

Occurrence of Intra-Ovum Infection and Effect of Egg-washing with Isotonic Solution on Elimination of Bacteria and Viruses before Fertilization in Salmon Aquaculture

M.Kohara^{1,2}, S.Ogawa², H.Kasai³, M.Yoshimizu³

¹ Graduate School of Fisheries Sciences, Hokkaido University

² Nagano prefectural Fisheries Experimental Station

³ Faculty of Fisheries Sciences, Hokkaido University

Introduction

● Problems of intra-ovum infection

Mechanism of intra-ovum infection	BKD <i>Renibacterium salmoninarum</i>	BCWD <i>Flavobacterium psychrophilum</i>
	* in the body	* out of the body
When	ovulation ? oogenesis ?	Fertilization and water-hardening
Where pass in in	Micropyle or pore canal ? Yolk , oocyte	? Perivitelline space
What condition	Contamination of egg-fluid	Contamination of egg surface (10 ⁶⁻⁷ CFU/ml) □
Reports	Evelyn <i>et al</i> (1984,1986) Bruno and Munro (1986) Lee and Evelyn (1989)	Kumagai and Nawata (2010) Kohara <i>et al</i> (2010)

The theme of this sturdy

- What is the true mechanism of intra-ovum infection ?
- Is the egg-washing method effective to prevent intra-ovum infection in salmon aquaculture ?

Immersion-challenge of rainbow trout eggs with *F. psychrophilum*

Exp.	Challenge dose (log ₁₀ CFU/ml)	Immersion-challenge of eggs		
		unfertilized eggs	before fertilization	after fertilization
		positive / examined (%)	positive / examined (%)	positive / examined (%)
1	8.3	11/60 (18.3)	16/60 (26.7)	24/60 (40.0)
	4.3	0/60	0/60	0/60
	-	0/60	0/60	0/60
2	8.8	4/60 (6.7)	2/60 (3.3)	5/60 (8.3)
	4.8	0/60	0/60	0/60
	-	0/60	0/60	0/60

The invasion of *F. psychrophilum* was not affected by fertilization.

Intra-ovum infection of water hardened eggs

Passage time of water hardening (min.)	Intra-ovum infection rate positive / examined (%)	Eyed-egg rate (%)
0 (before)	5/80 (6.3)	48.6
2	3/80 (3.8)	15.0
5	1/80 (1.3)	3.9
10	1/80 (1.3)	1.4
30	0/80 (0)	0
60	0/80 (0)	ND

There is the high possibility that *F. psychrophilum* invaded through the micropyle as well as the sperm.

The conditions for the occurrence of intra-ovum infection

Exp.	Challenge dose (log ₁₀ CFU/ml)	Challenge method	
		egg surface contamination	water contamination
		positive / examined (%)	positive / examined (%)
1	9.9	21/60 (35.0)	1/60 (1.7)
	8.9	3/60 (5.0)	0/60
	7.9	1/60 (1.7)	0/60
	6.9	0/60	0/60
	5.9	0/60	0/60
	-	0/60	0/60
2	9.9	14/60 (23.3)	2/60 (3.3)
	7.9	1/60 (1.7)	0/60
	5.9	0/60	0/60
	4.9	0/60	0/60
	3.9	0/60	0/60
	-	0/60	0/60

The Occurrence of intra-ovum infection is much affected by egg surface contamination than water contamination.

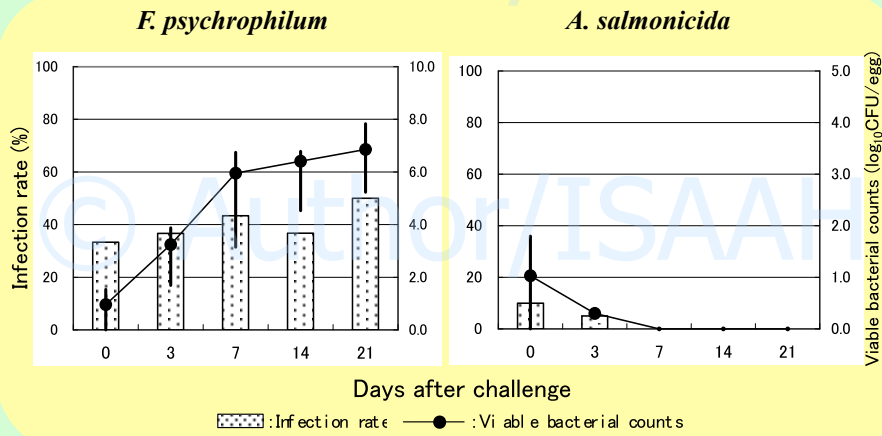
Intra-ovum infection of pathogenic bacteria and other bacteria

Bacteria	Challenge dose (log ₁₀ CFU/ml)	Intra-ovum infection rate positive/examined (%)
<i>R. salmoninarum</i>	10.2	11/60 (18.3)
	5.2	0/60
	-	0/60
	10.3	31/60 (51.7)
	2	5.3
<i>A. salmonicida</i>	-	ND
	9.0	0/60
	7.0	0/60
	5.0	0/60
	-	0/60

Bacteria	Challenge dose (log ₁₀ CFU/ml)	Intra-ovum infection rate positive/examined (%)	
<i>Pseudomonas</i>	No.27	9.5	16/60 (26.7)
		5.5	0/60
<i>Comamonas</i>	No.9	9.5	27/60 (45.0)
		5.5	0/60
<i>Sphigomonas</i>	No.25	9.4	16/60 (26.7)
	No.10	9.9	2/60 (3.3)
	No.11	9.6	4/60 (6.7)
Cont.	-	0/60	

Intra-ovum infection occurred by immersion-challenge with *R. salmoninarum* and other bacteria, but not with *A. salmonicida*.

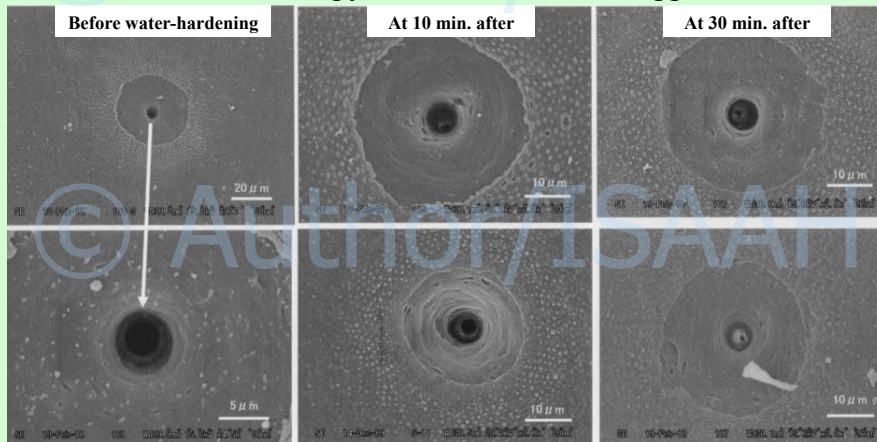
The increase and decline of *F. psychrophilum* and *A. salmonicida* in immersion-challenged eggs



F. psychrophilum was cultured in the egg, but *A. salmonicida* was not cultured at seven days after immersion-challenged.

Observation of the micropyle by SEM – 1

The micropyle of water-hardened egg

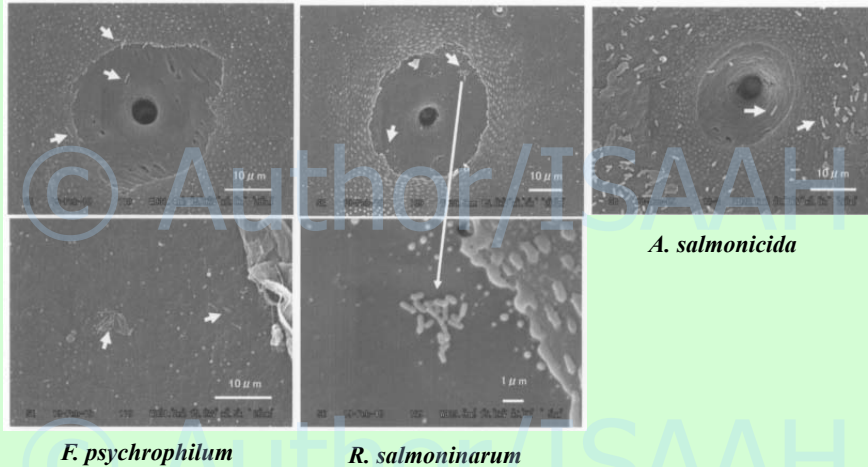


The micropylar canal was open until 10 min. had elapsed, but closed on and after 30 min.

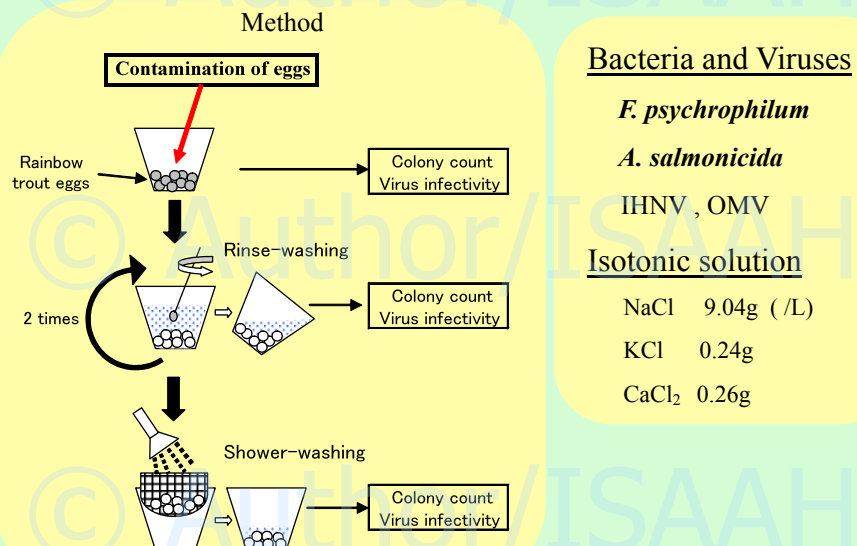
The pore canal was not observed on the egg surface without the micropyle.

Observation of the micropyle by SEM - 2

The micropyle of contaminated eggs with pathogens



Effects of egg-washing on elimination of bacteria and viruses



Results

Group	Process	<i>F. psychrophilum</i>			<i>A. salmonicida</i>		
		(log ₁₀ CFU/ml)			(log ₁₀ CFU/ml)		
		before	after	eliminated	before	after	eliminated
Egg-washing	1st. Rinse	6.2	5.7	0.5	8.3	7.3	1.0
	2nd. Rinse	5.7	5.2	0.5	7.3	6.3	1.0
	Shower	5.2	2.5	2.7	6.3	3.1	3.2
	Total			3.7			5.2
Shower egg-washing	Shower	6.1	3.7	2.4	8.4	5.7	2.7

Group	Process	IHNV			OMV		
		(log ₁₀ TCID ₅₀ /ml)			(log ₁₀ TCID ₅₀ /ml)		
		before	after	eliminated	before	after	eliminated
Egg-washing	1st. Rinse	7.4	6.1	1.3	5.4	4.6	0.8
	2nd. Rinse	6.1	5.4	0.7	4.6	3.9	0.7
	Shower	5.4	3.1	2.3	3.9	1.4	2.5
	Total			4.3			4.0
Shower egg-washing	Shower	7.1	4.6	2.5	5.1	1.9	3.3

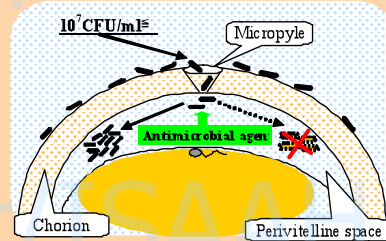
Effects of egg-washing on prevention for intra-ovum infection

Group	Treatment	Viable bacterial counts	Intra-ovum infection rate
		(log ₁₀ CFU/ml)	positive/examined (%)
Contaminated eggs	No-washing	9.1	13/60 (21.7)
	Rinse-washing	7.8	3/60 (5.0)
	Shower-washing	6.9	0/60
Uncontaminated eggs	No-washing	0	0/60

F. psychrophilum was clearly less in rinsed eggs than in non-treated eggs, and results of detection from showered eggs was negative.

Conclusions

- Intra-ovum infection occurs by invading of bacteria on the egg surface into the perivitelline space through the micropyle for the water-hardening period.



- The growth of bacteria is relevant to resistance to antimicrobial agents.

- Egg-washing with an isotonic solution is very effective to eliminate bacteria and viruses on the egg surface and is able to decrease the risk of intra-ovum infection.



My presentation is successfully over.
Thank you for your kind attention.