TRANSFORMING AUSTRALIAN SHELLFISH PRODUCTION

Lower Limeburners Creek Harvest Area, Hastings River.

Report on Stage 1, May 2018 - March 2021, Sydney, Australia

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

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Transforming Australian Shellfish Production: Lower Limeburners Creek Harvest Area, Hastings River. Report on Stage 1, May 2018 - March 2021

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Executive Summary

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

We installed a real-time sensor into Lower Limeburners Creek harvest area, Hastings River, recording high-resolution temperature, salinity and depth data. Oyster farmers collected weekly biological samples (654 environmental DNA samples and 273 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, nutrients etc.), to develop predictive models. Finally, we developed an additional model to link oyster growth with environmental variables and assessed its predictive capability.

MAJOR FINDINGS

2

Available data indicated that two harvest area closures and three harvest area downgrades could have potentially been avoided between May 2018 and May 2022

100%

Salinity was a more reliable predictor than rainfall for the 4 faecal bacteria types modelled, showing changed harvest area management would be safer and more discriminatory.

E. coli (and to a lesser extent, cow and human bacteria) increased after rainfall, but generally dissipated soon after.

Bird bacteria became elevated in warmer months

x2

Cumulative mortality in the Hastings River from August 2018 to February 2020 was 34%. This was significantly higher than the background farming mortality level, which is estimated at 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 improving production and harvest management and to reduce harvest closure days for farmers.

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

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

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

Preliminary results from this high frequency data have already demonstrated the link between salinity levels related to rainfall and *E. coli* levels. In 2019, the NSW Shellfish Program's Annual Sanitary Survey Report (DPI) stated that using this real-time, high frequency environmental data, the project provided the basis for a change to the management plans for the Pambula River harvest area and the Cromarty Bay harvest area (Port Stephens). These

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

Across the NSW shellfish industry, the potential economic benefit from the use of real-time sensors for harvest area management is conservatively estimated at up to $3 million annual farm gate value. Increased revenue will improve the confidence of the industry to further invest and drive more growth. As of August 2023, nineteen salinity-only management plans had been offered for harvest areas in participating NSW estuaries, with seven being taken up and the remaining twelve under consideration.

1.2 Hastings River

Hastings River (-31.40406°S, 152.8917°E) is an open and trained intermediate wave dominated barrier estuary located in the Mid North Coast region of New South Wales, approximately 380 km north of Sydney (Roy et al. 2001). It has a catchment area of 3659 km^2, a total estuary area of ~30 km^2 and a flushing rate of ~13 days (Roy et al. 2001, Roper et al. 2011) (Fig. 1). The surrounding catchment is mainly undeveloped (70% undisturbed forest) with the remaining ~30%, used for grazing and urban development. The aquatic system supports many significant areas of seagrass (1.5 km^2) as well as mangroves (3.4 km^2), saltmarsh (~2 km^2) (Roper et al. 2011). Tributaries entering Hastings River to the left include Forbes River, Pappinbarra River, Mortons Creek and Maria River, and to the right include Fenwicks Creek, Tobins River, Ralfes Creek, Ellenborough River and Thone River.

1.3 Oyster Production in the Hastings River

The Hastings River has long history of oyster harvesting and cultivation beginning as early as the 1830's (Hastings River Oyster Farmers EMS 2012). Hastings River produces Sydney Rock Oysters and production value today is estimated to be ~115K dozens and valued at ~$1.1 mil (NSW DPI 2023). Over the years there have been periods of oyster mortality, and water quality issues such as pollution/stormwater and acid sulphate soils have been identified and remedial work undertaken (Hastings River Oyster Farmers EMS 2012).

FINDINGS

2. Findings

2.1. The data assessment from this report supports implementing a harvest area management plan based on sensor salinity data for Lower Limeburners Creek harvest area, Hastings River, subject to agreement by the local shellfish industry. Available data indicated that two harvest area closures and three harvest area downgrades could have potentially been avoided between May 2018 and May 2022.

2.2. We developed rapid, efficient, and sensitive qPCR assays for *E. coli*, cow, bird, and human faecal indicators, and used these rapid genetic tools to track these sources of pollution in the Georges River over the biological sampling period, September 2018 to September 2020.

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

2.4. The maximum predictive capability for each bacterial group was 28% for *E. coli*, 65% for cow, 33% for bird, and 29% for human at the sensor site.

2.5 Where the models were predictive, they predominantly suggested bacterial abundance increased with decreasing salinity. The exception to this was avian bacteria which increased with increasing salinity and warmer months.

2.6. The greatest increase in shell length in Hastings River was recorded from August to December 2019. Whole oyster weight increased steadily over the trial, with the greatest increase occurring from February to June 2020. Oyster whole weight increased by 10.6g over this time.

2.7. Cumulative mortality in the Hastings River reached 34% by February 2020. This is above the level of mortality expected for background farming of the Sydney Rock Oyster (approximately 10% per annum). A major proportion of this mortality occurred during the warmer spring to summer months from January to February 2019 (20%) and November 2019 to February 2020 (9%). Mortality was low (<1%) between all other sampling occasions.

ACKNOWLEDGEMENTS

3. Acknowledgements

This project has been funded under the Bushfire Local Economic Recovery Fund, co-funded by the Australian and NSW Governments in association with the Food Agility CRC and the NSW Farmer's Association. The Food Agility CRC Ltd is funded under the Commonwealth Government CRC Program. The CRC Program supports industry-led collaborations between industry, researchers and the community. The Department of Primary Industries and the University of Technology also provided project funding. The project team would like to acknowledge the invaluable assistance of Mr Paul Wilson for his assistance with sample collection and Stuart Bale for site access. 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 Hastings River were funded by the NSW Food Authority and the shellfish industry. We thank Kyle Johnston and Brandt Archer (DPI) for oyster stock preparation and growth/survival data collection, and Dr Nahshon Siboni and Prof Justin Seymour (UTS) for source tracking assistance. Finally, we would like to thank Dr Torri Callan (UTS) for statistical analyses and Chris Komorek and Anika Berkman (Food Agility CRC) for report layout.

FEEDBACK

4. Feedback

In June 2018, the Oyster Transformation Team held an information workshop to allow farmers the opportunity to have their say in the project. The workshop was at the Manning Valley Visitor Information Centre in Taree, New South Wales. Farmers were asked to rate the following factors in order of importance and benefit to their business operations (Fig 4.1). Of highest importance to them was the prediction of harmful algal blooms and access to real time monitoring data, followed by reduced stock mortalities/disease, longer harvest opening times with forecasting ability, and access to real time tidal information. Group discussions followed, whereby additional issues that farmers raised were; if routine algal monitoring methods could be changed and if identifying sources of *E. coli* via genetics was possible. Remarks relating to direct harvest and management plan changes, pollution source tracking, and concerns about mudworm were also noted.

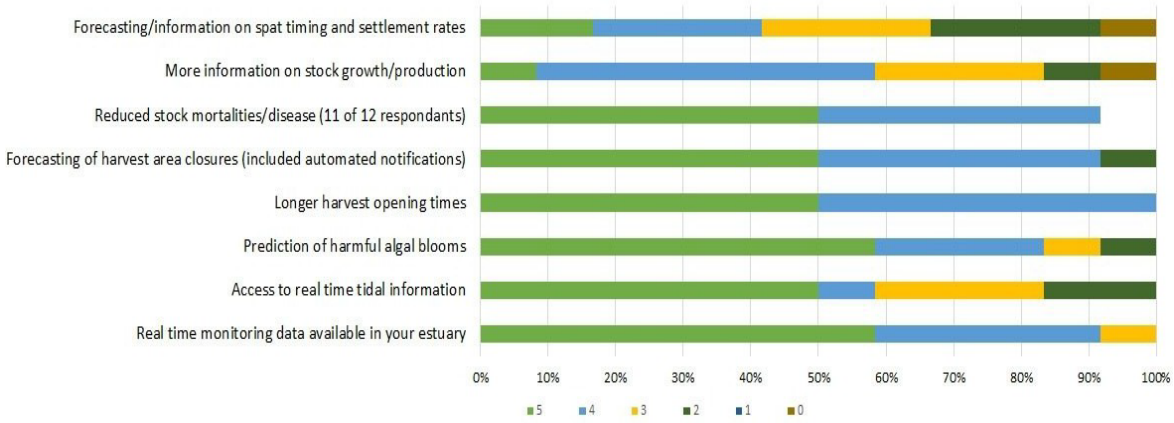


Figure 4.1. The importance of factors as rated by farmers in relation to their business operations. Light green is most important and brown is least important.

RESULTS

5. Results

5.1 High resolution temperature and salinity data

High-resolution real time data summaries for the Hastings River for the period 1 May 2018 to 14 Dec 2020 are shown in Figs. 5.1A-C. Data after 14 Dec 2020 (until Mar 2021) appeared erroneous, possibly due to debris after heavy rainfall in mid-December, and not included in any further processing. Depth recordings ranged from 0.3 m (21 Nov 2018) to 2.4 m (25 May 2020). The lowest and highest daily average salinity recordings were 4.1 ppt (10 Feb 2020) and 35.7 ppt (6 Dec 2020) respectively, while the lowest and highest daily average temperature recordings were 15.2℃ (11 Aug 2019) and 25.9℃ (25 Mar 2019) respectively.

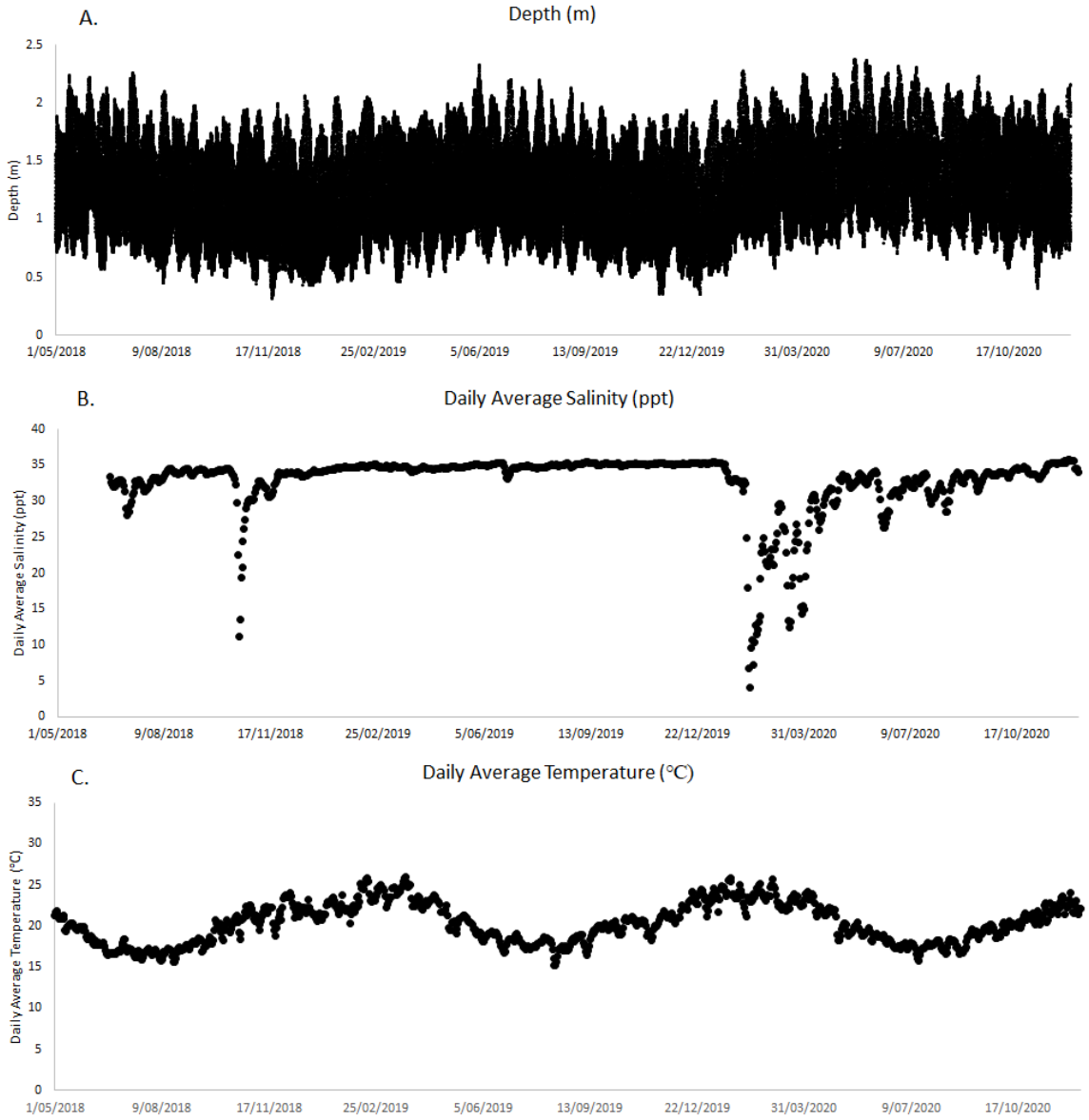


Figure 5.1A-C. Real time sensor data from the Hastings River sensor 1 May 2018 to 14 Dec 2020 A. Depth (m); B. Daily average salinity (ppt); and C. Daily average temperature (°C).

The maximum daily rainfall at the Port Macquarie BOM rainfall gauge (60139/60168) occurred on 20 Mar 2021 and was reported as 187 mm (Fig. 5.2).

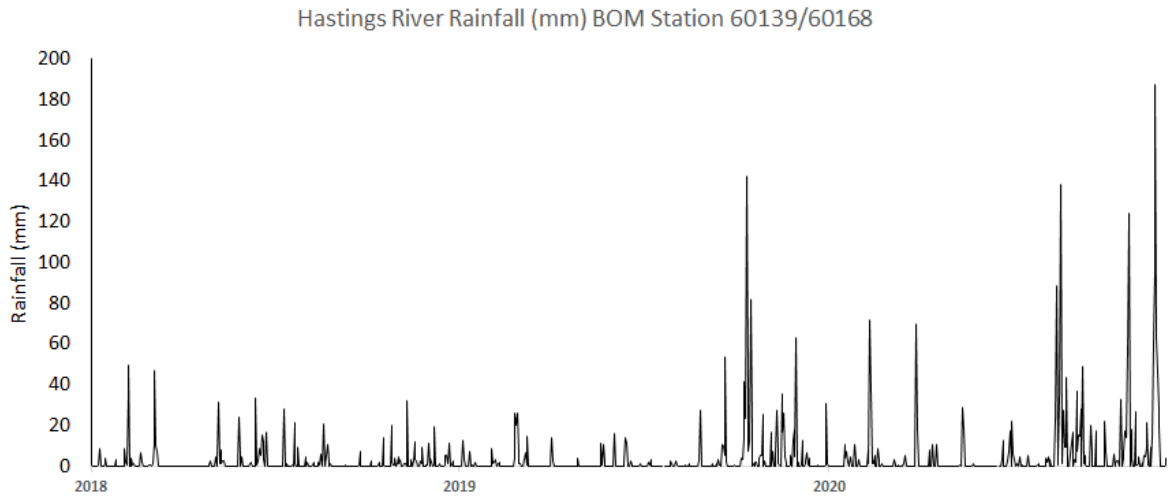


Figure 5.2. Daily rainfall (mm) from the Port Macquarie BOM rainfall gauge (60139/60168) (~-31.43° S, 152.87° E) from May 2018 to March 2021.

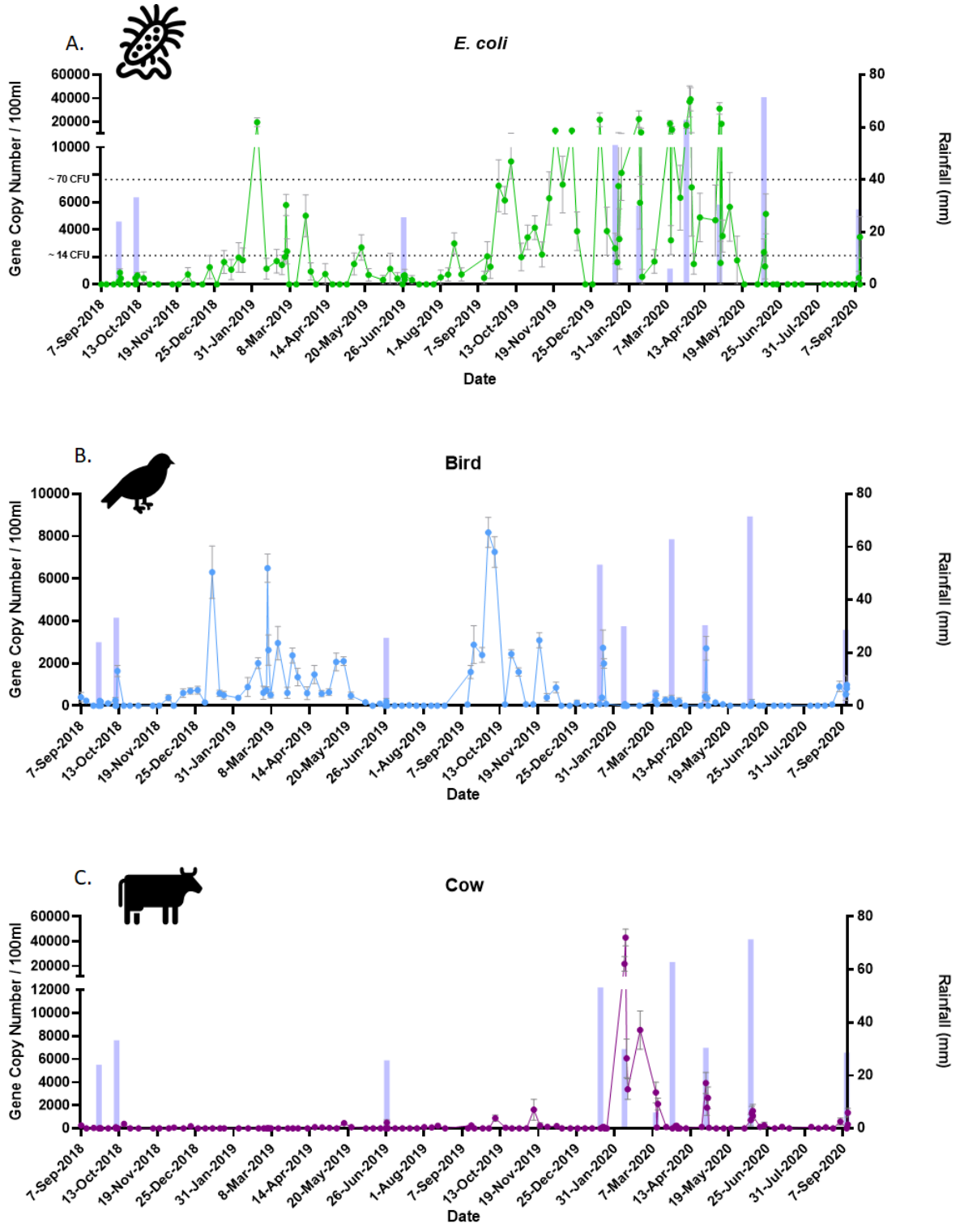
5.2 Management Plan

Data analysed during the 2022 annual review of Lower Limeburners Creek indicated that there could have been fewer harvest area closures and downgrades since the sensor was installed, if downgrades and closures were based on salinity sensor data. There were thirteen harvest area rainfall closures in Lower Limeburners Creek harvest area between May 2018 and May 2022. During the same period there were two harvest area salinity closures, as advised by the local program monitoring local conditions. Based on a management plan sensor salinity closure limit of 19 ‰, harvest area closures were reviewed focusing on available salinity sensor data and shellfish program microbiological results since May 2018. Ten harvest closure days occurred over two rainfall closures, although salinity sensor data did not decline below 19 ‰ and microbiological results from samples collected between 0 and 6 days post closure met Approved harvest criteria. During the same period, there were eight rainfall downgrades in Lower Limeburners Creek harvest area. A review of salinity sensor data and shellfish program microbiological results indicated that there were three rainfall downgrades where salinity as reported by the sensor was higher than 21 ‰ (downgrade salinity range 19-21 ‰), and available microbiological results from samples collected between 0 and 3 days post downgrade met Approved harvest criteria. Time periods where salinity is slower to recover may require additional sampling to meet management plan requirements. A review of available data also indicated that given fluctuations in salinity between high and low tides, particularly after prolonged wet periods, decisions on harvest area closures would consider salinity trends rather than point in time measurements.

5.3 Bacterial source tracking

A total of 654 water samples and 273 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 the Hastings River (Fig. A1).

For the Hastings River the maximum *E. coli* reached 39,159 gene copies 100 mL^{-1} on 31 Mar 2020, 8,180 copies 100 mL^{-1} for *Helicobacter* (bird) on 3 Dec 2019, 43,105 gene copies 100 mL^{-1} for bovine faecal pollution (cow) on 11 Feb2020, and finally, 220 copies 100 mL^{-1} for human faecal pollution also on 15 Nov 2019 (Fig. 5.3 A-D).



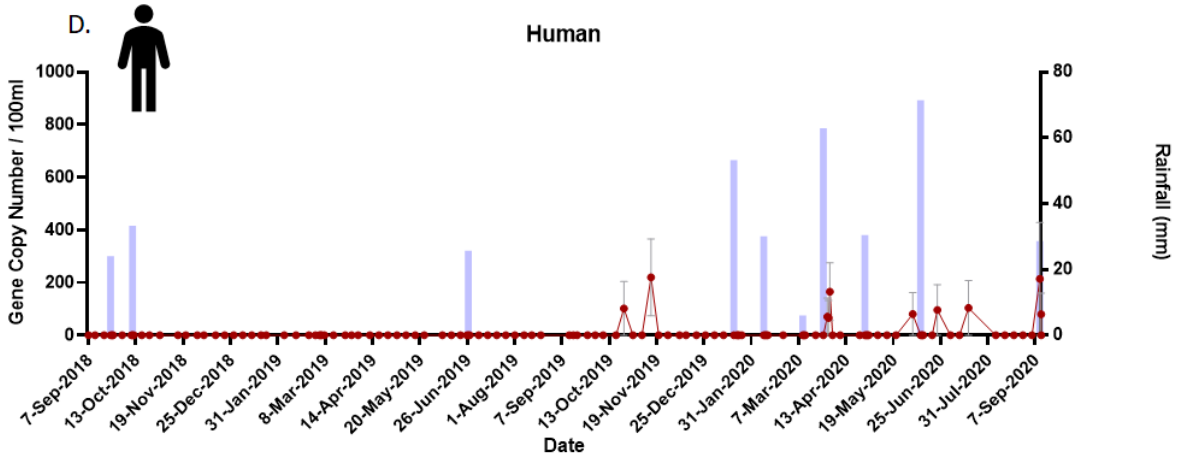


Figure 5.3 A-D. Weekly *E. coli* data from the sensor location, Hastings River, using A. *E. coli* assay; B. Bird assay; C. Cow assay; C2. Cow assay with different y-axis scale to show low levels of bovine contamination across sampling period; and D. Human assay. Dotted lines in Fig. A at 14 and 70 cfu/100 mL are the operational limits for direct or restricted (oysters must meet depuration requirement) harvest, respectively, depending on individual harvest area classification. Lower Limeburners Creek harvest area is classified as Conditionally Approved dual management. https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/industry/shellfish_industry_manual.pdf.

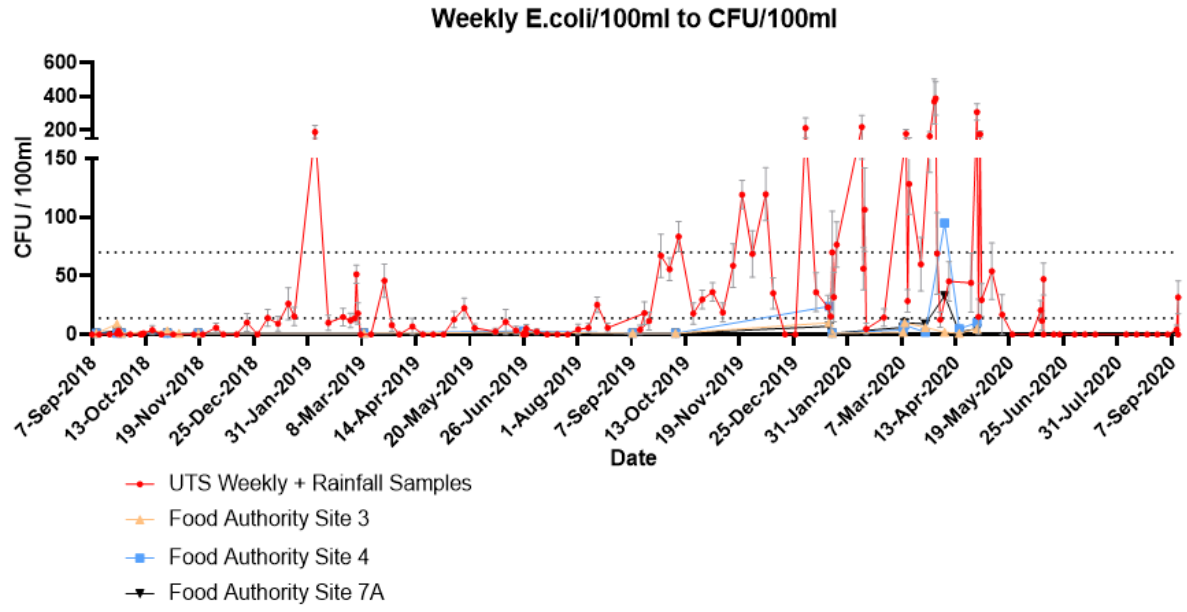
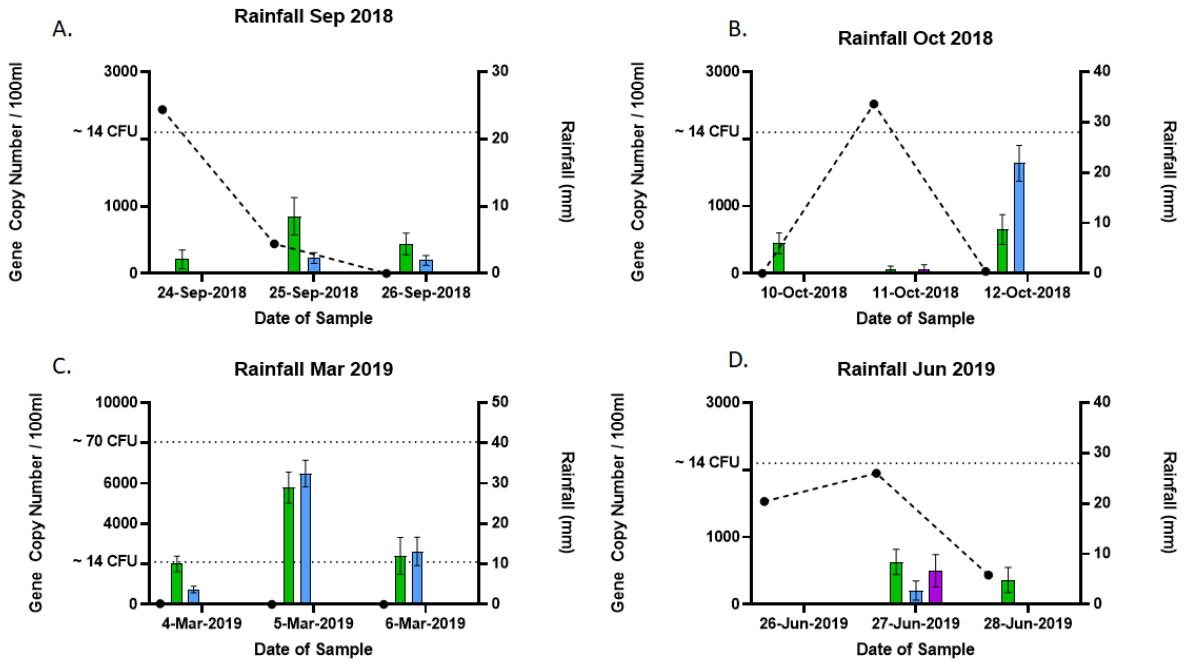


Figure 5.4 Weekly faecal coliform counts (cfu/100 mL) from water samples collected by DPI Food Authority at three sites in the Hastings River compared to Oyster Transformation Project weekly sampling results. Dotted lines at 14 and 70 cfu/100 mL (Fig. 5.4B) are the operational limits for direct or restricted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

Elevated faecal coliform counts reported by the DPI Food Authority often corresponded to elevated levels in samples collected by the CRC. The CRC project test methods appeared more sensitive than routine testing on some occasions (Fig. 5.4).

Eleven rainfall events were also sampled across the study period (see purple bars in Fig 5.3 A-D). These occurred on 24-26 Sept 2018, 10-12 Oct 2018, 4-6 Mar 2019, 26-28 Jun 2019, 20-22 Jan 2020, 10-12 Feb 2020, 11-13 Mar 2020, 30 Mar- 1 Apr 2020, 28-30 Apr 2020, 10-12 Jun 2020, 10-11 Sept 2020 (Fig. 5.5 A-K). *E. coli* was highly variable across rainfall sampling campaigns. In some instances, counts were highest on day 1, other times day 2, and sometimes highest on day 3. On most occasions, *E. coli* declined as rainfall declined, or soon after rainfall ceased. In rare instances, *E. coli* continued to be elevated after rainfall ceased (Mar 2020), but it is unclear without further sample collection, how quickly these levels would have dissipated. Bird bacteria was highly variable, but most often peaked when *E. coli* became elevated (Fig. 5.5B-C, E). Bovine contamination generally remained low, however during one rainfall event (Feb 2020), it peaked on day 2, but then dissipated by day 3. Human bacteria remained low or below detection limits during all events (Fig. 5.5A-K).



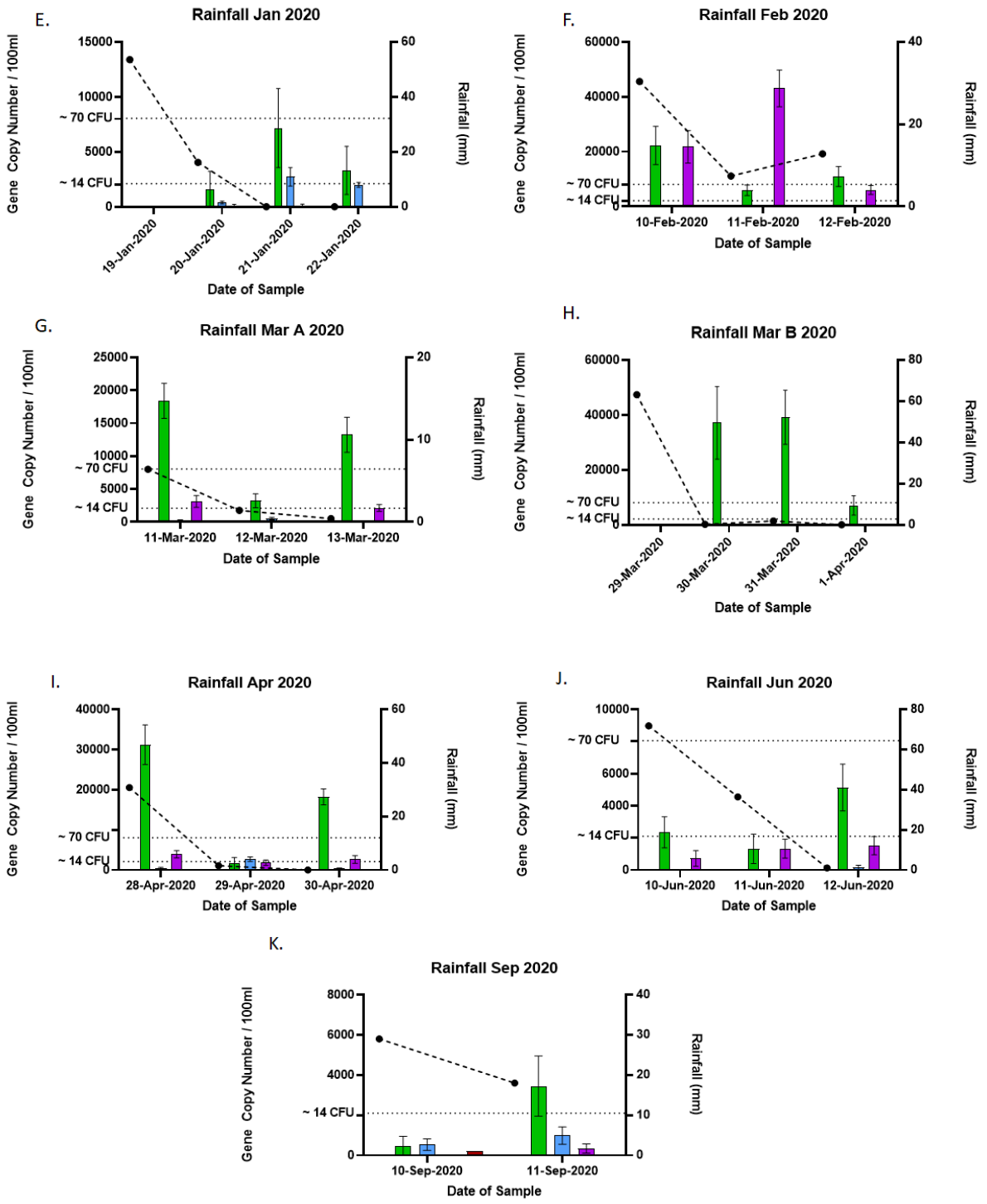


Figure 5.5 A-K. Sensor site (Hastings River) rainfall events sampled for *E. coli* assays. Green bar = 16S *E. coli*; blue bar = bird assay; purple bar – cow assay; red bar = human assay. Dotted line is rainfall (mm) obtained from the closest rainfall station (Port Macquarie BOM rainfall gauge (60139/60168). 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 (May 2018 to March 2021) occurred on 2 Feb 2020 (Fig. 5.6). Total cell concentrations reached 5.2E +06

cells L^{-1} and the sample was dominated by a mix of planktonic diatoms (*Leptocylindrus*), benthic diatoms (*Cylindrotheca, Nitzschia*) and small flagellates (cryptomonads, dinoflagellates, prasinophytes). Very high levels of sediment and organic detritus were observed. This bloom coincided with a total of 84 mm rainfall during the preceding week.

Only one potentially harmful bloom occurred cross the sampling period at the phytoplankton site closest to the sensor. This was due to the toxic diatom *Pseudo-nitzschia fraudulenta/australis* gp., with cells reaching a maximum cell density of 61,000 cells L^{-1} on 17 Oct 2020. NSW Food Authority trigger levels for flesh testing are 50,000 cells L^{-1} for this group. In addition, *Dinophysis acuminata* (a toxic dinoflagellate) reached cell densities of 2,900 cells L^{-1} on 23 Nov 2020 at site 27 (another phytoplankton site in the river adjacent to Settlement Point Rd). NSW Food Authority trigger levels for flesh testing are 1,000 cells L^{-1} for this species. No positive biotoxin results were reported in routine monitoring samples collected by HRSP during the same period

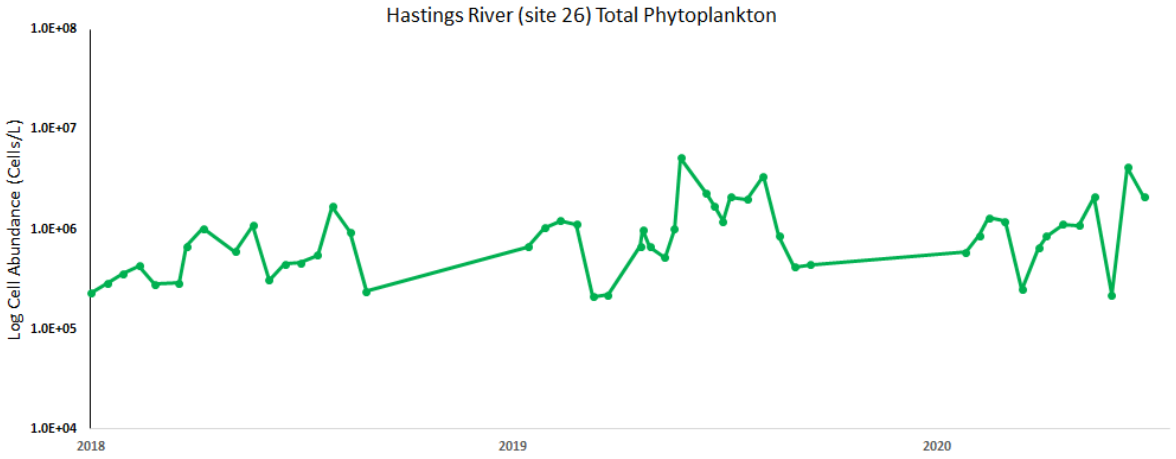


Figure 5.5 Log abundance of total phytoplankton sampled approximately fortnightly from 1 May 2018 to 31 Mar 2021.

5.6 Oyster Growth and Mortality

5.6.1 Oyster Growth

Average oyster whole weight increased by 29.9 g from deployment in August 2018 to June 2020 (Fig. 5.7A). Oyster whole weight was 52.5 ± 3.2 g at the end of the experiment (June 2020). Oysters deployed in Hastings River attained a large size grade, where average whole weight exceeded 50 g, in approximately May 2020, when they were 41 mo. old.

Oyster shell length was 55 ± 2 mm at the start of the experiment and increased to 65 ± 3 mm in June 2020 (Fig. 5.7 B). The greatest increase in shell length in Hastings River was recorded from August to December 2019. The increase in size through this period was 11 mm. Shell lengths were measured more frequently than whole weight and fluctuated throughout the experiment. Five periods of shell length decreases were recorded between August and

October 2018, November and December 2018, January to February 2019, June and August 2019 as well as December 2019 and February 2020. Oysters in Hastings River did not reach a large size grade with respect to shell length (>70 mm) by the end of the experiment.

5.6.2 Mortality

From August 2018 to February 2020, cumulative oyster mortality was 34% in Hastings River. Low levels of mortality were recorded throughout most of the experiment (Fig 5.7 C-D). Two periods of high mortality occurred from January to February 2019 and November 2019 to February 2020, with 20% and 9% mortality recorded over these periods, respectively. This level of mortality was greater than the background Sydney Rock Oyster farming mortality level which is estimated to be approximately 10% per annum. Oyster mortality was minimal (<1%) between all other sampling occasions. Oysters from this site remain frozen for future analyses.

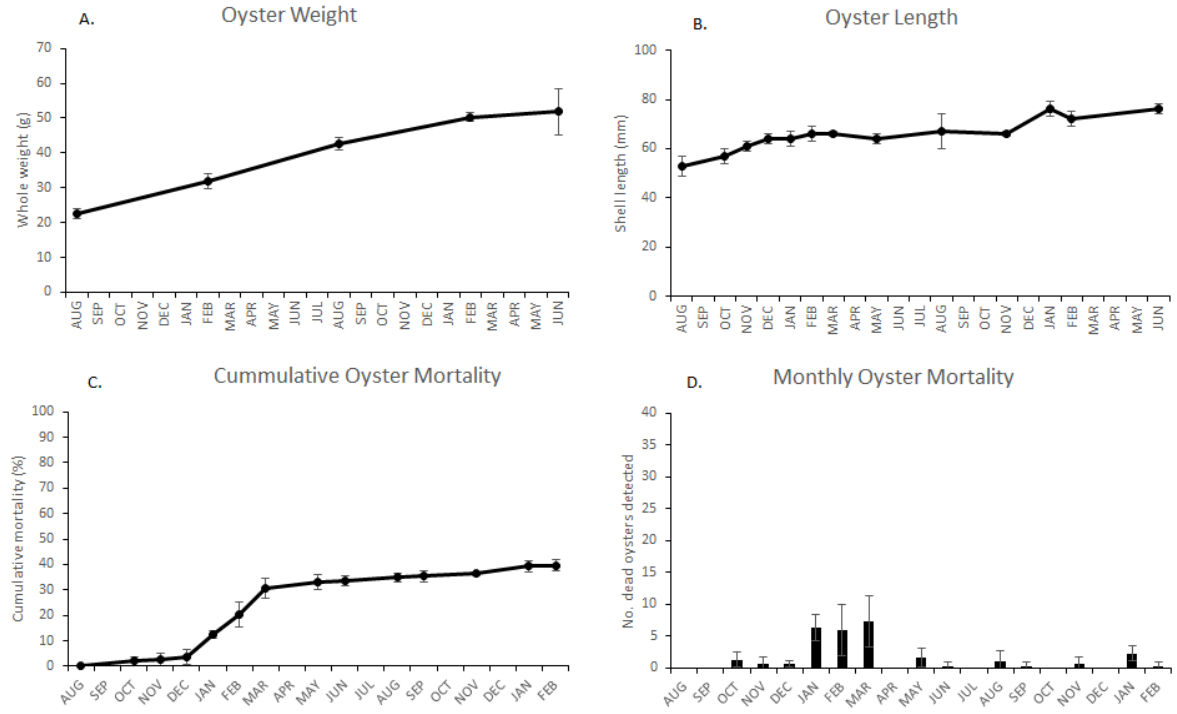


Figure 5.7 A-D. Oysters deployed at the sensor site, Hastings River. A. whole weight; B. shell height; C. cumulative mortality, and D. monthly mortality.

5.7 Modelling

5.7.1 Modelling of E. coli data

Summary statistics for all bacterial concentrations and environmental variables used in the general additive models are shown in Appendix 2. Correlation coefficients were calculated among every pair of environmental variables and suggested one strong negative relationship between (log) total phytoplankton and salinity over the past 72 hours (r = -0.78). 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

(logged or unlogged). Depth and week were included as response variables in all models. The maximum predictive capability for each bacterial group at the sensor site was: 28% for *E. coli* (sensor+ total phytoplankton), 65% for cow (sensor + total phytoplankton), 33% for bird (sensor + total phytoplankton) and 29% for human (sensor) (Table 1).

The abundance of *E. coli* at the sensor site was best explained by the sensor data compared to the rainfall data (28% deviance explained as compared to 17%) and was strongly linked to (decreasing) salinity and water temperature (peaking at ~23℃) over the past 72 hours (Table 1, Figures 5.8 A-D).

Cow bacterial abundance was also better predicted using salinity data compared to rainfall data (65% compared to 56%), and was linked with (decreasing) salinity and water temperature (peaking at ~21℃) over the past 72 hours (Table 1, Figures 5.8 A-D).

Faecal contamination from birds at the sensor site was significantly better explained by the salinity model (33% deviance explained, compared to 9% using rainfall data), with high salinity over the past 72 hours resulting in higher bacterial load. This also coincided with peak temperature between 22-23℃ (Table 1, Figures 5.8 A-D).

An increase in human bacteria abundance was also significantly better explained by the sensor data (29% compared to rainfall data at 7%), and was strongly linked salinity (peaking at 25 ppt) and (decreasing) water temperature over the past 24 hours (Table 1, Figures 5.8 A-D).

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. The best model to explain oyster shell length explained 64% of the deviance, with the week of the year being the best predictive variable of oyster growth.

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

Bacteria	Variables	No. of observations	Significant Variables	Deviance Explained
E. coli	Salinity, Depth, Temp	123	Depth72***, Salinity72***, Temp72***	26.6%
E. coli	Salinity, Depth, Temp, logPhytoplankton	123	logPhytoplankton***, Depth72***, Salinity72***, Temp72***	28.1%
E. coli	Rainfall72	123	Rainfall72***	2.21%
E. coli	Rainfall72, logPhytoplankton	123	Rainfall72***, logPhytoplankton ***	17.3%
Bird	Salinity, Depth, Temp	123	Salinity72***, Depth72***, Temp72***	32.9%
Bird	Salinity, Depth, Temp, logPhytoplankton	123	Salinity72***, Depth72***, Temp72***, logPhytoplankton ***	33.3%
Bird	Rainfall72	123	Rainfall72***	8.82%
Bird	Rainfall72, logPhytoplankton	123	Rainfall72***, logPhytoplankton***	9.32%
Cow	Salinity, Depth, Temp	123	Salinity72***, Depth72***, Temp72***	62.6%
Cow	Salinity, Depth, Temp, logPhytoplankton	123	Salinity72***, Depth72***, Temp72***, logPhytoplankton***	65.1%
Cow	Rainfall24	123	Rainfall24***	27.7%
Cow	Rainfall24, logPhytoplankton	123	Rainfall24***, logPhytoplankton***	55.9%
Human	Salinity, Depth, Temp	125	Salinity24***, Depth24***, Temp24***	29%
Human	Salinity, Depth, Temp, logPhytoplankton	123	Salinity24***, Depth24***, Temp24***, logPhytoplankton***	28.6%*
Human	Rainfall48	124	Rainfall48***	1.18%
Human	Rainfall48, logPhytoplankton	124	Rainfall48**, logPhytoplankton*	6.87%

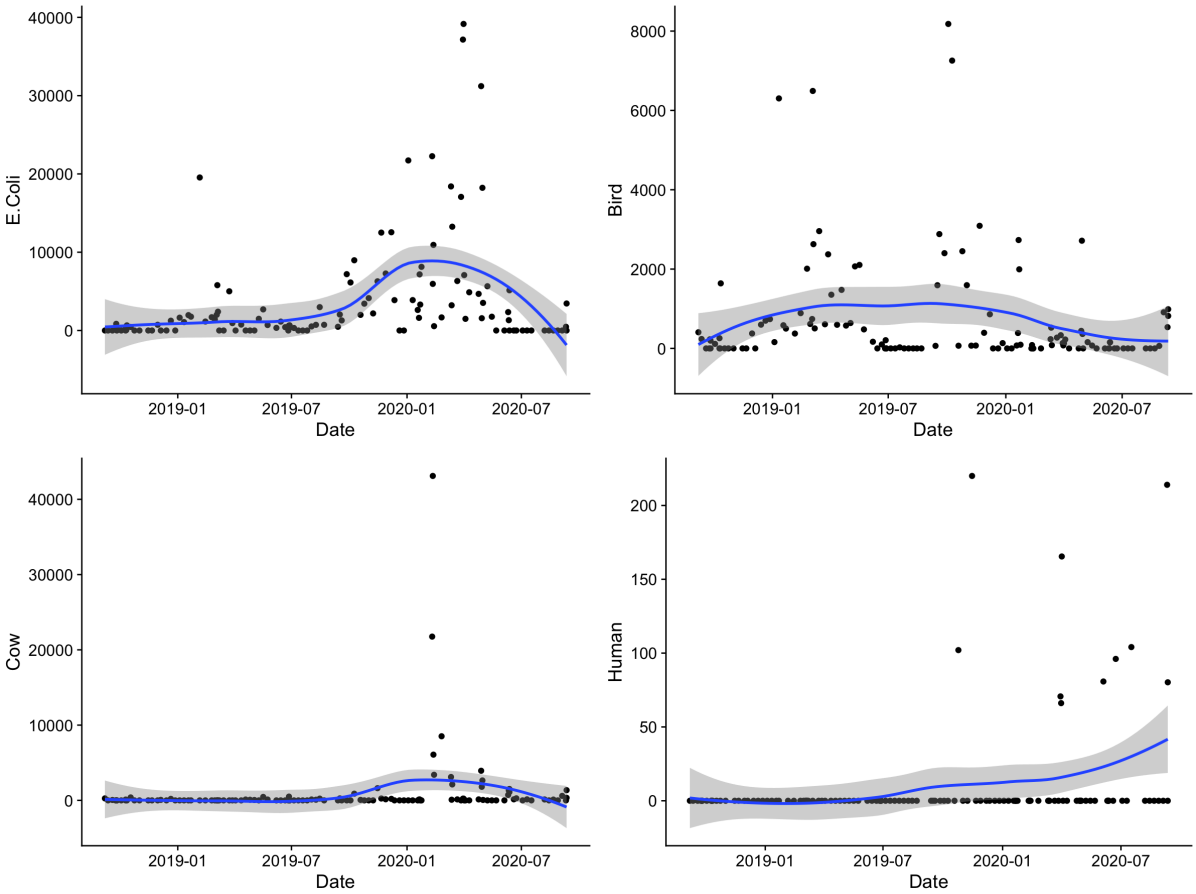


Figure 5.8 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. *E. coli*, B. Bird, C. Cow, and D. Human bacterial load as measured by weekly sampling at the sensor site, Hastings River.

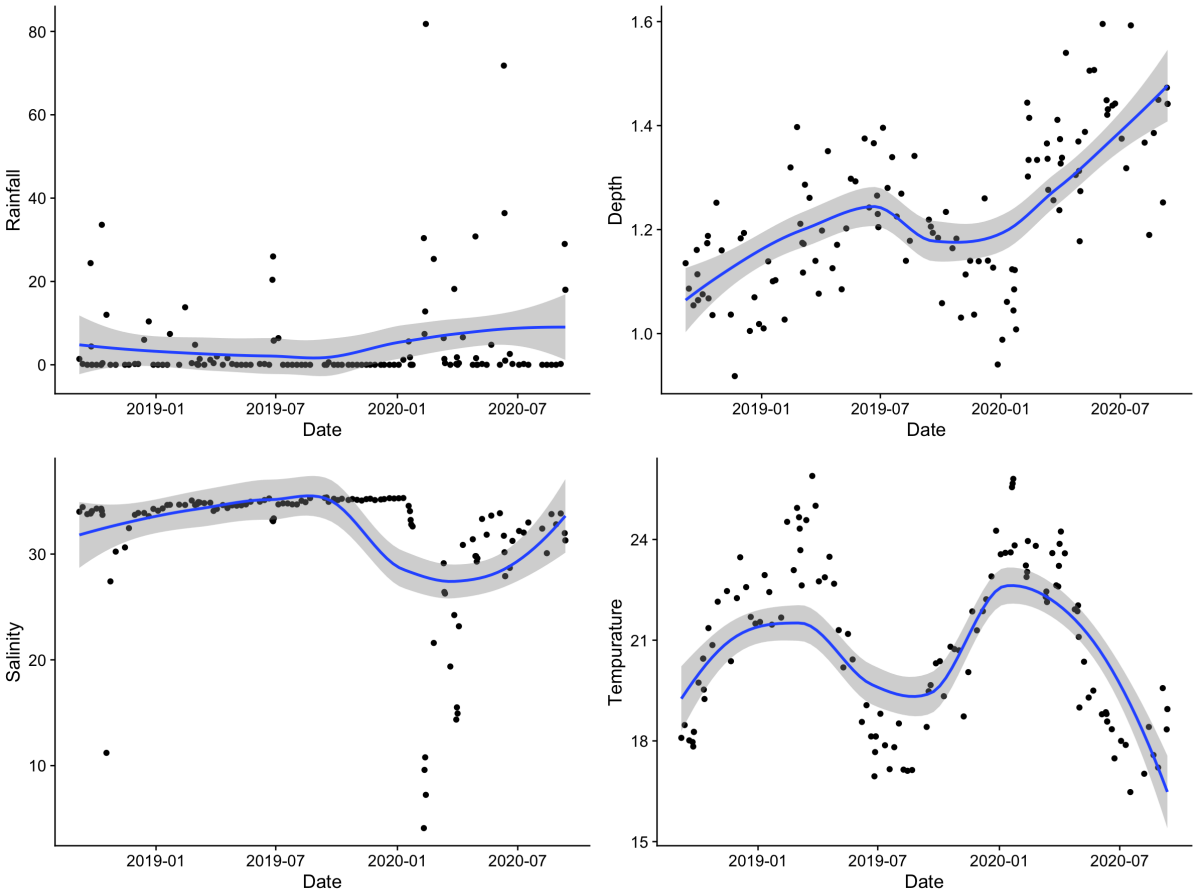


Figure 5.9 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. Rainfall, B. Depth, C. Salinity, and D. Temperature values measured in at the sensor site, Hastings River.

DISCUSSION

6. Discussion

6.1 High Resolution Sensor Data and Management Plan

Analysis of sensor data during the annual review process demonstrated that there is potential to implement a salinity sensor-based management plan for Lower Limeburners Creek harvest area. Based on the available data, up to two harvest area closures and three harvest area downgrades could have potentially been avoided between May 2018 and May 2022. During the initial implementation of such a management plan change, rainfall events would continue to be monitored to increase the database to support the change. Hastings River Shellfish Program (HRSP) were consulted about the option of a salinity-only management plan for Lower Limeburners Creek harvest area following the 2020 annual review, but a decision has not yet been reached. If HRSP 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 Limeburners Creek harvest area management plan would revert to the current management plan that is based on both rainfall and salinity closure limits.

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6.2 Phytoplankton and HABs

The most common HAB species that bloomed in the Hastings River during this study was *Pseudo-nitzschia*. Although this did not occur in significantly high numbers during our sampling period, *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., 2013, 2020). Blooms within the Hawkesbury River estuary (330 km south of Wallis River), a high-risk area in SE Australia for HAB events, recently experienced a very dense bloom of *P. delicatissima* gp., with one out of seven strains isolated to produce domoic acid (Ajani, 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 group to watch 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).

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

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 Hastings River

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

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

The second group of assays developed were those that were microbial source tracking as they detect bacteria of faecal origin specifically associated with a group of animals, i.e. bird, cow and human. Birds are a significant source of faecal contamination in estuarine/marine waters during dry periods, and increase faecal indicator bacteria load in catchments (Araujo et al. 2014, Converse et al. 2012). The marker we used was 100% avian specific, with gulls, geese, ducks and chickens being tested (Green et al. 2012) and has been successfully used in catchments across different continents (Ahmed et al. 2016, 2019; Li et al. 2019, Vadde et al. 2019). Our source tracking assay for cows had 100% sensitivity to bovine faecal samples, with little cross reactivity to other species (93% specific). When tested in a rural catchment, a high proportion of faecal contamination was attributable to cattle (Layton 2006). Finally, the human marker we used has demonstrated the best performance for the detection of human faecal contamination compared to all other assays since it was developed in 2000 (Boehm 2013, Shanks 2010).

In most coastal and estuarine systems, an increase in bacterial load is usually linked to an increase in rainfall and a decrease in water salinity. These events most likely lead to a concomitant increase in nutrients entering the waterway (Amato et al. 2020, Abimbola et al. 2021, Liang et al. 2019, Buszka & Reeves 2021), providing bioavailable nutrient forms for phytoplankton growth. *E. coli* pollution entering a waterway can also induce nutrient recycling and accelerate the decomposition of other organics like aquatic plants, further releasing nutrients into the system (Wu et al. 2021). The survival and proliferation of *E. coli* in the aquatic systems have also been found to be strain specific, with hydrological conditions, differing sources of pollution, selective pressures in the waters, and various land uses, all contributing to the community structure and diversity of *E. coli* in a waterway (Bong et al. 2021).

Salinity was a more reliable predictor than rainfall for all four faecal indicators tested in the Hastings River. Elevated *E. coli* was highly variable, and linked to a decreasing salinity and temperature, suggesting rainfall was the main contributor. When *E. coli* did become elevated with rainfall (event sampling), it generally decreased as rainfall declined. Similarly, cow and human bacteria occasionally became elevated in response to rainfall events (albeit low compared to other estuaries), however they dissipated quickly.

Avian faecal pollution was observed to peak during the warmer months. The first peak occurred in the summer of 2028/2019, while the second peak which was similar to many other 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.

The generally 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 present the highest impact/risk for human contamination in the Hastings River. It was suggested that, due to the wider range of human enteric viruses in a large number of oyster and sediment samples, the outbreak of hepatitis A linked to the consumption of oysters from Wallis Lake in 1997 was linked to significant sewage or faecal contamination. New legislation followed on from this event, tightening controls over septic maintenance, new sewerage management plans developed, and a mandatory notification system for sewage overflows introduced. Following this, mandatory membership for industry to Shellfish Quality Assurance Programs was implemented and an estuary classification system introduced (Conaty et al. 2000).

The future use of molecular tools such as qPCR for the detection and quantification of bacteria or HABs would require further validation in accordance with the Association of Official Agricultural Chemists (AOAC) procedures for the validation of such tests. This would include the validation of the sensitivity, precision and reliability of methods and a rigorous comparison to existing methods. Methodology and protocols for sampling accreditation and assurance of independence in testing and reporting for on farm testing would then follow.

Increases in whole oyster weight in Hastings River were greatest in the latter part of the experiment trial from February to June 2020. However, growth, in terms of shell length, was greatest from August to December 2019. The salinity level during the period of maximum shell growth was very stable and remained above 34 ppt. Higher salinities increase seawater alkalinity providing more calcium carbonate available for oyster shell deposition. The salinity level that promotes the greatest growth rates in Sydney Rock Oyster spat is 30 ppt for small spat (1.3 mg) and 35 ppt for larger spat (0.61 g) (Nell and Holliday, 1988). A period of reduced salinity was recorded from January to April 2020 with salinity falling to 4.1 ppt over this time, following episodes of heavy rainfall (Fig. 5.1B). This period of reduced salinity had no effect on whole oyster weight but coincided with a decrease in shell growth (December to February 2020). Limeburners Creek, where oysters were held in this study, can become slightly acidic after heavy rainfall (pH < 7; Dove and Sammut 2007). Such estuarine acidification can cause

significant reductions in shell growth, along with shell dissolution, in Sydney Rock Oysters (Dove and Sammut 2007; Parker et al. 2010) and may have contributed to the decreased shell growth recorded at this monitoring site during episodes of reduced salinity.

Low levels of mortality were recorded for most of the experiment, between August 2018 to January 2019 and February 2019 to November 2020. Mortality during this period was less than background mortality commonly experienced when farming Sydney Rock Oysters (approximately 10% per annum). High levels of mortality were recorded, however, between January to February 2019 and November 2019 to February 2020, with mortality reaching 20% and 9%, respectively during these periods. Cumulative mortality at the end of the sampling period (February 2020) was 34%. This was the second highest cumulative mortality recorded across the monitoring sites, behind Camden Haven, which had a cumulative mortality level of 40%.

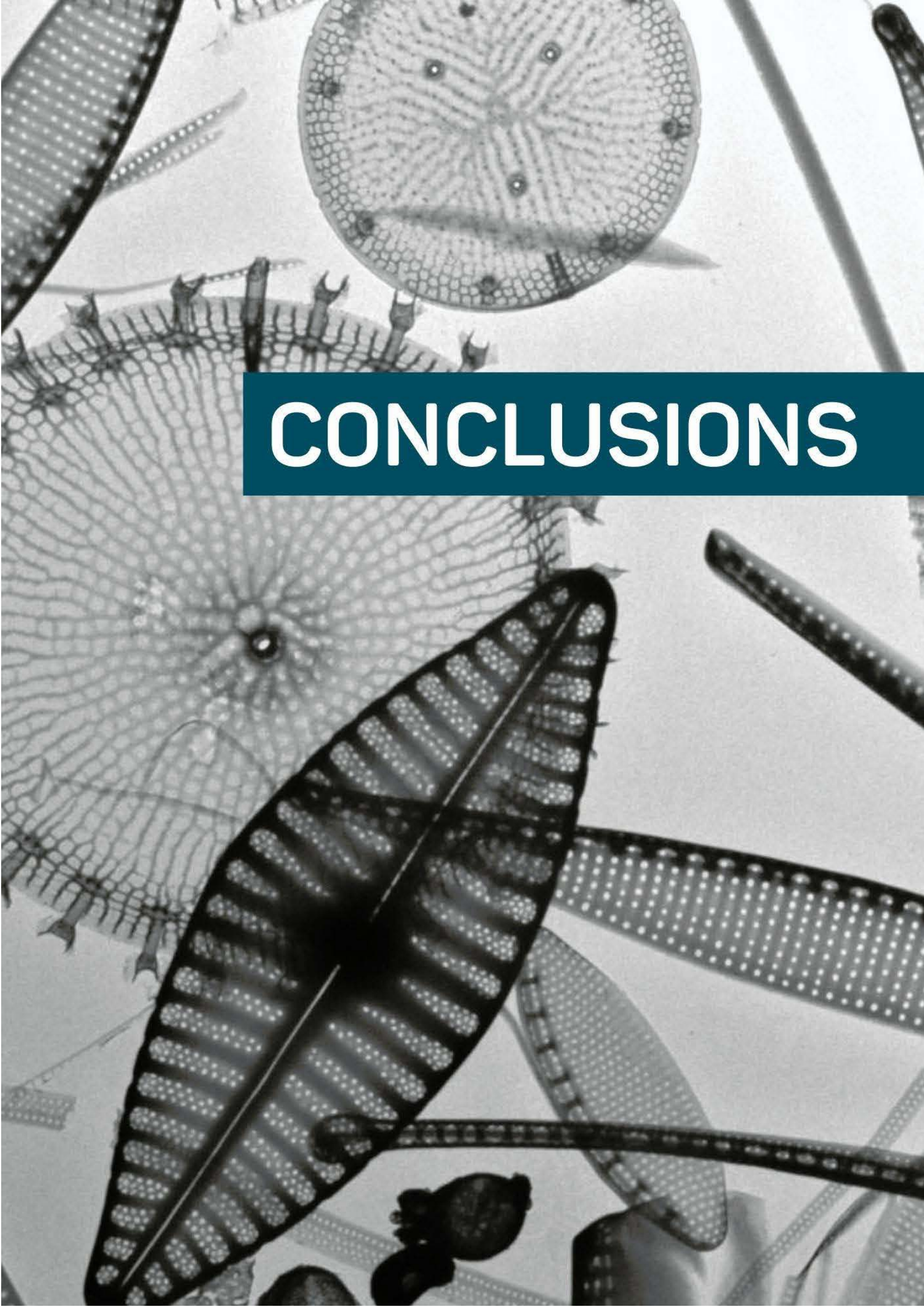
The cause of high cumulative mortality in the Hastings River during the experiment is unknown. Unlike estuaries such as the Georges River, the Hastings River does not experience known outbreaks of QX or winter mortality disease, both of which cause significant mortality in Sydney Rock Oysters (Nell et al. 2000; Dove et al. 2013). The two periods of high mortality events occurred in Hastings River when daily average temperatures were at their highest, but only partially coincided with a reduction in salinity, suggesting that salinity was unlikely to be the cause of mortality (Nell and Holliday, 1988; Sarwer 2020).

The batch of oysters used for this experiment was 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 approximately 3 years and 5 months to reach the large oyster size grade (>70 mm total length or >50 g whole weight) in the Hastings River. This was quite slow compared to other estuaries in the experiment, with the only estuaries where this same batch of oysters reached the large oyster size grade benchmark slower being Wallis Lake, Shoalhaven River, and Wonboyn River.

The Hastings River is ranked 11th in the state for Sydney Rock Oyster production with 114,840 dozen oysters sold annually worth $1.15 million (NSW Department of Primary Industries, 2023). Approximately half of all Sydney Rock Oysters sold in the Hastings River are at the medium size grade (>55 mm to <70 mm in length or >30g to <50g; NSW Department of Primary Industries 2023). This may reflect the slower growth rates of Sydney Rock Oysters in this estuary. The Hastings River is also a major supplier of juvenile oysters to other estuaries, with significant quantities leaving the river before harvest size.

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 Limeburners Creek harvest area, subject to the agreement by the local shellfish industry. Available data indicated that two harvest area closures and three harvest area downgrades could have potentially been avoided between May 2018 and May 2022. As of August 2023, nineteen salinity-only management plans had been offered for harvest areas in participating NSW estuaries, with seven being taken up and the remaining twelve under consideration.

Compared to the other monitoring sites in NSW, Hastings River was ranked 4th slowest in terms of oysters reaching the large size grade (>70 mm total length or >50 g whole weight). Low levels of mortality were recorded over most of the monitoring period, except for January to February 2019 and November 2019 to February 2020, where mortality was 20% and 9%, respectively. The cumulative mortality at the end of the experiment (February 2020) was 34%. This was the second largest cumulative mortality recorded among the monitoring sites and was more than double the level accepted as background farming mortality (approximately 10% per annum). Reduced salinity appeared to be a predictive indicator of reduced shell growth, but not whole-body weight or mortality.

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 showed a higher predictive capability than rainfall for all of the four faecal indicator bacteria. Elevated levels of *E. coli*, and to a lesser extent, cow and human bacteria, corresponded to rainfall (and a subsequent decline in salinity). Furthermore, while contamination from bird sources was observed at levels similar to other estuaries, 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 Hastings River.

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9. Appendices

A1. Methods

A1.1 Sampling locations in the Hastings River

Data used in this report originates from locations within the Hastings River over the period 1 May 2018 to 31 March 2021. High-resolution temperature, salinity and depth data were obtained from a sensor located in Lower Limeburners Creek harvest area, located within the Hastings River (Fig. A1). At this sensor location, oysters were both deployed and retrieved, and water samples for eDNA were collected. From here on, this location is referred to as the 'sensor site'. Phytoplankton was also collected at a second sampling location established as part of the DPI's Shellfish Quality Assurance program (Fig. A1).

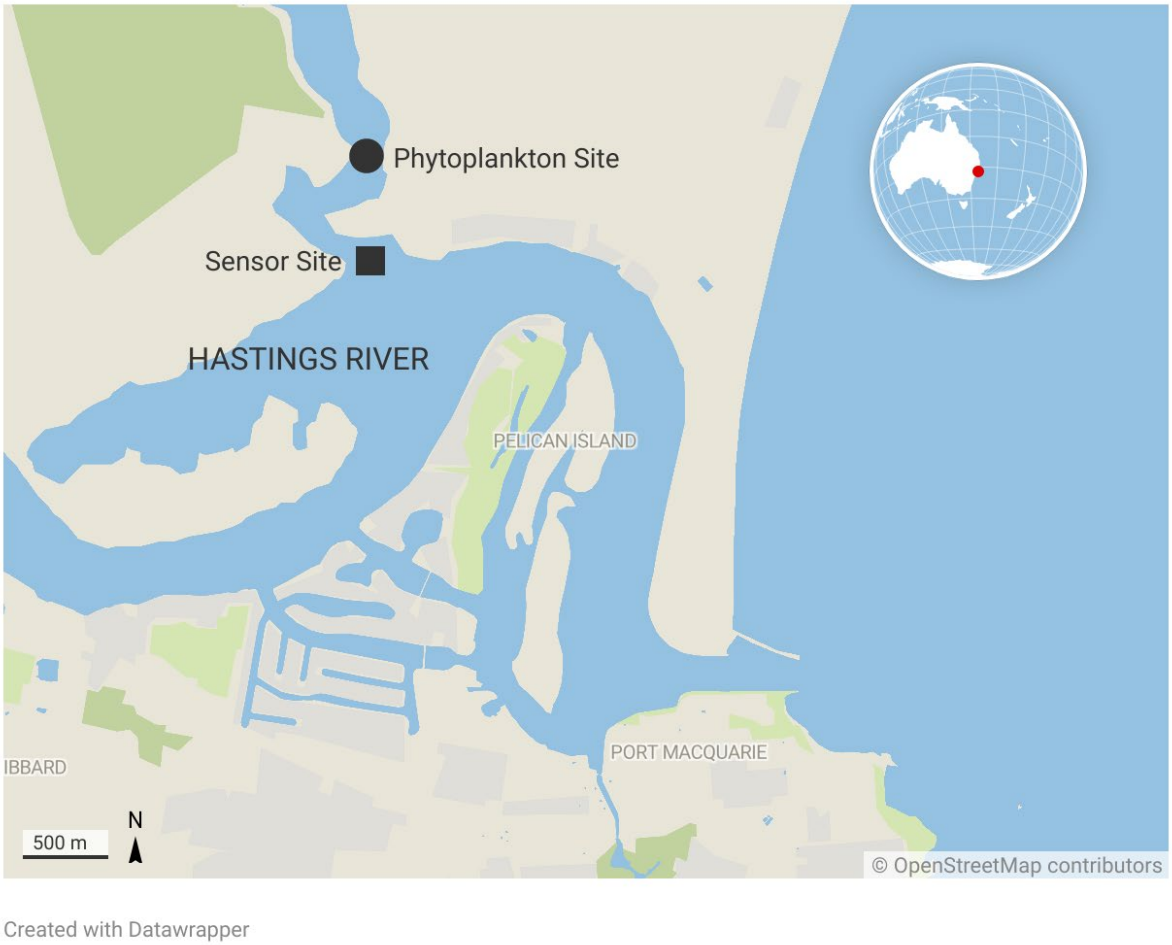

Figure A1: Map of the Hastings River highlighting the sensor located in Lower Limeburners Creek (black square) and the phytoplankton sampling location (black circle).

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 sensors. This sensor was deployed using a fixed installation, with the inlet 60 cm above the seabed and at least 30 cm below the estimated Lowest Astronomical Tide (LAT) (Fig. A2). This fully autonomous instrument collected and transmitted data every 10 minutes (24 h day^{-1}) to Microsoft Azure cloud storage before downstream quality checking and analysis. Sensor data was then packaged into RO-Crates by the e-Research team at UTS, which are then uploaded to an Arkisto-based website. This website allows for the filtering and downloading of these crates based on both time and location, for use in research and analysis (Fig. A3). Finally, rainfall data were obtained from the closest rainfall gauge at Port Macquarie BOM rainfall gauge (60139/60168) (~-31.43° S, 152.87° E) from May 2018 to March 2021.

Figure A2 Seabird MicroCAT high accuracy conductivity, temperature and depth (CTD) field sensor deployed in the Hastings River. Image Credit: Paul Wilson

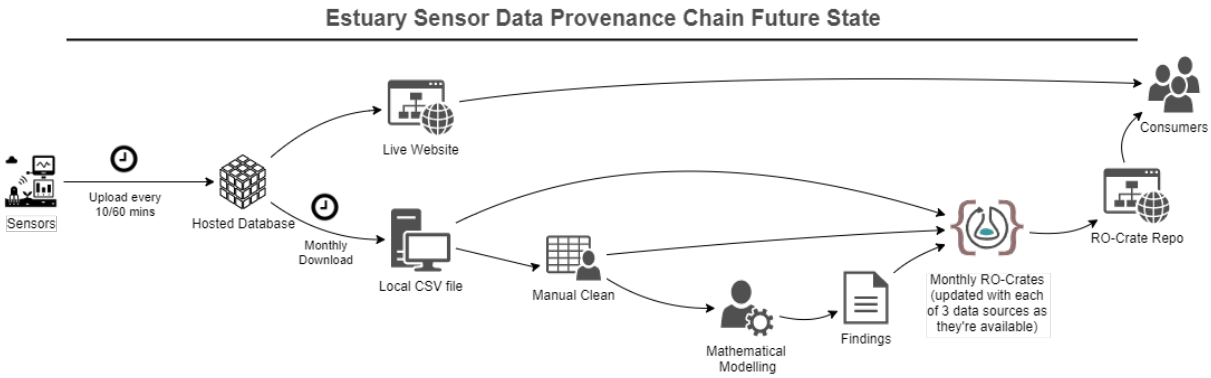

Figure A3. Hastings River data provenance chain from source of data (sensor), via quality assurance processes, data analyses, to consumers.

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 Hastings annual review is 1 June. As part of the most recent (2022) annual review for Lower Limeburners Creek harvest area, all salinity data from the monitoring sensors during the 2018, 2019, 2020, 2021 and 2022 annual review periods were assessed in relation to microbiological

samples collected by the local shellfish program during the same period. There was a gap in data collection between mid-December 2020 and late-April 2021, due to damage to the sensor during heavy rainfall/flooding, and while the sensor was transitioned to a new provider (28 April 2021). Other occasional salinity data points removed from the analysis were generally elevated above expected salinity levels, and indicative of debris interfering with the sensor reporting. It appeared that the outliers were due to the sensor coming out of the water during lower tides. More frequent outliers were observed after 2 December 2021, coinciding with wetter conditions. The HRSP reported very low salinity, as reported by the sensor from ~18 May 2022. This was a suspected issue with the instrument and data from 18 May onwards were not included in the analyses.

A1.4 Biological sampling, eDNA extraction and nutrient analyses

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

In the case of a rainfall event, water samples were collected for bacterial analysis (only) every 24 h over a two-day period commencing on the first day of rainfall and processed as described above. Daily rainfall measurements were taken from the closest available rainfall gauge at Port Macquarie BOM rainfall gauge (60139/60168) (~-31.43° S, 152.87° E) from May 2018 to March 2021.

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 (max. magnification x1000). Cell counts were undertaken to determine the abundance of individual HAB species and total phytoplankton cell (>5 mm) numbers. *Dinophysis* cells were counted to a minimum detection threshold of 50 cells L^{-1} while all other species were counted to a minimum detection threshold of 500 cells L^{-1}.

A1.8 Oyster Growth and Mortality

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

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

A1.8.1 Oyster Whole Weight

Whole weight was measured in August 2018, February 2019, August 2019, February 2020 and finally in June 2021. Thirty randomly sampled oysters from each replicate were pooled and weighed on each sampling date using a calibrated weight balance to the nearest 0.1 g. The average whole weight of oysters at the start of the experiment in August 2018 was 22.6 ± 1.4 g.

A1.8.2 Shell Length

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

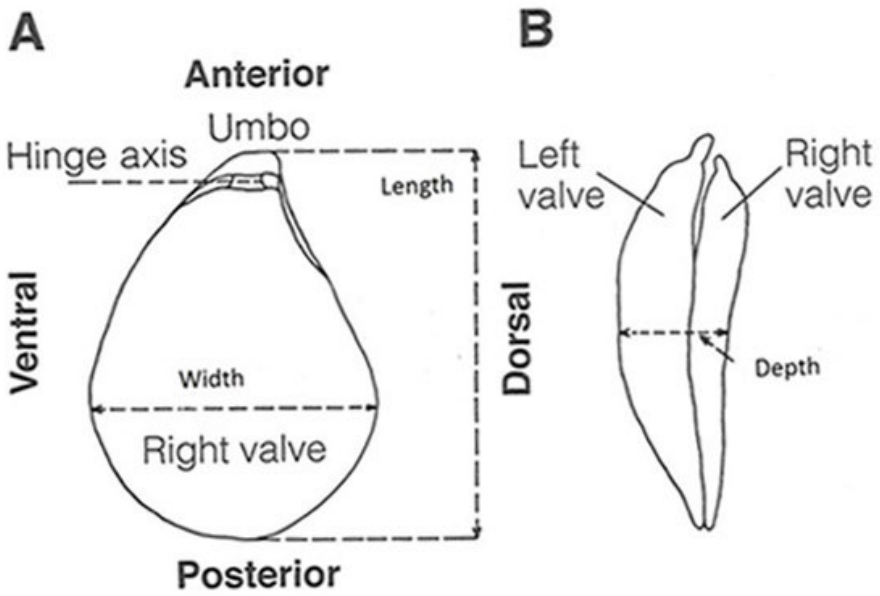

Figure A4. Oyster shell dimensions (Carriker 1996)

A1.8.3 Oyster Mortality

Oyster mortality was calculated by counting the number of empty oyster shells in each replicate approximately each month from August 2018 to 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 rainfall gauge at Port Macquarie BOM rainfall gauge (60139/60168) (~-31.43^{o} S, 152.87^{o} E) from May 2018 to March 2021, which was the official management plan gauge for this harvest area, were averaged over the 24 h, 48h, 72 h and 7 days prior to the water sampling each day, to incorporate a measure of exposure of the bacterial community and deployed oysters. Total phytoplankton (and log transformed total phytoplankton) from microscopic phytoplankton enumeration was also included in the modelling as a potential predictor variable. Finally, week of the year and water depth were included in the models to understand any seasonality or tidal variability that was present in the data.

To model the relationship between bacteria (*E. coli*, bird, cow, human) abundance and/or oyster growth (response variables) and environmental variables (temperature, salinity, week, depth, total phytoplankton and rainfall) at the sensor location within the Hastings River, correlation analyses were initially undertaken to explore the relationships between variables. Generalised additive models (GAMs) were then applied to the data. GAMs allow abundance data to be treated as count data (discrete integer values), and as such can handle zero counts. GAMs also allow for smoother functions to be incorporated into each model for the environmental variables that had a non-linear relationship with bacterial abundance.

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

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

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

Variable	Mean	Standard Error	Median	Standard Deviation	Minimum	Maximum	Count	Missing
average_cfu	35.09	6.12	9.29	68.40	-0.24	388.65	125	0
bird	711.14	125.41	153.11	1402.12	0.00	8180.00	125	0
cow	893.18	393.96	0.00	4404.56	0.00	43104.88	125	0
depth24	1.23	0.01	1.21	0.15	0.92	1.60	125	0
depth48	1.23	0.01	1.21	0.13	0.96	1.55	125	1
depth72	1.23	0.01	1.22	0.13	1.00	1.54	125	2
ecoli	3708.69	620.14	1077.29	6933.43	0.00	39159.08	125	0
human	9.60	3.24	0.00	36.24	0.00	219.99	125	0
logPhytoplankton	13.21	0.07	12.99	0.79	12.25	15.46	125	0
Phytoplankton	803680	87050	440000	973248	210000	5200000	125	0
rainfall24	4.88	1.09	0.00	12.19	0.00	81.80	125	0
rainfall48	4.84	0.88	0.60	9.79	0.00	54.10	125	1
rainfall72	4.80	0.75	0.80	8.41	0.00	40.00	125	2
salinity24	31.62	0.55	34.06	6.20	4.09	35.35	125	0
salinity48	31.61	0.52	34.04	5.84	6.84	35.33	125	1
salinity72	31.60	0.50	34.02	5.62	8.16	35.32	125	2
temp24	20.85	0.22	20.80	2.45	16.48	25.89	125	0
temp48	20.87	0.21	20.79	2.39	16.75	25.74	125	1
temp72	20.89	0.21	20.75	2.36	17.13	25.68	125	2

Appendix 3. Summary of project related publications, seminars, workshops, conference presentations and other project related public presentations.

Author(s)	Title	Bibliographic details	Status (Submitted, Accepted, Published)
Penelope Ajani, Hernan Henriquez-Nunez, Arjun Verma, Satoshi Nagai, Matthew Tesoriero, Hazel Farrell, Anthony Zammit, Steve Brett and Shauna Murray	Mapping the development of *Dinophysis* spp. HABs using a novel molecular qPCR assay	*Harmful Algae* 116 (2022) 102253	Published
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
Penelope A. Ajani, Michaela E. Larsson, Stephen Woodcock, Ana Rubio, Hazel Farrell, Steve Brett, &Shauna A. Murray.	Fifteen years of *Pseudo-nitzschia* in an Australian estuary, including the first potentially toxic *P. delicatissima* bloom in the southern hemisphere	*Estuarine, Coastal and Shelf Science*, 236 (2020) 106651.	Published

DPI Food Authority	Foodwise - Issue 46	https://www.foodauthority.nsw.gov.au Feb 2018	Published
Shauna Murray & Penelope Ajani	Ah shucks, how bushfires can harm and even kill our delicious oysters	The Conversation https://theconversation.com/ah-shucks-how-bushfires-can-harm-and-even-kill-our-delicious-oysters-131294 Aug 2020	Published

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

Wayne O'Connor	Macquarie University, Microbiomes Workshop, Epping, November 2018	Overview and Progress – Oyster Transformation Project
Shauna Murray, Arjun Verma, Penelope Ajani, Anthony Zammit, Hazel Farrell, Swami Palanisami & Wayne O'Connor	Australian Shellfish Quality Assurance Advisory Committee Science Day 2018	Building profitability and sustainability in the NSW oyster industry
Penelope Ajani, Michaela Larsson, Ana Rubio, Stephen Bush, Steve Brett, Stephen Woodcock, Hazel Farrell & Shauna Murray	Australian Shellfish Quality Assurance Advisory Committee Science Day 2018	Modelling harmful algal blooms in the Hawkesbury River, Australia
Hazel Farrell, Grant Webster, Phil Baker, Anthony Zammit, Penelope Ajani, Shauna Murray & Steve Brett	Australian Shellfish Quality Assurance Advisory Committee Science Day 2018	Developing phytoplankton and biotoxin risk assessments for both shellfish aquaculture and wild harvest shellfish in New South Wales.
Wayne O'Connor	SIMS, July 2017	Oyster Research Overview Presentation

Presenter(s)	**Event**	**Presentation title**
Shauna Murray & Arjun Verma	https://www.youtube.com/watch?v=cfAyjjnASy0&t=154s	Sept. 2019: PROJECT NEWS: Can World Leading Research Transform the NSW Oyster Industry?
Shauna Murray	https://www.youtube.com/watch?v=4NM_U_IKCEE&t=1s	Sept. 2020: Food Agility CRC – Cooperative Research Centre customer story
Arjun Verma & Penelope Ajani	https://www.youtube.com/watch?v=iRcRZkptpOY&t=46s	Feb. 2020: Food Agility Summit 2020: WE LOVE SCIENCE!
Anthony Zammit	https://www.cnbc.com/video/2017/03/05/one-of-the-most-sustainable-farming-enterprises-meets-hi-tech.html	Mar 2017: One of the most sustainable farming enterprises' meets hi-tech