ENVIRONMENTAL DIAGNOSTICS: THE USE OF MEDICAL DIAGNOSTIC TECHNIQUES TO ASSESS THE HEALTH OF THE MARINE ENVIRONMENT

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Aims of study

1. Investigate the potential of medical diagnostic technologies for use in environmental monitoring to assess the health status of animals (focus on *Mytilus spp.*)
   - Clinical chemistry
   - Immunoassay

2. Assess Impact of a contaminant on aquatic animals
   - Chronic effects of diclofenac on *Mytilus spp.* & rainbow trout
   - The effect of 17α-ethynylestradiol (EE2) on steroid levels in *Mytilus spp.*
‘The Piccolo xpress™ is a compact, portable, fully automated Point of Care (POC) clinical chemistry analyser’

- Developed for human & veterinary samples
- Fully automated, simple to use
- Complete clinical chemistry analysis
- Test response of liver function tests
- Fast, 12 min for 13 endpoints
- Internal quality control system
- Using validated techniques
Mussel Hemolymph

- Clinical chemistry endpoints normally measured in blood
- Mussel hemolymph very dilute
- Need to develop protocol for concentration of hemolymph for use in diagnostic testing
- Hemolymph advantages: ease of sampling, little sample prep, sample over time, ethically acceptable
Piccolo xpress™ endpoints investigated

• Rotor General Chemistry 13

• Alanine aminotransferase (ALT) – Liver function test (inflammation)
• Aspartate aminotransferase (AST) – Acute liver damage
• Alkaline phosphatase (ALP) – Liver and bile duct
• Gamma-glutamyltransferase (GGT) – Liver function
• Total Bilirubin (TBIL) – Liver disorders
• Amylase (AMY) – Inflammation of pancreas
• Creatinine (CRE) – Renal disease
• Glucose (GLU) – Metabolism
Exposure of *Mytilus spp.* to diclofenac

- Semi-static (water change every 24h)
- C, SC (DMSO), 1 µg/L & 1000 µg/L
- Tanks in Triplicate
- 14 d exposure
- Sampled after 24h, 96h, 7d & 14d
- Samples taken for
  - Chemical analysis*
  - 2D GE analysis*
  - ‘Traditional biomarkers’
  - Diagnostic endpoints

*Schmidt et al. 2014. Drug Testing Analysis, 6(3): 210-219
Clinical chemistry analysis of *Mytilus spp.* digestive gland exposed to diclofenac

**Mytilus ALT & AST expression**

- **ALT & AST U/L**
- **7 day exposure**
- **14 day exposure**

**Mytilus ALP, AMY, GLU, TBIL & GGT expression**

- **ALP, AMY, GLU, TBIL U/L & GGT uMol/L**
- **7 day exposure**
- **14 day exposure**
Exposure of Rainbow trout (*Oncorhynchus mykiss*) to diclofenac

- OECD guidelines 203 (fish acute toxicity test)
- Semi-static (water change every 24h)
- C, 1 µg/L & 1000 µg/L
- Tanks in Triplicate
- 96h exposure
- Blood sampled after 96h
- Centrifuged 2000g, 10 min
- Serum analysed using GC13 rotor
Clinical chemistry analysis of Rainbow trout serum exposed to diclofenac
Siemens Immulite 2000 Immunoassay analyser

Solid phase competitive chemiluminescent enzyme immunoassay system

- Developed for human samples
- Semi-automated, simple to use
- Throughput of up to 200 tests/hour
- Comprehensive menu > 100 assays
- Internal quality control system
- Using validated techniques
- Potential for environmental monitoring?
Steroid levels in *Mytilus spp.*

- Mussels sampled monthly over 12 month period
- Presenting results for May – Oct/Nov
- Mussel Digestive gland (DG) and gonad (Gd) dissected
- Homogenised in ice cold buffer (130 mM NaCl, 25 mM Hepes-NaOH containing 1 mM EDTA & 1 mM dithiothreitol, pH 7.4, at 4°C)
- Centrifuged at 15,000 rpm for 60 min @ 4°C
- S15 frozen at -80°C until analysis
- Samples defrosted & immediately run on Immulite
Endpoints

1. **Adrenocorticotropic hormone (ACTH);** Increases production & release of corticosteroids, ultimately results in steroidogenesis
2. **Estrogen;** Primary female sex hormone. Regulates functions of the reproductive system
3. **FSH Follicle-stimulating hormone (FSH);** Regulates the development, growth, maturation & reproductive processes of the body.
4. **Luteinizing hormone (LH);** In females triggers ovulation. In males stimulates production of testosterone. FSH & LH act synergistically
5. **Testosterone (TES);** Androgen steroid hormone. Principal male sex hormone. Primarily secreted in the testicles and ovaries
6. **Progesterone (PROG);** Involved in the female menstrual cycle, pregnancy & embryogenesis. Produced in the ovaries
**Mytilus spp. 17α-ethinylestradiol exposure**

- *Mytilus spp* taken from reference site (5-6 cm)
- Semi-static exposed to EE2 for 7 day using artificial seawater
- 60 mussels in 60 L tanks
- Concentration of 150 ng/L EE2
- Solvent (ethanol) concentration (0.00015%)
- Exposures:
  - Seawater Control (SW)
  - Solvent Control (SC)
  - Intertidal EE2 exposed (IT)
  - Submerged EE2 exposed (EE2)
Steroid levels in *Mytilus spp.*: Findings

- **E2 EE2 exposed *Mytilus***
  - E2 pmoL/L
  - SW, SC, IT, EE2

- **ACTH EE2 exposed *Mytilus***
  - ACTH pg/ml
  - SW, SC, IT, EE2

- **FSH EE2 exposed *Mytilus***
  - FSH IU/L
  - SW, SC, IT, EE2

- **TES EE2 exposed *Mytilus***
  - TES ng/dL
  - SW, SC, IT, EE2
Conclusion

1. Clinical chemistry endpoints measurable and impacted following diclofenac exposure in *Mytilus spp.*

2. Can measure steroid levels in *Mytilus* over time and the impact of EE2 exposure with human based immunoassays

3. Opportunity for direct inter-species and inter-phyla comparison – ecosystem level approach

4. More validation needed, but initial results indicate these diagnostic technologies suitable for environmental monitoring
Acknowledgements:

Thank you for your attention...
Questions?

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