Contaminant effects on fish: Responses at the individual and population levels

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SLU, 2014-11-19

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Outline

- Introduction
- Monitoring near point sources
- Integrated monitoring of coastal fish in Sweden
- Case study ”Focus Kvädöfjärden”, a follow-up project
- Future

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FISH TOXICOLOGY

- Mechanisms of chemical toxicity in fish
- Effects of pollutants/contaminants in fish
- Identify sources of hazardous substances
- Field studies (Field monitoring)

- Biomarkers

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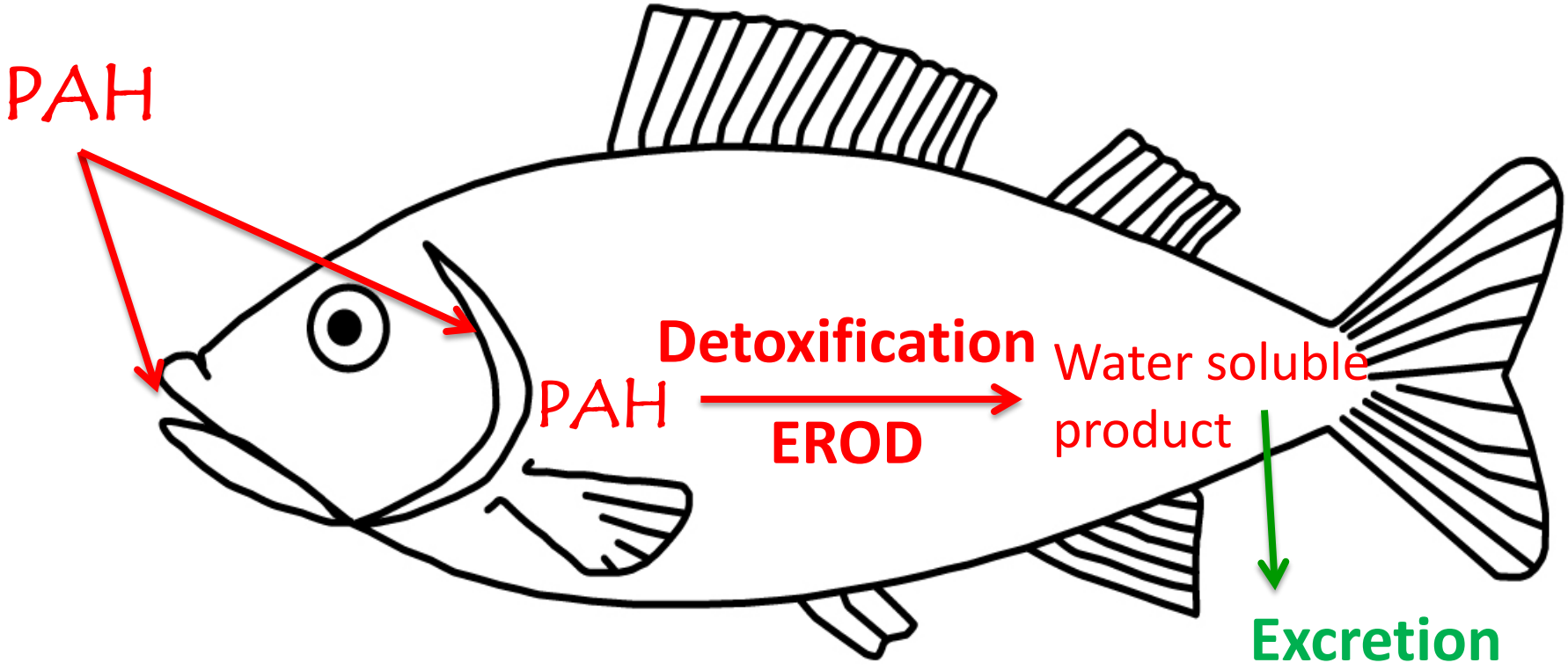
Biomarker: EROD activity

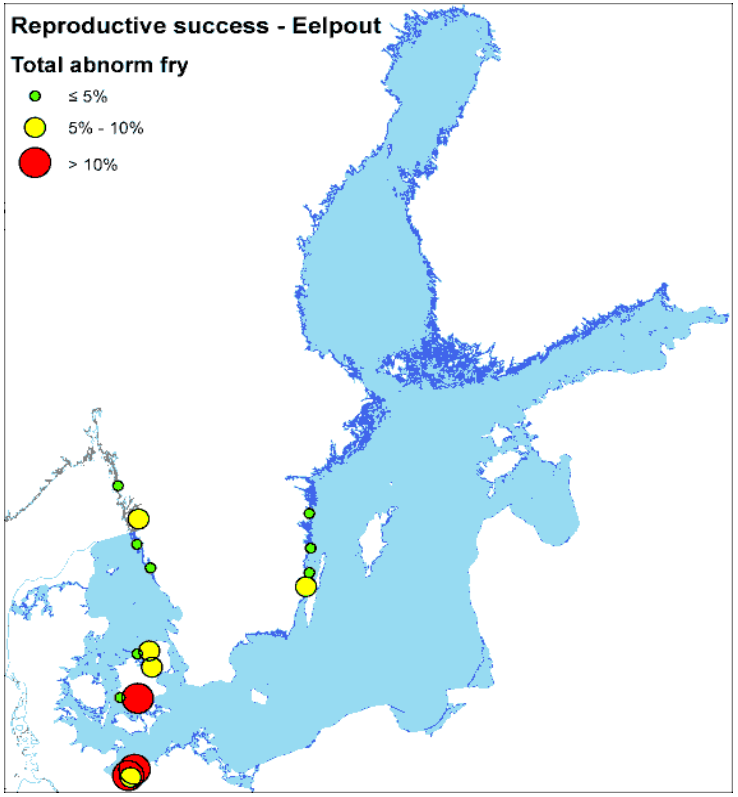


EROD activity can increase as a respons to exposure to PAH (polyaromatic hydrocarbons)

Biomarker:
Reproductive success in eelpout (Zoarces viviparus)

Indicator	Parameter	BAC	EAC
Reproductive success impairments caused by a range of contaminants	Malformed fry (mean frequency)	1%	2%
	Late dead fry (mean frequency)	2%	4%
	Early dead fry (mean frequency)	2.5%	5%
	Total abnormal fry (mean frequency)	**5%**	**10%**

Proposed Background Assessment Criteria (BAC) and/or Environmental Assessment Criteria (EAC) for reproductive success in eelpout

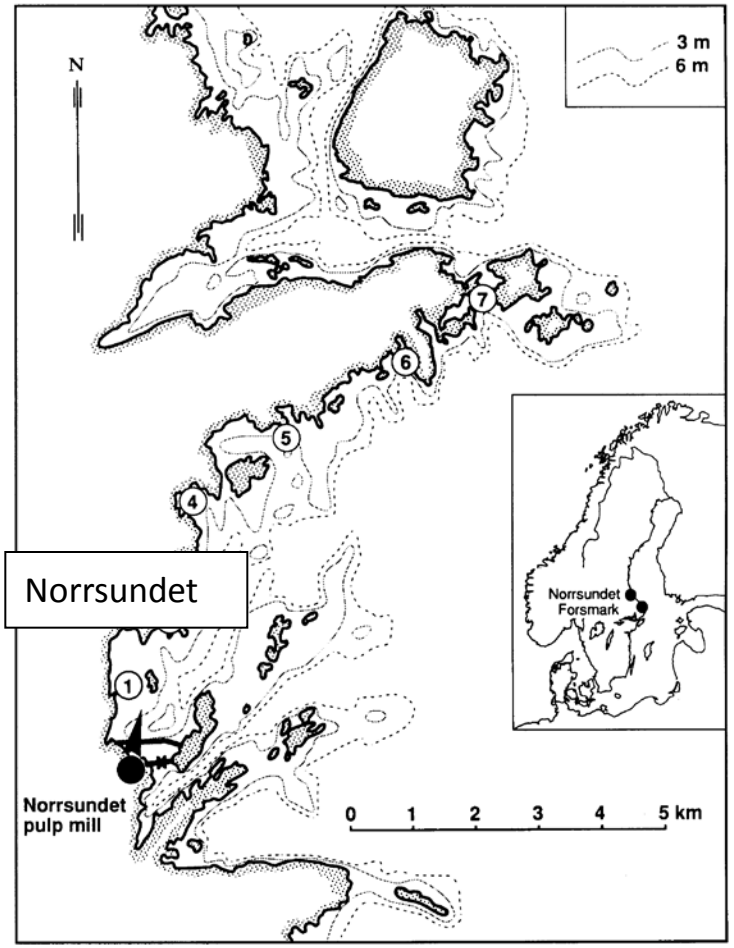


From Jakob Strand et al., Aarhus University

Studies of effects in fish by pulp mill effluents:

Swedish studies started in early 1980s in Norrsundet´s pulp mill

Perch were selected for the individual health and biomarker studies.



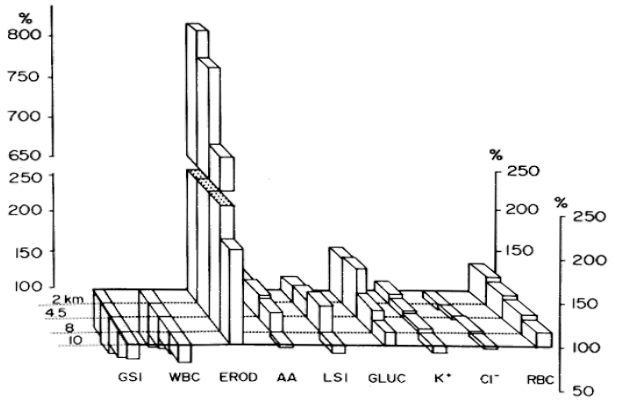
Health variables (biomarkers) studied in the early 1980s studies:

- Growth/condition/energy metabolism
  - growth rate and CF
  - LSI
  - glycogen and lipids
- Liver functions
  - detoxification enzymes (e.g. EROD)
  - Ascorbic acid
  - histology
  - glycogen and lipids
- Reproduction
  - GSI
  - Age/size at sexual maturity
  - Sex hormones
- Immune defence
  - White blood cell count
- Pathology/hematology
  - Fins and skeletal
  - Ht, Hb
  - Plasma ions

The results indicated that pulp and paper mill effluents:

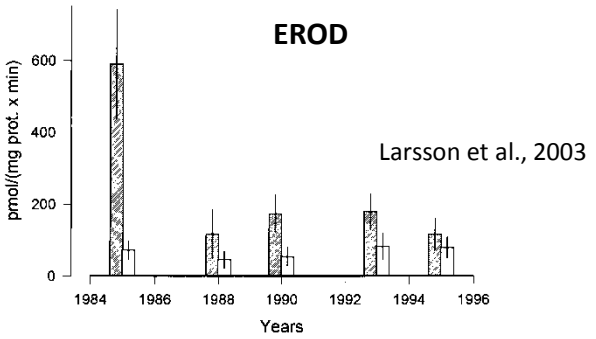
- caused a wide spectrum of responses/effects including
  - *reduced gonad weight and sex steroid levels*
  - *induction of EROD*
  - *metabolic disturbances*
  - *suppressed immune defence*
  - *disturbed plasma ion balance*
  - *affected red blood cell picture*
  - *pathological abnormalities*

- and indicated <u>disturbed perch population</u>
  - *delayed sexual maturity*
  - *impaired fry production*
  - *growth disturbance*
  - *increased fish mortality*
  - *low abundance*



Andersson et al., 1988

Continued studies with pulp and paper mill effluents indicate:

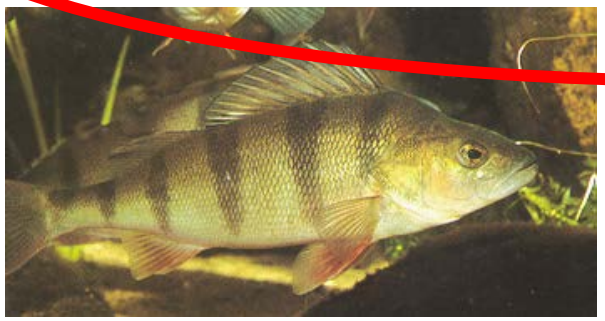


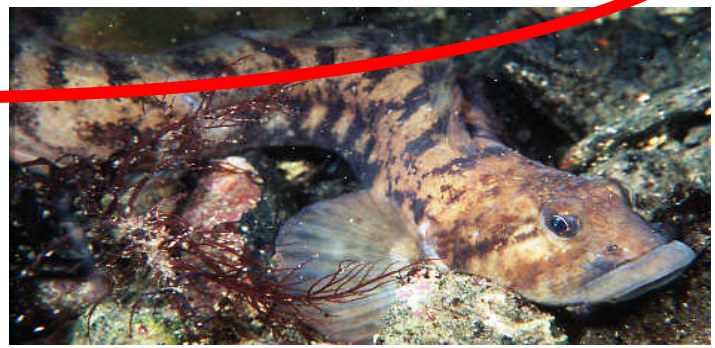
Larsson et al., 2003

- Improved fish health during 1990s.
- Remaining effects include disturbed
  - *growth*
    - stimulated and reduced
  - *immune defence*
    - stimulated
  - *reproduction*
    - Small gonads in females
    - Altered steroid levels
    - Delayed maturation
    - Masculinisation
    - Altered vitellogenin levels
  - *recruitment*
    - Still impaired fry production

Summary

- Severe effects during the 80's
- Large improvements in fish health during the 90's
- Still effects on reproduction, growth and immune defence
- These studies form the basis for national recommendations of fish health/biomarkers studies near pulp and paper mills



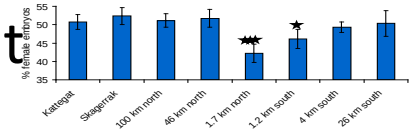


Case studies using eelpout for monitoring point sources

- Complex chemical industry effluents affect embryo development

Vetemaa et al. 1997. J Aquat. Ecosys. Stress Recov. 6, 33.

- Pulp mill effluents caused male biased eelpout embryo sex ratio



Larsson et al. 2000. Environ. Toxicol. Chem. 19, 2911.

Larsson and Förlin 2002. Environ Health Perspec. 110, 739.

- Dredging affected eelpout health-indices

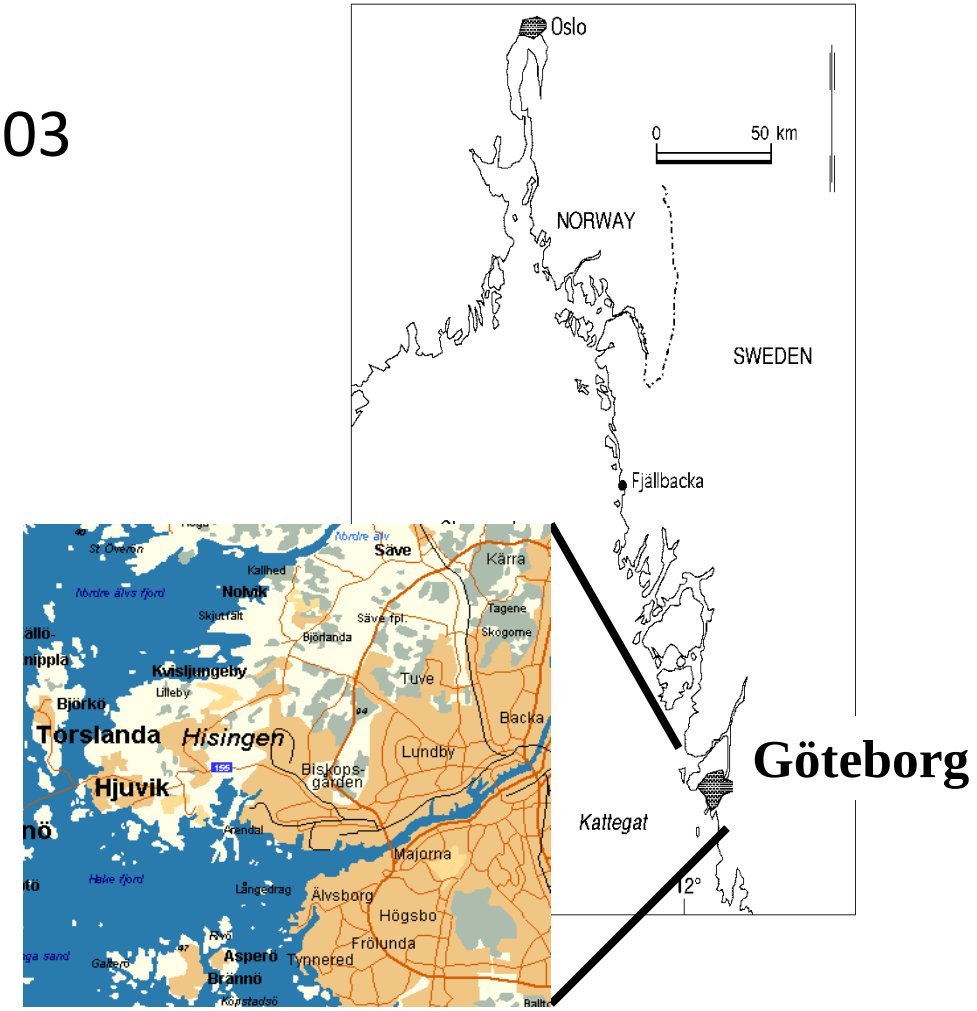
Sturve et al., 2005. Environ. Toxicol. Chem. 24, 1951.

- Bunker oil spill markedly affected fish health

Sturve et al., 2014. Environ. Sci. Pollut. Res.
DOI 10.1007/s11356-014-2890-z

Dredging activities in Göteborg harbour

- Started during winter 2003
- 12 milj tonnes of clay
- 0.5 milj tonnes of rock
- Ended November 2003

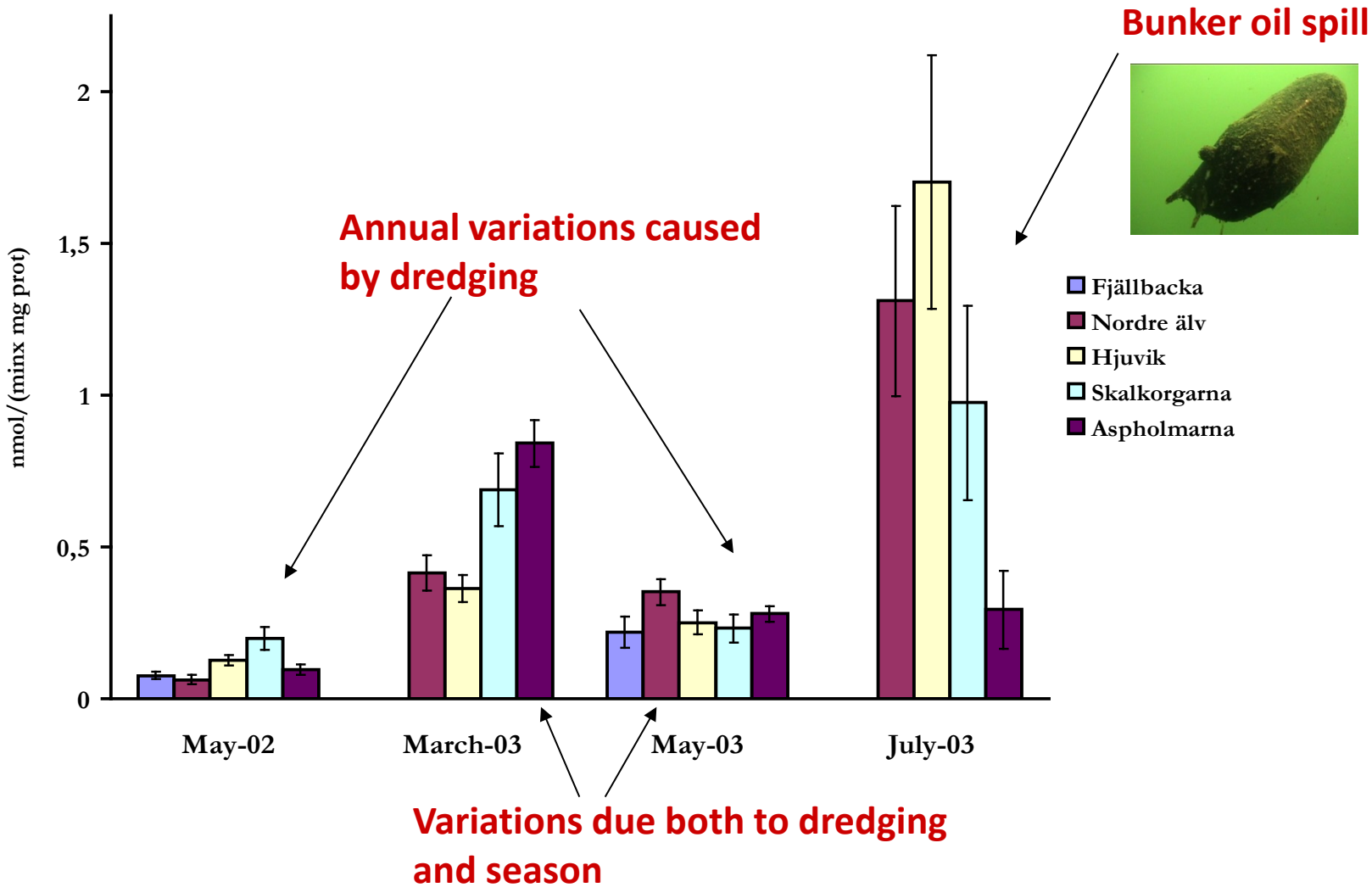


Bunker oil spill in Göteborg harbour

June 23, 2003

- 10-100 tons
- ca 25% PAH

EROD activities in female eelpout from Gothenburg harbour area and Fjällbacka



More results from the Gothenburg harbour studies

- Increased DNA adducts (genotoxicity)
- Increased metallothionein levels (metal exposure)
- Decreased lysosomal stability (general cellular toxicity)
- Oxidative stress symptoms (reactive compounds)
  - Increased glutathion reductase activities
  - Increased glutathion levels
  - Increased lipid- and protein oxidation

From these studies we concluded that

- The dredging activities had a an **impact in fish** indicated by responses in many biomarkers (EROD, DNA adducts, MT, lysosomal stability, protein oxidation etc.) in the Gothenburg harbour

- The bunker oil spill had a **marked impact on fish health** indicated by **strongly affected biomarker** responses (increased EROD activity, DNA adduct levels, lipid peroxidation, decreased lysosomal stability), in the Gothenburg harbour area. The high PAH exposure in the inner parts of the harbour probably inhibited the EROD activity

- Fjällbacka vs Gothenburg harbour: Significant biomarker responses (EROD, DNA adducts, lysosomal stability) at Fjällbacka during dredging may indicate a **large scale transport** of dredging materials along the Swedish west coast.

Integrated fish monitoring in coastal waters…

- is a biomonitoring strategy supported by the Swedish EPA including
  - Individual fish health studies (e.g. biomarkers)
  - Fish ecology (e.g. abundance, recruitment, reproductive success)
  - Environmental chemistry
- with Perch and Eelpout as selected fish species
- is annual studies that started in 1988

Participating laboratories are from:

University of Gothenburg

Swedish University of Agricultural Sciences (SLU)

Swedish Museum of Natural History

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Purpose…

is to provide a framework for assessment of ecosystem health:

- Monitor long term time trends
- Provide data for comprehensive/integrated intrepretations
- Provide data on natural variations
- Act as ”watchdog” for banned or new risk compounds
- Provide reference data for local and regional monitoring
-

Sandström et al., 2005. Wat. Qual. Res. J. Can. 40(3) 233.

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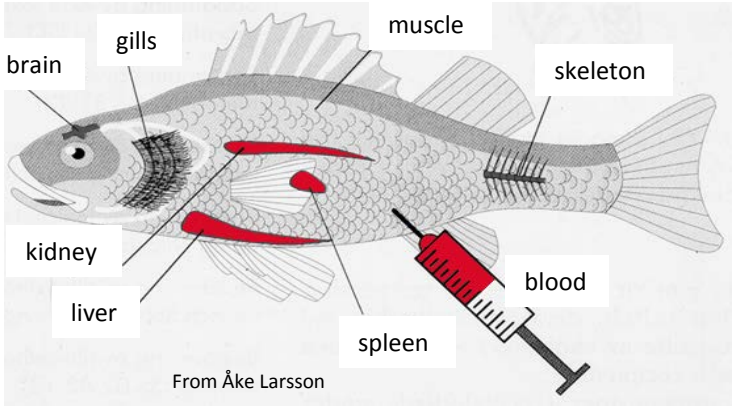
Investigations of fish health

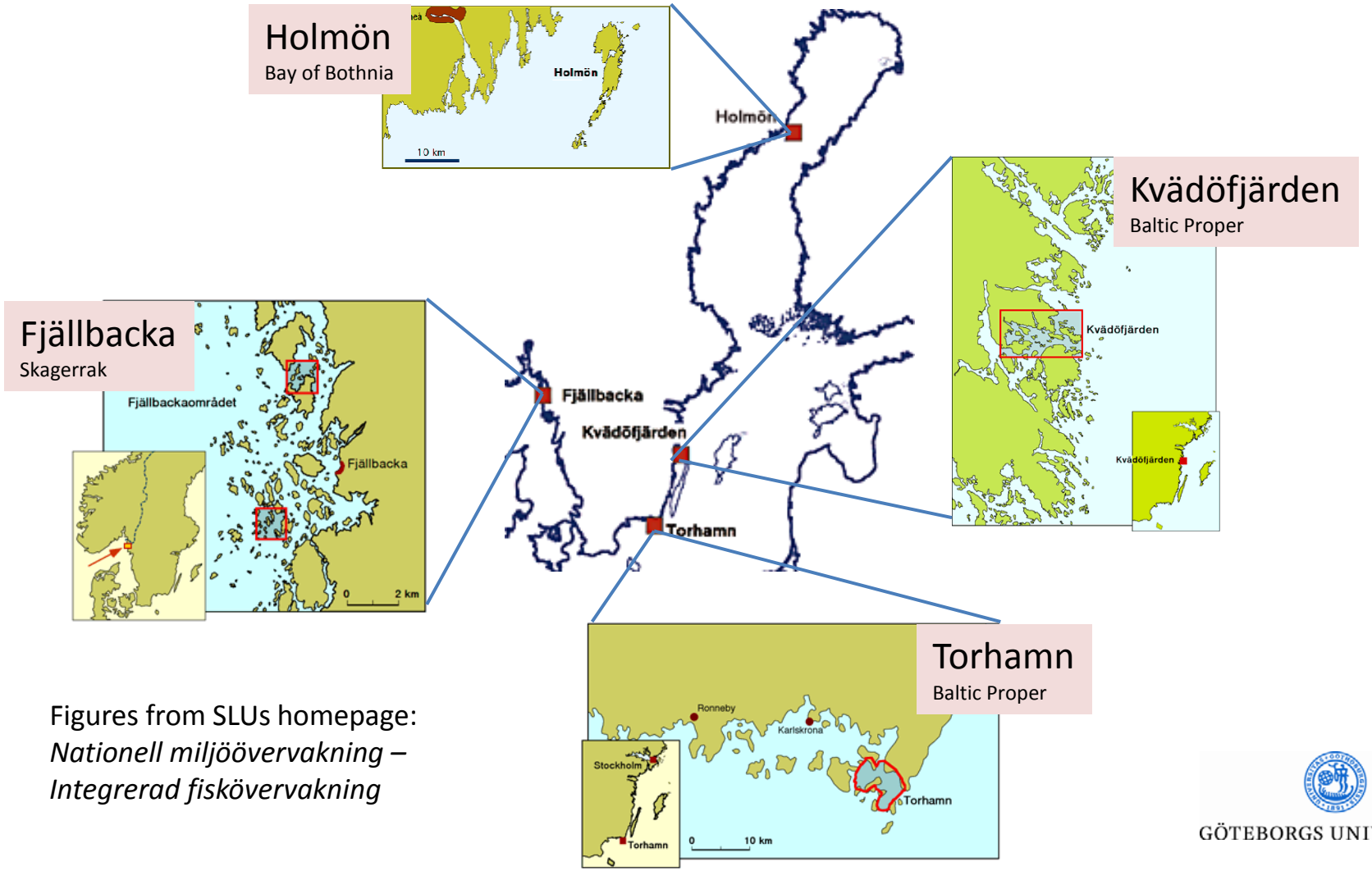


- Fish health investigations have been done for more than 30 years in Sweden to study effects of pollutants in the aquatic environment
- Analytical programme contains 20-30 established biochemical, physiological and histological variables so called biomarkers.
- The analytical programme makes it possible to trace ”early signals” of pollutants
- The biomarker approach can be used both in field studies in polluted sites and in studies in the laboratory where fish can be exposed to single chemicals/pollutants or mixtures of compounds including complex industry effluents etc.

The varibles (biomarkers) reflects different functions in the fish

Function	Variable / biomarker
Energi, growth, condition	Total body weigth, somatic weigth, lenght, age, condition index
Reproduction, endocrine disruption	Gonadsomatic index (GSI), vitellogenin i bloodplasma, sex ratio in eelpout larvae
Liver function, detoxification, oxidativ stress	Liver somatic index (LSI), liver histologi, EROD-activity, glutation reductase, glutation *S*-transferase, catalase.
Genotoxicitet	DNA-adducts in liver
Indicator of metal exposue	Metallothionein in liver
Corbohydrate metabolism / stress	Blood glucos, blod lactate
Oxygen transport	Hematocrite, hemoglobine, immature red blood cells
Immune defence, tissue damage	White blood cells; lymphocytes, granulocytes, trombocytes
Salt balans, cell damage	Chloride, sodium, potasium and calcium in blood plasma

Integrated monitoring in four coastal reference sites



Figures from SLUs homepage:
Nationell miljöövervakning –
Integrerad fiskövervakning

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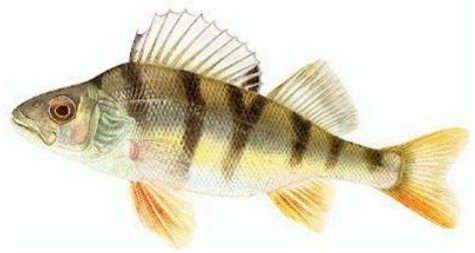
The health status in Perch and Eelpout from national coastal reference sites in Skagerrak, Baltic proper and Bay of Bothnia

- Many of the health indicators (biomarkers) do not indicate any changes or time trends in the four coastal sites during the time period, 1988-2013.
- BUT, more and more of the health indicators (biomarkers) show significant time trends during the last years. The changes seem to suggest that the fish are exposed to one or more pollutants and/or affected by other environmental factors.
- The effects/changes are most pronounced in Perch from Kvädöfjärden and Eelpout from Fjällbacka, but similar trends are seen in all four coastal sites.

Here follows some examples…



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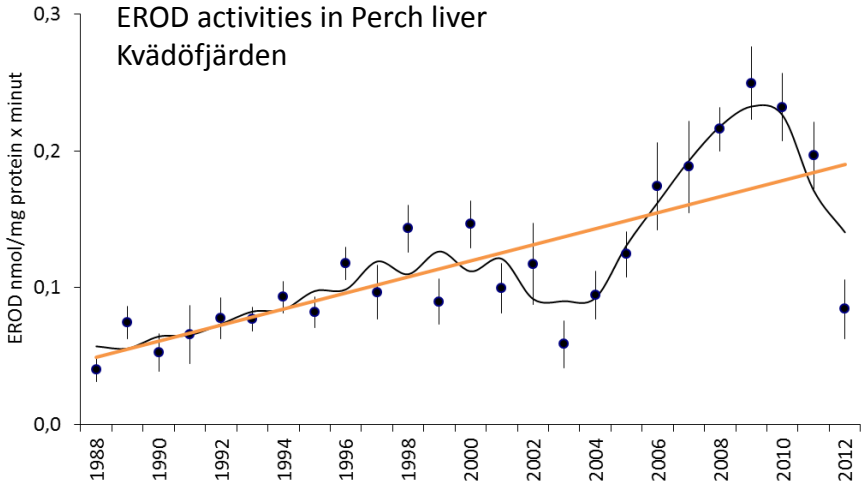
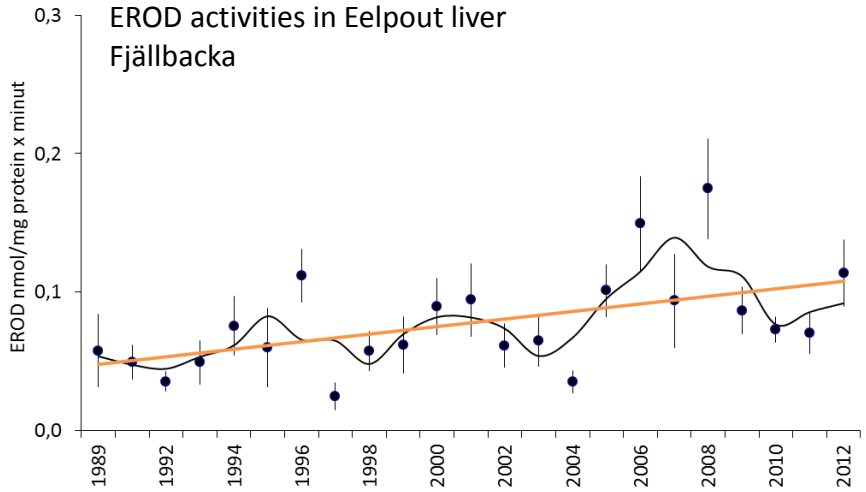
The EROD-activity changes over time seem to indicate increasing exposure to pollutants, and induced detoxification



EROD activities in Perch from Kvädöfjärden (Baltic Proper) were 4-5 times higher in 2010 than in 1988 (when the studies started). This may indicate exposure to potent chemicals (e.g PAHs). In 2012 a sharp trend change is seen.

Similar trend changes occur in Perch also from Holmön and Torhamn (the other two coastal reference sites).



EROD-activities in eelpout from Fjällbacka also show an increase over time (Skagerrak, The North Sea). Similar trend is also seen in eelpout from Kvädöfjärden.

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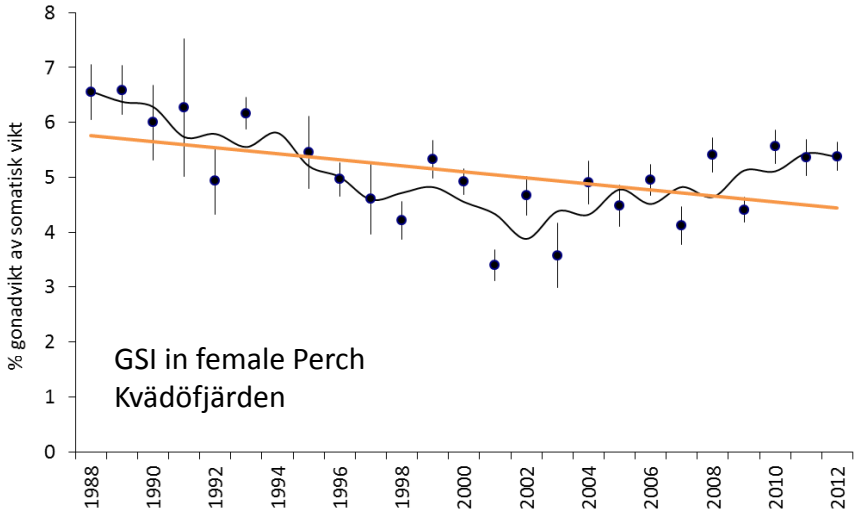
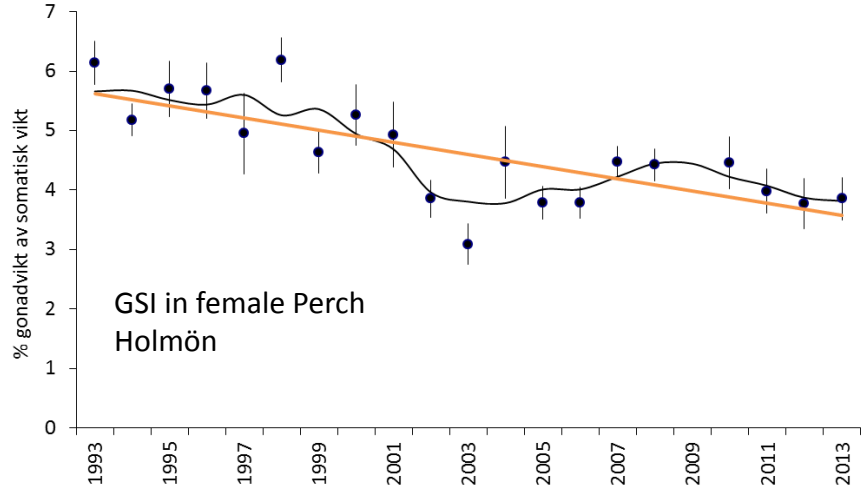
Since 1990:ties the relative gonad size (GSI) has decreased in female perch from Kvädöfjärden and Holmön





GSI decreased ca 30% in female perch from Kvädöfjärden during the period 1990-2004. From 2004 to today GSI show a tendency to increase again.

Smaller gonad sizes signal inhibited or delayed gonad (ovary) development.

Data also indicate fewer eggs i.e. impared fecundity

GSI has decreased significantly (25%) during the time period in female perch from Holmön.

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More biomarkers show significant time trends signalling that coastal fish (perch and eelpout) in Swedish national reference sites are affected….

- Significant **increase of lymphocytes** in eelpout from both Fjällbacka och Kvädöfjärden. Also in perch from the coastal sites show similar changes in the WBC picture. These changes seem to indicate activation of the immune defence.

- **Increases in blood plasma chloride**, in perch and eelpout from Kvädöfjärden, provide evidence for problem with salt (electrolyte) reglulation

- **Increase in calcium levels (blood plasma)** in coastal fish from all sites strengthen the suggestion that the fish show sign of impaired ion regulation

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More biomarkers show significant time trends signalling that coastal fish (perch and eelpout) in Swedish national reference sites are affected….

- **Smaller numbers of immature red blood cells** in perch from Kvädöfjärden and Torhamn indicate lower new production of red blood cells

- **Situation for eelpout is ”problematic” in the Fjällbacka area** suggested by smaller stocks, inferior condition, impaired larvae status (deformities, dead larvae) and impaired health

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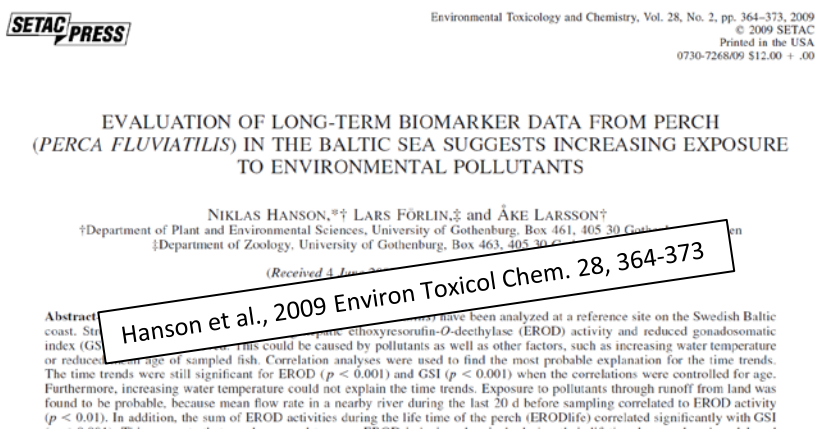

Are Swedish coastal fish healthy?

Fish from “clean” coastal reference areas show more and more signs of exposure to chemical substances and impacts on several physiological functions such as:

- Reduced gonad sizes (GSI)
- Induced detoxification system (EROD)
- Activated immune system (WBC)
- Increased oxidative stress
- Reduced formation of red blood cells
- Impacted salt regulation and metabolism
-

This multi-faceted symptom picture is observed in two coast fish species (perch and eelpout) and in four different reference areas with some variation in effect patterns and strengths

sources in the Baltic Sea. Fish were sampled at three coastal sites in Sweden: two in the Baltic Proper (since 1988) and one in the Bothnian Bay (since 1993). In all, 19 biochemical, physiological and histopathological variables were measured. By reflecting central functions of life, such as red blood cell functions, immune defence, liver function, biotransformation processes, intermediary metabolism, ion balance, and reproduction,

Focus Kvädöfjärden – What causes the deterioration of coastal fish health?

Project supported by HaV (Swedish Agency for Marine and Water Management)

Figure from SLUs homepage:
Nationell miljöövervakning – Integrerad fiskövervakning

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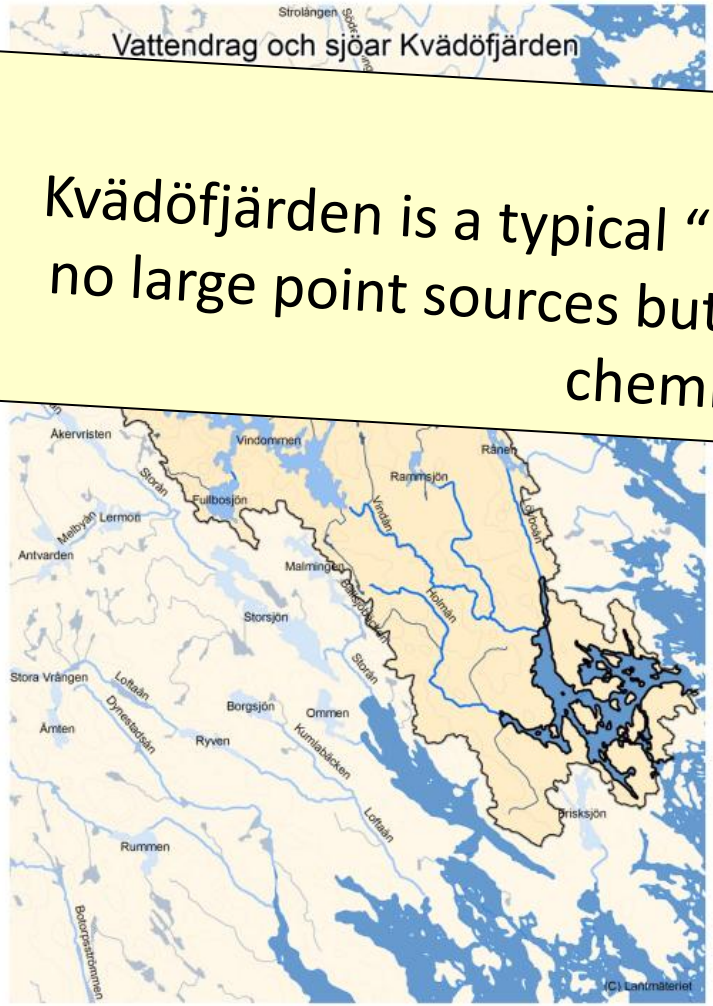
Mapping of catchment area, Kvädöfjärden



In short:

Kvädöfjärden is a typical “low burdened” catchment area with no large point sources but with many small diffuse sources of chemicals/pollutants

– Vindan – Ruggebosj
– Holmån – Lindödjupet

- Many water ways and lakes are eutroficated

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What pollutants are found in fish from Kvädöfjärden?

In short the inventory shows that

- the last 10 years Cd, Hg, Cu, fluorene och benzo(a)antracene have increased in fish or other organisms from Kvädöfjärden or Landsort (in Baltic Sea)
- Also many perfluorinated substances (PFAS), and polybrominated compounds (PBDE) (especially OH-PBDE) and siloxanes have increased
- In addition it is indicated that organophosphate esters and adipates must be monitored

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Toxic effects and effect patterns by chemical-mixtures and single chemicals in fish

Short about effects of **complex mixtures**

There are qualitative similarities between changes in physiological functions in fish from Kvädöfjärden and

- functional disturbances in fish in **complex polluted areas**
- known effects caused by ”old” pollutants such as **PCBs and DDT**
- and **PAHs** broad effect pattern

organiska te…

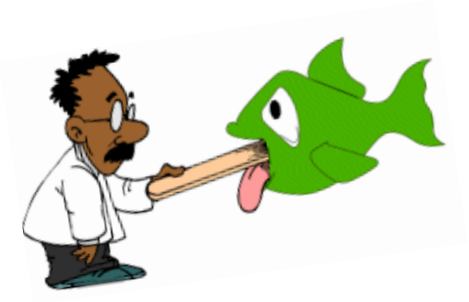
- **Slutsatser**

Overall assessment and conclusions

The results from the project show that, based on current knowledge, it is **not possible to find any simple** explanation/causation for the observed deterioration (impairment) of fish health in coastal fish from Kvädöfjärden or from the other three Swedish national reference sites (Holmön, Torhamn och Fjällbacka).

The different issues handled in the project have given the following conclusions/suggestions:

- It is **not likely that any single chemical** has caused the changes seen in health status in coastal fish. It is more likely that the observed changes in health status is caused by the continous and varied exposure to mixtures of chemicals acting together.



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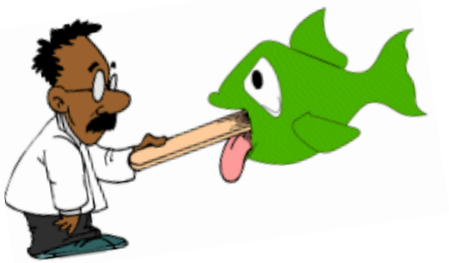
Overall assessment and conclusions

CONT.. ….have given the following conclusions/suggestions:

- there are no large point source of chemicals in Kvädöfjärden, but a number of diffuse sources that can result in distribution of chemicals in the area.
- there are a number of chemicals with known effect pattern, such as PAHs, PFAS-compounds and cadmium, that can cause the same/similar physiological changes as have been observed in the coastal fish (over time)
- exposure to biogenic and anthropogenic polybrominated diphenyleters (PBDE) and dioxins may affect the fish health status. It is important to study OH-PBDEs, because content of OH-PBDEs increase in the Baltic Sea



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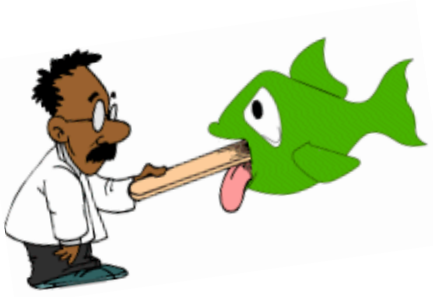
Overall assessment and conclusions

CONT.. ….have given the following conclusions/suggestions:

- an unexpected increase in **”old” pollutants** in fish from Kvädöfjärden during the last years, e.g. Hg and some PCBs, may indicate generally increased release of chemicals from e.g. sediments and deposits and thus resulted in an increased exposure of fish and other organisms
- many new chemical groups, such as **organophosphate esters, siloxanes and adipates**, increase in the aquatic environment, also in background/reference areas. Their contribution to the observed effects can not be assessed because lack of knowledge of their environmental effects



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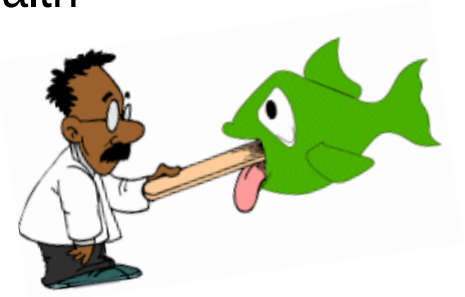
Overall assessment and conclusions

CONT.. ….have given the following conclusions/suggestions:

- also a number of **other environmental factors** such as climate and temperature changes, variation in salinity, bottom fauna changes and changes in food preference and availability can, in different ways, have affected the fish physiology and the pollutant transport in the environment.
- we believe that it is the **continuous and varied exposure to mixtures of chemicals together with changes in environmental factors** such as temperature, salinity and food availability that cause the changes seen in coastal fish health



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Examples how we can/will continue…

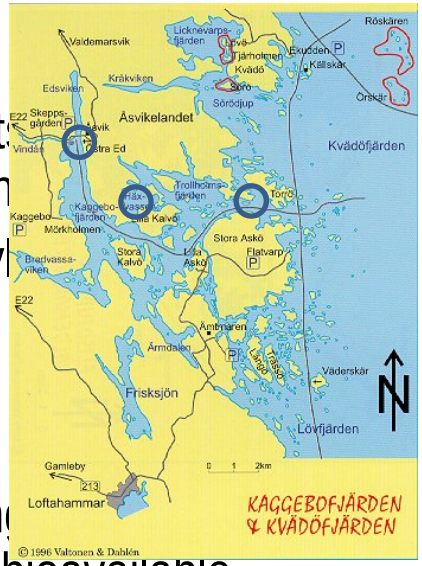
Analyses of stable isotopes (N, C) of two time series of perch from Kvädöfjärden have started

Work with land-sea-gradient has started

hat there still arc ocus to
served cha health in
and other coastal areas.

<u>One priority issue is</u> route of transport and exposure of pollutants
Kvädöfjärden area. This would require studies about the followin

- Are there a land-sea gradient in pollutant pressure respectiv fish?
- Has food availability/choice changed during the time period uptake of different pollutants?
- Has a changed bottom (benthic) fauna community and chan and release of pollutants in sediment made pollutants more bioavailable forcoastal fish?



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Examples how we can/will continue…

CONT:. … to further elucidate causality…:

Another priority issue is to weigh and sum up the significance of the different impact variables (biomarkers), including pollutants, different environmental factors and interaction in the food web, in the assessment of fish health

An additional priority issue is to find out the long term effects of the changed fish health on the fish population levels.

Examples how we can/will continue…

CONT:. … to further elucidate causality…:

It is also very important to retrospective analyse some of the pollutants, not measured today in fish from Kvädöfjärden, that we prioritized such as halogenated dioxins, PFAS-substances, OH-PBDEs, organophosphat esters, siloxanes and adipates.

Finally it is important to fill the knowledge gap of ecotoxicological effects of ”new” pollutants, such as organophosphate esters, siloxanes and adipates.

Finally…

Do pesticides have a role in the oserved health effects in perch from Kvädöfjärden or the other three reference sites (Holmö, Torhamn and Fjällbacka)?

We do not know, but we have not (yet) focused pesticides in these coastal reference sites

Thanks to many

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Elisabeth Nyberg
et al

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Helene Ek

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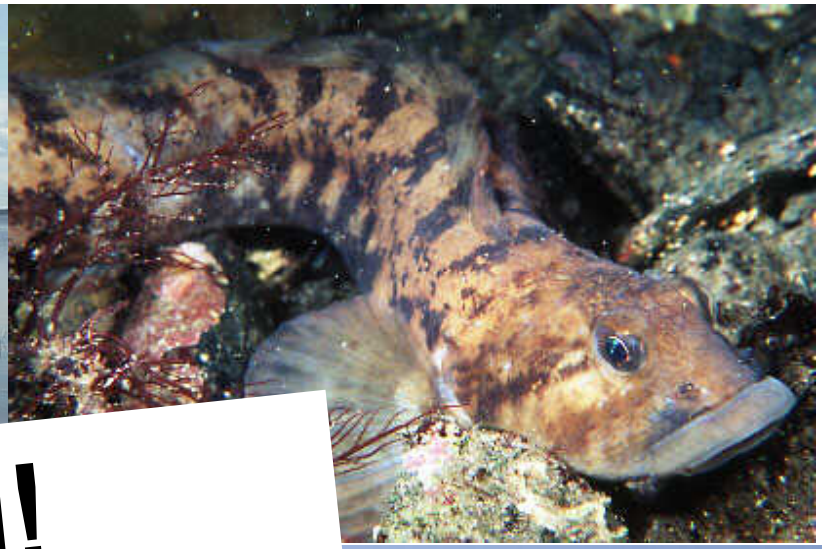
Anders Bryhn, Anna Gårdmark, Jens Olsson et al



Thank you!

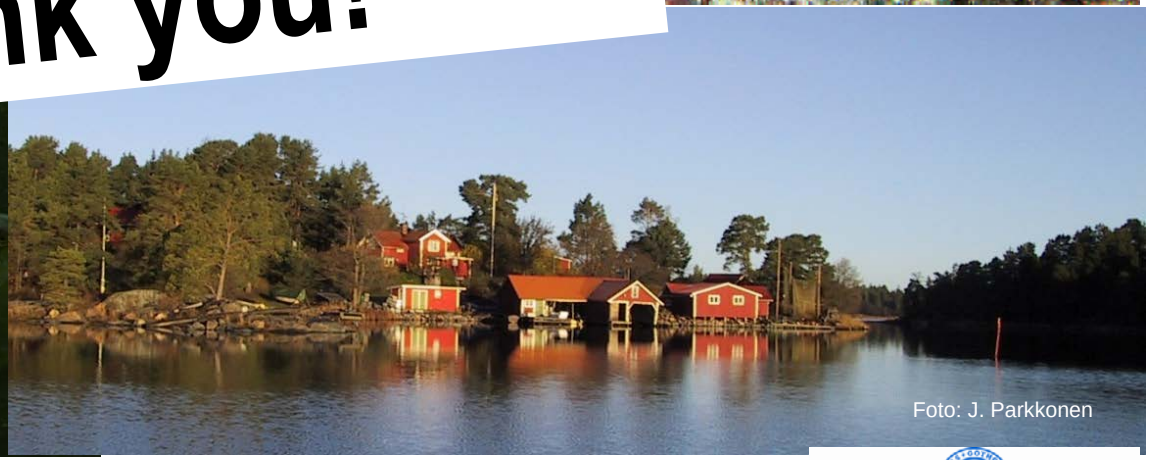


Foto: J. Parkkonen

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