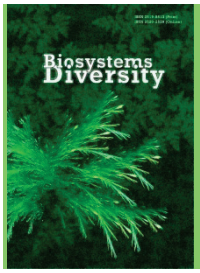

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Biofouling growth on plastic substrates: Experimental studies in the Black Sea

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Despite long-term research on marine litter there is still insufficient knowledge about benthic organisms associated with these substrates, especially experimental studies and methodology of sampling for complex biofouling assemblages. To predict the fate of plastic in the marine environment it is necessary to know how long the macrolitter can stay in different sea matrices and what are the steps of colonisation by marine organisms. The experiments were carried out during various seasons in situ in the north-western Black Sea coastal area. Three new types of the experimental constructions intended for different durations of exposure (1–10 months) were designed. This article is the first to present the methodology and the results of complex experiments investigating marine fouling (from microalgae to meio- and macrofauna) on plastic surfaces. Overall, 28 genera of microalgae, 13 major groups of meiobenthos and 36 species of macrofauna were found on plastic during the experiments. The microalgae fouling was mainly formed by representatives of genus *Cocconeis*. The species composition of microalgae was common for the research area. The average density and biomass of meiobenthos were the greatest on I construction type after 8 months of exposure. In the total macrozoobenthos biomass and density of Bivalvia and Crustacea dominated, respectively. The obtained results on the interaction between fouling organisms and plastic materials in the marine environment form an important contribution to the understanding of the "good ecological status" of the sea. Additional studies based on the tested methodology could be used as a component of ecological monitoring during development and implementation of the approaches of the Marine Strategy (descriptor 10).

Keywords: benthos; microalgae; meiobenthos; macrozoobenthos; marine litter; field studies; Marine Strategy.

Introduction

Plastic pollution of oceans, seas and continental water bodies raises increasing interest among the academic and environmental sector (Di Bartolo et al., 2021). To identify the sources of marine litter entering water bodies, the main efforts are currently focused on monitoring the pollution itself and quantifying the different types of marine litter, such as beach litter, floated litter and sea-floor litter (Galgani et al., 2013; Fleet et al., 2021). However, the problem of interaction of litter with living objects remains extremely understudied.

All objects that end up in the water environment become a substrate for hydrobionts. Therefore, plastic has become a new habitat for marine biota (Winston, 1982; Barnes, 2002; Aliani & Molcard, 2003). It is known that the diversity of microfilm on its surface is different in comparison to the microorganism communities in the outer water environment, which allows it to be distinguished as a "plastisphere" (Zettler et al., 2013). Moreover, plastic litter becomes a place of living not only for microorganisms. It is colonised by the range of multicellular organisms, in all water matrices. The rafting assemblages on floating litter (neuston litter) are documented the best (Póvoa et al., 2021) and counts 387 taxa at a global scale (Kiessling et al., 2015). The publications on dwellers on beach or benthic plastic litter are known to a minor extent (Subías-Baratau et al., 2022; Aytan et al., 2019). Regional studies show the high level of threat of plastic (as marine litter) to the diversity of marine areas, for example, 134 species in the Mediterranean were affected by marine litter (Deudero et al., 2015). Our previous studies in the north-western part of the Black Sea revealed 91 species as a part of the fouling on seafloor marine litter (Snigirova et al., 2020). Special attention is being paid to the impact of colonisers on sinking properties of the main components of plastic contamination – polyethylene and polypropylene (Pauli et al., 2017; Amaral-Zettler et al., 2021; Liu et al., 2022). There is a lot of evidence that large pieces of marine litter (Barnes, 2002; Kiessling et al., 2015; Maso et al., 2016) as well as microparticles (Jones et al., 2007; McCormick et al., 2014; Reisser et al., 2014) are a way for transportation and spreading of biota along the water pathways (Harrison et al., 2014). Understanding of processes of fouling formation on plastic may help in the study of the role of colonisers in the vertical transportation of the marine litter in the marine environment (Rummel et al., 2017).

Studies of biofouling on plastic have been intensifying recently, however the methodological approaches for these studies need to be developed and standardized (Eich et al., 2021; Póvoa et al., 2021). Most studies related to the formation of biofouling were set in laboratories and dealt with the microorganisms or microalgae (Tosin et al., 2012; Pinto et al., 2019; Lear et al., 2021). There is lack of biofouling studies that were conducted in natural environments (Oberbeckmann et al., 2016), and only scattered studies that include macroinvertebrate communities (Scanlon, 2021). To the best of our knowledge, there is no information of how the whole benthic community forms on plastic substrates, covering several steps of the succession – from microalgae- to meio- and macrobenthic communities. There is also lack of information on the experimental design organisation as itself. It is obvious that the organisation and design of the natural experiment depends on the specificity of the studied area, its hydrological regime and hydrodynamics. Taking this in account, the general methodological approach should be developed. Thus, the aim of this study is to represent the methodological approach for the complex study of biofouling communities on the plastic surfaces. This approach includes both the design of experimental constructions that can be used in natural seacoast environments, as well as the methods of sampling, processing, and quantifying of the fouling, including microphyto-, meio- and macrozoobenthic components.

Material and methods

The field experiments were conducted in the Gulf of Odessa in the area of the Malyi Fontan (Fig. 1) in different seasons during 2019–2021.

The three types of constructions were designed (Fig. 2) to study the biofouling formation. The constructions were installed in the sea at a depth of 3 m. The duration of exposure for the three constructions and the assemblages studied are shown in Table 1. The weekly data on temperature and salinity was provided by the Marine Research Station of the Odessa National I. I. Mechnikov University.

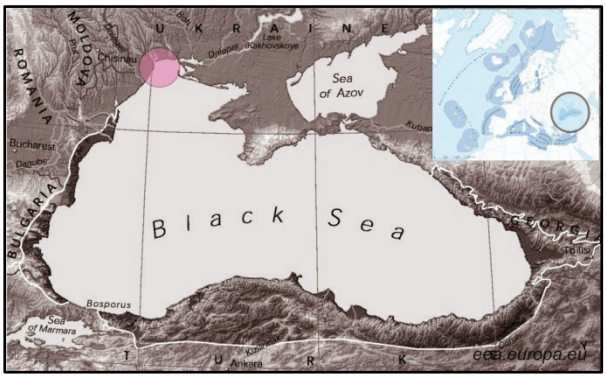

Fig. 1. Location of the field experiment in the Gulf of Odessa, the North-West Black Sea (47°48′06″ N; 29°31′09″ E)

I type of construction. The construction is composed of the cylinders cut from dark and transparent polyethylenterephthalat (PET) bottles. The parameters of the cylinders are as follows: a height of 10 cm, the diameters: dark PET (PETd) – 10 cm, and transparent (PETt) – 8.5 cm. The biofouling was studied both on the internal (PETt-in, PETd-in) and external (PETt-out, PETd-out) surfaces of the cylinders. Part of the cylinders was rubbed with sandpaper (P600 grit) to form a rough surface (PETr). Strips of the polyethylene (PE) and low density polyethylene (LDPE) were placed between the cylinders.

Table 1
Types of the experimental plastic constructions and duration of expositions in marine environment

Type	Start	End	Period, month	Community
I	November 2019	April 2020	5	microalgae
I	November 2019	July 2020	8	microalgae, meio-, macroozoobenthos
II	July 2020	September 2020	2	microalgae, meio-, macroozoobenthos
III	September 2020	October 2020	1	microalgae, meio-, macroozoobenthos
III	September 2020	July 2021	10	meio-, macroozoobenthos

II type of construction. In the centre of the construction a cube was placed, on which the bottles were screwed so that all the bottles were in the same plane. Dark and transparent PET bottles were used. Half of the bottles were rubbed with sandpaper as with the I type of the construction. The surface area of the bottle made up 940 cm^2.

III type of construction. The construction was made of a wooden frame (1.5 m long, 3 × 3 cm^2 thick). We fixed the 2 L volume bottles to the side surfaces of the frame in the horizontal plane. In addition, the elements with PET cylinders and PE strips similar to that one from construction I, were tied to the frame.

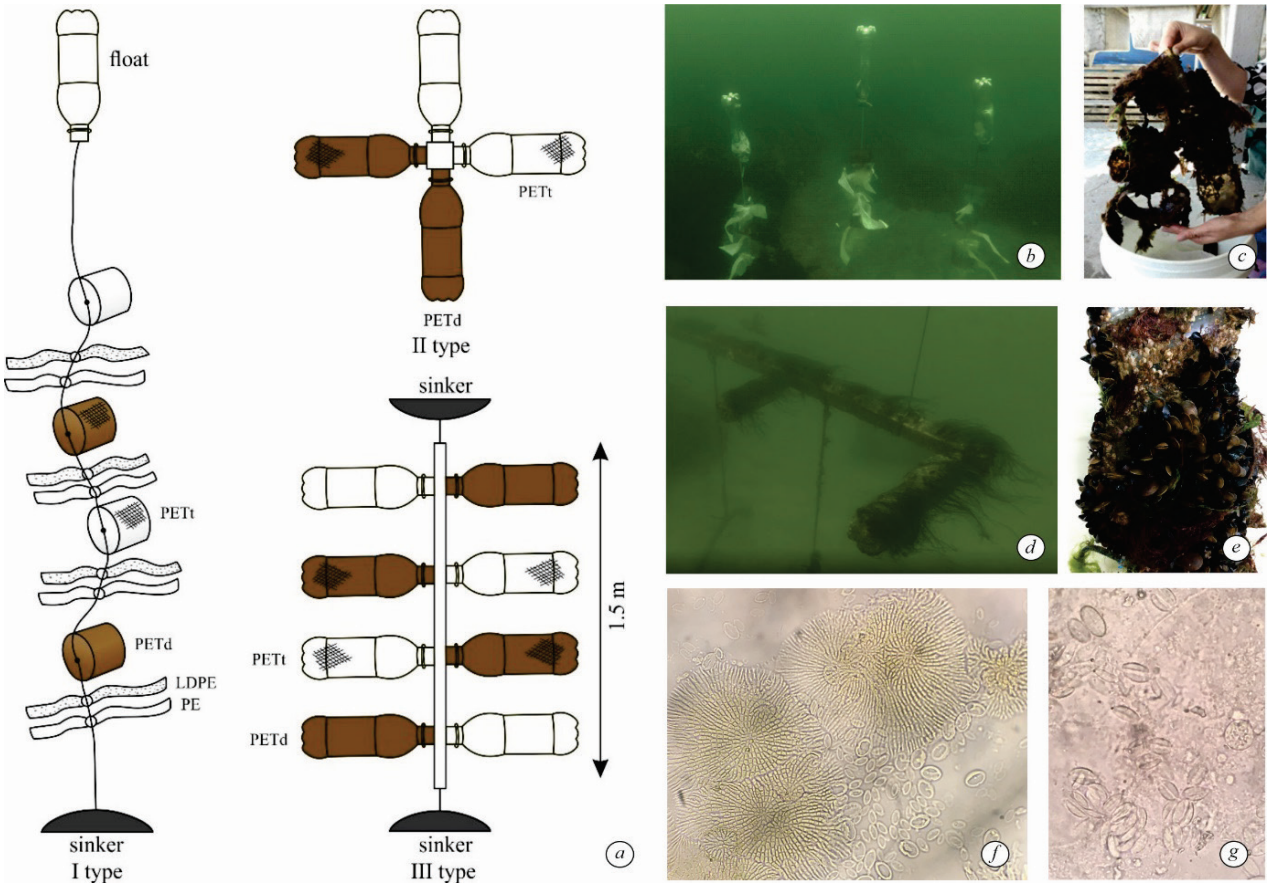

Fig. 2. Schemes and photos of constructions that were tested in the experimental polygon in the Gulf of Odessa in different seasons and exposures: *a* – construction schemes (PETd – dark polyethylenterephthalat, PETt – transparent, LDPE – low density polyethylene, PE – polyethylene); *b*, *c* – type I; *d* – type III; *e* – general view of macrofouling on the PET; *f*, *g* – microfouling represented by *Cocconeis*, *Amphora* and *Ulvella* spp.

The elements of the constructions were sampled by scuba diving. Under the water the samples were put separately into the polyethylene bags and then processed in the laboratory of the Institute of Marine Biology of the NAS of Ukraine. 18 samples were collected for meio- and macrozoobenthos analysis, and 34 samples for microalgae (24 PET, 6 LDPE, 4 PE). The sessile benthos from the construction elements` surface was scraped and washed through a system of sieves (1 mm upper to collect macrobenthic organisms) and then through the gas with mesh size 70 μm (for meio-

benthic organisms). Representatives of macrozoobenthos were not noted by the diver after the first exposure (5 months) and the constructions were examined only for microalgae.

Plastic experimental elements with microfouling were cut to subsamples (3 x 3 cm^2) and fixed with 96% alcohol solution with the addition of glycerol. The plastic subsamples with the microfouling were studied directly under the light microscope (Konus-Biorex-3, x160, x640 magnification). The calculation of the microalgae abundance was done directly on the plastic by choosing several counting sites. The quantity of the sites depended on the character of the biofouling and was no less than 15 ones per one subsample. The abundance of microalgae cells was determined per 1 cm^2. Biomass was calculated in accordance with the methods of Olenina et al. (2006) for real volume. For species identification and nomenclature of algae, we used the AlgaeBase (www.algaebase.org) and the following sources (Komárek & Anagnostidis, 1998, 2005; Witkowski et al., 2000).

Meiobenthos samples were preserved in 4% buffered formaldehyde solution and stained by Bengal Rose (Hullings & Gray, 1971). The meiobenthic organisms were calculated in a Bogorov chamber under stereomicroscope (×32 magnification). For meiobenthic taxa the density (thous. ind./m^2) and biomass (g/m^2) were calculated.

Macrozoobenthos samples were preserved in 4% formalin for further species identification. All benthic organisms were identified to the lowest possible taxonomic level and the density (N) and biomass (B) of each taxon was calculated to 1 m^2. The nomenclature of the zoobenthic taxa (meio-, macro-) is given according to the www.marinespecies.org.

The data on abundance of the microalgae community from all plastic constructions were fourth root transformed to normalise the data and similarity matrix was received for the community using the Bray-Curtis index. The hierarchical Cluster analysis, using the group average linkage option, with the SIMPROF test for the grouping of microalgae samples from different types of plastic was conducted (Clarke et al., 2014). For each average mean the standard error (SE) was calculated. We performed single-factor analysis of variance (ANOVA) and the Tukey test. The difference between the parameters from various exposures and type of constructions was considered significant when the probability of the difference was $P < 0.05$. The data were statistically analysed with the software Primer v7.0 (Primer-E Ltd, Plymouth, UK, 2016).

Results

Environmental parameters. Changes in salinity and temperature over the period of the experiments and the sampling time points are shown in Figure 3. Our experiment was designed to capture all the seasons in order to follow the stages of the fouling formations on the plastic. The variations of the measured parameters were in line with the usual course of salinity and temperature changes in the studied area.

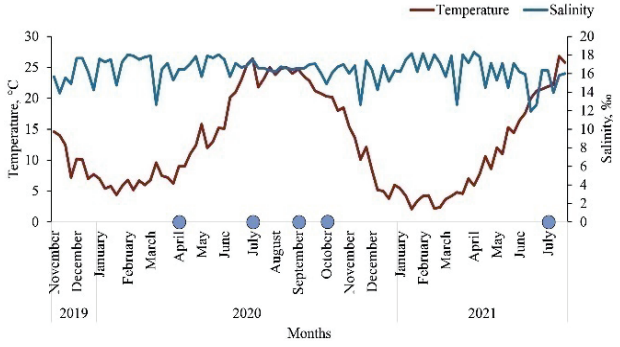

Fig. 3. Range of temperature and salinity during the field experiments with time of sampling indicating by circles on bottom axis

I type of construction, 5 months. A total of 18 species of microalgae were revealed on the experimental constructions of type I that were exposed for 5 months. Microfouling was formed mainly by diatoms (17 species). The species diversity of the polymers was comparable: 11 species were revealed on the PET, and 10 species revealed on the LDPE. The main component of the microfouling was represented by the complex of Cocconeis species (61.3 ± 17.3% of total average abundance), which formed mosaic spots on the surface of all types of plastic (Fig. 4). Some parts of the experimental surfaces were covered with a dense layer of the cells of these species. The other two representatives *Tabularia fasciculata* (18.4 ± 5.0%), *Amphora* sp. (9.0 ± 4.3%) and *Navicula* sp. (8.0 ± 4.0%) were lower in abundance but were common on almost all studied elements of the construction. *Grammatophora marina* and *Melosira moniliformis* were present only on PET cylinders, whereas *Synedra* sp. was noted only on LDPE (Table 2).

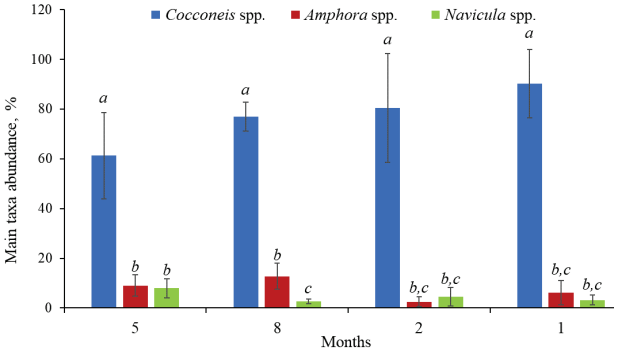

Fig. 4. The main taxa contribution to total abundance of microalgae on the surface of experimental constructions of three types during four exposures: 5 months exposure of the experiment, x ± SE, n = 11; 8 months exposure of the experiment, x ± SE, n = 16; 2 months exposure of the experiment, x ± SE, n = 5; 1 months exposure of the experiment, x ± SE, n = 15; the data were analysed with the Tukey test with Bonferroni correction; letters *a – c* indicate statistically significant differences in the abundance of microalgae within a group

Table 2
Microalgae taxa found on the plastic constructions during field experiment in the North-Western Black Sea

Phylum	Genus	Construction			
		Type I		Type II	Type III
		Exposure time, month			
		5	8	2	1
Cyanobacteria	*Jaaginema* sp.	0	0	1	0
	Merismopedia sp.	0	1	1	0
	Oscillatoria sp.	0	0	1	1
	Spirulina sp.	0	1	1	0
Bacillariophyta	*Achnanthes* spp.	1	1	0	1
	Amphiprora sp.	0	0	1	0
	Amphora spp.	0	1	1	1
	Climaconeis sp.	0	0	0	1
	Cocconeis spp.	1	1	1	1
	Coscinodiscus sp.	0	0	0	1
	Ceratoneis sp.	0	1	0	1
	Diploneis sp.	0	1	1	1
	Grammatophora sp.	1	1	0	1
	Halamphora spp.	1	1	1	1
	Hyalodiscus sp.	0	1	0	1
	Licmophora spp.	1	1	0	1
	Melosira sp.	1	0	0	1
	Navicula spp.	1	1	1	1
	Nitzschia spp.	0	0	0	1
	Parlibellus sp.	0	0	0	1
	Plagiotropis sp.	1	1	0	1
	Pleurosigma sp.	1	0	1	1
	Rhoicosphenia sp.	1	0	0	0
	Striatella sp.	0	1	0	0
	Synedra sp.	0	1	0	1
	Tabularia sp.	1	1	1	1
Chlorophyta	*Monoraphidium* sp.	1	0	0	0
	Ulvella spp.	0	1	1	1

Note: 0 – absence of the taxon; 1 – presence of the taxon.

The microalgae were observed on both sides of the cylinders, forming a layer of different intensity. The fouling of microalgae in the inner parts of the cylinders was much higher than on outer ones (Fig. 5). The abundance on the inner side differs by 17–30 times from that on the the outer side,

and the biomass in 9–36 times. It is likely that these differences are caused by better hydrodynamic conditions in the more protected parts inside the cylinders, which must be considered in the further planning of experimental constructions for the study of microalgae fouling.

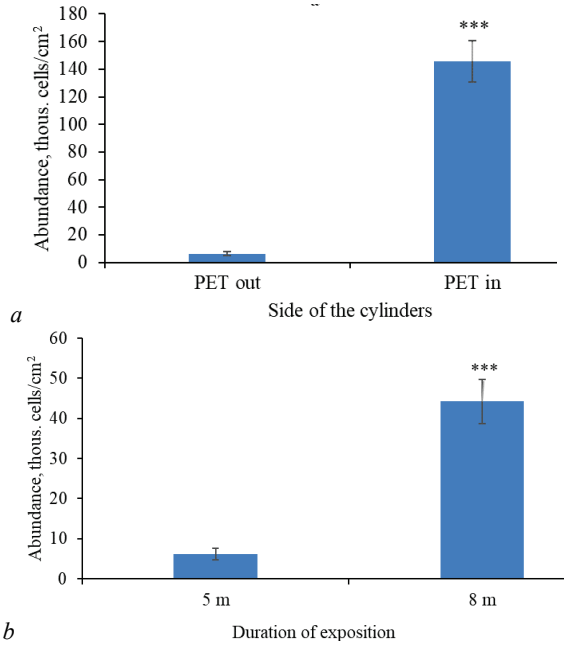

Fig. 5. The differences of microalgae abundance on the two sides of polyethylenterephthalat cylinders on the I type of experimental construction (*a*) and in two periods of exposure (*b*) (5 months exposure of the experiment x ± SE, n = 4; 8 months exposure of the experiment x ± SE, n = 8): PET out – external side of the polyethylenterephthalat cylinders; PET in – internal side of the cylinders; *** – P < 0.001

The microalgae assemblages grew more intensively on the LDPE compared with the outer parts of PET-cylinders. The lowest parameters were registered on the transparent cylinders PET-out (abundance 3.9 ± 1.8 thous. cells/cm^2; biomass 22.8 ± 13.2 mg/cm^2). We did not reveal any significant differences between the biofouling on transparent and dark, smooth or rough surfaces of the PET-cylinders. The cluster analysis shows the differentiation of three groups between the elements of the experiment in the construction I (Fig. 6). There is a distinct difference between the internal and external parts of the PET-cylinders. The LDPE surfaces had the higher values of microalgae abundance and were united with the internal parts of the cylinders (PET-in). The character of the surface (smooth or rough) or its colour did not match the significant influence on microfouling.

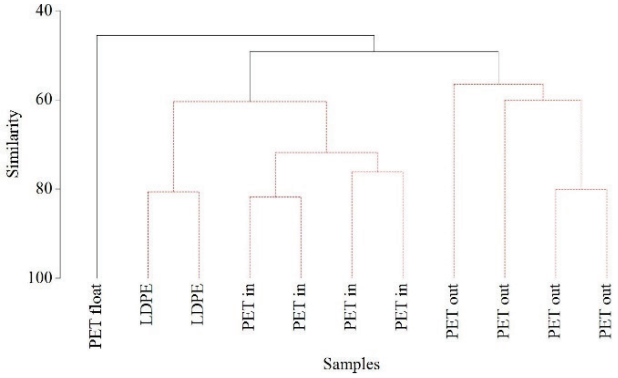

Fig. 6. Cluster analysis, based on resemblance matrix of Bray-Curtis similarity, representing the distribution of the microalgae abundance on polymer substrates on the construction type I after 5 months of exposure: PET float – float polyethylenterephthalat bottle of the construction; PET out – external part of the cylinders; PET in – internal part of the cylinders, LDPE – low density polyethylene. Red dot lines represent significant division on cluster proved by the SIMPROF test

The biofouling on PET-float was formed most actively (abundance 87.9 ± 37.1 thous. cells/cm^2; biomass 151.9 ± 67.9 mg/cm^2), probably because of the best light conditions, as it was located in a water layer close to the surface.

I type of construction, 8 months. A total of 24 species of microalgae were found on the experimental constructions of type I that were exposed for 8 months. Three groups were represented: Bacillariophyta (20 species), Cyanoprokaryota (3 species), Chlorophyta (1 species) (Table 2). The microalgae fouling was mainly formed by diatoms. In this experiment the dominants were also represented by the same species group, mainly by *Cocconeis* spp. (76.9 ± 5.7% of total average abundance), which covered the surfaces of all types of polymers with an entire layer. The following species also contributed on a large scale: *Amphora* sp. (12.7 ± 5.2%), *Navicula* sp. (2.6 ± 0.9%), *Tabularia fasciculata* (1.9 ± 0.5%), *Grammatophora marina* (2.1 ± 0.9%, Fig. 4). They were present on all elements of the construction. The greatest species diversity was found on transparent rough PET (15 species), the lowest was found on LDPE (9 species).

The total abundance of microalgae on PET cylinders was much higher after 8 months of exposure that at 5 months (Fig. 5b). The lowest abundance was found on PE and made 19.0 thousand cells/cm^2, the largest was 110.4 thousand cells/cm^2 on LDPE. The average values of abundance of microalgae were comparable on LDPE and PET-float. There was no significant difference in abundance of microalgae on various PET elements. LDPE has some of the highest rates, 3.4 times higher than PE and 1.4–1.8 times higher than PET.

The 4 permanent higher meiobenthos taxa, which included Nematoda, Harpacticoida (Copepoda), Ostracoda and Halacaridae, were registered on the I type plastic construction after 8 months of exposure (Table 3). Among the temporary meiobenthos presence of 7 taxa larvae was recorded: Oligochaeta, Polychaeta, Bivalvia, Cyrripedia, Amphipoda, Isopoda and Insecta. The average means of meiobenthos density and biomass on this type of construction were the largest, which made up 1124 ± 279 thous. ind./m^2 and 11.5 ± 2.3 g/m^2 respectively. Temporary taxa of meiobenthos prevailed. Their average density was 945 ± 262 thous. ind./m^2 and the average biomass was 10.0 ± 3.0 g/m^2 (Fig. 7). On this type of construction Bivalvia made up the largest percent contribution to the average density of the total meiobenthos, which were 64.4 ± 7.3% (Fig. 8a). The average biomass was formed mainly by Polychaeta, the percentage of which was 48.3 ± 7.6% (Fig. 8b).

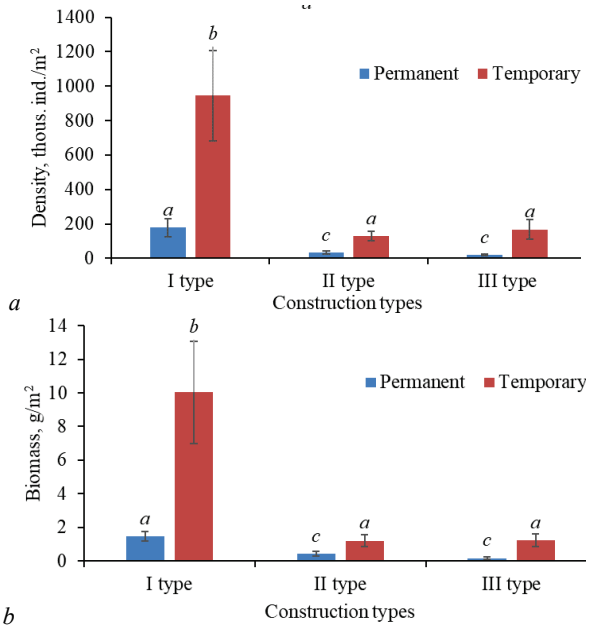

Fig. 7. The ratio of permanent and temporary meiobenthos taxa average density (*a*) and biomass (*b*) in the fouling of plastic substrate on three types of constructions: construction type I (8 months exposure of the experiment, x ± SE, n = 8); construction type II (2 months exposure of the experiment, x ± SE, n = 5); construction type III (1 month exposure of the experiment, x ± SE, n = 3); see Fig. 4

Table 3
List of meiobenthos higher taxa noted on the three types of plastic constructions during four exposures in the north-western Black Sea

Taxa	Construction			
	type I	type II	type III	
	exposure time, month			
	8	2	1	10
Amphipoda	1	1	0	1
Bivalvia	1	1	1	1
Cyrripedia	1	1	1	1
Gastropoda	0	1	1	1
Halacaridae	1	0	1	1
Harpacticoida	1	1	1	1
Insecta	1	1	0	0
Isopoda	1	0	0	1
Nematoda	1	1	1	1
Oligochaeta	1	1	1	0
Ostracoda	1	1	1	1
Platyhelminthes	0	0	1	0
Polychaeta	1	1	1	1

Note: 0 – absence of the taxon; 1 – presence of the taxon.

The macrozoobenthos community consisted of 21 taxa in July, after the 8 months of exposure of construction type I. The largest number of species was represented by crustaceans (Fig. 9). The average density of macrozoobenthos was 8.6 ± 2.1 thous. ind./m^2, biomass – 1.749 ± 0.554 kg/m^2. The main component of the abundance was formed by crustaceans and molluscs, whereas the main component of biomass was formed by molluscs. *Mytilus galloprovincialis* Lamarck, 1819 (4.2 ± 1.5 thous. ind./m^2) and *Amphibalanus improvisus* (Darwin, 1854) (2.3 ± 0.5 thous. ind./m^2) predominated; the mussels dominated in biomass (1.650 ± 0.534 kg/m^2). The fouling was dominated by individuals with a 5–20 mm shell length. The maximum length did not exceed 30 mm.

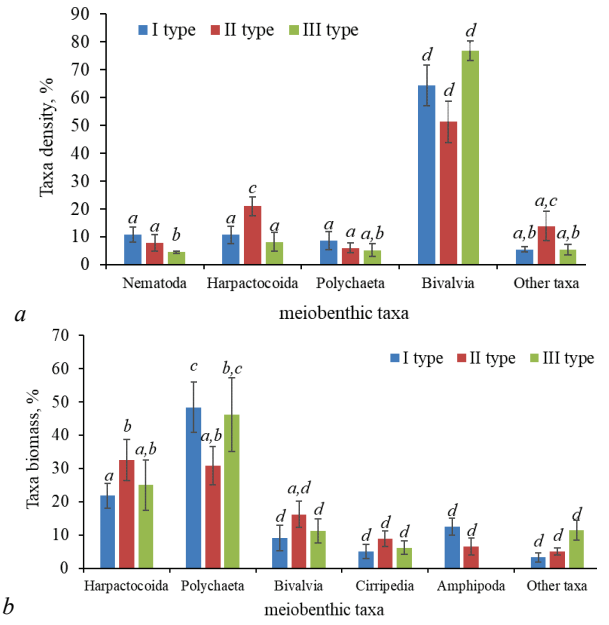

Fig. 8. Percentage of different taxa in the total meiobenthos density (*a*) and biomass (*b*) in the fouling of plastic substrate on three types of constructions (x ± SE): construction type I (8 months exposure of the experiment, n = 8); construction type II (2 months exposure of the experiment, n = 5); construction type III (1 month exposure of the experiment, n = 3); see Fig. 4

II type of construction, 2 months. The microalgae associations were represented by 14 species from 12 genera after 2 months exposure on the construction type II. Elements with dark and transparent PET were used in this experiment. The abundance varied from 1.5 up to 41.8 thous. cells/cm^2 (on average 21.7 ± 6.6 thous. cells/cm^2). The average biomass reached 34.6 ± 12.1 mg/cm^2. The 80.5 ± 21.9% of the abundance was presented by *Cocconeis* species that covered the plastic directly. The species from *Navicula* and *Amphora* genera accounted for around 4.5 ± 3.7% and 2.5 ± 1.9% each. 4 species from Cyanobacteria made up 12.1 ± 11.0% of abundance. Other species made up less than 1%. No significant variation was found on the transparent or dark PET.

Three permanent (Nematoda, Harpacticoida (Copepoda) and Ostracoda) and six temporary (Oligochaeta, Polychaeta, Bivalvia, Gastropoda, Cyrripedia and Amphipoda) meiobenthos taxa were registered on the II type of plastic construction (Table 3). The average density was the lowest among other construction types and made up 165 ± 37 thous. ind./m^2. The average biomass of the total meiobenthos was 1.6 ± 0.4 g/m^2. The average density of the temporary taxa was 131 ± 28 thous. ind./m^2 and the biomass was 1.2 ± 0.4 mg/m^2 (Fig. 7). On the II type of construction 51.3 ± 7.4% of meiobenthic assemblage density was represented by Bivalvia and 21.1 ± 3.4% by Harpacticoida (Fig. 8a). The total meiobenthos biomass was formed mainly by Harpacticoida (32.6 ± 6.1%) and Polychaeta (30.8 ± 5.7%, Fig. 8b).

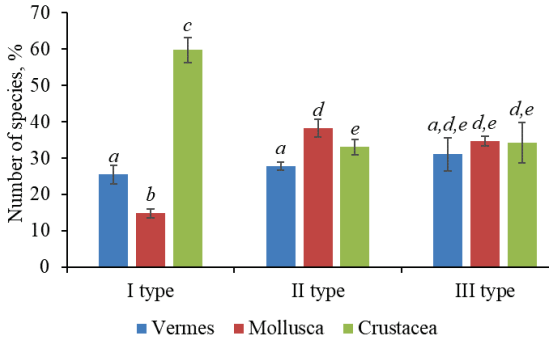

Fig. 9. The ratio of number of species for main marozoobenthos taxa in the fouling of plastic substrate on three types of constructions (x ± SE): *a* – construction type I (8 months exposure of the experiment, n = 8); *b* – construction type II (2 months exposure of the experiment, n = 5); *c* – construction type III (1 month exposure of the experiment, n = 3); see Fig. 4

After 2 months of exposure (August–September), 26 species of macrozoobenthos were present in the fouling of the plastic construction of II type (Table 4). The crustaceans dominated in the number of species. The average density of macroinvertebrates was 38.9 ± 3.7 thous. ind./m^2, biomass – 0.969 ± 0.077 kg/m^2. In this experiment, the dominant species in abundance and biomass of macrozoobenthic taxa was presented by *A. improvisus* (20.8 ± 1.6 thous. ind./m^2, and 0.702 ± 0.050 kg/m^2). Among mytilids, plastic was actively colonized by *Mytilaster lineatus* (Gmelin, 1791) (10.9 ± 2.3 thous. ind./m^2), the young specimens of *M. galloprovincialis* were absent. High abundance and biomass of the detritivore polychaete *Alitta succinea* (Leuckart, 1847) were revealed: 2.0 ± 0.2 thous. ind./m^2 and 0.015 ± 0.003 kg/m^2, respectively. Only in this experiment (two months exposure on the construction type II) were such invasive species as *Polydora cornuta* Bosc, 1802, *Corambe obscura* (A. E. Verrill, 1870), *Arcuatula senhousia* (Benson, 1842) registered. The young of the bivalve mollusc *Anadara kagoshimensis* (Tokunaga, 1906) was present in significant quantities, its abundance reached 3.1 ± 0.4 thous. ind./m^2, biomass – 0.170 ± 0.025 kg/m^2.

III type of construction, 1 month. After 1 month of the exposure of construction III (on PET cylinders and PE) we observed 29 species from 20 genera. Two types of plastic were used in this experiment – PET and PE – with different surface types (rough and smooth). The average abundance and biomass had the following values: 40.8 ± 8.8 thous. cells/cm^2 and 1.0 ± 0.3 mg/cm^2, respectively. The most common species were *Cocconeis* spp. (90.2 ± 13.7%), *Navicula* spp. (3.2 ± 3.9%) and *Amphora* sp. (6.3 ± 4.8%). *Achnanthes* cf. *lyrata* made a greater input than most other species, whose contribution was less than 1%.

5 permanent meiobenthos taxa were registered on the III type constructions after 1 month of exposure: Nematoda, Harpacticoida (Copepoda), Ostracoda, Halacaridae and Platyhelminthes (Table 3). From the temporary meiobenthos 5 taxa were noted: Oligochaeta, Polychaeta, Bivalvia, Gastropoda, Cyrripedia. The average means of the total meiobenthos density and biomass were 189 ± 43 thous. ind./m^2 and 1.4 ± 0.3 g/m^2 respectively. The average density of temporary meiobenthos was

168 ± 55 thous. ind./m^2 and biomass – 1.2 ± 0.4 g/m^2 (Fig. 7). The highest percent contribution to the total density was made by Bivalvia, whose percentage was 76.8 ± 3.4% (Fig. 8a). The average total meiobenthos biomass index was formed by Polychaeta and Harpacticoida, whose percentages were 46.1 ± 11.1% and 25.0 ± 7.5% respectively (Fig. 8b).

13 macrozoobenthos taxa were registered in the community that was formed in October after 1 month exposure on the construction type III. Their average density and biomass were 2.2 ± 1.6 thous. ind./m^2 and 0.115 ± 0.079 kg/m^2, respectively. As in the previous experiment, exhibited during August–September, *A. improvisus* dominated by density and biomass, accounting for 52.8% and 91.3% of the total, respectively. The density of *M. lineatus* was 6.4 ± 4.6 thous. ind./m^2. During the period of the last exposure the larvae of *M. galloprovincialis* settled, the length of the shells of its juveniles in the community did not exceed 5 mm, and the density reached 2.7 ± 2.4 thous. ind./m^2.

Table 4

List of macrofauna taxa found on the three types of plastic constructions during four exposures in the North-Western Black Sea

Phylum	Class	Species	Construction			
			Type I	Type II	Type III	
			Exposure time, month			
			8	2	1	10
Platyhelminthes	–	–	1	1	1	1
Annelida	Polychaeta	*Alitta succinea* (Leuckart, 1847)	1	1	1	1
		Harmothoe imbricata (Linnaeus, 1767)	0	1	0	1
		Harmothoe reticulata (Claparède, 1870)	1	1	0	1
		Mysta picta (Quatrefages, 1866)	1	1	0	1
		Nereis zonata Malmgren, 1867	0	0	0	1
		Perinereis cultrifera (Grube, 1840)	1	0	0	1
		Platynereis dumerilii (Audouin & Milne Edwards, 1833)	1	1	1	1
		Polydora cornuta Bosc, 1802	0	1	0	1
Mollusca	Gastropoda	*Bittium reticulatum* (da Costa, 1778)	0	1	0	1
		Corambe obscura (A. E. Verrill, 1870)	0	1	0	1
		Rissoa membranacea (J. Adams, 1800)	0	1	1	1
	Bivalvia	*Anadara kagoshimensis* (Tokunaga, 1906)	0	1	0	1
		Arcuatula senhousia (Benson, 1842)	0	1	0	1
		Mytilaster lineatus (Gmelin, 1791)	1	1	1	1
		Mytilus galloprovincialis Lamarck, 1819	1	0	1	1
		Parvicardium exiguum (Gmelin, 1791)	0	1	0	1
Arthropoda	Thecostraca	*Amphibalanus improvisus* (Darwin, 1854)	1	1	1	1
	Malacostraca	*Athanas nitescens* (Leach, 1814)	0	0	0	1
		Brachynotus sexdentatus (Risso, 1827)	0	1	0	1
		Palemon elegans Rathke, 1836	1	0	0	1
		Idotea balthica (Pallas, 1772)	1	1	1	1
		Stenosoma capito (Rathke, 1836)	1	0	1	1
		Apohyale perieri (Lucas, 1846)	0	0	0	1
		Ampithoe ramondi Audouin, 1826	1	1	0	1
		Chaetogammarus olivii (H. Milne Edwards, 1830)	0	1	0	1
		Crassicorophium bonellii (H. Milne Edwards, 1830)	1	1	0	1
		Dexamine spinosa (Montagu, 1813)	1	1	1	1
		Ericthonius difformis H. Milne Edwards, 1830	1	1	0	1
		Gammarus subtypicus Stock, 1966	0	1	0	1
		Melita palmata (Montagu, 1804)	1	1	0	1
		Microdeutopus gryllotalpa Costa, 1853	1	1	1	1
		Microprotopus longimanus Chevreux, 1887	1	0	0	1
		Plumulojassa ocia (Spence Bate, 1862)	1	0	0	1
		Stenothoe monoculoides (Montagu, 1813)	1	0	0	1
Chordata	Ascidiacea	*Molgula euprocta* (Drasche, 1884)	0	1	0	1

Note: 0 – absence of the taxon; 1 – presence of the taxon.

III type of construction, 10 months. At the end of 10 months exposure of the III type construction the data on meiobenthos and macrozoobenthos were received only from two samples. We registered such permanent meiobenthos taxa as Nematoda, Harpacticoida (Copepoda), Ostracoda and Halacaridae (Table 3). Larvae of the Polychaeta, Bivalvia, Gastropoda, Cyrripedia, Amphipoda and Isopoda were noted among the temporary meiofauna. The average density of the total meiobenthos was 312 thous. ind./m^2. The average biomass of all registered meiobenthos taxa on this construction type made up 4.3 g/m^2. The average density of permanent taxa was 292 thous. ind./m^2, which made up 93.4% of the total meiobenthos. In the biomass permanent taxa made up 88.6%, which was 3.8 g/m^2. The density of the meiobenthos assemblages was dominated by Harpacticoida (74.6%) and Nematoda (16.6%), the percentage contributions of which were the highest. The biomass of the meiobenthos was formed mainly by harpacticoid copepods, the percentage of which was 87.2% of the total.

According to available data, the average abundance of macrozoobenthos reached 0.06 thous. ind./m^2, biomass – 11.952 kg/m^2. 15 macrozoobenthos taxa were present in the fouling. In terms of abundance and biomass, *M. galloprovincialis* dominated – 80.1% and 97.6%, respectively. The maximum length of the individuals did not exceed 35 mm, juveniles up to 5 mm long were present in the settlement. 80.5% of the population were individuals with a shell length of 5–20 mm.

Discussion

The *Cocconeis* genus formed the 76–90% of the abundance on all the studied constructions. Together with other two genera *Amphora* and *Navicula*, which were present almost on all elements and made up 3.3–10.7% and 3.3–10.8%, respectively, this taxon is most often registered on the polymer surfaces (Reisser et al., 2014; Maso et al., 2016; Esensoy et al., 2020; Snigirova et al., 2020; Ryabushko et al., 2021; Du et al., 2022). Other researchers from the Northern Black Sea Region mentioned that *Ceratoneis closterium* and *Nitzshia sigma* made up 100% occurrence of diatom species (Ryabushko et al., 2021) and that the species from *Navicula*, *Halamphora*, *Nitzschia*, *Licmophora* genera form groups, regardless of the type of plastic (Ryabushko et al., 2021). Probably these differences are conditioned by different localities and methodological approaches to

processing the samples. The place of the deployment of the experimental constructions (on the bottom or in pelagic area) might affect the species composition of the fouling communities (Maso et al., 2016).

During the study of microalgae fouling, the necessity of direct observation using light microscopy is highlighted (Oberbeckmann et al., 2016). This approach gives the possibility to observe the structure of the microalgae communities and to reveal those species that attach directly to the plastic and most likely participate in processes of its degradation. Understanding of the participation of diatoms in the process of degradation of polymers that compose up to 80% of marine litter, is very relevant for developing the new technologies with combination synthesis of diatom algae and polymers (Sapozhnikov et al., 2020).

To the best of our knowledge this study presents the first data concerning meiobenthos on the plastic substrates during experimental conditions. Meiobenthos taxa widely inhabit both natural and artificial substrates in the North-Western Black Sea (Vorobyova, 1999; Vorobyova et al., 2016). In the Gulf of Odesa during our previous studies we analyzed the diverse meiobenthic assemblages associated with marine plastic litter of two types (PET and PE) (Snigirova et al., 2020; Uzun & Portianko, 2021). These were first steps to developing the methodological approach for the studies of meiobenthic organisms as a fouling component of plastic substrates.

In this study the taxonomic structure of meiobenthos on the different plastic constructions was similar to those on plastic marine litter, with some peculiarities (Snigirova et al., 2020). The average density of the meiobenthos on I and II plastic construction types during experiments was 3–4 times higher than on plastic litter, while biomass was almost the same, which is explained by a high presence of the lightweight permanent meiobenthic taxa. The average density and biomass on the III construction type are much higher than on litter, however a few times lower than those on the artificial substrate of protective coastal constructions (concrete piers, breakwaters, traverses, etc.) in the Gulf of Odesa (Vorobyova et al., 2016).

In terms of macrozoobenthos in the conditions of the north-western part of the Black Sea, in the experiments carried out on all types of constructions, the plastic substrate was most intensively settled by bivalve molluscs and *A. improvisus*. Over a relatively long period of time (8 and 10 months), a fouling community of macrozoobenthos was formed with the dominance of *M. galloprovincialis*. During shorter periods of exposure (1 and 2 months) in the summer-autumn period of 2020, *A. improvisus* and *M. lineatus* quantitatively dominated in the fouling macrozoobenthos community. The juvenile Mediterranean mussel appeared in the experiment in October. In the coastal zone of the Black Sea, as a rule, two peaks of reproduction of *M. galloprovincialis* are observed – spring and autumn, reproduction of *M. lineatus* occurs once a year in August–September (Zaika et al., 1990). It is known that the formation of fouling is affected by the reproduction of hydrobionts included in its composition and the features of the spatial distribution of larvae, their survival during the period of planktonic life (Aleksandrov, 2008).

Among marine litter fouling, Bryozoa, Hydrozoa, Ascidia, Cirripedia, Serpulidae, Bivalvia, which are known to foul on different types of artificial substrates, were the most numerous among attached organisms (Aliani & Molcard, 2003; Bravo et al., 2011; Pauli et al., 2017; Mancini et al., 2021). Bryozoans, sea anemones and barnacles, as well as calcareous tube worms, colonize solid substrates very quickly (Scanlon, 2021). Moreover, bryozoans are considered to be one of the most numerous colonizers of marine litter (Mancini et al., 2021), among which macrozoobenthos organisms colonize plastic litter most intensively (Mancini et al., 2021). Researchers note that the composition of marine litter fouling is formed by epibionts of dominant macrophytes in the study area and species that form coastal fouling communities (Aliani & Molcard, 2003; Pauli et al., 2017). In our studies, the composition of fouling on plastic substrates was formed by typical fouling species of natural stone substrate and artificial coastal protective structures. Among the colonisers *M. galloprovincialis* and *A. improvisus* are functional dominants of the fouling community in the coastal zone of the Gulf of Odesa (Aleksandrov, 2008).

The results of the experiments show that the design of the constructions should be resistant to the influence of abiotic factors of the environment and convenient for use at different depths. Because of different degrees of illumination, biofilm formation processes take place with different intensities. Further colonization by mobile benthic organisms and the possibility of their living on the substrate will be related to the degree of fouling and the architecture of the biofilm (Huang et al., 2007; Hadfield, 2011). The construction of the types I and III were good for long-term exposure of up to 10 months. The design of construction II is not reliable for the long periods of deployment. Moreover, each structure should be fixed at a distance from each other, so there should be enough space on the experimental polygon and fixing devices. Therefore, such structures can be used only for short-term exposure purposes (up to two months).

These experimental studies showed that the results depended on the duration of exposure. In our studies it was noticed that the microalgal communities were more diverse in shorter experiments. Our previous experiments on microalgal associations on polymers also prove this (Kapshyna et al., 2021). This is also proven in microbiological experimental studies in nature when the microbial compositions differed on various conditions and different types of succession. It is said in Pinto et al. (2019) that the variation of the composition of microorganisms was stronger in earlier deployment periods that in later ones. On the other hand, long-term experiments may help to reveal more contrasts for communities on different substrates, as was shown for mature biofilms that were exposed in marine environments for 12 and more months (Kirstein et al., 2018, 2019). The duration of the experiments is meaningful also in research that is aimed at studying the degradation of plastic (Harrison et al., 2014; Kirstein et al., 2019).

The application of the PET and PE in our experiments is explained by their suitability (thickness, transparency) to be used under a light microscope. On the other hand, these are the most common polymers that are spread in the ocean as marine litter (Subías-Baratau et al., 2022) and are often used in the experimental studies (Pinto et al., 2019). It is also mentioned that PET meets the best properties for the biofouling growth in comparison with PE and PP (polypropylene) (Subías-Baratau et al., 2022). The highest diversity has been observed on PET in the studies that were set in the Black Sea region (Esensoy et al., 2020; Snigirova et., 2020). For future experiments, other types of the soft polymers (HDPE, PP, etc.) can be easily added to the construction.

The results that were obtained in the present study will become the basis for the research strategy of plastic colonisation in the region. Depending on the aim of the study, the synchronisation of the deployments of three types of the constructions is planned. Besides, it is necessary to take into consideration the seasonality of marine organisms' living cycles. It should be taken into account that the formation of fouling depends on the reproduction of marine organisms that are included in its composition. Most of them have the pelagic stage of development in ontogenesis. The total duration of the planktonic life of invertebrates from the fouling communities ranges from 1 week to six months and is determined by their biological characteristics and natural factors (Aleksandrov, 2008). Therefore, we recommend studying the processes of plastic colonization by fouling organisms and the succession of this community together with the zooplankton community. To reveal the stages of individual growth, we highly recommend raising the sampling effort to weekly or twice a month. On the other hand, more frequent (every 3–7 days) observation of biofilm formation is obvious and can be very valuable in understanding of the species composition of the first settlers on various polymers (Rummel et al., 2017; Kirstein et al., 2019; Snigirova et al., 2020; Ryabushko et al., 2021; Sapozhnikov et al., 2021).

Despite the fact that the impact of plastic litter on individual marine organisms (mammals and birds) is well documented (Deudero & Alomar, 2015; Kühn et al., 2015), very little is known about the consequences of such pollution for the functioning of marine communities and ecosystems as a whole. In the maritime directive of the European Union, one of the descriptors of the state of the marine environment is proposed to be "marine litter" (D10), which includes both macro and microlitter and the thresholds that are needed to be developed for understanding the ecological status of marine environment (Van Loon et al., 2020).

The data from these experimental studies form the basis for describing the processes occurring on the surface of marine litter. Information about the stages of colonisation by marine organisms of polymer surfaces of various origins in the Black Sea region allows us to estimate the period of presence of marine litter in the natural environment, predict the nature

of fouling on marine litter and highlight the peculiarities of the biological successions on different types of plastic. It will be possible to analyse the species composition of litter communities, the presence of indicator species of plastic pollution and the participation of marine organisms in the degradation of this relatively new type of substrate in the marine environment. Another component of future studies is the spatial species structure of benthic communities and finding the relationships with the amount of marine litter, which will allow us to demonstrate which species try to avoid the plastic substrate or, on the contrary, increase their numbers on it.

Further research should be aimed at improvement and unification of the methods of studying complex communities on plastic substrates and comparing the structure of fouling on polymers with natural substrates. The combination of natural and experimental approaches will reveal the patterns of the communities functioning on the surface of marine litter and characterize their role in bottom marine ecosystems.

Conclusions

Complex experimental research on the colonization of plastic substrates was conducted for the first time in the Black Sea. This study describes the approach of complex investigation of biofouling from the microalgal settlement to macrofauna dwellers on the surface of new types of the substrates in the marine environment. The results could be used as a part of monitoring marine litter components. The obtained results form an important contribution to the development and implementation of Marine Strategy Framework Directive approaches (Descriptor 10) and the Marine Strategy of Ukraine. The data can help to estimate the duration of the presence of litter in the natural environment based on the analysis of the fouling formed on it, and to predict the nature of fouling on marine litter. All this together will highlight the peculiarities of successions on different types of plastic and contribute to the understanding of the degradation of plastics and their fate in the marine environment.

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