Jihočeská univerzita v Českých Budějovicích

Fakulta rybářství a ochrany vod

Výzkumný ústav rybářský a hydrobiologický

Bakalářská práce

Vliv teploty při krátkodobém uchování jiker jesetera malého, *Acipenser ruthenus*, *in vitro*

(Effect of temperature on *in vitro* short-term storage of sterlet, *Acipenser ruthenus*, eggs)

Autor: Marek Let

Vedoucí bakalářské práce: prof. Ing. Otomar Linhart, DrSc.

Konzultant bakalářské práce: MSc. Mohammad Abdul Momin Siddique

Studijní program a obor: Zootechnika, Rybářství

Forma studia: Prezenční

Ročník: Třetí

České Budějovice, 2016

Prohlašuji, že svoji bakalářskou práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že, v souladu s § 47b zákona č. 111/1998 Sb. v platném znění, souhlasím se zveřejněním své bakalářské práce, a to v nezkrácené podobě. Zveřejnění probíhá elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

V Českých Budějovicích, 5. 5. 2016

…….

Poděkování:
Na prvním místě bych rád poděkoval svému vedoucímu bakalářské práce, prof. Ing. Otomaru Linhartovi, DrSc., za trpělivé vedení a cenné rady. Rovněž děkuji svému konzultantovi, MSc. Mohammadu Abdulovi Mominovi Siddique, za pomoc, cenné rady a jazykovou korekci.
Dále bych chtěl poděkovat za pomoc Mgr. Vladimíře Tučkové a prof. MSc. Williamu L. Sheltonovi, Ph.D.
V neposlední řadě pak děkuji své přítelkyni, Haně Kratochvilové, rodině a všem kamarádům a známým, kteří mě psychicky podpořili při psaní této práce.

JIHOČESKÁ UNIVERZITA V ČESKÝCH BUDĚJOVICÍCH
Fakulta rybářství a ochrany vod
Akademický rok: **2014/2015**

ZADÁNÍ BAKALÁŘSKÉ PRÁCE

(PROJEKTU, UMĚLECKÉHO DÍLA, UMĚLECKÉHO VÝKONU)

Jméno a příjmení:	**Marek LET**
Osobní číslo:	**V13B051P**
Studijní program:	**B4103 Zootechnika**
Studijní obor:	**Rybářství**
Název tématu:	**Effect of temperature on *in vitro* short-term storage of sterlet, *Acipenser ruthenus*, eggs**
Zadávající katedra:	**Výzkumný ústav rybářský a hydrobiologický**

Zásady pro vypracování:

An overview of the literature on Short storage of sturgeon eggs will constitute minimally 50% of the thesis. The rest of the thesis will include experimental part. Overview literary section must be completed independently with MSc. Mohammad Abdul Mozuin Siddique. One experiment with Mark Let participation will be held in Vodňany in the period from March to June 2015.
Expected outcomes:
As we know, the fertilization ability of sturgeon eggs is decreased with the increasing of storage period. Our main objective of this study is to develop a method to short term storage of sterlet sturgeon ova without changing their fertilizing ability.
Materials and methods:
In this study, influence of four tempereture conditions (7°C, 11°C, 15°C, and 19°C) and of storage time post-stripping (Control = time 0 h, 2.5 h, 5.0 h, 7.5 h and 10.0 h) on viability of sterlet, *Acipenser ruthenus*, unfertilized eggs in vitro will be observed through percentage of hatching or other stages. An Ovulation and spermiation will be stimulated by using CPE in two dosages for females (first dose 0.5 mg.kg^{-1} b.w. and second dose 4.0 mg.kg^{-1} b.w.) and one dosage for males (4.0 mg.kg-1 b.w.), respectivelly. The ovulated eggs will be collected from three females through a small surgery, than mixed together. The good quality sperm will be selected from three males. The eggs from each temperature treatment groups will be inseminated with the semen and activate in dechlorinated water at 15°C. Then, eggs will be placed into experimental cage incubation system with separate chambers at 15°C in triplicate to plastic petri dishes for evaluation of fertilization rate during neuralization phase. Approximately, 5-6 days post-fertilization, the total number of succesfully hatched larvae will be acurately calculated for the final evaluation of storage success. All data will be analyzed using STATISTICA v 12 software.

Rozsah grafických prací:	**maximálně 10 grafů a tabulek**
Rozsah pracovní zprávy:	**35 - 60 stran**
Forma zpracování bakalářské práce:	**tištěná**
Jazyk zpracování bakalářské práce:	**Angličtina**

Seznam odborné literatury:

Linhart, O., Gela, D., Rodina, M., & Gutierrez, M. R. (2001). Short-term storage of ova of common carp and tench in extenders. *Journal of fish biology*, **59(3), 616-623.**

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. *Aquatic Living Resources*, *8(04)*, **317-322.**

Sohrabnezhad, M. A. H. D. L., Kalbassi, M. R., Nazari, R. M., & Bahmani, M. A. H. M. O. U. D. (2006). Short?term storage of Persian sturgeon (Acipenser persicus) ova in artificial media and coelomic fluid. *Journal of Applied Ichthyology*, **22(s1), 395-399.**

Siddique, M. A. M., Cosson, J., Psenicka, M., & Linhart, O. (2014). A review of the structure of sturgeon egg membranes and of the associated terminology. *Journal of Applied Ichthyology*, **30(6), 1246-1255.**

Niksirat, H., Sarvi, K., Amiri, B. M., Karami, M., & Hatef, A. (2007). In vitro storage of unfertilized ova of endangered Caspian brown trout (*Salmo trutta caspius***) in artificial media.** *Animal reproduction science*, **100(3), 356-363.**

Gisbert, E., & Williot, P. (2002). Influence of storage duration of ovulated eggs prior to fertilisation on the early ontogenesis of sterlet (Acipenser ruthenus) and Siberian sturgeon (Acipenser baeri). *International review of hydrobiology*, **87(5?6), 605-612.**

Vedoucí bakalářské práce:	**prof. Ing. Otomar Linhart, DrSc.**
	Výzkumný ústav rybářský a hydrobiologický
Konzultant bakalářské práce:	**MSc. Mohammad Abdul Momin Siddique**
	Výzkumný ústav rybářský a hydrobiologický

Datum zadání bakalářské práce:	**13. prosince 2014**
Termín odevzdání bakalářské práce:	**6. května 2016**

prof. Ing. Otomar Linhart, DrSc.
děkan

JIHOČESKÁ UNIVERZITA
V ČESKÝCH BUDĚJOVICÍCH
FAKULTA RYBÁŘSTVÍ A OCHRANY VOD
Zátiší 728/II
389 25 Vodňany

prof. Ing. Pavel Kozák, Ph.D.
ředitel

V Českých Budějovicích dne 29. dubna 2016

CONTENTS

1. INTRODUCTION

Sterlet sturgeon, *Acipenser ruthenus*, is an economically valuable species, evaluated as a suitable candidate for aquaculture due to their early sexual maturity than the other acipenserids (Baruš and Oliva, 1995; Gessner *et al.*, 2010a; Gela *et al.*, 2012). Sterlet is a potamodromous, zoobenthophagous and relativelly short-living sturgeon (Dettlaff *et al.*, 1993; Williot *et al.*, 2002; Kottelat and Freyhof, 2007; Chebanov *et al.*, 2011; Siddique *et al.*, 2014b). Sterlet eggs are 1.9–2.0 mm in diameter, in most cases have elliptical shape, after fertilization are strongly adhesive (Baruš and Oliva, 1995; Siddique *et al.*, 2014a, Siddique *et al.*, 2014b). Unovulated eggs are commonly used for pruduction of small-grained caviar (Bronzi *et at.*, 2011).

Most of the sturgeons are less or more endengered due to human impact, for example overfishing and blocking spawm migrations up to stream by resorvoirs (Baruš and Oliva, 1995; Billard and Lecointre, 2000; Kotellat and Freyhof, 2007). Recently, several conservation programes have been started to conserve these endengered species by culturing them in captive environment (Kottelat and Freyhof, 2007; Gela *et al.*, 2008; Gessner *et al.*, 2010a; Siddique *et al*, 2014a). The fertilization capacity of fish eggs is gradually decreasing in ovarian fluid and in water (Dettlaff et al., 1993). Knowledge and technology of short-term storage of ovulated unfertilized eggs can minimize the problems resulting from inbreeding and provide synchronous brooder maturation as well as improve hatchery management (Bromage and Roberts, 1995; Hajirezaee and Niksirat, 2009).

The successful technologies of short-term storage of sterlet eggs have not been developed yet. Attempt to cryopreserve fish eggs is out of the question, because of inadequate dehydration and toxicity of cryoprotectants (Rana, 1995; Boryshpolets *et al*, 2011). Sturgeon eggs can be preserved in natural or artificial coelomic fluid and in extenders. The optimum temperature for short-term storage of eggs was not determined for sturgeons. Temperature is one of the most important factors that influence the fertilizing ability of eggs during short-term storage. Gisbert and Williot (2002) stored sterlet eggs in coelomic fluid at 15°C, but eggs only retained their fertilizing ability for 2–4 h at 15°C.

Previous studies showed that ovulated eggs of Persian sturgeon, *Acipenser persicus*, can be held in artifical coelomic fluid at 18°C up to 3 hours without significant

loss of hatchability and fertilization rate (Sohrabnezhad *et al*., 2006; Hajirezaee and Niksirat, 2009). Unfertilized eggs of endangered Caspian brown trout, *Salmo trutta caspius*, were found successfully stored in artificial media for 48 h without significant loss of fertility (Niksirat *et al*., 2007). On contrary, eggs of polytermic species of fish often lose their fertility very quickly. For example eggs of grass carp, *Ctenopharyngodon idella*, is not able to retain their fertilizing ability in artificial coelomic fluid for more than 1 hour (Safarzadenia *et al*., 2013). Unfertilized eggs of common carp, *Cyprinus carpio*, and tench, *Tinca tinca*, had also retained their fertilizing ability for very short period during storage (Linhart *et al*, 2001).

However, knowledge on short-term storage of sterlet eggs are very limited in the literature. Therefore, the main objective of this study is to review the current knowledge on short-term storage of sterlet sturgeon and to determine the optimum temperature for short-term storage of sterlet sturgeon eggs in coelomic fluid *in vitro*.

2. REVIEW

2.1. Sterlet sturgeon, *Acipenser ruthenus*, Linnaeus, 1758

2.1.1. Taxonomic status

Phylum: Chordata

Subphylum: Vertebrata

Infraphylum: Gnathostomata

Superclass: Osteichthyes

Class: Actinopterygii

Actinopteri – subclass: Chondrostei

Order: Acipenseriformes

Family: Acipenseridae

Genus: *Acipenser*

Species: *Acipenser ruthenus*

(according to Betnacu-R *et al*., 2013)

2.1.2. Diagnostic signs of sterlet

Sterlet is one of the smallest species of sturgeon in the world, size up to 1250 mm TL and maximum weight recorded 16 kg (Gessner *et al.*, 2010a). The first dorsal scute is distinctly separated from the head. Inferior lip is interrupted in the middle**.** Barbels are fimriate and distinguished from other euroasian acipenserids by having 56–71 lateral scutes (Kottelat and Freyhof, 2007). The maximal reported life span for sterlet is 20 years (Baruš and Oliva, 1995**).**

2.1.3. Biology, distribution, and conservation status

Sterlet is fluvial fish that live in freshwater (Vostradovsky, 1973**)**, semi-anadromous and river-resident species (Dettlaff *et al.*, 1993). Sterlet is widely distributed in Euroasian rivers draining to Azov, Black, Caspian, Kara and White (Barents) Seas, introduced into Pechora drainage and into basins of Ladoga and Onega lakes (Dettlaff *et al.*, 1993; Kottelat and Freyhof, 2007). In recent years, sterlet have been introduced throughout Europe, usually without formation of self-sustaing populations (Williot *et al.*, 2002; Kottelat and Freyhof, 2007; Bronzi *et al.*, 2011). Gessner *et al.* (2010a) reported some population with spontaneous reproduction in the reservoirs in Russia. Sterlet feeds on planktonic and benthic invertebrates (Vostradovsky, 1973), also steals fish eggs (Baruš and Oliva, 1995). Maturity of sterlet usually occurs at 3–5 years for males and 5–8 years for females (Gessner *et al.*, 2010a). Relative fecundity of sterlet is 20 000 to 30 000 egg per kg of body weight (b.w.) and the massive hatching occurs after 5–7 days, according to incubation tempertature (Gela *et al.*, 2012).

In recent years, significant decline of wild population was reported. Sterlet was listed on CITES Appendix II in 1998, categorized like vulnerable (VU) on The IUCN Red List Categories and Criteria (2001) (Gessner *et al.*, 2010a).

2.1.4. Sturgeon aquaculture

The total production in sturgeon aquaculture has been increased multiply in recent years, in comparison with harvesting of open water, where the production has been drastically reduced (Williot *et al.*, 2002; Bronzi *et al.*, 2011). Sturgeon aquaculture still forms a minor part of total global aquaculture production (Gela *et al.*, 2012). Despite the fact that sterlet produce the smallest eggs than the other acipenserids, sterlet caviar is sold at a high price due to their good taste and high demand for cosmetic and medical purposes (Gela *et al.*, 2008; Gessner *et al.*, 2010a).

Nowadays, the breeding process of sterlet and other sturgeon has been improved and practical knowledges on reproduction and culture were reported by several authors (Gela *et al.*, 2008; Chebanov *et al.*, 2011; Gela *et al.*, 2012; Gela *et al.*, 2014). The succesful controled propagation with a high efficiency and low mortality is possible in

the hatchery (Gela *et al.*, 2012), rearing of fry and juveniles in controled conditions is convenient and profitable (Chebanov *et al.*, 2011). Hybrids of sterlet × beluga, *Huso huso,* or of sterlet × Russian sturgeon, *Acipenser gueldenstaedti*, are artificially formed to increase livestock performance (Baruš and Oliva, 1995; Bartley *et al.*, 2000)

2.2.Sterlet egg morphology

The structure of acipenserids eggs is complex due to having mutiple micropyles (Siddique *et al.*, 2014b), 5–13 in sterlet eggs (Siddique *et al.*, 2014a). The structure of egg membranes and micropyle structure of sterlet are similar with other acipenserids, but greatly different from the teleost species (Siddique *et al.*, 2014a,b).

2.2.1. Oocytes characteristic

The oocyte is a large cell characterized by a typically polarized structure, deviding to a vegetal region with presence of yolk granules (compact crystalline body structures surrounded by a thin granular layer, up to 15 μm in diameter according to species) (Dettlaff *et al.*, 1993) and large inclusions of lipid and an animal region with presence of a oval nucleus, often called the germinal vesicle (GV). The cytoplasm is less dense than the yolk granules and have a small lipid inclusion. The cytoplasm contains a large number of reserve nutrients to support embryonic developement. At the end of stage IV of ovarian maturity the GV is increased in size and moved towards the animal pole (Dettlaff *et al.*, 1993; Rodina, 2006; Gela *et al.*, 2008; Siddique *et al.*, 2014a). The GV contains small solid body of chromosomes, which are suspended in karyoplasm and numerous stained nucleoli are located closer to the nuclear membrane (Dettlaff *et al.*, 1993).

2.2.2. The structure of sterlet egg envelopes

In the ovarian cavity, unovulated oocytes are encased in three-layered external envelope (follicular epithelium, basement lamina, internal and external thecal cells) (Dettlaff *et al.*, 1993; Siddique *et al.*, 2014a). The innermost membranes of the ovulated sterlet oocyte are cortical granules, a layer of oocyte matrix, and thereafter four noticeable layers - zona radiata interna (ZRI), zona radiata externa (ZRE), alveolar layer, and an adhesive layer. An epilayer, thin layer arising between ZRI and ZRE after ovulation, has not been reported in this species of sturgeon (Siddique *et al.*, 2014a).

The proximal layer of the follicular epithelium is proximal to the adhesive layer of the oocyte (Dettlaff *et al.*, 1993). Beneath the ZRI, a thin electron-lucent membrane (less than 1 μm), extra-oocyte matrix, are located which contains several outgrowths of

the cytoplasmatic cortex and microvilli leading up to ZRE. It plays essential role in formation of micropilar canals (Aizenshtadt and Dettlaff, 1972; Dettlaff *et al.*, 1993; Siddique *et al.*, 2014a). Under the cortical granules a layer of rod shaped mitochondria and a layer of cortical granules are visible in the oolemma (Dettlaff *et al.*, 1993). The completely grown oocyte has many annulate lamellae forming large units, concentric rows (up to 10 μm according to species) in the animal polar region (Aizenshtadt and Dettlaff, 1972). The thickness of zona radiata decreases in the animal polar region during ovulation (Siddique *et al.*, 2014a), the length of microvilli is significantly reduced and micropilar canals are fully developed (Dettlaff *et al.*, 1993). The increased amount of micropiles probably causes increased risk of polyspermy (Cherr and Clark, 1982).

2.2.3.1. Structure of sterlet micropylar canal

Sterlet micropyle has a double-tapered, funnel shaped structure. The entrance to micropylar canal is covered by adhesive layer of the egg envelope (Pšenička *et al.*, 2010). The external opening of sterlet egg micropyle funnel has 20 μm in diameter and the inner opening of the canal is severalfold narrower (2.5–3.0 μm in diameter) than the opening of micropylar funnel (Vorob'eva and Markov, 1999) nevertheless a bit wider passageway for just one acrosome of sterlet spermatozoa (Pšenička *et al.*, 2010).

2.2.4. Characteristic of coelomic fluid

The coelomic fluid (CF) is clear viscous liquid, which accumulates gradually in abdominal cavities of females before the onset of ovulation (Dettlaff *et al.*, 1993). This liquid is formed by filtration from the blood plasma (Matsubara *et al.*, 1985; Niksirat *et al.*, 2007) but the exact origin of this fluid is unknown. The biochemical composition of sterlet coelomic fluid is shown in Table 1.

2.3. Fertilization process

2.3.1. Sterlet semen

Semen of sturgeon species is milky white in color containig sperm and seminal plasma. It differs from ejaculate of warm-blooded animal because the fish do not have the accessory sex glands (Linhart *et al.*, 2011). The total amount of semen which can be obtained from one male of chondrostean is enormous with comparison to teleostean (Pšenička, 2007), nevertheless from a single sterlet male is possible to obtain a several mililitres of sperm (Gela *et al.*, 2008). The duration of the motility of sturgeon spermatozoa is longer than the other freshwater species and can be motile up to several minutes (Lahnsteiner *et al.*, 2004).

2.3.1.1. Seminal plasma

Seminal plasma is the primary product of Sertoli cells and constitute the main component of males semen (50–90 %) (Linhart *et al.*, 2011). It contains Ca^{2+}, Na^{+}, K^{+} and Cl^{-} ions (Pšenička *et al.*, 2008) and also organic components (carbohydrates, proteins, lipids, enzymes and other substances which belong to metabolism). Potassium cations inhibit motility of spermatozoa (Linhart *et al.*, 1991). The osmolality of seminal plasma is high, therefore, sperm can not be activated in seminal plasma. Ionic effect together with decrease of osmolality (a hypo-osmotic shock) influances the activation of sperm motility in sturgeon species (Lahnsteiner *et al.*, 2004; Alavi and Cosson, 2006).

2.3.1.2. Morphology of sterlet spermatozoa

The size and structure of acipenserid spermatozoa are significantly different from that of teleost and other group of fishes, even a large variation has been found among acipenserids (McMillan, 2007; Pšenička, 2007; Pšenička *et al.*, 2008). The presence of acrosome distinguish sturgeon spermatozoa from the other freshwater species (Pšenička, 2007).

Sterlet sperm (Figure 1) (TL = 47.61 μm) (Pšenička *et al.*, 2008) consists of a narrow slightly tapering head with an acrosome (AL = 0.79±0.07 μm), a short midpiece

(ML = 0.97±0.23 μm), formed by a mitochondria part and a neck (a centriolar complex) and a long flagellum (FL = 42.47±1.89 μm). The nucleus (NL = 3.30±0,31 μm) is located in the head and occupies most of the space (Dettlaff *et al.*, 1993; Pšenička, 2007).

It contains three helical organised endonuclear canals (EC), passing through the centre of the nucleus and leading from the implantation fossa (small cavity linking EC with the centriole of midpiece) to the acrosome (Pšenička, 2007). Dettlaff *et al.* (1993) reported that these canals are 50–60 nm wide. The number of EC can vary rarely among spermatozoa from one male (Pšenička *et al.*, 2008).

The standing out acrosome posseses posterolateral projections (PPs) (Pšenička, 2007). One sperm of sterlet has 9–10 PPs and one projection is 550 nm long (Pšenička *et al.*, 2008). Pšenička *et al.* (2007) believe, that PPs diverge like fingers during the penetration and then serve to anchor when spermatozoon passes through the micropyle.

In the midpiece 3–6 mitochondria are symmetrically distributed around the flagellum, which is symmetrically connected to the head and two types of centrioles (proximal and distal) forming the neck. Nine triplets of peripheral microtubules compact each centriole (Pšenička, 2007; Pšenička *et al.*, 2008).

Flagellum originates from a centriolar complex and has a 9 + 2 organisation (Stoss, 1983) (nine peripheral doublets and one central pair of microtubules). As well, there is an extracellular cytoplasm canal between the midpiece and the flagellum. Movement of the sturgeon flagellum is not spiral as is the case of other fish species. It is equiped with two independent fins of different length, originate from flagellar plasma membrane and form together a fringe which is an average of 568 nm wide in sterlet sperm. The flagellum diameter of sterlet spermatozoon is 151.39 ± 20.06 nm (Pšenička, 2007; Pšenička *et al.*, 2008).

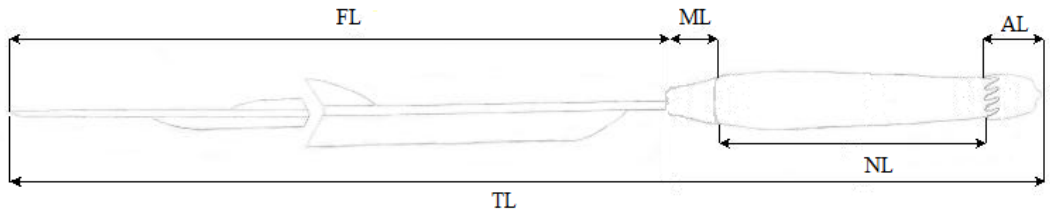

Figure 1: Sturgeon sperm according to Pšenička *et al.* (2007) adjusted by Let

2.3.2. Fertilization strategies

The duration of spermatozoa motility is several minutes for sturgeon species. Sturgeon eggs are much resistent in the water compared to other fish species and they can be fertilized by sperm after several hours released in water. The fertilizing ability of Russian sturgeon has been recorded 6 hours after activation in water. Ovulated eggs of vimba bream *Vimba vimba*, which completely lose their fertilizing ability within a minute (Dettlaff *et al.*, 1993). Sterlet eggs are activated upon contact with water. Siddique *et al.* (2015) reported that sterlet eggs can be activated in water within few seconds, able to be fertilize for several minutes. They did not found any evidence of cortical reaction or formation of perivitelline space in the egg cortisol 10 minutes after activation of eggs in freshwater.

2.4.Factors effect on short-term storage of eggs

Factors effect on egg storage are very closely related to each other. If one of these factors do not stay in optimum, a chain reaction of degradation will be triggered and the egg fertizability will be lost (Hajirezaee and Niksirat, 2009). The most important factor is biological quality of eggs without which, storage of eggs has no practical significance (Gisbert and Williot, 2002).

2.4.1. Biological quality of eggs

It can can be defined as the summary of the features which are a prerequisite of the egg to be fertilized and subsequently developed into a viable fry, able to ingest (Kjørsvik *et al.*, 1990; Bobe and Labbé, 2010). Due to great variability in eggs quality, this factor is very significat and naturaly, possible to improve (Stoss, 1983; Kjørsvik *et al.*, 1990; Brooks *et al.*, 1997; Bobe and Labbé, 2010; Chebanov *et al.*, 2011), nevetheless it is not possible to exactly categorize eggs quality before fertilization, but it can be estimated based on observable or measurable characteristics of eggs by microscopic observations after egg activation (Pšenička *et al.*, 2014). In sturgeon aquaculture, the observing criteria for the determination of egg quality is called biopsy, especially to undersatnd the factor of matureness (Rodina, 2006; Gela *et al.*, 2008). Upon the artificial spawning, good quality unfertilized eggs from the individuals are often selected by observing the uniformity of egg colour (differences can indicate erratic maturation), and size of eggs (differences can indicate erratic maturation) (Chebanov *et al.*, 2011; Pšenička *et al.*, 2014), surface drawing (overriped eggs have usually white spots on animal pole or white marbling), egg shape regularity, absence of activated and resorbed eggs, abnormalities, plugs, ovarian fluid transparency (Pšenička *et al.*, 2010; Chebanov *et al.*, 2011; Pšenička *et al.*, 2014) (grey coloration indicates foreign substances which can activate eggs prematurelly) and amount of coelomic fluid (optimal amount for quality is 5–10 % of weight of spawned eggs) (Pšenička *et al.*, 2014). Low quality of eggs might increase the abnormality of embryo, polyspermic clevage development and partenogenetic activation, which are typical for sturgeons (Pšenička *et al.*, 2014; Siddique *et al.*, 2016a). In aquaculture, good quality of sturgeon eggs is allowed certainly by many factors during artificial spawning and the developement of individual female ovaries at stage I–IV of maturity (Dettlaff *et al.*,

1993; Brooks *et al.*, 1997; Bayunova *et al.*, 2002; Bobbe and Labbé, 2010; Chebanov *et al.*, 2011).

2.4.2. Temperature

Temperature is one of the main factors, which influences time possibilities of ova storage (Sanches *et al.*, 2011) because eggs are heat sensitive (Dettlaff *et al.*, 1993). It is well measurable and is relatively easy to control in artificial conditions. As we know, increasing and decreasing of temperature conditions in water affect maturation and ovulation and subsequently incubation time. Bromage and Roberts (1995) called this changes as "ageing phenomenon" which is associated with metabolism (Lahnsteiner *et al.*, 2001) and over-ripening (Samarin *et al.*, 2015). However, exact and unambiguous ultrastructual analyse of the various temperature conditions which affects on changes in ovulated oocytes structure was never performed. Results of many previous works showed that the optimal temperature for short-term storage of ova is species-specific, but these temperature values are often near to values of spawning temperatures of the species and usually is stable without significant fluctuations (Dettlaff *et al.*, 1993; Sohrabnezhad *et al.*, 2006; Niksirat *et al.*, 2007). Salmonids eggs can retain their fertizability for the longest time from all investigated fish species, several days at 2–4°C (Stoss, 1983; Niksirat *et al.*, 2007; Samarin *et al.*, 2008), because natural spawning occures usually in cold tempertures and the incubation lasts longer (Randák *et al.*, 2006; Kottelat and Freyhof, 2007). Compared to that, polytermic species are able to conserve their initial fertizability at higher temperatures, the best storage temperatures are 2–4°C lower than their incubation temperatures (Linhart and Billard, 1995). In the case of sterlet, natural spawning occurs when temperature rises above 10°C (Kottelat and Freyhof, 2007) and incubation tempertures on hatcheries are usually ajusted on 12.8–17.8°C (Gela *et al.*, 2012).

2.4.3. pH value

Coelomic fluid pH variation during storage of ova is often reported by researchers. Lahnsteiner *et al.* (2001) observed significant decrease of fertilization rate with decrease or increase of pH value of common carp ovarian fluid (OF). It seems that eggs of

several species are very sensitive on pH variations. *In vivo*, reduction of OF pH in common carp occures by disturbances in the aerobic respiration process which leads to the production of lactic acid. It accumulates in the OF and causes ultimate loss of oocyte membrane integrity (Linhart *et al.*, 1995). *In vitro* fertilization, production of carbonic acid from CO_2 can leads to the drop in pH of storage media (Komrakova and Holtz, 2009) and probably, the decrease of pH can be due to the accumulation of compounds derived from the over-riped eggs during lysis of proteins, aspartate aminotransferase and esterified and non-esterified fatty acids (Lahnsteiner, 2000).

2.4.1. Oxygen

Effect of oxygen on short-term storage of eggs were not studied before. Several authors have described cellular and molecular changes during over-ripening, but most findings are based on studies of higher vertebrates oocytes (Samarin *et al.*, 2015). Most researchers were postulated that concentration of reactive oxygen species (ROS) increases during post-ovulatory oocyte ageing. The term ROS contains chemically reactive mollecules of oxygen, such as oxygen ions and peroxides (Samarin *et al*., 2015). ROS care responsible for mitochondrial oxidative stress and lipid peroxidation in cell membrane systems. Increasing mitochondrial oxidative stress can induce dysfunction of mitochondria, which consequently leads to decreasing of ATP production. These events result in further changes, for instance disturbances in intracellular Ca^{2+} regulation in endoplasmatic reticulum (Takahashi *et al.*, 2013). A slowdown or a prevention of these processes in eggs *in vitro* can be attained by treating them with some antioxidant chemical agents (Samarin *et al.*, 2015).

2.4.2. Osmolality

The factor of stable and strictly defined osmolality value of storage media is necessary for short-term storage of ova. Osmosis is the spontaneous diffuse solvent molecules through a semipermeable membrane into a region of higher solution concentration and it can cause osmotic pressure (Haynie, 2001). The osmolality value indicates amount of these osmotically active molecules in solvent and it is usually expressed in $mOsm.kg^{-1}$ units. CF/OF of many freshwater fish species including sturgeons, contains higher rate of osmolality and K^+ than seminal plasma and than

activating sollution (AS) during fertilization (Alavi and Cosson, 2006; Pšenička *et al.*, 2008). The total CF osmolality of sterlet, Siberian sturgeon, *Acipenser baerii*, and Russian sturgeon, *Acipenser gueldenstaedti*, is presented in Table 1.

The living eggs are not tolerable to changes of osmolality and these changes evoke some responses. In fertilization process, it is necessary to decrease osmolality value, in short-term storage of freshwater fishes ova, increase of storage media osmolality often causes rapid degradation of egg quality by plasmolysis and dehydratation (Dettlaff *et al.*, 1993; Hajirezaee and Niksirat, 2009). It can be attributed to the possible evaporation and loss of cellular material as a result of membrane damage caused by degradation of eggs during storage (Hajirezaee and Niksirat, 2009).

2.4.4. Ionic composition of coelomic fluid

The CF/OF acts as storage medium for many freshwater species because of its higher osmolality which maintains eggs fertilizing ability and protects them from drying out (Dettlaff *et al.*, 1993). Moreover, sturgeons CF contains higher rate of potassium cations which are inhibitors of sperm motility (Linhart *et al.*, 1991) and of acrosomal reaction (Pšenička *et al.*, 2010). In sturgeon species, it can prevent undesirable polyspermy by stabilizing the microenvironment around the micropyles. (Oda *et al.*, 1995; Rosengrave *et al.*, 2008; Gasparini *et al.*, 2012). Nevertheless, by nature spawning, the fertilization occurs in riverbed in strong-current (Kottelat and Freyhof, 2007), CF drifts downstream downward and, from this reason this can not be such an obstacle for spermatozoan. Therefore, it is necessary to remove CF from the eggs before insemination in controlled reproduction (Gela *et al.*, 2008).

Table 1: Physicochemical properties of coelomic fluid of sterlet, *Acipenser ruthenus*, (age 5-8 years), Siberian sturgeon, *Acipenser baerii*, (age 16-22 years), and Russian sturgeon, *Acipenser gueldenstaedtii*, (age 13-18 years). Data are presented as mean ± SEM. Different letters within columns indicate significant differences (Siddique *et al.*, 2016b).

	Sterlet, *Acipenser ruthenus* (N = 10 females)	Siberian sturgeon, *Acipenser baerii* (N = 7 females)	Russian sturgeon, *Acipenser gueldenstaedtii* (N = 4 females)
pH	7.92±0.03	7.98±0.03	7.96±0.04
Osmolality ($mOsm.kg^{-1}$)	190.0±6.28	208.43±9.20	213.50±8.03
Ca^{2+} (mM)	0.92±0.17	0.87±0.06	0.96±0.24
Na^{+} (mM)	104.68±7.74	126.37±6.19	123.01±5.86
K^{+} (mM)	6.11±0.55	5.42±0.42	4.39±1.06
Cl^{-} (mM)	89.80±6.41	98.00±5.33	94.00±5.72
Mg^{2+} (mM)	0.63±0.04	0.67±0.06	0.57±0.07
Glucose (mM)	1.80±0.29	2.46±0.19	1.35±0.39
Cholesterol (mM)	0.16±0.03	0.13±0.04	0.15±0.05
Total protein ($g.l^{-1}$)	2.98±0.35	2.41±0.30	3.57±1.41

2.5. Short-term storage of eggs in Acipenserids and other species

Preservation of eggs, for their later use, is very advantageous and useful practice in hatchery, where ovulation is artificially controled and stimulated. It minimizes problems resulting from inbreeding, provides synchronous brooder maturation, enables transportation of gametes, improves oportunities of selective programes and can serve well in studies of chromosome-set manipulations. Knowledge of egg storage for the fish species can help to save eggs post-mortem during emergency conditions in hatcheries when mature females die prematurelly due to the technical reasons and/or a mistake of the staff (Stoss, 1983; Bromage and Roberts, 1995; Rothbard *et al.*, 1996; Safarzadenia *et al.*, 2013; Samarin *et al.*, 2013).

Degradation of ova quality with time after manual stripping is one of the main difficulties which have been limiting for artificial reproduction and higher production of viable fry (Furuita *et al.*, 2003; Rizzo *et al.*, 2003; Samarin *et al.*, 2013). As a consequence, short-term storage protocols aimed at increasing post-spawning egg longevity, using positive temperatures in coelomic (CF) or ovarian fluid (OF) and in artificial media, have been developed for several fish species (Niksirat *et al.*, 2007).

2.5.1. Freezing procedures

Unlike the investigation with fish milt, attempts to freeze fish unfertilized ova for storage have been unsuccessful due to inadequate dehydration and toxicity of cryoprotectants (Stoss, 1983; Rana, 1995). As example dimethylsulfoxide, dimethylacetamide, ethyleneglycol and methanol can be presented, whose effect for cryopreservation of sterlet semen was experimentally tested by Boryshpolets *et al.* (2011).

2.5.2. Natural and artificial storage media

Eggs stored in CF/OF can retain their fertizability for longer time period, when temperature conditions is properly specified for the species. CF/OF is obtained during manual stripping together with ova (Dettlaff *et al.*, 1993) and acts well as storage medium because CF/OF creates a unique environment for unfertilized ova preventing from dryness and plasmolysis during *in vitro* and obviously *in vivo* conditions (Lahnsteiner *et al.*, 1995; Sohrabnezhad *et al.*, 2006). In many fish species including

sturgeons, CF/OF contains higher osmolality and K^+ concentration than seminal plasma (Turner and Montgomerie, 2002; Pšenička *et al.*, 2008). This high osmolality and K^+ concentrations in coelomic fluid can inhibit the spermatozoan motility (Chebanov *et al.*, 2011). Nevertheless, it can play an important role in the fertilization process by stabilizing the microenvironment around the micropyles and prevention of polyspermy (Oda *et al.*, 1995; Rosengrave et al., 2008; Gasparini *et al.*, 2012).

Researches have tried to substitute CF/OF for artificial media, which may affect positively and the duration of egg fertizability. Artificial media can be divided to extenders and artificial coelomic fluid or artificial ovarian fluid (ACF/AOF) unfortunately, the terminology is not standartized.

2.5.3. Extenders

Extenders are artificially synthetized saline sollutions which are designed to extend the egg viability and increase the fertizability (Glenn and Tiersch, 2002; Muchlisin and Siti-Azizah, 2010; Safarzadenia *et al.*, 2013). They should be stable environment for eggs, where the ratio of components is predetermined and their ionic composition (Ca^{2+}, Na^+, K^+, Cl^- and Mg^{2+}) is similar to natural CF/OF. The three best known extenders are Dettlaff extender (111.3 mM NaCl+3.3 mM KCl+2.1 mM $CaCl_2$+23.8 mM $NaHCO_3$), Ringer sollution (103 mM NaCl+1 mM KCl+1 mM CaCl2+1.1 mM NaHCO3) (Linhart *et al.*, 2001) and Cortland sollution (124.1 mM NaCl+5.1 mM KCl+1.0 mM $MgSO_4.7H_2O$+1.6 mM $CaCl_2.2H_2O$+5.6 mM glucose) (Wolf and Quimby, 1969; Niksirat *et al.*, 2007).

Dettlaff extender and Ringer sollution are usable for cyprinids and also for other species. Their advantages are easy preparation and low price, that is why they are predetermined for practical usage. These extenders can be modified by adding some substances for example bovine serum albumin (BSA) (Linhart *et al.*, 2001).

Cortland sollution was developed for salmonids (Wolf and Quimby, 1969). It is more complicated to prepare, but also usable (Niksirat *et al.*, 2007). Its inorganic composition corresponds with CF of salmonids at ovulation time (Lahnsteiner *et al.*, 1995).

2.5.4. Artificial coelomic or ovarian fluid

ACF/AOF is prepared exactly by biochemical composition of natural CF/OF of the species (Zhang *et al.*, 2010) and belongs to the most sophisticated artificial storage

media. Its basic iont composition (Ca^{2+}, Na^{+}, K^{+}, Cl^{-} and Mg^{2+}) is similar to extenders, moreover there are other organic additions for example proteins, aminoacids, carbohydrates etc. according to biochemical analysis (Sohrabnezhad *et al.*, 2006; Taati *et al.*, 2011).

2.5.5. Buffer addition

Very important parameter for ova storage in artificial medium is the pH value. CF/OF pH value of most of the fish freshwater species is around 7–9 (Lahnsteiner *et al.*, 2001; Niksirat *et al.*, 2007). Several studies were reported that pH value can vary during storage, and can decrease total ova fertizability. For this reason are both, extenders and ACF/AOF often enhanced by biochemical buffers. Their ability is to maintain stable pH value (Sohrabnezhad *et al.*, 2006; Niksirat *et al.*, 2007; Zhang *et al.*, 2010). Hepes sollution ($C_8H_{18}N_2O_4S$), Tris sollution ($C_4H_{11}NO_3$) (Figure 2) and their modifications are most commonly used because their pK_a value is near to the natural pH of CF/OF. Afterwards the pH value is adjusted by adding required amount of acid (H^{+}) or base (OH^{-}) (Niksirat *et al.*, 2007).

Preparation of this kind of medium is indeed costly and more difficult, and often it does not bring such good results, to be put into practice (Safarzadenia *et al.*, 2013).

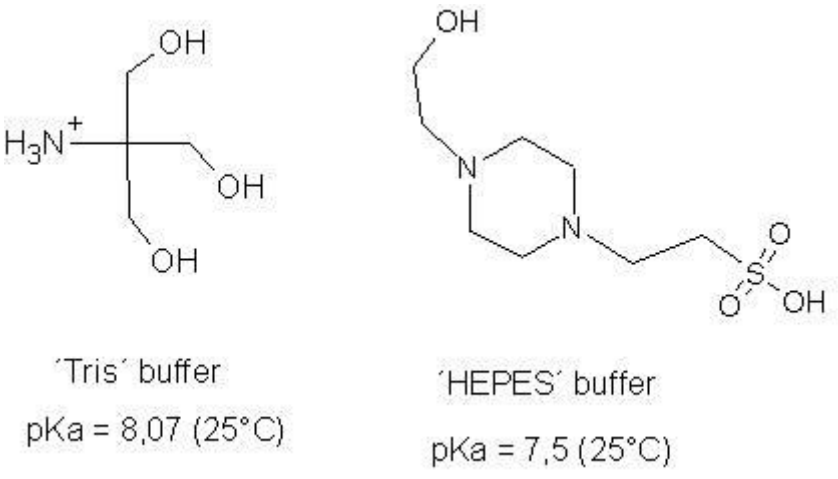

Figure 2: Examples of buffer agents (ChemSketch software)

2.5.6. Disinfectant additives

Niksirat *et al.* (2007) reported addition of 100 $IU.ml^{-1}$ penicillin and 0.1 $mg.ml^{-1}$ streptomycin sulfate to their Cortland artificial medium.

2.5.7. Previous works on short-term storage of eggs

The ability of eggs to remain viable, for certain period of time, after stripping, has been proved „to be different from some hours to some days for a variety of species" (Samarin *et al.*, 2013; Samarin *et al.*, 2015). In general, eggs of the oligotermic fish species, for instance species of family Salmonidae have longer duration of viability (Niksirat *et al.*, 2007) in compared to eggs of polytermic fishes, for example species of family Cyprinidae (Lahnsteiner *et al.*, 2001).

2.5.7.1. Acipenseridae

These species are able to hold their natural fertility for several hours in right conditions (Gisbert and Williot, 2002; Hajirezaee and Niksirat, 2009; Zhang *et al.*, 2010). Eggs of model fish of this theses, oligotermic sterlet, was succesfuly stored by Gisbert and Williot (2002) in CF at 15°C up to 2–4 hours.

Another species of sturgeon, whose eggs were experimentally stored for short-time period, Siberian sturgeon and Persian sturgeon were found similar to produce viable eggs without loss of fertility up to 4 hours (at 16°C) and 3 hours (at 18°C), respectively (Sohrabnezhad *et al.*, 2006; Hajirezaee and Niksirat, 2009; Zhang *et al.*, 2010). Sohrabnezhad *et al.* (2006) and Hajirezaee and Niksirat (2009), experimented with ova endangered Persian sturgeon from Iranian waters, tried out *in vitro* storage, using these media: CF and ACF designed by chemical composition of CF from 18 females.

Zhang *et al.* (2010) stored Siberian sturgeon eggs in Hepes solution prepared according to the biochemical composition of Siberian sturgeon CF, for 4 hours at 16°C with the high percentage of fertilization and no malformation rate. Significant malformation rate was observed by Gisbert and Williot (2002) for storage of this species above 4 hours.

2.5.7.2. Cyprinidae

Another researched species family Cyprinidae, which belong to the most breeded livestock species, were founded different for results of short-term preservation of their

eggs. For example ova of common carp, grass carp, silver carp, *Hypophthalmichthys molitrix*, and tench, *Tinca tinca*, were observed (Mikodina and Makeyeva, 1980; Linhart *et al.*, 2001; Lahnsteiner et al., 2001; Taati *et al.*, 2011; Samarin *et al.*, 2013; Safarzadenia *et al*., 2013).

In the comparison with eggs of sturgeon species, cyprinids species eggs were reported not to be able to hold their fertility for longer time period, because of auto activation after ovulation in many cases (Stoss and Donaldson, 1983, Samarin *et al.*, 2013). For instance eggs of polytermic common carp were preserved experimentally for short time period by Linhart et al. (2001) and Taati et al. (2011). It was researched that eggs of this species are able to hold their original viability for the longest time period at 21°C for only 30 minutes in non-modified Dettlaff extender. It was observed that preferable sollution for storage at 21°C for 60 minutes has been modified Dettlaff extender, nevertheless the hatching was significantly lower than in the control (Linhart et al., 2001). Taati et al. (2011) researched that maintaining of carp eggs at 4°C has higher percentage of hatching and eyeing rate than at 21°C. Higher rate of embryonic malformations of common carp was not observed (Linhart *et al.*, 2001; Taati *et al.*, 2011; Samarin *et al.*, 2013).

Linhart *et al.* (2001) experimented also with ova of polytermic tench at 21°C and noted significant decrease of fertility above 10 minutes period of time of storage in non-modified Dettlaff extender and also modified extenders and carp AOF.

Another livestock species of Cyprinidae, polytermic grass carp was observed too. For instance, its ova were experimentally stored in grass carp artificial coelomic fluid medium and Dettlaff extender by Safarzadenia *et al*. (2013), however it was discovered that eggs lost their viability comparatively quickly in this solutions. The best was grass carp AOF at 4°C with holding of original viability for 60 minutes.

Not all of eggs of family Cyprinidae species are able to hold their viability for only short time period. For example eggs of endangered kutum, *Rutilus frisii kutum*, (species which inhabits Caspian Sea drainage) are very resistively (or the technology of the storage is well developed) in the comparison with other Cyprinidae family species. The successful storage duration is to 8 hours at 4°C in natural OF of this fish (Samarin *et al.*, 2013).

2.5.7.3. Salmonidae

In comparison to species of family Cyprinidae and Acipenseridae, eggs of oligotermic species of family Salmonidae are much more viable in longer period of time (Niksirat *et al.*, 2007; Samarin *et al.*, 2008; Samarin *et al.*, 2013). All species of Salmonids have huge eggs and their count for one female is rather small, in order of hundreds to thousands, but often only to one hundred, according to size of the female (Baruš and Oliva, 1995; Kottelat and Freyhof, 2007; Gessner *et al.*, 2010a). Short-term storage of these eggs has been founded to be more successful because of occurence of post-spawning activation only after releasing oocytes in the water (Stoss and Donaldson, 1983; Samarin *et al.*, 2008). Time period of prior fertiability of salmonids eggs has been reported to be several days at cold temperatures. For instance, Caspian brown trout eggs retain their viability for 2 days (Niksirat *et al.*, 2007) and rainbow trout, *Oncorhynchus mykiss*, for 9 days at 2–3°C (Samarin *et al.*, 2008).

2.5.7.4. Siluridae

Linhart and Billard (1995) reported survival of European catfish, *Silurus glanis*, ova after *in vitro* storage under aerobic conditions. Their primal fertiability was hold up to 3 h at 17–18°C and they confirmed that larvae of this species used to have increased percentage rate of abnormalities (37 % after 8.5 hours at 19°C without significant decrease of hatching), nevertheless larvae of another economically important African catfish, *Clarias gariepinus*, from nearly related family Clariidae, had much higher percentage of abnormalities (60 % after 6 hours of storage) (Richter and Van Den Hurk, 1982).

3. MATERIALS AND METHODS

3.4.Ethics

All experimental procedures were performed according to National and Institutional guidelines on animal experimentation and care, and were approved by the Animal Research Committee of University of South Bohemia in Ceske Budejovice.

3.5. Animal husbandary and gamete collection

3.5.1. Animal husbandary

Three males ♂ and three females ♀ (age 6–8 years) of sterlet, *Acipenser ruthenus*

3.5.2. Materials

Tanks (0.8 m3); Carp pituitary homogenized-extract (CPE); Syringes; Equipment for artificial spawning; 3 × plastic cathether (4 mm o.d.); 3 × cell-culture flask (50 ml); Microscope; Accessories for microscopy; Automatic pipettes; Marker; Tools for microsurgery (tweezers, scalpel, spatula); 3 × plastic dish (1.5 l); Burner (BURNER J-2000 J.P. SELECTA S A); disinfectant sollution (1 g $KMnO_4$ per l); Polysterene ice box

3.5.3. Method

For the experiment, three males ♂ and three females ♀ of sterlet were selected during the spawning season at the beginning of May 2015 in hatchery of Faculty of fisheries and protection of waters in Vodňany. Fish were held in tanks (0.8 m^3) at 15 °C during controled propagation. For induction of spermiation in three males was used a single intramuscular injection CPE in dosage of 4.0 $mg.kg^{-1}$ b.w. Fresh semen was tapped at 42–48 hours after the injection, from the urogenital papilla by usage of a plastic catheter (4 mm o.d.), collected into separate cell-culture flasks (50 ml) (Gela *et al.*, 2008), and stored in the polystyrene ice box during whole experiment. Thereafter, males ♂ underwent preventative disinfectant bath (1 g $KMnO_4$ per 1 l). Three samples

of semen from three different males ♂ was analyzed by microscopy in laboratory (× 400). After spermatozoan activation caused by dilution sperm with water, selection based on assessment of factors of fairly good motility and high spermatozoan density (1.0×10^9 per ml) was done and single cell-culture flasks were labelled by the marker. The aim of the experiment was an observation of egg viability through hatching success rate, only good quality sperm from different males were pooled so as to minimize paternal effects (Stoss, 1983).

In females ♀, ovulation was induced with a total dose of 4.5 $mg.kg^{-1}$ b.w CPE. Two injections were given, a primary dose of 0.5 $mg.kg^{-1}$ b.w., and then 12 hours later a second dose of 4.0 $mg.kg^{-1}$ b.w. was dealt. The fish ♀ were manually stripped through microsurgery 18–20 hours after the second injection. The eggs were collected into three separate plastic dishes (1.5 l) and the burner (BURNER J-2000 J.P.SELECTA S A) was used for desinfection of surgical tools during manual stripping. Females ♀ were also placed into preventative disinfectant bath (1 g $KMnO_4$ per l). Time period from ovulation until the initial control insemination lasted about 1 hour.

CF obtained together with the eggs during manual stripping was used as a storage medium to protect eggs from dryness until beginning of experiment. However, during artificial fertilization, a small plastic spoon was used for a collection of the eggs from the CF.

3.6.Experimental design

3.6.1. Materials

Good quality sperm; Eggs from three famales ♀; Plastic bowl (0,5 l); Plastic spoons; Digital scale; Foil; Incubators (3 × UNIMED POLL LAB, Type: Q–CELL, Model: 140/2/40 Basict 1 × UNIMED POLL LAB, Type: Q–CELL, Model: 60/60 Std.); 16 × plastic bowl (250 ml) 30 × plastic bowl (75 ml); Automatic pipettes; Shaker table (IKA® KS 260 basic); 30 × plastic petri dish; Experimental cage incubation system with separate chambers (3.5 l); Marker; Binocular loupe; Calculator; Notepad

3.6.2. Method

The experiment was conducted using pooled eggs from three females ♀, placed into one plastic bowl (0,5 l). They were stored in CF in various storage times (Control = time 0 h, 2.5 h, 5.0 h, 7.5 h and 10.0 h). Sixteen samples of eggs with CF were created (cca 12 g for one sample) in sixteen plastic bowls (250 ml), covered with the foil, and labelled by the marker according to deviding to four temperature graded treatment groups (7°C, 11°C, 15°C, and 19°C) for storage in four incubators (3 × UNIMED POLL LAB, Type: Q–CELL, Model: 140/2/40 Basict 1 × UNIMED POLL LAB, Type: Q–CELL, Model: 60/60 Std.). Simultaneously, the control group of the eggs in CF (cca 4 g ~ 200–250 eggs) was placed into the small plastic bowl (75 ml) and inseminated in dechlorinated water at 15°C with 200 µl of semen with usage of pipette. The sperm was diluted with 8 ml of water and immediately added to the eggs in the small bowl on the shaker table (IKA® KS 260 basic) at 250 rpm kept in motion for 5 minutes. The same method of fertilization was repeated with the four remaining temperature treatment groups; 4 g of eggs from each temperature treatment group were always used for one insemination, according to the aforementioned procedure. Than, the smaller sample of fertilized eggs was allocated in triplicate to plastic petri dishes and other eggs was dispersed onto the network bottoms of each of three rectangular stainless steel incubating chambers (3.5 l). The total number of eggs was counted in each replicate and enrolled to the protocol. No de-sticking materials (clay, milk, tannic acid etc.) were used for the elimination of stickness although, fertilized sterlet eggs become strongly adhesive within a few minutes after coming in contact with water (Siddique *et al.*, 2014b). Water temperature during incubation was 16°C, in the petri dishes it was changed daily, while UV-treated water from hatchery was continuously circulated through the cage incubation system, and approximately one-third of the water volume was replaced each day.

Evaluation of fertilization rate was accomplished during neuralization phase in petri dishes at about 72 hours post-fertilization by viewing with a binocular loupe, counting non-viable and normal-developed eggs (Dettlaff *et al.*, 1993). Than, approximately 5–6 days post-fertilization, the total number of succesfully hatched larvae was acurately calculated for the final evaluation of storage success. The percentage of hatched larvae [H_r] was computed for each treatment from the total number of eggs [E_t] placed in the

incubation cage minus dead eggs [E_d] by the following equation: $H_r = (E_t - E_d) / E_t * 100$.

3.7. Statistical analyses

All data were analyzed using STATISTICA v 12 (Statsoft Inc., Tulsa, OK, USA) software. Residuals were tested for normality (Shapiro-Wilk test) and homogeneity of variance (plot of residuals vs predicted values). All the data were converted by angular transformation (arcsin $\sqrt{p}$) prior to analysis by ANOVA. Alpha was set at 0.05 for main effects and interactions.

Hatching success was analyzed using a factorial ANOVA model containing the egg storage time (0 h, 2.5 h, 5.0 h, 7.5 h and 10 h) and storage temperatures (7°C, 11°C, 15°C and 19°C). Analysis included main effects, as well as the egg storage time × egg storage temperature. Then, the model was decomposed into a series of lower-order models. Here, the decomposed ANOVA models were run to determine the effect of egg storage time for each temperature using a series of one-way ANOVA models.

4. RESULTS

4.1. Effect of temperature and storage time on hatching success

The saturated two-way ANOVA model indicated significant effects of storage time and storage temperature as well as storage time and storage temperature interaction effects on hatching rate ($p < 0.01$). Generally, eggs retained their hatchability when stored at 7°C and 11°C for up to 10 hours, but decreased percentage of hatching was observed at 15°C and 19°C (Figure 3 and 4). Egg viability was noticeably reduced at 7.5-h storage at 19°C compared to cooler temperatures, moreover viability decreased significantly after 10 hours at 19°C (Figure 3 and 4). Fertilization rates were estimated at the neurula stage and essentially mirrored these standard changes of egg viability observed in the hatching data, especially indicating that 19°C was not suitable for storage for longer than 2.5–5.0 hours.

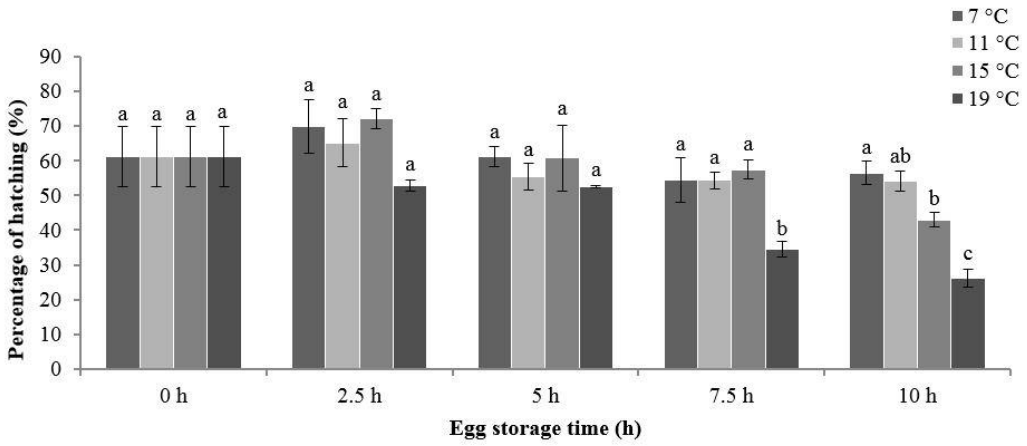

Figure 3

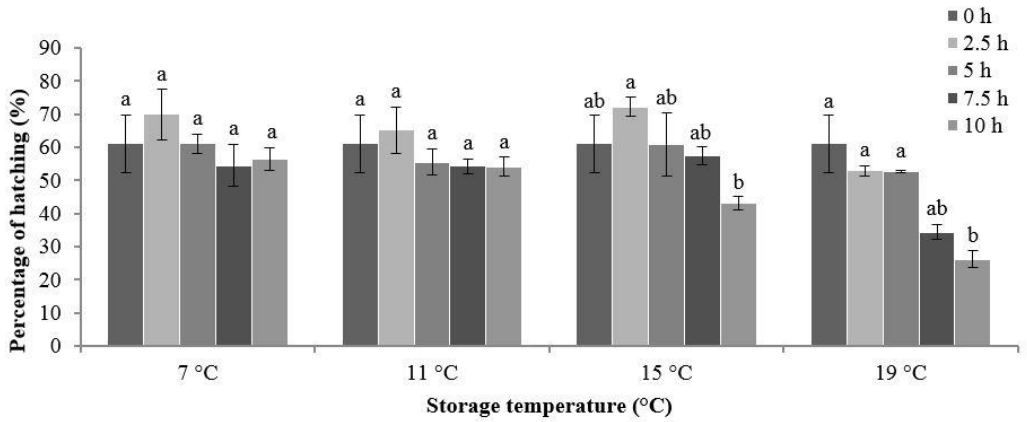

Figure 4

5. DISCUSSION

Several studies have coincided that the biological quality of eggs which is related to over-ripenig, affects on short-term storage of eggs (Linhart and Billard, 1995; Gisbert and Williot, 2002; Furuita *et al.*, 2003; Rizzo *et al.*, 2003; Bobe and Labbé, 2010; Safarzadenia *et al.*, 2013; Samarin *et al.*, 2015). According to Dettlaff *et al.* (1993), good quality sturgeon eggs can be polyspermic only rarely, but in the case of the poor physiological condition, the cortical reaction spreading which probably prevents penetration of multiple spermatozoa, can be delayed. Slowing or stoping of cortical reaction was also observed in overriped eggs of other fish species (Samarin *et al.*, 2015). Hajirezaee and Niksirat (2009) reported that dead and overripe eggs in storage medium *in vitro* can be a potential source of danger for the eggs, because they can induce fluctuation of pH value and increase the osmolality. It can be correlated with experimental findings of Lahnsteiner *et al.* (2001) who showed that eggs of several cyprinids species can be very sensitive to changes of pH and osmolality. Although, pH value can be managed and maintained by using bichemical buffers (Niksirat *et al.*, 2007; Zhang *et al.*, 2010). Several hatchery manuals about sturgeons described how to improve quality of eggs and predict and recognize over-ripening eggs (Gela *et al.*, 2008; Chebanov *et al.*, 2011; Pšenička *et al.*, 2014). However, a reliability of these over-ripening biomarkers is controversial (Samarin *et al.*, 2015, Bobe and Labbé, 2010). They argued that postovulatory ageing can cause a significant decrease in egg developmental capacities without any noticeable morphological changes in the appearance of the egg. Therefore, these uncertainties can significantly complicate the whole succesuful process of short-term preservation.

Fertilizability retention of good quality ova depends on the storage temperature as well as fish species (Stoss, 1983; Rizzo *et al.*, 2003; Niksirat *et al.*, 2007; Samarin *et al.*, 2015). Generally, egg over-ripening occurs faster in polytermic fishes than oligotermic species (Lubzens *et al.* 2010). Sturgeon species belongs rather to oligotermic species and concretely sterlet ecological valence is somewhere on the border of both groups (Kottelat and Freyhof, 2007; Gessner et al., 2010a; Gela *et al.*, 2012). Dettlaff *et al.* (1993) generalized retention of sturgeon eggs fertizability on 4–6 hours in the CF at the spawning temperatures and according to them, holding unovulated eggs at 0°C or 30°C causes activation of the eggs. Gisbert and Williot (2002) published data about

experimental storage of sterlet and Siberian sturgeon eggs at 15°C and reported lower time possibilities of their succesful storage, approximatelly 2–4 hours in both species, while results of our study showed that sterlet eggs retain their hatchability at 15°C for 7.5 hours. The hypothesis why eggs in our experiment held their hatchability for longer time then in previous study, can be just better quality of eggs, because Gisbert and Williot (2002) used older females (age 14–16 year × age 6–8 years used for our experiment) and unlike our experiment, they eliminated eggs stickness, probably because they dealt with higher amount of eggs, so some part of them could also be damaged. Nevertheless, temperature 15°C is quite inconvenient for scheduled storage up to 10 hours according result of our experiment but, this temperature condition can increase the percentage of hatching rate when storage is scheduled up to 2.5 hour.

Sohrabnezhad *et al.* (2006) and Hajirezaee and Niksirat (2009) dealt with short-term storage of Persian sturgeon and they found similar results. Sohrabnezhad *et al.* (2006) observed significant decline of eggs fertizability after 3 hours at 4°C in CF but, better results were received after storage at 18°C, particularly when they used Persian sturgeon CF with strong buffer agent, like a storage medium (Using this medium looks like a potential opportunity for improving technology of egg preservation of other sturgeon as well as sterlet). Then, Sohrabnezhad *et al.* (2006) suggested two reasonable hypotheses for their results, first was that the considerable part of these eggs could be autoactivated at 4°C, which corresponds with the finding of Dettlaff *et al.* (1993). Their second hypothesis related to high sensitivity of Persian sturgeon ova to low temperature. According to Gessner *et al.* (2010b) and Chebanov *et al.* (2011), this anadromous sturgeon prefers quite warmer temperature during spawning than sterlet, at about 16–25°C. Hajirezaee and Niksirat (2009) storaged eggs of this species in natural CF only at 18°C and observed changes in the pH and osmolality of the CF and found similar results in storage possibilities of eggs. Their control group of eggs had quite worse fertilization rate (approximatelly 74.3 %, according to the Figure) than fertilization rate in experiment of Sohrabnezhad *et al.* (2006) (87.0±1.0 %), but the fall of fertizability and hatchability curve was more rapidly in Sohrabnezhad *et al.* (2006) experiment, when they stored eggs in the same conditions, in CF at 18°C. It can be explained variations in reproductive ability of individual females because these two experiments varied significantly only in dosages of their preparations for ovulation induction: 3.7 $mg.kg^{-1}$ for Sohrabnezhad *et al.* (2006) and 2 $mg.kg^{-1}$ for Hajirezaee and Niksirat (2009),

respectively. This results shows that primary fertizability and hatchability of Persian sturgeon eggs are limited up to 3 hours in comparison with sterlet which is able to hold its primary hatchability probably till over 10 hours at 7°C according our results. However effect of more than two temperature conditions has been never determined for Persian sturgeon ova, so we can assume that retention time of viability can be more than 3 hours.

The retention of sturgeon eggs viability is probably several times lower in the comparison with ova of oligotermic salmonids which retain their fertizability generally for several days (Niksirat *et al.*, 2007; Samarin *et al.*, 2008). However incubation time of for example wild brown trout, *Salmo trutta morpha fario*, embryo lasts 260–520 degree-days (Randák *et al.*, 2006) whereas sterlet incubation time lasts approximately 82.5–114 degree-days (after conversion) according to Gela *et al.* (2012). The developement is therefore several times faster in sterlet than brown trout. The polytermic species like for example European catfish or tench have often shorter incubation time than sturgeons (Linhart *et al.*, 2002; Gela *et al.*, 2003) and also their eggs retain their original viability for shorter time period than salmonids (Linhart and Billard, 1995; Linhart *et al.*, 2001). Samarin *et al.* (2015) referr about very high concentration of ATP of Chinook salmon, *Oncorhynchus tschawytscha*, eggs. The concentration was approximately 100 times higher than in common carp eggs and did not decrease during five days *in vitro* storage unlike common carp eggs in which, the concentration of ATP decreased up to four hours. Nevertheless, faster decreasing of ATP concentration was observed at 17°C than 12°C, in eggs of rainbow trout (Aegerter and Jalabert, 2004) which corresponds with generally valid opinion that reduced temperature reduces the activity of the metabolism (Samarin *et al.*, 2015). ATP concentration in sterlet and or other sturgeon eggs has not been ever reported, but it can be presumed that it is lower than in eggs of salmonids and higher than in cyprinids eggs, generally.

We have to admit that the technology of egg preservation is at the begining of developement but in future it could serve very well. According to Samarin *et al.* (2015), an application of antioxidants for protection fish eggs from ROS has been never tried. On the other hand, the technology progression is significantly limited by lack of mature fish able to produce ova, especially in the case of sturgeon species. Samarin *et al.* (2015) inform also about inadequate knowledge of fish egg cellular and molecular

changes through oocyte ageing. According them, most findings in this field have been obtained from studies on higher vertebrates and it should be broken in a future for aquaculture developement and the protection of endangered fish species.

6. CONCLUSION

The aim of this thesis was to determine the effect of various temperature conditions on short-term storage of sterlet ova. Our findings showed that sterlet eggs succesfully retain their fertilizing ability for up to 10 hours at 7 to 11°C. The question which remains is, how much viability can be reduced in case of less capable sterlet females to produce quality eggs or in case of delayed stripping. The best temperature range has been determined as 7–11°C. Results of our study could be a baseline study for future research works as well as for sturgeon hatchery practise.

For technological improvement of short-term storage of sterlet unovulated eggs can be recommend clarifying the process of egg over-ripening by ultrastructual study of fertilization in dependence on time and temperature (for example 7°C and 19°C), measuring ATP concentration in eggs in dependence on concentration of ROS in medium and time duration, determining pH fluctuations in storage medium during experiment duration at 7–11°C and than synthesize several ACF based on ionic composition of sterlet CF with different antioxidants and various buffer agents.

7. ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic – projects ‘CENAKVA’ (No. CZ.1.05/ 2.1.00/01.0024), ‘CENAKVA II’ (LO1205 under the NPU I program).

8. REFERENCES

Aegerter, S., Jalabert, B., 2004. Effects of post-ovulatory oocyte ageing and temperature on egg quality and on the occurrence of triploid fry in rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 231, 59–71.

Aizenshtadt, T. B., Dettlaff, T. A., 1972. Ultrastructure of stellate sturgeon oocytes during maturation. I. Annulate lamellae and golgi complex. The Soviet journal of developmental biology. 3, 220–229.

Alavi, S. M. H., Cosson, J., 2006. Sperm motility in fishes.(II) Effects of ions and osmolality: a review. Cell biology international. 30, 1–14.

Bartley, D. M., Rana, K., Immink, A. J., 2000. The use of inter-specific hybrids in aquaculture and fisheries. Reviews in Fish Biology and Fisheries.10, 325–337.

Baruš, V., Oliva, O., 1995. Fauna ČR a SR: Mihulovci (Petromyzontes) a ryby (Osteichthyes) (1). Academia, Prague, 624 pp.

Bayunova, L., Barannikova, I., Semenkova, T., 2002. Sturgeon stress reactions in aquaculture. Journal of Applied Ichthyology. 18, 397–404.

Betancur-R, R., Broughton, R. E., Wiley, E. O., Carpenter, K., López, J. A., Li, C., Holcroft, N. I., Arcila, D., Sanciangco, M., Cureton, J. C., Zhang, F., Buser, T., Campbell, M. A., Ballesteros, J. A., Roa-Varon, A., Willis, S., Borden, W. C., Rowley, T., Reneau, P. C., Hough, D. J., Lu, G., Grande, T., Arratia, G., Ortí, G., 2013. The tree of life and a new classification of bony fishes. PLoS Curr. 5, 1–33.

Billard, R., Lecointre, G., 2000. Biology and conservation of sturgeon and paddlefish. Reviews in Fish Biology and Fisheries. 10, 355–392.

Bobe, J., Labbé, C., 2010. Egg and sperm quality in fish. General and comparative endokrinology. 165, 535–548.

Boryshpolets, S., Dzyuba, B., Rodina, M., Alavi, S. M. H., Gela, D., Linhart, O., 2011. Cryopreservation of sterlet (*Acipenser ruthenus*) spermatozoa using different cryoprotectants. Journal of Applied Ichthyology. 27, 1147–1149.

Bromage, N. R., Roberts, R. J., 1995. Broodstock management and egg and larval quality. Blackwell Science Ltd, Oxford, 424 pp.

Bronzi, P., Rosenthal, H., Gessner, J., 2011. Global sturgeon aquaculture production: an overview. Journal of Applied Ichthyology. 27, 169–175.

Brooks, S., Tyler, C. R., Sumpter, J. P., 1997. Egg quality in fish: what makes a good egg?. Reviews in Fish Biology and Fisheries. 7, 387–416.

Chebanov, M., Rosenthal, H., Gessner, J., van Anrooy, R., Doukakis, P., Pourkazemi, M., Williot, P., 2011. Sturgeon hatchery practices and management for release – Guidelines. Food and Agriculture Organization, Ankara, 110 pp.

Cherr, G. N., Clark, W. H., 1982. Fine structure of the envelope and micropyles in the eggs of the white sturgeon, *Acipenser transmontanus* Richardson. Development, Growth & Differentiation. 24, 341–352.

Dettlaff, T. A., Ginsburg, A. S., Schmalhausen, O. I., 1993. Sturgeon fishes: developmental biology and aquaculture. Springer-Verlag, Berlin, pp. 1–155.

Furuita, H., Ohta, H., Unuma, T., Tanaka, H., Kagawa, H., Suzuki, N., Yamamoto, T., 2003. Biochemical composition of eggsin relation to egg quality in the Japanese eel, *Anguilla japonica*. Fish Physiology and Biochemistry. 29, 37–46.

Gasparini, C., Andreatta, G., Pilastro, A., 2012. Ovarian fluid of receptive females enhances sperm velocity. Naturwissenschaften. 99, 417–420.

Gela, D., Linhart, O., Flajšhans, M., Rodina, M., 2003. Egg incubation time and hatching success in tench Tinca tinca (L.) related to the procedure of egg stickiness elimination. Journal of Applied Ichthyology. 19, 132–133.

Gela, D., Rodina, M., Linhart, O., 2008. Řízená reprodukce jeseterů (*Acipenser*). Edice Metodik, VÚRH JU Vodňany, 24 pp.

Gela, D., Kahanec, M., Rodina, M., 2012. Metodika odchovu raných stádií jeseterovitých ryb. Edice Metodik, VÚRH JU Vodňany, 46 pp.

Gela, D., Kahanec, M., Buřič, M. 2014. Technologie chovu jeseterů. Edice Metodik, VÚRH JU Vodňany, 49 pp.

Gessner, J., Freyhof, J., Kottelat, M., 2010a. *Acipenser ruthenus*. The IUCN Red List of Threatened Species 2010: e.T227A13039007. http://dx.doi.org/10.2305/IUCN.UK.2010-1.RLTS.T227A13039007.en. Downloaded on 24 April 2016.

Gessner, J., Freyhof, J., Kottelat, M., 2010b. *Acipenser persicus*. The IUCN Red List of Threatened Species 2010: e.T235A13043839. http://dx.doi.org/10.2305/IUCN.UK.2010-1.RLTS.T235A13043839.en. Downloaded on 24 April 2016.

Gisbert, E., Williot, P., 2002. Influence of storage duration of ovulated eggs prior to fertilisation on the early ontogenesis of sterlet (*Acipenser ruthenus*) and Siberian sturgeon (*Acipenser baeri*). International review of hydrobiology. 87, 605–612.

Glenn, D. W., Tiersch, T. R., 2002. Effect of extenders and osmotic pressure on storage of eggs of ornamental common carp *Cyprinus carpio* at ambient and refrigerated temperatures. Journal of the World Aquaculture Society. 33, 254–267.

Hajirezaee, S., Niksirat, H., 2009. In vitro storage of ova of Persian sturgeon, *Acipenser persicus* in ovarian fluid: the changes in the pH and osmolality of the ovarian fluid, fertilization and hatching rates. Australian Journal of Basic and Applied Sciences. 3, 3438–3442.

Haynie, D. T., 2001. Biological thermodynamics. Cambridge University Press, Cambridge, pp. 130–136.

Kjørsvik, E., Mangor-Jensen, A., Holmefjord, I., 1990. Egg quality in fishes. Advances in Marine biology. 26, 71–113.

Komrakova, M., Holtz, W., 2009. Factors responsible for successful chilled storage of unfertilized rainbow trout (*Oncorhynchus mykiss*) eggs. Aquaculture. 286, 156–163.

Kottelat, M., Freyhof, J., 2007. Handbook of european freshwater fishes. Publications Kottelat, Cornol and Freyhof, Berlin, 646 pp.

Lahnsteiner, F., Weismann, T., Patzner, R. A., 1995. Composition of the ovarian fluid in 4 salmonid species: *Oncorhynchus mykiss*, *Salmo trutta f lacustris*, *Salvelinus alpinus* and *Hucho hucho*. Reproduction Nutrition Development. 35, 465–474.

Lahnsteiner, F., 2000. Morphological, physiological and biochemical parameters characterizing the over-ripening of rainbow trout eggs. Fish Physiology and Biochemistry. 23, 107–118.

Lahnsteiner, F., Urbányi, B., Horvath, A., Weismann, T., 2001. Bio-markers for egg quality determination in cyprinid fish. Aquaculture. 195, 331–352.

Lahnsteiner, F., Berger, B., Horvath, A., Urbányi, B., 2004. Studies on the semen biology and sperm cryopreservation in the sterlet, *Acipenser ruthenus* L. Aquaculture Research. 35, 519–528.

Linhart O, Šlechta V., Slavík T., 1991. Fish sperm composition and biochemistry. Bulletin of Institut of Zoology. Academia Sinica, monograph. 16, 288–311.

Linhart, O., Kudo, S., Billard, R., Slechta, V., Mikodina, E. V., 1995. Morphology, composition and fertilization of carp eggs: a review. Aquaculture. 129, 75–93.

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. Aquatic Living Resources. 8, 317–322.

Linhart, O., Gela, D., Rodina, M., Gutierrez, M. R., 2001. Short-term storage of ova of common carp and tench in extenders. Journal of fish biology. 59, 616–623.

Linhart, O., Štěch, L., Švarc, J., Rodina, M., Audebert, J. P., Grecu, J., Billard, R., 2002. The culture of the European catfish, *Silurus glanis*, in the Czech Republic and in France. Aquatic Living Resources. 15, 139–144.

Linhart, O., Rodina, M., Boryshpolets, S., 2011. Hodnocení čerstvého spermatu ryb. Edice Metodik, VÚRH JU Vodňany, 13 pp.

Lubzens, E., Young, G., Bobe, J., Cerda, J., 2010. Oogenesis in teleosts: How fish eggs are formed. Gen Comp Endocrinol. 165, 367–389.

Matsubara, T., Hara, A., Takano, K., 1985. Immunochemical identification and purification of coelomic fluid-specific protein in chum salmon (*Oncorhynchus keta*). Com. Biochem. Physiol. Part B: Com. Biochem. 81, 309–314.

McMillan, D. B., 2007. Fish histology: female reproductive systems. Springer, London, 598 pp.

Mikodina, Y. V., Makeyeva, A. P., 1980. The structure and some properties of egg membranes in pelagophilous freshwater fishes. Journal of Ichthyology. 20, 86–94.

Muchlisin, Z. A., Siti-Azizah, M. N., 2010. Preliminary study on the natural extenders for artificial breeding of African catfish *Clarias gariepinus* (Burchell, 1822). AACL Bioflux. 3, 119–124.

Niksirat, H., Sarvi, K., Amiri, B. M., Karami, M., Hatef, A., 2007. In vitro storage of unfertilized ova of endangered Caspian brown trout (*Salmo trutta caspius*) in artificial media. Animal reproduction science. 100, 356–363.

Oda, S., Igarashi, Y., Ohtake, H., Sakai, K., Shimizu, N., Morisawa, M., 1995. Sperm activating proteins from unfertilized eggs of the pacific herring *Clupea pallasii*. Dev. Growth Differ. 37**,** 257–261.

Pšenicka, M., Alavi, S. M., Rodina, M., Gela, D., Nebesarová, J., Linhart, O., 2007. Morphology and ultrastructure of Siberian sturgeon (*Acipenser baerii*) spermatozoa using scanning and transmission electron microscopy. Biology of the Cell. 99, 103–115.

Pšenička, M., 2007. Ultrastructure of chondrostean spermatozoa (BRIEF REVIEW). Bulletin VÚRH Vodňany. 43, 3–15.

Pšenicka, M., Alavi, S. M. H., Rodina, M., Cicová, Z., Gela, D., Cosson, J., Nebesarová, J., Linhart, O., 2008. Morphology, chemical contents and physiology of chondrostean fish sperm: a comparative study between Siberian sturgeon (*Acipenser baerii*) and sterlet (*Acipenser ruthenus*). Journal of Applied Ichthyology. 24, 371–377.

Pšenicka, M., Rodina, M., Linhart, O., 2010. Ultrastructural study on the fertilisation process in sturgeon (*Acipenser*), function of acrosome and prevention of polyspermy. Animal reproduction science. 117, 147–154.

Pšenička, M., Saito, T., Rodina, M., 2014. Hodnocení oplozenosti a vývoje embryí divoce zbarvených a albinotických forem jesetera. Edice Metodik, VÚRH JU Vodňany, 29 pp.

Rana, K., 1995. Preservation of gametes. Broodstock management and egg and larval quality. Blackwell Science Ltd, Oxford, pp. 53–75.

Randák, T., Kocour, M., Žlábek, V., Policar, T., Jarkovský, J., 2006. Effect of culture conditions on reproductive traits of brown trout *Salmo trutta* L. Bulletin Français de la Pêche et de la Pisciculture. 383, 1–12.

Richter, C. J. J., Van Den Hurk, R., 1982. Effects of 11-desoxycorticosterone-acetate and carp pituitary suspension on follicle maturation in the ovaries of the African catfish, *Clarias lazera* (C. & V.). Aquaculture. 29, 53–66.

Rizzo, E., Godinho, H. P., Sato, Y., 2003. Short-term storage of oocytes from the neotropical teleost fish *Prochilodus marggravii*. Theriogenology. 60, 1059–1070.

Rodina, M., 2006. Application of image analysis for the determination of nucleus position in sturgeon oocytes. Journal of Applied Ichthyology. 22, 373–374.

Rosengrave, P., Gemmell, N. J., Metcalf, V., McBride, K., Montgomerie, R., 2008. A mechanism for cryptic female choice in Chinook salmon. Behav. Ecol. 19, 1179–1185.

Rothbard, S., Rubinshtein, I., Gelman, E., 1996. Storage of common carp, *Cyprinus carpio* L., eggs for short durations. Aquaculture Research. 27, 175–181.

Safarzadenia, M., Yazdanparast, T., Taati, M. M., Esfahani, H. K., 2013. Storage of unfertilized eggs of grass carp, *Ctenopharyngodon idella*, in artificial media. AACL Bioflux. 6, 478–485.

Samarin, A. M., Ahmadi, M. R., Azuma, T., Rafiee, G. R., Amiri, B. M., Naghavi, M. R., 2008. Influence of the time to egg stripping on eyeing and hatching rates in rainbow trout *Oncorhynchus mykiss* under cold temperatures. Aquaculture. 278, 195–198.

Samarin, A. M., Amiri, B. M., Soltani, M., Nazari, R. M., Kamali, A., Naghavi, M. R., 2013. Effects of storage duration and storage temperature on viability of stored ova of kutum (*Rutilus frisii kutum*) in ovarian fluid. African Journal of Biotechnology. 10, 12309–12314.

Samarin, A. M., Policar, T., Lahnsteiner, F., 2015. Fish oocyte ageing and its effect on egg quality. Reviews in Fisheries Science & Aquaculture. 23, 302–314.

Sanches, E. A., Neumann, G., Baggio, D. M., Bombardelli, R. A., Piana, P. A., Romagosa, E., 2011. Time and temperature on the storage of oocytes from jundiá catfish, Rhamdia quelen. Aquaculture. 319, 453–458.

Siddique, M. A. M., Cosson, J., Psenicka, M., Linhart, O., 2014a. A review of the structure of sturgeon egg membranes and of the associated terminology. Journal of Applied Ichthyology. 30, 1246–1255.

Siddique, M. A. M., Psenicka, M., Cosson, J., Dzyuba, B., Rodina, M., Golpour, A., Linhart, O., 2014b. Egg stickiness in artificial reproduction of sturgeon: an overview. Reviews in Aquaculture. 8, 1–12.

Siddique, M. A. M., Butts, I. A. E., Psenicka, M., Linhart, O., 2015. Effects of pre-incubation of eggs in fresh water and varying sperm concentration on fertilization rate in sterlet sturgeon, Acipenser ruthenus. Animal reproduction science. 159, 141-147.

Siddique, M. A. M., Butts, I. A. E., Cosson, J., Linhart, O., 2016a. First report on facultative parthenogenetic activation of eggs in sterlet sturgeon, *Acipenser ruthenus*. Animal reproduction science. 168, 110–115.

Siddique, M. A. M., Niksirat, H., and Linhart, O., 2016b. Comparative coelomic fluid biochemistry of three threatened species of sturgeon. Journal of Applied Ichthyology (In press).

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. Journal of Applied Ichthyology. 22, 395–399.

Stoss, J., 1983. Fish gamete preservation and spermatozoan physiology. Fish physiology. Academic press Inc., New York, pp. 305–340.

Stoss, J., Donaldson, E. M., 1983. Studies on cryopreservation of eggs from rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). Aquaculture. 31, 51–65.

Taati, M., Jafaryan, H., Mehrad, B., 2011. Effects of extenders, temperature and holding duration on short-term storage of unfertilized eggs of common carp "*Cyprinus carpio*". Australian Journal of Basic and Applied Sciences. 5, 146–152.

Takahashi, T., Igarashi, H., Amita, M., Hara, S., Matsuo, K., Kurachi, H., 2013. Molecular mechanism of poor embryo development in postovulatory aged oocytes: mini review. Journal of Obstetrics and Gynaecology Research. 39, 1431–1439.

Turner, E., Montgomerie, R., 2002. Ovarian fluid enhances sperm movement in Arctic charr. J. Fish Biol. 60, 1570–1579.

Vorob'eva, E. I., Markov, K. P., 1999. Specific ultra-structural features of eggs of Acipenseridae in relation to reproductive biology and phylogeny. J. Ichthyol. 39, 157–169.

Vostradovsky, J., 1973. Freshwater fishes. The Hamlyn Publishing Group Limited, London, 252 pp.

Williot, P., Arlati, G., Chebanov, M., Gulyas, T., Kasimov, R., Kirschbaum, F., Patriche, N., Pavlovskaya, L. P., Poliakova, L., Pourkazemi, M., Kim, Y., Zhuang, P., Zholdasova, I. M., 2002. Status and management of Eurasian sturgeon: an overview. International Review of Hydrobiology. 87, 483–506.

Wolf, K., Quimby, M. C., 1969. 5 Fish Cell and Tissue Culture. Fish physiology. 3, 253–305.

Zhang, T., Yan, S. W., Zhuang, P., Zhang, L. Z., Tian, M. P., Wang, B., Zhang, D. H., Yao, Z. F., 2010. Effects of storage medium and temperature on short-term storage of *Acipenser baerii* eggs. The journal of applied ekology. 21, 227–231.

9. ABBREVIATIONS

b.w. - body weight

o.d. - of diameter

GV - germinal vesicle

ZRI - zona radiata interna

ZRE - zona radiata externa

CF - coelomic fluid

ACF - artificial coelomic fluid

OF - ovarian fluid

AOF - artificial ovarian fluid

EC - endonuclear canals

ROS - reactive oxygen species

AS - activating sollution

PPs – Postlateral projections

BSA - bovine serum albumin

CPE - Carp pituitary homogenized-extract

10. ABSTRACT

Effect of temperature on *in vitro* short-term storage of sterlet, *Acipenser ruthenus*, eggs

The effects of temperature (7°C, 11°C, 15°C, and 19°C) and egg storage time (Control = time 0 h, 2.5 h, 5.0 h, 7.5 h and 10.0 h) on the fertilization and hatching success of sterlet, *Acipenser ruthenus*, eggs were studied. Ovulation and spermiation were stimulated by using CPE in two dosages for females (first dose 0.5 $mg.kg^{-1}$ b.w. and second dose 4.0 $mg.kg^{-1}$ b.w.) and one dosage for males (4.0 $mg.kg^{-1}$ b.w.), respectivelly. The ovulated eggs were collected from three females (age 6–8 years) through a small surgery, than mixed together. The good quality semen was selected from three males (age 6–8 years) and was stored in polystyrene ice box during the experiment. The eggs were placed separately into four incubators, where were stored in above-mentioned temperature conditions. Four grams of eggs from each temperature treatment groups were inseminated with 200 µl of the semen in dechlorinated water at 15°C. Then, eggs were placed into experimental cage incubation system with separate chambers at 15°C and a small sample of fertilized eggs was allocated in triplicate to plastic petri dishes for evaluation of fertilization rate during neuralization phase. Approximately, 5–6 days post-fertilization, the total number of succesfully hatched larvae was acurately calculated for the final evaluation of storage success. All data were analyzed using STATISTICA v 12 software. Hatching success was analyzed using a factorial ANOVA model containing the egg storage time and storage temperatures. Eggs retained their hatchability when stored at 7°C and 11°C for up to 10 hours. Egg viability was noticeably reduced at 7.5-h storage at 19°C compared to cooler temperatures, moreover the viability decreased significantly after 10 hours at 19°C. In contrast with the one previous study about shor-term storage of sterlet eggs, this experiment probably dealt with better quality eggs which can be a reason why they retained their hatchability for significantly longer time period.

Keywords: Biological quality of eggs, coelomic fluid, degeneration, osmolality, fertizability, hatchability

11. ABSTRAKT

Vliv teploty při krátkodobém uchování jiker jesetera malého, *Acipenser ruthenus, in vitro*

Studovali jsme vliv teploty (7 °C, 11 °C, 15 °C, and 19 °C) a doby uchování jiker (Kontrola = čas 0 hod, 2,5 hod, 5,0 hod, 7,5 hod and 10,0 hod) na úspěšnost oplození a líhnutí u jiker jesetera malého, *Acipenser ruthenus*. Ovulaci jsme u jikernaček stimulovali kapří hypofýzou dvěma dávkami (první dávka 0,5 $mg.kg^{-1}$ a druhá dávka 4,0 $mg.kg^{-1}$) a spermiaci u mlíčáků jednou dávkou (4,0 $mg.kg^{-1}$). Ovulované jikry jsme vytřeli z trojice jikernaček (věk 6–8 let) za pomoci mikrochirurgického řezu a následně smíchali. K oplozování jsme vybrali sperma s nejlepší kvalitou od 3 mlíčáků (věk 6–8 let), jež jsme po celou dobu experimentu uchovávávali v polystyrenovém boxu s šupinkovým ledem. Jikry jsme umístili odděleně do 4 inkubátorů při výše zmíněných teplotních podmínkách. Čtyři gramy jiker z každé teplotní skupiny jsme osemenili 200 µl spermatu a aktivovali v dechlorované vodě o teplotě 15 °C. Jikry jsme dále inkubovali v experimentálním inkubačním systému s oddělenými komorami při 15 °C a malý vzorek oplodněných jiker z každé skupiny byl umístěn do tří Petriho misek pro hodnodnocení oplozenosti během neurulace. Přibližně 5–6 dní po oplození jsme přesně spočítali celkový počet úspěšně vykulených larev a z toho vypočetli i úspěch uchování. Úspěšnost líhnivosti jsme analyzovali použitím faktoriálního ANOVA modelu, obsahujícího čas uchování jiker a teploty při uchovávání. Jikry si udržely svoji schopnost líhnivosti uchováním při 7 °C a 11 °C po dobu 10 hodin. Životaschopnost jiker se výrazně snížila 7,5 hodinovým uchováním při 19 °C ve srovnání s nižšími teplotami, a navíc se životaschopnost signifikantně snížila i po 10 hodinách při 19 °C. Na rozdíl od jediné předchozí studie, která se zabývá krátkodobým uchováním jiker jesetera malého, tento experiment pravděpodobně disponoval jikrami lepší kvality, což může být důvod, proč si jikry udržely svoji schopnost líhnivosti po významně delší časový úsek.

Klíčová slova: Biologická kvalita jiker, coelomová tekutina, degenerace, osmolalita, schopnost oplození, schopnost líhnivosti