Population genetic structure of natural and hatchery-raised populations of European abalone *Haliotis tuberculata tuberculata*: lessons for future restocking and stock-enhancement

Ronan Le Gall, Pierre Chauvaud, Sabine Roussel, Eric Pante, Amélia Viricel & Grégory Charrier

Abalone Symposium, Auckland, New Zealand
02 March 2023

PhD financing: 50% ARED UBO - 50% UBO EDSML
**Introduction**

**Material & Methods**

**Local Structure**

**Hatchery genetic diversity**

**Differentiation Wild/Hatchery**

**Conclusion**

_Haliotis tuberculata tuberculata_

Location

---

_Nicolas et al., 2002; Huchette et al., 2005; Lessard and Campbell, 2007; Travers et al., 2009; Lorenzen et al., 2012; Lachambre, 2017; Roussel et al., 2019_
Haliotis tuberculata tuberculata

Location

Populations decline

Vibrio harveyi

Nicolas et al., 2002; Huchette et al., 2005; Lessard and Campbell, 2007; Travers et al., 2009; Lorenzen et al., 2012; Lachambre, 2017; Roussel et al., 2019
Haliotis tuberculata tuberculata

Location

Restocking & stock enhancement program

Vibrio harveyi

Populations decline

Nicolas et al., 2002; Huchette et al., 2005; Lessard and Campbell, 2007; Travers et al., 2009; Lorenzen et al., 2012; Lachambre, 2017; Roussel et al., 2019
**Introduction**

*Material & Methods*  
*Local Structure*  
*Hatchery genetic diversity*  
*Differentiation Wild/Hatchery*  
**Conclusion**

**Haliotis tuberculata tuberculata**

- **Location**
- **Populations decline**
- **Vibrio harveyi**
- **Levels of Study**
- **Restocking & stock enhancement program**

*FRANCE HALIOTIS*

Nicolas et al., 2002; Huchette et al., 2005; Lessard and Campbell, 2007; Travers et al., 2009; Lorenzen et al., 2012; Lachambre, 2017; Roussel et al., 2019
Genetics

- Need to limit genetic differentiation between wild & hatchery individuals
- Study natural genetic structure

Price, 1984; Bell et al., 2008; Laikre et al., 2010; Rhode et al., 2012; Morvezen et al. 2016; Grant et al., 2017
Genetics

- Need to limit genetic differentiation between wild & hatchery individuals
- Study natural genetic structure

Genetic drift in hatchery

Genetic differentiation between wild & hatchery

Release: Potential Impact on wild population

Reduce local adaptation to local environmental pressures

Price, 1984; Bell et al., 2008; Laikre et al., 2010; Rhode et al., 2012; Morvezen et al. 2016; Grant et al., 2017
**Genetics**

- Need to limit genetic differentiation between wild & hatchery individuals
- Study natural genetic structure

**Aims:**
- Identify genetic structuring along French coast
- Assess genetic diversity of hatchery-reared individuals and compare with natural populations

---

*Genetic drift* in hatchery

*Genetic differentiation* between wild & hatchery

Release: **Potential Impact** on wild population

*Reduce local adaptation* to local environmental pressures

---

*Price, 1984; Bell et al., 2008; Laikre et al., 2010; Rhode et al., 2012; Morvezen et al. 2016; Grant et al., 2017*
Sampling

Wild Population

- 429 Individuals
- 10 sites
  - Covers natural range of the subspecies
- Several cohorts sampled
**Sampling**

**Wild Population**
- 429 Individuals
- 10 sites
  - Covers natural range of the subspecies
- Several cohorts sampled

**Hatchery Samples**
- 467 Individuals
- France Haliotis
- 14 subgroups
  - 3rd to 5th generation in hatchery (selection program)
SNP selection and laboratory genetic analyses

- Single-nucleotide polymorphism (SNP)
  - Variations of one single base pair throughout the genome

Harney et al., 2018
SNP selection and laboratory genetic analyses

- Single-nucleotide polymorphism (SNP)
  - Variations of one single base pair throughout the genome

Genotyping using Kompetitive Allele Specific PCR assays (KASP) by LGC Genomics

Harney et al., 2018
**SNP selection and laboratory genetic analyses**

- **Single-nucleotide polymorphism (SNP)**
  - Variations of one single base pair throughout the genome

  ![SNP examples](image)

- **Genotyping** using *Kompetitive Allele Specific PCR assays* (KASP) by LGC Genomics

- **158 bi-alleic nuclear SNPs**
  - Filtered to keep only neutral markers for connectivity analysis
  - Using Hardy-Weinberg Equilibrium, Linkage Disequilibrium, Outliers

=> **147 SNPS**

*Harney et al., 2018*
• **Index of genetic differentiation**
  (Pairwise Fst)

![Genetic differentiation heatmap]
**Index of genetic differentiation (Pairwise Fst)**

- **Genetic differentiation**
  - Low (0.00)
  - High (0.04)

*P-value* < 0.05
• **Index of genetic differentiation (Pairwise Fst)**

<table>
<thead>
<tr>
<th></th>
<th>BOB-1</th>
<th>BOB-2</th>
<th>BOB-3</th>
<th>MOL-1</th>
<th>MOL-2</th>
<th>MOL-3</th>
<th>MOL-4</th>
<th>ABW-1</th>
<th>ABW-2</th>
<th>ABW-3</th>
<th>GUI</th>
<th>SQP</th>
<th>SMA</th>
<th>CHA</th>
<th>BLA</th>
<th>CHE</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOB-1</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>BOB-2</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>BOB-3</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>MOL-1</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>MOL-2</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>MOL-3</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>MOL-4</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>ABW-1</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>ABW-2</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>ABW-3</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>GUI</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>SQP</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>SMA</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>CHA</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>BLA</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>CHE</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

Genetic differentiation

- Low (0.00)
- High (0.04)

*P-value < 0.05

*Genetic differentiation map showing significant genetic differentiation between different locations.*
• **Index of genetic differentiation (Pairwise Fst)**

![Genetic differentiation heatmap](image)

- Genetic differentiation
  - Low (0.00)
  - High (0.04)

*P-value < 0.05
• Discriminant Analysis of Principal Components (DAPC)
• Discriminant Analysis of Principal Components (DAPC)
Structure North/South

- Similar pattern found in many invertebrate species:

  - **Great Scallop** (*Pecten maximus*) (Handal, 2019)
  - **Polychaetes** (*Pectinaria koreni, Owenia fusiformis*) (Jolly et al., 2005; Jolly et al., 2006)
  - **Edible Cockle** (*Cerastoderma edule*) (Vera et al., 2021)

Jolly et al., 2005; Jolly et al., 2006; Ayata et al., 2010; Handal, 2019; Vera et al., 2021
Structure North/South

- Similar pattern found in many invertebrate species:
  - Great Scallop (*Pecten maximus*) (Handal, 2019)
  - Polychaetes (*Pectinaria koreni, Owenia fusiformis*) (Jolly et al., 2005; Jolly et al., 2006)
  - Edible Cockle (*Cerastoderma edule*) (Vera et al., 2021)

- Possible barriers (Ayata et al., 2010):
  - Ushant front
  - The Furrow of Talbert

Abalone Spawning

Seasonal Hydrodynamics

- J F M A M J J A S O N D

Jolly et al., 2005; Jolly et al., 2006; Ayata et al., 2010; Handal, 2019; Vera et al., 2021
Structure North/South

- Similar pattern found in many invertebrate species:
  - Great Scallop (*Pecten maximus*) (Handal, 2019)
  - Polychaetes (*Pectinaria koreni, Owenia fusiformis*) (Jolly et al., 2005; Jolly et al., 2006)
  - Edible Cockle (*Cerastoderma edule*) (Vera et al., 2021)

- Possible barriers (Ayata et al., 2010):
  - Ushant front

- The Furrow of Talbert

**Seasonal Hydrodynamics**

- Abalone Spawning

**North/South differentiation**

Broodstock must be chosen depending on the locality

*Jolly et al., 2005; Jolly et al., 2006; Ayata et al., 2010; Handal, 2019; Vera et al., 2021*
• Basic **population genetic statistics** (Ho)

\[
\text{Ho} = \text{observed heterozygosity, estimated from allele frequencies}
\]
• Basic **population genetic statistics** (Ho)

\[
\begin{array}{c}
0.37 \\
0.36 \\
0.35 \\
0.34 \\
0.33 \\
0.32 \\
0.31 \\
0.30 \\
0.29 \\
\end{array}
\]

**Ho** = observed heterozygosity, estimated from allele frequencies
Hatchery genetic diversity

- Retention of the genetic diversity in hatchery-raised stock
  - Similar between wild & hatchery samples

- Breeding protocol avoided any noticeable erosion

© Sébastien Hervé

Harney et al., 2018
Hatchery genetic diversity

- Retention of the genetic diversity in hatchery-raised stock
  - Similar between wild & hatchery samples

- Breeding protocol avoided any noticeable erosion

Limits
- Ascertainment bias
  - Markers developed for parentage assignment
  (Harney et al., 2018)
• **Index of genetic differentiation (Pairwise Fst)**

*All the comparison are significant*
• Index of genetic differentiation (Pairwise Fst)

\[ A = \text{Wild populations} / \text{14 hatchery subgroups} \]

*All the comparison are significant*
- **Index of genetic differentiation** (Pairwise Fst)

**A** = **Wild** populations / 14 **hatchery** subgroups

**B** = **Wild** populations / **Pooled all hatchery** samples

*All the comparison are significant*
Differentiation Wild & Hatchery

- Highly differentiated wild and hatchery samples
  - Similar pattern found in *H. midae* in South Africa and *H. rubra* in Australia (Evans et al., 2004; Rhode et al., 2012)

- The use of wild broodstock and the use of different cohorts from different parents ensures to minimize the loss of genetic diversity and adaptive potential (Hornick & Plough, 2019)

*Evans et al., 2004; Rhode et al., 2012; Hornick & Plough, 2019*
Differentiation Wild & Hatchery

• Highly differentiated wild and hatchery samples
  • Similar pattern found in *H. midae* in South Africa and *H. rubra* in Australia (Evans et al., 2004; Rhode et al., 2012)

• The use of wild broodstock and the use of different cohorts from different parents ensures to minimize the loss of genetic diversity and adaptive potential (Hornick & Plough, 2019)

Breeding practices appropriate in France Haliotis

⚠️ But strong differentiation suggests seeds released should be composed of several cohorts/generation

*Evans et al., 2004; Rhode et al., 2012; Hornick & Plough, 2019*
Now?

- **Local Structure between North and South**
  
  Spatial autocorrelation analysis to study recruitment patterns and gain a better understanding of connectivity (Miller et al., 2016)

*Araki et al., 2007; Christie et al., 2014; Miller et al., 2016*
Now?

- **Local Structure between North and South**
  Spatial autocorrelation analysis to study recruitment patterns and gain a better understanding of connectivity (Miller et al., 2016)

- **High genetic diversity in the hatchery**
  Confirm it with the use of genomics tools for a more complete analysis

_araki et al., 2007; christie et al., 2014; miller et al., 2016_
Now?

**Local Structure between North and South**
- Spatial autocorrelation analysis to study recruitment patterns and gain a better understanding of connectivity (Miller et al., 2016)

**High genetic diversity in the hatchery**
- Confirm it with the use of genomics tools for a more complete analysis

**High differentiation between hatchery & wild**
- Can it affect fitness? (Araki et al., 2007; Christie et al., 2014)
- Compare responses of natural and hatchery population to environmental variability
  - Common garden experiment
  - Poster
  - "Effect of domestication on the response of European Abalone to natural environmental variations and global change: a common garden experiment"

*Araki et al., 2007; Christie et al., 2014; Miller et al., 2016*
Acknowledgement

Thank You!

Pierre Chauvaud
Sabine Roussel
Eric Pante
Amélia Viricel
Grégory Charrier
Erwan Amice
Isabelle Bihannic

Thierry Le Bec
Emilie Grossteffan
Olivier Basuyaux
Françoise Calvez
Christian Aillet
Philippe Orveillon
Thank you for your attention


[Handal, W., 2019. Rôle de la connectivité et de l'adaptation locale dans la structure et le fonctionnement des populations de coquilles Saint-Jacques (*Pecten maximus*) en Manche, Mer d'Iroise et Rade de Brest (Doctoral dissertation, Brest).]


• Basic population genetic statistics (Ho, He)