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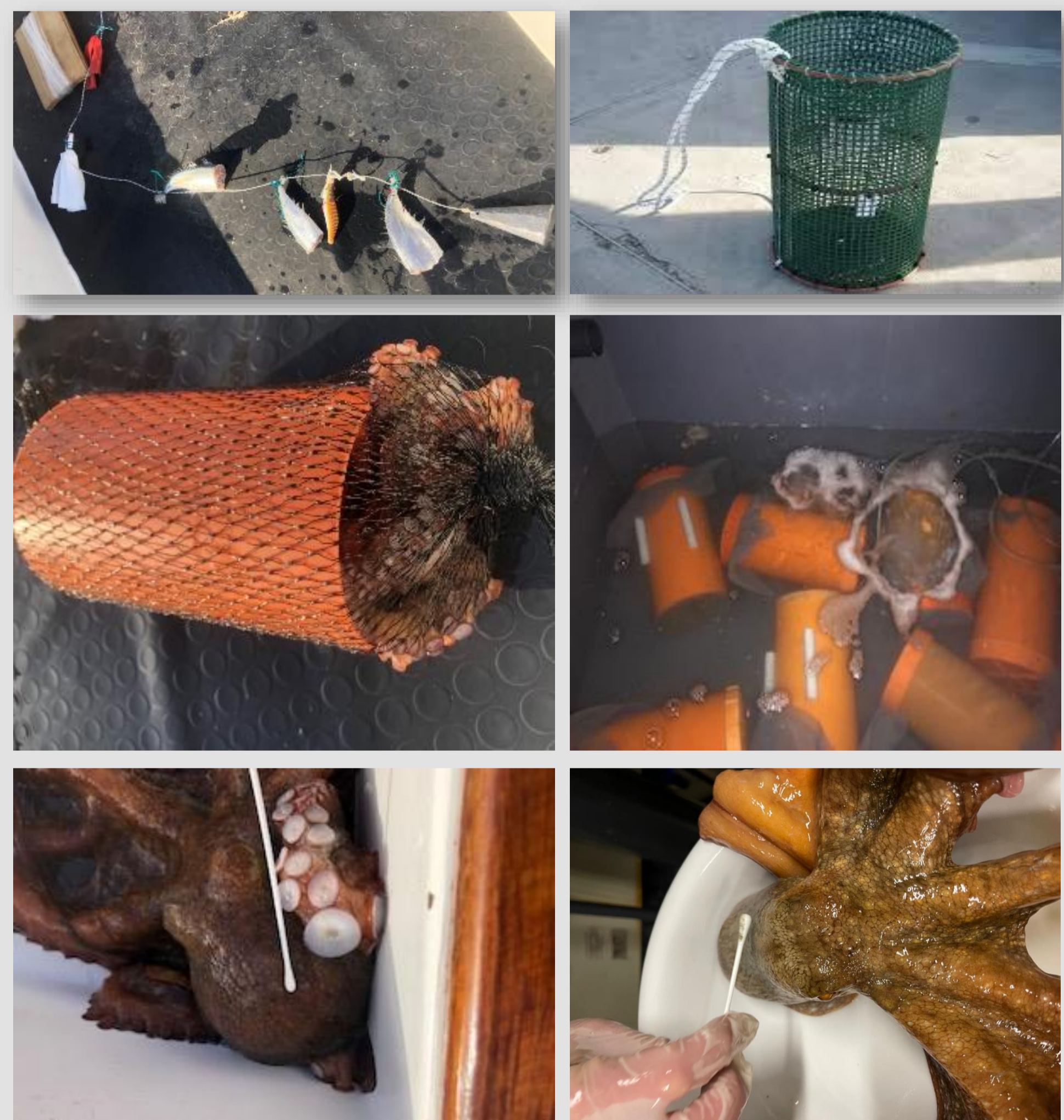
## INTRODUCTION

*Octopus vulgaris* (Cuvier, 1797) is a highly valued species worldwide and its demand has increased significantly in recent years (Iglesias et al., 2014). Specimens are caught in industrial and artisanal fisheries and have been overexploited in recent decades (Sauer et al., 2021). All steps involved in the capture of octopus should be able to avoid any form of PSDLH and should best reduce the stress associated with the severity of the capture technique (Pieroni et al., 2022). Similarly, the transport system (type of tank, isolation, number of individuals, water quality, temperature), timing and inter-species variability (e.g. body size) must be considered when preparing the specimen for the journey. Hence, continuous monitoring of hormones that respond to a stressor throughout can assist with interpretation of octopus behaviour observations. Developing techniques to measure hormones and repeat them on the same individual in a non-invasive way is essential to protect the welfare of the octopus (Chancellor et al., 2021).

The aim of this study was to develop a non-invasive method to collect and quantify cortisol levels from octopuses caught using two different fishing techniques (trap and handline) and transported to a facility.

## MATERIALS AND METHODS

Wild sub-adults of *O. vulgaris* were caught using a traditional handline (“polpara”) capture system (n.7: 735.2±35.7g) and traps (n.7: 855.4±84.1g) in the Ionian Sea. Each animal was placed in a PVC cylinder, which was netted to avoid aggression, and transported in an insulated tank (300 L) to the laboratory in Cesenatico. Animals were swabbed on capture and on arrival at the facility (after 10 h). The swab (Puritan Sterile Polyester Tipped Applicators) was applied to the dorsal part of the mantle and cut to fit into in a 2 mL tube containing 1 mL of 70% ethanol. Samples of skin mucus were collected by scraping the side of the octopus with cotton buds and samples were stored at -20°C until the analysis. Cortisol was measured with a specific microtitre radioimmunoassay (RIA), as described by Bertotto et al. (2010) with a few modifications eg incubation with the anti-cortisol serum solution lasting 2h at 37°C. Mucus was not extracted, but only sometimes diluted with PBS, and aliquots of 10 microliters were used for RIA.



## RESULTS AND DISCUSSION

Skin swabs have been used previously in fish (Santymire et al., 2022; De Mercado et al., 2018; Schultz et al., 2005) and amphibians (Santymire et al., 2018).

The results show that cortisol levels at the time of capture were not statistically different in trapped compared to handline captured subjects (0.793±0.204 vs. 0.675±0.249). After transport and arrival at the facility, cortisol levels decreased for both capture techniques. This shows that the mode of transport did not affect the stress level of the octopus, but contributed to a reduction in cortisol level.

The values obtained in this study are in line with those found by Chancellor et al. (2021) on other cephalopod species. In the future, more days of monitoring will be needed after the move to a new habitat in order to have evidence of full acclimatisation of the octopuses.

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