

Storage of unfertilized eggs of grass carp, *Ctenopharyngodon idella*, in artificial media

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Abstract. Two separate experiments were conducted to investigate the effects of artificial storage media, temperature and holding duration on the percentage of eyed embryos, hatching and malformed larvae of grass carp eggs, *Ctenopharyngodon idella*. Grass carp ova, outside the ovary cavity, were stored in GCACF (Grass Carp Artificial Coelomic Fluid) and Dettlaff extender at 4°C and 20°C for 30, 60, 120 and 180 min. GCACF medium was made base on composition of grass carp coelomic fluid. For this work and designing the GCACF medium, ova were collected from ten females and they were filtered and coelomic fluid was separated for chemical composition analysis. This study was done with 16 treatments and one control group in three replicates. The results shown that storage duration and artificial media had significant effects on eyed embryos rate and hatching percentage ($P < 0.05$) but their effect on rate of malformed larvae was not significant ($P > 0.05$). Also, temperature had significant effects on the percentage of eyed embryos and hatching ($P < 0.05$) but no significant effects on the number of malformed larvae ($P > 0.05$). Eyed embryo and hatching rates decreased with increasing storage duration. Highest eyed embryo and hatching rate between treatments were observed in GCACF, 30 min storage and 4°C and lowest of them were observed in Dettlaff extender at 20°C and 180 min storage.

Key words: artificial media, short-term storage, *Ctenopharyngodon idella*.

Introduction. Various species of carp have been domesticated and reared as food fish across Europe and Asia for thousands of years. These various species appear to have been domesticated independently, as the various domesticated carp species are native to different parts of Eurasia (Balon 1995ab; Molnar 2009).

Family *Cyprinidae* is one of the most successful families of fish, with more than 2000 species grouped in approximately 340 genera (Bănărescu & Coad 1991). Cyprinid fishes show a wide distribution around the world and occur in almost every freshwater environment. One of them is the grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) and except America and Australia it is produced worldwide in large quantities for human consumption.

Like in the case of reproduction of other Cyprinid fishes (Taati et al 2010a-e; Ebrahimzadeh Mousavi et al 2011), the best management and proper technologies in hatchery are important aspects of grass carp production. As it has been shown, the egg quality is the primary factor in determining embryo survival of numerous species of fish (Nagler et al 2000; Kabir et al 2011). As over-ripening of eggs will inevitably eventually occur in fish broodstocks, it is important to obtain and fertilize eggs at the correct time after ovulation. In fish hatcheries, over-ripening represents a problem when ovulated eggs are not stripped on time, especially for those fish whose eggs can only be obtained by manual stripping and fertilized artificially (Kjorsvik et al 1990). Oocyte viability refers to the time period during which fertilization of ovulated oocytes remains possible once they have been emitted by the female (Legendre et al 1996). Prolonging their viability becomes an important issue in aquaculture management. Fertility of fish gametes

undergoing external fertilization is limited to a few seconds or minutes in water because oocytes are activated and undergo cortical reaction leading to micropyle closure (Hart 1990). Mature ova may remain arrested for weeks in the second meiotic metaphase until ovulation and activation. Nevertheless, after ovulation of eggs, the storage time available for effective use and the options to prolong their viability becomes a crucial issue (Rothbard et al 1996). Still, the eggs must be fertilized within certain duration after ovulation to produce viable embryos and their time varies between species from hours to weeks (Hobby & Pankhurst 1997). The fertilizability of mature ovulated ova gradually decreases and then disappears altogether when they are retained in the body cavity of the female, kept in coelomic fluid or in water, or under in vitro conditions (Rothbard et al 1996). Fertility of the eggs is retained much longer in saline and especially in coelomic fluid (Dettlaff et al 1993). With the addition of an extender solution, storage at reduced temperatures can delay progressive declines in gamete viability. However, there is a lack of research regarding storage of eggs in extender solutions and different temperatures prior to fertilization (Donald et al 2002; Muchlisin et al 2010). According to Stoss (1983), successful short-term oocyte preservation is obtained in species whose post-spawning activation can be controlled so that it will only occur after the oocyte is released in the water, as in salmonids. In other fish groups such as *Carassius* and *Cyprinus* auto-activation occurs after ovulation, and also when oocytes are stored in Ringer solution. Thus, precluding mechanical activation of the oocyte appears to be critical for the success of short-term storage (Leung & Jamieson 1991).

Storage for short-term duration is generally a useful and advantageous practice in various hatchery operations, where ovulation is hormonally and/or environmentally controlled and stimulated (Rothbard et al 1996). Short term-storage is frequently applied in short-distance transport of gamete, in the synchronization of gamete collection from males and females during artificial propagation, in the over-ripening process of eggs (Kjorsvik et al 1990) and in studies of chromosome-set manipulations (Stoss 1983); this short-term storage improves accessibility to specific seedstocks and facilitates seedstock production, preserves genetic diversity, improves selective breeding and hybridization, and expands research opportunities (Donald et al 2002).

The post-ovulation storage of ova in the ovarian cavity (in vivo) of common carp *Cyprinus carpio* Linnaeus, 1758 is usually very short, ranging from 1 to 6 h (Jahnichen 1981). Ova stripped after ovulation and stored in vitro progressively lost their fertility after 4–6 h at temperatures of 15–20 °C (Zlabek & Linhart 1987). Eggs of *Oreochromis mossambicus* (Peters, 1852) stored in coelomic fluid at 20 °C for 19 h yielded 35% fertilization, while at temperatures below 18°C, the fertilizing ability was decreased after 1.5 h (Harvey & Kelley 1984). After dilution either with fresh water or saline solution, the duration of fertilizing capacity of ova is very short, decreasing to zero within a few minutes (Renard et al 1987).

The aim of the present study was to evaluate the effects of holding temperature (4°C and 20°C) and short-term storage time (30, 60, 120 and 180 min) as well as storage medium (Dettlaff extender and GCACF medium) in terms of eyed embryo, hatching and malformed larvae of grass carp.

Material and Method. Broodstocks (fifteen mature males and ten mature females) were captured from Sari hatchery, during the spawning season of grass carp, and transferred to the place of experiment.

Egg, milt and coelomic fluid collection. In order to stimulate the males to produce gametes, we injected intraperitoneally: 0.5 mL kg⁻¹ b.w. Ovaprim (sGnRHa+dompridon). Milt samples were collected during the 2010 spawning season from ten sexually mature three-years-old male carps (mean TW: 1486.73 ± 572.69 g). Semen samples were collected by massage from the anterior portion of the testis towards the genital papilla. Care was taken to avoid contamination of the semen with water, mucus, blood cells, faeces or urine. Semen of each male was collected by using 2 mL polyethylene syringes and sperm batches were stored in a refrigerator at (4°C) temperature until used for fertilization. At fertilization, sperm of all males was pooled in the same proportions by volume as that obtained by stripping. These mixtures were used for the fertilization

assay. Females were injected with 0.5 mg kg⁻¹ Ovaprim (sGnRH+Dompridon) and 12 hours after injection treatment females were stripped. Eggs were collected in a bowl and during collection, ova were held in their coelomic fluid at 20 °C.

Designing GCACF (Grass Carp Artificial Coelomic Fluid). In order to mimic ovarian fluid for practical operation the mean composition of ovarian fluid was determined in ten samples. Based on the results, a specific GCACF was designed that can be made available to hatchery operators. The ovarian fluid was pipetted gently out of the egg batch and into screw-cap tubes with minimal head space to minimize air equilibration. Ovarian fluids were centrifuged at 3000 rpm for 8 min. The pH of ovarian fluids was immediately determined using a laboratory pH meter (pH meter, Iran T.S. co 462) and samples were frozen at -20°C until the analysis moment. Two mineral (Ca⁺⁺ and Mg⁺⁺) and two biochemical parameters (total protein and Glucose) of the ovarian fluid were measured using the spectrophotometric method (WPA-S2000-UV/VIS Cambridge - UK). The concentration of Na⁺ and K⁺ were determined with flame photometer (Jenway PFP 7, England) (standard kits from Parsazmoon, Tehran, Iran).

After chemical analysis the composition of a specific GCACF was formulated as 174 mM NaCl, 1.78 mM KCl, 0.84 mM MgSO₄.7H₂O, 0.93 mM CaCl₂, 7.48 mM glucose, 1g bovine serum albumin, 20 mM NaHCO₃, 20 mM Hepes buffer (4-2-hydroxyethyl-1-piperazineethanesulfonic acid).

Storage of ovulated ova. All of the female ova were pooled. For each replicate 300 mg (about 300) eggs were placed in Petri dishes (10 cm diameter) and Dettlaff extenders (111.3 NaCl + 3.3 KCl + 2.1 CaCl₂ + 23.8 NaHCO₃) and GCACF were added to them. This study was done with sixteen treatments and one control group in three replicates. Half of Petri dishes of each medium were placed into a refrigerator (exposure to 4°C) and half of them with eggs submersed in to the other medium held at 20 °C. Batches of about 300 ova (300 × 3 replicates) were fertilized immediately after egg collection and was considered as control group.

Fertilization and incubation. Just before each insemination (after storage of 30, 60, 120 and 180 min), the excess of Dettlaff extender and GCACF was removed.

After storage for above time in the two test temperature, undiluted sperm (0.02 mL) was added, and water (10 mL) at ambient temperature (20 °C) was used to activate the gametes, allowing fertilization and adhesion of eggs to the Petri dishes, then transferred to aquarium (containing 80 L aerated water) and were placed at their bottom. The eggs were placed in a monolayer to ensure adequate oxygen supply. The percentage of eyed embryos, hatching rate and percentage of malformed larvae were recorded.

Statistical analysis. Data analysis of variance (two-way ANOVA) was done with Duncan test for the comparison of mean values resulting from the various treatments at a significance level of P<0.05. Before analysis by ANOVA, data was used for normality of data distribution and homogeneity of variance. Results are presented as mean ± SD. Statistical analyses were performed with SPSS 16 for Windows statistical package.

Results and Discussion

Chemical composition of the ovarian fluid. The mean chemical composition and the overall variation of the studied parameters in the ovarian fluid of ten samples collected from ten matured females of grass carp are shown in Table 1.

Table 1

Mean chemical composition of the ovarian fluid of ten mature females of grass carp

| Variable | Mean ± SD |
|---------------------------|----------------|
| Na ⁺ (mmol/L) | 174.56 ± 11.42 |
| K ⁺ (mmol/L) | 1.78±0.39 |
| Mg ⁺⁺ (mmol/L) | 0.84±0.41 |
| Ca ⁺⁺ (mmol/L) | 0.93±0.26 |
| Glucose (mmol/L) | 7.48±0.52 |
| Total protein (g/dL) | 5.67±0.29 |
| pH | 8.2±0.9 |

Effect of short-term storage of ova on eyed embryos and hatching rate. Results showed that the percentage of eyed embryo and hatching of stored ova in Dettlaff extender and GCACF, decreased with increasing time at 4 °C and 20 °C. Differences were observed in eyed embryos and hatching rate between experimental treatments in both extenders, but this difference was not significant between 20 and 30 min storage in GCACF media ($P>0.05$). Temperature had a significant effect on eyed embryo and hatching rate, so that these parameters at 4 °C were greater than at 20 °C. Effects of media storage resulted in a greater decrease of survival at high storage temperature than at low storage temperature, indicating that storage media may have damaged the fertilization capacity of ova more than storage temperature.

Effect of egg storage on malformed larvae rate. The rate of larvae malformed has not been significantly different in all treatments after 30, 60, 120 and 180 min storage. Literature shows that malformation occurs in fish larvae due to a wide range of combined environmental and genetic factors, many of them poor studied (Petrescu-Mag 2007; Petrescu-Mag et al 2008, 2011).

Effect of storage duration, holding medium and temperature on fertilization, hatching and malformed larvae rate. Result showed that the effect of storage time and medium on eyed embryos and hatching rate was significant ($P<0.05$). Storage temperature had a significant effect on fertilization rate ($P<0.05$). Fertilization and hatching rate decreased with increasing time (30, 60, 120 and 180 min) (Table 2) but malformed larvae rate had not changed with time in all treatments (Figure 1). Highest eyed embryo and hatching rate between treatments was observed in GCACF, 20 min storage and 4°C (GCACF + 20 min + 4°C) and lowest eyed embryo and hatching rates were observed in Dettlaff + 180 min + 20°C.

Table 2
Effects of storage medium, temperature and time on eyed embryos and hatching rate (Mean \pm SD) in grass carp

| Treatments | Eyed embryo | Hatching |
|-------------------------|-------------------------------|-------------------------------|
| Control | 72.29 \pm 2.67 ^a | 79.84 \pm 2.18 ^a |
| Dettlaff- 4°C- 30 min | 59.43 \pm 1.24 ^c | 74.58 \pm 4.37 ^c |
| Dettlaff- 4°C- 60 min | 34.51 \pm 3.78 ⁱ | 47.64 \pm 3.29 ⁱ |
| Dettlaff- 4°C- 120 min | 19.92 \pm 2.6 ^m | 25.71 \pm 2.65 ^m |
| Dettlaff- 4°C- 180 min | 14.37 \pm 2.68 ⁿ | 21.28 \pm 4.36 ⁿ |
| Dettlaff- 20°C- 30 min | 36.42 \pm 3.49 ^h | 49.76 \pm 2.39 ^h |
| Dettlaff- 20°C- 60 min | 29.67 \pm 5.19 ^j | 43.58 \pm 3.08 ^j |
| Dettlaff- 20°C- 120 min | 22.29 \pm 6.41 ^k | 37.26 \pm 5.63 ^k |
| Dettlaff- 20°C- 180 min | 10.84 \pm 7.12 ^p | 10.73 \pm 4.29 ^p |
| CACF- 4°C- 30 min | 62.54 \pm 8.37 ^b | 77.18 \pm 5.49 ^b |
| CACF- 4°C- 60 min | 62.19 \pm 2.23 ^b | 75.51 \pm 3.08 ^b |
| CACF- 4°C- 120 min | 47.82 \pm 4.06 ^f | 59.36 \pm 4.48 ^f |
| CACF- 4°C- 180 min | 19.52 \pm 2.71 ^l | 33.94 \pm 5.78 ^l |
| CACF- 20°C- 30 min | 55.68 \pm 4.3 ^d | 69.58 \pm 2.35 ^d |
| CACF- 20°C- 60 min | 51.22 \pm 3.49 ^e | 62.45 \pm 3.73 ^e |
| CACF- 20°C- 120 min | 42.35 \pm 1.93 ^g | 54.71 \pm 3.84 ^g |
| CACF- 20°C- 180 min | 11.64 \pm 3.87 ^o | 14.26 \pm 4.85 ^o |

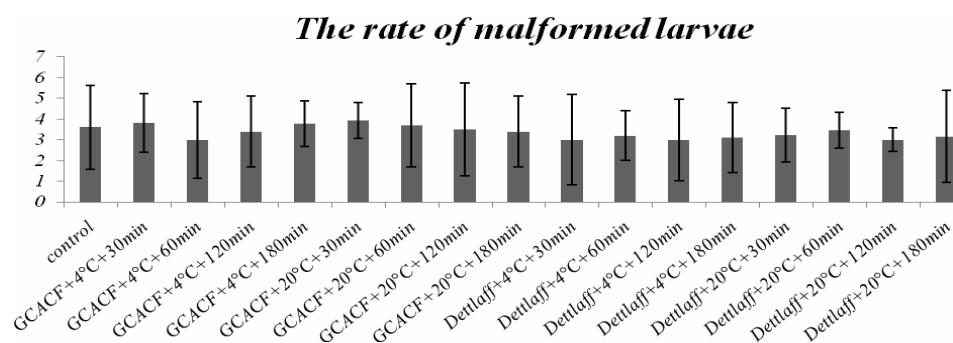


Figure 1. The percentage of larvae malformation in experimental groups (control, GCACF and Dettlaff extenders).

Few other studies have dealt with fertilizing capacity and hatching rate of warm-water fishes ova kept in various extenders. One of the parameters that had key role in short-term storage was a medium in which ova were stored. In our observations (Table 2) eyed embryo and hatching rate in GCACF medium is better than Dettlaff extender. It could be hypothesized that strong buffer (Hepes) that was used in building this artificial medium can ensure stable pH. Result of this study showed that the eyed embryo and hatching rate of carp ova stored in GCACF and Dettlaff extender was low and confirm the results of Rothbard et al (1999) and Linhart et al (2001) during androgenesis in common carp. Therefore, this medium is not very good for grass carp ova storage in durations longer than 20 and 30 min. Linhart & Billard (1995) found that European catfish *Silurus glanis* Linnaeus, 1758 ova were sensitive to fresh water, various saline solutions and urine. The survival of ova was very short, 40 s to 2 min in fresh water and 2–4 min in saline solution.

Hsu & Goetz (1993) preserved the fertilizing capacity of goldfish *C. gibelio* ova for 30 min in Ringer solution + Tris pH 7.3 and 0.5 mg mL⁻¹ soybean trypsin inhibitor compared with control (Ringer solution + Tris pH 7.3 and fresh water), which had a different osmotic concentration from the experimental solution.

In general, loss of fertility in water or saline solution is believed to result from sealing of the micropylar canal, as a consequence of oocyte activation following cortical reaction. Trypsin treatment of nonfertilized *Oryzias latipes* (Temminck & Schlegel, 1846) oocytes for 5 min produced total occlusion of the lower third of the micropylar canal and glycoprotein digestion in the mucous area of the micropylar region, even in the absence of cortical alveoli exocytosis (Iwamatsu et al 1997). According to Saad & Billard (1987) the fertilizing capacity of common carp ova was lost 3 min after immersion in fresh water, probably due to the closing of the micropyle (Kudo et al 1994). Nevertheless, these authors found that fertilizing capacity was retained for 8 min when the ova were held in spermatozoa-activating solution (40 mM NaCl, 5 mM KCl, 20 mM Tris-HCl pH 8).

In our experiments eyed embryo and hatching rate at 4°C were higher than at 20°C (Table 2). We can hypothesize two reasons for these performances. First auto activation at 20 °C is more probable than at 4 °C. Second apparently, grass carp ova were very sensitive to high temperature (20 °C). There are many probable reasons for decrease in the percentage of eyed embryo and hatching but none of them have been proved up to know. These results did not agree with ex situ storage of oocytes of *Prochilodus argenteus* Spix & Agassiz, 1829 at 18 °C, temperature that caused a drastic reduction in fertilization rate as compared with storage at 26°C (Rizzo et al 2003). However in some studies, the hatching performance declined with the increasing of temperature. In the South American catfish *Rhamdia quelen* (Quoy & Gaimard, 1824) oocyte viability starts decreasing after 9 h at 20 °C or after 5 h at 24 °C, and viable oocytes are totally absent after 15 h at 20 °C or after 8 h at 24 °C (Espinach et al 1984).

In this work, with increasing time, eyed embryo and hatching rate were decreased. This result agreed with several other works, such as Goetz & Coffman (2000) and Sohrabnezhad et al (2006). Oocyte overripening may be evaluated on the basis of the decline in fertilization and hatching rates (Formacion et al 1993). Since fertility and deformed larvae rate are strongly correlated, the fertilization rate alone may be sufficient to indicate the subsequent performance of embryos and larvae (Springate et al 1984). But in this work, the rate of deformed larvae in grass carp eggs stored in GCACF and Dettlaff extender at 4 °C and 20 °C was not correlated with eyed embryo and hatching rate.

Short-term preservation is only feasible with eggs from species in which activation is controllable (Stoss 1983). In cyprinid fish, like carps auto activation is not controllable and occurs in different ways like increase or decrease in water temperature or pH or other reasons.

In the present study it has been shown that under hatchery conditions, ova of grass carp can be stored in GCACF for 60 min or in Dettlaff extender for 30 min.

Conclusion. Results showed that using GCACF (Grass Carp Artificial Coelomic Fluid) and Dettlaff extender (111.3 NaCl + 3.3 KCl + 2.1 CaCl₂ + 23.8 NaHCO₃) is not very

appropriate for short-term storage of grass carp eggs longer duration than 30, 60, 120 and 180 min. Also, maintaining grass carp ova at 4 °C resulted in percentage of eyed embryo and hatching rate greater than at 20 °C. Storage duration had a significant effect on hatching performance, but media, temperature and time had no significant influence on percentage of malformed larvae.

References

- Balon E. K., 1995a Origin and domestication of the wild carp, *Cyprinus carpio*: from the Roman gourmets to the swimming flowers. *Aquaculture* 129: 3-48.
- Balon E. K., 1995b The common carp, *Cyprinus carpio*: its wild origin, domestication in aquaculture, and selection as colored nishigikoi. *Guelph Ichthyological Review* 3: 1-55.
- Bănărescu P., Coad B. W., 1991 Cyprinids of Eurasia. In: *Cyprinid fishes systematic, biology and exploitation*. Winfield I. J., Nelson J. S. (eds.), pp. 127-155. Chapman and Hall, London.
- Dettlaff T. A., Ginsburg A. S., Schmalhausen O. I., 1993 *Sturgeon Fishes. Developmental Biology and Aquaculture*. Springer-Verlag, Berlin, 300 pp.
- Donald W., Glenn M., Terrence T., 2002 Effect of extenders and osmotic pressure on storage of eggs of ornamental common carp *Cyprinus carpio* at ambient and refrigerated temperatures. *J World Aquacult Soc* 33(3): 254-267.
- Ebrahimzadeh Mousavi H. A., Behtash F., Rostami-Bashman M., Mirzargar S. S., Shayan P., Rahmati-holasoo H., 2011 Study of *Argulus* spp. infestation rate in Goldfish, *Carassius auratus* (Linnaeus, 1758) in Iran. *HVM Bioflux* 3(3): 198-204.
- Espinach R. A., Amutio V. G., Mestre A. J. P., Orti G., Nani A., 1984 Induced breeding of the South American catfish *Rhamdia sapo* (C. & V.). *Aquaculture* 37: 141-146.
- Formacion M. J., Hori R., Lam T. J., 1993 Overripening of ovulated eggs in goldfish. I. Morphological changes. *Aquaculture* 114: 155-168.
- Goetz F. W., Coffman M. A., 2000 Storage of unfertilized eggs of rainbow trout (*Oncorhynchus mykiss*) in artificial media. *Aquaculture* 184: 267-276.
- Hart N. H., 1990 Fertilization in teleost fishes: mechanisms of sperm-egg interactions. *Int Rev Cytol* 121: 1-66.
- Harvey B., Kelley R. N., 1984 Short-term storage of *Sarotherodon mossambicus* ova. *Aquaculture* 37: 391-395.
- Hobby A. C., Pankhurst N. W., 1997 Post-ovulatory egg viability in the snapper *Pagrus auratus*. *J Mar Freshw Resour* 48: 385-389.
- Hsu S. Y., Goetz F. W., 1993 Inhibition of chorion expansion and preservation of fertility in goldfish (*Carassius auratus*) eggs by protease inhibitor. *Canadian J Fish Aquat Sci* 50: 932-935.
- Iwamatsu T., Yoshizaki N., Shibata Y., 1997 Changes in the chorion and sperm entry into the micropyle during fertilization in the teleostean fish, *Oryzias latipes*. *J Dev Growth Differ* 39: 33-41.
- Jahnichen H., 1981 Kurzeitkonservierung von Karpfensperma als weitere Möglichkeit der Rationalisierung der künstlichen Vermehrung von Karpfen (*Cyprinus carpio* L.). *Zeitschrift für die Binnenfischerei DDR* 28: 244-245. [In German with English abstract].
- Kabir M., Imanpoor M. R., Asghari M., 2011 The effects of different times of reproductive migration on biochemical compounds of ovarian fluid and on fertilization rate of Persian sturgeon (*Acipenser persicus*) brood stocks. *AAFL Bioflux* 4(3): 351-360.
- Kjorsvik E., Mangor-Jensen A., Holmefjord I., 1990 Egg quality in fishes. *J Adv Mar Biol* 26: 71-113.
- Kudo S., Linhart O., Billard R., 1994 Ultrastructural studies of sperm penetration in the egg of the European catfish (*Silurus glanis* L.). *Aquat Living Resour* 7: 93-98.
- Legendre M., Linhart O., Billard R., 1996 Spawning and management of gametes, fertilized eggs and embryos in Siluroidei. *Aquat Living Resour* 9: 59-80.

- Leung L. K. P., Jamieson B. G. M., 1991 Live preservation of fish gametes. In: Jamieson BGM (ed.), *Fish evolution and systematics: evidence from spermatozoa*. Cambridge: Cambridge University Press, p. 245–69.
- Linhart O., Billard R., 1995 Survival of ovulated oocytes of the European catfish (*Silurus glanis*) after in vivo and in vitro storage or exposure to saline solutions and urine. *Aquat Living Resour* 8: 317–322.
- Linhart O., Gela D., Rodina M., Rodriguez G. M., 2001 Short-term storage of ova of common carp and tench in extenders. *J Fish Biol* 59: 616–623.
- Molnar K., 2009 Data on the parasite fauna of the European common carp *Cyprinus carpio carpio* and Asian common carp *Cyprinus carpio haematopterus* support an Asian ancestry of the species. *AACL Bioflux* 2(4): 391-400.
- Muchlisin Z. A., Nadiya N., Nadiyah W. N., Musman M., Siti-Azizah M. N., 2010 Preliminary study on the natural extenders for artificial breeding of African catfish *Clarias gariepinus* (Burchell, 1822). *AACL Bioflux* 3(2): 119-124.
- Nagler J. J., Parsons J. E., Cloud J. G., 2000 Single pairmating indicates maternal effects on embryo survival in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 184: 177-183.
- Petrescu-Mag I. V., 2007 [Sex Control in Guppyculture]. AcademicPres, Cluj-Napoca. [In Romanian]
- Petrescu-Mag I. V., Hărășan R., Csep L., Lozinsky L. R., 2008 Teratogenic effect of cloramphenicol in the guppy (*Poecilia reticulata* Peters, 1859). *Acta Ichtiologica Romanica* 3: 105-112.
- Petrescu-Mag I. V., Pășărin B., Hoha G., Hărășan R., Odagiu A. M., 2011 New contributions to knowledge of embryonic malformations in guppies. *AACL Bioflux* 4(2): 216-228.
- Renard P. H., Billard R., Christen R., 1987 Formation of the chorion in carp oocytes. An analysis of the kinetics of its elevation as a function of oocyte ageing, fertilization and the composition of the dilution medium. *Aquaculture* 62: 153–160.
- Rizzo E., Godinho H. P., Sato Y., 2003 Short-term storage off oocytes from the neotropical teleost fish *Prochilodus marggravii*. *Theriogenology* 60: 1059-1070.
- Rothbard S., Rubinish I., Gelman E., 1996 Storage of common carp, *Cyprinus carpio* L. eggs for short durations. *Aquac Res* 27: 175-181.
- Rothbard S., Rubinsthein I., David L., Shelton W. L., 1999 Ploidy manipulations aimed to produce androgenetic Japanese ornamental (KOI) carp, (*Cyprinus carpio* L.). *Israeli J Aquacult Bamidgheh* 51: 26–39.
- Saad A., Billard R., 1987 [The composition and use of a sperm diluent in the carp, *Cyprinus carpio*]. *Aquaculture* 66: 329–345. [In French, with English abstract].
- Sohrabnezhad M., Kalbassi M. R., Nazari R. M., Bahmani M., 2006 Short-term storage of Persian sturgeon (*Acipenser persicus*) ova in artificial media and coelomic fluid. *J Appl Ichthyol* 22(1): 395-399.
- Springate J. R. C., Bromage N. R., Elliot J. A. K., Hudson D.L., 1984 The timing of ovulation and stripping and their effects on the rates of fertilization and survival to eyeing, hatch and swim-up in the rainbow trout (*Salmo gairdneri* R.). *Aquaculture* 43: 313–322.
- Stoss J., 1983 Fish gamete preservation and spermatozoa physiology. In: W. S. Hoar, D. J. Randall, E. M. Donaldson (eds), *Fish Physiology*, vol. IXB, Academic Press, New York, pp. 305–350.
- Taati M. M., Mehrad B., Shabani A., 2010a The effect of ascorbic acid on hatching performance and tolerance against environmental stressor (high temperature) by immersion of Prussian carp (*Carassius gibelio*) fertilized eggs. *AACL Bioflux* 3(3): 219-225.
- Taati M. M., Mehrad B., Shabani A., Golpour A., 2010b Investigation of chemical composition of ceolomic fluid and its effect on sperm motility traits in Prussian carp, *Carassius gibelio*, during spawning season. *AACL Bioflux* 3(3): 227-232.
- Taati M. M., Mehrad B., Shabani A., Golpour A., 2010c Correlation between chemical composition of seminal plasma and sperm motility characteristics of Prussian carp (*Carassius gibelio*). *AACL Bioflux* 3(3): 233-238.

- Taati M. M., Jafaryan H., Mehrad B., Golpour A., 2010d Changes of ovarian fluid compositions and sperm quality parameters in koi (ornamental *Cyprinus carpio*) during spawning season. *ABAH Bioflux* 2(2): 83-90.
- Taati M. M., Mehrad B., Jafaryan H., 2010e Investigation of the hatching performance, growth, survival and tolerance against high ammonia concentration by enrich water incubation of carp (*Cyprinus carpio*) eggs with α -tocopherol. *ABAH Bioflux* 2(2): 91-98.
- Zlabek A., Linhart O., 1987 [Short-term storage of non-inseminated and unfertilized ova of the common carp, grass carp and silver carp]. *Bulletin VURH Vodnany* 23: 3–11. [In Czech, with English summary]

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