UDC 577.322: 577.152.11: 577.117: 57.017.32: 594.1

APPLICATION OF MULTI-MARKER APPROACH FOR ASSESSMENT OF STRESS SYNDROME IN TRANSPLANTED MUSSELS *DREISSENA POLYMORPHA*

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Zebra mussels *Dreissena polymorpha* were transferred for 14, 28, 60 and 120 days from their reference site (C) to sites with agricultural pollution (A), industrial pollution from the alcohol industry (I1), or manufactured wastes (I2). The aim for that was to determine their ability to reflect various types of stressful conditions. General temporal patterns of mussels were confirmed by Centroid grouping and Discriminant Function analysis of the battery of their biochemical markers. After 14 days of transplantation, mussels demonstrated most prominent inter-site differences. In site A, a decreasing of the activity of oxidative defense enzymes took place, demonstrating weak stress response. In both sites I, increased levels of lipid peroxidation, metallothioneins and ethoxyresorufin-O-deethylase indicated oxidative injury, pollution by heavy metals, and persistent organic compounds, respectively. Low cholinesterase activity was detected in both C and B sites, showing the pollution by agricultural discharges. Prolonged exposure to polluted environment provoked the endocrine disruption (high levels of vitellogenin-like proteins), oxidative stress and decrease of glutathione and metallothionein concentrations, especially in the industrial sites.

Key words: *Dreissena polymorpha*, oxidative stress, biomarkers of exposure, pollution, field transplantation.

INTRODUCTION

Caged molluscs have been widely used for active monitoring, for evaluating the effect of adaptation to polluted surroundings, or for inspecting sites at which indigenous molluscs are absent [31]. However, the relationship between temporal and spatial effects in caged molluscs has been poorly investigated [16, 22]. Therefore, we undertook a multi-marker approach in the widely used zebra mussel, *Dreissena polymorpha* [16], to determine general and specific responses in mussels caged at different field sites.

A set of biomarkers is usually explored to evaluate the effects of pollutants on health of living organisms [31]. Multi-marker battery includes the non-specific biomarkers of stress,

mainly the characteristics of the oxidative damage, and the biomarkers of exposure to specific kinds of pollution. The metallothioneins (MTs), rich in thiols low-molecular-weight cytosolic proteins, represent a constitutive part of this set, and their concentration is regarded as a marker of heavy metal pollution [25, 31]. Other markers of specific kinds of pollution include cholinesterase activity (ChE, marker of pollution by carbamate and organophosphate pesticides), vitellogenin-like protein level (Vtg-LP, marker of endocrine disruption caused by the environmental steroids), and ethoxyresorufin-o-deethylase activity (EROD, characteristic for microsomal biotransformation system). Despite criticisms concerning the utility of several markers of specific pollutants in molluscs [32], we included these indices in our study in order to compare their response to that in the indigenous bivalve molluscs [12].

The limitations of the biomarker approach, such as confounding factors that are not related to pollution, should be carefully considered when interpreting biomarker data. Particularly, some authors considered that biomarkers of molluscs are highly seasonally dependent and therefore not appropriate for monitoring [7, 12, 17, 31]. Therefore, more precise study of characteristics of zebra mussels in field conditions with respect to different kinds of pollution, season, and the effect of caging is needed.

The higher stream of the Dnister Basin (Ternopil region, Ukraine) is thought to be one of the most ecologically clean area in Ukraine [19]. However, it is difficult to select an impeccable unpolluted site due to the overall usage of chemical substances in small farming inherent to this area, and almost total destruction of water purification systems [1, 12, 13, 14]. These circumstances point to the need for the earlier identification of change and detection of several toxic compounds in this area. To our knowledge, the biological responses to the complex pollution in zebra mussels have never been studied in field situation in Ukraine.

MATERIALS AND METHODS

The experiments were started in July, 2008. The specimens of zebra mussels, *Dreissena polymorpha*, were sampled from a site in the upstream portion of river Seret (the tributary of Dnister, Western Ukraine,) upper the city of Ternopil where no industrial contamination could be detected; therefore, this was considered as reference site (C site). In our study, the indigenous population of *D. polymorpha* was found only at this site. In the same day, the specimens with the shell length between 2.4 and 3.0 cm were taken to the transplantation sites and placed here. The cages consisted of plastic net (mesh: 3 mm), highly perforated on the sides in order to ensure constant exchanges between the inside and the surrounding medium (30×30×20 cm). The upper part was fixed on the bank of water body. Approximately 100 specimens of *D. polymorpha* were placed into each cage; four cages were immersed at each station.

The localities for transplantation were selected at Dnister River tributaries near the boroughs of city Borschiv on the Nichlava River (A site), in the village of Scalat on the Gnylka River (I1 site), and in the village of Ostriv (I2 site) downstream from the city of Ternopil on the Seret River (Fig. 1). The A site was situated near the lower portion of the river in rural vicinity that received effluent from the city with destroyed water purification plant and highly developed agricultural region. At the I1 site, the molluscs were kept on the banks of a pond on the Gnylka River (Rotten River), downstream from an alcohol manufactory that flushes its sewage directly into the river. The I2 site was situated downstream the city of about 220,000 residents near a motorway and railway lines with intense traffic and a number of different manufacturers.
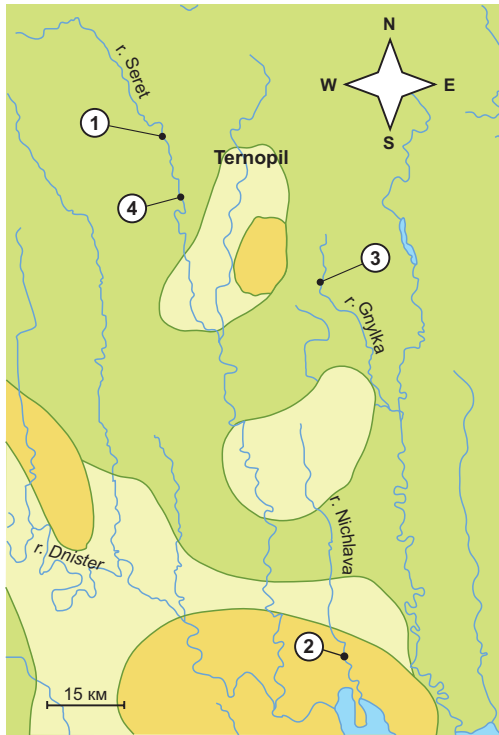
Fig. 1. Localization of the sampling sites on the left upper tributaries of Dnister River, in Western Ukraine. Sites:
1, reference (C); 2, A; 3, I1; 4, I2. The map from ukrainainkognita.org.ua/pzf/Ternopil.htm was utilized

Рис. 1. Розташування ділянок дослідження на лівих верхніх притоках р. Дністер, Західна Україна. Ділянки:
1, контрольна (С); 2, А; 3, I1; 4, I2. Було використано карту з ukrainainkognita.org.ua/pzf/Ternopil.htm

The residential and caged molluscs were studied simultaneously after 14 (July), 28 (August) and 60 (September) days of the transplantation. As far as the mortality rate at A site was comparatively low, the period of study in this site was continued till November (120 days of transplantation). At the I2 site, the cages disappeared in August and were renewed. So, second 14 days exposure instead 60 days was studied at this site in September.

At each sampling time, one cage was withdrawn from each site. Individuals were transported to the laboratory in cool boxes filled native water and constantly aerated and treated within a day after the sampling procedure. Mussels from the reference site served as controls. In each group, individuals' mortality, length, total and whole soft body weights were recorded. The condition index (CI) of soft tissues was calculated as the ratio: (drained mass of tissues/total mass of mussel) $\times$ 100. The condition factor (CF) of the animals was calculated on the next equation:

$$CF = [total\ mass\ (g)/total\ length^3\ (cm)] \times 100.$$

Acetylthiocholine iodide (ATCh), cytohrome *c*, EDTA, 7-ethoxyresorufin, Glutathione Reductase from baker's yeast (*S. cerevisiae*), 2,4-dinitrophenylhydrazine (DNPH), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), β-mercaptoethanol, β-NADPH, nitroblue tetrazolium (NBT), reduced glutathione (GSH), phenazine methosulfate, phenylmethyl-sulfonyl fluoride (PMSF), serum albumin, and thiobarbituric acid (TBA) were purchased from Sigma. All other chemicals were of analytical grade.

Whole soft tissues of *D. polymorpha* were homogenized (1/10 w/v) in 0.1 M pH 7.4 phosphate buffer containing 100 mM KCl and 1 mM EDTA as well 0.1 mM PMSF for the inhibition of proteolysis. For all measurements except the assay of microsomal enzyme activity and glutathione content, the homogenates were centrifuged at 6,000$\times g$ for

10 min and the resulting supernatant measured immediately. All steps were carried out at 4°C. The absorbance values were measured on UV/Vis spectrophotometer „Lomo-56" („Lomo", Russia).

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured according to the method of Beauchamp and Fridovich [4] based on aerobic reduction of NBT at 535 nm by superoxide radicals. In order to assess Mn-SOD activity, the supernatant was preincubated for 60 min at 0°C in the presence of 5 mM KCN, which produced total inhibition of Cu,Zn-SOD. The latter activity was calculated as the difference between the activities in the absence and the presence of KCN. SOD activities were expressed as units$\times$mg^{-1} soluble protein; 1 unit of SOD activity being defined as the amount of protein causing 50% inhibition of the rate of NBT reduction.

Catalase (CAT, EC 1.11.1.6) activity was measured by monitoring the decomposition of 10 mM H_2O_2 according to Aebi [2] at 240 nm (ε=40 M$^{-1}\times$cm^{-1}) in the medium containing 50 mM KH_2PO_4 (pH 7.0) and approximately 150 μg of protein. Results were related to soluble protein.

Total glutathione (GSH) concentration was quantified by the glutathione reductase recycling assay [3]. Standards were prepared from reduced glutathione, and concentrations were expressed as μmol per g wet weight.

Lipid peroxidation (LPO) was determined in supernatant by the production of TBA-reactive substances (TBARS) [27]. The absorbance of the chromogen was determined at 532 nm. A molar extinction coefficient of 1.56×10^5 M$^{-1}\times$cm^{-1} was used.

Metallothioneins (MTs) were determined on the basis of thiols measure, according to the method of Viarengo et al. [32]. Tissue samples were homogenized in three volumes of homogenizing buffer with 0.1% β-mercaptoethanol after the ethanol/chloroform extraction. After incubation with DTNB, the samples absorbance were read at 412 nm. The level of MT was calculated assuming the relationship: 1 mol MT = 20 mol GSH and expressed as μg of MT per gram of FW tissues.

Cholinesterase (ChE, EC 3.1.1.7) activity was determined according to the colorimetric method of Ellman et al. [11] at 25°C. The reaction was initiated by adding of 0.04 ml 0.075 M ATCh to the reaction mixture. Enzymatic activity was calculated using a molar extinction coefficient of 13.6×10^3 M$^{-1}\times$cm^{-1} and referred to soluble protein content.

Microsomal ethoxyresorufin O-deethylase (EROD) activity was measured by monitoring the formation of resorufin at 572 nm [18]. For this measurement, the homogenates were centrifuged at 10,000$\times$*g*, 10 min. A microsomal pellet was obtained by calcium precipitation of the postmitochondrial supernatant in 80 mM $CaCl_2$ in 10 mM Tris-HCl buffer, pH 7.4 [8]. The reaction was initiated by adding 0.5 mM NADPH. EROD activity was calculated using a molar extinction coefficient of 73.2 mM$^{-1}\times$cm^{-1} and referred to the microsomal protein content.

Vitellogenin-like proteins (Vtg-LP) were evaluated in tissue homogenate as alkali-labile phosphate level according to Blaise et al. [6]. The labile phosphates were measured by a colorimetric phosphomolybdenum method.

Protein concentration in the supernatant (soluble protein), or microsomal pellet (microsomal protein) was determined by the method of Lowry et al. [21] using bovine serum albumin as protein standard.

The morphological parameters were determined in 35 specimens, and all other measurements, in 8 specimens. The results were expressed as means $\pm$ standard deviation (SD). Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnoff and Levene's tests, respectively. Since data were not normally distributed

(Lilliefors' test), non-parametric tests (Kruskall–Wallis ANOVA and Mann–Whitney *U*-test) were performed (significant at $p < 0.05$). Data were subjected to Centroid grouping and Discriminant Functional analyses to differentiate the individual specimens by a set of their indices, both in different sites and different sampling periods. All statistical calculations were performed on the separate data from each individual with SPSS 15.0 software, Statistica v 7.0 and Excel for Windows-2000.

RESULTS

Morphological indices of mollusks

No mortality was observed in the caged groups 14 days after transplantation. After 28 days, the mortality rate was about 10% at site A and about 65% at site I1. After 60 days the mortality rates were 25% and 85% at sites A and I1 respectively, and after 120 days it was 30% at site A. Caging did not affect the morphological indices deleteriously within 120 days of incubation, since the mussels transplanted to site A had the same CI and CF as in the control group (Table 1). However, after 28 days of caging a decrease in body parameters was observed.

Table 1. Morphological indices of resident and transplanted zebra mussels *D. polymorpha*, M±SD, N=35

Таблиця 1. Морфологічні показники контрольних і переселених дрейсен *D. polymorpha*, M±SD, N=35

Parameters	Group	Month, time of transplantation			
		July, 14 days	August, 28 days	September, 60 days*	November, 120 days
Condition index, CI	C	27.3±8.0	22.6±4.3[b]	18.7±5.7[b]	17.7±5.3[b]
	A	29.2±9.2	24.5±4.7[b]	23.0±5.0[a,b]	18.9±3.2[b]
	I1	24.4±7.2	18.6±5.8[a,b]	22.5±3.7[a]	ND
	I2	26.8±7.2	ND	19.3±4.3[b]	ND
Condition factor, CF	C	11.0±2.8	14.0±2.9[b]	13.3±3.9[b]	20.3±5.4[b]
	A	12.7±2.7[a]	12.3±2.7[a]	13.0±2.9	18.0±3.2[b]
	I1	11.3±2.7	12.6±3.1	13.6±2.5[b]	ND
	I2	11.3±2.6	ND	14.0±2.8[b]	ND

In Tables 1, 2: * – repeated 14 days exposure at site I2; [a] – spatial significant difference compare to control, [b] – temporal significant difference in the same group compare to value in July; always $P < 0.05$; ND – not determined.

У таблицях 1, 2: * – повторне переселення на 14 діб у місцевості I2; [a] – просторова відмінність порівняно з контролем достовірна, [b] – часова відмінність порівняно зі значенням у липні в тій самій групі достовірна; завжди $P < 0,05$; ND – не визначали.

Oxidative stress markers

The data on the markers of oxidative stress are displayed in Fig. 2. Temporal changes were indicated for the majority of the markers with the lowest values in August. Spatial differences for each site were also recorded. The common features for all transplanted mussels were elevated TBARS levels in July, and low GSH levels in September. Typical features of the mussels at site A were low activities of SOD and CAT in June. At sites I1 and I2, high levels of SOD and CAT were observed in the mussels, especially in September. SOD activation was primarily provided by the Cu,Zn-SOD form and further,
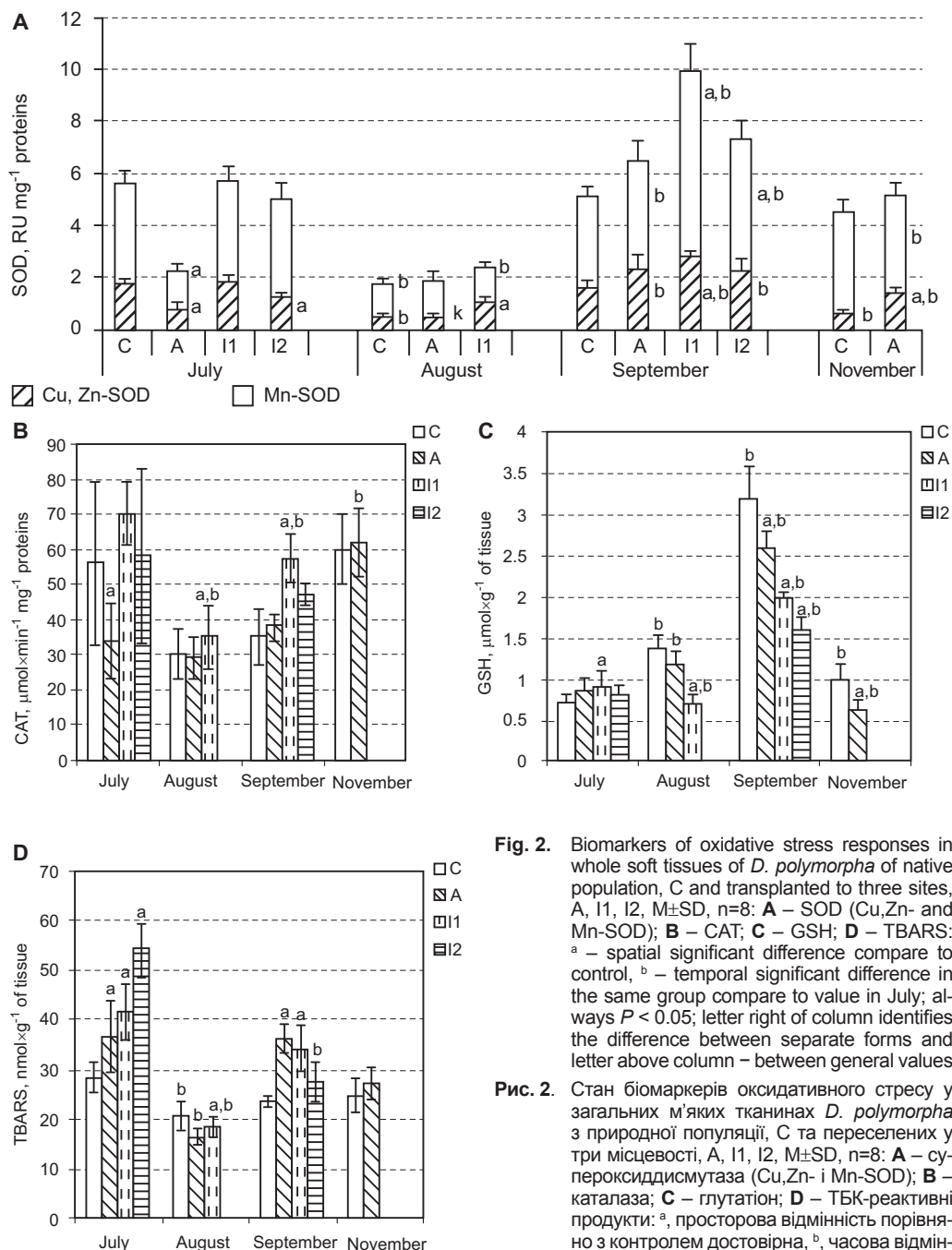
Fig. 2. Biomarkers of oxidative stress responses in whole soft tissues of *D. polymorpha* of native population, C and transplanted to three sites, A, I1, I2, M±SD, n=8: **A** – SOD (Cu,Zn- and Mn-SOD); **B** – CAT; **C** – GSH; **D** – TBARS: [a] – spatial significant difference compare to control, [b] – temporal significant difference in the same group compare to value in July; always $P < 0.05$; letter right of column identifies the difference between separate forms and letter above column − between general values

Рис. 2. Стан біомаркерів оксидативного стресу у загальних м'яких тканинах *D. polymorpha* з природної популяції, С та переселених у три місцевості, А, I1, I2, M±SD, n=8: **A** – супероксиддисмутаза (Cu,Zn- і Mn-SOD); **B** – каталаза; **C** – глутатіон; **D** – ТБК-реактивні продукти: [a], просторова відмінність порівняно з контролем достовірна, [b], часова відмінність порівняно зі значенням в липні у тій самій групі достовірна; завжди $P < 0,05$; буква справа від стовпчика характеризує відмінність між окремими формами, буква над стовпчиком − між загальними значеннями

by the Mn-SOD form. Concentrations of the products of oxidative damage, TBARS, were elevated especially at sites I1 and I2 in September and reached approximately double of control values.

Specific biochemical markers

The results of the assay of markers of exposure to specific kinds of pollution are presented in Table 2. The MTs concentration demonstrated a common temporal pattern. The transplanted mussels had higher MTs level than the controls in July and August (except at site A in July) but lower levels in September. Wave-like temporal changes were observed for ChE activity. In each period, its levels were lowest in the control and sometimes at site A.

Table 2.   Specific biomarkers in the tissues of zebra mussels, *D. polymorpha*, M±SD, N=8

Таблиця 2. Специфічні біомаркери у тканинах дрейсен, *D. polymorpha*, M±SD, N=8

Parameters	Site	Month, time of transplantation			
		July, 14 days	August, 28 days	September, 60 days*	November, 120 days
Metallothioneins, $\mu g \times g^{-1}$ FW	C	14.6±4.0	136.7±21.9[b]	194.9±40.9[b]	96.1±28.2[b]
	A	18.1±6.2	178.2±25.8[b]	121.6±22.1[b]	101.1±26.7[b]
	I1	32.8±11.2[a]	182.9±42.2[b]	106.4±17.1[b]	ND
	I2	61.8±21.1[a]	ND	108.0±19.9[b]	ND
ChE, nmol×min^{-1} mg^{-1} protein	C	8.6±2.0	0.7±0.2[b]	3.8±1.0[b]	2.4±0.3[b]
	A	7.5±1.9	1.1±0.8[b]	5.9±1.4[a]	3.4±1.1[b]
	I1	17.8±3.3[a]	4.0±2.7[a,b]	7.8±0.5[a,b]	ND
	I2	12.7±1.4[a]	ND	5.2±1.3[b]	ND
EROD, pmol min^{-1} mg^{-1} protein	C	0.1±0.0	0.89±0.09[b]	1.9±0.2[b]	2.6±0.4[b]
	A	0.1±0.0	0.88±0.03[b]	2.0±0.1[b]	3.6±0.9[a,b]
	I1	2.4±0.7[a]	0.84±0.02[b]	1.9±0.1	ND
	I2	1.0±0.4[a]	ND	2.5±0.2[b]	ND
Vtg-LP, μg Pi mg^{-1} protein	C	50.6±12.4	41.4±11.6	75.6±14.7	106.2±18.1[b]
	A	13.5±5.6[a]	70.4±7.6[a,b]	58.7±17.4[b]	226.3±55.6[ab]
	I1	13.2±5.3[a]	57.5±10.5[b]	91.2±3.5[a,b]	ND
	I2	21.0±7.4[a]	ND	59.2±20.4[b]	ND

In general, EROD activity and Vtg-LP content increased over time. However, some deviations from this trend were observed. Higher EROD activity was occasionally apparent in the transplanted groups. Vtg-LP levels were lower in all transplanted groups than in the controls in July but in later periods they exceeded the control value, especially at site I1 in September.

Statistical analysis

The application of centroid grouping analysis (Fig. 3) allowed the differentiation of three clusters corresponding to July, August, and September and November combined. In each period, except August, sites A and I1 were distinctly separated from C within the common temporal cluster. By Discriminant Function Analysis (Table 3), the groups were discriminated best of all after 14 days of exposure with the biggest distance between site A and I2. The least differences between groups were reflected after 28 days. However, all these differences were significant.
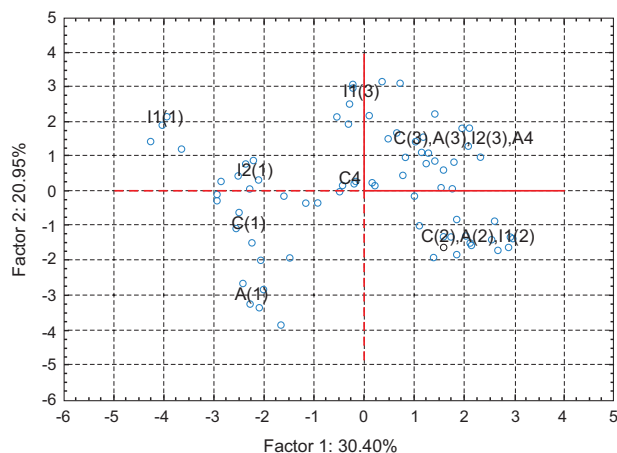
Fig. 3. Graphical representation of C, A, I1, I2 groups clusters derived from centroid grouping analysis in July (1), August (2), September (3), and November (4)

Рис. 3. Графічне зображення за допомогою центроїдного групового аналізу кластерів C, A, I1, I2 груп у липні (1), серпні (2), вересні (3) та листопаді (4)

Table 3.   Discriminant Function Analysis of the effect of site on the multi-marker response of zebra mussels, *D. polymorpha,* transplanted to three sites, P-levels

Таблиця 3. Дискримінантний функціональний аналіз впливу місцевості на мультимаркерну характеристику дрейсен, *D. polymorpha,* переселених у три місцевості, значення Р

Group	C	B	I1
		14 days	
C		0.006868	0.000003
B	0.006868		0.000006
I1	0.000003	0.000006	
I2	0.000075	0.000196	0.000646
		28 days	
C		0.000121	0.022264
B	0.000121		0.000194
I1	0.022264	0.000194	
		60 days	
C		0.000135	0.000088
B	0.000135		0.000500
I1	0.000088	0.000500	
I2	0.000230	0.000416	0,.003890

Significant at P < 0.05 / Достовірно при P < 0,05

DISCUSSION

The most frequently used transplantation time for *D. polymorpha* is about 14 to 60 days [16, 26, 29]. In our study, morphological indices confirm the absence of deleterious effects of caging technique itself over 120 days (at site A). High mortality had local reasons as it was reported for the polluted sites after 24–48 days [22]. General seasonal dependence so well known for mollusks [7, 12, 17, 25, 31] was confirmed in *D. polymorpha* by applying centroid grouping analysis suggesting the effect of natural factors, such as temperature, reproductive cycle and food availability. The unexpected second transplantation of mussels appeared to be a useful manipulation demonstrating the relation of this group to a corresponded season but not to time of caging.

In August, the specimens from all sites were characterized by the highest similarity. This similarity probably reflected the priority of strong global effect over local peculiarities during this period, whilst this period was characterized by high temperatures during two

previous weeks [30]. Other reports support a view that general seasonal factors might influence biomarker responses in molluscs to a greater extent than variations in pollution [23]. In any case, *D. polymorpha* demonstrated easily adaptation to new conditions.

According to Discriminant analyses, spatial peculiarities of biological response were also significant and best shown after 14 days of caging. It is known that, pollutants of different composition, including non-redox cycling xenobiotics, can induce oxidative stress in the aquatic animals [15, 25, 29]. At site A, the oxidative stress was less evident because a depletion in antioxidant parameters was not accompanied by a prominent increase in LPO [9]. At the industrial sites, despite the continuous activation of the antioxidant enzymes, enhanced oxidative damage showed similar effects to those demonstrated in the aquatic organisms exposed to high levels of pollution [7]. Prominent decrease in low weight thiols, both MTs and GSH, revealed in September, confirms the oxidative injury.

Composition of pollution specific for each site was tested by applying of specific biochemical markers. In present study, we consider the industrial sites as polluted by the heavy metals, according to early revealed elevation of MTs concentrations. Low levels of ChE at sites A and C may be the result of its inhibition by typical agricultural pollutants (carbamates or organophosphates) at rural area [25, 31]. The same low ChE activity was also reported in *Anodonta cygnea* at these two sites [12]. Initially, this evidence in the control site (C) seems to be curious. However, the reduction in ChE activity in *Mytilus galloprovincialis* at two control stations considered as „uncontaminated" controls was also observed [20]. That was explained by possible leaching of pesticides into sea water from the agricultural lands. High sensitivity of ChE activity to agricultural chemicals was reported in *D. polymorpha* and in the clam *Scrobicularia plana* [5, 28].

The increase in EROD activity indicates the pollution by organic contaminants, probably polychlorinated biphenyls [5, 10]. These substances are typical compounds of industrial and urban pollution. Values observed in this study were more than 10 times of that of naturally occurring variation, which strongly suggests an exposure to these contaminants at the industrial sites.

Despite the ambiguity in the detection of the sensitivity and origin of Vtg-LP in molluscs [24], the long-term caged mussels showed similar evidence that indigenous bivalve *Anodonta cygnea* confirming the pollution by endocrine disruptors at sites A and I1 [12, 24].

Prolonged exposure provoked also oxidative stress and decrease of glutathione and metallothionein concentrations, especially in the industrial sites. The alterations may be related to different strategies of stress response at each site. Similar to our results, Frenzilli et al. [15] showed a biphasic trend for single antioxidants and total oxy-radical scavenging capacity, with an increase during the first two weeks of exposure to the industrialized site, followed by a progressive decrease up to severe depletion after one month of caging in marine mussels.

CONCLUSIONS

The use of non-specific and toxicant-specific biomarkers in the transplanted zebra mussel allowed to distinguish the peculiarities of stress syndrome in agricultural (rural) and industrial types of sites. The combination of various biological measurements (multi-marker approach) demonstrated an integrating effect of a large number of individual and interactive processes in aquatic organisms in the field situation.

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ОЦІНКА СИНДРОМУ СТРЕСУ У ПЕРЕСЕЛЕНИХ МОЛЮСКІВ *DREISSENA POLYMORPHA* ЗА ДОПОМОГОЮ МУЛЬТИМАРКЕРНОГО ПІДХОДУ

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Визначали здатність дрейсени *Dreissena polymorpha* відобразити наявність стресорних умов, для цього молюсків переселяли з природної місцевості (С) на 14,

28, 60, 120 діб у інші водойми, що характеризуються аграрним (А) або індустріальним – відходи спиртзаводу (I1) чи технічного виробництва (I2) – забрудненням. Факторний і дискримінантний аналізи набору біохімічних маркерів дрейсени підтвердили спільні часові закономірності. Найбільш значні відмінності між групами були встановлені після 14-ти діб переселення. Зменшення активності ферментів антиоксидантного захисту спостерігалось у групі А, що було ознакою слабкої відповіді на стрес. В обох групах I встановлено окисну деструкцію, забруднення важкими металами та стійкими органічними сполуками згідно з підвищеними рівнями пероксидації ліпідів, металотіонеїнів і 7-етоксирезоруфін-О-деетилази відповідно. Низьку холінестеразну активність відзначено у групах С та А, що свідчить про забруднення аграрними стоками. Тривале переселення молюсків викликало ендокринні розлади (високий вміст вітелогенін-подібних білків), оксидативний стрес і зменшення концентрації глутатіону та металотіонеїнів, особливо в індустріальних місцевостях.

Ключові слова: *Dreissena polymorpha*, оксидативний стрес, біомаркери експозиції, забруднення, переселення.

ОЦЕНКА СИНДРОМА СТРЕССА У ПЕРЕСЕЛЕННЫХ МОЛЛЮСКОВ *DREISSENA POLYMORPHA* С ПОМОЩЬЮ МУЛЬТИМАРКЕРНОГО ПОДХОДА

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Определяли способность дрейсены *Dreissena polymorpha* отобразить наличие стрессорных условий, для чего моллюсков переселяли из естественной местности (С) на 14, 28, 60, 120 суток в другие водоемы, которые характеризуются аграрным (А) или индустриальным – отходы спиртзавода (I1) либо технического производства (I2) – загрязнением. Факторный и дискриминантный анализы набора биохимических маркеров дрейсены подтвердили общие временные закономерности. Наиболее значительные отличия между группами были отмечены после 14 суток переселения. В группе А наблюдалось снижение активности ферментов антиоксидантной защиты, что являлось признаком слабого ответа на стресс. В обеих группах I установлены окислительная деструкция, загрязнение тяжелыми металлами и стойкими органическими соединениями согласно повышенным уровням пероксидации липидов, металлотионеинов и 7-этоксирезоруфин-О-деэтилазы. В группах С и А отмечена низкая холинэстеразная активность, что свидетельствует о загрязнении аграрными стоками. Длительное переселение моллюсков вызвало эндокринные расстройства (высокое содержание вителогенин-подобных белков), оксидативный стресс и уменьшение концентрации глутатиона и металлотионеинов, особенно в индустриальных местностях.

Ключевые слова: *Dreissena polymorpha*, оксидативный стресс, биомаркеры экспозиции, загрязнение, переселение.