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Rapid Communication

Sometimes they come back: the re-colonization of the alien seagrass *Halophila stipulacea* (Forsskål) Ascherson, 1867 (Hydrocharitaceae) in the Palinuro Harbor (Tyrrhenian Sea, Italy)

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Abstract

We report the return of the alien seagrass *Halophila stipulacea* (Forsskål) Ascherson, 1867 (Hydrocharitaceae) in the harbor of Palinuro (Salerno, Tyrrhenian Sea, Italy), six years after the local extinction of this species in this area in 2011. The record of this new colonization event of *H. stipulacea* in Palinuro represents the northernmost location of this tropical alien seagrass in the Western Mediterranean. The species, when first studied in June 2007, had a bottom cover of 16 m^2, which was reduced to 4 m^2 in 2009, and to 2 m^2 in 2010. In 2011, the species was no longer detectable and was considered extinct in the area. In September 2017, the species was recorded again in the area in a few zones, close to where it was originally observed, and covering approx. 5.5 m^2. Seven patches were monitored, occurring between 1.5 and 4 m depth, and with a mean shoot density of 6,300 shoots/m^2, mean leaf length of 33.8 mm, and mean width of 5.3 m, while flowers were absent. Evidence of strong grazing (bite-marks) was observed on 57% of the *H. stipulacea* leaves by native fish species.

Key words: seagrasses, introduced species, geo-referenced distribution, phenology, grazing, Mediterranean Sea

Introduction

The alien seagrass *Halophila stipulacea* (Forsskål) Ascherson, 1867 (Hydrocharitaceae) is a Red Sea immigrant species that has been recorded in the Mediterranean since 1895 and in Italian waters since 1988 (Biliotti and Abdelaad 1990; Gambi et al. 2009). The species was apparently restricted to the Eastern Mediterranean Sea for several decades (Lipkin 1975; Van der Velde and Den Hartog 1992), where it was reported up to the Albania coast (Valona Gulf, Kashta and Pizzuto 1995), but subsequently showed a progressive and relatively rapid colonization of western areas through Malta, on the Ionian coast of Sicily (Biliotti and Abdelahad 1990; Alongi et al. 1992), and Tunisia (Sghaier et al. 2011). More recently, *H. stipulacea* was also introduced into the Caribbean Sea (Ruiz and Ballantine 2004) where it is progressively spreading (Willette and Ambrose 2009; Smulders et al. 2017).

In the western basin of the Mediterranean Sea, *Halophila stipulacea* was first observed in 1995 at Vulcano Island in the Aeolian Archipelago (Southern Tyrrhenian Sea) (Acunto et al. 1997; Procaccini et al. 1999). For more than 10 years, the species was not reported north of Vulcano Island, but was found in 2006 in the harbor of Palinuro (Salerno, Tyrrhenian Sea), approx. 180 km north of Vulcano Island, which represents the northernmost occurrence of the species in the Tyrrhenian Sea and Western Mediterranean (Gambi et al. 2009). In June 2007, several patches *H. stipulacea* in Palinuro were found from 1 to 5 m depth, covering an area of approximately 16 m^2 (Gambi et al. 2009). Since then, there was a progressive reduction in *H. stipulacea* colonization, possibly due to the construction of an artificial submerged rocky barrier for beach protection (approx. 45 m long under-water) in February 2008 that was close to the main seagrass patch. A survey in May 2008 revealed a similar number and cover of patches of *H. stipulacea*, as compared to the first survey in 2007 (Gambi and Barbieri 2013). By contrast, a dramatic reduction of seagrass cover down to 4 m^2 was observed one year later in June 2009. The largest reduction occurred in the largest patch, located at 2.5 m depth on a sandy-mud bottom just adjacent to the rocky artificial reef (Gambi and Barbieri 2013). By April 2010, the largest patch was completely gone, and the remains of dead shoots and uprooted rhizomes/roots were barely visible. Living plants were confined to two dense patches on dead *Posidonia oceanica* (an endemic Mediterranean seagrass) patches, with a total cover of about 2 m^2. By July 2011, *H. stipulacea* was no longer present, and only dead shoots and rhizomes/roots in a few small patches were observed (Gambi and Barbieri 2013). As the progressive decline and regression of *H. stipulacea* density in the area started following the construction of the artificial rocky reef, Gambi and Barbieri (2013) hypothesized that the decline of *H. stipulacea* was related to the negative effects of the barrier in reducing water circulation and modifying sediment features, thus leading to progressively unfavorable conditions for the survival of this seagrass. The area was evaluated again in 2012 and 2013, and, as no evidence of colonization was observed (authors' observations), the species was considered locally extinct and monitoring was halted. In the present study, we document the return/recolonization of *Halophila stipulacea* in the shallow zone of Palinuro Harbor in summer 2017, providing data on the features of the new patches and morphology of the shoots from a survey carried out in October 2017.

Study area

Palinuro is a small touristic village on the coast of the Cilento National Park (Salerno Campania, Italy), approx. 130 km south of Naples (40º01′47.89″N; 15º16′32.20″E). The harbor is characterized by a dock for local fishery boats and hydrofoil docking (approx. 180 m long), some floating docks (approx. 200 m long) and a dedicated area of bottom moorings for sailing and recreational touristic boats, which are particularly abundant during the summer season (Figure 1). The bottom of the harbor is impacted by these bottom moorings and is covered by disconti-nuous *Posidonia oceanica* meadows, often distributed in large patches, separated by wide zones of dead patches of *P. oceanica* and bare sandy bottoms. On the western side of the beach, there is a shallow thermal spring (25 °C) of brackish water (Gambi et al. 2009) (Figure 1).

Material and methods

Surveys were done by SCUBA diving on 29 and 30 October 2017, after having visually recorded the presence of *Halophila stipulacea* in September 2017 (by FB).The survey, authorized by the local Port's Authority, was conducted in the same area and along the same depth range (0.5–5 m) surveyed previously in June 2007 (e.g., Gambi et al. 2009). The GPS geographic coordinates of each identified patch were annotated and elaborated with Q-GIS (v.2.18.15) and projected according to the EPSG: 4326 (WGS 84) spatial reference to produce a geo-referenced map (Figure 1, Table 1).

In two of the largest patches found during the survey (patch A and B in Figure 1), shoot density and morphological variables were measured from three quantitative samples (10 × 10 cm quadrats) collected with a knife. Variables measured from 80 randomly selected shoots included the number of leaves per shoot, leaf length and width, leaf condition (i.e., broken or grazed), and presence of flowers. Leaf condition was annotated for each shoot, and the percentage in each condition calculated from the total number of leaves examined (Pergent et al. 1995; Buia et al. 2004). Data were then compared with shoots collected in June 2007 (Gambi et al. 2009). A Student t-test (based on means and standard deviations) was performed in order to detect signi-ficant differences ($P = 0.05$) among the measured morphological variables.

Results

In mid-April 2017, one of us (MCG) did a visual snorkel survey of the Palinuro shallow harbor area in zones previously occupied by *Halophila stipulacea* (Gambi et al. 2009); no *H. stipulacea* was found. In mid-September 2017, one of us (FB) observed two patches (A and B in Figure 1). Therefore, we hypo-thesize that the new settlement surveyed in October was a relatively recent and rapid colonization event which likely occurred at the beginning of the summer.

We visually censused seven uniquely defined patches of *H. stipulacea*, distributed between 1.5 and 4 m depth (Figure 1). Patch A (1.5 m depth) was the closest to the sandy beach, constituting the shallo-west and largest of the recorded patches, with a rounded shape and a maximum diameter of 2.5 m

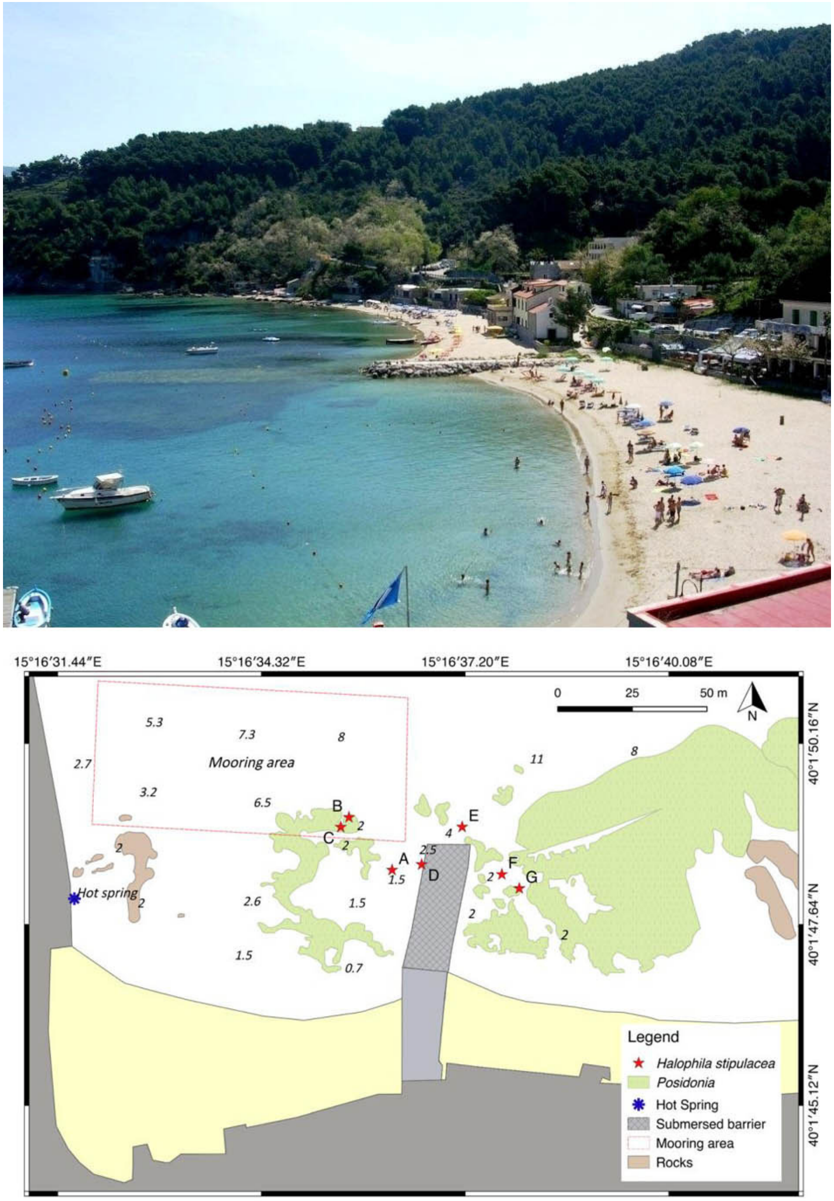

Figure 1. Top) Picture of the Palinuro Harbor beach and the rocky barrier that we surveyed for *Halophila stipulacea* patches in October 2017 (Photo: Gambi M.C.). Bottom) Geo-referenced (GIS) map of the Palinuro Harbor area with the location of the *H. stipulacea* patches (Capital letters) recorded in October 2017. The numbers inside the harbor indicate the depth in meters.

(approx. 3.5 m^2). The patch was located approx. 10 m from the submerged barrier (Figure 1) on a dead *Posidonia* matte covered with dense amounts of *Posidonia* leaf detritus (Figure 2A, 2B). Shoot density and cover was homogeneous. Patch B was the second largest patch, located at 2 m depth on the edge of a dead *P. oceanica* matte and with an approximately squared shape of 1.4 × 1.4 m. This patch was surrounded by living *P. oceanica* shoots on most of its margins, and the distribution of *H. stipulacea* shoots was more heterogeneous. Patch C was approx. 3 m away from patch B and located on the edge of the dead *Posidonia* matte forming a belt 80 cm long and 30 cm wide, mixed with living *P. oceanica* shoots. Patches B and C are the closest (approx. 130 m) to the thermal brackish water spring located at the western corner of the harbor's beach (Figure 1). Patch D, located on the left side of the submerged barrier at 2.5 m depth, is smaller than the other patches (approx. 90 cm in length) and irregularly shaped. Patch E was the deepest (4 m) patch recorded and smaller (approx. 50 cm linear belt) than the other patches, located at the head of the submersed barrier (approx. 3 m from the margin of the barrier

Table 1. Geographical coordinates of the *Halophila stipulacea* patches (capital letters as in Figure 1) recorded within Palinuro Harbor in October 2017.

Halophila patch	Lat (Y)	Long (X)	Depth (m)
A	40º01′48,40″N	15º16′36,17″E	1.5
B	40º01′49,13″N	15º16′35,56″E	2
C	40º01′49,00″N	15º16′35,44″E	2
D	40º01′48,47″N	15º16′36,58″E	2.5
E	40º01′49,00″N	15º16′37,16″E	4
F	40º01′48,34″N	15º16′37,72″E	2
G	40º01′48,14″N	15º16′37,97″E	2

Table 2. Mean values (± standard deviations) of shoot densities and leaf morphological features of *Halophila stipulacea* shoots collected in 2017 and 2007 (Gambi et al. 2009) in Palinuro Harbor. Student t-test based on means ± standard deviation; n.s. = not significant differences.

	Halophila shoots June 2007	*Halophila* shoots October 2017	Student t-test (P)
N. replicates	4	3	
N. shoots analysed	40	80	
mean density (n. shoots/m^2)	10,500 ± 2,700	6,230 ± 1,644	n.s.
mean n. of leaves × shoots	2.0 ± 0	2.0 ± 0	n.s.
mean % of male flowers	22.2 ± 7.5	0	
mean leaf lenght (mm)	33.3 ± 4.3	33.4 ± 6.8	n.s.
mean leaf width (mm)	4.4 ± 0.7	5.2 ± 0.1	P = 0.0001
% broken/grazed leaves	0	57	

rocks) and mixed with *P. oceanica* shoots. Finally, patches F and G were on the right side of the submerged barrier at 2 m depth and were characterized by small plants mixed with living *P. oceanica* shoots. Overall, we estimated a total cover of *H. stipulacea* of approx. 5.5 m^2. Surface water temperature during the October survey was 21 °C.

Shoot density in 2017 (6,300 shoots/m^2) was not significantly different from that measured in 2007 (10,500 shoots/m^2) (Table 2). Most leaf features were also comparable between the two periods, except for leaf width, which was significantly higher in 2017 (mean 5.2 mm *vs* 4.4 mm). In addition, no male flowers were observed in 2017 (Table 2). Evident signs of grazing (i.e., half-moon-shaped bites on the apex and sides of leaves) were recorded on 57% of the leaves (Figure 2E, 2F). Such morphology is typical of the bites of the herbivorous fish *Sarpa salpa* (salema) (Boudouresque and Meinesz 1982; Buia et al. 2004), a species which grazes heavily on *P. oceanica* leaves and also inflorescences (Piazzi et al. 2000), especially at shallow depths (Steele et al. 2014). While no salemas were visually observed during the snorkel surveys, they could be present in the area considering the occurrence of shallow *Posidonia* meadows within the harbor. Other omnivorous fishes, such as *Diplodus* spp., *Mugil cephalus*, and *Oblada melanura*, which may also occasionally graze on macrophytes (Caronni et al. 2015), were observed. Very few epibionts were recorded on *H. stipulacea* leaves, including juvenile *Gibbula* sp. (mesograzer gastropod), and nine specimens of holothurians (*Holothuria* sp.) (inside patch A only) were observed on the *P. oceanica* leaf detritus mixed with the *Halophila* shoots.

Discussion

Our study showed that the introduced seagrass *Halophila stipulacea* was recorded near previously colonized areas in Palinuro Harbor in 2017 at comparable depths and similar bottom features (i.e., *Posidonia oceanica* living and dead matte) after six years of apparent local extinction. The lack of any *H. stipulacea* in April 2017 but dense populations the following September–October supports the idea that this species can undergo rapid settlement and expansion under suitable conditions. Rapid colonization by this species *via* fragments of adult plants was recently documented in the Caribbean Sea (Smulders et al. 2017). It appears that *H. stipulacea* is again utilizing Palinuro Harbor, potentially reinitiating colonization. Gambi et al. (2009) hypothesized that the vector of the initial occurrence of *H. stipulacea* within Palinuro Harbor was anchors of pleasure boats that had visited Vulcano Island (Aeolian Archipelago), approximately 180 km south. Vulcano Island hosts a large population of *H. stipulacea* (Procaccini et al. 1999). We cannot exclude, however, that some *H. stipulacea* shoots or small patches survived at deeper depths in Palinuro Harbor or that shoots have been introduced from other, yet unknown,

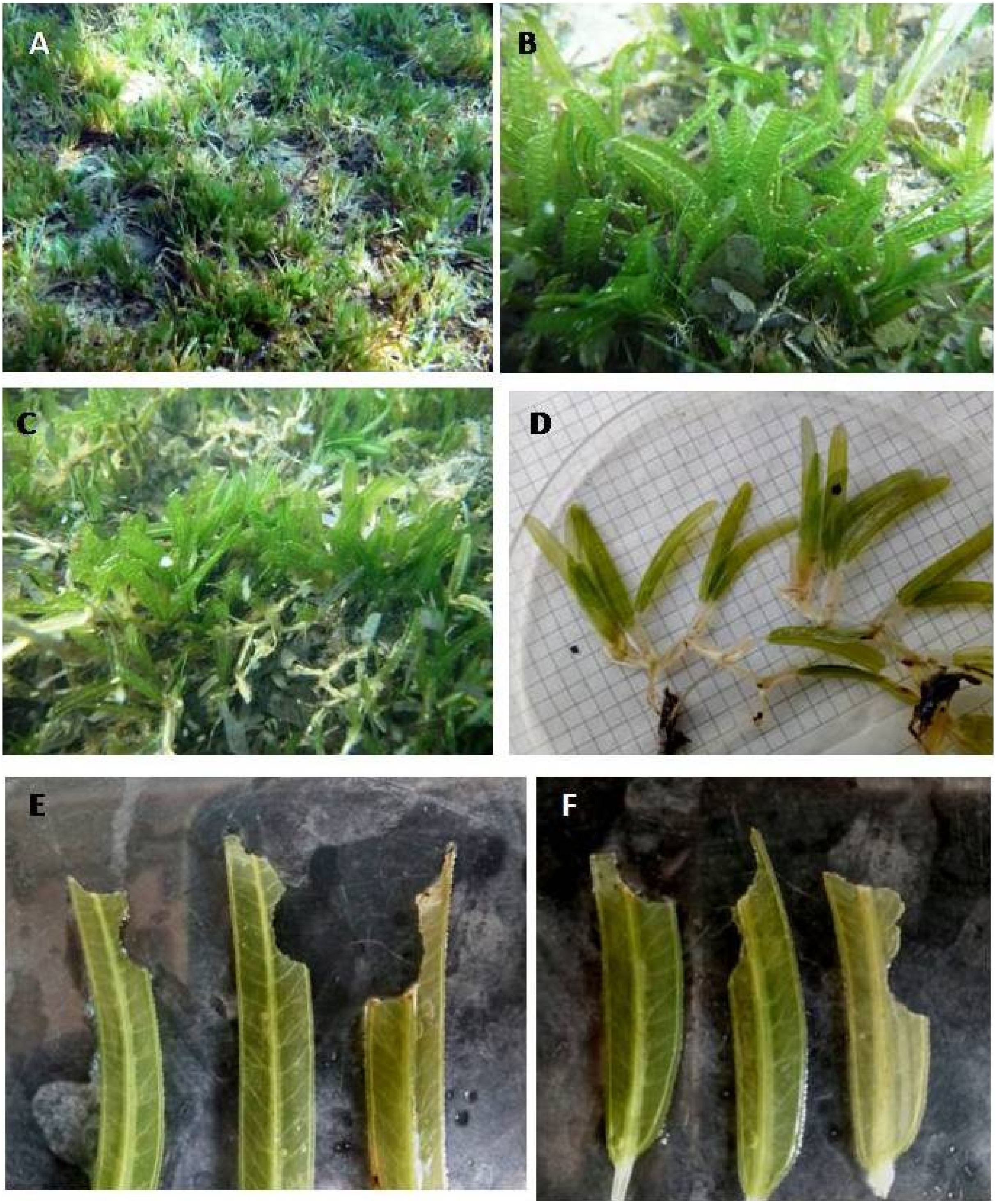

Figure 2. A) View of the largest patch of *Halophila stipulacea* recorded inside Palinuro Harbor in October 2017 (patch A in Figure 1) (depth 1.5 m); B) and C) Shoots of *H. stipulacea* with some uprooted rhizomes and *P. oceanica* leaf detritus on the bottom; D) Shoots of *H. stipulacea* collected for analyzing morphology; note the absence of flowers (leaf length 3.0 cm); E) and F) Marks of fish bites with a typical half-moon shape (likely *Sarpa salpa*, salema) on the tip and sides of *H. stipulacea* leaves from Palinuro Harbor (leaf width 6 mm). Photographs by M.C. Gambi.

nearby sites. Unfortunately, SCUBA diving at depth higher than 5 m is not permitted in the Palinuro Harbor. However, some floating *H. stipulacea* were observed near Palinuro, off the coast, in between Pisciotta (8 km from Palinuro) and Ascea (15 km) in the summer of 2012 (Gambi and Barbieri 2013).

While the shoot densities were the same between the two surveys (2007 and 2017), the leaves of 2017 *H. stipulacea* were wider and grazed more heavily (57%) than 2007 plants. In addition, 2017 *H. stipulacea* plants lacked male flowers. These differences could be due to differences in sampling season, as the 2007 sampling occurred in mid June (summer) and the 2017 sampling in September–October (fall). However, sexual reproduction and the occurrence of flowers (especially female flowers) in *H. stipulacea* is a relatively rare event and has been reported only twice in the Mediterranean Sea and indirectly documented by the presence of seeds (Gerakaris and Tsiamis 2015). Contrary to the high number of leaf grazing marks on *H. stipulacea* in 2017, no bite-marks were present on leaves in 2007, although schools of salemas were observed grazing on the patches (Gambi et al. 2009). We hypothesize that, in 2007, fishes fed on male flowers of *H. stipulacea*, possibly on seagrass pollen, as reported for *Thalassia testudinum* male flowers in the Caribbean Sea (van Tussenbroek et al. 2008). The exine coat, a pollen wall-protein highly resistant to degradation by digestive enzymes, is poorly developed in *Thalassia* spp. pollen and therefore the feeding efficiency of fish consuming this pollen may be higher (van Tussenbroek et al. 2008). In the Caribbean Sea, invasive *H. stipulacea* is grazed by native green turtles (*Chelonia midas*) (Becking et al. 2014). Our observation of leaf bite-marks provides the first evidence of grazing on *H. stipulacea* leaves by fishes in the Mediterranean Sea.

The recolonization of *H. stipulacea* in the Palinuro Harbor represents the northernmost occurrence of this alien seagrass in the Western Mediterranean, at a latitude comparable to the northernmost record of this species in the Eastern basin, off the coast of Albania (Valona Harbor, Kashta and Pizzuto 1995). Thus, *H. stipulacea* may experience a latitudinal limitation in its ability to colonize the Mediterranean Sea. However, a small patch of *H. stipulacea* was observed at a very shallow depth (< 2 m) in August 2016 on the north Sardinia coast (Santa Teresa di Gallura, "Rena Bianca" beach) (Zupo V., personal communication). If this anecdotal record is confirmed and documented, this fact, together with the rapid recolonization of the Palinuro Harbor, suggests that *H. stipulacea* is possibly undergoing a phase of expansion in the Western Mediterranean.

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