



AFRESH

Detecting invasive species with the eDNA method

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

Target species: *Gambusia holbrooki*



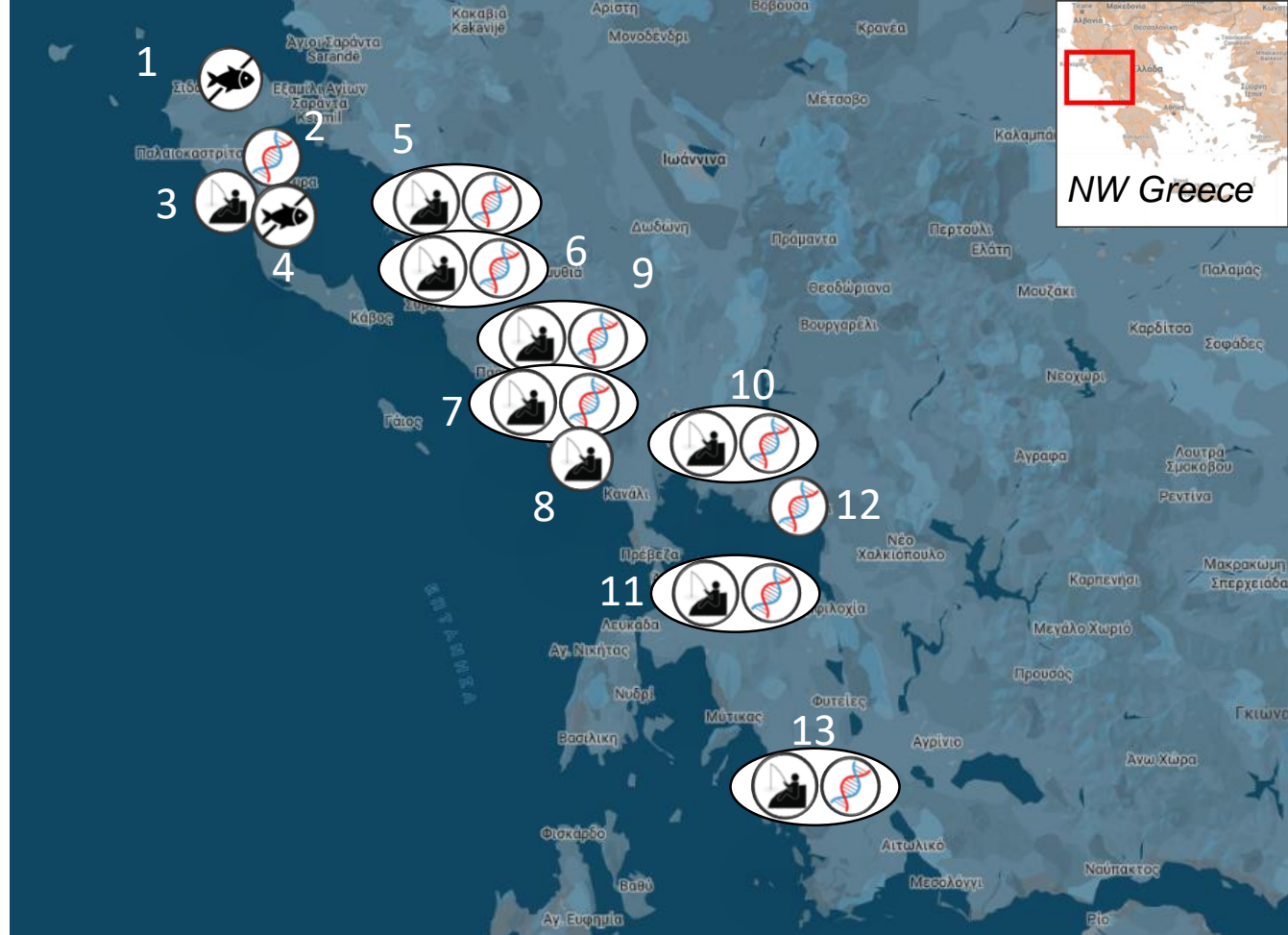
OBJECTIVE

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



G. holbrooki

- Fishing detection
- e DNA detection
- No detection

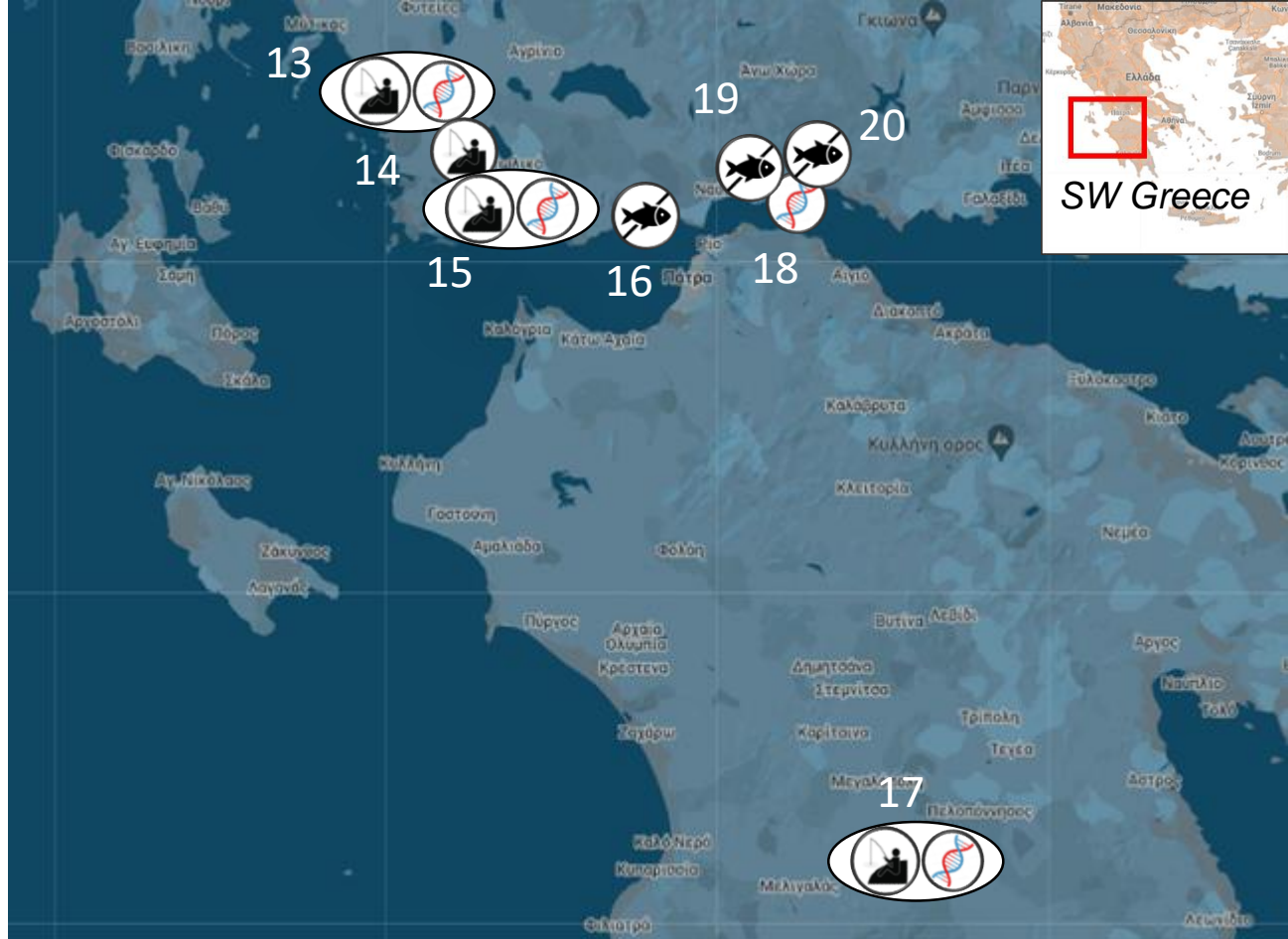


Map showing eDNA and electrofishing results of *G. holbrooki* at locations sampled in W. Greece (*V. Letourneuxi* distributional range) during the 2018 autumn survey



G. holbrooki

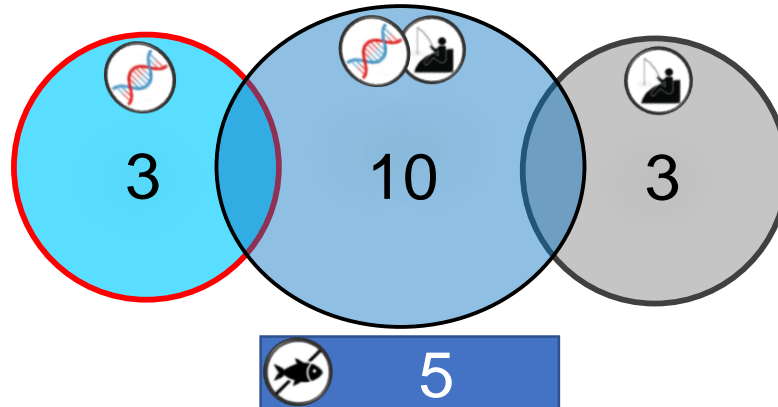
- Fishing detection
- e DNA detection
- No detection



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

Conclusions

- At 3 systems, *G.holbrooki* was detected through eDNA but **NOT through fish sampling**, → low densities, indicating the suitability of the eDNA method for species detection
- At 3 sites, *G. holbrooki* was detected through fish sampling but **NOT through eDNA (pseudonegatives)**, → limitations of the method (fast flow and/or turbidity)



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

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

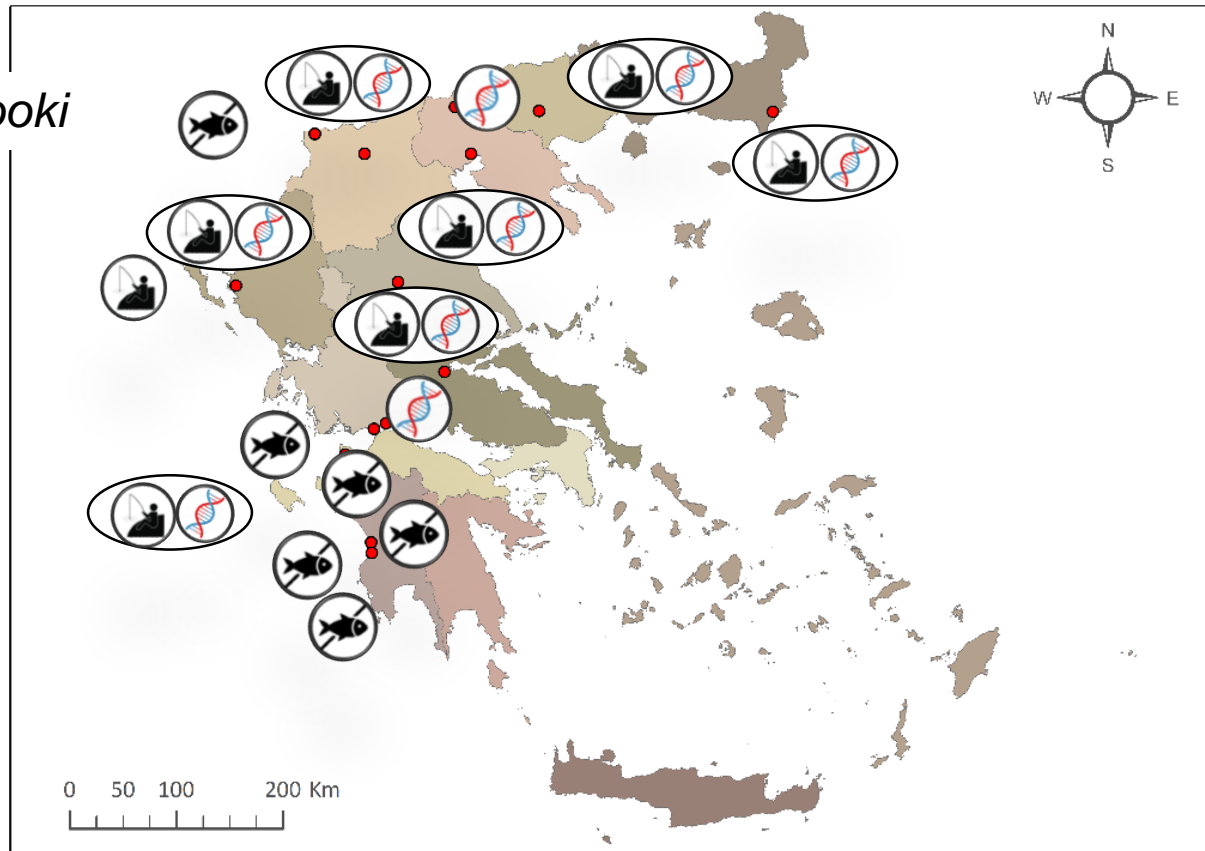
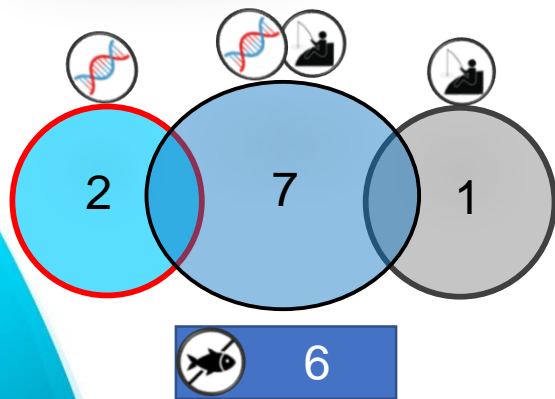
OBJECTIVE



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



G. holbrooki



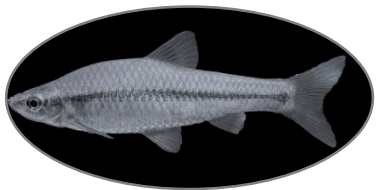
e DNA detection



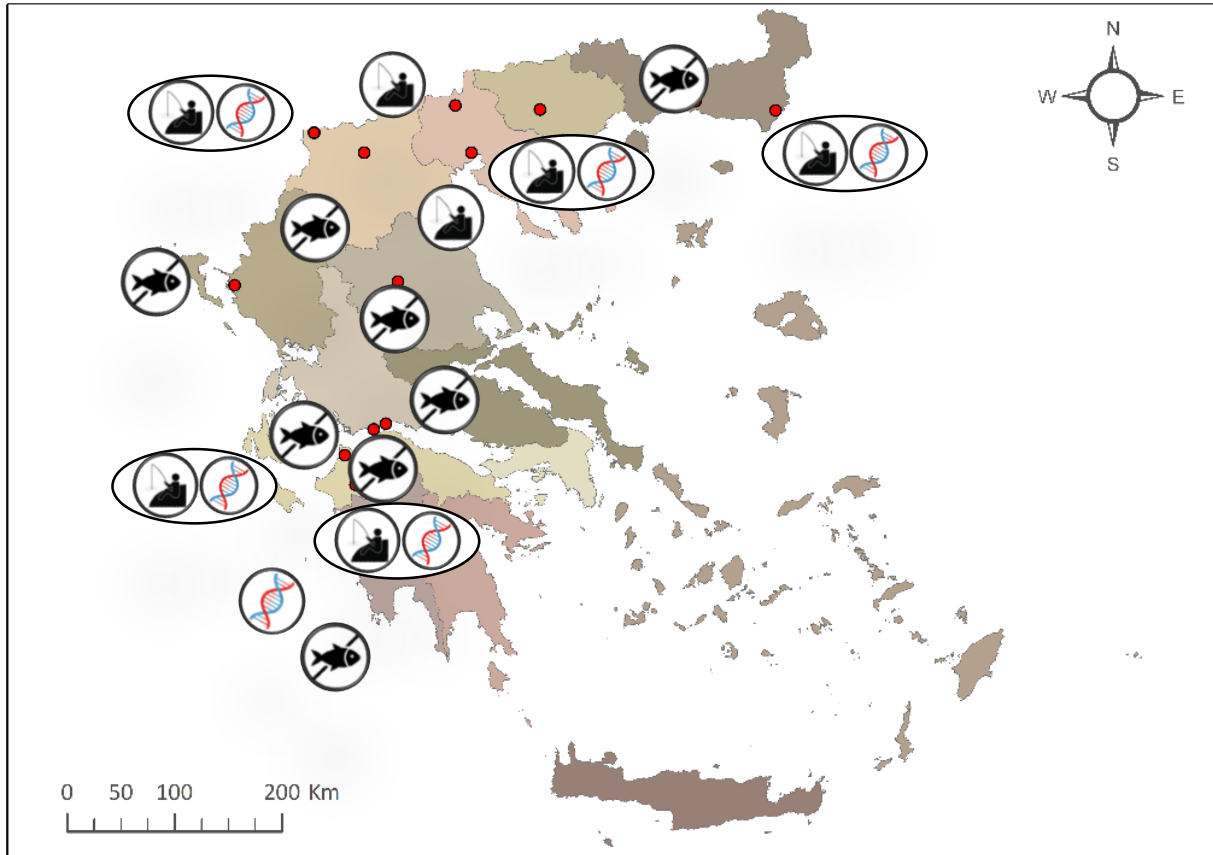
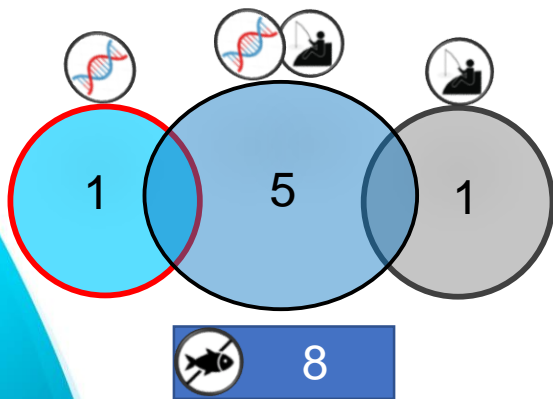
Fishing detection



No detection



P. parva



e DNA detection



Fishing detection

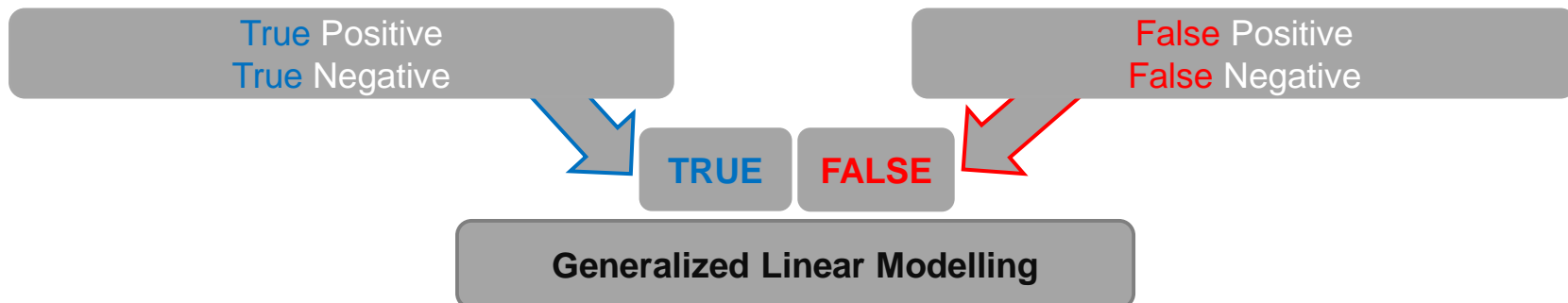


No detection

Conclusions

- At two systems (within known range), ***G.holbrooki* was detected through eDNA** but **NOT through fish sampling**, i.e. low densities, indicating the suitability of the eDNA method for species detection
- No eDNA pseudo-negatives for *G. holbrooki*
- At one system, ***P. parva* was detected through eDNA** but **NOT through fish sampling** (early detection of recent expansion?)
- Two eDNA pseudo-negatives for *P. parva*

Occupancy Modelling



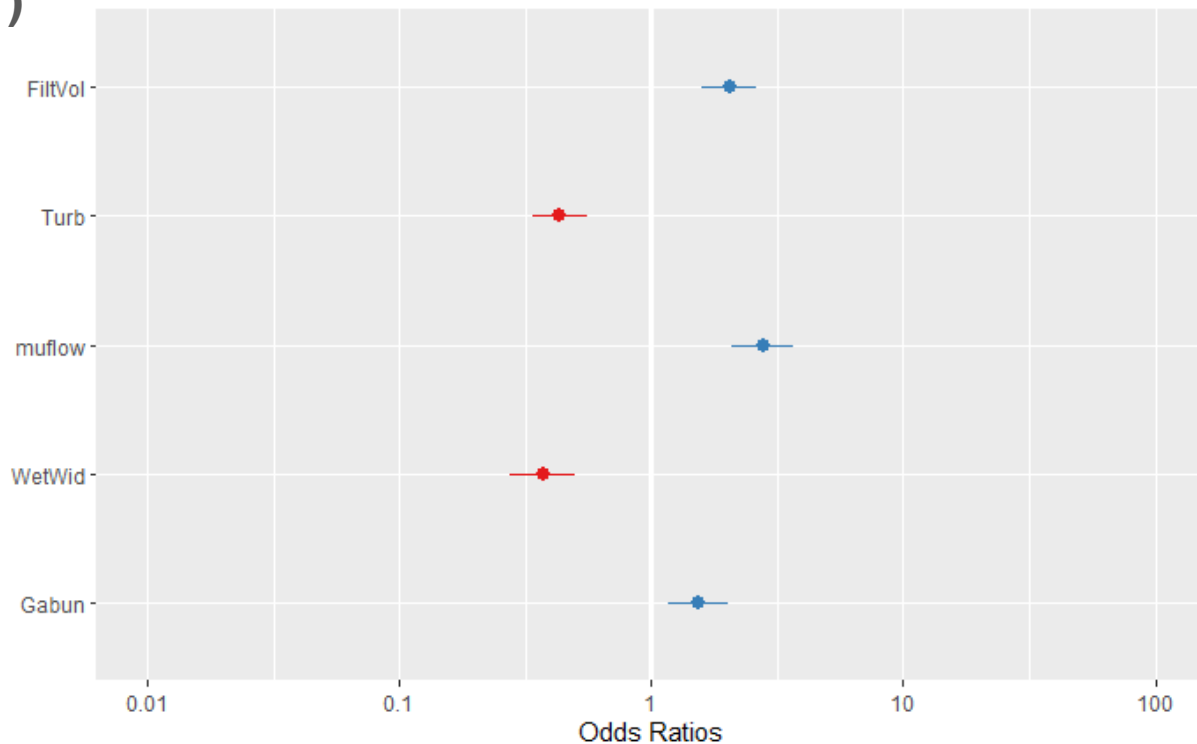
23 environmental, habitat and biological variables from 16 sampling locations were regressed against the outcome of the eDNA analysis in order to identify the most significant predictors

- | | | | |
|--------------------|--------------------|------------------------|---------------------------|
| 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 | |

RESULTS

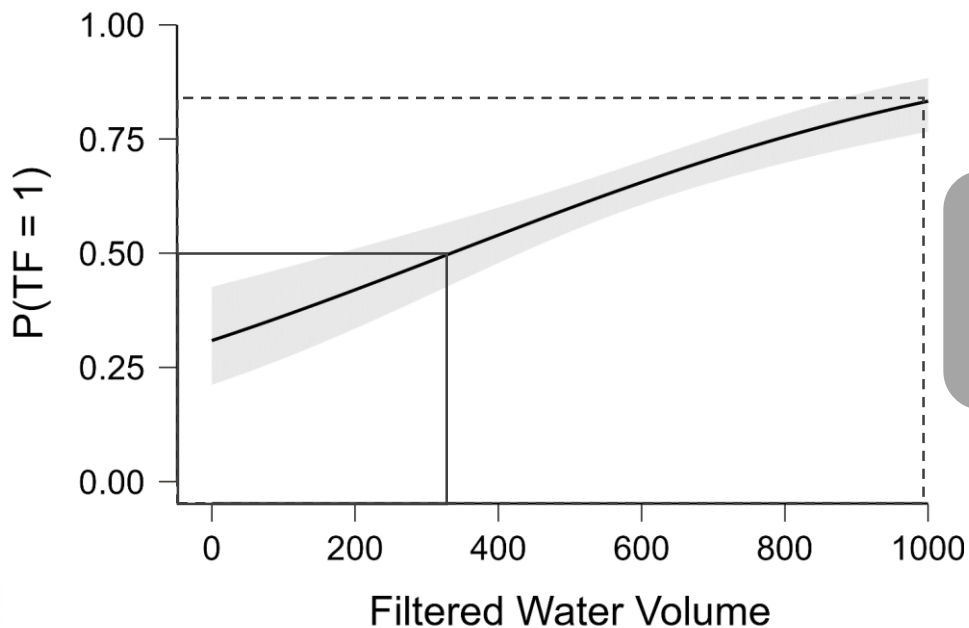
Effect of Standardized Coefficients

($P < 0.01$)



Conditional Plot of Filtered Water Volume

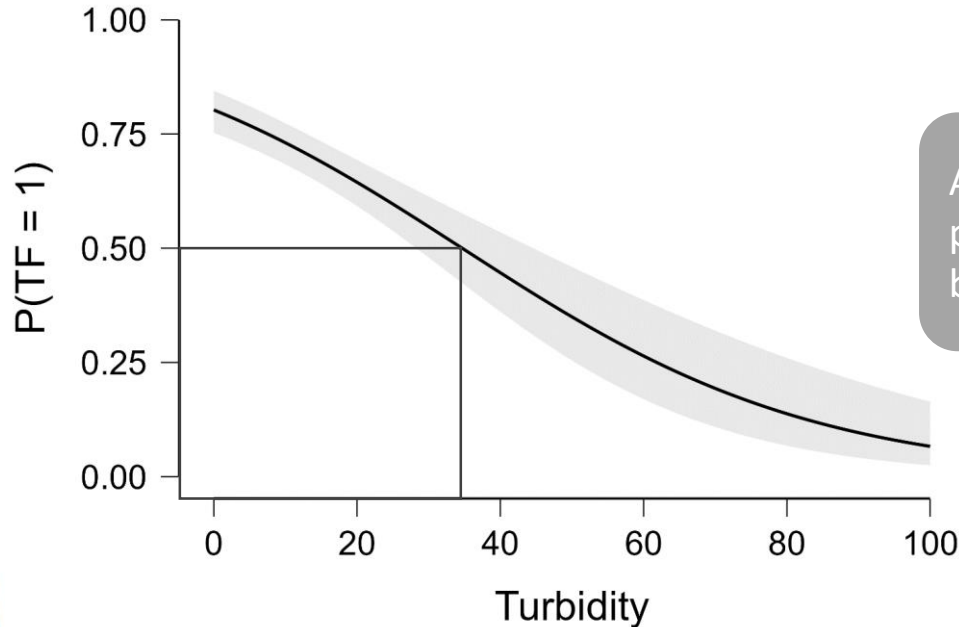
The odds of a TRUE replicate outcome occurring increased by 0.24% for every unit of increase in the Volume of Filtered Water per replicate sample.



At approximately 350 ml, the probability of a true outcome rises over 50%

Conditional Plot of Turbidity

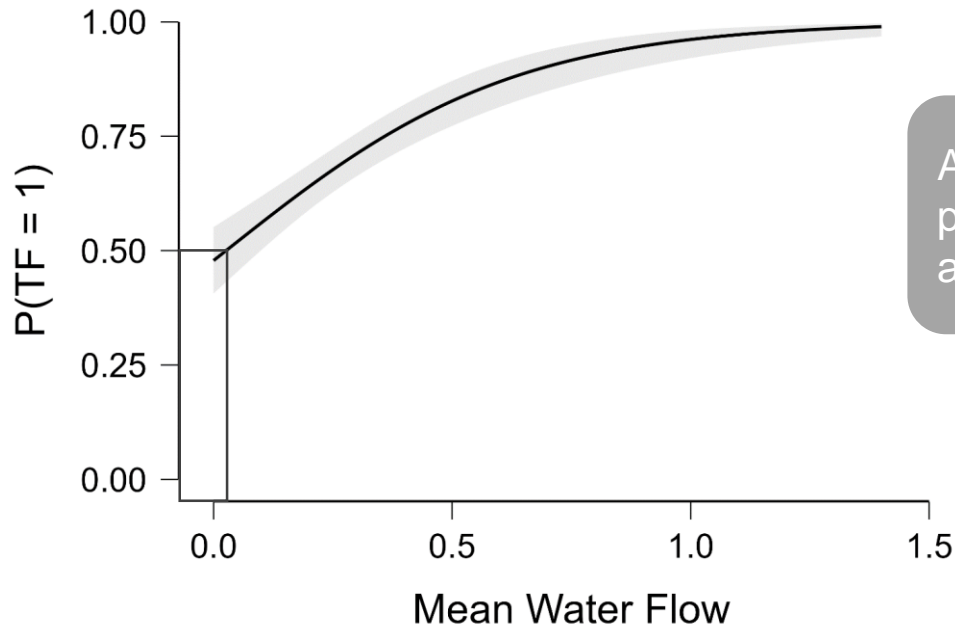
The odds of a TRUE replicate outcome occurring decreased by 3.97% for every unit of increase in water turbidity.



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

Conditional Plot of Mean Water Flow

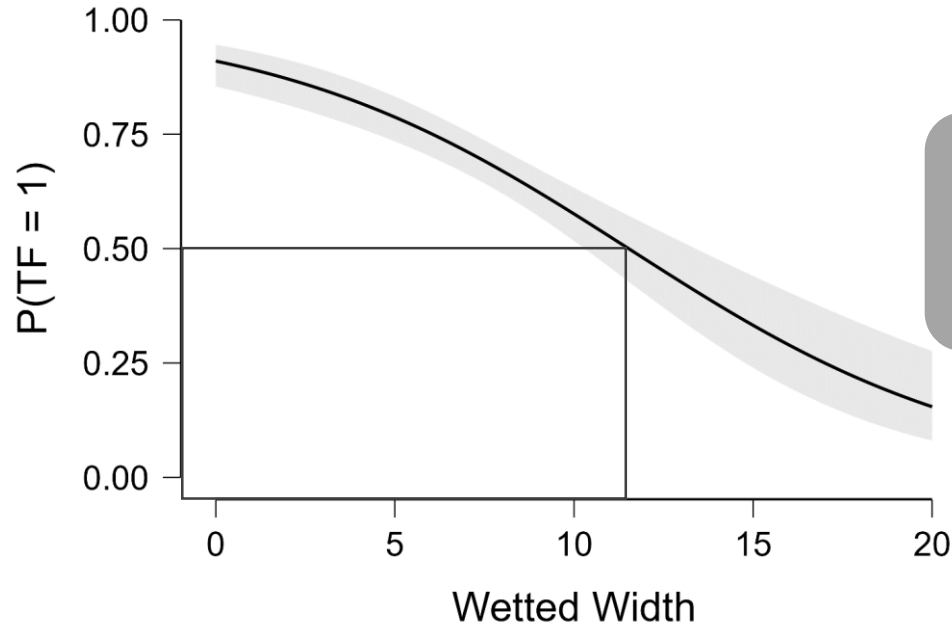
The odds of a TRUE replicate outcome occurring increased by 2618.91% for every unit of increase in water flow.



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

Conditional Plot of Wetted Width

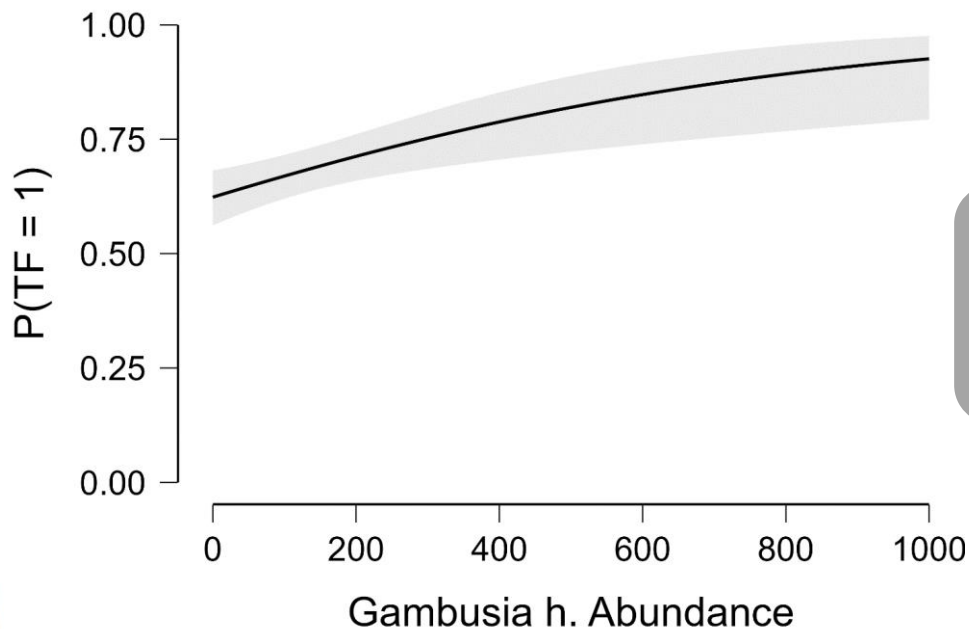
The odds of a TRUE replicate outcome occurring decreased by 18.18% for every unit of increase at wetted width.



At approximately 10 m. of wetted width, the probability of a true outcome fell below 50%

Conditional Plot of *G. holbrooki* Abundance

The odds of a TRUE replicate outcome occurring increased by 0.2% for every unit of increase in the abundance of *G. holbrooki*



On non-occupied sites (zero abundance), the probability of a true outcome was already over 50%

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

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

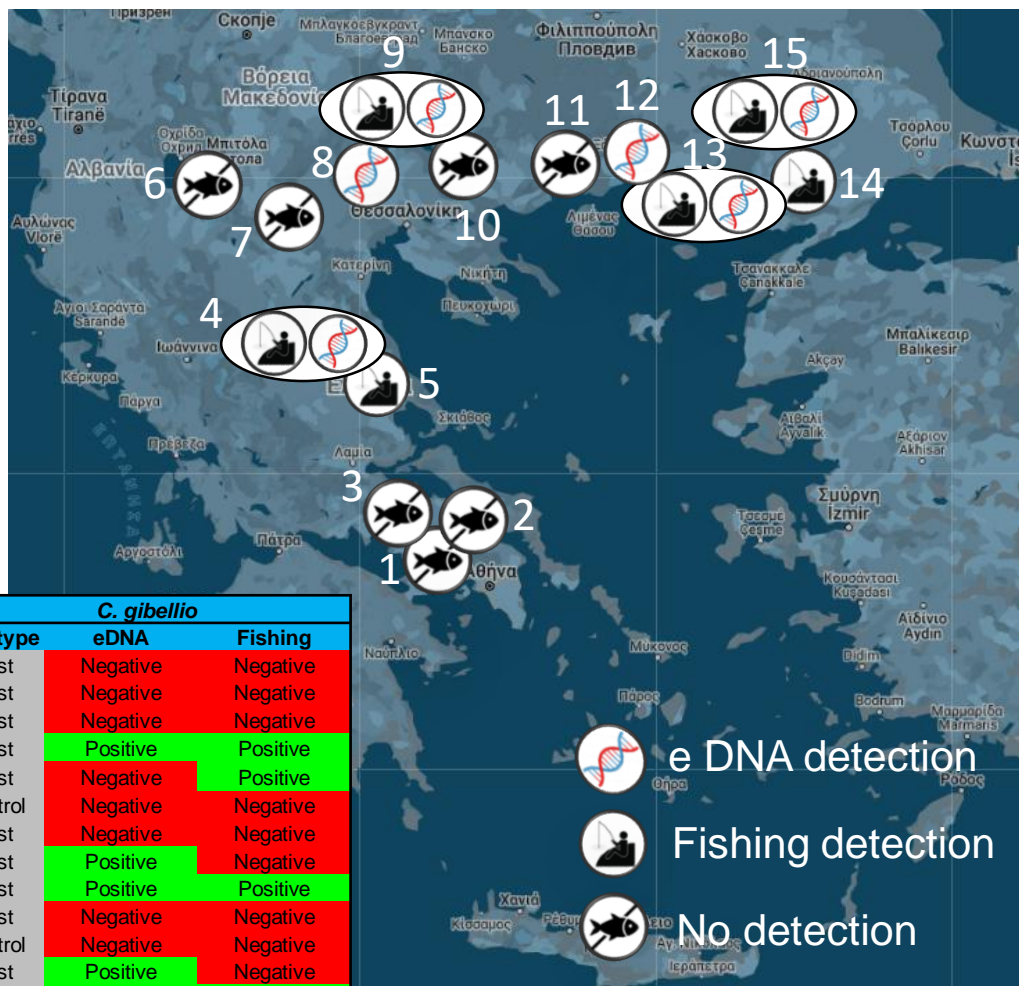
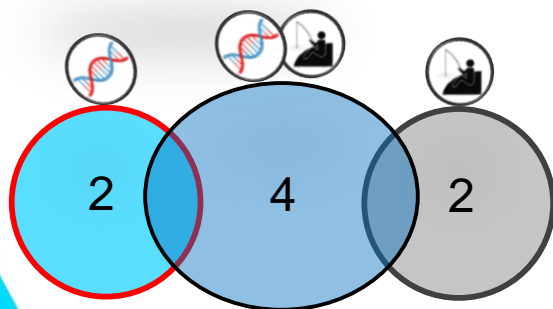
OBJECTIVE

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





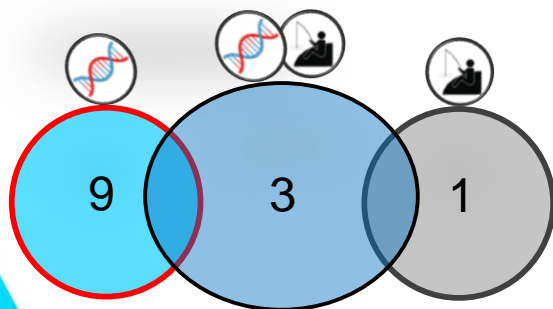
C. gibelio



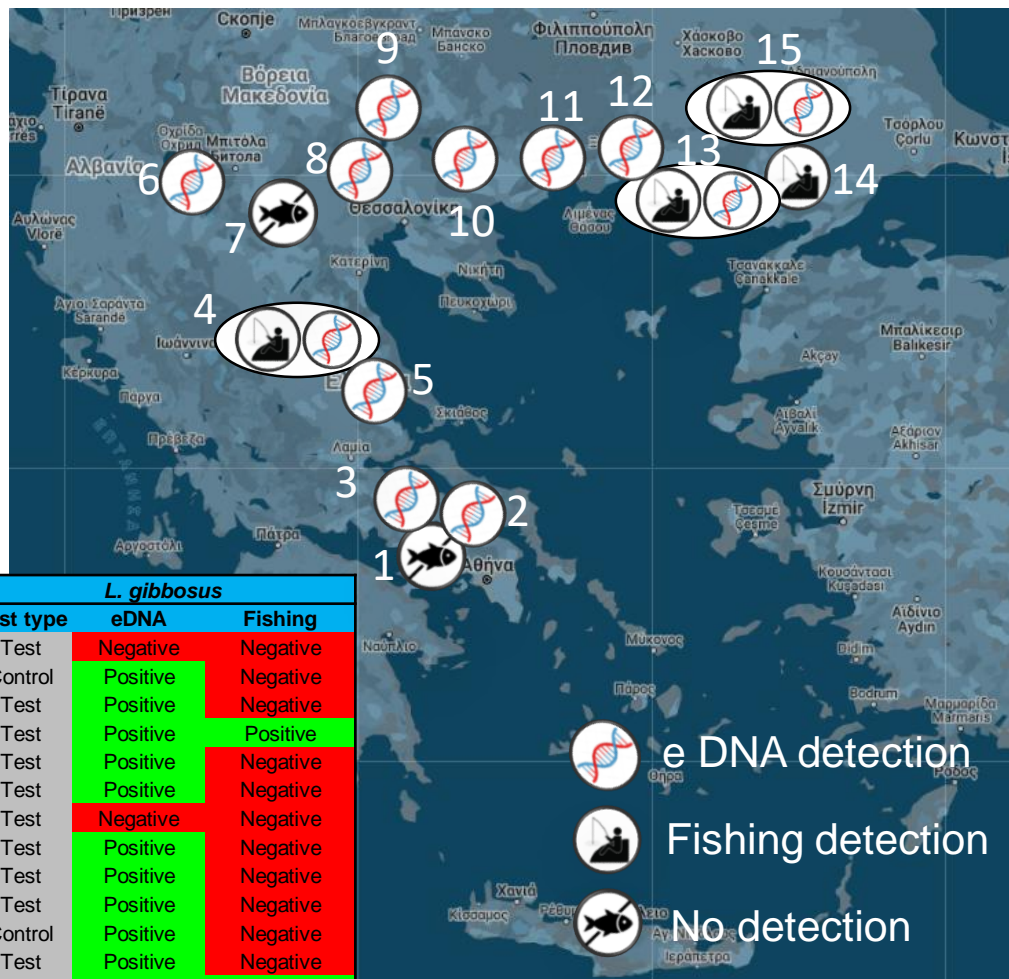
| River Basins | <i>C. gibelio</i> | | |
|-------------------------|-------------------|----------|----------|
| | Test type | eDNA | Fishing |
| 1 Assopos Beot. | Test | Negative | Negative |
| 2 Assopos Beot. | Test | Negative | Negative |
| 3 Kifissos Beot. | Test | Negative | Negative |
| 4 Pinios Thes. | Test | Positive | Positive |
| 5 Pinios Thes. | Test | Negative | Positive |
| 6 Prespa (Ag. Germanos) | Control | Negative | Negative |
| 7 Aliakmon | Test | Negative | Negative |
| 8 Axios | Test | Positive | Negative |
| 9 Doiran | Test | Positive | Positive |
| 10 Strymon | Test | Negative | Negative |
| 11 Nestos | Control | Negative | Negative |
| 12 Kossynthos | Test | Positive | Negative |
| 13 Filiouris | Test | Positive | Positive |
| 14 Evros | Test | Negative | Positive |
| 15 Evros | Test | Positive | Positive |



L. gibbosus



2



| River Basins | <i>L. gibbosus</i> | | |
|-------------------------|--------------------|----------|----------|
| | Test type | eDNA | Fishing |
| 1 Assopos Beot. | Test | Negative | Negative |
| 2 Assopos Beot. | Control | Positive | Negative |
| 3 Kifissos Beot. | Test | Positive | Negative |
| 4 Pinios Thes. | Test | Positive | Positive |
| 5 Pinios Thes. | Test | Positive | Negative |
| 6 Prespa (Ag. Germanos) | Test | Positive | Negative |
| 7 Aliakmon | Test | Negative | Negative |
| 8 Axios | Test | Positive | Negative |
| 9 Doiran | Test | Positive | Negative |
| 10 Strymon | Test | Positive | Negative |
| 11 Nestos | Control | Positive | Negative |
| 12 Kossynthos | Test | Positive | Negative |
| 13 Filiouris | Test | Positive | Positive |
| 14 Evros | Test | Negative | Positive |
| 15 Evros | Test | Positive | Positive |

eDNA detection

Fishing detection

No detection

Conclusions

- At 2 systems (within known range), ***C. gibelio* was detected through eDNA** but **NOT through fish sampling**, i.e. low densities, indicating the suitability of the eDNA method for species detection
- One eDNA pseudo-negative for *C. gibelio*
- At 5/9 systems, ***L. gibbosus* was detected through eDNA** but **NOT through fish sampling** (early detection of recent expansion?)
- One eDNA pseudo-negatives for *L. gibbosus*!



Ευχαριστώ πολύ για την προσοχή σας!