

PAH biomarkers in eelpouts from Danish waters

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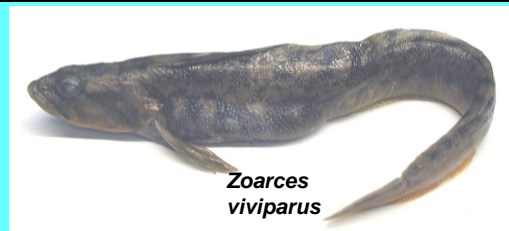
Introduction :

CYP1A enzymes - the group of enzymes, responsible for the metabolism of PAH compounds excreted in fish bile and urine. The enzyme activity assay, ethoxyresorufin-O-deethylase (EROD) as well as PAH metabolites are established biomarkers for PAH exposure and effects in fish.

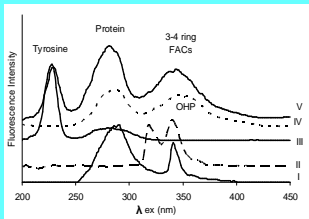
PAH-metabolites in gall bladder bile and urine of the eelpout *Zoarces viviparus* were investigated as potential biomarkers of PAH exposure. Levels of PAH-metabolites in fish urine and bile were compared with effects (CYP1A) in Danish coastal waters.

The aim :

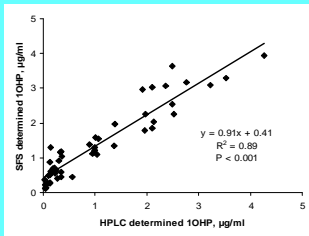
To establish cause and effect relationship between PAH exposure and effects in eelpout.



Methods:

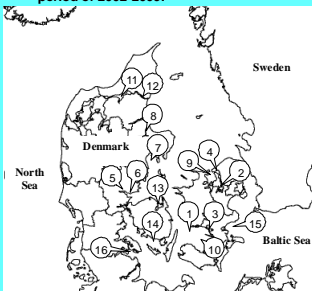


Examples of Synchronous fluorescence spectra (SFS) of: (I) fish bile sample SFS $\Delta\lambda = 42$ nm; (II) 1-hydroxypyrene standard) measured by SFS $\Delta\lambda = 70$ nm; (III) tyrosine standard measured by SFS $\Delta\lambda = 70$ nm; (IV) fish urine sample measured by SFS $\Delta\lambda = 42$ nm; (V) same fish urine sample measured by SFS $\Delta\lambda = 70$ nm.



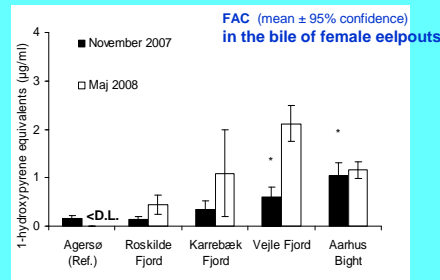
Validation of SFS method with HPLC/F method. Measurements of 1OHP in eelpout bile by both methods show very good correlation, thus SFS is a good screening tool, although much simpler than HPLC/F.

NOVANA sampling locations in Danish coastal waters during the period of 2002-2009:



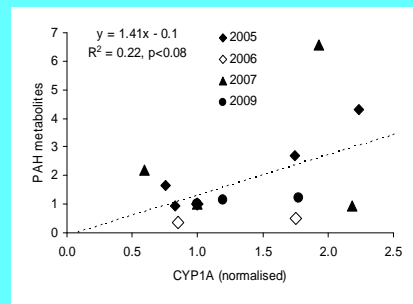
- 1) Agersø
- 2) Roskilde Fjord
- 3) Karrebæk Fjord
- 4) Frederiksværk
- 5) Inner Vejle Fjord
- 6) Outer Vejle Fjord
- 7) Aarhus Bight
- 8) Randers Fjord
- 9) Isefjord
- 10) Krudshoved Odde
- 11) Nibe Bredning
- 12) Langerak
- 13) Odense Fjord
- 14) Nakkebolle Fjord
- 15) Fakse Bight
- 16) Flensborg Fjord

Results:

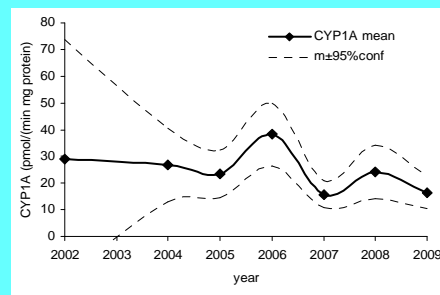


* Significantly different from the reference station Agersø ($p < 0.01$)

Bile from the spring season contained more than factor 2 higher concentrations of PAH metabolites than fish bile collected in the fall.

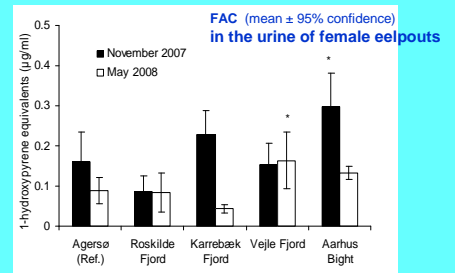


A weak relationship between CYP1A activities and PAH metabolites in bile of eelpouts from November 2005, 2006, 2007 and 2009 was observed, although not significant at 5% level.



Time trend figure of CYP1A activity in liver of female eelpouts sampled at 16 stations along Danish coast, in the period of time from year 2002 until 2009.

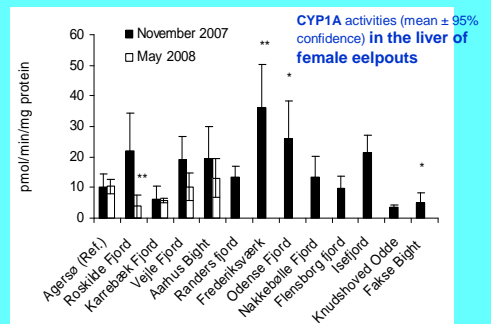
Acknowledgements
This project is part of BALCOFISH project, SFS/HPLC methods were validated in international intercalibration arranged by partners of BEAST project.



* Significantly different from the reference station Agersø ($p < 0.05$)

Urine from fall season contained significantly higher amount of PAH metabolites (1.9 times) than urine from spring season ($p < 0.01$).

Concentrations of FACs in urine samples from May 2008 were factor 15 lower than concentrations measured in bile from the same period.



* Significantly different from the reference station Agersø: * $p < 0.05$, ** $p < 0.01$.

Conclusions:

-Eelpout is useful indicator fish species for study of biological effects of PAH exposure

-eelpout bile and urine: important routes of PAH metabolites excretion

-Bile is most useful PAH metabolites containing matrix compared to urine

-urinary FACs: questionable potential as biomarkers of exposure

- When using CYP1A activity as biomarker: season of the year, sexual cycle-triggered changes and abiotic factors have to be taken into account.

-Potential links to effects on reproduction success in eelpout