

L. CASTELLANO¹, C. ARCURI^{1,2,3}, N. PEREZ¹, A. ATZORI², M. SPOTO^{2,4}, M.C. CATTÀ², G. CORRIERO³, T. LAZIC³, M. RAVISATO³, C. PIERRI³, M. GRISTINA²

¹Acquario di Genova – Costa Edutainment SpA - Area Porto antico 16128 Genova

²IAS-CNR, Lungomare Cristoforo Colombo, 4521 90149 - Palermo

³DBBA – Università di Bari, Via Orabona 4, 70125 Bari

⁴ BiGeA- Università di Bologna, Via Zamboni 67, 40126 Bologna

corresponding author: lcastellano@costaedutainment.com

HIPPOCAMPUS GUTTULATUS (CUVIER, 1829) AND H. HIPPOCAMPUS (LINNAEUS, 1758) CAPTIVE BREEDING PRELIMINARY DATA

DATI PRELIMINARI SULLA RIPRODUZIONE IN AMBIENTE CONTROLLATO DI HIPPOCAMPUS GUTTULATUS (CUVIER, 1829) E H. HIPPOCAMPUS (LINNAEUS, 1758)

Abstract - Mediterranean seahorses (*Hippocampus guttulatus* and *H. hippocampus*) are undergoing marked population declines due to habitat degradation, bycatch, and illegal harvesting linked to demand for traditional Chinese medicine. Conservation efforts require effective tools such as captive breeding programs to support population recovery. This study developed a standardized breeding protocol for both species, optimizing husbandry conditions, reproductive output, and animal welfare. Ten breeding pairs (seven *H. guttulatus*, three *H. hippocampus*) were collected from the Mar Piccolo of Taranto and acclimated at the Acquario di Genova. Diets, adjusted to developmental stages, included live prey (*Acartia tonsa*, *Artemia salina*, *Neomysis integer*) enriched with supplements and offered *ad libitum* under controlled water parameters. Overall, 159 *H. guttulatus* and 100 *H. hippocampus* survived to one year, reaching adult sizes. The results confirm the feasibility of captive breeding and provide a practical framework for population reinforcement and long-term conservation.

Keywords: *Hippocampus hippocampus*; *Hippocampus guttulatus*, captive breeding protocol

Introduction - Seahorse populations (*Hippocampus* spp.) worldwide are facing severe threats from habitat degradation, bycatch in commercial and artisanal fisheries, and overexploitation driven by demand in traditional medicine (Vincent, 1996). In recent years, both Mediterranean seahorse species *Hippocampus guttulatus* Cuvier, 1829 and *H. hippocampus* (Linnaeus, 1758) have been illegally harvested in several European regions, presumably to supply the traditional Chinese medicine market. This exploitation has likely contributed to population declines and underscores the urgent need for targeted conservation measures (Pierri *et al.*, 2021; Correia, 2022). Evidence of population reinforcement of threatened species is well-documented for many vertebrate groups, including freshwater fishes (Seddon *et al.*, 2014). In contrast, evidence for marine fishes (e.g., Welsh *et al.*, 2020) and, particularly, syngnathids remains sparse (Woods, 2020). The primary goal of the present study was to establish a standardized and comprehensive breeding protocol for the two Mediterranean seahorse species, ensuring optimal husbandry conditions, enhancing reproductive success, and prioritizing animal welfare. Defining best practices for captive breeding is crucial not only to ensure the sustainability of conservation programs but also to support future reintroduction initiatives and the reinforcement of local wild populations.

Material and methods - A total of ten breeding pairs (seven *H. guttulatus* and three *H. hippocampus*) were collected from the Mar Piccolo of Taranto (southern Italy) and

transported to the Acquario di Genova, where they were acclimated in dedicated rearing systems equipped with mating and rearing tanks (Fig. 1a). The physical and chemical water parameters were closely monitored and maintained as follows: temperature 22 ± 1 °C, salinity 37 ± 1 ppt, pH 8.0 ± 0.15 , with ammonia and nitrite maintained at undetectable levels. The seahorses were provided with a diet tailored to their developmental stage, established through multiple feeding trials involving different prey types. Individuals were fed *ad libitum* with live prey to ensure constant food availability. The live prey included *Acartia (Acanthacartia) tonsa* Dana, 1849-1852 (adult copepods), *Artemia salina* (Linnaeus, 1758) (nauplii, metanauplii, and adults), and *Neomysis integer* (Leach, 1814) (subadult and adult mysids) (Fig. 1b). All prey species were cultured under controlled environmental conditions and enriched with nutritional supplements (Easy DHA, Spresso, Red Algamac, and live microalgae) to optimize their nutritional content. Standard length (SL) and total weight (TW) of newborn seahorses was measured according to Lourie *et al.* (1999), from the first day post-parturition to adult age and the relationships between SL and age (days) were calculated (Fig. 2). Furthermore, egg development time was recorded and fertility, expressed as the number of juveniles per reproductive event, was calculated.

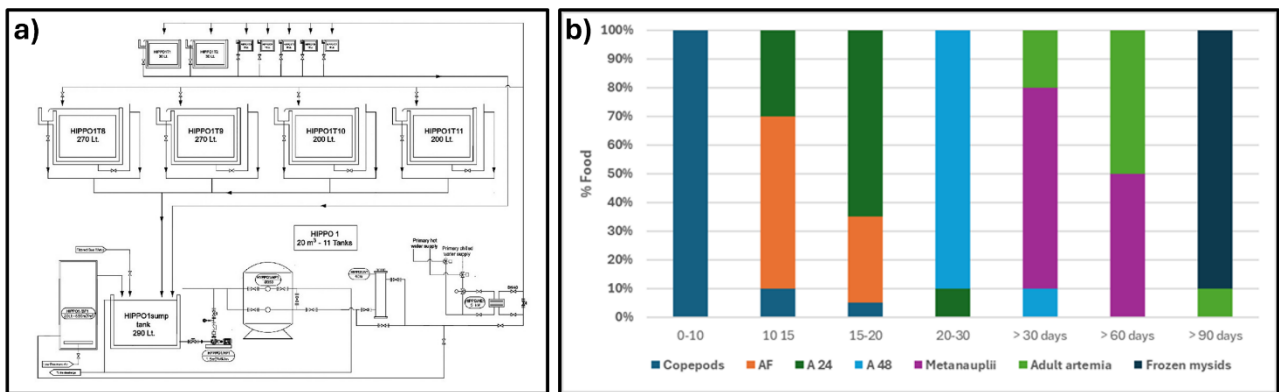


Fig. 1 - (a) Breeding and rearing Life support System; (b) Diet of newborn seahorses from day 0 to day 90, AF: smaller *Artemia* nauplia, A24 EG: 24h *Artemia* nauplia, A48 EG: 48h enriched *Artemia* nauplia.

(a) Life Support System per riproduttori e allevamento giovanili; (b) Dieta dei nuovi nati dal giorno 0 al 90, AF: nauplii di *Artemia* più piccoli, A24 EG nauplii *Artemia* 24h, A48: nauplii *Artemia* arricchita 48 h.

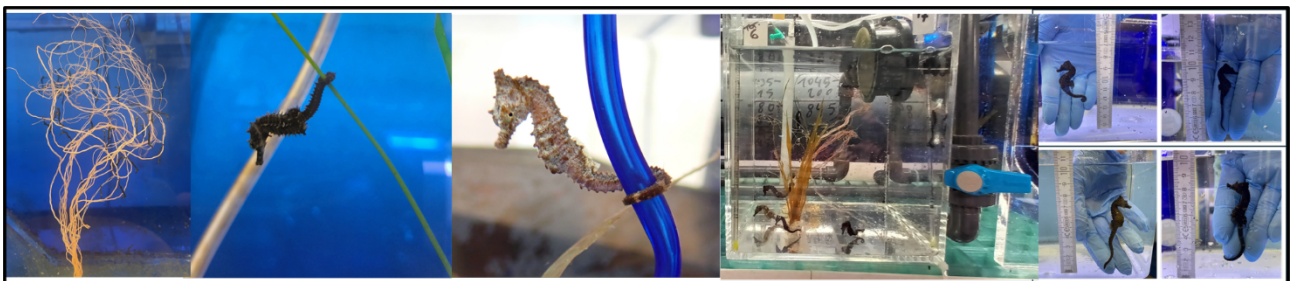


Fig. 2 - Seahorse juveniles at different ages; on the right: non-invasive measurement procedures. Giovanili di cavalluccio a diverse età; a destra: procedure per misure non invasive.

Results - The breeding pairs adapted well and rapidly to the new environment, readily feeding on both live and frozen prey and displaying courtship behaviors followed by matings (Fig. 3a). Egg development within the male’s brood pouch lasted from 18 to 24

days at 22 ± 1 °C in both species. Fecundity was highly variable: in *H. guttulatus* (Fig. 3b), it ranged from 100 to 635, whereas in *H. hippocampus* it ranged from 89 to 734. At birth, *H. guttulatus* exhibited a mean standard length (SL) of 9.16 ± 1.67 mm (mean \pm SD) and a mean total weight (TW) of 0.0028 ± 0.0008 g. One month after birth, juveniles reached a mean SL of 23.65 ± 0.21 mm and a mean TW of 0.0361 ± 0.031 g. After three months, they attained a mean SL of 57.81 ± 2.19 mm and a TW of 0.7970 ± 0.0212 g. *H. hippocampus* juveniles at birth exhibited a mean SL of 6.8 ± 0.41 mm and a TW of $0.000385 \pm 9.19 \times 10^{-5}$ g. After one month, their mean SL increased to 22.8 ± 4.2 mm and TW to 0.019 ± 0.013 g, while at three months they reached a mean SL of 64.5 ± 3.88 mm and a TW of 0.72 ± 0.15 g (Fig. 4a; Fig. 4b). No discernible sexual dimorphism was observed within the first three months of development; however, it became evident at around five months in both species. To date, 159 *H. guttulatus* and 100 *H. hippocampus* individuals have reached one year of age, attaining adult stages with total lengths exceeding 96.0 ± 8.0 mm and 66.1 ± 1.0 mm, respectively.

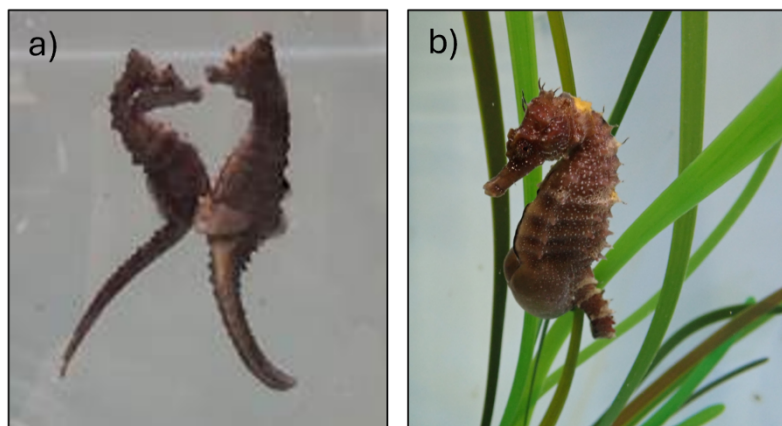


Fig. 3 - (a) *H. hippocampus* mating; (b) *H. guttulatus* pregnant male.

(a) *Accoppiamento di H. hippocampus* (b) *maschio incinto di H. guttulatus*.

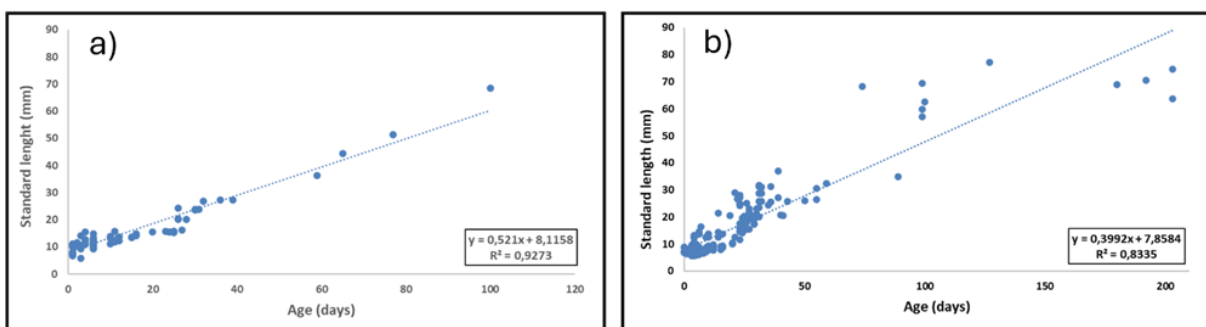


Fig. 4 - Length-age relationship for (a) *H. guttulatus* and (b) *H. hippocampus*.

Relazione lunghezza-età per (a) H. guttulatus e (b) H. hippocampus.

Conclusions - This study demonstrates that both *H. guttulatus* and *H. hippocampus* can successfully adapt, reproduce, and grow under controlled conditions when provided with optimized husbandry practices and appropriate nutritional regimes. The results highlight the importance of species-specific management protocols that account for the biological and ecological requirements of each species. By establishing a standardized captive breeding protocol, this study achieves its primary aim of providing a practical

framework for Mediterranean seahorse conservation. Such a study represents a crucial tool to ensure animal welfare in captivity and support wild population reinforcement, particularly in areas where populations have dramatically declined, such as the Mar Piccolo of Taranto, where losses have reached up to 90% (Pierri *et al.*, 2021). While captive breeding alone is not sufficient, when integrated with broader conservation strategies, including habitat protection, population monitoring, and public awareness initiatives, it provides a strong foundation for the sustainable management and long-term conservation of these emblematic marine species.

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