

MONITORING POLLUTION IN MARINE & FRESHWATER USING MUSSELS, THREE SPINED STICKLEBACK & qPCR

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OUTLINE

- What is Ecotoxicology?
- Why Environmental Genomics?
- Genomics Methods
- Project
- Results
- Summary

ECOTOXICOLOGY

- study of how biological systems respond to stress caused by environmental contaminants.
- incorporates aspects of ecology, toxicology, physiology, molecular biology, analytical chemistry and many other disciplines
- ultimate goal is to be able to *predict* the effects of pollution.
- so the most efficient and effective action to prevent or remediate any detrimental effect can be identified.

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ENVIRONMENTAL GENOMICS

- SEPA use standardised tests that produce values that can indicate if an organism has been affected by an external factor.
- results of such tests range from effect to lethality on the organism as a whole.
- 21st Century = knowledge of DNA
- Why not identify genes that have shown to be ‘stressed’ by certain environmental factors?
- Use them to monitor responses and effects of pollutants and stressors on organisms.

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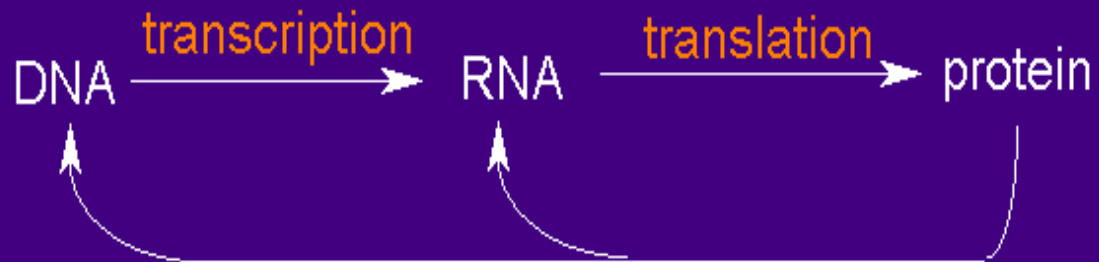
Results

Summary

mRNA

mRNA = A molecule that encodes for Proteins

Overall Reactions of Protein Synthesis



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PCR-Polymerase Chain Reaction

What is PCR?

- a technique that amplifies a single or few copies of a piece of DNA
- mRNA = fragile and easily broken down – Convert to cDNA.



wanted gene

3rd cycle

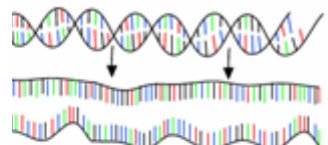
4th cycle

PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :

Step 1 : denaturation

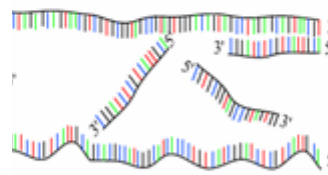
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

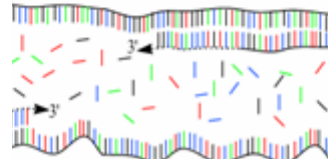
forward and reverse primers !!!



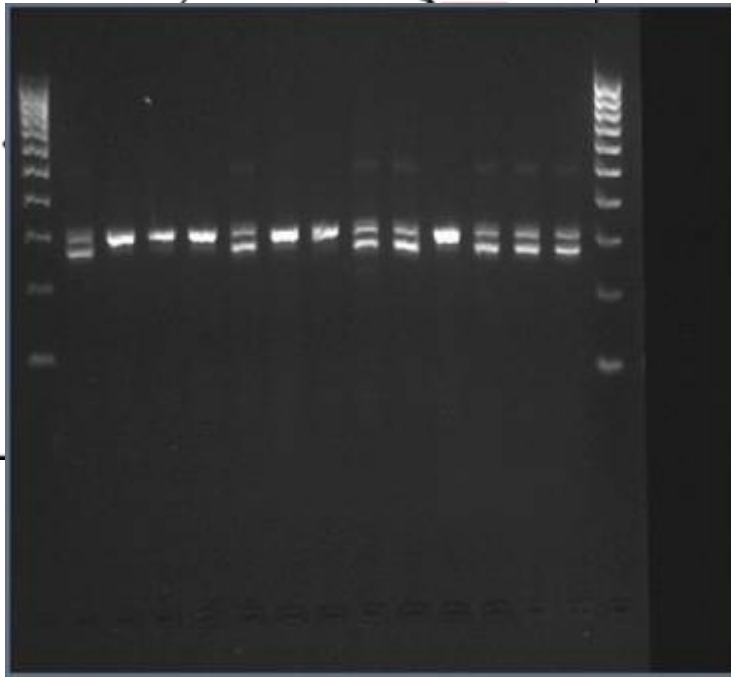
Step 3 : extension

2 minutes 72 °C

only dNTP's



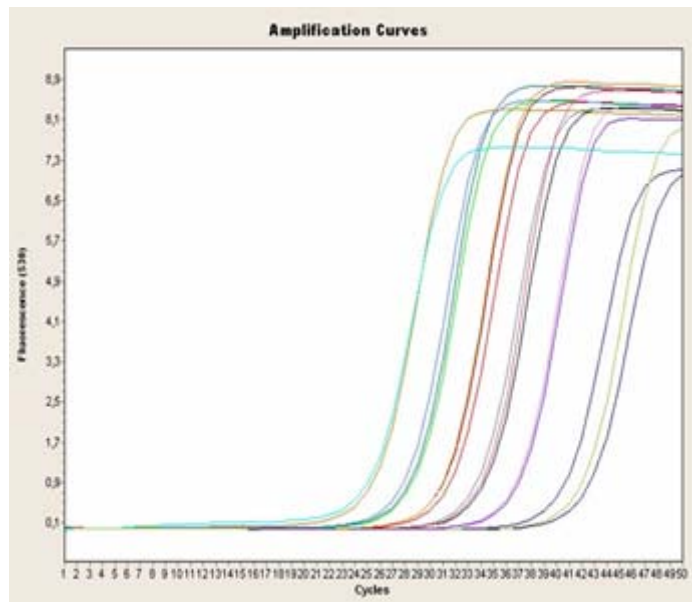
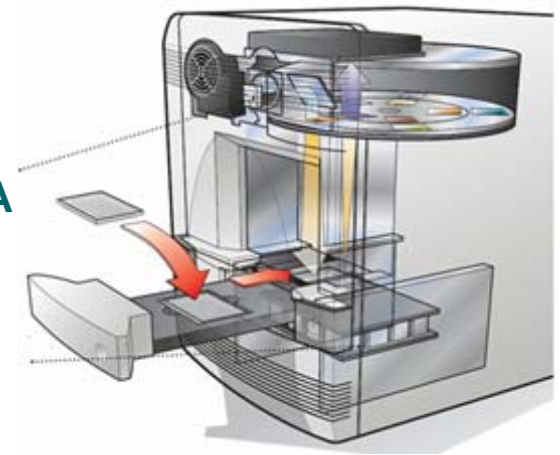
(Andy Vianzana 1999)



template DNA

qPCR

- **qPCR = Quantitative PCR or Real-Time PCR**
- **Uses a dye that fluoresces in presence of DNA**
- **Measured by Real-Time PCR Machine.**



Basically the results from the graph enables us to monitor changes in the levels of genes in each sample.

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- **Freshwater = Three-spined Stickleback, *Gasterosteus aculeatus* as a Sentinel Species.**
- **Marine = Mussel, *Mytilus Edulis* as a Sentinel Species.**



Genes of Interest?

- Sewage (Oestradiol)
- Metals
- Hydrocarbons

Identified various genes of each type within both Stickleback and Mussels.

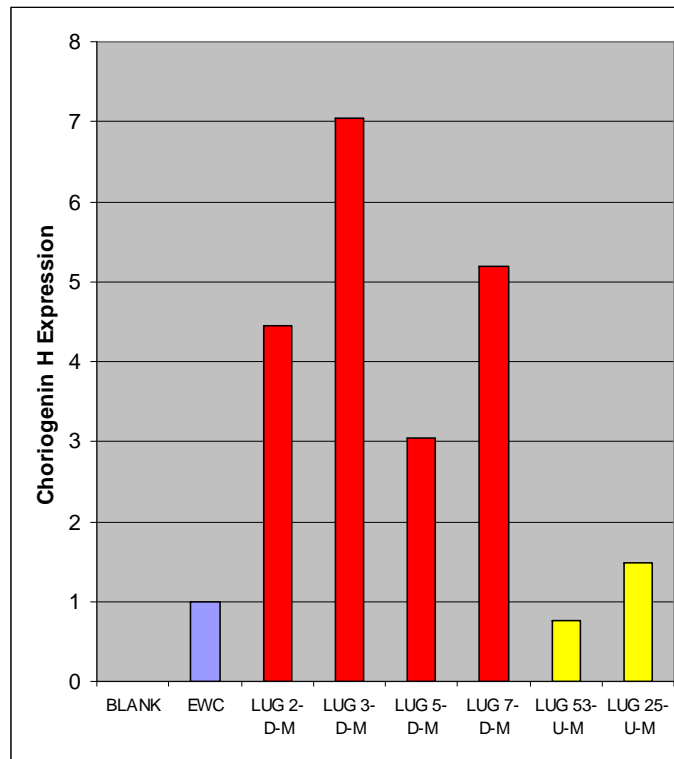
Using qPCR tried to establish if we could use these genes as 'stress indicators' when exposed to the contaminants of interest.

Did we succeed?

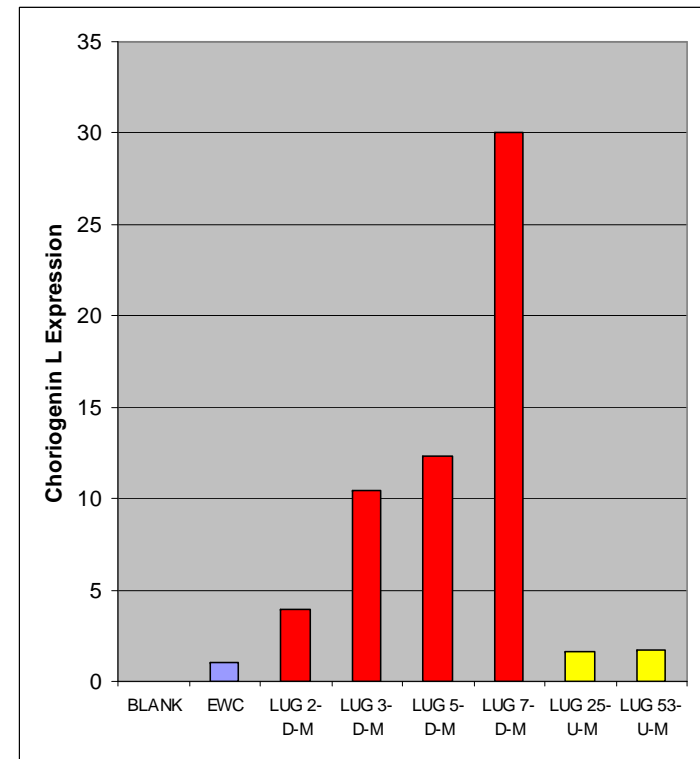
Stickleback

Choriogenin H & L- Males Lugar Water (Oestradiol)

Ch H



Ch L



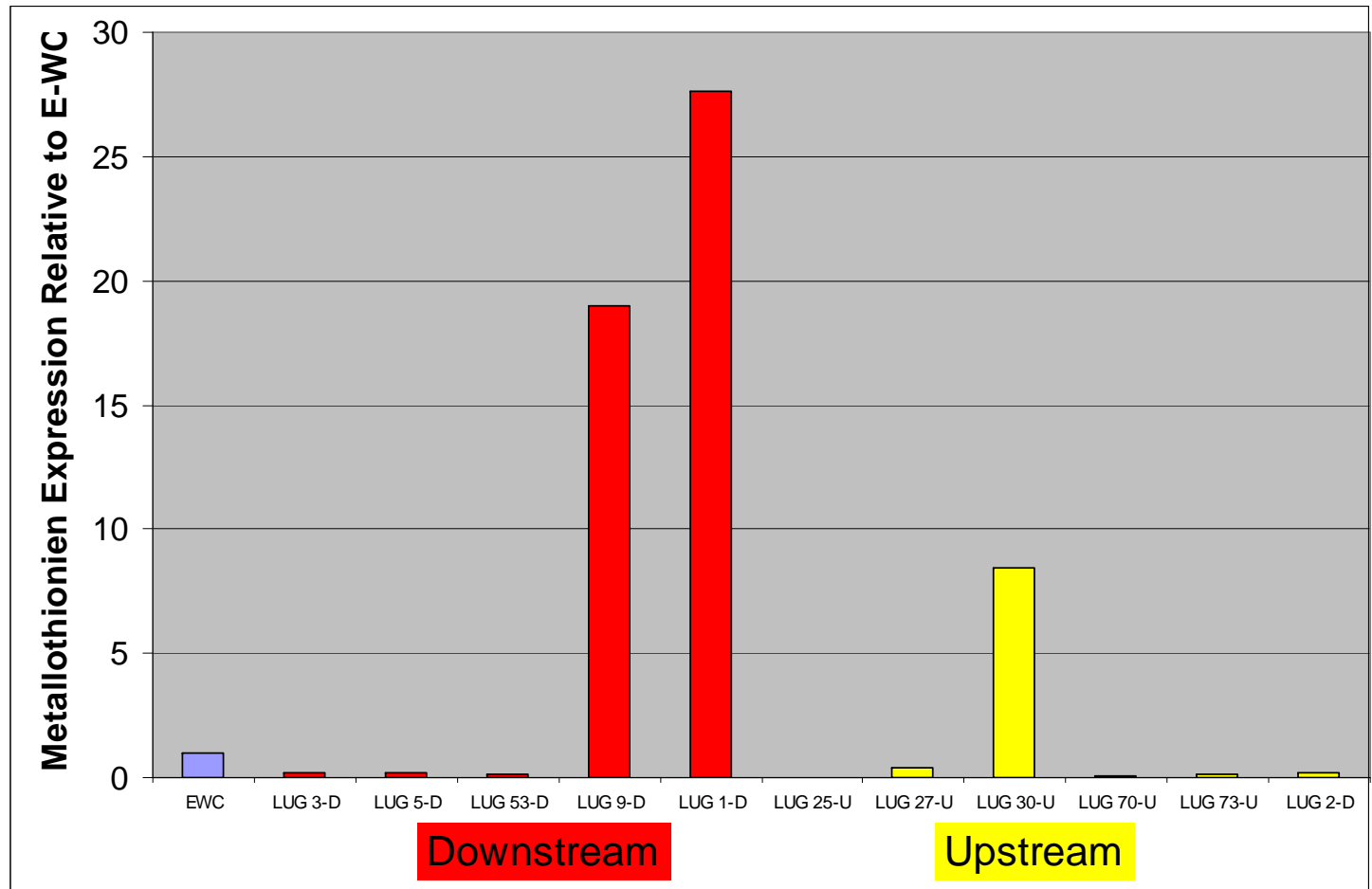
Downstream

Upstream

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Metallothionein Lugar Water Females

Up/Down stream



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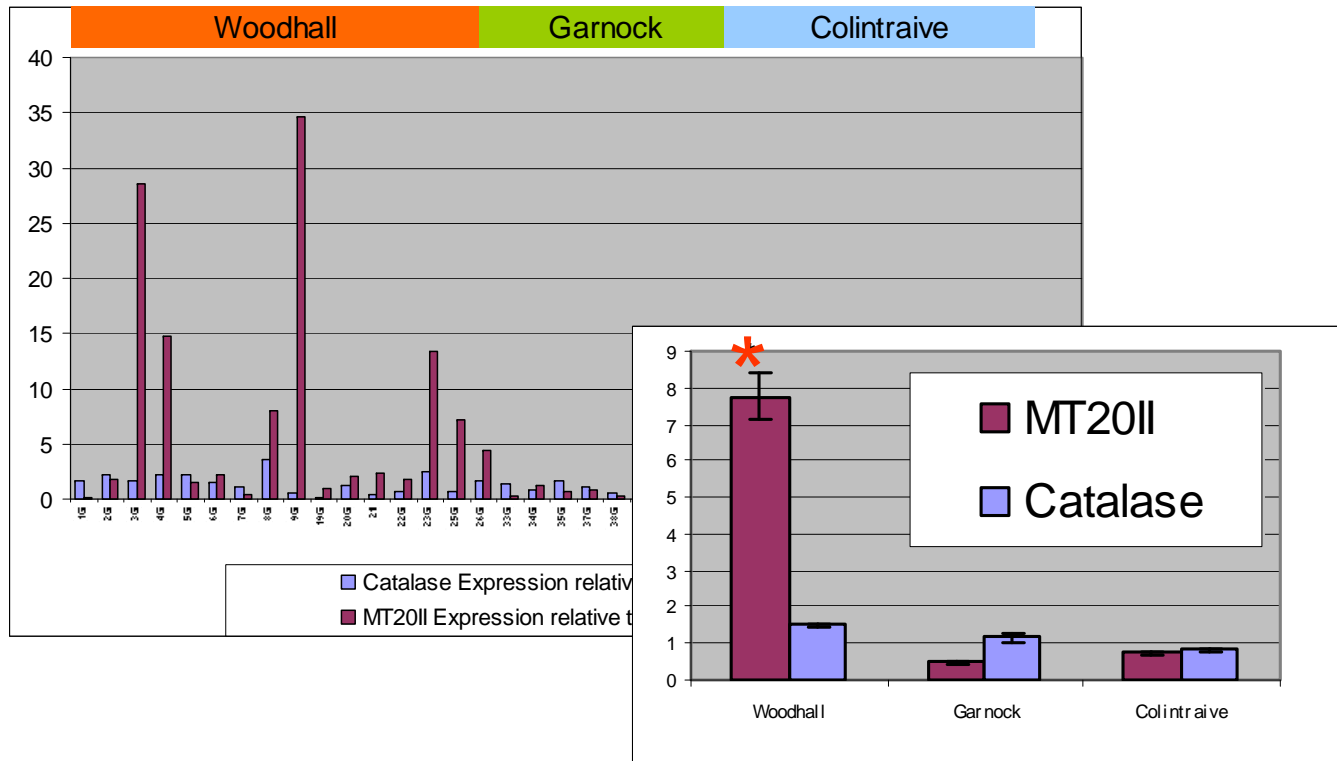
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Gill : Catalase & MT20II

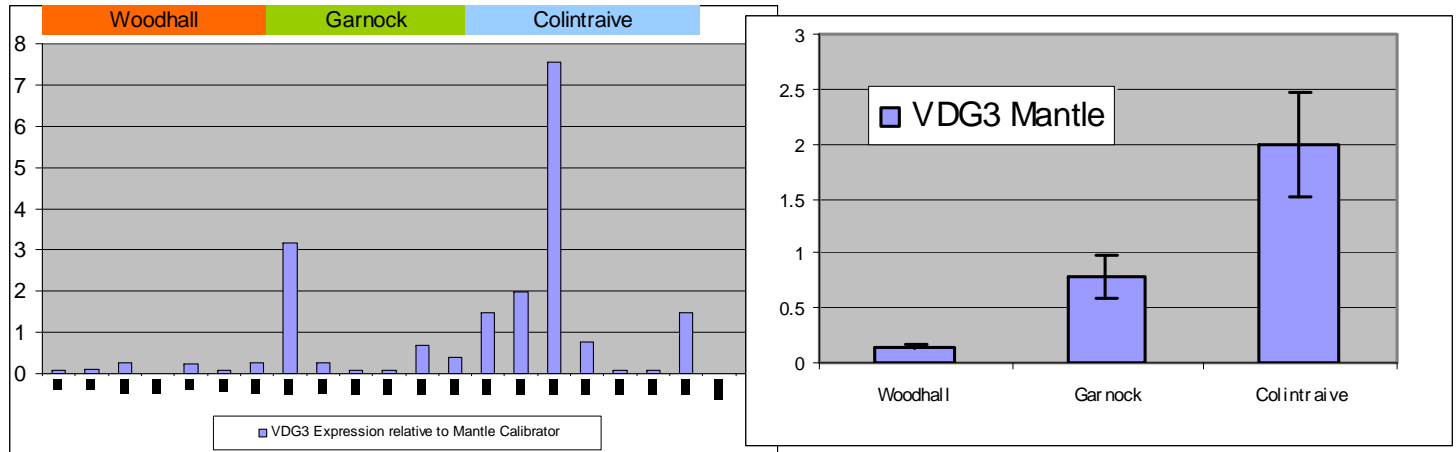


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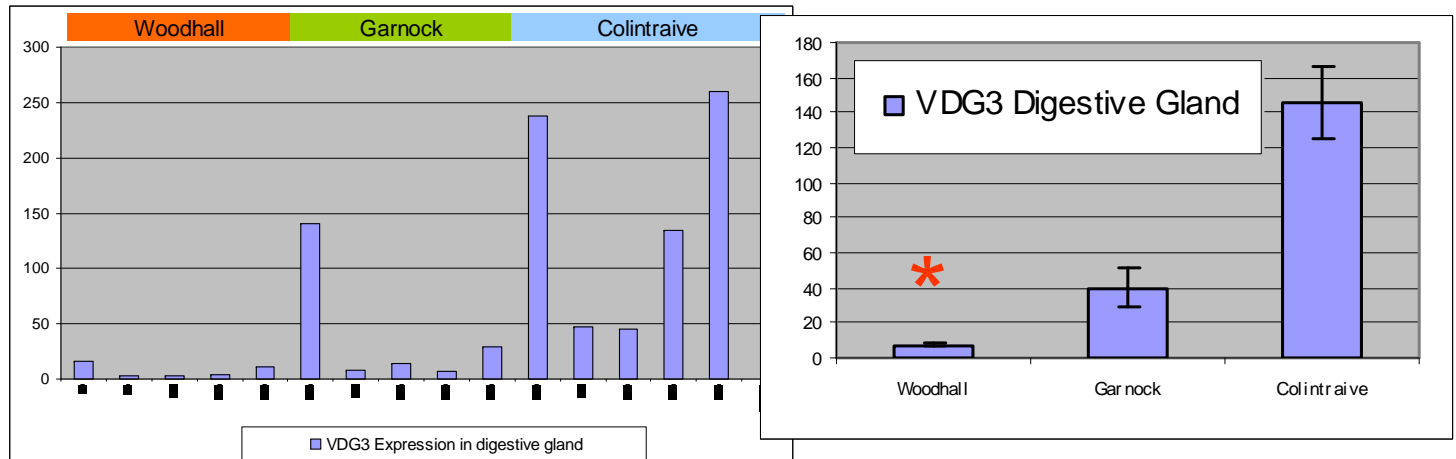
Genes:VDG3

Tissue-Mantle

Mean +/-SEM



Tissue-Digestive gland



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- Shown that gene expression can be measured in polluted sites.
- Genes that WERE induced matched the contamination present.
- Potential to infer pollution from the levels of the genes measured.

Further Work

- Look for more genes within mussels.
- Increase in numbers of mussels tested.
- Potential to apply to other organisms e.g. earthworms within soils



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