

PRELIMINARY STUDY REGARDING THE EFFICIENCY OF DIFFERENT HORMONES ON PIKEPERCH SPERMATION

STUDIU PRELIMINAR PRIVIND EFICIENȚA DIFERIȚILOR HORMONI ÎN INDUCEREA SPERMIAȚIEI LA ȘALĂU

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The aim of this study is to test the efficiency of different hormones on pikeperch spermiation and to establish a protocol for obtaining a high quantity of milt. Sixteen clinically healthy adult pikeperch males (3-4 years old) were used in our experiments. They were intraperitoneal injected with one of the following hormones: human chorionic gonadotropine - hCG (Pregnyl), GnRH analog (Receptal) and carp pituitary extract (CPE). These hormones were injected following two experimental protocols. The main differences between these two protocols are the number of stripings after the first injection (1 and 2 for the 1st protocol and the 2nd one, respectively) and the moment of thermal stimulation. The best results in stimulating spermiation were obtained with hCG and CPE. The protocols applicable for milt collecting in pikeperch males must include a thermal stimulation period.

Key words: pikeperch, hormones, spermiation

Introduction

Pikeperch (*Sander lucioperca*) has been suggested among the most promising species for possible intensive culture in Europe (Hilge & Steffens, 1996). Zakes (1999) and Kestemont & Mélard (2000) emphasize that pikeperch is one of the best percid species candidate for aquaculture.

In several countries producing pikeperch for restocking or food markets, breeders are caught from open waters a short time before spawning or reared in ponds during winter and early spring and fertilization occurs naturally. Some countries still use the traditional spawning methods on nests (Bodis, 2006). Although pikeperch is a promising species for inland aquaculture, few information regarding its controlled reproduction with high efficiency are available. Controlled reproduction is the most reliable method for obtaining a high number of pikeperch larvae. The maturity of sexual products 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.

Hormonal stimulation, in the case of pikeperch, can be applied using two different ways. One of them is preparing a single injection. 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 (Kucharczyk, 2008).

The aim of this study is to test the efficacy of different hormones on pikeperch spermiation and establishing of a protocol for high quantity of milt obtaining.

Materials and Methods

Sixteen clinically healthy adult pikeperch males (3-4 years old) were used in our experiments. They were collected at the beginning of the breeding season of the year 2008, from Ineu Piscicultural Farm and B.H.M Hungary Ltd. (Fonyód, Ungaria). They were kept in the ponds at Banat's University of Agricultural Sciences and Veterinary Medicine from Timisoara until the water temperature reached 10.5°C. Other water parameters in pond were: pH = 8; dH° = 5; O₂ = 11.65 mg/l (104,6%); NO₂⁻ <0.3 mg/l; NO₃⁻ = 6 mg/l. Males were then moved into a concrete tank with about 7000 liters of water. The water was recirculated 0.3 times per hour and an aerator device was used to maintain the oxygen level at 7 ppm / liter. The male breeders were kept for 24 hours at a temperature of 11°C and than were randomly splitted in experimental variants. They were intraperitoneal injected at the base of the pelvic fin (fig. 1) with one of the following hormones: human chorionic gonadotropine - hCG (Pregnyl), GnRH analog (Receptal) and carp pituitary extract (CPE). Carp pituitary extract was homogenized in mortar and then dissolved in 0.9% NaCl.



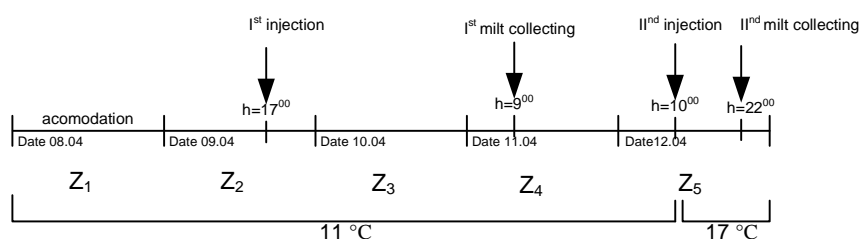
Fig. 1. Intraperitoneal injection of the males



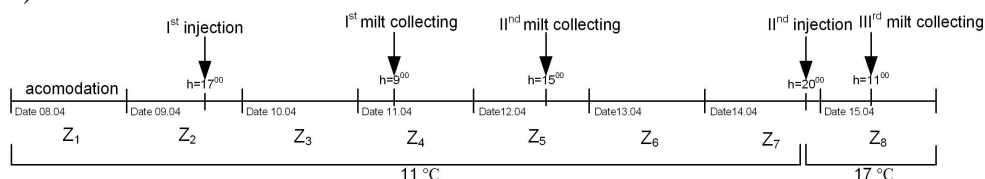
Fig. 2. Milt collection with catheter

These hormones were injected following two experimental protocols:

1)



2)



The main differences between these two protocols are the number of stripings after the first injection (1 and 2 for the 1st protocol and the 2nd one, respectively) and the moment of the thermal stimulation.

At the **first injection**, 4 groups with 4 males each were established and fish received:

- V₁: 500 UI hCG / kg body weight (b.w.);
- V₂: 0.25 ml Receptal / kg b.w.;
- V₃: 0.5 pituitary gland in 0.5 ml of 0.9% NaCl / kg b.w.;
- Control: 0.5 ml of 0.9% NaCl / kg b.w.

The fish received the **second injection** after one (Protocol 1) or two (Protocol 2) milt collecting, as follows:

Protocol 1 for 1st group (8 males):

V₁, V₂, V₃ and Control: 0.5 pituitary gland in 0.5 ml of 0.9% NaCl / kg b.w.;

Protocol 2 for 2nd group (8 males):

V₁: 500 UI hCG / kg body weight;

V₂, V₃ and Control: 0.5 pituitary gland in 0.5 ml of 0.9% NaCl / kg b.w.;

Pikeperch males were handled very gently. Before injection and milt collection, they were anaesthetized with MS222 (Tricaine Methane Sulphonate) at a concentration of 1:10000. Fish were cleaned and dried before milt collecting using a soft towel.

Milt was collected using sterile plastic syringes and a catheter (fig. 2) in order to avoid contamination with water or urine.

Results and Discussions

The efficiency of hormones and protocols applied in our experiments were established according with the moment when milt was easy to be striped and with the milt quantity.

In the first phase, both protocols revealed that hCG (V_1) and CPE (V_3) were the best for stimulating males. Anyway, no male released more than 1 ml of sperm, which is a very small quantity. GnRH had just a partial efficacy, because just a male from three released sperm, in a very small quantity, like in the other variants. In the control variant, no males released collectable sperm.

After the second injection and thermal stimulation, all males from group 1 (protocol 1) released milt very easily, at a gentle striping. We collected different quantities of milt, as it is shown in table 1.

Table 1

The milt volume striped from males in experimental variants				
Specification	Milt volume per male ($\bar{X} \pm S\bar{x}$) (ml)			
	V_1	V_2	V_3	Control
<i>1st milt collecting after 1st injection</i>				
1 st and 2 nd group				
Male 1	+	++	+	-
Male 2	+	-	+	-
Male 3	+	-	+	-
Male 4	+	-	+	-
<i>2nd milt collecting after 1st injection</i>				
2 nd group	1.667±0.914		0.800±0.354	0.096±0.058
<i>milt collected after 2nd injection</i>				
1 st group	2.625±0.106	3.600±0.671	1.216±0.246	
2 nd group	1.517±0.143	0.350±0.224	1.250±0.949	0.176±0.054

„-“ no milt; „+“ very small quantity of milt; „++“ small quantity of milt

In the table no. 1 volumes of milt striped from males stimulated with different hormones in two protocols are presented. It is relevant that hCG (V_1) and

CPE (V₃) had good effect in stimulating males, thus the males from the variants treated with these hormones were striped more easily. Therefore, at 1st milt collecting after the 1st injection, all males from variants V₁ and V₃ were able to release a very small quantity of milt. No male from control variant was prepared to release milt at the moment of 1st striping. In variant 3, only a male released a small quantity of milt.

After the second injection, all males from the 1st group were striped easily and different average volumes of milt were obtained per male, according with the variant. The highest volume was obtained in variant 3 (3.600±0.671 ml), suggesting that a first injection with GnRH, followed after 56 hours with the second injection with CPE, could be a good way to obtain a high quantity of sperm.

The data obtained in our experiment emphasize that in any protocol applicable for milt collecting in pikeperch males must have a thermal stimulation period, because only after this phase males are prepared to be striped and they release an important quantity of sperm.

Anyway, more data are necessary to be obtained in the next breeding season to find the best protocol that allows obtaining the highest quantity of milt with the minimum manipulation of the males.

Conclusions

- Human chorionic gonadotropine - hCG (Pregnyl) and carp pituitary extract (CPE) were the best for stimulating males;
- The highest volume of milt per male, in average, was obtained in variant 3 (3.600±0.671 ml) suggesting that a first injection with GnRH followed after 56 hours with a second injection with CPE, could be a good way to obtain a high quantity of sperm;
- The protocols applicable for milt collecting in pikeperch males must have a thermal stimulation period.

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