

## Theme Session I

### Effects of hazardous substances on ecosystem health in coastal and brackish-water ecosystems: present research, monitoring strategies and future requirements

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#### ICES CM 2007/I:01

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##### **Can biomarkers measure environmental health of estuaries?**

Josephine A. Hagger, Malcolm B. Jones, Tamara S. Galloway, Richard Owen, David Lowe, and D. R. Paul Leonard

The European Commission's Water Framework Directive (WFD) has emphasised the need for biological-effects end points that can be used to classify the ecological health of aquatic ecosystems. Accepting the premise that a healthy ecosystem is reflected in the 'health' of the constituent biota, we have advocated the application of using a suite of biomarkers (a biological response that signals exposure to and/or adverse effects of potential chemical, physical or biological hazards) to measure environmental health through an integrated assessment of the health status of individual organisms (and thereby the ecosystem). As 95% of animal species are invertebrates (and include commercially-exploited species), we propose that it is reasonable to use them as surrogates of all coastal biota. We have developed a tool box of biomarkers (including molecular, cellular, physiological and behavioural endpoints) for a range of invertebrate species inhabiting different estuaries around the UK coastline. We have used the results in a pragmatic, weight-of-evidence, holistic approach to devise a Biomarker Response Index (BRI) (a relative set of criteria based upon a 'traffic-light system') to give a measure of the general health status of invertebrates. In this presentation, we report the underlying basis for the BSI approach and discuss the results from *Mytilus edulis* collected from different transitional water bodies (estuaries) along the southern coastline of the UK whose risks of failing the WFD has been classified with regards to point-source pollution. In eight of the ten transitional water bodies, mussels were healthier than predicted based on the risk classification for point source pollution from that particular estuary. Mussels from the other two water bodies showed a similar health status to that of the predicted risk classification. Present results indicate that the BSI offers a potential measure of organism health that can be used in monitoring under the WFD to reduce uncertainty in defining risk classification and to provide evidence of impact.

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#### ICES CM 2007/I:02

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##### **Modelling Driver-Pressure-State changes for TBT in coastal ecosystems**

Georgi Daskalov, Peter Kershaw, Eva Garnacho, Andrew Kenny, and John Thain

The impact of tributyltin (TBT) on marine organisms, in the form of endocrine disruption and induction of Imposex, has been well documented. TBT persists in estuarine and coastal waters of NW European shelf seas, despite a ban of the use of TBT-based antifouling coatings on vessels of < 25m. This is due to a combination of the legacy of past practises, dredging contaminated sediment and the continued use of TBT on larger vessels, with the delay in implementation of the total ban proposed by IMO. We have developed a Bayesian Belief Network model to examine the occurrence of TBT, within the DPSIR framework (Driver Pressure State Impact Response). We have included a variety of Drivers and Pressures and populated the model with data, using information based on proxies (e.g. number of ship arrivals) in cases where specific data sources are inadequate. For ecosystem State we included both concentrations of TBT in water, sediments and biota and the incidence of Imposex in the dogwhelk *Nucella lapillus*, using the VDSI index (Vas Deferens Sequence Index). The model was used to describe current state and to predict the state changes in TBT and Imposex under 4 future scenarios of economic growth and social organisation: World Markets, National Enterprise, Global Community and Local Responsibility. Finally, we discuss the potential of using the BBN approach to examine potential trends in emerging 'lifestyle' chemicals, to inform monitoring and assessment programmes.

Keywords: tributyltin, TBT endocrine disruption, imposex, antifouling, marine monitoring.

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**ICES CM 2007/I:03**

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**Are biomarkers useful in environmental management?**

M. J. Bebianno, V. Sousa, B. Lopes, L. Barreira, R. Company, and A. Serafim

The use of biomarkers (of exposure and/or effect to xenobiotic contamination) as surrogate measures of biological impact of contaminants within the environment has been studied in several European coastal areas. However, the incorporation of biomarkers into regulatory legislation for environmental risk assessment has rarely been used. Moreover, the approval of the Water Framework Directive by the European Union offers the potential for the incorporation of biomarkers as an integrated approach in environmental management. With this aim several biomarkers of exposure and/or effect were measured in several tissues of the mussel *Mytilus galloprovincialis* collected from several hot spots along the South Coast of Portugal. The biomarkers used were: superoxide dismutase (SOD), catalase (CAT), glutathion peroxidases (total and selenium dependent) cytochrome P450 (CYP450), glutathione-S-transferases (GST), acetylcholinesterase (AChE), metallothioneins (MT),  $\delta$ -aminolevulinic acid dehydratase (ALA-D), along with the condition index to assess the mussels health status. The data of this battery of biomarkers was used in an integrated manner to classify the health status of these invertebrates and consequently of this coastal ecosystem. A biomarker index was calculated using several approaches. The use of this index enabled to highlight that despite metals and organic contaminants present in their tissues, mussels from six of the eight sites, were in good health while those from the two sites were seriously affected by environmental contamination. Therefore, the use of this index provides a useful tool of assessment, since it integrates a suite of biomarkers of exposure and/or effect, facilitating the definition of risk sorting and ultimately offering an easier way for "decision makers" to assess the quality of the aquatic environment.

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**ICES CM2007/I:04**

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**Toxic chemicals and their impacts in the St. Lawrence Estuary and Saguenay Fjord, Quebec, Canada: from a chemical- to an ecosystem-based risk management**

C. M. Couillard and M. Lebeuf

The St. Lawrence Estuary (SLE) and Saguenay Fjord (SF), Quebec, Canada, have received world-wide attention in the early 1980s when high concentrations of contaminants and high prevalence of lesions including neoplasia, hermaphroditism and infection by opportunistic agents were reported in beluga whales (*Delphinapterus leucas*). Both persistent organic pollutants (POPs) such as PCBs mainly originating from the upstream industrialized sectors of the Great Lakes and the upper St. Lawrence River and local contamination by polycyclic aromatic hydrocarbons (PAHs) and mercury have been incriminated. The release of these chemicals has been successfully reduced through environmental regulations and restoration of contaminated areas. Since 1970s, declines in mercury, PAHs and PCBs have been observed in sediments and in biota. However, organisms remain exposed to complex mixtures of contaminants including regulated persistent compounds remaining in the ecosystem and newer compounds which also have the potential of causing deleterious effects. Interactions between toxic chemicals and other environmental stressors may increase the risk of deleterious impacts. New concerns include: chronic inputs of tributyltin from ship transportation associated with reproductive/immune disturbances, interaction between nutritional condition and POPs, increasing concentrations of brominated flame retardants in beluga tissues and inputs of agricultural chemicals and nutrients from SLE tributaries. Several fish populations historically used as preys by the beluga are declining as a consequence of multiple anthropogenic factors. Moreover hypoxic area in the bottom of the SLE is increasing and could act as an additional stressor. An ecosystem-based approach is being developed to pursue the protection the SLE/SF ecosystems facing multiple stressors and variable environmental conditions.

Keywords: St. Lawrence Estuary Saguenay Fjord toxic chemicals beluga whale.

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**ICES CM 2007/I:05**

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**Environmental genotoxicity studies in marine fish and mussels**

Janina Baršienė, Aleksandras Rybakovas, and Laura Andreikėnaitė

The micronucleus (MN) test has been widely used *in vivo* assay and was proved as simple to perform, sensitive enough and fast test to detect genomic alterations due to clastogenic effects and impairments of mitotic spindle caused by aneuploidogenic poisons. Main objective of the present study is to identify

regularities of genotoxicity in marine indigenous organisms *in situ*, under experimental caging, deployment and laboratory conditions. Peculiarities of MN formation were investigated in various cells of fish and mussel species inhabiting geographically and ecologically different zones of the Baltic and North Seas. Active monitoring approach (fish and mussel caging) applied to assess MN induction in certain polluted areas of the North Sea. MN test validation was performed in multiple controlled exposures at RF Akvamiljo (Norway) marine experimental centre. The wide-range MN investigations indicated specific responses in relation to species, tissue, environmental temperature, contaminant type and concentration, duration of exposure, distance from contamination source. Furthermore micronuclei formation in the blue mussels was approximately 10-fold higher than in studied fish species (cod, flounder, turbot, perch, eelpout, wrasse and others). Results of environmental genotoxicity will be discussed regarding target species, organs, cells, sex, mitotic activity as well as exposure and sampling procedures.

Keywords: micronucleus test, genotoxicity, Baltic, North Sea, fish, mussels.

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## ICES CM 2007/I:06

### Peculiarities of environmental genotoxicity in offshore zones of the Baltic and North Seas

Aleksandras Rybakovas, Janina Baršienė, and Thomas Lang

Micronucleus test in different fish species has been considered a sensitive tool for the evaluation of genotoxic effects in coastal zones of the Baltic and North Seas. In the present study, micronuclei (MN) and other nuclear abnormalities in erythrocytes from peripheral blood and cephalic kidney were analyzed in flounder (*Platichthys flesus*) and cod (*Gadus morhua*) from 9 offshore locations in the Baltic Sea and in cod and dab (*Limanda limanda*) inhabited 9 zones in the North Sea. The outputs of the current study revealed that micronuclei frequency 0.05-0.1 MN/1000 cells could be defined as reference level in fish from the offshore zones in the Baltic Sea and 0.1 MN/1000 cells – in the North Sea. The highest response (0.34 MN/1000 cells) was found in dab females from areas potentially impacted by contamination from the River Elbe (Germany). Comparatively high levels of micronuclei (0.31 and 0.28 MN/1000 cells) and fragmented-apoptotic (FA) cells (0.34 and 0.5 FA/1000 cells) were observed in dab from areas close to oil and gas platforms in the North Sea. Increased genotoxicity was identified in the Baltic flounders from the Arkona Basin (0.3 MN/1000 cells and 0.25 FA/1000 cells). In fish cephalic kidney, the response to genotoxic/mutagenic compounds was higher than in peripheral blood, in flatfish females was higher than in males. Flounder and dab are more sensitive to action of genotoxins compared to cod, thus, the flatfish species could be used in the further monitoring of environmental genotoxicity in offshore zones.

Keywords: genotoxicity, micronucleus test, offshore zones, Baltic, North Sea, flatfish.

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## ICES CM 2007/I:07

### Is p,p'-DDE still affecting Baltic Sea cormorants?

Thomas Kellner and Rolf Schneider

During a cormorant (*Phalacrocorax carbo sinensis*) population control campaign in May 2005, eggs were collected from a ground breeding colony on the small island Heuwiese (N 54°25', E 13°7') at the Baltic Sea shore of Western Pomerania, Germany. The hatching status of all eggs was scaled as hatched, probably hatched, probably not hatched and unhatched. Eggshell thickness was measured with an electronic precision micrometer, and indices of eggshell thickness were calculated. Levels of 10 PCB congeners and several organochlorine pesticides (HCB, HCHs, dieldrin and DDT and its metabolites DDE and DDD) were analysed to test if their concentrations are correlated with eggshell thickness parameters. Contents of organochlorines were measured in probably hatched and unhatched eggs (homogeneous content without eggshell) by high resolution capillary gas chromatography. Concentrations of several compounds (PCBs as well as pesticides) in cormorant eggs were often more than 1000fold higher than in commercial chicken eggs, and frequently exceeded safety limits for human consumption. Significant negative correlations were found between p,p'-DDE in eggs and eggshell thickness as well as its index. Highly DDE-contaminated eggs had significantly thinner eggshells and significantly lower indices of eggshell thickness than less contaminated eggs. Any impact of hatching status on eggshell thickness or on its index could be excluded, and no significant correlations were found between PCB contents and eggshell thickness parameters. It is concluded that present p,p'-DDE levels can still cause eggshell thinning in Baltic Sea cormorant, although DDT and PCBs were banned all around the Baltic Sea three decades ago.

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#### ICES CM 2007/I:08

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### Historical and recent contents of PCB and organochlorine pesticides in sediments from Baltic Sea basins

Rolf Schneider and Thomas Leipe

Lipophilic contaminants dissolved in the water column have a strong tendency to accumulate not only in organisms, but also in sediments. Undisturbed, datable sediment cores can therefore be used to reconstruct the pollution history in the Baltic Sea. Nine recent sediment cores from different basins (2 Arkona, 2 Bornholm, 1 Gdansk, 3 Gotland, 1 Landsort) were sliced (2cm layers), dated and analysed for 10 PCB congeners and several organochlorine pesticides (HCB, HCHs, dieldrin and DDT and its metabolites DDE and DDD). The cores from the central and western Baltic Sea showed remarkably similar patterns in the vertical distribution, starting from zero in the beginning of the 20th century. Increasing values mirror the anthropogenic impact, reaching a maximum around the 1960ies and 70ies. The deposits of the past 30 years are mostly characterized by decreasing trends, indicating the success of the measures to ban the compounds. However, some cores showed intermediate peaks and even increasing levels up to the surface, reflecting recent inputs. A comparison of the calculated inventories of the anthropogenic contaminants results in partly surprising similarities of the different basins (PCBs). This shows that these pollutants were widespread and nearly uniformly distributed all over the whole area before final deposition. Particularly DDT values were higher in the near coastal and shallower southwestern basins in comparison to the central and deeper basins of the Baltic Sea. Recent contaminant contents in the sediments are likely to be still high enough to cause effects in biota particularly in coastal shallow waters.

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#### ICES CM 2007/I:09

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### A comprehensive methodology for the assessment of the health status of estuarine ecosystems

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In the framework of the French National Program of Ecotoxicology, environmental quality was assessed in the multi-polluted Seine estuary and the comparatively clean Authie estuary, France (2002–2004, quarterly sampling) by determining contaminant levels in water, sediments and the infaunal worm *Nereis diversicolor*. Contamination effects were examined in different constituents of the biota. A higher abundance of cadmium and mercury resistant bacteria was shown in Seine mudflats. The physiological status, abundance and population structure of *N. diversicolor* were degraded in the Seine estuary despite its tolerance likely due to genetic adaptation to contamination. Chemical stress might act directly, through lower food availability in the Seine (diatoms, foraminiferans, nematofauna). In return, the influence of biota on the fate of contaminants was focussed on metals and interactions with the sulphur cycle. Biogeochemical transformations in the upper layers of sediments were examined considering inorganic forms of sulphur, fatty acids and *dsrAB* gene (which codes for an enzyme responsible for the production of H<sub>2</sub>S) used as markers of microbial activity. Early diagenesis modelling has shown the influence of bioturbation due to *N. diversicolor* on the profiles of dissolved compounds (oxygen, sulphates, H<sub>2</sub>S). The main achievements for coastal zone management and society include: i) the development of analytical tools for the determination of speciation and bioavailability of metals in interstitial water; ii) the validation of biochemical and physiological markers in an estuarine species representative of the sedimentary compartment; iii) a proposal for a comprehensive methodology to assess the health status of estuarine ecosystems.

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**ICES CM 2007/I:10**      **Poster****Impact of Cyanobacteria blooming on the ecosystem health in the Curonian Lagoon of the Baltic Sea**

Sergey Aleksandrov and Natalia Chukalova

The Curonian Lagoon is the largest coastal lagoon of the Baltic Sea. Hydrochemical and hydrobiological monitoring (phytoplankton, zooplankton, primary production, chlorophyll, nutrients concentrations and others) was carried out at 12 stations from 1991 to 2006. Morphopathological and histopathological changes were studied in bream (*Abramis brama*) in summer of 2004–2006. At present the Curonian Lagoon may be characterized as a hypertrophic water basin. The Lagoon eutrophication affects all trophic levels and primarily the intensity of Cyanobacteria development. In the most part of the Lagoon the strong summer water warming-up combined with freshwater conditions, slow water exchange and high nutrients concentrations creates conditions for blooming of toxic Cyanobacteria species (*Aphanizomenon flos-aquae*, *Microcystis aeruginosa*). In 1980s–2000s Cyanobacteria biomass in summer was always at the level of intensive blooming and during 10 seasons it reached the hyperblooming state (above 100 g/m<sup>3</sup>). Hyperblooming of Cyanobacteria affects seriously the Lagoon ecosystem. The toxic species biomass during several months (July–October) exceeded the level at which the secondary eutrophication (biological pollution) of the water is observed. The recurrent accumulation and decomposition of Cyanobacteria results in oxygen deficiency and death of fish in the coastal zone. In the period of Cyanobacteria blooming the morphopathological changes on skin, gill, in liver and kidneys were found in 70–79% of mature bream. The detected histopathological signs in fish liver and kidneys were similar to the symptoms in fish affected by toxins of *Microcystis aeruginosa* and *Aphanizomenon flos-aquae* dominating in the Lagoon in summer. The above considerations indicate the possible toxic impact of Cyanobacteria on fish and pollution effect on the ecosystem health of the Curonian Lagoon.

Keywords: hyperblooming of Cyanobacteria, eutrophication, ichthyopathological researches, the Curonian Lagoon.

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**ICES CM 2007/ I:11****Biomonitoring strategy to assess the effects of chemical pollution along the Iberian Mediterranean Coast: Present state and future development.**

J. Concepción Martínez-Gómez, J. Benedicto, J. A. Campillo, V. León V, and B. Fernández

Since 2001, the Oceanographic Centre of Murcia (COMU, IEO) started to include selected biomarkers within the chemical pollution monitoring activities conducted along the Iberian Mediterranean coast. The main objectives of this biomonitoring programme are: (1) the determination of spatial distribution and temporal trends of chemical pollution in coastal and reference areas; (2) to seek evidence of detrimental biological effects. Sediment samples, feral fish (*Mullus barbatus*) and wild mussels (*Mytilus galloprovincialis*) are analysed yearly for selected pollutants (trace metals, organochlorinated compounds and polycyclic aromatic hydrocarbons) and selected biomarkers are measured in fish and/or mussels (EROD activity, metallothionein content, micronuclei frequency, genotoxic damages and lysosomal membrane stability). An integrated chemical-biological effect assessment approach is being conducted at five selected areas since 2006. Due to its geographical location, Spain contributes to both the CEMP and MEDPOL programmes and future strategy will be focused to achieve the harmonization of criteria among different programmes and to meet the monitoring requirements in a cost-effective and cost-efficient way. Main results obtained up till now and future development (use of caged mussels) of this biomonitoring programme will be presented and discussed.

Keywords: Biomonitoring, biological effects, *Mytilus galloprovincialis*, *Mullus barbatus*, Western Mediterranean.

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**ICES CM 2007/I:12****Organotin bioaccumulation and imposex in female *Nassarius reticulatus* and *N. nitidus* in port and marina areas of the Basque Country**

J. G. Rodríguez, M. J. Belzunce, A. Borja, J. Franco, J. M. Garmendia, I. González Herraiz, I. Muxika, O. Solaun, I. Tueros, and V. Valencia

Tributyl tin (TBT) is a biocide that has been widely used in anti-fouling paints for ships. It is one of the most toxic xenobiotics ever produced and deliberately introduced into the environment, causing toxic effects in

many different organisms. These effects have been monitored in many areas by analysing the imposex development (superimposition of male characters upon females). The use of TBT in European waters started to be partly restricted in 1982 and will be highly restricted in 2008. Studies in the Iberian Peninsula have been carried out mainly in the Atlantic and Mediterranean coast; no studies on imposex existed in the Basque Country. In order to assess the present status of TBT pollution, before future restrictions in 2008, biological effects in biota and the content of TBT in biota and sediments were studied in several ports and marina areas of the Basque Country. Imposex was studied in two species of neogastropods: *Nassarius reticulatus* and *N. nitidus*. While the imposex in *N. reticulatus* has been already studied in several European countries, almost no studies exist for *N. nitidus*. Results within the studied area showed that TBT concentration and imposex development are, generally, higher in zones with high intensity of large vessel traffic or presence of shipyards. Some exceptions were found due to unexpected high values in small harbours, and vice versa. Areas with very low imposex development were identified; these sites could be used in future transplanting experiments. Comparative results for the two studied species will be presented.

Keywords: imposex, TBT, *Nassarius reticulatus*, *Nassarius nitidus*.

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## ICES CM 2007/I:13

### Assessing ecosystem health in the Baltic Sea: requirements and strategic outline of an integrated biological-chemical monitoring

Kari K. Lehtonen and Doris Schiedek

The implementation of the ecosystem approach to monitor the marine environment and to assess “ecosystem health” requires a re-evaluation of the current strategies and methodologies in use. Within the EU this has already been accepted with an attempt to shift from chemical to biological monitoring parameters in order to assess the status of coastal areas (cf. Water Framework Directive). The European Marine Strategy foresees regional approaches for the protection of the environment and, as a follow-up, the HELCOM Baltic Sea Action Plan is under development. Within this general framework the main goals are good ecological status and healthy wildlife. In order to assess the health status of organisms indicators are needed that reflect disturbances on different biological levels. Hazardous substances are still of concern having an impact on biota in the Baltic Sea. Regarding the assessment of contaminant effects, a large number of methods have been under evaluation and validation in the OSPAR and MEDPOL areas during the last decade. In the Baltic Sea, important baseline data and experiences were recently obtained in the EU BEEP project, and these have been put forward within the ICES Study Group on Ecosystem Health. Practical application of the biological effects methods in the Baltic Sea is still limited, partly because of lack of validation and a missing integrated monitoring strategy. This presentation gives a brief overview on the current status and future options concerning the development of an integrated monitoring and assessment of chemical pollution in the Baltic Sea.

Keywords: Baltic Sea; biological effects; chemical pollution; ecosystem health; monitoring.

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## ICES CM 2007/I:14

### Deployment of caged mussels (*Mytilus* spp.) as a method to study chemical pollution in the Baltic Sea: a case study in the Archipelago Sea (SW Finland)

Kari K. Lehtonen, Hélène Budzinski, Raisa Turja, Mirja Leivuori, and Marie Hélène Devier

In late April 2006, blue mussels (*Mytilus* spp.) collected from a clean reference area were transplanted in three steel cages along a suspected pollution gradient in the Archipelago Sea (SW Finland, Baltic Sea). The study focused on the accumulation of concentrations of different contaminant groups (polycyclic aromatic hydrocarbons, heavy metals, organotins, organochlorines and brominated flame retardants) and biological endpoints [biomarkers (acetylcholinesterase activity, oxidative stress enzymes and metallothionein), shell growth and body condition] in the target organisms. In early July (ca. 2 mo from deployment) half of the mussels were taken from the cages for chemical and biological analyses, and the rest were sampled in late August (ca. 4 mo). Seawater temperature at the caging depth (ca. 8 m), measured continuously with sensors connected to data loggers fixed to the cages, developed from ca. 0.5 °C in April to ca. 20 °C in early July, remaining high until the end of the experiment. The results demonstrate marked differences in regard to accumulation of contaminants and biological effects, with marked correlations between tissue concentrations of contaminants and selected biological parameters. Temporal changes in accumulation and effects were also

observed, partly related to changes in abiotic factors (e.g. temperature). The mussel caging approach has never been used to this extent in the Baltic Sea, and the results strongly support the use of caged organisms in environmental studies also in this sea area.

Keywords: Accumulation; Baltic Sea; biomarkers; chemical pollution; mussels; *Mytilus* spp.; transplantation

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#### ICES CM 2007/I:15

##### **Fiber-optic remote biosensor systems for permanent biological monitoring of the surface waters quality and bottom sediments in the real time**

S. V. Kholodkevich, V. P. Fedotov, A. I. Ivanov, T. V. Kuznetsova, A. S. Kuraskin, and E. L. Kornienko

Today marine ecosystems especially in coastal regions of industrial active zones are subjected to permanent anthropogenic impact and risk of sudden emergency pollution. The level of ecological danger and economical losses in any technogenic emissions highly depends on how fast governing decision directed to its elimination could be made. That is why the problem of developing and improving methods and tools for early determination and express assessment of emergency pollution level dangerous for the environment and living beings is now of great importance.

Any anthropogenic impact on the environment should be considered from the point of view of its ecological danger for species inhabiting such territories or water bodies. The world practice shows that monitoring of the environmental components (air, surface water, bottom sediments etc.) based on automatic on-line registration stations is the most reliable and economically effective method to obtain objective and necessary information.

In 2005 in the laboratory of experimental ecology of water systems RAS there was developed a System for Industrial Biological Water Quality Monitoring for “Vodokanal St.Petersburg”. Heart rate and variational pulsometry characteristics, for example stress-index, are used there as biomarkers.

This system was designed to provide on-line monitoring for toxicity level changes in the Neva River water intakes of St.Petersburg’s drinking water supply stations (WSS). To the present, such automatic systems have already been mounted in all 11 WSSs of St.Petersburg and put in industrial process.

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#### ICES CM 2007/I:16

##### **Responses of metabolic pathways to polycyclic aromatic compounds in flounder following oil spill in northwest of Estonian**

Randel Kreitsberg, Irina Zemit, Rene Freiberg, and Arvo Tuvikene

In January 2006 oil spill involved approximately 40 tons of heavy fuel oil unknown origin affected more than 30 km of northwest coast of Estonia. The aquatic pollution of coastal area of Baltic Sea was monitored by measuring the content of selected polycyclic aromatic hydrocarbons (PAHs) in flounder (*Platichthys flesus trachurus*) muscle and liver tissues, as well as in water, sediment and water plants.

Four types of polycyclic aromatic hydrocarbon equivalents were analyzed from flounder bile as well as from urine: 2, 3, 4 and 5- ringed PAH represented by naphthalene, phenanthrene, pyrene and benzo(a)pyrene. Fluorescence analyzes were carried out using fixed wavelength pairs 290/380, 256/380, 341/383 and 380/430, respectively.

In time scale slight decrease of PAH concentrations in bile were noticed. Moreover, urine samples formed approximately 75 % of total bile fluorescence concentrations. Obtained results indicate remarkable buffer capacity of hydrodynamically active sea as well as considerable importance of kidney-urine metabolic pathways in flounder physiology.

Keywords: PAH, FAC, oil spill, flounder.

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**ICES CM 2007/I:17**

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**The use of biomarkers to assess the effects of environmental contamination in coastal and estuarine ecosystems: what questions remain? An example of the Portuguese NW coast**

L. Guilhermino

In the last decades, biomarkers have been widely used to diagnose environmental contamination and to assess its effects on wild populations. The so called “biomarker” approach has been proven to be very efficient in detecting early effects of pollutants that may have reflexes later in time in higher levels of biological organization levels. However, since these are parameters measured at a sub-individual level, its use in ecological risk assessment and other types of environmental studies has been questioned because they were considered as having low ecological relevance. Therefore, recently, a top line of research in the field was devoted to try to find relationships between biomarkers and ecological relevant parameters such as reproduction, feeding and growth. Several works were able to find relationships that can be used in real scenarios and, thus, they have been giving an important contribution to the question. Another important aspect related with biomarkers use in coastal and brackish-water areas is to investigate why populations from close and similar ecosystems seem to respond differently to pollution and which mechanisms and life strategies have been developed to face and survive in polluted environments. Here, these and other important questions related with the advantages and limitations of using biomarkers to assess environmental contamination and its effects in coastal and brackish-water areas are discussed taking some examples from the Portuguese NW coast.

Keywords: biomarkers, ecological relevant parameters, cDNA microarrays, coastal and estuarine ecosystems.

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**ICES CM 2007/I:18**

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**Effects of pollutants in estuarine and coastal animals: from genes to populations**

Gravato, C.; Guimarães, and L; L. Guilhermino

Some pollutants are able to induce genetic alterations on wild populations by several mechanisms, including fixation and spreading of favourable mutations, differential selection and gene expression. In the present study, the effects of polycyclic aromatic hydrocarbons on estuarine and coastal organisms were investigated using parameters and approaches at different levels of biological organization, including gene expression, biomarkers, behaviour and growth. Animals were exposed to different concentrations of benzo[a] pyrene (BaP), a PAH that has been used as reference substance. At the end of the assay, several biomarkers involved in functions determinant for the survival and performance of the animals were determined, gene expression was analysed using cDNA microarrays, and selected genes were used to population studies. Significant alterations of oxidative parameters, behaviour and growth were found in exposed animals. Furthermore, several differentially expressed genes were found in these animals. Results were analysed and discussed in relation to physiology, life strategies and population evolution.

Keywords: biomarkers, ecological relevant parameters, cDNA microarrays, coastal and estuarine ecosystems.

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**ICES CM 2007/ I:19**

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**Macrophyte communities quality in the Gulf of Riga, Baltic sea and heavy metal bioaccumulation in key species (1997–2007).**

Elmira Boikova, Z. Seisuma, I. Kulikova, Z. Dekere, U. Botva, and N. Petrovics

The underwater meadows in the Gulf of Riga are responsible for wealthy fish population development both from the Gulf and from the coastal waters of open southeastern Baltic. Therefore three reference sites with different eutrophication impact were chosen. Investigations with scuba diving and according to HELCOM recommendations were done. Ecology of macrophyte communities continuously was studied and availability of key species to accumulate heavy metals was estimated. The correlation between the macrophyte communities wealthy (biodiversity, the ratio of annual/perennial species, vertical distribution) and integrated trophic index under different eutrophication impact was demonstrated. The bioaccumulation of heavy metals (Cd, Pb, Zn Cu) was determined by AAS method (model Spektra A880), but Hg content by Flow Injection Mercury system (Perkin Elmer). According to long term investigations the bioaccumulation of metals in three species *Chara aspera*, *Fucus vesiculosus* and *Cladophora glomerata* differs by following trend. Only for *Chara aspera* Hg was accumulated in higher level as other metals in comparison with two

other key species. Cd and Zn values were higher in *Fucus vesiculosus*, but Pb and Cu – in *Cladophora glomerata*.

Keywords: macrophytes, eutrophication, trophic index, heavy metals, bioaccumulation, Baltic sea.

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**ICES CM 2007/I:20      Poster****Effects of sediment contaminants on winter flounder in estuaries along the east coast of the US**

D. Wieczorek, A. F. J. Draxler, A. D. Deshpande, Y. Y. Waguespack, R. C. Chambers, and T. H. Cleary

Sediments in estuaries along the east coast of the United States (e.g., Chesapeake, Raritan, and Newark Bays) are contaminated with an array of anthropogenic compounds including PCBs, PAHs and pesticides. Young-of-the-year winter flounder (*Pseudopleuronectes Americanus*) that settle on such sediments run the risk of sub-lethal as well as lethal effects. Accumulation of these toxic compounds through respiration, ingestion, and transdermal uptake has the potential to alter behavior and reduce the ability of fish to perform essential ecological functions. In controlled experiments using both wild-caught and laboratory reared fish, we examined survival and behavioral responses of winter flounder (20–30 mm) that were exposed to sediments for 7 to 14 days and then measured contaminants and effects. Fish held on sediment from Elizabeth River, Virginia suffered significantly higher mortality and higher predation by bay shrimp (*Crangon septemspinosa*) than fish held on (relatively) uncontaminated York River, Virginia sediment. Fish held on Newark Bay, New Jersey sediment had a decreased ability to exploit an available food resource (*Artemia* sp.). Alteration of such essential behaviors is expected to reduce growth, increase susceptibility to predation, and limit long-term viability of a local population. Ongoing work seeks to emphasize variation among life history stages.

Keywords: winter flounder juvenile sediments contaminants effects behavior survival estuaries.

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**ICES CM 2007/I:21      Poster****Examination reciprocal relationship of blood profile in great sturgeon (*Huso huso*) cultured in brackish water**

T. Bagheri and S. A. A. Hedayati

Recognition of blood profile exchange and awareness of their reciprocal relationship is important in aquaculture, especially in recognition of disease, abnormal condition and determination time of injection. The present work reports on blood profile measured in 4–5 years old Great sturgeon cultured in brackish water pools in BAFGH-Iran. Blood sampling was performed in caudal vein every three month and plasma was frozen until future analyses. Hormone levels measured by Radio Immunoassay (RIA), Glucose with Autoanalyser, calcium and magnesium with spectrophotometer and sodium and potassium with film photometer, in Yazd central laboratory. Statistical result show that some biochemical parameter had direct significant correlation, include: glucose/ calcium; sodium/ potassium; sodium/ magnesium; potassium/ calcium and potassium/ magnesium, which with increase of someone, amount of other will be increase. Among hormonal profile, testosterone/ estradiol had direct significant correlation. Also Cortisol with glucose, sodium and potassium had direct significant correlation, but Cortisol hadn't significant correlation with other hormone and this correlation was invert. Eventually definite that blood relationship in Great sturgeon is like other sturgeons.

Keyword: reciprocal relationship, blood profile, Great sturgeon, aquaculture, plasma.

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**ICES CM 2007/I:22      Poster****“Towards a Healthy Baltic Sea” – putting theory into practice**

Johanna Ikävalko, Ilkka Herlin, and Eeva Mäkelä

The project “Towards a Healthy Baltic Sea” is an initiative that focuses on practical efforts to diminish nutrient input to the Baltic Sea. It is coordinated by the Cargotec Corporation, a Finnish company that wants to support actions for a better future of the Baltic Sea. The core idea is to put relevant scientific knowledge about the environmental status of the Baltic Sea and the reasons behind it into practice by identifying vital causes for eutrophication and finding solutions how to control them. Once identified, technical solutions will be searched to diminish its nutrient input to a sustainable level.

Financing is achieved by a “common pot” approach, where enterprises may donate funds or other substance (such as volunteers, advertisement space/time) for e.g. technical solutions on the spot or for the promotion of the project.

“Towards a Healthy Baltic Sea” project represents a new way of thinking, where research, policymaking and enterprises unite their strengths towards a common goal, better future of mare nostrum, the Baltic Sea.

Keywords: Baltic Sea, nutrient limitation, research, enterprises, policymaking/.

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## ICES CM 2007/I:23      Poster

### **B(a)P: Risk assessment and Challenge**

M. I. Hanna; I. M. K. Abumourad; A. M. Kenawy; and S. M. Tawfeek

The hazard assessment of mutagens and carcinogens requires sensitive specific tests to establish the maximum allowable chemical concentrations prior to the release to the environment and affect the environmental organisms. BaP proved to be a mutagenic and carcinogenic PHC. Probiotics were indicated as a protective immunostimulant towards different varieties of pathogenic and environmental stress factors. In this study, Evaluation of Bap hazards was applied on *Clarias gariepinus* in association with studying the validity of Diamond V probiotic as an immunostimulant factor against Bap adverse effects. Histopathological, immunological and cytological analyses were conducted and revealed the following: Histopathological alterations exemplified by degenerative tissue changes, tissue necrosis and hyperplasia with no detectable histopathological probiotic role of improvement. Using of in vitro single cell gel assay indicated the hazard implementation of BaP on derived erythrocyte cells, sharing the same result of MN assay. The role of action of Diamond V showed a reduction in the induced micronuclei when challenged the Bap, this also was indicated through the immunological outcomes that revealed the suppressive effect of Bap on serum lysozyme, NO, globulin and total proteins levels associated with the long term BaP exposure, this reduced levels were found to elevate in case of Diamond V treatment and to some extent in case of mixed Bap and Diamond V treatment.

Keywords: bap, risk assessment, genotoxicity, biological control, probiotics.

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## ICES CM 2007/I:24      Poster

### **Anthropogenic nitrogen inputs to littoral ecosystems of NW Spain traced by stable isotopes**

Antonio Bode, Nicolás González, and Manuel Varela

Urban population growth enhances nitrogen inputs to littoral ecosystems, mostly through sewage and wastewater disposal. One major concern is the fate of the increased nutrient loads that may lead to eutrophication or to the production of harmful algal blooms. Because of isotopic fractionation during the remineralization of terrestrial organic matter, inorganic nitrogen derived from wastewater is more enriched in heavy isotopes than nitrogen from marine sources. This study analyzes the presence of wastewater nitrogen in coastal ecosystems of NW Spain through the natural abundance of stable isotopes in intertidal organisms. The macrophytes *Ascophyllum nodosum* and *Fucus vesiculosus* were selected as indicator species because of their wide distribution and relatively long life span. Similarly, accumulation of heavy isotopes was analysed in wild mussel (*Mytilus galloprovincialis*) populations. Significant differences in isotopic signatures between ocean-exposed and wave-protected sites inside the rias indicate the assimilation of large amounts of wastewater nitrogen, despite the study area is naturally enriched in nutrients by the seasonal upwelling. The isotopic enrichment increases non-linearly with the size of the urban population near the sampling sites, as wastewater disposal systems improve near large urban areas. Nevertheless, large differences in the efficiency of local wastewater disposal since 1999 are estimated from species-specific growth rates and stratified isotopic analysis.

Keywords: nitrogen, stable isotopes, wastewater, coastal, eutrophication, NW Spain.

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## ICES CM 2007/I:25

### **An integrated approach to biological effects measurements using the Blue mussel, *Mytilus edulis*.**

Steven Brooks, Brett Lyons, John Bignell, and John Thain

The Blue mussel, *Mytilus edulis* is an important biomonitoring species due to its ecological importance, geographical coverage and the wide range of biological effects techniques available. Biological effects techniques have been used with the aim to further integrate biological effects measurements with chemical analysis and to apply these methods to environmental monitoring scenarios. Live native mussels were collected from selected coastal and estuarine sites around the British Isles including the Rivers Test, Thames, Tees, Clyde and Lunderston Bay. A selection of biological effects techniques were carried out on these mussels including whole organism responses (scope for growth), tissue responses (histopathology) and subcellular responses (lysosomal stability, multi-xenobiotic resistance (MXR) and comet assay). In addition, whole mussel homogenates were used to measure organic (PAHs, PCBs) and metal concentrations. The results of these biological response techniques in relation to contaminant burdens for the different mussel populations will be presented. The benefits of using an integrated approach for the assessment of environmental status in marine and estuarine habitats using native mussel populations will be discussed.

Keywords: Mussels, Biomarkers, Monitoring, Comet, Scope for Growth, MXR.

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**ICES CM 2007/I:26      Poster****Physiological and health condition of yellow eels developing in polluted and reference estuaries from NW Portugal**

Laura Guimarães, Joana Santos, Carlos Gravato, and Lúcia Guilhermino

The considerable decrease of yellow eel's populations observed during the last decades in rivers and estuaries all over Europe raised concerns on how the exposure to pollution in estuaries under anthropogenic pressures can impact the health of eels and eventually interfere with their capacity to initiate the catadromous migration, attain the sexual maturation and/or reproductive success. Therefore, a seasonal comparative study of physiological and health condition of yellow eels from the estuaries of rivers Minho, Douro and Lima was carried out using sensitive "early warning" biomarkers (neurotoxic, biotransformation and anti-oxidative stress parameters), health condition indices, morphometric parameters, and abiotic factors (e.g. temperature, salinity, pH). The population of Minho estuary was used as a reference one, while those from Lima and Douro were populations developing in polluted estuaries, under the influence of a harbour, and of untreated domestic sewage and effluents from urban and industrial sources. A redundancy analysis was performed to assess the relationship of physiological condition indices and biomarkers activities with environmental parameters. The gradients identified result in a striking distinction of the estuary of Minho River, from those of Lima and Douro rivers. Exposure of eels to pollution during their continental life-cycle development in the estuaries of Lima and Douro Rivers is inducing changes in biomarkers involved in physiological functions determinant for their survival and performance and, therefore, may reduce their contribution to next generations. This study was funded by the FCT and FEDER EU funds through the project EELEANORA (POCTI/BSE/47918/2002).

Keywords: yellow eels, estuarine ecosystems, biomarkers, biomonitoring, redundancy analysis.

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**ICES CM 2007/I:27      Poster****Integrated biomarker response in transplanted mussels along the French Côte d'Azur**

Michèle Roméo, G. Damiens, and M. Gnassia-Barelli

*Mytilus galloprovincialis* mussels from a clean area were transplanted to several stations in the Bay of Cannes and Nice (North-Western Mediterranean Sea) including a site considered as reference, for one month at the end of spring (May) or in autumn (September). Several biomarkers (acetylcholinesterase AChE, glutathione transferase GST and catalase CAT activities, thiobarbituric acid reactive substances TBARS and metallothionein MT concentrations) were measured in the transplanted organisms. The concentrations of metals (Cd, Cu and Zn) were determined in whole soft tissues. PAH and PCB analyses were performed only in the mussels caged in 2004. The integrated biomarker response (IBR) was calculated; pollutant concentrations in mussels were displayed as star plots and compared to IBR star plots. Visualization was thus possible between sites for comparison with exposure conditions. Results obtained in the bay of Cannes demonstrated that the mussels from the old harbour site (VP) are characterized by elevated copper and PCB concentrations, those from Canto harbour (PC) presented high PCB contents and those from the mouth of the Siagne River (ES) high PAH concentrations compared to the animals transplanted in the reference site (IL). The agreement between the copper gradient and the PCB gradient measured in the caged mussels and the IBR variation was good whereas the PAH gradient did not seem to contribute to the IBR demonstrating that

the chosen biomarkers did not respond to PAHs. GST and CAT protein expressions were measured using western blot in some transplanted mussels, they were correlated with the respective enzyme activities in most cases, demonstrating the specificity in the mussel antibodies and in particular the anti-GST-pi one.

Keywords: biomarkers, chemical pollutants; Mediterranean mussel, transplantation experiment

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**ICES CM 2007/I:28      Poster**

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**Isolation of primary liver cell cultures of harbour seals (*Phoca vitulina*) for identification of novel biomarkers of pollutant influence**

Veronika Hellwig, Antonia Wargel, Annika Behr, and Ursula Siebert

Marine mammals like harbour seals are considered as sensible indicators for environmental changes in the marine ecosystem. They still accumulate various pollutants as they are top predators in the food web. These contaminants influence a broad range of physiological processes on a cellular and molecular level in the body. An in-vitro strategy to study pollutant-induced alterations in the protein expression profile based on incubation of primary liver cells with ecological relevant pollutants and subsequent gel-based proteome analysis is presented. We developed a non-perfusion technique for the isolation of primary seal hepatocytes adapted to the conditions when sampling stranded (that means randomly died) free-ranging harbor seals. Several times, we managed to perform the sampling within the first hour after the death of the animals to avoid cellular damages. After Percoll density gradient centrifugation we obtained pure parenchymal cell populations. The first incubation experiments were performed with polychlorinated biphenyls (PCBs). Aliquots of cell cultures treated only with the solvent were used as blank samples in the subsequent proteome analysis. The amino acid sequences of up- or downregulated expression after incubation with pollutants are elucidated using mass spectrometric techniques and compared with sequence data of other mammalian species. Data from the cell culture experiments as well as preliminary results from the protein sequencing will be shown. Studying these effects on the level of protein expression aims at the identification of novel species-specific biomarkers regarding their pollutant impact.

Keywords: marine mammals, harbour seals, hepatocytes, biomarkers, pollutants, cell isolation.

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**ICES CM 2007/I:29      Poster**

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**Integrative assessment of sediment contamination. A case of study in a coastal area affected by wastewater discharges in the Basque Country (N. Spain).**

J. Franco, M. J. Belzunce, A. Borja, J. M. Garmendia, I. Menchaca, I. Muxika, M. Revilla, and I. Tueros

In the Basque Country (N. Spain) an integrative approach is used for the assessment of the environmental quality of the sediments in estuaries and coastal areas. In this study the results obtained in a coastal area affected by wastewater discharges (submarine outfall) are presented.

The information comes from a monitoring program in operation since 2000; three main lines of evidence have been considered for the assessment: (i) the contamination measured by chemical analyses (heavy metals and organic compounds in sediments); (ii) the toxicity determined by ecotoxicological tests (Microtox and the amphipod *Corophium multisetosum*); and (iii) the biological alteration of soft-bottom benthic communities (AMBI and structural parameters).

According to the chemical analyses the sediments present low-moderate contamination. The Microtox test indicate no apparent toxicity at any of the stations, whereas the amphipod acute test indicate significant toxicity at the station located in the waste discharging site. The biological communities in the area reflect clear differences in the degree of alteration both spatially (distance to the discharging point) and temporally. The processes of degradation and recovery of the soft-bottom benthic communities in this area occur at different temporal scales.

It is concluded that although sediments present signs of contamination, they do not cause relevant toxicity in most of the area, and the changes in the biological communities reflect the impacts from organic enrichment. Integrative assessment is considered as an useful approach to evaluate the health status of the marine environment and the effects of anthropogenic impacts.

Keywords: integrative assessment, sediments, toxicity, biological communities, organic enrichment, Basque Coast.

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**ICES CM 2007/I:30      Poster**

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**Comparison of contaminant contents and some biochemical parameters in salmon (*Salmo salar* L.) caught in the Baltic Sea and experimentally fed with feed made of Baltic herring or sprat**

Pekka J. Vuorinen, Marja Keinänen, Susanna Eerola, Hannu Kiviranta, Mikko Kiljunen, Ulla Perttilä, Rune Waagbø, Kari Ruohonen, and Juha Karjalainen

Atlantic salmon (*Salmo salar* L.) were sampled in three areas of the Baltic Sea for analysis of polychlorinated dibenzo-p-dioxins (PCDD), dibenzofurans (PCDF) and biphenyls (PCB), and polybrominated flame-retardants and analysis of some biochemical parameters like liver 7-ethoxyresorufin-O-deethylase (EROD) activity. To study the significance of different feeds in accumulation of toxicants and manifestation of their biochemical effects, salmon were fed in net cages in brackish-water for three years with feeds were made of Baltic herring (*Clupea harengus* L.) or sprat [*Sprattus sprattus* (L.)], which are the main prey species of the Baltic salmon, and respective commercial control feeds. PCDD/Fs and PCBs accumulated into the experimental salmon at similar levels as in wild salmon caught from the Baltic Sea. Relationships between contaminant concentrations and some biochemical parameters as well as differences in them between wild and experimental salmon and between the different diet groups in experimental salmon were compared.

Keywords: salmon, organochlorines, dioxin, biomarker, EROD.

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**ICES CM 2007/I:31      Poster**

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**Suitability of enzymatic biomarker responses in bivalve *Macoma balthica* from the southern Baltic Sea for environmental monitoring**

Ewa Mulkiewicz, Dorota Napierska, and Magdalena Podolska

The development of anthropogenic activities is the main factor leading to increasing levels of contaminants in the marine environment. The measurement of cellular and sub-cellular responses to environmental contaminants (referred to as biomarkers) in organisms allows the detection of early biological effects of their exposure to toxic chemicals before acute responses are observed. Over the past decade, biochemical responses in bivalves have been extensively used in pollution monitoring of aquatic environments. The aim of the study was to evaluate suitability of selected enzymatic biomarkers in bivalve *Macoma balthica* for monitoring biological effects of contaminants in the southern Baltic Sea. The bivalves were collected in several sites along the Polish coast. The biomarkers selected for the study were: antioxidant enzyme catalase (CAT), the phase II detoxifying enzyme glutathione S-transferase (GST) and neurotoxicity marker acetylcholinesterase (AChE). The Generalized Linear Models (GLM) were used to analyze the dependence of the enzyme activities on sampling area. The area effect was significant in models of the biomarkers activities in *M. balthica*. In addition, correlations between the enzyme activities and hydrological parameters measured in sampling areas (water depth, temperature, salinity, O<sub>2</sub> content) were estimated. For AChE the area effect was highest for open sea where the highest salinity, the lowest temperature and O<sub>2</sub> content were recorded. In models for GST and CAT the highest area effects were noticed in Swinoujście (western side of the Polish coast) characterized by the highest temperature and the lowest O<sub>2</sub> content. For GST area effect decreased eastwards, reaching the lowest level in Gulf of Gdansk.

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**Integrated use of biomarkers in flounder *Platichthys flesus* from the Polish coastal area of the Baltic Sea**

Ewa Mulkiewicz, Dorota Napierska, and Magdalena Podolska

Recent studies have shown that fish populations are being exposed to an extensive mixture of contaminants in their natural environment. It is particularly difficult to establish a causal link between exposure to pollutants and its effects on fish populations. The impact of a multitude of environmental variables, the influence of human activities, and the inherent attributes of fish biology make the relative contribution of xenobiotic exposure to fish population "health" extremely difficult to assess. Integrated use of several biomarkers can provide more adequate information about actual condition of environment and therefore has been recommended for biomonitoring of environmental pollutions. The aim of the present study was field application of several biomarkers in flounder, *Platichthys flesus*, from the Polish coastal area of the Baltic Sea. Fish samples were collected in September 2005 from known pollution gradients and from reference area regarded as relatively free of anthropogenic input. Acetylcholinesterase (AChE), glutathione S-transferase

(GST), catalase (CAT), alanine transaminase (ALT), aspartate transaminase (AST), creatyne kinase (CK), and lactate dehydrogenase (LDH) were measured in each sampled specimen of flounder. Additionally body condition factor (CF) and somatic indices for liver (HSI) and gonads (GSI) were determined. Differences in enzyme activities were detected between fish caught in the area of the Gulf of Gdansk and the reference area. Higher values for AChE and lower activity levels of GST, CAT, ALT and AST were evident in samples taken from well-flushed coast with lower contaminant inputs.

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**Exposure to diesel oil prevents recovery from *Prorocentrum minimum* induced pathological changes in Baltic blue mussels, *Mytilus trossulus***

Annika Aalto, Johanna Ikävalko, and Inke Sunila

Increasing incidences of oil spills and Harmful Algae Blooms (HABs) are of great concern in the Northern Baltic. Blue mussels (*Mytilus trossulus*) were collected in the Gulf of Finland in the Baltic Sea in September of 2005. Mussels were exposed to diesel fuel in the laboratory. Cotton grass sorbent and tanks with no oil served as controls. Tissues were fixed in Davidson's, embedded in paraffin and stained with Haematoxyline-Eosine. In situ hybridization was performed by Apoptag®. Due to prior exposure at the sampling location, a toxic dinoflagellate, *Prorocentrum minimum*, was detected at high numbers in the mussels associated with multifocal granulomas. Apoptag® revealed several apoptotic hemocytes in the granulomas. During one-month laboratory experiment control animals and those treated with cotton grass after the oil exposure, had a lower prevalence of granulomas. Exposure to only diesel caused the mortality of all animals. Ocadaic acid, the toxin produced by *P. minimum*, has been demonstrated to induce apoptosis in a neuroblastoma cell line. Algae toxins appear to impair the mussel's immune system by killing hemocytes via apoptosis. Our results suggest that oil exposure disables mussels from clearing an infection to *P. minimum*.

Keywords: mussels, oil, *Prorocentrum minimum*.

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