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Research Article

The invasive *Anadara transversa* (Say, 1822) (Mollusca: Bivalvia) in the biofouling community of northern Adriatic mariculture areas

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Abstract

The composition of biofouling communities on colonised terracotta tiles was assessed in mariculture areas of the northern Adriatic Sea (Croatia) according to a hierarchical nested sampling design. Significant differences in the composition of biofouling assemblages were detected between depths (1 and 5 m) and at the larger spatial scale (tens of km). Additionally, the biofouling community showed different patterns in relation to the time of immersion (2, 4, 6 and 8 months). The most abundant taxa were *Spirobranchus triqueter*, *Spirorbis spirorbis*, *Ostrea edulis*, *Mytilus galloprovincialis*, *Schizoporella* sp., *Balanus* sp. and *Botryllus* sp. The invasive *Anadara transversa* was consistently an important component of these biofouling assemblages. It was able to spawn throughout the duration of a survey (April to January) showing peak recruitment during the summer. A maximum density, up to 500 individuals m^{-2}, were assessed at the depth of 5 m. Maximum shell length of 30 mm was recorded at the end of the experiment. These results suggest that, besides the native fouling community species, the invasive and opportunistic *A. transversa* might cause additional problems in mariculture facilities of the northern Adriatic Sea.

Key words: NIS, shellfish, mariculture, settlement, Adriatic Sea, Croatia

Introduction

Biofouling represents a serious and costly problem for mariculture (Lane and Willemsen 2004; Braithwaite and McEvoy 2005; Adams et al. 2011; Fitridge et al. 2012). Fouling communities on artificial structures are invasion hotspots for non-indigenous species (NIS). Monitoring fouling communities on these structures can be valuable for detecting new introductions to an area, including those that aim to mitigate the ecological and economic impacts of NIS (Gartner et al. 2016). The negative impact of non-indigenous species is related to the degradation of native communities and to the creation of novel habitats through competition for resources, predation, release of toxins, disease transmission and ecosystem engineering (Stachowicz et al. 1999; Galil and Zenetos 2002; Zenetos et al. 2005).

Although some papers have discussed biofouling communities in the Adriatic Sea, Croatian waters (Jelić-Mrčelić et al. 2006, 2012), the knowledge related to the biofouling communities associated with aquaculture facilities of the Croatian coastal area is both scarce (Slišković et al. 2011) and requires updating. Igić (1984, 1986, 1991, 1995, 1999) provided the most comprehensive data set. Therefore, there is an urgent need to update the existing knowledge on the temporal and spatial variability of the biofouling communities' composition along the Adriatic Coast (Zenetos et al. 2012; Crocetta et al. 2013; Spagnolo et al. 2017; Nerlović et al. 2016, Gartner at al. 2016). Recently, increasing numbers of NIS have been recorded along the Adriatic Coast highlighting the need for new surveys of the biofouling community in this area (Pećarević et al. 2013). In particular, it is necessary to assess the temporal succession and the distribution of NIS within the native biofouling community in areas devoted to aquaculture activities.

Aquaculture-related trade of infested equipment, seed-stock or crop represents a well known mode of introduction of new, NIS fouling species to coastal habitats (Ferrario et al. 2016; Gartner et al. 2016), which influences the diversity and quantity of biofouling organisms within aquaculture areas. Among alien species, *Anadara transversa* (Say, 1822) has been highlighted as a component of biofouling communities, which negatively affects shellfish aquaculture (Gartner et al. 2016; Ferrario et al. 2016).

A. transversa is among the top 100 "worst invasive species threatening biodiversity in Europe" (Streftaris and Zenetos 2006). It is an opportunistic species with the capability to thrive in heavily polluted ecosystems (Çinar et al. 2006). *A. transversa*, formerly *Anadara demiri* (Piani 1981), was first recorded in the Mediterranean in 1972 in the Bay of Izmir (Turkey) as *Arca amygdalum* (Demir 1977) and, twenty years later, in the Gulf of Thermaikos and the Bay of Thessaloniki (Aegean Sea) (Zenetos 1994). The introduction pathway into the Mediterranean Sea is uncertain, although shipping seems to be the most likely vector of introduction (Zenetos et al. 2005). This species was probably introduced into the Mediterranean Sea either as plankton larvae in the ship ballast waters or as benthic stages within shipments of other bivalves related to mariculture (Lodola et al. 2011). For the Adriatic Coast, *A. transversa* was firstly recorded in the Lim Bay (northern Adriatic Sea), an area where aquaculture activities are well developed. Nerlović et al. (2012a) suggested that *A. transversa* was introduced in the Lim Bay throughout the import of mariculture products.

Impoverished communities, such as those inhabiting polluted and physically degraded environments, are particularly vulnerable to invasions by alien species than species rich communities characterising pristine environments. Moreover, *A. transversa* is able to live both attached to a substratum and free within the sediment (Solustri et al. 2003; Çinar et al. 2006). Artificial substrate was used in the study since it can be easily manipulated and placed under a variety of environmental conditions (Richmond and Seeds 1991; Cook et al. 2006). To evaluate the possibilities for application of alternative mitigation strategies, it is necessary to determine the temporal and spatial variations of biofouling communities at a particular location.

This study aims to assess the relevance of *A. transversa* in the biofouling community characterising areas of the west Istrian Coast (northern Adriatic coast) almost devoted to aquaculture purposes. These areas are usually bays sheltered from the exposure to wave action located in protected sites with a very low level of urbanisation. The expected results are focused on (1) *A. tranversa* growth assessment in the northern Adriatic Sea, which represents the northernmost part of the Mediterranean Sea, (2) assessing the spawning season and the potential recruitment of this NIS species and (3) determining the biofouling community components associated with *A. transversa*.

Materials and methods

Study area

The structure of biofouling assemblage was examined at two locations of the northern Adriatic (Figure 1) approximately 50 km apart by a straight line transect. The locations Limski Kanal (LK) and Pomer Bay (PB) were randomly selected among a dozen of mariculture farms scattered along the Istrian Coast. The first location was the inner part of Limski Kanal (LK) (45º07′48.50″N; 13º44′12.63″E). The Limski Kanal is a fjord shaped bay (ria) 11 km long and 200–500 m wide, with a maximum depth of about 33 m. The bottom is mainly muddy or sandy-muddy. This karst formation has numerous underwater freshwater springs, especially throughout the inner area, which contribute to its high productivity. It is a marine protected area where shellfish aquaculture dates back to the 19th century. Furtherore, it is one of the most important sites for *Mytilus galloprovincialis* and *Ostrea edulis* production in the Republic of Croatia. The Limski Kanal, has great importance as a natural shellfish spawning ground. The second location was the Pomer Bay (PB) (44º49′24.91″N; 13º54′58.52″E), also an important shellfish aquaculture area. It is a moderate sheltered bay with a slight development of tourism resorts. The shoreline is mainly rocky. The bottom is muddy or sandy-muddy and reaches maximum depths of around 15 m.

Set-up of experiments

At these two locations, the biofouling community was investigated exposing terracotta tiles of 12 × 12 cm that were inspected after a given time of immersion. In both locations, terracotta tiles were placed at 1 and 5 m depth, on 15 May 2011. They were sampled after 2, 4, 6 and 8 months of immersion. Within each location, two sites, at the distance of several hundred meters from each other were randomly chosen. At each depth, within each site, 40 tiles were deployed, so the total number of tiles was 320. Tiles were attached to ropes suspended horizontally from longlines placed next to mussels' polyethylene mesh sleeves (locally known as *pergolari*). At each sampling time, 10 randomly chosen tiles for each depth and site were collected and transferred to the laboratory in containers filled with seawater. The density of molluscs, polychaetes and barnacles was determined by counting the organisms, whereas the abundance of

Figure 1. Investigated area showing the position of 2 locations: Limski Kanal (LK) and Pomer Bay (PB).

algae, tunicates and bryozoans was estimated as area coverage, expressed as percent of the tile surface. The length of each recorded *A. transversa* specimen was measured using a Vernier calliper to the nearest 0.1 mm.

Species identification

Species were identified according to Hamel (1931–1939), Funk (1955), Gayral (1966), Coppejans (1983), Riedl (1991) and Costello et al. (2001). The abundance of molluscs, polychaetes and barnacles was estimated as the number of individuals per tile. Bivalve species were identified according to Tebble (1966), Nordsieck (1969), Parenzan (1974, 1976), and polychaets according to Fauvel (1927) and Fauchald (1977). The taxonomic nomenclature followed the World Register of Marine Species (WoRMS; http://www.marinespecies.org).

Statistical analyses

Multivariate data were graphically explored by non-metric multidimensional scaling (MDS) and the effects of factors depth, location and site were tested using permutational multivariate analysis of variation (PERMANOVA). Both analyses were performed using a software package PERMANOVA+ for PRIMER (PRIMER-E Ltd). Data collected for each sampling occasion were analysed separately. Both coverage and density data were included in the analyses (see Anderson et al. 2005). Data expressed as number of individuals (per tile) were fourth-root transformed. For abundance assessed as coverage, an ordinal semiquantitative score from 0 to 3 was assigned correspondingly to the relative coverage of organisms: 0 = absent, 1 = rare, 2 = fairly frequently encountered and 3 = very common. After this separate pre-treatment, multivariate analyses were done on the basis of Bray-Curtis dissimilarity matrix. The analyses were performed according to a three factor linear model with each immersion sampling time analysed separately. The factors were: (1) Depth (fixed, 2 levels: 1 and 5 meters' depth), (2) Location (random, 2 levels: LK and PB), and (3) Site (random and nested in Location, 2 levels, *i.e.,* the two sites randomly chosen with each level of factor Location).

Results

Altogether 41 biofouling taxa were identified on the 320 tiles (Table 1). The most frequent taxa were the polychaetes *Spirobranchus triqueter* (LK = 92.09%, PB = 94.19%) and *Spirorbis spirorbis* (LK = 99.44%, PB = 65.16%), the bryozoan *Schizoporella* sp. (LK = 78.53%, PB = 80.65%) and the tunicate *Botryllus* sp. (LK = 55.37%, PB = 82.58%). The phyla Mollusca

Table 1. The frequency of sessile invertebrates taxa that recruited during the study period at stations LK = Limski Kanal, PB = Pomer Bay.

Phylum	Frequency (%)	
	LK	PB
Annelida		
Spirobranchus triqueter (Linnaeus, 1758)	92.09	94.19
Spirorbis spirorbis (Linnaeus, 1758)	99.44	65.16
Filograna implexa Berkeley, 1835	0.00	37.42
Echinodermata		
Paracentrotus lividus (Lamarck, 1816)	0.56	0.00
Ophiuroidea	1.13	0.00
Mollusca		
Modiolus barbatus (Linnaeus, 1758)	1.69	0.00
Anomia ephippium Linnaeus, 1758	12.99	26.45
Mimachlamys varia (Linnaeus, 1758)	6.21	1.29
Mytilus galloprovincialis Lamarck, 1819	23.16	16.13
Monia patelliformis (Linnaeus, 1761)	0.00	0.65
Pecten sp.	1.13	0.65
Flexopecten glaber (Linnaeus, 1758)	0.00	1.29
Musculus subpictus (Cantraine, 1835)	0.56	1.94
Anadara transversa (Say, 1822)	34.46	2.58
Ostrea edulis Linnaeus, 1758	48.59	30.32
Barbatia barbata (Linnaeus, 1758)	0.56	0.00
Hiatella sp.	6.78	2.58
Placophora indet.	1.69	4.52
Gastropoda indet.	1.13	0.65
Flabellina sp.	0.56	1.29
Bolinus brandaris (Linnaeus, 1758)	1.69	0.00
Nemertina		
Turbellaria sp.	0.56	1.94
Bryozoa		
Schizoporella sp.	78.53	80.65
Cryptosula pallasiana (Moll, 1803)	0.00	21.29
Crustacea		
Balanus sp.	63.28	5.16
Tunicata		
Botryllus sp.	55.37	82.58
Ciona intestinalis (Linnaeus, 1767)	22.60	5.81
Phallusia mammillata (Cuvier, 1815)	14.69	18.71
Microcosmus vulgaris Heller, 1877	7.91	0.65
Pyura microcosmus (Savigny, 1816)	10.17	6.45
Diplosoma listerianum (Milne Edwards, 1841)	25.42	39.35
Phallusia fumigata (Grube, 1864)	2.26	3.23
Phallusia sp.	2.26	0.00
Tunicata indet.	11.86	1.94
Porifera		
Spongia officinalis Linnaeus, 1759	0.56	0.00
Ochrophyta		
Sphacelaria sp.	37.29	45.16
Rhodophyta		
Polysiphonia sp.	31.64	25.16
Ceramium sp.	0.56	0.00
Chlorophyta		
Acetabularia sp.	0.00	5.81

(16 taxa) and Tunicata (9 taxa) displayed the highest diversity of species (Table 1). Within Mollusca, the most frequent species were oysters *Ostrea edulis* (LK = 48.59%, PB = 30.32%), *Mytilus galloprovincialis* (LK = 23.16%, PB = 16.13%) and *A. transversa* (LK = 34.46%, PB = 2.58%). Within Tunicata, besides *Botryllus* sp., the most frequent taxa were *Diplosoma listerianum* (LK = 25.42%, PB = 39.35%) and *Ciona intestinalis* (LK = 22.60%, PB = 5.81%). The most frequent macroalgae were *Sphacelaria* sp. (LK =

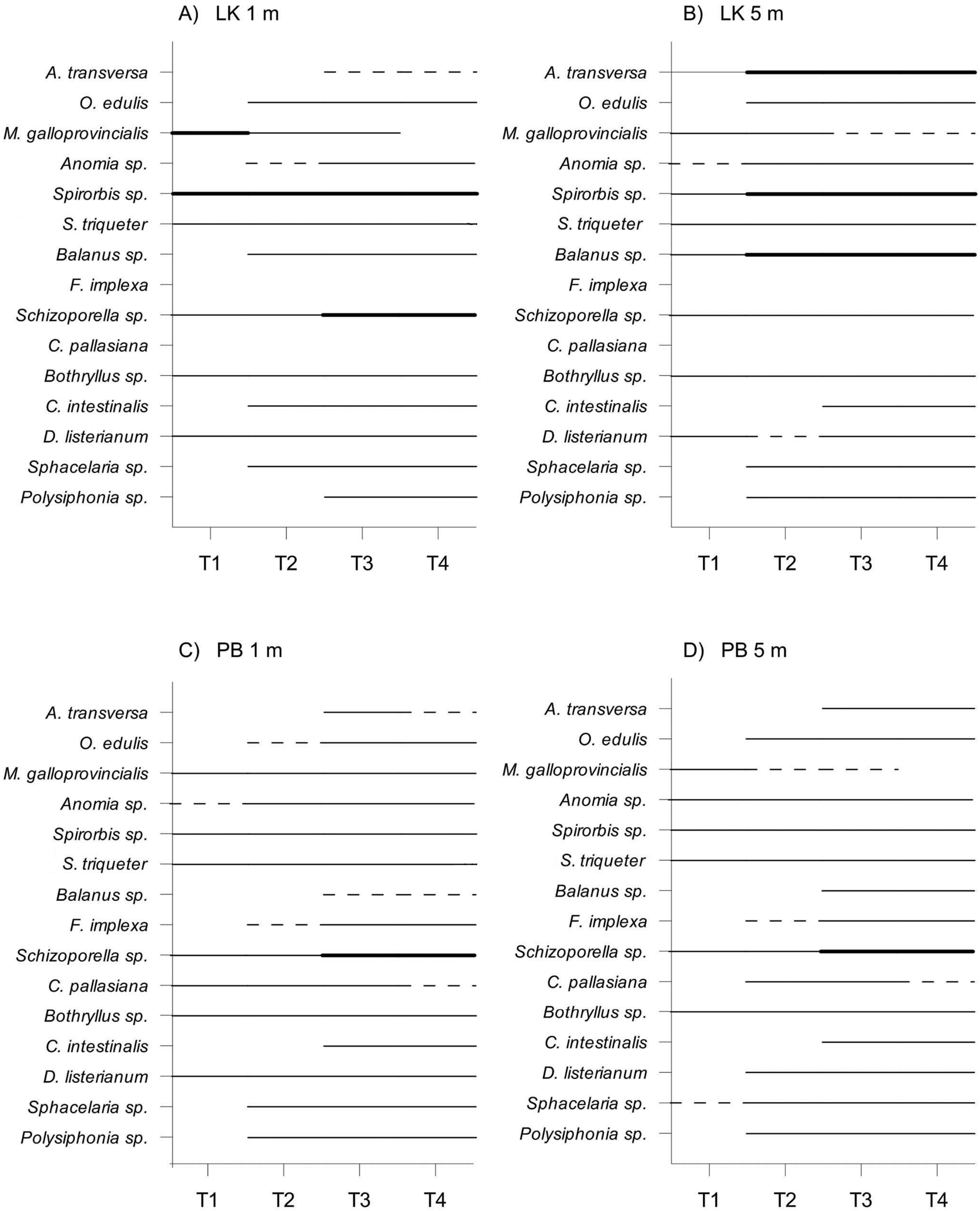

Figure 2. Species abundance over the 4 time lags of immersion in the two locations: intense: 50–100% (▬▬), moderate: 11–49% (——), weak: 1–10% (- - - - - -).

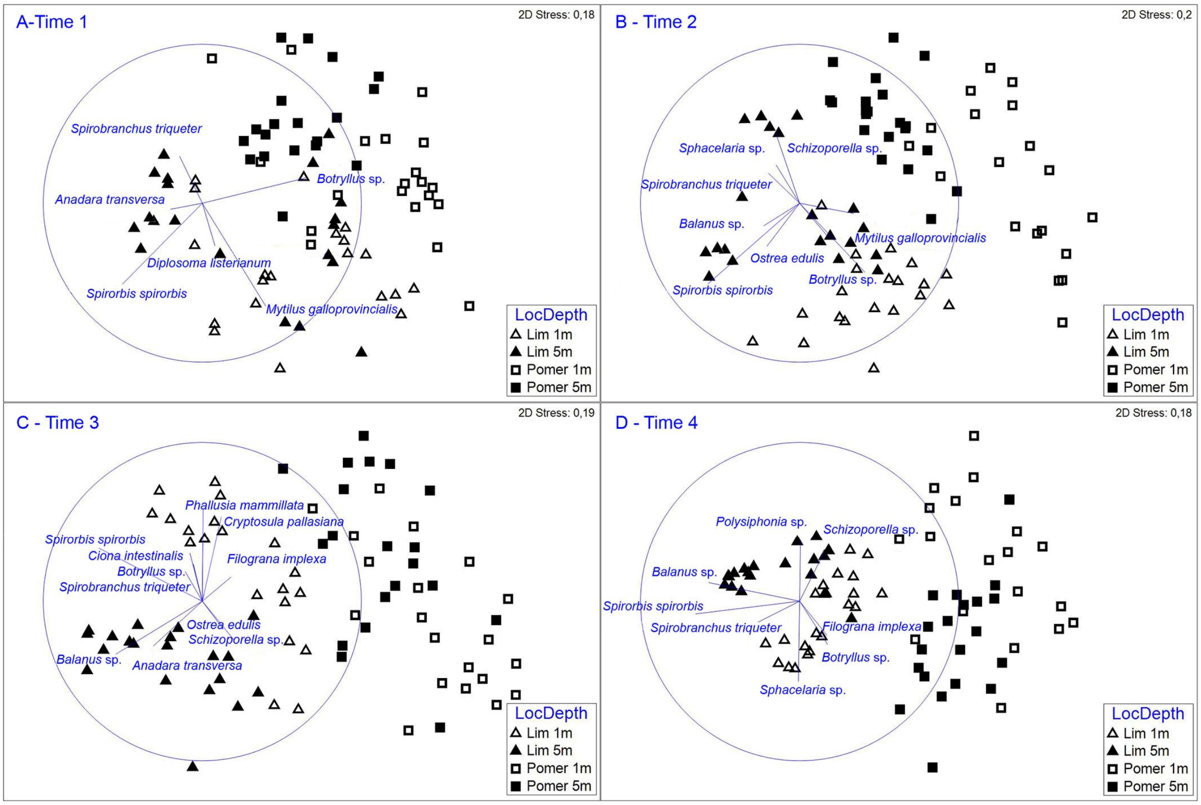

Figure 3. Non-metric multidimensional scaling (nMDS) for biofouling communities forming on terra-cotta tiles based on Bray-Curtis dissimilarity. Abundance data were four-root transformed; cover data were converted as semi-quantitative scores. Tiles were placed in two locations of the west Istrian Coast (LK and PB). Time 1 = 15 Jun (A), Time 2 = 15 August (B) and Time 3 = 15 November 2011 (C) and Time 4 = 15 January 2012 (D). Symbols indicate replicate tiles within each combination of time and location levels: n = 40 (tiles at each site), N = 320 (number of tiles in total). Complete scientific names are provided in Table 1.

37.29%, PB = 45.16%) and *Polysiphonia* sp. (LK = 31.64%, PB = 25.16%).

For the location LK at 1 m depth, taxa which were present across the whole sampling period were *S. spirorbis*, *S. triqueter*, *Schizoporella* sp., *Botryllus* sp. and *D. listerianum*. *Spirorbis* sp. was very abundant across all four sampling times of immersion; the remaining four taxa were abundant across all four times, with the exception of *Schizoporella* sp., which was very abundant at T3 and T4 (Figure 2A). Other abundant taxa were found after 4, 6 and 8 months of immersion with the exception of *A. transversa* and *Polysiphonia* sp. which colonized terracotta tiles after 6 and 8 months of immersion (Figure 2A).

For the location LK at 5 m depth, *A. transversa* appeared immediately after 2 months of immersion at moderate abundances; for the other three sampling times of immersion it was very abundant (Figure 2B). Other taxa present across all times of immersion were *M. galloprovincialis*, *Anomia ephippium*, *S. spirorbis*, *S. triqueter*, *Balanus* sp., *Schizoporella* sp., *Botryllus* sp., *D. listerianum*. Other abundant taxa colonised the substrate starting from 4 months of immersion with exception of *C. intestinalis*, which was recorded after 6 months of immersion (Figure 2B).

For the location PB at 1 m depth, *A. transversa* appeared on terracotta tiles after 6 months of immersion (Figure 2C). Abundant taxa present across entire sampling period were *M. galloprovincialis*, *A. ephippium*, *S. spirorbis*, *S. triqueter*, *Schizoporella* sp., *C. palassiana*, *Botryllus* sp. and *D. listerianum.* Other abundant taxa colonised terracotta tiles after 4 of 6 months of immersion (Figure 2C).

For the location PB at 5 m depth, *A. transversa* showed similar colonisation pattern as at 1 m depth. Abundant taxa present across the complete sampling period were *Anomia ephippium*, *S. spirorbis*, *S. triqueter*, *Schizoporella* sp., *Botryllus* sp. and *Sphacellaria* sp. Other taxa were found at T2 or T3 (Figure 2D).

Table 2. PERMANOVA testing differences among biofouling communities forming on terra cotta tiles for two depths (1 and 5 m) at two locations (Lim Kanal and Pomer Bay) and two sites nested among locations sampled after 2, 4, 6 and 8 months of immersion.

After 2 months of immersion **(A)**				
Source	***DF***	***MS***	**Pseudo-*F***	***P* (perm)**
Location = L	1	19685	14.638	**0.0064**
Depth = D	1	11647	21.42	**0.0046**
Site(L)	2	1344.8	1.7008	0.126
L × D	1	1279	2.3521	0.1744
D × Site(L)	2	543.76	0.6877	0.6556
Residual	72	790.7		
After 4 months of immersion **(B)**				
Source	***DF***	***MS***	**Pseudo-*F***	***P* (perm)**
Location = L	1	28075	33.564	**0.0001**
Depth = D	1	17982	21.498	**0.0001**
L × D	1	3688.3	4.4095	**0.0002**
Pooled	76	836.44		
After 6 months of immersion **(C)**				
Source	***DF***	***MS***	**Pseudo-*F***	***P* (perm)**
Location = L	1	29578	46.79	**0.0001**
Depth = D	1	5095	8.0599	**0.0001**
L × D	2	7059.3	11.167	**0.0001**
Pooled	76	632.15		
After 8 months of immersion **(D)**				
Source	***DF***	***MS***	**Pseudo-*F***	***P* (perm)**
Location = L	1	40285	65.14	**0.0001**
Depth = D	1	3965.9	6.4127	**0.0002**
L × D	1	6838.2	11.057	**0.0001**
Pooled	76	618.44		

Biofouling after 2 months of immersion

A two-dimensional MDS ordination (2D Stress = 0.18) differentiated samples for LK from those for BP (Figure 3A) showing that the composition of the biofouling community differed between the two bays. The distinction between 1 and 5 m samples within the same location was less evident in the two dimensional ordination plot; however, the separation of samples according to factor depth was evident on a 3D ordination plot (Stress = 0.12, ordination not shown). A vector superimposition (the length and direction of each vector indicate the strength and sign of the relationship between species abundance and the first two MDS axes) suggested that the most influential species were *S. triqueter*, *Botryllus* sp., *A. transversa*, *D. listerianum*, *S. spirorbis* and *M. galloprovincialis* (Figure 3A). PERMANOVA analysis confirmed the patterns suggested by MDS ordination: significant differences between the two locations across the two depths and between the two depths across the two locations were detected (Table 2A).

A. transversa was recorded exclusively at LK 5m; i.e., it was not found in the other location × depth combinations. In total, 11 individuals were counted on 20 tiles immersed at this depth, resulting in a 41 individuals per m^2 density. However, all the *Anadara* juveniles were found at one site (10 tiles – replicate). Therefore, 84 individuals per m^2 density was reported for this site. The shell length of *A. transversa* colonising tiles ranged from few mm to 12 mm (Figure 4A) suggesting a rapid growth in the northern Adriatic Sea. The presence of *A. transversa* after two months of immersion indicated the shellfish spawning capacity during the April–June period; however, during this period the production of larvae was likely rather low.

Biofouling after 4 months of immersion

A two dimensional MDS plot (2D plot stress = 0.20, 3D plot stress = 0.11) clearly showed the separation between LK and PB samples as well as between the two depths within the same location (Figure 3B). Among influential species, *Balanus* sp., *O. edulis* and

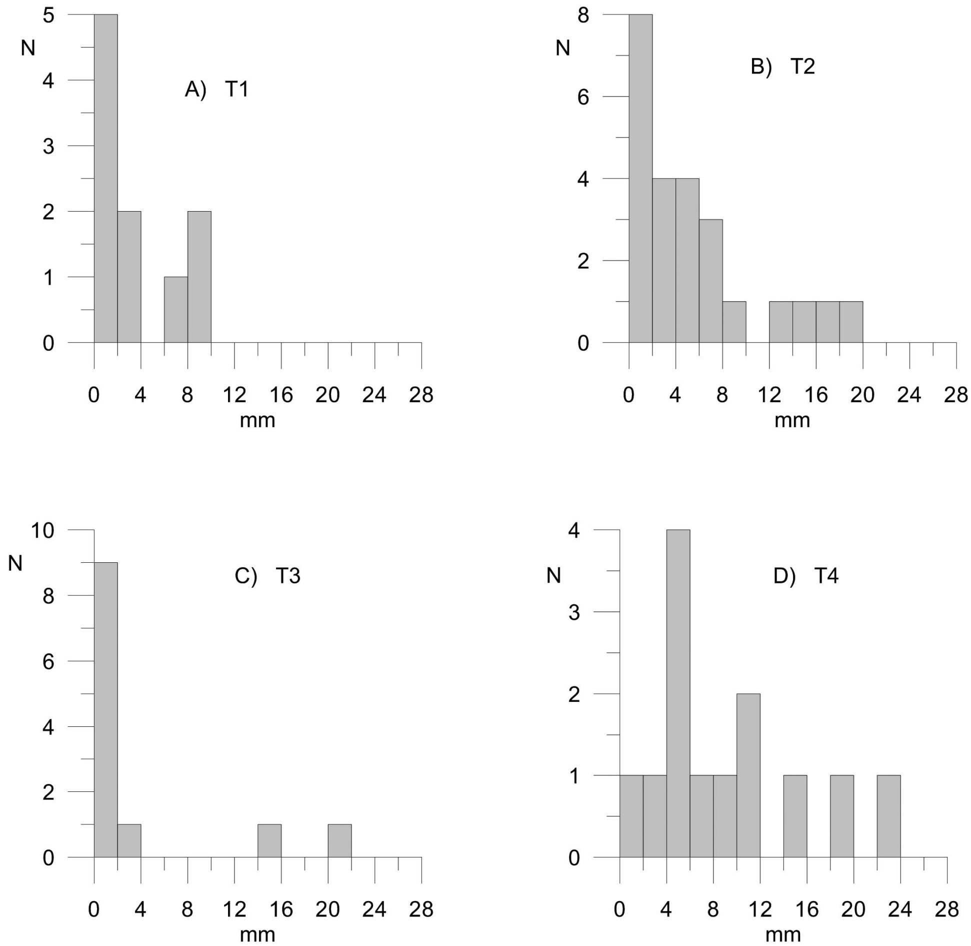

Figure 4. Length frequency of *Anadara transversa* at both locations: Limski Kanal (LK) and Pomer Bay (PB): Time 1 = 15 June, Time 2 = 15 August and Time 3 = 15 November 2013 and Time 4 = 15 January 2014.

S. spirorbis were more abundant at LK. *Botryllus* sp., *D. listerianum* and *M. galloprovincialis* were more abundant at 1 m, whereas *Schizoporella* sp., *S. triqueter* and *Sphacellaria* sp. were more abundant at 5 m depth only (Figure 3B). PERMANOVA analysis revealed the significance of factors Location and Depth. Additionally, the interaction Location × Depth was significant (Table 2B) showing that within the two locations (LK and PB) the biofouling community composition differed with depth (1 and 5 m).

After 4 months of immersion, *A. transversa* was recorded exclusively at LK 5 m. In total, 123 individuals were counted on the 20 tiles immersed at this depth resulting in 500 individuals per m^2 density. However, all the *Anadara* juveniles were found at one site (10 tiles) confirming that *Anadara* larvae showed gregarious colonisation patterns. Therefore, the abundance was 1000 individuals per m^2 for this site. The shell length ranged from a few mm to 21 mm. The distribution of the shell length values was markedly skewed to the right with the modus located in the 4–6 mm class, which indicated that the recruitment of *A. transversa* was intense during the period June–August (Figure 4B).

Biofouling after 6 months of immersion

After 6 months of immersion, the biofouling community at sites LK and PB continued to differentiate as shown in a two dimensional MDS plot (Figure 3C; 2D stress = 0.19). The differentiation was even more evident in a 3D plot (stress = 0.11). In total 11 influential species were identified according to a multiple correlation coefficient of 0.20 (Figure 3C). *A. transversa* and *Balanus* sp. were more abundant at LK whereas *Filograna implexa* was more abundant at PB. *Botryllus* sp., *C. intestinalis*, *Cryptosula pallasiana*, *P. mammillata*, *S. triqueter* and *S. spirorbis* were more abundant at LK 1 m than at LK 5 m. In contrast, *Ostrea edulis* and *Schizoporella* sp. were more abundant at LK 5 m than at LK 1 m. The PERMANOVA analysis revealed the significance of factors Location and Depth as well as of the interaction Location × Depth (Table 2C).

In total, 478 specimens of *A. transversa* were recorded on all tiles. *A. transversa* was recorded at both depths at LK at 1 m depth; 7 specimens were recorded at one site whereas at the other site none were found. The *A. transversa* density was 24 individuals per m^2, or when considering only the colonised site 48 individuals per m^2. At 5 m depth, both LK sites were colonized by *A. transversa* reaching high densities of 1576 individuals per m^2. *A. transversa* colonised also tiles at PB at both depths. Densities of 14 individuals per m^2 and 45 individuals per m^2 were assessed at 1 and 5 m depth, respectively. Recorded specimen sizes ranged from a few mm to 24 mm (Figure 4C). The shell length distribution was prominently skewed to the right with the modus located in the 0–3 mm length class. Since the number of recorded specimens was very high, it can be suggested that the main spawning period of *A. transversa* in the study area occurred from August to October.

Biofouling after 8 months of immersion

After 8 months of immersion, in the MDS plot (2D stress = 0.18), samples for LK grouped together; however, the two depths were well differentiated (Figure 3D). Samples for PB were more dispersed. The separation according to depth was very evident (Figure 3D). These patterns were more visible in the 3D MDS plot (3D stress = 0.13). The two locations were separated mainly because of a higher abundance of *Balanus* sp., *S. triqueter* and *S. spirorbis* at LK. *Filograna implexa* and *Botryllus* sp. being more abundant at LK 1 m were influential in separating LK 1 m from LK 5 m. *Polysiphonia* sp., *Schizoporella* sp. and *Sphacellaria* sp. were influential in separating PB 1 m from PB 5 m: the first two were more abundant at PB 1 m, the last one being more abundant at PB 5 m. The PERMANOVA analysis revealed the significance of factors Location and Depth as well as of the interaction Location × Depth (Table 2D).

In total, 147 *A. transversa* specimens were recorded on all tiles. *A. transversa* was recorded at both depths at LK whereas it was found on tiles only at 5 m depth at PB. The density was 10 individuals per m^2 and 465 individuals per m^2 at LK 1 m and LK 5 m, respectively. At PB 5 m, the density was 35 individuals per m^2. The shell length of *A. transversa* varied from a few mm to 30 mm (Figure 4D). The shell length distribution was skewed to the right; however, the modus was located in the 6–9 mm size class suggesting that during early winter the recruitment of *A. transversa* was lower than during summer.

Discussion

Most dominant taxa were present at both locations and both depths of 1 and 5 m, indicating that the composition of the biofouling community in mariculture areas of the northern Adriatic Sea was rather stable. There were only two exceptions, *F. implexa* and *C. pallasiana*, which were recorded only at PB (Figure 2). Historical data for the eastern Adriatic Sea show that the same species, except the alien bivalve *A. transversa*, were recorded as dominant components of the biofouling community on artificial substrates in the past (Igić 1984, 1984, 1986, 1991, 1995, 1999). This suggests that the dominant taxa of the biofouling communities of the eastern Adriatic Sea have remained stable throughout decades.

However, PERMANOVA revealed significant differences in the composition of the biofouling community between two locations (LK and PB) two investigated depths (1 and 5 m) for the whole period of immersion (2, 4, 6 and 8 months), which can be related to differences in abundance of the dominant species. Moreover, results showed clear differences among periods of immersion, which can be mainly related to differences in settlement peaks and to regressions of some taxa likely due to intraspecific competition for space on the terracotta tiles.

The two commercially important bivalves' *O. edulis* and *M. galloprovincialis* settled on terracotta tiles at two different times. *O. edulis* settled on the substrate between June and September whereas *M. galloprovincialis* colonised the substrate earlier *i.e.* between April and June in both LK and PB. Hrs-Brenko (1974, 1977, 1980, 1983) assessed similar settlement peaks for these two species in LK. However, along the coast of northern Adriatic Sea the most intensive settlement of both *O. edulis* and *M. galloprovincialis* takes place in April and May with the potential to spawn also during autumn (Hrs-Brenko 1974, 1989). Concerning *O. edulis*, our results reflected an increased recruitment in autumn compared to the results of Hrs-Brenko (1974, 1989). However, *M. galloprovincialis* density tended to decrease in autumn. Hrs-Brenko (1974) recorded that after May the number of mussels decreased in the Limski kanal. It could be suggested that the spring mussel spat settlement was overgrown by other fouling organisms that also impeded further recruitment of this species (Igić 1984). Comparing the two depths, *M. galloprovincialis* was more abundant at 1 m depth; these patterns were also reported in Adriatic waters (Hrs-Brenko 1989) and in the Mediterranean Sea (Raby et al. 1994; Hammond and Griffiths 2004). Among bivalves, *Anomia* sp. was also very abundant. It is known that the two *Anomia* species, namely *Anomia ephippium* and *Pododesmus patelliformis*, are permanently present in the biofouling community in the Mediterranean Sea (Saldanha et al. 2003; Sarà et al. 2007) and the Adriatic Sea (Igić 1986). In our study *Anomia* sp. was present throughout the whole experiment duration at both depths except for LK at 1 m depth where it settled the substrate during the period June–August.

The polychaetes *S. triqueter* and *S. spirorbis* were particularly abundant throughout the whole experiment at both locations and at both depths. Maximum density values were assessed in LK. Comparable results for these species were obtained for biofouling communities of the Adriatic and Mediterranean Sea (Raby et al. 1994; Saldanha et al. 2003; Hammond and Griffiths 2004; Sarà et al. 2007).

The barnacle *Balanus* sp. was also an abundant taxon and it is recognised as an important member of the biofouling community in the Adriatic and Mediterranean Sea (Koçak et al. 1999; Sarà et al. 2007; Fitridge et al. 2012). There were marked differences in settlement patterns between locations and depths. At LK, the first settlement of *Balanus* sp. occurred during June–August period and in the April–June period at 1 and 5 m of depth, respectively. The *Balanus* density was higher at 5 m depth and tended to increase over time suggesting that this taxon was able to spawn throughout the whole investigated period. At PB, the density of *Balanus* was similar at both depths and lower than at LK. The settlement of *Balanus* at PB initiated during the period August–October as it was previously observed by Raimondi (1988) and Benedetti-Cecchi (2000).

Schizoporella sp. was substantially more abundant than the other bryozoan *Cryptosula pallasiana*. It settled on the terracotta tiles during the initial period of immersion increasing it coverage throughout time at both locations and depth. *Balanus* sp. and *Schizoporella errata* were determined as dominant foulers in the observations on fouling organisms in the Eastern Mediterranean coast of Turkey (Bobat 2000). Furthermore, Bobat (2000) indicated that *S. errata* have settled on the test panels after the slime layer of diatoms. Later on, tunicate *Ciona intestinalis* and polychaete *Hydroides norvegica* appeared on the test panels.

The most abundant tunicates were *Botryllus* sp., *C. intestinalis* and *D. listerianum*. Only *Botryllus* sp. settled on the substrate during the first period of immersion at each location and depth. *C. intestinalis* settled on terracotta tiles during the period June–August at LK 1 m depth and during the period August–October in all other combinations of factors Location × Depth. *D. listerianum* settled on the substrate during the first period of immersion except for PB at 5 m depth where it was recorded for the first time during the period June–August. These results suggest that *D. listerianum* was able to produce recruits at least from April to August and that along the northern Adriatic coast the time of settlement of important biofouling tunicate varied in relation to locations and depths. The vase tunicate *C. intestinalis* fouled with high density and caused low diversity (Blum et al. 2007).

The most abundant macroalgae were those of the genera *Sphacellaria* and *Polysiphonia*. In general, these macroalgae characterize impacted ecosystems according to Jelić-Mrčelić et al. (2012). Those authors reported that after 6 months of immersion of test panels in oligotrophic waters (the Vela Luka Bay, Croatia), the total percentage coverage was 100% at both depths of the panels. *S. cirrosa* and *P. scopulorum* were dominant algal species. In our study, both species were also recorded among the first settlers on the new substrate. *Polysiphonia scopulorum* is among the dominant algal species in the Adriatic Sea (Igić 1995; Špan et al. 1990; Slišković et al. 2011).

NIS *A. transversa* can be considered as an established component of the coastal benthic community along the Italian Coast of the northern Adriatic Sea (Morello et al. 2004). Results obtained in this study revealed that the establishment of this invasive species might have occurred also along the eastern coast of the northern Adriatic Sea. The fact that *A. transversa* was present on terracotta tiles achieving lengths up to 30 mm after 8 months of immersion indicates that it is potentially able to compete for space with the native fouling community on hard substrata. This result contradicts previous studies showing that, in spite of *A. transversa* capability to produce byssus threads also when adult, only smaller size classes dwell on hard substrata including shells of other species (Solustri et al. 2003) and immersed mariculture structures (Morello et al. 2004). These authors ascertained that *A. transversa* is a year-round spawner that display two spawning peaks, one in early spring and another in summer. In addition, all the *Anadara* juveniles were found at one site, suggesting that *Anadara* larvae showed gregarious colonisation patterns. Our results are in accordance with this finding, as we recorded a recruitment peak of *A. transversa* on terracotta tiles in summer and juveniles were found throughout the duration of the experiment (from April to January). Apparently, the early spring recruitment peak could not be detected due to delayed immersion of tiles. In mariculture, the best timing for spat collector deployment and to maximize recruitment is associated with a high abundance of shellfish larvae in the water column. Premature deployment of collectors—prior to the period of highest abundance of shellfish larvae competent for settlement—usually results in preferential settlement of sessile marine invertebrate larvae: mussels, crustaceans, polychaetes and bryozoans (Lök and Acarli 2006). In general, it can be concluded that *A. transversa* as a new member of the biofouling community raises the concern for possible negative effects on mariculture facilities in terms of competition for resources. Beside the documented deleterious effects on soft bottom natural assemblages (Zenetos 1994; Zenetos et al. 2005), we suggest that *A. transversa* might also affect hard bottom assemblages and potentially might impact larval recruitment of commercial shellfish species of interest as well. That is particularly important for the northern Adriatic Sea, which is a shallow semi-enclosed basin subject to periodic hypoxia events due to variation of euthrophication levels (Degobbis et al. 1995, 2000; Nerlović et al. 2011, 2012a). *A. transversa* can withstand hypoxic conditions, which could confer it a competitive advantage over native benthic organisms (Zenetos 1994). In contrast to previous reports on the sporadic findings of no more than few specimens along the Eastern Adriatic coastal area without evidences of permanent population (Peharda et al. 2010; Nerlović et al. 2012b; Despalatović et al. 2013), the current report of the continuous presence of *A. transversa* over a longer period of almost one year, might indicate the possibility of its spreading within the investigated habitat.

Artificial structures, including shellfish aquaculture installations, provide novel habitats for a multitude of marine organisms (Powers et al. 2007). Unfortunately, the effect of biofouling accumulation is reflected in damage to the cultured shellfish species; biofouling also has a negative impact on the aquaculture installations as well as on the local ecosystem (Fitridge et al. 2012). Biofouling organisms are usually removed at later developmental stages by air exposure and mechanical cleaning; however, both methods are often laborious, time-consuming and have an unfavorable cost-benefit ratio. Aquaculture equipment and installations, as well as the cultured shellfish themselves, provide an attachment surface for various invasive species that have a high potential for proliferation, which can negatively impact aquaculture production (Forrest et al. 2009). The accidental or unwitting introduction of NIS species could potentially have a negative impact on the aquaculture of native commercial shellfish species. Monitoring NIS larvae to detect unwanted recruitment peak, and optimizing spat collector deployment accordingly could be an option to reduce side effects on aquaculture facilities and production. Overall, advances in early detection and NIS monitoring could facilitate and improved sustainable aquaculture management. Considering the presence and fast spreading within ecologically impoverished areas, further research is needed on the occurrence of NIS *A. transversa* – to ensure the continuation of well-established aquaculture activities within investigated area.

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