

Project title: Potential of fossil DNA (ancient DNA) and contemporary DNA to built evolutionary history of diadromous fish

Manager: INRAE

Partners (in progress): MNHN, Hades, CNRS, Göteborgs Naturhistoriska Museum - University of Göteborg, University of Cambridge, University of the Highlands and Islands, Institute of Archaeology - Nicolaus Copernicus University, Leibniz Institut für Biodiversität – ZFMK Koenig Museum, Centre for Baltic and Scandinavian Archaeology, Jurrasica Museum, University of Toulouse, University of Oviedo, Polytechnic University of Valencia, University of Vigo, Complutense University of Madrid, NIBIO, NINA, University of Oslo, University of Science and Technology of Trondheim, Natural Resources Institute of Finland, Salmon and Trout Research Centre - Game and Wildlife Conservation Trust ,University College of Cork, Agri-Food and Biosciences Institute, Inland Fisheries Institute of Olsztyn, BFS, University of Liège, Laval University.

Summary :

This study will address the major questions on the effects of human, the nature and the climate, at time scales and on different taxa, in order to study past, current and future adaptation capacity. For that, the study will reconstruct the evolutionary history of 3 diadromous fish (*Anguilla anguilla*, *Salmo salar* and *Salmo trutta*) and address the question of their adaptation through time and space scale (so by using ancient and contemporary DNA, in different countries).

This project will take place in 3 phases which are described in the figure below (Figure 1). The two first parts of the project will be carried out in 2021 for the laboratory manipulations and 2022 for the analysis and interpretation of results.

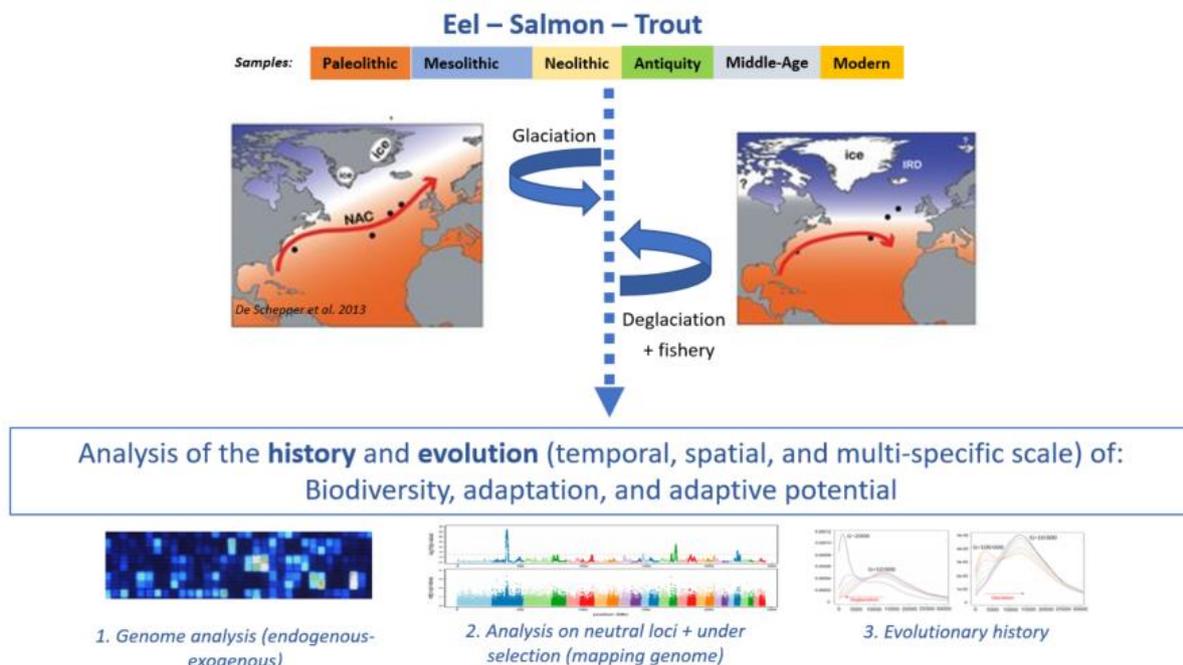


Figure 1. Summary of the links between the three parts of the project.

This study is thus innovative on several levels: 1) technical (acquisition and potential improvement of techniques relating to very old fish DNA), 2) theoretical (by reconstructing and comparing evolutionary

histories over contrasting and overlapping time steps, possibility of measuring the sensitivity of models in population genetics - eg coalescence models), and 3) conceptual (by approaching the study of the pressure exerted on species through a global approach including human and environmental dimensions).

Part 1:

The aim of this part is to conduct a pilot study in the field of ancient DNA (aDNA) on the 3 diadromous fish (Atlantic salmon, trout, European eel) from samples with various times and geographic areas. This study is intended to verify if the aDNAs are quantitatively and qualitatively likely to provide data / nucleic sequences likely to enlighten us on the structure and the evolutionary history of the populations to which these individuals belonged before initiating a study on a larger scale.

To carry out this study, we optimize the protocols for extracting DNA from subfossil remains (mainly vertebrae), and we will build genomic aDNA libraries and sequence approximately 120 samples (40 libraries per species) in low coverage on a Next Generation Sequencing (NGS) platform (NextSeq500 130 Millions reads PE). We will rely on the experience of two French teams recognized in the field of paleogenetics (Technical platform "Paleogenomics and human molecular genetics" of MNHN Paris and UMR AMIS Toulouse). The extraction DNA protocol of Dabney Korlevic/Roche improved by Regis DeBruyne (specialist in Ancient DNA, Natural History Museum in Paris) will be used and the library preparation, amplification and target enrichment from the protocol of Kircher et al. (2012) improved by MNHN. These sequences will be used to authenticate aDNAs (nuclear and mitochondrial), to reveal the levels of endogenous and exogenous DNA (DNA foreign to the targeted or introduced species), to study the degradation patterns of endogenous DNAs and to characterize the exogenous DNA. The results of the screening will make it possible to characterize the environments that are favorable to good preservation of fish aDNAs, which will make it possible to reason and optimize future sampling. This screening will provide the basis for identifying in future projects promising specimens from which we can generate complete genomes (Whole Genome Sequencing or Whole Genome Enrichment) or capture sets of SNPs (Target Capture) for which modern reference data exist already. already. The samples will be selected from contrasting archaeological periods from the Paleolithic to the last centuries to i) better understand degradation processes and encompass all of the potential genetic variability and ii) optimize the construction of the marker bank.

Part 2:

The goal of this part is to conduct new technology sequencing to build an SNP library using DART-seq technology (collaboration with Andrzej Kilian) from contemporary DNA (minimum 32 samples by species), older (2-4 scales from the last decades), and ancient (2-4 archeology). For this, we plan to use individuals from different countries to have a global representativeness with the most genetic variants. Following this SNP library, we will produce a coding gene library. However, the oligos of the coding genes are more expensive and for the moment we obtained the funding only for the SNP library. We have to find others fundings to cover this library on loci under selection.

Part 3:

It concerns the main project (Figure 1, Part 3) : analysis and comparison of ancient and contemporary DNA to retrace the evolutionary and adaptive history of species. The implementation of this project will be carried out following the results obtained by the first two parts (part 1 and 2) and discussions with partners. We will probably need around 30 samples per geographic site and per species to perform these analyzes. For contemporaries, we believe this is doable. For archaeologists, we think it

will depend on the countries and the sites. For the moment, we have a promising number of archaeological eel samples in France. We are in the process of acquiring samples of the other two species in France. Our partners are in the process of finding out about the possibilities in their countries.

Information on wild contemporary samples

Tissue for eel; tissue or scales for salmon and trout (a minimum of 2 scales/individual if it's possible).
To deal with the Part 2, we need only 5-10 individuals per area or country.
The deadline to get the samples is the end of June 2021.

Information on the individuals will be welcome as :

Date of sampling

Localisation (Latitude and Longitude)

Size and Weight of individuals

Sex

People to acknowledge for the sampling

Tissue samples:

Get a piece of tissue (minimum 0.5 cm square - skin, fine, muscle, etc.) per individual. Store the tissue in a tube with ethanol (95-96%).

Scales samples :

Take 2 untreated scales per individual and put them directly in a paper envelope.

DNA samples

A minimum of 20 µl with 50-100 ng/µl.

Information on archeological samples

Bones (the best is vertebra to obtain the body size relationship). The bones should be stored in a box to avoid DNA degradation by UV rays and high temperatures. All information on the sample is welcome (date, localisation, people to acknowledge for the sampling, etc.) and information on conservation context (plastic bag, in a black box, in a light room during 10 years ?, etc.). This last point is to understand if certain conditions are unfavorable for the preservation of DNA (ex. high temperature and U.V degrade DNA). Morphological measurements to return to the size of the individual will also be taken. All these informations will then be provided to the person who provided the sample and to the deposite structure.

To obtain a good quality of DNA, we will take at least 20mg of bone from the sample. The archaeological samples will be digitized (in 2D) because their use is partly (large vertebra) or completely (small vertebra) destructive.

To deal with the part 1, we need only 5-10 individuals per archeological area and per species.
The deadline to get the samples is the end of August 2021.

Adress to send the samples

INRAE
ECOBIOIP
Aurélie Manicki et Natacha Nikolic
173 Route de Saint-Jean-de-Luz
RD918
64310 Saint-Pée-sur-Nivelle
France

Please add the customs letter (french and english), we will send you before sending the package, inside and outside the package.

Information on permits

A MTA (Material Transfert Agreement) - MTA-type INRAE - is designed to meet regulatory requirements for ABS. We will send you the document in english. This document will be share between all the parties (the recipient and provider).

For archeological samples additional reglementations can exist and can be different per countries and/or depose site, so please inform us about the supplementary documentation(s) to be completed.

For contemporary samples, we will also contact the ABS National focal point (<https://absch.cbd.int/search/nationalRecords>) when you will provide the list of informations on samples (please see the typical email we have to send to the ABS National focal point (Annexe 1)). Concerning the European eel, the species is included in Appendix II at CoP14 (<https://cites.org/fra/node/48606>) since 2009 and we have to completed CITES permits : https://cites.org/eng/prog/Permit_system. Let us know if you want to fill this permit or if we do.

Scientific people involved in this project:

A network of competent and motivated collaborators from various backgrounds has already been established for this project (alphabetical classification; (*) Designation of project leaders):

- Juan Asturiano, biology physiology and ecology (Polytechnic University of Valencia)
- Michel Barbaza, archaeologist (UMR TRACES, University of Toulouse)
- Philippe Béarez, archaeo-ichthyologist (UMR AASPE, MNHN, Paris)
- Rafał Bernaś, population geneticist (Inland Fisheries Institute, Olsztyn, Poland)
- James H. Barrett, archaeologist, archaeo-ichthyologist (University of Cambridge, UK)
- Geir Bolstad, Evolutionary biologist (Norwegian Institute for Nature Research, NINA, Norway)
- Joelle Chat *, molecular geneticist (UMR ECOBIOIP, INRAE, Saint-Pée-sur Nivelle)
- Jan Grimsrud Davidsen, ecologist (Norwegian University of Science and Technology, Trondheim, Norway)
- Régis DeBruyne, paleogeneticist (DGD-REVE, MNHN, Paris)
- Dennis Ensing, Evolutionary biologist (Agri-Food and Biosciences Institute, Belfast, UK)
- Brice Ephrem, archaeo-ichthyologist (Hades, Bordeaux)
- Jaakko Erkinaro, Evolutionary biologist (Natural Resources Institute Finland (Luke))

- Matthias Geiger (Leibniz Institut für Biodiversität –ZFMK Koenig Museum, Bonn, Germany)
- Stephen D Gregory, Fisheries Scientist (Salmon and Trout Research Centre, Game and Wildlife Conservation Trust, Wareham, UK)
- Jennifer Harland, archaeologist (Archaeology Institute, University of the Highlands and Islands; Orkney College UHI, Orkney. Scotland)
- Jose Luis Hórreo Escandón, population geneticist (Complutense University of Madrid (UCM))
- Leif Jonsson, Osteoarchaeologist (Göteborgs Naturhistoriska Museum, University of Göteborg , Sweden)
- Andrzej Kilian, geneticist and platform manager (Diversity Arrays Technology Pty Ltd, Australia)
- Cornelya Klutsch, geneticist and ecologist (NIBIO, Norway)
- Gonzalo Machado Schiaffino, eco - evolutionary geneticist (University of Oviedo, Spain)
- Daniel Makowiecki, archeologist (Institute of Archaeology, Faculty of History, Nicolaus Copernicus University, Torun Poland)
- Aurélie Manicki *, molecular geneticist (UMR ECOBIOP, INRAE, Saint-Pée-sur Nivelles)
- Phil McGinnity, Evolutionary biologist (University College of Cork, Ireland)
- Paloma Morán, population geneticist (Universidad de Vigo, Spain)
- Marina Morini, molecular geneticist (Polytechnic University of Valencia)
- Natacha Nikolic *, molecular and population geneticist (UMR ECOBIOP, INRAE, Saint-Pée-sur Nivelles)
- Billy Nzau Matondo, biologist ecologist (LDPH, university of Liège, Belgium)
- Ulrich Schmölcke, archaeologist (Centre for Baltic and Scandinavian Archaeology (ZBSA), Germany)
- Jörg Schneider, biologist and ecologist (Büro f. fisch- und gewässerökologische Studien – BFS, Germany)
- Bastiaan Star, eco - evolutionary geneticist (Department of Biosciences, University of Oslo, Norway)
- Myriam Sternberg, archaeologist-ichthyologist (CNRS-UMR 7299, Aix-en-Provence)
- Davit Vasilyan, osteo-paleontologist (Jurassic Museum, Porrentruy, Switzerland)
- Stefanie Wagner, molecular geneticist (INRAE, AMIS, Toulouse)

Annexe 1 :

Dear Sir or Madam,

We contact you in your capacity as national focal of the Nagoya Protocol because we wish to access genetic resources from **[Country]** for their utilization in the framework of the research project described below.

We would be grateful if you could help us to answer the following questions:

- › Are genetic resources listed below subject to prior consent procedures? We have already established an MTA (Material Transfer Agreement designed to meet regulatory requirements for ABS) but do we need to add documents?
- › If any, are there any applicable Benefit-sharing terms?
- › If any, what is the competent national authority in charge of receiving this authorization request and negotiating any benefit-sharing terms?

You will find below the relevant information regarding the concerning resources and the intended utilization within the framework of the research project described further.

-Species name and sampling sites: *Indicate the list of resources, the date and their place of sampling (type of environment, geographical location) or attach this information to the email.*

Description of the resource

Date of access

Localisation sampling

- **Project objectives and applications:** It is a strictly academic research without commercial development objective.

- The aim of the project is to analyse the DNA quality on old samples and to built a SNP library from sequencing technology and test the potential to apply the library on contemporary and old samples.
- Partners will be included in the publication process (results) as coauthors.
- The results will open to the possibilities of developing a project with partners around population genetics analysis and more particularly to retrace the evolutionary history of diadromous fish.

- **Funding bodies:** This project is part of a « Pari-Scientifique» call for tender from the French Ministry of Research and Higher Education led by the National Research Agency.

Many thanks for your support,

Yours sincerely,