Genetic improvement of hatchery propagated bivalve stocks : prospects and constraints

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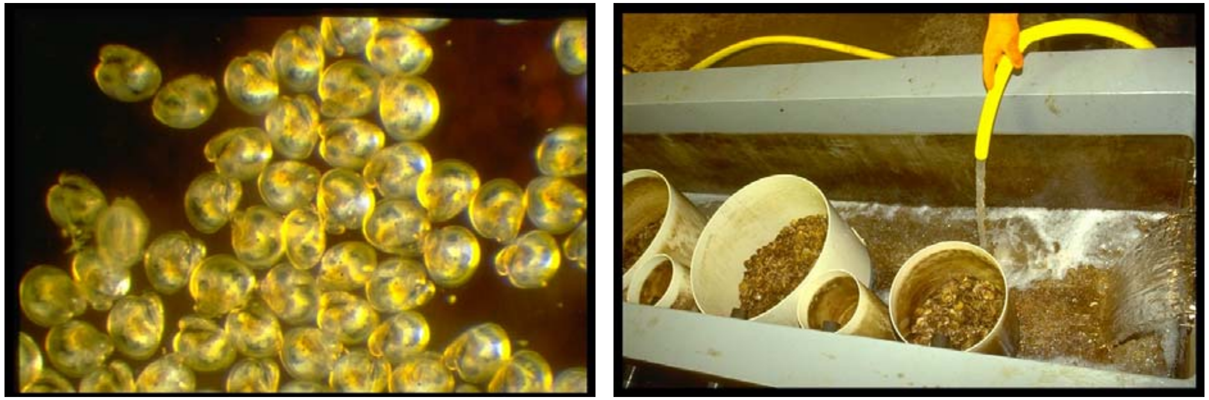
Two possible sources of oyster spat

(1) natural settlement (native or introduced species)

Natural selection ?
Local adaptation ?
Gene flow ?

(2) hatchery propagation



Genetic drift ?
Domestication ?
Selective breeding ?

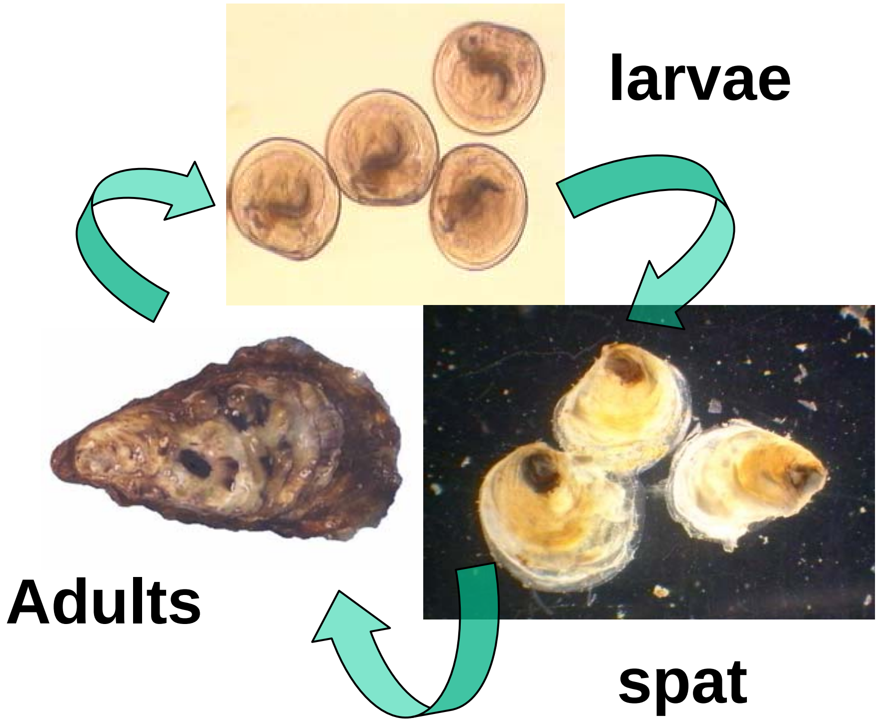
Why and when to worry about genetic diversity of hatchery-propagated stocks ?

- **Open stocks** (i.e. new « wild » genitors at each generation) :

✓ Stability of performance (« buffer effect »)
✓ Reduce the potential impact on the diversity of wild stocks

- **Closed stocks** :

✓ inbreeding depression
✓ limited response to selection



N_e estimates in shellfish broodstock

(Hedgecock et al., 1992)

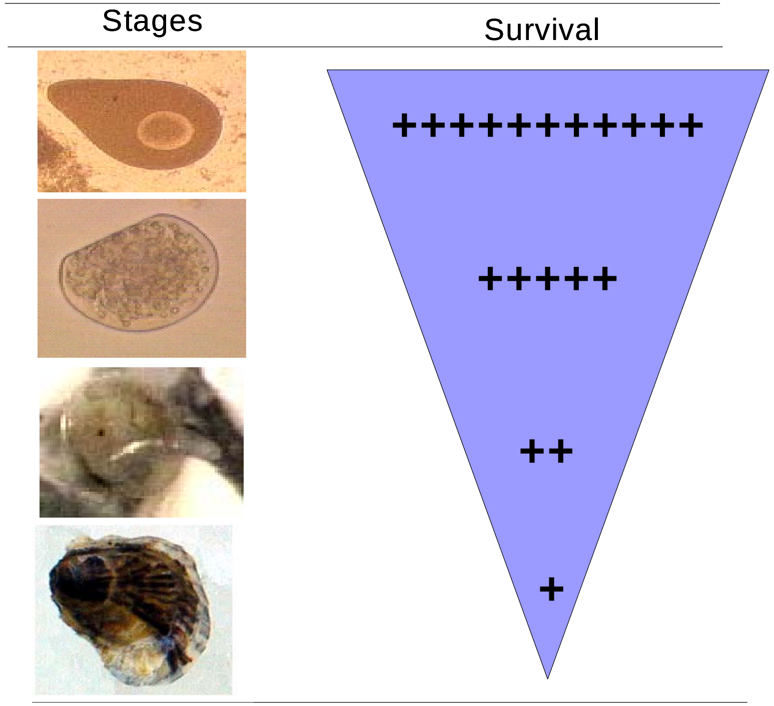
➢ **Estimates based on temporal variance in allelic frequencies of neutral markers among generations (Waples, 1989)**

e.g. in *O. edulis* :

- ✓**Saavedra & Guerra (1996): *Ne* ≈ 4**
- ✓**Launey et al. (2001): *Ne* = 3 to 20**

High fecundity

High mortality at early stages



“elm-oyster model”

G. C. Williams 1975

How to maximize the genetic diversity and N_e of a hatchery-propagated stock ?

1) **High number of genitors**

- ✓how many genitors really spawned ?

2) **Balanced sex-ratio**

$$N_e = \frac{4Nm\,Nf}{Nm + Nf}$$

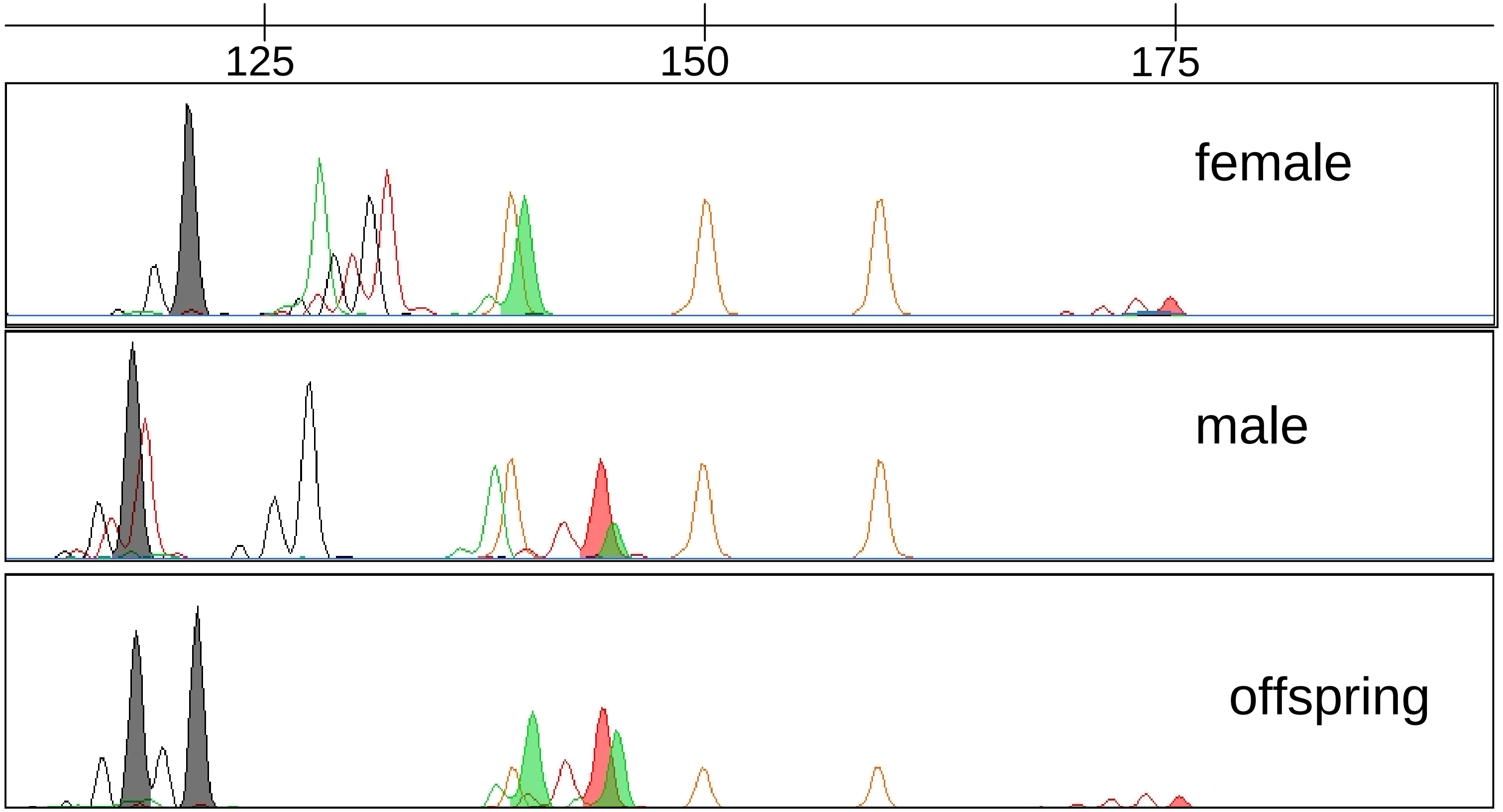
- ✓non destructive sex determination prior to spawning ?

3) **Equal representation of the genitors in the progeny**

- ✓same number of gametes / genitor ?
- ✓same number of offspring / genitor ?

$$N_e = \frac{(\Sigma n_{ij})^2}{\Sigma n_{ij}^2}$$

How to estimate variance in reproductive success ?

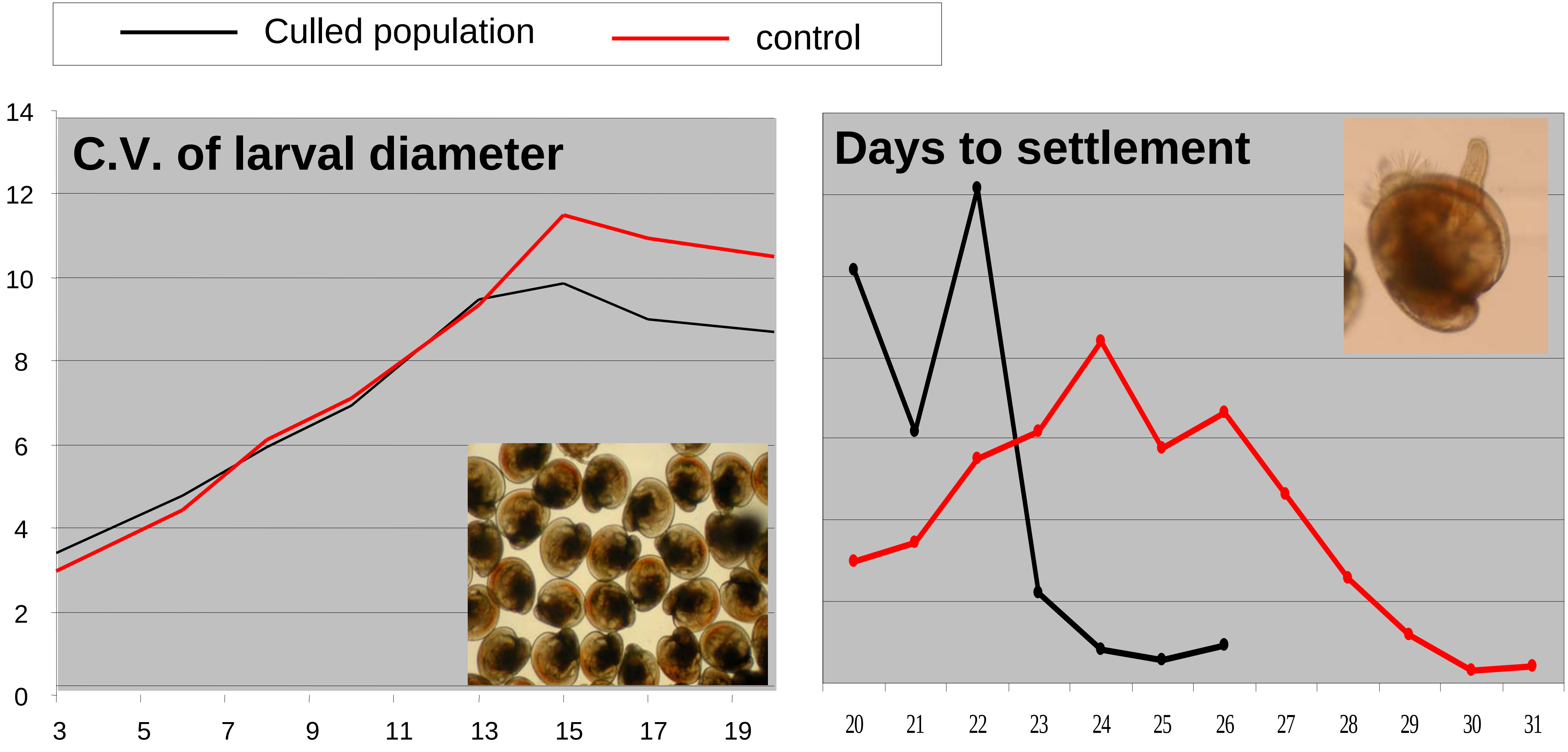


PCR-multiplexed microsatellite loci

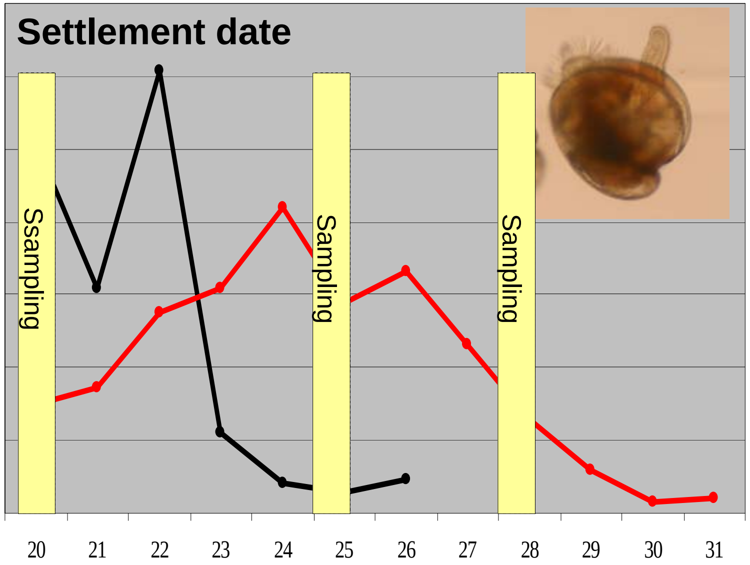
(Taris et al., Aquac. Res. 2005)

Experimental examination of factors affecting N_e :

Effect of culling 50% of the larvae in a 10 males x 3 females cross

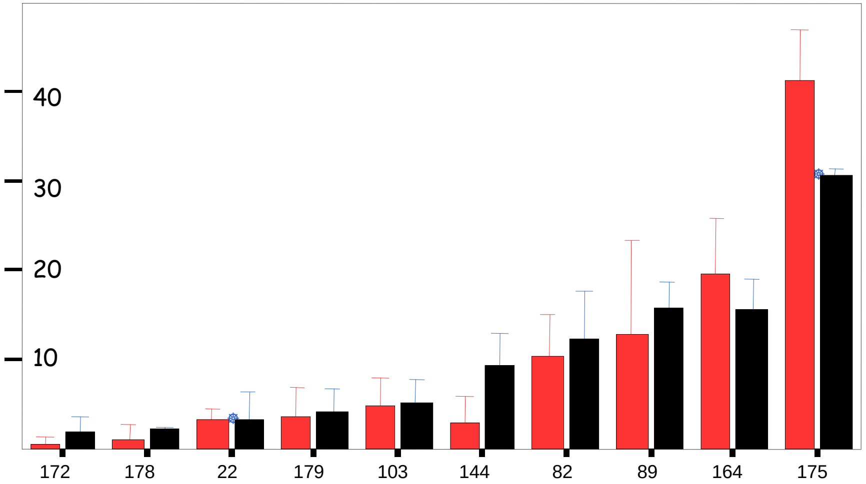


Temporal variation of male reproductive success in 3 successive settlement cohorts

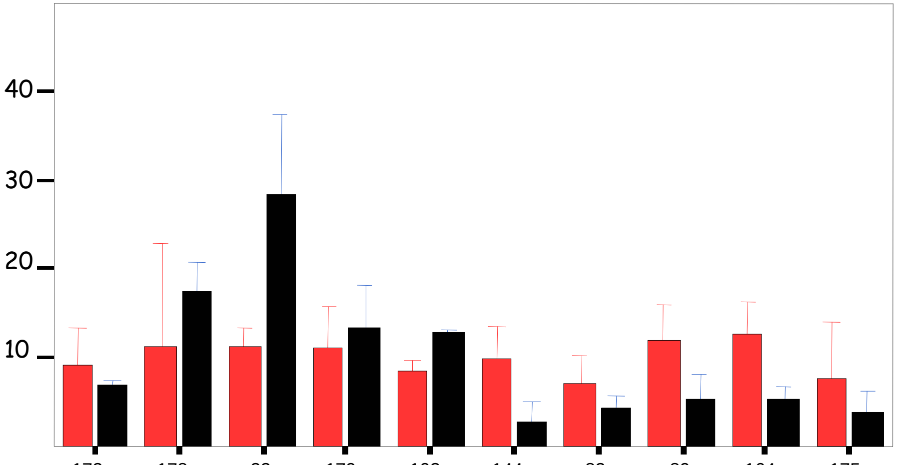


	A	B	C
Ne =	8.2	12.3	
	6.3	15.2	15.9

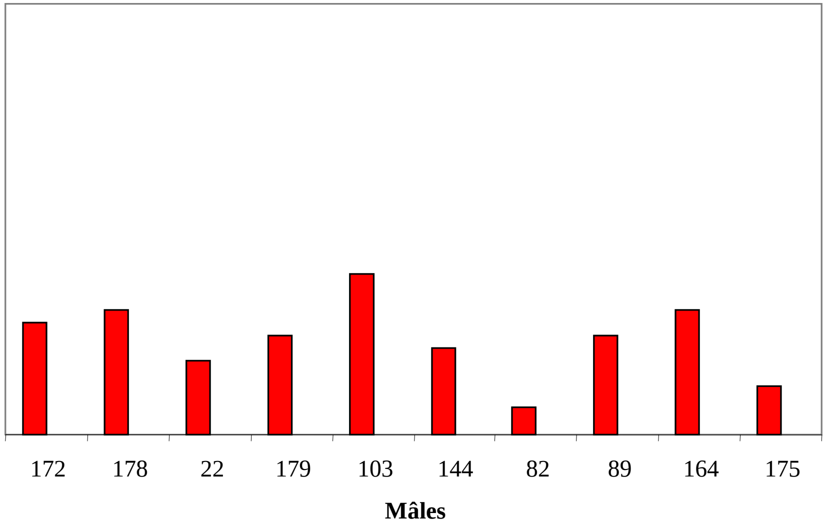
A



B



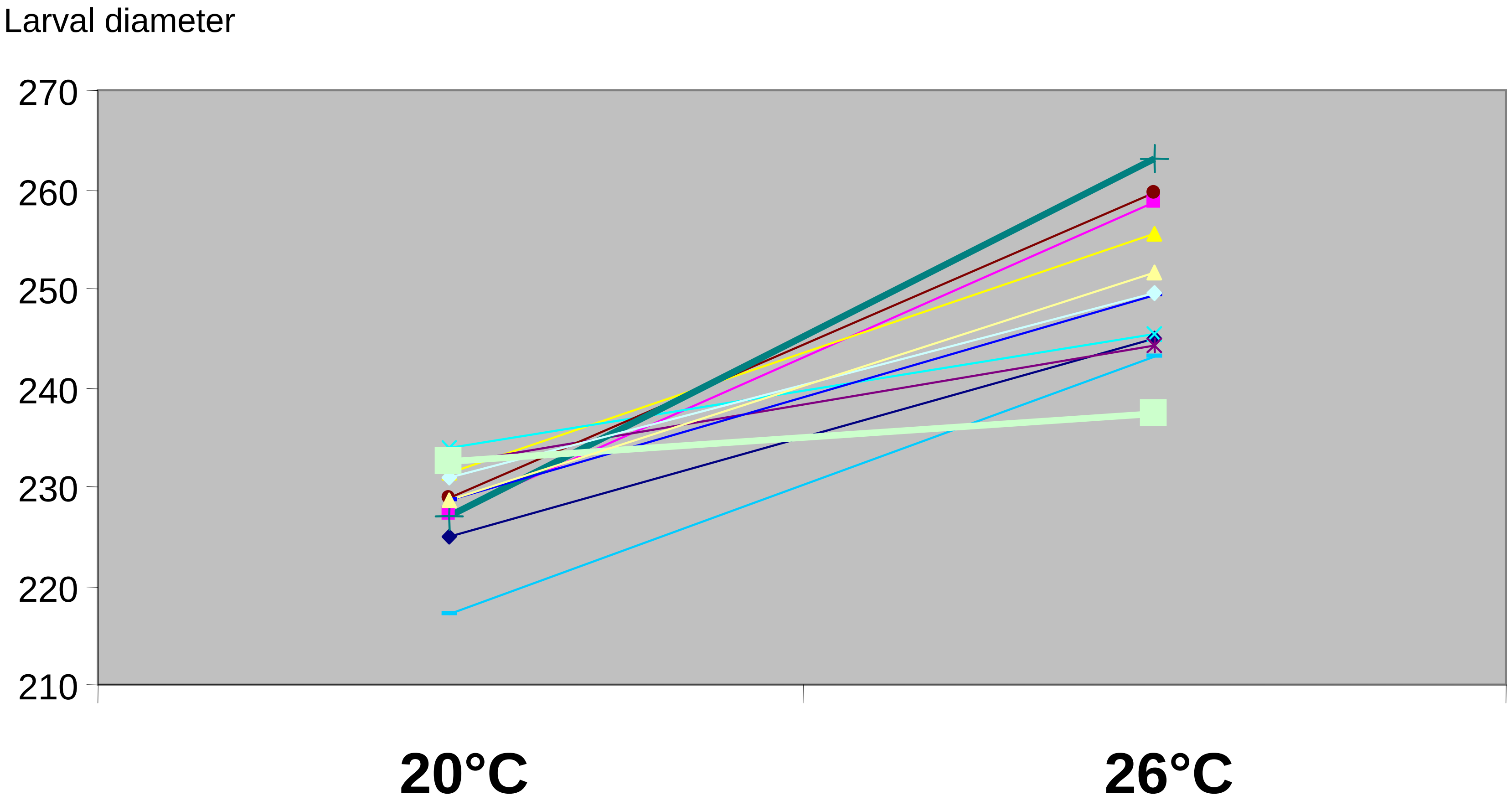
C



(Taris et al., in prep)

Experimental examination of factors affecting larval growth :

Effect of temperature in a 12 males x 4 females cross

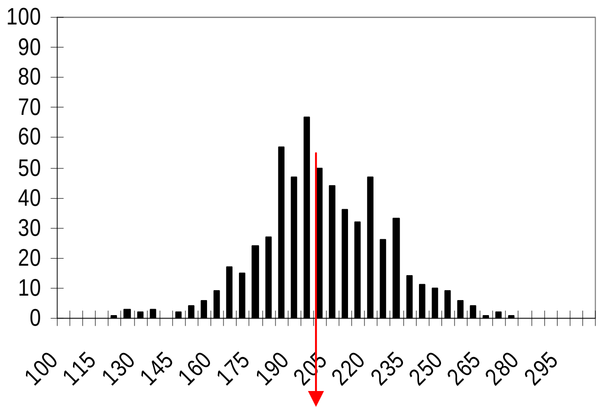


Male	ns	p<0.05
Female	ns	p<0.05

Is there selection for fast growing larvae in hatcheries ?

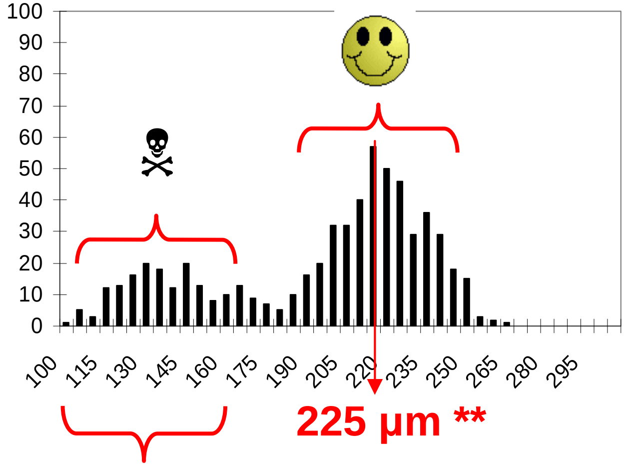
Hatchery stock :
7 generations of “domestication”
Loss in allele diversity ≈ 70%
Loss in heterozygosity ≈ 20%

Wild stock (= control)



205 µm

Day 15
24°C
No culling

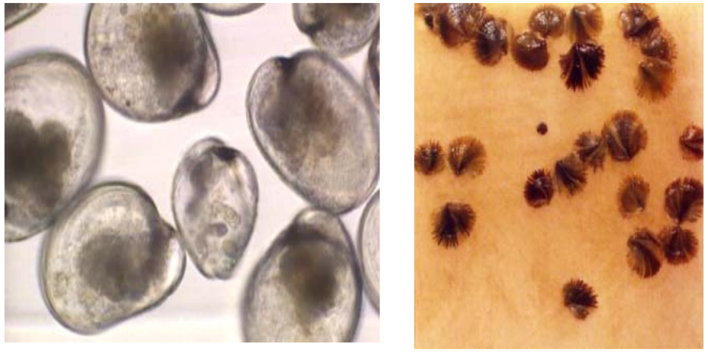


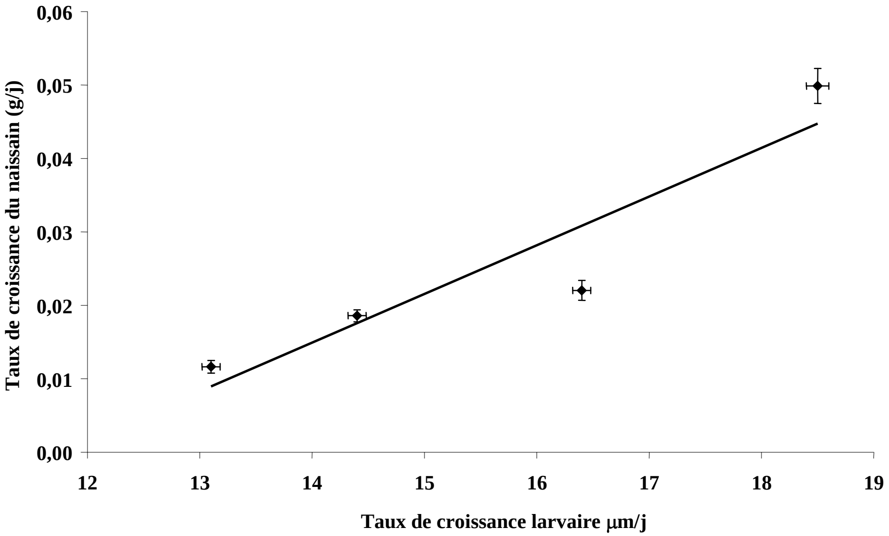
225 µm **

Inbred larvae ?

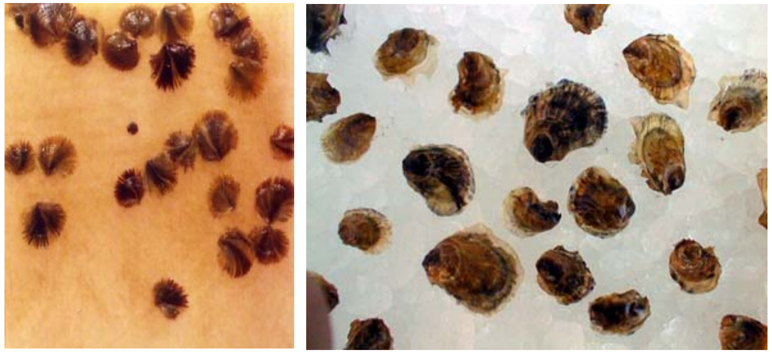
Is larval growth important ?

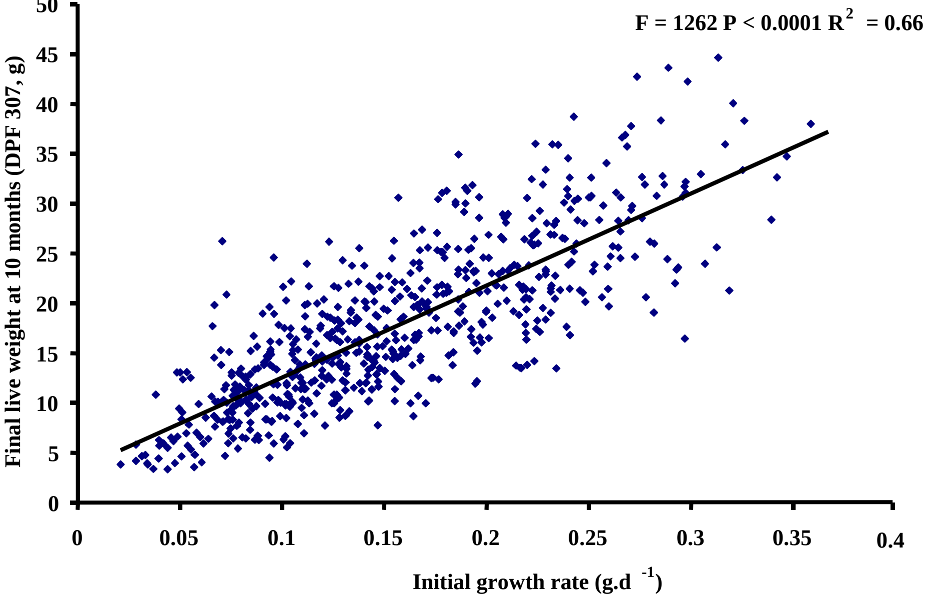
Temporal phenotypic correlations





Collet et al., Aquaculture 1999

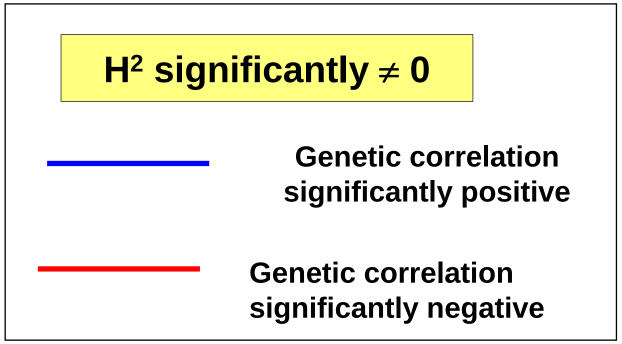


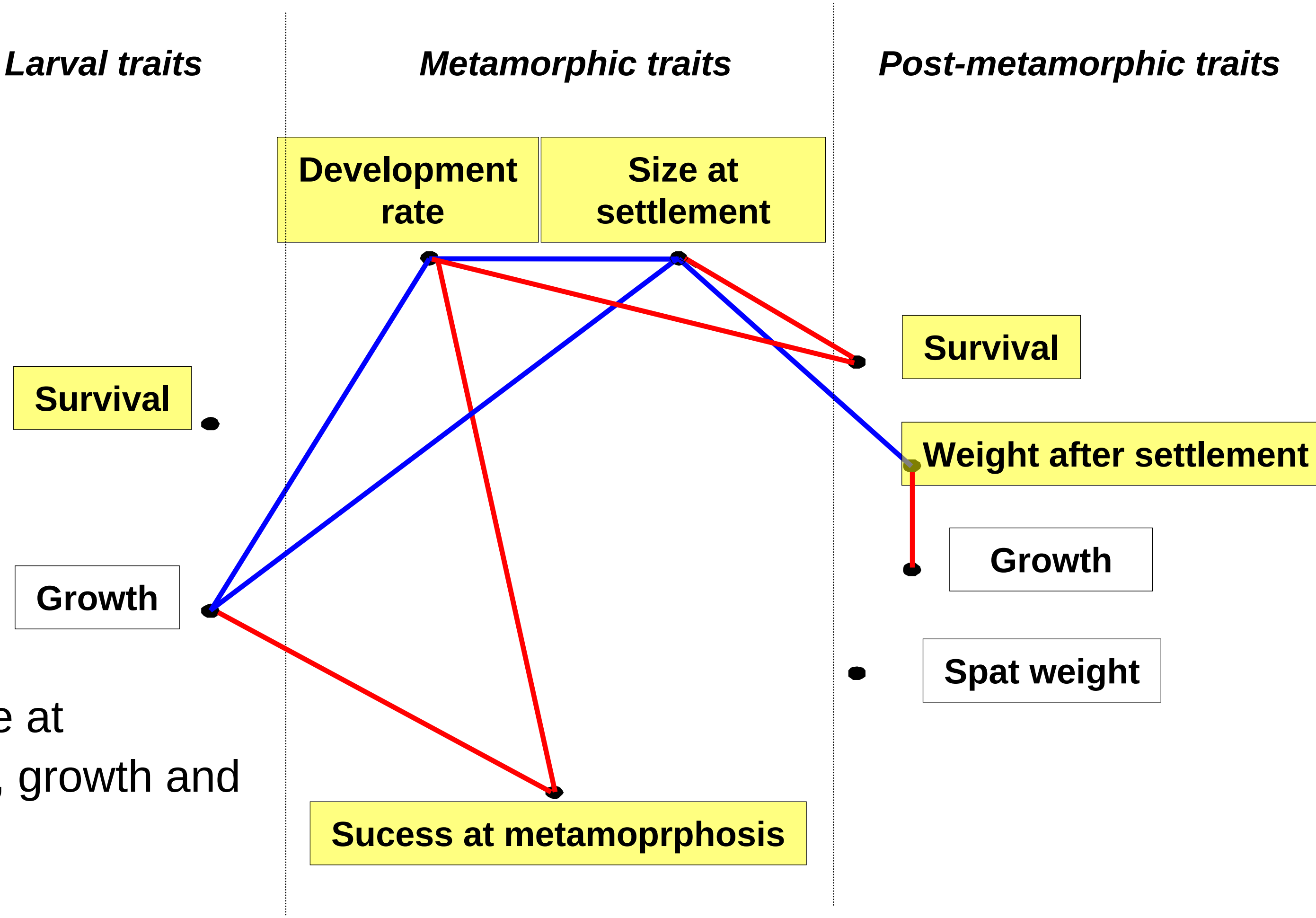


Boudry et al., Aquac. Int. 2003

Is larval growth important ?

Genetic correlations with other early life traits





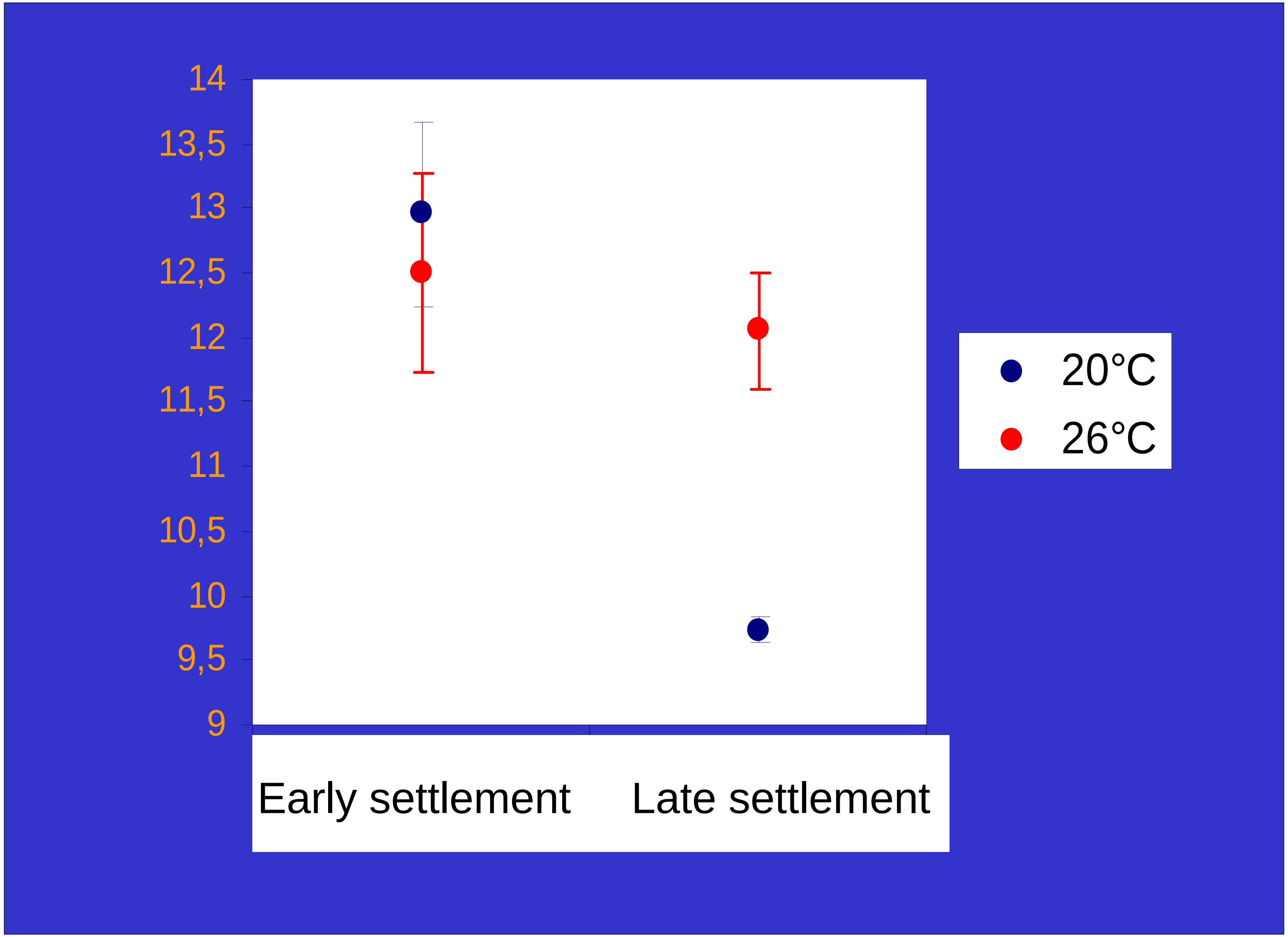
Two extreme «strategies » :

- High larval growth rate and larval size at settlement but low settlement success, growth and survival after settlement

- Lower larval growth rate and larval size at settlement but higher settlement success, growth and survival after settlement

Ernande et al., J.E.B. 2003

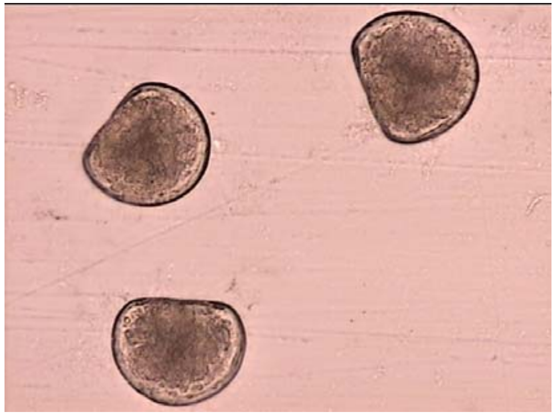
Effect of temperature during larval rearing on spat growth : early *versus* late settlement



Conclusions *(first part)*

Genetic diversity and N_e should be taken into account in the management of hatchery propagated bivalve stocks, especially in closed/selected stocks.

Intensive hatchery practices (temperature, culling) can directly or indirectly influence variance of reproductive success and lead to significant genetic changes in the populations.



Genetic improvement of bivalve production ?

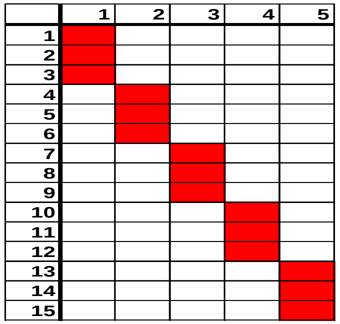
Ploidy manipulations:

- triploidy induction
- tetraploids :

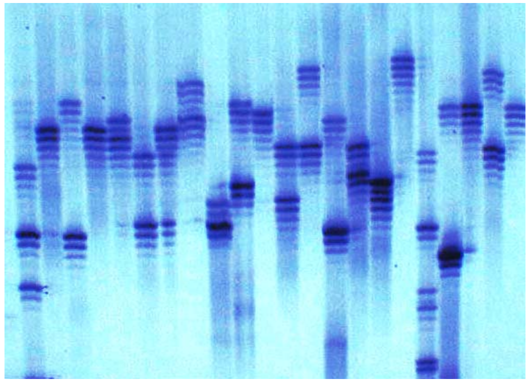
4n x 2n = 3n

Selective breeding:

- heritability estimates
- genetic correlations and trade-offs
- familly-based or mass selection programs ?
- inbreeding and heterosis
- genome mapping and QTLs



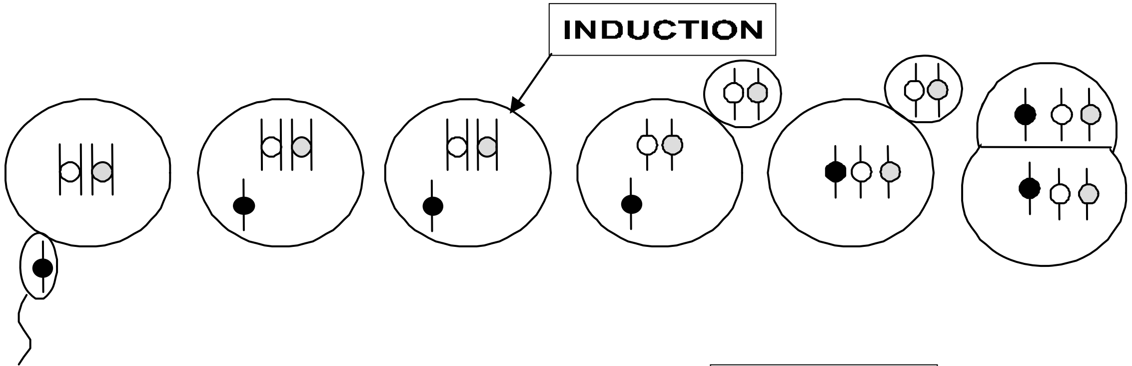
	1	2	3	4	5
1	🟥				
2	🟥				
3	🟥				
4		🟥			
5		🟥			
6		🟥			
7			🟥		
8			🟥		
9			🟥		
10				🟥	
11				🟥	
12				🟥	
13					🟥
14					🟥
15					🟥



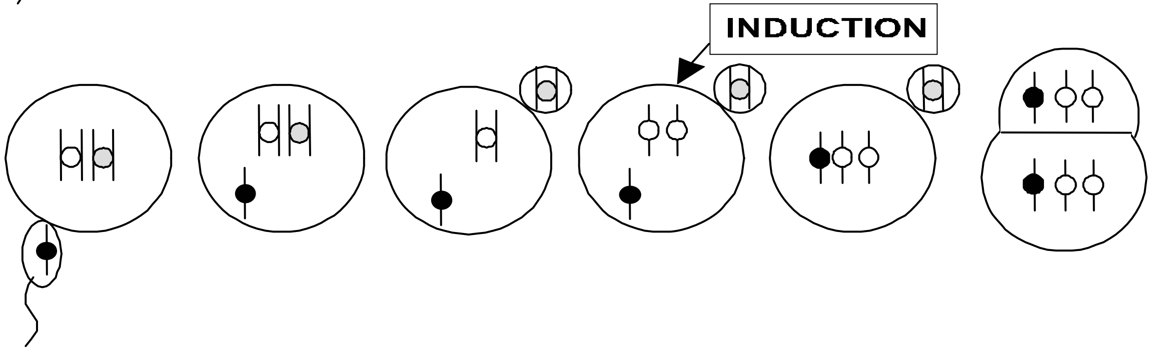
Ways to produce triploid bivalves

1) Chemical treatment of fertilized eggs using Cytochalasine B or 6-DMAP

Inhibition of the expulsion of the __first__ polar body



Inhibition of the expulsion of the __second__ polar body

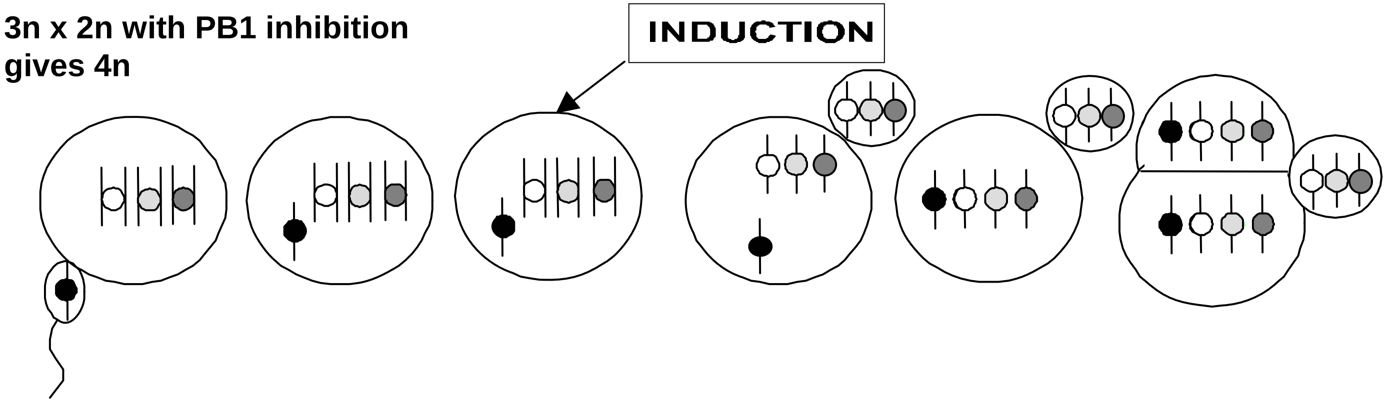


Successfully applied on oysters, pearl oysters, mussels...
Dose and timing are key factors for successful production

Ways to produce tetraploid bivalves

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

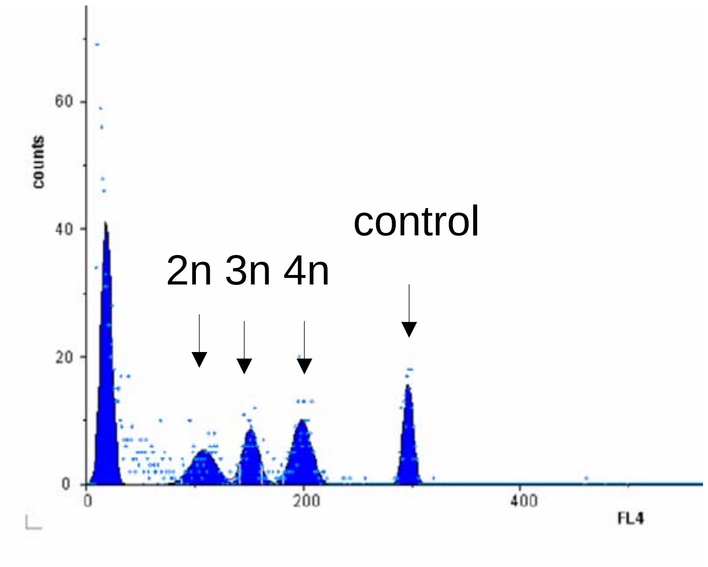
- First method to produce viable tetraploid oysters published in 1994 (Guo & Allen, 1994)

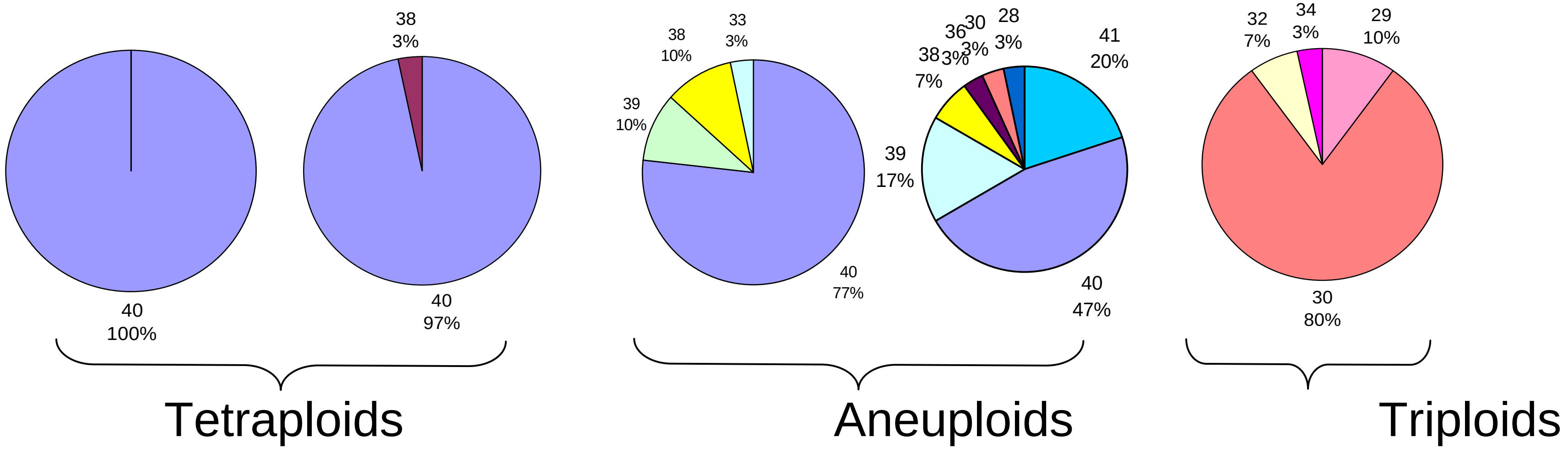


Successfully applied on C. gigas, C. ariakesis & C. virginica

Difficulties associated with tetraploids

- Success of induction methods varies between species
- chromosome set instability and reversion
- need to score ploidy on tetraploid genitors
- confinement of tetraploids is recommended (ICES).

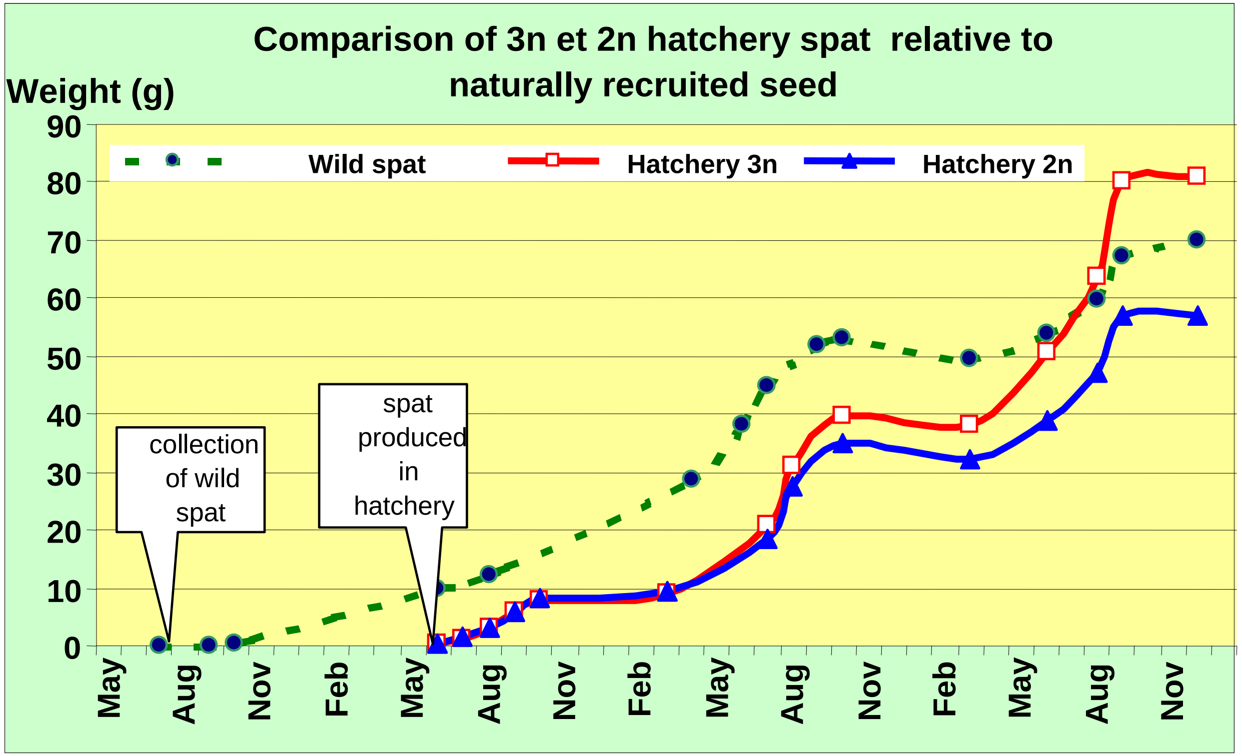




Variation in chromosome number in 4n x 4 n C. gigas progeny (McCombie et al, Aquaculture 2005)

Triploidy : a “single step” improvement

Re-allocation of energy from reproduction to maintenance and growth in triploid oysters



CREAA

- ➢ Nell, J.A. (2002). Farming triploid oysters. Aquaculture 210: 69-88
- ➢ “Natural triploids” are superior to “chemically induced” triploids (Eudeline, 2004)

Selective breeding of oysters

- U.S.A. : **yield**
  - WRAC: « Crossbreeding » and heterosis
  - MBP (http://www.hmsc.orst.edu/projects/mbp)

  disease resistance
  - VIMS
  - Rutgers University

- Australia: **growth**
  - CSIRO

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

- France : **Stress and disease resistance**
  - Ifremer

Mass (= individual) selection

- Targeted traits : growth, disease resistance
    - Bonamiosis resistance in *O. edulis* (Naciri-Graven et al., 1998; Culloty et al., 2001)
    - MSX and dermo resistance in *C. virginica*
    - Growth in *S. commercialis* (Nell et al., 2000)

- Main advantages :
    - Relatively easy to manage
    - Possibility of strong selective pressures

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

    - Selection under a single environment
        - **genotype x environment interaction ?**

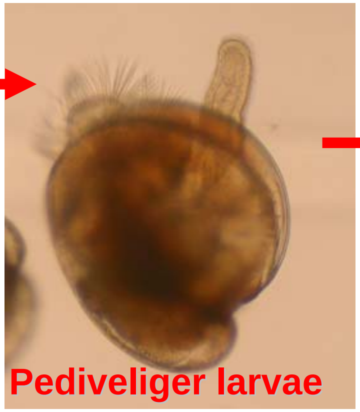
Family-based selective breeding

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

		Male					
		1	2	3	4	5	6
Female	1	Half-Sib 1					
	2						
	3						
	4						
	5		Half-Sib 2				
	6						
	7						
	8						
	9			Half-Sib 3			
	10						
	11						
	12						
	13				Half-Sib 4		
	14						
	15						
	16						
	17					Half-Sib 5	
	18						
	19						
	20						
	21						Half-Sib 6
	22						
	23						
	24						

		Male					
		7	8	9	10	11	12
Female	25	Half-Sib 7					
	26						
	27						
	28						
	29		Half-Sib 8				
	30						
	31						
	32						
	33			Half-Sib 9			
	34						
	35						
	36						
	37				Half-Sib 10		
	38						
	39						
	40						
	41					Half-Sib 11	
	42						
	43						
	44						
	45						Half-Sib 12
	46						
	47						
	48						

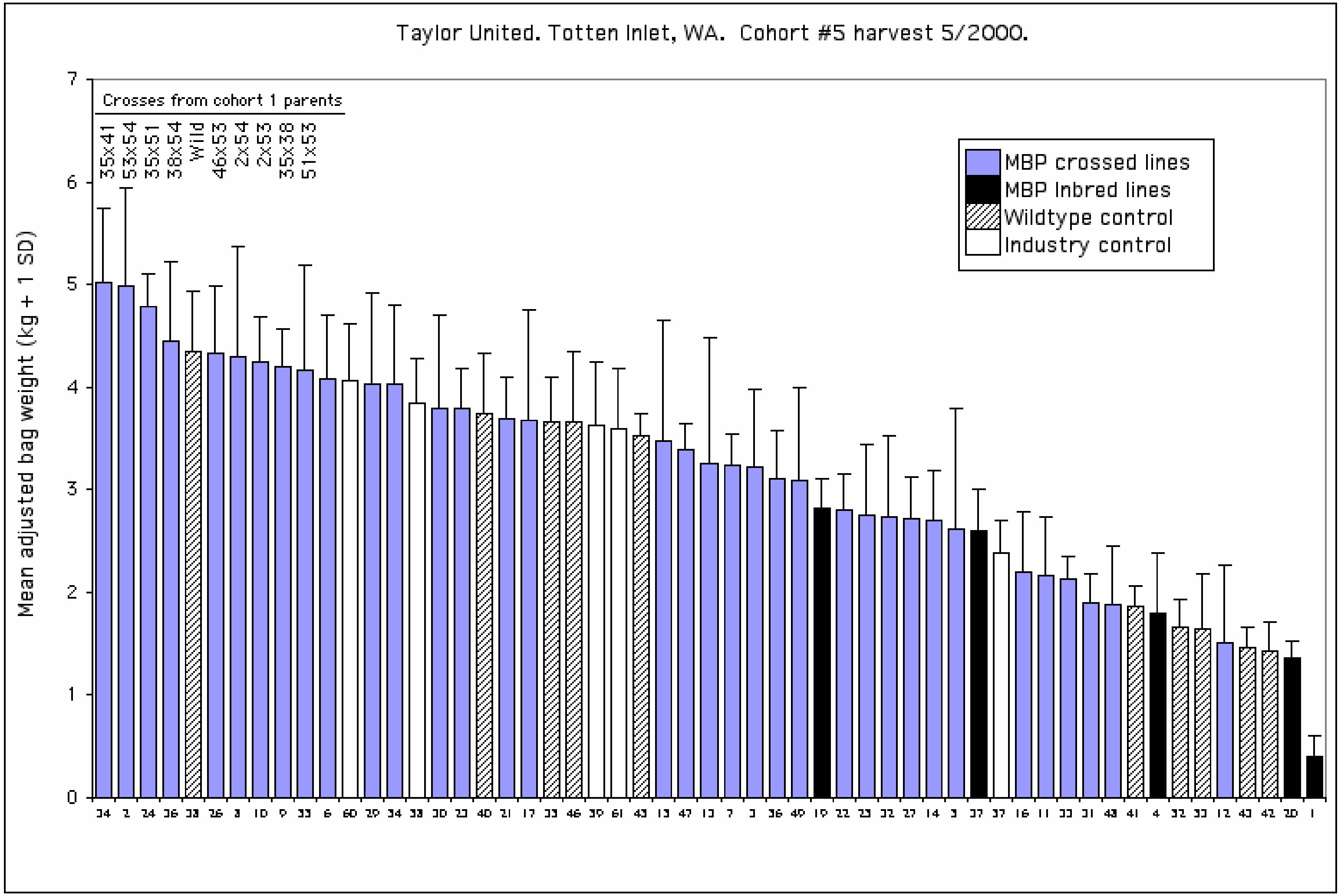
		Male					
		13	14	15	16	17	17
Female	49	Half-Sib 13					
	50						
	51						
	52						
	53		Half-Sib 14				
	54						
	55						
	56						
	57			Half-Sib 15			
	58						
	59						
	60						
	61				Half-Sib 16		
	62						
	63						
	64						
	65					Half-Sib 17	
	66						
	67						
	68						
	69						Half-Sib 18
	70						
	71						
	72						



Family-based selective breeding programs

- Molluscan Broodstock Program (MBP): selection for yield

Molluscan Broodstock Program · In Partnership With Industry · Oregon State University—Hatfield Marine Science Center



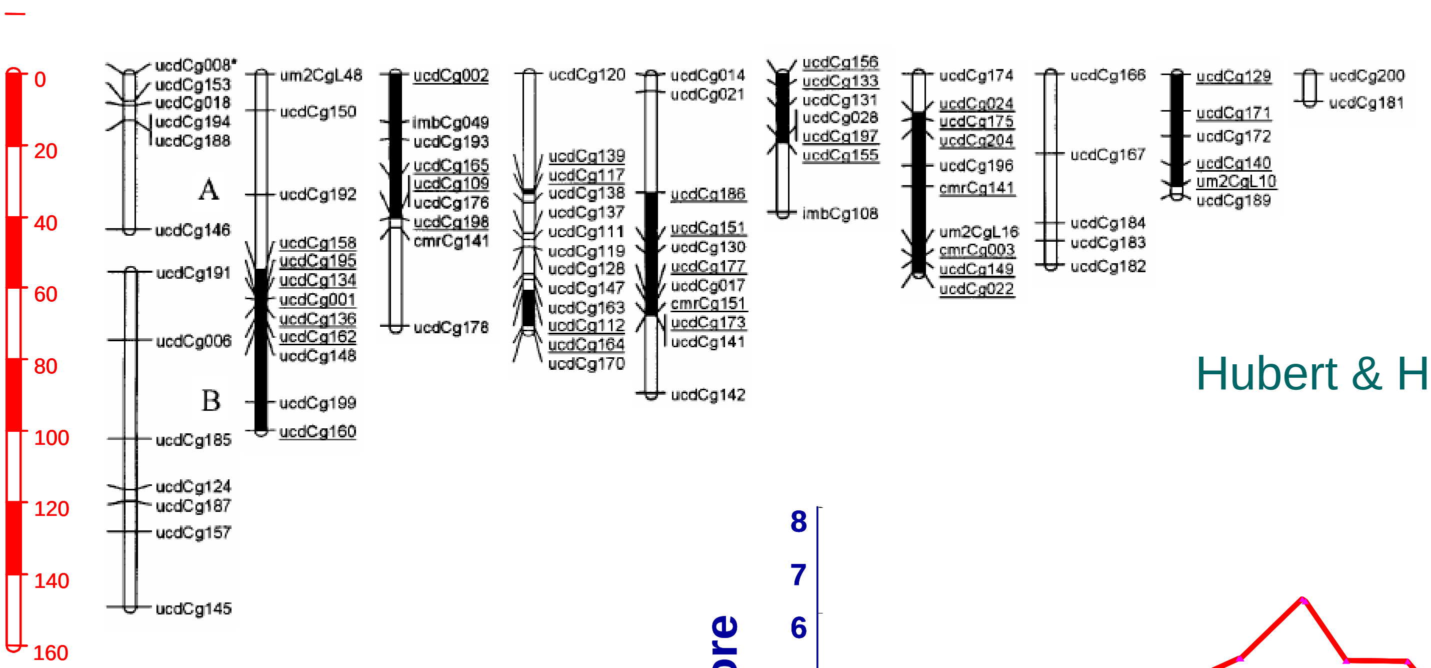
http://www.hmsc.orst.edu/projects/mbp

Family-based selective breeding programs

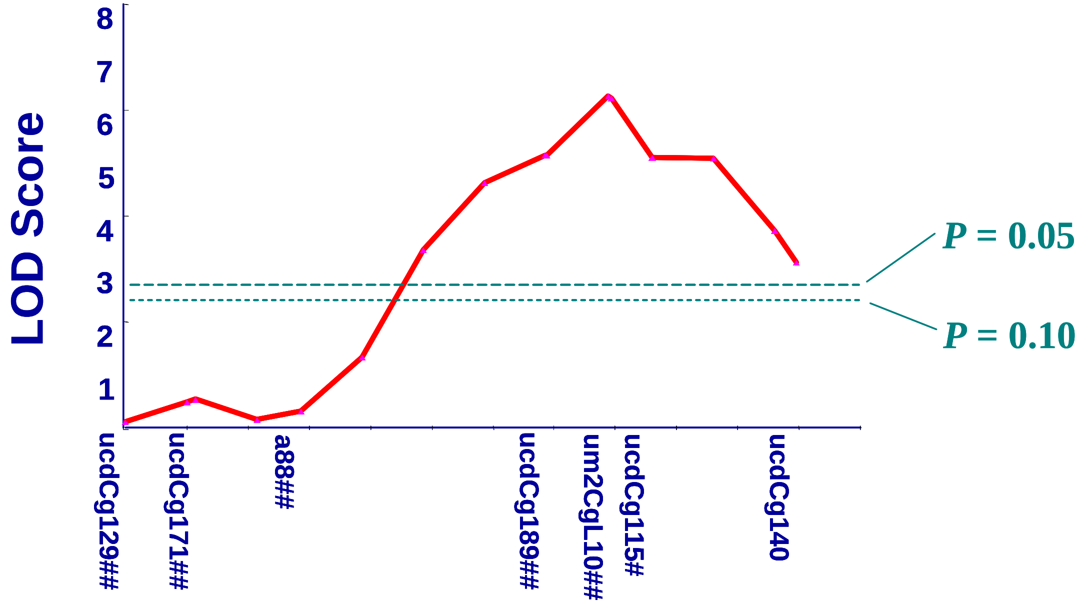
- “WRAC” : development of inbreed lines and crossbreeding

http://hmsc.oregonstate.edu/projects/wrac/

QTLs for heterosis and marker-assisted selection

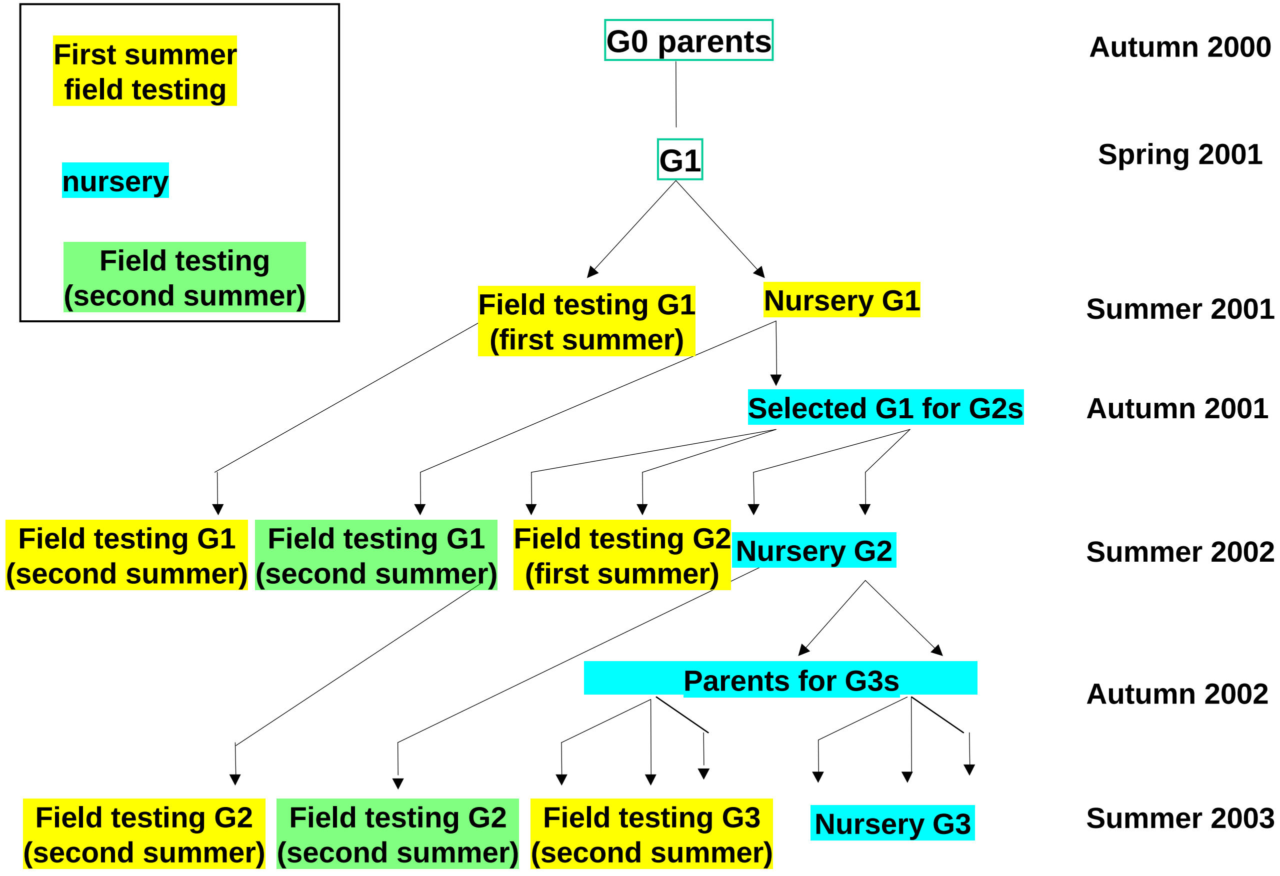


Hubert & Hedgecock, Genetics 2004



Hedgecock et al., Univ. California

Experimental selective breeding on spat survival



First summer field testing

nursery

Field testing (second summer)

G0 parents — Autumn 2000

G1 — Spring 2001

Field testing G1 (first summer)

Nursery G1 — Summer 2001

Selected G1 for G2s — Autumn 2001

Field testing G1 (second summer)

Field testing G1 (second summer)

Field testing G2 (first summer)

Nursery G2 — Summer 2002

Parents for G3s — Autumn 2002

Field testing G2 (second summer)

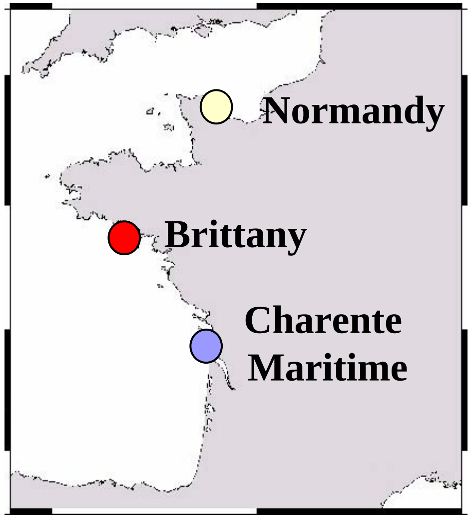
Field testing G2 (second summer)

Field testing G3 (second summer)

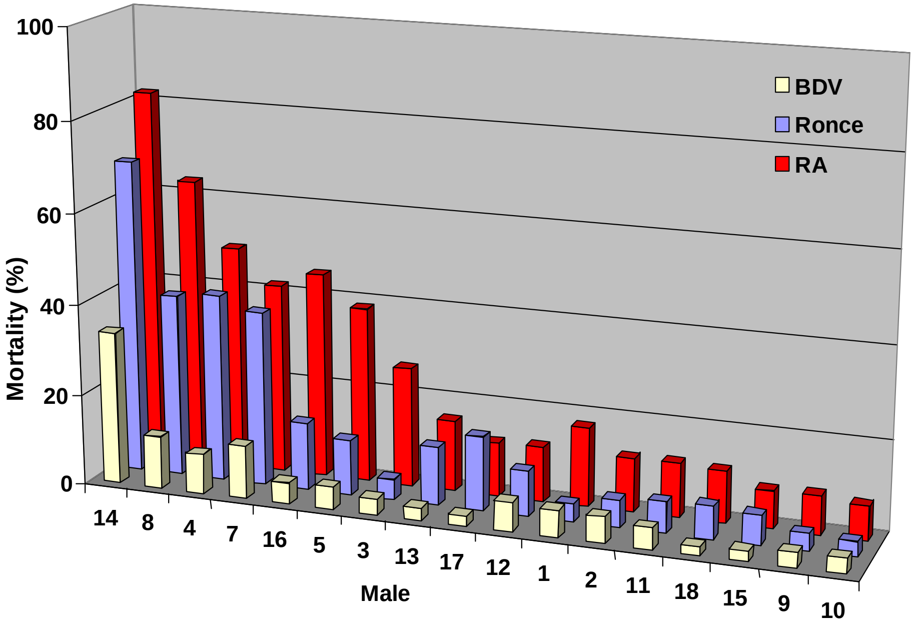
Nursery G3 — Summer 2003

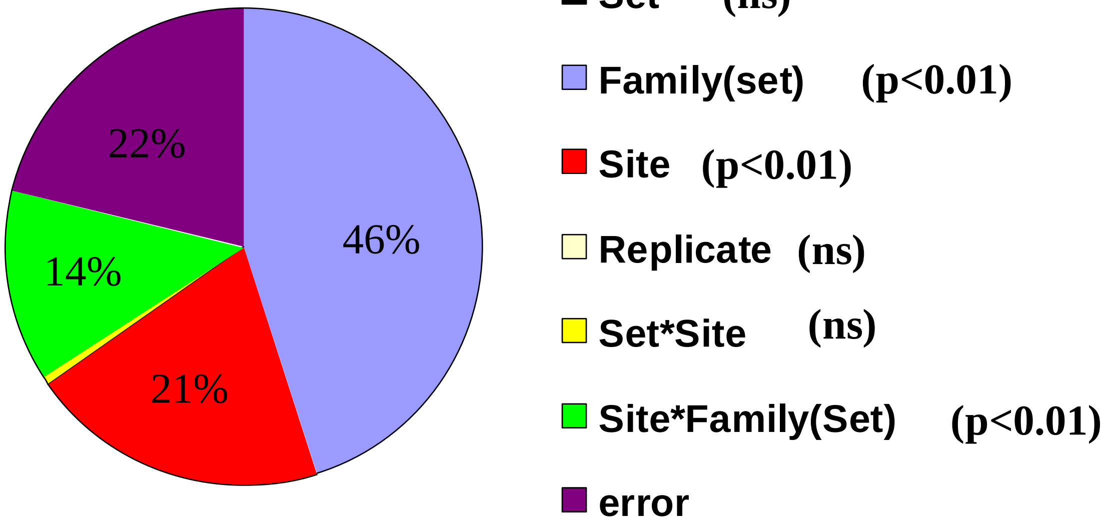
MOREST

Samain et al, 2001-2005



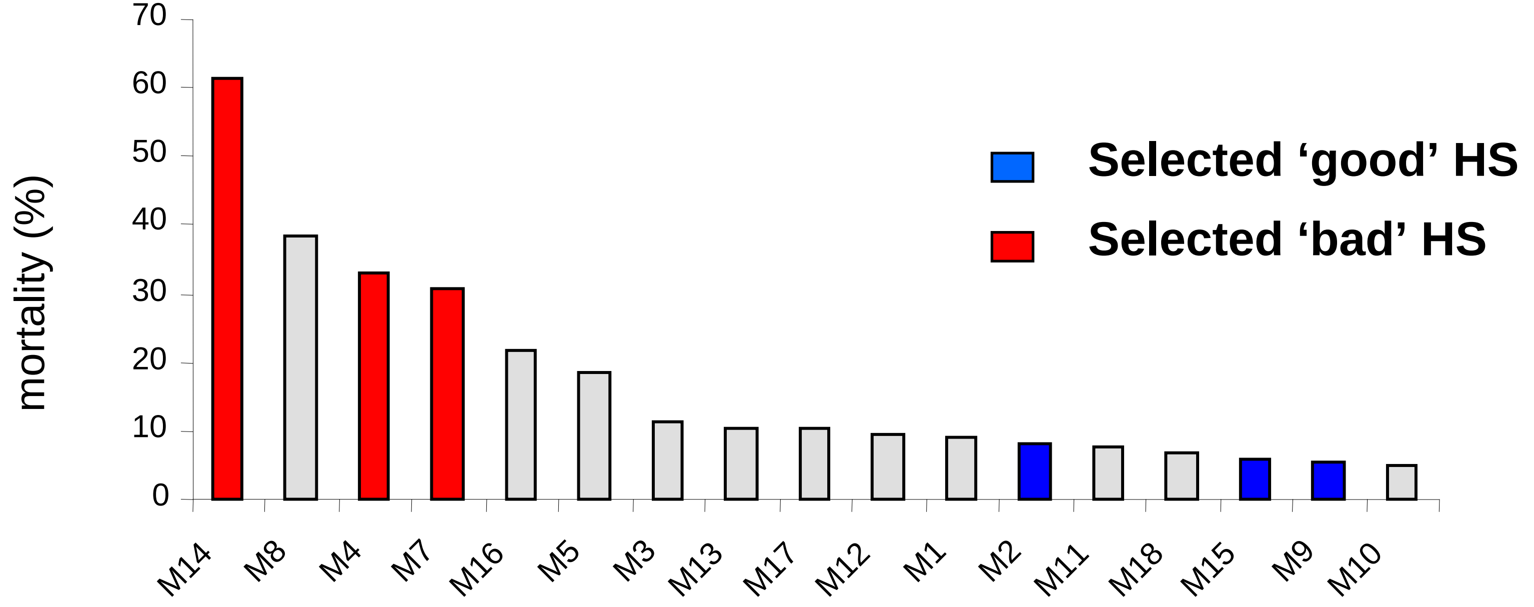
G1 half-sib families: mortality in the field





Degrémont et al., Aquaculture in press

Second generation (G2SD): divergent selection



Low selected group ‘S’

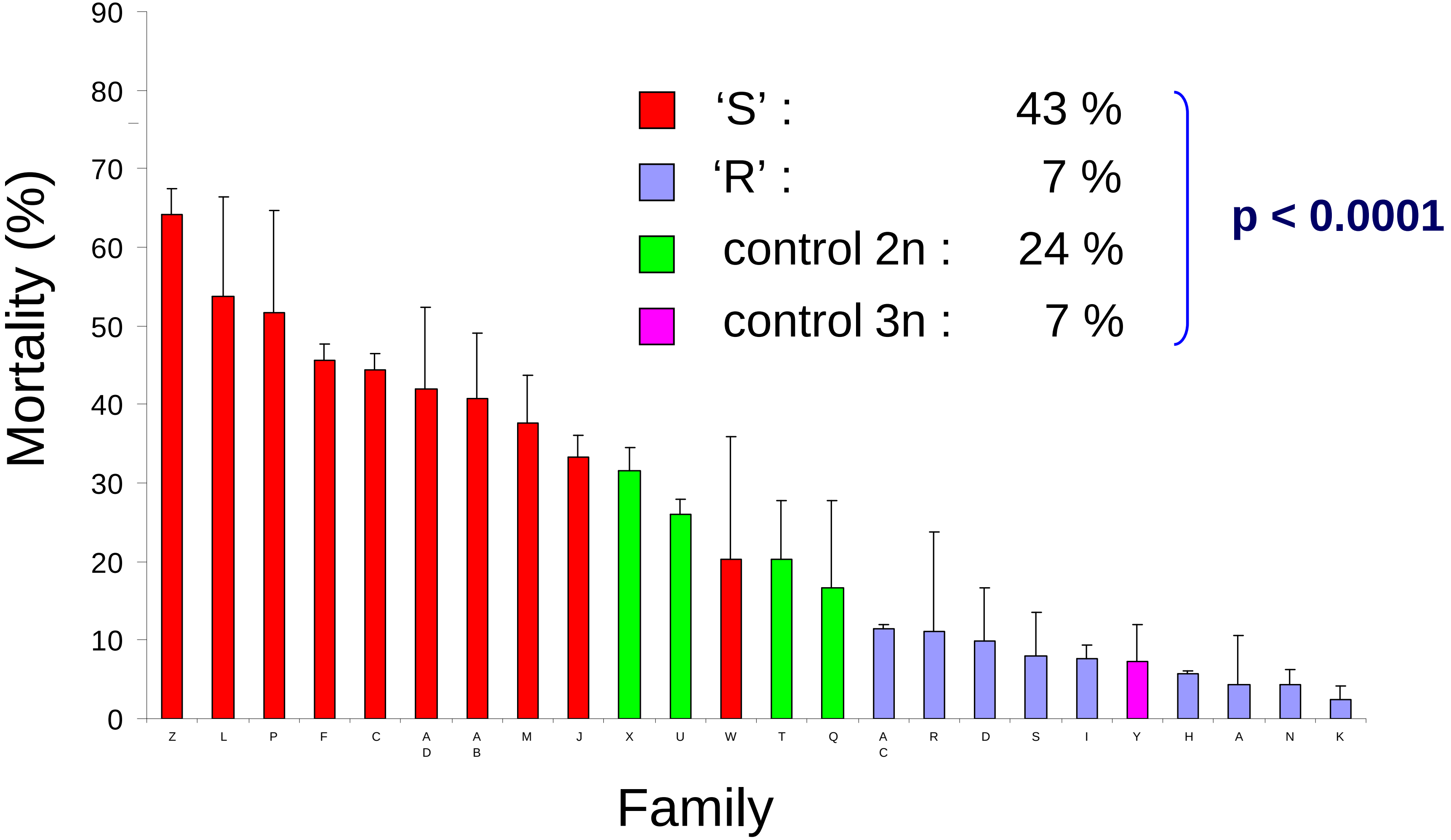
Male		*4*		*7*		*14*	
	Family	**F4-15**	**F4-16**	**F7-25**	**F7-26**	**F14-54**	**F14-55**
4	**F4-15**			13	14	17	18
	F4-16			15	16	19	20
7	**F7-25**					21	22
	F7-26					23	24
14	**F14-54**						
	F14-55						

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						

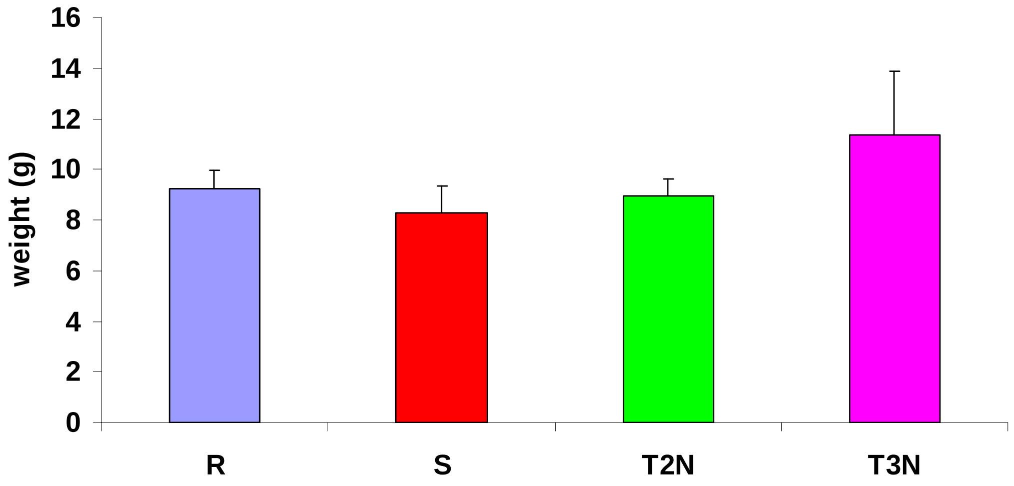
+ Controls : 2N and 3N

G2SD: Summer mortality in Brittany



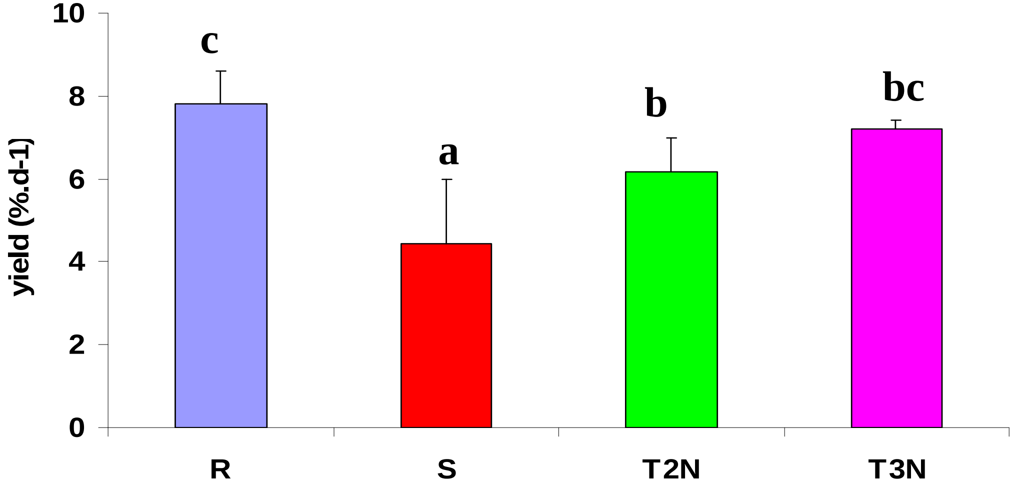
S > control 2n > control 3n = R

G2SD: Response to selection for survival on growth and yield



Growth

ns

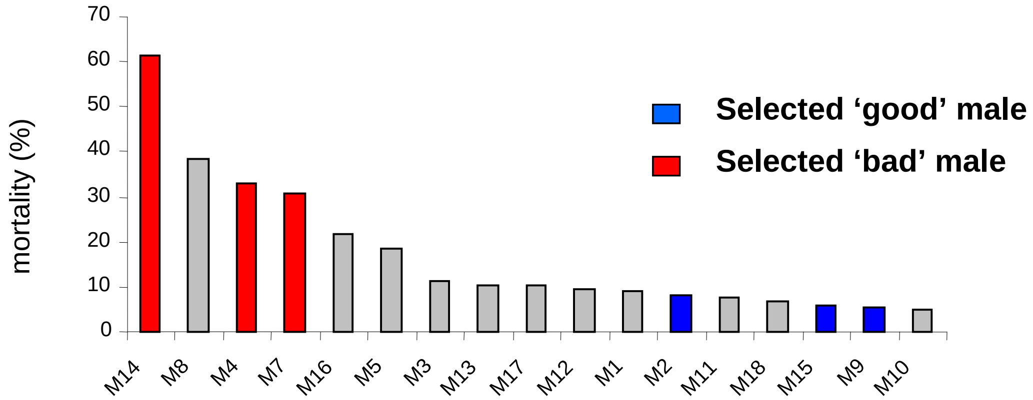


Yield

****

$R^2 = 0.94$

Third generation (G3)



G1

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						

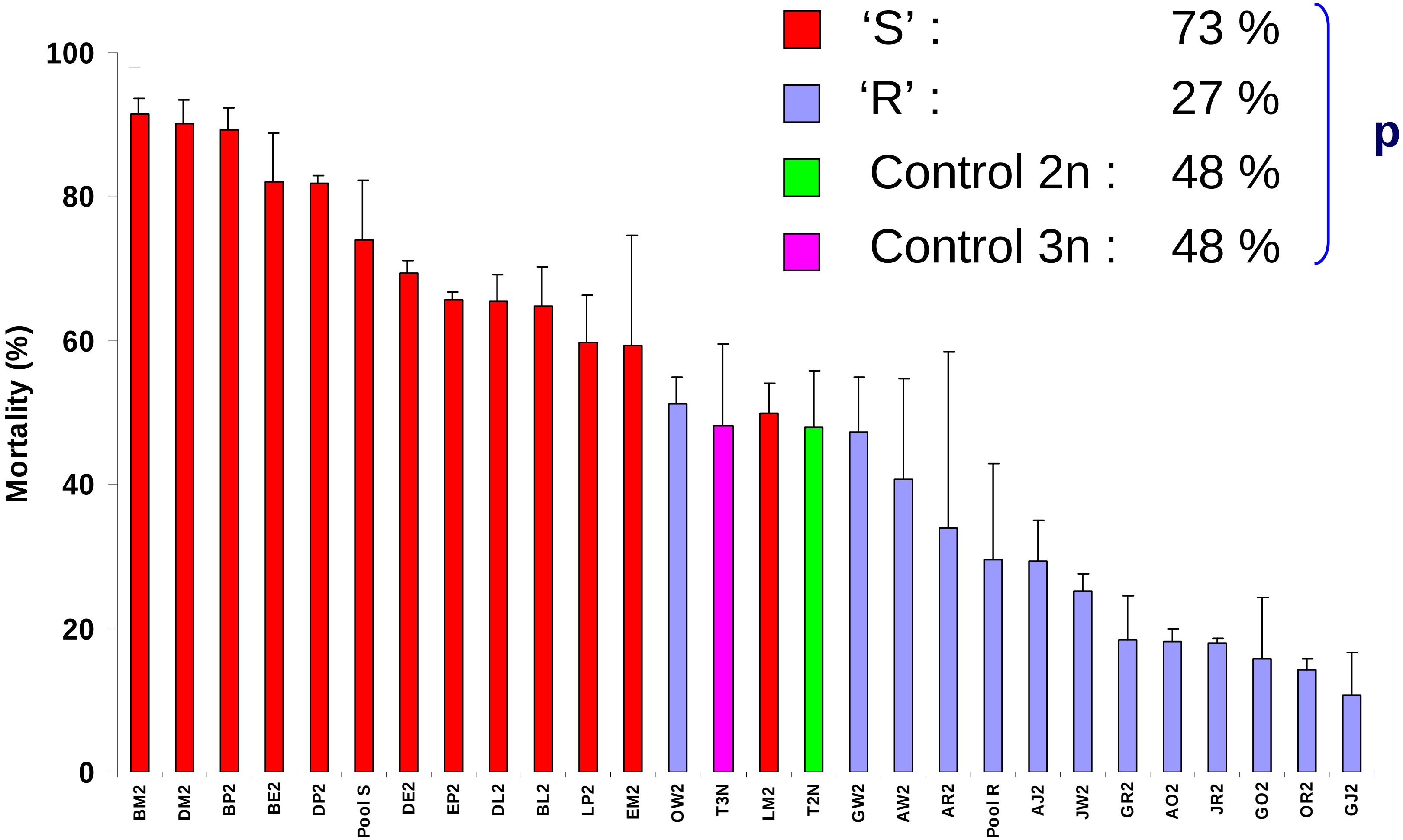
G2 inbred

G0		7		14	
	G2C	E2	L2	M2	P2
4	B2	BE2	BL2	BM2	BP2
	D2	DE2	DL2	DM2	DP2
7	E2			EM2	EP2
	L2			LM2	LP2

G3

G0		9		15	
	G2C	J2	O2	R2	W2
2	A2	AJ2	AO2	AR2	AW2
	G2	GJ2	GO2	GR2	GW2
9	J2			JR2	JW2
	O2			OR2	OW2

G3 : Summer mortality in Brittany



🟥 ‘S’ : 73 %

🟦 ‘R’ : 27 %

🟩 Control 2n : 48 %

🟪 Control 3n : 48 %

$p < 0.0001$

S > control 2n = control 3n > R

Mortality during the second summer

		'R'		'S	Control	
Summer 2001	6 months	7	<	52	21	
Summer 2002	18 months	8	=	7		G1
Global mortality	18 months	14	<	55		
Summer 2002	6 months	6	<	48	24	
Summer 2003	18 months	6	=	7		G2
Global mortality	18 months	12	<	52		

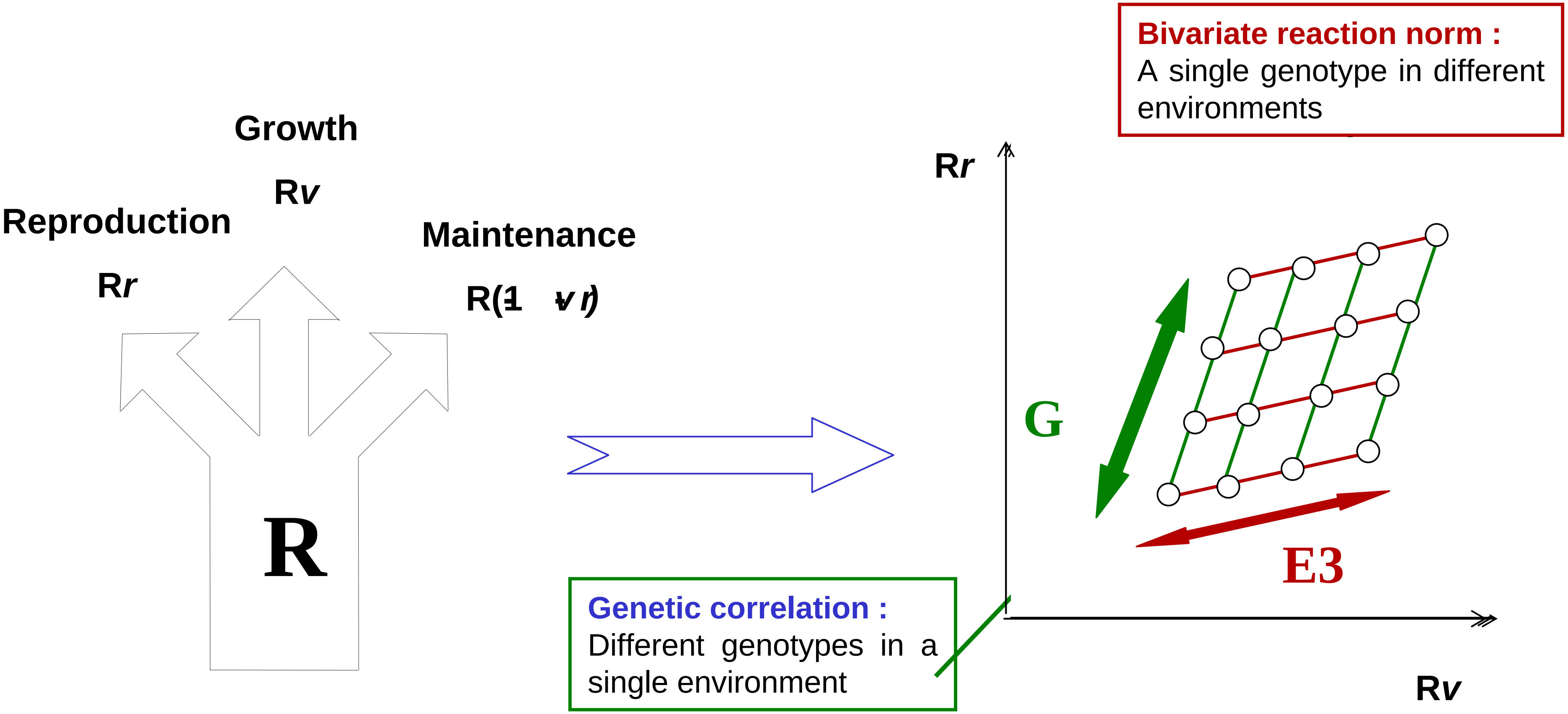
- Mortality occurs mostly during the first summer (in 2 of the 3 sites)
- 'S' and 'R' oysters show similar performance during their second summer
- Global survival of 'R' oysters is much higher than 'S' oysters

Why so much additive variance for spat survival ?

- Impact of hatchery propagation on life cycle / resource allocation ?
- Maintenance of genetic polymorphism due to spatially and/or temporally variable selective pressures ?
- Trade-off between survival and another fitness-related trait ?

→ **Characterization of ‘ S ’ and ‘ R ’ oysters using physiological and genomic approaches**

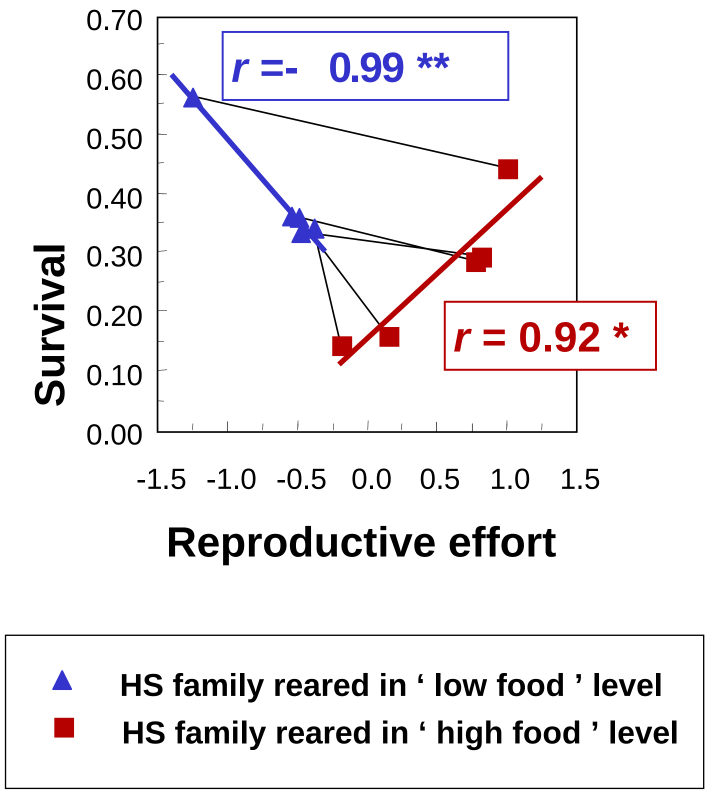
Genetic variability of resource allocation traits: trade-offs between growth, reproduction and survival ?

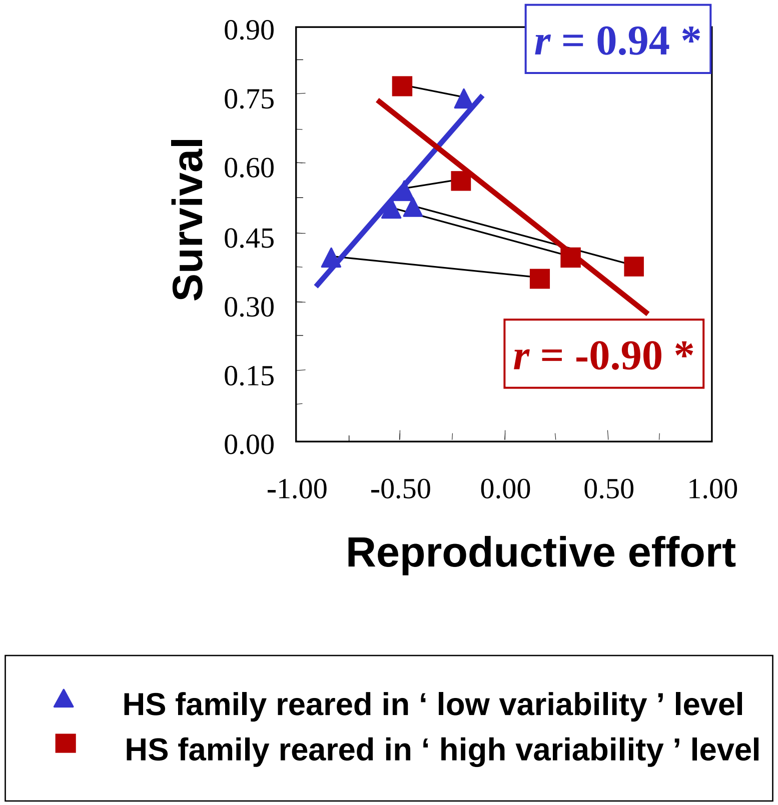


Bivariate reaction norm :
A single genotype in different environments

Genetic correlation :
Different genotypes in a single environment

Trade-offs ? Evidence in one-year old oysters



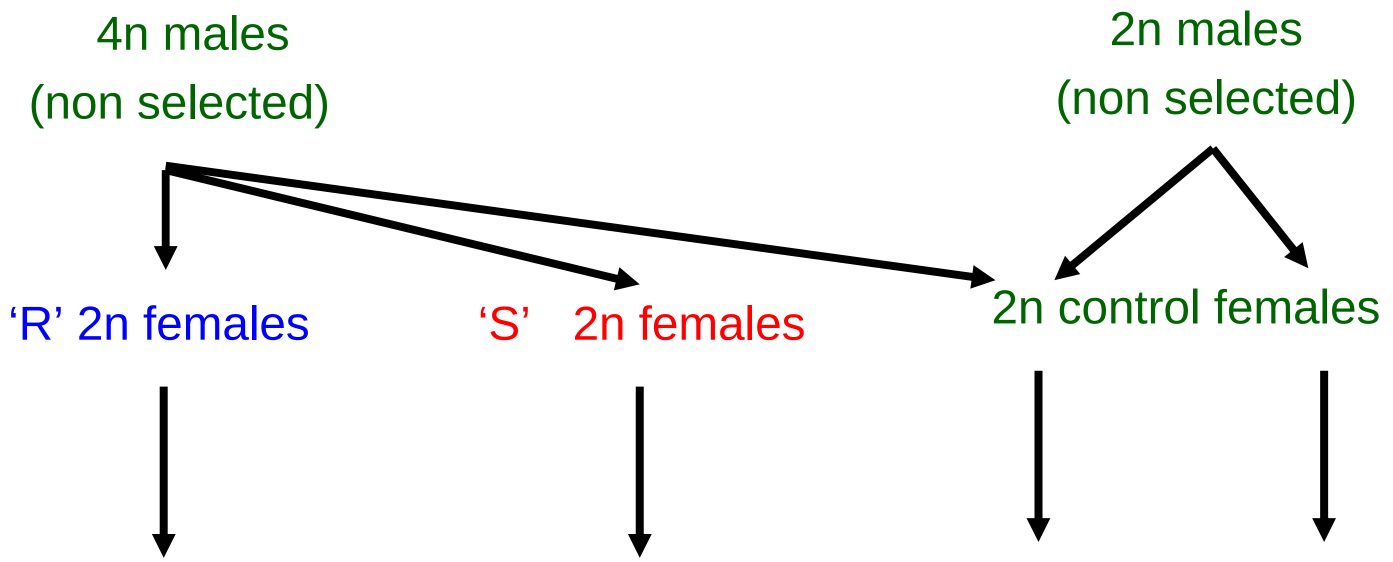


+ Significant positive genetic correlation between plasticity of reproductive effort and survival

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

Ernande et al., in prep.

Towards ‘selected’ triploids ?



4n males
(non selected)

2n males
(non selected)

‘R’ 2n females

‘S’ 2n females

2n control females

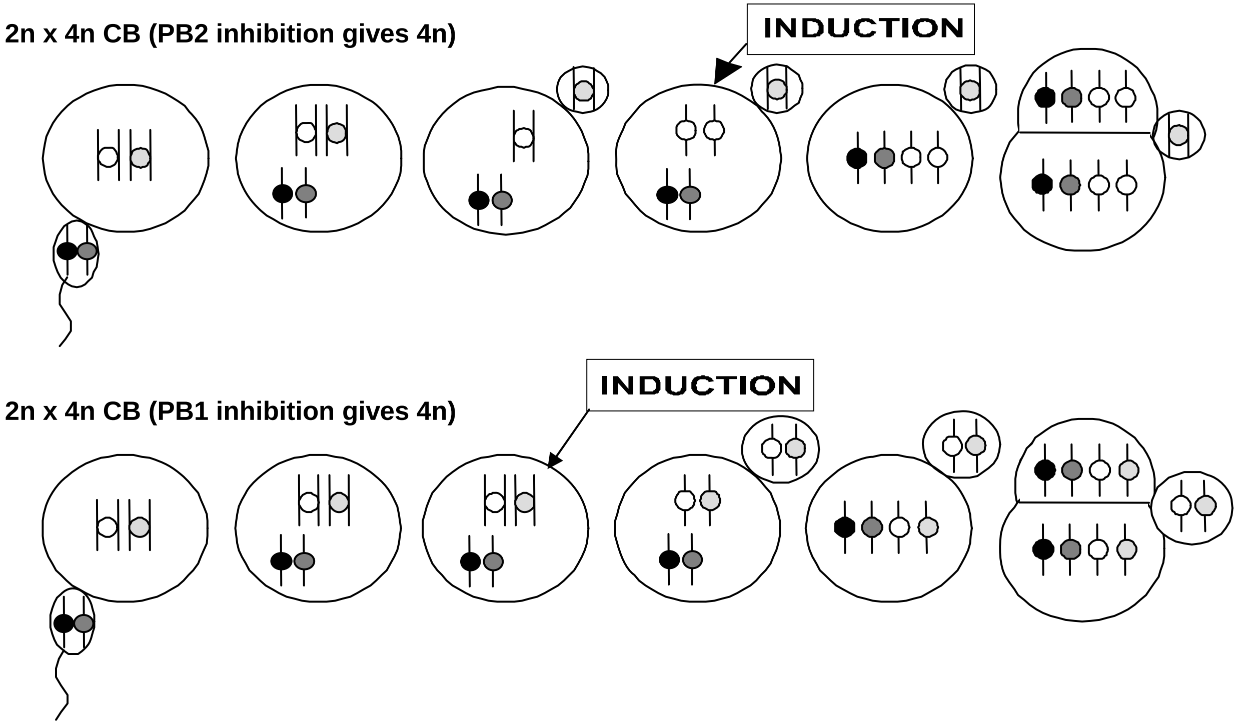
	3n R	3n S	3n	2n
Mortality :	**36 %**	**58 %**	**50 %**	**48 %**

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

Towards ‘selected’ tetraploids ?

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

Direct introgression of selected traits from diploids to tetraploids :



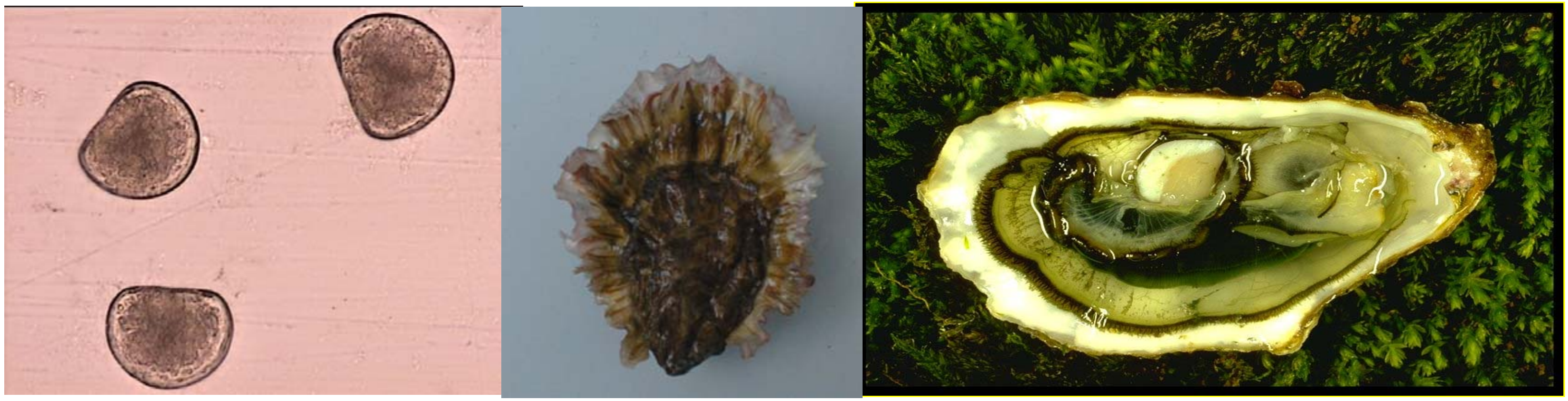
McCombie et al., Marine Biotech. 2005

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 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

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