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ORIGINAL PAPERS

RECENT DATA ON ZOOBENTHIC NON- INDIGENOUS SPECIES PRESENT IN ROMANIAN PORT MARINE AREAS IN 2023

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Abstract. *Under the Marine Strategy Framework Directive (MSFD), Member States are required to report to the European Commission on the various stages of their marine strategies, assess the state of marine waters, determine the good ecological status and set the environmental objectives. Following Article 11 of the Directive regarding the need to update the monitoring programs, the Member States must establish and carry out the monitoring of the marine environment by applying these requirements. In order to respond to the requirements of the MSFD and to update the monitoring program for the qualitative pressure Descriptor D2- non indigenous species, in 2023 a monitoring of the Romanian port areas considered "hot spots" for the introduction of non-indigenous species was carried out to observe potential newly introduced species through maritime transport. The purpose of the paper is to present the results of the evaluation of the non-indigenous species present and identified in the three maritime port areas chosen for monitoring, such as Touristic Tomis Port, Constanța and Mangalia Ports. The investigations carried out on different types of habitats (collectors with artificial habitat and a system of biofouling plates) introduced into the sea led to the identification of 11 non-indigenous species from the total of 49 macrozoobenthic species that composed the benthic community present on the artificial substrate in the three port locations. The non-indigenous benthic species identified in 2023 are already established in the Black Sea basin, respectively near the Romanian coast, being periodically identified in populations with varying abundances.*

Keywords: MSFD, monitoring port areas, non-indigenous species, Romanian waters

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Introduction

In recent years, marine bio invasions have become one of the greatest global threats to the diversity and integrity of indigenous communities. These invasions are considered as factors of irreversible impact in the host environments, affecting at the same time the diversity and stability of native habitats [6].

Maritime transport and intentional introduction are the main vectors of introducing non-indigenous species into the Black Sea. Due to the semi-closed nature and low biodiversity of the Black Sea, several non-indigenous species represent threats to the native biota, the dispersion of these species being a dynamic and frequent process that may continue in the future [7].

Considering *Descriptor 2 - Non-indigenous species* of the MSFD, as biological pressure focuses on the assessment of the extent of pressure and impact of non-indigenous marine species introduced as a result of human activities, in relation to the main vectors and pathways of introduction into the marine environment, in 2023, for the first time, a specific strategy dedicated to the monitoring of non-indigenous species in maritime port areas was implemented to the Romanian coast [2].

Ports are considered as one of the most disturbed coastal ecosystems due to increasing anthropogenic pressures (shipping activities, pollution and dredging). In addition, harbors are considered the major entry ways for non-indigenous species due to their special characteristics being considered primary host regions where non-indigenous species are most likely to establish, mainly because an adequate diversity of habitats is found in these marine ecosystems.

The purpose of this paper is to provide updated information on the occurrence, presence and distribution of non-indigenous zoobenthic species in the Romanian port areas, in order to establish, in the near future, the ecological state of the port marine waters environment.

Material and methods

The monitoring of benthic macroinvertebrates included in the evaluation of *Descriptor 2 - Non-indigenous species* was carried out by collecting samples from areas considered at high risk of introducing non-indigenous species, such as maritime ports. In 2023, for the identification of non-indigenous species, the following port areas were investigated: Touristic Tomis Port, Constanta and Mangalia ports.

For sampling of vagile epifauna, such as crabs, shrimps and other organisms (polychaetes, amphipods, isopods, mysides, decapods), the artificial habitat collectors, connected to existing structures (buoys, docks), were used. Artificial habitat collectors are efficient at capturing smaller mobile fauna and consist of plastic boxes filled with either mussel shells (pre-sterilized in an autoclave or oven) or mixed contents (e.g. fragments of ceramic pots or tubes) (see Figure 1). The collectors are selective and therefore provide only relative measures of species abundance [11]. The collectors with artificial substrate were placed at an average depth of 1.5 m.

Fig. 1 - Recommended trap for sampling epifauna - collector with artificial habitat filled with: broken pots, decorative stones and pieces of rubber tubes (original photo, NIMRD).

For the inventory of fouling organisms, systems consisting of three fouling fixation plates were used, which provide a surface for colonization/adhesion of species with sessile behavior by attaching to these surfaces immersed in water for a longer period [11] (see Figure 2). These organisms can be collected by scraping with a special scraping tool.

Both artificial habitat collectors and fouling systems were recovered after a period of time (minimum 6 weeks) to allow time for organisms to colonize those artificial structures (see Figure 2-B; Figure 3). According to the standard methodology the collected samples were stored in plastic bags and buffered with formaldehyde 4%. In the laboratory, the macrozoobenthos samples were gently washed using a set of stainless steel gauze sieves with mesh sizes of 1.0 × 1.0 mm and 0.5 × 0.5 mm, in order not to damage the more fragile organisms.

After the organisms were identified and determined under the stereomicroscope, qualitative and quantitative parameters of abundance (ind/m^2) were calculated. All taxa were identified to the lowest possible taxonomic level (e.g., species) using specific identification keys, and all names were updated according to the World Register of Marine Species (WoRMS) (www.marinespecies.org).

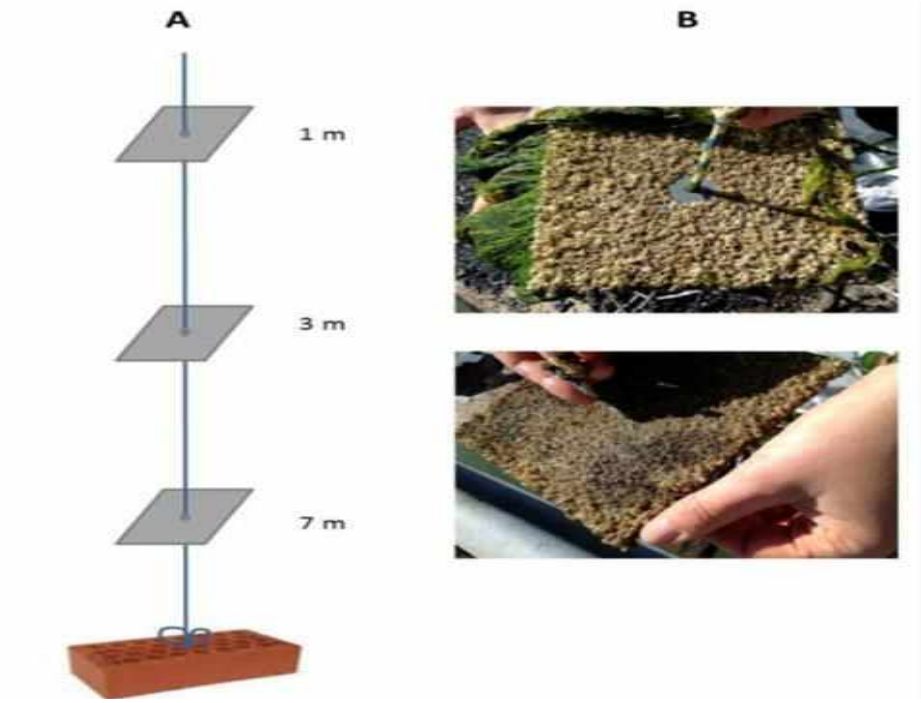


Fig. 2 - Suggested configuration for fouling plates (A) and recovered fouling plates (B) after six weeks of installation/immersion (B- original photo E. Pantea)

Fig. 3 - The image of a collector with artificial habitat after it has been removed from the water (original photo, E. Pantea)

The result of the evaluation was materialized in information regarding the presence of non-indigenous species identified in the analyzed period, in the three maritime port areas.

Results and discussion

Following the sample processing, 49 macrozoobenthic species were identified on the entire studied area belonging to the following groups: Polychaeta (27%), Malacostraca (37%), Mollusca (16%), and "Other groups" (20%), which are represented by Coelenterata, Tunicates, Hallacarida, Turbellaria, Bryozoans (see Table 1; Figure 4).

Table 1. List of macrozoobenthic species identified in port areas in 2023

Crt. no.	Species
1	*Actinia equina* (Linnaeus, 1758)
2	*Diadumene lineata* Verill, 1769
3	*Leptoplana tremellaris (*Muller,O.F.1773
4	*Stylochus tauricus* Jacubowa, 1909
5	*Alitta succinea* (Leuckart, 18470
6	*Capitella capitata* (Fabricius, 1780)
7	*Exogone naidina* Oersted, 1845
8	*Eteone picta* Quatrefages, 1866
9	*Eumida sanguinea* Oersted, 1843
10	*Fabricia stellaris,* Muller, 1774
11	*Ficopomatus enigmaticus,* Fauvel, 1923
12	*Platynereis dumerilii* (Audouin&Milne Edwards, 1833)
13	*Polydora cornuta* Bosc, 1802
14	*Streblospio shrubsolii* Buchanan, 1890
15	*Syllis gracilis* Grube, 1840
16	*Salvatoria clavata* (Claparede, 1863)
17	*Salvatoria limbata* (Claparede 1868)
18	*Lepidochitona cinerea* (Linnaeus, 1767)
19	*Corambe obscura* (A.E. Verrill, 1877)
20	*Chrysallida* sp.
21	*Embletonia pulchra* (Alder &Hancock, 1844)
22	*Arcuatula senhousia* (Benson, 1842)
23	*Anadara kagoshimensis* (Tokunaga, 1906)
24	*Mytilus galloprovincialis* Lamarck, 1819
25	*Mytilaster lineatus* Gmelin, 1791
26	*Amphibalanus improvisus* Darwin, 1854)
27	*Apherusa bispinosa* (Spence Bate, 1857)
28	*Stenothoe monoculoides* Montagu, 1813
29	*Condrochelia savignyi* (Kroyer, 1842)
30	*Hyale pontica* Rathke, 1836
31	*Melita palmata* Montagu, 1804
32	*Microdeutopus gryllotalpa* A. Costa, 1853

Crt. no.	Species
33	*Monocorophium acherusicum* A. Costa, 1853
34	*Nototropis guttatus* Costa, 1853
35	*Tanais dulongii* Audouin, 1826
36	*Siriella jaltensis* Czerniavsky 1868
37	*Athanas nitescens* Leach, 1814
38	*Crangon crangon* (Linnaeus, 1758)
39	*Palaemon adspersus* Rathke, 1836
40	*Eurypanopeus depressus* Smith, 1869
41	*Pilumnus hirtellus* Linnaeus, 1761
42	*Rhithropanopeus harisii* (Gould, 1814)
43	*Eriphia verrucosa* (Forskal, 1775)
44	*Rhombognathus magnirostris* (Trouessart, 1899)
45	*Botryllus schlosseri* Pallas, 1766
46	*Molgula manhattensis* De Kay, 1843
47	*Cryptosula pallasiana* (Moll, 1803)
48	*Conopeum seurati* Canu, 1928
49	*Membranipora* sp.

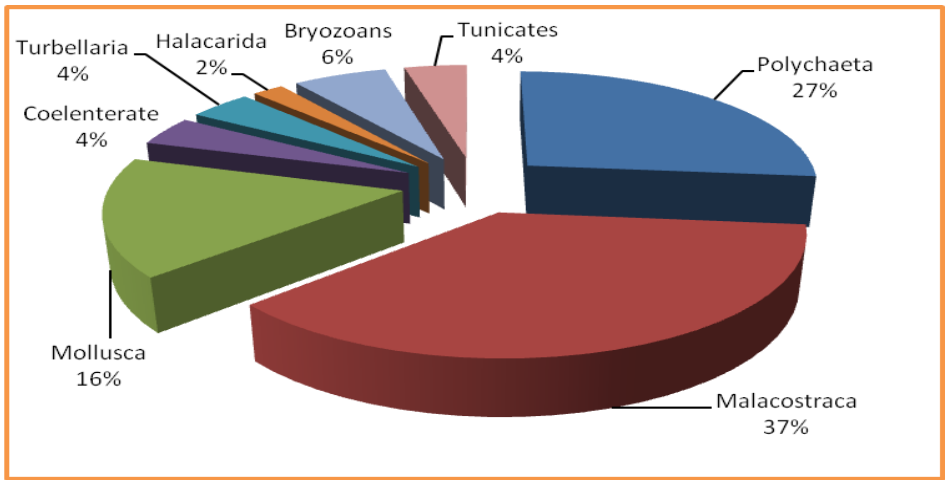


Fig. 4 – Distribution of benthic invertebrate's groups identified in port areas, 2023

From the total number of macrozoobenthic species (49) which constituted the benthic community present on the artificial habitat collectors and fouling plates in the studied locations, 11 non-indigenous species were identified, with a percentage of 18% (see Figure 5). The zoobenthic species identified as non-indigenous belong to the following taxa: Polychaeta (*Alitta succinea, Polydora cornuta, Ficopomatus enigmaticus*), Ascidiacea (*Molgula manhattensis*), Anthozoa (*Diadumene lineata*), Cirripedia (*Amphibalanus improvisus*), Decapoda

(*Eurypanopeus depressus, Rhithropanopeus harrissi*), Bivalvia (*Anadara kagoshimensis*, *Arcuatula senhousia*), gastropoda (*Corambe obscura*) (see Table 1). All the mentioned species are included in the list of non-indigenous species from the Romanian coast [1,8,9].

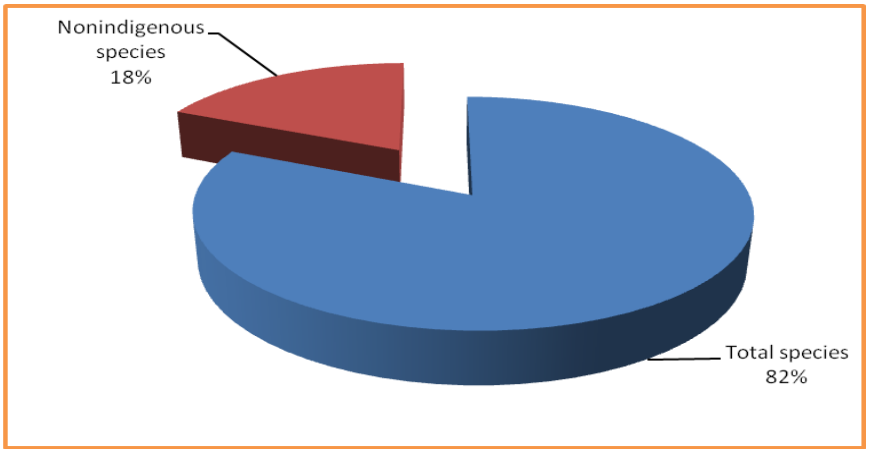


Fig. 5 - The percentage of occurrence of non-indigenous zoobenthic species in the harbor waters, 2023

The non-indigenous sessile zoobenthic species, which have successfully colonized the system of fouling plates immersed in port areas, have developed compact and numerous populations, such as the ascidian *Molgula manhattensis*, the cirriped, *Amphibalanus improvisus* and the tubiculous polychaete *Ficopomatus enigmaticus.* The highest abundance was recorded by *F. enigmaticus* in the Touristic Tomis port, registered an average value of 13,434 ind/m^2 forming compact populations, being also present in all sites, with lower abundances. Due to their special environmental requirements, *Ficopomatus* reefs are bound to exist only in limited and disjunct areas. Thus, as a neozoon species, *Ficopomatus* does not have an invasive behavior and does not pose a threat to native species [3].

Besides *Ficopomatus*, the other two sessile species populated all the fouling plates in the studied locations, but with a relatively lower abundance (see Figure 6). *M. manhattensis*, a sessile filter species, is usually present in harbours or near them, forming agglomerations of tens of individuals [3]. These fouling-forming species are considered already established in hard or artificial harbour substrate habitats.

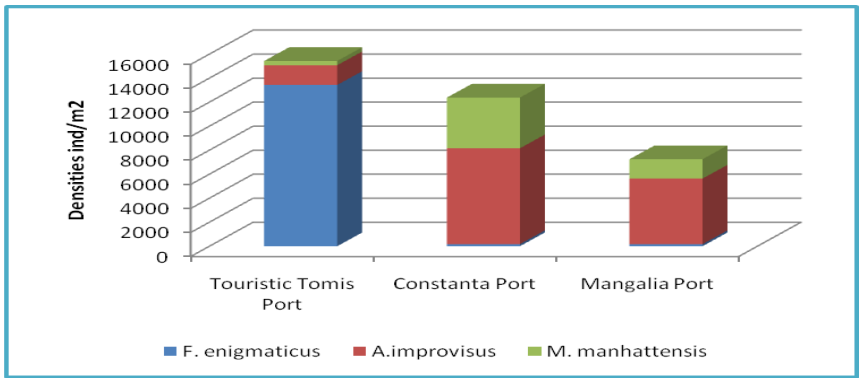
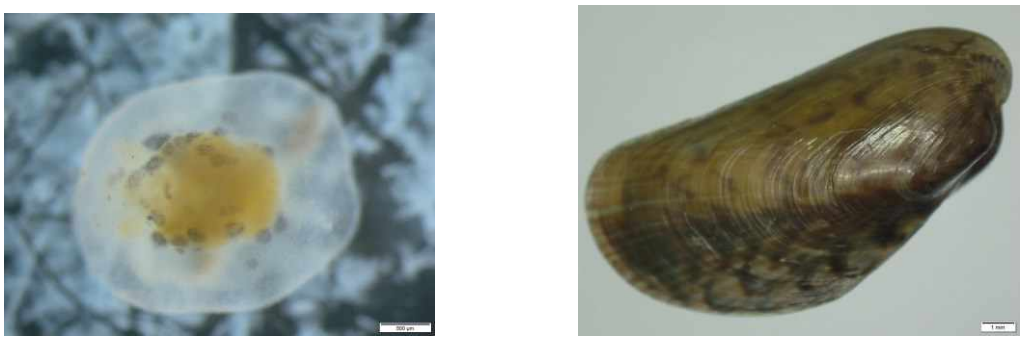


Fig. 6- The average densities of non-indigenous species on fouling plates

Non-indigenous species such as the decapods *Eurypanopeus depressus*, *Rhithropanopeus harrissi*, the polychaetes *Polydora cornuta, Alitta succinea*, which constitute the mobile epifauna were also widely distributed, being present in all investigated port locations.

The nudibranchiat predatory gastropod, *Corambe obscura*, a tolerant species both for salinity and temperature was more abundant in Constanta Port area, indicating the presence of a stable population, with an average abundance of 1, 320 ind/m^2 (see Figure 7 a). Feeding strictly on bryozoans, *e.g. Membranipora sp.*, *Conopeum seurati* which were present both on the fouling plates and the collectors with artificial substrate, *Corambe* developed numerous populations occupying an ecological niche insufficiently exploited by indigenous predator species.

Another non-indigenous species from the Bivalvia group was also identified in the analyzed samples, belonging to Mytilides family, namely, *Arcuatula senhousia*, known as the Asian mussel, an opportunistic, invasive species that can be found in habitats occupied by marine phanerogame, in the port area (see Figure 7, b). The bivalve has the qualities of an opportunistic species. It is very fertile with a long larval stage, during which the bivalve spreads over considerable distances. In addition, it has a rapid growth forming numerous agglomerations, building dense and extensive "carpets" of shells that are caught between them. These properties help *A. senhousia* to occupy new habitats by competing quickly and successfully with native species. With all these characteristic features of an invasive species, *A. senhousia* failed to form stable populations, being still in the stage of adaptation to the new conditions of the Black Sea, even if the species was reported at the beginning of the 21st century [4,5,10]. *A. senhousia* was present in the Constanta port area forming agglomerations of 2,112 ind/m^2 and Mangalia Port, in small quantities, 88 ind/m^2.



a.Corambe obscura (Verrill, 1877) b. *Arcuatula senhousia* (Benson, 1842)

Fig. 7 – Non –indigenous species, gastropod (a), bivalvia (b), in 2023 (original photo M. Teodorof)

The anthozoan *Diadumene lineata* was also identified on the fouling plates in the Constanta Port area forming agglomerations of 1,144 ind/m^2 being an extremely adaptable species in new habitats.

The decapods, *Rhitropanopeus harrissi* and *Eurypanopeus depressus* were caught in submerged collectors with artificial habitat in Constanta and Mangalia harbours, in small quantities, 4-14 individuals per sample, due to their greater mobility (see Figure 8, a,b).

*a.Rhitropanopeus harrissi (*Gould, 1814) (Smith, 1869) b. *Eurypanopues depressus*

Fig.8. – Non –indigenous species, decapods, in 2023 (original photo M. Teodorof)

The non-indigenous species identified in 2023 are already established in the Black Sea basin, respectively near the Romanian coast, being periodically identified in populations with varying abundances.

Conclusions

(1) A monitoring of maritime ports considered areas with a high risk of introduction of non-indigenous species was carried out.

(2) Samples were taken in three Romanian maritime ports, Touristic Tomis Port, Constanta and Mangalia Ports.

(3) From the total of 49 macrozoobenthic species identified in the port areas, 11 are non-indigenous species.

(4) The non-indigenous sessile zoobenthic species which colonized the fouling plates system developed compact and numerous populations, such as *Molgula manhattensis*, *Amphibalanus improvisus*, *Ficopomatus enigmaticus*.

(5) The decapods *Eurypanopeus depressus*, *Rhithropanopeus harrissi*, polychaetes *Polydora cornuta, Alitta succinea*, which constitute the mobile epifauna were widely distributed, being present in all investigated port locations.

(6) The non-indigenous polychaetes, *A. succinea* and *P. cornuta*, are periodically identified, inhabiting other habitats with sandy, silty substrate, at depths of over 20 m, forming abundant populations.

(7) The nudibranchiat gastropod, *Corambe obscura* was more abundant in Constanta Port area, indicating the presence of a stable population, with an average abundance of 1, 320 ind/m^2.

(8) *Arcuatula senhousia* was present in the Constanta port area, forming agglomerations of 2,112 ind/m^2 and Mangalia Port, in small quantities, about 88 ind/m^2.

(9) All the 11 non-indigenous species identified in 2023 are already established in the Black Sea basin, respectively near the Romanian coast, being periodically identified in populations with varying abundances.

(10) In order to know the impact of established non-indigenous species on the marine ecosystem, permanent long-term monitoring is necessary, as well as a series of extensive studies on the interrelationships with the other components.

Acknowledgment

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