Atlantic eels: a transatlantic cause

Professor of Ecotoxicology at the University Bordeaux/CNRS, **Magalie Baudrimont-Larue** and colleagues discuss their current Franco-Quebecois collaborative project, IMMORTEEL, which investigates the impact of contaminated estuaries on two endangered eel species using several innovative approaches

MAGALIE BAUDRIMONT-LARUE

PATRICE COUTURE

FABIEN PIERRON

European and American eels are culturally, economically and ecologically significant species under natural and anthropogenic threat. What are the objectives of the 'Impacts of metallic and organic contaminations of the Gironde and St Laurent systems on two threatened species, the European and American eels' (IMMORTEEL) project?

Among the different hypotheses advanced to explain such decline of Atlantic eels, the possible contribution of pollution has been little explored. Results from comparative *in situ* studies often show that eels accumulate large quantities of persistent contaminants compared to other fish species.

In the Gironde estuary, *Anguilla anguilla* is the second most contaminated fish by cadmium. The monitoring system developed in the framework of the National Action Plan on polychlorinated biphenyls (PCBs) has also shown that PCB levels are higher than the European average in eels living in the Garonne river (an affluent of the Gironde estuary). Studies carried out in the St Lawrence river have shown that the American eel is one of the fish species most contaminated by mercury, PCBs and Mirex.

In this new project we will assess, through complementary and multidisciplinary approaches, the effects of pollutants in these two water systems on resident eel populations.

Why did you decide to conduct your investigation on eels in these estuaries?

These two estuaries are particularly impacted by contaminants, representing a specific signature in each of them.

From an ecological standpoint, the reproduction of the entire North American eel population is essentially supported by females from the St Lawrence. In Europe, the Gironde estuary is one of the rare estuaries that still has a cluster of migratory amphihaline fish species including eels.

Possible reasons for Atlantic eel decline are global climate change, overfishing, obstacles to freshwater migration, parasitism and exploitation. Why have you chosen to focus on metallic and organic contamination?

American and European eels can accumulate significant amounts of these types of contaminants compared to other fish species living in the same environment, due to their particular biological lifecycle, which leads to long-term exposure of sexually immature individuals. They are particularly rich in lipids, thus readily accumulate lipophilic contaminants such as methylmercury and PCBs.

The Gironde and the St Lawrence receive particularly high levels of metallic and organic pollutants originating from anthropogenic activities.

To what extent does pollution inhibit eel reproduction?

Eels reproduce only once in their life, in the Sargasso Sea, after a period of somatic growth, the yellow phase, lasting an average of 10-15 years.

During reproductive migration eels stop feeding, instead using the energy reserves accumulated in the yellow stage for swimming and gonad maturation.

Contaminants accumulated during the growth phase can be remobilised and transferred to the gonads. Thus, even in the absence of significant external sources of contamination, toxic events may still occur. These events can compromise the spawning migration of the species.

IMMORTEEL is a joint initiative between French and Quebecois researchers. How will international collaboration benefit your project?

The teams involved in this project have lengthy experience in our principal lines of enquiry. This new international consortium will pool this expertise, developing integrated, complementary approaches to respond to the unique problem of eel decline.

By what means do you intend to assess the relationship between pollution, both organic and inorganic, and the health of Atlantic eels?

All results will contribute to a shared database. At first, the statistical treatment will involve multidimensional exploratory analyses which will identify the correlations between quantitative and qualitative variables.

Tools of hierarchical clustering will allow us to visualise typologies of individuals according to their physiological, transcriptomic and genetic (SNP) status and contaminant loads. Depending on the predefined variables, these analyses will be completed by non-parametric and robust regression analyses to identify the nature of underlying mechanisms leading to these typologies.

What techniques could be used to develop sensitive and efficient tools to determine the health status of eels in different systems?

It is always difficult to unequivocally link pollution monitored in field studies to effects observed in organisms. In this context, -omic approaches, which allow the simultaneous measurement of a large number of endpoints in biological samples, open the door to understanding mechanisms of toxicity and the interactions among contaminants and natural stressors.

Safeguarding a species

Researchers engaged in the transatlantic **IMMORTEEL** project are employing a multidisciplinary approach to investigate the impact of organic and metal contamination on the population. This sustainable project seeks to eventually provide information vital for habitat and species conservation

THE ATLANTIC EEL is suffering a dangerous decline. The American eel species (*Anguilla rostrata*) has been classified as of particular concern in Canada, currently experiencing a rate of decline of 40-80 per cent in the St Lawrence estuary compared to figures from the 1980s. *Anguilla anguilla*, the European eel, is in even further dire straits: a 90-99 per cent decline compared with the 1970s puts the species at serious risk of extinction. The Atlantic eel is of major import – economically, ecologically and in terms of natural heritage.

UNIQUE SPECIES

In both the North American region and in Europe, exploitation of the Atlantic eel is a vital component of fisheries. Statistics demonstrate that during the glass eel stage of its metamorphosis the Atlantic eel is the third most valuable catch between the Loire in France and the south of Portugal. Across the ocean, commercial value of the American eel from the St Lawrence river was almost four times that of the average for fish and shellfish as a whole.

To scientists, the unique and complex lifecycle of the Atlantic eel is most important. These fish go through two metamorphoses (from larvae into glass eels and from the juvenile growth or 'yellow' phase into mature silver eels), two transatlantic migrations and reproduce only once a lifetime. Although it has been possible to induce the reproductive cycle of the Atlantic eel and to achieve fertilisation in captivity through hormonal injections, the larvae thus far produced have always died. Professor Magalie Baudrimont-Larue of the University Bordeaux reports that to date no scientific explanation for this has been found. "The best attempts have so far failed to identify why the process of maturation does not succeed enough to ensure the growth of larvae," she says. Preserving the eels in their natural habitat is thus vital.

Due to their lifecycle, which requires massive energy reserves to be accumulated during the 'yellow' phase, Atlantic eels must be able to store large amounts of lipids. Up to 30 per cent of the body weight of an Atlantic eel can be lipids, used up in gonad maturation, migration to the Sargasso Sea and the spawning which takes place there. Successful migration and reproduction is therefore directly dependent on the quantity of lipids stored. Unfortunately, these factors are also what make the Atlantic eel so threatened by pollutant contamination and bioaccumulation.

A TRANSATLANTIC RESPONSE

The 'Impacts of metallic and organic contaminations of the Gironde and St Laurent systems on two threatened species, the European and American eels' (IMMORTEEL) project was set up in response to these drastic population declines. Funded by the Agence Nationale de la Recherche et Région Aquitaine (France) and the Conseil de recherche en sciences naturelles et en génie (Canada), and run by research teams in France and Quebec, IMMORTEEL has three central objectives. Firstly, to test the central hypothesis that organic and metal contamination results in differences in phenotypic and genotypic (via SNPs markers) composition between eel populations from clean and contaminated sites. This should in turn help researchers to interpret the influence of habitat contamination on the yellow or growth phase of the eels' lifecycle in terms of energy accumulation, metabolic capacities, growth, tissue and cell damage and histopathology. As eels migrate to spawn, dormant accumulated contaminants can be remobilised. Second, the team also evaluates the effects of this remobilisation on migratory capacities and gonad maturation of female silver eels. Finally, general health of eel populations at all stages of the lifecycle is monitored.

In order to meet these objectives, IMMORTEEL have devised a rigorous, five-stage programme supported by novel technologies and divided into two working groups: one French, with UMR CNRS EPOC at the University of Bordeaux, UMR BOREA in Paris and IRSTEA in Bordeaux-Cestas, and one Quebecois, with the Institut national de la recherche scientifique, centre Eau Terre Environnement (INRS-ETE), the Université Laval of Quebec and Pêches et Océans Canada. Sampling took place at at least one clean and two to three contaminated sites in both France and Quebec to provide scope for comparison. The group is also keen to stress the non-invasive nature of their sampling and analysis on these endangered specimens. At each site, only 30 eels will be sacrificed per 400 sampled, and part of the project is also aiming to develop the most sustainable strategies for preparing samples for analysis.

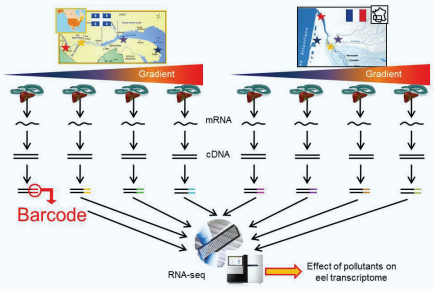
MULTI-LEVEL, MULTIDISCIPLINARY APPROACHES

IMMORTEEL conducts sample analysis at many different levels – genomic, transcriptomic, chemical, ecotoxicological, genotoxic, physiological and endocrine – to reveal and interpret the mechanisms of toxic contamination in eels, as well as to gain a precise understanding of the health status of the Gironde and St Lawrence populations.

At both sites, yellow and silver eel samples are at first subject to the same analyses. Using next-generation sequencing techniques, notably the new Illumina HiSeq2000 sequencing technology, a DNA microarray and single nucleotide polymorphism (SNP) markers are developed. The latter refers to the variation of a single base pair in the DNA sequence within a species; in functional genes, polymorphisms could affect response to chemicals and disease. Developing SNP markers is therefore a vital part of understanding how organic and metal contamination could impact eels.

The novelty of the RNA-seq technology is that it allows for the rapid calculation of gene expression by enabling the simultaneous procurement of both the sequencing information required to identify specific probes as well as the candidate genes for the DNA microarray and the project's ongoing population genomics approach. Researchers such as Fabien Pierron, who heads the DNA microarray for IMMORTEEL, find that the genes identified in this multi-pronged enquiry are those involved in energy metabolism and cell cycle regulation. Data gathered at this stage allows for transcriptomic comparison of populations in contaminated and non-contaminated habitats and is in turn linked to studies into the overall health status of the fish, which measure not only according to traditional weight indices (the Fulton condition factor and the hepatosomatic index), but also take into account external and international pathological conditions caused by oxidative stress and resulting cell damage.

Recently, IMMORTEEL researchers have measured Ag, As, Cd, Cr, Cu, Hg, Ni, Pb, Se and Zn concentrations in kidney and muscle of both species of Atlantic eel. They found that metal concentrations in the kidneys of eels were significantly higher than those measured in muscle. The highest concentrations of Ag, As, Cd, Hg, Pb and Se were found in fish from the contaminated site in France. However, in Canada only Hg and Se concentration in muscle was markedly higher in contaminated sites than elsewhere. When these results were pooled, they indicated a wide gradient of metal contamination. They demonstrate the value of IMMORTEEL's comprehensive, multidisciplinary approach, as well as validating the project's second key line of enquiry.



Total hepatic mRNA from fish sampled along a contamination gradient was extracted, reverse transcribed, labelled with unique barcode sequences and sequenced. This approach aimed to identify correlations between the transcription level of single genes and the hepatic or muscle concentrations of individual metals and organic pollutants.

SAFEGUARDING FUTURE GENERATIONS

Professor Patrice Couture, Sylvie Dufour, Pierre Elie and Catherine Couillard are responsible for the assessment of the silver eels. To evaluate the impact of pollutants of the two water systems on their migration and reproductive capacities, female silver eels are forced to swim and are subjected to artificial gonad maturation. At this stage many factors are tested: maternal transfer of contaminants to gonads and eggs, migratory capacity (especially metabolic capacity and energy reserves), reproductive capacity (gonad development and histopathology) and levels of endocrine disruption. The results of this stage of analysis naturally inform research into yellow eels, helping to indicate the extent to which contamination is an inherited factor.

As a complete reproductive cycle has been shown to fail in captivity, such research into reproduction in the eel's natural environment is vital for the survival of the species. As the impact of organic and metal contaminants on this stage of the Atlantic eel's lifecycle is elucidated, it will be possible for IMMORTEEL to instigate improvement in the conservation of the Gironde and St Lawrence habitats and to retain their suitability for this unique and important species.

AMERICAN EELS IN THE ST LAWRENCE RIVER SYSTEM IN NORTH AMERICA.

INTELLIGENCE

IMMORTEEL

IMPACTS OF METALLIC AND ORGANIC CONTAMINATIONS OF THE GIRONDE AND ST LAURENT SYSTEMS ON TWO THREATENED SPECIES, THE EUROPEAN AND AMERICAN EELS

OBJECTIVES

To assess the impacts of pollution on European and American eels inhabiting the Gironde and St Laurent systems. The relations between *in situ* accumulation of pollutants, genetic diversity, health and reproductive potential of these species are examined, in order to limit their disappearance.

KEY COLLABORATORS

Fabien Pierron, CNRS researcher, UMR CNRS EPOC 5805 – University Bordeaux **f.pierron@epoc.u-bordeaux1.fr**

Professor Patrice Couture, Institut national de la recherche scientifique, centre Eau Terre Environnement (INRS-ETE) **patrice.couture@ete.inrs.ca**

FUNDING

Agence Nationale de la Recherche et Région Aquitaine, France

Conseil recherche en sciences naturelles et génie (CRSNG), Québec, Canada

CONTACT

Professor Magalie Baudrimont-Larue
Professor of Ecotoxicology

UMR CNRS EPOC 5805 – University Bordeaux
Aquatic Ecotoxicology team
Station Marine – Place du Dr Peyneau
33120 Arcachon
France

E m.baudrimont@epoc.u-bordeaux1.fr

www.agence-nationale-recherche.fr/projet-anr/?tx_lwmsuivibilan_pi2%5BCODE%5D=ANR-10-INTB-1704

MAGALIE BAUDRIMONT-LARUE received her PhD in ecotoxicology at the University of Bordeaux in 1997. She is currently Professor of Ecotoxicology at the University of Bordeaux/CNRS. Her research concerns the cellular mechanisms of metals detoxification and the adaptive response of aquatic organisms at the physiological, biochemical and genetic levels.