Mapping **threatened** & **invasive** species with the eDNA method

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afresh
Bristol Workshop

December 2nd, 2022

Outline

1st PART

Threatened species

- Application of e-DNA for mapping threatened species in Greek freshwater ecosystems

2nd PART

Invasive species

- Application of e-DNA for mapping invasive (top invaders) species in Greek freshwater ecosystems

…during past projects (RESILIENT, PACIM) and the AFRESH project

First application of eDNA to map threatened native species in Greek freshwaters (project RESILIENT)

Target species: *Valencia letourneuxi* & *Valencia robertae*

CR

OBJECTIVE

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

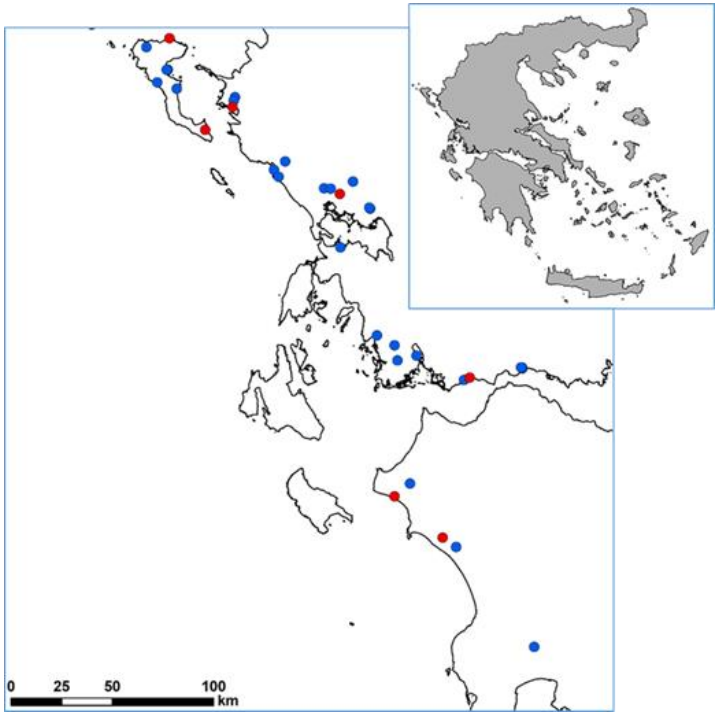
using ***BOTH*** conventional fish sampling methods and eDNA sampling

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 one site outside its native range (to be used as outlier for eDNA analysis)

Total sites sampled with electrofishing or netting:

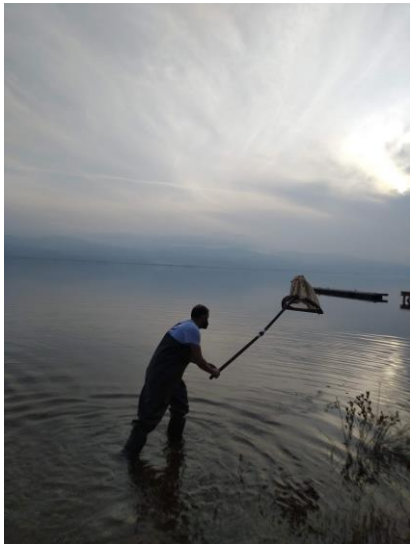
28 sites



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

Methodology – Conventional fish sampling

- Electrofishing or netting
- HCMR protocols used



HCMR // Rapid Ichthyo-Assessment Protocol

1. Researcher:	**2. Fisher:**	**3. Completed by:**
4. Sampling Site: (Name / Code)		**5. Date:**
6. Hydrographic Basin:	**7. Course:**	
8. Location Description: (nearest village, distance from bridges, etc. ...)		**9. Reference site** Yes ☐ Near ☐ No ☐ estim. on site
10. GPS Coordinates	**11. Time:** Start: Finish:	**12. Altitude:** **13. Slope:**
14. Sampling Equipment: equipment type: ... ... manufacturer: ... ... electricity: DC ☐, PDC ☐, other: ... ... mean Volt: ... ... , mean frequency:	**15. Sampling Effort:** A B C D comments: ... ... * if "C": sampling data will be used under provision, "if "D": sampling data will be considered incomplete, or qualitative, * regarding sampling conditions e.g. equipment efficiency, complete habitat cover, difficulties due to flow regime, deep water, turbidity that reduce fishing efficiency etc	
16. Sampling strategy: a) whole ☐ partial whole ☐ one bank ☐ ambient ☐ other: ... ... b) wading ☐ boat ☐ wading+boat ☐ other:		
17. Fished length (m):	**18. Fished area** (m²):	**19. Flow regime:** Permanent ☐ Intermittent ☐ Ephemeral ☐

20. SITE DIMENSIONS

Width (m)	
Wetted width	
Left bank up to water	
Right bank up to water	

* mean of the site

21. WIDTH (m)

<1	%
1≤L<5	%
5≤L<10	%
10≤L<20	%
≥20	%

* in braided channels refer to the sampling area

22. DEPTH (m)

	sampling area	site area
<0,25	%	%
0,25≤ P <0,5	%	%
0,5≤ P <1	%	%
≥1	%	%
	Mean: (m)	Mean: (m)
	Max: (m)	Max: (m)

23. SUBSTRATE (%)

Rock continuous		Sand <2mm	
Boulder >256mm		Silt	
Cobble 64-256mm		Clay	
Pebble 16-64mm		Organic	
Gravel 2-16mm		Artificial	

* reference to the sampling area

24. SHADEDNESS (%)

* canopy cover over sampling area

25. WEATHER

Sunny ☐ Cloudy ☐ Rainy ☐ other: ... ... * prevailing conditions of the last few days

26.VELOCITY (m/sec)

< 0,1	
0,1 - 0,25	
0,25 - 0,5	
0,5 -0,75	
0,75 -1	
> 1	

* estim. mean velocity

27. PHYSICOCHEMICAL MEASUREMENTS

Conductivity (mS/m)		T° of air (°C)	
Diss.Oxygen		T° of water (°C)	
pH		Salinity	

Turbidity:clear ☐ slight turbid (>1m) ☐ turbid (<1m) ☐ very turbid ☐

28. HELOPHYTES

Missing	
Isolated Rare	
Sparce	
Intermidiate	
Rich	

Dominating sp.:

29.BOTTOM VEGETATION

Missing	
Sparce	
Intermidiate	
Rich	

Dominating:

30. HABITAT TYPE (%)

Pool (deep/still)	
Glide (shallow/move)	
Run (deep/move)	
Riffle (shallow/rough)	
Rapid (steps/fast)	
Other.....	

*reference to sampling area

31.Important Pressures:

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,

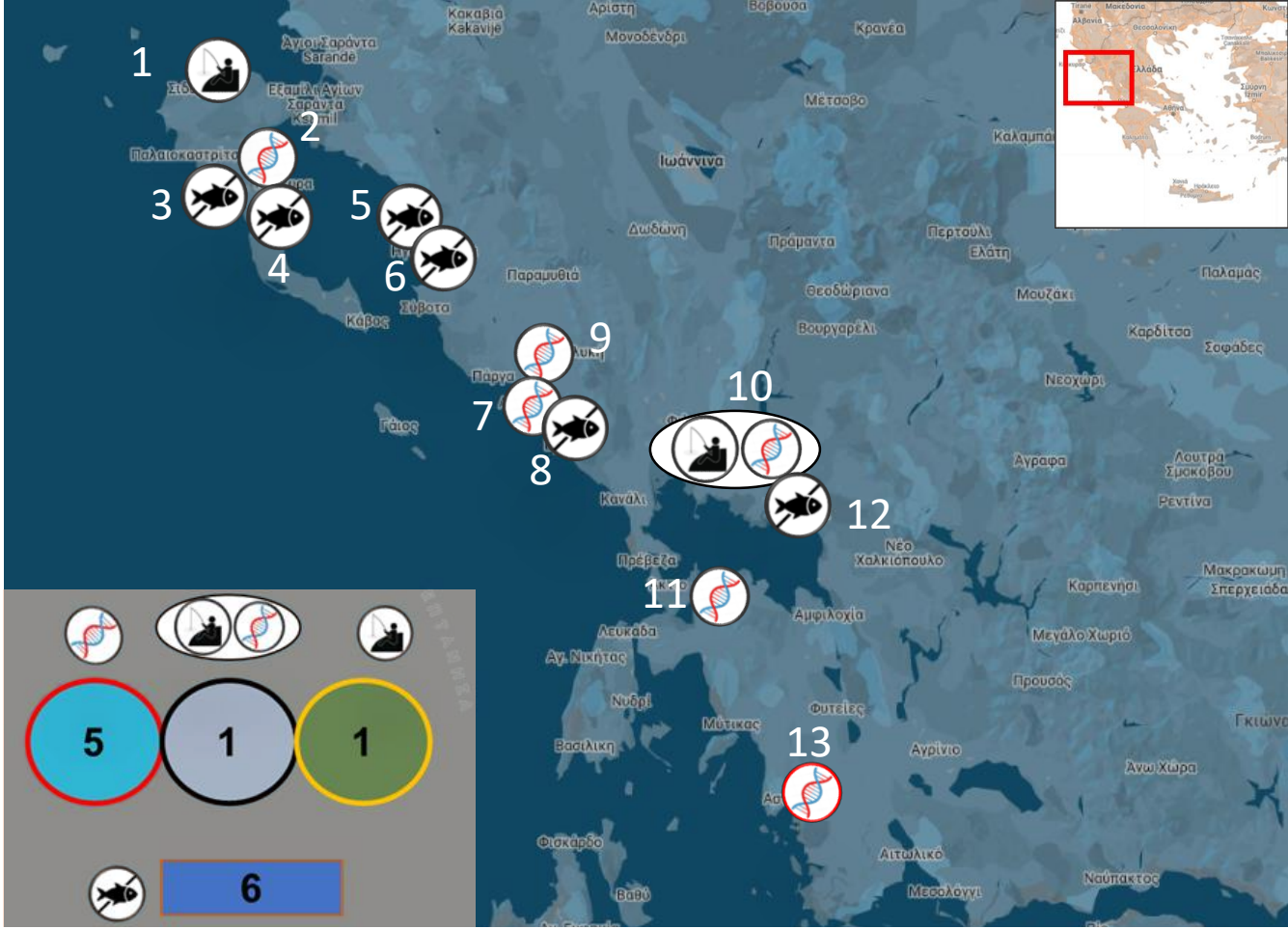
Methodology – Water sample collection for eDNA

- 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

Results

V. letourneuxi

- Fishing detection
- e DNA detection
- No fishing/eDNA detection

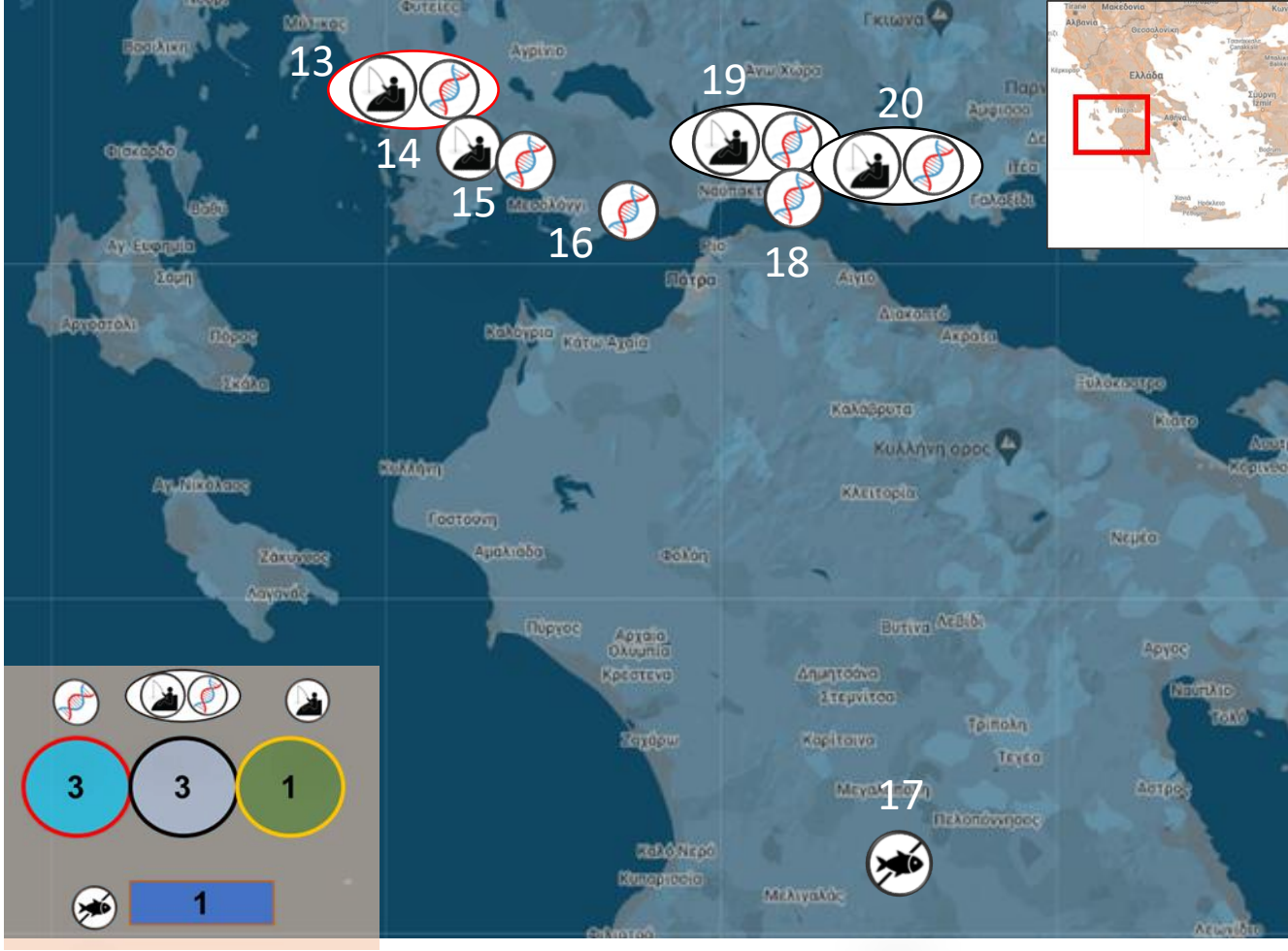


Fish sampling and eDNA results for *V. letourneuxi* at locations sampled in W. Greece during the 2018 autumn survey

Results

V. robertae

- Fishing detection
- e DNA detection
- No detection



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

Conclusions

• At 7 sites, *Valencia* spp **were detected by using eDNA** but **NOT** through conventional fish sampling methods

eDNA → effective in low densities of the targeted species

• At 2 sites, *Valencia* spp. **was detected through fish sampling** but **NOT** through eDNA (pseudonegatives)

limitations of eDNA → large volume of water, or flow or turbidity

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

Target species: Evia barbel *Barbus euboicus*

&

the Greek stickleback *Pungitius hellenicus*

CR

OBJECTIVE

Assessment of the current population status and range of the threatened Evia barbel *Barbus euboicus* & the Greek stickleback *Pungitius hellenicus* *using* ***BOTH*** *c*onventional fish sampling methods and eDNA sampling

hcmr ΕΛΚΕΘΕ

Bristol Zoological Society

UWE Bristol | University of the West of England

Methodology – Sampling network for *B. euboicus*

- Field work was conducted in August 2019 and January 2022
- Sampling conducted in six basins in Evia Island and in the Sperchios basin in Central Greece (total 15 sites)
- Same methods for fish and e-DNA sampling

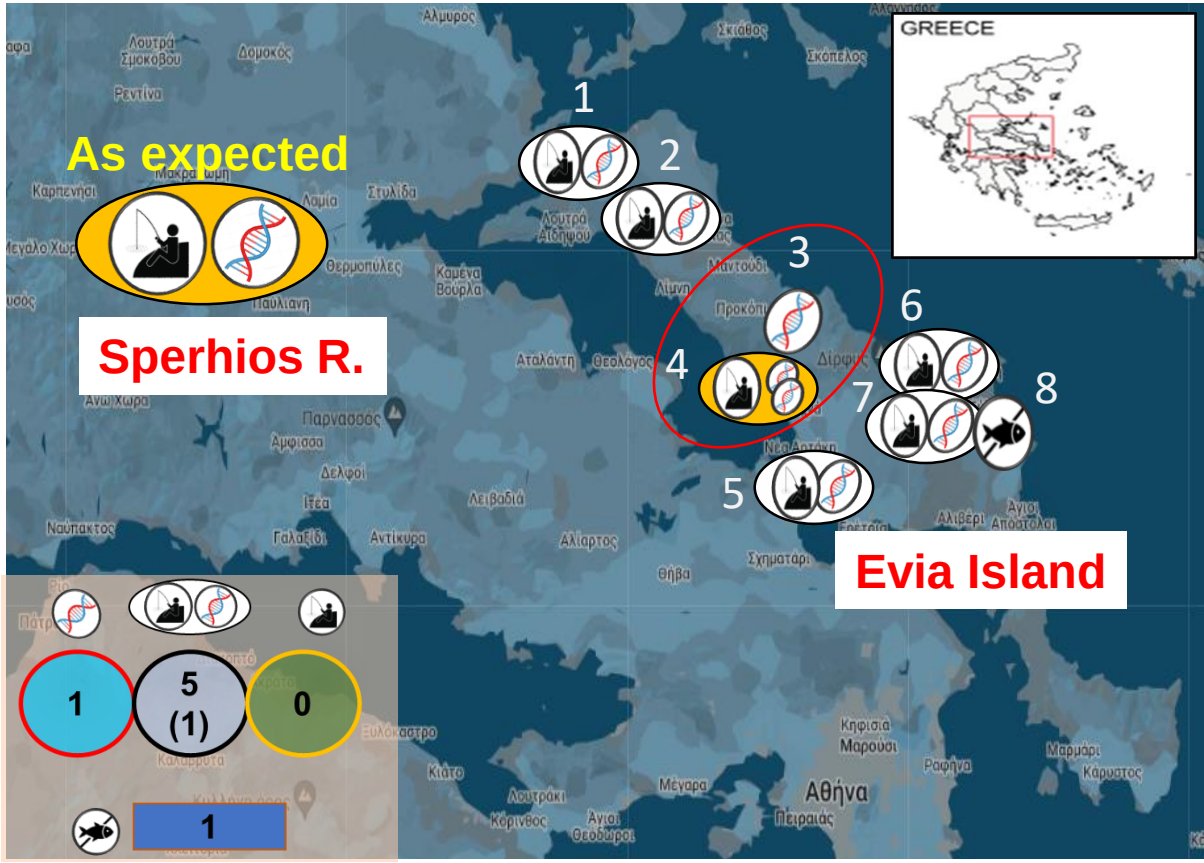
Methodology – Sampling network for *P. hellenicus*

- Field work was conducted in August 2019 and January 2022
- Sampling conducted in the Sperchios basin in Central Greece

2. Results *B. euboicus*

Barbus euboicus

Barbus sperchiensis



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

Results *P. hellenicus (pending)*

CR

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

OBJECTIVE

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

METHODOLOGY

: out of 34 fish species (CR, EN) located in Greece

we selected **6** species since:

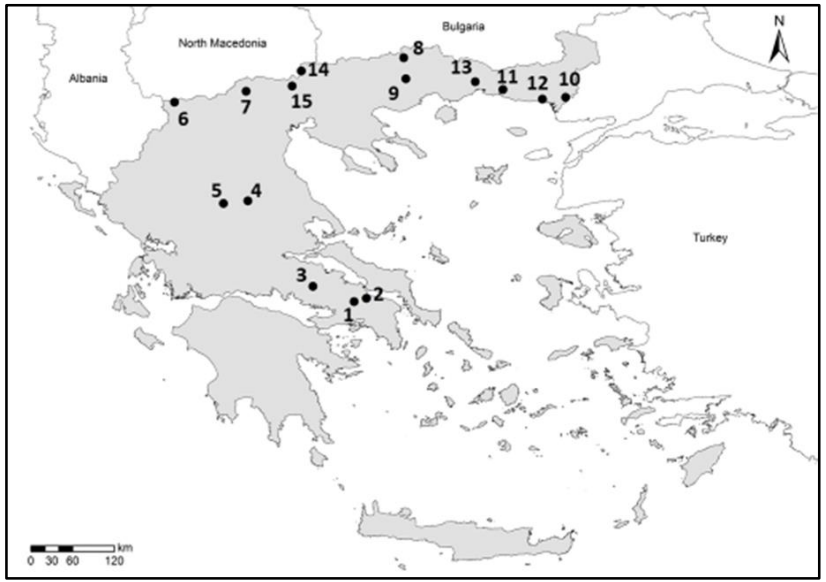
a) some species had been previously investigated (e.g. *Valencia* spp)

b) absence of type locality specimens (e.g. sturgeons)

c) Issues with co-occurrence with other species of the same genus (e.g. *Alburnus* spp)

METHODOLOGY

- Field work was conducted in 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

Results as expected

K. thesalla

Only at Pinios Thes.

T. beoticus

Only at Attico-Beotia

A. macedonicus

Only at Doiran Lake

Results

A. vistonicus CR

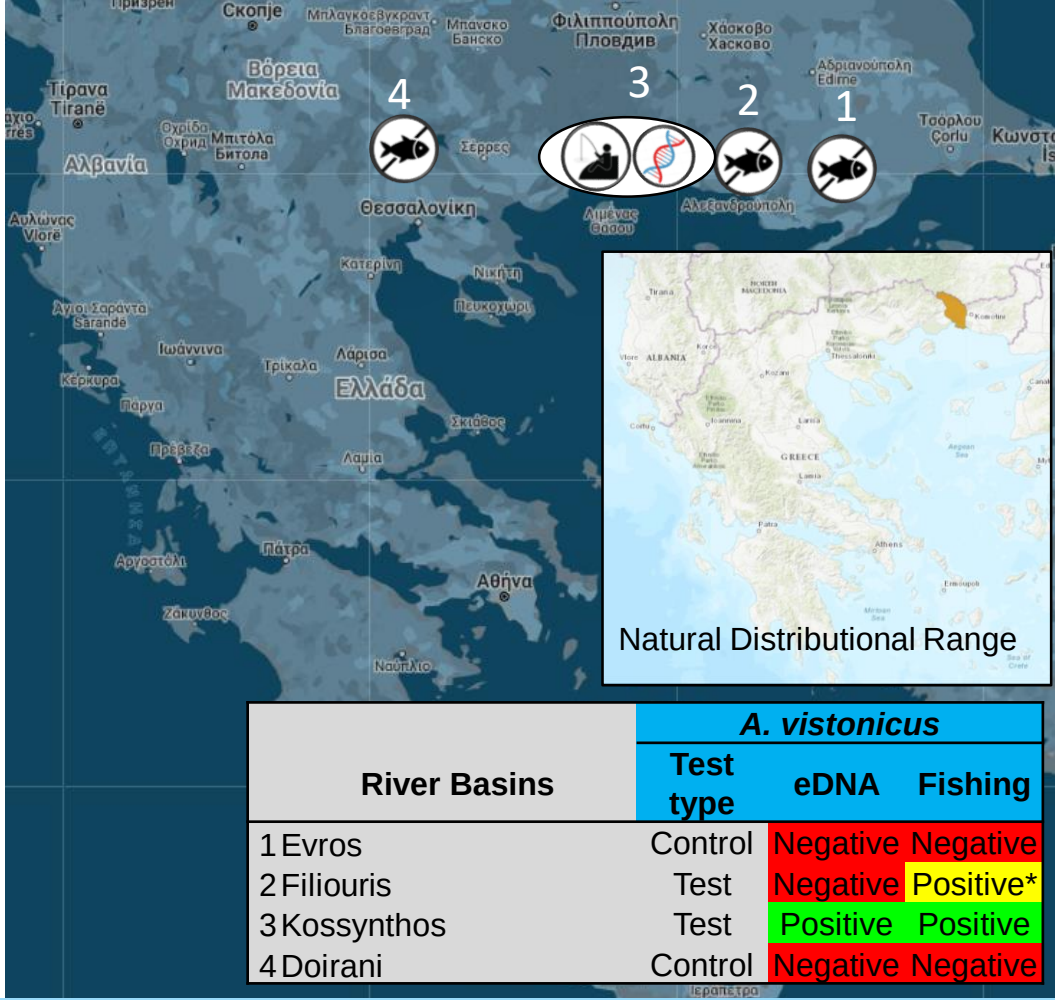
According to Barbieri et al.

Alburnus vistonicus Freyhof & Kottelat, 2007 ENDEMIC

Αλάϊα, 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

Unexpected Results



River Basins	*A. vistonicus* Test type	eDNA	Fishing
1 Evros	Control	Negative	Negative
2 Filiouris	Test	Negative	Positive*
3 Kossynthos	Test	Positive	Positive
4 Doirani	Control	Negative	Negative

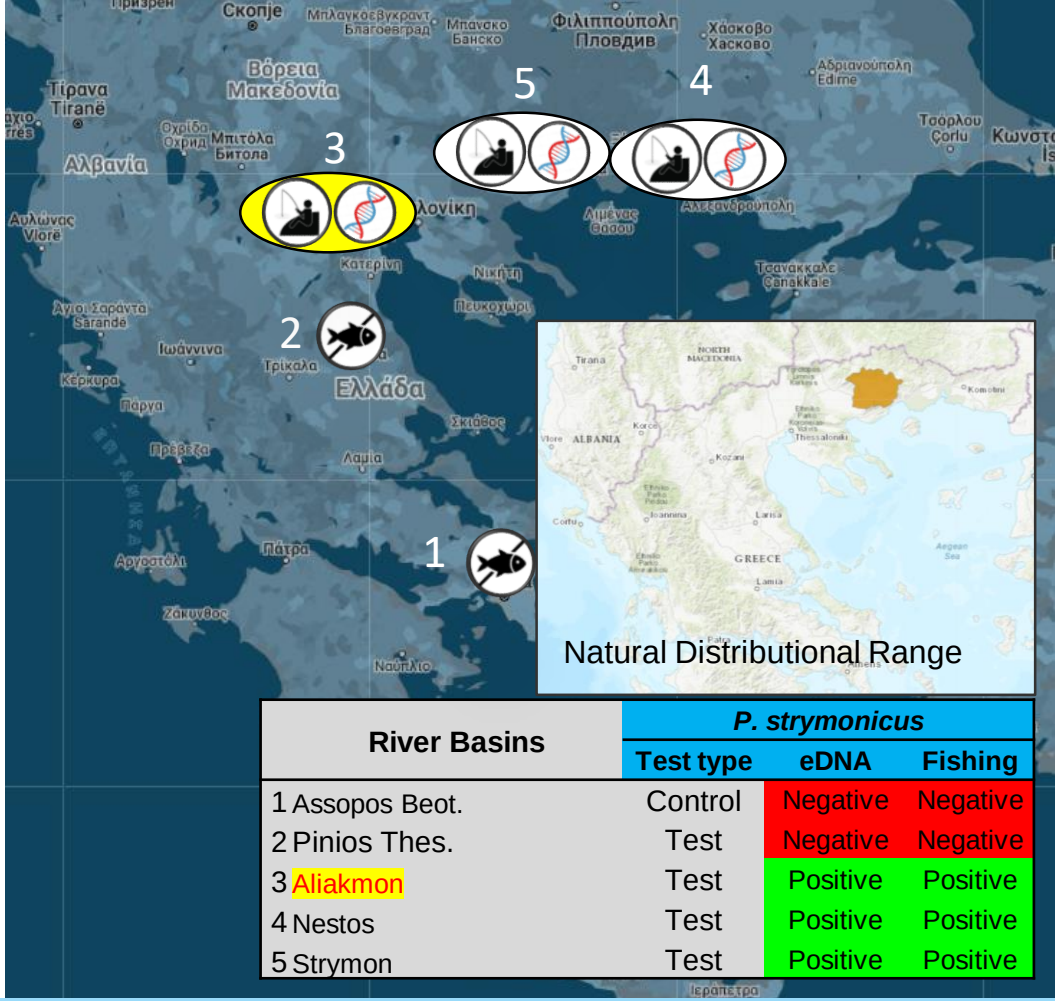
Results

P. strymonicus EN

Unexpected Results

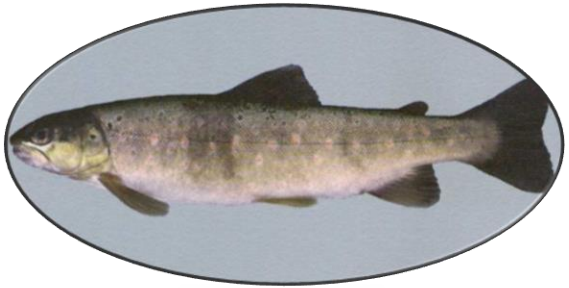
Recorded at Aliakmon R. & Nestos R.

According to Sanda et al.
Aliakmon R. → *P. lumaireul*



River Basins	*P. strymonicus*		
	Test type	eDNA	Fishing
1 Assopos Beot.	Control	Negative	Negative
2 Pinios Thes.	Test	Negative	Negative
3 Aliakmon	Test	Positive	Positive
4 Nestos	Test	Positive	Positive
5 Strymon	Test	Positive	Positive

RESULTS (AFRESH)

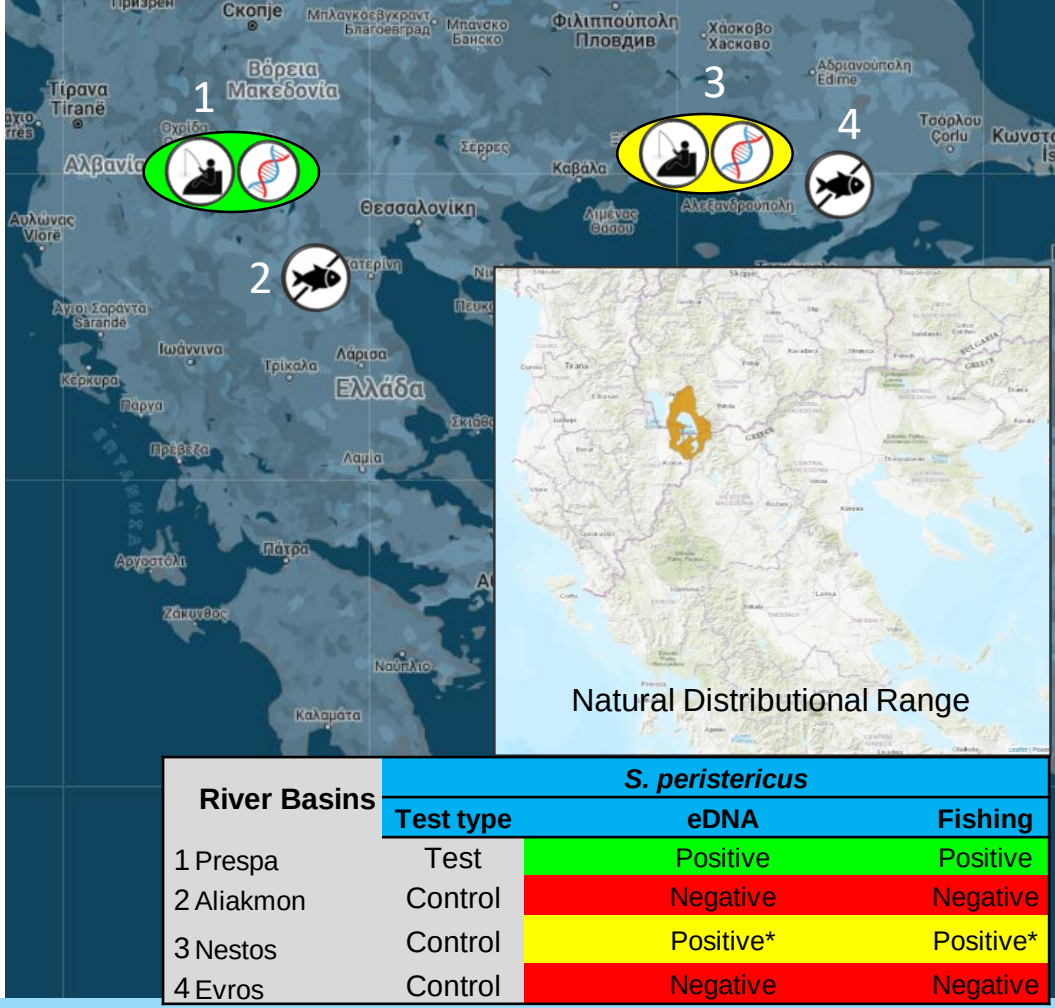


S. peristericus EN

Surprising Results

Strong signal in Nestos R. (pseudopositive)

- **Problematic issues with salmonids**



River Basins	*S. peristericus*		
	Test type	eDNA	Fishing
1 Prespa	Test	Positive	Positive
2 Aliakmon	Control	Negative	Negative
3 Nestos	Control	Positive*	Positive*
4 Evros	Control	Negative	Negative

First application of eDNA to map alien, invasive species in Greek freshwaters (project RESILIENT)

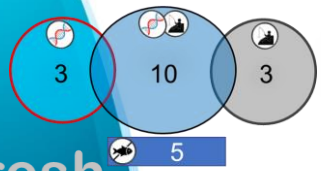
Target species: *Gambusia holbrooki*

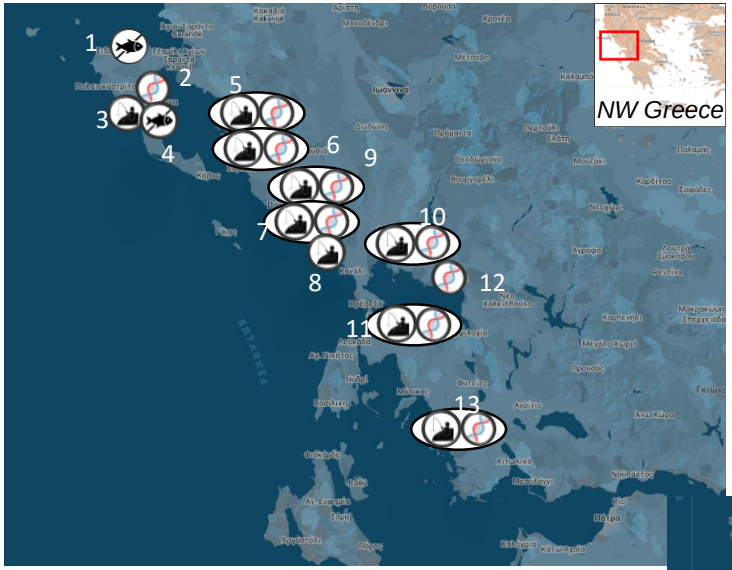
OBJECTIVE

Mapping the alien invasive Eastern mosquitofish *G. holbrooki* in Valencia habitats, *using BOTH c*onventional fish sampling methods and eDNA sampling

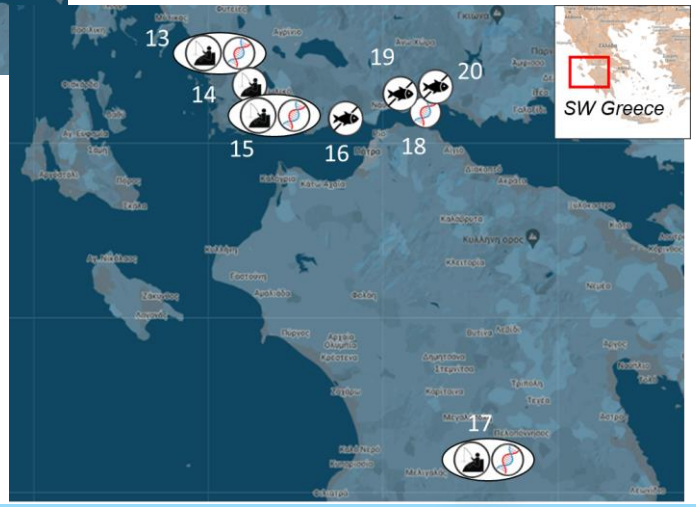
G. holbrooki

- Fishing detection
- e DNA detection
- No detection





Map showing eDNA and fish sampling results of *G. holbrooki* at locations sampled in W. Greece (*V. letourneuxi* distributional range) during the 2018 autumn survey



Map showing eDNA and fish sampling results of G. holbrooki at locations sampled in W. Greece (*V. robertae* distributional range) during the 2018 autumn survey

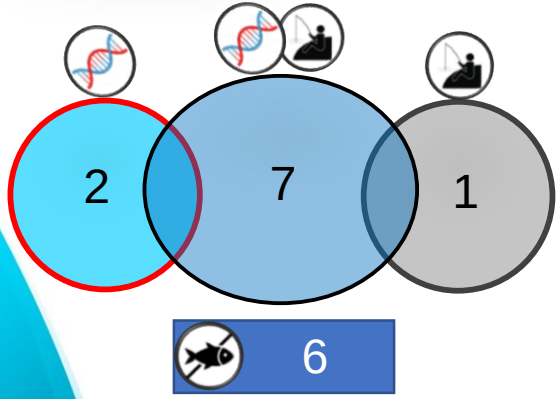
Targeting two top invaders/nation-wide survey (project PACIM)

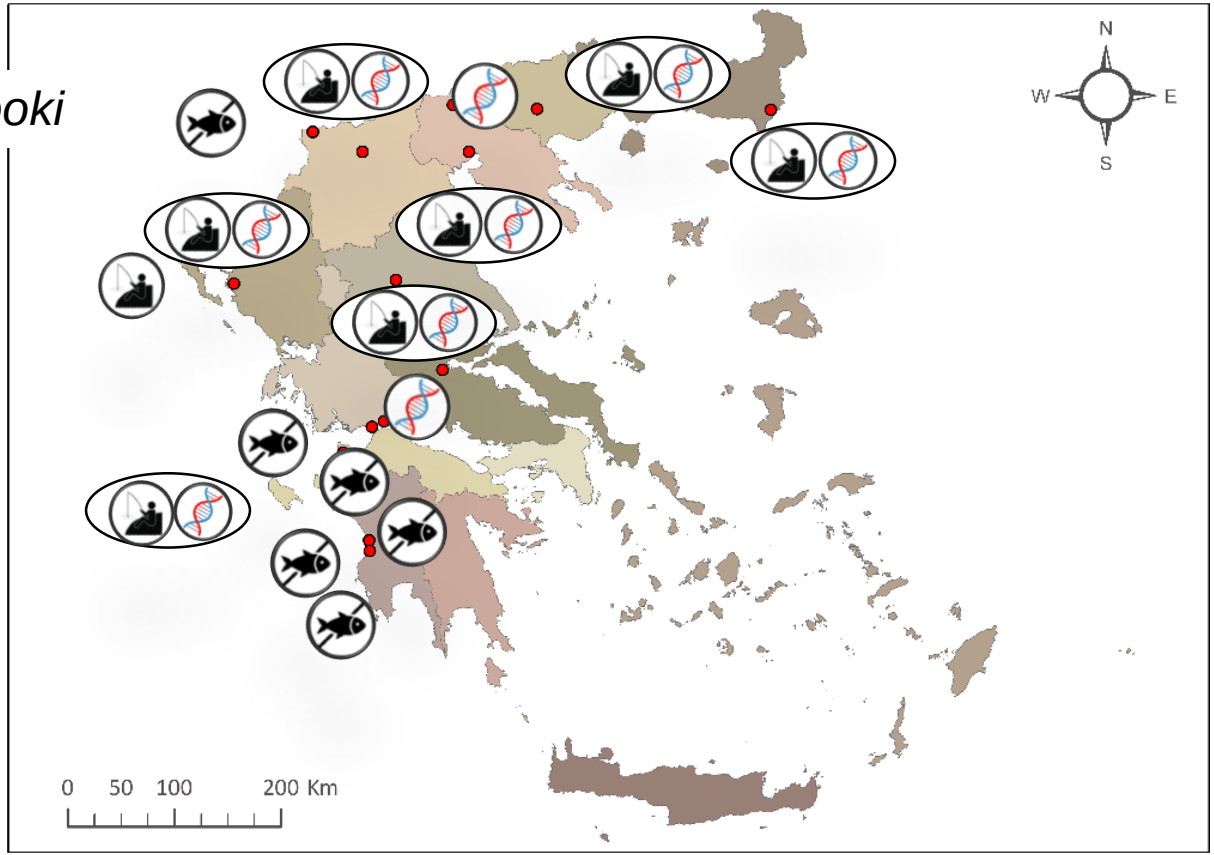
Target species: *Gambusia holbrooki* and *Pseudorasbora parva*

OBJECTIVE

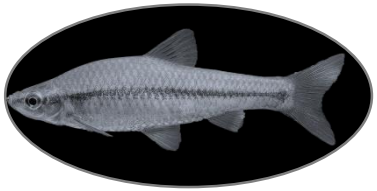
Nation-wide survey targeting two top freshwater fish invaders,
*using BOTH c*onventional fish sampling methods and eDNA sampling

G. holbrooki

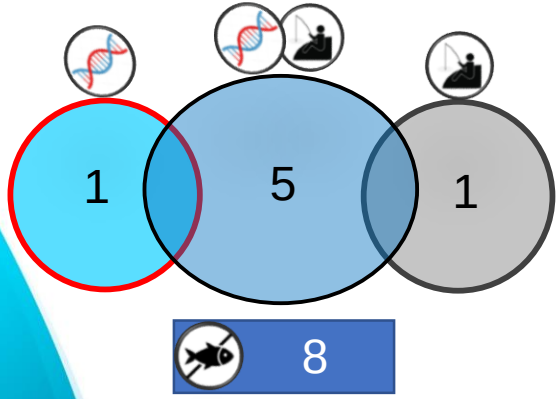


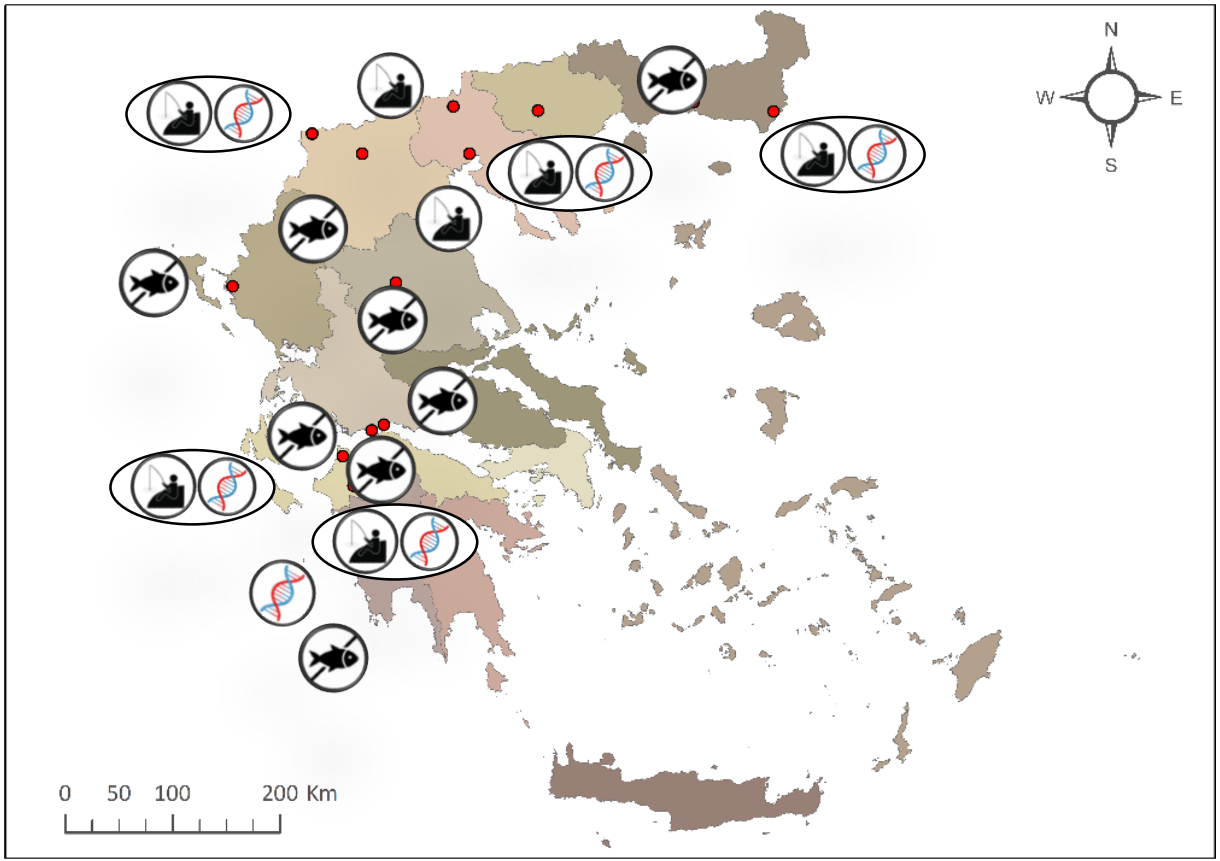


e DNA detection Fishing detection No detection



P. parva





e DNA detection Fishing detection No detection

Occupancy Modelling

True Positive
True Negative

False Positive
False Negative

TRUE **FALSE**

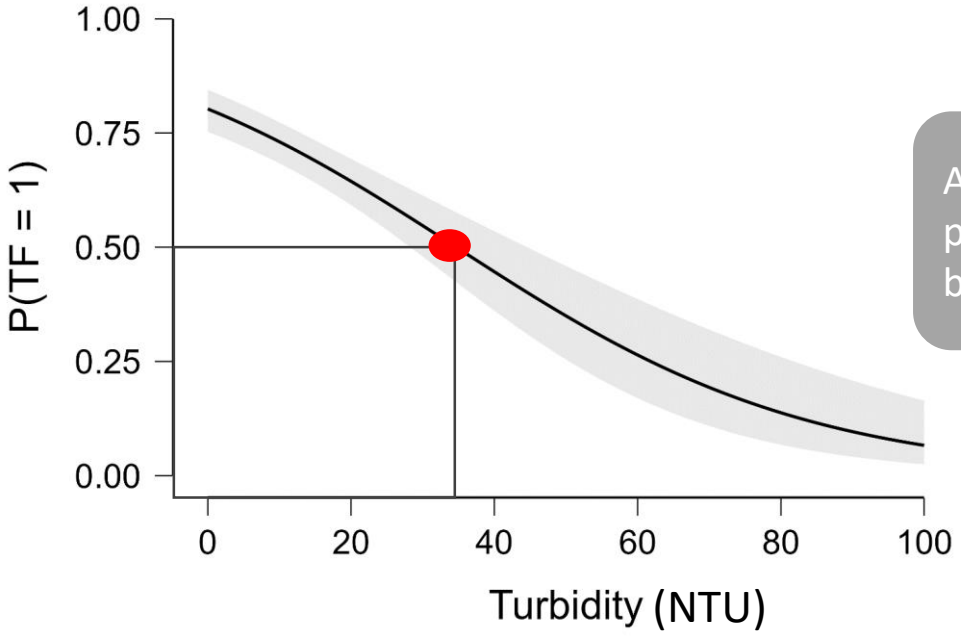
Generalized Linear Modelling

23 environmental, habitat and biological variables from 16 sampling locations were regressed against the outcome of the eDNA analysis in order to identify the most significant predictors

1.	Fish species	7.	pH	13.	Depth	19.	G.hol. density
2.	**Filtered Volume***	**8.**	**Turbidity***	14.	Coarse Substrate %	20.	G. hol. abundance/Min
3.	Ecoregion	9.	D.O. Saturation	15.	Shadedness	21.	P. par. abundance
4.	Latitude	**10.**	**Flow ***	16.	Helophytes	22.	P. par. density
5.	Longitude	11.	Fast habitat %	17.	Bottom Vegetation	23.	P. par abundance/Min
6.	Temperature	**12.**	**Wetted Width***	**18.**	**G. hol. Abundance***		

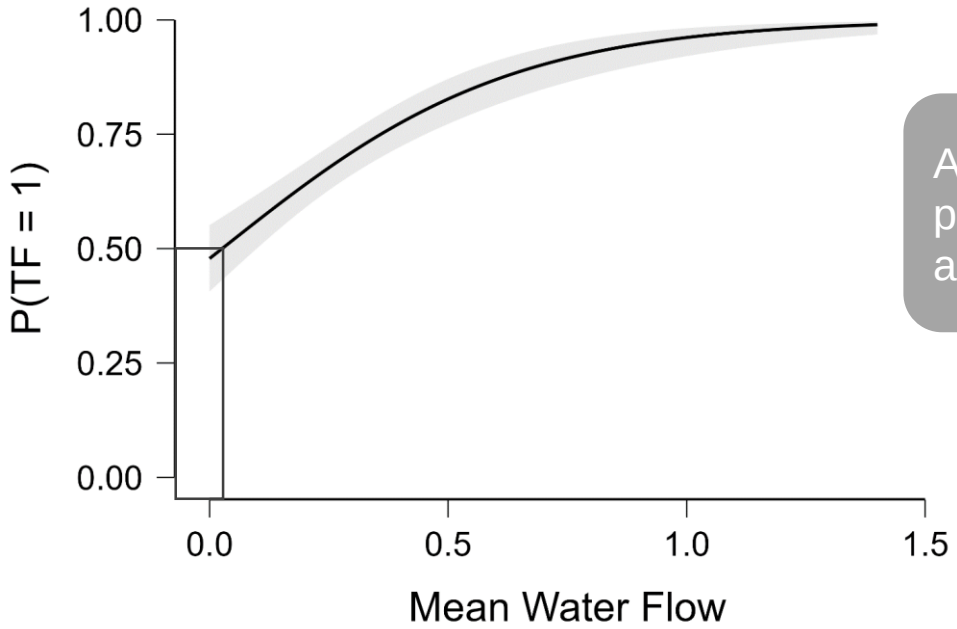
* Statistically significant

Conditional Plot of Turbidity



At approximately 35 NTU, the probability of a true outcome fell below 50%

Conditional Plot of Mean Water Flow



Already at zero flow, the probability of a true outcome was almost 50%

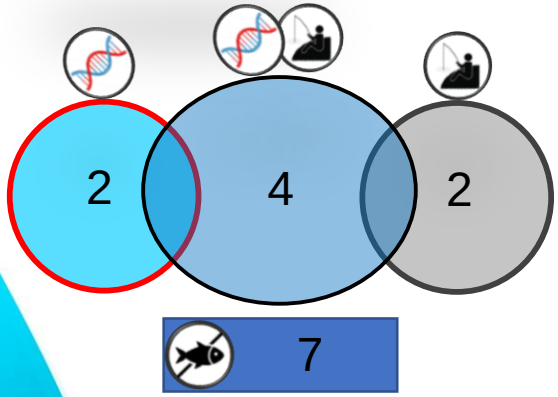
Widening the scope, two more top invaders/<u>nation-wide</u> survey in Greek freshwaters (project AFRESH)

Target species: *Carassius gibelio* and *Lepomis gibbosus*

OBJECTIVE

Nation-wide survey targeting two more top freshwater fish invaders,
*Using BOTH c*onventional fish sampling methods and eDNA sampling

C. gibelio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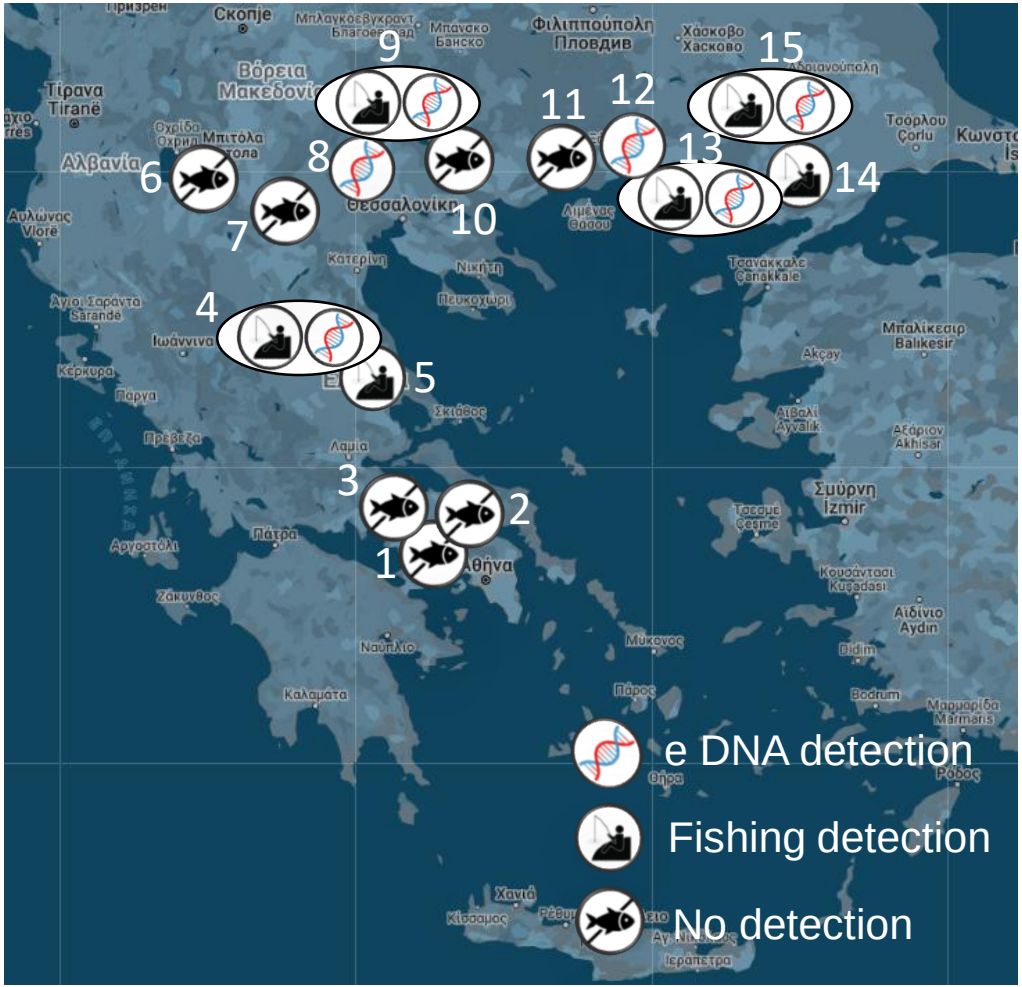
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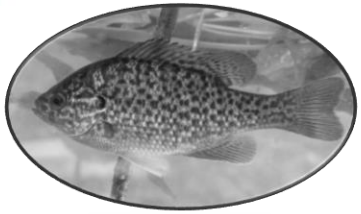
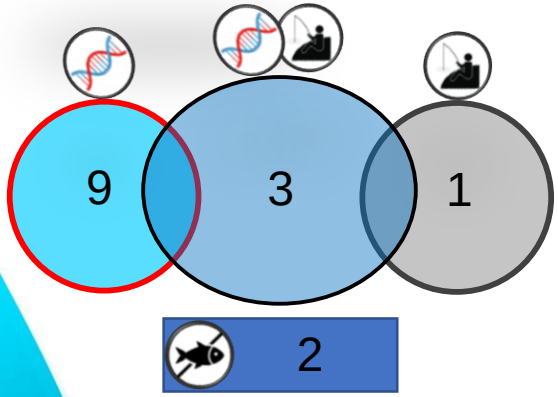
e DNA detection
Fishing detection
No detection



L. gibbosus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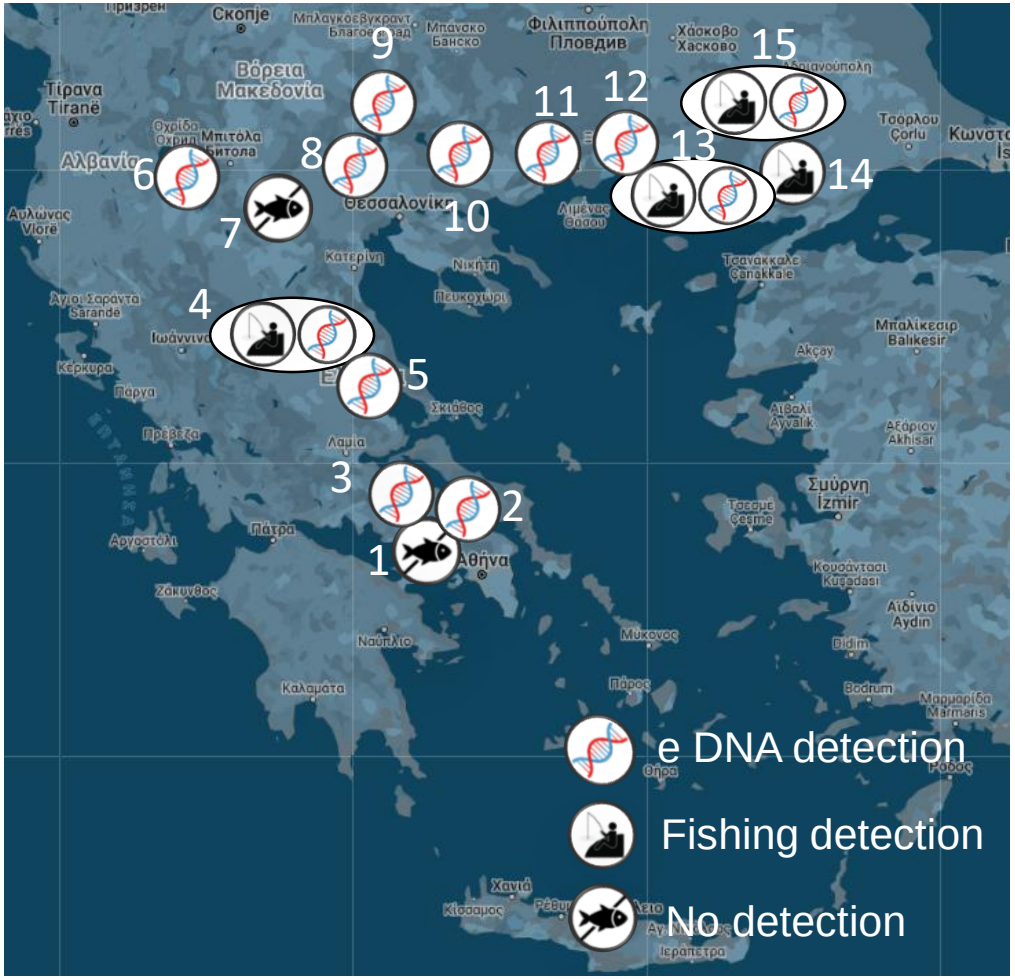
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e DNA detection
Fishing detection
No detection

Take-home message

1. e-DNA is **effective** in cases where low population densities of the target species are observed (however with some limitations)
2. Can monitor changes in distributional ranges of threatened species
3. Can be used of early detection of invasive species
4. Repetition of e-DNA sampling and analysis is required

Thank you for your attention