

Pyceze® as a disinfectant of marine finfish eggs



Research Objectives

Bacterial contamination of eggs is a major issue in marine larviculture as it is widely believed to lead to reduced survival whilst also being a potential route of disease transmission both vertically within a hatchery and horizontally between hatcheries.

Pyceze, manufactured by Novartis, is currently licensed for use as a treatment for fungal infections in salmonids. Its active ingredient, Bronopol, has broad spectrum anti-bacterial and preservative properties, and for this reason is currently included in a wide range of health care products

This poster therefore describes trials using Pyceze to disinfect the eggs of cod, *Gadus morhua*, and haddock *Melanogrammus aeglefinus* eggs as a potential means of improving survival through to hatch.

Fish Egg Disinfection

Egg surfaces are highly favourable to bacterial growth (Hansen 1989, Morrison 1999). Any such contamination may not only result in poor survival to hatch but also act as potential route for pathogen transmission to emerging larvae. For this reason good hygiene is required to maximise the survival of eggs, and to this end egg disinfection has



become a common practice in most marine finfish hatcheries. A range of materials are presently used for this purpose, but Pyceze was seen as a potentially more suitable alternative as it is both an effective fungicide and also an efficient bactericide. The active ingredient of Pyceze exhibits its effect on bacteria by catalytic oxidation of accessible thiols.

Methods

Single Dip Treatment. Cod eggs were dip treated with Pyceze at concentrations of 50, 500 and 1000 mg I⁻¹ active bronopol for 45 seconds as soon as possible after fertilisation, and this was compared with a traditional treatment, and a seawater control. Replicate samples of eggs from each treatment were homogenised and 0.1 ml plated on marine agar, and then incubated at 10°C. The number of colony forming units (CFU) ml⁻¹ on each plate was counted after 5 and 10 days and the total bacterial numbers assessed. Similarly replicate samples of 6-8 eggs from each treatment were placed into 5 ml volume well plates at 10°C, and survival was also assessed daily through to hatch,

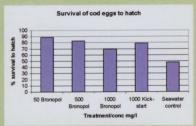
Daily Treatment. Haddock eggs were stocked un-disinfected. After 30 minutes the water flow was stopped and aeration provided. Pyceze was then added to give a concentration of 50 mg I⁻¹ bronopol for 30 minutes after which the water flow was restarted. The disinfection procedure was repeated daily for 8 days, with similarly handled untreated eggs acting as controls. Any dead eggs were removed before each disinfection. Samples of eggs were taken on day 1 and day 8, plated, and bacterial numbers assessed. On day 8, five replicate egg samples were taken, and observed in well plates for survival and development.





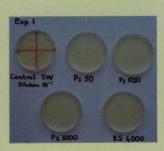
Results

<u>Dip Treatment</u>. For the cod, egg survival to hatch after dip treatment with Pyceze was 89% compared to 48% for untreated eggs (P<0.05). For haddock, survival to hatch was 72% compared to 62% in untreated controls (NS). Dip immersion of cod eggs for 45 seconds in 50 mg/ litre bronopol resulted in 30 CFU / ml compared with 14.000 CFU for untreated controls





Daily Treatment. The egg survival through the incubation period was 63% compared to 35% in untreated eggs. There was a 100 fold reduction in bacterial numbers, from 892 (+/-20SE) CFU/ml disinfected compared with 91,400 (+/-4,676SE) P<0.05. Under the microscope there was a difference in the appearance of the eggs, with treated eggs having a noticeably smoother and cleaner chorion.



Conclusions

- Survival of cod and haddock eggs was improved following disinfection with Pyceze
- · Survival was higher than with the current treatment methods
- · Pyceze significantly reduced bacterial loading
- A static daily disinfection technique in incubators was successful and does not involve handling eggs