

### Tracking the impacts of sewage overflows on ecosystem function using novel techniques




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### Estuaries

- Among most productive and diverse ecosystems in the world
- Most impacted of coastal habitats (Clark et al., 2015).
- Subject to multiple anthropogenic pressures
  - Habitat loss, contamination by pollutants



Source: Clarke (2011)



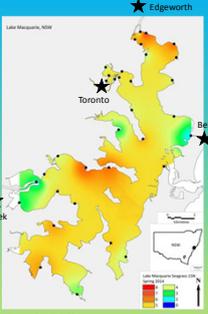
### Sewage Pollution

- Increased urbanisation has led to sewage becoming a major stressor
- Impacts of sewage pollution
  - Alter structure and function of biological communities → repercussions for entire ecosystem
- Difficult to assess ecological impacts
  - Past focus on water quality alone



### Lake Macquarie

- Largest estuary in NSW
- Extensive shoreline and catchment development
- Hunter Water
  - Four wastewater treatment works servicing Lake Macquarie
- Previous research found evidence of ongoing sewage inputs at specific locations around the lake
  - Ideal system to investigate impacts of sewage pollution



Source: Clarke (2016)

## Stable Isotope Analysis

- Provides a tool to identify nitrogen sources based on characteristic nitrogen isotope values
- Sewage derived nutrients distinguishable on the basis of ratios of nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) isotopes
- Secondary treatment of sewage enriches concentration of  $^{15}\text{N}$  (Gaston et al., 2004).
  - Results in enriched  $\delta^{15}\text{N}$  signature



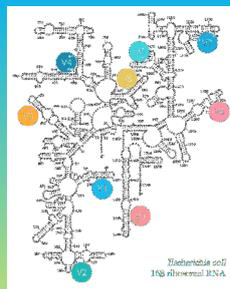
## Benthic Macroinvertebrates

- Most widely used bioindicators
  - Reliable and often predictable responses to anthropogenic pollution
- Moderate pollution → elimination of sensitive taxa and dominance of tolerant taxa with high densities (Dauer & Conner, 1980).



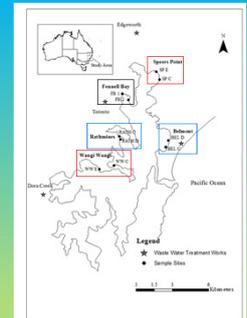
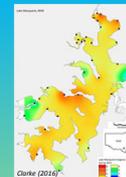
## Benthic Microbial Communities

- High potential as bioindicators in coastal systems
  - Rapid response to environmental disturbances
  - Present in high densities in sediments
- Current knowledge of benthic microbial response to pollutants quite limited
  - 16S rDNA amplicon sequencing!



## Study Design

- Four locations
  - Impacted and control site within each
- Fennell Bay used as 'true control'
- Sampling carried out over May and July
  - Dry weather sampling → capture background conditions



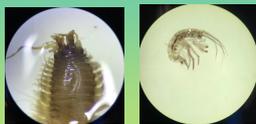
## Aims

- Assess the effectiveness of a combination of tools to investigate the ecological impact of anthropogenic pollution in coastal ecosystems
  - 1) Establish if stable isotope signatures of sewage are detectable in organisms
  - 2) Examine how benthic microbial and macroinvertebrate community composition is altered in response to sewage inputs

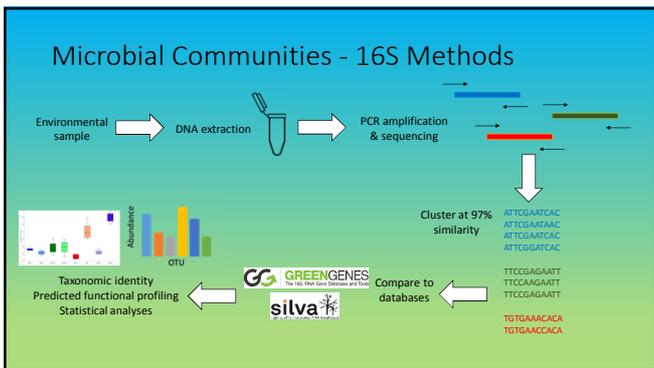


## Methodology

- Stable Isotope Analysis**
  - Nitrogen taken up first by primary producers
  - Seagrass *Zostera capricorni* & associated epiphytes
- Macroinvertebrates**
  - Sampled from seagrass and bare sediments
  - Counted and identified to lowest possible taxonomic level
  - Abundance and diversity analysed


## Microbial Communities - 16S Methods



Environmental sample → DNA extraction → PCR amplification & sequencing → Cluster at 97% similarity → Compare to databases (GREENGENES, SILVA) → Taxonomic identity, Predicted functional profiling, Statistical analyses

Sequence motifs shown: ATTCGATCAC, ATTCGATAAC, ATTCGARTCAC, ATTCGGATCAC, TTCGGAGAAT, TTCGAAGAAT, TTCGGAGAAT, TGTGAACACA, TGTGAACACA

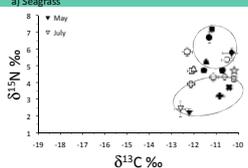
## Stable Isotope Data

- Seagrass and epiphyte  $\delta^{15}\text{N}$  reflected inputs of treated and untreated sewage
  - Consistent with previous research

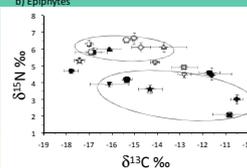


Source: Jones (2016)

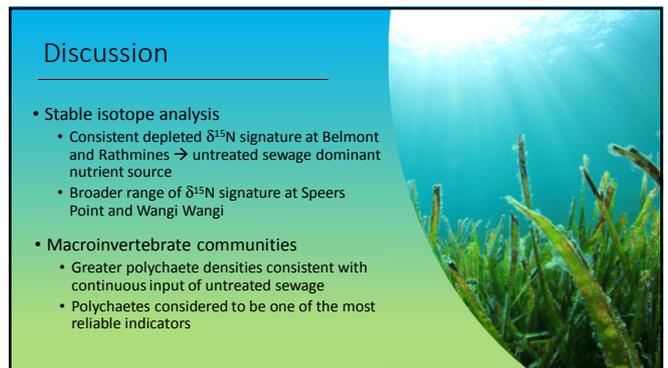
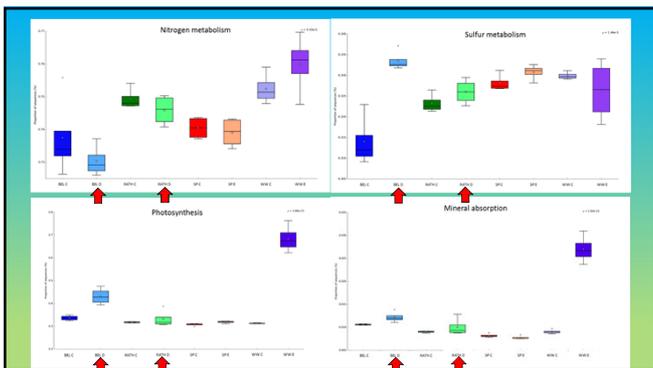
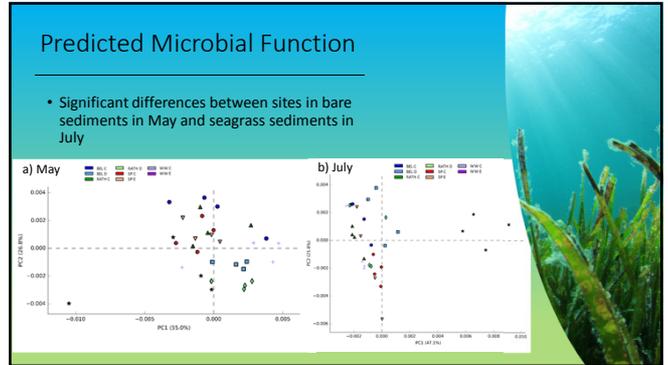
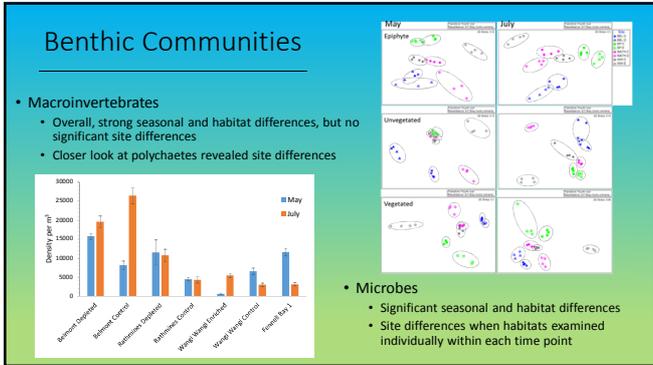
a) Seagrass



b) Epiphytes



- ◆ Belmont-Degraded
- ◆ Belmont-Control
- ◆ Rathmines-Degraded
- ◆ Rathmines-Control
- ◆ Sperrin-Point-Degraded
- ◆ Sperrin-Point-Control
- ◆ Wangi-Wangi-Control
- ◆ Fernhill-Bay-1
- ◆ Fernhill-Bay-2



## Microbial Communities

- Most shifts at Belmont and Rathmines sites
- Shifts in communities stronger when exposed to constant than intermittent stress (Birrer et al., 2018).
  - Nitrogen and sulfur have major role in sediment productivity— changes likely to have repercussions for entire ecosystems (Nogales et al., 2011; Birrer et al., 2018).

## Summary & Conclusion

- Constant untreated input at Belmont and Rathmines → evidence that these inputs impact benthic communities here
- Rapid urbanisation to coastal systems → need to find practical methodologies to monitor and assess ecological impacts of anthropogenic pollution e.g. sewage
- Integrated use of techniques used provides a tool to rapidly and cost-effectively investigate ecological impact of sewage pollution in coastal systems

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Thank you!

