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 3.9: Hematology - blood glucose level in fish

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What is it?

The blood glucose level increases with acute, repeated or sustained stress. It has been used as indicator for monitoring, is easy to measure, relatively inexpensive and the most commonly measured indicator of stress response in fish. (Barton, 2002)

The easiest way to measure blood glucose level is to use a common human medical blood glucose meter (e.g., Contour® XT, Bayer, Germany) with corresponding sensors (Contour® Next, Bayer, Germany). The detection is based on an amperometric measurement. (Yoo et al., 2010).

What does it tell you?

The blood glucose level is considered as a generic non-specific indicator of habitat quality and environmental health, reflecting the acute status of the fish's well-being. Repeated or prolonged exposure of fish to stressful stimuli can result in reduced growth, impaired reproductive performance and immunosuppression (Barton, 1997). The responsiveness of the indicator to stressors has been reported repeatedly for fish (Pickering and Pottinger, 1989; Pickering et al., 1993; Di Marco et al., 2008; Kubilay et al., 2002).

Acute stress events have a high impact on hormonal regulation of catecholamines (adrenalin) and glucocorticoids (cortisol) and constitute a complex process involving several factors. The hypothalamic portion of the brain stimulates the release of adrenocorticotropic hormone (ACTH). A stress-induced increase of catecholamines induces a rapid rise of blood glucose level (Wedemeyer, 1996; Reddy and Leatherland, 1998; Reid et al., 1998; Pottinger and Carrick, 1999).

A high blood glucose level indicates that fish has an increased stress level. Due to a chronic high level of stress, ACTH is chronically increased. This implies a chronic high level of, amongst others, cortisol, adrenalin and glucose. Stimulatory actions of cortisol on hepatic glucose production may be limited to gluconeogenesis. This can results in a chronically higher blood glucose level and a lower increase of glucose level in acute stress events as usually seen in non-stressed fish. The changed hormonal levels in chronic stress situations can also contribute to a weight loss. (Wendelaar Bonga, 1997; Mommsen et al., 1999).

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?

For measuring just blood glucose level with human medical blood glucose meter + sensors, it is sufficient to take a drop of blood during processing of the fish. It is important that fish is anaesthetized or sacrificed directly before taking blood samples and while blood is still not coagulated.

If other blood parameters are to be measured in addition to blood glucose, the blood sampling should be performed by puncture of the caudal vein. The puncture site must be wiped dry to avoid possible contamination of the blood sample. The blood has to be collected in a tube pre-filled with anticoagulant (e.g., 500 µl Microvette®, Sarstedt). For all measurements, the same anticoagulant must be used as it can have a strong influence on the results (Tavares-Dias & Sandrim, 1998). The most widely used and validated anticoagulant is lithium heparin (e.g., 500 µl Microvette®, Sarstedt).

For measuring with an amperometric measurement system (e.g., Contour® XT and sensors Contour® Next, Bayer, Germany), the inserted sensor is to be hold to a drop of blood and the sensor will absorb it. The result is usually given in mg/dl or in mmol/l.

The conversion factor for glucose is:

mmol/l * 18.02 = mg/dl

mg/dl * 0.0555 = mmol/l

How to analyse and assess the data?

For the measurements of the blood glucose level, different issues have to be taken into account when interpreting the data. The blood glucose levels must be considered separately for each fish species. The blood glucose is subject to the natural influences of season, physical influences such as water temperature, water depth (pressure) and salinity as well as chemical inserts in relation to the anticoagulant used and the measurement method (Houston 1996). After trawling and before sampling the fish, there may be changes in blood glucose level. The causes are complex. In addition to the changed physical parameters of the holding water compared to the original environment, an adaptation of the fish to the new conditions or a stress response is possible. It is important that the trawling time as well as the sampling time of the caught fish should be kept as short as possible in order to keep an influence of stress on the blood glucose as low as possible.

From the individual glucose values, mean values per sample and sampling site can be calculated; e.g., arithmetic means and standard deviation or arithmetic means and 95 % confidence intervals.

Depending on the distribution of the data and the form of the mathematical relationship, medians with percentiles are also applicable.

For the assessment of effects on the blood parameters, two commonly applied approaches can be used:

(1) Statistical comparison of mean blood parameter values obtained from impacted areas (e.g. a munitions dumpsite) and from un-impacted reference areas,

(2) The use of assessment criteria (BAC: background assessment criteria; EAC: environmental assessment criteria) reflecting a good, medium or bad fitness status.

So far, no generally applicable assessment criteria for blood parameters in fish have been established. One reason is that each criteria has to be species-specific, because the mean of each blood glucose values and the range of such values occurring in a population differ by species.

In the data analysis and assessment for the DAIMON project, assessment criteria (BAC and EAC values) for glucose levels were defined. Due to the complex physiological and pathophysiological releasing of hormones and glucose in stress situations, the values need to be a more complex assessment, because the high and low values indicate stress. The no-effect level = BAC is between the quantile 20-80 %, the effect level 1 it is defined between BAC and EAC, quantile 5-20 % and 80 – 95 %, and effect level 2 = EAC corresponds to quantile 5 % and quantile 95 %. The following assessment criteria were used in DAIMON:

Dab (*Limanda limanda*):

- BAC: ≥ 1,57 – 6,08
- Effect level 1_{below}: ≥ 0,85 – 1,57
- Effect level 1_{top}: > 6,08 – 7,47
- EAC_{below}: < 0,85
- EAC_{top}: > 7,47

Cod (*Gadus morhua*):

- BAC: ≥ 2,49 – 6,71
- Effect level 1_{below}: ≥ 0,70 – 2,49
- Effect level 1_{top}: > 6,71 – 9,02
- EAC_{below}: < 0,70
- EAC_{top}: > 9,02

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