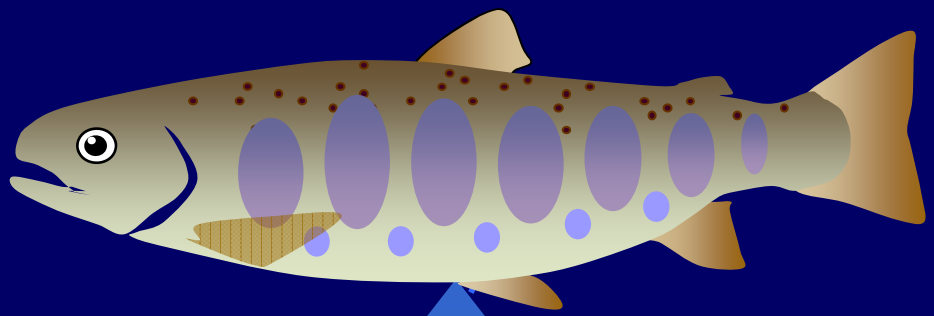
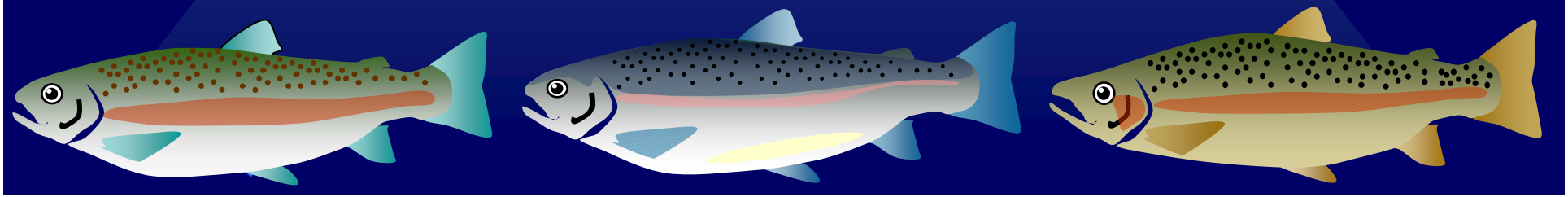
Larvi 2009



Germ Cell Transplantation in Fish

Goro Yoshizaki

(Tokyo University of Marine Science and Technology, SORST/JST)

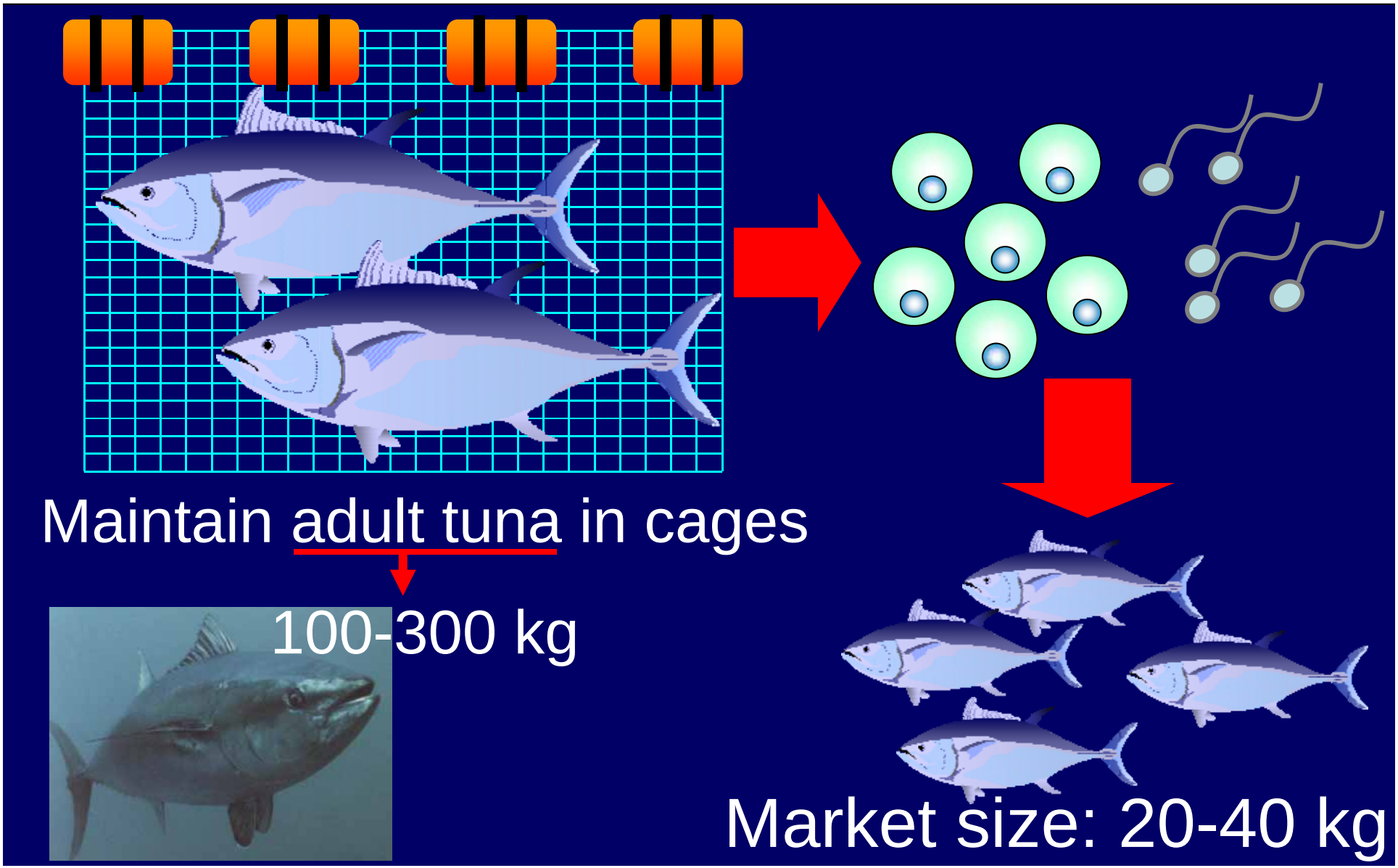


	Tuna	Mackerel
Body weight;	300 kg	300 g
Body length;	3 m	30 cm

Scombridae family

Aquaculture of tuna

***conventional aquaculture is capture-base**

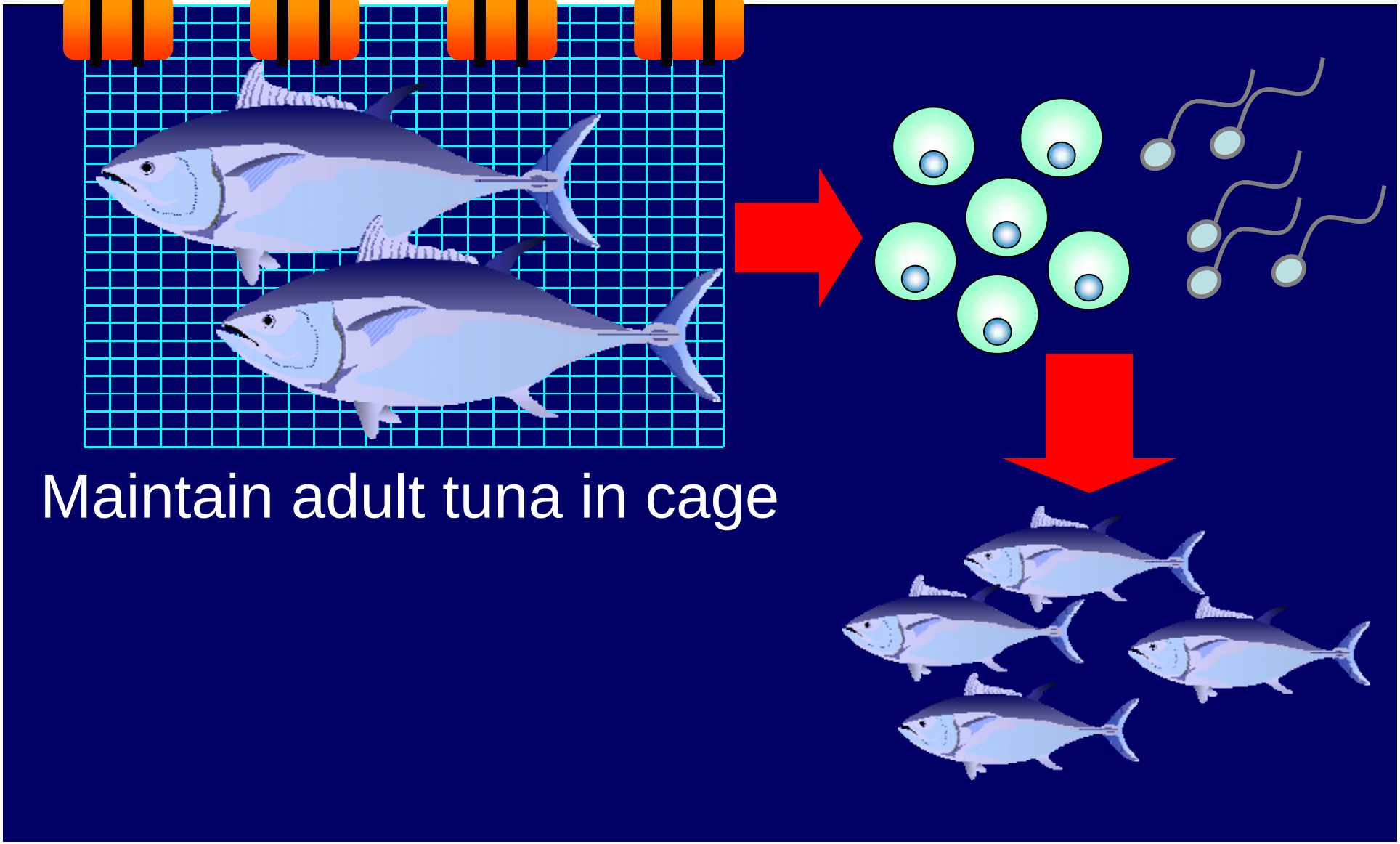


Maintenance of adult tuna requires a lot of space, cost, and labor

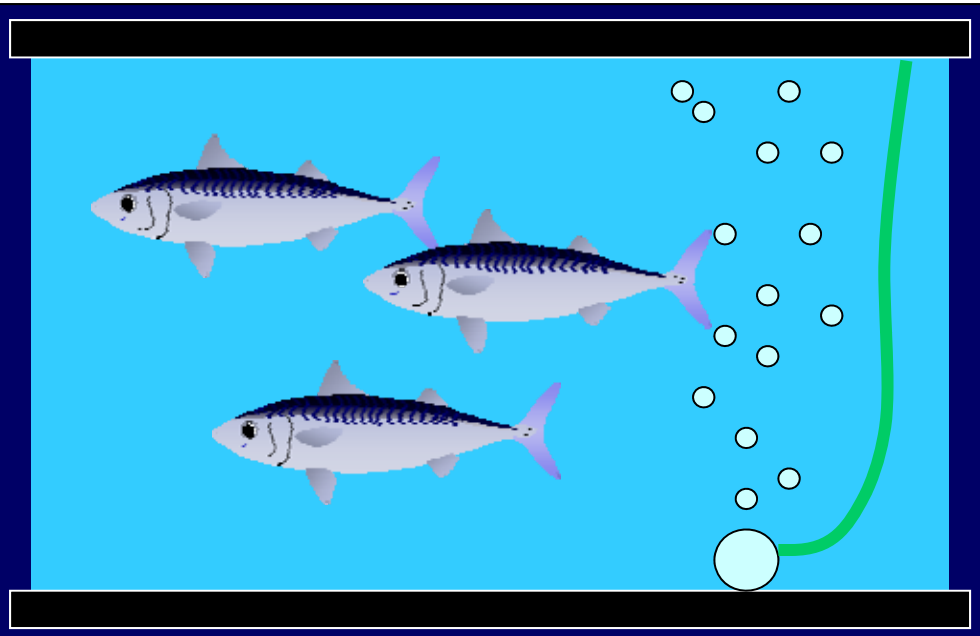
Mackerel can spawn in a small fish tank

If we can obtain mackerel that produce tuna gametes

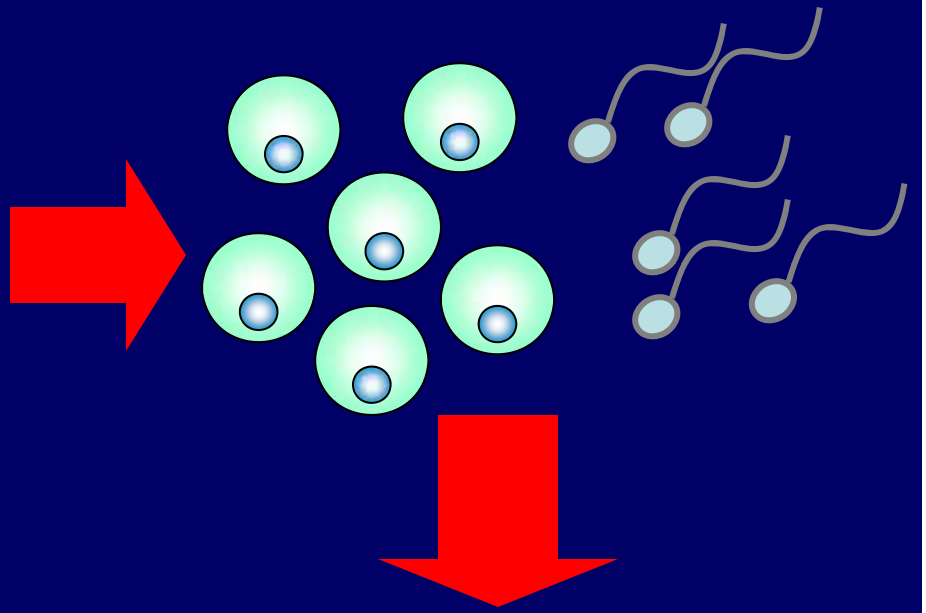
Aquaculture of tuna



Mackerel as surrogated broodstock

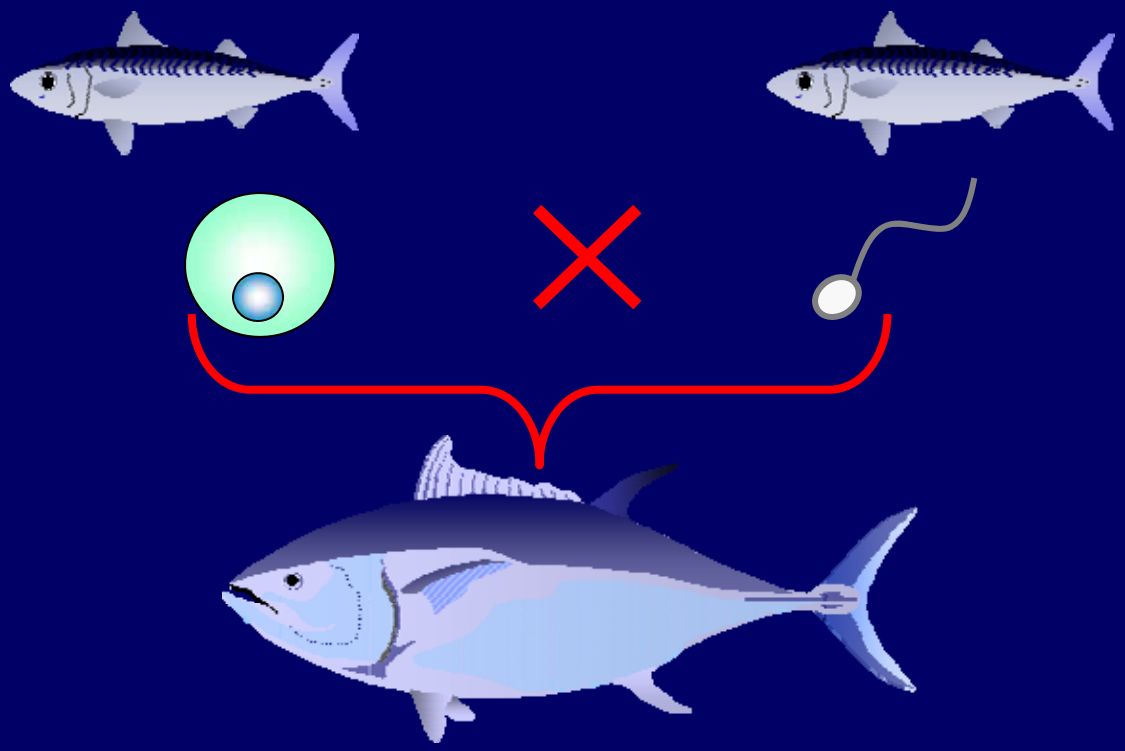


Egg taking in small tanks



*save a lot of space, labor, and cost.

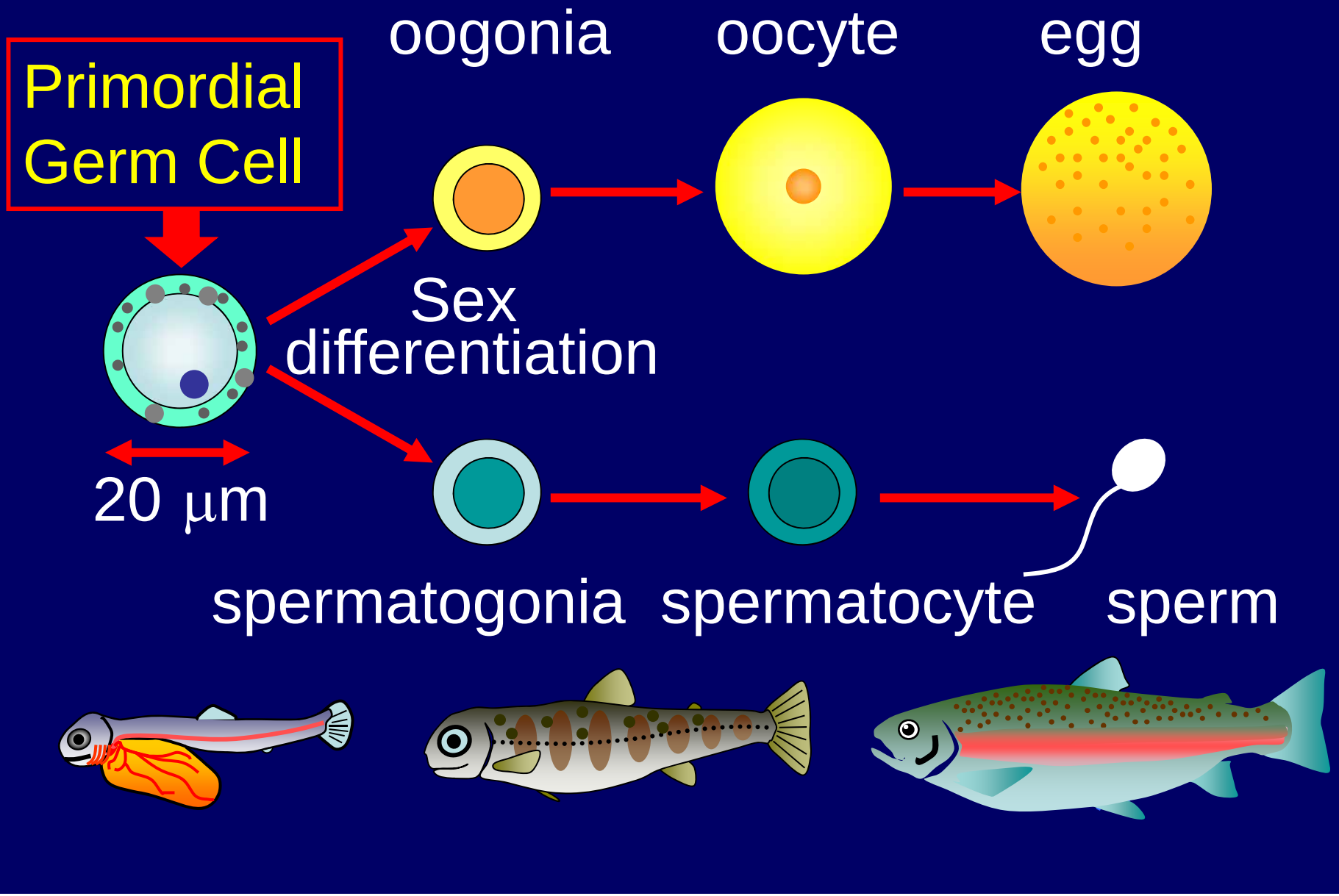
*environmental manipulation and hormonal treatment can be easily performed.

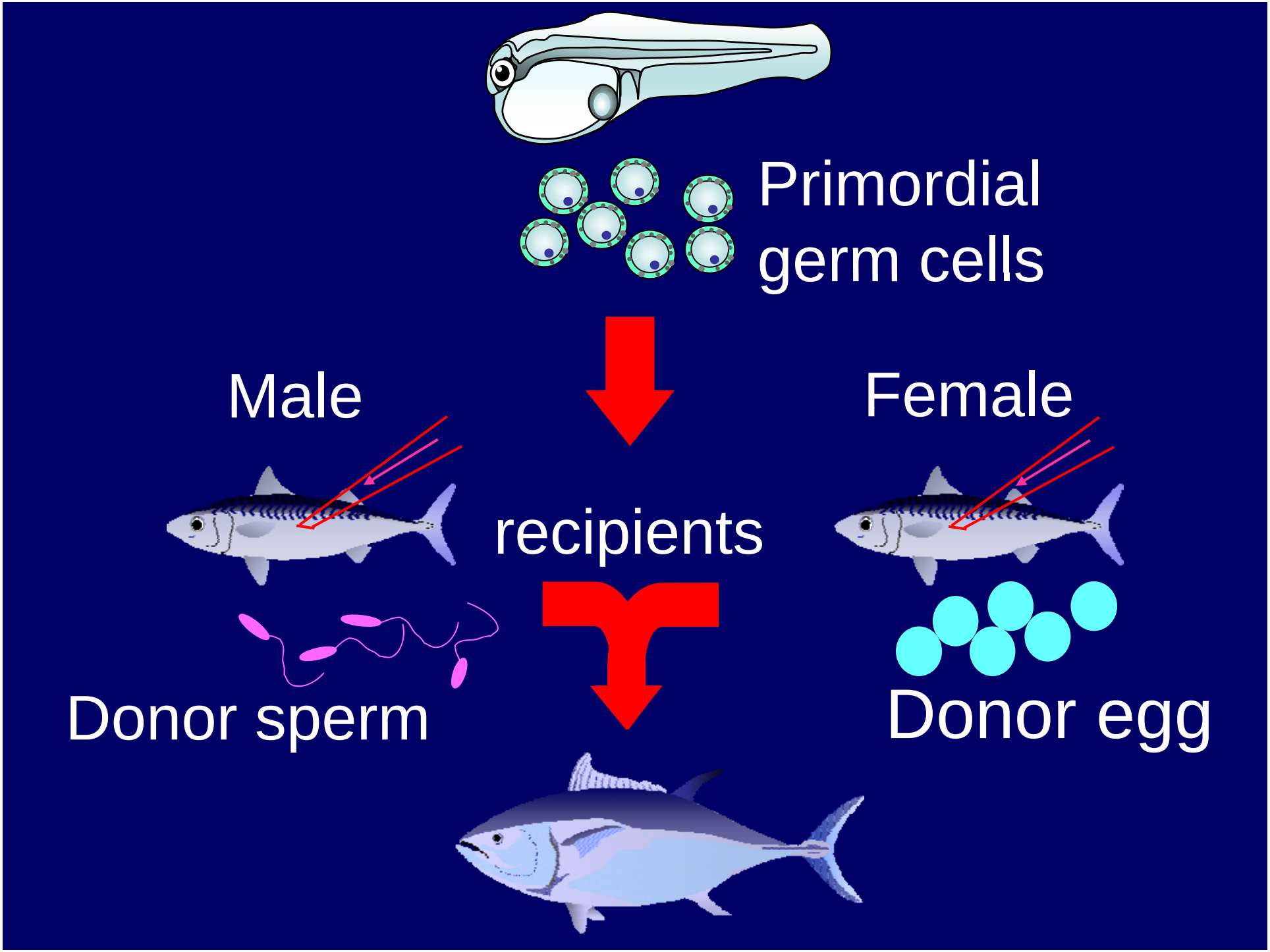


How can we obtain mackerel that can produce tuna eggs and sperm???

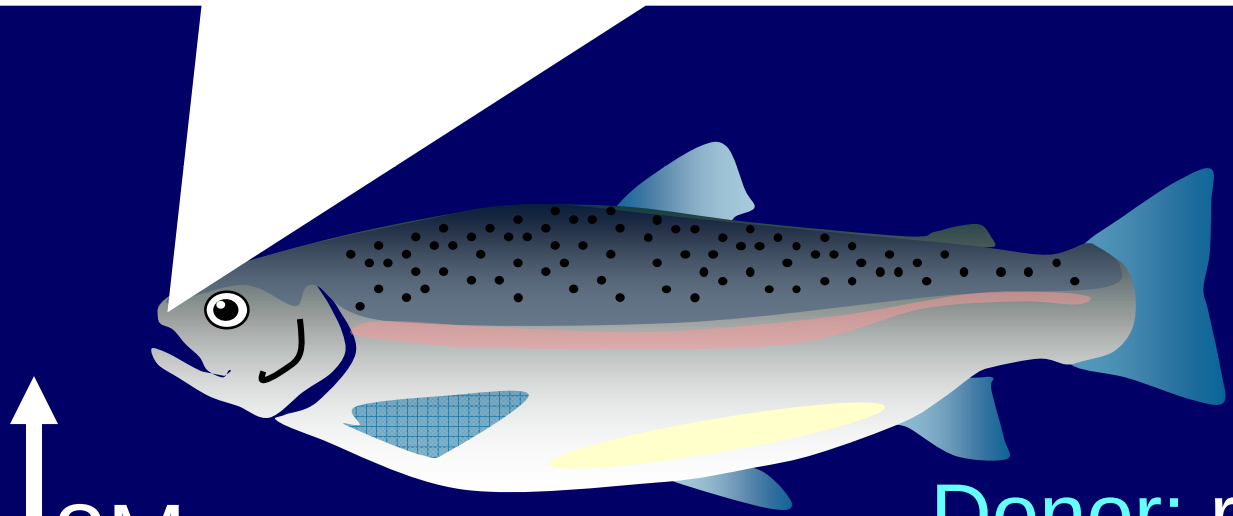
1. Isolate the progenitor cells of eggs and sperm from tuna.
2. Transplant them into recipient mackerel.

What are the progenitor cells of eggs and sperm?



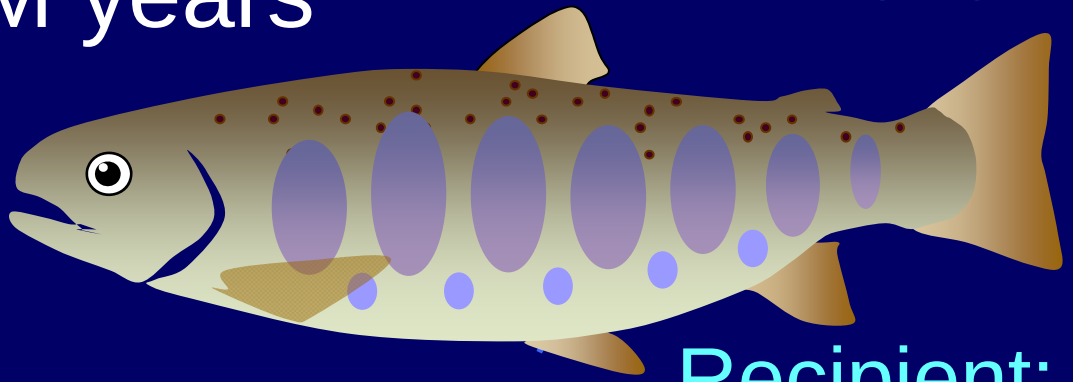

Primordial
germ cells
Male
Female
recipients
Donor sperm
Donor egg

How can we transplant primordial germ cells into recipients?

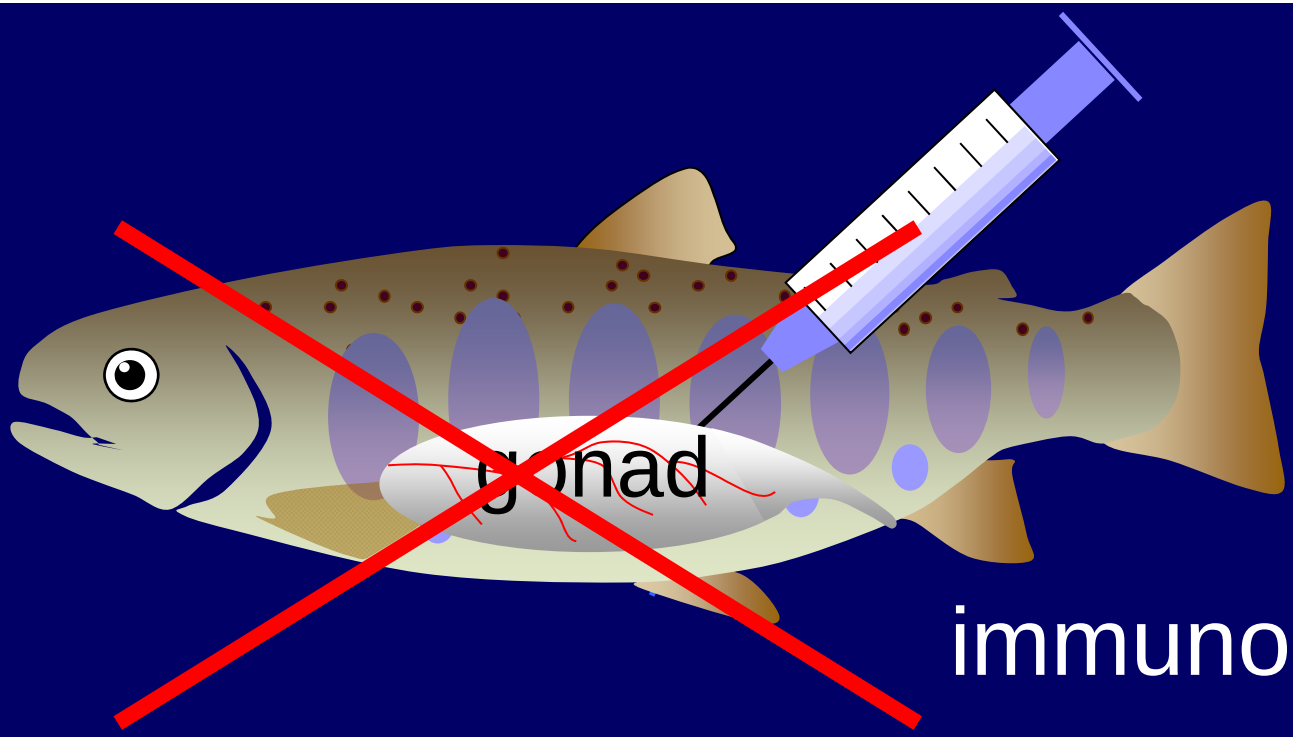


8M years

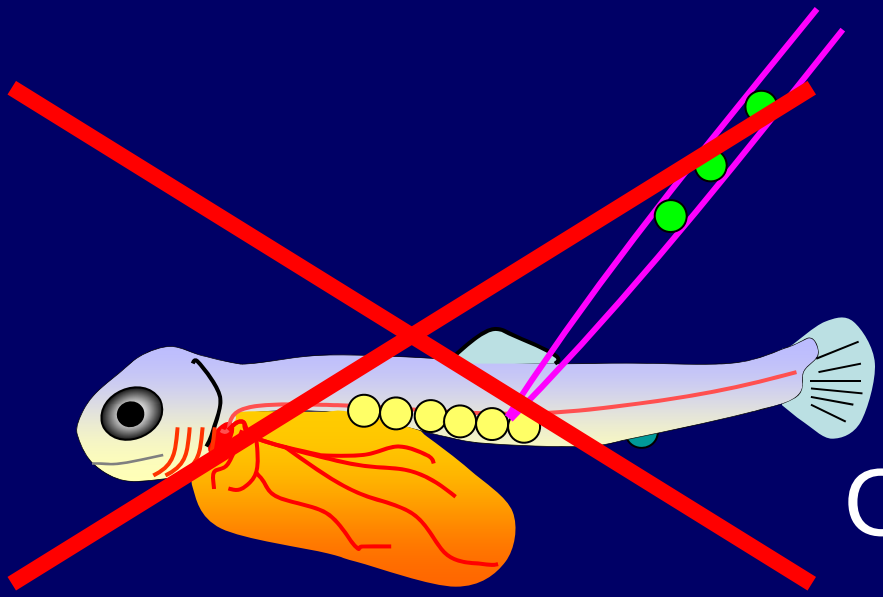
Donor: rainbow trout



Recipient: masu salmon



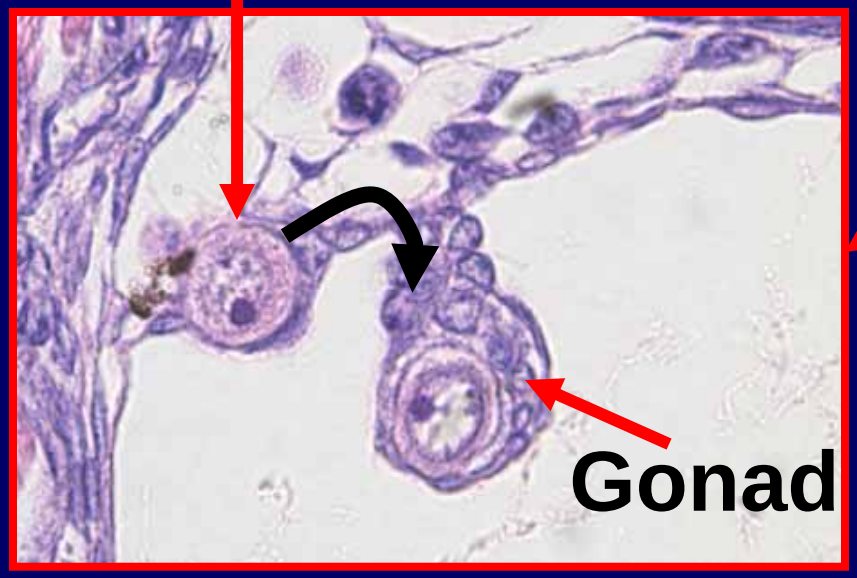
immunorejection

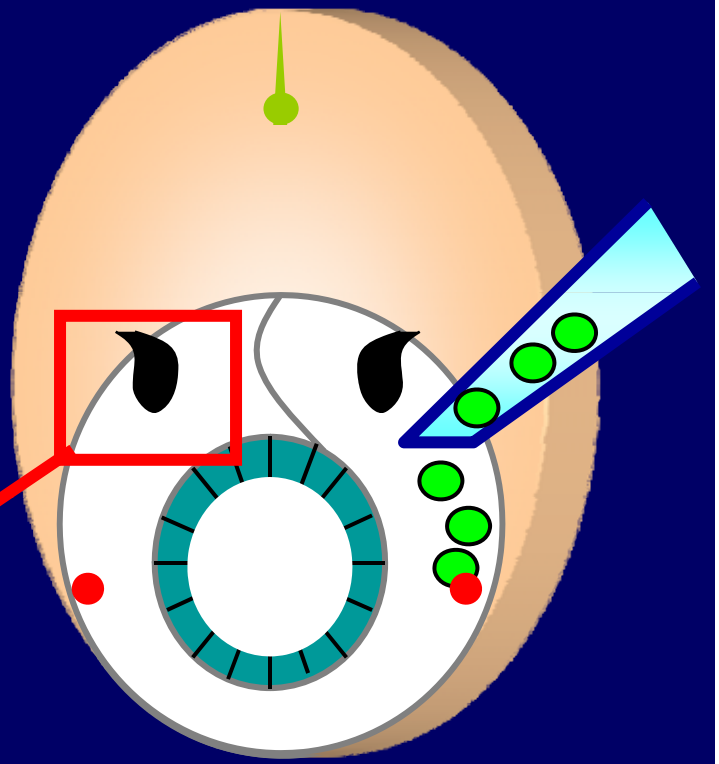


Gonads are too small

Migration of Primordial Germ Cells

Migrating primordial germ cells

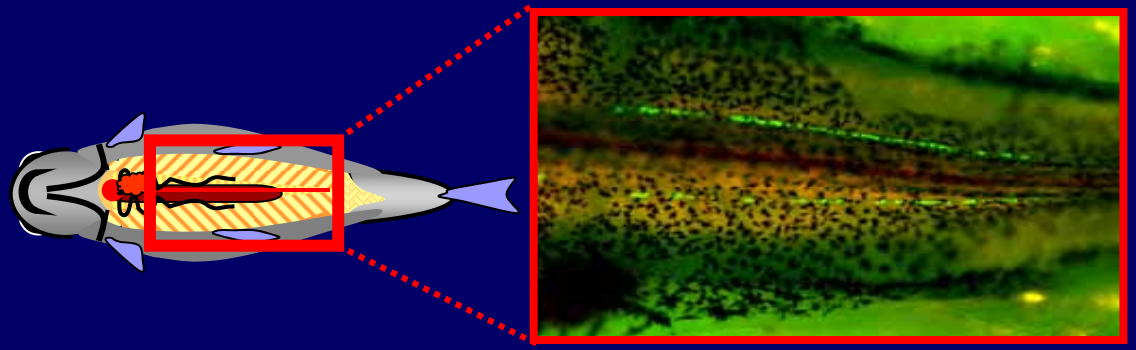




Cross section of a hatchling

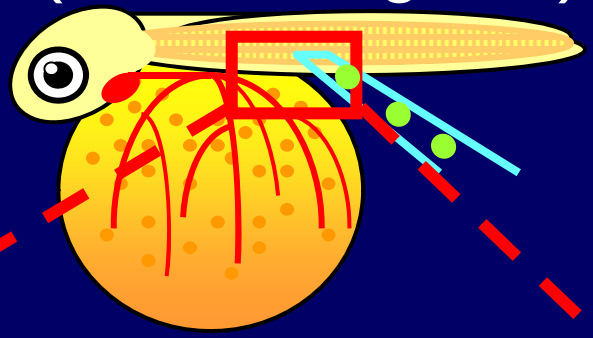
Primordial germ cells emerge in extragonadal areas and then migrate to the immature gonads

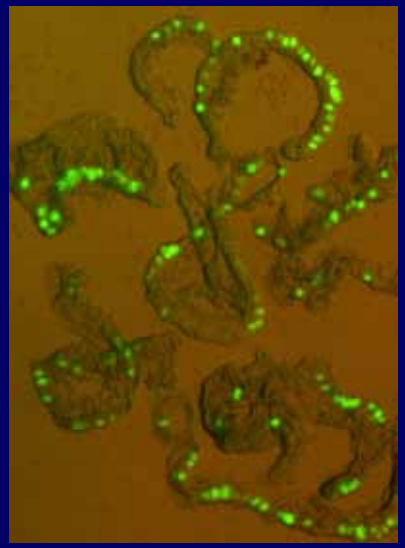
PGCs Transplantation into the Peritoneal Cavity of Hatched Embryos

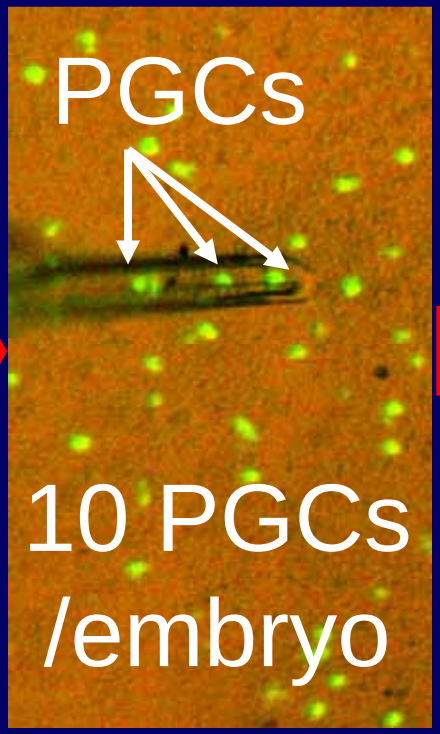


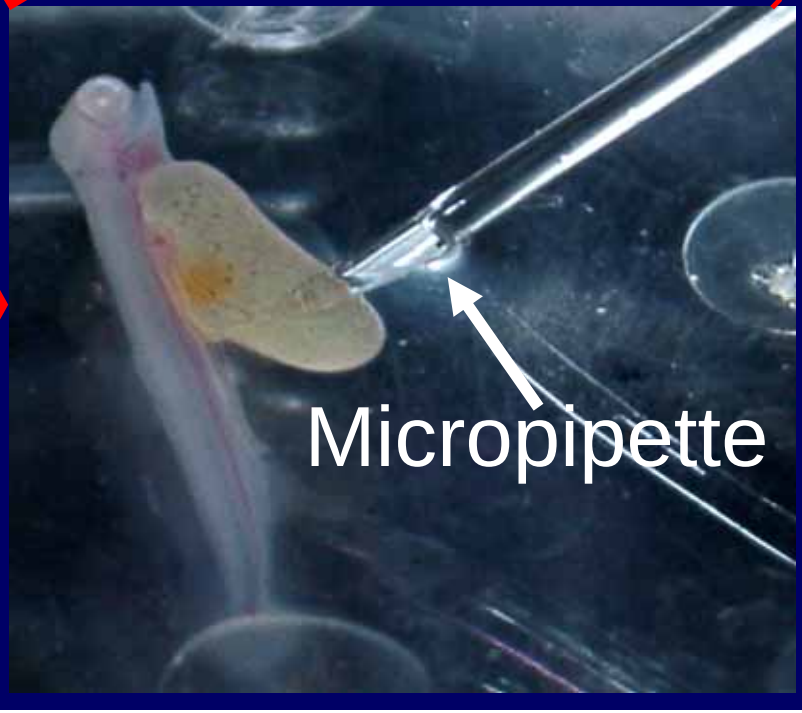
vasa-Gfp rainbow trout

Xenogenic recipient (non-transgenic)

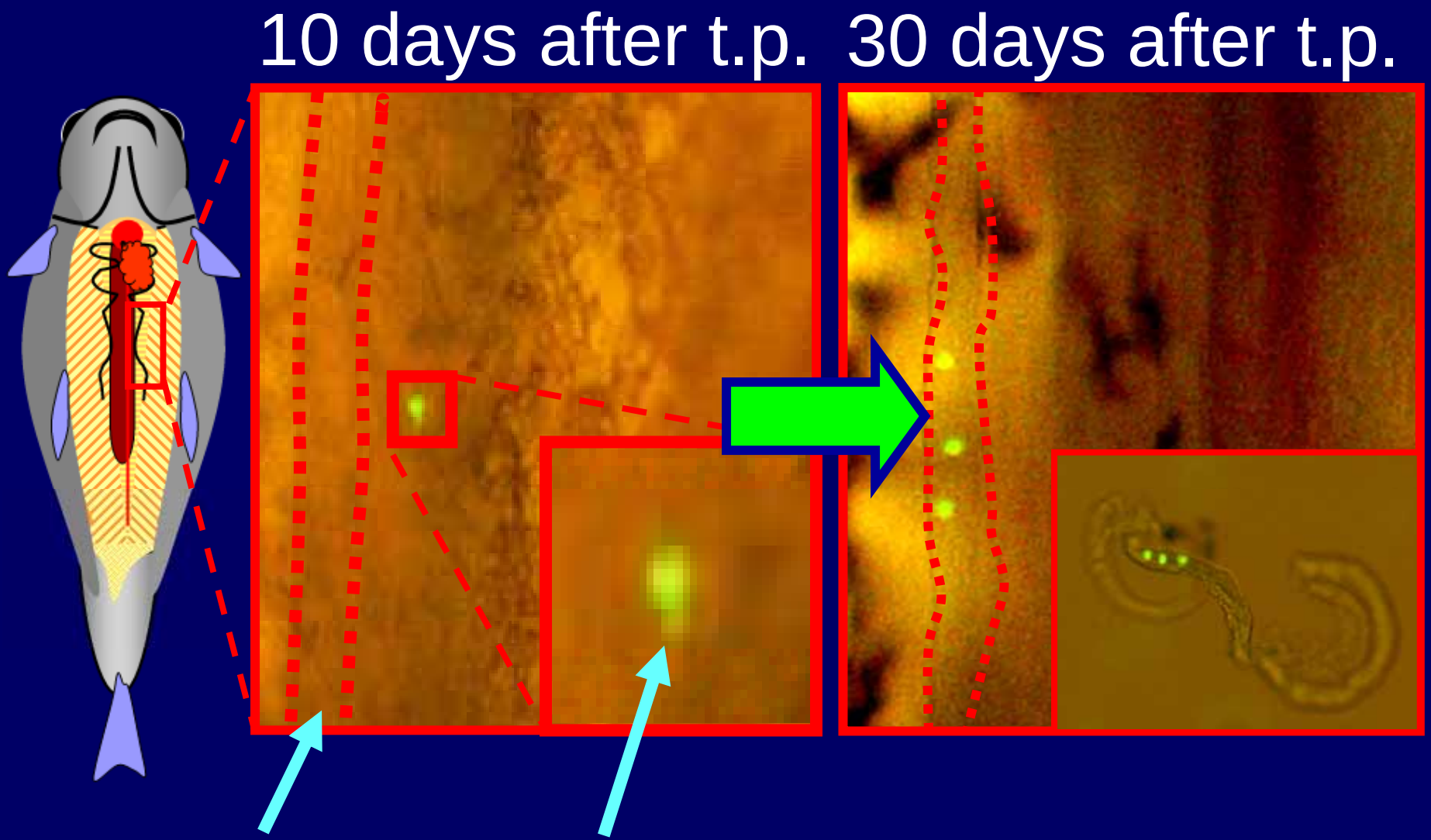




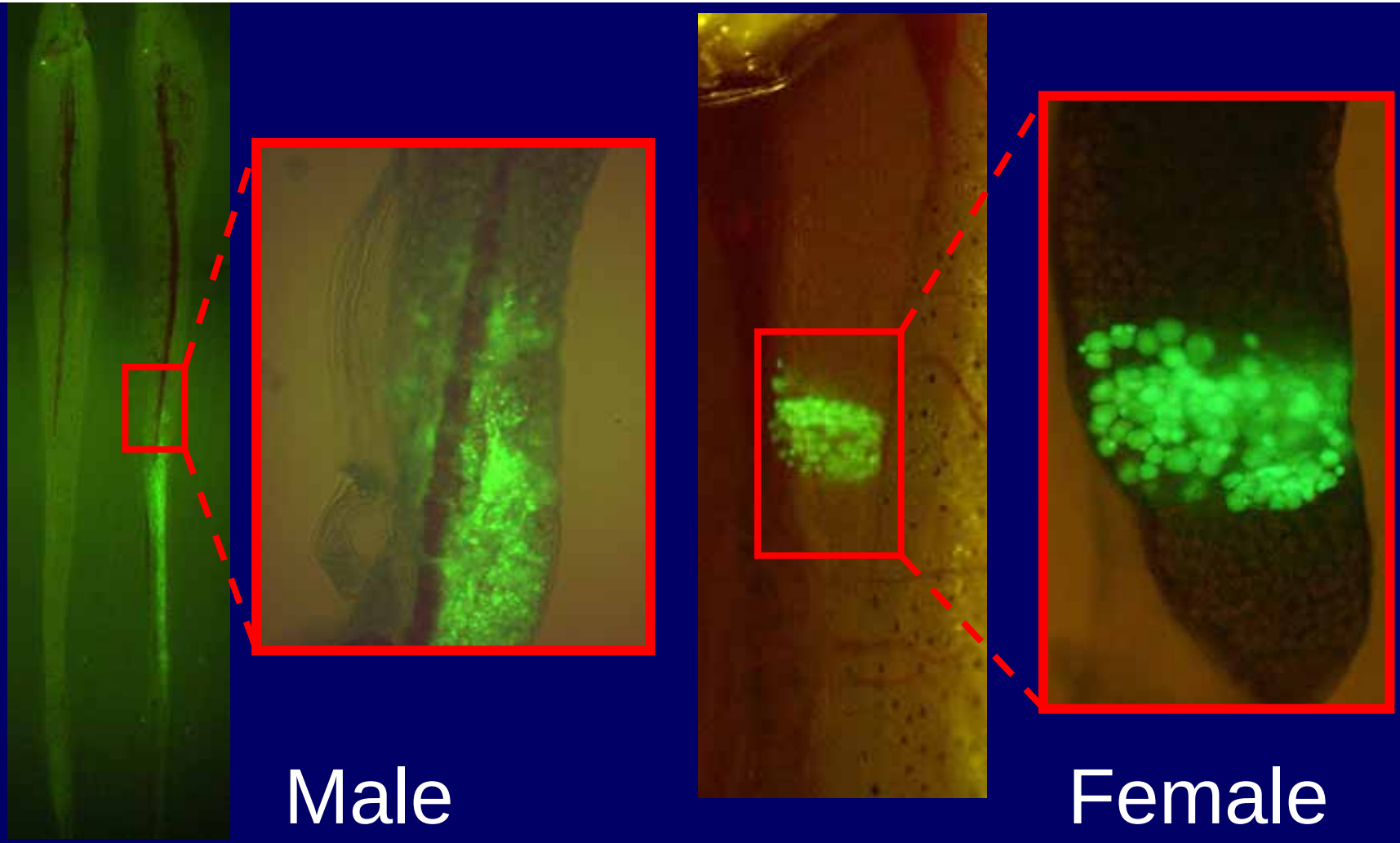




Behavior of Transplanted PGCs in Recipient Fish

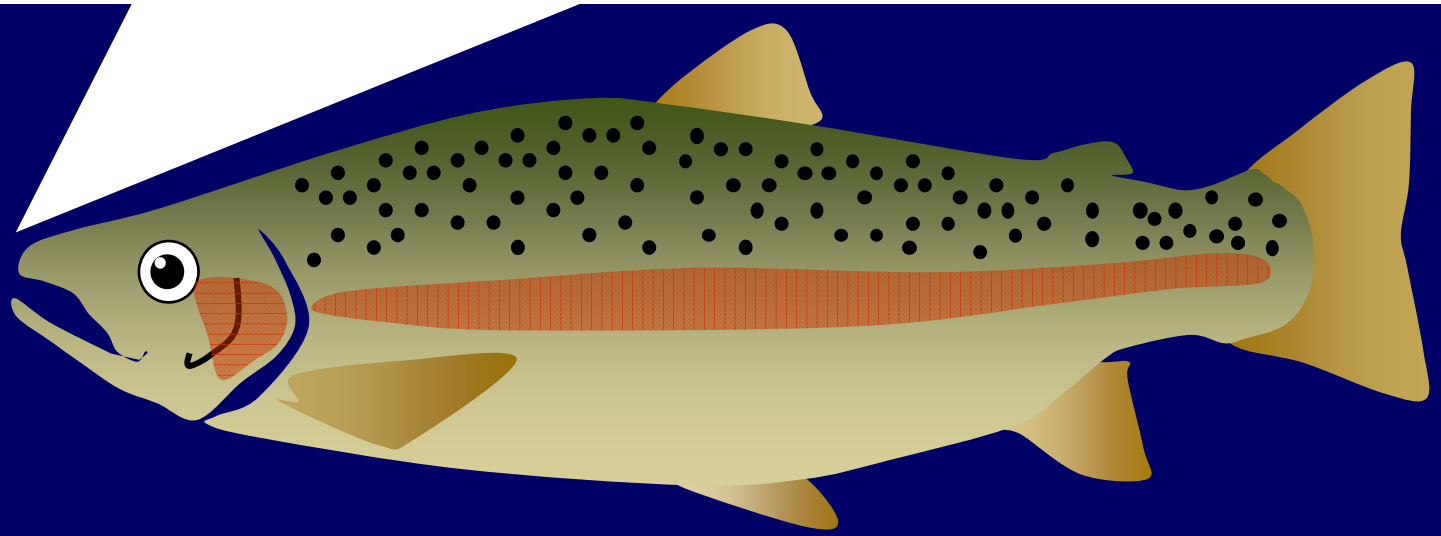


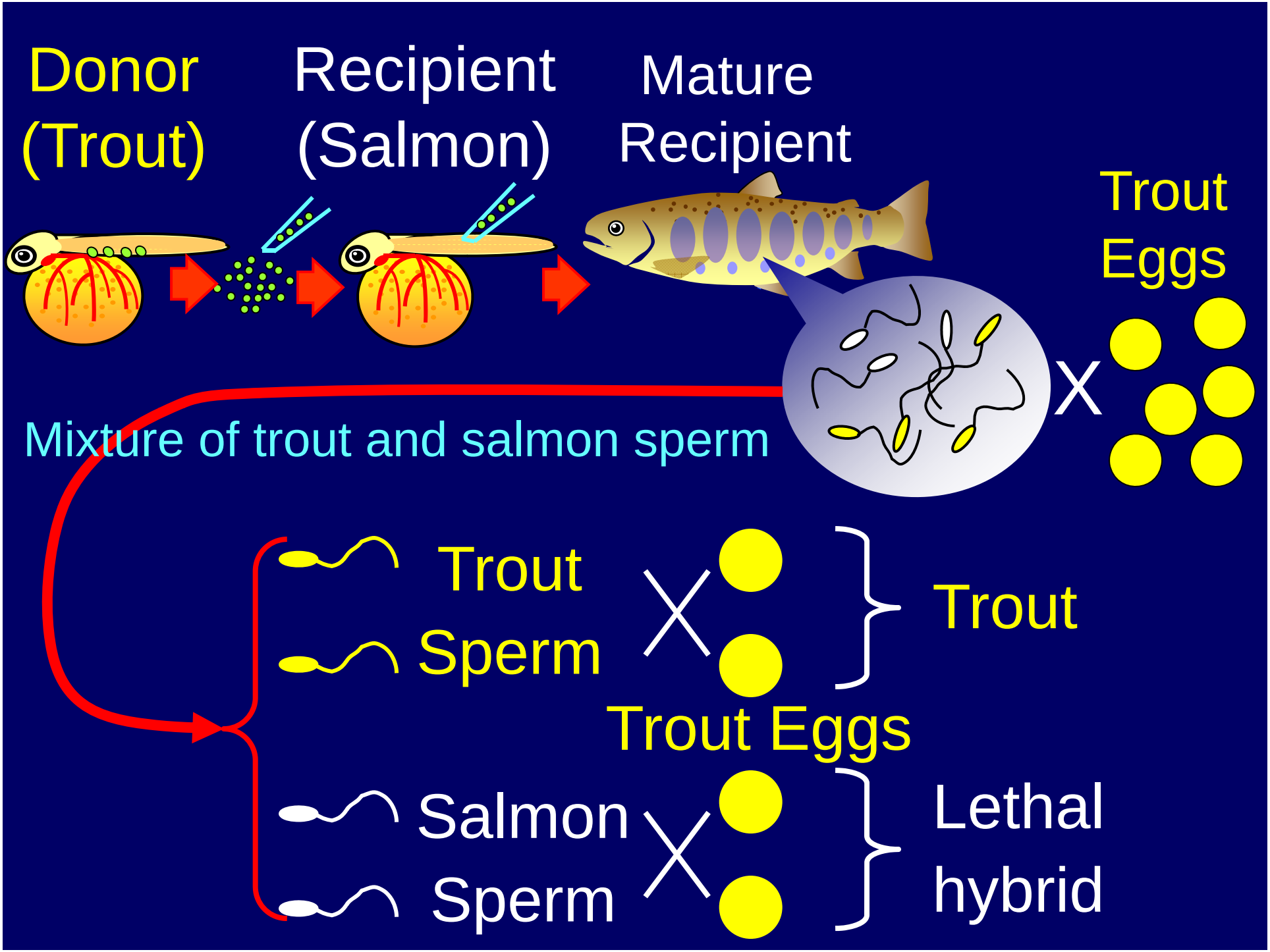
Takeuchi et al., Biol Reprod, 2003



Transplanted primordial germ cells proliferated and started meiosis in gonads of xenogeneic recipients

Can the transplanted PGCs differentiate into functional gametes in the gonads of xenogenic recipients?



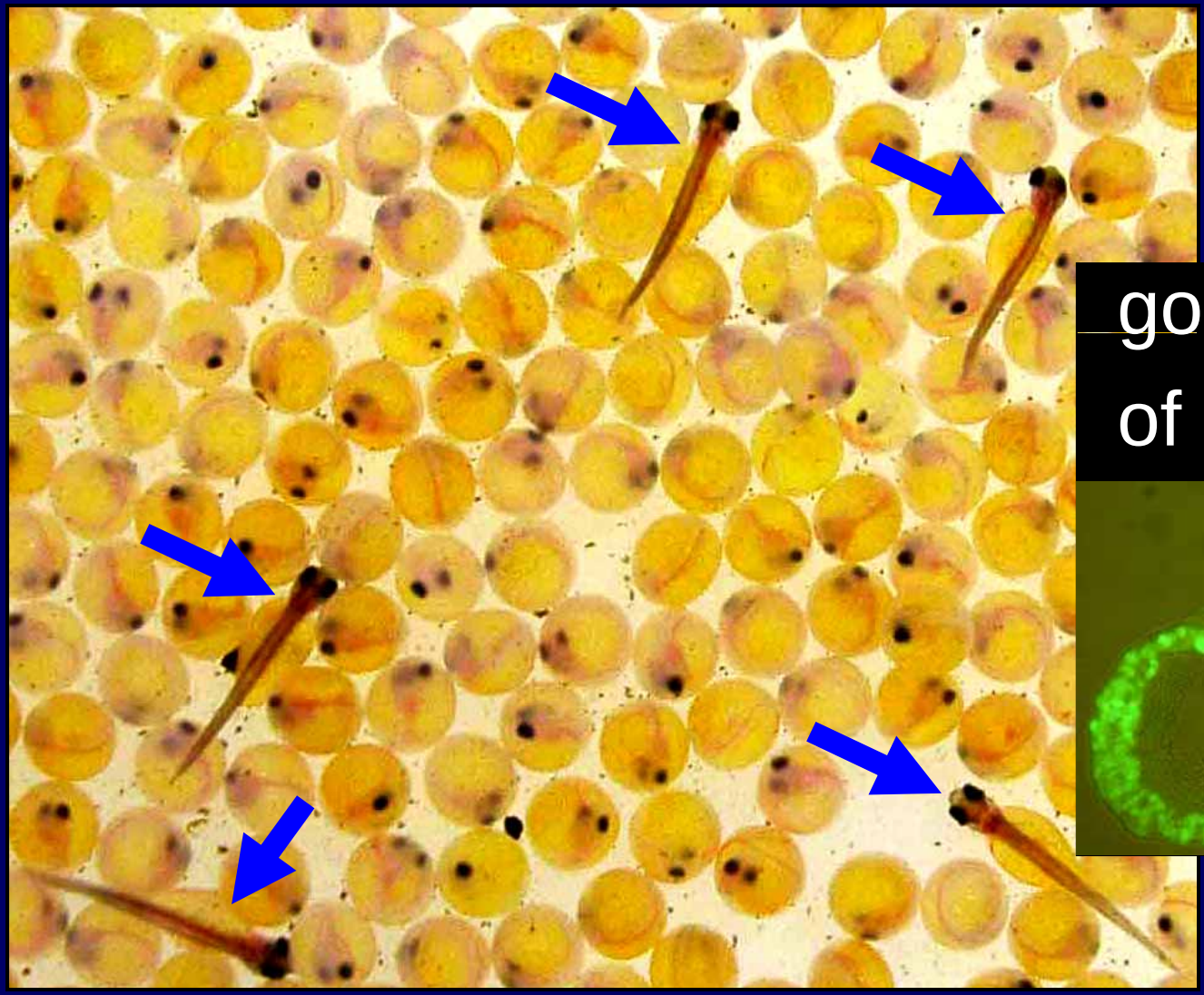

Donor
(Trout)
Recipient
(Salmon)
Mature
Recipient
Trout
Eggs
X
Mixture of trout and salmon sperm
Trout
Sperm
Trout
Trout Eggs
Salmon
Sperm
Lethal
hybrid

34 days post-fertilization at 10°C

Trout embryos

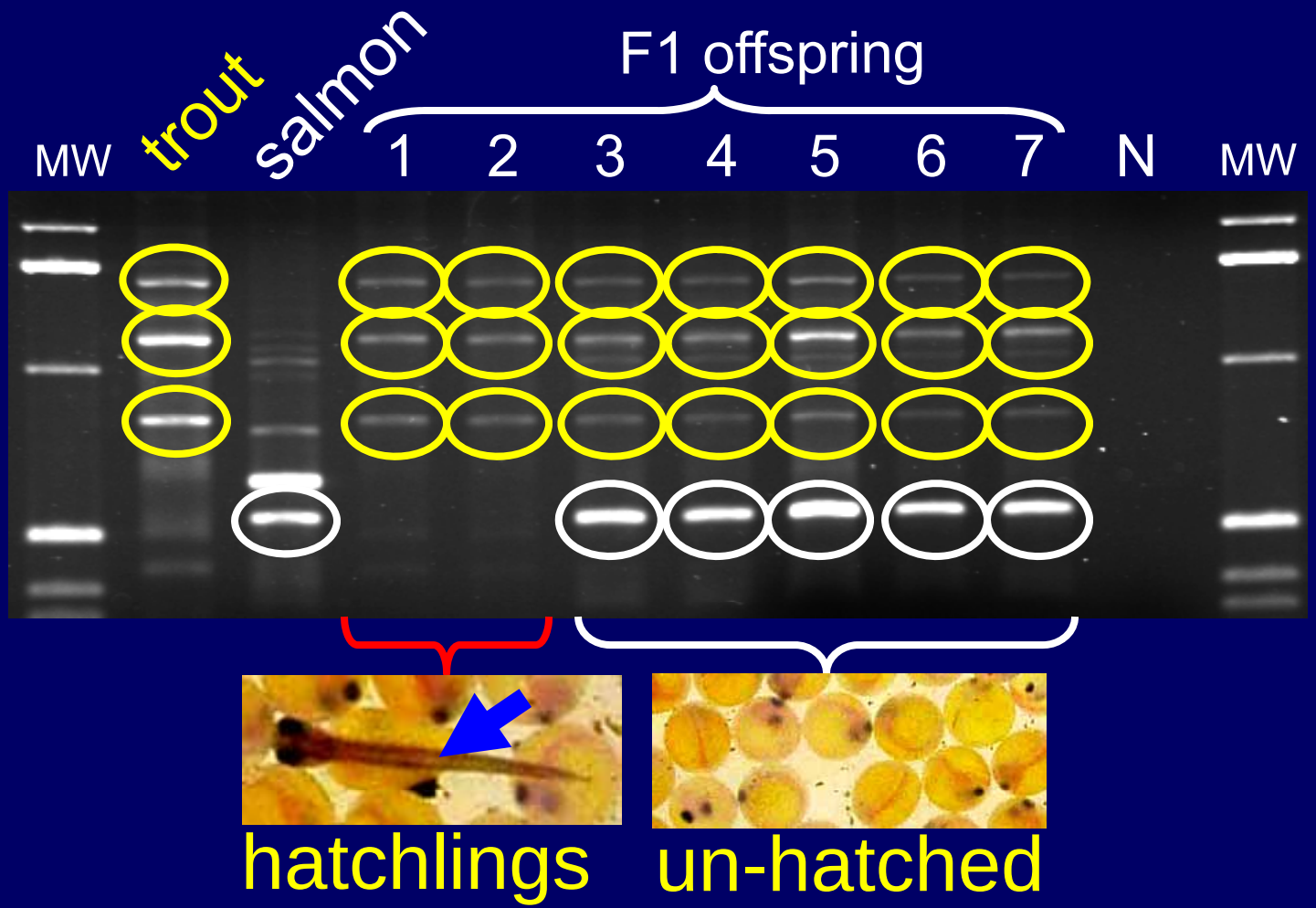
Trout/salmon hybrid

F1 embryos (34 dpf) derived from recipient salmon



gonad
of hatchlings

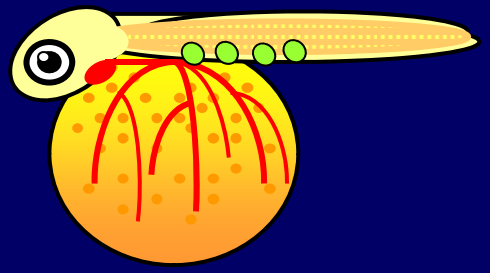
Takeuchi et al., Nature, 2004



Takeuchi et al., Nature, 2004

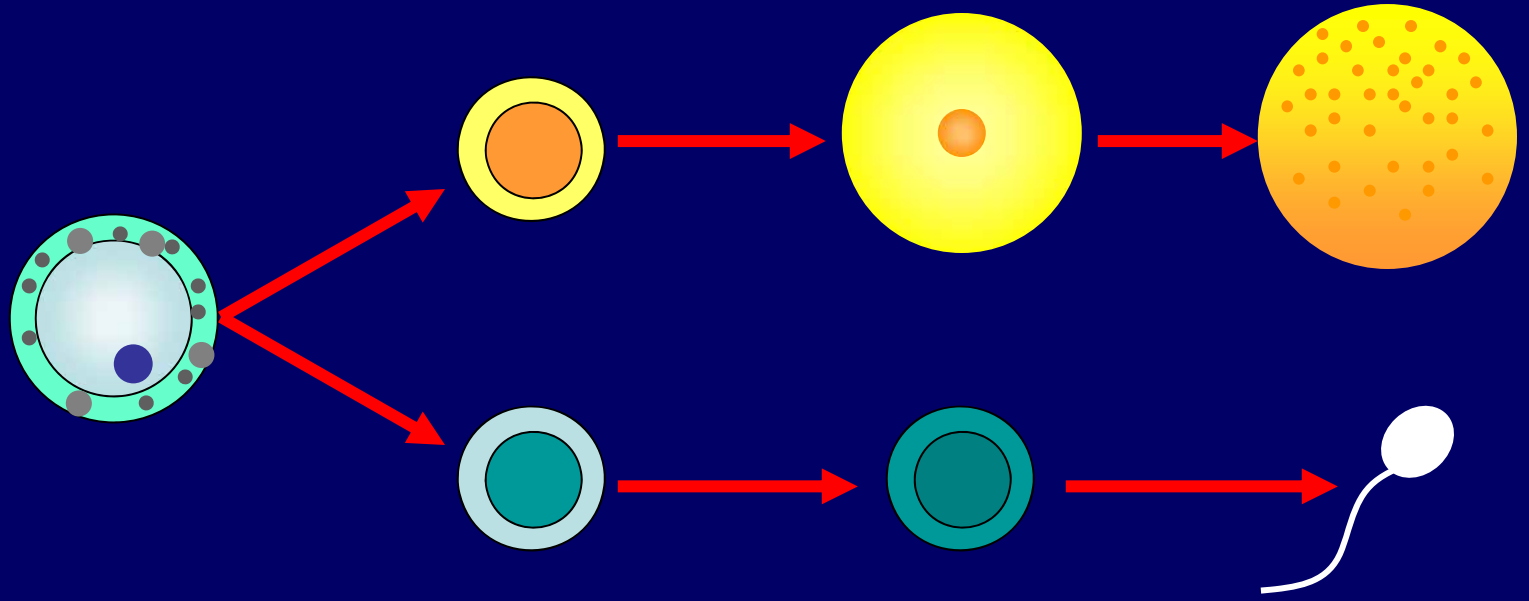
Donor-derived offspring from inter-species germ-cell transplantation were successfully produced

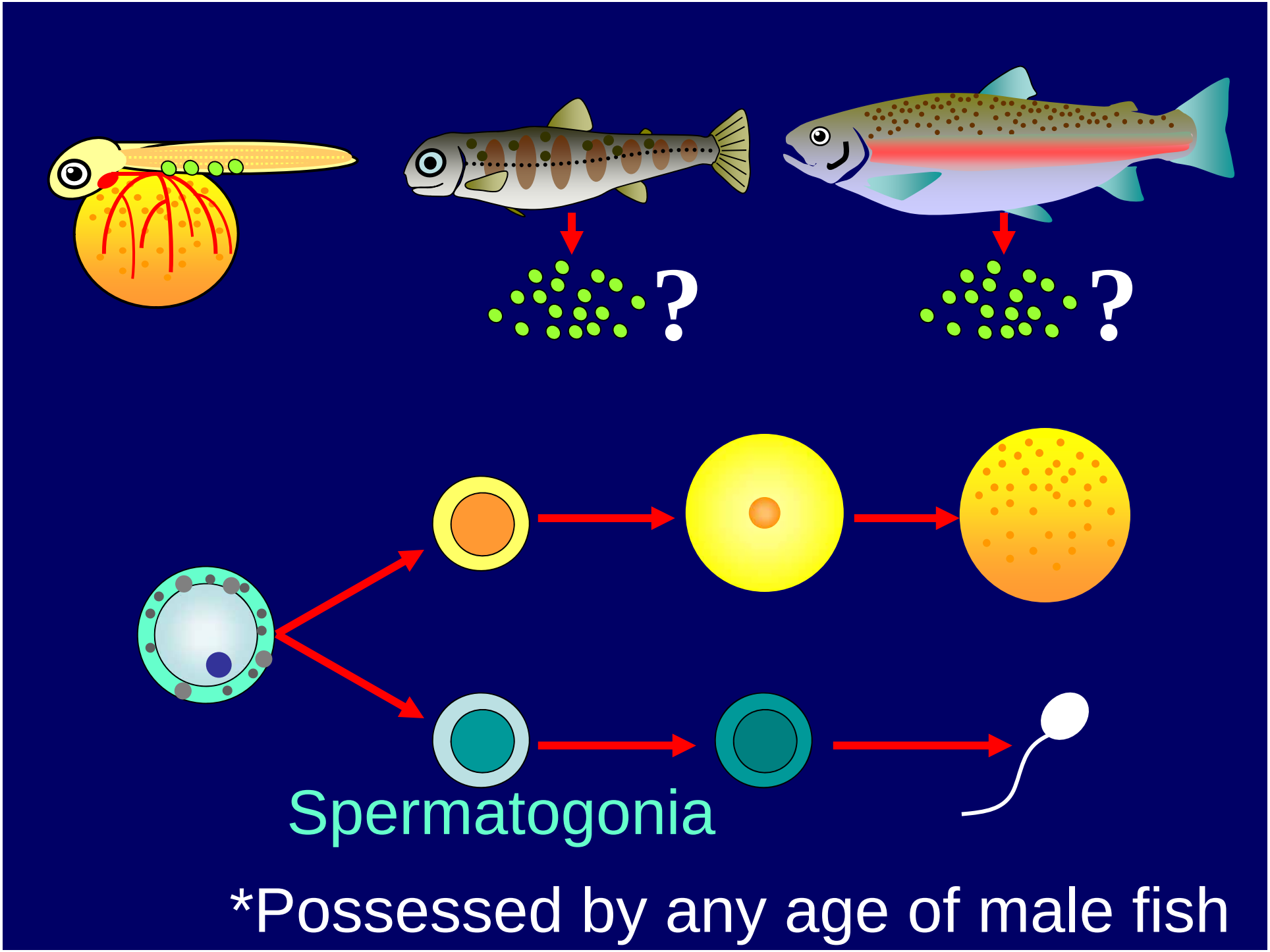
Takeuchi et al., Nature, 2004



1)Number of PGCs is limited (20-30 from one embryo)

2)Difficult to capture newly hatched embryos of commercially important species

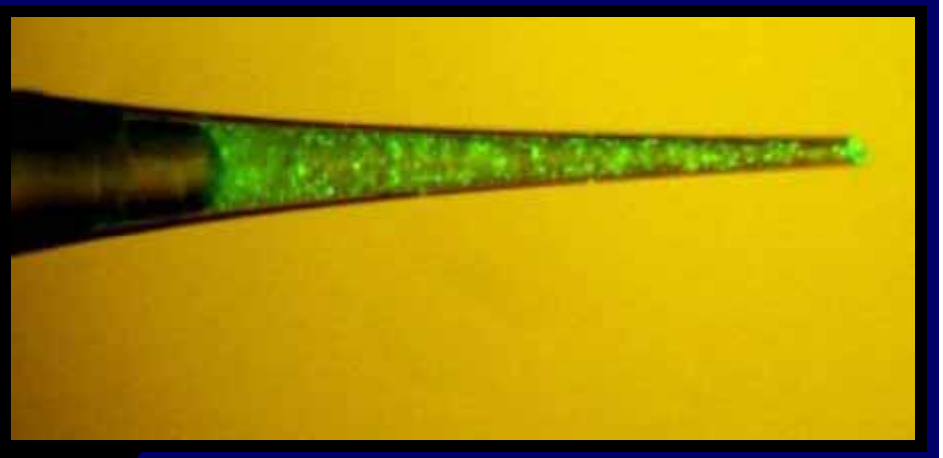


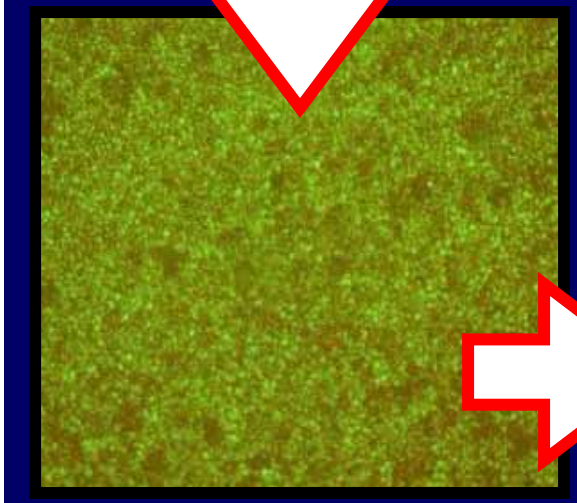


*Possessed by any age of male fish

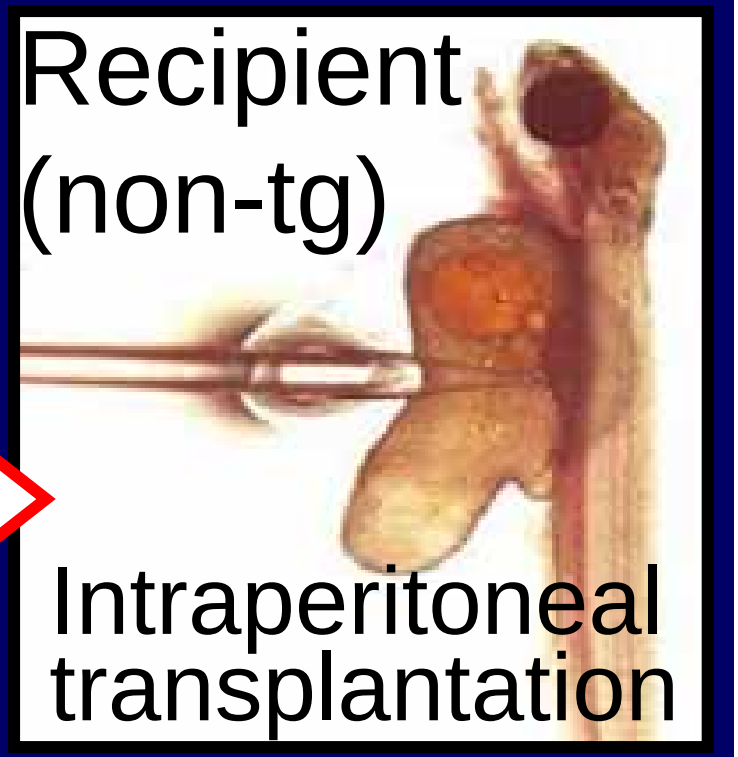
Intraperitoneal Transplantation of Testicular Germ Cells

Testes of vasa-GFP trout





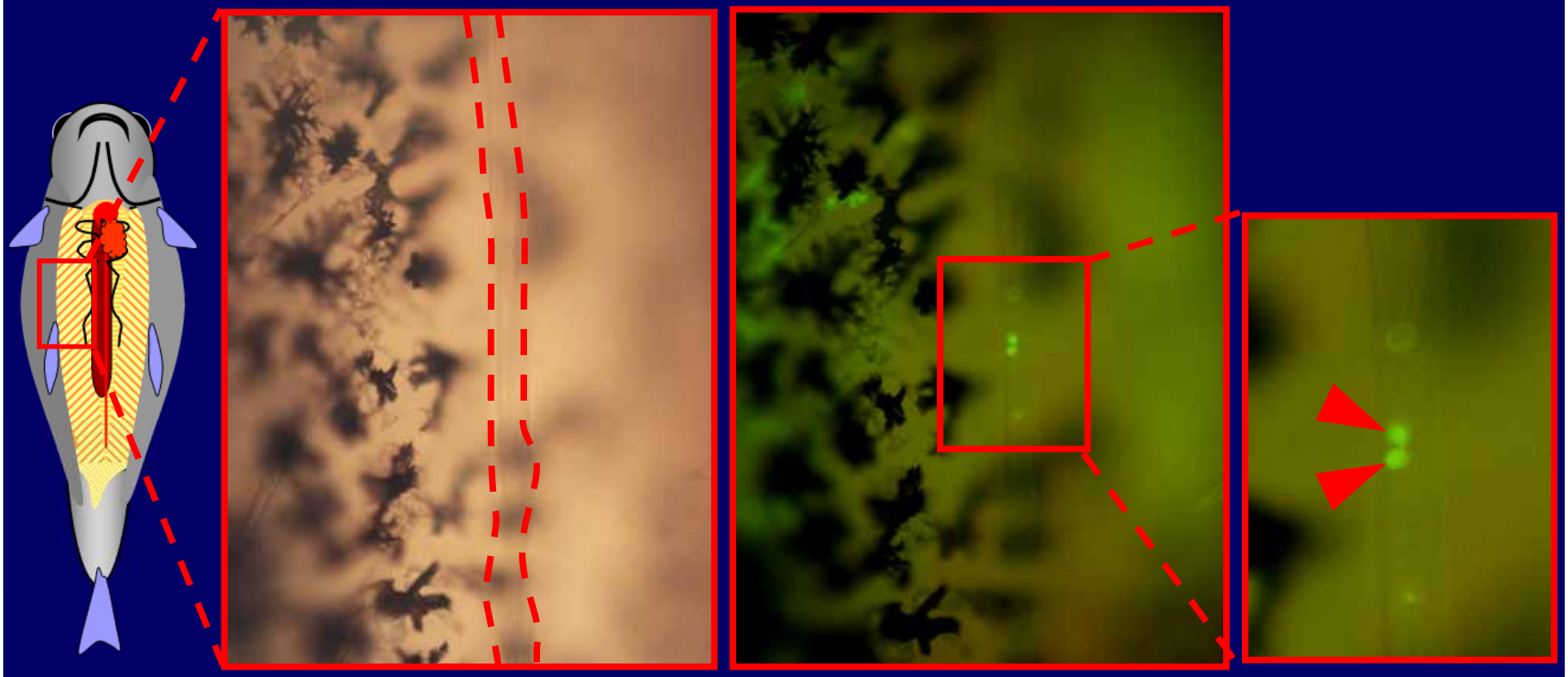
Testicular cell suspension



Recipient (non-tg)

Intraperitoneal transplantation

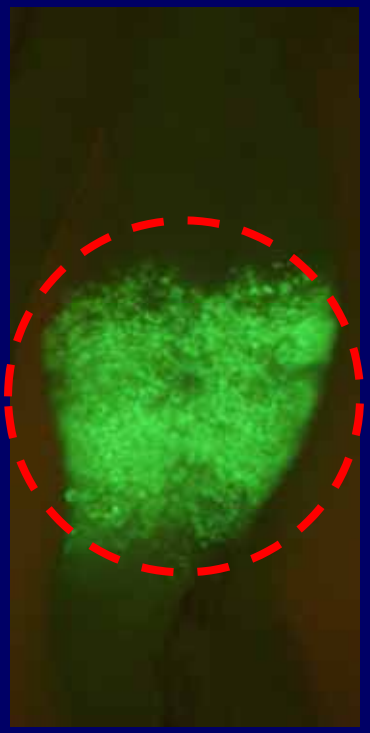
Incorporation of Spermatogonia in Allogenic Genital Ridges



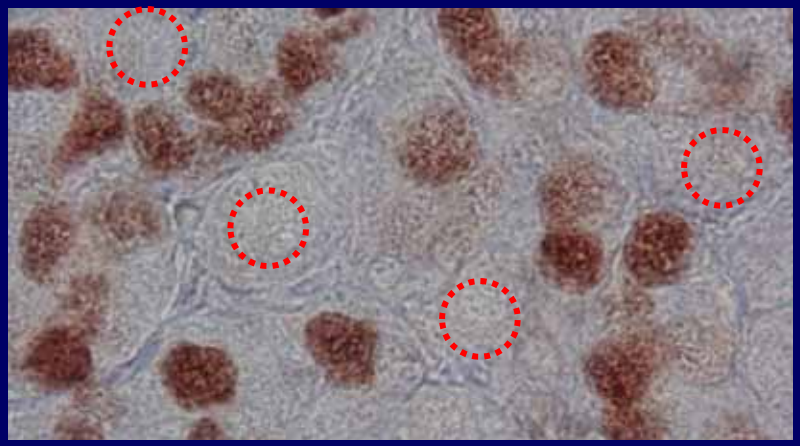
Bright field view Fluorescent view

18 days after transplantation

Proliferation of Donor-Derived Spermatogonia in a Recipient Testis



Testis of 6-month-old recipient

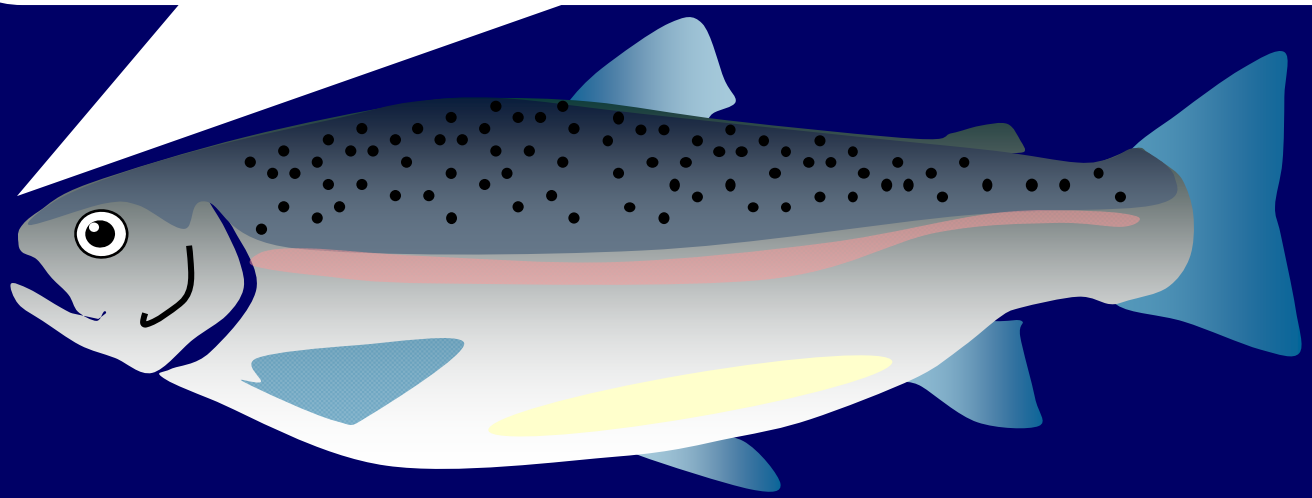


IHC with GFP-specific antibody

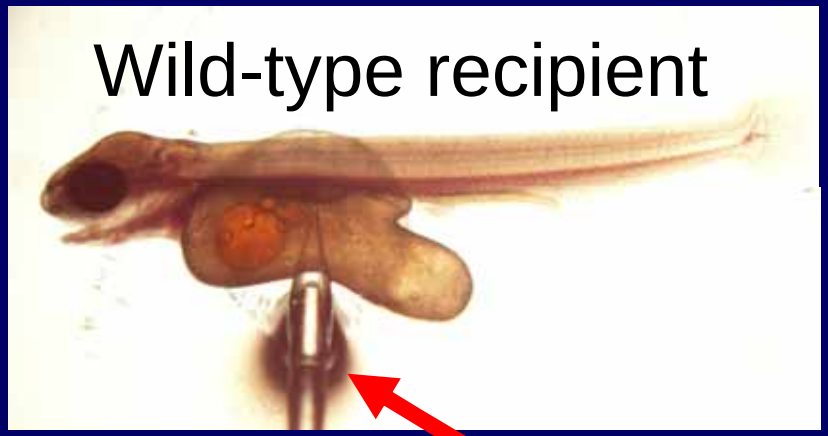
: Endogenous germ cell

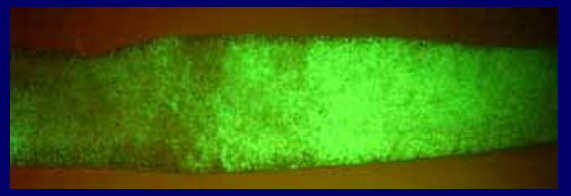
Okutsu et al., PNAS, 2006

Can donor-derived spermatogonia mature and produce normal sperm in recipient gonads?



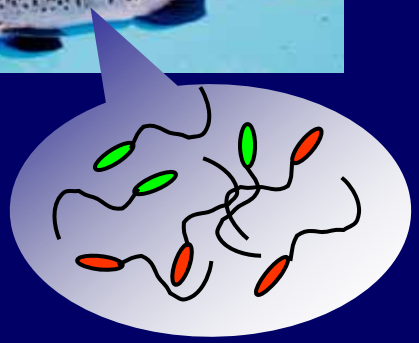
Wild-type recipient



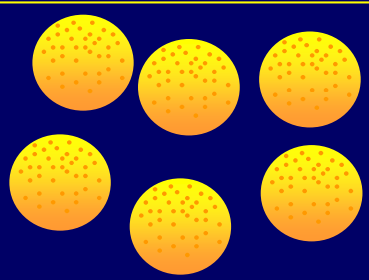


vasa-GFP transgenic Albino

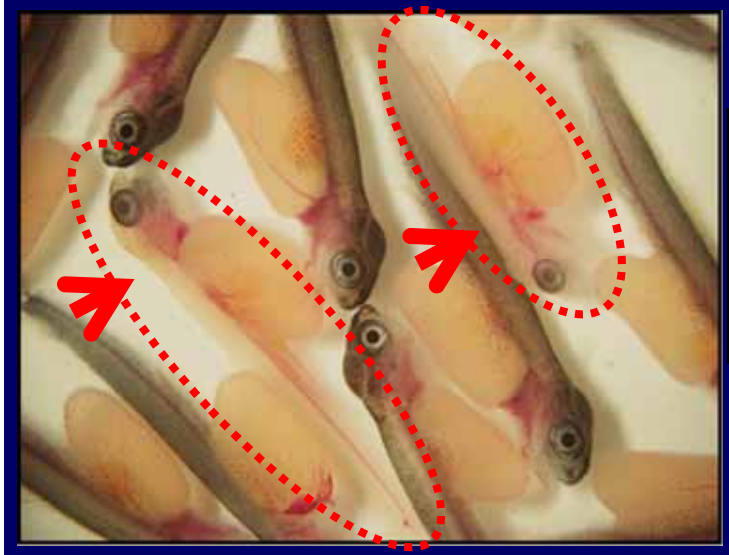
If the recipients produce donor-derived sperm, F1 embryos with albino body color and green germ cells will be obtained.

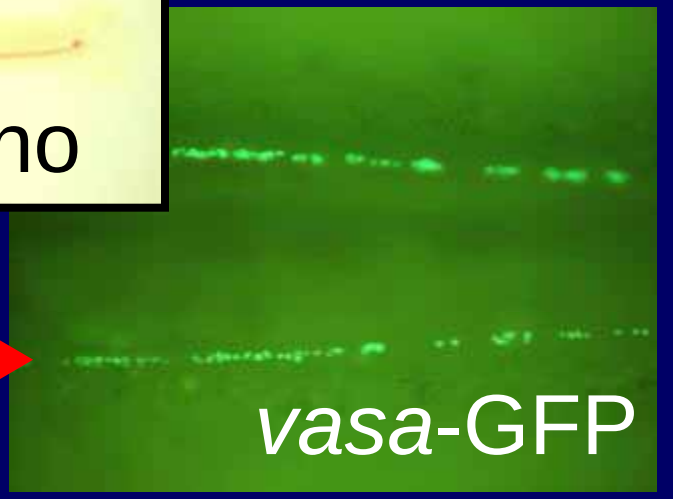


X



Wild-type eggs

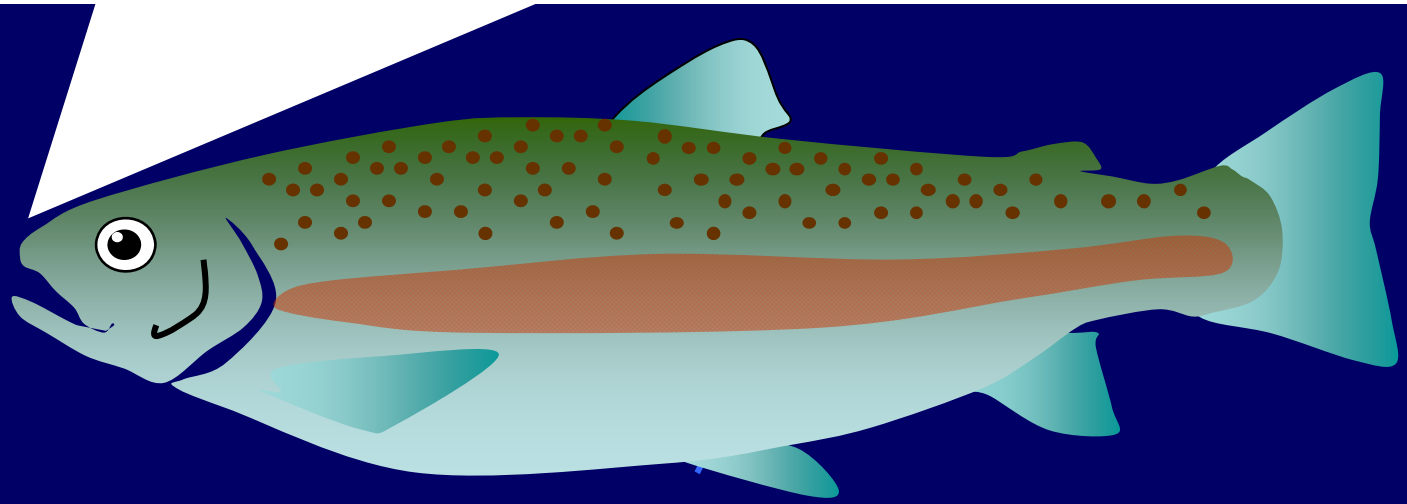




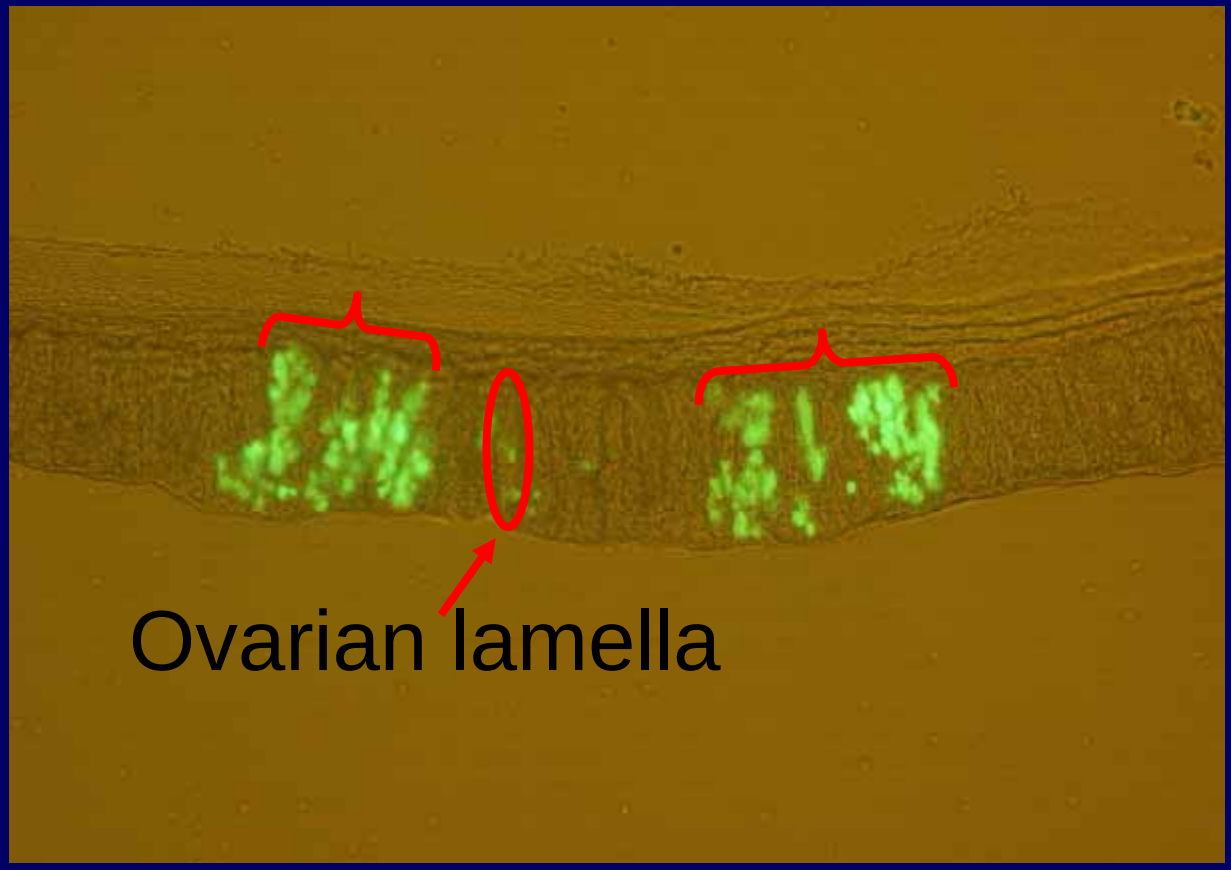
Donor-derived spermatogonia resumed spermatogenesis and produced functional sperm in the recipient testes

Okutsu et al., PNAS, 2006

Can donor-derived testicular germ cells colonize in female recipient gonads?

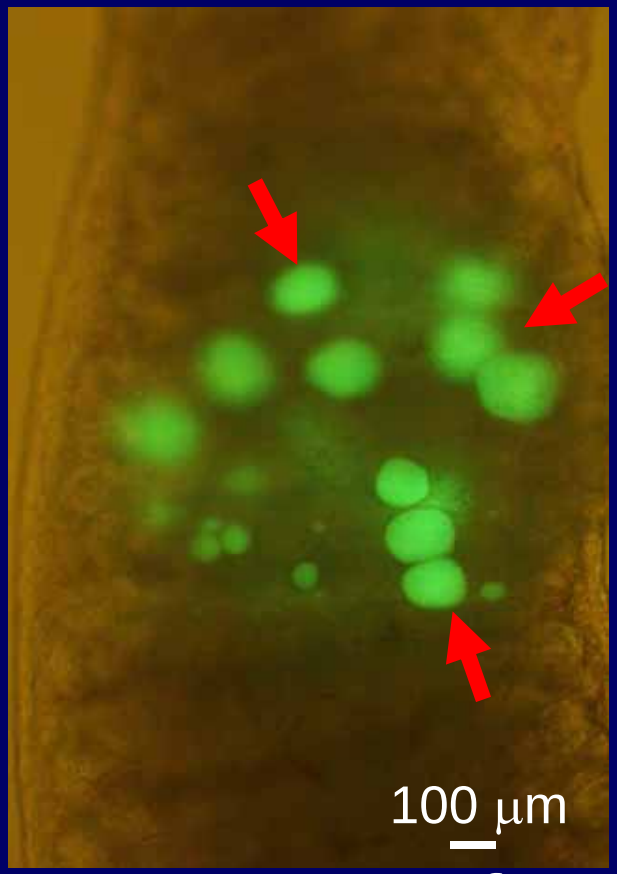


Proliferation of Spermatogonial Stem Cells in Ovaries

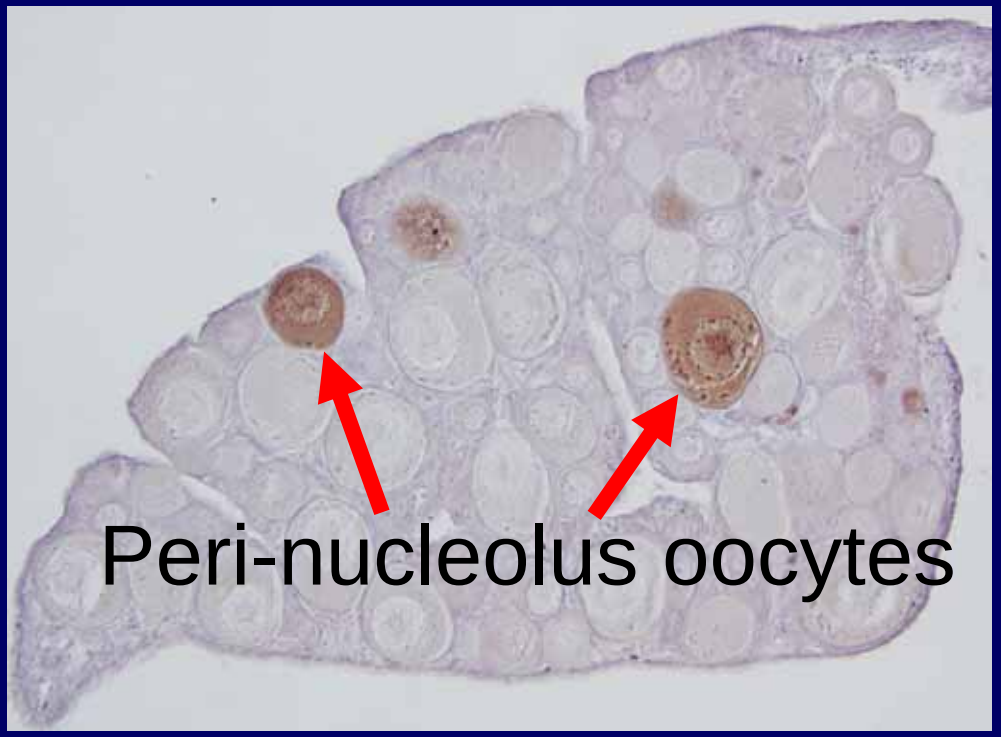


2 months after transplantation

Differentiation of Spermatogonial Stem Cells in Ovaries

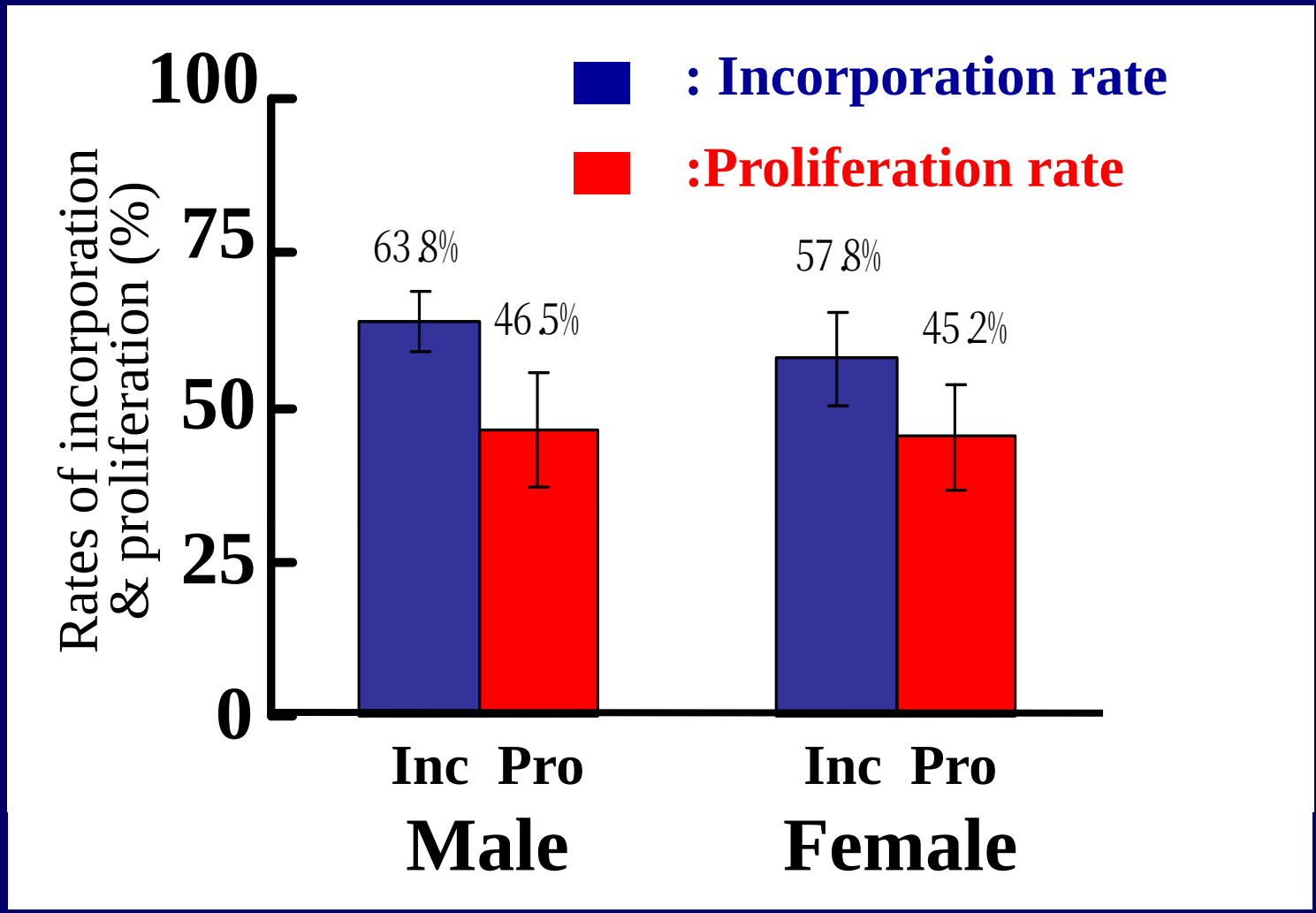


Ovary of
6-month-old recipient



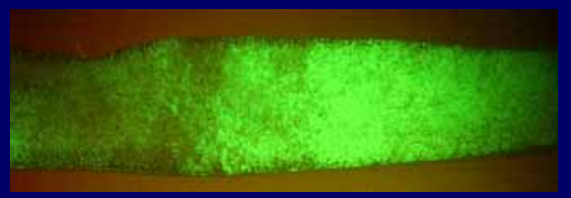
IHC with antibody specific to GFP

Okutsu et al., PNAS, 2006



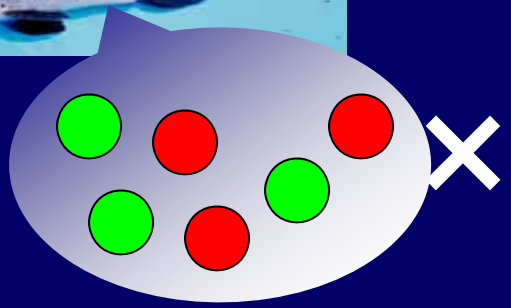
There were no significant differences in incorporation and proliferation efficiency of spermatogonia in male and female recipients

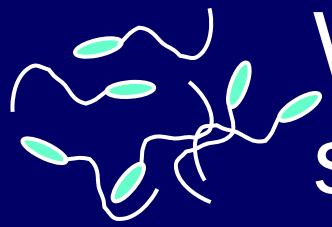
Wild-type recipient



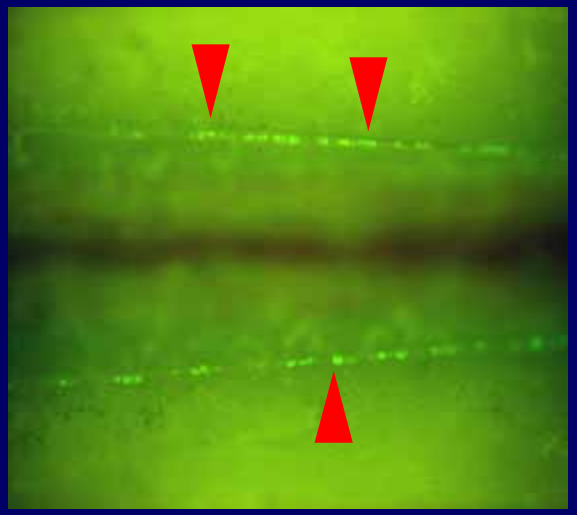
vasa-GFP transgenic

Albino





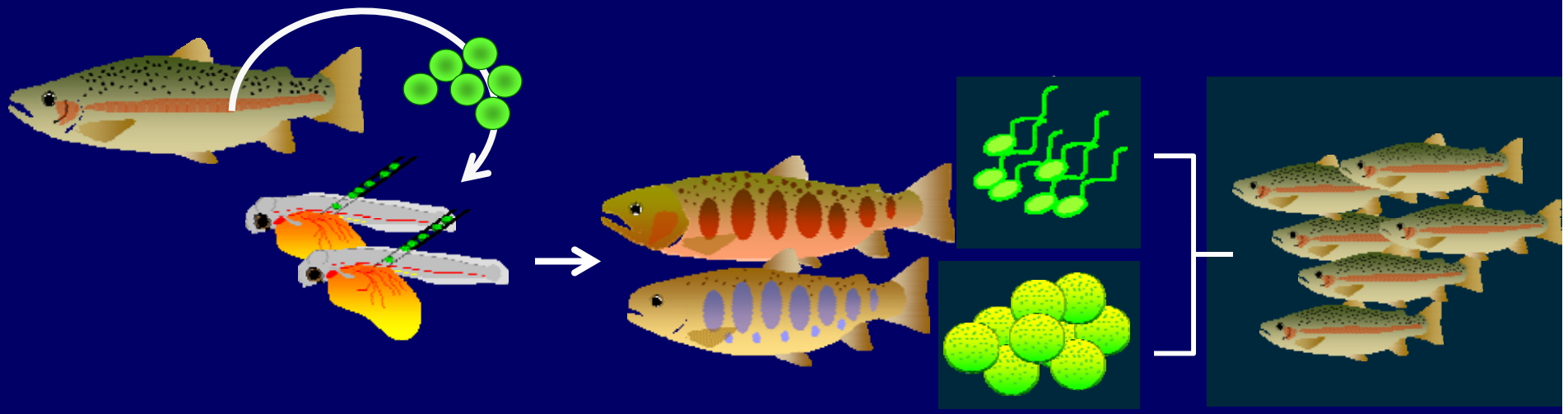
Wild-type sperm



F1 offspring developed from spermatogonia-derived eggs

Spermatogonia can produce functional eggs

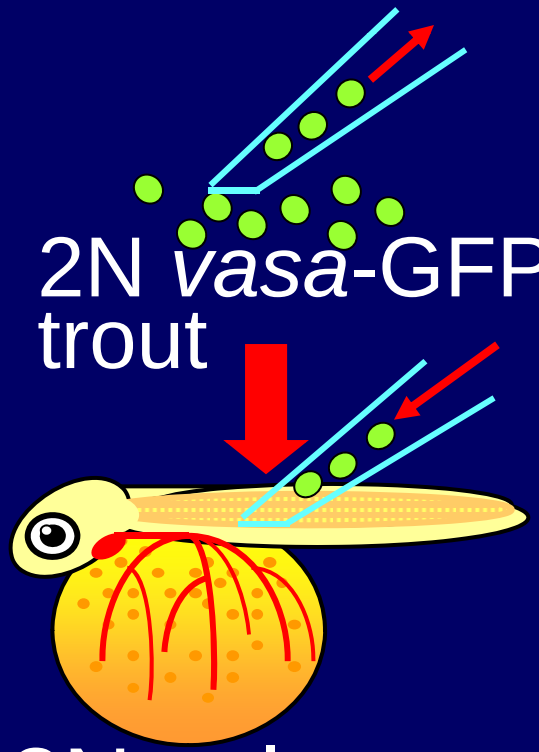
Okutsu et al., PNAS, 2006



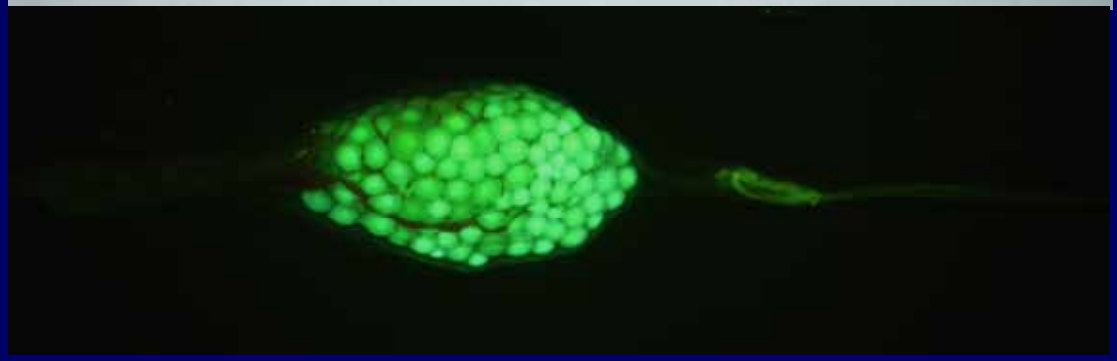
Making salmon that produces only trout gametes

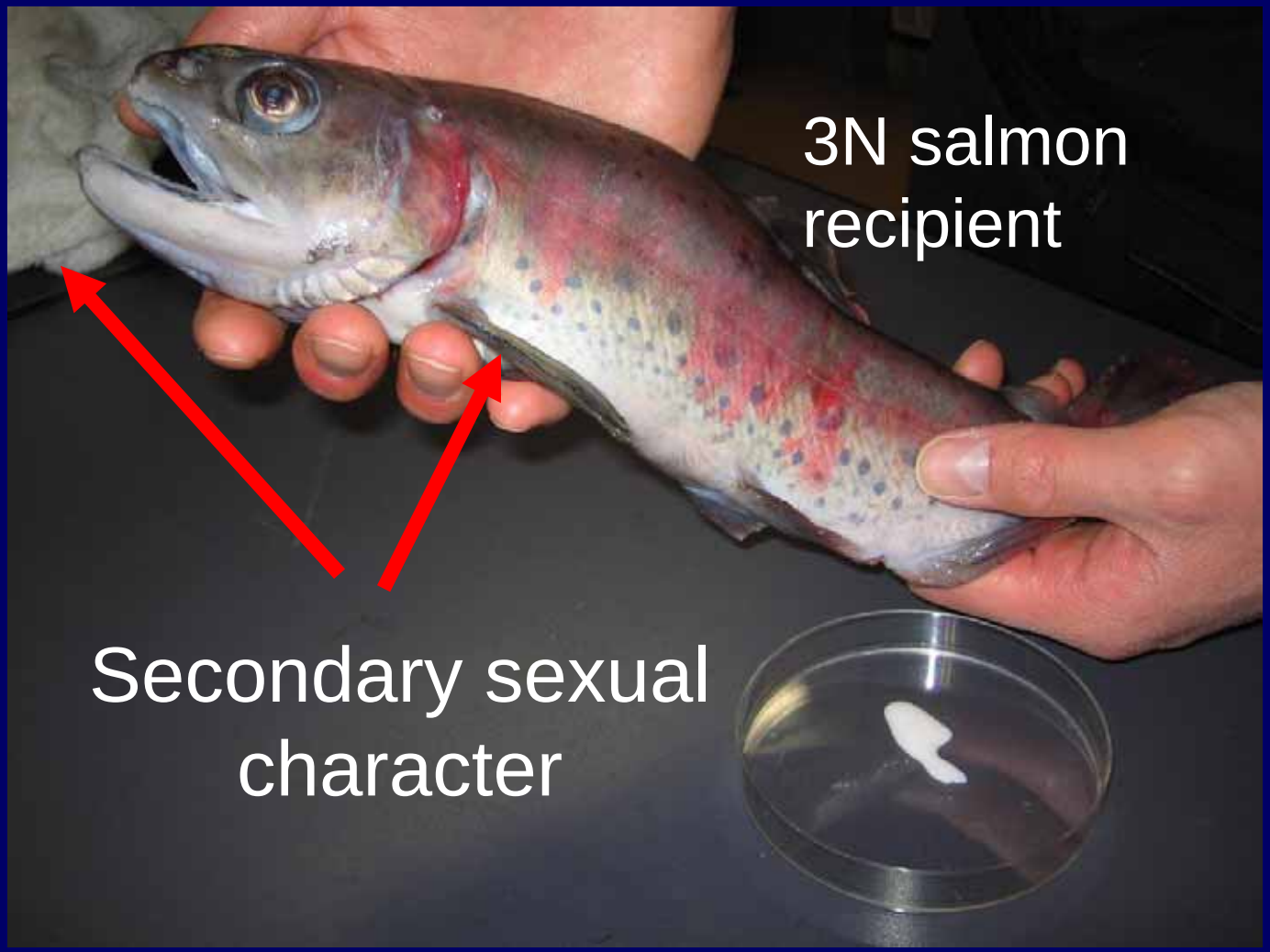

2N

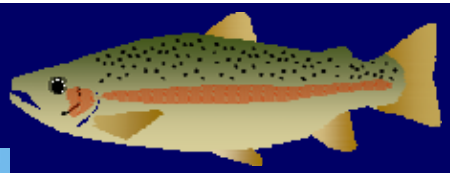

3N


2N *vasa*-GFP trout
3N salmon


2N→3N

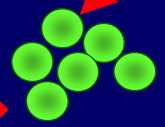


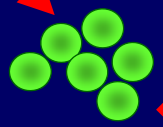

3N salmon
recipient
Secondary sexual
character



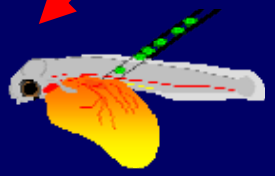
2N male trout (fertile)

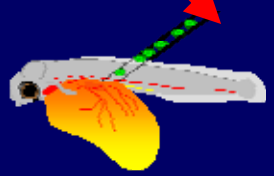
3 N control salmon





2N trout spermatogonia





3N salmon recipients

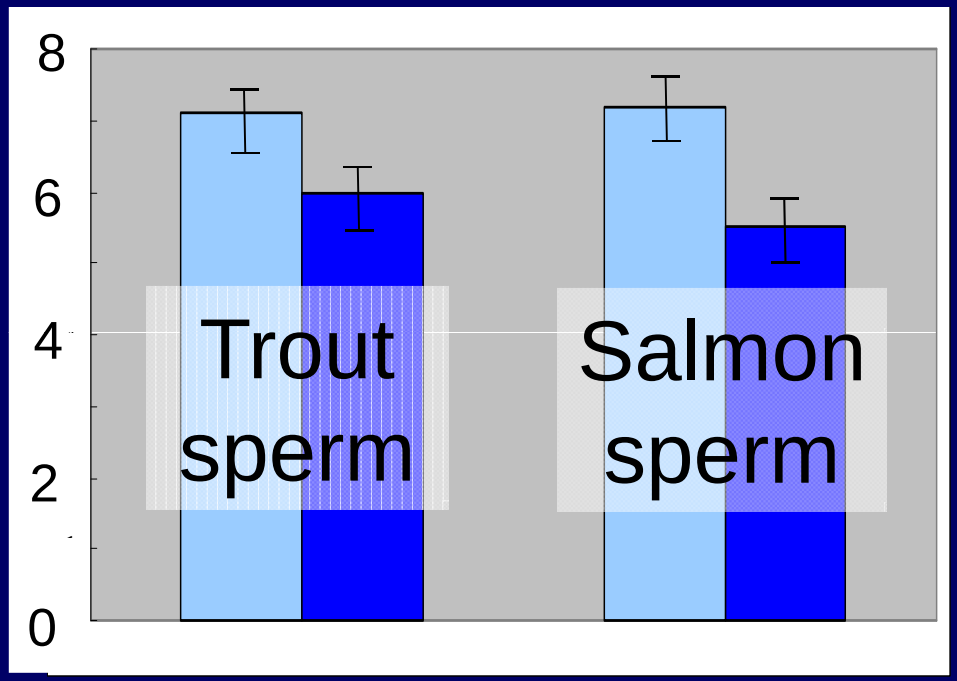
3N female salmon

3N male salmon

Progeny test

Gametes obtained from masu salmon

Male salmon

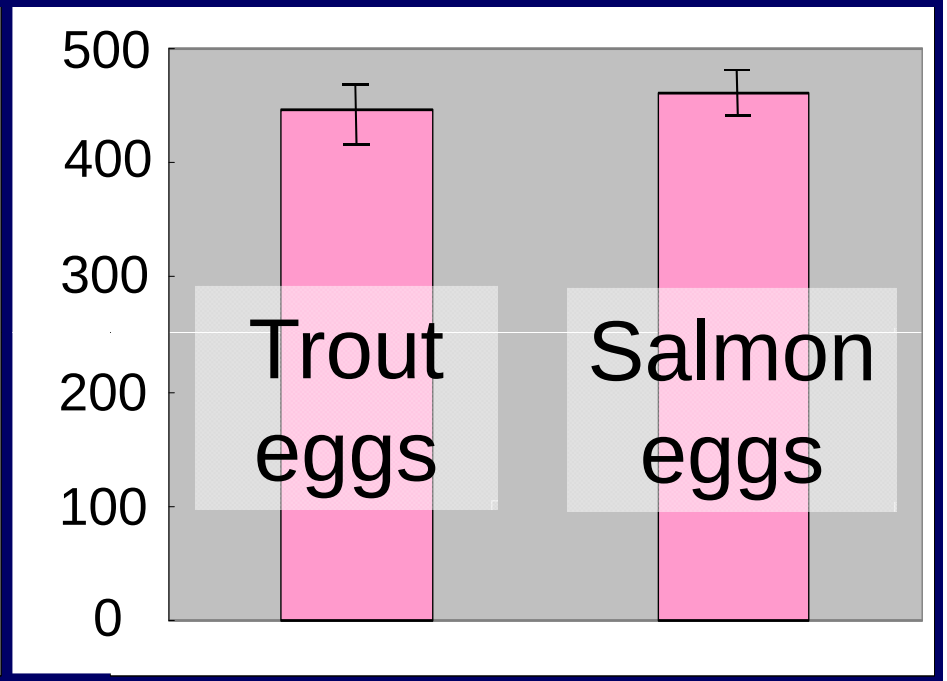


3 N recipients Control

Milt volume (ml)

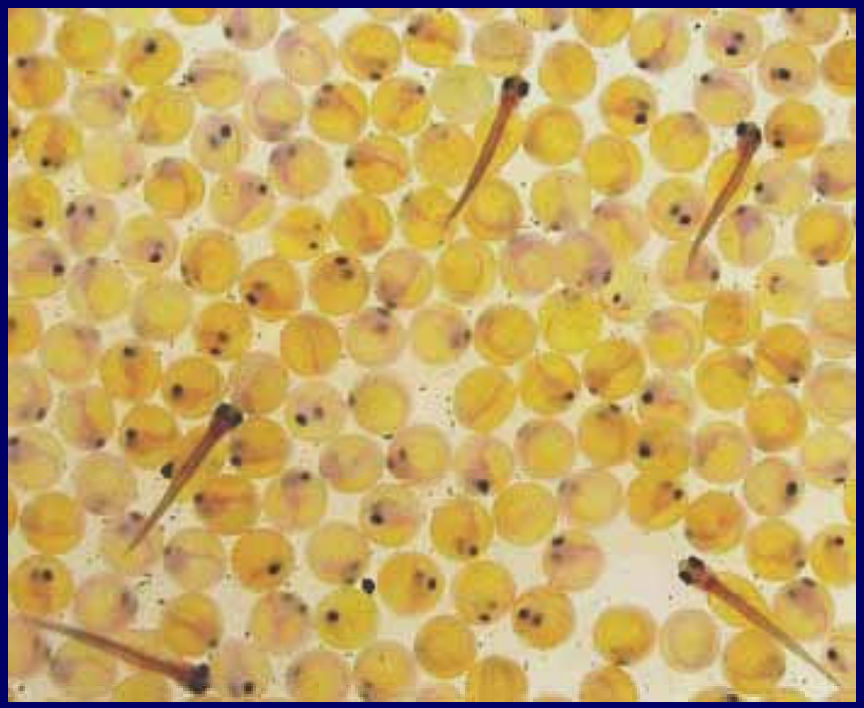
Sperm number ($X10^9$)

Female salmon



3 N recipients Control

Egg number

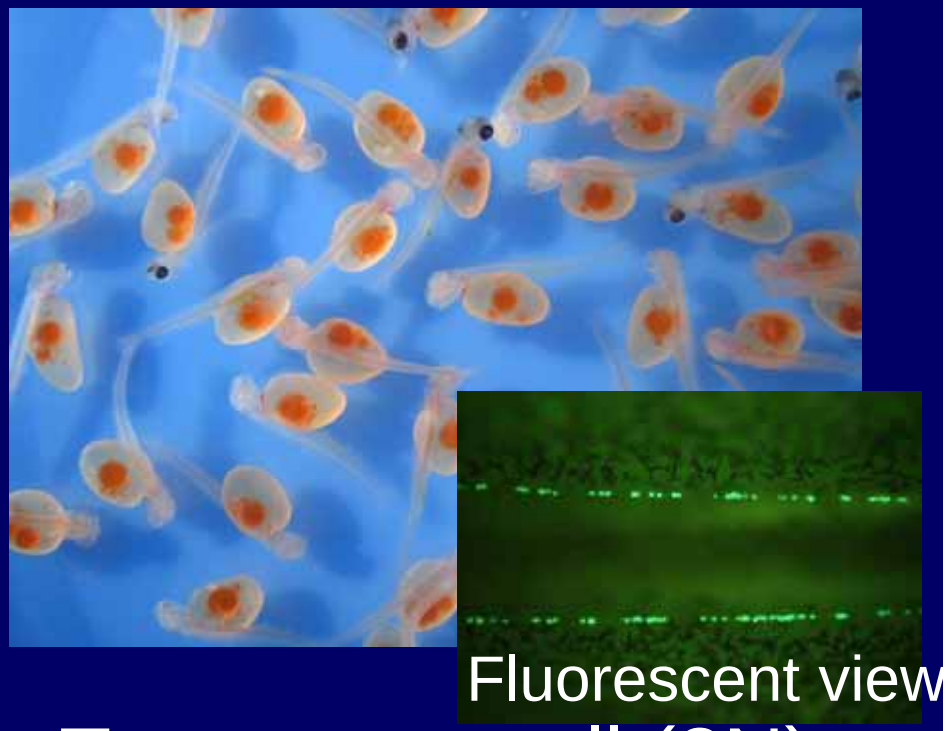


Trout germ cell (2N)

↓

2N-salmon recipient

(Donor-derived F1 : 0.4%)



Trout germ cell (2N)

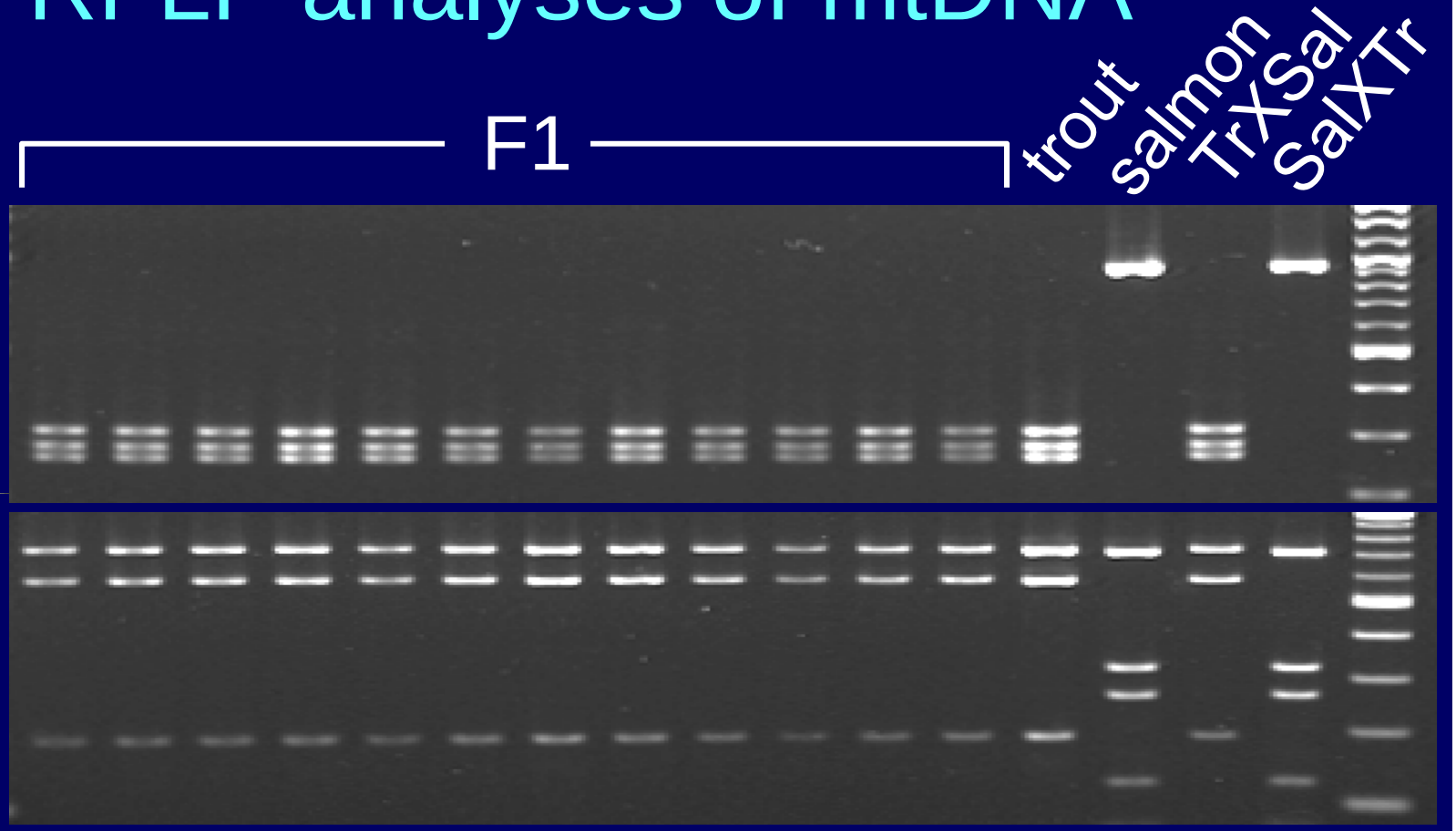
↓

3N-salmon recipient

(Donor-derived F1 : 100%)

Okutsu et al., Science, 2007

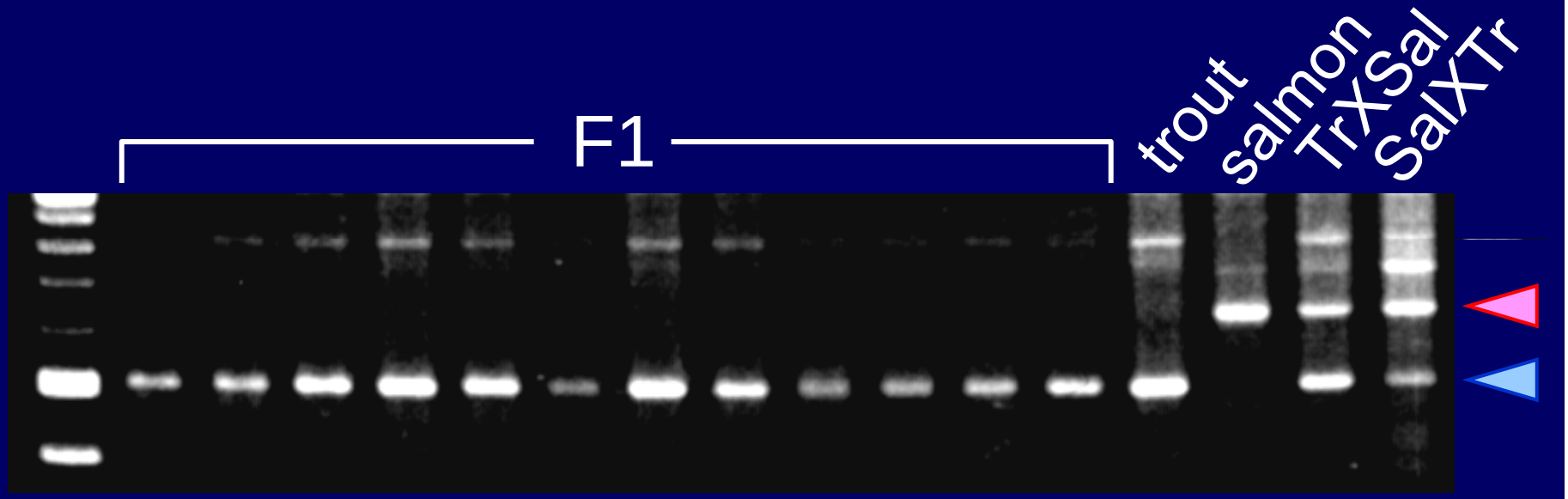
RFLP analyses of mtDNA



All F1 fish derived from salmon recipients possess rainbow trout-derived mitochondria

Triploid salmon recipients produce only trout eggs

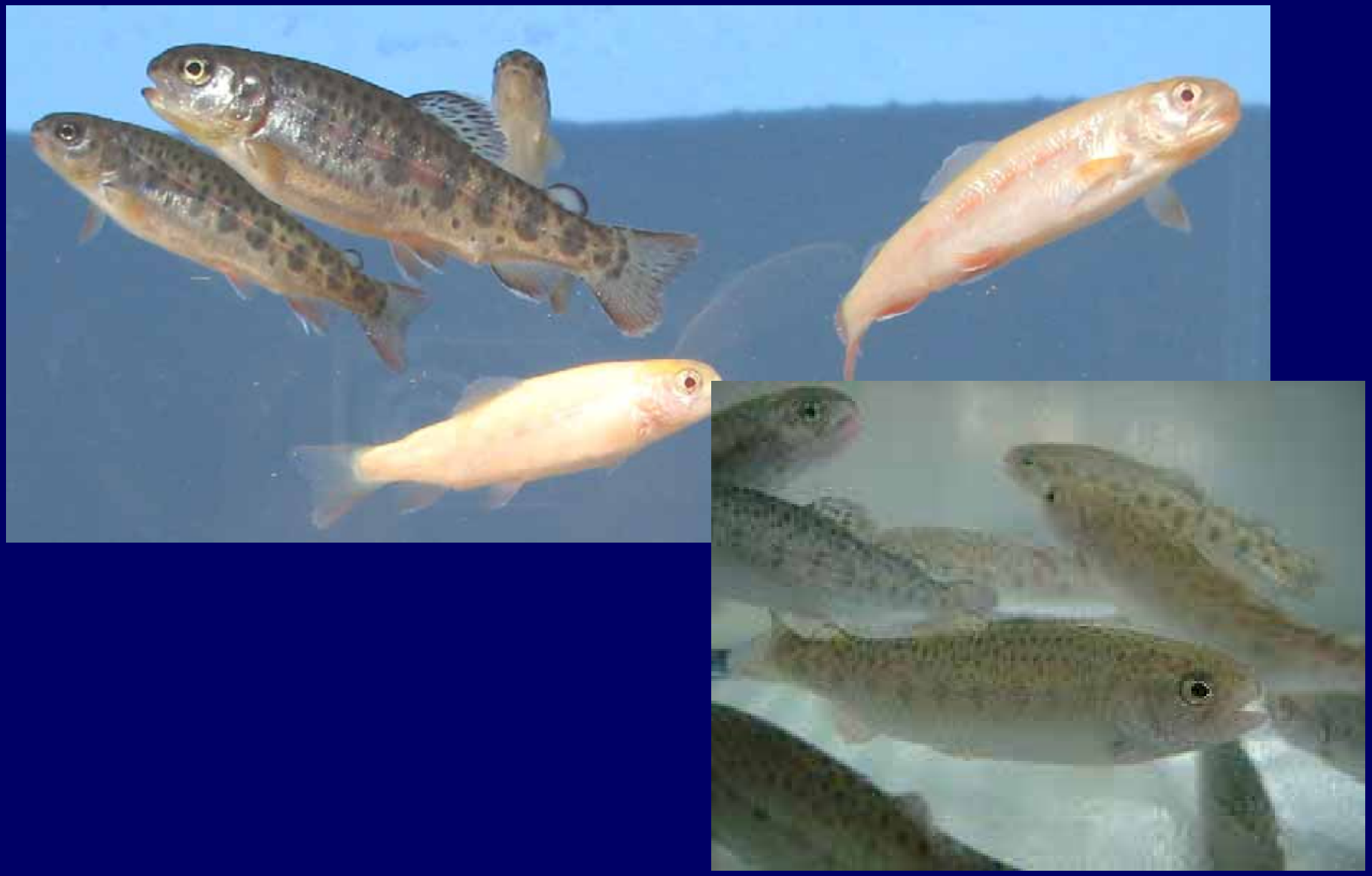
Random Amplified Polymorphic DNA (RAPD) analysis of 3N-recipient salmon-derived F1



Triploid salmon recipients received trout germ cells produced only trout offspring

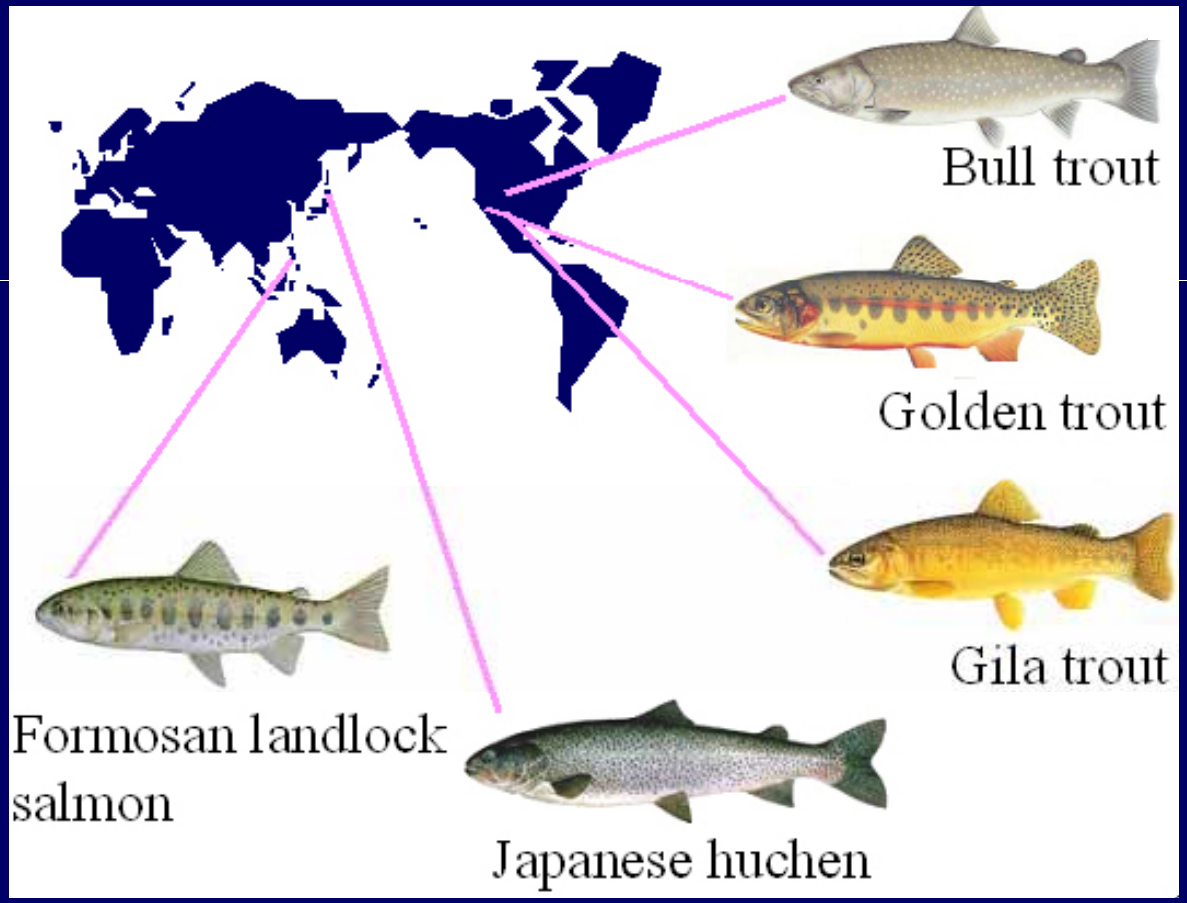
Okutsu et al., Science, 2007

Trout offspring from triploid salmon parents



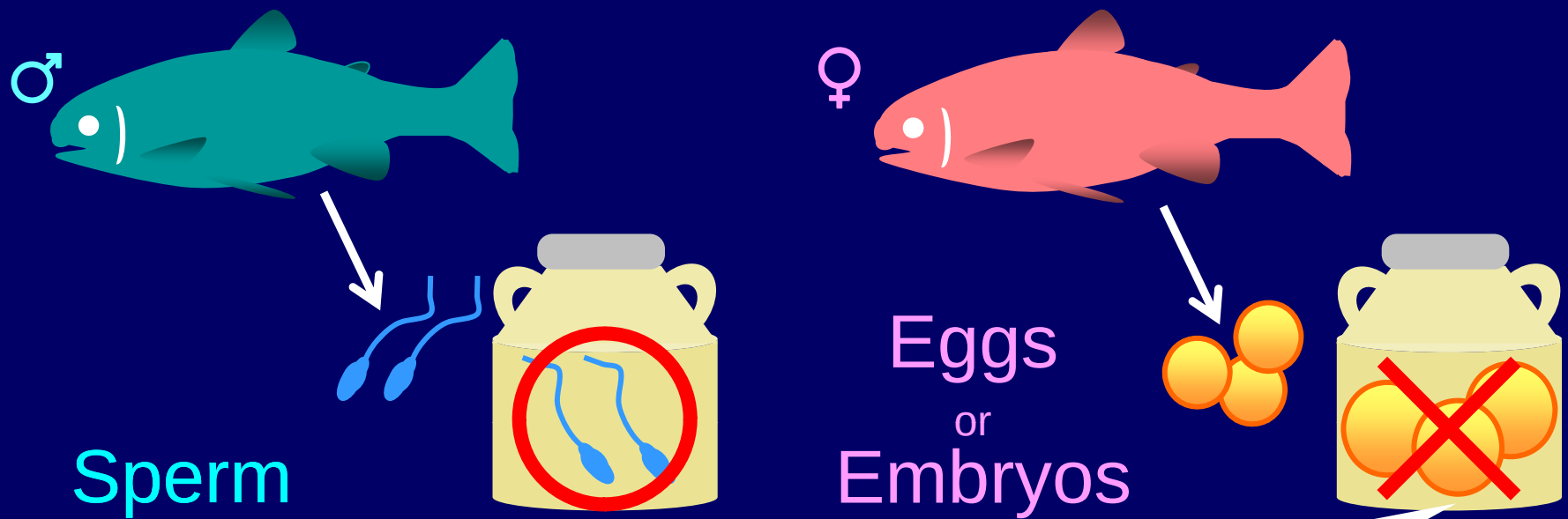
Okutsu et al., Science, 2007

World salmon crisis



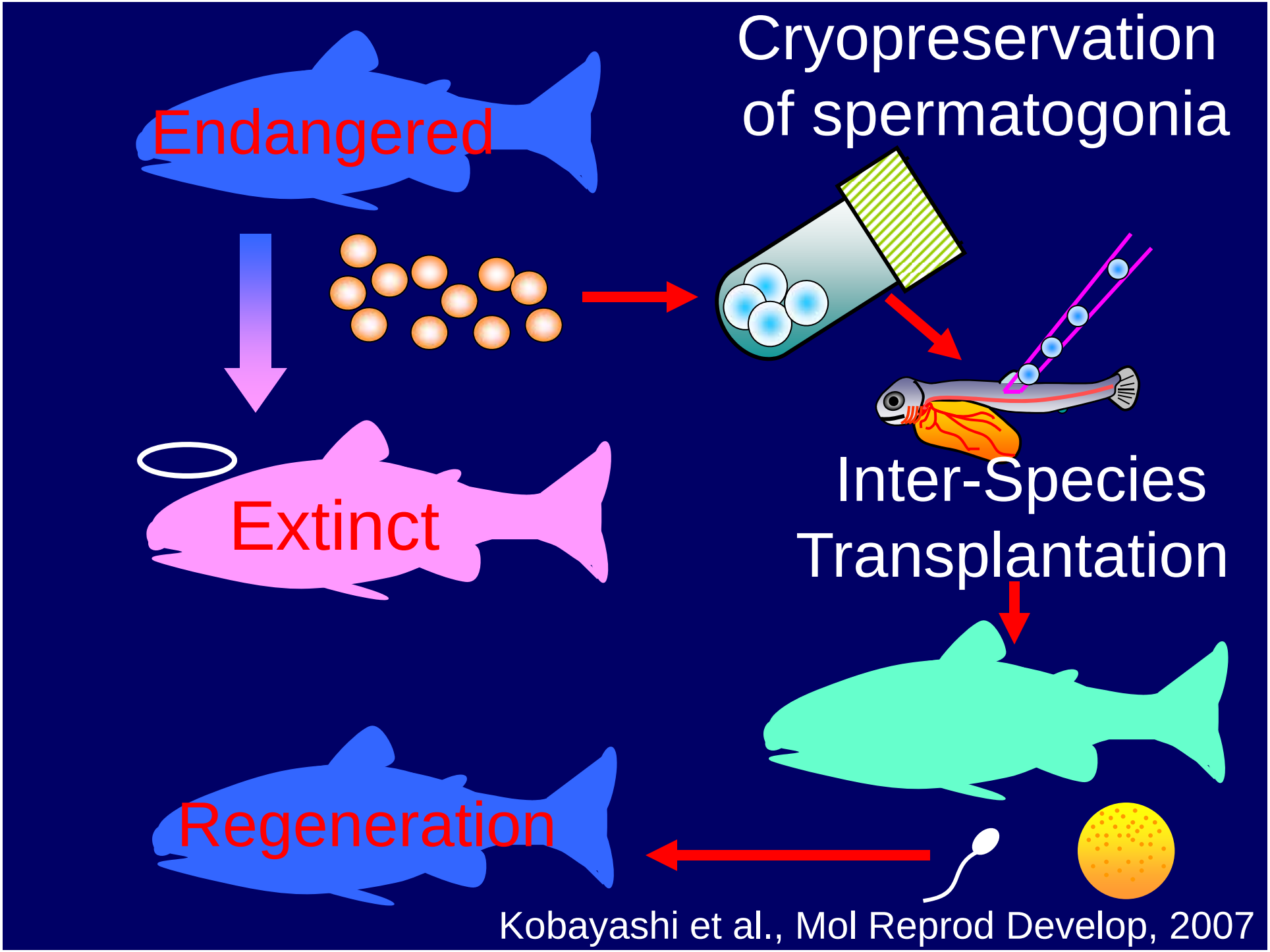
- Over-fishing
- Habitat destruction

Preservation of genetic information in fish



Due to their large size and high yolk content

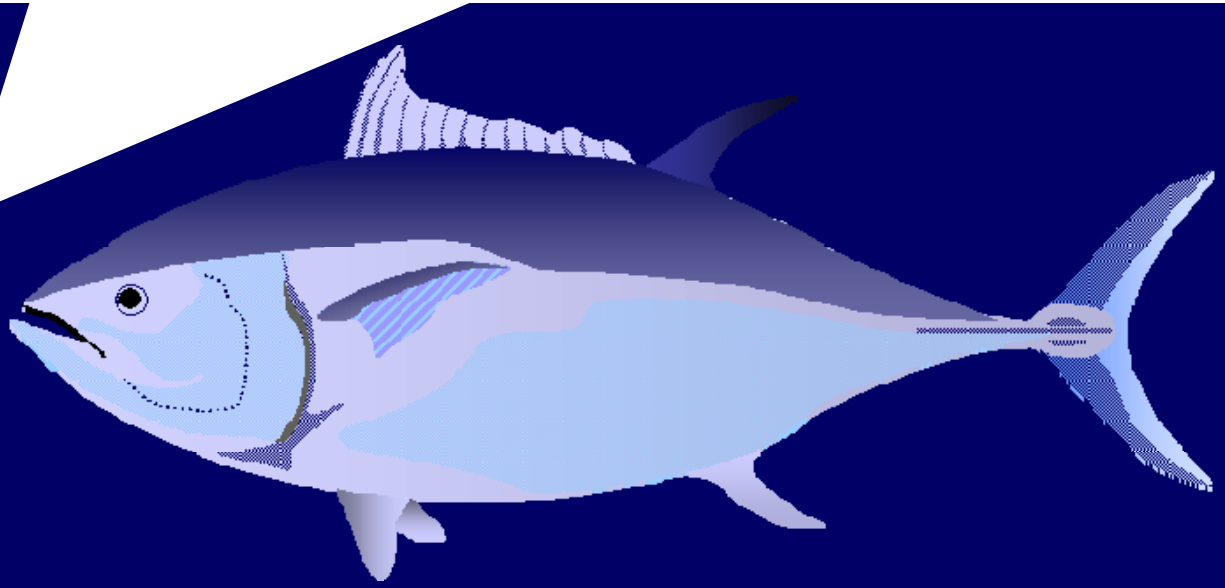
Maternally-inherited cytoplasmic compartments (mitochondrial DNA) can not be preserved!


Endangered
Cryopreservation
of spermatogonia
Extinct
Inter-Species
Transplantation
Regeneration
Kobayashi et al., Mol Reprod Develop, 2007

Sockeye

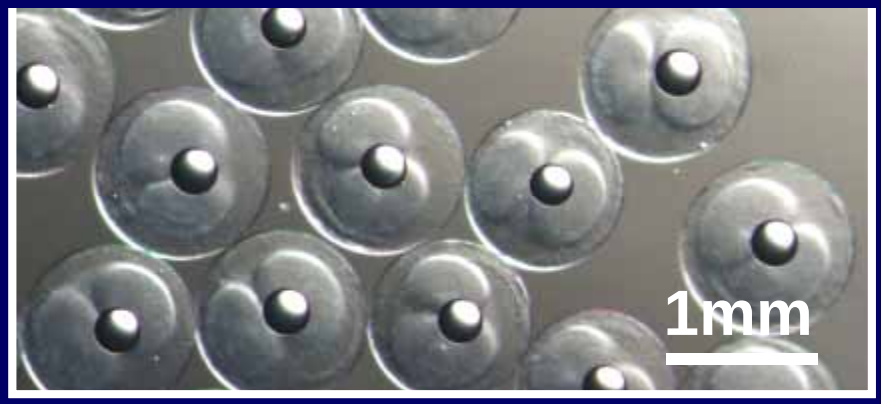
	Male	Female	Total
2006	1	2	3
2007	2	2	4

Can spermatogonial transplantation be applicable to marine fish?



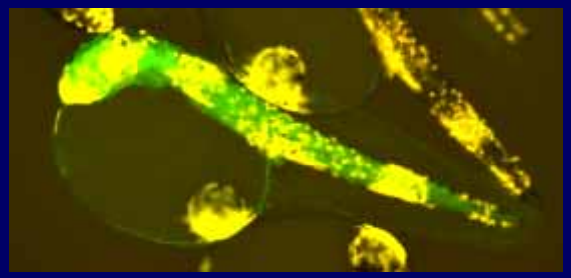
Application of germ cell transplants to marine fishes

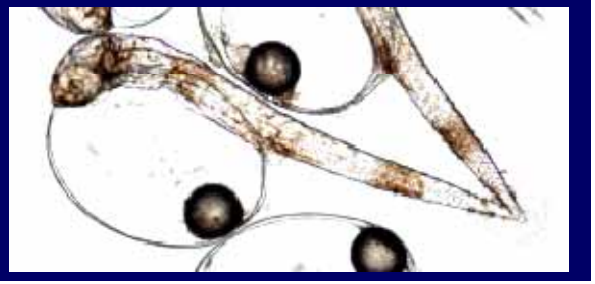
Nibe croaker (*Nibea mitsukurii*)



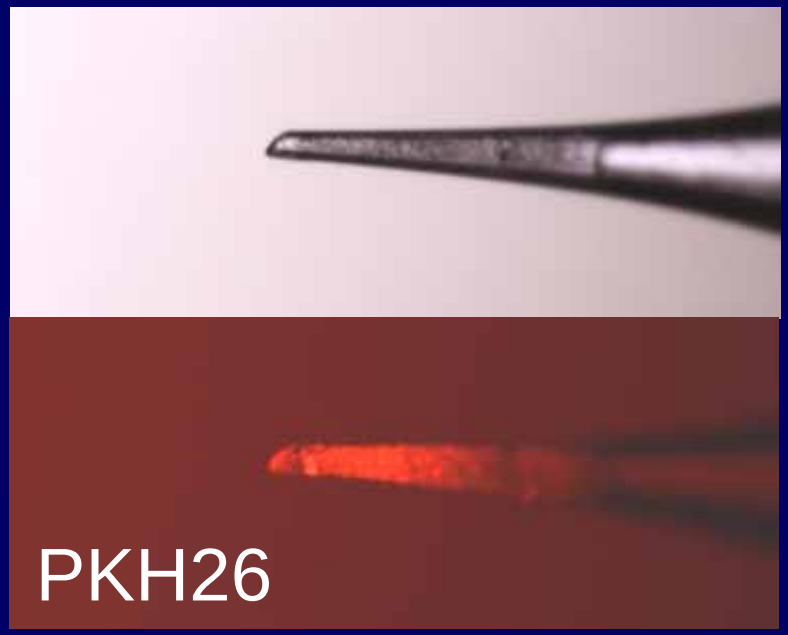
Donor: HSC-GFP-transgenic (heterozygous)

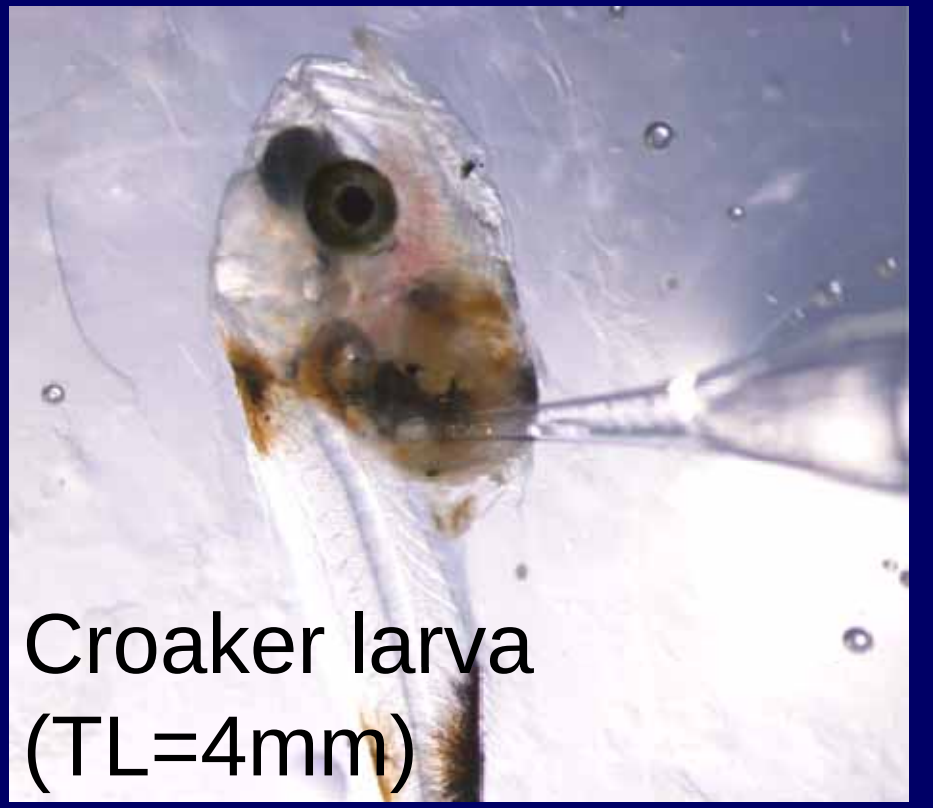
Recipient: triploid sterile non-transgenic





donor testicular cells labeled with PKH26



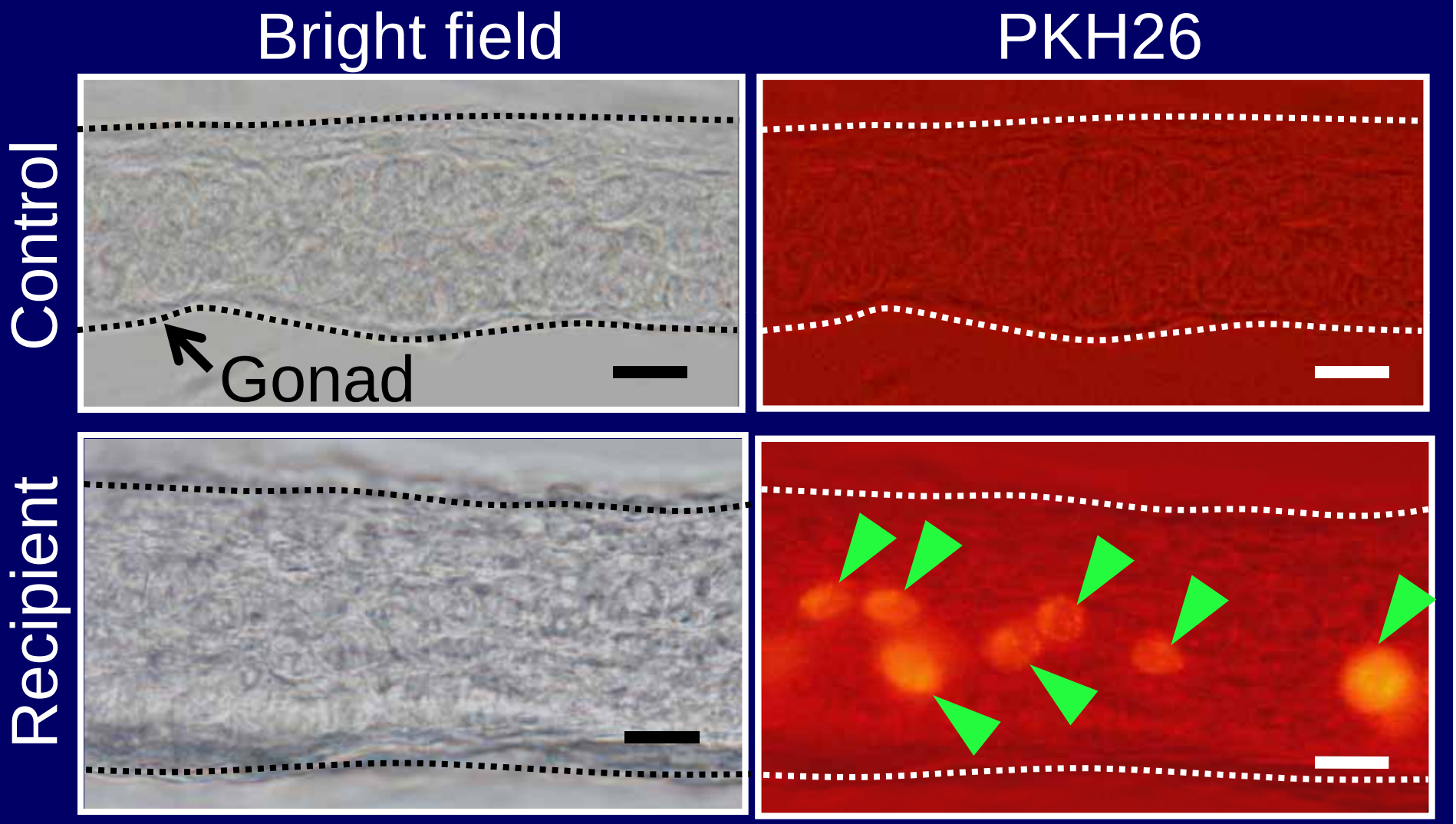


Croaker larva (TL=4mm)

Microinjection into body cavity

Survival: 30-50% (2 weeks after TP)

Fluorescent observation of gonads at 3 wks after TP

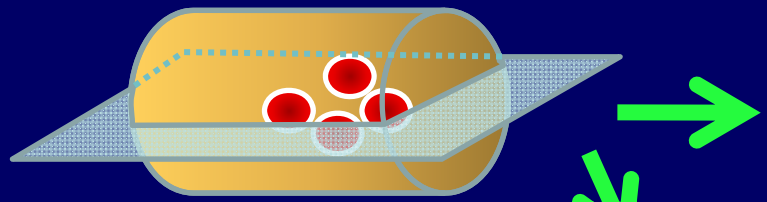


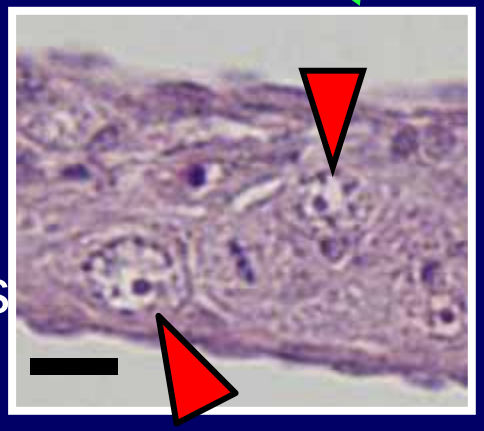
(Scale bars : 20 μm)

Takeuchi et al., Biol Reprod, 2009

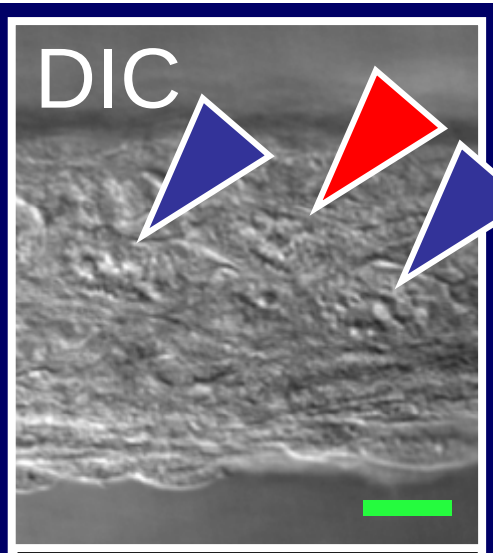
PKH-positive cells were germ cells?

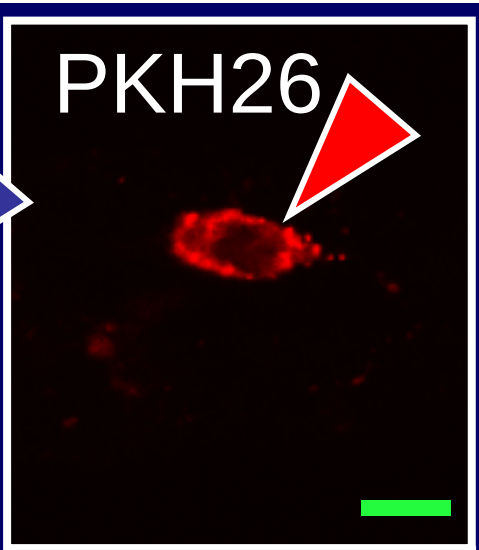
Confocal microscopic analysis

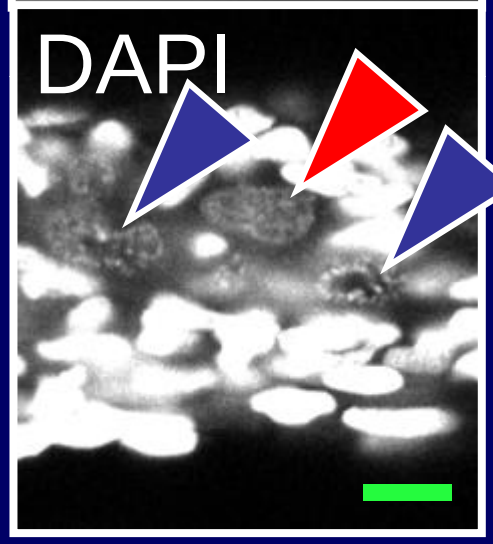


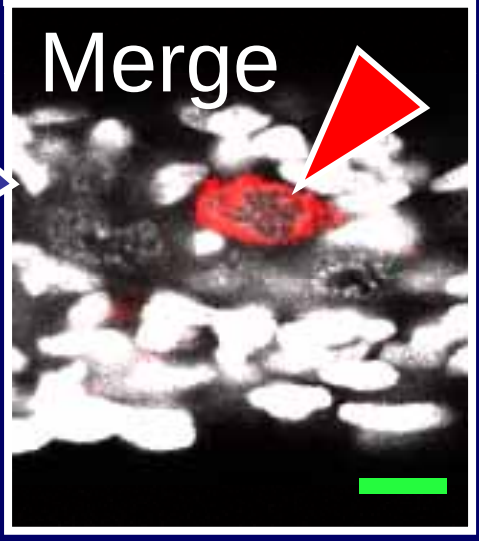


(Scale bars : 10 μm)









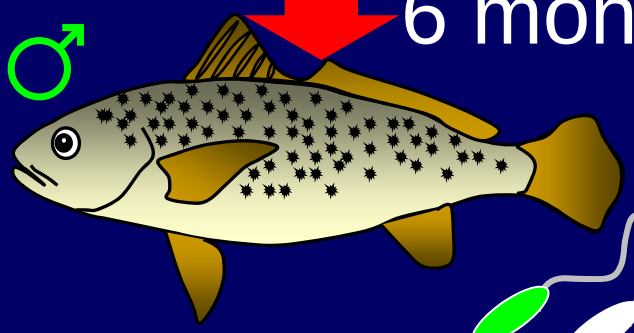
*no PKH(+) somatic cells were observed

PKH (+) cells found in the recipient gonad were surely donor-derived germ cells

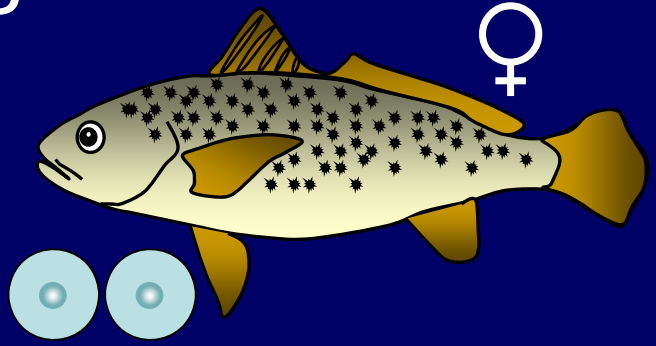
Progeny test using ♂ recipient and wild-type ♀

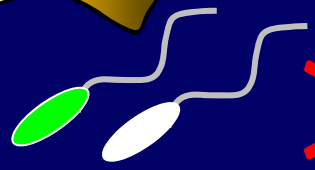
6 month after TP

♂



♀





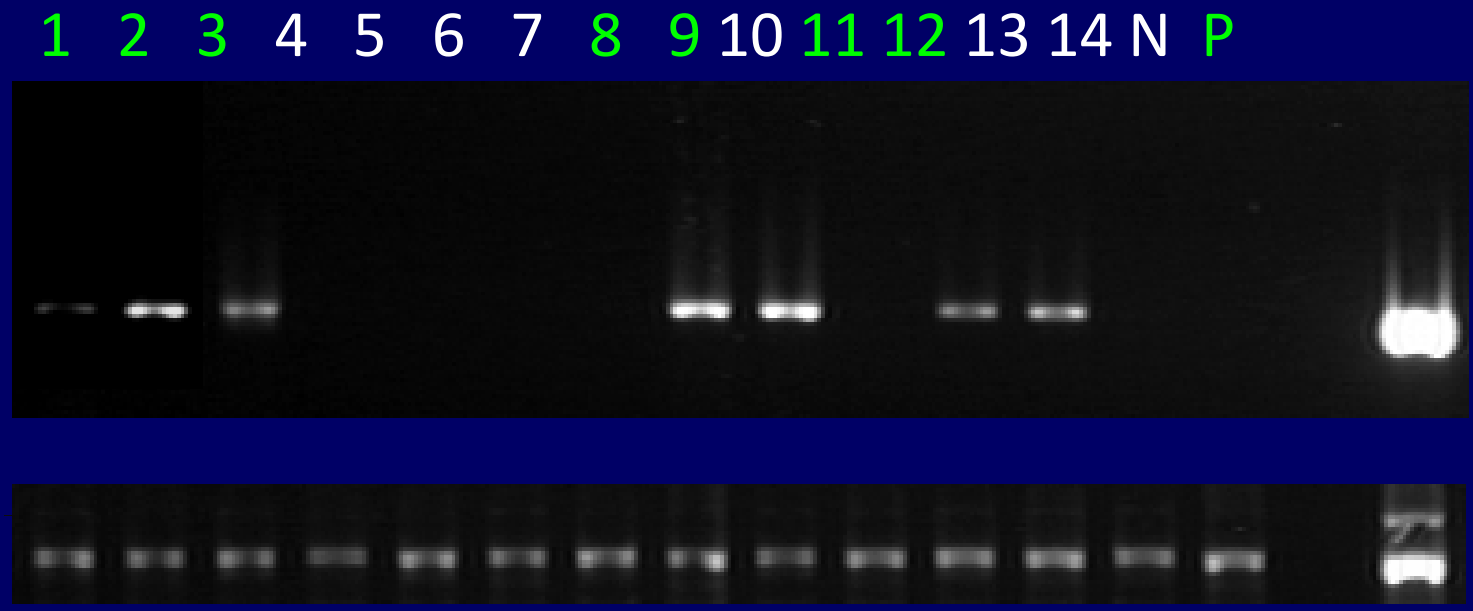
Donor-derived sperm
(hetelozygous GFP)

Wild-type eggs

F1 embryos

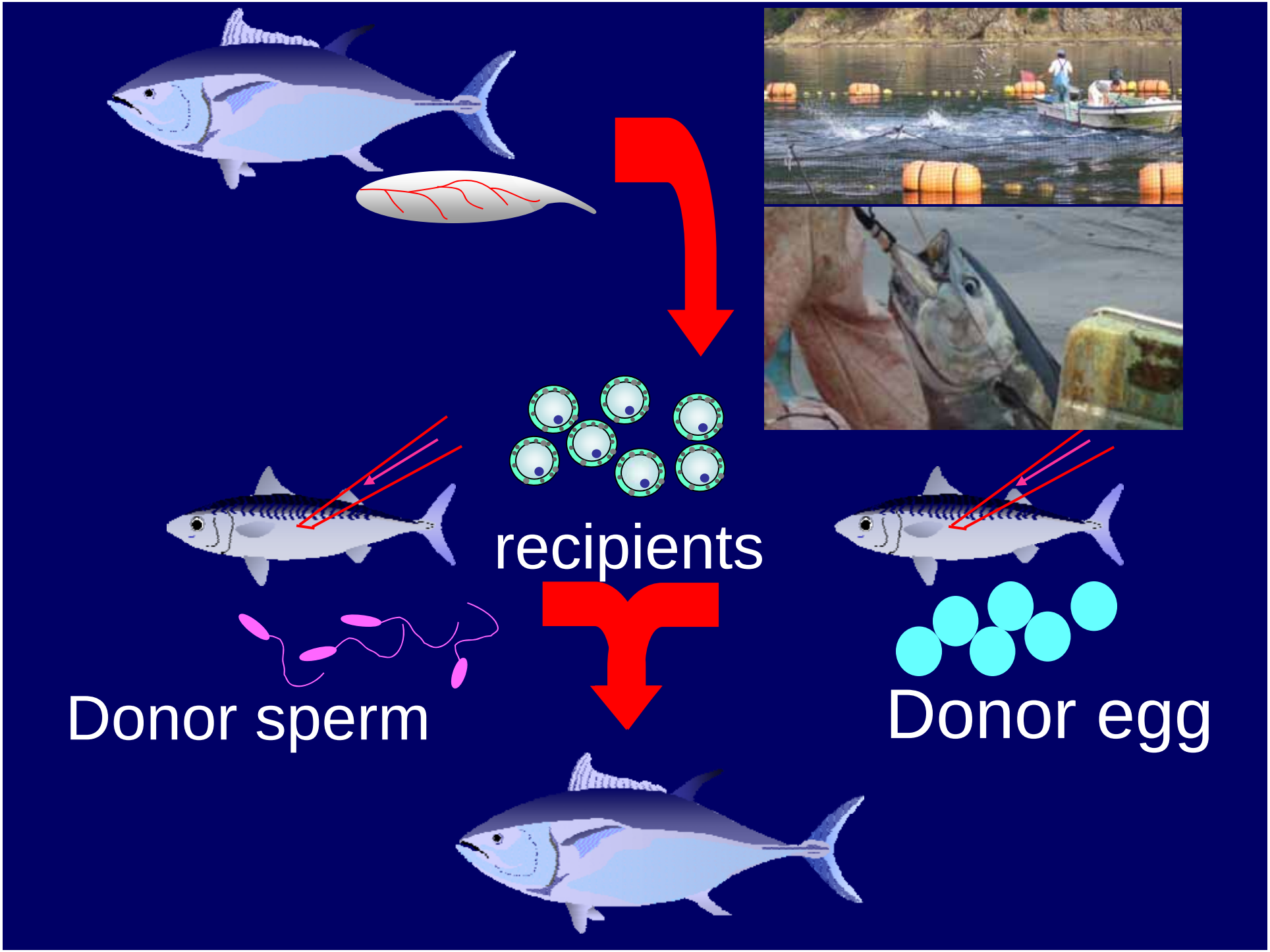
Genomic DNA extraction & PCR for GFP gene

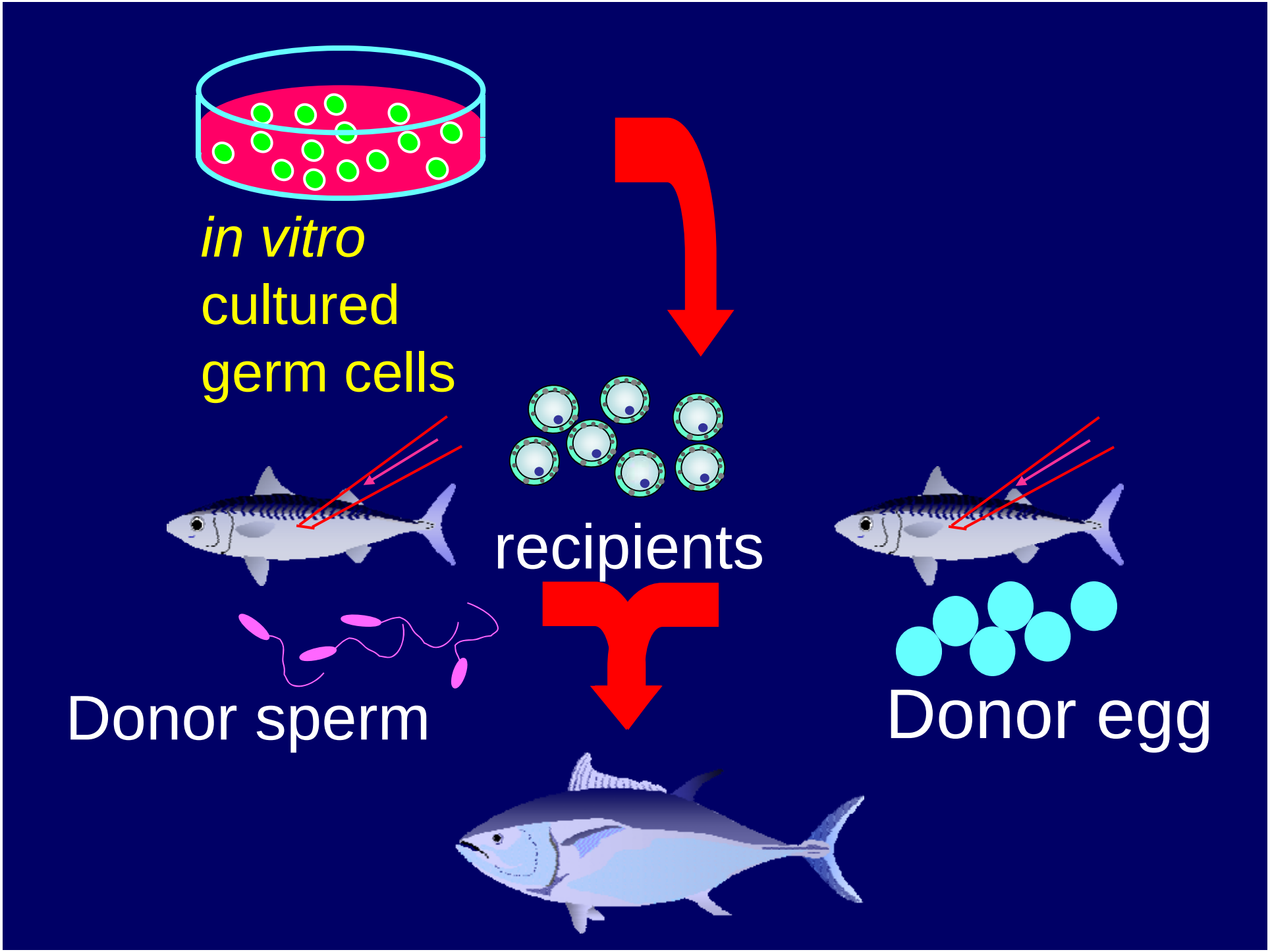
If the male recipient produces only donor-derived sperm, 50% of F1 embryos should be GFP-positive



Triploid nibe croaker recipients only produced donor-derived functional sperm

Germ cell transplantation is applicable to marine fish species


recipients
Donor sperm
Donor egg


in vitro
cultured
germ cells
recipients
Donor sperm
Donor egg

Yutaka Takeuchi

Tomoyuki Okutsu

Naoki Kabeya

Ryosuke Yazawa

Kentaro Higuchi