



## Anthropogenic factors influencing invasive ascidian establishment in natural environments



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### ABSTRACT

Marine environments are constantly impacted by bioinvasions. Invasive ascidians (Chordata, Tunicata) are well-known for their ability to rapidly overgrow any available substrate. While the majority of studies have investigated the factors contributing to the successful establishment of ascidians on artificial substrates, the anthropogenic factors that contribute to such establishment on natural substrates have rarely been investigated. Here, we studied non-indigenous ascidians presence on natural substrate for the first time, using underwater field surveys at eight natural sites along the Israeli Mediterranean coast, in order to provide an analysis of factors assisting their establishment. The findings revealed that sites exposed to extended sewage-spill events experienced a reduction in native ascidian species. Understanding which factors alter ascidian population is essential for further monitoring efforts, to protect areas that are more susceptible to invasion, and for developing effective management tools to control further spread of invasive species in natural environments.

### 1. Introduction

Invasive species have long been noted for their adverse effects, contributing to biodiversity loss (Bax et al., 2003; Worm et al., 2006). Combined with other anthropogenic stress factors such as pollution, coastal development, marine vessel movement, and temperature rise, their negative effect on natural ecosystems can become significantly enhanced. Marine vessels, for example, are well known for their contribution to the spread of invasive species, facilitating their arrival to new regions (Bax et al., 2003; Molnar et al., 2008). Together with the rise in sea-water temperature, which enables exotic species, from tropical areas to survive in temperate areas (Dijkstra et al., 2011; Lord, 2016), and the proliferation of artificial substrates following coastal development, which creates favorable conditions for non-indigenous species, additional species are able to establish sustainable populations in new areas (Tyrrell and Byers, 2007; Bulleri and Chapman, 2010). Among the man-made stressors, sewage effluent is a major contributor to altering nutrient regimes in coastal environments, causing organic enrichment (Ryther and Dunstan, 1971; Gappa and Tablado, 1990; Vitousek et al., 1997; Soltan et al., 2001; Muniz et al., 2011). This fluctuation in organic input may be considered as one of the main causes of faunal change in near-shore benthic environments (Pearson and Rosenberg, 1978; Borja et al., 2006; Jaubet et al., 2011, 2013;

Elías et al., 2015; Becherucci et al., 2016b), often leading to an increase in the abundance of opportunistic species, and a reduction of previously established species (Littler and Murray, 1975; Chapman et al., 1995; Stark et al., 2003). Several invasive species that are known to be opportunistic (Cloern, 2001; Stachowicz et al., 2002) are also sewage-tolerant organisms, such as the introduced mollusk *Crepidula fornicata* (Cloern, 2001), the tube dwelling diatom *Berkeleya* sp. (Becherucci et al., 2016a), the non-indigenous macroalgae *Undaria pinnatifida* (Morelissen, 2012) and many species of polychaetes (Pearson and Rosenberg, 1978; Borja et al., 2006; Jaubet et al., 2011; Elías et al., 2015).

Ascidians (Phylum: Chordata, Sub-phylum: Tunicata) are sessile filter-feeders with several species successfully established worldwide, and represent one of the main biofouling taxa, particularly on marine vessels (Lambert, 2002; Davidson et al., 2010). Ascidians are known for their resilience to wide ranges of salinity and temperature (Nagar and Shenkar, 2016), as well as to high levels of pollution (Naranjo et al., 1996; Beiras et al., 2003; Pineda et al., 2012). Moreover, they tend to flourish in areas characterized by high organic enrichment (Naranjo et al., 1996; Marins et al., 2010). Introductions of non-indigenous ascidians (NIA) into harbors are now commonplace in every marine ecosystem, and severely impact the native fauna, aquaculture and marine infrastructures (Lambert and Lambert, 2003; Castilla et al.,

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2004; Aldred and Clare, 2014). Once established, introduced ascidians are able to rapidly overgrow any available substrates, including other organisms, and are very challenging to eradicate (Bullard et al., 2007).

In many cases NIA will appear first in harbors and marinas, mainly on artificial substrates, and only later will be observed to inhabit natural environments too (Lambert and Lambert, 1998; Gewing et al., 2014). This phenomenon can be explained by their main mode of introduction – marine vessels (Gewing and Shenkar, 2017), and their preferences for an artificial substrate (Lambert, 2002; Glasby et al., 2007). The majority of studies investigating NIA establishment have been conducted on settlement plates placed on artificial structures, and concentrated on monitoring marinas and harbors (Stachowicz et al., 1999; Lambert, 2002; Dijkstra et al., 2007; Glasby et al., 2007; Roche et al., 2015; Lord, 2017). However, the conditions assisting to NIA successful establishment in natural environments have largely remained overlooked. Understanding which factors contribute to the successful establishment of NIA in natural ecosystems is thus essential for developing effective management tools and mitigate the impact of NIA on the environment. In the current study, we recorded the distribution of NIA at eight natural sites along the Mediterranean coast of Israel. Our aim was to characterize the sites that showed the highest numbers of NIA, and to investigate potential factors that may contribute to their establishment. Our hypotheses were: (1) NIA population will increase in high proximity to international ports; and (2) prolonged sewage spill events encourage NIA establishment.

## 2. Methods

### 2.1. Data collection method and sites

Ascidian abundance and richness were assessed by a series of field surveys, carried out by SCUBA divers at eight different sites along the Mediterranean Israeli coast (see Fig. 1). Numbers of individuals and species were recorded by belt transects of 10 m length, and 1.5 m width. Transects were located on the natural rocky reef, at a depth of 2–5 m, and a distance of 150–250 m offshore, and were repeated four times per site. Species were defined as “indigenous” or “non-indigenous” according to Pérès (1958) and Shenkar (2008). Surveys were carried out during two seasons - fall (October–December) 2015 and spring (May–June) 2016, in order to avoid incorrect diversity data due to seasonal appearance. Several biotic and abiotic factors that may contribute to NIA successful establishment in the natural environment were examined: (1) distance from the Suez Canal (as the main gateway to non-indigenous species (Coll et al., 2010)); (2) proximity to international ports (km); (3) duration of sewage spill events (accumulated number of days where water contained more than 400 bacterial colonies of Fecal Coliform and Fecal Enterococci in 100 ml), based on open to the public information published by the Ministry of Health, Israel ([http://www.health.gov.il/Subjects/Environmental\\_Health/Pages/ShoresMap.aspx](http://www.health.gov.il/Subjects/Environmental_Health/Pages/ShoresMap.aspx)), for the six months prior to date of survey, and (4) diversity and abundance of native ascidian species. The latter was examined in order to investigate the paradigm in which species-rich communities should be less susceptible to invasion (Stachowicz et al., 1999). The full dataset is provided in Fig. 1 and Appendix A.

### 2.2. Data analysis

Analysis was conducted in R (R Core Team, 2016), using the ‘vegan’ package (Oksanen et al., 2015). Rarefaction curves were built for every sampled site to determine whether complete species richness sampling had been achieved. Further analyses were held only for the sites where the sampling method had been able to capture the actual ascidian species richness.

To determine the differences among sites we used one-way analysis of similarity (ANOSIM, permutation = 9999). The ANOSIM statistic ranges between 0 and 1, where a score closer to 1 indicates that all

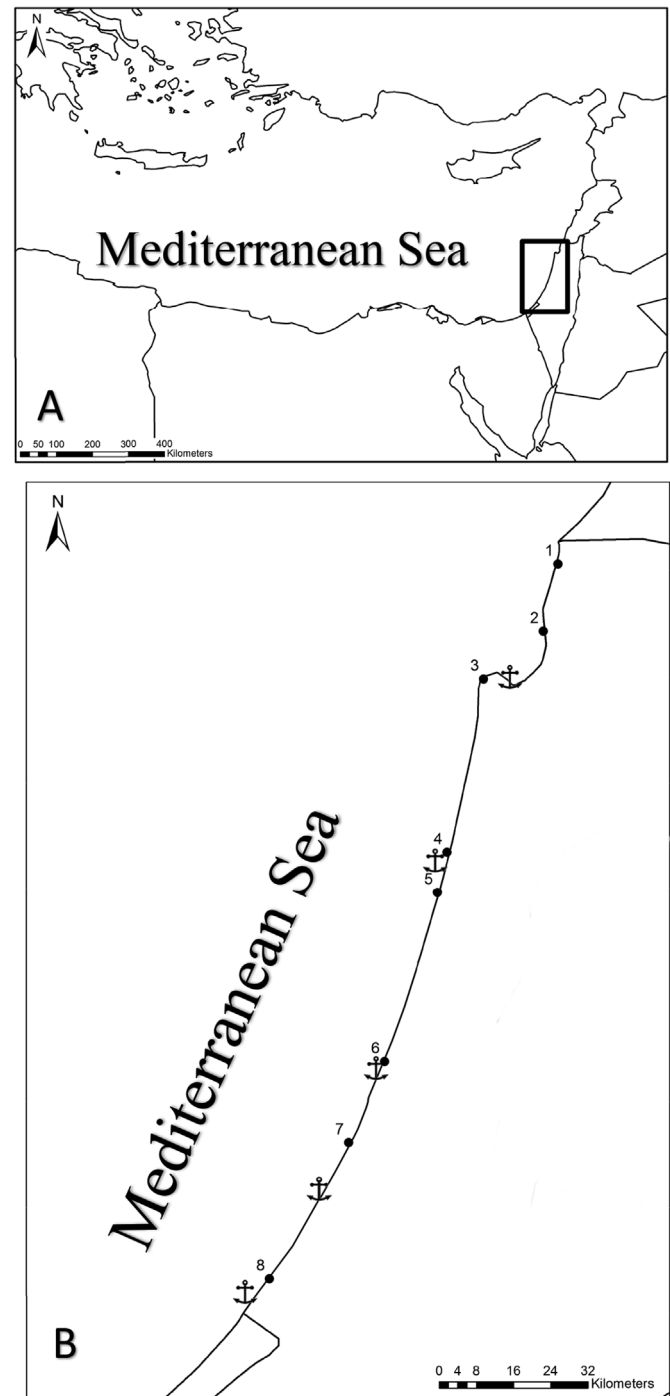


Fig. 1. Study area A. The Levant Basin, B. Study sites along the Mediterranean coast of Israel. Sites from north to south: (1) Achziv, (2) Akko, (3) Haifa, (4) Sdot-Yam, (5) Michmoret, (6) Tel-Aviv, (7) Palmachim, and (8) Ashqelon. Anchor symbols represent international ports locations (coordinates are provided in Appendix A.).

dissimilarities between clusters are larger than any dissimilarity among samples within each cluster (Clarke and Warwick, 2001). Non-metric multidimensional scaling (nMDS) was carried out based on the Bray-Curtis similarity measure (Anderson, 2005), in order to quantify and examine the similarity and differences among ascidian populations at each site.

Similarity percentage analysis (SIMPER) was performed to identify which species best explains the similarity and dissimilarity between ascidians population (richness and abundance) in the sampled sites. Rényi entropy was used to quantify the diversity of each one of the sites

(Hill, 1973). This latter function allows a continuum of possibilities of diversity measurement according to change in the parameter  $\alpha$  in the equation

$$H\alpha = \log \sum_{i=1}^s \frac{P\left(\frac{\alpha}{i}\right)}{(1-\alpha)^i}$$

Where  $\alpha_0 = \log N$ ,  $N$  is the total number of a community's species;  $\alpha_1 =$  is Shannon's entropy (Shannon, 2001), and  $\alpha_2 =$  Simpson's index (Simpson, 1949).

We analyzed the relationship between the investigated biotic and abiotic factors, and NIA abundance and richness using linear regression models. The regression tests were performed from permutation tests, which are suitable for data with limited sample size or that is non-normally distributed. We also used Pearson correlation coefficient to test the correlation between native ascidian abundance and richness. Analysis was conducted in R (R Core Team, 2016), using the *vegan* and *lmPerm* R packages (Oksanen et al., 2015; Wheeler, 2010).

### 3. Results

Species recorded during field surveys included the NIA *Herdmania momus*, *Microcosmus exasperatus*, *Phallusia nigra*, *Styela canopus*, *Rhodosoma turcicum*, and *Symplegma brakenhielmi*, and the indigenous species *Botrylloides leachii*, *Botryllus* sp., *Didemnum* sp., *Cystodytes del-lechiajei*, and *Pyura dura*. *P. nigra* was the most common NIA, while *Didemnum* sp. was the most common indigenous species. *Botryllus* sp. and *Didemnum* sp. found in the survey have long been known from our region, with samples in “The Steinhardt Museum of Natural History, Israel National Center for Biodiversity Studies” in Tel Aviv University (Shenkar, 2008). However, they are yet to be identified to species level. Rarefaction curve of the entire sampling area showed a successful capture of the actual ascidian species richness ( $n = 11$ , Fig. 2a). Examination of each site separately revealed that the sites Achziv, Akko, Haifa, and Michmoret, were insufficiently sampled to capture the true ascidians richness (Fig. 2b–e). The sampling method was found sufficient for Ashqelon, Palmachim, Tel-Aviv, and Sdot-Yam (Fig. 2f–i), and they were included in further analysis.

These latter four sites also revealed a significant difference in ascidian abundance and richness, according to ANOSIM analysis ( $p$ -value = 0.01,  $R = 0.476$ ). nMDS analysis demonstrated a similarity between Ashqelon and Tel-Aviv, and an even greater similarity between Sdot-Yam and Palmachim (Fig. 3).

The largest dissimilarity in ascidians population, revealed by SIMPER analysis, was 91% between Ashqelon and Sdot-Yam, and the smallest was 57% between Palmachim and Sdot-Yam. The presence of the local species *Didemnum* sp. in Palmachim and Sdot-Yam, and its absence in Ashqelon and Tel-Aviv, contributed greatly to the dissimilarity between these sites (Appendix B); while the presence of the NIA *P. nigra* contributed greatly to the dissimilarity between Ashqelon and Tel-Aviv (Appendix B). Rényi entropy revealed that the sites' species richness was the highest in Palmachim and Sdot-Yam, but with no difference in diversity among all the four sampled sites (see Fig. 4). We found a significant negative relationship between sewage spill event duration, and native ascidian richness ( $p$ -value < 0.05,  $y = -0.086x + 1.46$ ,  $R^2 = 0.87$ ). Distance from the Suez Canal and proximity to international ports did not reveal any significant relationship with NIA abundance or richness. In addition, no correlation was found between indigenous species and NIA richness and abundance.

### 4. Discussion

One of the main questions that remains unsolved is that of the mechanism that enables introduced species to succeed in new environments. Here, we investigated possible biotic and abiotic factors

contributing to introduced species' ability to establish in new habitats. The impact of threats and diversity of native species, on habitat resilience to invasion, has been examined elsewhere (Torchin et al., 2003; Olyarnik et al., 2009). The effect of different pollutants and physical disturbances on environment invasiveness has also been extensively investigated (Crooks et al., 2011; Canning-Clode et al., 2011). However, most of the above-mentioned studies have been conducted based on observations of artificial man-made substrates, while research of natural substrates has remained mostly overlooked.

This study sought to characterize the anthropogenic factors affecting NIA establishment in the natural environment. The data we obtained in our work provide valuable information regarding the spread of NIA along the Israeli Mediterranean coast, and can contribute to effective future monitoring efforts. More importantly, the findings will enable us to focus on the elements we indicated as having a significant impact on invasive sessile filter-feeders. The Mediterranean Sea is highly disturbed by anthropogenic threats (Bas, 2009; Coll et al., 2010), many of which are known to encourage marine bioinvasions (Occhipinti-Ambrogi and Savini, 2003; Galil, 2007; Seebens et al., 2013). Dealing with such an enormous extent of potential hazards is highly challenging, if not impossible. Uncovering some of the factors that may influence NIA establishment will enable us to focus our efforts on a strategy that may allow their efficient removal and/or control. Finally, it will hopefully lead to development of effective management tools to control further spread of invasive species in natural environments.

The human-mediated factors examined in our study included the duration of sewage spill events, the proximity to international ports, and the distance from the Suez Canal. Only the first one demonstrated a significant effect on ascidians population in natural environments. The effect of sewage run-offs on the marine environment is well known. Sewage pollution strongly influences the benthic fauna and impacts its diversity in cold, temperate, and tropical environments (Anger, 1975; Littler and Murray, 1975; Pastorok and Bilyard, 1985). Indeed, in our study we witnessed similar phenomena: the sites that were exposed to extended sewage-spill events (Ashqelon and Tel-Aviv) showed lower overall ascidian diversity, with a particularly negative effect on indigenous ascidian richness. The sites that were exposed to short events, or were not exposed at all, demonstrated the highest ascidian richness (Palmachim and Sdot -Yam, respectively). Furthermore, in Ashqelon, where the site was exposed to a constant sewage flow, no native species were observed, only NIA. *Didemnum* sp., which is considered native to the region, contributed greatly to the dissimilarity between the sites, probably due to it being detected only at sites with low or no sewage presence. A difference in its abundance was shown too between Palmachim and Sdot Yam, being higher in Sdot-Yam, where there were no records of any sewage spills. The species that contributed most to the dissimilarity between Ashqelon and Tel Aviv was the solitary ascidian *P. nigra* (native to the tropical Red Sea, introduced to the Mediterranean via the Suez Canal (Shenkar and Loya, 2009);). Bokn et al. (2002), suggested that species that are expected to survive or even thrive in sewage contamination are usually considered as dominant organisms and demonstrate a rapid growth rate and high survivability. *P. nigra* is known for its ability to grow rapidly in large aggregates (Rocha et al., 1999). Moreover, it has also demonstrated the largest geographic distribution along the Mediterranean coast, with populations detected from the Suez Canal to the Aegean Sea (Kondilatos et al., 2010). This expansion to new regions indicate that *P. nigra* is a dominant and opportunistic species, which could explain its presence and dense population in Ashqelon, in contrast to its presence in Tel-Aviv.

Some Lessepsian species have based their spread in the Mediterranean Sea on a “stepping stone” distribution, and demonstrate a decreasing gradient with greater geographic distance from the Suez Canal (Por, 1971; Golani, 1998). In contrast, for ascidians able to travel with marine vessels as their main mode of introduction (Clarke Murray et al., 2011), the effect of proximity to the Canal is not correlated with

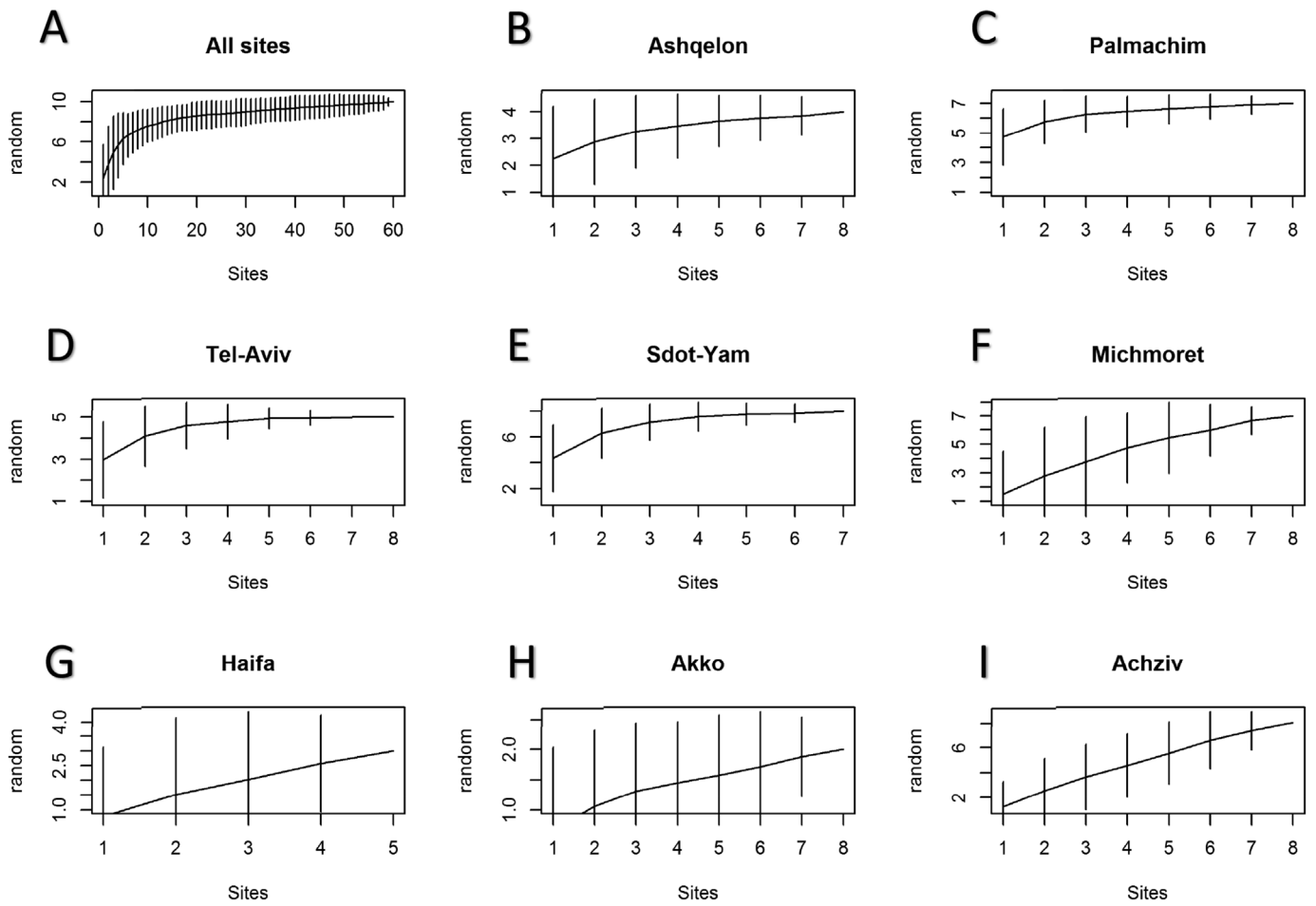


Fig. 2. Rarefaction curves of sampled sites: A. Number of species recorded in each transect, in the sampled area. Ascidian richness was revealed after 30 transects. B–C. Sites sufficiently sampled to capture species richness. F–I. Sites insufficiently sampled to capture species richness.

their distribution.

Proximity to international ports was another factor that was not significantly correlated with ascidians population at the different sites. As noted above, marine vessels are the main vector of ascidian introduction and dispersal; nonetheless, the Israeli Mediterranean coastline is very small, sites are close to each other and constantly connected by vessels movement. Therefore, this factor did not demonstrate

significant difference in NIA population.

Palmachim and Sdot-Yam ascidian richness was significantly higher compared to Ashqelon and Tel-Aviv, mainly due to the native species that were present at the two former sites but not at the latter ones. However, no significant correlation was found between indigenous and NIA abundance and richness. This correlation was the only biotic factor that was examined in order to investigate the local indigenous

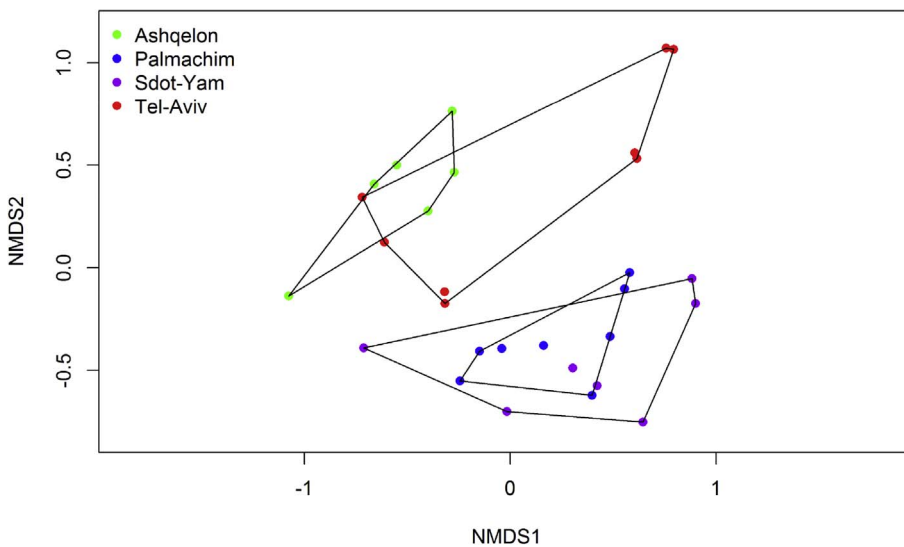


Fig. 3. NMDS ordinations, based on ascidian populations (richness and abundance), across four different sites.

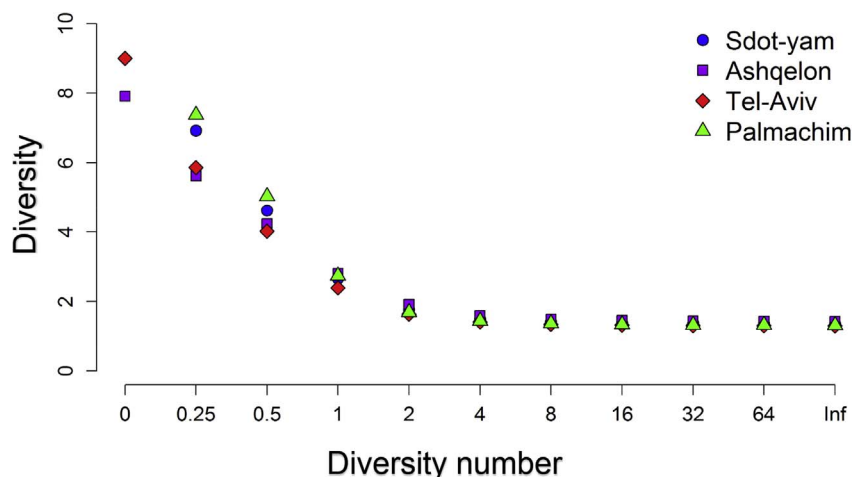


Fig. 4. Rényi entropy demonstrates ascidian diversity at the sampled sites. The X axis represents diversity values in each site, while the Y axis represents  $\alpha$  values. Where  $\alpha = 0$ , X values represent the total species richness in each site; where  $\alpha = 1$ , X values represent the Shannon's entropy;  $\alpha = 2$ , X values equals the Simpson's index; and when  $\alpha = \text{inf}$ , it reciprocal of the proportional abundance of the commonest species.

biodiversity role in invasion success. Unfortunately, our findings may have been affected by the low number of indigenous species along the Mediterranean coast of Israel. Since it is likely that the presence of native ascidians species is not the only factor effecting NIA settlement, other associated taxa should be taken into consideration. We recommend incorporation of an ecological tool with a wider perspective, such as the BENTIX index (Simboura and Zenetos, 2002; Kalkan et al., 2007), or Shannon diversity (Shannon et al., 1963), which will investigate whether higher species diversity (Shannon) or better ecological quality status (Bentix) is related to successful establishment of NIA. Our results reveal that at least half of the sites examined are dominated by NIA. Unfortunately, no previous data on ascidian diversity from the natural habitat along the Israeli Mediterranean Sea are available. Consequently, we are unable to follow the changes that may have taken place in native ascidian populations in correlation to NIA

introduction and establishment. Nonetheless, the data obtained in our study provides a solid dataset for comparison for future monitoring efforts.

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**Appendix A**

Full dataset obtained during underwater field surveys, including number of specimens detected of each species: (a) *Microcosmus exasperatus*, (b) *Herdmania momus*, (c) *Pyura dura*, (d) *Phallusia nigra*, (e) *Styela canopus*, (f) *Rhodossoma turcicum*, (g) *Symplegma brakenhielmi*, (h) *Didemnum* sp., (i) *Botrylloides leachi*, (j) *Cystodytes dellechiaiei*.

j	i	h	g	f	e	d	c	b	a	Nearest international port			Proximity to the nearest port (km)	Distance from the Suez Canal (km)	Duration of sewage-spill events (days)	Site
										Longitude,°E	Latitude,°N	Port				
0	0	0	0	1	34	153	0	0	37	34° 33' 20.89"	31° 38' 15.15"	Oil Port	3.5	213	73	Ashqelon
62	0	141	1	0	12	15	23	0	33	34° 31' 11.99"	31° 49' 55.54"	Ashdod Port	11	235	21	Palmachim
2	0	11	3	0	14	8	48	0	29	34° 47' 35.32"	32° 9' 39.79"	Tel-Aviv Marina	0.2	245	40	Tel-Aviv
0	2	2	3	0	0	36	5	1	11	34° 51' 53.36"	32° 28' 15.47"	Hedera Power Station	6	270	2	Michmoret
139	0	147	1	0	14	5	38	7	22	34° 51' 53.36"	32° 28' 15.47"	Hedera Power Station	3	275	0	Sdot-Yam
6	0	0	0	0	2	0	1	0	0	35° 0' 35.01"	32° 49' 28.12"	Haifa Port	5	300	7	Haifa
2	0	0	0	0	0	1	0	0	15	35° 0' 35.01"	32° 49' 28.12"	Haifa Port	12	315	0	Akko
1	0	1	2	0	1	1	4	1	1	35° 0' 35.01"	32° 49' 28.12"	Haifa Port	26	325	0	Achziv

## Appendix B

The average similarity between the sampled sites, obtained by similarity percentage analysis (SIMPER).

Palmachim vs. Tel-Aviv, Average dissimilarity			Ashqelon vs. Palmachim, Average dissimilarity		
dissimilarity = 84%			dissimilarity = 87%		
Contribution (%)		Species	Contribution (%)		Species
0.37	37.24	<i>Didemnum</i> sp.	0.34	33.72	<i>Didemnum</i> sp.
0.16	15.95	<i>Cystodytes dellechiajei</i>	0.20	20.18	<i>Phallusia nigra</i>
0.13	12.65	<i>Pyura dura</i>	0.14	14.39	<i>Cystodytes dellechiajei</i>
0.09	8.88	<i>Microcosmus exasperatus</i>	0.07	6.81	<i>Styela canopus</i>
0.04	4.24	<i>Phallusia nigra</i>	0.06	6.42	<i>Microcosmus exasperatus</i>
0.04	4.04	<i>Styela canopus</i>	0.05	5.09	<i>Pyura dura</i>
0.01	0.78	<i>Symplegma baharini</i>	0.00	0.40	<i>Symplegma baharini</i>
			0.00	0.10	<i>Rhodosoma turcicum</i>

Palmachim vs. Sdot-Yam, Average dissimilarity			Ashqelon vs. Tel-Aviv, Average dissimilarity		
dissimilarity = 57%			dissimilarity = 79%		
Contribution (%)		Species	Contribution (%)		Species
0.20	20.48	<i>Didemnum</i> sp.	0.30	29.83	<i>Phallusia nigra</i>
0.18	17.85	<i>Cystodytes dellechiajei</i>	0.20	19.56	<i>Pyura dura</i>
0.07	7.30	<i>Pyura dura</i>	0.16	16.03	<i>Microcosmus exasperatus</i>
0.05	4.50	<i>Microcosmus exasperatus</i>	0.12	12.45	<i>Styela canopus</i>
0.03	3.35	<i>Styela canopus</i>	0.01	0.92	<i>Symplegma baharini</i>
0.03	2.66	<i>Phallusia nigra</i>	0.00	0.12	<i>Rhodosoma turcicum</i>
0.01	0.83	<i>Herdmania momus</i>			
0.00	0.46	<i>Symplegma baharini</i>			

Tel-Aviv vs. Sdot-Yam, Average dissimilarity			Ashqelon vs. Sdot-Yam, Average dissimilarity		
dissimilarity = 87%			dissimilarity = 91%		
Contribution (%)		Species	Contribution (%)		Species
			0.30	30.42	<i>Didemnum</i> sp.
0.33	33.29	<i>Didemnum</i> sp.	0.20	19.81	<i>Cystodytes dellechiajei</i>
0.22	21.55	<i>Cystodytes dellechiajei</i>	0.19	19.28	<i>Phallusia nigra</i>
0.14	14.27	<i>Pyura dura</i>	0.07	7.09	<i>Styela canopus</i>
0.08	7.81	<i>Microcosmus exasperatus</i>	0.07	6.82	<i>Pyura dura</i>
0.06	5.60	<i>Styela canopus</i>	0.06	5.83	<i>Microcosmus exasperatus</i>
0.02	2.46	<i>Phallusia nigra</i>	0.01	1.01	<i>Herdmania momus</i>
0.01	1.10	<i>Herdmania momus</i>	0.01	0.70	<i>Symplegma baharini</i>
0.01	0.97	<i>Symplegma baharini</i>	0.00	0.09	<i>Rhodosoma turcicum</i>

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