Environmental diagnostics

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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*.

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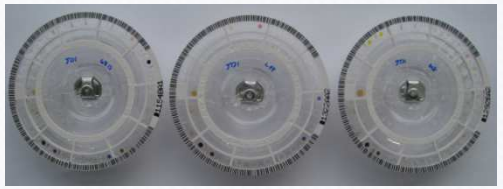
Abaxis Piccolo xpress™ clinical chemistry analyser

'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



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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

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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

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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

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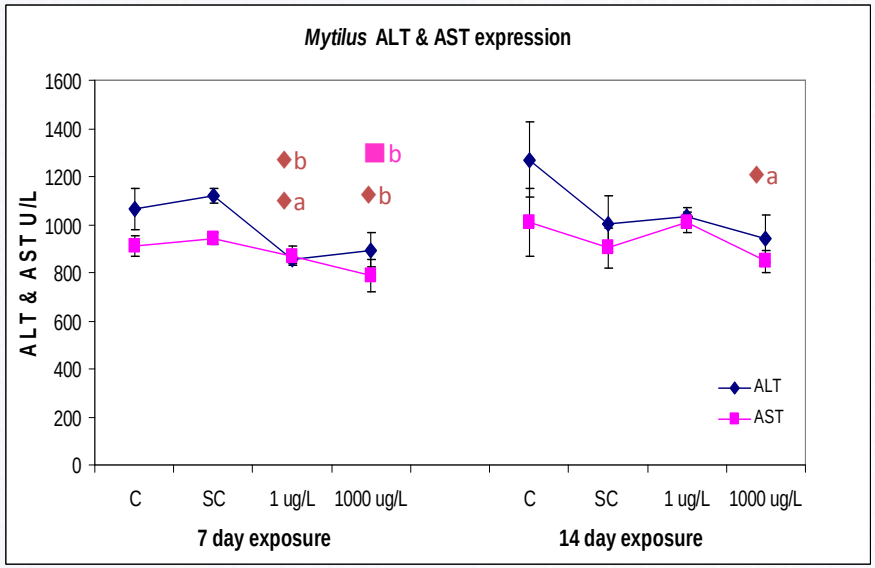
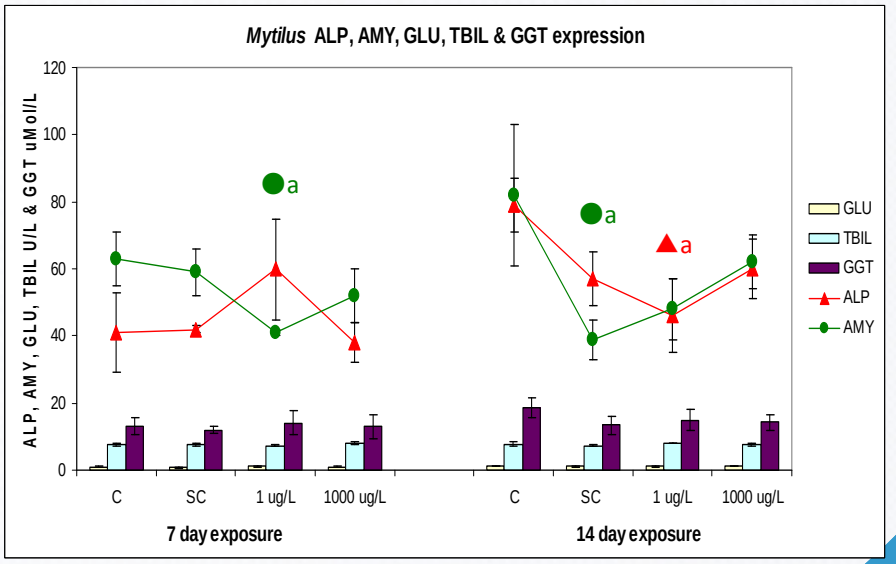
*Schmidt et al. 2014. Drug Testing Analysis, 6(3): 210-219

Clinical chemistry analysis of *Mytilus spp.* digestive gland exposed to diclofenac





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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

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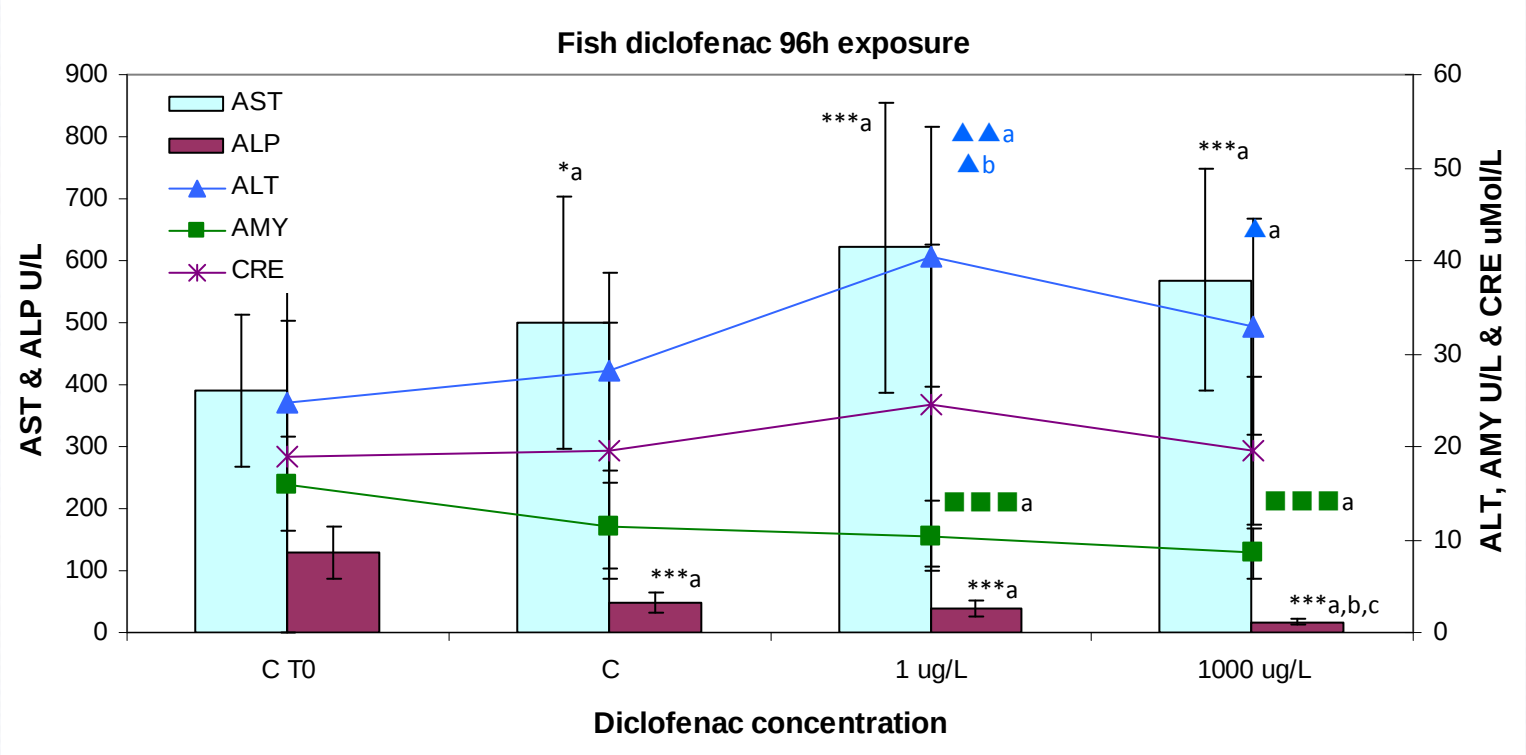
Clinical chemistry analysis of Rainbow trout serum exposed to diclofenac



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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?

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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^{o}C)
- Centrifuged at 15,000 rpm for 60 min @ 4^{o}C
- S15 frozen at -80^{o}C until analysis
- Samples defrosted & immediately run on Immulite

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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

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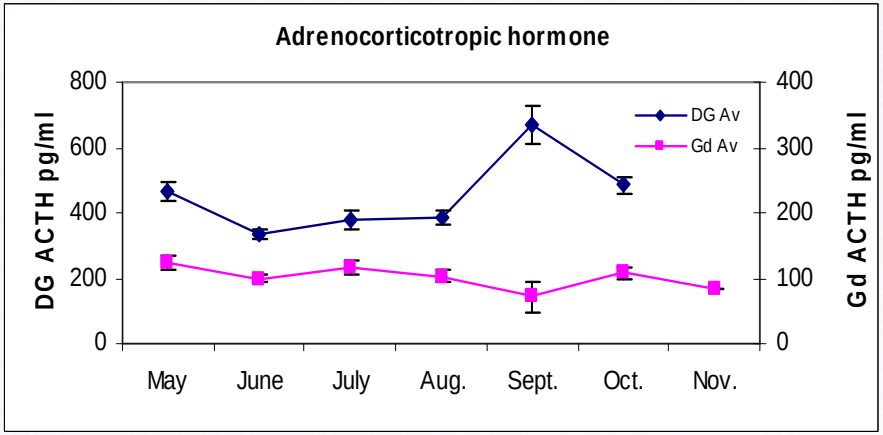
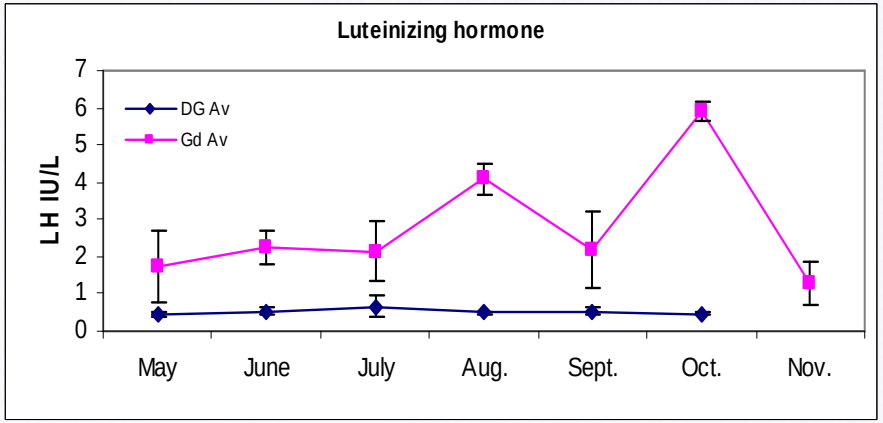
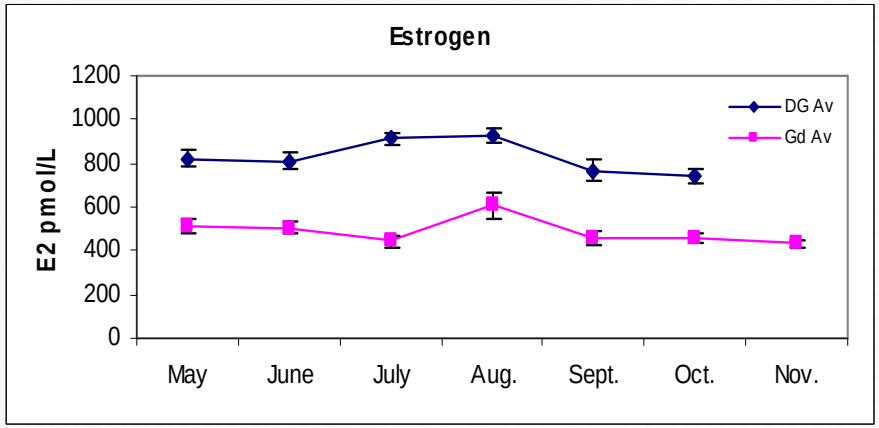
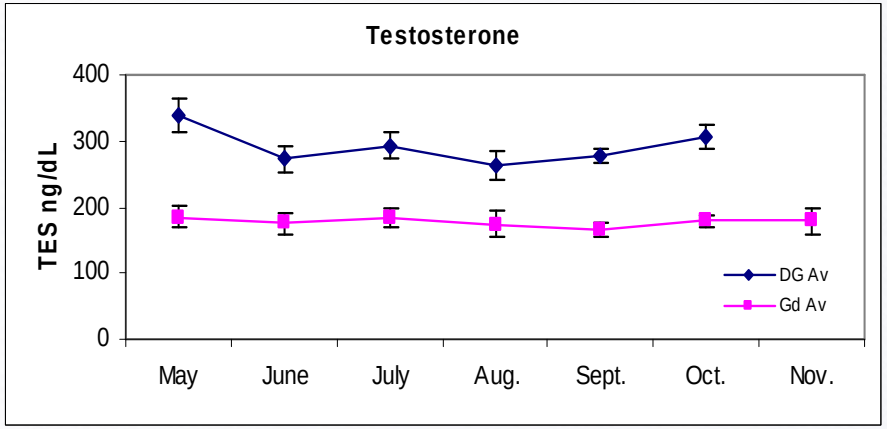
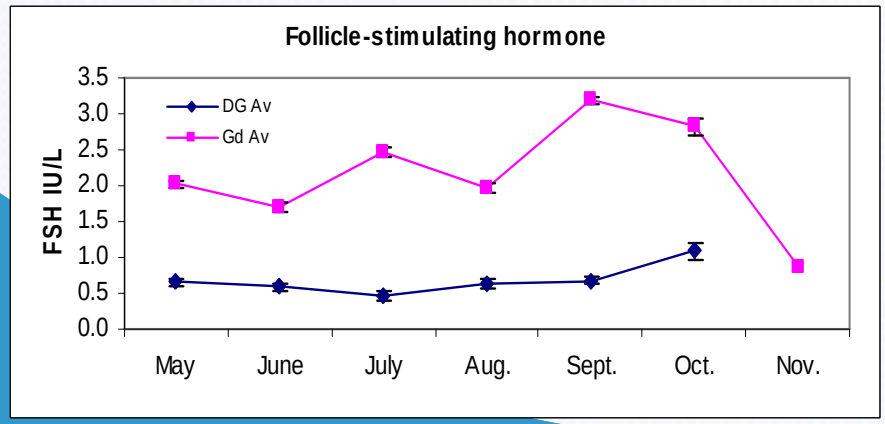
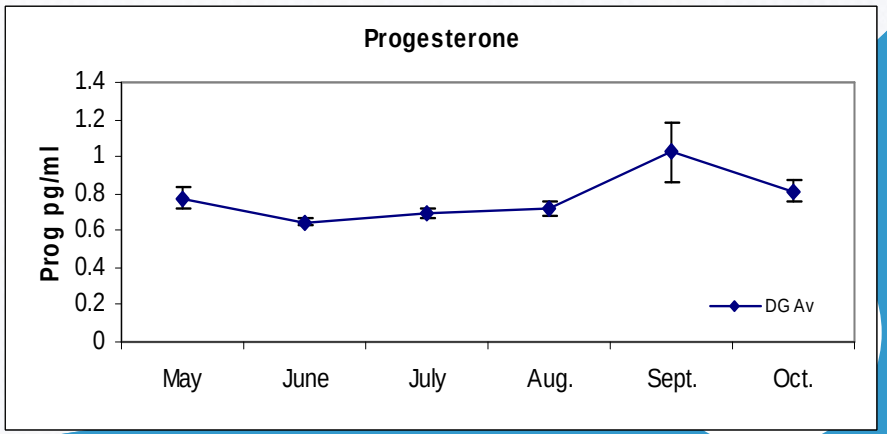

Adrenocorticotropic hormone
DG ACTH pg/ml
Gd ACTH pg/ml
DG Av
Gd Av
800
600
400
200
0
400
300
200
100
0
May
June
July
Aug.
Sept.
Oct.
Nov.


Luteinizing hormone
LH IU/L
DG Av
Gd Av
7
6
5
4
3
2
1
0
May
June
July
Aug.
Sept.
Oct.
Nov.


Estrogen
E2 pmol/L
DG Av
Gd Av
1200
1000
800
600
400
200
0
May
June
July
Aug.
Sept.
Oct.
Nov.


Testosterone
TES ng/dL
400
300
200
100
0
DG Av
Gd Av
May
June
July
Aug.
Sept.
Oct.
Nov.


Follicle-stimulating hormone
FSH IU/L
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
DG Av
Gd Av
May
June
July
Aug.
Sept.
Oct.
Nov.


Progesterone
Prog pg/ml
1.4
1.2
1
0.8
0.6
0.4
0.2
0
DG Av
May
June
July
Aug.
Sept.
Oct.

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 (0.000015%)
- Exposures:
    - Seawater Control (SW)
    - Solvent Control (SC)
    - Intertidal EE2 exposed (IT)
    - Submerged EE2 exposed (EE2)

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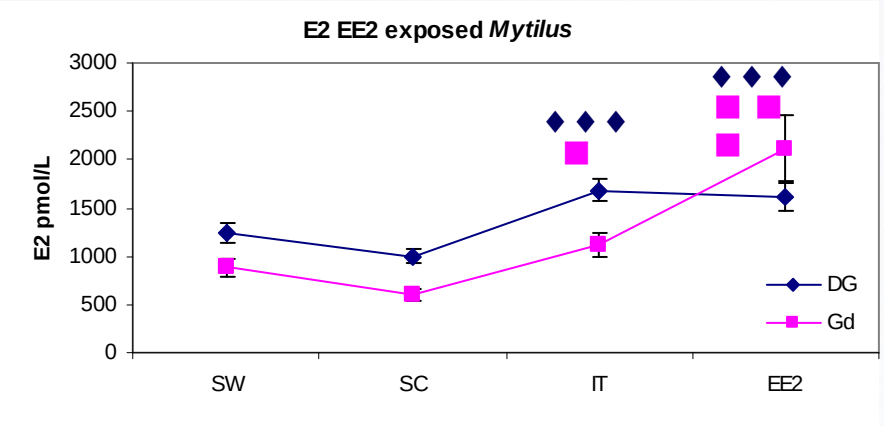
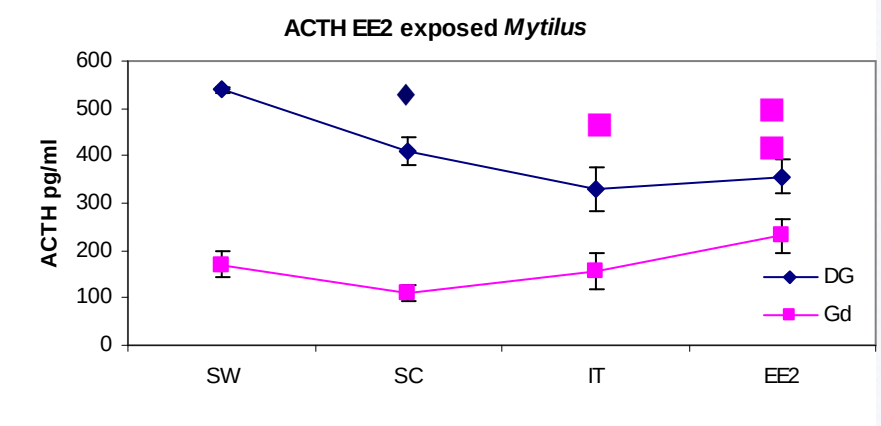
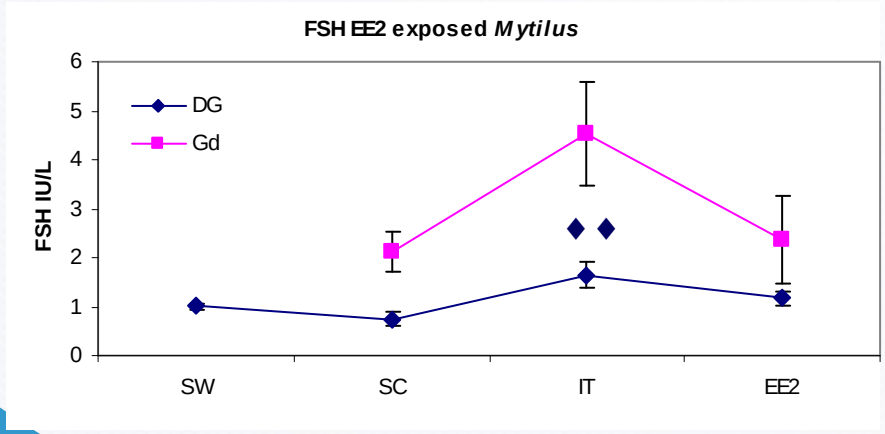
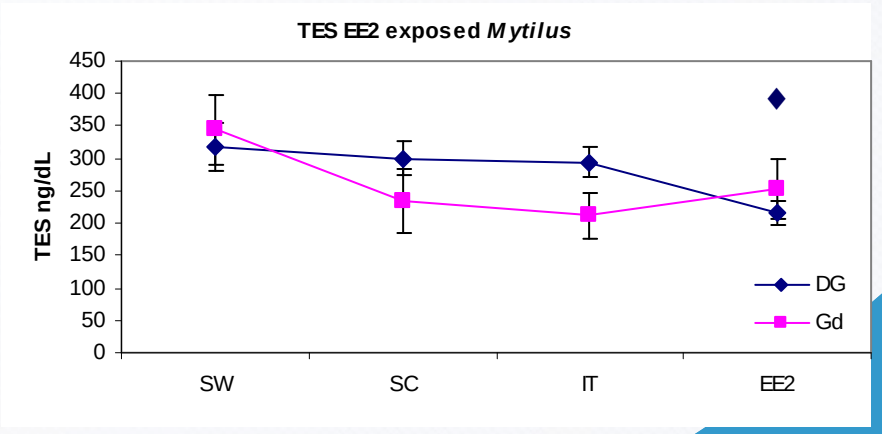
Steroid levels in *Mytilus spp.*: Findings









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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

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Cruinn Diagnostics Limited

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epa
Environmental Protection Agency

Thank you for your attention…
Questions?

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