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Implementation of DNA-based Environmental Monitoring in Marine Systems

Maylene G K Loo¹, Kathy Ophel Keller¹, Alan McKay¹
and Anthony Cheshire²

¹SARDI and Aquafin CRC

²Science to Manage Uncertainty

presented at

ICE WaRM Living Laboratories:

Emerging Technologies in Environmental Monitoring Workshop

12th February 2008



Tuna Environmental Monitoring Programme (TEMP)

- TEMP is required as part of licensing conditions, implemented by PIRSA Aquaculture
- 1996 – industry-wide TEMP
 - Broad regional approach
 - Water quality
 - Phytoplankton community structure
 - Epibenthic and infaunal communities
- 2001 – compliance-based TEMP
 - Farm site approach
 - Farm Management component
 - Benthic Assessment component



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Benthic Assessment Component

- Common practice for environmental assessment and monitoring of effects of aquaculture
 - Wide use of macrobenthic infauna, accepted as an excellent indicator
 - Collect sediment samples
 - Sort through sediment
 - Identify and enumerate sorted animals
 - Analyse data
 - patterns of community structure
 - measures of community stress




Traditional Approach

- Advantages
 - Diverse with both sensitive and tolerant species
 - Closely associated with the sediments
 - Integrate conditions over time and space
 - Respond to changes in water quality, physico-chemical status of the benthos, nutrient and organic loading to the seafloor
- Disadvantages
 - require high level of technical expertise
 - time consuming
 - difficult to apply in a routine manner



The Need for Change

- Need recognised for a new assessment system
 - rapid
 - cost effective
 - can be used routinely
- Aquafin CRC project 
 - “Development of novel methodologies for cost effective assessment of the environmental impact of aquaculture”

Key Challenges

- Select indicator taxonomic groups
- Assess number of DNA assays required
- Develop each DNA assay
 - DNA sequencing
 - develop probes in diagnostic region
- Calibrate and validate assays to manual count technique



DNA Assay System

- 9 taxonomic groups of infauna from 5 polychaete families were selected for use in Port Lincoln region
- Assays developed using quantitative PCR based on existing soil DNA diagnostic



Lumbrineridae



Spionidae



Capitellidae



Nephtyidae



Cirratulidae



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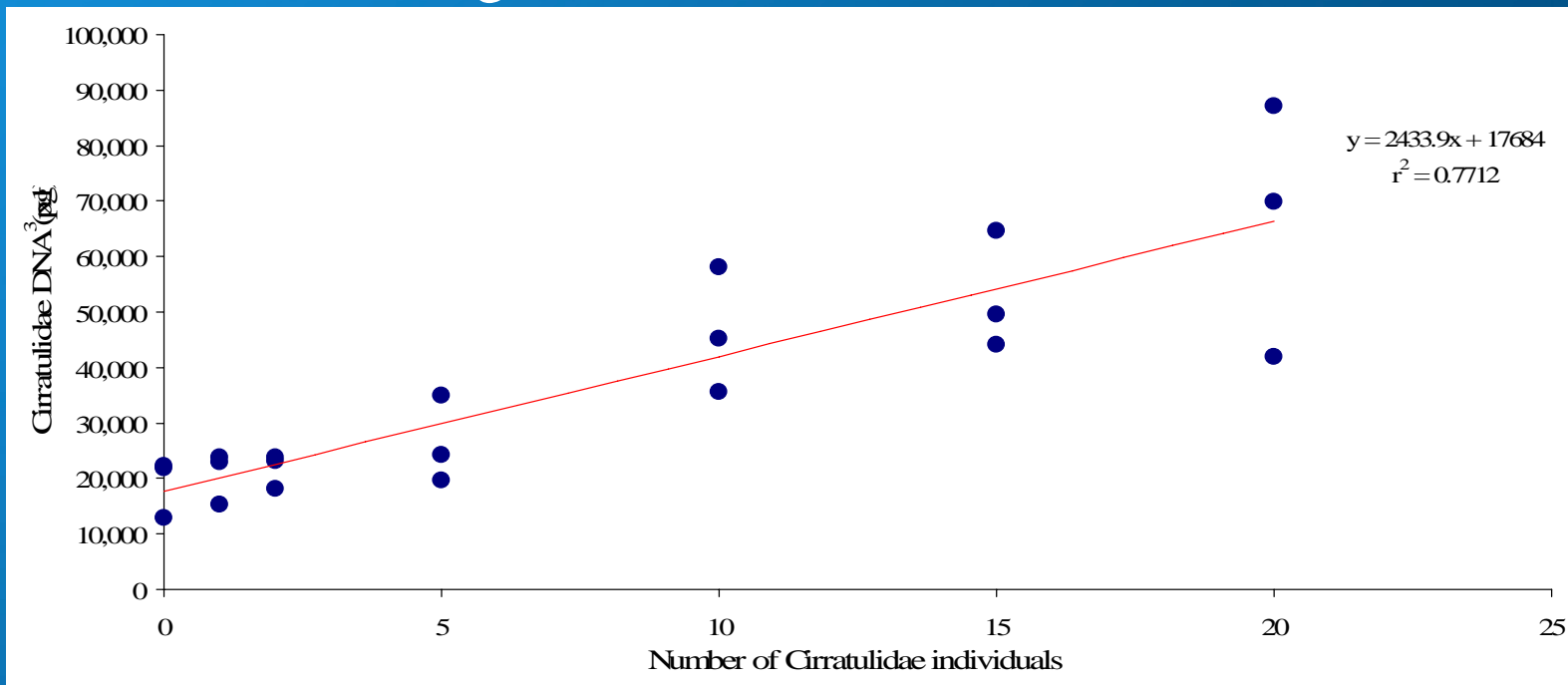
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Validation of Assays

- Quantitative extraction of DNA from marine sediments
 - use of 500g samples (biologically relevant)
- Calibration and validation of real-time PCR assays
 - linear calibration of assays over abundance ranges up to 5 orders of magnitude



Proof of Application

- Validation
 - Comparative assessment of farmed versus control samples
- Manual count versus DNA-based quantification
 - Count of all taxa used as benchmark for comparative analysis and evaluation

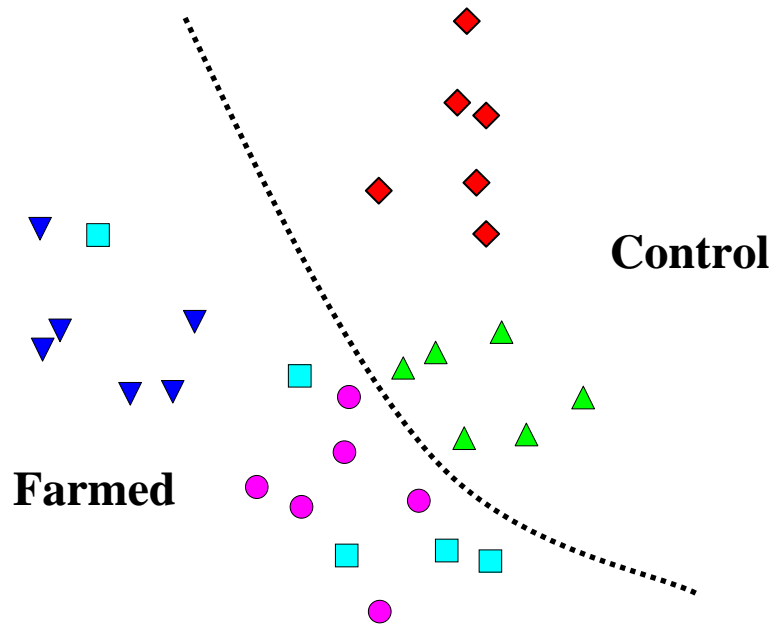


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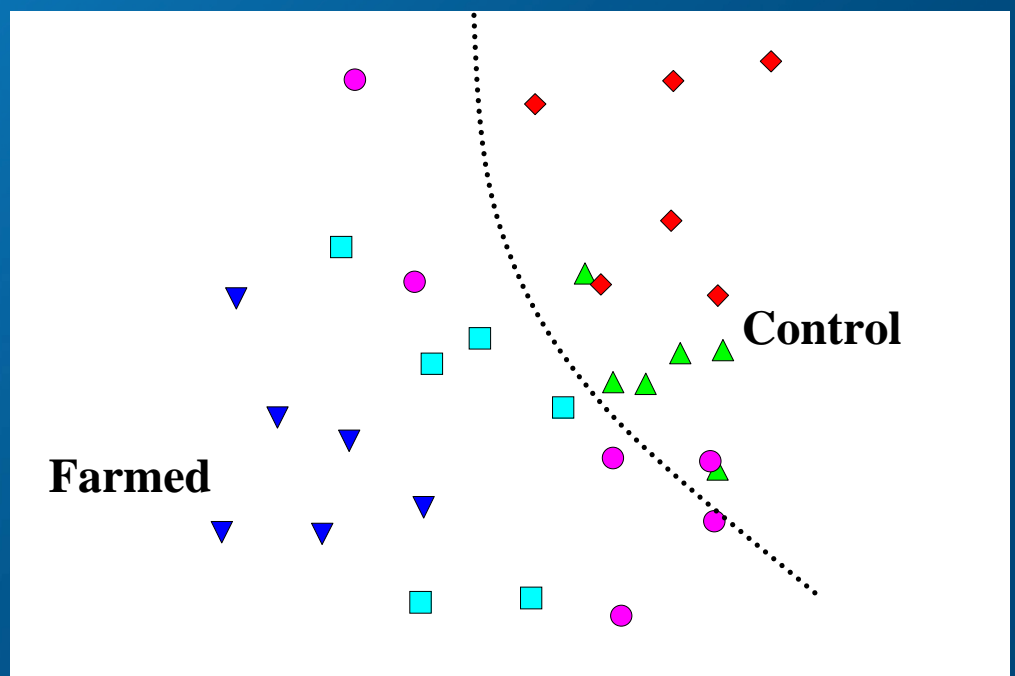


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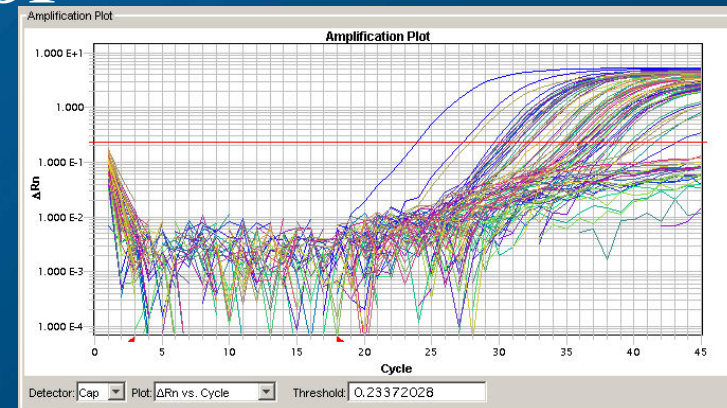
Benchmark
MDS ordination of
Count data (58 taxa)
(stress=0.17)

MDS ordination DNA data
(stress=0.18)



DNA Assay System

- Quantitative
- Capable of clearly distinguishing between organically enriched sites versus non-impacted sites
- Provides comparable resolution to the traditional technique of sorting and enumeration of taxa



Environmental Compliance Scorecard (ECS) System

- Provides a packaged set of statistical and mathematical routines for analysing, integrating and summarising results from the DNA assay system
- Developed to support decision making for environmental compliance



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Explanation of indicator colours

A green indicator means that control and compliance samples are not different

Current environmental management practices are working well

100% full score

A yellow indicator demonstrates a situation where, although there are some differences between control and compliance points, the magnitude or significance of the difference is relatively low

Environmental management practices are not consistent with industry best-practice

80% score

An orange indicator demonstrates a situation where, although there is a significant difference between control and compliance points, the magnitude or significance of the difference is low

Environmental management practices need to be changed to prevent further deterioration of the supporting environment

60% score

A red indicator demonstrates a situation where there is a significant difference between compliance and control sites and where this difference has a magnitude that warrants immediate remedial action

This outcome, is technically in breach of licence conditions and immediate changes should be made to farming practices to prevent further environmental harm

40% score

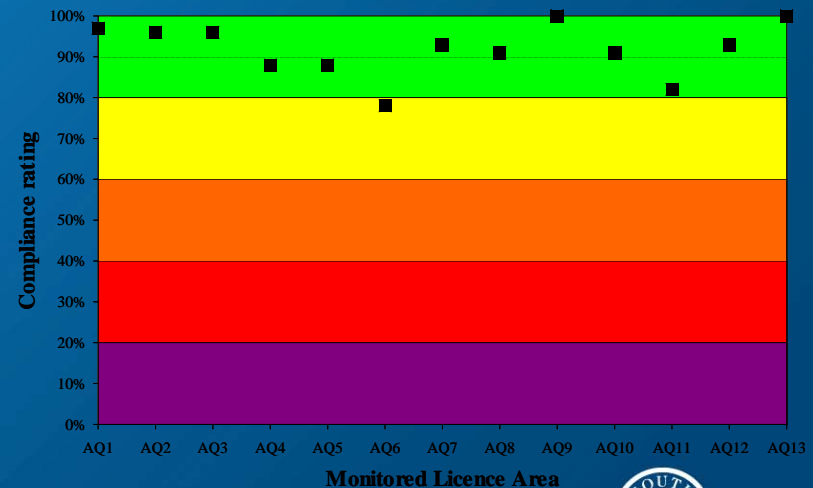
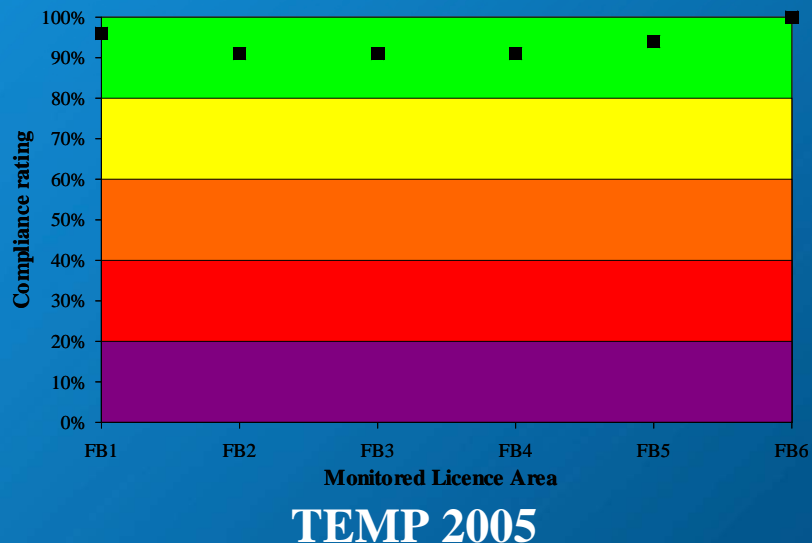
A violet indicator demonstrates a situation where there is a substantial difference between compliance and control sites and where this difference has a magnitude that warrants immediate remedial action

This outcome is in breach of licence conditions. Farming should cease on this site to prevent further environmental harm

20% score

Implementation

- This complete system was trialed for the Tuna Environmental Monitoring Programme (TEMP) in 2005 by PIRSA Aquaculture and the southern bluefin tuna industry
- Used again for a second year for TEMP 2006



Extension to Other Aquaculture Systems

- Environmental Monitoring Programmes of other finfish aquaculture in Fitzgerald, Arno and Boston Bays
 - Potentially additional DNA assays may be needed
 - Advantage is increased resolution of the DNA assay system
 - Cost reduction with increase number of samples processed



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Application to Fish Health

- DNA assays has been developed for various disease organisms such as blood fluke, gill fluke and swimmers disease in southern bluefin tuna
- Allows for cost effective screening of same sediment samples collected for TEMP



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Application to Biosecurity

- Need identified for marine pest monitoring
- Highly sensitive assays developed for *Sabella spallanzanii*, *Corbula gibba*, *Musculista senhousia*
- Can “add on” assays of other pests
- Can be used on water/sediment/plankton tow samples
- Useful for port and ballast water monitoring



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Application to Other Systems

- Environmental monitoring of freshwater systems
 - Discussion has been held with SA Water
 - Potential to identify target taxa using data from previous studies
 - Funding required for scoping study
- Can be applied to terrestrial systems, e.g. monitoring of soil samples



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Conclusion

- High throughput DNA-based monitoring system
- Applicable to soil/sediments/water/plankton samples
- Quantification of multiple targets in one sample
- Can be linked to ECS system for interpretation and management decision support



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Acknowledgements

The authors would like to thank the following:

- *Members of ASBTIA, in particular Brian Jeffriess and David Ellis*
- *PIRSA Aquaculture*
- *Sharon Drabsch*
- *Russell Burns*
- *Herdina*



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