

Conservation behaviour, translocation and captive breeding

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Overview

- 1. Introduction definitions
- 2. Behavioural interactions between killifish and mosquitofish (DECAGON)
- 3. Effects of turbidity on the behaviour of a native minnow
- 4. Behavioural effects of turbidity: killifish vs mosquitofish (AFRESH)
- 5. Captive breeding of two threatened native killifish species (DECAGON)
- 6. Conservation translocation: creating a refugia population (AFRESH)
- 7. Creating breeding stocks of 2 range-restricted threatened fishes (AFRESH)
- 8. Future perspectives



Workshop

1. Introduction - definitions

Behaviour refers to animal responses to stimuli (external or internal) and includes all the ways animals interact with one another and with the environment

Ethology → the scientific study of animal behaviour (in the light of evolution)

Conservation aims to prevent biodiversity loss and mitigate the impact of anthropogenic pressures

Lack of knowledge of key behavioural characteristics of the target species can lead to problems in the implementation of conservation plans



Conservation behaviour: the application of ethology to biodiversity conservation efforts



2. Behavioural interactions between killifish and mosquitofish (DECAGON)

29 Pairs of *Valencia letourneuxi* and *Gambusia holbrooki* → in 3 consecutive phases:

60-min acclimation

- 1. In an **empty** tank (15 min)
 5-min interval
- 2. In the presence of **artificial cover** (15 min)
 5-min interval
- 3. In the presence of **food** (15 min)

We measured various behaviours including aggressive and stress-related behaviours

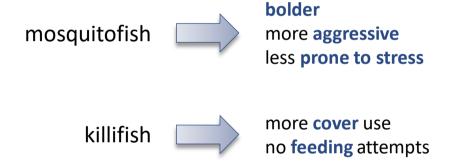
 2^{nd} stage \rightarrow cover use

3rd stage → **feeding attempts**



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2. Behavioural interactions between killifish and mosquitofish (DECAGON)





Article submitted to Animal Behaviour by Kapakos et al. (2022)

Turbidity

Natural (e.g. flash floods)

Impacts expected to intensify!

Human-induced (e.g. quarries)





70 *Pelasgus stymphalicus*, a native minnow with 'least concern' status

→ model for other more vulnerable congeners (e.g. *Pelasgus laconicus*) and other small-sized cyprinids

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Three tests conducted in succession:

5-min acclimation

- 1. Emergence test (5 min)
- 2. Open field test (5 min)
- 3. Predator response test (3 + 3 min)

Two turbidity levels

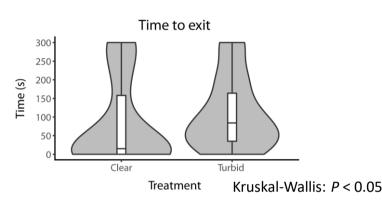
Clear: 0 NTU Turbid: 30 NTU

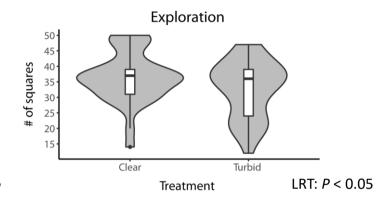
Behavioural measures:

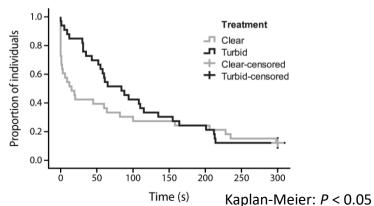
- Time to exit
- Activity & exploration
- Time immobile
- Time in the centre
- Dashing/erratic behaviour

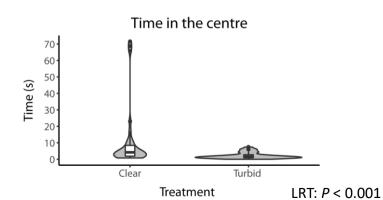


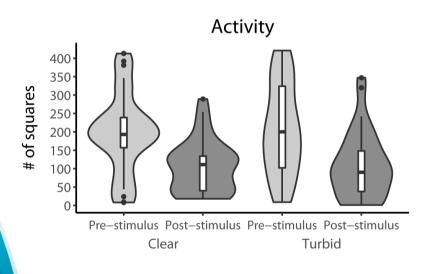
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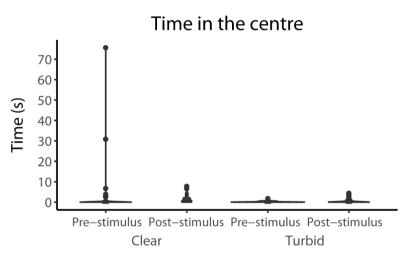












GLM: Activity ~ Turbidity treatment + Stimulus addition





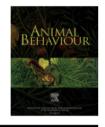
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Turbidity and predation risk: behavioural responses of a freshwater minnow



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Two tests conducted in succession:

2-min acclimation

1. Open field test (5 min)

2-min interval

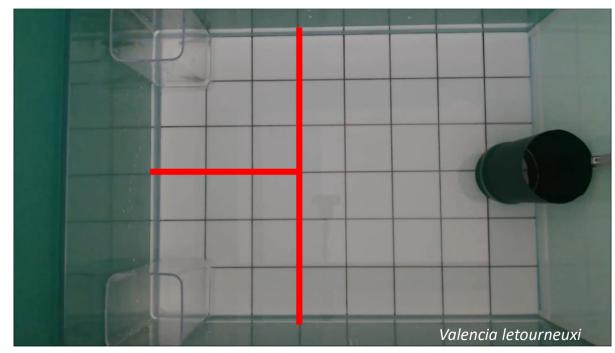
2. Sociability test (5 min)

Two turbidity levels

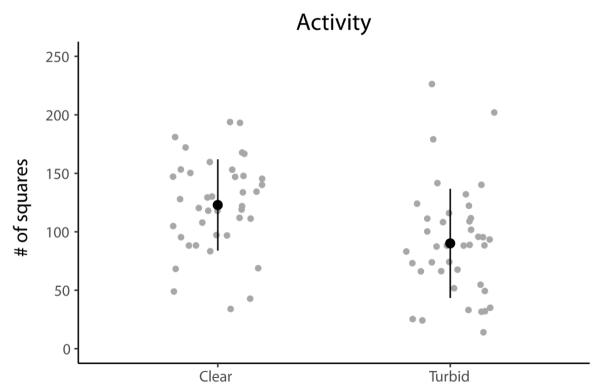
Clear: 0 NTU Turbid: 30 NTU

Behavioural measures:

- Activity & exploration
- Time immobile
- Time in the centre
- Time shoaling
- Dashing/erratic behaviour

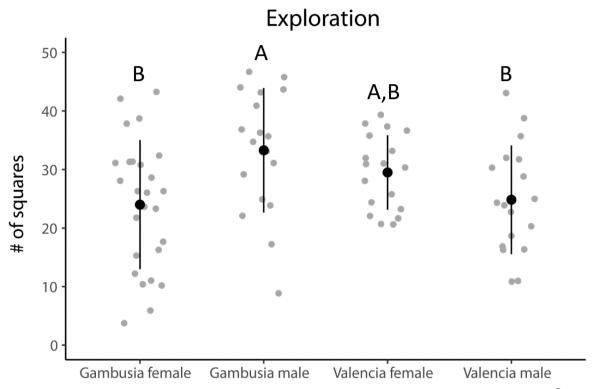


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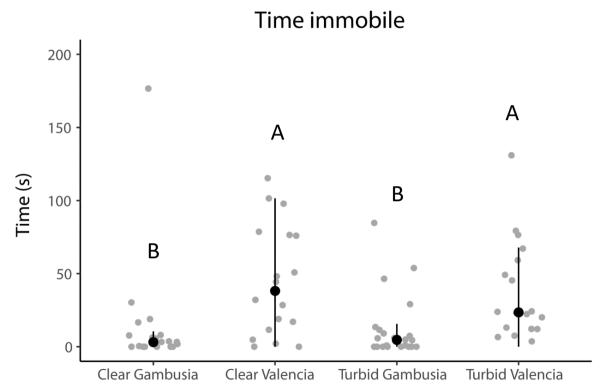


Bristol Workshop 2-way ANOVA: *P* < 0.01



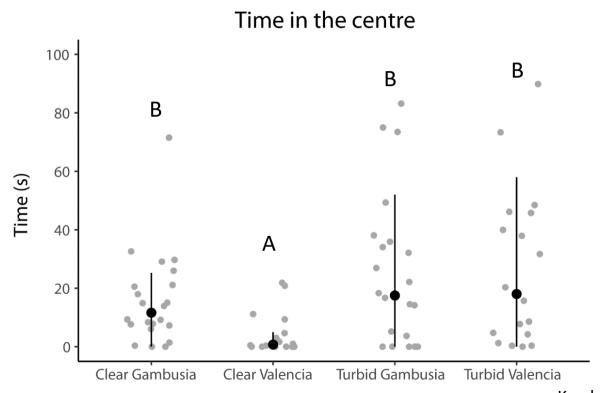


Bristol Workshop 2-way ANOVA: *P* < 0.01





Bristol Workshop Kruskal-Wallis: *P* < 0.001



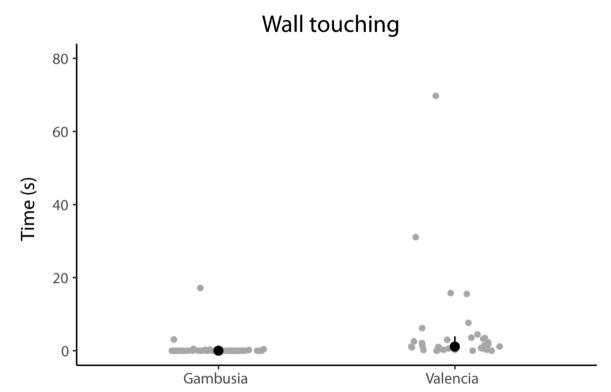


Bristol Workshop Kruskal-Wallis: *P* < 0.01



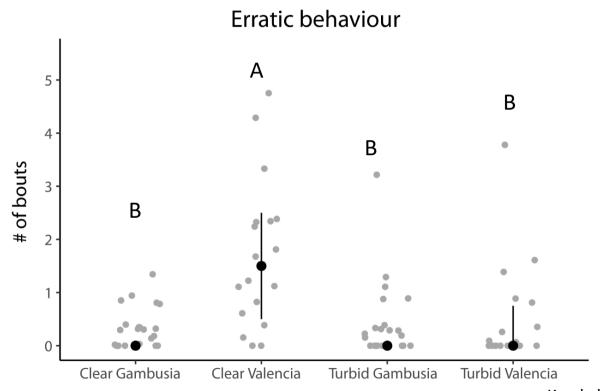


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Bristol Workshop Kruskal-Wallis: *P* < 0.001





Bristol Workshop Kruskal-Wallis: *P* < 0.001

Development of breeding protocols for Valencia robertae and Valencia letourneuxi

Study of their reproductive behaviour and larval development

3 aquaria of 350 l (dimensions 100x70x50 cm) and 3 aquaria 280 l (dimensions 80x70x50 cm) were established at **HCMR facilities** in 2018





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103 *V. robertae* (2018) and 45 *V. letourneuxi* (2019) were transferred to the aquarium facilities





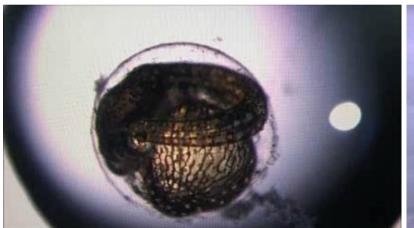
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Both *V. letourneuxi & V. robertae* reproduced in aquaria conditions





Eggs hatched and larvae reared to adult stage F1 generation reproduced in the following year







6. Conservation translocation: creating a refugia population (AFRESH)

The aim was to move adult fish into a safe habitat to act as a **refugia** in the wild for the threatened Corfu killifish *Valencia letourneux*i





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6. Conservation translocation: creating a refugia population (AFRESH)

September 2021

48 adult (>1.7 cm) killifish were transferred to the release site with no mortalities







6. Conservation translocation: creating a refugia population (AFRESH)

June 2022

Larvae presence was confirmed (with hand nets) + enhancement with 36 adult fish

September 2022

Population of various sizes was detected (with electrofishing)





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Bristol

Workshop

7. Creating breeding stocks of 2 range-restricted threatened fishes (AFRESH)

Economidichthys trichonis
Economidis & Miller 1990

Pungitius hellenicus Stephanidis, 1971





November 2021 (1st attempt)

Seine net → 107 fish collected, sorted and transported to HCMR





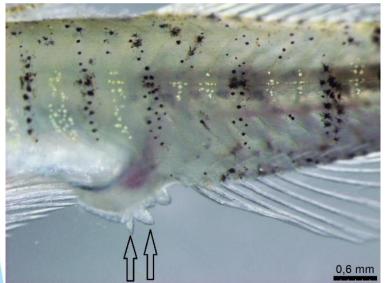
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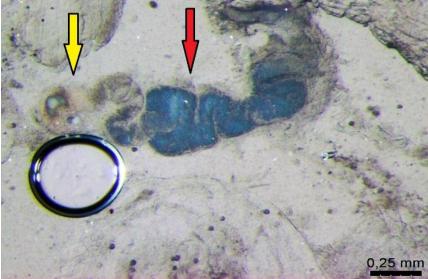
After 4 hours, 58 live fish arrived in the laboratory (46% mortality)

Mortality continued in the following days

histopathological examination of fish

Treated with Emamectin benzoate





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After the treatment!









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ABSTRACT

Yiannis Kapakos, Ioannis Leris, Leonidas Vardakas, Eleni Kalogianni: Collection, transfer and acclimatization of the dwarf goby *Economidichthys trichonis* in a closed circuit system



November 2022 (2nd attempt)

We collected 89 gobies (seine net) → 500 ml plastic bottles + pure oxygen + ammonia detoxifier Transport with 0% mortality + preventive treatment with hemamectin benzoate (same day) Fish consumed food within 24h





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7. Creating breeding stocks of 2 range-restricted threatened fishes (AFRESH) *Pungitius hellenicus*

January 2022 (1st attempt)

We collected 40 sticklebacks (electrofishing), transported in plastic container

Tcollection: ~8 °C → Taquaria: ~20 °C

Fish were not feeding and some males assumed breeding colours

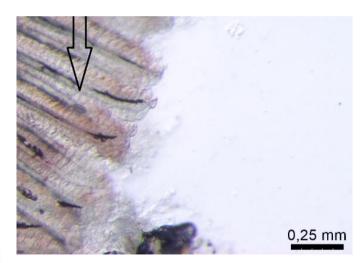


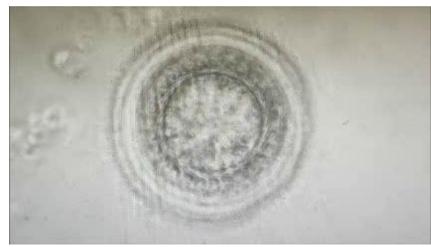


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7. Creating breeding stocks of 2 range-restricted threatened fishes (AFRESH) Pungitius hellenicus

Mortality recorded after three weeks
Infected by *Trichodina* parasites
Treatment with malachite green oxalate & formaldehyde solution
Five individuals eventually survived





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7. Creating breeding stocks of 2 range-restricted threatened fishes (AFRESH) Pungitius hellenicus

November 2022 (2nd attempt)

We collected 32 sticklebacks (electrofishing), transported in plastic container

Tcollection: ~12 °C → Taquaria: ~16 °C

Preventive treatment in the field: malachite green oxalate & formaldehyde solution bath (30 min)

Extended treatment in the lab (3 days)

Use of live food (Tubifex, Artemia, white mosquito larvae, Daphnia)





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8. Future perspectives

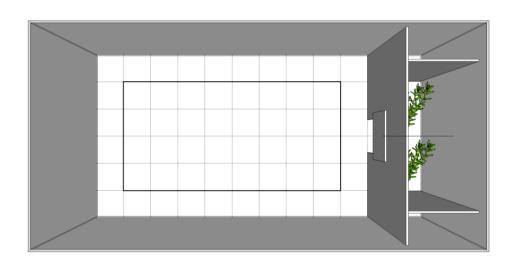
Comparative (killifish – mosquitofish) and new species studies

Behavioural tests

- Emergence test
- Predator response test
- Novel object test

Environmental factors

- Temperature
- Salinity
- Turbidity





8. Future perspectives

In HCMR facilities there is now a modern laboratory for captive breeding and rearing threatened fish species and for the study of their behavior (social, reproductive etc)



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Thank you for your attention!



