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The hidden invasion of the alien seagrass *Halophila stipulacea* (Forsskål) Ascherson along Southeastern Italy

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Abstract

The seagrass *Halophila stipulacea* (Forsskål) Ascherson (Hydrocharitaceae) is a non-indigenous species (NIS) for the Mediterranean Sea. This species is expected to have a high potential for invasiveness based on models predicting the present and future suitability of the Mediterranean coasts with respect to the plant requirements. This species was recorded for the first time along the Apulian coast in the Otranto harbor in 2011. Such a record was not considered in recent publications dealing with the species distribution and its forecasted invasion pathway, probably due to the scant information provided. One decade after its first record in the area, we confirmed the presence of the *H. stipulacea* meadow in the same locality (Otranto), and we identified four new *H. stipulacea* meadows along the Ionian coast are between 1 and 30 m deep in touristic areas. Indirect evidence suggests that the plant is actively spreading along the southeastern Italian coast, and monitoring programs aiming at mapping its distribution in the area, as well as its potential effect on the native biota, are required.

Keywords: Non-indigenous species; invasive species; Lessepsian species; Mediterranean Sea.

Introduction

The seagrass *Halophila stipulacea* (Forsskål) Ascherson (Hydrocharitaceae) is a small dioecious plant native to the Indian Ocean, Red Sea, and Persian Gulf (Den Hartog, 1970). It is among the earliest Lessepsian migrants since it entered the Mediterranean Sea only 25 years after the opening of the Suez Canal in 1869 (Fritsch, 1895). *H. stipulacea* is a non-indigenous species (NIS) with a high potential for invasiveness in the Mediterranean Sea (Georgiou *et al.*, 2016; Winters *et al.,* 2020; Nguyen *et al*., 2020; Beca Carretero *et al*., 2020a;b). Few observations of male and female flowers and fruits, limited in the southern part of the basin suggest that sexual reproduction occurs seldom in the invaded area (Gerakaris & Tsiamis 2015; Nguyen *et al.,* 2018). In the other parts of the Mediterranean Sea, only male flowers were reported, suggesting that its westward spread is probably due to asexual reproduction by fragments, possibly enhanced by shipping through touristic vessels among marinas (Gambi *et al.*, 2009; Pica *et al.,* 2021; Thibaut *et al.,* 2022).

The invasiveness success of this plant is likely also due to its wide thermal tolerance (10-30° C), and, based on its physiological requirements, it is forecasted to colonize most of the Mediterranean basin within the next 100 years (Nguyen *et al.,* 2020; Beca Carretero *et al.,* 2020a). In fact, after 125 years since it entered the Mediterranean Sea, *H. stipulacea* is found in Egypt, Lebanon, Syria, Turkey, Cyprus, Greece, Albania, Tunisia, Libya, Malta, Italy (Sicily and Campania), and more recently, has also reached the northern coast of Sardinia (Pica *et al.,* 2021) and the coast of Cannes in France, that is the northmost record in the basin (Thibaut *et al.,* 2022).

Following the predicted habitat suitability of *H. stipulacea* in the Mediterranean Sea, based on its thermal and salinity tolerance and physiology, the southeastern coast of Italy (Apulia) appears suitable for the invasion of this NIS (Winters *et al.,* 2020, Beca-Carretero *et al.,* 2020a). The area is the natural crossroad between the Ionian and the Adriatic Seas, with the Ionian coast of Sicily and the Adriatic coast of Albania representing the closest locations where the plant was recorded since 1995 (Winters *et al*., 2020, Beca Carretero *et al.*, 2020a). It is worth mentioning that *H. stipulacea* was already observed in the area at 1-3 m depth on fine sand within the port of Otranto (Italy) in August 2011 (Olivieri, 2012); however, the short note by Olivieri was overlooked in recent publications dealing with the distribution of the plant and the forecasting of its spread in the Mediterranean Sea. Thus, nothing is known about the eventual persistence of the

NIS more than 10 years after its first record (Olivieri, 2012) and its spread along the southeastern coast of Italy.

The aim of this study is to check for the persistence of *H. stipulacea* along the Apulian coast and to evaluate its potential for further spreading in the area.

Materials and Methods

Our sampling strategy was based on the evidence that shipping by touristic vessels may be responsible for *H. stipulacea* spreading, and asexual reproduction by fragments favors its spread (Gambi *et al.,* 2009; Pica *et al.,* 2021; Thibaut *et al*., 2022). From December 2021 to November 2022, in the framework of the project MOSSHA ("Monitoraggio Specie Siti e Habitat Natura 2000 in Puglia" FESR-FSE 2014-2020), supported by the Apulian Region, we surveyed the south Apulian coast following two different strategies: 1) indirectly, by moving randomly in 9 sites (beaches and ports) along the coast of the Salento Peninsula (South Apulia) to check for beached or floating *H. stipulacea* fragments; 2) based on their vicinity to popular touristic marinas, we randomly selected 22 diving sites searching for *H. stipulacea* meadows (Fig. 1). Among the diving sites, we also surveyed the Otranto Port for confirming the presence of the NIS after 11 years from its first record in the area (Olivieri, 2012). Underwater, at each diving location, we randomly selected 1-3 transects 50-200 meters long based on the coastal geomorphology. The transects were perpendicular to the coast. Based on the geomorphological features of each diving site, the depth of the surveys varied from 1 to 30 m.

Once detected, *H. stipulacea* meadows were georeferenced and sampled to calculate their maximum length and width (when possible) using a line meter and to calculate the shoot density using a 20x20 centimeters square with 5 replicates per meadow.

Results

A total of 31 sites were investigated, among them 6 beaches (surveyed from the coastline) and 7 ports (Otranto, Tricase, Santa Maria di Leuca, Gallipoli, Santa Caterina, Porto Cesareo, Taranto) (surveyed from the coastline and/or by diving). Among the sites, 22 were analyzed by scuba diving.

In December 2021, tens of *H. stipulacea* rhizomes were observed beached and floating in the harbor of Otranto two days after an intense storm; the survey of other ports and beaches of the Salento Peninsula (from Taranto to Torre Guaceto) from the ground did not reveal the presence of any plant fragments nor floating or washed ashore, except for the port of Santa Maria di Leuca where two floating rhizomes were observed. Diving inside the port of Santa Maria di Leuca was impossible due to logistic constraints, while the underwater investigation outside the port did not detect any meadow/patch. (Fig. 1; Table 1).

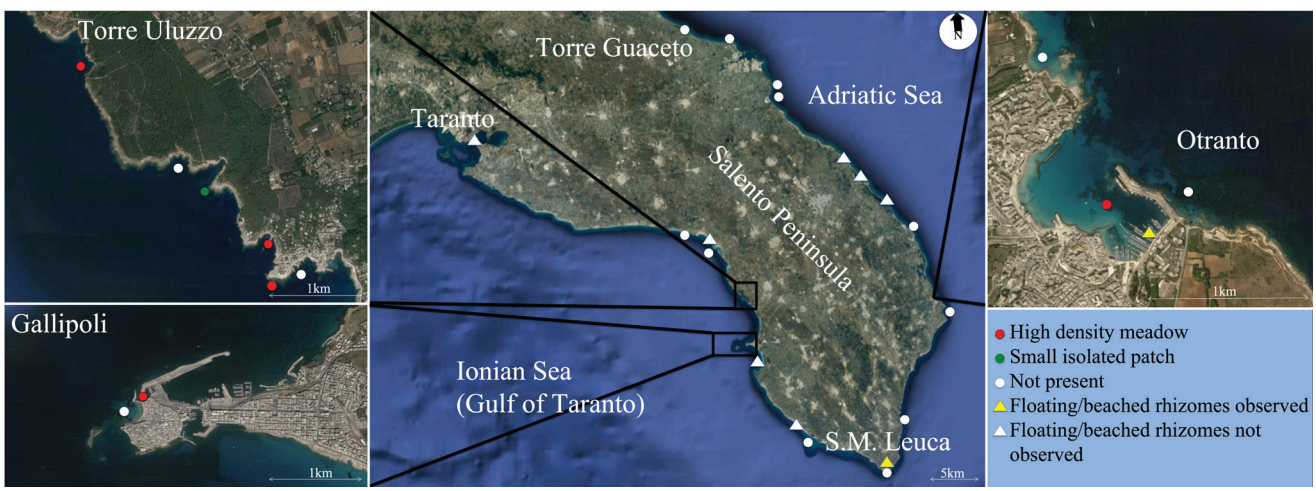

Fig. 1: Map of the study area and sampling sites along the southeastern coast of Italy. Triangle = observation from the coastline; circle = diving site.

Table 1. Summary table of *Halophila stipulacea* records along the Salento Peninsula coast.

Locality	Coordinates	Depth (m)	Shoot density m^{-2} (mean ± SD)	Length/width (m)	Substrate
Gallipoli (port)	40.05712° N, 17.97445° E	1.5-2	425±64	4.5/4	dead algae
Otranto (port)	40.14812° N, 18.49314° E	3.5	697±234	35/>10	sand
Santa Maria di Leuca (port)	39.79714° N, 18.36201° E	*	*	*	*
Santa Caterina	40.13834° N, 17.97904° E	20 to >30	490±133	80/>35	sand
Santa Caterina	40.14083° N, 17.97960° E	12.5-13	585±148	4.7/4.4	sand
Torre Uluzzo	40.15550° N, 17.95941° E	20	685±205	3.2/2.1	sand

(* floating/beached rhizomes).

The underwater survey inside the port of Otranto in November 2022 revealed the persistence of a wide meadow in front of the San Nicola deck at 3.5 m depth. The maximum measurable length was 35 m, and the maximum observable width was 10 m. Since the meadow colonized an area of the port where any diving activity is forbidden, measuring its actual dimensions was impossible. The shoot density was 697±234 m^{-2} (average ± standard deviation) (Fig. 2A).

The underwater sampling on both the left and the right side of the Otranto Port mouth did not detect *H. stipulacea* patches/meadows. Further underwater visual census along the eastern coast of the Salento Peninsula did not detect any evidence of colonization (Fig. 1).

Along the Ionian coast, within the Gulf of Taranto from Santa Maria di Leuca to Taranto, four meadows between 1.5 and >30 m depth were observed between June 2022 and October 2022 (Fig. 1). Flourishing meadows were recorded at Gallipoli, at Santa Caterina di Nardò and Porto Selvaggio (Table 1; Fig. 2). In June 2022 one patch was observed at Santa Caterina di Nardò with a maximum density of about 585±148 shoots m^{-2} surrounded by small patches formed by 20-40 shoots each, altogether covering an area 40 m long and 10 m wide (Fig. 2B). It is worth mentioning that male flowers were observed in this last location and that and several isolated rooted rhizomes were also observed in the areas of Santa Caterina and Porto Selvaggio. Further surveys in the same area carried out during October 2022 detected a large meadow from 20 to 30 meters depth (maximum diving depth reachable for security reasons). The maximum measurable length was 80 m, and the maximum measurable width was 35 m. The meadow clearly extended behind 30 meters in depth. The shoot density of this last meadow was 490±133 m^{-2} (Fig. 2C).

In July 2022, *H. stipulacea* plants were observed in the small touristic port of San Giorgio in Gallipoli, in the area hosting small artisanal fishery vessels; the patch was 4.5 m long and 4 m wide, growing among algal detritus surrounded by rocks with a shoot density of 425±64 m^{-2} (Fig. 2D). During September 2022 *H. stipulacea* plants were detected growing at 20 m depth along the Porto Selvaggio coast in front of Torre Uluzzo forming a meadow 3.2 m long and 2.1 m wide, with the maximum density of about 685±205 (SD) shoots m^{-2} (Fig. 2E). In the area between Santa Caterina and Torre Uluzzo, two small patches formed by 10-30 shoots were also detected (Fig. 1; Table 1).

The survey (both underwater and on the ground) of the Marine Protected Area of Porto Cesareo, the largest municipality hosting artisanal fishing vessels and popular marinas immediately north of Porto Selvaggio, did not reveal the presence of *H. stipulacea*.

Discussion

This study confirms the persistence of *H. stipulacea* along the southeastern Italian coast 11 years after its first record (Olivieri, 2012). Moreover, our random survey along the Salento Peninsula detected the spreading of this NIS, which has now moved from the eastern to the western coast, thus entering the Gulf of Taranto in the Ionian Sea. Our findings align with the forecasts on the

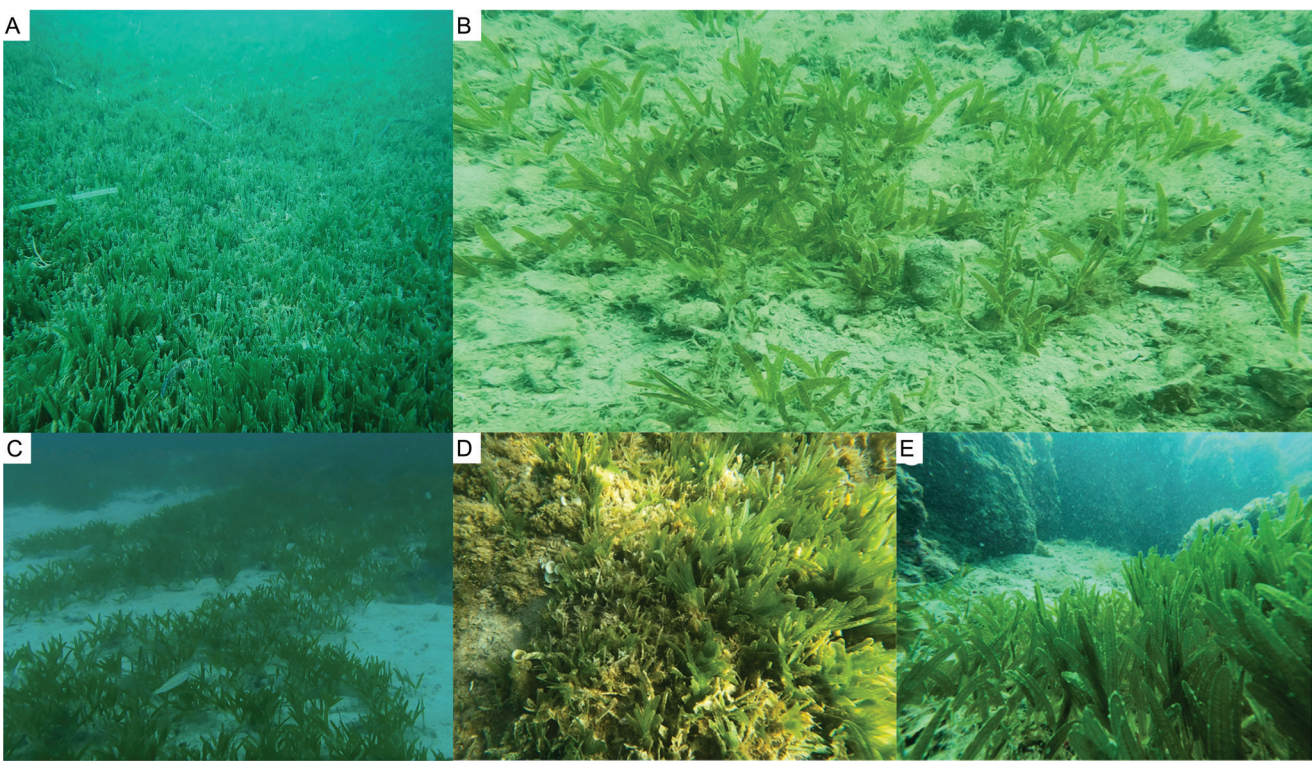

Fig. 2: *Halophila stipulacea* was found at three sites along the Salento Peninsula. A. Meadow at 3.5m depth in the Otranto port. B. Isolated small patch growing on small rocks and sand at 13 m depth at Santa Caterina di Nardò. C. Meadow at 26 m depth in Santa Caterina di Nardò. D. Meadow at 1.5 m depth in Gallipoli port. E. Meadow at 20 m depth at Torre Uluzzo.

suitability of the southeastern Italian coast to be invaded by *H. stipulacea* (Gamliel *et al.,* 2020; Winters *et al.,* 2020, Beca Carettero *et al.,* 2020a).

Our field observations revealed a high invasive potential of *H. stipulacea* in the Ionian area since, in the surveyed locations, together with relatively large patches, several isolated rhizomes rooted in the sandy bottom were detected, probably indicating the ongoing active spreading. One of the sites at present colonized by small patches of the NIS in Santa Caterina is the same location of other diving surveys routinely carried out at 10-15 m depth from 2019 to 2021 by one of the authors (Toso *et al.,* 2022) and *H. stipulacea* was never observed there.

Although it is difficult to identify the pathway of the plant spreading along the southeastern coast of Italy, the fact that floating rhizomes were abundant in the port of Otranto during the winter of 2021 after an intense storm and that fragments were also observed in the harbor of Santa Maria di Leuca, may suggest that both natural and anthropogenic factors may have co-facilitated the invasion of the area. Touristic sailing was suspected to be among the main drivers of *H. stipulacea* spread in the Mediterranean area (Gambi *et al.,* 2009; Pica *et al.,* 2021; Thibaut *et al.,* 2022). In fact, our finding of *H. stipulacea* in the little San Giorgio marina in Gallipoli, as well as its establishment in the Otranto harbor (Olivieri, 2012), indicate the potential role of shipping in the first arrival of the plant in Southeast Italy (possibly from Albania or Greece), and its further spread in the area. However, natural spread due to fragments dislodged by wave action and currents cannot be excluded, as possibly indicated by our finding of three large patches alternating with isolated rhizomes along a 2.3 km stretch of coast between Santa Caterina and Torre Uluzzo.

Moreover, the actual distribution of *H. stipulacea* in the area is probably underrated due to the limited sampling effort since our analysis was based on the inspection of 31 locations along c.a. 280 km of the Salento Peninsula coast. This might also explain the lack of stepping points of invasion from Otranto on the eastern side, which should be considered as the first invaded location, and Gallipoli on the western coast. This gap in the NIS distribution and the observation of floating fragments in the harbor of Santa Marina di Leuca suggest that further sampling efforts should be applied to enable us to reconstruct the invasion route of *H. stipulacea* in the area.

In conclusion, our survey sheds light on the hidden invasion of the southeastern coast of Italy that has probably been occurring for at least one decade (Olivieri, 2012) but has remained unnoticed so far. Further studies are needed to better understand the extent of *H. stipulacea*'s spread, its phenology in the study area, and the eventual production of female flowers that might allow sexual reproduction since male flowers were already recorded. Moreover, the potential impact of the ongoing spread of *H. stipulacea* on the local biota needs to be assessed. Setting up a long-term monitoring plan in the region, including proper technological tools such as multibeam, side scan sonar, and ROV to effectively map the meadows' dimensions, is required to address these topics.

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