

# BEEP project

## “**B**iological **E**ffects of **E**nvironmental **P**ollution in Marine Coastal Ecosystems”

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# BEEP

- **1 Feb. 2001 – 31 Jan 2004**
- **30 Participating Institutes**
- **From 12 countries**
- **>70 different biomarkers measured**



# BEEP

- **Core biomarkers**
  - NRR, ACH, MT, EROD & FAC's
- **Immune function**
- **Oxidative stress**
- **Genotoxicology**
- **Detoxification**
- **Genomics & Proteomics**
- **Histology**
  - Tissue and cell alterations
  - Immunohistology
- **Chemical**
  - Body burden
  - Metabolites



# QA and Intercalibration

- Intercalibration exercises were undertaken for all core biomarkers
- Several intercalibration exercises were undertaken between labs for non core biomarkers
- SOPs were produced for all core biomarkers and for some new biomarkers



# *The specific objectives of the BEEP*

- **Develop new biological markers** ranging over different levels of biological organizations
- **Validate** the use of selected biomarkers in specific sites for both routine assessment of chemical contamination and for the improvement of national and international monitoring programmes
- Validate a methodology for the biomarker exploration in **ecological risk assessment**
- Prepare information and **advices for user group**, policy-makers and fishery institutions about biological effects of chemical contamination on coastal marine resources
- Establish a **network of biomarker researchers** throughout Europe





**OBJECTIVES**

**TASKS**

**DELIVERABLES**

**ACHIEVEMENTS**

**WP0**  
Management / Coordination / Communication

**WP1**  
Novel Biomarkers  
Research/Development

**WP2, WP3, WP4**  
Regional sites WP  
Developping in situ  
Biomarkers

**WP2, WP3, WP4**  
Regional sites WP  
Common Biomarkers in  
Coastal Biomonitoring

- Dose/response effects
- Pollutant(s) specificity
- Validation in the lab/field

- Validation in the field
- Comparison between biomarkers

- Validation in the field of selected biomarkers
- QA/QC and Intercalibration exercises
- Multibiomarker approach
- Definition of GBI

- Recommendations/Guidelines for Biomarkers of reproduction
- Test kits and probes
- New Biomarker methods

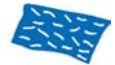
- Guidelines on validated methods

**WP5**  
Data Management Qa/QC

- Intercalibration – Database
- Multivariate analysis
- Multimarker based guidelines
- Global Biomarker Index for pollution assessment

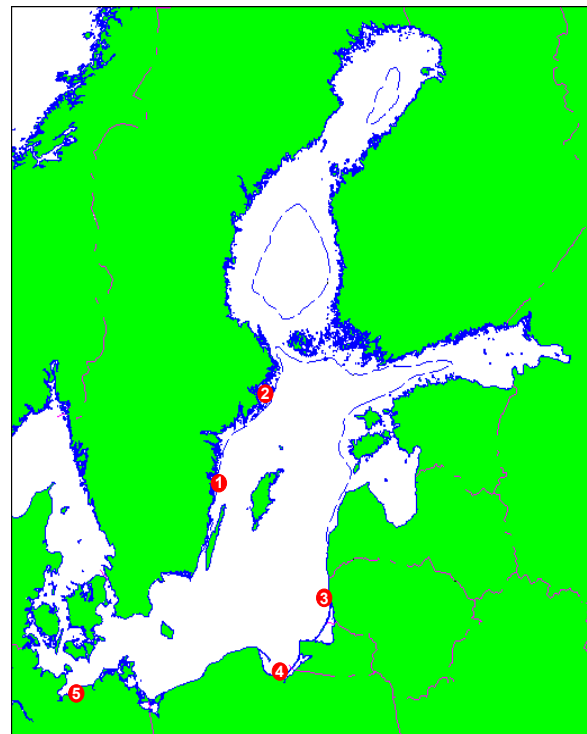
**WP0**  
Management/Coordination

- Biomonitoring Programmes
- Ecological Risk Evaluation





WP4 North Atlantic Sea



WP2 Baltic Sea

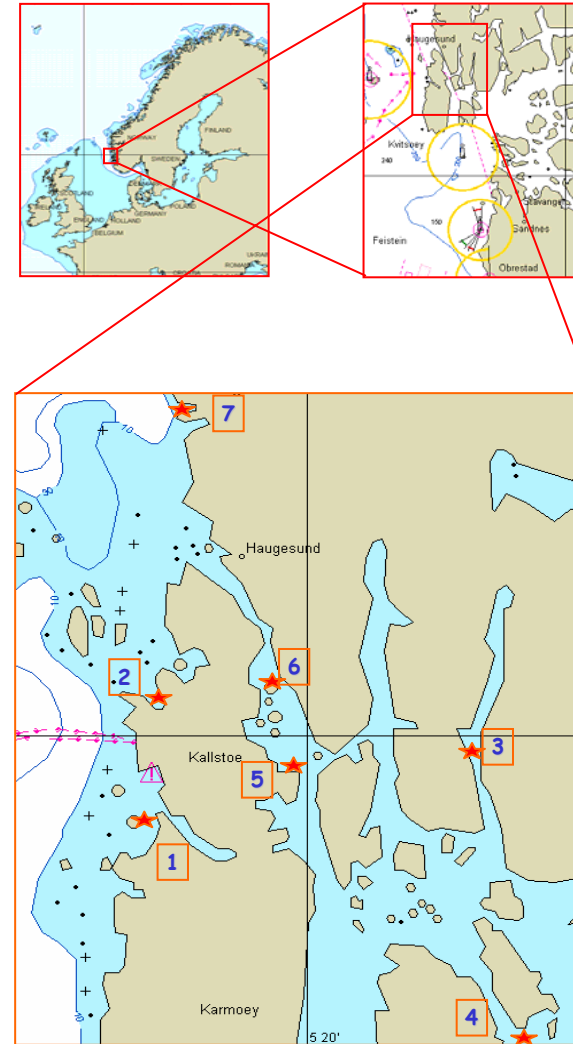


WP3 Mediterranean Sea



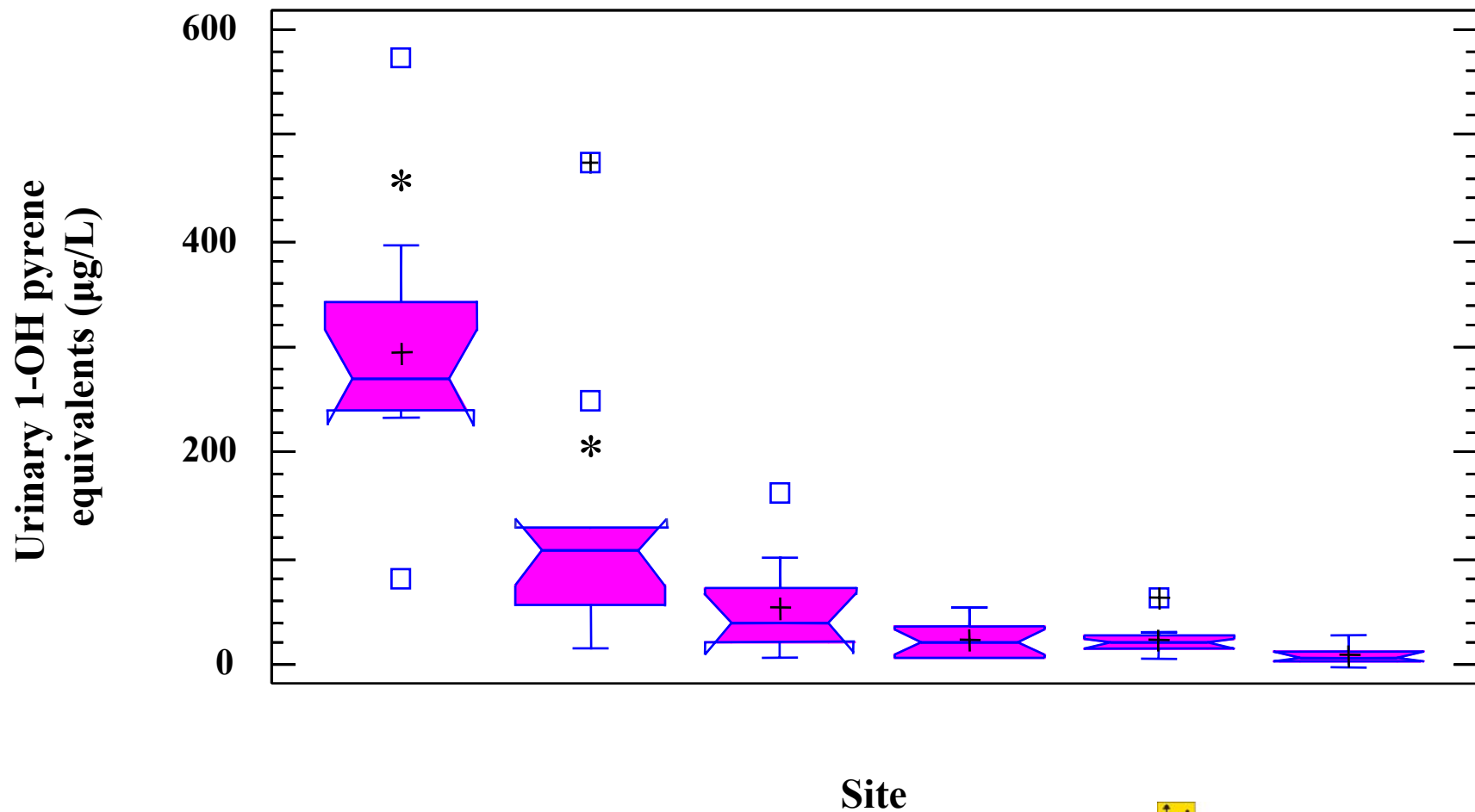
# WP4 - Field samples

- **3 sites**
  - Førlandsfjorden - Reference
  - Høgevarde – PAH
  - Visnes – Metals (Cu+ Zn)
- **Blue mussels (*Mytilus edulis*)**
  - Haemolymph
  - Gills
  - Digestive gland
- **60 individuals per site**
  - 30 males and 30 females





# PAH pollution gradient



# WP1 Novel biomarkers - Laboratory exposures

- **Continuous flow, steady state exposure system**
- **Sample of cod, turbot, shore crab, and mussels**
  - Serum
  - Gills
  - Liver/hepatopancreas
- **30 males and 30 females per group**



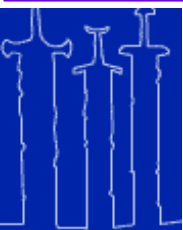
# WP1 - Novel biomarkers exposures

- **Produced water type exposure**
  - Control
  - 0,5 ppm oil
  - 0,5 ppm oil + 0,1 ppm alkylated phenols
  - 30 ppb nonylphenol
- **Exposure to potential endocrine disrupters**
  - Control
  - Bisphenol A - 50 µg/l
  - DAP - 50 µg/l
  - PBDE 47 - 5 µg/l (0.23 µg/l)
- **Copper gradient exposure**

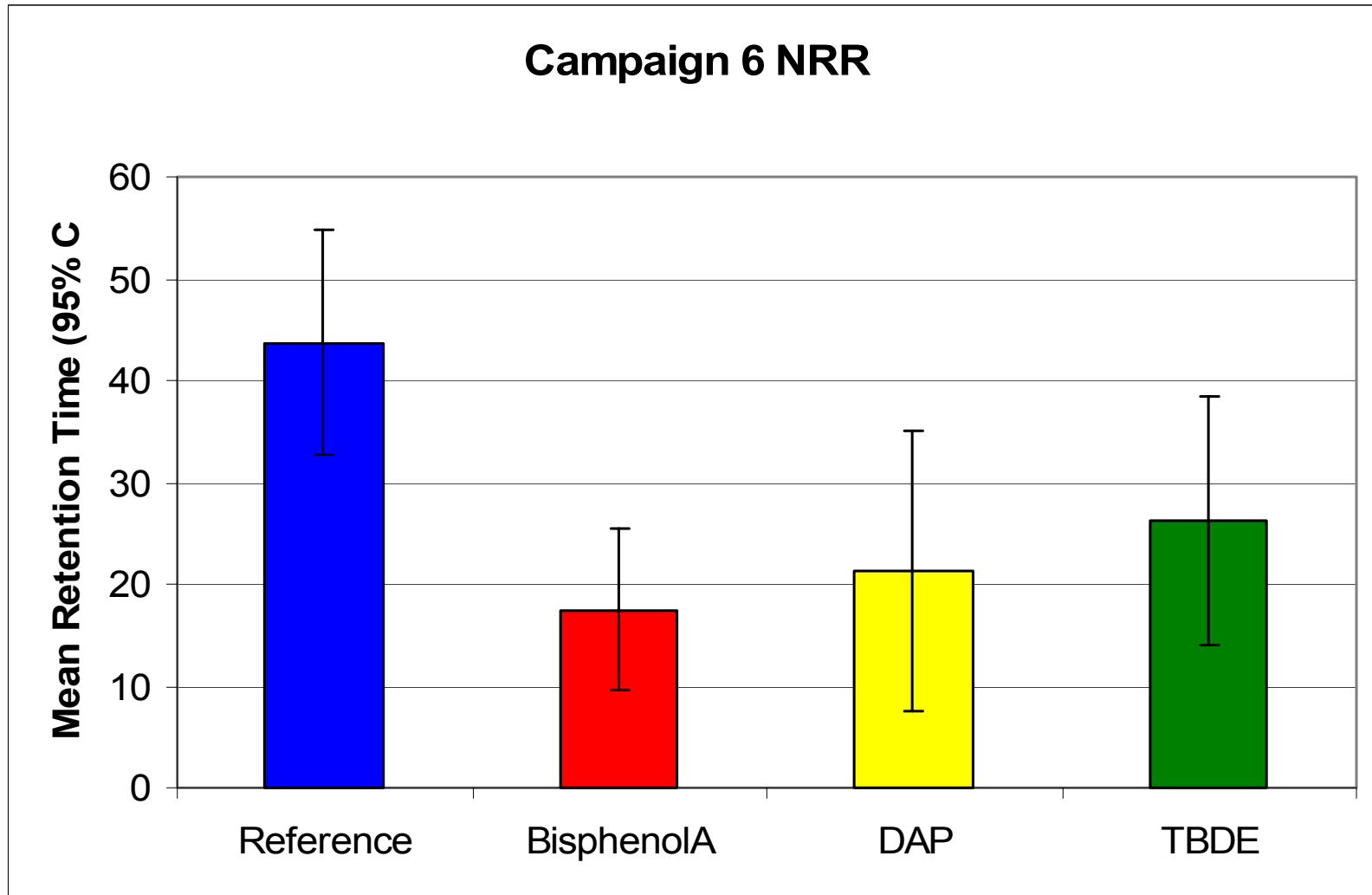


# Bioconsentration of PBDE

Biota	No. analysis	Tissue mg/g	BCF direct	% lipid	mg/mg tissue lipid normalized	BCF lipid
Cod (liver)	2	49,3 ± 2,7	216 200	63,1	81,8 mg/g	351 000
Turbot (liver)	2	15,1 ± 0,7	65 500	7,4	209,0 mg/g	897 000
Mussel (soft tissues)	1 pool of 2	2,9	12 430	1,3	225,1 mg/g	966 000



# Lysosomal membrane stability

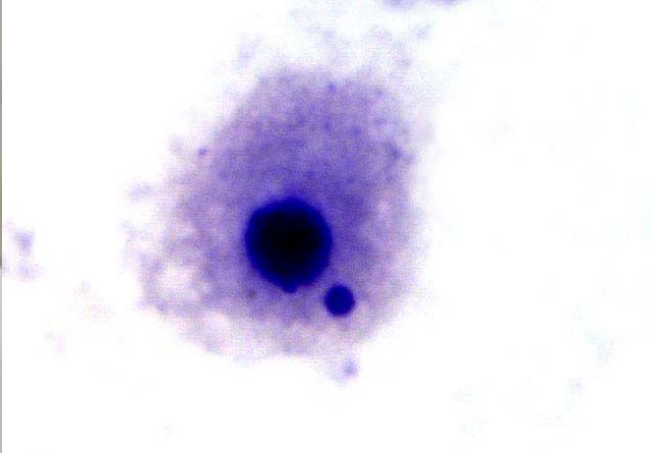


David Lowe, PML

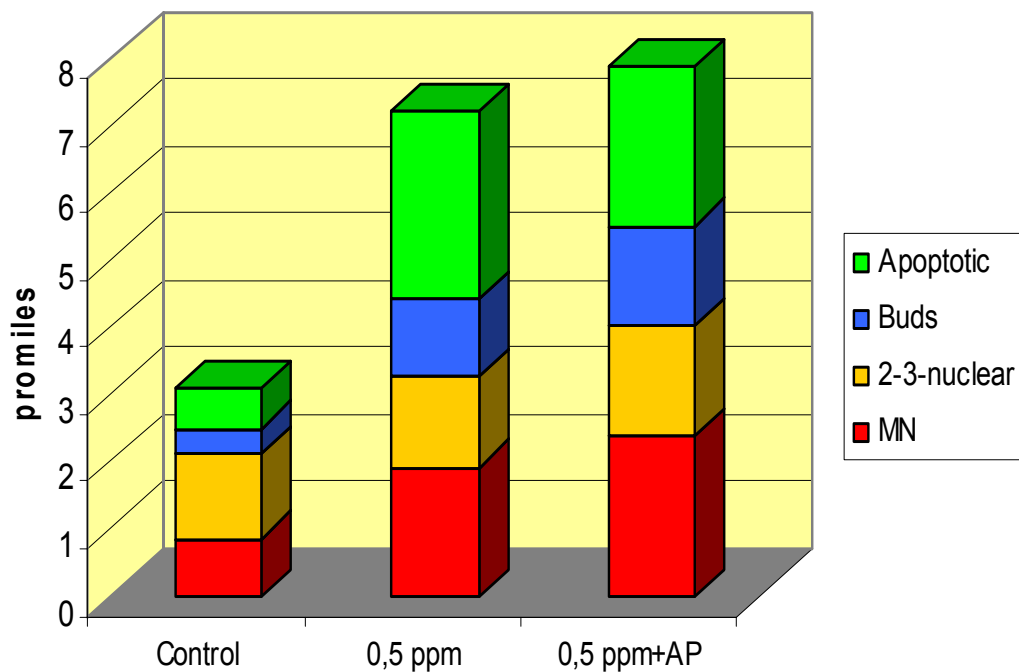


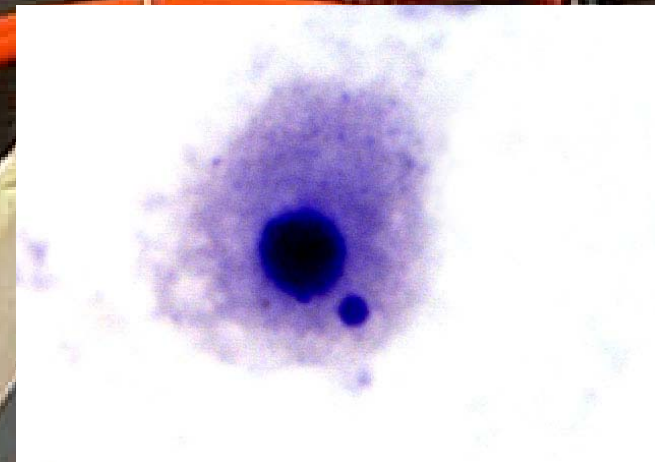
RF - Akvamiljø



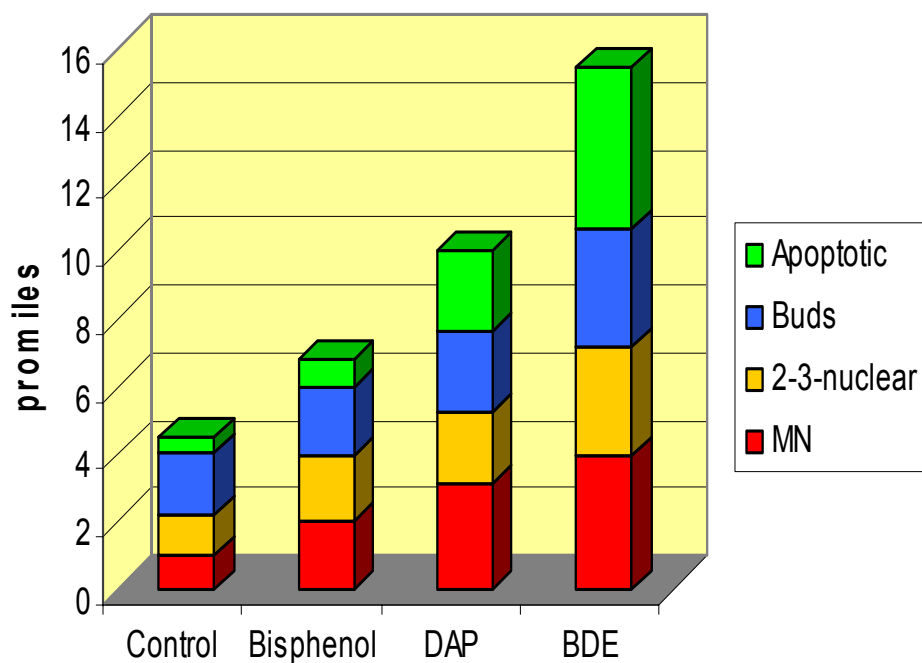


**Fig. 1 Frequency of MN, 2-3-nuclear and apoptotic cells and nuclear buds in mussels exposed to crude oil**





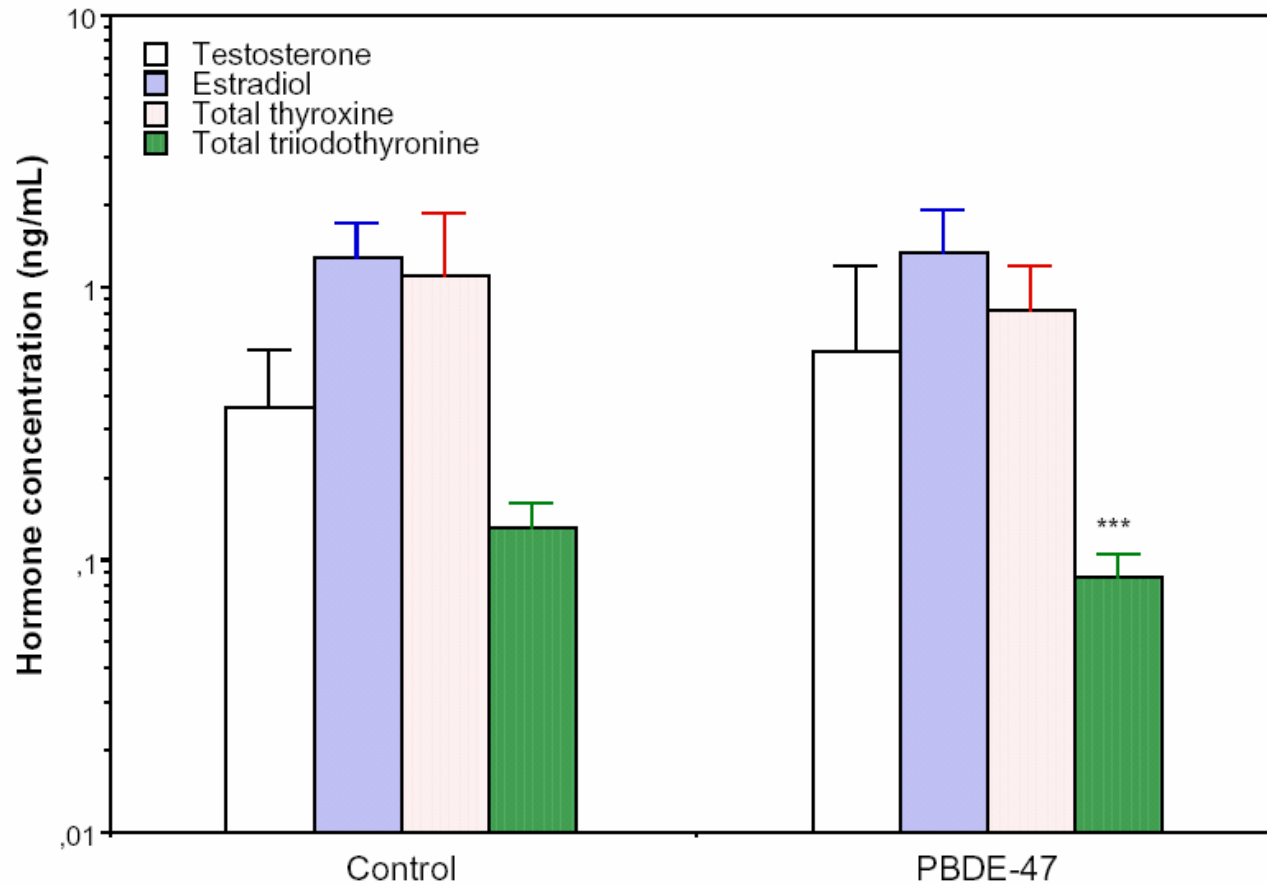
**Fig. 2** Frequency of MN, 2-3-nuclear and apoptotic cells and nuclear buds in mussels exposed to organic compounds



**J. Baršienė**



# Hormone effects



\*\*\* indicates a significant difference ( $P < 0.001$ ) between the control and the exposed group.

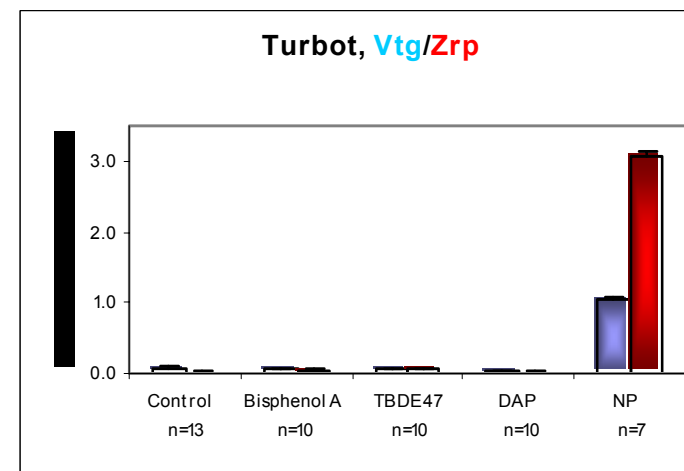
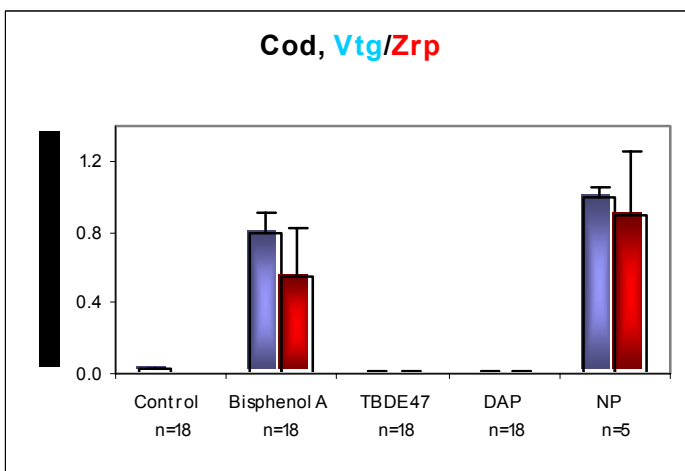
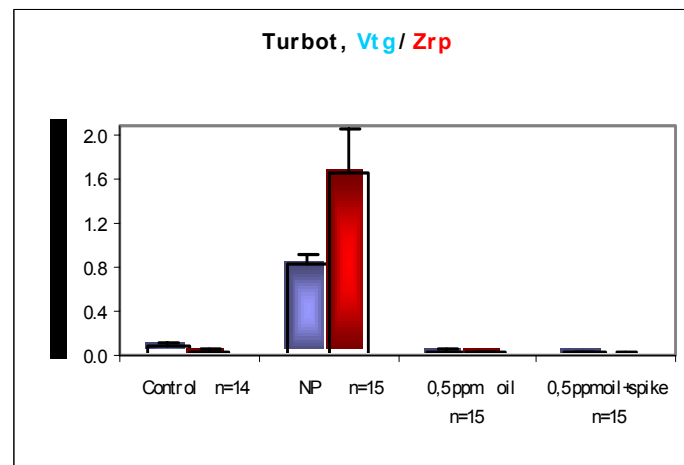
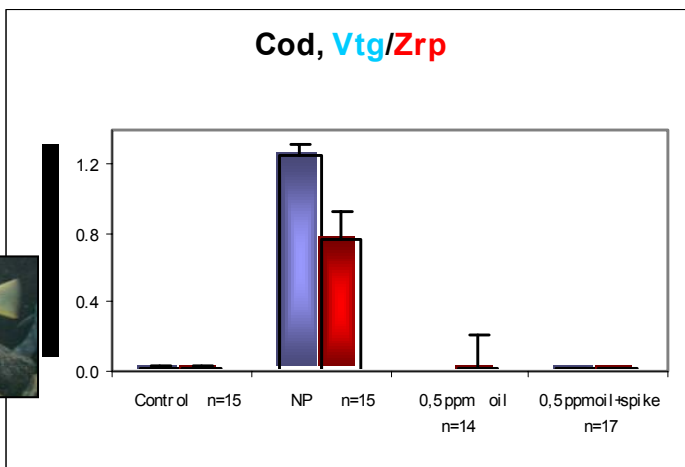
Figure 1. Concentrations (mean  $\pm$  standard deviation) of testosterone, estradiol, total thyroxine and total triiodothyronine in juvenile turbot (*Scophthalmus maximus*) exposed to a water concentration of 5 ppm PBDE-47 for 3 weeks.





# Stavanger workshop 1 & 3

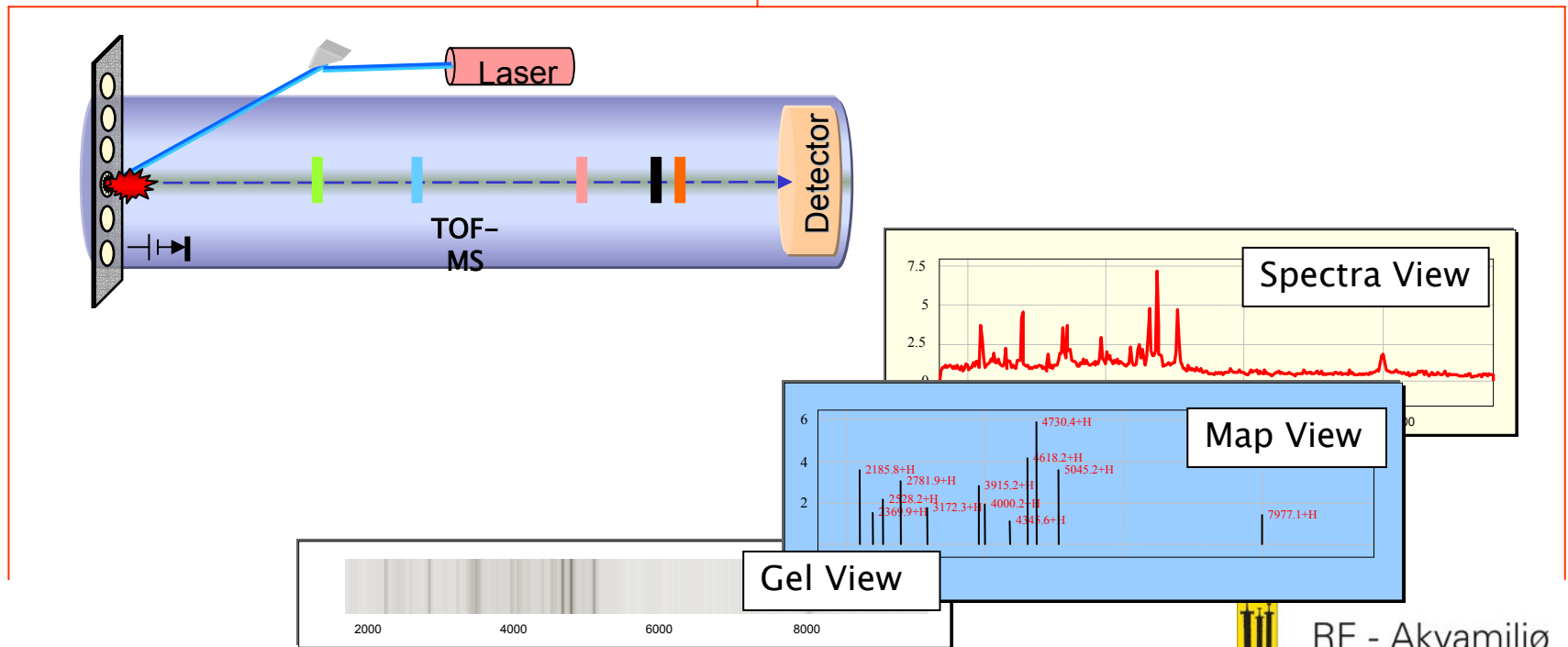
## Plasma vtg/zrp in juvenile cod and turbot



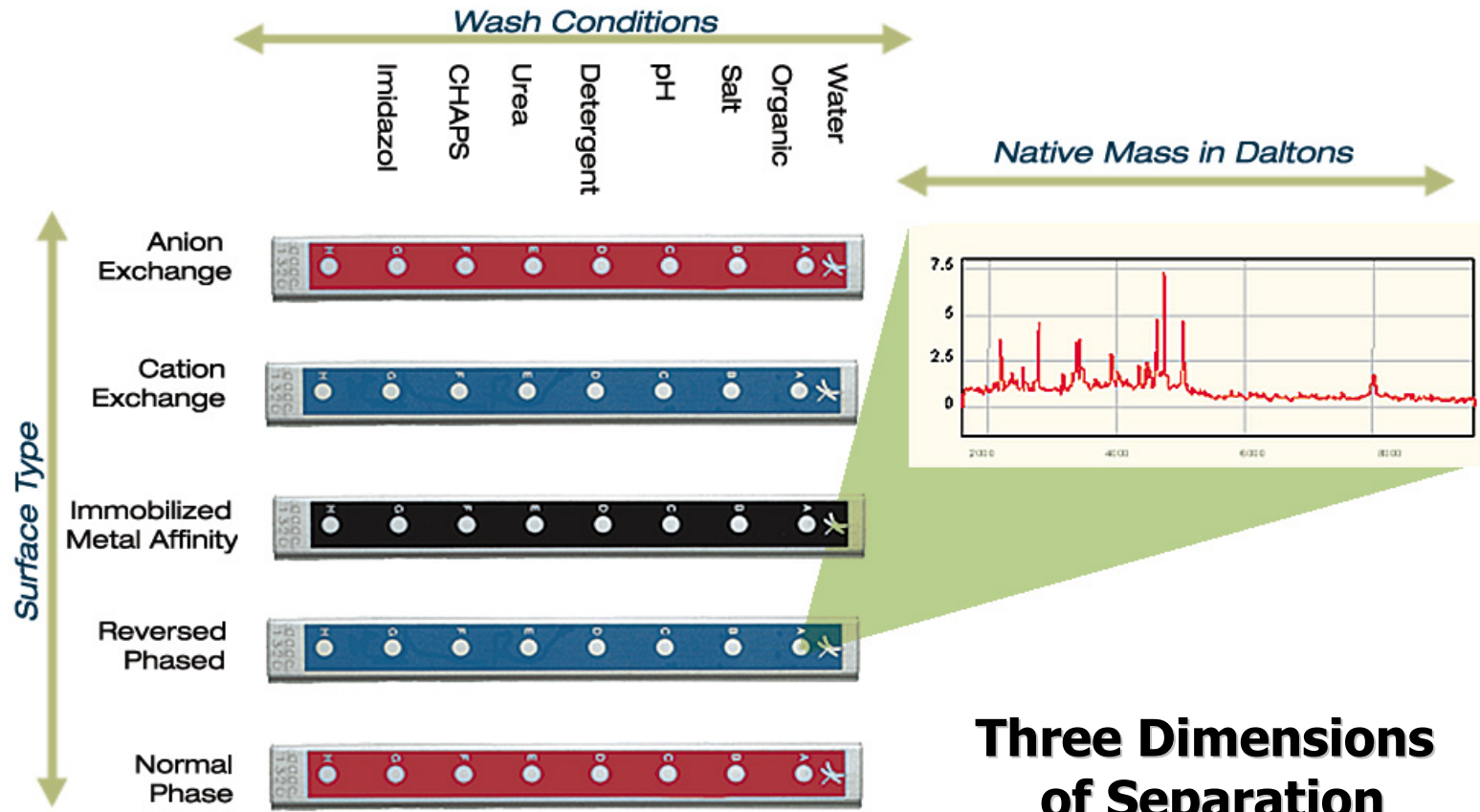
# SELDI - Surface Enhanced Laser Desorption and Ionization



TOF-Mass Spectrometer



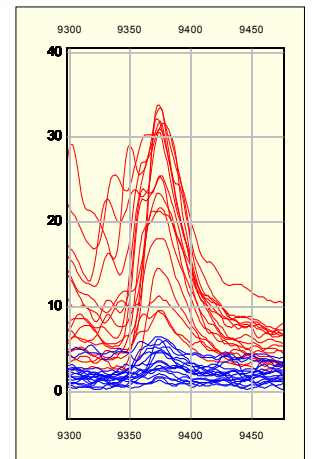
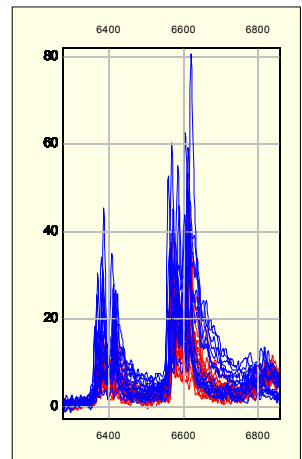
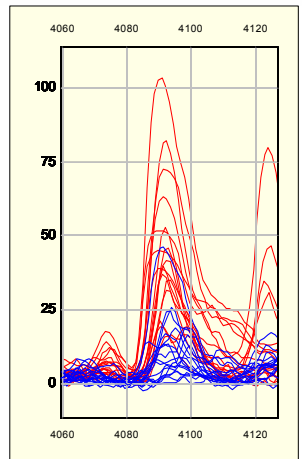
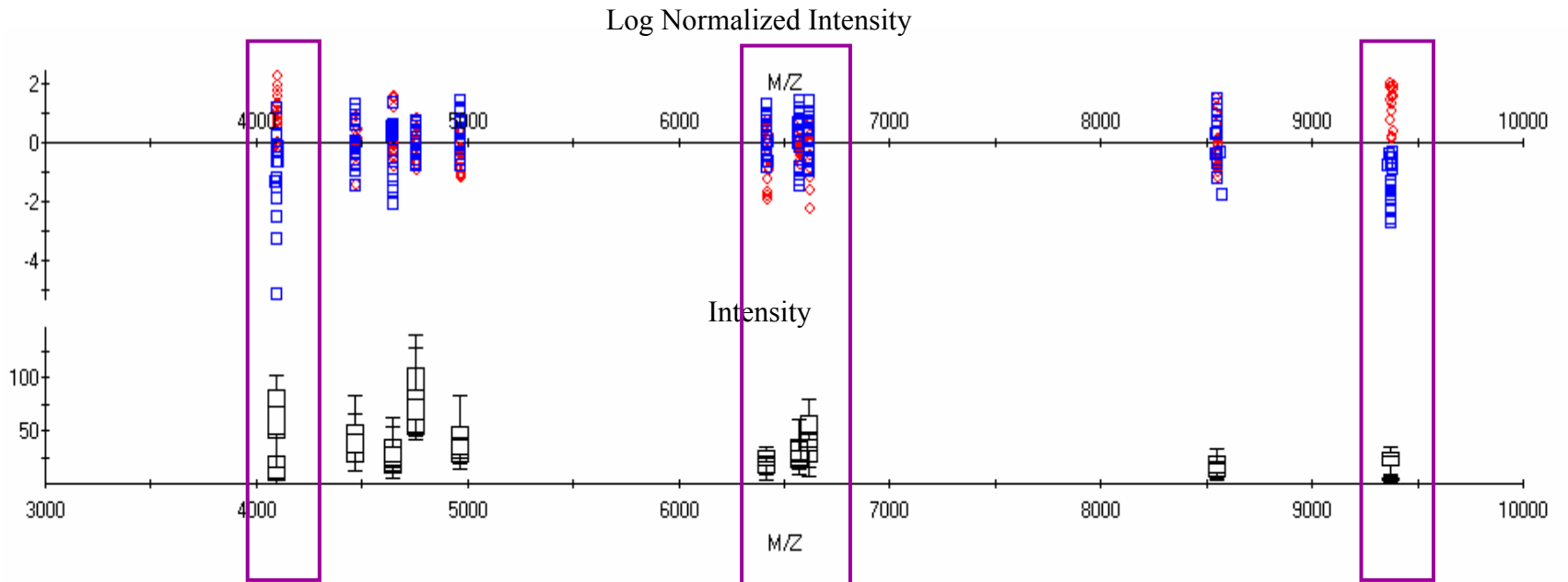
# Protein Separation Procedure



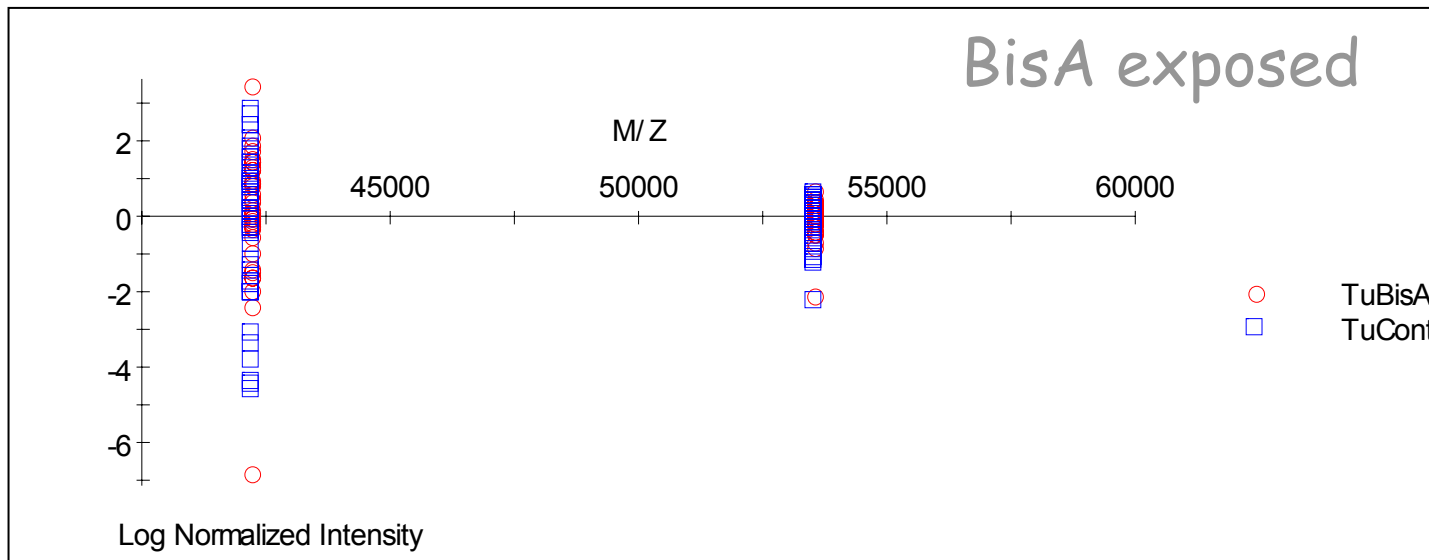
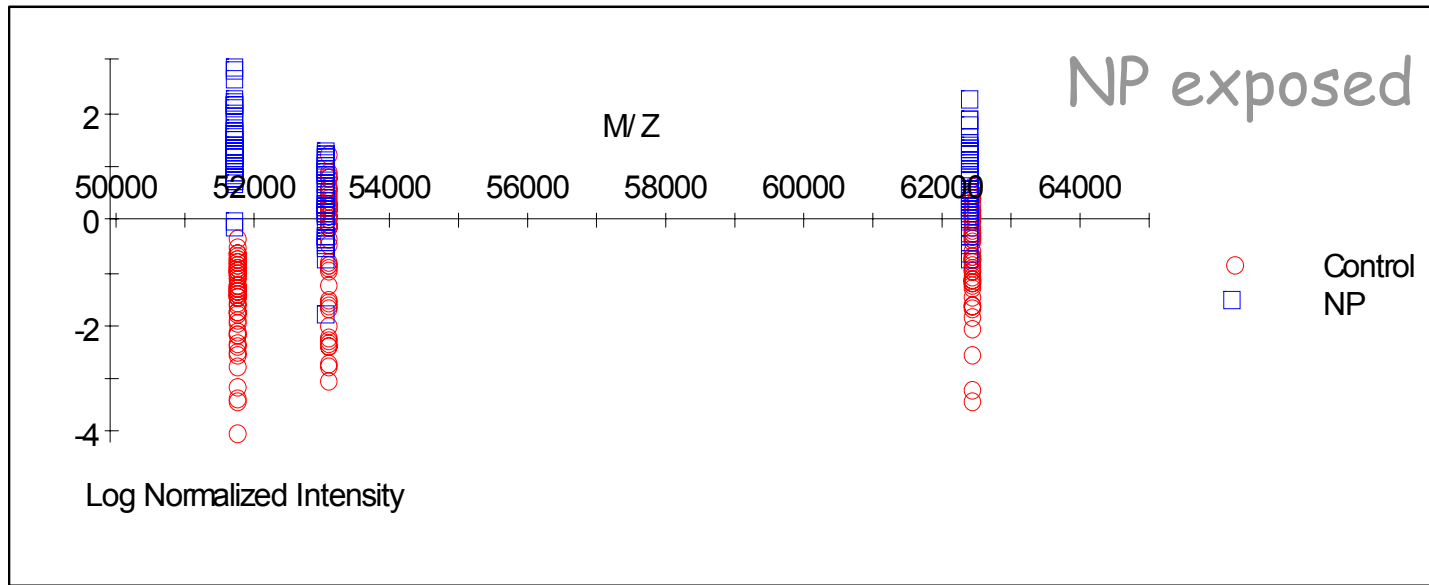
**Three Dimensions  
of Separation**



# Biomarker wizard

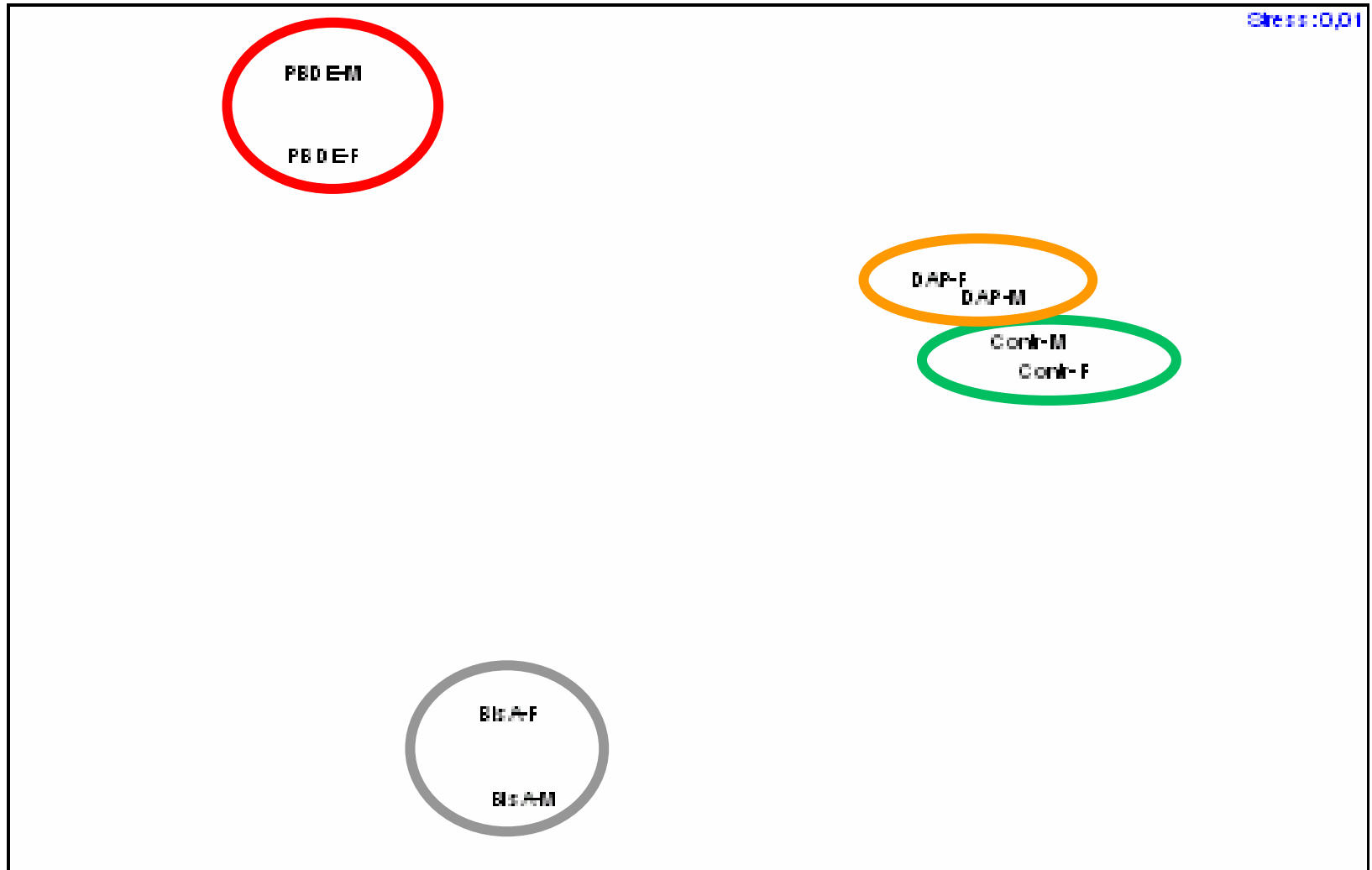


# Zrp in turbot



# MDS plot of proteom response in plasma of blue mussels

Plasma exp study



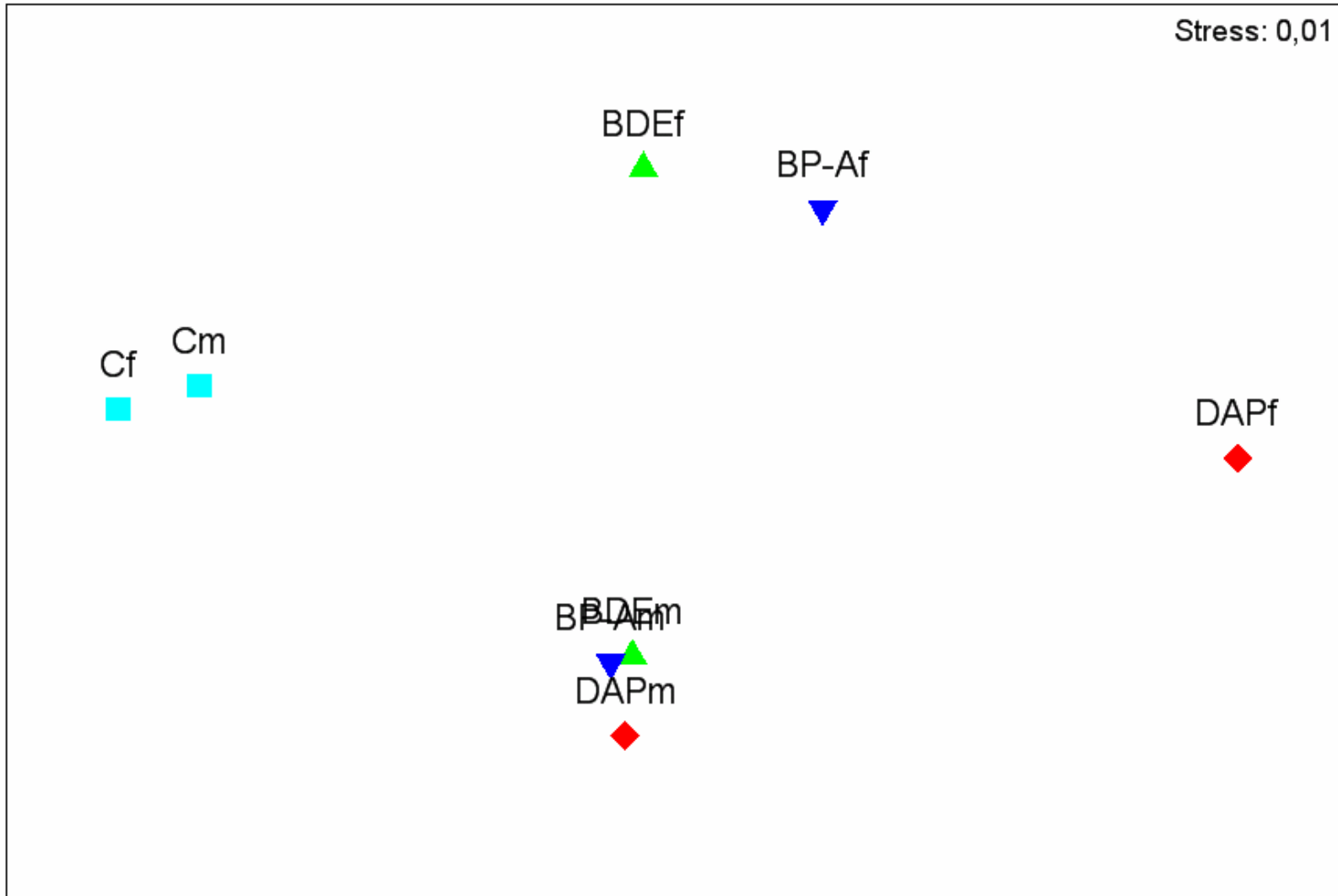
# MDS plot of proteom response in spider crabs (*Hyas araneus*)

Resemblance: D1 Euclidean distance

Stress: 0,01

Exposure

- ▲ BDE
- ▼ BP-A
- C
- ◆ DAP



# Response types

- Species specific expression patterns
- Sex determination
  - Mature and juvenile organisms
- Sex independent response
  - Toxicological response
- Sex dependent response
  - Indication of endocrine disruption?





# Acknowledgements

**All participants in BEEP**

**RF-Akvamiljø Staff**

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