



HRO



Artificial Propagation of Arctic Lamprey in Japan

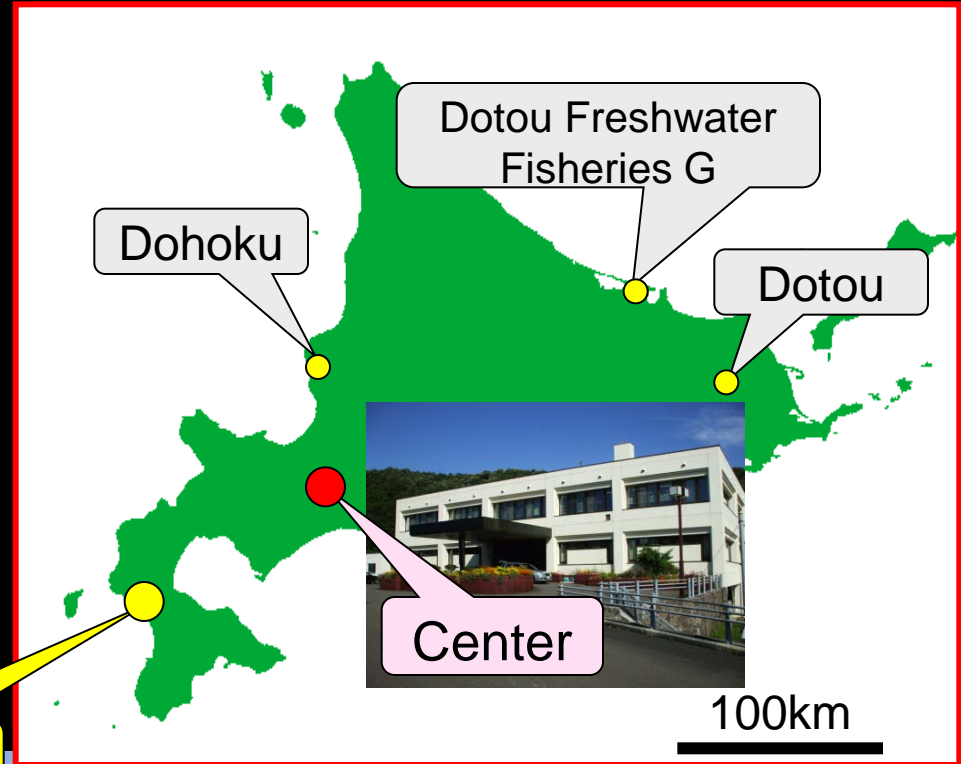
Satoshi Kusuda

Salmon and Freshwater Fisheries Research Institute
Donan Research Branch

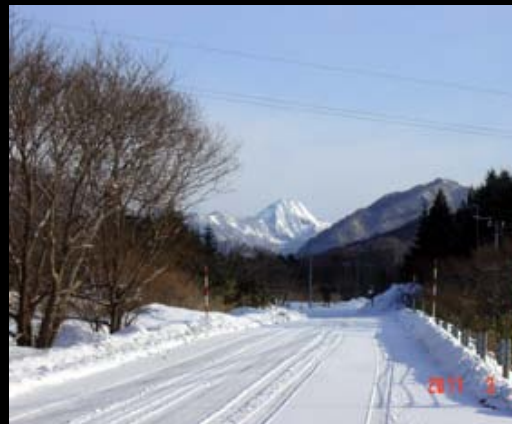
Local Independent Administrative Agency Hokkaido Research Organization

Photo: Tomohiro Watanabe

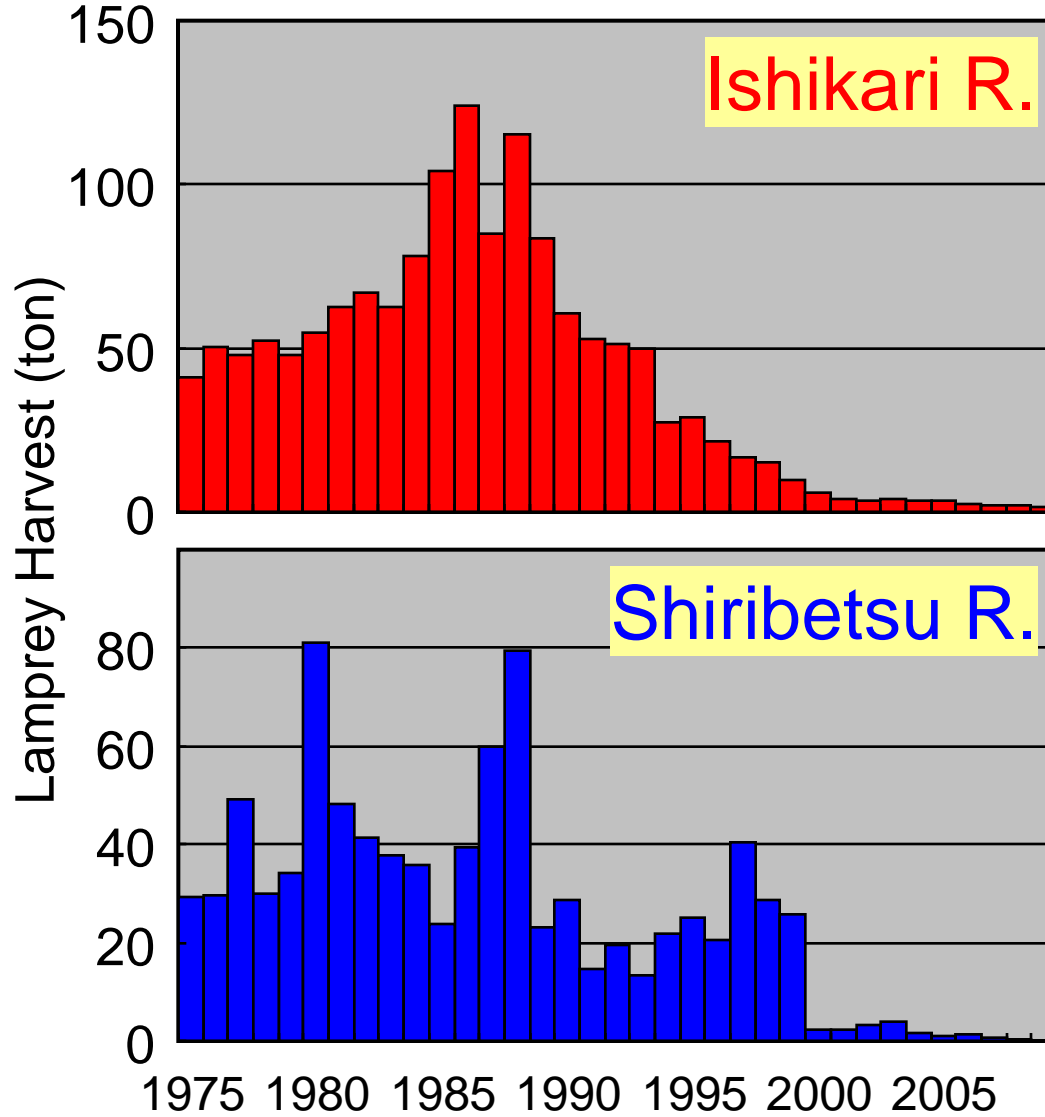
Salmon and Freshwater Fisheries Research Institute ●



Donan



Annual Harvest of Arctic Lamprey



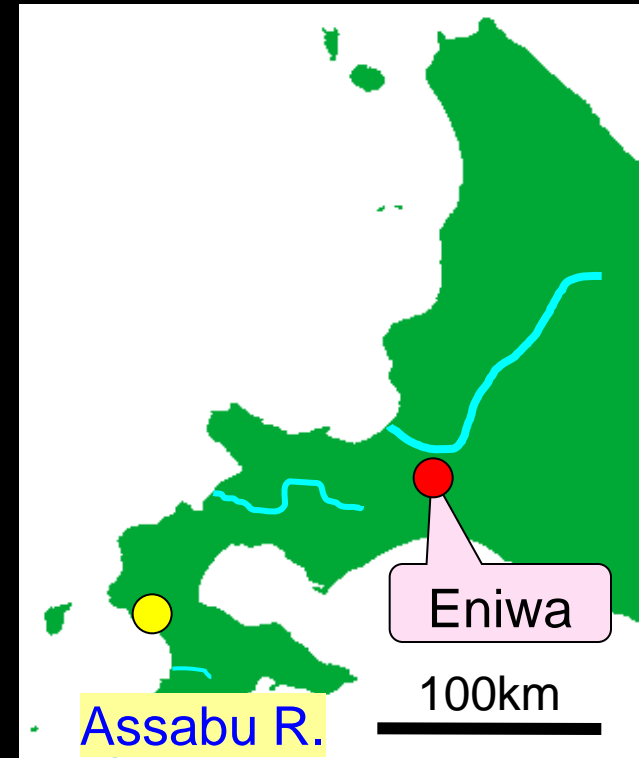
Presentation Outline

- Artificial Insemination and Egg Incubation

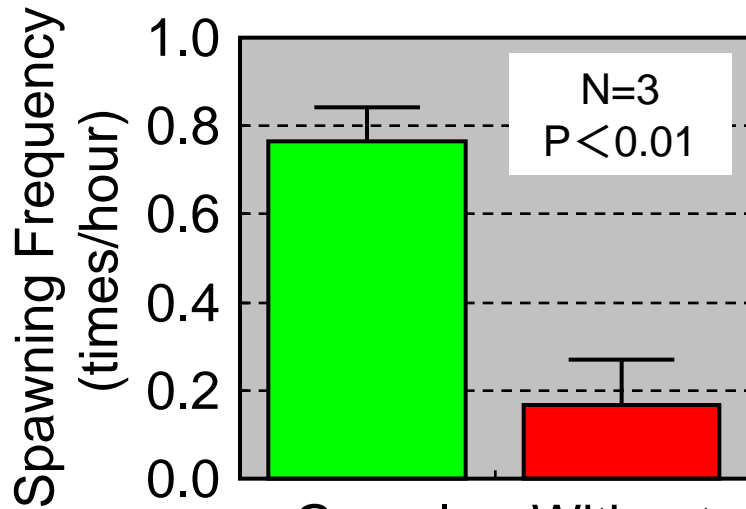
Assessed optimum conditions (Eniwa)

- Larvae after Release

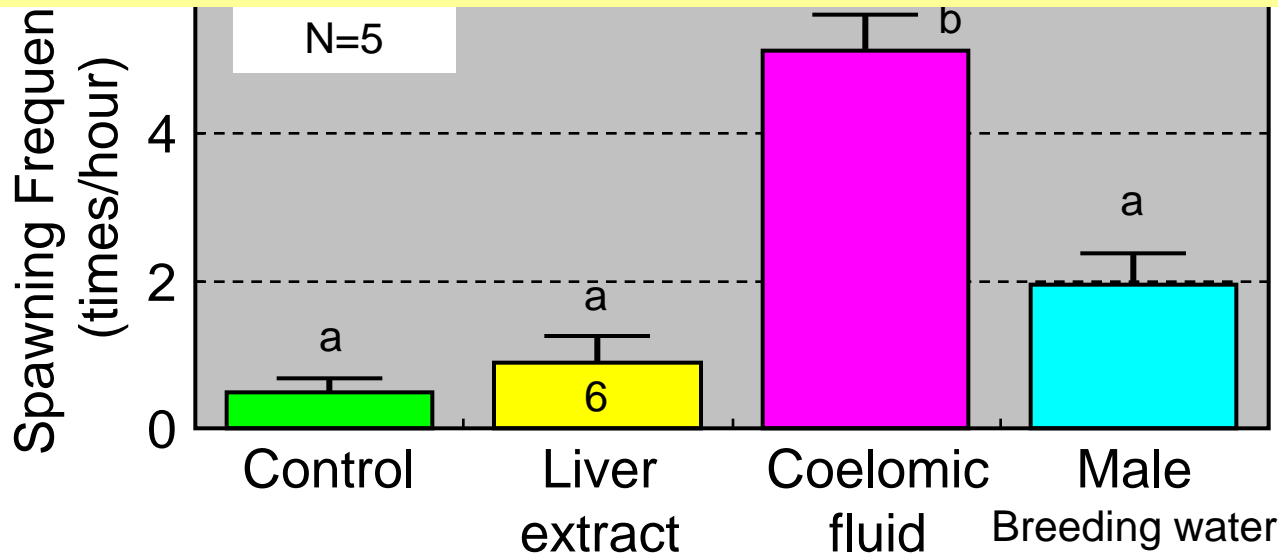
Examined the habitat and conditions of larvae after release (Assabu R.)



Spawning Condition

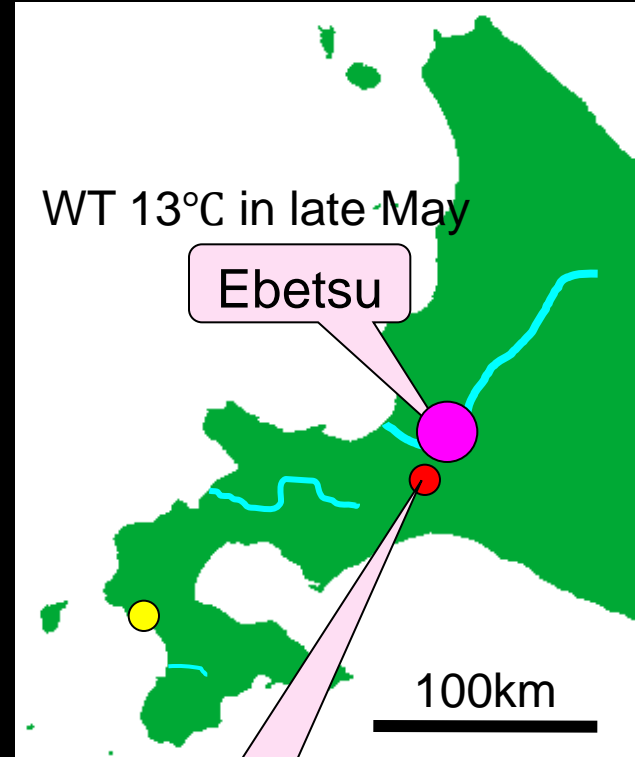


Gravel is vital for spawning.
The spawning behavior was stimulated by coelomic fluid.



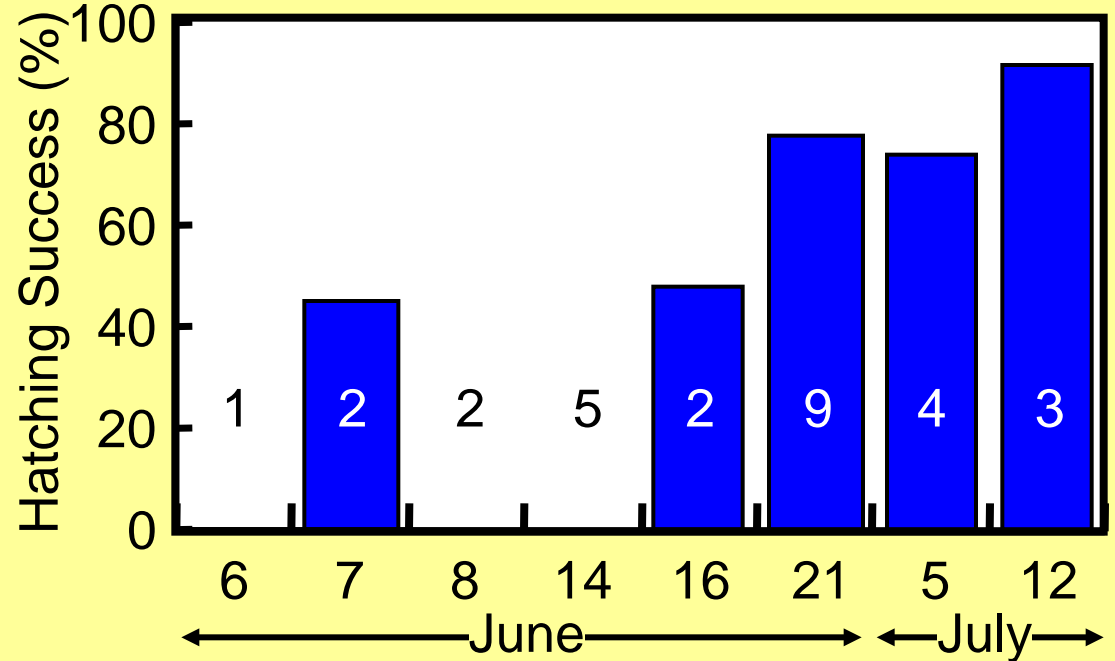
The upper line above the column indicates the standard error.

Artificial Insemination & Egg Incubation



WT 8°C (Spring Water)

Hatching Success of Fertilized Eggs

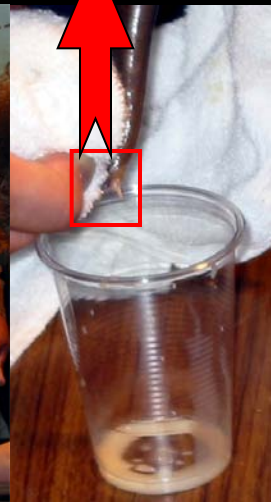


Total number of females are displayed inside the graph.

Hatching rate is different for each female. → Why ?

The criteria for egg quality were investigated;
volume of coelomic fluid,
rate of adhesive eggs.

Collection of Gametes



Fertilization Assays

Egg batches of 1g



50 μ l of milt



5ml of 1/10ASP



ASP; Artificial seminal plasma
107.2 mM NaCl
14.2 mM KCl
1.7 mM MgCl₂
1.0 mM CaCl₂
10 mM HEPES
pH7.4

Mixed and stirred

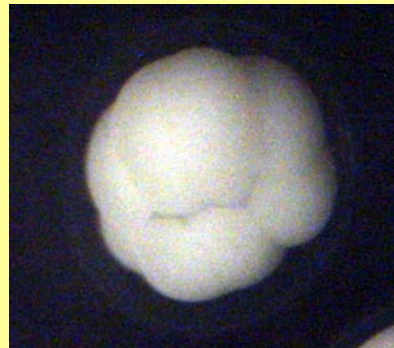


10°C

rinsed several times

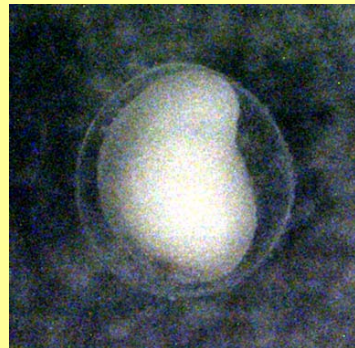


Fertilization rate



32-cell embryo
1 day after fertilization (daf).

Incubation at 15°C
Embryo
formation rate



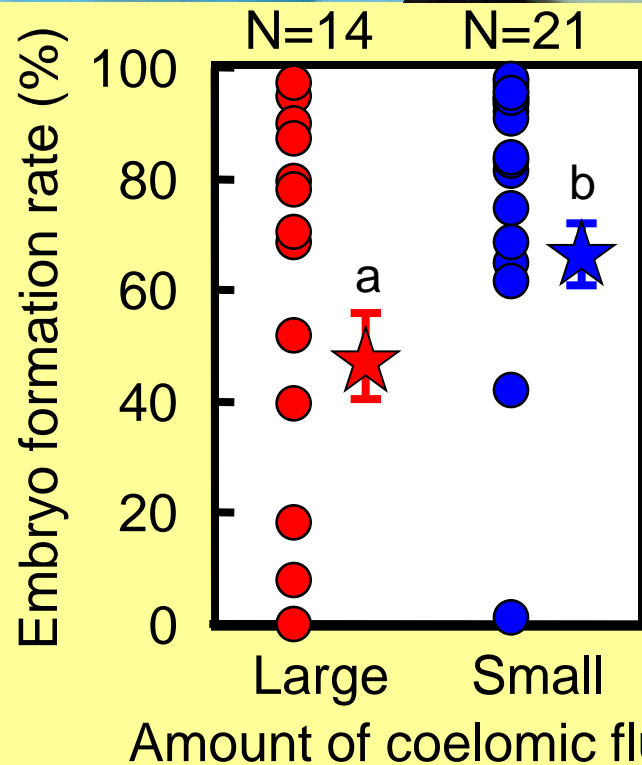
Head protruding embryo
7 daf

Hatching rate



14 daf

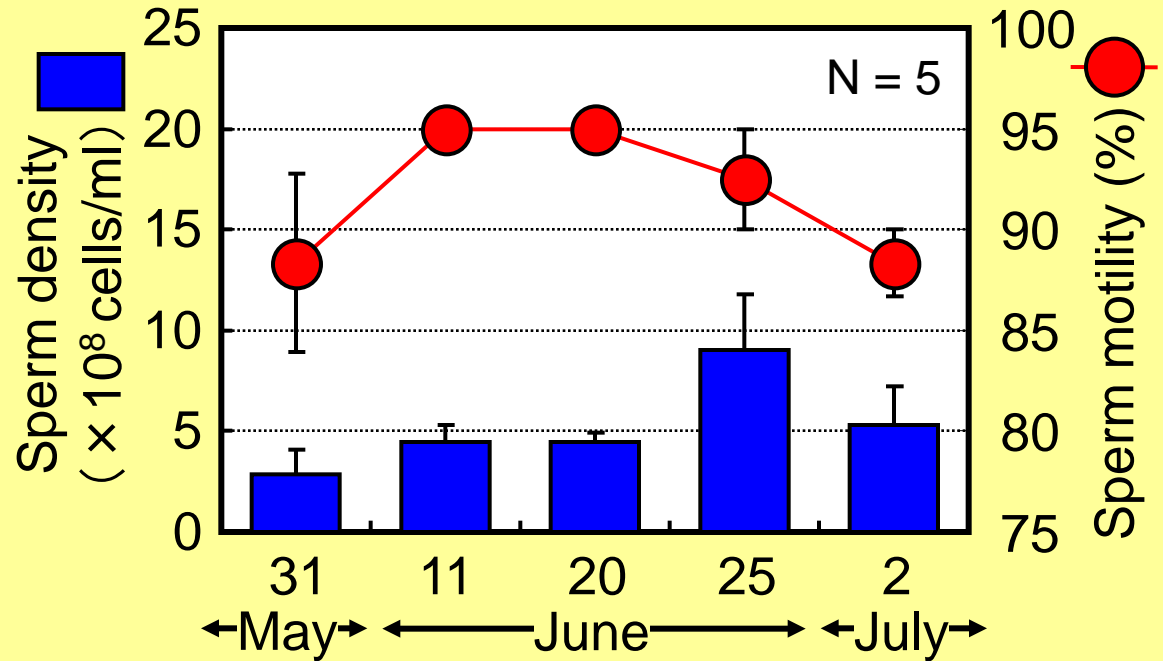
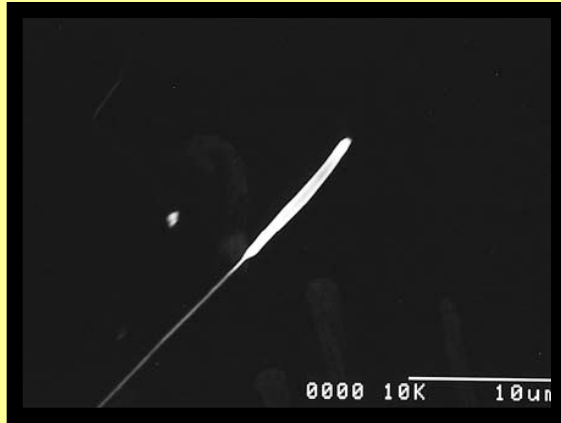
Amount of Coelomic Fluid & Embryo formation rate



$P < 0.05$

The stars and bars represent the means and SE, respectively.

Amount of Milt & Sperm Motility



Sperm density and motility are different for each male.

Optimal conditions were examined;

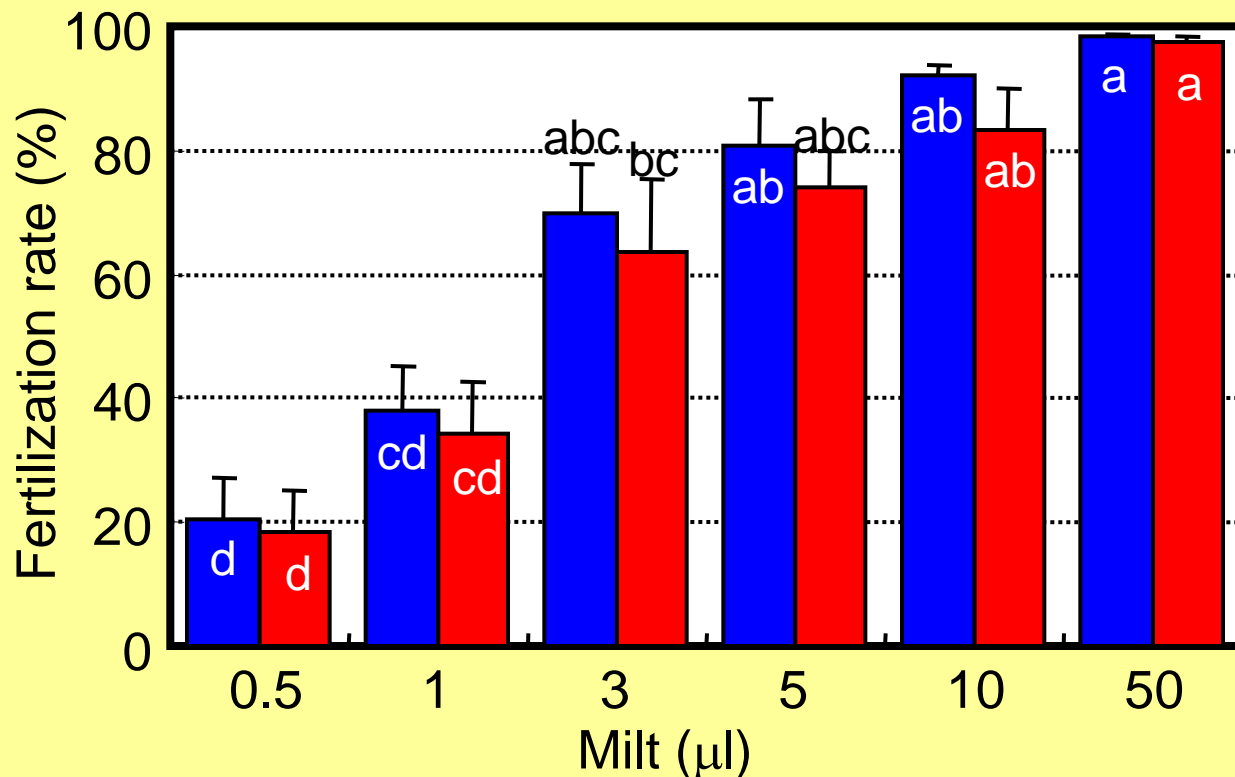
volume of milt to egg, efficacy of ASP,
incubation temperature, short term preservation,
and density of eggs.

Minimal Amount of Milt for Fertilization



■ Milt
■ Milt+ASP

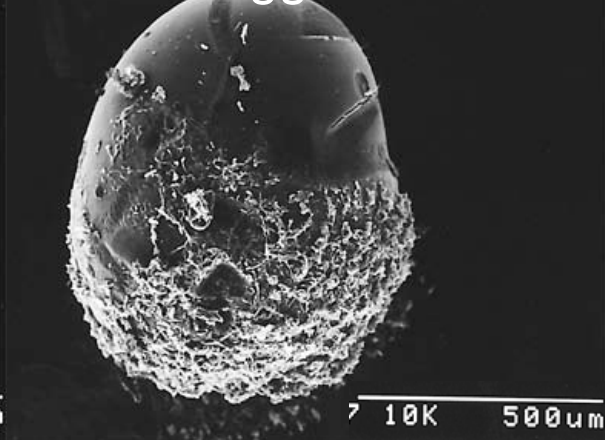
N = 5



Spermatozoa



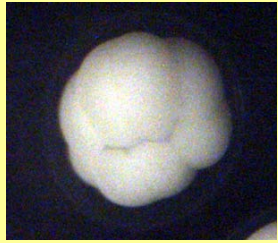
Matured egg



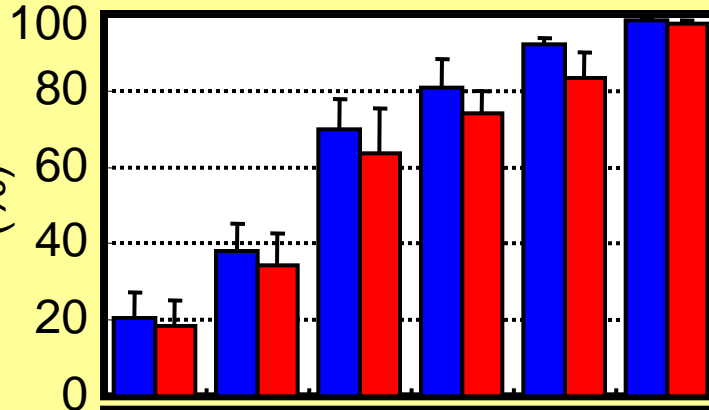
Egg batches of 1g



Hatching Success using Milt & Milt+ASP

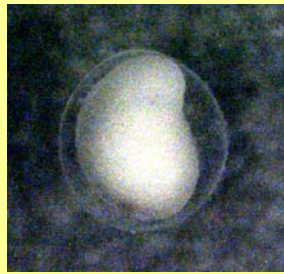


Fertilization rate (%)

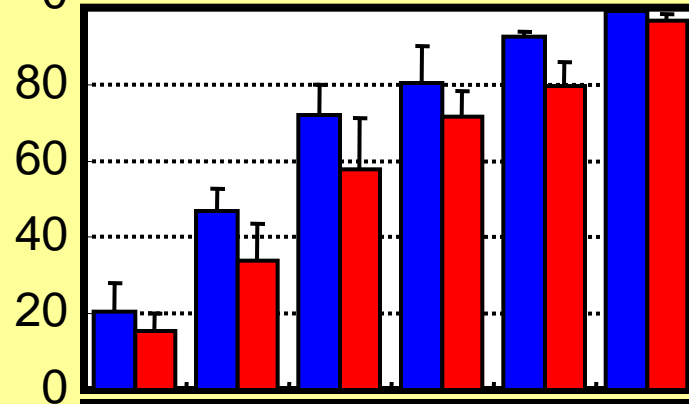


■ Milt
■ Milt+ASP

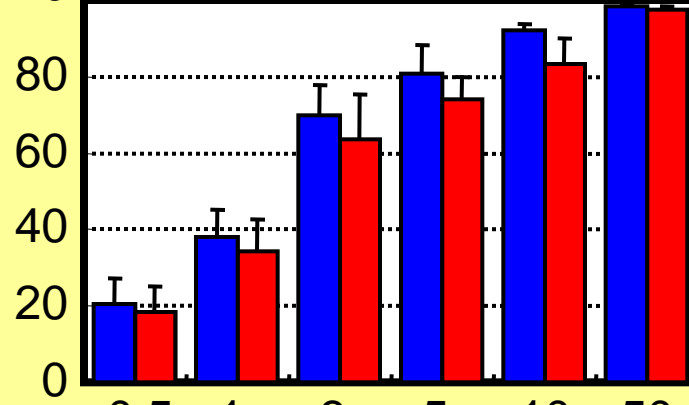
N = 5



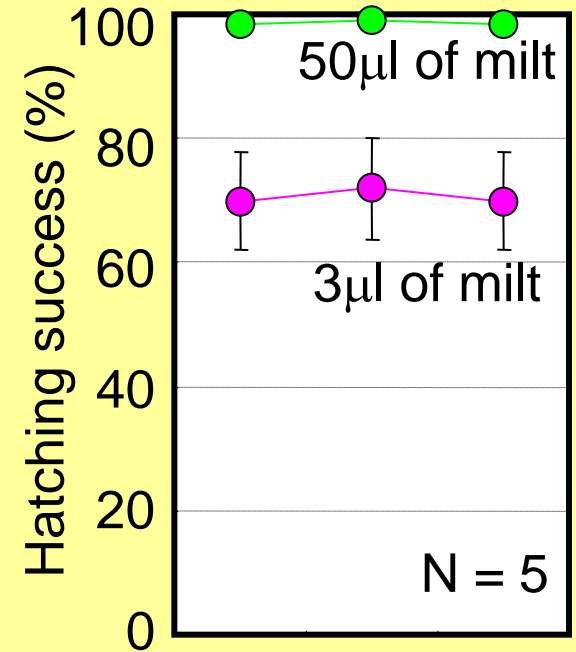
Embryo formation rate (%)



Hatching rate (%)



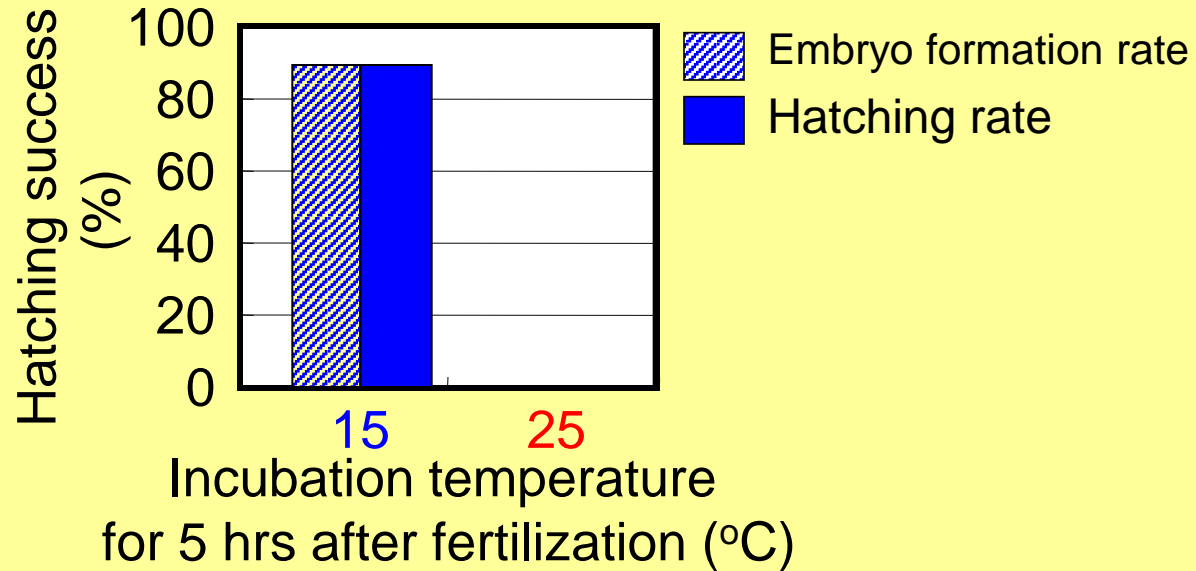
Milt (μl)



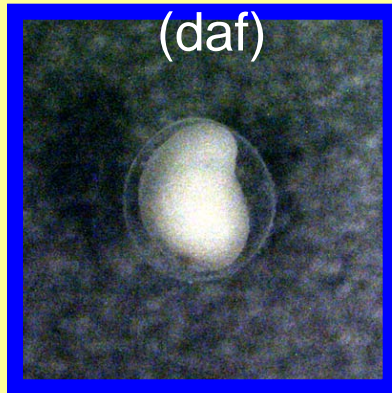
N = 5

Fertilization
Embryo formation
Hatching

Incubation Temperature



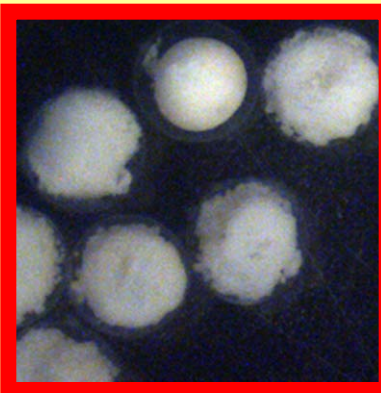
15°C
7days after fertilization



25°C → 15°C
1daf

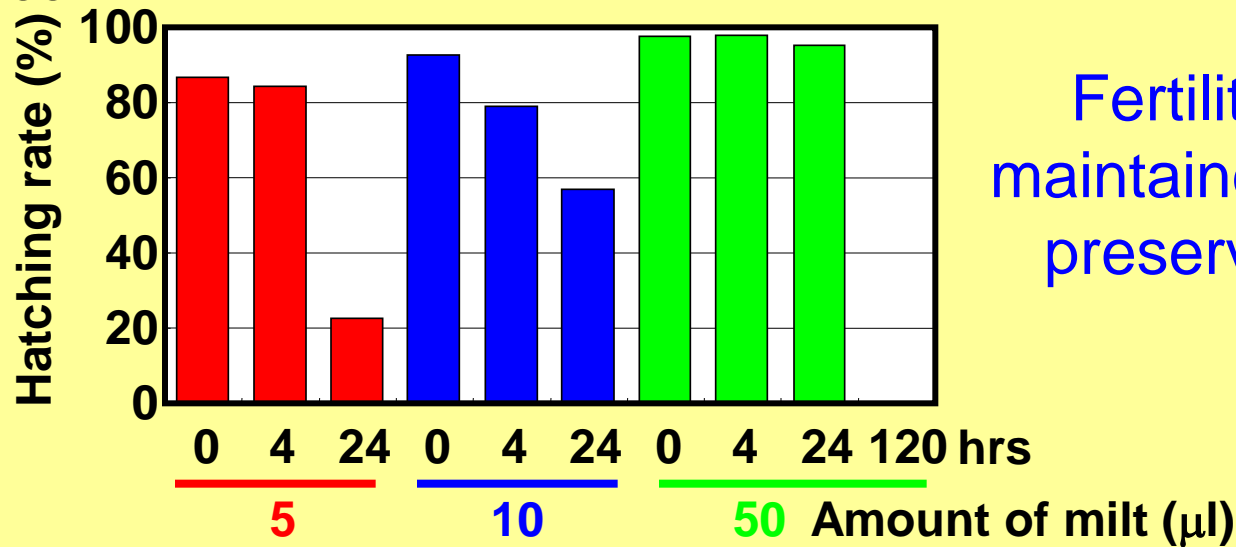


6daf



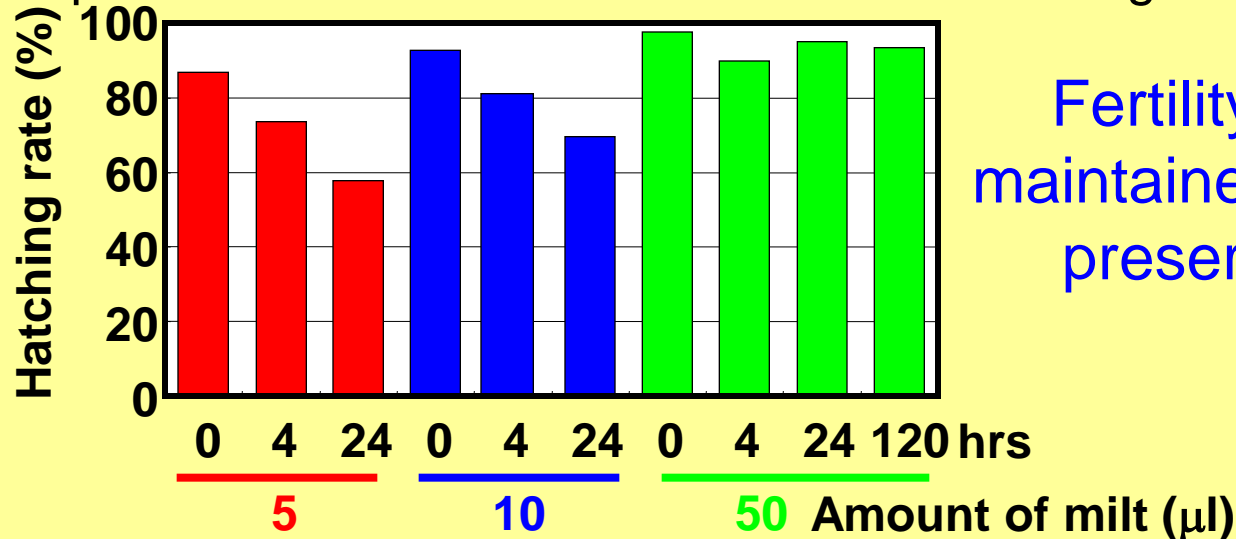
Short Term Preservation of Gametes

Egg preserved at 10 °C were fertilized with various quantities of fresh milt.



Fertility of eggs was maintained after 24 hrs of preservation at 10 °C.

milt preserved at 4 °C were each fertilized with 1 g of eggs.



Fertility of sperm was maintained after 120 hrs of preservation at 4 °C.

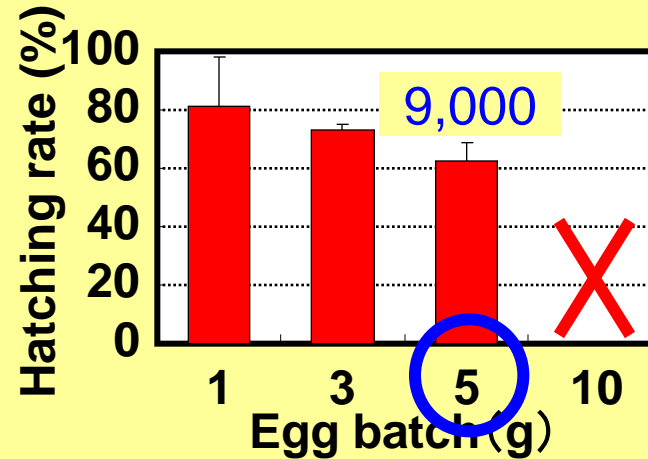
Density of Eggs in Container

Container of 2.4 L

A base area (BA) is
294 cm²



$$5\text{g} \div 294\text{cm}^2 \times 100 = 1.7\text{g}/100\text{cm}^2$$

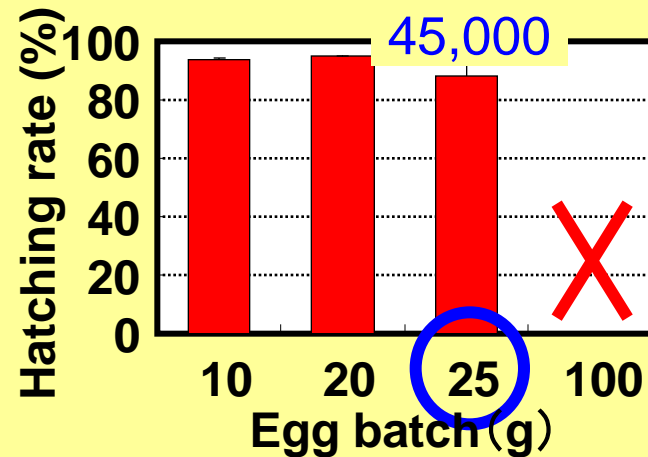


Container of 16 L

A BA is 1,350cm²
(4.6 times)



$$25\text{g} \div 1,350\text{cm}^2 \times 100 = 1.9\text{g}/100\text{cm}^2$$



Adequate density of eggs is about 2 g per BA 100cm²
(3,600 eggs)

Conclusion



• Spawning Behavior

Gravel is vital for spawning.

The spawning behavior was stimulated by coelomic fluid.

• Artificial Insemination and Egg Incubation

Matured female ovulated at 13°C for 3 days.

Egg batch with small volume of coelomic fluid and higher egg adhesion rates show higher hatching success.

Fifty μl of milt is necessary and sufficient in order to fertilize 1g of eggs (i.e., egg batches obtained from 2 female X milt obtained from 2-3 male).

Hatching success did not improve using the mixed milt with ASP.

Incubation temperature of fertilized eggs may be optimum at 10-18 °C.

Eggs preserved for 24 hrs at 10 °C and milt preserved for 120 hrs at 4 °C maintained fertility.

Adequate egg density is about 2 g / 100 cm² container base area.

Mass production of larvae was possible using these criteria and recommended conditions.

• Future Directions for Artificial Propagation

We should understand the structure and the function of ammocoete habitats and conserve the habitat of ammocoetes.

Riverine Lagoon like this is found in other rivers as well



Collaborators

Noboru Kasahara ¹

Kouji Araya ¹

Hajime Omori ^{1·2}

Yoshitaka Sasaki ^{1·2}

Katsumi Takeuchi ^{1·2}

1; Hokkaido Fish Hatchery

2; Salmon and Freshwater Fisheries
Research Institute

Cooperators

Ebetsu Fisheries Cooperative Association

Assabu River Resource Conservation Council

Setana-gune Freshwater Fisheries Cooperative
Association

Ralph Lampman

Thank you for listening