



PAH biomarkers in eelpouts from Danish waters

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Introduction

The fish eelpout (*Zoarces viviparus*), or viviparous blenny, is mainly found in the littoral zone of northeast Atlantic and in most of coastal areas of the Baltic Sea. CYP1A enzymes - the group of enzymes, responsible for the metabolism of PAH compounds excreted in fish bile and urine. The enzyme activity assay, ethoxresorufin-O-deethylase (EROD) as well as PAH metabolites are established biomarkers for PAH exposure and effects in fish.

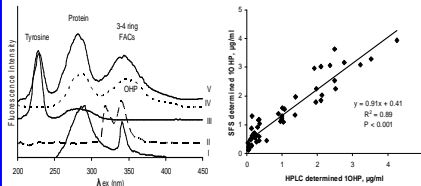
PAH-metabolites in gall bladder bile and urine of the eelpout *Zoarces viviparus* were investigated as potential biomarkers of PAH exposure. Levels of PAH-metabolites in fish urine and bile were compared with effects (CYP1A) in Danish coastal waters.

Objective

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

Methods

SFS on bile and urine validated by



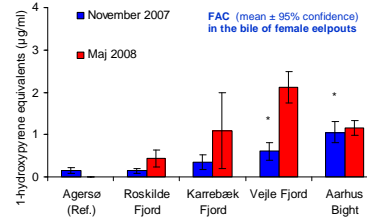
Examples of Synchronous fluorescence spectra (SFS) of: (I) fish bile sample SFS $\Delta\lambda = 42$ nm; (II) 1-hydroxypyrene standard measured by SFS $\Delta\lambda = 70$ nm; (III) tyrosine standard measured by SFS $\Delta\lambda = 70$ nm; (IV) fish urine sample measured by SFS $\Delta\lambda = 42$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

Validation of SFS method with HPLC method. Measurements of 10HP in eelpout bile by both methods show very good correlation, thus SFS is a good screening tool, although much simpler than HPLC.

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

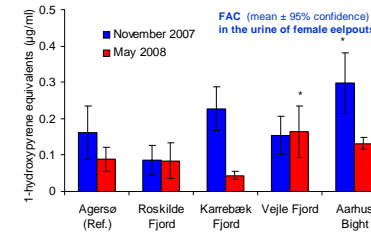


More biliary PAH metabolites in fish caught in the spring



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

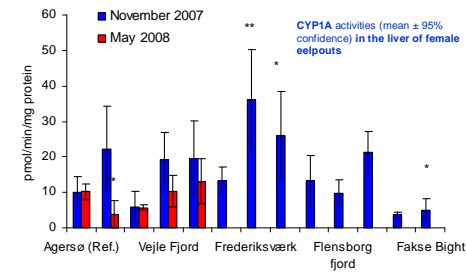
More urinary PAH metabolites in fish caught in the fall



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

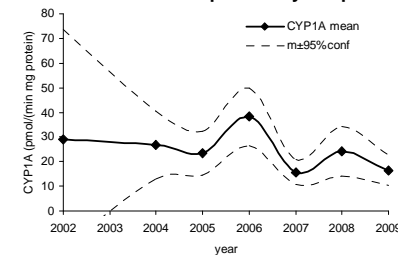
Concentrations of FACs in urine samples from May 2008 were factor 15 lower than concentrations measured in bile from the same period.

Higher CYP1A activity in fish caught in the fall



* Significantly different from the reference station Agersø: * $p < 0.05$, ** $p < 0.01$. Male fish had significantly higher CYP1A activity than females from sampling of November 2002 and 2004 (data not shown), however there was no significant difference between sexes in fish caught in May 2008.

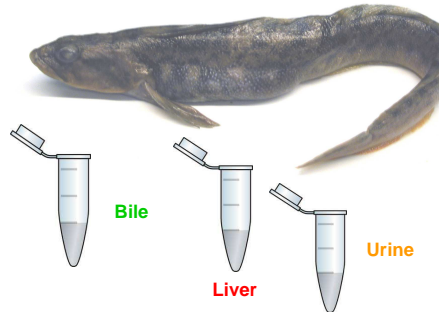
Temporal trends in CYP1A activity can not be explained by temperature



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 (data from NOVANA project).

Acknowledgements

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



Conclusions

- Eelpout is useful indicator fish species for study of PAH specific biomarkers
- However, there was found no correlation between levels of CYP1A activity in liver and PAH metabolites in bile
- Eelpout bile and urine: important routes of PAH metabolites excretion, while urine contained by factor 15 lower amounts of metabolites
- Bile is most useful PAH metabolites containing matrix compared to urine
- Normalisation methods (to biliverdin, protein and tyrosin contents) did not reduce CV% of the data when applied in both urine and bile
- 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 reproductive success in eelpout