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Report

PIKEPERCH ARTIFICIAL REPRODUCTION AND LARVAE REARING

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Table of Content

Introduction 4
Short description of the method 4
Pikeperch broodstock management for artificial reproduction 4
Determination and stimulation of pikeperch female maturity 5
Fertilization 7
Incubation of eggs 9
Hatching of eggs 9
Larvae development 10
Feeding 10

Introduction

Pike perch is considered as one of the most promising freshwater fish species for domestic aquaculture in Europe. It has the highest potential in aquaculture diversification and market opportunities; however, it is particularly important to improve and adapt existing farming methods to ensure the future successful development of freshwater aquaculture on a European scale. The bio-economic feasibility of intensive breeding of pike perch is based on various national-scale projects of European countries. Intensive fish farming systems need to look for commercially high-value fish species that can reach commodity size in an economically acceptable short time, and pike perch is one of the fish species that meets both conditions if the latest approaches and adapted methods are used for its propagation and cultivation. In order to ensure the successful and economical production of this fish species under artificial intensive breeding conditions, it is necessary to first learn the methods of its reproduction, then to obtain suitable breeding material, and to raise the fish to the desired market size. These juvenile fish must be tamed and adapted to rearing in fully controlled artificial environments. Reproduction of the natural resources of pike perch is also important.

In Europe, aquaculture farmers are increasingly focusing on rearing pike perch in recirculating aquaculture systems. However, the complicated reproduction of pike perch, artificial feeding of juveniles and production of non-competitive products due to the energy-intensive production process are still the main hindering factors for the rapid development of the production of this fish species.

Short description of the method

Evaluation and sorting of breeding fish by sex. Examination of the stage of maturity of females. Application of spawning stimulators. Egg stripping, evaluation of egg quality (cortical reaction), artificial fertilization, desticking of eggs and incubation. Egg hatching, fry and fingerling feeding.

Historically, pikeperch were reproduced by natural and semi-natural methods. The natural method is to place the breeding fish in a pond where natural spawning, egg incubation and juvenile growth occur. The semi-artificial method is when fish spawn in spawning nests on an artificial substrate, and later the spawned eggs are transferred to juvenile rearing ponds, where the process continues naturally.

Pikeperch broodstock management for artificial reproduction

Broodstock is immediately after being caught in nature in the beginning of spawning season, the fish are transported in tanks to the hatchery. They are placed in adjustable tanks with adjustable temperature (12-14°C) and photoperiod. Males and females are kept separately.

The temperature in the tanks is set at 12°C. The photoperiod is constant throughout the time when the fish are kept in the hatchery and is equal to 12 h (12 h light : 12 h dark).

Determination and stimulation of pikeperch female maturity

After 12 h of acclimation or immediately, a sample of oocytes is taken from each female and immersed in clarifying solution (70% ethanol, 40% formaldehyde and 99.5% glacial acetic acid in the ratio of 6 : 3 : 1). After cytoplasm clarification, eggs are placed under a stereoscopic microscope and the maturity of each female has to be determined according to the classification provided for pikeperch. The classification covered four pre-ovulatory stages or some methods have six stages:

Stage I – germinal vesicle (GV) situated in the oocyte center, oil droplets poorly visible or beginning of GV migration (GV mostly located very close to the center of oocyte), beginning of coalescence of oil droplets, which are easily visible.

Stage II – migrating GV (reached half the oocyte diameter), oil droplets clearly visible or GV located above half the oocyte, a large oil droplet clearly visible (droplet diameter greater than GV diameter and reaches the size of 1/3 of the oocyte diameter) with visible smaller droplets.

Stage III – GV located above half the oocyte, some or one large oil droplet clearly visible (size of about half the oocyte diameter).

Stage VI – oocyte samples taken for analysis are macroscopically transparent; GV close to the oocyte membrane or no visible GV after being placed in Serra's solution (following GV breakdown (GVBD)), oocytes at the pre-ovulation stage.

Pikeperch males are kept in a separate tank and can be used to obtain milk in sufficient quantity if necessary.

The females are classified to be at Stages III and IV of maturity. Subsequently, all the females are labeled and subjected to hormonal stimulation. The marking of fish, with the method of cutting off a small part of the fin is applied (1. spiny dorsal, cranial part; 2. spiny dorsal, caudal part; 3. soft dorsal fin; 4. caudal, dorsal part; 5. caudal, ventral part; 6. anal; 7. right pelvic; 8. left pelvic; 9. right pectoral; 10. left pectoral).



Figure 1. Hormonal stimulation of the female pikeperch and necessary instruments for the artificial fertilization.

The fish in stage III and IV are receiving intramuscular injection of human chorionic gonadotrophin (hCG) at 500 IU kg. The injection is made in two steps, the first 1/2 and after 24 hours remaining 1/2 of the total dose. The spawning stimulant is injected intramuscularly (i/m) in the dorsal part of the body caudal to the dorsal fin. As well as is performed suturing of genital opening to prevent spontaneous spawning. In stage IV fish after the first and stage III after the second injection, ovulation (spawning) is checked every 4 hours.

Females in stages I and II can be receiving a pre-stimulation injection of chorionic gonadotrophin (hCG) at 100 IU kg at one time. Males can also be stimulated with this dose of hormone.

Fertilization

After ovulation, eggs from each female are collected separately into dry plastic containers. The eggs are weighed to determine the amount of milk required for insemination. The containers are subsequently covered tightly and stored until further procedures (not longer than 15 min). Small egg samples from each female are placed on separate Petri dishes and covered. The remaining eggs are used for incubation. Pour a little water on each egg sample in a Petri dish and observe the cortical reaction under a microscope after 1-3 minutes. Gametes are activated with clean hatchery water at a temperature of 12°C. The Petri dishes are put under a stereoscopic microscope. Upon contact with water, fish eggs are activated, which leads to a cortical reaction. In this reaction, the chorion separates from the vitelline membrane, creating the perivitelline space, into which the contents of cortical alveoli are released. The occurrence of egg deformations within 3 to 5 minutes after activation is shown to be closely correlated with their biological quality. A much lower percentage of swollen eggs is found among eggs with the lowest percentage of deformations (below 30%). If the cortical reaction is weak, such a batch of eggs is discarded and not fertilized.



Figure 2. Egg stripping and cortical reaction under the optical microscope.

Batches of eggs with a good (>30%) cortical reaction are fertilized separately. Sperm is collected in dry syringes. Eggs are inseminated with a mixture of freshly collected spermatozoa taken from two to four males (5ml/1kg eggs).

I Fertilization in clean hatchery water takes 2-4 minutes
II Short wash
III Ungluing or desticking the eggs (several methods)

1. Milk : Water 1:5 (30 min)

Milk quality matters UHT 3.5% (as more fat as better). Eggs should be properly covered with milk. No problems with sticking for half an hour, add the mixture as the eggs are swelling.

Drain the milk, with a caution.

Add the clay 20 - 25 g per litter of water. Solution amount depends on the number of eggs ≈0.5 L per 100g of eggs. Mix the solution for 2.5 - 3 minutes, add a lot of water. Properly clean from clay.

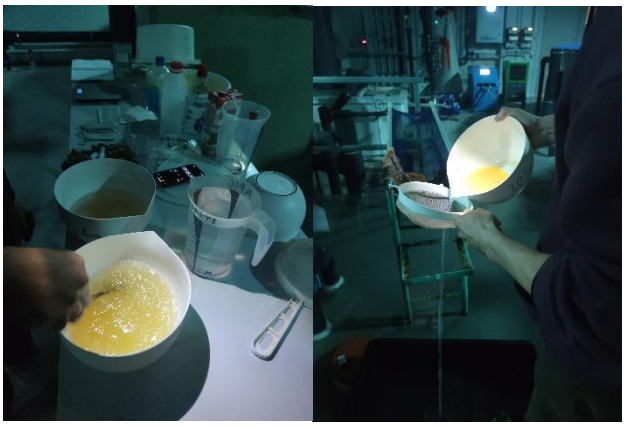

Figure 3. Processing of the eggs

2. Tannic acid

Fertilize the eggs. Wash the eggs. Mix in the water, change the water every 5 - 10 minutes. 30 minutes after putting the tannic acid solution. There is different concentration of tannic acid and duration of bath.

A) Tannin solution concentration 2g/5L. Washing last for 2 minutes, after which the eggs are rinsed with water and checked for sticking, leaving it at rest. If, after the first washing, the sticking of eggs remained, then a similar repeated desticking procedure can be performed. After the eggs are washed and placed for incubation.

B) Tannin solution concentration 5g/5L. Washing takes 1 minute.

Sometimes could be problems with hatching, hard egg shell.

3. Enzymes

Protease enzyme, alcalase (Merck EC 3.4.21.14 The number is important!) 25°C and pH 7.5

Apply the enzyme 4 - 5 minutes after fertilization. Concentration 1 - 1.5 ml/L. Temperature of the bath 20°C, perhaps 15 - 16°C is good enough. Add the salt or not, depends on water, perhaps from need. Bath the eggs for 5 minutes. Could be problems, possibilities digestion of the chorion.

4. Enzyme + Tannin

Perhaps the most promising method to do it fast offers high survival and preserves the chorion structure. Rather complicated needs concentration. The same problems with hatching as in usual tannic acid treatment.

Prepare enzyme 1 mL/L. Fertilize the eggs and wash. Add the enzyme to the water and straight to the eggs 200 mL per 100g of eggs mix for 10 - 15 seconds. Add the tannic acid solution to the eggs 200 mL per 100g of eggs mix for 30 second. Wash. Usually it is not a necessity to repeat.

After the ungluing procedure, the eggs should be incubated at 14 - 16°C in Weiss or McDonald hatching jars.

Incubation of eggs

Pikeperch eggs develop on average in 90-120 degree days. Between 60 and 90 degree days, the volume of eggs in incubators is measured to determine their number (~600,000/L). At around 80-90 degree-days, a fine mesh (200 µm) larval trap is placed in the larval catchment tank.



Figure 4. Incubation process

Hatching of eggs

The hatching of pike perch larvae usually takes place around 24 hours. If the eggs do not hatch by themselves until 140 degree-days, need to stimulate for their hatching by stopping

the water flow in the incubation jars for 10-15 min. Or put in a separate container for hatching. Sunlight is preferred.

Larvae development

After hatching, the larvae attach to the walls of the tank and then begin to float freely in the water mass in a horizontal direction. Later hatched larva swims better and starts feeding earlier good to reduce the gap between the hatching and start of exogenous feeding. The 180 – 200 degree days old larvae has to be fed or released into ponds for further rearing.

Feeding

Environmental parameters:

- Temperature: 15-18°C;
- Light: dispersed and non-intensive;
- Flow: depends on the tank size;
- p-welling should be most appropriate;
- as low as possible, -25-35% of water exchange.

In the Institute BIOR larvae are fed according to the program developed in previous years, live food - artemia (*Artemia salina*) - are used for 21 days. During the first two days, it is grinded and fed at two-hour intervals. During the last 19 days, together with artemia, artificial granulated starter feeds are used, gradually increasing their volume and the size of the granules, and from the 22nd day, larvae completely switch to granulated feed.