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CAMPUS DI RAVENNA

FANO MARINE CENTER

FishMed-PhD

Molecular physiology of marine organisms under climate changes and emerging anthropogenic threats

Silvia Franzellitti

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Dept. Biological, Geological and Environmental Sciences (BiGeA - Ravenna)

The Physiology of Global Change: Linking Patterns to Mechanisms

George N. Somero

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Somero (2012) - ANNU. REV. MARINE SCI. 4:39

The scope of physiological anaysis and its utility in the study of global changes

*The term **physiology** is essentially synonymous with **function***

The science of physiology explains how organisms work and how these workings are influenced by the changing environments in which organisms live

Adaptation

the **processes** by which natural selection **adjusts** the frequency of genes that code for traits affecting fitness

Acclimatization

the process of **tuning physiology** of **organisms within their lifetime** allowing them to cope with varying environments, and it is also referred to as **phenotypic plasticity**

Biomarker

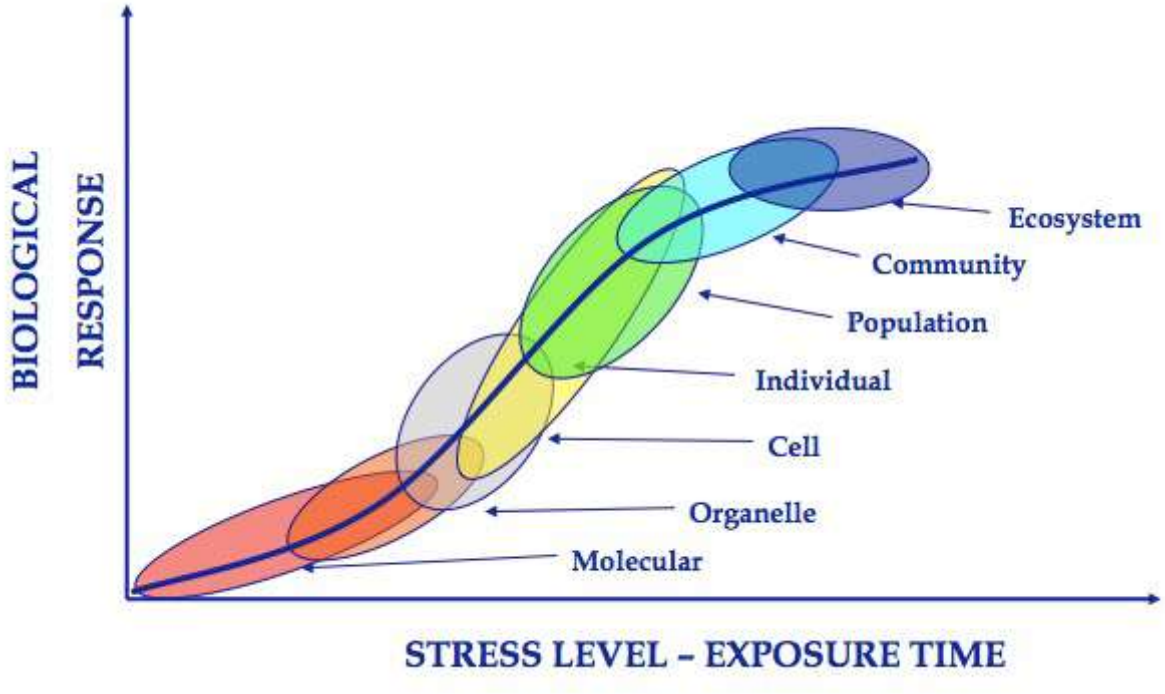
The simplest definition … a biomarker is a change in a molecular, cellular, tissue, orgnaims and even behavioral component that indicates an alteration in physiology from normal

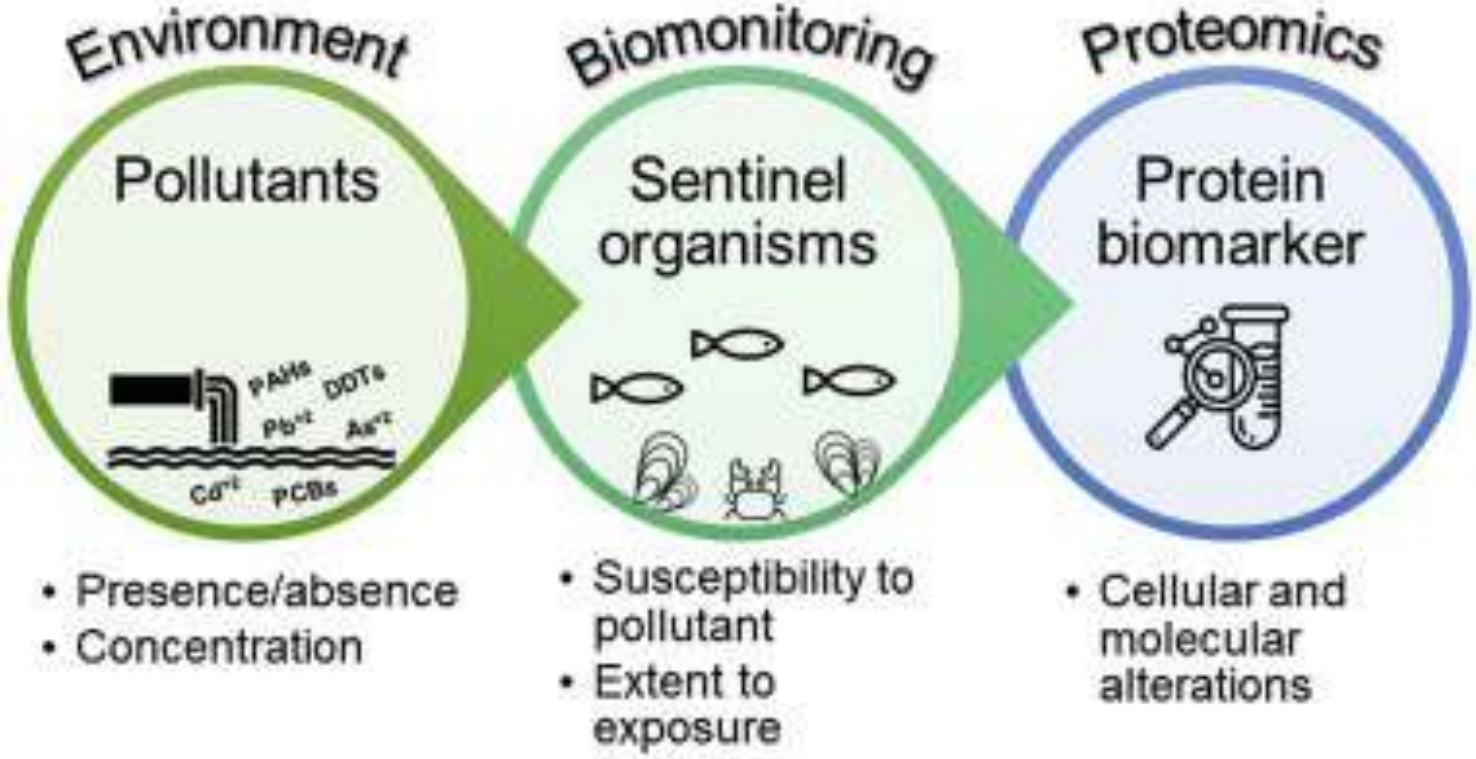
Why looking at the molecular responses?

They are the first mechanisms to be activated in the biological respose to enviornmental stimuli

They are the indication of animal-environment interaction

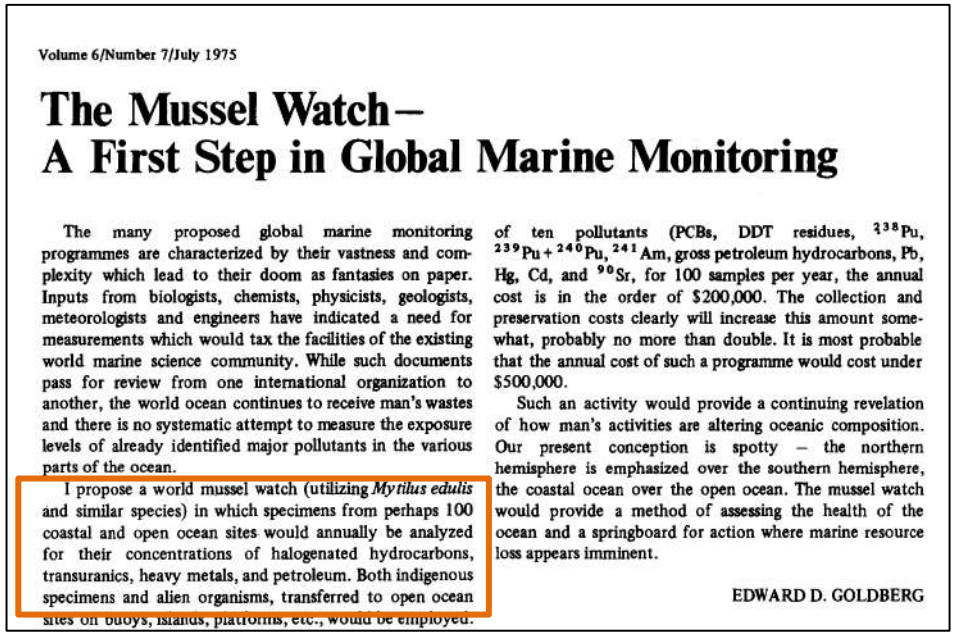
They may forecast changes at higher biologcal levels





Biomonitoring using the biomarker approach and sentinel organisms

Goldberg (1975) – The Mussel Watch



Volume 6/Number 7/July 1975

The Mussel Watch— A First Step in Global Marine Monitoring

The many proposed global marine monitoring programmes are characterized by their vastness and complexity which lead to their doom as fantasies on paper. Inputs from biologists, chemists, physicists, geologists, meteorologists and engineers have indicated a need for measurements which would tax the facilities of the existing world marine science community. While such documents pass for review from one international organization to another, the world ocean continues to receive man's wastes and there is no systematic attempt to measure the exposure levels of already identified major pollutants in the various parts of the ocean.

I propose a world mussel watch (utilizing *Mytilus edulis* and similar species) in which specimens from perhaps 100 coastal and open ocean sites would annually be analyzed for their concentrations of halogenated hydrocarbons, transuranics, heavy metals, and petroleum. Both indigenous specimens and alien organisms, transferred to open ocean sites on buoys, islands, platforms, etc., would be employed.

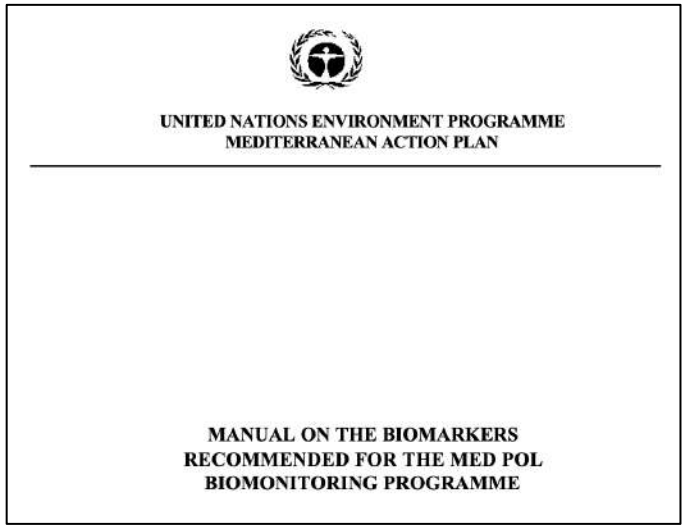
of ten pollutants (PCBs, DDT residues, ^{238}Pu, $^{239}Pu + ^{240}Pu$, ^{241}Am, gross petroleum hydrocarbons, Pb, Hg, Cd, and ^{90}Sr, for 100 samples per year, the annual cost is in the order of $200,000. The collection and preservation costs clearly will increase this amount somewhat, probably no more than double. It is most probable that the annual cost of such a programme would cost under $500,000.

Such an activity would provide a continuing revelation of how man's activities are altering oceanic composition. Our present conception is spotty — the northern hemisphere is emphasized over the southern hemisphere, the coastal ocean over the open ocean. The mussel watch would provide a method of assessing the health of the ocean and a springboard for action where marine resource loss appears imminent.

EDWARD D. GOLDBERG

Pollutant bioaccumulation

UNEP_MAP (1999)

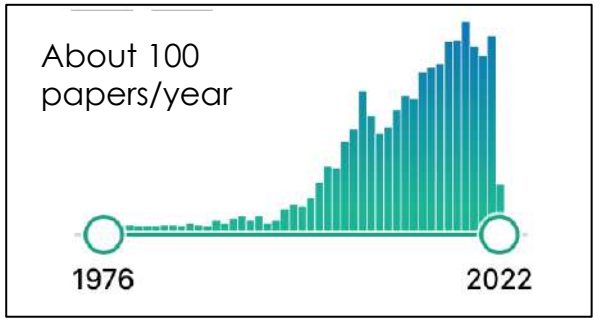


UNITED NATIONS ENVIRONMENT PROGRAMME
MEDITERRANEAN ACTION PLAN

MANUAL ON THE BIOMARKERS RECOMMENDED FOR THE MED POL BIOMONITORING PROGRAMME

Few biomarkers considered, mainly related to metal or oil spill pollution

Today



Broad spectrum of pollutant sources considered (natural toxins, physical stressors, emerging pollutants)

Mixed exposure scenarios

Incorporating -omic technolgies, whole life-cycle analyses

Integrating biological and chemical datasets ➔ **Weight Of Evidence**

Addressing whole pathway perturbations ➔ **Adverse Outcome Pathways**

Sentinel organisms

Indicator or sentinel species are those species that are sensitive to environmental disturbance and develop measurable biological responses that provide an early warning that more severe water quality conditions are likely to occur unless mitigation measures are taken.

Main features:

- Sensitive enough to develop sublethal responses towards environmental stress
- Widespread in the study area and simple to be sampled
- Fast and repeatable biological responses
- Good knowledge about their physiology and acclimatory features

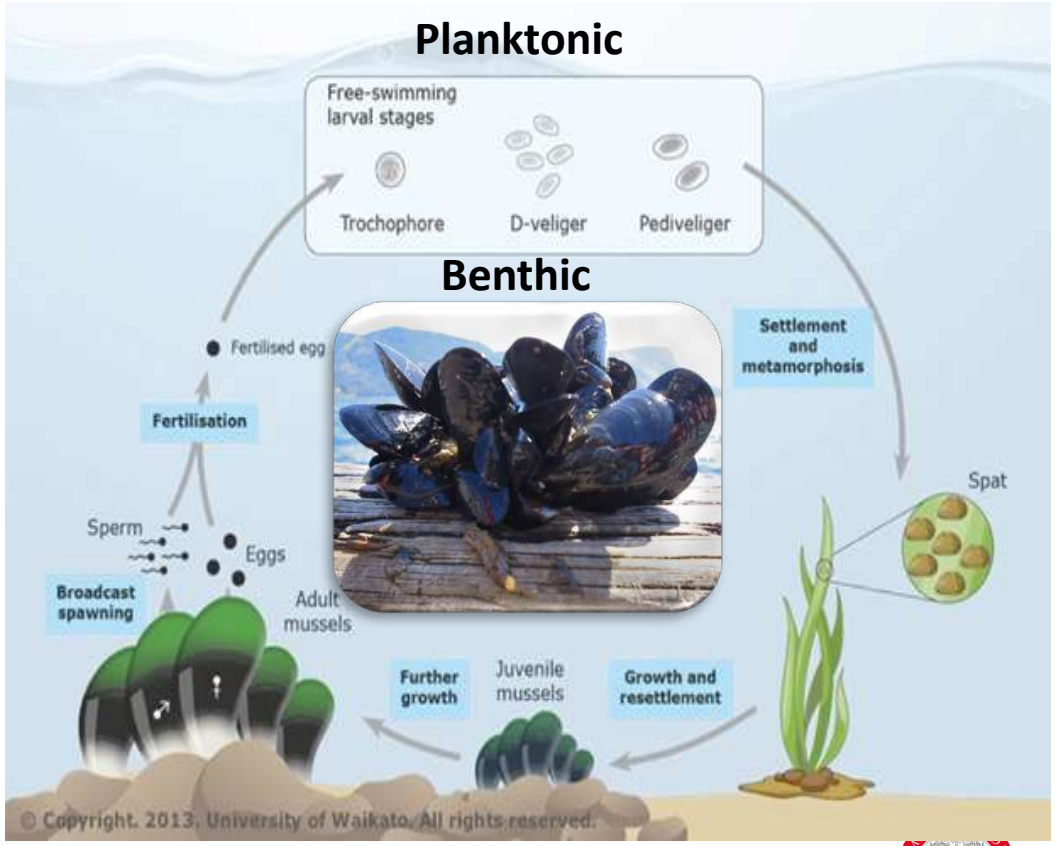
Acknowledged sentinel species …

MUSSELS	**CLAMS**	
		
Mytilus galloprovincialis	***Chamelea gallina***	***Tapes philippinarum***
Water column	sediment	sediment
Good survival	Low survival	Good survival
High sensitivity	High sensitivity	Low sensitivity
Measurable responses	Measurable responses	Few measurable responses
Good reproducibility	low reproducibility	low reproducibility

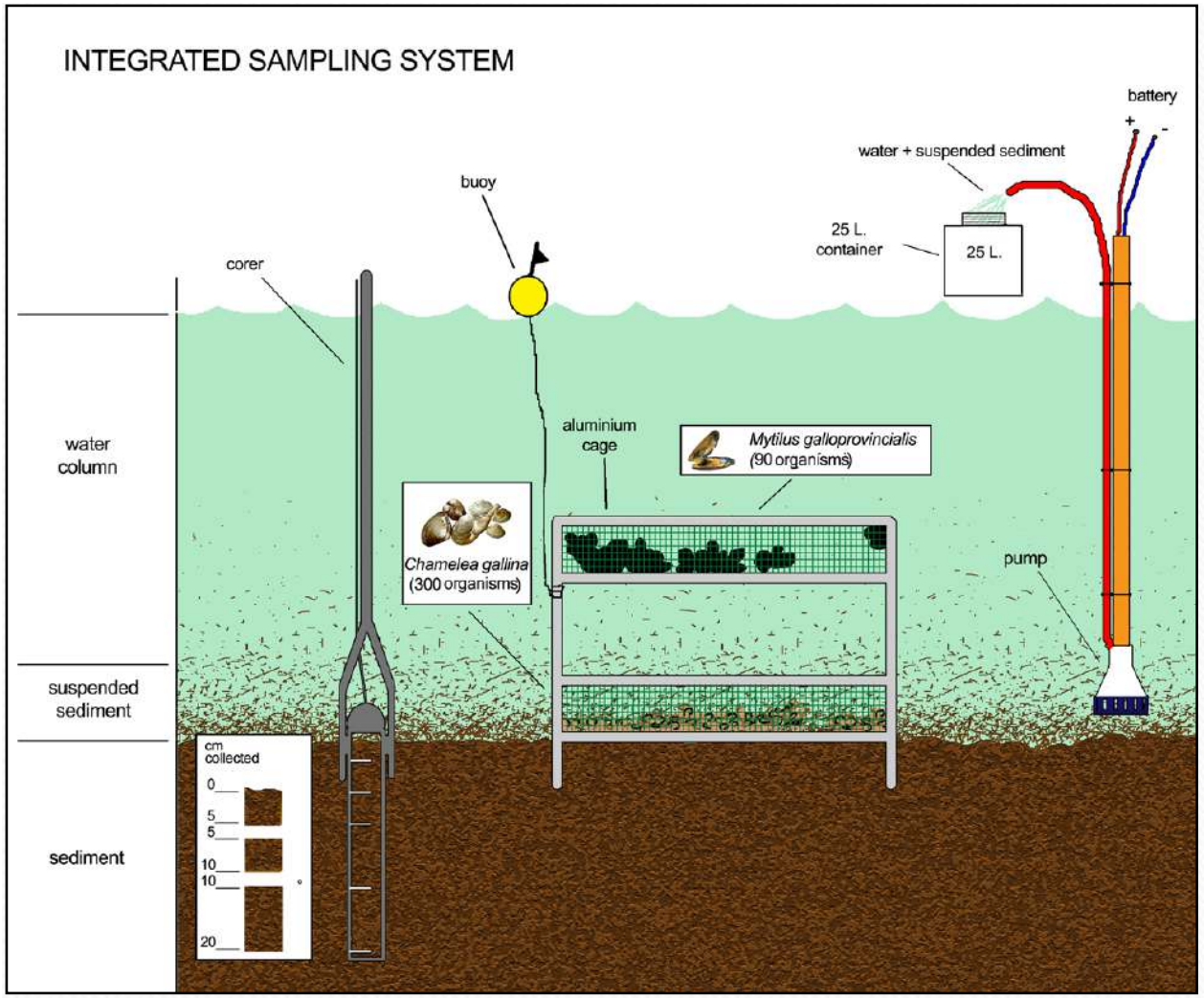
Marine mussels (*Mytilus* spp.) as model organisms in environmental physiology

- Sessile
- Benthic filter-feeder
- Lives in coastal marine environments characterized by:
    - high daily/seasonal variability of environmental variables (temperature, salinity, pH …)
    - continuous exposure to natural and anthropogenic toxins

Effective mechanisms of protection and adaptation are needed to cope with environmental challenges



Experimental setup

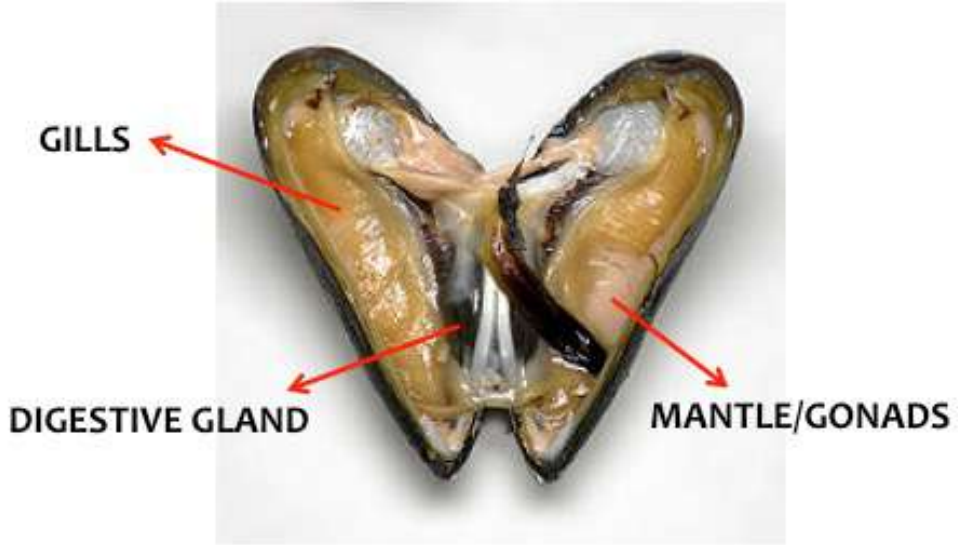


Caging experiments

about 30 days

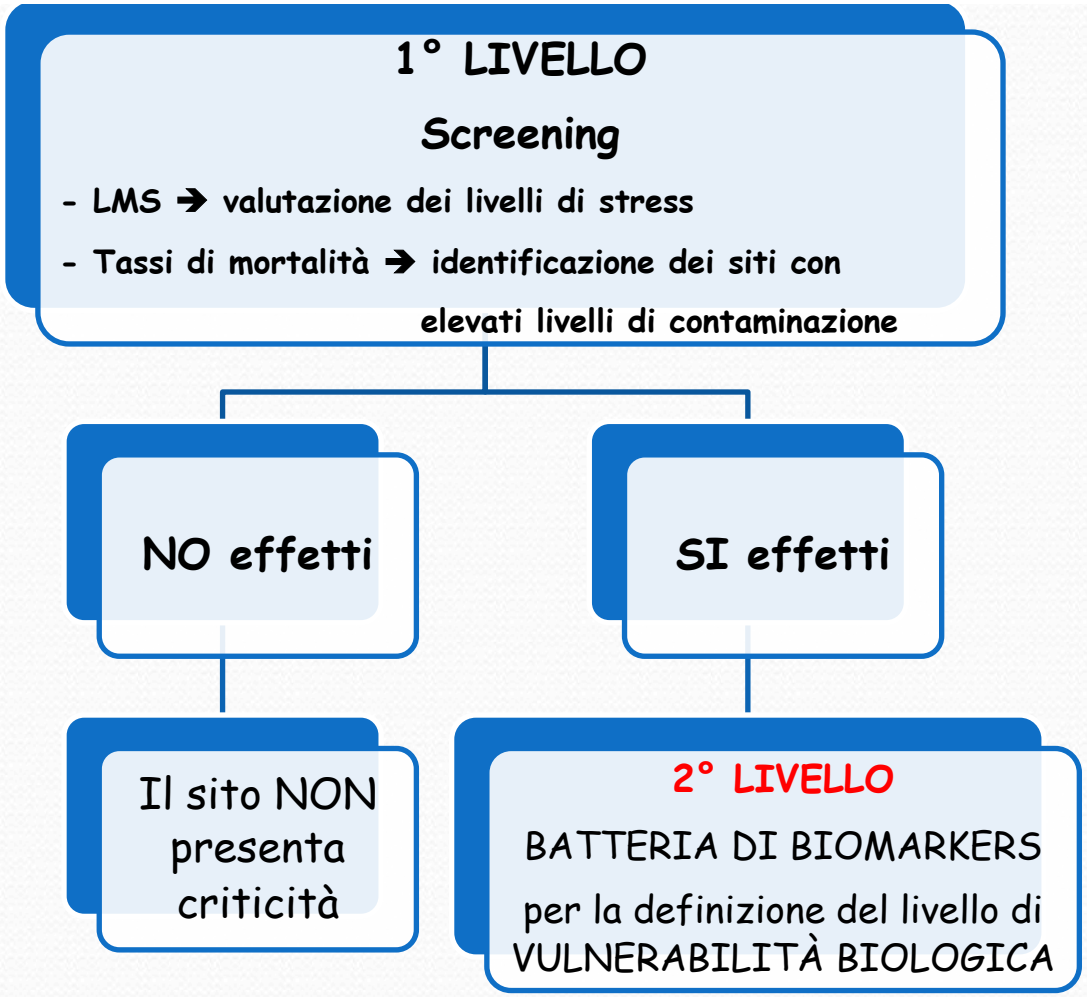
Sampled tissues :

- Haemolimph
- digestive gland
- mantle/gonads



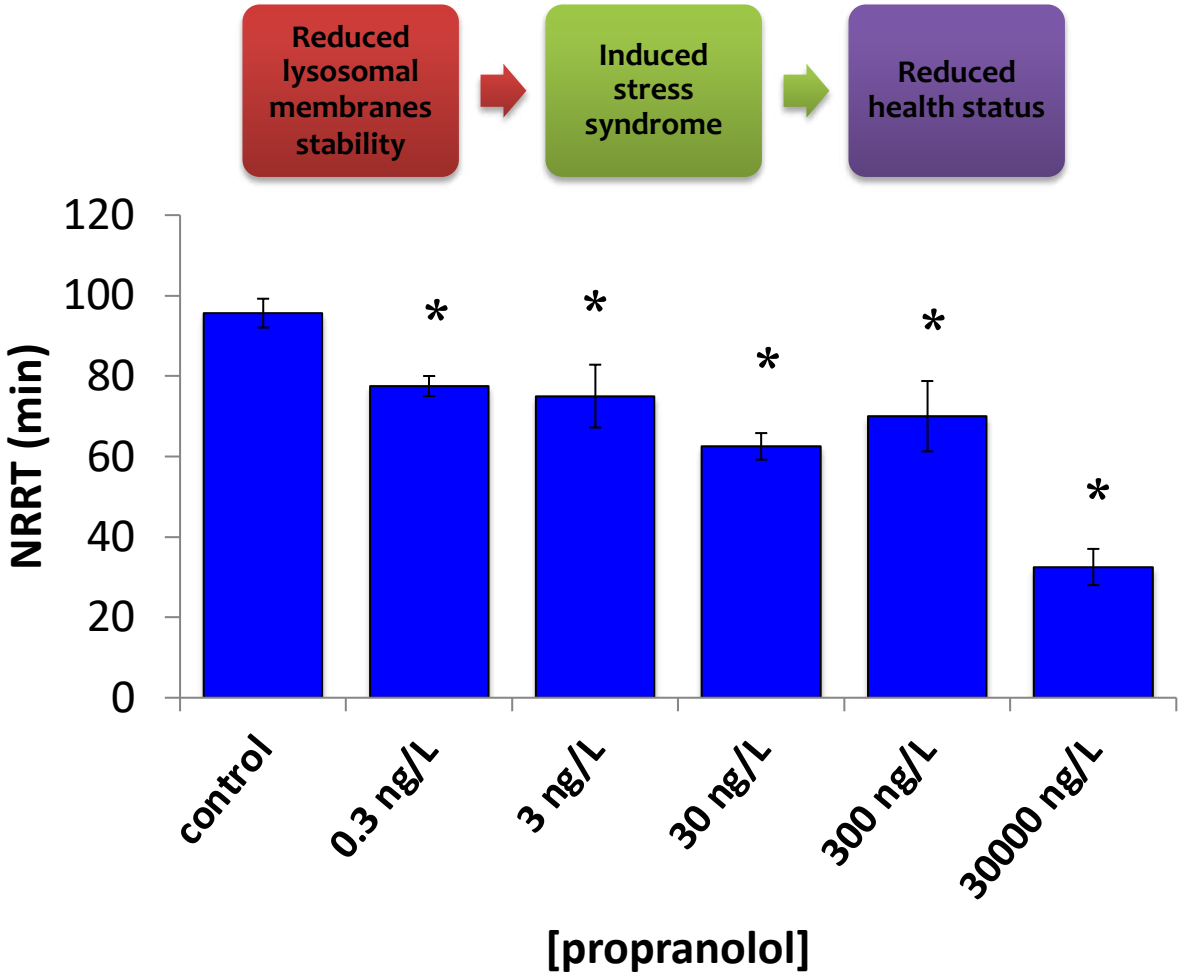
Which biomarkers?

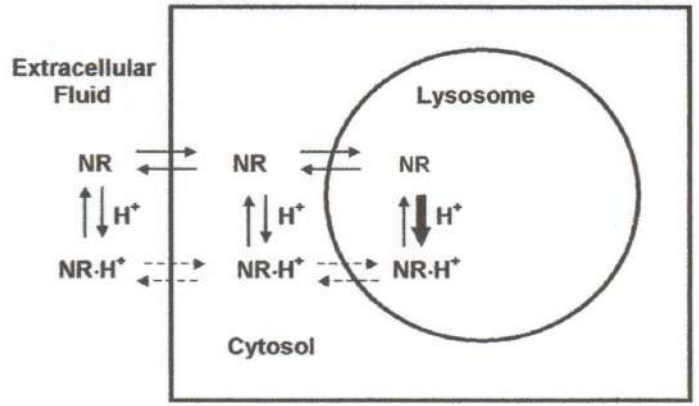
(Viarengo et al., 2007 – Biochem. and Physiol.)

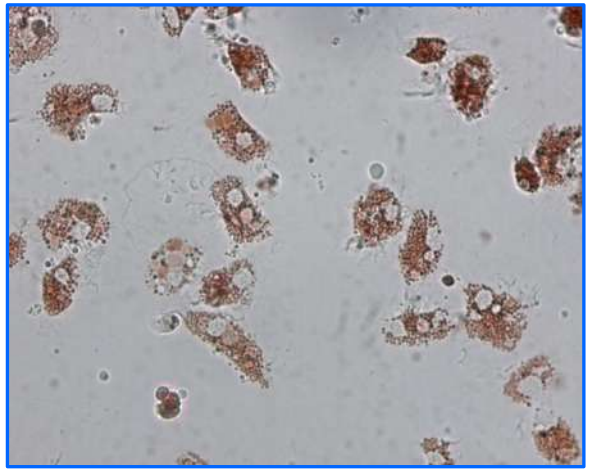


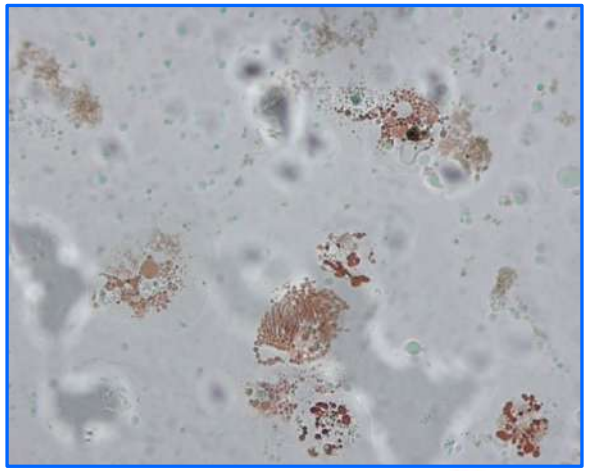
Lysosomal membrane stability assay

early-warning parameter predicting effects at the population level









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Evaluating the quality of transitional environments through sentinel organisms and the biomarker approach

- Laguna costiera salmastra situata a nord-est della città di Ravenna (Italia).
- E' inclusa nella convenzione di Ramsar tra le come zona umida di interesse internazionale e nel Parco del Delta del Po.
- La circolazione delle acque dolci è regolata artificialmente.
- Gli scambi d'acqua col mare avvengono grazie all'escursione di marea.

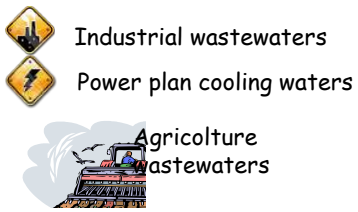
Tipologia di contaminanti riversati nella Pialassa Baiona:

Negli anni '50 – '70

- Mercurio
- IPA, PVC, PVA

Oggi

- Scarichi urbani
- Acque di scolo da zone agricole
- Scarichi industriali depurati



Sorgenti di inquinamento nella Pialassa Baiona.

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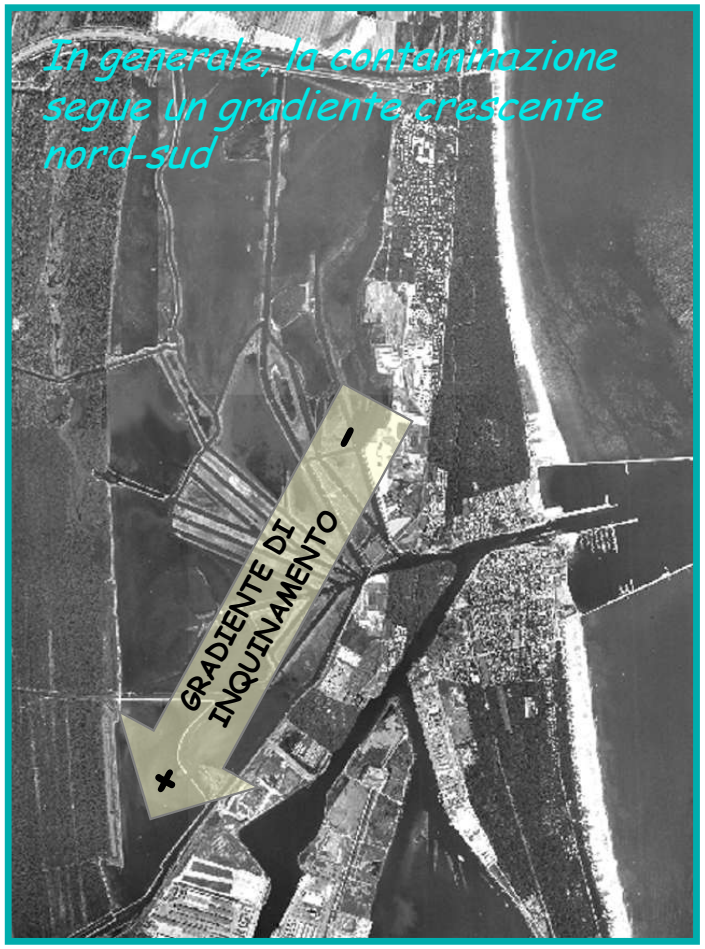
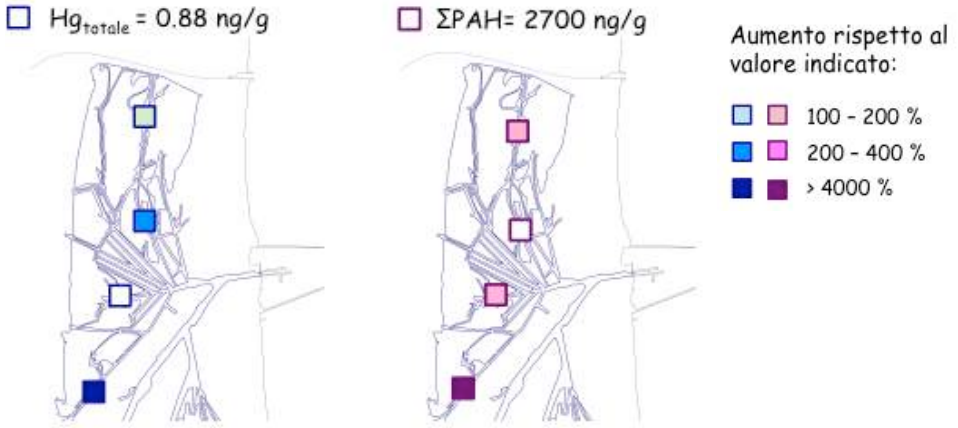
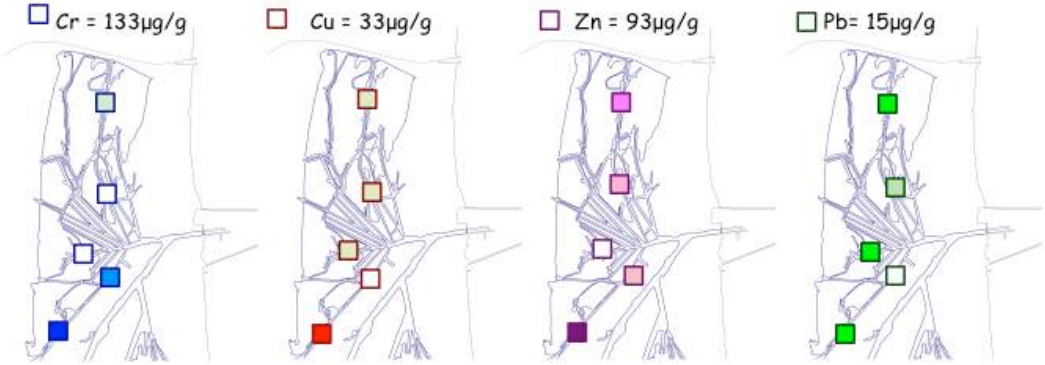


GRADIENTE NORD-SUD NELLA CONTAMINAZIONE DA Hg E IPA



CONTAMINAZIONE DIFFUSA DA METALLI PESANTI



From the end of the '50s to the '70s the lagoon has been impacted by poorly treated industrial waste waters, produced mainly by chemical plants manufacturing synthetic polymers. The lagoon is nowadays affected by a widespread contamination of the sediments by metals, and a north–south increasing gradient was observed for Hg and organic pollutants

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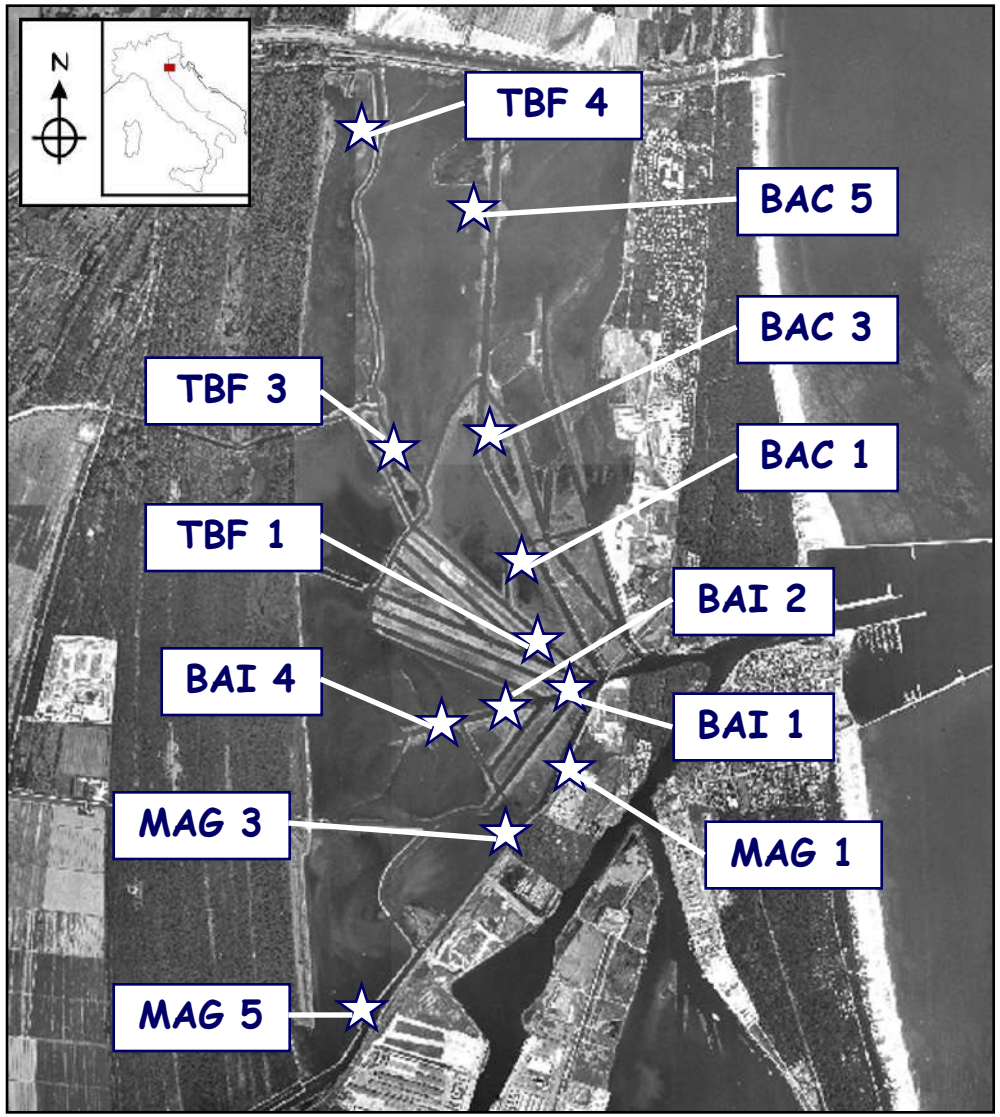

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A biological and geochemical integrated approach to assess the environmental quality of a coastal lagoon (Ravenna, Italy)

Filippo Donnini [a], Enrico Dinelli [a], Francesca Sangiorgi [a,b], Elena Fabbri [a,*]

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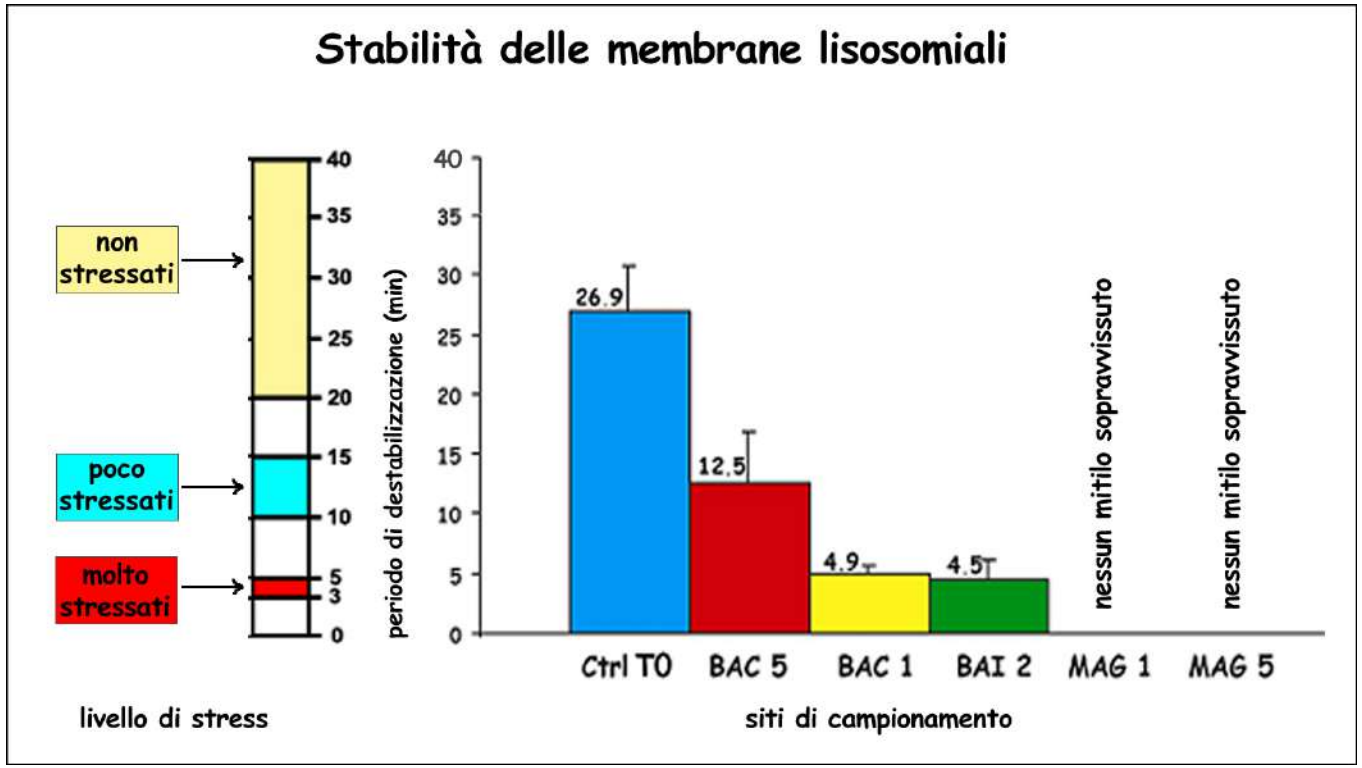

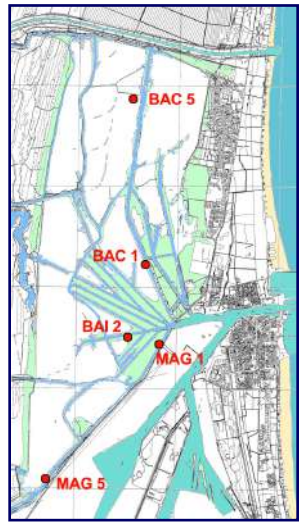

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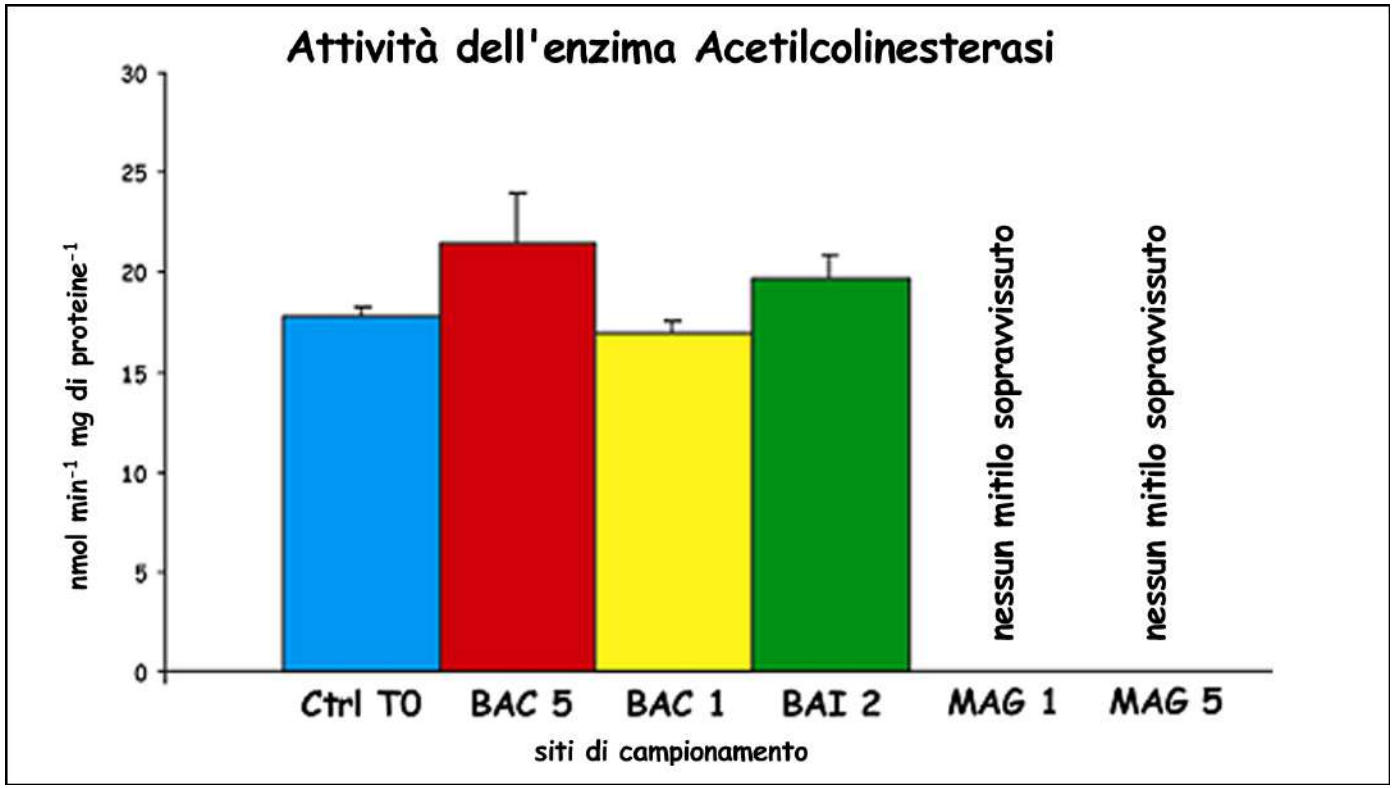

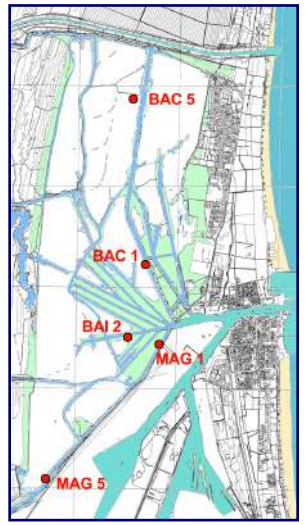

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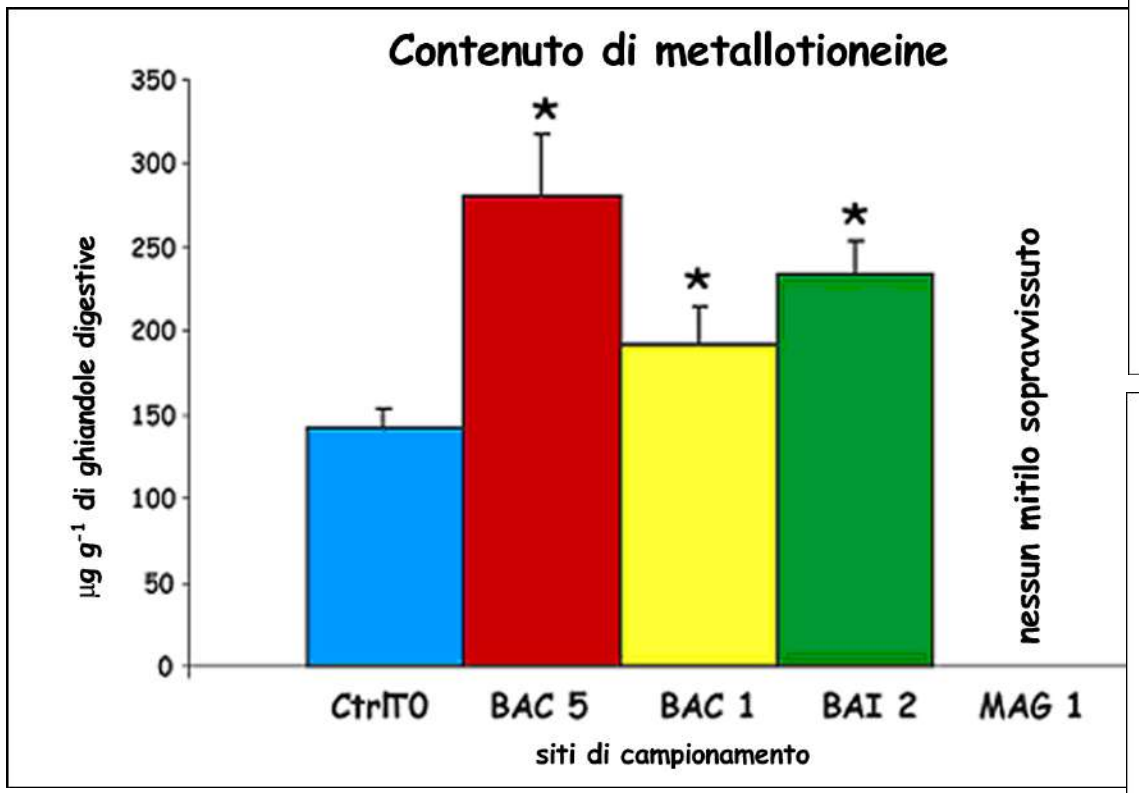
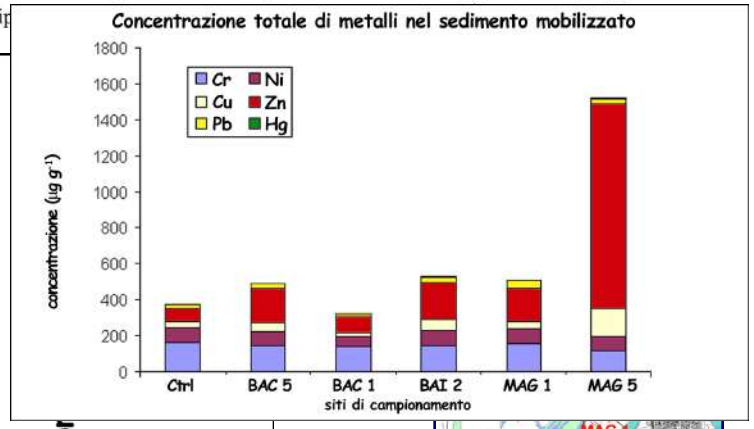
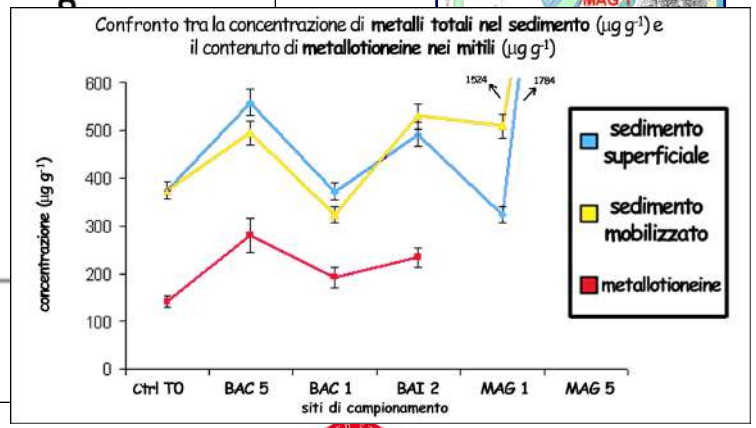
Filip







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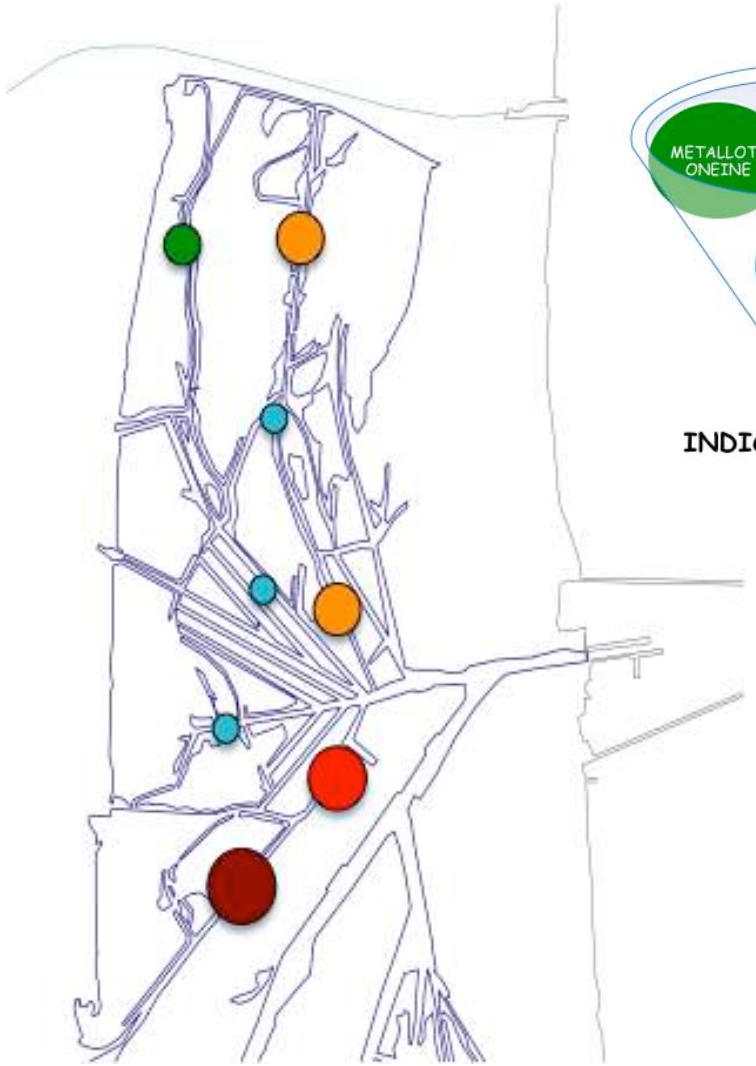
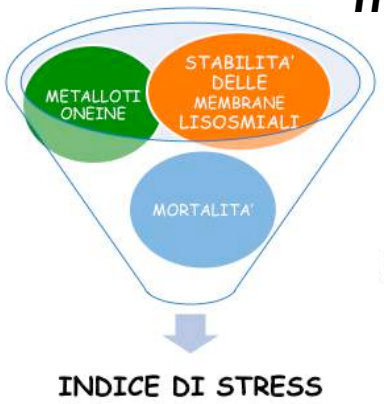
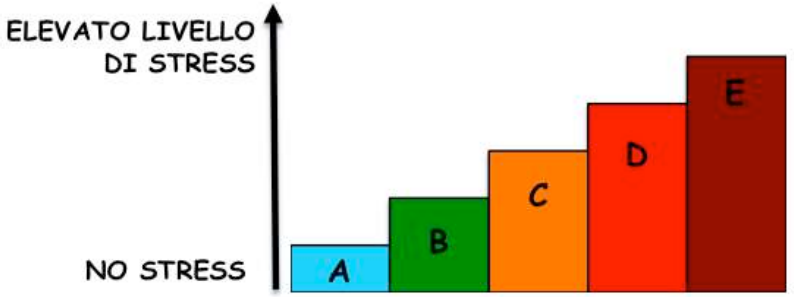


Combining the biomarker responses

The Expert Decision Support System





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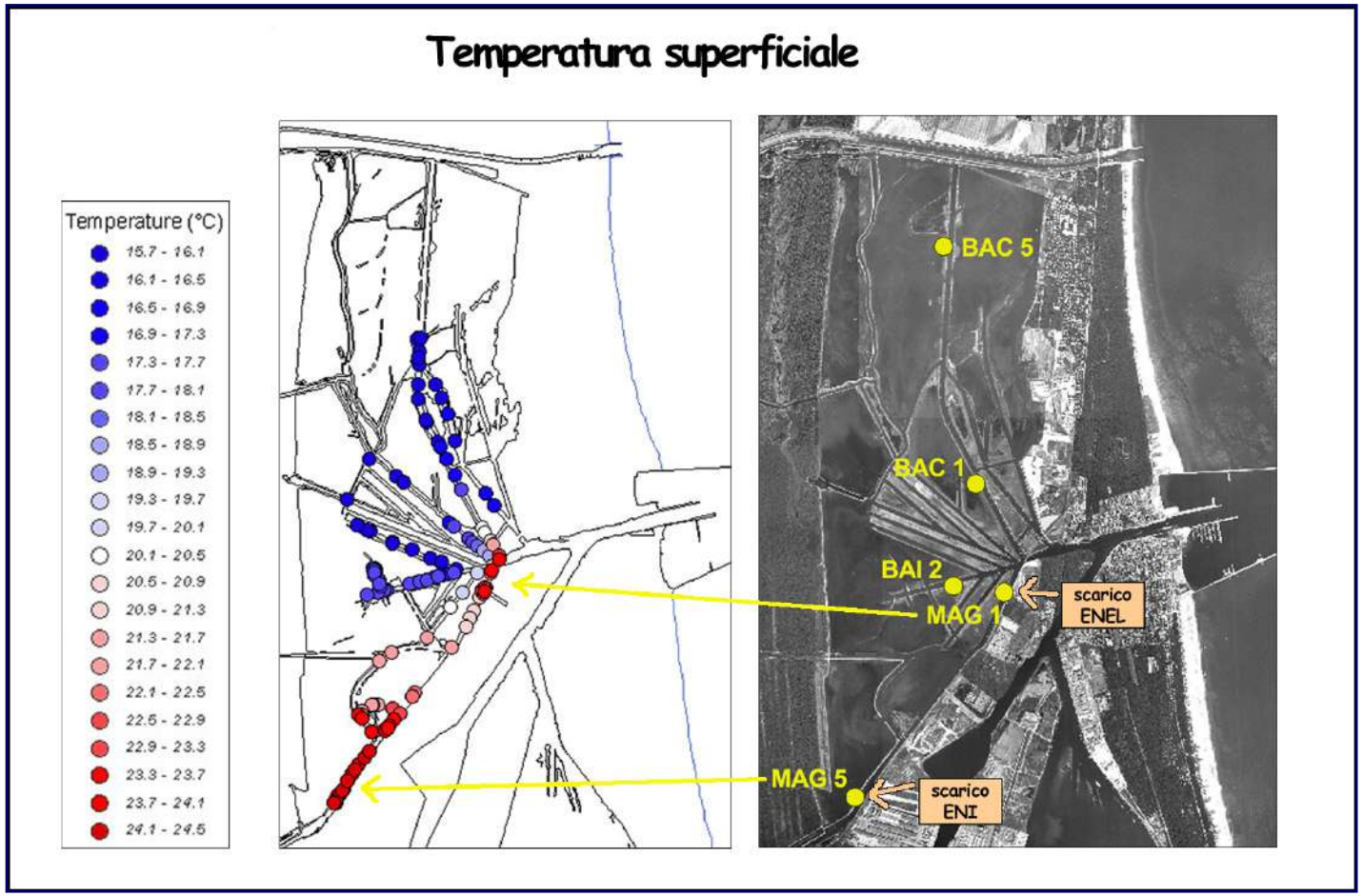
Evaluating the quality of transitional environments through sentinel organisms and the biomarker approach

How to explain the high mortality at the MAG sites?

Chemical pollution + thermal pollution



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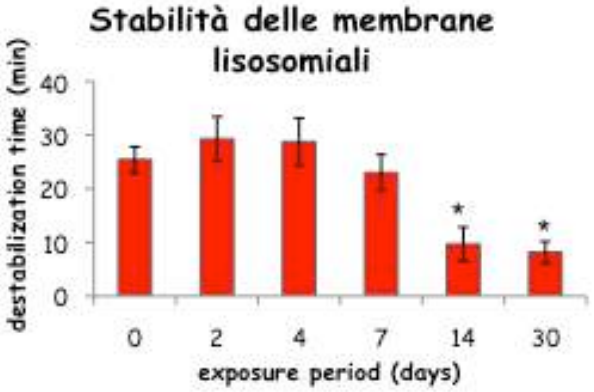
How to explain the high mortality at the MAG sites?

Autumn 2006
Molecular biomarkers to study the development of a stress syndrome at the MAG sites



Comparative Biochemistry and Physiology, Part C 152 (2010) 24–33

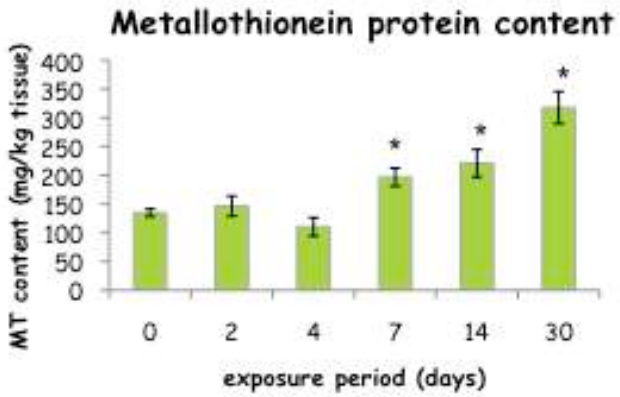
Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Exposure of mussels to a polluted environment: Insights into the stress syndrome development

Silvia Franzellitti, Sara Buratti, Filippo Donnini, Elena Fabbri *



Biomarkers indicate a physioloigical alteration from day 7

Evaluating the quality of transitional environments through sentinel organisms and the biomarker approach

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Autumn 2006
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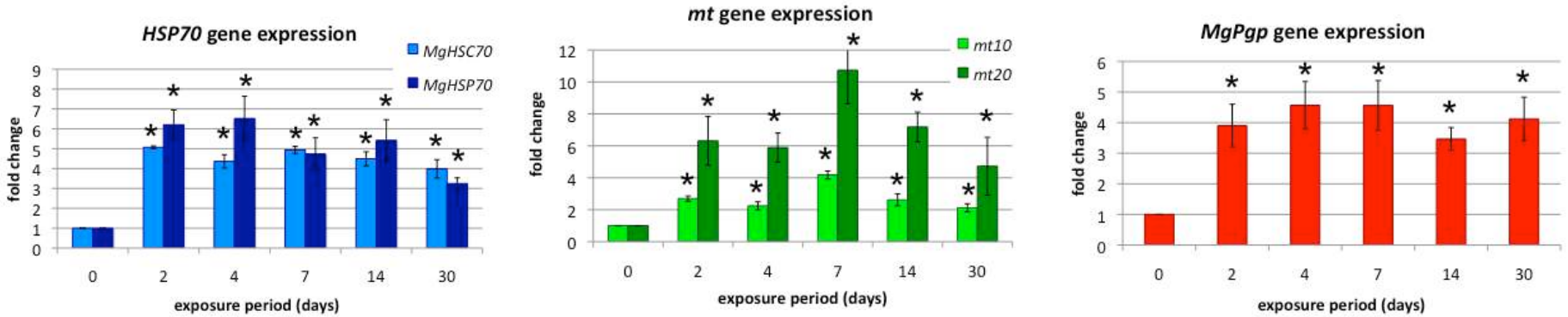

Biomarkers indicate a physioloigical alteration from day 7

Trasncripional profiles significantly altered from day 2

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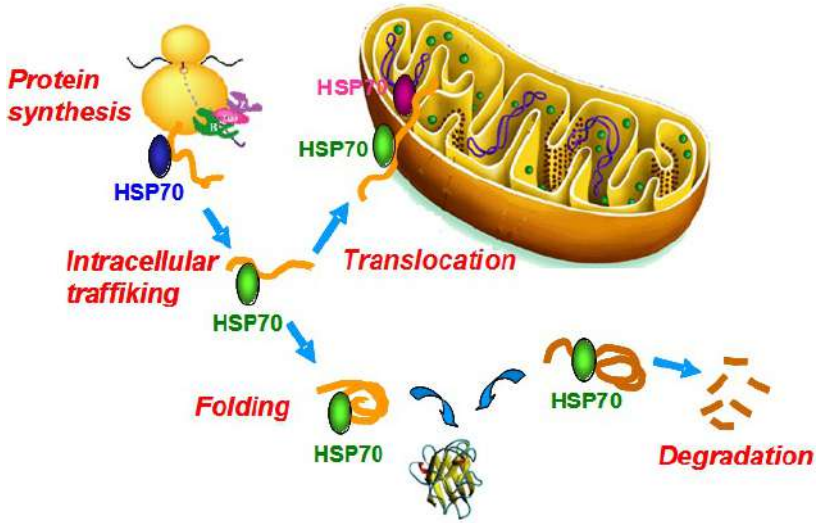
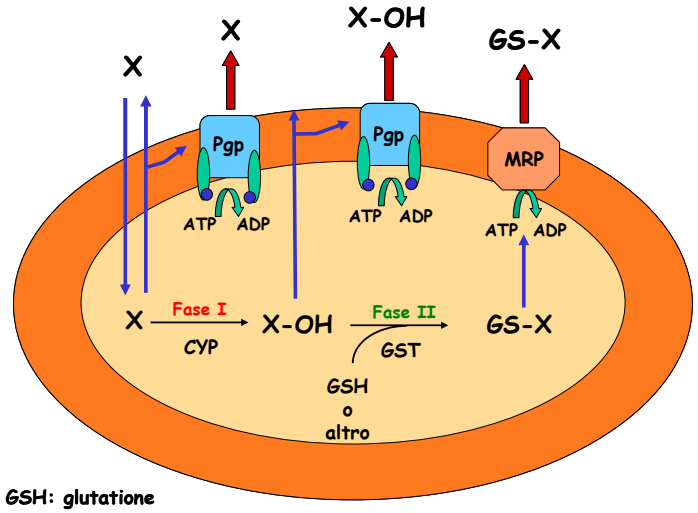
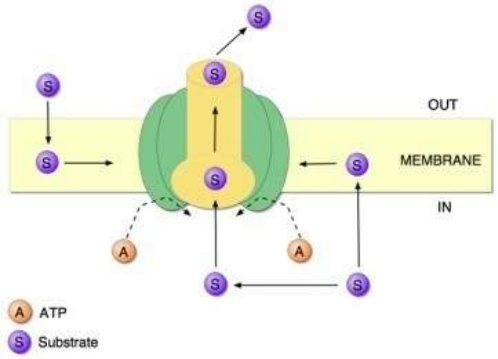
How temperature may affect cytoprotective responses in marine ectoterms





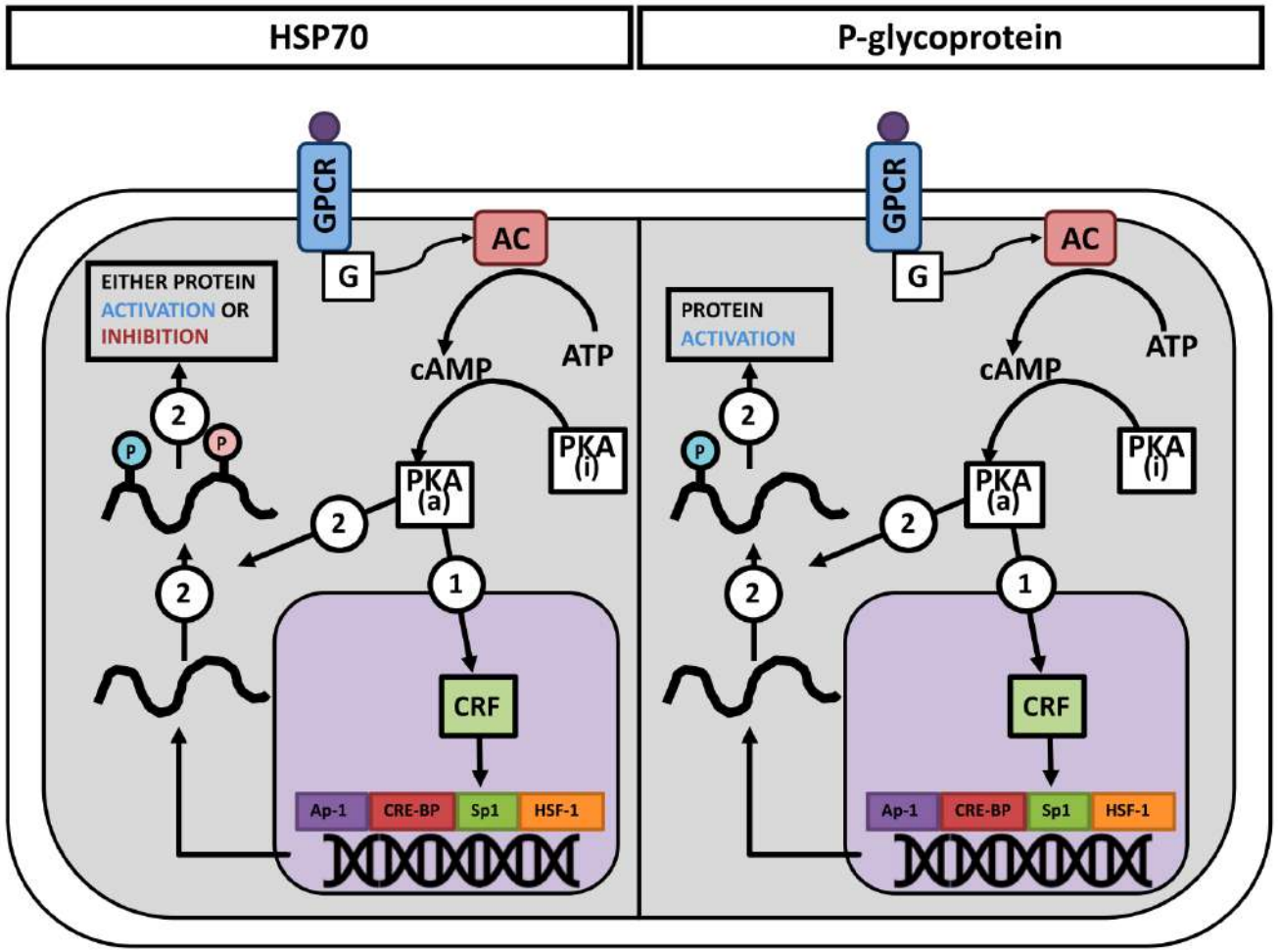
GSH: glutatione
CYP: citocromo P450
GS-X: glutatione-coniugati



70 kDa heat shock proteins (Hsc70, Hsp70)

MXR-related active transporters (Pgp, Mrp2)

- Both displaying cytoprotective functions
- Highly conserved across evolution
- May be co-regulated



Science of the Total Environment 720 (2020) 137733

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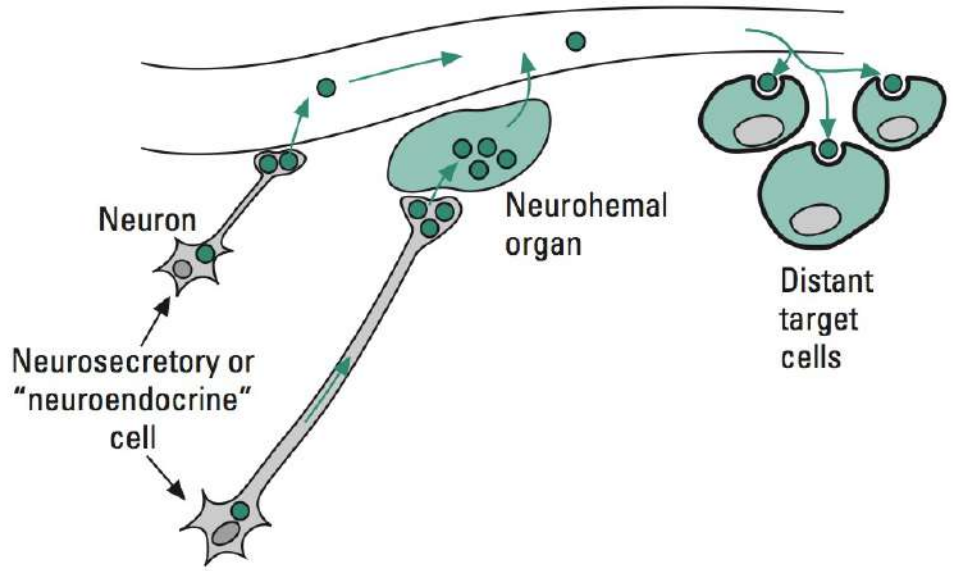
Evaluating bivalve cytoprotective responses and their regulatory pathways in a climate change scenario

Silvia Franzellitti [a,b,*], Fiorella Prada [b,c], Aldo Viarengo [d], Elena Fabbri [a]

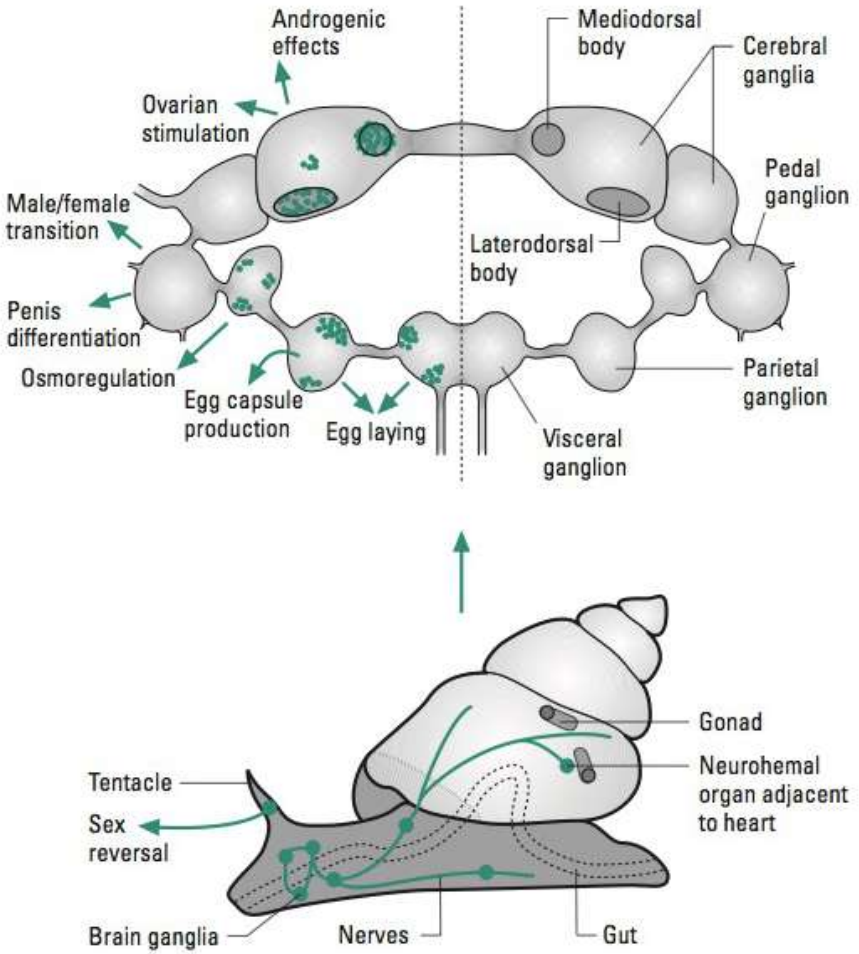
- Both displaying cytoprotective functions
- Highly conserved across evolution
- **May be co-regulated**

The bivalve neuroendocrine control



Nei molluschi non è possibile identificare un vero e proprio sistema endocrino.

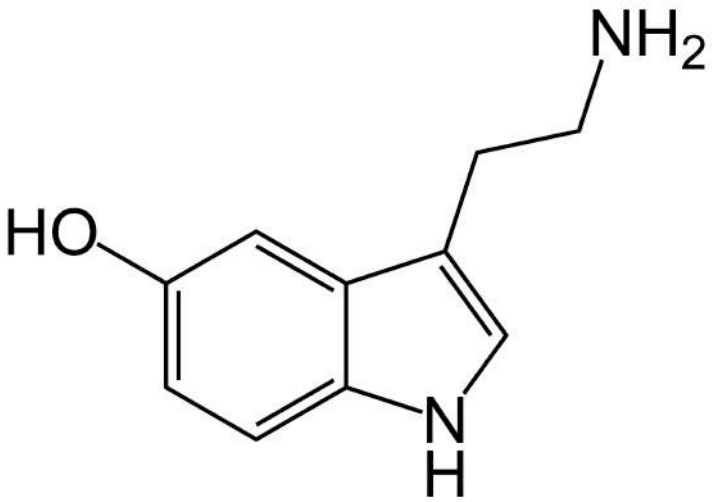
Neurosecretory cells and ganglia that release in the open circulatory system molcecules acting as **neuroendocrine modulators**



The location of the main neurosecretory structures in a gastropod mollusc, with the nervous ganglia enlarged to show the main neurosecretory centers (green dots) on the left, and the known neuroendocrine effects elicited from different ganglia (green arrows).

Serotonin (5-HT) is and high-tier physiolopgical controller in bivalves

Cardiac output



Respiration and feeding

- Cilia beeting in gill cells
- Catch muscle

Reproduction

- Gametogenesis and spawning

Growth

- Metabolism
- Larvae development

Shell biogenesis

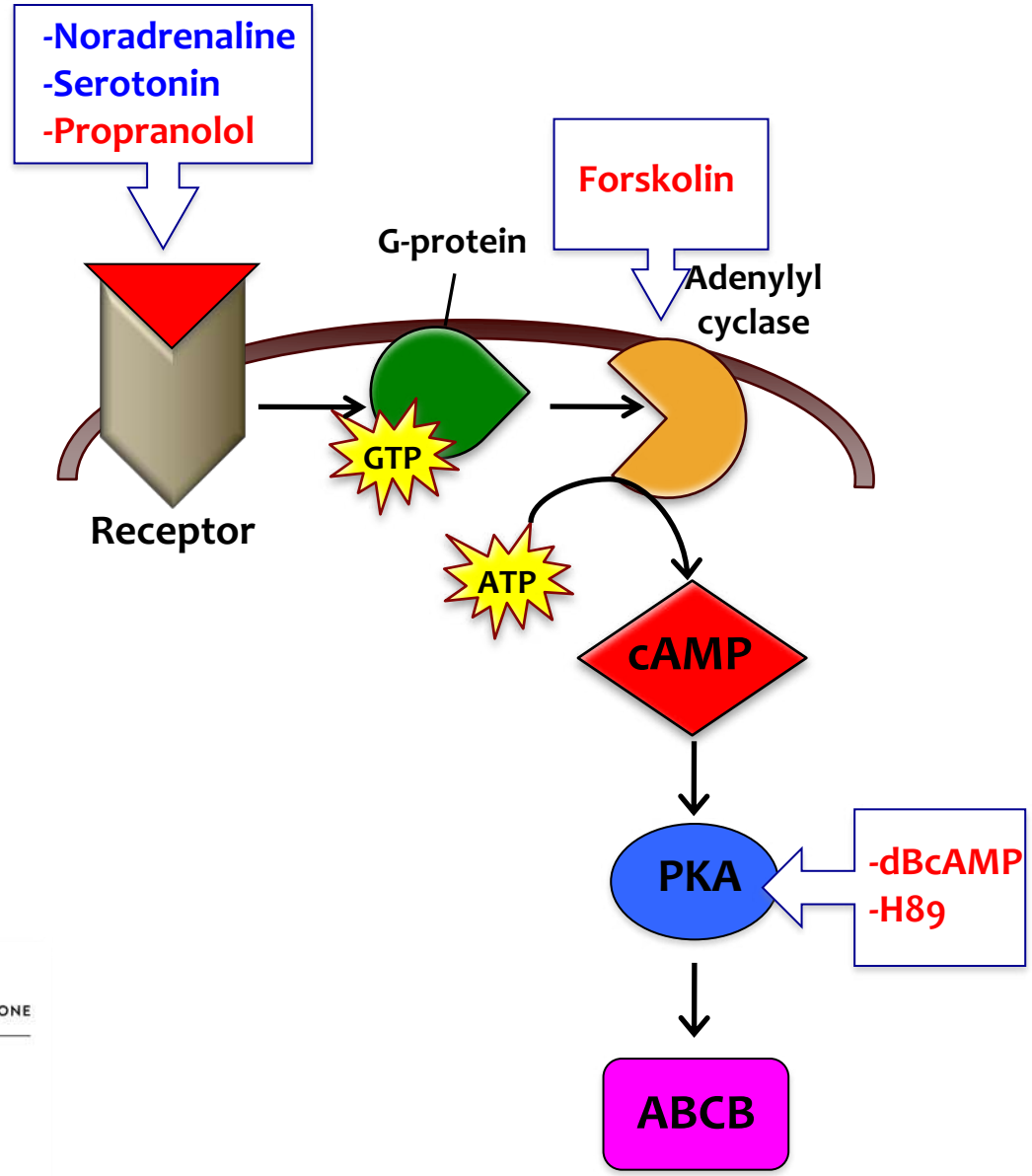
- Mantle tissue physiology

Is it possible to assess a regulatory pathway in a marine invertebrate?

***In vitro* experiments with *Mytilus galloprovincialis* living haemocytes exposed to:**

pharmacological modulators

physiological agonists



OPEN ACCESS Freely available online

PLOS ONE

Cyclic-AMP Mediated Regulation of *ABCB* mRNA Expression in Mussel Haemocytes

Silvia Franzellitti[1]*, Elena Fabbri[1,2]

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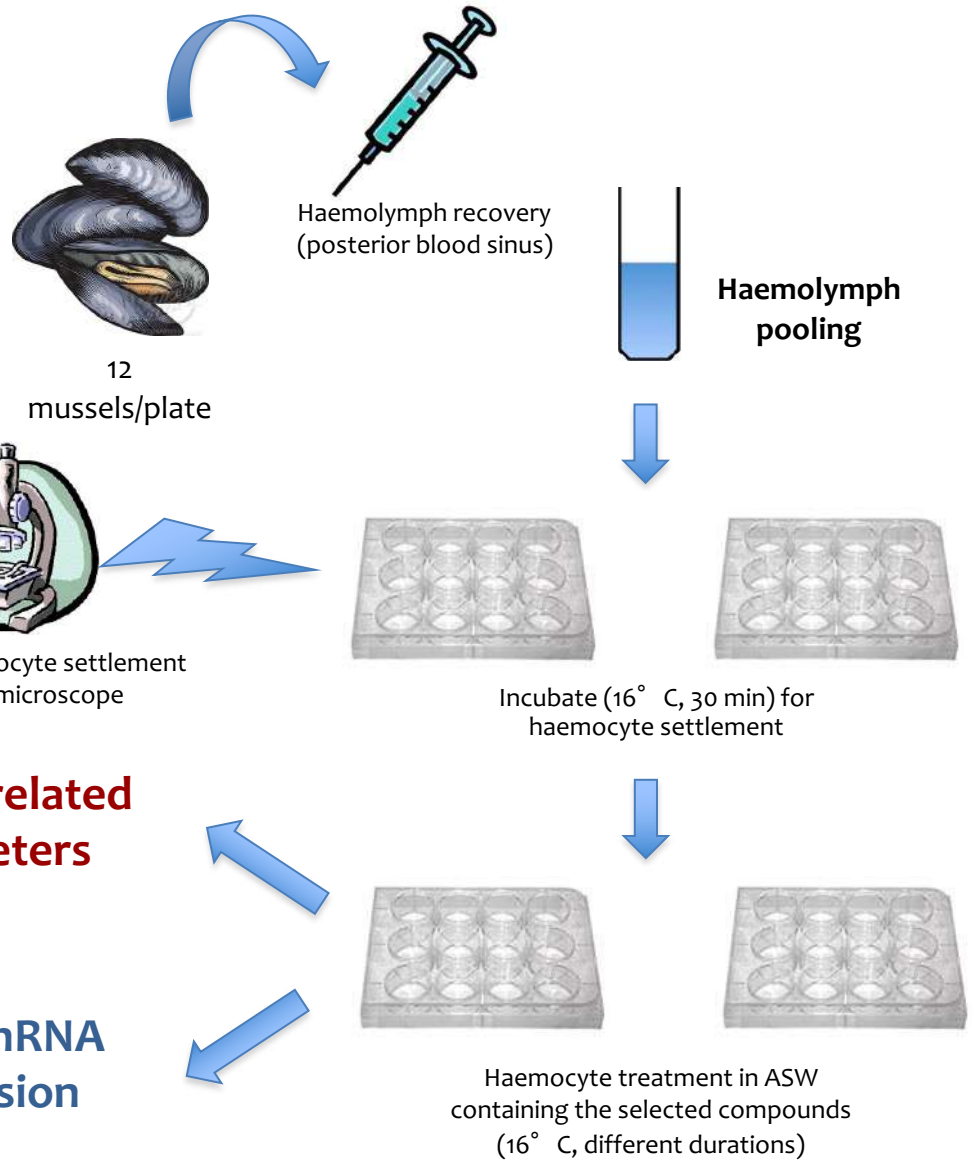
Is it possible to establish a regulatory pathway in a marine invertebrate?



- cAMP cell content➔ ELISA assay
- PKA activity ➔ spectrophotometric assay

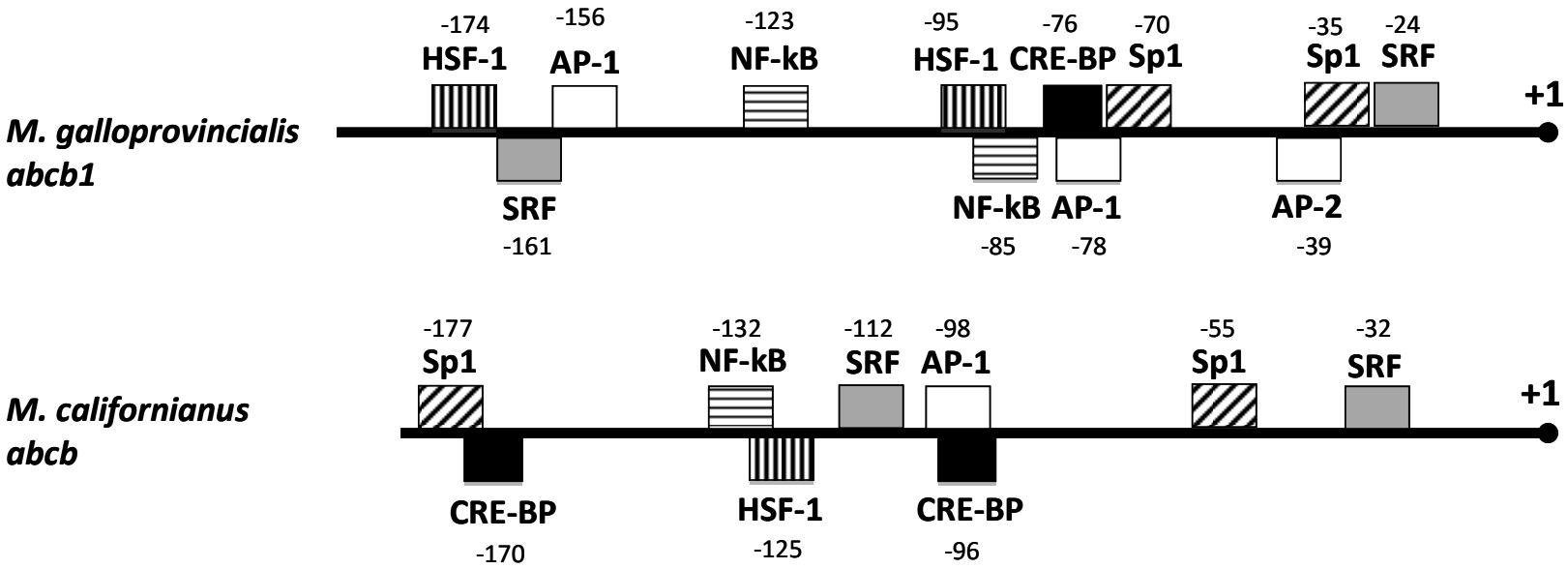
cAMP-related parameters

- Total RNA extraction
- cDNA preparation
- real-time PCR

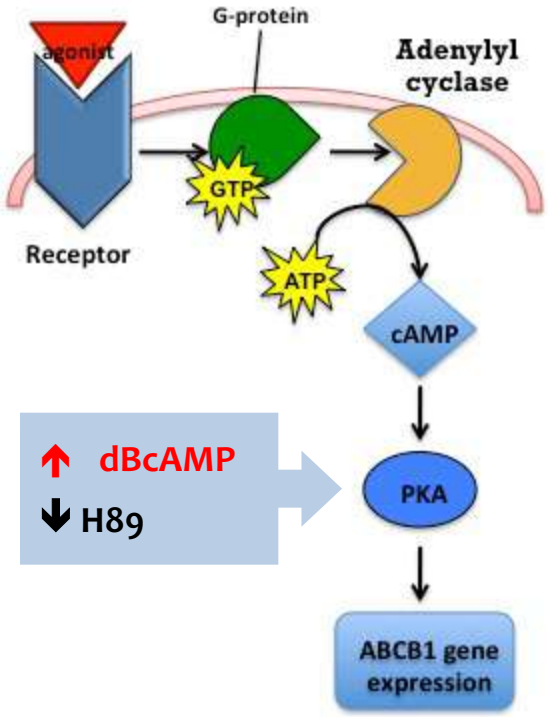
***ABCB* mRNA expression**

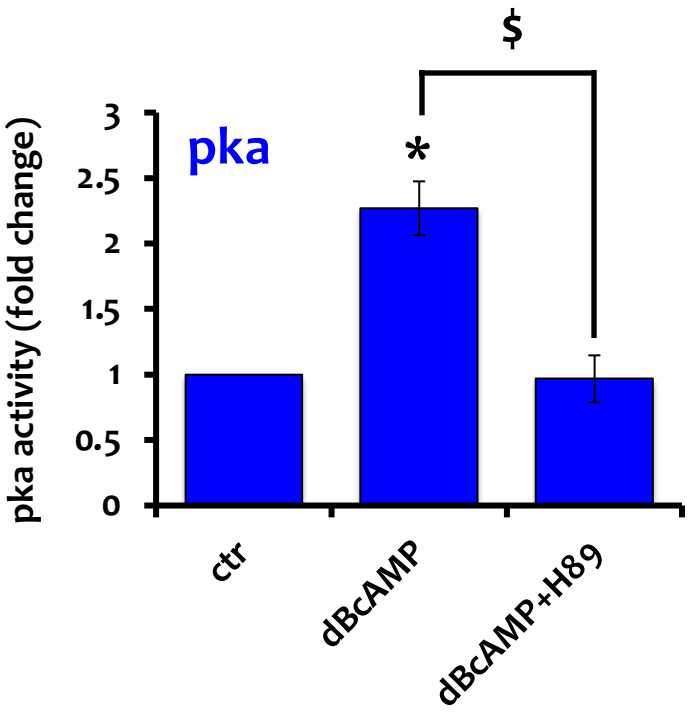
Is PKA involved in the control of ABCB gene transcription in mussels?

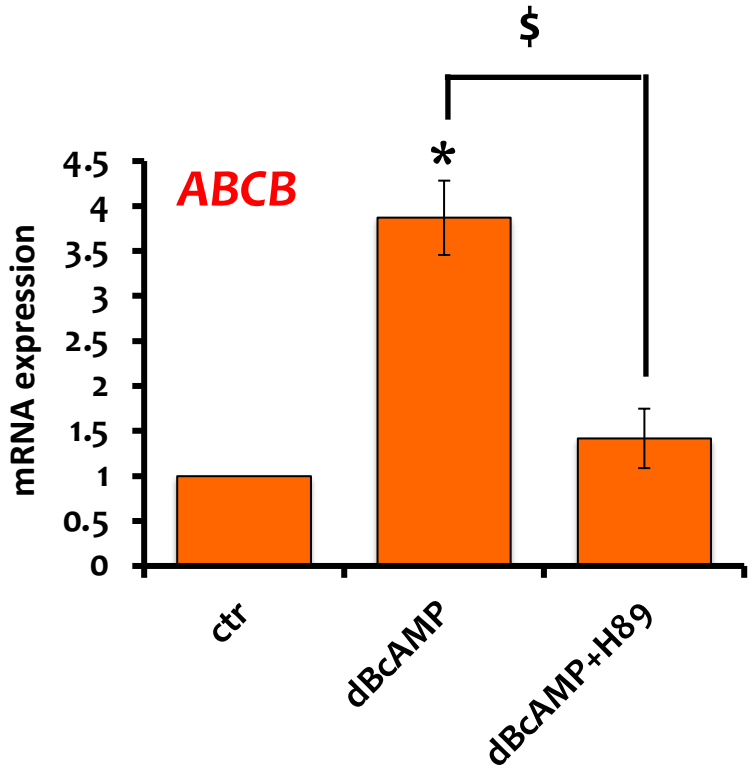
Putative binding sites for PKA-activated transcription factor within the unstranslated 5' regulatory regions of mussel ABCB genes



Is PKA involved in the control of ABCB gene transcription in mussels?







*p<0.05 *vs* CTR
$p<0.01

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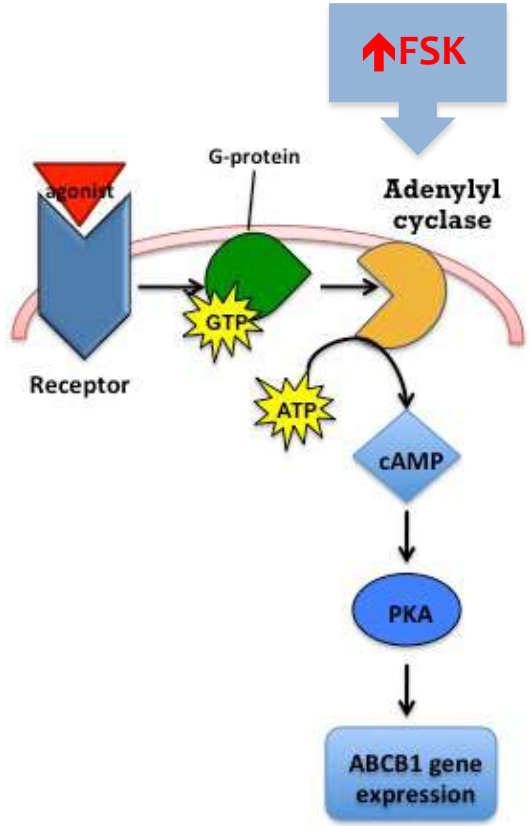
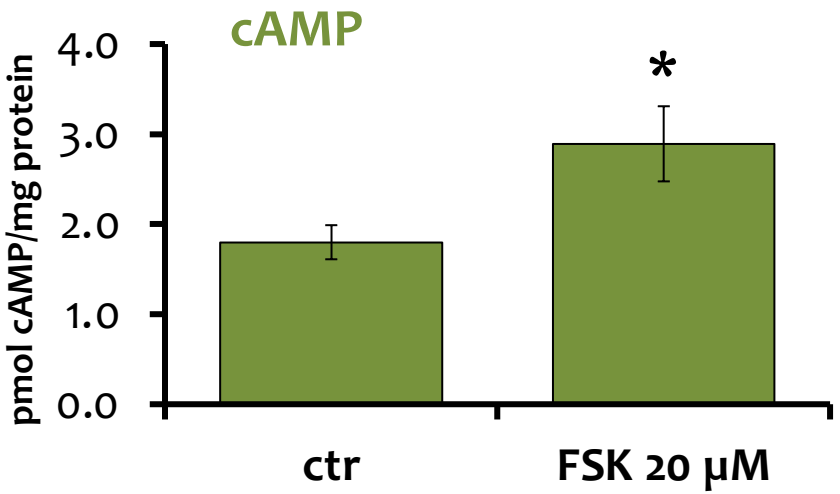
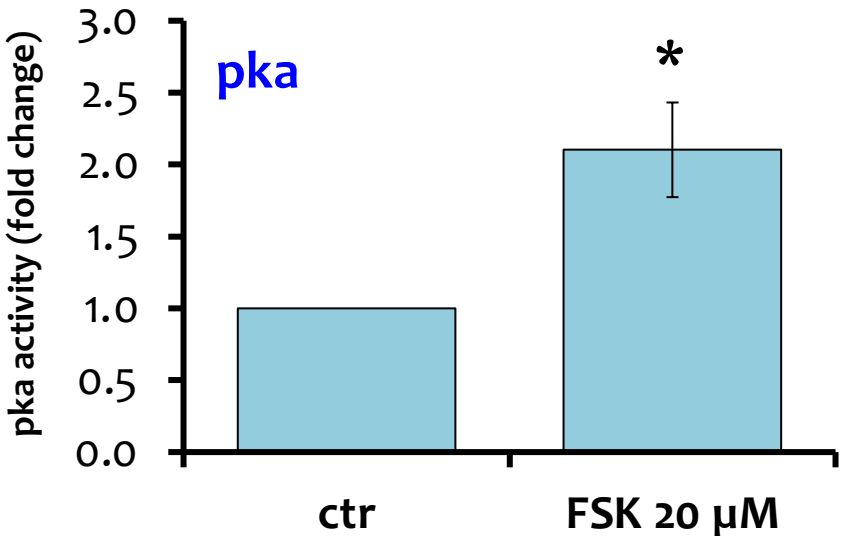
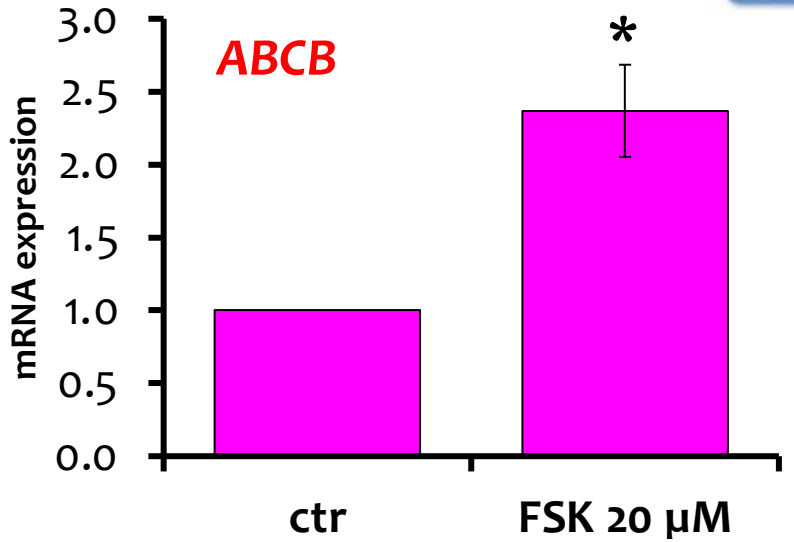
Is PKA involved in the control of ABCB gene transcription in mussels?

Forskolin









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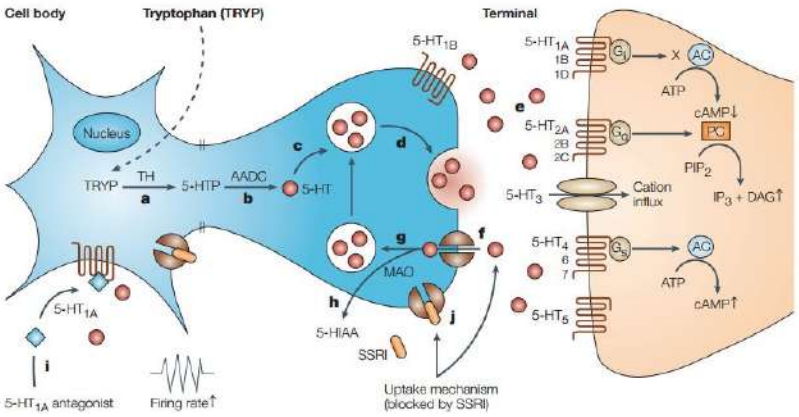
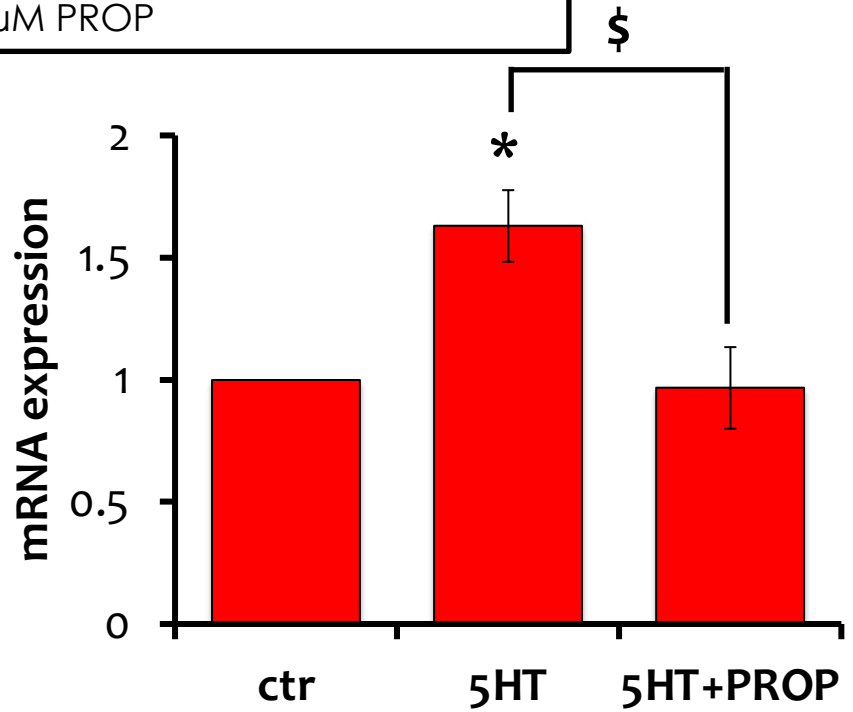
A 5-HT1 receptor is expressed in mussel hemocytes

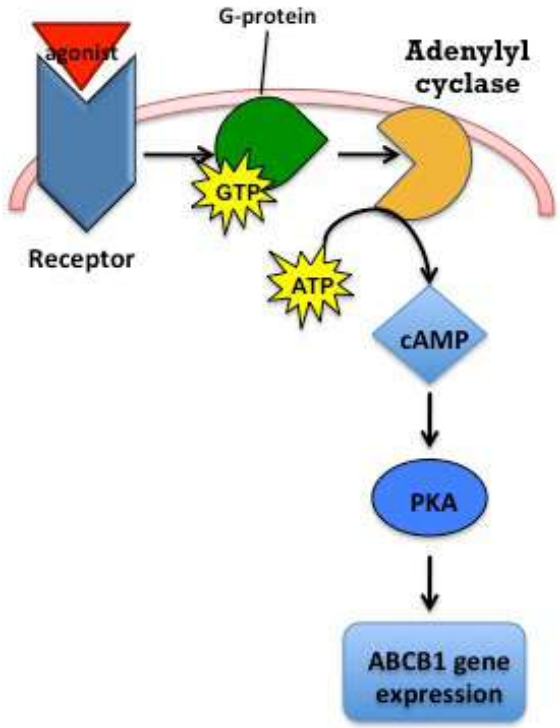
recettore	Proteina G accoppiata	Pathway di trasduzione
5-HT1	Gi	cAMP ↓
5-HT2	Gq	IP↑ & Ca^{++}↑
5-HT4	Gs	cAMP ↑
5-HT5	??	??
5-HT6	Gs	cAMP ↑
5-HT7	Gs	cAMP ↑

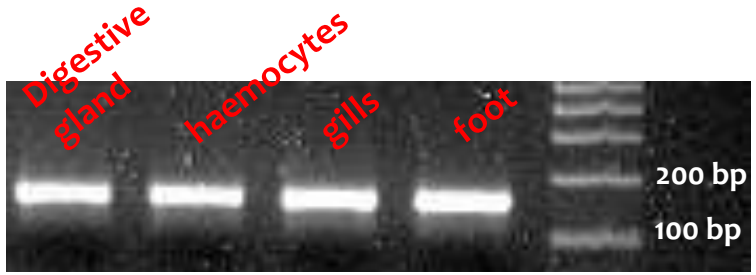


1-h exposure to 1 µM 5HT + 15 min pre-incubation with 100 µM PROP



↑ 5HT
X PROP





*p<0.05 *vs* CTR
$p<0.01

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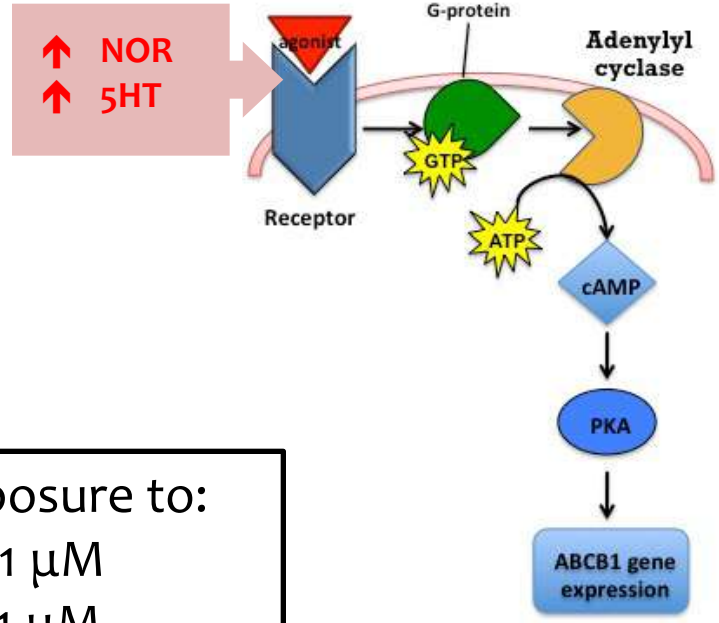
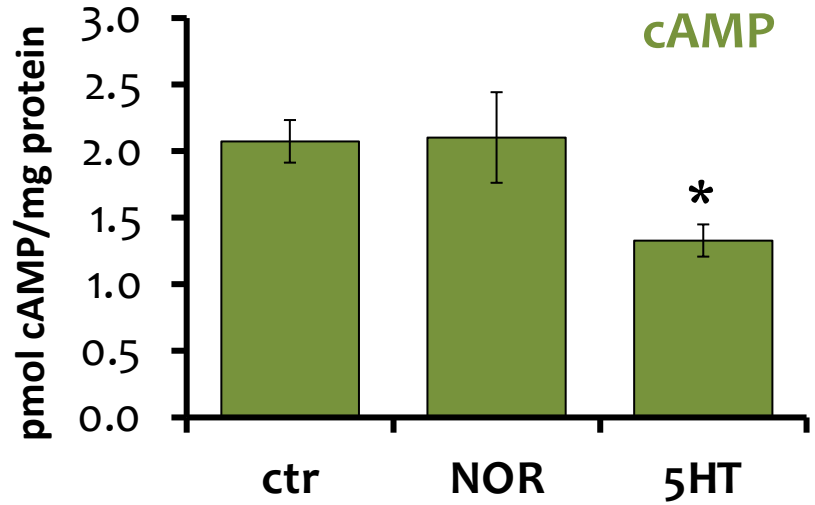
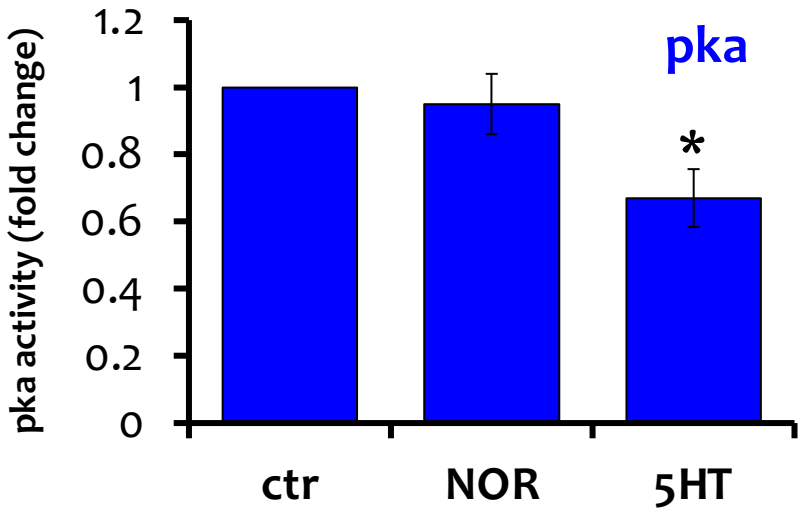
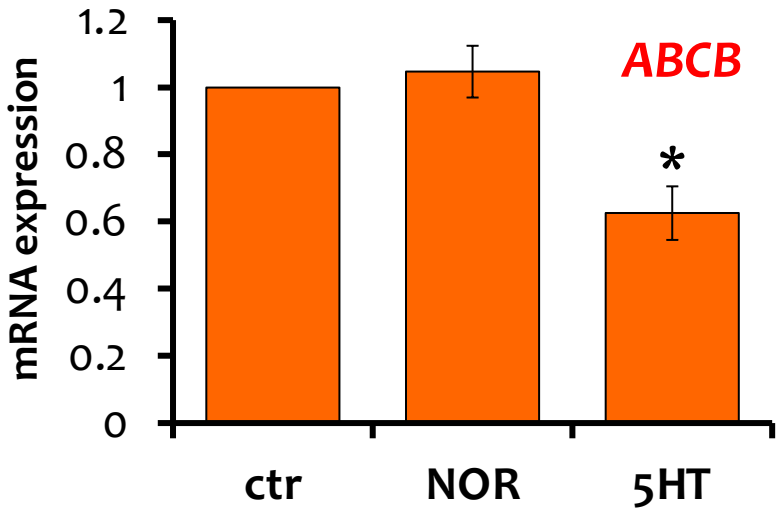
Effects of agonists NOR and 5-HT





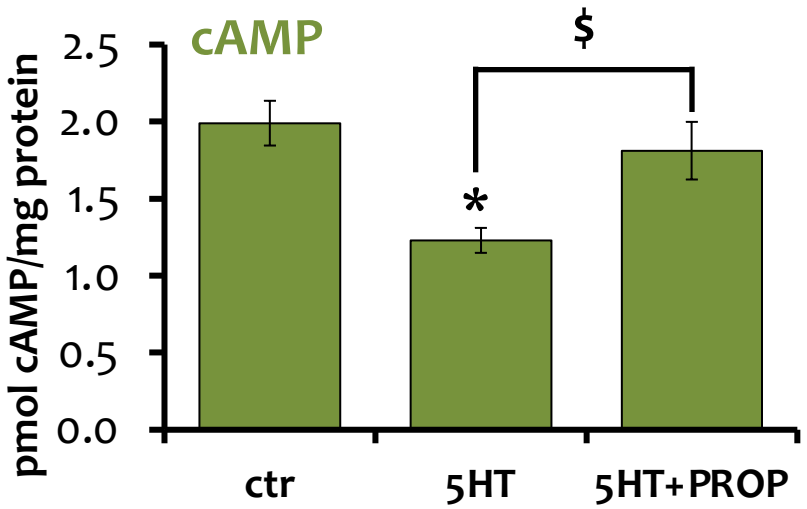
1-h exposure to:
- NOR 1 µM
- 5-HT 1 µM

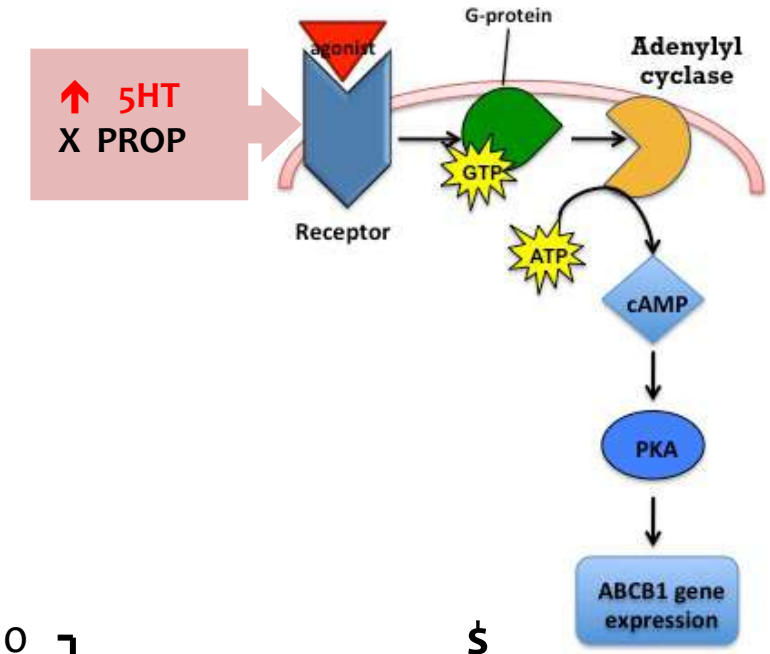


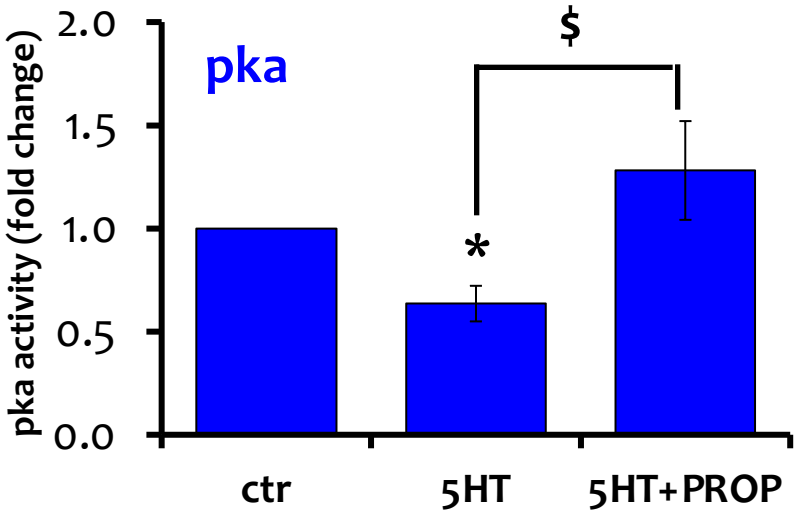


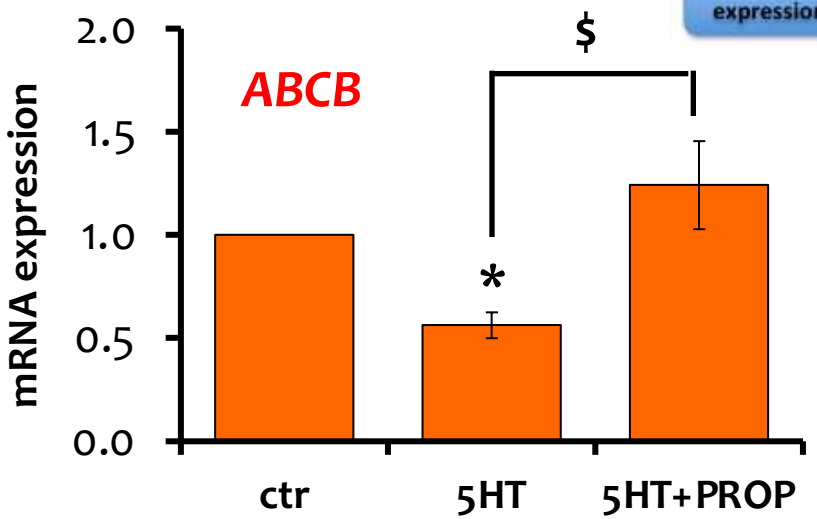
*p<0.05 *vs* CTR

PROP blocks 5-HT effects









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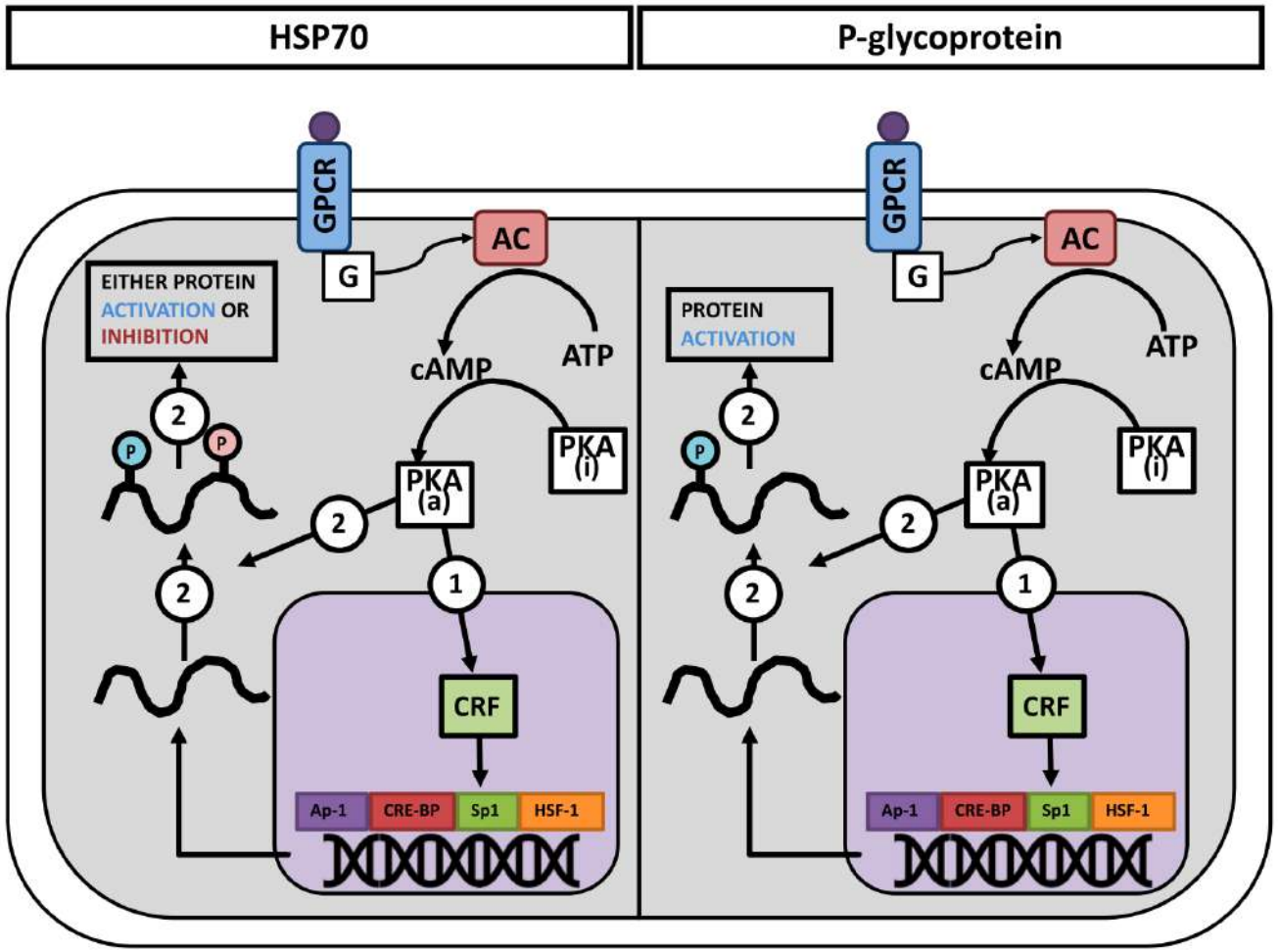

Science of the Total Environment 720 (2020) 137733

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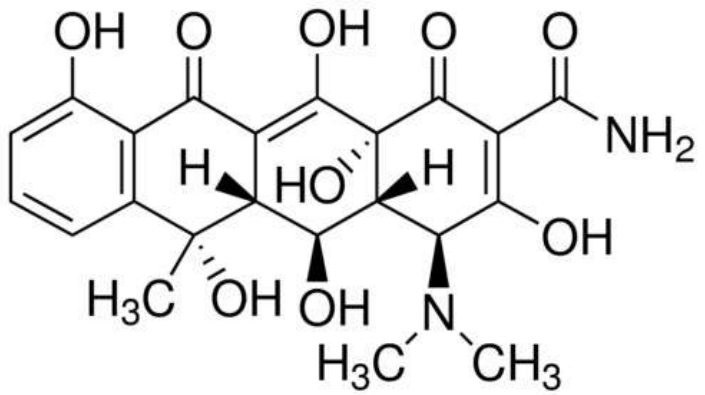
Evaluating bivalve cytoprotective responses and their regulatory pathways in a climate change scenario

Silvia Franzellitti [a,b,*], Fiorella Prada [b,c], Aldo Viarengo [d], Elena Fabbri [a]



OXYTETRACYCLINE

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Evaluating bivalve cytoprotective responses and their regulatory pathways in a climate change scenario

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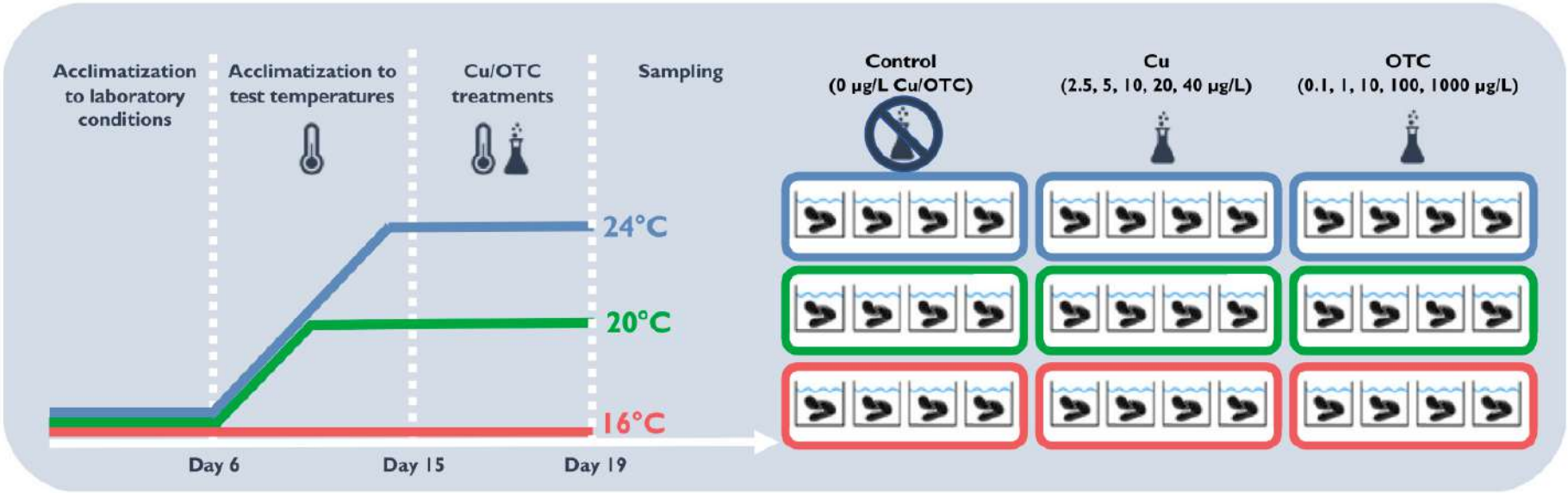
Experimental setup



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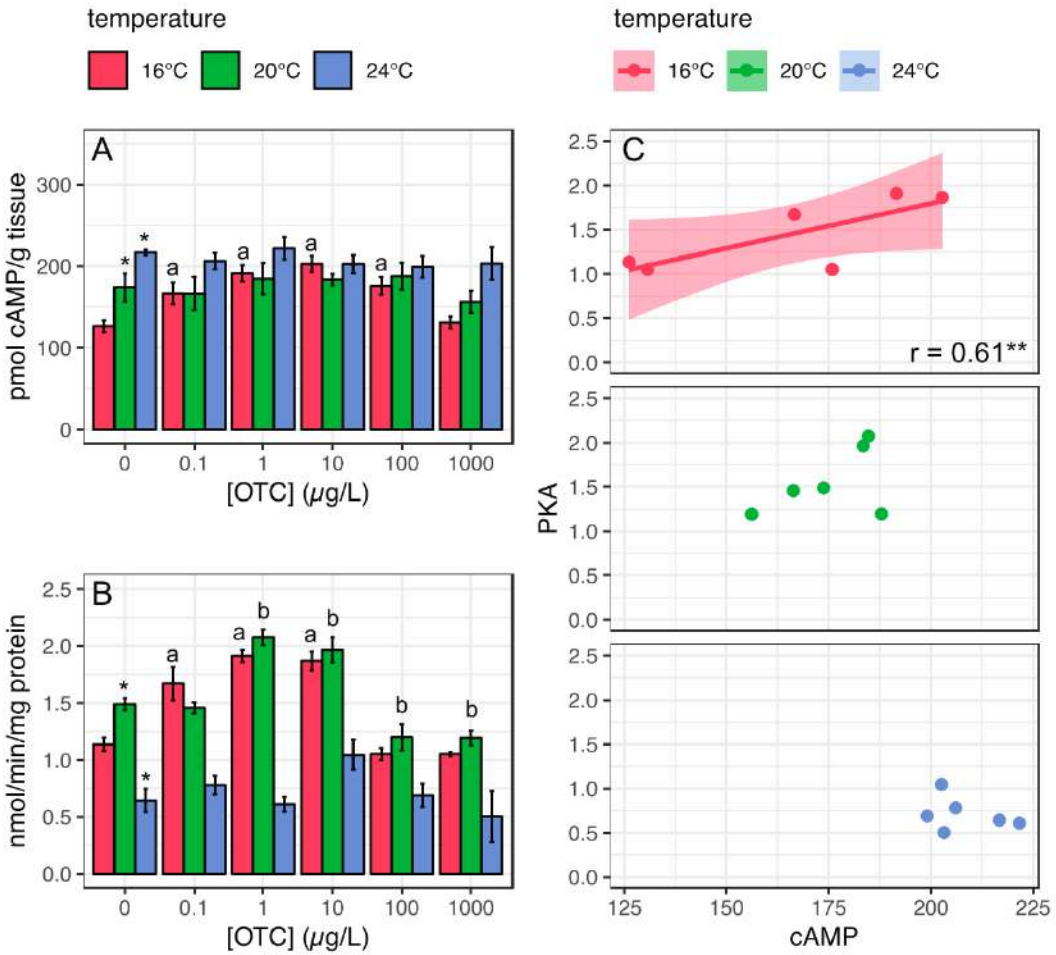
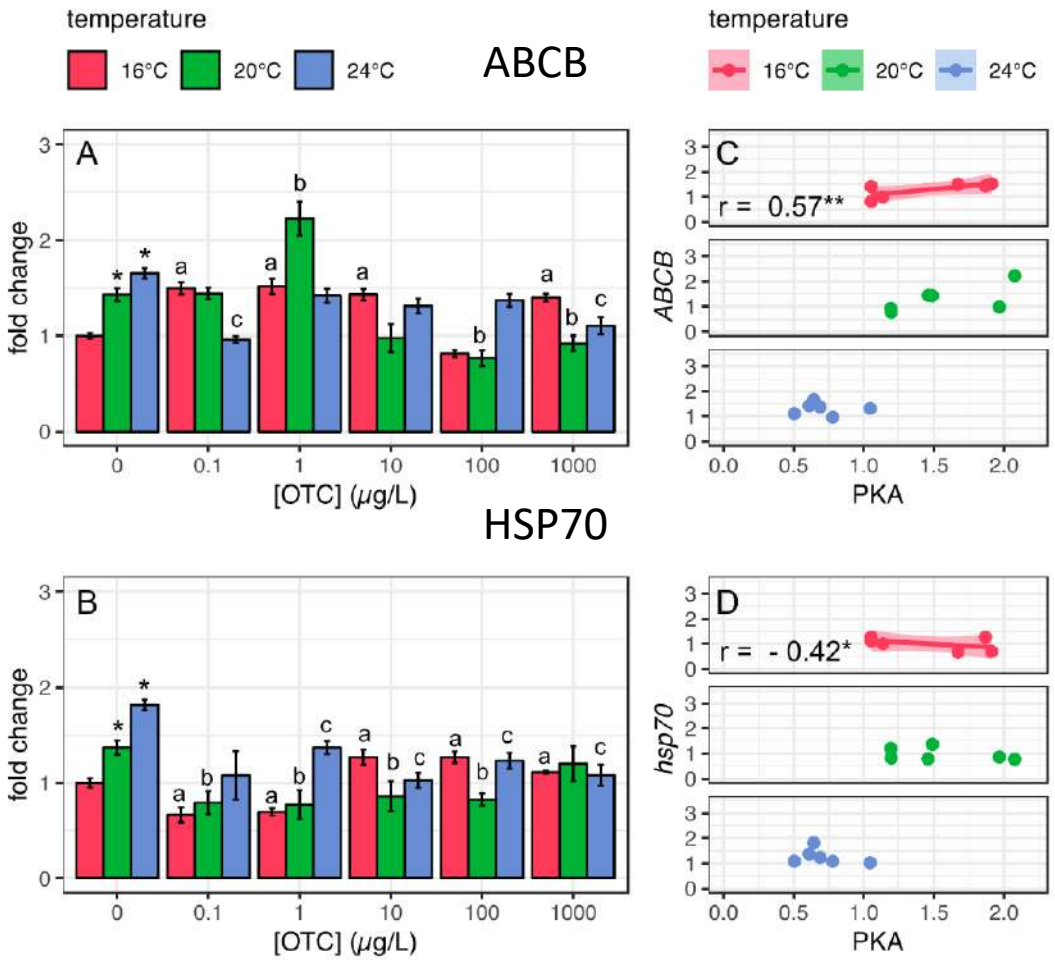
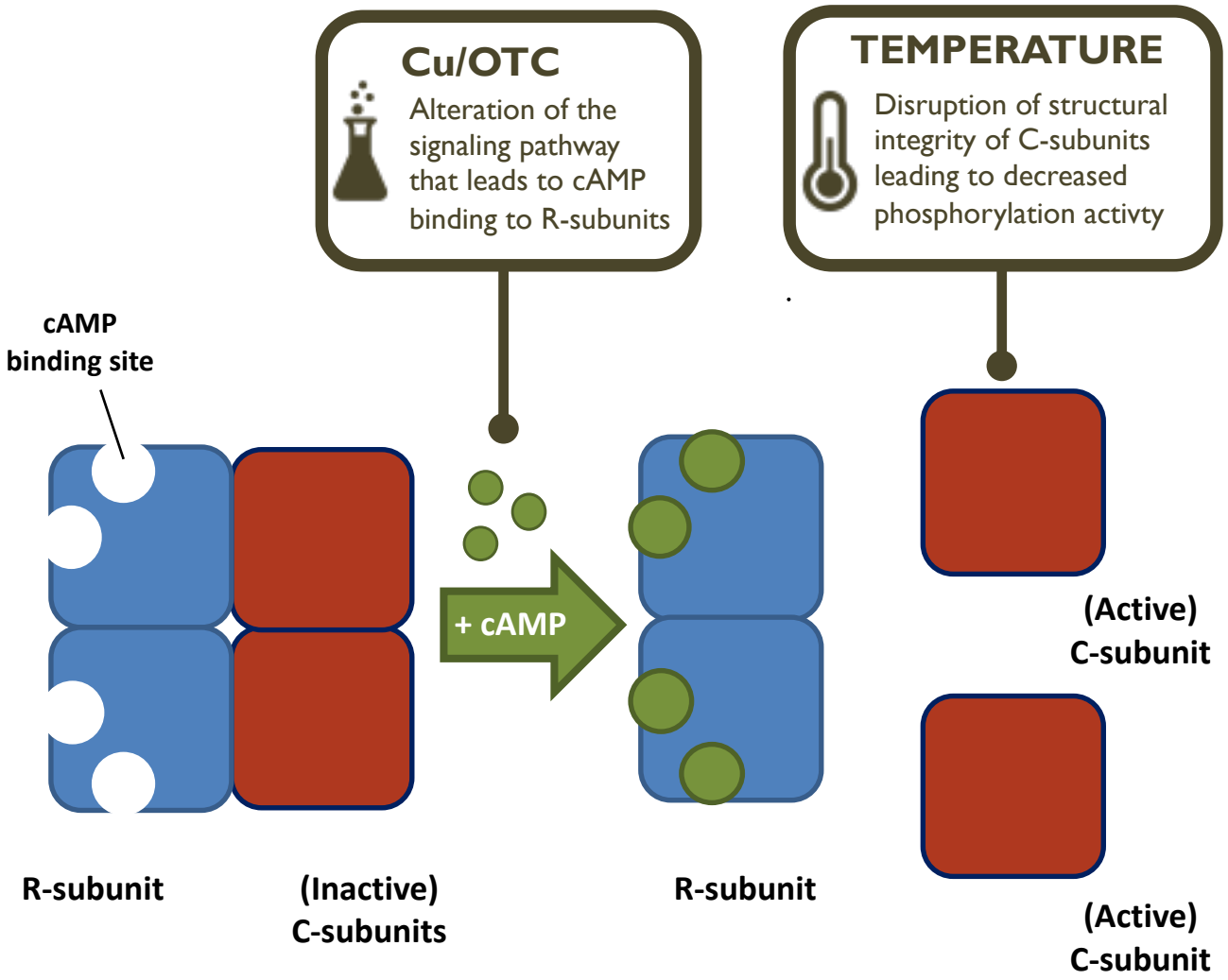
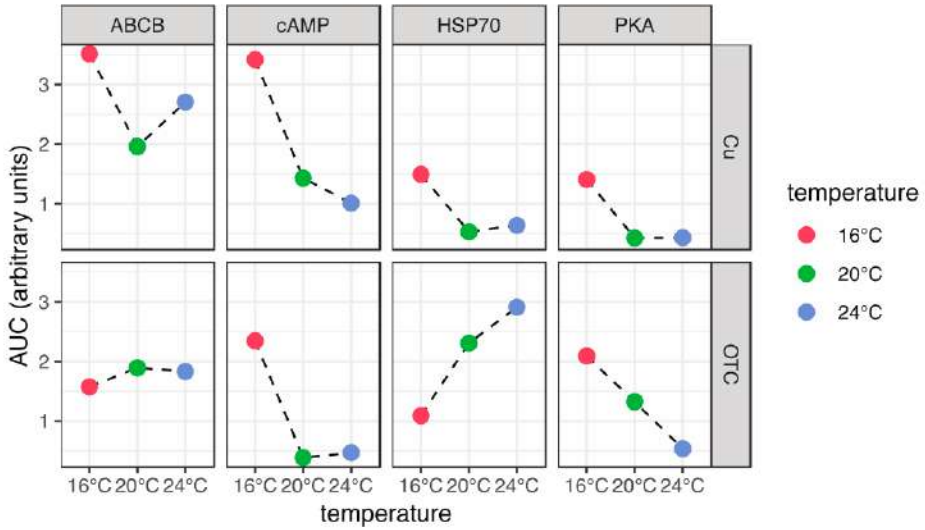


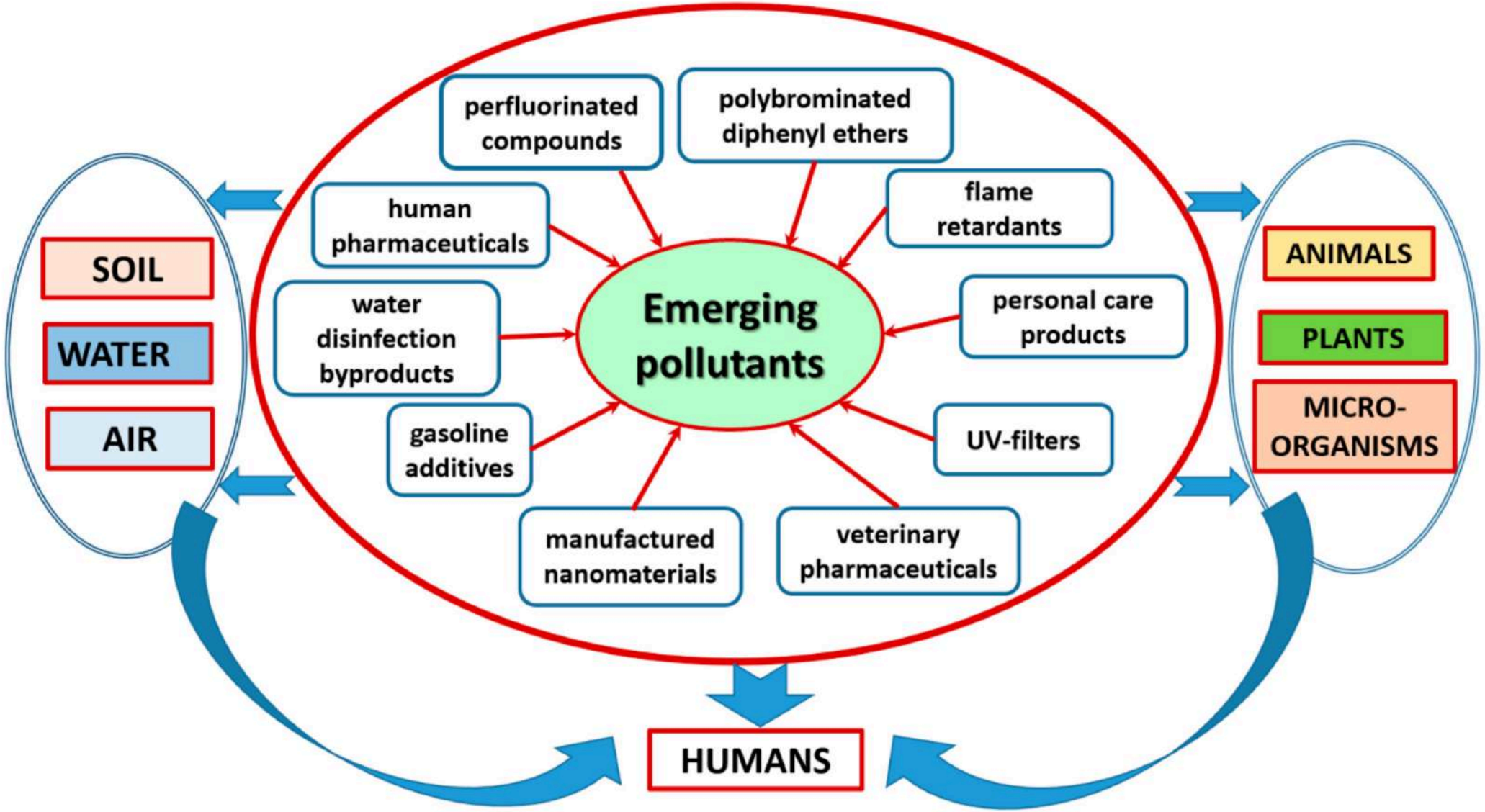




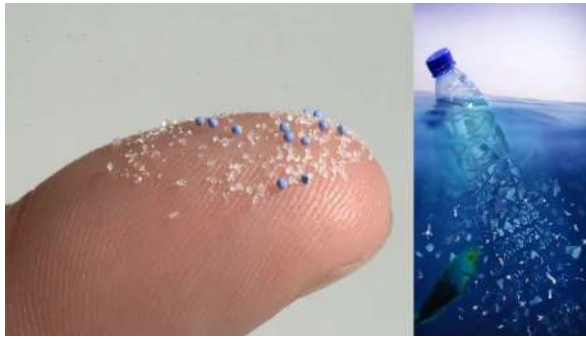
Cu or OTC concentration-related variation at each temperature expressed by the Area Under the Curve (AUC)\



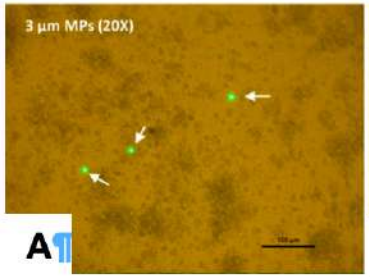
The threats of emerging pollutants

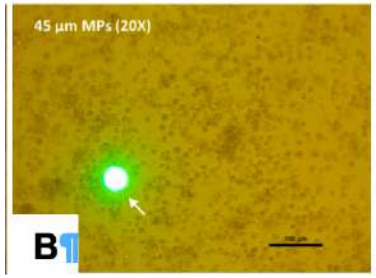


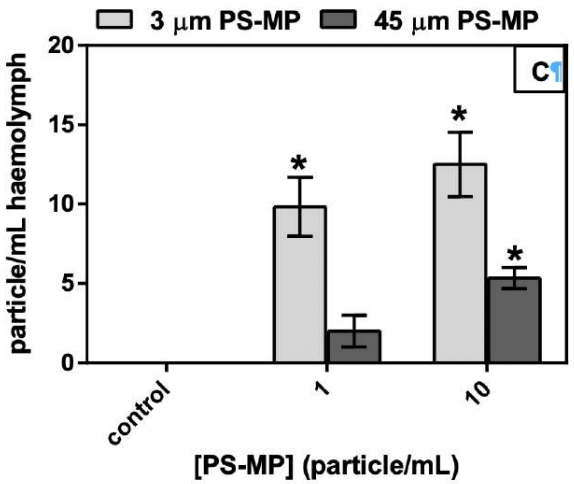
Mussels as microplastic bioccumulator



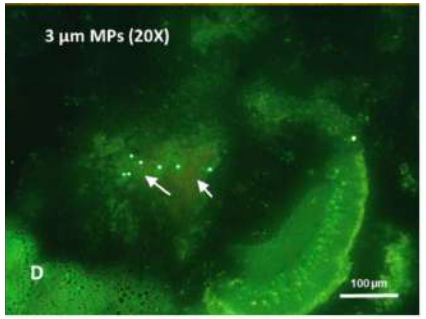
From Haemolymph..

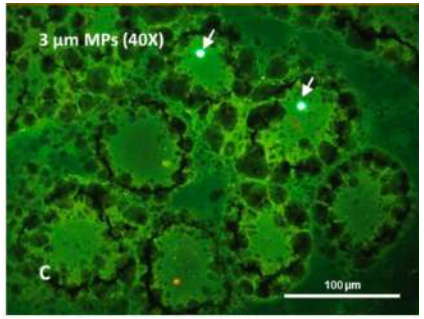


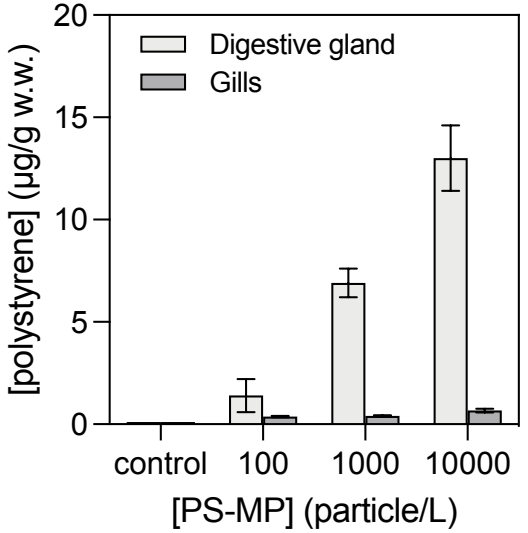




… to other tissues/organs







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Pharmaceuticals as enviornmental pollutants

EUROPEAN COMMISSION

Brussels, 11.3.2019

COM(2019) 128 final

COMMUNICATION FROM THE COMMISSION

European Union Strategic Approach to Pharmaceuticals in the Environment

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COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE COUNCIL AND THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE

European Union Strategic Approach to Pharmaceuticals in the Environment

EU recognizes pharmaceutical as an environmental issue

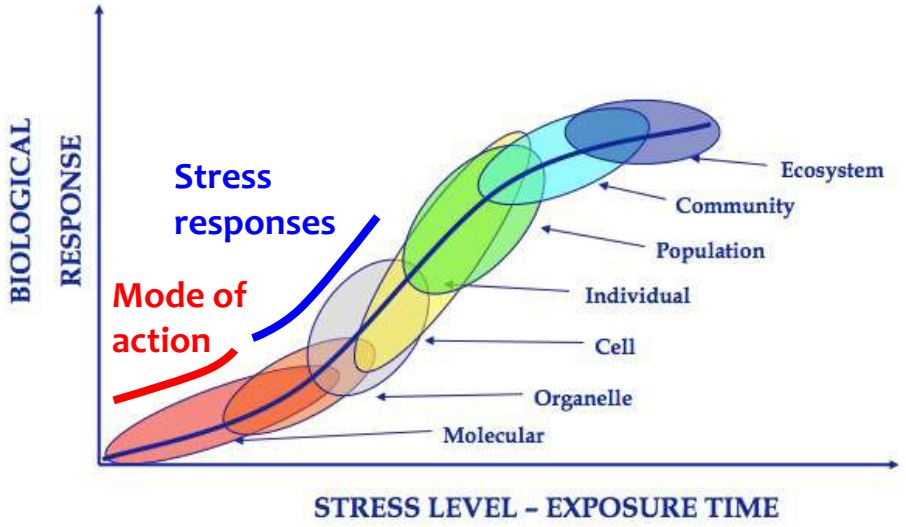
improve environmental monitoring approaches by coupling analytical (chemical) and complementary (biological *in vivo* *in vitro* and *in silico* testing) techniques, and to fill knowledge gaps on ecotoxicity of pharmaceuticals

https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52019DC0128

1. To assess whether a pharmaceutical at concentrations found in coastal waters induces consistent stress responses in marine organisms

PHARMA as emerging contaminants ⇨ *biomarkers of stress response*

- Lysosomal membrame stability
- Activity of Antioxidant enzymes
- ...



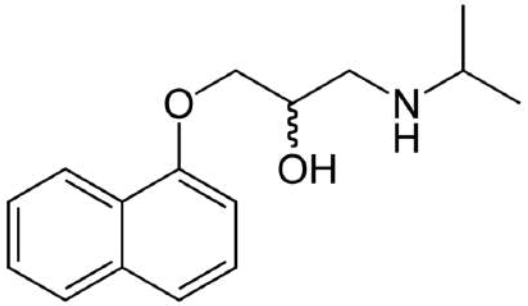
2. To assess the MODE OF ACTION (MoA) of a pharmaceutical in marine mussels in relation with its molecular targets

PHARMA as therapuetic agents ⇨ *Target specifc endpoints*

Identification of highly active compounds among environmental pharmaceuticals (Christen et al., 2010 – Aquat. Toxicol.)

✓The mode of action of the pharmaceutical;
✓The degree of homology between the human drug target and the potential target in aquatic organisms;
✓The relevance of the affected pathways

PROPRANOLOL



A β-adrenergic receptor blocker used in human therapy against cardiovascular diseases

- ✓ Highly prescribed
- ✓ 10% excreted unchanged
- ✓ variably removed by treatment plants (60- 96%)
- ✓ fairly persistent, scarcely biodegraded
- ✓ bioaccumulative,
- ✓ rather water soluble,
- ✓ low tendency for adsorption to organic matter
- ✓very stable in the range of pH values typical of seawater

Water sol = 100 g/L

Log Kow = 0.78 at pH 7; 3.5 at pH 9

Suitable candidate as emerging pollutant for the aquatic environment

Propranolol effects on aquatic organisms

Organism	Experimental conditions	Biological endpoints	Reference
Invertebrates			
Mytilus edulis trossulus	1–10,000 μg/L different periods of exposure (7–21 days) depending on the biological endpoint	• Decreased scope for growth;	Ericson et al. (2010)
Mytilus galloprovincialis	11 and 147 μg/L 10-day exposure	• Decreased strength and abundance of byssus threads;	
Daphnia magna		increased in gills.	
		• LOEC for growth and fecundity of 0.44 and 12 mg/L, respectively;	Dorne et al. (2007)
Hyalella azteca, Daphnia magna, Daphnia lumholtzi, and *Ceriodaphnia dubia*	Acute tests (48 h) and chronic tests (7, 14, or 27 days depending on the target organism)		
Thamnocephalus platyurus	0.1–33 mg/L acute test (24 h)		
Vertebrates			
Pimephales promelas	0.001–10 mg/L (I exp) 0.001–1 mg/L (II exp) 21-day exposure	• Increased gonad somatic index; • Decreased hatchability; • Significant bioaccumulation of PROP in plasma.	
Oncorhynchus mykiss	0.0001–10 mg/L 40-days exposure	• Altered growth-related parameters; • Significant bioaccumulation of PROP in fish plasma.	Owen et al. (2009)
Oncorhynchus mykiss hepatocytes (*in vitro* exposure)	31.25–500 μM 24-h exposure		
Oryzias latipes	Acute tests (48 h) and chronic tests (7, 14, or 27 days depending on the target organism)		
Oryzias latipes (larval stage)	4.4–22.2 mg/L acute test (96 h)	• Survival (LC_{50} = 11.4 mg/L).	Kim et al. (2009)
Various	Various	• EC_{50} for different endpoints and LC_{50} between 1 and 1000 mg/L.	Fent et al. (2006)

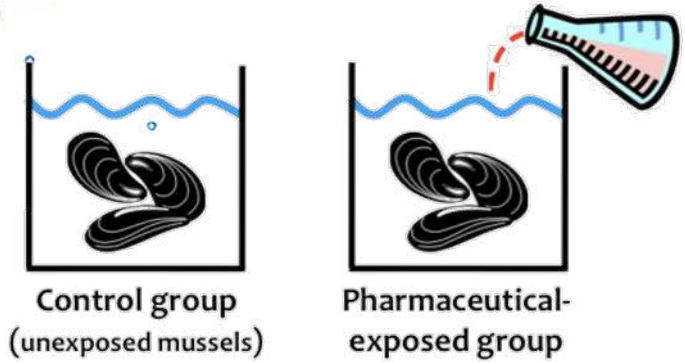
NOEC, no-observed effect concentration; LOEC, lowest observed effect concentration; LPO, lipid peroxidation levels; CbE, carboxylesterase activity; AChE, acetylcholinesterase activity; GST, glutathione-S-transferase activity; LC_{50}, median lethal concentration; EC_{50}, median effective concentration; EROD, ethoxyresorufin-O-deethylase activity.

Effective concentrations far exceeds PROP levels measured in aquatic environments

BUT pharmaceuticals are designed to affect specific molecular targets, and to be effectives at low concentrations

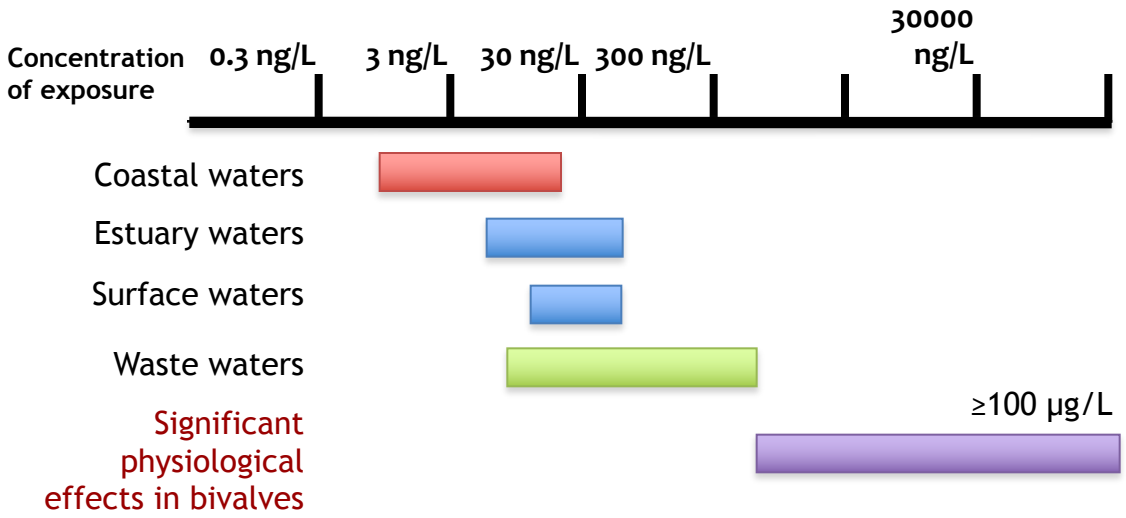
Is PROP really not dangerous for marine wildlife?

Experimental set-up

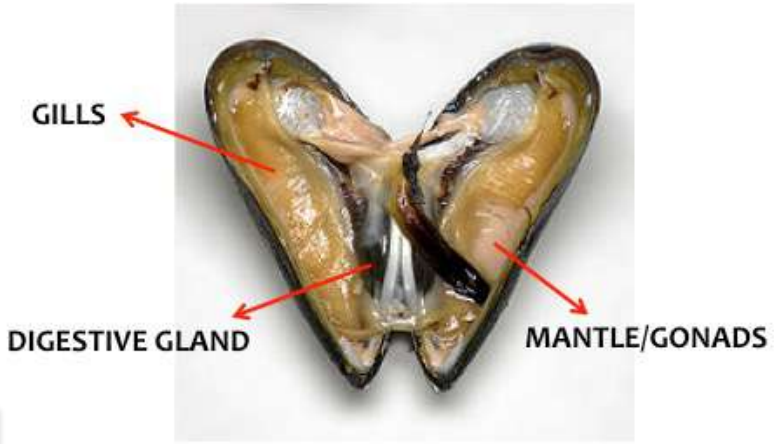


- 3-days acclimatization
- 7-days exposure
- 35-psu seawater
- Continuous aeration
- Constant water temperature (16° C)

5 concentrations of (DL)-propranolol encompassing the environmental range



Sampled tissues : -haemolimph
- digestive gland
- mantle/gonads



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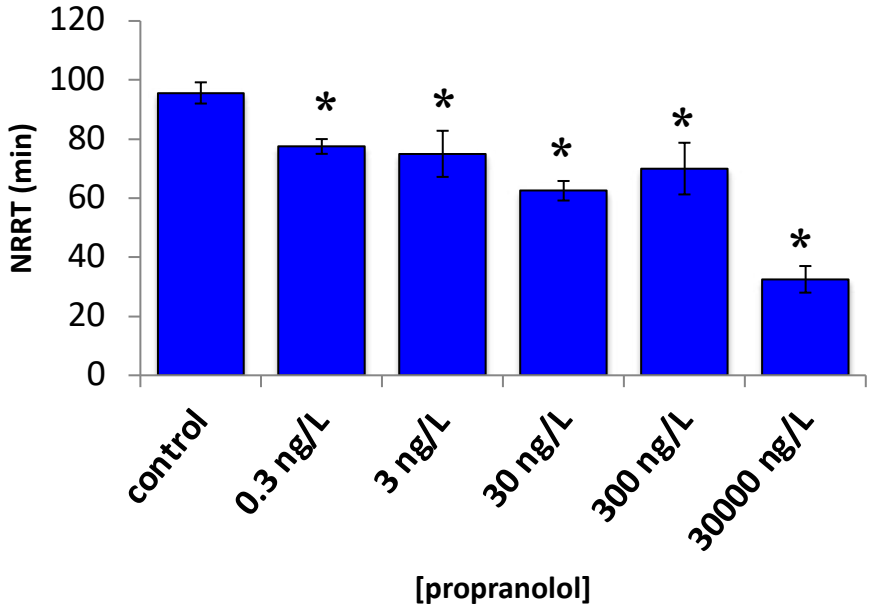
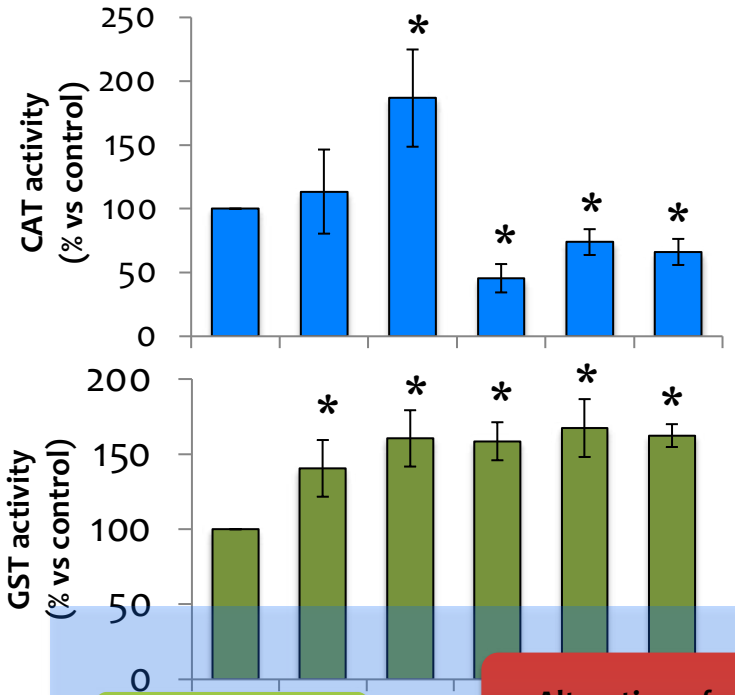
The biomarker responses

Lysosomal membrane stability



Antioxidant enzyme activities



Reduced NR retention time → Reduced lysosomal membrane stability → Reduced health status

Oxidative stress → Alteration of antioxidant enzyme activities

p<0.05 vs control

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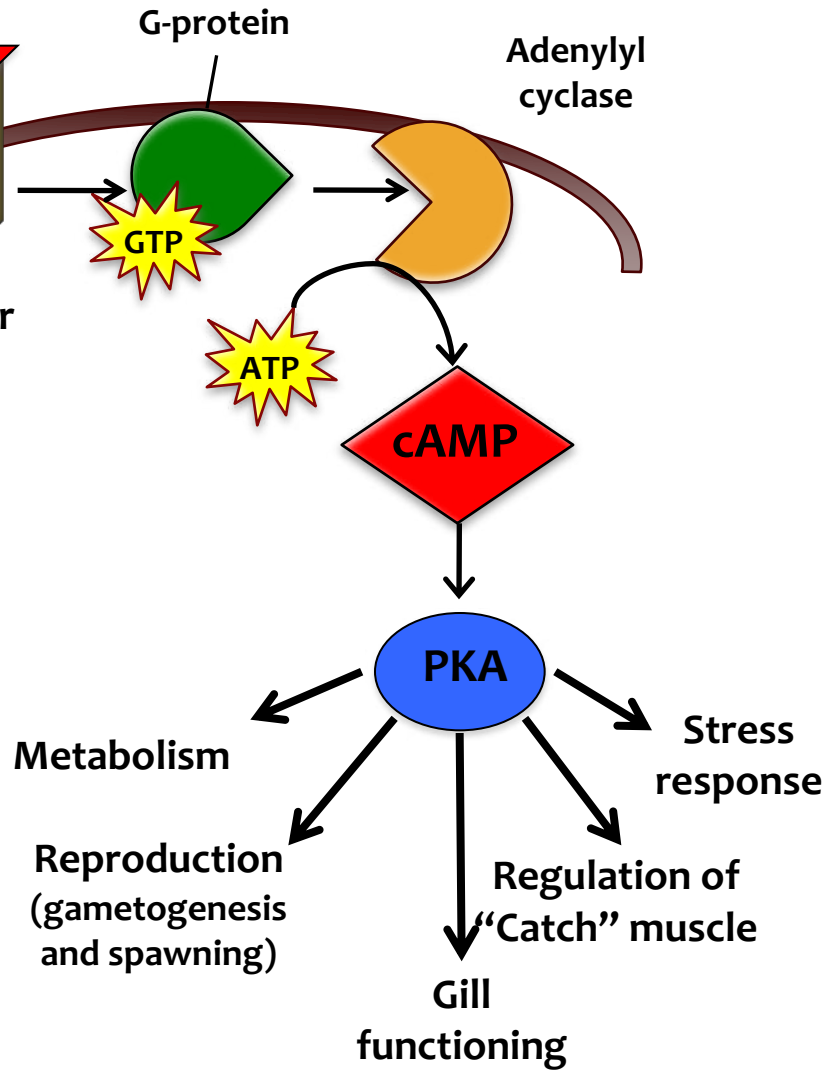
Propranolol interaction with its specific molecular targets

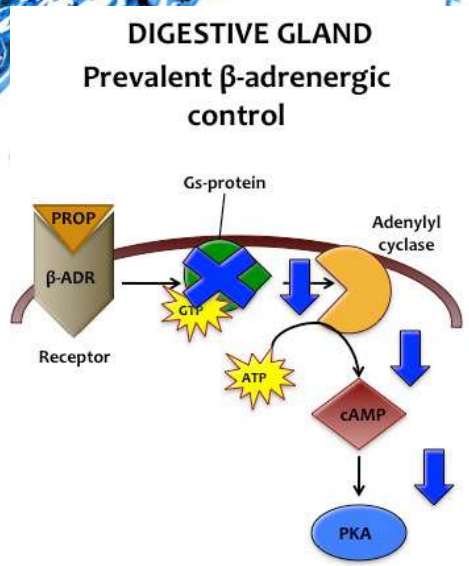
PROP acts as an antagonist for 2 families of membrane receptors

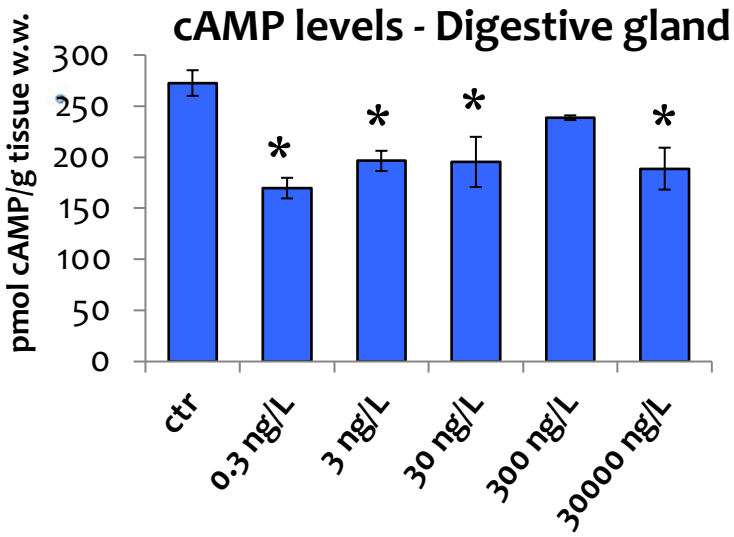
	Receptor control on the cAMP synthesis	Putative effect following receptor blockage by PROP
β adrenergic receptor (β-ADR)	Enanched cAMP synthesis rate	Decreased cAMP levels
Serotonin receptor (5HT1/2 rec)	Decreased cAMP synthesis rate	Increased cAMP levels

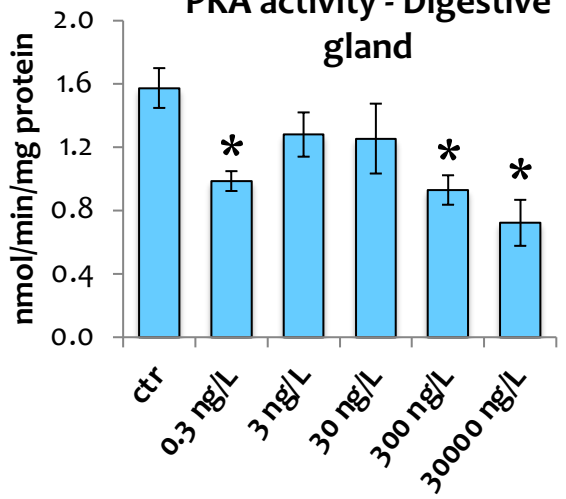


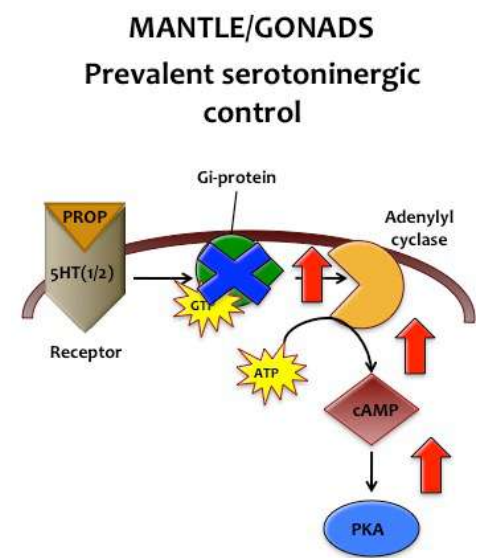
PROP interaction with its specific molecular targets

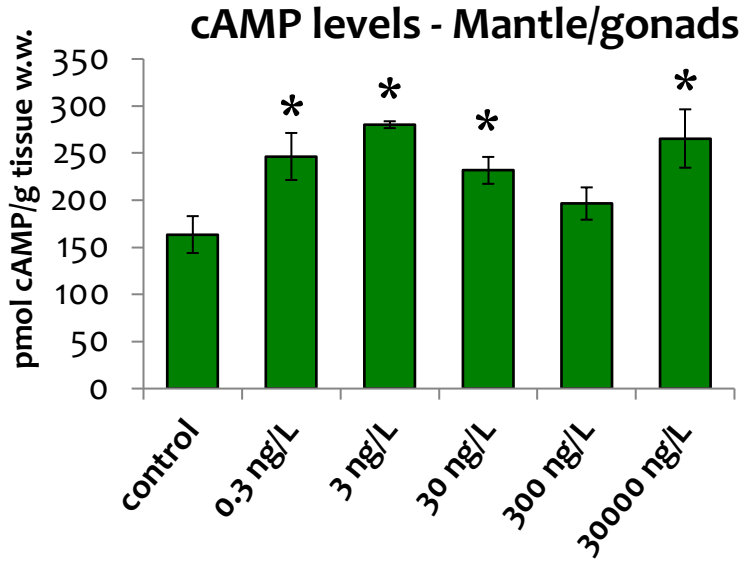
The cAMP/PKA system

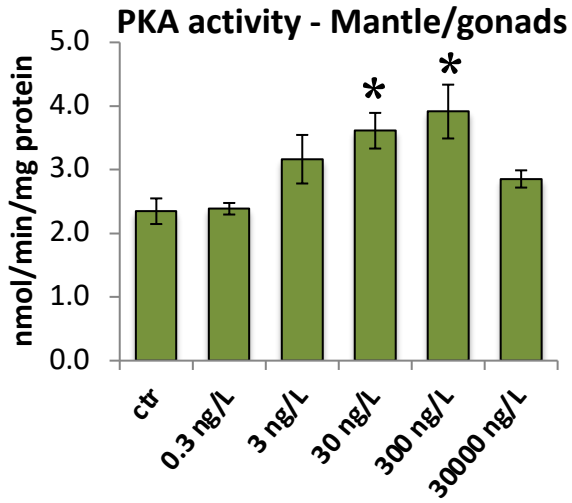












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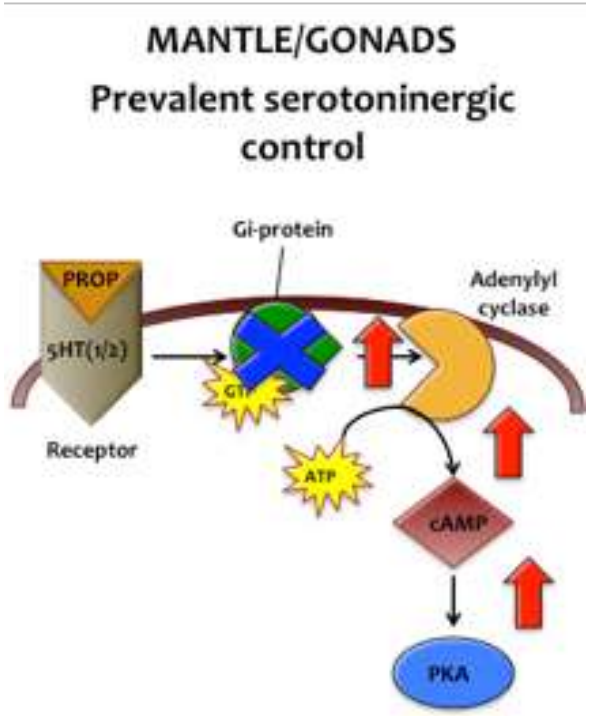
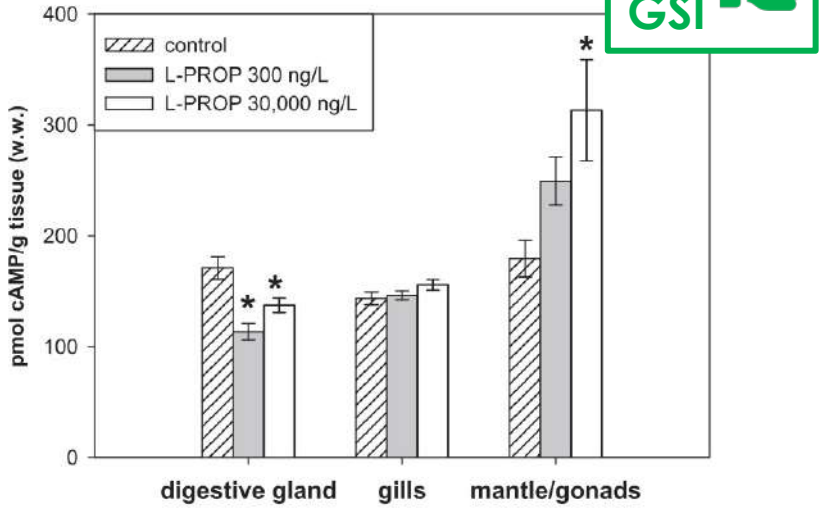
Gender bias and seasonal patterns

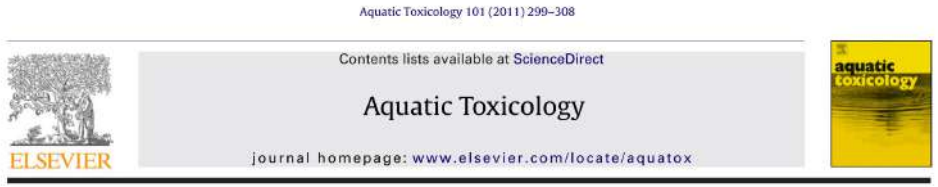
Dealing with propranolol effects on mussel cell signaling



February-March (Pre-spawning)

LMS GSI



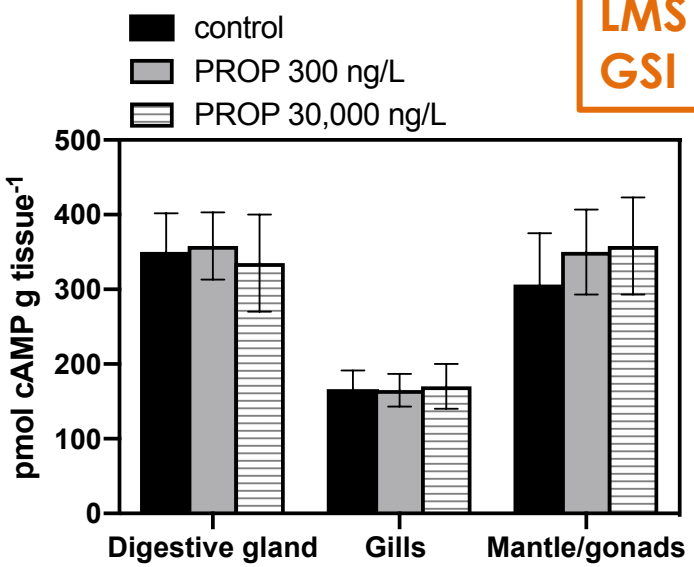


The β-blocker propranolol affects cAMP-dependent signaling and induces the stress response in Mediterranean mussels, *Mytilus galloprovincialis*

Silvia Franzellitti[a], Sara Buratti[a], Paola Valbonesi[a], Antonio Capuzzo[b], Elena Fabbri[a,*]

October-November (gonad development and gametogenesis)

LMS GSI



Preliminary experimental trials

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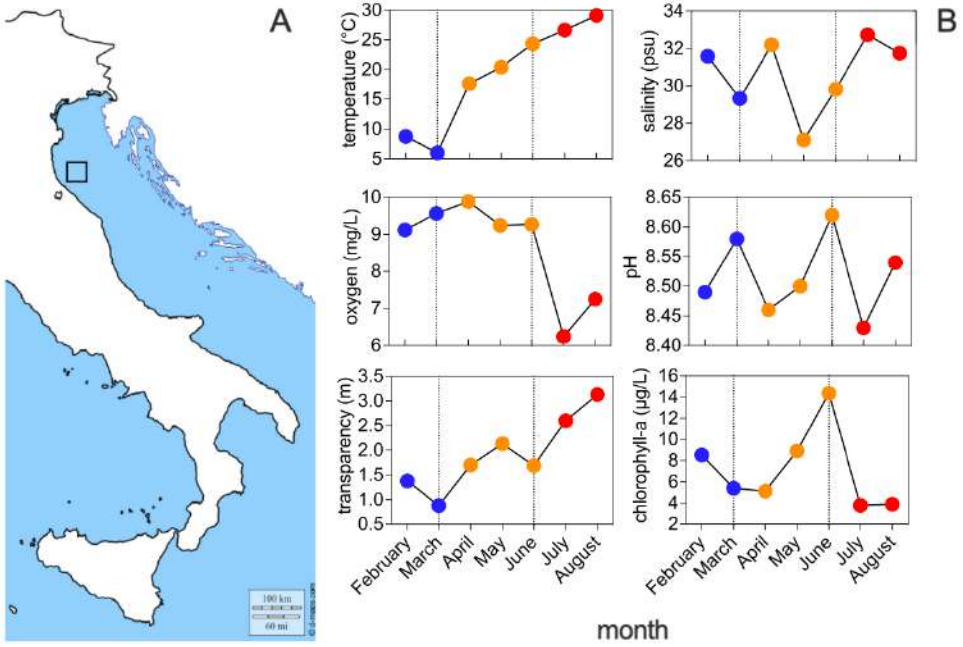
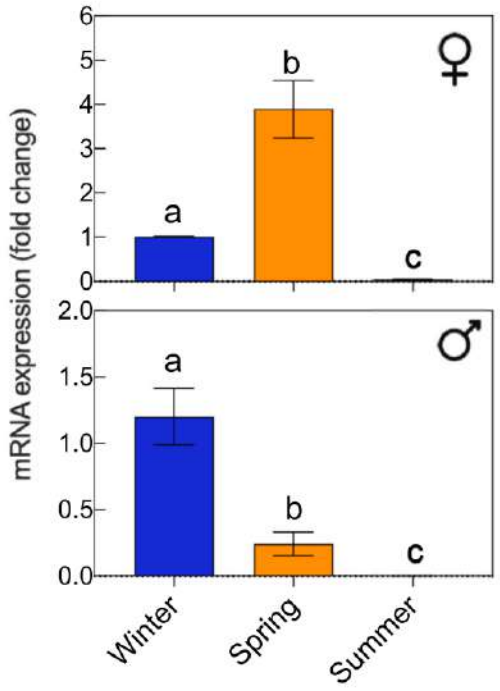
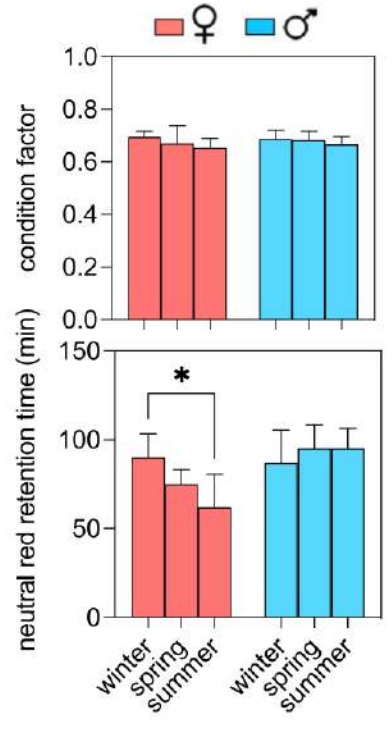
Gender bias and seasonal patterns







Marine Pollution Bulletin 172 (2021) 112847

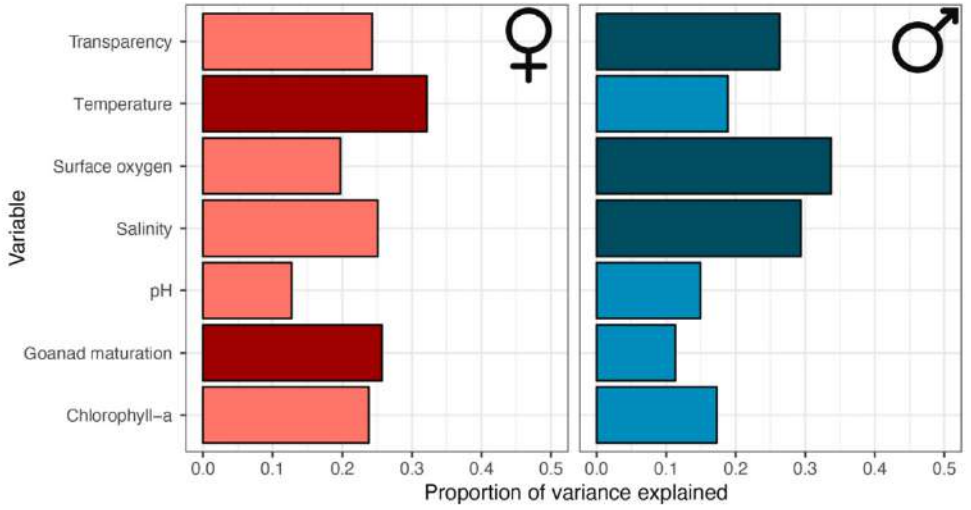
Contents lists available at ScienceDirect

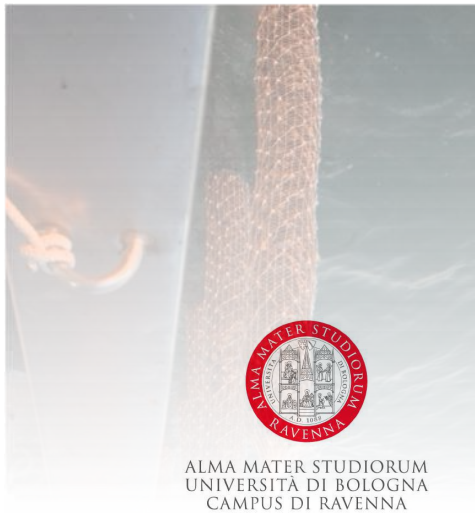
Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Variability of metabolic, protective, antioxidant, and lysosomal gene transcriptional profiles and microbiota composition of *Mytilus galloprovincialis* farmed in the North Adriatic Sea (Italy)

Rajapaksha Haddokara Gedara Rasika Wathsala [a], Margherita Musella [b,c], Paola Valbonesi [a], Marco Candela [b,c,*], Silvia Franzellitti [a,c,**]





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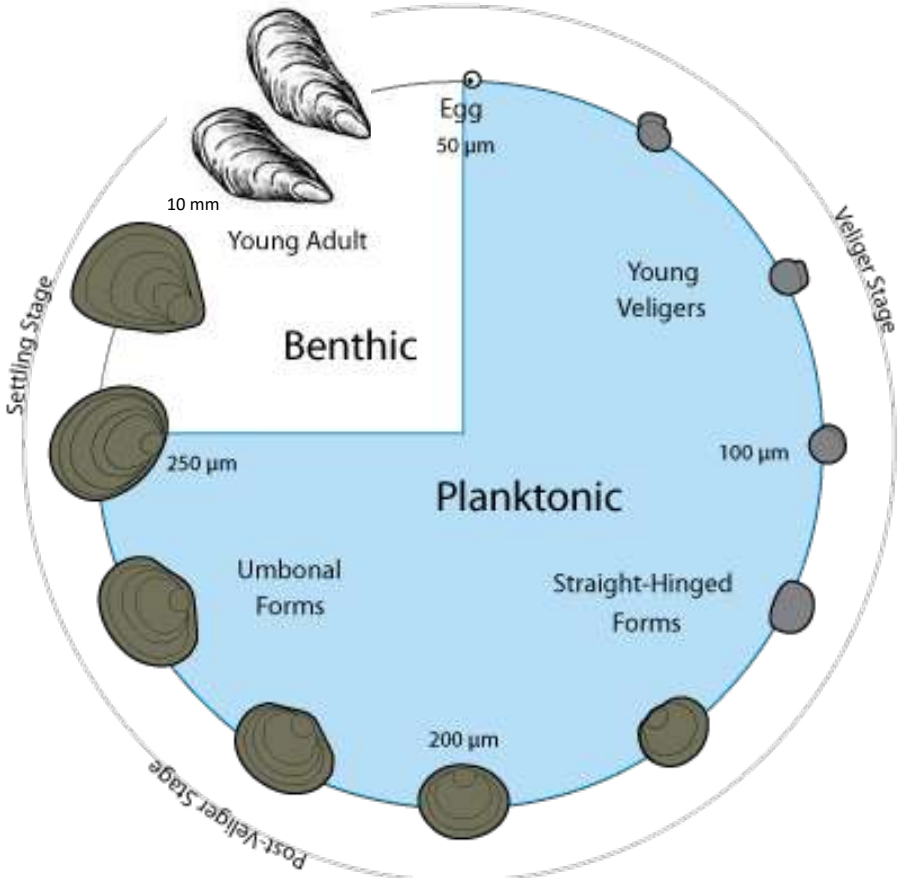
Unexpected effects on embryo-larval development…



- **Mussels have complex life cycle with a planktonic development up to the juvenile stage, when larvae complete their metamorphosis, settle to the sea bottom and grow up as a benthonic adult.**
- **Early planktonic stages are considered the most sensitives to environmental stressors.**
- **Fast evolving adaptation**

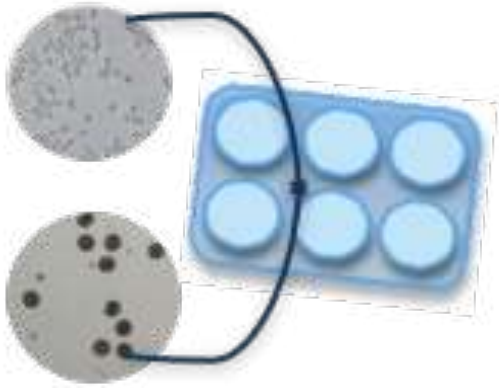
Embriotoxicity and morphological effects of pharmaceuticals

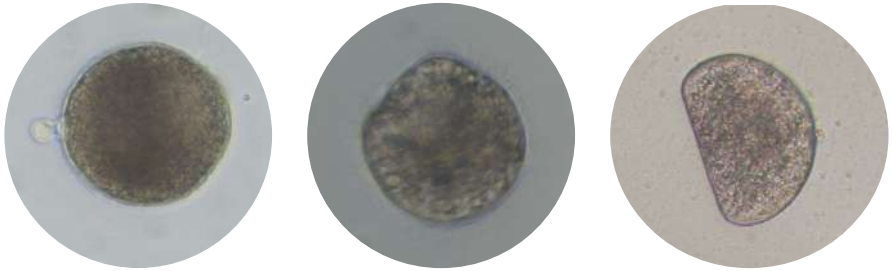
Natural spawning of sexually mature mussels

Sperm:oocyte mixing under controlled conditions (16°C)

check for fertilization success (> 85% fertilized eggs)

Larvae grown for 48 h in the presence of PHARMA TESTED CONCENTRATIONS





30 min pf

24 h pf

48 h pf

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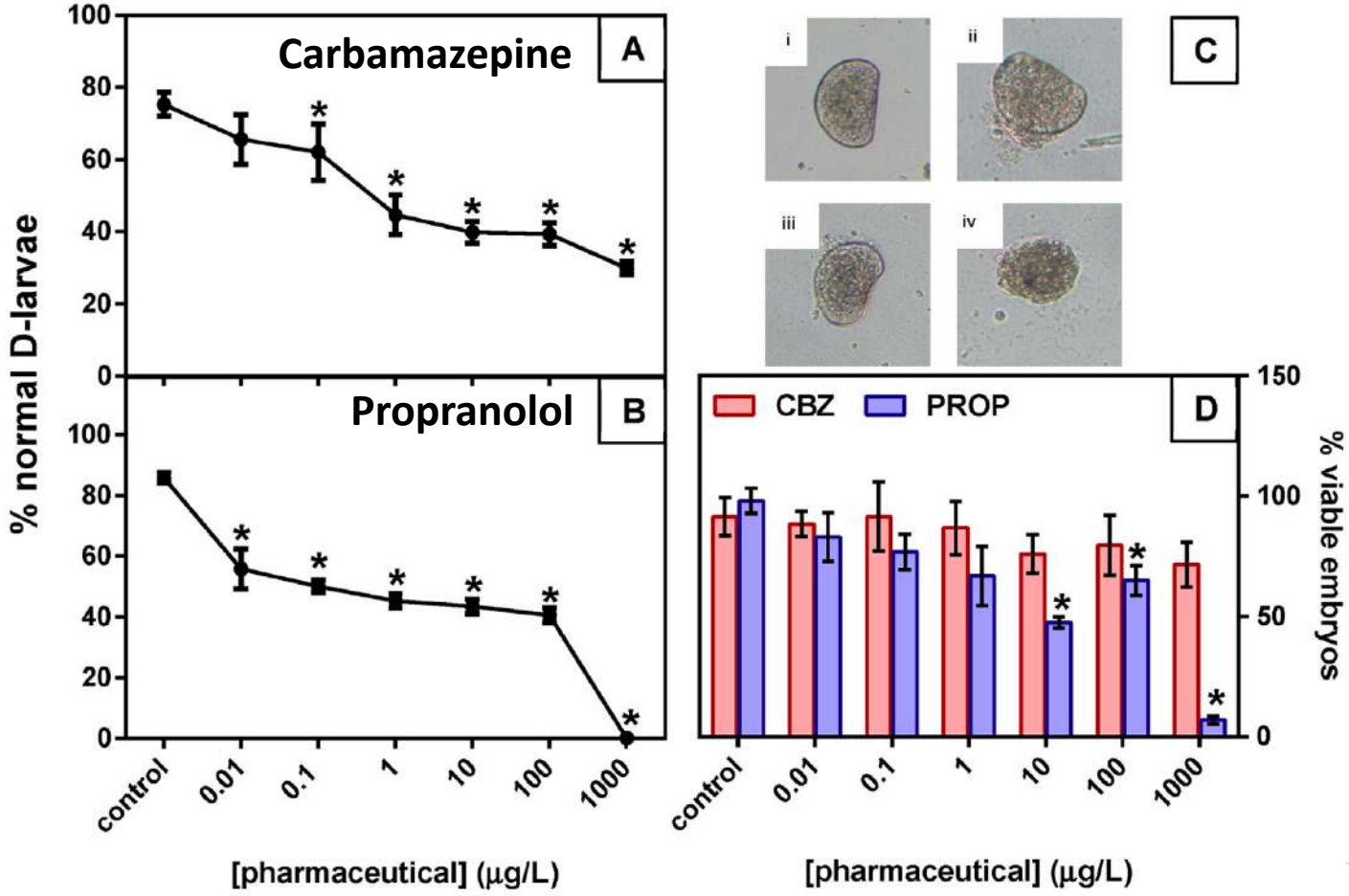
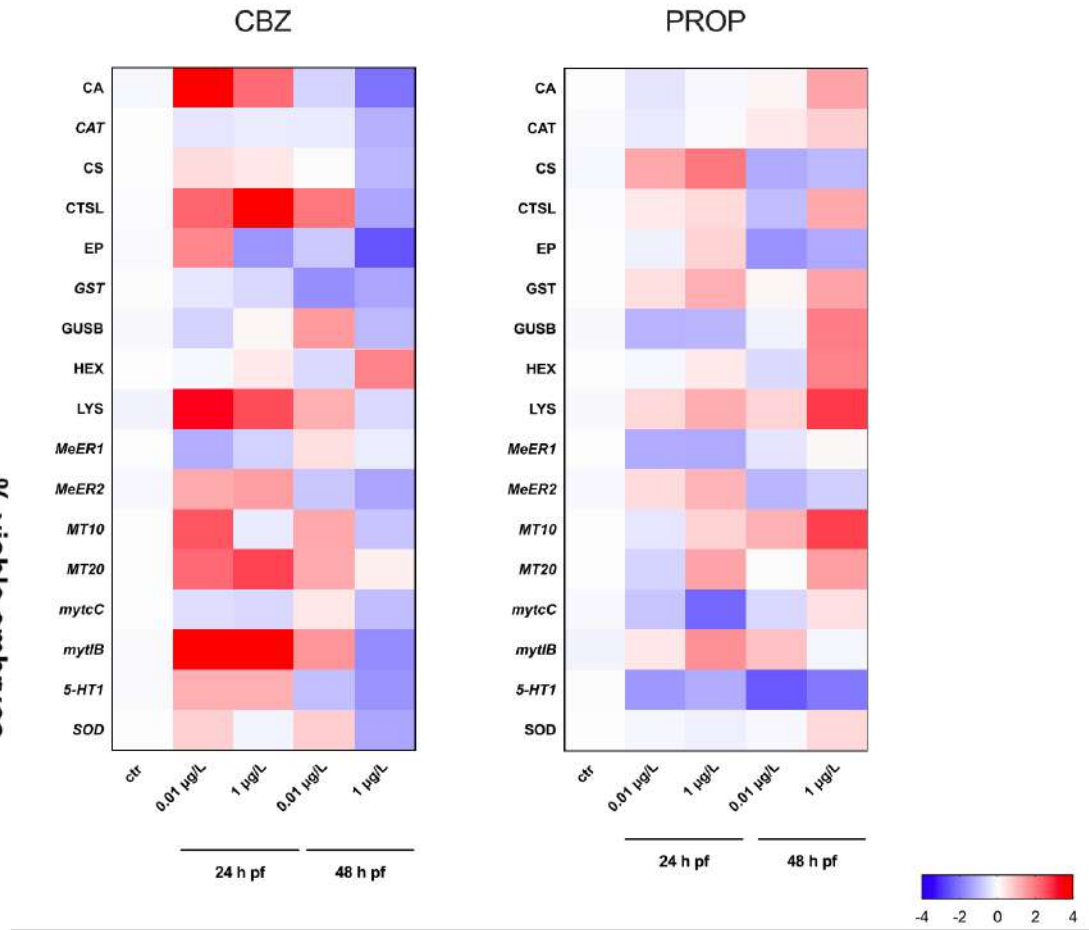
Embriotoxicity and morphological effects of pharmaceuticals





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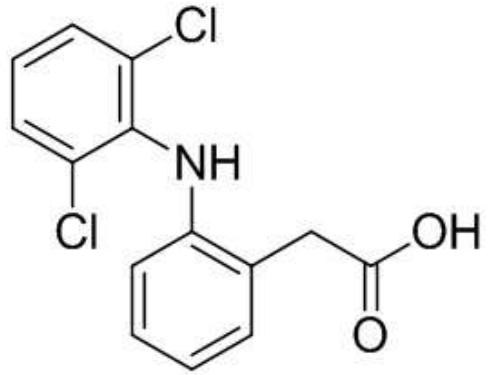
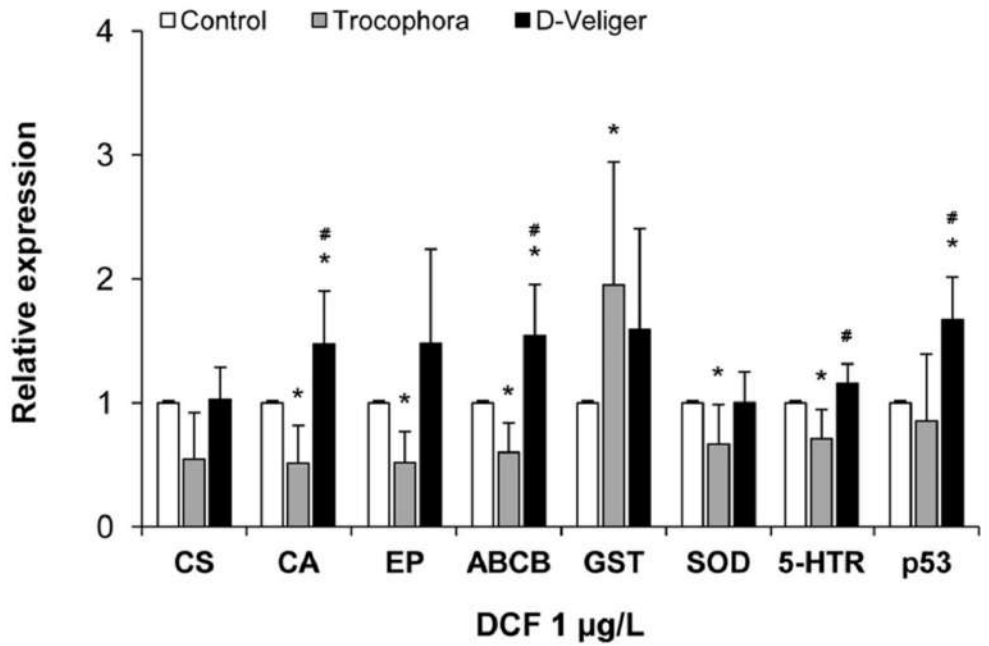
Embriotoxicity and morphological effects of pharmaceuticals

DICLOFENAC





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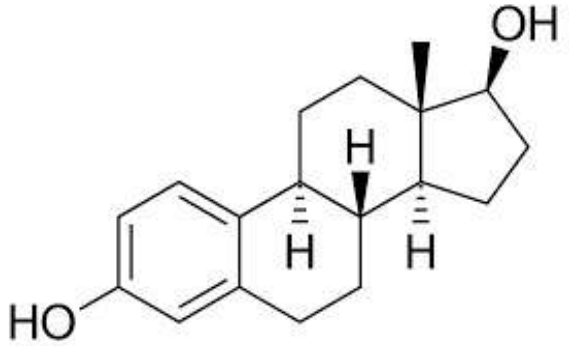
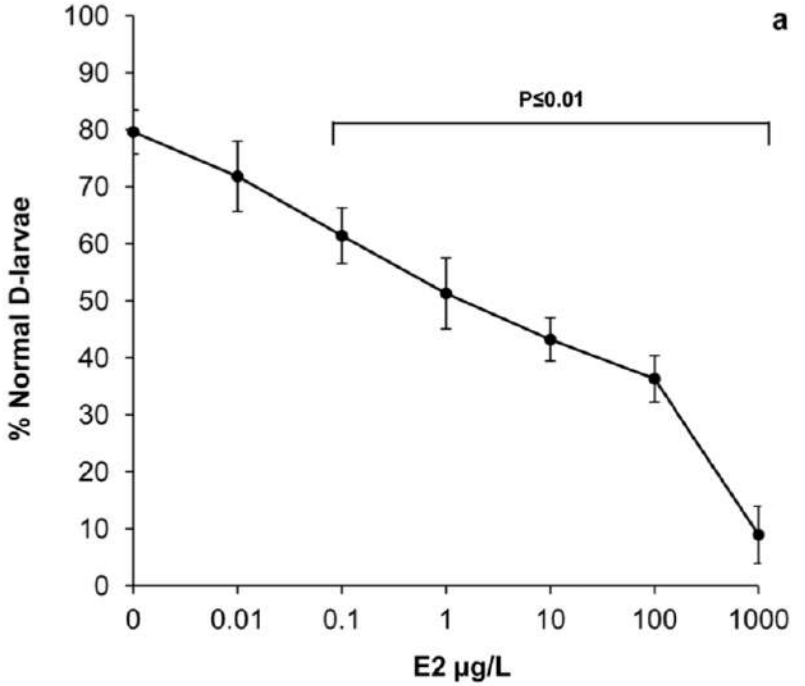
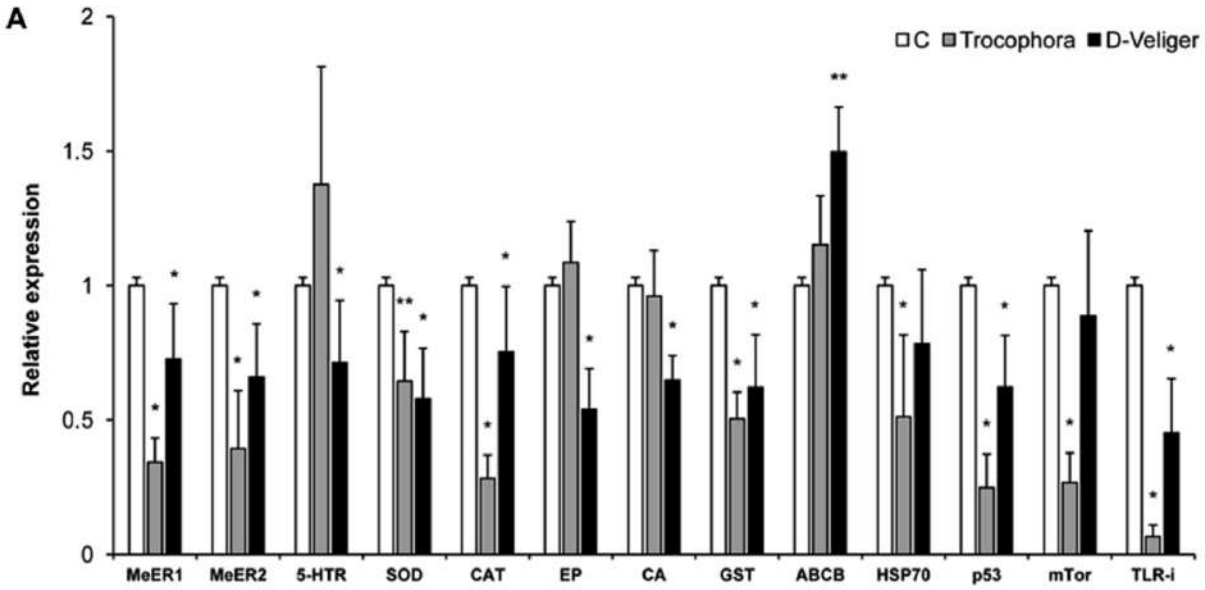
Embriotoxicity and morphological effects of pharmaceuticals

Estradiol







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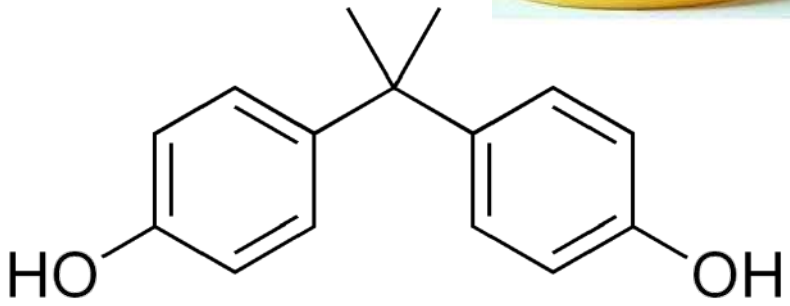
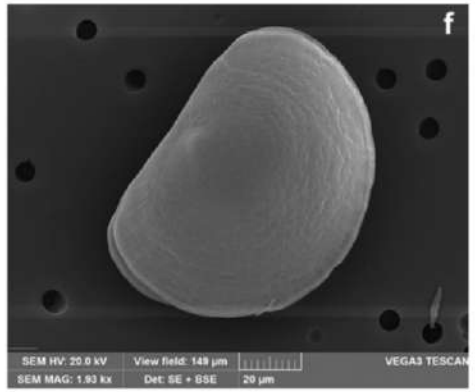
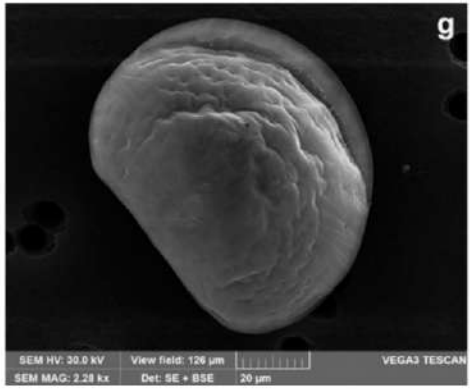
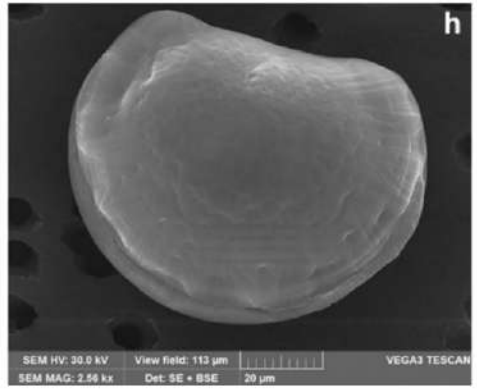
Not only pharmaceuticals … plastic polymers and additives

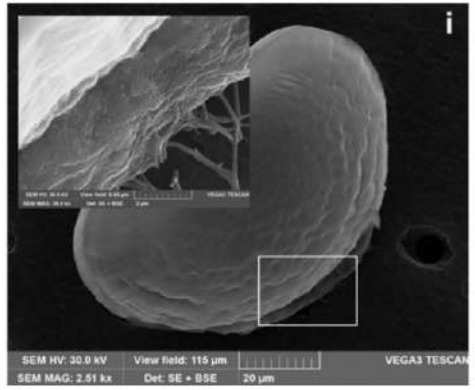
BISPHENOLS

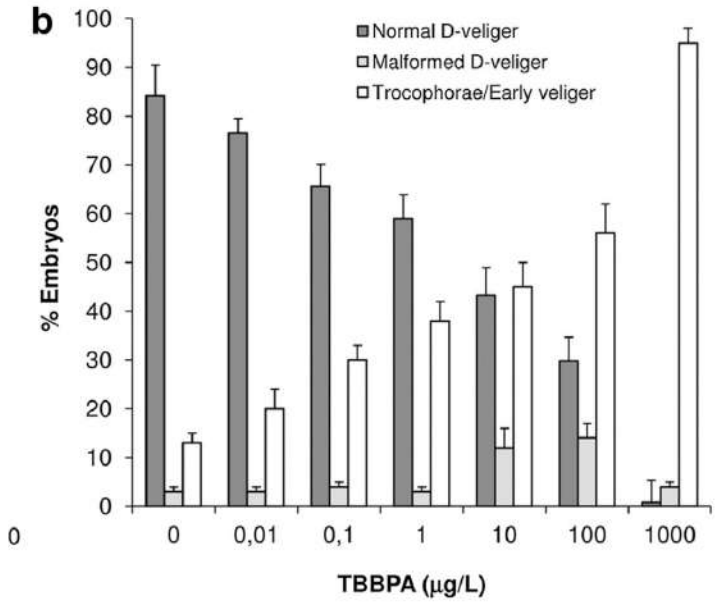


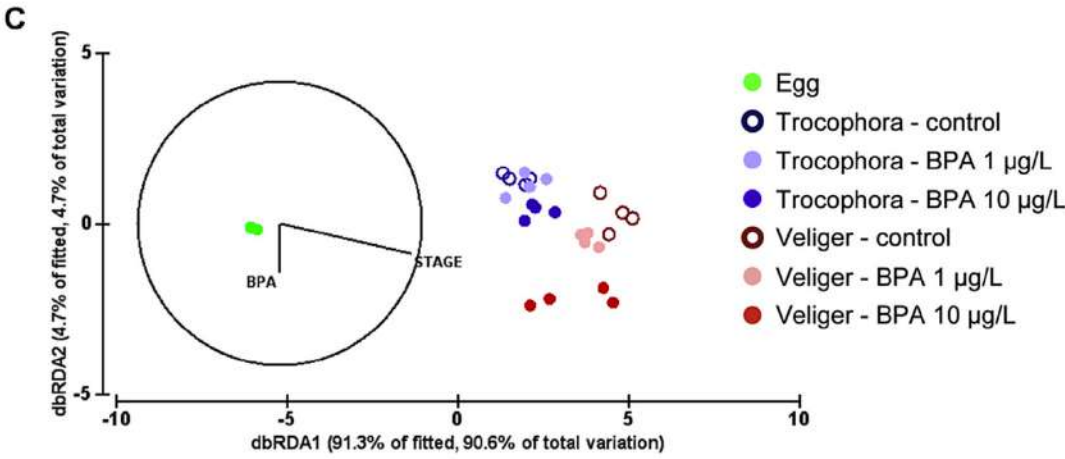












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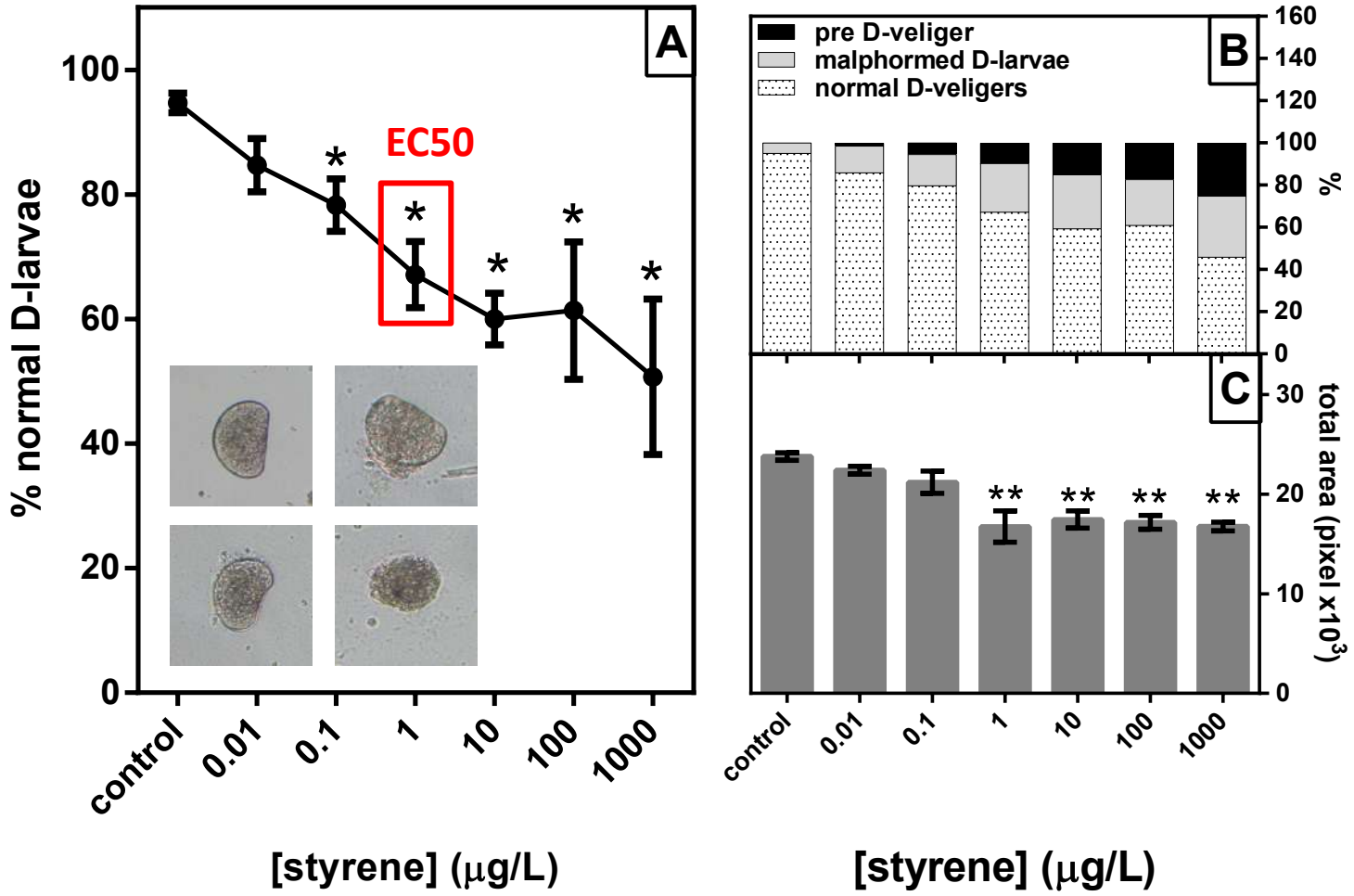
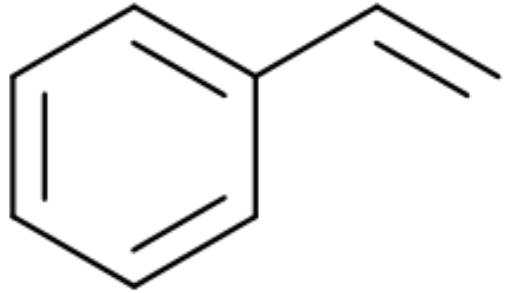
Not only pharmaceuticals … plastic polymers and additives



STYRENE



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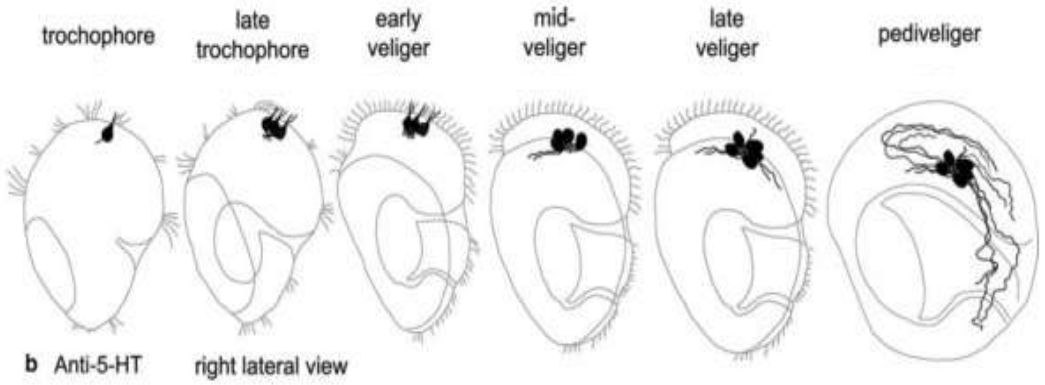
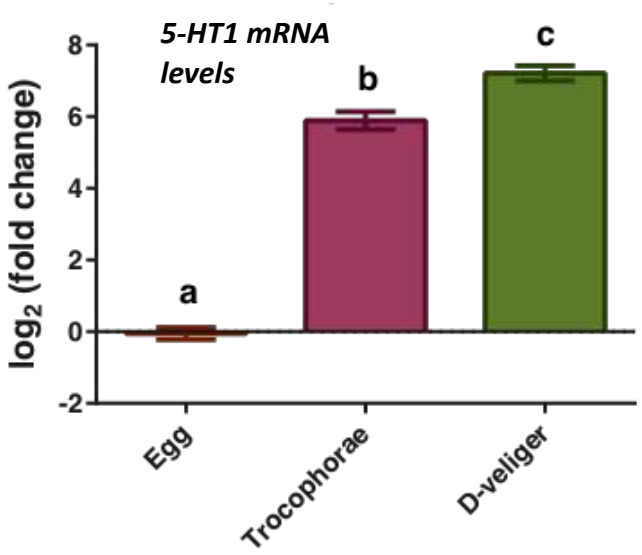
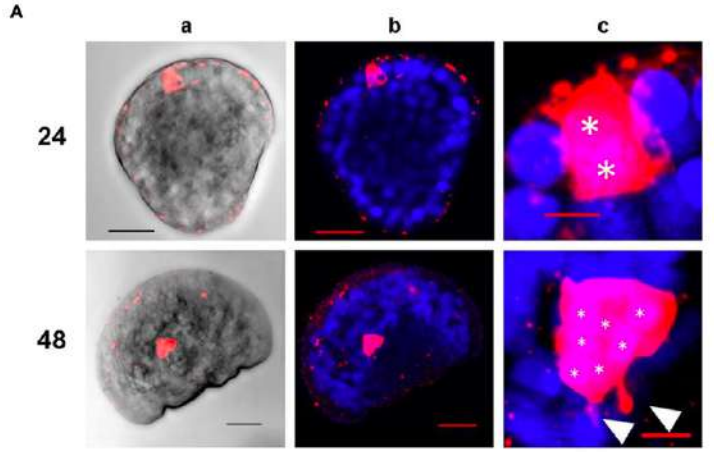
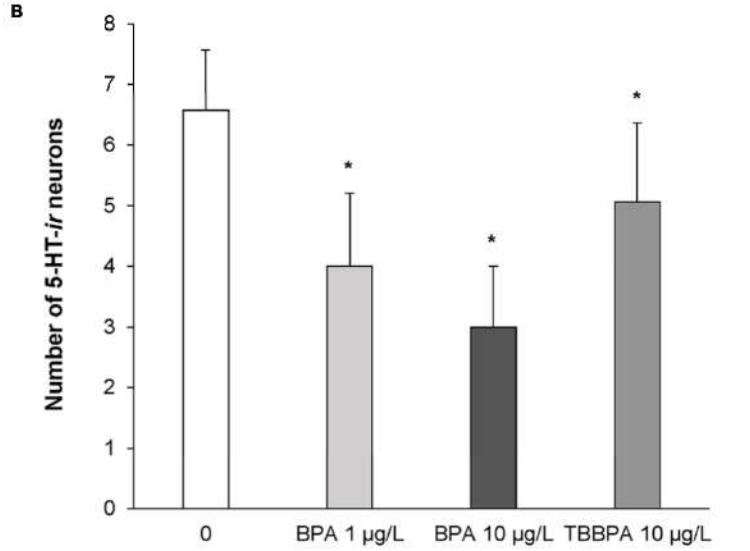
Ontogeny of the serotoninergic neuromodulatory system in mussel embyos







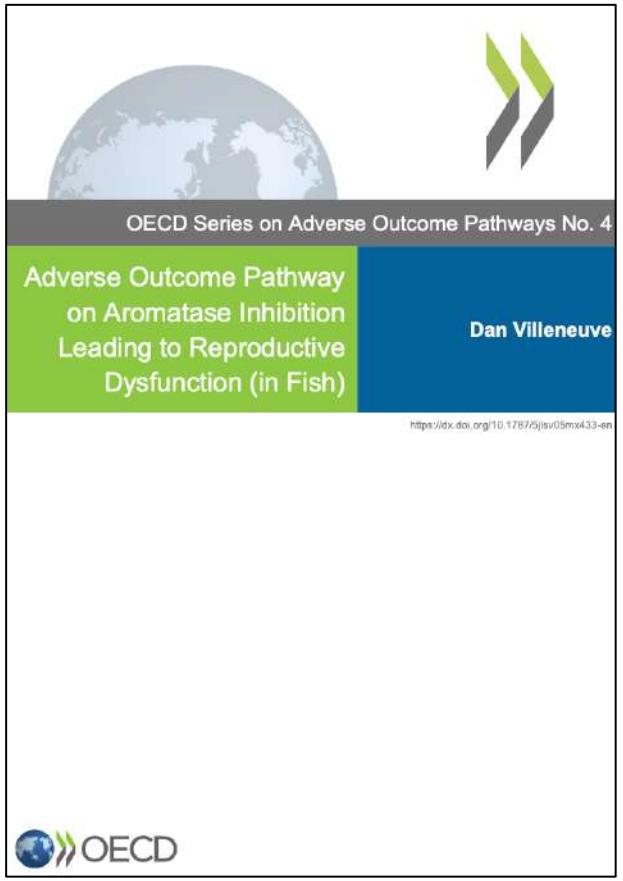


Science of The Total Environment
Volume 793, 1 November 2021, 148596

Tetrabromobisphenol A acts a neurodevelopmental disruptor in early larval stages of *Mytilus galloprovincialis*

A. Miglioli [a, b], T. Balbi [a] ✉, M. Montagna [a], R. Dumollard [b, 1], L. Canesi [a, 1]

Pharmaceuticals as enviornmental pollutants: the recent mthodological approach



The Adverse Outcome Pathway (AOP)

an Adverse Outcome Pathway (AOP) describes a sequence of events commencing with the initial interaction of a stressor with a biomolecule within an organism that causes a perturbation in its biology, which can progress through a series of intermediate key events up to and apical adverse outcome relevant to risk assessment or regulatory decision-making