

Shark and Ray Areas globally will transform shark conservation and contribute to reducing mortality in this highly threatened group.

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Creating safety stock populations of two of the smallest threatened freshwater fishes of Europe

The dwarf goby *Economidichthys trichonis* and the Greek stickleback *Pungitius hellenicus* are both endemic to Greece, have narrow geographical ranges and are categorized as Endangered and Critically Endangered, respectively, on the IUCN Red List. The creation of safety stock populations is a top priority for future conservation translocations of the two species. Therefore, within the framework of conservation project AFRESH (afresh.hcmr.gr), we collected, transferred and acclimatized individuals of both species to closed circuit systems in 2021 and 2022.

The first attempt to transfer *E. trichonis* (maximum length 3 cm), on 10 November 2021, involved the collection with a seine net of 107 individuals from Lake Trichonis, at a water temperature of 19 °C. Fish were transported in an 80 l aerated tank. Mortality upon arrival following a transport time of 4 h was 46%, attributed to the mode of transport and/or to intestinal parasitosis. Despite subsequent anti-parasite treatment, only 12 individuals survived. We identified transport stress and intestinal parasitosis as the causes of mortality, to be addressed through modification of the collection and transport protocol. On 16 November 2022, 89 fish were caught with a seine net at a water temperature of 20 °C, and transported in groups of 1–4 fish in plastic bottles supplied with pure oxygen from a portable cylinder. Upon arrival, with no mortalities, fish were acclimatized to 17.7 °C and then treated with anti-helminthic medication. They were kept initially in low lighting and provided with live *Artemia* nauplii daily. Fish were observed feeding



A dwarf goby *Economidichthys trichonis* (total length 2.5 cm) guarding its nest in a reed. Photo: Y. Kapakos.

after 48 h, and 3 weeks after transfer all fish appeared healthy, with no mortalities.

The first attempt to transfer *P. hellenicus* (maximum length 5 cm), on 19 January 2022, involved the collection of 40 fish by electrofishing, from an irrigation channel at a water temperature of 8 °C, in Sperchios basin, and transfer in an 80 l aerated tank, with a transport time of 3 h. Transport was completed with no mortalities and fish were then acclimatized to 20 °C. However, there were primary and secondary parasite infections, and the fish did not feed, with mortalities starting after 3 weeks. Only five individuals survived. We identified the temperature difference between collection site and housing aquaria, and absence of feeding, with subsequent parasitosis, as the main issues to be addressed. On 9 November 2022, 32 *P. hellenicus* were collected, at a water temperature of 12.2 °C, and, following anti-parasite treatment, they were transferred to the laboratory and acclimatized to 15.6–16.6 °C. They were offered live *Artemia* nauplii and *Tubifex* worms, and were observed feeding soon after transfer. Four weeks after transfer mortalities were zero.

This work highlights the need to address a wide array of issues related to handling, mode of transfer, acclimatization issues, disease prevention and dietary differences when creating fish stock populations of threatened species.

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