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**Evaluation of the antioxidant defense of the freshwater bryozoan
Cristatella mucedo CUVIER, 1798 (Bryozoa, Phylactolaemata)
of Lake Piediluco (Italy)**

A.C. ELIA, G. PIERONI & M.I. TATICCHI

A b s t r a c t : The aim of this study was to get information about the detoxificant responses and heavy metal accumulation in *Cristatella mucedo* CUVIER, 1798, a freshwater bryozoan. Total glutathione content and enzymatic activities of glutathione peroxidase, glutathione reductase, catalase, glutathione S-transferase, and heavy metal accumulation of lead, cadmium, chromium and nickel were performed on samples of *Cristatella mucedo*. The colonies were collected from Lake Piediluco (Umbria, Italy) during the summer 1997 and 1999. As a general rule, higher levels of biochemical parameters were evidenced in specimens sampled during 1999, probably in consequence to the highest metal concentration in tissues of this species.

Key words : Freshwater bryozoan, Heavy Metals, Total Glutathione, Detoxifying Enzymes.

Introduction

Environmental pollutants, such as heavy metals, can act as prooxidant forces rising the levels of reactive oxygen species (ROS). Thus, to prevent oxidation-induced damage, caused by ROS, organisms induce some defense mechanisms to counteract their harmful effects. Glutathione content and antioxidant enzymes are involved in the detoxification processes of environmental pollutants, and have been considered biomarkers of contaminant-mediated oxidative stress in aquatic organisms (WINSTON & DI GIULIO 1991; SHEENA & POWER 1999). Biomarkers are defined as "xenobiotically-induced variations in cellular or biochemical components or processes, structures or functions that are measurable in a biological system or sample" (NRC 1987).

Reduced glutathione (GSH) is important as a non enzymatic scavenger of oxyradicals, and is involved in the metabolism of many toxic compounds and endogenous substances (MEISTER 1989). In this form, glutathione is a substrate or cofactor for several detoxifying enzymes (glutathione S-transferases, glutathione peroxidases, and glutathione reductase), and thus its variations can indicate changes of these enzymatic activities. Glutathione S-transferase (GST; EC 2.5.1.18) is involved in conjugation processes of the reactive electrophilic centers of different substrates with the thiol group of GSH. Glutathione peroxidases (GPx Se-dependent enzyme, EC 1.11.1.9; GPx Se-independent enzyme, EC 2.5.1.18) catalyze the reduction of hydrogen peroxide and organic peroxides.

Glutathione reductase (GR; EC 1.6.4.2) maintains the reduced glutathione level. Catalase (EC 1.11.1.6), an enzyme not glutathione-dependent, is involved in the cellular defense system through the elimination of hydrogen peroxide.

Data about the antioxidant defense of bryozoans are reported only for *Lophopus crystallinus* Pallas (ELIA et al. 2001) and no information is available for antioxidant parameters and environmental contaminants of *Cristatella mucedo* CUVIER, 1798.

Cristatella mucedo (Bryozoa, Phylactolaemata) is a freshwater filter feeding bryozoan and as all freshwater bryozoans is a sessile, hermaphroditic, colonial species. The colonies are gelatinous and globular; commonly grows on roots, submerged branches, aquatic macrophytes, and other substrata. The freshwater Bryozoa may be considered as biosensor of environmental disturbance and pollution (BUSHNELL 1974; WÖSS 1994; CECCAGNOLI et al. 1997).

Therefore, the aim of the study was to gather information about the detoxificant response and heavy metal accumulation of *Cristatella mucedo*. Total glutathione content and enzymatic activities of glutathione peroxidase, glutathione reductase, catalase, glutathione S-transferase, and lead, cadmium, chromium and nickel levels were performed on samples collected from Lake Piediluco (Umbria, Italy) during summer 1997 and 1999.

Material and methods

Lake Piediluco is the second largest hydroelectric basin in Umbria, with an area of 1.52 km² (Fig. 1). It receives continuous water inflow (about 20 m³/s) from the Nera River through an artificial channel, and is also connected by another artificial channel to the Velino River; water inflow and outflow from the latter are artificially regulated for power production (GIANOTTI et al. 1988), so hydrological regulation determines a significant daily variation of water levels (ca. 90 cm).

Colonies of *Cristatella mucedo* were sampled monthly from Lake Piediluco (Umbria, Italy) at the station of Braccio di Capolozza from July to September 1997 and 1999 (Fig. 1). In order to eliminate the detritus from the gut, the colonies were rinsed with filtered lake water for 24 h and most associated organisms were eliminated using pointed needles under the stereomicroscope. The living colonies were grouped in pools, subdivided into aliquots for biochemical and chemical analyses, and stored at -80 °C until used.

The samples of *Cristatella mucedo* (2-3 g) were placed in a Teflon vessel (100 ml capacity) with water (7 ml) and concentrate nitric acid (3 ml). They were therefore subjected to treatment for 20 min, in a Milestone Digestion System Model ETHOS 900. After cooling, the digests were diluted to 50 ml with ultrapure water and analysed by atomic absorption spectrometry (Perkin Elmer 5100). Cd, Cr, Cu, Ni and Pb measurements were performed using a flameless graphite oven with an autosampler (AS-60). The standard addition method was used to control for matrix effects.

Colonies of *Cristatella mucedo* (0.5 g) were homogenized for total glutathione content (GSH+2GSSG) as previously described in ELIA et al. (2001) and total thiol content was determined by the GR recycling assay at 412 nm according to AKERBOOM & SIES (1981). Samples analyzed for enzyme activities (0.5 g) were homogenized in 5 volumes of 100 mM Tris buffer, pH 7.8, containing 100 µM phenylmethylsulphonyl fluoride (PMSF).

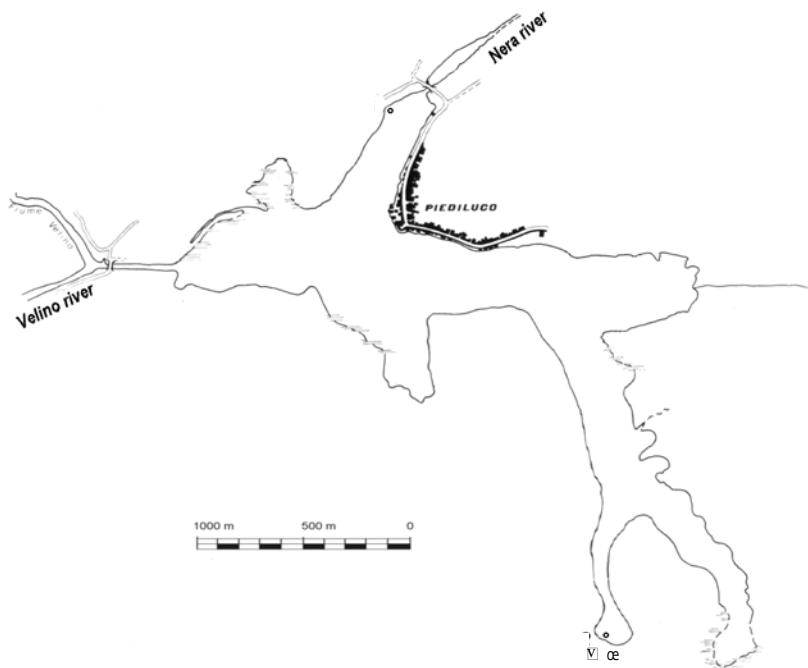


Fig. 1. Lake Piediluco (● = Braccio di Capolozza)

The cytosolic fractions were analyzed according to the original assays and under conditions described previously (ELIA et al. 2001). GST activity, with the substrate 1-chloro-2,4-dinitrobenzene (CDNB), was measured according to HABIG et al. (1974). The assay of GST activity with CDNB measured the formation of the conjugate with GSH at 340 nm ($\epsilon = 9.6 \text{ mM}^{-1} \text{ cm}^{-1}$). GPx activity toward CuOOH as substrate was determined by the coupled enzyme method of LAWRENCE & BURK (1976). The oxidation of NADPH was followed at 340 nm ($\epsilon = - 6.22 \text{ mM}^{-1} \text{ cm}^{-1}$). G R activity was assayed according to CHUNG et al. (1991) by following the decrease in absorbance at 340 nm ($\epsilon = - 6.22 \text{ mM}^{-1} \text{ cm}^{-1}$) due to oxidation of NADPH. Catalase activity was measured according to GREENWALD (1985), following the decrease in absorbance at 240 nm due to H_2O_2 consumption ($\epsilon = - 0.04 \text{ mM}^{-1} \text{ cm}^{-1}$). Protein concentration of supernatant fractions was determined according to LOWRY et al. (1951) employing bovine serum albumin (BSA) as a protein standard.

The biochemical and chemical data obtained by samples collected monthly were grouped for each year. The variables were log-transformed in order to minimize the impact of outliers. Statistical analyses was carried out using a single-factor ANOVA followed by TUKEY HSD test to evidence the significance of differences during both cycles. The experimental data matrices were submitted to the simple linear PEARSON correlation analysis. In all cases, statistical significance was taken at $P < 0.05$.

Results

The heavy metal concentration in tissues of *Cristatella mucedo* collected from both vegetative periods are reported in Table 1. The results evidenced higher levels of Cd, Cr and Ni (ca 2-3 times) and of Pb (ca 27 times) in specimens of 1999, as to those of 1997. Antioxidant enzymes and total glutathione of *Cristatella mucedo* are reported in Table 2. As shown by the results, differences between specimens of both two cycles were recognized for almost all biochemical indicators, except for glutathione level, which did not show statistically significant differences. The comparison among the two vegetative periods evidenced an higher GST activity (ca 2 times) in specimens of cycle II. The same trend was displayed also by CAT, GR, and GPx activities.

The levels of biochemical parameters and of metals in specimens of *C. mucedo* were examined by using PEARSON moment correlation analysis (Tab. 3). The analysis expresses positive correlations of GSH+2GSSG and GPx with Pb and Cd, of GST with Pb, Cr and Ni, of GR only with Ni and of CAT with all metals investigated in this study.

Discussion

Previous results of laboratory studies performed on some freshwater bryozoans, *Pectinatella magnifica*, *Plumatella emarginata* and *Lophopodella carteri* demonstrated that these species are responsive to copper, cadmium, chromium and zinc. According to the authors these bryozoans can be employed as bioindicators of water quality (PARDUE & WOOD 1980). However, the results of a field study performed on other freshwater bryozoans, *Fredericella sultana* and *Plumatella fungosa*, designated these species as poor heavy metal accumulators (HENRY et al. 1989). Until now, no data are available in the literature about normal and contaminated heavy metal content in *Cristatella mucedo*. In the present research, the colonies of *C. mucedo* showed an higher metal accumulation in colonies of 1999. However these concentrations are very low if compared to those evidenced in *Fredericella sultana* and *Plumatella fungosa* collected from River Meuse, highly polluted by heavy metals (HENRY et al. 1989). Our previous study evidenced that colonies of *Lophopus crystallinus* collected from Lake Piediluco during the same years (1997 and 1999), exhibited lower Pb and Cd levels and higher Cr level than those recorded in colonies of *Cristatella mucedo*. In addition, the heavy metal concentrations of Cd, Cr, Cu, Fe, Pb, Ni and Zn in water samples of Lake Piediluco during 1999 were low and generally below to the method detection limits (data not shown). From these results it is possible to assume that this species may be able to accumulate heavy metals, even if they were recorded in low content in tissue.

Until now, few studies have been performed on the antioxidant response of freshwater bryozoans (ELIA et al. 2001). The evaluation of antioxidant enzymes in *Lophopus crystallinus* of Lake Piediluco suggested the existence of an annual cyclic path for these enzymatic activities (ELIA et al., 2001). In the present study, specimens of *Cristatella mucedo* of cycle II evidenced elevated levels of biochemical parameters, probably as a result of the highest metal concentration. In fact, the thiol level in these specimens, was positively correlated with Pb and Cd content as indicated by Pearson moment correlation. It is also interesting to note that thiol level of *Cristatella mucedo* was higher than that recorded in *Lophopus crystallinus* collected from the same lake and year (ELIA et al.,

2001). This result might indicate a greater ability of *Cristatella mucedo* to assure protection against oxidative stress induced by pollutants. GPx activity showed similar correlation of total glutathione with metals and highest activity in samples of 1999. Elevated GST value was also evidenced in samples of 1999, and the activity was positively correlated with tissue metal concentration. Since the biological role of the enzyme is to detoxify many electrophilic compounds, it is not surprising that a significant correlation was detected for the enzymatic activity and tissue Pb, Cr and Ni levels.

GR enzyme has an important role in cellular antioxidant protection because it catalyses the regeneration of GSH from GSSG (STAGEMAN et al. 1992). In fact, a decrease in this enzymatic activity may cause GSH depletion if compensatory synthesis of thiol is not adequate to assure its redox status. Therefore, elevated GR catalyzing activity of *C. mucedo* specimens of cycle II, might indicate an enhancement in total glutathione consumption, used as defense line against prooxidant factors.

CAT enzyme is involved in the detoxification process of ROS and is also employed as biomarker of oxidative stress in many aquatic invertebrates (WINSTON & DI GIULIO 1991). In the present study, the increased enzyme activity of *C. mucedo* of cycle II can be induced by the highest metal concentration in tissues, as evidenced by the positive correlation with all metals recorded in these specimens.

In conclusion, the results of biochemical and chemical analyses carried on *C. mucedo* evidenced an higher ability to counterbalance the oxidative stress of specimens of cycle II. This study may provide information on the role of glutathione and its associated enzymes in the detoxification processes of freshwater Bryozoa. In addition, these biochemical variations may provide background information for the implementation of biomarker-based monitoring programs, using this bryozoan species.

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Tab. 1: Heavy metals concentrations in *Cristatella mucedo* of Lake Piediluco (mean \pm SD); number of pools = 4. Significant differences between the two cycles are reported with the asterisk (*) at $P < 0.05$.

	Cycle I 1997	Cycle II 1999
Pb ng/g wet weight	36.25 \pm 14.17	976 \pm 369*
Cd ng/g wet weight	8.0 \pm 3.46	17.75 \pm 5.74*
Cr ng/g wet weight	32 \pm 16.95	93.50 \pm 20.63*
Ni ng/g wet weight	78.75 \pm 9.0	121.25 \pm 25.57*

Tab. 2: Total glutathione content and enzymatic activities in *Cristatella mucedo* of Lake Piediluco (mean \pm SD); number of pools =14 (cycle I) and 28 (cycle II). Significant differences between the two cycles are reported with the asterisk (*) at

	Cycle I 1997	Cycle II 1999
GSH+2GSSG nmol/g tissue	38.66 \pm 8.10	75.48 \pm 31.42
GST nmol/min/mg prot	55.53 \pm 10.02	102.24 \pm 22.90*
GPx nmol/min/mg prot	27.03 \pm 7.68	49.26 \pm 21.29*
GR nmol/min/mg prot	6.08 \pm 1.42	12.40 \pm 3.32*
Catalase μ mol/min/mg prot	8.36 \pm 1.88	26.79 \pm 9.72*

Tab. 3: Pearson moment correlation for biological data and metal content in *C. mucedo* of Lake Piediluco. Significant correlations are reported with the asterisk (*) at $P < 0.05$.

	Pb	Cd	Cr	Ni
GSH+2GSSG	0.96*	0.85*	0.74	0.50
GST	0.84*	0.75	0.83*	0.90*
GPx	0.95*	0.80*	0.71	0.41
GR	0.52	0.36	0.62	0.86*
CAT	0.96*	0.90*	0.91*	0.78*

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