DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota

Assessment category: Biological Effects

Toolbox component: Immunotoxicity

Fact Sheet 3.22: Hematology - differential white blood cell count (WBC)

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What is it?

The hematology parameters erythrocytes, hemoglobin and hematocrit give an overview about the abundance of red blood cells (erythrocytes), leucocrit the percentage of white blood cells (WBC) (leucocytes) in the blood. The differential quantification of white blood cells (white blood cell count = WBCC) can be used as an indicator of the health and stress status of fish, because these cells are the effector of the immune system and circulate throughout the bloodstream and the lymphatic system. Changes may have an infectious (e.g., viral, bacterial, parasitic) or non-infectious (various causes, like contamination with toxic substances or other environmental stressors) etiology (Ellis, 1977).

What does it tell you?

Differential white blood cells counts determine the exact number or the relative proportion of each type of WBC that is present in an individual stained blood smear. The differentiation of WBC types is made based on their morphological characteristics which have to be known before counting can be carried out. A combination of counting WBC and checking the general health condition of the same fish is helping to identify causes that affect one or more types of WBCs and is useful in confirming the diagnosis of specific disorders. (Ellis, 1977; Pund, 1998).

The results of WBC are considered as a generic non-specific indicator of habitat quality and environmental health, reflecting the well-being of fish and the status of their non-specific immune system. Changes in the percentage of WBC may be caused by a variety of natural and anthropogenic stressors, including exposure to hazardous substances (Witeska et al., 2005; Adedeji et al., 2009; Dey et al., 2013).

In addition to the quantification of WBCs, the inspection of smears under the microscope can be used for identifying blood parasites or the presence of bacteria in blood (sepsis).

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?

Blood smears are prepared by dropping a blood drop on one end of a clean lipid-free glass object slide, and smearing the blood drop gently over the slide by using a bevelled slide. Slides should be produced at least as duplicate. Blood smear slides are air-dried and subsequently fixed in methanol for 10 minutes before staining it with a commercial available fast staining kit or traditional Giemsa staining (Pund, 1998). For the examination, the stained object slides are inspected under a light microscope. First, the smears are inspected at low magnification to assess the distribution of cells and to select an area where blood cells are evenly distributed and do not overlap. Erythrocytes are dominating and should be well separated, while leucocytes are rarer, but should also be evenly spread. All further examinations are executed using an oil immersion objective with 100 times magnification. For quantification of the different WBC types, at least two times 200 leucocytes should be counted for each slide and classified according to their staining reactions, nuclear morphology and characterisation of any cytoplasmic granules that may occur. Here a differentiation is made between following leukocytes: lymphocytes, granulocytes (eosinophil, basophil and neutrophil) and monocytes. The values of each cell type are expressed as percentage of the total amount of leucocytes.

Because of the high species-specific morphological variability of the WBC types, all types of WBC have to be analysed for the fish species under study before quantitative analyses can be made (Tandon & Joshi, 1976; Vázquez & Guerrero, 2007).

How to analyse and assess the data?

For the measurement of the WBC, different issues have to be taken into account when interpreting the data. The WBC must be considered separately for each fish species. The WBC is subject to the natural influences of season, maturity, physical influences, such as water temperature, water depth (pressure) and salinity, as well as chemical effects.

If there are no data available for the selected fish species to be studied, it is important to first establish the “normal” healthy status of WBC. For this purpose, it is recommended to also generate data on other haematological parameters (erythrocytes, haemoglobin, haematocrit, leucocrit etc.) and on the general health status of the fish species.

For the data analysis, , mean values per sample and sampling site can be calculated from the individual WBC; e.g., arithmetic means and standard deviation or arithmetic means and 95 % confidence intervals.

References

Adedeji, OB, OK Adeyemo, and SA Agbede. 2009. Effects of diazinon on blood parameters in the African catfish (Clarias gariepinus), *African Journal of Biotechnology*, 8: 3940.

Dey, Sangeeta, Manabendra Dutta Choudhury, and Suchismita Das. 2013. 'A review on toxicity of paper mill effluent on fish', *Bull. Environ. Pharmacol. Life Sci*, 2: 17-23.

Ellis, A_E. 1977. 'The leucocytes of fish: a review', *Journal of fish biology*, 11: 453-91.

Lugowska, K, E Kondera, and M Witeska. 2017. 'Leukocyte count in fish–possible sources of discrepancy', *BULLETIN OF THE EUROPEAN ASSOCIATION OF FISH PATHOLOGISTS*, 37: 94-99.

Pund, Ralf Peter. 1998. 'Anwendung hämatologischer Untersuchungsmethoden für Fischblut und Beeinflussung des Blutbildes von Bachforellen (Salmo trutta f. fario) durch Haltungs-und Umwelteinflüsse sowie endogene Faktoren'.

Tandon, RS, and Bishwambhak Datt Joshi. 1976. 'Total red and white blood cell count of 33 species of fresh water teleosts', *Zeitschrift für Tierphysiologie Tierernährung und Futtermittelkunde*, 37: 293-97.

Vázquez, G Rey, and GA Guerrero. 2007. 'Characterization of blood cells and hematological parameters in Cichlasoma dimerus (Teleostei, Perciformes)', *Tissue and cell*, 39: 151-60.

Witeska, Małgorzata. 2005. 'Stress in fish-hematological and immunological effects of heavy metals', *Electronic Journal of Ichthyology*, 1: 35-41.