



Hatchability of eggs from Atlantic cod, turbot and Atlantic halibut after disinfection with ozonated seawater

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Received 10 May 2002; received in revised form 2 December 2002; accepted 4 December 2002

Abstract

In aquaculture the risk of transmission of fish pathogens via eggs is reduced by disinfection in ozonated seawater, but this treatment may delay or reduce hatching. The objective of this study was to investigate the tolerance of Atlantic cod, turbot and Atlantic halibut eggs to ozonated seawater. Groups of eggs were treated with different concentrations and exposure times 2 days before hatching, and the effects on hatchability were observed. The groups of eggs of all three species that had been exposed to 2 mg O₃/l for 2 min or less showed normal hatching. In the groups with high total exposure (4 mg O₃/l for 1 min or higher), a clearly lower percentage of hatching was found. Interspecies differences in tolerance were observed, with turbot eggs displaying higher tolerance than eggs of either halibut or cod. Due to the interspecies differences, the tolerance of eggs to ozonated seawater should be carefully evaluated in controlled laboratory-scale experiments in order to establish a basis for disinfection protocols. Two milligrams O₃ per liter for 2 min and lower exposures ought to be sufficient to ensure an excess of oxidants for efficient inactivation of fish pathogens while avoiding negative effects on the hatchability of halibut, cod and turbot eggs.

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Keywords: Disinfection; Ozone; Fish egg; Hatchability; Atlantic cod; Turbot; Atlantic halibut

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1. Introduction

Microorganisms such as bacteria and viruses may be found on the surface of teleost eggs. Several sets of observations suggest that the intensive incubation techniques employed in aquaculture may lead to a heavier and often detrimental microbial load on eggs and larvae (Kusuda et al., 1986; Perez Benavente and Gatesoupe, 1988). Some of these microorganisms may be major fish pathogens that are transferred with eggs (Yoshimizu et al., 1989). In addition, regional and international trade in live fish eggs provides potential routes for parallel movements of pathogens. Disinfection of the egg surface may reduce the negative effects of the microbial load and offer a barrier to the transfer of pathogens both between broodstock and their offspring and between geographical regions. Egg disinfection may thus contribute to a more stable production.

Several authors have addressed the effects of disinfectants such as iodophores, chloramine, hypochlorite and glutaraldehyde on teleost eggs, with most attention being paid to bacterial inactivation (Harboe et al., 1994; Salvesen and Vadstein, 1995; Bergh and Jelmert, 1996). Major fish pathogens such as nodaviruses and infectious pancreatic necrosis virus (IPNV) are small naked viruses, and are far more resistant to disinfectants than bacteria (Arimoto et al., 1996; Frerichs and Tweedie, 1997). Nodavirus for instance are resistant to pH 2–9, heating at 56 °C for 30 min (Frerichs et al., 1996) and retain their infectiousness after storage in seawater for more than one year at 15 °C (Frerichs and Tweedie, 1997). This virus has been shown to cause the disease viral encephalopathy and retinopathy (VER) in more than 30 maricultured teleost species from most parts of the world (Munday and Nakai, 1997), including the Atlantic cod (Starkey et al., 2001), turbot (Bloch et al., 1991) and Atlantic halibut (Grotmol et al., 1997). Furthermore, in some teleost species, nodavirus are shed from broodstock during spawning probably adhering to the egg surface and infecting offspring at hatching (Mushiake et al., 1994).

When a powerful oxidant such as ozone (O₃) is introduced into seawater, it reacts with a wide array of compounds, and new oxidants are formed. Some of these are powerful disinfectants like bromine, bromamine and hypobromide. Ozonated seawater therefore efficiently inactivates bacteria (Sugita et al., 1992; Liltved et al., 1995), nodavirus (Yoshimizu et al., 1995; Arimoto et al., 1996; Grotmol and Totland, 2000), IHNV (Yoshimizu et al., 1989) and IPNV (Liltved et al., 1995). However, the oxidants formed may also react with compounds in the eggshell (chorion), altering its functional properties and thus possibly influencing hatchability. Our study was designed to reveal the effects of disinfection with ozonated seawater on the hatchability of eggs of cod, turbot and halibut.

2. Materials and methods

2.1. Eggs

A mixed batch of fertilized eggs derived from 5 to 10 spawners of Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*) were obtained from the Institute of Marine Research (Austevoll, Norway). An equivalent batch of turbot eggs was supplied by Stolt Sea Farm (Øye, Norway).

2.2. Experimental set-up

2.2.1. Ozonation of seawater

Seawater (80 l) was held in a reaction chamber connected to a circulation pump. Ozone gas, produced by a commercial ozone generator by high-voltage corona discharge (PK Ozone, Molde, Norway), was mixed with the seawater using a gas injector connected to the seawater circulation system. The temperature of the seawater was kept at 6 °C.

2.2.2. Measurement of oxidative power expressed as mg O₃/l

The oxidative power of the ozonated seawater was measured by iodometry according to the method of the Franson (1989), and the concentration of total residual oxidant (TRO) was expressed as mg Cl₂/l. In this study the oxidative power was converted to mg O₃/l by means of the following formula: [O₃]=0.68 × [Cl₂].

2.2.3. Egg disinfection

Eggs from Atlantic cod, turbot and Atlantic halibut were disinfected 2 days before expected hatching. From each species, 16 groups of eggs were disinfected using different concentrations of ozonated seawater and different exposure times. Four different concentrations of ozone per liter, ranging from 0.2 to 10 mg O₃/l, were combined with four different exposure times (0.5, 1, 3 and 5 min) (Figs. 1–3). Each of the 16 ozone-treated groups consisted of 120 eggs. An additional group was left untreated (control group, C).

2.2.4. Rearing conditions

The eggs and larvae were kept in complete darkness in a climate-regulated room except during periods of rearing and observation, when the room was illuminated by a dim red light (2.5 lx). The room temperature was kept at 6 °C for the cod and halibut, while the turbot were held at 14 °C. The halibut eggs were incubated in polystyrene six-well tissue-culture plates (Multidish 6, Nunc, Roskilde, Denmark). The wells were filled with 10 ml autoclaved, diluted seawater (25‰ salinity), which had been temperature equilibrated. Each of the 120 eggs from each of the experimental groups was transferred to a separate well (Grotmol et al., 1999). The cod and turbot eggs were incubated in 24-well tissue culture plates (Multidish 24, Nunc) containing 2.5 ml seawater in each well. Each egg was transferred to a separate well.

Day 0 was defined as the day by which at least 50% of the eggs in the untreated control group (C) had hatched. On the day after hatching, most of the water was removed from each well together with the eggshell debris and replaced with fresh autoclaved diluted seawater (25‰ salinity).

2.2.5. Recording of hatching and development

During the first week of the experiment, hatching and any signs of abnormal development of the larvae were observed and recorded daily and thereafter every second day until day 13. The observations were performed through an Olympus stereomicroscope.

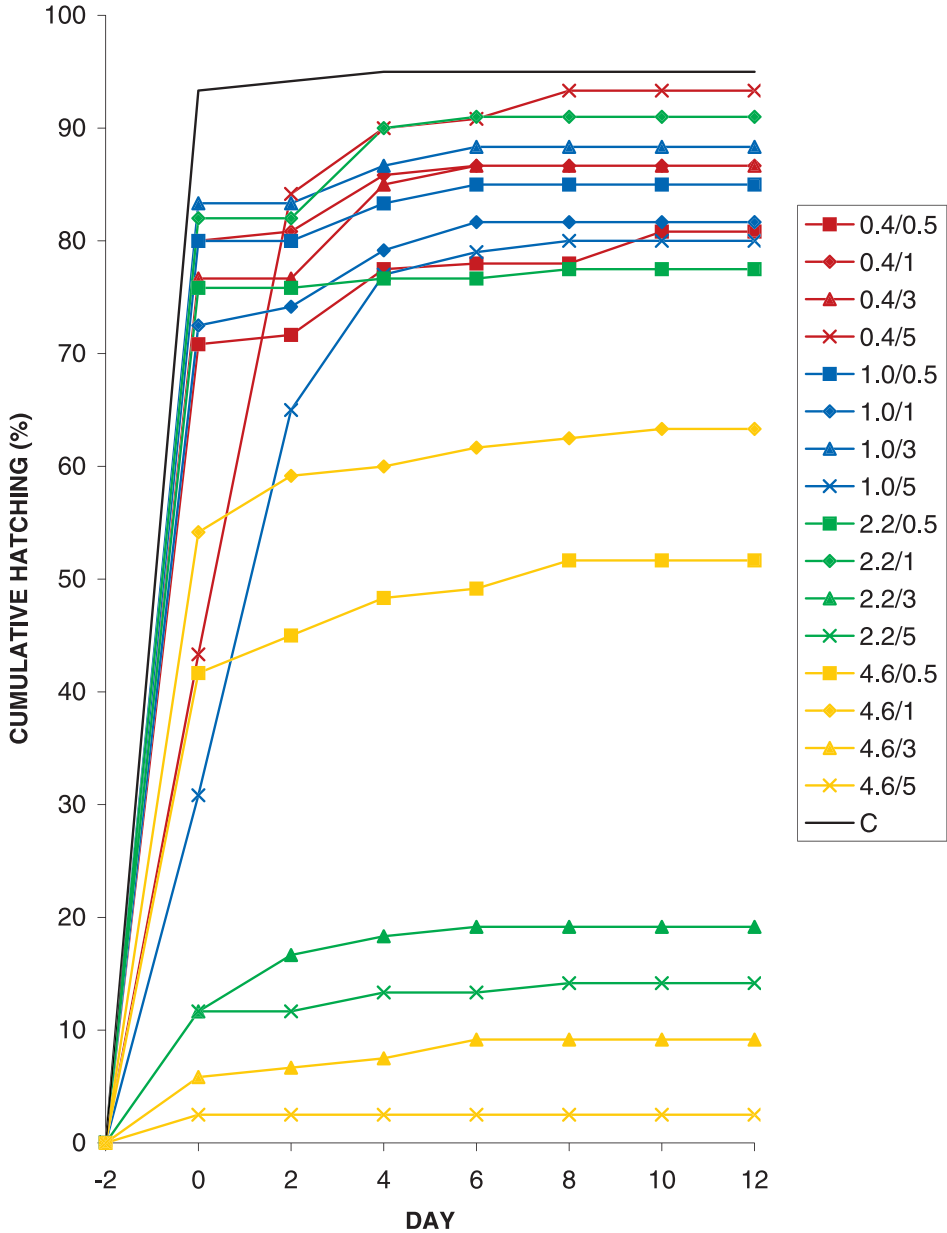


Fig. 1. Cumulative hatching in individual groups of Atlantic cod eggs. The hatching rates of the control groups were normal, reaching more than 90% in one night. In the groups exposed to 2.2 mg O₃/l for 30 s or lower exposures, most hatching occurred in the same period as in the control group, but the cumulative hatching rate was from 2% to 15% lower. The experimental groups are identified by the ozone concentration and the exposure time (mg O₃/min).

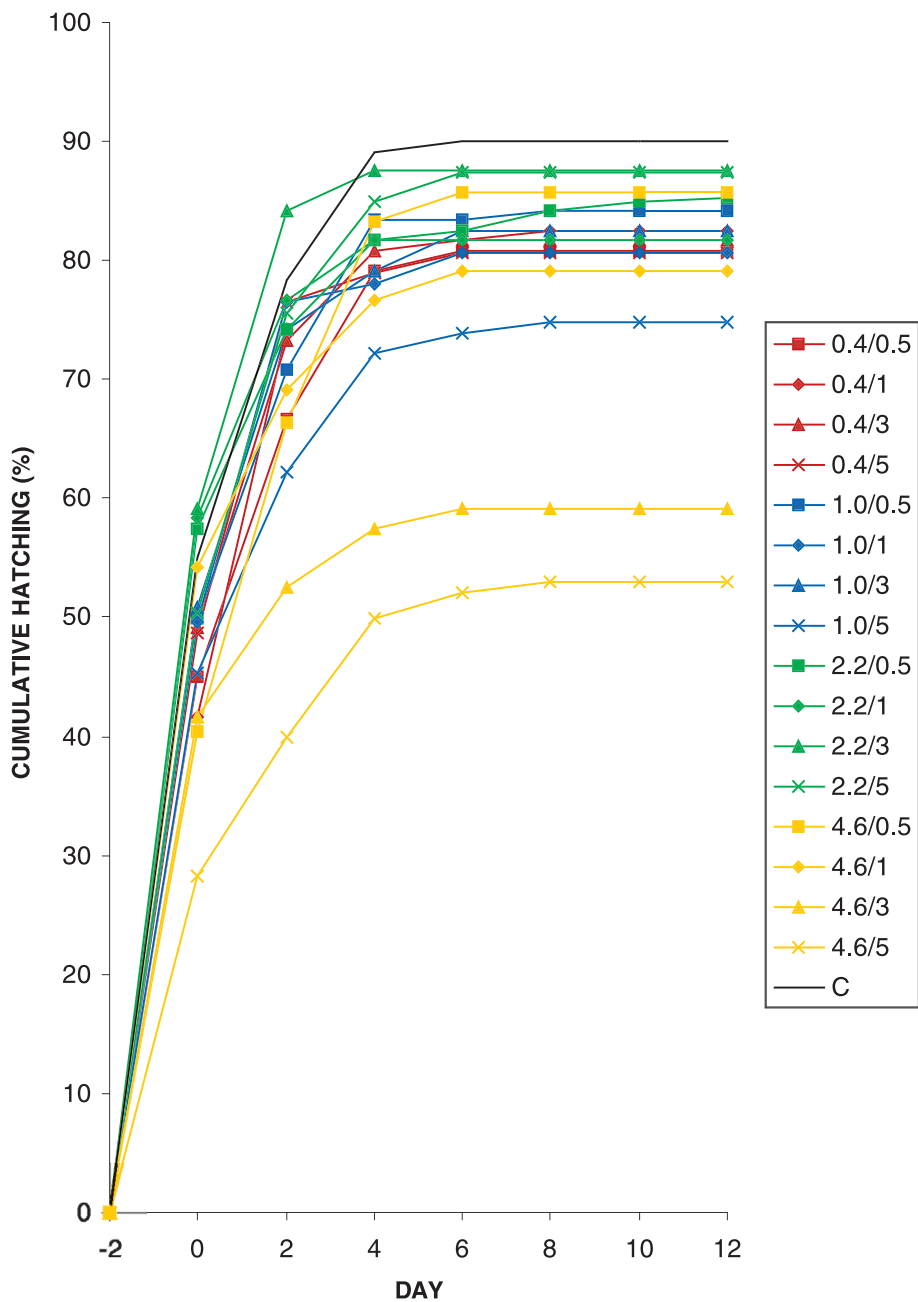


Fig. 2. Cumulative hatching in individual groups of turbot eggs. Hatching in all these groups was asynchronous, but was completed within 7–8 days. Only the two groups with the highest exposure showed delayed hatching and reduced hatchability. The experimental groups are identified by the ozone concentration and the exposure time (mg O₃/min).

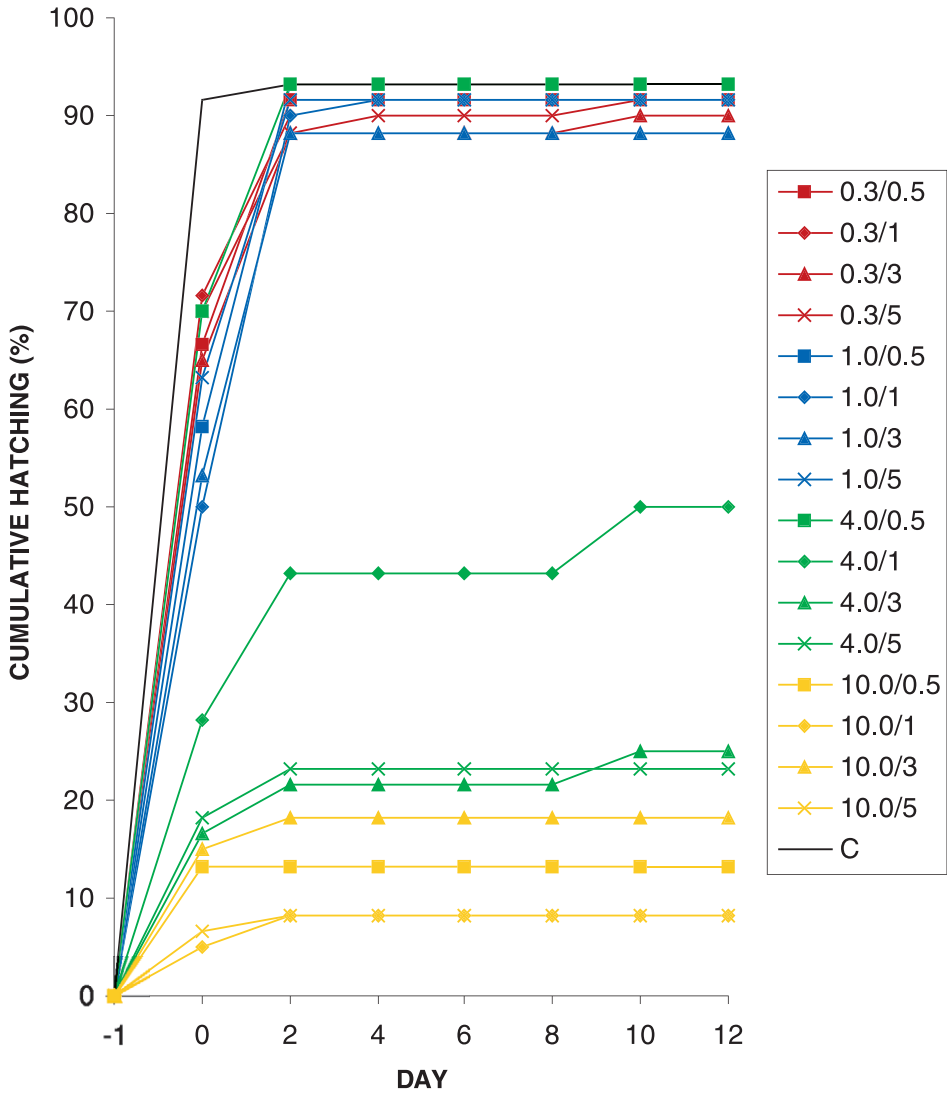


Fig. 3. Cumulative hatching in individual groups of Atlantic halibut eggs. The hatching rates of the control groups were normal, reaching more than 90% in one night. In the groups exposed to 4 mg O₃/l for 30 s or less, hatching was asynchronous and delayed for up to 2 days. In the groups exposed to 4 mg O₃/l for 3 min or more, 75–92% of the eggs did not hatch, but developed inside the eggshell. Due to overlap of the curves from the groups exposed to 4 mg O₃/l for 30 s or less, not all curves are visible. The experimental groups are identified by the ozone concentration and the exposure time (mg O₃/min).

3. Results

3.1. Atlantic cod

Most of the larvae of the control group hatched in the course of 2 days (Fig. 1). In the egg groups exposed to 2.2 mg O₃/l for 30 s or lower total exposures, most of the hatching occurred in the same period as in the control group, but the cumulative hatching rate was from 2% to 15% lower. In general, within each experimental group, cumulative hatching rates fell as exposure times increased. In the groups exposed to 2.2 mg O₃/l for 3 min or greater exposures, rates of hatching fell below 20% (Fig. 2). Intermediate cumulative hatching rates were observed in the groups exposed to 1 mg O₃/l for 5 min and to 4.6 O₃/l for 30 s and 1 min. The larvae hatched from ozone-treated eggs displayed normal patterns of swimming and development.

3.2. Turbot

In all groups, hatching was asynchronous and in most it was completed in 7–8 days (Fig. 2). The different levels of ozone exposure had little influence on hatchability. Only the two egg groups with the highest ozone exposures, 4.6 O₃/l for 3 min and 4.6 O₃/l for 5 min, showed significant detrimental effects on hatching. The larvae hatched from ozone-treated eggs showed normal patterns of swimming and development.

3.3. Atlantic halibut

Most of the larvae of the control groups hatched synchronously during a single night (Fig. 3). In the groups of eggs exposed to 4 mg O₃/l for 30 s or lower total exposures, hatching was asynchronous, but after 2 days the cumulative hatching was close to that of the control group. In the groups exposed to 4 mg O₃/l for 3 min or higher exposures the hatching ranged from 25% to 8%, and related to the total exposure. The group exposed to 4 mg O₃/l for 1 min formed a group of intermediate hatchability with a cumulative hatching rate of about 50%. Where high exposure to oxidants inhibited hatching, the larvae continued to grow within the chorion during the period of observation. The larvae hatched from ozone-treated eggs showed normal patterns of swimming and development.

4. Discussion

In the laboratory-scale model employed in our study, each well is an independent experimental unit for monitoring individual eggs and larvae, making it an accurate tool for studying the effects of disinfection on eggs. When scaling up ozone disinfection to large volumes of eggs, the content of organic compounds in the water may be critical (Elliott and Amend, 1978). When disinfection protocols are being established, it is important to bear in mind the ability of fish pathogenic bacteria and viruses to adhere to organic substances in the water, thereby avoiding inactivation. In order to obtain an adequate

effect, surplus organic materials should be removed prior to disinfection by washing the eggs with filtered seawater with low organic load. In addition, to be sure of a surplus of ozone-produced oxidants, it is essential to optimize the quantity of eggs relative to the volume of disinfectant.

Nodavirus and IPNV are capable of infecting Atlantic cod (Starkey et al., 2001), turbot (Bloch et al., 1991; Novoa et al., 1993) and Atlantic halibut (Grotmol et al., 1997; Biering and Bergh, 1996), and successful hatchery production is dependent on keeping these infections under control. Since vertical transmission of these viruses cannot be ruled out, treating eggs of susceptible species with disinfectants capable of inactivating viruses is therefore of prime importance. Several studies have shown that disinfection with ozonated seawater inactivates these types of viruses (Liltved et al., 1995; Arimoto et al., 1996; Grotmol and Totland, 2000). The conclusion of this study is that the concentration of ozonated seawater needed to inactivate nodavirus and IPNV is far lower than the concentrations that reduce the hatchability of eggs of the three species tested.

Turbot eggs display considerably higher tolerance of ozonated seawater than those of halibut and cod, and eggs of all three species have a higher tolerance than those of striped jack (Arimoto et al., 1996). These results indicate that there are species variations in ozone tolerance of eggs, and that laboratory-scale tests with careful evaluation of concentration and exposure time should be performed in order to establish a basis for designing disinfection protocols.

In our study, all the larvae that hatched from ozone-treated eggs were observed through a stereomicroscope and appeared to develop normally. This may indicate that the exposures to ozonated seawater that we employed had no severe toxic effects. However, further pathological and toxicological studies are needed to confirm this. The adverse effects of high levels of ozone on hatching recorded in this study may be due to modification of the eggshell protein polymer by oxidants, rendering it more resistant to the hatching enzyme. On the other hand, the secretion of enzyme from the hatching gland may have been inhibited. Similar side effects of ozonated seawater on hatchability have been observed in other teleost species (Hall et al., 1981; Arimoto et al., 1996; Mimura et al., 1998). The negative effects of excess oxidants make it essential to follow a strict protocol and to monitor the concentration of ozone employed closely in hatchery-scale disinfection.

Fish eggs may transmit pathogens originating from the broodstock or the surroundings. Disinfecting eggs with ozonated seawater efficiently inactivates even the most resistant viral fish pathogens. This procedure may thus help to reduce the geographical spread of disease and the transfer of pathogens from broodstock to their offspring. In hatchery production, egg disinfection should be an integral part of the hygienic measures that should also include stringent control of water quality and other potential routes of disease transmission, such as feed, equipment and personnel.

Acknowledgements

We thank the Institute of Marine Research and Stolt Sea Farm for the supply of eggs. Pål Kristiansen of PK Ozone (Molde, Norway) is thanked for supplying the ozone

generator. The technical assistance of Ingrid Uglenes, Tove Boge Eriksen and Teresa Cieplinska is highly appreciated.

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