Development of cytoprotective responses in mussels exposed to stress factors, alone or in combination.

MEECE WP1 coordinated by R. Bellerby
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Laboratory of Environmental Physiology and Biochemistry: KEY WORDS

Selected PAPERS
- The beta-blocker propranolol affects cAMP-dependent signaling and induces the stress response in Mediterranean mussels, Mytilus galloprovincialis Aquatic Toxicology 101 (2011) 299–308
- Effects of environmental concentrations of the antiepileptic drug carbamazepine on biomarkers and cAMP-mediated cell signaling in the mussel Mytilus galloprovincialis Aquatic Toxicology 94 (2009) 177–185
- Cytoprotective responses in the Mediterranean mussel exposed to Hg2+ and CH3Hg+ Biochem Biophys Res Comm 351 (2006) 719–725

Selected REVIEWS
**General AIM:** contribute to the prediction models by

1) exploration of the mechanistic relationship between changes in abiotic variables and changes experienced by the organisms (organism sensitivity to environmental changes)

2) quantitative understanding of the physiological mechanisms underlying tolerance to abiotic stress (organism vulnerability to environmental changes)

Evaluating cytoprotective responses elicited by chemicals and temperature changes, at the basis of mussel survival and/or adaptation.

**Cytoprotective responses: activation of the multidrug resistance (MXR) system**

[Diagram showing the ABC transporter (P-gp) and metabolic pathways]
Cytoprotection: activation of the Heat Shock Protein response

Environmental Stress

![Graphs and charts illustrating temperature and environmental stress on cytoprotection and heat shock protein response.](Image)
The stress factors

**Oxytetracycline**

In Italian rivers, OTC detected up to 250 ng/L; international literature: values up to 50 µg/L.

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**Copper**

**Temperature**

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The experiments

**OXYTETRACYCLINE (OTC)**

0, 0.1, 1, 10, 100, 1000 µg/L

**COPPER (Cu)**

0, 2.5, 5, 10, 20, 40 µg/L

Mediterranean mussels exposed at 16°C, 20°C and 24°C; 4 days

- 4 replicates per concentration
  - 12 mussels per replicate
- 48 animals per concentration per each temperature tested
- 864 mussels per each experiment

**Applied Methodologies**

Fluorimetric assays

Western blotting

PCR, RT-PCR and Q-PCR

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Gills
**Cu/temperature co-exposure on cell signalling**

*16 °C: Cu (up to 10 μg/L) activates cAMP signaling*
*20 °C: the activation is mainly due to increase in T ° C*
*24 °C: cAMP levels is rised by Cu, however PKA is not activated*

Interaction between Cu and T ° C on PKA was indicated by 2-way ANOVA

**The role of cAMP in mussels**

- Energetic “fuels” availability for gonad development
- Heart beating
- Movements of gill cilia
- Pg-P expression (MultiXenobiotic Resistance)

**Modulation of cAMP levels by endogenous or exogenous factors**
Cu/temperature co-exposure on stress response
(P-gP transporters)

ABCB1 gene expression

* p< 0.05 vs ctr at 16° C

Cu increases PgP gene expression at the different temperatures
24° C increases PgP expression
2 way-ANOVA shows interaction between Cu and temperature

Cu/temperature co-exposure on stress response
(heat shock proteins)

HSC70 and HSP70 gene expression

* p< 0.05 vs ctr at 16° C

16° C: MgHSC70 expression increased by Cu
20° C and 24° C: 2-way ANOVA shows interaction between Cu and T° C

MgHSP70 expression is affected both by temperature and Cu
Further insights....

**HSP70 AJ624615**

Q-PCR showed an increased expression at 20°C and relatively lower at 24°C. In the presence of 40 ug/L Cu the hsp70 response is the highest at 20°C.

**HSP70 AJ624323**

A further HSP70 gene was tested by QPCR. In this case Cu was able to suppress the thermal stress effects.
Cu/temperature co-exposure on stress response (heat shock proteins)

**HSP70 protein expression**

* p<0.05 vs control at 16°C

HSP70 expression is increased by Cu and by temperature, independently. At 24°C the T°C it is clearly prevailing.

**HSC70 protein expression**

HSC70 expression is increased by temperature and not by Cu. No interaction is revealed by 2-way ANOVA.
OTC/temperature co-exposure on cell signaling

16 °C: OTC increased cAMP levels, and not PKA activity
20 °C: OTC increased cAMP levels, and not PKA activity
24 °C: only T °C seems to affect cAMP and PKA activity.
No interaction between the two factors was revealed by 2-way ANOVA

*p<0.05 vs control at 16 °C

OTC/temperature co-exposure on stress response (P-gP transporters)

OTC slightly increases Pgp gene expression; 2-way ANOVA shows interaction between OTC and temperature at 20 °C and 24 °C

*p<0.05 vs ctr at 16 °C
OTC/temperature co-exposure on stress response (heat shock proteins)

MgHSC70 and MgHSP70 expression is affected by T° C and slightly by OTC. 2-way ANOVA shows interaction between OTC and T° C on MgHSP70.

HSC70 protein expression

HSC70 exp increased by T° C and not by OTC.
**OTC/temperature co-exposure on stress response**  
**HEAT SHOCK PROTEINS**

HSP70 exp is increased by OTC at 16°C and 20°C; not at 24°C when T°C effect prevails. Interaction between the two is revealed by 2-way ANOVA.

**OVERVIEW (2-way ANOVA)**

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*p<0.05 and **p<0.01 according to 2-way ANOVA
Conclusions

**Copper** induces cytoprotective responses (MXR system and Hsp response) that may underline adaptation phenomena. Cu interacts with temperature on some parameters (e.g. reduces the HSP expression).

The antibiotic has moderate effects, that are overwhelmed by the thermal effects. OTC interacts with temperature on several parameters.

Per se, temperature increases (from 16 to 20 and 24°C) affects all examined parameters (except PgP).

Note, temperature changes and Cu effects on cAMP pathway may alter several physiological functions, including reproduction, heart beating and gill cilia movements.

Conclusions

Pollutant exposure may hinder the ability of organism to acclimate and make them more susceptible to other stressors.

This is more problematic for species living at the edge of their physiological tolerance range.

In general, the effect of temperature increases on pollutant effects seems not linear; it appears the existence of tipping points in which interaction of stressors lead to relevant effects on living organisms.
Aknowledgments

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