Hybridization between *Alosa alosa* and *A. fallax*: causes and consequences for conservation

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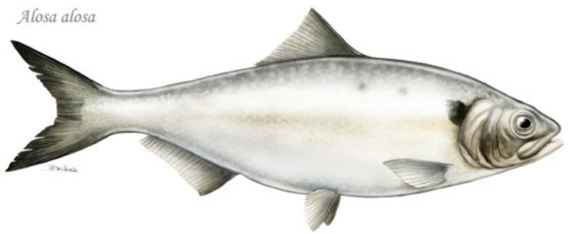
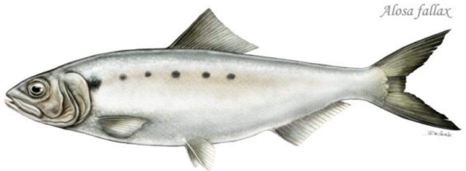
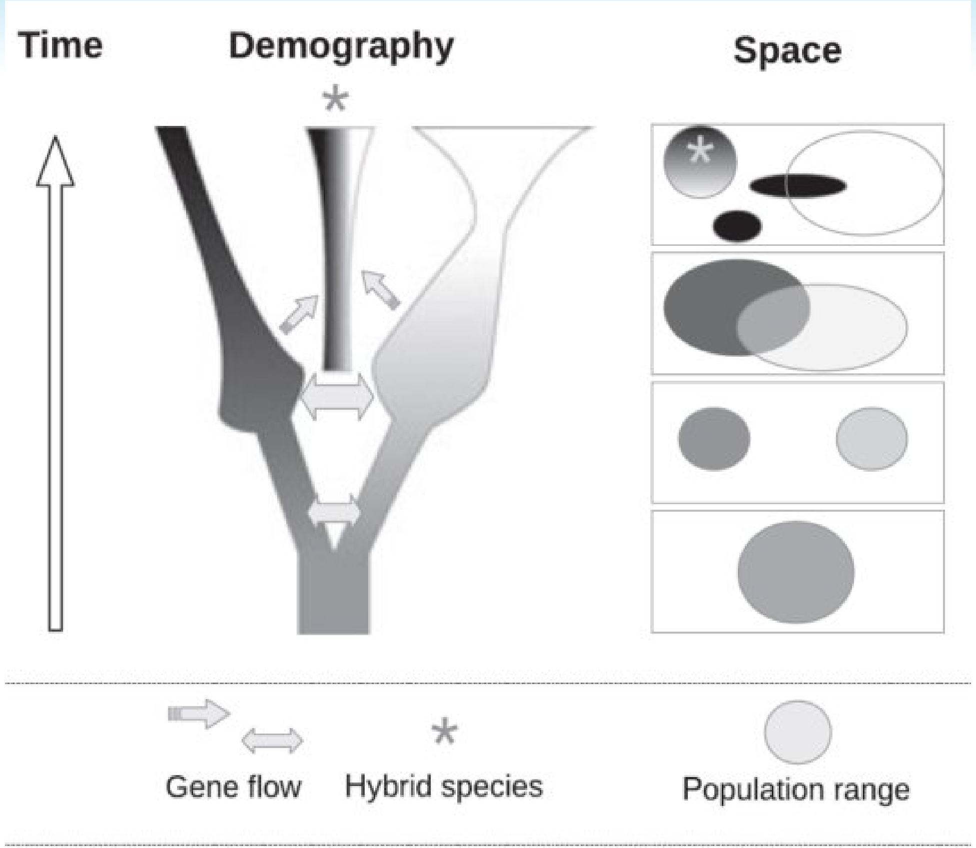




LOCAL AND GLOBAL INITIATIVES:
HOW SCIENCE SUPPORTS MANAGEMENT ACTIONS ON DIADROMOUS FISH

Speciation, hybridization: what is this all about?



Adapted from Abbott et al., 2013 (J. Evol. Biol.)

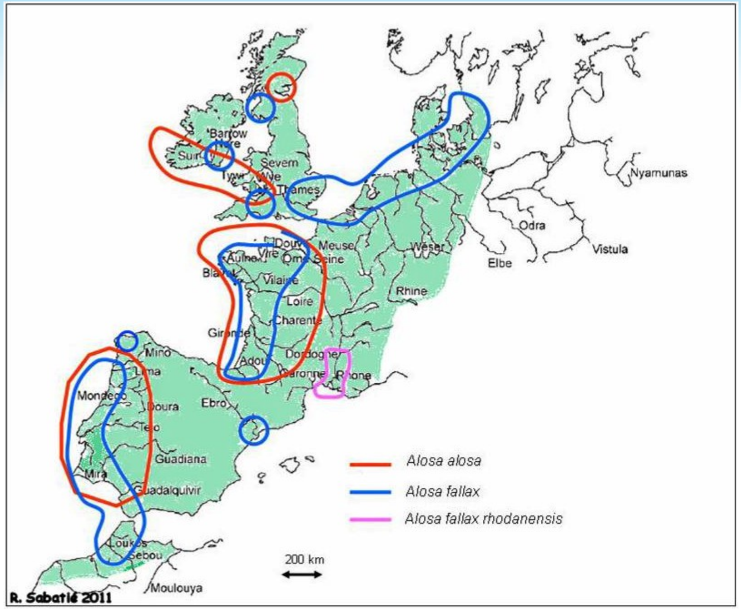
Evolution in action: hybridization impacts speciation process.

Human activities can increase hybridization by modifying species distribution, phenology, habitat degradation.

Hybridization consequences:

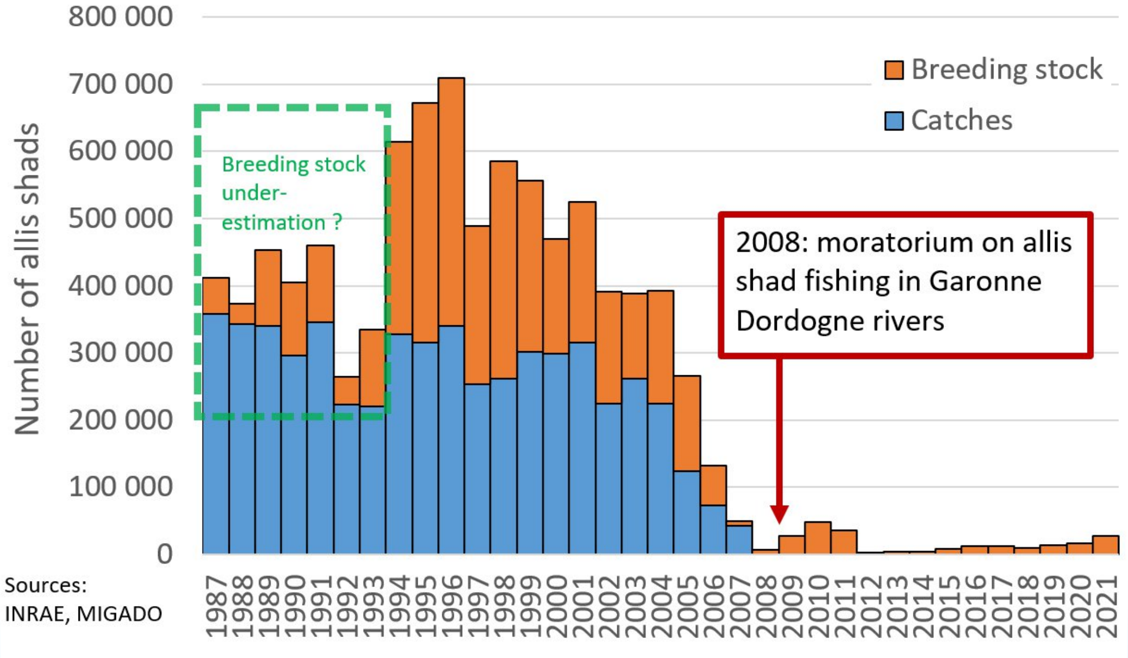
- Reproductive loss if unviable hybrids
- Interspecific gene flow and introgression if hybrid viable: threat to species maintenance or increase in adaptive potential.

European shad in an anthropogenized world



R. Sabatié 2011

- Anadromous fish impacted by human activities since the 19th century: dam construction, gravel extraction, hydropower electricity production, water pollution, climate change…



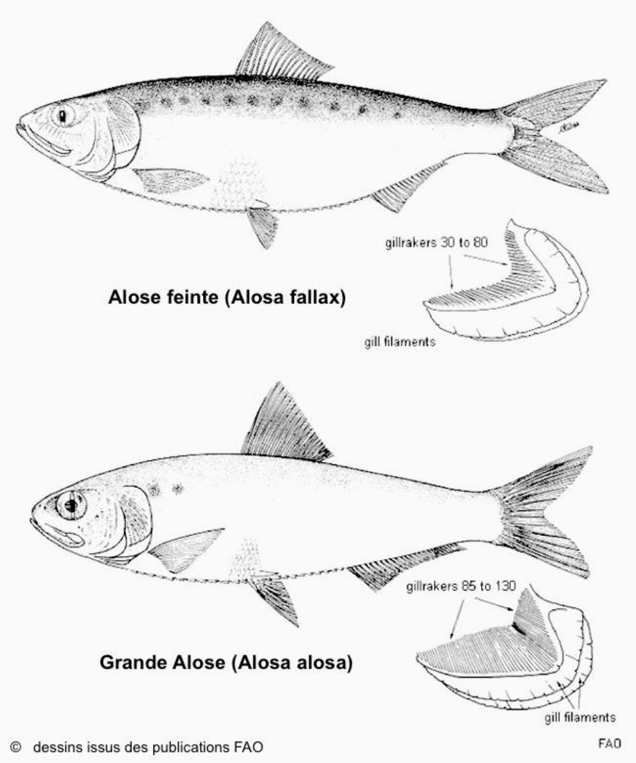
Breeding stock under-estimation ?

2008: moratorium on allis shad fishing in Garonne Dordogne rivers

Sources:
INRAE, MIGADO

- Allis shad distribution area reduction
- Restricted access to upstream spawning grounds

Why studying shad hybridization?



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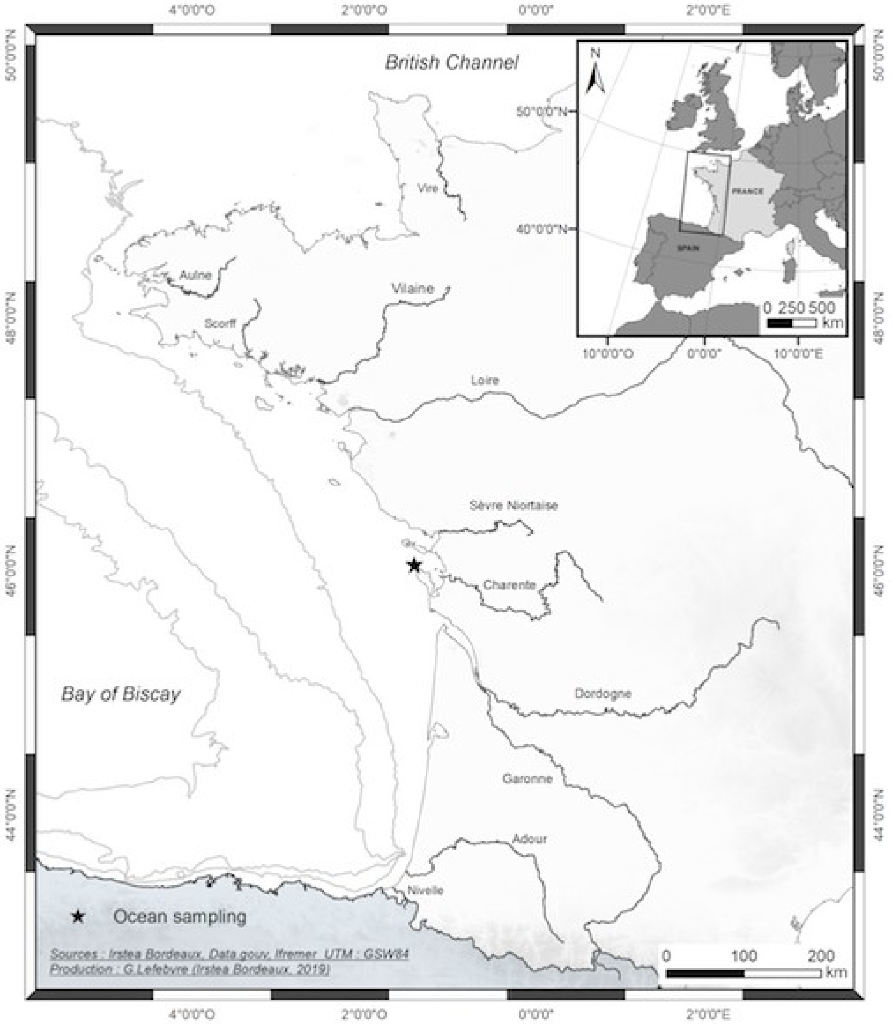
- Hybrids between allis and twait shads observed for a long time
    - Number of gill rakers has a morphological trait to identify species: allis shad > 90, twait shad < 60, and hybrids intermediate.
    - Genetic characterization: limited number of genetic markers, difficult to study hybridization dynamics in detail and its consequences
- Scientific issues and questions
    - Need for more precise molecular tools for hybrid assessment
    - Can hybridization be a cause of allis shad decline?
    - What is the effect of habitat perturbation on species reproduction?
    - Is hybridization driven by anthropogenic pressures or a evolutionary process?

Materials and methods

River / Locality	Year of collection	Sample size	Nuclear SNP	Mito SNP	Nuclear + Mito SNP
Vire	2013	29	26	29	26
Aulne	2013	15	12	15	12
Scorff	2013	20	19	18	17
Vilaine	2013	18	17	18	17
Loire	2013, 2017	53	51	50	49
Sèvre Niortaise	2016, 2017	9	7	4	4
Charente	2013 - 2018	68	59	16	13
Ocean	2018	49	47	46	44
Dordogne	2015, 2017	126	123	105	103
Garonne	2015 - 2017	132	119	105	97
Adour	2017	88	87	56	56
Nivelle	2016	27	26	3	3
TOTAL		634	593	465	441



Sources : Irstea Bordeaux, Data.gouv, Ifremer UTM : GSW84
Production : G.Lefebvre (Irstea Bordeaux, 2019)

Nuclear SNP: biparental inheritance

- Transcriptomic sequences: selection of 77 diagnostic SNP = **contemporary hybridization**
- Genotyping using Agena Biosystem MassArray (PGTB)

PGTB
SEQUENCAGE | GENOTYPAGE

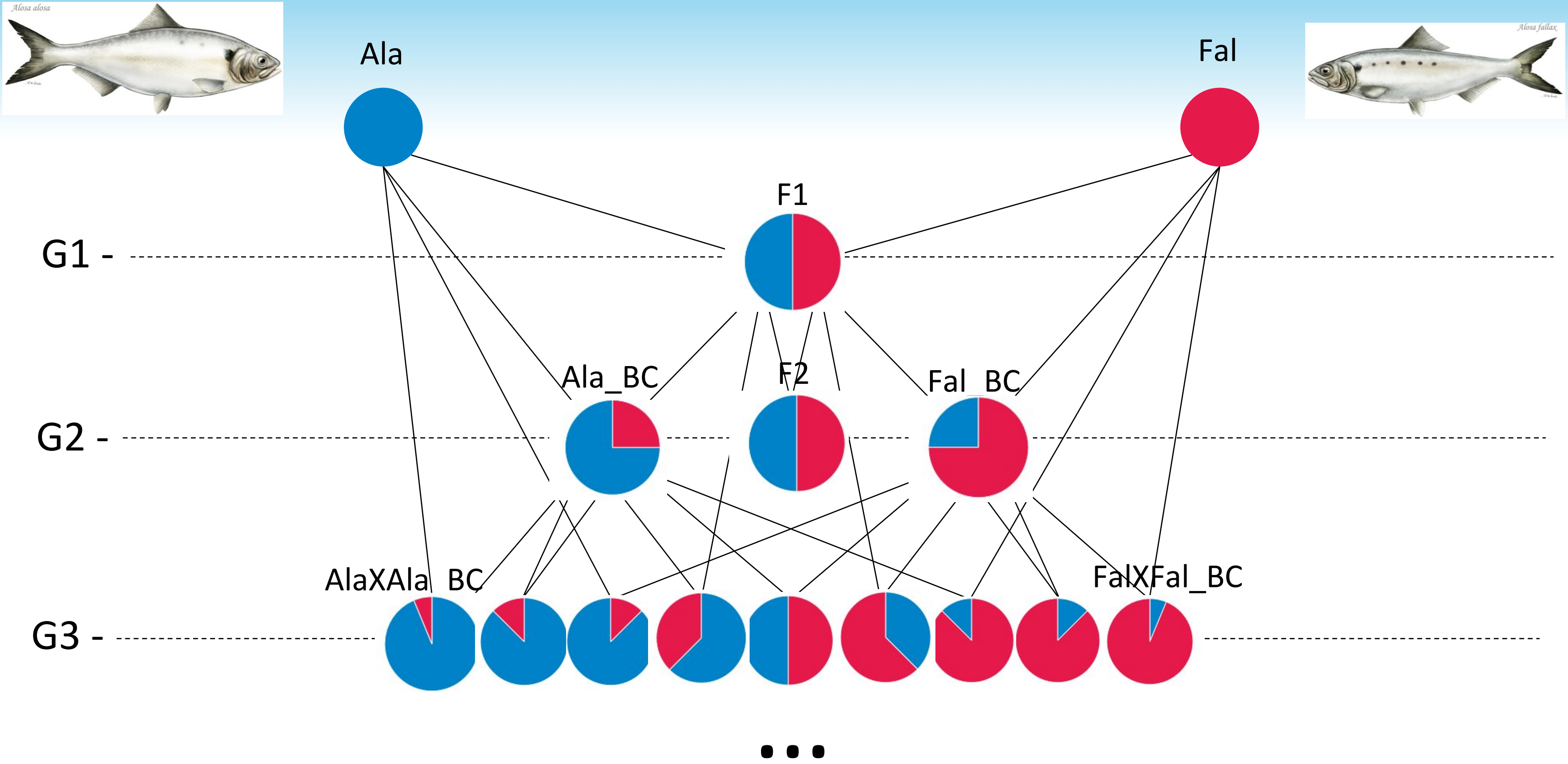
Mitochondrial sequences: maternal inheritance

- 10 « diagnostic » SNP available from the bibliography / public databases (NCBI)
- Genotyping by amplicon sequencing on Illumina MiSeq = **sex-biased and historical hybridization**

Morphological diagnostic trait: phenotypic identification of species

- Gill raker counts: hybrids are expect to be intermediate between species.

Hybridization: beyond the first generation

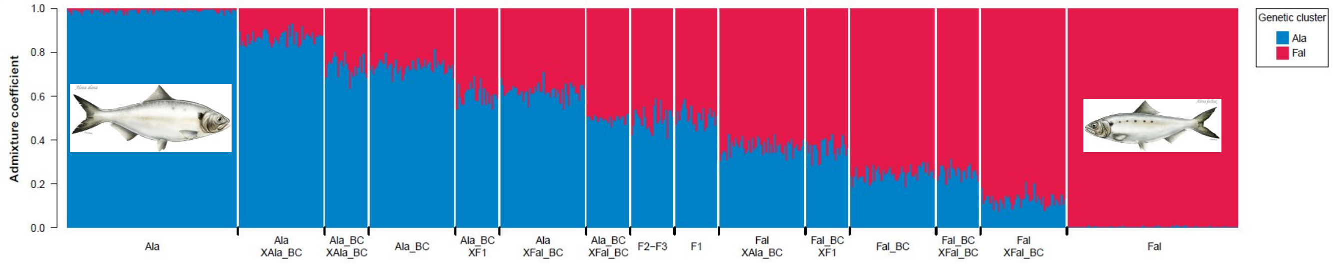


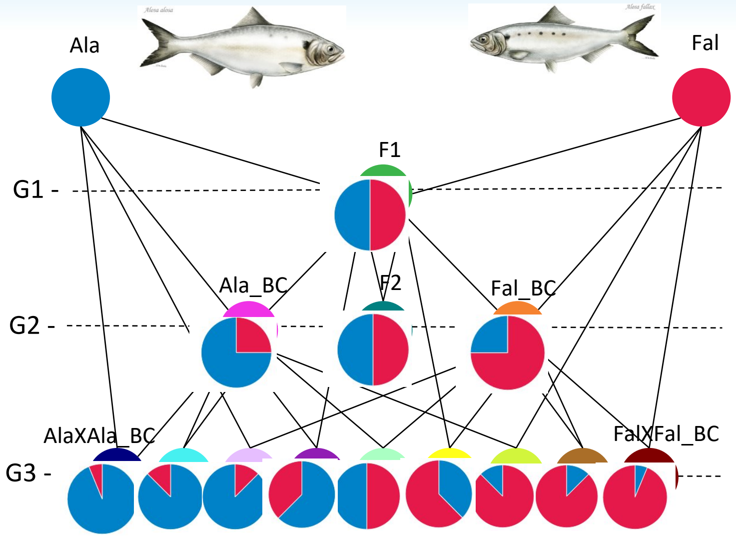
- Usually, hybrid genetic assignment is limited at best to the first two generations
- Challenge to go further to better understand hybridization consequences.

Species and hybrid genetic assignment

Information power of the 77 diagnostic nuclear SNP? → genetic assignment of simulated hybrids genotypes

STRUCTURE – simulated genotypes

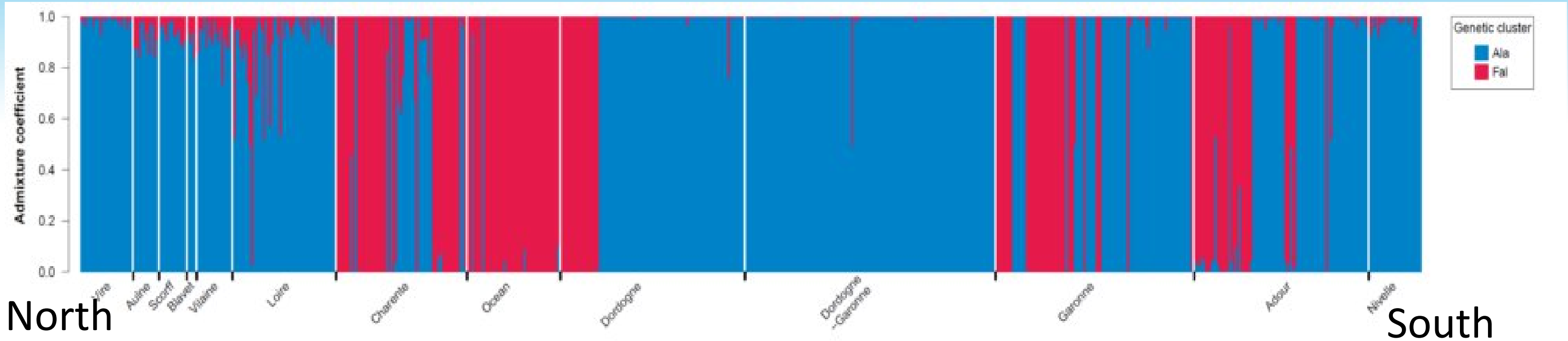




- Overall, good accuracy (98%), efficiency (90%) and power (83%).
- Lower power to identify very similar hybrid classes (likely rare in natural populations).

Species and hybrids assignments in natural populations

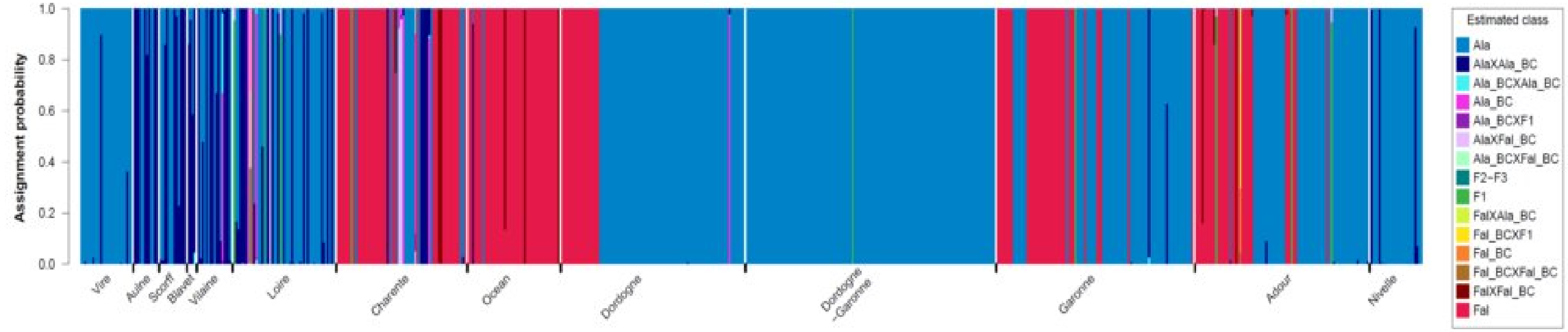
STRUCTURE – observed genotypes

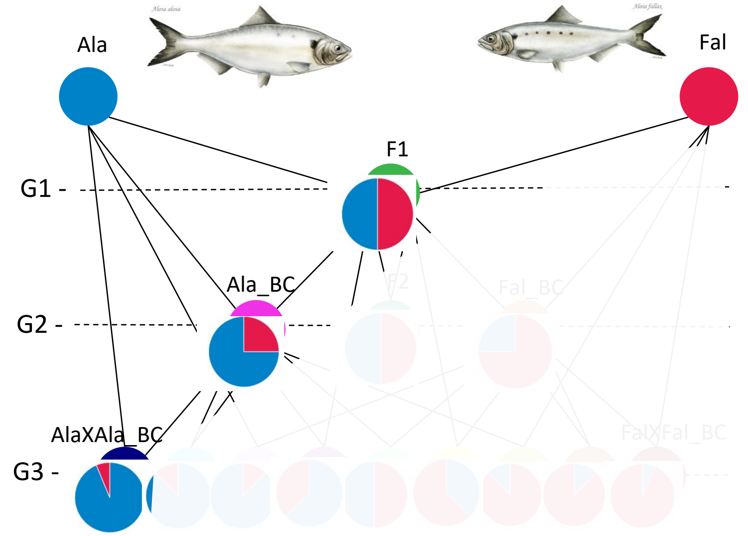


North

South

NEWHYBRID – observed genotypes



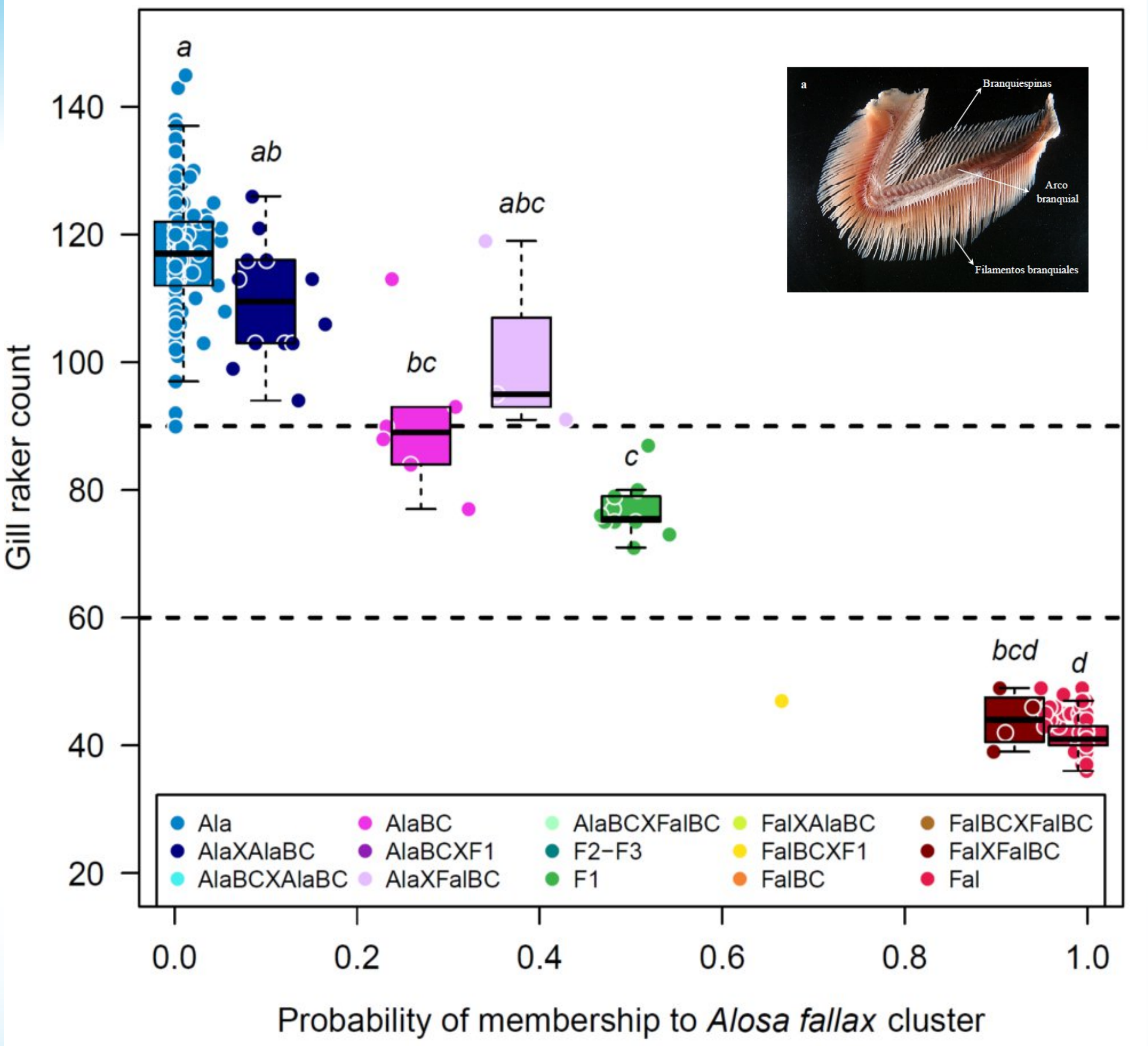


- 86% of purebred individuals, 14% of hybrids
- 7 different hybrid classes, 67% are 3rd generation hybrids Ala x Ala_BC

→ *Hybridization events three generations ago…*

…followed by contemporary introgression of genes from A. fallax into A. alosa

Comparison of morphological and genetic assignment



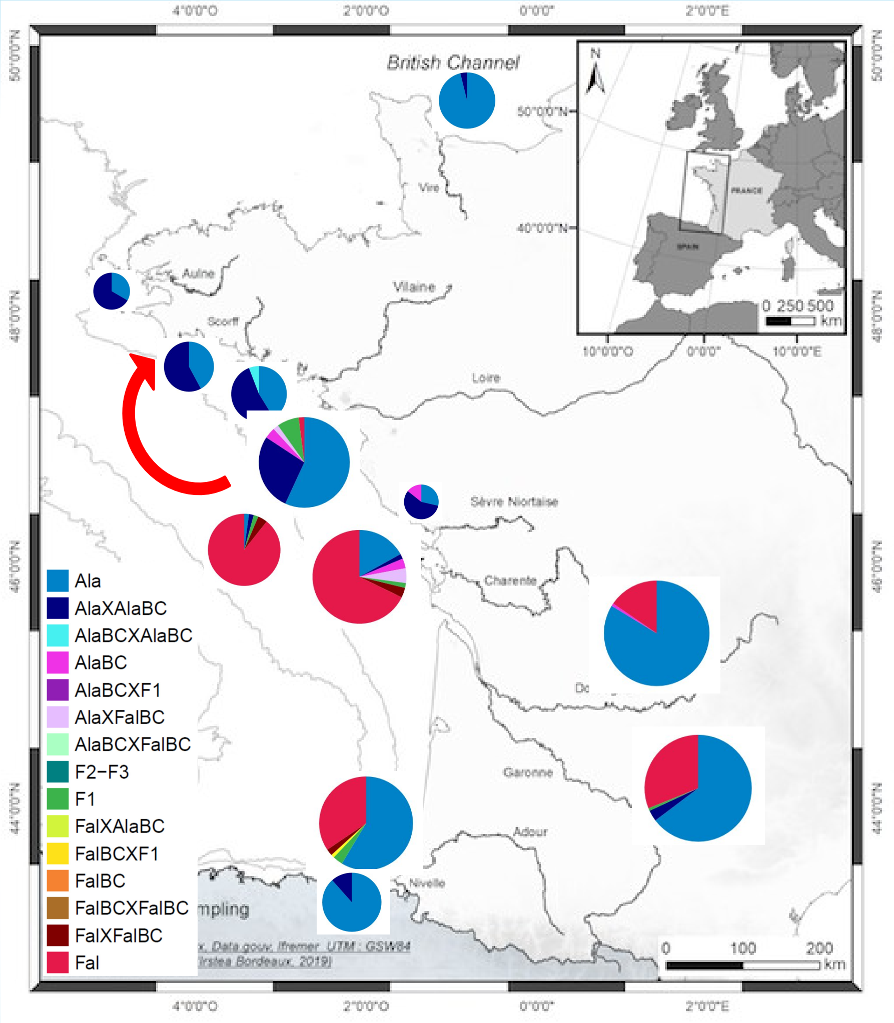
- F1 hybrids are morphologically intermediate
- Other hybrids form a continuum between parental species morphology.

Consequences:

- Limits of morphology to study hybridization dynamics
- Ecological functions of gill raker ?

→ adaptive value of intermediate gill raker count?

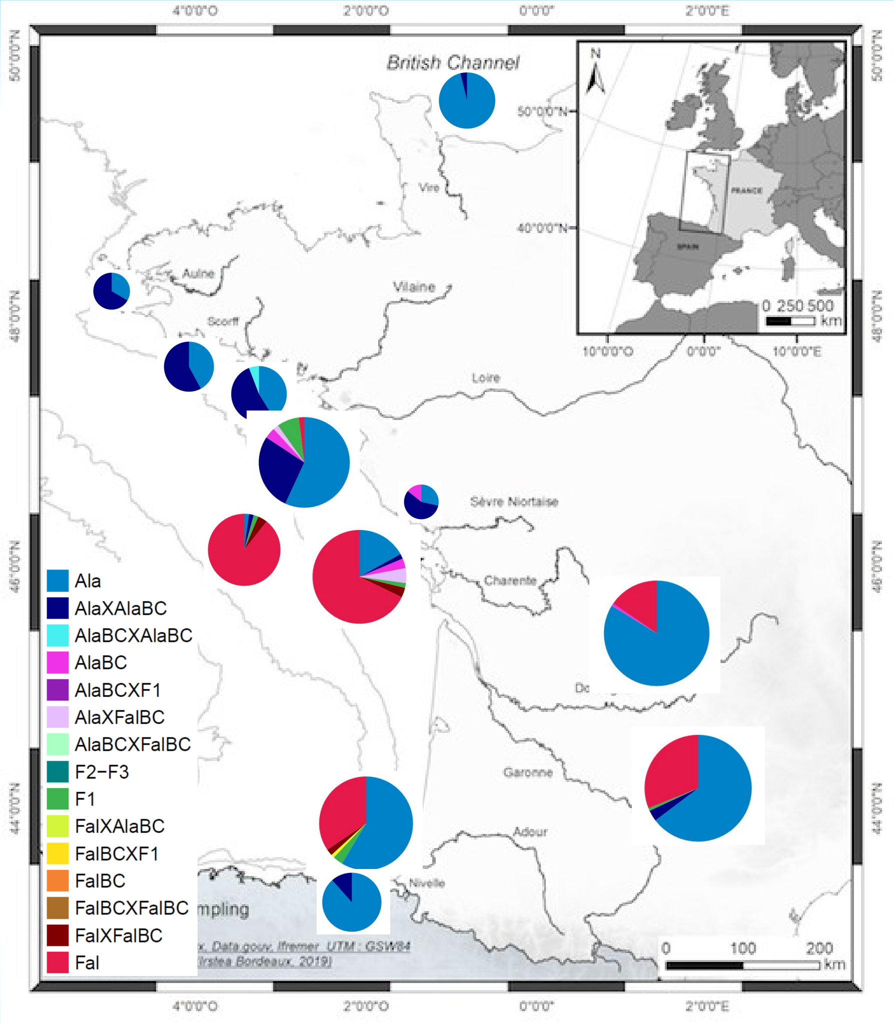
Hybridization linked to demographic processes



Brittany: >50% of 3rd generation Ala x Ala_BC hybrids

- Recent (20 years) recolonization of species
- Hybridization during recolonization
- Followed by backcrossing with the more numerous *A. alosa*
- Hybridization: introgression linked to a specific demographic processes. Unknown role of selection.

Hybridization linked to environmental contexte



- High spatial segregation of *A. alosa* and *A. fallax* reproductive ground (Garonne / Dordogne):

→ Only a few hybrids (2.5%)

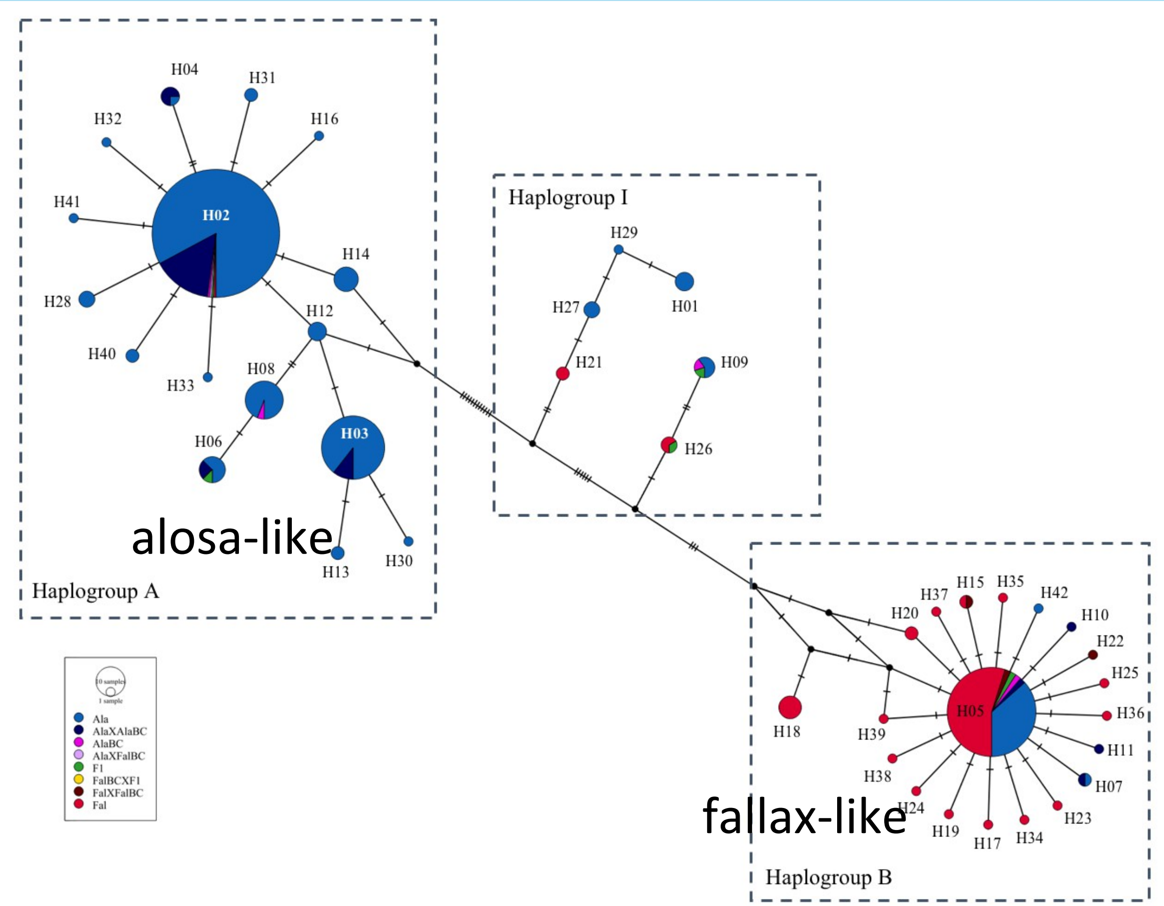
- Low spatial segregation of *A. alosa* and *A. fallax* reproductive ground (Charente / Adour and Loire):

→ More hybrids (10% to 40%), with specific hybridization dynamics (hybrid types).

Shared spawning groups due to:

- Dams / barriers to upstream migration
- Peculiar upstream migration of *A. fallax* in Loire

Historical sex-biased directional hybridization



Mitochondrial haplotypes (maternally inherited) of purebred:

- 12% of *A. alosa* have a fallax-like mt haplotype
- Only 1% of *A. fallax* have an alosa-like mt haplotype

→ Ancient hybridization involving ♀ *A. fallax* X ♂ *A. alosa* followed by recurrent backcrossing with ♂ *A. alosa*

→ Consequences: ancient introgression of genes from *A. fallax* into *A. alosa.*

Mitochondrial haplotypes (maternally inherited) of hybrids:

- As much F1 with alosa-like and fallax-like mt haplotypes
- 5x more 3rd generation hybrids with alosa-like mt haplotypes

→ Recent hybridization involving ♀ *A. alosa* X ♂ *A. fallax* followed by recurrent backcrossing with ♂ *A. alosa*

Conclusion and perspectives

- Hybridization in Atlantic shads: an historical and contemporary processes
- Anthropogenic pressures increase opportunity for hybridization

Consequences for conservation:

- Hybridization is not a threat for the species persistence.
- On the contrary, it might be an evolutionary processes allowing species to adapt to environmental changes (new combination of genes = raw material for selection to act upon).
- Variable hybridization dynamics between catchment area → response to specific environmental context → specific management by river catchment needed

Perspectives

- Monitoring hybridization as an indicator of environmental health or efficient management actions
- Molecular tools usable in the field for quick species and hybrids genetic assignment
- Is hybridization and gene introgression adaptive? Much opportunities for interspecific gene flow, and high variation in phenotypic trait linked to adaptation → a model to study adaptive introgression?

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Variable outcomes of hybridization between declining *Alosa alosa* and *Alosa fallax*

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