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

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Received: 28 November 2017 / Accepted: 21 June 2018 / Published online: 24 July 2018

Handling editor: Richard Piola

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 et 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. 2012). Moreover, 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. transversa 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) (45°07′48.50″N; 13°44′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. Furthermore, 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) (44°49′24.91″N; 13°54′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
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               | Taxon                  | Frequency (%) |
|----------------------|------------------------|---------------|
| Phylum               |                        | LK | PB |
| Annelida             | Spirobranchus triqueter (Linnaeus, 1758) | 92.09 | 94.19 |
| Phylum               | Spirobranchis spirorbis (Linnaeus, 1758) | 99.44 | 65.16 |
| Phylum               | Filograna implexa Berkeley, 1835 | 0.00 | 37.42 |
| Echinodermata        | Paracentrotus lividus (Lamarck, 1816) | 0.56 | 0.00 |
| Phylum               | Ophiuroidea            | 1.13 | 0.00 |
| Mollusca             | Modiolus barbatus (Linnaeus, 1758) | 1.69 | 0.00 |
| Phylum               | Anomia ephippium Linnaeus, 1758 | 12.99 | 26.45 |
| Phylum               | Mimachlamys varia (Linnaeus, 1758) | 6.21 | 1.29 |
| Phylum               | Mytilus galloprovincialis Lamarck, 1819 | 23.16 | 16.13 |
| Phylum               | Monia patelliformis (Linnaeus, 1761) | 0.00 | 0.65 |
| Phylum               | Pecten sp. | 1.13 | 0.65 |
| Phylum               | Flexopecten glaber (Linnaeus, 1758) | 0.00 | 1.29 |
| Phylum               | Musculus subpictus (Cantraine, 1835) | 0.56 | 1.94 |
| Phylum               | Anadara transversa (Say, 1822) | 34.46 | 2.58 |
| Phylum               | Ostrea edulis Linnaeus, 1758 | 48.59 | 30.32 |
| Phylum               | Barbatia barbata (Linnaeus, 1758) | 0.56 | 0.00 |
| Phylum               | Bistella sp. | 6.78 | 2.58 |
| Phylum               | Placophor9 indet. | 1.69 | 4.52 |
| Phylum               | Gastropoda indet. | 1.13 | 0.65 |
| Phylum               | Flabellina sp. | 0.56 | 1.29 |
| Phylum               | Bolinus brandaris (Linnaeus, 1758) | 1.69 | 0.00 |
| Nemertina            | Turbellaria sp. | 0.56 | 1.94 |
| Bryozoa              | Schizoporella sp. | 78.53 | 80.65 |
| Phylum               | Cryptosula pallastiana (Moll, 1803) | 0.00 | 21.29 |
| Crustacea            | Balanus sp. | 63.28 | 5.16 |
| Tunicata             | Botryllus sp. | 55.37 | 82.58 |
| Phylum               | Ciona intestinalis (Linnaeus, 1767) | 22.60 | 5.81 |
| Phylum               | Phallusia manmilla (Cuvier, 1815) | 14.69 | 18.71 |
| Phylum               | Microcosmus vulgaris Heller, 1877 | 7.91 | 0.65 |
| Phylum               | Pyura microcosmus (Savigny, 1816) | 10.17 | 6.45 |
| Phylum               | Diplosoma listerianum (Milne Edwards, 1841) | 25.42 | 39.35 |
| Phylum               | Phallusia fumigata (Grube, 1864) | 2.26 | 3.23 |
| Phylum               | Phallusia sp. | 2.26 | 0.00 |
| Phylum               | Tunicata indet. | 11.86 | 1.94 |
| Porifera             | Spongia officinalis Linnaeus, 1759 | 0.56 | 0.00 |
| Phylum               | Sphacelaria sp. | 37.29 | 45.16 |
| Rhodophyta           | Polysiphonia sp. | 31.64 | 25.16 |
| Phylum               | 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 =
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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 terracotta 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. Spirobranchus 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 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. ephiippium, 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 ephiippium, S. spirorbis, S. triqueter, Schizoporella sp., Botryllus sp. and Sphacellaria sp. Other taxa were found at T2 or T3 (Figure 2D).

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. ephiippium, 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 ephiippium, S. spirorbis, S. triqueter, Schizoporella sp., Botryllus sp. and Sphacellaria sp. Other taxa were found at T2 or T3 (Figure 2D).
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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      | 5905  | 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 m² density. However, all the Anadara juveniles were found at one site (10 tiles – replicate). Therefore, 84 individuals per m² 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 m² 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 m² 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 pallasianna, P. mammillata, S. triqueter and S. spirorbis...
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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 m², or when considering only the colonised site 48 individuals per m². At 5 m depth, both LK sites were colonized by A. transversa reaching high densities of 1576 individuals per m². A. transversa colonised also tiles at PB at both depths. Densities of 14 individuals per m² and 45 individuals per m² 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 m² and 465 individuals per m² at LK 1 m and LK 5 m, respectively. At PB 5 m, the density was 35 individuals per m². 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 taxai 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. triquetra 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 Hydrodies norwegica 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
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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 Acařlı 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 eutrophication 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.

Acknowledgements

We wish to thank Professor Massimo Devescovi for suggestions on earlier version of the manuscript. We also thank the owner and staff of Istrida d.o.o. (LK) and Rak d.o.o. (PB) mariculture enterprises for providing technical assistance and the use of all facilities.

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