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CHILLED STORAGE OF RAINBOW TROUT, *Oncorhynchus mykiss*, EGGS

Igor Babiak, Jerzy Strzeżek*, Krzysztof Goryczko***

*University of Agriculture and Technology in Olsztyn, Poland
**The Stanisław Sakowicz Inland Fisheries Institute, Poland

A B S T R A C T. Unfertilized eggs of rainbow trout, *Oncorhynchus mykiss*, were refrigerated (1°C) in a single layer, in a moistened oxygen atmosphere. Batches of eggs were inseminated with milt every two days. Fertilization success in fresh, non-stored eggs was 84.0±3.3% of eyed eggs. Fertilization rate of stored eggs did not significantly decrease until the 8th day of storage. A rapid decrease in egg viability was observed between the 12th (67.6±1.1%) and 16th day of storage (19.6±1.7%); after 24 days of storage egg survival to eyed-egg stage was 0.9±0.1%.

Keywords: EGGS, GAMETES, PRESERVATION, STORAGE, TROUT

INTRODUCTION

Ova of rainbow trout, *Oncorhynchus mykiss*, may be kept *in vivo* for 15-30 days without loss of their fertilizability (Escaffre et al. 1977; cited by Billard 1992). However, preservation attempts *in vitro* resulted in a limited success with the survival of stored eggs not exceeding a few days (reviewed by Billard 1992). Barrett (1951) noted that rainbow trout eggs kept in sealed jars in an icebox (1.5-4°C) had lost 25% of their initial fertilizing capacity after 7 days of storage. Stoss (1983) has mentioned unpublished data of H. Pueschel, W. Holtz and J. Stoss which were much more better (high level of fertility after 10 days of storage), but up to date Barrett's classic studies remain the most successful among the published results on short-term storage of rainbow trout.

Temperature of storage (Jensen and Alderdice 1984), oxygen supply, and proper gaseous exchange (Stoss 1983) are the main factors determining preservation efficiency of salmonid eggs These factors were probably the reason for short viability of stored salmonid eggs reported in the literature (Withler and Humphreys 1967; Poon and Johnson 1970; Takano et al. 1973). The use of lower (3°C) storage temperature resulted in prolonged viability of stored eggs of sockeye (*Oncorhynchus nerka*), pink (*Oncorhynchus gorbuscha*, Withler and Morley 1968) and chum salmon (*Oncorhynchus keta*, Jensen and Alderdice 1984).

The purpose of this study was to develop a simple, efficient method of short-term

chilled storage of unfertilized eggs of rainbow trout and to observe the dynamics of decrease in egg fertilizability during storage.

MATERIALS AND METHODS

Eggs were collected from three ripe autumn-spawner females reared in the Inland Fisheries Institute, Department of Salmonid Research, Rutki, Poland. After pooling, eggs were put into a wide glass beaker (7-8 egg layers), which was covered (90% of surface) with "Parafilm M". The beaker was placed in a black plastic bag (75 L) inflated with pure oxygen, and transported within 3 h (3-4°C) on ice-water bath to Olsztyn University of Agriculture and Technology, Olsztyn, Poland.

Eggs (single layer, immersed in ovarian fluid) were refrigerated (>0-1°C) in glass beakers covered with parafilm (90% of surface). Beakers were placed into a plastic tray filled with water and put into the black plastic bag, which was inflated with pure oxygen and sealed (Fig. 1). The oxygen was replenished every two days. Since the 6th day of storage, eggs showing morphological ageing changes were counted and removed every two days. Stored eggs were qualified as "showing ageing changes" if: they were swollen, the breakdown of cortical alveoli and oil droplets occurred, and the egg membranes became transparent. These eggs were unfertilizable.

Milt was collected three times (each time from 5 males), on day 0, 8, and 16 of chilled storage of eggs. After collection, samples of milt were diluted 1:1 with modified Cortland solution (Truscott et al. 1968) and supplemented with antibiotics at a final concentration of 500 U penicillin + 0.5 mg streptomycin per 1 mL (Sigma Chemical Co.). Diluted samples (layer not exceeding 3 mm) were refrigerated separately in an oxygen atmosphere similarily to the stored eggs. According to our preliminary observations (Babiak, unpublished data), such a storage of rainbow trout milt should maintain spermatozoa fertilization ability for ten days. Motility of spermatozoa was assessed in 0.7% NaCl activating solution under a microscope (x 500) and expressed as a percentage of motile spermatozoa (Terner 1986; Babiak et al. 1996). Samples showing less than 60% of motile spermatozoa were discarded. For fertilization tests, eggs were inseminated with milt lots with more than 60% of motile spermatozoa.

Eggs were inseminated at two-day intervals. Batches of eggs (approximately 100 eggs per batch) were supplemented with 5 mL of fertilization diluent (0.9% NaCl and 1 mM Ca^{2+}, buffered to pH 9.0 with 20 mM Tris + 30 mM glycine; recommended by Billard 1992), then 0.1 mL of sperm (pooled just before insemination) was added.

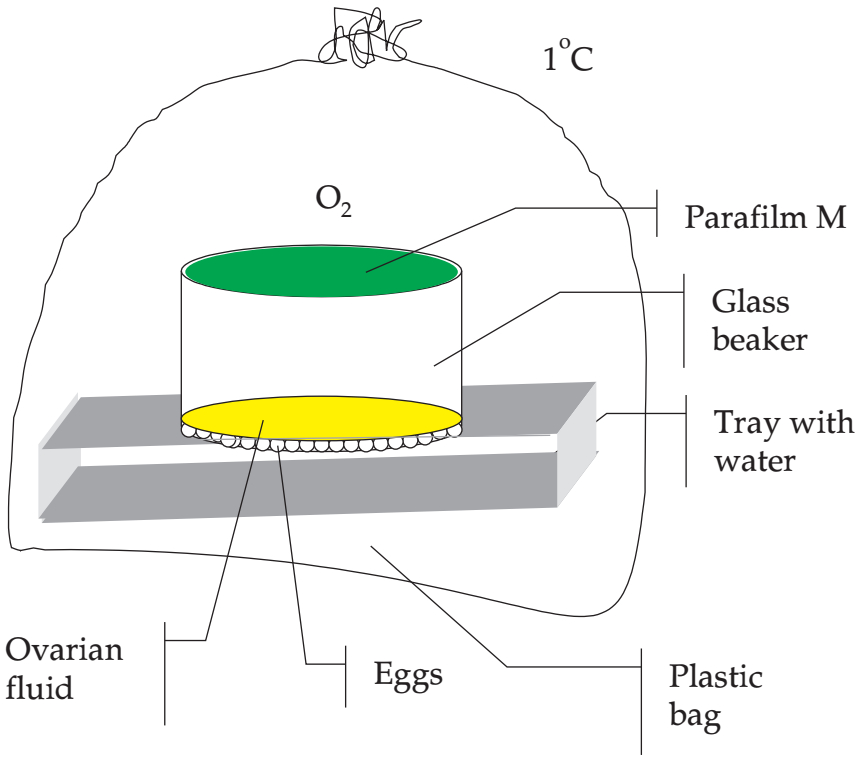

Fig. 1. Schematic diagram of the refrigeration of unfertilized rainbow trout (*Oncorhynchus mykiss*) ova.

Three replicates per each fertilization test were made. Inseminated eggs were gently swirled, left for 20 min, followed by the addition of fresh tap water (5°C). Water-hardened (1 - 2 h) eggs were rinsed and incubated at 5°C until the eyed-egg stage. Live and dead eggs were counted and the results were corrected by the percentage of the eggs showing ageing changes, which were removed from stored batch.

Since the percentage of eyed eggs and ageing eggs showed binomial distribution, those values were transformed ($T = \arcsin \sqrt{\bar{P}}$, where P is proportion of eyed eggs), as recommended by Parker (1973). The homogeneity of variance was tested using Cochran's and Bartlett's test. One-way analysis of variance (ANOVA) was used to evaluate differences between groups. Significance of differences between groups was estimated by multiple range analysis (Tukey HSD intervals at $P < 0.05$). Spearman's correlation coefficient was used for finding the relationship between decreasing fertilization ability and ageing in stored eggs.

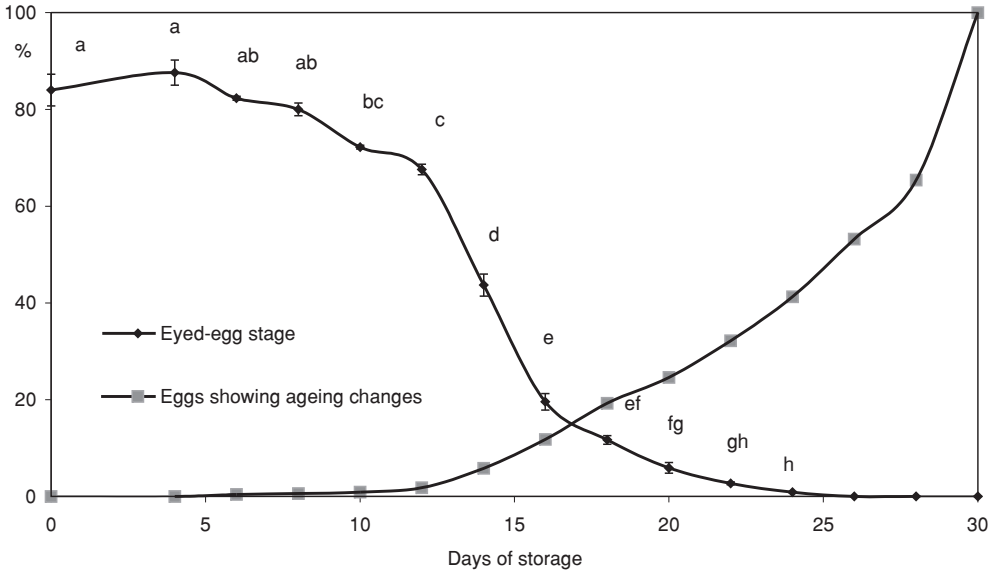

Fig. 2. Fertilization ability of stored (0-1°C) rainbow trout (*Oncorhynchus mykiss*) eggs, expressed as percentage (±SE) of eyed eggs and percentage of eggs showing morphological ageing changes during storage. Values having the same letter did not differ significantly (Tukey HSD intervals at $P < 0.05$).

RESULTS

Loss of viability of eggs during storage is presented on Figure 2. ANOVA showed significant differences between tested groups (F-ratio = 327.291 at 11 d.f.; P < 0.0001). No significant heterogeneity within variances was detected (Cochran's C test: 0.3780, P > 0.05; Bartlett's test: 2.6123, P > 0.05). Fertilization success in fresh untreated eggs was 84.0±3.3% of eyed eggs. Multiple range analysis (Tukey HSD Intervals at P < 0.05) revealed that fertilization ability of stored eggs did not significantly decrease until the 8th day of storage. A rapid decrease in egg viability was observed between the 12th (67.6±1.1%) and 16th day of storage (19.6±1.7%), resulting in a loss of 57% of initial fertilization ability during 4 days. After 24 days of storage, survival to the eyed-egg stage was 0.9±0.1%. A complete loss of fertilization ability of eggs was noted on the 26th day of storage.

Ageing of eggs was observed since the 6th day of storage. On the 30th day, all stored eggs were qualified as ageing. A very high significant negative correlation between fertilization success of stored eggs and their ageing rate (r = -0.9885; $N = 12$; $P < 0.0001$) was noted.

DISCUSSION

Simple method of short-term preservation of rainbow trout ova resulted in maintenance of their full fertilization ability for up to 8 days. Rapid loss of egg viability was observed from the 12th day of storage. Low temperature (>0-3°C), oxygen atmosphere, and low number of layers of stored eggs (not exceeding 4 layers) are most important for short-term preservation of salmonid ova (Stoss 1983; Jensen and Alderdice 1984). In our study, eggs were arranged in a single layer, enabling proper gaseous exchange. Desiccation was reduced by partial covering of a storage beaker with parafilm and by presence of water in tray (moisture). As broken eggs reduce fertilization success in salmonids (Wilcox et al. 1984; Van Heerden et al. 1996), the removal of ageing eggs probably prolonged viability of stored cells. The use of antibiotics can also be beneficial in salmonids (Stoss 1983), as it was in a case of storage of tilapia, *Oreochromis mossambicus*, eggs (Harvey and Kelley 1984).

As fresh sperm was not available every two days, we had to use stored milt. The effect of milt storage on its fertilization ability was minimized by the use of milt from several donors (spermatozoa motility prior to insemination was always higher than 60%) and by the excessive numbers of spermatozoa used (more than 1×10^7 spermatozoa per egg) which were several-fold higher than the minimum suggested by Moccia and Munkittrick (1987). Stoss and Holtz (1983) demonstrated that similar chilled storage (undiluted, with antibiotics added) in moistened oxygen atmosphere did not reduce fertilizing capacity of rainbow trout sperm until day 34.

Quality of rainbow trout eggs is affected by several factors, such as chemical composition, size, time of stripping, husbandry, and, above all, elapsed time from ovulation to stripping (Billard 1992). The highest egg viability is achieved 4-6 days after ovulation (Springate et al. 1984). Egg donors in the present study were stripped a few days after ovulation, but at the end of autumn spawning season. This time of spawning could adversely affect fertilization ability of stored eggs.

A method described here is very simple and it does not require qualified person for handling. It can be a practical tool for a few day storage of salmonid oocytes in laboratory for research purposes. Also, it can be helpful in hatchery selection works if there is a need to cross chosen individuals and their gametes are unavailable at the same time.

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STRESZCZENIE

KRÓTKOOKRESOWE PRZECHOWYWANIE NIEZAPŁODNIONEJ IKRY PSTRĄGA TĘCZOWEGO, *Oncorhynchus mykiss*

Oocyty uzyskano od trzech owulujących samic. Próbę zbiorczą ikry umieszczono w płynie owaryjnym w pojedynczej warstwie na dnie szklanej zlewki zakrytej Parafilmem (90% powierzchni). Następnie przechowywano ją w temperaturze 1°C w atmosferze tlenu. Co dwa dni porcje oocytów były zapładniane nasieniem. Nasienie uzyskiwano trzy razu w trakcie doświadczenia: na początku, po ósmym dniu i po szesnastym dniu przechowywania oocytów i przechowywano je w atmosferze tlenu (1°C).

Po inseminacji świeżych oocytów uzyskano 84,3±3,3% zarodków w stadium zaoczkowania. Do ósmego dnia przechowywania oocytów nie odnotowano istotnego spadku ich jakości biologicznej. Znaczący spadek jakości oocytów nastąpił między dwunastym (67,6±1,1% zarodków w stadium zaoczkowania) a szesnastym dniem przechowywania (19,6±1,7% zarodków w stadium zaoczkowania). Po 24 dniach przechowywania odsetek zaoczkowanych zarodków wyniósł 0.9±0.1%.

Uzyskane wyniki pozwalają zastosować tę prostą i tanią metodę krótkookresowego przechowywania oocytów pstrąga tęczowego przede wszystkich w pracach badawczych (możliwość kilkudniowego przechowywania oocytów w każdym laboratorium, bez konieczności przetrzymywania dojrzałych tarlaków) jak również w wylęgarnianych pracach selekcyjnych, gdy asynchronizm tarłowy często uniemożliwia skrzyżowanie dwóch konkretnych osobników.

ADRESY AUTORÓW:

Dr I. Babiak
Prof. dr hab. J. Strzeżek
Katedra Biochemii Zwierząt, Akademia Rolniczo – Techniczna w Olsztynie, 10-718 Olsztyn

Prof. dr hab. K. Goryczko
Zakład Hodowli Ryb Łososiowatych Rutki,
Instytut Rybactwa Śródlądowego w Olsztynie, 83-330 Żukowo