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SERBIA

POLJOPRIVREDNI FAKULTET
UNIVERZITET U BEOGRADU
SRBIJA

VII INTERNATIONAL CONFERENCE

VII MEĐUNARODNA KONFERENCIJA

WATER & FISH

CONFERENCE PROCEEDINGS
June 10 - 12, 2015

ZBORNIK RADOVA
10. - 12. Jun 2015.

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UNIVERZITET U BEOGRADU, POLJOPRIVREDNI FAKULTET - SRBIJA

UZ PODRŠKU
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I
PAN-HELENSKOG DRUŠTVA TEHNOLOGA IHTIOLOGA

U OKVIRU PROGRAMA
EU FP7 PROJEKTA AREA

VII MEĐUNARODNA KONFERENCIJA ”VODA I RIBA”

ZBORNIK PREDAVANJA

Poljoprivredni fakultet, Beograd– Zemun, Srbija
10. – 12. jun, 2015. godine

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Univerzitet u Beogradu, Poljoprivredni fakultet

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– 10% PG (20% total concentration), 15% MeOH – 15% PG (30%) and 20% MeOH – 20% PG (40%). Chemicals were purchased from Reanal (Budapest, Hungary) and Sigma-Aldrich (Budapest, Hungary). The sperm suspension was plunged directly into liquid nitrogen without pre-cooling in its vapour. For all vitrification experiments Cryotops (Fig. 2.) were used as cooling device (Kitazato-Dibimed, for 2 µl of solution). Motility of vitrified samples was determined using CASA following thawing of Cryotops directly into the activating solution under the microscope.

We have collected eggs from Eurasian perch females (Fig. 3.). After the use of single hormonal injection (hCG, 500 IU/g fish) the females were checked daily by ovarian biopsy, enable to predict the accurate time of the ovulation. One day before the planned ovulation the genital pore were sutured to avoid spontanous spawning into the tank. After removing the suture and drying the genital area the eggs were stripped into a dry dish.

For fertilization tests approximately 100 eggs were used for each sample. Sperm was vitrified in the presence of cryoprotectants at a final concentration of 30%. We thawed the vitrified Cryotops directly into 10 ml of activating solution (50 mm NaCl) in a petri dish containing the eggs. Fresh sperm was used for a control fertilisation test. Fertilized eggs were incubated in a floating system (styrofoam boards with filters on a tank, Fig.4.).

Three trials were realized to find the most appropriate number of cryotops per egg batch. During the experiments we have used 1, 6 and 18 cryotops for the fertilisation of one portion of eggs. The 2 µl diluted sperm on one cryotop contained approximately 0,33 µl sperm. Ferilization ratios were counted with a steromicroscope (Fig. 5.).

To analyze the results, statistical software GraphPad Prism 5.0 for Windows (GraphPad Software, La Jolla, California, USA) was used.

Figure 1. *Sperm stripping*

Figure 2. *Cryotops*

Figure 3. *Stripping the eggs*

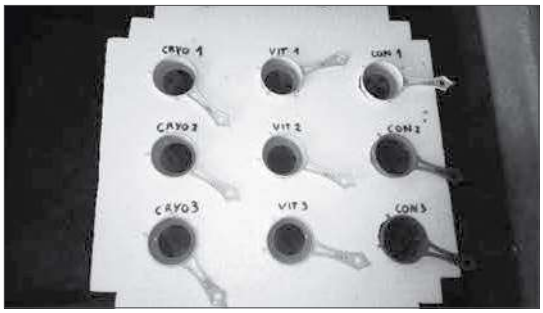

Figure 4. *Floating incubation*

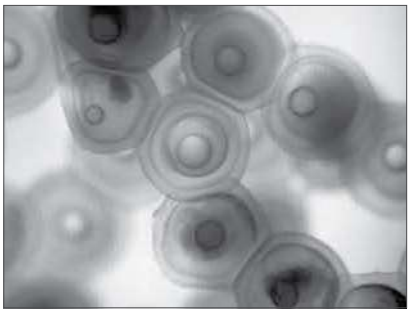

Figure 5. *Developing embryos*

RESULTS AND DISCUSSION

According to the motility data, the use of 20% cryoprotectant (10% methanol and 10% PG), the measured average progressive motility after thawing was 10,31% (±1,92). In the case of 30% cryoprotectant (15% methanol and 15% PG) this value was 13,95% (±1,67), and with using 40% total cryoprotective agent we reached 7,07% (±4,05). Several studies suggest that fish spermatozoa can tolerate high cryprotectant concentrations when the proportion of the chemicals is appropriate (Cuevas-Uribe et al., 2011a, b). During previous experiments with other fish species we have also found that the most appropriate cryoptotectant concentration is around 30% of the total solution, because with lower alcohol content the creation of harmful ice crystals is not fully inhibited, and higher concentrations of cryoprotectants are toxic for spermatozoa.

Thus we can conclude that best vitrification method was carried out with using the following protocol: 1:5 dilution ratio, Tanaka extender, 30% cryoprotectant (15% methanol + 15% propylene-glycol), cooling device: Cryotop, 2 µl droplets (Fig. 6.).

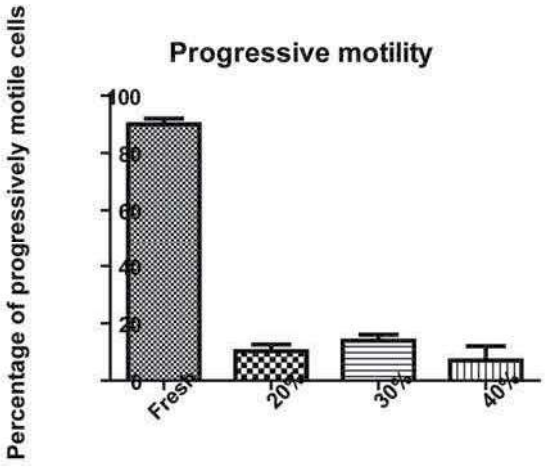

Figure 6. Progressive motility measured in fresh sperm, and sperm vitrified with 20%, 30% and 40% cryoprotectant. Columns represent the datas from 3 experiments, vitrified 3 samples in 4 replicates.

According to the fertilisation rates of the three trials, we can conclude that increasing the number of used Cryotops enhances the fertilisation ratio: fertilising with one Cryotop resulted 1,44% (±1,58) fertilisation, 6 Cryotops per egg batch resulted 4,9% ±4,77), and 18 Cryotops resulted 1,27% (±1,51). In the case of the third experiment (with 18 Cryotops), egg quality was moderate (under 70% fertilisation in the control group), thus further studies are needed to achieve more accurate results.

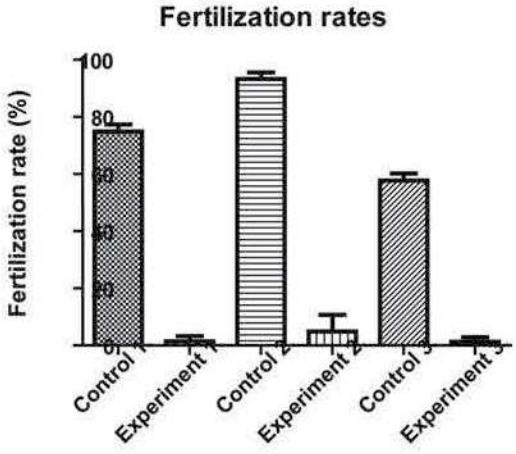

Figure 7. Fertilizaton rates measured in controlls, and vitrified sperm with 1/6/18 cryotops per egg batch. Columns represent the datas from 3 experiments.

CONCLUSION

Successful vitrification of Eurasian perch sperm was conducted for the first time. Motile spermatozoa were recovered following vitrification of sperm and fertilization of eggs with vitrified sperm resulted in developing embryos in this species. Thus, vitrification of sperm is feasible in the Eurasian perch, although futher studies are needed to improve this technique.

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REFERENCES

Cuevas-Uribe, R., Leibo, S. P., Daly, J., Tiersch, T. R. (2011/a). Production of channel catfish with sperm cryopreserved by rapid non-equilibrium cooling. Cryobiology 63 (3): 186-197.

Cuevas-Uribe, R., Yang, H., Daly, J., Savage, M. G., Walter, R. B. Tiersch, T. R. (2011/b). Production of F1 Offspring with vitrified Sperm from a Live-Bearing Fish, the Green Swordtail (*Xiphophorus hellerii)*. Zebrafish 8 (4): 167-169.

Figueroa, E., Risopatrón, J., Sánchez, R., Isachenko, E., Merino, O., Isachenko, V., Valdebenito, I. (2013). Spermatozoa vitrification of sex-reserved rainbow trout *(Oncorhyncus mykiss):* Effect of seminal plasma on physiological parameters. Aquaculture 372-375: 119-126.

Figueroa, E., Merino, O., Risopatrón, J., Sánchez, R., Effer, B., Isachenko, E., Farias J. G., Valdebenito, I. (2015). Effect of seminal plasma on Atlantic salmon *(Salmo salar)* sperm vitrification. Theriogenology 83: 238-245.

F. Lahnsteiner, Spermatozoa of the teleost fish *Perca fluviatilis* (perch) have the ability to swim for more than two hours in saline solutions, Aquaculture 314 (2011) 221–224.

G. Szabó, T. Müller, M. Bercsényi, B. Urbányi, B. Kucska, Á. Horváth: Cryopreservation of European eel (Anguilla anguilla) sperm using different extenders and cryoprotectants, Acta Biologica Hungarica 56 (2005) 173–175.

Hsun-Heng Tsai, Chien-Hsiung Tasi, Wei-Te Wu, Fu-Zen Chen, Pei-Ju Chiang: Numerical investigation into thermal effect of pre-cooling zone in vitrification-based cryopreservation process. Cryobiology 70 (2015) 32-37.

THE RELATION BETWEEN TANK COLOR AND EUROPEAN SEABASS (*DICENTRARCHUS LABRAX*) JUVENILES GROWTH PERFORMANCE

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ODNOS IZMEĐU BOJE BAZENA I PRIRASTA MLAĐI EVROPSKOG BRANCINA (*DICENTRARCHUS LABRAX*)

Apstrakt

Dizajn sistema za gajenje riba je veoma bitan za održivu i visoko profitabilnu proizvodnju u akvakulturi. Različitim vrstama riba potrebani su drugačije dizajnirani sistemi i veštačke sredine. Sistemi u zatvorenom prostoru su korisni za mrestilišta a tankovi su veštačka staništa za vrste gajene u tim sistemima. Prethodna istraživanja pokazuju da boja zida bazena utiče na nivo stresa kod riba (Rotlant et al., 2003) i parametre koji utiču na rast, a dobrobit riba može da bude ugrožena u stresnim uslovima (De Silva and Anderson 1994). Cilj ovog istraživanja je da ispita efekte koje različite boje zidova tankova imaju na prirast mlađi Evropskog brancina (*Dicentrarchus labrax*).

480 jedinki mlađi nasumice su raspoređene u 12 identičnih plastičnih tankova (40 jedinki po tanku). Zapremina svakog bazena iznosila je 40 litara. U triplikatu su korišćene četiri različite boje bazena (crvena, zelena, plava i svetlo žuta). Riba je hranjena komercijalnom hranomn za brancina 2 puta dnevno u period od 60 dana.

Najveći prirast dostigla je riba gajena u crvenim bazenima, dok je riba gajena u žutim bazenima imala najmanji prirast.

Prethodna istraživanja su pokazala da boja zida bazena utiče na prirast ribe u uslovima gajenja i da je različitim vrstama riba potrebna drugačija boja bazena da bi postigle najbolji

prirast (Duray et al., 1996; Rotland et al., 2003; Imanpoor and Abdollahi, 2011). Rezultati pokazuju da boja bazena utiče na prirast riba u uslovima gajenja.

INTRODUCTION

System design is very important for sustainable and high profit aquaculture production. Different species need various system design and artificial area. Indoor aquaculture systems are useful for hatcheries and tanks are artificial habitats for culture species in these systems. Previous studies reported that, tanks wall color can affected stress level of fishes (Rotlant et al., 2003) and growth-related parameters and welfare of the fish may be negatively affected under stressful conditions (De Silva and Anderson 1994). In this study it was intended to research the effects of different tank colors on growth performance of Seabass (*Dicentrarchus labrax*) juveniles.

MATERIAL AND METHODS

A total of 480 juveniles were randomly distributed in 12 identical plastic tanks (40 fish per tank) with a water volume of 40 L. Four different tank colors (red, green, blue and light yellow) with triplicate treatments were used. Fish were fed on commercial seabass diet 2 times a day for 60 days.

RESULTS

The best growth performance was obtained in red color, while the lowest growth of fish was recorded in the yellow colored tanks.

DISCUSSION

Previous studies showed that fish growth performance under culture conditions were affected by tank wall color and different species need different tank color for best growth performance (Duray et al., 1996; Rotland et al., 2003; Imanpoor and Abdollahi, 2011). According to results, tank color affects growth performance in fish under culture conditions.

REFERENCES

De Silva SS, Anderson TA (1994) Fish nutrition in aquaculture. Chapman & Hall, London, p 319

Rotllant J, Tort L, Montero D, Pavlidis M, Martinez SE, Wendelaar B, Balm PHM (2003) Background colour influence on the stress response in cultured red porgy Pagrus *pagrus*. Aquaculture 223:129–139

Duray MN, Estudillo CB, Alpasan LG (1996) The effect of bacground color and rotifer density on rotifer intake, growth and survival of the grouper (*Epinephelus suillus*) larvae. Aquaculture 146:217–224

Imanpoor MR, Abdollahi M (2011) Effects of tank color on growth, stress response and skin color of juvenile caspian kutum *Rutilus frisii Kutum*. Glob Vet 6(2):118–125.