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EVALUATION OF FOUR CHEMICALS FOR SURFACE-DISINFECTION OF MARINE FISH EGGS[1]

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Introduction

High mortality of marine fish eggs and larvae under intensive rearing is due to a wide range of causes, including high bacterial densities. In addition fish eggs are considered as a possible vector for transmission of diseases, because pathogens may be included in the microflora (Bergh *et al.*, 1990). In this study four chemicals were tested for their ability to reduce the bacterial load on the egg surface without having toxic effects on the embryo.

Materials and methods

Four chemicals were tested: Buffodine (iodophor; 1.06% free iodine), buffered glutaraldehyde, chloramine-T and sodium-hypochlorite (5% free chlorine). All solutions were prepared with sterile, filtered (0.2µm) seawater. Concentrations of Buffodine and sodium-hypochlorite refer to the amount of free iodine and chlorine added to seawater. Eggs of plaice (*Pleuronectes platessa* L.), at the stage were the embryo surrounds approximately 180° of the yolk mass, were washed with sterile, filtered seawater before and after treatment to remove organic materials and residual disinfectants. Disinfection was carried out at 5°C for 10 min. Single eggs (N = 60) were incubated at 10°C in M-65 seawater agar (1% agar). The amount of eggs with bacterial growth was recorded after 7 days. Egg samples were kept in darkness at 5-6°C in sterile, filtered seawater to observe mortality and hatchability. At the end of the yolk sac period, 14 to 23 day old larvae were placed in small volumes of concentrated seawater (N = 3) with a salinity of approximately 70.0ppt and the cumulative mortality was recorded over time. The results were analyzed using a chi-square (χ^2) test.

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Results

Disinfection with 400 ppt concentrations of other experiments. Co hypochlorite showed bacterial growth. Rep a clear correlation bet (data not shown)

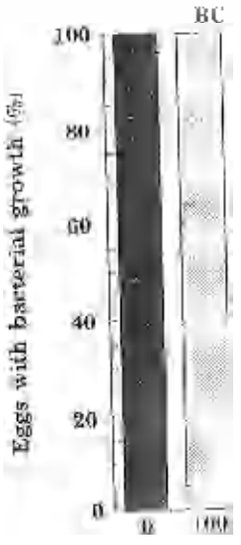

Fig. 1. Reduction

The dose of 400pp exceed e.g. 250ppm Treatment for 5 mi mortality. A signifi in the number of 200ppm free iodine and died after a w not have any negat was confirmed for

Larvae that hatche (P<0.01), and > 2 after 3h in concen ner the lowest dos larvae to salinity rest of the groups

Results

Disinfection with 400ppm Buffodine reduced the bacterial load to some extent (Fig. 1), but concentrations of 100 to 200ppm free iodine had no effect. This was confirmed in other experiments. Contrarily, all groups treated with glutaraldehyde, chloramine-T and hypochlorite showed an approximately 90% reduction in the number of eggs with bacterial growth. Replicate experiments with a standard disinfection procedure revealed a clear correlation between the bactericidal effect and the initial bacterial load on the egg (data not shown).

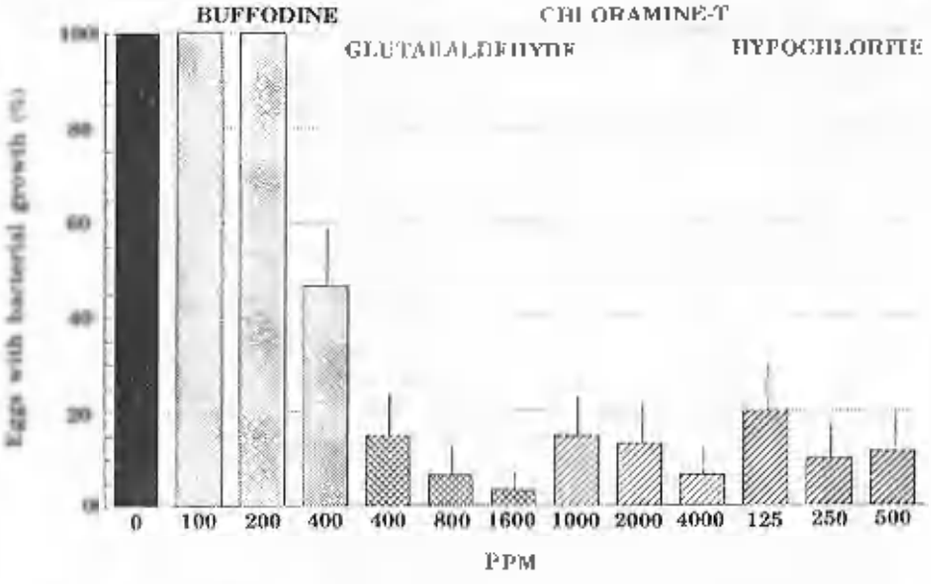

Fig. 1. Reduction in number of eggs with bacterial growth (+ 95% confidence limits).

The dose of 400ppm Buffodine was highly toxic (data not shown) having a level exceeding 250ppm free iodine, which in other experiments caused 100% mortality. *Treatment for 5 min treatment with 400ppm Buffodine did, however, not result in acute* mortality. A significantly higher mortality ($P<0.01$) at hatching and a significant reduction in the number of eggs that hatched ($P<0.05$) was observed for groups treated with 200ppm free iodine. Eggs disinfected with concentrations of hypochlorite did not hatch and died after a week. Disinfection with doses of glutaraldehyde and chloramine-T did not have any negative effect on survival during incubation or on hatchability. This trend was confirmed for all chemicals in other experiments.

Larvae that hatched from eggs disinfected with concentrations > 800ppm glutaraldehyde ($P<0.01$) and > 2 000ppm chloramine-T ($P<0.001$) had a significantly higher mortality after 3h in concentrated seawater than the untreated groups (Fig. 2). Neither Buffodine nor the lowest doses of glutaraldehyde and chloramine-T influenced the tolerance of the larvae to salinity-stress. These larvae also survived for a longer period of time than the rest of the groups (> 54 *versus* 20–24h).

Conclusions

Doses of Buffodine that reduced the bacterial load were above the toxic level. The interval between toxic and non-toxic dose appears to be very narrow. A large number of surface-sterile eggs was observed for all doses of hypochlorite, but hatching was inhibited and all eggs eventually died. Doses of glutaraldehyde and chloramine-T had good bactericidal effects and did not affect survival of incubating eggs or hatchability. Some groups had a higher cumulative mortality at hatching or a lower hatchability, but no relation to doses was found. The lowest doses of these two disinfectants did not reduce the salt tolerance of the larvae.

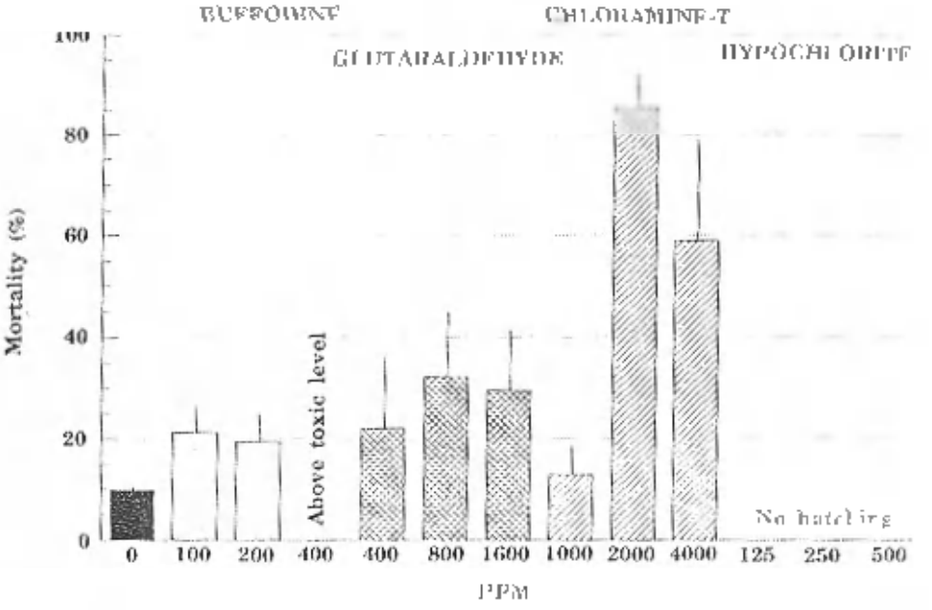

Fig. 2. Cumulative mortality of larvae (+ standard error) after 3h in seawater of approximately 30ppt.

References

Bergh Ø., G.H. Hansen, and A. Jelmert. 1990. Bacterial diseases of eggs and yolk sac larvae of halibut (*Hippoglossus hippoglossus* L.): characterization and experimental infection. ICES CM 1990, F:28.