2015･6/9

海洋資源利用促進技術開発プログラム

生殖幹細胞操作によるクロマグロ等の新たな受精卵供給法の開発

東京海洋大学
吉崎悟朗

クロマグロ資源の減少を食い止めるために・・・

人工種苗生産の効率化が必須

サバにマグロを生ませる！

	科	親魚体重	成熟年齢	飼育施設
クロマグロ	サバ科	70-250kg	3-5歳	海面イケス
マサバ	サバ科	0.3-0.5kg	1歳	小型水槽

小型水槽で代理親サバからクロマグロを生産することで・・・

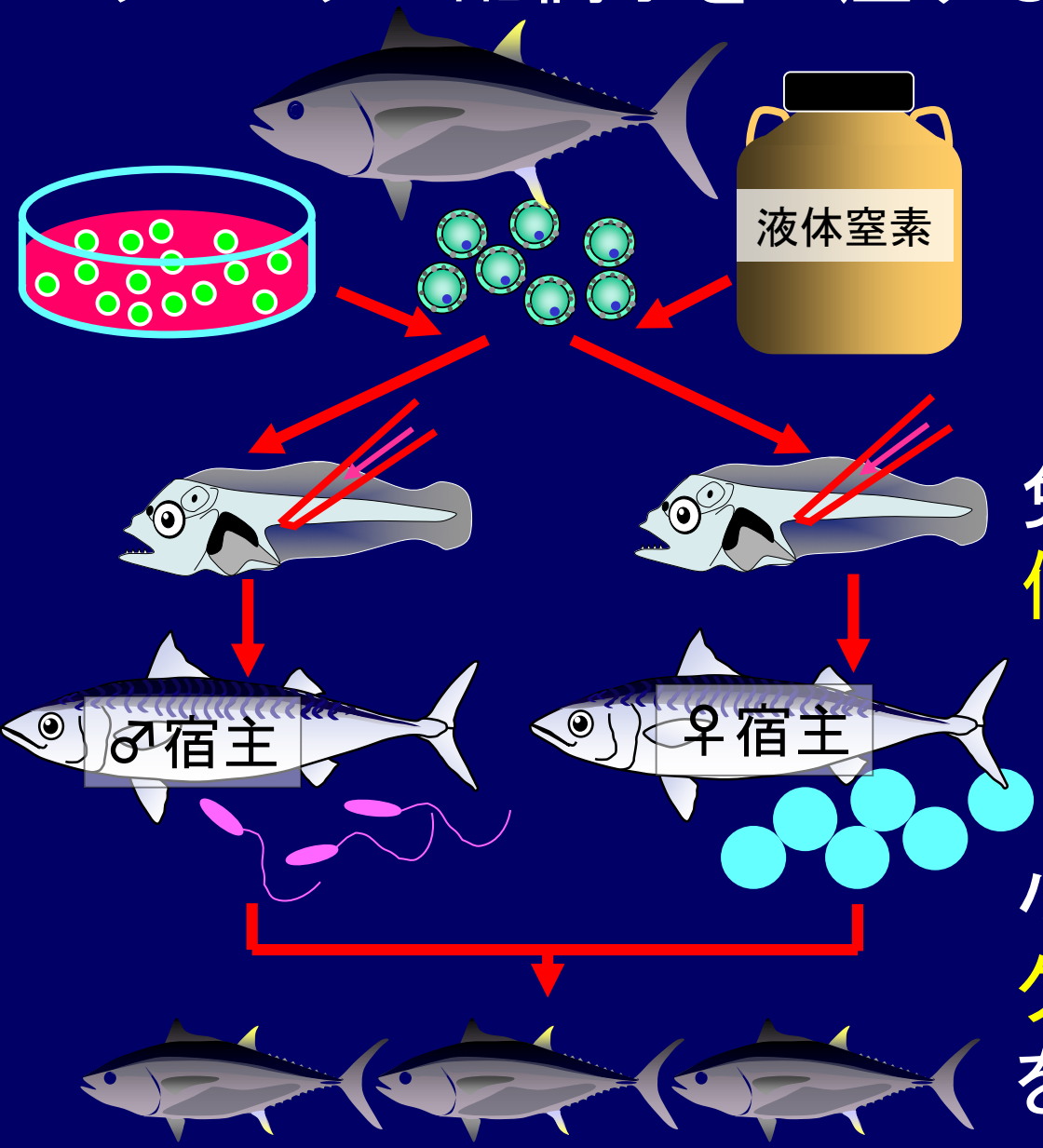
*スペース、労力、コストの節約

*環境調節→周年採卵

*育種の加速（JAPANブランドのマグロ品種の創出）

どのようにしてサバにマグロを生ませるか？

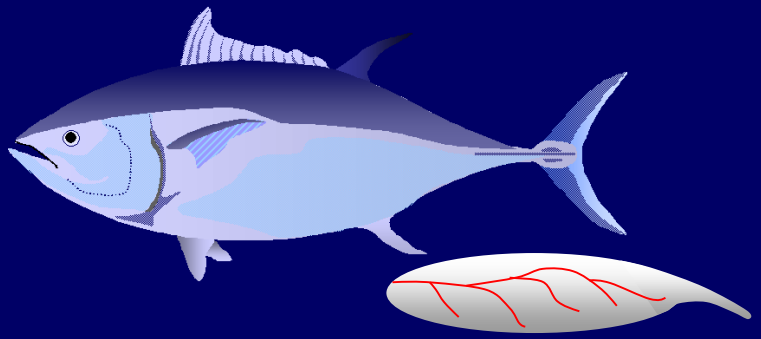
1.クロマグロ配偶子を生産する小型代理親魚の作出



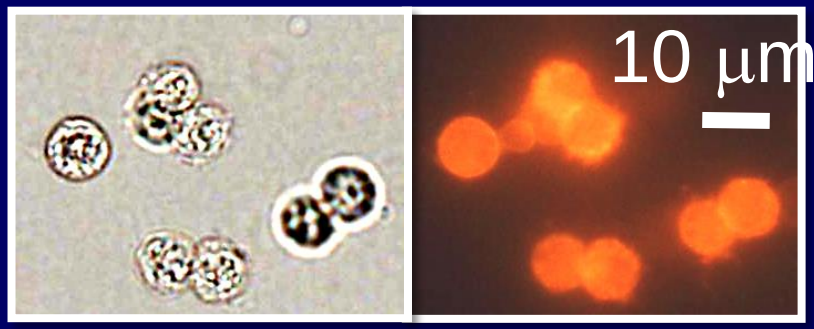
卵・精子のおおもとの細胞：生殖幹細胞

免疫的に未熟な孵化仔魚腹腔内へと移植

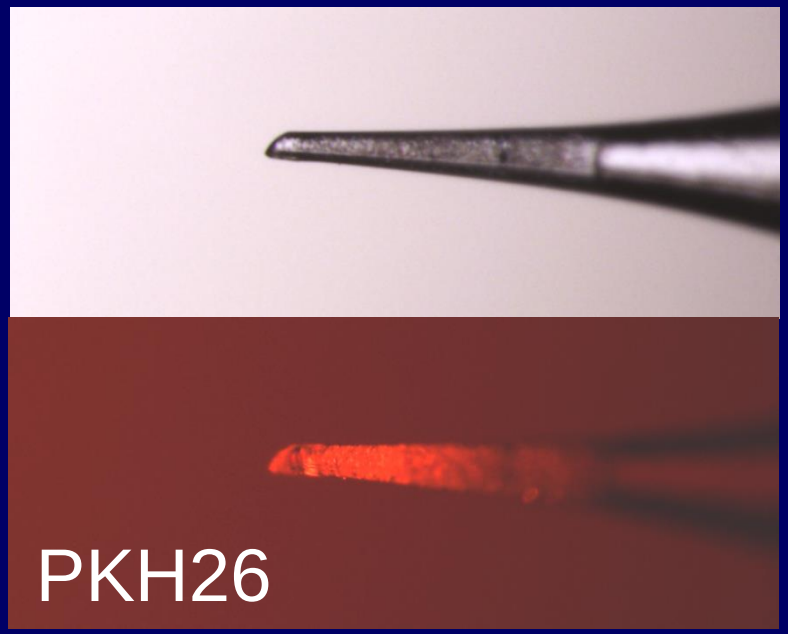
小型のサバ科魚類がクロマグロの配偶子を生産



ドナーマグロ



PKH26で標識した
クロマグロ精巣細胞

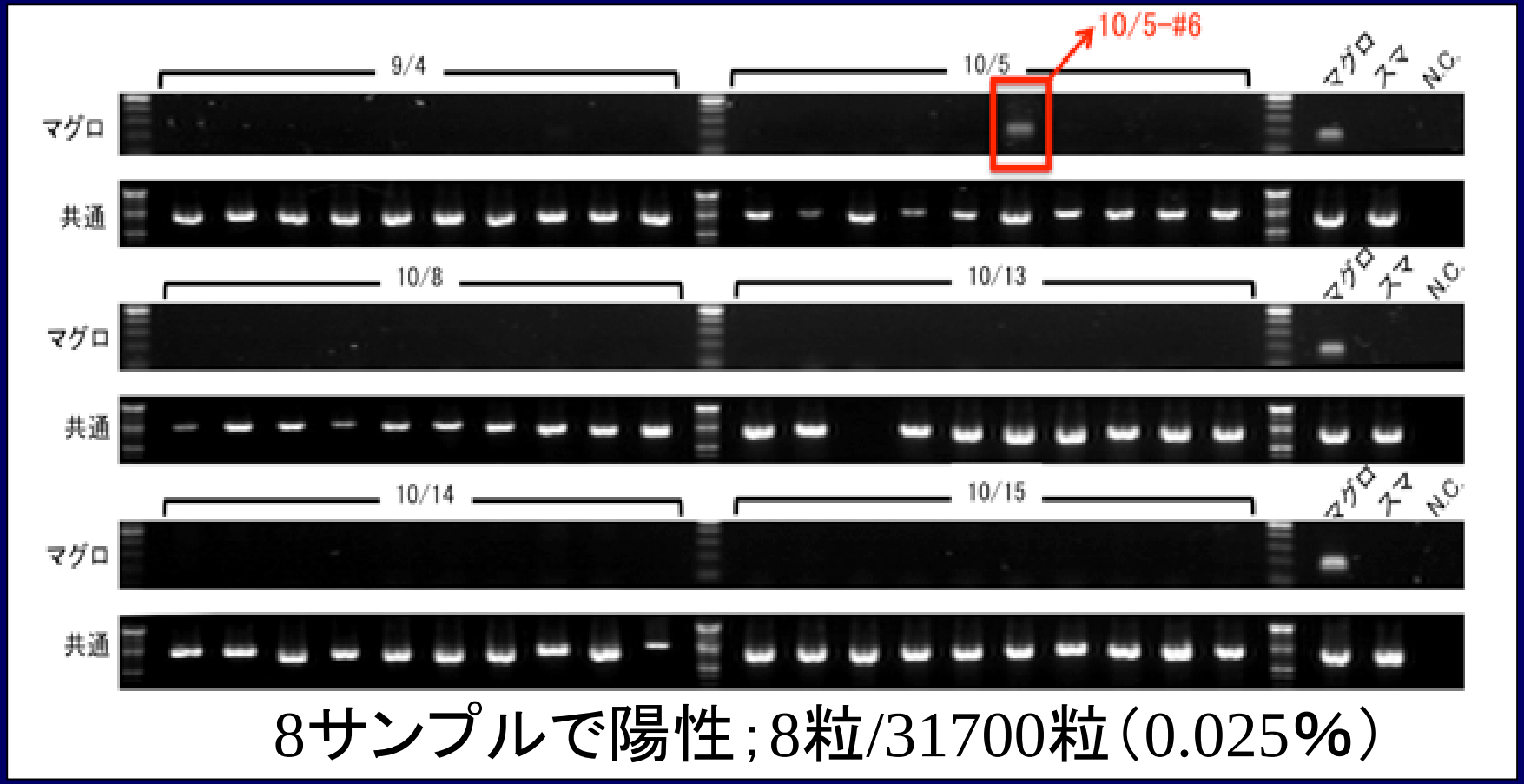


移植技術の改良・宿主種の選定

スマ宿主由来次世代のPCR解析

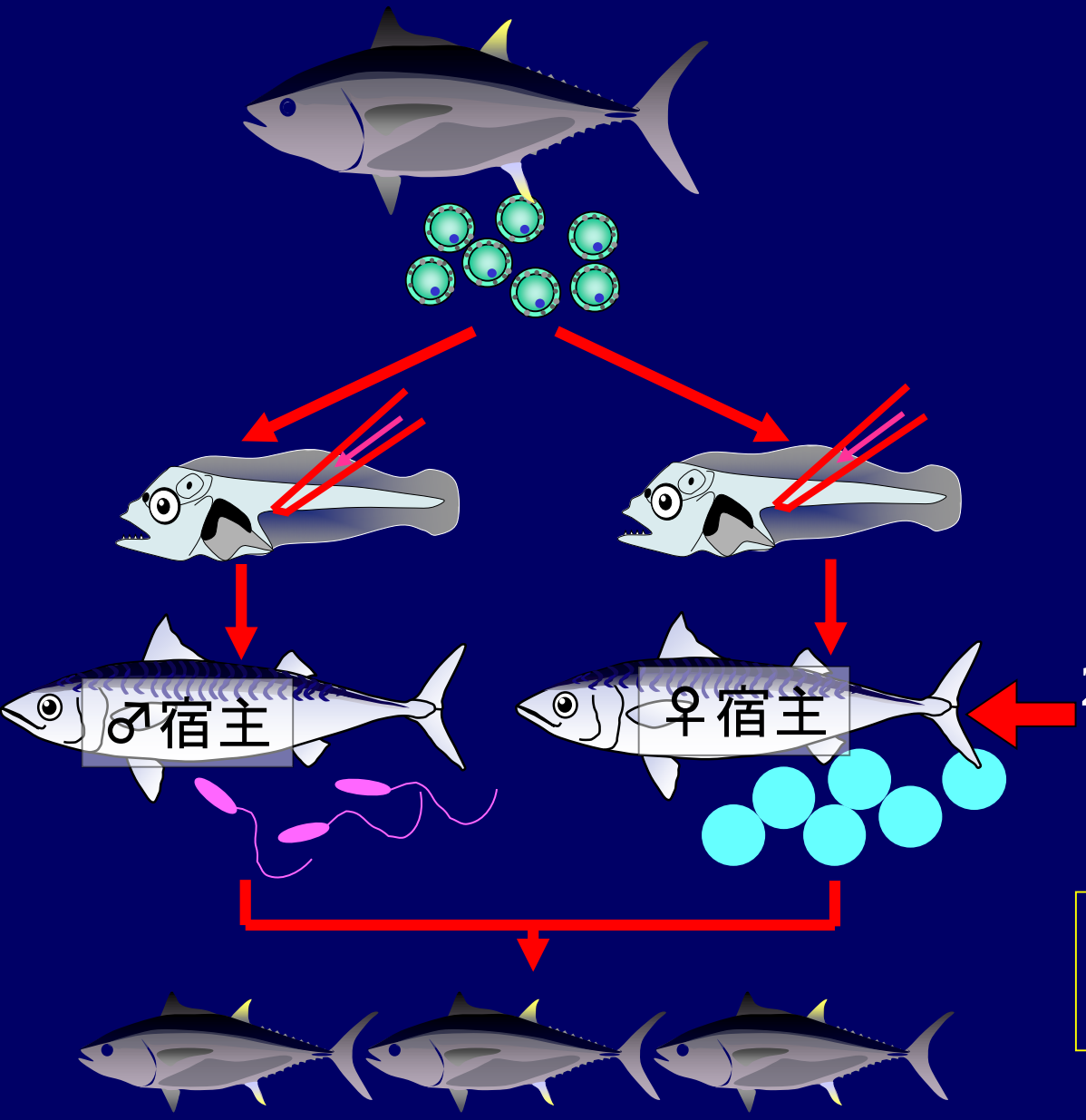
満一歳（♂4：♀5）

産卵日数：24日　　PCR：100粒プールで317回（31700粒）



8サンプルで陽性；8粒/31700粒（0.025%）

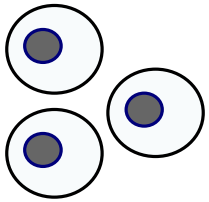
スマ宿主がクロマグロ配偶子を生産したことを強く示唆

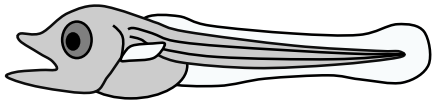


2.不妊宿主の作出とその
代理親魚としての利用

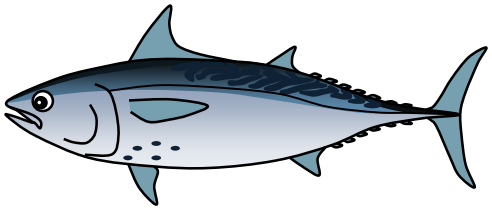
小型サバ科魚類の
三倍体化条件の至適化

三倍体出現頻度
70%

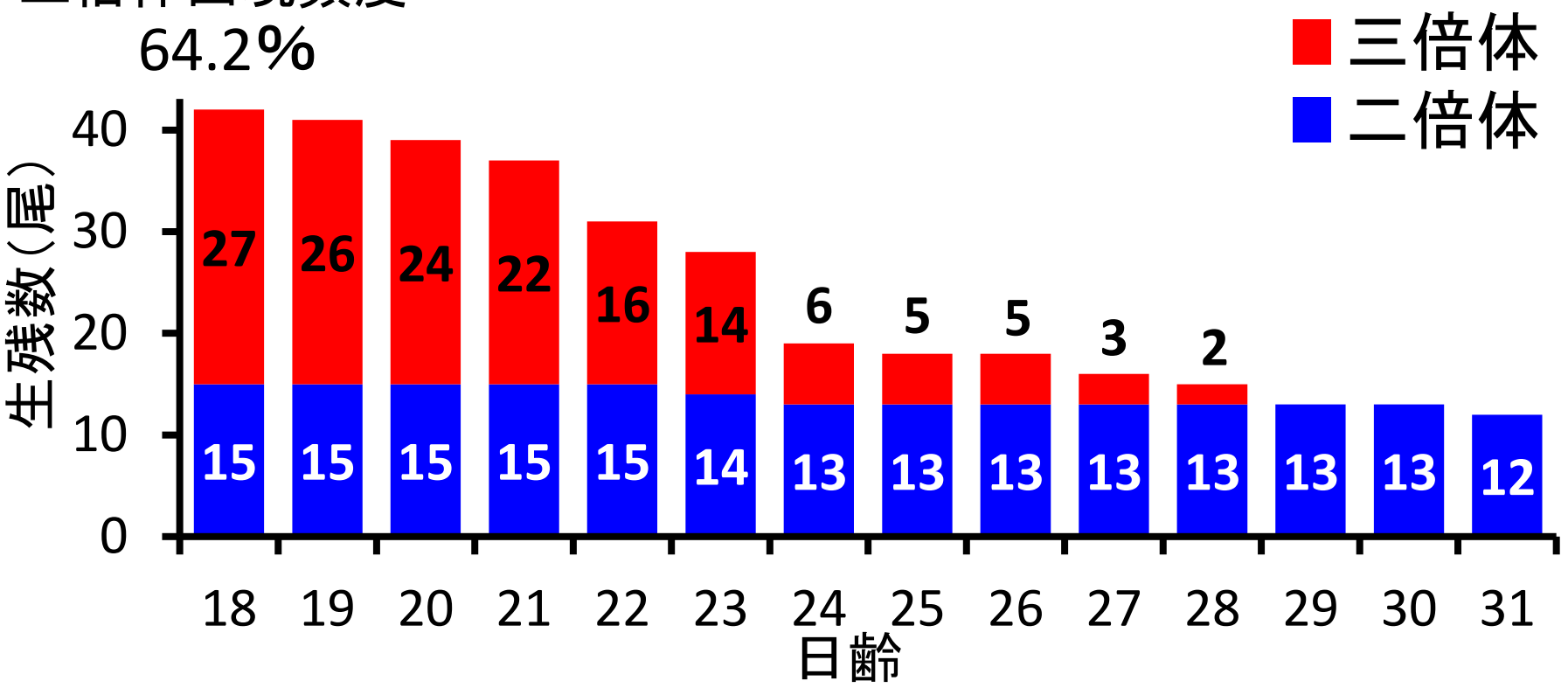




42-97日齢
三倍体 0%



三倍体出現頻度
64.2%



三倍体個体は、二倍体との競合に勝てない

高生残性の100%不妊集団を如何に作るか？

雑種不妊の利用！

↓

多くの雑種不妊は減数分裂不全が要因

そこで・・・

ゴマサバ♀

Scomber australasicus

×

マサバ♂

Scomber japonicus

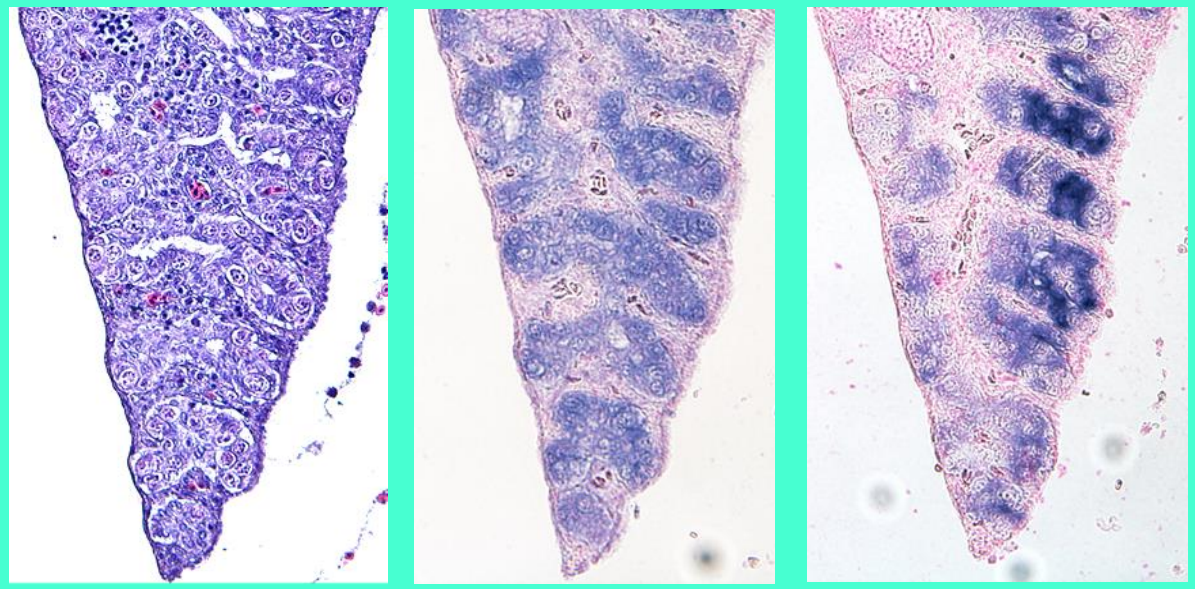
サケ科を用いた研究から・・・三倍体よりも生殖細胞を持たない宿主のほうが移植細胞は効率的に増殖、分化

HE

vasa
生殖細胞

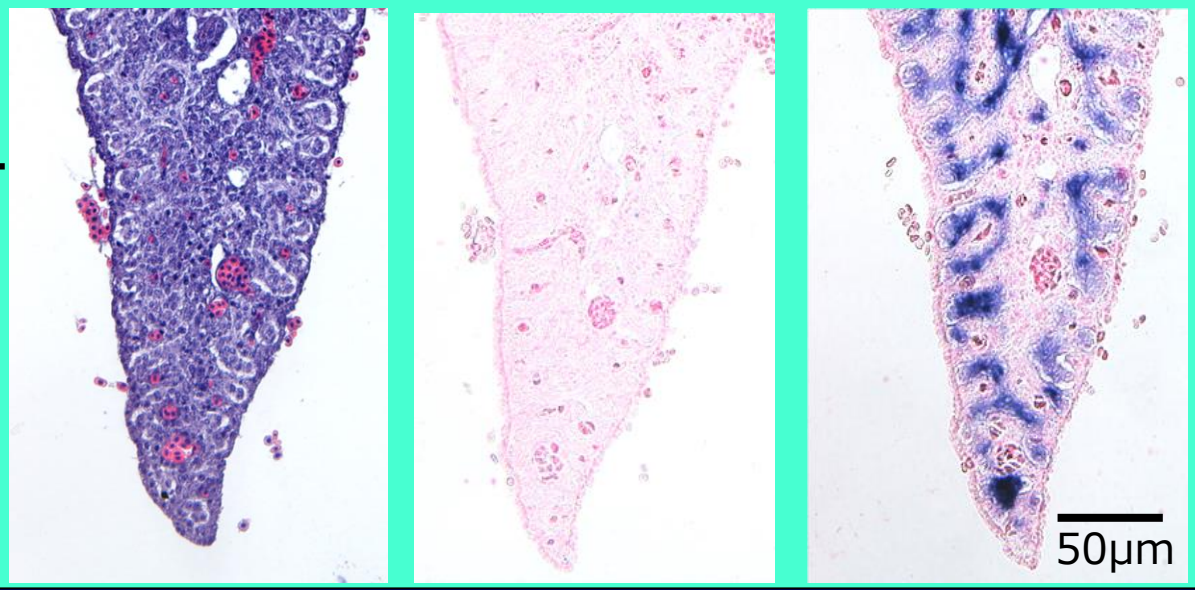
gsdf
支持細胞

ゴマサバ

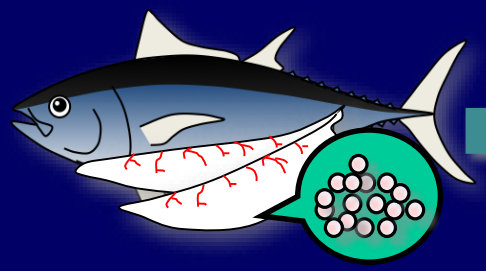


ゴマサバ♀
×
マサバ♂

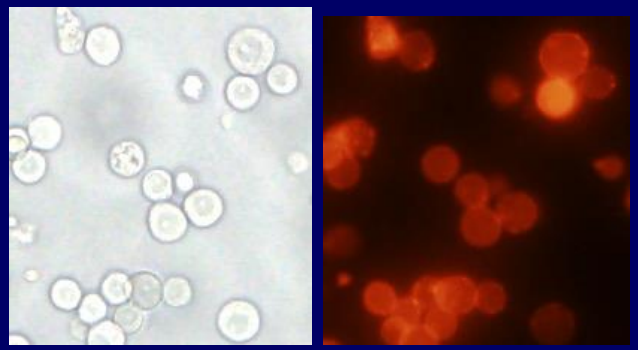
雑種



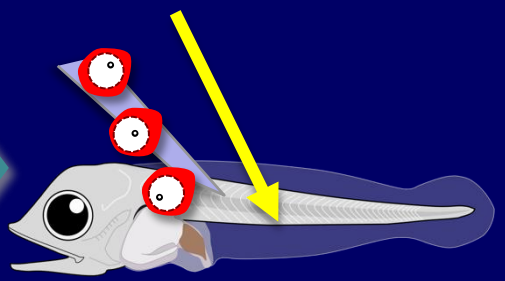
ゴマサバxマサバ宿主：移植後生殖腺の観察



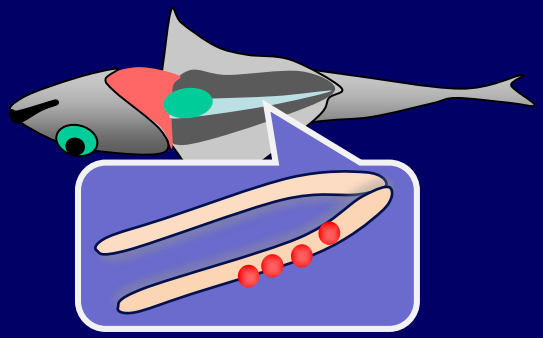
クロマグロ精巣細胞



PKH26による蛍光ラベル

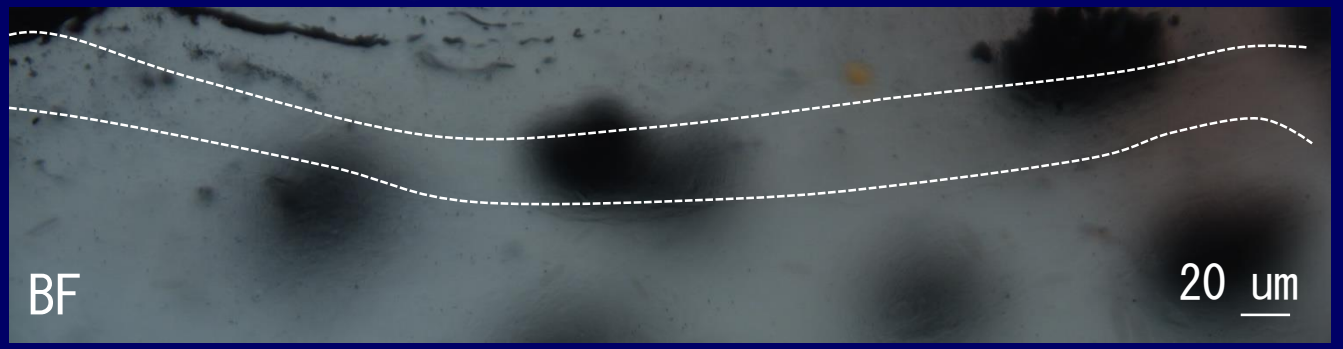


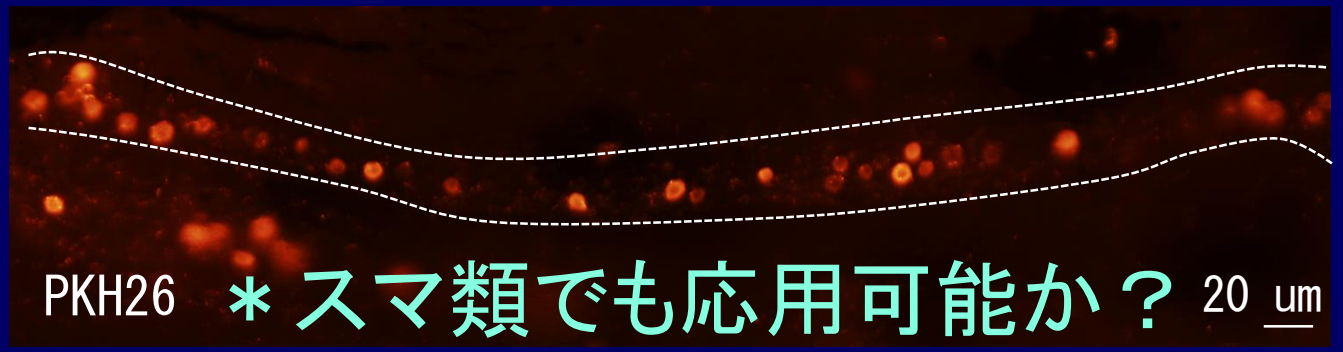
ゴマサバxマサバ
へ移植（10 dpf）



移植後２週間

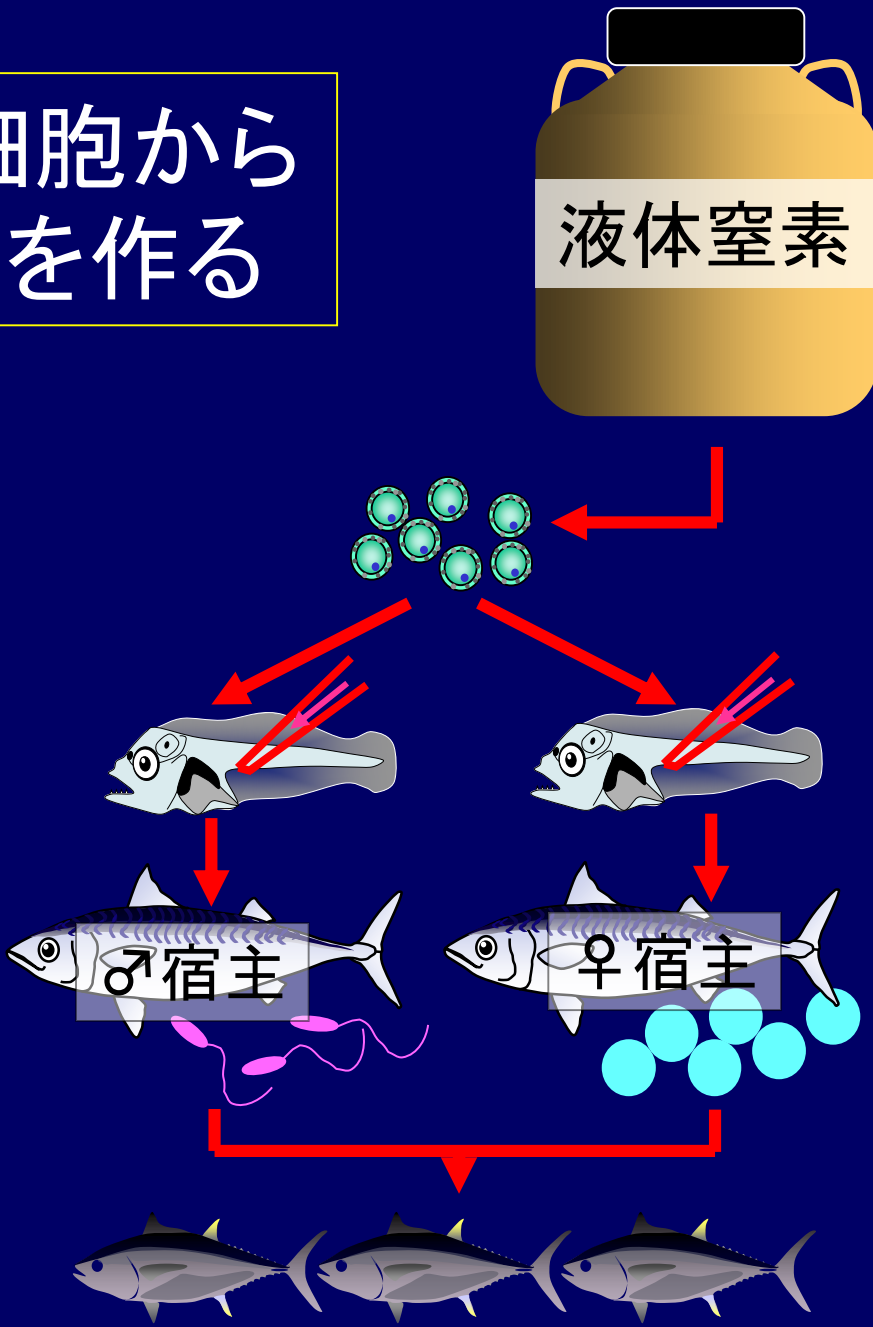
14/14で
取り込み





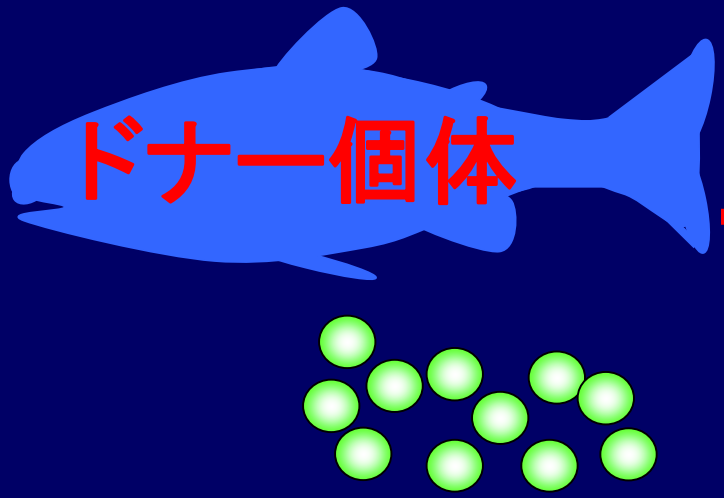
3.移植用生殖幹細胞の試験管内培養とその凍結保存

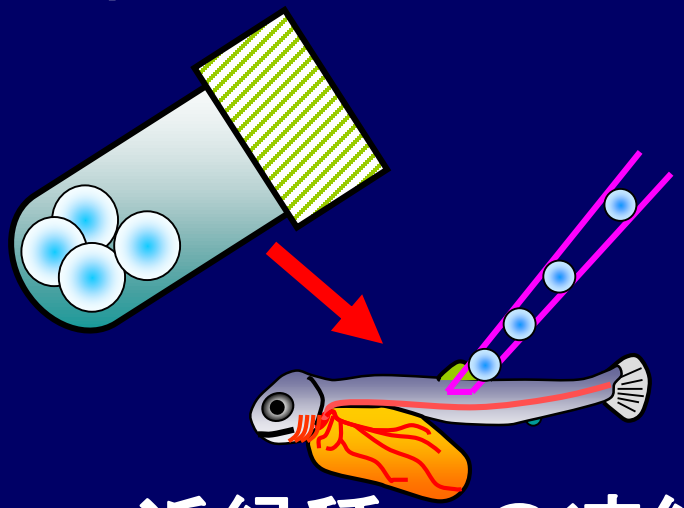
凍結細胞から
マグロを作る



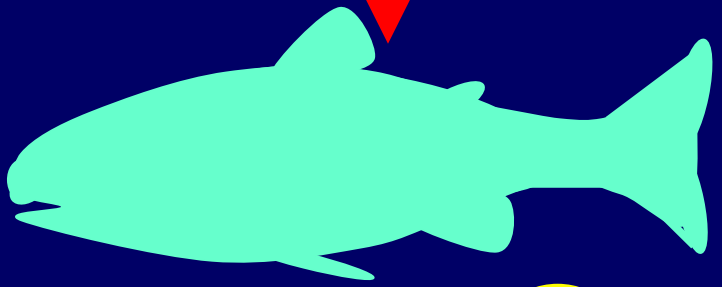
ニジマスをモデルに凍結精巣から個体を作出

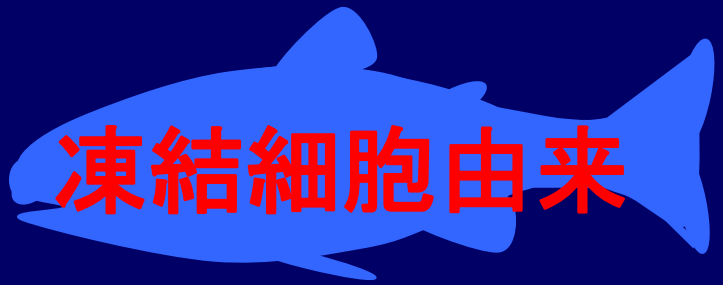
精巣の凍結保存

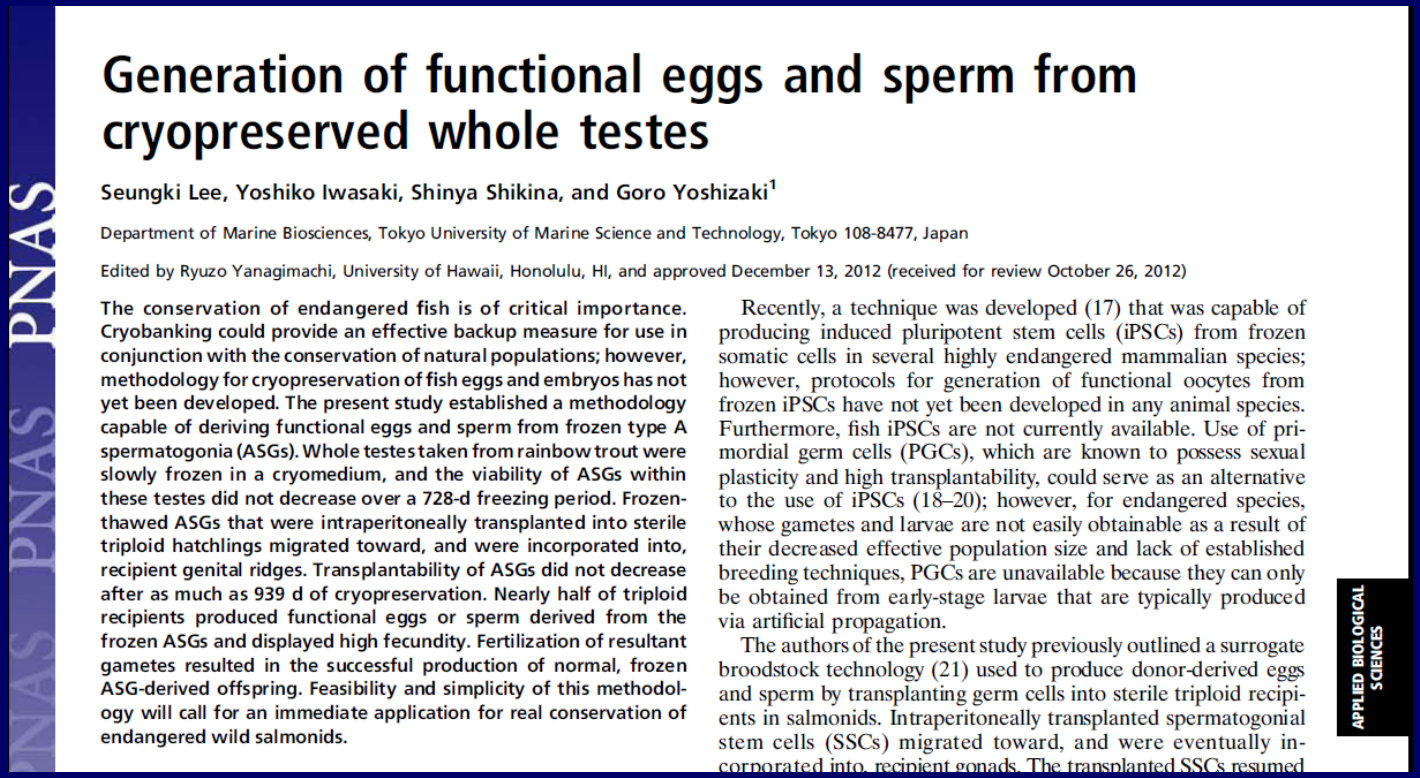




近縁種への凍結
精原細胞移植







Generation of functional eggs and sperm from cryopreserved whole testes

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Edited by Ryuzo Yanagimachi, University of Hawaii, Honolulu, HI, and approved December 13, 2012 (received for review October 26, 2012)

The conservation of endangered fish is of critical importance. Cryobanking could provide an effective backup measure for use in conjunction with the conservation of natural populations; however, methodology for cryopreservation of fish eggs and embryos has not yet been developed. The present study established a methodology capable of deriving functional eggs and sperm from frozen type A spermatogonia (ASGs). Whole testes taken from rainbow trout were slowly frozen in a cryomedium, and the viability of ASGs within these testes did not decrease over a 728-d freezing period. Frozen-thawed ASGs that were intraperitoneally transplanted into sterile triploid hatchlings migrated toward, and were incorporated into, recipient genital ridges. Transplantability of ASGs did not decrease after as much as 939 d of cryopreservation. Nearly half of triploid recipients produced functional eggs or sperm derived from the frozen ASGs and displayed high fecundity. Fertilization of resultant gametes resulted in the successful production of normal, frozen ASG-derived offspring. Feasibility and simplicity of this methodology will call for an immediate application for real conservation of endangered wild salmonids.

Recently, a technique was developed (17) that was capable of producing induced pluripotent stem cells (iPSCs) from frozen somatic cells in several highly endangered mammalian species; however, protocols for generation of functional oocytes from frozen iPSCs have not yet been developed in any animal species. Furthermore, fish iPSCs are not currently available. Use of primordial germ cells (PGCs), which are known to possess sexual plasticity and high transplantability, could serve as an alternative to the use of iPSCs (18–20); however, for endangered species, whose gametes and larvae are not easily obtainable as a result of their decreased effective population size and lack of established breeding techniques, PGCs are unavailable because they can only be obtained from early-stage larvae that are typically produced via artificial propagation.

The authors of the present study previously outlined a surrogate broodstock technology (21) used to produce donor-derived eggs and sperm by transplanting germ cells into sterile triploid recipients in salmonids. Intraperitoneally transplanted spermatogonial stem cells (SSCs) migrated toward, and were eventually incorporated into, recipient gonads. The transplanted SSCs resumed

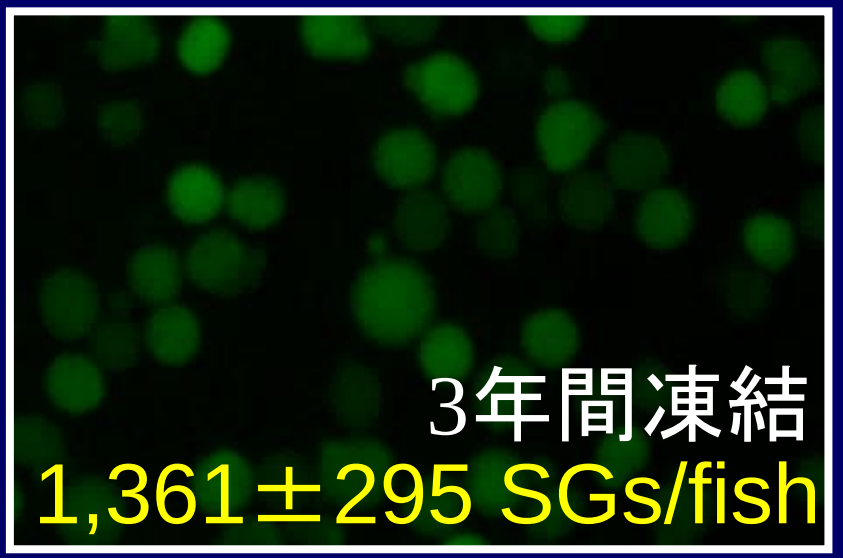
APPLIED BIOLOGICAL SCIENCES

2013年1月15日　日本経済新聞（科学面）・東京新聞（科学面）・毎日新聞（科学面）・読売新聞（科学面）・朝日新聞（科学面）・AFP通信（インターネット版）・ Times・NHK・民放各社

同様の方法でクロマグロ生殖細胞も凍結保存可能

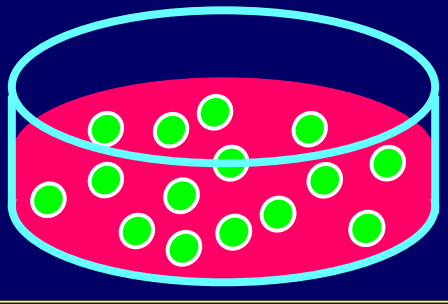
・20-200gの魚は精巣が緩慢凍結状態に
・魚類血清は高い凍結保護効果

ジップロップバッグに入れたまま超低温庫（-80℃）で凍結



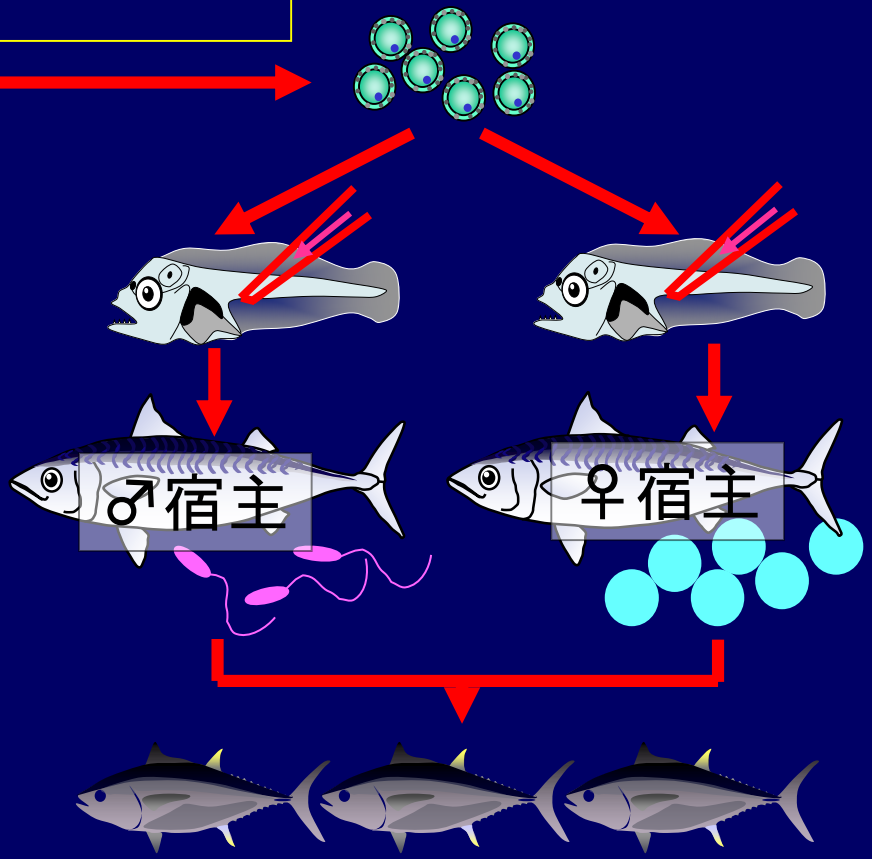
移植により機能的な卵精子ひいては個体に改変可能

3.移植用生殖幹細胞の試験管内培養とその凍結保存

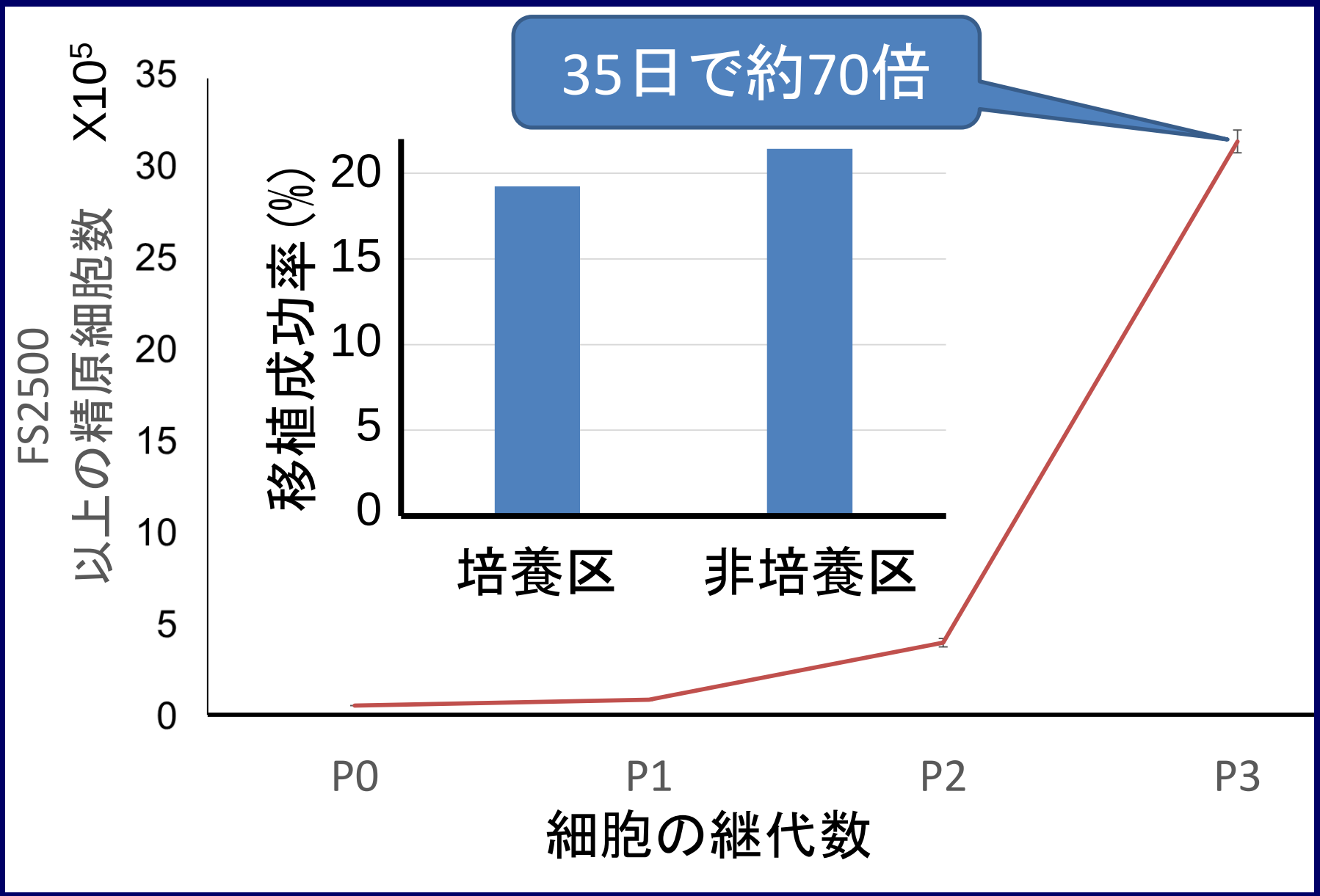


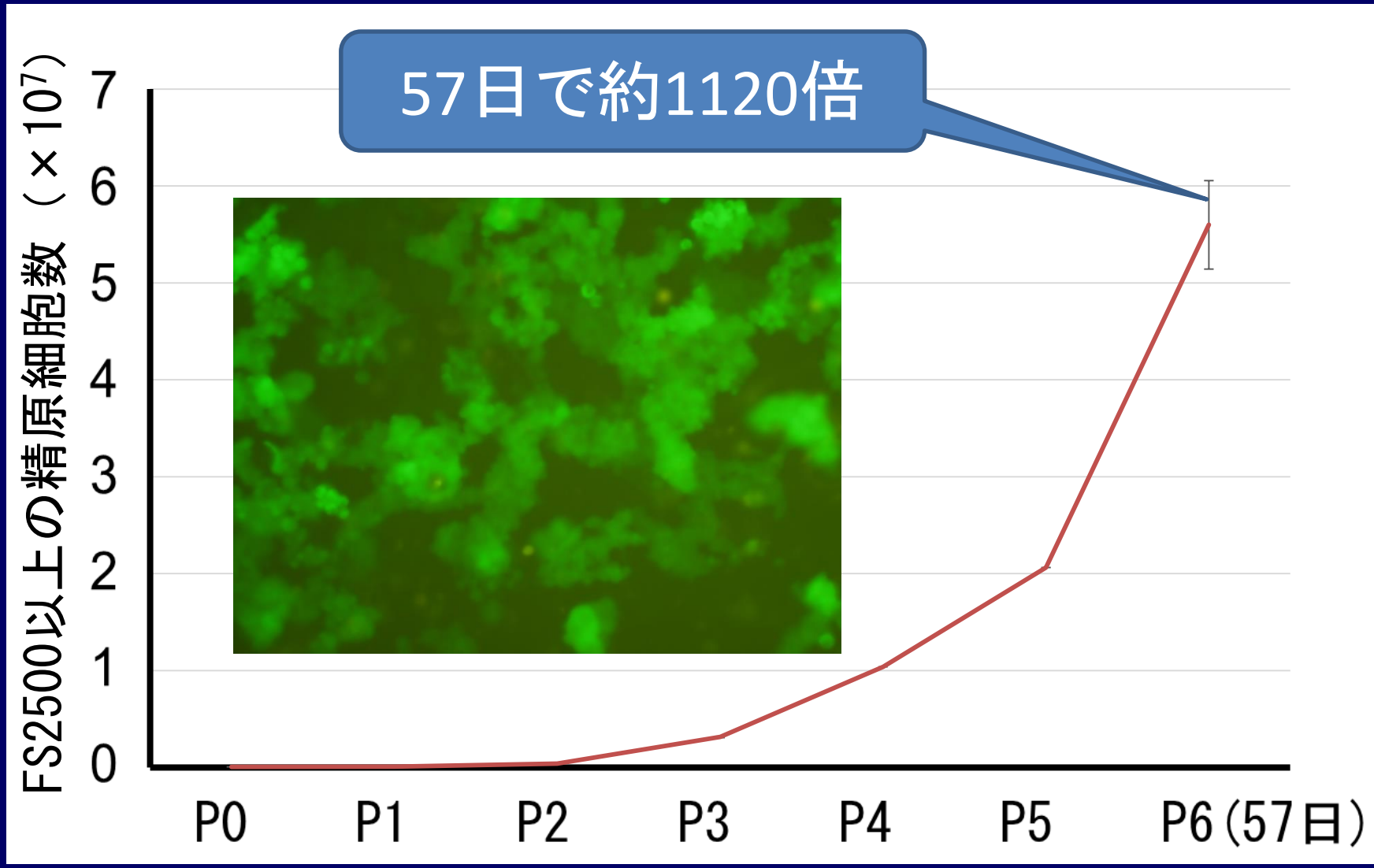
培養細胞からマグロを作る

宿主へと移植可能なニジマス生殖幹細胞の培養技術構築

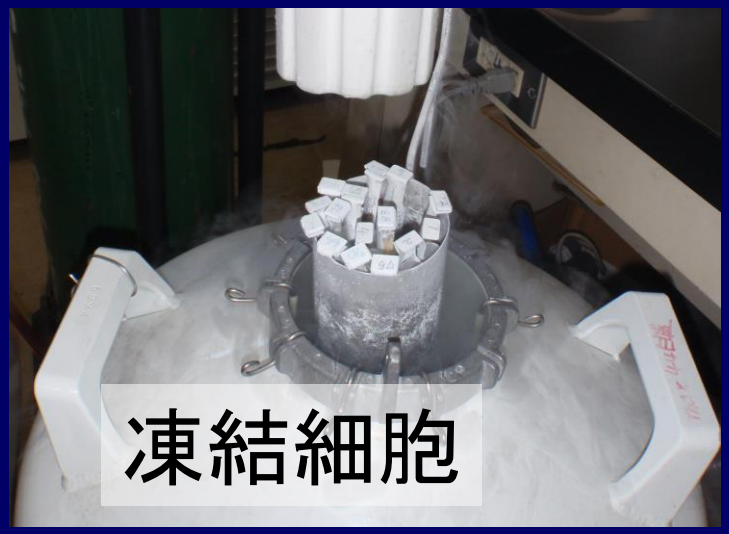


ニジマス精原細胞のin vitro培養と移植





培養細胞から魚を作ることが現実的に・・・
（クロマグロへと応用中）



個々の基盤技術、基礎情報は充実、如何に組み合わせるか・・・　飼育施設（含インフラ）、
マンパワー（PD, teck＋安定した雇用体制）