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Ecotoxicogenomics: Validation of a quantitative PCR macroarray for use as a biomarker of exposure to pollutants in flounder

MASTS VISITING FELLOWSHIP
Project summary

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Introduction

For many years, there has been increasing concern over the effects of hazardous substances present in the marine environment. Chemical and effects monitoring of these pollutants has proven difficult due to low environmental concentrations, variable bioavailability, and the generalised nature of ecological responses to these substances. Biological response techniques (biomarkers) are rapidly increasing in relevance as tools to overcome problems with low chemical analysis detection limits and in linking of organismal responses to contaminant exposure.

With a combined interest in linking biological effects and chemical analysis, the MASTS fellowship was used to support the visit of Dr. Michelle Giltrap, postdoctoral researcher at TCD, to visit MSS and IOA and work with two collaborative groups between October 2014 and March 2015. The visit was used to validate a quantitative PCR macroarray for use as a biomarker of exposure to pollutants in flounder. mRNA extraction and gene expression quantification was performed on flounder samples previously exposed to model contaminants at the IOA and these results were used to validate the macroarray. Model contaminants included 17β-estradiol (E2), 3-methylcholanthrene (3-MC), commercial mixture of polychlorinated biphenyls (PCB; Arochlor), perfluoroctanoic acid (PFOA) and lindane. Statistical analysis of data previously obtained by MSS from wild-caught flounder data was then performed in order to link up/down regulated genes to biological effects and chemistry data. The development of gene expression arrays offer the potential to determine changes in multiple relevant genes simultaneously and can infer pathways of toxicity and adverse outcomes in sentinel organisms used for bio-monitoring.

Research objectives:

- to further validate a quantitative PCR (qPCR) macroarray for determining the expression of flounder genes diagnostic of exposure to environmental contaminants,
- to analyse existing gene expression data in wild-caught flounder and investigate links between gene expression and other biomarker and chemistry data

Preliminary results:

Fish exposures and candidate biomarker genes

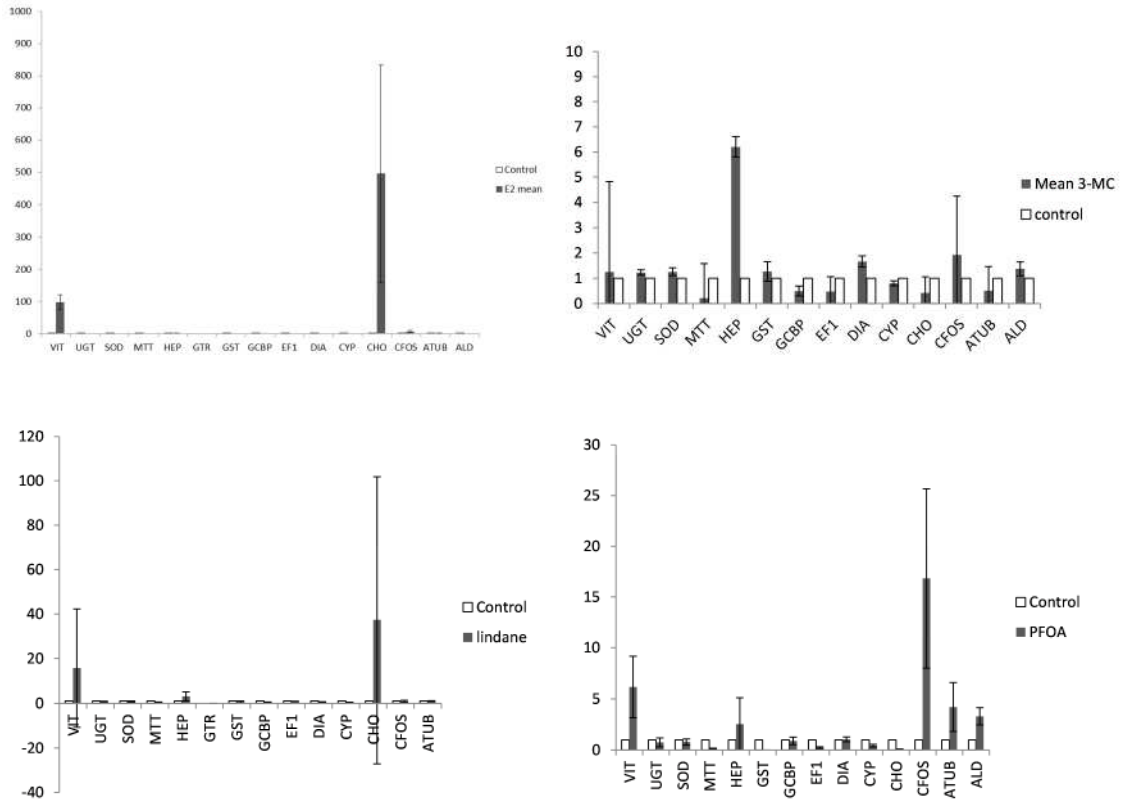
After 8 days exposure, differences in gene expression were observed between model contaminant treatments (Figure 1). Vitellogenin (VTG) is the egg yolk precursor protein produced by female fish during gametogenesis. The production of this protein by male or immature fish serves as a marker for detecting the presence of environmental pollutants with estrogenic activity (Tyler et al. 1996). 17β-estradiol (E2) is a natural estrogen that can be found in the environment as a component of sewage effluents and farmland runoff, amongst other sources. In this study, E2 treatment resulted in strongly expressed vitellogenin and choriogenin (CHO) genes. Choriogenin is the precursor gene of the inner layer subunits of the egg envelope and both VTG and CHO are regarded as sensitive biomarkers for estrogenic pollutants (Chen et al., 2008).

Lindane is commonly used as a pesticide, and fish exposed to it also showed a similar pattern with gene expression of vitellogenin and choriogenin genes, although less extreme than those exposed to E2. Williams et al. (2008) previously reported lindane to be an oxidative stressor and to have altered other processes such as amino acid metabolism, co-factors and oxido-reductases, although effects on VTG and CHO expression were not noted.

Treatment with 3-MC, which is a planar polycyclic aromatic hydrocarbon, resulted in the least extreme changes in gene expression. With this chemical, expression of hepcidin (HEP; an immune precursor gene) was evident. Expected expression of the CYP1A gene was not observed with this compound however. In fact, CYP1A was down regulated in all exposures.

PFOA is a C-8 fatty acid analogue used in production of non-stick coatings (e.g. Teflon) and as a fat and water resistant coating for fabrics and paper (Key et al., 1997). Exposed fish resulted in increased expression of VTG, HEP, c-FOS (a transcription factor who's dysregulation may be associated with carcinogenesis) and aldehyde dehydrogenase (ALD, a detoxification enzyme).

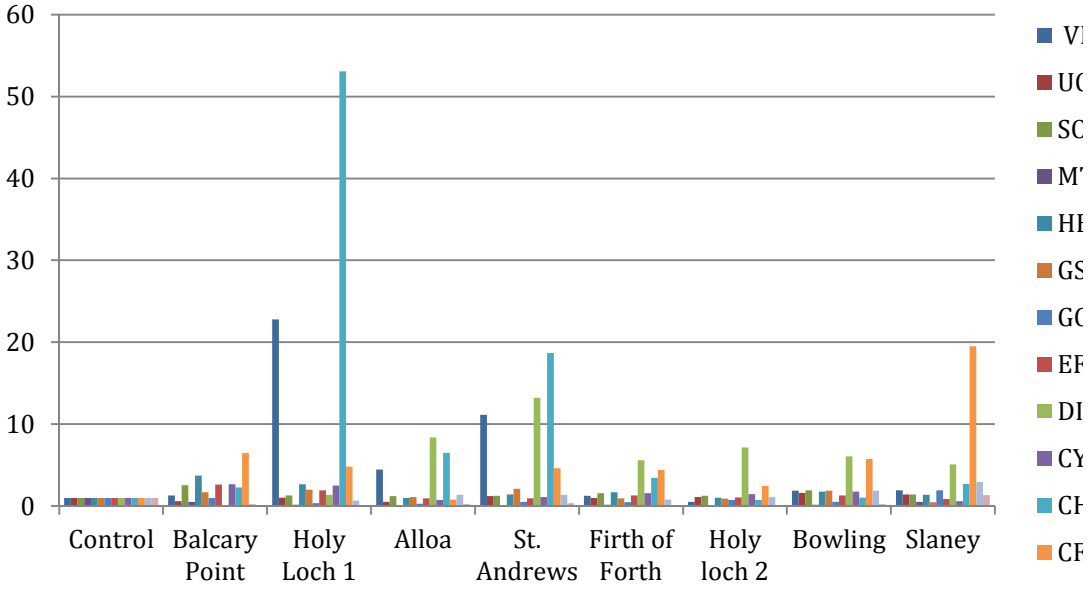
Figure 1 Gene expression data for fish treated with (a) E2, (b) 3-MC methylcholanthrene, (c) Arochlor, (d) PFO and (e) lindane after 8 days exposure. Significant up regulation is determined by x>2 and down regulation x<0.5.



Wild caught Scottish and Irish fish data

Flounder had previously been collected from the Solway Firth (Balcary Point), the Clyde (Holy Loch and Bowling), the Forth (Alloa and Tancred Bank), St Andrews Bay and from the Slaney estuary, in South East Ireland. Flounder from the inner Firth of Clyde showed strong expression of vitellogenin and choriogenin genes in 2009 (Holy Loch 1), indicating estrogenic exposure occurring at this site, that was not observed in 2010 (Holy Loch 2; Figure 2). Estrogenic exposure was also evident for St. Andrews and Alloa, but to a lesser extent. However, this was not consistent with below detection limit measurements of plasma VTG levels at these sites. Overall, there seemed to be less up-regulation of genes at other sites such as Balcary Point (Solway Firth), Bowling (Clyde estuary) and the Firth of Forth. The CFOS gene was significantly up-regulated in the Irish Slaney site.

Figure 2 Gene expression data in wild caught flounder from Ireland and the West and East coasts of Scotland



Networking with Scottish Researchers

During the visit, the VF presented in the MSS Seminar series. Collaborations were established between the VF and associates at both IOA and MSS. For example, the VF is currently working on barcoding of meiofauna in environmental samples as a method of community analyses with colleagues at IOA. Also, collaborations were made with phytoplankton biologists at MSS and important links and collaborators established for future research. It is predicted that the initiated collaborations which were developed between TCD, MSS and IOA during the course of the MASTS fellowship with the host institutions, will be continued in future work.

Future prospects

We showed that exposure to model contaminants such as E2 in the environment can be detected with this macroarray tool. However, this may not be so

straightforward in wild caught fish. Genes included on the macroarray were selected from gene sequences found to be differentially expressed in previous studies involving IoA (e.g. Falciani *et al.,* 2008; Williams *et al.,* 2008). These included both experimentally exposed and wild-caught fish. Here we confirm the utility of the macroarray to determine expression of these genes in environmentally-exposed fish from Scotland and Ireland. For the chemical exposures, male fish data showed clearer links between contaminant exposure and gene expression, than female fish. Further links between biological effect data and gene expression will be investigated and a final paper is in preparation.

References

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