GENETICAL INACTIVATION OF PIKEPERCH (*SANDER LUCIOPERCA)* SPERM USING UV IRRADIATION

INACTIVAREA GENETICĂ A SPERMATOZOIZILOR DE ŞALĂU (*SANDER LUCIOPERCA*) PRIN UTILIZAREA RADIATIILOR UV

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Although pikeperch is a promising species for intensive aquaculture and some experiments regarding all-female production in this species were made, there are no specifically data regarding genetical inactivation of pikeperch sperm. The aim of this study is to test two different UV lights (15 and 30 Watts) and exposure times of the diluted milt at UV irradiation for sperm genetical inactivation, in order to establish the first step in the gynogenesis protocol for pikeperch. The milt collected from 4 clinically health adult pikepearch males (3-4 years old) was diluted 1:9 with Ringer solution, after that being placed into Petri dishes (Ø = 3 cm) in a thin layer of 1 mm. Two UV lights (λ = 253.7 nm) at 15 Watts (variant V_1) and 30 Watts (variant V_2) were used in our experiments. These were placed 20 cm above Petri dishes, and were established for each variant, 5 different periods for exposure to the irradiation: 8 min. (T_1), 11 min. (T_2), 14 min. (T_3), 17 min. (T_4) and 20 min. (T_5). The successful of irradiation was assessed by percent of fertilization (at 2 hours post fertilization), daily percent of survival, percent of hatching and percent of haploids (embryos/larvae with severely curved backbones and other deformities). After this study we could conclude that genetically inactivation of the pikeperch sperm can be performed with 15 Watts and 30 Watts UV lights at an exposure time which can vary between 8 and 20 minutes.

Keywords: pikeperch, sperm, genetical inactivation, UV

Introduction

Pikeperch (*Sander lucioperca*) is a relatively fast growing species in temperate freshwater, living in most of the large rivers and lakes of continental Europe, having high economical value. It has been suggested among the most promising species for possible intensive culture in Europe (Hilge & Steffens, 1996). Up to now, most market size pikeperch come from open waters (lakes, rivers, ponds or lagoons) and relatively few are produced under intensive and/or indoor conditions (Kucharczyk et al., 2007).

There are a lot of fishes which show sexual dimorphism. The most widespread type of sexual dimorphism is size, and very often in these species the female is larger or at least achieves a larger size than male (Moyle and Cech, 2000). Pikeperch show sexually related dimorphic growth in which females grow faster and reach a larger size than males. Therefore, production of monosex female stocks in this species is an important fishery management tool (Demska-Zakes and Zakes, 1997, 2008).

The methods most commonly employed to manipulate the sex of fish are based on exogenous hormone treatment, chromosome manipulation or a combination of both (Strüssmann et al., 2005). Gynogenesis (all-maternal inheritance) is a way which allows producing monosex population, and it is accomplished by activating cell division with irradiated sperm and then restoring diploidy to the developing zygote (Dunham, 2004). The irradiation of the sperm in order to brake or destroy its DNA, can be performed either using ultraviolet (UV) irradiation or gamma irradiation (Dunham, 2004; Ranga and Shammi, 2002), but UV irradiation has advantages and is more effective than gamma irradiation (Dunham, 2004). Ultraviolet (UV) irradiation has been successfully used in a number of fish species to inactivate paternal DNA and produce genetically-inactivated sperm with adequate motility and capacity for eggs activation (Felip et al., 2001; Grozea et al., 2006; Morgan et al., 2006).

Although pikeperch is a promising species for intensive aquaculture and some experiments regarding all-female production in this species were made, there are no specifically data regarding genetical inactivation of pikeperch sperm.

The aim of this study is to test two different UV lights (15 and 30 Watts) and exposure times of the diluted sperm for irradiation, in order to establish the first step in the gynogenesis protocol for pikeperch.

Materials and Methods

Four clinically health adult pikepearch males (3-4 years old) were captured from the pond in March 24, 2009 and were kept indoor for 7 days, until the experiment starts, into a tank (5.65 m^3) in aquaculture facility from Banat's University of Agricultural Sciences and Veterinary Medicine from Timisoara. The water temperature rose from 10°C to 11.5°C. The water was recirculated 0.3 times per hour and an aerator device was used to maintain the oxygen level above 8 ppm. The males were then moved in aquaculture laboratory and they where anaesthetized in ethyl 3-aminobenzoate methanesulfonic acid salt 98% (E10521, Sigma-Aldrich) (0.1 mg l^{-1}), and then they were intraperitoneal injected at the base of the pelvic fin with 300 UI hCG / kg body weight (hCG-Chorulon, Intervet). After resuscitation, males were introduced into 500 l aquarium at a temperature of 17°C for 48 hours. Pikeperch males were then anaesthetized and were cleaned and dried before milt collecting using a soft towel. Milt was collected using plastic syringes and a catheter in order to avoid contamination with water or urine.

Before UV irradiation the milt was diluted 1:9 with Ringer solution (Braun Melsungen), after that being placed into Petri dishes (Ø = 3 cm) in a thin layer of 1 mm. The control variant was also prepared with diluted milt and was maintained in the same condition as experimental ones (17°C) until all variants was exit from irradiation not more than 20 minutes.

Two UV lights (λ = 253.7 nm) at 15 Watts (variant V_1) and 30 Watts (variant V_2) were used in our experiments. These were placed 20 cm above Petri dishes, and were established for each variant, 5 different periods for exposure to the irradiation: 8 min. (T_1), 11 min. (T_2), 14 min. (T_3), 17 min. (T_4) and 20 min. (T_5).

The milt from all variants and the control one was used to fertilize fresh eggs (1 ml of eggs per variant) in the same time. The adhesive layer was removed from the eggs surface using alcalase (protease from *Bacillus licheniformis*, Sigma). Eight minutes post-fertilization the eggs were bathed in alcalase (2 ml l^{-1}), for 12 minutes. Then the fist enzyme bath was removed and the second enzyme bath (10 ml l^{-1}) was applied for 60 seconds. After the second bath the eggs are washed 3 times for the removal of the enzyme. Fertilized eggs were incubated in 400 ml plastic flasks (NUNC easy flask) which assured good monitoring conditions, and 4 times per day, 50% of the water from each flask was changed with the fresh water having the same quality. At that time dead embryos were removed.

The successful of irradiation was assessed by percent of fertilization (at 2 hours post fertilization), daily percent of survival, percent of hatching and percent of haploids (embryos/larvae with severely curved backbones and other deformities).

Results and Discussion

The fertilization was partially affected by irradiation of the sperm as it is shown in table 1, but in all variants the fertilization percent was above 57%.

Table 1

The percent of fertilized eggs in the control and experimental variants

Variant – timing	Total eggs	No. of fertilized eggs	% of fertilization
Control	1465	1082	73.86
V_1 - T_1	1205	826	68.55
V_2 - T_1	1371	838	61.12
V_1 - T_2	1146	797	69.55
V_2 - T_2	1133	655	57.81
V_1 - T_3	1478	1066	72.12
V_2 - T_3	1281	740	57.77
V_1 - T_4	1523	994	65.27
V_2 - T_4	1255	885	70.52
V_1 - T_5	811	580	71.52
V_2 - T_5	1332	833	62.54

V_1 – 15 Watt; V_2 – 30 Watt; T_1 – 8 min; T_2 – 11 min; T_3 – 14 min; T_4 – 17 min; T_5 – 20 min;

Table 2

The embryos survival rate after the first 3 days of incubation

Variant – timing	Total no.	Survival after							
		12 h		Day 1		Day 2		Day 3	
		no.	%	no.	%	no.	%	no.	%
Control	1465	552	37.68	365	24.91	274	18.70	191	13.04
V_1 (15 Watts)									
8 min	1205	159	13.20	63	5.23	36	2.99	29	2.41
11 min	1146	131	11.43	48	4.19	41	3.58	35	3.05
14 min	1478	139	9.40	23	1.56	16	1.08	13	0.88
17 min	1523	101	6.63	27	1.77	20	1.31	18	1.18
20 min	811	92	11.34	9	1.11	6	0.74	4	0.49
V_2 (30 Watts)									
8 min	1371	150	10.94	132	9.63	64	4.67	30	2.19
11 min	1133	116	10.24	65	5.74	29	2.56	21	1.85
14 min	1281	95	7.42	47	3.67	31	2.42	28	2.19
17 min	1255	71	5.66	17	1.35	15	1.20	15	1.20
20 min	1332	82	6.16	8	0.60	6	0.45	6	0.45

Table 3

The embryos survival, hatching and haploid rates between 4 and 6 days of incubation

Variant – timing	Total no.	Day 4				Day 5				Day 6					
		Survival		Hatching		Survival		Hatching		Survival		Hatching		Haploids	
		no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%*
Control	1465	179	12.22	60	4.10	155	10.58	155	10.58	152	10.38	152	10.38	-	-
V_1 (15 Watts)															
8 min	1205	25	2.07	1	0.08	17	1.14	6	0.50	9	0.75	8	0.66	9	100
11 min	1146	35	3.05	2	0.17	29	2.53	15	1.31	26	2.27	18	1.57	17	94.44
14 min	1478	10	0.68	1	0.07	10	0.68	3	0.20	11	0.74	6	0.41	6	100
17 min	1523	18	1.18	1	0.07	12	0.79	2	0.13	9	0.59	5	0.33	5	100
20 min	811	4	0.49	-	-	4	0.49	-	-	4	0.49	3	0.40	3	100
V_2 (30 Watts)															
8 min	1371	21	1.53	2	0.15	16	1.17	4	0.29	10	0.73	8	0.58	8	100
11 min	1133	17	1.50	-	-	9	0.79	2	0.18	8	0.70	4	0.35	4	100
14 min	1281	21	1.64	3	0.23	15	1.17	9	0.70	9	0.70	9	0.70	8	88.88
17 min	1255	14	1.12	5	0.40	11	0.88	6	0.48	10	0.80	8	0.64	8	100
20 min	1332	6	0.45	-	-	5	0.38	1	0.07	5	0.38	2	0.15	2	100

* % of haploids is calculated as percent from hatched larvae at 6 days

These results suggest that sperm motility and their capacity for eggs activation was not seriously affected, even they were irradiated with 30 Watts UV lights for 20 minutes, percent of fertilization being 62.54 for this variant.

The percent of survival was evaluated daily until hatching as it is shown in tables 2 and 3 and in chart 1-5. The data from table 1 emphasize high mortality in all experimental variants. The survival rate in the first 3 days of incubation vary between 0.45 (V_2 –T_5) and 3.05 (V_1 –T_2). We cold speculate that high mortality

percent in experimental variants is due to haploids that couldn't continue their development and died.

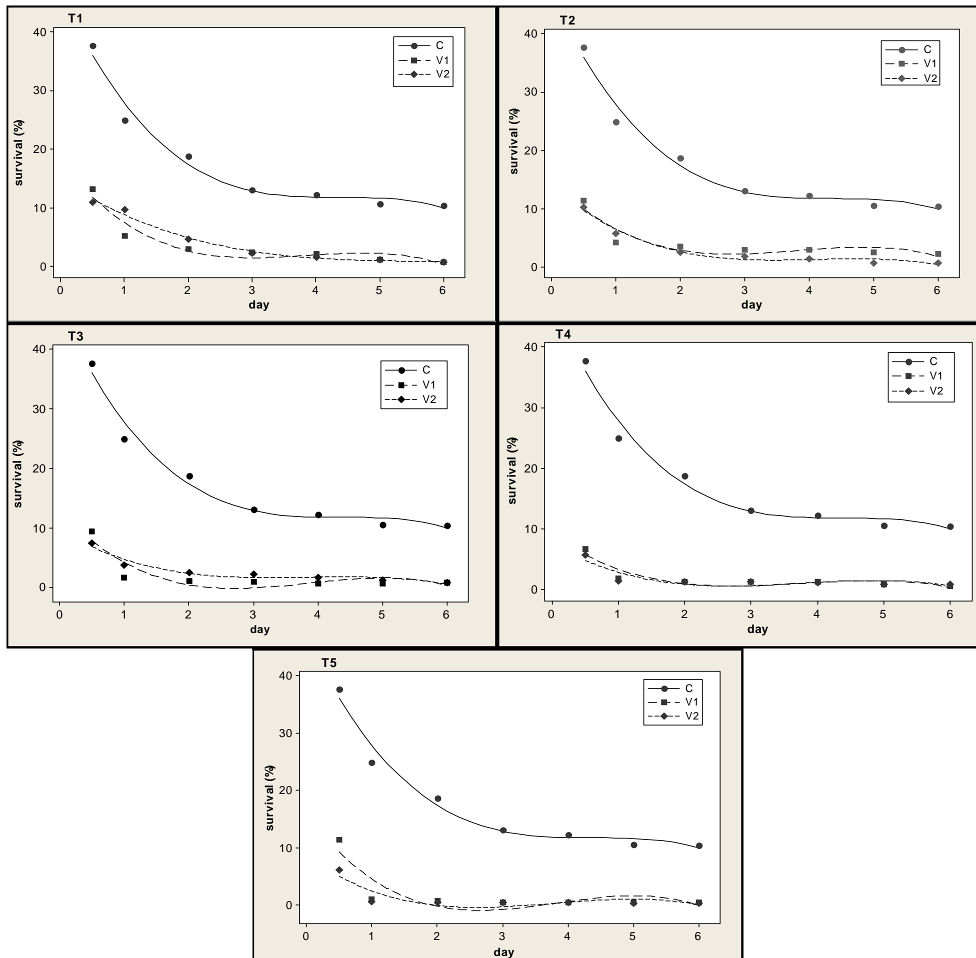

Chart 1-5. Graphical representation of survival percent modulated with third degree polynomial regression

After 4 days post-fertilization the larvae start to hatch almost in all variants but in higher percent (4.10%) in control one. No hatch was registered in the 4th day post-fertilization in experimental variants V_1 - T_5, V_2 - T_2 and V_2 - T_5. Until the 6th day post-fertilization in all variants were hatched larvae and the percent of hatching varied between 0.15% (V_2 - T_5) and 1.57% (V_1 – T_2) from initial number of incubated eggs, but in control variant the hatching percent reached 10.38%.

Hundred percent of larvae which survived 6 days post-fertilization hatched in control variant and in one of experimental variants (V_2 - T_3). In the other variants there were larvae which couldn't hatch because abnormal development as a result

of haploidy (fig. 1). Anyway, 100% from hatched larvae in experimental variants shown clearly signs of morphological deformities, being haploids (fig. 2). Only one normal developed larva in each from experimental variants $V_1 – T_2$ and $V_2 - T_3$ was observed, that suggest hat not all sperm in these variants were genetically inactivated.

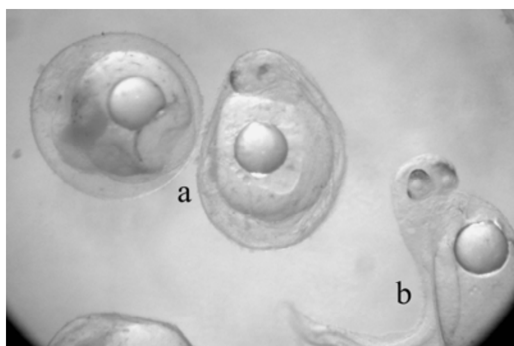

Figure 1. Two unhatched and abnormal developed pikeperch embryos (a) and one hatched larvae with curved backbone and other morphological abnormalities as a result of haploidy (b)

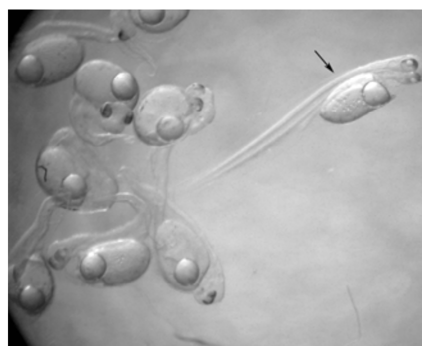

Figure 2. Group of pikeperch haploid larvae and one normal developed larva (arrow to it) from experimental variants.

Corroborating all data we can conclude that genetically inactivation of the pikeperch sperm can be performed with 15 Watts and 30 Watts UV lights and the exposure time to the irradiation could vary between 8 and 20 minutes. Hundred percent of genetically inactivated sperm we obtained in the variants with 8, 14, 17, 20 minutes exposure time to the 15 Watts UV light and also in the variants with 8, 11, 17 and 20 minutes exposure time to the 30 Watts UV light.

Conclusions

- Sperm motility and their capacity for eggs activation was not seriously affected in experimental variants after UV irradiation of the milt;
- High mortality rate of the embryos was registered in all experimental variants, this being related with irradiation of the milt;
- Genetically inactivation of the pikeperch sperm can be performed with 15 Watts and 30 Watts UV lights and the exposure time to the irradiation could vary between 8 and 20 minutes.

References

1. Demska-Zakes K., Zakes Z., 1997, Effect of 17α-methyltestosteroneone on gonadal differentiation in pikeperch, Stizostedion lucioperca, *Aquaculture Research*, **28**, 59-63.

2. Denska-Zakes K., Zakes Z., 2008, Endocrine sex control strategies of pikeperch (Sander lucioperca), in Fontaine P. et al, *Percid Fish Culture – From Research to Production. Abstracts and short communications*.
3. Dunham R.A., 2005, Aquaculture and Fisheries Biotechnology. Genetic Approaches, CABI Publishing, Cambrige, MA.
4. Felip, A., Zanuy, S., Carrillo, M., Piferrer, F., 2001, Induction of triploidy and gynogenesis in teleost fish with emphasis on marine species. *Genetica*, **111**, 175-195
5. Grozea A., Muscalu-Nagy C., Banatean-Dunea I., 2006, Chromosomes inactivation in common carp spermatozoa in order to obtain all-female population by means of gynogenesis, *Proceeding Abstr., „Animal Husbandry, Veterinary and Agroeconomy in transitional processes"*, University of Novi Sad, Herceg Novi, p. 88.
6. Hilge V., Steffens W., 1996, Aquaculture of fry and fingerling of pike-perch (Stizostedion lucioperca L.) – a short review, *Journal of applied Ichthyology*, **12**, 167-170.
7. Kucharczyk D., Kestemont P., Mamcarz A., (Ed.), 2007, Artificial reproduction of pikeperch, Mercurius Kaczmarek Andrzej, Olsztyn.
8. Morgan, A.J., Murashige, R., Woolridge, C.A., Luckenbach, J.A., Watanabe, W.O., Borski, R.J., Godwin, J., Daniels, H.V., 2006, Effective UV dose and pressure shock for induction of meiotic gynogenesis in southern flounder (Paralichthys lethostigma) using black sea bass (Centropristis striata) sperm, *Aquaculture*, **259**, 290-299.
9. Moyle P.B., Cech Jr. J.J., 2000, Fishes. An Introduction to Ichthyology 4th ed., Prentice-Hall Inc., NJ.
10. Rhanga M.M., Shammi Q.J., 2002, Fish Biotechnology, Ed. Agrobios, Jodhpur.
11. Strüssmann C.A., Karube M., Miranda L.A., Patino R., Somoza G.M., Uchida D., Yamashita M., 2005, Methods of Sex Control in Fishes and an Overview of Novel Hypotheses concerning the Mechanisms of Sex Differentiation in Pandian T.J. et al. editors, *Fish Genetics and Aquaculture Biotechnology*, Science Publishers Inc, Enfield, NH.

Acknowledgements

This study was supported by Program 4 „Parteneriate", projects 61-020 (SANDERBIOT) and 51-096 (SANDERTEH) – www.sander.ro