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Research Article

Massive colonization by the solitary ascidian *Microcosmus exasperatus* Heller, 1878, on the sandy bottom of the Israeli littoral

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Abstract

The rapid increase in the arrival of tropical-origin species into the Levant region has dramatically changed local ecosystems. Non-indigenous species are known for their ability to utilize available ecological niches and in some events expand their non-native niche over time. Here, as an example of such expansion, we report on a massive colonization by the non-indigenous solitary ascidian, *Microcosmus exasperatus* (Heller, 1878), on soft bottoms along the Mediterranean coast of Israel. While this tropical-origin species is well-known for its ability to form dense aggregations on rocky substrates and artificial structures, only limited reports exist from soft-bottom habitats. In September 2022, a massive settlement of *M. exasperatus* was sighted on the sandy bottom (15–22 m depth) in front of Mikhmoret, Israel. *M. exasperatus* had settled on miniature "islets" of hard substrates, such as polychaete tubes, shells, or pebbles. By October, the population had reached a peak density, with a mean of 1.8±1.3 individuals m^{-2} (±95% confidence interval for the mean). Longshore visual surveys by towed divers revealed similar populations scattered along the central Israeli coast. Monthly compass surveys monitoring the population density, revealed a gradual population decline during late fall and winter, leading to a complete eradication in February 2023, probably due to a severe winter storm. No population was detectable throughout the spring but in August 2023 a few specimens were again detected on the sandy bottom, albeit at densities several orders of magnitude lower than the previous year. It is postulated that the ephemeral colonization of soft-bottom areas serves as "stepping stones" for the species' dispersal into new habitats, potentially amplifying its invasive potential. Long-term monitoring across a more comprehensive depth range will reveal whether the observed massive colonization was a singular event or a recurring phenomenon that had previously remained unnoticed.

Key words: Tunicates, marine bioinvasion, suspension feeding, soft bottom, lessepsian invasion, epifauna

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Introduction

The global dispersion and distribution of non-indigenous species are of worldwide concern. In the Mediterranean Sea, as of the early 2020s, a comprehensive inventory has identified the establishment of over 500 non-indigenous species (Zenetos et al. 2022). Their distribution exhibits a notable concentration in the eastern Mediterranean basin, primarily attributed to the ongoing influx of Indo-Pacific species through the Suez Canal (Por 1978; Koukouras et al. 2010; Tzomos et al. 2012; Galil et al. 2018). Anthropogenic activities, including maritime traffic, canals, and aquaculture, are principal vectors for the introduction and subsequent dispersal of non-indigenous marine species (Minchin et al. 2009). During the dispersal phase, anthropogenic vectors operate together with natural vectors, such as current-driven larval dispersion, the active or passive movement of adult individuals, and the translocation of adult sessile organisms together with the substrate to which they are attached (Johnson and Carlton 1996; Johnson and Padilla 1996; Shefer et al. 2004; Bryan et al. 2012; Præbel et al. 2013; Ivkić et al. 2019).

Ascidians (Chordata, Ascidiacea) are sessile suspension feeders with a global distribution in all marine environments (Shenkar and Swalla 2011). Some species are highly invasive and are able to impact aquaculture facilities (MacNair 2005; Clarke and Therriault 2007), and reduce local biodiversity (Coutts and Sinner 2004). Typically, non-indigenous ascidians initially colonize harbors and marinas, where they thrive on artificial surfaces, possibly due to lower competition for space (Lambert and Lambert 1998; Shenkar and Loya 2009; López-Legentil et al. 2015).

Soft-bottom inhabiting ascidians are well-documented around the globe in low-energy environments (Allen 1953; Macginitie 1955; Sanders 1960; Millar 1962; Dragovich and Kelly 1964; Tatian et al. 1998; Yakovis et al. 2005; Rimondino et al. 2015). Although reports of soft-bottom ascidians and "ascidian bottoms" habitats are common from the western Mediterranean and the Adriatic Sea (Harant and Vernieres 1933; Parenzan 1959; Pérès et al. 1964; Monniot 1965; Turon 1988; El Lakhrach et al. 2012; Ramos-Espla et al. 2013; Arroyo et al. 2021), their occurrence has been scarce and notably less studied in the eastern Mediterranean, where only a few instances have been documented (Voultsiadou et al. 2011). Observations in the Levant basin are also scarce (Stamouli et al. 2022), and we are not aware of any report of "ascidian bottoms" from the exposed Israeli coastal shelf.

In the Anthropocene, with most of the shelf area being routinely trawled, soft-bottom benthic macrofauna communities predominantly comprise infauna, including annelids, crustaceans, mollusks, echinoderms, and other phyla, albeit often in lower abundances (Warwick and Clarke 1993; Olsgard and Somerfield 2000; Labrune et al. 2008). In soft-bottom habitats, the benthos faces challenges such as limited organic resources, continuous sedimentation, and abrasion by loose particles (Bonsdorff et al. 1996; Chou et al. 2004). The structure, spatial distribution, and biomass of these communities are tightly coupled to environmental variables (Saulnier et al. 2019; Foulquier et al. 2020), with anthropogenic and natural disturbances contributing to modifying both the soft-bottom habitats and their macrobenthic populations (Warwick and Clarke 1993; Bourcier 1996; Sparks-McConkey and Watling 2001). In nearshore regions, characterized by elevated wave energy levels relative to deeper areas, the consequential sediment instability leads to reduced levels of both species' abundance and diversity (Dolbeth et al. 2007; Martins et al. 2013).

Microcosmus exasperatus (Heller, 1878) (Family: Pyuridae) is a solitary ascidian with a global distribution, primarily in tropical and subtropical waters, and is considered non-indigenous to the Mediterranean Sea (Nagar and Shenkar 2016). Turon et al. (2007), revealed a very restricted distribution of this species in the eastern Mediterranean basin, attributed to introductions via the Suez Canal. It was first documented along the Israeli coastline in 2003, both on artificial substrates and in the natural rocky reefs (Shenkar 2008; Shenkar and Loya 2009).

Marine non-indigenous species are often characterized by opportunistic life-history traits alongside a versatile habitat preference (Belmaker et al. 2013; Safriel 2013; Cardeccia et al. 2018). Upon introduction into a new environment, non-indigenous species typically exploit available niches (Givan et al. 2017), which may not completely represent their ecological niche (Strubbe et al. 2015). Certain non-indigenous species may nonetheless manage to expand their realized niche in the new environment over time (Pack et al. 2022; Iseli et al. 2023). The observed colonization of the non-indigenous solitary ascidian, *M. exasperatus*, on soft-bottom areas along the Mediterranean coast of Israel (Fig. 1), offers an example of such niche expansion. We investigated the distribution of *M. exasperatus* along the shallow Israeli soft-bottom areas over an annual cycle, and discuss the potential effects of this niche expansion.

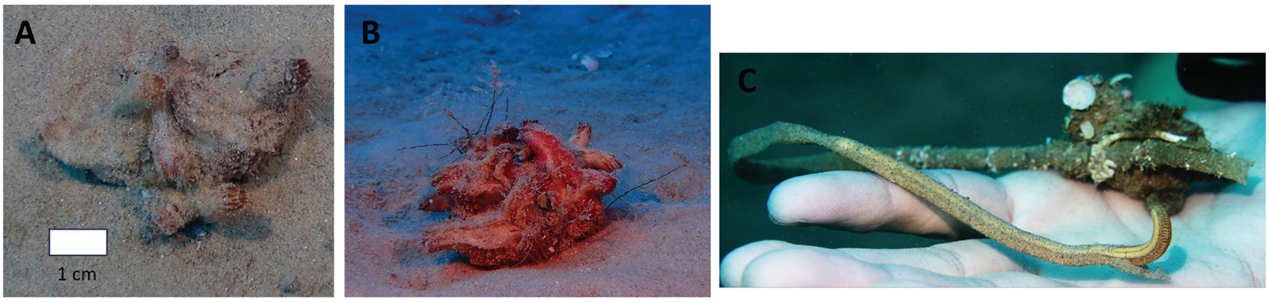

Figure 1. *Microcosmus exasperatus* aggregations on the soft bottom in front of Mikhmoret (17 m bottom depth) and settlement substrate: (A) Aggregation of three specimens covered in sand (top view); (B) aggregation of at least six specimens with hydrozoans epibionts (side view); (C) The polychaete *A. vesiculosum* and its tube, used as settlement substrate for *M. exasperatus*.

Methods

Study site

The Israeli coastline is elongated and exposed, located in the easternmost region of the Mediterranean Sea. It features oligotrophic conditions (Suari and Krom 2015), marked by summer stratification, and typically experiences several severe winter storms, with maximum significant wave height reaching up to seven meters (Bitton 2022). Israel's continental shelf predominantly comprises unconsolidated sediment (Nir 1984; Almogi-Labin et al. 2012), considered as 'soft-bottom', and representing the largest benthic habitat along the coast. The nearshore sediment is dominated by quartz sand originating from the Nile Delta. Below 25 m, grain size gradually decreases; with increasing percentages of silt and clay replacing the sand.

Underwater visual surveys by divers were conducted in several locations along the Israeli coast (Fig. 2) where, due to logistic constraints, they were limited to the sand belt (<22 m depth). Surveys encompassed an entire annual cycle (September 2022 to September 2023) in order to monitor the populations and settlement of the non-indigenous solitary ascidian *M. exasperatus* on the soft bottom. The presence and abundance of these populations were determined using two different methodologies: 1) To follow the fate of the population over time, we carried out monthly compass surveys around a fixed, subsurface, taut mooring line (32°24'28.96"N, 34°51'16.38"E); To minimize the bottom footprint of the anchor, we used a helix anchor designed according to Eco-mooring Systems (https://www.ecomooringsystems.com/helix-anchors). 2) To delineate the extent of the ascidian settlement on the sandy bottom, and its survival over time, we initially undertook compass surveys in four locations in front of Mikhmoret. These were followed by systematic towed-diver surveys along the Israeli coast (see Fig. 2).

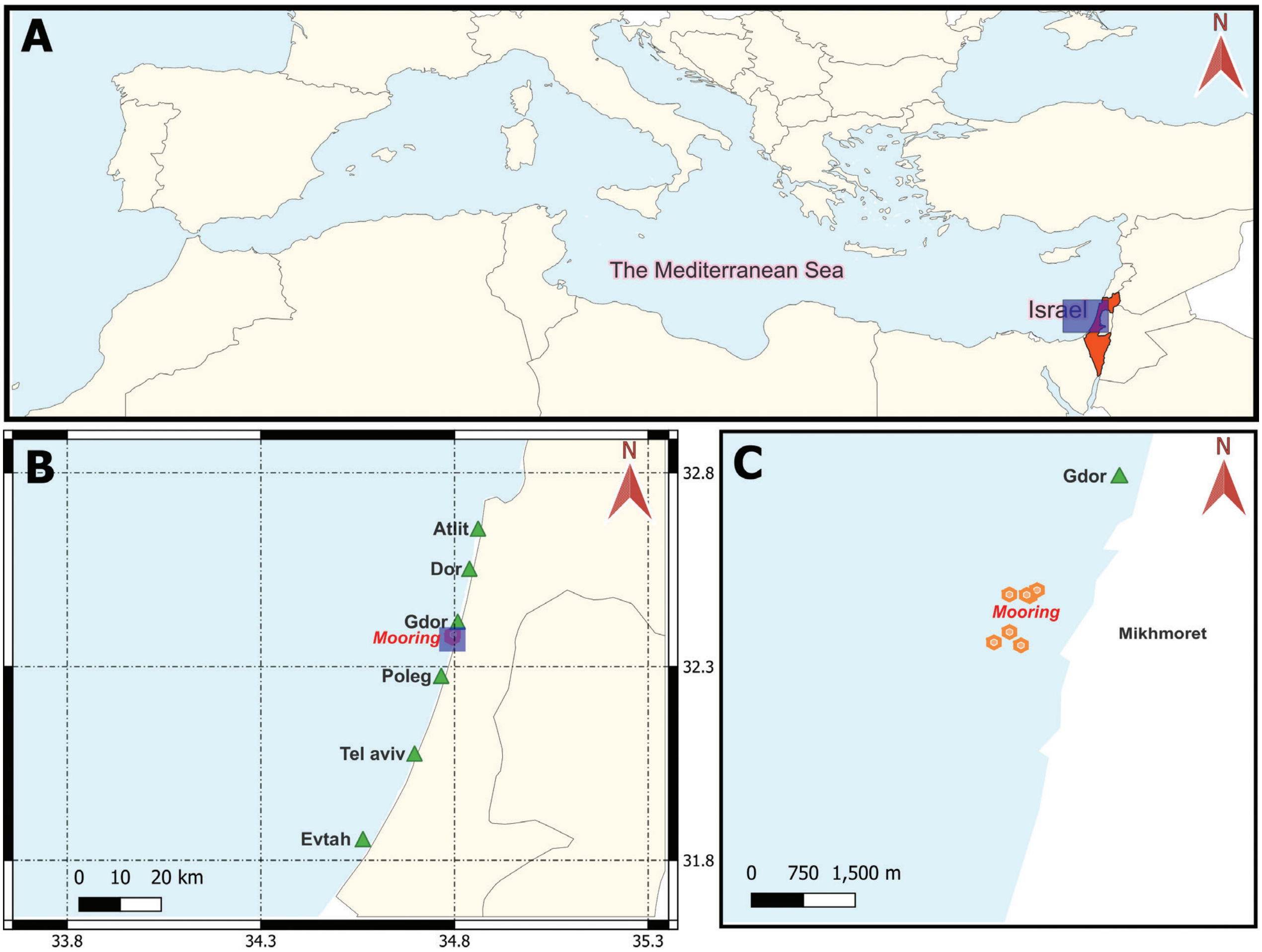

Figure 2. Survey sites along the Israeli coast (see Suppl. material 1: table S1): (A) Israel's geographical position along the Mediterranean coast; (B) the study area where systematic surveys were carried out: green triangles denote the sites where towed-diver surveys were conducted, and orange hexagons denote the locations of compass surveys; (C) specific positions of compass surveys conducted in front of Mikhmoret. The blue rectangles denote zoomed-in areas on each map for more detailed examination.

Monthly compass surveys

Visual SCUBA surveys utilizing a concentric belt methodology were systematically conducted around the mooring at the designated study site (Fig. 3). Every month, and also following significant storm events, surveys were carried out by affixing a measuring tape to the mooring line, positioned approximately one meter above the sea floor. Two to three divers were stationed at one-meter intervals along the tape, commencing the survey at a four-meter radius from the mooring point. The four-meter interior range was left unsurveyed due to its small area. The divers traversed the seabed while systematically scanning for ascidian specimens within a one-meter-width concentric belt. Upon completing one circuit, the measuring tape was extended outward by the same number of meters as the number of divers, and the process was repeated. With the expanding radius, the surveyed area proportionally increased, and this expansion was taken into consideration when calculating mean population densities. This method efficiently facilitated the coverage of an extensive area within a single dive, while maintaining high accuracy in density measurements. The surveys spanned an average area of 346 m^2, ranging from 160 m^2 to 480 m^2, depending on the number of divers, air consumption, and decompression limits.

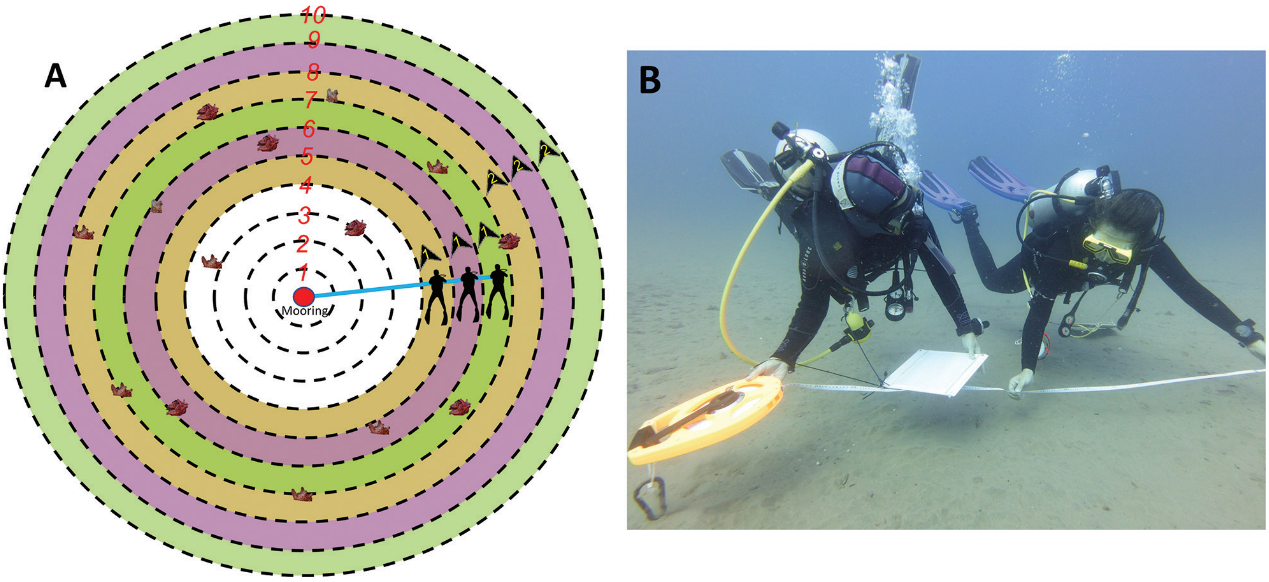

Figure 3. Illustration of the compass surveys: (A) A schematic representation of the compass survey technique. The red dot symbolizes the mooring line, with each circle denoting distance from the mooring in meters, as indicated by the red numerals. The blue line denotes the measuring tape used for the survey. The white area depicts a "dead zone", excluded from the survey due to its relatively small size. Each color-coded concentric belt corresponds to a survey performed by the same diver during different rounds, as indicated by the yellow numerals within the arrows; (B) a pair of divers conducting a survey, counting specimens between their outstretched hands within the one-meter-width belt area.

Towed diver surveys

Towed-diver visual surveys were conducted during the fall, winter, and spring (October 2022 to May 2023). The six survey sites comprised the Haifa-Atlit coastline, Dor-Habonim nature reserve, Gdor nature reserve, Poleg coastline, Tel-Aviv coastline, and the proposed Evtah marine reserve (Fig. 2). Within each of these locations, two separate transects were made: a deeper survey at approximately 20 m depth; and a shallower survey at approximately 12 m. During each survey, a pair of divers was towed behind a skiff using a 70 m rope, about 1–2 meters above the bottom. During these surveys, the divers meticulously documented the presence or absence of ascidians, groundfish, and seagrass on a minute-by-minute basis (25–66 m intervals). Groundfish and seagrass data were analyzed by Hepner et al. (in prep.). The location, distance, and time of each transect were determined according to the GPS on the boat. A communication system (UDI-14 underwater communication system) between the boat and the divers was used to ensure the divers' safety and to determine the start and end of each transect (Lino et al. 2018). Divers were towed longshore (north-south) for approximately one hour at 1.5–2.5 knots along a 1,500–4,000 m long transect. The surveys were photographed continuously using a downward-facing GoPro 10 camera. Uncertainty in regard to location was low, and estimated as being within a radius of up to 70 m.

Taxonomic identification

Samples of *M. exasperatus* from the Israeli coast had been kindly and professionally identified in the past by Prof. Xavier Turon. Following Prof. Turon's confirmation, *M. exasperatus* individuals could be compared to those deposited at the Steinhardt Museum of Natural History and identified by Prof. N. Shenkar, a co-author of the current paper, following Kott (1985) and Turon et al. (2007). A detailed description of the taxonomic tools by which to identify *M. exasperatus* samples, including molecular data, is provided in Gewing et al. (2016). In the Levant basin, *M. exasperatus* stands out as the sole ascidian species characterized by elongated siphons widely distanced from each other, as illustrated in Fig. 1. During SCUBA surveys, the identification of *M. exasperatus* was facilitated by this distinctive morphological feature. Sedentary polychaetes that were part of the *M. exasperatus* complex were identified by Dr. L. Goren, a professional taxonomist from the Steinhardt Museum of Natural History, Tel-Aviv University. The material is deposited at the Steinhardt Museum of Natural History and National Research Center under voucher numbers SMNHTAU-Vr-25362 and SMNHTAU-Vr-25363.

Settlement substrate analysis

In November 2022, 30 ascidian specimens were arbitrarily selected for settlement substrate analysis. We carefully excavated around each specimen to expose the settlement substrate and subsequently lifted the specimens and their settlement substrates from the soft bottom for examination and documentation (Fig. 1c).

Sediment particle size analysis

The disaggregated particle size distribution of the sediment from our study site was measured using standard methods with a laser diffraction analyzer (Mastersizer 3000, Malvern) following digestion of the organic matter with a hydrogen peroxide solution.

Data analysis

Since the area of the concentric belts in each compass survey increased with their increasing radii, calculating the mean density and the variance around this mean using a simple arithmetic mean could be biased by the unequal size of the sampling units. To account for this potential bias, we employed two complementary methods to calculate the mean density and the confidence interval for the mean: 1) A simple arithmetic mean, calculating the confidence interval for the mean while ignoring the size differences between the belts; and 2) A linear regression of the number of ascidians observed in each belt, over its area. The regression slope was used as an estimator of the mean density, and the number of belts (n) and the standard error of the slope (SE) were used to calculate the 95% confidence interval for the mean ascidian density. The results of the regression analysis are provided in the text and the arithmetic mean data are provided as supplementary data in Table 1.

For the towed-diver surveys, we calculated the percentage of 1-minute intervals during which ascidians were observed within each transect.

R (version 4.2.0) was used for statistical analysis and data visualization was performed using ggplot2 and JMP (version 16.1.0). Geographic data visualization was performed using QGIS (version 3.32.0).

Results

Throughout the course of this study, *M. exasperatus* settlements were observed in five of the seven locations surveyed (using either compass or towed-diver surveys) along the Israeli coastline: Mikhmoret, Dor-Habonim nature reserve, Gdor nature reserve, Poleg, and Tel-Aviv (see Suppl. material 1: table S1).

Table 1. *M. exasperatus* densities based on the compass surveys and two alternative methods used to calculate the density: (A) The regression slope of the number of individuals observed in each belt, over its area in each compass survey; and (B) the arithmetic mean of the densities in each concentric belt. Asterisks denote surveys in which only a few specimens were observed. Hashtag represents compass surveys carried out at a site 100 meters south to the study site.

Date	N	Slope ± CI 95% [individuals m²]	Arithmetic mean ± CI 95% [individuals m²]
02/10/2022#	9	0.1 ± 0.2	0.1 ± 0.1
02/10/2022	9	0.9 ± 0.6	1.1 ± 0.2
27/10/2022	6	1.1 ± 1.2	0.8 ± 0.3
27/10/2022#	9	1.8 ± 1.3	0.8 ± 0.3
22/11/2022	9	1.4 ± 1	0.5 ± 0.2
01/12/2022	9	0.1 ± 0.3	0.1 ± 0.1
19/01/2023	9	0.0	0.0
16/02/2023	4	0.0	0.0
22/03/2023	3	0.0	0.0
09/04/2023	3	0.0	0.0
18/05/2023	6	0.0	0.0
12/06/2023	6	0.0	0.0
16/07/2023	6	0.0	0.0
21/08/2023	9	0.0*	0.0*
19/09/2023	9	0.0*	0.0*

Monthly compass surveys revealed maximal mean population densities during the fall season in front of Mikhmoret (1.8 ± 1.3 individuals m^{-2}, mean ± 95% confidence interval) at a bottom depth of >15 m. A continuous decline in this population was observed throughout the winter, marked by significant reductions following major storm events. Notably, the population had been completely eradicated from the sampling site by January 2023 and no specimens were detected there during the winter and subsequent spring months. In the following late summer (August – September 2023), a small number of specimens was observed at the sampling site, with mean densities close to zero, greatly differing from the high densities of the previous year (Table 1 and Fig. 4).

The towed-diver surveys revealed that the massive colonization of *M. exasperatus* was not limited to the Mikhmoret area but, rather, had spread in a patchy manner throughout the survey area, from Tel Aviv in the south to Dor in the north (Figs 5, 6b). Similarly, large-scale surveys confirmed that the seasonal trend in density observed in the compass surveys was also not limited to the Mikhmoret area but, rather, extended to all locations where ascidians had previously been observed (Fig. 6c), peaking during the fall months and subsequently declining throughout the winter until becoming absent from these locations, with no specimens detected during the subsequent spring surveys (data not shown). Towed-diver survey data also reveal that *M. exasperatus* was exclusively found at depths below 10 meters, with a notably higher presence observed at bottom depths of 17 to 20 meters (Fig. 6a).

Settlement substrate analysis

Most of the studied ascidians had settled on polychaete tubes (60%), some of which were identified as *Diopatra cf. neapolitana* (Delle Chiaje, 1841) (family: Onuphidae) and *Acromegalomma vesiculosum* (Montagu, 1813) (family: Sabellidae)

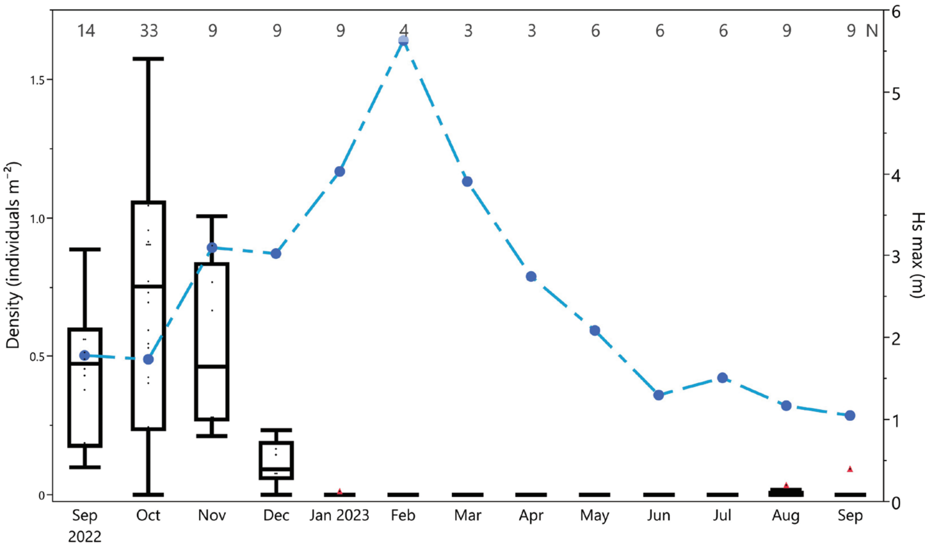

Figure 4. *Microcosmus exasperatus* population density and significant wave height measurements throughout the sampling period. The boxplots visually convey the distribution of *M. exasperatus* density, with black dots denoting individual samples and red triangles denoting outliers. The dashed line and filled dots present the monthly maximum significant wave height values. The numbers on top of the boxplots represent the number of belts surveyed each month Wave data courtesy of the Israel Oceanographic and Limnological Research.

(Fig. 1c). The rest were attached to other hard substrates, such as broken bivalve shells and gastropods (37%) or a combination of both (4%). The *M. exasperatus* aggregations were often found together with the colonial ascidian *Polyclinum constellatum* (Savigny, 1816), an emerging non-indigenous ascidian in the Mediterranean (Montesanto et al. 2021).

Sediment particle size analysis

The D50 (median sediment grain size) value of the sediment particle size distribution at the study site was 166 µm, which categorizes the sea bottom of the study site as being of sandy composition.

Discussion

In September 2022, massive colonizations by *M. exasperatus* on sandy bottom areas (15–22 m depth) were observed, extending for at least 65 kilometers along the Israeli coastline. This marks the first report of sandy-bottom colonization of *M. exasperatus* in the Levant.

Turon et al. (2007) revised the distribution of *Microcosmus squamiger* (Michaelsen, 1927) and *M. exasperatus* in the Mediterranean. According to their revision, the first confirmed record of *M. exasperatus* in the Mediterranean occurred in 1998 in Tunisia (Méliane 2002). Since then, the species has also been reported from Lebanon, Turkey, Israel, Cyprus, and from additional locations in Tunisia (Bitar et al. 2007; Shenkar 2008; El Lakhrach et al. 2012; Ramos-Espla et al. 2013; Gewing et al. 2016). The only previous reports of this species' settlement on soft bottom were documented in the Gulf of Gabès, Tunisia, using SCUBA and bottom trawling (El Lakhrach et al. 2012; Ramos-Espla et al. 2013) and in Lebanon, where it was found attached to *Caulerpa taxifolia* (M.Vahl) C.Agardh 1817 (Ramos-Espla A, pers. comm.). It remains uncertain as to whether the massive

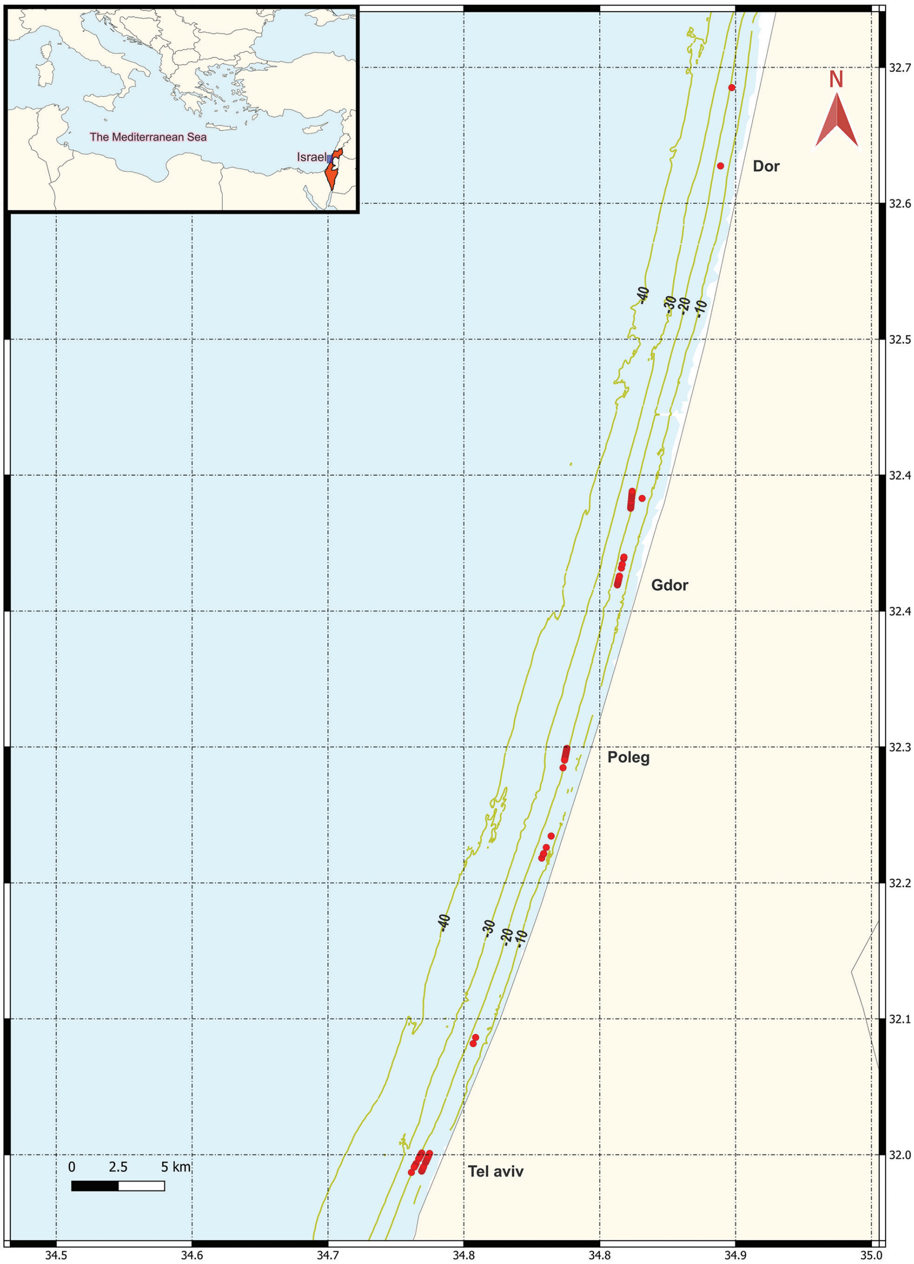

Figure 5. Spatial distribution of *Microcosmus exasperatus* settlements along the sandy seabed (see Suppl. material 1: table S2). The red dots denote the presence of *M. exasperatus* and the green lines represent bathymetry, with each line indicating a depth interval of 10 meters.

colonization event reported here from along the Israeli coast is a recent seasonal phenomenon, but it is certainly the first time the species has been observed during fall surveys of the soft bottom in front of Mikhmoret (Israel) that have been conducted since 2007 (Yahel, unpublished data).

Various ascidian species and even different species within the *Microcosmus* genus have demonstrated the ability to utilize isolated areas of rigid substrate within soft-bottom habitats for settlement (Monniot 1968; Arroyo et al. 2021). However, these prior occurrences have not been perceived as events of niche expansion. *M. exasperatus* was first recorded along the Israeli coastline in 2003. It was observed both in the natural rocky reefs and on artificial substrates and became quickly established all along the coastline (Shenkar and Loya 2009). The current report of its colonization in soft-bottom habitats constitutes an important niche expansion,

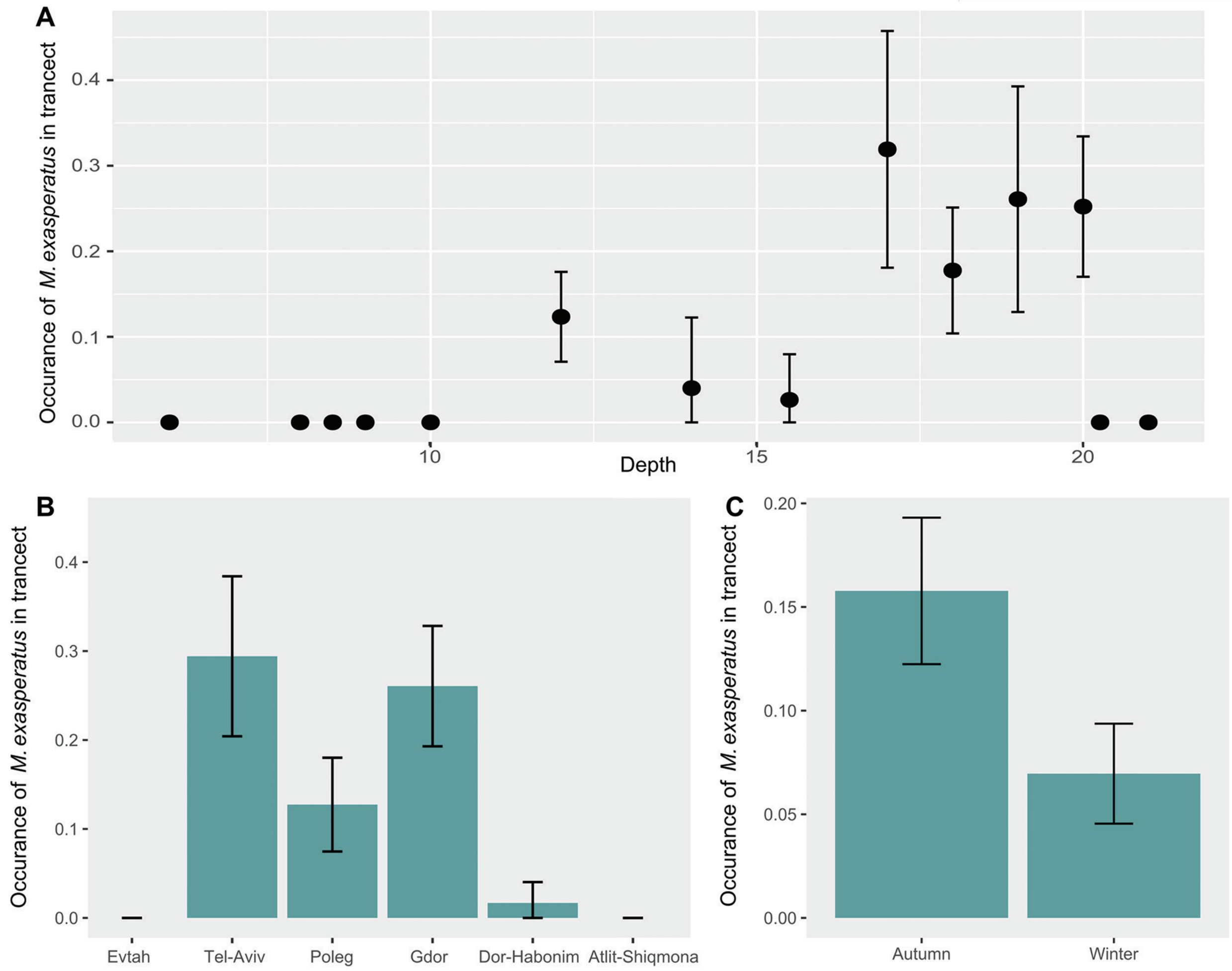

Figure 6. Spatio-temporal occurrence of *Microcosmus exasperatus* in the towed-diver surveys (October 2022 – February 2023). (A) Depth, (B) Locations, and (C) Seasons. The Y-axis values are expressed as the fraction of the transect in which *M. exasperatus* was observed (1 = 100%); Error bars signify a confidence interval of 95%.

20 years after its first report. This sequence of events may reflect the establishment of the species in Tunisia, initially reported on an artificial substrate in 1998 (Méliane 2002) and subsequently documented on the natural soft bottom of the Gulf of Gabes, 14 years later (El Lakhrach et al. 2012; Ramos-Espala 2013).

The only earlier account we are aware of pertaining *M. exasperatus* on a soft bottom is that by El Lakhrach et al. (2012) who reported a maximum density of 0.031 individuals m^{-2} (313.5 individuals ha^{-1}) for *M. exasperatus* in Tunisia, two orders of magnitude lower than the population seasonal peak (1.8 ± 1 individuals m^{-2}) reported here. This substantial difference led us to characterize the current observation as “massive colonization”. Populations of *M. exasperatus* on hard substrates in Israel exhibited considerably higher maximum densities than soft-bottom populations (21.5 individuals m^{-2}; Nagar and Shenkar 2016). Analogous to the hard-bottom population, the density of the soft-bottom population experienced a decline over the winter, but with no significant recovery following improved environmental conditions. The observed collapse of the soft-bottom population suggests that it is not yet firmly established. Although the causes behind the latter’s niche expansion remain unclear, the potential impacts of this expansion should be duly considered.

In July 2023, following a seven-month absence, a new settlement of *M. exasperatus* was observed on the mooring line at the study site. Subsequently, in August and September 2023, a few specimens were discovered settling on empty shells on the soft bottom in two distinct locations: one within the study site itself and another approximately one kilometer to the south. These subsequent settlement events reinforce the notion that this settlement ability is not a sporadic occurrence but a genuine and previously undocumented feature of *M. exasperatus* in the invaded area.

M. exasperatus colonization of the sandy bottom in the fall of 2022 resulted in massive populations along the Israeli coastline. The source of the larvae that eventually comprised the documented colonization remains obscure. However, several different habitats with known populations of *M. exasperatus* are located within a short distance (a few kilometers) from the observed sandy-bottom populations. Those habitats include the natural rocky reef, marinas, ports, and artificial structures (Nagar and Shenkar 2016). It is postulated that, consequently, the propagule pressure of *M. exasperatus* was particularly high, especially during the fall of 2022, when the population reached the highest densities. The fact that this putative propagule pressure did not translate into new settlements or larger populations in the following year suggests that recruitment is not limited by propagule supply, especially in the soft-bottom habitats. Previous studies have suggested that the closure depth (the maximal depth for wave-induced sediment transport) of the Israeli coastline is between 10 and 15 meters (Golik 1997; Bitan and Zviely 2019). However, even a relatively calm winter such as that in 2022, with only one severe storm (February 2022, $H_s > 5$ m), seems to have significantly impacted the documented population along the coast, potentially via abrasion and dislodgment by the strong bottom currents.

The ability of *M. exasperatus* to form massive populations on the sandy bottom reveals a potential dispersal pathway for the species. According to Zhan et al.'s (2015) invasion model for ascidians, *M. exasperatus* is currently at its spread stage in the Mediterranean Sea. The duration of *M. exasperatus* pelagic dispersal time is estimated to be around three days, from gamete spawning to larval metamorphosis (Shenkar N, unpublished data). Such a period should enable the larvae to disperse tens of kilometers from their origin, even under moderate near-bottom currents (5–15 cm s^{-1}). The combination of massive settlement over large swathes of the soft-bottom sediment and the extended planktonic larval stage of *M. exasperatus*, facilitates the use of soft-bottom habitats as "stepping stones" for its dispersal to new areas, given favorable sea and environmental conditions. Such ability significantly enhances its invasive potential in the Levant basin and can extend its dispersal range to across soft-bottom habitats.

Sediment covers the majority of the ocean floor, establishing the soft bottom as the world's most extensive marine habitat (Snelgrove 1999). Stamouli et al. (2022) noted that there has been a restricted research effort focused on large invertebrate species inhabiting soft-bottom environments in the southeastern Mediterranean. The current discovery of *M. exasperatus* populations on the soft bottom along the Israeli coast calls for further studies to determine the presence of ascidians in this less explored habitat, in particular the presence of non-indigenous opportunistic species.

Conclusions

The discovery of the ability of the non-indigenous ascidian *M. exasperatus* to form aggregations on soft-bottom substrates represents a significant increase in its known invasive potential in the Levant basin of the Mediterranean. This ability provides a "stepping stones" dispersal pathway for similar benthic species, which may be able to exploit miniature "islets" of hard substrate in the expansive soft-bottom environment, and hence significantly increase propagule pressure along the coastline during the reproduction seasons. Despite their status as the largest marine habitat globally, the soft-bottom habitats of the southeastern Mediterranean have been overlooked in the context of ascidian ecology.

It remains unclear as to whether the observed massive colonization of the soft bottom in the Levant was a singular event or is a recurring phenomenon that has gone unnoticed to date. It is also unclear as to what depths this phenomenon extends. The dynamics of the soft-bottom environment nonetheless remain a critical determinant of *M. exasperatus* population persistence, at least within the studied depth range. Given the extensive nature of the soft-bottom habitat and the inherently patchy distribution of *M. exasperatus* populations, the combination of towed-diver and compass surveys has enabled the acquisition of detailed data and provided a broader perspective of its population dynamics. Long-term monitoring across a more comprehensive depth range is undoubtedly needed in order to fully answer the still unresolved questions.

Authors contribution

DBG, AN, GK, AF, OUH, and GY designed the study and participated in field experiments, data analysis, and manuscript preparation. NS participated in the study design and manuscript preparation.

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References

Allen JA (1953) Observations on the epifauna of the deep-water muds of the Clyde Sea area, with special reference to *Chlamys septemradiata* (Müller). Journal of Animal Ecology 22(2): 240–260. https://doi.org/10.2307/1816

Almogi-Labin A, Clavo R, Elyashiv H, Amit R, Harlavan Y, Herut B (2012) Sediment Characterization of the Israeli Mediterranean Shelf (10–100 m). The Geological Survey of Israel (GSI) and Israel Oceanographic & Limnological Research (IOLR). GSI report GSI/27/2012, IOLR report H68/2012, 33 pp.

Arroyo E, Moya-Urbano E, García-Ruíz C, Esteban A, Ramos-Esplá AA (2021) Ascidians (Chordata: Tunicata) from circalittoral and upper-bathyal soft bottoms sampled by experimental trawling in the Iberian Mediterranean Sea. Regional Studies in Marine Science 43: 101669. https://doi.org/10.1016/j.rsma.2021.101669

Belmaker J, Parravicini V, Kulbicki M (2013) Ecological traits and environmental affinity explain Red Sea fish introduction into the Mediterranean. Global Change Biology 19(5): 1373–1382. https://doi.org/10.1111/gcb.12132

Bitan M, Zviely D (2019) Lost value assessment of bathing beaches due to sea level rise: a case study of the Mediterranean coast of Israel. Journal of Coastal Conservation 23(4): 773–783. https://doi.org/10.1007/s11852-018-0660-7

Bitar G, Ocaña Vicente O, Espla AA (2007) Contribution of the Red Sea alien species to structuring some benthic biocenosis in the Lebanon Coast (Eastern Mediterranean). Rapp Comm int Mer Médit 38: 437.

Bitton Y (2022) Sandy beach profile morphodynamics and closure depth along the Mediterranean coast of Israel. MSc Thesis, Ruppin Academic Center, Israel, 92 pp.

Bonsdorff E, Diaz RJ, Rosenberg R, Norkko A, Cutter GR (1996) Characterization of soft-bottom benthic habitats of the Åland Islands, northern Baltic Sea. Marine Ecology Progress Series 142(1): 235–245. http://doi.org/10.3354/meps142235

Bourcier (1996) Long-term changes (1954 to 1982) in the benthic macrofauna under the combined effects of anthropogenic and climatic action (example of one Mediterranean Bay). Oceanologica Acta 19(1): 67–78.

Bryan SE, Cook AG, Evans JP, Hebden K, Hurrey L, Colls P, Jell JS, Weatherley D, Firn J (2012) Rapid, Long-Distance Dispersal by Pumice Rafting. PLoS ONE 7(7): 1–13. https://doi.org/10.1371/journal.pone.0040583

Cardeccia A, Marchini A, Occhipinti-Ambrogi A, Galil B, Gollasch S, Minchin D, Narščius A, Olenin S, Ojaveer H (2018) Assessing biological invasions in European Seas: Biological traits of the most widespread nonindigenous species. Estuarine, Coastal and Shelf Science 201: 17–28. https://doi.org/10.1016/j.ecss.2016.02.014

Chou LM, Yu JY, Loh TL (2004) Impacts of sedimentation on soft-bottom benthic communities in the southern islands of Singapore. Hydrobiologia 515(1): 91–106. https://doi.org/10.1023/B:HYDR.0000027321.23230.2f

Clarke CL, Therriault TW (2007) Biological synopsis of the invasive tunicate *Styela clava* (Herdman 1881). Canadian Manuscript Report of Fisheries and Aquatic Sciences, 2807, vi + 23 pp.

Coutts A, Sinner J (2004) An updated benefit-cost analysis of management options for *Didemnum vexillum* in Queen Charlotte Sound (New Zealand). Prepared for Marlborough District Council. Cawthorn Report No. 925. 29 pp.

Dolbeth M, Ferreira Ó, Teixeira H, Marques JC, Dias JA, Pardal MA (2007) Beach morphodynamic impact on a macrobenthic community along a subtidal depth gradient. Marine Ecology Progress Series 352: 113–124. https://doi.org/10.3354/meps07040

Dragovich A, Kelly JA (1964) Ecological observations of macro-invertebrates in Tampa bay, Florida 1961–1962. Bulletin of Marine Science 14(1): 74–102.

El Lakhrach H, Hattour A, Jarboui O, Elhasni K, Ramos-Espla AA (2012) Spatial distribution and abundance of the megabenthic fauna community in Gabes gulf (Tunisia, eastern Mediterranean Sea). Mediterranean Marine Science 13(1): 12–29. https://doi.org/10.12681/mms.19

Foulquier C, Baills J, Arraud A, D'Amico F, Blanchet H, Rihouey D, Bru N (2020) Hydrodynamic Conditions Effects on Soft-Bottom Subtidal Nearshore Benthic Community Structure and Distribution. Journal of Marine Sciences 2020: 1–16. https://doi.org/10.1155/2020/4674580

Galil B, Marchini A, Occhipinti A (2018) East is east and West is west? Management of marine bioinvasions in the Mediterranean Sea. Estuarine, Coastal and Shelf Science 201: 7–16. https://doi.org/10.1016/j.ecss.2015.12.021

Gewing MT, Bronstein O, Nagar LR, Granot I, Frid O, Shenkar N (2016) First record of the non-indigenous ascidian *Microcosmus exasperatus*, Heller 1878, in Cyprus. Marine Biodiversity 46(4): 937–941. https://doi.org/10.1007/s12526-015-0442-5

Givan O, Parravicini V, Kulbicki M, Belmaker J (2017) Trait structure reveals the processes underlying fish establishment in the Mediterranean. Global Ecology and Biogeography 26(2): 142–153. https://doi.org/10.1111/geb.12523

Golik A (1997) Dynamics and management of sand along the Israeli coastline. Bulletin de l'Institut oceanographique 18: 97–110.

Harant H, Vernieres P (1933) Tuniciers. Fasc. 1. Ascidies. In: Fauna de France. Paris, 101 pp.

Iseli E, Chisholm C, Lenoir J, Haider S, Seipel T, Barros A, Hargreaves AL, Kardol P, Lembrechts JJ, McDougall K, Rashid I, Rumpf SB, Arévalo JR, Cavieres L, Daehler C, Dar PA, Endress B, Jakobs G, Jiménez A, Küffer C, Mihoc M, Milbau A, Morgan JW, Naylor BJ, Pauchard A, Ratier

Backes A, Reshi ZA, Rew LJ, Righetti D, Shannon JM, Valencia G, Walsh N, Wright GT, Alexander JM (2023) Rapid upwards spread of non-native plants in mountains across continents. Nature Ecology and Evolution 7(3): 405–413. https://doi.org/10.1038/s41559-022-01979-6

Ivkić A, Steger J, Galil BS, Albano PG (2019) The potential of large rafting objects to spread Lessepsian invaders: the case of a detached buoy. Biological Invasions 21(6): 1887–1893. https://doi.org/10.1007/s10530-019-01972-4

Johnson LE, Carlton JT (1996) Post-Establishment Spread in Large-Scale Invasions: Dispersal Mechanisms of the Zebra Mussel *Dreissena polymorpha*. Ecology 77(6): 1686–1690. https://doi.org/10.2307/2265774

Johnson LE, Padilla DK (1996) Geographic spread of exotic species: Ecological lessons and opportunities from the invasion of the zebra mussel *Dreissena polymorpha*. Biological Conservation 78(1): 23–33. https://doi.org/10.1016/0006-3207(96)00015-8

Kott P (1985) The Australian Ascidiacea. Part 1, Phlebobranchia and Stolidobranchia. Memoirs of the Queensland Museum 23: 1–439.

Koukouras A, Kitsos MS, Tzomos TH, Tselepides A (2010) Evolution of the entrance rate and of the spatio-temporal distribution of Lessepsian Crustacea Decapoda in the Mediterranean Sea. Crustaceana 83(12): 1409–1430. https://doi.org/10.1163/001121610X539498

Labrune C, Grémare A, Amouroux JM, Sardá R, Gil J, Taboada S (2008) Structure and diversity of shallow soft-bottom benthic macrofauna in the Gulf of Lions (NW Mediterranean). Helgoland Marine Research 62(3): 201–214. https://doi.org/10.1007/s10152-008-0108-9

Lambert CC, Lambert G (1998) Nonindigenous ascidians in southern California harbors and marinas. Marine Biology 130(4): 675–688. https://doi.org/10.1007/s002270050289

Lino K, Asher J, Ferguson M, Gray A, McCoy K, Timmers M, Vargas-Angel B (2018) Ecosystem Sciences Division Standard Operating Procedures: Data Collection for Towed-diver Benthic and Fish Surveys. Pacific Islands Fisheries Science Center. National Marine Fisheries Service, National Oceanic and Atmospheric Administration (NOAA). Report H-18-02, 76 pp. https://doi.org/10.25923/59sb-sy51

López-Legentil S, Legentil ML, Erwin PM, Turon X (2015) Harbor networks as introduction gateways: contrasting distribution patterns of native and introduced ascidians. Biological Invasions 17(6): 1623–1638. https://doi.org/10.1007/s10530-014-0821-z

Macginitie GE (1955) Distribution of ecology of the marine invertebrates of Point Barrow, Alaska. Smithsonian Miscellaneous Collections 128(9): 1–201.

MacNair N (2005) Invasive tunicates of concern for Prince Edward Island aquaculture. Charlottetown, Aqua Info Aquaculture Note. Prince Edward Island Department of Agriculture, Fisheries and Aquaculture, AIN 19.2005.

Martins R, Quintino V, Rodrigues AM (2013) Diversity and spatial distribution patterns of the soft-bottom macrofauna communities on the Portuguese continental shelf. Journal of Sea Research 83: 173–186. https://doi.org/10.1016/j.seares.2013.03.001

Méliane I (2002) Contribution to the knowledge of the ascidian fauna in the south-east of Tunisia. MSc thesis, University of Alicante, Spain, 65 pp.

Millar RH (1962) Some ascidians from the Caribbean. Studies on the Fauna of Curaçao and other Caribbean Islands 13: 61–77.

Minchin D, Gollasch S, Cohen AN, Hewitt CL, Olenin S (2009) Characterizing Vectors of Marine Invasion. In: Rilov Gil & Crooks JA (Ed) Biological Invasions in Marine Ecosystems: Ecological, Management, and Geographic Perspectives. Springer Berlin Heidelberg, Berlin, Germany, 109–116. https://doi.org/10.1007/978-3-540-79236-9_5

Monniot C (1965) Les "blocs a Microcosmus" des fonds chalutables de la région de Banyuls-sr-mer. Vie et Milieu 16: 819–850.

Monniot C (1968) Les Ascidies et la faune des zones chalutables du golfe du Lion. Commission Internationale Exploration Scientifique de la mer Méditerranée 19: 187–188.

Montesanto F, Chimienti G, Gissi C, Mastrototaro F (2021) *Polyclinum constellatum* (Tunicata, Ascidiacea), an emerging nonindigenous species of the Mediterranean Sea: integrated taxonomy and the importance of reliable DNA barcode data. Mediterranean Marine Science 23(1): 69–83. https://doi.org/10.12681/mms.28311

Nagar LR, Shenkar N (2016) Temperature and salinity sensitivity of the invasive ascidian *Microcosmus exasperatus* Heller, 1878. Aquatic Invasions 11(1): 33–43. https://doi.org/10.3391/ai.2016.11.1.04

Nir Y (1984) Recent sediments of the Israel Mediterranean continental shelf and slope. PhD Thesis, University of Gothenburg, Gothenburg, Sweden, 211 pp.

Olsgard F, Somerfield PJ (2000) Surrogates in marine benthic investigations - which taxonomic unit to target? Journal of Aquatic Ecosystem Stress and Recovery 7(1): 25–42. https://doi.org/10.1023/A:1009967313147

Pack KE, Mieszkowska N, Rius M (2022) Rapid niche shifts as drivers for the spread of a non-indigenous species under novel environmental conditions. Diversity and Distributions 28(4): 596–610. https://doi.org/10.1111/ddi.13471

Parenzan P (1959) Biocenologia bentonica: il fondo ad Ascidie. Thalassia jonica 2: 1545.

Pérès JM, Picard J, Picard J (1964) Nouveau manuel de bionomie benthique de la mer Méditerranée. Recueil des travaux de la Station Marine d'Endoume 31(47): 1–137.

Por FD (1978) Lessepsian Migration: The Influx of Red Sea Biota Into the Mediterranean by Way of the Suez Canal. Ecological studies. Springer Berlin, Heidelberg, Berlin, Germany, 228 pp. https://doi.org/10.1007/978-3-642-66728-2

Præbel K, Gjelland KØ, Salonen E, Amundsen PA (2013) Invasion genetics of vendace (*Coregonus albula* (L.)) in the Inari-Pasvik watercourse: revealing the origin and expansion pattern of a rapid colonization event. Ecology and Evolution 3(5): 1400–1412. https://doi.org/10.1002/ece3.552

Ramos-Espla AA, Izquierdo A, Çinar ME (2013) *Microcosmus exasperatus* (Ascidiacea: Pyuridae), current distribution in the Mediterranean Sea. Marine Biodiversity Records 6(e89): 1–5. https://doi.org/10.1017/S1755267213000663

Rimondino C, Torre L, Sahade R, Tatián M (2015) Sessile macro-epibiotic community of solitary ascidians, ecosystem engineers in soft substrates of Potter Cove, Antarctica. Polar Research 34(2015): 1–9. https://doi.org/10.3402/polar.v34.24338

Safriel UN (2013) The "Lessepsian invasion" – a case study revisited. Israel Journal of Ecology & Evolution 59(4): 214–238. https://doi.org/10.1080/15659801.2013.930994

Sanders HL (1960) Benthic studies in Buzzards bay III. the structure of the soft-bottom community. Limnology and Oceanography 5(2): 138–153. https://doi.org/10.4319/lo.1960.5.2.0138

Saulnier E, Brind'Amour A, Tableau A, Rufino MM, Dauvin JC, Luczak C, Bris H Le (2019) Seasonality in coastal macrobenthic biomass and its implications for estimating secondary production using empirical models. Limnology and Oceanography 64(3): 935–949. https://doi.org/10.1002/lno.11086

Shefer S, Abelson A, Mokady O, Geffen E (2004) Red to Mediterranean Sea bioinvasion: natural drift through the Suez Canal, or anthropogenic transport? Molecular Ecology 13(8): 2333–2343. https://doi.org/10.1111/j.1365-294X.2004.02227.x

Shenkar N (2008) Ecological aspects of the ascidian community along the Israeli coasts. PhD Thesis, Tel Aviv University, Tel-Aviv, Israel, 120 pp.

Shenkar N, Loya Y (2009) Nonindigenous ascidians (Chordata: Tunicata) along the Mediterranean coast of Israel. Marine Biodiversity Records 2: e166. https://doi.org/10.1017/s1755267209990753

Shenkar N, Swalla BJ (2011) Global diversity of Ascidiacea. PloS ONE 6(6): e20657. https://doi.org/10.1371/journal.pone.0020657

Snelgrove PVR (1999) Getting to the Bottom of Marine Biodiversity: Sedimentary Habitats: Ocean bottoms are the most widespread habitat on Earth and support high biodiversity and key ecosystem services. BioScience 49(2): 129–138. https://doi.org/10.2307/1313538

Sparks-McConkey PJ, Watling L (2001) Effects on the ecological integrity of a soft-bottom habitat from a trawling disturbance. Hydrobiologia 456(1): 73–85. https://doi.org/10.1023/A:1013071629591

Stamouli C, Zenetos A, Kallianiotis A, Voultsiadou E (2022) Megabenthic invertebrate diversity in Mediterranean trawlable soft bottoms: a synthesis of current knowledge. Mediterranean Marine Science 23(3): 447–459. https://doi.org/10.12681/mms.29165

Strubbe D, Beauchard O, Matthysen E (2015) Niche conservatism among non-native vertebrates in Europe and North America. Ecography 38(3): 321–329. https://doi.org/10.1111/ecog.00632

Suari Y, Krom MD (2015) Eastern Mediterranean sea. A natural laboratory for studying marine biogeochemical processes RIME-restoration of Israeli micro estuaries View project The role of estuaries in controlling pollution of the Mediterranean Sea: The Alexander River as a case study View project. Ocean Challenge 21(1): 46–52.

Tatian M, Sahade RJ, Doucet ME, Esnal GB (1998) Ascidians (Tunicata, Ascidiacea) of Potter Cove, South Shetland Islands, Antarctica. Antarctic Science 10(2): 147–152. https://doi.org/10.1017/s0954102098000194

Turon X (1988) Ascidias de los caladeros de arrastre de la costa catalana (Mediterraneo noroccidental). Investigacion Pesquera (Barcelona), 467–481.

Turon X, Nishikawa T, Rius M (2007) Spread of *Microcosmus squamiger* (Ascidiacea: Pyuridae) in the Mediterranean Sea and adjacent waters. Journal of Experimental Marine Biology and Ecology 342(1 SPEC. ISS.): 185–188. https://doi.org/10.1016/j.jembe.2006.10.040

Tzomos T, Kitsos MS, Koutsoubas D, Koukouras A (2012) Evolution of the entrance rate and of the spatio-temporal distribution of Lessepsian Mollusca in the Mediterranean Sea. Journal of Biological Research 17: 81–96.

Voultsiadou E, Fryganiotis K, Porra M, Damianidis P, Chintiroglou C (2011) Diversity of invertebrate discards in small and medium scale Aegean Sea fisheries. The Open Marine Biology Journal 5: 73–81. https://doi.org/10.2174/1874450801105010073

Warwick R, Clarke K (1993) Comparing the severity of disturbance: A meta-analysis of marine macrobenthic community data. Marine Ecology Progress Series 92: 221–231. https://doi.org/10.3354/meps092221

Yakovis EL, Artemieva AV, Fokin MV, Grishankov AV, Shunatova NN (2005) Patches of barnacles and ascidians in soft bottoms: Associated motile fauna in relation to the surrounding assemblage. Journal of Experimental Marine Biology and Ecology 327(2): 210–224. https://doi.org/10.1016/j.jembe.2005.06.015

Zenetos A, Tsiamis K, Galanidi M, Carvalho N, Bartilotti C, Canning-Clode J, Castriota L, Chainho P, Comas-González R, Costa AC, Dragičević B, Dulčić J, Faasse M, Florin AB, Gittenberger A, Jakobsen H, Jelmert A, Kerckhof F, Lehtiniemi M, Livi S, Lundgreen K, Macic V, Massé C, Mavrič B, Naddafi R, Orlando-Bonaca M, Petovic S, Png-Gonzalez L, Carbonell Quetglas A, Ribeiro RS, Cidade T, Smolders S, Stæhr PAU, Viard F, Outinen O (2022) Status and Trends in the Rate of Introduction of Marine Non-Indigenous Species in European Seas. Diversity 14(12): 1077, https://doi.org/10.3390/d14121077

Zhan A, Briski E, Bock DG, Ghabooli S, MacIsaac HJ (2015) Ascidians as models for studying invasion success. Marine Biology 162: 2449–2470. https://doi.org/10.1007/s00227-015-2734-5

Supplementary material 1

Spatial distribution of *M. exasperatus* settlements along the sandy seabed of Israel

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