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Antibacterial treatment procedures of eggs of halibut (Hippoglossus hippoglossus L.)

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ABSTRACT

Halibut eggs were surface disinfected with a iodophor one day before hatching, and mortality and larval development was recorded until the time of first feeding. The groups exposed to higher concentrations of disinfectant had lower mortalities and lower fraction of larvae with developmental deformities compared to groups exposed to lower concentrations and untreated control groups.

INTRODUCTION

The surface of marine fish eggs constitutes a habitat well suited for many epibiotic bacteria (Hansen and Olafsen 1989), and some of these are pathogens (Bergh et al. 1990). Intensive aquaculture systems implies monoculture of species at artificially high population densities, favoring parasitizing bacteria. The need for efficient egg disinfection procedures is thus obvious.

Iodophors, which are in use in hatcheries of several freshwater fish species, particularly salmonids (Schnick 1987), are attractive compounds for fish egg disinfection, as they have beneficial pathogen/host differential of toxicity (Amend and Pietsch 1972 Amend 1974, Ross and Smith 1972). Physiological differences among fish eggs are large (Davenport et al. 1986, Lønning et al. 1988), thus tolerance for disinfectants may be highly variable. Also, the marine environment constitutes a chemical and microbial environment different from freshwater. The pharmacological effects of a given compound can therefore not be directly inferred from freshwater application and dosages.

In this study, we have tested the use of a iodophor disinfectant on eggs of the Atlantic halibut (*Hippoglossus hippoglossus* L.), which is an attractive candidate for marine cold-water aquaculture.

METHODS AND MATERIALS

Treatment of eggs and water

Halibut eggs from the broodstock at Austevoll Aquaculture Research Station were reared in 250 l open-circulation incubators as described by Rabben and Jelmert (1987). One day before hatching, eggs were divided into 4 groups, each of which were transferred to autoclaved 1 l glass bottles, each containing approximately 100 eggs in 200 ml seawater. The iodophor disinfectant (Buffodine; Evans Vanodine International Ltd., Preston, U.K.) was added to three of the bottles, obtaining final concentrations of 0.5, 0.05 and 0.005%, respectively (v/v). The fourth bottle served as a control. All bottles, including the control, were carefully agitated. After 10 minutes, water was siphoned off all four bottles and eggs were carefully washed with approximately 200 ml sterile 70% seawater, which was then immediately siphoned off again. After being washed by this procedure three times, 60 eggs were randomly chosen from each group and transferred to polystyrene multiwell dishes (NUNC, Roskilde, Denmark). Each well contained 11 ml sterile 70% seawater and one egg Jelmert and Naas (1990). The multiwell dishes was incubated in darkness in a thermostatted room at 5.5°C. Within 1 day after hatching, visible remainings of the eggshell was removed in all wells together with 10 ml of the water with a pipette. Within 1 minute, 10 ml sterile 70% seawater was added to each well. To avoid cross-contamination among groups, one sterile pipette was used for each group.

Activity measurements

Two assays based on similar principles were used to measure enzymatic activity of bacterial origin. Both methods are based on fluorochrome-coupled substrates which are cleaved by bacterial enzyme systems. Only the free fluorochrome is fluorescent, and the enzymatic activity in the samples can be recorded as the fluorescence increase. (I.e. the slope of a line, dimensionless).

Proteolytic activity was measured by a method adapted from Somville and Billen (1983). The water of 4 wells were pooled after removing the larvae. The samples were distributed in autoclaved 10ml test tubes in triplicate, and 125 ul of a 40 mM solution of L-Leucine-ß-Naphtylamide (LLbNA) was added to 5 ml of the samples. (Sigma Chemical Co., St. Louis, Mo.). The reaction was performed at 20°C in darkness. Autoclaved 70% seawater served as blank (spontaneous hydrolysis) and was subtracted from the measurements. After mixing, the fluorescence was

measured after approximately 2 and 62 minutes. The measurements were made in a Perkin Elmer LS-3 spectrofluorometer at exitation/emission wavelengths of 340/410 nm ,respectively. CV of the measurements ranged from 0 - 7%. When measuring individual wells, the measurements were made in duplicate.

"General" enzymatic activity was measured by a method modified from Schnürer and Rosswall (1982). The substrate fluorescine diacetate ((3',6'diacetylfluorescin), FDA) is hydrolyzed by a number of different enzymes, such as proteases, lipases and esterases. The water from the wells were pooled and distributed as above in autoclaved 10 ml test tubes. 1.0 ml (blank) or 0.5 ml (samples) of 0.6mM EDTA was added to each tube. 25 ul of a stock solution (0.2mg/ml of FDA (Sigma) in *pro analysi* acetone) was added. The tubes were immediately agitated in a vortex mixer and the fluorescence was measured after 2 min. and 1h,2min. The incubation was similar to the incubation for proteolytic activity measurements and the exitation and emission wavelengths was 480 and 513 nm, respectively.

Monitoring of larvae

Mortality and development of the larvae was recorded every third or fourth day from Day 1 after hatching until Day 37, which is well into the halibut's window of first feeding (Skiftesvik et al. 1990). After termination of the experiment, samples were taken for dry weight measurements, and recording of deformities. The samples for enzymatic activity was taken before the treatment (Day 0) and at Day 37.

RESULTS AND DISCUSSION

Cumulative mortality of the larvae is shown in Figure 1. The group exposed to 0.5% Buffodine had highest mortality during the early phase of the yolk sac stage. After 15 days however, all but one larvae survived to the end of the experiment. In the group exposed to 0.05% Buffodine, all larvae survived until Day 20. After this point, however, cumulative mortality raised to approximately the same level as the group exposed to 0.5% Buffodine. Cumulative mortality at the end of the experiment was 12.5 and 15 % in the groups exposed to 0.5% and 0.05% Buffodine, respectively. The group exposed to 0.005% Buffodine showed a mortality pattern very similar to the control group, with very low mortalities in the early phase of the yolk sac stage, but increasing mortality towards the end of the experiment. When the experiment was terminated, cumulative mortality had increased to 38 and 33% in the 0.005% and control groups, respectively.

Figure 2 shows the number of surviving larvae at the end of the experiment, together with the number of deformed larvae.

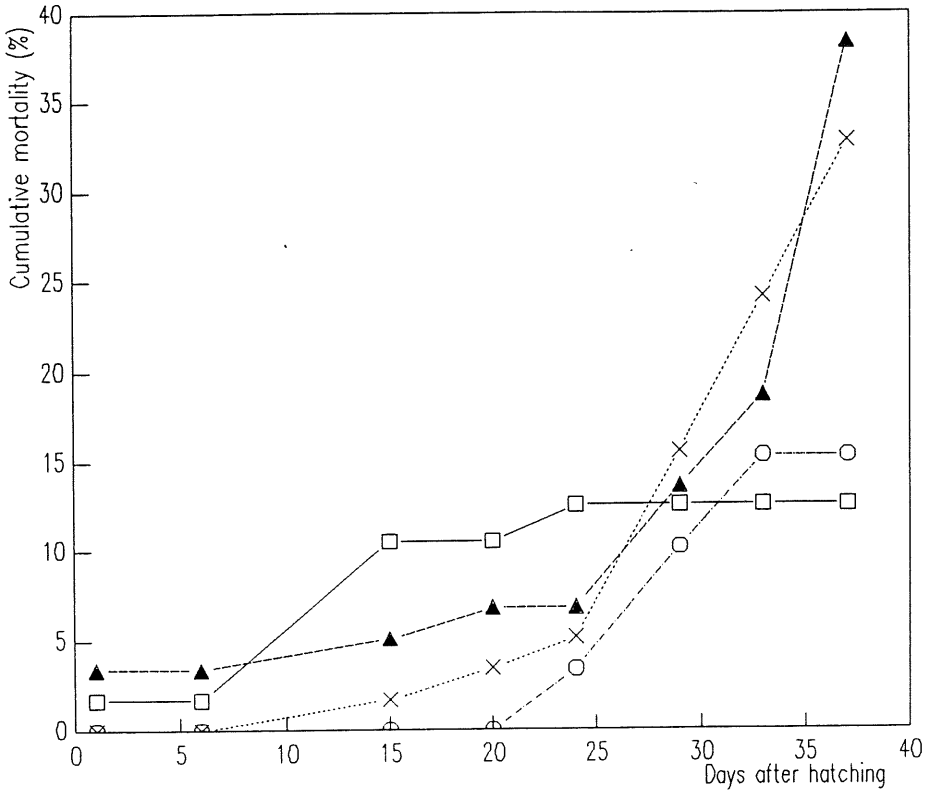

Figure 1. Cumulative mortality (in%) of the four groups throughout the yolk sac stage. The groups are: eggs exposed to 0.5% Buffodine (squares), 0.05% Buffodine (circles), 0.005% Buffodine (triangles) and control group (crosses)

In the control group as well as the group exposed to 0.005% Buffodine, virtually all larvae showed developmental disorders, notably seriously under-developed hearts, lockjaws (Pittman et al. 1990) and necrotic tissue, especially in the gill and heart region. In the two groups that had been exposed to higher concentrations of Buffodine, the fraction of deformed larvae were lower (32 and 10 % in the 0.05 and 0.5% Buffodine groups, respectively).

Figure 3 shows the bacterial activity in the three treatments and the control (4 wells with living larvae from each group pooled). Measurements of water from individual wells showed large variation. This is reflecting the large differences in bacterial growth and attack which could be observed within each experimental group.

The activity was measured in a few wells with dead and decaying larvae. One well with a dead larvae from the medium concentration group(0.05%) showed a FDA-activity of 1313 units/h. Corresponding wells from medium concentration and control showed a LLbNA-activity of 583.2 and 129.6 units/h, respectively. The activity in wells with dead larvae were thus approximately 10 times greater than the activity in wells with living larvae.

The tolerence of halibut egg to physical stressors are highly variable during different phases of the egg development, and eggs are more sensitive before blastopore closure (Holmefjord and Bolla 1988). Egg disinfection with Buffodine

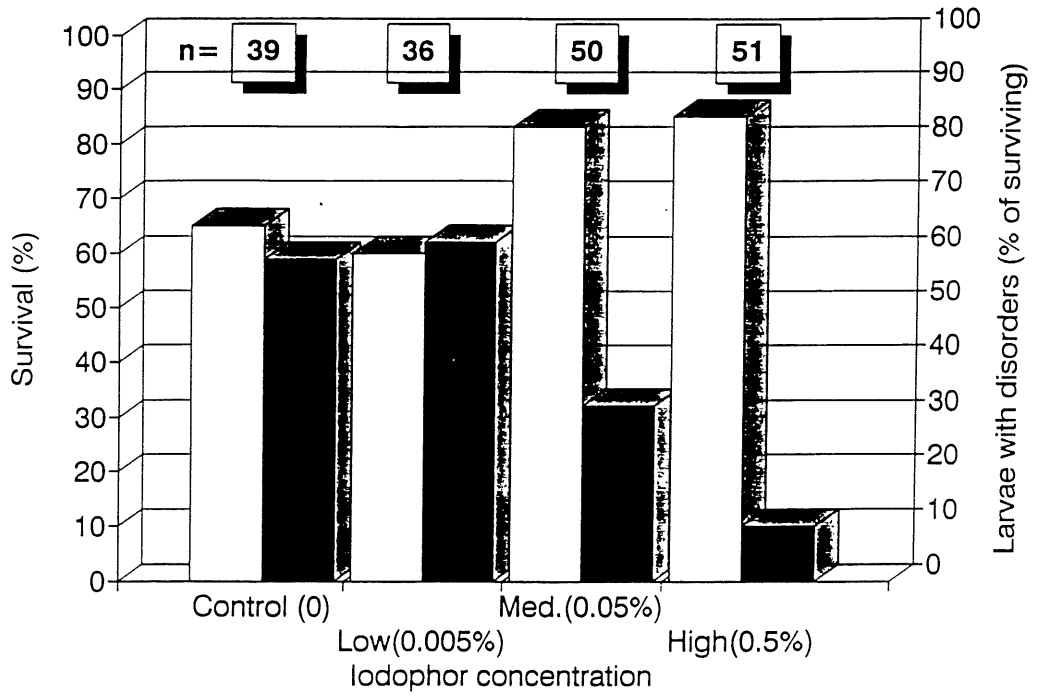

Figure 2. Percentage of surviving larvae (white bars) and larvae with developmental disorders (black bars) at the end of the experiment.

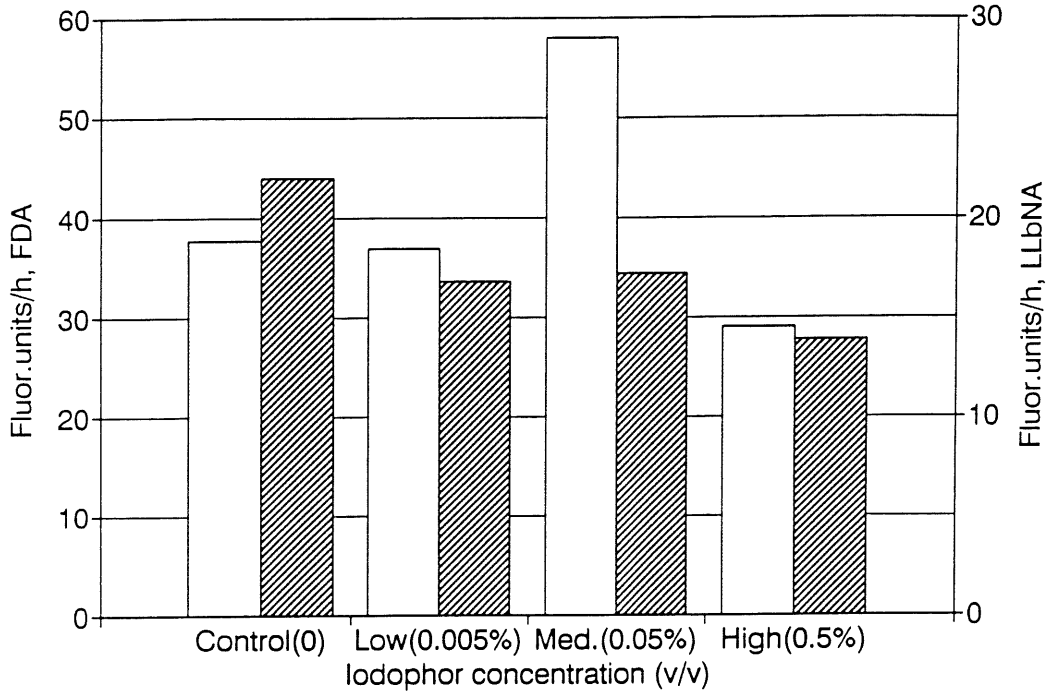

Figure 3. Bacterial activity (FDA, white bars) and LLbNA (scattered bars)

of newly fertilized eggs led to very high mortalitiesduring the egg phase compared to control groups (Bergh and Jelmert, unpublished data). When looking for optimal desinfection procedures for marine fish larvae both the time of application and concentration of desinfectant should be addressed.

The results indicates that the epibiotic microorganisms at the egg surface are

closely involved in the pathogenesis of many of the developmental disorders encountered. Surface disinfection of eggs with iodophors should be an adequate prophylactic treatment.

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