Anthropogenic influence on mussels in marinas of the Adriatic Sea: distribution of heavy metals, metallothioneins and Cellular Energy Allocation

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Introduction

• Antifouling paints are applied to the hulls of boats and to static structures to prevent the growth of fouling organisms. With the ban on organotin compounds, most antifouling paints are based on copper as biocides, boosted by other chemicals like zinc oxide.
• As the result of slow and controlled leaching of biocides, semi-enclosed marine systems, such as marinas and harbours, are characterised by elevated concentrations of metals.
•Non-essential metals (e.g. Hg, Pb) can be highly toxic to organims, but essential metlas (e.g. Fe, Zn, Cu) can be toxic at high doses.
• Metals are not biodegradable and can accumulate in sediment and biota, making metal pollution environmental issue for decades.
• Adverse effect of contaminants can be first observed at cellular level as biological and physiological changes, so combination of physiological and biological parameters can give better insight in toxicity.
• Metal exposure is reflected as induction of **metallothioneins (MT)**, low molecular proteins with high content of cysteine residues responsible for binding of metals. Its main roles are homeostasis of essential and detoxification of toxic metals.

• Investigation of metals bound to proteins other than MT pool could provide a more complete understanding of potential mechanisms of toxicity. Distribution of metals bound to cytosolic biomolecules of different size can be determined by **size-exclusion high performance liquid chromatography (SEC-HPLC)** in combination with **inductively coupled plasma mass spectrometry (ICP-MS)**.
•Metal detoxification and the maintenance of detoxification mechanisms may be energetically expensive. Therefore, organisms in better condition with more energy stores may invest more energy in metal detoxification.
• Physiological state of organism, including energy stores, may be estimated using physiological biomarkers like **Cellular Energy Allocation (CEA)**. Decrease of CEA indicates either a reduction in available energy or higher energy expenditure, both resulting in a lower amount of energy available for growth, reproduction or defence against stresses (e.g. natural stress, pollution).
•The **aim** of our work is to determine differences in physiological state, distribution of cytosolic metals and metallothioneins between mussels from marinas and clean area.

Materials and method

• Selected bioindicator is indigenous mussel *Mytilus galloprovincialis*, a sessile filter feeder and good accumulator of metals. Two indicator tissues are dissected; **digestive gland** as metal storage and **gills** as metal uptake site.

• Samples are collected in winter and summer season from two marinas with different number of berths (A being larger than B) and reference station (RS).
• Biometric measurements and sex determination are performed.

CEA – general physiological biomarker

•Homogenates of individual samples of digestive glands are used.

• The content of proteins, lipids and carbohydrates were determined by biochemical measurements and transformed to energetic equivalents. The sum of these components represents **available energy Ea**.

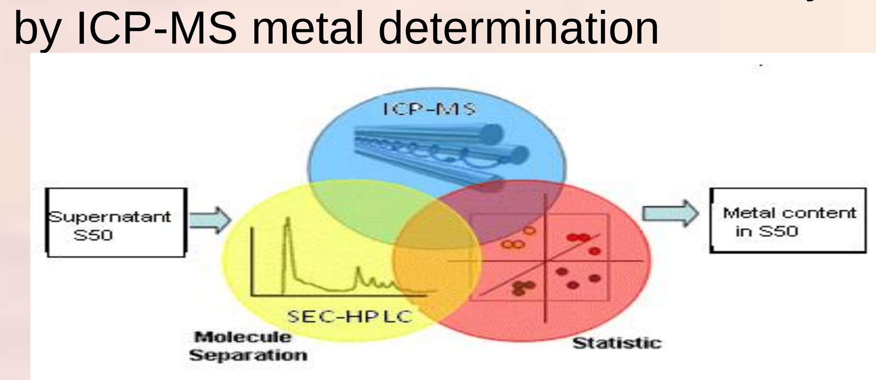
•The **energy consumption Ec** is estimated by measuring the activity of mitochondrial electron transport system and transformed to energetic equivalents.

$$CEA = \frac{Ea}{Ec}$$

Decrease of CEA indicates either a reduction in available energy or higher energy expenditure

***Homogenization and centrifugation**:* digestive gland tissue samples were diluted 6 times with cooled homogenization buffer (20 mM Tris-HCl/Base, pH 8.6 at 4°C) supplemented with reducing agent (DTT) and protease inhibitors (PMSF and Leupeptine). Homogenates were centrifuged at 50000g for 2h at 4°C to obtain supernatant S50 which contains cytosol.

Analysis in S50 fraction
• Metal levels were measured by ICP-MS.
• MT levels were measured by differential pulse voltammetry
• Total proteins were measured by Lowry procedure
• Fractionation of biomolecules of different size by SEC-HPLC, followed by ICP-MS metal determination



Results

Table 1.Concentrations of Zn,Cd,Pb and Cu in the sea water (data are shown as mean ± standard deviation)

Sampling site	**Zn** (µg/L)		**Cd** (ng/L)		**Pb** (ng/L)		**Cu** (µg/L)	
	winter	summer	winter	summer	winter	summer	winter	summer
Marina A	2.89 ±0.18	2.69 ±0.40	8.2 ±1.8	10.71 ±2.06	33.1 ±8.8	57.81 ±3.89	2.65 ±0.04	0.22 ±0.02
Marina B	1.89 ±0.14	2.46 ±0.30	7.3 ±0.2	9.58 ±1.05	13.7 ±1.6	43.63 ±3.72	1.30 ±0.06	0.16 ±0.01
RS	0.68 ±0.04	1.21 ±0.34	8.7 ±1.4	8.82 ±0.31	10.5 ±5.4	41.44 ±4.59	0.24 ±0.02	0.44 ±0.07

What about the rest of the results?!

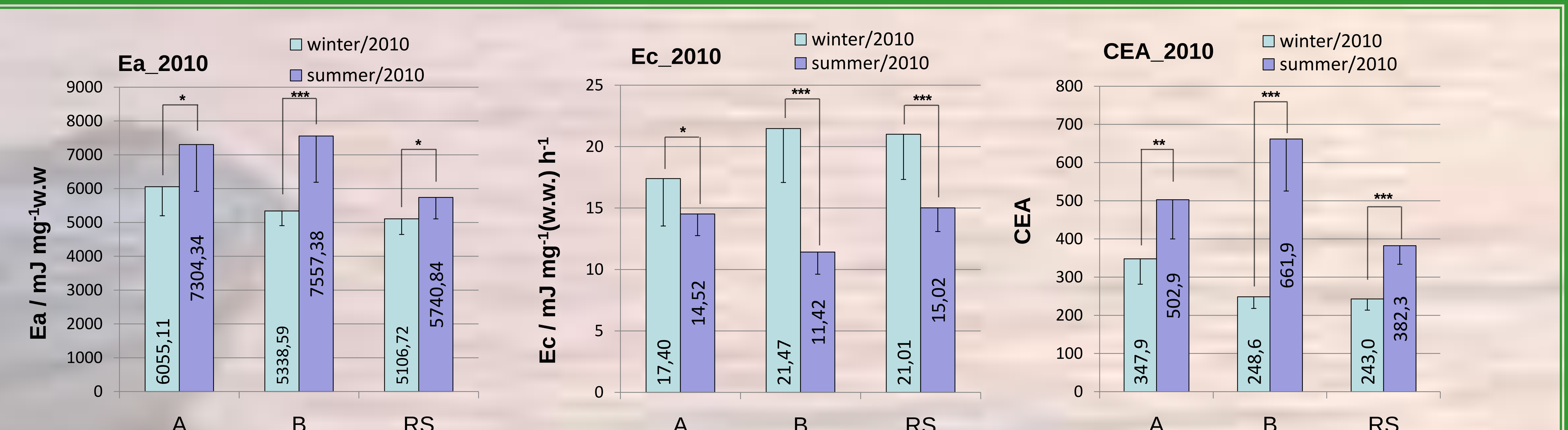


Figure 1. Total energy available (Ea), energy consumption (Ec) and CEA in digestive gland of *M.galloprovincialis* determined at three sampling sites (mean values and standard deviations are presented; Significant differences between seasons are indicated with probability levels *p<0,05, **p<0,02, ***p<0,001). Since there were no significant differences between male and female individuals, Ea, Ec and CEA results are considered together for each site.

Concluding remarks

• There were no significant differences between male and female mussels regarding CEA parameters
• As expected marina A, which has larger number of berths has higher concentrations of metals in sea water. Both marinas have higher concentration of Cu in winter than in summer, what is probably due to painting of vessels performed in winter period.
• CEA parameters show seasonal variation for all stations. In summer period mussels have higher available energy than in winter due to higher lipid and carbohydrates energy levels, what may be a consequence of increased food after phytoplankton blooms. Additionally, higher CEA values in that period are contributed by lower energy consumption.
• Evident seasonal variation in all CEA parameters (except proteins) recorded at all stations indicate the necessity to take into account the sampling period.
• Mussels from reference station had lower CEA than from marinas what is not in accordance with available contamination data, but can be a result of rich food sources in the marinas.

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