

***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 3.19: Lipofuscinosis – pathological accumulation of lysosomal lipofuscin**

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**What is it?**

Lipofuscinosis is a pathological accumulation of age/stress pigment in the lysosomes of molluscan cells of the digestive gland.

**What does it tell you?**

Lysosomes are accumulating trace metals and organic xenobiotics. This uptake of metals and pollutants lead to the generation of oxy-radicals and peroxidation processes (Au et al. 1999, Davies & Vethaak, 2012). As a result a pathological accumulation of the age/stress pigment lipofuscin may be detected (Viarengo et al., 1985).

**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:** Lipofuscin can be measured in lysosomes of mussel digestive gland cells

**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 lysosomes. Slides are photographed through a microscope and evaluated according to maximum staining reaction within cells of digestive tubuli. Lipofuscin 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 more effected and might have experienced more peroxidation events than tissues of individuals with lower concentrations of lipofuscin in their tissues. Assessment criteria for lipofuscin accumulations have not been developed yet. Results of the lipofuscin assessment should be used for internal comparison of the study results only.

#### References

- Au, D.W.T., Wu, R.S.S., Zhou, B.S., Lam, P.K.S., 1999. Relationship between ultrastructural changes and EROD activities in liver of fish exposed to benzo[a] pyrene. *Environmental Pollution*, 104, 235-247
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