DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota

Assessment category 3: Biological effects

Toolbox component: Disease/Pathology

Fact Sheet 3.18: Lysosomal membrane stability (LMS)

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What is it?

Lysosomes are cytoplasmic, single-membrane organelles ubiquitous to animal cells. Lysosomes play an important role in nutrient transport and recycling of larger cell molecules. Further, Lysosomes are involved in detoxification processes by entrapping and metabolizing pollutants and toxins. Lysosome membrane stability (LMS) can be altered by physiological or pathological conditions induced by pollutants, resulting in the activation or liberation of hydrolytic enzymes to the cytosol.

What does it tell you?

LMS is used as a biomarker of the general health status of an individual organism. A reduction in LMS is a non-specific stress response that can be caused by various factors, including exposure to pollutants.

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

***Matrix*:** Fish liver tissue, mussel digestive gland tissue, mussel haemocytes.

***Equipment*:**

Histochemical method: cryostat; equipment for the preparation of cryohistological slides; microscope equipped with camera. For detailed protocols and reagents, see ICES (2004) and Martínez-Gómez et al. (2015).

In vitro method using haemocytes (Neutral Red Retention test, NRR): syringes and needles; slides; incubation chamber; microscope. For detailed protocols and reagents, see ICES (2004) and Martínez-Gómez (2015).

***Measurements and units*:**

Histochemical method: Serial cryosections of the target tissue are prepared and treated according to a protocol leading to the visualization of a defined marker enzyme activity within the lysosomes. Slides representing the different post-dying intervals are photographed through a microscope and evaluated to determine the time (in min) of maximum colour intensity resulting from reactions involving the marker enzymes, and the time is used as the endpoint parameter.

NRR: Living (mussel) haemocytes are observed at fixed intervals under a microcope after the introduction of the Neutral Red dye solution. When 50% of the cells in an individual sample show the predefined alterations, the observation time (min) is recorded as the endpoint parameter.

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

How to analyze and assess the data?

Histochemical method: Different species show slightly different baseline LMS levels and also different response sensitivity to pollutants. In the Baltic Sea, fish and mussels are considered to have no toxically-induced stress if LMS is >15 min, are stressed but compensating between 15 and 8 min, and severely stressed and probably exhibiting irreversible toxicopathic alterations if <8 min.

NRR: In Baltic Sea mussel populations, mean NRR times <50 indicate a poor health status, values between 50 and 90 a moderate health status, and values >120 a good health status.

References

Martínez-Gómez, C., Bignell, J. Lowe, D. 2015. Lysosomal membrane stability in mussels. *ICES Techniques in Marine Environmental Sciences*, No. 56. 41 pp.

ICES. 2004. Biological effects of contaminants: Measurement of lysosomal membrane stability. By M.N. Moore, D. Lowe, and A. Köhler. *ICES Techniques in Marine Environmental Sciences*, No. 36. 31 pp.