TRANSFORMING AUSTRALIAN SHELLFISH PRODUCTION

Goodnight Island Harvest Area - Shoalhaven and Crookhaven Rivers

Report on Stage 1, December 2017-March 2021

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

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Transforming Australian Shellfish Production: Goodnight Island Harvest Area, Shoalhaven and Crookhaven Rivers. Report on Stage 1, December 2017-March 2021

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Executive Summary

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

We installed a real-time sensor in the Shoalhaven and Crookhaven Rivers within Goodnight Island Harvest Area, recording high-resolution temperature, salinity and depth data. Oyster farmers collected weekly biological samples (666 environmental DNA samples and 276 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 and an additional model to link oyster growth with environmental variables and assessed its predictive capability.

MAJOR FINDINGS

4

Available data indicated that four harvest area closures and one harvest area downgrade could have potentially been avoided between December 2017 and June 2020.

The relationship between salinity fluctuations and harvest area status was less clear between July 2020 and May 2022 due to wetter conditions.

100%

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

E. coli increased with rainfall, less so with cow and human bacteria, while bird bacteria fluctuated seasonally

0

Very low levels of mortality were recorded throughout the experiment and mortality was less than the standard background mortality level commonly experienced for Sydney Rock Oyster production in New South Wales (<10% per annum)

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

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

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

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

1.2 Shoalhaven and Crookhaven Rivers

The Shoalhaven River (-34.86°S, 150.69°E) rises on the east side of the Great Diving Range and travels ~327 km east to Greenwell Point, where it meets the Crookhaven River (-34.92°S, 150.73°E), and becomes an open mature wave dominated barrier estuary system (Fig. A1). The Shoalhaven River has a catchment area of ~7500 km^2, an estuary area of ~32 km^2, and a flushing rate of ~78.2 days (Roper et al. 2011). The Crookhaven River on the other hand, is significantly smaller, and has a water area of only ~8 km^2. Both the Shoalhaven and Crookhaven Rivers are important mangrove (0.7 km^2 and ~3 km^2 for Shoalhaven and Crookhaven Rivers respectively), seagrass (0.3 km^2 and 0.7 km^2) and saltmarsh areas (0.15 and 1.4 km^2) (Roy et al. 2001). The land use surrounding the Shoalhaven-Crookhaven estuary is 63% forested, 32% rural and 3.5 % urban. In terms of estuary health, urban and agricultural runoff, soil erosion, sedimentation and nutrient/bacterial loads from riverbank cattle, are all major environmental problems (Shoalhaven CMP Scoping Study, Shoalhaven City Council 2020).

1.3 Oyster Production in the Shoalhaven and Crookhaven Rivers

The Shoalhaven and Crookhaven Rivers are significant producers of Sydney Rock Oysters in Australia. Production values for 2020/21 financial year in the Crookhaven River is estimated to be $1.77 million (NSW DPI 2022). While production value in the Shoalhaven River is not available for confidentiality reasons (≤ 5 current permit holders in the estuary), the total production value across the 11 estuaries with ≤ 5 permit holders is estimated to be ~224,672 dozens and valued at ~$2,208,619 (NSW DPI 2022).

FINDINGS

2. Findings

2.1. The data assessment from this report supports implementing a harvest area management plan based on sensor salinity data for Goodnight Island harvest area, subject to the agreement by the local shellfish industry. Available data indicated that four harvest area closures and one harvest area downgrade could have potentially been avoided between December 2017 and June 2020. More recent salinity data (July 2020 – May 2022) showed a higher level of variability due to more frequent rainfall events, and the relationship between salinity fluctuations and harvest area status was less clear.

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 the Shoalhaven and Crookhaven Rivers over the biological sampling period, September 2018 to September 2020.

2.3. The real time sensor data showed a substantially higher predictive capacity than rainfall data for all four faecal bacteria indicators.

2.4. The maximum predictive capability for each model/bacterial group were 23.9% for *E. coli*, 34.8% for cow, 46.1% for bird, and 73% for human at the sensor site. *E. coli* most often increased with rainfall, while cow and human were more variable (sometimes becoming elevated with rainfall. On the other hand, bird bacteria increased seasonally, corresponding to warmer surface water temperatures.

2.6. The greatest oyster growth rates in the Shoalhaven and Crookhaven Rivers occurred during the first 3 months of this experiment, with the best model explaining a moderate amount of the ~36.9% of the deviance. Strongest predictor variables were the daily average salinity (decreasing, with optimal growth at ~35.6 ppt) and weekly rainfall (optimal growth occurring when little rainfall occurred over the previous week).

2.7. The cumulative mortality measured at the Shoalhaven River site between August 2018 and February 2020 was 8.3% which was the lowest level recorded at all estuarine sites monitored in this study.

ACKNOWLEDGEMENTS

3. Acknowledgements

This project is proudly funded by the NSW Government in association with the Food Agility CRC and the NSW Farmer's Association. The Food Agility CRC Ltd is funded under the Commonwealth Government CRC Program. The CRC Program supports industry-led collaborations between industry, researchers and the community. The Department of Primary Industries, the University of Technology and NSW Farmers also provided project funding. The project team would like to acknowledge the invaluable assistance of the Shoalhaven and Crookhaven Rivers oyster farmers for collecting weekly samples. Specifically, we thank Angela and Leon Riepsamen for their assistance and co-ordination of sample collection. We also wish to acknowledge the assistance of staff from The Yield Technology Solutions for facilitating access to the water salinity and temperature data used in the analysis. Routine phytoplankton monitoring sample data for the Shoalhaven and Crookhaven Rivers 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.

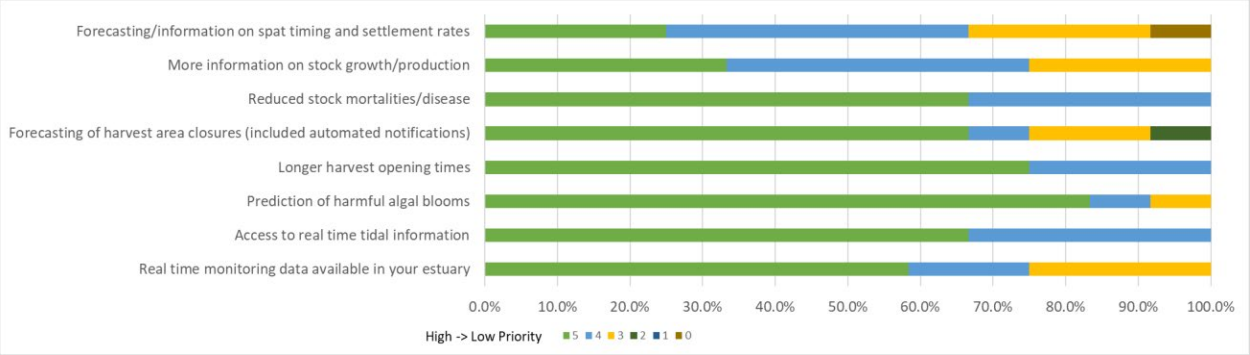


Figure 4.1. The importance of factors as rated by farmers in relation to their business operations. Green is most important and brown is least important.

RESULTS

5. Results

5.1 High resolution temperature and salinity data

High-resolution real time data summaries for the Shoalhaven and Crookhaven Rivers sensor for the period 21 Dec 2017 to 31 Mar 2021 are shown in Figs. 5.1A-C. Depth recordings ranged from 0.15 m (22 Nov 2019) to 2.2 m (10 Feb 2020). The lowest and highest daily average salinity recordings were 1.4 ppt (12 Aug 2020) and 35.9 ppt (11 Jan 2020) respectively, while the lowest and highest daily average temperature recordings were 11.5℃ (9 Aug 2020) and 26.0 ℃ (31 Jan 2020) respectively.

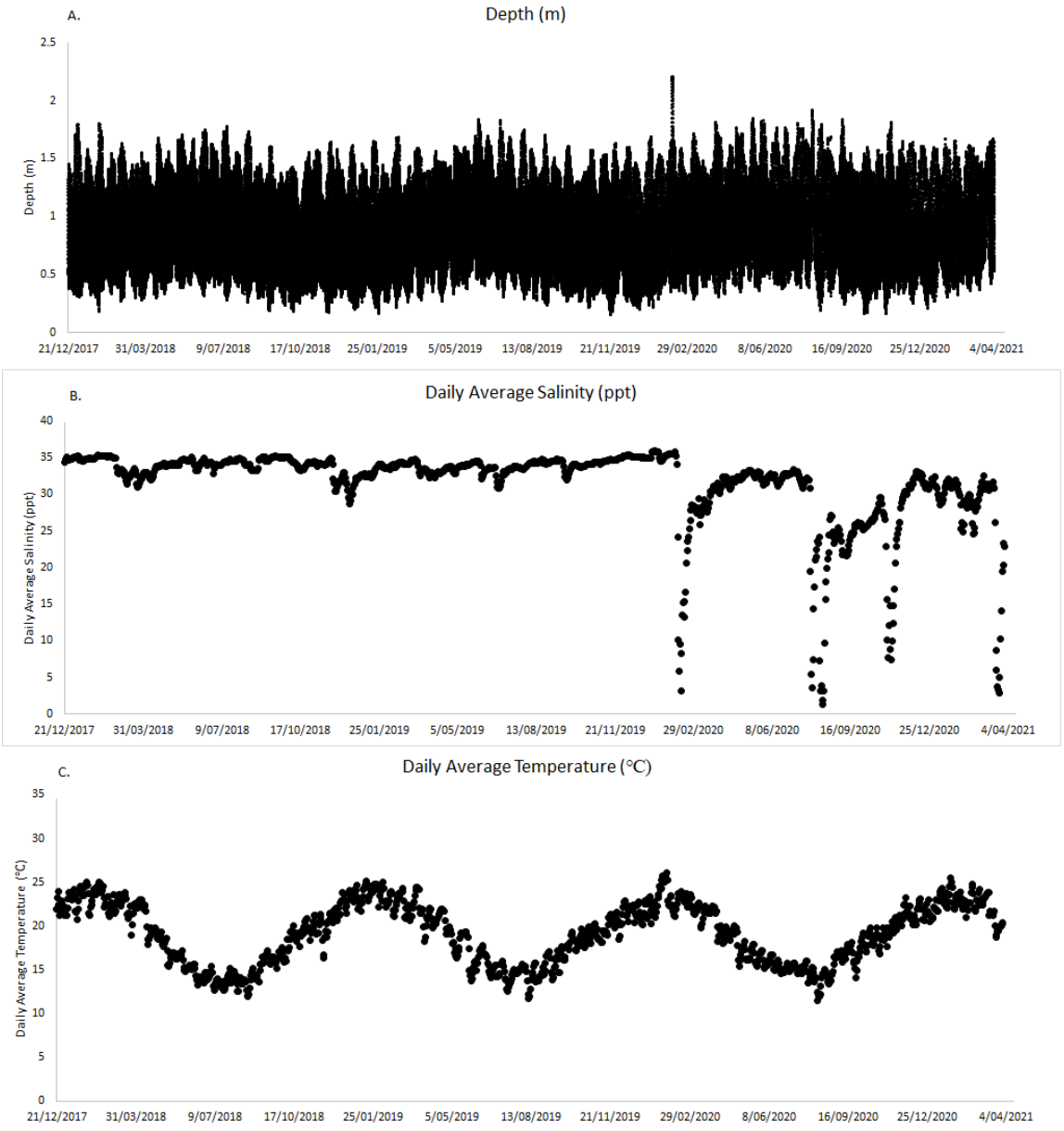


Figure 5.1A-C. Real time sensor data from the Shoalhaven and Crookhaven Rivers 21 Dec 2017 to 31 Mar 2021 A. Depth (m); B. Daily average salinity (ppt); and C. Daily average temperature (°C).

The maximum daily rainfall at the Greenwell Point Bowling Club rainfall gauge (BOM Station No. 068080, ~-34.158°S and 150.7312°E) occurred on 27 July 2020 and was reported as 129 mm (Fig. 5.2). Rainfall data was absent from 1/5/2020 to 1/6/2020 from this rainfall gauge and in its place rainfall data was taken from Greenwell Point (Council) station no. 568180 for modelling purposes.

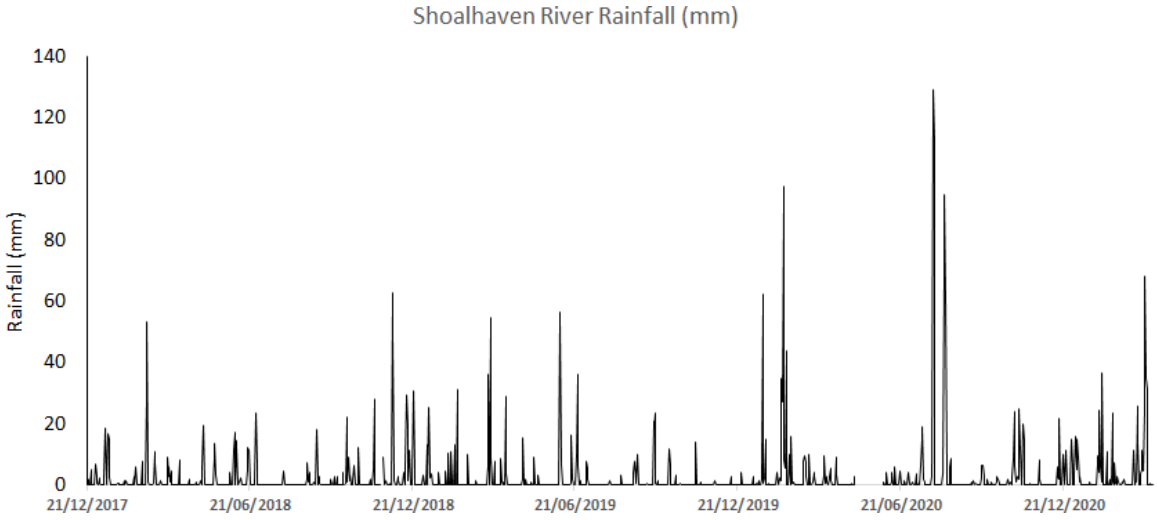


Figure 5.2. Daily rainfall (mm) from the Greenwell Point Bowling Club rainfall gauge (BOM Station No. 068080 from Dec 2017 to March 2021.

5.2 Management Plan

Salinity data recorded by the sensor between December 2017 and June 2020, indicated that there could have been less harvest area closures or downgrades since the sensor was installed, if closures were based on salinity sensor data. During that time period, there were six harvest area closures and six harvest area downgrades due to rainfall. Based on a management plan sensor salinity closure limit of 22 ‰, harvest area closures were reviewed focusing on available salinity sensor data and shellfish program microbiological results since December 2017. Twenty-seven harvest closure days occurred over four rainfall closures, although salinity sensor data did not decline below 22 ‰ and microbiological results from samples collected between 1-7 days post closure met Approved or Restricted harvest criteria. A review of salinity sensor data and shellfish program microbiological results indicated that there was one rainfall downgrade where salinity as reported by the sensor was higher than 26 ‰ (downgrade salinity range 22-26 ‰), and microbiological results from samples collected 5 days post downgrade met Approved harvest criteria.

Salinity data recorded by the sensor from July 2020 onwards showed a higher level of variability due to more frequent rainfall events, and the relationship between salinity fluctuations and harvest area status was less clear. It should also be considered that there is some possible lag in salinity changes due to inputs from the larger catchment upstream of Goodnight Island harvest area, following severe wet weather events. Similarly, there may be groundwater inflows impacting the salinity profile of Goodnight Island harvest area after flood conditions. Available shellfish program data collected during various salinity conditions, including when salinity minima were below management plan limits, generally met

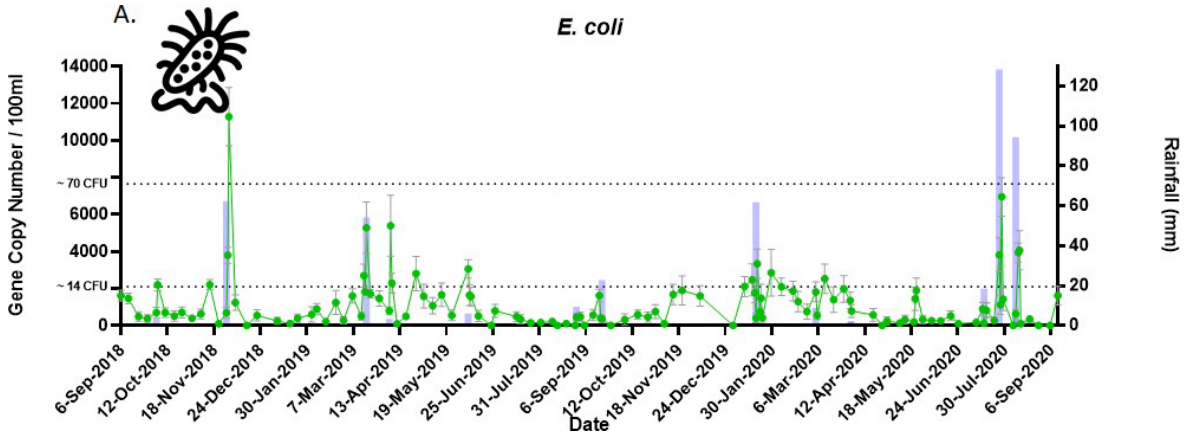
operational microbiological criteria. Time periods where salinity is variable or slower to recover may require additional sampling to meet management plan requirements. If a salinity sensor-based harvest management plan was in place in Goodnight Island harvest area between July 2020 and May 2022, it could have resulted in additional harvest area closures and/or a higher sampling frequency. The potential negative impact of this may be offset somewhat by more harvest days due to fewer closures over the longer term, and decisions on harvest area management following prolonged rainfall events would generally consider salinity trends rather than point in time measurements.

The potential benefits of real-time salinity-based management plans to improve food safety outcomes while also providing lower total closure days during periods of low to moderate rainfall are clearly demonstrated by the data collected under this project. However, the operation of salinity only management plans in this estuary during periods of repeated severe wet weather events requires further exploration.

5.3 Bacterial source tracking

A total of 666 water samples and 276 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 Shoalhaven and Crookhaven Rivers (Fig. A1).

For Shoalhaven and Crookhaven Rivers the maximum *E. coli* reached 11,281 gene copies 100 mL^{-1} on 30 Nov 2018, 2,010 copies 100 mL^{-1} for *Helicobacter* (bird) on 16 Mar 2019, 16,386 gene copies 100 mL^{-1} for bovine faecal pollution (cow) on 30 Nov 2018, and finally, 2188 copies 100 mL^{-1} for human faecal pollution on again on 30 Nov 2018 (Fig. 5.3 A-D).



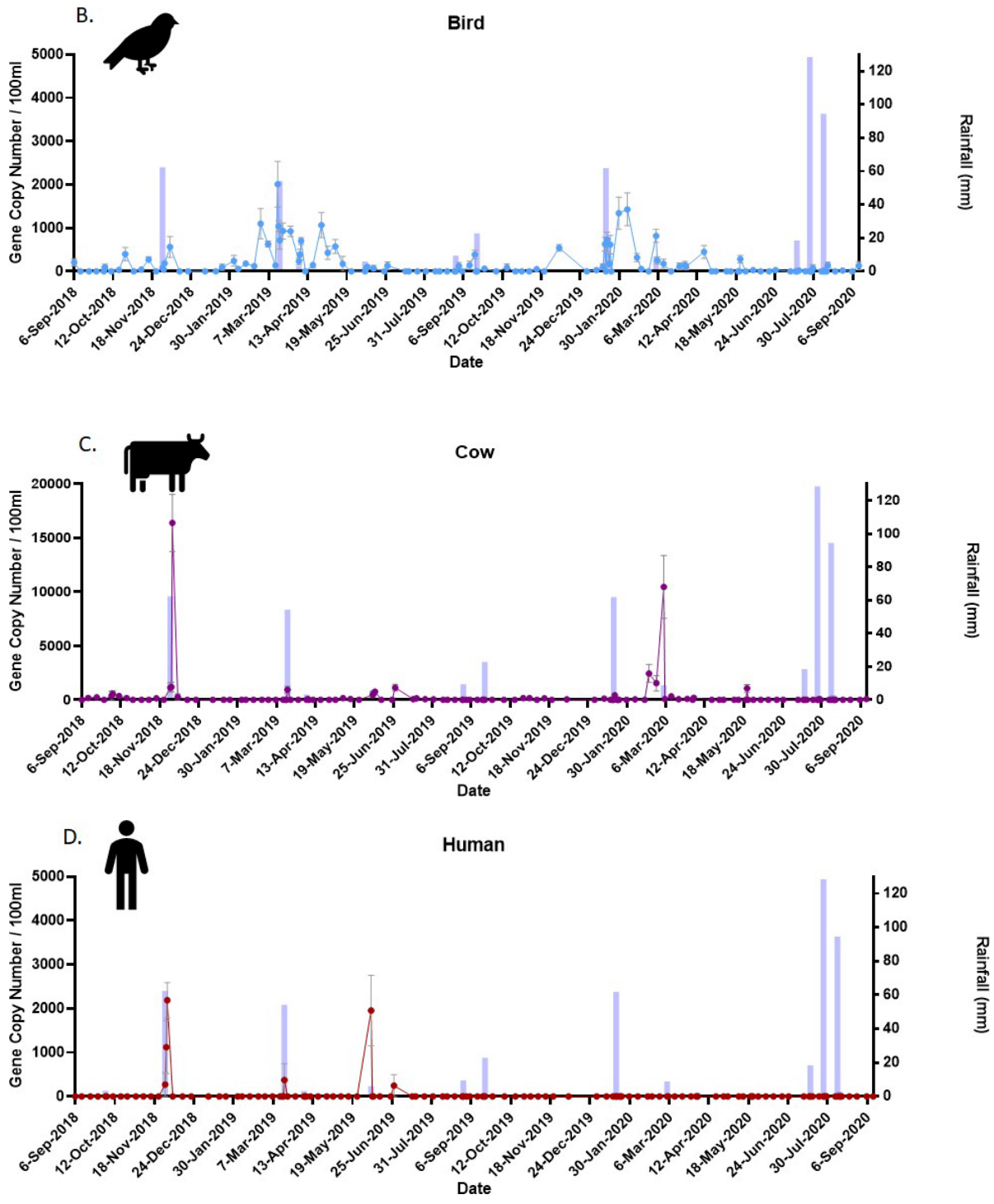


Figure 5.3 A-D. Weekly *E. coli* data from the sensor location, Shoalhaven and Crookhaven Rivers, using A. *E. coli* assay; B. Bird assay; C. Cow assay; and D. Human assay. Purple bars represent rainfall events that were sampled. Dotted lines in Fig. A at 14 and 70 cfu/100 mL are the operational limits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

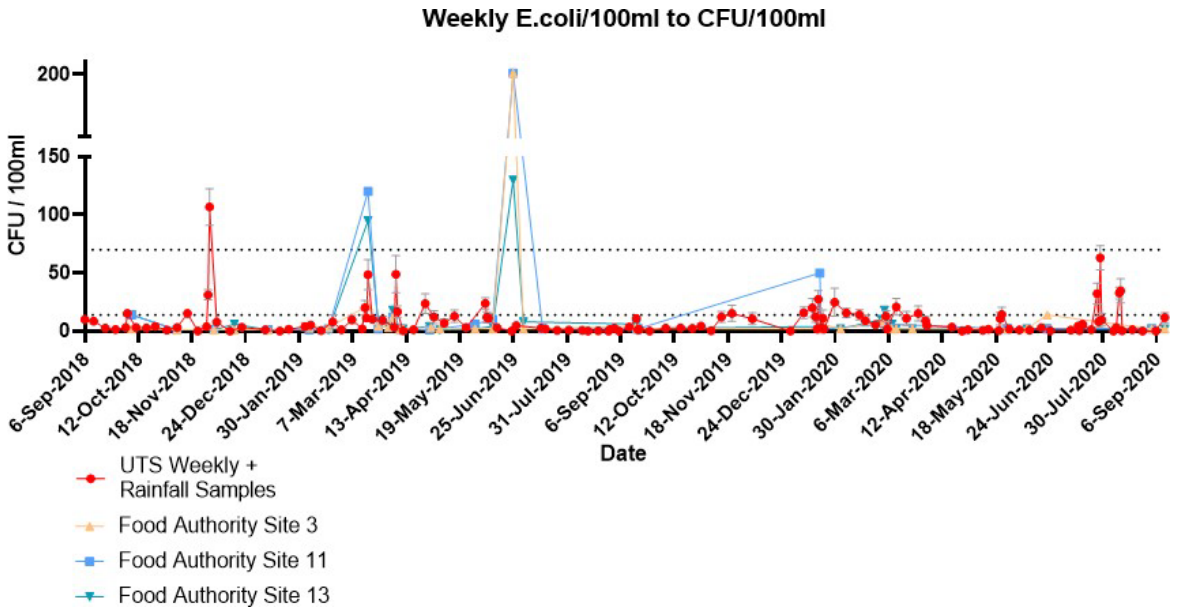


Figure 5.4 Weekly faecal coliform counts (cfu/100 mL) from water samples collected by DPI Food Authority at three sites in the Shoalhaven and Crookhaven Rivers compared to Oyster Transformation Project weekly sampling results (including rainfall sampling). Dotted lines at 14 and 70 cfu/100 mL are the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

Faecal coliform counts in samples from the current project samples generally corresponded to DPI Food Authority counts when collected at the same time (Fig. 5.4). There was one occasion (Jun 2019), when the DPI samples were elevated (Jun 2019), but this was not observed in the current project's samples. The reason for this may be that the current project sampled on the 24 Jun, while the DPI sampling occurred the day after. In between these two sampling days significant rainfall (34 mm) fell, which may have resulted in the subsequent spikes in *E. coli* at the DPI sites.

Eleven rainfall events (3 days sampled) were also sampled across the study period in the Shoalhaven and Crookhaven Rivers (see purple bars in Fig 5.3 A-D). These were: 28-30 Nov 2018, 16-18 Mar 2019, 5-7 Apr 2019, 6-8 Jun 2019, 17-19 Sep 2019, 17-19 Jan 2020, 21-23 Jan 2020, 21-23 May 2020, 14-17 Jul 2020, 27-29 Jul 2020, and 9-12 Aug 2020 (Fig. 5.5 A-K). *E. coli* concentrations were highly variable across rainfall events, with peak concentrations observed on day 1, day 2 or day 3 depending on the event. Without further sample collection, it is unclear how quickly the high concentrations on day 3 would dissipate. Bird bacteria generally remained low during rainfall events, while cow bacteria showed one significant peak on day 3 of a Nov 2018 rainfall event. Similarly, human bacteria peaked on day 1 of a rainfall event in Jun 2019, but remained low during all other events (Fig. 5.4 D).

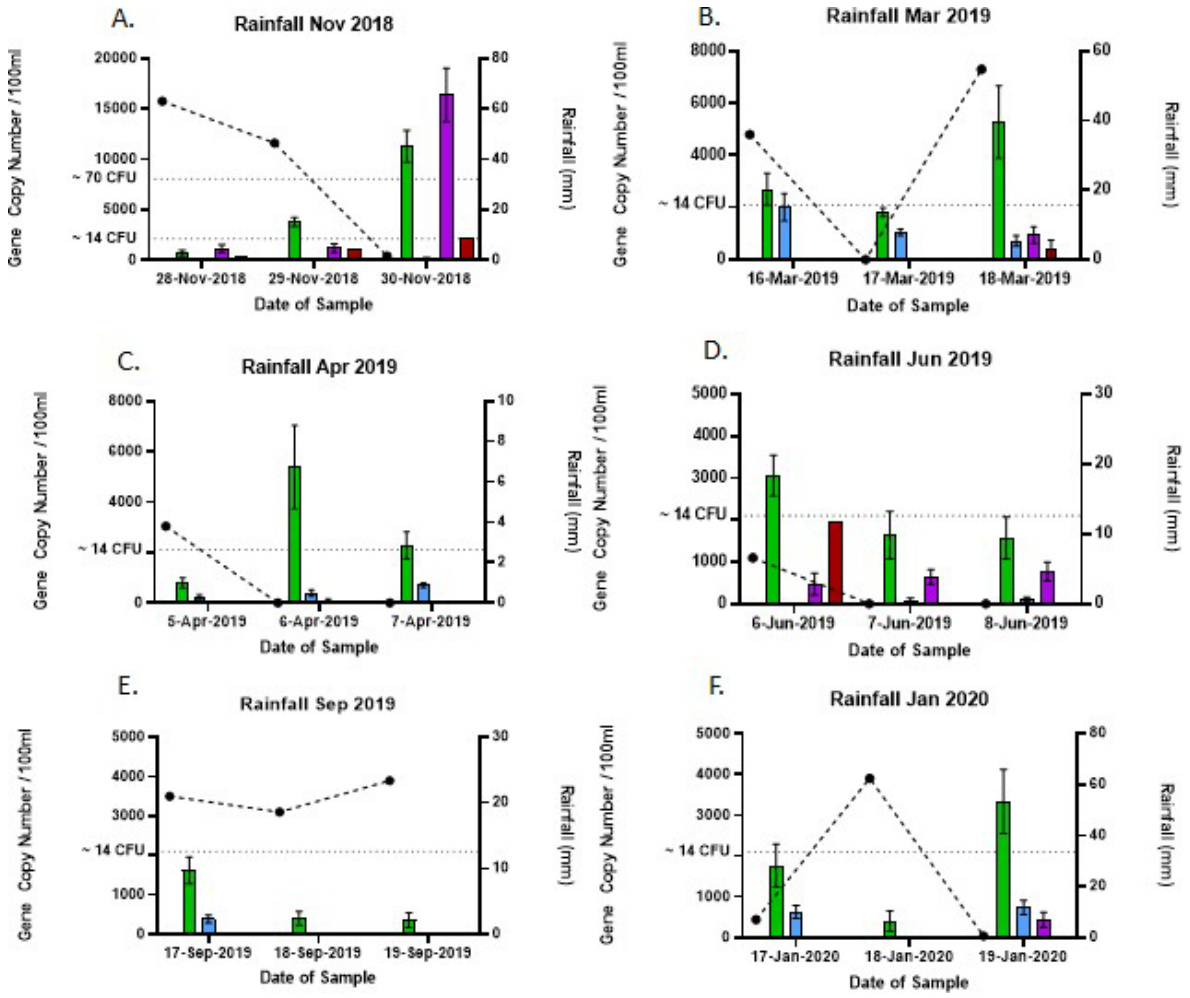


Figure 5.5 A-F. Sensor site (Shoalhaven and Crookhaven Rivers) rainfall events sampled for *E. coli* assays. Green bar = 16S *E. coli*; blue bar = bird assay; purple bar – cow assay; red bar = human assay. Dotted line is rainfall (mm) obtained from the closest rainfall station (Greenwell Point BOM Station No. 068080). 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.

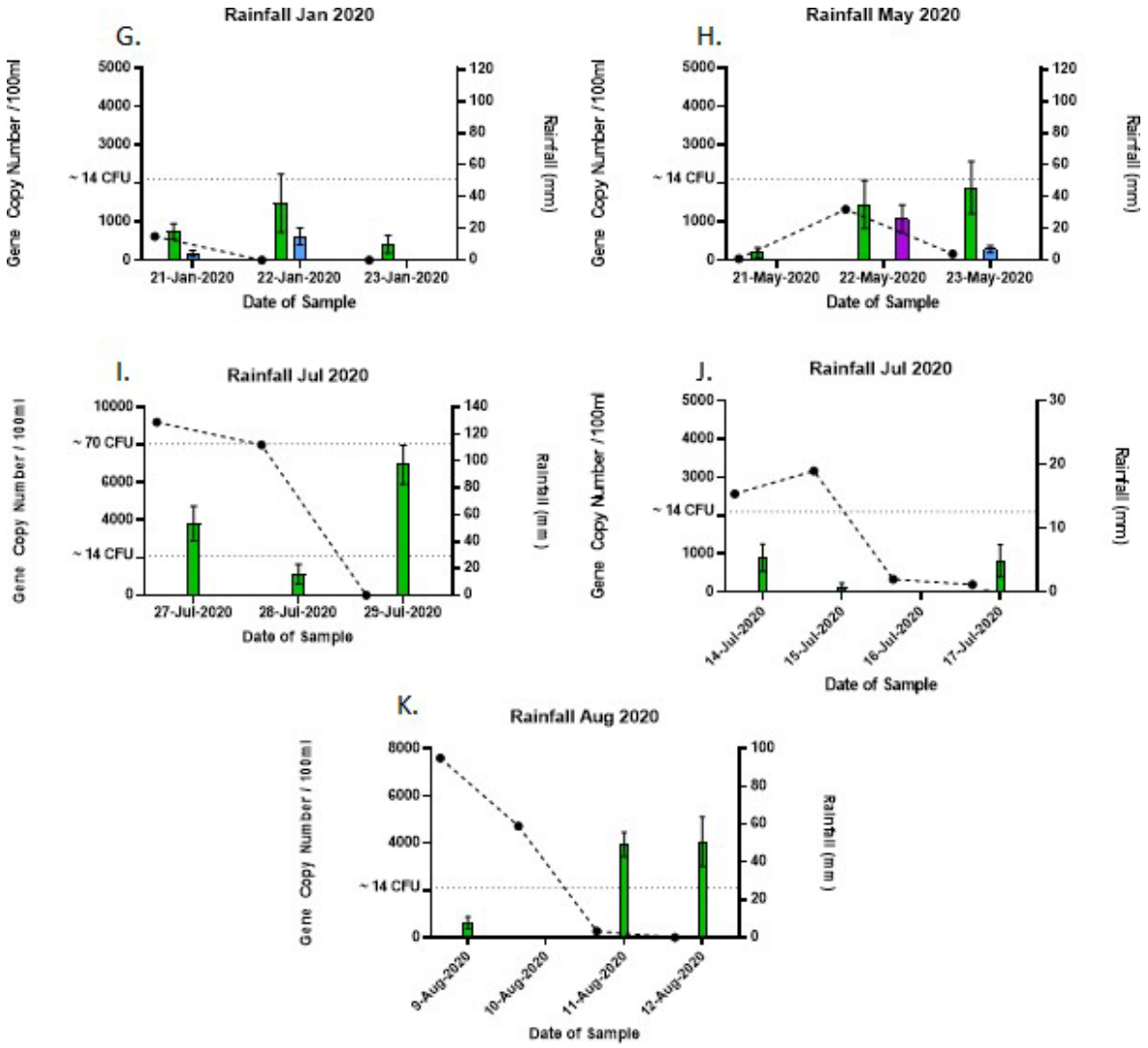


Figure 5.5 G-K. Sensor site (Shoalhaven and Crookhaven Rivers) rainfall events sampled for *E. coli* assays. Green bar = 16S *E. coli*; blue bar = bird assay; purple bar – cow assay; red bar = human assay. Dotted line is rainfall (mm) obtained from the closest rainfall station (Greenwell Point BOM Station No. 068080). 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 (Dec 2017 to March 2021) occurred on 16 Sept 2020 (Fig. 5.6). Total cell concentrations reached 4.4E +06 cells L^{-1} and sample was dominated by planktonic diatoms (*Melosira, Chaetoceros*) and flagellates (*cryptomonads, dinoflagellates, prasinophytes,* and *prymnesiophytes*). The sample also contained sediment and organic detritus. This bloom did not coincide with any significant rainfall event.

A water sample collected on 22 Oct 2018 revealed elevated concentrations of the toxic dinoflagellate *A. pacificum* at 1400 cells L^{-1}. On 10 April 2019, another toxic dinoflagellate

Dinophysis acuminata increased in cell density, reaching 500 cells L^{-1}. In Oct 2019, *A. pacificum* (700-1100 cells L^{-1}), *D. acuminata* (450-1500 cells L^{-1}), and a third species *Dinophysis caudata* (200 cells L^{-1}) all reached elevated concentrations on the same sampling day (17 Oct 2019). A few days later (22 Oct 2019), *A. pacificum* was still elevated across sites (520-750 cells L^{-1}). Two weeks on, these species decreased in cell densities, and the potentially toxic diatom *Pseudo-nitzschia fraudulenta/australis* became elevated, reaching a maximum cell concentration of 5.2E +04 cells L^{-1} on 4 Dec 2019. The NSW Food Authority's Phytoplankton Action Limits to trigger biotoxin testing are 50,000 cells L^{-1} for *Pseudo-nitzschia (australis & multiseries),* 200 cells L^{-1} for *Alexandrium pacificum* and 500 cells L^{-1} for *Dinophysis acuminata* (NSWFA 2015). No biotoxins were detected in association with shellfish samples collected during the *Pseudo-nitzschia* or *Dinophysis* blooms noted above. Positive detections of paralytic shellfish toxins (PSTs) of 0.26 and 0.23 saxitoxin equivalent (STX eq.) mg/kg total PST were reported in shellfish samples collected 24 and 29 Oct 2018 coinciding with the bloom *A. pacificum* during Oct 2018. Shellfish samples collected 5 Nov 2018 were negative for PSTs. Positive detections of PSTs of 0.11, 0.16, 0.11 and 0.048 saxitoxin equivalent (STX eq.) mg/kg total PST were reported in shellfish samples collected 10, 17, 22 and 28 Oct 2019 coinciding with the bloom *A. pacificum* during Oct 2019. Shellfish samples collected 14 Nov 2019 were negative for PSTs.

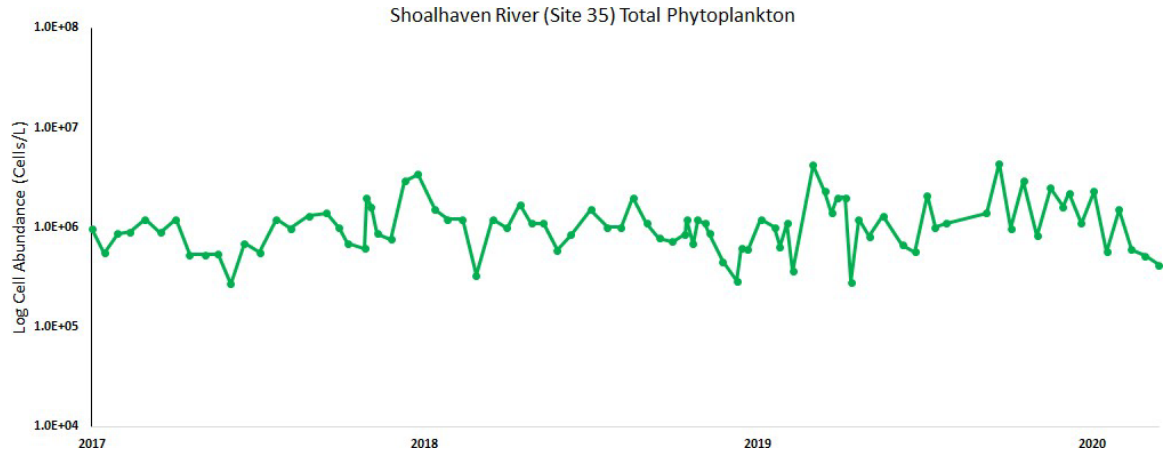


Figure 5.6 Log abundance of total phytoplankton sampled approximately fortnightly from 27 Dec 2017 to 10 March 2021.

5.6 Oyster Growth and Mortality

5.6.1 Oyster Growth

Oyster whole weight increased by 28 g in the experimental period (August 2018 to June 2020) (Fig. 5.7 A). Oyster whole weight increases were greatest in the final 3 months of this experiment (February 2020 to June 2020) when oysters increased their average weight by 10.3 g. Average oyster whole weight was 50.6 ± 1.4 g at the end of the experiment (June 2020). Oysters deployed in Shoalhaven River reached the large size grade (> 50 g whole weight) in June 2020 (50.6 g) and were 42 mo on this date.

Oyster shell length was 53 ± 2 mm at the start of the experiment and increased to 69 ± 1 mm in June 2020 (Fig. 5.7 B). The greatest increase in shell length in the Shoalhaven River was recorded from August to October 2018. The increase in size through this period was 11 mm. Shell lengths were measured more frequently than whole weight and fluctuated throughout the experiment. Periods of shell length decreases were recorded between October 2018 and November 2018, January and February 2019, September and November 2019 as well as December 2019 and February 2020.

5.6.3 Mortality

Low levels of mortality were recorded throughout the experiment (Fig 5.7 C-D). The period where the highest levels of oyster mortality occurred was between September and December 2019. However, the maximum number of dead oysters removed from a basket on a sample date was two in November 2019 and June 2020. Oyster mortality over the study period in Shoalhaven River did not exceed background Sydney Rock Oyster farming mortality (~10% per annum). Oysters from this site remain frozen for future analyses.

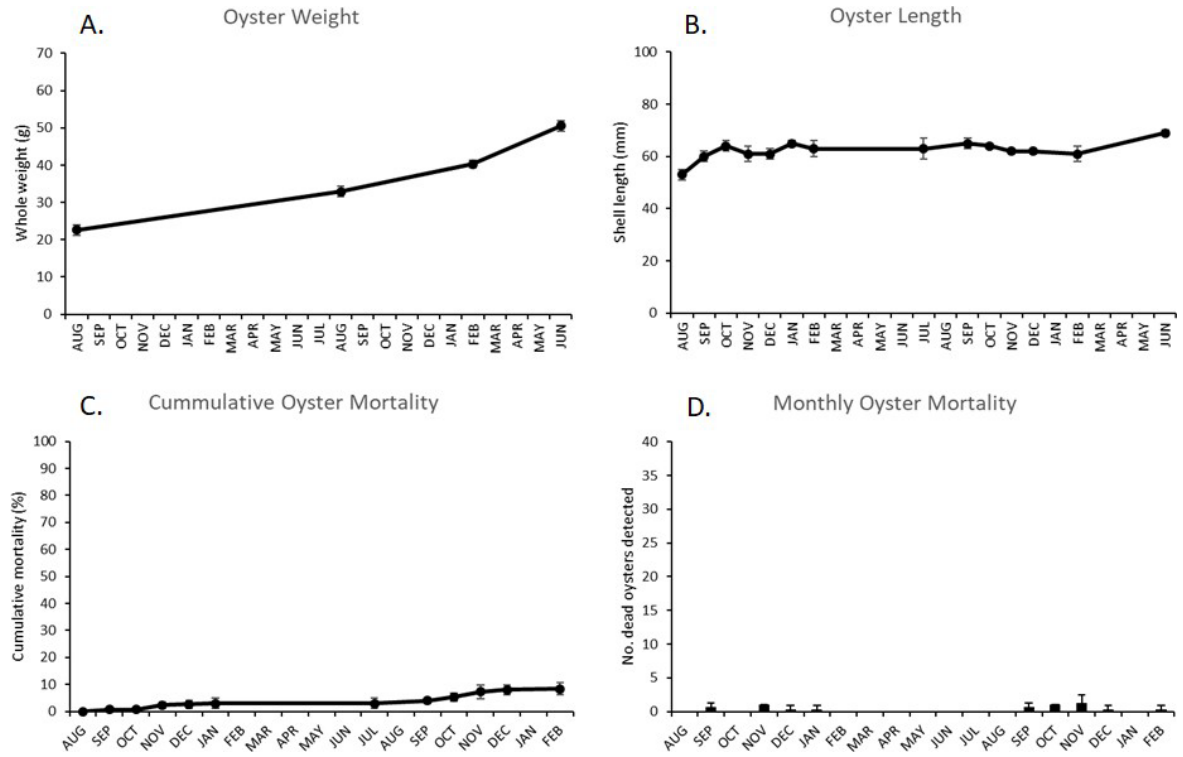


Figure 5.7 A-D. Oysters deployed at the sensor site, Shoalhaven and Crookhaven Rivers. A. whole weight; B. shell height; C. cumulative mortality, and D. monthly mortality.

5.7 Modelling

5.7.1 Modelling of bacterial 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 all best explained by the sensor models as compared to the rainfall models: 23.9% for *E. coli* (sensor + total phytoplankton), 34.8% for cow (sensor + total phytoplankton), 46.1% for bird (sensor + total phytoplankton) and 73% for human (sensor + total phytoplankton) (Table 1A). Conversely, rainfall models only explained 8.66% of the deviance for *E. coli*, 4.38% for cow, 8.88% for bird, and 17.4% for human bacterial loads.

Peak abundance of *E. coli* at the sensor site was linked decreasing salinity over the 72 hours prior to sampling, and a surface water temperature between ~20-24℃ (Table 1 A-B) (Figures 5.7 A-D, 5.8 A-D).

Cow bacteria was also linked to decreasing salinity (again over the previous 72 hours), with a peak in *E. coli* observed at 22 ppt and a surface water temperature of 19℃ (Table 1A).

Faecal contamination from birds was linked with salinity (variable), but increased with an increasing surface water temperature, with maximum load corresponding to >24℃ (Table 1A).

An increase in human bacteria was linked to rainfall on one occasion, but across all samples was best predicted by a salinity of 20-22 ppt and a surface water temperature of 18-20℃ (Table 1A).

5.7.2 Modelling of oyster growth and mortality

While there was insufficient oyster weight data to model (only 4 data points across the sampling period), there was sufficient shell length data to model. The best model explained a moderate ~36.9% of the deviance, with the strongest predictor variables being the daily average salinity (decreasing, with optimal growth at ~35.6 ppt) and weekly rainfall (optimal growth occurring when little rainfall occurred over the previous week).

Table 1 A. Modelling results for bacterial source tracking at the sensor site in the Shoalhaven and Crookhaven Rivers. Only significant variables are shown for each model.

Bacteria	Variables	No. of observations	Significant Variables	Deviance Explained
E. coli	Salinity, Depth, Temp	125	Depth72**, Salinity72***, Temp72***	23%
E. coli	Salinity, Depth, Temp, logPhytoplankton	125	logPhytoplankton ***, depth**, salinity***, temp***	23.9%
E. coli	Rainfall72	111	Rainfall72***	7.49%
E. coli	Rainfall72, logPhytoplankton	111	Rainfall72***, logPhytoplankton ***	8.66%
Bird	Salinity, Depth, Temp	125	Salinity***, Depth***, Temp***	45.9%
Bird	Salinity, Depth, Temp, logPhytoplankton	125	Salinity***, Depth***, Temp***, logPhytoplankton ***	46.1%
Bird	Rainfall72	111	Rainfall72***	6.93%
Bird	Rainfall72, logPhytoplankton	111	Rainfall72***, logPhytoplankton***	8.88%
Cow	Salinity, Depth, Temp	125	Salinity***, Depth***, Temp***	34.7%
Cow	Salinity, Depth, Temp, logPhytoplankton	125	Salinity***, Depth***, Temp***, logPhytoplankton***	34.8%
Cow	Rainfall24	119	Rainfall48***	3.38%
Cow	Rainfall24, logPhytoplankton	119	Rainfall48***, logPhytoplankton***	4.38%
Human	Salinity, Depth, Temp	125	Salinity***, Depth***, Temp***	62.5%
Human	Salinity, Depth, Temp, logPhytoplankton	125	Salinity***, Depth***, Temp***, logPhytoplankton***	73%
Human	Rainfall24	111	Rainfall24***	11.3%
Human	Rainfall24, logPhytoplankton	111	logPhytoplankton***	17.4%

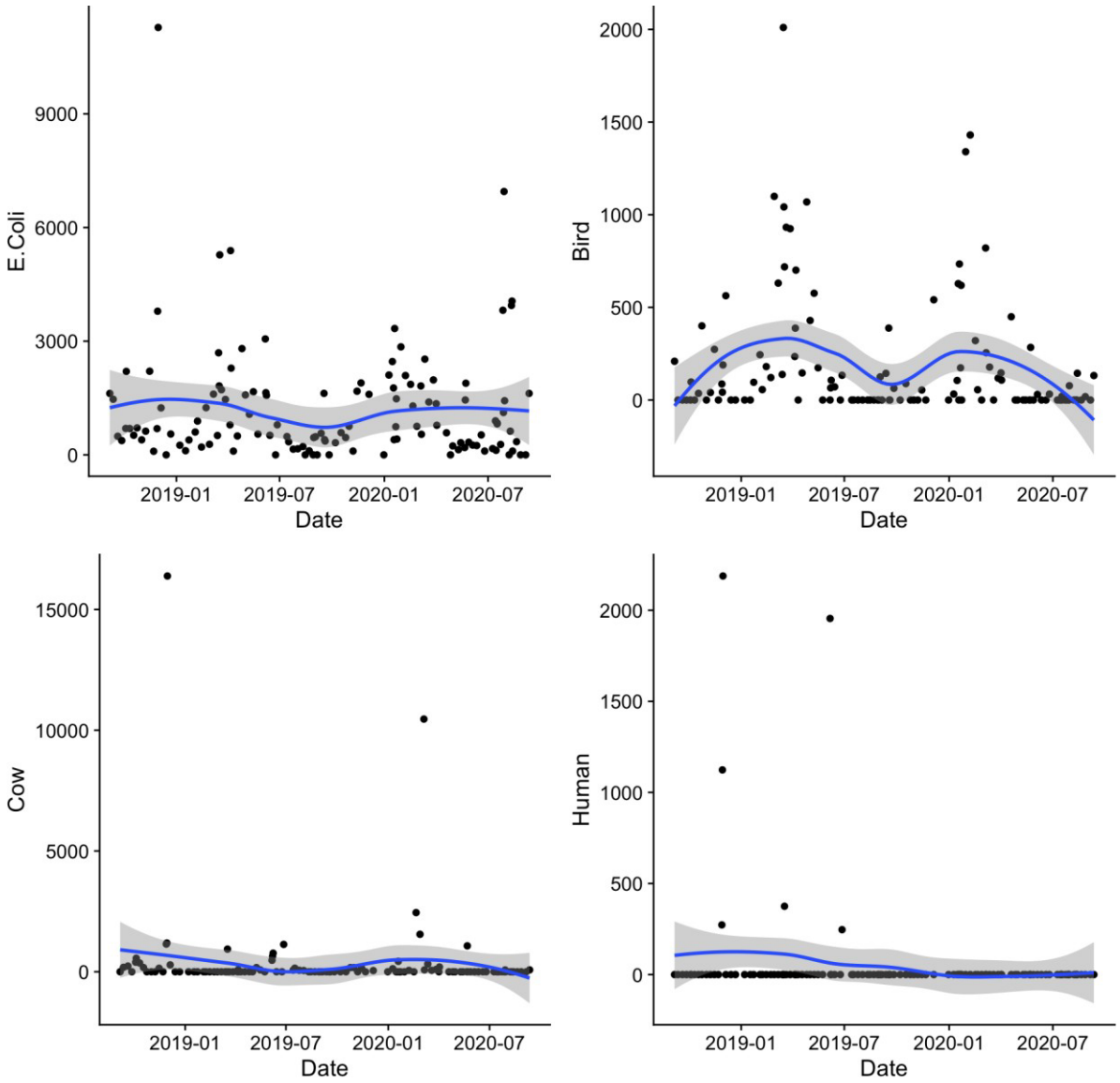


Figure 5.7 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. *E. coli*, B. Bird, C. Cow, and D. Human bacterial load as measured by weekly sampling at the sensor site, Shoalhaven and Crookhaven Rivers.

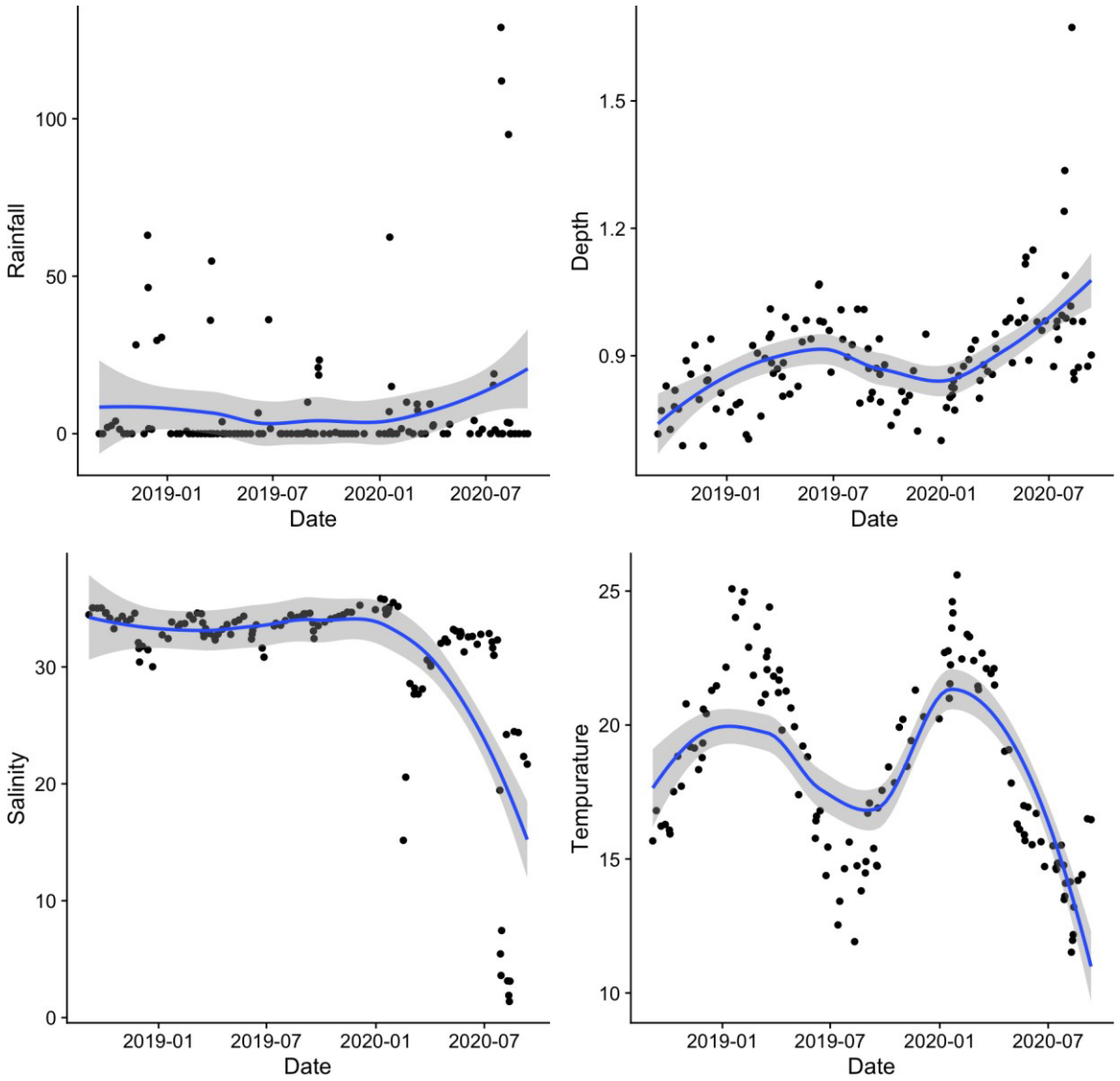


Figure 5.8 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. Rainfall, B. Depth, C. Salinity, and D. Temperature values measured in at the sensor site, Shoalhaven and Crookhaven Rivers.

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 Goodnight Island harvest area, but a distinct pattern was apparent between drought and flood conditions. Data collected prior to July 2020, during a drought period, indicated that up to four harvest area closures and one harvest area downgrade could have potentially been avoided. Salinity data from July 2020 onwards showed a higher level of variability due to more frequent rainfall events, and the relationship between salinity fluctuations and harvest area status was less clear. Further data collection may provide insight to support management plan changes in estuaries that have substantial fresh water impacts. Shoalhaven and Crookhaven Rivers Shellfish Program (SCRSP) were consulted about the option of a salinity-only management plan for Goodnight Island harvest area following previous annual reviews, but a decision has not yet been reached. If SCRSP 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 Goodnight Island harvest area management plan would revert to the current management plan that is based on both rainfall and salinity closure limits.

6.2 Phytoplankton and HABs

Pseudo-nitzschia is a high-risk HAB group in SE Australia for the shellfish aquaculture industry, and both estuaries and coastal waters in this area remain under threat (Ajani et al., 2013a, 2020). Blooms within the Hawkesbury River estuary (~290 km north of the Shoalhaven and Crookhaven Rivers), 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, 2020). Fifteen years of modelled data in the Hawkesbury River estuary revealed that *Pseudo-nitzschia* was linked to an increase in soluble reactive phosphorus and a decrease in nitrogen at all six sites sampled (via rainfall/nutrient runoff). There is contrasting evidence, however, of which environmental conditions promote the blooming of the different species complexes (Dermastia et al., 2020). In response to a toxic bloom of *Pseudo-nitzschia delicatissima* gp. (dominated by *P.* cf. *cuspidata)* in Wagonga Inlet in April 2019, we have now successfully developed a rapid, sensitive and efficient quantitative real-time polymerase chain reaction (qPCR) assay to detect *P. pseudodelicatissima* complex Clade I, to which *P.* cf. *cuspidata* belongs (Ajani et al. 2021).

Another HAB species that bloomed in the Shoalhaven and Crookhaven Rivers during this study was *Alexandrium pacificum.* Approximately 33 species of *Alexandrium* have been recorded worldwide, of which around 10 species can potentially produce Paralytic Shellfish Toxins (PSTs). These are *A. affine, A. andersonii, A. pacificum* (= *A. catenella* Group IV ribotype); *A. australiense* (= *A. tamarense* Group V ribotype), *A. minutum, A. ostenfeldii, A. catenella, A. tamiyavanichii* and *A. taylori* (Anderson et al. 2012, Tomas et al. 2012, John et al. 2014). PSP was first reported in Australia in 1935, when typical PSP symptoms were observed following the consumption of wild mussels collected from Batemans Bay, NSW (Le Messurier et al. 1935). In 1986, the first PSP outbreak in Australia was recorded in Port Philip Bay, Victoria,

with *A. pacificum* (as *A. catenella*) as the causative organism (Hallegraeff et al. 1992). A. *pacificum* is also the main causative agent of PSTs in NSW (Ajani et al. 2013). In October 2016, high cell densities of this species were detected in the coastal waters of Twofold Bay, NSW, an unprecedented event for this location in south eastern Australia. With a maximum cell density (89,000 cells L^{-1}) and a concentrations of 7.2 mg/kg PST STX equivalent in blue mussels (*Mytilus galloprovincialis*) from the bay, a four-month shellfish harvest closure ensued (Barua et al. 2020). Another unprecedented bloom of this species occurred early in Tasmania in 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).

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 the Shoalhaven and Crookhaven Rivers

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

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

The second group of assays developed were those that were microbial source tracking as they detect bacteria of faecal origin specifically associated with a group of animals, i.e. bird, cow and human. Birds are a significant source of faecal contamination in estuarine/marine waters during dry periods, and increase 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. 2021).

E.coli, cow and human bacterial contamination were all (in most cases) linked to decreasing salinity (increased rainfall) with the sensor being significantly more sensitive to this change in the water characteristics than rainfall data. The exception to this was faecal contamination from birds which again, was significantly more predictable using the sensor data compared to the rainfall

data, but was also linked to water temperature, with the warmer waters of summer and autumn revealing high loads.

Similarly, avian faecal pollution in the Manning River and Wagonga Inlet was linked to increasing salinity and temperature, but was observed to peak during the autumn and summer months. Peak levels in all these estuaries 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.

While levels of human bacterial contamination observed in this study are variable, the link of elevated concentrations to rainfall in Nov 2018 may suggest that water quality management efforts in regard to sources of human contamination in this estuary should be reviewed. Sewer overflows and septic tank seepage present the highest impact/risk for human contamination in the Shoalhaven/Crookhaven Rivers. After an outbreak of hepatitis A which was linked to the consumption of oysters from Wallis River in 1997 (via a sewage spill), a wide range of human enteric viruses were detected in a large number of oyster and sediment samples at this location. Since this time, new legislation which tightens controls over septic maintenance was created, new sewerage management plans were 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 the Shoalhaven River were greatest during the last 3 months of this experiment (February 2020 to June 2020). Growth, in terms of shell length, was greatest in the first 3 months of the experiment (August to October 2018). This was a period characterised by high salinity (> 32 ppt) and increasing water temperature levels. Higher salinities increase seawater alkalinity providing more calcium carbonate available for oyster shell deposition. The salinity level that promotes the greatest growth rates in Sydney Rock Oyster spat is 30 ppt for small spat (1.3 mg) and 35 ppt for larger spat (0.61 g) (Nell and Holliday, 1988). Fastest growth of Sydney Rock Oyster spat occurs at 30 °C. However, the optimal water temperature and salinity combination for spat survival is 23 °C and 30 ppt, respectively (Dove and O'Connor, 2009).

Survival of oysters during the experiment was very high from deployment in August 2018 through to February 2020. Cumulative mortality through this period was 8.3% which was the lowest level recorded for all sites monitored and was below the background farming mortality (approximately 10% per annum) commonly experienced when farming Sydney Rock Oysters. The cumulative mortality measured in the Shoalhaven River was comparable to cumulative mortality measured in Wagonga Inlet (10.7%) over the study period. Cumulative mortality measured in the

Shoalhaven River in a previous study was 20% over a 26-month period from April 2004 to June 2006 (Dove and O'Connor, 2009).

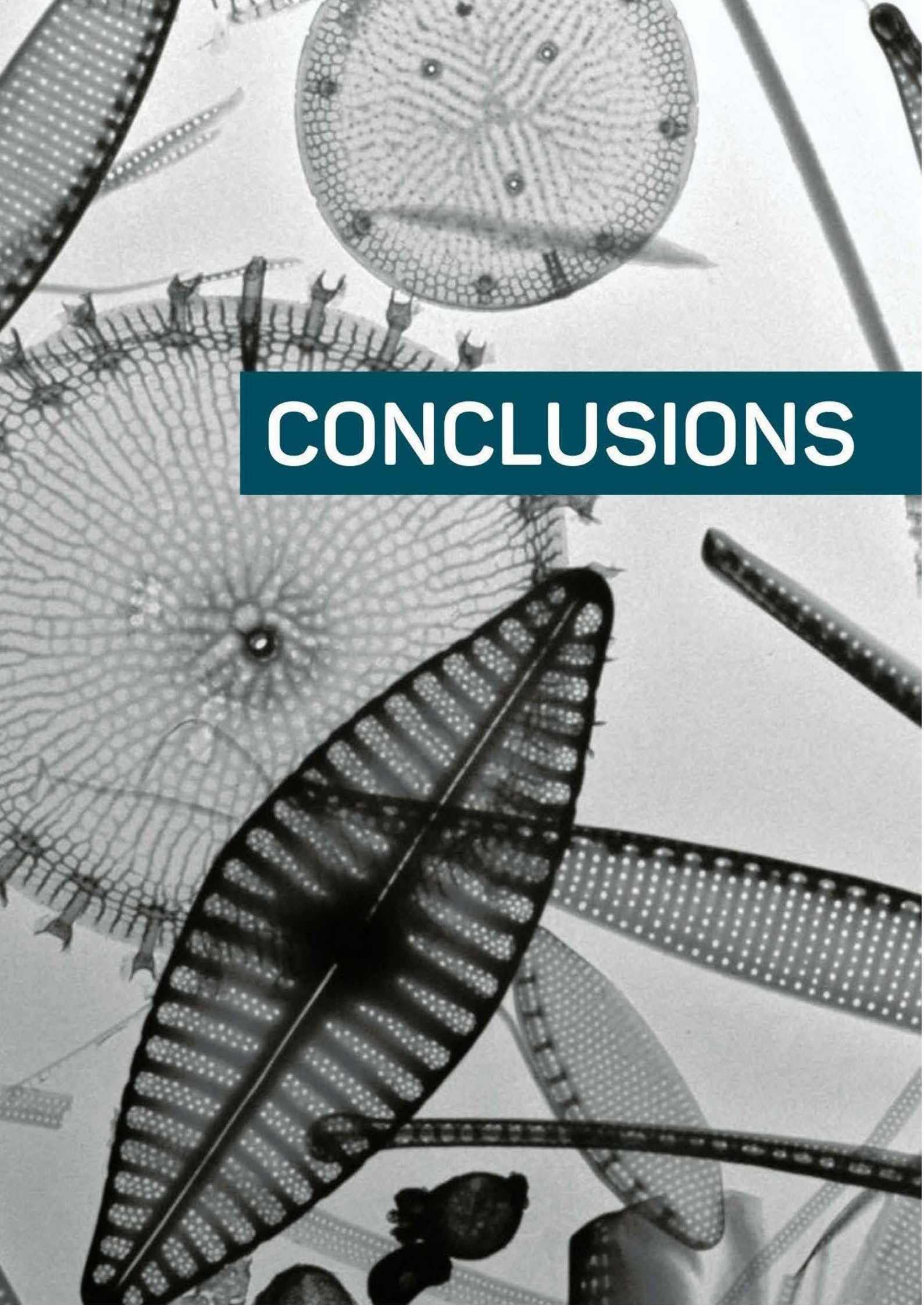
The batch of oysters used for this experiment were a random mix of families taken from the 2016-year class of the Sydney Rock Oyster Breeding program. This particular year class had 86% of the parents selected from wild and QX disease resistant genetic groups. Only 14% of the parents for this year class were sourced from the fast growth genetic group. It took this year class 3 years and 6 months to reach the large oyster size grade (> 70 mm total length or > 50 g whole weight). Estuaries where this same batch of oysters reached the large oyster size grade benchmark at the same time were Hastings River (52.5 g), Port Stephens (58.5 g), Wallis Lake (50.6 g) and Pambula River (59.4 g).

When oyster growth measured at the conclusion of the experiment (June 2020) was compared between the twelve estuarine sites in this study, the Shoalhaven River ranked 10th and 9th in terms of whole oyster weight and shell length, respectively. The growth of oysters in Shoalhaven River was very similar to what was measured in Wallis Lake during this experiment.

The Shoalhaven/Crookhaven River is the 9th largest oyster producing estuary in NSW (NSW DPI 2022) with Sydney Rock Oyster production worth approximately $1.6 million in the 2020/21 financial year (NSW DPI 2022). Most Sydney Rock Oysters produced in this estuary are sold as a medium size grade. Triploid Pacific Oysters are also produced in Crookhaven River and contributed an extra $160,000 to farm gate income.

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 suggested that the current harvest area management plan salinity may be conservative after some rainfall events. Implementing a harvest area management plan based on sensor salinity data for Goodnight Island harvest area, subject to the agreement by the local shellfish industry is possible. Available data indicated that up to four harvest area closures and one harvest area downgrade could have potentially been avoided between December 2017 and June 2020. From July 2020 onwards, the relationship between salinity fluctuations and harvest area status was less clear, and the local program should consider that more closures or additional testing may be necessary depending on the severity of rainfall events. As of August 2022, seventeen salinity-only management plans had been offered for harvest areas in participating NSW estuaries, with six being taken up and the remaining eleven under consideration.

Compared to the other monitoring sites in NSW, oyster growth in the Shoalhaven River ranked 10th and 9th in terms of whole oyster weight and shell length, respectively. The Shoalhaven River had the best oyster survival out of all estuarine sites that were monitored for 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 however, showed a significantly higher predictive capability than rainfall for all of the four faecal indicator bacteria. Furthermore, while contamination from bird sources was observed at low levels, a distinct presence throughout the black summer bushfires 2019-2020 was observed.

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

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

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9. Appendices

A1. Methods

A1.1 Sampling locations in the Shoalhaven and Crookhaven Rivers

Data used in this report originates from locations within the Shoalhaven and Crookhaven Rivers over the period Sept 2017 to March 2021. High-resolution temperature, salinity and depth data were obtained from a sensor location from 21 Dec 2017 to 31 March 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 the Shoalhaven and Crookhaven Rivers highlighting the sensor location (black square) and the phytoplankton sampling location (black circle).

A1.2 High-resolution sensor data

High-resolution temperature (°C), salinity and water depth (m) data were collected from the sensor site using 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 BOM rainfall gauge at Greenwell Point (068080, -36°56'29.78"S and 149°48'37.73"E).

Figure A2 Seabird MicroCAT high accuracy conductivity, temperature and depth (CTD) field sensor deployed in the Shoalhaven and Crookhaven Rivers. Image supplied by Angela Riepsamen.

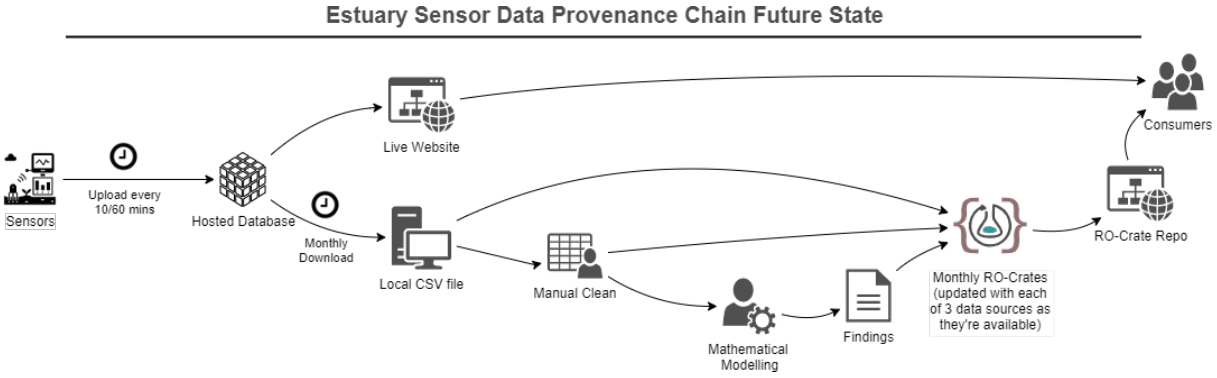

Figure A3. Shoalhaven and Crookhaven Rivers data provenance chain from source of data (sensors), via quality assurance processes, data analyses, to consumers.

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 Shoalhaven and Crookhaven Rivers annual review is 1 July. As part of the most recent (2022) annual review for Goodnight Island harvest area, all salinity data from the monitoring sensors during the 2018, 2019, 2020, 2021 and 2022 annual review periods were assessed in relation to microbiological samples collected by the local shellfish program during the same period. Data via a new sensor and provider commenced on 15 April 2021. During the transition period, there was a gap in data between 1 and 15 April 2021. During the 2022 annual review period there were occasional and sporadic gaps in data collection due to telecommunications issues. After 1 January 2022, there were occasional spikes in salinity data to above 35 ‰. It is likely that these data were a result of debris during large freshwater events impacting sensor readings. These points have not been removed from the dataset, so that the salinity profile from that time onwards could be examined, however, the high values should be interpreted with caution. Salinity data were unavailable due to instrument error after 1 June 2022.

A1.4 Biological sampling and eDNA extraction

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

In the case of a rainfall event, water samples were collected for bacterial analysis (only) every 24 h over a two-day period commencing on the first day of rainfall and processed as described above. Daily rainfall measurements were taken from the closest available weather stations at PLSP, Lot 5 Robinson Road Lochiel 2549, NSW, which is approximately ~8 km from the of sensor site.

A1.5 qPCR assays for bacterial source tracking

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

A1.6 Phytoplankton enumeration

Water samples (500 mL) were collected at approximately 2-weekly intervals from a depth of 0.5 m closest to the sensor for microscopic phytoplankton identification and enumeration in accordance with the NSW Marine Biotoxin Management Plan (NSW MBMP) and the Australian Shellfish Quality Assurance Program (ASQAP). Once collected, samples were immediately preserved with 1% Lugol's iodine solution, and returned to the laboratory for concentration using gravity-assisted membrane filtration. Detailed cell examination and counts were then performed using a Sedgewick Rafter counting chamber and a Zeiss Axiolab or Standard microscope equipped with phase contrast. Cells were identified to the closest taxon that could be accurately identified using light microscopy (maximum magnification x1000). Cell counts were undertaken to determine the abundance of individual HAB species and total phytoplankton cell (>5 mm) numbers. *Dinophysis* cells were counted to a minimum detection threshold of 50 cells L^{-1} while all other species were counted to a minimum detection threshold of 500 cells L^{-1}.

A1.7 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.

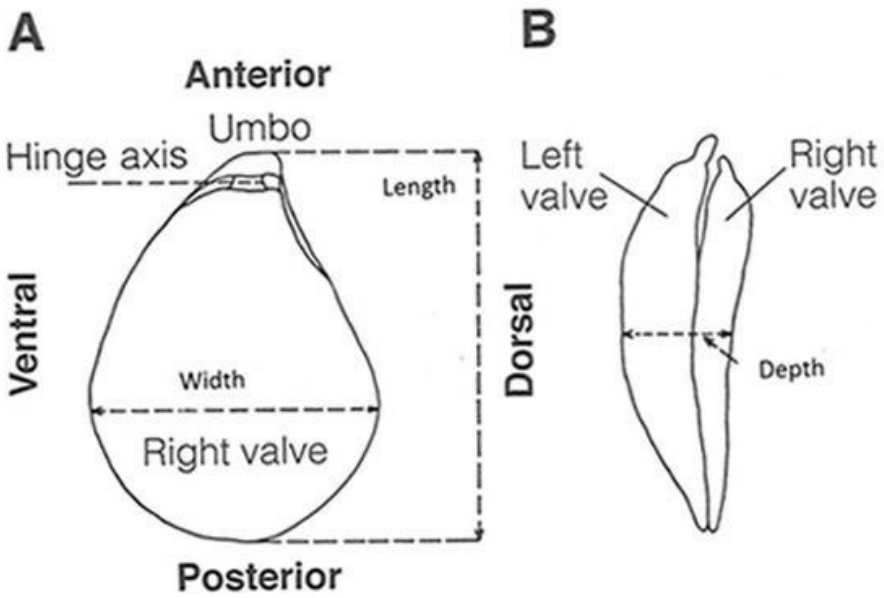

Figure A4. Oyster shell dimensions (Carriker 1996)

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.

A1.8 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 BOM rainfall gauge at Greenwell Point Bowling Club (~-34.158°S and 150.7312°E), which was the official management plan gauge for this harvest area, were averaged over the 24 h, 48h, 72 h and 7 days prior to the water sampling each day, to incorporate a measure of exposure of the bacterial community and deployed oysters. Total phytoplankton (and log transformed total phytoplankton) from microscopic phytoplankton enumeration was also included in the modelling as a potential predictor variable. Finally, week of the year and water depth were included in the models to understand any seasonality or tidal variability that was present in the data.

To model the relationship between bacteria (*E. coli*, bird, cow, human) abundance and/or oyster growth (response variables) and environmental variables (temperature, salinity, week, depth, total phytoplankton, rainfall) at the sensor location within 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 time periods, including depth, week and total phytoplankton (logged or unlogged). For comparison to current (non-sensor-based) practice, models were also run using only rainfall data. Again, these included depth, week and total phytoplankton. As total phytoplankton data is not available in real time, and therefore not considered a predictor variable by definition, models were run both with and without this variable. In summary, four models were developed for each of the bacterial sources: rainfall only, rainfall and total phytoplankton; sensor only; and sensor and total phytoplankton.

To model the relationship between oyster growth various GAMs models were also investigated using the sensor/total phytoplankton/rainfall data for the same time period. These models were then fitted in version 3.4.3 of the R statistical package (Team R Core, 2013), using the GLM function in version

1.8–22 of the 'mgcv' package (Wood, 2006). Models were then compared using the Akaike information criterion (AIC) and the model with the lowest AIC selected.

Appendix 2A. Summary Statistics for Bacterial Modelling – Sensor site, Shoalhaven and Crookhaven Rivers

Variable	Mean	Standard Error	Median	Standard Deviation	Minimum	Maximum	Count	Missing
average_cfu	8.72	1.22	3.27	13.76	0.00	106.71	127	0
bird	188.03	30.27	31.94	341.16	0.00	2010.37	127	0
cow	340.77	154.15	0.00	1737.17	0.00	16386.03	127	0
depth24	0.90	0.01	0.88	0.13	0.69	1.67	127	0
depth48	0.90	0.01	0.89	0.11	0.71	1.35	127	1
depth72	0.90	0.01	0.88	0.10	0.74	1.23	127	2
ecoli	1190.00	133.44	692.63	1503.78	0.00	11281.13	127	0
human	48.51	24.81	0.00	279.59	0.00	2188.15	127	0
logPhytoplankton	13.85	0.04	13.91	0.48	12.58	15.25	127	0
Phytoplankton	1172755.91	60171.57	1100000.00	678099.21	290000.00	4200000.00	127	0
rainfall24	7.91	1.86	0.00	20.95	0.00	129.00	127	8
rainfall48	8.03	1.50	0.70	16.87	0.00	120.50	127	12
rainfall72	8.05	1.26	1.87	14.17	0.00	80.33	127	16
salinity24	30.80	0.65	33.11	7.38	1.37	35.84	127	0
salinity48	30.82	0.63	33.10	7.09	1.64	35.81	127	1
salinity72	30.84	0.61	33.01	6.86	2.13	35.51	127	2
temp24	18.49	0.31	18.43	3.52	11.52	25.60	127	0
temp48	18.51	0.31	18.51	3.46	11.74	24.89	127	1
temp72	18.52	0.30	18.73	3.44	11.89	24.80	127	2

Appendix 3. Outreach: 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
DPI Food Authority	Foodwise - Issue 60	https://www.foodauthority.nsw.gov.au Winter 2022	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
DPI Food Authority	Foodwise - Issue 56	https://www.foodauthority.nsw.gov.au Autumn 2021	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	Published

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

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