TRANSFORMING AUSTRALIAN SHELLFISH PRODUCTION

Pelican Point Harvest Area - Manning River

Report on Stage 1, December 2017-March 2021

A Food Agility CRC collaboration project partnering with the University of Technology Sydney and the New South Wales government.

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Executive Summary

This report presents results from the Manning River, one of the estuaries selected as part of Stage 1, the NSW Oyster Industry Transformation Project 2017-2021. To predict the impact of rainfall on faecal indicator organisms, Harmful Algal Blooms (HABs) and oyster disease, precise environmental data with a high temporal frequency were collected and modelled. Combined with state-of-the-art molecular genetic methods, this information will help to improve efficiency and transparency in food safety regulation, provide predictive information and provide insights for more informed and responsive management of shellfish aquaculture.

We installed a real-time sensor at Pelican Point in the Manning River, recording high-resolution temperature, salinity and depth data. Oyster farmers collected weekly biological samples (615 environmental DNA samples and 252 deployed/retrieved oysters for growth assessment) from this same location. We developed a rapid molecular qPCR (quantitative polymerase chain reaction) assay for the harmful algae *Dinophysis*, and another for *E. coli*, which could directly compare to the currently used plate count by commercial laboratories. We also developed specific qPCR assays that could determine which animals were contributing to the *E. coli* load in the river system. We used these assays to observe trends in faecal pollution and modelled these in relation to environmental variables (salinity, temperature, rainfall etc), to developed predictive models. Finally, we tested the capability of a further model to determine its predictive capability to link oyster growth with these environmental variables.

MAJOR FINDINGS

8

Available data indicated that eight harvest area closures could have potentially been avoided between December 2017 and August 2021

75%

Salinity was a more reliable predictor than rainfall of faecal bacteria (3 out of 4 indicators tested), showing changed harvest area management would be safer and more discriminatory

E. coli and cow faecal bacteria increased with rainfall, and generally dissipated after three days

Human bacteria were very low across sampling period

0

No oyster mortality events that exceed background farming mortality occurred over the study period

1. Introduction

1.1 Transforming Australian Shellfish Production

The Transforming Australian Shellfish Production Project (TASPP) follows on from the success of the NSW Oyster Industry Transformation Project (NSWOITP), which is a UTS led, multidisciplinary collaboration between oyster farmers (NSW Farmers Association), researchers (UTS, DPI Aquaculture and Fisheries), regulators (DPI Biosecurity and Food Safety) and the Food Agility CRC. Hunter Local Land Services (LLS) has been an important partner in the Manning, Wallis Lake and Port Stephens estuaries, funding the installation of hardware in the Manning and working in partnership with MidCoast Council to improve catchment management and reduce water quality impacts on the oyster industry. The project uses real time, high-resolution salinity, temperature and depth sensing, combined with novel molecular genetic methods (eDNA), to model oyster food safety, pathogenic bacteria, harmful algae, and oyster growth and disease, with the aim of changing harvest management and to reduce harvest closure days for farmers.

As filter feeders, shellfish like oysters and mussels actively remove particles from surrounding waterways. Following high-risk events such as heavy rainfall or harmful algal blooms, regulators like the NSW Food Authority implement precautionary harvest area closures to manage potential food safety risks or implement shellfish movement restrictions to manage potential biosecurity risks. Shellfish farmers in Australia are not currently able to predict the likelihood of a harvest area closure due to these high-risk events. If farmers were aware of imminent closure, they could take meaningful action such as harvesting early, or moving stock to lower risk areas of their lease. The same environmental variables that influence food safety can also impact on oysters' health and can increase the risk of certain diseases. Understanding these relationships and monitoring these variables could be used to reduce the risk and severity of disease outbreaks.

This project will deliver functioning, estuary-specific models relating to oyster growth, disease risk, harmful algal bloom risk, sources of contamination, and other supporting factors influencing industry productivity. Each of these models will relate biological data to high frequency water quality metrics as measured by real-time sensors deployed in situ.

Stage 1 (2018-2021) of the program has been successfully completed, with ~5000 water and 3000 oyster samples collected across 13 NSW estuaries engaged in the project. Stage 2 (2021-2024) is now underway, with two further NSW estuaries engaged, and expansion of the project into Western Australia. Sample processing, data analysis and report writing will continue during this second phase, with modelling to predict oyster growth and mortality rates, including key oyster diseases such as *Marteilia sydneyi* (QX), and Winter Mortality, and the intensity of harmful algal blooms planned. As part of these analyses, novel qPCR assays for *E. coli* (bird, cow, human) and harmful algal species (*Pseudo-nitzschia* spp., *Dinophysis* spp., *P. minimum*), which were developed during Phase 1, will also be implemented.

Preliminary results from this high frequency data have already demonstrated the link between salinity levels related to rainfall and *E. coli* levels. In 2019, the NSW Shellfish Program's Annual Sanitary Survey Report (DPI) stated that using this real-time, high frequency environmental data,

the CRC project provided the basis for a change to the management plans for the Pambula Lake harvest area and the Cromarty's Bay harvest area (Port Stephens). These management plan changes mean that harvest area openings and closures can be based on salinity-only data, with unnecessary extra harvest closure days avoided. As early adopters of the technology for harvest area management, an independent economic assessment by NSW DPI completed in January 2021 evaluated Pambula Lake and Cromarty's Bay. The report highlighted the positive benefits for industry through using salinity-based management plans. Focusing on the six-month period where oysters were at peak marketable condition, it was estimated that up to two extra weeks of harvest could be achieved, with a projected annual net profit boost of $15,344 (Cromarty's Bay) and $95,736 (Pambula Lake) for the study areas, based on current lease area used. The full report is available on the NSW Food Authority website.

Across the NSW shellfish industry, the potential economic benefit from the use of real-time sensors for harvest area management is conservatively estimated at up to $3 million annual farm gate value. Increased revenue will improve the confidence of the industry to further invest and drive more growth. As of February 2022, thirteen salinity-only management plans had been offered for harvest areas in participating NSW estuaries, of which six were taken up and seven are under consideration.

1.2 Manning River Estuary

The Manning River Estuary is a mature, wave dominated barrier estuary, with a largely non-agricultural (forested) catchment area of 8420 km^2. The estuary has 4.83 and 0.13 km^2 of mangrove and saltmarsh cover respectively, 16 major contributing tributaries, and an average flushing time of 31.6 days (Roy et al. 2001, Roper et al. 2011). This relatively long residence time means this estuary particularly sensitive to the risk of contamination from pathogens (stock and human sewage). Land management issues impacting on water quality in the catchment include hill-slope and streambank/bed erosion, run-off from acid sulphate soils and extensive livestock agriculture which is often situated adjacent to the oyster production areas in the lower catchment/floodplain (Swanson 2019).

1.3 Oyster Production in the Manning River

The Manning River is an important oyster-growing estuary. The industry was established in this river in 1871 and now produces ~67K dozen oysters per year (DPI 2021). Significant threats to this production include pathogens (faecal coliforms and *E. coli*) from sewerage and septic systems particularly in Pelican Bay, Scott's Creek and the South Channel. As part of DPI's Shellfish Quality Assurance program, Pelican Point harvest area is classified as Conditionally Restricted[1], and the harvest area is closed when >30 mm of rainfall[2] occurs within 24 h, or salinity measured at any site at mid-ebb tide is <19 ‰. These closures are based on the increased risk of faecal coliforms in the waterway resulting from significant rainfall run-off. Faecal coliforms entering the river most likely originate from stock as well as human sources with many of the surrounding subcatchments being unsewered (Swanson 2019).

[1] When the harvest area is open, any shellfish stock harvested must meet depuration requirements prior to sale.
[2] The official management plan rainfall gauge for Pelican Point harvest area is BOM Taree Airport

FINDINGS

2. Findings

2.1. The data assessment supports implementing a harvest area management plan based on sensor salinity data, subject to the agreement by the local shellfish industry. Available data indicated that eight harvest area closures could have potentially been avoided between December 2017 and August 2021.

2.2. The real time sensor data showed a higher predictive capacity than rainfall data for three out of the four faecal indicator bacteria.

2.3. Modelling using real time sensor data was highly predictive of *E. coli*/bacteria related to cow (32-74%), and human faecal pollution (60-78%). *E. coli* and cow faecal contamination became elevated with increasing rainfall (decreasing salinity), but generally dissipated after three days. We developed rapid, efficient, and sensitive qPCR assays for *E. coli*, cow, bird, and human faecal indicators, and used these rapid genetic tools to track these sources of pollution in the Manning River over the biological sampling period, September 2018 to September 2020.

2.4. Bird bacteria was highly variable across rainfall events and not strongly correlated with any environmental indicators, while human bacteria remained in relatively low concentrations during rainfall events.

2.5 We developed a rapid, sensitive and efficient quantitative real-time polymerase chain reaction (qPCR) assay to detect *Dinophysis* and evaluated its effectiveness by successfully comparing environmental samples to microscopy-based cell counts across the same time period.

2.6. The greatest oyster growth occurred during late winter to spring (August to November). General Additive Modelling revealed that the maximum predictive capability of oyster growth was 79%, with the weekly maximum salinity being the most significant predictor variable. Higher weekly salinity peaks predicted larger shell lengths.

2.7. No oyster mortality events that exceeded background farming mortality (approximately 10% per annum) occurred at the Manning River monitoring site over the study period.

ACKNOWLEDGEMENTS

3. Acknowledgements

This project is proudly funded by the NSW Government in association with the Food Agility CRC and the NSW Farmer's Association. The Food Agility CRC Ltd is funded under the Commonwealth Government CRC Program. The CRC Program supports industry-led collaborations between industry, researchers and the community. The Department of Primary Industries, the University of Technology and NSW Farmers also provided project funding. Hunter Local Land Services (LLS) provided funding for sensor deployment and maintenance through the NSW Government's Catchment Action NSW program, enabling the Manning River to be included in the state-wide project. The project team would like to acknowledge the invaluable assistance of the Manning River oyster farmers for collecting weekly samples. Specifically, we thank Ian and Rose Crisp for their assistance and co-ordination of sample collection. We also wish to acknowledge the assistance of staff from The Yield Technology Solutions for facilitating access to the water salinity and temperature data used in the analysis. Routine phytoplankton monitoring sample data for the Manning River were funded by the NSW Food Authority and the shellfish industry. We thank Kyle Johnston and Brandt Archer (DPI) for oyster stock preparation and growth/survival data collection, and Dr Nahshon Siboni and Prof Justin Seymour (UTS) for source tracking assistance. Finally, we would like to thank Prof Stephen Woodcock (UTS) and Prof. Manfred Lenzen (USyd) for statistical analyses, Phil Baker (DPI) for the map, and Chris Komorek (Food Agility CRC) for layout.


FEEDBACK
MERCURY
60

4. Feedback

A meeting with key Manning River growers was held on the 7 March 2018 at Manning Point. This meeting included the local shellfish quality coordinator, key growers and representatives from the Food Authority, NSW DPI and LLS. At the meeting topics discussed were:

- the spectrum of potential benefits arising from the program;
- the potential benefits specifically relating to salinity-based estuary closures and harvest area classification;
- general scientific requirements and aims of the project;
- the positioning of the sensor unit for greatest gain; and
- further opportunities for growers to consider future sensor placement.

As a direct result of this meeting, LLS began a sub-catchment program around Pelican Bay with the aim of improving water quality for oyster growers. This ongoing program has been rehabilitating coastal wetlands by reinstating tidal flows, stock exclusion and weed control. This work is not a direct part of the Oyster Transformation Project, but it highlights the flow on benefits that can arise when engaging growers and other stakeholders on water quality issues. Results from the Oyster Transformation Project continue to be fed back to landholders, MidCoast Council and other stakeholders, providing impetus to keep improving water quality.

In June 2018, the Oyster Transformation Team held an information workshop to give farmers the opportunity to have their say in the project. The workshop was at the Manning Valley Visitor Information Centre in Taree, New South Wales. Farmers were asked to rate the following factors in order of importance and benefit to their business operations (Fig 4.1). Of highest importance to them was the prediction of harmful algal blooms and access to real time monitoring data, followed by reduced stock mortalities/disease, longer harvest opening times with forecasting ability, and access to real time tidal information. Group discussions followed, whereby additional issues that farmers raised were; if routine algal monitoring methods could be changed and if identifying sources of E. coli via genetics was possible. Remarks relating to direct harvest and management plan changes, pollution source tracking, and concerns about mudworm were also noted.

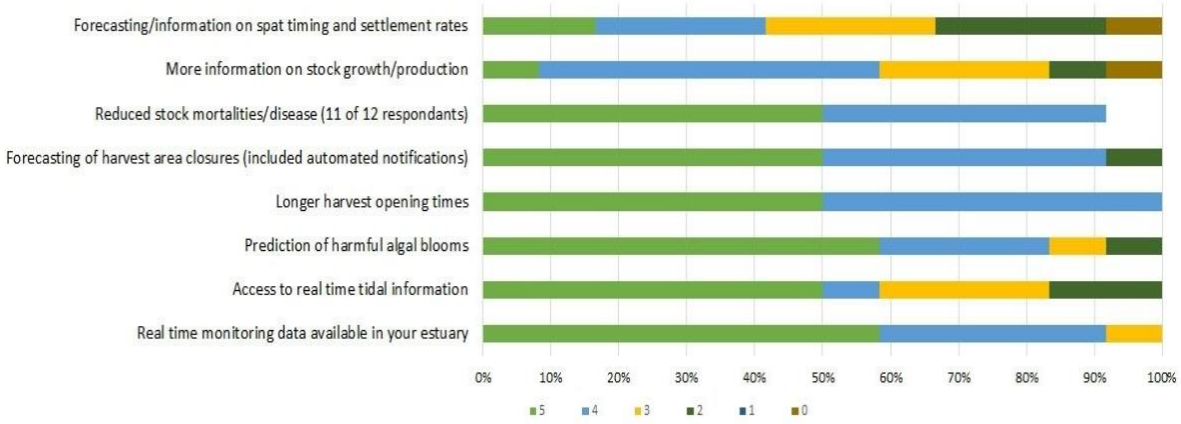


Figure 4.1. The importance of factors as rated by farmers in relation to their business operations. Light green is most important and brown is least important.


RESULTS

5. Results

5.1 High resolution temperature and salinity data

High-resolution real time data summaries for the Manning River are shown in Figs. 5.1A-C. Data between 11 Jul 2019 and 16 Aug 2019 was removed from the dataset due to sensor fault/odd salinity data. Depth recordings ranged from 0.17 m (2 Jan 2019) to 2.8 m (20 Mar 2021). The lowest and highest daily average salinity recordings were 0.1 ppt (21 Mar 2021) and 37.3ppt (7 Jan 2020) respectively, while the lowest and highest daily average temperature recordings were 13.6℃ (20 Aug 2018) and 29.9 ℃ (14 Feb 2018), respectively.

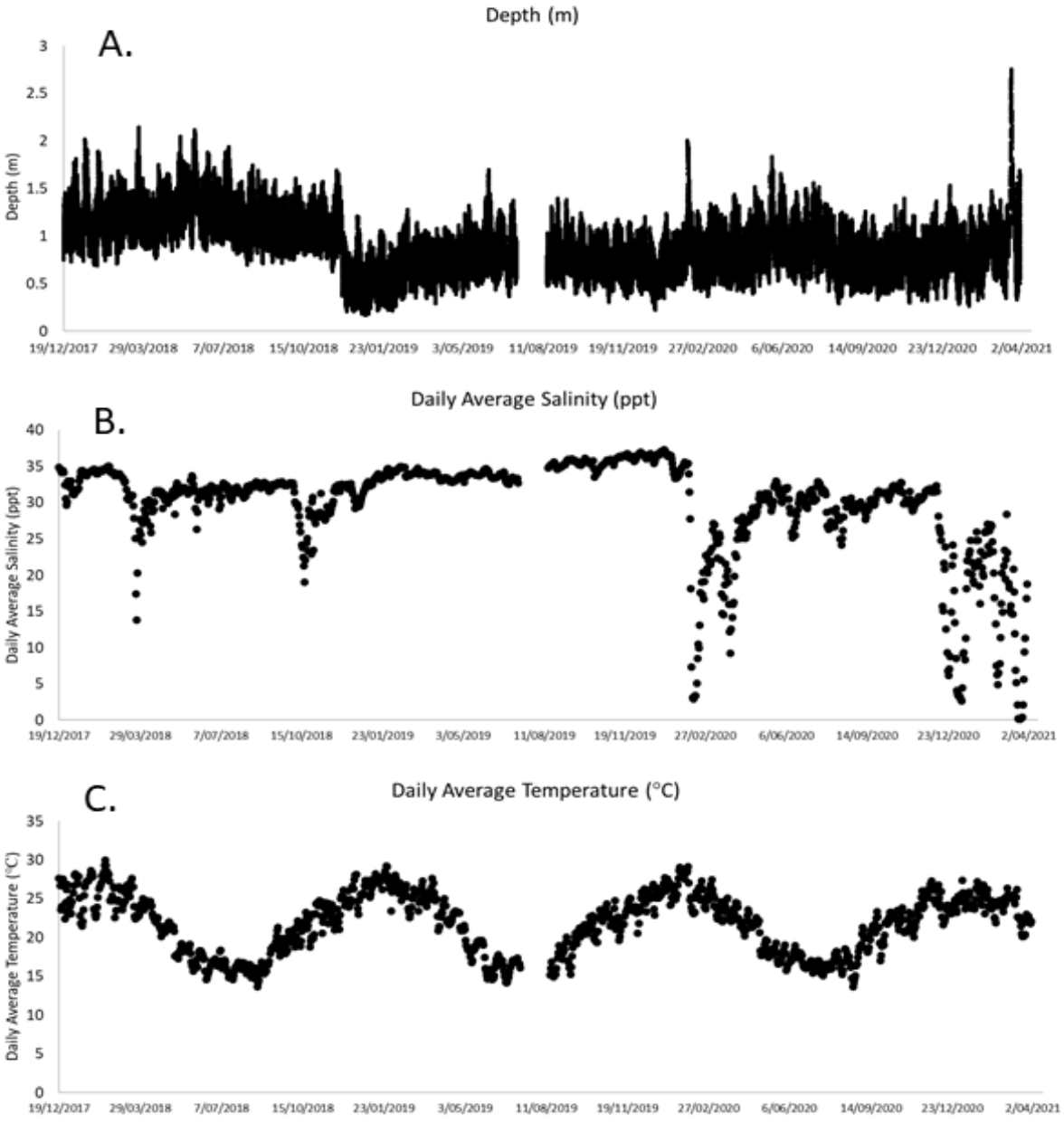


Figure 5.1A-C. Real time sensor data from Manning River 19 Dec 2017 to 31 Mar 2021. A. Depth (m); B. Daily average salinity (ppt); and C. Daily average temperature (℃).

Ten moderate rainfall events were sampled across the study period. These occurred on 27-29 Sept 2018; 29 Nov- 1 Dec 2018; 11-12 Mar 2019; 2-4 April 2019; 5-7 June 2019; 20-22 Sept 2019; 13-15 Oct 2019; 16-18 Mar 2020; 10-12 June 2020; and 27-29 July 2020. The maximum daily rainfall occurred on 22 March 2018 and was reported as 165.6 mm at Taree Airport rain station (Number: 60141, Lat: 31.89° S, Lon: 152.51° E) (Fig. 5.2).

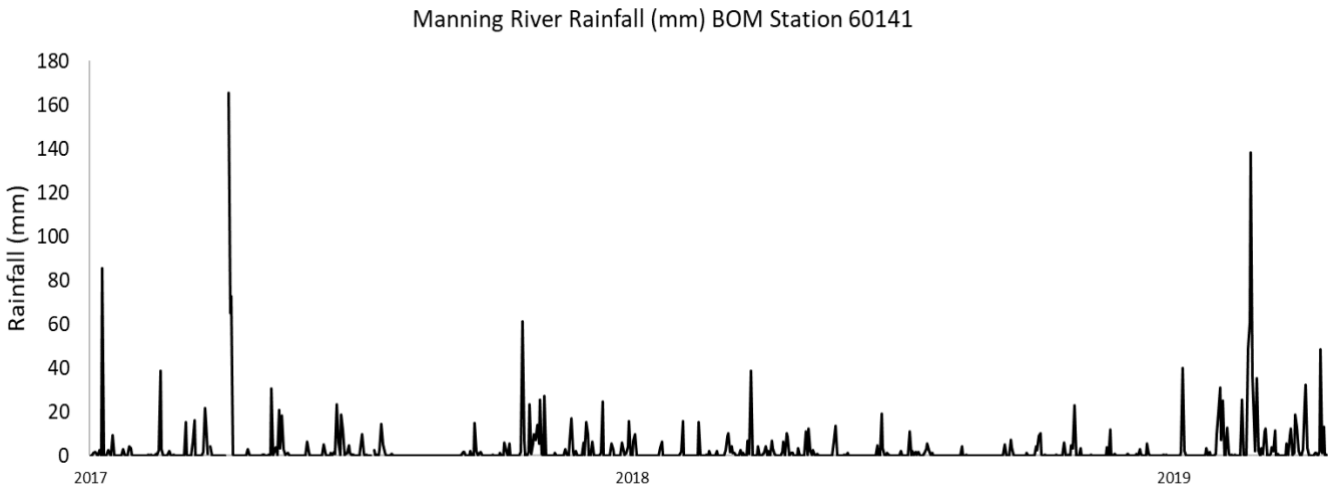


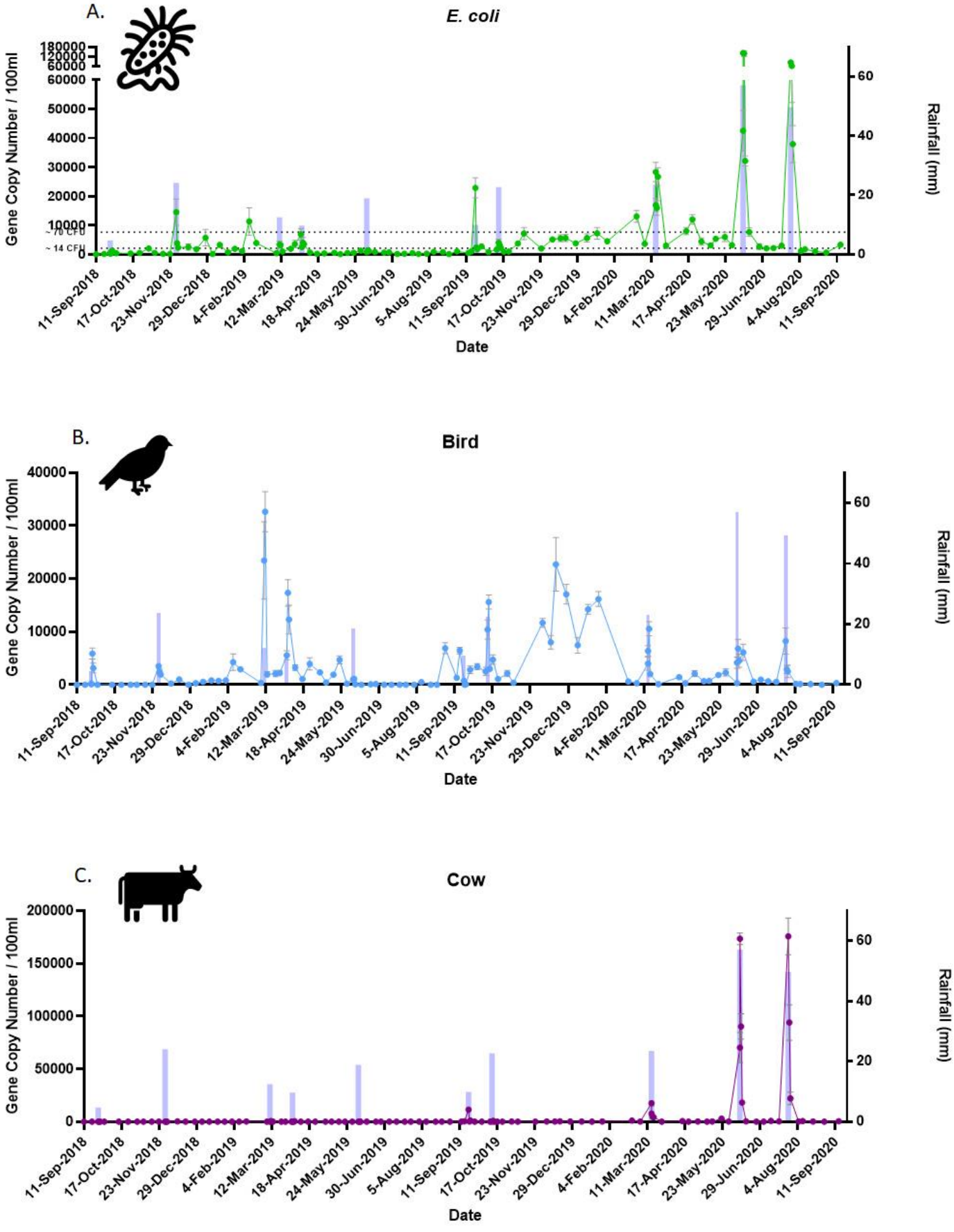
Figure 5.2 Daily rainfall (mm) from the Bureau of Meteorology rainfall gauge site at Taree Airport (Station No. 60141).

5.2 Management Plan

Data analysed during the 2020 and 2021 annual reviews of Pelican Point harvest area indicated that there could have been fewer harvest area closures since the sensor was installed, if closures were based on salinity sensor data. Seventeen harvest area rainfall closures occurred between December 2017 and August 2021). Based on a management plan closure limit of 19 ‰, harvest area closures were reviewed based on available salinity sensor data and shellfish program microbiological results since December 2017. Fifty-four harvest closure days occurred over eight rainfall closures, although salinity sensor data did not decline below 19 ‰ and microbiological results from samples collected between 0-16 days post closure met Restricted harvest criteria. Time periods where salinity is slower to recover may require additional sampling to meet management plan requirements.

5.3 Bacterial source tracking

A total of 615 water samples and 252 oysters were collected over a two-year period (a subset of the entire sensor data collection time) from Sept 2018 to Sept 2020 from the sensor location in the Manning River. The maximum *E. coli* reached 141,841 gene copies 100 mL^{-1} on 10 Jun 2020, 32,632 gene copies 100 mL^{-1} for *Helicobacter* (bird) on 12 Mar 2019, 175,694 gene copies 100 mL^{-1} for bovine faecal pollution (cow) on 10 Jun 2020, and finally, 5,649 gene copies 100 mL^{-1} for human faecal pollution on 27 Jul 2020 (Fig. 5.3 A-D).


A.
E. coli
Gene Copy Number / 100ml
Rainfall (mm)
~ 70 CFU
~ 14 CFU
Date
B.
Bird
Gene Copy Number / 100ml
Rainfall (mm)
Date
C.
Cow
Gene Copy Number / 100ml
Rainfall (mm)
Date

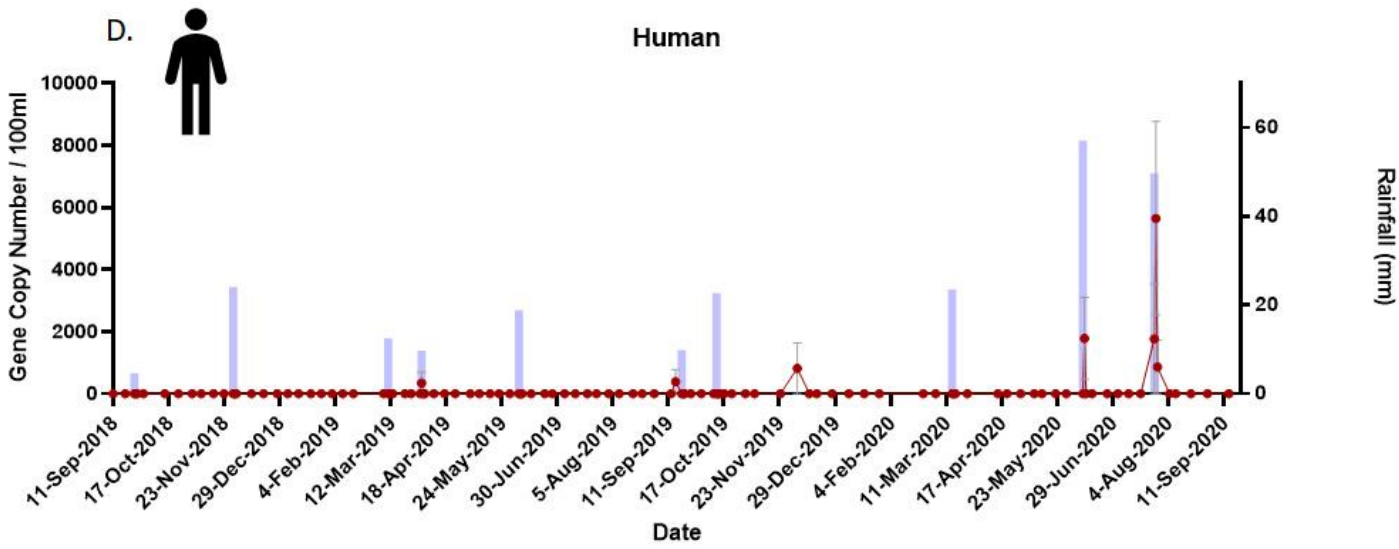


Figure 5.3 A-D. Weekly *E. coli* data from the sensor location, Manning River, using A. *E. coli* assay; B. Bird assay; C. Cow assay; and D. Human assay. Purple bars represent rainfall events. Dotted lines in Fig. A at 14 and 70 cfu/100 mL are the operational limits for direct or restricted (oysters must meet depuration requirement) harvest, respectively, depending on individual harvest area classification. Pelican Point harvest area is classified as Conditionally Restricted. https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/industry/shellfish_industry_manual.pdf.

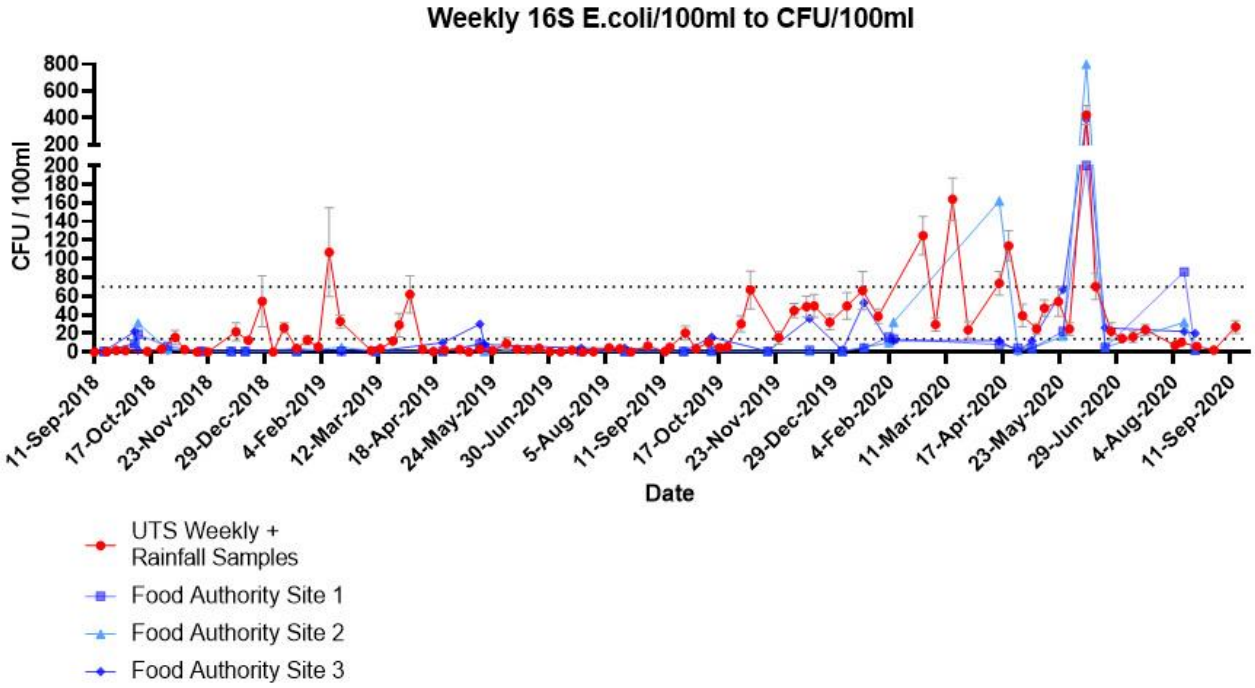
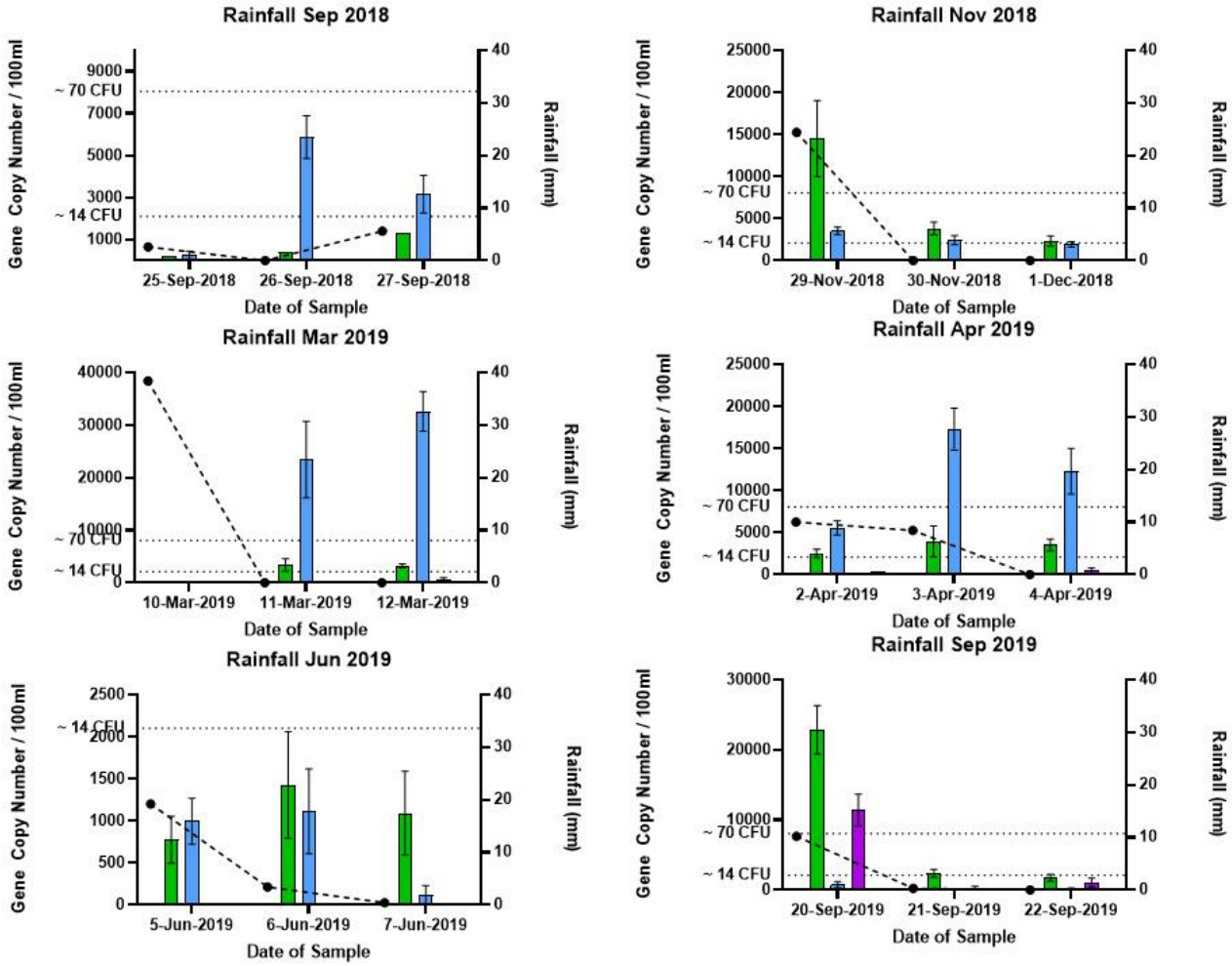


Figure 5.4 Weekly faecal coliform counts (cfu/100mL) from water samples collected by DPI Food Authority at three sites in the Manning River compared to Oyster Transformation Project weekly sampling results (including rainfall sampling). Dotted lines at 14 and 70 cfu/100 mL are the operational limits for direct or restricted (oysters must meet depuration requirement) harvest, respectively, depending on individual harvest area classification (see above).

Maximum faecal coliform counts in samples reported from CRC project samples (Figure 4A-D) corresponded to maximum DPI Food Authority counts within the Manning River at the same time ~June 2020 (Fig. 5.4).

Ten rainfall events were also sampled across the study period (see purple bars in Fig 5.3 A-D). These included: 27-29 Sept 2018; 29 Nov- 1 Dec 2018; 11-12 Mar 2019; 2-4 Apr 2019; 5-7 Jun 2019; 20-22 Sept 2019 (Fig. 5.5 A-F); and 13-15 Oct 2019; 16-18 Mar 2020; 10-12 Jun 2020; and 27-29 Jul 2020 (Fig. 5.5 G-J).

Overall, *E. coli* became elevated with increasing rainfall, but generally dissipated by day 3 of sampling (Fig. 5.5 A-J). There was one exception to this in March 2020, whereby the *E. coli* was still elevated on day 3 after rainfall had decreased (Fig. 5.5 H). Cow bacteria became elevated during fewer rainfall events, however when it did, it also generally went back to background concentrations by day 3. Bird bacteria was highly variable across rainfall events, sometimes peaking on day 1 and day 2, and at other times on day 2 and day 3. Human bacteria remained in relatively low concentration across all rainfall events, with a minor spike in July 2020 (Fig. 5.3 D).



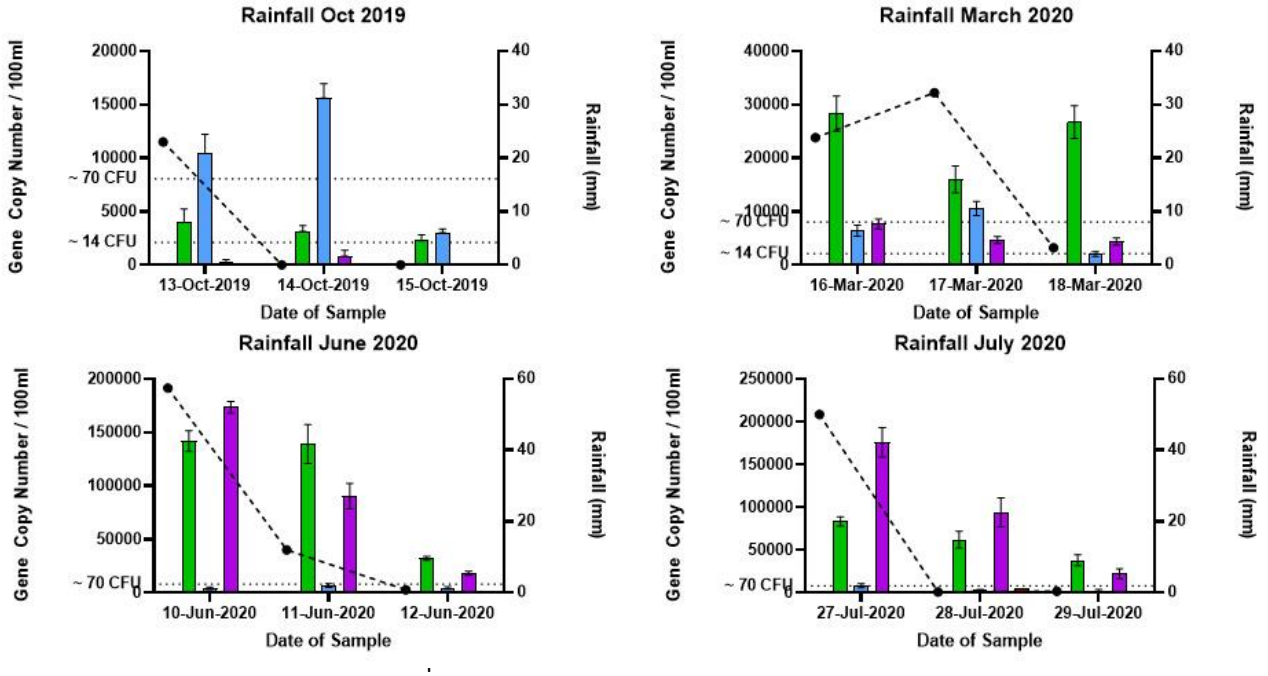


Figure 5.5 (A-J) Rainfall events sampled for E. coli assays. Green bar = E. coli; blue bar = bird assay; purple bar = cow assay; grey bar = human assay. Dotted line is rainfall (mm) obtained from the closest Bureau of Meteorology weather station at Taree (BOM 060141). All bars are the mean value of nine replicate samples (3 biological x 3 technical) and the error bars are the standard error of all nine replicates.

5.4 Phytoplankton enumeration and HAB events

The maximum phytoplankton cell concentration across the sampling period (at the sampling site closest to the sensor - site '39') from December 2017 to March 2021 occurred on 15/3/21 (37 mm rainfall) (Fig. 5.6). Total cell concentrations reached 2.3E +07 cells L^{-1} and samples contained very high abundance of a small *Heterocapsa/Azadinium*-like cell (1.4E +07 cells L^{-1}). An attempt was made to examine the thecal plates of this cell using fluorescence microscopy to provide further information on its identity, but the condition of the cells made this impossible. Further sampling of this event was not possible due to a large flood event which commenced 19 & 20 March 2021 (103mm & 140mm rainfall per day, respectively). Future molecular analysis using the qPCR methodology may delineate this species, but this is outside the scope of the present study.

Other bloom events across the sampling period included *Pseudo-nitzschia fraudulenta/australis* and *P. pungens/multiseries* on 28/10/18 (1.3E +05 and 7.5E +04 cells L^{-1} respectively), and *Pseudo-nitzschia fraudulenta/australis* again on 2/6/20 (2.2E +05 cells L^{-1}). *Dinophysis acuminata* cell densities were elevated on 5/11/2018 at 1,600 cells L^{-1}, on 17/2/2019 at 5,300 cells L^{-1} (see qPCR assay results), and again on 7/10/2019 at 1,300 cells L^{-1}. NSW Food Authority trigger levels for flesh testing are 50,000 cells L^{-1} for *Pseudo-nitzschia (australis & multiseries)* and 500 cells L^{-1} for *Dinophysis acuminata* (NSWFA 2015).

While these concentrations of HAB species are elevated, they are not considered extremely high cell densities. Additionally, no HAB species showed recurring high cell densities across the biological sampling period (Sept 2018 - Sept 2020). For these reasons, no statistical modelling was carried out on HAB species during Stage 1 of the project in this estuary.

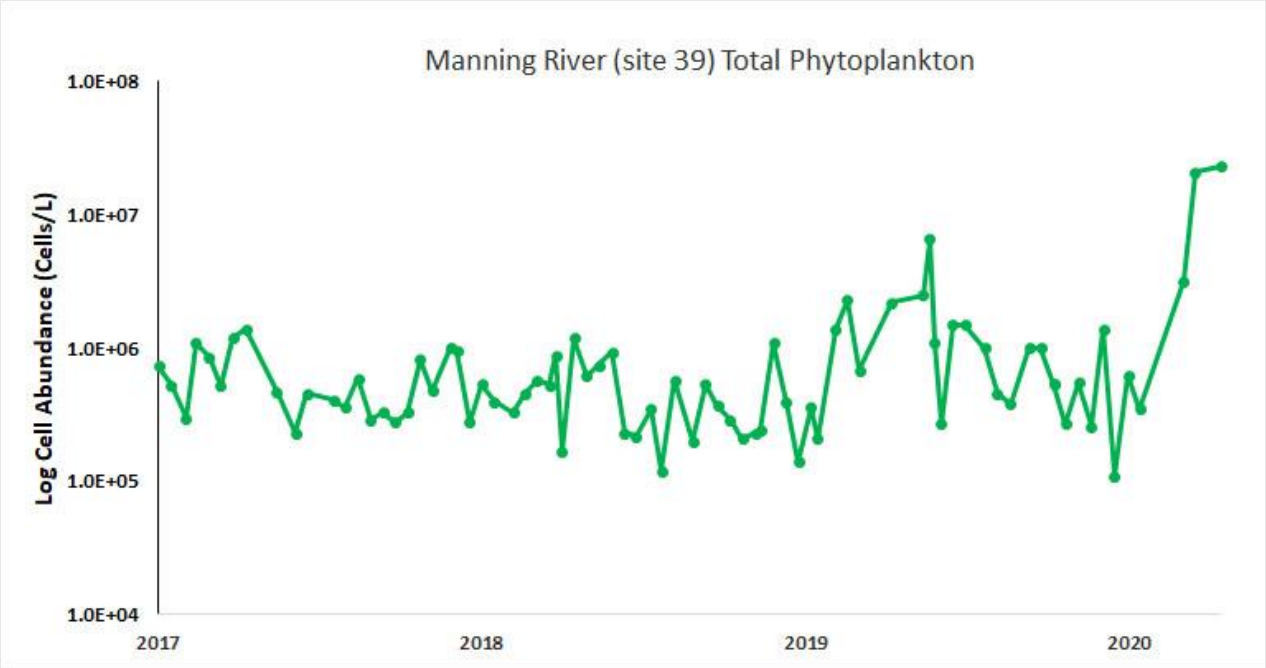


Figure 5.6 Log abundance of total phytoplankton sampled approximately fortnightly from December 2017 to March 2021 from site 39, located closest to the sensor.

5.5 *Dinophysis* bloom assessment using qPCR assay

To evaluate the effectiveness of the *Dinophysis* qPCR assay previously developed for the detection of *Dinophysis* in environmental samples, we compared microscopy-based *Dinophysis* spp. *(D. acuminata* and *D. caudata*) cell counts with eDNA samples collected from the Manning River across the same time period. Sixteen water samples collected from 10 Sept 2018 to 31 Mar 2019 showed that *Dinophysis spp.* peaked on 19 Feb 2019 at a cell concentration of 5,400 cells L^{-1} (Fig. 5.7). Using the *Dinophysis* assay developed in this study, we then screened twenty-four eDNA samples (in triplicate) across this similar time period (11 Sept 2018 to 26 Mar 2019) and successfully detected gene copies of *Dinophysis* in 62 out of 72 replicate samples. Mean gene copy number peaked on 9 Feb 2019 and corresponded to 364,591 gene copies L^{-1} (Fig. 5.7). Assuming the bloom was dominated by *D. acuminata* (as reported by microscopy) at this time, we then used the x factor for *D. acuminata* (x 49) to determine the peak cell concentration of *D. acuminata* to be ~6,316 cells L^{-1}. A Lomb Scargle Periodogram (Lomb 1976; Scargle 1982) was applied to the microscopy and qPCR data to compare the periodicity in the unevenly sampled time-series. The correlation coefficient generated from this analysis was 0.31 which was significant with a student's t-test ($P<0.1$) (Fig. 5.8 A-B) (Ajani et al. under review). This means there was reasonable corroboration between the two methods.

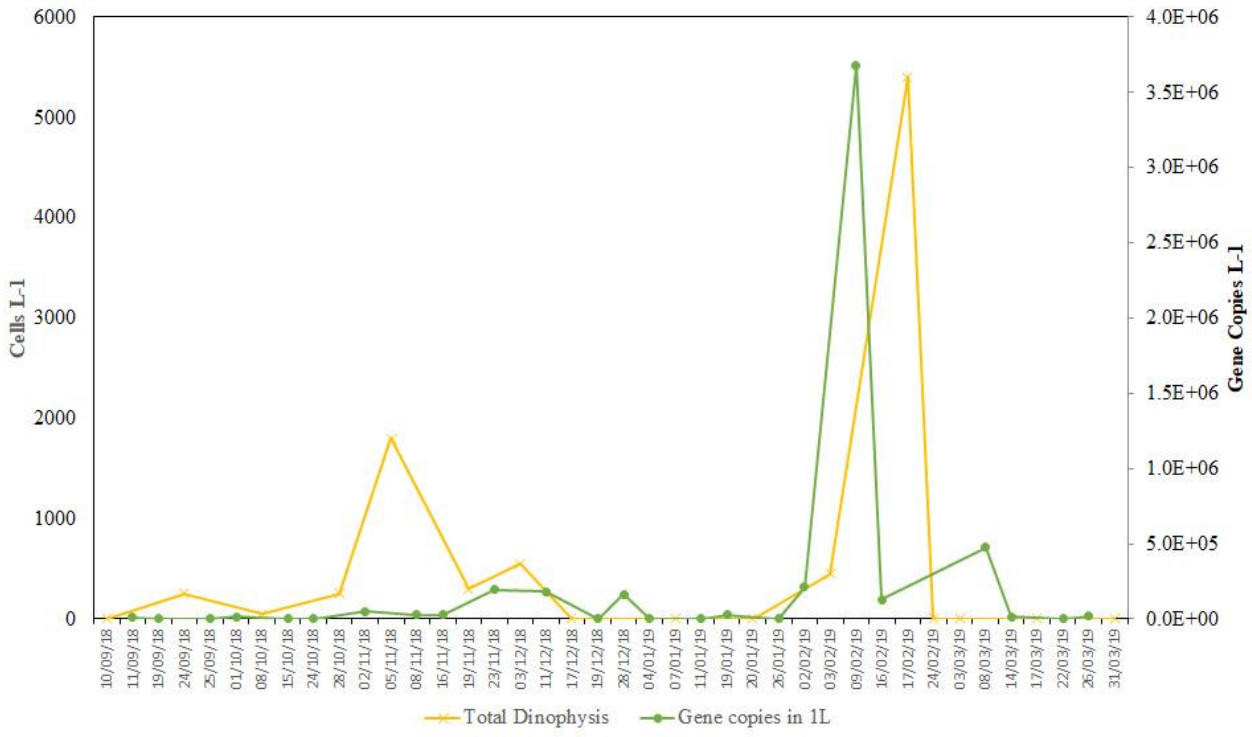


Figure 5.7 Comparative quantification of total *Dinophysis* spp. (cells L^{-1}) using A. light microscopy (cells L^{-1}, yellow line); and B. using qPCR (gene copies L^{-1}, green line) for *Dinophysis* spp. in the oyster-growing Manning River estuary.

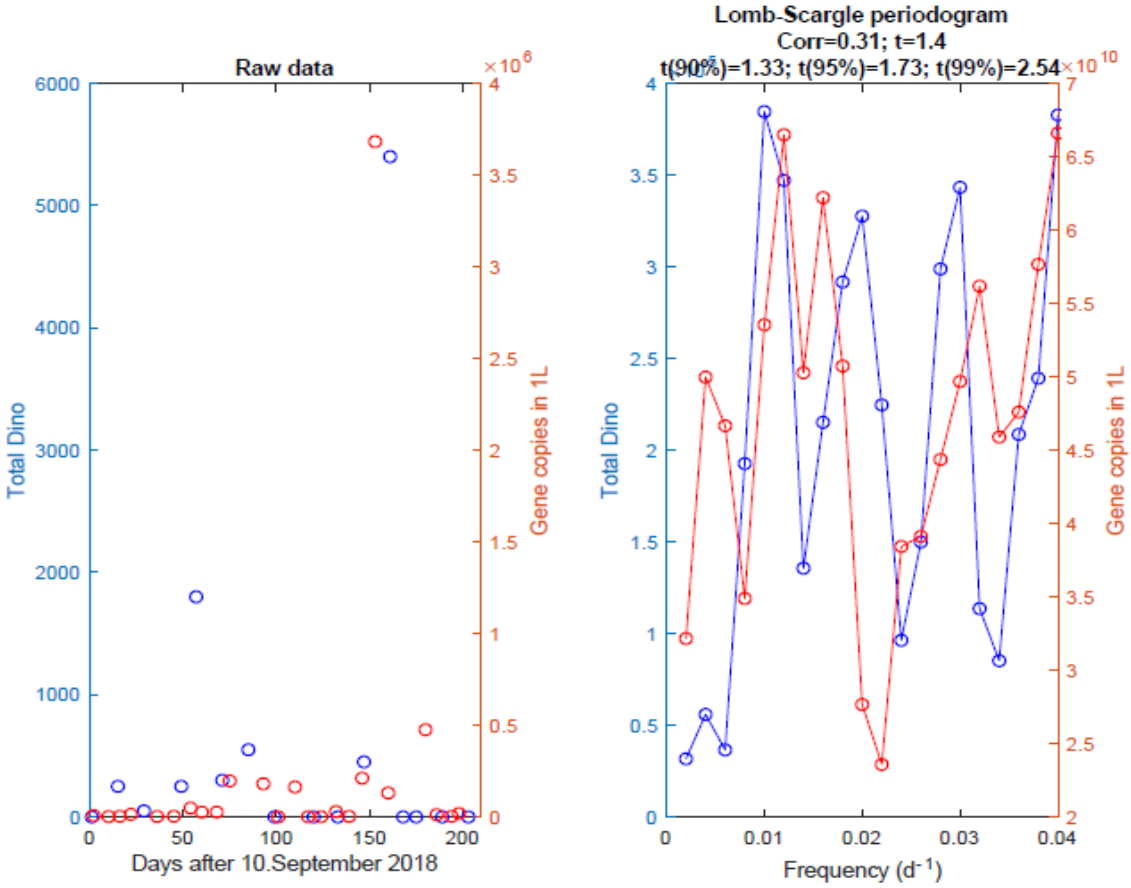


Figure 5.8 Lomb–Scargle periodogram (B) computed from the raw data (A) of both total *Dinophysis* enumeration using microscopy (blue) and gene copies/L (red) and qPCR (blue).

5.6 Oyster Growth and Mortality

5.6.1 Oyster Growth

Oyster whole weight increased by 29.5 g in the experimental period (August 2018 to February 2020) (Fig. 5.9 A). Oyster whole weight increases were greatest in the first spring and summer period of this experiment. Oyster whole weight was 52.1 ± 8.2 g at the end of the experiment (February 2020) which is classified as plate grade (> 40 g) market size. Oysters were 38 mo when they had reached this weight.

Oyster shell height was 54 ± 7 mm at the start of the experiment and increased to 70 ± 4 mm in February 2020 (Fig. 5.9 B). Greatest increases in shell height were recorded during the months of August through to November in the Manning River. Shell heights were measured more frequently than whole weight and fluctuate throughout the experiment. Periods of decreasing shell length were recorded in this experiment between January and April 2019, June and August 2019, Oct and November 2019 as well as December 2019 and January 2020.

5.6.3 Mortality

No mortality events that exceeded background farming mortality (~10% per annum) occurred at the Manning River monitoring site over the study period (Fig. 5.9C-D). The period of highest oyster mortality in this experiment was between February and April 2019 when oysters were approximately 29 mo. (Fig. 5.9 C). Oysters from this site remain frozen for future analyses.

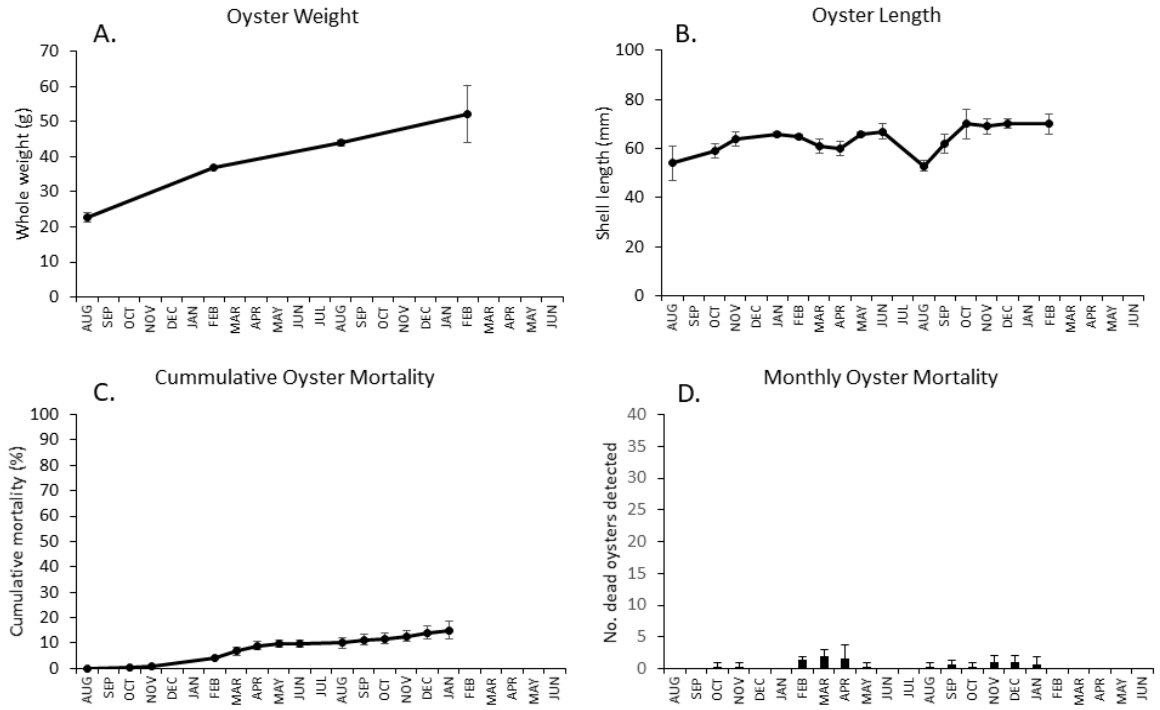


Figure 5.9 A-D. Oysters deployed at the sensor site, Manning River. A. whole weight; B. shell height; C. cumulative mortality and D. monthly mortality.

5.7 Modelling

5.7.1 Modelling of E. coli data

Summary statistics for all bacterial concentrations and environmental variables used in the general additive models are shown in Appendix 2. Correlation coefficients were calculated among every pair of environmental variables and suggested very few strong positive relationships ($r > 0.7$) overall, with the exception of E. coli and rainfall over 48 h and 72 h and cow bacteria and rainfall over 24 h and 48 h. A total of 4 models were developed for each of the bacterial sources: sensor only; sensor and total phytoplankton (logged or unlogged); rainfall only; and rainfall and total phytoplankton. Depth and week were included as response variables in all models. The maximum predictive capability for each bacterial group is: 62% for *E. coli* (rainfall+totalphyto), 74% for cow (sensor+totalphyto), 19% for bird (sensor) and 78% for human (sensor+totalphyto). Each of the model results is shown in Table 1 and summarised as follows:

1. An increase in *E. coli* abundance was significantly linked ($P<0.001$) to a decrease in salinity over the previous week and an increase in water temperature over the past 24 h (45% deviance explained; number of observations = 91). When log total phytoplankton is included in the model, the model's predictive capability increased slightly to 47%, with log total phytoplankton becoming a marginally significant variable ($P<0.05$), together with salinity and temperature.

   Rainfall predicted *E. coli* abundance with 58% of the deviance explained (number of observations = 111), with both rainfall over the previous 72 h and week of year (summer maximum) being significant variables in the model ($P<0.001$). When log total phytoplankton was added to the model, its predictive capability increased to 62%, with log total phytoplankton (increasing) becoming a significant variable ($P<0.001$), along with rainfall and week of year.

2. An increase in cow bacteria abundance was linked to a decrease in salinity ($P<0.001$) and temperature ($P<0.01$) over the past 24 h (32% deviance explained; number of observations =106). When total phytoplankton was added to the model, the model's predictive capability again increased to 74%, with total phytoplankton (increasing), depth (0.7 max), and week of year (winter maximum) significantly linked ($P<0.001$) to cow bacterial abundance.

   An increase in cow bacteria was significantly linked to rainfall over the past 72 h ($P<0.001$), with 40% of the deviance explained (no. of observations = 111). When total phytoplankton was added to the model, its predictive capability remained unchanged at 40%, with rainfall (over 72 hours, $P<0.001$) and total phytoplankton ($P<0.01$) linked to an increase in cow bacteria.

3. An increase in bird bacterial abundance was not very well predicted by either the sensor model (19% deviance explained; no. of observations = 91) or the rainfall model (14%), with the addition of total phytoplankton making little improvement to either model. Interestingly

when salinity was a significant factor in the model, it was increasing in relation to bird bacteria abundance ($P<0.001$).

4. An increase in human bacteria abundance was weakly linked to an increase in salinity and temperature over the past 24 h ($P<0.05$), with 60% of the deviance explained (number of observations = 91). When total phytoplankton was added to the model its predictive capability increased to 78%, with increasing salinity being a significant predictor ($P<0.001$) and log total phytoplankton ($P<0.01$) marginally influential.

   An increase in human bacteria was not very well predicted with the rainfall model (15% deviance explained; no. of observations = 111), with the inclusion of total phytoplankton only marginally improving the model (29% deviance explained). In both models, rainfall over the past 48 h was significantly linked to human bacterial load ($P<0.01$ without total phytoplankton and $P<0.001$ with log total phytoplankton included).

5.7.2 Modelling of oyster growth and mortality

While there was insufficient oyster weight data to model (only 4 data points across the sampling period), there was sufficient shell length data to model. During the modelling process however, it was noted that there was a gap in the sensor data in July 2019, with a concurrent missing length measurement. Both these data gaps were immediately prior to the drop in shell length in August.

The best model explained ~79% of the deviance. The strongest predictor in this model was the weekly maximum salinity. Higher weekly salinity peaks predicted larger shell lengths. This was dissipated by a negative coefficient on the daily max salinity variable. That is, shell lengths were greater during weeks which reached more saline conditions, although if the maximum was reached on the day of measurement, this effect was somewhat diminished, maybe because insufficient time had passed for the oyster to respond fully to these beneficial conditions. There was a nonlinear response to rainfall, with lengths appearing greater when rainfall was neither excessively large nor small. Total phytoplankton did nothing to improve the models so was dropped from the final model.

Table 1. Modelling results for bacterial source tracking in the Manning River. Only significant variables are shown for each model; #model result is the same with or without total phytoplankton; colored arrows indicate variable is increasing or decreasing; varying yellow lines indicate bimodal variable (this may be due to cow bacteria peaking ~ 25 ppt and bird/human ~35 ppt within the E. coli assay).

Bacteria	Variables included in model	No. of obs. in model	Significant variables	Variable Direction	Deviance Explained %
E. coli	Sensor	91	SalWk***		45
			Temp24***		
	Sensor+TPhyto	91	SalWk***		47
			Temp24***		
			LogTPhyto*		
	Rainfall	111	R72***		58
			Week***	Summer Max	
	Rainfall+TPhyto	111	R72***		62
			LogTPhyto***		
			Week***	Summer Max	
Cow	Sensor	106	Sal24***		32
			Temp24**		
	Sensor+TPhyto	106	TPhyto***		74
			Depth24***	0.7 m Max	
			Week***	Winter Max	
	Rainfall	111	R72***		40
	Rainfall+Tphyto	111	R72***		40
			LogTPhyto*		
Bird	Sensor#	91	Temp24***		19
			SalWk***		
			Depth24**	0.9 m Max	
	Rainfall#	111	RWk***		14
			Week***	Summer Max	
Human	Sensor	91	SalWk*		60
			TempWk*		
	Sensor+Tphyto	91	SalWk***		78
			LogTPhyto*		
	Rainfall	111	R48*		15
	Rainfall+TPhyto	111	R48**		29
			LogTPhyto**		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DISCUSSION

6. Discussion

6.1 High Resolution Sensor Data and Management Plan

Analysis of sensor data during the annual review process demonstrated that there is potential to implement a salinity sensor-based management plan for Pelican Point harvest area. Based on the available data, up to eight harvest area closures could have potentially been avoided between 19 December 2017 and August 2021. During the initial implementation of such a management plan change, rainfall events would continue to be monitored to increase the database to support the change. Manning River Shellfish Program (MRSP) were consulted about the option of a salinity-only management plan for Pelican Point harvest area following the 2020 annual review but a decision was not reached at that time. Further consultation with MRSP occurred as part of the 2021 annual review process, and MRSP indicated that they would like to adopt a salinity only management plan on a trial basis for a period of 12 months commencing February 2022. If MRSP did not wish to pursue the implementation of a management plan that is based on sensor salinity, or if the salinity sensor data were not accessible, the Pelican Point harvest area management plan would revert to the current management plan that is based on both rainfall and salinity closure limits.

6.2 Phytoplankton and HABs

The biggest increase in phytoplankton growth throughout our project was observed prior to the major flood event of March 2021. While the majority of rainfall occurred during this event, there was some significant rain in the preceding weeks that most likely contributed to this excessive phytoplankton growth. This unprecedented growth was most likely a response to nutrients entering the waterway during this rainfall. Other minor pulses in the potentially toxic diatom *Pseudo-nitzschia* were also observed throughout the study, but to a far lesser extent. The only other notable harmful bloom was that caused by the toxic dinoflagellate *Dinophysis acuminata*. Species belonging to the genus *Dinophysis* (and more rarely benthic *Prorocentrum)* are the most problematic Diarrhetic Shellfish Toxin (DST) producers worldwide. With over 100 species represented worldwide, ten have been unambiguously found to be toxic (*Dinophysis acuminata*, *D. acuta*, *D. caudata*, *D. fortii*, *D. infundibulum*, *D. miles*, *D. norvegica*, *D. ovum*, *D. sacculus* and *D. tripos*), producing DSTs (okadaic acid and dinophysistoxins) even at low cell densities ($<10^3$ cells L^{-1}) (Reguera et al., 2014; Reguera et al., 2012; Simoes et al., 2015).

Diarrhetic Shellfish Poisoning (DSP) was first described after a large toxin event occurred in Japan in 1976 (Yasumoto et al., 1980; Yasumoto et al., 1978), whereby many people became sick after eating scallops (*Patinopecten yessoensis*). This contamination was linked to toxins produced by *D. fortii*. Following this event, further toxic episodes occurred in Japan, Spain and France, with several thousands of cases of human poisonings occurring over the 1970s and 1980s, leading to the development of many regional monitoring programs. This monitoring has seen a gradual increase in reported DSP episodes in countries including Chile, Argentina, Mexico, the east coast of North America, Scandinavia, Ireland, Great Britain, Spain, Portugal, Italy, Greece, India, Thailand, Australia and New Zealand (Lembeye et al., 1993; Taylor et al., 2013; Whyte et al., 2014; Yasumoto et al., 1978).

Dinophysis is common in Australian waters, with 36 species reported (Ajani et al., 2011; Hallegraeff and Lucas, 1988; McCarthy, 2013). Toxic species include *D. acuminata*, *D. acuta*, *D. caudata*, *D. fortii*, *D. norvegica*, and *D. tripos*. There have been three serious human DSP poisoning events in Australia. The first episode was caused by contamination of Pipis (*Plebidonax deltoides*) in New South Wales

in 1997 (NSW) by *D. acuminata* (Quaine et al., 1997). One hundred and two people were affected and 56 cases of gastroenteritis reported. A second episode occurred again in NSW in March 1998, this time with 20 cases of DSP poisoning reported (Madigan et al., 2006). The final event occurred in Queensland in March 2000, in which an elderly woman became seriously ill after eating local Pipis (Burgess and Shaw, 2001). While no human fatalities from DSP are known globally, DSTs continue to be a major food safety challenge for the shellfish industry.

In response to elevated cell densities of a toxic algal species *Dinophysis* in February 2019 in the Manning River, we have now successfully developed a rapid qPCR assay to detect species belonging to the genus *Dinophysis* in environmental samples. Quantitative PCR can be an efficient and powerful tool to identify and enumerate HAB species, especially those that are difficult to distinguish using routine methods (Handy et al. 2008, Penna and Galluzzi 2013). For this reason, this method is used routinely in certain monitoring programs around the world (Clarke & Gilmartin 2020). The qPCR assay can be used on-farm, allows for automation, is easy to use without specialist knowledge, and provides an early warning that harmful algae are present in the water column.

6.3 Assay Development and Faecal Pollution in Manning River

Molecular assays for the detection of faecal bacterial contamination in the Manning River were determined with two main aims. The first was to design a faster method for the currently used place count methodologies for the detection of faecal indicator bacteria by commercial laboratories and secondly, for source tracking. This later assay would be used to identify which animals might be contributing to any *E. coli* in the river system. Assays needed to be sufficiently specific to only the target organism, to have a sufficiently low level of detection, and finally have a high level of efficiency, in line with the best practice guidelines for qPCR assays (Bustin et al. 2009).

E. coli is the primary faecal indicator bacterial species, and is most commonly used for detecting faecal contamination using culture-based methods (Odonkor & Ampofo 2013, NHMRC 2008, 2011). Although there are assays that target genes which detect faecal coliforms (Isfahani 2017), genetic variability between coliforms makes it a challenge for accurate assessment (Maheux et al. 2014). As *E. coli* is tested for in oyster meat (NSWFA 2015, 2017). *E. coli* was considered to be a more targeted approach to also detect in estuarine waters. In this study, several primer pairs were trialled which targeted 3 different genes within *E. coli*, with the final *E. coli* assay selected being the most efficient and specific only to the target organism (Tesoreiro 2020).

The second group of assays developed were those that were microbial source tracking as they detect bacteria of faecal origin specifically associated with a group of animals, i.e. bird, cow and human. Birds are a significant source of faecal contamination in estuarine/marine waters during dry periods, and increase FIB load in watersheds (Araujo et al. 2014, Converse et al. 2012). The marker we used was 100% avian specific, with gulls, geese, ducks and chickens being tested (Green et al 2012), and has been successfully used in watersheds across different continents (Ahmed et al 2016, 2019; Li et al. 2019, Vadde et al. 2019). Our source tracking assay for cows had 100% sensitivity to bovine faecal samples, with little cross reactivity to other species (93% specific). When tested at a rural watershed, a high proportion of faecal contamination was attributable to cattle (Layton 2006). Finally, the human marker we used has demonstrated the best performance for the detection of human faecal contamination compared to all other assays since it was developed in 2000 (Boehm 2013, Shanks 2010).

Bacterial contamination, specifically *E. coli* and bovine faecal pollution entering the Manning River was most often linked to rainfall and associated declines in salinity. These events most likely lead to a concomitant increase in nutrients entering the waterway (Amato et al. 2020, Abimbola et al. 2021, Liang et al. 2019, Buszka & Reeves 2021), providing bioavailable nutrient forms for phytoplankton

growth. *E. coli* pollution entering a waterway can also induce nutrient recycling and accelerate the decomposition of other organics like aquatic plants, further releasing nutrients into the system (Wu et al. 2021). The survival and proliferation of *E. coli* in the aquatic systems has also been found to be strain specific, with hydrological conditions, differing sources of pollution, selective pressures in the waters, and various land uses, all contributing to the community structure and diversity of *E. coli* in a waterway (Bong et al. 2021).

Avian faecal pollution in the Manning River on the other hand, was not correlated to salinity or rainfall, but was observed to peak during the autumn and summer months (with an absence in winter). The mouth of the Manning River is home to many migratory shore birds and their breeding periods fall over these summer months (Stuart 2008). However, the infrequent detection of bird pollution during the first summer sampling period (2018/19) could also suggest that there was an influx of birds during the second summer period (2019/2020). Also, the molecular marker used in this study does not discriminate between avian species (gulls, geese, chickens, ducks etc), so it is uncertain what percentage of the bacterial load is attributable to terrestrial birds and that of aquatic birds. Further discrimination into the breakdown of this faecal load would be required for this elucidation.

Human faecal pollution was weakly linked to an increase in water temperature and salinity (as predicted by the sensor model), although rainfall did not increase this predictability. Very few measurable events occurred during the sampling campaign including more intensive sampling during rainfall events, suggesting that water quality management efforts in regard to sources of human contamination are working. Partially treated effluent from septic tanks or caravan parks presents the highest impact/risk for human contamination in the Manning River (Swanson 2019). It was suggested that, due to the wider range of human enteric viruses in a large number of oyster and sediment samples, the outbreak of hepatitis A linked to the consumption of oysters from Wallis Lake in 1997 was linked to significant sewage or faecal contamination. New legislation followed on from this event, tightening controls over septic maintenance, new sewerage management plans developed, and a mandatory notification system for sewage overflows introduced. Following on from this, mandatory membership for industry to Shellfish Quality Assurance Programs was implemented and an estuary classification system introduced (Conaty et al. 2000).

The future use of molecular tools such as qPCR for the detection and quantification of bacteria or HABs would require further validation in accordance with Association of Official Agricultural Chemists (AOAC) procedures for the validation of such tests. This would include the validation of the sensitivity, precision and reliability of methods and a rigorous comparison to existing methods. Methodology and protocols for sampling accreditation and assurance of independence in testing and reporting for on farm testing would then follow.

6.4 Oyster growth in the Manning River

Shell length increases were greater in periods characterised by more saline conditions. Seawater alkalinity increases as salinity increases providing more calcium carbonate available for oyster shell deposition. The salinity level that promotes the greatest growth rates in Sydney Rock Oyster spat 30 ppt for small spat (1.3 mg) and 35 ppt for larger spat (0.61 g) (Nell and Holliday, 1988). Seawater temperature also influences oyster growth rates and the best growth rates in this experiment were recorded in the spring and summer months. This is a period where NSW estuary water temperatures incrementally increase (Wolf 1979). Shell length decreases were also recorded in the experimental oysters deployed in the Manning River. This occurs when there are periods of high wind and wave action causing excessive oyster movement within baskets resulting in loss of the shell fringe.

The batch of oysters used for this experiment were a random mix of families taken from the 2016-year class of the Sydney Rock Oyster Breeding program. This particular year class had 86% of the parents selected from wild and QX disease resistant genetic groups. Only 14% of the parents for this year class were sourced from the fast growth genetic group. It took this year class 3 years and 2 months to reach the premium oyster grade (>50 g) for sales. Estuaries where this same batch of oysters reached the premium plate grade benchmark at the same time were Camden Haven (50.3 g), Hawkesbury River (52.8 g), Georges River, Wagonga Inlet (51.3 g) and Wapengo Inlet (54.6 g).

The period of the highest oyster mortality (February to April 2019) coincided with a period of high rainfall in the lower Manning River catchment. The cumulative mortality measured in oysters at the Manning River in January 2020 was 15% which was below the average measured across all assessment sites used in the project (18%).

The cumulative mortality in January 2020 was comparable to Wallis Lake (14%) and Port Stephens (15.7%). Wallis Lake and Port Stephens are the two oyster producing estuaries located 37 km and 100 km, respectively, south of Manning River. Manning River cumulative mortality in January 2020 was much lower compared to the two oyster producing estuaries situated to the north. Cumulative mortality levels in January 2020 were 39% in the Camden Haven River and 31.7% in the Hastings River. Camden Haven River is 30 km north of Manning River and the Hastings River is 56 km north of Manning River.

Low levels of mortality have been measured near the Manning River monitoring site in previous studies where there was only 4% mortality in Sydney Rock Oysters over an 8-month period starting in May 1999 and concluding in January 2000 (Dove 2003).

This site in the Manning River, which is proximate to the mouth of this estuary, experiences good oyster growth, above the historic average expected for the NSW industry. In particular, the Manning River has demonstrated comparatively high levels of oyster survival, well above many other tested estuaries. The rapid recovery of this site from decreased salinity increases both the number of oysters available for harvest and the number of days oyster harvesting is permitted under a salinity-based management plan.

6.5 Outreach

Outreach and project materials developed during Stage 1 of this project include two scientific publications - *Harmful Algae* (international scientific journal) and *The Conversation,* and a further one in preparation; one Department of Primary Industry Report; three newsletters/factsheets; sixteen seminars/conferences/workshop presentation and four videos/YouTube posts (Appendix 3). Regular program progress reports were provided to the NSW Shellfish Committee and the NSW

Aquaculture Research Advisory Committee. Researchers also discussed the results of the project at Seafood Industry field days hosted by LLS held in Port Stephens, Wallis Lake and the Manning River in June 2021.

CONCLUSIONS

7. Conclusions

The data assessment from this report supports implementing a harvest area management plan based on sensor salinity data, subject to the agreement by the local shellfish industry. Available data indicated that eight harvest area closures could have potentially been avoided between December 2017 and August 2021. As of February 2022, thirteen salinity-only management plans had been offered for harvest areas in participating NSW estuaries, with six being taken up and the remaining seven under consideration.

Oyster growth in the Manning River over the study period was relatively good, with highest growth during more saline conditions, and highest mortality during high rainfall periods.

The pollution source tracking results were highly variable across the study period, most likely attributable to the extreme variation in environmental conditions experienced (drought, bush fires, floods). Real time sensor data however, showed a higher predictive capability than rainfall for three out of the four faecal indicator bacteria.

Specifically, elevated concentrations of E. coli were strongly linked to rainfall events and declines in salinity, the most significant of these observed in the first half of 2020. Contamination from cattle was observed sporadically at elevated levels, but again was strongly linked to rainfall that resulted in a salinity decline. Data showed that these events generally dissipated after three days.

Contamination from bird sources was observed regularly throughout the study period at low to moderate levels, with a distinct presence throughout the black summer bushfires 2019-2020. Contamination from human sources was observed rarely (at low levels), and was also linked to wet weather events.

PCR based assays demonstrate significant potential to supplement and/or replace classical environmental sample analytical methods. The benefits of PCR based analysis includes reduced cost, faster sample turnaround time and potentially the ability to analyse samples on-site, removing the need for the cost and delay of sample transport. Sample transport often comprises >50% of the delay between sample collection and result reporting. These delays cost industry money and reduce the utility of samples for risk management purposes. Future work should focus on validating qPCR methods in accordance with AOAC procedures.

Overall these results demonstrate the utility of salinity-based management plans for predicting potential contamination events and managing water quality risks. Real time sensor data, combined with rapid molecular tools, can help predict optimal conditions for harvesting and growth. This has the potential to improve regulatory and management outcomes and enhance the productivity and profitability of oyster farming in the Manning River.

We encourage you to submit your feedback or questions after you've completed reading this report. You can do so via Penelope.Ajani@uts.edu.au

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9. Appendices

A1. Methods

A1.1 Sampling locations in Manning River

Project data used in this report originates from two locations within the Manning River estuary over the period December 2017 to March 2021. High-resolution temperature, salinity and depth data were obtained from a sensor deployed at Pelican Point harvest area (-31.89S, 152.64E) (Fig. A1). At this location, oysters were both deployed and retrieved, and water samples for eDNA were collected. From here on, this location is referred to as the 'sensor site'. Phytoplankton was also collected at a second sampling location established as part of the DPI's Shellfish Quality Assurance program (labelled 'site 39' as part of this program) (Fig. A1).

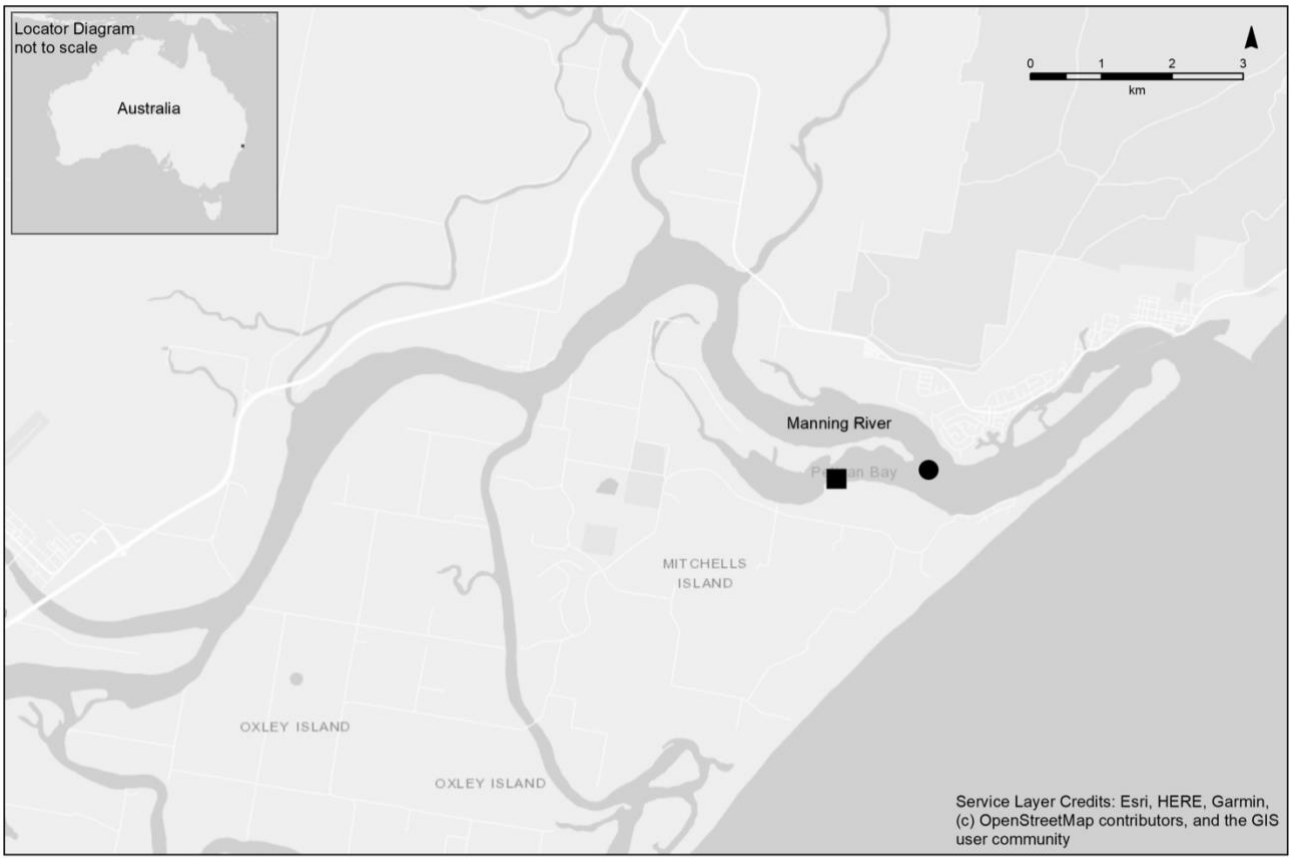

Figure A1: Map of the Manning River Estuary highlighting the sensor and eDNA sampling location (black square) and the phytoplankton sampling location – site '39' (black circle).

A1.2 High-resolution sensor data

High-resolution temperature (°C), salinity and water depth (m) data were collected from 19 Dec 2017 to 31 March 2021 using a Seabird SBE 37-SM/SMP/SMP-ODO MicroCAT high accuracy conductivity, temperature and depth (CTD) field sensor. This sensor was deployed using a fixed installation, with the inlet 60 cm above the seabed and at least 30 cm below the estimated Lowest Astronomical Tide (LAT) (Fig. A2). This fully autonomous instrument collected and transmitted data every 10 minutes (24 h day^{-1}) to Microsoft Azure cloud storage before downstream quality checking and analysis. Sensor data was then packaged into RO-Crates by the e-Research team at UTS, which are then uploaded to an Arkisto-based website. This website allows for the filtering and downloading of these crates based on both time and location, for use in research

and analysis (Fig. A3). Finally, rainfall data were obtained from the closest Bureau of Meteorology weather station at Taree (Bureau of Meteorology Station No. 060141, - 31.89S 152.51E).

Figure A2 Seabird MicroCAT high accuracy conductivity, temperature and depth (CTD) field sensor deployed in Manning River estuary. Image: Ian Crisp.

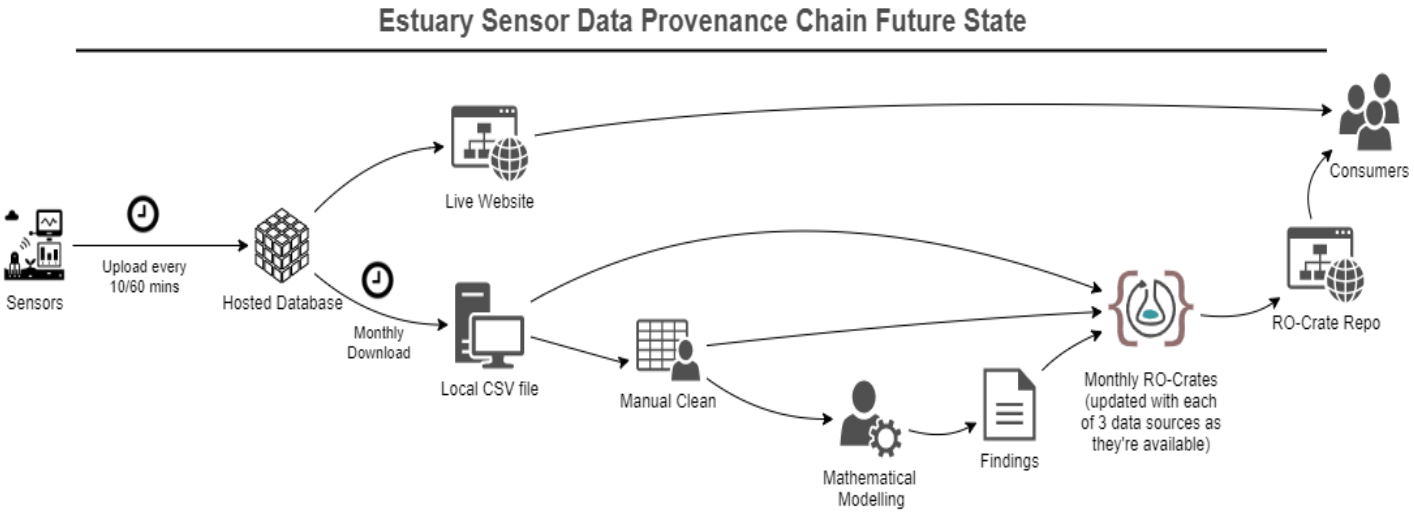

Figure A3. Manning River Data provenance chain from source of data (sensors), via quality assurance processes, data analyses, to consumers.

A1.3 DPI Management Plan review

Evaluation of the harvest area management plans for each NSW harvest area occurs annually. This is carried out by the NSW Shellfish Program (NSW DPI Food Authority). The date of the Manning River annual review is 1 September. As part of the recent (2020 and 2021) annual reviews for Pelican Point harvest area, all salinity data from the monitoring sensor during the 2018, 2019, 2020 and 2021 annual review periods were analysed and assessed in relation to microbiological samples collected by the local shellfish program during the same period. Due to a hardware issue with the sensor, a portion of data between 11 July and 16 August 2019 was unavailable.

A1.4 Biological sampling and eDNA extraction

Estuarine water samples were collected weekly by oyster farmers working at Manning Rock Oysters located in Croki, New South Wales from September 2018 - September 2020 for both phytoplankton and bacteria. For algal samples, 3L sub-surface water samples (0.5 m, in triplicates) were collected and filtered using a specially made PVC sampler. Samples were then stored at 4 ℃ until further downstream processing. DNA was then extracted using the DNeasy 96 PowerSoil Pro QIAcube HT Kit (Qiagen) and DNA stored at -20℃ until further analysis.

In the case of a rainfall event, water samples were collected for bacterial analysis (only) every 24 h over a three-day period commencing on the first day of rainfall and processed as described above. Daily rainfall measurements were taken from the closest available weather station, Taree airport, 17.5 km upstream.

A1.5 qPCR assays for bacterial source tracking

Realtime qPCR tests were carried out on all water samples in triplicate for bacterial source tracking of E. coli, bird, cow and human faecal indicators.

A1.6 Phytoplankton enumeration

Water samples (500 ml) were collected at approximately 2-weekly intervals from a depth of 0.5 m closest to the sensor (site ‘39’) for microscopic phytoplankton identification and enumeration in accordance with the NSW Marine Biotoxin Management Plan (NSW MBMP) and the Australian Shellfish Quality Assurance Program (ASQAP). Once collected, samples were immediately preserved with 1% Lugol's iodine solution, and returned to the laboratory for concentration using gravity-assisted membrane filtration. Detailed cell examination and counts were then performed using a Sedgewick Rafter counting chamber and a Zeiss Axiolab

or Standard microscope equipped with phase contrast. Cells were identified to the closest taxon that could be accurately identified using light microscopy (maximum magnification x1000). Cell counts were undertaken to determine the abundance of individual HAB species and total phytoplankton cell (>5 mm) numbers. *Dinophysis* cells were counted to a minimum detection threshold of 50 cells L^{-1} while all other species were counted to a minimum detection threshold of 500 cells L^{-1}.

A1.7 *Dinophysis* qPCR assay for environmental bloom dynamics

As part of Stage 1 of the NSW Oyster Industry Transformation Project, we developed a rapid and quantitative polymerase chain reaction (qPCR) assay to detect species belonging to the genus *Dinophysis* in environmental samples (Ajani et al. submitted). With no cross-reactivity to other closely related species, and an efficiency of 91.5% for *D. acuminata*, 91.3% for *D. fortii*, 92.4% for *D. caudata*, and 97.9% for gene fragment based serial dilutions, this novel assay was then evaluated for its potential to detect *Dinophysis* in environmental samples from the Manning River during a bloom which occurred on 17 Feb 2019 (5,300 cells L^{-1}).

A1.8 Oyster Growth and Mortality

At the sensor site, we also deployed two types of experimental Sydney Rock Oysters (*Saccostrea glomerata*). The first group of oysters were all the same age and used to collect weekly samples at the sensor site when water samples were collected for downstream processing. Three oysters were removed on each sampling occasion and placed whole and live into a freezer for preservation.

The second group of experimental oysters were obtained from the NSW DPI Sydney Rock Oyster Breeding Program and were deployed at the sensor site to measure shell length (Fig. A4), whole weight and mortality. These oysters were from the 2016-year class and were the same age, size and originated from a single genetic group. Three replicate floating baskets were placed on the designated oyster sampling lease and each replicate unit contained approximately 70 oysters.

A1.8.1 Oyster Whole Weight

Whole weight was measured in August 2018, February 2019, August 2019 and February 2020. Thirty randomly sampled oysters from each replicate were pooled and weighed on each sampling date using a calibrated weight balance to the nearest 0.1 g. The average whole weight of oysters at the start of the experiment in August 2018 was 22.6 ± 1.4 g.

A1.8.2 Shell Length

Oyster shell length was measured monthly from August 2018 to June 2020 (Fig. A4). A subsample of 30 oysters from each replicate were measured on each sampling occasion. The 30 oysters from each replicate were arranged on a measuring board that included a scale bar. A digital image was taken and GrabIt software (MyCommerce Inc, Minnetonka, MN, USA) was used to estimate the shell length (mm) of oysters in the images provided.

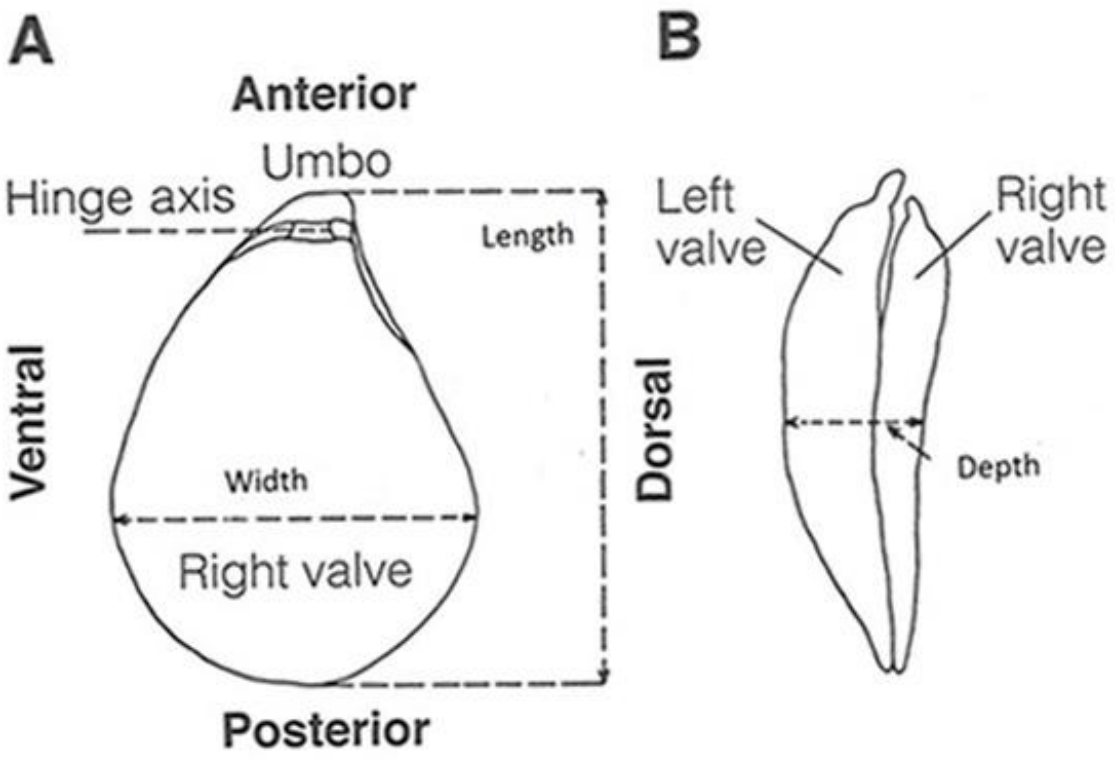

Figure A4. Oyster shell dimensions (Carriker 1996)

A1.8.3 Oyster Mortality

Oyster mortality was calculated by counting the number of empty oyster shells in each replicate each month from August 2018 to June 2020. After empty oyster shells were counted, they were removed from the experimental baskets. Oyster farmers performed the counts and recorded this information during the experiment.

A1.9 Modelling

To model the relationship between pathogens and oyster growth in this estuary, a series of models were run to investigate firstly the predictors of faecal bacteria abundance and secondly, oyster growth.

Daily averages for all sensor measurements taken on a calendar day, midnight to midnight, were then calculated. A simple unweighted average was taken over all observations. Data for a day was regarded as missing if fewer than 96 observations were made. 24 h, 48 h, 72 h and weekly salinity and temperature averages were then calculated by taking the simple unweighted averages of each day's daily average. Where a day's data were missing, all other variables which relied on this were classified as missing. For example, if no observations were recorded on 1 June, then the 1 June 24 h average was missing, the 1 June and 2 June 48 h average was missing, the 1 June, 2 June and 3 June 72 h average were missing (Appendix 2).

Rainfall data from the closest Bureau of Meteorology weather station at Taree (BOM 060141), which was the official management plan gauge for this harvest area, were averaged over the 24 h, 48h, 72 h and 7 days prior to the water sampling each day, to incorporate a measure of exposure of the bacterial community and deployed oysters. Total phytoplankton (and log transformed total phytoplankton) from microscopic phytoplankton enumeration was also included in the modelling as a potential predictor variable. Finally, week of the year and water depth were included in the models to understand any seasonality or tidal variability that was present in the data.

To model the relationship between bacteria (*E. coli*, bird, cow, human) abundance and/or oyster growth (response variables) and environmental variables (temperature, salinity, week, depth, total phytoplankton, rainfall) at the sensor location within the Manning River, correlation analyses were initially undertaken to explore the relationships between variables. Generalised additive models (GAMs) were then applied to the data. GAMs allow abundance data to be treated as count data (discrete integer values), and as such can handle zero counts. GAMs also allow for smoother functions to be incorporated into each model for the environmental variables that had a non-linear relationship with bacterial abundance.

Input data (predictor variables) were the sensor observations for both salinity and temperature, including aggregation over several different time periods, including depth, week and total phytoplankton (logged or unlogged). For comparison to current (non-sensor-based) practice, models were also run using only rainfall data. Again, these included depth, week and total phytoplankton. As total phytoplankton data is not available in real time, and therefore not considered a predictor variable by definition, models were run both with and without this variable. In summary, four models were developed for each of the bacterial sources: rainfall only, rainfall and total phytoplankton; sensor only; and sensor and total phytoplankton.

To model the relationship between oyster growth various GAMs models were also investigated using the sensor/total phytoplankton/rainfall data for the same time period. These models were then fitted in version 3.4.3 of the R statistical package (Team R Core, 2013), using the GLM function in version 1.8–22 of the 'mgcv' package (Wood, 2006). Models were then compared using the Akaike information criterion (AIC) and the model with the lowest AIC selected.

Appendix 2. Summary Statistics for Bacterial Modelling

	Mean	Standard Error	Median	Standard Deviation	Minimum	Maximum	Count	Missing
Salinity24	31.8	0.4	32.8	3.9	17.4	37.2	106	7
Salinity48	31.8	0.4	32.9	3.9	17.9	37.2	104	9
Salinity72	32.0	0.4	33.1	3.7	18.7	37.2	101	12
SalinityWk	32.1	0.4	33.5	4.0	17.6	37.0	91	22
Temp24	21.0	0.4	20.9	3.7	14.3	29.3	106	7
Temp48	21.1	0.4	21.2	3.7	14.6	28.9	104	9
Temp72	21.1	0.4	21.2	3.7	14.8	28.6	101	12
TempWk	21.7	0.4	22.0	3.5	14.7	28.5	91	22
Depth24	0.8	0.0	0.8	0.2	0.4	1.3	106	7
Rainfall24	4.9	1.2	0.0	12.4	0.0	71.6	111	2
Rainfall48	8.9	1.6	0.6	16.7	0.0	72.6	111	2
Rainfall72	12.1	1.8	3.2	19.2	0.0	76.4	111	2
RainfallWk	19.0	2.1	10.7	21.7	0.0	77.2	111	2
TotalPhyto	750541	75283	540000	793157	120000	6600000	111	2
LogTotalPhyto	13.2	0.1	13.2	0.8	11.7	15.7	111	2
Week	28	1	28	14	1	52	111	2
Ecoli	8072.2	2013.2	2073.2	21400.8	0.0	141841.6	113	0
Bird	3435.2	518.7	1102.8	5513.4	0.0	32633.0	113	0
Cow	6247.6	2512.0	51.5	26703.4	0.0	175694.2	113	0
Human	102.9	55.3	0.0	588.1	0.0	5649.2	113	0

Appendix 3. Summary of project related publications, seminars, workshops, conference presentations and other project related public presentations.

Author(s)	Title	Bibliographic details	Status (Submitted, Accepted, Published)
Penelope Ajani, Hernan Henriquez-Nunez, Arjun Verma, Satoshi Nagai, Matthew Tesoriero, Hazel Farrell, Anthony Zammit, Steve Brett and Shauna Murray	Mapping the development of *Dinophysis* spp. HABs using a novel molecular qPCR assay		In prep.
Penelope Ajani, Arjun Verma, Jin Ho Kim, Hazel Farrell, Anthony Zammit, Steve Brett & Shauna Murray	Using qPCR and high-resolution sensor data to model a multi-species *Pseudo-nitzschia* (Bacillariophyceae) bloom in southeastern Australia	*Harmful Algae* 108 (2021) 102095	Published
NSW DPI	Net Returns of Real-Time Sensors and Salinity-Based Management Plans in NSW Oyster Production - Report	https://www.foodauthority.nsw.gov.au/about-us/science/science-in-focus/real-time-sensors-shellfish-harvest-area-management	Published
NSW DPI	Net Returns of Real-Time Sensors and Salinity-Based Management Plans in NSW Oyster Production - Factsheet	https://www.foodauthority.nsw.gov.au/about-us/science/science-in-focus/real-time-sensors-shellfish-harvest-area-management	Published
The Team	Oyster Transformation Project	NSW Oyster Newsletter https://www.nswoysters.com.au/nsw-oyster-newsletter.html July 2020	Published
DPI Food Authority	Foodwise - Issue 46	https://www.foodauthority.nsw.gov.au Feb 2018	Published
Shauna Murray & Penelope Ajani	Ah shucks, how bushfires can harm and even kill our delicious oysters	The Conversation https://theconversation.com/ah-shucks-how-bushfires-can-harm-and-even-kill-our-delicious-oysters-131294 Aug 2020	Published

Appendix 4. Summary of project related seminars, workshops and conference presentations

Presenter(s)	Event/Activity	Presentation title
Matthew Tesoriero (Supervisors: Arjun Verma and Shauna Murray)	Final Hons Seminar, School of Life Sciences, UTS, 2020	Abundance and distribution of pathogenic bacteria in NSW oyster producing estuaries

Shauna Murray, Penelope Ajani, Arjun Verma, Rendy Ruvindy, Jin Ho Kim & Kate McLennan	Australasian Society for Phycology and Aquatic Botany Annual Conference 2020	Using molecular genetic techniques to detect harmful algal bloom-forming species impacting aquaculture
Arjun Verma & Matt Tesoriero	Catchment, Estuary and Wetland Mapping, Modelling and Prioritisation Workshop 2020	Oyster Transformation Project
Shauna Murray & Matt Tesoriero	Manning River Estuary CMP Discussion Group - Sewerage and Septic Pathogen Risks 2020	Discussion Group
Wayne O'Connor	Aust & NZ Biotechnology Conference, May, 2019, Sydney	Plenary Address: The future of NSW Aquaculture: the need for clever solutions
Shauna Murray, Arjun Verma, Swami Palanisami & Penelope Ajani	Australia New Zealand Marine Biotechnology Conference (ANZMBS) 2019	The use of eDNA and arrays for precise estuarine water quality assessment
Arjun Verma, Swami Palanisami, Penelope Ajani & Shauna Murray	Australian Marine Science Association Conference 2019	Novel molecular ecology tools to predict harmful algal blooms in oyster- producing estuaries
Arjun Verma and Matthew. Tesoriero	Trade table, NSW Oyster Conference, Forster NSW 2019	Oyster Transformation Project
Penelope Ajani, Arjun Verma & Shauna Murray	NSW Oyster Conference, Forster NSW (Poster Presentation) 2019	Common harmful algae in the oyster growing estuaries of New South Wales.
Wayne O'Connor	DPI, Senior Scientist Symposium. EMAI, Camden, November 2018	Overview and Progress – Oyster Transformation Project
Penelope Ajani, Michaela Larsson, Ana Rubio, Stephen Bush, Steve Brett, Stephen Woodcock, Hazel Farrell & Shauna Murray	Estuarine Coastal Shelf Science Conference 2018	Modelling harmful algal blooms in the Hawkesbury River, Australia
Wayne O'Connor	Macquarie University, Microbiomes Workshop, Epping, November 2018	Overview and Progress – Oyster Transformation Project
Shauna Murray, Arjun Verma, Penelope Ajani, Anthony Zammit, Hazel Farrell, Swami Palanisami & Wayne O'Connor	Australian Shellfish Quality Assurance Advisory Committee Science Day 2018	Building profitability and sustainability in the NSW oyster industry
Penelope Ajani, Michaela Larsson, Ana Rubio, Stephen Bush, Steve Brett, Stephen Woodcock, Hazel Farrell & Shauna Murray	Australian Shellfish Quality Assurance Advisory Committee Science Day 2018	Modelling harmful algal blooms in the Hawkesbury River, Australia
Hazel Farrell, Grant Webster, Phil Baker, Anthony Zammit,	Australian Shellfish Quality Assurance Advisory Committee Science Day 2018	Developing phytoplankton and biotoxin risk assessments for both shellfish aquaculture and wild harvest shellfish in New South Wales.

Penelope Ajani, Shauna Murray & Steve Brett		
Wayne O'Connor	SIMS, July 2017	Oyster Research Overview Presentation

Presenter(s)	**Event**	**Presentation title**
Shauna Murray & Arjun Verma	https://www.youtube.com/watch?v=cfAyjjnASy0&t=154s	Sept. 2019: PROJECT NEWS: Can World Leading Research Transform the NSW Oyster Industry?
Shauna Murray	https://www.youtube.com/watch?v=4NM_U_lKCEE&t=1s	Sept. 2020: Food Agility CRC – Cooperative Research Centre customer story
Arjun Verma & Penelope Ajani	https://www.youtube.com/watch?v=iRcRZkptpOY&t=46s	Feb. 2020: Food Agility Summit 2020: WE LOVE SCIENCE!
Anthony Zammit	https://www.cnbc.com/video/2017/03/05/one-of-the-most-sustainable-farming-enterprises-meets-hi-tech.html	Mar 2017: One of the most sustainable farming enterprises' meets hi-tech