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# Antibiotic treatment and dose-response of bacterial activity associated with flatfish eggs.

by

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ABSTRACT.

Newly stripped and fertilized eggs from Plaice (Pleuronectes platessa) and Atlantic Halibut (Hipppoglossus hippoglossus) were incubated in 34 ppt sea water.
150 eggs(Plaice), 30 eggs(Halibut) or 20 glass beads were incubated in 30 ml seawater in light at 5.5°C. The antibiotics Oxytetracycline(-HCl) and Flumiquil were added to end concentration ranges 0 - 105 ppm and 0 - 60 ppm, respectively.

With a method modified from Somville and Billen (1983) it was possible to measure the activity both in the incubation water and on the chorion of the incubated eggs.
The activity on egg surfaces was significant higher than the activity of a comparable surface aerea of the glass beads. The activity on the egg surfaces was greater than the activity in a water volume corresponding to the combined egg volume.

Introduction

At present it is considered important to domesticate more species suitable for aquaculture.
In spite of subtsantial efforts, it has been difficult to produce a larger quantity of fish larvae of several species. This is in particular true for several species of flatfish e.g. The Atlantic Halibut (Hippoglossus hippoglossus L.) Solemdal et.al.(1974), Blaxter et.al. (1983).

It is thought that bacterial activity might be responsible, directly or indirectly, for parts of the high mortality observed. Rabben and Jelmert (1986), Rabben et.al.(1986).
A pilot experiment was carried out to monitor the bacterial activity associated with fish eggs in closed water systems. Eggs from the Atlantic Halibut and Plaice (Pleuronectes platessa) were chosen as suitable experimental material.

MATERIALS AND METHODS.

Water.

Unfiltered seawater was taken from the deepwater supply(50 m depth) at Austevoll Marine Aquaculture Station. Salinity was 33.8 ppt and temperature was 5.5 ± 0.1°C.
This water was used as incubation water(IW),and the bacterial activity at t=0 was 0.9 relative fluorescence units (RFU).

Eggs and incubation.

Newly stripped and fertilized eggs from the Atlantic Halibut and Plaice were diluted approximately 10.000-fold in IW to remove exess sperm. 30 eggs (Halibut) or 150 eggs (Plaice) were transferred to 50 ml polystyrene beakers(Nunc Intermed Denmark, hygenic quality) containing 30 ml IW.
In jars without eggs or antibiotics, 10 glass beads with average diameter 4.0 ± 0.4 mm (St.Dev., n=10) were used as measure of "unspecific" surface growth.
All jars were incubated in light(Osram 140 W/32, giving an intensity of approx. 60 lux) in a termostatted room at 6.1 ± 0.1°C. Cumulative death (day 0 trough 7)is shown in tables 1 a and b.

Antibiotics.

Stock solutions of 2000 ppm oxytetracycline-HCl (Norsk Medisinaldepot, Norway) and 40.000 ppm Flumiquil (Reg. Trademark, Clin Midy Vetrinaire, St-Jean de La Ruelle France) were made up with o.2 um-filtered IW.
To 30 ml IW the following amounts of stock soloutions

were added: 0.1, 0.2, 0.3, 0.4, 0.8 and 1.6 ml. (1.6 ml, oxytetracycline only).
For Oxytetracycline, this yields end concentrations of: 6.6, 13.2, 19.8, 26.3 51.9 and 101.3 ppm . "Flumiquil" contains 3% active compound. This yields end concentrations:4.0, 8.0, 15.8, 31.2, 60.8 ppm.
Two jars for each concentation of antibiotics, and the control were incubated as described above.

Bacterial activity in incubation water.

Bacterial activity was measured with a method adapted from Somville and Billen (1983).
A stock soloution of 40 mM of substrate, L-Leucine-ß -Naphtylamide HCl(Sigma Chem.Company St.Lois, USA) were made up with autoclaved destilled water. This soloution was stord at -18°C and thawed before use.
2 ml sample and 50 ul substrate was added to a rinsed quartz cuvette and the development of fluorescence was monitored in an Shimadzu RF-530 HPLC-monitor and a Tarkan W+W recorder 600. The slope of the line represented developed fluorescence (relative fluorescence units, RFU).

Bacterial activity on surfaces.

4 glass beads or 5 eggs (halibut) were transferred to sterile 3.6 ml cryotubes. Surplus IW were removed by means of a sterile pasteur pipette. 2 ml sterile seawater and 50 ul substrate was added and incubated for 4 minutes at 20°C. The seawater was decanted from the cryotube into a rinsed cuvette and measured as described above.
The total surface area and volume of the reincubated eggs was 160.8 $mm^2$ and 0.0172 ml, respectively. The total surface area and volume on the glass beads was 200.1 $mm^2$ and 0.0335 ml, respectively. The ratio of surface area to water volume, was calculated as added sterile water (2 ml) divided with egg (glass bead) volume.
The results of surface related activity is shown in table 2.

RESULTS.

Table 1 a. Cumulative death day 0 to 7, Halibut with "Flumiquil". Doses as ppm end concentration.

| Day 0 | | | | Day 7 | | |
|---|---|---|---|---|---|---|
| Dose | Alive | Dead | %Dead | Alive | Dead | %Dead |
| 0 | 30 | 0 | 0 | 0 | 30 | 100 |
| 4.0 | 30 | 0 | 0 | 0 | 30 | 100 |
| 8.0 | 30 | 0 | 0 | 0 | 30 | 100 |
| 15.8 | 30 | 0 | 0 | 15 | 15 | 50 |
| 31.2 | 30 | 0 | 0 | 19 | 11 | 36.7 |
| 60.8 | 30 | 0 | 0 | 20 | 10 | 33.3 |

Table 1 b. Cumulative death day 0 to 7, Plaice with Oxytetracycline-HCl. Doses as ppm end concentration.

| Day 0 | | | | Day 7 | | |
|---|---|---|---|---|---|---|
| Dose | Alive | Dead | %Dead | Alive | Dead | %Dead |
| 0 | 150 | 0 | 0 | 0 | 150 | 100 |
| 6.6 | 150 | 0 | 0 | 0 | 150 | 100 |
| 13.2 | 150 | 0 | 0 | 0 | 150 | 100 |
| 19.6 | 150 | 0 | 0 | 142 | 8 | 5.3 |
| 26.3 | 150 | 0 | 0 | 140 | 10 | 6.7 |
| 51.9 | 150 | 0 | 0 | 140 | 10 | 6.7 |
| 101.3 | 150 | 0 | 0 | 143 | 7 | 4.7 |

The bacterial activity in the incubation water is shown in figs. 1a and b.

Table 2. Bacterial activity (as RFU) on halibut egg, and glass bead surfaces; e=eggs, g=glass beads. The area/volume corrected activity is compared to activity in the incubation water.

| Surface | Dose(PPM) | RFU | RFU, Corrected | RFU, Water |
|---|---|---|---|---|
| 5e | 0 | 2.4 | 279.1 | 51.2 |
| 5e | 4.0 | 2.7 | 313.9 | 46.7 |
| 5e | 8.0 | 1.7 | 197.7 | 23.0 |
| 5e | 15.8 | 1.1 | 127.9 | 19.9 |
| 5e | 31.2 | 0.4 | 46.5 | 5.1 |
| 5e | 60.8 | 0.2 | 23.3 | 3.0 |
| 4g | 0 | 0.8 | 47.8 | 3.6 |

DISCUSSION.

The results show that substantial amount of bacterial activity after 7 d of incubation were concentrated on the egg surface and in the watershell surrounding each egg.
The higher concentrations of both antibiotics yilded a bacterial activity approximately like the incubation water at time = 0.
The lower cocentrations did show a close to linear dose-response to the antibiotics added.
Eventually released chorionase from the eggs is not considered to interfere with the measurements.
The eggs were only 0 to 8 days old in the experiment.
The vesicles containing chorionase does not develop before approx. one week before hatching (J.V.Helvik, Dep.Biochem., Univ.of Bergen, pers.comm.).
The close to linear dose-response observed for both the antibiotics tested, made it very unlikely that the chorionase had an influence on the results.

The administration of antibiotics to the incubation water did have a clear positive effect on mortallity of the eggs. Minimizing bacterial activity in egg and larval incubation systems would be a major contribution to higher survival in halibut fry production. The use of antibiotics in a production process should be avoided if possible, and other methods to minimize bacterial activity should therefore be sought for .

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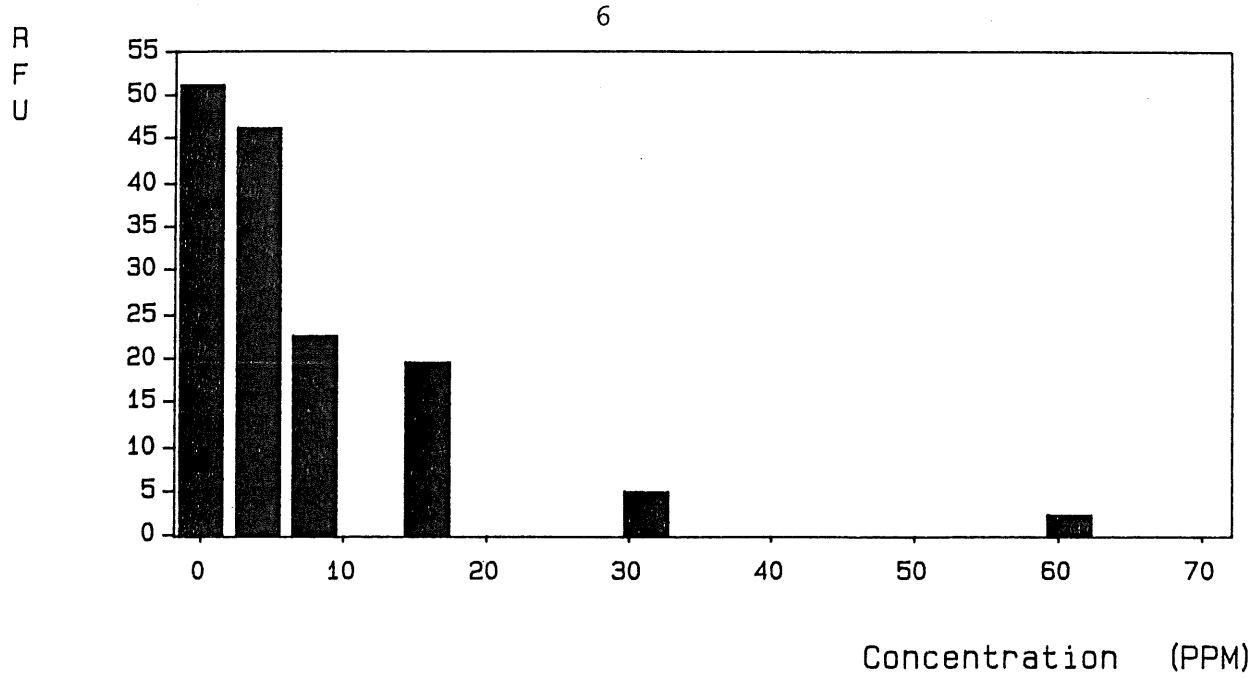


Figure 1a. Bacterial activity, halibut eggs and "Flumiquil"

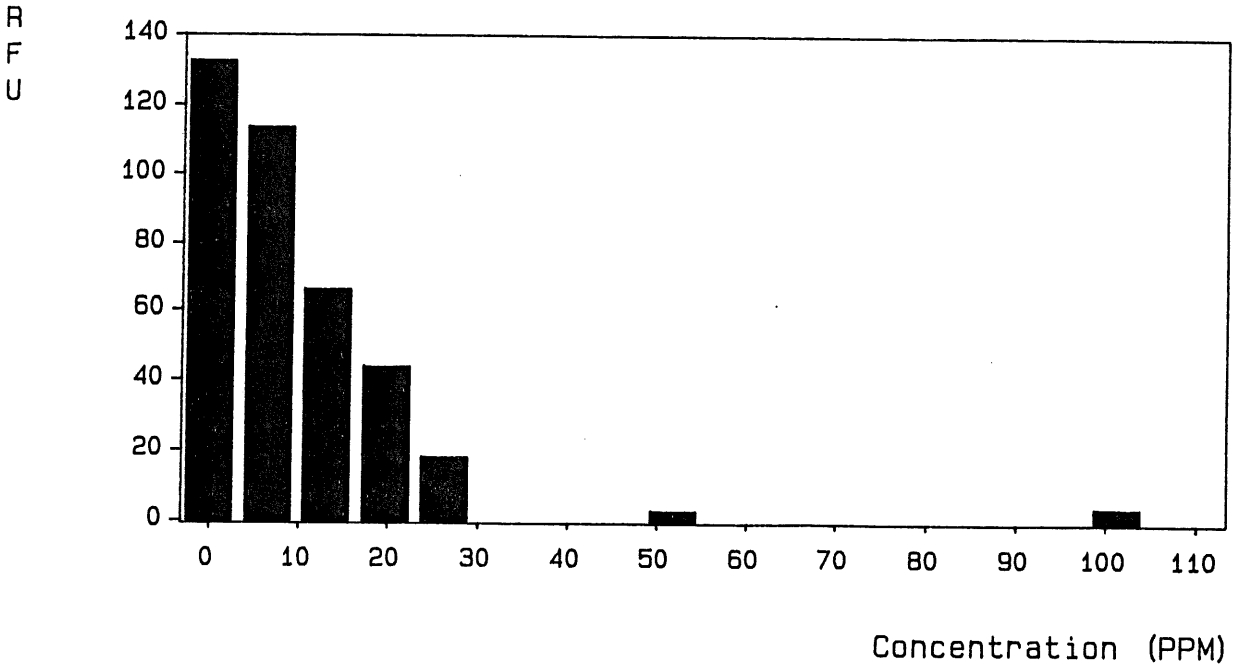


Figure 1b. Bacterial activity, plaice eggs and Oxytetracycline-HCl.