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BONUS briefing

SCIENCE FOR A BETTER FUTURE OF THE BALTIC SEA REGION

BALCOFISH Investigates chemical pollution by developing fish monitoring

Modern society utilises more man-made chemicals than ever before with the aquatic environment ending up as the ultimate sink for many hazardous substances. There is a continuous need to improve monitoring of biological effects caused by hazardous chemicals in the environment as hundreds of new chemicals reach the market every year. The BALCOFISH project investigates chemical pollution by developing fish monitoring. The viviparous eelpout *(Zoarces viviparus)* is the selected species in these studies.

The project generates a large set of data of profound importance. High frequency of malformed and dead fish larvae which is found in the south of the Baltic Sea can markedly affect population development and can even lead to extinction of populations. Feminised male eelpout expressing intersex (testis containing egg cells) that has been observed relatively often indicates the presence of endocrine disruptors in the coastal environment. The biomarker approach signals for example "early warning" impact of contaminants in eelpout of harbour area in Sweden and neurotoxicity in German and Danish polluted coastal sites. Moreover, the genetic technologies have provided new insight of impaired functions in fish from polluted sites and hence suggested new useful biomarkers. The genetic work has also given evidence that eelpout seem to be more mobile than thought earlier which is of major practical importance in designing field monitoring programmes for eelpout.

OVERVIEW

The BALCOFISH project applies an integrated approach i.e. combination of contaminant concentrations and biological effect measurements to develop ideas and techniques in order to improve the tools to assess impact of hazardous chemicals in coastal fish populations. Scientists from Germany, Denmark and Sweden are working together to improve biological effect monitoring. The ultimate purpose is to provide input of solid information to develop appropriate measures in the management of the Baltic Sea environment to protect it against human induced chemical pollution. It is important to unravel causal links between the current pollution situation and effects observed in the field. To establish such links BALCOFISH has developed novel toxicogenomic approaches and integrated these with existing early effects biomarkers. These responses are of critical importance when considering the effects relevant to the sustainability of coastal fish populations of the Baltic Sea, such as impaired reproduction.

OUTLINE OF KEY RESULTS

Eelpout is a very suitable "model species" to identify responses and mechanisms of action of individual chemicals and their mixtures on coastal fish populations in the Baltic Sea. The BALCOFISH work has included development of new and application of existing tools in biological effect monitoring, field sampling campaigns, microarray technology and population modelling. In order to standardise the

methodology, BALCOFISH has arranged several workshops on sampling and dissection of fish, assessment of reproductive success and work with the common data base. Two large sampling campaigns covered eight Swedish, five Danish and three German off the coasts sites.

BONUSHAZ, THE BALCOFISH METADATABASE

BALCOFISH has assembled both new and old eelpout monitoring data in a metadatabase, called BonusHAZ. The BonusHAZ contains data on biometrics, biomarkers, reproductive success, chemistry data and other. Eelpout data generated in the BALCOFISH project will be submitted to the ICES Data Centre. Moreover, it provides an important base for the development of guidelines for biological effects monitoring in eelpout including integration of biomarker responses, levels of contaminants and disturbances on the different level of fish population. A dedicated monitoring guideline will be submitted to ICES working group for biological effects of contaminants.

ECOTOXICOGENOMICS

Ecotoxicogenomics refers to large-scale genome-wide molecular analyses within ecotoxicological research. While studying polluted harbour site BALCOFISH scientists noted an increase in programmed cell death and DNA damage: a probable effect of the polluted environment and potential biomarkers for stress. Some of the suppressed genes were found to take part in the innate immune system. So far, biomarkers assessing the status of the immune system are not a part of monitoring surveys and this finding could indicate the need for such assays to be included in future studies - 160 arrays on samples from 16 different sampling sites are currently being analysed. When completed, the results from these studies will provide a unique gene expression profiles for eelpout caught in different sites in the Baltic Sea that can in turn be combined with the large data set from the joint sampling campaigns. The gene expression oligonucleotide microarray is an important compliment to traditional environmental monitoring in the search for new biomarkers and for the understanding of molecular mechanisms resulting from exposures to toxicants.

REPRODUCTIVE STUDIES AND INTERSEX

BALCOFISH observations at polluted sites have repeatedly shown disorders and malformations among eelpout fry resulting in a classification system developed for these malformations. Furthermore, skewed sex ratios in contaminated areas have been observed. Histopathological assessment of the testis of male fish has shown higher occurrence of intersex (in the eelpout sampled from German and Danish sites compared to Swedish sites. Although intersex (testis tissue containing egg cells) in eelpout is not a novel finding, the results show that intersex is relatively common in eelpout from coastal sites at least in some areas of the Baltic Sea. Frequent occurrence of feminised male eelpout suggests the exposure to endocrine disrupting substances i.e. estrogenic compounds in the coastal environment.

EELPOUT POPULATION MODEL

Age classed Leslie matrix population models have been developed for four reference sites, representing a gradient in salinity and climate. Compiled life history data reveal marked differences among sites in several biological properties: growth rate, fecundity, age at maturity and longevity being the most important. Results show that, despite depending on different life history characteristics, the survival of early life stages is most important for population growth and persistence. Low survival in early life stages is observed in contaminated sites, with higher frequencies of malformation and dead larvae as compared to reference sites. The range of change in survival of larvae necessary to affect population dynamics (i.e. growth) is well within the range documented at contaminated costal sites. Hence, induced malformation from contamination can have a large effect on population dynamics, and even lead to extinction depending on the dynamics of the populations before. This study adds important knowledge linking individually measured effects to population level and explaining how populations can be affected by contaminants in the environment.



GENE FLOW AND POPULATION GENETICS

BALCOFISH scientists use molecular gene markers, so called microsatellite markers that have shown to be the most suitable to study genetic differentiation, diversity and gene flow among eelpout populations. The microsatellite loci characterised in this study will contribute to a better knowledge of migration processes and gene flow between different populations of this species. The results suggest that gene flow among populations and therefore the migration of eelpout is stronger than usually thought. This is an important discovery for planning and evaluating monitoring activities in this species and for the interpretation of ecotoxicological studies. Strong migration might lead to wrong conclusions concerning the pollution in a given area. We, therefore, recommend placing reference stations at a larger distance from the impact area than presently practiced.

EFFECTS OF HAZARDOUS CHEMICALS OF CONCERN

Brominated dioxins, in a mixture reflecting the concentration found in the Baltic Sea was used in laboratory exposure studies on adult zebrafish. The results indicate that all brominated dioxins were taken up by the female fish and transferred to eggs. Exposure of brominated dioxins reduced spawning success, altered ovarian morphology and reduced hepatic vitellogenin gene expression which indicate that brominated dioxins may impair reproductive function in fish. Another compound tested in exposure studies on zebrafish was 2, 4, 6- tribromophenol, a widely used as industrial chemical. Our results show that dietary exposure to tribromophenol can interfere with reproduction and affects the fertility. Overall, these results provide data for risk analysis of chemicals and contribute to the identification of chemicals possibly affecting reproduction in Baltic fish species.

A REVIEW ARTICLE ON EELPOT

As initiated at a workshop arranged by the German Environmental Specimen bank in Berlin, 2009, a review article on eelpout in marine environmental monitoring was recently completed (1). This publication was jointly financed by the German Environmental Specimen bank and the BALCOFISH project.

NEXT STEPS AND FUTURE PLANS

There is a continuous need to improve monitoring of biological effects caused by contaminants in the environment. The immediate perspectives beyond the BALCOFISH project are to develop and implement potential biomarkers obtained from the large sets of gene expression profiles of eelpout from polluted regions in the Baltic Sea into the environmental monitoring programmes.

It is also necessary to link molecular responses to pollutants with reproductive performance in the eelpout in its natural environment, and to further investigate gonadal disorders (e.g. intersex) possibly caused by endocrine disruptors. The development in "omics" technologies will in future make it possible to find out if the chemical pollution in the Baltic eelpout can drive genetic variation which is not only of scientific interest but also has practical implications, for example, in planning and designing future regional monitoring programmes.

Making use of new technologies to develop common techniques and assessment criteria for national- and regional-scale assessment of the marine environment is also necessary. This in order to establish monitoring programmes and set assessment criteria of the marine environmental statues within the Marine Strategy Framework Directives. The development of such technologies and strategies will in future be an aid in assessments of environmental impact of mixtures of toxic chemicals occurring in the Baltic Sea region.

1. Hedman JE, Rüdel H, Gercken J, Bergek S, Strand J, Quack M, Appelberg M, Förlin L, Tuvikene A, Bignert A, 2011. Eelpout *(Zoarces viviparus)* in marine monitoring. Marine Pollution Bulletin. In press. Doi: 10.1016/j.marpolbul.2011.06.028.

IN BRIEF

BALCOFISH
Investigates chemical pollution by developing fish monitoring

Modern society utilises more chemicals than ever before. Hundreds of new chemicals are reaching market every year and many of them potentially end up in the aquatic environment. Fish has a top position in the food chain, accumulating chemicals from prey organisms, and then forwarding them to us as consumers. The BALCOFISH project investigates and maps the impact of chemical pollution by developing fish monitoring. The project generates important information to develop appropriate measures in the management of the Baltic Sea environment to protect it against human induced chemical pollution.

KEY RESULTS

- Collection of eelpout data into the BonusHAZ database, to be reported to ICES, provides a unique source of information that will enable us to follow chemical impact on fish in the Baltic Sea from both historical and geographical perspectives.
- Ecotoxicogenomics provides new insight of impaired functions in fish from polluted sites and have as well provided suggestion of new biomarkers.
- Laboratory exposure studies of zebrafish using chemicals at concentrations found in the Baltic Sea contributes to the identification of compounds possibly affecting reproduction in Baltic fish species.
- Observations of intersex (egg cells within the testis) of male eelpout indicate the presence of endocrine disruptors in the costal environment.
- Eelpout population modelling indicates that the survival of early life stages is the most important factor for eelpout population growth and persistence. An important fact to consider as a high frequency of malformed and dead larvae is repeatedly found the south of the Baltic Sea.
- Genetic work gives evidence that eelpout has a stronger migration behaviour than earlier believed which is of major practical importance in designing field monitoring programs for eelpout.
- BALCOFISH provides a platform for scientists working with fish to exchange information, to establish common standardised procedures in eelpout.

WHO NEEDS THE INFORMATION

Direct stakeholders of the BALCOFISH project are national, regional and international authorities and organisations responsible for development of marine environmental monitoring programmes and assessment of the contamination status of marine environment, e.g. national environmental protection agencies, ICES, HELCOM, OSPAR etc. In broad sense, by developing the monitoring and revealing so far neglected, but threatening effects of pollution our achievements support management and policy making to take proper action for a cleaner Baltic Sea.

PROJECT PARTNERS AND COORDINATOR

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