# Current situation in stock enhancement of barfin flounder in Japan


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# CONTENTS

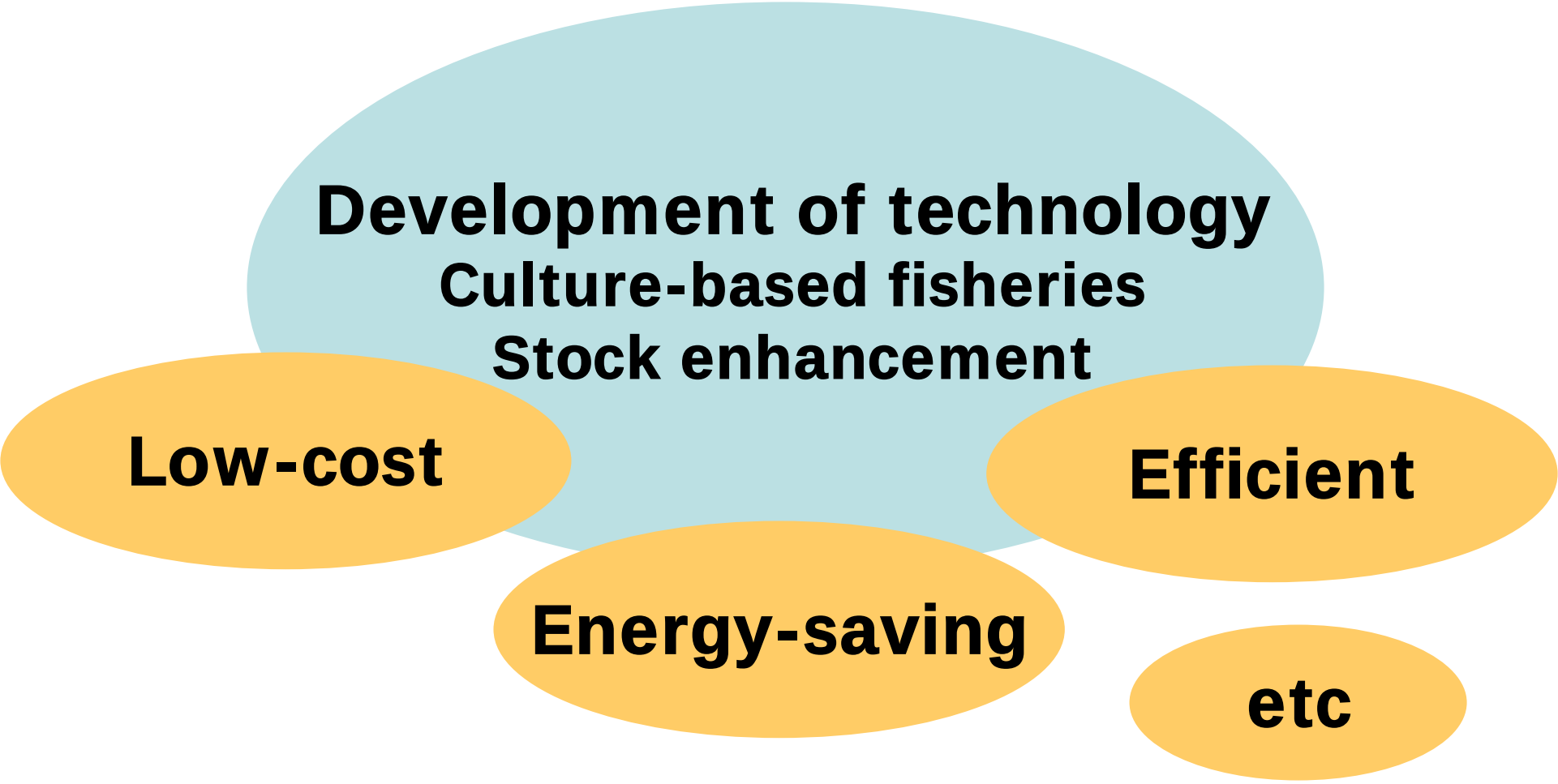
- For the Eco-Development
  (Earth-friendly and Fish-friendly way)
- Technique for maintaining genetic diversity in stock enhancement
- Current situation in stock enhancement of flatfish(Barfin Flounder) in Japan

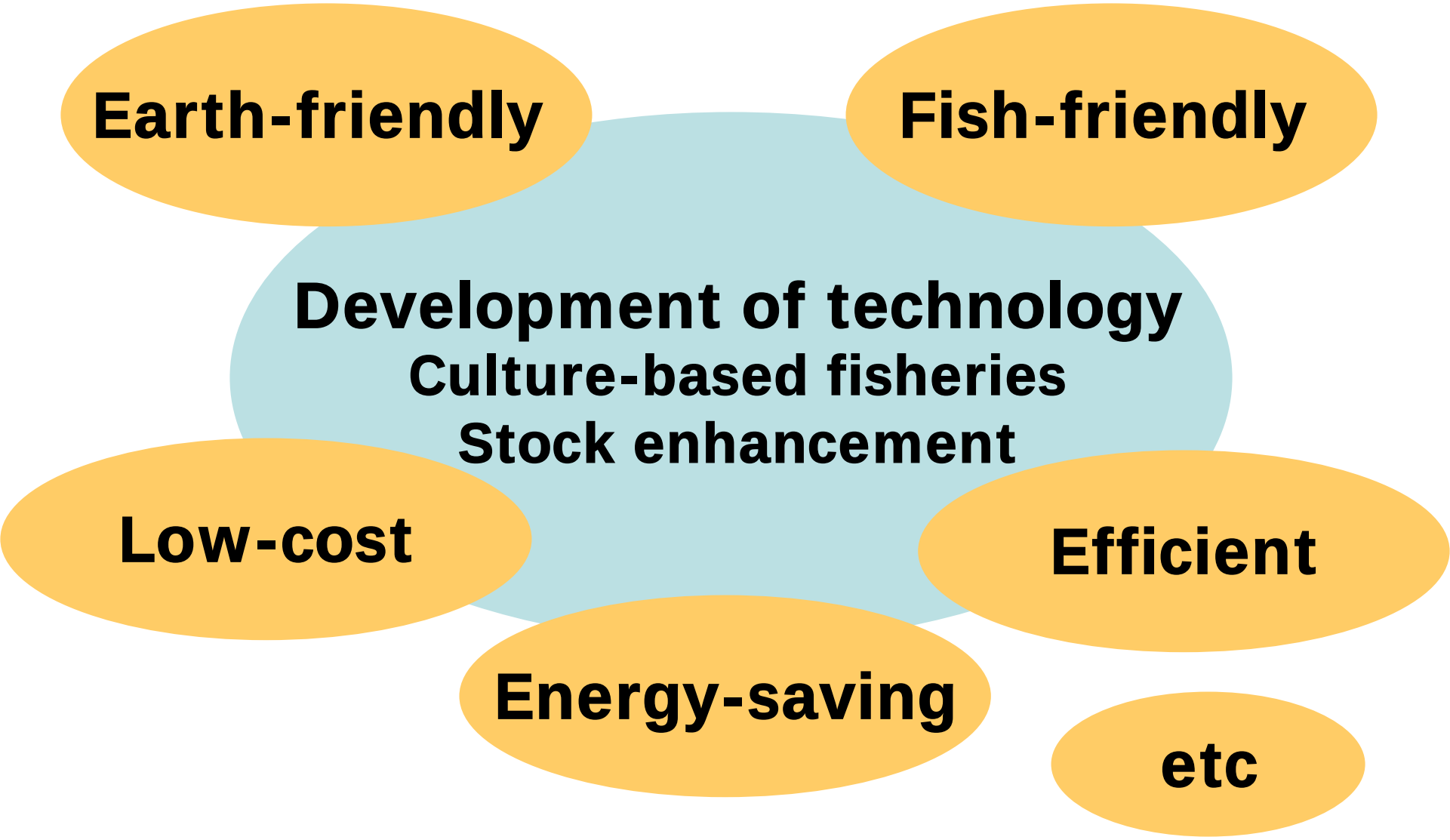

Salmon


Prawn

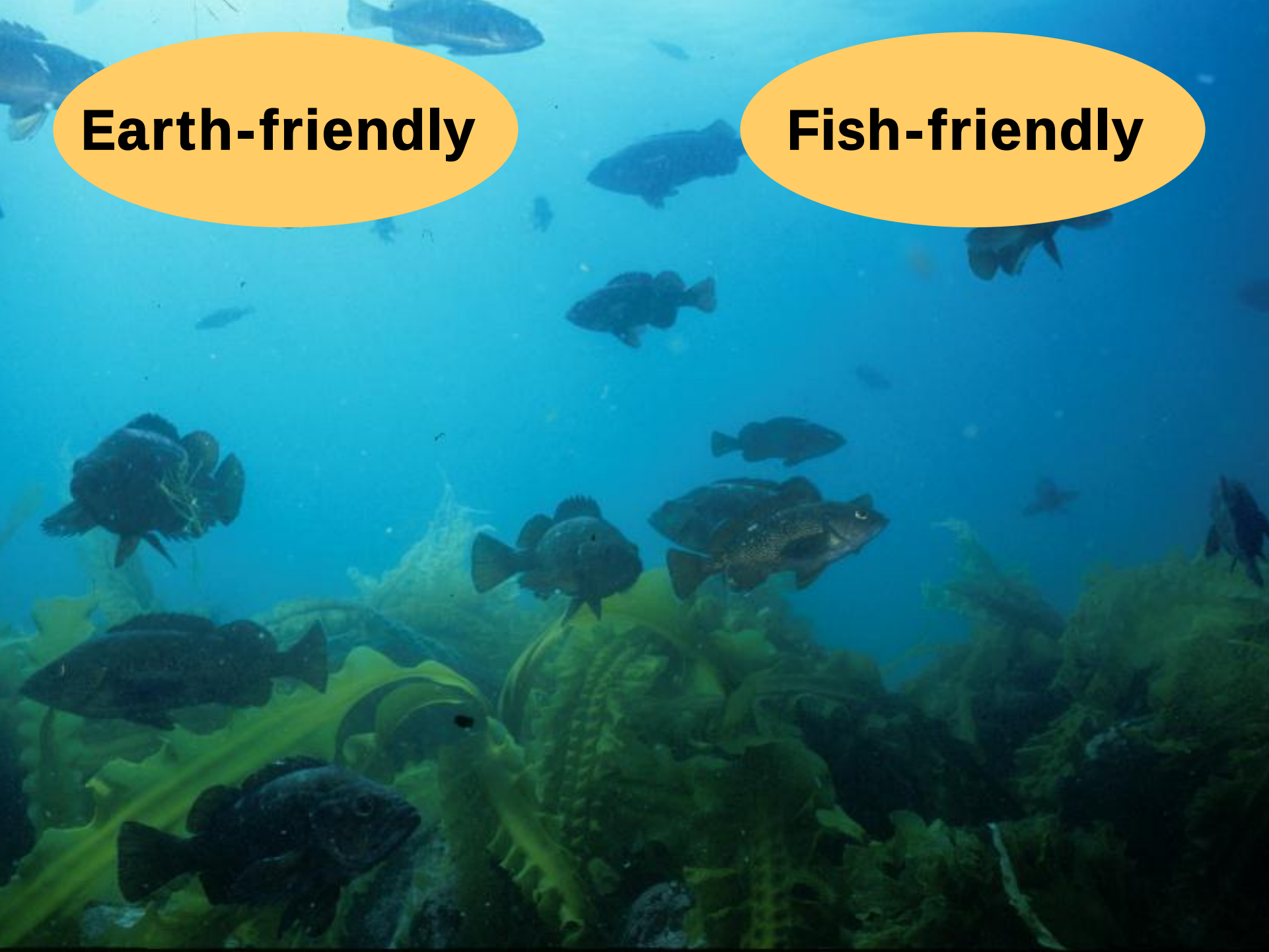

Kelp


Scallop


Development of technology
Culture-based fisheries
Stock enhancement
Low-cost
Efficient
Energy-saving
etc




**We must develop well-balanced Eco-friendly techniques**


Earth-friendly
Fish-friendly

**Earth-friendly**

**Fish-friendly**

***Environmental rehabilitation technologies**

**regeneration**

***Fisheries management technologies**

**(for assurance of reproduction)**

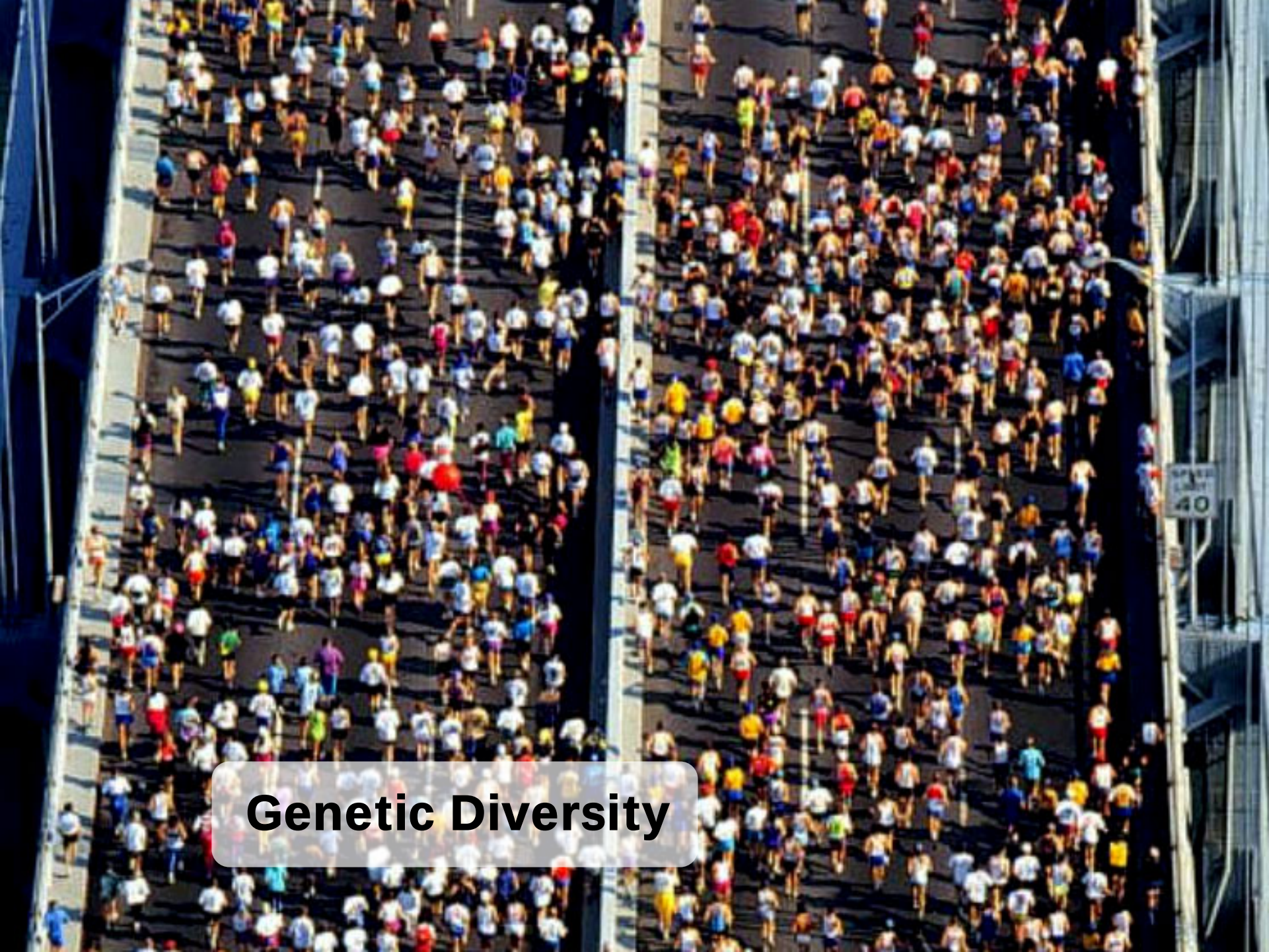
***Maintenance technologies of diversity**

**Environmental diversity**

**Species diversity**

**Genetic diversity**

***and so on**


Genetic Diversity

# Genetic Diversity

- **Genetic diversity is necessary for populations to evolve and to adapt to environmental change.**

- **The inherent capability of organisms is indispensable to maintain stable viability.**

# Technique for maintaining genetic diversity in stock enhancement

## Introduction

Endangered species “Matsukawa”

## Broodstock management

Progeny selection with assistance of microsatellite DNA markers

## Seed production

Mating technique for maintaining genetic diversity

# Brafin Flounder

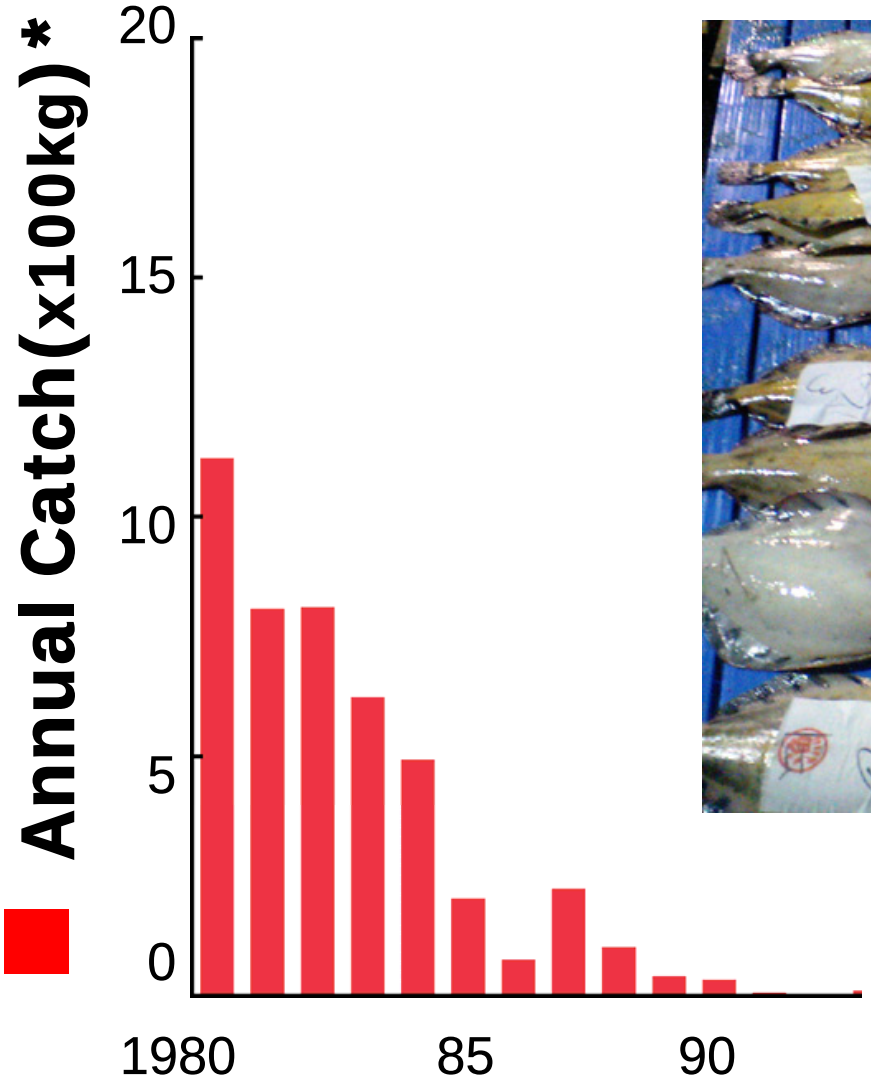
*Verasper* moseri

"Matsukawa" in Japanese

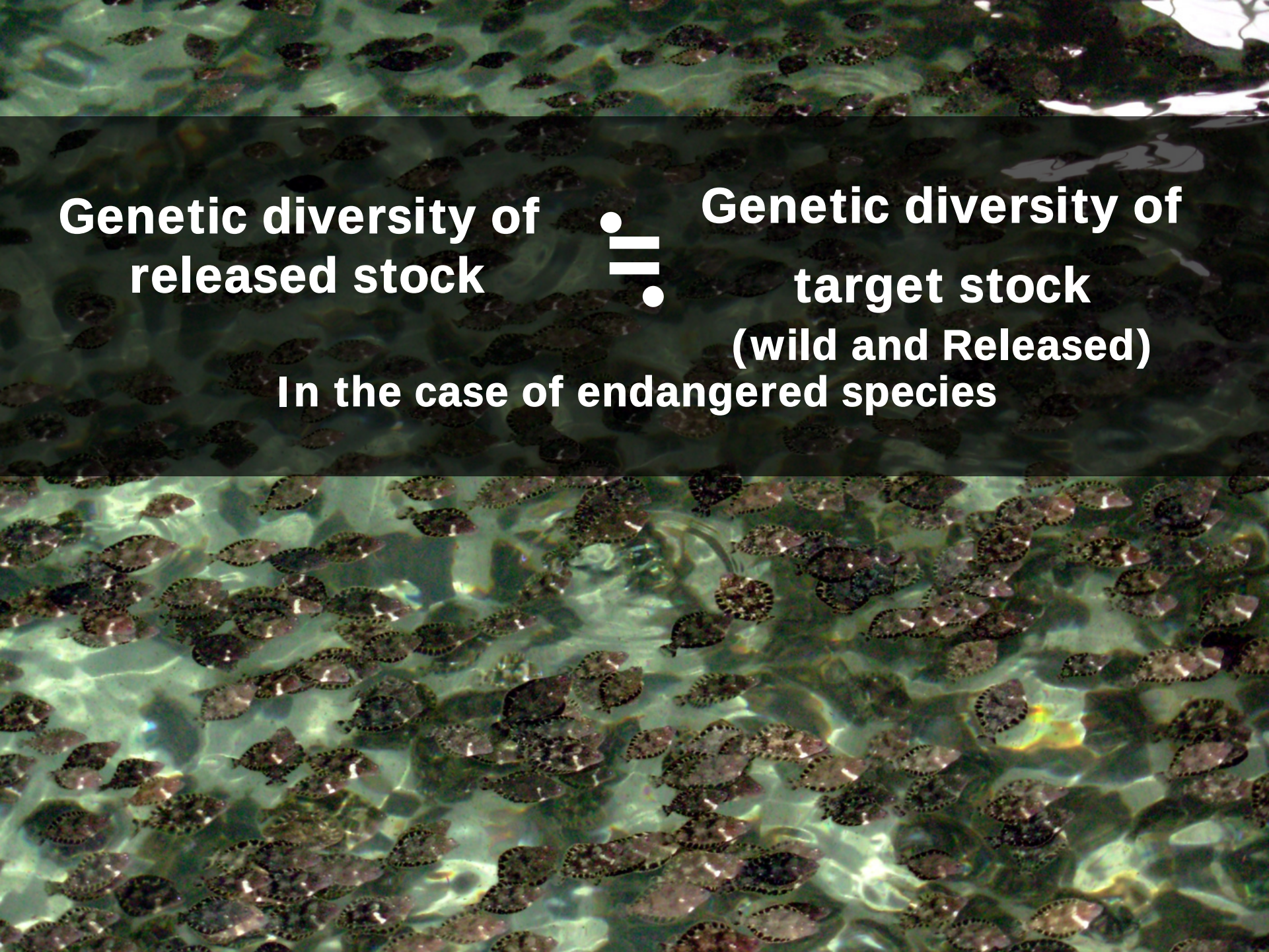
**Large Flatfish**
**Tasty Fish**
**High-Value Fish**

**Endangered Species**

# Trend in Annual Catch of Matsukawa




*Total catch of Fisheries Cooperative Association of Hiroo, Mitsuishi and Nemurowan-Chubu.


Genetic diversity of released stock
÷
Genetic diversity of target stock
(wild and Released)
In the case of endangered species

# Number of captive wild Matsukawa is only 37!

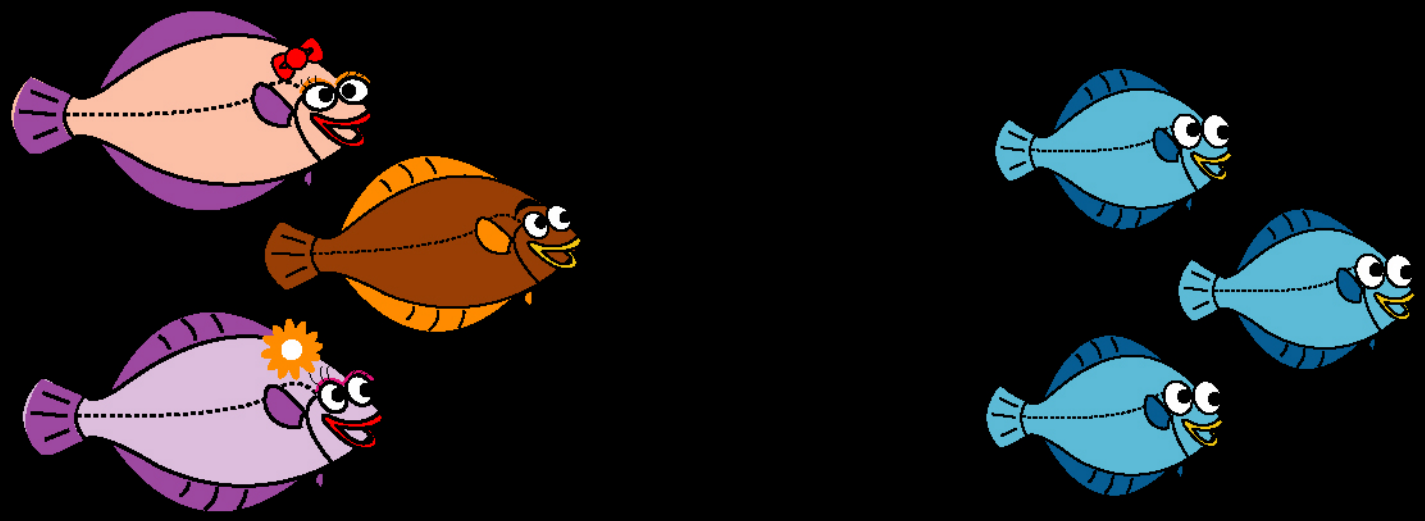
# Information of microsatellite DNA markers developed for "Matsukawa"

| Locus | Primer pair sequence(5' -3') | Repeat sequence | No.of observed alleles | Size range (bp) |
|---|---|---|---|---|
| *Vmo2* | F: ATGCTTTTACTCACGGCTCAGT<br>R: AGTGGCAGTTCATTTGGATTC | $(CA)_{17}$ | 33 | 189-318 |
| *Vmo17* | F: GTGACCTGCCTCCGAATA<br>R: ACTGTCGCTTCAAGATTTCAA | $(GA)_{29}$ | 27 | 167-242 |
| *Vva7* | F: GGTGCTTGCGTTCTTGG<br>R: ACAAAGGCTTCAATCAGGATG | $(GT)_{12}TT(GT)_{2}$ | 24 | 148-286 |

Maria Del Mar Ortega-Villaizan Romo (2003)

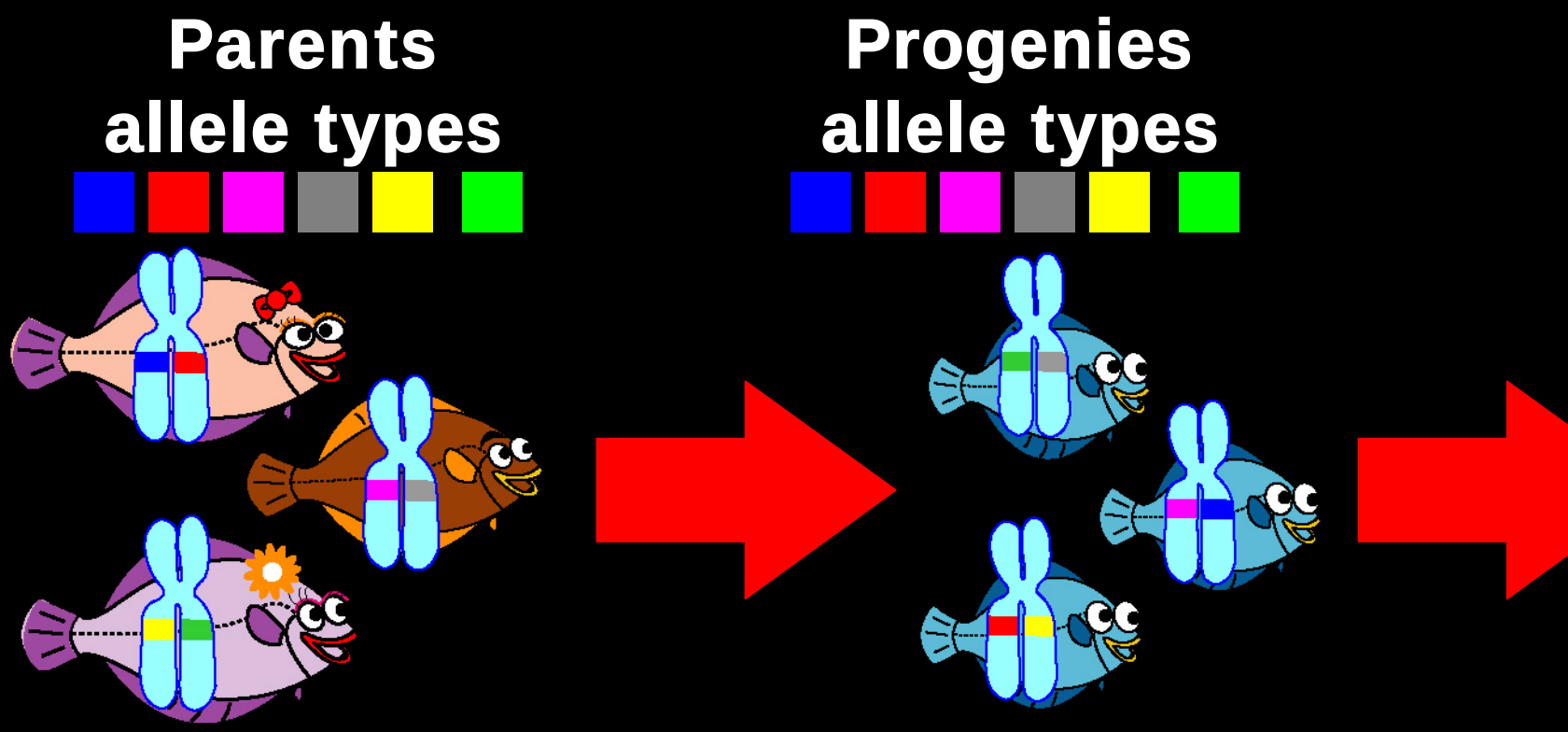
# Broodstock management with assistance of microsatellite DNA marker

# Strategy for Broodstock Management



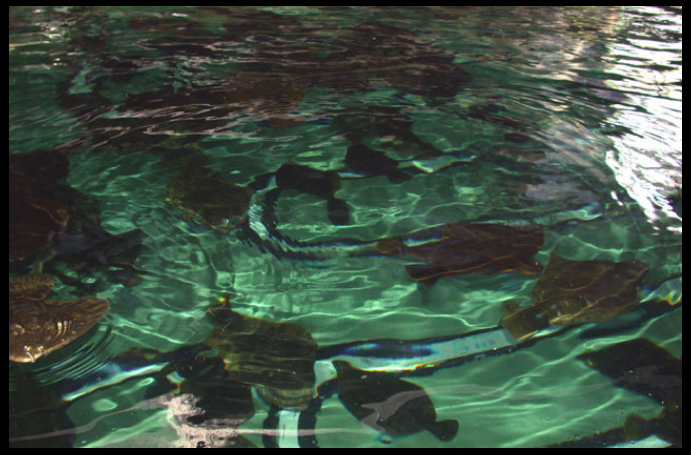
# Strategy for Broodstock Management

## Conservation of allelic diversity



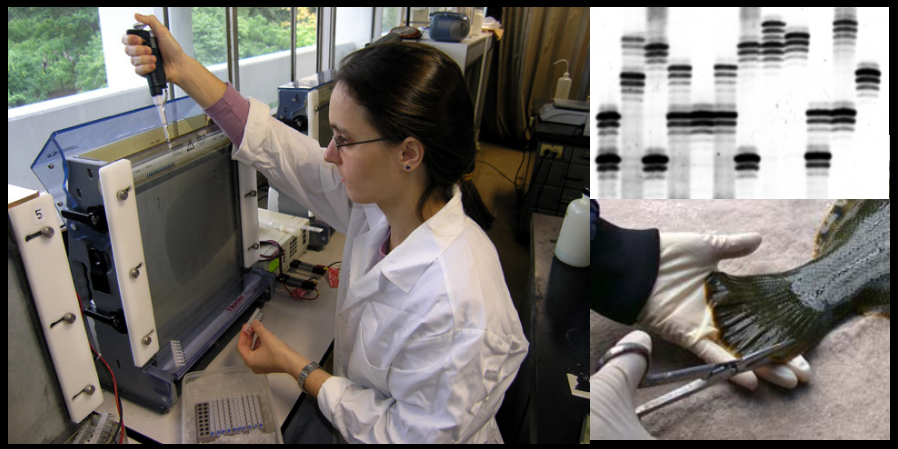
# Progeny Selection for conservation of allelic diversity

## Rearing of Artificial Seeds

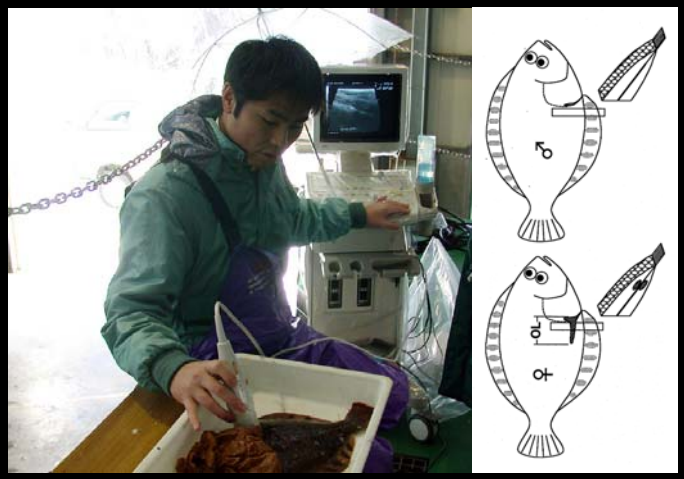


## Tagging for Individual Identification

## Genotyping Using msDNA Markers



## Ultrasonographic Sexing

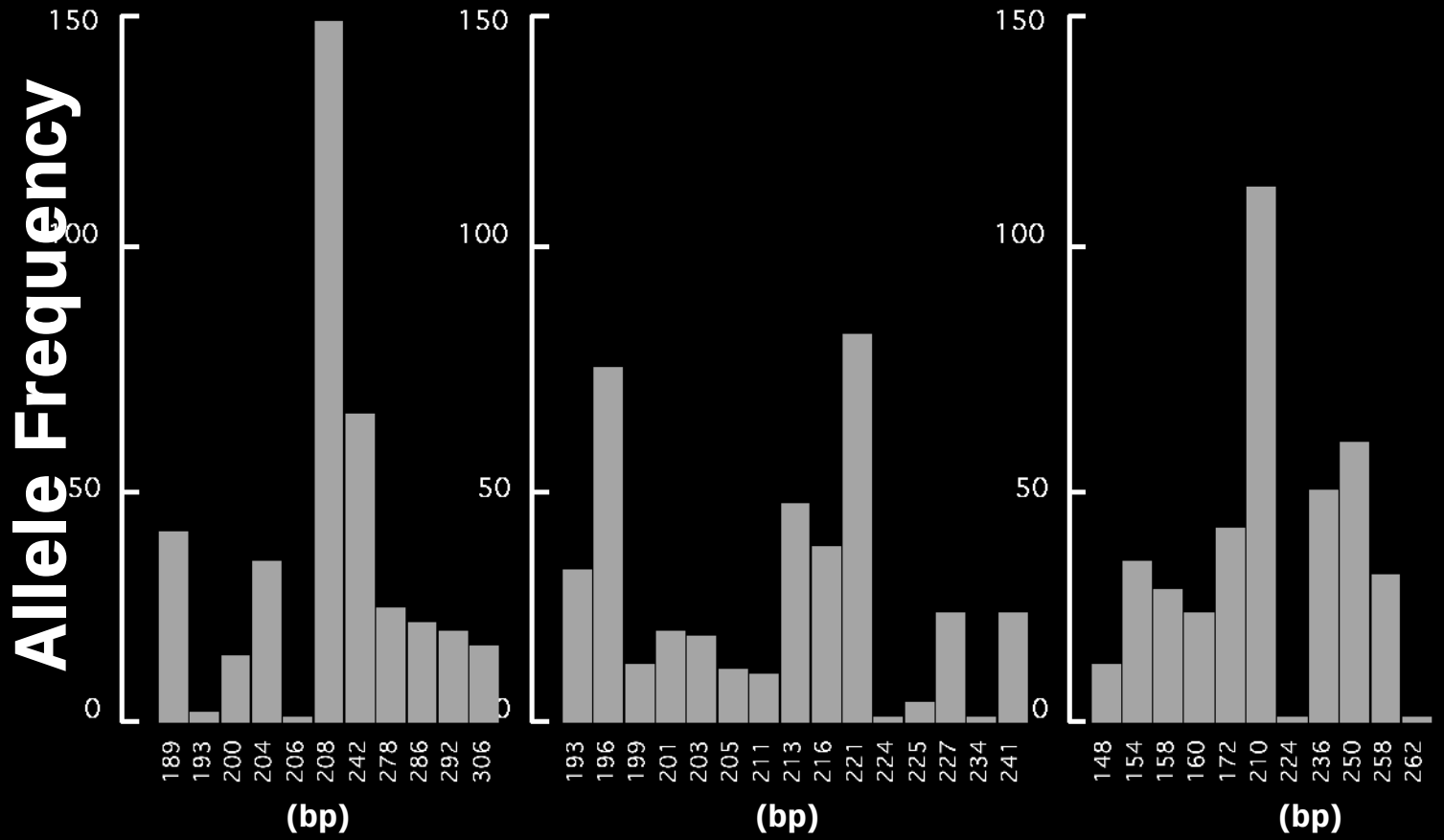


# Data on progeny selection

| | ID | sex | Allele Type(bp) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Vmo2 | | Vva7 | | Vmo17 | |
| 1 | 1 D 1 E | Female | 193 | 278 | 158 | 262 | 225 | 224 |
| 2 | 6 7 1 B | Male | 204 | 208 | 154 | 172 | 216 | 216 |
| 3 | 0 8 0 2 | Male | 208 | 306 | 158 | 210 | 199 | 199 |
| 4 | 4 4 5 E | Female | 208 | 306 | 158 | 236 | 196 | 196 |
| 5 | 4 A 1 5 | Male | 278 | 306 | 158 | 210 | 205 | 201 |
| 6 | 2 7 0 F | Female | 278 | 306 | 210 | 258 | 201 | 196 |
| 7 | 0 F 1 4 | Male | 208 | 306 | 250 | 258 | 205 | 199 |
| 8 | 7 B 5 B | Female | 278 | 306 | 210 | 258 | 205 | 199 |
| 9 | 6 4 2 C | Male | 208 | 278 | 158 | 250 | 205 | 199 |
| 10 | 2 5 7 9 | Male | 208 | 242 | 148 | 258 | 203 | 196 |
| . | . | . | . | . | . | . | . | . |
| . | . | . | . | . | . | . | . | . |
| 196 | 4 C 2 A | Male | 204 | 286 | 160 | 172 | 241 | 193 |
| 197 | 6 5 3 E | Female | 286 | 292 | 154 | 236 | 241 | 193 |

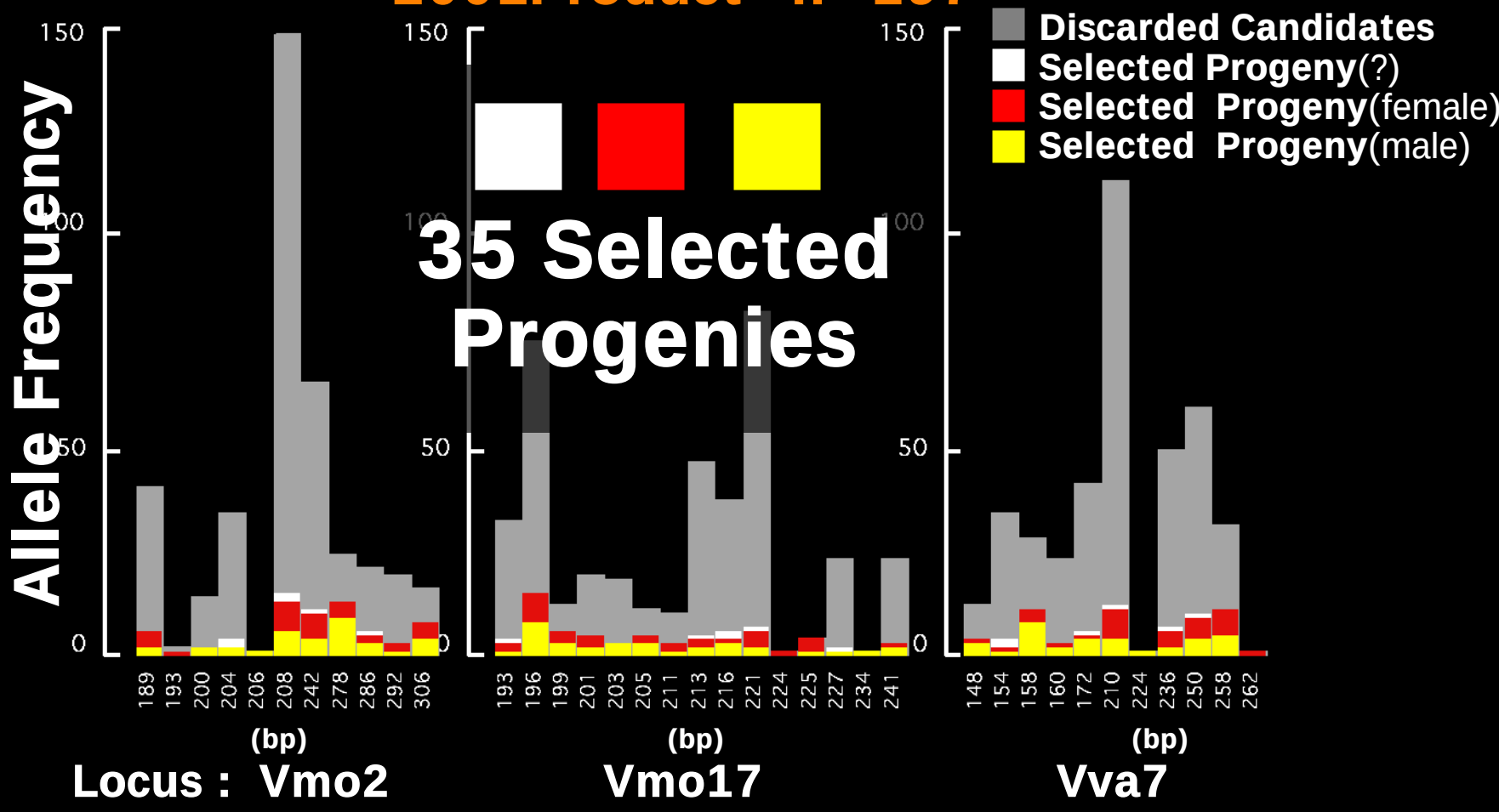
# Allele frequency distribution at 3 msDNA loci of candidate for progeny

## 2001Product n=197

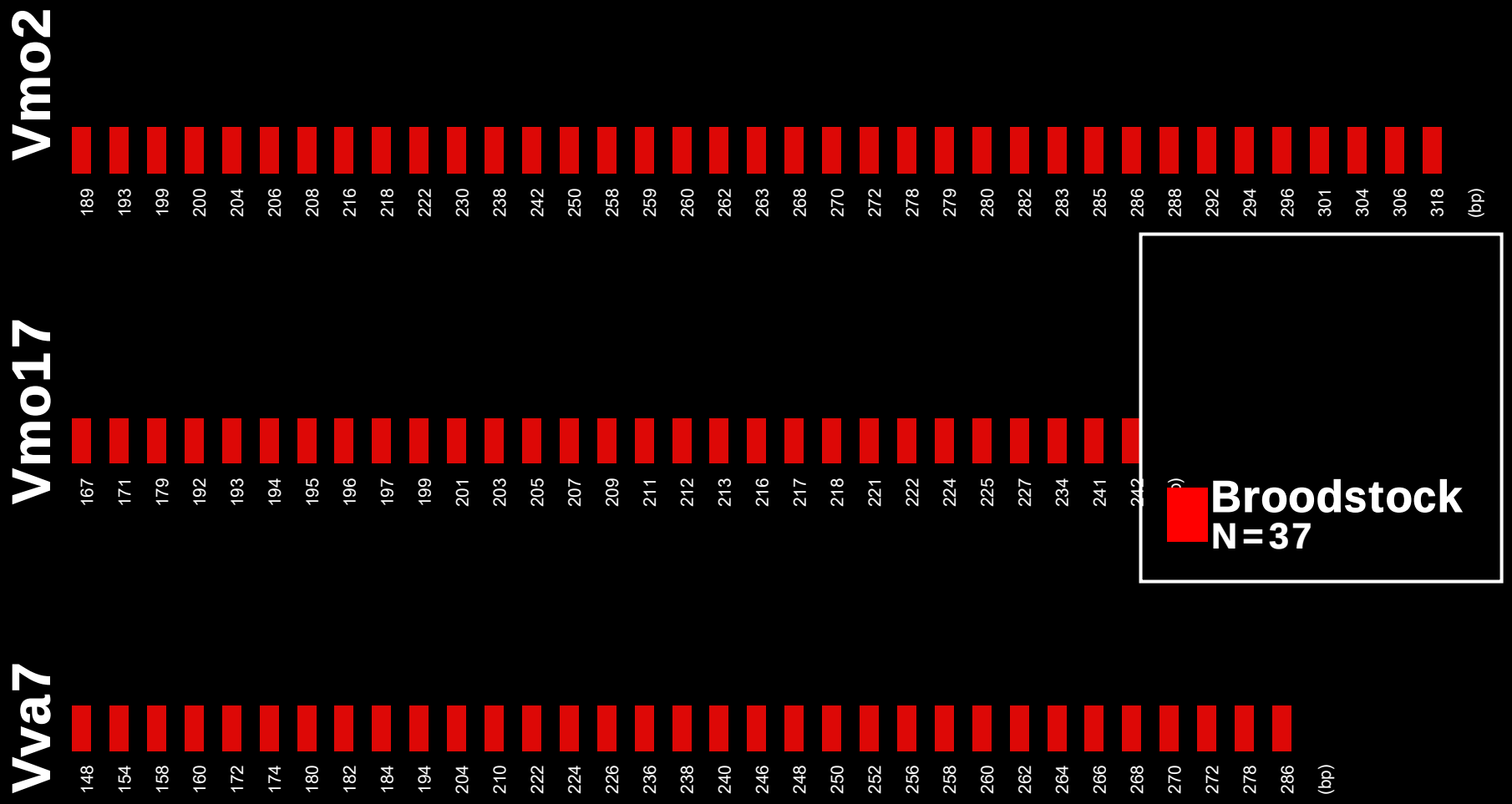


# Allele frequency distribution at 3 msDNA loci of candidate for progeny

## 2001Product n=197



# Comparison of allelic diversity between wild broodstock and selected progeny



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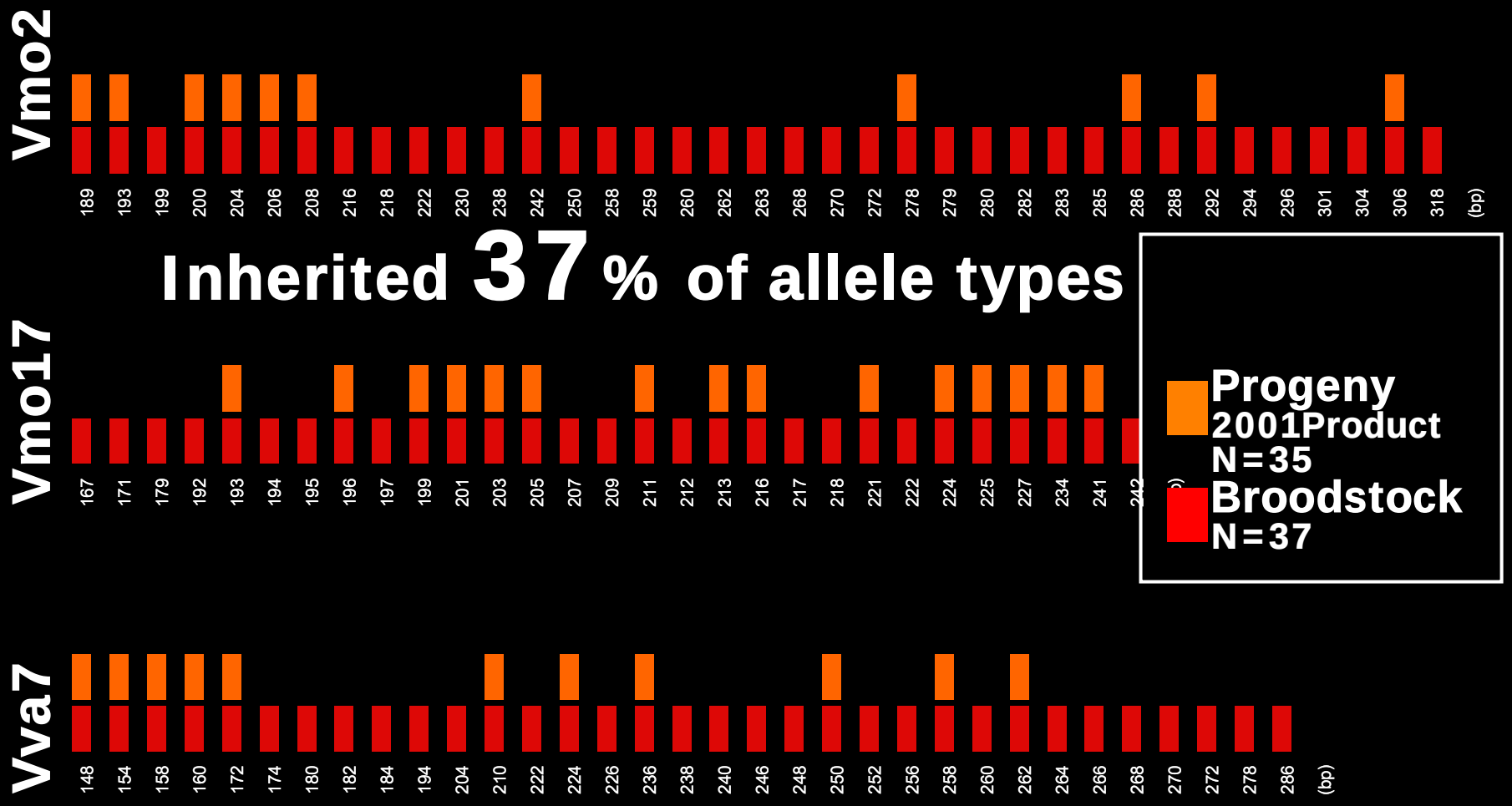

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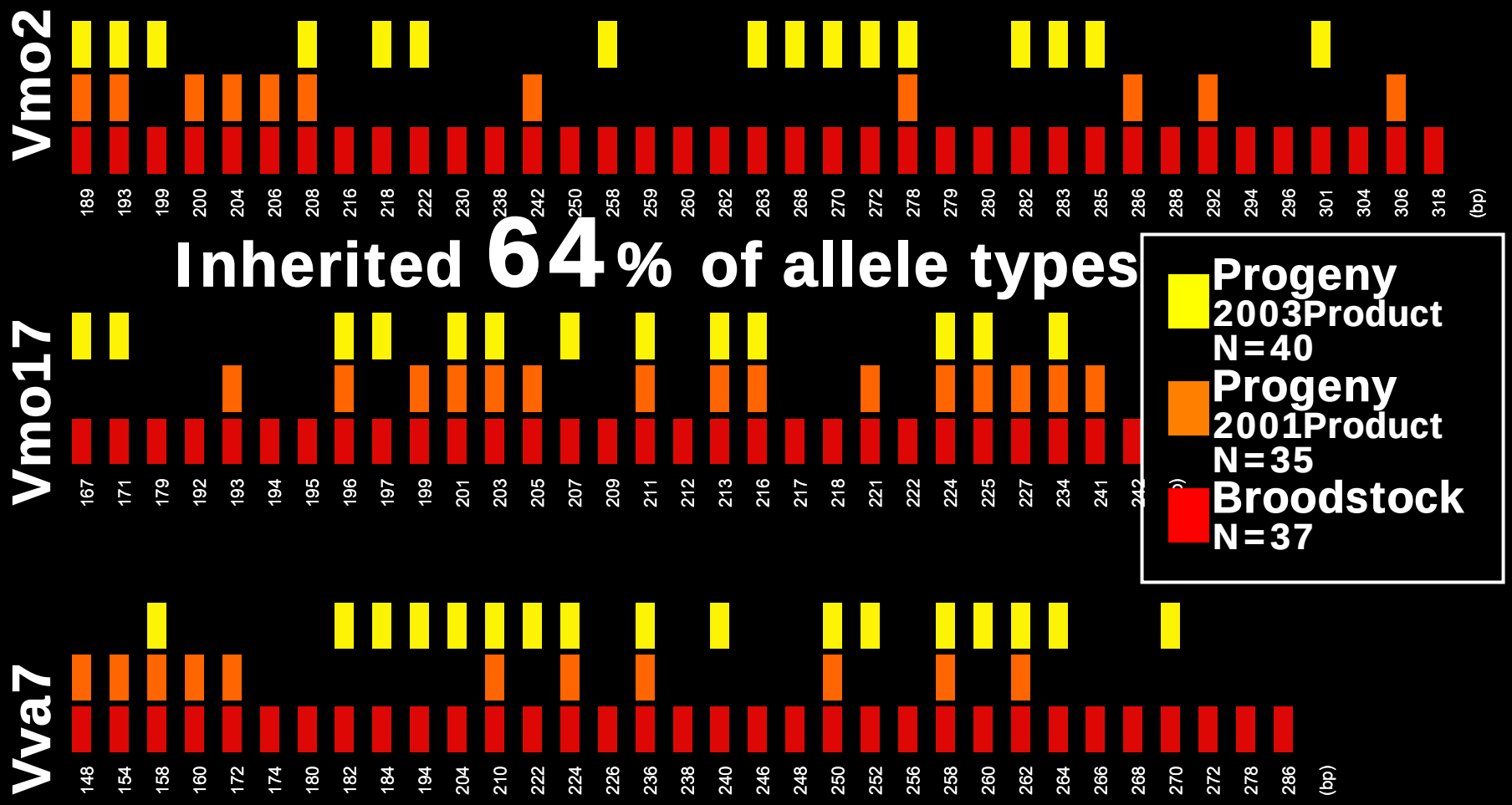
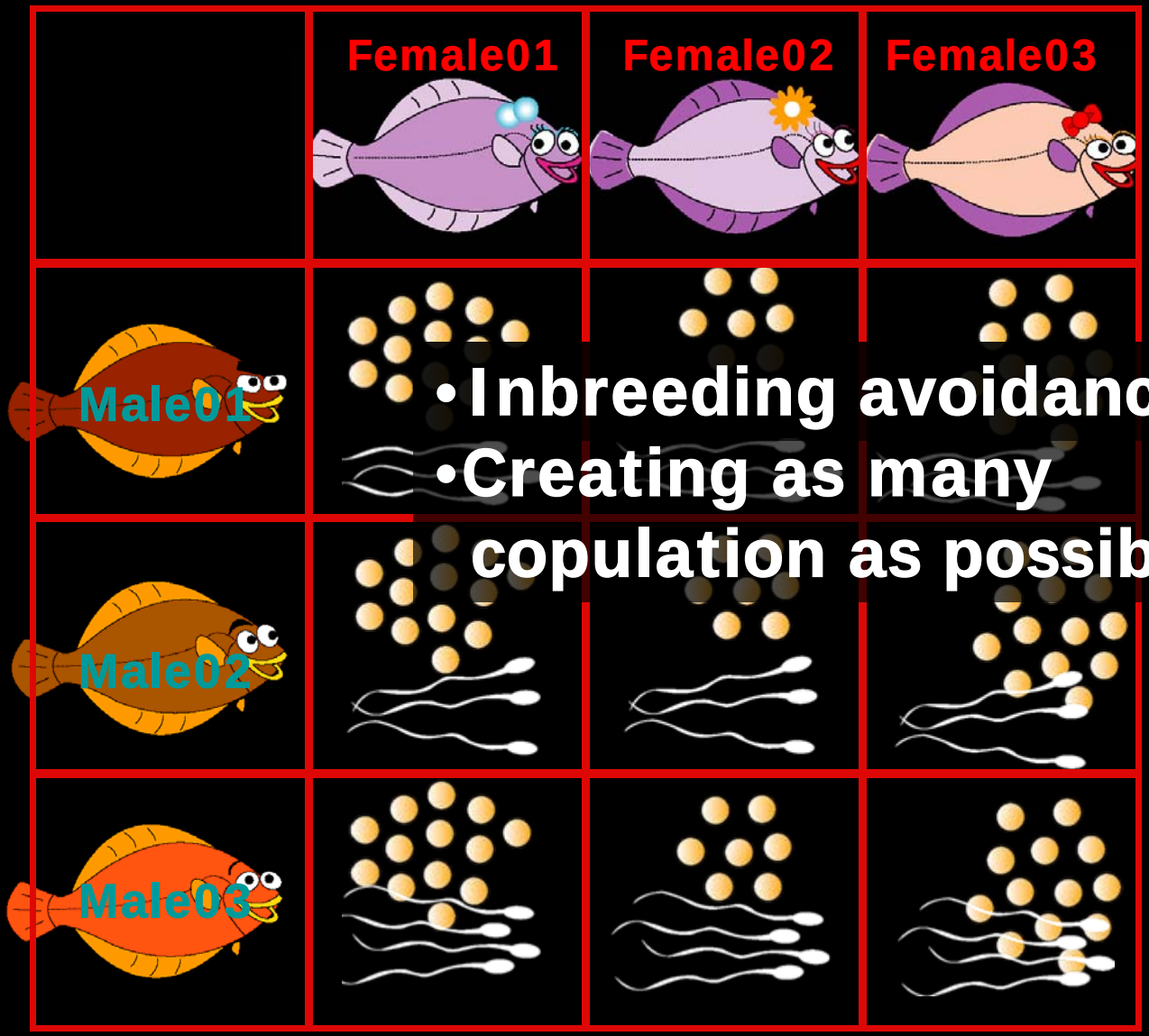


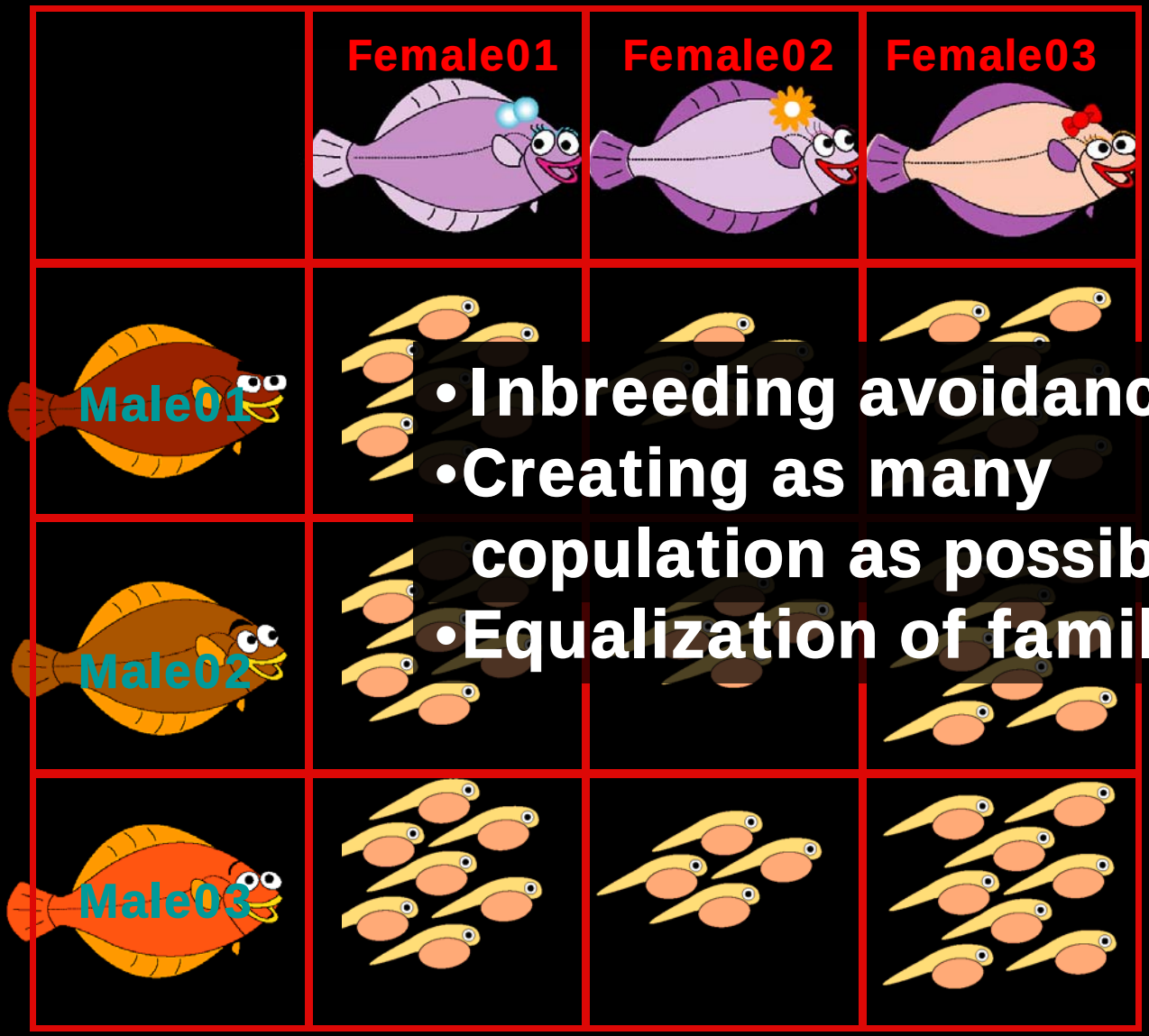
## Seed production

**Mating technique for maintaining genetic diversity with assistance of microsatellite DNA marker**

# Mating Strategy

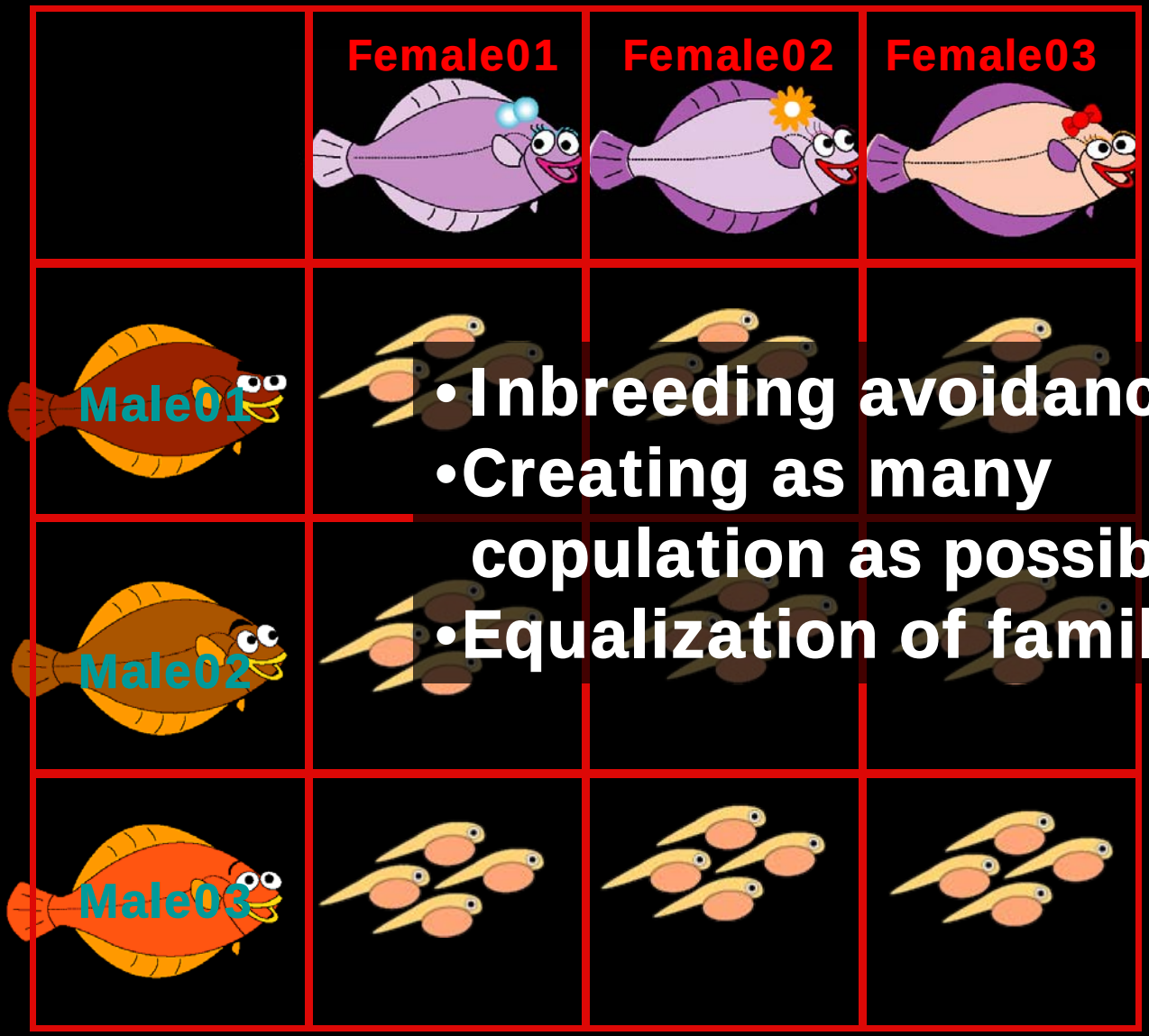


# Mating Strategy




- Inbreeding avoidance
- Creating as many copulation as possible
- Equalization of family size

# Mating Strategy

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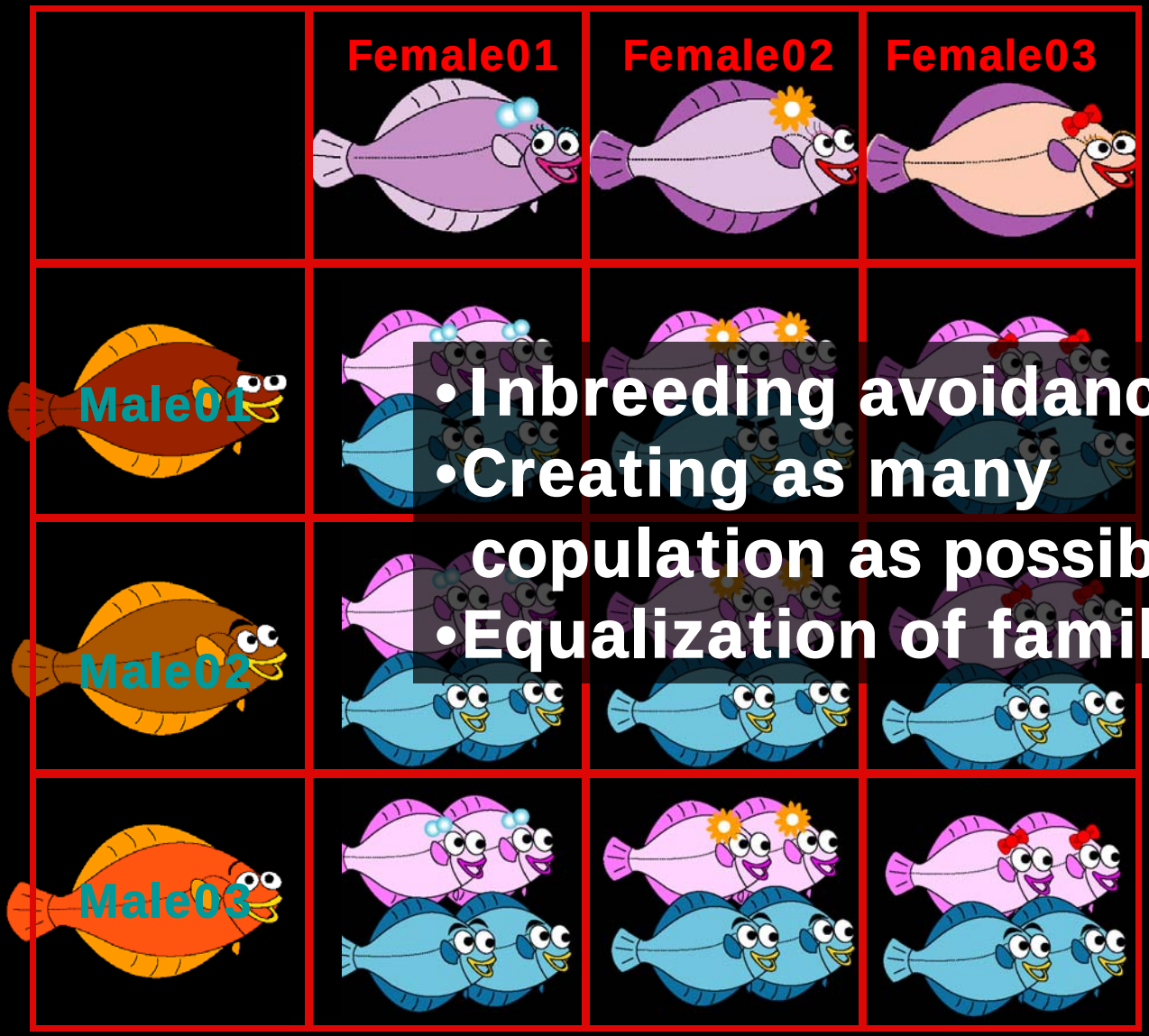
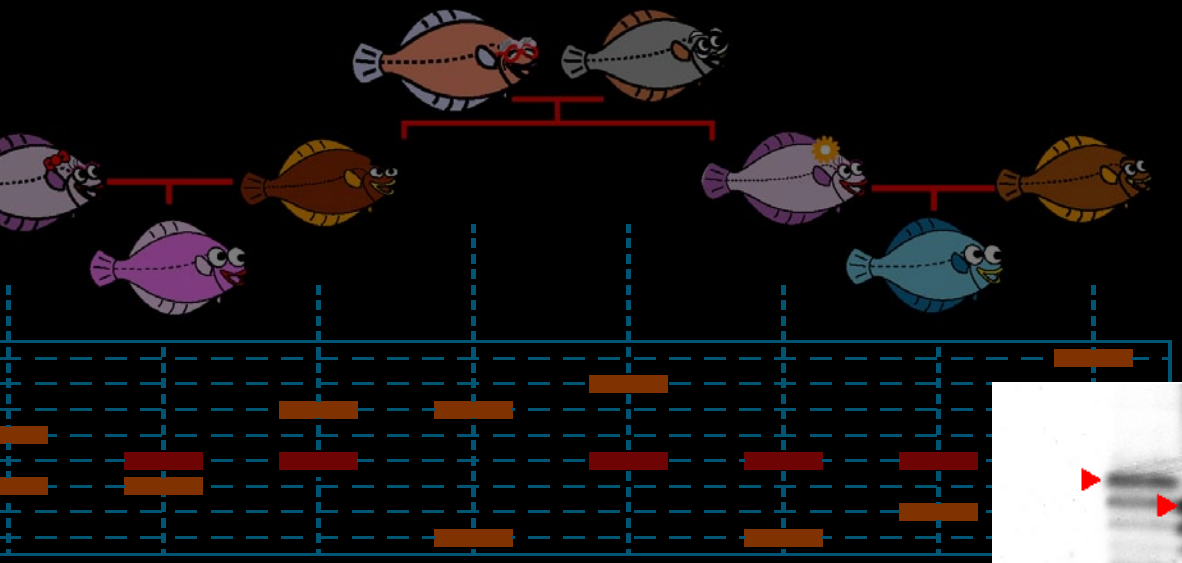
# Mating Strategy

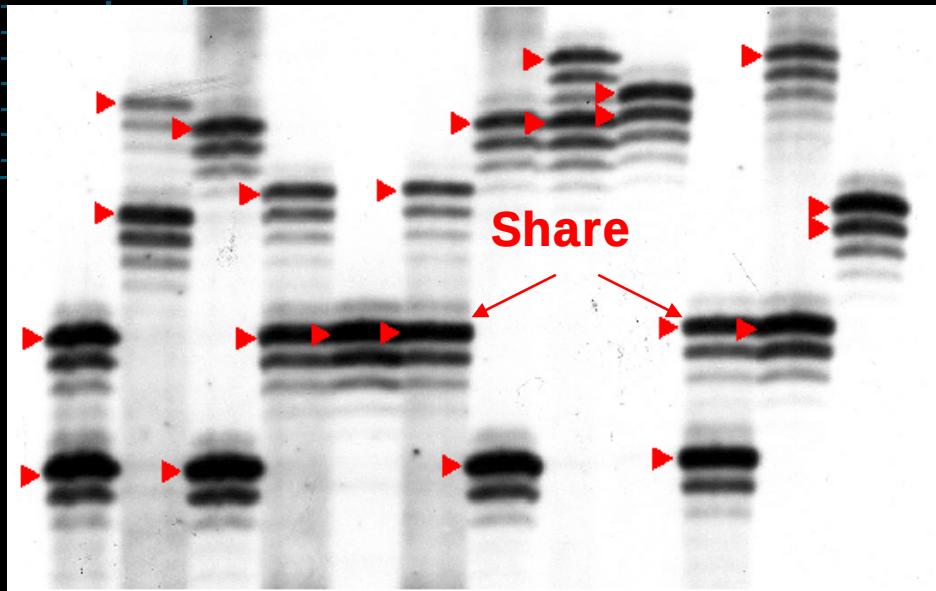


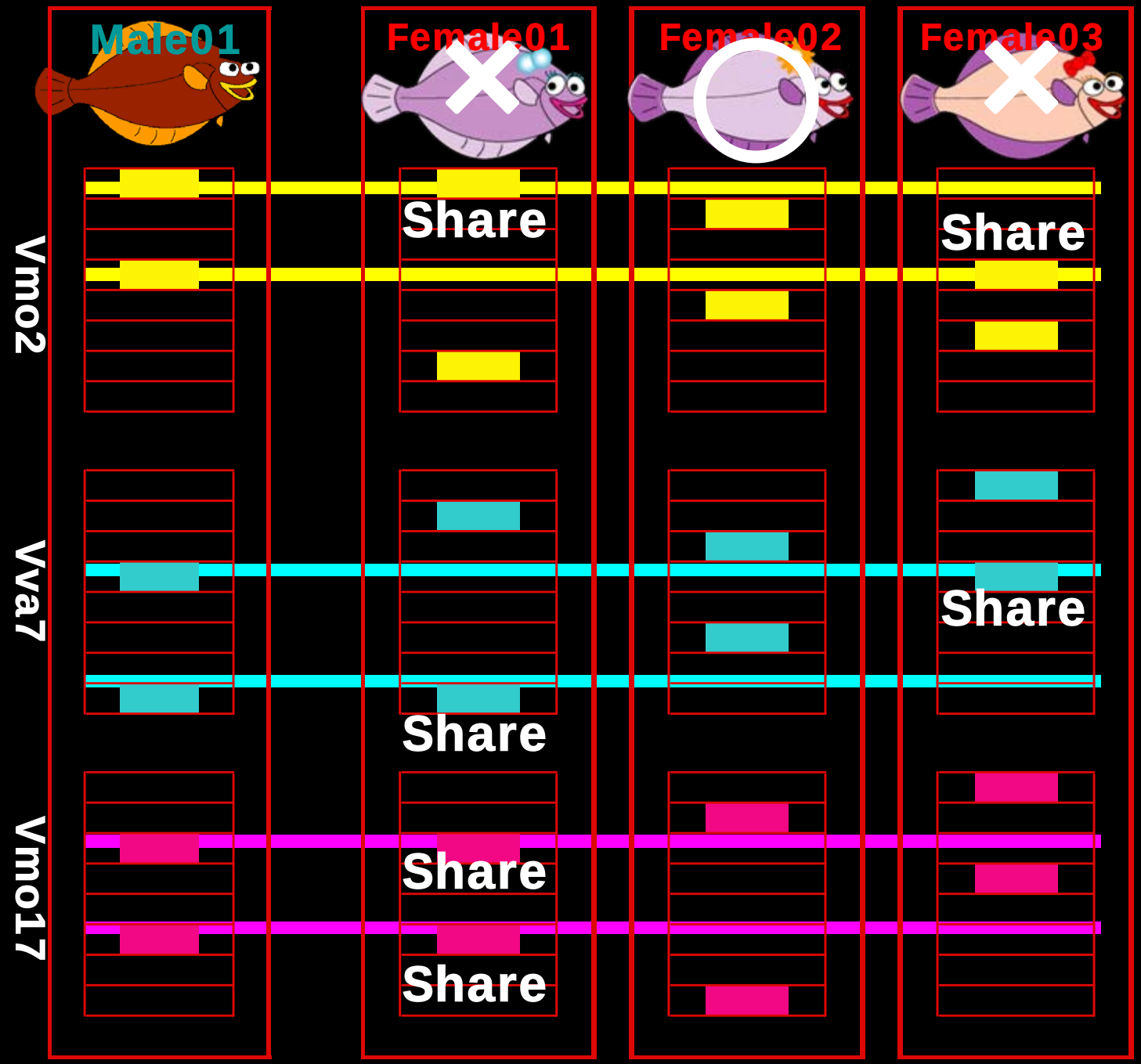

- Inbreeding avoidance
- Creating as many copulation as possible
- Equalization of family size

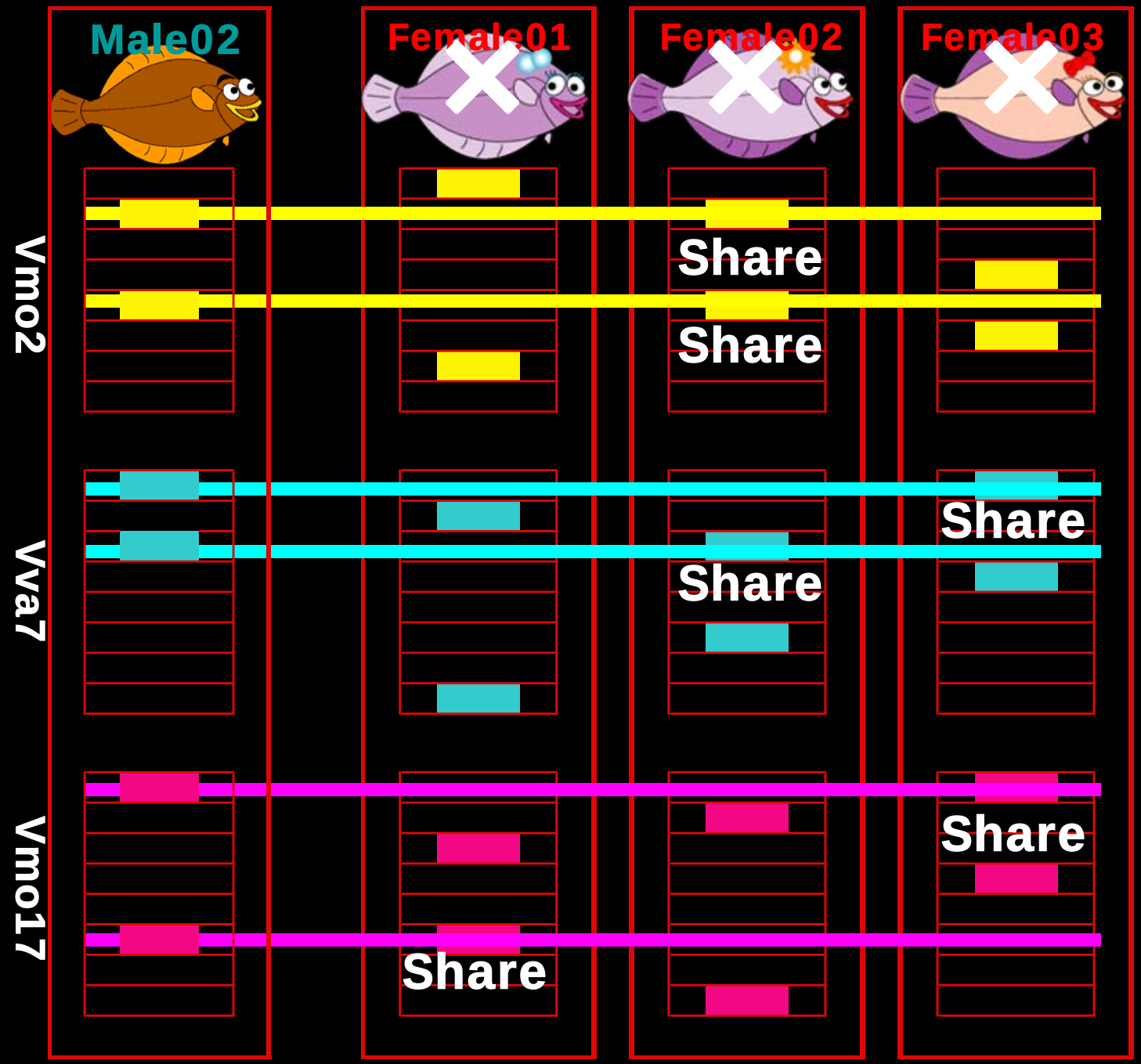
First Step

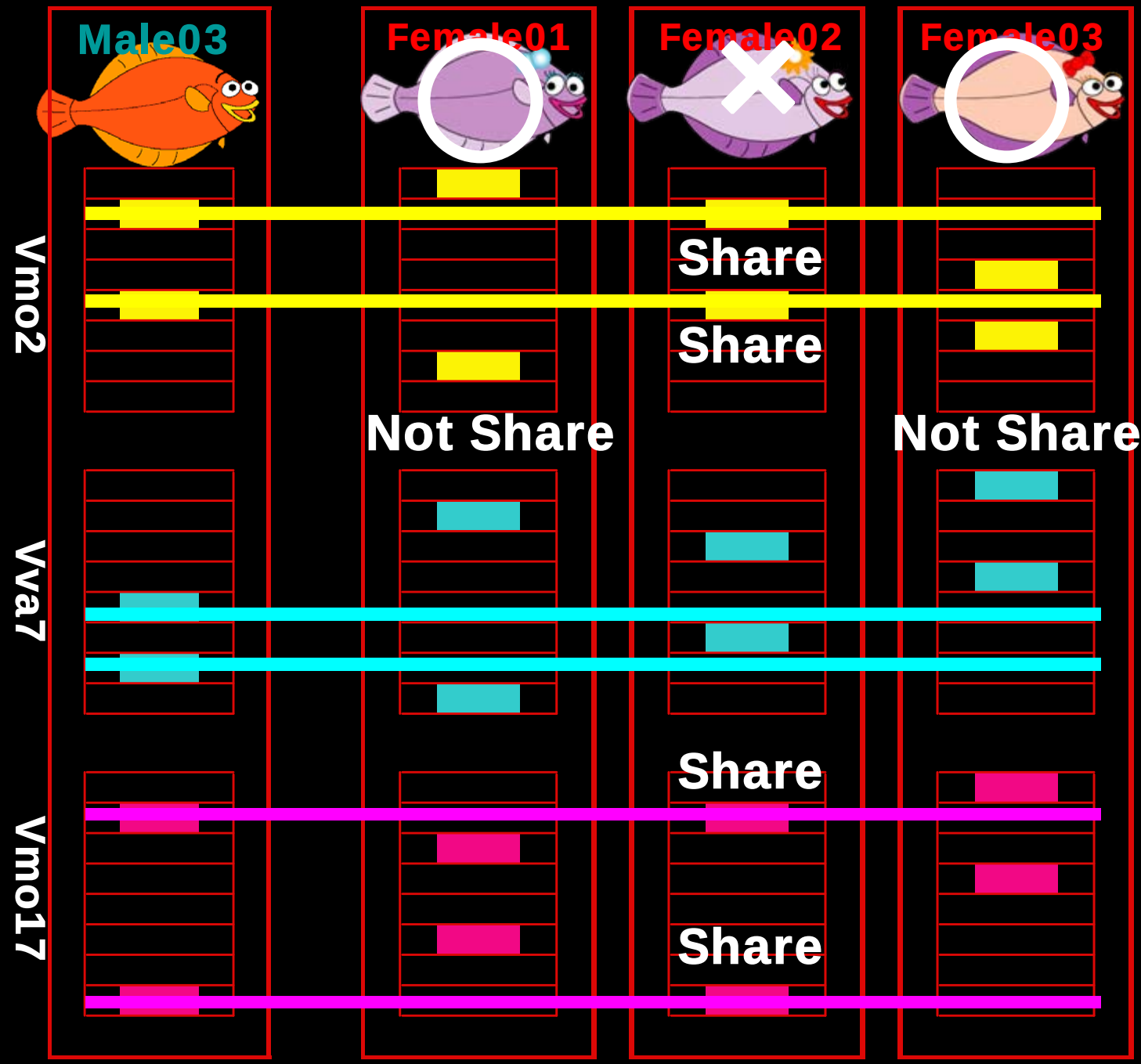
# Inbreeding avoidance






Male01
Female01
Female02
Female03
Vmo2
Vva7
Vmo17
Share
Share
Share
Share
Share
Share


Male02
Female01
Female02
Female03
Vmo2
Vva7
Vmo17
Share
Share
Share
Share
Share


Male03
Female01
Female02
Female03
Vmo2
Share
Share
Not Share
Not Share
Vva7
Share
Vmo17
Share

× :Pair which Shares Same Allele
○ :Pair which does not Share Allele

| | Female01  | Female02  | Female03  |
|---|---|---|---|
|  Male01 | × | ○ | × |
|  Male02 | × | × | × |
|  Male03 | ○ | × | ○ |

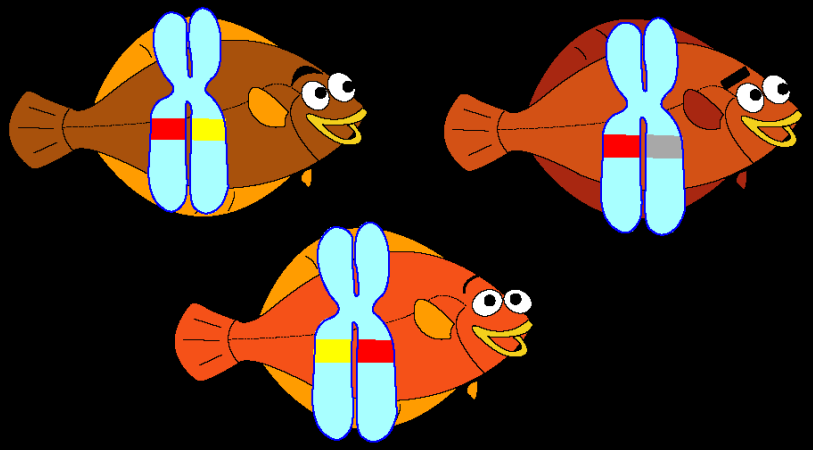
| | Female02 | Female03 | Female04 | Female05 | Female07 | Female08 | Female22 | Female29 | Female32 | Female38 | Female46 | Female47 | Female48 | Female06 | Female09 | Female10 | Female11 | Female12 | Female13 | Female14 | Female15 | Female16 | Female17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Male02 | × | × | × | × | × | × | ○ | ○ | ○ | × | × | × | ○ | ○ | ○ | × | × | × | × | ○ | × | ○ | × |
| Male03 | × | × | × | × | × | × | ○ | ○ | ○ | × | ○ | ○ | × | × | × | × | × | × | × | ○ | × | × | ○ |
| Male09 | ○ | ○ | ○ | ○ | ○ | ○ | × | ○ | × | ○ | × | ○ | ○ | ○ | ○ | ○ | ○ | × | ○ | × | ○ | ○ | × |
| Male11 | ○ | ○ | ○ | ○ | ○ | ○ | × | ○ | ○ | × | ○ | ○ | ○ | × | ○ | ○ | ○ | × | × | × | ○ | ○ | × |
| Male12 | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | × | ○ | ○ | ○ | × | ○ | ○ | × | ○ | ○ | ○ | × | ○ | ○ | × |
| Male13 | ○ | ○ | × | ○ | × | × | ○ | ○ | ○ | ○ | ○ | ○ | ○ | × | ○ | × | × | × | × | ○ | × | × | ○ |
| Male17 | ○ | ○ | ○ | ○ | ○ | ○ | × | ○ | ○ | × | ○ | ○ | ○ | ○ | ○ | × | ○ | × | ○ | × | ○ | × | ○ |
| Male18 | ○ | ○ | × | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | × | ○ | ○ | ○ | × | ○ | ○ |
| Male21 | ○ | ○ | ○ | ○ | ○ | ○ | × | ○ | ○ | × | × | ○ | ○ | ○ | × | ○ | ○ | ○ | ○ | ○ | × | ○ | ○ |
| Male22 | ○ | ○ | ○ | ○ | ○ | ○ | × | ○ | × | ○ | × | ○ | ○ | × | ○ | ○ | ○ | ○ | × | ○ | ○ | ○ | ○ |
| Male23 | ○ | ○ | ○ | ○ | ○ | ○ | × | ○ | × | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Male27 | × | × | ○ | × | × | × | × | ○ | ○ | × | × | ○ | ○ | × | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Male28 | ○ | ○ | ○ | ○ | ○ | ○ | × | × | × | ○ | ○ | ○ | ○ | × | ○ | ○ | × | ○ | × | ○ | ○ | ○ | ○ |
| Male29 | ○ | ○ | × | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | × | × | × | × | × | × | ○ | × | × | ○ |

All couples were checked

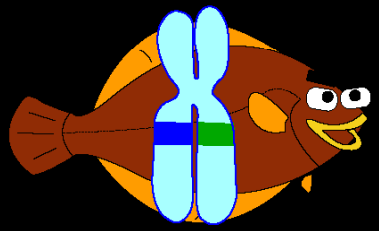
## Second Step

# Selection of sire
## for Planned Artificial Fertilization

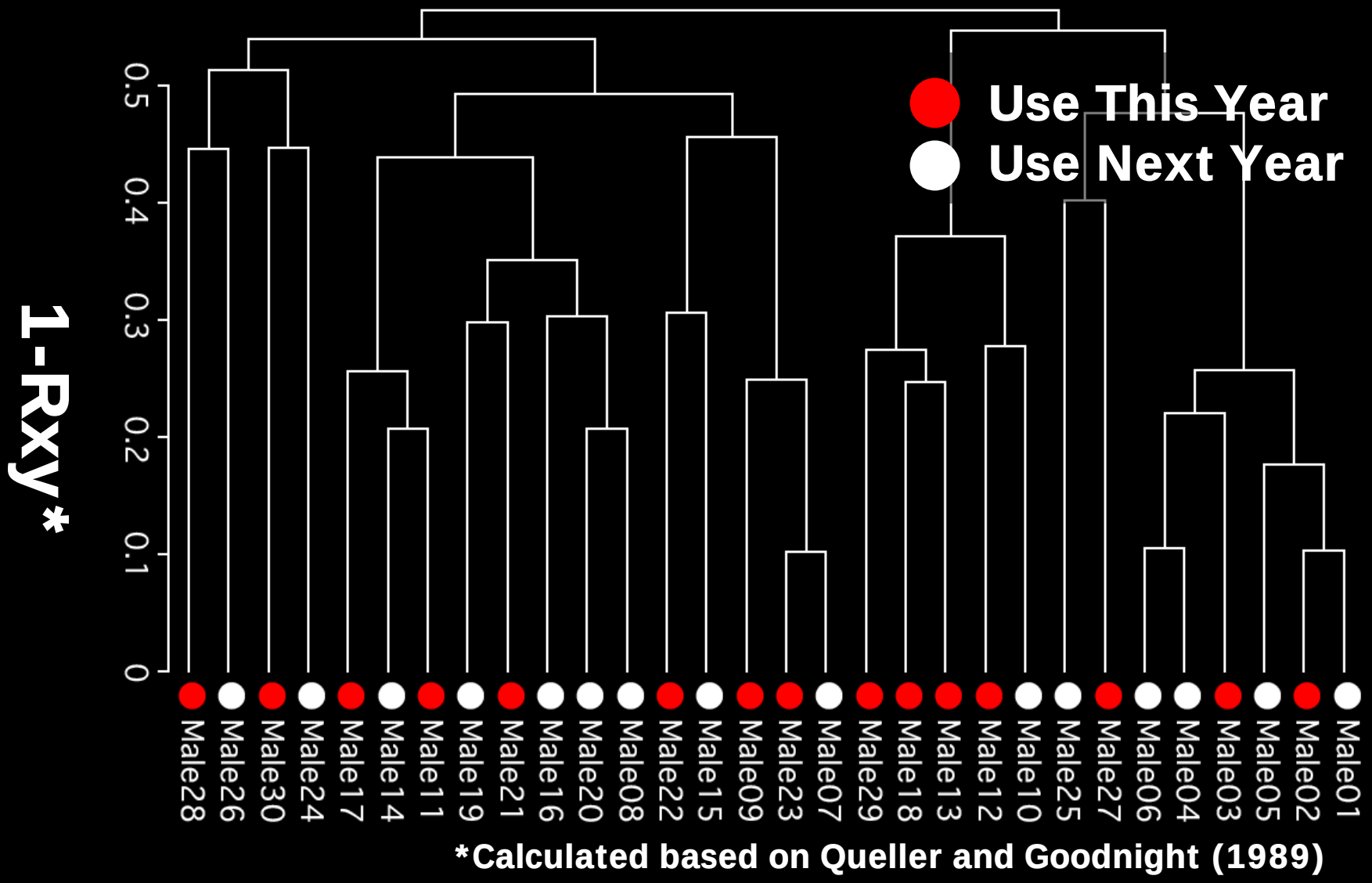
Unremarkable Type



Rare Type



# Genetic relationships among male parents constructed by relatedness



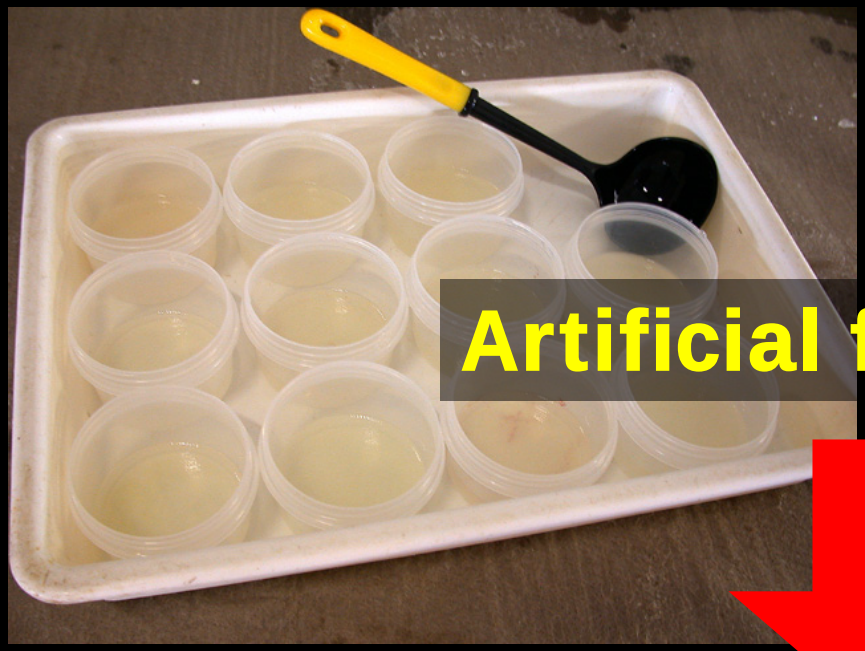

*Calculated based on Queller and Goodnight (1989)

Sperm collected by syringe

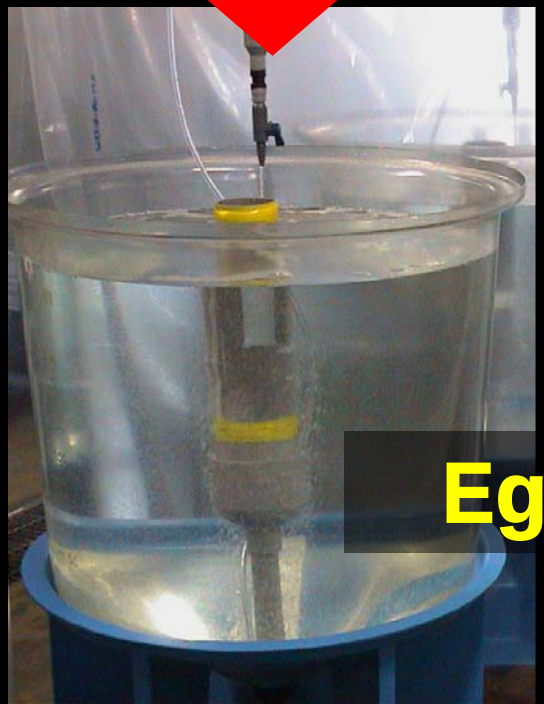

Sperm diluted with artificial seminal plasma

Egg Stripping

Equally divided eggs




Artificial fertilization



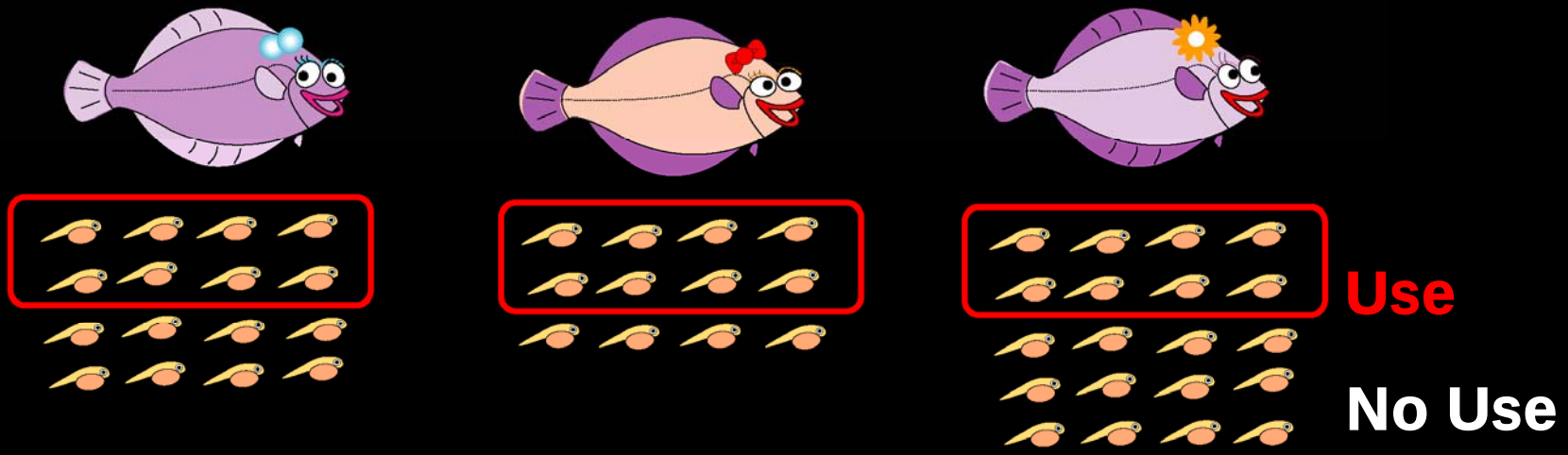
Egg incubation

**Eggs incubated by each female in separate tanks**

Third Step

# Culling of hatched larva

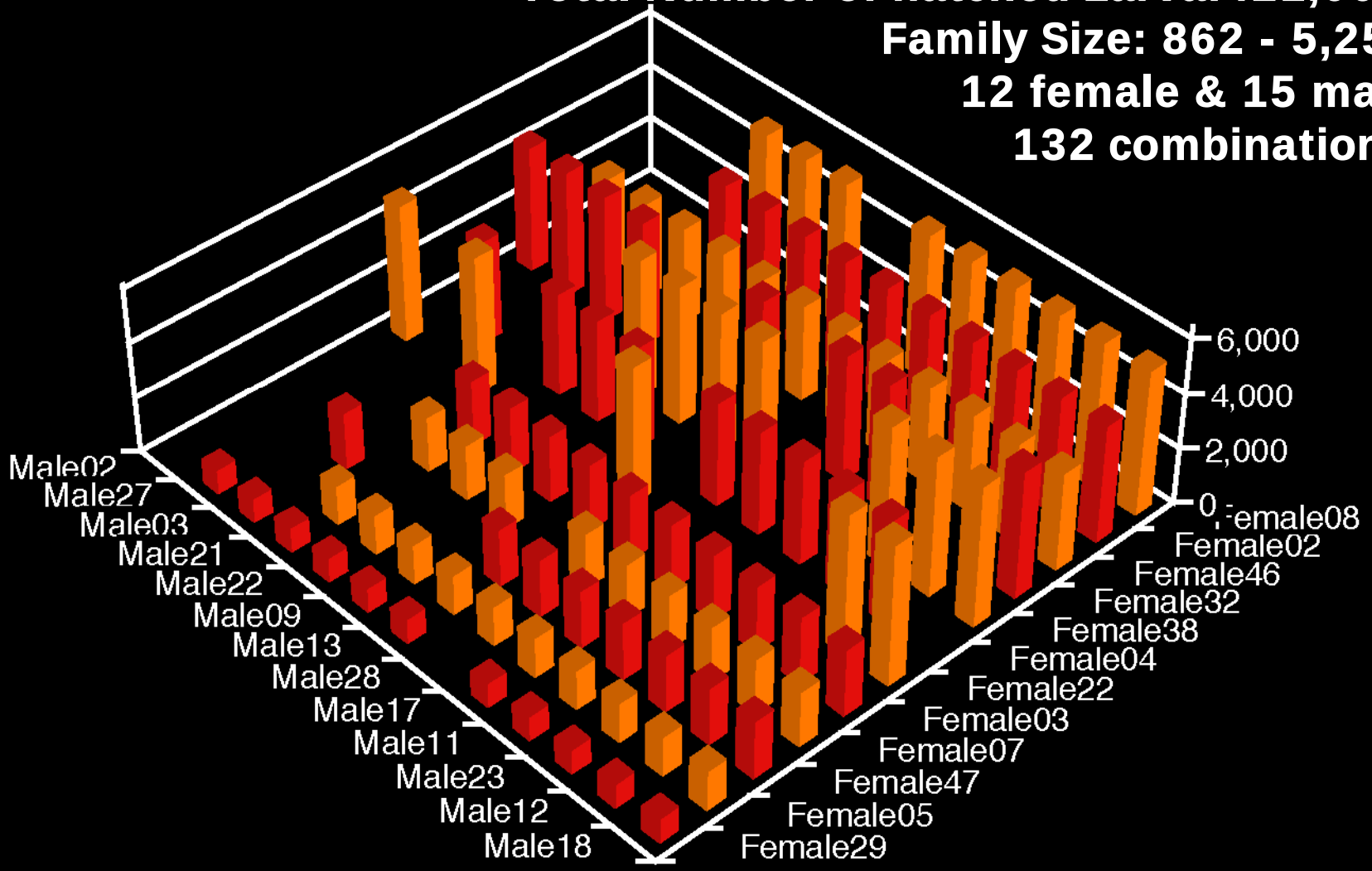
## - Equalization of family size -



# Number of hatched larvae in each family

## Before culling (2005 Seed Production)

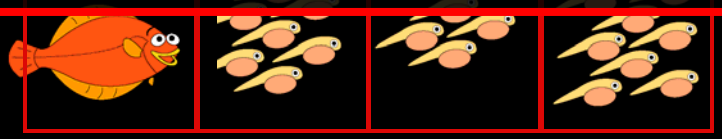
**Total Number of hatched Larva:421,000**
**Family Size: 862 - 5,255**
**12 female & 15 male**
**132 combinations**



# Culling of hatched larva for equalization of family size

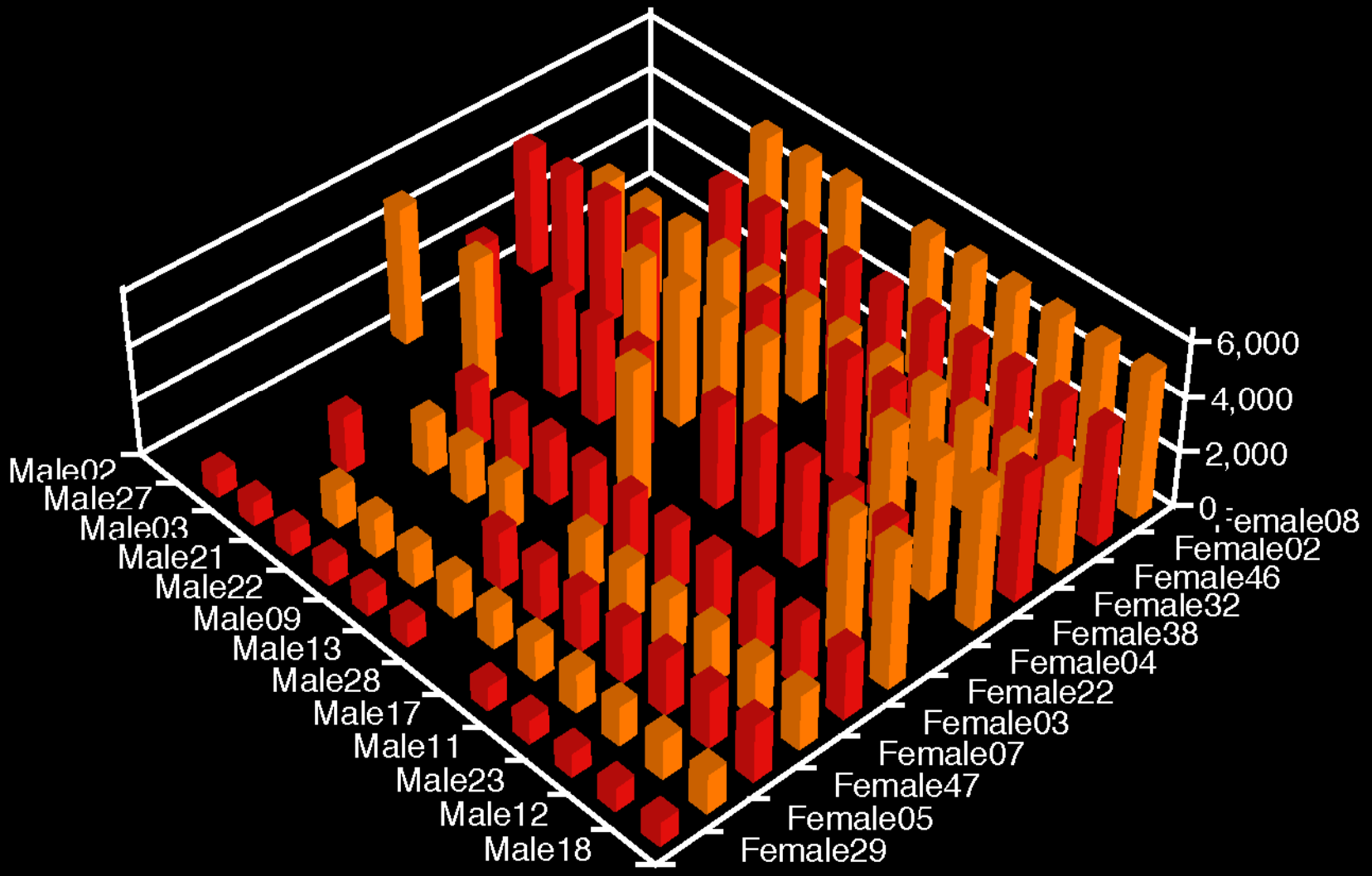
## Calculation method

- Minimization of family size variance
- Objective number of larvae is 200,000
- Calculated using SOLVER in microsoft EXCEL



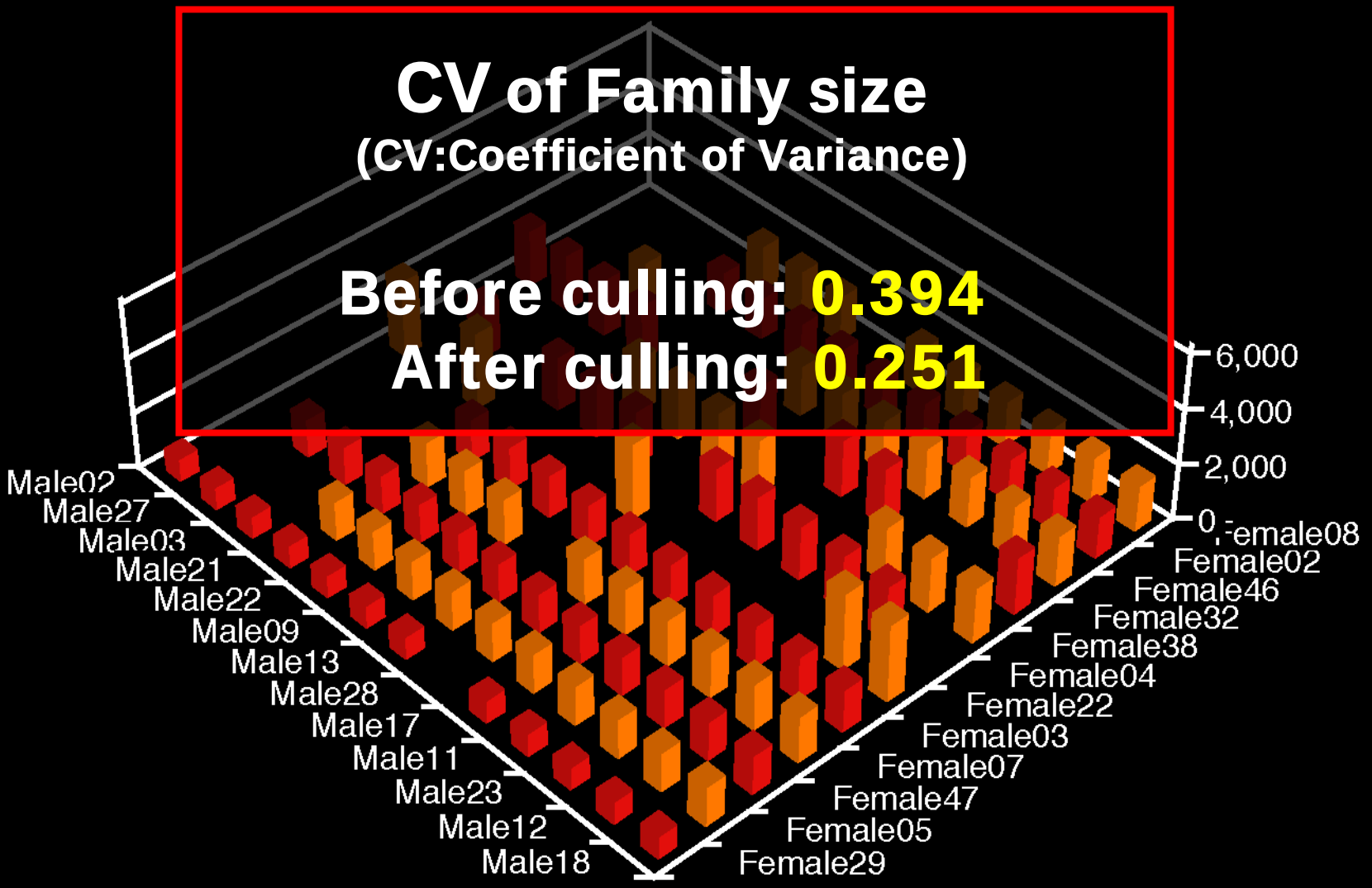
# Number of hatched larvae in each family

## Before culling (2005 Seed Production)



# Number of hatched larvae in each family

## After culling (2005 Seed Production)



# Genetic diversity maintained through seed production ?

**Start**

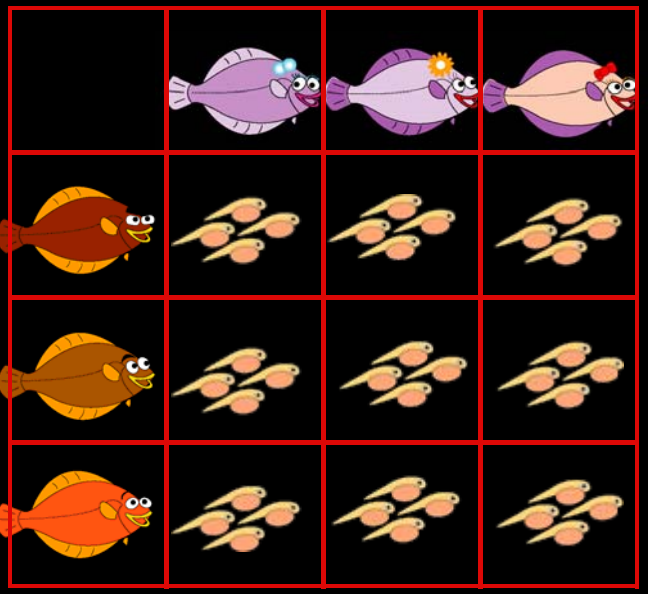
**Seed Production**

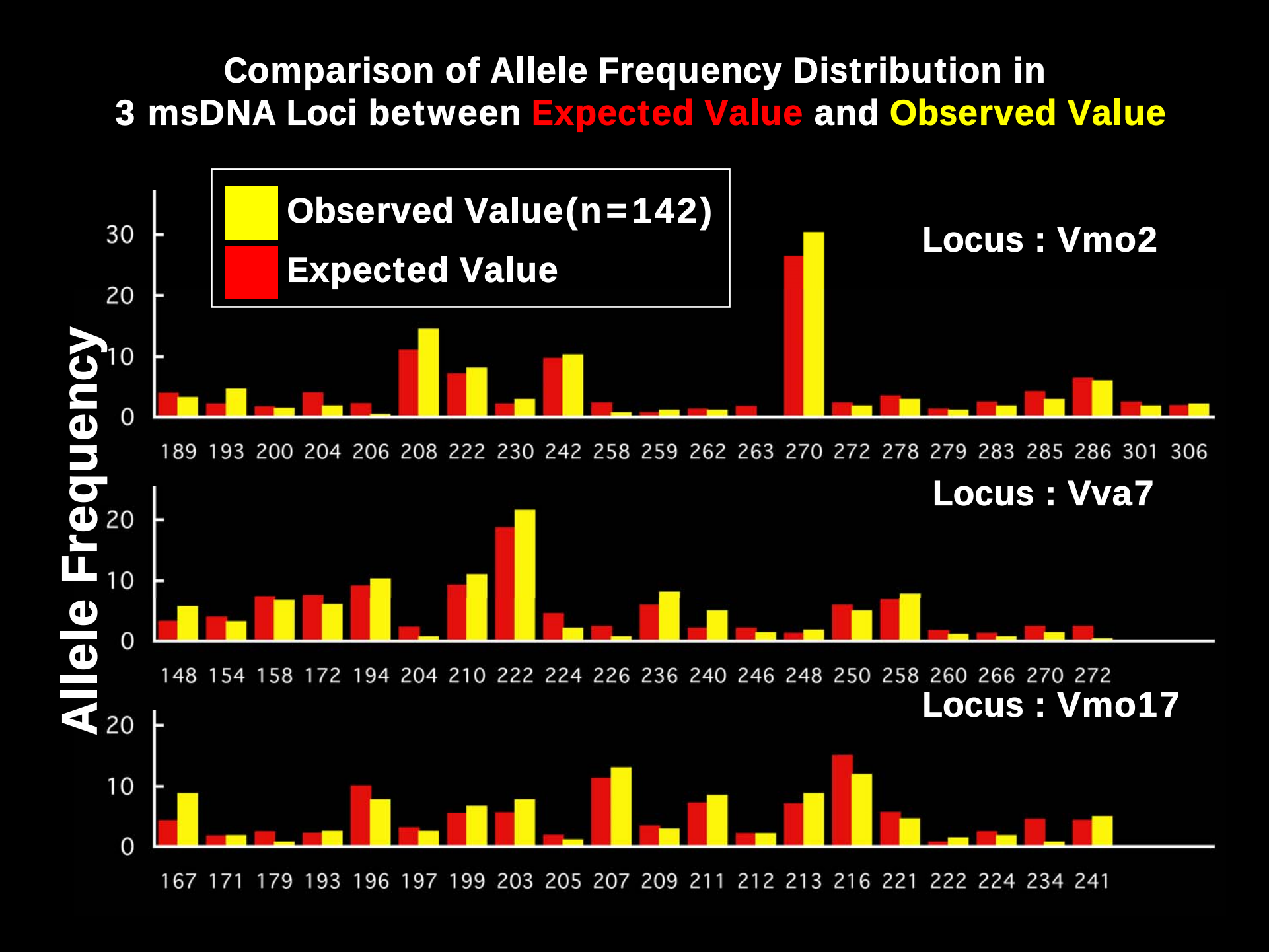
**End**

**( 68days)**

**Hatched larva**
**200,000**

**Juvenile**
**33,000**




Comparison of Allele Frequency Distribution in
3 msDNA Loci between Expected Value and Observed Value
Observed Value(n=142)
Expected Value
Allele Frequency
Locus : Vmo2
30
20
10
0
189 193 200 204 206 208 222 230 242 258 259 262 263 270 272 278 279 283 285 286 301 306
Locus : Vva7
20
10
0
148 154 158 172 194 204 210 222 224 226 236 240 246 248 250 258 260 266 270 272
Locus : Vmo17
20
10
0
167 171 179 193 196 197 199 203 205 207 209 211 212 213 216 221 222 224 234 241

# Conclusion

- **Broodstock Management**
  We developed the basic technique for conserving the hatchery gene pool.

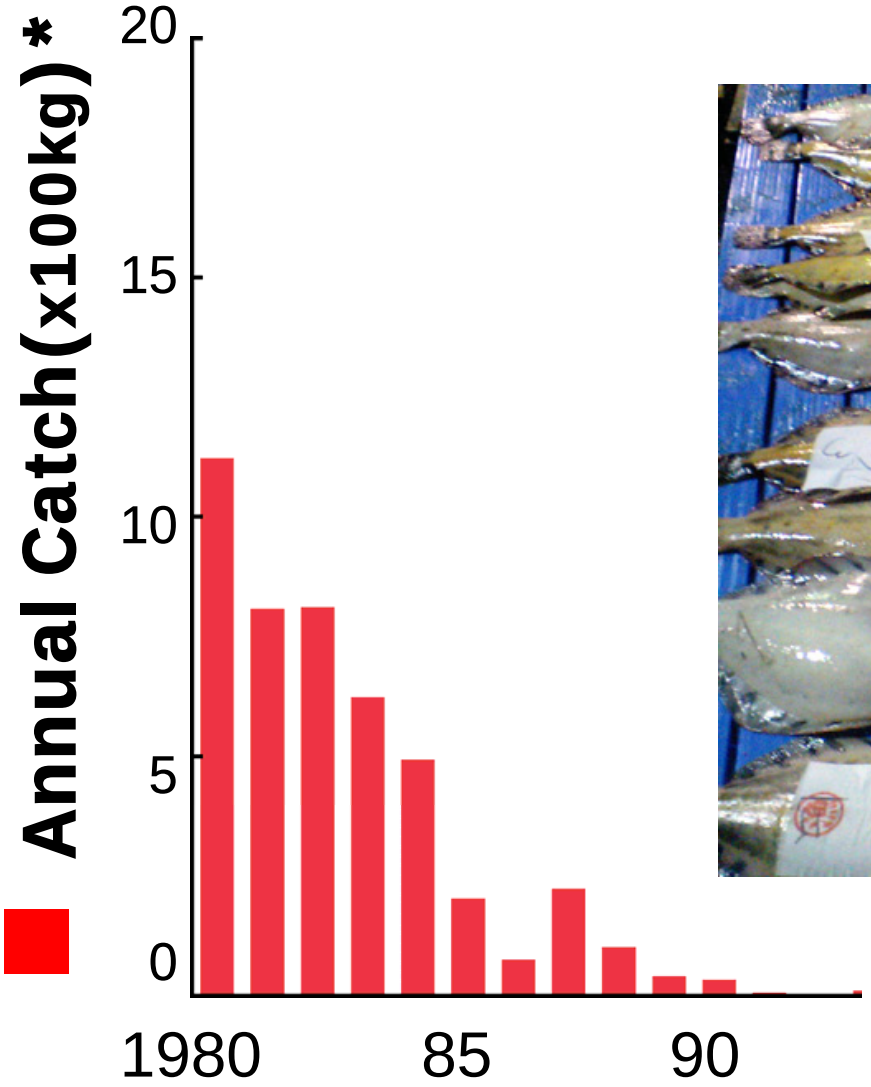
- **Mating Technique**
  We developed the base of a mating technique to enhance genetic diversity of seed concurrently with inbreeding avoidance.

# Future issue

- **Mating technique**
  Devising the mating strategy for long-term conservation of genetic diversity

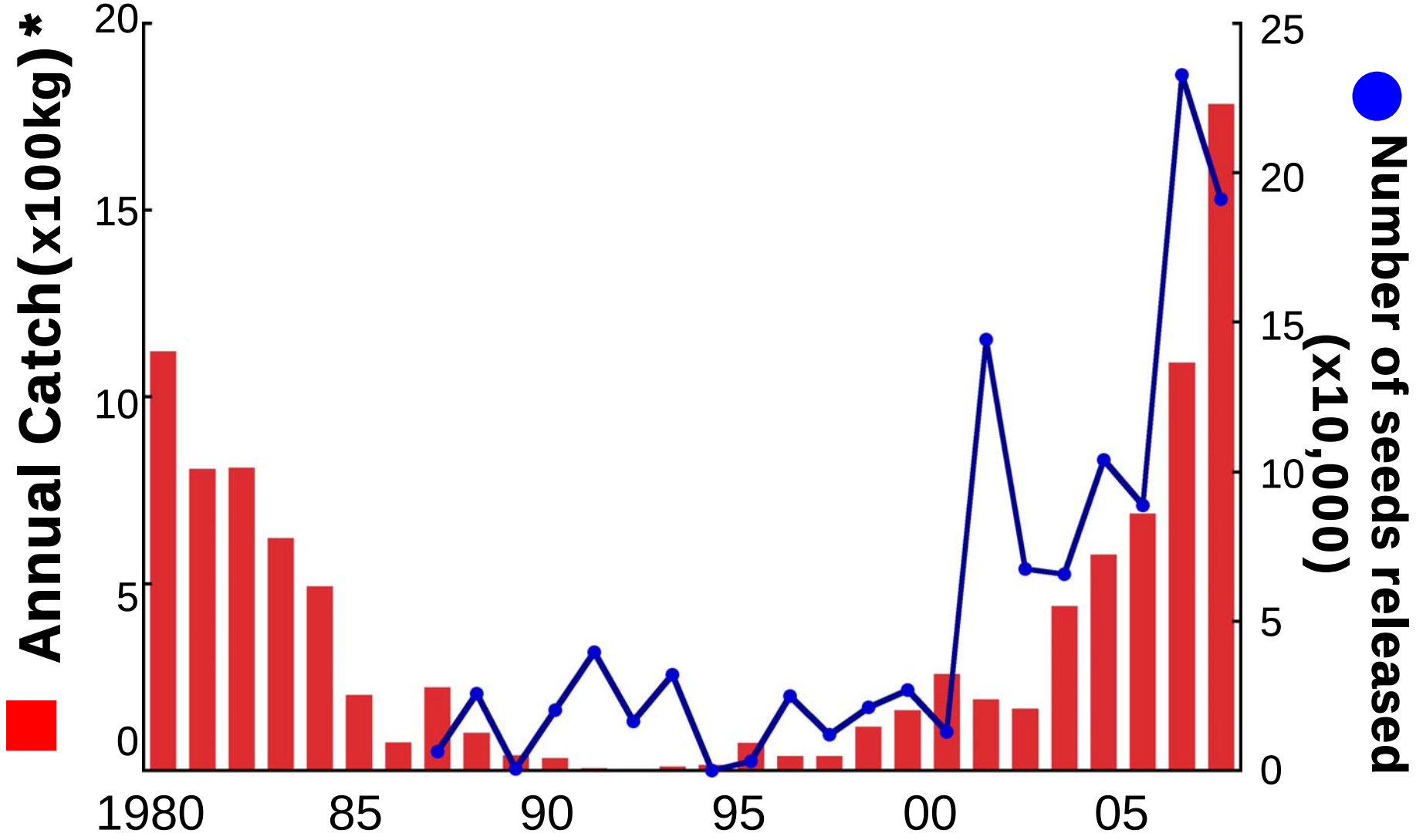
# Current situation in stock enhancement of Matsukawa

# Changes in Annual Catch of Matsukawa




*Total Catch of Fisheries Cooperative Association of Hiroo, Mitsuishi and Nemurowan-Chubu.

# Changes in the Number of Seeds Released and Annual Catch of Matsukawa

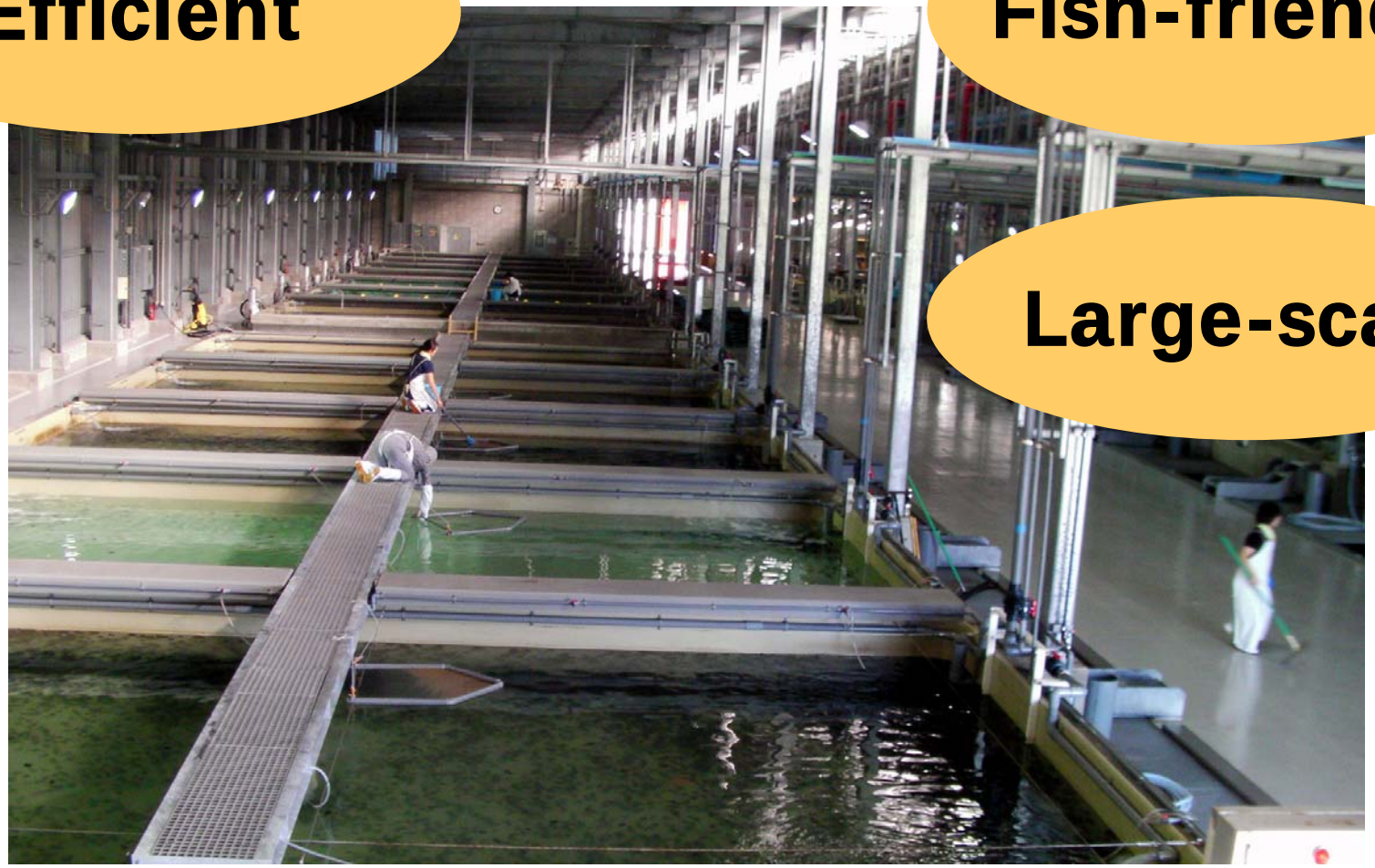



*Total Catch of Fisheries Cooperative Association of Hiroo, Mitsuishi and Nemurowan-Chubu.

# Newly-built hatchery customized for Matsukawa

**Efficient**

**Fish-friendly**



**Large-scale**

**Hokkaido Aquaculture Promotion Corporation. Matsukawa Center**

# Large-scale enhancement program has started since 2006

# It should be remembered that our target is organism !

**Inanimate products**

**Animate beings**