TRANSFORMING AUSTRALIAN SHELLFISH PRODUCTION

Pambula Lake Harvest Area, Pambula River

Report on Stage 1, September 2016-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 Pambula River, 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 Pambula River harvest area, recording high-resolution temperature, salinity and depth data. Oyster farmers collected weekly biological samples (690 environmental DNA samples and 306 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

9

Available data indicated that nine harvest area closures could have potentially been avoided between September 2016 and September 2019

Between September 2019 and March 2022 an estimated additional 55 days of harvest opportunities have been achieved using sensor salinity data for harvest area management

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 increased with rainfall, and to a lesser extent cow and human bacteria, while bird bacteria fluctuated

0

Oyster mortality during the study did not exceed background farming mortality (estimated at 10% per annum) in Pambula River.

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. 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 ~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 project provided the basis for a change to the management plans for the Pambula River 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 River 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 River) 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 August 2022, seventeen salinity-only management plans had been offered for harvest areas in participating NSW estuaries, with six being taken up and the remaining eleven under consideration.

1.2 Pambula River

Pambula River (-36.93° S, 149.87°E) an open, semi-mature wave dominated barrier estuary, with a catchment area of ~296 km^2, an area of ~4.72 km^2, and a flushing rate of ~13.6 days (Roy et al. 2001, Roper et al. 2011) (Fig. 1). The river catchment extends across forested (82%) and rural areas and flows east for 15 km before reaching the mouth at Pambula Beach. The estuary itself supports a diverse range of recreational and commercial uses, yet retains good to excellent estuary health and water quality (Bega Shire Valley Council 2015).

1.3 Oyster Production in Pambula River

Pambula River is a significant producer of Sydney Rock Oysters in Australia, with production in 2018/19 of 273K dozens valued at $2.5 Mil (Gippel, 2021). Pambula Lake also has significant Aboriginal oyster middens highlighting the importance of this area to Aboriginal peoples and their connection to aquaculture. Threats include the modifying actions of direct human use: poor soil, weed and stock management; storm water pollution; and other broad scale drivers of change such as climate change (Bega Shire Valley Council 2015).

FINDINGS

2. Findings

2.1. The data assessment from this report supports implementing a harvest area management plan based on sensor salinity data for Pambula Lake harvest area, which was agreed by the local shellfish industry during September 2019. Data between September 2016 and 2019 indicated that nine harvest area closures could have potentially been avoided. Between September 2019 and March 2022, an estimated additional 55 days of harvest opportunities have been achieved using of sensor salinity data for harvest area management.

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 Pambula River 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 three out of the four faecal indicator bacteria.

2.4. While the abundance of cow, bird and human bacteria were very low across the sampling period, the maximum predictive capability for each bacterial group were 24.2% for *E. coli*, 39.9% for cow, 37.4% for bird, and 38.8% for human at the sensor site.

2.5 Where the models were predictive, they often suggested bacterial abundance increased with decreasing salinity (increasing rainfall), which is not unexpected for this estuary.

2.6. The greatest oyster growth in terms of whole oyster weight occurred during the last 10 months of the experiment (August 2019 to June 2020), however none of the environmental variables measured/modelled were predictive of oyster growth.

2.7. No oyster mortality events that exceeded background farming Sydney Rock Oyster mortality (approximately 10% per annum) occurred in the Pambula River 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 Pambula River oyster farmers for collecting weekly samples and monitoring oyster growth. Specifically, we thank Rod McIntyre, Stacey Phelan, Peter Ferguson and Anna Simonds for your ongoing involvement and assistance. We also thank Sue and Greg from Broadwater Oysters for storing the sample collections for the duration of the project. 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 Pambula 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 Dr Torri Callan (UTS) for statistical analyses and Chris Komorek (Food Agility CRC) for report layout.

FEEDBACK

4. Feedback

In May 2018, the Oyster Transformation Team held information workshops to allow farmers to have their say in the project. The workshops were held in Pambula (Pambula Fishing Club) and Bateman's Bay (Catalina Country Club).

Farmers were asked to rate the following factors in order of importance and benefit to their business operations (Fig. 4.1). In order of importance (highest to lowest) was the potential to predict algal blooms, longer harvest opening times, reduced stock mortality/disease, forecasting of harvest area closures, and access to real time tidal and monitoring data.

Group discussions followed, whereby additional issues that farmers raised were: the suitability of the sensor location and BOM rainfall gauge; and the breakdown of bacterial data into human and animal sources.

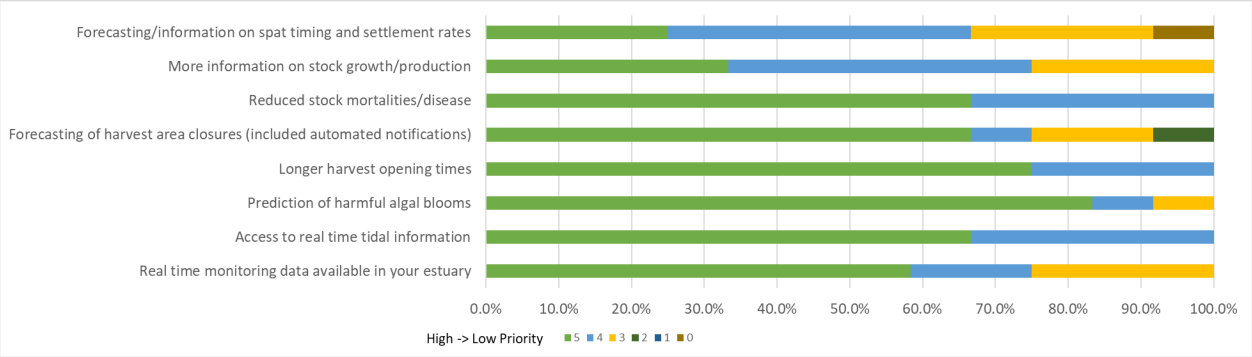


Figure 4.1. The importance of factors as rated by farmers in relation to their business operations. Green is most important and brown is least important.

RESULTS

5. Results

5.1 High resolution temperature and salinity data

High-resolution real time data summaries for Pambula River (upstream sensor) for the period 19 Sept 2016 to 12 Feb 2018 are shown in Figs. 5.1A-C. Depth recordings ranged from 0.3 m (14 Nov 2017) to 2.2 m (24 Jun 2017). The lowest and highest daily average salinity recordings were 25.5 ppt (24 May 2017) and 36.1 ppt (3 Oct 2017) respectively, while the lowest and highest daily average temperature recordings were 11.6℃ (21 Jul 2017) and 27.1 ℃ (28 Jan 2018) respectively. This sensor was decommissioned and moved to Wonboyn River on 14 Feb 2018.

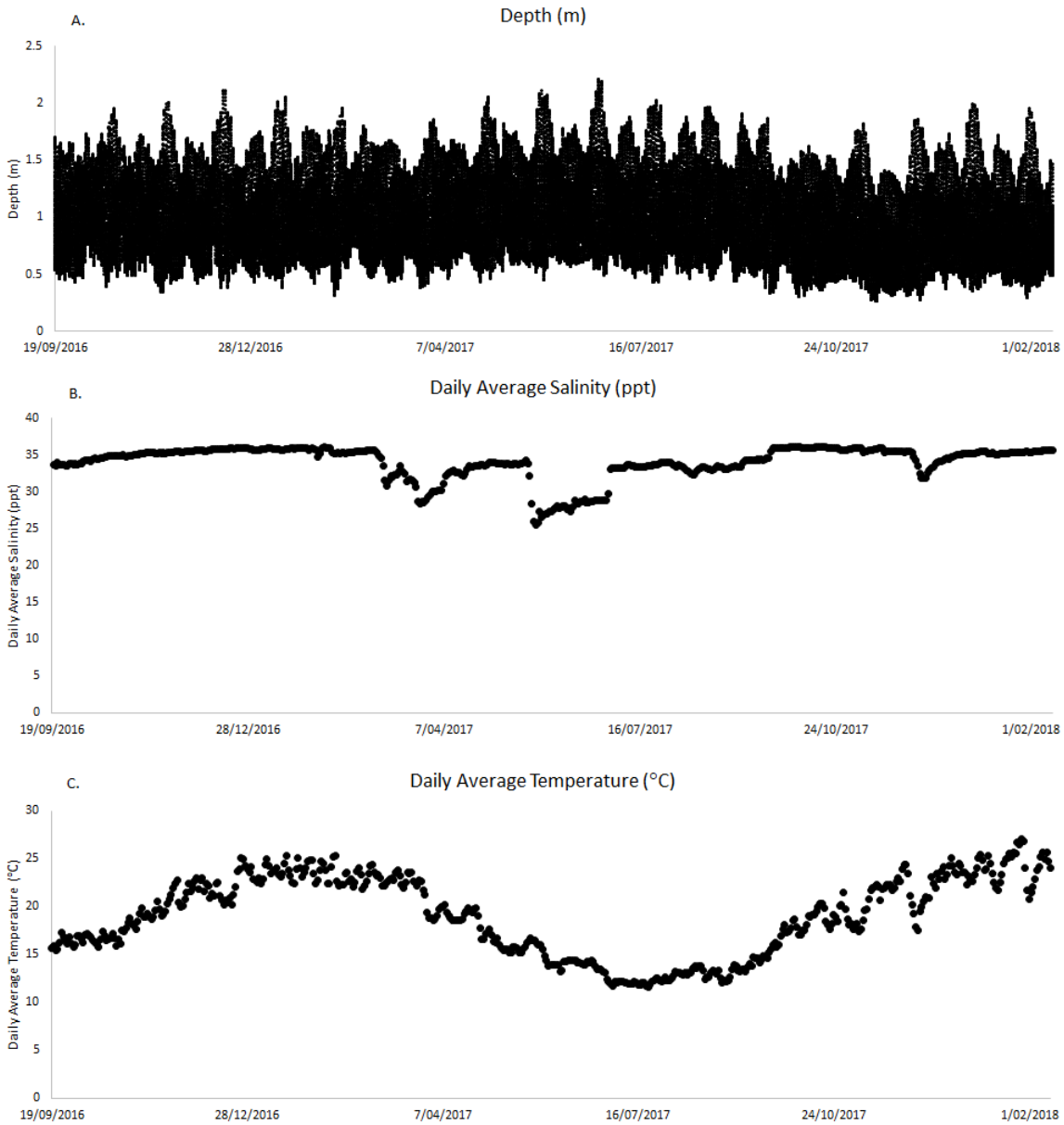


Figure 5.1A-C. Real time sensor data from Pambula River (upstream sensor) 19 Sept 2016 to 12 Feb 2018 A. Depth (m); B. Daily average salinity (ppt); and C. Daily average temperature (°C).

High-resolution real time data summaries for Pambula River (downstream sensor) for the period 20 Sept 2016 to 31 March 2021 are shown in Figs. 5.1D-F. Salinity data between 11 and 14 Jan 2020 appeared to be erroneous and data was patchy during March 2021. This may have been due to telecon issues or flood impacts/debris.

Depth recordings ranged from 0.1 m (28 Feb 2018) to 2.4 m (23 Mar 2021). The lowest and highest daily average salinity recordings were 0.6 ppt (24 Mar 2021) and 36.1 ppt (24 Jan 2017) respectively, while the lowest and highest daily average temperature recordings were 11.2℃ (21 Jul 2018) and 25.8 ℃ (28 Jan 2018) respectively.

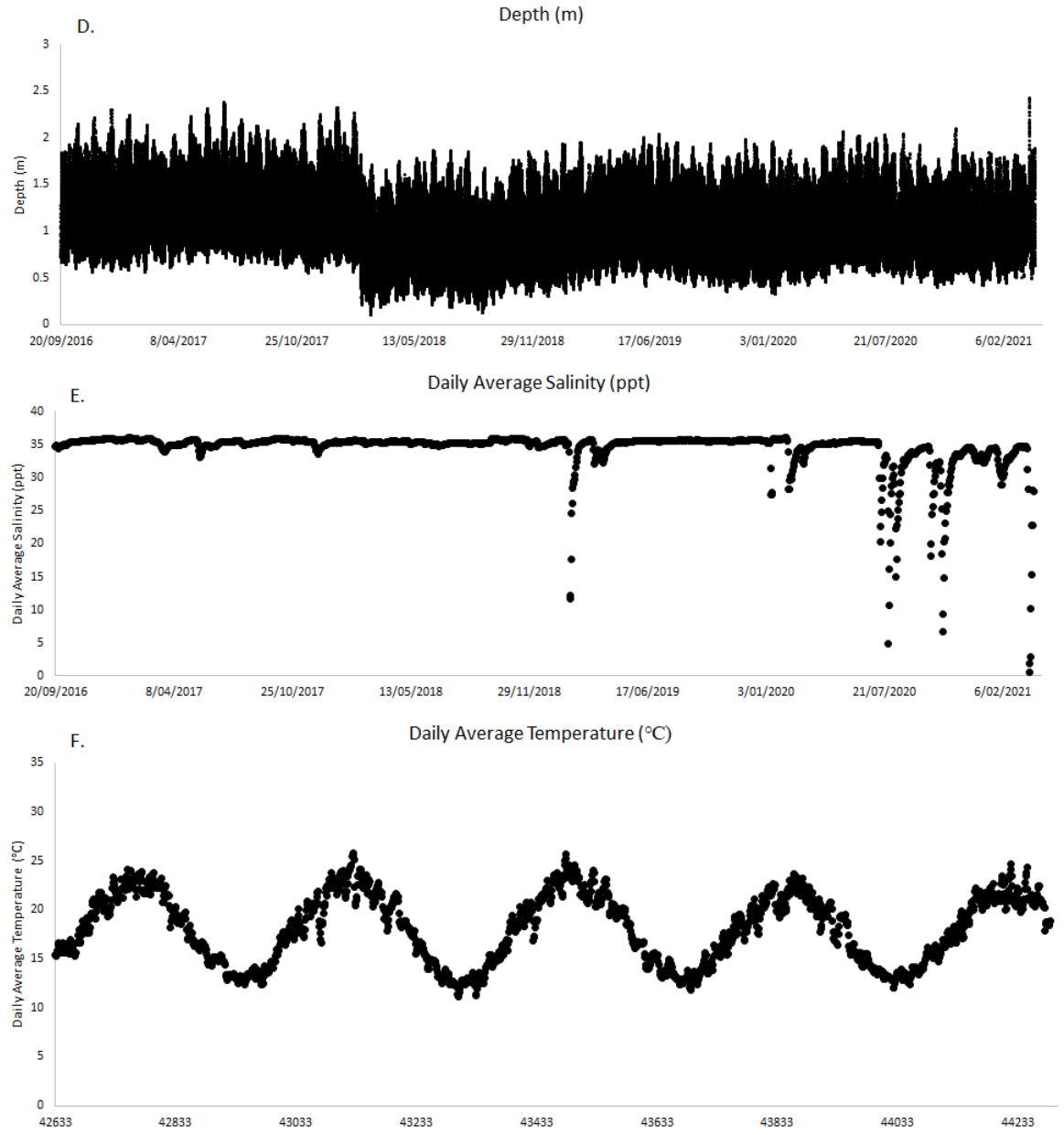


Figure 5.1D-F. Real time sensor data from Pambula River (downstream sensor) 20 Sept 2016 to 31 March 2021 D. Depth (m); E. Daily average salinity (ppt); and F. Daily average temperature (°C).

The maximum daily rainfall at the PRSP gauge occurred on 24 March 2021 and was reported as 148 mm (Fig. 5.2).

Nine rainfall events were sampled across the study period. These occurred on 15-17 Dec 2018, 31 Jan-2 Feb 2019, 6-8 Feb 2019, 17-19 Mar 2019, 21-23 Mar 2019, 8-11 Feb 2020, 5-7 Mar 2020, 13-15 Jul 2020, and 27-29 Jul 2020.

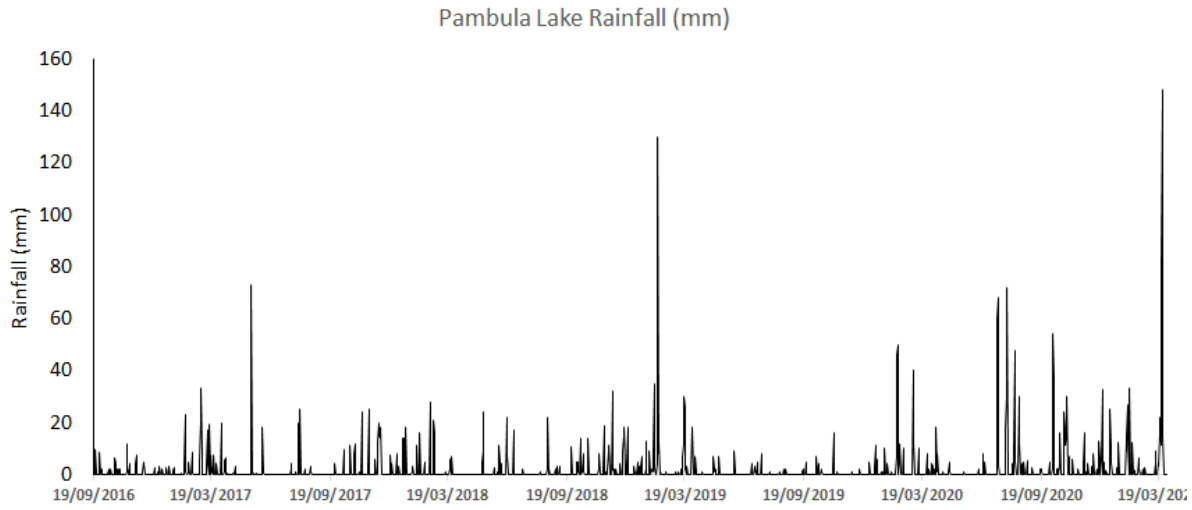


Figure 5.2. Daily rainfall (mm) from PLSP rainfall gauge (~-36°56'29.78"S and 149°48'37.73"E) from Sept 2016 to March 2021.

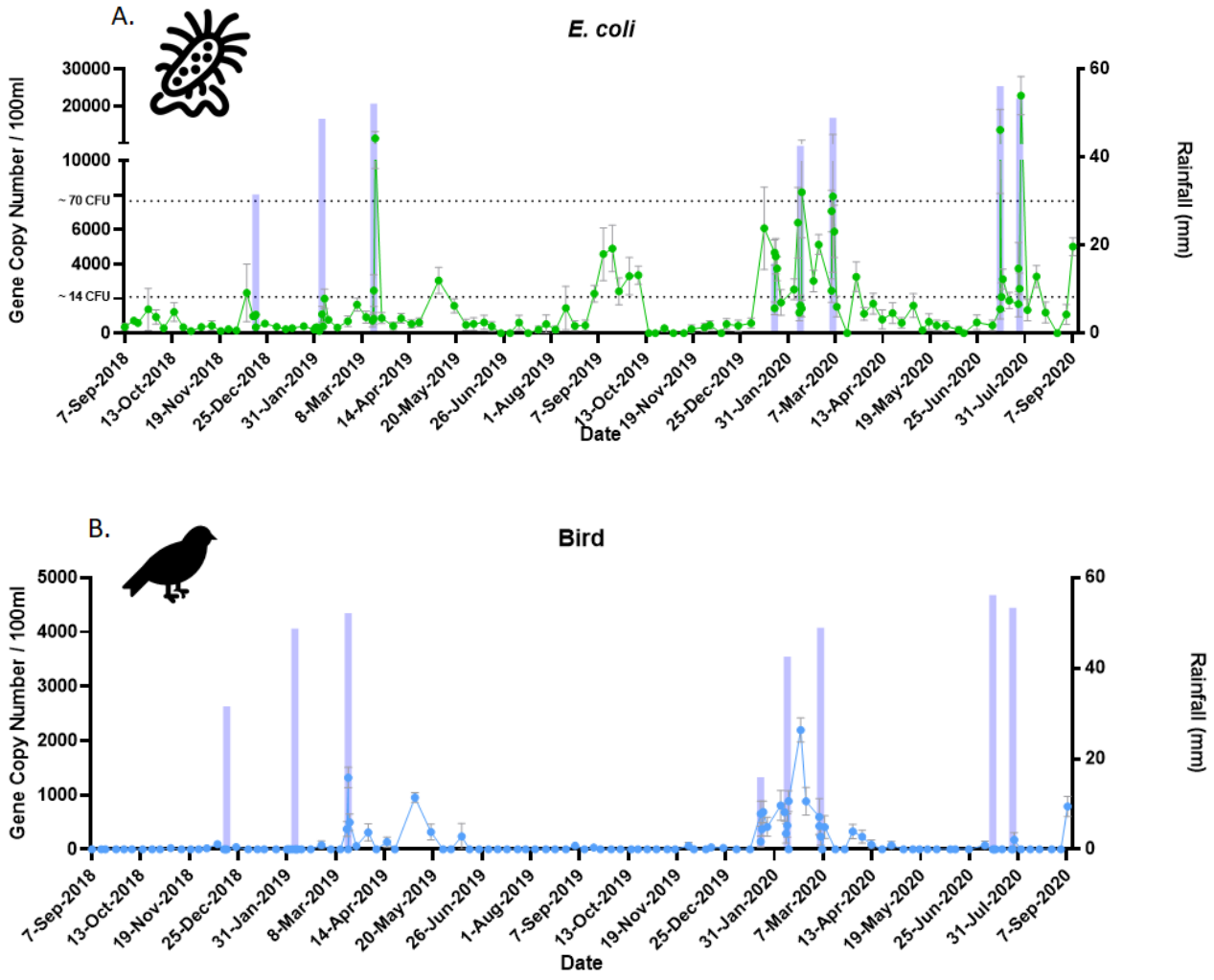
5.2 Management Plan

Data analysed during the 2019 annual review of Pambula Lake harvest area (see Fig. A1) indicated that there could have been less harvest area closures since the sensor was installed, if closures were based on salinity sensor data. There were eleven harvest area rainfall closures in Pambula Lake harvest area between September 2016 and March 2019. Based on a management plan sensor salinity closure limit of 29 ‰, harvest area closures were reviewed focusing on available salinity sensor data and shellfish program microbiological results since September 2016. Nine harvest area closures, of 50 days duration, could have potentially been avoided during this period. During the 2020 annual review period (9 September 2019), a salinity based management plan was implemented for Pambula Lake harvest area. There were no harvest area closures between April and September 2019. It is estimated that up to 55 days (2020 review: 1.5 days, 2021 review 26 days, 2022 review 27.25 days) of additional harvest have been achieved since the commencement of this plan. In addition, a benefit of having access to high frequency, real time data is that management plans can be updated to reflect the extra information available. Following the 2021 and 2022 annual reviews, the available data supported a change to the management plan opening limit (target >28 ‰) and closure limit (29 to 28 ‰), respectively. Both changes are more advantageous to industry. During the 2022 annual review assessment, it was noted that more recent salinity data was variable due to more frequent rainfall events. 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 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.

5.3 Bacterial source tracking

A total of 690 water samples and 306 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 Pambula River (Fig. A1).

For Pambula River the maximum *E. coli* reached 22,843 gene copies 100 mL^{-1} on 29 Jul 2020, 2,196 copies 100 mL^{-1} for *Helicobacter* (bird) on 20 Feb 2020, 36,780 gene copies 100 mL^{-1} for bovine faecal pollution (cow) on again on 29 Jul 2020, and finally, 990 copies 100 mL^{-1} for human faecal pollution on 7 Jul 2020 (Fig. 5.3 A-D).



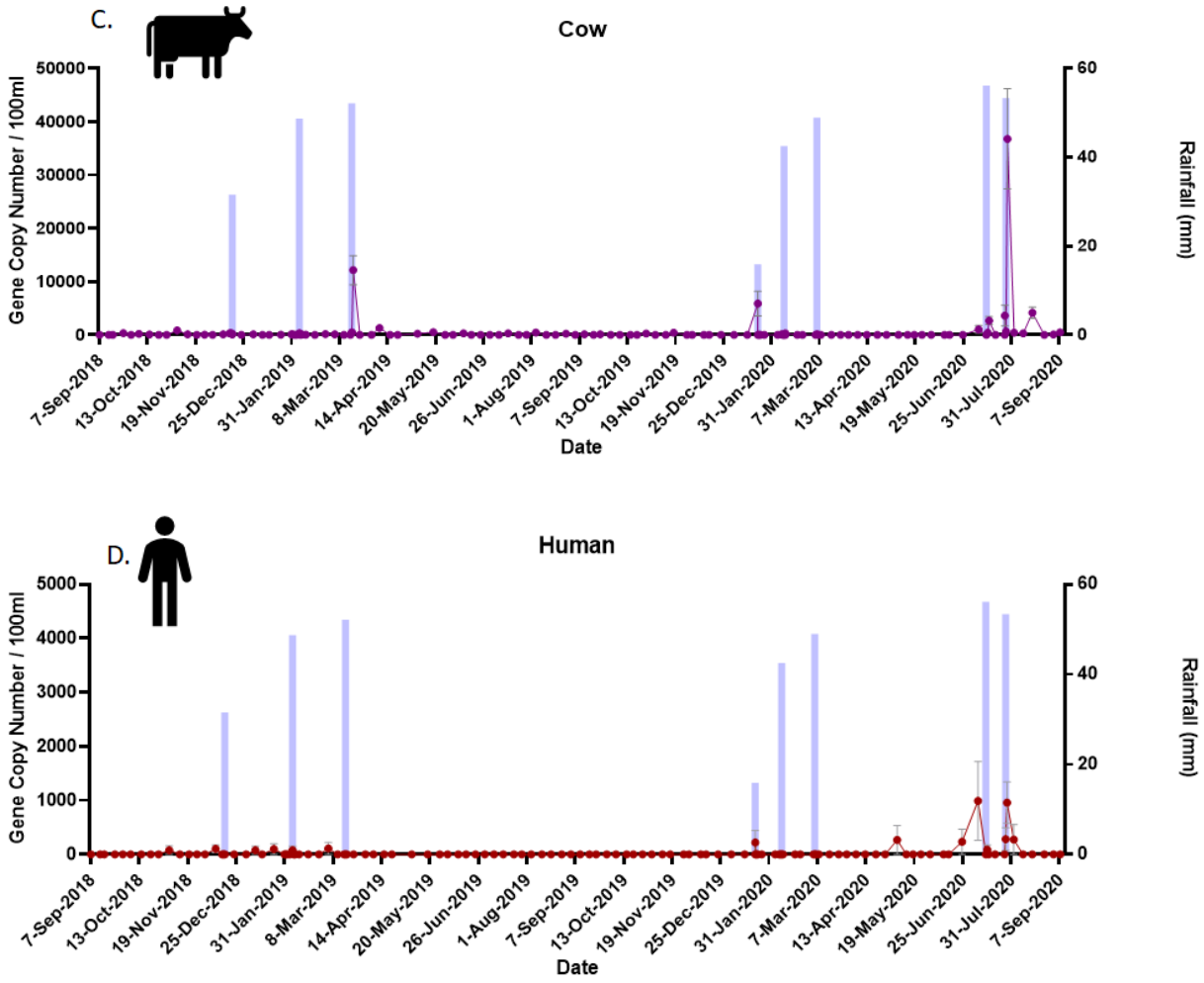


Figure 5.3 A-D. Weekly *E. coli* data from the sensor location, Pambula River, using A. *E. coli* assay; B. Bird assay; C. Cow assay; and D. Human assay. Purple bars represent rainfall events that were sampled. Dotted lines in Fig. A at 14 and 70 cfu/100 mL are the operational limits for direct or restricted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

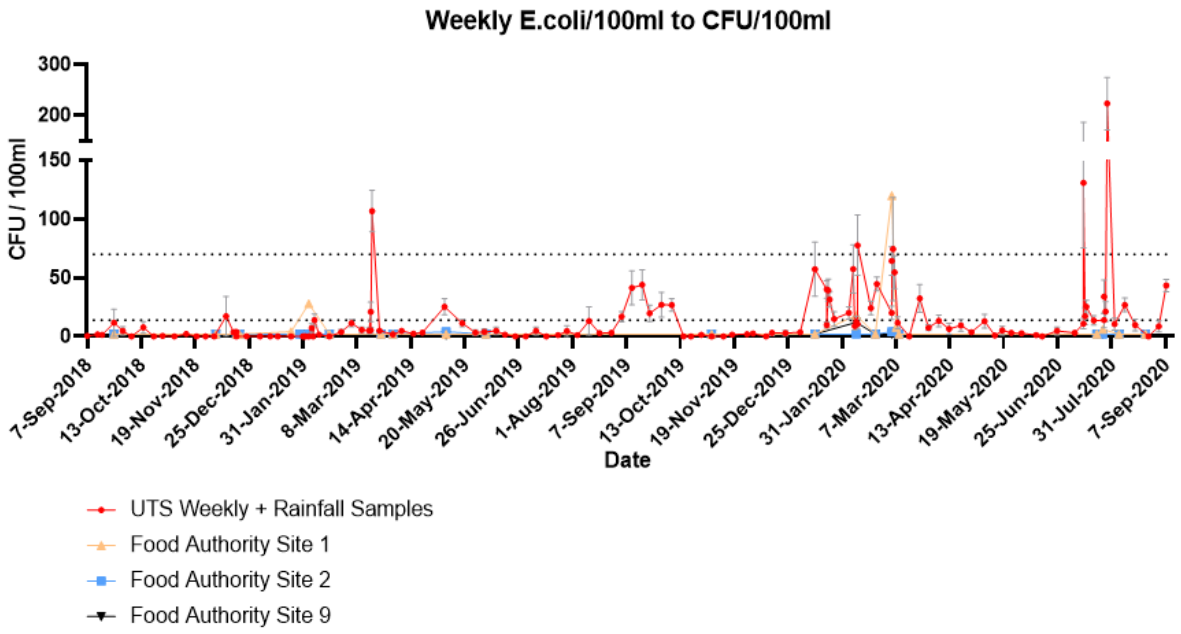


Figure 5.4 Weekly faecal coliform counts (cfu/100 mL) from water samples collected by DPI Food Authority at two sites in Pambula River sensor site compared to Oyster Transformation Project weekly sampling results (including rainfall sampling). Dotted lines at 14 and 70 cfu/100 mL are the operational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

Elevated faecal coliform counts reported by the DPI Food Authority often corresponded to elevated levels in samples collected by the CRC, however at other times the CRC samples often revealed significantly higher counts compared to those collected by the Food Authority at the same time suggesting it may be a more sensitive assay that the traditional plate count method (Fig. 5.4A-B).

Nine rainfall events were also sampled across the study period (see purple bars in Fig 5.3 A-H). These occurred on 15-17 Dec 2018, 31 Jan-2 Feb 2019, 6-8 Feb 2019, 17-19 Mar 2019, 21-23 Mar 2019, 8-11 Feb 2020, 5-7 Mar 2020, 13-15 Jul 2020, and 27-29 Jul 2020 (Fig. 5.5 A-I). *E. coli* was highly variable across rainfall sampling campaigns. In some instances, counts were highest on day 1, sometimes highest on day 3, and sometime remaining constant across all three days of sampling. On the one occasion when 4 consecutive rainfall days were sampled, *E. coli* declined as time progressed (Fig. 5.5F). It is unclear without further sample collection, how quickly these levels would have dissipated. Bovine contamination generally remained low, however during two rainfall events (17-19 Mar 2019 and 27-29 Jul 2020), it peaked on day 3. Again, it is unclear how quickly these levels would have dissipated without further sampling. Bird and human bacteria remained low or below detection limits during all events (Fig. 5.5A-I).

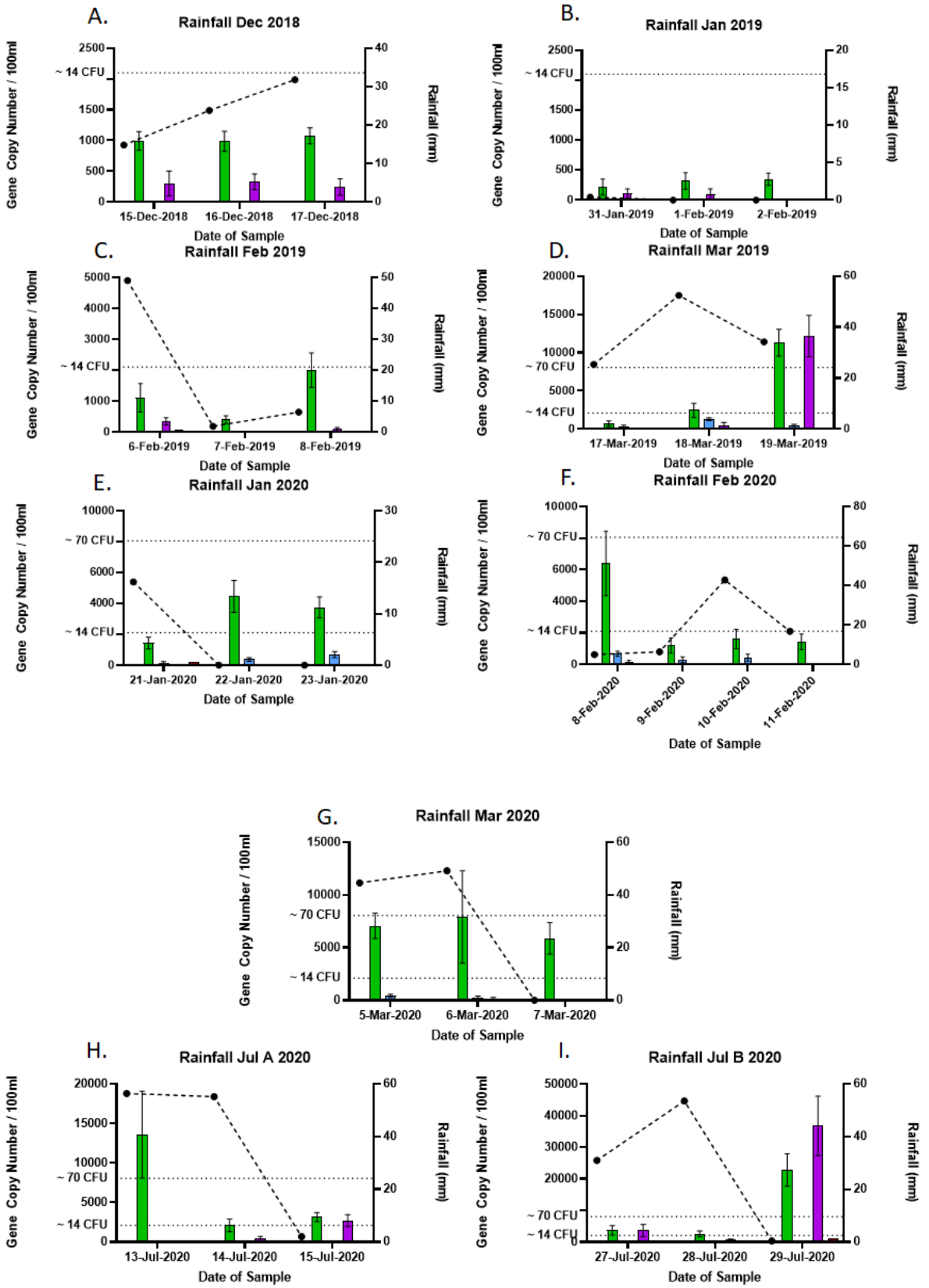


Figure 5.5 A-I. Sensor site (Pambula River) rainfall events sampled for *E. coli* assays. Green bar = 16S *E. coli*; blue bar = bird assay; purple bar – cow assay; red bar = human assay. Dotted line is rainfall (mm) obtained from the closest rainfall station (PLSP = Lot 5 Robinson Road Lochiel 2549, NSW). 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 (Sept 2016 to March 2021) occurred on 17 July 2017 (Fig. 5.6). Total cell concentrations reached 5.7E +07 cells L^{-1} and sample was dominated by a massive bloom of small unicelluar green algae (cf. *Monoraphidum*) and a mix of planktonic diatoms (*Chaetoceros* spp.), benthic diatoms (Cylindrotheca sp.) and small flagellates (cryptomonads, dinoflagellates). This bloom did not coincide with any significant rainfall event.

Other potentially harmful bloom events across the sampling period included blooms of the diatom *Pseudo-nitzschia fraudulenta/australis* which reached a maximum cell concentration of 7.5E +04 cells L^{-1} on 27 Mar 2017. The toxic dinoflagellate *Alexandrium pacificum* reached elevated cell densities from 19 Oct through to 15 Nov 2016 with a maximum count of 1,300 cells L^{-1}. The NSW Food Authority's Phytoplankton Action Limits to trigger biotoxin testing are 50,000 cells L^{-1} for *P. australis & multiseries* and 200 cells L^{-1} for *Alexandrium pacificum* (NSWFA 2015). No biotoxins were detected in association with shellfish samples collected during the *Pseudo-nitzschia fraudulenta/australis* bloom. A positive detection of paralytic shellfish toxins (PSTs) of 0.14 saxitoxin equivalent (STX eq.) mg/kg total PST was reported in a shellfish sample collected 15 Nov 2016 coinciding with the report of maximum *A. pacificum*. Shellfish samples collected 19 Oct, 8 Nov and 21 Nov 2016 were negative for PSTs.

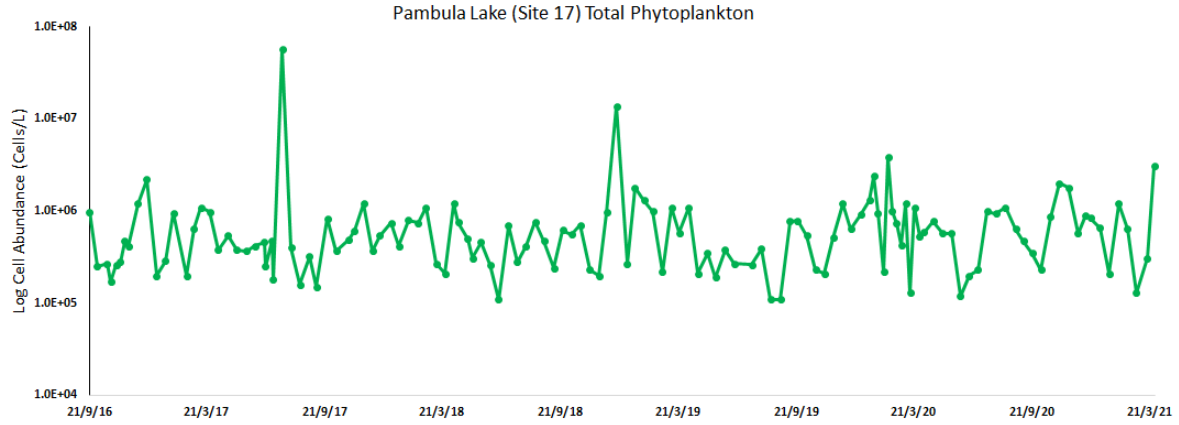


Figure 5.6 Log abundance of total phytoplankton sampled approximately fortnightly from 21 Sept 2016 to 29 March 2021.

5.6 Oyster Growth and Mortality

5.6.1 Oyster Growth

Average oyster whole weight increased by more than 36 g from deployment in August 2018 to June 2020 (Fig. 5.7 A). Oyster whole weight increases were greatest from August 2019 to June 2020 when oysters increased their weight by 24.6 g in 10 months. Oyster whole weight was 59.4 ± 7.0 g at the end of the experiment (June 2020). Oysters deployed in Pambula River reached the large size grade (> 70 mm total length or > 50 g whole weight) in June 2020 and were 42 mo on this date.

Oyster shell length was 54 ± 2 mm at the start of the experiment and increased to 74 ± 4 mm in June 2020 (Fig. 5.7 B). The greatest increase in shell length in Pambula River was recorded from August to November 2018. The increase in shell size through this period was 8 mm. Shell lengths were measured more frequently than whole weight and fluctuated throughout the experiment. Periods of shell length decreases were recorded on two occasions and were between May and July 2019 and October and December 2019.

5.6.3 Mortality

From August 2018 to February 2020, cumulative oyster mortality was 16% in Pambula River. Low levels of mortality were recorded throughout the experiment (Fig 5.7 C-D). The month that had the highest level of mortality recorded was November 2018, however, mortality on this date was less than 5%. There were no losses of oysters recorded in the period from February 2019 to July 2019. Oyster mortality over the study period in Pambula River was less than the background Sydney Rock Oyster farming mortality level which is estimated to be approximately 10% per annum. Oysters from this site remain frozen for future analyses.

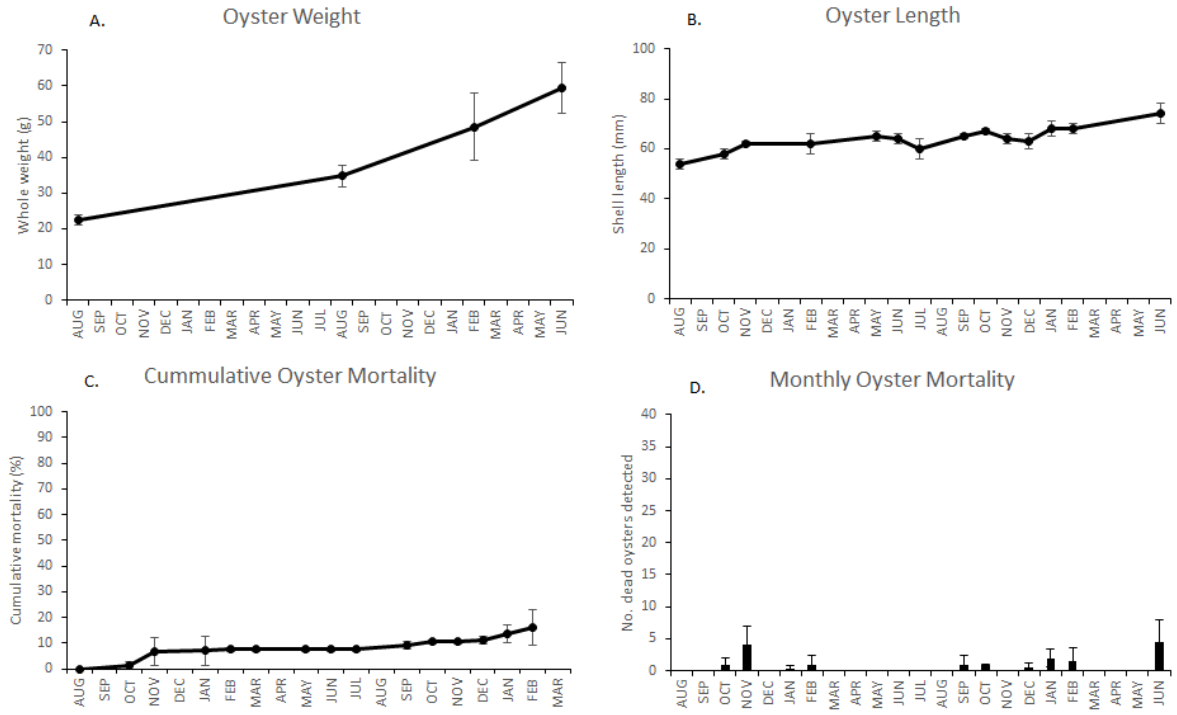


Figure 5.7 A-D. Oysters deployed at the sensor site, Pambula River. A. whole weight; B. shell height; C. cumulative mortality, and D. monthly mortality.

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 2A-B. Correlation coefficients were calculated among every pair of environmental variables and suggested very few strong positive relationships ($r > 0.7$) overall. The only notable positive correlation across the entire dataset

was *E. coli* and cow bacteria (r = 0.72). 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: 24.4% for *E. coli* (rainfall + total phytoplankton), 39.9% for cow (sensor + total phytoplankton), 37.4% for bird (sensor + with/without total phytoplankton) and 38.8% for human (sensor + with/without total phytoplankton) (Table 1).

The abundance of *E. coli* was only marginally better predicted using rainfall data compared to sensor data (24.2% compared to 20% deviance explained), and appeared to be significantly linked to an increase in rainfall over the past 72 hours and a concomitant decrease in salinity. Data indicated that a peak *E. coli* coincided with a peak surface water temperature of ~18℃ (Table 1) (Figures 5.7 A-D, 5.8 A-D). Adding phytoplankton data to the model did not change its predictive ability.

The prediction of bovine bacterial abundance was significantly improved using the sensor data (39.9%) compared to a model with rainfall data (13.8%), with total phytoplankton only marginally improving this predictive capability. Modelling showed peak contamination was linked to decreasing salinity and an increasing surface water temperature (peaking at ~24℃) (Table 1).

Faecal contamination from birds was again best explained using the sensor data (37.4% deviance explained, compared to 22.9% using rainfall data), with a peak salinity of 25 ppt and surface temperature of ~24℃. Adding phytoplankton data to the model made no change to its predictive ability (Table 1).

An increase in human bacteria abundance was again best explained by the sensor data (38.8%) compared to rainfall (1.6%), and was linked to a decreasing salinity and an increasing surface water temperature. Similarly adding phytoplankton data to the model did not improve its predictive capability (Table 1).

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. The best model explained a very high ~89.9% of the deviance (no. of observations = 98), with the strongest predictor variables being the maximum average salinity for the week prior to the oyster length observation (greatest growth at 35.8 ppt), and to a lesser extend the daily average salinity (increasing). The effect of salinity 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.

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

Bacteria	Variables	No. of observations	Significant Variables	Deviance Explained
E. coli	Salinity, Depth, Temp	127	Depth72**, Salinity72***, Temp72***	20%
E. coli	Salinity, Depth, Temp, logPhytoplankton	127	logPhytoplankton ***, depth**, salinity***, temp***	20%
E. coli	Rainfall72	124	Rainfall72***	22%
E. coli	Rainfall72, logPhytoplankton	124	Rainfall72***, logPhytoplankton ***	24.2%
Bird	Salinity, Depth, Temp	127	Salinity***, Depth***, Temp***	37.4%
Bird	Salinity, Depth, Temp, logPhytoplankton	127	Salinity***, Depth***, Temp***, logPhytoplankton **	37.4%
Bird	Rainfall72	124	Rainfall72***	22.9%
Bird	Rainfall72, logPhytoplankton	124	Rainfall72***, logPhytoplankton***	23.1%
Cow	Salinity, Depth, Temp	129	Salinity***, Depth***, Temp***	39.2%
Cow	Salinity, Depth, Temp, logPhytoplankton	129	Salinity***, Depth***, Temp***, logPhytoplankton***	39.9%
Cow	Rainfall24	128	Rainfall24***	0.852%
Cow	Rainfall24, logPhytoplankton	128	Rainfall24***, logPhytoplankton***	13.8%
Human	Salinity, Depth, Temp	129	Salinity***, Depth***, Temp***	38.8%
Human	Salinity, Depth, Temp, logPhytoplankton	129	Salinity***, Depth***, Temp***	38.8%
Human	Rainfall24	128	Rainfall24***	1.5%
Human	Rainfall24, logPhytoplankton	128	logPhytoplankton***	1.63%

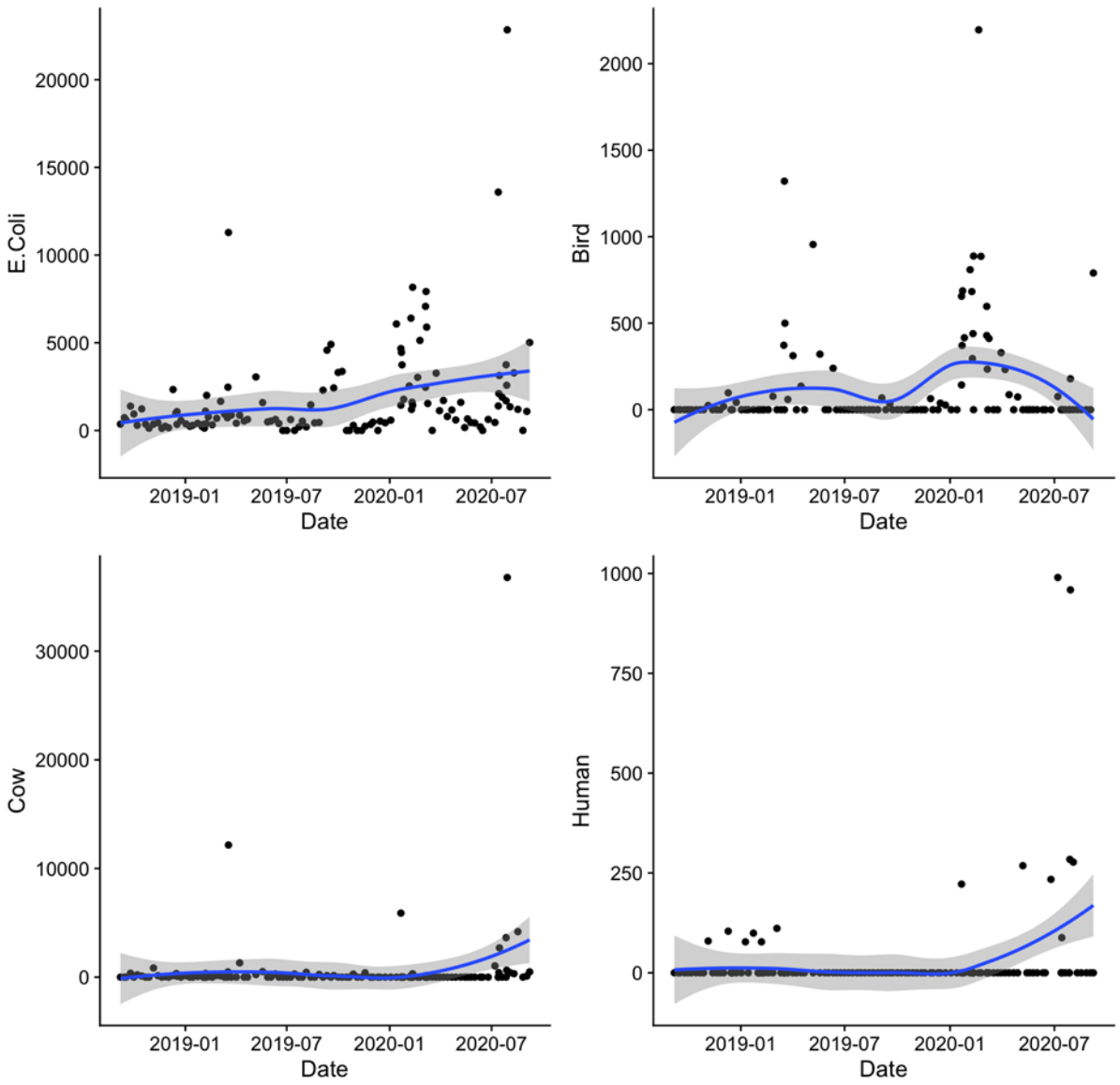


Figure 5.7 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. *E. coli*, B. Bird, C. Cow, and D. Human bacterial load as measured by weekly sampling at the sensor site, Pambula River.

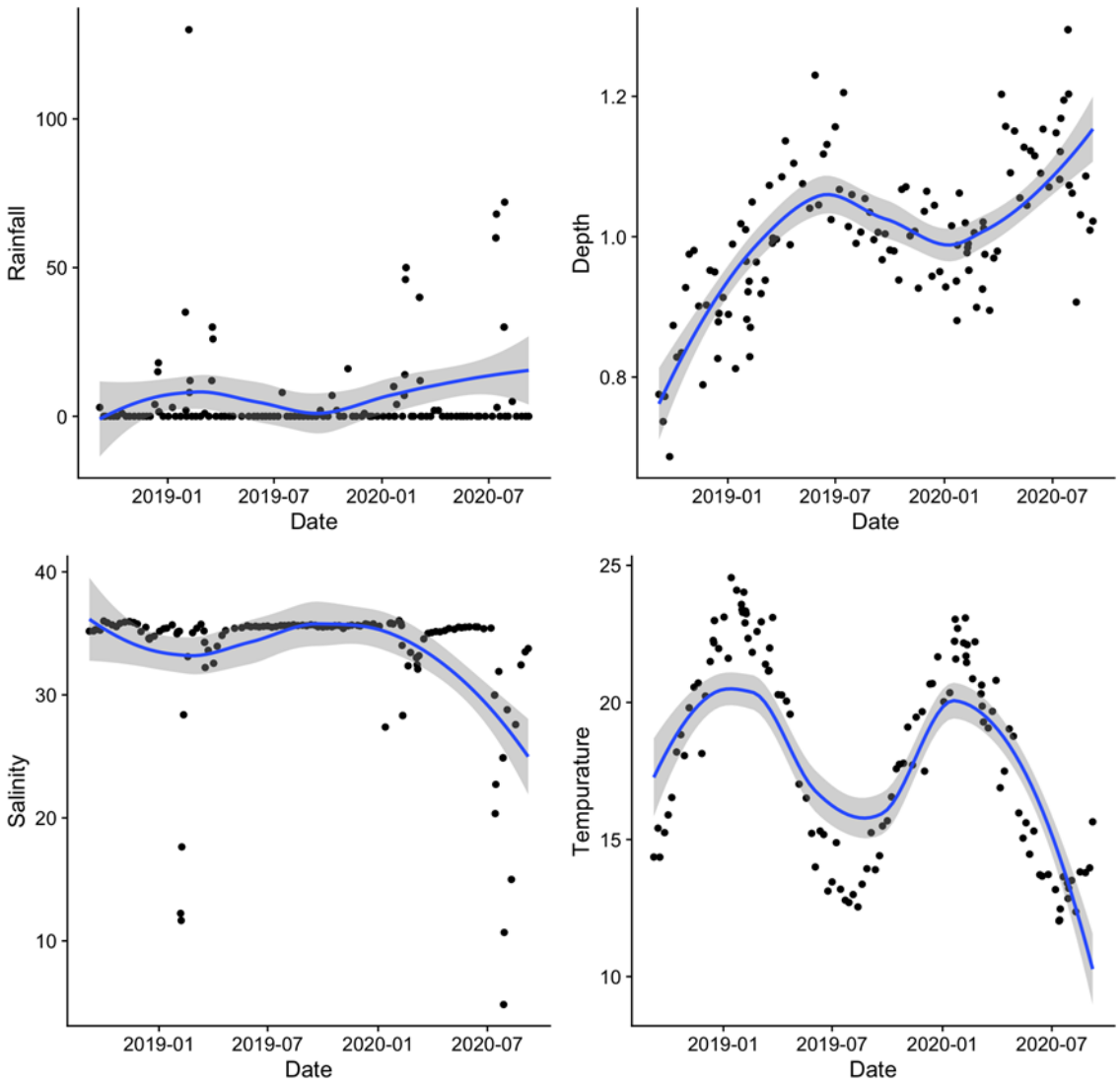


Figure 5.8 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. Rainfall, B. Depth, C. Salinity, and D. Temperature values measured in at the sensor site, Pambula River.

DISCUSSION

6. Discussion

6.1 High Resolution Sensor Data and Management Plan

Analysis of sensor data during the annual review process demonstrated that there was potential to implement a salinity sensor-based management plan for Pambula Lake harvest area. During the 2019 annual review assessment, results to date from the sensor supported a change to a salinity only based management plan closure limit for Pambula Lake harvest area. Based on the available data at that time, nine harvest area closures could have potentially been avoided between 21 September 2016 and 31 March 2019. PRSP were consulted about this option and PRSP requested the management plan change, which was implemented 9 August 2019. There were no harvest area closures between April and September 2019. Since the implementation of a salinity based management plan in Pambula Lake, there has been up to 55 additional harvest days gained (estimated between 9 August 2019 and 31 March 2022). If PRSP 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 Pambula Lake 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

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, 2021). Blooms within the Hawkesbury River estuary (330 km south of Wallis River), a 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, 2021). 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. 2021).

Another HAB species that bloomed in Pambula during this study was *Alexandrium pacificum.* 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 2012. 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).

Another HAB group to watch in NSW is the toxic dinoflagellate genus *Dinophysis*. Species belonging to this genus (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).

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 Pambula River

Molecular assays for the detection of faecal bacterial contamination in the Pambula 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 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 bird and human bacterial contamination was extremely low across the sampling period in Pambula River, modelling revealed that *E. coli*, and to a lesser extent the bovine bacterial load entering Pambula River were not unexpectedly linked to rainfall events (and decreasing salinity).

Avian faecal pollution in Pambula River, however was linked to a specific salinity and water temperature, and was observed to peak during the autumn and summer months. This peak coincided with the Australian forest mega-fires of 2019/2020 (Boer et al. 2020), whereby coastal areas may have been a relatively safer refuge during that extreme period. The molecular marker used in this study, however, 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 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 Pambula River. 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 Pambula River were greatest in the second half of the experiment from August 2019 to June 2020. However, growth, in terms of shell length, was greatest during the first 3 months of the experiment. The salinity level during the period of maximum shell growth was very stable and remained above 32 ppt. This period was also characterised by increasing water temperatures. 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). The period of maximum whole weight increase occurred over the last 10 month of the experiment which was also characterised by stable salinity levels above 32 ppt other than in February 2020 where salinity dropped rapidly to approximately 25 ppt and then quickly recovered to levels above 30 ppt (Figure 5.1E).

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. Oyster mortality measured on each sampling occasion did not exceed 5%. Cumulative mortality in February 2020 was 16% and comparable to cumulative mortality measured on the same date in Wallis Lake (14%), Manning River (15%), Port Stephens (16%), Georges River (16%) and Wapengo Lake (15%). The cumulative mortality in Pambula River over the 18 months of this experiment was similar to that measured in a previous study which ran for 18 months (8/5/2014 to 19/11/15) in Pambula River (Hall-Aspland et al. 2015) which found that cumulative mortality of Sydney Rock Oysters ranged from 10-23%. In Pambula River, there were no sampling events where mortality exceeded 5 % which corresponds with data presented in Hall-Aspland et al. (2015).

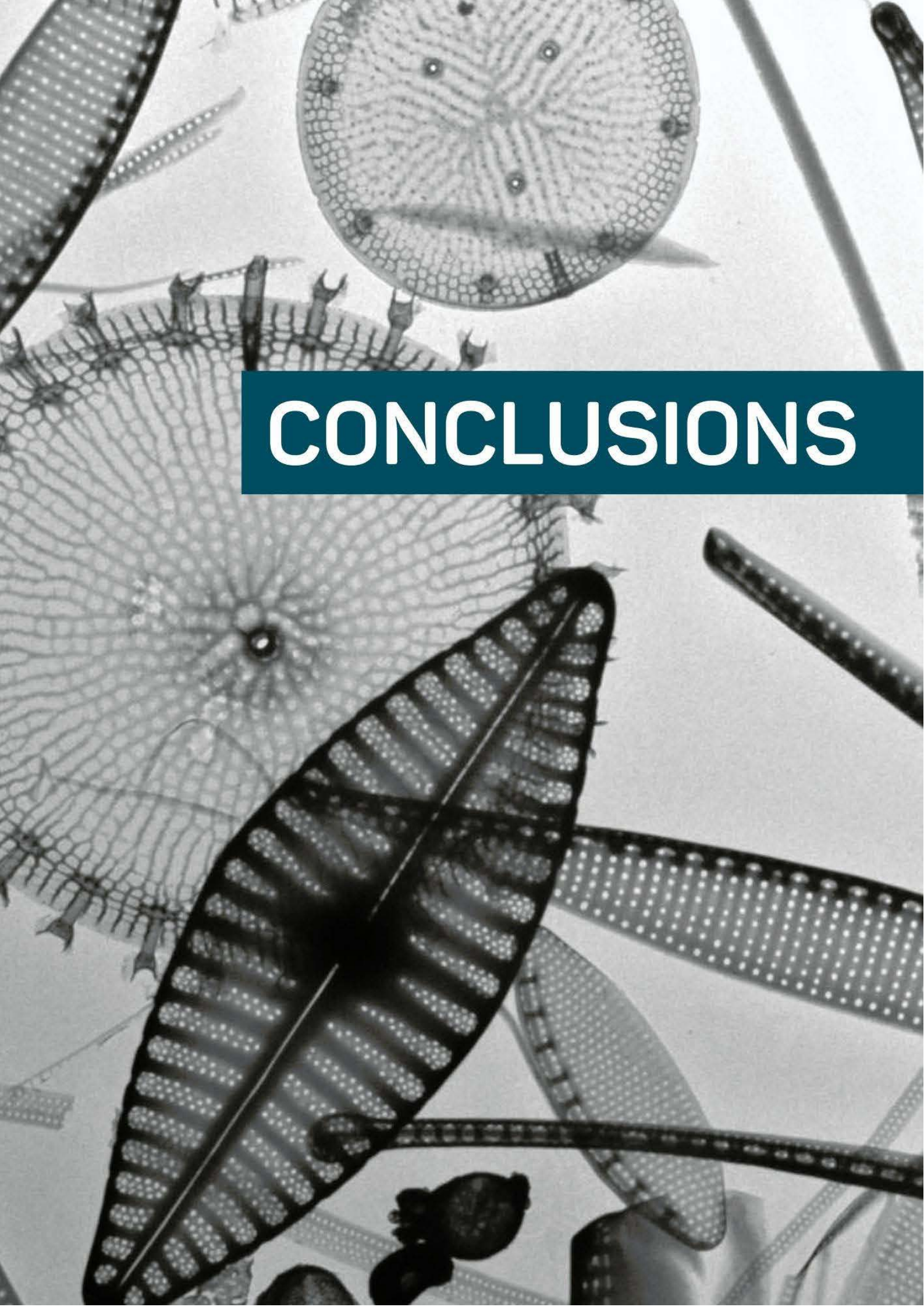
The batch of oysters used for this experiment were a random mix of families taken from the 2016-year class of the Sydney Rock Oyster Breeding program. This particular year class had 86% of the

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 Wallis Lake (50.6 g).

Pambula River is the state's 6th largest oyster producing estuary with 255,000 dozen oysters sold annually worth $2.3 million (NSW Department of Primary Industries, 2022). When oyster growth measured at the conclusion of the experiment (June 2020) was compared between the other twelve estuarine sites used for this study, Pambula River ranked 4th and 6th in terms of whole oyster weight and shell length, respectively. Most Sydney Rock Oysters in Pambula Lake are sold at the medium size grade. The medium size grade for Sydney Rock Oysters is specified as 55-70 mm total length or 30-50 g whole weight (NSW Department of Primary Industries 2022). Oysters in Pambula River reached the medium size benchmark for whole weight in August 2019 when they were 33 months in age from the date they were spawned.

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 Pambula Lake harvest area. This was agreed by the local shellfish industry, and implemented during September 2019. Available data indicated that nine harvest area closures could have potentially been avoided between September 2016 and September 2019 and 55 additional harvest days were gained between September 2019 and March 2022. As of August 2022, seventeen salinity-only management plans had been offered for harvest areas in participating NSW estuaries, with six being taken up and the remaining eleven under consideration.

Compared to the other monitoring sites in NSW, oyster growth in Pambula River ranked 4th and 6th in terms of whole oyster weight and shell length, respectively. Low levels of mortality were recorded over the period from August 2018 to February 2020 and mortality was below the level accepted as background farming mortality (approximately 10% per annum). Most oysters in Pambula River are marketed at the medium size grade and oysters were 33 mo when they reached this benchmark for whole oyster weight.

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 three out of the four faecal indicator bacteria.
Furthermore, while contamination from bird sources was observed at low levels, a distinct presence throughout the black summer bushfires 2019-2020 was observed. Finally, contamination from human sources was observed rarely, and at very low levels.

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 Pambula River.

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9. Appendices

A1. Methods

A1.1 Sampling locations in Pambula River

Data used in this report originates from locations within Pambula River over the period Sept 2016 to March 2021. High-resolution temperature, salinity and depth data were obtained from two sensor locations – one located downstream from 20 Sept 2016 to 31 March 2021 (sensor site 1) and one located upstream from 19 Sept 2016 to 12 Feb 2018 (sensor site 2) (Fig. A1). At the downstream sensor 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 third sampling location established as part of the DPI's Shellfish Quality Assurance program (Fig. A1).

Figure A1: Map of Pambula River highlighting the two sensor locations in Pambula River (black squares), and the phytoplankton sampling location (black circle).

A1.2 High-resolution sensor data

High-resolution temperature (°C), salinity and water depth (m) data were collected from the two sensor sites using Seabird SBE 37-SM/SMP/SMP-ODO MicroCAT high accuracy conductivity, temperature and depth (CTD) field sensors. These sensors were 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). These fully autonomous instruments 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 rainfall PRSP gauge at -36°56'29.78"S and 149°48'37.73"E.

Figure A2 Seabird MicroCAT high accuracy conductivity, temperature and depth (CTD) field sensor deployed in Pambula River.

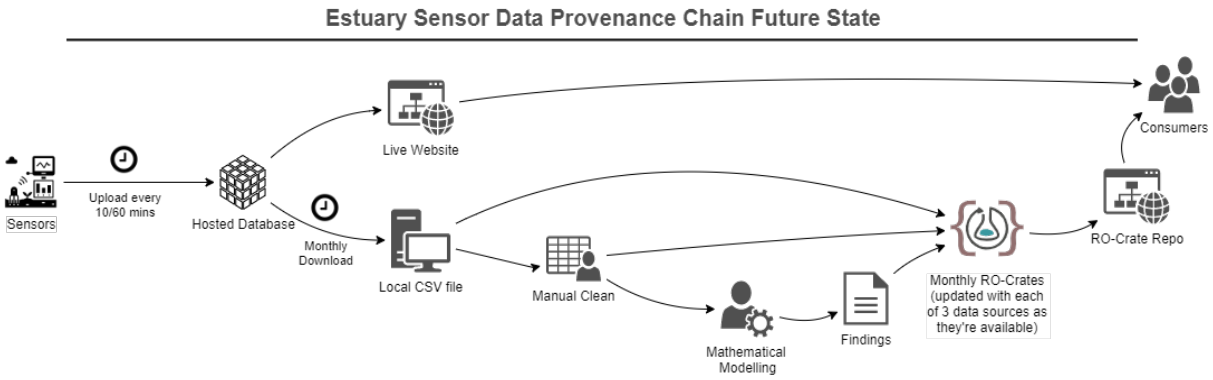

Figure A3. Pambula River data provenance chain from source of data (sensors), via quality assurance processes, data analyses, to consumers.

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 Pambula Lake annual review is 1 April. As part of the most recent (2022) annual review for Pambula Lake harvest area, all salinity data from the monitoring sensors during the 2017, 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. Salinity data collected between 11 and 14 January 2020 appeared to be erroneous. Salinity data were lower than expected (~27 ‰) during this period, with no apparent corresponding change in temperature or depth data or any substantial rainfall (12 January 2020: 22 mm/week). This suggested that there may have been a physical disruption impacting the sensor during that period (i.e., marine debris). There was a gap in data collection between 1 and 13 April 2021 due to a change in sensor provider. Low salinity data from 28 September (30 data points) were excluded from the analysis, due to an issue of instrument error/maintenance.

A1.4 Biological sampling and eDNA extraction

Estuarine water samples were collected weekly by oyster farmers working at Broadwater Oysters 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 PLSP, Lot 5 Robinson Road Lochiel 2549, NSW, which is approximately ~8 km from the of sensor site.

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.

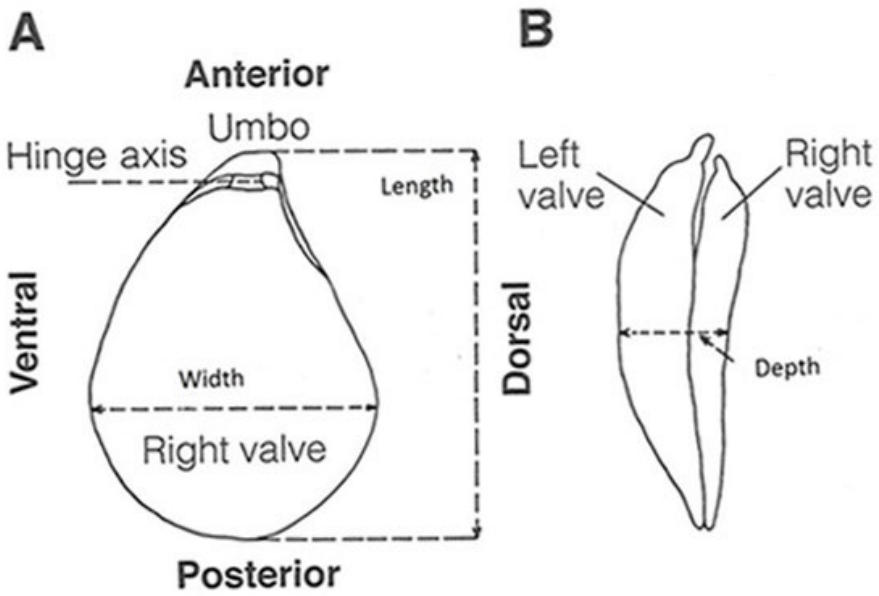

Figure A4. Oyster shell dimensions (Carriker 1996)

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 PRSP rainfall gauge (~-36°56'29.78"S and 149°48'37.73"E), 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 Pambula 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 2A. Summary Statistics for Bacterial Modelling – Sensor site, Pambula River

Variable	Mean	Standard Error	Median	Standard Deviation	Minimum	Maximum	Count	Missing
average_cfu	14.74	2.46	4.75	27.95	0.00	223.31	129	0
bird	128.82	26.78	0.00	304.22	0.00	2195.50	129	0
cow	608.47	304.65	0.00	3460.12	0.00	36780.12	129	0
depth24	1.00	0.01	1.00	0.11	0.69	1.29	129	0
depth48	1.00	0.01	1.00	0.10	0.73	1.29	129	1
depth72	1.00	0.01	1.00	0.09	0.73	1.26	129	2
ecoli	1795.77	253.83	776.16	2883.00	0.00	22843.11	129	0
human	30.01	11.48	0.00	130.39	0.00	989.93	129	0
logPhytoplankton	13.31	0.08	13.34	0.93	11.61	16.43	129	0
Phytoplankton	1059147.29	180925.26	620000.00	2054915.99	110000.00	13700000.00	129	0
rainfall24	7.69	1.64	0.00	18.64	0.00	130.00	129	1
rainfall48	7.28	1.27	0.50	14.45	0.00	69.00	129	3
rainfall72	7.20	1.11	1.00	12.64	0.00	62.67	129	5
salinity24	33.22	0.48	35.38	5.50	4.83	36.04	129	0
salinity48	33.21	0.45	35.34	5.09	7.76	35.94	129	1
salinity72	33.20	0.42	35.32	4.81	13.47	35.92	129	2
temp24	18.24	0.33	19.03	3.73	12.03	24.55	129	0
temp48	18.27	0.32	18.87	3.68	12.03	24.32	129	1
temp72	18.29	0.32	18.90	3.66	12.04	24.22	129	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