Heliyon

Endobiotic communities of Marine Sponges in Cyprus (Levantine Sea)

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Abstract

Sponges host cryptic endobionts within their network of canals, with representatives from all major animal phyla. This study investigates the endobiotic community of four sponge species (*Spongia officinalis, Sarcotragus spinosulus, Ircinia* cf. *variabilis* and *Ircinia oros*) that were collected during scientific trawl surveys in the coastal area of Cyprus. Moreover, it examines the endobiotic community composition of *S. spinosulus* in relation to sponges' volume, and various environmental variables. In general, the four sponge endobiotic communities were similar; *S. officinalis* had a significantly different community composition to *I.* cf. *variabilis* and *I. oros*. The phyla identified followed the general infauna composition of sponges, with the relative abundances of the dominant phyla, Arthropoda and Annelida, ranging from 66.9 - 83.7 % and 4.8−26.5 %, respectively. The highest intensity (*I*) corresponded to the isopod *Cymodoce truncata* in *S. officinalis* (*I* = 85 individuals/sponge) and *S. spinosulus* (*I* = 27.2 individuals/sponge). A general linear model also suggested that distance from shore influenced the total endobiotic abundance of

S. spinosulus. This is the first sponge endobiotic community baseline study that covers the whole coastal area of the Republic of Cyprus and is particularly important due to potential changes of Eastern Mediterranean endobiotic communities due to the invasion through the Suez Canal of non-indigenous species.

Keywords: Ecology, Environmental science

1. Introduction

Sponges comprise a major part of the global marine benthos (Taylor et al., 2007; Fan et al., 2012). They increase environmental heterogeneity and can act as microhabitats for vertebrates, invertebrates and a rich microbiome (Abdo, 2007; Fiore and Jutte, 2010; Pita et al., 2018). They are also contemplated as important ecological contributors in reef habitats as they perform several vital ecosystem functions for both the benthic and pelagic environments, compete for space with other benthic organisms, as well as support and enhance biodiversity through bottom-up coupling (Brown et al., 1995; Rutzler, 2003; Trussell et al., 2006; de Goeij et al., 2013; Webster and Thomas, 2016). As autogenic ecosystem engineers, they assist in the survival of productive ecosystems in low nutrient areas like the ultra-oligotrophic regions of Eastern Mediterranean (Gerovasileiou and Voultsiadou, 2012; Miller et al., 2012; Rix, 2015).

Sponge endobiotic communities have been described and investigated extensively around the world, including within regions of Southern US (i.e. Brazil and Argentina), Antarctica, Australia, the Faroe Islands and the Mediterranean Sea (Klitgaard, 1995; Ribeiro et al., 2003; Abdo, 2007; Fiore and Jutte, 2010; Schejter et al., 2012; Kersken et al., 2014; Gerovasileiou et al., 2016). Sponges found in these locations tend to primarily comprise an endobiotic community with organisms from the phyla Arthropoda, Annelida, Echinodermata, Mollusca and Chordata (Klitgaard, 1995; Ribeiro et al., 2003; Abdo, 2007; Fiore and Jutte, 2010; Schejter et al., 2012; Kersken et al., 2014; Gerovasileiou et al., 2016).

Even though the Mediterranean is considered an ecological hotspot for sponge biodiversity with a total of 681 species recorded (48 % of them endemic; Coll et al., 2010), there are still regions and habitats within the Mediterranean, and especially the eastern Levantine Basin (e.g. deep-sea habitats and submarine caves) that need further investigation (Pansini and Longo, 2003; Voultsiadou, 2005, 2009; Coll et al., 2010; Gerovasileiou and Voultsiadou, 2012). More precisely, over the years, the Levantine Sea has had only a handful of investigations concentrating on sponges and their endobiotic community compositions, with only one of these just briefly considering the coastal area of Cyprus (Özcan and Katağan, 2011; Pavloudi et al., 2016). The most studied aspects of Mediterranean sponges are ecology (46.6 % of

available publications), taxonomy (24.7 %) and molecular biology (17 %) (Becerro, 2009).

Koukouras et al. (1996), indicated that endobiotic sponge communities can be either similar or unique between sponge species, as well as that the volume of the sponge can affect the presence, abundance and richness of associated sponge fauna. Further-more, endobiotic associates of the sponges *A. oroides, Petrosia ficiformis*, *Ircinia variabilis* and *A. aerophoba*, in the North Aegean Sea, were found not to be host specific (Koukouras et al., 1992). This leaves the question of whether the endobiotic communities of the sponge species in Cyprus are similar or different, and whether they are affected by their associated fauna, the host, the environmental variables, or a combination of these factors.

Due to the crucial role sponges have within ecosystems, the more we know about their ecology, their associated communities, and the biotic and abiotic factors that affect their ability to thrive, the better our understanding can improve on how they potentially assist the increased abundance and biodiversity of an area. This is espe-cially important for the Eastern Mediterranean because, even though it is an ultra-oligotrophic region, it once was one of the most productive sponge banks (Perez and Vacelet, 2014). Moreover, Cyprus has had a long history of sponge fishery and harvest, activities that have increasingly become more difficult due to the decreased population of commercial sponges from over-exploitation and global warming (Economou & Konteatis 1988, 1990; Perez and Vacelet, 2014).

This baseline study was conducted to tackle the lack of data on sponges and their associated endobiotic communities within the coastal area of Cyprus, as well as their interaction with their environment. In this paper the endobiotic communities of four sponge species are compared as well as the relation between the endobiotic commu-nities with the sponge species, sponge morphology and abiotic factors (depth, salinity, temperature and distance from the shore).

2. Materials and methods

2.1. Study area

The sponges (n = 43) were collected in the Republic of Cyprus using the Trawler R/V 'MEGALOHARI', during two scientific trawler surveys: August 2015 and June 2016 MedITS (Mediterranean International Bottom Trawling Survey) and June 2016 PROTOMEDEA (Protecting Mediterranean East) project (Fig. 1). The samples were retrieved from a depth of 45−100 meters, at a towing speed of 3 knots and duration 30 minutes. The trawler specifications complied with the 'International Bot-tom Trawl Survey in the Mediterranean', Instruction Manual, Version 8 (Spedicato-
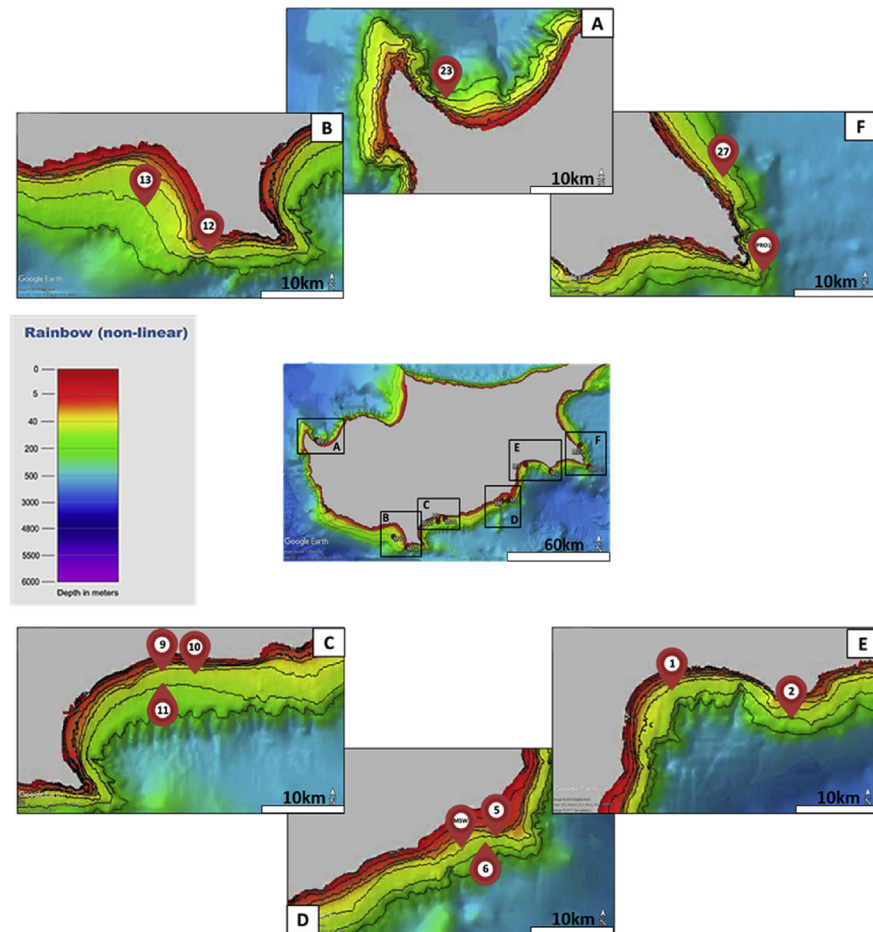
Fig. 1. Haul locations from which sponges were obtained within the coastal area of the Republic of Cyprus; M: 2015−2016 MedITS; PRO: 2016 PROTOMEDEA; MSW: the Mazotos ship wreck (Mean Depth Rainbow Coloured Ramp attained by EMODnet Bathymetry Consortium, 2018).

NB, 2016). One sample was also collected by scuba diving at 43 m depth during a PROTOMEDEA survey at the Mazotos ancient ship wreck (Fig. 1).

2.2. Sponge Collection and Identification

Due to limited time available between hauls, only 43 sponges in total were collected and inspected. Scaled top and side-view photos of each sponge were taken in order to calculate maximum length, width and height (cm). These measurements were then used to calculate the approximate volume (cm^3) of the sponge using the most appropriate formula. A small section of sponge was cut and preserved in 70 % ethanol for later analysis and identification. The remaining sponge was sliced into thin equal strips (2 ± 0.5 cm), and the endobionts (those visible to the eye, >1 mm) were carefully removed with forceps and preserved in 70 % ethanol.

The sponge and associate organism identification was achieved to the lowest taxonomic resolution using the standard identification method and an assortment of identification keys, research papers and online databases (van Soest et al., 1912, Day, 1967, Demetropoulos, 1976, Abel et al., 1986, Tornaritis, 1987, Ruffo, 1989, van Soest et al., 1994a,b, Debelius, 1999, Hooper and Van Soest, 2002, Hooper, 2003, van Soest, 2003, Fauvel, 2004, Pillai, 2009, Fiore and Jutte, 2010, Manconi et al., 2013, San Martín and Worsfold, 2015).

2.3. Environmental data

Salinity was recorded at the end of each haul using a CTD (SBE 19plus Thermosalinometer), while a MiniCTD (SBE 39 Temperature-Pressure Recorder) attached to the trawler-net, logged the depth (m) and temperature (°C). Additionally, the average distance from the shore was calculated using the start and end coordinates of each haul.

2.4. Statistical analysis

2.4.1. Description and comparison of different sponge associated communities

Percentage of relative abundance (*R.A.*) of the endobiotic organisms were calculated both at the Phylum and Family level. Prevalence (*P*) and Intensity (*I*) of each phylum were also calculated to determine their encounter rate and average number of individuals per host sponge species respectively, by using the following formulas:

$$R.A. \, (\%) = \frac{\text{total n of phylum (or Family)individuals within all samples} \, \times 100}{\text{total n of individuals within all samples}} \quad (1)$$

$$P \, (\%) = \frac{\text{n of times encountered in the samples}}{\text{total n of samples}} \times 100 \quad (2)$$

$$I = \frac{\text{total n of individuals per endobiont species}}{\text{total n of host sponge samples}} \quad (3)$$

Species Richness, Shannon-Wiener Diversity Index (*H'*), Pielou evenness (*J'*) and Species Accumulation Curves were calculated per sponge species. Additionally, an analysis of similarity (ANOSIM) based on Bray-Curtis similarity was conducted in 'PastV3' (Version 3.20), to compare the endobiotic community between the sponge species, where initially an overall analysis was performed on all sponges, followed by pairwise tests to identify similarities between individual species. A similarity percentage analysis (SIMPER) was then used to identify the species contributing to the most differences between the sponges. Additionally, a Venn diagram was created to show the distribution of the unique endofauna sponge

associates, versus those that were common in more than one species. A permutational multivariate analysis of variance (PERMANOVA) was also conducted in 'PastV3' to investigate the relationship between the endobiotic community of different sponge species and year.

2.4.2. Sarcotragus spinosulus associated community abundance

A General Linear Model (GLM) was fitted to the data in R (Version 0.98.1091), to examine if maximum depth, and average salinity, temperature and distance from the shore had any significant effect on the total endobiotic abundance of the sponge *Sarcotragus spinosulus*. Similarly, a GLM was also used to investigate whether volume had a significant effect on the total endobiotic abundance of *S. spinosulus*. Variables were only tested on *S. spinosulus* as it was the only species with a large enough sample pool. This was accomplished by first testing the model for normality of residuals (histogram and Q-Q plot), independence of variance, homoscedasticity and collinearity (VIF). Significance of the variables, and their interactions, was determined using the F-ratio and the P-values (significance level $= 0.05$) calculated by ANOVA.

The relationship between the environmental variables and total endobiotic abundance of *S. spinosulus* was calculated using Pearson's correlation coefficient (r) in Microsoft Excel 2010. The same procedure was followed for the volume.

3. Results

3.1. Endobiotic sponge associated community descriptions

Forty four sponge specimens in total were inspected: *Spongia officinalis* (survey 2015 = 2, survey 2016 = 4), *S. spinosulus* (2015 = 11, 2016 = 11), *Ircinia* cf. *variabilis* (survey 2016 = 9) and *Ircinia oros* (2015 = 2, 2016 = 5).

The phylum with the highest relative abundance (*R.A.*) in all four sponge species was Arthropoda (Families: Alpheidae and Sphaeromatidae), followed by Annelida (Families: Nereididae and Terebellidae; except in *I. oros*) (Table 1 and Fig. 2.). A small percentage of organisms were too damaged to be identified.

The Arthropoda species, *Pilumnus hirtellus* (Decapoda) and *Cymodoce truncata* (Isopoda) had both genders present in all sponge species, except in *I. oros* which had only one male. Additionally, *Gnathia phallonajopsis* (Isopoda) had both males and females within *S. spinosulus*, only females in *S. officinalis* and one male in *I. cf. variabilis*. Furthermore, there were a total of seven gravid *P. hirtellus* (four in *S. officinalis*, and three in *S. spinosulus*), four *C. truncata* (in *S. officinalis*), 89 *Synalpheus gambarelloides* (Decapoda; 51 in *S. spinosulus*, 23 in *I. cf. variabilis*, and 15 *I. oros*), one *Pandalina brevirostris* (Decapoda; in *S. spinosulus*), and one *Galathea intermedia* (Decapoda; in *S. spinosulus*) within the samples.

Table 1. Prevalence (P), and Intensity (I) of the endobiotic organisms inhabiting the four sponge species; *Spongia officinalis, Sarcotragus spinosulus, Ircinia* cf. *variabilis* and *Ircinia oros*, collected within the coastal area of Cyprus. '+' = indicates presence of the specific gender within the species.

Species	Spongia officinalis		Sarcotragus spinosulus		Ircinia cf. variabilis		Ircinia oros	
	P	**I**	**P**	**I**	**P**	**I**	**P**	**I**
Annelida								
Clitellata								
Rhynchobdellida								
Stibarobdella moorei (Oka, 1910)			4.6%	1				
Polychaeta								
Amphinomida								
Hermodice carunculata (Pallas, 1766)			4.6%	1				
Eunicida								
Lysidice ninetta Audouin & H Milne Edwards, 1833							14.3%	1
Marphysa sanguinea (Montagu, 1813)	16.7%	1	4.6%	2	11.1%	1		
Phyllodocida								
Phyllodocida sp. 1	16.7%	5	22.7%	1.6				
Bylgides groenlandicus (Malmgren, 1867)			9.1%	1.5				
Hediste diversicolor (O.F. Müller, 1776)	83.3%	8	59.1%	3.9	66.7%	3.3	28.6%	1
Lepidasthenia elegans (Grube, 1840)			4.6%	1				
Odontosyllis sp. 1					11.1%	1		
Hesionidae sp. 1			4.6%	1				
Syllis armillaris (O.F. Müller, 1776)					11.1%	2		
Sabellida								
Sabella pavonina Savigny, 1822					11.1%	1		
Serpula cf. *vermicularis* (Linnaeus, 1767)					11.1%	1		
Vermiliopsis infundibulum (Phillipi, 1844)			4.6%	1	11.1%	1		
Terebellida								
Eupolymnia nesidensis (Delle Chiaje, 1828)					11.1%	3		
Nicolea zostericola (Örsted, 1844)	50.0%	2	13.6%	2.7	22.2%	1		
Terebellidae sp. 1			4.6%	2				
Arthropoda								
Malacostraca								
Amphipoda								
Aristias cf. *tumidus* (Kroyer, 1846)	16.7%	3			11.1%	1		
Colomastix pusilla (Grube, 1861)	16.7%	3	13.6%	4				
Dexamine spiniventris (Costa, 1853)			4.6%	1				
Quadrimaera inaequipes (A. Costa, 1857)			13.5%	5.7				

(*continued on next page*)

Table 1. (*Continued*)

Species	Spongia officinalis		Sarcotragus spinosulus		Ircinia cf. variabilis		Ircinia oros	
	P	**I**	**P**	**I**	**P**	**I**	**P**	**I**
Decapoda								
Athanas nitescens (Leach, 1813)	66.7%	2.5	13.6%	2				
Galathea intermedia Lilljeborg, 1851	16.7%	2.5	18.2%	1.3				
Pandalina brevirostris (Rathke, 1843)			9.1%	2.5				
Pilumnus hirtellus (Linnaeus, 1761)	66.7%	4	54.6%	2.8	33.3%	3.3	14.3%	1
Pilumnus hirtellus − FEMALE	+	3.3	+	2	+	1		
Pilumnus hirtellus − MALE	+	1.5	+	2	+	1	+	1
Synalpheus gambarelloides (Nardo, 1847)			59.1%	22.4	66.7%	9.5	100%	6.1
Isopoda								
Anilocra physodes (Linnaeus, 1758)			4.6%	1				
Cymodoce truncata (Leach, 1814)	16.7%	85	40.9%	27.2	33.3%	3.7		
Cymodoce truncata − FEMALE	+	45.5	+	10.9	+	3		
Cymodoce truncata − MALE	+	39.5	+	18.4	+	2		
Gnathia phallonajopsis Monod, 1925	16.7%	5	22.7%	5	22.2%	1		
Gnathia phallonajopsis − FEMALE	+	5	+	2.2				
Gnathia phallonajopsis − MALE			+	2.8	+	1		
Chordata								
Actinopterygii								
Bony fish sp. 1	16.7%	1						
Perciformes								
Spicara smaris (Linnaeus, 1758)							14.3%	2
Ascidiacea								
Stolidobranchia								
Herdmania momus (Savigny, 1816)			4.6%	1				
Echinodermata								
Asteroidea								
Forcipulatida								
Marthasterias glacialis (Linnaeus, 1758)			4.6%	1				
Ophiuroidea								
Ophiurida								
Amphiura chiajei (Forbes, 1843)	16.7%	5	13.3%	2				
Mollusca								
Bivalvia								
Limida								
Lima lima (Linnaeus, 1758)							14.3%	12
Arcida								

Table 1. (*Continued*)

Species	Spongia officinalis		Sarcotragus spinosulus		Ircinia cf. variabilis		Ircinia oros	
	P	I	P	I	P	I	P	I
Barbatia barbata (Linnaeus, 1758)			13.3%	1	11.1%	1	14.3%	1
Gastropoda								
Neogastropoda								
Raphitoma contigua (Monterosato, 1884)			4.6%	1				

The dominant organisms within the sponges *S. spinosulus*, *I. cf. variabilis* and *I. oros* was the snapping shrimp *S. gambarelloides*, followed by the polychaete *Hediste diversicolor* (Phyllodocida). In *S. officinalis*, *S. gambarelloides* was absent and the dominant species was *H. diversicolor*.

The endobiotic species with the highest intensity included *C. truncata* (in *S. officinalis* and *S. spinosulus*), *S. gambarelloides* (in *S. spinosulus*, *I. cf. variabilis* and *I. oros*), and the bivalve *Lima lima* (Limida; in *I. oros*).
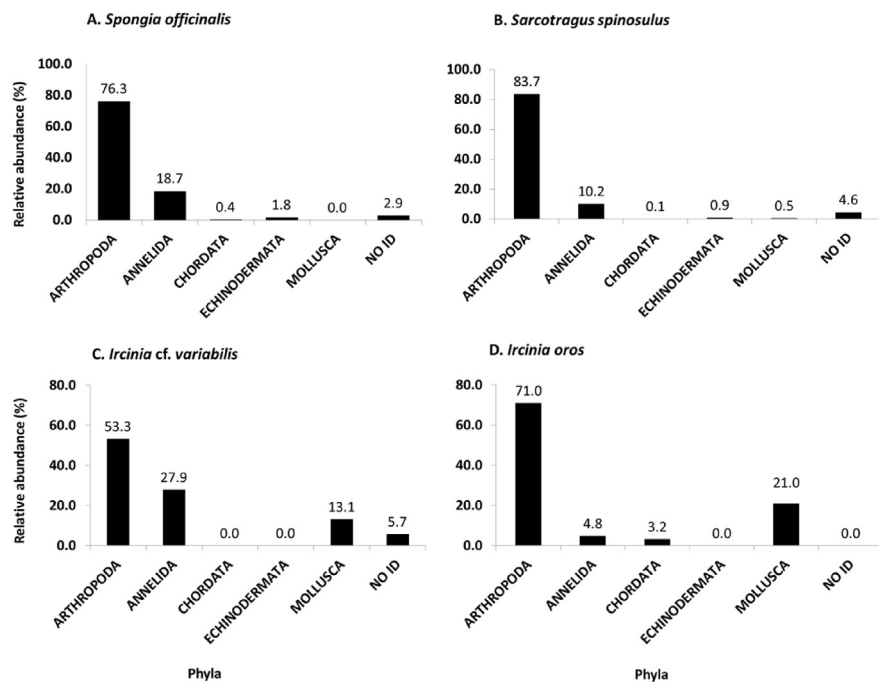

Fig. 2. Relative abundances of all phyla established within the four sponge species collected during the Mediterranean International Bottom Trawling Survey (MedITS) and Protecting Mediterranean East (PROTOMEDEA) projects, in the coastal area of Cyprus. **A.** *Spongia officinalis*, **B.** *Sarcotragus spinosulus*, **C.** *Ircinia* cf. *variabilis* and **D.** *Ircinia oros*.

3.2. Species richness, diversity, evenness and accumulation curve

The highest species richness (n) and diversity (H') were obtained by *S. spinosulus* (n = 30; H' = 1.74), followed by *Ircinia* cf. *variabilis* (n = 15; H' = 1.69), *S. officinalis* (n = 14; H' = 1.38), and lastly *I. oros* (n = 5; H' = 0.93). Additionally, the endobiotic evenness for the sponges *I. oros* (J' = 0.52), *S. spinosulus* (J' = 0.53) and *S. officinalis* (J' = 0.54) was around 0.5. The species that displayed the most evenness was *Ircinia* cf. *variabilis* (J' = 0.62).

The species accumulation curves of *S. spinosulus* and *I. oros* (Fig. 3) demonstrated that enough samples were obtained for an accurate endobiotope description. However, *S. officinalis* and *Ircinia* cf. *variabilis* lacked samples for a complete endobiotic community account.

3.3. Similarities amongst sponge associated communities

The endobiotic community composition was similar between the four sponge species (ANOSIM; overall R = 0.15, p = 0.014; Table 2). Furthermore, the pairwise comparisons (*pw*) indicated that the sponge *S. officinalis* had a relatively different endobiotic community in relation to *I.* cf. *variabilis* (*pw*; R = 0.22, p = 0.040; Table 2), and an almost completely different community composition to the species *I. oros* (*pw*; R = 0.93, p = 0.0004; Table 2). The SIMPER analysis indicate that the associated endobiotic species that most contributed to the dissimilarity between *S. officinalis* and *I.* cf. *variabilis,* and *S. officinalis* and *I. oros* were *C. truncata* (27.46 and 22.22 %), *S. gambarelloides* (19.78 and 22.47 %) and *H. diversicolor* (19.94 and 21.67 %). Additionally, *S. spinosulus* was the only species that had several mutual organisms with all four sponge species (Fig. 4).
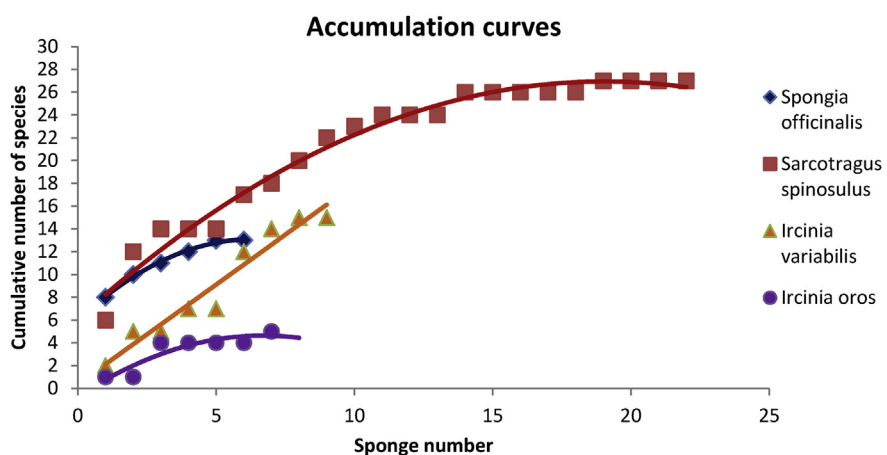

Fig. 3. Species Accumulation Curves for the four studied sponge species; *Sarcotragus spinosulus* (red), *Spongia officinalis* (blue), *Ircinia* cf. *variabilis* (orange) and *Ircinia oros* (purple), within the coastal area of the Republic of Cyprus.

Table 2. Summary of analysis of similarity (ANOSIM) indicating both the R-statistic and p-values for the overall test (similarity of the endobiotic community of the four sponges species), and pairwise test comparing the four sponge species between themselves. '*' = indicates 95% significance.

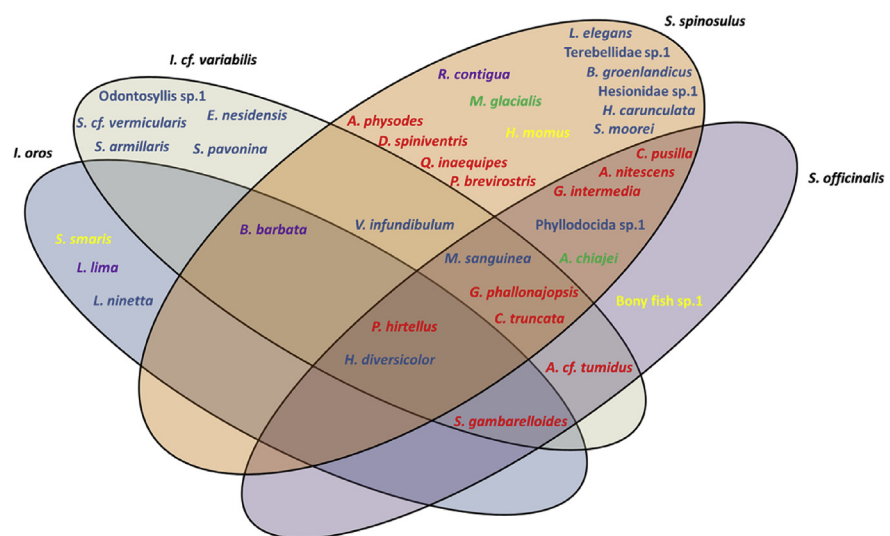| Sponge species	R-statistic	P-value
Overall	0.1534	0.0139*
S. spinosulus vs S. officinalis	0.1556	0.0897
S. spinosulus vs I. cf. variabilis	0.0011	0.4482
S. spinosulus vs I. oros	0.1823	0.0586
S. officinalis vs I. cf. variabilis	0.2211	0.0403*
S. officinalis vs I. oros	0.9253	0.0004*
I. cf. variabilis vs I. oros	0.0666	0.1795

Fig. 4. Venn diagram illustrating the distribution of the endobiotic associates within the four sponge species; *Sarcotragus spinosulus*, *Spongia officinalis*, *Ircinia cf. variabilis* and *Ircinia oros*. Shown are the phyla Annelida (blue), Arthropoda (red), Chordata (yellow), Echinodermata (green) and Mollusca (purple).

The analysis also illustrated that the endobiotic associated community of the sponges was affected by sponge species (PERMANOVA; F = 1.46, p = 0.0005; Table 3), but not the year (PERMANOVA; F = 1.01, p = 0.09; Table 3).

3.4. Total endobiotic abundance of *Sarcotragus spinosulus*, in relation to environmental variables

Distance from the shore (GLM; $F_{1,9}$ = 5.68, p = 0.041; Table 4) significantly affected the endobiotic abundance of *S. spinosulus*, while depth, salinity and

Table 3. Summary of permutational multivariate analysis of variance (PER-MANOVA) for the endobiotic community composition of the sponge samples; *S. spinosulus*, *S. officinalis*, *I.* cf. *variabilis* and *I. oros*, and Year. '*' = indicates 95% significance.

	d.f.	F-value	p-value
Sponge	3	1.456	0.0005*
Year	1	1.013	0.0863
Interaction	3	-4.024	0.3823
Residuals	35		

Table 4. Summary of the General linear model (GLM) fitted to the data to analyse the relationship between the environmental variables in relation to the total endobiotic abundance of the sponge *Sarcotragus spinosulus*. Depth = Maximum depth; Distance = Distance from the shore; Temperature = Average temperature at depth and Salinity; '*' = indicates 95% significance.

	d.f	F-value	P-value
Depth	1	5.0462	0.05131
Distance	1	5.6823	0.04097*
Salinity	1	0.4680	0.51115
Temperature	1	1.3261	0.27918
Depth: Distance	1	0.7316	0.41455
Depth: Salinity	1	8.9508	0.01516*
Distance: Salinity	1	0.1696	0.69006
Depth: Temperature	1	0.9568	0.35355
Distance: Temperature	1	0.0030	0.95747
Salinity: Temperature	1	7.4930	0.02295*
Depth: Distance: Salinity	1	0.5333	0.483781
Depth: Distance: Temperature	1	3.2584	0.10454
Residuals	9		

temperature didn't (GLM, Depth; $F_{1,9} = 5.05$, p = 0.051, Salinity; $F_{1,9} = 0.47$, p = 0.51, Temperature; $F_{1,9} = 1.32$ p = 0.28; Table 4). Moreover, total endobiotic abundance had a very weak negative correlation with maximum depth ($r^2 = -0.10$), and a weak relationship with salinity ($r^2 = -0.40$; Fig. 5A & B). In contrast, average temperature and distance from the shore displayed a weak ($r^2 = 0.31$), and a very weak positive ($r^2 = 0.13$) relationship with total abundance respectively (Fig. 5C & D).

The variable interaction effect (Table 4) presented an interaction between depth and salinity (GLM, $F_{1,9} = 8.95$, p = 0.015), and one between salinity and temperature (GLM, $F_{1,9} = 7.49$, p = 0.023).
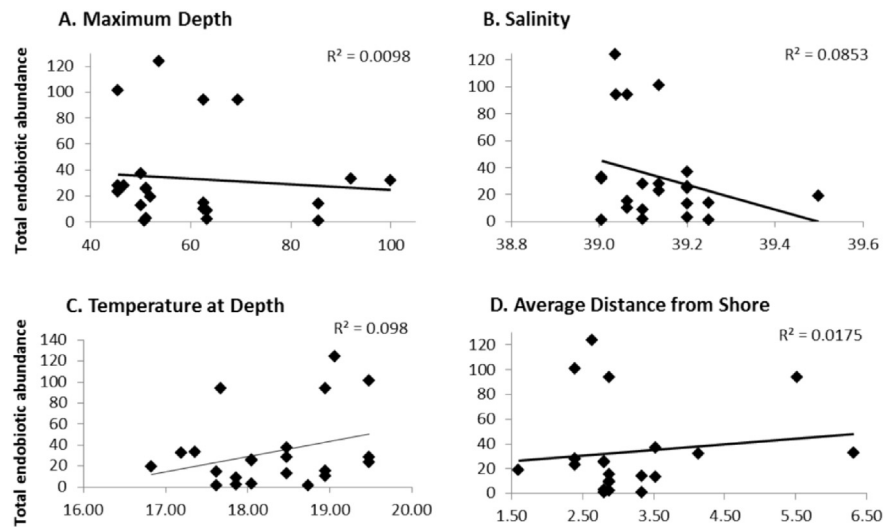
Fig. 5. Correlation between the total endobiotic abundance of *Sarcotragus spinosulus* and the environmental variables **A.** Maximum depth, **B.** Salinity, **C.** Temperature at depth and **D.** Average distance from the shore.

3.5. Total sponge endobiotic abundance in relation to sponge volume

A significant relationship between sponge volume and total endobiont abundance (GLM: $F_{1,25} = 14.3$, p = 0.0009; Table 5), but not species (GLM: $F_{3,25} = 2.77$, p = 0.063) was found. Additionally, the GLM produced a positive correlation ($r^2 = 0.48$) between total endobiotic abundance and volume. The endobiotic phyla abundance increased proportionally to the percentage of the sponge that they already inhabited i.e. at a volume of 591.29 cm^3 the highest abundance was obtained by the Arthropoda phylum (n = 10), followed by the Annelida (n = 5); at a volume of 9599.44 cm^3 the highest abundance was obtained again by the Arthropoda (n = 89), followed by the Annelida (n = 13) (Table 6).

4. Discussion

The four sponge species follow the general faunal composition (Annelida, Arthropoda, Chordata, Echinodermata and Mollusca) that usually forms the infaunal

Table 5. Summary of the General linear model (GLM) fitted to the data to analyse the relationship between the volume and "Total endobiotic community abundance" of the sponge samples.

	d.f	F-value	P-value
Species	3	2.7657	0.0628576
Volume	1	14.3295	0.0008578*
Residuals	25		

Table 6. Sponge samples collected during the Mediterranean International Bottom Trawling Survey (MedITS) and Protecting Mediterranean East (PROTO-MEDEA) projects, in the coastal area of the Republic of Cyprus, along with their corresponding dimensions (maximum length, width and height), approximate volume and their total endobiotic abundance.

Sample name	Sponge Species	Sponge length (cm)	Sponge width (cm)	Sponge height (cm)	Approximate volume (cm^3)	Total Endobiotic Abundance
H1SP1-M16	*S. officinalis*	30.51	22.60	15.50	5596.04	81
H1SP3-M16	*S. officinalis*	22.20	17.26	10.30	2066.47	17
H1SP4-M16	*S. officinalis*	42.17	32.35	13.50	9642.96	150
H10SP2- M16	*S. officinalis*	15.50	12.75	9.50	983.02	14
H1SP2-M16	*S. spinosulus*	23.03	20.03	13.10	3164.06	124
H6SP3-M16	*S. spinosulus*	35.10	27.00	20.50	10172.40	94
H9SP2-M16	*S. spinosulus*	36.48	29.05	17.30	9599.44	189
H9SP4-M16	*S. spinosulus*	20.42	15.85	11.20	1898.02	23
H9SP5-M16	*S. spinosulus*	23.04	20.99	14.80	3747.62	28
H10SP3-M16	*S. spinosulus*	17.53	14.32	8.50	1117.23	94
H10SP4-M16	*S. spinosulus*	12.70	11.60	7.60	591.29	10
H10SP5-M16	*S. spinosulus*	10.68	10.00	8.55	478.12	15
H11SP1-M16	*S. spinosulus*	24.84	23.43	16.50	5028.13	32
H13SP1-M16	*S. spinosulus*	40.50	27.27	10.20	5898.46	46
H1SP5-M16	*I.* cf. *variabilis*	17.18	13.02	9.40	1100.93	13
H7SP2-M16	*I.* cf. *variabilis*	9.44	8.30	8.10	332.30	8
H9SP3-M16	*I.* cf. *variabilis*	13.95	12.86	9.40	882.96	22
H10SP1-M16	*I.* cf. *variabilis*	19.29	16.70	10.70	1804.81	33
H10SP6-M16	*I.* cf. *variabilis*	11.80	11.00	4.80	326.22	8
H10SP7-M16	*I.* cf. *variabilis*	11.20	10.30	4.30	259.73	10
H11SP2-M16	*I.* cf. *variabilis*	9.48	8.60	4.60	196.36	6
H6SP2-M16	*I. oros*	-	11.00	28.06	2666.63	16
H7SP1-M16	*I. oros*	-	5.52	38.15	912.98	6
H1SP4-M16	*I. oros*	-	6.48	29.17	962.00	2
H1SP5-M16	*I. oros*	-	6.00	12.00	339.29	1
DIVESP1- M16	*I. oros*	-	3.00	11.70	82.70	4

community of sponges worldwide (Ribeiro et al., 2003; Abdo, 2007; Fiore and Jutte, 2010; Gerovasileiou et al., 2016). The same synthesis at the phylum level was also found for the communities associated with two species of *Haliclona* sp. in Southwest Australia, and the sponges *Agelas oroides* and *Aplysina aerophoba* in the North Aegean Sea (Abdo, 2007; Gerovasileiou et al., 2016). Moreover, the sponge *Mycale*

microsigmatosa from Southeast Brazil, had an associated endobiotic community that comprised species mainly from the phyla Crustacea, Polychaeta, Mollusca, Cnidarian and Echinodermata (Ribeiro et al., 2003).

The dominant phylum (*R.A.* > 50%) within all sponge species was Arthropoda, and more specifically the obligate sponge-dweller shrimp *Synalpheus gambarelloides* (Duffy et al., 2000). Similarly, *S. gambarelloides* (*R.A.* = 86.6%) was the dominant organism in the sponge *Sarcotragus muscarum* in a study conducted in Turkey - Levantine Basin (Özcan and Katağan, 2011). The high abundance of *S. gambarelloides* could be explained by its bigger size, which possibly provides it with a competitive advantage of obtaining more space and nutrients (Özcan and Katağan, 2011). The same concept can probably also be applied to the isopod *Cymodoce truncata*, where in the absence of the *Synalpheus* shrimp, its intensity was observed to be three times higher.

The Arthropoda had the most common infaunal associates within the four sponge species, with the hairy crab *Pilumnus hirtellus* being identified in all of them (Table 1). The presence of *P. hirtellus* in numerous sponge species was confirmed by several studies, including an investigation conducted in the Gulf of Morbihan, France on the sponge *Celtodoryx girardae* (Perez et al., 2006).

Annelida are almost always second or third most dominant phylum in sponges (Ribeiro et al., 2003). In *Spongia officinalis*, *Sarcotragus spinosulus* and *Ircinia* cf. *variabilis*, Annelida was the second most abundant phylum. This was also observed in two studies conducted in the North Aegean Sea using the sponges *I. variabilis* and *S. officinalis* (Koukouras et al., 1985, 1992). The Annelida also varied greatly in their frequency and abundance within the sponges, with the exception of *Hediste diversicolor* which obtained the highest dominance and prevalence within all sponge species. Similarly, *H. diversicolor* was identified as dominant in sponge communities in the Bay of Izmir, Aegean Sea (Çinar and Ergen, 2001).

Moreover, the bivalve *Barbatia barbata*, which was present in all sponge species except *S. officinalis* was also identified in *Aplysina* (*Verongia*) *aerophoba*, North Aegean Sea, while the other two species, *Lima lima* (Bivalvia) and *Raphitoma contigua* (Gastropoda), haven't been associated with any other sponge so far (Voultsiadou-Koukoura et al., 1987). The Ophiurida *Amphiura chiajei* was also present in the sponges *A. aerophoba* and *Axinella cannabina*, but there are no studies indicating the starfish *Marthasterias glacialis* as an endobiotic sponge associate (Koukouras et al., 1996). There is however some evidence demonstrating a feeding relationship between *M. glacialis* and various sponge associates, such as the bivalve *Arca noae*, which is a host mollusc for the sponge *Crambe crambe* (Marin and Belluga, 2005). It is possible that *M. glacialis* was lodged into the sponge by the force of the haul. Similarly, it's possible that the *Spicara smaris* were also accidentally lodged into *I. oros* during the trawl, because even though fish have been

identified within sponges before, juvenile *S. smaris* usually inhabit soft bottoms or *Posidonia* meadows (Tunesi et al., 2005; Gerovasileiou et al., 2016).

The differences between the prevalence and the intensity of *H. diversicolor*, *S. gambarelloides* and *C. truncata* could be a consequence of several biotic and abiotic factors that affect either the sponge (host) or the associates (endobionts) directly, including environmental, biological or morphological variables (Iyaji et al., 2009; Khidr et al., 2012; Sherrard-Smith et al., 2012). An interesting case to point out is the hairy crab *P. hirtellus* where no matter its prevalence within sponges, it seems to have a constant intensity of about 2.7 individuals per host sponge. This could be a result of the life history of the endobiont itself. Examples of where the sponge morphology might be playing a role in the number of individuals per host sponge are the endobionts *C. truncata*, *S. gambarelloides* and *H. diversicolor*. This is because these species are relatively large organisms compared to the rest of the sponge associates, and in the sponge species identified they always seem to follow the same trend: highest numbers of individuals were identified within the sponge *S. officinalis*, followed by *S. spinosulus*, *I.* cf. *variabilis* and then *I. oros,* (Table 1).

Many of the infauna associates were common between more than one sponge species (Table 1), and all four sponges shared at least two endobiont species. Many of the endobionts were also shared with various other sponge associate communities i.e. the species, *Lepidasthenia elegans* (Polychaeta), *Athanas nitescens* (Decapoda), *Colomastix pusilla* (Amphipoda), *C. truncata* (Isopoda), *Dexamine spiniventris* (Amphipoda) and *B. barbata* (Bivalvia), were also identified in the sponge *Aplysina* (*Verongia*) *aerophoba* in the North Aegean Sea (Voultsiadou-Koukoura et al., 1987). A recent investigation into the endobiotic community of the sponge *Sarcotragus foetidus* in Greece and Cyprus, revealed common endobiotic species of Annelida (6 species), Arthropoda (5 species) and Mollusca (1 species) with the sponge samples from the current investigation (Pavloudi et al., 2016). To be more precise, it had the most mutual species from the Annelida phylum with the sponge *I.* cf. *variabilis*, from the Arthropoda with *S. spinosulus*, and from the Mollusca with *I. oros*.

Distance from the shore was the only variable that affected the total endobiotic abundance of *S. spinosulus*, and after further analysis the following pattern arose: at closer distances there was lower species richness with higher abundance, but as distance increased the abundance of those species decreased and species richness across all phyla increased.

Depth is a known factor influencing sponges and the abundance of endosymbionts (Ribeiro et al., 2003). In the current study depth did not significantly affect the endobiotic abundance of *S. spinosulus*, however, that might be because of the depth range present within our study area; no shallow zones present. Moreover, since there is no known direct association between depth and salinity, the interaction is most likely

driven by a third unknown variable that could be triggered by a number of factors including currents, oxygen availability or turbidity (Bell, 2007).

The sponge volume also affected the total endobiotic community abundance. As the volume of the sponge increased and there was more available space (i.e. niche) for the organisms to use, the total endobiotic abundance increased too.

5. Conclusions

The sponge endobiotic communities identified in Cyprus seem to have similarities in their general structure with sponge associated infauna reported from other areas. Moreover, *Synalpheus* shrimp dominated the samples it inhabited, probably because of its bigger size and competitive advantage over other endobionts. From all four sponge species, *Sarcotragus spinosulus* is most likely the species that can endure the most external pressures. Depth did not significantly affect the endobiotic abundance of *S. spinosulus*, but that might be due to the depth range used in the study, rather than the significance of depth. Bigger sponges were able to facilitate higher community abundances, while at the same time the number of organisms increased proportionally to their relative abundance within the sponge specimens. Based on the present investigation and the arrival of the non-indigenous species (NIS) through the Suez Canal, we suggest that the endobiotic research areas that need prompt attention include the distribution and abundance of phyla within sponges, and how these interact with each other and their host. The potential effects of these NIS, which more often than not establish successfully in the Levantine Sea, a well-known hot spot of bioinvasions, need to be documented (Katsanevakis et al., 2014; Giakoumi et al., 2016; Galil et al. 2017, 2018; Rilov et al., 2017).

Declarations

Author contribution statement

Magdalene Papatheodoulou: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Carlos Jimenez: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Antonis Petrou: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Ioannis Thasitis: Performed the experiments.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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