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ORIGINAL ARTICLE

Early detection of potentially invasive invertebrate species in *Mytilus galloprovincialis* Lamarck, 1819 dominated communities in harbours

Cristina Preda · Daniyar Memedemin · Marius Skolka · Dan Cogălniceanu

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Abstract Constanţa harbour is a major port on the western coast of the semi-enclosed Black Sea. Its brackish waters and low species richness make it vulnerable to invasions. The intensive maritime traffic through Constanţa harbour facilitates the arrival of alien species. We investigated the species composition of the mussel beds on vertical artificial concrete substrate inside the harbour. We selected this habitat for study because it is frequently affected by fluctuating levels of temperature, salinity and dissolved oxygen, and by accidental pollution episodes. The shallow communities inhabiting it are thus unstable and often restructured, prone to accept alien species. Monthly samples were collected from three locations from the upper layer of hard artificial substrata (maximum depth 2 m) during two consecutive years. Ten alien macro-invertebrate species were inventoried, representing 13.5% of the total number of species. Two of these alien species were sampled starting the end of summer 2010, following a period of high temperatures that triggered hypoxia, causing mass mortalities of benthic organisms. Based on the species accumulation curve, we estimated that we have detected all benthic alien species on artificial substrate from Constanţa harbour, but additional effort is required to detect all the native species. Our results suggest that monitoring of benthic communities at small depths in harbours is a simple and useful tool in early detection of potentially invasive alien species. The selected habitat is easily accessible, the method is low-cost, and the samples represent reliable indicators of alien species establishment.

Keywords Constanţa harbour · Black Sea · Invertebrates · Alien species · Recolonization · Monitoring

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C. Preda · D. Memedemin · M. Skolka (✉) · D. Cogălniceanu
Faculty of Natural and Agricultural Sciences,
Ovidius University of Constanţa, Aleea Universităţii 1,
Building B, 900470 Constanţa, Romania
e-mail: mskolka@univ-ovidius.ro

Introduction

Globalization and increasing trade lead to a higher rate of transfer of organisms around the world, some of which may have significant ecological or economical impact in the recipient area. Monitoring is a valuable tool that provides the opportunity to detect early potential crises such as biological invasions (Elzinga et al. 2001). Species accumulation theory offers information on the required sampling effort to detect the species from a studied area and is useful for testing various biodiversity sampling approaches (Gotelli and Colwell 2001). Species richness increases rapidly as more samples are collected due to the presence of common species, but this increase slows down as rare species are added (Bunge and Fitzpatrick 1993). Thus, the probability of detecting alien species including rare ones can be computed (Chao et al. 2009) and can be an effective measure of testing the efficiency of early detection systems.

As many other seas, the Black Sea is severely affected by anthropogenic changes (Leppäkoski and Mihnea 1996), biological invasions being one of the most important (Gomoiu et al. 2002; Paavola et al. 2005). The number of alien species inventoried in the Black Sea is continuously rising (e.g., Aleksandrov 2010; Alexandrov and Zaitsev 2000; Leppäkoski et al. 2002; Skolka and Gomoiu 2004). For example, Zaitsev and Öztürk (2001) reported 59 alien species in the Black Sea, but recent efforts of updating the status of European marine alien species increased the number to 184 including established, casual and cryptogenic species (Zenetos et al. 2009). We use the term 'alien' according to Richardson et al. (2000) to describe taxa present in an area outside their historical native range, following human-mediated introduction.

The benthic hard substrate has usually a complex structure and high species richness. The dominant species in the Black Sea is the mussel (*Mytilus galloprovincialis* Lamarck, 1819), which provides a secondary substrate for sessile species as well as appropriate conditions for many vagile species. Organisms living in the north-western part of the Black Sea are constantly subjected to environmental stressors (e.g., variable freshwater inflow from the Danube, fluctuating temperatures and oxygen levels) that cause mass mortality of shallow benthic communities followed by rapid recolonization.

In marine and brackish water environments, shipping is considered the main vector of alien species introductions (Reise et al. 1999). Constanţa harbour is the major Romanian transit hub providing connections between Central and Eastern Europe, Caucasus and Central Asia. Previous studies of the benthic communities on mobile substrate and planktonic associations inside Constanţa harbour showed that these are species poor, with a simplified structure composed of few resistant and/or opportunistic species (Petran 1984; Ţigănuş 1982).

Considering the great importance of shipping as a vector of alien species, monitoring programmes developed within the harbour are essential, since usually harbours have impoverished communities of organisms and are more prone to invasions (Galil 2000). We hypothesized that the benthic community on the harbours hard substrate would be unstable and has low species richness. The aim of our study was (1) to investigate the species composition of the shallow mussel beds inside the Constanţa harbour and the proportion of alien species and (2) to test the usefulness of the method in the early detection of newly established alien species.

Materials and methods

Study site

Located on the western coast of the Black Sea, Constanţa harbour (44°09′N 28°39′E) is situated at a distance of 157 km from the Danube (Sulina Branch) and 331 km from the Bosphorus Strait. Placed at the crossing of two Pan-European transport corridors, Constanţa harbour is among the first ten major European harbours. It has 140 functional berths and a handling capacity of over 100 million tons per year (Administration of Constanţa Port 2007). We selected three sampling sites from the entrance of Constanţa North harbour (Site 1) towards the inner part of the harbour (Site 2 and Site 3) (Fig. 1; Table 1). We used a Garmin 60CSx receiver to locate the sampling sites and Garmin MapSource version 6.15.6 for mapping.

Sampling

For a period of 25 months starting with December 2008, benthic communities were sampled monthly from hard substrate represented by vertical flat concrete structures. Samples were scraped from 0 to 2 m depths, weighing 1,500–2,000 g each. We chose the upper layer for this study because this area is dynamic, affected by extremely high (in summer) or low temperatures (in winter), occasional chemical pollution events (e.g., with hydrocarbons) and by oxygen deficiency. All these induce mass mortalities followed by recolonization. Each sample was stored in a plastic container, labelled and brought to the laboratory for washing and sieving (0.071 mm). Invertebrates were identified to the lowest taxonomic level possible using a Nikon SMZ645 stereomicroscope, based on the available field guides and monographs (e.g., Băcescu 1967; Cărăuşu et al. 1955; Grossu 1962; Marinov 1977; Morduhai-Boltovskoi 1968; Prenant and Bobin 1966). Nomenclature of species is in agreement with the World Register of Marine Species (Appeltans et al. 2011). Mussels *M. galloprovincialis* with shell lengths larger than 12 mm were measured individually with a digital calliper. Data on the physical and chemical properties of the seawater were provided by the National Institute for Marine Research and Development 'Grigore Antipa' in Constanţa.

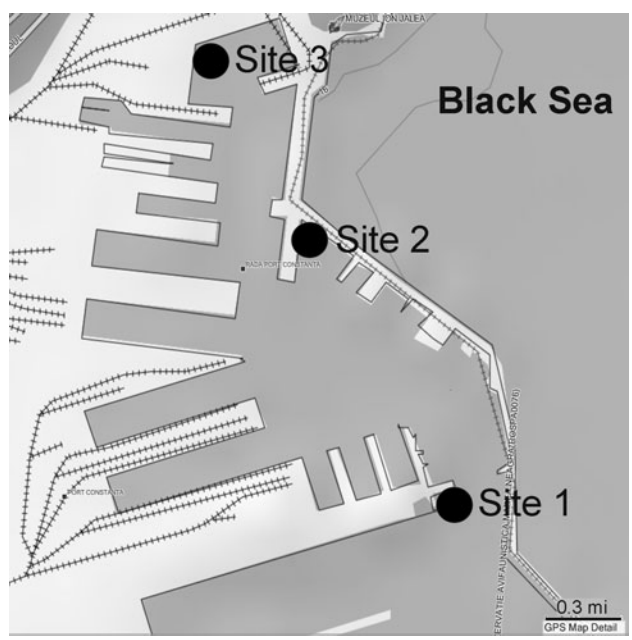

Fig. 1 Location of sampling sites in Constanţa harbour

Table 1 Characteristics of the selected sampling sites

Sampling site	Location	Depth (m)	Activities	Vessel type	Number of monthly samples
Site 1	N 44.145701° E 28.667786°	12	Oil trading berths	Tankers	25
Site 2	N 44.160670° E 28.658235°	5	Technical berths	Auxiliary	24
Site 3	N 44.169762° E 28.650945°	8	Cereals and general cargo	Bulk carriers	21

Data analysis

Following identification, the species were classified in native and alien species, based on the available data in the literature regarding native ranges, historical and present distribution, pathways of introduction in the Black Sea. Nematodes and harpacticoides were not identified at species level and were not included in the following analyses. Polychaetes of the genus *Polydora* were considered a complex of sibling species and were referred to as '*Polydora* complex'. Seasons were established as periods of 3 months as follows: 'winter' (December–February), 'spring' (March–May), 'summer' (June–August) and 'autumn' (September–November).

The assumptions of normality were tested using Shapiro–Wilk test, and ANOVA was used to calculate the statistical significance of differences between samples. The relation between the monthly species richness and the average temperature was tested with Pearson's product moment correlation coefficient. For tests and correlations, we rejected the null hypothesis if $p \leq 0.05$. To estimate whether our inventory was complete, we computed species accumulation curves (SAC) using EstimateS 8.2 (Colwell 2009). Based on the presence–absence matrix, we computed five non-parametric incidence-based estimators of species richness using EstimateS 8.2 (Colwell 2009): ICE, Chao2, 1st and 2nd order jackknife estimators and the Bootstrap estimator.

The incidence-based coverage (ICE) estimator assumes that the detection probabilities vary among species (Eq. 1) (Chao and Shen 2009; Lee and Chao 1994). The Chao2 estimator (Eq. 2) uses the number of uniques (species detected in only one sample) and duplicates (species detected in only two samples) for the same purpose (Chao 1987).

$$S_{\text{ice}} = S_{\text{freq}} + \frac{S_{\inf r}}{C_{\text{ice}}} + \frac{Q_1}{C_{\text{ice}}}\gamma^2_{\text{ice}} \quad \text{where}$$
$$\gamma^2_{\text{ice}} = \max\left[\frac{S_{\inf r}}{C_{\text{ice}}}\frac{m_{\inf r}}{(m_{\inf r-1})}\frac{\sum_{j=1}^{10} j(j-1)Q_j}{(N_{\inf r})^2} - 1, 0\right] \quad (1)$$

$$\hat{S}_{\text{Chao2}} = S_{\text{obs}} + \left(\frac{m-1}{m}\right)\left(\frac{Q_1(Q_1-1)}{2(Q_2+1)}\right) \quad (2)$$

The frequency of uniques is used by 1st order jackknife estimator (Eq. 3), while the 2nd order jackknife (Eq. 4) uses the frequency of uniques and duplicates to estimate the number of undetected species (Burnham and Overton 1978). The Bootstrap (Eq. 5) is a reliable method related to the jackknife and has a wider applicability (Smith and van Belle 1984).

$$S_{\text{jack1}} = S_{\text{obs}} + Q_1\left(\frac{m-1}{m}\right) \quad (3)$$

$$S_{\text{jack2}} = S_{\text{obs}} + \left[\frac{Q_1(2m-3)}{m} - \frac{Q_2(m-2)^2}{m(m-1)}\right] \quad (4)$$

$$S_{\text{boot}} = S_{\text{obs}} + \sum_{k=1}^{S_{\text{obs}}}(1-p_k)^m \quad (5)$$

The variables used are defined as follows: S estimated species richness, S_{obs} observed species richness, Q_j number of species that are observed in exactly j samples, m number of samples, C_{ice} sample incidence coverage estimator, γ^2_{ice} estimated coefficient of variation of the Q_i for infrequent species, p_k proportion of samples that contain species k.

The sampling effort for the detection of the total number of species (or a determined percentage of the estimated species richness) was calculated using the non-parametric method proposed by Chao et al. (2009).

Results

We identified 74 species belonging to 15 taxonomic groups in the 70 samples collected from the harbour. Amphipod crustaceans and polychaets were the groups with the highest number of species (a list of species is provided in 'Appendix'—Table 5). We also inventoried 10 alien species from seven higher taxa (Fig. 2). The investigated sites differed in species richness and composition, the highest number of species being registered at Site 1 (Table 2).

Regarding the seasonal dynamics, the highest number of species per sample was recorded in the cold season and the lowest species richness was registered in summer 2010

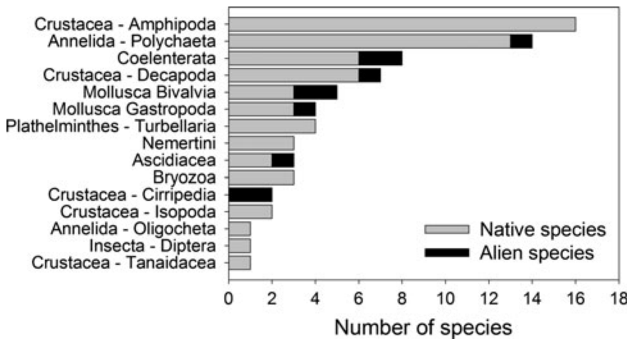

Fig. 2 Taxonomic groups and species (*native* and *alien*) identified in the samples from sampling sites

(Fig. 3). One-way ANOVA detected significant differences between seasons ($F_{3,66} = 7.74$, $p < 0.001$). Holm-Sidak multiple pairwise comparison indicated that the number of species detected in winter was significantly higher than in summer ($t = 4.64$, $p < 0.001$), spring ($t = 3.47$, $p < 0.001$) or autumn ($t = 2.78$, $p = 0.007$) (Fig. 3). A negative correlation was observed between the average temperature and species richness recorded monthly ($r = -0.459$, $p = 0.021$).

Of the ten alien macro-invertebrate species identified in the harbour, eight were present in all sites: *Diadumene lineata* (Verrill, 1869), *Ficopomatus enigmaticus* (Fauvel, 1923)*, Corambe obscura* (Verrill, 1870)*, Rhithropanopeus harrisii* (Gould, 1841)*, Balanus improvisus* Darwin, 1854*, Anadara* sp.*, Mya arenaria* Linnaeus, 1758 and *Molgula manhattensis* (De Kay, 1843). The hydrozoan *Blackfordia virginica* Mayer, 1910 was present at the harbour entrance (Site 1) only in December, while the barnacle *Balanus amphitrite* Darwin, 1854 was encountered inside the harbour only at Site 3 in December 2010.

Diadumene lineata was present both on natural and artificial hard substrata including mussels' valves, sometimes in large numbers. In general, the abundance of *D. lineata* individuals exceeded that of native sea anemones like *Actinia equina* (Linnaeus, 1758) or *Actinothoe clavata* (Ilmoni, 1830). The presence of *F. enigmaticus* was limited to single individuals or groups of maximum 15 specimens living on mussels' shells. *C. obscura* was present only during the cold season. *R. harrisii* was collected in most samples (89%) and had a higher relative abundance than the combined number of individuals of native brachyuran decapods. The barnacle *B. improvisus* was a dominant species in the harbour together with the native species of bivalves *M. galloprovincialis* and *Mytilaster lineatus* (Gmelin, 1791). Several juveniles of *M. arenaria* were observed in 10% of the samples collected. *M. manhattensis* was found mostly as single individuals inside the harbour at Sites 2 and 3 (in 12 samples) and less at the harbour entrance (in two samples). The majority (98%) of *Anadara* sp. individuals were sampled at Site 1 starting September 2010 (the rest of 2% was divided between S2 and S3) after a hot summer (27°C average air temperature between June and August 2010). The size distribution of the mussels sampled from the harbour varied seasonally (Fig. 4). During the autumn and winter of 2010, the percentage of mussels with shell lengths greater than 51 mm was lower compared to 2009. *B. improvisus*, *R. harrisii* and *F. enigmaticus* are the alien species easiest to detect in the harbour, with a probability of more than 80% as shown in Table 3. The proportion of alien species was similar for the three sites: 13.4% for Site 1, 13.8% for Site 2 and 15.8% for Site 3.

The species accumulation curves have similar shapes for the three sampling sites but do not reach an asymptote (Fig. 5). The sampling effort required to detect 95% of the observed species richness is 51, while three samples are sufficient for the detection of 50% of the species (Fig. 6). Almost half of the species inventoried (~48%) were present in ten or less samples (out of 70) with 16% encountered in just one sample, suggesting a high species turnover. We then estimated the number of species using estimators of species richness (Table 4). The probability of a new alien species to be observed in the next sample is 0.0035, and the additional number of samples required to detect a new alien (S_{est}) is 0. However, additional sampling

Table 2 Species richness and composition for the investigated sites

Sampling site	Site 1	Site 2	Site 3
No. of observed species	67	58	57
No. of higher taxa	15	15	13
Monthly average no. and standard deviation of species	24 ± 5.7	20 ± 5.1	21 ± 4.9
Month of highest observed species richness	January 2009	November 2009	December 2009
Month of lowest observed species richness	April 2009, June 2009, March 2010	July 2010	July 2010
Taxonomic groups with high number of species	Amphipoda (19%)	Polychaeta (21%)	Polychaeta (19%)
	Polychaeta (19%)	Amphipoda (14%)	Amphipoda (18%)
	Coelenterata (12%)	Coelenterata (12%)	Decapoda (11%)
	Decapoda (10%)	Decapoda (10%)	Coelenterata (11%)

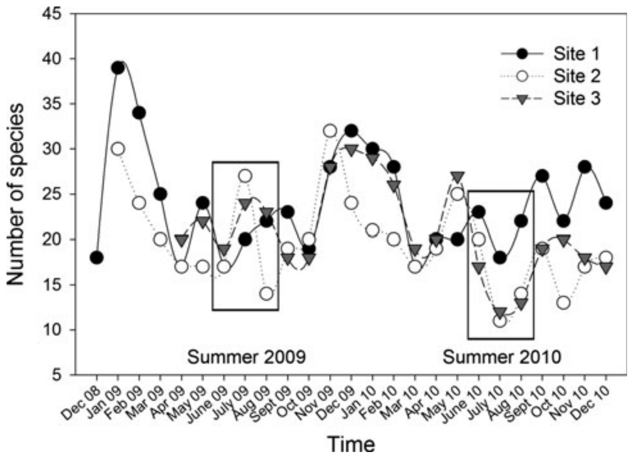

Fig. 3 Seasonal variation of species richness in the harbour

effort is necessary for inventorying at least 90% of the total S_{est} in the harbour (Table 4).

Discussion

Our monitoring system of benthic communities on hard artificial substrate at small depths proves useful in the early detection of potentially invasive alien species in harbours. On natural hard substrata the community of the stone mussels dominated by *M. galloprovincialis* has two components that are bathymetrically delimited and are characterized by specific assemblages of invertebrates (Băcescu et al. 1971). In the harbour area, these two components are combined and several species described as characteristic and abundant in natural areas are absent in the harbour. Instead, pollution resilient species are more common. The mussels *M. galloprovincialis* form a bed of 5–15-cm thickness providing interstitial space for other sessile organisms depending on their size. Overall, the mussels and the associated fauna in Constanţa harbour form a complex community, but the species richness is lower when compared with similar communities at the Romanian coast outside the harbour (Băcescu et al. 1971; Teacă et al. 2006a, b). The relationship between species richness and invasibility of a system is still widely debated (e.g., Borges et al. 2006; Herben et al. 2004; Kennedy et al. 2002; Levine and D'Antonio 1999). An efficient use of resources can contribute to the community's resistance to the establishment of new alien species (Stachowicz et al. 1999). *M. galloprovincialis*, as the dominant species on the artificial substrate in the harbour, leaves little available space for the establishment of other sessile organisms.

At local scale, the invasibility of a given habitat is influenced by physical factors and the level of disturbance (Zaiko et al. 2007). At the Romanian coast of the Black Sea, the average salinity in 2010 was close to the minimum recorded during 1959–2010. The lowest salinity for the same year was observed in Constanţa area in July (10.09 PSU). Average sea water temperature was 13.6°C in 2009

Fig. 4 Size distribution of mussels (*M. galloprovincialis*) sampled from Constanţa harbour: **a** spring, $n = 3{,}213$ in 2009, $n = 2{,}345$ in 2010; **b** summer, $n = 4{,}626$ in 2009, $n = 1{,}411$ in 2010; **c** autumn, $n = 3{,}305$ in 2009, $n = 510$ in 2010; and **d** winter, $n = 2{,}956$ in 2009–2010, $n = 130$ in 2010–2011

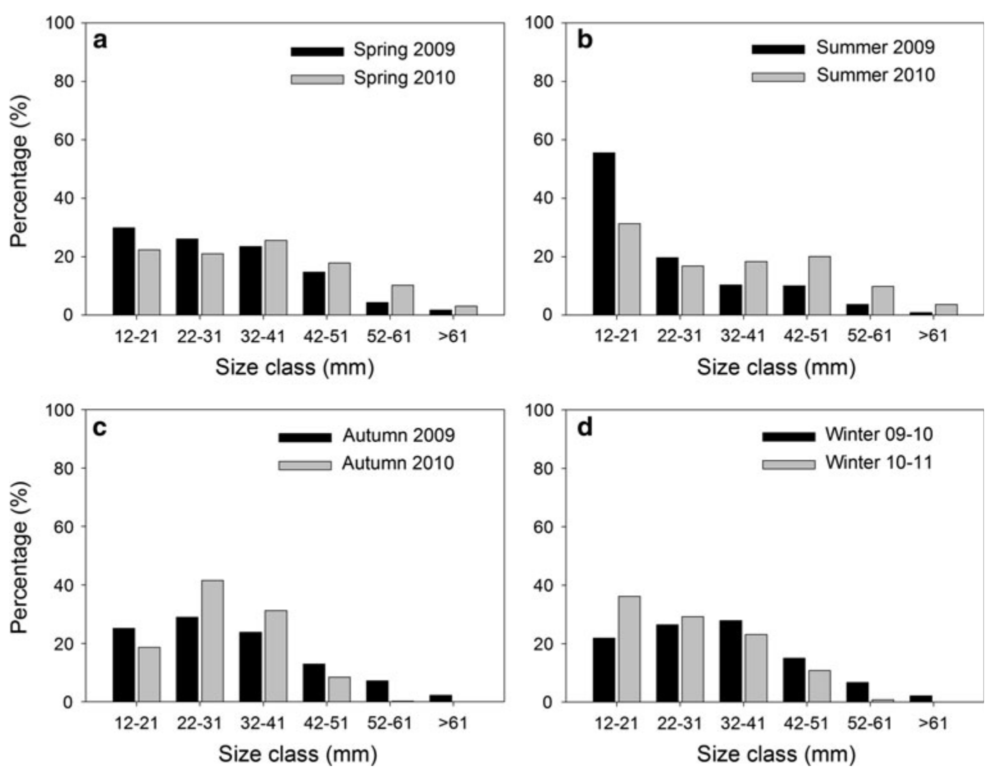

Table 3 Frequency of occurrence of benthic alien species within Constanta harbour monitoring network (%)

Species	Site 1 $n = 25$	Site 2 $n = 24$	Site 3 $n = 21$	Total $n = 70$
Balanus improvisus	100	100	100	100
Rhithropanopeus harrisii	96	79	90	89
Ficopomatus enigmaticus	84	67	90	80
Corambe obscura	56	58	52	56
Diadumene lineata	32	25	43	33
Molgula manhattensis	8	21	33	20
Anadara sp.	16	13	5	11
Mya arenaria	12	4	14	10
Blackfordia virginica	8	0	0	3
Balanus amphitrite	0	0	5	1

and 14.1°C in 2010, close to the maximum value recorded between 1959 and 2010 at the Romanian coast. The highest value for Constanţa area in 2010 reached 29.8°C on 17th of August. The high surface water temperatures recorded during summer coupled to decaying organic matter produced by algal blooms and wastewater cause episodes of hypoxia that can affect even surface water. During the summers of 2009 and 2010, five hypoxia events were recorded in the water column. The lowest oxygen value (1.55 cm^3/l) was registered in September 2010 at 20 m depth, in the southern part of the Romanian coast (Lazăr 2011). Inside Constanţa harbour at the end of July 2009, average values of salinity, temperature and dissolved oxygen at the surface were 12.1 PSU, 25°C and 14 mg/l, respectively. At the end of August 2010, average values of the parameters registered in the same locations inside the harbour were 13.8 PSU, 27.3°C and 9.4 mg/l (Lazăr 2011). High temperatures and low oxygen levels in summer led to changes in species composition and to significant differences between seasons.

These extreme events induce the mass mortality of shallow benthic communities and make the hard substrate available for recolonization by native and/or non-native species. The success of establishment of alien species is further facilitated in harbours by the constant flow of potential settlers. An optimal timing of arrival increases the chances of the alien species to become established. The occasional presence of alien species like *Musculista senhousia* (Benson in Cantor, 1842), *Styela clava* Herdman, 1881, *Palaemon macrodactylus* Rathbun, 1902, *Hemigrapsus sanguineus* (De Haan, 1835) and *Dyspanopeus sayi* (Smith, 1869) was recently recorded in other areas of the Romanian Black sea coast, including Constanţa South-Agigea and Midia Harbors, as well as Tomis and Eforie marinas (Micu and Micu 2004; Micu and Niţă 2009; Micu et al. 2010a, b). The percentage of alien species inventoried in the harbour was 13.5%, almost three times higher compared to other similar communities on natural and artificial hard substrata at the Romanian coast, outside the harbour. Cohen et al. (2005) conducted a rapid assessment

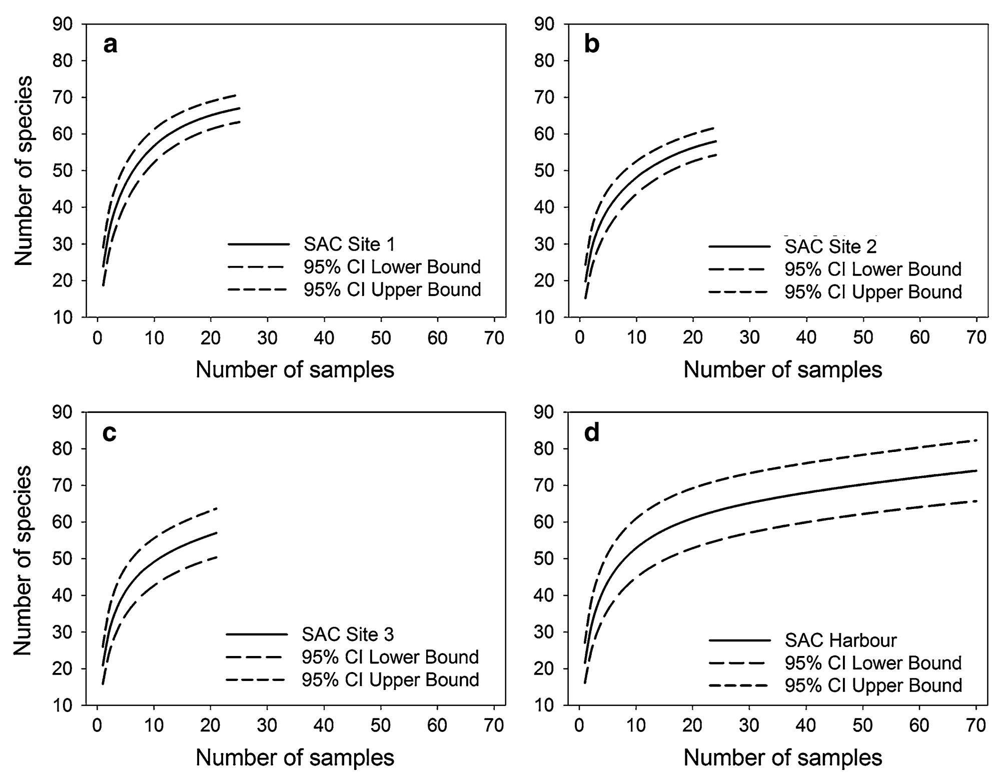

Fig. 5 Species accumulation curves (SAC) and 95% confidence interval (CI) for: **a** Site 1, **b** Site 2, **c** Site 3 (see Fig. 1) and **d** Constanţa harbour

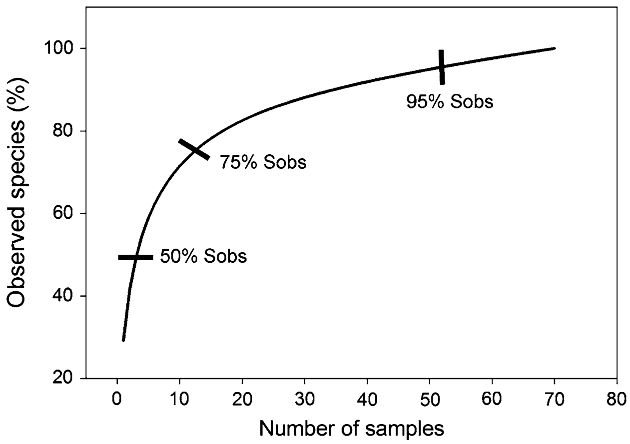

Fig. 6 The estimated number of samples required to detect 50, 75 and 95% of the observed species richness (S_{obs}) in the harbour

Table 4 Estimated species richness (mean, standard error and 95% confidence interval) based on non-parametric estimators of species richness computed using EstimateS 8.2 (Colwell 2009)

Estimator	Total	Native species	Alien species
No. of observed species	74	64	10
Chao2	90.3 ± 12.7	82.1 ± 14.9	10 ± 0.3
	(78.2–137.1)	(68.4–138.1)	(10.0–10.0)
ICE	82.5 ± 0.01	72.2 ± 0.01	10.6 ± 0
1st order jackknife	85.8 ± 3.1	74.8 ± 3.0	11 ± 1
2nd order jackknife	94.6	83.6	11
Bootstrap	78.9	68.5	10.5

survey for alien organisms in southern California and concluded that the number or proportion of alien/cryptogenic taxa is not significantly higher in harbour areas in comparison with non-harbour ones. However, we underline the importance of harbours as reservoirs for alien species, similar to other findings (e.g., Arenas et al. 2006; Ashton et al. 2006; Minchin 2007; Paavola et al. 2008).

The alien species identified in Constanţa harbour are euryhaline and tolerate wide ranges of salinity in their native areas. Most of them originate from the North Atlantic area while 30% are of Indo-Pacific origin or cosmopolite. With the exception of the nudibranch gastropod *C. obscura*, which is a preferential feeder on bryozoans and covered a previously empty niche, the other alien species observed are in the same trophic and functional groups as the native species (i.e., mainly suspension feeders). To understand their presence in Constanţa harbour, a brief description of these species is provided below.

The barnacle *B. improvisus*, one of the oldest documented introductions in the Black Sea, is a suspension feeder that facilitates the establishment success and development of the alga *Enteromorpha intestinalis* (L.) (Gomoiu and Skolka 1996; Kotta et al. 2006).

The anthozoan *D. lineata* originates in the north Pacific area. Its presence was mentioned from the Black Sea area since the 1960 under the name *Aiptasiomorpha luciae* (Verrill, 1898) (Băcescu et al. 1971). Due to its increased capacity to tolerate variable abiotic factors, *D. lineata* is a very widespread species.

Ficopomatus enigmaticus, a serpulid polychaete, was discovered in France and described by Fauvel (1922) who presumed it had arrived there on ships' hulls. However, the origin of this species is still uncertain. In the Black Sea, *F. enigmaticus* was reported in the late 1920s (Annenkova 1929). *F. enigmaticus* tolerates a wide range of salinities (from 0 to ~55PSU) (Hill 1967), temperatures (0–35°C) and pH (4–9) (Bianchi 1981) and has the potential of building reef-like structures. The growth and spread of *F. enigmaticus* is dependent on environmental conditions, higher biomasses being reported in brackish waters (Schwindt et al. 2004). A *F. enigmaticus*-dominated community was reported from the Belona marina at the Romanian Black Sea coast (Micu and Micu 2004).

Corambe obscura is a small nudibranch gastropod of North Atlantic origin that most probably arrived in the Black Sea around 1980 by shipping (Roginskaya and Grintsov 1997). Observations performed at the Romanian coast showed a preference of the mollusk for feeding and depositing eggs on *Conopeum seurati* (Canu, 1928) colonies rather than *Cryptosula pallasiana* (Moll, 1803) (Gomoiu and Skolka 1997). It tolerates a wide range of temperatures and salinities, but experimental studies showed that *C. obscura* has a reduced resistance in hypoxic conditions (Sagasti et al. 2001).

The crab *R. harrisii* is present in the Black Sea region since the 1930s, probably arrived by shipping from the Netherlands (Makarov 1939). The species is highly adaptable, living in waters of various salinities (preferable below 15PSU), temperatures and substrates (Băcescu 1967).

A few years after its arrival in the Black Sea in the 1960s, *M. arenaria* became the dominant species in the soft sediment associations at the Romanian littoral exceeding in some areas 1,500 g/m^2 (Gomoiu 1981; Gomoiu and Porumb 1969). The bivalve was reported from soft benthic sediments in the harbour, but only as small individuals, the estimated biomass being about 1 g/m^2 (Pecheanu et al. 2002).

Molgula manhattensis, a solitary ascidian, is commonly found in benthic communities in harbours. The species is resistant to polluted waters and tolerates various salinities and temperatures (Lambert and Lambert 1998).

The hydrozoan *Blackfordia virginica* was mentioned from the Black Sea since 1925 (Valkanov 1936). Even though Mills and Sommers (1995) consider the species native to the Black Sea, we consider it of North Atlantic origin similar to Zaitsev and Mamaev (1997) and Shiganova et al. (2005).

The striped barnacle, *B. amphitrite,* a widespread species found also in brackish waters, is a typical component of the fouling communities. The species is tolerant to pollution and other physical stress, being quite abundant in some harbours; for example, in Oostende *B. amphitrite* outcompetes *B. improvisus* (Kerckhof and Cattrijsse 2001).

Starting with September 2010, more than 900 specimens of *Anadara* sp. were sampled. It is possible to be *A. transversa* (Say, 1822) (syn. *A. demiri* Piani, 1981)*,* a bivalve recorded for the first time in the Mediterranean basin in Izmir harbour in the 1970s (Demir 1977). Our identification was based on morphological traits but due to the fact that only immature individuals were sampled so far, further investigations are necessary. The presence of *Anadara* sp. in our samples corresponded with a sharp decrease in abundance of *M. galloprovincialis* adults (Fig. 4). Juveniles of *M. galloprovincialis* are more resistant to thermic shock, but temperatures of 30°C are lethal for the adults (Mirza and Crăciun 1989). The juveniles of *Anadara* sp. were interspersed between *B. improvisus* shells, *M. lineatus* and *M. galloprovincials* valves. By reducing the surface occupied by dominant native species, high temperatures and low levels of dissolved oxygen facilitate in some cases the establishment and spread of aliens.

The species accumulation curves for the sampling sites do not reach a plateau suggesting that more species will be inventoried with increasing sampling effort. We anticipate that the increase in the species richness from additional sampling will be mostly due to natives since the estimated number of alien species is close to the observed one.

The situation of alien species in the harbours of the Black Sea is poorly known. Investigations carried out in Odessa harbour (Ukraine) emphasized the need for baseline standardized surveys in the Black Sea harbours (Alexandrov et al. 2004). Increased attention is given to ballast water as vector for alien species, but fouling on ship's hulls should not be neglected either. Information on species presence and abundance in harbours can improve predictions of invasion pathways and contribute to ranking of different vectors based on their importance. These aspects are equally important for the Black Sea basin, both as recipient and as donor region. Early detection of potentially invasive species is necessary for applying control/eradication measures and for testing the efficiency of such actions.

Conclusions

Given the fact that Constanţa harbour is a major transit hub, we investigated the structure of the benthic community on hard artificial substrate. The habitat selected for this study is dynamic and periodically subjected to extreme environmental conditions that destroy shallow benthic communities, leading to frequent recolonizations. Our results show that these communities are impoverished and unstable. As harbours are constant recipients of alien species, a good timing of arrival of these species increases the chances of establishment. Monitoring of benthic communities at small depths in harbours proved useful in the early detection of potentially invasive alien species. The selected habitat is easily accessible; the method is low-cost, and the samples represent reliable indicators of alien species establishment. We recommend it as a routine monitoring technique for harbours with a high transit.

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Appendix

See Table 5.

Table 5 Species identified in the samples (*S* = sampling site, 1 = presence, 0 = absence, *n* = 70 samples)

No.	Species	*S*1	*S*2	*S*3	Overall frequency (%)
1	*Mytilus galloprovincialis* Lamarck, 1819	1	1	1	100
2	*Mytilaster lineatus* (Gmelin, 1791)	1	1	1	100
3	*Cerastoderma edule* (Linnaeus, 1758)	0	1	1	21
4	*Anadara* sp.	1	1	1	11
5	*Mya arenaria* Linnaeus, 1758	1	1	1	10
6	*Hartlaubella (Obelia) gelatinosa* (Pallas, 1766)	1	1	1	14

Table 5 continued

No.	Species	*S*1	*S*2	*S*3	Overall frequency (%)
7	*Laomedea (Obelia) exigua* M. Sars, 1857	1	1	0	11
8	*Obelia longissima* (Pallas, 1766)	1	1	1	41
9	*Obelia* sp.	1	1	1	7
10	*Blackfordia virginica* Mayer, 1910	1	0	0	3
11	*Actinothoe clavata* (Ilmoni, 1830)	1	1	1	26
12	*Actinia equina* (Linnaeus, 1758)	1	1	1	54
13	*Diadumene lineata* (Verrill, 1869)	1	1	1	33
14	*Leptoplana tremellaris* (Müller OF, 1773)	1	1	1	54
15	*Convoluta albomaculata* (Pereyaslawzewa, 1892)	1	1	1	10
16	*Stylochus tauricus* Jakubova, 1909	1	1	0	4
17	Turbellaria varia	1	1	1	6
18	*Emplectonema gracile* (Johnston, 1837)	1	0	0	4
19	*Tetrastemma* sp.	1	1	0	3
20	Nemertea varia	1	0	0	1
21	*Tergipes tergipes* (Forskål, 1775)	1	1	1	14
22	Opisthobranchia varia	1	1	1	27
23	*Corambe obscura* (Verrill, 1870)	1	1	1	56
24	*Pusillina (Rissoa) lineolata* (Michaud, 1832)	1	1	1	10
25	*Alitta (Neanthes) succinea* (Leuckart, 1847)	1	1	1	67
26	*Hediste diversicolor* (O.F. Müller, 1776)	1	1	1	77
27	*Nereis rava* Ehlers, 1864	1	1	1	11
28	*Platynereis dumerilii* (Audouin & Milne-Edwards, 1834)	1	1	1	46
29	*Perinereis cultrifera* (Grube, 1840)	1	1	1	10
30	*Polydora* complex	1	1	1	59
31	*Polynoe scolopendrina* Savigny, 1822	0	1	0	3
32	*Sphaerosyllis bulbosa* Southern, 1914	1	0	0	1
33	*Harmothoe reticulata* (Claparède, 1870)	1	1	1	34
34	*Harmothoe imbricata* (Linnaeus, 1767)	1	1	1	11
35	*Ficopomatus enigmaticus* (Fauvel, 1923)	1	1	1	80
36	*Spirobranchus triqueter* (Linnaeus, 1758)	1	0	0	1
37	*Phyllodoce lineata* (Claparède, 1870)	1	1	1	17
38	*Polychaeta* varia	1	1	1	11
39	*Enchytraeus albidus* Henle, 1837	1	1	1	26
40	*Palaemon elegans* Rathke, 1837	1	1	1	17
41	*Athanas nitescens* (Leach, 1813)	1	1	1	40
42	*Rhithropanopeus harrisii* (Gould, 1841)	1	1	1	89
43	*Pilumnus hirtellus* (Linnaeus, 1761)	1	1	1	27
44	*Xantho poressa* (Olivi, 1792)	1	1	1	19
45	*Pisidia longicornis* (Linnaeus, 1767)	1	1	1	14
46	*Pachygrapsus marmoratus* (Fabricius, 1787)	1	0	0	1
47	*Stenothoe monoculoides* (Montagu, 1815)	1	1	1	66
48	*Melita palmata* (Montagu, 1804)	1	1	1	77
49	*Microdeutopus gryllotalpa* Costa, 1853	1	1	1	67
50	*Microdeutopus damnoniensis* (Bate, 1856)	1	0	0	1
51	*Dexamine spinosa* (Montagu, 1813)	1	1	1	47
52	*Echinogammarus olivii* (Milne-Edwards, 1830)	1	1	1	36
53	*Obesogammarus obesus* (G.O. Sars, 1894)	1	1	1	7
54	*Hyale pontica* Rathke, 1847	1	1	1	36

Table 5 continued

No.	Species	*S*1	*S*2	*S*3	Overall frequency (%)
55	*Synchelidium maculatum* Stebbing, 1906	1	0	1	7
56	*Synchelidium* sp.	0	0	1	1
57	*Ampithoe gammaroides* (Bate, 1856)	1	0	0	7
58	*Ampithoe ramondi* Audouin, 1826	1	0	0	1
59	*Corophium volutator* (Pallas, 1766)	0	1	0	1
60	*Corophium mucronatum* G.O. Sars, 1895	1	0	0	4
61	*Amathillina cristata* G.O. Sars, 1894	1	0	0	1
62	*Dikerogammarus villosus* Sowinsky, 1894	0	0	1	1
63	*Idotea balthica* (Pallas, 1772)	1	1	1	63
64	*Exosphaeroma pulchellum* Colosi, 1921	1	1	1	10
65	*Tanais dulongii* (Audouin, 1826)	1	1	1	73
66	*Balanus improvisus* Darwin, 1854	1	1	1	100
67	*Balanus amphitrite* Darwin, 1854	0	0	1	1
68	*Cryptosula (Lepralia) pallasiana* (Moll, 1803)	1	1	1	49
69	*Conopeum seurati* (Canu, 1928)	1	1	1	99
70	*Bowerbankia gracilis* Leidy, 1855	1	1	1	30
71	*Molgula manhattensis* (De Kay, 1843)	1	1	1	20
72	*Ctenicella* sp.	0	0	1	1
73	*Botryllus schlosseri* (Pallas, 1766)	1	1	1	49
74	*Chironomus* sp.	1	1	0	16

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