Update on the NSW Oyster Transformation Project August 2020

1. Introduction

The NSW Oyster Transformation Project is a collaborative initiative of oyster farmers, the Food Agility CRC, University of Technology Sydney (UTS), NSW Hunter LLS and the NSW DPI (Aquaculture Research and Biosecurity and Food Safety). Oyster farming is sensitive to water quality problems such as pathogenic bacteria and harmful algae. The recent bushfires and subsequent run off have highlighted the need to understand how oyster producing estuaries respond to extreme events. Access to high frequency data is key to developing tools for farm management. The project is using real time salinity and temperature sensing and novel molecular genetic methods (eDNA) to model oyster food safety, pathogenic bacteria, harmful algae, oyster growth and disease, with the aim of changing harvest management plans to reduce harvest closure days.

2. High Resolution Sensor Data

What have we learned from the sensor data?

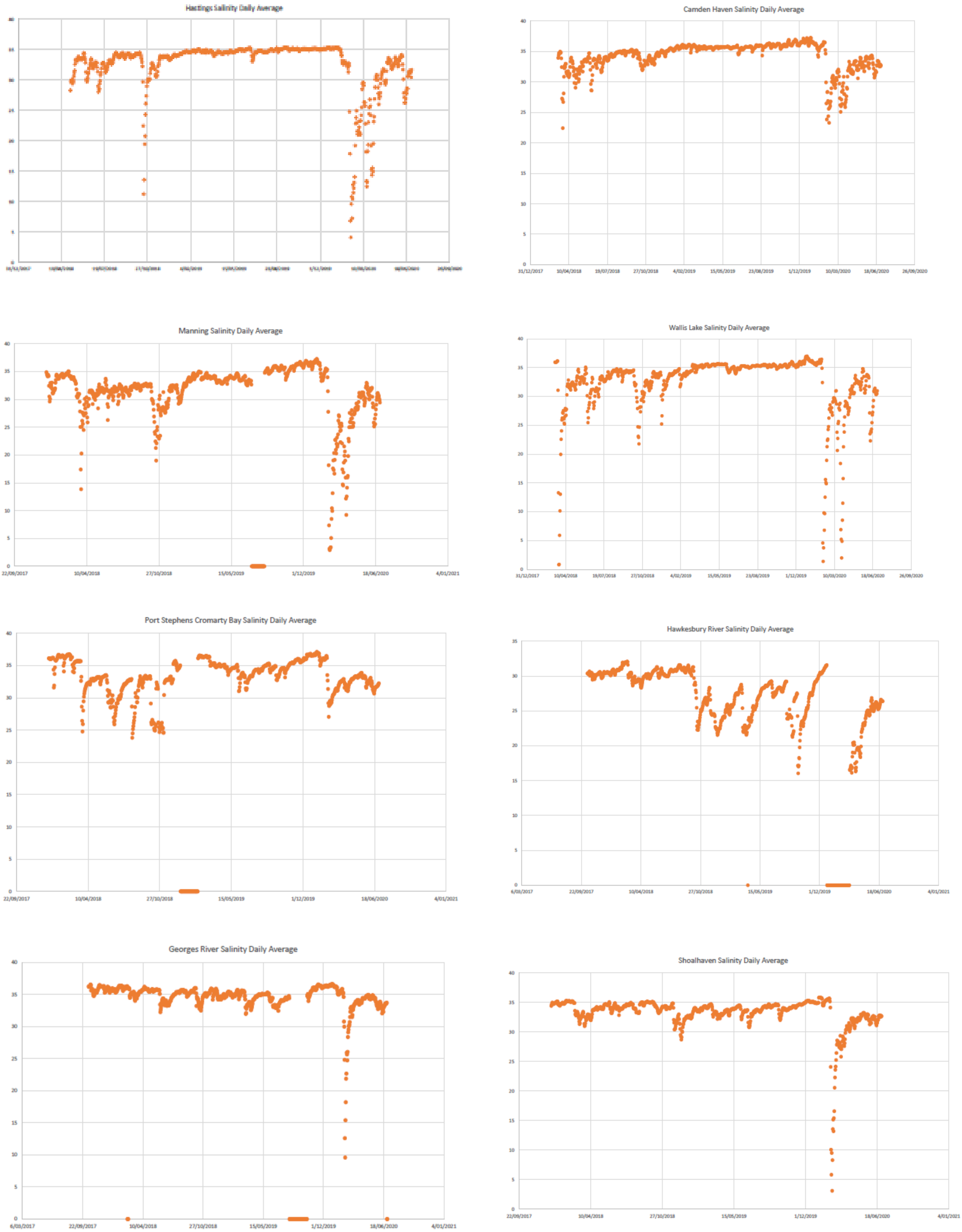
Preliminary results have shown links between salinity, rainfall and *E. coli.* High-resolution data has been collected for 18 months in 13 estuaries using fully autonomous instruments which collect and transmit data on water level, temperature and salinity every 10 minutes (24 hrs per day) to the cloud before downstream quality checking and analysis. The dataset is currently being quality checked and analysed.

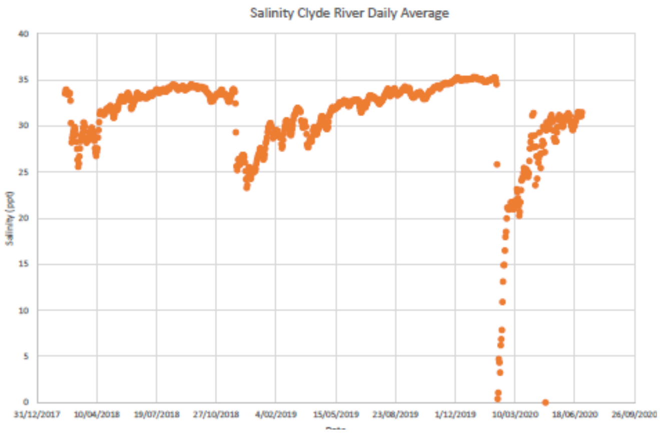
Sensor data has provided the basis for a change to the management plans for the Pambula River Oyster Growing Area and the Cromarty Bay Oyster Growing Area (Port Stephens) in reviews conducted by NSW DPI. These management plan changes mean that harvest area openings and closures can be based on real time salinity sensing.

The extreme drought conditions during the study made it difficult to model rainfall for all estuaries. While salinity only management may not be suitable for all areas yet, these tools in Cromarty Bay and Pambula Lake have shown significant financial benefit. Hindcasting the salinity based management plan for Pambula Lake demonstrated that it would provide 3 additional weeks of harvest opening per year. At a conservative estimate of the Pambula mean annual farm gate production value, this would lead to $137,721 in additional revenue, representing 6% of current revenue per year. In Cromarty Bay, the ~6 months of salinity only management has shown that there is a significant delay between rainfall and runoff impacting the harvest area, providing additional harvest opportunity for farmers in this harvest area.

If these benefits were replicated across the oyster producing estuaries in NSW, that would lead to an estimated revenue increase of $3.48 million and 87 FTE jobs.

Figure 1: Daily salinity averages from 2017- June 2020 as recorded by the sensors at the Hastings River, Camden Haven, Manning River, Wallis Lake, Port Stephens, Hawkesbury River, Georges River, Shoalhaven-Crookhaven River, Clyde River, Pambula Lake, Wagonga Inlet, Wapengo and Wonboyn Lake.




Salinity Clyde River Daily Average
Salinity (ppt)
40
35
30
25
20
15
10
5
0
31/12/2017
10/04/2018
19/07/2018
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4/02/2019
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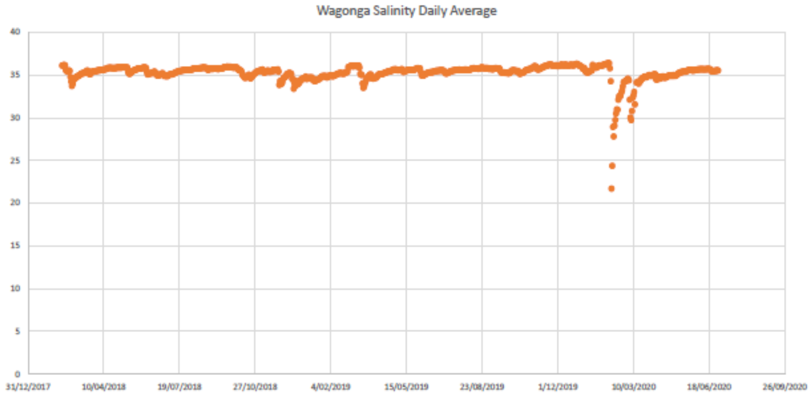
18/06/2020
26/09/2020


Wagonga Salinity Daily Average
40
35
30
25
20
15
10
5
0
31/12/2017
10/04/2018
19/07/2018
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4/02/2019
15/05/2019
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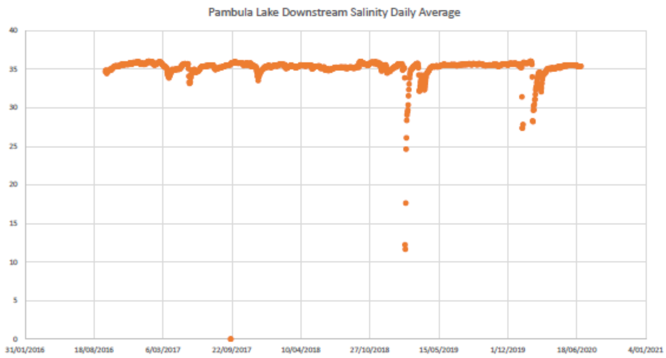
Pambula Lake Downstream Salinity Daily Average
40
35
30
25
20
15
10
5
0
31/01/2016
18/08/2016
6/03/2017
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27/10/2018
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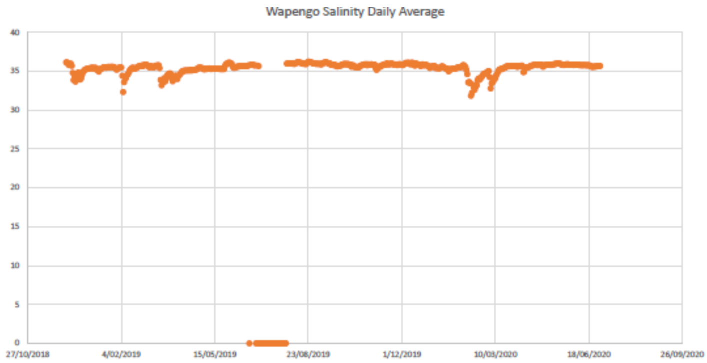
18/06/2020
4/01/2021


Wapengo Salinity Daily Average
40
35
30
25
20
15
10
5
0
27/10/2018
4/02/2019
15/05/2019
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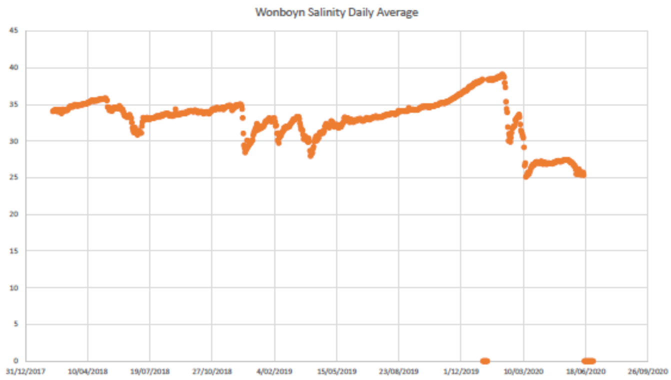
10/03/2020
18/06/2020
26/09/2020


Wonboyn Salinity Daily Average
45
40
35
30
25
20
15
10
5
0
31/12/2017
10/04/2018
19/07/2018
27/10/2018
4/02/2019
15/05/2019
23/08/2019
1/12/2019
10/03/2020
18/06/2020
26/09/2020

3. eDNA sampling and data analysis

In this project, we are using (a method called environmental DNA (or eDNA) and quantitative PCR (qPCR) to measure bacterial and algal concentrations. These methods look at genetic material to distinguish different species and strains.

This method has many advantages over traditional water quality analysis methods, in that it is more accurate, cheaper, and can be adapted to be run on site with portable equipment, with results available within 1-2 hours. In addition, DNA samples can be stored in freezers, and therefore it is possible to archive them for future research studies in years to come.

In this project, we have collected the largest known database of oyster and water samples from NSW estuaries. We are now in the process of analysing them, looking for harmful algae, *E.coli* and oyster diseases.

At the end of June 2020, a total of 6,662 filtered water samples and 2,828 oyster samples have been collected by shellfish industry members since the start of the project in September 2018 – what an effort!

Why do we need so many samples and what will the samples tell us?

Having a large number of samples means that we have enough data to model the impacts of temperature, salinity, and seasonality on *E.coli*, oyster growth, disease, and harmful algae, and be able to start to predict what will occur under certain sets of conditions. The ability to predict these factors impacting oyster farming can be incorporate into tools for improved farm management.

qPCR assay development for *E.coli* and other microbiological indicators

The qPCR (quantitative-polymerase chain reaction) technique monitors DNA amplification in real time by reading increments of fluorescence signals as DNA is amplified over cycles of temperature changes. This method is being increasingly used to quantify microbes in water samples.

In February 2020, water samples from Quibray Bay (Georges River estuary) were collected before and after a rainfall event (total rainfall greater 300 mm over 7-10 February 2020) (see Figure 2B) to monitor the amounts of *E. coli* and *Enterococci* (faecal coliform forming bacteria) and other harmful microbial agents that originate from humans, cow and birds (microbial source tracking).

We successfully detected and quantified different pathogenic bacteria in the estuary samples after the rainfall event (shown in red in Figure 2A-B) as compared to prior to rainfall (highlighted in green in Figure 2A-B). Such genetic assays have previously been used for monitoring the quality of recreational water bodies, and we are now optimising them to detect low quantities of microbial contaminants (up to 8-9 CFU/100ml).

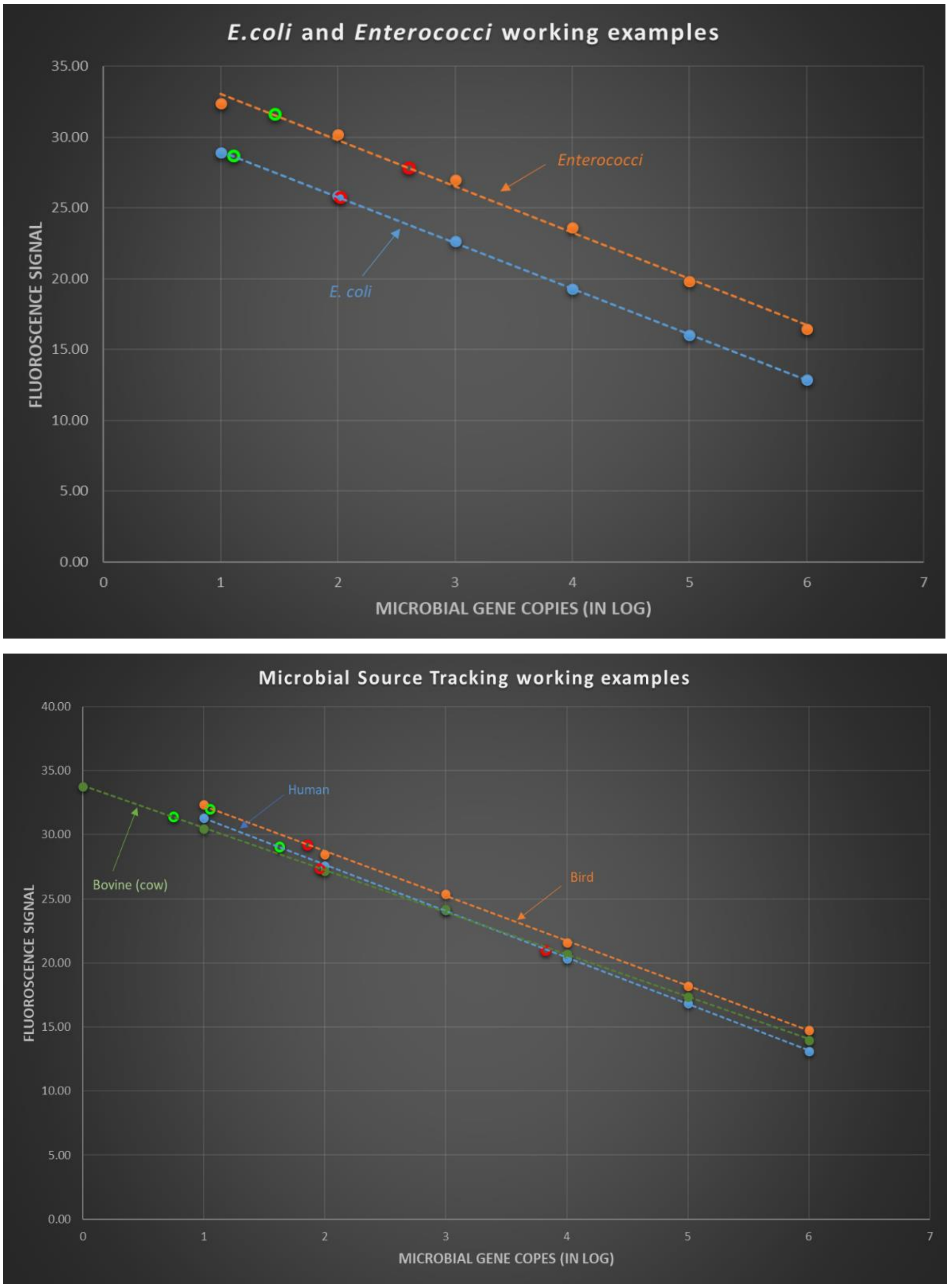
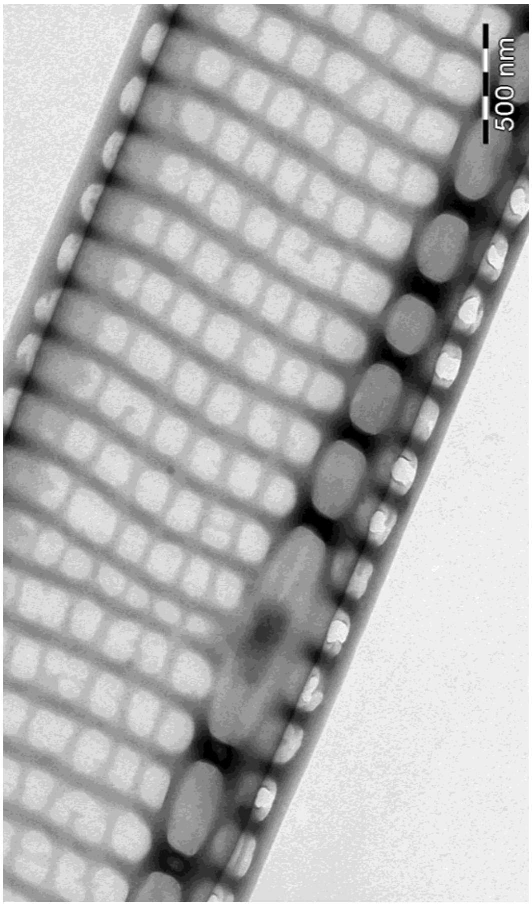


Figure 2. qPCR results showing ~10x more *E. coli*, *Enterococci* and harmful microbial pathogens originating from cow, bird and humans in Quibray Bay water samples after (in red) as compared to before (in green) the rainfall event in February 2020.

4. Case Study: Harmful Algal Bloom Modelling in Wagonga Lake

Pseudo-nitzschia is a diatom, a type of phytoplankton common in marine waters, with approximately 52 species identified worldwide. Of these, 26 have proven to produce the neurotoxin domoic acid (DA) which can bioaccumulate through the food web and cause Amnesic Shellfish Poisoning (ASP) in consumers of seafood. Symptoms range from gastrointestinal (nausea, vomiting, diarrhoea) to neurological (headaches, dizziness, disorientation, seizures, short-term memory loss, permanent brain damage), to death in some cases.



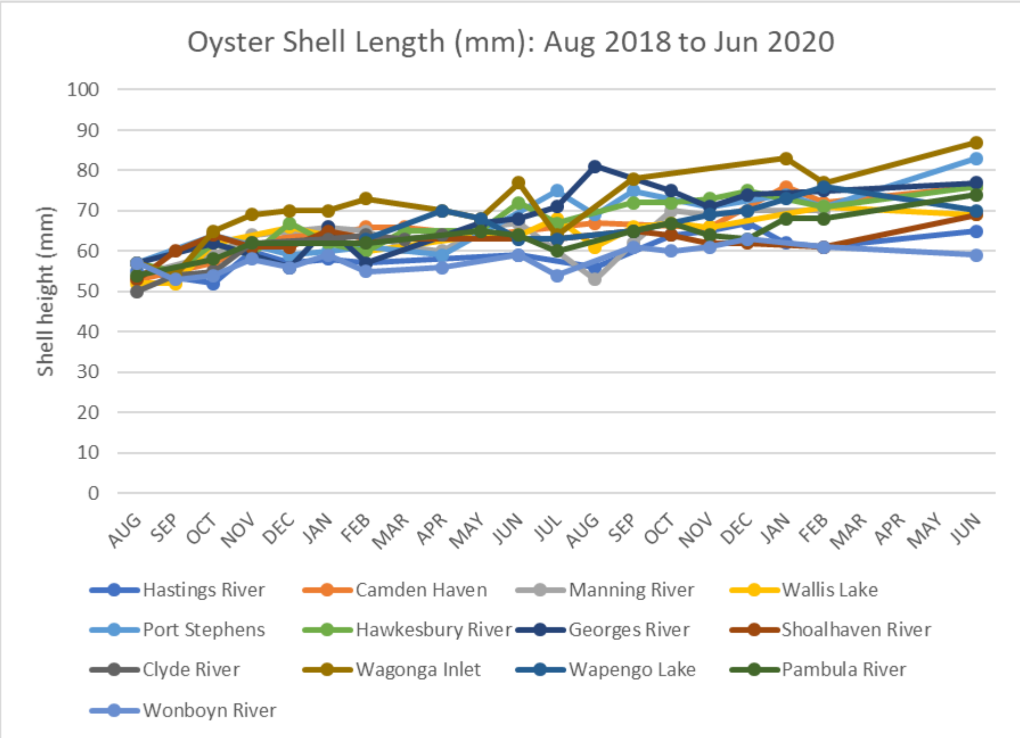
Pseudo-nitzschia cuspidata (Fig. 3) was responsible for a significant toxic episode and closure of oyster harvest areas in Wagonga Inlet in 2010. The closure lasted 16 weeks, the longest ever experienced in south-east Australia, and resulted in significant financial loss to the industry.

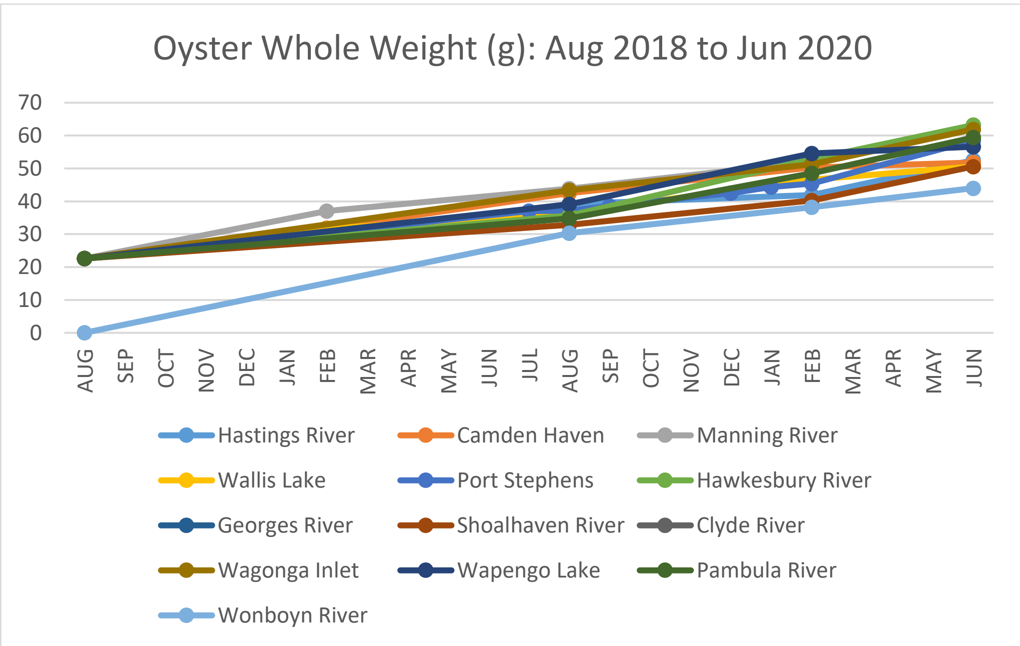
In April 2019, during the Oyster Transformation Project, a subsequent *Pseudo-nitzschia* bloom occurred in Wagonga Lake. Using novel molecular methods, four species were identified, including the 2010 toxic culprit *P. cuspidata*. Subsequent mathematical modelling (Generalised Linear Modelling) using the sensor data confirmed that salinity data was significantly more predictive of the bloom than rainfall data, with 48hr to weekly salinity averages being significantly more predictive of the bloom than any other variable. Presently, qPCR assays (similar to as described above) are being developed to track the bloom dynamics and populations of *Pseudo-nitzschia* species in Wagonga Lake as well as other sampling estuaries.

Figure 3. An electron microscope image of the toxic diatom *Pseudo-nitzschia cuspidata* from Wagonga Inlet

5. Oyster Health

During this project the oyster farmers involved have provided a huge data set related to oyster health. Oyster shell length, whole weight and mortality data were collected by participants at each site from August 2018 to June 2020. The oysters sent to each site were from a genetically similar group and were 21 months old at the start of the experiment. These data are currently undergoing analysis/modelling. Some draft preliminary graphs have been produced to identify specific trends for oyster shell length (mm), whole weight (g) and cumulative morality (%) at each of the sites before further analysis occurs. These preliminary graphs have been included below and further dissemination of these results will shortly follow.





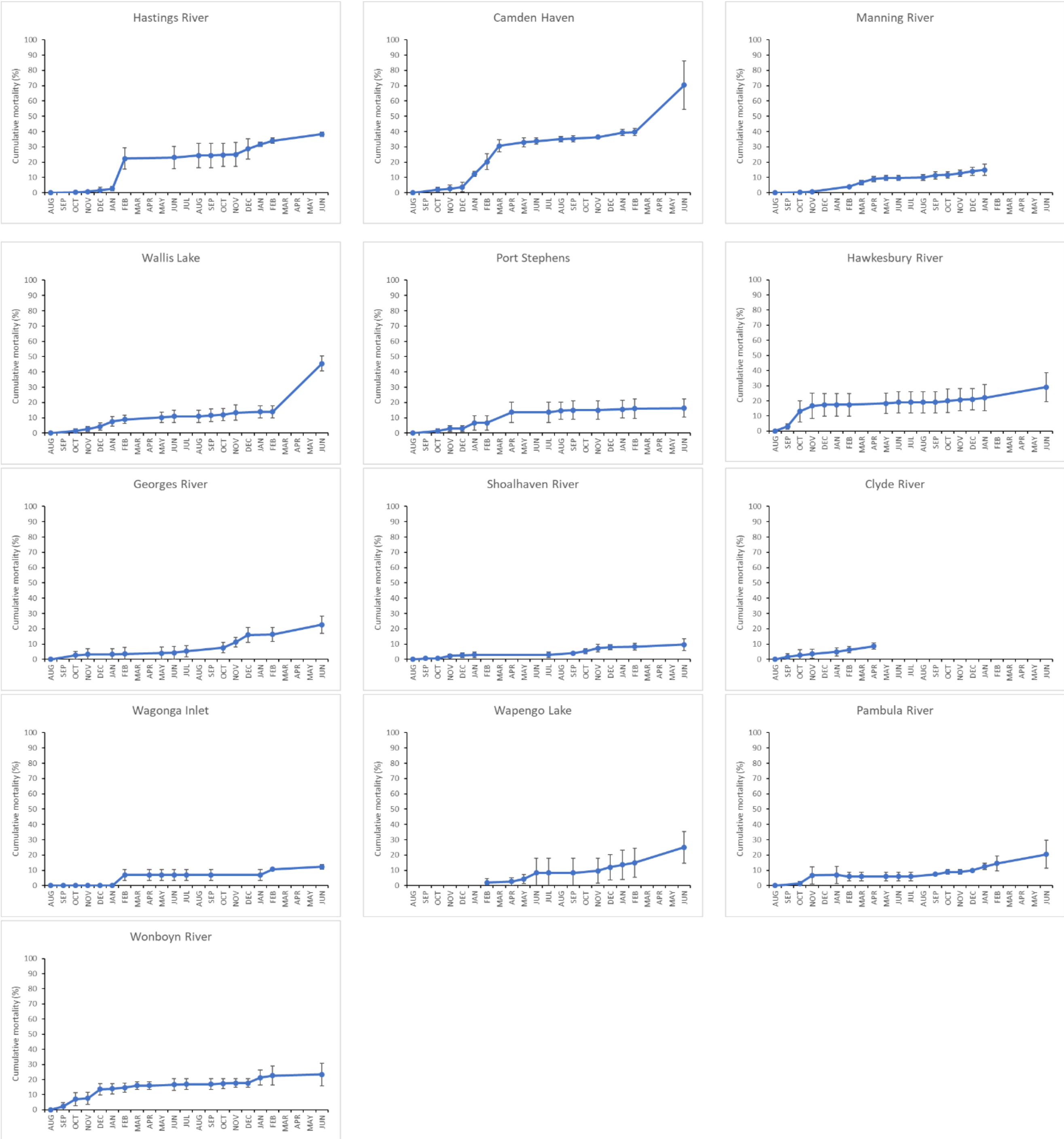


Figure 5a-c. Oyster shell length (mm), whole weight (g) and cumulative mortality data from 13 NSW oyster growing estuaries.

Next Steps

Outcomes from the current project

Once the project team has finished processing the samples and analysing the data generated from this work they will present their findings to industry and other stakeholders. Given the large amount of information generated it is anticipated that this process will continue for the next 1-2 years. This will be an on-going process that will happen in consultation with industry. If funding is secured for Stage 2 of the project the new data generated will expand on the information generated in Stage 1.

Stage 2 of the project

The CRC FA Oyster Transformation project was designed to run for a limited period, ending in December 2020, and the Yield will be removing their sensors at this time. Having established their potential benefit to industry, funding has now been sought to provide sensors for 2021-2022. Funding is being discussed with collaborators, NSW Farmers, Food Agility CRC, UTS, councils, and the NSW DPI.

The funding sought will be to install and operate salinity/temperature/water level sensors for two additional years in each estuary (2021-2022). These sensors will have a publicly available interface for data sharing, with not only the oyster industry, but all interested end users and beneficiaries including the local community, councils, other government agencies and stakeholders. Sampling for harmful algae and bacterial indicators in oyster harvest areas will continue as a regulatory requirement by DPI Biosecurity and Food Safety.

Key questions that we aim to answer:

- What is the link between estuarine salinity and microbiological contaminant loads in relation to rainfall and runoff?
- Can we track the sources of pathogenic microbes (e.g distinguishing sources such as from kangaroos, cattle, birds, humans)?
- Can we predict the prevalence and intensity of harmful algal blooms?
- Can we predict oyster growth and mortality rates from QX and Winter Mortality disease?
- Can we better understand water quality for estuary management, for example, openings of Intermittently Closed and Open Lakes and Lagoons (ICOLLs)?
- Can we work towards a goal of rapid, on site testing for pathogenic microbes and harmful algal blooms, with results available in hours instead of days?

Answering these questions will move us along on our long term goal of improving the way that industry can operate, to allow for rapid information flow for decision making, and regulatory systems that are equally responsive to short term environmental changes that impact oyster farming.