Enzyme tools to determine the well-being of biological communities

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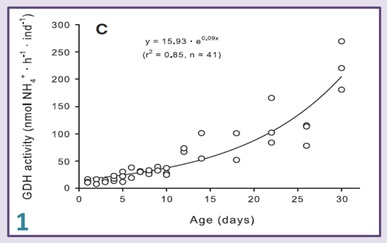
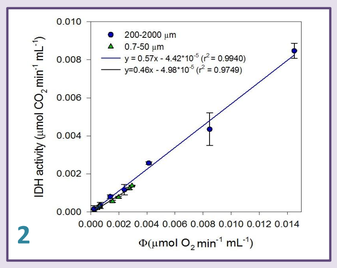
The marine environment is subject to numerous human activities, with significant pressures and impacts from fishing, alterations derived from climate change, and discharges (urban and/or industrial), among others, with consequent loss of environmental quality, and the elimination or alteration of habitat 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.

ENZYMES





- 1. GDH activity measured in the mysid *Leptomysis lingvura* 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)

ETS — Electron transport system

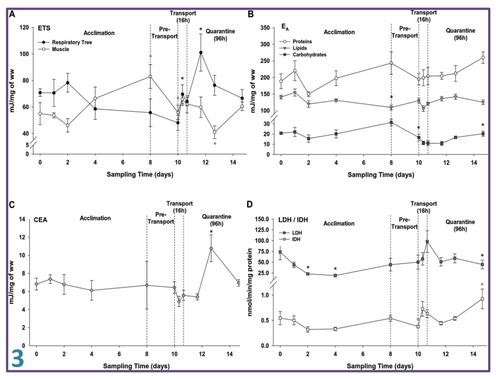
- A combination of enzymes that control the respiratory oxygen consumption
- Measure of activity: production of formazan, from INT (a tetrazolium salt) reduction

IDH — Isocitrate dehydrogenase

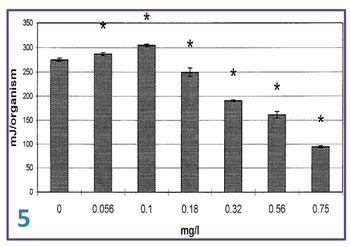
- Enzyme responsible for respiratory CO_2 production
- Measure of activity: production of NADPH, from $NADP^+$ reduction

GDH — Glutamate dehydrogenase

- The main role of this enzyme consists of glutamate deamination, producing α-ketoglutarate, feeding the tricarboxylic acid cycle
- Measure of activity: production of NADH, from NAD^+ reduction



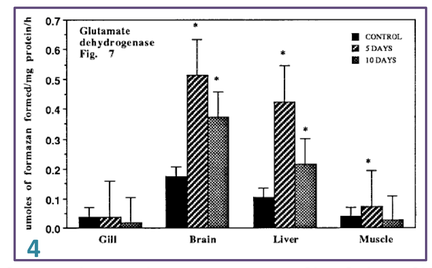
- 3. Energy metabolism-related biomarkers in *Holothuria forskali.* Fig 2 in Tonn et al. (2016). Measures of ETS activity, E_a, CEA and IDH in the muscle tissue.



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

- Proteins
- Lipids
- Carbohydrates

→ Energy available → Intermediary metabolism (IDH, GDH) → ETS → Energy consumption



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

CELLULAR ENERGY ALLOCATION

Based on the metabolic costs hypothesis (Calow and Sibly, 1990), in the last decades, a biomarker technique to assess the effect of stress on the energy budget of organisms has been developed → the cellular energy allocation (CEA)

$CEA = E_a / E_c$

- ✓ E_a : energy reserves available = $E_{protein} + E_{carbohydrate} + E_{lipid}$
- ✓ E_c: energy consumption = ETS activity

Intermediary metabolism + CEA = ENERGY METABOLISM

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

METHODS

*** s.h. : sample homogenate**

ETS activity	Based on Owens & King (1975) and Kenner & Ahmed (1975) 100µl s.h. + 300µl substrate solution + 100µl INT Read the absorbance during 8min at 490nm
IDH activity	According to Tames-Espinosa et al. (2017) 100µl s.h. + 300µl ($MgCl_2$+Isocitrate) solution + 100µl $NADP^+$ solution Read the absorbance at 340nm during 10min
GDH activity	As described in Fernández-Urruzola et al. (2011) 200µl s.h. + 300µl NAD^+ solution + 250µl ADP solution + 500µl Glutamate Read the absorbance at 340nm during 8min
Proteins	According to Smith et al. (1985) 25µl s.h. + 500µl work reagent / Incubate 30' at 37.5ºC Bovine serum albumin as standard Read the absorbance at 562nm
Lipids	Based on Bligh and Dyer (1959) 200µl s.h. + 500µl chloroform + 500µl methanol + 250µl bidistilled water → centrifugation → 100µl supernatant + 500µl sulfuric acid → Charred → diluted 1:6 in bidistilled water Glyceryl tripalmitate as standard Read the absorbance at 340nm
Carbohydrates	According to Nahrgang et al. (2010) 50µl s.h. + 200µl sulfuric acid + 50µl phenol Glycogen as standard Read the absorbance at 490nm

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