs for further procedures. Additionally, such egg quality indicator would be very useful for scientists who working on the improvement of reproductive protocols and strict breeding procedures (e.g. gene banking, selective breeding). Application of high quality eggs for fertilization trials may save time and very often precious material (e.g. cryopreserved sperm). To date, the embryonic survival was the most widely applied egg quality indicator in pikeperch (e.g. Kucharczyk et al. 2007, Zakęś and Demska-Zakęś 2009, Źarski et al.

2012a). However, until recently the earliest possible time allowing determination of egg quality was 72 h post fertilization (Žarski et al. 2012a). The most recently Žarski et al. (2012b), during observation of the egg swelling process, recorded that cortical reaction intensity (observed between 3 and 5 min post activation) was an objective and reliable egg quality indicator. It allowed for considerably precise evaluation of egg quality before fertilization of particular egg batches and facilitated hatchery work with pikeperch eggs (for details see: Žarski et al. 2012b).

#### *Eggs fertilization and adhesiveness elimination*

The effectiveness of *in vitro* fertilization protocols depends, among others, on the activating medium used and time of contact sperm with water (e.g. Žarski et al. 2012d) as well as sperm-to-egg ratio (e.g., Kristan et al. 2012b). In the case of pikeperch only the latter variable was studied to date. It was found that during fertilization protocol 100,000 of spermatozoa should be used per each egg (Kristan et al. 2012b). There is still missing many data considering different activating medium as well as necessary time of contact of gametes with each other. Until now, only 'hatchery water' was used for *in vitro* fertilization and no data on the egg activation period (what was found to be crucial for fertilization effectiveness in perch by Žarski et al. 2012d) or other fertilization protocols (e.g. different activating solutions) are available.

In the case of pikeperch eggs, before the incubation in commonly used incubating devices (commonly known as 'Weiss' or 'Zug' jars), it is necessary to remove the adhesive layer of the eggs. For that purpose few methods were developed, where the most commonly applied were tannic acid (sometimes preceded by immersion in Woynarovich solution), talc with salt or milk, milk or clay (see e.g. Zakęs and Demska-Zakęs et al. 2005, Bokor et al. 2008, Žarski et al. 2013). All the methods were based on the treatment of the eggs few minutes following fertilization. However, Žarski et al. (2013) reported, that the effectiveness of the eggs unsticking procedures depends on the time following egg activation. The authors has proven that the tannic acid was the most effective after the egg finished their swelling period (water-hardening period), i.e. 30 minutes following activation. Application of the tannic acid for the elimination of the adhesion in pikeperch eggs 30minutes following egg activation allowed to shorten the period of eggs immersion (down to 1 min) with the average concentration ( $0.75 \text{ g L}^{-1}$ ) of tannin. Until that time the usual immersion time (allowing moderate effectiveness of eggs unsticking) was 5min with concentration of 0.5 to  $10 \text{ g L}^{-1}$  (Demska-Zakęs et al. 2005) what was reported to affect hatching rate (due to the chorion hardening caused by the tannic acid). However, the most recently the enzyme (Alcalase) treatment was successfully applied in laboratory condition (Kristan et al. 2012c). This proofs that the methods of eggs unsticking is still developing. Existence of few effective methods will create very convenient situation in the future where farmers will be having choice to use the most preferable by them protocol.

#### SOME PROSPECTS OF THE RESEARCH AND DEVELOPMENT IN CONTROLLED REPRODUCTION OF PIKEPERCH

Despite quite intensive research activities at improvement of the pikeperch controlled reproduction it is still very little known about some aspects. The results published within last three years has shown that there is still many work to be done as considering this phase of aquacultural production of this species.

One of the main problem is still the optimization of hormonal stimulation of both, males and females. The development of the protocols for hormonal induction of ovulation may allow not only the higher synchronization and prediction of moment of ovulation but also the control over the egg quality. According to the latest findings of hormonal stimulation (Kristan et al. 2012a) and temperature effect on latency time (Żarski et al. 2012c) it may be suggested that combined stimulation with temperature and hormonal agents may become a valuable tool in control of ovulation in this species. However, it require more detailed investigation of final oocyte maturation (process involving among others germinal vesicle migration and its break-down [GVBD]; Nagahama and Yamashita 2008) and the effect of different doses and different preparations at a given temperature and culture conditions. This aspect is even more important in cultured stocks which are totally subjected to the conditions provided by the farmers.

As considering the cultured stocks it has to be pointed out, that in the future research a specific reproductive protocols should be developed for each culture conditions. Despite the importance of stimulation of ovulation a special attention should be paid to the stimulation of spermiation, since the sperm of cultured fish is very often characterized by low motility rate (20-30%) (unpublished data).

The general reproduction outcome of pikeperch may be also improved by the application of different activating solutions for *in vitro* fertilization. This step of reproduction was not studied to date in this species. Additionally, the egg treatment protocols following fertilization needs improvements as well. In some specific cases, during semi-commercial conditions even 1 min immersion in tannic acid (as described for laboratory conditions by Żarski et al. 2013) was reported to have negative effect on the hatching rate (U. Ljubobratovic, personal communication). On the other hand, the trials under semi commercial conditions of the Alcalase treatment proved also to be less efficient as compared to that reported by Kristan et al. (2012c) for laboratory conditions (personal observation). It is therefore needed to make the critical trials on the transfer of the methods developed under laboratory conditions to the commercial scale.

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