BALCOFISH

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Helsinki, January 2009

Balcofish participants

- Lars Förlin, Department of Zoology, University of Gothenburg, Sweden
- Jakob Strand, Department of Marine Ecology, National Environmental Research Institute, University of Aarhus, Denmark
- Magnus Appelberg, Institute of Coastal Research, Swedish Board of Fisheries, Sweden
- Jens Gercken, Institute for Applied Ecology Ltd, Germany
- Leif Norrgren, Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Sweden
- Anders Bignert, Department of Contaminant Research, Swedish Museum of Natural History, Sweden
- Joakim Larsson, Institute for Neuroscience and Physiology, the Sahlgrenska Academy at University of Gothenburg, Sweden

Chemicals/contaminants/pollutants

- We use more chemicals tody than ever before. More than 100.000 chemicals are present in the technosphere
- 20-30.000 ”every-day chemicals” (present in daily chemical products and goods), and a few thousand chemicals in pharmaceuticals
- Generally too little is known about these chemicals fate and effects in the environment
- Chemicals of special concern for health and environment are persistant chemicals, carcinogenic chemicals and endocrine disrupters which may for example disturb reproduction

Fish in monitoring

Fish are included in several research projects and environmental monitoring programs in Northen Europe.
For example in the Swedish projects “Integrated Fish Monitoring” in the Baltic Sea and Skagerrak areas environmental chemistry, fish ecology and functional performance of fish are integrated to assess long term trends of selected pollutants and fish “health” at individual, population and ecosystem levels.

What is Balcofish about

- To provide science-based input to foster the development of appropriate measures in the management of the Baltic Sea environment to protect it against anthropogenic chemical pollution.

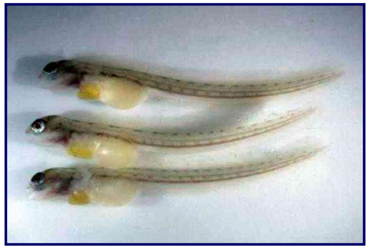
- This is a very urgent issue because modern society utilizes more man-made chemicals than ever before.
- Many of these end up in the coastal environment as chemical mixtures, impacting the well-being of organisms (including ourselves).

What is Balcofish about

- For this purpose it is important to unravel causal links between the current pollution situation and effects observed in the field.
- To establish such links we will in Balcofish develop toxicogenomics approaches and integrate these with existing early effects biomarkers. These responses will be anchored to effects relevant to the sustainability of coastal fish populations of the Baltic Sea, such as impaired reproduction.
- We will study the indigenous viviparous eelpout and other relevant coastal fish species. Approaches will be explored and strategies will be tested in the 6 work packages (WPs)

Eelpout, *Zoarces viviparus*

- *Relatively stationary during its entire life-cycle*
- *Viviparous - field studies on early life stages are feasible*
- *Common in coastal waters of the north-east Atlantic and the Baltic Sea*
- *Used in Swedish, Danish and German (and some other Baltic countries) monitoring programs for several years*

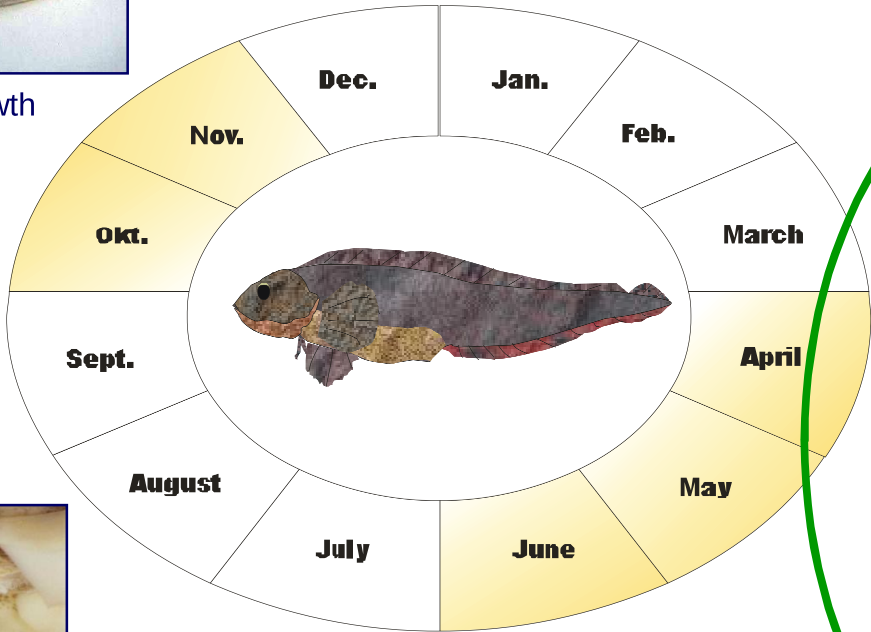


Larval Growth

Larval Malform.

Sex Ratio

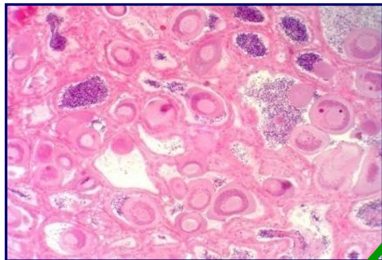
Reproductive Success



Muscle and Liver Samples

Gonadal Disorders

Atresia



Intersex

Umwelt Bundes Amt
Für Mensch und Umwelt

Jens Gercken

IfAÖ

Example of eelpout monitoring studies

- Integrated fish monitoring (Reference site studies)
- Male biased sex ratio in eelpout embryos near pulp mill effluents
- Complex chemical industry effluents affect embryo development
- Bunker oil spill markedly affected fish health
- Dioxins and biological effects in eelpout
- Intersex and atresia in eelpout
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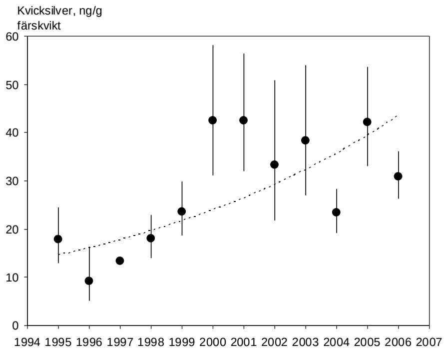
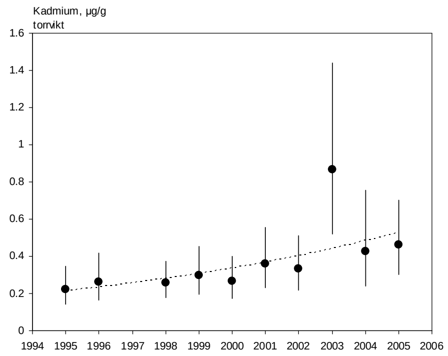
Some metals and biomarkers in Eelpout from a reference site (Swedish west coast)

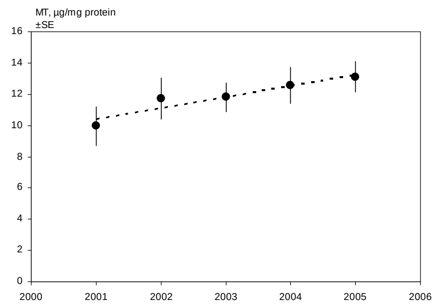
Hg content in Eelpout



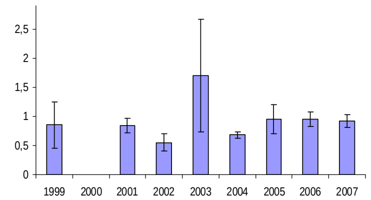
Cd content in Eelpout



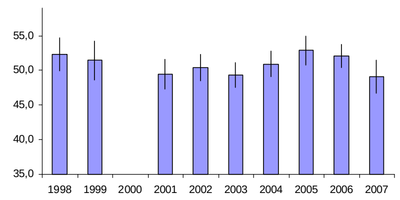
MT in Eelpout



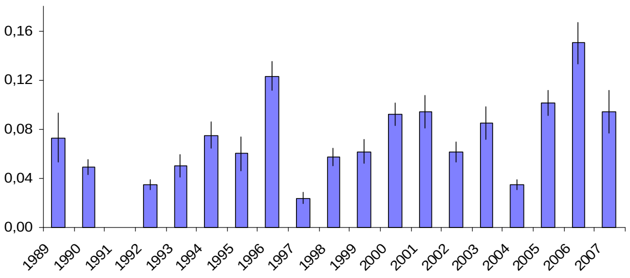
DNA adducts in Eelpout



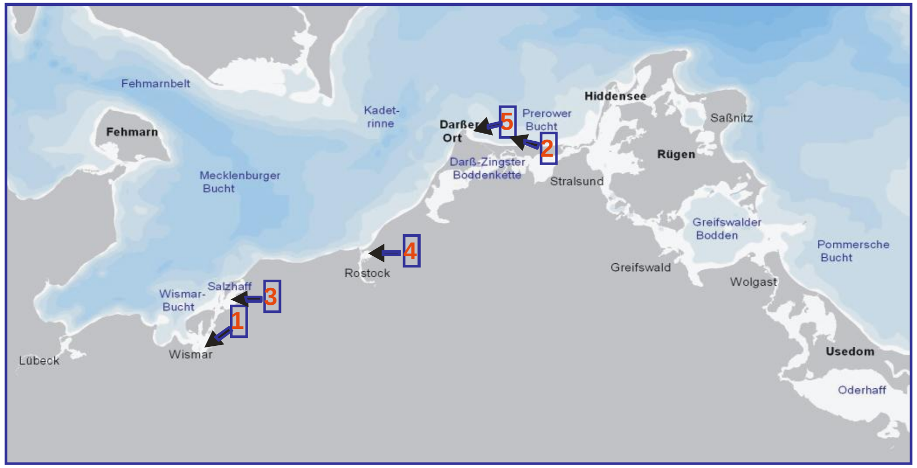
Embryo sex ratio in Eelpout



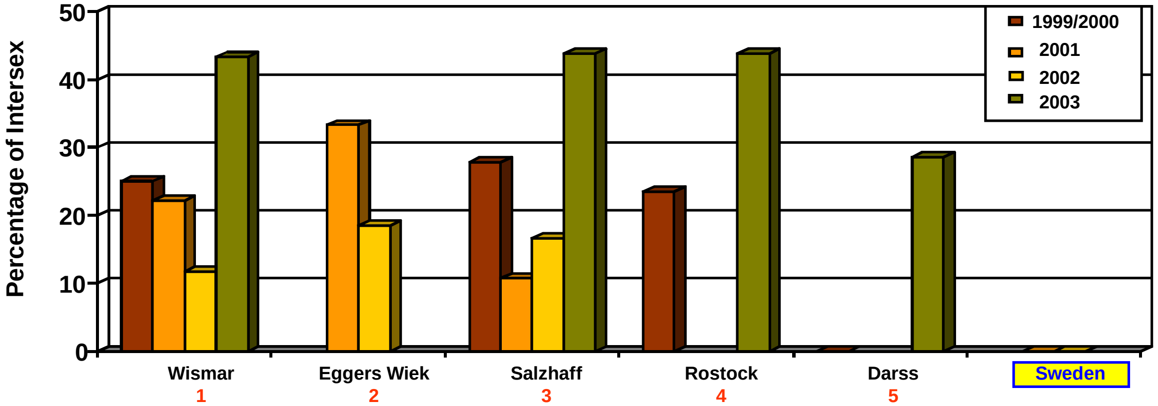
EROD in Eelpout



Historical Data Intersex



Percentage of male *Zoarces viviparus* with Intersex Condition



German Baltic Sea

Reference sites of the Swedish coastal monitoring programme

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Many examples reproductive effects in fish from the Baltic Sea

- Intersex and atresia in eelpout in the southern Baltic Sea
- Pulp mill industry:
  - Reproductive disturbances and testis abnormalities
  - Masculinisation in eelpout
- Infertile burbots in the Bay of Bothnia
- Intersex in roach along the Baltic coast
- Mortality in Baltic salmon yolk sac larvaes (M74)
- Delayed sexual maturation in perch

WPs in Balcofish

- 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 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…

- **<u>Task 1.1</u> Deliver existing data and access to ongoing monitoring activities applying biomarkers and reproduction parameters to eelpout in Baltic coastal waters.**

  Responsible partner: **P2** (NERI). *Contributing partner:* **P1, P3, P4, P6**

  <u>Aim:</u> To bring forward existing national and regional eelpout data and to provide a platform for new field sampling.

  <u>Methods:</u> Access to national monitoring data bases in Denmark and Sweden. In addition, data from other relevant environmental studies on eelpouts and contaminants in the Baltic Sea will be included when using adequate reporting formats and information.

  <u>Expected outcome:</u> Submission of existing and new data to the project data base.

- **<u>Task 1.2</u> Developing a data bank and relevant and quality assured information**

  Responsible partner: **P2** (NERI). *Contributing partner:* **P1, P3, P4, P6**

  <u>Aim:</u> To develop a single report format for eelpout as monitoring organism and for supporting variables.

  <u>Methods:</u> The project database as MS-SQL-Server, 2005. Data reports will be submitted as Access.

  <u>Expected outcome:</u> A substantial data matrix on eelpout physiology, reproduction and effects and linked with relevant abiotic variables like contaminants, temperature, oxygen levels etc., which will be important for analyses on retrospective studies of environmental effects in eelpout and development of environmental assessment tools for biological effects in eelpout in WP 4 and 5.

WP2. Develop new tools for studying effects of contaminants on eelpout in Baltic Sea

- **<u>Task 2.1</u> Development of gene expression assays to analyze eelpout from various coastal sites**

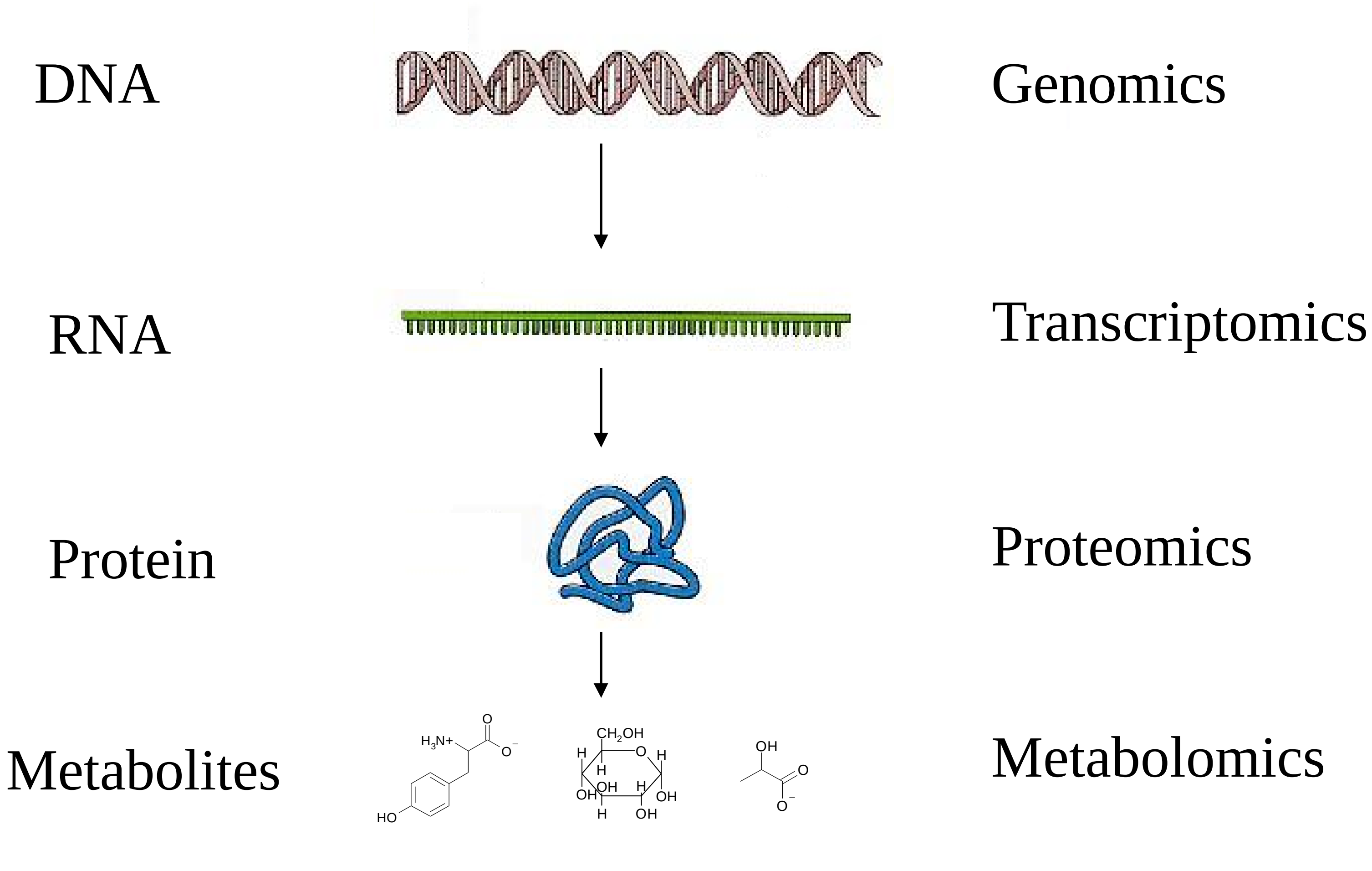
  Responsible partner: **P7** (SA). *Contributing partner:* **P1**

  <u>Aim:</u> To deliver a genome-wide approach to measure mRNA responses in eelpout.

  <u>Methods:</u> We have recently used massively parallel 454-pyrosequencing to characterize the liver trancriptome of the eelpout. We have sequenced 89 million basepairs and assembled transcripts (Kristiansson et al, in prep. 1), which will be the foundation for designing probes on a Geniom array with high coverage. General probe design for Geniom arrays have been optimized by a comparison with the expression of 160 mouse genes in two samples measured in parallel by qPCR (Kristiansson et al, in prep. 2). The developed probe design strategy will be used for the eelpout, and validated with qPCR for specific eelpout genes.

  <u>Expected outcome:</u> A validated, sensitive and specific eelpout mRNA microarray.

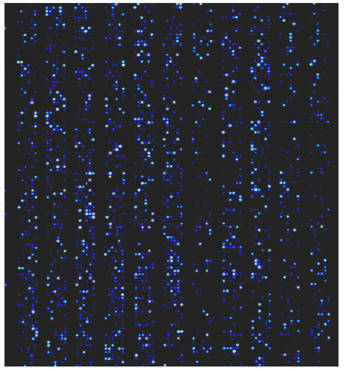
”Omics”-technics



Development of an eelpout gene expression microarray

In order to generate better understanding of the impact of pollutants on coastal fish we have sequenced the eelpout liver transcriptome and developed a gene expression array (Kristiansson et al. Manuscript in prep.).

An eelpout microarray with 120,000 probes divided into 8 different sub-arrays



Ecotoxicogenomics

Ecotoxicogenomics is the use of large scale analyses of mRNA, proteins and metabolites (transciptomics, proteomics and metabolomics) together with bioinformatics to address ecotoxicological questions

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  <u>Expected outcome:</u> A validated, sensitive and specific eelpout mRNA microarray.

WP2. Develop new tools for studying effects of contaminants on eelpout in Baltic Sea

- **Task 2.2 Population genetics in different Baltic eelpout populations.**

  Responsible partner: **P2** (NERI). *Contributing partner:* **P1, P7**

  Aim: to optimise genetic population tools before field sampling.

  Methods: To screen for diversity in the whole genome, the AFLP-technique will be used (Vos et al. 1995). In AFLP the genome is digested followed by selective PCR-amplification of the resulting fragments. Enough fragments (50-100) are scored using a sequencing gel which gives sufficient power to resolve diversity. Development includes selection of digestion enzymes and primers for optimal resolution of the eelpout genome. PCR-conditions will be optimised for existing primers for mitochondrial and nuclear genes (cytochrome b, rhodopsin, CYP1A). As a complement we will use the data set of 89 million basepairs (from task 2.1) generated from a large number of fish from different sites, to look for the presence of SNPs (single nucleotide poymorfism) in coding regions of eelpout genes. The extreme accuracy of 454-pyrosequening makes such a strategy feasible. A selection of the most variable SNPs will then be validated by PCR in fish from several locations.

  Expected outcome: Validated markers to assess genetical variability of eelpout between study sites.

WP2. Develop new tools for studying effects of contaminants on eelpout in Baltic Sea

- **Task 2.3 Development of sex specific genetic markers in eelpout.**

  Responsible partner: **P7** (SA). *Contributing partner*: **P1, P2**

  Aim: Search for a genetic basis for sex determination in eelpout.

  Method: As skewed sex ratios of eelpout embryos have been found frequently at certain polluted sites, we will search for a genetic basis for sex determination which can be used to investigate if there are indeed are phenotypic males (induced by pollutants) with a female genetic background. Amplified fragment length polymorphism (AFLP) will be used as a primary strategy to find such markers. A complementary strategy is to analyze genes known in other species to be sex specific by using PCR with primers in conserved regions.

  Expected outcome: Sex specific genetic markers in eelpout.

WP3. Apply existing and new tools in field studies of eelpout…

- **Task 3.1 Contaminant /congener patterns in fish from various coastal sites.**

  Responsible partner: **P6** (SMNH). *Contributing partner:* **P1, P2, P4**

  Aim: To explore contaminant exposure by analysing fish samples from various coastal sites, regarding congener patterns of PCDD/F's, brominated flame retardants i.e. PBDE's, PCB's, DDT's, PFC's, TBT and metals.

  Methods: High quality chemical analyses of both adults and larvae. Statistical evaluation by means various multivariate statistical techniques.

  Expected outcome: Identification of congener patterns of for the above mentioned contaminants in "hot spot" areas as well as in reference sites suitable for coastal field studies of eelpout in the Baltic Sea

- **Task 3.2 Monitoring biomarker responses in eelpout from different coastal sites.**

  Responsible partner: **P1** (ZGU). *Contributing partner:* **P2, P3, P4, P7**

  Aim: Combine research resources and test a broad set of biomarkers in eelpout in differently polluted coastal sites in the Baltic Sea.

  Methods: The set biomarkers include measures for detoxifications defences such as CYP1A/EROD, endocrine disruption such as vitellogenin, oxidative stress including antioxidant defences system and oxidative damages (see for example Sturve et al., 2005). In addition also retinoids/vitamin A, and gene arrays in eelpout.

  Expected outcome: To compare classical biomarkers approach and a genome-wide approach to assess the environmental effects of pollutants in indigenous fish species of the Baltic Sea.

WP3. Apply existing and new tools in field studies of eelpout…

- **<u>Task 3.3</u> Larvae malformation and pathology in eelpout.**

  Responsible partner: **P4** (IAE). *Contributing partner:* **P1, P2, P3, P5, P7**

  <u>Aim:</u> To assess prevalence and severity of reproductive disorders of male and female eelpout at different stages of the reproductive cycle.

  <u>Methods</u>: Sampling of eelpout gonads is conducted during the time of gonadal maturation. In male testis the presence of female gametes (intersex) is investigated by means of histopathology. Degeneration of oocytes (atresia) will be assessed macroscopically and by application of histology. In autumn the brood of pregnant eelpout will be inspected for mortalities, malformations and growth retardation of embryos.

  <u>Expected outcomes:</u> To compare reproductive disorders of parent fish and developmental disorders of the embryos with classical biomarkers and a genome-wide approach. To link maternal transport of pollutants or other routes or causes to eelpout embryo malformation and mortality.

- **<u>Task 3.5</u> Population genetics in different Baltic eelpout populations.**

  Responsible partner: **P2** (NERI). *Contributing partner:* **P1, P4, P7**

  <u>Aim:</u> To map genetic variations and abnormalities in different Baltic Sea regions, and to explore if pollution can drive genetic variations by comparing changes in three levels of genetic diversity to degree of contamination.

  <u>Methods:</u> DNA from fish from nine sites with be extracted and genetic diversity on whole genome (AFLP), a fast evolving mitochondrial gene (*cyt b*-sequencing), CYP1A , rhodopsin (allele frequency) and SNPs will be determined. Diversity expressed with different diversity indices (phylogenetic distance, allele frequency, diversity richness and evenness) will be correlated to contamination load and the other bioindicators used in the project.

  <u>Expected outcome</u>: An assessment of the use of genetic diversity as a tool for monitoring.

WP3. Apply existing and new tools in field studies of eelpout…

- **<u>Task 3.6</u> Application of gene arrays in eelpout in different Baltic populations**

  Responsible partner: **P1** (ZGU). *Contributing partner:* **P3, P4, P6, P7**

  <u>Aim:</u> To explore gene responses to pollutants in female eelpout and larvae with reproductive performances in the eelpout in its natural environment.

  <u>Methods:</u> The viviparous eelpout are collected from selected polluted coastal sites during autumn when the females carry embryos. We will apply the validated eelpout mRNA microarray developed in Task 2.1.

  <u>Expected outcome:</u> To link molecular responses to pollutants with the individual reproductive performance in a Baltic fish species in its natural environment, thus anchoring the significance of molecular changes, and if feasible to compare expression profiles in eelpout mother fish and their offspring.

  <u>Task 3.7</u> Comparative studies with stickleback in different coastal sites in the Baltic.

  Responsible partner: **P5** (SLU). *Contributing partner:* **P1, P2, P3, P4**

  <u>Aim:</u> To investigate if different three-spined stickleback (*Gasterosteus aculeatus*) populations are affected by chemical exposure and if this can be linked to reproductive health.

  <u>Method:</u> Three-spined stickleback are sampled at selected sites. Gonads are examined by microscopy to verify the reproductive status. The histopathology is based on relatively pragmatic endpoints such as intersex and staging of germ cells in ovaries and testis.

  <u>Expected outcome:</u> The results from histopathology of gonads will provide important information on the reproductive health in polluted and “clean” sites.

WP3. Apply existing and new tools in field studies of eelpout…

- **Task 3.8 Comparative studies with flounder in different coastal sites in the Baltic.**

  Responsible partner: **P1** (ZGU). *Contributing partner:* **P2, P3, P4, P7**

  Aim: To study pollutant responses in flounder (*Platichthys flesus*) from different costal sites in the Baltic Sea.

  Method: Flounder is a common indicator fish species in European monitoring of contaminants and pollutant impact. Here we will apply a set of classical biochemical and histopathological biomarkers.

  Expected outcome: To compare and anchor significant responses to pollutants between eelpout and flounder in field studies

WP4. Confirm laboratory studies and validate extrapolations between species

- **Task 4.1 Laboratory exposure studies using three-spined stickleback.**

  Responsible partner: **P5** (SLU). *Contributing partner*: **P1, P6, P7**

  Aim. To provide information to explain observations from field studies by experimental exposure in order to provide data for risk analysis of chemicals.

  Methods: Three-spined stickleback will be exposed during the period of sexual maturation either trough contaminated feed or via the water. When the fish are sexually mature reproductive health is evaluated by studies of nesting, spawning frequency, fecundity, fertilisation, embryonic development, hatching and survival. The adult fish are sampled for studies of gonad histopathology, and other relevant biomarkers.

  Expected outcome: Identification of risk chemicals that affects reproductive health of Baltic fish.

- **Task 4.2 Laboratory exposure studies using zebrafish.**

  Responsible partner: **P5** (SLU). Contributing partner: **P1, P7**

  Aim. To explain observations from field studies by experimental exposure in order to provide data for risk analysis of chemicals.

  Methods: Zebrafish will be exposed during the period of sexual maturation either trough contaminated feed or via the water. When the fish are sexually mature reproductive health is evaluated by studies of spawning frequency, fecundity, fertilisation, embryonic development, hatching and survival. After the spawning period the adult fish are sampled for studies of gonad histopathology, and other relevant biomarkers.

  Expected outcome: Contribute to the knowledge why certain Baltic fish species are affected by poor reproduction by identification of risk chemicals.

WP5. Link gene responses to population effects

- **Task 5.1 Individual-based population dynamic model for eelpout.**

  Responsible partner: **P3** (SBF). *Contributing partner:* **P1, P2, P4, P5**

  Aim: Develop a population model to simulate changes in eelpout population dynamics under different environmental scenarios.

  Methods: Based on existing and additionally sampled data on reproduction, growth and mortality an individual based model (IBM) for eelpout population dynamics will be developed. The model will be verified in at least two areas in the Baltic Sea. It will be used for simulating the effects of different types of disturbances on population dynamics.

  Expected outcome: A population model for predicting the effects of different substances at different complexity levels (organ, individual) at the eelpout population dynamics.

WP6. Bridge the gap between scientists, stakeholders and managers

- **<u>Task 6.1</u> Environmental assessment tools for biological effects in eelpout**

  Responsible partner: **P2** (NERI). *Contributing partner:* **P1, P3, P4, P5, P6**

  <u>Aim:</u> To develop and evaluate environmental indices including derivation of reference values/base lines and assessment criteria for biological effects in eelpout.

  <u>Methods:</u> Applying principles for derivation of assessment criteria suggested for integrated monitoring of contaminants and their effects in coastal and open-sea areas (ICES 2006).

  <u>Expected outcome:</u> 3 – 5 indicators for biological effects in eelpout including reproduction and an integrative eelpout health index.

- **<u>Task 6.2</u> Workshops in conjunction with field sampling campaigns and coordination meetings**

  Responsible partner: **P1** (ZGU). *Contributing partner:* **P2, P3, P4, P5, P6, P7**

  <u>Aim:</u> Coordinate and when nessecary intercalibrate current and new methods applied in field sampling, analyses, data handling and reporting for BALCOFISH consortium participants.

  <u>Method:</u> All BALCOFISH partners contribute with their respective competence and expertise in a mutual field sampling campaign in a selected Baltic coastal site for example at the Danish and German coasts (e.g. Roskilde Fjord and Wismar Bay) where high frequencies of malformed eelpout embryos, intersex and atresia have been detected or close to a pulp mill along the Swedish coast where male biased brood of eelpout embryos repeatedly have been found.

  <u>Expected outcome:</u> Transfer and exchange of best available knowledge to monitor contaminants and effects in eelpout in polluted coastal sites in the Baltic Sea.

WP6. Bridge the gap between scientists, stakeholders and managers

- **Task 6.3 Retrospective analyses of existing contaminants data in eelpout stored in biobanks**

  Responsible partner: **P6** (SMNH). *Contributing partner:* **P1, P2, P3, P4**

  Aim: Study temporal changes that will increase the chance to find causal relationships between contaminant exposure and physiological effects.

  Method: Retrospective analyses of existing contaminants data in eelpout stored frozen in specimen banks. The results will be evaluated by pattern analyses and other multivariate techniques.

  Expected outcome: Indications or significant correlations indicating causal relations between specific xenobiotics and physiological effects.

WP6. Bridge the gap between scientists, stakeholders and managers

- **<u>Task 6.4</u> Workshops for scientists and environmental managers,**

  Responsible partner: **P1** (ZGU). *Contributing partner:* **P2, P3, P4, P5, P6, P7**

  <u>Aim:</u> Transfer and exchange of knowledge between the BALCOFISH partners and a broad audience of Baltic scientists, managers and stakeholders.

  <u>Method:</u> Arranging workshops with themes related to integrated approaches for monitoring contaminants and effects using the viviparous eelpout and other fish species in the Baltic Sea. The plan is to invite key representatives from academia, authorities and other interested organisation from the Baltic region to participate and discuss and identify and unravel possible cultural dilemmas between and in science and management of the Baltic Sea. This is planned in autumn 2011. A joint workshop with the the German UBA is scheduled for March 2008.

  <u>Expected outcomes:</u> To form a basis to establish a network of fish scientists and environmental managers

- **<u>Task 6.5</u> Special session at SETAC conference**

  Responsible partner: **P1** (ZGU). *Contributing partner:* **P2, P3, P4, P5, P6, P7**

  <u>Aim:</u> Present data at SETAC conferences.

  <u>Method:</u> Members of the BALCOFISH consortium participate in the organisation of SETAC 2009 to be held in Göteborg, Sweden. This conference will most likely have some Nordic oriented themes. We will apply for sessions focusing on the use of indicators species such as eelpout for assessing acceptable impact of pollutants in Baltic coastal areas.

  <u>Expected outcome</u>: Sessions in future SETAC meetings.

WP6. Bridge the gap between scientists, stakeholders and managers

- **Task 6.6 Disseminate data at meetings on endocrine disruption, ecosystem health and coastal fisheries.**

  Responsible partner: **P1** (ZGU). *Contributing partner:* **P2, P3, P4, P5, P6, P7**

  Aim: Present results and achievements from the BALCOFISH project.

  Method: To meet interested groups in meetings and workshops to discuss science-based knowledge in the management of the sea, and science handling of urgent needs and "real world" problems formulated by managers and societal awareness.

  Expected outcome: Closing gaps between science and management about environmental issues in the Baltic Sea.

- **Task 6.7 BALCOFISH website**

  Responsible partner: **P1** (ZGU). *Contributing partner:* **P2, P3, P4, P5, P6, P7**

  Aim: Create a website.

  Method: Prepare a website with all relevant information and results from the BALCOFISH project. Update the website continuously.

  Expected outcome: Communicating results and other information to the general public.

WP6. Bridge the gap between scientists, stakeholders and managers

- **<u>Task 6.8</u> ICES guideline for eelpout monitoring within HELCOM COMBINE**

  Responsible partner: **P2** (NERI). *Contributing partner:* **P1, P3, P4, P5, P6, P7**

  <u>Aim:</u> To publish a guideline for biological effects monitoring in eelpout with focus on the methods recommended for HELCOM COMBINE.

  <u>Method</u>: The guideline will be reviewed and approved by ICES working group for biological effects of contaminants (WGBEC) before publishing.

  <u>Expected outcome</u>: Published as an ICES Techniques in Marine Environmental Sciences (TIMES), online version.

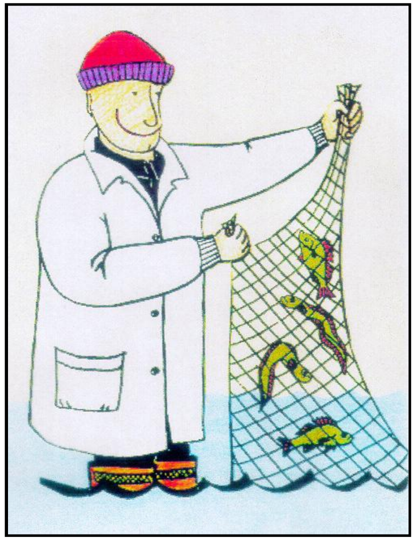
- **<u>Task 6.9</u> Submission of relevant eelpout data to ICES's data base**

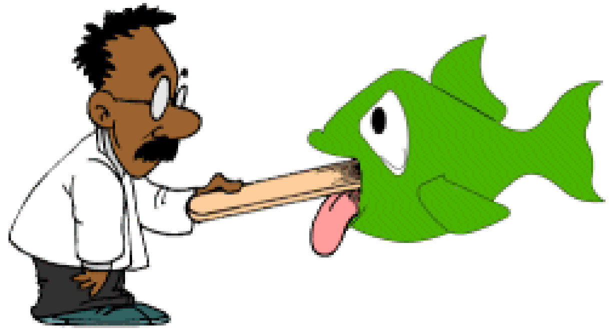
  Responsible partner: **P2** (NERI). *Contributing partner:* **P1, P3, P4, P5, P6**

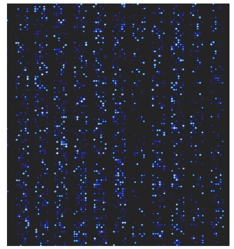
  <u>Aim:</u> To submit relevant eelpout data compiled and generated in the BALCOFISH project to the ICES Data Centre Environment Data.

  <u>Method:</u> Translation of the project data base into corresponding ICES codes. <u>Expected outcome:</u> Delivery of Danish, Swedish and German data on biological effect monitoring in eelpout to the ICES database.

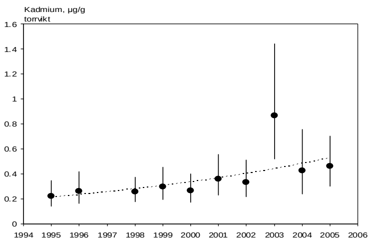
- **<u>Task 6.11</u> Yearly Balcofish reports: Year 1 report.**
- **<u>Task 6.12</u> Yearly Balcofish reports: Year 2 report.**
- **<u>Task 6.13</u> Yearly Balcofish reports: Year final report.**

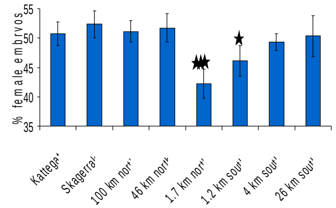






Thank You!


Kadmium, µg/g torrvikt
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006


% female embryos
55
50
45
40
35
Kattegat
Skagerrak
100 km nort
46 km north
1.7 km nort
1.2 km sout
4 km sout
26 km sout