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Adaptability of the alien Pacific oyster to the coastal marine environment of the Bulgarian Black Sea and potential implications for ecosystem conservation

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Running title: Adaptability of Pacific oyster in the Black Sea

Abstract

Feral individuals of Pacific oysters (*Magallana gigas* Thunberg, 1793) in natural habitats are increasingly being reported from the Bulgarian coast. Studies on the interactions of the Pacific oyster with native species in local ecosystems are contradictory, and it is not yet definitely established to what extent ecological conditions in the Black Sea are favorable for the Pacific oyster growth and reproduction.

For the first time in this study, an assessment has been made of the adaptive capacity and resilience of the alien Pacific oyster allowing its development in the ecological conditions of the Bulgarian Black Sea ecosystems and the risk of a future mass invasion.

Oysters were gathered manually by SCUBA diving from natural habitats or obtained from shellfish farms. A set of major oxidative stress (OS) indicators: lipid peroxidation, protein oxidation, glutathione level, and activity of antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase, were measured spectrophotometrically, using commercially available kits. The adaptive capacity and resilience of the Pacific oysters were assessed by the integral Specific Oxidative Stress (SOS) index.

The results indicate that *M. gigas* from more polluted localities have higher OS, but it was effectively compensated by their antioxidant system. A comparison was also made between *M. gigas* OS and that of the native species *Mytilus galloprovincialis* Lamarck, 1819, using the SOS index. In some localities, Pacific oysters had even lower SOS index than the native black mussels.

The level of adaptive capacity of the oysters, as indicated by the SOS index, was compared to the phases of Holling's adaptive cycle theoretical model and showed that the Bulgarian Black Sea oyster population is in an initial growth stage (resource exploitation), which confirms its high adaptive potential and resilience.

In conclusion, this study confirms that the Pacific oyster possesses the capacity to cope with the marine environment and the native species, which allows further expansion of the oyster population in the Bulgarian Black Sea part.

More research and monitoring of the *M. gigas* population, along with assessment of their impact on biodiversity and the local ecosystems, are urgently needed for taking adequate management decisions.

Keywords: adaptability, Bulgarian Black Sea, *Magallana gigas*, *Mytilus galloprovincialis,* oxidative stress

Introduction

The Pacific oyster, *Magallana gigas* Thunberg, 1793 (=*Crassostrea gigas*), originates from the Pacific coast of northeastern Asia but has been widely introduced elsewhere for aquaculture following the collapse of native oyster cultures (e.g., *Ostrea edulis* Linnaeus, 1758). Due to their high nutritional value, Pacific oysters are classified by the European Union as a valuable resource for the aquaculture industry (Council

Regulation (EC) No 708/2007, 2022). Besides high exploitation and production in aquaculture in many countries, *M. gigas* is also spreading unintentionally to new areas, mainly via ships. Currently, this species is expanding its distribution in European coastal waters across the former ranges of native oyster taxa, whose habitats have suffered extermination for various reasons (McAfee and Connell 2021). However, studies on the interactions of Pacific oyster with native species in local ecosystems give contradictory results, depending primarily on the habitat, species communities, and climatic conditions (Ruesink et al. 2005, Padilla 2010, Troost 2010).

Pacific oysters typically inhabit intertidal and shallow subtidal zones, preferring hard substrates, littoral and circalittoral rock in sheltered coastal waters. It is a sessile-burrower, primarily herbivorous or detritivorous suspension feeder (Poutiers 1998). Being highly invasive and variable, *M. gigas* has been considered a pest or a noxious species in several countries (Holm et al. 2015) and is classified as a nuisance for several EU member states (Hansen et al. 2023).

The initial introduction of *M. gigas* into the Black Sea occurred in the 1980s along the Crimean coast for aquaculture purposes (Zolotarev 1996). Since then, individuals of the species have also been reported outside aquaculture zones in natural areas (Skolka and Gomoiu 2004; Krapal et al. 2019; Mitov et al. 2020; Aydin and Gül 2021). Feral *M. gigas* specimens have also been found in the Bulgarian Black Sea part, with the first discovery in 2010 from the coast of Sts. Constantine and Helena Resort, and since then, an increasing number of feral specimens are being found. In addition to the spread of isolated individuals, two relatively small wild colonies of *M. gigas* were registered for the first time in the coastal areas of Burgas and Kiten. (Mitov et al. 2020).

There is limited information on the growth and development of the Pacific oyster under the influence of variable marine stress factors (Ferreira et al. 2011). Since the first findings and descriptions of *M. gigas* in the Bulgarian Black Sea no specific studies have been carried out concerning the eco-physiological adaptability of the species to the variable conditions of the local marine environment. This further limits the risk assessment of a possible future mass reproduction of *M. gigas* and colonization of coastal habitats, which concerns competition with native species and the different physiological responses both within and between the species. Here, the black mussels

(*Mytilus galloprovincialis* Lam.) are the native primary consumers in the water food chain and filter feeders that play a key role for the resilience of the whole Bulgarian Black Sea ecosystem.

The vulnerability of organisms to environmental pressure is related to their capacity to resist and recover from physiological stress. Cellular and physiological resilience refers to the organism's ability to withstand stress, which involves various cellular processes, including antioxidant defenses, DNA repair, and cellular reprogramming (Pfau et al. 2015). The coordinated activation of biochemical and physiological pathways is a key aspect of resilience, enabling the organism to adapt and cope with stressors (Basile et al. 2021).

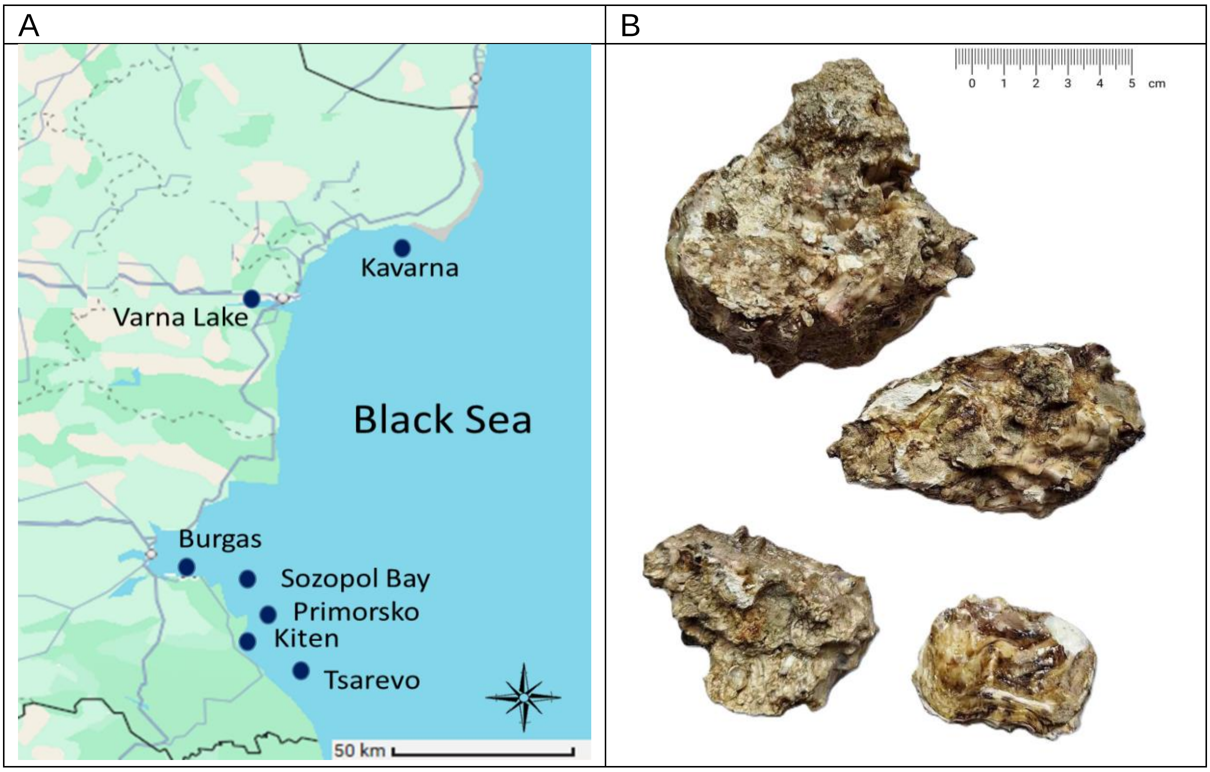
Ecological interaction changes and other stressful conditions can disrupt the balance between pro- and antioxidant processes in the organism, leading to oxidative stress (OS). During pro-oxidant processes, reactive oxygen species (ROS) are produced and can trigger key signaling pathways closely related to adaptation. However, in excess, their high reactivity can damage cellular structures and interfere with the body’s normal physiological functions. It has been shown that OS underpins molecular mechanisms that can reduce the reproduction capacity, growth, abundance, and survival of marine mussels and hence their adaptability (Coppola et al. 2020).

The present study aims to evaluate the specific adaptive capacity of the alien Pacific oyster to the ecological conditions of the Bulgarian Black Sea using OS to indicate the organism's response to the multiple stressors of the marine environment and its potential for resilience and sustainable development. A comparison is also made with the OS in the native species *M. galloprovincialis,* which has long adapted to living in the same habitats, and after a mass *M. gigas* invasion, will be under the risk of competitive suppression.

Materials and methods

Sampling

A total number of 70 specimens morphologically and genetically identified as *M. gigas* (Otero et al. 2013; Amaral and Simone 2014; Mitov et al. 2020) were collected from 7 characteristic localities along the Bulgarian Black Sea coast (Fig.1). Feral individuals of Pacific oysters were gathered manually from their attachment sites by SCUBA diving. Some specimens were obtained from a black mussel farm (Kavarna) where they were attached to mussel collectors. The sampling locations included Kavarna (black mussel farm), Varna Lake, Burgas (Port of Burgas), Sozopol Bay (different sites in the region of Chernomorets and Sozopol), Primorsko, Kiten, and Tsarevo coastal areas. The collected specimens were transferred to the laboratory in thermocontainers with dry ice and stored at -80°C for further analysis.



Location	Coordinates	
	Longitude	Latitude
Kavarna	43°36'77"N	28°34'01"E
Varna Lake	43°19'55"N	27°87'45"E
Burgas	42°46'05"N	27°45'56"E
Sozopol Bay	42°42'98"N 42°35'47"N	27°65'19"E 27°39'07"E
Primorsko	42°27'14"N	27°78'50"E
Kiten	42°24'47"N	27°77'70"E
Tsarevo	42°10'12"N	27°51'23"E

Figure 1. Sampling localities along the Bulgarian Black Sea coast (A) and sampled specimens of *M. gigas* from Sozopol Bay (B).

Samples of *M. galloprovincialis* were also collected from several of the same habitats where Pacific oysters have also settled, for comparative analyses.

Tissue preparation

The soft tissues of each oyster from each site, as well as black mussels from several sites where they co-occur, were dissected on ice and homogenized separately in 0.15 M KCl using a Potter-Elvehjem homogenizer fitted with a Teflon pestle (Thomas Scientific, USA). The homogenates were then centrifuged at 3000 g for 10 min to obtain a post-nuclear fraction, which was used to determine the lipid peroxidation (LPO), protein oxidation (PO), and glutathione levels (GSH). Then a portion of the fraction was re-centrifuged at 12 000 g for 20 min to obtain a post-mitochondrial supernatant, in which the activities of enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST) were measured. All work was carried out at 4°C.

Measurement of oxidative stress biomarkers

All tested OS biomarkers were measured spectrophotometrically using commercially available kits (Sigma-Aldrich Co. LLC, USA): Lipid Peroxidation (MDA) Assay Kit MAK085, Protein Carbonyl Content Assay Kit MAK094, Glutathione Assay Kit CS0260, Superoxide Dismutase Assay Kit MAK528, Catalase Assay Kit CAT100, Glutathione Peroxidase Cellular Activity Assay CGP1, Glutathione Reductase Assay Kit GRSA, and Glutathione-S-Transferase Assay Kit, CS0410.

Protein content was measured according to Lowry et al. (1951) and calculated against a standard curve prepared with bovine serum albumin.

Specific Oxidative Stress (SOS) index

The Specific Oxidative Stress (SOS) index is an integral OS measure calculated according to Yakimov et al. (2018) with some modifications. It is obtained by subtracting the value of the antioxidant (AntiO) score from the Pro-oxidant (Pro) score: SOS = PrO – AntiO, as the PrO score was the average value of the Z-scores of the prooxidant markers tested (LPO and PO), and the AOE score was the average value of the Z-scores of the antioxidants (GSH, SOD, CAT, GPX, GR, and GST). Values near zero correspond to the average (z = 0). Negative and positive values indicate deviation (above or below) from the average for the given site, reflecting the OS level. The SOS index can also serve as an indicator of adaptability.

To visualize the distribution of the SOS index levels employing the relations of pro-oxidant and anti-oxidant indices, a distribution model coordinate system of 4 quadrants was constructed (Fig. 2). In quadrant Q1 (Pro-<0 and Anti-<0) the levels of pro-oxidant and antioxidant processes are below the average values, which indicates very little stress (Underload); in quadrant Q2 (Pro-<0 and Anti->0), the levels of pro-oxidant processes are below the average values, due to the initial activation of the antioxidant defense system (optimum stress, Eustress); in quadrant Q3 (Pro->0 and Anti->0), the levels of pro-oxidant and antioxidant processes are above the average values, which suggests exceeding the capacity of the antioxidant system to effectively neutralize pro-oxidant processes, i.e. too much stress (Overload); in quadrant Q4 (Pro->0 and Anti-<0) the levels of the pro-oxidant processes are increased above the average values, due to depletion of non-enzymatic and/or significant inhibition of enzymatic antioxidants (burnout, Distress).

Statistical analyses

Meaningful post hoc and other two-group comparisons of means were made depending on datasets via the Student’s t-test or the Mann-Whitney test (statistical significance of differences at $p<0.05$). The calculations and statistical analyses of data were carried out using the STATISTICA 10 package (StatSoft Inc., Tulsa, Oklahoma, USA).

Results

The upper and lower shell length and width of *M. gigas* specimens gathered from the studied littoral habitats of the Bulgarian Black Sea were measured, as well as the thickness and wet weight, both in summer and autumn (Table 1). In addition, the ratios of Upper shell width/Upper shell length and Lower shell width /Lower shell length were also calculated (Table 1).

Table 1. Morphometric characteristics (Mean±SD) of Pacific oyster from the studied sites (na - not accessed).

	Upper shell length (mm)	Upper shell width (mm)	Lower shell length (mm)	Lower shell width (mm)	Thickness (mm)	Wet Weight (g)	Upper shell width/Upper shell length	Lower shell width/Lower shell length
Kavarna	**53.7** ±11.5	**43.1** ±10.9	**59.5** ±8.4	**45.1** ±11.4	**25.6** ±4.5	**31.7** ±16.8	**0.81** ±0.17	**0.75** ±0.12
Varna Lake	**76.60** ±14.00	**71.59** ±1.11	**na**	**na**	**30.55** ±1.25	**na**	**0.97** ±0.19	**na**
Burgas	**48.1** ±5.7	**38.5** ±8.8	**50.9** ±7.4	**38.8** ±7.9	**16.6** ±3.3	**17.7** ±8.9	**0.79** ±0.12	**0.76** ±0.10
Sozopol Bay	**94.3** ±16.4	**71.1** ±11.1	**98.3** ±15.6	**72.7** ±10.6	**29.5** ±6.3	**116.6** ±51.8	**0.76** ±0.14	**0.75** ±0.12
Primorsko	**57.70** ±13.64	**60.64** ±16.63	**46.53** ±11.3	**50.34** ±13.80	**18.80** ±7.67	**45.52** ±34.46	**1.04** ±0.04	**1.07** ±0.04
Kiten	**76.1** ±11.5	**62.4** ±10.9	**77.1** ±8.4	**62.2** ±11.4	**34.1** ±4.5	**133.2** ±16.8	**0.83** ±0.17	**0.81** ±0.12
Tsarevo	**67.6** ±22.6	**58.1** ±26.8	**68.6** ±20.6	**59.5** ±28.9	**34.7** ±11.3	**114.2** ±97.8	**0.84** ±0.16	**0.82** ±0.15

Size of the Pacific oyster can approximately indicate age. The size of the Pacific oysters studied by us varied among the sampling localities. Individuals of *M. gigas* having the largest mean length were present in the samples from Sozopol Bay, Kiten, and Varna Lake, corresponding to approximately more than 3-year age. The mean upper shell length of the Pacific oysters from the other studied localities was: Burgas 48.1 mm (1-year age), Kavarna 53.7 mm (1-year age), Primorsko 57.7 mm (1-year age), and Tsarevo 67.6 mm (2-year age) (Table 1). The mean ratios of shell width/shell length were very similar for the oysters from all the studied localities.

The smallest measured mean shell thickness of the oysters was 16.6 mm (Burgas), and the maximum thickness was 34.7 mm (Tsarevo). The mean wet weight of the oysters varied from a minimum of 17.7 g (Burgas) to a maximum of 132.2 g (Kiten). The least mean wet weight was measured for oysters from Burgas. The greatest mean wet weight had the oysters from Kiten (133.2 g), Sozopol Bay (116.6 g), and Tsarevo (114.2 g). Specifically, the high wet weight of the oysters from Sozopol Bay was likely related to their relatively large shell length, but with low shell thickness (Table 1).

The measured values of the basic OS biomarkers of the Pacific oysters from the studied locations varied depending on the region, season and the biomarker (Table 2).

Table 2. Oxidative stress biomarkers (Mean±SD) in *M. gigas* from studied sites (* - difference significant at p<0.05; ** - difference significant at p<0.1).

Sampling Site	LPO	PO	GSH	SOD	CAT	GPx	GR	GST
	nM MDA/ mg protein	nM PC/ mg protein	ng/mg protein	U/mg protein	U/mg protein	U/mg protein	U/mg protein	U/mg protein
Summer (July-August)								
Kavarna (n=6)	**3.61** ±1.35	**7.13** ±1.61	**430.05**** ±115.5	**9.07** ±2.88	**5.49** ±2.75	**7.80** ±0.83	**14.84** ±3.90	**15.77** ±3.42
Varna Lake (n=6)	**4.22** ±0.31	**19.21*** ±3.90	**905.38*** ±430.95	**3.84** ±1.36	**9.41*** ±4.71	**16.30** ±0.79	**16.40** ±3.51	**15.64** ±2.92
Burgas (n=9)	**9.05*** ±2.14	**5.17** ±1.23	**128.71** ±48.02	**162.31*** ±108.45	**1.44** ±0.75	**34.19*** ±16.84	**37.11*** ±17.77	**47.73*** ±20.06
Sozopol Bay (n=14)	**3.79** ±1.08	**5.52** ±0.66	**117.12** ±29.61	**53.00** ±22.43	**0.95** ±0.17	**15.74** ±3.53	**25.44** ±3.41	**17.73** ±3.76
Primorsko (n=3)	**4.07** ±2.93	**2.89** ±0.33	**229.73** ±41.12	**95.95** ±8.61	**1.29** ±0.58	**12.83** ±6.53	**21.01** ±2.92	**22.47** ±5.27
Kiten (n=15)	**2.38** ±0.33	**7.82** ±2.22	**217.12** ±67.42	**33.58** ±23.80	**1.35** ±0.77	**7.13** ±5.57	**26.30** ±7.71	**28.75** ±6.86
Tsarevo (n=4)	**10.12*** ±1.78	**15.13*** ±6.87	**134.01** ±26.62	**259.07*** ±148.64	**0.67** ±0.32	**22.51*** ±8.18	**20.67** ±4.39	**40.21*** ±19.17
Autumn (September)								
Kavarna (n=7)	**3.00** ±0.65	**8.27** ±1.27	**179.01** ±25.99	**5.80** ±0.39	**1.09** ±0.16	**7.14** ±3.30	**15.06** ±2.17	**15.61*** ±1.71
VarnaLake (n=3)	**6.00*** ±1.21	**18.20*** ±4.86	**244.80** ±36.2	**4.70** ±2.1	**4.41*** ±2.6	**15.12*** ±12.1	**7.80** ±1.4	**11.52** ±2.78
Kiten (n=3)	**2.095** ±0.01	**7.80** ±2.55	**374.63*** ±30.44	**9.47*** ±1.80	**1.25** ±0.02	**3.41** ±1.28	**9.28*** ±0.85	**13.67**** ±1.52

The two pro-oxidative biomarkers, LPO and PO, reflect the cellular oxidative changes in the studied organisms in response to environmental pro-oxidant stressors. The highest LPO values in summer were observed in oysters from Tsarevo (10.118±1.78 nmoles MDA/mg protein) and Burgas (9.05±2.14 nmoles MDA/mg protein), while in autumn, the highest values were in oysters from Varna Lake (6.00±1.21 nmoles MDA/mg protein). The highest PO was found in oysters from Varna Lake, both in summer and autumn (19.21±3.90 and 18.20±4.86 PC/mg protein, respectively) and in samples from Tsarevo (15.13±6.87 nmoles PC/mg protein) during summer. Overall, the results indicate that, in summer, oysters from Tsarevo and Varna Lake experienced the highest stress, with oxidative processes dominating, and in autumn, the most stressed were oysters from Varna Lake.

The antioxidant defense system includes the non-enzymatic GSH and enzymes such as SOD, CAT, GPx, and GR. The highest GSH levels were observed in the soft tissues of oysters from Varna Lake during summer and in samples from Kiten in autumn (Table 2). The lowest GSH concentrations were found in oysters from Sozopol Bay, Burgas, and Tsarevo. Extremely high activities of SOD were seen in summer specimens from Tsarevo and Burgas. High CAT activities were recorded in oyster individuals from Varna Lake in both summer and autumn. Notably, the activities of the enzymes GPx, GR, and GST were the highest in summer samples from Burgas. This suggests that the oysters there were strongly fighting against pro-oxidant pressure, but the stress was too intense, leading to significant oxidative changes, especially in cellular lipids. Similarly, in summer, oysters from Tsarevo showed high activities of GPx and GST (Table 2) together with high GST activities in the oysters from Burgas and Tsarevo, which suggested the presence of high levels of contamination, as GST is essential for cellular detoxification by various xenobiotics. In addition, GST catalyzes the degradation of OS products.

The OS biomarkers in *M. galloprovincialis* individuals (4.9–6.0 cm length) from several of the habitats where the Pacific oysters have also settled were measured for comparative purposes (Table 3).

Table 3. Oxidative stress biomarkers (Mean±SD) in *M. galloprovincialis* from the studied sites (* - difference significant at p<0.05; ** - difference significant at p<0.1).

Sampling Site	LPO	PO	GSH	SOD	CAT	GPx	GR	GST
	nM MDA/ mg protein	nM PC/ mg protein	ng/mg protein	U/mg protein	U/mg protein	U/mg protein	U/mg protein	U/mg protein
Summer (July-August)								
Kavarna (n=10)	**4.26** ±1.49	**6.02** ±0.97	**1374.75** ±380.2	**6.50** ±7.07	**0.27** ±0.12	**4.25** ±2.39	**12.18** ±1.57	**19.26** ±2.40
Varna Lake (n=6)	**4.46** ±1.33	**18.68** ±2.25	**234.91** ±128.71	**4.58** ±1.55	**1.39*** ±0.28	**8.54** ±0.84	**27.21**** ±10.86	**21.91** ±13.68

Sozopol Bay (n=12)	**2.02** ±0.49	**4.85** ±1.54	**406.93** ±46.01	**1.72** ±0.44	**0.20** ±0.06	**4.05** ±1.53	**3.80** ±0.83	**18.72** ±3.92
Primorsko (n=6)	**2.07** ±0.86	**16.19** ±1.49	**184.17** ±31.18	**2.47** ±0.59	**0.24** ±0.05	**3.33** ±0.92	**5.66** ±2.70	**12.98** ±3.24
Autumn (September)								
Kavarna (n=6)	**4.56** ±0.88	**8.39** ±1.07	**1760.33*** ±75.55	**1.36** ±0.06	**0.50** ±0.18	**2.65** ±0.83	**11.94** ±3.54	**14.92** ±3.13
Varna Lake (n=6)	**4.73*** ±0.99	**8.34** ±5.25	**163.28** ±86.90	**1.86** ±0.87	**0.41** ±0.12	**10.96** ±1.86	**8.95** ±5.47	**15.60** ±9.92

The analysis of the OS biomarkers in *M. galloprovincialis* revealed varying OS responses depending on habitat and season (Table 3). During both summer and autumn, relatively high LPO levels were observed in black mussels from Kavarna and Varna Lake. Herein, similar values were measured for oysters from the same locations. (Table 2). The highest levels of protein oxidation PO were recorded in summer in black mussels from Varna Lake and Primorsko (18.68±2.25 and 16.19±1.49 nmol PC/mg protein, respectively). In *M. gigas*, the highest values of PO were also found in individuals from Varna Lake (19.21±3.90 nmol PC/mg protein, Table 3).

In the black mussels from Varna Lake, relatively low levels of GSH were measured in both seasons, while in those from Kavarna, GSH concentrations were 5 to 10 times higher. In the specimens from Primorsko in summer, low GSH levels were also detected. Notably, elevated activities of CAT, GPx, and GR were found in mussels from Varna Lake in summer, while GPx activity remained high in black mussel specimens from Varna Lake in autumn, as well (Table 3). Similarly, in autumn, the GPx activity in the oysters from this location was also high (Table 2).

Based on the measured OS biomarkers of both co-occurring species, the integral SOS index was calculated to assess and make a comparison of the physiological resilience and adaptability of the alien *M. gigas* and the local *M. galloprovincialis* individuals developing in one and the same habitat (Table 4).

Table 4. Pro-oxidant, anti-oxidant indexes and the Specific Oxidative Stress (SOS) index (z-transformed) of *M. gigas* in comparison with *M. galloprovincialis* from the studied sites.

Magallana gigas				***Mytilus galloprovincialis***			
Site	**PRO-oxidant index**	**ANTI-oxidant index**	**SOS index**	**Site**	**PRO-oxidant index**	**ANTI-oxidant index**	**SOS index**
Summer (July-August)							
Kavarna	-0.518	-0.493	**-0.025**	**Kavarna**	0.755	0.665	**0.090**
Varna Lake	1.107	0.678	**0.429**	**Varna Lake**	1.414	-0.698	**2.112**
Burgas	1.111	0.864	**0.247**				
Sozopol Bay	-0.805	-0.747	**-0.058**	**Sozopol Bay**	-1.766	-1.659	**-0.107**
Primorsko	-1.176	-0.425	**-0.751**	**Primorsko**	1.882	1.890	**0.008**
Tsarevo	1.197	0.529	**0.667**				
Kiten	-0.752	-0,569	**-0.182**				
Autumn (September)							
Kavarna	-0.909	-0.177	**-0.732**	**Kavarna**	0.170	1.019	**-0.849**
Varna Lake	2.421	0.322	**2.101**	**Varna Lake**	0.234	-0.499	**0.734**
Kiten	-2.244	-0.589	**-1.654**				

The calculated SOS index allows, among others, an integrated and comparable assessment of the effects of multiple stress factors of the local marine environment on the biota, including cell and physiological resilience and adaptability.

The data of the calculated SOS index (Table 4) showed that there were differences in the stress response of *M. gigas* among localities. The results of the differences in the pro-oxidant and anti-oxidant indexes, indicated by the SOS index of the invasive Pacific oysters and the native black mussels, are presented in the coordinate system in Fig. 2. The SOS of the Pacific oysters sampled from both seasons indicated two groups of localities – one group where oysters were with low stress (Q1) (Fig. 2A) and one group of localities with higher SOS index of oyster but with effective antioxidant system functioning (Q3) (Fig. 2A). The summarized and standardized SOS indexes clearly showed that none of the oysters from the studied localities falls into the red stress risk zone (quadrant Q4) (Fig. 2).

A) B)

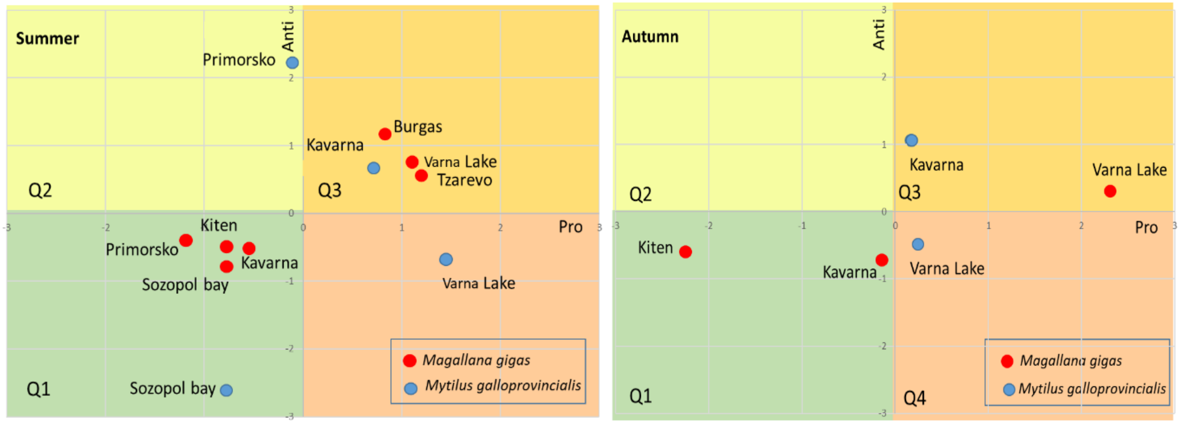


Figure 2. Visualization of the specific oxidative stress index values in the coordinate system of the pro-oxidant and anti-oxidant indexes of *M. gigas* in comparison with *M. galloprovincialis* from the studied infralittoral sites in A) summer and B) autumn.

By comparison, the SOS index of the local *M. galloprovincialis* in summer was much higher for several localities where *M. gigas* was also present. The SOS index of *M. gallorovincialis* from Primorsko indicated initial activation of the antioxidant defense system (Q2=optimum stress), unlike *M. gigas* from the same locality, which showed too little stress (Q1=underload) (Fig. 2A). In both seasons, the SOS index of oysters from Kavarna showed very little stress (Q1=underload) while the black mussels from this same locality were more stressed (Q3=overload), which suggests exceeding the capacity of their antioxidant system to neutralize the pro-oxidant processes (Fig. 2A). In Varna Lake, the black mussels were at the limit of their resilience capacity, i.e. near the burnout stage (Q4=burnout) in both seasons. This was most likely due to excessive environmental pressure, which suppressed the activity of cellular non-enzymatic antioxidants. In contrast, *M. gigas* from this habitat had lower stress (Q3=overload) (Fig. 2A and B). Interesting results were present for Sozopol Bay, where both species were not stressed and fell in the Q1 quadrant with the black mussels having even lower OS, which could be expected as this is the native species of this ecosystem (Fig. 2A).

Discussion

After the introduction of *M. gigas* to the Black Sea for aquacultural purposes in the 1980s (Zolotarev 1996), findings of feral individuals in natural habitats are increasingly being reported (Mitov et al. 2020) for the Bulgarian coast. This coincided with the registered beginning of the mass decline (the 1980s and 2000s) of the Black Sea oyster (*Ostrea edulis* L., 1758) (Todorova et al. 2009) and raises the possibility of a *C. gigas* mass invasion.

Given that the Black Sea has initially been considered unfavorable for the development of *M. gigas* without artificially created hydrobiological conditions (Fabioux et al. 2005; Anglès d'Auriac et al. 2017; Ibarra et al. 2017; Zaharia and Crivăţ 2017; Krapal et al. 2019), recent findings of Pacific oysters in natural habitats raise the question of their adaptive capacity and growing and developing in the ecological conditions of the Bulgarian Black Sea.

For the first time in this study, the adaptability of Pacific oysters from different locations in the Bulgarian Black Sea infralittoral was evaluated, using the "stress-response" approach. Feral individuals of Pacific oysters were collected and analyzed from both northern and southern sites. The size of the Pacific oyster can indicate the approximate age class (Diederich 2006; Cardoso et al. 2007; Walles et al. 2015) although this inference should be cautiously accepted, since the growth may differ across habitats and regions. The studied Pacific oysters varied in size among the sampling locations and their approximate age appeared to be mainly between 1 and 3 years.

The largest *M. gigas* individuals were found in Sozopol Bay (near Chernomorets) and Kiten, which corresponds to an age of approximately 3-4 years. This suggests that the presence of the *M. gigas* feral population in the Bulgarian Black Sea part is relatively recent.

For a better evaluation of the adaptive capacity of *M. gigas* to the environment of the Bulgarian Black Sea, we compared their OS level with that of the local *M. galloprovincialis* populations, as native inhabitants of the same infralittoral rocks where the oysters have also settled. The OS levels of *M. gigas* individuals indicated the stressfulness of the local environmental conditions of the habitats. The calculated SOS index was the integrated indicator of the physiological resilience and adaptability of the Pacific oysters to the marine environment of the Bulgarian Black Sea part. The SOS index strongly indicated the presence of shifts in *M. gigas* pro-antioxidant balance depending on the locality and season. The *M. gigas* individuals settled in the infralittoral of the coast of Kavarna, Sozopol Bay, Kiten, and Primorsko seemed to have little OS, thus showing their high cell resilience and adaptive capacity to the ecological conditions of the habitats.

Although *M. gigas* and *M. galloprovincialis* live in the same habitats, comparing their SOS indexes demonstrated differences in their stress response reactions to the environmental conditions of the same localities and season (Fig. 2). The studied *M. gigas* from Kavarna had lower SOS index levels, compared to *M. galloprovincialis* from the same site. Since here the analyzed Pacific oyster individuals were gathered from the collectors of the mussel farm, they likely had found there favorable conditions for development, very probably associated with the characteristics of the local marine environment and its maintenance following the norms of Bulgarian and European requirements, standards, and directives on the cleanliness and quality of coastal marine waters for shellfish farming, as well as the cleanliness and quality of the production itself (Black Sea Shells Ltd: https://www.blackseashells.com). In contrast, the black mussels from Kavarna exhibited high OS levels (Fig. 2), likely due to their intensive commercial exploitation for food purposes, particularly toward the end of the active tourist season.

Sozopol Bay is a specific ecosystem, and here, together with the abundant black mussels on the rocky habitats, a relatively large number of feral Pacific oysters was also found, which were the largest in size. The bay of Sozopol is located on Bulgaria's southern Black Sea coast and encompasses a variety of marine habitats, including rocky substrates favorable for Pacific oyster development and also a submarine "petrified forest" with many trunks rising from the bottom of the bay, representing a new specific habitat (Chipev et al. 2024). Here, the individuals of the two studied species were found to be unstressed with SOS index corresponding to the Q1 quadrant in our model (Fig. 2), while the co-inhabiting native black mussels had even lower values of the SOS index. These results may be due to the fact that Sozopol Bay is a biologically diverse marine ecosystem, which is also under protection as part of the European Natura 2000 network (sites BG0000146 and BG0001001). Hence, it seems possible that here the presence of *C. gigas* may induce a form of species niche separation.

On the other hand, Pacific oysters from Varna Lake, Tsarevo, and Burgas showed high levels of OS, indicated by the higher values of pro-oxidant markers LPO and PO, despite the positive response of the antioxidant defense system (Table 4). Significant difference was observed when comparing the OS response of *M. gigas* and *M. galloprovincialis* collected from Varna Lake. Here, the *M. gigas* specimens showed lower OS and higher cell resilience to the traditionally adverse environmental conditions in this area. In fact, Varna Lake is known for the presence of significant pollution with heavy metals and petroleum products, as well as a high degree of eutrophication (Ganchev et al. 2023). Pacific oysters are known to have high tolerance to environmental pollutants similar to those in Varna Lake (Boutet et al. 2004; Wang et al. 2018; Shulkin et al. 2003). In addition, their tolerance to hypoxia has also been well documented (Johnson et al. 2009; Steffen et al. 2023; Adzigbli et al. 2024). It is established that the Pacific oyster cell resilience is supported by effective detoxification mechanisms, including the production of metallothioneins, activation of cytochrome P450 enzymes, and elevated antioxidant enzyme activity, which together help mitigate the toxic effects of accumulated metals and petroleum-derived polycyclic aromatic hydrocarbons, enabling Pacific oysters to persist in contaminated and low-oxygen habitats (Boutet et al. 2004; Wang et al. 2018). It was also reported that Pacific oysters possess a higher number of stress-responsive genes compared to most other marine animals, likely resulting from evolutionary gene duplication events associated with their adaptation to the dynamic intertidal environments (Wang et al. 2018).

The multicomponent antioxidant system of Pacific oysters integrates several lines of defense (Ighodaro and Akinloye 2018) and plays a vital role in maintaining redox homeostasis. The increased activity of the antioxidant system, with both enzymatic and non-enzymatic components, can overlap with the vulnerability of the organism to environmental load, expressed by OS development. This helps oysters to preserve their physiological resilience and expands their adaptation to local marine environmental conditions. The increase of physiological adaptive capacity improves the opportunity of organisms to manage varying ranges and magnitudes of stressogenic impacts while also providing the flexibility to adjust their strategies if future conditions reveal that a current strategic trajectory is unsustainable or undesirable (Smit et al. 2001; Engle 2011). Thus, it seems that Pacific oysters, besides the other, are also genetically equipped to cope with environmental stressors and changes, in advance. In this respect, their adaptability, i.e., their ability to adjust and respond to effects caused by stress (Smit et al. 2001; Engle 2011), is definitely high. In contrast, the black mussels from Varna Lake showed significantly higher values of SOS, which place them in the Q4 quadrant risk red zone (Overload) (Fig. 2).

The presence of not only single feral individuals but also of initial colonies of *M. gigas* along the Bulgarian Black Sea coast (Mitov 2020) raises questions concerning the increasing development of oyster colonies and their potential ecological consequences. The environmental repercussions of coexistence and competition between native and invasive species are believed to be a powerful driver of ecological change in both a community and ecosystem context (Grosholz, 2002). It can be expected that *C. gigas, as an invasive species,* can induce changes in local plankton composition, habitat heterogeneity and biodiversity, carrying capacity, food webs, and parasite life cycles (Diederich 2006; Troost 2010; Waser et al. 2016). High survival and growth rates of the introduced Pacific oysters may cause restrictions on habitat use by the native *M. galloprovincialis* mussels, as they may outcompete them by invading their niche, or alternatively, the partitioning of the niche can allow coexistence of the two species. On the other hand, the development of Pacific oyster reefs can lead to higher biodiversity (van Broekhoven 2005) and also provide ecosystem services such as protecting shorelines from erosion and flooding (Bongarts Lebbe et al. 2021; Gonzalez et al. 2021; NOAA Fisheries 2022).

Measures and management of Pacific oyster invasions of European coastal and marine ecosystems have long been discussed, including preventing further spread of this species and colonies' destruction, followed by restoration of invaded local ecosystems (Herbert et al. 2016; Hansen et al. 2023).

Recent research on the Romanian Black Sea coast led to the conclusion that the newly established population of *M. gigas* seems to fill the ecosystem niche vacated by the native Black Sea oyster (*Ostrea edulis* L., 1758) due to the invasion of *Rapana venosa* (Valenciennes 1846), and this is believed to have a positive ecological effect (Krapal et al. 2019).

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In an attempt to seek compliance with system theory stages of a possible massive increase in Pacific oyster population abundance in the Bulgarian Black Sea habitats based on resilience and adaptability we imposed the calculated values of the SOS indicators distributed within the four quadrats of our stress-response model (Fig. 2) on the corresponding phases of Holling's theoretical system adaptive cycle (Holling's Loop) model scheme (Holling 2001; Holling and Gunderson 2002; Sundstrom and Allen 2019) (Fig. 3).

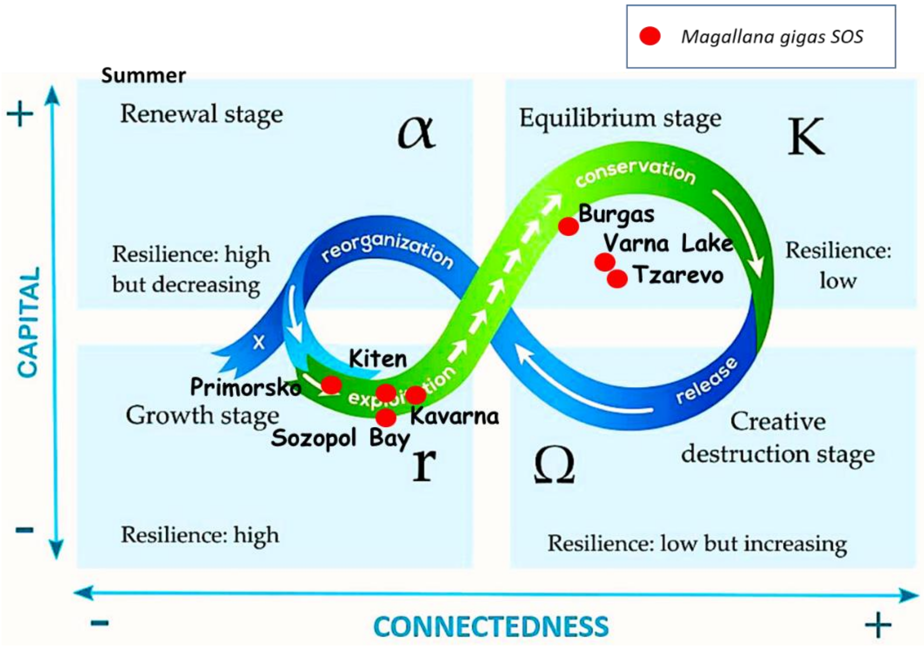

Figure 3. Schematic presentation of Holling's adaptive cycle four-phase model with transferred points of Pacific oyster stress response intensity level measured in the present study.

In general, the calculated here SOS response indexes, which position the Pacific oysters from all the studied localities in two quadrats (Q1 and Q3) of our model (Fig. 2) also correspond to two squares (stages) of Hill's theoretical model, i.e. the growth-equilibrium stage of the expansion process (Fig. 3). Thus, the newly obtained figure indicates and clearly visualizes that the Pacific oyster population in the Bulgarian Black Sea is in a stage of growth (resource exploitation) which confirms its high adaptive potential and resilience.

Conclusions

Since the first findings of *M. gigas* in the Bulgarian Black Sea part no specific research has been carried out concerning the eco-physiological adaptability of this invasive species to the local marine ecological conditions.

The present study is the first to analyze and provide initial data on the adaptive capacity of *M. gigas* to the specific conditions of the Bulgarian Black Sea part. The obtained results confirmed that high survival and growth rate of *M. gigas* in the Bulgarian Black Sea infralittoral is entirely possible and is in progress.

The Pacific oysters obviously possess the adaptive capacity and resilience to cope with the native species and the marine environment, and their population to expand in the Bulgarian Black Sea part, which is in agreement with other views (Orlenko 2012; Hansen et al. 2023) defining the Pacific oyster as a permanent allochthonous species for the Black Sea fauna.

Further detailed studies on the dispersal and reproduction of *M. gigas* in the Bulgarian Black Sea coastal region are urgently needed. Proper monitoring is still not carried out, as well. The lack of scientific data and monitoring greatly limits the possibility of estimating objectively enough the possible effects of a future alien Pacific oyster population expansion on the local marine biodiversity and the native ecosystems. Only objective scientific research data can provide a sound basis for any future actions, whether to implement measures preventing *M. gigas* further spreading and abundance or, conversely, to support a regulated development of their population for ecosystem engineering and/or commercial purposes.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Albena Alexandrova and Nesho Chipev conceived and designed the study. Plamen Mitov and Lyubomir Kenderov obtained the samples and contributed to data analyses and summaries. Elina Tsvetanova, Almira Georgieva, Madlena Andreeva, Georgi Petrov, and Georgi Pramatarov performed the biochemical analysis and contributed to data analyses and summaries. Albena Alexandrova and Nesho Chipev supervised the data analysis and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability

Researchers wishing to access the raw data used in this study can make a request to the corresponding author

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