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Stress Ecology of Wedge Clams *Donax trunculus* Linnaeus, 1758 from Sandy Habitats of the Bulgarian Black Sea Coast

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Abstract: The aim of the present study was to assess the multiple stress or effects and adaptive capacity of wedge clams *Donax trunculus* Linnaeus, 1758 from nine typical localities of the Bulgarian Black Sea coast. The accumulation of trace metals, polycyclic aromatic hydrocarbons (PAH), microbiome composition and contents of microplastic particles were analysed. The levels of lipid peroxidation (LPO), protein oxidation (PO), glutathione (GSH) and activity of glutathione-S-transferase (GST) and acetylcholine esterase (AChE) in wedge clams were measured. The relationships among environmental stressor variables and values of oxidative stress biomarkers were analysed by Cluster and Principal Components Analysis. The results showed that the induction of LPO and activation of GST were associated with levels of metal accumulation in wedge clams' tissues. PO was more strongly related to increased levels of PAH, while activity of AChE was associated with the abundance of microorganism species present in the wedge clams. In conclusion, we demonstrated the presence of specific stress response of the wedge clams to the state variables of the marine environment of their habitats. Within their tolerance range, the wedge clams were able to manage and effectively compensate the different pressures of multiple environmental stressors in their local habitats.

Key words: oxidative stress, wedge clams, xenobiotics, adaptive capacity

Introduction

It is currently accepted that environmental factors can exert a background of physiological stress that can influence the toxic effects of pollutants, thus causing multiple stressor effects (Crain et al. 2008). The organisms respond to the stress with a cascade of internal changes leading to adaptation or death, depending on the strength and duration of the impact (Steinberg 2012). Although the stress concept can be defined at various levels of ecological integration (Parker et al. 1999), it is most commonly studied in the context of individual organisms, whereas stress responses are studied on cellular and biochemical levels (Korsloot 2002).

Bivalves are essential for maintaining the functionality and stability of marine ecosystems since they are a key element in them. Like any organism, mussels are in constant contact with the environment and changes in it have a significant impact on their

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physiology. Being filter and suspension-feeders, bivalves are able to accumulate large amounts of various xenobiotics such as metals, polycyclic aromatic hydrocarbons, pesticides and microplastics. The accumulated inorganic and organic contaminants can affect the health status of bivalves. Many studies underline the importance of measuring different biomarkers in the same organism at the same time, to evaluate the effect of environmental impacts (Hook et al. 2014). In modern ecotoxicology two types of biomarkers are conditionally distinguished - those that reflect the exposure of organisms to environmental stressors (Exposure Biomarkers) and those that show the adverse effect of environmental stressors on the state of organisms (Effects Biomarkers) (Hook et al. 2014). Widely used exposure biomarker in marine organisms is the activity of glutathione-S-transferase (GST). GST activation has been reported in bivalves exposed to pesticides, surfactants and polychlorinated biphenols (Khessiba et al. 2001, Amira et al. 2011). Acetylcholinesterase (AChE) inhibition is used in biomonitoring programs as marker for both exposure and effect mainly in fish. The most widely used effect biomarkers in aquatic studies are the markers of oxidative stress (OS), such as lipid peroxidation, protein oxidation and the state of antioxidant defense. Changes in the pro/antioxidant balance of the cell are the most important ways by which chemical, physical and biological agents contribute to the health state of organisms (Sies 2019).

The Black Sea has limited water exchange and the inflow of large rivers make it a subject to high pollution (Moncheva et al. 2016). The Bulgarian Black Sea part makes no exception and a number of pollutants have been identified there. Wedge clams are one of the most common species inhabiting the shallow sandy bottoms along the Black Sea coast. They are suspension-feeders and like other bivalves, are capable to bioaccumulate different xenobiotics in their soft tissues. Significant amounts of metals (Stancheva & Ivanova 2012, Peycheva et al. 2021), polycyclic aromatic hydrocarbons (PAH) (Georgieva et al. 2022), polychlorinated biphenyls (PCB), organochlorine pesticides and their metabolites (Georgieva et al. 2016) have been found in bivalves from the Bulgarian Black Sea coast. Recently, different bacterial species, incl. pathogenic, were also established (Ignatova-Ivanova et al. 2018). Data on oxidative stress in wedge clams inhabiting the Bulgarian Black Sea are very scares. The first preliminary study was that by Yakimov et al. (2018).

The aim of the present study was to assess the level of stress in wedge clams (*Donax trunculus* Linnaeus, 1758) caused by multiple stressors effects of their habitats of the Bulgarian Black Sea coast by evaluation the reduction of their health condition and their adaptive capacity. In order to accomplish this aim, the levels of lipid peroxidation (LPO), protein oxidation (PO), glutathione (GSH) and activity of glutathione-S-transferase (GST) and acetylcholinesterase (AChE) of clams, their microbiome composition, concentration of certain trace metals and polycyclic aromatic hydrocarbons in soft tissues were analysed.

Materials and Methods

Sampling and sample preparation

The wedge clams were gathered manually from their natural habitats in the upper subtidal zone along the Bulgarian Black Sea coast (Table 1). Adult wedge clams of similar shell length (23-40 mm) were

Table 1. Localities of *D. trunculus* collection along the Bulgarian Black Sea coast with GPS coordinates, depth and distance from the shore

Code	Locality	Coordinates		Depth	Distance from the shore
		N	E	[m]	[m]
S1	Kranevo	43.2667	28.0266	1.50-2.00	50-100
S2	Varna Bay	43.2667	28.0266	3.00-3.50	100-150
S3	Shkorpilovtsi	42.9603	27.8970	3.00-3.50	20-50
S4	Sveti Vlas	42.7090	27.7595	3.00-3.50	10-20
S5	Slanchev Bryag	42.6906	27.7137	1.50-2.00	10-20
S6	Arkutino	42.3311	27.7368	2.50-3.00	50-100
S7	Primorsko	42.2577	27.7520	1.50-2.00	50-100
S8	Tzarevo	42.1666	27.8533	2.50-3.00	50-100
S9	Ahtopol	42.1140	27.9243	1.50-2.00	20-50

placed in plastic bags with seawater and transported to the laboratory, shock frozen and kept at -20°C until analysis. Subsamples of clams (~250 g) were provided for analyses at the Department of Chemistry of the Medical University – Varna (PAH and trace metals) and the Department of Biology of the Shumen University (microbiological analyses).

Physicochemical characteristics of the seawater

During the clams' sampling the temperature, total salinity (by using YSI Model 33 salinity meter, Xylem Analytics, USA), and pH (by using Portable pH meter Five easy, METTLER TOLEDO, USA) were measured in situ.

Chemical analysis

Analysis of PAH was performed on gas chromatograph (GC/MS) GC FOCUS (Thermo Electron Corporation, USA) using POLARIS Q Ion Trap mass spectrometer (MS), equipped with an AI 3000 autosampler and splitless Injector according to Georgieva et al. (2016). Elements' measurement was made by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) after digestion of the clams' tissues with nitric acid (Peycheva et al. 2021).

Biochemical analysis

On the day of the analysis, the shells of the individual wedge clams were opened and the soft tissues were removed. The tissue samples were homogenized with 0.1 M potassium buffer, pH 7.4 and centrifuged at 3000 g for 10 min at 4°C. The obtained post-nuclear fraction was used for determination of LPO, PO and GSH levels and AChE activity. A portion of the post-nuclear fraction was re-centrifuged at 12000 g for 20 min at 4°C for obtaining a post-mitochondrial supernatant, used for measurement of the activity of GST. Commercially available kits were used for spectrophotometrical measurement of the biomarkers: Lipid Peroxidation (MDA) Assay Kit MAK085, Protein Carbonyl Content Assay Kit MAK094, Glutathione Assay Kit CS0260, and Glutathione-S-Transferase Assay Kit CS0410 (Sigma-Aldrich Co. LLC, USA).

Acetylcholinesterase activity was measured in reaction mixture contained 0.1 M potassium buffer pH 8.0, 0.045 M acetylthiocholine iodide, and 0.008 M 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) (Ellman et al. 1961). The appropriate amount of clam's tissue homogenate was added to the mixture and the enzyme hydrolization of acetylthiocholine produced thiocholine, which reacted with DTNB. The absorbance of the resulting yellow coloured product, 5-thio-2-nitrobenzoic acid was measured at 412 nm. The enzyme activity was calculated as U/mg protein. Protein concentration was measured according to Lowry et al. (1951) using a standard curve, obtained with bovine serum albumin.

Microbiological analysis

For microbiological analyses, the wedge clams were washed in sterile hypochlorite water solution (0.02%) and extracted from the shells. Tissue samples were homogenized and culture suspensions were prepared after Ignatova-Ivanova et al. (2018). Microbial identification was performed by a 3rd generation identification technology with Manual Microbial Identification system (MicroLog M® BIO45101 Biolog Inc) and GEN III software.

Screening for the presence of microplastics

The content of microplastic particles (MPs) in clams was evaluated after soft tissue degradation by H_2O_2, flotation and filtration with saline through a nitrate cellulose membrane filter (Roch & Brinker 2017) and subsequent analysis of the obtained filtrates with a stereomicroscope (MicroImaging GmbH, Germany). The MPs were visually identified by their physical characteristics and by using DinoCapture 2.0 software for size quantifying based on their largest cross section.

Statistical analyses

Statistical analyses were carried out using the software package STATISTICA Version 10 (Data analysis software system), StatSoft Inc. Comparisons between variables were made using Mann-Whitney test. Patterns of similarities between sites in xenobiotics bioaccumulation in clam tissues were studied by cluster analysis. Multidimensional Scaling (MDS) was carried out to detect underlying similarities (dissimilarities) in the studied objects.

Results

The obtained results from the biochemical analyses and xenobiotics measurement for the studied localities are presented in Table 2.

The investigated wedge clams were found to accumulate different levels of metal elements depending on the localities (Table 2). Higher levels of Pb and Fe were found in the tissues of clams from the northern localities compared to those from the southern. In clams from Sveti Vlas and Slanchev Bryag high concentrations of all measured metals were detected. The highest concentrations of Pb and Cd were accumulated in the clams from Sveti Vlas.

Table 2. Biomarkers' values and accumulated xenobiotics (Mean±SD) in wedge clams from sandy habitats of different localities of the Bulgarian Black Sea coast (na – not available)

Characteristics	**Kranevo**	**Varna Bay**	**Shkorpilivtsi**	**Sveti Vlas**	**Slanchev Bryag**	**Arkutino**	**Primorsko**	**Tsarevo**	**Ahtopol**
LPO nM/ mg prot	0.755±0.187	0.885±0.111	0.362±0.040	0.990±0.147	0.892±0.121	0.437±0.051	0.558±0.071	0.650±0.045	0.980±0.267
PO nM PC/ mg prot	8.844±1.904	10.008±2.542	7.43±0.793	9.066±1.143	11.168±2.494	10.406±2.822	5.371±0.621	13.312±1.890	9.767±2.773
GSH ng/ mg prot	334±40.8	460±53.3	392±58.1	370±87.1	330±63.2	357±118.1	349±29.6	322±127.8	265±42.9
GST U/ mg prot	67±11.315	162±31.635	110±30.636	153±17.544	180±19.350	100±21.921	115±28.906	112±18.842	75±20.445
AChE U/ mg prot	31.299±7.317	10.648±2.270	38.878±2.973	11.677±2.891	14.793±1.970	31.29±7.317	19.151±3.523	18.493±6.395	19.207±4.720
Pb mg/kg ww	0.237±0.137	0.257±0.026	0.349±0.086	2.511±0.834	0.371±0.088	0.112±0.044	0.018±0	0.019±0.001	0.187±0.014
Cd mg/kg ww	0.054±0.008	0.057±0.004	0.077±0.009	0.322±0.203	0.161±0.014	0.056±0.003	0.077±0.001	0.097±0.004	0.116±0.015
Cu mg/kg ww	19.231±1.328	25.89±1.631	18.144±2.683	29.754±0.477	23.580±0.647	5.807±0.133	6.404±0.090	4.916±0.025	18.708±1.234
Fe mg/kg ww	137.271±62.413	60.317±8.348	114.659±34.335	186.564±19.632	213.71±27.077	42.087±3.961	45.279±0.453	50.753±0.482	41.554±0.417
PAH/Sum4 ng/g ww	1.04	3.43	0.54	3.56	1.09	2.13	1.13	4.10	3.33
PAH/Sum13 ng/g ww	36.56	17.17	30.63	31.33	19.09	36.36	11.84	87.29	12.74
MPs (≤25 μm) n/clam	na	7.33	6.33	5.0	2	2	na	na	12.7
Enterococcus hirae cells/ml	$6.8x10^5$				$0.22x10^5$	$11.33x10^5$	$1x10^5$		$3.87x10^5$
Escherichia vulneris cells/ml					$0.49x10^5$	$16x10^5$	$1.03x10^5$		$2.97x10^5$
Pseudomonas alcaligen cells/ml			$12.3x10^5$				$0.34x10^5$		$461x10^5$
Vibrio cincinnatiensis cells/ml		$2x10^5$							
t^0C	22.5	23.2	22.7	23.4	24.2	23.3	24.3	24.6	24.8
Salinity $^0/_{00}$	15.2	15.7	14.1	15.9	14.7	16.8	17.1	16.6	17.3
pH	8.5	8.3	8.0	8.2	8.1	8.4	8.6	8.5	8.5

Relatively high concentrations of Pb and Cd were measured in the samples from Ahtopol, of Pb and Fe in the samples from Kranevo and Shkorpilovtsi. Higher content of Cu was found in the mussels from Varna Bay. There were no higher concentrations of the studied metals in the samples from Primorsko compared to the clams sampled from the other studied localities.

The estimated PAH levels (PAH Sum4 and PAH Sum13) in the wedge clams differed significantly among the studied localities (Table 2). PAH Sum 4 were higher in the samples from Varna Bay, Sveti Vlas, Tsarevo and Ahtopol, and PAH Sum13 - in those from Kranevo, Shkorpilovtsi, Sveti Vlas, Primorsko and Tsarevo. The highest concentrations of PAH (Sum4 and Sum13) were measured in the samples from Tsarevo (Table 2). The PAH Sum13 in clams from Tsarevo exceed more than 2 times the measured values in the samples from Kranevo, Shorpilovtsi, Sveti Vlas and Arkutino and 4 to 7 times more than those from Varna Bay, Slanchev Bryag, Primorsko and Ahtopol.

We carried out an initial assessment of the presence of MPs in the soft tissues of wedge clams. In all wedge clamps tested MPs particles of different size were observed in the soft tissues. The MPs grain counts (≤25 μm) in the wedge clams were used in the analyses. Higher mean numbers of microplastics were found in the tissues of wedge clams from Varna Bay, Shkorpilovtsi and Sveti Vlas. The highest average number of MPs contained the wedge clams from Ahtopol (Table 2).

The presence of several species of microorganisms was identified in soft tissues of the studied wedge clams. Their frequency and abundance differed significantly among the studied localities (Table 2). The most frequent and abundant species was *Enterococcus hirae,* established in wedge clams from Kranevo, Slanchev Bryag, Arkutino, Primorsko and Ahtopol. The second abundant species was *Escherichia vulneris*, found in the wedge clams from Slanchev Bryag, Arkutino, Primorsko and Ahtopol. The species *Pseudomonas alcaligene* was present in wedge clams from Shkorpilovtsi, Primorsko and Ahtopol. Among the species of microorganisms, pathogenic bacteria of the genus *Vibrio* were established only in wedge clam specimens from Varna Bay (Table 2).

For the assessment of the stressfulness of the marine environment for the wedge clams specific OS biomarkers were used (Table 2). The measured biomarkers showed characteristic pattern of variations among the studied localities (Table 2). The wedge clams collected from Varna Bay, Sveti Vlas, Slanchev Bryag, Ahtopol and Tsarevo demonstrated signs of OS, characterized by significantly higher values of LPO, together with relatively high PO compared to the wedge clams from other localities. The lowest average value for LPO was measured in the wedge clams from Shkorpilovtsi. The lowest value of PO was present in the clams from Primorsko. Comparatively lower values of PO were also measured in the clams from Shkorpilovtsi and Kranevo, although the differences were not considerable. As a whole, GSH concentrations in the studied clams did not differ significantly between the samples from the studied localities. The lowest GSH values were measured in wedge clams from Ahtopol and Tsarevo. Significantly higher activities of GST were found in wedge clams from Slanchev Bryag, Varna Bay and Sveti Vlas. Significantly higher AChE activity was present in wedge clams from the localities Shkorpilovtsi, Kranevo and Arkutino.

In order to study the relationships among the measured multiple stressor variables and the values of wedge clams OS bioimarkers we applied Principal Components Analysis (PCA), which allows to analyse variables that are heterogeneous with respect to their means or to both their means and standard deviations. Stress biomarkers were set as active variables and the measured stressor variables of the marine environment at the studied localities were set as supplementary variables, for the purpose of interpretation (Fig. 1). The PCA clearly indicated the presence of relationships (correlation) among the studied variables. The induction of LPO seemed to be associated with the levels of metal accumulation in the tissues of wedge clams. Similarly, the activation of GST was also related to metals concentrations in wedge clams. On the other hand, PO appeared to be more strongly related to the levels of accumulated PAH4 SUM. The activity of AChE appeared to be related to the abundance of the identified microorganism species present in the wedge clams.

The similarity and grouping of the studied localities, based on the accumulated zenobiotics in the wedge clams and the state of the marine environment, was assessed using cluster analysis (Euclidean distance) (Fig.2A). The cluster diagram showed two main groups of localities according to the similarity in the level of stressors present in the marine environment there. The first cluster (upper part of the diagram) included localities situated in the more northern coastal zone. The second main cluster (lower part of the diagram) included localities situated in the southern coastal zone with the exception of Varna Bay. The main difference between these main clusters was the higher accumulation of metal

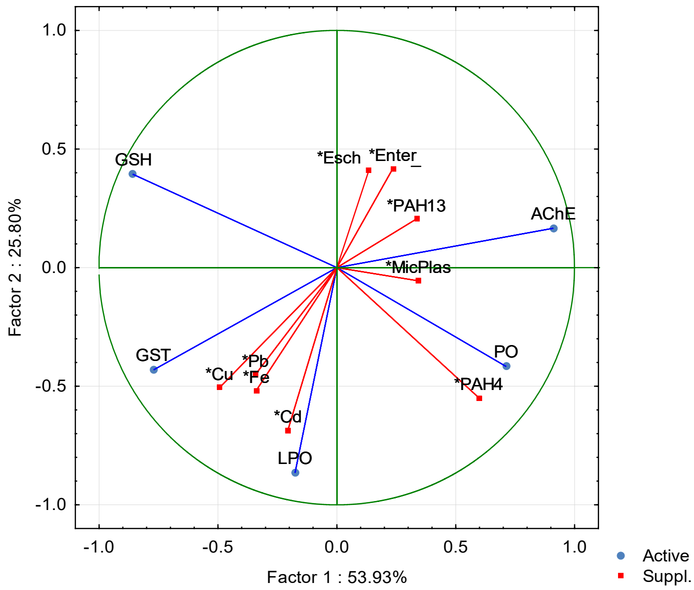

Fig. 1. Factor plot of principle component analysis of active and supplementary variables relations

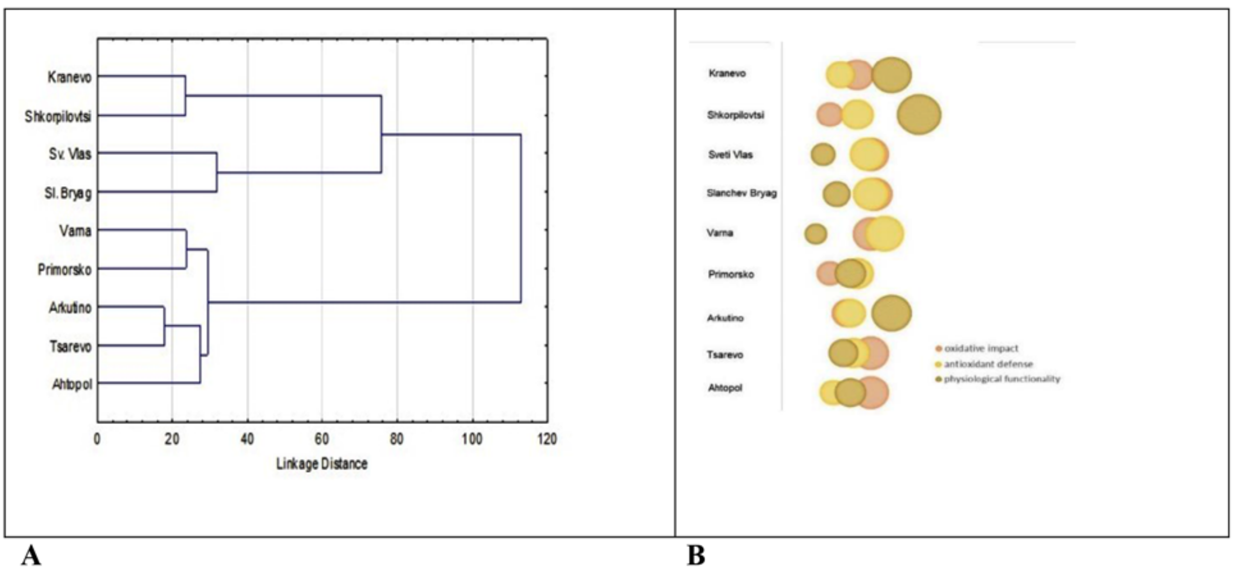

Fig. 2. Similarity grouping (cluster) of localities, based on state of the environment and accumulated pollutants (A) and the corresponding stress effects and level of tolerance of the wedge clams (B)

elements (Pb, Cu and Fe) in the wedge clams inhabiting northern localities. On the other hand, in wedge clams from the southern coastal zone (Tsarevo and Ahtopol) higher levels of PAH as well as more abundant and diverse microbiota was present, compared to the other localities.

The stress effects and the response of the wedge clams (range of tolerance) to the multiple stressor pressure at the corresponding localities are presented in Fig. 2B. The tolerance of wedge clams to the environmental stressors was estimated by the measured OS biomarkers, which, in combination, indicated the levels of oxidative impact, antioxidant defence and physiological functionality of the wedge clam organism. The range of tolerance of the wedge clams for pressures of environmental stressors by locality can be arranged in the following order (from high to low): Shkorpilovtsi > Arkutino > Kranevo > Primorsko > Slanchev Bryag > Sveti Vlas > Tsarevo > Varna Bay > Ahtopol. The clams from Shkorpilovtsi were characterized by low prooxidative impact, good antioxidant protection and high physiological functionality (Fig. 2B), and it can be assumed the wedge clams are well adapted and develop "normally" in the marine environment of this locality. A similar pattern of biomarker levels, with pro/antioxidant balance and high physiological functionality, was observed in wedge clams from habitats in the regions of Kranevo (typical northern) and Arkutino (typical southern), but in the wedge clams from Kranevo, the oxidative impact partially exceeded the antioxidant protection (Fig. 2B). Such fluctuations in the pro/antioxidant balance of marine bivalves are common and as a whole reflect the variations in the state of the local environment, and are normally tolerated. The wedge clams inhabiting the region of Primorsko also showed the presence of low oxidative impact and good antioxidant protection, but here they had relatively lower physiological functionality, which may be a prerequisite for reduced fitness and resistance capacity (stress alarm phase). In the wedge clams from Slanchev Bryag, Sveti Vlas and Tsarevo somewhat higher oxidative impact was present with induced compensatory activation of the antioxidant defence, together with reduced physiological functionality. The condition of wedge clams inhabiting Varna Bay was characterized by high oxidative impact (high level of prooxidants) and, in response, high antioxidant protection level, necessary for compensating and tolerating pressure effects of the changing state variables of the marine environment. Additionally, the wedge clams living here showed also lowered physiological functionality, which was probably due to the mobilization of energy resources for the adaptive response, which caused energy shortage. The health condition of the wedge clams from habitats in the region of Ahtopol was characterised by the presence of higher oxidative impact, very low antioxidant defence activity and reduced physiological functionality. This makes them vulnerable to further/additional stressors pressure and thrives them closer to the limits of their tolerance range to changes in the environmental state variables.

Discussion

In the present study, an attempt was made to access the health condition and adaptive capacity of wedge clams inhabiting natural sandy habitats along the Bulgarian Black Sea coastal zone. Much of the basic knowledge of the individual effects of different stressors, incl. various xenobiotics (metals, PAH, PCB, pesticides, etc.), on marine organisms was the result of laboratory tests. Under natural conditions however, multiple stressors are the rule, with different duration and intensities of impact, and may have cumulative and even antagonistic effects (Steinberg 2012). On the other hand, the phenomenon of induced cross-tolerance in the simultaneous exposure to stressors is believed to prepare the organism to the impact of the next stressor and is crucial for the survival and adaptation of individuals and populations under changing environmental conditions (Crain et al. 2008, Steinberg 2012).

A common effect of stressors on cross-exposed marine organisms is the induced OS, which corresponds to an imbalance between pro-oxidative processes and antioxidant defence mechanisms in the cells, with levels depending also on organisms' sensitivity (i.e. sensitive, tolerant or resistant organisms) (Steinberg, 2012). The tolerance and resistance of organisms allows them to cope with OS stress within some limits. In this study, we showed that the health condition of wedge clams living in different marine environmental conditions, as those in the studied localities, and exposed to different xenobiotics, also differed correspondingly, as indicated by the measured OS biomarkers of damage. Specifically, high values of LPO were found in wedge clams inhabiting Varna Bay, Slanchev Bryag, Sveti Vlas and Ahtopol (Table 2). Our data strongly suggested that this effect was the result of the relatively high contents of Pb and Cd accumulated in the tissues of clams from these locations. A number of previous studies on marine bivalves have similarly shown increased LPO in tissues after exposure to metal elements. Such examples are the increased LPO in the gills and mantle of *M. galloprovincialis* in response to exposure to Cu, Cd, Pb and Fe (Vlahogianni & Valavanidis 2007) as well as in the gills and digestive gland of *M. edulis* after exposure to Cd, Ag and Hg (Geret et al. 2002). Redox-sensitive metal elements, such as Cu, Zn, Mn, and Ni, generate reactive oxygen species (ROS) by the mechanism of the Fenton reaction, while other metal elements, such as Cd and Pb, generate ROS indirectly (Stainberg 2012, Le Saux et al. 2020). In general, the induction of GST activity in different bivalves was established in experiments after exposure to PAH, PCB, dioxins (Van der Oost et al. 2003) and metal pollution (Vidal-Linan et al. 2014). Our results clearly showed that in the studied wedge clams from localities with higher metal content (Slanchev Bryag, Sveti Vlas and Varna Bay) antioxidant mechanisms (for ROS neutralization) were activated, as indicated by the significantly higher activity of GST, which is involved in detoxification processes in cells, eliminating not only metals, but also organic pollutants, as well as OS products. In this context, it can be speculated that the health condition of the wedge clams from Ahtopol was not optimal and their adaptive capacity was lowered, as indicated by the high levels of LPO, PO, relatively low GSH concentrations and low activity of GST. This corresponds well with the high concentration of PAH Sum4, Pb, Cd, bacterial coliforms and MPs found in the tissues of wedge clams from this locality (Table 2).

In the present study, for the first time an assessment of the presence of MPs in wedge clams' tissues was conducted. Given the established significant pollution of the Bulgarian Black Sea part with macroplastics (Berov et al. 2020), it was not surprising that in all wedge clams tested MPs were found. The highest mean number of particles (≤25 µm) in our samples was present in soft tissues of the wedge clams from Ahtopol. Although a significant increase of GSH in response to increased number of microplastics has been reported in a laboratory test (Wang et al. 2020), our study showed somewhat reduced level of GSH in wedge clams with high number of MPs. Although our data on MPs was very preliminary, the observed relatively low concentration of GSH in the clams from Ahtopol together with high number of MPs could be explained by the combined overlapping effects of PAH detoxification, presence of significant amounts of microorganisms, some metals, all of which can contribute to GSH depletion.

Our results demonstrated specific variations in the level of response of the wedge clams to effects of multiple stressors of the marine environment of their habitats in the different studied localities of the Bulgarian Black Sea coastal zone. The wedge clams were found to manage and effectively compensate for various pressures of the environmental stressors, specific for the local marine environment of their habitats, within their range of tolerance. However, the state of the marine environment of the habitats in the region of Ahtopol presented an example of a very high oxidative stress impact, inhibited antioxidant defence capacity and reduced physiological functionality by energy depletion, thus thriving the organism of wedge clams to the limits of their tolerance to environmental stress and making them vulnerable to further/additional stressors.

Conclusion

The main conclusion of our study was that the wedge clams could successfully tolerate, within their adaptive capacity range, the changes in the environmental conditions of their habitats along the Bulgarian Black Sea coast. However, this involved complex mechanisms of antioxidant reaction and detoxification, triggered by specific effects of pressures caused by the multiple stressors of the marine environment. Changes in the pro/antioxidative balance of wedge clams, as bioindicators, can serve as an early warning system for deterioration of the marine environment. Further studies, also on other marine organisms, are necessary to incorporate the antioxidant reactions of suitable marine bioindicator organisms into the methodology of the state of the marine environment assessment and into monitoring programs.

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