

# Tracing glass eels by multi-element pattern and strontium isotopes

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10 Years of the Sustainable Eel Group. Where next for eel protection? Natural History Museum June 27 – 28, 2019, London UK



Federal Ministry Republic of Austria Digital and Economic Affairs  Federal Ministry Republic of Austria Transport, Innovation and Technology







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### Project idea & objectives

#### Identify potential chemical markers for glass eel traceability

- Glass eel samples from different sites, caught at river mouths
- Multi-element pattern and Sr isotopes by (MC)-ICP-MS in
  - water samples (n=3 per site, in river)
  - digested eel bodies (prior half, after otolith extraction n=45 per site)
  - digested eel otoliths (n=5 per site)
  - LA-ICP-MS line scans across otoliths (n=45/50 per site)



- Stable isotope ratios d<sup>13</sup>C, d<sup>15</sup>N d<sup>34</sup>S in
  - eel bodies (posterior half, n=50 per site)
- Non-targeted metabolomics
  - "add on" exploration (n=5 per site)

### Sampling sites & sample types

- Orio, Spain
- Vilaine, F
- Parret, UK
- Severn, UK
- Planned: samples
   from Marocco

- Water samples (n=3 per site)
- Eel bodies & otoliths (n=50 per site)



# Analytical methodology – ICP-MS (after sample preparation)



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### Discrimination of eel bodies by multi-element fingerprint

Stepwise discriminant analysis

- 75.8 % correct classification by

- Sr/Ca
- Rb/Ca
- S/Ca
- Mg/Ca
- Sr/Ba



#### Classification Results<sup>a</sup>

|          |       | Predicted Group Membership |       |      |      |      |       |  |  |  |
|----------|-------|----------------------------|-------|------|------|------|-------|--|--|--|
|          |       | ggroup                     | ORI   | PAR  | SEV  | VIL  | Total |  |  |  |
| Original | Count | ORI                        | 37    | 0    | 0    | Ó    | 37    |  |  |  |
|          |       | PAR                        | 0     | 32   | 17   | 0    | 49    |  |  |  |
|          |       | SEV                        | 0     | 8    | 30   | 10   | 48    |  |  |  |
|          |       | VIL                        | 0     | 1    | 7    | 36   | 44    |  |  |  |
|          | %     | ORI                        | 100,0 | ,0   | 0,   | ,Ö   | 100,0 |  |  |  |
|          |       | PAR                        | ,0    | 65,3 | 34,7 | 0,   | 100,0 |  |  |  |
|          |       | SEV                        | ,0    | 16,7 | 62,5 | 20,8 | 100,0 |  |  |  |
|          |       | VIL                        | 0,    | 2,3  | 15,9 | 81,8 | 100,0 |  |  |  |

a. 75,8% of original grouped cases correctly classified.

### Main elemental discriminators between sites eel bodies vs. water



### Discrimination of eel bodies by multi-element fingerprint & <sup>87</sup>Sr/<sup>86</sup>Sr isotope ratios (initial results)

Stepwise discriminant analysis – 91.4 % correct classification by

- Sr/Ca
- Rb/Ca
- S/Ca
- Mg/Ca
- Sr/Ba
- <sup>87</sup>Sr/<sup>86</sup>Sr isotope ratios

French, UK and Spanish samples differentiated by almost 99 %!



#### Classification Results<sup>a</sup>

|          |       | Predicted Group Membership |       |      |      |      |       |  |  |
|----------|-------|----------------------------|-------|------|------|------|-------|--|--|
|          |       | gr                         | ORI   | PAR  | SEV  | VIL  | Total |  |  |
| Original | Count | ORI                        | 26    | Ó    | 0    | 0    | 26    |  |  |
|          |       | PAR                        | O     | 33   | 1    | 1    | 35    |  |  |
|          |       | SEV                        | Û     | 4    | 3    | 0    | 7     |  |  |
|          |       | VIL                        | 0     | 1    | 0    | 12   | 13    |  |  |
|          | %     | ORI                        | 100,0 | ,Ö   | ,0   | ,0   | 100,0 |  |  |
|          |       | PAR                        | ,0    | 94,3 | 2,9  | 2,9  | 100,0 |  |  |
|          |       | SEV                        | 0,    | 57,1 | 42,9 | 0,   | 100,0 |  |  |
|          |       | VIL                        | 0,    | 7,7  | 0,   | 92,3 | 100,0 |  |  |

a. 91,4% of original grouped cases correctly classified.

<sup>87</sup>Sr/<sup>86</sup>Sr isotope ratio in eel bodies vs. water

Slight increase in <sup>87</sup>Sr/<sup>86</sup>Sr isotope ratio in Vilaine eels (but only a limited number of samples analysed so far).



Stable isotope approach

Carbon  $(\delta^{13}C)$ 

Nitrogen  $(\delta^{15}N)$ 

Sulfur  $(\delta^{34}S)$ 

Phytoplankton type Local carbon sources Local nitrogen source Sewage / fertiliser input

Sediment Anoxia





Earth Science

### Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IR-MS)

- 1. Eel body (lower half) frozen
- 2. Ca. 1 cm portion removed
- 3. Dried, ground, ca. 3.0 mg weighed
- 4. Analysed by EA-IR-MS







#### Stable isotope results



- d<sup>13</sup>C and d<sup>15</sup>N values remarkably consistent among the four sites -> muscle tissue synthesised from a relatively consistent diet source.
- d<sup>34</sup>S values differed among regions, with eels from Orio having lower d<sup>34</sup>S values -> incorporation of sulfur from anoxic / wetland sediments?

#### Stable isotope results

Eels from Orio had lowest d<sup>34</sup>S values



# Non-targeted metabolomics (molecular fingerprinting)



# Non-targeted metabolomics (separation of molecules with different m/z ratio)



RT Abund m/z ... ... ... .... ... ... 2.11 195,1745 21000 2.11 257 2566 33550 234 224,2134 11784 .... .... .... ... .... ....

Compound List

List of molecular features with retention time, accurate mass of all aligned features and intensity

### Multivariate data evaluation

#### Unsupervised methods (pattern recognition)

• Principal Component Analysis (PCA)



#### Supervised methods (examine structure)

 Discriminant analysis (Orthogonal Projections to Latent Structures-Discriminant Analysis (OPLS-DA); marker identification and prediction)



#### Unsupervised - PCA

- Indication for discrimination of Spanish glass eels (blue) from the rest (in both, positive and negative mode).
- More pronounced clustering and significantly better differentiation amongst sample groups was obtained for negative ionisation data.



Supervised – OPLS-DA (Orthogonal partial least squares discriminant analysis)

- Positive ionization mode metabolite data prediction rate ullet-> 67.1% (R2 = 0.794; Q2 = 0.671)
- Negative ionization metabolite data prediction rate • ->72.3% (R2 = 0.832; Q2 = 0.723)



Positive

#### **Conclusion & recommendation**

- 1. Overall the combined data showed that in order to provide geographic discrimination among glass eels sampled recently on entry into river systems, tracers must ideally be (1) rapidly introduced into the tissue under study and (2) must differ widely among sites in order to yield sufficient signal with short times available for uptake.
- Elemental ratios (Sr/Ca, Rb/Ca, S/Ca, Mg/Ca and Sr/Ba) and stable isotopic patterns (d<sup>13</sup>C, d<sup>15</sup>N, d<sup>34</sup>S, <sup>87</sup>Sr/<sup>86</sup>Sr) of eel bodies together with metabolomic fingerprints should be further explored in combination for the geographic differentiation of glass eels.
- Reference data specific to the year and estuary in question are likely to be required for any applied assignment.

#### Contact details

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