

# Field studies of seahorse population density, structure and habitat use in a semi-closed north-eastern Mediterranean marine area (Stratoni, north Aegean Sea)

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## Abstract

The present study was carried out in the marine area of Stratoni, Greece, where two seahorse species are present (*Hippocampus hippocampus* and *H. guttulatus*). Two surveys were conducted (September 2016, May 2019) to gather information regarding seahorse species' abundance, distribution and habitat characteristics. Four different seahorse natural and artificial habitat types were identified. Results revealed that the abundance of *H. hippocampus* was relatively high, especially at sites with artificial structures, while the presence of *H. guttulatus* was rare. Data collected can provide baseline information for future population assessments.

**Keywords:** coastal fish, habitat use, artificial structures, threatened species, Mediterranean Sea.

## Significance Statement

Information regarding seahorse species distribution in the Mediterranean is scarce and this study aims to add important data in order to allow a better understanding and inform local decision makers to support further protection measures.

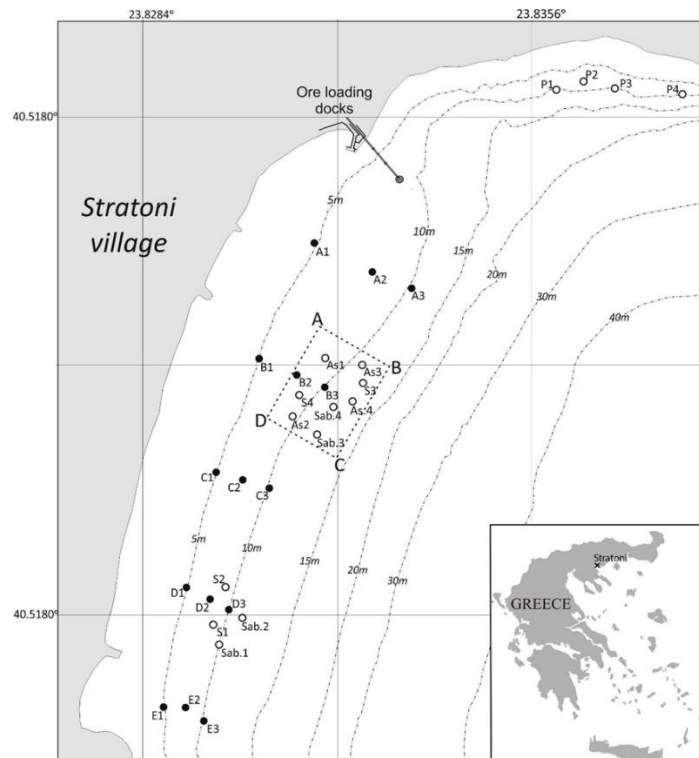
Seahorses (*Hippocampus* spp.) are charismatic and iconic marine fishes, often used as flagship species for conservation issues, that live in some of the most vulnerable marine habitats in shallow areas around the world (Vincent *et al.*, 2011). They are characterized

by sparse distribution, low mobility, small home ranges, low fecundity, lengthy parental care and mate fidelity (Foster and Vincent, 2004). Seahorse life history and behaviour renders them vulnerable to population decline (Vincent *et al.*, 2011), which lead to the inclusion of many seahorse species in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES Convention) and in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species. The short-snouted seahorse *Hippocampus hippocampus* Linnaeus, 1758 and the long-snouted seahorse *Hippocampus guttulatus* Cuvier, 1829 are two species present in the Mediterranean Sea. Although both species have been assessed as ‘Data Deficient’ at a global level, they have recently been re-assessed as ‘Near Threatened’ in the Mediterranean Sea (Pollom, 2014; 2017). They are both typically present in coastal environment, and associated with habitats of different complexity (Correia *et al.*, 2015a; Correia *et al.*, 2018; Woodall *et al.*, 2018). Despite the extended geographical distribution of both species there are only a few inshore locations where seahorse abundance, distribution and habitat use have been studied within the Mediterranean Sea (Louisy, 2011; Gristina *et al.*, 2015; Ape *et al.*, 2019). To our knowledge, ecological data has been rare in Greek waters and especially in the Aegean Sea focusing on wild seahorse population structure (Kitsos *et al.*, 2008; Woodall *et al.*, 2018) including ichthyofaunal assessments (Koutrakis *et al.*, 2005; Lamprakis *et al.*, 2008).

The present study was carried out in the marine area of Stratoni (Chalkidiki Peninsula, north Aegean Sea) which seems to be a refuge for seahorse populations, initially reported by professional divers during fieldwork activities in November 2007. The objectives of the present study were to: (i) describe the seahorses’ habitat types in the Stratoni marine area; (ii) estimate the seahorses’ population density and describe the population structure; (iii) investigate seahorses’ habitat use.

The marine area of Stratoni (Figure 1, latitude from 40°30’ to 40°32.5’ and longitude from 23°45’ to 23°32.5’), lies within Ierissos Gulf, a semi-closed water body, affected by small river inputs, characterized also by soft sediments and high biodiversity (Koutrakis *et al.*, 2003). A first pilot survey of the area was conducted in September 2016 to gather information regarding seahorse abundance, distribution and habitat characteristics using SCUBA underwater visual census. A total of 15 100m-long and 4m-wide transects were laid parallel to the shoreline, at three different depths (5m, 7.5m and 10m), covering a total area of 6,000 m<sup>2</sup>. Whenever an individual of a seahorse was found, species, sex and size (height), was recorded along with four environmental

variables: water depth and water temperature at depth, holdfast at first sighting (structure grasped by a seahorse's tail) and the predominant habitat (i.e. benthos observed within a 1m<sup>2</sup> quadrat centred around the seahorse). This data was used to identify and classify the most representative habitats found according to the European Nature Information System (EUNIS) habitat code (<http://eunis.eea.europa.eu>). In May 2019, four sites were chosen per habitat type and registered in a GPS unit: (a) sand (*Cymodocea* beds. EUNIS A5.5313), characterized by substrates of fine sand, previously covered by *Cymodocea nodosa* beds; (b) *Sabella* sp., (Infralittoral mixed sediments. EUNIS A5.432), dominated by *Sabella* sp. in soft-bottom substrates; (c) *Posidonia oceanica*, (*Posidonia* beds. EUNIS A5.535); (d) artificial structures (ropes) deployed a few months before the first pilot survey. Each site was surveyed by laying a 50m-belt transect, following a bearing parallel to the shoreline to allow constant depth, and a 2m wide strip on each side was covered by two divers. Overall, 16 sites were surveyed for a total area of 3,200 m<sup>2</sup> (Figure 1). The same data was collected as described in the previous survey. In addition, information regarding predominant habitat type was recorded each 10 m along the transect line.



**Figure 1:** Map of the study area (X) of Stratoni (Chalkidiki Peninsula, north Aegean Sea) depicting the arrangement and the code numbers of sampling visual census stations-transects (ABCD delimits the area selected for the deployment of artificial structures).

In 2016, a total of 19 seahorses were sighted (Table I). The seahorse *H. hippocampus* was the most sighted species and female-biased (18 individuals; 5 males, 13 females), while only one *H. guttulatus* male was sighted. Maximum density for *H. hippocampus* was estimated in site C2 (0.015 ind.m<sup>-2</sup>). The one individual of *H. guttulatus* was found in site D3. Almost half of the seahorses were found not grasping any holdfast (52.6%), while 26.3% were observed grasping *Sabella* sp., 15.8% artificial structures and 5.3% shells. All seahorses were found between the 5 m and 10 m depth range. The height of adult individuals of *H. hippocampus* varied from 4.0 to 12.0 cm (mean size of 8.8 ± 2.1 cm), while *H. guttulatus* was 12 cm in height. Temperature of the water column was 22°C.

**Table I:** Abundance indices for *Hippocampus hippocampus* and *Hippocampus guttulatus*.

	2016						2019				
	A	B	C	D	E	pooled	S	Sab	P	As	pooled
<i>Hippocampus hippocampus</i>											
N total	2	0	10	4	2	18	1	5	0	15	21
Males	0	0	2	2	1	5	0	1	0	7	8
Females	2	0	8	2	1	13	0	2	0	6	8
Juveniles	0	0	0	0	0	0	1	2	0	2	5
Mean density (m <sup>-2</sup> )	0.002	0.000	0.008	0.003	0.002	0.003	0.001	0.006	0.000	0.019	0.007
SE	0.001	0.000	0.003	0.003	0.002	0.001	0.001	0.005	0.000	0.008	0.004
<i>Hippocampus guttulatus</i>											
N total	0	0	0	1	0	1	0	1	0	3	4
Males	0	0	0	1	0	1	0	0	0	1	1
Females	0	0	0	0	0	0	0	1	0	2	3
Mean density (m <sup>-2</sup> )	0.000	0.000	0.000	0.001	0.000	0.0002	0.000	0.001	0.000	0.004	0.001
SE	0.000	0.000	0.000	0.001	0.000	0.0002	0.000	0.001	0.000	0.002	0.001

Along the 16 transects surveyed in 2019, a total of 25 seahorses were sighted. The seahorse *H. hippocampus* was the most sighted species (n = 21), with a relative abundance 5 times higher than *H. guttulatus* (n = 4). After pooling all sites, the mean density was 0.007 (SE ± 0.004) ind.m<sup>-2</sup> for *H. hippocampus* and 0.001 (SE ± 0.001) ind.m<sup>-2</sup> for *H. guttulatus*. Maximum density for *H. hippocampus* was estimated in site As3 (0.035 ind.m<sup>-2</sup>), while for *H. guttulatus* in site As4 (0.010 ind.m<sup>-2</sup>). A total of four juveniles of *H. hippocampus* were found which corresponded to 19% of total observations for this species. Juveniles varied in size between 4.0 to 5.0 cm. The seahorse *H. hippocampus* was marginally female-biased (52.9%). The sex ratio was not calculated for *H. guttulatus* due to low sighting numbers. Adult individuals of *H. hippocampus* varied in size from 6.0 to 11.5 cm (mean size of 8.9 ± 1.9 cm), while *H. guttulatus* size ranged from 6.5 to 13.5cm (mean size of 8.6 ± 3.0 cm). Temperature varied from 16 to 17°C. Individuals of *H. guttulatus* were absent in Sand and *Posidonia* habitats, while *H. hippocampus* were found in all habitats, excluding *Posidonia*. Most

of the seahorses were sighted grasping artificial structures (73%) and *Sabella* sp. (23%), while just a few were not grasping any holdfast (~4%). Individuals of *H. hippocampus* and *H. guttulatus* were observed in Artificial habitats at a mean density of 0.019 (SE  $\pm$  0.008) ind.m<sup>-2</sup> and 0.004 (SE  $\pm$  0.002) ind.m<sup>-2</sup>, respectively.

This study is the first one to describe population density and structure as well as investigate habitat use of the two seahorse species in Greek seas. Results of the two field surveys and previous underwater observations (Mentogiannis, pers. comm.) indicate that the population of *H. hippocampus* appears to be present over time in the marine area of Stratoni, while individuals of *H. guttulatus* are rare. Considering that both species are classified as “Near Threatened” in the IUCN Red List, it is of the utmost urgency that this baseline information is made available to contribute to appropriate location-specific conservation strategies.

The seahorse *H. hippocampus* mean size is within the range documented for other areas of the Mediterranean Sea (Woodall *et al.*, 2018), while the juvenile fraction seems to reflect a good population status (Woodall, 2009). Furthermore, the high density value of *H. hippocampus* estimated from the second survey at this site is the highest described in the Mediterranean (Woodall *et al.*, 2018), which indicates that the species might find the necessary environmental conditions to settle even in relatively limited areas. Both seahorse species occur in the study area most probably due to high availability of food resources observed (e.g. dense populations of hyperbenthic crustaceans; Koulouri, pers. comm.). The presence of artificial structures (ropes) deployed a few months prior to the 2016 survey might have contributed to aggregate seahorses as they provide much needed holdfast. Seahorse species have been reported as typically associated with seagrass habitats (Foster and Vincent, 2004). However, no seahorse was found in the *Posidonia oceanica* meadows of the study area, which might indicate that other important environmental variables are at play. Considering that seagrass meadows have been found to be a very important habitat for seahorses as they provide shelter and food availability (Woodall *et al.*, 2018), it is worth further exploring the presence/absence of seahorses in *Posidonia* beds of the study area, while investigating the associated biotic and abiotic factors. Unlike previous studies (Woodall *et al.*, 2018), *H. hippocampus* was much more abundant than *H. guttulatus* in both surveys. This might be explained by the type of low complexity habitats in the study area, muddy substrate and water depth (Woodall *et al.*, 2018). The use of artificial structures as habitat enrichment for seahorses, firstly observed in 2016, has apparently contributed to seahorse settlement in

the deployment area, as also documented in other studies (Correia *et al.*, 2013; Correia *et al.*, 2015b; Simpson *et al.*, 2020). In fact, most of the seahorses of both species were found grasping artificial structures (e.g. ropes) in the study area. While these structures may act as fish aggregation devices (Correia *et al.*, 2015b), they provide holdfasts and enhance the habitat complexity, providing a long-term beneficial effect on the recovery of seahorse populations, particularly as a major component of a wider rehabilitation and management plan.

The marine area of Stratoni seems to support seahorse populations by providing a substratum of artificial holdfasts and relatively high food sources. The results of our study demonstrate that the population of seahorses is well adapted to this particular environment. However, there is an urgent need of substantially further research on the local population distribution, structure and ecology of *H. hippocampus* and *H. guttulatus* through a long-term monitoring plan. Population genetic studies may also contribute to assess the genotypic health of local seahorses' population, which might be facing genetic isolation. Future research will contribute to inform decision-makers and stakeholders in order to protect and conserve these charismatic fishes in the marine area of Stratoni, used also for other coastal areas of the Aegean Sea.

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