



RESEARCH ARTICLE

EVALUATING THE EFFECT OF AN ARTIFICIAL HABITAT ON THE IMPROVEMENT OF THE BULGARIAN BLACK SEA BIODIVERSITY

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ABSTRACT

In the present study the effect of an artificial habitat construction on the biodiversity of the coastal waters of the "Vromos" Bay, Bulgarian Black Sea was investigated for the period 2011- 2012. Several environmental factors such as water temperature, salinity, pH, dissolved oxygen, nutritional conditions, phytoplankton taxa were observed. The fouling of the habitat by macrozoobenthos, especially mussels was reported. Individual counts and dimensions (mm) of mussels, shell length and asymmetry, intermediate size of the shell (mm), etc. were presented. Dominants of *Dinophyceae* - 36.96 % and *Bacillariophyceae* - 23.91% with total of 60.87% of the phytoplankton taxonomic composition were recorded as well as blooms of small flagellates from class *Cryptophyceae* with higher levels at the bottom horizon -1881.89 x10⁶ cells/m³; 212.84 mg/m³; 83,85 % (numbers) and 58.28 % (biomass). Prevailing species of *Mytilus galloprovincialis* with 64 mm maximum shell size and considerable number of 20-30- 40 mm sized mussels were registered on the surface of the artificial habitat.

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INTRODUCTION

The Bulgarian Black Sea high anthropogenic load, pollution of marine waters, destruction of the natural habitats and intensive exploitation of marine resources during the last years has become a reason for the degradation of marine environment. The increasing eutrophication, hypoxia and anoxia in the bottom layers, invasive species, overfishing, bottom trawling, shipping, climatic changes, etc. have posed a threat to the balance and integrity of the marine ecosystems and have led to the loss of biodiversity and significant reductions in populations of many pelagic and benthic species of economic importance (Trayanova et al., 2007; Petrova and Gerdzhikov 2009; Oguz and Velikova 2010; Moncheva et al., 2010; Trayanova et al., 2011; IO - BAS 2012; Berov 2012; Klisarova 2015). The disruption of the Bulgarian Black Sea ecological balance has become an issues of great scientific concern. According to investigations of Bulgarian scientists the Bulgarian Black Sea basin is characterized with suitable

climatic, physico- chemical and nutritional conditions for the development of some aquatic species which habitats plays a key, ecological role for the functioning and resilience of the marine ecosystem as a whole (Todorova and Konsulova 2009; Dencheva 2010; Shtereva et al., 2012). One of the solutions to reduce the negative effect of the exploitation of marine resources and to improve the recovery of benthic and pelagic communities is the construction of artificial habitats (reefs). Experiments are being carried out in many parts of the world in deliberately constructing artificial reefs using a variety of materials. Most of them are submerged bottom or surface constructions - old ships, concrete elements, flexible linear marine equipment, raft systems, etc.(Seamen and Jensen 2000; Deysher et al., 2002; Relini 2004; Seamen 2007; Maura et al., 2007; Karayücel et al., 2010). The aim is often to encourage a greater variety and abundance of fish life for the benefit of local fishermen or divers. Shipwrecks are well known for their ability to act as a focus for marine life and new wrecks soon become colonized. Bottom constructions are characterized by good storm resistibility but destroy most of the benthic flora and fauna, sink in the sandy and muddy bottom and within the

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time become a barrier against the smooth movement of debris. The main disadvantage of the surface installations is their strong influence by the wind and waves, which requires numerous reconstructions and maintenance. In order to improve the recovery of marine communities in the Bulgarian Black Sea an installation of benthic - pelagic type was constructed as an artificial habitat and deployed in the "Vromos" bay during 2011. The aim of the research was to investigate the effect of the habitat on the conservation and sustainability of marine ecosystems. The specific objective was to assess the colonization of the artificial substrata by marine species, especially the black mussels (*M. Galloprovincialis*) which are among the most common opportunistic species in such assemblages.

MATERIALS AND METHODS

Study area

According to the requirements of the European Water Framework Directive 2000/60 (WFD) and the Bulgarian Regulation N^oH 4/2012 -13 water bodies were differentiated along the Bulgarian Black Sea coast (EU 2000; MOEW2012). Seven aquatic regions, situated in different water bodies were provided for the purpose of the investigation. The region selected as an experimental area for elaboration of the artificial habitat was the "Vromos" bay (region V) (Fig.1). The "Vromos" bay is situated in the coastal waters of Chernomorets town, located between Nos Atiya and Nos Akin and associated to water body- Burgas coastal waters (ID BG2BS000C008). The "Vromos" bay was selected as an experimental area due to the anthropogenic pressure of the Burgas coastal waters-one of the most polluted marine areas as well as Chernomorets town untreated sewerage discharge (Simeonova 2012; Simeonova *et al.*, 2012; Toneva *et al.*, 2012). The bay was influenced by the dredging, carried out in the past, which destroyed the natural habitats of many benthic hydrobionts. On the other hand the hydrological, hydrographic, meteorological and hydrodynamic conditions of the selected area are suitable for the positioning and exploitation of the artificial habitat (Jekova *et al.*, 2012, Toneva *et al.*, 2012).

Artificial habitat main characteristics

For the purpose of the investigation underwater habitat was designed, constructed and installed in the "Vromos" bay during 2010 – 2011 by scientific group of the Technical University -Varna. The positioning of the artificial habitat was carried out in May 2011. The installation was benthic - pelagic hydro facility located in an area of $6.5 \cdot 10^3 \text{ m}^2$ at a depth of about 15 m. The main elements of the construction were arranged above the bottom and below the water in order to avoid some of the disadvantages of the existing artificial reefs (Nikov 2012; Nikov *et al.*, 2012). It was composed of special flexible elements: anchors (basic and intermediate)- 3.5 - 4 t each; baselines; triangular collectors- 3 - 4 m in length; floats, attached to the upper part of the triangular collectors and traps for *Rapana*, mounted to the lower part (Fig. 2). These elements were allocated in three lines with a length of 250 m each. The main elements were arranged 3 m above the sea bed and 9 m below the water surface. The specifics of the terrain (shallow and almost flat sea bed) and the geographical location of the "Vromos" bay favored the high resistibility of the construction.

Physico - chemical parameters

Background physico - chemical research was conducted - before the positioning of the habitat May - October 2011. Water samples were collected from the sea surface sampling point (SP 1.0) and 14 m depth (SP 1.14) by portable surface sampler and bathometer respectively. The following water indices were analyzed: temperature, salinity, pH, dissolved oxygen (DO), ammonium (NH_4^+), nitrites (NO_2^-), nitrates (NO_3^-) and phosphates (PO_4^{3-}). The water chemistry was analyzed by standard methods.

Sampling procedure and taxonomic composition of phytoplankton

Investigations of phytoplankton were conducted in the region of the "Vromos" bay in May 2011. Phytoplankton samples were collected at water surface and depth of 15 m by bathometer Niskin - 5L. The samples were fixed with 2% formaldehyde, concentrated by sedimentary method and identified taxonomically, using microscope technique (Olympus BX41) with magnification from 100x to 400x in a composite chambers "Sedgwick Rafter" (1 ml volume) and "Palmer - Maloney" (0.05 ml) (Moncheva and Parr 2010). The cell volume was calculated using Edler equation according to the dimensions of each size class. In order to specify the phytoplankton group and class besides the traditional Black Sea Identifier, electronic database "WoRMS" and "Algaebase" were applied (WoRMS2013; Guiry MD and Guiry GM 2013).

Sampling procedure and morphological measurement of mussels

Field investigations concerning the degree of spreading of mussels over the habitat surface were conducted within the period 2011 - 2012 year, after the positioning of the construction. Ten months after the positioning underwater photographs of the fouling were taken, using digital camera. In May 2012 three samples were collected by a diver from one segment of the construction (20 cm section of the segment for each sample). First sample was collected from the upper part of the segment - the nearest to the water surface (sample 1); second sample - from the middle part of the segment (sample 2) and third (sample 3) - from the bottom part. Mussels and associated organisms were removed from the segments' surface, transferred to a plastic bag with 10% formaldehyde and washed through a sieve of 0.25 mm mesh. The following indices were determined: individual counts and dimensions (mm) of the individuals (shell length); intermediate size of the shell (mm); asymmetry of the shell length and intermediate size of the species with dimensions >P95 (the intermediate shell length of the largest 5 percentile species of the population). The asymmetry of the shell length (an indicator of survival and filling of the population) was assessed as deviation of the dimensions compared to the intermediate size of mussels.

RESULTS

Physico - chemical characteristics

The average values of the physico - chemical indices - temperature, pH and salinity of the samples collected from the sea surface (SP 1.0) and 14 m depth (SP 1.14) during 2011

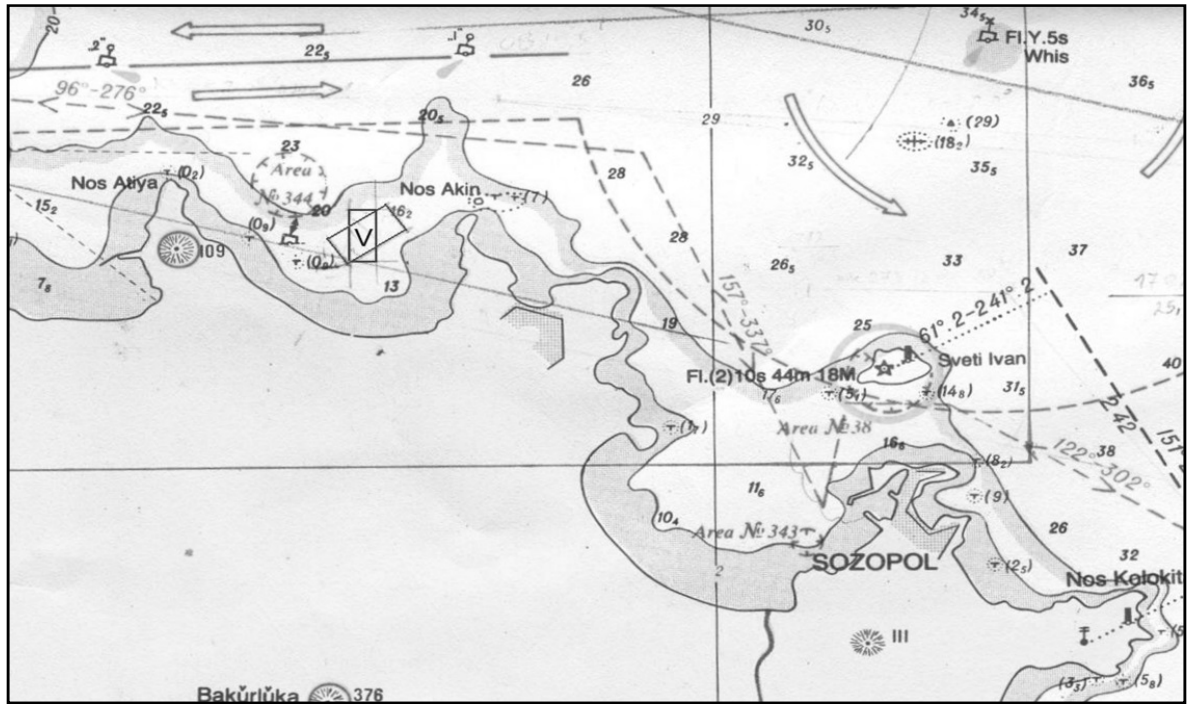


Fig. 1. Map of the “Vromos” bay, including the selected area (region V) for the elaboration of the artificial habitat, located between Nos Atiya and Nos Akin

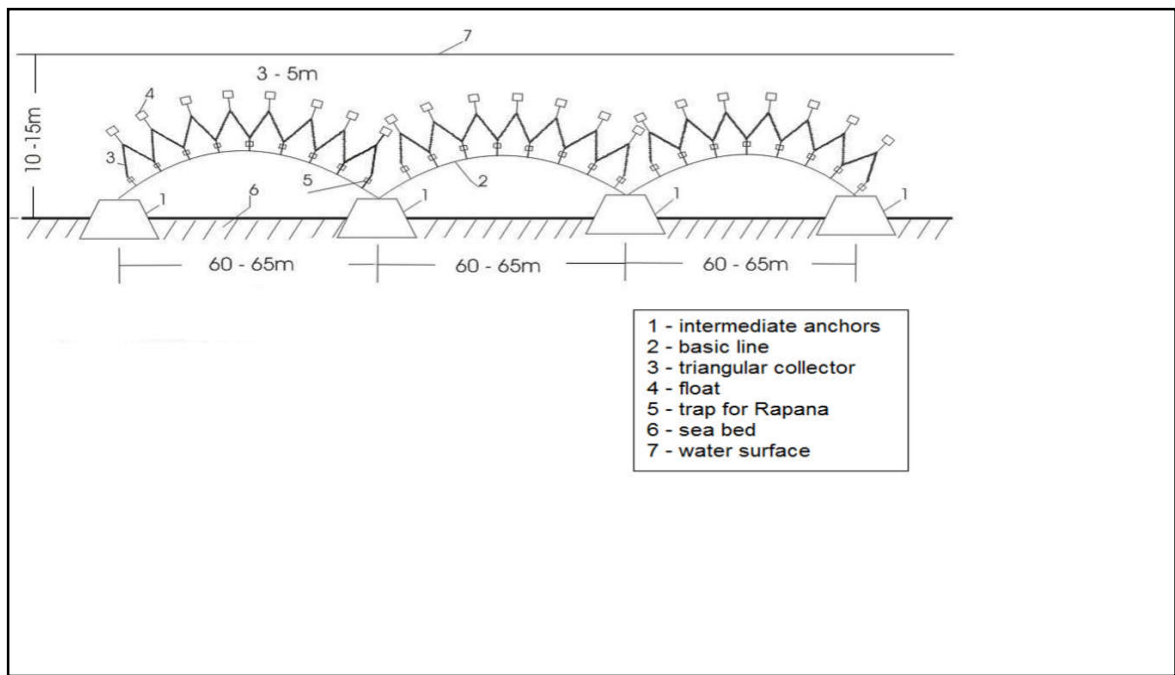


Fig.2. Simple schematic diagram of one segment from the main line of the artificial habitat in the “Vromos” bay

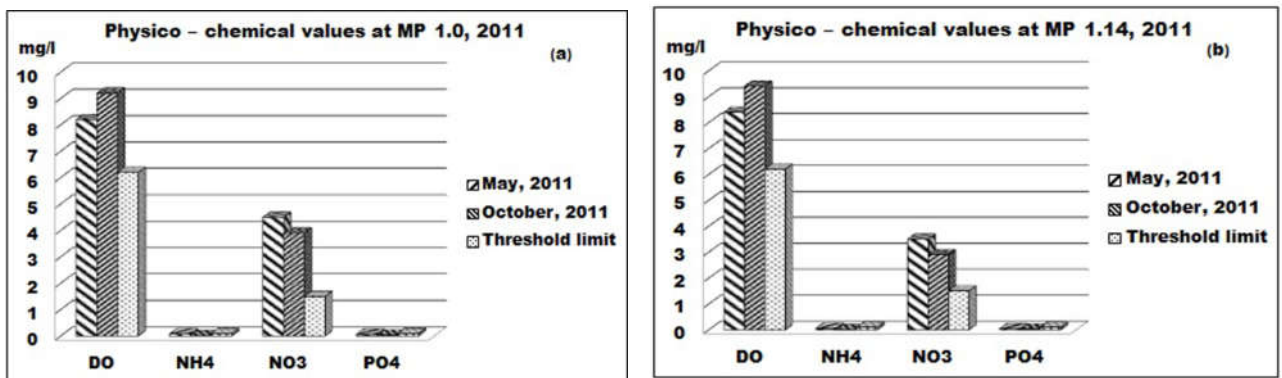


Fig. 3. DO and nutrient concentrations in the “Vromos” bay at MP 1.0 (a) and MP 1.14 (b), 2011

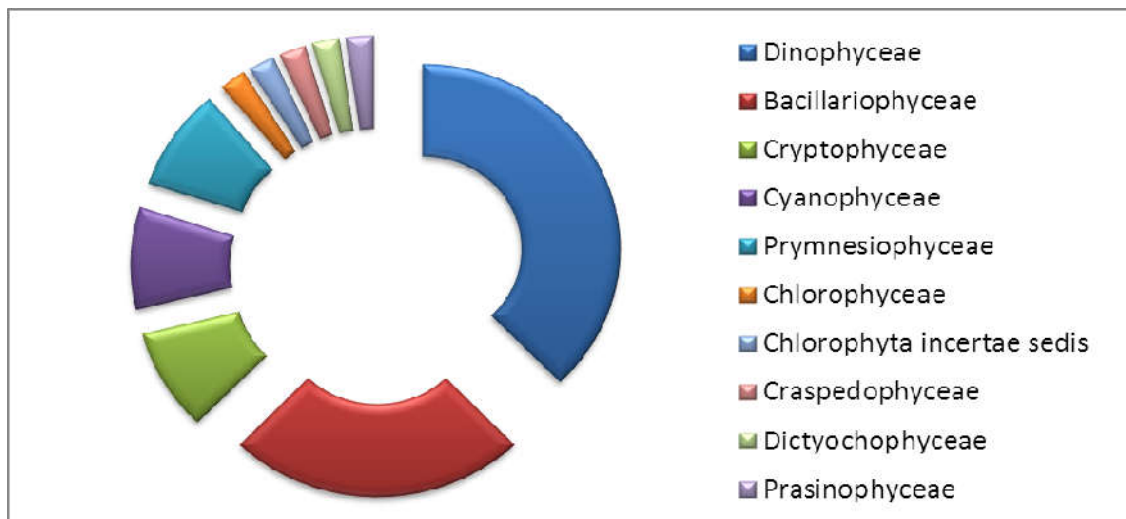


Fig.4. Basic taxonomic classes in phytoplankton composition in the “Vromos” bay, 2011 (Guiry and Guiry, 2013; WoRMS, 2013)

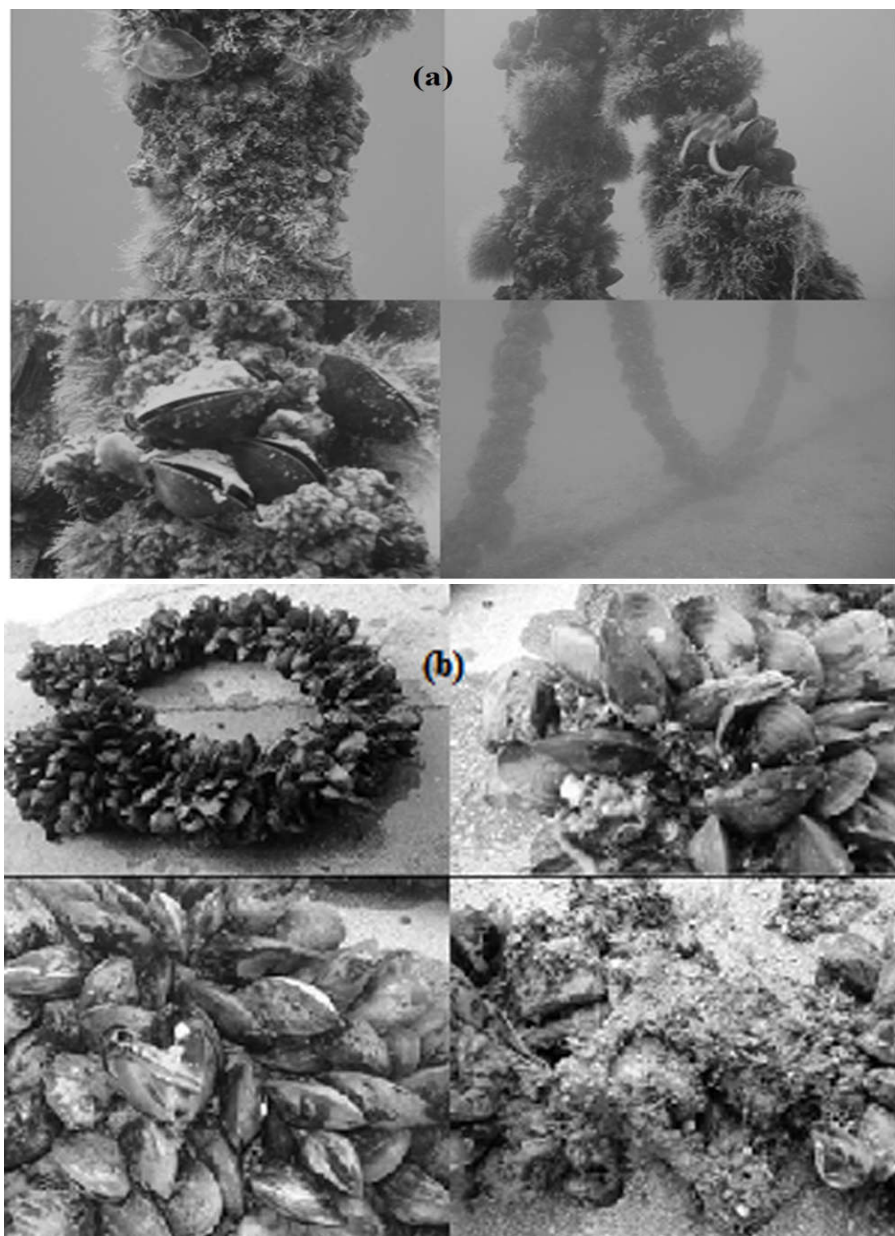


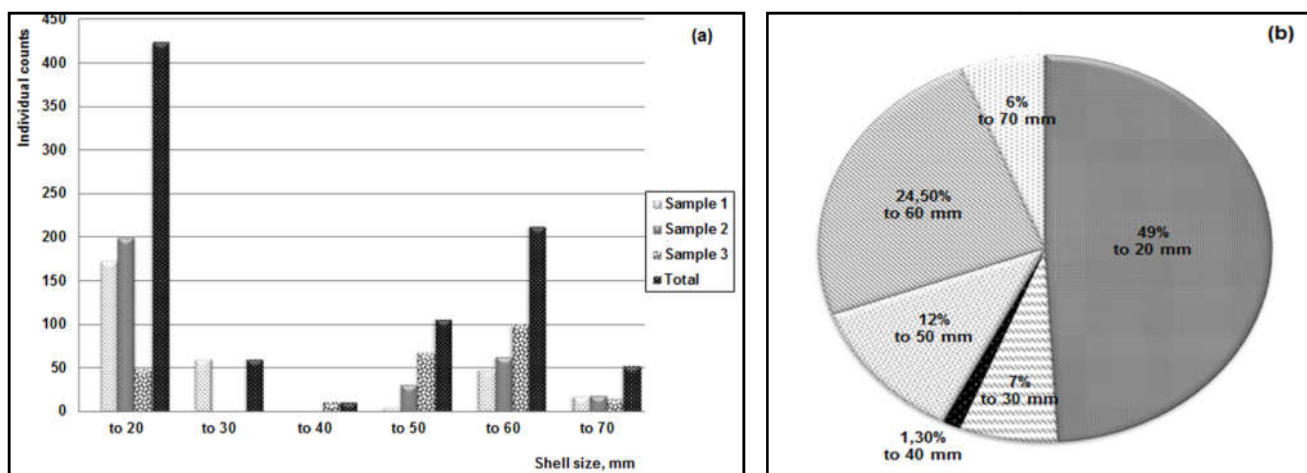
Fig. 5. Underwater photos of mussels occupancy on some elements of the habitat construction, 10 months after positioning in the “Vromos” bay, 2011 (a); mussels communities from one segment of the artificial habitat, 2012 (b)

Table 1. Main characteristics of the phytoplankton (number of species ($\times 10^6$ cells/m³); Biomass (mg/m³)) and Shannon-Weaver index of biodiversity by number of species and by biomass in the “Vromos” bay, 2011

Horizon, m	Number of species ($\times 10^6$ cells/m ³)	Biomass (mg/m ³)	Shannon index (number of species)	Shannon index (biomass)
0	1401.76	574.87	1.36	3.08
14	2244.42	365.19	1.05	2.59
Average for the water column	1823.09	470.03	1.21	2.83

Table 2. Individual counts and demographic characteristics of the mussels communities- *Mytilus galloprovincialis*, *Mytilus edulis* and *Conearca cornea* in the “Vromos” bay, 2012

Samples from one segment of the construction -20 cm section of the segment	sample 1 - the nearest to the water surface	sample2 - from the middle part of the segment	sample3 -from the bottom part of the segment
Dimensions – number of species	64mm- 18 numbers 55mm- 38 53mm- 11 48mm- 6 21mm - 61 5-12-13mm- 173	62mm- 20 numbers 60mm- 12 58mm- 11 55mm- 26 53 mm- 12 52 mm- 3 50 mm- 14 48 mm- 18 5-12-13 mm - 200	62mm-16 numbers 60mm-28 56mm-3 55mm -6 54 mm -6 53 mm-6 52 mm-51 50 mm-34 48 mm-22 45 mm-4 44 mm-3 43 mm-4 42 mm -2 40 mm -5 38mm-4 32 mm -3 5-12-13mm - 50
Total individual counts	307 numbers	316 numbers	247numbers
Intermediate size, mm	28	33	45
Asymmetry of the shell length	(-) 56% (0) 21% (+) 23%	(-) 63% (0) 0% (+)37%	(-) 25% (0) 28% (+)47%
Intermediate size>P95, mm	64	62	62
Individuals with size >80mm	none	none	none

**Fig. 6. Quantitative characteristics of mussels from one segment of the habitat in the “Vromos” bay according to the shell size - individual counts (a); percentage of the total number of the species (b)**

showed insignificant differences. The sea surface temperatures (SP 1.0) during the sampling period varied from 16.0 °C in May to 13.4 °C in October 2011. The temperatures measured at 14 m depth (SP 1.14) were lower varying from 14.0 °C in May to 11.0 in October 2011. The salinity at the surface horizon was within 17.70 - 17.85 ‰ in May and October 2011. The values registered at depth of 14 m were as follows: 17.76 ‰ in May and 18.08 ‰ in October. Regarding pH relative stability was registered during the sampling period at both SP. The recorded values of pH at the sea surface varied in a narrow range from 8.56 in May to 8.37 in October 2011. At 14 m depth pH was from 8.35-8.31 in May and October 2011 respectively. The pH

values were in compliance with the threshold limits of the National Regulation 8/2001 (pH threshold limit - 6.5 - 9.0) (MOEW 2001). DO concentrations showed that the aquatic area was well oxygenated. The average concentration at the surface (MP 1.0) was 8.4 mg/dm³ in May and 9.2 mg/dm³ in October and at 14 m depth (MP 1.14) - 8.4 mg/dm³ in May and 9.4 mg/dm³ in October, in compliance with the threshold limit (DO threshold limit - 6.2 mg/dm³) (Fig.3a, b). With reference to the nutrients, ammonium concentrations below the threshold limit were measured at both SP: SP 1.0 - 0.08 mg/dm³ in May and 0.01 mg/dm³ in October and at SP 1.14 - 0.06 mg/dm³ in May and 0.01 mg/dm³ in October. (ammonium threshold limit -

0.1 mg/dm³) (Fig. 3 a, b). Nitrate concentrations above the limit were observed in 2011 at both SP. The highest were the values measured in May 2011 at SP1.0- 4.50 mg/dm³ - 3 times higher than the limit (nitrates threshold limit - 1.5 mg/dm³). The phosphates were below the limit, ranging from 0.03 - 0.06 mg/dm³ at both SP for the whole sampling period (phosphates threshold limit - 0.1 mg/dm³).

Phytoplankton characteristics

The phytoplankton samples collected in 2011, before positioning of the artificial habitat were divided into 10 classes, presented in Figure 4. The largest share belonged to classes - *Dinophyceae* - 36.96 % (17 species) and *Bacillariophyceae*-23.91% (11 species) with total of 60.87% of the taxonomic composition. During the investigations blooms of small flagellates from class *Cryptophyceae* were registered through the whole water column with higher levels at the bottom horizon -1881.89 x10⁶ cells/m³; 212.84 mg/m³; 83,85 % (numbers) and 58.28 % (biomass). The small flagellates were ≥ 25% of the phytoplankton community. High number of small sized representatives (3µm) of the blue - green algae *Merismopedia sp.*, Meyen 1839 were registered either -185.04 x10⁶ cells/m³, 2.62 mg/m³, 8.24% (number of species), 0.72% (biomass). Except the small flagellates another dominant species by biomass were *Prorocentrum micans* peridines, Ehrenberg 1833 - 33.65%. The main characteristics of phytoplankton community - number of species, biomass, and Shannon-Weaver index were identified and presented in Table 1. Shannon-Weaver index, calculated by biomass exhibited higher values than Shannon-Weaver index calculated by number of species. The phytoplankton community exhibited lower values of biomass (365.19mg/m³) and higher number of species (2244.42x10⁶cells/m³) at 14 m depth.

Mussels characteristics

The investigation conducted in “Vromos” bay showed that within ten months after the positioning of the installation considerable development of mussels population with maximum shell length of 64 mm and surface occupancy above 50% was registered (Fig.5a). During 2012 considerable increase of the number of species and dimensions from each segment of the artificial habitat was recorded (Fig. 5b). Representatives of the following mussels species were registered: *Mytilus galloprovincialis*, *Mytilus edulis* and *Cunearca cornea*. Quantitative and demographic characteristics of the mussels are presented in Figure 6 and Table 2. The shell size varied from 5 to 64 mm. Mussels with very small size lower than 20 mm were prevailing in sample 1 (the upper part of the segment) and sample 2 (the middle part of the segment). In sample 1 were registered 173 small sized species -56% of the total and in sample 2 - 200 species (63%) (Fig. 6 a). The number of the small sized species in sample 3 (the bottom part of the segment) was the lowest - 50 species only (20% of the total number, found in sample 3). In general at the bottom part of the segment mussels with shell size above 40 mm were 75% of the total number. The abundance of species with size 20 - 30- 40 mm from the three samples exhibited good filling of the population. The species with size up to 20 mm were 49% of the total number of the mussels (found in the three samples), those with size up to 30 mm- 7% and with size up to 40 mm-1.3% (Fig. 6 b). The intermediate size of the mussels shell was 28 mm in sample 1, 33 mm in sample 2 and 45 mm in sample 3 (Table 2). According to this

indicator the colony at the bottom part of the construction showed best status. The asymmetry of the shell size was as follows: in sample 1 and 2 prevailed (-) asymmetry - 56% and 63% respectively of the mussels number. In sample 3 - 25% of the population was with (-) and 47% with (+) asymmetry. The negative asymmetry can be interpreted as good filling of the population, but poor survival, whereas positive asymmetry - as better survival but poor filling of the population (IO - BAS 2012). Therefore sample 1 exhibited good filling of the population and sample 3- low filling but good survival. With reference to the intermediate size of the individuals with dimensions >P95 (the largest 5 percentile species) - 64 mm sized individuals (18 numbers) were recorded in sample 1 and 62 mm in samples 2 (20 numbers) and 3 (16 numbers). Larger sized classes ≥ 80 mm were missing. The total number of the mussels from one segment was 870 species, indicating good space occupancy and development rates. The dominant mussels taxa registered in all samples was *Mytilus galloprovincialis*.

DISCUSSION

Growth of marine species strongly depends on several environmental factors such as water temperature, salinity, changes in pH, dissolved oxygen near the bottom, food supply, nutritional conditions, phytoplankton availability as well as hydrographic, hydrological and hydrodynamic characteristics of the aquatic areas (Melek et al., 2012). Water temperature and salinity are the main abiotic factor which may affect the rates of biochemical reactions in marine organisms. Water temperature exerts a major control over the distribution and activities of aquatic organisms, since they live and reproduce only within certain temperature ranges (Tait and Dipper 1998; Sîrbu and Benedek, 2017). The mussel *M. galloprovincialis* is one of the marine species with very good capacity of adaptation and resistance to large fluctuations in temperature and salinity. The mussel could be successfully cultivated in salinity range 8-38 ‰ and temperature 5-28°C. When temperature is above 10°C and food is available, growth rate of mussels is high. At salinities below 8‰, the mussels isolate themselves by closing the valves and stop filtering water, resulting in mortality. In addition, the degree of fixation by the byssus on the substrate decreases (Karayücel et al., 2002; Rosioru, 2014). The water temperature in the “Vromos” bay during the sampling period followed a seasonal cycle similar to the temperatures of the Burgas coastal waters, part of which is the investigated area. The average monthly and annual values of Burgas water temperature are - 16.3°C in May and 17.2°C in October (MOEW 2011). The insignificant variations of temperature at the sea surface and at depth of 14 m during spring and autumn are typical for the thermocline layers of the Black Sea (Ivanov and Samodurov, 2001). Surface cooling of the water in autumn and warming in spring and almost the uniform temperature to the bottom produce little difference between the surface and the deep layers. That was the reason for the constant temperature through the whole depth of water in the investigated area. The salinity measured in the “Vromos” bay during the investigation was similar to the salinity of the Burgas coastal water which annual average value according to Burgas synoptic stations is 16.11‰ with maximum in October 17-18%. Bearing in mind the optimum thermal and salinity range for the physiology of mussels, evidently these parameters in the area of the habitat were suitable for the growth and survival of the mollusk. The active reaction and DO concentrations of the marine water are very

important for the development of benthic communities. pH can affect the spreading and development of many benthic communities because they can survive in relatively narrow range. When DO is reduced many bivalve species are able to close their shells tightly and use the oxygen reserves retained in their tissues which makes them very tolerant to harsh environmental conditions, hypoxia included. When oxygen at the bottom layers is reduced significantly *Mytilus galloprovincialis* can be replaced by other mussel (*Cunearca cornea*). *M. galloprovincialis* requires DO concentrations of the marine water above 5mg/dm^3 (Tait and Dipper 1998). The recorded pH values in the "Vromos" bay (8.35 - 8.90 pH) showed low alkalinity, typical for the marine waters during phytoplankton growth. The DO concentrations at the sea surface and underwater layers (14 m depth) were above the threshold limit (higher in October 9.2 - 9.4 mg/dm^3) and exhibited good water oxygenation. Both pH and DO values measured in the investigated area exhibited suitable conditions for the growth and development of mussels. Phytoplankton productivity and detritus are the main nutritional factors for the development of mussels communities, boosted by nutrient-rich waters. Unicellular phytoplankton forms the base of food chains and serves as a primary food source for the marine fauna. Phytoplankton species in the Bulgarian Black Sea in the last decade has grown more than twice (up to 1621 confirmed and 48 unconfirmed species on the list of the Black Sea Phytoplankton Checklist compared to 80s (700 species identified to 1978) (Petrova and Gerdzиков 2015; Petrova *et al.* 2014). Regular researches of the phytoplankton at the Burgas Bay from the beginning of the 90's allowed to be characterized many of phytoplankton most important parameters at the end of the eutrophication period in Black Sea. In the last years in Burgas Bay is observed a tendency of biomass reduction of phytoplankton coenoses and not been observed large and harmful "blooms" (Klisarova *et al.*, 2015). The highest quantitative indicators of the phytoplankton stock (number and biomass) were registered in May and October. Regarding "Vromos" bay there was a pronounced trend of higher concentrations of all nutrients in May and lower in October during 2011, which might be due to the more intensive nutrient utilization by the phytoplankton during the autumn period. The defined percentage of phytoplankton groups - *Dinophyceae* - 36.96 % (17 species) and *Bacillariophyceae* - 23.91% (11 species) in the taxonomic composition was in line with the trend of increasing species diversity of phytoplankton communities in direction of representatives of "other" taxonomic classes established for the entire Black Sea basin since 2000 (Petrova and Gerdzиков, 2015). During the investigation the small flagellates blooming was under the level of the "red tide" (5000×10^6 cells/ m^3) and due to the small sizes of the cells ($6\mu\text{m} \pm 3$) didn't produce high levels of biomass. The blooms were under the level of the "red tide". Such phytoplankton blooms are typical for the so called post eutrophication period in the development of the Black Sea ecosystem (from the middle of the 90 till now) (Moncheva 2010). Bearing in mind the requirements of WFD 2000/60 and Bulgarian Regulation N₀H 4/2012 the values of *Shannon-Weaver* index and the number of species ($\times 10^6$ cells/ m^3) were assessed as high (EU 2000; MOEW 2012) and the ecological status of the "Vromos" bay as "moderate", which means that phytoplankton productivity was suitable for the development of mussels.

The black mussel *Mytilus galloprovincialis* is the most common bivalve along the Bulgarian Black Sea coast. In open

sea areas it can be found to 65 meters and in shallow waters to 15-20 meters. The maximum size of mussel reached 12-13 centimeters and the sizes in the catches vary from 4-5 to 7-8 cm (Petrova and Stoykov 2011a). Unfortunately since 1970 the mussel's abundance along the Bulgarian Black Sea shelf has undergone considerable reduction, the main reason being the *Rapana thomasiana* invasion, oxygen deficiency at the bottom layers and the extensive dragging (Todorova and Konsulova 2009). After the destructive effects caused by *Rapana* and eutrophication of waters in the 1970s and 1980s, the main mussel fields along the Bulgarian Black Sea coast are in good condition. Investigations of some bivalve mollusks in several mussel fields along the Burgas Bay showed that mussels size varied from 3.5 to 8.0 cm (Petrova and Stoykov, 2011b). In the "Vromos" aquatic area the mussels are less prevalent with relatively low coefficient of perseverance or are almost missing (IO- BAS, 2012). Due to the artificial habitat, positioned in the investigated area mussels showed a marketable minimum size of 50 - 60 mm within 10 months of culture. The values are consistent with the findings reported for Burgas Bay and other regions where mussel raft farming represents a matured industry (Petrova and Stoykov 2011a). The total number of mussels showed good space occupancy, development and survival especially at the bottom part of the construction. One of the reasons probably is the larger area for attachment and coverage as well as the lack of strong underwater currents. Furthermore the greater the depth the lower the sun lighting and the quantity of the detritus is higher which is important for the mussels development. The dominant mussels taxa was *Mytilus galloprovincialis*. The exotic immigrant *Cunearca (Anadara) cornea* is a typical indicator for eutrophication. The fact that representatives of *Cunearca cornea* were not dominant species exhibited that eutrophication processes were not available in the experimental area.

Conclusion

The construction of the artificial habitat in the "Vromos" bay was intended to create suitable conditions for biodiversity conservation and sustainability of marine ecosystems, combined with appropriate development of black mussels. As a result of the positioning of the habitat considerable development and spreading of mussels with maximum dimensions of 64 mm were registered for one year period. The dominant mussels taxa was *Mytilus galloprovincialis* which plays a key, ecological role for the functioning and resilience of the marine ecosystem due to the strong bio - filtration capacity which is expected to improve the physico- chemical status of the water in the investigated area. At the same time the mussel beds will provide substrate for a diverse epifauna and increase the ecosystems diversity and sustainability.

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