

Standard Operating Procedure (SOP)

Aim of SOP (tick box)

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|---|---|
| <input type="checkbox"/> Munition detection or identification | <input type="checkbox"/> Toxicity |
| <input type="checkbox"/> Sampling | <input type="checkbox"/> In situ exposure studies |
| <input type="checkbox"/> Chemical analysis | <input type="checkbox"/> Bioassays |
| <input checked="" type="checkbox"/> Bioindicators/biomarkers | |

27. Hepatosomatic Index (HSI) in fish

version 1.1

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Scope

This Standard Operating Procedure describes the hepatosomatic index (HSI) in fish. This method is used to determine the relationship between body weight and liver weight and can be applied to any desired fish species. The HSI is used as an indicator of the nutritional status and the energy metabolism of fish.

Summary of the method

In addition to its use as an indicator of nutrients and metabolism, the HSI is also generally regarded as a non-specific indicator of habitat quality and the environmental stress to which the fish have been exposed. Often the term liver somatic weight (LSI) is used instead of HSI which has to be taken into account when referring to other references. Changes in the HSI can be caused by various natural and anthropogenic stressors, such as hazardous substances¹⁻³. The mathematical relationship of the HSI is described by the following formula, where the body weight is based on the gutted weight of the fish:

$$HSI = \frac{Body\ Weight\ (g) \times 100}{Liver\ Weight\ (g)}$$

The gutted weight is used to avoid an influence of the intestinal fullness, the weight of the inner organs or the gonad maturity status on the HSI. High values of the HSI generally describe a good status/condition of the fish and low values a poor status respectively.

In the context of dumped munitions, the HSI can be applied in screening studies or detailed studies on the effects of conventional munitions or chemical warfare agents on fish. However, it should only be used in combination with selected, more specific biomarkers, as it is considered a generic stress indicator.

Safety aspects

When working onboard research vessels and with pointed or sharp objects, the safety instructions of the responsible safety officer must be followed.

Documentation

Data should be noted directly after weighing in spreadsheets or with appropriate software. It is advisable to note down the data line by line per fish. In addition, geographical coordinates must be logged for each sampling site and hydrographic data such as temperature and salinity must be measured so that the sampling site can be clearly identified afterwards.

Methods

Equipment:

- Dissection tools: Forceps, scalpel, knife etc.
- Gloves.
- Balance that is suitable for working on unstable platforms, such as research vessels.

Chemicals:

- Ethanol for cleaning, CAS 64-17-5.

Species:

HSI can be measured in all fish species that are used in studies on the effects of dumped munitions on fish. For calculating HSI the whole fish and the liver are needed.

Sample size:

Ideally, HSI should be calculated in 100 specimens per sampling site. These should be the same fish that are also used for calculating the somatic condition factor (CF, see SOP on Fulton's Condition Factor) and other biomarkers.

Measurement:

For each fish, the somatic/body weight is recorded after dissection (gutting) of the fish and removal of inner organs. The digestive tract, adherent liver, spleen, gall bladder and gonads have to be removed. In addition, the weight of the liver is determined to calculate the HSI.

Data evaluation:

The individual HSI values per fish can be used to calculate means values per sampling site, e.g. arithmetic means and standard deviation or arithmetic means and 95% confidence interval. Depending on the sample size or distribution of the data, medians with percentiles are also applicable.

For the detection of effects by the HSI, usually two approaches are used:

1. Statistical comparison of mean HSI values obtained from possibly impacted areas (e.g. munition dumpsites) and from reference areas,
2. The use of assessment criteria, such as background assessment criteria (BAC) and environmental assessment criteria (EAC), that are describing a good, medium or poor fitness status.

To date, there are no generally applicable assessment criteria for HSI in fish. One reason for this is that such criteria must be species-specific, as the mean HSI values and the variance of HSI values occurring in a population differ depending on the species. During data analysis and assessment in the DAIMON project, the BAC and EAC values for HSI in cod (*Gadus morhua*) were defined on the basis of the highest 25% percentile (BAC) and the lowest 10% percentile (EAC) of all HSI values measured in cod from reference areas during the winter. The following criteria were derived for cod, where an HSI value greater than the BAC indicates good status and an HSI value smaller than the EAC indicates poor status:

$$BAC: HSI \geq 7.15$$

$$EAC: HSI \leq 4.0$$

References

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- (2) Morado, C. N.; Araújo, F. G.; Gomes, I. D. The Use of Biomarkers for Assessing Effects of Pollutant Stress on Fish Species from a Tropical River in Southeastern Brazil. *Acta Sci. Biol. Sci.* **2017**, *39* (4), 431–439.
- (3) Hansson, T.; Thain, J. E.; Martínez-Gómez, C.; Hylland, K.; Gubbins, M. J.; Balk, L. Supporting Variables for Biological Effects Measurements in Fish and Blue Mussel. *ICES Tech. Mar. Environ. Sci.* **2017**, *60*.

Change history

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