# "First class oysters": progress and constraints in genetic improvement of the Pacific oyster

(*Crassostrea gigas*)


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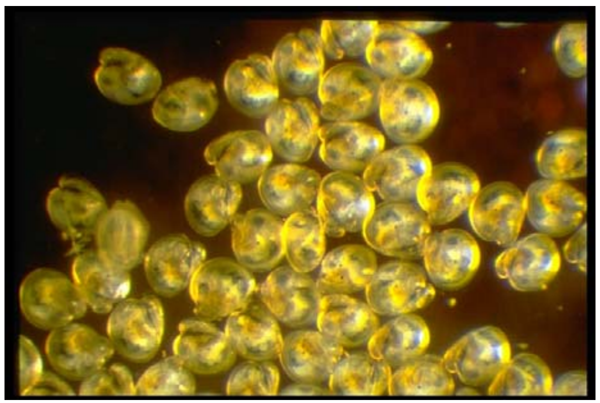
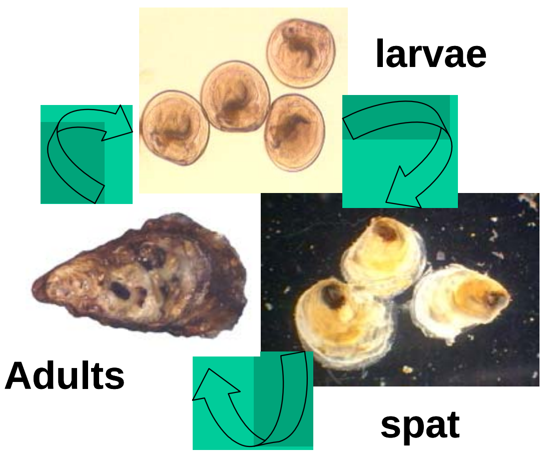
# Two possible sources of oyster spat

**(1) natural settlement (native or introduced species)**

**(2) hatchery propagation**



**Which broodstock ?**




**Genetic variability ?**
**Domestication ?**
**Selective breeding ?**

# Genetic improvement of bivalve production ?

**<u>Ploidy manipulations</u>:**

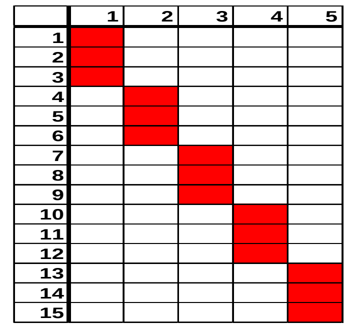
- triploidy induction
- tetraploids :

**4n x 2n = 3n**

**<u>Selective breeding</u>**

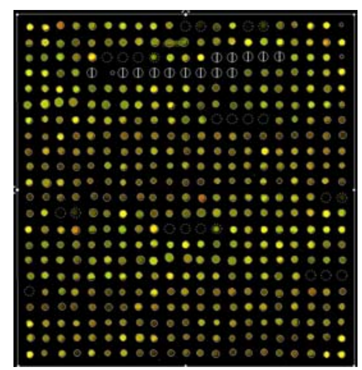
***Today:***

- heritability estimates
- genetic correlations and trade-offs
- family-based *vs* mass selection programs
- inbreeding and heterosis




***Tomorrow:***

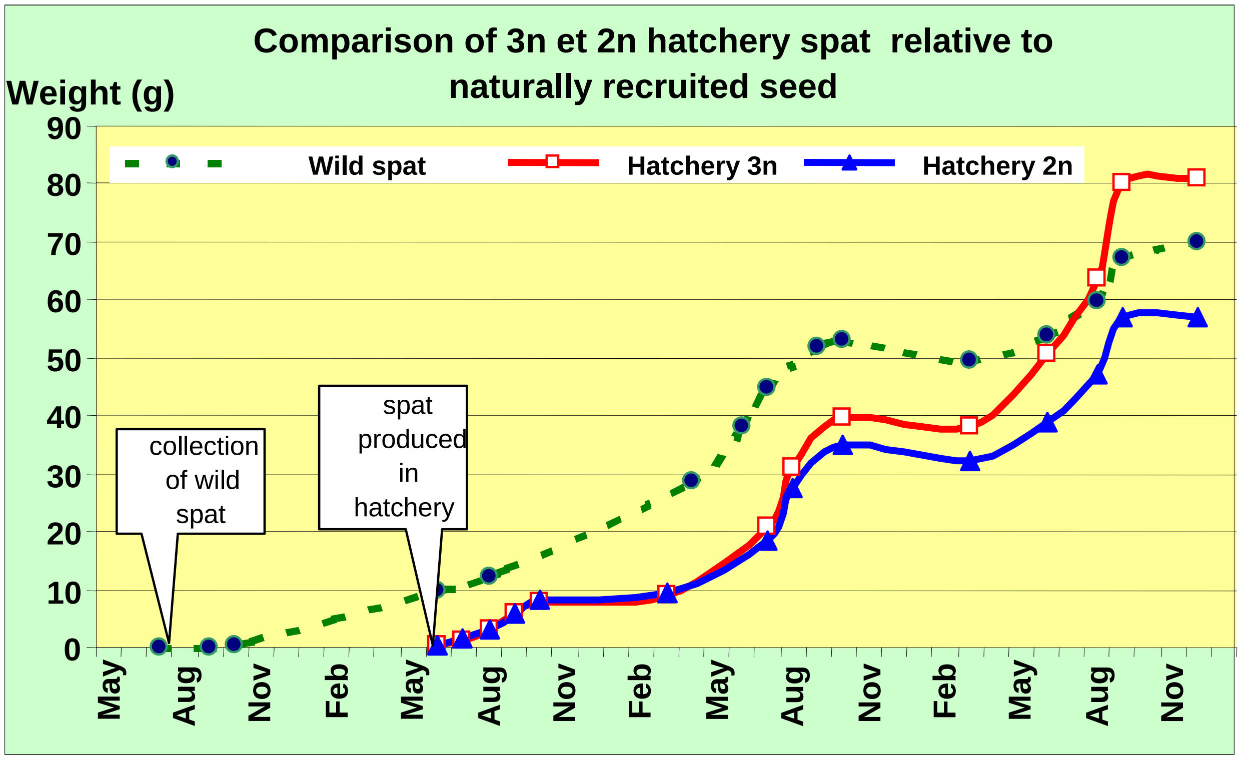
- mixed-family approach
- marker assisted selection and QTLs
- gene expression profiling



# Triploidy : a “single step” improvement

**Lower reproductive allocation**

**Better growth and survival**



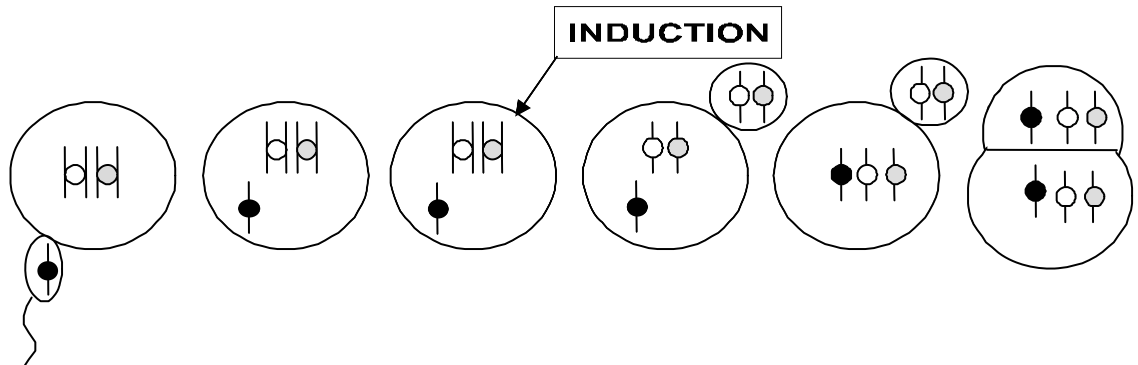

CREAA

- Nell, J.A. (2002). Farming triploid oysters. Aquaculture 210: 69-88

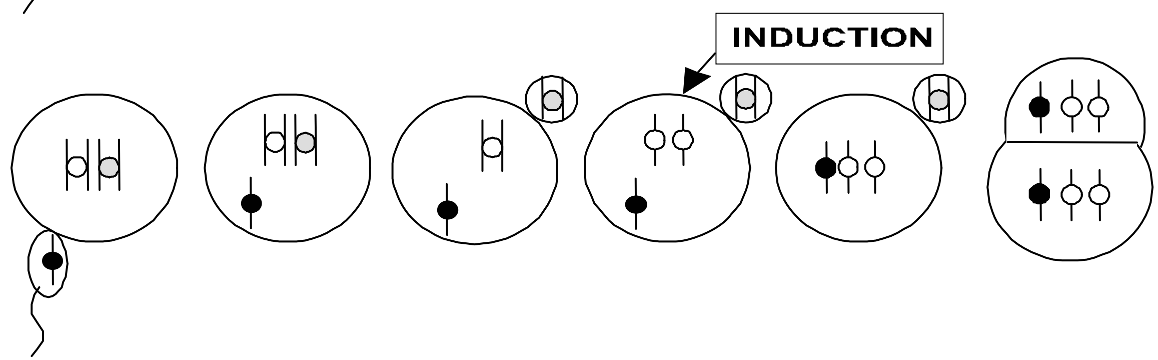
# Methods to produce triploid bivalves

## 1) Treatment of fertilized eggs (Cytochalasine B or 6-DMAP)

***Inhibition of the expulsion of the <u>first</u> polar body***




***Inhibition of the expulsion of the <u>second</u> polar body***

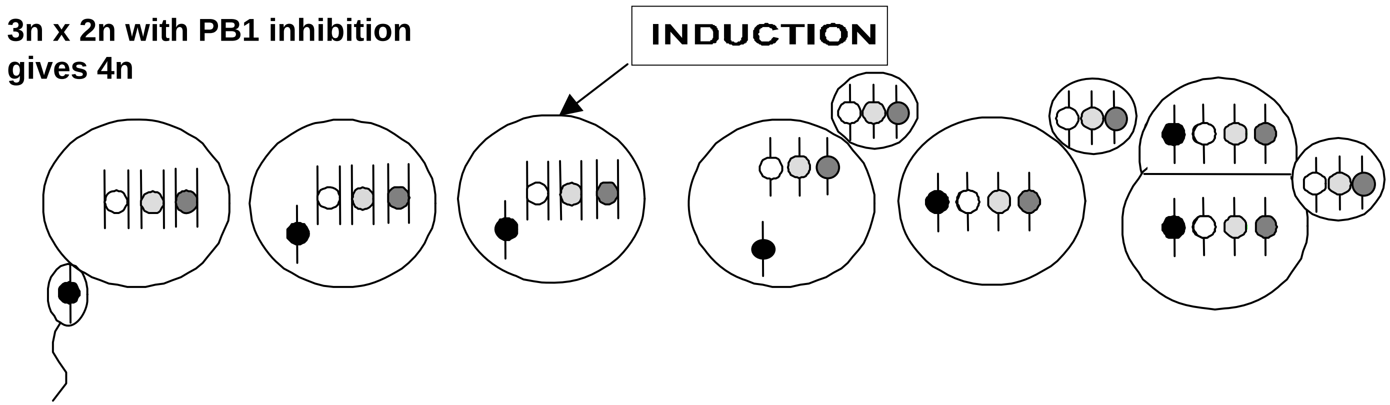



***Successfully applied on oysters, pearl oysters, mussels...***
***Dose and timing are key factors for successful production***

# Methods to produce tetraploid bivalves

## 2) Tetraploid x diploid  = 100 % ‘natural’ triploid

- First method to produce viable tetraploid oysters published in 1994 (Guo & Allen, 1994)

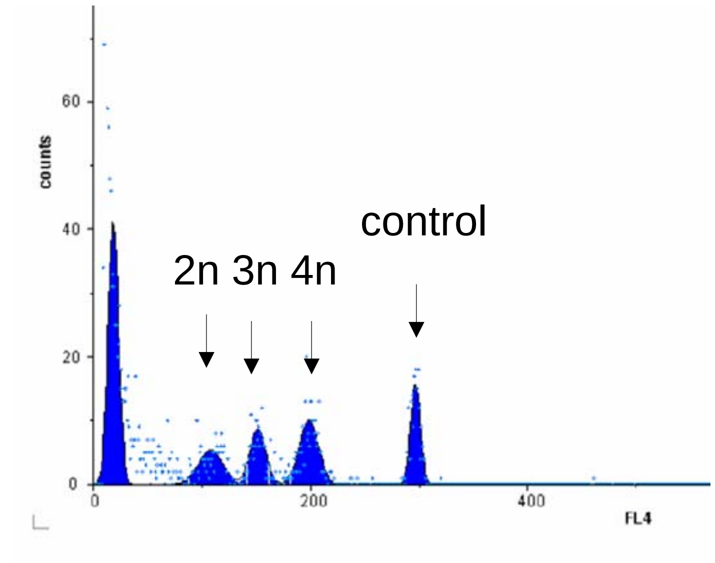


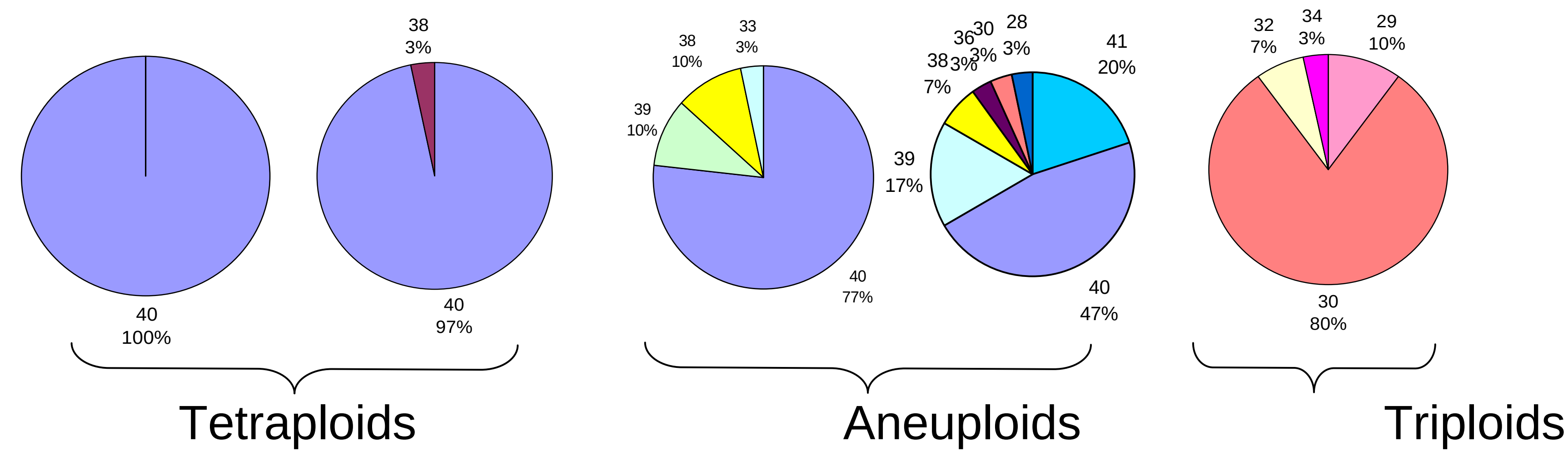

- ***Successfully applied in C. gigas, C. ariakensis & C. virginica***
- ***Patented***

•“Natural” triploids are superior to “chemically induced” triploids (Eudeline, 2004)

# Constraints associated with tetraploid oysters

- Success of induction methods varies between species
- Confinement of tetraploids is recommended (ICES).
- Chromosome set instability and reversion
- Need to score ploidy on all tetraploid genitors




***Variation in chromosome number in 4n x 4 n C. gigas progeny (McCombie et al, Aquaculture 2005)***

# Selective breeding of *C. gigas* worldwide

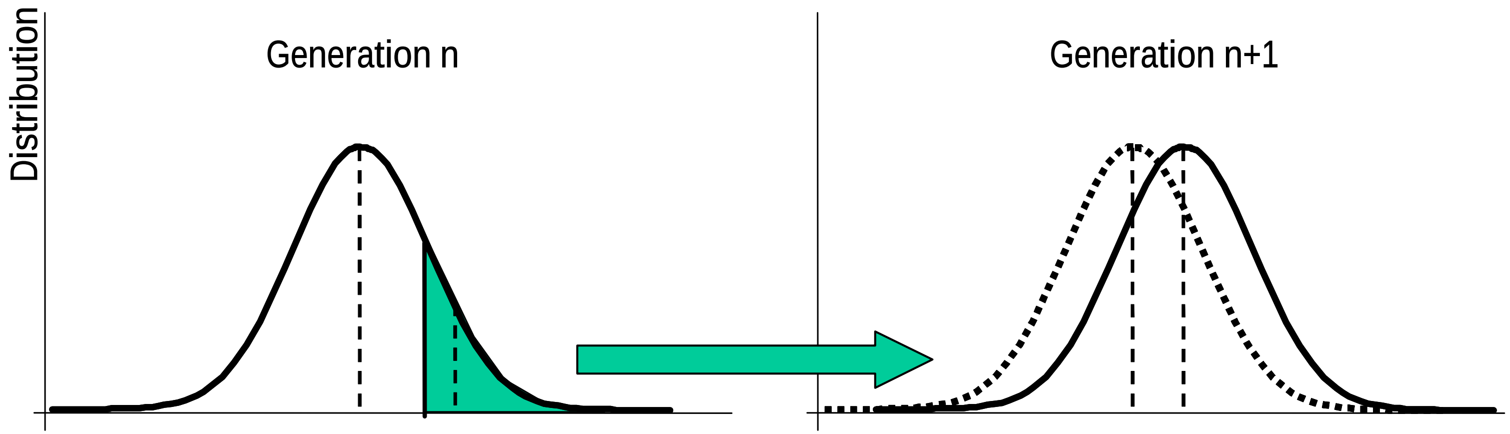
- U.S.A. : **yield (= growth x survival)**
  - WRAC
  - MBP

- Australia: **growth**
  - CSIRO / ASI

- New Zeland: **growth and quality**
  - Cawthron Institute

- France : **Resistance to summer mortality**
  - Ifremer

# Individual selection




➢ <u>Targeted traits</u> : growth, survival to disease

- **Bonamiosis resistance in *O. edulis* (Naciri-Graven et al., 1998; Culloty et al., 2001)**
- **MSX and dermo resistance in *C. virginica* (Ford et Haskin 1987; Calvo et al 2003)**
- **Growth in *S. commercialis* (Nell et al., 2000)**

➢ <u>Main advantages</u> :

- **Relatively easy to manage**
- **Possibility of strong selective pressures**

➢ <u>Main constrains</u> :

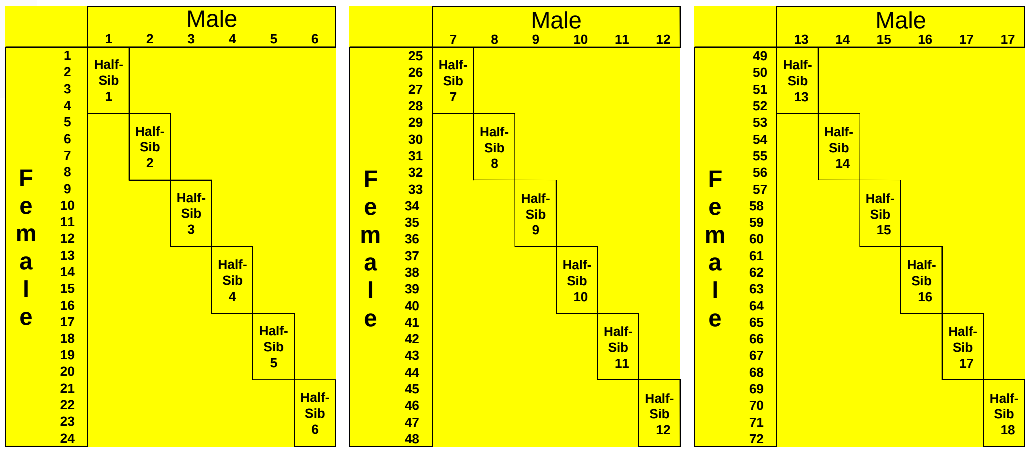
- **Selection under a single environment : genotype x environment interaction ? (Evans et al., 2006; Swan et al., 2007)**

- **Rapid loss of genetic variability:inbreeding ?**

# Family-based selective breeding

**Relative performance of (many) families reared under <u>common</u> environment(s) to estimate their genetic value**







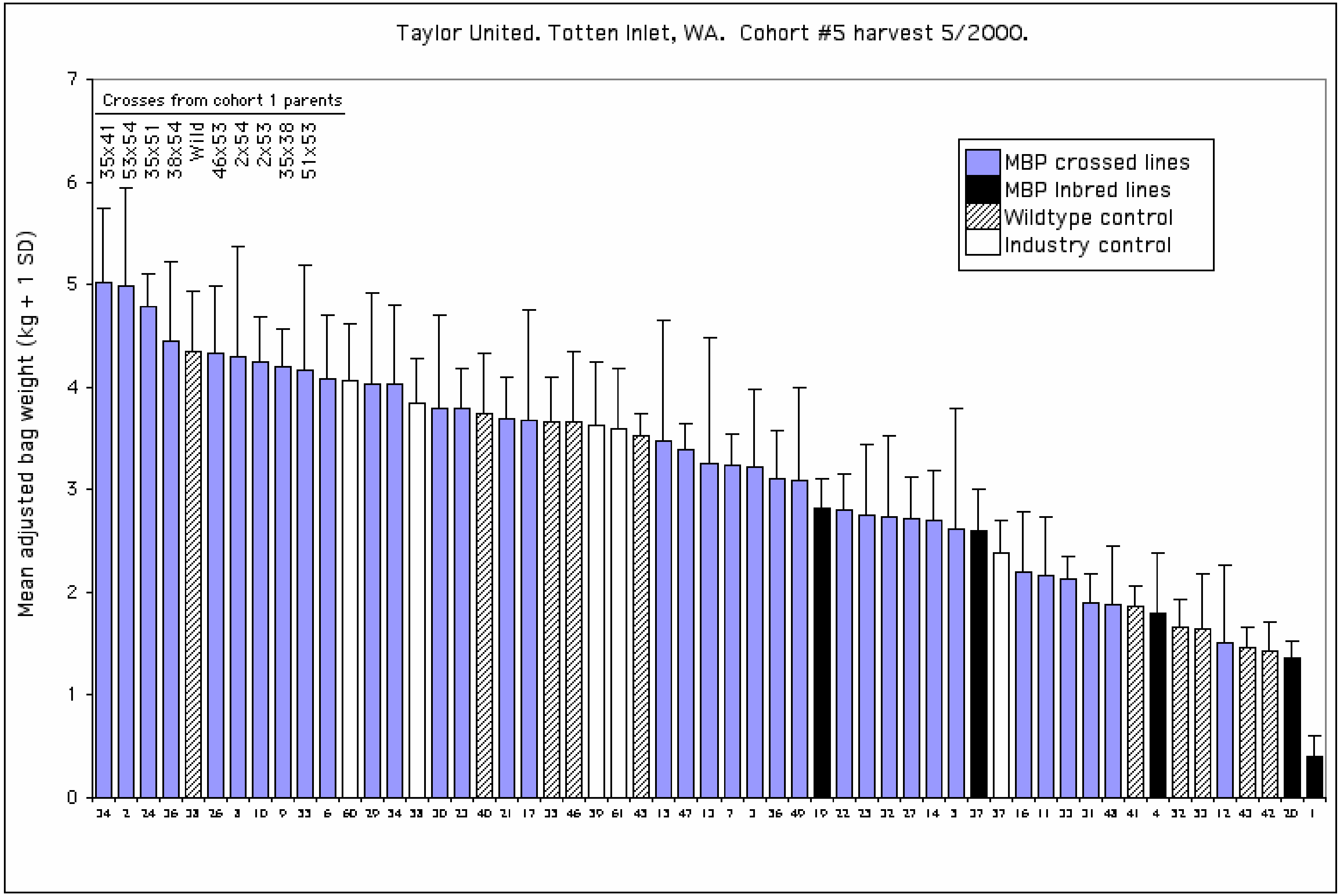



# Family-based selective breeding programs

- Molluscan Broodstock Program (MBP): selection for yield

Initiated in 1996

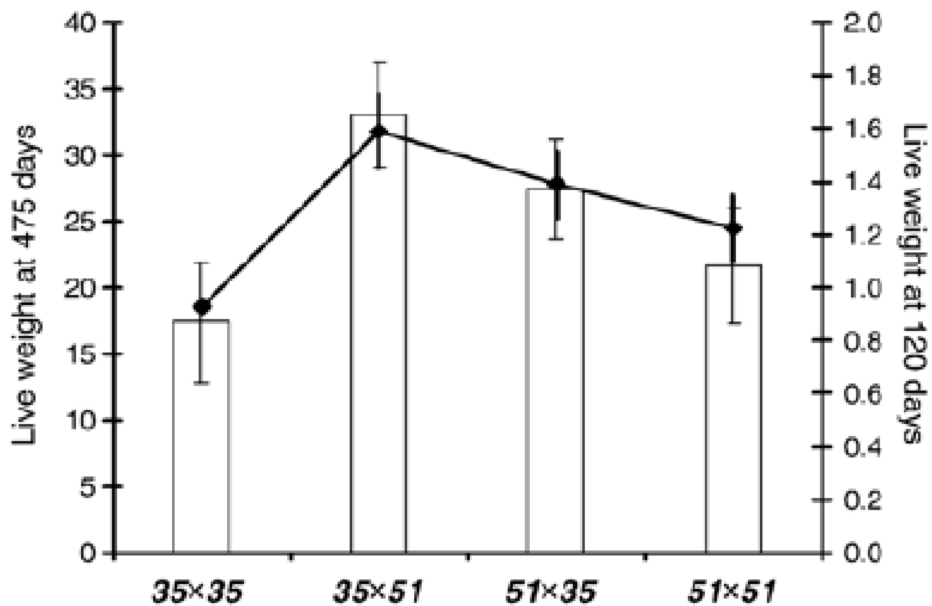
+10% / generation




www.hmsc.orst.edu/projects/mbp

# Family-based selective breeding programs

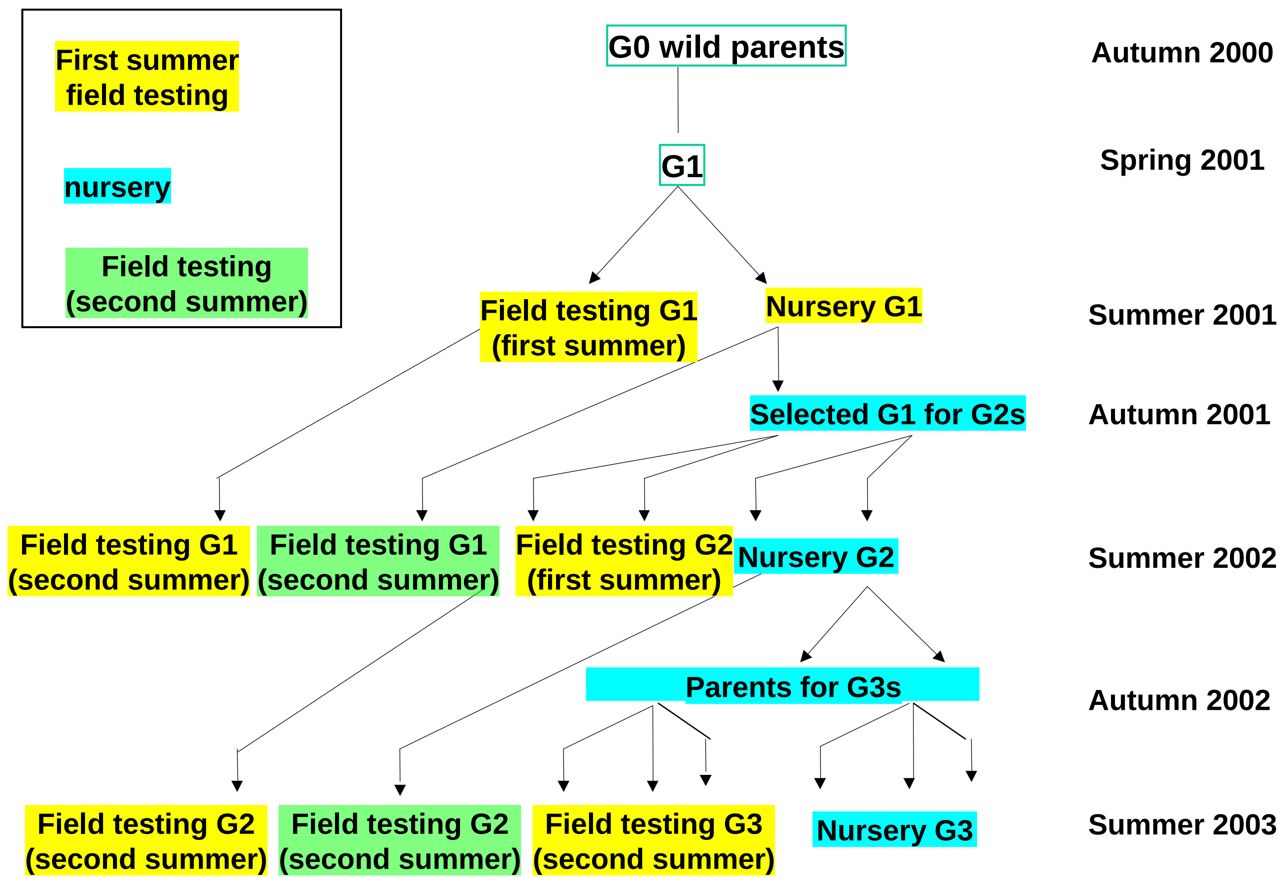
- "USDA Western Regional Aquaculture Center (WRAC) : hybrid vigor for yield by crossbreeding of inbred lines.

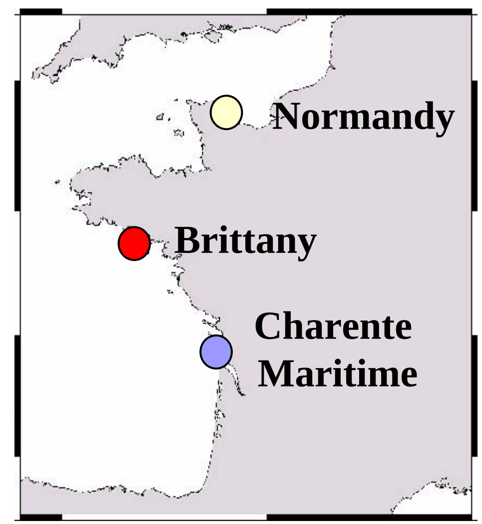

Hedgecock et al., 1995
*Hedgecock & Davis, Aquaculture in press*

www.fish.washington.edu/wrac

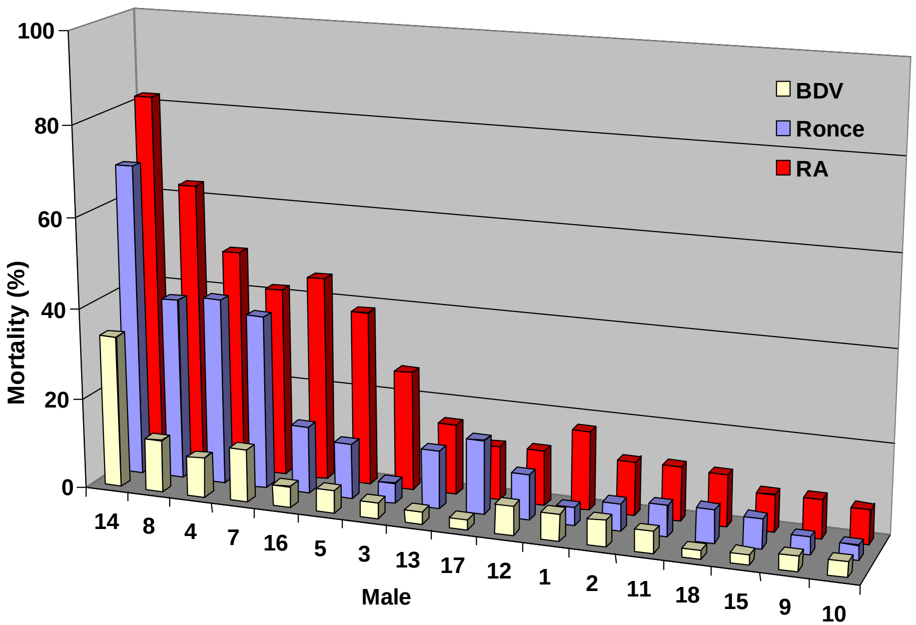
# Experimental selective breeding on spat survival

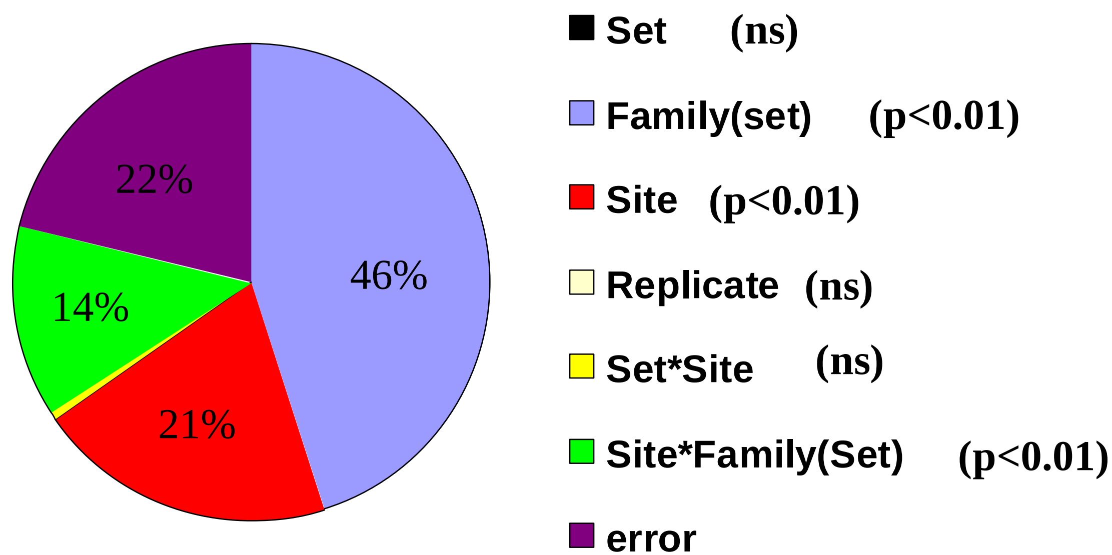



*Samain et al, 2001-2005*



# G1 (half-sib families): mortality in the field

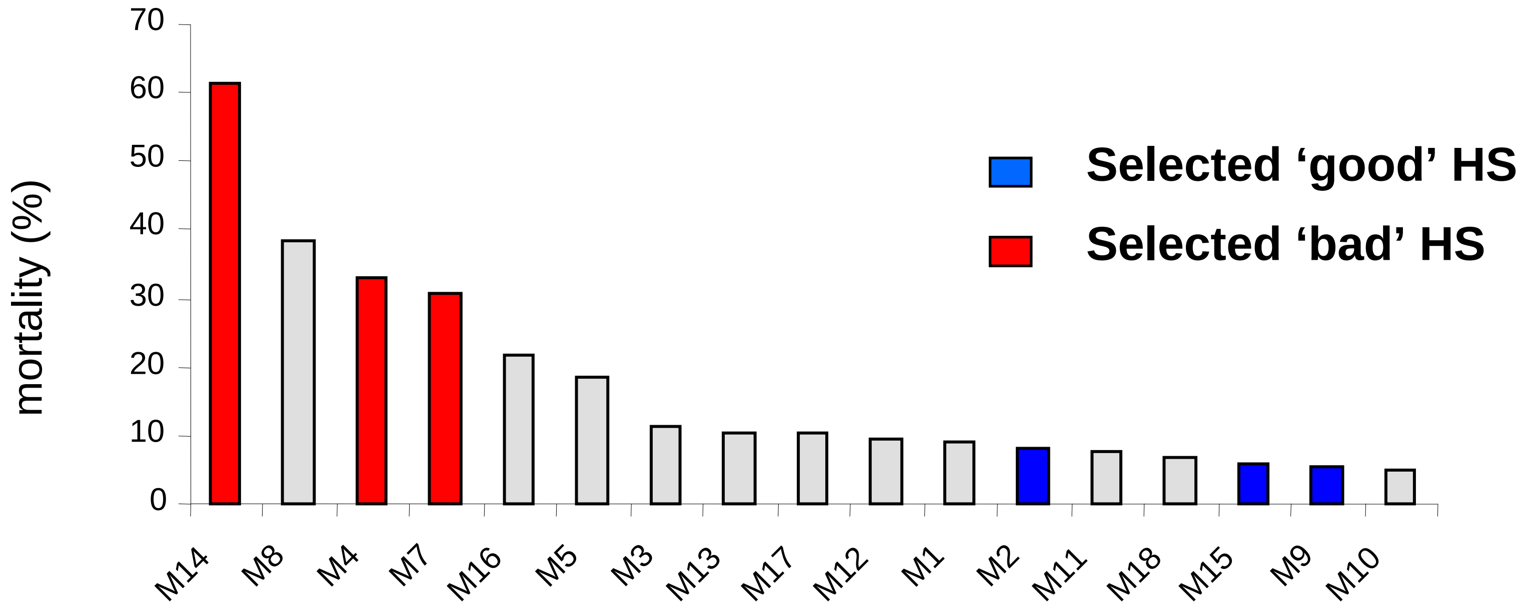



**Heritability = 0.83 ± 0.40**

*Degrémont et al., Aquaculture 2005; 2007*

# Second generation by divergent selection




## Low selected group 'S'

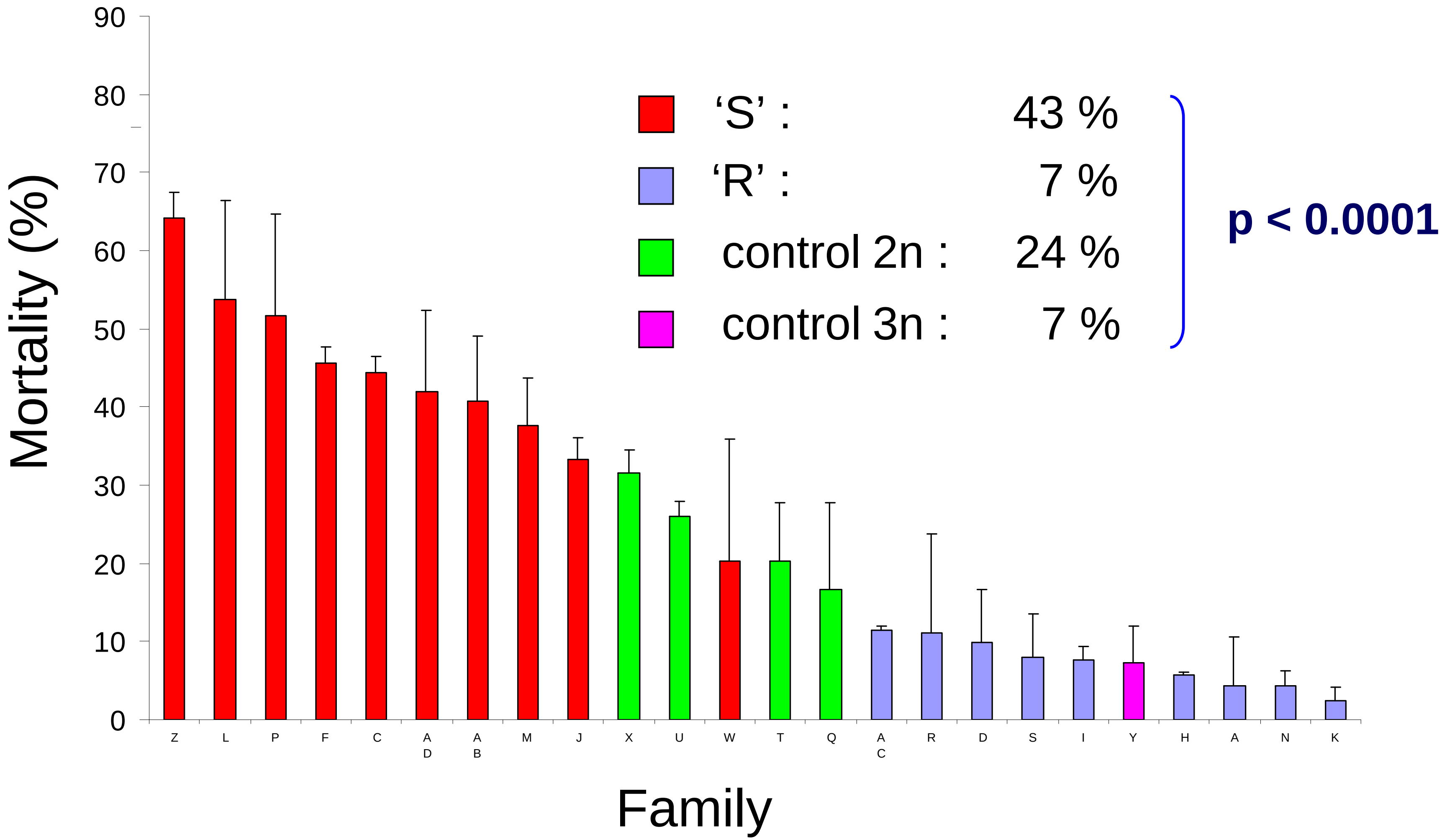
| Male | | 4 | | 7 | | 14 | |
|---|---|---|---|---|---|---|---|
| | Family | F4-15 | F4-16 | F7-25 | F7-26 | F14-54 | F14-55 |
| 4 | F4-15 | | | 13 | 14 | 17 | 18 |
| | F4-16 | | | 15 | 16 | 19 | 20 |
| 7 | F7-25 | | | | | 21 | 22 |
| | F7-26 | | | | | 23 | 24 |
| 14 | F14-54 | | | | | | |
| | F14-55 | | | | | | |

## High selected group 'R'

| Male | | 2 | | 9 | | 15 | |
|---|---|---|---|---|---|---|---|
| | Family | F2-5 | F2-8 | F9-35 | F9-36 | F15-57 | F15-58 |
| 2 | F2-5 | | | 1 | 2 | 5 | 6 |
| | F2-8 | | | 3 | 4 | 7 | 8 |
| 9 | F9-35 | | | | | 9 | 10 |
| | F9-36 | | | | | 11 | 12 |
| 15 | F15-57 | | | | | | |
| | F15-58 | | | | | | |

**+ Controls : 2N and 3N**

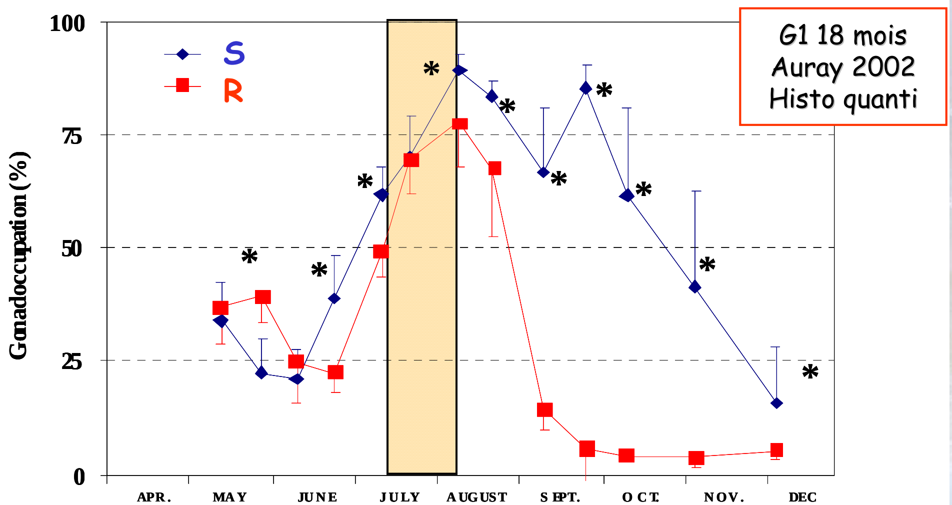
# Second generation:
# Summer mortality in Brittany




**S > control 2n > control 3n = R**

# Comparative physiology of oysters selected as « resistant » or « susceptible »

## Reproductive behavior



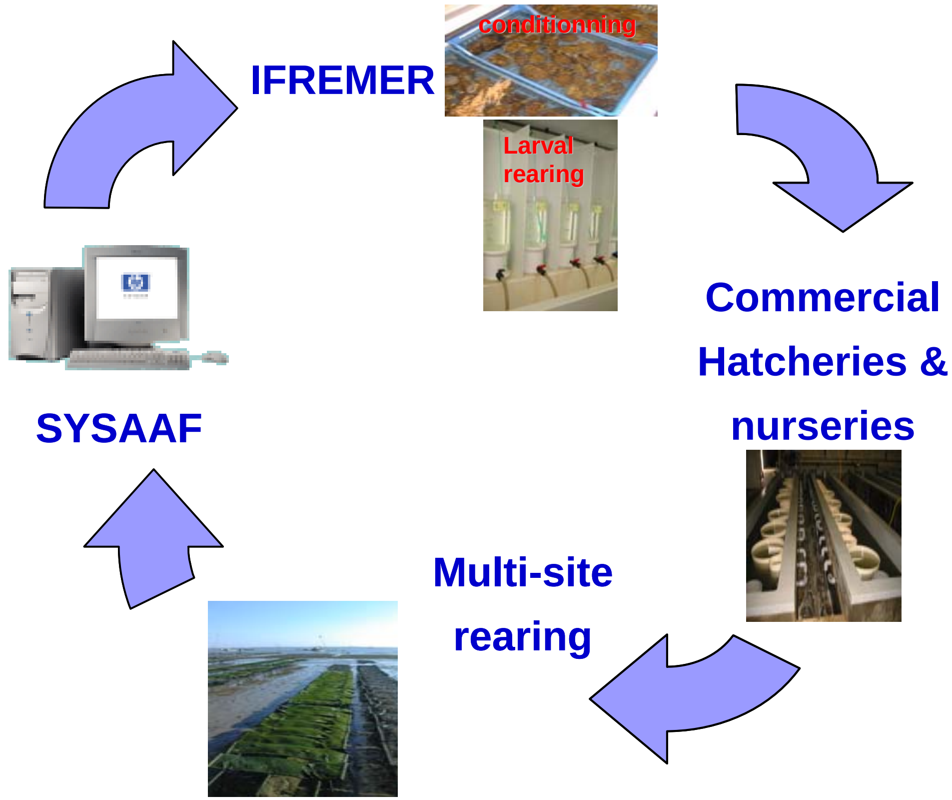


*Samain et al., Aquaculture in press*

**Trade-off between survival and reproduction**

*Ernande et al., JEB 2004*

# GIGAS+, a selection program to improve summer survival of the Pacific oyster Crassostrea gigas in France




Soon available as a book
Editors: J.-F. Samain & H. McCombie

# Towards selected triploids ?

## Combining gains of triploidisation and selective breeding

4n males (non selected)

2n males (non selected)

'R' 2n females

'S' 2n females

2n control females

**3n R** | **3n S** | **3n** | **2n**

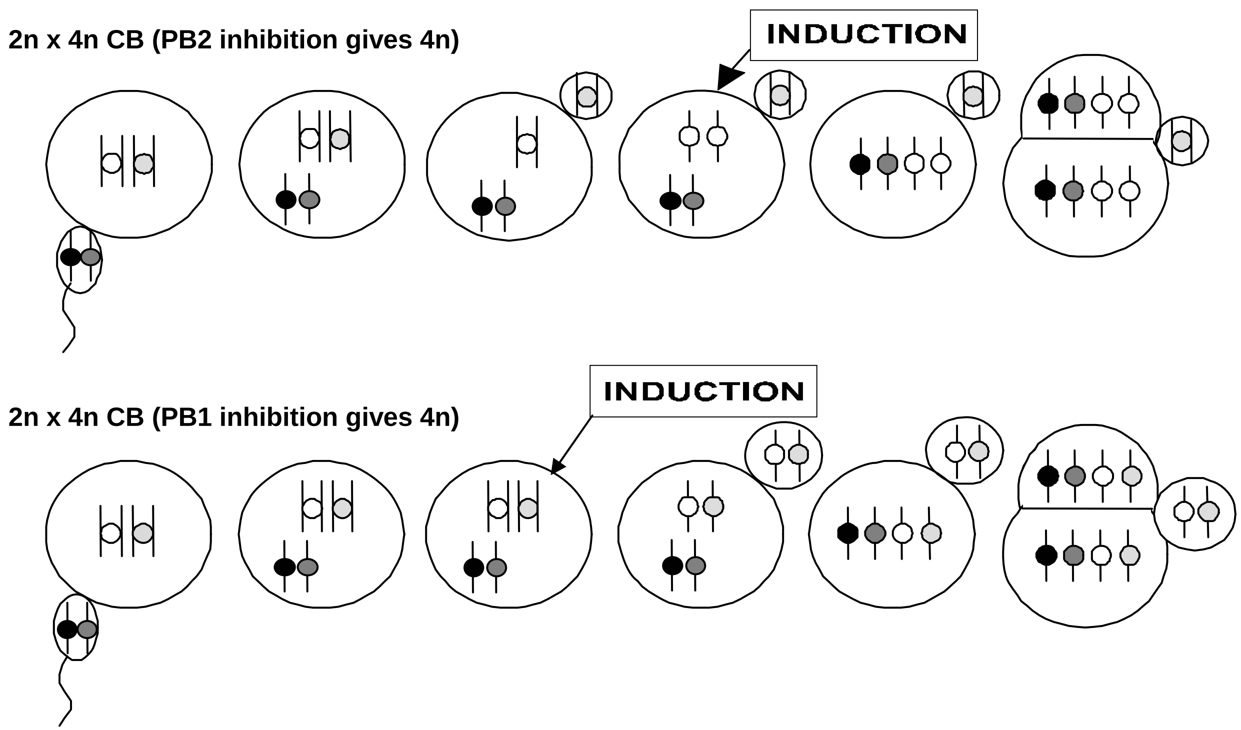
**Mortality :** **36 %** | **58 %** | **50 %** | **48 %**

**3n R < 2n = 3n = 3n S**

# Towards selected tetraploids ?

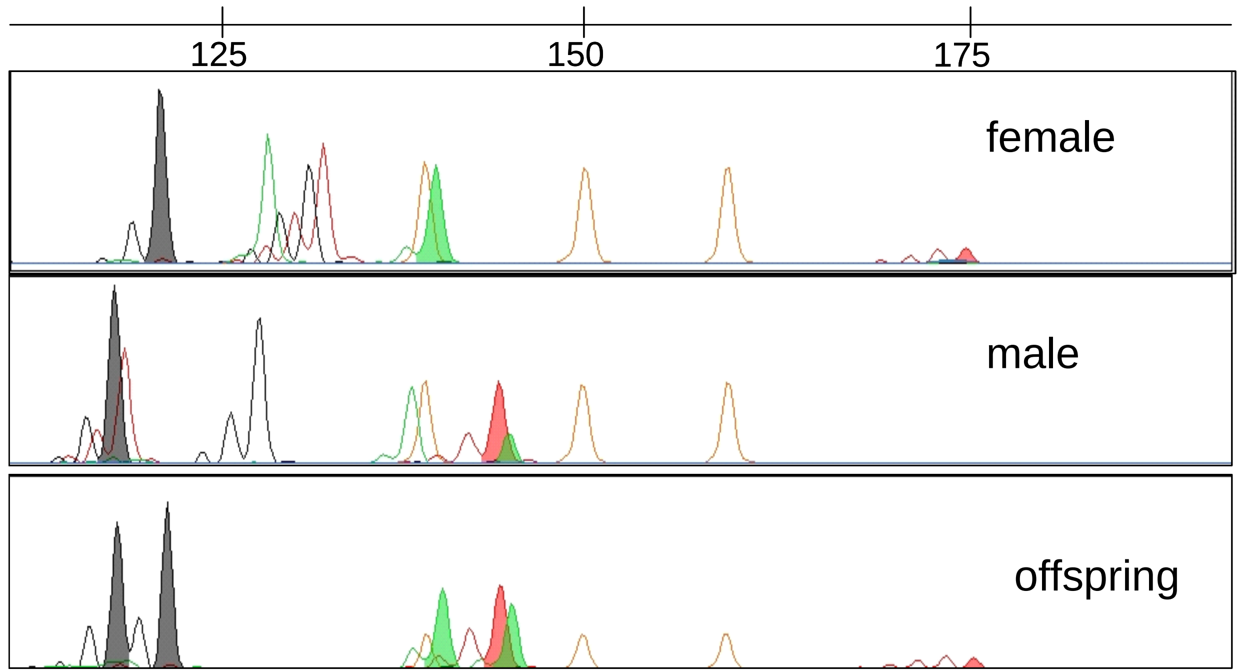
- **Production of tetraploid oysters is rather difficult**
    - production of improved tetraploids from improved diploids ?
    - direct selection at the tetraploid stage ?

**Introgression of selected traits from diploids to tetraploids :**




***McCombie et al., Marine Biotech. 2005***

# Parentage analysis using DNA markers



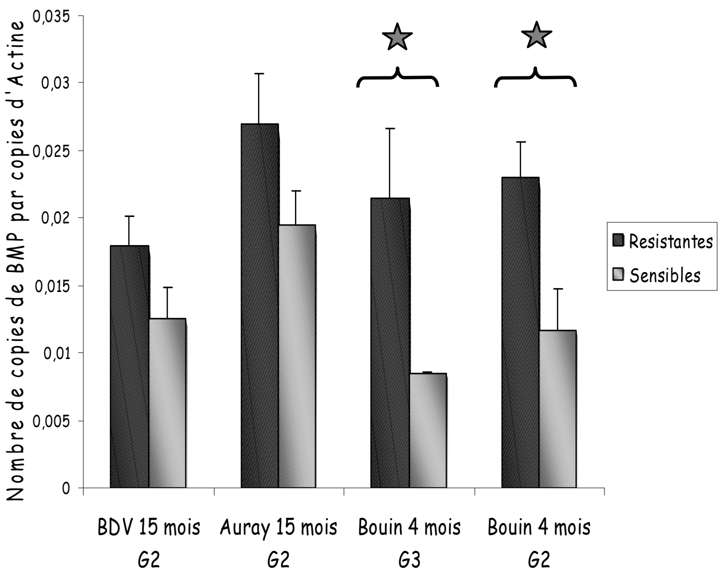

***Taris et al., Aquacuture. Research 2005***

- **Monitoring of genetic diversity in broodstock**
- **Confirmation of parentage and pedigree of broodstock**
- **Mixed-family approach : “walk-back selection” or “animal model”-based methods**

# Genomics:

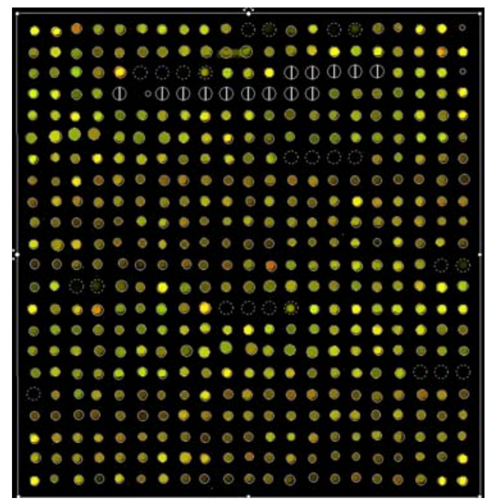
Study of gene expression of oysters selected as resistant or susceptible to summer mortality

**Candidate genes:**
***e.g.*** **Transforming Growth Factor β**



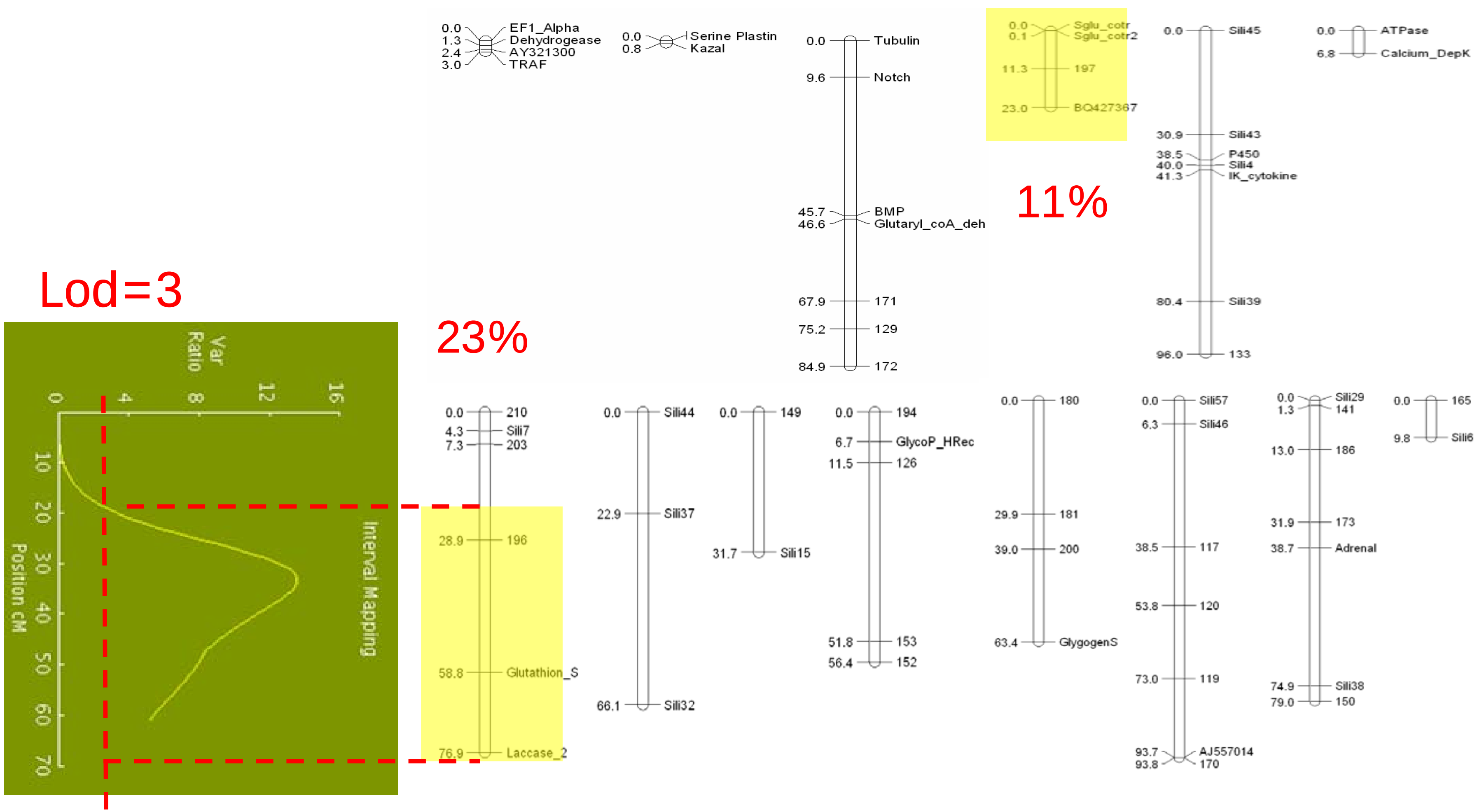

*Fleury et al., submitted*

**Microarrays:**
9058 unigenes spotted
(Max Plank Institute,Berlin)



# QTL mapping:

Towards a better understanding of the genetic basis of resistance to summer mortality and marker assisted selection



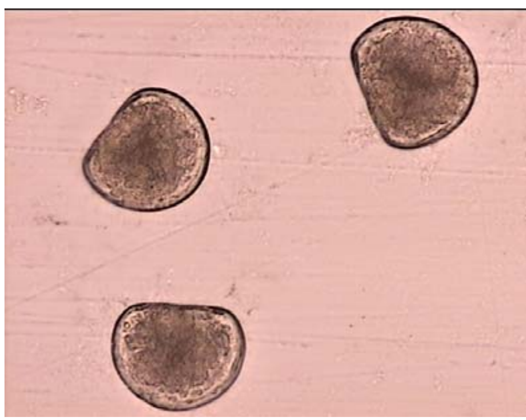

*Aquafirst EU supported project - Sauvage et al., in prep*

# Conclusions:

Until now , polyploidy is the most significant method to genetically improve bivalve production

Selective breeding programs based on individual selection can be efficiently established but should include monitoring of genetic variation in the selected population using genetic markers

Family-based selective breeding programs are more difficult and expensive to establish but they are likely to provide durable and long term multi-traits genetic improvement



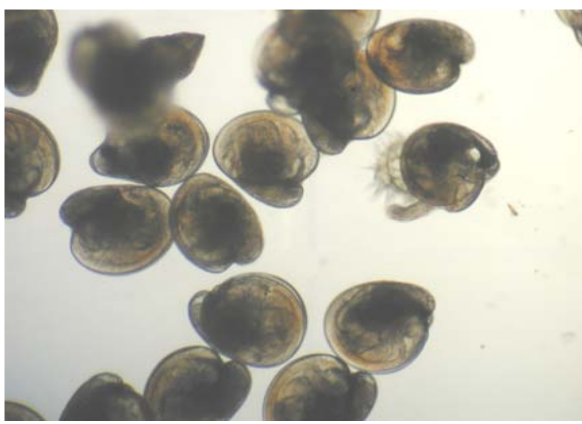
# Suggested recommandations for future bivalve breeding programs:

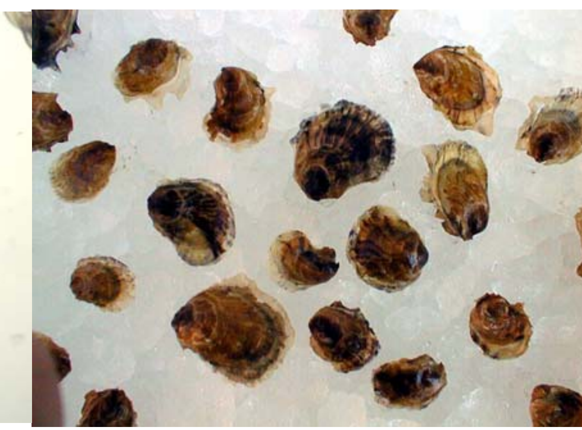
‘Full-scale’ breeding programs should be established as a collaborative effort between industry and research

‘Full-scale’ breeding programs should consider multi-trait heritabilities, genetic correlations, reaction norms and trade-offs in different rearing environments (G x E interactions).

Selection of diploids and polyploid breeding should be integrated

Genomics and QTL mapping should be supported as they open many promising perspectives to improve breeding strategies





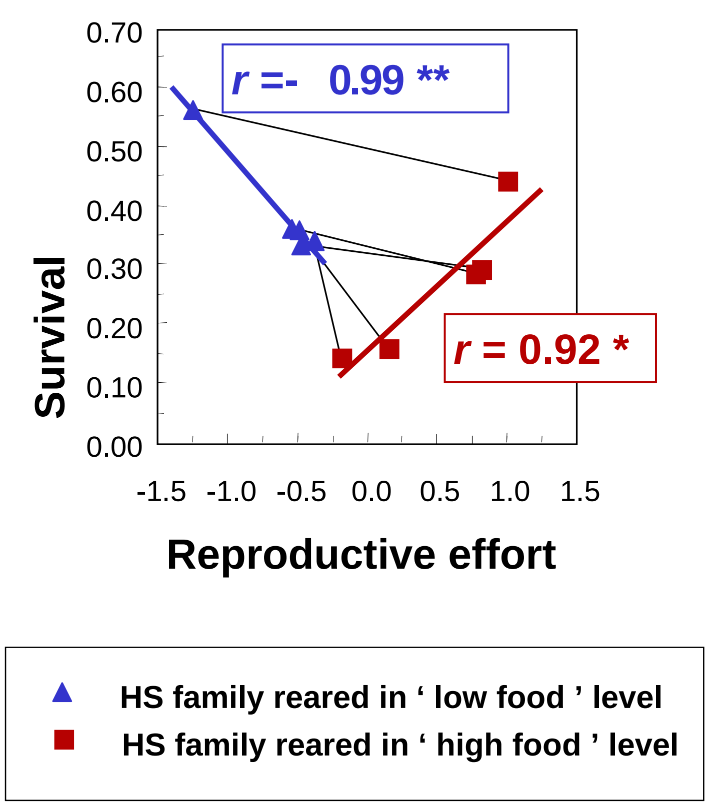
# Acknowledgments :

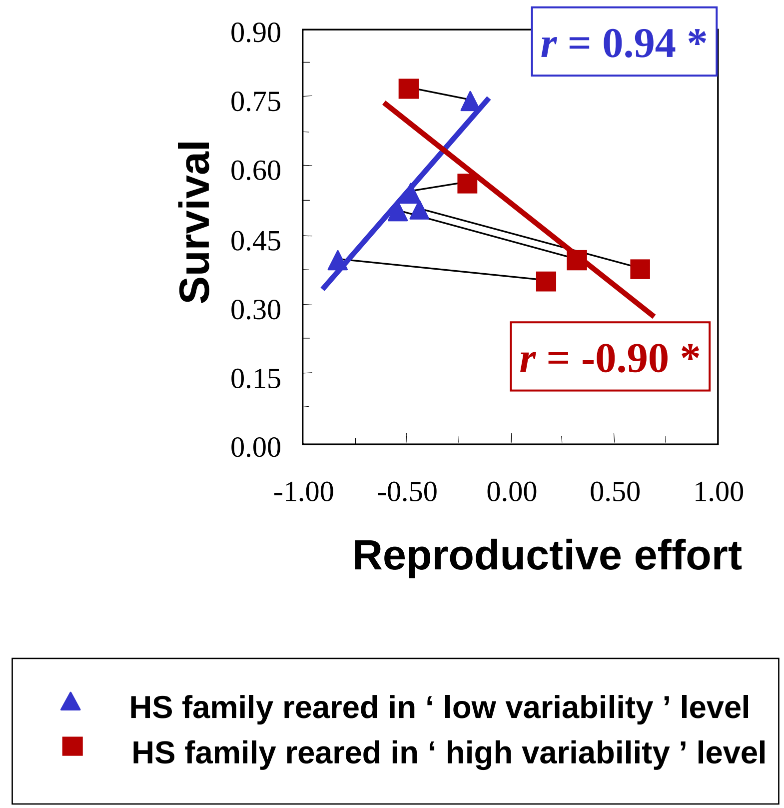
**Ifremer *La Tremblade : Lionel Degrémont, Nicolas Taris, Helen McCombie, Bruno Ernande***

***SYSAAF : Pierrick Haffray***

***MOREST : Jean François Samain et al.***


# Trade-offs ? Evidence in one-year old oysters




+ Significant positive genetic correlation between plasticity of reproductive effort and survival

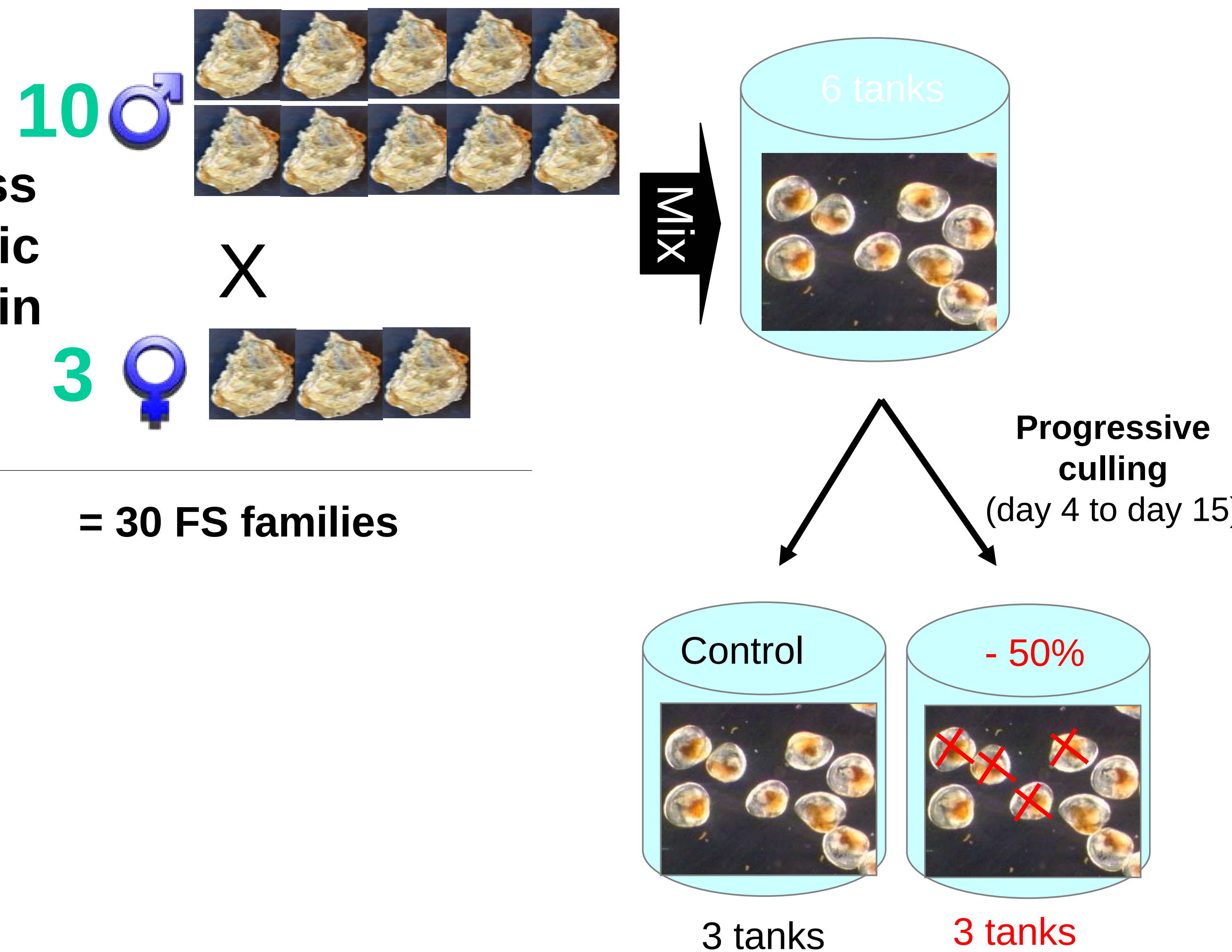
*Ernande et al., J.E.B. 2004*

*Ernande et al., in prep.*

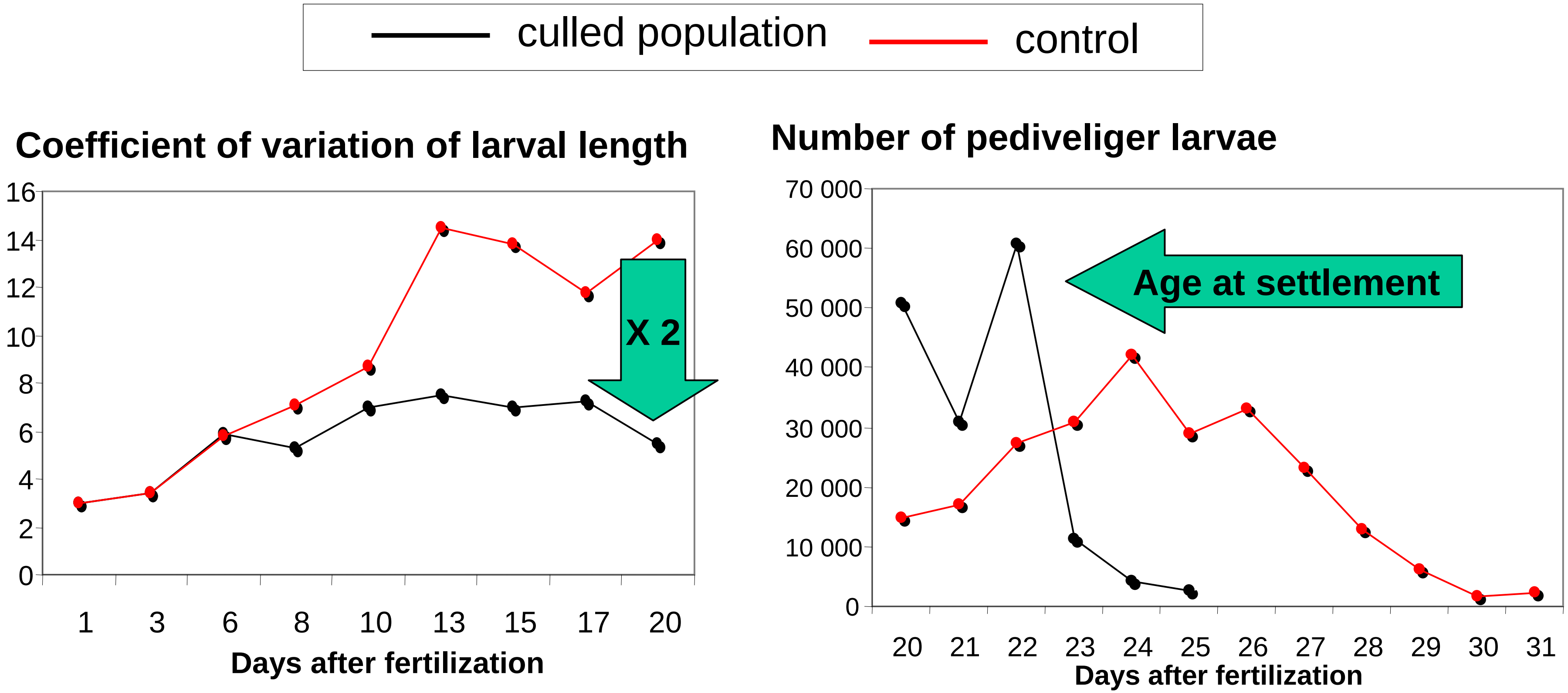
# Mixed-family approach:

## genetic effect of culling at larval stage

**Full factorial cross with <u>equal</u> gametic contribution within each sex**



# Phenotypic effect of culling

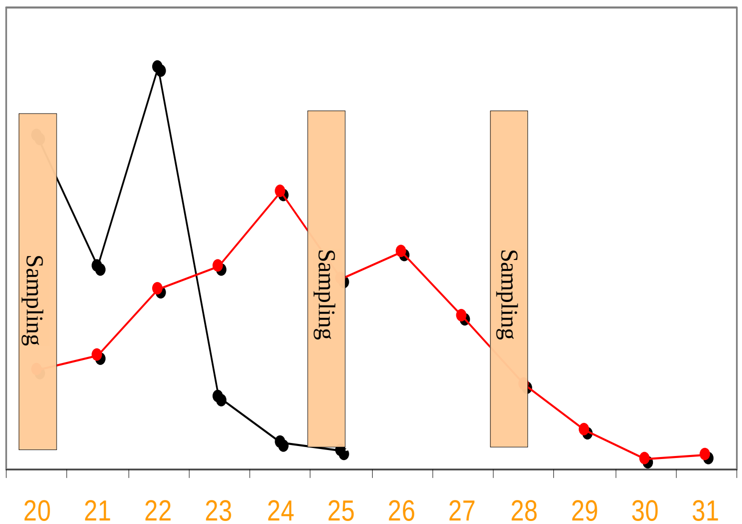



**Limited effect on <u>yield</u>:**

-30 % of ready-to-settle larvae (higher survival of fast growing larvae)

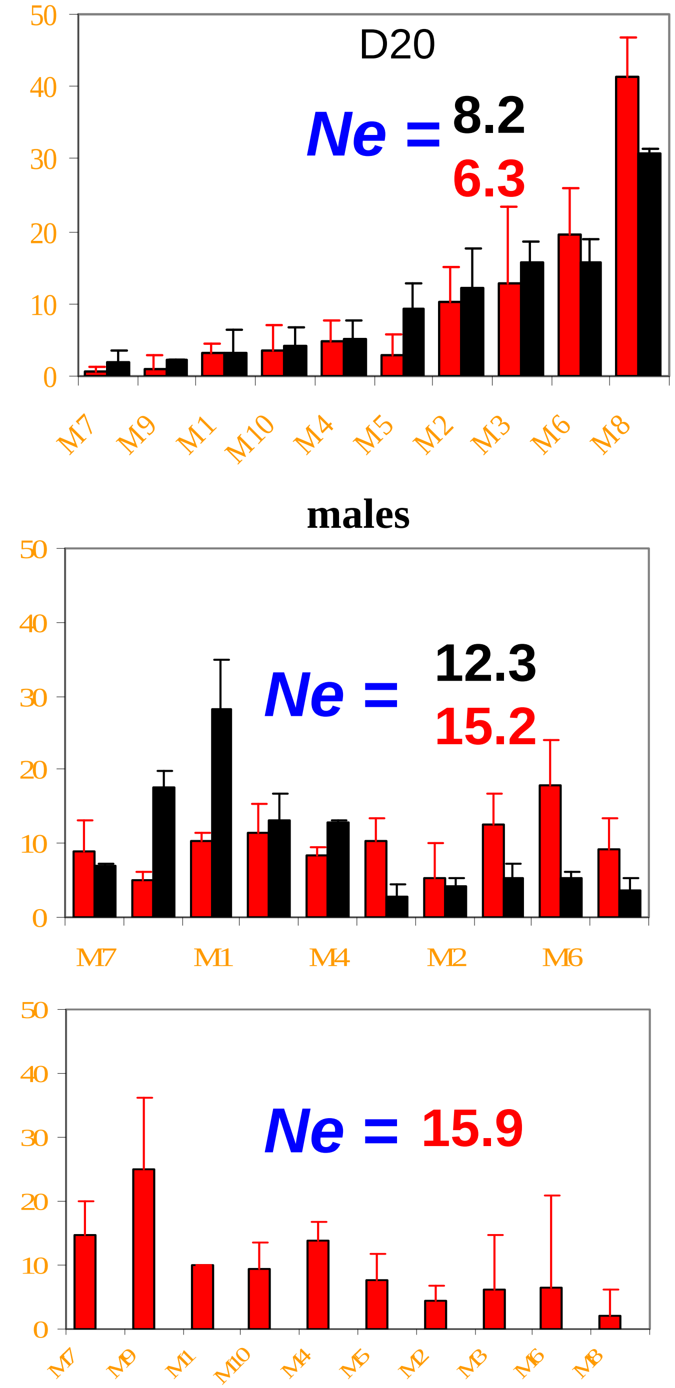
-15 % of spat (higher settlement success of fast growing larvae)

# Mixed-family approach:
## genetic effect of culling at larval stage

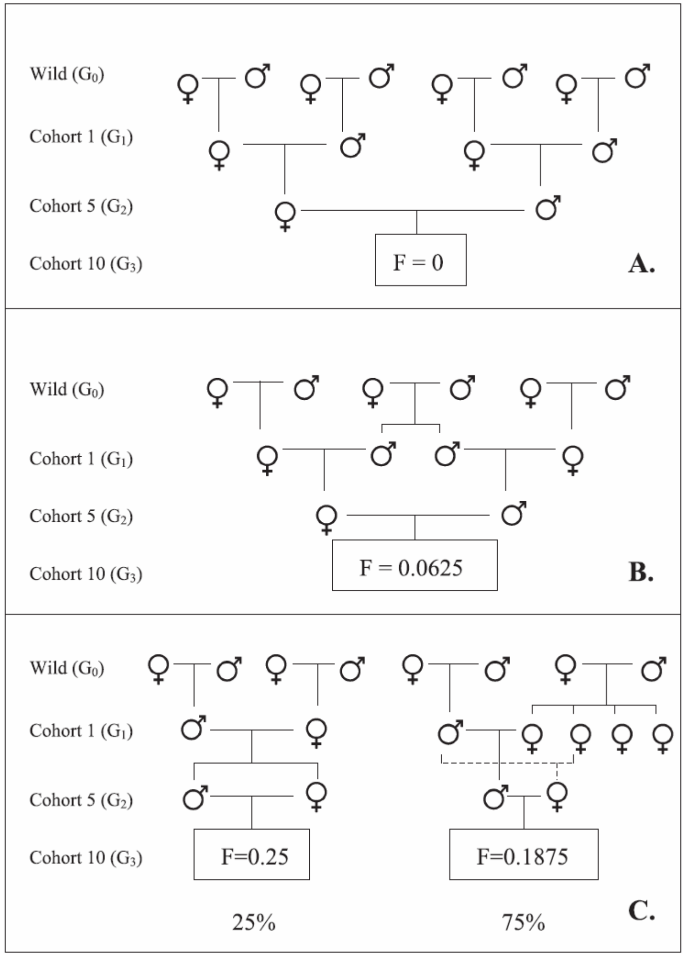


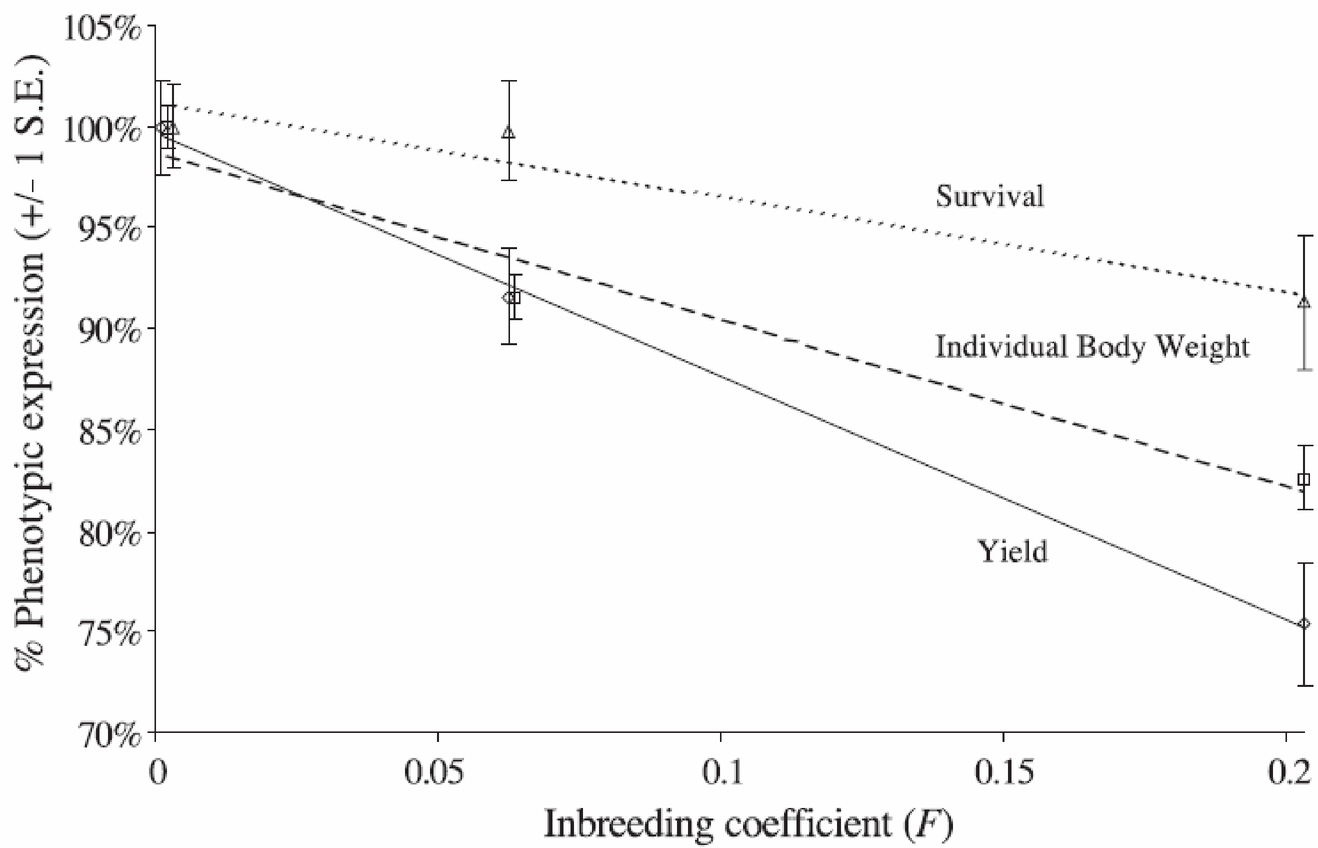

**The effect of culling on genetic diversity is mediated through its effects on the timing of settlement**

(Taris *et al*., JEMBE 2006)



# Inbreeding depression of survival, growth and yield resulting from mating related parents



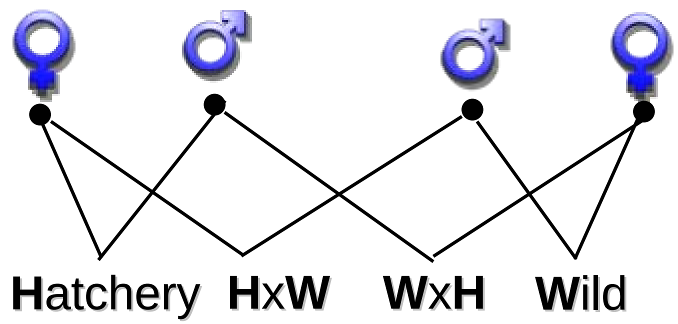




*Evans et al, Aquaculture 2004*

# « Hidden » inbreeding depression at larval stage in a domesticated oyster broodstock

**Oysters from a commercial hatchery broodstock selected over 7 generations for growth and shell shape with high culling and high temperature at larval stage**

**Oysters from a French natural bed**

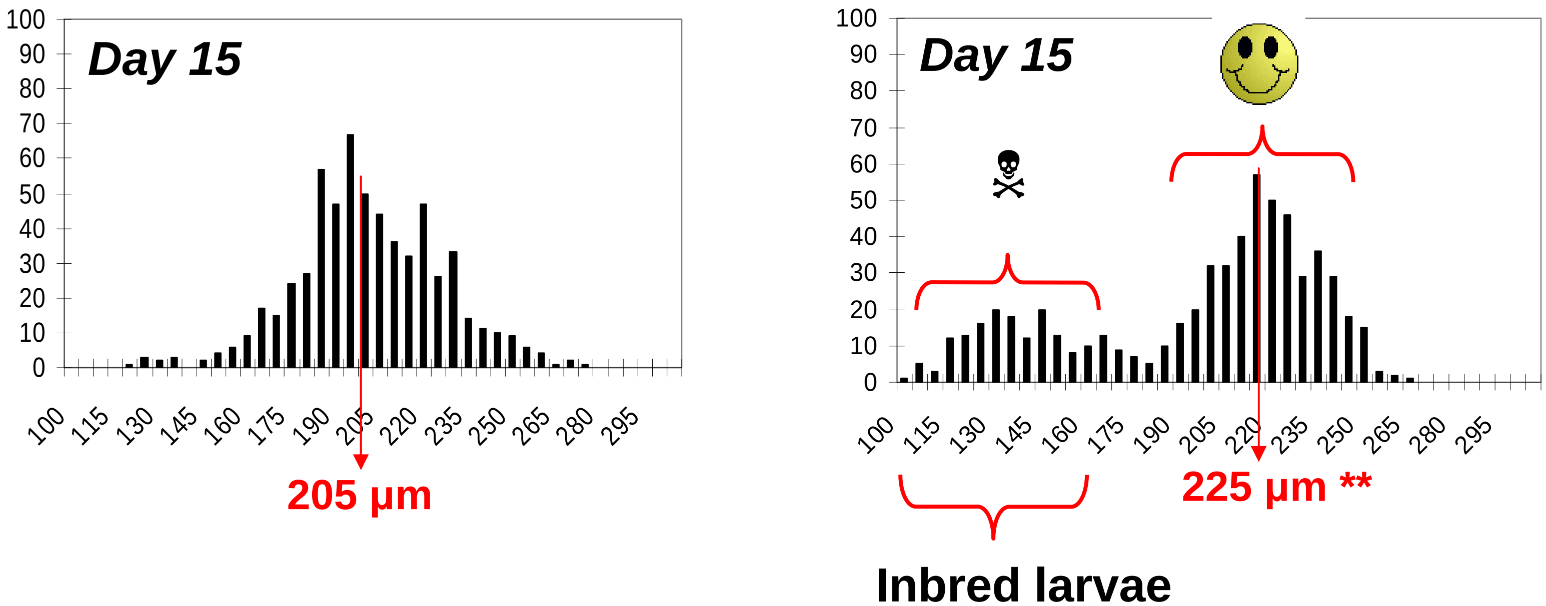



*Taris et al, Aquaculture in press*

# « Hidden » inbreeding depression at larval stage in a domesticated oyster broodstock

| | Wild broodstock | | Hatchery broodstock | |
|---|---|---|---|---|
| **Microsatellite markers :** | | | | |
| **Mean nb. of allele / locus** | **34** | **>** | **10** | **- 70%** |
| **Observed heterozygosity** | **0.86** | **<** | **0.66** | **- 20%** |

## Wild larvae / Domesticated larvae




*Taris et al, Aquaculture in press*