

BACTERIAL DISEASES OF EGGS AND YOLK SAC LARVAE OF HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS* L.)

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Introduction

Halibut is an attractive species for marine cold-water aquaculture. High mortality at early life stages has, however, so far hindered commercial success. Infections by bacteria are believed to be a major cause (Bolinches and Egidius, 1987; Hansen and Olafsen, 1989; Pittman *et al.*, 1990; Opstad and Bergh, in press). Here we present studies considering experimental infection, treatment and characterization of the morphological, ultrastructural and behavioural consequences of infections at early life stages.

Materials and methods

Eggs and larvae were reared in 10ml autoclaved seawater in polystyrene multiwell dishes at 5°C in darkness. Eggs were experimentally infected 4 days before hatching by a *Flexibacter* sp. isolated from halibut eggs, *Vibrio anguillarum*, or other *Vibrio* spp. Mortality was recorded until day 37 when larvae were fixed for ultrastructural studies by transmission electron microscopy. In addition, eggs were fixed for investigation by scanning electron microscopy. In an otherwise identical infection experiment, larvae were filmed by a video camera for behavioural studies; and transferred to a buoyancy column for larval buoyancy measurements every 3rd or 4th day.

In disinfection experiments, the eggs were treated with Buffodine (Evans Vanodine, Preston, UK) for 10min at different concentrations 24h before hatching, before transfer to multiwell dishes. Mortality was recorded throughout the experiment. At day 37 after hatching, the larvae were examined by microscope for developmental disorders.

Results

Cumulative mortality in the infection experiments is shown in Table I. The *Flexibacter*-infected individuals showed high mortality at the egg stage, at hatching and early yolk-sac stage, whereas larvae infected by *Vibrio* spp. had low mortality at these early stages,

followed by a high mortality throughout the yolk-sac stage. In the uninfected control group, only 5 to 60 larvae died during the experiment. Examination by scanning electron microscopy in *Flexibacter*-infected eggs showed wounds colonized by large amounts of bacteria. The chorion was penetrated and the zona radiata was severely damaged. Transmission electron microscopy of larvae infected with *Vibrio* spp. revealed bacteria present in large amounts between gill arches, in the heart region and in the blood vessels. Uninfected control larvae appeared healthy, both on cellular and subcellular level, and no deformities or developmental disorders were observed. Research is in progress characterizing the infection-induced changes in behaviour and buoyancy.

Table I. Cumulative mortality in the disinfection experiments

Bacterium	Hatching (%)	Day 37 (%)
<i>Flexibacter</i> sp.	74	-
<i>V. anguillarum</i>	7	95
Control	0	8

As shown in Table II, surface-disinfection caused significantly increased survival as well as an increased percentage of larvae without developmental disorders of any of the kinds that could be identified.

Table II. Mortality and percentage of larvae with deformities in the disinfection experiments

		Mortality (day 37, %)	Deformed (day 37, %)
Buffodine concentration (%)	0.5	13	10
	0.05	15	32
	0.005	41	61
Control		33	59

Discussion

The infection experiment showed that the bacteria that were tested could be pathogenic to halibut eggs or larvae. The *Flexibacter* sp. is able to penetrate the chorion, and probably the zona radiata as well. This bacterium, which has been shown to be abundant on halibut eggs and larvae, as well as in the water in the incubator tanks, has not previously been described (Hansen *et al.*, in prep.).

The disinfection experiments prove that surface disinfection is an adequate way to increase survival and developmental success in halibut aquaculture. Further work is

necessary to determine optimal concentrations and application procedures. The results presented emphasize the importance of the bacterial microflora of eggs and larvae on the survival and developmental success.

References

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