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

e.g. in *O. edulis*:
✓ Saavedra & Guerra (1996): $Ne \approx 4$
✓ Launey et al. (2001): $Ne = 3$ to 20

<table>
<thead>
<tr>
<th>Stages</th>
<th>Survival</th>
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<tbody>
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<td>+</td>
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</table>

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?
     \[ N_e = \frac{4 Nm Nf}{Nm + Nf} \]

2) **Balanced sex-ratio**
   - 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

C.V. of larval diameter

Days to settlement

- Culled population
- Control
Temporal variation of male reproductive success in 3 successive settlement cohorts

\[ \text{Settlement date} \]

\[ \text{Sampling} \]

\[ \text{Sampling} \]

\[ \text{Temporal variation of male reproductive success in 3 successive settlement cohorts} \]

\[ \text{(Taris et al., in prep)} \]
Experimental examination of factors affecting larval growth:

Effect of temperature in a 12 males x 4 females cross

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>20°C</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>26°C</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>
Is there selection for fast growing larvae in hatcheries?

Wild stock ( = control)

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

*Day 15*
*24°C*
*No culling*

**205 $\mu$m**

*Inbred larvae?*

**225 $\mu$m**
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:
  \[4n \times 2n = 3n\]

Selective breeding:
- heritability estimates
- genetic correlations and trade-offs
- family-based or mass selection programs?
- inbreeding and heterosis
- genome mapping and QTLs
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*
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 4n 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

- “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 **common** condition(s) to estimate their genetic value

![Maturation](image1)

![Fertilization](image2)

![Larval rearing](image3)

![Pediveliger larvae](image4)

![Setting](image5)

![Nursing](image6)

![Field testing](image7)
Family-based selective breeding programs

- Molluscan Broodstock Program (MBP): selection for yield

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

G1

Field testing G1 (first summer)

Nursery G1

Selected G1 for G2s

Field testing G1 (second summer)  Field testing G1 (first summer)

Nursery G2

Parents for G3s

Field testing G2 (second summer)  Field testing G2 (first summer)  Field testing G3 (second summer)

Nursery G3


Samain et al, 2001-2005

Normandy  Brittany  Charente Maritime
G1 half-sib families: mortality in the field

Degrémont et al., Aquaculture in press
Second generation (G2SD): divergent selection

Low selected group ‘S’

High selected group ‘R’

+ Controls: 2N and 3N
G2SD: Summer mortality in Brittany

- 'S': 43%
- 'R': 7%
- control 2n: 24%
- control 3n: 7%

p < 0.0001

S > control 2n > control 3n = R
G2SD: Response to selection for survival on growth and yield

Growth

\[ R^2 = 0.94 \]

Yield

\[ *** \]

\[ R^2 = 0.94 \]
# Third generation (G3)

## Low selected group ‘S’

<table>
<thead>
<tr>
<th>Family</th>
<th>Male</th>
<th>4</th>
<th>7</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>G0</td>
<td>G2</td>
<td>E2</td>
<td>L2</td>
<td>M2</td>
</tr>
<tr>
<td>G2C</td>
<td>B2</td>
<td>BE2</td>
<td>BL2</td>
<td>BM2</td>
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<tr>
<td></td>
<td>D2</td>
<td>DE2</td>
<td>DL2</td>
<td>DM2</td>
</tr>
<tr>
<td>G1</td>
<td>E2</td>
<td>EM2</td>
<td>EP2</td>
<td>LM2</td>
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</table>

## High selected group ‘R’

<table>
<thead>
<tr>
<th>Family</th>
<th>Male</th>
<th>2</th>
<th>9</th>
<th>15</th>
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<tbody>
<tr>
<td>G0</td>
<td>G2</td>
<td>J2</td>
<td>O2</td>
<td>R2</td>
</tr>
<tr>
<td>G2C</td>
<td>A2</td>
<td>AJ2</td>
<td>AO2</td>
<td>AR2</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>GJ2</td>
<td>GO2</td>
<td>GR2</td>
</tr>
<tr>
<td>G3</td>
<td>J2</td>
<td>JR2</td>
<td>JW2</td>
<td>O2</td>
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G3 : Summer mortality in Brittany

- ‘S’ : 73 %
- ‘R’ : 27 %
- Control 2n : 48 %
- Control 3n : 48 %

S > control 2n = control 3n > R

p < 0.0001
## Mortality during the second summer

<table>
<thead>
<tr>
<th></th>
<th>‘R’</th>
<th>‘S’</th>
<th>Control</th>
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</thead>
<tbody>
<tr>
<td><strong>Summer 2001</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>7</td>
<td>&lt;52</td>
<td>21</td>
</tr>
<tr>
<td><strong>Summer 2002</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 months</td>
<td>8</td>
<td>= 7</td>
<td></td>
</tr>
<tr>
<td><strong>Global mortality</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 months</td>
<td>14</td>
<td>&lt;55</td>
<td></td>
</tr>
<tr>
<td><strong>Summer 2002</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>6</td>
<td>&lt;48</td>
<td>24</td>
</tr>
<tr>
<td><strong>Summer 2003</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>= 7</td>
<td></td>
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<tr>
<td><strong>Global mortality</strong></td>
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</tr>
<tr>
<td>18 months</td>
<td>12</td>
<td>&lt;52</td>
<td></td>
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- 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?

- Growth
- Reproduction
- Maintenance

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)

‘R’ 2n females

3n R

Mortality: 36%

‘S’ 2n females

3n S

58%

2n males
(non selected)

2n control females

3n

50%

2n

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-trait genetic improvement.
Suggested recommendations 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.
Acknowledgments:

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MOREST: Jean François Samain et al.