



Biomonitoring strategy to assess the effects of chemical pollution along the Iberian Mediterranean Coast: Present state and future development

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**OSPAR
Convention
CEMP**



**Barcelona
Convention
MEDPOL**

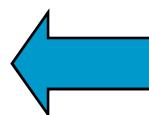
**MCBE research
group (IEO)**



BARCELONA CONVENTION

**Environmental assessment
component**

MEDPOL PROGRAMME



**Mediterranean
Action Plan**

MEDPOL Phase III (1996-2005):

Selected
contaminants

+

Biomarkers

(UNEP/RAMOGÉ, 1999)

EBCs (Biomarkers)



Biomarkers recommended for the MED POL biomonitoring Programme (UNEP/RAMOGÉ, 1999)

Biomarkers recommended	Methodology	Protocol provided
Lysosomal Membrane Stability	Cytochemical assay Neutral Red Retention Assay	Fish/mussels mussels
Genotoxic effects	Alkaline filter elution Micronuclei frequency	Fish/mussels Mussels
Mixed Function Oxidase activity (EROD)	Fluorimetric method	Fish
Metallothionein Level	Spectrophotometric method	<i>Fish/mussels</i>



Mullus barbatus

Since 2001 incorporating biomarkers (OC of Murcia, IEO)



Mytilus galloprovincialis

MEDPOL Phase IV (2006-2013):



Strategy for the development of Mediterranean Marine Pollution Indicators (**MPIs**), (UNEP, 2003)

MPIs recommended	Methodology	Protocol provided
Stress on stress	Survival in air	mussels
Evaluation of Lysosomal Membrane Stability	Cytochemical assay	<i>Fish</i> /mussels
	Neutral Red Retention Assay	mussels
Lipofuscin lysosomal accumulation	Cytochemical assay	Fish/mussels
Neutral lipid lysosomal accumulation	Cytochemical assay	Fish/mussels
Peroxisome proliferation	Cytochemical assay	Fish/mussels
Evaluation of Metallothionein Level	Spectrophotometric method	<i>Fish</i> /mussels
Acetylcholinesterase activity	Spectrophotometric method	mussels
Evaluation of Mixed Function Oxidase activity (EROD)	Fluorimetric method	Fish
Evaluation of genotoxic effects	Alkaline filter elution	Fish/mussels
	Micronuclei frequency	Fish/mussels

EBCs (Biomarkers)



Marine pollution indicators (MPIs) according UNEP (2005) in molluscs and fish target species

MPIs recommended	Methodology	Protocol provided
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	Micronuclei frequency	Fish/mussels

Two-tier approach: Level of pollutant-induced stress syndrome in target species

(Viarengo et al., 2007. *Biochem. And Phisio*)



Biomonitoring programme conducted by MCBE (IEO) along the Mediterranean coast: Main goals

- Determination of **spatial distribution** and **temporal trends** of **chemical contamination** in coastal and references areas by using **target organisms** (red mullet and mussels) and **sediments**.
- To seek evidences of detrimental **biological effects** in **target species** and monitor them over time.



Sampling fields

Sampling strategy: UNEP/FAO/IOC/IAEA, 1984; UNEP, 2006 and ICES, 2005

FREQUENCY AND SAMPLING SEASON

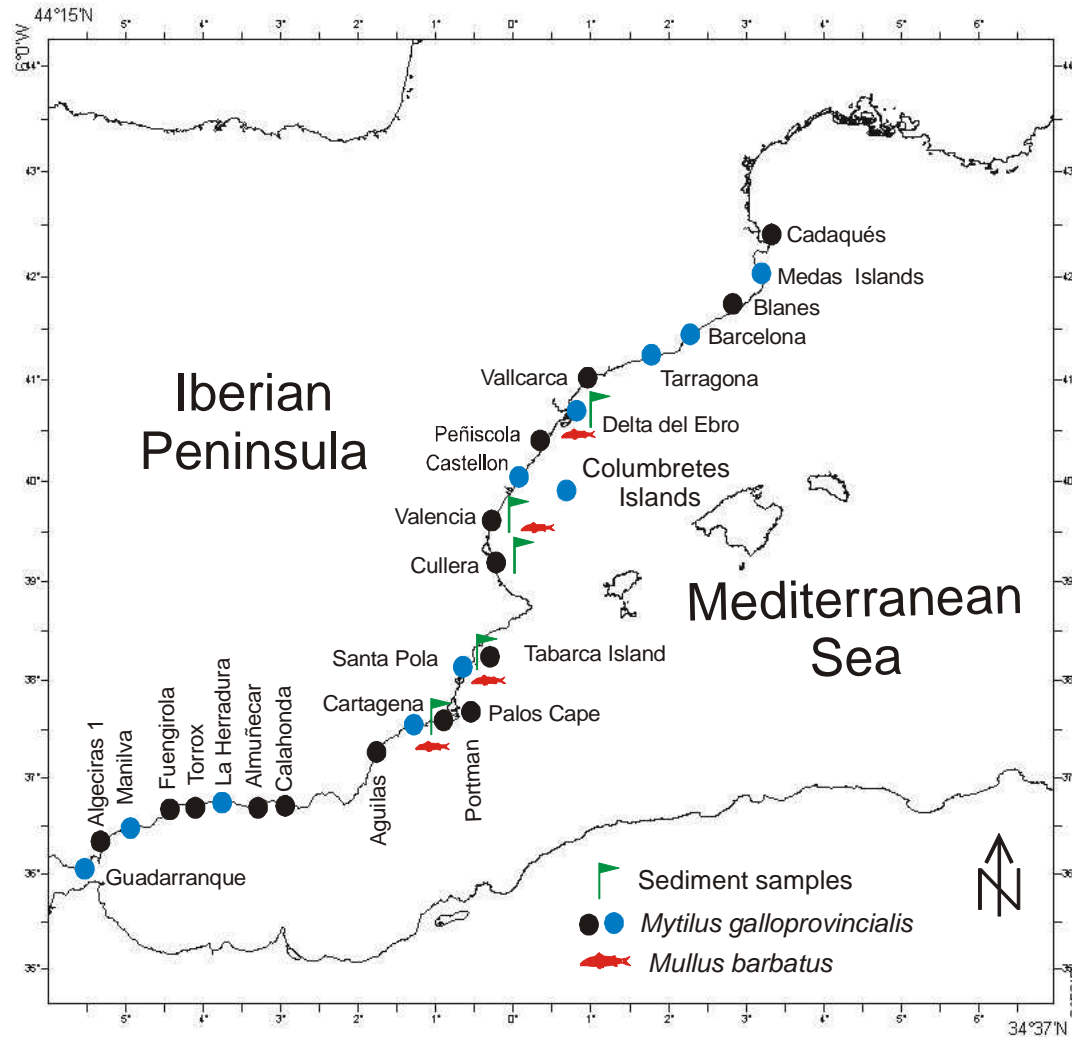
Temporal trend monitoring in biota: Yearly

Temporal trend monitoring in sediment: Yearly

Spatial monitoring: Once every 5 years

- The samples of *Mullus barbatus* and sediments are collected in **April** (non-matured specimens).
- The samples of *Mytilus galloprovincialis* are collected from **mid May to mid June** (no-spawning period).

Field samplings 2006

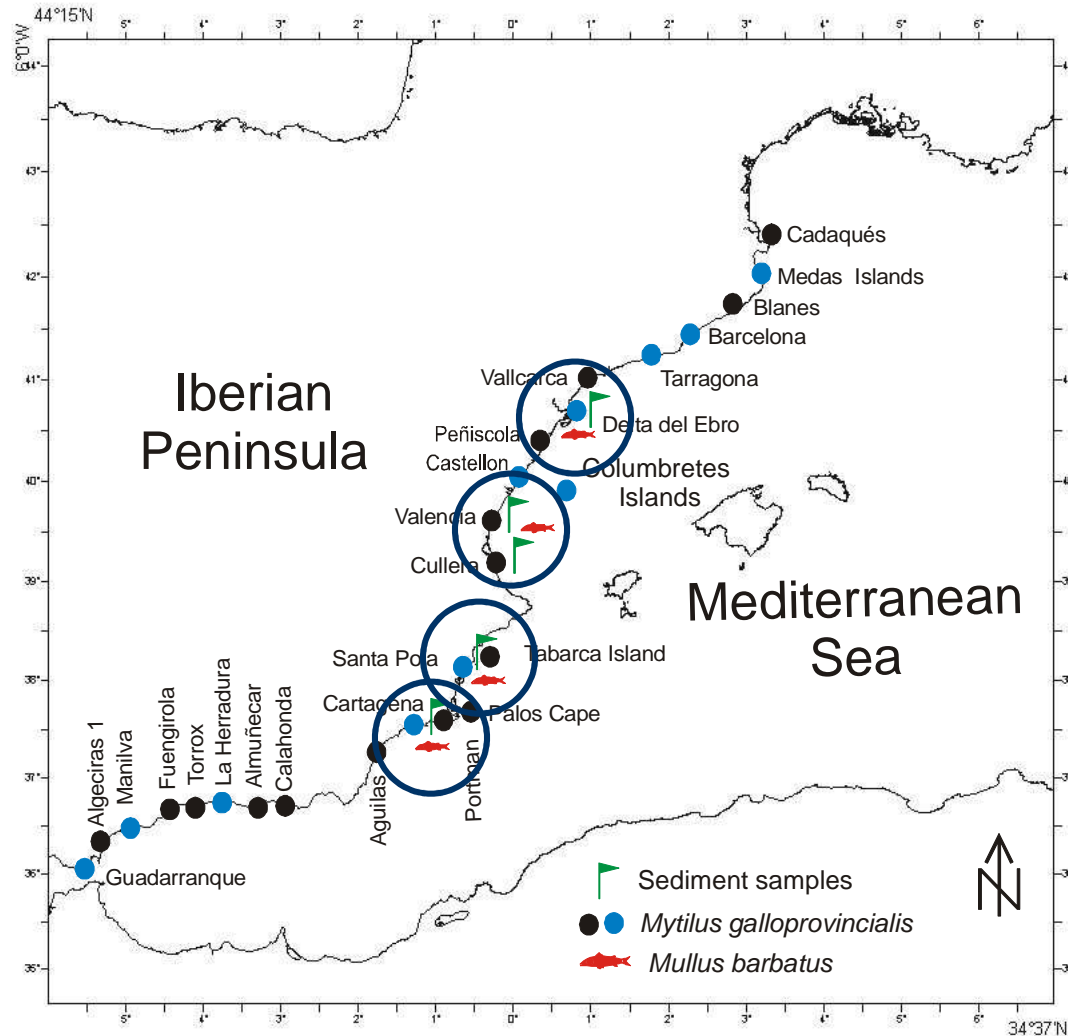


BECs and chemical analyses are performed in same specimens/populations

Field samplings 2006



Integrated
assessment



Sediment samples were collected same in areas where fish were caught

CHEMICAL CONTAMINANTS



Recommendations of MEDPOL Programme and also considering OSPAR.

Use of reference materials, **QUASIMEME** and **IAEA** intercomparison exercises



TRACE METALS	Mercury
	Lead
	Cadmium
	Copper
	Zinc
	Arsenic
	Nickel
Aluminium	
ORGANOCHLORINATED COMPOUNDS	Polychlorinated byphenyls: CB28, CB52, CB101, CB105, CB118, CB138, CB153, CB156, B180.
	<i>pp'</i> DDE, <i>pp'</i> DDT y <i>op'</i> DDT
	γ -Hexachlorocyclohexane
	α -Hexachlorocyclohexane
	Hexachlorobencene
	Transnonachlor
	Dieldrin
	Aldrin
POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)	Phenanthrene
	Anthracene
	Fluoranthene
	Pyrene
	Benzo[a]anthracene
	Chrysene
	Benzo[e]pyrene
	Benzo[b]fluoranthene
	Benzo[k]fluoranthene
	Benzo[a]pyrene
	Benzo[g,h,i]perylene
	Dibenzo[a,h]anthracene
	Indeno[1,2,3-c,d]pyrene

AAS: Graphite furnace, flameless atomic.

Cold vapour technique

GC: ECD-MS detector, helium carrier gas

HPLC: UV detector, water-methanol gradient phase

BIOMARKERS



Analysed following recommendations of MEDPOL Programme.

BEQUALM and **MEDPOL** (Prof. A. Viarengo)



Mussel



Red mullet

Stress on Stress

Lysosomal Membrane

Micronuclei

AChE

Metallothioneins

Two- tier
approach

Genotoxic damages

Micronuclei

EROD

Metallothioneins

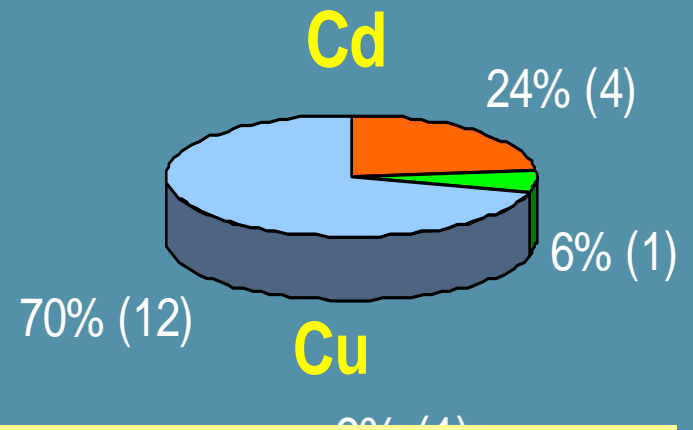
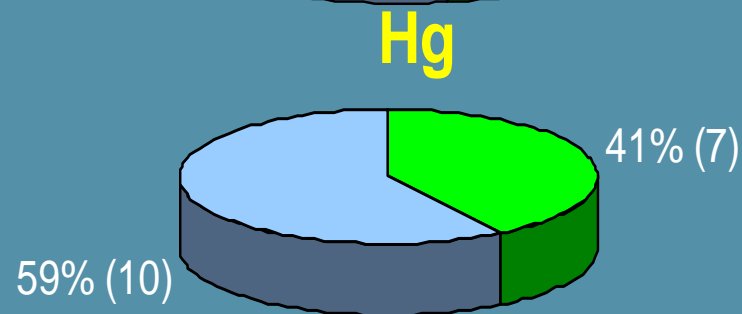
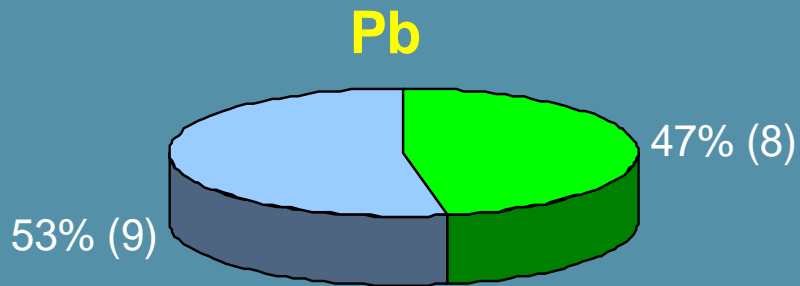


Some Preliminary Results

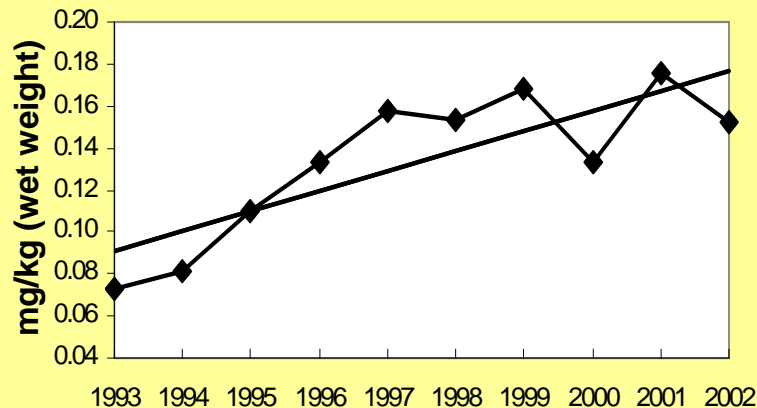
Metal content in mussels from Catalanian



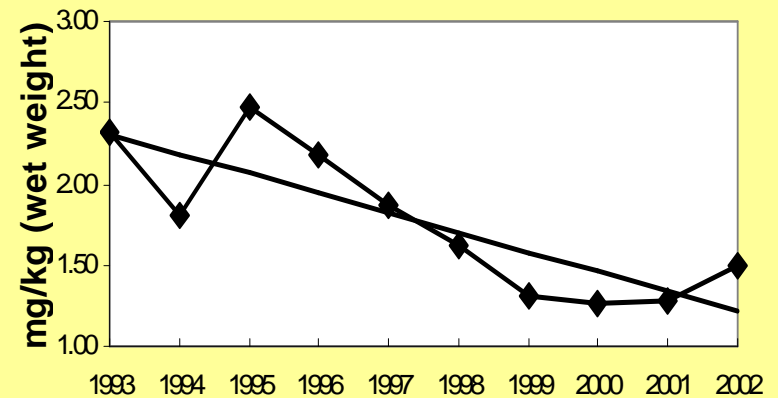
- No significant trends
- Significant upward trends
- Significant downward trends



Cadmium at Medas Islands $R=0,600$

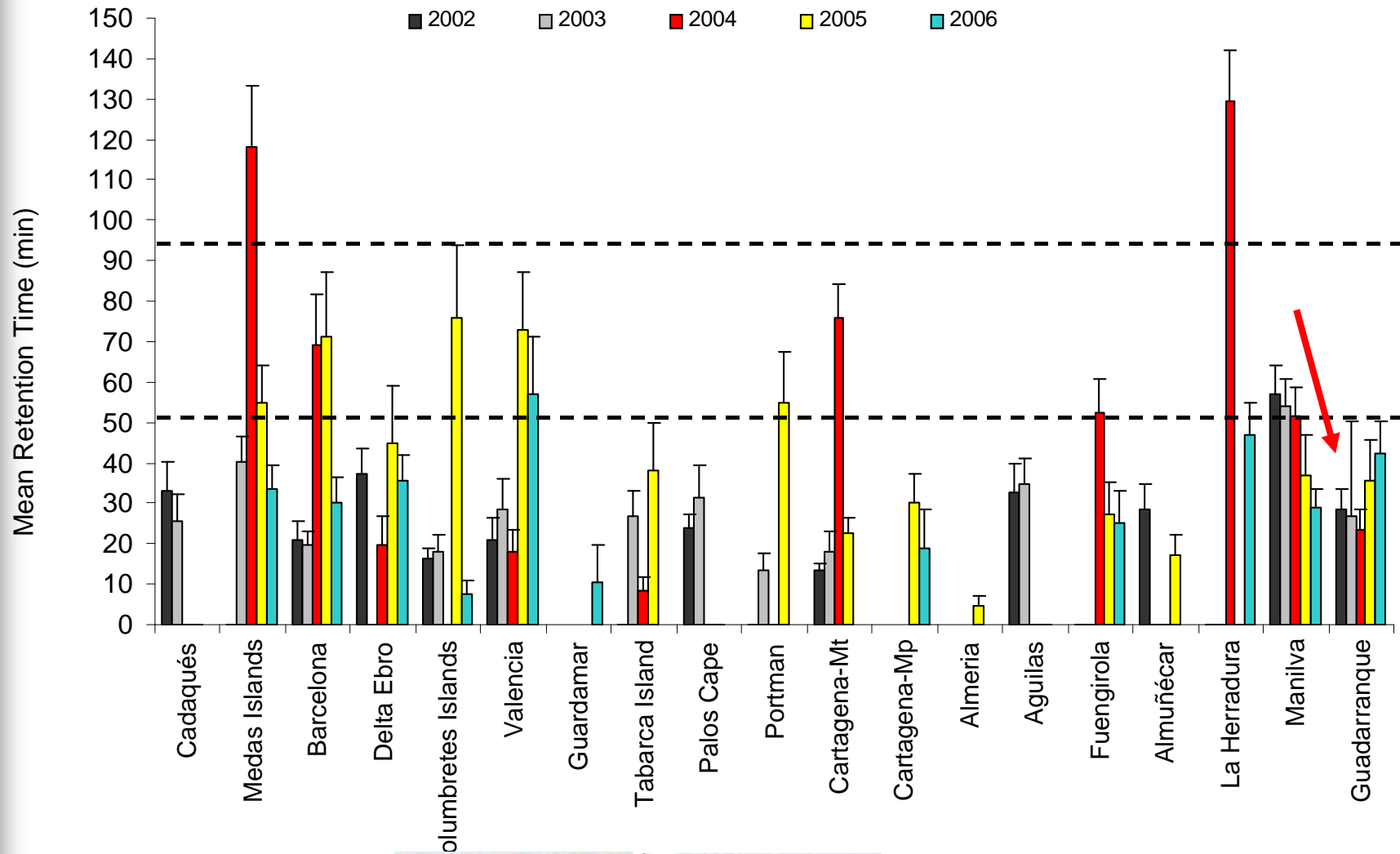


Lead at Barcelona $R=-0,644$



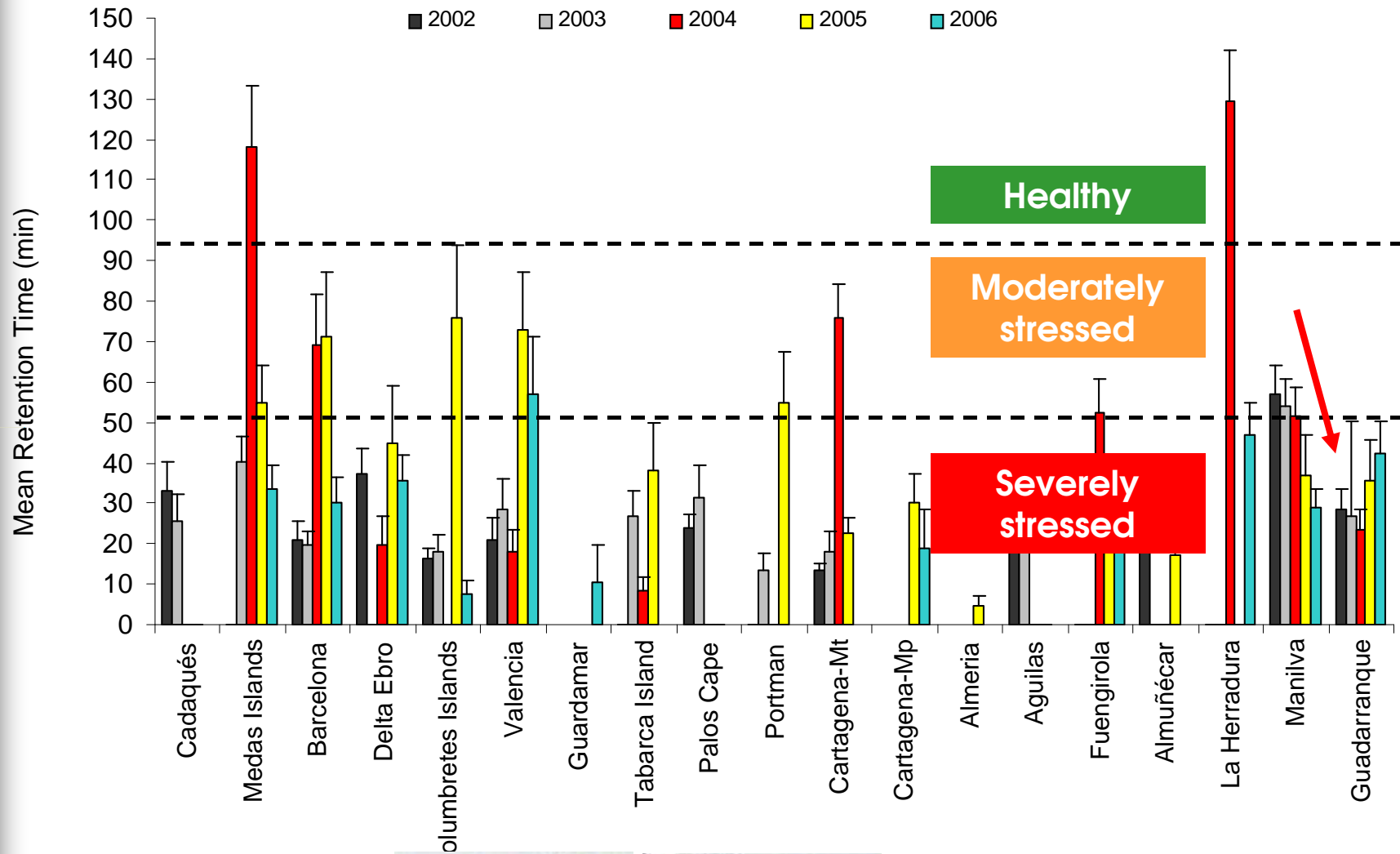
Benedicto et al., 2004. *Rapp. Comm. Int Mer. Medit.*, 37

Lysosomal membrane stability in mussels



Martínez-Gómez *et al.*, 2006. *Mar. Environm. Res.*, 62

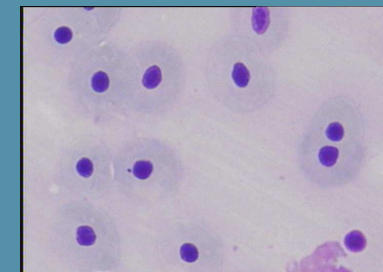
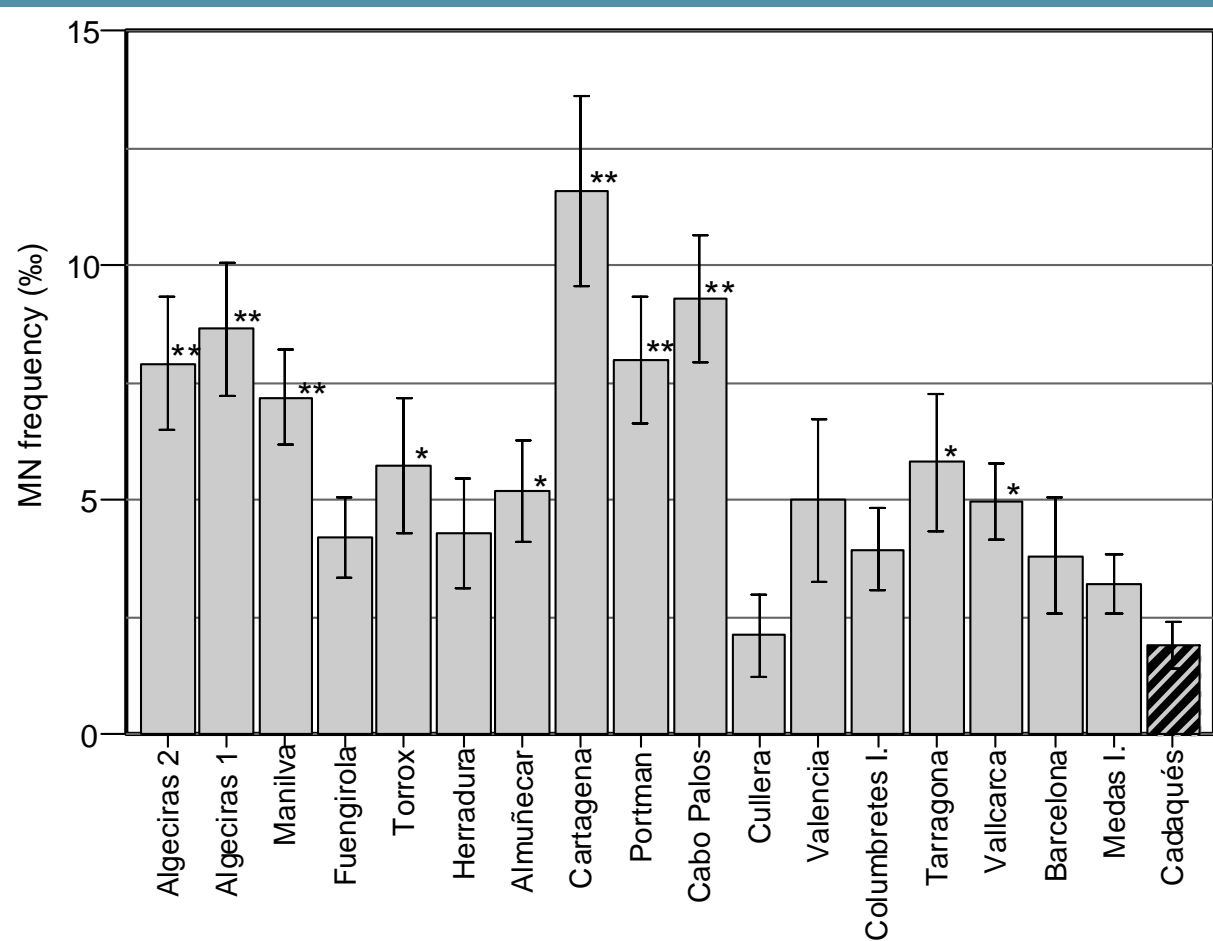
Lysosomal membrane stability in mussels



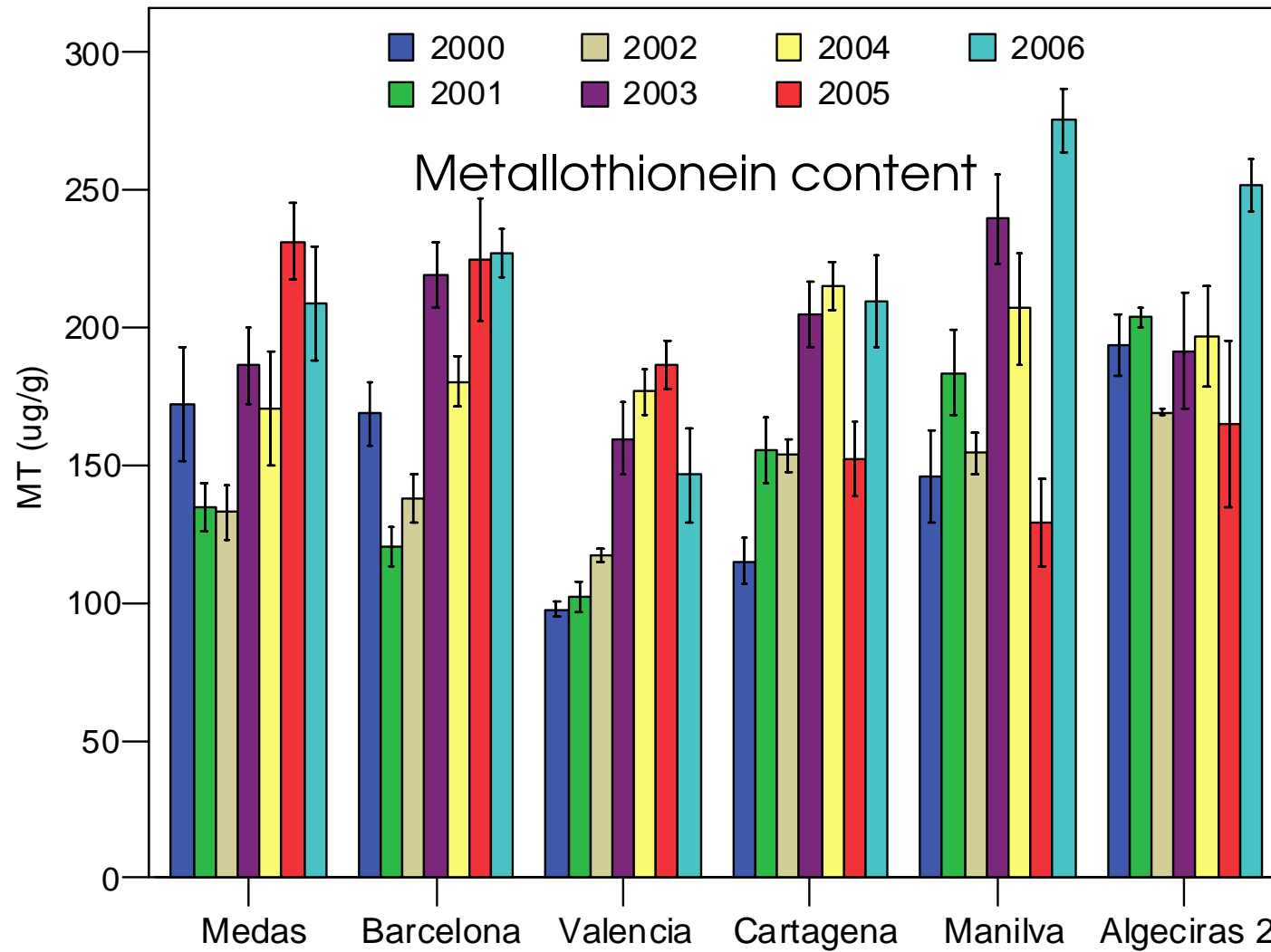
Micronuclei frequency in mussels, 2003



Fernández et al., 2007. *Rapp. Comm. Int Mer. Medit.*, 38



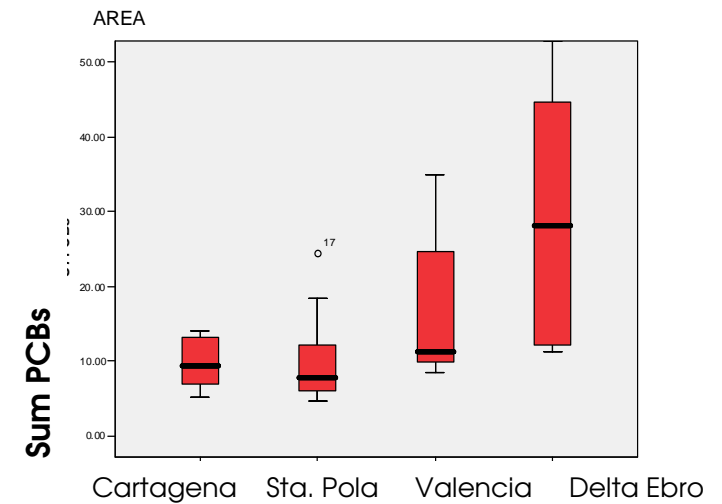
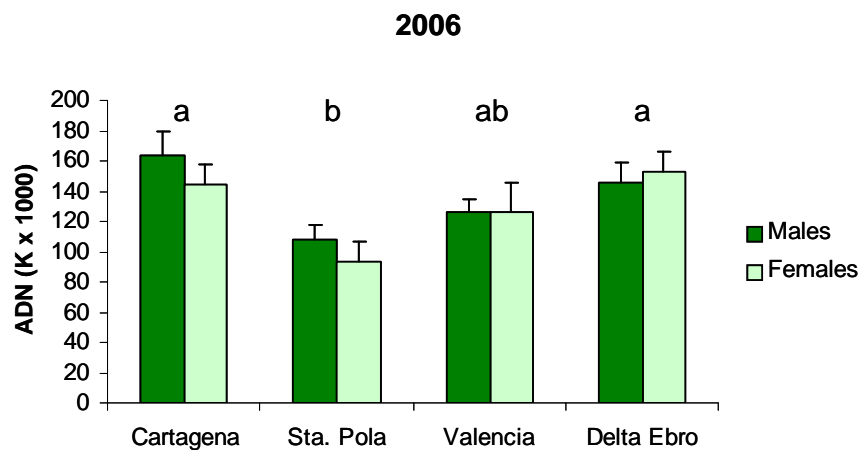
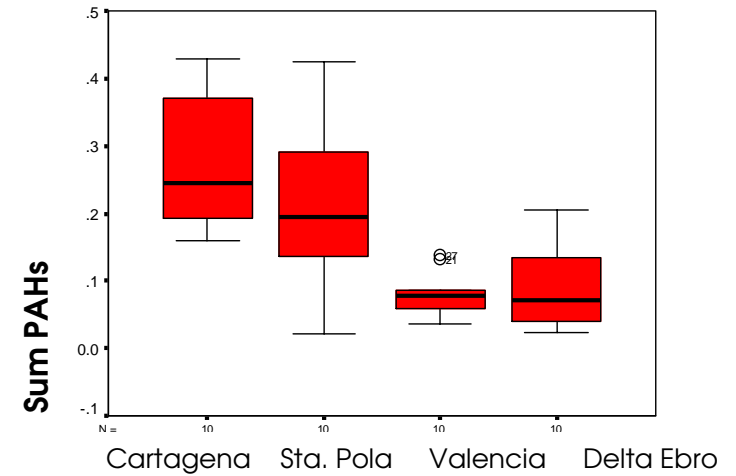
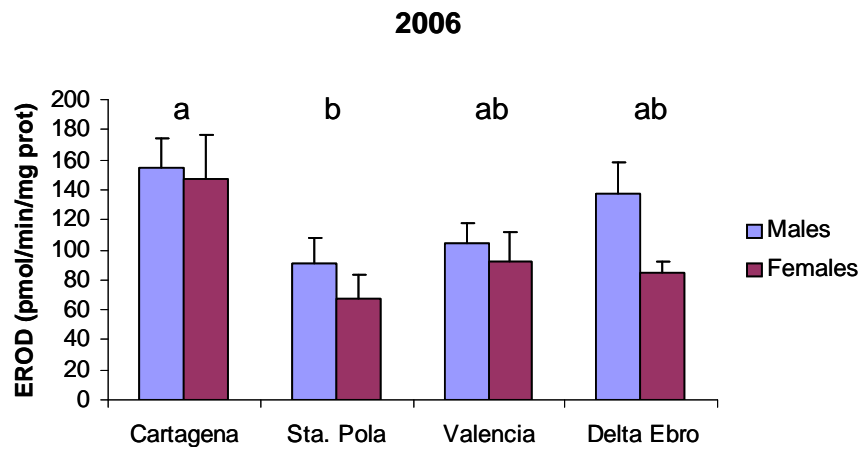
Metallothionein content in mussels



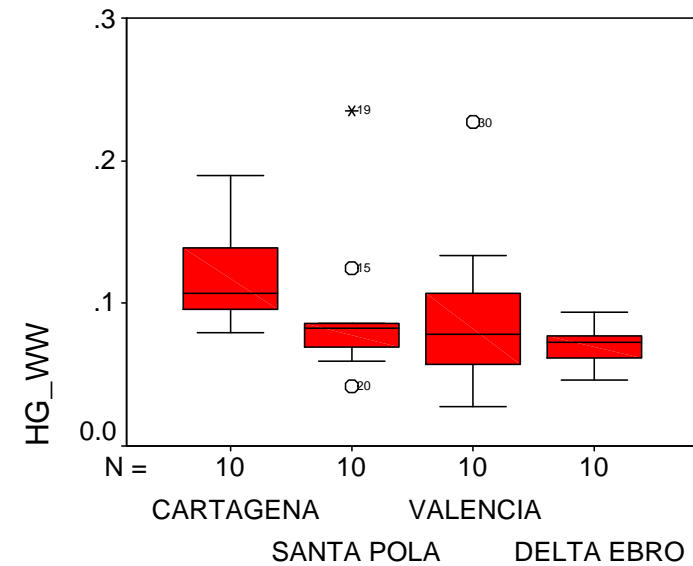
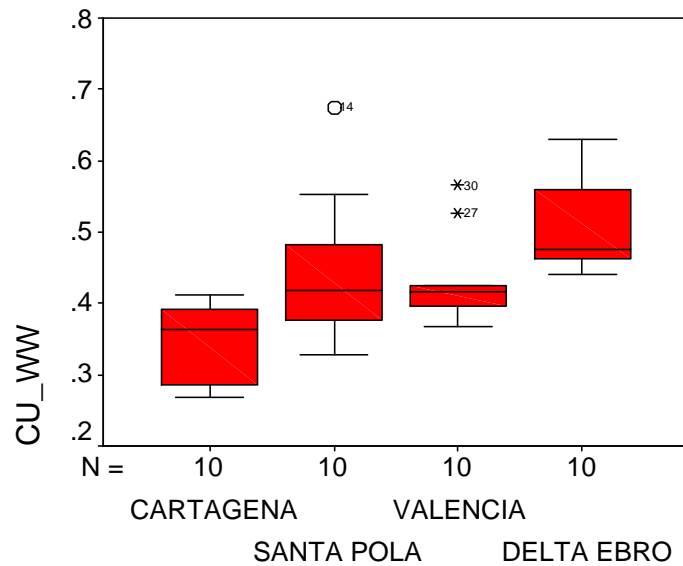
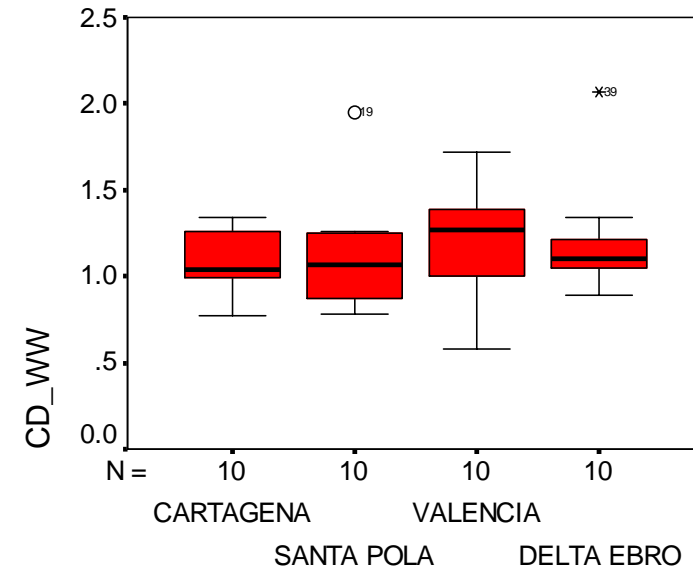
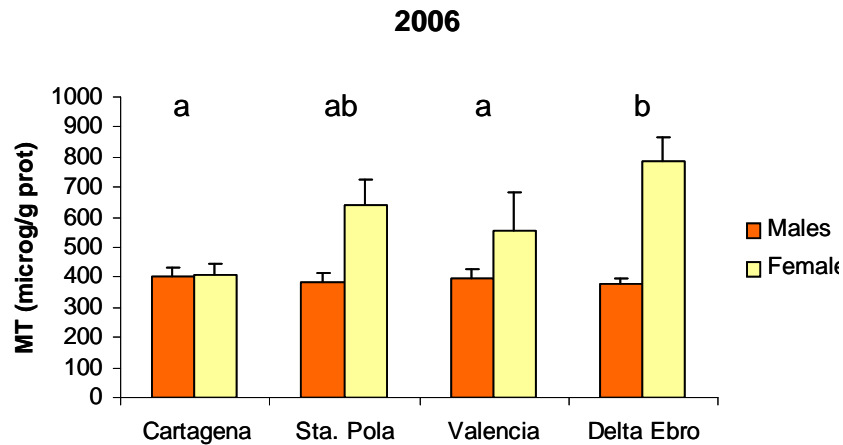
Error bars: +/- 1,00 SE

Fernández et al., 2003. CICTA.

EROD activity , Genotoxic damages, PAHs and PCBs concentrations in red mullet, 2006



Metallothionein content, Hg, Cu and Cd concentrations in red mullet, 2006.





FUTURE DEVELOPMENTS AND CONSIDERATIONS

- Scarcity of natural mussel stocks.
- Attachment of wild mussels to artificial beds
- Oligotrophic water conditions

During last Workshop on the MED POL Biological Effects programme, the use of caged specimens was highly recommended (Alexandria, 2006).



Use of caged mussels: Considerations....

- Sampling desing “*a la carté*”



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- Longer exposure period lasting several months to cover bioaccumulation and subtle chronic effects: to identify changes over time
- **Higher cost strategy** for wide area biomonitoring programmes: Two field sampling and recovery is not guaranteed

Future development using mussels



- To continue using wild mussels
- To implement the use of caged mussels
 - to solve the problem of scarcity
 - to assess the water quality in pelagic ecosystems



Immersion period ¿?

Cost effective and cost-efficient way



Efforts on biomarkers demonstrating damage at the tissue level



Future development using fish and sediments

- **To continue sampling in different fishing grounds:** Cartagena and Delta del Ebro suitable areas for temporal trend study
- **To initiate the use of bioassays using whole sediments**



Efforts on biomarkers demonstrating damage at the tissue level



ECOLOGICAL AND CHEMICAL STATUS

WFD

Monitoring
AACC

(Biological effects methods not prescribed)



ECOLOGICAL AND CHEMICAL STATUS

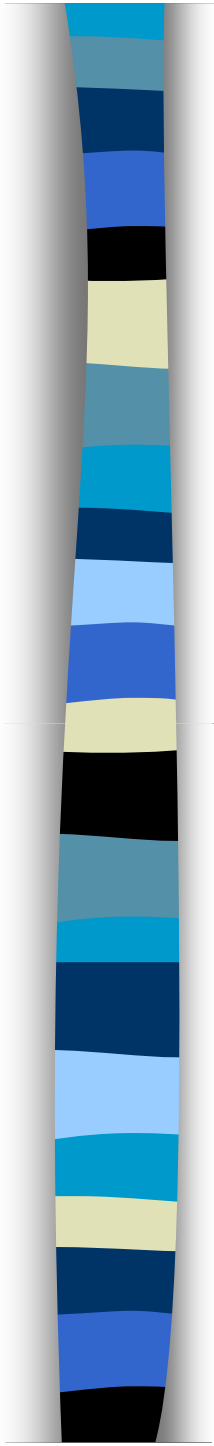
WFD

Monitoring
AACC

Biom. Prog.
MEDPOL

Biom. Prog.
CEMP

(Biological effects methods prescribed)





ECOLOGICAL AND CHEMICAL STATUS

WFD

EU Marine Strategy

(Assessment of Biological effects)

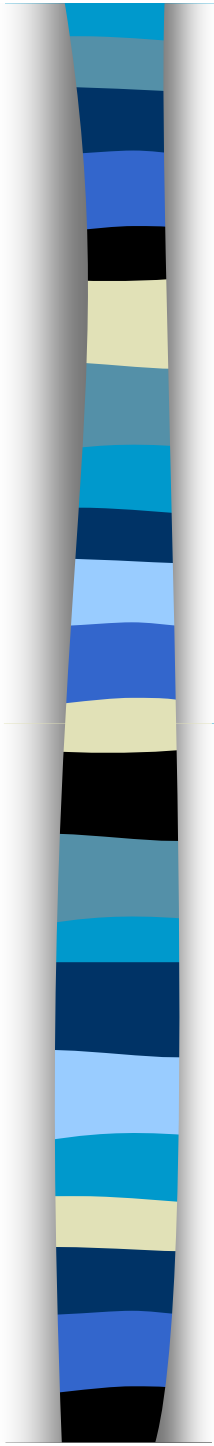
Reducing costs
More realistic picture

Biom. Prog.
MEDPOL

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ECOLOGICAL AND CHEMICAL STATUS

WFD

EU Marine Strategy

(Assessment of Biological effects)

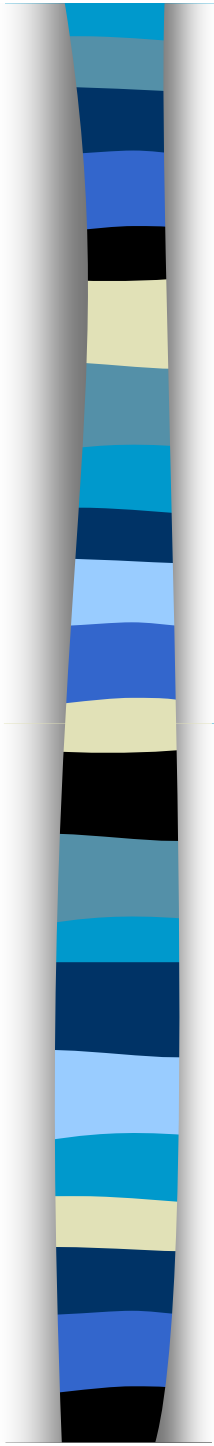
Reducing costs
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Biom. Prog.
MEDPOL

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Need for an integrated assessment



Tools for data integration:

- **Expert system** (Viarengo et al., 2000)
- **Fullmonti** (ICES, 2006)
- **Waterproff index** (Van der Oost, 2005)

Higher harmonization of criteria among different regional programmes would be desirable

ICON Workshop: Link with MEDPOL activities



Demonstrate the usefulness of the OSPAR integrated monitoring approach and guidelines

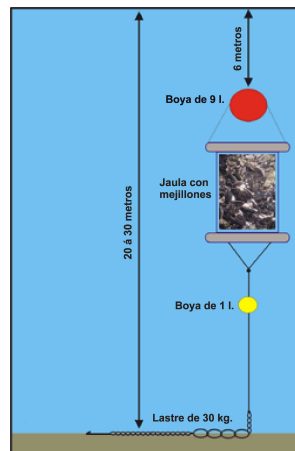


IEO (MCBE) will participate

PILOT STUDY USING CAGED MUSSELS



To conduct an initial integrated chemical-biological effect assessment



- Comparison between the MEDPOL integrated approach with that conducted by OSPAR
- Participating in harmonization exercises



Thank you!



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