Enzyme tools to determine the well-being of biological communities
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The marine environment is subject to numerous human activities, with significant pressures and impacts from fishing, alterations derived from climate change, and discharges (urban and/or industrial), among others, with consequent loss of environmental quality, and the elimination or alteration of habitat and species populations. The possible alterations of biological communities can cause changes in physiological states, species diversity, abundance and biomass. Because of this, we have aligned our research with the objectives of environmental management. Using enzymes as biomarkers helps us understand and correct the effects of anthropogenic stresses on marine ecosystems.

Enzymes are central to every biochemical process. They catalyze the hundreds of stepwise reactions that degrade nutrient molecules, conserve and transform chemical energy.

To assay the enzyme activities, the velocity of the specific-enzyme reaction is determined, measuring the generation of product per time using different chemical techniques.

**1. GDH activity measured in the mysid Leptomysis lingüvora over a month of experimentation (Fernández-Urruzola et al., 2011)**

**2. IDH vs φ (potential respiration from ETS activity), in 200-2000µm and 0.7-50µm plankton samples (Tames-Espinosa et al., 2017)**

**3. Energy metabolism-related biomarkers in Holothuria forskali. Fig 2 in Tonn et al. (2016). Measures of ETS activity, E₆, CEA and IDH in the muscle tissue.**

**4. Alterations in GDH studied in different tissues of Laboe rohita exposed to sublethal concentration of cypermethrin. Fig 7 in Philip and Rajastree (1996).**

**5. Effect of lindane on the energy budget (CEA values) of Daphnia magna. Fig 1 in DeCoen and Janssen (1997).**

**METHODS**

* s.h.: sample homogenate

<table>
<thead>
<tr>
<th>ETS activity</th>
<th>Based on Owens &amp; King (1975) and Kenner &amp; Ahmed (1975) 100µl s. + 300µl substrate solution + 100µl INT Read the absorbance during 8min at 490nm</th>
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<tbody>
<tr>
<td>IDH activity</td>
<td>According to Tames-Espinosa et al. (2017) 100µl s. + 300µl (MgCl₂:isocitrate) solution + 100µl NADP⁺ solution Read the absorbance at 340nm during 10min</td>
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<tr>
<td>GDH activity</td>
<td>As described in Fernández-Urruzola et al. (2011) 200µl s. + 300µl NAD⁺ solution + 250µl ADP solution + 500µl Glutamate Read the absorbance at 340nm during 8min</td>
</tr>
</tbody>
</table>

- **E₆**: energy reserves available = Eₚrotein + Eₖarbohydrate + Eₖ lipid
- **E₆**: energy consumption = ETS activity

**ENERGY METABOLISM**

(a key goal in defining metabolic stress in marine organisms)

**REFERENCES**

- Bligh E.G. and Dyer W.J. (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37(8), 911-917