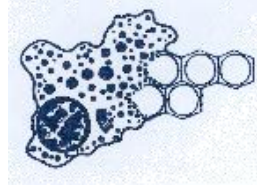


25TH ANNIVERSARY

22nd July 2022



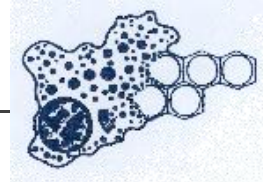
Stressors in marine organisms: understanding exposures and population-level impacts of chemical contamination on fish populations

Anthony Nzioka

Cell Biology in Environmental Toxicology (CBET) consolidated research group
Department of Zoology and Animal Cell Biology
Faculty of Science & Technology
University of the Basque Country (UPV/EHU)

<https://www.ehu.eus/es/web/cellbiologyinenvironmental toxicology/home>

Introduction: CBET+ Research Group



- Develop of **early warning** cell and molecular **biomarkers** of pollution exposure and effects



- **Toxicity testing** of environmental pollutants using standard and novel toxicity tests in cells *in vitro*, algae, invertebrates and vertebrates

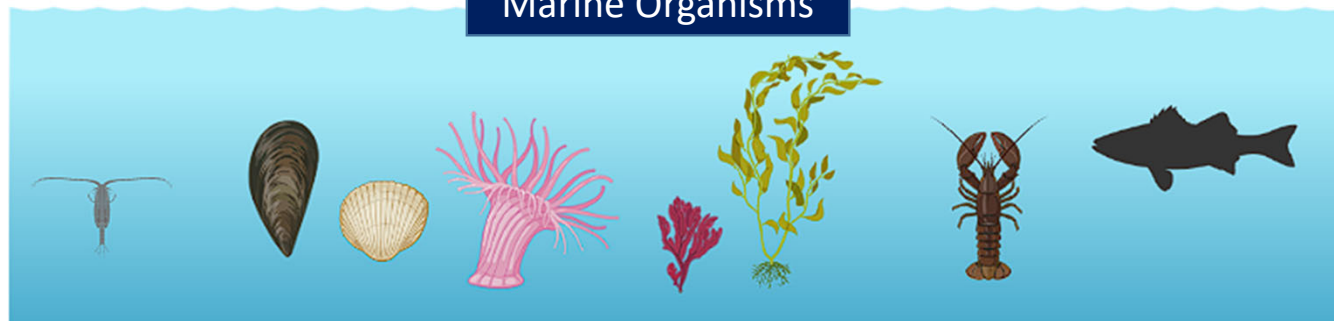
One Ocean, One Health

- Pollution
- Multiple stresses
- Disease
- Animal models

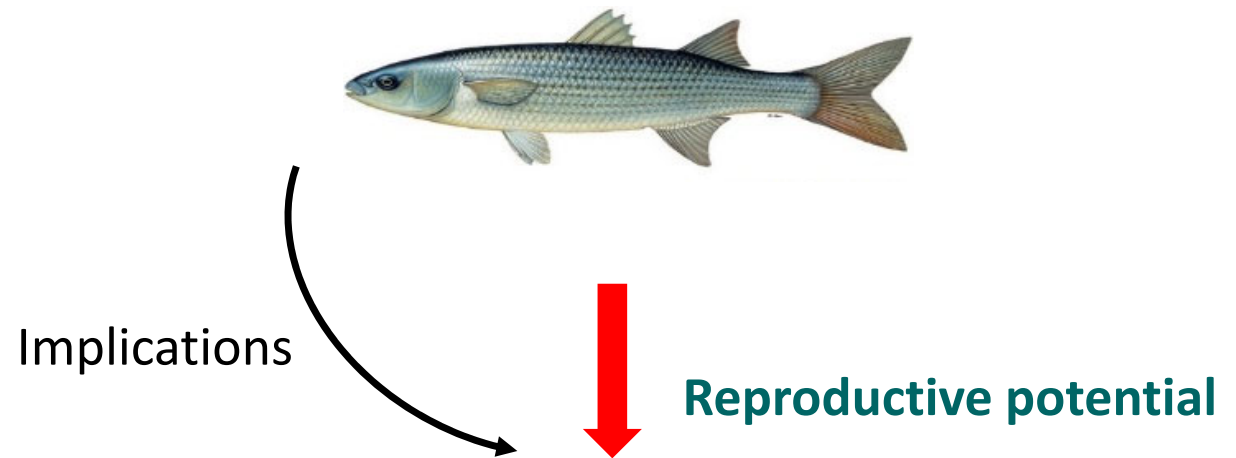
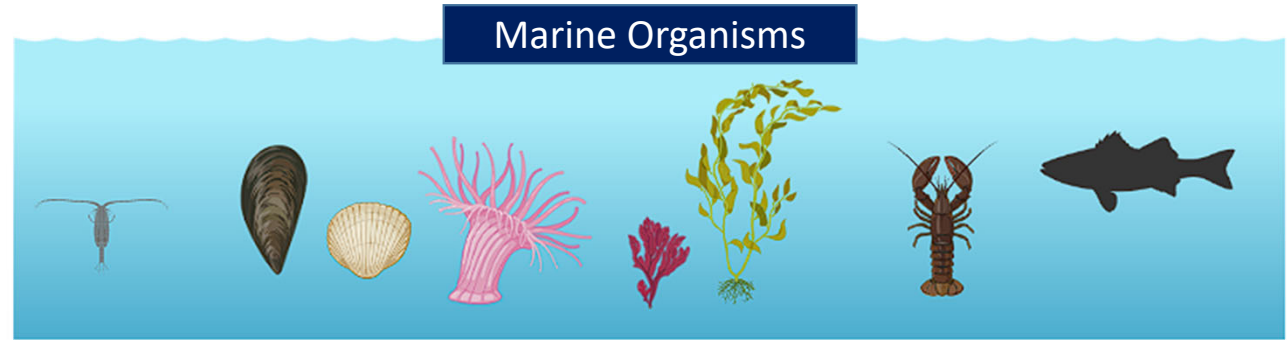
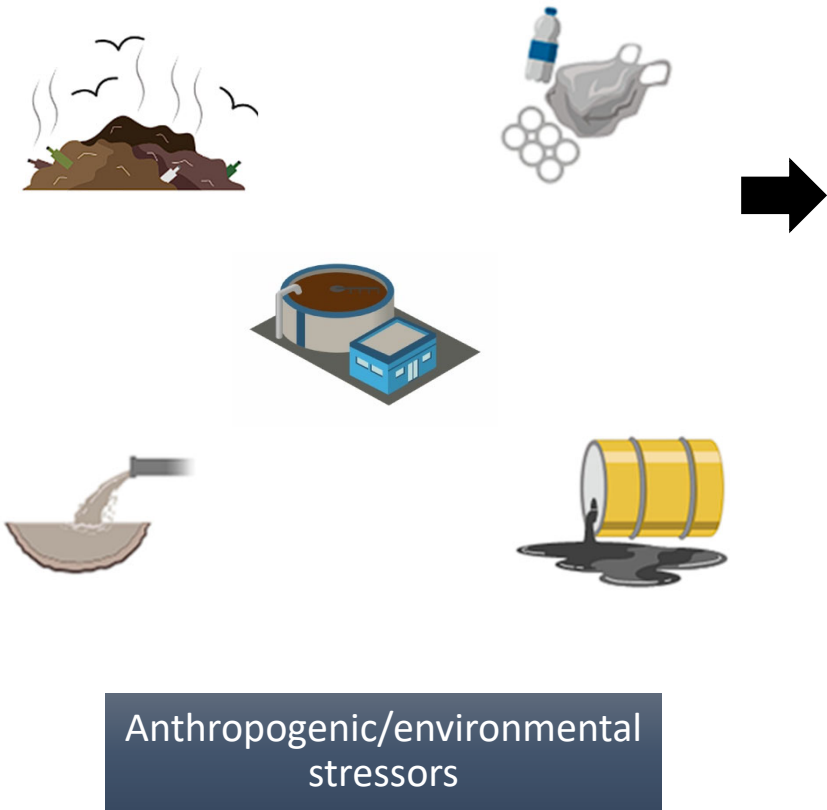
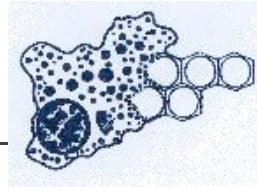
Research lines:

- Ecosystem health assessment
- Effects of chemical contaminants (oil spills, wastewater, etc)
- Endocrine disruption
- Toxicogenomics
- Nanotoxicology

Marine Organisms



Stressors in Marine Organisms

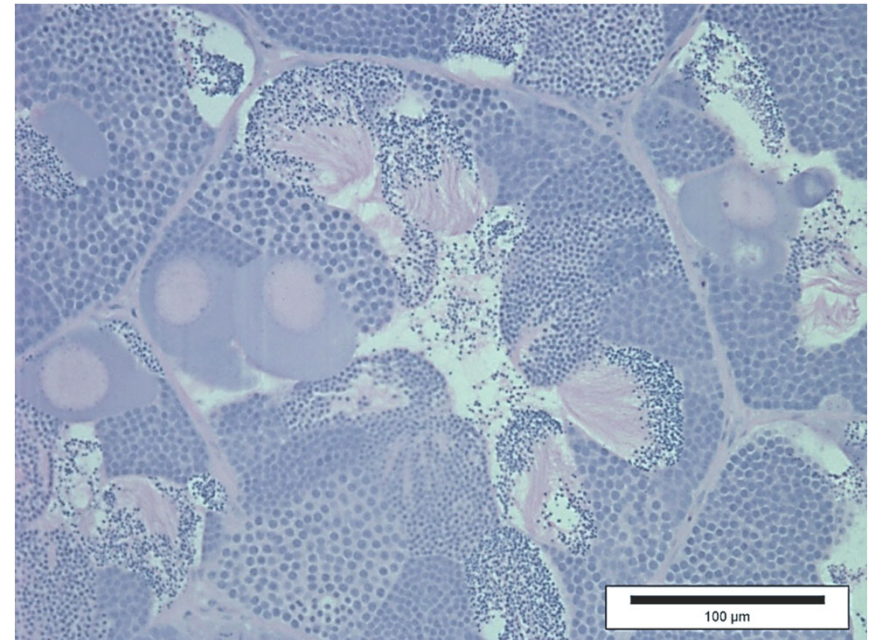


Intersex condition and atresia in fish



- **Intersex condition** – simultaneous presence of testicular and ovarian tissue in gonads of gonochoristic fish species (**ovotestis**).

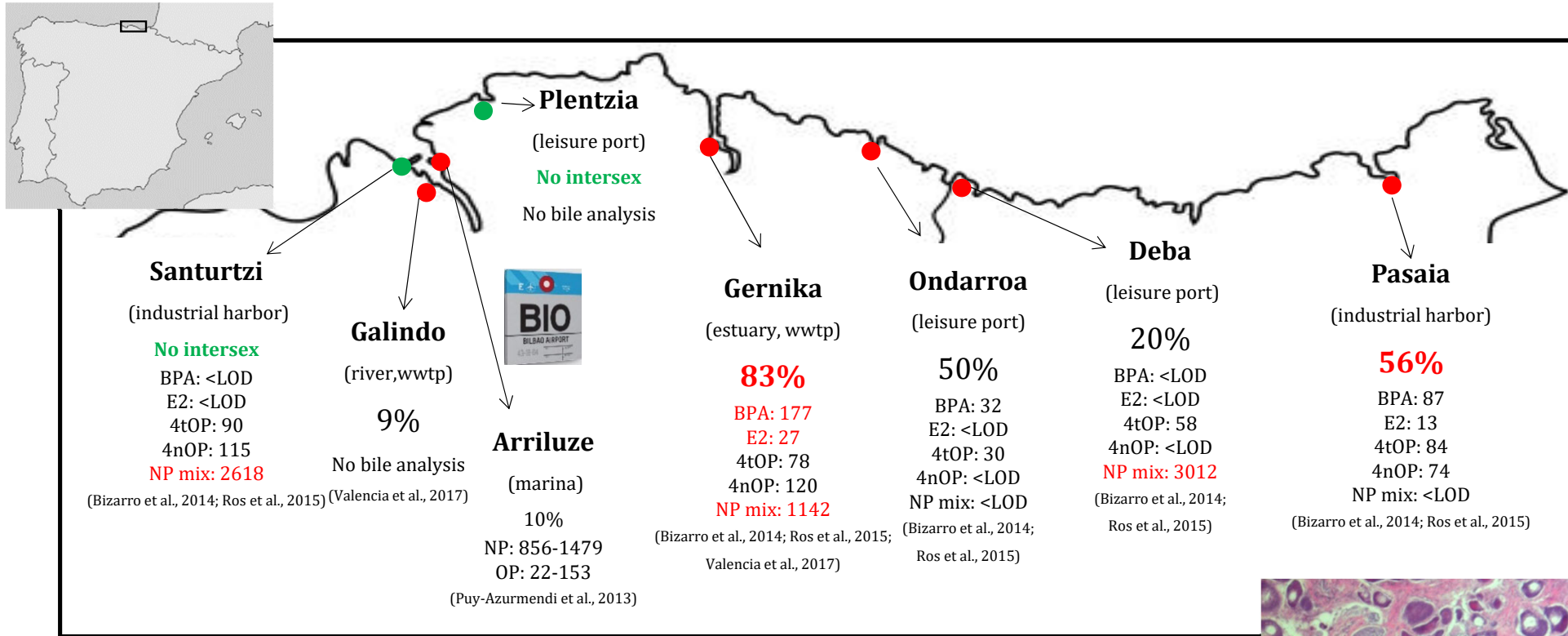
.= Normally occurs in species exposed to **xenoestrogenic EDCs** while living in contaminated habitats.



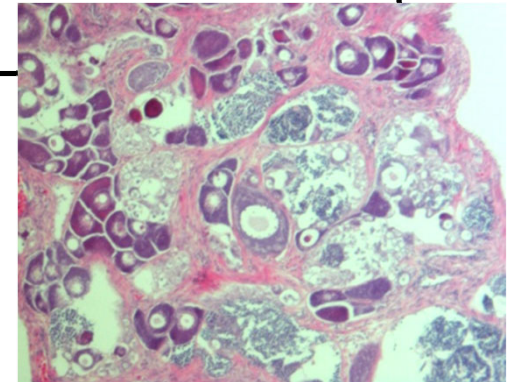
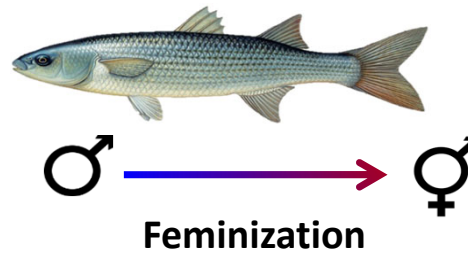
- **Follicular atresia** – degenerative process in which oocyte follicles are reabsorbed (physiological/pathological)

.= **Triggered** as a result of **environmental stress** (contaminants, starvation, etc) when environmental conditions for spawning are suboptimal.

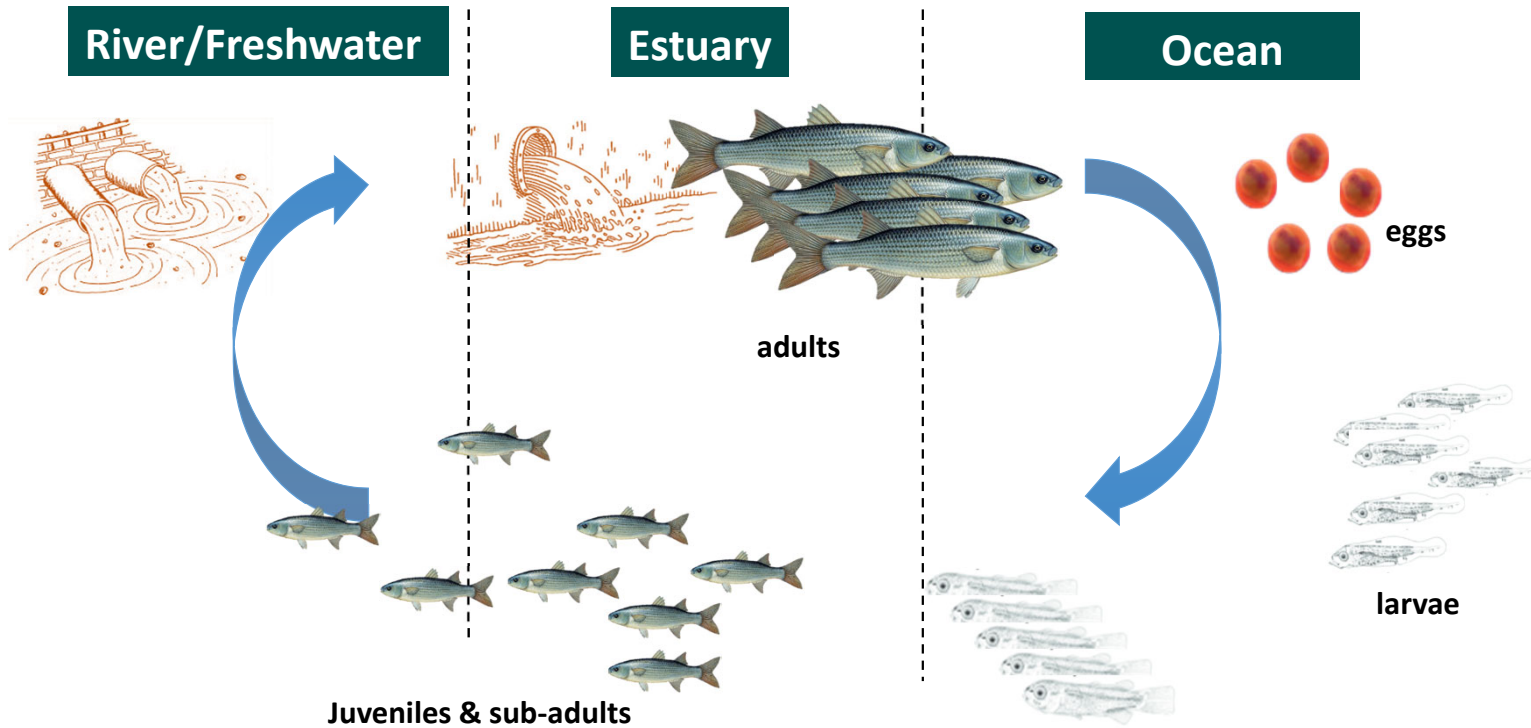
Xenoestrogenic effects in the Basque coast



High incidence of **intersex** condition of *Chelon labrosus* in Basque **estuaries**



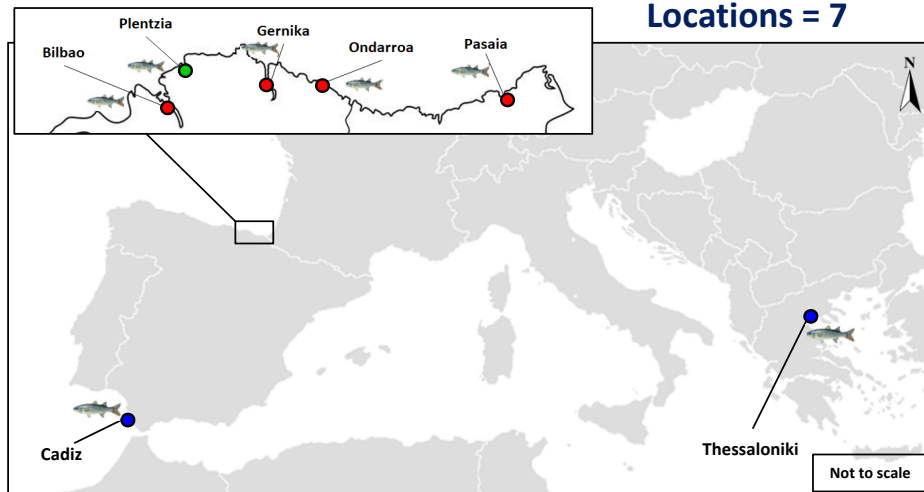
Life history of thicklip grey mullets: Catadromy



Implications in their history of xenoestrogenic exposure and development of intersex condition

Are newly hatched larvae recruited into estuaries for life, or do the adults move between estuaries after each reproductive migration?

Study area and microsatellite analysis

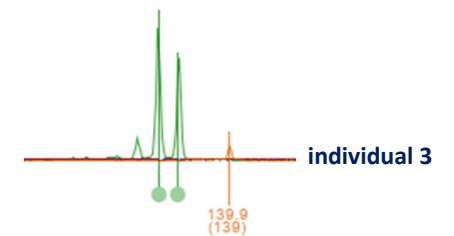
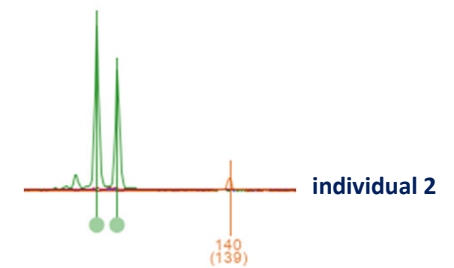
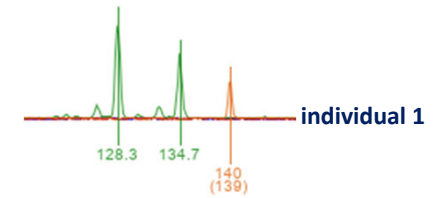


Microsat Amplification (Multiplex PCR)

Flourescent marked primers!!!



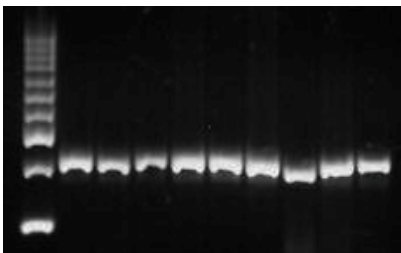
Sequence Analysis (reads base pairs!!!)



Genotyping



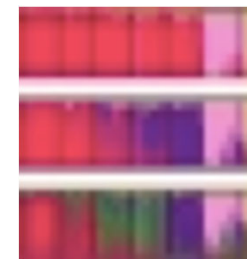
DNA extraction,
amplification (PCR),
quality checks



Primer Design

Reference sequences
(46 microsatellite loci
screened)

15 microsatellite loci
selected
(≥ 6 microsatellites)



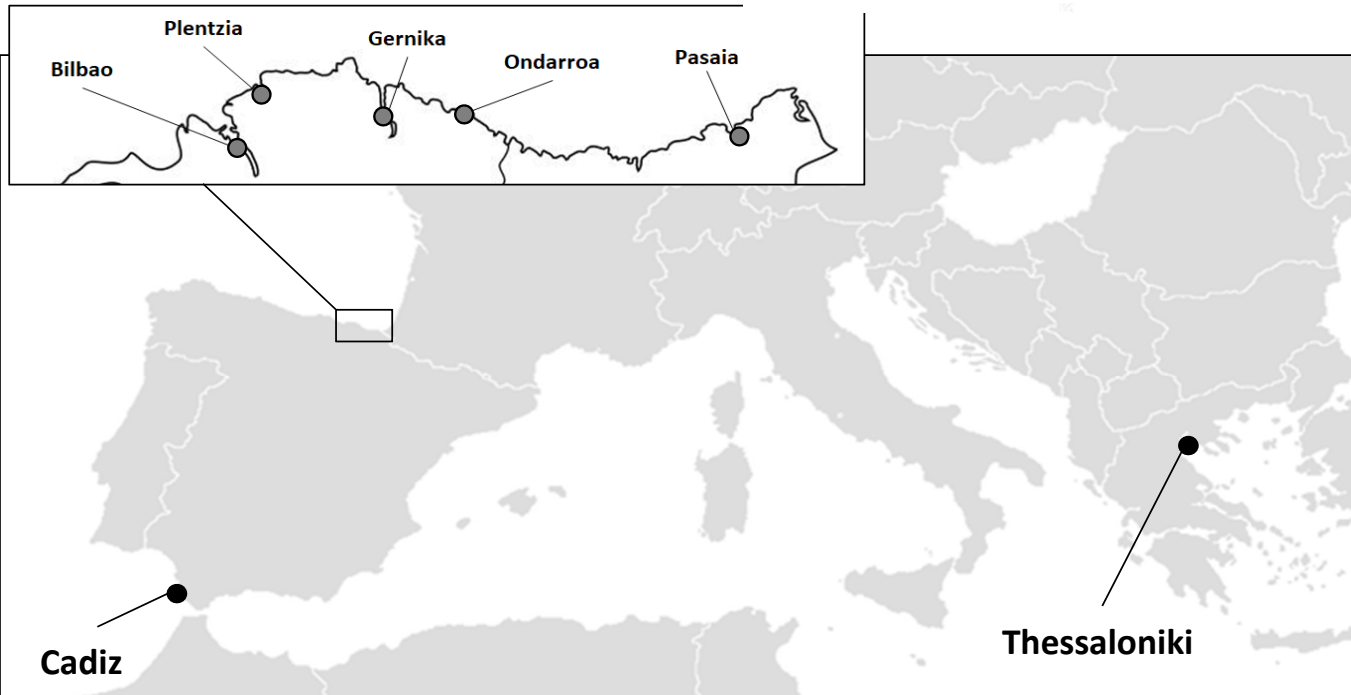
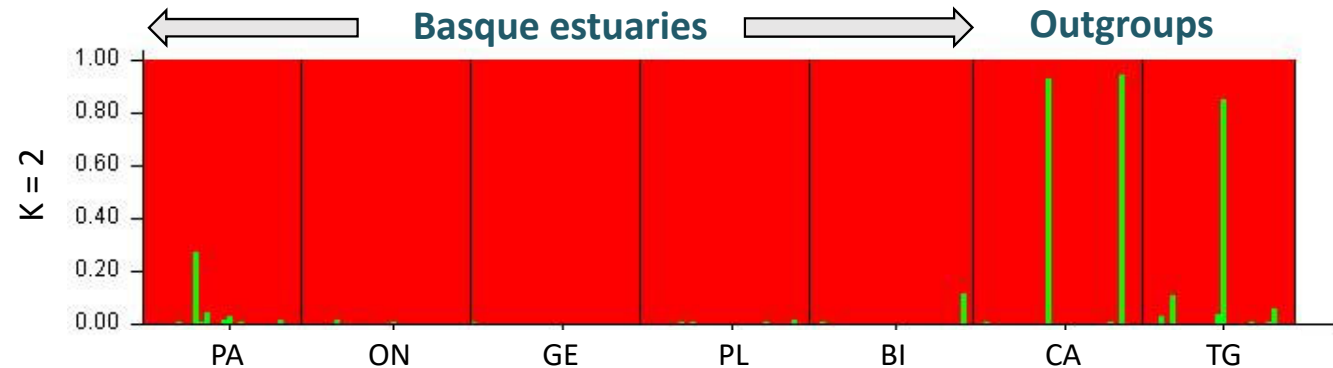
Bayesian Clustering Analysis:
Structure 2.3.4

Genetic structure in mullets from Basque estuaries



KEY:

PA = Pasaia
ON = Ondarroa
GE = Gernika
PL = Plentzia
BI = Bilbao
CA = Cadiz
TG = Thessaloniki Gulf



STRUCTURE plots for *Chelon labrosus* data set using admixture model with LOCPRIOR.

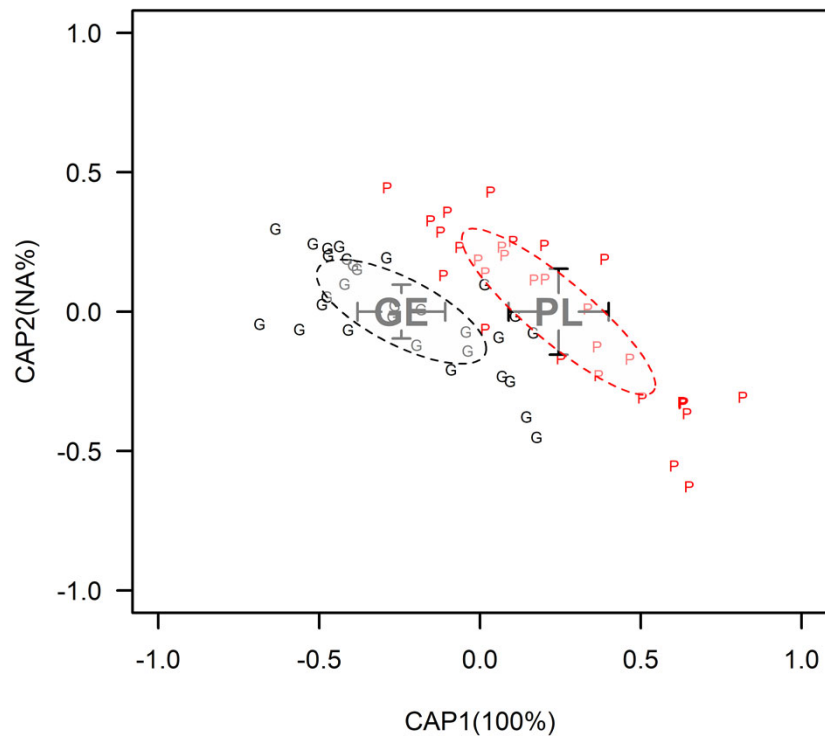
Between the Atlantic Ocean and the Mediterranean Sea

- Admixture observed with no cluster formed by the populations
- No evidence genetic structuring/differentiation
- No evidence of restricted gene flow

Mullet otolith shape analysis GE vs PL



Canonical clustering



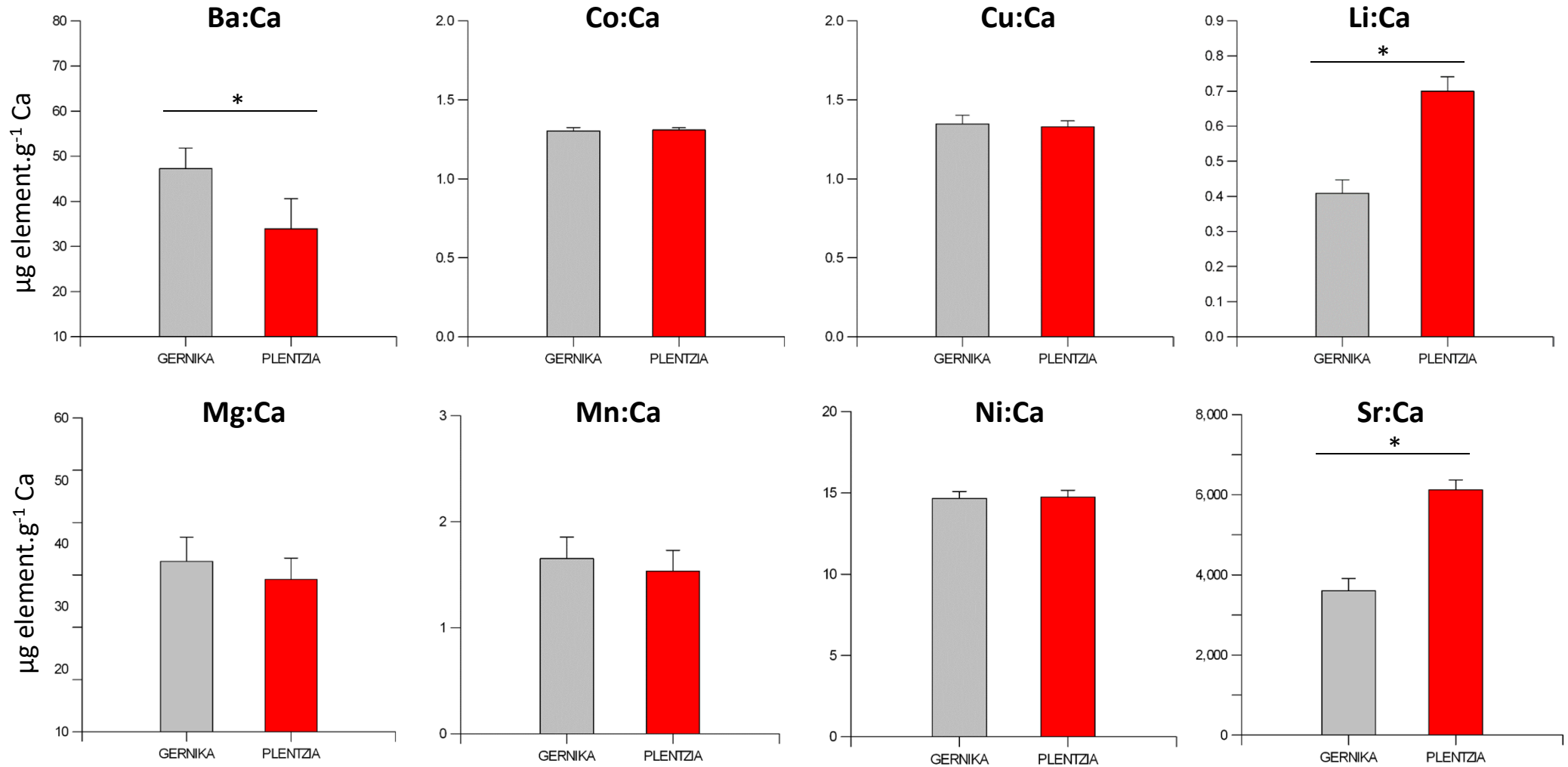
Canonical analysis of CAP plots for otolith shape signatures GE vs PL. Ellipses represent 95% confidence intervals. Each point represents an individual fish.

Stepwise discriminant linear function (SLDFA)

Original Location	N	Predicted Location		% correct
		Gernika	Plentzia	
Shape Analysis				
Gernika	30	20	10	67
Plentzia	30	5	26	83
Total	60	30	30	75
Elemental Signatures				
Gernika	30	28	2	93
Plentzia	30	1	29	97
Total	60	29	31	95
Shape & Elemental Signatures combined				
Gernika	30	28	2	93
Plentzia	30	0	30	100
Total	60	30	30	97

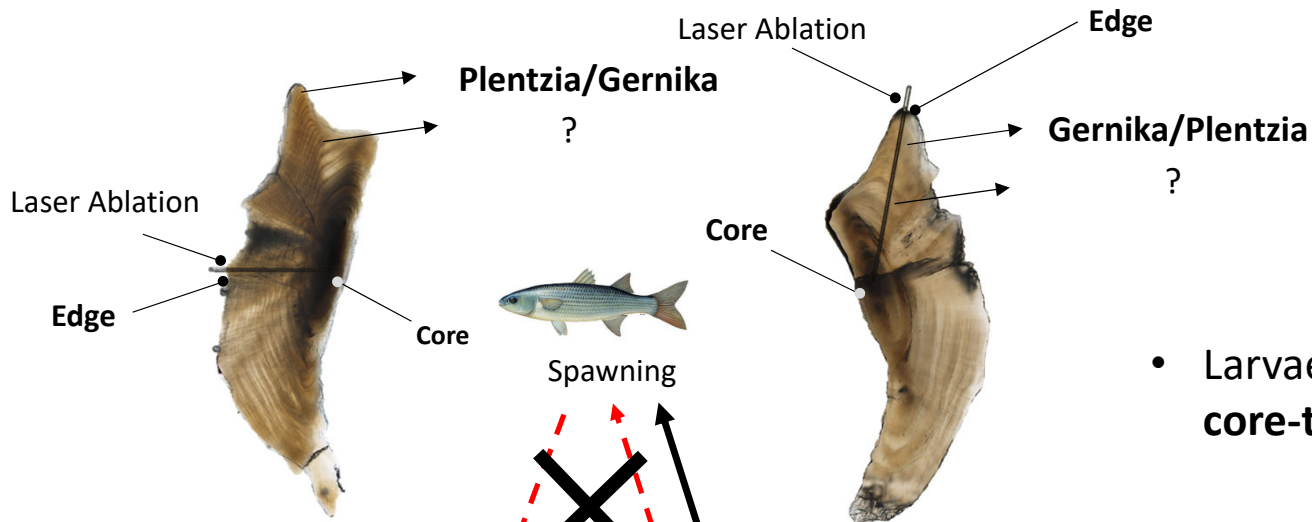
- Otolith shape analysis showed significant variation among the two locations, Gernika and Plentzia
- Suggests that there is more than one local population unit along the Basque coast and that mullets remain attached to one estuary

Mullet elemental analysis GE vs PL



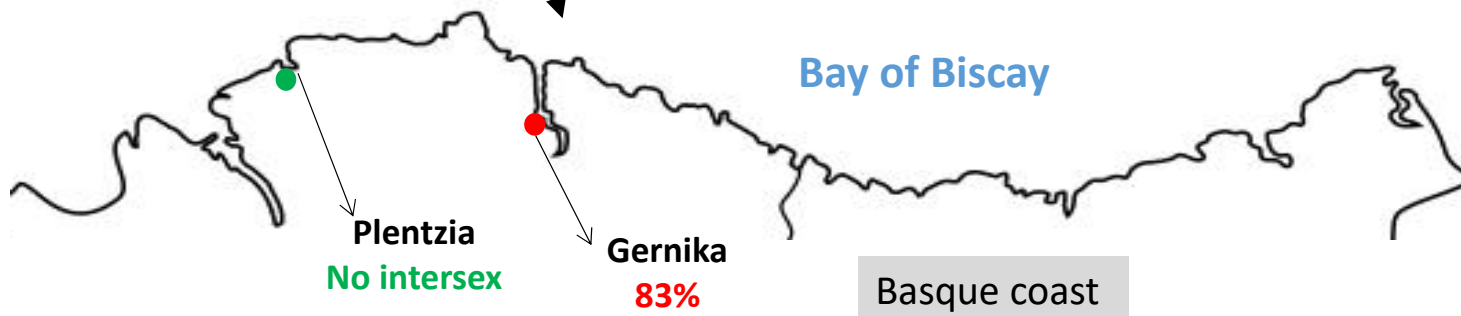
Element:Ca ratios (Mean \pm SE) in whole otoliths of *C. Labrosus* (Gernika and Plentzia). Unit of measurement $\mu\text{g element g}^{-1} \text{Ca}$. Asterisk indicate statistically significant different results ($p < 0.05$).

Otolith core-to-edge elemental signatures GE vs PL



- Larvae to adult life history can be observed from **core-to-edge elemental composition (LA-ICPMS)**

Similar core chemical composition = **homogeneous marine environment**



Finalizing Results & Data Analysis

Highlights



- Microsatellite analysis points towards **genetic homogeneity** and **panmixia**, suggesting the **existence of significant gene flow** among the mullet sub-populations in the SBB. This does not rule out the possibility of adult mullets always return to their estuary of origin after reproductive migration to the sea.
- Otolith shape and chemical composition showed **significant differences** between mullets of Gernika and Plentzia suggesting that individuals from both estuaries passed enough time geographically separated and could be regarded as **two different population units**.
- This fits the hypothesis that **gonad alterations in mullets** develop due to **exposure to xenoestrogens** along their life history and **after young-age homing in a polluted estuary**.

Thank you

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Barrio Sarriena s/n
48940 Leioa - Bizkaia