BALCOFISH, Annual report, Year 2, 2010

January 30, 2011.

Lars Förlin
Department of Zoology
University of Gothenburg
Gothenburg, Sweden

Content

1. Gained scientific results during the reporting period.

In the Balcofish project the work is divided in six WPs. Below is reported year 2 (2010) results subdivided into these WPs:

WP1. Provide a data matrix on contaminant levels, effects and population descriptors in eelpout, and supporting environmental variables from Baltic coastal waters
WP2. Develop new tools for studying effects of contaminants on eelpout in the Baltic Sea
WP3. Apply existing and new tools in field studies of eelpout in contaminated coastal sites in the Baltic Sea
WP4. Confirm laboratory studies and validate extrapolations between species
WP5. Link gene responses to population effects
WP6. Bridge the gap between scientists, stakeholders and managers

WP1. Provide a data matrix on contaminant levels, effects and population descriptors in eelpout, and supporting environmental variables from Baltic coastal waters.

Task 1.1 Deliver existing data and access to ongoing monitoring activities applying biomarkers and reproduction parameters to eelpout in Baltic coastal waters.
Comment: The existence of data on biological effects in eelpout has been identified by BALCOFISH partners and it includes national and regional monitoring and research data in Sweden, Denmark and Germany. The Danish data include several years monitoring data on biometrics, CYP1A/protein, reproductive success, and some surveys of contaminants and biomarker studies e.g. intersex, PAH-metabolites. Swedish data include several years monitoring data on various biometrics, biomarkers, reproductive success and contaminants. Also research data is available. German data include regional survey and monitoring data on reproductive success and intersex and additional biomarker data from research projects. At the moment more than 23000 eelpout results have been submitted to the BonusHaz database, but the data set will expand even more during 2011!

Task 1.2. Developing a data bank and relevant and quality assured information
Comment: The project database is named "BONUSHAZ" has been developed. ICES code lists has been implemented in the BonusHAZ structure, but also additional codes for parameters not present in the ICES code list has been added, for instance most codes related to the brood layer. It has from the beginning been intended to utilise the ICES code lists as a foundation for data exchange and consistency between institutes. As of 2010 we have made a formal arrangement with ICES so we can use their code lists from their RECO database http://www.ices.dk/datacentre/reco/reco.asp
This also means that ICES knows the existence of the Balcofish project, and they are interested in helping so data can be submitted to them at a later stage.

WP2 Development of new tools for studying contaminants effects in eelpout in the Baltic Sea

Task 2.1 Development of gene expression assays to analyze eelpout from various coastal sites.

Comment: This task was completed Y1.

Task 2.2 Population genetics in different Baltic eelpout populations.
Comments: Protocols for several different methods to study eelpout population genetics have now been developed. These methods are now applied in Task 3.5.

Task 2.3 Development of sex specific genetic markers in eelpout.
Comment: The planned methodology turned out to be impossible to apply to develop sex specific markers in the eelpout. Work is however in progress to test an other strategy to study sex specific marker at molecular level.

WP3 Applying existing and new tools in field studies of eelpout in contaminated coastal sites in the Baltic Sea

Task 3.1 Contaminant /congener patterns in fish from various coastal sites.
Comment: Larvae, muscle and liver tissue from female eelpout collected at 15 sites (reference and polluted) around the southern Baltic Sea in November 2009 has been analysed for a wide range of relevant environmental contaminants (dioxins, brominated flame retardants, phenolic, perfluorinated and organo-tin compounds, and metals, including mercury). The laboratory results will be statistically evaluated and reported before June 2011.

Task 3.2 Monitoring biomarker responses in eelpout from different coastal sites.
Comment: As indicated in Balcofish year 1 report two large sampling campaigns with eelpout have been performed. In May 2009 including four sites in Sweden, five in Denmark and three in Germany, and in November 2009 seven sites in Sweden, four in Denmark and three in Germany. The data includes various biometrics, many biomarkers such as EROD and PAH metabolites, oxidative stress, reproductive success and inter sex and more, aal of which will be reported during 2011. In addition to investigate and analyse several fish biomarkers and reproduction variables, it is also included parasitological studies in the eelpout. It includes gastrointestinal investigation in eight sampling sites in Sweden, three in Germany and five in Denmark and whole fish studies in eight sites in Sweden."

Task 3.3 Larvae malformation and pathology in eelpout.
Comment: The eelpout gonads from the 2009 spring sampling campaigns in Germany, Denmark and Sweden were examined for the presence of developmental disorders. In male eelpout the histological analysis of the testes showed a quite common occurrence of intersex in samples from German and Danish stations compared to a low frequency at Swedish stations. The analysis of ovaries revealed different kinds of degenerations of follicles either resulting from a normal process or as a consequence of putative environmental stress. At present efforts are made to discriminate between these forms of degeneration. The synoptic analysis of the broods sampled from gravid female eelpout in autumn 2009 revealed that malformations of larvae was more frequent at German and Danish stations in relation to Swedish stations.

Task 3.5 Population genetics in different Baltic eelpout populations.
Comment: Work are in progress to apply the methods indicated in Task 2.2 on eelpout collected at several coastal sites during the large 2009 autumn sampling campaign in Denmark, Germany and Sweden.

Task 3.6 Application of gene arrays in eelpout in different Baltic populations.
Comment: As mentioned in Balcofish Year 1 report the gene arrays will mainly be run on the November 2009 samples. As indicated below (P 2: "Comparison with original research and financial plan") we have had to change to the NimbleGene microarray platform. This has delayed the measurements, but it is planned that all will be analysed during spring 2011.

Task 3.7 Comparative studies with stickleback in different coastal sites in the Baltic.
Comment: Sticklebacks have been sampled in several coastal sites in Germany, Denmark and Sweden in 2009, 2010 and will also be sampled in 2011. The work include recording of basic biometric data, and processing of the gonads of both male and female specimens for histological analysis of developmental disorders like intersex in testis and degeneration of oocytes in ovaries. The microscopical inspection of the samples will take place in 2011.

Task 3.8 Comparative studies with flounder in different coastal sites in the Baltic.
Comment: Only small numbers of appropriate sizes of flounders can be collected at the coastal sites we have selected. Therefore we will increase our fishing effort, but we will also increase our focus on the stickleback in our comparative studies (Task 3.7).

WP4 Confirming laboratory studies and species comparison

Task 4.1 Laboratory exposure studies using three-spined stickleback.
Comment: Juvenile three-spined stickelback were caught in the Baltic sea in the vicinity of Öregrund in November 2010 and brought to our laboratory for exposure. Based on the results from the zebrafish the stickleback will be exposed to the same mixture of brominated dioxins used in the zebrafish study (Task 4.2). Exposure will be performed during sexual maturation.

Task 4.2 Laboratory exposure studies using zebrafish.
Comment: Adult zebrafish were exposed to feed spiked with a mixture of structurally diverse BFRs to investigate accumulation from feed, maternal transfer, and effects on reproduction and early life-stage development. One of the compounds in the BFR mixture, i.e. 2,4,6-tribromophenol, was tested separately. The BFRs were also tested individually in an embryo toxicity test, to screen for effects of waterborne BFRs on early life stages. To investigate effects of PBDDs on reproduction, early life-stage development, and on the aryl hydrocarbon receptor (AHR) pathway, adult zebrafish were exposed to feed spiked with 2,3,7,8-tetraBDD (TBDD), or a mixture of PBDDs that was designed to reflect relative concentrations found in Baltic Sea biota. Most brominated chemicals exposed via feed were detected in females and in their offspring. Ovarian morphology was altered in all studies, and the PBDDs induced AHR-regulated genes and ethoxyresorufin-*O*-deethylase activity. Effects on early life-stage development were seen after parental and water exposure, although at concentrations generally higher than in the environment. However, compared to several other fish species, zebrafish have a relatively low sensitivity to AHR agonists regarding effects on early life stages. To be able to evaluate the risk of PBDDs for fish in the Baltic Sea, future studies should focus on fish species native in the Baltic Sea. Overall, the results suggest that maternal transfer is an important exposure route for several brominated environmental chemicals, and that these compounds may interfere with reproduction and early life-stage development in fish.

WP5 Linking gene to population

Task 5.1 Individual-based population dynamic model for eelpout.
Comment: Within WP 5, age structured Leslie matrix population models has been developed at four reference sites have been used (Skagerrak, W Baltic Proper, E Baltic Proper and Bothnian Sea), representing a gradient in salinity and climate. Compiled life history data reveal marked differences among sites in several of the measured variables, growth rate, fecundity, age at maturity and longevity being the most important. The model will be used to analyse population dynamics of eelpout under various conditions. Effect data from field and experimental eelpout studies have been compiled in order to uncover the effect on population dynamics. Additionally, possible density dependent effects and environmental noise will be compensated for in the model. In conjunction, a specific study on oocytes was performed in 2010 to add the egg stage into the population model.

WP6 Bridging the gap between scientists, stakeholders and environmental managers

Task 6.1 Environmental assessment tools for biological effects in eelpout.
Comment: Work in progress. Potential indicators for setting evaluation criteria for the response levels have been identified. Biological response levels for derivation of environmental evaluation criteria will be developed for reproductive success and 1 - 2 supplementary eelpout biomarkers i.e. intersex and PAH-metabolites based on the data in the BonusHaz database. An integrative eelpout health index will be suggested, when the other ACs are in place during 2011. Cooperation with the BEAST project.

Task 6.2 Workshops in conjunction with field sampling campaigns and coordination meetings.
Comment: Below is listed workshops held within the project:

> Balcofish Workshop at Institute of Coastal Research, Swedish Board of Fisheries, Öregrund, Sweden, March 10-11, 2010. The most important issues handled were updates of current activities, work in progress, discussion and decision about chemical analyses.
> http://www.balcofish.science.gu.se/balcofish/Workshops___meetings/Oeregrund_10/
>
> Balcofish Workshop at Villa BelParc, Gothenburg Sweden, December 1-2, 2010. The meeting was hosted by University of Gothenburg. At the meeting all WPs and tasks were discussed and current statues updated.
> http://www.balcofish.science.gu.se/balcofish/Workshops___meetings/gothenburg-10/

Task 6.3 Retrospective analyses of existing contaminants data in eelpout stored in biobanks
Comment: Data available from long-term (>15 years) concurrent monitoring of biological effects and chemical load in eelpout from Swedish coastal stations are being statistically evaluated to find potential correlations between contaminant exposure and biological and physiological effects in a historical perspective. The information will be used to find causal relationships for the overall interpretation of results obtained from the project. Results are aimed at being summarized in a scientific paper. With the available budget, it was prioritized to increase the geographical span of samples for new chemical analyses (see 3.1) and instead

of retrospective chemical analyses focus on the large amount of historical chemical data already available. A review paper on the use of eelpout in environmental monitoring in the Baltic Sea and the results thereof has been completed and will be submitted shortly.

Task 6.4 Workshops for scientists and environmental managers.
Comment: Science faculty at University of Gothenburg arranged a BONUS Day, October 8, 2010. The day was initiated by the five BONUS coordinators at University of Gothenburg. The purpose of the day was to discuss and develop common and new research strategies and look beyond BONUS+. For program details see http://www.gu.se/infoglueCalendar/digitalAssets/1771012588_BifogadFil_BONUS_program 8%20okt.pdf

Task 6.5 Special session at SETAC conference
Comment: No activities during 2010.

Task 6.6 Disseminate data at meetings on endocrine disruption, ecosystem health and coastal fisheries.
Comment: Below are listed dissemination activities related to Balcofish at scientific meetings, meetings with stakeholders as well as teaching in courses given to undergraduate and graduate students.

BONUS Annual Conference 2010, Vilnius Lithuania, January 19-21 2010.

Integrated fish monitoring. Lars Förlin, University of Gothenburg, Sweden.

Assessment of reproductive disorders of fish and invertebrates as part of monitoring biological effects of pollution. Jens Gercken et al. Institute for Applied Ecology Ltd., Germany.

Multi-endpoint studies on Zoarces viviparus, using gene expression oligonucleotide microarray. Noomi Asker, Erik Kristiansson, D.G. Joakim Larsson and Lars Förlin. University of Gothenburg, Sweden

Eelpout – a fish indicator of biological effects in Danish coastal waters. Jakob Strand, Ingela Dahllöf, & Zhanna Tairova. National Environmental Research Institute, Denmark

27th ESCPBnew Congress: Biological Effects of Climate and Pollution: from Biomarkers to System Biology, Alessandria, Italy, September 5-7, 2010.

Environmental biomonitoring of Zoarces viviparus in combination with large scale gene expression profiling. N. Asker, E. Kristiansson, D.G.J. Larsson and L. Förlin, University of Gothenburg, Sweden

Nordic Marine Science Conference 2010, September 13-16, Strömstad, Sweden,

Integrated fish ecology and ecotoxicology in Baltic coastal fish and fisheries –BALCOFISH project. Sara Bergek and Magnus Appelberg. Swedish Board of Fisheries, Sweden

International Conference for Environmental Specimen Banks, 15-16 November 2010, Berlin

Eelpout in Marine Environmental Monitoring. Jenny Hedman et al. Swedish Museum of Natural History, Sweden

Aquatic ecotoxicology – can we improve its influence on policies and risk management? May 6-7, 2010 Copenhagen, Denmark.

Biomarkers in Environmental Monitoring. Lars Förlin, University of Gothenburg, Sweden.

Temamøde, Danish Water Forum, April 21, 2010, Hørsholm, Denmark.

Hormonforstyrrende og miljøfremmede stoffers påvirkning af biologien. Jakob Strand. National Environmental Research Institute, Denmark

1st General Meeting of the BONUS BEAST Project, April 12-15, 2010, Zoological Institute RAS, Scientific Research Centre for Ecological Safety RAS, St. Petersburg, Russia

Eelpout – a fish indicator of biological effects in Danish coastal waters. Jakob Strand, Ingela Dahllöf, & Zhanna Tairova. National Environmental Research Institute, Denmark

The Research Day for the Faculty of Veterinary Medicine and Animal Sciences 15 December 2010.

Effects of 2,4,6-tribromophenol on reproduction and early development in zebrafish. Anna Norman Haldén, Henrik Holbech, Jenny Rattfelt Nyholm, Patrik L. Andersson, Leif Norrgren. University of Agricultural Sciences, Uppsala, Sweden

SETAC Europe 20th Annual Meeting, Seville, Spain, 23-27 May 2010.

Effects of 2,4,6-tribromophenol on reproduction and early development in zebrafish. Anna Norman Haldén, Henrik Holbech, Jenny Rattfelt Nyholm, Patrik L. Andersson, Leif Norrgren. University of Agricultural Sciences, Uppsala, Sweden

Courses, lectures, seminars where materials with links to the Balcofish project have been used for example.

Ecotoxicology with emphasis on physiology, Noomi Asker and Lars Förlin. Undergraduate course at the Department of Zoology, Gothenburg University.

Ecotoxicology with emphasis on ecology, Lars Förlin. Undergraduate course at the Department of Zoology, Gothenburg University.

Fish population dynamics and the effects of environmental changes. Sara Bergek. Seminar at the Department of Mathematics, Uppsala University.
Swedish Board of Fisheries.

The BALCOFISH project. Information at HELCOM FISH meeting in Tallinn. Magnus Appleberg Swedish Board of Fisheries.

Task 6.7 BALCOFISH website
Comment: The Balcofish websites (www.balcofish.science.gu.se/english) is regularly updated by the webmaster Noomi Asker.

Task 6.8 ICES guideline for eelpout monitoring within HELCOM COMBINE
Comment: Work in progress. A draft guideline prepared for august 2011 for BALCOFISH partners, and with submission of revised draft to ICES WGBEC in the end of 2011. This work will be about one year delayed, because it will have to wait for the next meeting in the ICES working group for biological effects of contaminants (WGBEC). The guideline was not ready for submission to ICES WGBEC in January 2011, but a final draft will now be prepared to be finalised for month 32, so it will be ready for submission to next years ICES WGBEC meeting.

Task 6.9 Submission of relevant eelpout data to ICES's data base
Comment: Work in progress. Potential parameters to be submitted to the ICES database have been identified and will at least include data on biometrics, reproductive success, EROD, PAH-metabolites. Submission to ICES will be done during the last quarter of 2011.

2. Comparison with the original research and financial plan

The Balcofish project follows the original research and financial plan. However two research issues have been delayed. The first concerns the gene expression studies of the eelpout using oligonucleotide microarrays (task 3.6). The original microarray platform was not developing as fast as other competing platforms and we decided to change to the NimbleGen microarray platform. This change has now made it possible for us to study 135,000 genes per array instead of 15,000, and this for the same price. The switch in microarray platforms has however given us extra work to optimise all analytical steps, and has delayed us by about half a year. It is still our aim that all planned measurements can be done. The second delayed project concerns the development of sex specific genetic markers in eelpout (task 2.3). Because of the large effort we have put into the microarray work we have had to down prioritise the development of sex marker, but it is in our plan to be able to perform this work during first half of 2011.

3. Statement if the research plan and schedule of deliverables had to be adapted

As indicated in Task 2.3 (above) the work with sex specific genetic markers in eelpout could not be finalised 2010. Therefore work needs to continue during spring 2011 and reported during 2011.

4. Will results of third parties have influence on the working plan expected?

We do not currently expect influence of third parties.

5. Are there any changes in the future plan working plan expected?

Although we have had to change microarray platform (see point 2 above) we do not foresee any major changes in the future plans.

6. Are there any changes expected for the deliverables?

See point 3, above.

7. Additional information

Three Highlights of 2010

In Balcofish scientists are working together with fish to exchange knowledge and data, and to develop new techniques to better understand contaminant impact in coastal fish populations. Classical biomonitoring approaches are combined with genetic technologies. Below is highlighted some important findings and/or activities within the Balcofish project.

Eelpout population model

Age classed Leslie matrix population models has been developed for four reference sites (Skagerrak, W Baltic Proper, E Baltic Proper and Bothnian Sea), representing a gradient in salinity and climate. Compiled life history data reveal marked differences among sites in several of the measured variables, growth rate, fecundity, age at maturity and longevity being the most important. The Skagerrak population, in the more saline environment, shows the highest growth rate, highest fecundity, become mature earliest and dies earliest. Preliminary results show that, despite expressing different life history characteristics, the survival of early life stages, i.e. larvae and juvenile fish, are most important for population growth and persistence. Low survival in early life stages is also what has been 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 recipient sites and experimental studies. Hence, induced malformation from contamination can have a large effect on population dynamics, and even lead to extinction depending on dynamics in the populations before. This study adds important knowledge linking individually measured effects to population level, explaining how populations can be affected by contaminants in the environment.

Intersex

Histopathological assessment of the gonad of male fish was performed in the eelpout gonads from the 2009 spring sampling campaigns in Germany, Denmark and Sweden. The analyses of the testes showed higher occurrence of intersex in the eelpout sampled from German and Danish sites compared to Swedish sites. The intersex condition means presence of primary oocytes within the testis tissue. Intersex in male eelpout was found at known contaminated coastal sites as well as at sites with apparently little pollution (reference sites). Although intersex 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. The occurrence of feminized male eelpout suggests the exposure to endocrine disrupting substances i.e. estrogenic compounds in the coastal environment.

Metadata base

Very important achievement is the collection of new and old eelpout monitoring data in a metadata base, including biometrics, biomarkers, reproductive success, chemical data etc. Not only will it be the platform for the report format for eelpout as monitoring organism, but provides important and interesting possibilities to compare and assess health statues of the eelpout from different sub regions of the Baltic Sea and Skagerrak. Preliminary evaluation of biomarkers data e.g. CYP1A/EROD and vitellogenin seems to suggest higher exposure to contaminants in certain areas in the Baltic compared to the Skagerrak area. Moreover, it provides important base for the development of guideline for biological effects monitoring in eelpout including integration of biomarker responses, contaminants levels and fish population level disturbances.

Educational activities

Using EST data in biological applications, Noomi Asker. Workshop at the Dept of Marine Ecology, University of Gothenburg, autumn 2009, including PhD-students, postdocs and senior scientists. Approx. 20 participants

From unsequenced species to analyzed gene expression microarray. Noomi Asker and Erik Kristiansson. Short course at SETAC international meeting in Göteborg, May 2009. The course was attended by 10 international participants.

Large scale genomics techniques, analysis and modelling, Erik Kristiansson and Noomi Asker. Undergraduate course at the Dept of Math. Sciences, Chalmers School of Technology, Spring 2009.Approx 15 participants.

Ecotoxicology with emphasis on physiology, Noomi Asker and Lars Förlin. Undergraduate course at the Dept of Zoogogy, Gothenburg University. 2009: 17 students; 2010: 18 students

Ecotoxicology with emphasis on ecology, Lars Förlin. Undergraduate course at the Department of Zoology, Gothenburg University. 2009: 13 students; 2010: 16 students

Fish population dynamics and the effects of environmental changes. Sara Bergek. Seminar at the Department of Mathematics, Uppsala University. Swedish Board of Fisheries. Approx. 15 participants.

Stakeholder events

The BALCOFISH project. Information at HELCOM FISH meeting in Tallinn. Magnus Appleberg Swedish Board of Fisheries.

BONUS Day, October 8, 2010. The was arranged by the Science faculty at University of Gothenburg. The day was initiated by the five BONUS coordinators at University of Gothenburg. The purpose of the day was to discuss and develop common and new research strategies and look beyond BONUS+. Approximately 40 participants. For program details see http://www.gu.se/infoglueCalendar/digitalAssets/1771012588_BifogadFil_BONUS_program 8%20okt.pdf

Meeting with the group for Integrated coastal fish monitoring, supported by the Swedish EPA. 1-2 times each year the group meets to discuss current and future activities. Representatives from the Swedish EPA regularly participate in these meetings.

EELPOUT MONITORING, Environmental Specimen Bank Workshop. September 7-8 2009. Berlin Germany. The meeting was organised by the German Federal Environment Agency (UBA). Approximately 30 participants.