

## Introduction

The primary aim of this survey was to investigate the occurrence of the presumed-extinct Cyprus freshwater blenny (*Salariopsis sp.*) in Cyprus, in watercourses with the highest probability of occurrence based on habitat requirements. Between the 24th and 28th of October 2022, the Freshwater Life Project team collected and filtered 20 samples from 19 coastal sites and 1 inland site, which were subsequently tested for the presence of teleost eDNA [See Map 1]. The sample sites are representative of 17 river systems in Cyprus comprising 19 both lotic and lentic estuaries of both fresh and brackish water, and 1 inland freshwater river at higher altitude (site 20).

Site 20 was selected as an upstream replacement for the estuary of the Amathos River, which has been highly developed, and now functions as a road and parking area and beach access for boating/tourism activities. The Amathos River is one of only two perennial rivers situated within the historic geographic range of the Cyprus freshwater blenny and thus is highly likely to have been one of the 3 known locations where Roland L. N. Michell first collected the fish in 1909. Site 20 is considerably further inland and situated at a higher altitude, but due to being easily accessible, relatively undisturbed, and at a significant distance from the Germasogeia dam, a larger dam that contains high levels of non-native predatory fishes, it was selected as a sample site for finding the target species.

The used sample kits were received by the analytic laboratory in November 2022, and the results were provided to Freshwater Life Project in January 2023.

The selected sites were chosen based on the presence of one or more of the following criteria:

- Perennial and quasi-perennial river systems (flowing or pools at end of driest period)
- Similar habitats to *S renatorum* and *S. burcucae* occurring in countries neighbouring Cyprus (Turkey, Syria, Lebanon, Israel) [See Map 2]
- Habitats within the known historic range of the species (Limassol district)
- Appropriate aquatic habitat and conditions suitable for the survival and reproduction of freshwater blenny

With these results, we also gathered valuable information on the distribution of other native species of conservation concern, including the critically endangered European eel (*Anguilla anguilla*), and non-native fishes.

## Key Findings

- The eDNA results did not identify the presence of the Cyprus freshwater blenny *Salariopsis sp.* in the samples taken.
- The most commonly detected species across the samples was the European eel *Anguilla anguilla*. This is significant as this species is listed as Critically Endangered on the International Union for the Convention of Nature (IUCN) Red List of Threatened Species. The European eel is also listed in Annex II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). European eel was recorded in 13 of the total 19 sites analysed for eDNA (metabarcoding was unsuccessful for site 16). Zogaris (2012) found that European Eel is the native fish most widespread across the Island, and the current results confirm this is still the case.
- A total of 18 taxa were recorded across the 19 survey sites. Species richness ranged from 1 to 8, and the average richness was 3.2. This indicates a low diversity in taxa across the sites.
- With regards to taxa families, the best-represented families were Mugilidae, with 5 species present, and Cyprinidae, with 4 species present.
  - For the Mugilids, also known as the Mullet family, the species recorded were the Golden grey mullet *Chelon auratus*, recorded at site 5 and 14, Thicklip grey mullet *C. labrosus*, recorded at sites 5, 8, 9, 11, 14, and 18, and Flathead grey mullet *Mugil cephalus*, recorded at sites 5, 9, 11, 14, 15 and 18. These species are native to the Mediterranean Sea and thus are native to the waters surrounding the islands. These species are also listed with a conservation status of “Least Concern” on the IUCN Red List. Another *Chelon* sp. was also recorded at site 14 but could not be analysed to species level. At site 14 there was also Mugilidae sp. recorded which again could not be analysed to species level. This may be due to limitations on databases.
  - For the Cyprinids, also known as the carp or minnow family, the 3 species recorded were the Amur carp *Cyprinus rubrofasciatus*, recorded at site 17, Common roach *Rutilus rutilus*, recorded at sites 17 and 20, and Goldfish *Carassius auratus*, recorded at sites 11, 17 and 20. These species are non-native to the Island and are each listed as “Least Concern” on the IUCN Red List.
- The Centrarchidae family is represented in the eDNA results by two species: Pumpkinseed pond perch *Lepomis gibbosus* and Largemouth bass *Micropterus salmoides*. Both species are non-native, listed as Least Concern on the IUCN Red List, and were only recorded at one location, site 17.
- Other families that were represented by one species in each were Atherinidae, Poeciliidae, Cichlidae, Moronidae, and Ictaluridae.
  - Atherinidae was represented by the Red sea hardy head silverside *Atherinomorus forskalii*. This species is not evaluated on the IUCN Red List and was recorded at site 9

- and 12. Another Atherinidae sp. was also recorded at site 5, with the highest proportion of DNA in that sample, but could not be analysed to species level.
- Poeciliidae was represented by the invasive Eastern mosquitofish *Gambusia holbrooki*. This species was recorded at sites 4, 8, 9, and 14 and is listed as Least Concern on the IUCN Red List.
  - Cichlidae was represented by the Blue tilapia *Oreochromis aureus* / Nile tilapia *Oreochromis niloticus*. The eDNA results show either/or species recorded at site 17. Both species are listed as Least Concern on the IUCN Red List.
  - Moronidae was represented by the European bass *Dicentrarchus labrax*. Recorded just at site 9, this species is native to the Mediterranean waters surrounding the island. European bass is also listed as Least Concern on the IUCN Red List.
  - Ictaluridae was represented by the Channel catfish *Ictalurus punctatus*, also listed as Least Concern on the IUCN Red List. This species was recorded at site 17 and accounted for the highest proportion of DNA within the sample.
- The order Perciformes was also represented by a record at site 17, which could not be analysed to family level.
  - Outside the class of bony fish, Actinopterygii, some DNA was also picked up for class Aves, comprising birds, and class Mammalia, comprising mammals. In class Aves, Moorhen *Gallinula* sp. was recorded at site 17. In class Mammalia, Brown rat *Rattus rattus* were recorded at sites 6, 12, 13, and 20 and Donkey *Equus acinus* was recorded at site 4.
  - Visual observations during the sampling period also included the Annex II EU protected species the Mediterranean killifish *Aphanius fasciatus* at sites 1 and site 5.

**Results**

| Observations                | Site 1                     | Site 2        | Site 3 | Site 4                    | Site 5                    |
|-----------------------------|----------------------------|---------------|--------|---------------------------|---------------------------|
| <b>eDNA (Teleost)</b>       |                            |               |        | <i>Anguilla anguilla</i>  | <i>Anguilla anguilla</i>  |
|                             |                            |               |        | <i>Gambusia holbrooki</i> | <i>Atherinidae sp</i>     |
|                             |                            |               |        |                           | <i>Chelon labrosus</i>    |
|                             |                            |               |        |                           | <i>Mugil cephalus</i>     |
|                             |                            |               |        |                           |                           |
| <b>eDNA (Non-Teleost)</b>   |                            |               |        | <i>Equus asinus</i>       |                           |
| <b>Visual (Teleost)</b>     | <i>Aphanius fasciatus</i>  | Mugilidae sp. |        |                           | <i>Aphanius fasciatus</i> |
| <b>Visual (Non-Teleost)</b> | <i>Callinectes sapidus</i> |               |        |                           |                           |
|                             |                            |               |        |                           |                           |

| Observations                | Site 6                       | Site 7                       | Site 8                    | Site 9                         | Site 10                    |
|-----------------------------|------------------------------|------------------------------|---------------------------|--------------------------------|----------------------------|
| <b>eDNA (Teleost)</b>       | <i>Anguilla anguilla</i>     | <i>Anguilla anguilla</i>     | <i>Anguilla anguilla</i>  | <i>Anguilla anguilla</i>       | <i>Anguilla anguilla</i>   |
|                             |                              |                              | <i>Gambusia holbrooki</i> | <i>Atherinomorus forskalii</i> |                            |
|                             |                              |                              | <i>Chelon labrosus</i>    | <i>Chelon labrosus</i>         |                            |
|                             |                              |                              |                           | <i>Dicentrarchus labrax</i>    |                            |
|                             |                              |                              |                           | <i>Gambusia holbrooki</i>      |                            |
|                             |                              |                              |                           | <i>Mugil cephalus</i>          |                            |
| <b>eDNA (Non-Teleost)</b>   | <i>Rattus rattus</i>         |                              |                           |                                |                            |
| <b>Visual (Teleost)</b>     |                              |                              |                           |                                |                            |
| <b>Visual (Non-Teleost)</b> | <i>Melanopsis sp</i>         | <i>Motacilla cinerea</i>     | Macrophyte                | Corixidae sp                   | <i>Carduelis carduelis</i> |
|                             | <i>Motacilla cinerea</i>     | <i>Pelophylax cypriensis</i> |                           |                                | <i>Melanopsis sp</i>       |
|                             | <i>Pelophylax cypriensis</i> |                              |                           |                                |                            |
|                             | <i>Potamon potamios</i>      |                              |                           |                                |                            |

| Observations                | Site 11                  | Site 12                        | Site 13                   | Site 14                  | Site 15                  |
|-----------------------------|--------------------------|--------------------------------|---------------------------|--------------------------|--------------------------|
| <b>eDNA (Teleost)</b>       | <i>Anguilla anguilla</i> | <i>Anguilla anguilla</i>       | <i>Anguilla anguilla</i>  | <i>Anguilla anguilla</i> | <i>Anguilla anguilla</i> |
|                             | <i>Carrasius auratus</i> | <i>Atherinomorus forskalii</i> | <i>Gambusia holbrooki</i> | <i>Chelon auratus</i>    | <i>Mugil cephalus</i>    |
|                             | <i>Chelon labrosus</i>   |                                |                           | <i>Chelon labrosus</i>   |                          |
|                             | <i>Mugil cephalus</i>    |                                |                           | <i>Chelon sp.</i>        |                          |
|                             |                          |                                |                           | <i>Mugil cephalus</i>    |                          |
|                             |                          |                                |                           | Mugilidae sp             |                          |
| <b>eDNA (Non-Teleost)</b>   |                          |                                | <i>Rattus rattus</i>      |                          |                          |
| <b>Visual (Teleost)</b>     |                          |                                |                           |                          |                          |
| <b>Visual (Non-Teleost)</b> | <i>Melanopsis sp</i>     | <i>Melanopsis sp</i>           | <i>Alcedo atthis</i>      |                          |                          |
|                             |                          | <i>Pelophylax cypriensis</i>   | Aquatic snail             |                          |                          |
|                             |                          |                                | Macrophyte                |                          |                          |

| Observations                | Site 16                      | Site 17                             | Site 18                   | Site 19                    | Site 20                      |
|-----------------------------|------------------------------|-------------------------------------|---------------------------|----------------------------|------------------------------|
| <b>eDNA (Teleost)</b>       | *Failed to amplify*          | <i>Carrasius auratus</i>            | <i>Anguilla anguilla</i>  |                            | <i>Carrasius auratus</i>     |
|                             |                              | <i>Cyprinus rubrofuscus</i>         | <i>Chelon labrosus</i>    |                            | <i>Rutilus rutilus</i>       |
|                             |                              | <i>Rutilus rutilus</i>              | <i>Mugil cephalus</i>     |                            |                              |
|                             |                              | <i>Lepomis gibbosus</i>             |                           |                            |                              |
|                             |                              | <i>Micropterus salmoides</i>        |                           |                            |                              |
|                             |                              | <i>Oreochromis aureus/niloticus</i> |                           |                            |                              |
|                             |                              | <i>Ictalurus punctatus</i>          |                           |                            |                              |
| <b>eDNA (Non-Teleost)</b>   |                              | <i>Gallinula sp.</i>                |                           |                            | <i>Rattus rattus</i>         |
| <b>Visual (Teleost)</b>     |                              |                                     | Atherinidae sp            | <i>Anguilla anguilla</i> * |                              |
| <b>Visual (Non-Teleost)</b> | <i>Pelophylax cypriensis</i> |                                     | 2 x Aquatic snail         |                            | <i>Pelophylax cypriensis</i> |
|                             |                              |                                     | Macrophyte                |                            | <i>Potamon potamios</i>      |
|                             |                              |                                     | <i>Trithemis annulata</i> |                            |                              |

\*Eel were visually observed at this site 7 days prior to the day of eDNA sampling. Construction works were taking place instream on the day of eDNA sampling.  
Exact locations not disclosed for protection of species with threatened and/or protected status

## Limitations and Challenges

Whilst this survey did provide a good indication of the absence of freshwater blenny from all of the sites sampled, due to the significant and diverse habitats encountered, further sampling would be wise to provide a more definitive conclusion. As eDNA provides results for a snapshot of an area at a certain time, in dynamic coastal habitats with temperature and habitat fluctuations, there are limitations with representative sampling and limits of detection. Increasing the number of sample locations will increase the level of certainty regarding presence/absence of a particular species.

Site 16 is also a candidate site for retesting as it is situated within the historic geographic range of the blenny and failed to amplify despite the troubleshooting methods employed by the laboratory team.

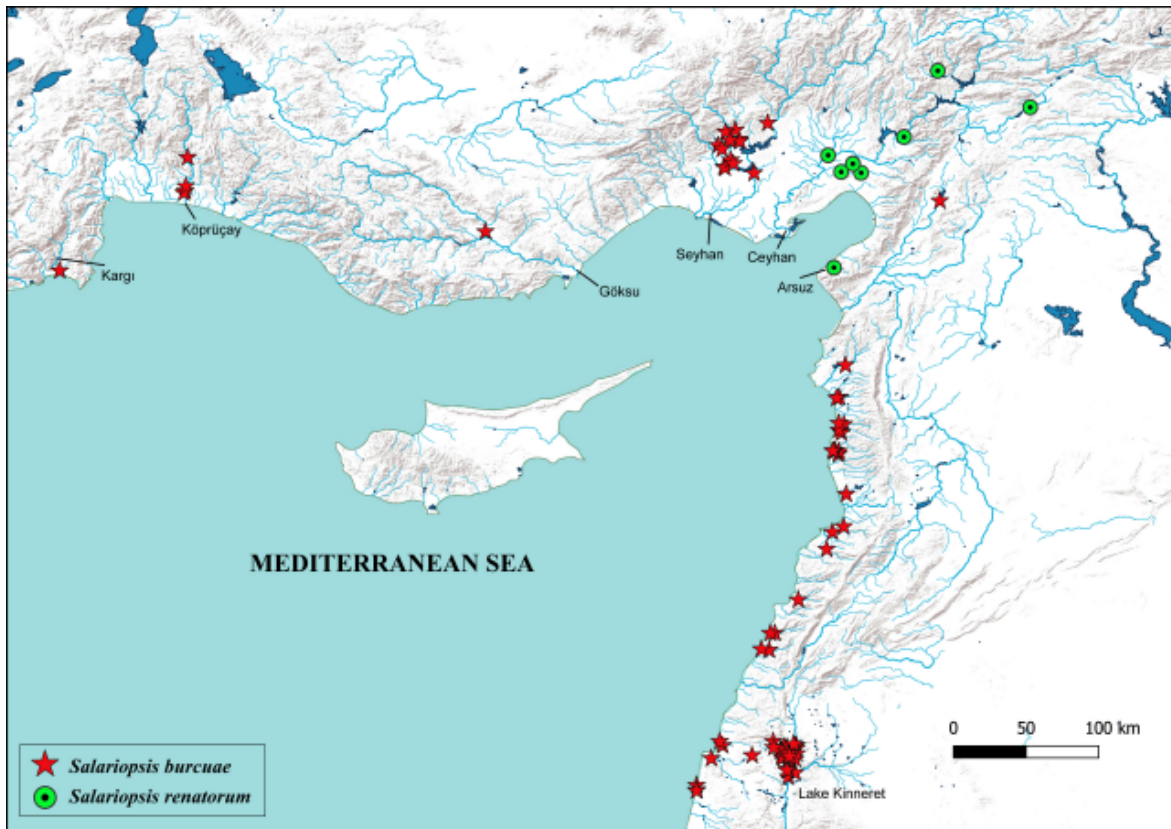
There are a number of non-coastal sites situated further inland which have not previously been sampled for eDNA, but qualify for sampling based on the suitability of habitat and their equivalence with freshwater blenny (*Salariopsis sp.*) occurrences in other countries not already mentioned; this includes sites at higher altitudes and in lakes.

A number of visual observations and dip net collections of teleosts were made at some sample sites, that were not detected by the test kits, inferring that although a complete island-wide survey will provide a very strong indication of the presence/absence of the freshwater blenny in Cyprus, all avenues and methods should be explored, especially at sites with a high diversity of species/habitats, prior to confirmation of extirpation. Multiple testing and/or slightly altering testing methods to accommodate for the elusive and benthic nature of the blenny in some locations could also allow for a higher likelihood of capturing any otherwise undetectable DNA.

## Surveying



**Map 1** ○ Sites successfully surveyed using eDNA kits in October 2022 (Source: google maps)



**Map 2** *Salariopsis* populations occurring in coastal habitats in neighbouring middle eastern countries (Source: Yoğurtçuoğlu et al, 2023)



## **Discussion**

### The Cyprus Freshwater Blenny

The results of this research infer the absence of the Cyprus freshwater blenny (*Salariopsis sp.*) from all of the sampled waterbodies.

### The European Eel

Our research also finds that although critically endangered, the European eel (*Anguilla anguilla*) is abundant at coastal sample sites, being present at 70% of the sites sampled. This corroborates the findings by Griffiths et al (2021) which reported an abundance of European eel at lowland elevations in Cyprus versus a notable absence in perennial systems at higher elevations. Throughout the survey period, repeatedly observed threats to European eel included a lack of access to suitable perennial freshwater habitats, such as destroyed, blocked, or modified estuaries and fragmented river systems, unsuitable physicochemical water conditions, and the lack of ecologically diverse freshwater streams/rivers that provided either sufficient water, sufficient food resources (complete trophic webs), or both. These factors likely significantly reduce the survival rate of the species during its freshwater phase.

### Non-Native Species

Our results indicate that native species represented approximately 52% of all recorded aquatic species from both eDNA and field observations at sample sites, with the remaining ~48% of aquatic species comprising non-native species.

### Conclusion

In addition to the extensive research that was conducted that failed to identify any evidence of the Cyprus freshwater blenny across 53 sites in 18 river basins between 2009-2011 (Zogaris et al 2012), between 2012-2022, the Freshwater Life Project team has conducted sporadic search-find surveys of perennial, intermittent, and ephemeral rivers and streams across over 25 river basins, including extensive research within the historic geographic range of the Cyprus freshwater blenny that also revealed no sign of the species. That being said, with the emergence and availability of eDNA technology that continues to provide tangible and largely accurate insight into the biodiversity of Cypriot aquatic ecosystems, we deduce that the search for the Cyprus freshwater blenny cannot be concluded without further eDNA sampling at other sites not already sampled for eDNA, specifically, those that correspond with freshwater blenny (*Salariopsis sp.*) habitats in other countries not already mentioned. These should include sites further inland, lake sites, and sites at higher altitudes. Pooling existing research already conducted by the competent authorities and other local organisations will maximise the efficiency of this process.

## **Solutions**

### Ecological Impact Assessment

With non-native fishes comprising a considerable portion of the species recorded during the survey period, assessing their ecological impact and how they interact with native species, particularly threatened species, is crucial to ensure the success of any conservation measures implemented for native species. Native species richness, particularly in the Mediterranean, has been observed to decline at sites with more non-native piscivores (Clavero et al, 2013). Equally, removing non-native species can be extremely difficult to achieve, and in some cases can be counter-productive, especially in already degraded habitats where they can occasionally uphold struggling ecosystems by serving as part of a depleted food web. Ascertaining the overall impact of interactions between native and non-native species will identify if or what mitigation actions are necessary for managing non-native species, better ensuring the long-term viability of any subsequent conservation strategy.



### Habitat Restoration Plan

A strategic habitat restoration plan that highlights precise restoration measures for lowland stretches of perennial and even semi-perennial rivers, including their estuaries, will seek to provide guidance for creating healthy refuges with enriched food webs capable of sustaining healthy populations of European eel, and juvenile food-fish species such as the sea bass *Dicentrarchus labrax* and gilt-head bream *Sparus aurata*, among others, which both rely heavily on estuaries and freshwater habitats for their survival (Kelley, 1988), (Isnard et al, 2015). Additionally, if executed in collaboration with planning departments, these habitat enhancements can function as key features in sustainable urban development planning and contribute to overall citizen welfare and well-being. In this way, prioritising habitat restoration in lowland river stretches has the potential to simultaneously enhance urban communities, support local fisheries, and provide conservation sites for species at risk.

### References

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