DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota

Assessment category 3: Biological effects

Toolbox component: General stress

Fact Sheet 3.14: Glutathione reductase activity (GR)

Authors: Aino Ahvo, Kari Lehtonen and Raisa Turja, Finnish Environment Institute (SYKE)

What is it?

Glutathione reductase (GR) catalyzes the reduction of oxidized glutathione (GSSG) back to its reduced form (GSH), thus helping in maintaining the glutathione system that protects against reactive oxygen species (ROS). Thus, GR is a key enzyme in the antioxidant defence system (ADS).

What does it tell you?

Elevated levels of GR activity indicate that the cellular ADS has been activated due to an accelerated production of ROS that need to be neutralized to prevent oxidative damage to macromolecules. On the other hand, lowered GR levels may indicate that the ADS is already overloaded and the organism cannot properly cope with the excessive amounts of ROS (the so-called bell-shape response).

Type of Indicator (tick box)

☒ non-specific stress indicator
☐ specific for groups of contaminants incl. CWA or explosives
☐ CWA-specific indicator
☐ specific for substances related to explosives (e.g. TNT)

How to measure it?

***Species*:** GR can be measured in a large variety of organisms, including fish and mussels.

***Matrix*:** Fish liver tissue and mussel digestive gland and gill tissue homogenates.

***Equipment*:** Spectrophotometer/microplate reader able to measure at 340 nm in intervals; basic laboratory equipment (pipettes, microplates, decanters). For reagents, see, e.g., Turja et al. (2014) and Vuori et al. (2015).

***Measurements and units*:** For a detailed desicription, see, e.g., Mannervik and Carlberg (1975), Turja et al. (2014) and Vuori et al. (2015). Briefly, the activity of GR is measured as the change in absorbance in a mixture containing 1 mM GSSG (oxidized glutathione), 0.75mM DTNB and 0.1 mM NADPH at a final concentration in EDTA-phosphate buffer (100 mM K-PO_4 + 2 mM EDTA, pH 7.5.

The activity of GR (OD/min) is adjusted to the protein concentration of the sample, measured with, e.g., the Bradford method (Bradford, 1976).

***Calculations*:** Change in absorbance (OD/min). Subtract blank measurement values from the sample measurements.

GR activity is calculated with the formula

$$activity = \frac{\text{absorbance change} * analysis\ volume}{molar\ attenuation\ factor\ of\ DTNB\ * light\ path * sample\ volume * sample\ protein\ concentration}$$

where molar attenuation factor of DTNB is $1.36 * 10^4$ mM^{-1} cm^{-1}.

***Sample size*:** Measurements are made from at least 15-20 individual specimens from each study site.

How to analyze and assess the data?

Compare the GR activity levels measured from organisms collected from the target area to those from the reference area. An elevated or lowered activity level (bell-shape response) compared to the reference area indicate a negative effect. If the difference in mean activity level is more than one standard deviation (SD) of the mean values measured in the reference area, stress is considered moderate. If the level differs more than two SDs, stress is severe.

References

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 72:248-254.

Mannervik, I., Carlberg, B. 1975. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *Biological Chemistry* 250: 5475-5480.

Turja, R., Höher, N., Snoeijs, P., Baršienė, J., Butriavičienė, L., Kuznetsova, T., Kholodkevich, S.V., Devier, M.H., Budzinski, H., Lehtonen., K.K. 2014. A multibiomarker approach to the assessment of pollution impacts in two Baltic Sea coastal areas in Sweden using caged mussels (*Mytilus trossulus*). *Science of The Total Environment*. 473-474:398-409.

Vuori, K.A., Lehtonen, K.K., Kanerva, M., Peltonen, H., Nikinmaa, M., Berezina, N.A., Boikova, E. 2015. Oxidative stress biomarkers in the copepod *Limnocalanus macrurus* from the northern Baltic Sea: effects of hydrographic factors and chemical contamination. *Marine Ecology Progress Series* 538:131-144.