ACTA ZOOLOGICA BULGARICA

Acta Zool. Bulg., June 2024, 76 (2): 235-239

Aquatic Ecology
Research Article

Published online 25 May 2024
DOI: https://doi.org/10.71424/azb76.2.002780

Assessment of Seasonal Changes of the Oxidative Status of Red Mullet *Mullus barbatus* L. (Actinopterygii: Mullidae) from the Bulgarian Black Sea Coastal Area

Elina R.Tsvetanova*, Albena V. Alexandrova, Almira P. Georgieva, Madlena N. Andreeva & Nesho H. Chipev

Laboratory of Free Radical Processes, Institute of Neurobiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria; elinaroum@yahoo.com

Abstract: Oxidative stress (OS) in aquatic organisms is associated with pressures within the marine environment by both anthropogenic and natural factors like pollution, climate change, ecological interactions, etc. These pressures can induce irreversible changes in the organism's status and health influencing communities and ecosystems as well. Red mullet is a widely distributed and commercially important demersal fish species in the Black Sea. This makes it a promising bioindicator for the state of the marine environment and ecosystem health. This study aimed to investigate the effect of seasonal variations in the marine environment on OS in red mullet from four sites on the Bulgarian Black Sea coast – Kavarna, Kamchiya, St. Vlas and Sozopol. Most of the measured OS biomarkers displayed seasonal variations in the two studied fish organs – liver and gills. In autumn, higher lipid peroxidation levels and activities of superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST) were presented in the liver of fish from all studied sites. In gills, higher activity of SOD, CAT, GST, and acetylcholinesterase were measured in the summer. In conclusion, the significant local variability of the sea environment quality induced different levels of OS in autumn in the fish liver and in summer in the gills for all studied sites. The measured seasonal fluctuations in the red mullet's OS biomarkers can be successfully used for biomonitoring and environmental marine risk assessment.

Key words: Black Sea, oxidative stress, red mullet, seasonal variations

Introduction

The state of the marine environment is a result of complex effects of both natural and anthropogenic origin, including pollution. They can have additive, synergistic, and even antagonistic effects on the environment and marine biota. Traditionally, the assessment of the marine coastal ecosystems state is based on heavy metals and persistent organic pollutants measurement in water, sediments, and some indicator organisms (Kouali et al. 2022). To provide more reliable information about the health of marine fish and ecosystems as a whole, it is becoming necessary to combine traditional environmental state measurements with more specific biological responses of organisms. One way to assess these

[*]Corresponding author: elinaroum@yahoo.com

[**]Paper presented at the International Seminar of Ecology – 2023 (Sofia, Bulgaria, 29-30 September 2023) organized by the Institute of of Biodiversity and Ecosystem Research (Bulgarian Academy of Sciences) and the Biology Section of the Union of Scientists in Bulgaria.

complex responses is by using oxidative stress (OS) biomarkers in the fish organism. All pollutants are capable of intensifying the intracellular reactive oxygen species (ROS) generation, of disturbing antioxidant efficiency, and of inducing OS (Van der Oost et al. 2003).

Data demonstrate significant seasonal alterations in pollutants' concentrations in marine water, sediments, and organisms (Recabarren-Villalón et al. 2021). Additionally, the seasonal variations, such as temperature, UV radiation, oxygen content, salinity etc. in water bodies influence important physiological processes in animals such as reproduction, hibernation, aestivation, immune functions, behaviour, which are all practically governed by metabolic activities (Lehninger et al. 2008). This may lead to changes in the pro/antioxidant balance of the organisms and affect OS parameters in marine biota.

Red mullet *Mullus barbatus* Linnaeus, 1758 is a Mediterranean immigrant in the Black Sea with 48,6% estimated frequency of occurrence in the Bulgarian shelf (2015). It is a demersal fish with commercial importance, which also plays a significant role in marine and estuarine trophic webs. Hence, the red mullet can be considered as key indicator species of the ecological state of its habitats in the Bulgarian Black Sea shelf. Fish gills and liver have been recently recommended as suitable target organs for environmental monitoring (Yancheva 2020; Shah et al. 2022). Gills have uninterrupted contact with the environment and thus are the first to uptake and accumulate contaminants. Hence, fish gills respond earlier than the liver and kidneys to contamination exposure (Pereira et al. 2010). The liver is a major metabolic organ and responsible for the detoxification of various endogenous and exogenous substances (Ardeshir et al. 2017). Specifically, fish liver and gills have proved to be important as short-term indicators of long-term biological environmental effects (Restivo et al. 2021).

Thus, the present study aimed to investigate the effect of seasonal variations in the marine environment on *M. barbatus* inhabiting the Bulgarian Black Sea shelf by the fish responses to stress. Our approach included estimation of the main OS biomarkers: lipid peroxidation (LPO), glutathione content (GSH), as well as antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and acetylcholinesterase (AChE).

Materials and Methods

Study areas and sampling sites

The fish were sampled during monitor trawling in summer (10-15 July) and autumn (5-10 November) 2021-2022 from four selected sites with intensive fishing activities – Kavarna (S1), Kamchiya (S2), St. Vlas (S3) and Sozopol (S4) (Table 1). On board, the fish were immediately shock frozen.

Tissue preparation

After defrosting each individual organ (n=12 for each site) was dissected and the liver and gills were removed on an ice bath. Both types of organs were homogenized in 0.1M K-PO_4 (pH 7.4) using a Potter Elvehjem homogenizer with a Teflon pestle (Thomas Scientific, USA). A post-nuclear fraction for determination of LPO and GSH levels was obtained by centrifugation of the homogenates for 10 min at 3000g and the post mitochondrial supernatant, used for measurement of the antioxidant enzyme activities, by re-centrifugation of the post-nuclear fraction at 12 000 g for 20 min. All operations were performed at 4°C.

Biochemical analysis

The biomarkers were measured spectrophotometrically using kits - purchased from Sigma-Aldrich Co. LLC(USA): Lipid peroxidation (MDA) Assay

Table 1. Sampling sites, coordinates, and condition index (CI).

Code	Site	Depth	N initial-final	E initial-final	Temp. July	Temp. November	CI summer	CI autumn
S1	Kavarna	15 m	43.392290°-43.388565°	28.172389° - 28.180401°	25°C	15°C	50,81	41,50
S2	Kamchiya	19m	43.022311° - 43.021683°	27.890776° - 27.891044°	25°C	15°C	58,89	55,00
S3	St. Vlas	24m	42.694399° - 42.680229°	27.760862° - 27.768114°	26°C	17°C	45,52	56,27
S4	Sozopol	35m	42.433997° - 42.433272°	27.705534° - 27.710062°	26°C	17°C	48,49	45,24

Kit MAK085,Glutathione Assay Kit CS0260,SOD Assay Kit-WST 19160,CatalaseAssay Kit CAT100,Glutathione Peroxidase Cellular Activity Assay CGP1,Glutathione-S-Transferase Assay Kit CS0410, Acetylcholinesterase Activity Assay KitCS0003.

Statistical analyses

Statistical analyses were carried out by ANOVA. The significance of differences in the values of every OS biomarker was compared using Student's t-test ($p \leq 0.05$).

Results

Seasonal differences were established in most of the biomarkers in both organs (Tables 2, 3). In the liver elevated LPO levels were observed in fish from points S1 and S4 in autumn, and in those from S2 in summer (Table 2). In the gills, significant differences were not detected. In samples from the summer in comparison to those from the autumn, the liver GSH content was lower in fish from S1 and S3 (Table 2) and in gills it was higher in fish from S1, S3, and S4 (Table 3). In the livers of fish, caught in autumn, higher activities of SOD were measured in the samples from all sites; of CAT – in samples from S1and S2, of GST – in samples from S4, and of AChE – in samples from S3 (Table 2). In autumn samples in comparison to summer samples, significantly lower liver activities were established for CAT in fish from S3, for GPx in fish from S4, and for GST in fish from S2 (Table 2). Significant differences were also present in gills: higher enzyme activities in the summer were established for SOD in fish from S1, S3, and S4, for CAT in fish from S2 and S4, and for GPx in fish from S1. Lower activities were measured in SOD of fish from S1, S3, and S4, in CAT of fish from S3, in GST of fish from S2,S3,and S4, and in AChE - from S1,S3,and S4 (Table 3).

Discussion

Climate changes, both seasonal and global, are suggested to affect physicochemical parameters of water like salinity, acidification, temperatures etc., which inevitably disrupts the intracellular mechanisms of aquatic organisms, including distress in regular biological processes and redox-balance, resulting in raised metabolite rates, oxygen consumption and overproduction of ROS (Birnie-Gauvin 2017). In an attempt to assess the influence of seasonal changes on the health condition of *M. barbatus* and the environmental health of the Bulgarian Black Sea costal ecosystems we investigated responses of main pro/antioxidant OS biomarkers in the gills and liver of the fish from 4 sites, exposed to the impacts of different stressors.

The extent of existing physiological tolerance and adaptive mechanisms of *M. barbatus* against stressors and their fluctuations, together with their collective impacts, is still unclear. Researchers reported seasonal variations of red mullet OS responses in gills and liver, which demonstrated different pro-oxidant challenge in winter and summer (Pavlović et al. 2010; Pereira et al. 2010). Latest data reported that red mullet exhibited seasonal-related gene expression of some antioxidant enzymes (Frappicini et al. 2021). It could be suggested that the seasonal downregulations in the synthesis of some antioxidants determines greater susceptibility of red mullets to the environmental pressure.

The overall results obtained in this study indicated a significant influence of seasonal variations of different factors on the pro/antioxidant balance in the liver and gills of red mullet from the Bulgarian Black Sea shelf area. Our data showed higher OS in the liver of fish from Kavarna and Sozopol in autumn compared to the fish caught in summer, judged by increased LPO and activated SOD. In general, a more active liver antioxidant defence was observed in the autumn fish samples (Table 2). This suggests the presence of pressure from the environment, which is compensated by the activation of the cellular defence system of the fish. Similar observations on the influence of seasonality on the activities of antioxidant enzymes in the liver were also found in red mullets from the Adriatic Sea (Pavlović et al. 2010). In gills, the opposite trend was observed, namely lower levels of antioxidant defence in fish caught in autumn compared to fish caught in summer (Table 3). Depletion of non-enzymatic antioxidant (GSH) and the inhibition of the enzymatic antioxidant (SOD) may be the result of prolonged pressure during the summer season, possibly as a result of household pollution due to tourist activity. The relatively worse health condition of the red mullet was also confirmed by the low activity of AChE. Although inhibition of AChE is assumed as a marker for the neurotoxic effects of pesticides in marine organisms, it has been shown that AChE activity can be reduced under exposure to treated municipal effluents (Kamel et al. 2012).

Our observation of tissue specific changes in antioxidant enzymes in relation to season was in line with data of da Rocha et al. (2009). In general, the use of enzyme activities as bioindicators in marine

Table. 2. Seasonal changes in red mullet liver oxidative stress biomarkers (± error of mean) in Summer vs Autumn in Bulgarian Black sea costal area (Shapiro-Wilk test; *- significant differences: $p \leq 0.05$*; $p \leq 0.01$**; $p \leq 0.001$***)

OS marker	LPO (nm MDA/mg protein)		GSH (ng/mg protein)		SOD (U/mg protein)		CAT (U/mg protein)		GPX (U/mg protein)		GST (U/mg protein)		AChE (U/mg protein)	
Site	**Summer**	**Autumn**	**Summer**	**Autumn**	**Summer**	**Autumn**	**Summer**	**Autumn**	**Summer**	**Autumn**	**Summer**	**Autumn**	**Summer**	**Autumn**
S1	0,53± 0,14**	0,87± 0,2	413,2± 129,14**	633,85± 129,24	25,70± 5,83***	68,51± 6,28	5,14± 1,97**	7,91± 0,83	3,45± 0,86	4,38± 1,49	85,54± 19,94	113,72± 64,66	70,62± 14,10	58,03± 12,72
S2	1,28± 0,49*	0,53± 0,06	393,3± 52,06	459,17± 145,15	6,78± 1,00***	45,89± 10,13	5,92± 2,66**	17,86± 4,47	5,70± 1,83	4,19± 1,85	203,44± 64,18*	120,08± 57,74	107,78± 44,06	73,11± 13,40
S3	0,93± 0,16	0,95± 0,15	239,13± 50,71**	376,62± 110,80	18,29± 4,61***	72,90± 15,41	9,70± 2,18*	5,93± 2,06	3,39± 1,48	3,03± 0,61	276,48± 123,88	255,71± 90,30	72,96± 17,43**	135,87± 45,30
S4	0,77± 0,39*	1,24± 0,34	441,43± 42,22	467,89± 80,24	31,02± 19,05**	58,11± 9,70	6,96± 2,56	7,77± 0,62	5,86± 2,28*	2,85± 0,30	85,90± 43,06***	327,17± 76,87	60,29± 34,37	89,86± 19,05

S1 – Kavarna; S2 – Kamchiya; S3 – St.Vlas; S4 - Sozopol

Table. 3. Seasonal changes in red mullet gill oxidative stress biomarkers (± error of mean) in Summer vs Autumn in Bulgarian Black sea costal area (Shapiro-Wilk test; *- significant differences: $p \leq 0.05$*; $p \leq 0.01$**; $p \leq 0.001$***)

OS marker	LPO (nm MDA/mg protein)		GSH (ng/mg protein)		SOD (U/mg protein)		CAT (U/mg protein)		GPX (U/mg protein)		GST (U/mg protein)		AChE (U/mg protein)	
Site	**Summer**	**Autumn**	**Summer**	**Autumn**	**Summer**	**Autumn**	**Summer**	**Autumn**	**Summer**	**Autumn**	**Summer**	**Autumn**	**Summer**	**Autumn**
S1	11,20± 9,20	12,91± 5,21	449,09± 122,76***	186,94± 35,37	23,62± 8,66**	11,37± 2,75	1,60± 0,39	1,49± 0,39	6,74± 1,92*	9,10± 2,37	37,16± 9,74	28,42± 5,92	292,93± 76,30**	165,06± 50,44
S2	3,31± 1,44	2,64± 0,23	315,91± 43,80	350,85± 33,82	3,20± 0,70***	23,08± 4,40	1,02± 0,17***	1,88± 0,14	9,54± 3,76	16,32± 5,80	68,32± 10,47***	14,97± 3,21	300,07± 36,52	355,86± 92,27
S3	6,32± 7,48	8,32± 6,20	211,68± 49,84**	137,64± 22,51	13,38± 2,72***	7,61± 2,29	4,54± 0,28*	1,46± 0,26	13,46± 2,30	13,60± 2,39	63,05± 10,62*	49,44± 9,35	294,33± 101,59*	182,18± 37,66
S4	14,75± 6,58	16,07± 3,01	411,63± 52,06***	158,89± 38,79	36,82± 8,83**	6,76± 1,76	0,88± 0,29***	2,00± 0,28	9,55± 2,10	14,34± 6,14	24,14± 5,56**	39,61± 7,80	268,54± 69,35***	101,93± 55,73

S1 – Kavarna; S2 –Kamchiya; S3 – St.Vlas; S4 – Sozopol biomonitoring studies is often complicated. On one hand this is because the levels of chemical pollutants in the environment often display wide seasonal variations in response to climate and other factors (Sheehan & Power 1999) and this affects variously the activity and expression of antioxidant enzymes. On the other hand, in marine water, dominant seasonal differences are temperature and concentration of dissolved oxygen, and the antioxidative status in species of marine fish seems to be related to tissue oxygen consumption or to organism activity level (Wilhelm Filho et al. 2000).

In conclusion, the significant local variability, incl. seasonal, of the sea environment quality at the studied sites induced different pro/antioxidant changes of OS biomarker responses in fish liver and gills. Basic knowledge of seasonal OS fluctuations in *M. barbatus* can be successfully used to provide complementary data for the state and monitoring of the ecological condition of the marine ecosystem and national environmental policies for protection.

Acknowledgements: This study was supported by grant КП-06-H41/7 of the Bulgarian National Science Fund.

References

Ardeshir R. A., Movahedinia A. & Rastgar S. 2017. Fish liver biomarkers for heavy metal pollution: A review article. Am J Toxicol 1: 1–8.

Birnie-Gauvin K., Costantini D., Cooke S. J. & Willmore W. G. 2017. A comparative and evolutionary approach to oxidative stress in fish: a review. Fish and Fisheries 18 (5): 928–942.

Da Rocha A. M., Salomão de Freitas D. P., Burns M., Vieira J. P., de la Torre F. R. & Monserrat J. M. 2009. Seasonal and organ variations in antioxidant capacity, detoxifying competence and oxidative damage in freshwater and estuarine fishes from Southern Brazil. Comparative Biochemistry and Physiology 150 (4): 512–520.

Frapiccini E., Cocci P., Annibaldi A., Panfili M., Santojanni A., Grilli F., Marini M. & Palermo F. A. 2021. Assessment of seasonal relationship between polycyclic aromatic hydrocarbon accumulation and expression patterns of oxidative stress-related genes in muscle tissues of red mullet (*M. barbatus*) from the Northern Adriatic Sea. Environmental toxicology and pharmacology 88: 103752.

Kamel N., Jebali J., Banni M., Ben Khedher S., Chouba L. & Boussetta H. 2012. Biochemical responses and metals levels in *Ruditapes decussatus* after exposure to treated municipal effluents. Ecotoxicological Environmental Safety 82: 40-46.

Kouali H., Chaouti A., Achtak H., Elkalay K. & Dahbi A. 2022. Contamination and ecological risk assessment of trace metals in surface sediments from coastal areas (El Jadida, Safi and Essaouira) along the Atlantic coast of Morocco. Journal of African Earth Sciences 186: 104417.

Lehninger L., Nelson D. L. & Cox M. M. 2008. Lehninger Principles of Biochemistry. W. H. Freeman & Co, New York, NY, USA, 5th edition.

Panayotova M. & Todorova V. 2015. Diversity and community structure of fish in the Bulgarian Black Sea shelf area. Journal of Research in Agriculture and Animal Science 3 (3): 7–14.

Pavlović S. Z., Mitić S. S. B., Radovanović T. B., Perendija B. R., Despotović S. G., Gavrić J. P. & Saičić Z. S. 2010. Seasonal variations of the activity of antioxidant defence enzymes in the red mullet (*Mullus barbatus* L.) from the Adriatic Sea. *Marine Drugs* 8 (3): 413–428.

Pereira P., Pablo H., Vale C. & Pacheco M. 2010. Combined use of environmental data and biomarkers in fish inhabiting a eutrophic and metal-contaminated coastal system – gills reflect environmental contamination. Marine Environmental Research 69 (2): 53.

Recabarren-Villalón T., Ronda A. C., Oliva A. L., Cazorla A. L., Marcovecchio J. E. & Arias A. H. 2021. Seasonal distribution pattern and bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) in four bioindicator coastal fishes of Argentina. Environmental Pollution 291: 118125.

Restivo V. E., Kidd K. A., Surette M. G., Servos M. R. & Wilson J. Y. 2021. Rainbow darter (*Etheostoma caeruleum*) from a river impacted by municipal wastewater effluents have altered gut content microbiomes. Science in the Total Environment 10 (751): 141724.

Shah Z. U. & Parveen S. 2022. Oxidative, biochemical and histopathological alterations in fishes from pesticide contaminated river Ganga, India. Science Reports 12 (1): 3628.

Sheehan D. & Power A. 1999. Effects of seasonality on xenobiotic and antioxidant defence mechanisms of bivalve molluscs. Comparative Biochemistry and Physiology, C, 123: 193–199.

Van der Oost R., Beyer J. & Vermeulen N. P. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology 13 (2): 57–149.

Wilhelm Filho D., Torres A., Marcon L., Fraga G. & Boveris A. 2000. Comparative antioxidant defences in vertebrates – emphasis on fish and mammals. Trends in Comparative Biochemistry and Physiology 7: 33–45.

Yancheva V., Stoyanova S., Velcheva I. & Georgieva E. 2020. Fish as indicators for environmental monitoring and health risk assessment regarding aquatic contamination with pesticides. International Journal of Zoology and Animal Biology 3 (1): 210.

Received: 10.12.2023
Accepted: 15.05.2024