TRANSFORMING AUSTRALIAN SHELLFISH PRODUCTION

Wonboyn Lake A Harvest Area, Wonboyn River.

Report on Stage 1, February 2018-February 2021, Sydney, Australia

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

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Transforming Australian Shellfish Production: Wonboyn Lake A Harvest Area, Wonboyn River. Report on Stage 1, February 2018-February 2021

2022

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Executive Summary

This report presents results from Wonboyn 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 Wonboyn Lake A harvest area, Wonboyn River, recording high-resolution temperature, salinity and depth data. Oyster farmers collected weekly biological samples (669 environmental DNA samples and 303 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.

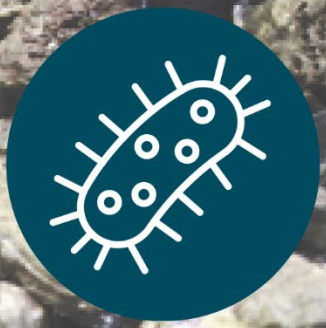
MAJOR FINDINGS

6

Available data indicated that six harvest area closures, three in Wonboyn Lake A harvest area and six in Wonboyn Lake B harvest area, could have potentially been avoided between February 2018 and March 2022

100%

Salinity was a more reliable predictor than rainfall of faecal bacteria for all four indicators tested, showing changed harvest area management would be safer and more discriminatory.



Cow and human bacteria were low across the sampling period however, E. coli and bird bacteria were high on some occasions.

Oyster mortality in Wonboyn River was 22.7% which is just above the background farming mortality (estimated at 10% per annum), however it did not exceed 5% between sampling occasions

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.*, Prorocentrum 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 January 2023, eighteen salinity-only management plans had been offered for harvest areas in participating NSW estuaries, with six being taken up and the remaining twelve under consideration.

1.2 Wonboyn River

Wonboyn River (-37.2° S, 149.9°E) is a barrier river with an intermittently closed entrance. It has a catchment area of ~335 km^2, an estuary area of ~4.2 km^2, and a flushing rate of ~66.4 days (Roy et al. 2001, Roper et al. 2011) (Fig. 1). The river has important seagrass (0.8 km^2) and saltmarsh areas (0.5 km^2) (Roy et al. 2001), with the surrounding catchment mainly forested. The exception to this is a small area of urban and grazing land associated with the townships of Wonboyn and Narrabarba (https://www.dpie.nsw.gov.au/).

1.3 Oyster Production in Wonboyn River

Wonboyn River is a mid-range producer of Sydney Rock Oysters in Australia, with production in 2020/21 of 101K dozen and valued at ~$1.1 Mil (NSW Department of Primary Industries, 2023). Wonboyn River is located toward the southern extent of the main growing range for Sydney Rock Oysters on the east coast of Australia. The 2019-2020 mega bushfires resulted in large areas of damage to the Wonboyn catchment. Significant threats to this industry now include catchment runoff resulting in increased turbidity, altered pH, reduced dissolved oxygen, and a potential increase in harmful algal blooms.

FINDINGS

2. Findings

2.1. The data assessment from this report supports implementing a harvest area management plan based on sensor salinity data for Wonboyn Lake harvest areas, subject to agreement by the local shellfish industry. Available data indicated that up to six harvest area closures, three in Wonboyn Lake A harvest area and six in Wonboyn Lake B harvest area, could have potentially been avoided between February 2018 and March 2022.

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 Wonboyn 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 all four faecal indicator bacteria.

2.4. The abundance of cow and human bacteria were generally low across the sampling period; however, the abundance of *E. coli* and bird bacteria were on occasions, very high. The maximum predictive capability for each bacterial group were 40% for *E. coli*, 41% for cow, 30% for bird, and 61% for human at the sensor site.

2.5 Where the models were predictive, they often suggested bacterial abundance increased with varying salinity which may be linked to a lack of flushing within the River and/or a lag with input from the upper catchment.

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. Cumulative oyster mortality in Wonboyn River over the study period was 22.7% which is just above the background farming Sydney Rock Oyster mortality level (approximately 10% per annum), however, mortality did not exceed 5% between sampling occasions.

ACKNOWLEDGEMENTS

3. Acknowledgements

This project has been funded under the Bushfire Local Economic Recovery Fund, co-funded by the Australian and NSW Governments 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 and the University of Technology also provided project funding. The project team would like to acknowledge the invaluable assistance of Caroline and Kel Henry for their ongoing involvement and assistance with sample collection and storage during 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 the Georges 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.

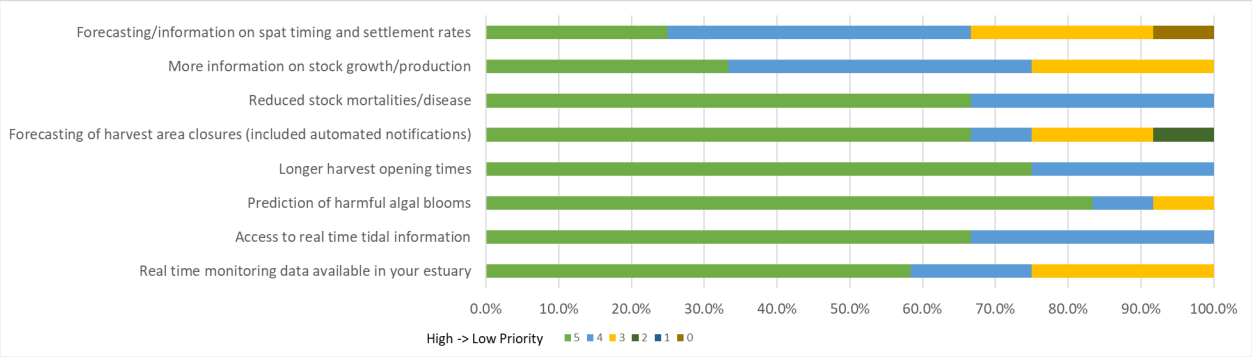


Figure 4.1. The importance of factors as rated by farmers in relation to their business operations. Green is most important and brown is least important.

RESULTS

5. Results

5.1 High resolution temperature and salinity data

High-resolution real time data summaries for Wonboyn River for the period 13 Feb 2018 to 4 Feb 2021 are shown in Figs. 5.1A-C. Limited data is available between 15 Jun 2020 and 17 Jul 2020, when sensor stopped working and then restarted independently (the cause was not determined). Depth recordings ranged from 0.3 m (23 Jan 2020) to 1.6 m (28 Jul 2020). The lowest and highest daily average salinity recordings were 0.6 ppt (31 Jul 2020) and 39.1 ppt (5 Feb 2020) respectively, while the lowest and highest daily average temperature recordings were 11.3℃ (16 Jul 2019) and 29.3 ℃ (1 Jan 2020) respectively.

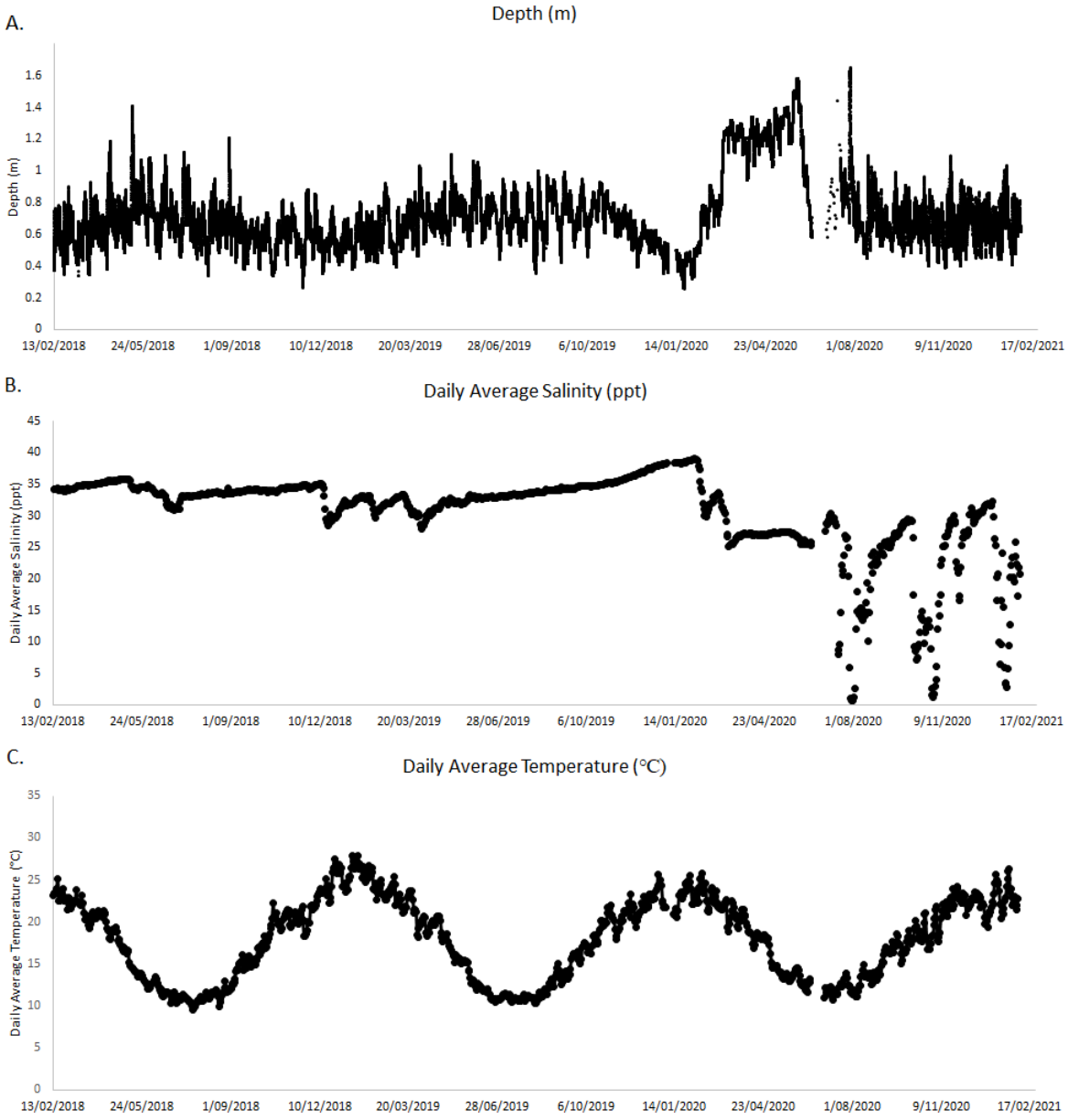


Figure 5.1A-C. Real time sensor data from Wonboyn River 13 Feb 2018 4 Feb 2021 A. Depth (m); B. Daily average salinity (ppt); and C. Daily average temperature (°C).

The maximum daily rainfall at the WLSP gauge occurred on 28 July 2020 and was reported as 80 mm (Fig. 5.2).

Eleven rainfall events were sampled across the study period. These occurred on 17-19 Dec 2018, 6-8 Feb 2019, 19-21 Mar 2019, 31 Mar-2 Apr 2019, 11-13 May 2019, 9-12 Feb 2020, 7-9 Mar 2020, 23-25 May 2020, 13-15 Jul 2020, 27-29 Jul 2020, and 17-19 Aug 2020. The maximum daily rainfall occurred on 28/7/2020 and was reported as 80 mm at WLSP/Henry's Gauge (37.25°S, 149.919°E) (Fig. 5.2).

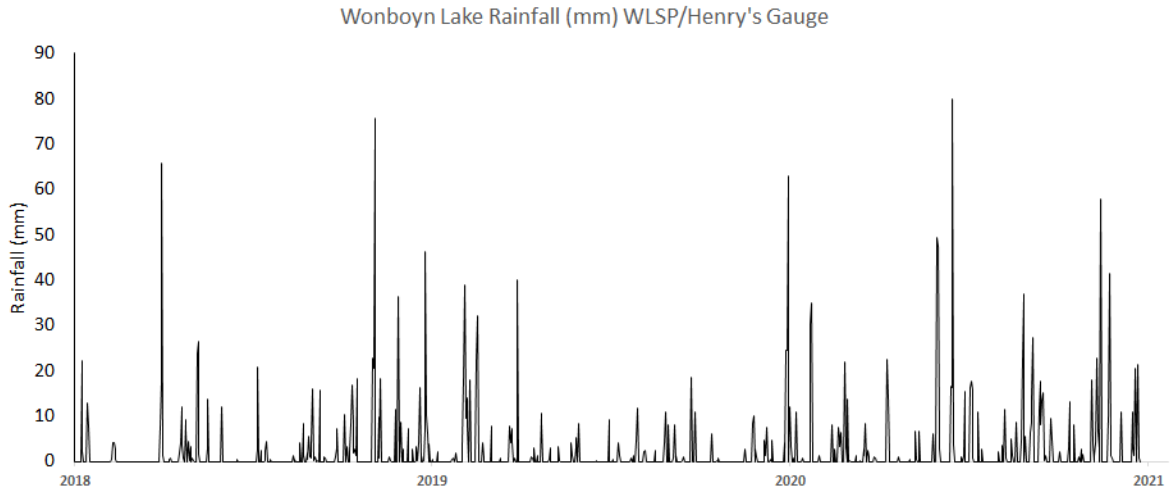


Figure 5.2. Daily rainfall (mm) from WLSP/Henry's gauge 37.25°S, 149.919°E from Feb 2018 to Feb 2021.

5.2 Management Plan

Data analysed during the 2019 annual reviews of Wonboyn Lake harvest areas indicated that there could have been fewer harvest area closures since the sensor was installed, if closures were based on salinity sensor data. There were nine harvest area rainfall closures between February 2018 and March 2020 in Wonboyn Lake A harvest area. Based on a management closure limit of 27 ‰, harvest area closures were modelled based on available salinity sensor data and shellfish program microbiological results since February 2018. For Wonboyn Lake A, up to 20 harvest closure days occurred over three rainfall closures, although salinity sensor data did not decline below 27 ‰ and microbiological results from samples collected between 3-7 days post closure met Approved harvest criteria. Wonboyn Lake B harvest are is situated downstream of Wonboyn Lake A harvest area There were ten harvest area rainfall closures between February 2018 and March 2020 in Wonboyn Lake B harvest area. Based on a management closure limit of 27 ‰, harvest area closures were modelled based on available salinity sensor data and shellfish program microbiological results since February 2018. For Wonboyn Lake B, up to 26 harvest closure days occurred over six rainfall closures, although salinity sensor data did not decline below 27 ‰ and microbiological results from samples collected between 0-4 days post closure met Approved harvest criteria.

During the more recent 2021 and 2022 annual review periods (April 20 – March 22), there were seven harvest area rainfall closures and two harvest area salinity closures in Wonboyn Lake A harvest area. There were two additional rainfall closures in Wonboyn Lake B harvest area during the same period. Data analysed during the 2021 and 2022 annual reviews

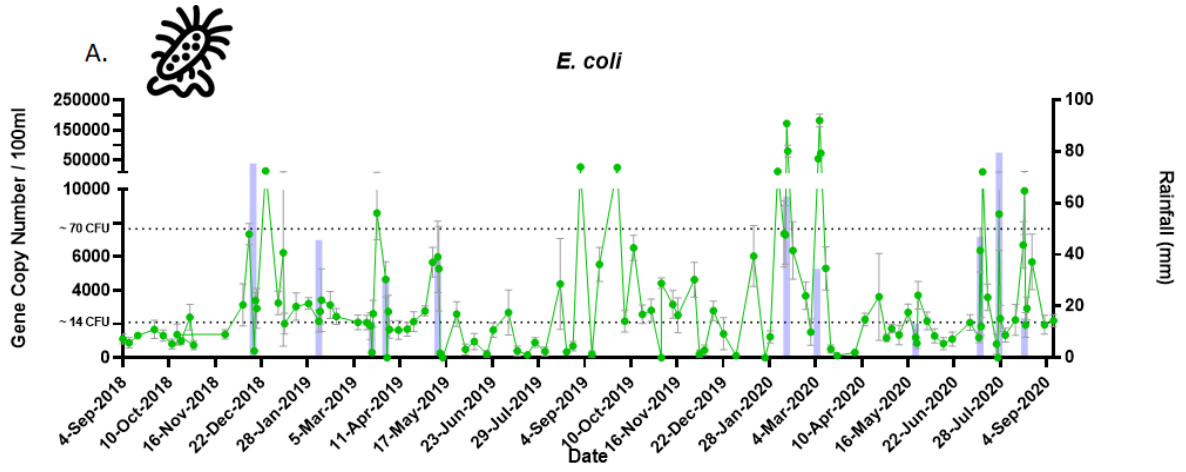
indicated there were no occasions when the harvest area would have remained open for harvest when comparing operations under a rainfall or sensor salinity-based management plan. This was due to wetter conditions that occurred during this time. A division of Wonboyn Lake A harvest area into two harvest areas, Wonboyn Lake A and Wonboyn Lake C, came into effect on 15 August 2022. The use of sensor salinity for harvest area management is also possible, based on the previous analysis of data from Wonboyn Lake A harvest area.

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 669 water samples and 303 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 Wonboyn River (Fig. A1).

For Wonboyn River the maximum *E. coli* reached 182,188 gene copies 100 mL^{-1} on 8 Mar 2020, 47,038 copies 100 mL^{-1} for *Helicobacter* (bird) on 7 Feb 2019, 19,469 gene copies 100 mL^{-1} for bovine faecal pollution (cow) again on 8 Mar 2020, and finally, 411 copies 100 mL^{-1} for human faecal pollution on 17 Mar 2020 (Fig. 5.3 A-D).



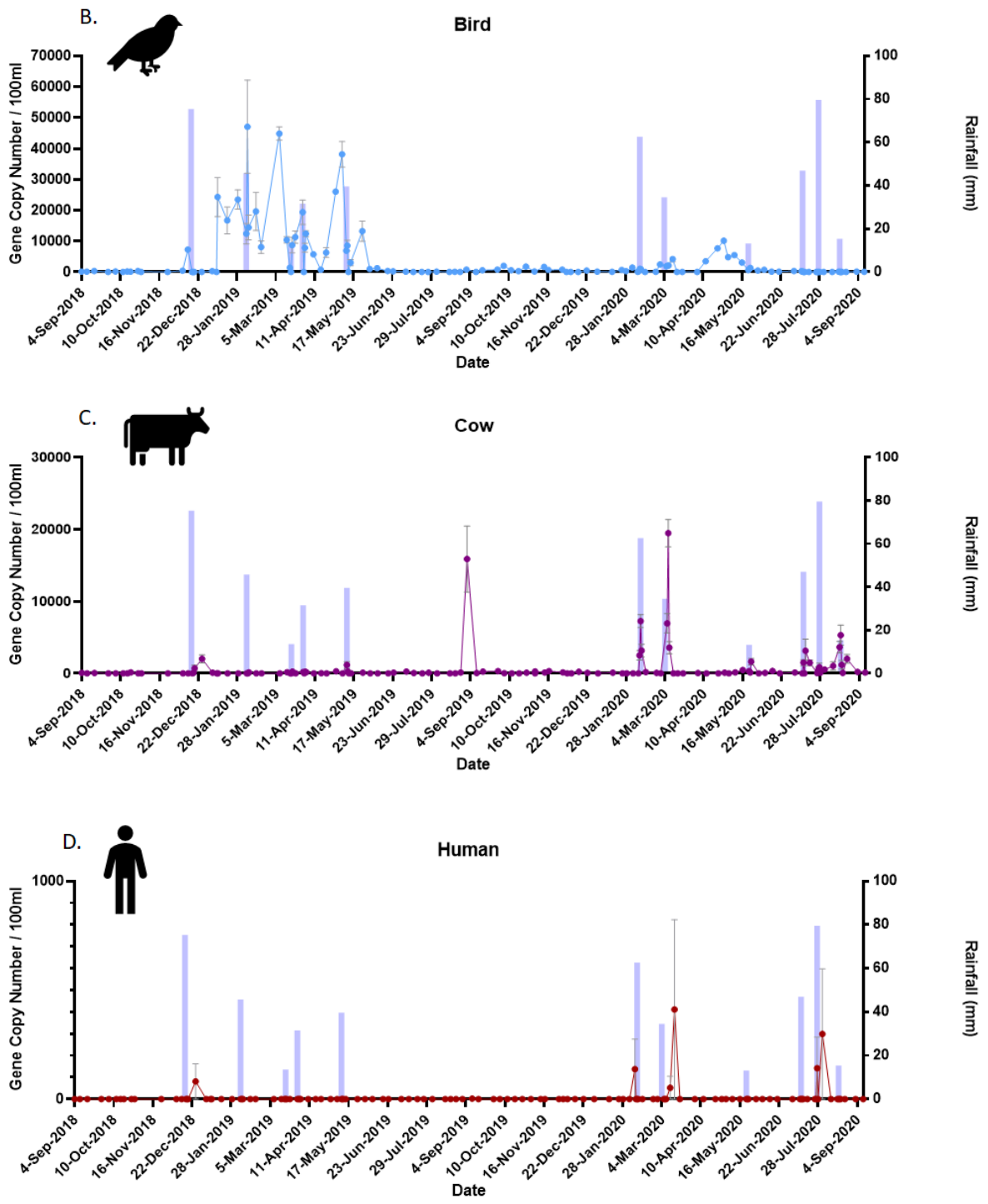


Figure 5.3 A-D. Weekly *E. coli* data from the sensor location, Wonboyn River, using A. *E. coli* assay; B. Bird assay; C. Cow assay; and D. Human assay. Purple bars represent rainfall events that were sampled. Dotted lines in Fig. A at 14 and 70 cfu/100 mL are the operational limits for direct or restricted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

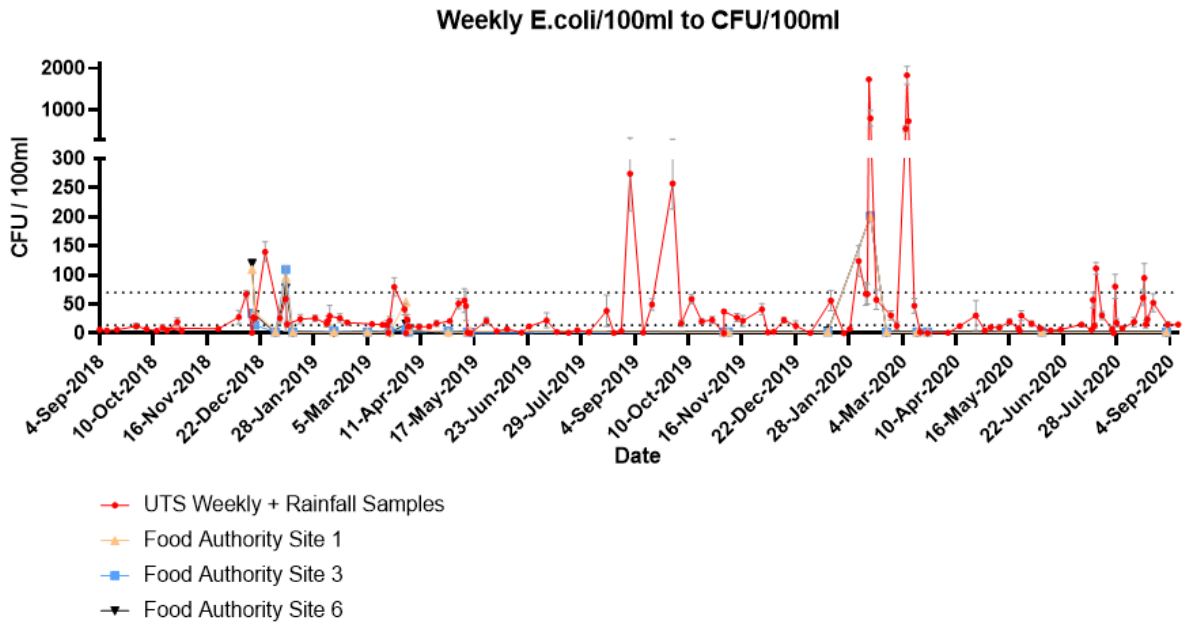
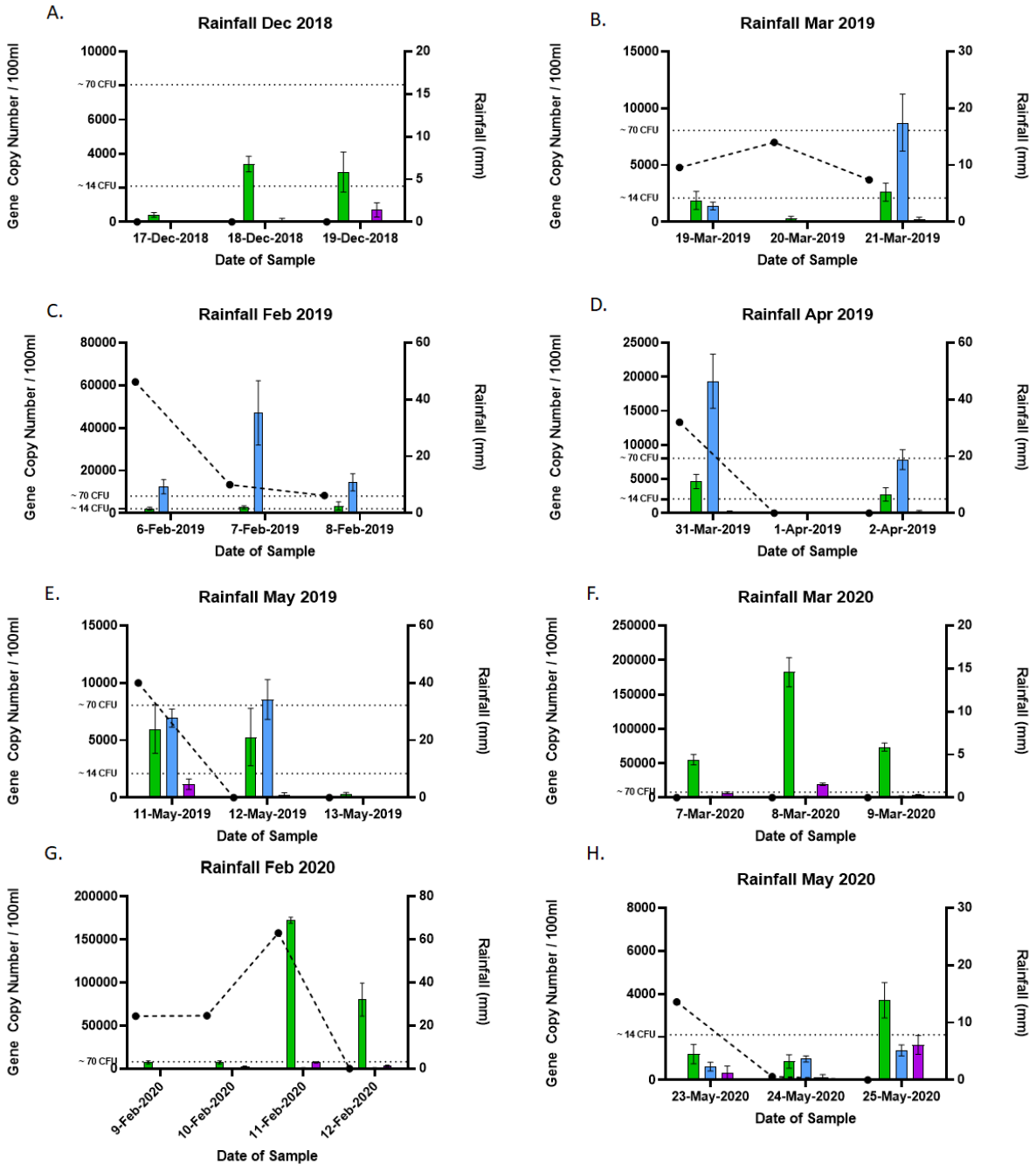


Figure 5.4 Weekly faecal coliform counts (cfu/100 mL) from water samples collected by DPI Food Authority at three sites in Wonboyn River compared to the Oyster Transformation Project sensor site weekly sampling results (including rainfall sampling). Dotted lines at 14 and 70 cfu/100 mL are the operational 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. 5.4).

Eleven rainfall events were sampled across the study period (see purple bars in Fig 5.3 A-D). These occurred on 17-19 Dec 2018, 6-8 Feb 2019, 19-21 Mar 2019, 31 Mar-2 Apr 2019, 11-13 May 2019, 9-12 Feb 2020, 7-9 Mar 2020, 23-25 May 2020, 13-15 Jul 2020, 27-29 Jul 2020, and 17-19 Aug 2020. *E. coli* was highly variable across rainfall sampling campaigns. In some instances, highest counts coincided with peak rainfall days, while at other times counts were highest after rainfall had declined (Fig. 5.5 A-K). It is unclear without further sample collection, how quickly these levels would have dissipated. Bird contamination was also highly variable across rainfall sampling, but was observed most often during the summer - autumn months. Bovine generally remained low, while human bacteria was very low or below detection limits during all events (Fig. 5.5A-K).


A.
Rainfall Dec 2018
Gene Copy Number / 100ml
Rainfall (mm)
~ 70 CFU
~ 14 CFU
17-Dec-2018
18-Dec-2018
19-Dec-2018
Date of Sample
B.
Rainfall Mar 2019
19-Mar-2019
20-Mar-2019
21-Mar-2019
C.
Rainfall Feb 2019
6-Feb-2019
7-Feb-2019
8-Feb-2019
D.
Rainfall Apr 2019
31-Mar-2019
1-Apr-2019
2-Apr-2019
E.
Rainfall May 2019
11-May-2019
12-May-2019
13-May-2019
F.
Rainfall Mar 2020
7-Mar-2020
8-Mar-2020
9-Mar-2020
G.
Rainfall Feb 2020
9-Feb-2020
10-Feb-2020
11-Feb-2020
12-Feb-2020
H.
Rainfall May 2020
23-May-2020
24-May-2020
25-May-2020

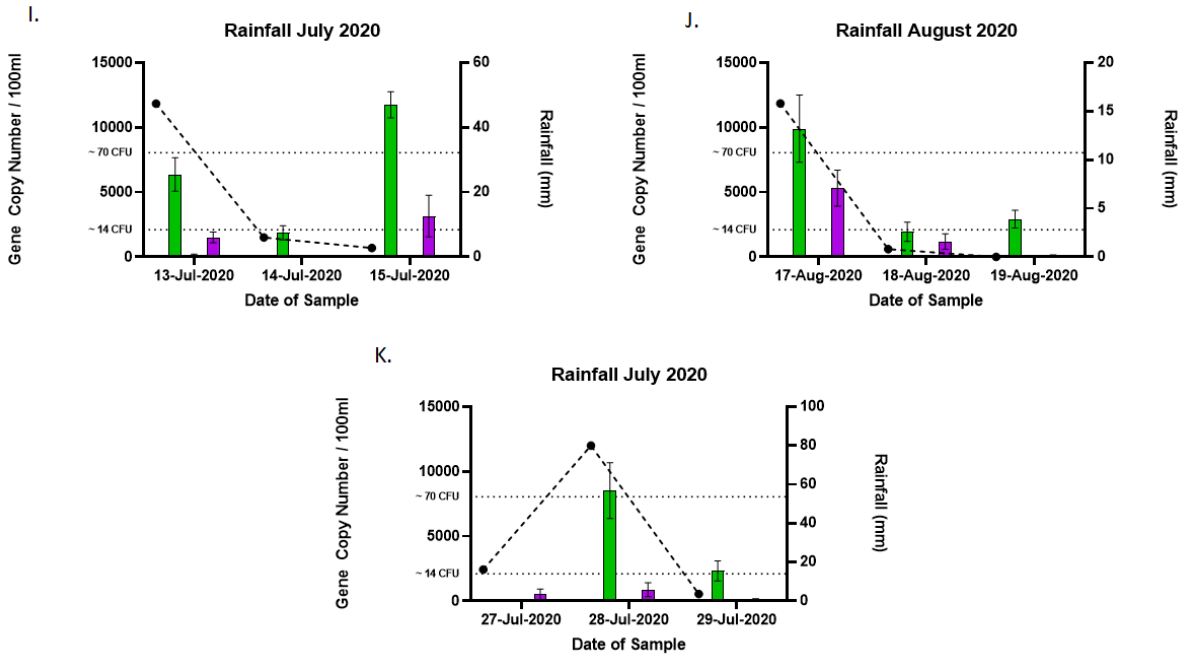


Figure 5.5 A-K. Sensor site (Wonboyn River) rainfall events sampled for *E. coli* assays. Green bar = 16S *E. coli*; blue bar = bird assay; purple bar – cow assay; red bar = human assay. Dotted line is rainfall (mm) obtained from the closest rainfall station (WLSP/Henry's gauge 37.25°S, 149.919°E). All 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 (Feb 2018 to Feb 2021) occurred on 26 May 2018 (Fig. 5.6). Total cell concentrations reached 4.4E +07 cells L^{-1} and the sample was dominated by the planktonic diatom *Minidiscus* with a small variety of small flagellates (cryptomonads, dinoflagellates, prasinophytes) but few other diatoms. This bloom did not coincide with any significant rainfall event on the day, but was preceded with elevated rainfall (66 mm on 13 May 2018) which may have been a contributing factor.

Potentially harmful bloom events across the sampling period included blooms of the diatom *Pseudo-nitzschia delicatissima* gp. which reached a maximum cell concentration of 2.1E +06 cells L^{-1} on 31 Nov 2018 (elevated levels were reported from 27 Oct 2018 to 9 Nov 2018), and *Pseudo-nitzschia fraudulenta/australis,* which reached 5.5E +04 cells L^{-1} on 22 March 2020. The NSW Food Authority's Phytoplankton Action Limit to trigger biotoxin testing for *P. delicatissima* is 500,000 cells L^{-1} and 50,000 cells L^{-1} for *P. australis & multiseries.*

Other bloom events were caused by the toxic dinoflagellate *Alexandrium australiense* which reached a maximum cell density of 300 cells L^{-1} on 27 Sept 2020, and *Alexandrium minutum* which peaked on 27 Jan 2020 at 850 cells L^{-1}. The NSW Food Authority's Phytoplankton Action Limits to trigger biotoxin testing for any toxic *Alexandrium* species is 200 cells L^{-1} (NSWFA 2015).

Finally, the toxic dinoflagellate *Dinophysis caudata* was reported above guideline levels (NSW Food Authority's Phytoplankton Action Limit of 500 cells L^{-1}) in many instances across the sensor deployment period. From early to late Nov 2018, cell densities were elevated, reaching a maximum cell density of 2,200 cells L^{-1} on 24 Nov 2018. Again, on 2 March 2019, cell

densities reached 500 cells L^{-1}. During February to April the following year, *D. caudata* was observed to be elevated, with a maximum cell count of 2,300 cells L^{-1} on 1 March 2020. Similarly, in Sept that same year, *D. caudata* became elevated and peaked at 2,700 cells L^{-1} on 5 Oct 2020. High cell densities were reported again on 7 Dec 2020 (950 cells L^{-1}). Routine biotoxin tests collected by WLSP reported 0.035 (5 April 2020) and 0.029 (5 October 2020) mg/kg pectenotoxin 2 (PTX2). Traditionally, regulation of diarrhetic shellfish toxins (DSTs) included okadaic acids, dinophysistoxins and pectenotoxins, and when detected and quantified, their concentrations have been combined. To date, there is no evidence that pectenotoxins are toxic to humans, although acute toxicity has been demonstrated in animals (Munday and Reeve 2013). In 2021, the pectenotoxin group was deregulated in the European Union, and removed from their grouping with okadaic acid and dinophysistoxins in reference to their maximum concentrations in bivalve molluscs. No other positive biotoxin results were reported in routine monitoring samples collected by WLSP during the same period.

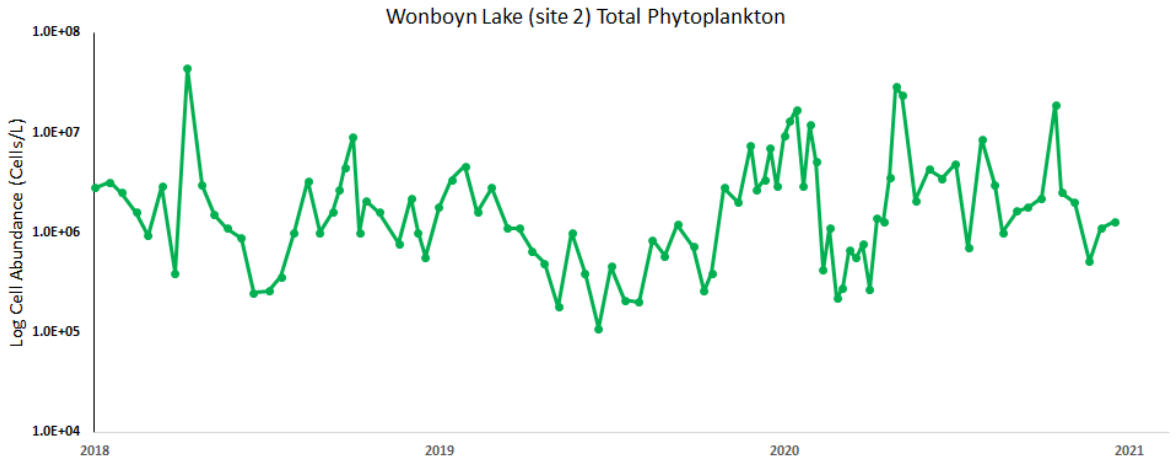


Figure 5.6 Log abundance of total phytoplankton sampled approximately fortnightly from 13 Feb 2018 to 4 Feb 2021

5.6 Oyster Growth and Mortality

5.6.1 Oyster Growth

Average oyster whole weight increased by 21.4 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 13.7 g in 10 months. Oyster whole weight was 44 ± 3.2 g at the end of the experiment (June 2020). Oysters deployed in Wonboyn River did not reach the large size grade (> 70 mm total length or > 50 g whole weight) when measured at the conclusion of the experiment. The average oyster size at the end of the experiment in June 2020 was 'Medium' grade (> 55 mm and < 70 mm total length and > 30 g and < 50 g whole weight) and were 42 mo on this date.

Oyster size, in terms of shell length, did not increase over the duration of this experiment. Average shell length was 57 ± 1 mm at the start of the experiment and was 59 ± 2 mm at the end of the experiment in June 2020 (Fig. 5.7 B). Shell lengths were measured more frequently than whole weight and fluctuated throughout the experiment. The maximum average shell

length measured during the experiment was 63 mm in December 2019 and the minimum was 53 mm measured in September 2018. Slight shell length increases in Wonboyn River were recorded from October to November 2018 and July to September 2019. However, the increase in shell size through these periods was only 4 mm and 7 mm, respectively. Periods of shell length decreases were recorded on six occasions and were between August and September 2018, November and December 2018, January and February 2019, June and July 2019, October and November 2019 and February and June 2020.

5.6.3 Mortality

From August 2018 to February 2020, cumulative oyster mortality was 23.3% in Wonboyn River. Low levels of mortality were recorded in most months throughout the experiment except for April 2019, September 2019 and December 2019 where no dead oysters were found in any of the replicate baskets (Fig 5.7 D). The month that had the highest level of mortality recorded was December 2018 (4.3%), however, mortality on this date was less than 5%. Oyster mortality over the study period in Wonboyn River exceeded the background Sydney Rock Oyster farming mortality level which is estimated to be approximately 10% per annum. Most mortalities occurred in the first four months of the experiment (Figures 5.7 C and D) where cumulative mortality reached 13.7% in December 2018. Oysters from this site remain frozen for future analyses.

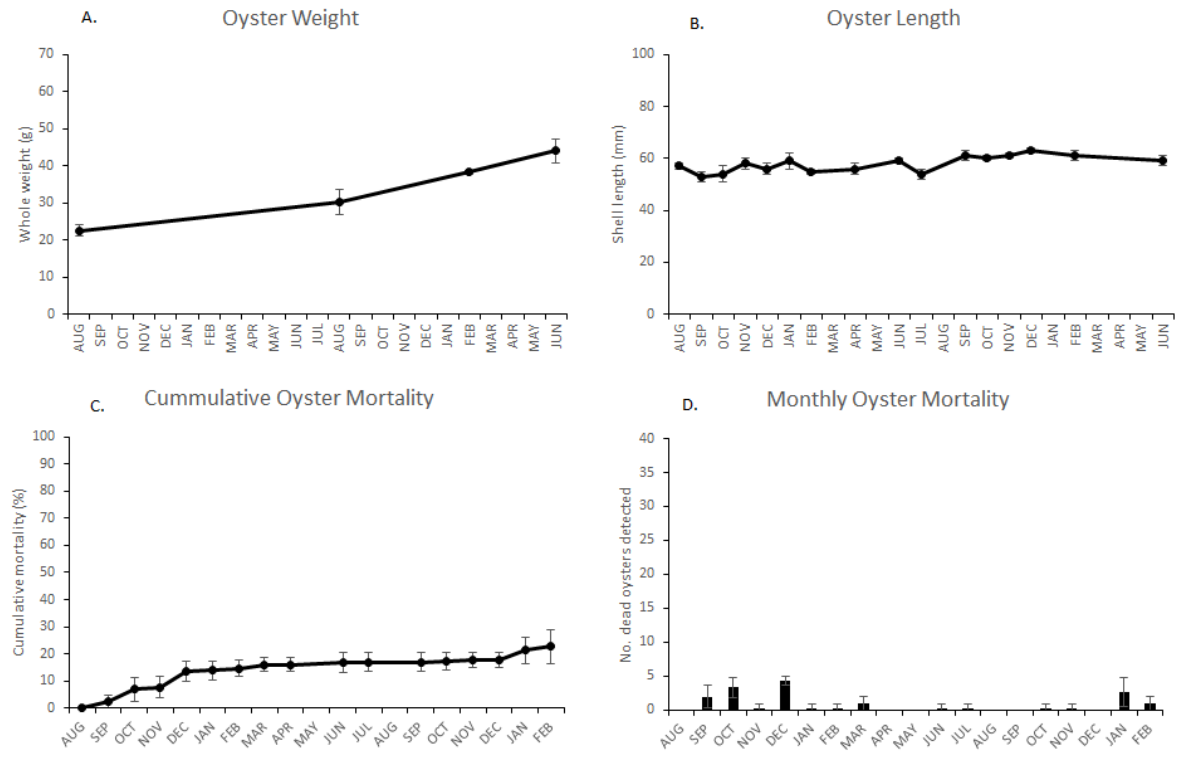


Figure 5.7 A-D. Oysters deployed at the sensor site, Wonboyn River. A. whole weight; B. shell height; C. cumulative mortality, and D. monthly mortality.

5.7 Modelling

5.7.1 Modelling of E. coli data

Summary statistics for all bacterial concentrations and environmental variables used in the general additive models are shown in Appendix 2. Correlation coefficients were calculated among every pair of environmental variables and suggested very few strong positive relationships ($r > 0.7$) overall. 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: 40.3% for *E. coli* (sensor + total phytoplankton), 29.9% for cow (sensor + total phytoplankton), 41.1% for bird (sensor + total phytoplankton) and 61.4% for human (sensor + total phytoplankton) (Table 1).

The abundance of *E. coli* was significantly better predicted using sensor data compared to rainfall data (40.3% compared to 14% deviance explained), and appeared to be significantly linked to an increase in water temperature, varying salinity over the past 72 hours as well as increasing phytoplankton. Data indicated that a peak *E. coli* coincided with a peak surface water temperature of ~>25℃ and was lowest with a salinity ~20 ppt (Table 1) (Figures 5.7 A-D, 5.8 A-D).

The prediction of bovine bacterial abundance was significantly improved using the sensor data (29.9%) compared to a model with rainfall data (2.2%), with total phytoplankton only marginally improving this predictive capability. Modelling showed peak contamination was linked to varying salinity (high or low) and an increasing surface water temperature (peaking at ~>25℃) (Table 1).

Faecal contamination from birds was again significantly better explained using the sensor data (41.1% deviance explained, compared to 7% using rainfall data), with a peak salinity of 30 ppt and surface temperature of ~>25℃. Adding phytoplankton data to the model made little difference to its predictive ability (Table 1).

An increase in human bacteria abundance was again best explained by the sensor data (61.4%) compared to rainfall (25.3%), and was linked to a decreasing salinity and an increasing surface water temperature. Adding phytoplankton data to the model only marginally improved 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 ~47.5% of the deviance, with the strongest predictor variables being the week of the year.

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

Bacteria	Variables	No. of observations	Significant Variables	Deviance Explained
E. coli	Salinity, Depth, Temp	119	Depth72**, Salinity72***, Temp72***	37.6%
E. coli	Salinity, Depth, Temp, logPhytoplankton	119	logPhytoplankton ***, Depth**, Salinity***, Temp***	40.3%
E. coli	Rainfall72	125	Rainfall72***	6.07%
E. coli	Rainfall72, logPhytoplankton	125	Rainfall72***, logPhytoplankton ***	14.1%
Bird	Salinity, Depth, Temp	119	Salinity***, Depth***, Temp***	40.6%
Bird	Salinity, Depth, Temp, logPhytoplankton	119	Salinity***, Depth***, Temp***, logPhytoplankton ***	41.1%
Bird	Rainfall72	125	Rainfall72***	7.01%
Bird	Rainfall72, logPhytoplankton	125	Rainfall72***, logPhytoplankton***	7.06%
Cow	Salinity, Depth, Temp	119	Salinity***, Depth***, Temp***	28.2%
Cow	Salinity, Depth, Temp, logPhytoplankton	119	Salinity***, Depth***, Temp***, logPhytoplankton***	29.9%
Cow	Rainfall24	127	Rainfall24***	0.113%
Cow	Rainfall24, logPhytoplankton	127	Rainfall24***, logPhytoplankton***	2.23%
Human	Salinity, Depth, Temp	125	Salinity***, Depth***, Temp***	54.6%
Human	Salinity, Depth, Temp, logPhytoplankton	125	Salinity***, Depth***, Temp***, logPhytoplankton***	61.4%
Human	Rainfall48	126	Rainfall48***	0.547%
Human	Rainfall24, logPhytoplankton	126	Rainfall24***, logPhytoplankton***	25.3%

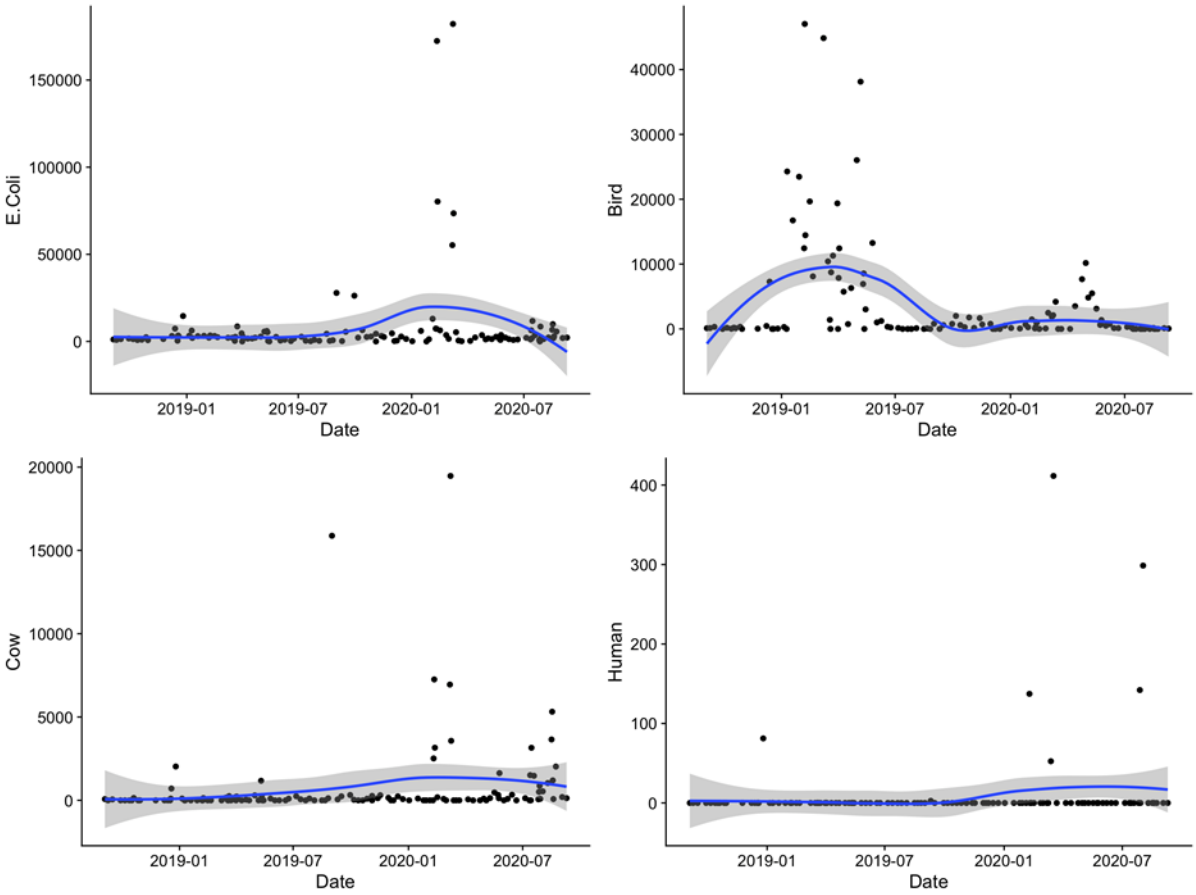


Figure 5.7 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. *E. coli*, B. Bird, C. Cow, and D. Human bacterial load as measured by weekly sampling at the sensor site, Wonboyn River.

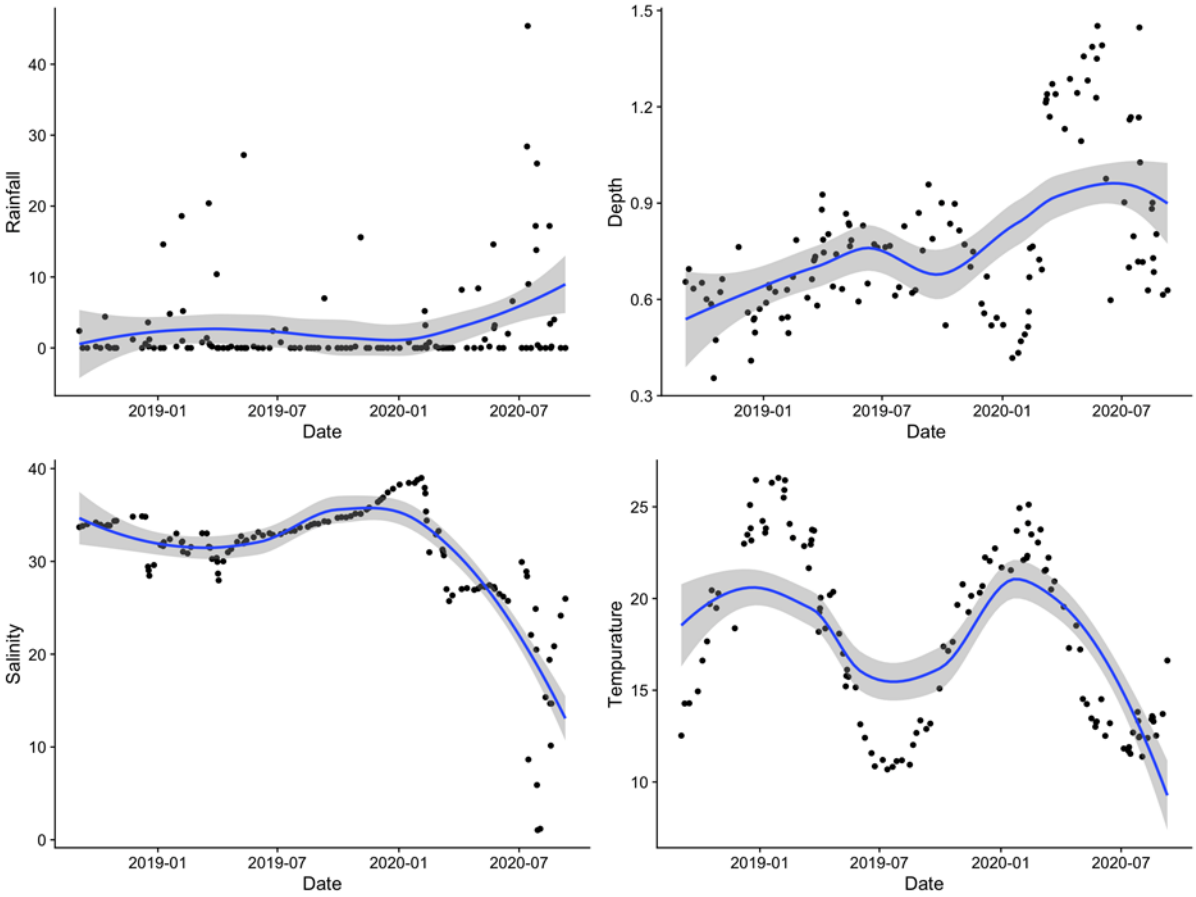


Figure 5.8 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. Rainfall, B. Depth, C. Salinity, and D. Temperature values measured in at the sensor site, Wonboyn River.

DISCUSSION

6. Discussion

6.1 High Resolution Sensor Data and Management Plan

Analysis of sensor data during the annual review process demonstrated that there is potential to implement a salinity sensor-based management plan for Wonboyn Lake harvest areas. Based on the available data, up to six harvest area closures, three in Wonboyn Lake A harvest area and six in Wonboyn Lake B harvest area, could have potentially been avoided between 14 February 2018 and 31 March 2022. 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. Wonboyn Lake Shellfish Program (WLSP) were consulted about the option of a salinity-only management plan for Wonboyn Lake harvest areas following the 2019 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 Wonboyn Lake harvest area management plans would revert to the current management plana that are 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 Wonboyn River during this study was *Alexandrium australiense.* 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. 2013b). 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 Wonboyn River

Molecular assays for the detection of faecal bacterial contamination in Wonboyn 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 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 cow and human bacterial contamination was extremely low across the sampling period in Wonboyn River, modelling revealed that *E. coli*, and to a lesser extent the bovine bacterial load entering the estuary were linked to a varying salinity, which in turn may be linked to a lack of flushing and/or a lag with input from the upper catchment.

Wonboyn River is a relatively small catchment of <340 km^2. The upper catchment is primarily steep forested terrain. It may be the case that the pattern of increasing salinity and increasing bacterial load reflects more influence from the wider catchment (i.e., localised rainfall has ceased, salinity is recovering but inputs from further upstream are still influencing water quality). It may also be confounded with the closing of the lake mouth. During the study period the mouth closed

on 6 Oct 2019 and reopened on 12 Feb 2020. It closed again on 5 Mar 2020 and reopened on 1 June 2020. The mouth remained opened at other times across the study period.

Avian faecal pollution in Wonboyn 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 in Wonboyn 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 Wonboyn River were greatest in the second half of the experiment from August 2019 to June 2020. However, oysters did not grow significantly larger in terms of their shell size over the entire experiment. Salinity levels throughout the experimental period remained above 30 ppt for most of the experiment other than three occasions in late 2019, early 2020 and after February 2020. On these occasions' salinity did not drop below 26 ppt. 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 30 ppt other than in the period after February 2020 where salinity dropped rapidly to approximately 25 ppt and remained around this level until June 2020 (Figure 5.1 B). Water temperature levels during the 2018 and 2019 winter months were dropping to levels below 12 °C. Growth of Sydney Rock Oyster at temperatures below this level would be minimal to non-existent.

Low levels of oyster mortality were recorded in most months during the experiment. However, oyster mortality between assessments did not exceed 5% at any time. Cumulative oyster mortality over the study period did exceed the background Sydney Rock Oyster farming mortality level

which is estimated to be approximately 10% cumulative mortality in February 2020 was 22.7% and comparable to cumulative mortality measured on the same date in the Hawkesbury River (22%). However, Hawkesbury River is an estuary that is affected by QX disease which is likely to increase Sydney Rock Oyster mortalities over the study period. Mortality in Wonboyn River was greatest in the first four months of the experiment (August to December 2018). This is generally the time period when losses attributable to winter mortality disease are detected. The only monitoring sites that experienced more oyster mortality than Wonboyn River over the study period were Camden Haven River (39.7% cumulative mortality) and Hastings River (34.0% cumulative mortality). There were no oyster sampling events where mortality exceeded 5 % in Wonboyn River.

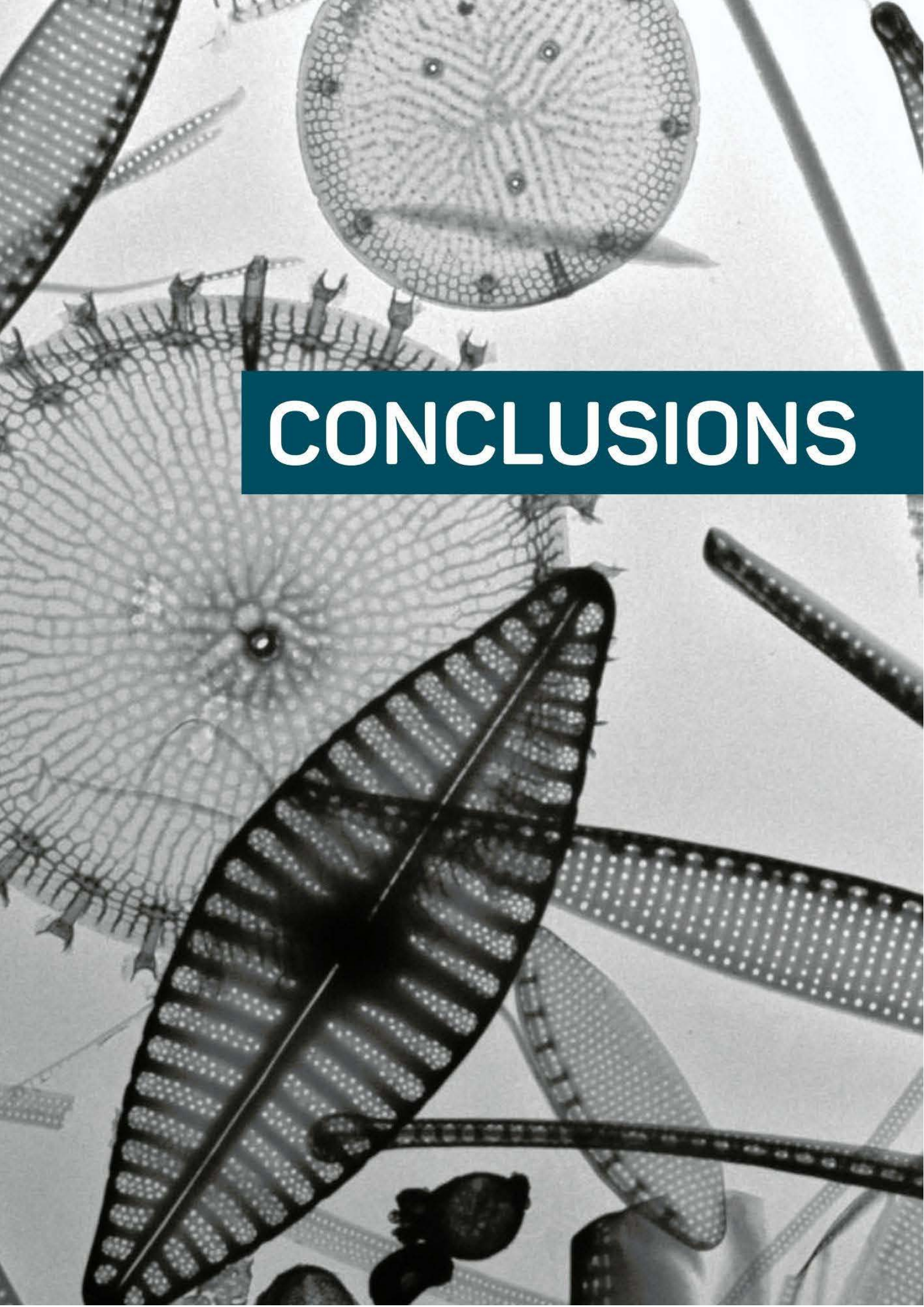
The cumulative mortality in Wonboyn River over the 18 months of this experiment was very similar to that measured in a previous study which also ran for 18 months (8/5/2014 to 19/11/15) in Wonboyn River (Hall-Aspland et al. 2015). Hall-Aspland et al. 2015 measured a combined average cumulative mortality of 21%in wild Sydney Rock Oysters deployed at 4 sites in Wonboyn River over an 18-month deployment. Wonboyn had the highest mortality compared to all other NSW south coast estuaries (Shoalhaven River, Clyde River, Wagonga Inlet, Wapengo Lake, Merimbula River, Pambula River) monitored in Hall-Aspland et al. 2015. Oyster shell length increases were recorded and were 12 mm for wild oysters over the 18-month study 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 12 months to reach the 'medium' oyster size grade with respect to oyster whole weight (30 – 50 g). However, oysters in Wonboyn River did not reach the 'large' size grade (> 70 mm total length or > 50 g whole weight) by the end of the experiment (June 2020).

Wonboyn River is ranked 14th in the state for Sydney Rock Oyster production with 101,000 dozen oysters sold annually worth $1.1 million (NSW Department of Primary Industries, 2023). Oyster growth in Wonboyn River, in terms of size and weight, was ranked lowest amongst all estuaries monitored for this study. Most Sydney Rock Oysters in Wonboyn River 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 Wonboyn 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 Wonboyn Lake harvest areas, subject to agreement by the local shellfish industry. Available data indicated that up to six harvest area closures, three in Wonboyn Lake A harvest area and six in Wonboyn Lake B harvest area, could have potentially been avoided between February 2018 and March 2022. As of January 2023, eighteen salinity-only management plans had been offered for harvest areas in participating NSW estuaries, with six being taken up and the remaining twelve under consideration.

Compared to the other monitoring sites in NSW, oyster growth in Wonboyn River was ranked last in terms of whole oyster weight and shell length. Low levels of mortality (< 5%) were recorded over the period from August 2018 to February 2020, however cumulative mortality over the entire experiment exceeded the level accepted as background farming mortality (approximately 10% per annum). Most oysters in Wonboyn 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 all four faecal indicator bacteria. Furthermore, the contamination from bird sources was observed at high levels, with 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 Wonboyn River.

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9. Appendices

A1. Methods

A1.1 Sampling locations in Wonboyn River

Data used in this report originates from locations within Wonboyn River over the period Feb 2018 to Feb 2021. High-resolution temperature, salinity and depth data were obtained from a sensor located in Wonboyn Lake harvest area A, Wonboyn River, from 13 Feb 2018 to 4 Feb 2021 (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 Wonboyn River highlighting the sensor location in Wonboyn Lake A (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 at the sensor site using Seabird SBE 37-SM/SMP/SMP-ODO MicroCAT high accuracy conductivity, temperature and

depth (CTD) field sensors. 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 rainfall WLSP/Henry's gauge at 37.25°S, 149.919°E

Figure A2 Seabird MicroCAT high accuracy conductivity, temperature and depth (CTD) field sensor deployed in Wonboyn River. Image Credit: ICT International

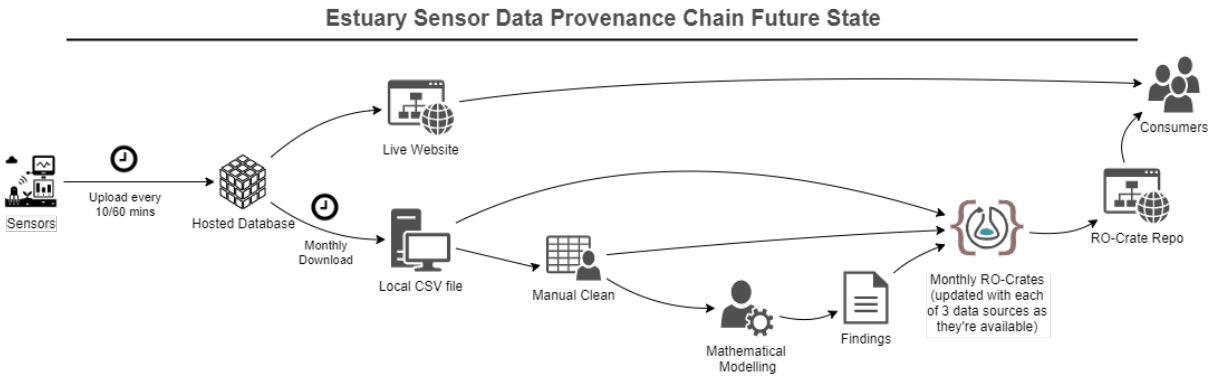

Figure A3. Wonboyn River data provenance chain from source of data (sensors), via quality assurance processes, data analyses, to consumers.

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 Wonboyn Lake annual review is 1 April. All available 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. There was a gap in salinity data between 4 and 14 January 2020 due to a disruption to the local telecommunication network, following an extensive bushfire event. Salinity data were limited between 15 June 2020 and 17 July 2020 due to instrument error. The original sensor ceased reporting on 4 February 2021 and data collection resumed with a new sensor on 13 April 2021. There was a gap in salinity data between 1 and 28 February 2022 due to instrument error.

A1.4 Biological sampling and eDNA extraction

Estuarine water samples were collected weekly by oyster farmers working at Wonboyn Rock Oysters (Caroline and Kel Henry) 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 °C until further downstream processing. DNA was then extracted using the DNeasy 96 PowerSoil Pro QIAcube HT Kit (Qiagen) and DNA stored at -20°C 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 WLSP/Henry's gauge (37.25°S, 149.919°E) NSW, which is approximately ~1.2 km from the 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 2015) 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.

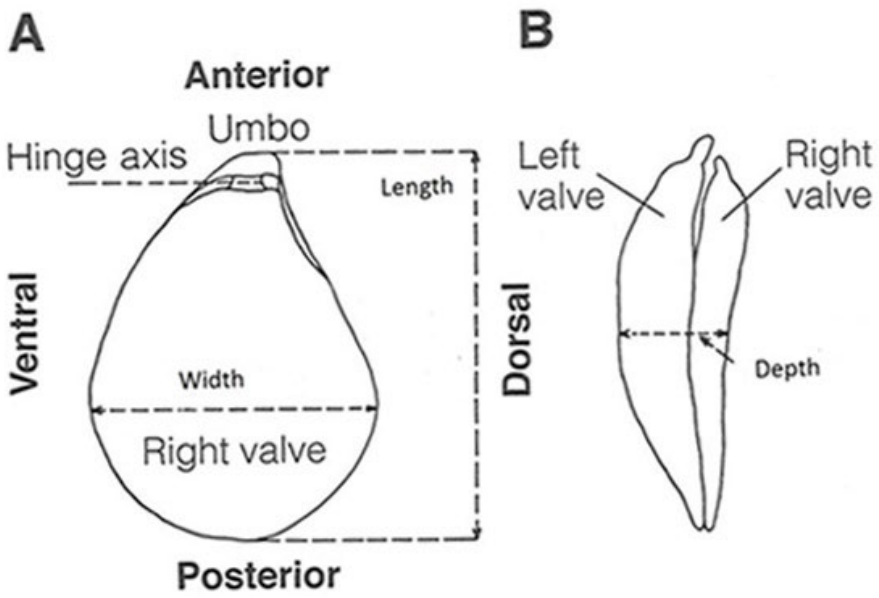

Figure A4. Oyster shell dimensions (Carriker 1996)

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 WLSP/Henry's gauge (37.25^{o}S, 149.919^{o}E from Feb 2018 to Feb 2021), 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 Wonboyn River, correlation analyses were initially undertaken to explore the relationships between variables. Generalised additive models (GAMs) were then applied to the data. GAMs allow abundance data to be treated as count data (discrete integer values), and as such can handle zero counts. GAMs also allow for smoother functions to be incorporated into each model for the environmental variables that had a non-linear relationship with bacterial abundance.

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

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

Appendix 2. Summary Statistics for Bacterial Modelling – Sensor site, Wonboyn River.

Variable	Mean	Standard Error	Median	Standard Deviation	Minimum	Maximum	Count	Missing
average_cfu	70.9	21.6	16.6	243.7	0.0	1830.4	127	0
bird	3722.7	727.6	296.6	8199.1	0.0	47038.5	127	0
cow	733.8	218.9	62.5	2467.2	0.0	19468.6	127	0
depth24	0.8	0.0	0.7	0.3	0.4	1.5	127	2
depth48	0.8	0.0	0.7	0.2	0.4	1.4	127	5
depth72	0.8	0.0	0.7	0.2	0.4	1.4	127	8
ecoli	7505.9	2149.5	2186.8	24223.4	0.0	182187.6	127	0
human	8.9	4.3	0.0	48.5	0.0	411.5	127	0
logPhytoplankton	14.2	0.1	14.2	1.1	11.6	17.2	127	0
Phytoplankton	2653779.5	365496.1	1400000.0	4118931.7	110000.0	29000000.0	127	0
rainfall24	3.0	0.6	0.0	7.0	0.0	45.4	127	0
rainfall48	3.1	0.5	0.5	5.6	0.0	36.9	127	1
rainfall72	3.1	0.4	1.3	4.7	0.0	27.6	127	2
salinity24	30.3	0.6	32.1	6.9	1.0	39.0	127	2
salinity48	30.4	0.6	32.3	6.7	1.1	38.9	127	5
salinity72	30.5	0.6	32.3	6.6	2.7	38.7	127	8
temp24	18.1	0.4	18.4	4.7	10.7	26.6	127	2
temp48	18.2	0.4	19.0	4.7	10.8	26.4	127	5
temp72	18.3	0.4	19.3	4.6	10.9	26.1	127	8

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
Penelope A. Ajani, Michaela E. Larsson, Stephen Woodcock, Ana Rubio, Hazel Farrell, Steve Brett, &Shauna A. Murray.	Fifteen years of *Pseudo-nitzschia* in an Australian estuary, including the first potentially toxic *P. delicatissima* bloom in the southern hemisphere	*Estuarine, Coastal and Shelf Science*, 236 (2020) 106651.	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	Published

		https://theconversation.com/ah-shucks-how-bushfires-can-harm-and-even-kill-our-delicious-oysters-131294 Aug 2020	

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_IKCEE&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