Broodstock management and larvi-culture in marine species reared in Polynesia and New Caledonia: genetic and health approach

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Aquaculture productions

- Shrimp: *Penaeus stylirostris*
  - New Caledonia and French Polynesia
- Pearl oyster: *Pinctada margaritifera*
  - French Polynesia,
- Fish: *Lates calcarifer*
  - French Polynesia
Shrimp: Historic

- **1970-1980**
  - Selection of species for their reproductive ability in captivity and growth performances.

- **1980-1990**
  - Broodstock maturation: nutritional improvement
  - Larval rearing:
    - replacement of live food by microparticulate diet,
    - water management (biological filter)
  - Sanitary status: genetic resistance to IHHNV (1987)

- **1990-2000**
  - Starting of a genetic program
    - characterisation and improvement of strains domesticated in Polynesia and Caledonia
    - development of tools: molecular markers and method of gametes conservation
Main French shrimp farming areas

- **New Caledonia**
  - 10 farms
  - 4 hatcheries
  - Ifremer-SASV

- **French Polynesia**
  - 3 farms
  - 1 hatchery
  - Ifremer-COP

*Yield (tons)*

- New Caledonia
- French Polynesia

*Location*

- Noumea
- Papeete

*Maps*

- New-Caledonia
- French Polynesia

*Scale*

- 50 km.
**P. stylirostris** shrimp : Broodstock management

- **Maturation**
  - unilateral eyestalk ablation in females
  - males kept at 25-26°C
  - improved feeding
  - artificial insemination (2 males X 1 female)

- **Gamete preservation I**
  - refrigeration at 4°C in conservation medium at pH 7 containing antibiotic allow to use sperm during 6 days
  - transfer from Ecuador to Tahiti (2 to 6 days from collection to mating), **165 wild males X 87 domesticated females** (17% success)

- **Gamete preservation II**
  - cryo-preservation in liquid nitrogen : **P. vannamei** nauplii obtained in 1991 and 1996, non reproducible probably due to breeder and gamete of insufficient quality, no results obtained with **P. stylirostris**
**P. stylirostris** shrimp: Genetic improvement

- Resistance to disease
  - spontaneous resistance to IHHNV
- Selection for growth
  - an improvement of 34% of weight at the sixth generation
P. stylirostris shrimp: Genetic resources

- Micro-satellite and intronic markers have allowed to characterise the strain and found:
  - a positive relation between heterozygocity and growth
  - the loss of genetic variability during the domestication process (20 generations) compared to the wild stock from Ecuador

- These markers allow to check shrimp pedigree and now to implement a strategy to preserve residual variability
  - maintenance of several independent populations
  - structuring of each population into 2 sub-populations

- Importation of genetic variability
  - Using refrigerated spermatophores: 15 F1 families were produced in quarantine facility
**P. stylirostris** shrimp: Genetic resources

Evidence of genetic variability loss during the domestication process (20 generations) compare to the wild stock from Ecuador

<table>
<thead>
<tr>
<th>P. stylirostris strains</th>
<th>Allelic number / locus</th>
<th>Heterozygocity level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tahiti</td>
<td>1-3</td>
<td>0.1</td>
</tr>
<tr>
<td>New Caledonia</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>Wild Ecuador</td>
<td>14-25</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Checking of pedigrees

![Genetic resources diagram](image)
**P. stylirostris shrimp**: Sanitary management

**Sanitary status of breeders**
- Except the presence of IHHNV, Polynesia and Caledonia territories keep a favourable sanitary status probably related to the use of closed broodstock
- Perspectives
  - Health state management (SPF)
  - Immune criteria for health status determination in relation with environmental, nutritional and genetic state

**Control of disease in larval rearing**
- Antibiotherapy: erythromycin, oxytetracycline (furazolidone replacement)
- Alternative: probiotic (*Pseudoalteromonas piscicida*)
Pearl oyster industry in French Polynesia

Historic

- First episode: mother-of-pearl era in XIXth century
  - mother-of-pearl was exploited to supply the button industry
  - appearance of synthetic button is the death of this industry in the middle of XXth century

- Second episode: fast-growing of pearl oyster industry
  - 1960: research on pearl production with the help of Japanese experts especially on spat collection and graft trials
  - 1980: start of pearl oyster industry
  - 1999: 6 metric tons of pearl, 200 million US$, 1076 farms-7000 employment
  - 2000: difficulties due to overproduction,
French Polynesia
Annual environmental change in French Polynesia lagoons according to the latitude

- Temperature (°C)
- Day length (h)

- North. Tuamotu
- Gambier
- Tahiti (Vairao)
### Hydrobiological characterisation of lagoons

<table>
<thead>
<tr>
<th>Location</th>
<th>Organic matter (mg.L(^{-1}))</th>
<th>Chlorophyll (a) (µg.L(^{-1}))</th>
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</thead>
<tbody>
<tr>
<td>Makemo</td>
<td>0.35</td>
<td>0.21</td>
</tr>
<tr>
<td>Manihi</td>
<td>0.50</td>
<td>0.46</td>
</tr>
<tr>
<td>Takaroa</td>
<td>0.48</td>
<td>0.30</td>
</tr>
<tr>
<td>Takapoto</td>
<td>0.35</td>
<td>0.40</td>
</tr>
<tr>
<td>Raiatea</td>
<td>0.42</td>
<td>0.54</td>
</tr>
<tr>
<td>Vairao</td>
<td>0.50</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Distribution spatiale des pigments à Manihi  
Pearl oyster: sex and reproductive cycle

Sex and reproductive cycle are evaluated with

- Spawning (induced with thermal shock)
- biopsy
- histology
- gonadal weight changes
Sex ratio and gametogenesis

[Bar chart and images related to sex ratio and gametogenesis]
Gametogenesis *in situ*

Gonadal weight and gonadal index (northern Tuamotu) (Pouvreau, 1999)

Spawning rate related to the temperature change (Vairao)

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Ifremer
Spat collection

100 metre longline suspended 3-4 metres deep

Surface of water

Sea floor 8-25 metres deep

1 metre

"Bowtie" shademesh collector

"Full shade" shademesh collector

Float

Headline

Dropper line

Ifremer
Genetic resources of *P. margaritifera*
Genetic resources of *P. margaritifera*

**Genetic markers**

**DALP Method (Direct Amplification of Length Polymorphism)**

- 3 markers:
  - Pin 1: 6 alleles
  - Pin 2: 5 alleles: A, B, C, D, E
  - Pin 3: 5 alleles

**EPIC Method (Exon Primers Intron Crossing)**

*use of primers defined in exonic sequence (amylase, aldolase)*

- Amplification of intronic sequence

- 2 markers:
  - U4: 6 alleles
  - Aldo: 5 alleles
# Development of hatchery

## Objective
- Supply spat when the collecting is insufficient
- Produce genetically improved spat (triploïd, selected for growth, pearl colour)
- Supply spat to restock some atolls by respecting the genetic structure of the natural populations

## Method
- **Broodstock conditioning**
  - Synchronism of breeders to control crossbreeding
  - Control of sex ratio (increase female percentage)
  - Improve larval quality with breeder feeding
- **Larval rearing**
  - Feeding
  - Water management
  - Disease control
Sanitary rules and zoo-sanitary surveillance network

• 1985: mass mortality (60% of oysters)
• Context still favourable to the emergence of disease (uncontrolled transfert)
• Outbreak in Japan (Akoya virus)

• Zoo-sanitary regulations
  – banning of importation of *P. margaritifera* from other areas and new pearl oyster species
  – surveillance network to prevent outbreak of disease
Conclusion

The industrial rearing of marine species in French Polynesia and New Caledonia are the subject of genetic and sanitary follow-up

- Preservation of the genetic resources
- Genetic improvement and creation of selected lines
- Saving of the sanitary state with the enforcement of zoo-sanitary regulations
- Suppression of antibiotic use