

**DAIMON Toolbox Fact Sheets:**

*Methods to Study the Impact of Dumped Munitions on Marine Biota*

**Assessment category 3: Biological effects**

**Toolbox component: Fitness/General stress**

**Fact Sheet 3.8: Glycogen – accumulation of primary energy reserve in mussels**

*Author: Matthias Brenner, Alfred Wegener Institute (AWI)*

**What is it?**

In bivalves glycogen is the primary energy reserve (Patterson et al., 1999). It can be quickly activated in cases of need and provides energy in situations of food shortage in winter or during gametogenesis, when it is transferred into the gametes.

**What does it tell you?**

A decrease in glycogen can be observed in situations of stress, like sudden temperature changes, anaerobiosis, starvation or pollutants (Hummel et al., 1989). As a reflection of the energetic and reserves status, which may undergo rapid changes, the glycogen level has been considered a useful biomarker of general stress (Ansaldo et al., 2006; Brenner et al., 2014).

**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:** Glycogen can be measured in cells of mussel digestive gland

**Matrix:** mussel digestive gland tissue

**Equipment:** cryostat; equipment for the preparation of cryo-histological slides and stainings; microscope equipped with camera (for details refer to Viarengo et al. 1985, Brenner et al. 2014).

**Measurements and units:** Cryo-sections of the target tissue are prepared and treated according to a protocol leading to the visualization of a defined staining reaction within the cells. Slides are photographed through a microscope and evaluated according to maximum staining reaction within cells of digestive tubuli. Glycogen accumulation is displayed in relation to the area investigated [area %].

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

#### How to analyse and assess the data?

Tissue sections with high staining intensity covering better parts of the investigated cell volumes are regarded as having more energy reserves than tissues of individuals with lower concentrations of glycogen in their tissues. Assessment criteria for lipofuscin accumulations have not been developed yet. Results of the glycogen assessment should be used for internal comparison of the study results only.

#### References

- Ansaldo, M., Nahabedian, D.E., Holmes-Brown, E., Agote, M., Ansay, C.V., Guerrero, N.R.V, Wider, E.A. (2006). Potential use of glycogen level as biomarker of chemical stress in *Biomphalaria glabrata*, *Toxicology*, 224(1-2), 119–127
- Brenner, M., Broeg, K., Frickenhaus, S., Buck, B.H. and Koehler, A. (2014). Multi-Biomarker approach using the blue mussel (*Mytilus edulis* L.) to assess the quality of marine environments: Impacts of habitat structure and season, *Marine Environmental Research*, 95, pp. 13-27
- Hummel, H., de Wolf, L., Zurburg, W., Apon, L., Bogaards, R.H., van Ruitenburch, M. (1989). The glycogen content in stressed marine bivalves: The initial absence of a decrease, *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 94(4), 729–733
- Patterson, M.A., Parker, B.C., Neves, R.J. (1999). Glycogen concentration in the mantle tissue of freshwater mussels (*Bivalvia*: Unionidae) during starvation and controlled feeding, *American Malacological Bulletin*, 15(1), 47–50.
- Viarengo, A., Moore, M. N., Pertica, M., Mancinelli, G., Zanichchi, G. and Pipe, R. K. (1985). Detoxification of copper in the cells of the digestive gland of mussel: the role of lysosomes and thioneins. *Science of the Total Environment*, 44, 135–145