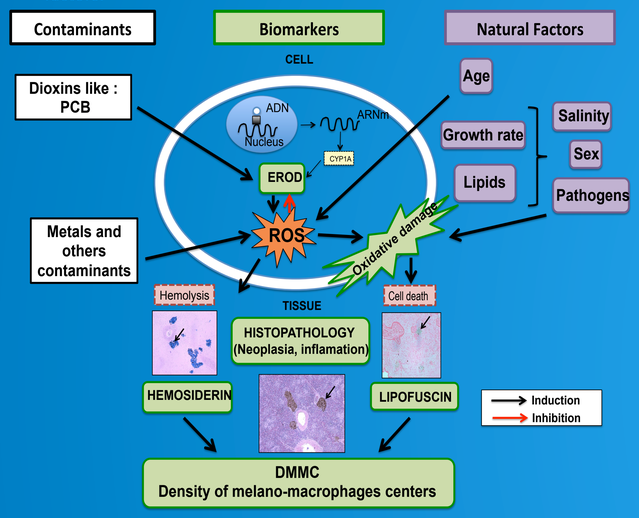
Multivariate Analysis of Biomarker Responses in European and American Yellow Eels in the Gironde and St. Lawrence Estuaries

Géraldine Patey [1,2], C. M. Couillard [1], G. Verreault [3], H. Budzinski [4], F. Pierron [5], M. Baudrimont[4], I. Boulangeat [6], P. Couture[2]

Introduction

- The American eel (*anguilla rostrata*) and the European eel (*anguilla anguilla*) have suffered a sharp decline.
- During their long growth period (yellow stage) in fresh or brackish water, PCBs, metals and other contaminants accumulate in their tissues.
- Contaminants can affect eels health alone or in interaction with natural factors.



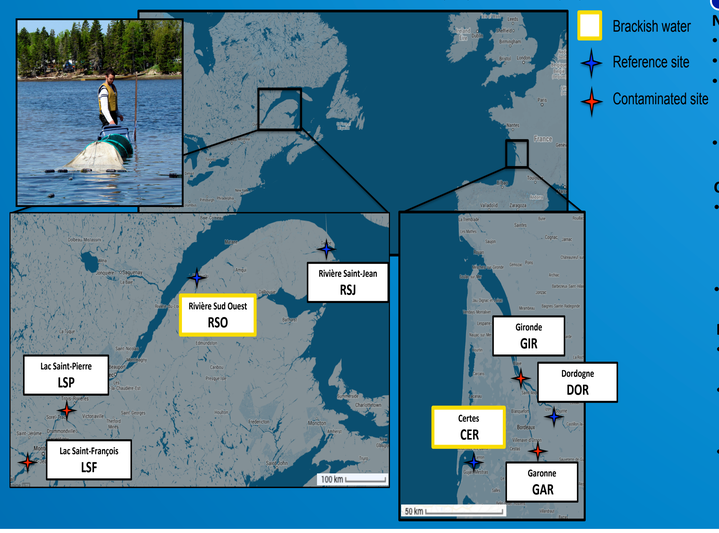
Objective & hypotheses

What are the effects of contaminants and naturals factors on biomarkers in Atlantic yellow eels in Quebec (QC) and France (FR)?

Hyp 1: Patterns of contaminants, biomaker responses and natural factors do not differ among sites.
Hyp 2 : Biological variables do not differ between countries.
Hyp 3 : Biomarker responses do not differ among sites.

Sampling

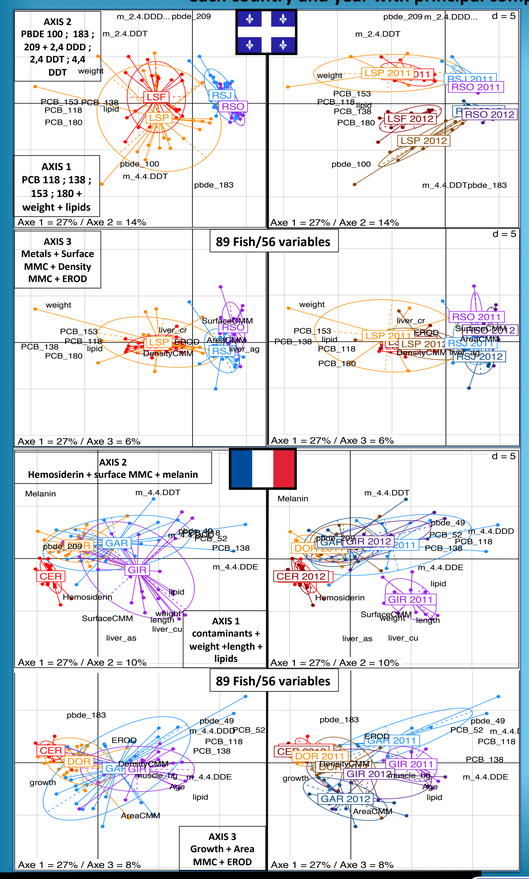
240 yellow eels (15 yellow eels / site / country / year) were captured in May-June 2011 and 2012 in 2 contaminated sites and 2 reference sites in Quebec (QC) and France (FR).



RESULTS & DISCUSSION

Multivariate analysis

Comparison of patterns of contamination, biomarker responses and natural factors among sites in each country and year with principal component analysis (PCA)



AXIS 1

- In Quebec, the sites LSF and LSP differ mainly from the reference sites RSJ and RSO by higher PCBs concentration, higher lipids and higher body condition.
- Eels from LSP and LSF are located near sources contamination and traveled across the geographical gradient of the Saint Lawrence river

AXIS 3

- Surface MMC is linked to the reference site RSO

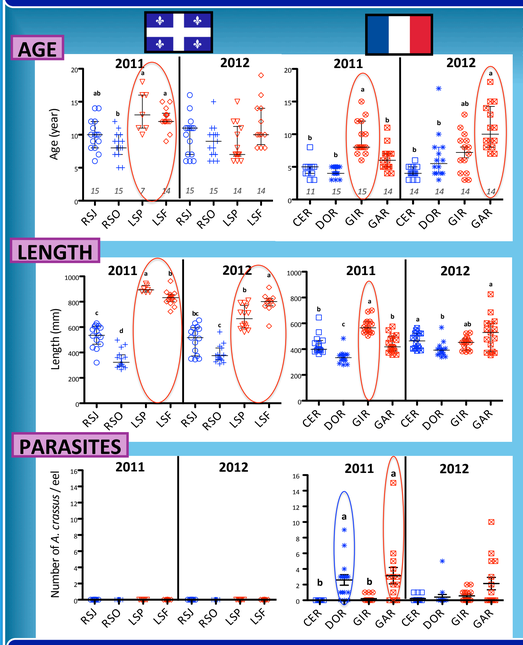
AXIS 1

- In France, the sites GAR and GIR differ mainly from the reference sites CER and DOR by higher PCBs concentration, organochlorine pesticides as 4.4 DDT and 4.4 DDE and polybrominated diphenyl ethers as PBDE 47 and PBDE 49.
- A part of eels from the site GIR seems to be highest lenght, weight and lipid content.

AXIS 2

- hemosiderin is still linked to a reference site CER (brackish water).
- An inter-annual difference in the site GIR is observed. In 2011, eels differ from GIR 2012 by higher length, weight, lipid content and surface MMC.

Natural factors

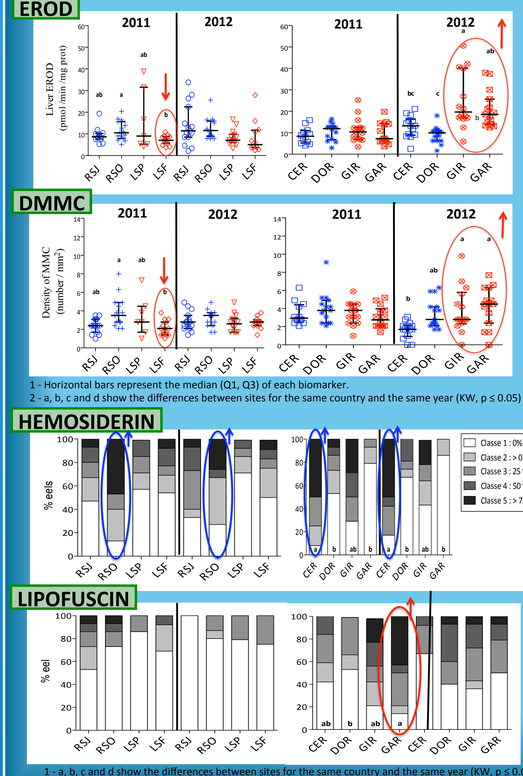


All eels ≤ 2 years old (CER, GAR and GIR) and those ≥ 20 years old (LSP and LSF) were removed from the analysis to keep age ranges comparable among sites.

- In 2011 and 2012, Eels from Quebec site are older than those sampled in France.
- Therefore, we conducted the analysis for each country separately.
- In France, in 2011 and 2012, eels from the reference sites CER and DOR are younger than those of contaminated sites GAR and GIR.
- In Quebec, there is a marked difference size at age between contaminated sites and reference sites in relation to a large spatial scale.

- In France, only in 2011, eels from the sites GAR and DOR are significantly most infested with *A. crassus*.

Biomarkers



1 - Horizontal bars represent the median (Q1, Q3) of each biomarker.
2 - a, b, c and d show the differences between sites for the same country and the same year (KW, $p \leq 0.05$)

- In Quebec, only in 2011, these 2 biomarkers are lower at the contaminated site LSF compared to RSO.
- In France, only in 2012, hepatic EROD activity and DMMCs are higher at the contaminated sites GIR and GAR.

- In Quebec, in 2011 and 2012, the brackish site RSO tends to have more hemosiderin depositions but it is not significant.
- In France, in 2011 and 2012, Eels from the reference site CER (brackish water) are those with the most hemosiderin depositions.

- In Quebec, lipofuscin depositions did not differ among sites in QC.
- In France, only in 2011, eels from contaminated sites GIR and GAR are more lipofuscin depositions than reference sites (DOR).

1 - a, b, c and d show the differences between sites for the same country and the same year (KW, $p \leq 0.05$).

Materials & methods

Natural factors:

- Weight and length were measured.
- Age was determined from otoliths.
- The dry-basis lipid content of each sample was estimated in muscle of fish by microwave assisted extraction (MAE) (Bodin et al., 2009).
- The presence and the number of the parasite *Anguillicoloides crassus (A. crassus)* were determined in the swimbladder.

Contaminants:

- Concentrations of organic contaminants (PCBs, PBDEs and organo-chlorinated pesticides) were measured in muscle by GC / MS (mass spectrometer) coupled to an ECD (electron capture detector).
- Metal concentrations were measured in liver and muscle by ICP / MS.

Biomarkers:

- The ethoxyresorufin-O-deethylase (EROD) was measured in the liver by spectrofluorimetry (Fragoso et al.,1998).
- The density (D) and the surface (S) of theMMCs were mesured using image analysis in spleen on histological slides stained with hematoxylin and eosin (H & E) (Luna, 1968).
- The presence of hemosiderin, lipofuscin and melanin in the MMCs were detected by staining with Prussian blue (Perls). The proportion of macrophages within MMCs stained positively with each of these stains was graded from classe 1 to classe 5.

Discussion

- In Quebec and in France, multivariate analysis does not clearly show the effect of contaminants measured on biomarker responses.
- For each country, the PCA identify chemical contaminants concentration and biological variables that differ among sites and also help to identify 'tracer' contaminants.
- Because of differences in biological variables and contamination patterns between countries, the analysis of the relationship between biomarkers and contaminants must be done for each country separately :
  1. In Quebec, biomarker responses not differ clearly among sites and years.
  2. In France, only in 2012, eels from GIR and GAR are higher EROD activity and DMMCs than eels from reference sites. This inter-annual variability can be linked to other contaminants not measured yet as HAPs for example.
  3. In France, in 2011 and 2012, eels from the most brackish water site CER reveal high hemosiderin deposition. This surprising response can be linked to a different parasite from *A. crassus.*
- Future analyzes will take into account additional biological variables such as sex, growth rate and salinity and biomarkers of oxidative damage.

(1) Institut National de la Recherche Scientifique, Centre Eau Terre Environnement
(2) Fisheries and Oceans Canada
(3) Ministère des Forêts, de la Faune et des Parcs
(4) Université de Bordeaux 1
(5) Université du Québec à Rimouski