BACTERIAL ENVIRONMENT OF GAMETES AND TURBO T (*Scophthalmus maxi mus*) EGGS

M.H. OMNES, Y. TEMEY, J. Y. DANIEL, and J. L. NICOLAS
Laboratoire Ressources Aquacoles BP 70, 29280 PLOUZANE (FRANCE)

INTRODUCTION

Bacterial infections have been suspected to be one cause of variable success in the incubation of turbot eggs, as it was the case in the incubation of halibut eggs (Bergh *et al.*, 1990). From an other source, it has been shown for salmonids, that ova were intraovularly infected and sperm contaminated by severals bacteria (Sauter *et al.*, 1987), suggesting these should be as possible contributors to diseases or losses of early development.
The assessment of bacterial flora associated with ova, sperm and developing eggs was conducted just at stripping gametes and during incubation.

MATERIALS AND METHODS

Before gametes were collected, genital papilla of fishes were carefully dried with soft tissue paper and disinfected to avoid contamination during stripping. Ova and sperm were collected respectively in sterile glass tubes or syringes.
After fertilization, eggs were hold in an open-circulation 40 l. incubator, at 13°C during 6 days until hatching. Viable eggs and incubation water were collected every other day throughout incubation.

Sperm collection

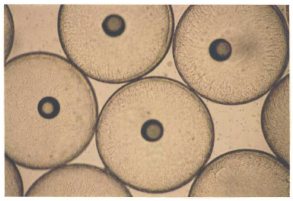
Incubator

Samples were rinsed several times with sterile seawater, homogenized, diluted and plated on agar.
Viable counts of bacteria were determined on two media, Zobell and TCBS agar. Epibiotic bacteria on egg surface and impact on the chorion were observed at scanning electron microscope (SEM).

RESULTS

Scanning electron observations were carried out only on ova, eggs and larvae.
- on the typical structure with small pores forming the outer egg surface (chorion), adherent bacteria were present, attached by fimbriae (fig. a and b).
- during incubation, massive colonization of chorion could occured prior hatching. Pores were no more visible at day 5 (fig. c and d).
- after hatching, low density of bacteria was observed on larvae.

No correlation was found between numbers of bacteria and losses of eggs in these observations, and the hatching rates achieved variable levels (from 15 % to 46 % viable larvae).



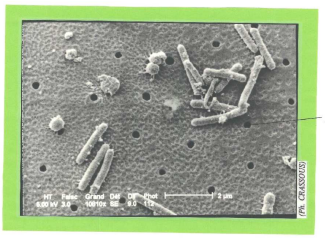
Ova newly ovulated

High variability in counts of bacteria was observed between samples. Ova and sperm were both infected within gonads. Over the developmental stages, bacterial flora was associated with eggs and was present in incubator water (table and figure).

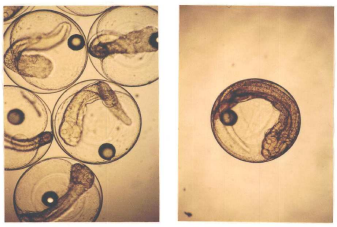
Source			Counts	
Ova	(n=8)		$<10^1$ to 4 10^3	CFU/ova
Coelomic Fluid	(n=3)		$<10^1$ to 8 10^2	CFU/ml
Sperm	(n=7)		$<10^1$ to 3 10^6	CFU/ml
Embryos	(n=3)	Day 3	$<10^1$ to 3.8 10^3	CFU/egg
Incubator water			2.4 10^3 to 9 10^3	CFU/ml
Embryos	(n=3)	Day 5	2.1 10^3 to 3.3 10^4	CFU/egg
Incubator water			4.7 10^3 to 9 10^4	CFU/ml
Larvae	(n=1)	Day 6	3.9 10^4	CFU/larva
Incubator water			6.5 10^4	CFU/ml

Bacterial counts on Zobell agar.

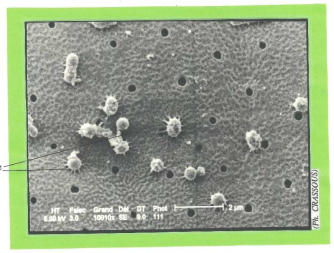
Phenotypic identification of bacteria revealed always a diversified bacterial flora associated with incubated eggs. Vibrios isolated on TCBS remained present at low level, and their pathogenicities were not established.



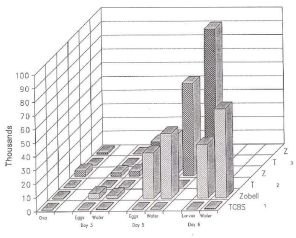
View of bacteria on ova surface (SEM): rods (a),



Embryos prior hatching Day 4, Day 5 at 13°C



cocci (b).



Bacterial growth during three incubations (CFU /ova, eggs, ml SV

Massive colonization of bacteria; bacteria are embedded in organic

matter at day 5 (c), others details (d).

DISCUSSION

Massive colonization occured five days after insemination. On embryos, adherent colonies may dissolve and strip off the outer chorion layer perhaps by exoenzyme activity (Holm-Hansen, 1973 in Alfonso, 1991). At high density, microorganisms could lead to restrict gazeous exchange across the embryonic surface.
Since this study, as bacterial growth is prevalent throughout incubation, seawater is cartridge filtered (5 μm pore size) and U.V. sterilized in ours facilities prior to admission at incubators.
Some authors preconise antibacterial treatments, but others have shown its could be ineffective in completely inhibiting bacterial growth in the incubation medium.

Questions about origin and type of bacteria with develop fimbriae for attachment on ova are asked: are they very specific and adapted bacteria or are they ubiquious?
Finally, in studies about gametes and developing eggs it is important to take into account their contamination by bacteria.

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