FIELD APPLICATION REPORT

EnSpire Multilabel Plate Reader

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# Studying environmental stress levels in Baltic Sea zooplankton *Limnocalanus macrurus* using biochemical markers

## Introduction

The Baltic Sea is a large brackish water sea in Northern Europe surrounded by Sweden, Finland, Russia, Estonia, Latvia, Lithuania, Poland, Germany and Denmark. The natural characteristics of the Baltic Sea, such as a slow exchange of water, make it especially vulnerable to both human impact and natural changes. Finding ways to monitor environmental changes and to study their effects throughout the food-chain and across the Baltic basin is a challenge. In this report we present one method using EnSpire™ Multilabel Plate Reader to detect environmental stress levels in organisms at the base of the marine food web.


## Environmental stressors in the Baltic Sea

Animals and plants inhabiting the Baltic Sea are subjected to multiple stressors, from considerable changes in temperature and salinity, to human generated harmful substances and nutrient loads. The Baltic Sea has one of the longest histories of contamination in the world; from the advent of modern industrialization it has been subjected to anthropogenic pressure and today the Baltic Sea has been described as the most polluted sea in the world (HELCOM 2010). The Baltic Sea is highly eutrophicated and suffering from excess loads of nutrients, nutrient enrichment harmful algal blooms and adverse effects at the ecosystem level (HELCOM 2009). Climate change has affected the area. Over the past century, it has been subject to greater than the reported global mean temperature increase (The BACC Author Team 2008).

The levels of different environmental stressors in different types of wildlife vary around the Baltic basin and change with time. They affect the Baltic Sea aquatic ecosystem in ways that are not yet fully understood.

## Zooplankton

Zooplankton is an important part of marine food webs. Zooplankton consumes other organisms such as phytoplankton. The abundance and nutritional value of zooplankton and the accumulation of harmful substances in zooplankton have derived consequences to the subsequent food web. By studying the effects that various environmental stressors have on zooplankton, we can better understand the secondary effects to subsequent food web.

*Limnocalanus macrurus* is a dominant copepod zooplankton species found in the pelagic regions in the northern parts of Baltic Sea, in the Gulfs of Bothnia and Finland. It is thus a suitable example species for studying variation and biological effects of environmental stressors in these sea areas.

*Limnocalanus macrurus* female.
Image © Siru Tasala.

## Oxidative stress biomarkers

Oxidative stress is essentially an imbalance between production of reactive oxygen species (ROS) and the detoxification of reactive intermediates. Excess ROS can damage cell's lipids, protein or DNA, inhibiting normal function. Changes in redox status and oxidative stress are common to many stress conditions and oxidative stress defence plays a central role in responses to various environmental stressors. Oxidative stress is an important component of the stress response in marine organisms exposed to a variety of environmental changes such as thermal stress or pollution (Lesser 2006). Certain evolutionarily conserved biochemical markers of oxidative stress defence and damage are used to indicate environmental stress levels in many organisms. In this report we present methods and results from three different oxidative stress biomarker assays on *L. macrurus* samples.

## Materials and Methods

## Sample collection


R/V Aranda is a modern, ice-reinforced research vessel adapted to multidisciplinary marine research around the year.
Image © Finnish Environment Institute

The pan-Baltic research project BEAST (Biological Effects of Anthropogenic Chemical Stress: Tools for the Assessment of Ecosystem Health) aims to develop integrated chemical-biological monitoring of chemical pollution, and assessment methods in regards to the Baltic Sea marine ecosystem (BEAST Project website, 2010). The parameters examined included hydrographic parameters, concentrations and indicators of biological effects of hazardous substances in sediments and in biota, phyto- and zooplankton community composition, and antioxidant pigments and vitamins in biota. During the BEAST 2010 Expedition, samples of *L. macrurus* were collected from marine areas around Finland, Sweden and Latvia. The expedition was carried out aboard R/V *Aranda* belonging to the Finnish Environment Institute.

Individual *L. macrurus* are less than 2 mm in size. For this study a total of 30 individuals were pooled in order for each sample to yield a sufficient sample volume for the projected measurements. Collecting 30 individuals per sample in field conditions is laborious, manual work and yields less than 100 µl of sample homogenate for all the measurements. In this report we show example results from 8 *L. macrurus* samples collected from one study site.

## Measurements

A set of biomarkers was measured to study oxidative stress in the *L. macrurus* samples. Altogether seven measurements were performed for each sample in three replicates. Three of the measurements were conducted on board during the cruise using the EnSpire Multilabel Plate Reader fitted with a prototype reagent dispenser unit.

EnSpire on board R/V Aranda
Image © Kristiina Vuori

In order to perform all the projected measurements with the small sample volumes, all the assays needed to be miniaturized. In this report we show three different miniaturized oxidative stress biomarker level assays: (1) glutathione-peroxidase enzyme (GP) activity; (2) glutathione reductase enzyme (GR) activity; and (3) superoxide dismutase enzyme (SOD) activity. All measurements were carried out using 384-well plates due to the small sample volumes.

**Experiment 1**

GP activity was measured using Glutathione Peroxidase Cellular Activity Assay Kit (Sigma Chemicals, St. Louis, Missouri, USA) according to manufacturer's instructions using $H_2O_2$ as a substrate. The total volume of the assay was reduced by 40 times from 1000 µl to 25 µl.

**Experiment 2**

GR activity was measured according to the protocol of Smith et al. (1988). The total volume of the assay was reduced by 20 times from 1000 µl to 50 µl.

**Experiment 3**

The inhibition rate of SOD activity was measured using a SOD Determination Kit (Fluka, Buchs, Germany). The total volume of the assay was reduced by 8 times from 240 µl to 30 µl.

## Results

The example GP, GR and SOD results from 8 *L. macrurus* samples collected from one study site are presented in Figure 1.

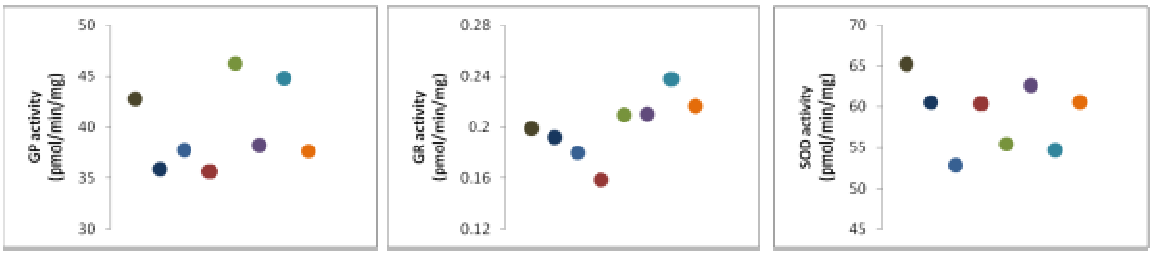


Figure 1. GP, GR and SOD results from eight *Limnocalanus macrurus* samples collected from one study site. Each sample is identified with the same colour in all the three measurements

The results obtained demonstrate the usefulness of the instrument for instant at-sea measurements. Temperature control, plate shaking, versatile protocol programming capabilities and small reagent volume dispensing capability were valuable for all these three experiments. Average CVs of 5.6, 5.5 and 5.0% were calculated for GP, GR and

SOD (respectively), after removing outlier wells. These CV values demonstrate the accuracy and precision of the EnSpire dispenser.

## Conclusions

The results demonstrate the ability to analyze a set of oxidative stress biomarkers from zooplankton specimen, including field samples. We were able to conduct seven different biomarker assays from pooled samples of 30 *L. macrurus* individuals (<100µl homogenate). The samples were prepared and three biomarkers measured on board marine research vessel. The rapid availability of results is a highly useful asset for outreach purposes. From an instrument viewpoint, we can conclude that even under highly unusual laboratory conditions, i.e. measuring samples aboard a research vessel and with prototype reagent dispenser data with exceptionally good CV values was produced. Reliable and precise data is essential for showing differences between the chosen markers in geographically distinct locations. The *L. macrurus* oxidative stress biomarker results will be used in the BEAST project to evaluate geographical variation and effects of environmental stressors in assessing the state of the ecosystem in different sub-regions of the Baltic Sea.

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