

Mapping threatened species with the eDNA method

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≪fresh e-Workshop First application of eDNA to map threatened native species in Greek freshwaters (project RESILIENT)

Target species: Valencia letourneuxi & Valencia robertae



OBJECTIVE

Assessment of the current population status of the threatened Greek killifishes Valencia letourneuxi and Valencia robertae

using BOTH conventional fish sampling methods and eDNA sampling



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Methodology – Fish sampling network

- 15-day survey → 36 locations in Western Greece (October 2018)
- Fish sampling → 27 sites with historical or suspected presence of Valencia spp. and
- Fish sampling → at 1 site outside its native range (to be used as outlier for eDNA analysis)
- Total sites sampled with electrofishing or netting: 28 sites



Map with sites surveyed/not sampled (red dots) in Western Greece and sites where fish sampling was conducted (blue dots)

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Methodology – Conventional fish sampling

- Modified Point-Abundance Sampling by Electrofishing (PASE) or netting
- HCMR protocols used (IMBRIW, 2013)





IMBRIW (2013). INLAND WATERS FISH MONITORING OPERATIONS MANUAL: ELECTROFISHING HEALTH AND SAFETY / HCMR RAPID FISH SAMPLING PROTOCOL. Hellenic Centre for Marine Research - HCMR Special Publication,

Methodology – Water sample collection for eDNA

- Water sampling was conducted at **20** sites (all fished)
- Volume: two 1 L samples per location
- Several subsamples across the river width
- Filtering with 0.45 µm Sterivex[™] HV filter
- Fixation with ethanol
- At room temperature until shipment



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detection

Electrofishing and eDNA results for *V. letourneuxi* at locations sampled in W. Greece during the 2018 autumn survey



Electrofishing and eDNA results for *V. robertae* at locations sampled in W. Greece during the 2018 autumn survey

Conclusions

At 7 sites, Valencia spp. <u>was detected through eDNA but NOT through</u>
 <u>fish sampling</u>, → low densities, indicating the suitability of the eDNA method for species detection

 At 2 sites, Valencia spp. <u>was detected through fish sampling</u> but NOT through eDNA, → limitations of the method (water volume, flow and/or turbidity)

• At 1 stream, at the border of the two species expansion ranges both *Valencia letourneuxi* (weak signal) and *Valencia robertae* (strong signal). Due to the higher strength of the *V. robertae* signal, we attribute this population to this species

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Targeting two more range restricted, threatened species (project PACIM)

Target species: Evia barbel *Barbus euboicus* & the Greek stickleback *Pungitius hellenicus*



OBJECTIVE

Assessment of the current population status and range of the threatened Evia barbel *Barbus euboicus* & the Greek stickleback *Pungitius hellenicus*

using BOTH conventional fish sampling methods and eDNA sampling









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Methodology – Sampling network for B. euboicus



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- Field work was conducted in August 2019 and January 2022, using both conventional electrofishing and eDNA sampling
- Sampling conducted in six basins in Evia Island and in the Sperchios basin in Central Greece (total 15 sites)

Methodology – Sampling network for *P. hellenicus*

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- Field work was conducted in August 2019 and January 2022, using both conventional electrofishing and eDNA sampling
- Sampling conducted in in the Sperchios basin in Central Greece

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⁵ Electrofishing and eDNA results for *B. euboicus* and *B. sperhiensis* at locations sampled in Evia & C. Greece

Barbus euboicus

2. Results *B. euboicus*



Results B. euboicus

- The known range of the Evia Barbel on Evia island expanded (confirmed presence in five basins)
- In a single river there was also a positive eDNA signal for *Barbus sperchiensis*
- All Sperchios sites tested **negative** for *B. euboicus* with eDNA (positive for *B. sperchiensis*)

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CR

Results P. hellenicus (preliminary)

- Detected through eDNA at five sites and though fish sampling at two sites in the Sperchios basin (preliminary)
- Analyses of 2022 samples are continued





SITE NAME	Sample for eDNA
Agios Dimitrios	YES
Agia Paraskevi	YES
Paliouri	YES
Mexiates	YES
Ioli 2	YES

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Widening the scope (several threatened native species) (project AFRESH)

Target species:

- Salmo peristericus --- Prespa trout
- Phoxinus strymonicus --- Aegean minnow
- Alburnus vistonicus --- Vistonida shemaya
- Alburnus macedonicus --- Doiran bleak
- Knipowitschia thesalla --- Thessaly goby
- Telestes beoticus --- Boeotian riffle dace

CR EN

OBJECTIVE

To provide data on the current status of six threatened freshwater species of Greece



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METHODOLOGY

IUCN Red List Status: out of 34 fish species (CR, EN) located in Greece

we selected 6 species since:

a) some species had been previously investigated (e.g. *Pelasgus* spp, *Valencia* spp)
b) absence of type locality specimens (e.g. *C. stephanidisi*, *S. moreoticus*, sturgeons)
c) Issues we co-occurrence with other species of the same genus (e.g. *Alburnus* in Volvi L.)

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METHODOLOGY

- Field work was conducted October-November 2021, using both conventional electrofishing and eDNA sampling
- Sampling conducted in 13 basins of Greece located in Central and Northern Greece (total 15 sites)





Sampling sites (total 15) in 13 basins, targeting six range-restricted threatened native species

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Fishing

Positive









Results

Τίρανα Tiranë



A. vistonicus CR

According to Barbieri et al.

Alburnus vistonicus Freyhof & Kottelat, 2007 ENDEMIC

Aλάïa, Vistonis shemaja+

Endemic to Lake Vistonis basin, including Kossinthos and Kompsatos rivers. The populations from the adjacent Filiouris and Vosvozis river basins probably belong to this species. A lacustrine species that migrates to the upper reaches of stream tributaries to spawn in riffles with strong currents. Dams and weirs block its upward migration, endangering localized populations. In the Vistonis basin, populations have sharply declined particularly due to anthropogenic salinity changes in the lake and are

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Unexpected Results



Results



P. strymonicus Unexpected finding

Recorded at Aliakmon R. & Nestos R.

EN

According to Sanda et al. Aliakmon R. \rightarrow *P. lumaireul* Filiouris R. \rightarrow *P. strandjae*



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Future perspectives

- Expand to other threatened freshwater fish species (i.e., usage of museum samples for genetic studies)
- Standardization of e-DNA protocols for regular monitoring

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Thank you for your attention



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