The role of two non-indigenous serpulid tube worms in shaping artificial hard substrata communities: case study of a fish farm in the central Mediterranean Sea

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ABSTRACT: Understanding the spread and establishment of non-indigenous species (NIS) is one of the primary areas of focus in bioinvasion science and is essential for generating appropriate management strategies in aquaculture. Here we investigated the role of 2 non-indigenous serpulid tube worms (*Hydroides elegans* and *H. dirampha*) in shaping the hard substrata communities around a fish farm in the Strait of Sicily over 1 yr (June 2014–June 2015), at 3 mo intervals (September and December 2014, March and June 2015). The mean density values of serpulids were significantly different at each sampling time. The density of serpulids on submerged panels showed a peak in spring (March 2015, after 9 mo) and decreased drastically in summer (June 2015). *Hydroides* shaped the entire associated macrobenthic community, which showed the highest density in the first month of observations. The lowest density was recorded in proximity to the highest density of serpulids. Margalef's species richness of the associated macrofaunal community peaked at the end of monitoring (summer). Our results will add new insights into the role of aquaculture as a vector and pathway for NIS.

KEY WORDS: Non-indigenous species · Annelida · Serpulidae · Macrofouling · Pathway · Aquaculture cages

1. INTRODUCTION

Non-indigenous species (NIS) are recognised as one of the major threats affecting biodiversity worldwide (Didham et al. 2007, Molnar et al. 2008) and since the last decade have become one of the focus areas of bioinvasion science (Pyšek & Richardson 2010, Vilà et al. 2011, Kumschick et al. 2015). NIS abundance and diversity vary on both regional and global scales, in both terrestrial and aquatic environments (Molnar et al. 2008, Byers et al. 2015, van Kleunen et al. 2015, Fletcher et al. 2016), according to a complex combination of differences in life histories and biological traits, timing and type of transport vectors, and abiotic and interactive biotic factors in the recipient environment (Wonham & Carlton 2005, Katsanevakis et al. 2014, Ojaveer et al. 2014, Hulme et al. 2015, Cardeccia et al. 2018, Sarà et al. 2018c). Invasions can generate further cascading effects, such as the alteration of local inter- and intraspecific relationships within native species (Shea & Chesson 2002, Wallentinus & Nyberg 2007) and the alteration of resource dynamics, which facilitate NIS spreading and further enhance the effects on biodiversity at a local scale (Meiners et al. 2001, Stachowicz et al. 2002, Yurkonis et al. 2005).

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Although some authors have recently focused on the study of seasonal NIS succession and macro-fouling community development (Lezzi et al. 2018, Martell et al. 2018), to date, the facilitation processes that regulate the spatial and temporal settlement, growth, population size and features of NIS are poorly known, especially in a Mediterranean context (Galil et al. 2018). Within fouling communities, more attention has been paid to sessile NIS, including a various array of solitary and colonial marine invertebrates (e.g. ascidians, bryozoans, bivalve molluscs, tube-building polychaete worms, sponges, barnacles and sea anemones; Wallentinus & Nyberg 2007, Lezzi et al. 2018, Sarà et al. 2018a). These organisms not only shape local metabolic and trophic interactions, by altering the physical or biological environment in the system, they can exert a strong habitat-modifying effect, making it more suitable for some species and less so for others (Stachowicz et al. 2002, Floerl et al. 2004, Sarà et al. 2018a,b). NIS habitat modifications might have a pronounced ecosystem impact and may cause changes in ecosystem goods and services (Wallentinus & Nyberg 2007, Sarà et al. 2018b). To disentangle the set of factors that can determine larval settlement and intra- or interspecific interactions along successive stages of colonisation (from recruitment to the maximum density step), it may be crucial to have an in-depth understanding of sessile NIS distribution and density (Bramanti et al. 2003). Such information is valuable when setting up modelling exercises (Sarà et al. 2018c) or when shaping new regional and cross-border NIS management strategies and plans and, more specifically, to facilitate the evaluation of risks and impacts associated with future spreading (Ojaveer et al. 2015).

A call for more efforts in reporting and monitoring NIS species is still widely advocated from many sides worldwide and from one of the most demanding European legislative instruments, the Marine Strategy Framework Directive (e.g. MSFD Descriptor 2; ICES 2016, Zenetos et al. 2017, Galil et al. 2018). To effectively deal with risk and impact evaluations on local biodiversity and ecosystem functioning, scientists, legislators, stakeholders and policy makers have pointed out the need for (1) an improved NIS database, (2) integrated checklists and databases based on geo-referenced occurrence and (3) proactive monitoring programmes and modelling exercises (Molnar et al. 2008, Hewitt et al. 2009, Ojaveer et al. 2014, Sarà et al. 2018a,b). To date, the MSFD (EC 2008), among other descriptors, includes the 'impacts of non-indigenous invasive species at the level of species, habitats and ecosystems', acknowl-

edging the critical role of vectors in biological invasions and considering the importance of managing the pathways of invasion to guarantee Good Environmental Status. This becomes particularly compelling in the Mediterranean Sea, a melting pot of bioinvasions showing the highest number of records of species introduced compared with other European seas (Galil et al. 2014).

In this regard, the aquaculture industry has been recognised as one of the main pathways of NIS introduction to Europe (Fernandez-Gonzalez & Sanchez-Jerez 2014). Furthermore, the hard substrata of aquaculture plants (e.g. nets, mooring structures and polyvinyl finfish cage collars) provide novel suitable habitat for colonisation and settlement of a wide variety of marine organisms (Sarà et al. 2007). Many NIS find easy housing among the encrusting benthic faunal communities, hereafter called biofouling or macrofouling (Molnar et al. 2008, Nunes et al. 2014, Floerl et al. 2016, Katsanevakis et al. 2016). Once again, little is known about the mechanisms that regulate and promote the distribution of NIS across these artificial surfaces. Another topic that remains poorly investigated is the temporal aspect of such colonisations, in particular the long-term consequences of NIS introductions that remain unknown (Ramsay et al. 2008, Rius et al. 2011). The study of the temporal dynamics of fouling communities could help researchers to understand whether they are more or less permanent and whether they can act as a source that can inoculate natural communities in the surrounding area.

Here we planned an extensive 1 yr manipulative experiment around aquaculture cages to provide quantitative and qualitative data on the occurrence and density of serpulid species. Serpulids constitute one of the most important groups in the marine fouling biota (Koçak et al. 1999, Çinar 2006), and our data will offer useful insights into the investigation of the potential role of an aquaculture plant in becoming a NIS hotspot. Our target species were 2 serpulid tube-worm foulers, *Hydroides elegans* (Haswell, 1883) and *H. dirampha* (Mörch, 1863), considered invasive marine aliens in the Mediterranean Sea (Zenetos et al. 2010). The 2 tubeworm species are known to colonise hard substrate from intertidal to sublittoral zones (Zibrowius 1971, Ben-Eliahu & Fiege 1996, Çinar 2006, Çinar et al. 2006). They are generally gregarious organisms but sometimes can form small colonies (Çinar 2006). Serpulids are one of the most represented groups in encrusting benthic fouling communities, as well as a well-recognised component of NIS, showing a high structuring role in biofouling encrusting com-

munities and a high degree of invasiveness (Çinar 2006, 2013, Çinar et al. 2014; see also Hove & Kupriyanova 2009 and references therein). *H. elegans* and *H. dirampha* are recognised as major biofoulers on artificial hard substrata and are described as being among the most important threats to both local biodiversity and the socio-economy due to the damage they (i.e. calcareous tubes) can cause to human infrastructure and buildings (Zenetos et al. 2012, Çinar 2013). We investigated a 1 yr settlement of these 2 non-indigenous serpulid species and their role in shaping the hard substrate communities settled around fish farm cages. For this purpose, by using artificial panels close to the aquaculture cages, we also analysed the development of macrofouling communities when they undergo colonisation pressure exerted by the 2 tube-building serpulid species.

2. MATERIALS AND METHODS

2.1. Experimental design and sampling

The sampling collection phase took advantage of an experimental biofouling monitoring project (INNO-VAQUA) performed in a system of aquaculture fish cages located in the northern part of the Strait of Sicily (Licata Harbour, Sicily) from 2014 to 2015 (Fig. 1). A total of 144 double-faced settlement panels (10 × 10 cm surface) were attached to 2 cages of the aquaculture plant. The experiment started in June 2014, and every 3 mo, 18 double-faced panels
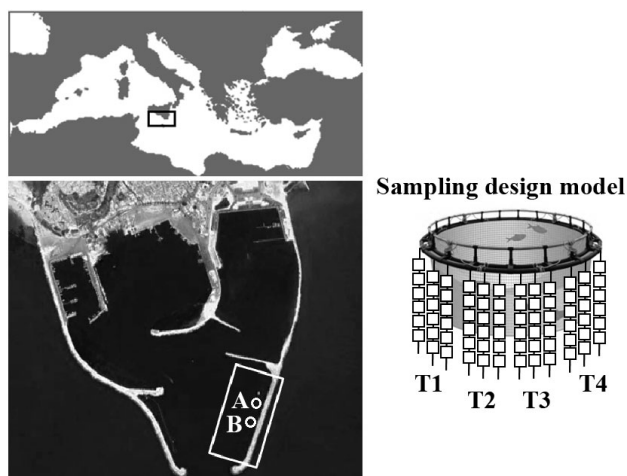

Fig. 1. Study area, Licata Harbour (northern side of Sicily Strait), showing the 2 cages (A and B; 100 m apart), and sampling design (each panel is double faced). Panels were collected every 3 mo (n = 18). T1: September 2014; T2: December 2014; T3: March 2015; T4: June 2015

were analysed (3 lines of 6 double-faced panels; 18 panels × 4 periods = 72 panels, repeated in 2 cages, i.e. 144 panels [thus 288 sides; see Fig. 1]). The panels were collected at 4 sampling times: in September 2014 (T1), December 2014 (T2); March 2015 (T3) and in June 2015 (T4), corresponding to 4 seasons (autumn, winter, spring, and summer, respectively). The 2 cages under investigation were located approx. 100 m apart and respectively contained about 75 000 and 77 000 specimens of *Dicentrarchus labrax* (Linnaeus, 1758) from June 2014 (3.8 and 3.6 kg m^{-3}, respectively) to June 2015 (11.8 and 11.3 kg m^{-3}, respectively). Continuous recorders of water temperature (°C) and dissolved oxygen (mg l^{-1}) were deployed at the 2 sites during the entire sampling period to detect any confounding effect due to the heterogeneity of local environmental variables.

2.2. Macrofouling community analysis

Once collected, the panels were transferred to the laboratory, and the fouling organisms were collected from the hard surfaces by scraping, fixed in a 10% formaldehyde-seawater solution and preserved in a 70% alcohol solution. Once washed through a 0.5 mm mesh sieve and sorted, the macrofouling specimens were individually identified to the lowest possible taxonomic level. A Leica M80 stereomicroscope and a Leica ICC50 HD optical microscope were used for taxonomic identification. Serpulid specimens were gently extracted from the tubes, identified (following Çinar 2006, Hove & Kupriyanova 2009) and counted. The number of analysed specimens was standardised over the total sampling surface (i.e. serpulid density: serpulids m^{-2}, mean ± SE).

Density and Margalef's species richness values (mean ± SE) were evaluated for the whole associated macrofouling community at each sampling time per each cage.

To assess the impact of NIS on macrofouling communities, we used the ALien Biotic IndEX (ALEX), proposed by Çinar & Bakir (2014) and widely applied in the eastern Mediterranean basin. Species were categorised into 4 biogeographic groups: Group I (native species), Group II (casual species), Group III (established species) and Group IV (invasive species). The index, based on the percentages of abundance of each group within the community, classified the ecological status of studied area as follows:

$$ALEX = \{(0 \times \%GI) + [3 \times (\%GII + \%GIII)] + (5 \times \%GIV)\}/100 \qquad (1)$$

We calculated the index considering *Hydroides elegans*, *H. dirampha*, *Anadara transversa* (Say, 1822), *Balanus trigonius* Darwin, 1854, and *Caprella scaura* Templeton, 1836, in Group III (i.e. established species sensu Zenetos et al. 2010); *Monocorophium acherusicum* (Costa, 1853) and *Ericthonius punctatus* (Spence Bate, 1857) in Group II (i.e. casual species sensu Rumbold et al. 2016); and the other species in Group I (i.e. native species). The values of this index ranged from 0 to 5. The value 0 indicates a community containing no alien species, whereas the value 5 indicates a community totally invaded by alien species (sensu Çinar & Bakir 2014).

2.3. Serpulid morphometric measurements

Additionally, the (1) total body length from collar to pygidium (to the nearest 0.1 mm), (2) operculum plus peduncle length (to the nearest 0.1 mm) and (3) body weight (wet weight, to the nearest 0.1 mg) were measured. Trends in class sizes of abundance and biomass were presented for the total amount of sampled serpulids; the length–weight relationship was analysed for every sampling time, using the following allometric equation (Gould 1966, Dulčić & Kraljević 1995):

$$TW = a \times TL^b \tag{2}$$

(linearised through base 10 logarithms; TW refers to the total body weight and TL to the total body length), together with the TL and operculum plus peduncle length relationship. The parameter *b* in the linearised form was taken as an allometric coefficient equal to 3 in the case of isometric growth (sensu Gould 1966). Differences among both body length and body weight frequency distributions between the 2 serpulid species were tested through a coupled series of *t*-test combinations.

2.4. Statistical analysis

To test differences taking into account the 3 factors cage, species and time, a 3-way ANOVA test was performed considering the density of serpulids as a response variable. Differences between the associated fouler macrofaunal species at each of the 4 sampling times were assessed using multivariate techniques in the PRIMER (v.6) software package. A similarity matrix was calculated using the Bray-Curtis similarity index computed from the abundance data after a square root transformation to downweigh the influ-

ence of extremely abundant species. Differences in abundance were related to sampling time categories and tested for significant differences using an ANOSIM test. A SIMPER routine was run to determine the species that contributed most to the average similarity among sampling times. A principal component analysis (PCA) was performed to highlight differences in macrofaunal community composition among sampling times. The average abundance of the serpulids, the density of associated macrofaunal species and the Margalef's index at each of the 4 sampling times were statistically tested with univariate analysis using 1-way ANOVA. All the ANOVA tests were applied after checking that the data conformed to the necessary assumptions regarding homogeneity of variance and normality using Levene's and Anderson-Darling tests, respectively; data were $\log_{10}(x + 1)$ transformed when necessary. Differences among categories were tested using the Student-Newman-Keuls (SNK) test (significant interaction terms were considered at $p < 0.05$, $p < 0.001$, $p < 0.0001$).

A distance-based linear model routine (DistLM, Legendre & Anderson 1999), based on Bray-Curtis dissimilarities (using the R^2 selection criterion and stepwise selection procedure), was applied to disentangle potential effects of the presence of serpulid NIS or by the environmental variables (i.e. *in situ* measured sea temperature and dissolved oxygen) in shaping macrofaunal abundance, community composition and diversity.

Values of temperature, oxygen and serpulid abundance were normalised and used as the predictor variable worksheet in DistLM analysis after a draftsman plot (Clarke & Gorley 2006) was carried out to detect possible skewness and/or strong correlation ($R > 0.7$) between environmental variables and abundance of serpulid species.

3. RESULTS

3.1. Environmental parameters

No differences in temperature and dissolved oxygen were detected between the 2 cages for each sampling time (Fig. 2).

3.2. Serpulid densities and morphometric results

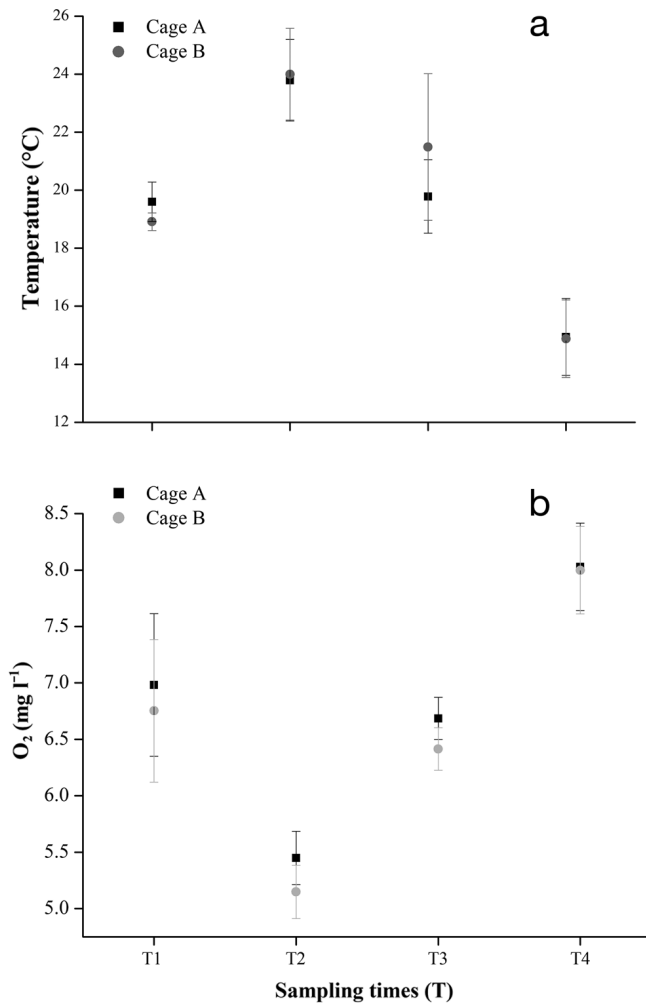No differences in density were detected between the 2 serpulid species sampled at each cage at each

Fig. 2. Mean ± SE (a) temperature and (b) dissolved oxygen for the 4 sampling times (see Fig. 1)

sampling time; significant differences were shown only for the last factor, time, with the same response trend for the 2 species (3-way ANOVA, factor cage, $F_{1,1}$ = 1.50, p = 0.235; factor species, $F_{1,1}$ = 0.16, p = 0.690; factor time, $F_{1,3}$ = 10.26, p = 0.001).

Differences were detected in the length and weight frequency distributions (Figs. 3 & 4) of the 2 serpulid species (Table 1); all were significant (t-tests, TL *Hydroides dirampha* ≠ *H. elegans*, t = −1.55, p = 0.135; TW *H. dirampha* ≠ *H. elegans*, t = −1.39, p = 0.176).

Linear regressions between total body length and operculum plus peduncle length were all significant and indicated a negative allometry (b < 1) for both serpulid species examined, *H. dirampha* (b = 0.677) and *H. elegans* (b = 0.064) (Table 1). Also, growth appeared to show a tendency toward a negative allometry for *H. dirampha* (b = 1.185) (b < 3; Table 1), while *H. elegans* did not show a significant allometric

relationship between biometric variables (p = 0.121; b = 0.493).

The mean density values of serpulids analysed along the 4 sampling times showed different trends at each sampling time (ANOSIM, R = 0.729, p < 0.05; Fig. 5).

Between the 2 sampled serpulids, *H. dirampha* was always the most abundant (Fig. 5; ANOVA, factor sampling time, $F_{1,3}$ = 6.77, p = 0.007; SNK test, T3 > T1, T2, T4, p = 0.043), followed by *H. elegans* (ANOVA, factor sampling time, $F_{1,3}$ = 8.05, p = 0.032; SNK test, T3 > T1, T2, T4, p = 0.036). The density pattern between the 2 species through the sampling time was the same, or rather, both started settling 9 mo after panel displacements, during spring (T3; Fig. 5). Densities drastically decreased in summer, favouring some of the associated macrofaunal species such as the crustaceans *Elasmopus rapax* Costa, 1853, and *Jassa marmorata* Holmes, 1905; the polychaete *Syllis gracilis* Grube, 1840; and the ascidian *Styela plicata* (Lesueur, 1823) (Table 2, Supplement at www.int-res. com/articles/suppl/q011p041_supp.pdf).

3.3. Macrofouling community structure

Peaks of associated macrofauna density values were recorded at both T1 and T2, ANOVA test, $F_{1,3}$ = 10.84, p < 0.05; pairwise test, T1, T2 > T3, T4, p < 0.05; Fig. 5); a visible decrease was recorded at T3, the sampling time at which we observed a dominance of Serpulidae (maximum serpulid density values *H. dirampha* = 535 ind. m^{-2}, *H. elegans* = 270 ind. m^{-2}). The highest values for Margalef's species richness calculated for the associated macrofaunal community were recorded at both T2 and T4, with the maximum value recorded at sampling time T4 (ANOVA, $F_{1,3}$ = 18.65, p < 0.01; pairwise test, T4 > T1, T3, p < 0.001, T4 > T2, p < 0.05; Fig. 6). Among the 16 most abundant associated macrofaunal species (SIMPER outcomes, Table 2), there were 2 small non-indigenous peracaridan (amphipod; crustaceans) species, *Monocorophium acherusicum* and *Ericthonius punctatus*, both natives from the North Atlantic. The second most abundant associated macrofaunal species was the tanaidacean *Tanais dulongii* (Audouin, 1826) that showed significantly higher abundance at sampling time T2 (winter) compared to T4 (summer). *Jassa marmorata* and the peracaridan gammarid *Elasmopus rapax* showed increasing trends, with the highest average abundance values at T4. The polychaete *Syllis gracilis* was more abundant at T4 compared to T2. The barnacle *Balanus trigonus* showed a peak
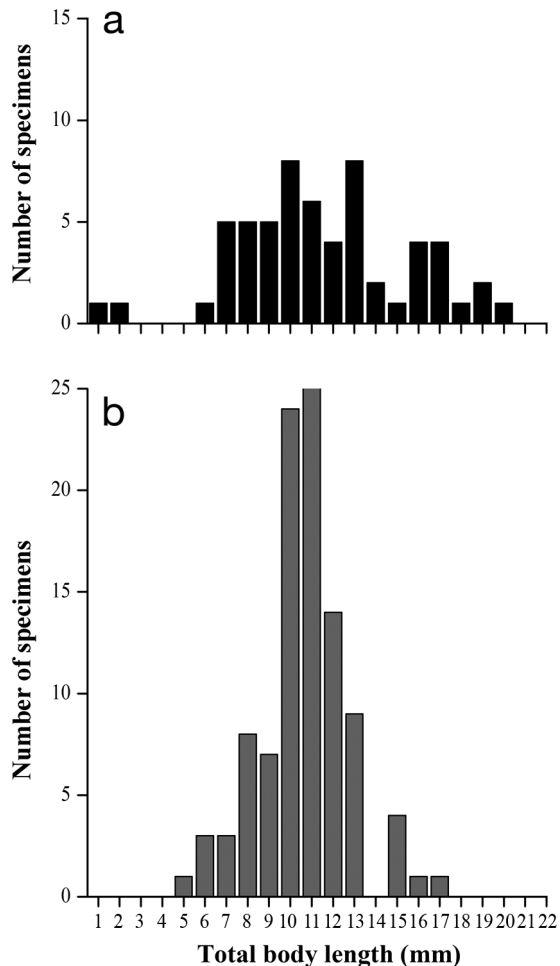
Fig. 3. Body length frequencies of (a) *Hydroides dirampha* and (b) *H. elegans*
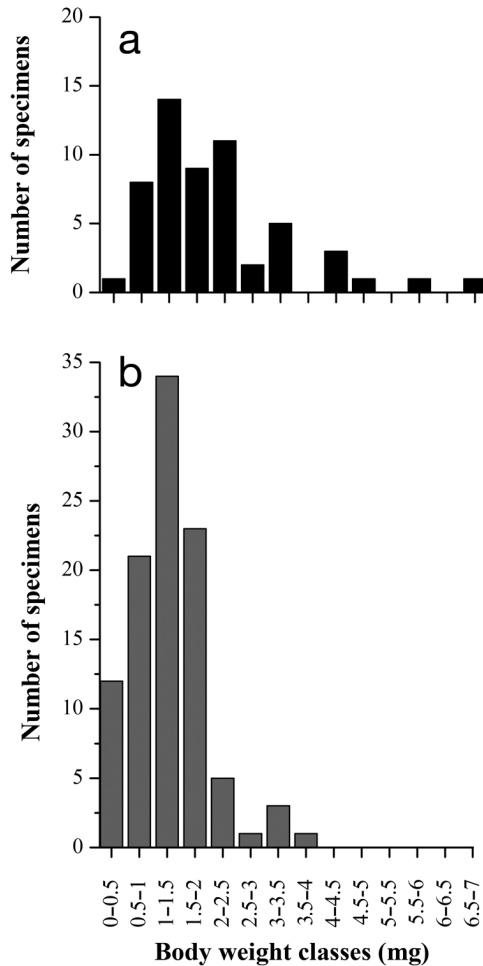

Fig. 4. Body weight frequencies of (a) *Hydroides dirampha* and (b) *H. elegans*

corresponding with the highest densities of serpulids. The solitary ascidian *Styela plicata* showed a significant increasing trend from T1 to T4. Both of the 2 most abundant caprellid associated native species, *Caprella dilatata* and *C. equilibra*, showed no differences among the sampling periods, similar to the tanaidacean *Chondrochelia savignyi* (Kroyer, 1842) and the 3 most abundant polychaetes, *Spirobranchus*

triqueter (Linnaeus, 1758), *Nereis* sp. and *Branchiomma bombyx* (Dalyell, 1853), and the bivalve *Musculus discors* (Linnaeus, 1767). Another serpulid species, *Hydroides dianthus* (Verril, 1873), was found; previously considered a NIS, it was only recently identified as indigenous according to a molecular study (Sun et al. 2017, Zenetos et al. 2017). This species showed a density pattern similar to the other 2

Table 1. *Hydroides dirampha* and *H. elegans* total length (TL, mm) and total weight (TW, mg) (range and mean ± SE) and linear regression between TW and TL and between TL and operculum plus peduncle (OP) length

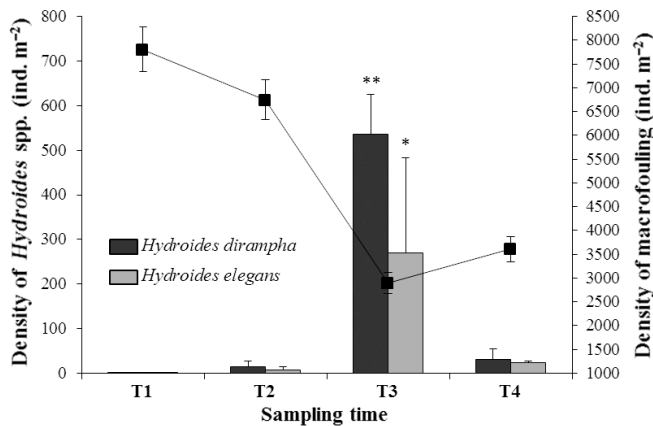| Species	TL range	TL mean ± SE	TW vs. TL	R	p	n
H. dirampha	6–20	12 ± 3.74	TW = 1.185 − 1.01 TL	0.55	<0.0001	58
H. elegans	5–17	11 ± 2.12	TW = 0.494 − 0.493 TL	0.155	0.121	100
	TW range	TW mean ± SE	TL vs. OP			
H. dirampha	0.3–9.2	2.2 ± 1.7	TL = 0.677−0.278 OP	0.76	<0.0001	58
H. elegans	0.2–4.0	1.2 ± 0.6	TL = 0.064−0.268 OP	0.62	<0.0001	100

Fig. 5. Mean (±SE) densities of non-indigenous *Hydroides* spp. (bars) and of other macrofouling species (black squares) at different sampling times (see Fig. 1). Significant highest values of density were reported for the 2 serpulids at T3. *p < 0.05, **p < 0.01

NIS serpulids, with a peak in abundance at T3 (SIM-PER, Table 2). In PCA, performed on the 16 most abundant associated macrofaunal species, the first 2 axes explained 86.6% of the variance. We observed that T1 and T2, characterised by higher abundance of *Monocorophium acherusicum*, *Ericthonius punctatus* and *Tanais dulongii*, were clearly separated from T3, where we observed higher abundance of *Balanus trigonus*, *H. dianthus* and *Branchiomma bombyx,* and T4, characterised by higher abundance of *Styela plicata* and *Syllis gracilis* (Fig. 7).

The DistLM analysis showed that the environmental variables did not explain macrofouling abundance, diversity and composition. The presence of the 2 serpulids did not significantly influence the abundance and diversity of macrofauna; only the variations in community composition were significantly explained by the abundance of *H. dirampha* (32%, Table 3).

A score of 2, a Good status for fouling communities in terms of the impact of alien species (sensu Çinar & Bakir 2014), was obtained by evaluating the ALEX index.

4. DISCUSSION

Here we have provided morphometric measurements of 2 NIS serpulids and evidence supporting their well-recognised enhanced capacity for colonisation (Relini 1993, Denitto & Licciano 2006). The density values of the serpulids in the present study are comparable with other studies performed in the

eastern Mediterranean basin (Alsancak Harbour at Izmir Bay; Çinar et al. 2008). Our novel data for the study area will integrate the available information on the presence of *Hydroides elegans* and *H. dirampha* for the whole basin. The gathered information may also confirm the westward spread pattern across the central Mediterranean Sea, a hydrodynamically crucial area with 90% of nautical traffic traversing and human activities through the Malta–Sicily Channel (Katsanevakis et al. 2014, 2016, Deidun et al. 2016, Mangano & Sarà 2017, Capodici et al. 2018). Ship hulls are considered the most probable vectors of translocation, and pier fouling, harbour docks and aquaculture infrastructures are considered the main hotspots of aggregation for these species (Ben-Eliahu & Hove 2011). *H. elegans* is one of the most studied serpulids in the Mediterranean Sea and is considered the primary fouling organism in the Mediterranean Sea on both hard and soft substrata (Koçak et al. 1999, Çinar et al. 2006, Cabanellas-Reboredo et al. 2010, Ben-Eliahu & Hove 2011), including aquaculture structures and equipment (Katsanevakis et al. 2014). The settlement patterns of the 2 serpulids where similar, with a peak during spring followed by a decline in abundance in summer, which also has been recorded for other Lessepsian immigrant serpulid polychaetes (Belal & Ghobashy 2012).

Our results highlight the significant role of the 2 serpulid NIS, in particular of the most abundant species, *H. dirampha*, in shaping the macrofouling community (DistLM analysis) and confirm the community-shaping power of these species. In fact, most of the macrofaunal species showed a visible decrease in abundance during the period dominated by the 2 serpulid species (spring) and a subsequent increase during summer. Our results are consistent with previous observations by Lezzi et al. (2018), which suggested that *H. elegans* and *H. dirampha* behave as r-selected pioneer species, structuring components of early communities, but after settlement, they are not able to develop in late communities. We observed that some sessile components of the community, principally represented by the native serpulid *H. dianthus*, the sabellid *Branchiomma bombyx* and the balanid *Balanus trigonius* (NIS according to Zenetos et al. 2010), showed a peak of abundance during spring, corresponding with the highest densities of the 2 serpulid NIS in this study. This suggests that sessile NIS recruitment on hard substrate can co-occur with that of other sessile species (Lezzi et al. 2018), and that *H. elegans* and *H. dirampha* are able to settle despite high competition for space with other sessile organisms during their recruitment. On the other hand, the

Table 2. SIMPER analysis for the 16 most abundant species at each of the considered 4 sampling times (see Fig. 1). C: crustacean; A: ascidian; P: polychaete; M: mollusc

Code	Species	Avg. abundance (ind. m^{-2})	Contribution (%)
T1 (avg. similarity = 70.58)			
C	*Monocorophium acherusicum* (Costa, 1853)	5682.0	42.5
C	*Tanais dulongii* (Audouin, 1826)	1158.0	19.47
C	*Ericthonius punctatus* (Spence Bate, 1857)	364.0	11.92
P	*Spirobranchus triqueter* (Linnaeus, 1758)	266.0	6.64
C	*Chondrochelia savignyi* (Kroyer, 1842)	100.5	2.75
P	*Nereis* sp.	36.0	2.75
C	*Caprella equilibra* Say, 1818	10.0	2.32
M	*Musculus discors* (Linnaeus, 1767)	72.0	2.32
C	*Balanus trigonus* Darwin, 1854	43.0	1.87
T2 (avg. similarity = 76.36)			
C	*Monocorophium acherusicum* (Costa, 1853)	3982.0	39.00
C	*Tanais dulongii* (Audouin, 1826)	2028.0	22.85
P	*Spirobranchus triqueter* (Linnaeus, 1758)	81.0	5.26
C	*Ericthonius punctatus* (Spence Bate, 1857)	274.0	3.72
P	*Nereis* sp.	45.0	3.55
A	*Styela plicata* (Lesueur, 1823)	35.0	3.49
C	*Balanus trigonus* Darwin, 1854	33.5	3.11
C	*Chondrochelia savignyi* (Kroyer, 1842)	30.0	2.82
C	*Jassa marmorata* Holmes, 1905	42.5	2.34
P	*Branchiomma bombyx* (Dalyell, 1853)	27.5	2.05
C	*Caprella dilatata* Kroyer, 1843	26.5	2.05
C	*Elasmopus rapax* Costa, 1853	15.0	1.75
C	*Caprella equilibra* Say, 1818	13.0	1.66
P	*Syllis gracilis* Grube, 1840	7.0	1.52
T3 (avg. similarity = 65.87)			
C	*Balanus trigonus* Darwin, 1854	1119.0	22.46
C	*Monocorophium acherusicum* (Costa, 1853)	622.0	16.75
C	*Tanais dulongii* (Audouin, 1826)	514.0	15.23
P	*Branchiomma bombyx* (Dalyell, 1853)	103.0	6.82
A	*Styela plicata* (Lesueur, 1823)	95.0	5.82
P	*Syllis gracilis* Grube, 1840	73.5	5.58
P	*Hydroides dianthus* (Verrill, 1873)	161.5	5.54
C	*Ericthonius punctatus* (Spence Bate, 1857)	39.0	3.29
P	*Spirobranchus triqueter* (Linnaeus, 1758)	32.0	2.93
C	*Caprella equilibra* Say, 1818	37.5	2.90
P	*Nereis* sp.	45.5	2.85
C	*Elasmopus rapax* Costa, 1853	74.0	2.69
C	*Jassa marmorata* Holmes, 1905	15.0	2.51
T4 (avg. similarity = 73.08)			
C	*Monocorophium acherusicum* (Costa, 1853)	1603.0	20.54
A	*Styela plicata* (Lesueur, 1823)	346.5	9.12
C	*Tanais dulongii* (Audouin, 1826)	361.0	8.29
C	*Elasmopus rapax* Costa, 1853	227.0	7.73
C	*Jassa marmorata* Holmes, 1905	208.0	7.4
C	*Ericthonius punctatus* (Spence Bate, 1857)	33.0	6.84
P	*Spirobranchus triqueter* (Linnaeus, 1758)	123.0	5.33
P	*Nereis* sp.	78.0	3.94
C	*Chondrochelia savignyi* (Kroyer, 1842)	74.0	3.66
C	*Balanus trigonus* Darwin, 1854	103.0	3.59
C	*Caprella equilibra* Say, 1818	143.0	3.4
P	*Branchiomma bombyx* (Dalyell, 1853)	65.5	3.36
P	*Syllis gracilis* Grube, 1840	83.0	2.29
C	*Caprella dilatata* Kroyer, 1843	35.0	1.99

highest abundance of mobile organisms and the high diversity level of associated macrofauna recorded at T4 could suggest a facilitating effect exerted by NIS. By modifying the habitat properties, the NIS may attract more diverse and abundant mobile native and non-native species (e.g. offering shelter and refuge within their encrusting calcareous tubes; Floerl et al. 2004, Stachowicz & Byrnes 2006).

Within the macrofouling community, interestingly, the 2 small non-indigenous peracaridan species, *Monocorophium acherusicum* and *Ericthonius punctatus*, have been recorded as dominant compared to native species, especially in harbour areas and in other Mediterranean areas (Rumbold et al. 2016). Hard substrata provided by fish farm components seem to represent the perfect artificial habitat to investigate the settlement of benthic NIS (Maughan 2001, Bowden et al. 2006) and are confirmed as NIS hotspots by our results. The result of the calculation of ALEX, a new and recently applied biotic index in the western Mediterranean basin (e.g. monitoring macroalgae, Piazzi et al. 2015, 2018) and applied here for the first time on a fouling community in the central basin, showed a fouling community which has alien species, but the majority of organisms belong to native species (Good status sensu Çinar & Bakir 2014). The occurrence of the 2 serpulid species on fish farm structures may also depend on the high food resources availability on the local scale, i.e. the large amount of particulate waste output from sea bream/sea bass farming. As a result of all these observations, fish aquaculture might be confirmed as a main driver in favouring the spread of both the investigated serpulids and the macrobenthic community primarily structured by these. The obtained results will integrate, update and complete the current status of knowledge regarding these small migrant serpulids in the Mediterranean basin by adding evidence on their settlement
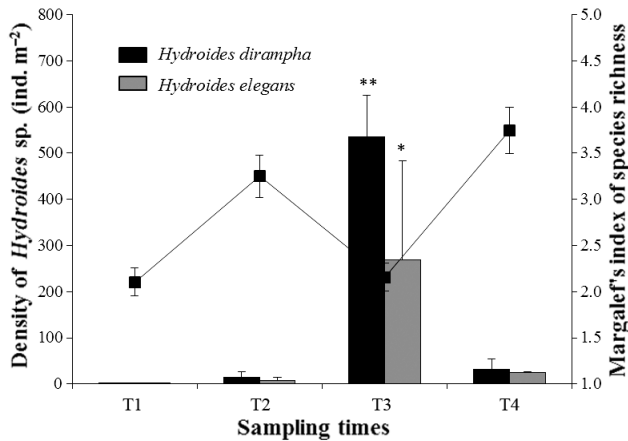
Fig. 6. Mean (±SE) densities of non-indigenous *Hydroides* spp. (bars) and the Margalef's species richness index (calculated excluding the 2 serpulid species) for the 4 sampling times (see Fig. 1). Significant highest values of density were reported for the 2 serpulids at T3 (*p < 0.05, **p < 0.01)

Table 3. DistLM analysis to ascertain the role of environmental variables (temperature and oxygen) and abundance of the 2 serpulid species (*Hydroides dirampha* and *H. elegans*) on macro-faunal community composition. ns: not significant. **p < 0.001

Variable	SS	*F*	p	Var. (%)	Cumulative %
H. dirampha	1828.9	2.8	**	0.32	0.32
Temperature	1119.2	2.0	ns	0.20	0.52
Dissolved oxygen	788.8	1.6	ns	0.14	0.66
H. elegans	542.0	1.1	ns	0.09	0.75

along a 1 yr time series and showing differences in the associated macrofaunal diversity. Serpulids, in fact, can easily remain undetected (Zenetos et al. 2005), and even if the number of observations of invasive migrants has greatly increased in the last 3 decades, our knowledge about Lessepsian serpulids is still scant. These small creatures have been sampled only sporadically, and information about them remains presumably limited to the taxa that have already built up sizeable populations (Çinar 2006), as in our case.

The preliminary data discussed here on the diversity and density of the associated macrofaunal community suggest a founder facilitating effect promoted

by serpulids worthy of further future investigation through setting up a more focused monitoring plan (extended in time and space) in relation to local environmental variables and to species resistance. NIS may negatively interact with local biodiversity or cause risks and threats to human activities, generating high economic costs (e.g. farm cage cleaning in aquaculture sectors; Floerl et al. 2016). In this regard, monitoring plans, as the one presented here, should be encouraged and promoted as a main solution to improve the management and regulation of introduced fauna in the Mediterranean region (Lehtiniemi et al. 2015, Ojaveer et al. 2015, Galil et al. 2018).
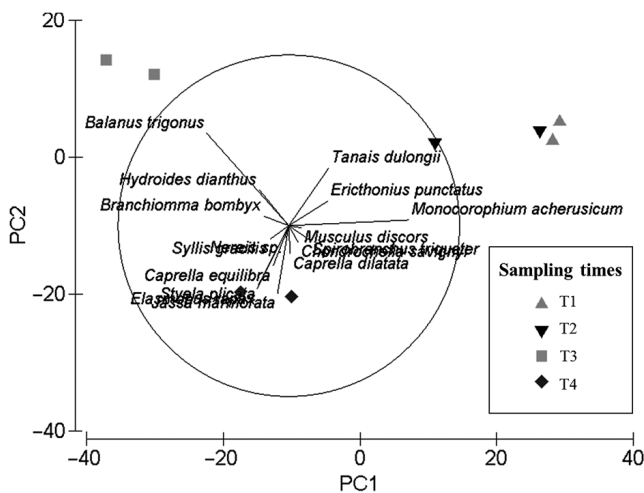
Fig. 7. Principal component analysis performed on square root transformed data of 16 most abundant macrofaunal species during the 4 sampling time periods (see Fig. 1). The first 2 axes explained 86.6 % of the variance

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