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RESEARCH ARTICLE

Iodine disinfection of sea trout, *Salmo trutta* (L.), eggs and the affect on egg surfaces

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Abstract. The aim of the research was to investigate the impact iodine solution disinfection had on *Salmo trutta* (L.) egg survival rates during incubation, and to determine the effect of the disinfection procedure on egg shells using scanning microscopy. The study groups were bathed in a Desamar K30 solution at a concentration of 100 ml per 10 dm^{-3} for 10 m once after the eggs had hardened and four times after the eyed stage. Egg samples for scanning analyses were collected on day 30 of incubation at the eyed stage after the first bath in the iodophor solution, and then at the end of incubation. Egg surface images were analyzed for the number of bacteria, the presence of hyphae, and the egg surface area covered with sediments. No statistically significant differences were noted in embryo survival rates in the groups that were disinfected. The highest number of bacteria was observed on egg surfaces which had not been disinfected prior to hatching. A significant amount of sediment was observed on the eggs during incubation. On day 90 of incubation, all of the egg surfaces were covered with sediments. Disinfection was not noted to have had a significant impact on the presence of hyphae. Iodophor preparations can be used for routine disinfection of trout eggs; however, other means of disinfection should be applied before the eyed egg stage.

Keywords: iodophor disinfection, salmonid eggs, scanning electron microscopy

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Introduction

Disinfection is one of basic means to prevent losses caused by microorganisms in animal production. Research into the use of various biocides in aquaculture was initiated many years ago. The disinfection of the eggs of Salmonidae and other economically valuable fish cultured in hatcheries can result in higher survival rate percentages, however the choice of appropriate preparations for dissinfection is problematic (Evelyn et al. 1984, Schreier et al. 1996, Peck et al. 2004, Khodabadeh and Abtahi 2006, Katharios et al. 2007, Overton et al. 2010). The influence of bacteria and fungi linked with aquatic organisms on egg survival rates observed in hatcheries is well known. The presence of high numbers of bacteria on egg surfaces can increase the mortality of developing embryos, and some bacteria can digest egg shells, which permits them to penetrate into the embryos. High egg density in incubators and water recirculation systems can foster excessive bacterial development (Antychowicz 2007). To date, formalin and malachite green are thought to be the most effective substances to limit the development of potentially harmful, pathogenic organisms (Barnes et al. 2000, Alderman 2002, Gieseker et al. 2006, Wagner et al. 2008). However, the use of malachite green in fish and aquatic animal production has been banned in both the European

Union and the United States of America because of its high toxicity, and its carcinogenic, mutagenic, and teratogenic impact on early development stages of fish. The pigment and its metabolites accumulate readily in tissues, while its affinity with organic sludge causes it to persist in breeding environments (Srivastava et al. 2004, Mitrowska and Posyniak 2005). Formalin, another effective agent, is suspected of being carcinogenic to humans (Duong et al. 2011). Therefore, new efficient, environmentally friendly disinfectants are needed.

Iodophores have long been known as disinfectants in aquaculture. The biocidal activity of active iodine stems from the denaturation of structural and enzymatic proteins in cells through the oxidation of the S-H groups in amino acids such as cysteine and methionine, and hydrogen blocking in the N-H groups of arginine, histidine, and lysine. Iodine also reacts with fatty acids and nucleotides. Changes occurring in cell walls, membranes, and in cytoplasm result in the rapid mortality of microorganism cells. The iodophors in current use are complexes of povidone-iodine that is stabilized additionally with iodides or iodates. During disinfection, free elemental iodine is slowly released in an aqueous solution so that the amount of the active agent (I_2) remains stable and does not reach high, irritating concentrations associated with the compounds discussed above. These formulations are liquids, but they are used as antiseptic ointments in human medicine (Atemnkeng et al. 2006, Cooper 2007). Their unique features allow them to be used in aquaculture: they are strong biocides at low temperatures, even at approximately 0°C; they are effective virucides, fungicides, and bactericides; they mix readily with water in unlimited ratios; they do not affect metals, plastic, or rubber; they do not irritate skin, mucous membranes; they are more efficient disinfectants than chlorine compounds. Povidone-iodine could be useful for egg disinfection (Siwicki et al. 2002, Terech-Majewska et al. 2010).

The aim of this research was to learn about the impact of iodophor solution baths on the survival of salmonid eggs during incubation in hatcheries, and to determine how the disinfectant affected egg shells.

Materials and methods

The following research was performed in the Szczodre Hatchery of the Polish Angling Association from November 2011 to March 2012. The study material comprised sea trout, *Salmo trutta* (L.), eggs obtained from fish spawning in the Rega River. Group size was determined with the volumetric method by calculating the mean of three measurements of 50 cm^3 of hardened eggs. Two adjacent refills of the incubators, the first and second before the runoff, were divided with plastic bars. Each apparatus was divided into nine parts. The test and control groups (n = 9), which were repeats, were located subsequently in the dams. Samples comprised 3,100 eggs. Desamar K30 was the disinfectant used in the research (Foodtech AG, Uster, Switzerland), with a declared active iodine content of 1.35 mg g^{-1} and pH 7.50. The sample groups were bathed in the iodophore solution at a concentration of 100 ml per 10 dm^{-3} (≈ 13.5 mg of active iodine dm^{-3}) for 10 m once after the eggs had hardened and four times after the eyed stage. The following rule was applied to ensure comparative activity of the disinfectant solution during subsequent baths: 1 dm^3 of solution per 2,000 eggs. The control groups were subjected to the same procedures as the test groups, but bathing was done in clean water without the addition of disinfectant. The procedure was conducted by lifting the eggs with a flexible hose, they were drained, and after the procedure they were rinsed with clean water. Dead eggs were removed and counted throughout incubation. The physicochemical properties of the water supplying the incubation apparatuses were as follows: temperature: 0.5-7.5°C; pH – 7.46-7.70; total hardness – 300-335 mg $CaCO_3$ dm^{-3}; chemical oxygen demand – 6.8-9.1 mg O_2 dm^{-3}; ammonium compounds – 0.01-0.1 mg $NH_4^+ dm^{-3}$; nitrites – 0.018-0.03 mg NO_2^- dm^{-3}; water oxygen saturation – 58-64%.

Eggs samples for scanning microscope analyses were collected on day 30 of incubation at the eyed stage immediately after the first bath in the iodophor

solution. The eggs were preserved in a 2.5% solution of glutaraldehyde. The preparation procedure involved rinsing the samples in a phosphate buffer of pH 7.4 and dehydrating them in increasing series of ethyl alcohol. A Scancoat 6 device was used to spray the samples with gold. The ultrastructure of the sample surfaces was examined under an Evo LS 15 Zeiss scanning microscope.

The statistical analyses of the results was performed with Statistica 10. Student's t-test, P = 0.05, was applied to verify differences among study groups in which the impact of disinfection on embryo survival rates was examined, while Levene's test was used to verify data variance homogeneity. Eight pictures taken of a group of four eggs from the control and four from the test groups, and two pictures of randomly chosen parts of each egg were used for the analysis. The magnification was 2000 ×, and the surface area of the individual segments observed was 2,166 μm^2. Assessments included the number of bacteria, the total length of visible mold hyphae, and the surface area covered with sediments. Zeiss Axiovision 4.8 software was used to assess the variables numerically. Differences among groups were verified using single factor analysis of variance (ANOVA) and Tukey's test, P = 0.05.

Results

Sea trout egg incubation lasted 105 days from the beginning of hatching and 115 days until all of the larvae had hatched. The eggs moved into the eyed stage on day 57 of development. No differences in the beginning and end of hatching were observed between the control and experimental groups, and no differences in larval appearance or behavior were noted among the various groups.

The application of the disinfection bath had no significant impact on embryo survival rates in the experimental groups. The number of hatched larvae in the control group was 2,509 eggs or 80.9%, (SD = 160, min = 2,231, max = 2,736). The average survival rate in the experimental groups was 2,467 eggs or 79.6% (SD = 198, min = 1,987, max = 2,674). The embryo mortality rate was also calculated after the eyed stage was reached, and it was 2.6% in the control group and 2.3% of the initial number of eggs. The highest number of bacteria was observed on the surfaces of the control group eggs just before hatching (Fig. 1). The differences among the experimental groups were slight and none was statistically significant. Changes in the amount of sediments on the sea trout egg surfaces during incubation are presented in Figure 2. On day 90 of

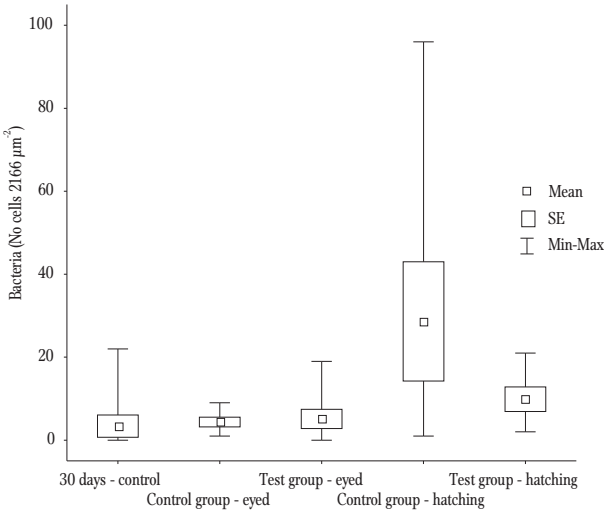

Figure 1. Number of bacteria cells observed on the surface of *S. trutta* eggs.

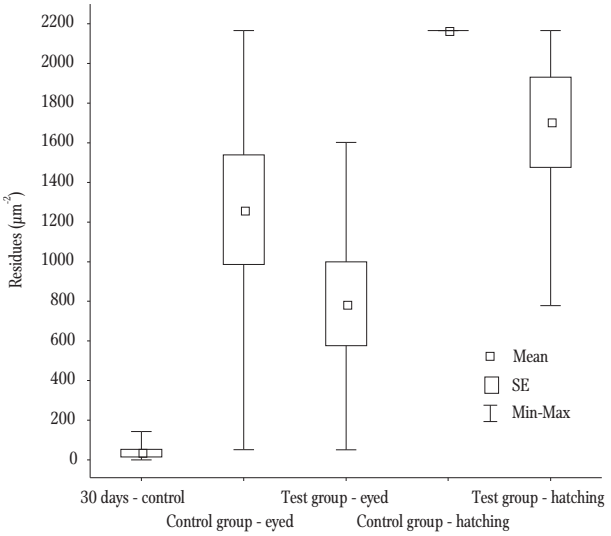

Figure 2. Amount of *S. trutta* egg shells covered with sediments. Surface area observed – 2,166 μm^2.

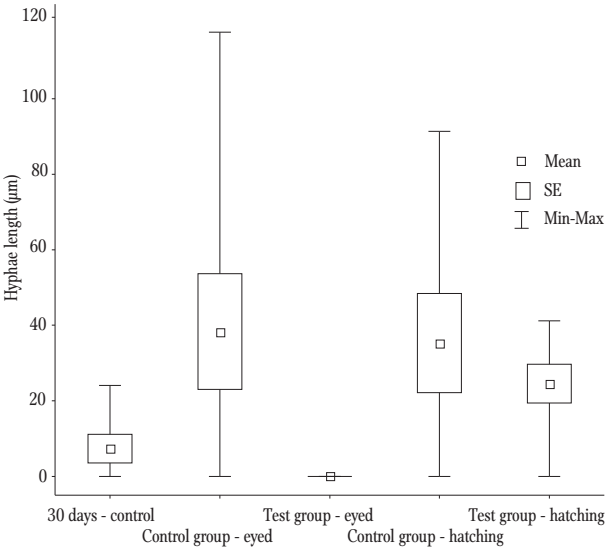

Figure 3. Total length of hyphae observed on the surface of *S. trutta* egg shell.

incubation, all of the eggs from the control group were covered with a thick layer of sediments (Photo 1a). The difference in the amount of sediments between the early and later incubation periods was statistically significant (30 days – control vs control group – hatching; Tukey test, P = 0.0001). The iodophorus bath after the embryos reached the eyed stage had a minor impact on the area covered by sediments at an average of 79%, and there were no significant differences between the control and experimental groups at the same stage of incubation; however, the microscopic image (Photo 1b) reveals differences in sediment thickness on the egg shells from the experimental group at the end of incubation. The first disinfectant bath reduced considerably the number of hyphae on the surface of the egg shells (Fig. 3). No statistically significant differences among the experimental and control groups were noted in the later incubation period.

Discussion

The initial factor that impacts incubation efficiency is spawn quality, and this is largely dependent on the spawning period. The highest fertilization percentage is obtained when the spawn sample is collected on the day of ovulation. Survival rates decrease significantly 14 days after ovulation (Goryczko 2012). The toxicity of iodophor to Salmonidae has been studied very well. Alderman (1984) indicates that during baths of 10 m not only is the active iodine concentration potentially harmful, but the pH of the solution can also be a factor. Solutions with 150 mg of active iodine in dm^{-3} at pH 3.0 , 800 mg in dm^{-3} at pH 6.0 and 3000 mg in dm^{-3} at pH 7.0 were similarly toxic (LD_{25}) to eyed stage rainbow trout eggs. The most commonly used concentrations, which are thought to be efficient disinfectants, were 50-100 mg dm^{-3} (Stuart et al. 2010, Overton et al. 2010, Katharios et al. 2007, Peck et al. 2004). The minimal recommended concentration of active iodine for limiting fungus development is about 10 mg dm^{-3} (Verner-Jeffreys et al. 2007).

The number of bacteria living on egg surfaces is usually positively correlated with increased mortality among developing embryos. Generally, the bacteria identified occurring most frequently on spawn are *Pseudomonas*, *Aeromonas*, *Cytophyta*, and *Chromobacterium.* Bacteria usually inhabit only a small area of egg surfaces (1 to 7.5%). They effect developing embryos through the excretion of enzymes and toxins, but above all they limit the amount of oxygen reaching embryos (Trust 1972, Sauter et al. 1987); however, it is improbable that even in high numbers they are responsible

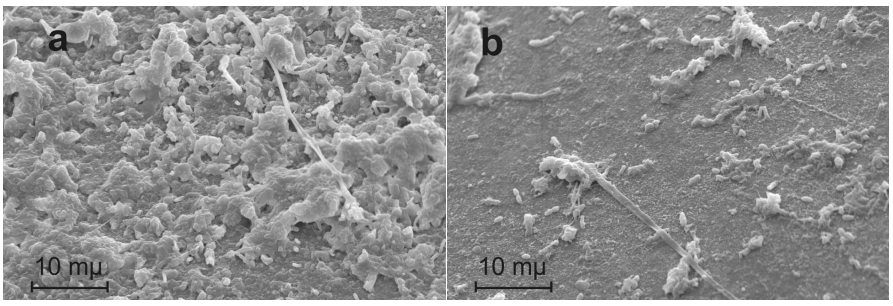

Photo 1. Microscope images of *S. trutta* egg surfaces on day 90 of incubation. a) control group, b) test group. Magnification 2000 ×.

for mass mortality among Salmonidae embryos. Embryo survival rates depend on a number of environmental factors linked with the quality of water supplying incubation fillers; these include water flow, temperature, and pH. Trout eggs are also very vulnerable to harmful bacterial activity immediately following fertilization, and eggs infected with bacteria such as *Pseudomonas fluorescens* and *Cytophyta* during hardening might have lowered embryo survival rates (Baker et al. 1989, 1991). The Salmonidae egg shell is highly immune to the activity of most proteolytic enzymes, and it constitutes good protection for the developing embryo (Bell et al. 1969). The destructive influence of bacteria developing on egg surfaces has been observed in other fish species. Pavlov and Moksness (1993) indicate that bacteria causing egg shell perforations was the main reason for embryo mortality during wolfish, *Anarhichas lupus* (L.), incubation.

Aquatic molds are particularly troublesome microorganisms and can cause considerable losses in hatchery production. The most frequently occurring genera are *Achlya* and *Saprolegnia* (Czeczuga et al. 2005). Their spores develop on the surface of dead eggs, but they cannot attack live embryos. However, hyphae relocating from dead to nearby live eggs also quickly results in mortality. It is widely known that hyphae entangling eggs cause mortality (Meyer 1991). The latest research indicates that even small numbers of hyphae, which do not limit gas exchange but damage egg shells, can cause embryo mortality. Details of resistance mechanisms of live eggs to mold spore invasions or the pathogenicity mechanisms of hyphae to embryos remain unknown (Paxton and Willoughby 2000, Thoen et al. 2011). Despite this, the present research did not confirm the results mentioned above that egg coverage with sediments can have a negative impact on embryo gas exchange (Greig et al. 2005). Iodophor solutions are currently the primary disinfectant used in aquaculture, and research on their use in various incubation conditions to prevent losses caused by microorganisms and to determine the influence it has on the breeding of other fish species is ongoing. The efficacy of iodophores is comparable with other preparations used in aquaculture at appropriate concentrations and operation times (Peck et al. 2004, Varner-Jeffreys et al. 2007, Overton et al. 2010, Mainous et al. 2010). Iodophores are applied in the hatchery production of Salmonidae fish for the routine disinfection of eggs once they reach the eyed stage; however, it should be noted that their use is limited. Preventing mold development also requires disinfection before embryos become eyed, and, at this time, it is not possible to remove eggs from incubators in order to bathe them.

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