THE SPAWNING EFFICIENCY INDEX AS A TOOL IN AQUACULTURE RESEARCH AND PRODUCTION

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INDEKS EFIKASNOSTI MRESTA KAO ALATKA ZA ISTRAŽIVANJE I PROIZVODNJU U AKVAKULTURI

Apstrakt

Intenzivno gajenje u akvakulturi je vrsta proizvodnje koja zahteva strogu kontrolu celog proizvodnog procesa. Ovo takođe važi za protokol reprodukcije, koji predstavlja prvi korak u intenzivnoj akvakulturi.

Efikasan protokol za reprodukciju je obično zasnovan na jednom broju veoma detaljnih eksperimenata, gde se svi faktori odgovorni za efikasnost mresta proveravaju. Međutim, glavna slaba tačka ovog procesa je ta što su zaključci o efikasnosti pojedinačnih protokola zasnovani na kratkoročnim ekperimentima, čiji su glavni indikatori preživljavanje embriona, stopa izleganja ili uopšteni kvalitativni parametri ranih stupnjeva larvi. Cilj ove studije je da proveri da li specijalno dizajniran indeks efikasnosti mresta (koji predstavlja broj mlađi proizveden u odnosu na jedinicu težine ženki) može da pokaže značajne razlike i slabosti najčešće korišćenih markera za kvalitet jaja, koji su indikatori efikasnosti mresta. Izabrana vrsta za model bio je Evroazijski grgeč, koji je danas jedan od najboljih kandidata za gajenje u intenzivnom sistemu u slatkim vodama.

Ribe su, u toku jeseni uzete iz zemljanog ribnjaka. Riba je bila izložena hladnim vremenskim uslovima u periodu od 40 dana. Ženke su zatim, kada je temperatura vode ponovo dostigla 10°C, nasumično raspodeljene u 6 tretmana (n=10 za svaku grupu). Svaka grupa bila je podvrgnuta različitim protokolima stimulacije hormona sa lososovim GnRHa (10, 25, 50 i 100 μg kg^{-1} u toku prvog ubrizgavanja i sa100 μg kg^{-1} u toku drugog, sedam dana posle prve aplikacije). Nakon toga, ribe su stavljene u odvojene tankove od 300 l.

Posle drugog ubrizgavanja, temperatura je podignuta na 12°C a svakom tanku dodato je 10 muških jedinki (koje su 4 dana ranije primile injekciju hCG u dozi od 500 IU kg^{-1}).

Riba je ostavljena da se spontano mresti. Jaja su sakupljana u narednih 10 dana i inkubirana na 14°C. Stopa preživljavanja embriona ustanovljena je u stadijumu očnog mehura. Posle izvaljivanja larve grgeča su gajene (iz svake grupe odvojeno) prateći isti protokol u toku od 48 dana. Larve su prvo hranjene sveže izvaljenim *Artemia* nauplii. 28og dana, gajene larve su nasilno odviknute i prebačene (bez uporedog hranjenja) na komercijalnu hranu.

Ustanovljena je stopa preživljavanja mlađi iz svake grupe posle odvikavanja i ukupna dužina tela (TL, mm). Izračunat je i indeks efikasnosti mresta (SEI) za svaku grupu, i on predstavlja broj odviknutih riba dobijenih od 1 kg ženki.

U kontrolnoj grupi 1 (koja je dva puta tretirana placebom) ovulacija se nije desila. U kontrolnoj grupi 2, dve ribe su ovulirale. Primena procesa u nekoliko ponavljanja izazvala je ovulaciju u 40 i 60%. Stopa preživljavanja embriona kod sakupljenih jaja bila je najniža u kontrolnoj grupi II. Među ostalim grupama zabeležena je slična stopa preživljavanja embriona. Nije bilo razlika među izlegnutim larvama, ako se uzme u obzir efikasnost punjenjaribljeg mehura kao i krajnja TL proizvedene ribe. Najniže SEI vrednosti zabeležene su u kontrolnoj grupi II. Najviše SEI vrednosti zabeležene su kod ribe koja je inicijalno tretirana sa 10 i 25 $\mu g\ kg^{-1}$.

Rezultati ove studije pokazuju da je SEI vrednost jako dobar i siguran indicator koji dozvoljava pouzdanu verifikaciju reproduktivnih protokola. Međutim, čini se da broj proizvedenih larvi sa napunjenim mehurom takođe može da posluži kao dobar indikator kvaliteta mresta. Ovakav indikator bi načinio process verifikacije efikasnosti reproduktivnih protokola mnogo kraćim i manje napornim, nego što se to ranije mislilo.

Takođe, rezultati ove studije pokazuju da ponavljana primena GnRHa može da poboljša mrest izvan sezone. Međutim, potrebno je raditi na optimizaciji doza i intervalima u kojima se daju injekcije da bi se proverila korisnost ovog procesa.

Ključne reči: hormonski tretman, efikasnost reprodukcije, gajenje percida, kvalitet jaja
Key words: hormonal treatment, reproduction effectiveness, percid culture, egg quality

INTRODUCTION

Intensive aquaculture is a type of production which requires strict control over the entire production process. This applies also to the reproductive protocol, which is the first step of intensive aquaculture (Mylonas et al. 2010, Żarski et al. 2011a).

Effective reproductive protocol is usually based on a number of very detailed experiments. However, the main weakness is that the conclusions are very often drawn on the base of short-term experiments where main indicators were embryonic survival, hatching rate or general qualitative parameters of early larval stages (Ronyai and Lengyel 2010, Żarski et al. 2011a, 2011b). Up to now there is no data clearly showing how different reproductive protocols affecting larvae quality from the perspective of further rearing procedures.

Eurasian perch, *Perca fluviatilis* L., is one of the most perspective freshwater fish species dedicated to the intensive aquaculture. Although huge progress has been made the seasonality of production is still one of the main problems in intensive production. To this end, many efforts have been undertaken at the development of out-of season reproduction which would allow production of stocking material year round. However, variable spawning effectiveness is usually observed. This probably stem from the fact that the research on re-

production very rarely consider the suitability of the produced larvae for further production process. In effect, many protocols considered survival rate of embryos and general quality of freshly hatched larvae (Kucharczyk et al. 1996, 1998, Kouril et al. 1997, Ronyai and Lengyel 2010, Żarski et al. 2011a, 2011b) as an egg quality indicators. It should be, however, emphasized that in the case of Eurasian perch even very low quality egg may exhibit developmental competence until hatching (Żarski et al. 2011b). Therefore, the aim of the study was to verify whether the specifically designed spawning efficiency index (which represents the number of weaned juveniles produced from a weight unit of females) may reveal the significant differences and weaknesses of typically used markers of egg quality being the indicators of spawning effectiveness.

MATERIALS AND METHODS

The fish (100 females and 75 males with an average weight of ~120 g) were obtained from the earthen ponds during autumn. Fish were kept in a recirculating aquaculture system (RAS) in 1000 l tank where they were subjected to 40 day-long vernalization period during which fish were kept in constant dimness (<5 lx). Next, when temperature was raised back to 10°C the females were divided randomly into 6 treatment groups (n=10 for each group). Each group were subjected to a various hormonal stimulation protocols (Tab. 1) and next they were placed separately to a 300 l tanks. After the second injection temperature was raised to 12°C and to each tank 10 males (injected 4 days in advance with the hCG at a dose of 500 IU kg^{-1}) were placed. The fish were left to spawn spontaneously. The eggs were collected for the next 10 days and incubated at 14°C. At the eyed-egg stage the survival rate of embryos was determined. After hatching, larvae were reared (from each group separately, in triplicates) with the same protocol for 48 days. Briefly, larvae were reared for the first 12 days at 15°C. At that time the swim bladder inflation effectiveness (SBIE - representing percentage of larvae with inflated swim bladder) was determined under the stereoscopic microscope. Next the temperature was gradually increased up to 25°C. Larvae were exposed to constant light conditions (24L:0D) for the entire rearing period. Larvae were fed first with freshly hatched *Artemia* nauplii twice a day *ad libitum* (the food was available for the larvae all the time). On day 28 of rearing larvae were transferred to the other RAS, were they were placed in 50 l tanks with the same stocking density (20 ind. l^{-1}). Then, fish were then weaned sharply (without co-feeding) into commercial diet. The tanks were cleaned twice a day. All the dead fish were counted in order to estimate the final survival rate of larvae. At the last day of rearing the number as well as total length (TL, mm) of juveniles produced from each group was determined. The spawning efficiency index (SEI), representing the number of weaned fish obtained from 1 kg of females, was calculated for each group.

The results obtained for different groups were analysed with one-way analysis of variance (ANOVA) and Tukey post-hoc test ($p<0.05$) was applied.

Table. 1. The hormonal treatment protocol applied during the experimental out-off season spawning of Eurasian perch. As a spawning agent pure salmon gonadoliberine analog was used (sGnRHa). Placebo treatment refers to the injection with 0.9% NaCl solution.

Group	1st injection	2nd injection
Control I	Placebo	Placebo
Control II	Placebo	100 μg kg^{-1}
E1	10 μg kg^{-1}	100 μg kg^{-1}
E2	25 μg kg^{-1}	100 μg kg^{-1}
E3	50 μg kg^{-1}	100 μg kg^{-1}
E4	100 μg kg^{-1}	100 μg kg^{-1}

RESULTS

In control group I (which was twice treated with placebo) no ovulation was certified. In control group II 2 fish were found to ovulate. The application of repeated administration caused ovulation in 40 and 60% of ovulation (Tab. 2). Embryonic survival rate of the collected eggs was the lowest ($p<0.05$) in control group II. Among the remaining groups similar ($p>0.05$) survival rate of embryos was noted (Tab. 2). There was no differences ($P>0.05$) among all the hatched larvae as considering the swim bladder inflation effectiveness as well as the final TL of the fish produced (Tab. 2).

The lowest SEI values were recorded for the control group II. The highest ($P<0.05$) were in groups E1 (Tab. 2).

Table 2. The results of the out-of season spawning of Eurasian perch after the application of different hormonal treatments and larvae characteristic obtained during the rearing period. SBIE – swim bladder inflation effectiveness observed in larvae obtained from different treatment groups, TL – total length of the body of juveniles at the end of the rearing period, SEI – spawning effectiveness index. Survival rate of larvae refer to the final survival of larvae from the start of weaning period until the end of experiment. Data in rows marked with different letter superscript were statistically different ($p<0.05$).

Group	Control II	E1	E2	E3	E4
Ovulation rate (%)	20	60	60	40	40
Embryonic survival rate (%)	22.6 ±16.0^{b}	63.0 ±39.2^{a}	46.4 ±51.1^{a}	60.9 ±40.8^{a}	67.8 ±26.6^{a}
SBIE (%)	31.3 ±9.1	38.6 ±6.1	36.2 ±6.4	32.9 ±7.8	35.4 ±9.2
Survival rate of larvae (%)	26.5 ±5.1	34.6 ±4.8	25.8 ±6.2	31.0 ±6.2	26.5 ±4.3
Final TL (mm)	132.5 ±11.6	131.9 ±10.7	134.3 ±11.0	131.1 ±15.4	129.0 ±9.5
SEI (No. of fish per kg of females)	29 ±6^{c}	265 ±23^{a}	194 ±41^{b}	173 ±46^{b}	181 ±43^{b}

DISCUSSION

Hormonal induction of ovulation in percids was usually conducted by a single injection of the spawning agent what usually allowed to obtain high ovulation rate and embryonic survival (Kucharczyk et al. 1996, 1998, Ronyai and Lengyel 2010, Żarski et al. 2011a). However, in the present study single injection (applied in control group II) was not enough to obtain satisfactory results. This could probably stem from too short vernalization period. Szczerbowski et al. (2009) reported that at least two months of wintering should be applied. However, shorter period of vernalization allowed to exhibit very clear differences between the treatment groups in terms of ovulation and embryonic survival rate. It allowed also to proof that repeated administration of GnRHa alone may improve the ovulation rate and enhance the egg quality obtained. Until now, the administration of the GnRHa was applied only in a single injection and very often together with dopamine antagonists (Szczerbowski et al. 2009, Targońska et al. 2014). To the best of our knowledge only Kouril et al. (1997) reported that application of GnRHa alone can be successfully applied in Eurasian perch. These authors reported that high dose (125 μg kg^{-1}) resulted in high ovulation rate. In the present study hormone dose applied in a single injection was also very high (100 μg kg^{-1}). However, the reaction of the fish organism was significantly weaker. But it stem probably from the much less advanced maturation stage of the fish in this study than those used by Kouril et al. (1997).

In the present study the efficiency of spawning, when ovulation and embryonic survival rates were reconsidered, was similar to the other studies on out-of season propagation of this species (Szczerbowski et al. 2010, Żarski et al. 2011a, Targońska et al. 2014). However, high standard deviation of the embryonic survival data made impossible to find any differences between the groups as considering egg quality. Finally, the calculated SEI indicated that groups treated with the initial dose of 10 and 25 μg kg^{-1} exhibit the highest efficiency of spawning. It is worth mentioning that the larval performance was quite similar among the groups after the inflation of the swim bladder. This suggests, that the larvae with inflated swim bladder can be considered as a fish with quality high enough to be suitable for further rearing procedure. The similar conclusion was also drawn by Żarski et al. (2011a) who also reported that SBIE was a good quality indicator of larvae in Eurasian perch. Therefore, it could be expected that the number of larvae with inflated swim bladder, not a weaned juveniles, can be reliable indicator of spawning efficiency.

CONCLUSIONS

The result of the present study suggests, that the SEI is a very good and reliable indicator allowing credible verification of the reproductive protocols. However, it seems that the number of produced larvae with inflated swim bladder can also constitute reliable indicator of spawning quality. Such an indicator would allow making the process of verification of effectiveness of reproductive protocols much shorter and less laborious, than initially expected.

Additionally, the results of the present study indicates that the repeated administration of GnRHa may improve the out-off season spawning. However, more work is needed at optimization of the doses and injection intervals in order to verify its usefulness.

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