TRANSFORMING AUSTRALIAN SHELLFISH PRODUCTION

Long Island Harvest Area - Wallis Lake

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 Wallis Lake, one of the estuaries selected as part of Stage 1 of the NSW Oyster Industry Transformation Project 2017-2021. To predict the impact of rainfall on potentially pathogenic bacteria, 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 in the Long Island harvest area, recording high-resolution temperature, salinity and depth data. Oyster farmers collected weekly biological samples (459 environmental DNA samples and 198 deployed/retrieved oysters for growth assessment) from the sensor site. We developed a rapid molecular qPCR (quantitative polymerase chain reaction) assay 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 develop predictive models. Finally, we developed an additional model to link oyster growth with environmental variables and assessed its predictive capability.

MAJOR FINDINGS

1

Available data indicated that one harvest area closure could have potentially been avoided between March 2018 and April 2021

100%

Salinity was a more reliable predictor than rainfall for the 3 faecal bacteria types modelled, showing changed harvest area management would be safer and more discriminatory

E. coli and cow bacteria increased soon after rainfall, but remained below regulatory limits suggesting efficient flushing

Bird, cow and human bacteria were very low across sampling period. Human bacteria was only detected on one occasion and was linked to rainfall

0

No oyster mortality events that exceeded background farming mortality occurred in Wallis Lake 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), natural resource managers (Local Land Services) and the Food Agility CRC. 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 improving production and 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. The same environmental variables that influence food safety can also impact on oyster 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 (2017-2021) of the project has been successfully completed, with ~5,000 water and 3,000 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 project provided the basis for a change to the management plans for the Pambula Lake harvest area and the Cromarty 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 Bay. The report highlighted positive benefits for industry 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 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 Wallis Lake

Wallis Lake (-32.27° S, 152.49°E) is an open, moderately large yet relatively shallow, coastal barrier estuary covering an area of ~99 km^2 with a catchment area of ~1197 km^2 and a flushing rate of ~76 days (Roy et al. 2001, Roper et al. 2011) (Fig. 1). It extends ~25 km upstream of the ocean and has two broad topographic units: the coastal plain and the inland ridges and valleys.

Based on drainage networks, the Lake's catchment is divided into seven primary subcatchments: Wallamba River, Lower Wallamba River, Wang Wauk River, Minimbah Sandbed, Coolongolook River, Wallingat River, and Wallis Lake Body. While land use is still largely agricultural (beef production/grazing ~36%) and forestry (40%), there has been a shift in recent years towards urban and rural residential development (Great Lakes Council 2014).

The estuary itself supports a diverse range of marine life, including important fisheries such as blue swimmer and mud crabs, prawns, bream, flathead and mullet. These are intrinsically linked with abundant seagrass (the largest area in NSW), saltmarsh (second largest in NSW) and mangrove habitats, which in turn support food webs, and provide habitat and fish nursery grounds (Great Lakes Council 2014). Wallis Lake also supports a unique and diverse assemblage of sponges with a greater number of species than other coastal lakes and lagoons in NSW (Barnes 2010).

Over recent years, Hunter LLS has initiated a number of studies into water quality in Wallis Lake aimed at improving understanding of the dynamic nature of the system and the impact on oysters. Two publications (Fitzer et al. 2018; Fitzer et al. 2019) investigated the impact of water quality on oyster shell biomineralization and potential adaptation responses such as the use of selectively bred Sydney Rock Oysters (*Saccostrea glomerata*). Decreasing salinity, pH and alkalinity in the mid-upper estuary was associated with reduced oyster growth indicating a reduced capacity of oysters to biomineralise (i.e. form calcium carbonate crystals) under acidified conditions. Acidification can occur from freshwater inputs (which have a lower pH than seawater) or as a result of sulfuric acid associated with acid sulfate soils.

A study by Department of Planning and Environment (2022) in collaboration LLS demonstrated that the total number of plate-sized Sydney Rock Oysters (SROs) harvested was negatively correlated with the 4-year antecedent annual rainfall total. The study investigated environmental conditions associated with SRO production in Wallis Lake by measuring salinity, temperature, water level and atmospheric pressure continuously during three periods between March 2017 and April 2019 at four sites located at the approximate boundaries of oyster aquaculture in Wallis Lake.

Spatial and temporal patterns in salinity are complex in Wallis Lake, being influenced by freshwater inputs, tidal exchange and sea level anomalies. Large rainfall events cause the Wallamba River to flush fresh to its confluence with the lake system, with estuary recovery (i.e. salinity returning to marine/brackish conditions) proceeding rapidly due to tidal exchange. Tidal exchange has increased as a result of the construction/extension of entrance training walls in the 1960s with a greater marine influence significantly increasing tidal range, increasing channel velocity and scouring of sediments (Neilsen and Gordon 2016) all factors that have impacted on oyster aquaculture in Wallis Lake.

The trajectory of estuarine recovery is determined by factors such as follow-up rainfall, groundwater inputs, tidal mixing, the spring/neap tidal cycle, and the mean water level anomalies. In contrast, hypersalinity can develop in the lake basin in response to high sea level anomalies coincident with extended dry periods. The data collected during the study allowed the development of an empirical rainfall-salinity model for the Wallamba River.

1.3 Oyster Production in Wallis Lake

Wallis Lake is one of the most significant producers of Sydney Rock Oysters in Australia, with production in 2020/21 of 1.1 Mil dozens valued at $9.5 Mil, ~18% of NSW's total oyster production (NSW Department of Primary Industries 2022). Key threats to water quality in the lake include elevated sediment and nutrient levels, pollutants (including litter, pathogens, chemical and oil spills and leachate), loss of ecosystem function and associated services, detrimental soil and ground cover management, invasive species, climate change and associated sea-level rise, acid sulfate soils and increasing development (Great Lakes Council 2009).

FINDINGS

2. Findings

2.1. The data assessment from this report supports implementing a harvest area management plan based on sensor salinity data for Long Island harvest area, subject to the agreement of the local shellfish industry. Available data indicated that one harvest area closure and three harvest area downgrades could have potentially been avoided between March 2018 and April 2021. If additional sensors were being considered by the Wallis Lake Shellfish Program (WLSP), a sensor situated within harvest area boundaries would provide more insight on salinity conditions.

2.2. 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 Wallis Lake over the biological sampling period, September 2018 to September 2020.

2.3. The real time sensor data showed a higher predictive capacity than rainfall data for all faecal indicator bacteria modelled.

2.4. Overall, the abundance of bacteria was low and highly variable across the sampling period, with the maximum predictive capability of models being 15% for E. coli, 27% for cow, and 36% for bird.

2.5 Human bacterial contamination was detected on only one occasion and followed on from a moderate rainfall event. There was insufficient data to model/predict its prevalence.

2.6 Where the models were predictive, they suggested *E. coli* and bird bacterial abundance increased with increasing salinity. This may be, in part, linked to a lag in input from the upper catchment. Cow bacterial abundance rose with decreasing salinity (linked to rainfall). All models indicated that higher bacterial concentrations occurred when surface water temperatures were between 20-22℃.

2.6. The greatest oyster growth occurred during the spring and summer months, with the sensor variables - daily salinity (increasing) and weekly rainfall (increasing) - resulting in a highly predictive model performance of ~92%.

2.7. No oyster mortality events that exceeded background farming mortality (approximately 10% per annum) occurred in Wallis Lake over the period from August 2018 to February 2020.

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. The project team would like to acknowledge the invaluable assistance of the Wallis Lake oyster farmers for collecting weekly samples. Specifically, we thank Brad Verdich for his 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 depth, salinity and temperature data used in the analysis. Routine phytoplankton monitoring sample data for Wallis Lake 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 Dr Torri Callan (UTS) for statistical analyses and Chris Komorek (Food Agility CRC) for report layout.

FEEDBACK

4. Feedback

In June 2018, the Oyster Transformation Team held an information workshop to allow 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.

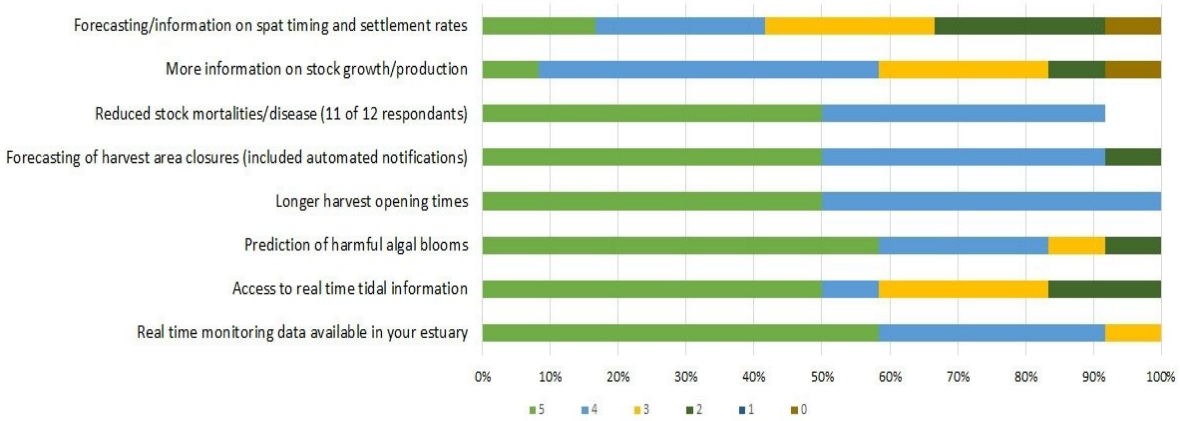


Figure 4.1. The importance of factors as rated by farmers in relation to their business operations. Light green is most important and brown is least important.

5. Results

5.1 High resolution temperature and salinity data

High-resolution real time data summaries for Wallis Lake for the period 13 March 2018 to 31 March 2021 are shown in Figs. 5.1A-C. Depth recordings ranged from 0.3 m (21 Nov 2018) to 1.9 m (21 Mar 2021). The lowest and highest daily average salinity recordings were 0.07 ppt (22 Mar 2021) and 36.98 ppt (30 Dec 2019) respectively, while the lowest and highest daily average temperature recordings were 13.7℃ (20 Aug 2018) and 27.3 ℃ (25 Jan 2021), respectively.

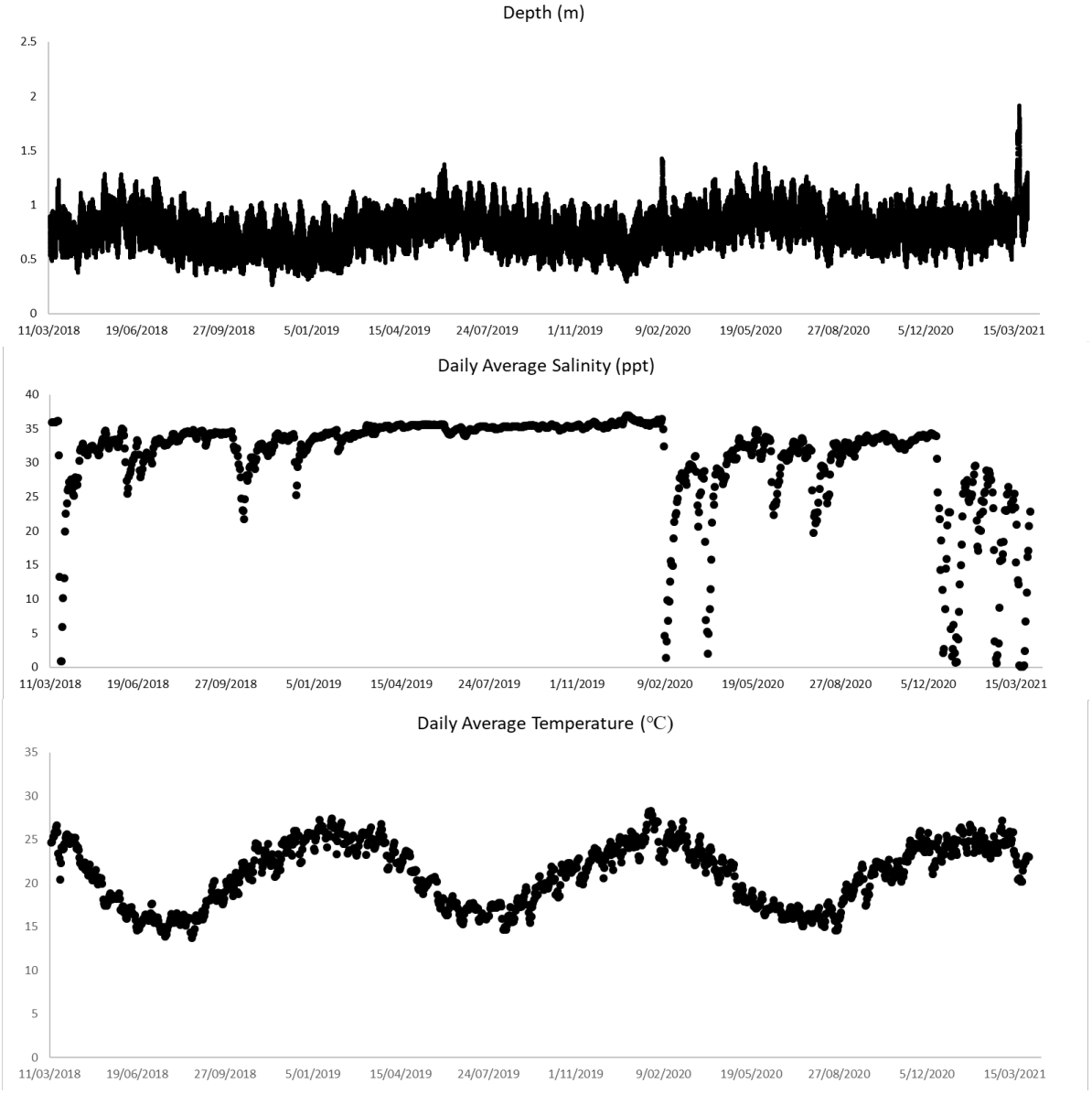


Figure 5.1A-C. Real time sensor data from Wallis Lake 13 March 2018 to 31 March 2021. A. Depth (m); B. Daily average salinity (ppt); and C. Daily average temperature (°C).

The maximum daily rainfall across both rain stations (BOM Station No. 060013 and MHL Station No. 209401D) occurred on 22 Mar 2018 and was reported as 174 mm (Fig. 5.2).

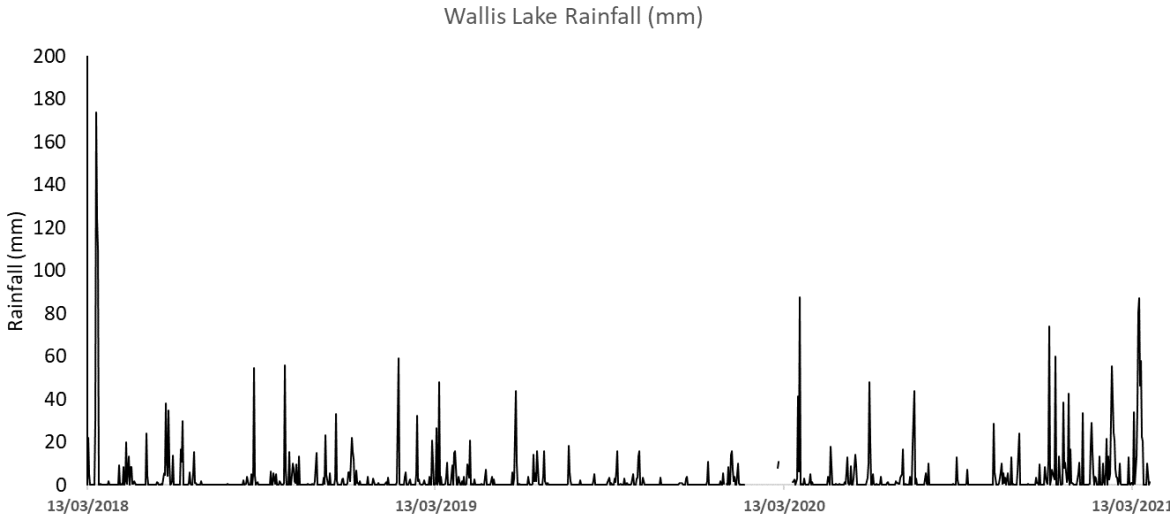


Figure 5.2. Daily rainfall (mm) from rainfall gauge sites at Forster (BOM Station No. 060013) and Tuncurry (MHL Station No. 209401D)

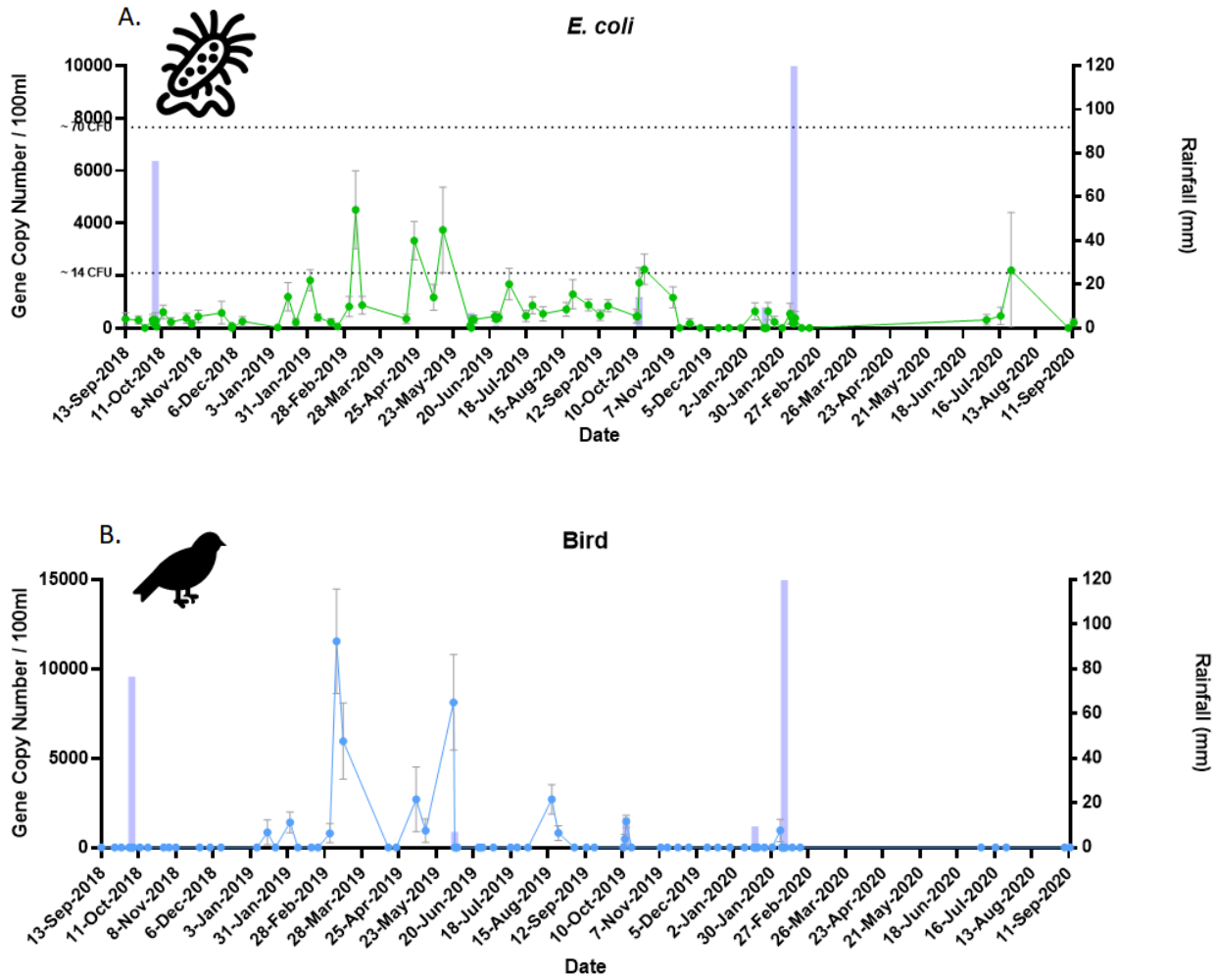
5.2 Management Plan

Data analysed during the 2021 annual review of Long Island harvest area (see Fig. A1) indicated that there could have been one less harvest area closure and fewer downgrades since the sensor was installed, if closures were based on salinity sensor data. There were ten harvest area rainfall closures in Long Island harvest area between March 2018 and April 2021. During the same period there were three harvest area salinity closures, as advised by the local program monitoring local conditions. Based on a management plan sensor salinity closure limit of 24 ‰, harvest area closures were reviewed focusing on available salinity sensor data and shellfish program microbiological results since March 2018. One harvest area closure, of two days duration, could have potentially been avoided. During the same time period, there were six rainfall downgrades in Long Island harvest area. A review of salinity sensor data and shellfish program microbiological results indicated that there were three rainfall downgrades where salinity as reported by the sensor was higher than 28 ‰ (downgrade salinity range 24-28 ‰), and microbiological results from samples collected 0-6 days post downgrade met Approved harvest criteria. It should be noted that salinity data analysed between May 2020 and May 2021 had a higher variability due to more frequent rainfall events, and time periods where salinity is slower to recover may require additional sampling to meet management plan requirements. A review of the available data also indicated that given high fluctuations in salinity between high and low tides, particularly after prolonged wet periods, decisions on harvest area closures would consider salinity trends rather than point in time measurements. It is also noted that the current location of the sensor is upstream of Long Island harvest area, and it was recommended that if additional sensors were being considered by WLSP, a sensor situated within harvest area boundaries would provide more insight on salinity conditions, which may lead to a more advantageous management regime.

5.3 Bacterial source tracking

A total of 459 water samples and 198 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 Wallis Lake (Fig. A1).

The pollution source tracking results were highly variable across the study period, with the majority of cow and human results just above detection limits. The maximum *E. coli* abundance was recorded as 4,509 gene copies 100 mL^{-1} on 9 Mar 2019, for bird as 11,534 copies 100 mL^{-1} on 9 Mar 2019, for bovine faecal pollution (cow) as 229 gene copies 100 mL^{-1} on 5 Feb 2020, and finally, 787 copies 100 mL^{-1} for human faecal pollution on 8 Jun 2019 (Fig. 5.3 A-D).



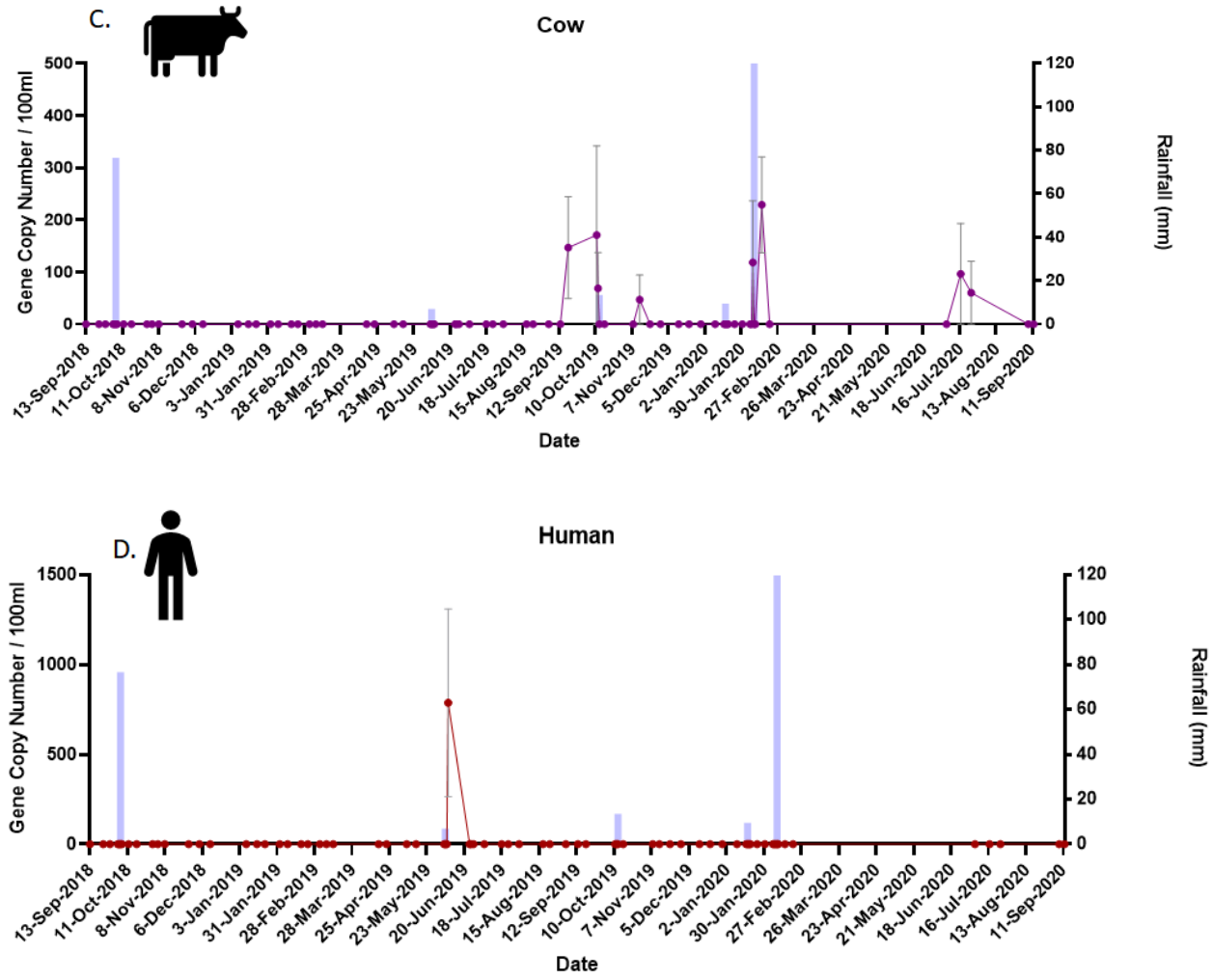


Figure 5.3 A-D. Weekly *E. coli* data from the sensor location, Wallis Inlet, using A. *E. coli* assay; B. Bird assay; C. Cow assay; and D. Human assay. Purple bars represent rainfall events that were sampled. Dotted lines in Fig. A at 14 and 70 cfu/100 mL are the operational limits for direct or restricted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

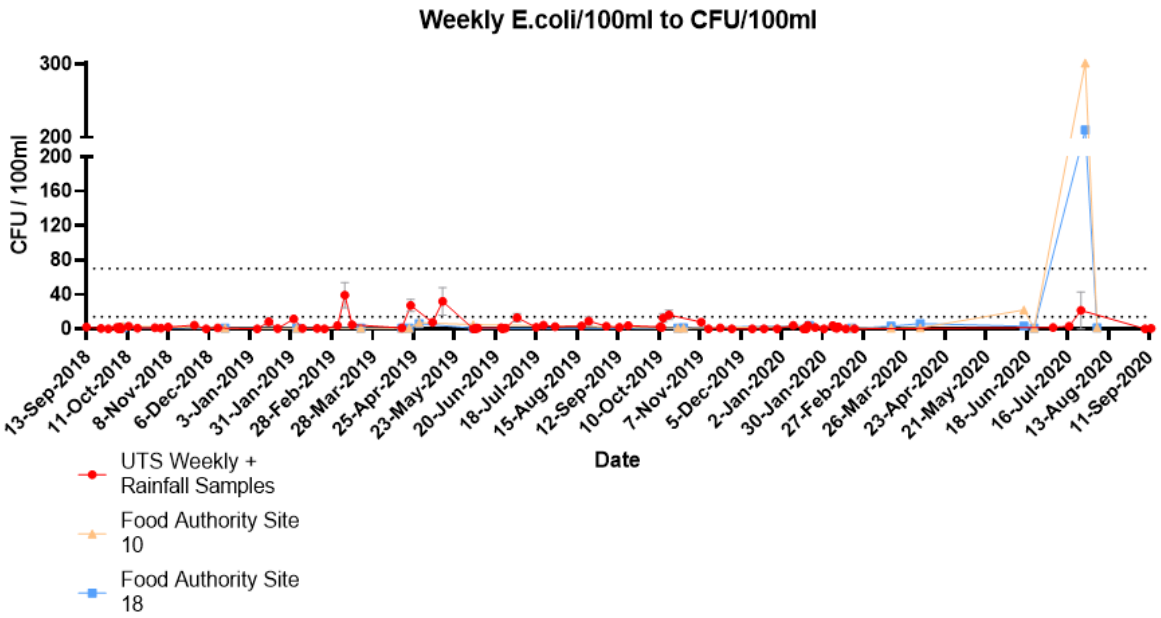


Figure 5.4 Weekly faecal coliform counts (cfu/100 mL) from water samples collected by DPI Food Authority at two sites in Wallis Lake sensor site compared to Oyster Transformation Project weekly sampling results (including rainfall sampling). Dotted lines at 14 and 70 cfu/100 mL are the operational limits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

Faecal coliform counts reported by DPI Food Authority generally corresponded well with those examined using qPCR as part of the CRC project. The maximum faecal coliform counts reported in Jul 2020 by the Food Authority, however, did not have corresponding samples collected by the CRC project, so a comparison of methods/results at this time is not possible (Fig. 5.4).

Six rainfall events were sampled across the study period. These occurred on 5-7 Oct 2018, 6-8 Jun 2019, 25 and 27 Jun 2019, 12 and 13 Oct 2019, 18-20 Jan 2020, and 7-10 Feb 2020 (Fig. 5.5A-F). Overall *E. coli* increased marginally after rainfall, but remained below regulatory limits on all occasions. Bird bacteria was only detected during one rainfall event (12-13 Oct 2019), increasing from day 1 to day 2 (no sampling occurred on day 3) (Fig. 5.5D). Cow bacteria was detected during one rainfall event (8 Feb 2020), however, it went back to background concentrations by day 3 (Fig. 5.5F). Human bacterial contamination was detected on one occasion (8 Jun 2019) and was linked to a moderate rainfall event. Without further sample collection after this event, it is unclear how quickly this contamination would have dissipated (Fig. 5.5B).

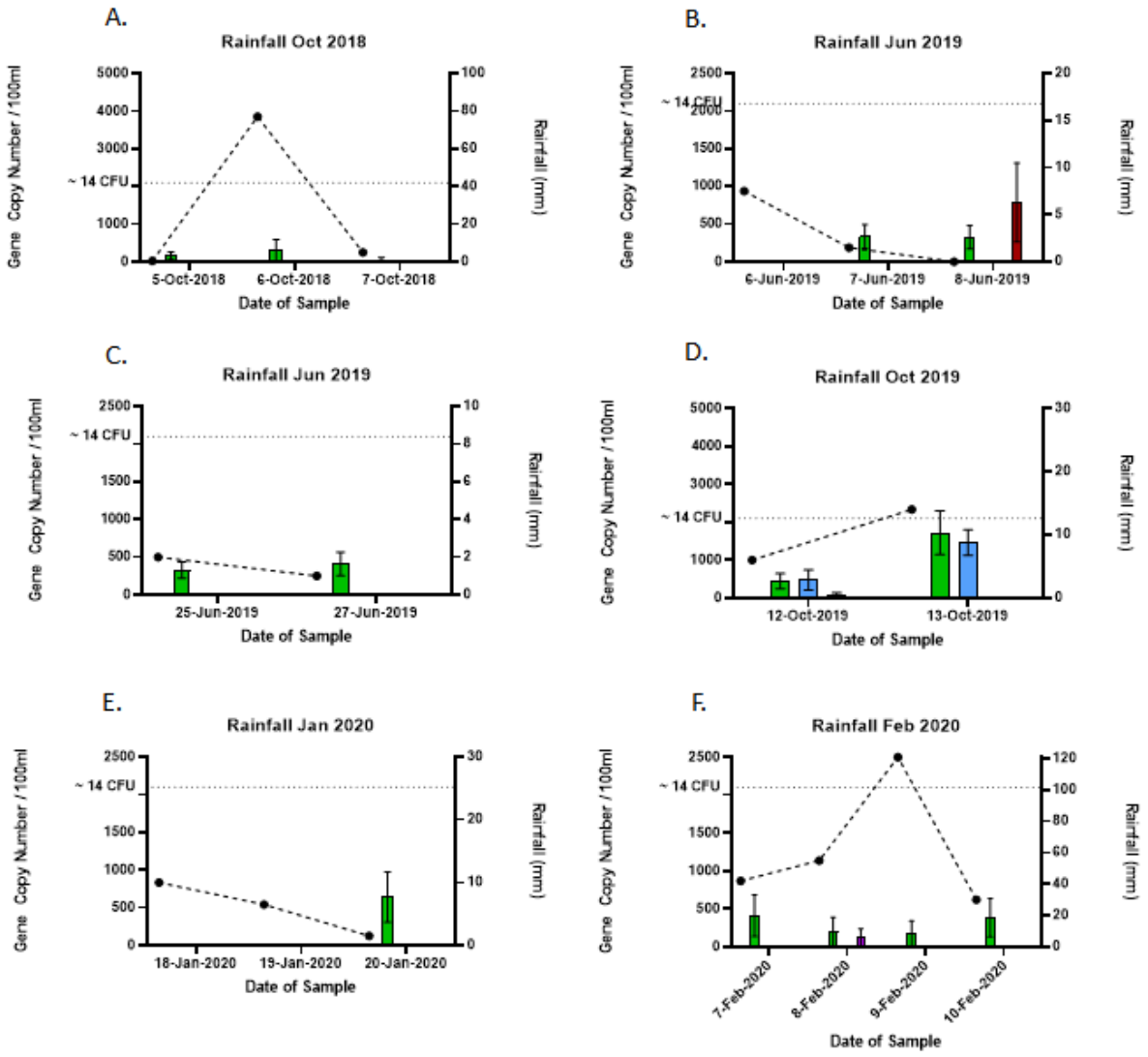


Figure 5.5 A-F. Sensor site (Wallis Lake) rainfall events sampled for *E. coli* assays. Green bar = 16S *E. coli*; blue bar = bird assay; purple bar – cow assay; red bar = human assay. Dotted line is rainfall (mm) obtained from the closest Bureau of Meteorology weather station at Forster (BOM Station No. 060013). 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 (Mar 2018 to Mar 2021) at the site closest to the sensor (site 3), occurred on 19 Feb 2020 (Fig. 5.6). Although rainfall data is not available for this date and in the lead up to this time, salinity dropped to an average daily of 1.4 ppt a week prior (10 Feb 2020) indicating a freshwater influx had occurred. Total cell concentrations reached 4.8E +06 cells L^{-1} and samples contained planktonic diatoms (particularly a small, solitary *Chaetoceros* sp., with other *Chaetoceros* spp. and *Leptocylindrus*), benthic diatoms (*Cylindrotheca*) and small flagellates (cryptomonads, dinoflagellates, euglenoids, ochromonads). High levels of sediment and organic detritus were also reported.

Other potentially harmful bloom events across the sampling period included blooms of the diatom *Pseudo-nitzschia delicatissima* gp. These occurred during Jun 2018, May and Oct 2020, with a maximum concentration of 2.2E +06 cells L^{-1} reported (21 Oct 2020). Another harmful diatom group, *Pseudo-nitzschia fraudulenta/australis*, bloomed in Jan/Feb 2019, reaching a maximum cell concentration of 2.3E +05 cells L^{-1}. The toxic dinoflagellate *Dinophysis caudata* reached elevated cell densities on 9 Apr 2018 and 23 Oct 2019, at 700 and 580 cells L^{-1} respectively. The NSW Food Authority's Phytoplankton Action Limits to trigger biotoxin testing are 500,000 cells L^{-1} for *Pseudo-nitzschia delicatissima* gp., 50,000 cells L^{-1} for *P. australis & multiseries* and 500 cells L^{-1} for *Dinophysis caudata* (NSWFA 2015). No biotoxins were detected in association with any of these blooms.

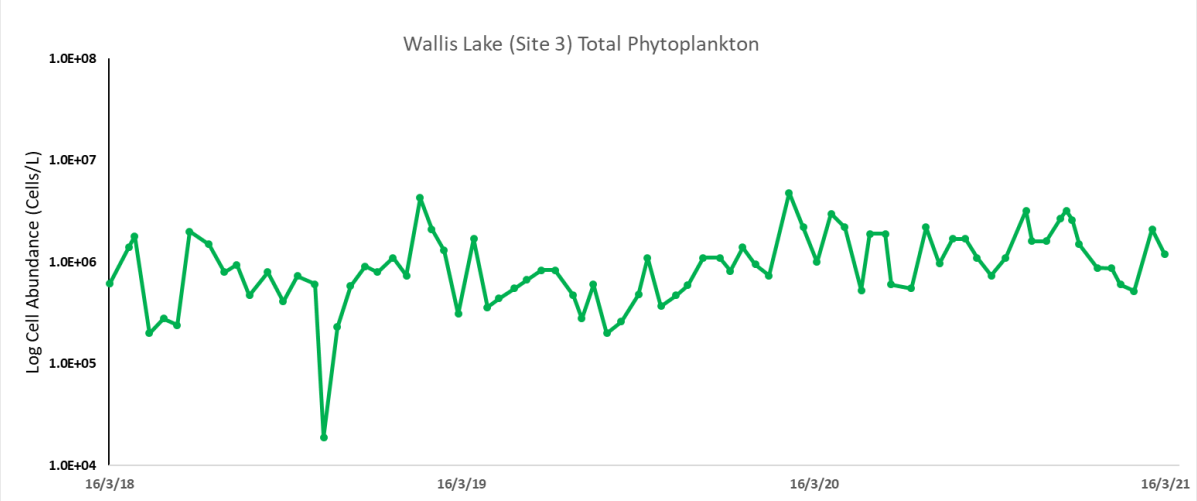


Figure 5.6 Log abundance of total phytoplankton sampled approximately fortnightly at site 2 (closest to the sensor) from 13 March 2018 to 31 March 2021.

5.6 Oyster Growth and Mortality

5.6.1 Oyster Growth

Oyster whole weight increased by 28 g in the experimental period (August 2018 to June 2020) (Fig. 5.7 A). Oyster whole weight increases were greatest in spring and summer months of this experiment when oysters increased their weight by 9.1 g over 6 months in 2018/2019 and 10 g over 6 months in 2019/2020. Oyster whole weight was 50.6 ± 4.9 g at the end of the experiment (June 2020). Oysters deployed in Wallis Lake attained a large size grade (> 70 mm total length or > 50 g whole weight) in June 2020 (50.6 g) and were 42 mo on this date.

Oyster shell length was 52 ± 2 mm at the start of the experiment and increased to 69 ± 2 mm in June 2020 (Fig. 5.7 B). The greatest increase in shell length in Wallis Lake was recorded from September to December 2018. The increase in size through this period was 14 mm. Shell lengths were measured more frequently than whole weight and fluctuated throughout the experiment. Periods of shell length decreases were recorded between December 2018 and February 2019, July and August 2019, October and November 2019 as well as February and June 2020.

5.6.3 Mortality

No mortality events that exceeded background farming mortality (<10% per annum) occurred in Wallis Lake over the period from August 2018 to February 2020 (Fig 5.7 C-D). The period of highest mortality in this experiment was between October 2018 and February 2019 when oysters were 25 mo (Fig 5.7 C). Oysters from this site remain frozen for future analyses.

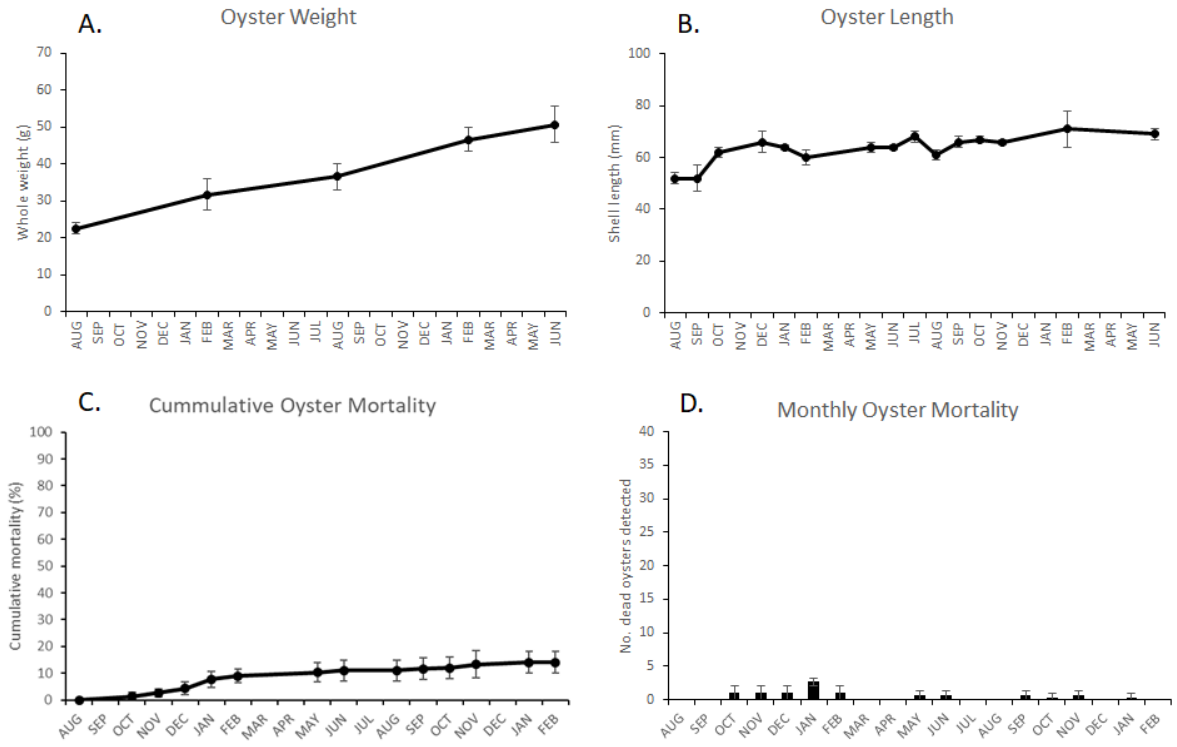


Figure 5.7 A-D. Oysters deployed at the sensor site, Wallis Lake. A. whole weight; B. shell height; C. cumulative mortality, and D. monthly mortality.

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 2A-B. Correlation coefficients were calculated among every pair of environmental variables and suggested very few strong positive relationships ($r > 0.7$) overall. 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 at the sensor site were: 15% for *E. coli* (sensor + total phytoplankton), 26.7% for cow (sensor + total phytoplankton), and 35.9% for bird (sensor + total phytoplankton) (Table 1). There was only one human bacterial detection across the entire sampling program, which occurred three days after significant rainfall of 44 mm on 5 Jun 2019. Considering this low occurrence, there was insufficient data to model/predict its prevalence.

The abundance of *E. coli* at the sensor site was only marginally explained by the models (15% deviance explained using sensor data compared to 6% using rainfall data), but appeared to be linked to increasing salinity. Data also indicated that peak *E. coli* coincided with a surface water temperature of ~20-22℃ (Table 1) (Figures 5.7 A-D, 5.8 A-D).

On the other hand, cow bacterial abundance was far better explained by the models (~27% using sensor data compared to 17% using rainfall), with both a decreasing salinity and decreasing temperature significantly contributing to these outcomes (Table 1). Data suggested, however, that a peak in cow bacteria still coincided with a surface temperature of ~20℃ (Figures 5.7 A-D, 5.8 A-D).

Faecal contamination from birds at the sensor site was twice as well explained by the salinity data compared to the rainfall data (36% and18% respectively), with salinity (increasing) and again a maximum temperature of ~22℃ significantly contributing to the prediction of this bacterial source (Table 1).

5.7.2 Modelling of oyster growth and mortality

While there was insufficient oyster weight data to model (only 5 data points across the sampling period), there was sufficient shell length data to model. The best model explained a very high ~92% of the deviance, with the strongest predictor variables being the daily average salinity (increasing) and the maximum average salinity for the week prior to the oyster length observation (decreasing). The negative effect of this latter variable suggests that the impact of daily salinity on oyster length is attenuated when relatively high salinity is present for an extended period. This suggests that if salinity has been relatively high during the previous week, the oysters will have grown, whereas a single day of elevated salinity might see all the growth at that particular point in time. There was also a linear response to rainfall, with lengths appearing greater as weekly rainfall increased, but this effect was not large, and could be due to increases in nutrient availability during periods of low to moderate rainfall that did not result in major prolonged reductions in salinity.

Table 1 A. Modelling results for bacterial source tracking at the sensor site in Wallis Lake. Only significant variables are shown for each model.

Bacteria	Variables	No. of observations	Significant Variables	Deviance Explained
E. coli	Salinity, Depth, Temp	74	Depth72**, Salinity72***, Temp72***	12.7%
E. coli	Salinity, Depth, Temp, logPhytoplankton	74	logPhytoplankton ***, depth**, salinity***, temp***	15%
E. coli	Rainfall72	65	Rainfall72***	5.14%
E. coli	Rainfall72, logPhytoplankton	65	Rainfall72***, logPhytoplankton ***	5.79%
Bird	Salinity, Depth, Temp	74	Salinity***, Depth***, Temp***	34%
	Salinity, Depth, Temp, logPhytoplankton	74	Salinity***, Depth***, Temp***, logPhytoplankton ***	35.9%
Bird	Rainfall72	65	Rainfall72***	17.2%
Bird	Rainfall72, logPhytoplankton	65	Rainfall72***, logPhytoplankton***	17.7%
Cow	Salinity, Depth, Temp	74	Salinity***, Depth***, Temp***	26.6%
Cow	Salinity, Depth, Temp, logPhytoplankton	74	Salinity***, Depth***, Temp***, logPhytoplankton***	26.7%
Cow	Rainfall24	65	Rainfall48***	16.6%
Cow	Rainfall24, logPhytoplankton	65	Rainfall48***, logPhytoplankton***	17.4%

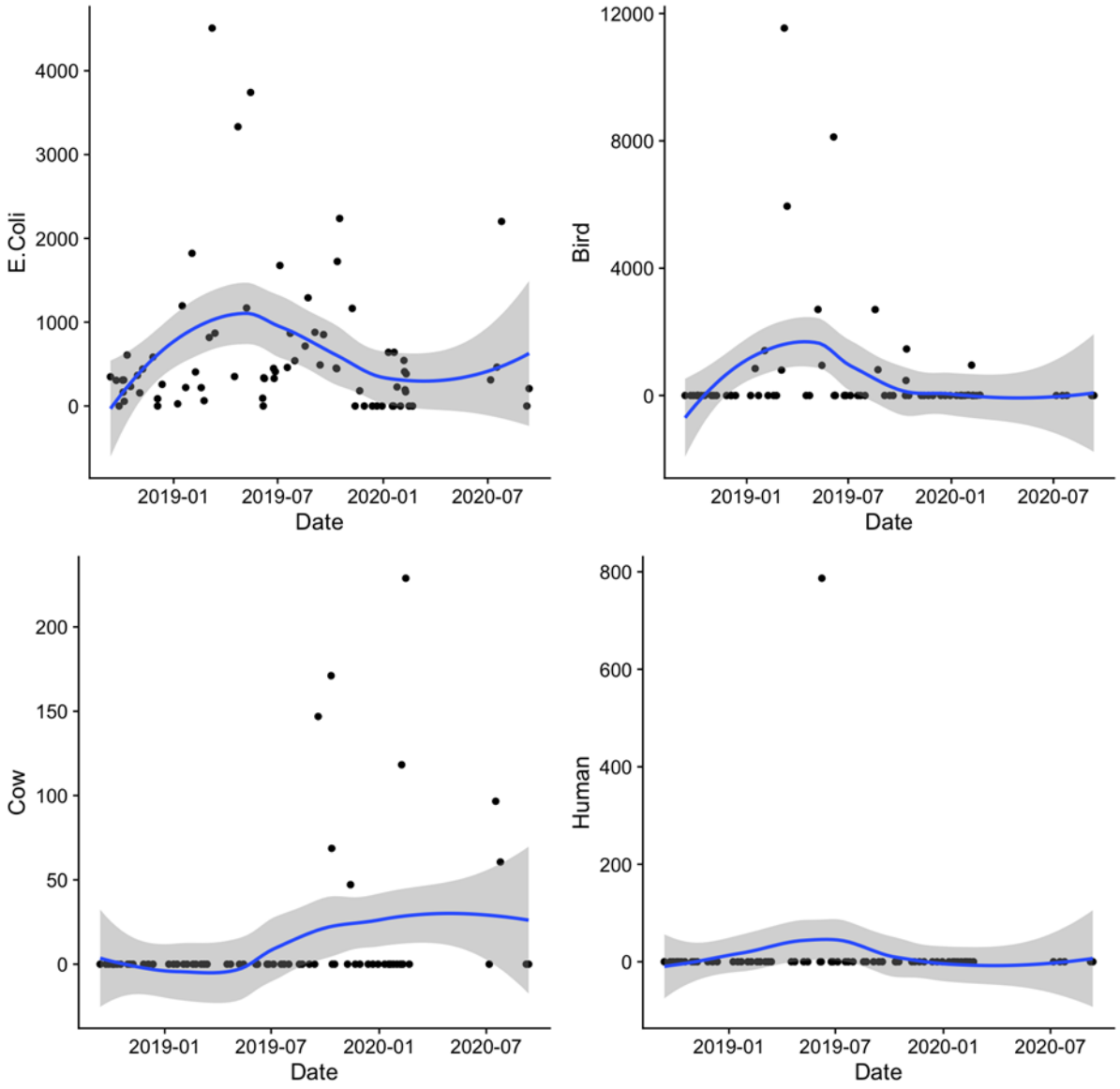


Figure 5.7 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. *E. coli*, B. Bird, C. Cow, and D. Human bacterial load as measured by weekly sampling at the sensor site, Wallis Lake.

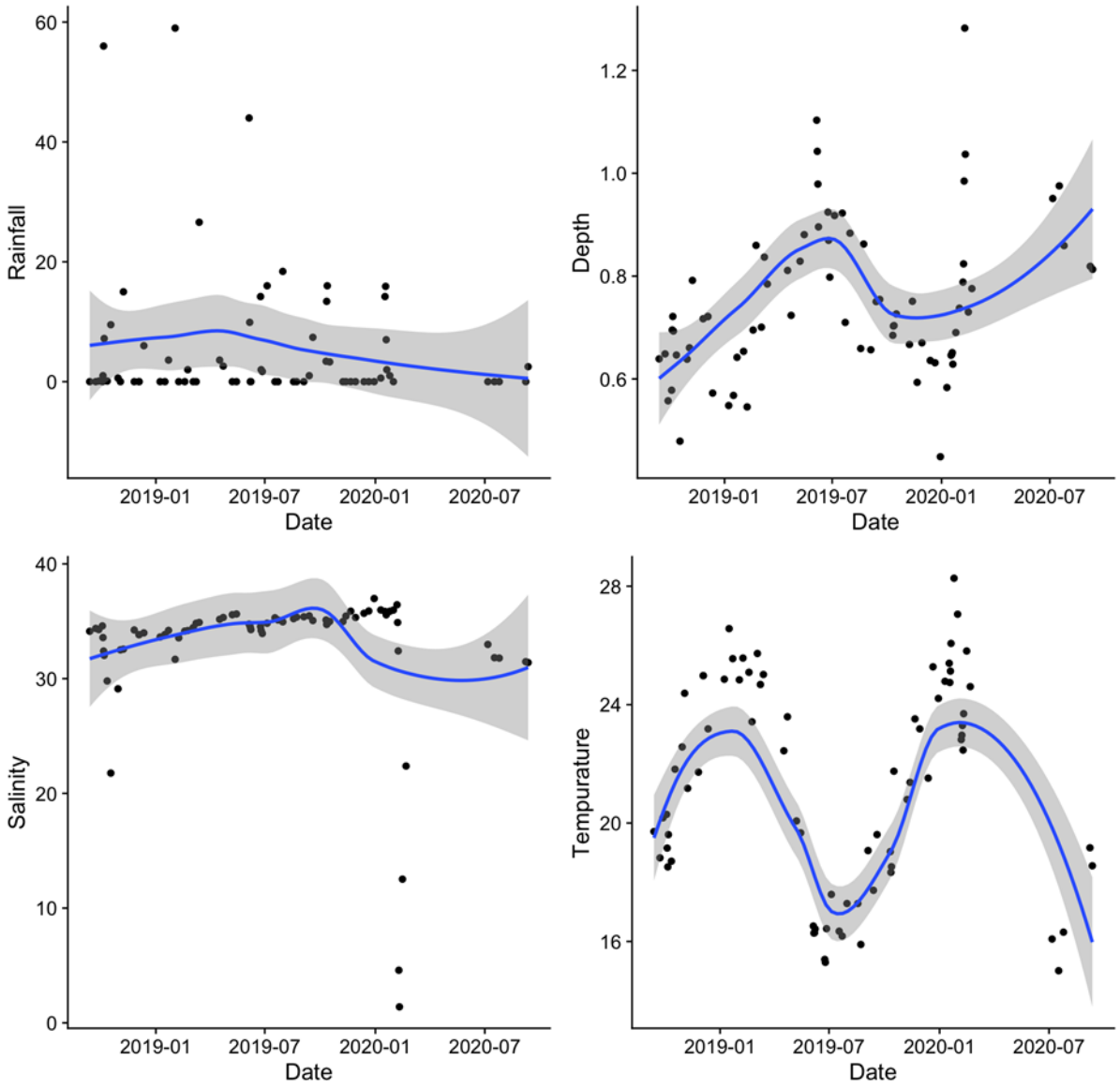


Figure 5.8 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. Rainfall, B. Depth, C. Salinity, and D. Temperature values measured in at the sensor site, Wallis Lake.

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 Long Island harvest area. Based on the available data, at least one harvest area closure and three harvest area downgrades could have potentially been avoided between March 2018 and April 2021. The option of a salinity based management plan based on the sensor in its current position is still feasible for Long Island harvest area, however, greater insight would be gained from sensor data within a harvest area boundary. 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. Wallis Lake Shellfish Program (WLSP) were consulted about the option of a salinity-only management plan for Long Island harvest area following the 2021 annual review, but a decision has not yet been reached. If WLSP 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 Long Island 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 the sampling period was observed prior to a significant drop in salinity. This growth was most likely a response to a rainfall event (data not available) and subsequent nutrients entering the waterway. Apart from *Pseudo-nitzschia* (discussed below), other HAB events were those caused by the dinoflagellates *Dinophysis caudata*, although no biotoxins were associated with any of these blooms.

Pseudo-nitzschia is a high-risk HAB group in SE Australia for the shellfish aquaculture industry, and both estuaries and coastal waters in this area remain under threat (Ajani et al., 2013a, 2020). Wallis Lake has been identified as a high-risk estuary, with maximum cell densities of (total) *Pseudo-nitzschia* spp. reported anytime across the austral winter, spring or summer, with an autumn minimum (Ajani et al., 2013a). Blooms within the Hawkesbury River estuary (330 km south of Wallis Lake), another high-risk area in SE Australia for HAB events, recently experienced a very dense bloom of *P. delicatissima* gp., with one out of seven strains isolated to produce domoic acid (Ajani, 2020). Fifteen years of modelled data in the Hawkesbury River estuary revealed that *Pseudo-nitzschia* was linked to an increase in soluble reactive phosphorus and a decrease in nitrogen at all six sites sampled (via rainfall/nutrient runoff). There is contrasting evidence, however, of which environmental conditions promote the blooming of the different species complexes (Dermastia et al., 2020). In response to a toxic bloom of *Pseudo-nitzschia delicatissima* gp. (dominated by *P.* cf. *cuspidata)* in Wagonga Inlet in April 2019, we have now successfully developed a rapid, sensitive and efficient quantitative real-time polymerase chain reaction (qPCR) assay to detect *P. pseudodelicatissima* complex Clade I, to which *P.* cf. *cuspidata* belongs (Ajani et al. 2020).

Species belonging to the genus *Dinophysis* (and more rarely benthic *Prorocentrum)* are the most problematic Diarrhetic Shellfish Toxin (DSTs) 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).

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, when 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 also successfully developed a rapid qPCR assay to detect species belonging to the genus *Dinophysis* in environmental samples (Ajani et al. 2022).

Another HAB group to watch in NSW is the toxic dinoflagellate genus *Alexandrium*. Approximately 33 species of *Alexandrium* have been recorded worldwide, of which around 10 species can potentially produce Paralytic Shellfish Toxins (PSTs). These are *A. affine, A. andersonii, A. pacificum* (= *A. catenella* Group IV ribotype); *A. australiense* (= *A. tamarense* Group V ribotype), *A. minutum, A. ostenfeldii, A. catenella, A. tamiyavanichii* and *A. taylori* (Anderson et al. 2012, Tomas et al. 2012, John et al. 2014). PSP was first reported in Australia in 1935, when typical PSP symptoms were observed following the consumption of wild mussels collected from Batemans Bay, NSW (Le Messurier et al. 1935). In 1986, the first PSP outbreak in Australia was recorded in Port Philip Bay, Victoria, with *A. pacificum* (as *A. catenella*) as the causative organism (Hallegraeff et al. 1992). A. *pacificum* is also the main causative agent of PSTs in NSW (Ajani et al. 2013). In October 2016, high cell densities of this species were detected in the coastal waters of Twofold Bay, NSW, an unprecedented event for this location in south eastern Australia. With a maximum cell density (89,000 cells L^{-1}) and a concentrations of 7.2 mg/kg PST STX equivalent in blue mussels (*Mytilus galloprovincialis*) from the bay, a four-month shellfish harvest closure ensued (Barua et al. 2020). Another unprecedented bloom of this species occurred early in Tasmania in 2010. This toxic event led to a worldwide product recall and it was estimated that this toxic event cost the Australian industry AUD ~$23 M in lost revenue (Campbell et al. 2013).

Quantitative PCR is 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). We have now developed qPCR assays for *Alexandrium* (sxtA gene) (Ruvindy et al. 2018), *Dinophysis* spp. (Ajani et al. 2022) and *Pseudo-nitzschia pseudodelicatissima* complex Clade 1 (Ajani et al. 2021). The qPCR assays can be used on-farm, allow for automation, are easy to use without specialist knowledge, and provide an early warning that harmful algae are present in the water column. It is envisaged

that high-resolution, real-time environmental data, combined with sensitive, specific and efficient molecular tools such as we have developed in the current study, will enable us to effectively predict and manage these blooms into the future.

6.3 Assay Development and Faecal Pollution in Wallis Lake

Molecular assays for the detection of faecal bacterial contamination in Wallis Lake 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 that 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 faecal indicator bacteria load in catchments (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 catchments 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 in a rural catchment, 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).

In most coastal and estuarine systems, an increase in bacterial load is usually linked to an increase in rainfall and a decrease in water 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 have 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).

While bacterial contamination (cow, human and *E. coli*) was relatively low across the sampling period in Wallis Lake, it was the avian faecal load that was best explained by the predictive

modelling (~36%), with increasing salinity and warmer surface water temperatures being the best predictors. The correlation with increasing salinity may be, in part, linked to a lag in input from the upper catchment. Like other estuaries examined so far in this program (Manning River, Wagonga Inlet), the bird contamination in Wallis Lake was observed during the Autumn months, but unlike these other estuaries, elevated, albeit lower, bird contamination was also observed during some winter months in Wallis Lake. This may be a result of some winter migrant (food migrant) species such as pigeons, doves, egrets and kestrels which travel for the ripening fruits of Cabbage Palms in the surrounding areas (Turner 2020). The molecular marker used in this study for avian bacteria, does not, however, 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 the faecal load would be required for this elucidation.

The low levels of human bacterial contamination observed in this study may suggest that water quality management efforts in regard to sources of human contamination over the past two decades are working. Sewer overflows and septic tank seepage present the highest impact/risk for human contamination Wallis Lake. 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 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 the 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.

Increases in whole oyster weight in Wallis Lake were greatest during the spring and summer months. Growth, in terms of shell length, was greatest during this same period in the first year of the experiment. The spring and summer months of this experiment were characterised by high salinity (> 30 ppt) and temperature levels (> 23 °C) which is optimal for Sydney Rock Oyster growth and survival. Higher salinities increase seawater alkalinity providing more calcium carbonate available for oyster shell deposition. The salinity level that promotes the greatest growth rates in Sydney Rock Oyster spat is 30 ppt for small spat (1.3 mg) and 35 ppt for larger spat (0.61 g) (Nell and Holliday, 1988). Fastest growth of Sydney Rock Oyster spat occurs at 30 °C. However, the optimal temperature and salinity combination for spat survival is 23 °C and 30 ppt, respectively (Dove and O'Connor, 2007).

Survival of oysters during the experiment was high from deployment until February 2020. Mortality during this period was below the background farming mortality (approximately 10% per annum) commonly experienced when farming Sydney Rock Oysters. Cumulative mortality in February 2020 was 14% and comparable to cumulative mortality measured on the same date in the two oyster producing estuaries located to the north and south of Wallis Lake, Manning River

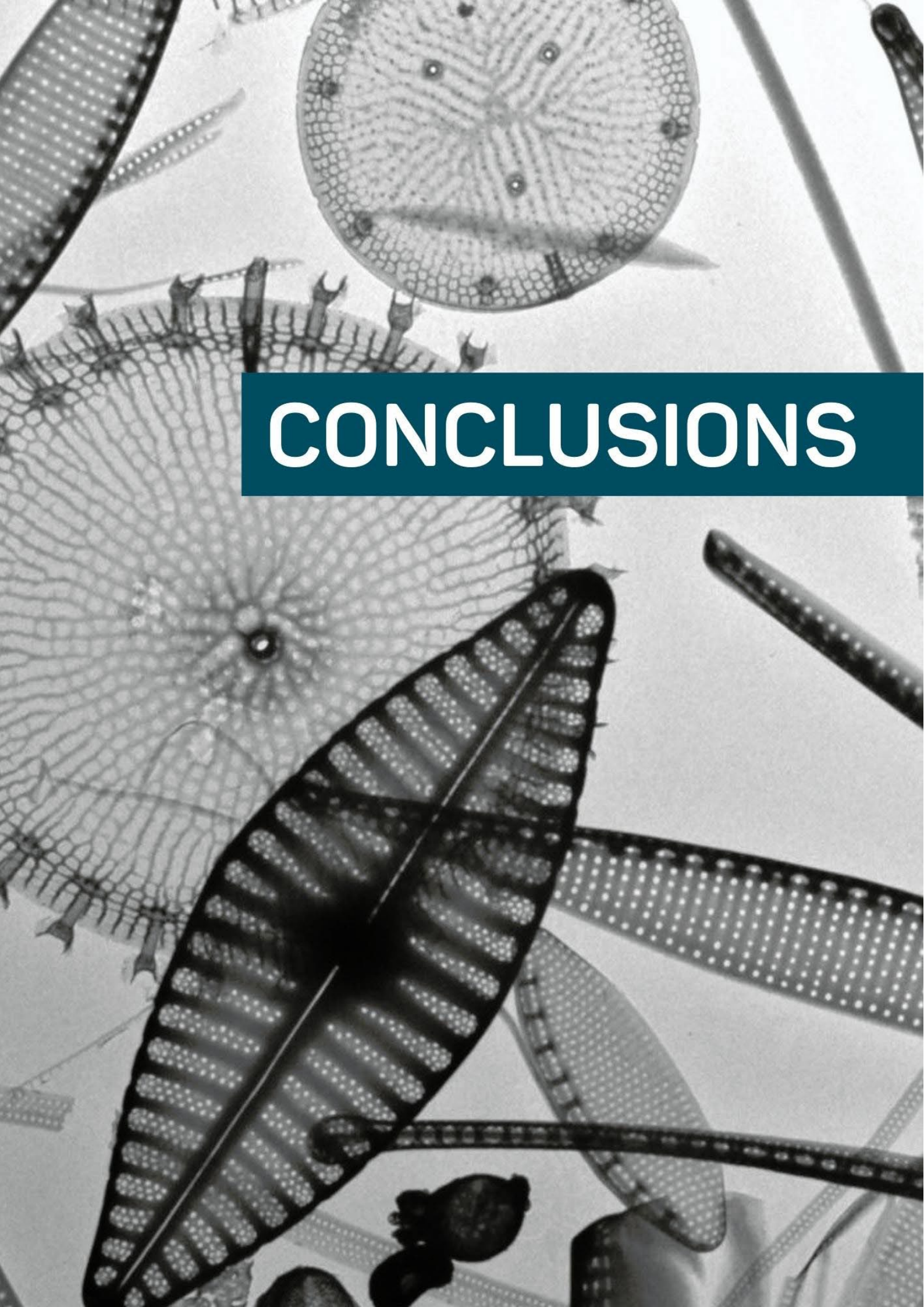
(15%) and Port Stephens (15.7%). Cumulative mortality measured at this site in a previous study was 25.3% over a 26-month period from April 2004 to June 2006 (Dove and O'Connor, 2009).

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 6 months to reach the large oyster size grade (> 70 mm total length or > 50 g whole weight). Estuaries where this same batch of oysters reached the large oyster size grade benchmark at the same time were Hastings River (52.5 g), Port Stephens (58.5 g), Shoalhaven River (50.6 g) and Pambula River (59.4 g).

When oyster growth measured at the conclusion of the experiment (June 2020) was compared between the twelve estuarine sites in this study, Wallis Lake ranked 10th and 9th in terms of whole oyster weight and shell length, respectively. Most oysters produced in Wallis Lake are sold at the 'small' size grade where oysters are less than 55 mm in shell size and less than 30 g in whole weight (NSW Department of Primary Industries 2022). Oysters at Wallis Lake reached this benchmark in February 2019 when they were just over 2 years in age. Wallis Lake is the state's largest oyster producing estuary with one million dozen oysters sold annually (NSW Department of Primary Industries, 2022). Wallis Lake is also the most valuable estuary in the state and is worth approximately $9.5 million annually despite selling most oysters at the 'small' size grade.

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.



CONCLUSIONS

7. Conclusions

The data assessment from this report supports implementing a harvest area management plan based on sensor salinity data for Long Island harvest area, subject to the agreement by the local shellfish industry. Available data indicated that one harvest area closure and three harvest area downgrades could have potentially been avoided between March 2018 and April 2021. During May 2022, NSW Hunter Local Lands Services facilitated the deployment of seven sensors in Wallis Lake. Data from these sensors can be included in future annual reviews for Wallis Lake harvest areas. As of April 2022, sixteen salinity-only management plans had been offered for harvest areas in participating NSW estuaries, with six being taken up and the remaining ten under consideration.

Compared to the other monitoring sites in NSW, oyster growth in Wallis Lake ranked 10th and 9th in terms of whole oyster weight and shell length, respectively. Very low levels of mortality were recorded over the period from August 2018 to February 2020 and was below the level accepted as background farming mortality (approximately 10% per annum). Wallis Lake is an important estuary for Sydney Rock Oyster culture in NSW as it is the most productive in terms of oyster sales and the most valuable (NSW Department of Primary Industries, 2022).

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 (increasing salinity) however, showed a higher predictive capability than rainfall for all of the four faecal indicator bacteria.

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 Wallis Lake.

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9. Appendices

A1. Methods

A1.1 Sampling locations in Wallis Lake

Data used in this report originates from two locations within Wallis Lake over the period March 2018 to March 2021. High-resolution temperature, salinity and depth data were obtained from a sensor deployed in Wallamba River, Long Island (-32.18S, 152.48E) (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 (Fig. A1).

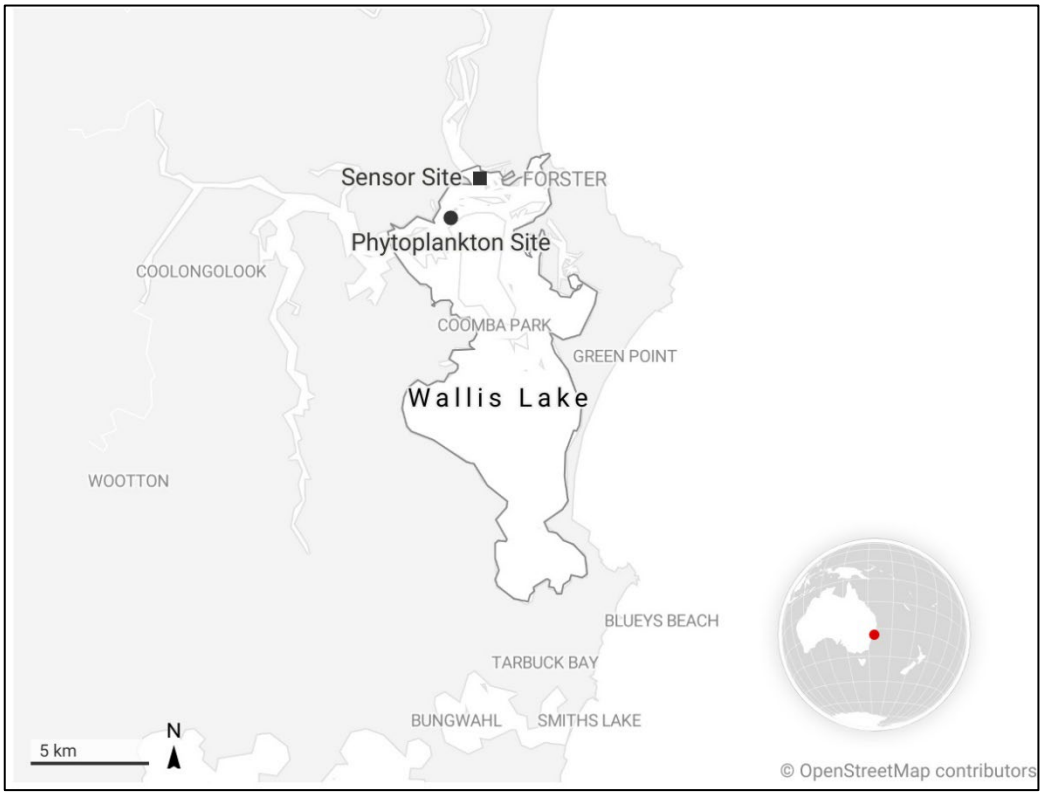

Figure A1: Map of Wallis Lake highlighting the sensor (black square) in Long Island harvest area and phytoplankton sampling location (black circle).

A1.2 High-resolution sensor data

High-resolution temperature (°C), salinity and water depth (m) data were collected from 13 March 2018 – 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 stations at Forster (BOM 60013, 32.21S, 152.53E).

Figure A2 Seabird MicroCAT high accuracy conductivity, temperature and depth (CTD) field sensor deployed in Wallis Lake. Image: Brian Hughes

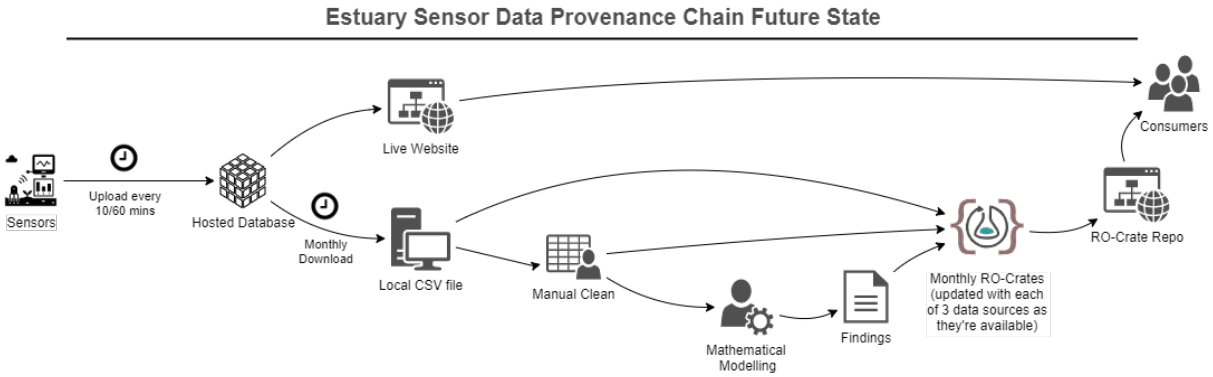

Figure A3. Wallis Lake data provenance chain from source of data (sensors), via quality assurance processes, data analyses, to consumers.

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 Wallis Lake annual review is 1 May. As part of the most recent (2022) annual review for Long Island harvest area, all salinity data from the monitoring sensors during the 2018, 2019, 2020, 2021 and 2022 annual review periods were assessed in relation to microbiological samples collected by the local shellfish program during the same period. There was a gap in data collection between 1 and 16 April 2021 due to a change in sensor provider. Data after 1 May 2021 (2022 annual review period) were only available up to 20 June 2021, and were not included in the analyses, as salinity reported by the sensor appeared to be drifting >40 ‰, which was not representative of this location and indicated a possible issue with the sensor.

A1.4 Biological sampling and eDNA extraction

Estuarine water samples were collected weekly by oyster farmers working at Verdich and Sons Pty Ltd 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 two-day period commencing on the first day of rainfall and processed as described above. Daily rainfall measurements were taken from the closest available weather stations at Forster (BOM Station No. 060013, 13 Mar 2018 - 24 Mar 2020) and Tuncurry (MHL Station No. 209401D, 25 Mar 2020 to 31 Mar 2021), which are approximately ~4 km downstream and 2 km upstream of sensor site respectively.

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 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}.

A1.8 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, February 2020 and finally in June 2021. 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.

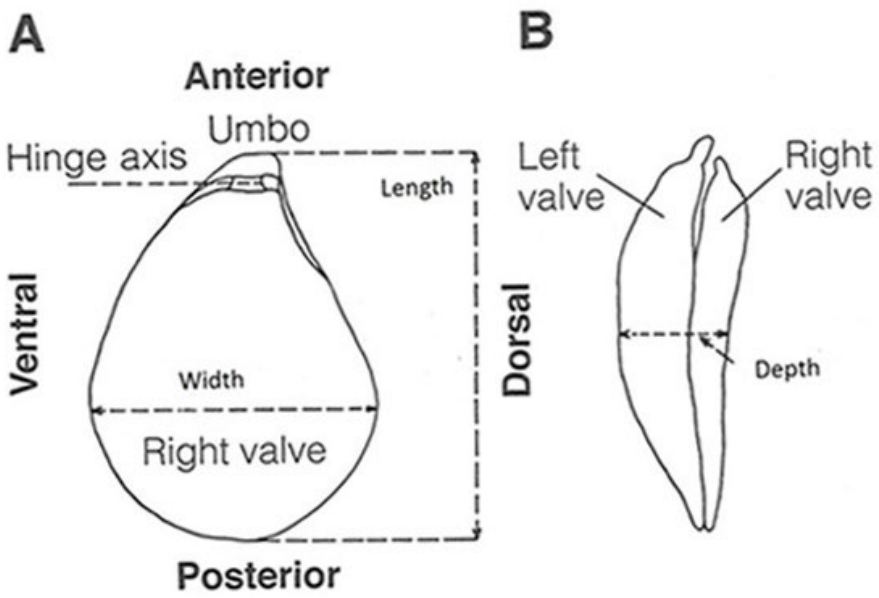

Figure A4. Oyster shell dimensions (Carriker 1996)

A1.8.3 Oyster Mortality

Oyster mortality was calculated by counting the number of empty oyster shells in each replicate approximately each month from August 2018 to February 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 Forster (BOM Station No. 060013), 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 Wallis Lake, 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 2A. Summary Statistics for Bacterial Modelling – Sensor site, Wallis Lake

Variable	Mean	Standard Error	Median	Standard Deviation	Minimum	Maximum	Count	Missing
average_cfu	3.86	0.82	1.41	7.18	0.00	39.19	76	0
bird	509.77	204.16	0.00	1779.87	0.00	11542.83	76	0
cow	12.34	4.69	0.00	40.92	0.00	228.89	76	0
depth24	0.75	0.02	0.72	0.15	0.45	1.28	76	0
depth48	0.75	0.02	0.72	0.14	0.52	1.16	76	1
depth72	0.75	0.01	0.72	0.13	0.55	1.10	76	2
ecoli	595.87	96.62	342.54	842.35	0.00	4508.50	76	0
human	10.35	10.35	0.00	90.26	0.00	786.84	76	0
logPhytoplankton	13.29	0.10	13.16	0.86	11.51	15.62	76	0
Phytoplankton	900578.95	131502.54	520000.00	1146412.55	100000.00	6100000.00	76	0
rainfall24	5.61	1.35	0.10	11.80	0.00	59.00	76	7
rainfall48	5.75	0.96	1.80	8.34	0.00	31.60	76	9
rainfall72	5.92	0.73	4.73	6.38	0.00	21.40	76	11
salinity24	32.90	0.69	34.56	6.05	1.39	36.98	76	0
salinity48	32.90	0.66	34.43	5.73	2.99	36.48	76	1
salinity72	32.90	0.62	34.54	5.37	6.17	36.29	76	2
temp24	21.27	0.41	21.62	3.55	15.01	28.27	76	0
temp48	21.30	0.39	21.83	3.43	15.34	27.66	76	1
temp72	21.33	0.38	22.10	3.35	15.70	27.13	76	2

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	*Harmful Algae* 116 (2022)102253	Published
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, Penelope Ajani, Shauna Murray & Steve Brett	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.
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