TRANSFORMING AUSTRALIAN SHELLFISH PRODUCTION

Lower Honeymoon Bay Harvest Area - Wagonga Inlet

Report on Stage 1, December 2017-March 2021

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

Penelope Ajani, Mike Dove, Hazel Farrell, Wayne O'Connor, Matt Tesoriero, Arjun Verma, Anthony Zammit, Brian Hughes and Shauna Murray

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Researcher Contact Details
Name: Dr Penelope Ajani
Address: PO Box 123, Broadway NSW 2007 NSW
Phone: 02 9514 2000
Email: Penelope.Ajani@uts.edu.au

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Executive Summary

This report presents results from Wagonga Inlet, one of the estuaries selected as part of Stage 1, the NSW Oyster Industry Transformation Project 2017-2021. To predict the impact of rainfall on 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 Lower Honeymoon Bay harvest area, recording high-resolution temperature, salinity and depth data. Oyster farmers collected weekly biological samples (570 environmental DNA samples and 348 deployed/retrieved oysters for growth assessment) from the sensor site, and 378 samples from a second site in Hobbs Bay (Upper Lavender Point harvest area) starting in July 2019. We developed a rapid molecular qPCR (quantitative polymerase chain reaction) assay for a toxin producing species of the harmful algal genus *Pseudo-nitzschia*, and another for *E. coli*, which could directly compare to the currently used plate count by commercial laboratories. We also developed specific qPCR assays that could determine which animals were contributing to the *E. coli* load in the river system. We used these assays to observe trends in faecal pollution and modelled these in relation to environmental variables (salinity, temperature, rainfall etc), to develop predictive models. Finally, we tested the capability of a further model to determine its predictive capability to link oyster growth with these environmental variables.

MAJOR FINDINGS

Available data indicated that four harvest area closures could have potentially been avoided between February 2018 and September 2021

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

Bird, cow and human bacteria were very low across sampling period

No oyster mortality events that exceed background farming mortality occurred at the Wagonga Inlet monitoring site over the study period

1. Introduction

1.1 Transforming Australian Shellfish Production

The Transforming Australian Shellfish Production Project (TASPP) follows on from the success of the NSW Oyster Industry Transformation Project (NSWOITP), which is a UTS led, multidisciplinary collaboration between oyster farmers (NSW Farmers Association), researchers (UTS, DPI Aquaculture and Fisheries), regulators (DPI Biosecurity and Food Safety) and the Food Agility CRC. The project uses real time, high-resolution salinity, temperature and depth sensing, combined with novel molecular genetic methods (eDNA), to model oyster food safety, pathogenic bacteria, harmful algae, and oyster growth and disease, with the aim of changing harvest management and to reduce harvest closure days for farmers.

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

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

Stage 1 (2017-2021) of the program has been successfully completed, with ~5000 water and 3000 oyster samples collected across 13 NSW estuaries engaged in the project. Stage 2 (2021-2024) is now underway, with two further NSW estuaries engaged, and expansion of the project into Western Australia. Sample processing, data analysis and report writing will continue during this second phase, with modelling to predict oyster growth and mortality rates, including key oyster diseases such as *Marteilia sydneyi* (QX), and Winter Mortality, and the intensity of harmful algal blooms planned. As part of these analyses, novel qPCR assays for *E. coli* (bird, cow, human) and harmful algal species (*Pseudo-nitzschia* spp.*, Dinophysis* spp.*, P. minimum*), which were developed during Phase 1, will also be implemented.

Preliminary results from this high frequency data have already demonstrated the link between salinity levels related to rainfall and *E. coli* levels. In 2019, the NSW Shellfish Program's Annual Sanitary Survey Report (DPI) stated that using this real-time, high frequency environmental data, the CRC project provided the basis for a change to the management plans for the Pambula Lake harvest area and the Cromarty Bay harvest area (Port Stephens). These management plan changes mean that harvest area openings and closures can be based on salinity-only data, with unnecessary extra harvest closure days avoided. As early adopters of the technology for harvest area management, an independent economic assessment by

NSW DPI completed in January 2021 evaluated Pambula Lake and Cromarty Bay. The report highlighted the positive benefits for industry through using salinity-based management plans. Focusing on the six-month period where oysters were at peak marketable condition, it was estimated that up to two extra weeks of harvest could be achieved, with a projected annual net profit boost of $15,344 (Cromarty Bay) and $95,736 (Pambula Lake) for the study areas, based on current lease area used. The full report is available on the NSW Food Authority website.

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

1.2 Wagonga Inlet

Wagonga Inlet (-36.13°S, 150.07°E) is a permanently open, wave-dominated drowned river valley estuary, with a catchment area of ~100 km^2, an area of ~7 km^2, and a flushing rate of ~35 days (Roy et al. 2001, Roper et al. 2011) (Fig. 1). It extends ~9 km upstream of the ocean, and due to its large connection to the ocean and low freshwater input, is similar in salinity to the ocean. As such, Wagonga Inlet supports an abundant and diverse range of marine life, including important estuarine vegetation such as saltmarshes, mangroves and seagrass beds, and associated fish nursery areas (Wainwright et al. 2019). It is largely surrounded by forest (90%), but also with urban (5%), rural (4%) and other (1%) land uses. The estuary supports aquaculture (oyster harvesting), recreational activities such as swimming, boating and fishing, and provides important ecosystem services such as fish nurseries within mangrove, seagrass and saltmarsh habitats.

1.3 Oyster Production in Wagonga Inlet

Wagonga Inlet is an important oyster-growing estuary. The industry produces ~340 K dozen oysters per year (Gippel 2021). While water quality is generally considered high quality in this estuary (chlorophyll, turbidity, dissolved oxygen, pH etc.), potential threats include pathogens (faecal coliforms and *E. coli*) from urban stormwater, sewer overflows, septic tank seepage, and rural runoff. Environmental programs over the past two decades have increased monitoring and awareness of septic systems, improved sewer overflows, and identified priority urban stormwater drains for treatment installation systems (ESC, 2010).

Sydney rock oysters (*Saccostrea glomerata*) in Wagonga Inlet, however, have previously been contaminated with toxins from harmful algal blooms (HABs), with the most significant bloom caused by the Domoic Acid-producing, *Pseudo-nitzschia* cf. *cuspidata* (Bacillariophyceae), formerly reported as *P. cuspidata* (Ajani et al. 2013b). A large toxic bloom of this species in 2010 closed the shellfish industry in this estuary for 16 weeks, and resulted in one of the longest and most financially detrimental toxic algal events in SE Australia. During this event, a maximum cell concentration of 6 x 10^6 cells L^{-1} of *P.* cf. *cuspidata* was found in water samples and 34 mg DA kg^{-1} in oyster tissue (Ajani et al. 2013b).

FINDINGS

2. Findings

2.1. The data assessment supports implementing a harvest area management plan based on sensor salinity data, subject to the agreement by the local shellfish industry. Twenty-six harvest closure days occurred over four rainfall closures, although salinity sensor data did not decline below 18 ‰ and microbiological results from samples collected between 2-7 days post closure met Approved harvest criteria.

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 Wagonga Inlet 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 12.8% for *E. coli*, 21.4% for cow, 33.2% for bird, and 32.5% for human at the sensor site, and 29% for *E. coli*, 50.5% for cow, 44.8% for bird, and 77.3% for human within Hobbs Bay.

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

2.6 We developed a rapid, sensitive and efficient quantitative real-time polymerase chain reaction (qPCR) assay to detect a problematic diatom *P. pseudodelicatissima* complex Clade I, and evaluated the assay's effectiveness by successfully comparing environmental samples to microscopy-based cell counts across the same time period.

2.6. Wagonga Inlet had the largest oysters, with respect to shell height, and the second heaviest oysters at the end of this experiment compared to the 11 other estuaries monitored in this study. However, none of the environmental variables measured/modelled were predictive of oyster growth.

2.7. Wagonga Inlet had one of the lowest levels of oyster mortality recorded compared to the other estuaries that were evaluated in this study. No oyster mortality events that exceeded background farming mortality (approximately 10% per annum) occurred at the Wagonga Inlet sensor site over the study period.

ACKNOWLEDGEMENTS

3. Acknowledgements

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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.

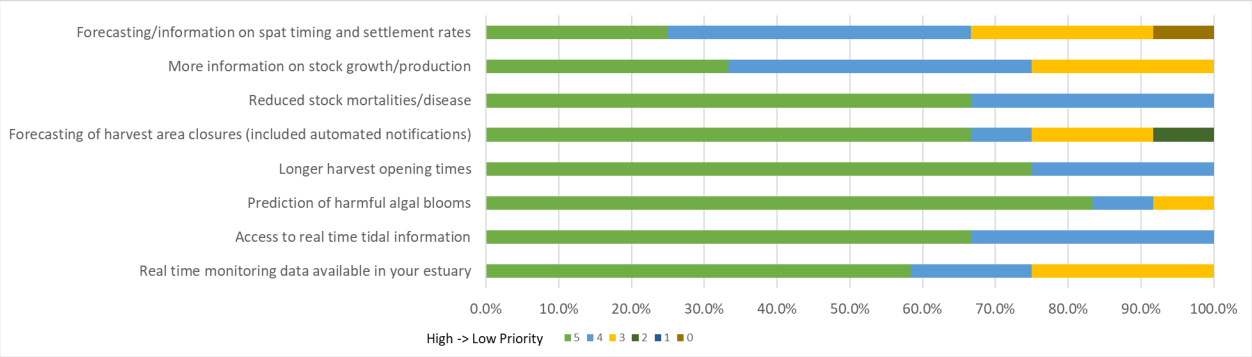


Figure 4.1. The importance of factors as rated by farmers in relation to their business operations. Green is most important and brown is least important.

RESULTS

5. Results

5.1 High resolution temperature and salinity data

High-resolution real time data summaries for Wagonga Inlet for the period 14 February 2018 to 31 March 2021 are shown in Figs. 5.1A-C. Data between 4 and 23 September 2020 was unavailable due to Telstra outage. Depth recordings ranged from 0.3 m (10 Jan 2020) to 2.1 m (20 Feb 2019). The lowest and highest daily average salinity recordings were 0.43 ppt (23 Mar 2021) and 36.4 ppt (7 Feb 2020) respectively, while the lowest and highest daily average temperature recordings were 10.3°C (21 Jul 2018) and 27.8 °C (25 Jan 2021) respectively.

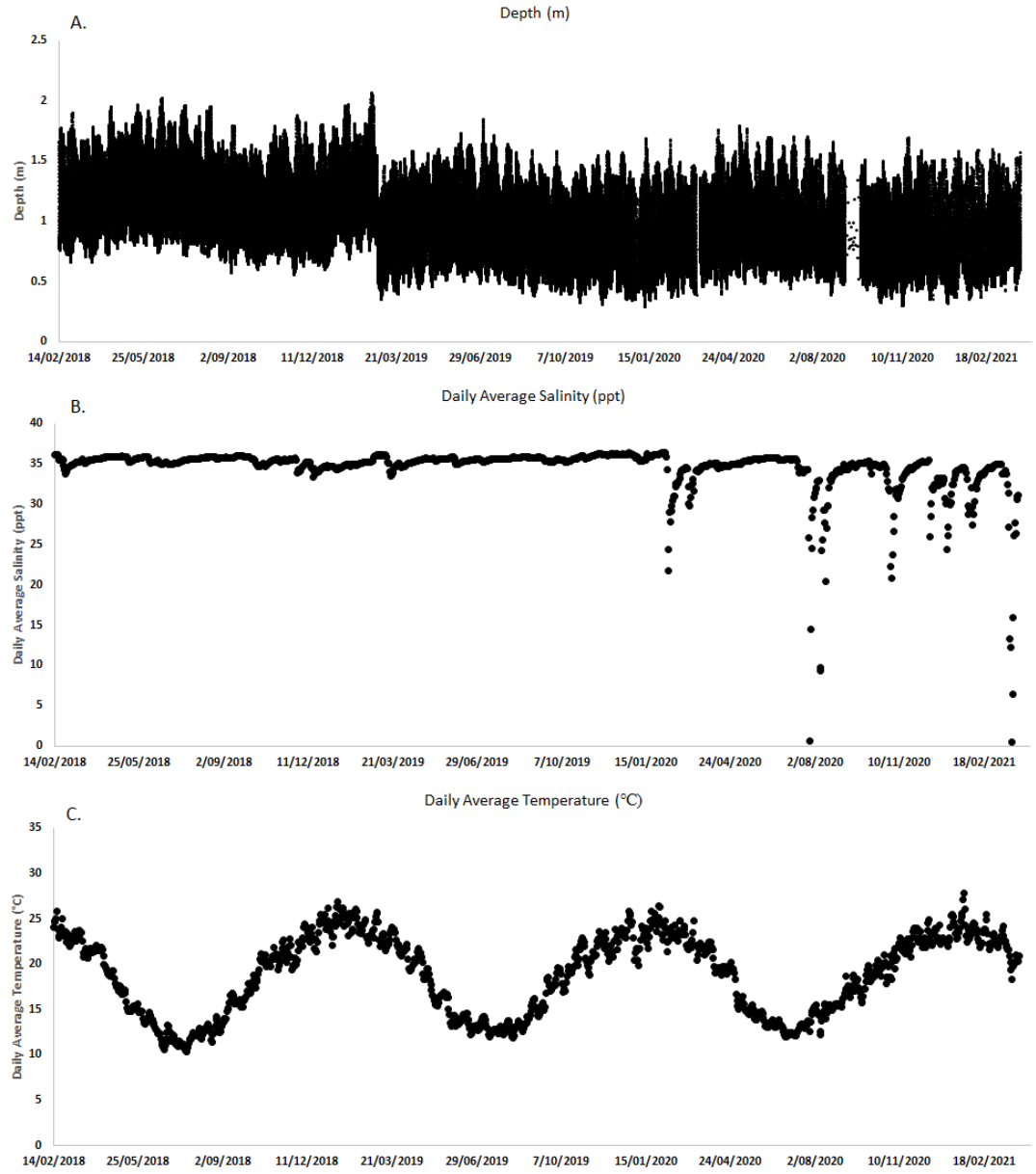


Figure 5.1A-C. Real time sensor data from Wagonga Inlet 14 February 2018 to 31 March 2021. A. Depth (m); B. Daily average salinity (ppt); and C. Daily average temperature (°C).

Only two moderate rainfall events were sampled across the study period. These occurred on 11-12 Oct 2018 (only sensor site sampled) and 13-14 Jul 2020, when both the sensor site and Hobbs Bay were sampled. The maximum daily rainfall occurred on 27 July 2020 and was reported as 80.6 mm at Narooma rain station (Number: 069022, Lat: 36.22° S, Long: 150.13° E) (Fig. 5.2).

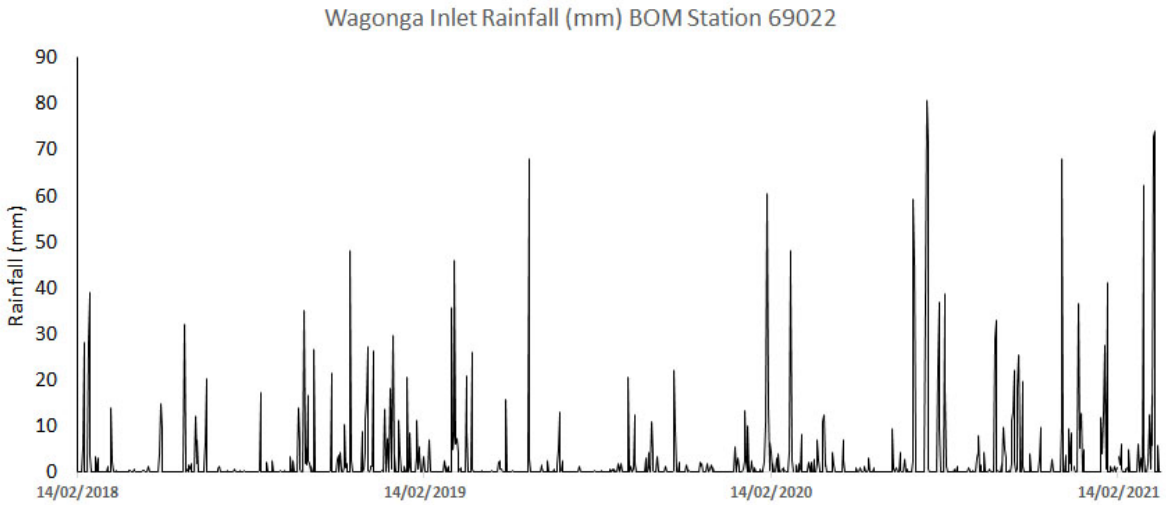


Figure 5.2. Daily rainfall (mm) from the Bureau of Meteorology rainfall gauge site at Narooma (BOM Station No. 069022).

5.2 Management Plan

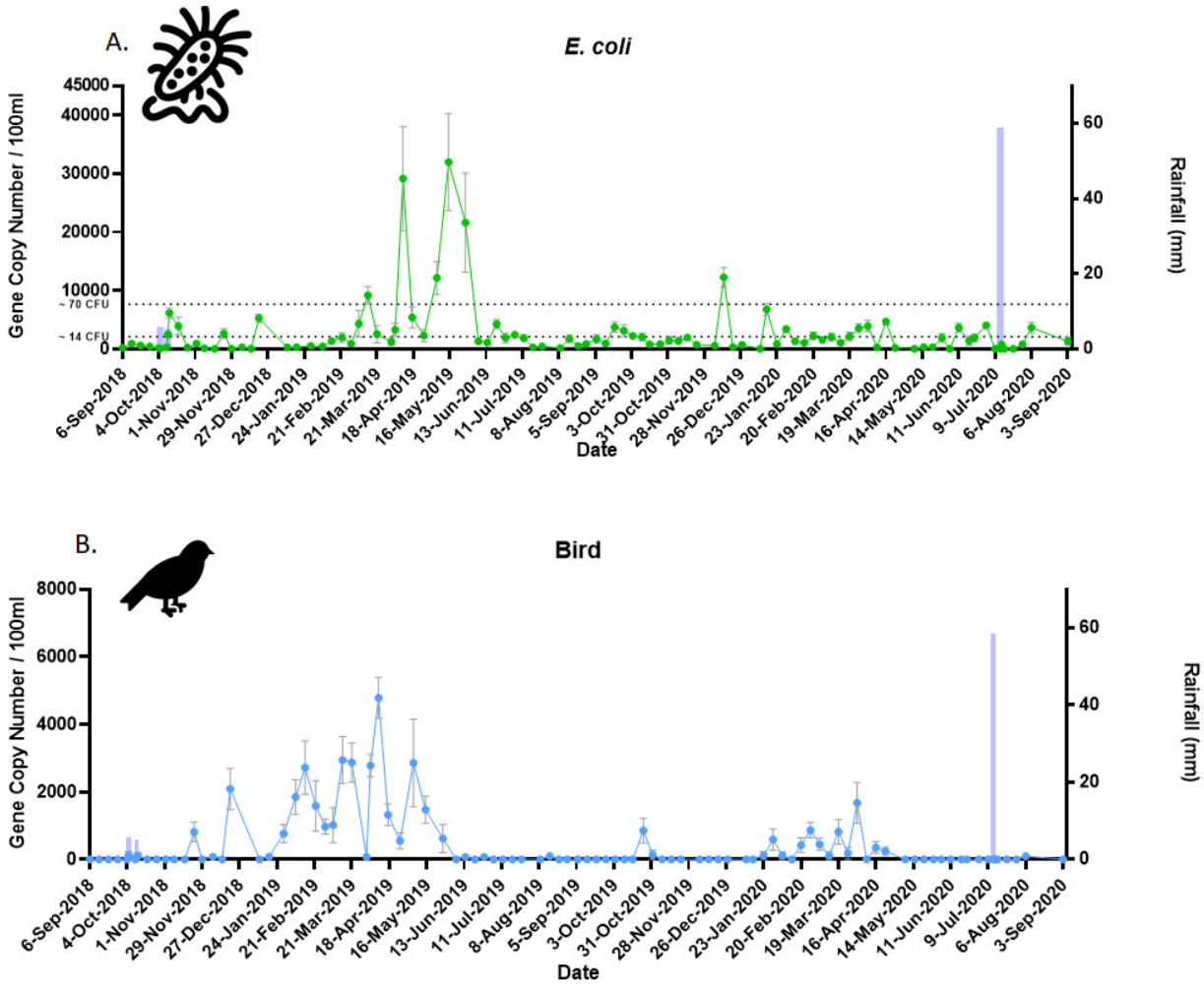
Data analysed during the 2021 annual review of Lower Honeymoon Bay harvest area (Fig. A1) indicated that there could have been fewer harvest area closures and downgrades since the sensor was installed, if closures were based on salinity sensor data. There were seven harvest area rainfall closures in Lower Honeymoon Bay harvest area between February 2018 and September 2021. During the same period there were five harvest area salinity closures, as advised by the local program monitoring local conditions. Based on a management plan sensor salinity closure limit of 18 ‰, harvest area closures were reviewed focusing on available salinity sensor data and shellfish program microbiological results since February 2018. Twenty-six harvest closure days occurred over four rainfall closures, although salinity sensor data did not decline below 18 ‰ and microbiological results from samples collected between 2-7 days post closure met Approved harvest criteria. During the same time period, there were seventeen rainfall downgrades in Lower Honeymoon Bay harvest area. A review of salinity sensor data and shellfish program microbiological results indicated that there were ten rainfall downgrades where salinity as reported by the sensor was higher than 25 ‰ (downgrade salinity range 18-25 ‰), and microbiological results from samples collected 1-7 days post downgrade met Approved harvest criteria. Time periods where salinity is slower to recover may require additional sampling to meet management plan requirements.**5.3**

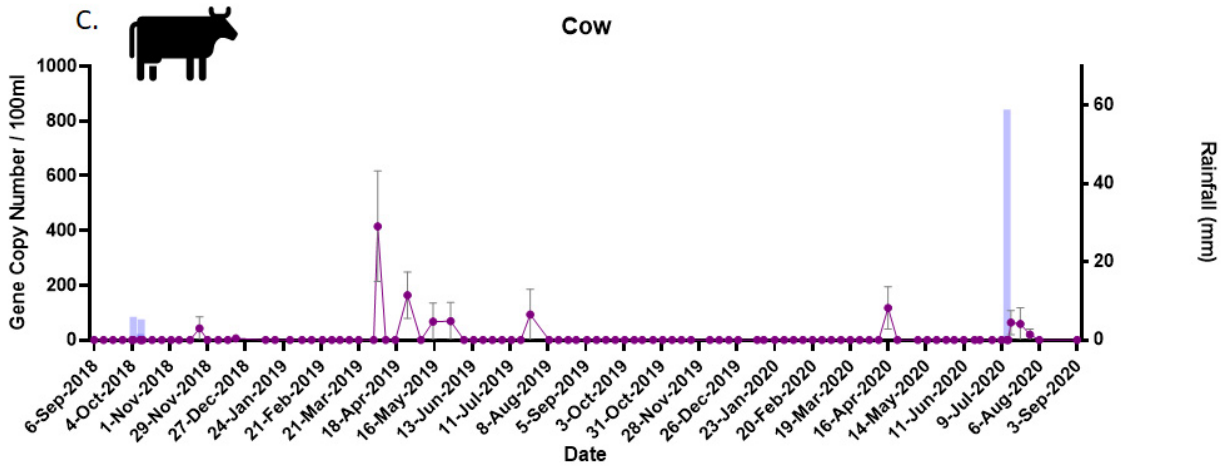
5.3 Bacterial source tracking

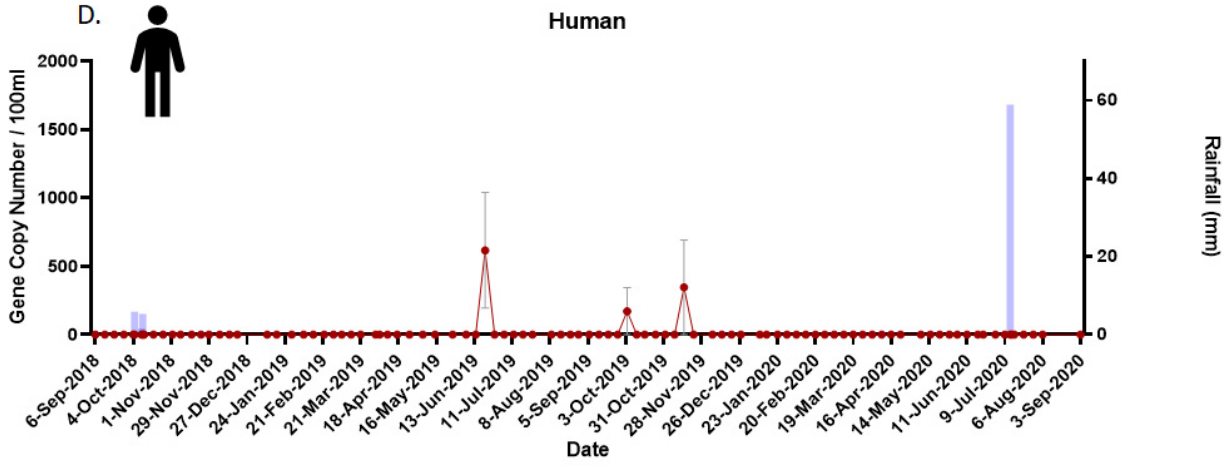
A total of 570 water samples and 348 oysters were collected over a two-year period (a subset of the entire sensor data collection time) from Sept 2018 to Sept 2020 from the sensor location in Wagonga Inlet. In 2019, an additional site was sampled at Hobbs Bay to investigate *E. coli* source tracking issues, and over the period June 2019 to Sept 2020 an additional 378 water samples were collected (Fig. A1).

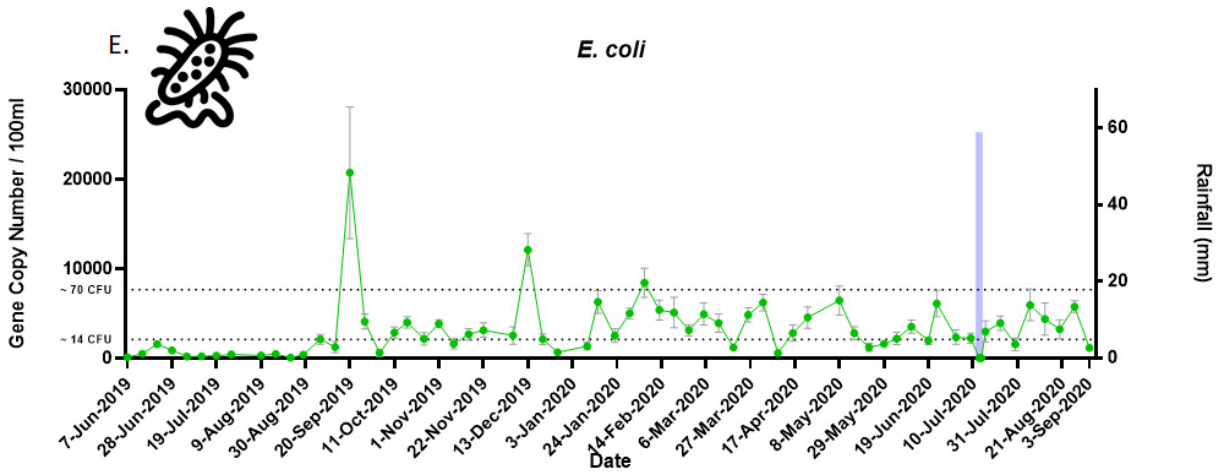
For Wagonga Inlet the maximum *E. coli* reached 31,390 gene copies 100 mL^{-1} on 15 May 2019, 4,780 copies 100 mL^{-1} for *Helicobacter* (bird) on 10 Apr 2019, 415 gene copies 100 mL^{-1} for bovine faecal pollution (cow) on 4 Apr 2019, and finally, 617 copies 100 mL^{-1} for human faecal pollution on 21 Jun 2019 (Fig. 5.3 A-D).

For Hobbs Bay the maximum *E. coli* reached 20,739 gene copies 100 mL^{-1} on 20 Sept 2019, 2,452 copies 100 mL^{-1} for *Helicobacter* (bird) on 2 Apr 2020, 12,147 gene copies 100 mL^{-1} for bovine faecal pollution (cow) on 23 Jul 2020, and finally, 183 gene copies 100 mL^{-1} for human faecal pollution on 21 Jun 2019 (Fig. 5.3E-H).




C.
Cow
Gene Copy Number / 100ml
Rainfall (mm)
Date


D.
Human
Gene Copy Number / 100ml
Rainfall (mm)
Date


E.
E. coli
Gene Copy Number / 100ml
~ 70 CFU
~ 14 CFU
Rainfall (mm)
Date

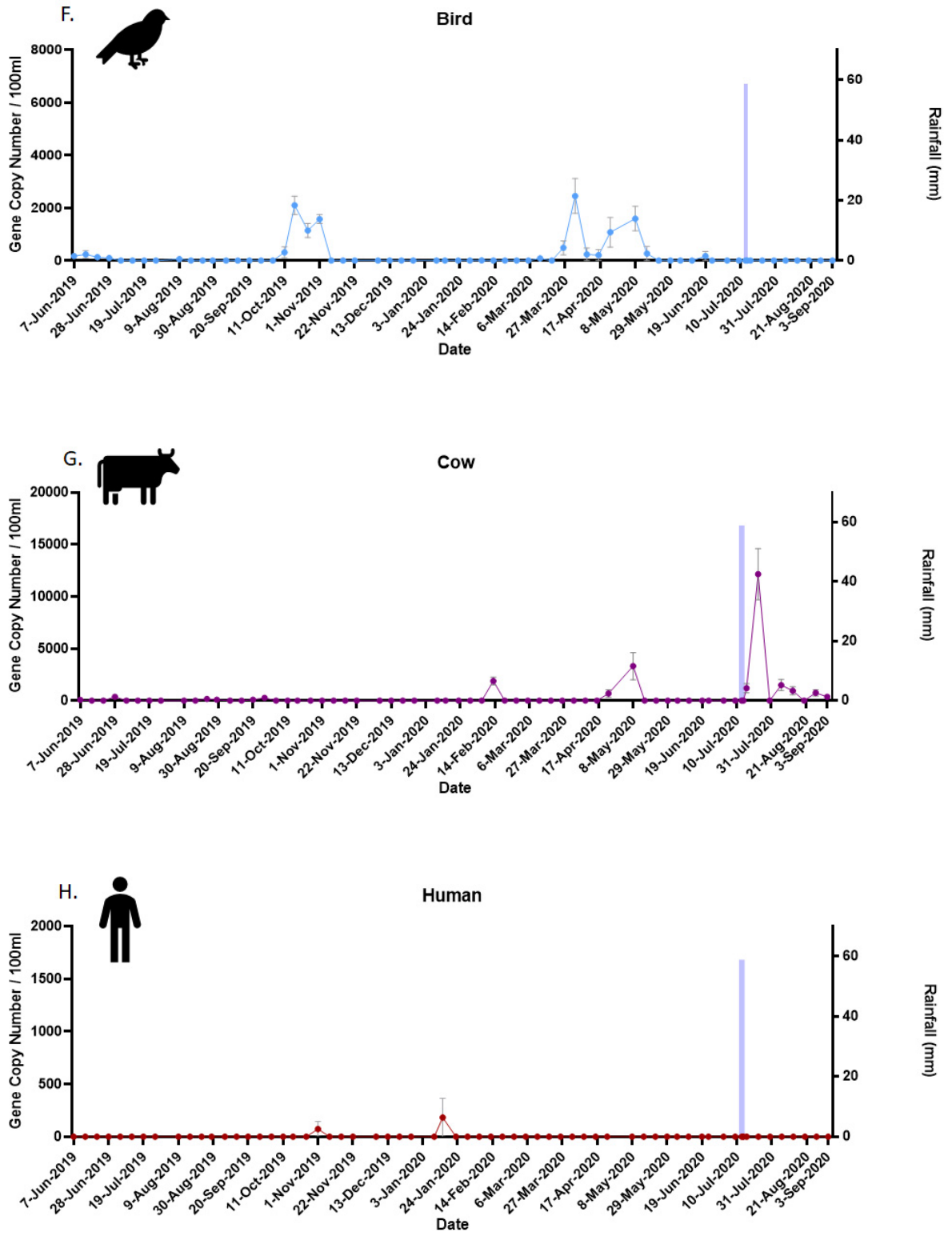


Figure 5.3 A-D. Weekly *E. coli* data from the sensor location, Wagonga Inlet, and **Figure 5.3 E-H.** Hobbs Bay, using A. *E. coli* assay; B. Bird assay; C. Cow assay; and D. Human assay. Purple bars represent rainfall events that were sampled. Dotted lines in Fig. A at 14 and 70 cfu/100 ml are the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

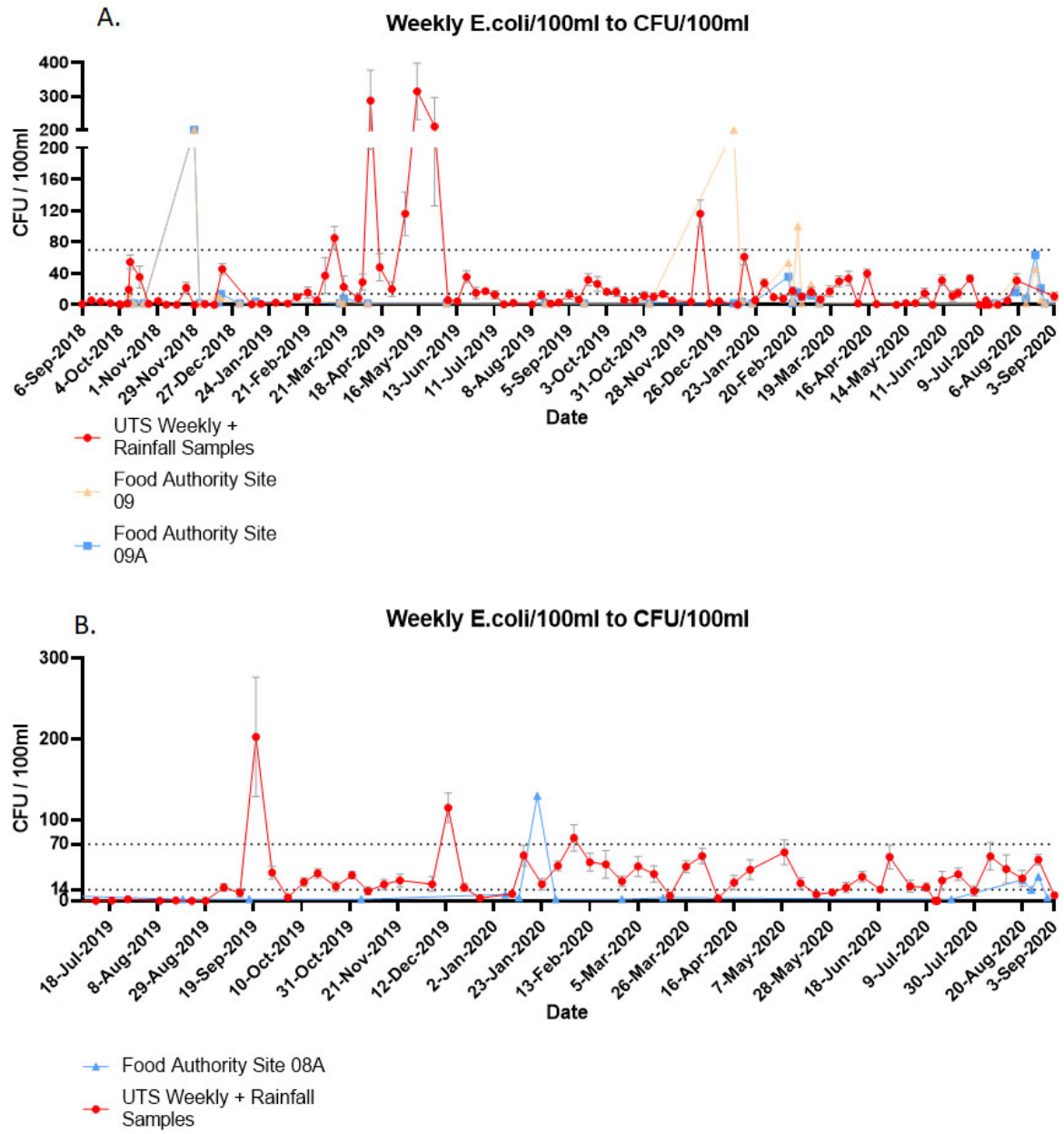


Figure 5.4 Weekly faecal coliform counts (cfu/100mL) from water samples collected by DPI Food Authority at two sites in Wagonga Inlet **A.** Sensor site and **B.** Hobbs Bay, compared to Oyster Transformation Project weekly sampling results (including rainfall sampling). Dotted lines at 14 and 70 cfu/100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

Maximum faecal coliform counts in samples reported from CRC project samples for both the sensor site and the Hobbs Bay site did not correspond to maximum DPI Food Authority counts when collected at the same time, however the number of paired samples was low (Fig. 5.4A-B).

Two rainfall events were also sampled across the study period in Wagonga Inlet, and one in Hobbs Bay (see purple bars in Fig 5.3 A-H). These were: 11-12 Oct 2018 and 13-14 Jul 2020 for Wagonga Inlet and 13-14 Jul 2020 for Hobbs Bay (Fig. 5.5 A-C). Generally, *E. coli* increased

by the second day of rainfall sampling at the sensor site, but without further sample collection, it is unclear how quickly these levels dissipated. Bird, cow and human bacteria remained below the detection limit during all events (Fig. 5.4 D). *E. coli* was detected during the Hobbs Bay event.

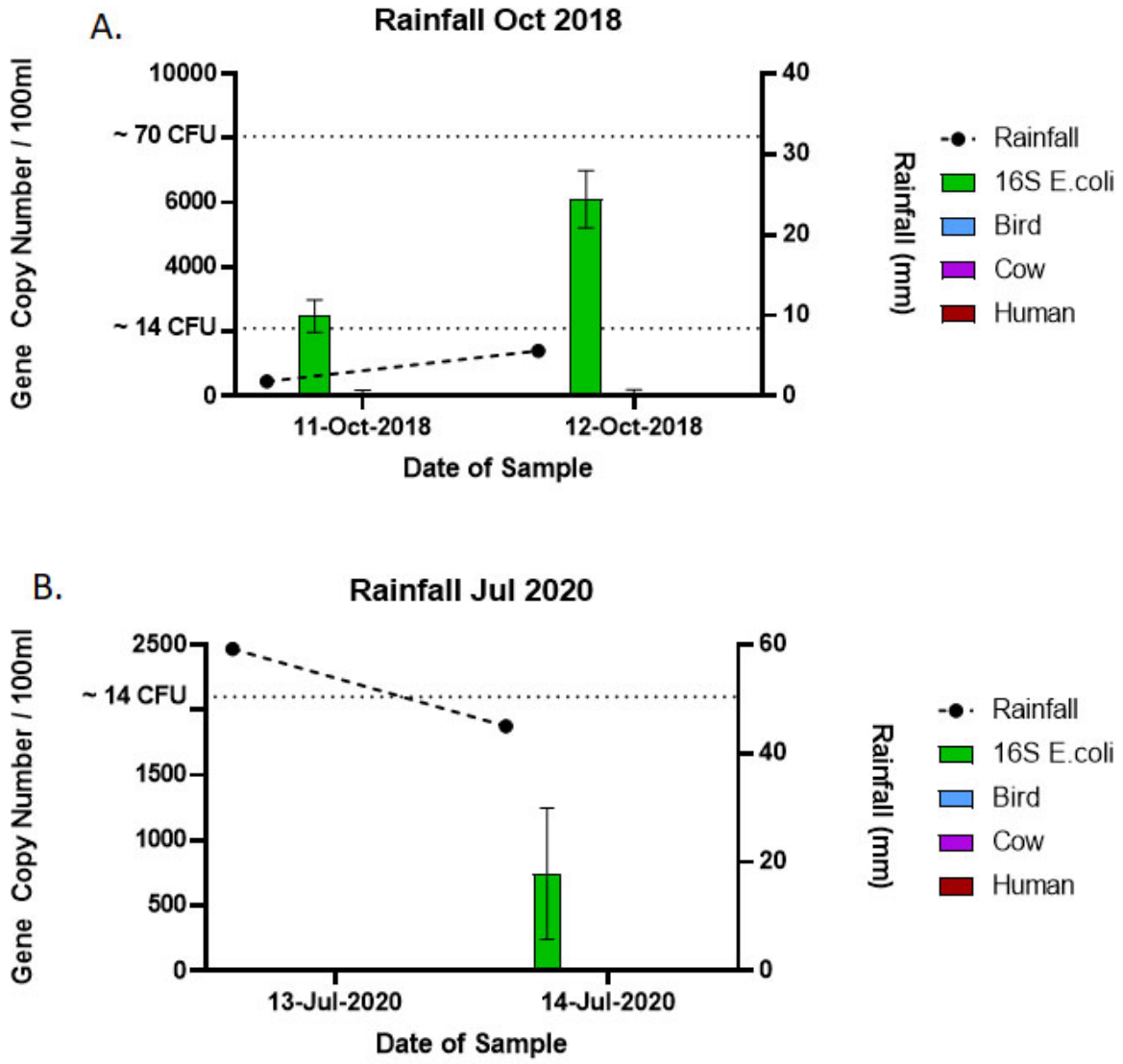


Figure 5.5 A-B. Sensor site (Wagonga Inlet) rainfall events sampled for *E. coli* assays. Green bar = 16S *E. coli*; blue bar = bird assay; purple bar – cow assay; grey bar = human assay. Dotted line is rainfall (mm) obtained from the closest Bureau of Meteorology weather station at Narooma (BOM Station No. 069022). 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 (at the sampling site closest to the sensor - WAG2) across the sampling period from February 2018 to March 2021 occurred on 12/2/20 (up to 60mm rainfall two days prior to this sampling day) (Fig. 5.6). Total cell concentrations reached 8.7E +06 cells L^{-1} and samples contained a very high abundance of small flagellates

including cryptomonads, dinoflagellates, prasinophytes and prymnesiophytes. Very few diatoms were reported although there was a high level of sediment and organic detritus.

Other potentially harmful bloom events across the sampling period included blooms of the diatom *Pseudo-nitzschia delicatissima* gp. These occurred during Oct/Nov 2018, April 2019, Feb /Mar 2019 and Sept 2019, with a maximum concentration of 1.5E +05 cells L^{-1} reported. Another harmful diatom, *Pseudo-nitzschia fraudulenta/australis*, bloomed on 18 Oct 2020 reaching a maximum cell concentration of 6.6E +04 cells L^{-1}. The toxic dinoflagellate *Alexandrium pacificum* reached 7.0E +02 cells L^{-1} on 17 Dec 2018 and *Dinophysis caudata* cell densities were elevated on 23 Nov 2020 at 1.2E +02 cells L^{-1}, and again on 7 Dec 2020 at 1.4E +02 cells L^{-1}. NSW Food Authority's Phytoplankton Action Limits to trigger biotoxin testing are 500,000 cells L^{-1} for *Pseudo-nitzschia delicatissima,* 50,000 cells L^{-1} for *P. australis & multiseries,* 500 cells L^{-1} for *Dinophysis caudata* and 200 cells L^{-1} for *Alexandrium pacificum* (NSWFA 2015). No biotoxins were detected in association with any of these blooms in samples collected across sampling sites in Upper and Lower Honeymoon Bay harvest areas.

During September 2020 however, results from samples collected from the downstream harvest areas (Upper Lavender Point, Lower Lavender Point A and B) reported low levels of domoic acid (DA) (13, 21 and 27 Sept 2020, 1.1, 1.6 and 2.4 mg/kg DA, respectively). These levels were associated with a bloom of *Pseudo-nitzschia delicatissima* gp, reported at cell concentrations above the Phytoplankton Action Limit (500,000 cells L^{-1}) at site 1 in two samples collected on 13 Sept 2020 (5.9E +05 cells L^{-1}) and 27 September 2020 (8.9 +05 cells L^{-1}).

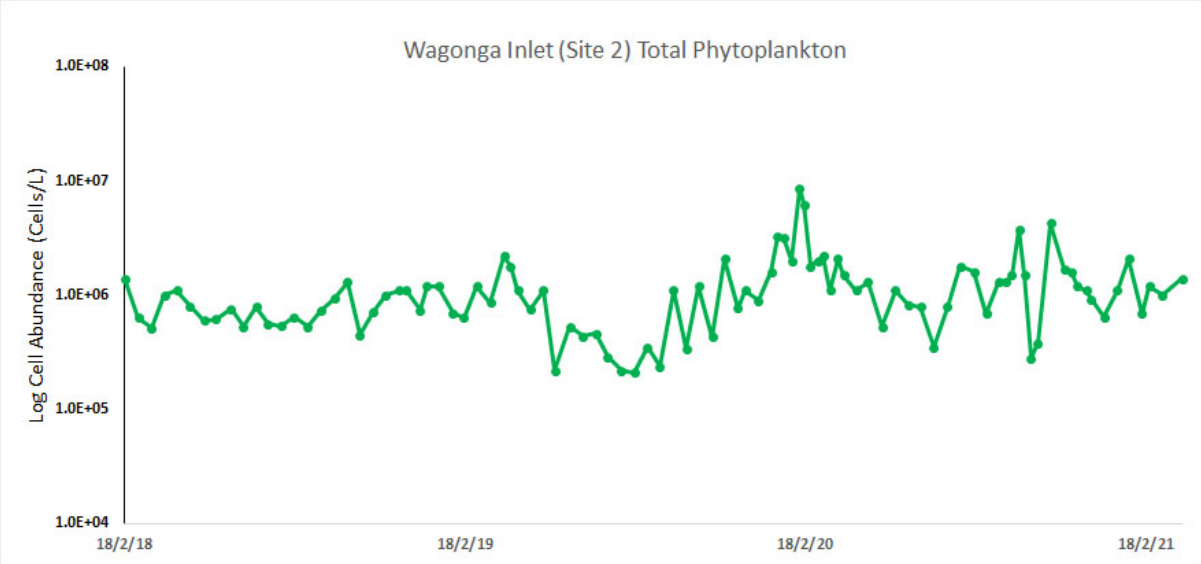


Figure 5.6 Log abundance of total phytoplankton sampled approximately fortnightly at site 2 (closest to the sensor) from 14 February 2018 to 31 March 2021.

Case Study - The bloom of *P. delicatissima* gp. which occurred in April 2019 also extended downstream to another phytoplankton sampling location (WAG1), which is also sampled in accordance with the NSW Marine Biotoxin Management Plan (NSW BMP) and the Australian Shellfish Quality Assurance Program (ASQAP). Maximum cell density of *P. delicatissima* gp. at this site was reported as 4.3E +05 cells L^{-1}. This bloom was subsequently investigated further

to determine the causative species, as well as assess the use of real time salinity/temperature data as a useful and timely predictor of *Pseudo-nitzschia* abundance in this estuary compared to rain-based management systems that are currently used (Ajani et al. 2021).

Using light microscopy, combined with molecular (ITS/5.8S and LSU D1-D3 rDNA regions) and toxicological evidence, this bloom was found to consist of multiple species of *Pseudo-nitzschia* including *P.* cf. *cuspidata, P. hasleana, P. fraudulenta* and *P. multiseries*, with *P.* cf. *cuspidata* being the only species that produced domoic acid (3.1 pg DA per cell). As several species of *Pseudo-nitzschia* co-occurred, only one of which produced DA, we then developed a rapid, sensitive and efficient quantitative real-time polymerase chain reaction (qPCR) assay to detect only species belonging to the *P. pseudodelicatissima* complex Clade I, to which *P.* cf. *cuspidata* belongs, and this indicated that *P.* cf. *cuspidata* or closely related strains may have dominated the *Pseudo-nitzschia* community at this time.

Finally, using high resolution water temperature and salinity sensor data, we modelled the relationship between light microscopy determined abundance of *P. delicatissima* group and environmental variables (temperature, salinity, rainfall) at the two phytoplankton sites within the estuary. A total of eight General Linear Models (GLMs) explaining between 9 and 54% of the deviance suggested that the temperature (increasing) and/or salinity (decreasing) data were generally more predictive of high cell concentrations than the rainfall data at both sites, and that overall, cell concentrations were more predictive at the more oceanic site than the more upstream site, using this method. We conclude that the combination of rapid molecular methods such as qPCR and real-time sensor data modelling, can provide a more rapid and effective early warning of harmful algal blooms of species of *Pseudo-nitzschia*, resulting in more beneficial regulatory and management outcomes (Ajani et al. 2021).

5.6 Oyster Growth and Mortality

5.6.1 Oyster Growth

Oyster whole weight increased by 39.3 g in the experimental period (August 2018 to June 2020) (Fig. 5.7 A). Oyster whole weight increases were greatest in autumn 2020 when oysters increased their weight by approximately 10 g over a period of four months. Oyster whole weight was 61.9 ± 1.4 g at the end of the experiment (June 2020). Oysters deployed in Wagonga Inlet attained a plate grade (> 40 g) market size in August 2019. Oysters were 32 mo when they had reached this weight.

Oyster shell height was 57 ± 1 mm at the start of the experiment and increased to 87 ± 4 mm in June 2020 (Fig. 5.7 B). The greatest increase in shell height was recorded after deployment to Wagonga Inlet during the period from September 2018 to November 2018. Shell heights were measured more frequently than whole weight and fluctuated throughout the experiment. Periods shell length decreases were recorded in Wagonga Inlet during this experiment between August and September 2018, February and May 2019, June and July 2019 as well as January and February 2020.

5.6.2 Mortality

Oyster mortality in Wagonga Inlet was only detected in February 2019, February 2020 and June 2020 (Fig. 5.7 C-D). Baskets were stocked with approximately 55 oysters at the start of the experiment. In February 2019, two baskets contained 4 dead oysters and a third contained 8 dead oysters. Oyster mortality over the study period in Wagonga Inlet was well below the expected background Sydney Rock Oyster farming mortality which is approximately 10% stock loss per annum.

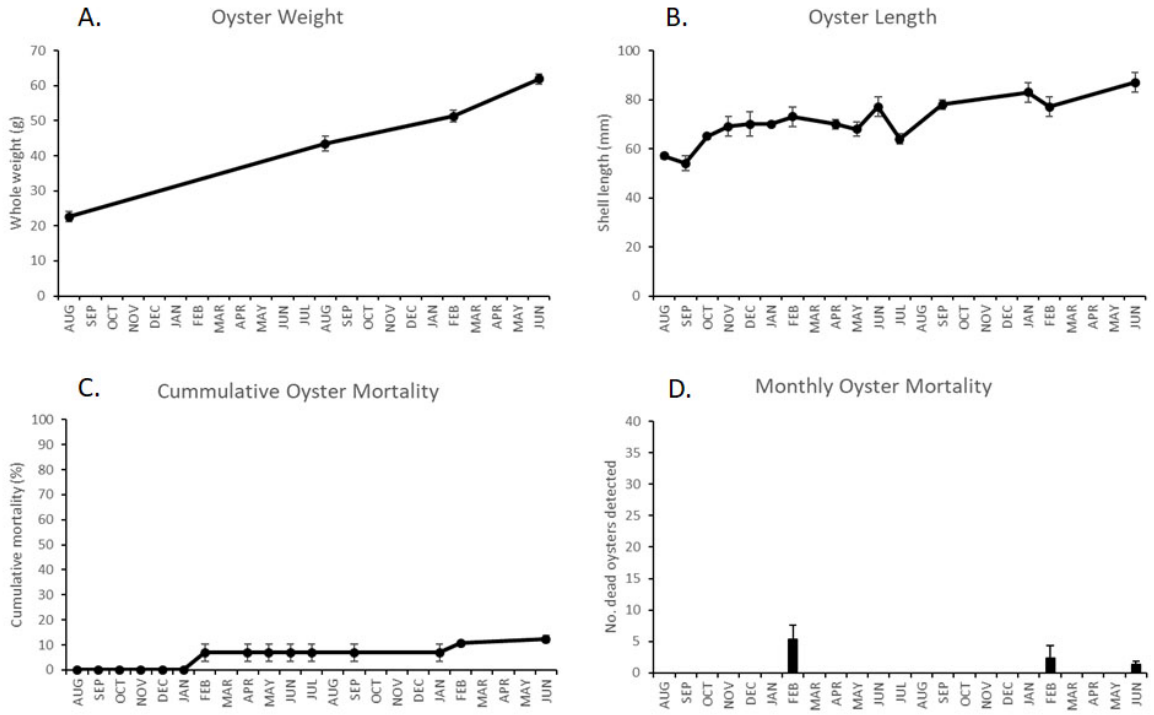


Figure 5.7 A-D. Oysters deployed at the sensor site, Wagonga Inlet. A. whole weight; B. shell height; C. cumulative mortality and D. monthly mortality.

5.7 Modelling

5.7.1 Modelling of E. coli data

Summary statistics for all bacterial concentrations and environmental variables used in the general additive models are shown in Appendix 2A-B. Correlation coefficients were calculated among every pair of environmental variables and suggested very few strong positive relationships ($r > 0.7$) overall. A total of 4 models were developed for each of the bacterial sources: sensor only; sensor and total phytoplankton (logged or unlogged); rainfall only; and rainfall and total phytoplankton. Depth and week were included as response variables in all models. The maximum predictive capability for each bacterial group at the sensor site were: 12.8% for *E. coli* (sensor + total phytoplankton), 21.4% for cow (rainfall + total phytoplankton), 33.2% for bird (sensor + t total phytoplankton) and 32.5% for human (sensor + total phytoplankton). For Hobbs Bay, the maximum predictive capability for each bacterial group were: 29% for *E. coli* (sensor + total phytoplankton), 50.5% for cow (sensor + total

phytoplankton), 44.8% for bird (sensor + total phytoplankton) and 77.3% for human (sensor + total phytoplankton) (Table 1A-B).

The abundance of *E. coli* at the sensor site was not very well explained by the models (<13% deviance explained), but appeared to be linked to decreasing rainfall and increasing salinity. Similarly, at the Hobbs Bay sampling site, the best model to explain *E. coli* abundance (29% deviance explained, compared to 22% using rainfall data) included total phytoplankton and salinity (both increasing). Data also indicated that peak *E. coli* coincided with a surface water temperature of ~18℃ (Table 1 A-B) (Figures 5.7 A-D, 5.8 A-D, 5.9 A-D, 5.10 A-D).

Cow bacterial abundance at the sensor site was marginally better predicted using rainfall data (21.4% compared to 17.1% with sensor data), although none of the models were very predictive (all <21.4%). By comparison, the best model at Hobbs Bay was predicted by the sensor data (50.5% compared to rainfall 16.8%), with increasing phytoplankton, salinity and temperature (peaking ~18℃) (Table 1A-B).

Faecal contamination from birds at the sensor site was best explained by the salinity model (33.2% deviance explained, compared to 12.4% using rainfall data), with total phytoplankton and salinity (increasing) significantly contributing to predict this variable. Similarly, the best model to explain bird faecal load was using the sensor at Hobbs Bay (44.8%, compared to 9.3% using rainfall), again included increasing total phytoplankton, salinity and temperature (peaking ~22℃) (Table 1A-B).

An increase in human bacteria abundance was best explained by the salinity model (32.5% compared to rainfall at 21.5%), and was linked to an increase in total phytoplankton, salinity and temperature. At Hobbs bay the best model, which explained 77.3% of the deviance was strongly linked to salinity (max 36 ppt) (Table 1A-B).

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 modelling process was carried out on both the raw scale, and the growth of the oysters as a ratio of the last measurement, but no model was significant or with high deviance, meaning that the data examined does not appear to be predictive of oyster growth.

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

Bacteria	Variables	No. of observations	Significant Variables	Deviance Explained
E. coli	Salinity, Depth, Temp	98	Salinity***, Depth***, Temp***	10.8%
E. coli	Salinity, Depth, Temp, logPhytoplankton	98	logPhytoplankton ***, depth**, salinity***, temp***	12.8%
E. coli	Rainfall72	96	Rainfall72***	10.2%
E. coli	Rainfall72, logPhytoplankton	96	Rainfall72***, logPhytoplankton ***	12.3%
Bird	Salinity, Depth, Temp	98	Salinity***, Depth***, Temp***	33.1%
	Salinity, Depth, Temp, logPhytoplankton	98	Salinity***, Depth***, Temp***, logPhytoplankton ***	33.2%
Bird	Rainfall24	96	Rainfall24***	4.2%
Bird	Rainfall24, logPhytoplankton	96	Rainfall24***, logPhytoplankton***	12.4%
Cow	Salinity, Depth, Temp	98	Salinity***, Depth***, Temp***	17%
Cow	Salinity, Depth, Temp, logPhytoplankton	98	Salinity***, Depth***, Temp***, logPhytoplankton***	17.1%
Cow	Rainfall48	97	Rainfall48***	17.5%
Cow	Rainfall48, logPhytoplankton	97	Rainfall48***, logPhytoplankton***	21.4%
Human	Salinity, Depth, Temp	98	Salinity***, Depth***, Temp***	28.5%
Human	Salinity, Depth, Temp, logPhytoplankton	98	Salinity***, Depth***, Temp***, logPhytoplankton***	32.5%
Human	Rainfall24	98	None	17.7%
Human	Rainfall48, logPhytoplankton	98	logPhytoplankton***	21.5%

Table 1 B. Modelling results for bacterial source tracking in Hobbs Bay, Wagonga Inlet. Only significant variables are shown for each model.

Bacteria	Variables	No. of observations	Significant Variables	Deviance Explained
E. coli	Salinity, Depth, Temp	64	Salinity***, Depth**, Temp***	20.9%
E. coli	Salinity, Depth, Temp, logPhytoplankton	64	logPhytoplankton ***, depth**, salinity***, temp***	29%
E. coli	Rainfall72	62	Rainfall72***	19.9%
E. coli	Rainfall72, logPhytoplankton	62	Rainfall72***, logPhytoplankton ***	22%
Bird	Salinity, Depth, Temp	64	Salinity***, Depth***, Temp***	42.7%
	Salinity, Depth, Temp, logPhytoplankton	64	Salinity***, Depth***, Temp***, logPhytoplankton ***	44.8%
Bird	Rainfall24	64	Rainfall24***	8.8%
Bird	Rainfall24, logPhytoplankton	64	Rainfall24***, logPhytoplankton***	9.3%
Cow	Salinity, Depth, Temp	64	Salinity***, Depth***, Temp***	49.5%
Cow	Salinity, Depth, Temp, logPhytoplankton	64	Salinity***, Depth***, Temp***, logPhytoplankton***	50.5%
Cow	Rainfall48	63	Rainfall48***	8.9%
Cow	Rainfall48, logPhytoplankton	63	Rainfall48***, logPhytoplankton***	16.8%
Human	Salinity, Depth, Temp	64	Salinity*, Temp**	76.7%
Human	Salinity, Depth, Temp, logPhytoplankton	64	Salinity*	77.3%
Human	Rainfall24	98	None	10.5%
Human	Rainfall24, logPhytoplankton	64	logPhytoplankton***	24.1%

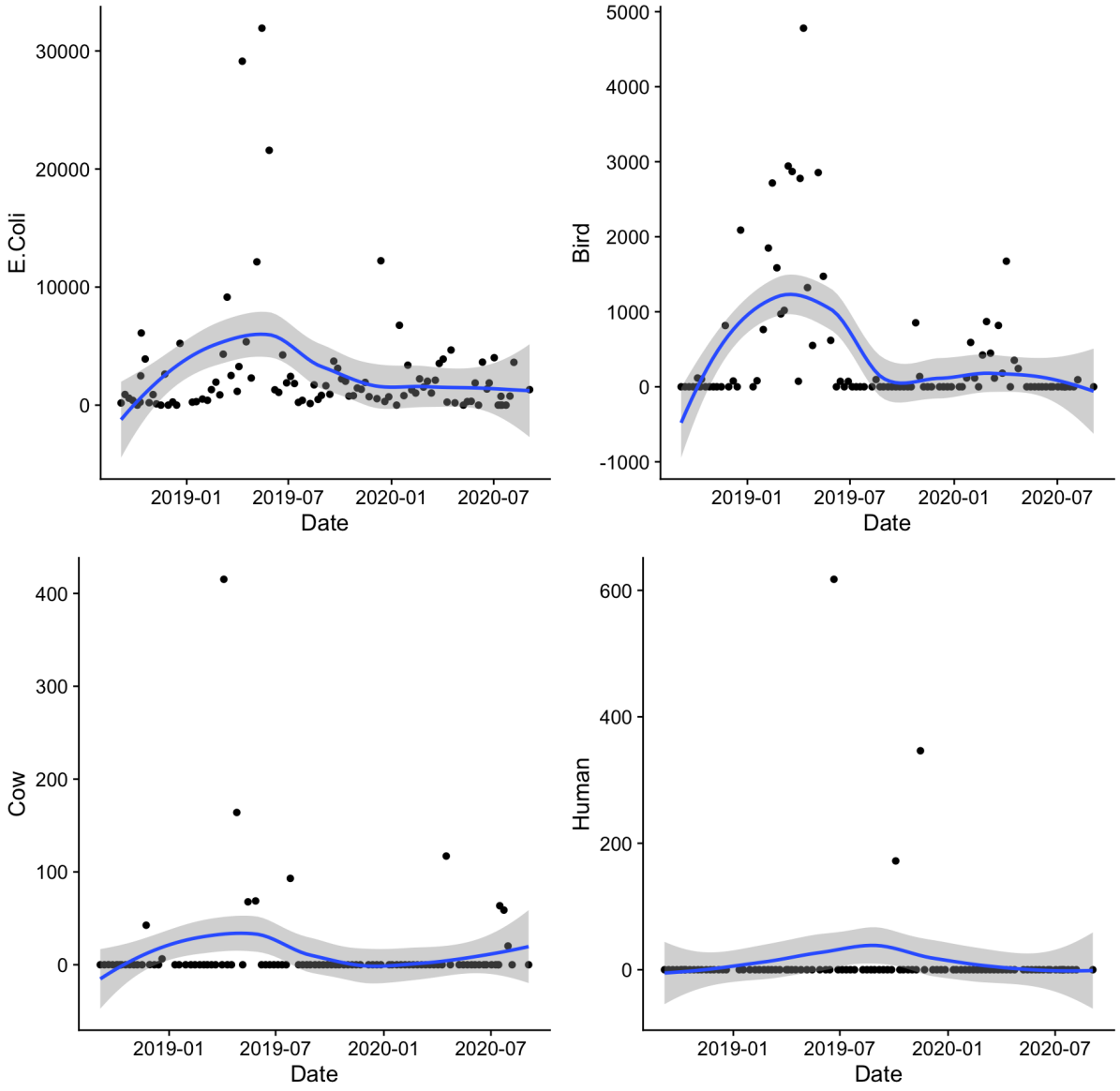


Figure 5.7 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. *E. coli*, B. Bird, C. Cow, and D. Human bacterial load as measured by weekly sampling at the sensor site, Wagonga Inlet.

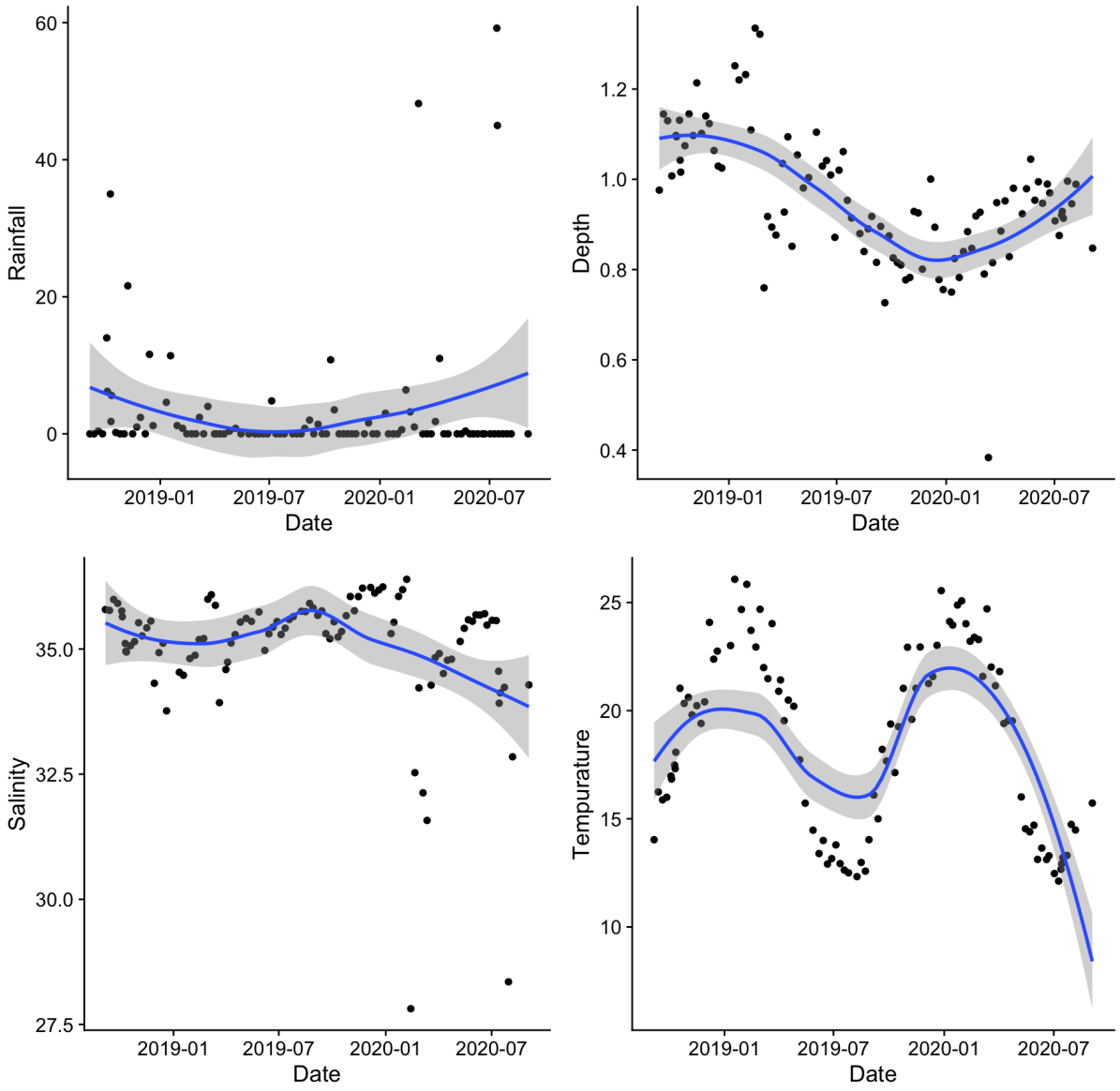


Figure 5.8 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. Rainfall, B. Depth, C. Salinity, and D. Temperature values measured at the sensor site, Wagonga Inlet.

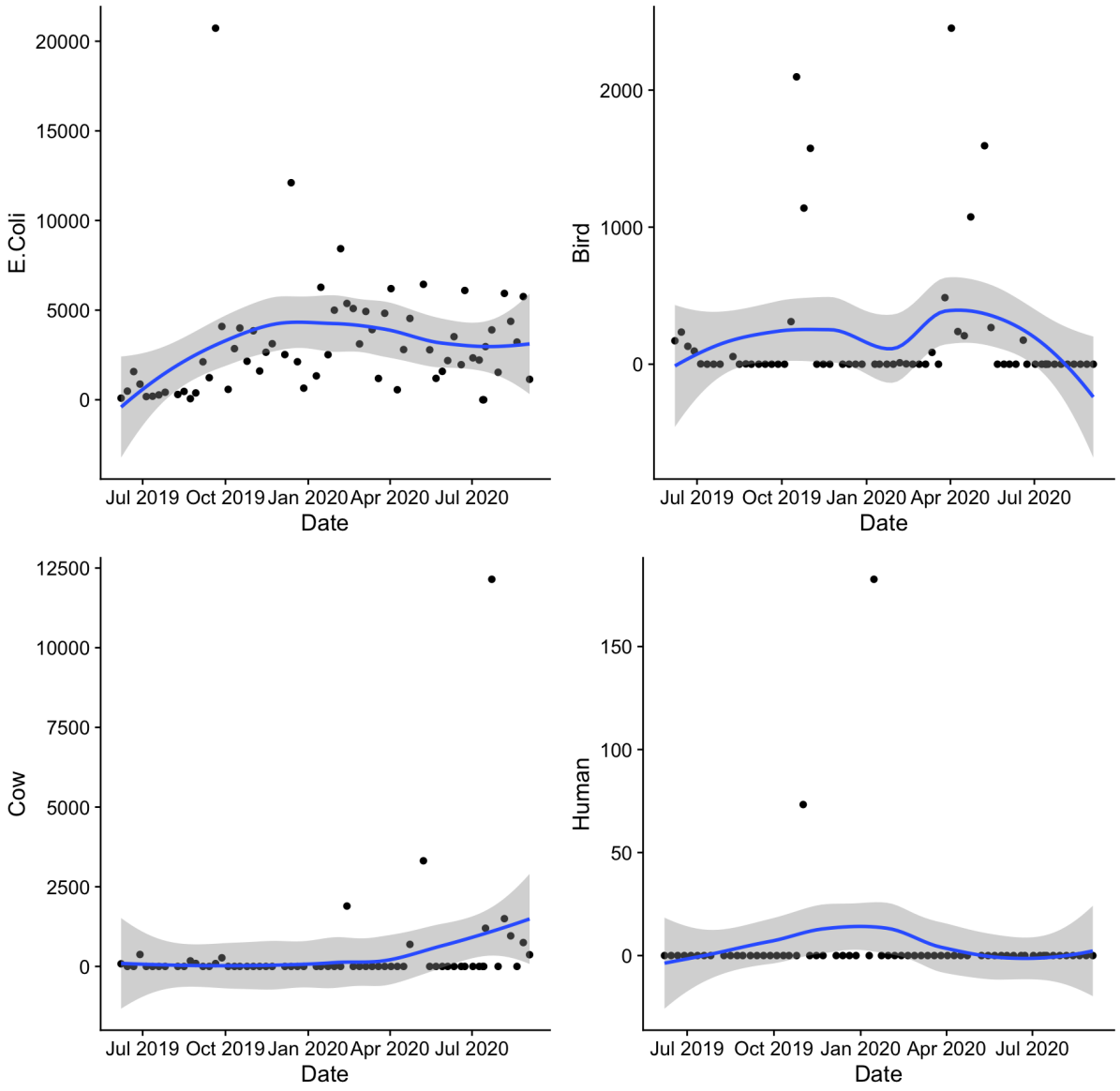


Figure 5.9 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. *E. coli*, B. Bird, C. Cow, and D. Human bacterial load as measured by weekly sampling in Hobbs Bay, Wagonga Inlet.

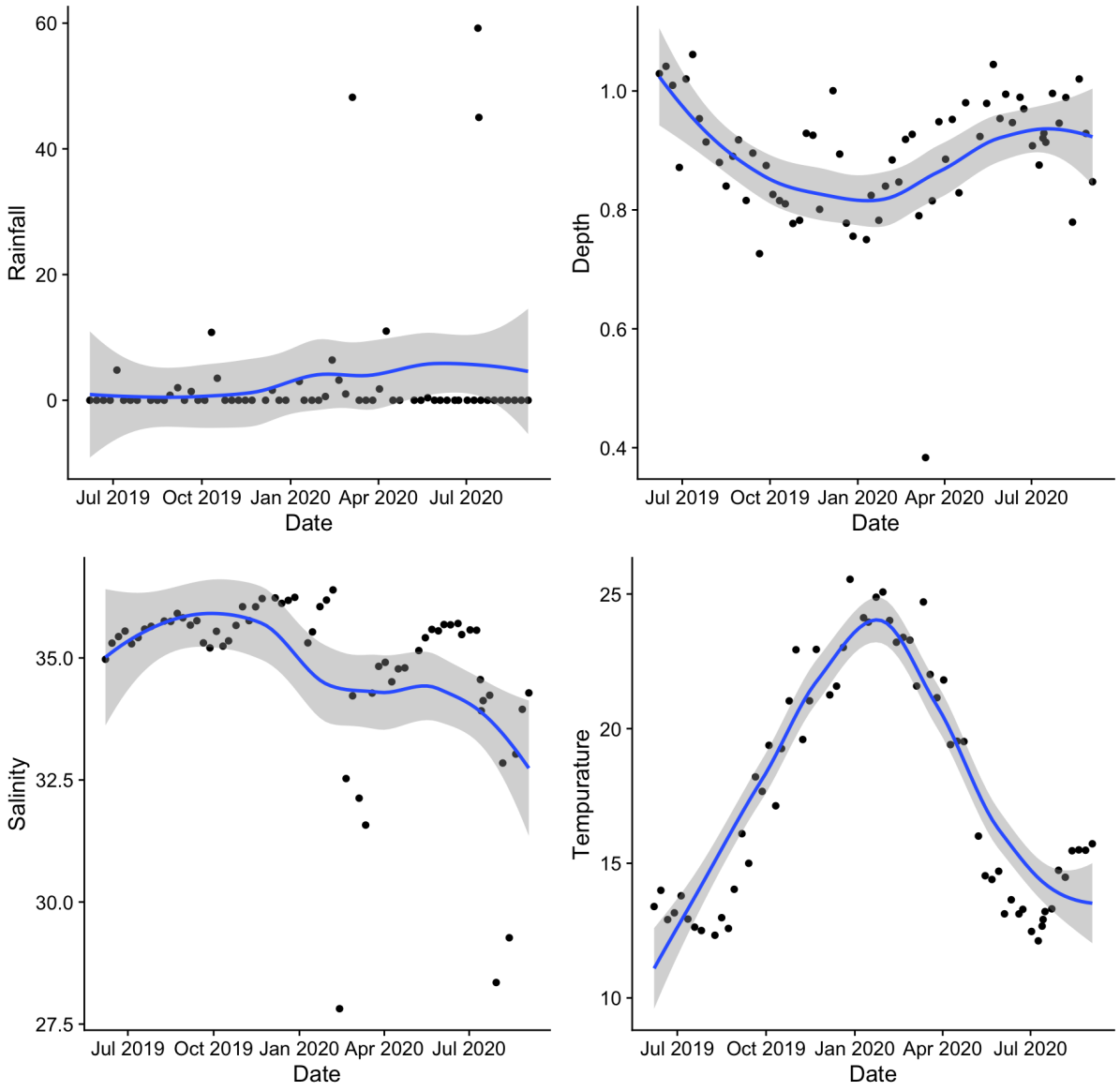


Figure 5.10 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. Rainfall, B. Depth, C. Salinity, and D. Temperature values measured in Hobbs Bay, Wagonga Inlet.

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 Lower Honeymoon Bay harvest area. Based on the available data, up to four harvest area closures and ten harvest area downgrades could have potentially been avoided between February 2018 and September 2021. During the initial implementation of such a management plan change, rainfall events would continue to be monitored to increase the database to support the change. Wagonga Inlet Shellfish Program (WISP) were consulted about the option of a salinity-only management plan for Lower Honeymoon Bay harvest area following the 2021 annual review, but a decision has not yet been reached. If WISP 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 Lower Honeymoon Bay harvest area management plan would revert to the current management plan that is based on both rainfall and salinity closure limits.

6.2 Phytoplankton and HABs

The biggest increase in phytoplankton growth throughout the sampling period was observed prior to significant rainfall. This growth was most likely a response to nutrients entering the waterway during this rainfall. Apart from *Pseudo-nitzschia* (discussed below), other harmful events were those caused by the dinoflagellates *Alexandrium pacificum* and *Dinophysis caudata*, although no biotoxins were associated with any of these blooms. Three detections of domoic acid however, were associated with *Pseudo-nitzschia* cf. *cuspidata*, in September 2020 albeit in the Lavender Point harvest area, downstream of sensor location.

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

Species belonging to the genus *Dinophysis* (and more rarely benthic *Prorocentrum)* are the most problematic Diarrhetic Shellfish Toxin (DSTs) producers worldwide. With over 100 species represented worldwide, ten have been unambiguously found to be toxic (*Dinophysis acuminata*, *D. acuta*, *D. caudata*, *D. fortii*, *D. infundibulum*, *D. miles*, *D. norvegica*, *D. ovum*, *D. sacculus* and *D. tripos*), producing DSTs (okadaic acid and dinophysistoxins) even at low cell densities (<10^3 cells L-1) (Reguera et al., 2014; Reguera et al., 2012; Simoes et al., 2015).

Dinophysis is common in Australian waters, with 36 species reported (Ajani et al., 2011; Hallegraeff and Lucas, 1988; McCarthy, 2013). Toxic species include *D. acuminata*, *D. acuta*, *D. caudata*, *D. fortii*, *D. norvegica*, and *D. tripos*. There have been three serious human DSP poisoning events in Australia. The first episode was caused by contamination of Pipis (*Plebidonax deltoides*) in New South Wales in 1997 (NSW) by *D. acuminata* (Quaine et al., 1997). One hundred and two people were affected and 56 cases of gastroenteritis reported. A second episode occurred again in NSW in March 1998, this time with 20 cases of DSP poisoning reported (Madigan et al., 2006). The final event occurred in Queensland in March 2000, when an elderly woman became seriously ill after eating local Pipis (Burgess and Shaw, 2001). While no human fatalities from DSP are known globally, DSTs continue to be a major food safety challenge for the shellfish industry. In response to elevated cell densities of a toxic algal species *Dinophysis* in February 2019 in the Manning River, we have now successfully developed a rapid qPCR assay to detect species belonging to the genus *Dinophysis* in environmental samples.

Pseudo-nitzschia is another high-risk HAB group in SE Australia for the shellfish aquaculture industry, and both estuaries and coastal waters in this area remain under threat (Ajani et al., 2013a, 2020). Wagonga Inlet has been identified as a high-risk, trained entrance estuary, with maximum cell densities of (total) *Pseudo-nitzschia* spp. reported across the austral winter and spring (Ajani et al., 2013a). Blooms within the Hawkesbury River estuary (450 km north of Wagonga Inlet), another high-risk area in SE Australia for HAB events, recently experienced a very dense bloom of *P. delicatissima*, with one out of seven strains isolated to produce domoic acid (Ajani et al., 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). While nutrient data was not available for Wagonga Inlet in our study, it was the modelled, high resolution temperature and salinity (sensor) data that proved to be more predictive of *Pseudo-nitzschia* blooms than rainfall, most likely responding in a similar way to the Hawkesbury, but on a shorted time scale. The high-resolution temperature and salinity sensor data resulted in a gain in predictive capability of the various models, when compared to available rainfall data. This is not unexpected given the potential for lag, or no impact on water quality, after rainfall events depending on other variables such as soil moisture content and/or intensity and distribution of rainfall.

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. under review)

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 Wagonga Inlet

Molecular assays for the detection of faecal bacterial contamination in the Wagonga Inlet 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 FIB load in watersheds (Araujo et al. 2014, Converse et al. 2012). The marker we used was 100% avian specific, with gulls, geese, ducks and chickens being tested (Green et al 2012), and has been successfully used in watersheds across different continents (Ahmed et al 2016, 2019; Li et al. 2019, Vadde et al. 2019). Our source tracking assay for cows had 100% sensitivity to bovine faecal samples, with little cross reactivity to other species (93% specific). When tested at a rural watershed, a high proportion of faecal contamination was attributable to cattle (Layton 2006). Finally, the human marker we used has demonstrated the best performance for the detection of human faecal contamination compared to all other assays since it was developed in 2000 (Boehm 2013, Shanks 2010).

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. 2020).

While cow, bird and human bacterial contamination was extremely low across the sampling period in Wagonga Inlet, modelling revealed that the bacterial load entering Wagonga Inlet was unexpectedly, linked to an increase in salinity. The exception to this was bovine contamination which was marginally more related to rainfall than salinity in Hobbs Bay.

Wagonga Inlet is a relatively small catchment of <100 km2. The slopes throughout the majority of the catchment tend to be steep (~10deg), but gentler towards the inlet. 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).

Faecal contamination from birds at the sensor site was best explained by the salinity model (33.2% deviance explained, compared to 12.4% using rainfall data), with total phytoplankton and salinity (increasing) significantly contributing to predict this variable. Similarly, the best model to explain bird faecal load was using the sensor at Hobbs Bay (44.8%, compared to 9.3% using rainfall), again included increasing total phytoplankton, salinity and temperature (peaking ~22℃) (Table 1A-B).

Similarly, avian faecal pollution in Wagonga Inlet was linked to increasing salinity and temperature, but 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 this 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 Wagonga Inlet. 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.

6.4 Oyster growth in Wagonga Inlet

At the conclusion of this study, Wagonga Inlet had the largest oysters, with respect to shell height, and the second heaviest oysters compared to the 11 other estuaries monitored in this study. Additionally, Oyster mortality in Wagonga Inlet was minimal during the entire experimental period and this estuary recorded the second best survival level compared to the 11 other estuaries monitored in this study. Most oyster mortality was recorded in February 2019 and February 2020. However, the level of mortality recorded on these dates was low and not considered unusual. The cumulative mortality measured in oysters at Wagonga Inlet in June 2020 was 12% which was below the average measured across all assessment sites used in the project (18%). The cumulative mortality at this time point was the second lowest recorded across all 12 experimental sites and comparable to Shoalhaven River (10%) located 150 km north of Wagonga Inlet. The mortality in Wagonga Inlet was much lower than in the three oyster producing estuaries measured to the south where cumulative mortality levels in June 2020 were 25% in Wapengo Lake and 21% in Pambula River and 23% in Wonboyn River.

Salinity remained above 32 ppt in Wagonga Inlet and was similar to oceanic levels for the first 17 months of this trial. This extended period of stable and high salinity facilitated excellent growth in shell height and whole weight. Seawater alkalinity increases as salinity increases providing more calcium carbonate available for oyster shell deposition. The salinity level that promotes the greatest growth rates in Sydney Rock Oyster spat 30 ppt for small spat (1.3 mg) and 35 ppt for larger spat (0.61 g) (Nell and Holliday, 1988). Shell length decreases were also recorded in the experimental oysters deployed in Wagonga Inlet. This can occur when there are periods of high wind and wave action which creates more oyster movement along with shell abrasion within trays resulting in loss of the shell fringe.

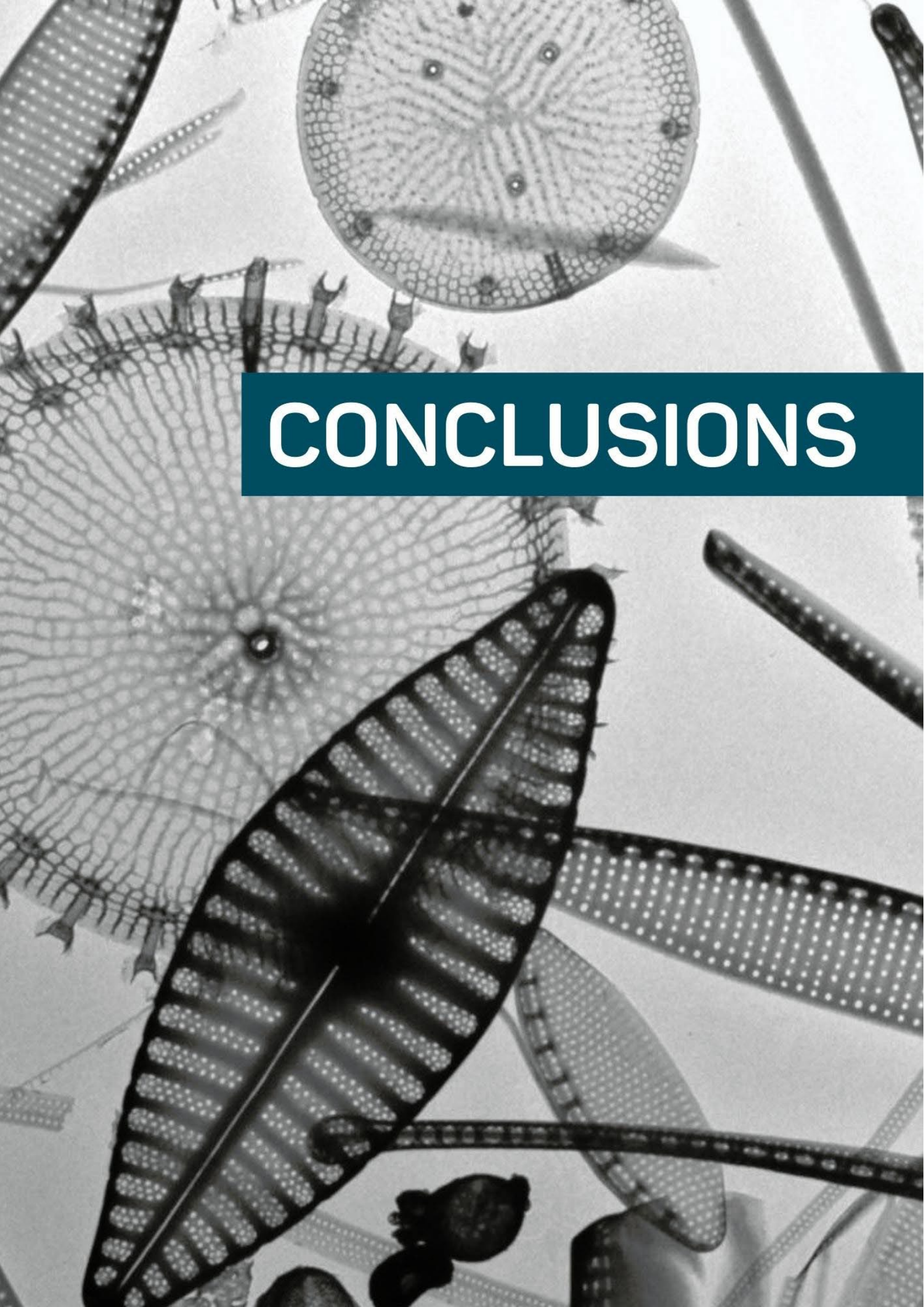
The batch of oysters used for this experiment were a random mix of families taken from the 2016-year class of the Sydney Rock Oyster Breeding program. This particular year class had 86% of the parents selected from wild and QX disease resistant genetic groups. Only 14% of the parents for this year class were sourced from the fast growth genetic group. The average weight of this batch of oysters in February 2020 was 51.3 g. Therefore, oysters were approximately 3 years and 2 months when they attained the premium oyster grade (>50 g) for sales. Estuaries where this same batch of oysters reached the premium plate grade benchmark at the same time were Camden Haven (50.3 g), Manning River (52.1 g), Hawkesbury River (52.8 g), Georges River (approx. 60g) and Wapengo Inlet (54.6 g).

Good growth rates for Sydney Rock Oyster have been recorded in Wagonga Inlet in a previous study. Seven estuaries in NSW were used to compare oysters selected for faster growth with non-selected oysters from April 2004 to April 2006 (Dove & O'Connor, 2009). The seven sites used for comparison were Kalang River, Camden Haven River, Wallis Lake, Shoalhaven River, Lake Conjola, Wagonga Inlet and Merimbula Lake. Wagonga Inlet had the 3rd best growth rate in terms of whole weight and ranked equal second in terms of the increase in oyster shell height for both oyster types. Wagonga Inlet and Shoalhaven had the same overall cumulative mortality (20%) for both selected and non-selected oysters between April 2004 to April 2006 (Dove & O'Connor, 2009). Additionally, Wagonga Inlet and Shoalhaven River recorded the lowest mortality level compared to the other five sites assessed in Dove & O'Connor (2009).

At the conclusion of this trial, this site in Wagonga Inlet had the largest oysters, the second heaviest oysters and the second-best survival level out of all 12 experimental sites. The results from this study are similar with previous comparisons of estuaries in terms of oyster growth and survival that used Wagonga Inlet as a study site. Excellent oyster growth rates coupled with high levels of oyster survival increases the productivity and profitability of this lease site compared to nearly all others measured in this study.

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 Lower Honeymoon Bay harvest area, subject to the agreement by the local shellfish industry. Available data indicated that four harvest area closures and ten harvest area downgrades could have potentially been avoided between February 2018 and September 2021. As of April 2022, sixteen salinity-only management plans had been offered for harvest areas in participating NSW estuaries, with six being taken up and the remaining ten under consideration.

Wagonga Inlet had the largest oysters, with respect to shell height, and the second heaviest oysters at the end of this experiment compared to the 11 other estuaries monitored in this study. Additionally, Oyster mortality in Wagonga Inlet was minimal during the entire experimental period and this estuary recorded the second-best survival level compared to the other estuaries monitored in this study.

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. The only notable exception to this was bovine bacteria in Hobbs Bay which was linked to an increase in rainfall. 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 Wagonga Inlet.

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9. Appendices

A1. Methods

A1.1 Sampling locations in Wagonga Inlet

Data used in this report originates from two locations within Wagonga Inlet over the period February 2018 to March 2021. High-resolution temperature, salinity and depth data were obtained from a sensor deployed in Lower Honeymoon harvest area (-36.22S, 150.07E) (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'. In June 2019 a second site in Hobbs Bay was sampled for eDNA. Phytoplankton was also collected at a second sampling location established as part of the DPI's Shellfish Quality Assurance program (labelled 'WAG2' as part of this program) (Fig. A1).

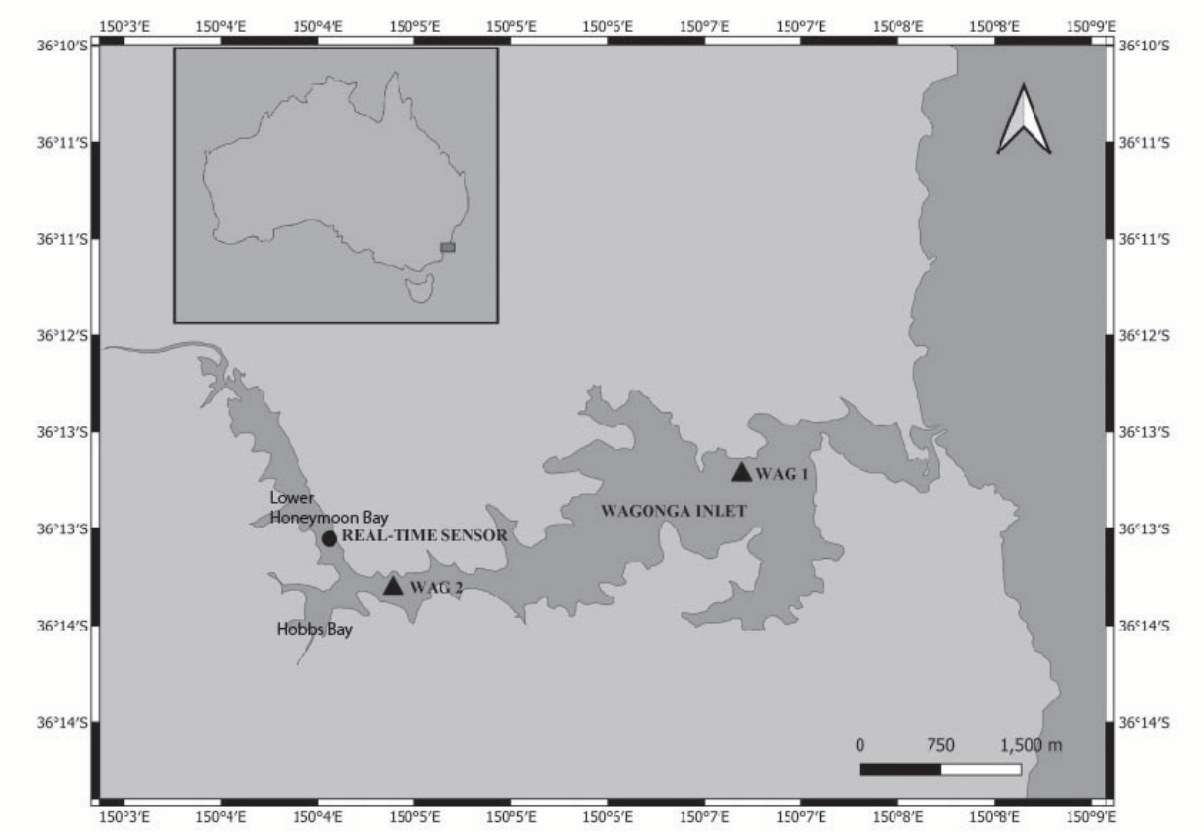

Figure A1: Map of Wagonga Inlet Estuary showing Lower Honeymoon Bay harvest area, Hobbs Bay, the sensor location (black circle), and phytoplankton sampling locations (WAG1 and WAG 2 - black triangles).

A1.2 High-resolution sensor data

High-resolution temperature (°C), salinity and water depth (m) data were collected from 14 February 2018 – 31 March 2021 using a Seabird SBE 37-SM/SMP/SMP-ODO MicroCAT high accuracy conductivity, temperature and depth (CTD) field sensor. This sensor was deployed using a fixed installation, with the inlet 60 cm above the seabed and at least 30 cm below the estimated Lowest Astronomical Tide (LAT) (Fig. A2). This fully autonomous instrument collected and transmitted data every 10 minutes (24 h day^{-1}) to Microsoft Azure cloud storage before downstream quality checking

and analysis. Sensor data was then packaged into RO-Crates by the e-Research team at UTS, which are then uploaded to an Arkisto-based website. This website allows for the filtering and downloading of these crates based on both time and location, for use in research and analysis (Fig. A3). Finally, rainfall data were obtained from the closest Bureau of Meteorology weather station at Narooma (BOM 069022, **-** 36.22S 150.13E).

Figure A2 Seabird MicroCAT high accuracy conductivity, temperature and depth (CTD) field sensor deployed in Wagonga Inlet. Image: Brian Coxon.

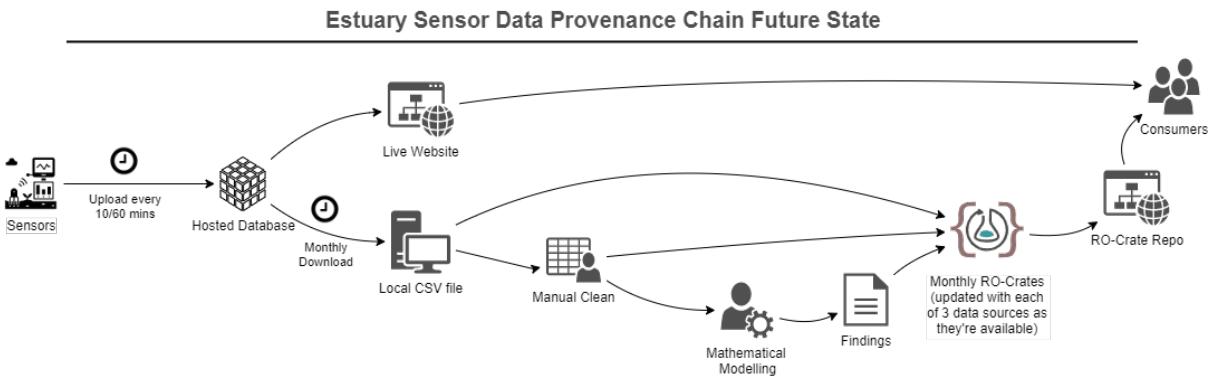

Figure A3. Wagonga Inlet Data provenance chain from source of data (sensors), via quality assurance processes, data analyses, to consumers.

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 Wagonga Inlet annual review is 1 October. As part of the most recent (2021) annual review for Lower Honeymoon harvest area, all salinity data from the monitoring sensor during the 2018, 2019, 2020 and 2021 annual review periods were analysed and assessed in relation to microbiological samples collected by the local shellfish program during the same period. During September 2020, there were some gaps in data due to a telecommunications issue. There was also a gap in data collection between 1 and 14 April 2021, while a new sensor was established. Salinity data collected between 20 and 27 June 2021 were excluded from the analyses, as the data appeared erroneous during this period, and readings returned to normal following cleaning/maintenance.

A1.4 Biological sampling and eDNA extraction

Estuarine water samples were collected weekly by oyster farmers working at Coxon's Oyster Farmers from September 2018 - September 2020 for both phytoplankton and bacteria. In June 2019, to investigate source tracking, a second site was sampled at Hobbs Bay. 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 station at Narooma Marine Rescue (BOM Station No. 069022), ~7 km downstream 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}.

A1.7 Pseudo-nitzschia qPCR assay for environmental bloom dynamics

As part of Stage 1 of the NSW Oyster Industry Transformation Project, we developed a rapid and quantitative polymerase chain reaction (qPCR) assay to detect species belonging to the *P. pseudodelicatissima* complex Clade I (see Case Study above). With no cross-reactivity to other closely related species, this novel assay was then evaluated for its potential to detect *Pseudo-nitzschia cf. cuspidata* in environmental samples from Wagonga Inlet during a bloom that occurred on 8 Apr 2019 (4.3E +05 cells L-1) (Ajani et al. 2021).

A1.8 Oyster Growth and Mortality

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

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

A1.8.1 Oyster Whole Weight

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

A1.8.2 Shell Length

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

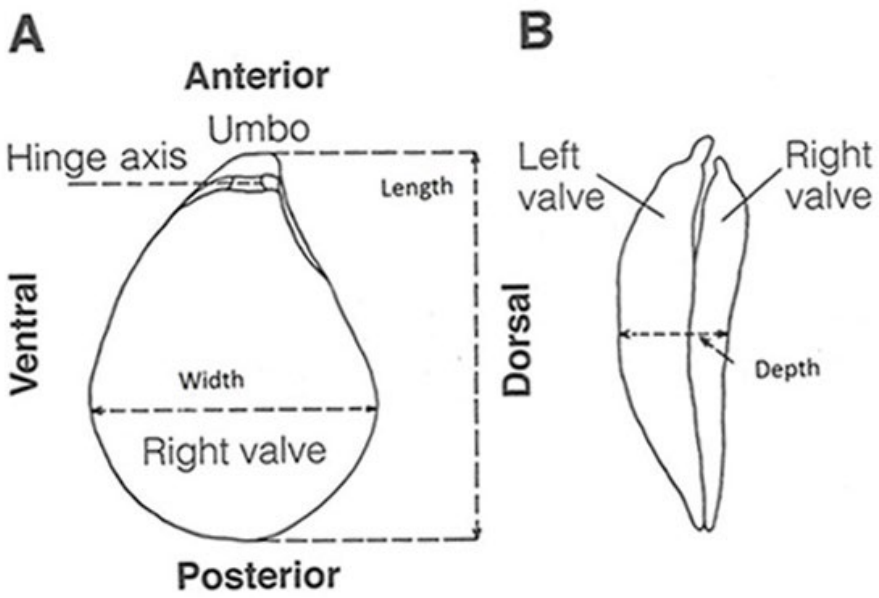

Figure A4. Oyster shell dimensions (Carriker 1996)

A1.8.3 Oyster Mortality

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

A1.9 Modelling

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

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

Rainfall data from the closest Bureau of Meteorology weather station at Narooma (BOM Station No. 069022), 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 Wagonga Inlet, 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 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, Wagonga Inlet

Variable	Mean	Standard Err	Median	Standard Deviation	Minimum	Maximum	Count	Missing
average_cfu	23.27	5.02	8.28	49.69	0.00	314.96	98	0
bird	406.38	86.42	0.00	855.47	0.00	4780.45	98	0
cow	11.41	4.90	0.00	48.49	0.00	415.27	98	0
depth24	0.96	0.01	0.95	0.14	0.38	1.33	98	0
depth48	0.96	0.01	0.95	0.13	0.59	1.33	98	1
depth72	0.96	0.01	0.95	0.12	0.66	1.26	98	2
ecoli	2636.28	509.99	1225.10	5048.60	0.00	31930.59	98	0
human	11.59	7.38	0.00	73.05	0.00	617.50	98	0
logPhytoplankton	13.61	0.07	13.69	0.72	12.25	15.98	98	0
Phytoplankton	1087346.94	113145.69	880000.00	1120085.19	210000.00	8700000.00	98	0
rainfall24	3.38	0.99	0.00	9.82	0.00	59.20	98	0
rainfall48	3.42	0.78	0.40	7.73	0.00	52.10	98	1
rainfall72	3.45	0.66	0.80	6.51	0.00	34.73	98	2
salinity24	35.06	0.13	35.42	1.32	27.81	36.39	98	0
salinity48	35.06	0.11	35.38	1.13	30.17	36.29	98	1
salinity72	35.06	0.10	35.39	1.02	31.52	36.21	98	2
temp24	18.61	0.43	19.41	4.22	12.12	26.07	98	0
temp48	18.65	0.42	19.53	4.15	12.29	25.37	98	1
temp72	18.68	0.42	19.48	4.12	12.42	25.53	98	2

Appendix 2B. Summary Statistics for Bacterial Modelling – Hobbs Bay, Wagonga Inlet

Variable	Mean	Standard Error	Median	Standard Deviation	Minimum	Maximum	Count	Missing
average_cfu	26.23	3.94	19.27	31.50	0	202.78	64	0
bird	193.85	63.25	0.00	506.04	0	2451.93	64	0
cow	373.60	198.73	0.00	1589.83	0	12146.74	64	0
depth24	0.89	0.01	0.91	0.11	0.383413	1.06	64	0
depth48	0.89	0.01	0.90	0.09	0.586811	1.04	64	1
depth72	0.89	0.01	0.90	0.08	0.662982	1.03	64	2
ecoli	3076.88	404.20	2426.40	3233.64	0	20729.52	64	0
human	4.00	3.06	0.00	24.46	0	182.62	64	0
logPhytoplankton	13.59	0.11	13.60	0.84	12.25486	15.98	64	0
Phytoplankton	1174843.75	169177.07	810000.00	1353416.60	210000	8700000.00	64	0
rainfall24	3.20	1.37	0.00	10.94	0	59.20	64	0
rainfall48	3.25	1.10	0.00	8.83	-1.39E-15	52.10	64	1
rainfall72	3.30	0.92	0.47	7.40	-1.48E-16	34.73	64	2
salinity24	34.83	0.22	35.43	1.74	27.81478	36.39	64	0
salinity48	34.83	0.19	35.42	1.51	30.17215	36.29	64	1
salinity72	34.83	0.18	35.43	1.42	30.15565	36.21	64	2
temp24	17.52	0.55	15.87	4.37	12.11986	25.55	64	0
temp48	17.57	0.54	15.60	4.33	12.294	24.98	64	1
temp72	17.62	0.54	16.56	4.32	12.41805	24.66	64	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		In prep.
Penelope Ajani, Arjun Verma, Jin Ho Kim, Hazel Farrell, Anthony Zammit, Steve Brett & Shauna Murray	Using qPCR and high-resolution sensor data to model a multi-species *Pseudo-nitzschia* (Bacillariophyceae) bloom in southeastern Australia	*Harmful Algae* 108 (2021) 102095	Published
NSW DPI	Net Returns of Real-Time Sensors and Salinity-Based Management Plans in NSW Oyster Production - Report	https://www.foodauthority.nsw.gov.au/about-us/science/science-in-focus/real-time-sensors-shellfish-harvest-area-management	Published
NSW DPI	Net Returns of Real-Time Sensors and Salinity-Based Management Plans in NSW Oyster Production - Factsheet	https://www.foodauthority.nsw.gov.au/about-us/science/science-in-focus/real-time-sensors-shellfish-harvest-area-management	Published
The Team	Oyster Transformation Project	NSW Oyster Newsletter https://www.nswoysters.com.au/nsw-oyster-newsletter.html July 2020	Published
DPI Food Authority	Foodwise - Issue 46	https://www.foodauthority.nsw.gov.au Feb 2018	Published
Shauna Murray & Penelope Ajani	Ah shucks, how bushfires can harm and even kill our delicious oysters	The Conversation https://theconversation.com/ah-shucks-how-bushfires-can-harm-and-even-kill-our-delicious-oysters-131294 Aug 2020	Published

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

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

Penelope Ajani, Michaela Larsson, Ana Rubio, Stephen Bush, Steve Brett, Stephen Woodcock, Hazel Farrell & Shauna Murray	Australian Shellfish Quality Assurance Advisory Committee Science Day 2018	Modelling harmful algal blooms in the Hawkesbury River, Australia
Hazel Farrell, Grant Webster, Phil Baker, Anthony Zammit, 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