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Population dynamics of the long-snouted seahorse (*Hippocampus guttulatus* Cuvier, 1829) in the Mar Menor coastal lagoon

M. Vivas[1] | J. Peñalver[2] | J. A. Oliver[3] | J. D. López Giraldo[4] | C. Mena[4]

[1]Spanish Institute of Oceanography, C/Varadero 1, San Pedro del Pinatar, Murcia, Spain

[2]Fisheries and Aquaculture Service. Regional Department of Water, Agriculture, Livestock, Fisheries and Environment, Cartagena, Spain

[3]Department of Physiology, Faculty of Biology, University of Murcia, Murcia, Spain

[4]Hippocampus Association, San Pedro del Pinatar, Murcia, Spain

Correspondence
C. Mena, Hippocampus Association, San Pedro del Pinatar 30740, Murcia, Spain.
Email: miguel.vivas@ieo.csic.es

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Directorate General for the Natural Environment, Murcia (Spain)

Abstract

A 10-year monitoring program was developed to quantify the population dynamics of the long-snouted seahorse population in the Mar Menor coastal lagoon. Based on 985 underwater visual censuses, we estimated the long-snouted seahorse (*Hippocampus guttulatus* Cuvier, 1829) population size in the Mar Menor lagoon and its reduction in size in the last decades, as well as the effect of eutrophication crises in 2016 and 2019 on the species. The annual recruitment for the 2013–2020 period was estimated by comparing the relative abundance of early seahorse life stages in the ichthyoplankton. The density ranged from 0.0458 specimens/m^3 at the beginning of the sampling period to 0.0004 at the end, showing a statistically significant difference between the three analyzed periods ($H_{gl=2} = 14.0$, $p = 0.001$). The long-snouted seahorse population from the Mar Menor lagoon exemplifies the impact of fishing activities and human pressure, especially euxinic episodes and habitat destruction. As a result of this, the Mar Menor population has decreased from several million specimens to a few thousand, in only three decades. This species showed considerable resilience, the seahorse population began to recover once fishing activity stopped. In contrast, the long-snouted seahorse showed high vulnerability to habitat loss and an episodic flooding event. Adult seahorses showed preferences for highly complex habitats, especially *Caulerpa prolifera*–*Cymodocea nodosa* mixed meadows and habitats of high complexity and anthropogenic origin, such as harbors, jetties, or breakwaters. In contrast, juvenile seahorses preferred monotonous seabeds with low complexity, such as the sandy beds that are characteristic of the Mar Menor lagoon littoral.

KEYWORDS
estuarine ecosystem, eutrophication crisis, habitat preferences, *Hippocampus guttulatus*, population size

1 | INTRODUCTION

The European long-snouted seahorse, *Hippocampus guttulatus* Cuvier, 1829, is a characteristic species of Mediterranean shallow waters, including littoral lagoons (Lelong, 1995), whose populations have suffered a worrying decline in recent decades. The decline of many wild populations of seahorses and other syngnathids as a result of uncontrolled exploitation and habitat degradation has been causing worldwide concern since the 1990s (Martin-Smith & Vincent, 2005; Vincent, 1996).

To enact effective management measures aimed at the recovery of this species, numerous studies on *H. guttulatus* have already been conducted examining the ecology and structure of populations (Curtis & Vincent, 2005, 2006; Foster & Vincent, 2004; Vincent et al., 2005), as well as growth rate and biology (Caldwell et al., 2011; Gurkan et al., 2011; Kitsos et al., 2008; Palma et al., 2014), including reproduction and fecundity (Curtis, 2007; Faleiro et al., 2008; Nauda et al., 2009; Planas et al., 2008).

Moreover, other studies have been conducted on the effect of fisheries activity on populations of the seahorse and other syngnathids (Curtis et al., 2007; Salin & Yohannan, 2005; Vincent, Foster, & Koldewey, 2011; Vincent, Giles, et al., 2011). One of the long-snouted seahorse populations that has suffered a steep decline as a result of fisheries activity and habitat degradation is located in the Mar Menor lagoon (south-eastern Spain, western Mediterranean). There is evidence that seahorses have been commercialized in the Mar Menor lagoon, at least since 1980. There are incomplete records since 1994, when around six tons of dried seahorses were sold and this decreased to 126 kg of dried seahorses being recorded on the market in 1999. It is estimated that during the period 1994–1999 at least 6 million seahorses were dried and sold (M. Vivas, personal observations, December 12, 2020).

The Mar Menor is the biggest coastal hypersaline lagoon in the Mediterranean basin. This protected system was considered a Place of Community Interest (PCI), according to the EU Habitat Directives 92/43 CEE, as the PCI ES6200030 Mar Menor, RAMSAR and ZEPIM area. This ecosystem is naturally oligotrophic, and its main distinguishing feature is the transparency of its waters compared to the great majority of coastal lagoons (Pérez-Ruzafa et al., 2019). However, the lagoon has suffered major impacts in recent decades such as eutrophication from agricultural runoff, urban development, seasonal tourism, habitat degradation, jellyfish blooms, and predatory invasive species (see Conesa & Jiménez-Cárceles, 2007; Jiménez-Martínez et al., 2016; Velasco et al., 2006). In the spring of 2016, the Mar Menor lagoon endured a strong ecological imbalance caused by an uncontrolled input of inorganic nitrogen from agriculture. As a result, the water turned turbid green and light did not reach the bottom for 9 months. Consequently, 85% of the area covered by benthic macrophytes and its associated community was completely lost. The ongoing lack of light caused the death of a large amount of plant biomass and sessile marine life (Ruiz-Fernández et al., 2019). Once this episode had passed, the system began a relatively rapid recovery, which was perceptible around mid-2018 (Pérez-Ruzafa et al., 2019). However, in September 2019, a severe storm led to the discharge of 60–70 Mm^3 of freshwater transporting large amounts of nutrients to the lagoon (Álvarez-Rogel et al., 2020). The particular limnological conditions over the subsequent weeks resulted in water stratification, where fresh, nutrient-rich water sat firmly on top of salty water. In the absence of gas exchange, the organisms, especially the bacteria, consumed the oxygen, causing an unprecedented euxinic episode, which finally resulted in the death of a large number of animals, especially sessile organisms, but also fish.

Among its fauna, the Mar Menor lagoon is noted for the presence of Spanish tooth carp (*Aphanius iberus*) and fan mussel (*Pinna nobilis*), included in Annexes II and IV of the UE Habitat Directives, respectively, and 10 species included in Annex II of the Barcelona Convention (Abdul Malak et al., 2011), including long-snouted seahorses. Despite its importance and the fact that it is a protected species by the 1973 Convention on International Trade in Endangered Species, no population studies have been published on this species in the lagoon so far.

Underwater visual census (UVC) has been widely used for population density estimation since Brock (1954) proposed the use of this technique. Since then, this noninvasive method has been applied to a large number of fish species, including seahorses, in different marine habitats (see Sale & Sharp, 1983; Samoilys, 1997; Bell et al., 2002; Curtis et al., 2004). One of these syngnathid species is *Hippocampus guttulatus*, whose populations have been studied through UVC to assess local abundance, habitat preferences, and trends in population size (Caldwell & Vincent, 2012; Correia et al., 2015; Curtis & Vincent, 2005; Gristina et al., 2015; Lazic et al., 2018).

For the first time, through the use of an optimal stratified sampling and UVC, we estimated seahorse density and the long-snouted seahorse population size in the Mar Menor lagoon and its reduction in size over the past decade, as well as the effect of both eutrophication crises in 2016 and 2019 on the species.

2 | MATERIALS AND METHODS

2.1 | Study area

The Mar Menor lagoon is located on the Spanish Mediterranean coast (Figure 1). This protected ecosystem has a total surface area of 135 km^2 and 73 km of coastline, a mean depth of 4.5 m, and a maximum depth of 6.5 m. It was considered an oligotrophic system with scarce and uneven nutrient inputs until agricultural practices changed from rainfed agriculture to irrigation farming in the 1980s. Since then, the lagoon has endured an increasing input of nutrients, mainly nitrates from intensive agriculture and ammonium and phosphorus when the human population increased in the zone as a result of tourism (Álvarez-Rogel et al., 2006). As a result of this more consistent and elevated nutrient input, the lagoon has gradually changed from a markedly oligotrophic to a eutrophic state. Beyond that, two strong ecological imbalances in 2016 and 2019 caused ecosystem degradation, with the loss of benthic macrophytes and countless fishes and invertebrates (Álvarez-Rogel et al., 2020; Ruiz-Fernández et al., 2019).

This hypersaline lagoon was listed as a PCI (PCI ES6200030 Mar Menor) in the EU Habitat Directive 92/43 CEE.

2.2 | Sampling plan

To estimate the relative population changes of *H. guttulatus*, as well as the effect of both the 2016 and 2019 eutrophic events, an optimal stratified-random design was employed. To this effect, a total of 985 UVCs were performed from August 2011 to October 2020. The planned study was established for three successive survey periods: 2011–2015, 2017–2019, and 2020. The UVCs were performed from March to October in each of these three survey periods. The number of sampling stations, UVCs, and total sample area per survey period are shown in Table 1.

Sampling was undertaken using UVC techniques. For each 2.4 × 120 m linear transect, stratum, depth, and habitat type were recorded. Linear transects were performed in pairs, through scuba diving, each one covering a width of 1.2 m. The width of the transect was determined by a PVC tube in which the compass and the measuring tape were placed (Figure 2). UVCs were performed with a minimum visibility of 1 m. If such conditions were not fulfilled, the sampling was suspended. To avoid detectability problems, the swimming speed of the divers was adapted to the habitat type. For all specimens sighted, standard length (SL), sex, and maturity stage (juvenile/adult) were recorded. SL was measured as the distance from the snout to the middle of the operculum and then to the end of the tail. Size at first maturity (L_{50}) was considered to be 109 mm (Curtis, 2004). The stratified sampling plan was used, in which three strata were defined: coastal strip, central zone and rocky stratum.

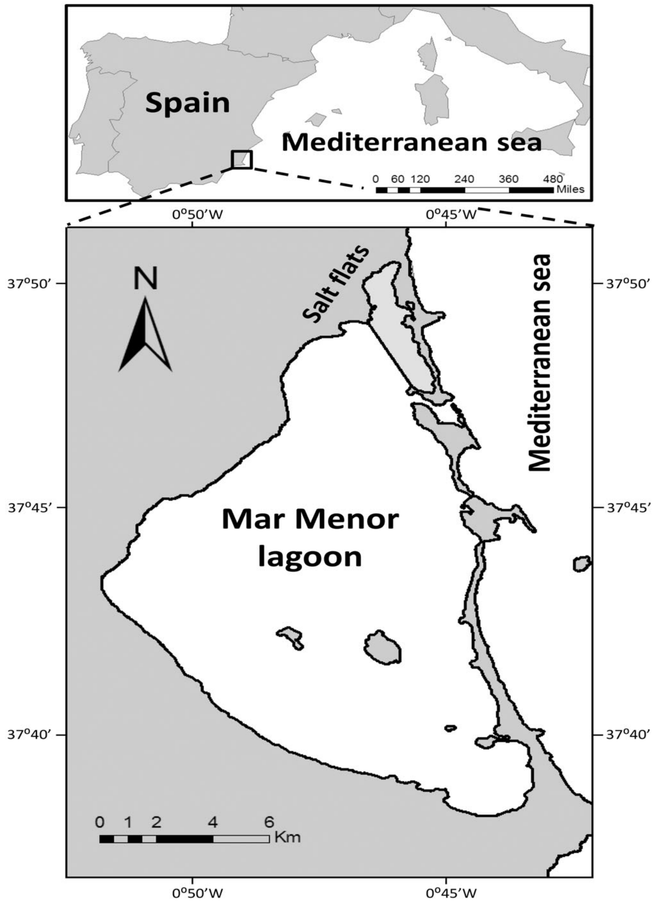

FIGURE 1 Map of the study area. The Mar Menor lagoon is located in the western Mediterranean Sea.

2.2.1 | Coastal strip (A)

This comprised 400-m wide strips for the coastline and 300-m wide strips for the inner islands. This stratum shows great habitat variability, with *Caulerpa prolifera* meadows widespread and interspersed with marine phanerogams comprising mainly *Cymodocea nodosa* or *Ruppia cirrhosa*. The total area is 26.6 km^2 of the lagoon.

2.2.2 | Central zone (B)

This is the most extensive stratum, covering most of the lagoon (107.9 km^2). The main constituent is *C. prolifera* and before 2016 these meadows were a mixture of *C. nodosa* and *C. prolifera*. The maximum depth of this stratum is 6.5 m.

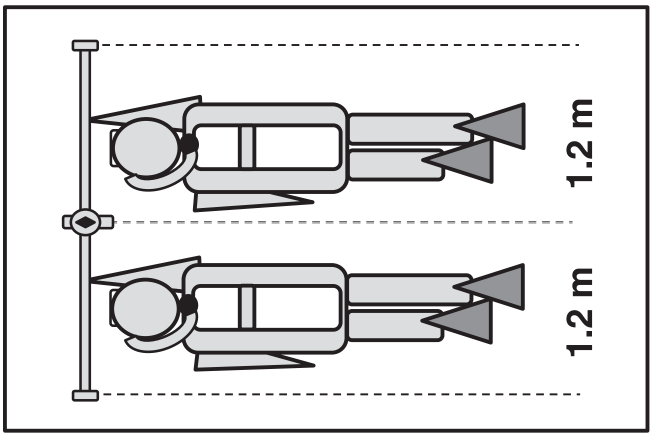

FIGURE 2 Scheme of the underwater visual census showing 2.4 x 120 m linear transectts. The width of the transect was determined by a PVC tube in which the compass and the measuring tape were placed. Both divers held in their hands the PVC tube and pushed it along the linear transect, helping them to define the linear transect area.

TABLE 1 Summary of the sample design

Stratum	A			B			C		
Period	STAT	UVC	TSA	STAT	UVC	TSA	STAT	UVC	TSA
2011–2015	23	203	47,375	11	144	34,152	9	37	6110
2017–2019	28	271	64,992	12	94	22,290	16	42	9882
2020	18	114	31,402	11	70	19,728	6	10	2304

Abbreviations: STAT, number of sampling stations; UVC, number of underwater visual censuses; TSA, total sample area.

2.2.3 | Rocky stratum (C)

The least prevalent habitat in the lagoon consists of the scarce rocky outcrops and anthropic structures such as harbors, jetties, and breakwaters (0.5 km^2). Preliminary sampling showed that seahorse distribution was heterogeneous and stratum related. In this sense, the achieved data dispersion was greater for the rocky stratum. Because of this, the sampling effort was proportionally higher in this habitat.

Concerning habitat type, a distinction was made between sand (SAN), mud (MUD), brown seaweed, mainly *Laurencia obtusa* (ALG), densely covered grassland of *C. prolifera* (DCA), sparsely covered grassland of *C. prolifera* (SCA), meadows of phanerogams, mainly *C. nodosa* (CYM), *C. prolifera*–*C. nodosa* mixed meadows (MIX), and rocky outcrop/anthropic structures (ROC).

2.3 | Annual recruitment estimation

Annual recruitment for the 2013–2020 period was estimated through sampling plankton. To this end, a plankton net (mouth diameter 50 cm, mesh size 500 μm, towing speed 2.5 knots) was horizontally towed once every 15 days during the breeding season (April to July) at seven sampling stations (Figure 3). The total number of samples through these years was 285. We computed the sample seawater volume per plankton net as:

$$V = \pi * r^2 * L$$

where V is the volume of sampled water (m^3), r^2 is the radius of net opening squared (m^2), and L is the towed distance (m). The juvenile specimens caught were counted and released back into the water.

2.4 | Data analysis

To graph the distribution patterns for each studied period, density maps were used. To this end, the deterministic spatial interpolation method (Spline) was used to calculate a smooth surface from sampling stations. For that purpose, the average density of each sampling station was calculated as the mean average of the different density values obtained for each strip transect, according to the formula:

$$D_s = \sum n_i/(z * N).$$

where D_s is the density of the station, z is the sampling area per strip transect, and N is the number of strip transects surveyed.

To explore differences in juvenile seahorse density according to the three different periods, a nonparametric Kruskal–Wallis test was conducted. In the same way, a one-way ANOVA was conducted to test these differences among sampling stations for the whole period.

A chi-square test was used to examine whether the density of *H. guttulatus* was independent of stratum and habitat type, and to analyze whether the sex proportion in the lagoon was 1:1. A two sample t-test was performed to compare mean depth between juvenile and mature seahorses. A significance level of 0.05 was set for all statistical analyses. All multivariate analyses were conducted in SPSS Statistics 17.0 software (SPSS, 2008).

2.5 | Ethical statement

The care and use of experimental animals complied with Spanish animal welfare acts, guidelines and policies as approved by the Natural Directorate of the Natural Environment, Ministry for Water,

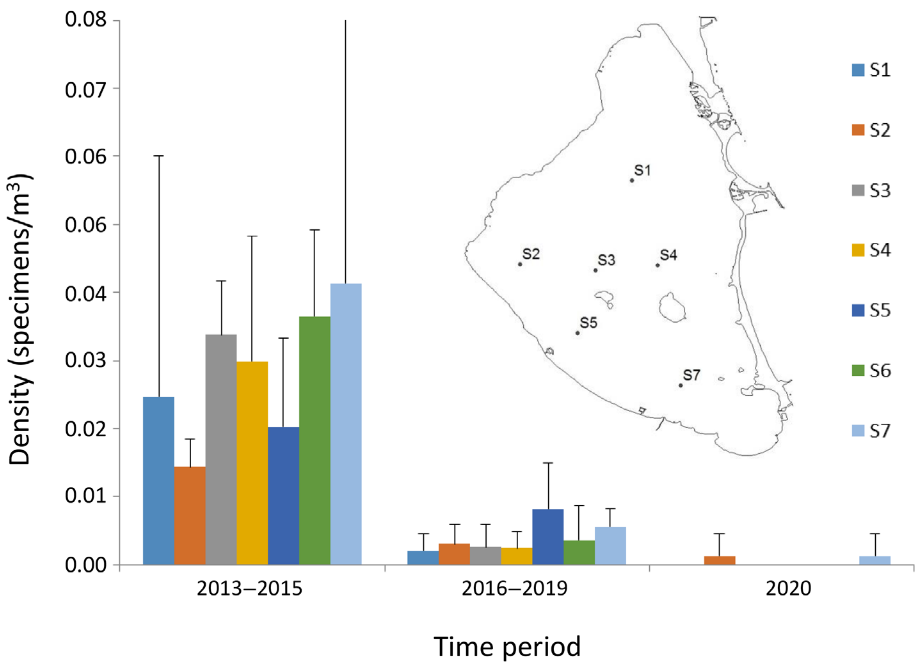

FIGURE 3 Sampling stations (S1–S7) and juvenile seahorse density according to the three time periods.

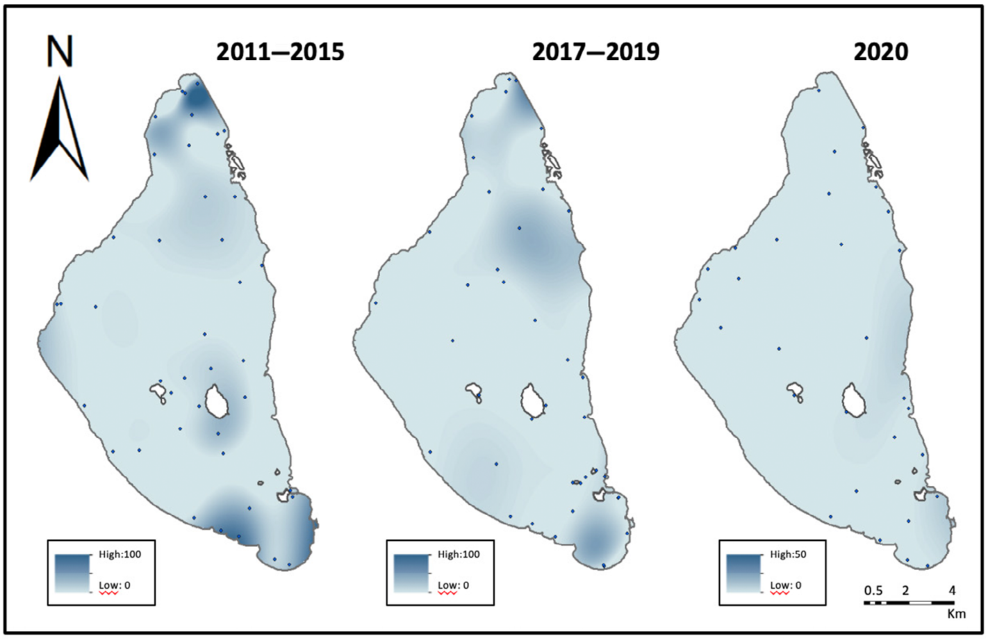

FIGURE 4 Density maps of the Mar Menor seahorse population for the three studied periods. The deterministic spatial interpolation method (Spline) was considered. Blue dots indicate sampling stations.

TABLE 2 Seahorse density (number of specimens per hectare) in the Mar Menor lagoon per stratum and time period

Stratum	A		B		C	
Period	Density (n°/ha)	SE	Density (n°/ha)	SE	Density (n°/ha)	SE
2011–2015	16.4	6.5	9.5	2.5	61.5	25.9
2017–2019	1.4	0.6	4.1	2.7	10.2	5.5
2020	0.5	0.4	0.0	–	0.0	–

Agriculture, Livestock, Fisheries and Environment (N/Rfa: 2020_0236_ AC3_MEN_ATZ). The sampling did not cause distress and no sample specimen died or was harmed during the whole study.

3 | RESULTS

Out of a total of 160 *H. guttulatus* specimens recorded during 985 underwater transects, 44 were females, 52 were males, and 64 were undetermined. There were no differences in proportions between sexes (chi-square test: $\chi^2_{gl=1} = 0.67$, $p = 0.4028$). Sizes ranged from 3.6 to 18.0 cm. The average size for females was 12.3 ± 2.5 cm (mean ± SD), for males was 12.5 ± 2.4 cm, and for undetermined sex was 6.4 ± 1.7 cm.

3.1 | 2011–2020 population size

The population size was estimated for three different periods: 2011–2015, 2016–2019 and 2020 (Figure 4). The percentages of sampling stations with the presence of seahorses for each period were 61.9%, 28.6%, and 6.7%, respectively. For the first period, the population density was 0.0011 specimens/m^2, with an estimated population size of 148,688 specimens. The interim period showed a density of 0.00036 specimens/m^2, with an estimated population size of 48,344 specimens. For the last period, the population density was 0.00001 specimens/m^2, with an estimated population size of 1347 specimens (Table 2).

3.2 | Annual recruitment estimation

A total of 364 juvenile seahorses was caught through sampling plankton from 2013 to 2020 (Table 3). Density values during the breeding season (April–July) ranged from 0.0458 specimens/m^3 at the beginning of the sampling period to 0.0004 at the end (2013–2020). Nonparametric Kruskal–Wallis tests showed statistically significant differences between the three analyzed periods ($H_{gl=2} = 14.0$, $p = 0.001$). For the whole period, the highest juvenile seahorse density was recorded in May (0.014 ± 0.028 specimens/m^3, mean ± SD), while the lowest density was recorded in July (0.003 ± 0.005).

In contrast to what happened with the three different periods, a statistically significant difference ($F[6279] = 0.519$, $p = 0.794$) did not exist between the different stations (Figure 3). Nevertheless, the most southern sampling station (S7) showed the highest juvenile seahorse density values, with 0.0181 specimens/m^3 for the whole period.

TABLE 3 Summary of the sampling design for annual recruitment estimation. Specimens; number of juvenile seahorses caught.

Year	Specimens	Number sampled	Volume (m^3)	Density (specimens/m^3)	SD
2013	143	23	2927.3	0.04476	0.05485
2014	66	28	3563.7	0.01852	0.00701
2015	86	35	4454.6	0.01929	0.01842
2016	13	45	5727.3	0.00233	0.00368
2017	8	42	5345.5	0.00150	0.00169
2018	18	35	4454.6	0.00404	0.00303
2019	28	35	4454.6	0.00629	0.00686
2020	2	42	5345.5	0.00035	0.00055

Note: Sampling took place between April and July.
Abbreviations: Density, density value per year; Number sampled, sample number per year; SD, standard deviation; Volume, sample volume per year.

3.3 | Habitat preferences

A total of 160 seahorses was recorded in the 985 UVCs performed for the whole period. Of these, 102 were located in the coastal strip stratum (A), 32 in the central zone stratum (B), and 26 in the rocky stratum (C). In 2020, seahorses were only recorded in stratum A. In terms of stratum abundance, seahorses preferred the rocky strata (chi-square test: $\chi^2_{gl=2} = 23.7$, $p < 0.001$) and stratum B was less widely preferred. In terms of habitat type, seahorses were recorded in all defined habitats, with the exception of brown seaweed beds, mainly *Laurencia obtusa* (ALG). In this regard, seahorses preferred meadows of *C. nodosa* (CYM), *C. prolifera*–*C. nodosa* mixed meadows (MIX), and rocky outcrop/anthropic structures (ROC) (chi-square test: $\chi^2_{gl=7} = 64.9$, $p < 0.001$).

In terms of maturity, juvenile seahorses showed preferences for sandy beds (SAN) and meadows of *C. nodosa* (CYM) (chi-square test: $\chi^2_{gl=7} = 28.3$, $p < 0.001$), while males showed preferences for *C. prolifera*–*C. nodosa* mixed meadows (MIX), and rocky outcrop/anthropic structures (ROC) (chi-square test: $\chi^2_{gl=7} = 71.7$, $p < 0.001$). Females showed preferences for *C. prolifera*–*C. nodosa* mixed meadows (MIX) and meadows of *C. nodosa* (CYM) (chi-square test: $\chi^2_{gl=7} = 21.4$, $p = 0.004$).

In terms of mean depth, *t*-tests revealed significant differences between juvenile (1.7 ± 1.1 m, mean ± *SD*) and mature seahorses (2.5 ± 1.7 m) ($t(146) = -3.956$, $p < 0.001$). On the contrary, there was no significant difference between males (2.5 ± 1.7 m) and females (2.9 ± 1.9 m) ($t(88) = -1.161$, $p = 0.054$).

4 | DISCUSSION

Our results provide for the first time an evaluation of the current state of the seahorse population in the Mar Menor coastal lagoon and its evolution over the course of the past decade. For a long time it was believed that the Mar Menor seahorse population was the largest *H. guttulatus* population in the Iberian Peninsula, with several million specimens. Nevertheless, despite its relevance and significant population decline, no studies had been carried out to analyze the current state of this species in the lagoon.

Over the past century, the lagoon suffered several impacts that adversely affected the long-snouted seahorse population (see Conesa & Jiménez-Cárceles, 2007; Jiménez-Martínez et al., 2016)), most importantly the enlargement of the Estacio Canal. This canal became navigable in the early 1970s, significantly increasing the limited exchange of waters with the Mediterranean Sea, causing a progressive salinity decrease and thus the emergence of new marine predators that, little by little, were able to colonize these waters, which were becoming less and less saline (Pérez-Ruzafa et al., 1991). Later, in the 1980s, the population was subject to fishing exploitation, triggering a significant reduction in its size in a few years. There are no precise data for the overall number of seahorses sold during the 1980s and 1990s, but it is estimated that fishermen caught and sold around 20 million specimens (data estimated based on the partial sales statistics for 1994–1999). Thus, when the seahorse fishery was closed at the beginning of the millennium, the population size was the lowest ever recorded.

In 2011–2015, for the first time in the Mar Menor lagoon, the long-snouted seahorse population size was estimated. The data obtained for this first period showed that the population size was considerably less than that estimated during the 1980s and 1990s. Nevertheless, despite the lack of population data between 2000 and 2005, the seahorse population seemed to be recovering from the effects of fishing in the recent past. These data confirm the adverse impact of fishing on *H. guttulatus* and support the findings of the negative impact of fishing on this particular species (Curtis et al., 2007) or seahorse populations and other syngnathids in general (Salin & Yohannan, 2005; Vincent, Foster, & Koldewey, 2011; Vincent, Giles, et al., 2011).

The strong nutrient pollution episode, because of an uncontrolled input of inorganic nitrogen in spring of 2016, caused the loss of 85% of the area covered by benthic macrophytes (Ruiz-Fernández et al., 2019). As a result of that, the *H. guttulatus* population was reduced by nearly a third of what it was prior to this episode. Long-snouted seahorses show preferences for complex vegetated habitats and an increase in habitat cover appeared to favor higher densities of this species (Curtis et al., 2007). Habitat destruction stopped *H. guttulatus* recovery and affected its population size. Likewise, as a result of the reduction in population size, the recruitment process was affected, declining by one order of magnitude.

In October 2019, the high nutrient levels associated with a large intake of freshwater associated with high precipitation caused an unprecedented euxinic episode, which finally resulted in the death of a large number of invertebrates and fish, especially sessile organisms (Álvarez-Rogel et al., 2020). *H. guttulatus* was significantly affected due to its low mobility and small home ranges compared to other fish species (Foster & Vincent, 2004) and thousands of specimens died due to hypoxia and later habitat destruction. In this way, population size declined to a few thousand specimens and juvenile densities were at the lowest values ever recorded.

Overall, the pollution episodes caused a significant population decline of 99% for this species in just 5 years. A similar decrease in abundance (94%) was reported by Caldwell and Vincent (2012) for this species in the Ria Formosa (southern Portugal). In the Mar Menor lagoon *H. guttulatus* showed resilience capacity, recovering from fishing activity once that finished, as well as after the first episode of pollution. This ability was due to high growth rates, maturity at young ages, and short generation times (Foster & Vincent, 2004). Nevertheless, the long-snouted seahorse showed high vulnerability to habitat lost and euxinic episodes.

Adult seahorses showed preferences for highly complex habitats, especially *C. prolifera–C. nodosa* mixed meadows and habitats of high complexity, including those of anthropogenic origin such as harbors, jetties, or breakwaters. In contrast, they were less frequently recorded in sandy or muddy bottoms and monotonous grasslands. Similar habitat preferences have been reported in other Mediterranean marine areas, such as Mar piccolo di Taranto in Italy (Gristina et al., 2015), the Apulian coast in Italy (Lazic et al., 2018), and the Rio Formosa in Portugal (Correia et al., 2015). On the other hand, juvenile seahorses showed preferences for more uniform benthic substrata with low complexity, such as sandy bottoms, and to a lesser extent meadows of *C. nodosa*, both characteristics of shallow waters.

The European long-snouted population from the Mar Menor lagoon is a good example of how this species is highly vulnerable to fishing activities and human pressure, especially euxinic episodes and habitat destruction. As a result of this, the Mar Menor seahorse population has decreased from several million specimens to a few thousand in only three decades.

H. guttulatus showed resilience capacity by recovering from anthropogenic impacts when they occurred sufficiently separated in time. Nevertheless, the survival of this population will depend on how we are able to limit the input of pollutants in the Mar Menor lagoon. Otherwise, this species is in real danger of disappearing from this estuarine ecosystem.

AUTHOR CONTRIBUTIONS

M. Vivas wrote the manuscript and conducted all statistical analyses. J. Peñalver sampled the water column to estimated annual recruitment for the 2013-2020 period through sampling plakton. J.A. Oliver, J.D. López, C. Mena and M. Vivas sampled the seahorse population to estimated the population size in Mar Menor lagoon and its reduction in size in the last decades. All authors reviewed the final manuscript.

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ORCID

M. Vivas https://orcid.org/0000-0003-4684-8202

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