# TRANSFORMING AUSTRALIAN SHELLFISH PRODUCTION

## Coba Bay Harvest Area, Hawkesbury 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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Australian Government

foodagility

UTS

NSW GOVERNMENT

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**Transforming Australian Shellfish Production: Coba Bay Harvest Area, Hawkesbury River. Report on Stage 1, Oct 2017 - March 2021**

**2023**

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# Contents

# Executive Summary

This report presents results from the Hawkesbury 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 Coba Bay harvest area, Hawkesbury River, recording high-resolution temperature, salinity and depth data. Oyster farmers collected weekly biological samples (624 environmental DNA samples and 294 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

**5**

Available data indicated that five harvest area closures could have potentially been avoided between October 2017 and June 2020. Since July 2020, and following the adoption of a salinity-based management plan in December 2020, there were no occasions when the harvest area would have remained open when comparing operations under a rainfall or salinity-based management plan.

**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 was highly variable, at times increasing with rainfall across the sampling period, while bird bacteria fluctuated seasonally.

Cow and human bacteria were generally low across the sampling period but significantly increased during one rainfall event.

**0**

Cumulative mortality was high in the first 3 months of the experiment from August to November 2018, reaching 17% over this period. Low levels of mortality (<5% per annum) were recorded for the remainder of the assessment period.

# 1. Introduction

## 1.1 Transforming Australian Shellfish Production

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

The Hawkesbury River (-33.5443°S, 151.1365°E) is a tide dominated, drowned valley estuary located northwest of Sydney. It originates at the confluence of the Nepean River and the Grose River and travels ~120 kilometres in a north–easterly and then a south–easterly direction to its mouth at Broken Bay (Roy et al. 2001). It has a catchment area of 21624 $km^2$, a total estuary area of ~114.5 $km^2$ and a flushing rate of ~49 days (Roy et al. 2001, Roper et al. 2011) (Fig. 1). The surrounding catchment is mainly undeveloped (71% undisturbed forest) with the remaining ~29%, used mostly for grazing and urban development. The aquatic system supports many significant areas of seagrass (1 $km^2$), mangroves (10 $km^2$) and saltmarsh (~3 $km^2$) (Roper et al. 2011).

### 1.3 Oyster Production in the Hawkesbury River

The Hawkesbury River has a long history of oyster harvesting - both as a source of food, and later for oyster-derived lime (Boon 2017). In 1884, new legislation led the way to allow for cultivation of Sydney Rock Oysters, replacing the harvesting of wild oysters, and the leasing of areas for oyster farming along the shoreline soon followed (Boon 2017). There was a gradual increase in production until the mid-1970's when the industry was impacted by several oyster diseases. Between 2004-2006 the industry nearly collapsed due to QX disease and a significant number of farmers shifted to growing Pacific Oysters. The production of triploid Pacific Oysters was initially successful and production increased to the value of $2,500,000 in 2010/2011 but then rapidly declined to about 1% of this value in 2015/2016 due to the January 2013 outbreak of ostreid herpesvirus which causes rapid and extensive Pacific Oyster mortality. Sydney Rock Oyster and Pacific Oyster production reached ~$1,900,000 in 2019/20, but dropped back to $276,000 in 2021/2022 due to impacts caused by heavy rainfall and flooding experienced in 2021/2022. Oyster production in Hawkesbury River is slowly increasing in value and produced ~49,000 dozen (~$660,000) of Sydney Rock Oysters and ~46,000 dozen (~$665,000) of Pacific Oysters (NSW DPI 2023).

# FINDINGS

# 2. Findings

2.1. The data assessment supports implementing a harvest area management plan based on sensor salinity data for Coba Bay harvest area, which was agreed by the local shellfish industry during December 2020. Data collected between October 2017 and June 2020 indicated that five harvest area closures could have potentially been avoided. Between July 2020 and June 2022, there were no occasions when the harvest area would have remained open when comparing operations under a rainfall or salinity-based management plan.

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 Hawkesbury River 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 bacterial group was 36% for *E. coli*, 91% for cow, 58% for bird, and 98% for human at the sensor site.

2.5 Where the models were highly predictive (>90%), they suggested bacterial abundance dramatically increased with increasing rainfall (decreasing salinity) and varying nutrients.

2 .6. Shell length of oysters increased steadily throughout the assessment period, reaching 76 mm by June 2020. Hawkesbury River had the second heaviest oysters (63.2 g) overall when compared to all other estuary monitoring sites measured for this project, with the greatest increase in whole weight occurring from August 2019 to June 2020 (27.5 g). Salinity appeared to be a predictive indicator of oyster shell length but not whole weight.

2.7. Cumulative mortality was high in the first 3 months of the experiment, with 17% mortality observed from August to November 2018. No oyster mortality events that exceeded background farming Sydney Rock Oyster mortality (approximately 10% per annum) occurred in Hawkesbury River for the remainder of the assessment period (November 2018 to February 2020).

# 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 Bruce Alford for his assistance with 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 Hawkesbury River were funded by the NSW Food Authority and the shellfish industry. We thank Kyle Johnston and Brandt Archer (DPI) for oyster stock preparation and growth/survival data collection, and Dr Nahshon Siboni and Prof Justin Seymour (UTS) for source tracking assistance. Finally, we would like to thank Dr Torri Callan (UTS) for statistical analyses and Chris Komorek (Food Agility CRC) for report layout.

# FEEDBACK

# 4. Feedback

In 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.

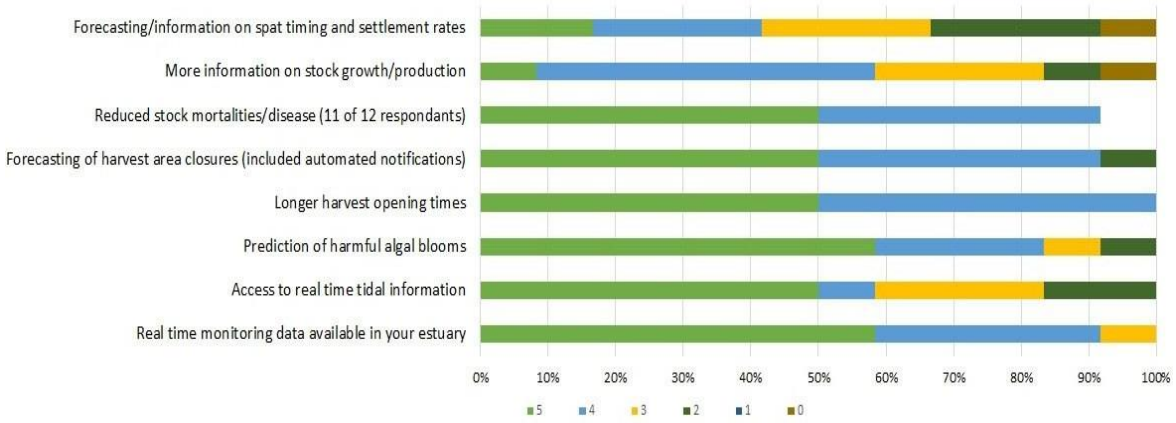



**Figure 4.1.** The importance of factors as rated by farmers in relation to their business operations. Light green is most important and brown is least important.

# RESULTS

# 5. Results

## 5.1 High resolution temperature and salinity data

High-resolution real time data summaries for the Hawkesbury River for the period 13 October 2017 to 31 Mar 2021 are shown in Figs. 5.1A-C. A gap in the data occurred between Jan and Mar 2020 and was due to sensor/gateway issues. Depth recordings ranged from 0.1 m (19 Aug 2019) to 2.8 m (3 Jan 2018). The lowest and highest daily average salinity recordings were 0.6 ppt (23 Mar 2021) and 32.1 ppt (23 Feb 2018) respectively, while the lowest and highest daily average temperature recordings were 11.3℃ (16 Jul 2018) and 30.5℃ (18 Jan 2019) respectively.

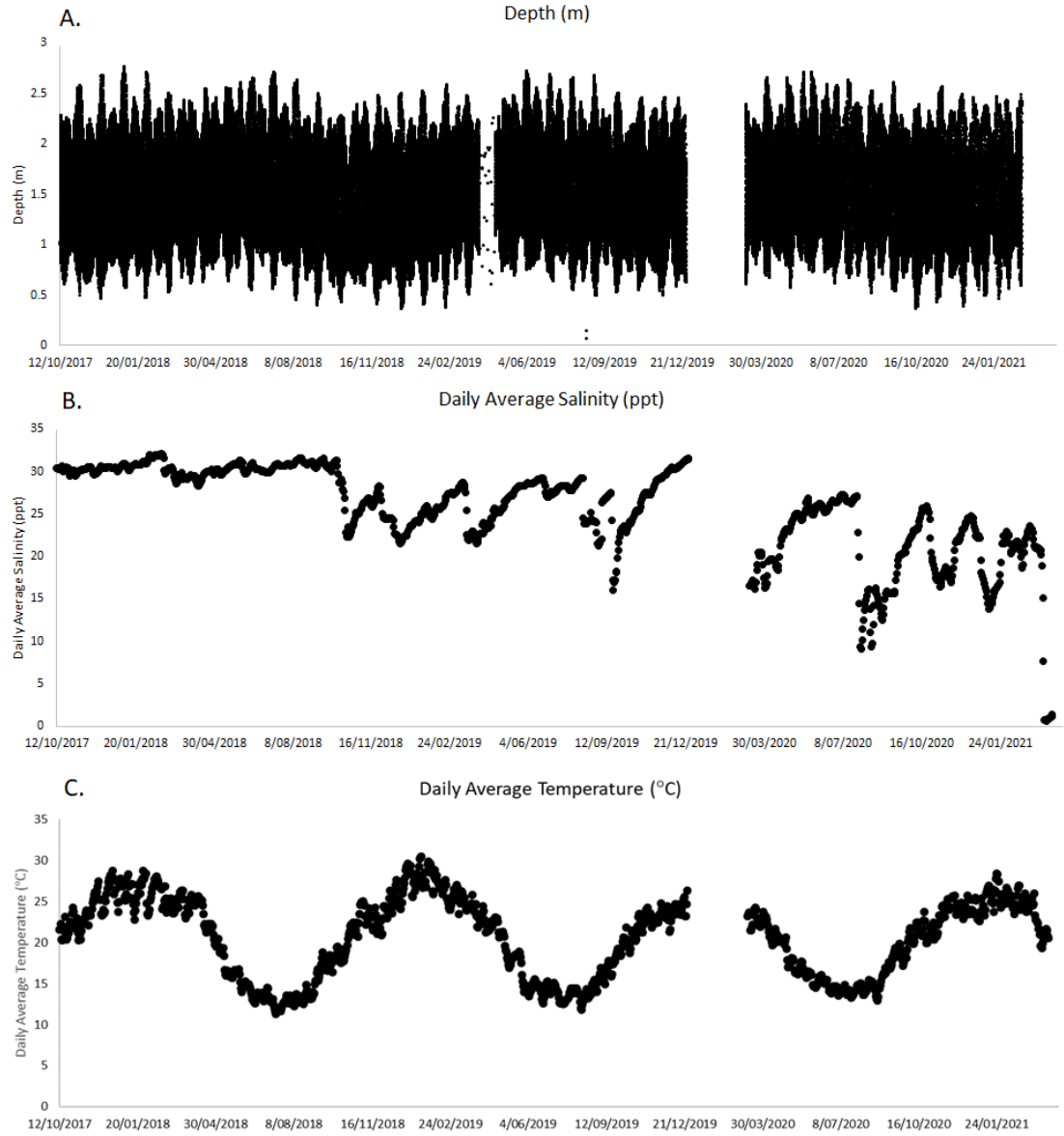



**Figure 5.1A-C.** Real time sensor data from the Hawkesbury River sensor 13 Oct 2017 to 31 Mar 2021 A. Depth (m); B. Daily average salinity (ppt); and C. Daily average temperature (°C).

The maximum daily rainfall at the HRSP rainfall gauge (Mooney) occurred on 10 Feb 2020 and was reported as 231 mm (Fig. 5.2).

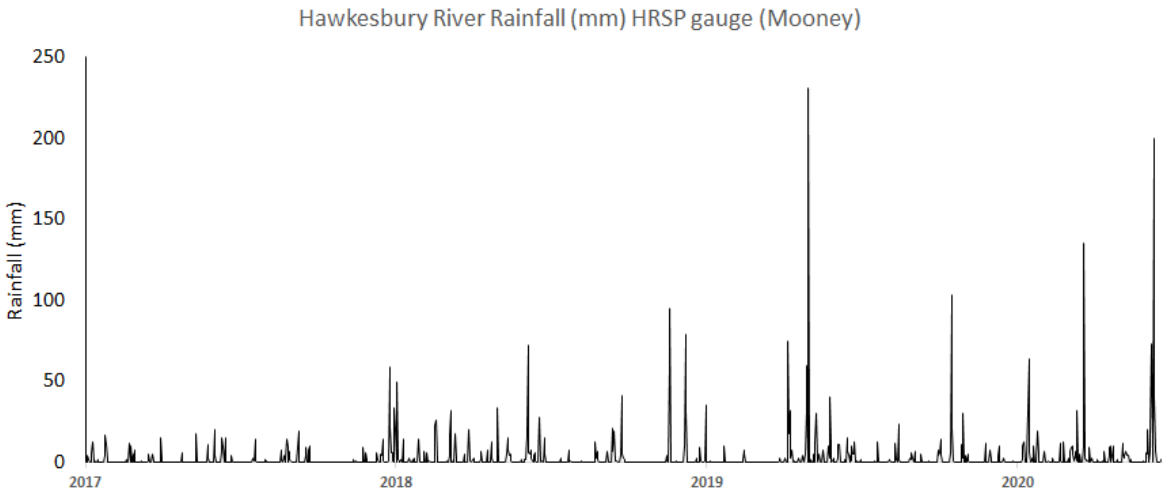



**Figure 5.2.** Daily rainfall (mm) from the HRSP rainfall gauge (Mooney) (~-33.52 °S, 151.20° E) from 13 Oct 2017 to 31 March 2021.

## 5.2 Management Plan

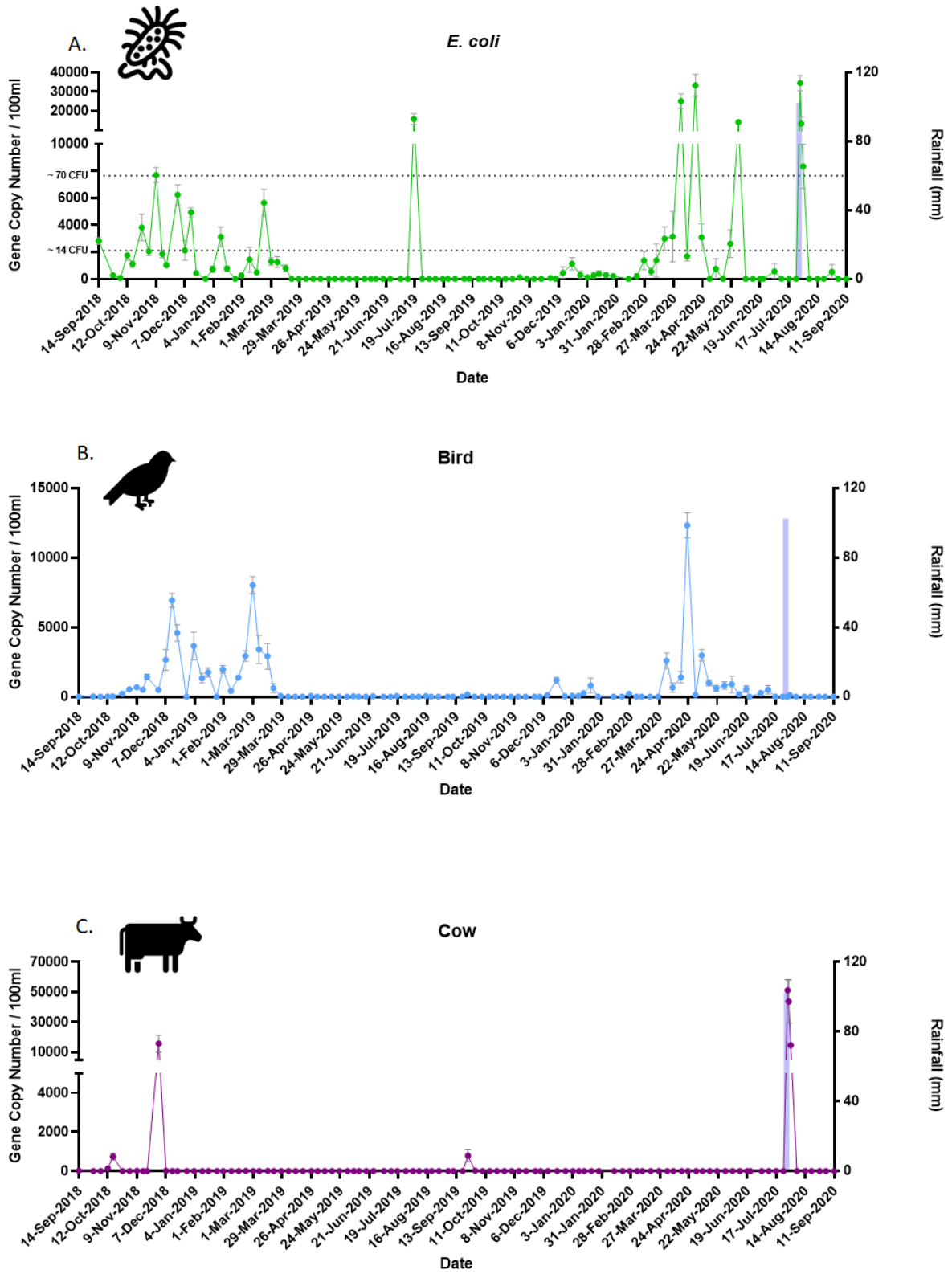
Data analysed during the 2020 annual review of Coba Bay harvest area (see Fig. A1) indicated that there could have been less harvest area closures since the sensor was installed, if closures were based on salinity sensor data. There were five harvest area rainfall closures in Coba Bay harvest area between October 2017 and June 2020. Based on a management plan sensor salinity closure limit of 20 ‰, harvest area closures were reviewed focusing on available salinity sensor data and shellfish program microbiological results since October 2017. Five harvest area closures, of 39 days duration, could have potentially been avoided during this period. During the 2020 annual review period (7 December 2021), a salinity-based management plan was implemented for Coba Bay harvest area. Since July 2020, and following the adoption of a salinity-based management plan in December 2020, there were no occasions when the harvest area would have remained open when comparing operations under a rainfall or salinity-based management plan. Time periods where salinity is slower to recover may require additional sampling to meet management plan requirements. A review of the available data also indicated that given fluctuations in salinity between high and low tides, particularly after prolonged wet periods, decisions on harvest area closures would consider salinity trends rather than point in time measurements.

## 5.3 Bacterial source tracking

A total of 624 water samples and 294 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 Hawkesbury River (Fig. A1).

For the Hawkesbury River the maximum *E. coli* reached 34,456 gene copies 100 $mL^{-1}$ on 28 Jul 2020, 12,324 copies 100 $mL^{-1}$ for *Helicobacter* (bird) on 23 Apr 2020, 51,180 gene copies 100

$mL^{-1}$ for bovine faecal pollution (cow) on 28 Jul 2020, and finally, 39,896 copies 100 $mL^{-1}$ for human faecal pollution also on 28 Jul 2020 (Fig. 5.3 A-D).



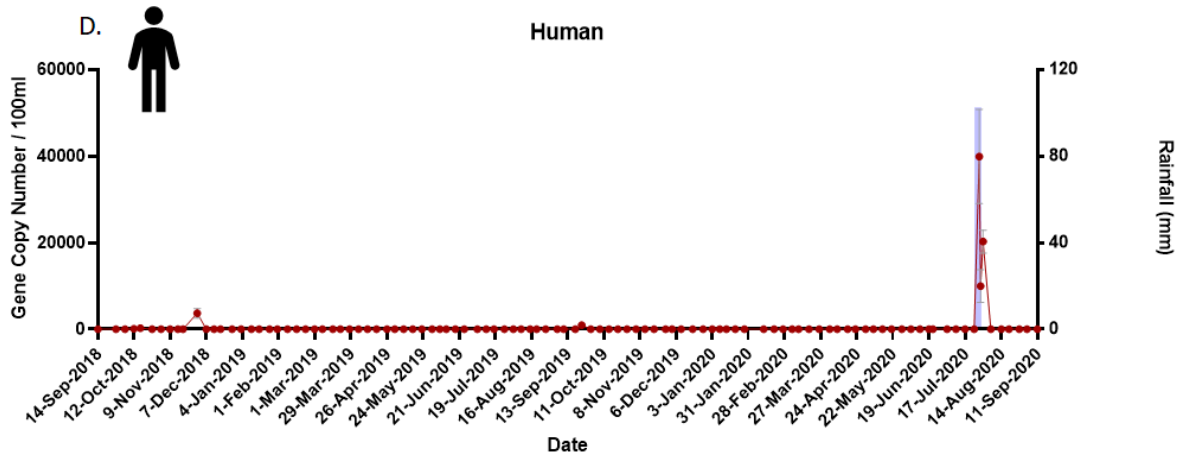



**Figure 5.3 A-D.** Weekly *E. coli* data from the sensor location, Hawkesbury River, using A. *E. coli* assay; B. Bird assay; C. Cow assay; C2. Cow assay with different y-axis scale to show low levels of bovine contamination across sampling period; and D. Human assay. Dotted lines in Fig. A at 14 and 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. Coba Bay harvest area is classified as Conditionally Approved. https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/industry/shellfish_industry_manual.pdf.

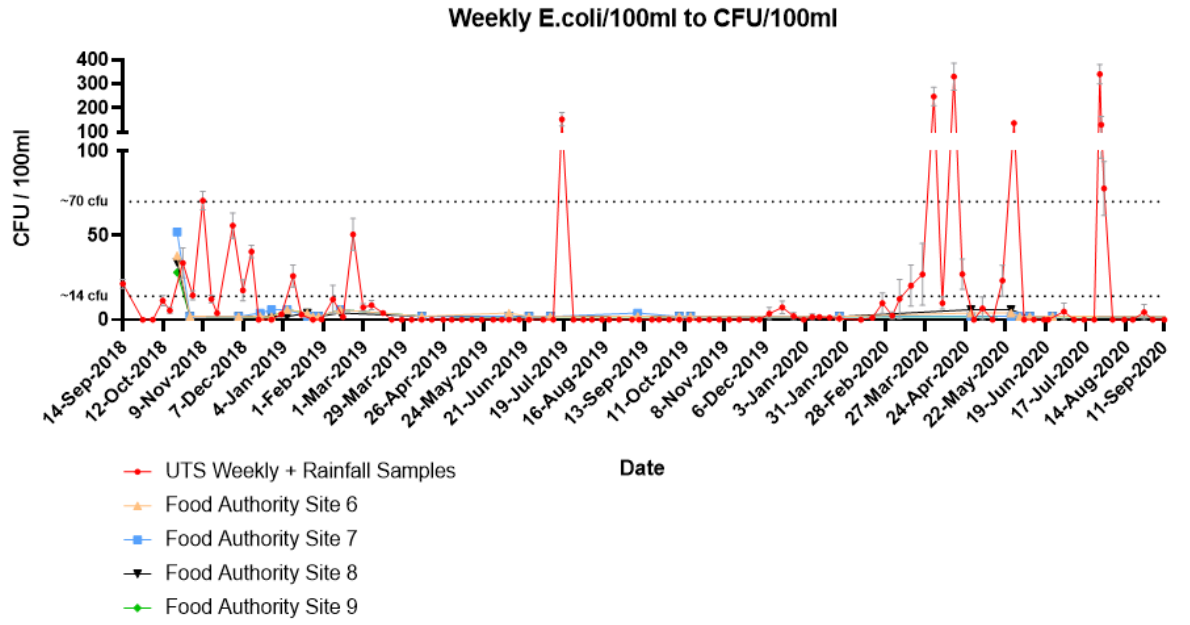



**Figure 5.4A** Weekly faecal coliform counts (cfu/100 mL) from water samples collected by DPI Food Authority at four sites in the Hawkesbury River compared to Oyster Transformation Project weekly sampling results. Dotted lines at 14 and 70 cfu/100 mL (Fig. 5.4B) are the operational limits for direct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

Sampling days with elevated faecal coliform counts reported from the DPI Food Authority corresponded to elevated levels as measured by the CRC project. On some occasions however, the CRC project test methods appeared more sensitive than routine testing (Fig. 5.4).

Only one rainfall event was sampled across the study period (see purple bars in Fig 5.3 A-D). This occurred on 28-29 Jul 2020 (Fig. 5.5). Rainfall on the previous day (27 Jul 2020) reached 103 mm, followed by 20 mm on the next day (28 Jul 2020) and down to 1mm on the third day (29 Jul 2020). Sampling for bacteria did not occur on the 27 Jul 2020, but results for the 28 Jul 2020 show elevated *E. coli*, cow and human bacteria. All three of these declined on the 29 Jul but without subsequent sampling it is unclear how quickly these levels would have dissipated.

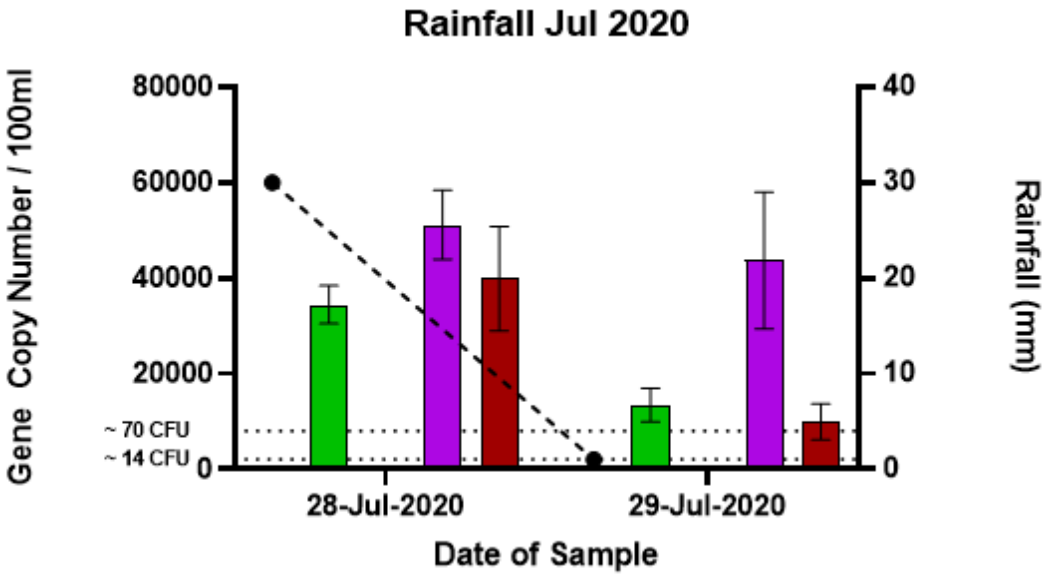



**Figure 5.5.** Sensor site (Hawkesbury River) rainfall event sampled for *E. coli* assays. Green bar = 16S *E. coli*; blue bar = bird assay; purple bar – cow assay; red bar = human assay. Dotted line is rainfall (mm) obtained from the closest rainfall station (HRSP rainfall gauge (Mooney)). All bars 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 (13 Oct 2017 to 31 March 2021) occurred on 13 Feb 2020 (Fig. 5.5). Total cell concentrations reached 2.2E +07 cells $L^{-1}$ and the sample was dominated by mainly freshwater species with the planktonic diatom *Aulacoseira*; flagellates (cryptomonads, dinoflagellates, euglenoids, ochrophytes); a variety of green algal species and some cyanobacteria; with high levels of sediment and organic detritus. This bloom coincided with the maximum rainfall reported over the sampling campaign, which was 231 mm on 10 Feb 2020.

Potentially harmful blooms occurred cross the sampling period at the phytoplankton site closest to the sensor on 16 Oct 2018 and again on 16 Apr 2019. These was due to the toxic diatom *Pseudo-nitzschia delicatissima* gp., with cells reaching a maximum cell density of 180,000 and 560,000 cells $L^{-1}$ respectively. NSW Food Authority trigger levels for flesh testing are 50,000 cells $L^{-1}$ for this group.

On 1 Oct 2019 and 7 Oct 2020, the toxic dinoflagellate *Alexandrium pacificum* was above alert levels in the samples at 450 cells/L and 400 cells $L^{-1}$ respectively. From Jan to April 2018, and Jan to Mar 2019 another *Alexandrium* species, *A. minutum* was above alert levels with

maximum cell densities reaching 4,900 cells $L^{-1}$ in 2018 and 2500 cells $L^{-1}$ in 2019. NSW Food Authority trigger levels for flesh testing are 200 cells $L^{-1}$ for toxic *Alexandrium* species. On 7 Apr 2020, *Dinophysis caudata* reached the NSW Food Authority trigger level for this species of 500 cells $L^{-1}$. On 7 Jan 2021 *Prorocentrum cordatum* was elevated in the sample from Hawkesbury 32 at 79, 000 cells $L^{-1}$. There is no trigger level for *P. cordatum*, as its toxic status is yet to be confirmed (McLennan et al. 2021). Finally, the potentially toxic group, *Takayama* spp. reached 8,000 cells $L^{-1}$ on 9 Dec 2019.

No positive biotoxin results were reported in routine monitoring samples collected by HRSP during the same period at Coba Bay harvest area.

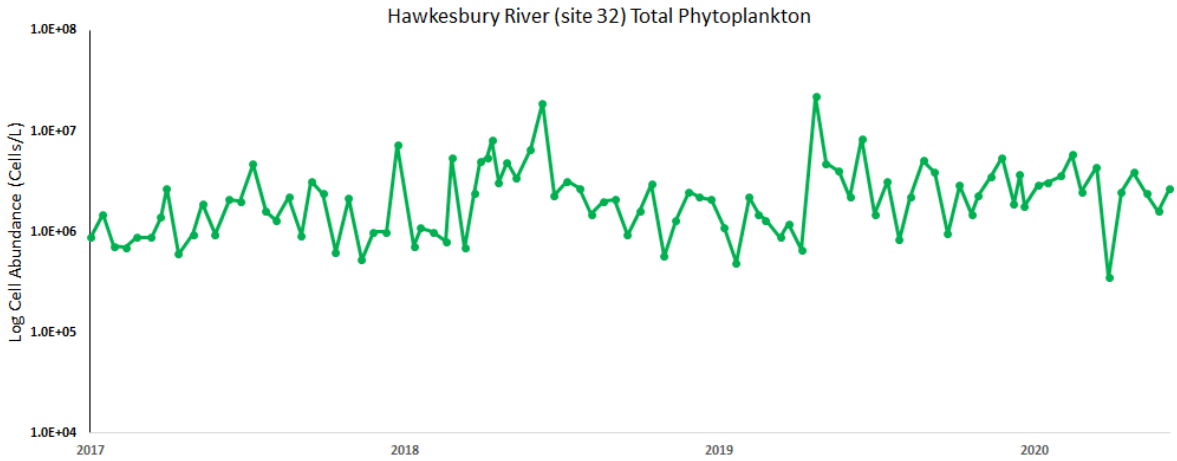
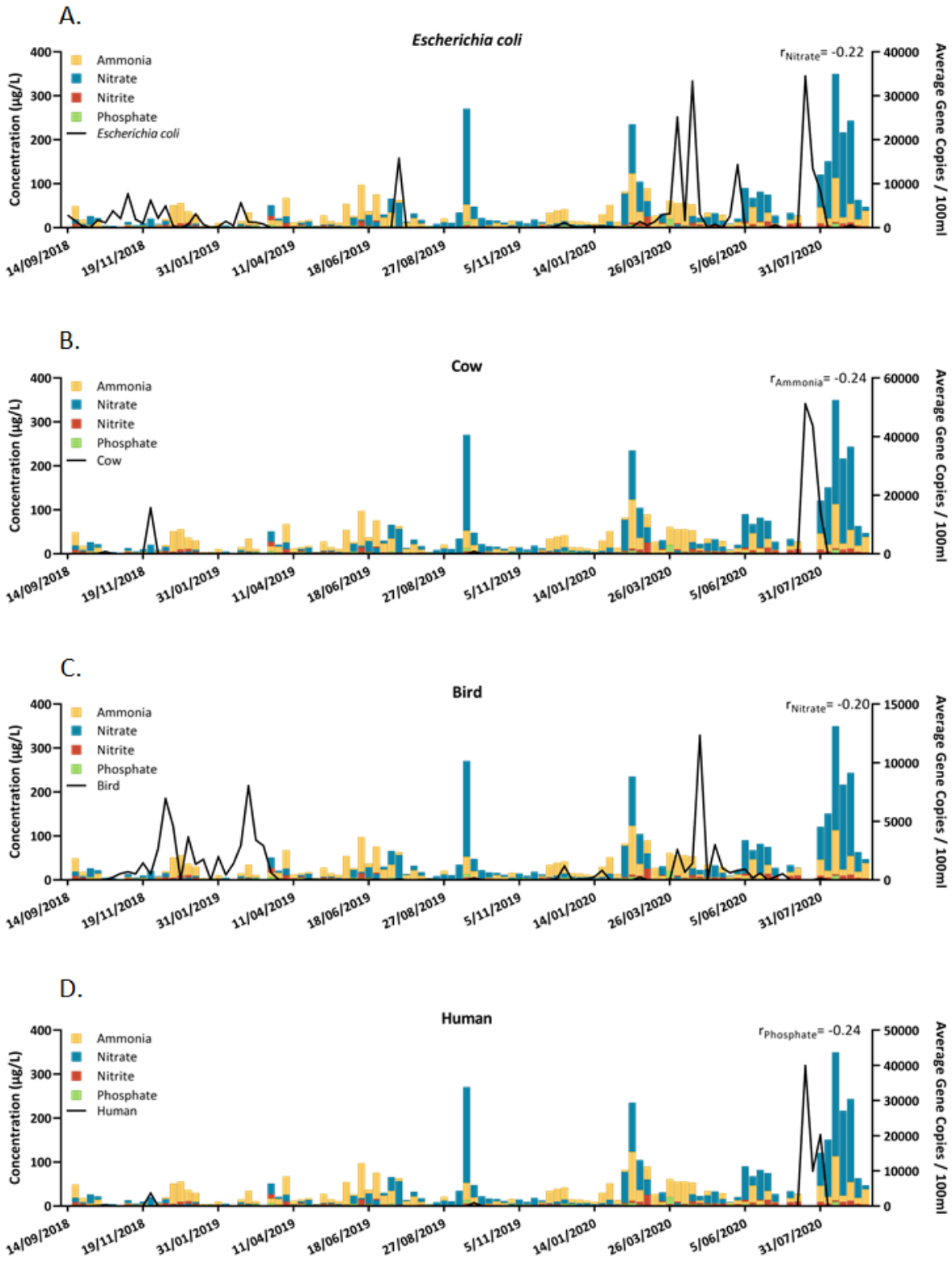



**Figure 5.6** Log abundance of total phytoplankton sampled approximately fortnightly from 13 Oct 2018 to 31 Mar 2021.

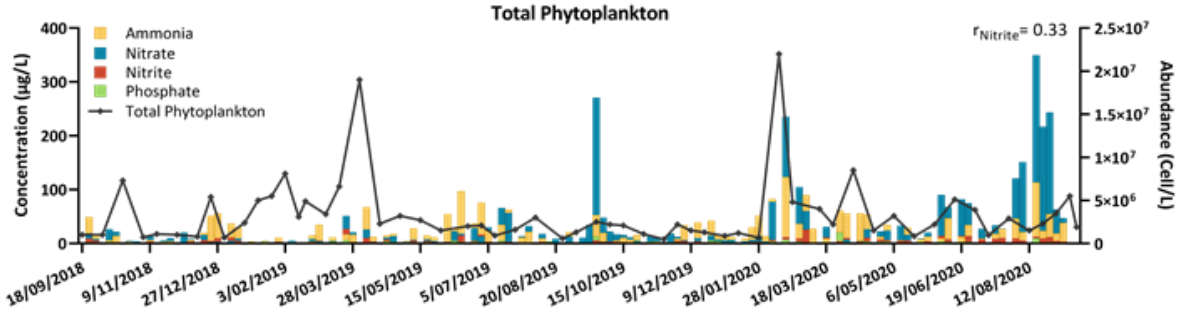
## 5.5 Nutrients

A total of 300 nutrient samples were collected over the sampling period, 21 Sept 2018 to 11 Sept 2020. Mean phosphate concentrations ranged from as low as the detection limit, to a maximum of 20.4 µg $L^{-1}$ on 26 Mar 2020. Mean nitrate concentrations ranged from 1.8 µg $L^{-1}$ on 26 Mar 2020 to a maximum of 349.7 µg $L^{-1}$ on 14 Aug 2020. Nitrite concentrations ranged from the detection limit to a maximum of 26.8 µg $L^{-1}$ on 21 Mar 2019. Significant, yet very weak, negative correlations were observed between *E. coli* and nitrate (r = -0.22); cow bacteria with ammonia (r = -0.24); bird bacteria and nitrate (r = -0.2), and human bacteria with phosphate (r = -0.24). No bacterial indicators correlated with nitrite concentrations (Fig. 5.6 A-D).

Where phytoplankton and nutrient samples were collected over the same 48-hour period, correlations were calculated. Total phytoplankton had a significant, yet weak positive correlation with nitrite (r = 0.33), *Pseudo-nitzschia* spp. and *Alexandrium* spp. had weak negative correlations with nitrate (r = -0.34 and -0.32 respectively), while *Dinophysis* spp. *Prorocentrum* spp. (predominantly *P. minimum*) and *Takayama* spp. showed no significant correlations with nutrients (Fig. 5.6 E-I).


A.
Escherichia coli
Ammonia
Nitrate
Nitrite
Phosphate
Escherichia coli
$r_{Nitrate}$= -0.22
Concentration (µg/L)
Average Gene Copies / 100ml
B.
Cow
Ammonia
Nitrate
Nitrite
Phosphate
Cow
$r_{Ammonia}$= -0.24
Concentration (µg/L)
Average Gene Copies / 100ml
C.
Bird
Ammonia
Nitrate
Nitrite
Phosphate
Bird
$r_{Nitrate}$= -0.20
Concentration (µg/L)
Average Gene Copies / 100ml
D.
Human
Ammonia
Nitrate
Nitrite
Phosphate
Human
$r_{Phosphate}$= -0.24
Concentration (µg/L)
Average Gene Copies / 100ml
14/09/2018
19/11/2018
31/01/2019
11/04/2019
18/06/2019
27/08/2019
5/11/2019
14/01/2020
26/03/2020
5/06/2020
31/07/2020

E.


Total Phytoplankton
Ammonia
Nitrate
Nitrite
Phosphate
Total Phytoplankton
$r_{Nitrite}$= 0.33
Concentration (µg/L)
Abundance (Cell/L)
18/09/2018
9/11/2018
27/12/2018
3/02/2019
28/03/2019
15/05/2019
5/07/2019
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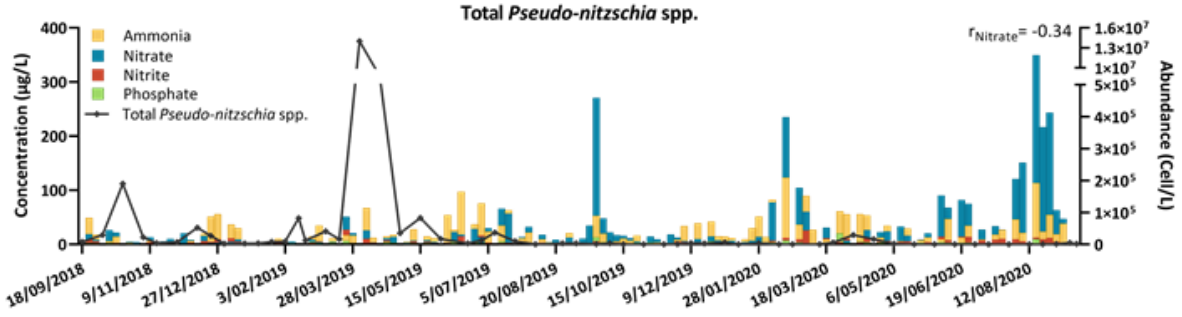
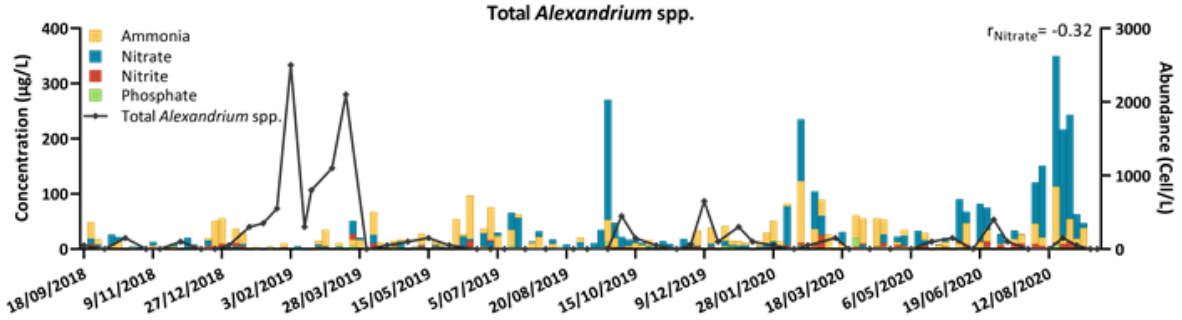
6/05/2020
19/06/2020
12/08/2020

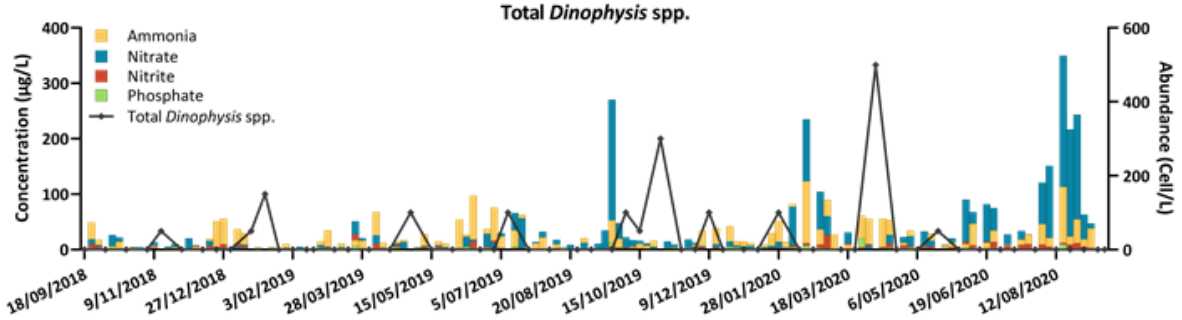
F.


Total *Pseudo-nitzschia* spp.
Ammonia
Nitrate
Nitrite
Phosphate
Total *Pseudo-nitzschia* spp.
$r_{Nitrate}$= -0.34
Concentration (µg/L)
Abundance (Cell/L)
18/09/2018
9/11/2018
27/12/2018
3/02/2019
28/03/2019
15/05/2019
5/07/2019
20/08/2019
15/10/2019
9/12/2019
28/01/2020
18/03/2020
6/05/2020
19/06/2020
12/08/2020

G.


Total *Alexandrium* spp.
Ammonia
Nitrate
Nitrite
Phosphate
Total *Alexandrium* spp.
$r_{Nitrate}$= -0.32
Concentration (µg/L)
Abundance (Cell/L)
18/09/2018
9/11/2018
27/12/2018
3/02/2019
28/03/2019
15/05/2019
5/07/2019
20/08/2019
15/10/2019
9/12/2019
28/01/2020
18/03/2020
6/05/2020
19/06/2020
12/08/2020

H.


Total *Dinophysis* spp.
Ammonia
Nitrate
Nitrite
Phosphate
Total *Dinophysis* spp.
Concentration (µg/L)
Abundance (Cell/L)
18/09/2018
9/11/2018
27/12/2018
3/02/2019
28/03/2019
15/05/2019
5/07/2019
20/08/2019
15/10/2019
9/12/2019
28/01/2020
18/03/2020
6/05/2020
19/06/2020
12/08/2020

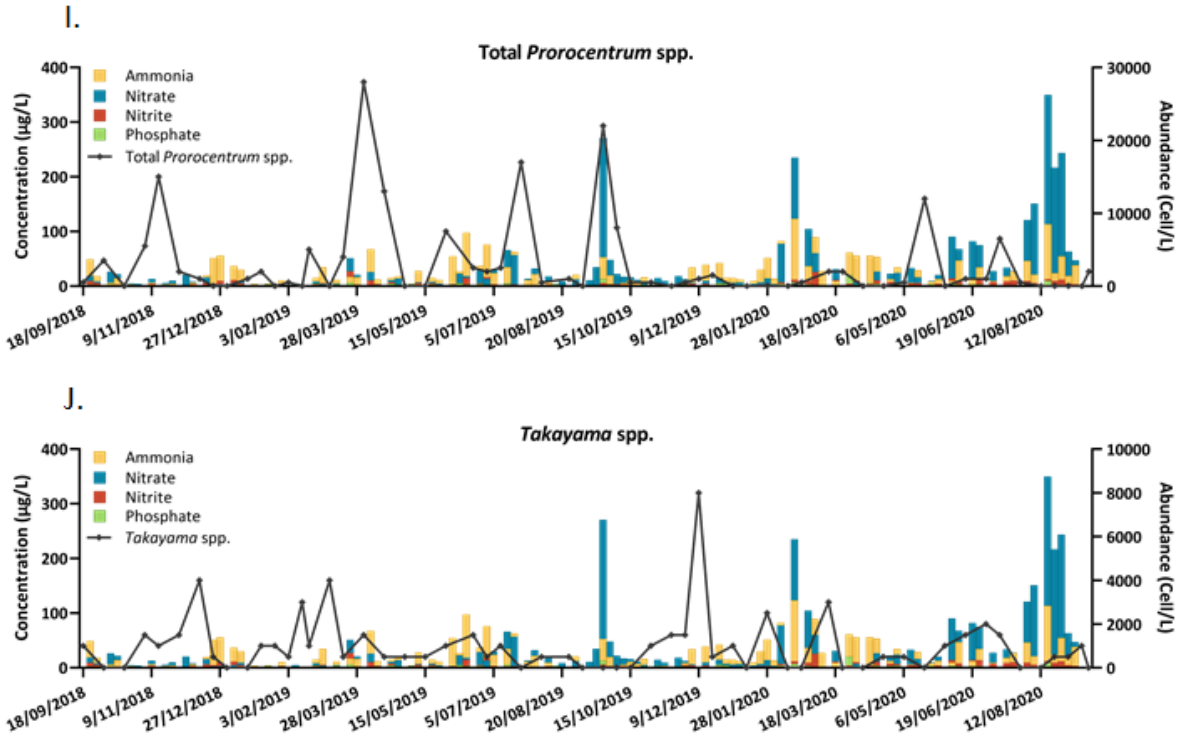



**Figure 5.7 A-D** Abundance of E. coli, bird, cow, and human bacteria; and **Figure 5.7 E-J** Total phytoplankton, total *Alexandrium* spp., A. *pacificum*, total *Pseudo-nitzschia* spp., and total *Dinophysis* spp., each with nutrient concentrations (phosphate and NOx) over the sampling period (2018-2020) in the Hawkesbury River.

## 5.6 Oyster Growth and Mortality

### 5.6.1 Oyster Growth

Average oyster whole weight increased by 40.6 g from deployment in August 2018 to June 2020 (Fig. 5.8A). Oyster whole weight was 63.2 ± 6.4 g at the end of the experiment (June 2020). Oysters deployed in Hawkesbury River attained a large size grade where average shell length was > 70 mm in September 2019 and exceeded 50 g whole weight in approximately January 2020. The age of oysters at each of these milestones was 33 mo and 37 mo, respectively.

Oyster shell length was 57 ± 2 mm at the start of the experiment and increased to 76 ± 3 mm in June 2020 (Fig. 5.8 B). The greatest increase in shell length in Hawkesbury River was recorded from August to December 2018. The increase in size through this period was 10 mm. Shell lengths were measured more frequently than whole weight and fluctuated throughout the experiment. Periods of shell length decreases were recorded from August to September 2018, October to November 2018, December 2018 to February 2019, June to July 2019 and December 2019 to February 2020.

### 5.6.2 Mortality

From August 2018 to January 2020, cumulative oyster mortality was 22% in Hawkesbury River. This was slightly above the level of mortality expected for background farming of the

Sydney Rock Oyster (approximately 10% per annum). A major proportion of this mortality (17%) occurred from August to November 2018 (Figure 5.8 C-D). Mortality was low (5%) for the remainder of the assessment period. Oysters from this site remain frozen for future analyses.

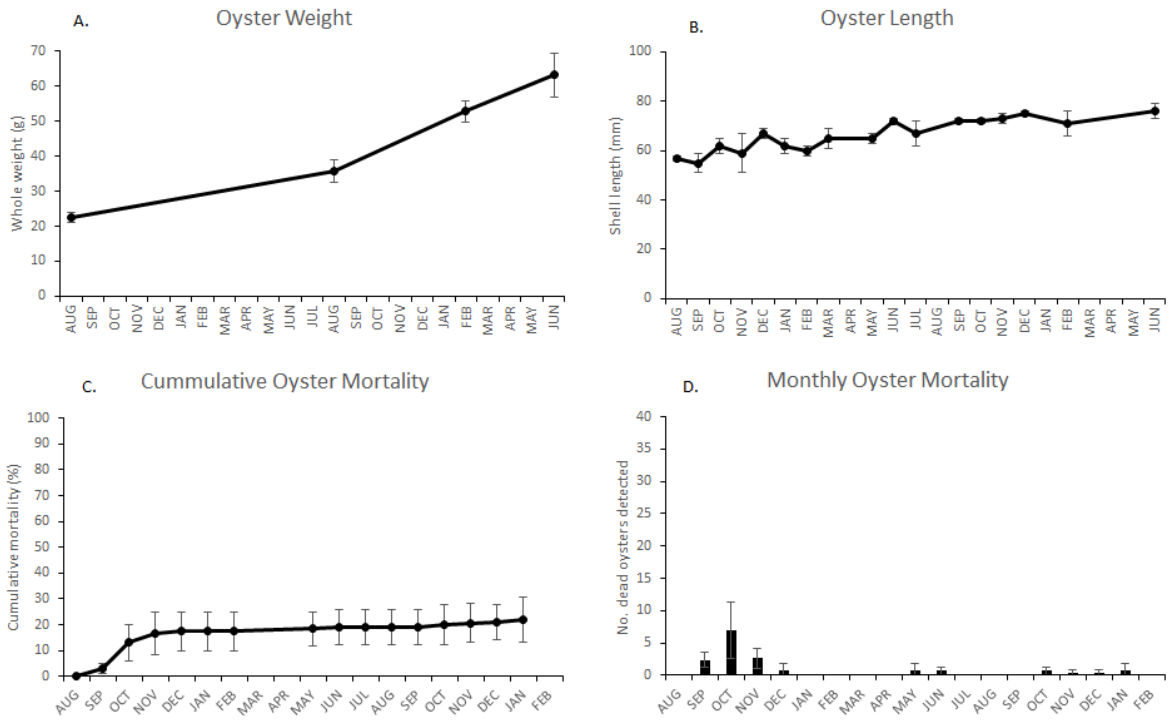



**Figure 5.8 A-D.** Oysters deployed at the sensor site, Hawkesbury River. A. whole weight; B. shell height; C. cumulative mortality, and D. monthly mortality.

## 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 positive relationship with cow and human bacteria (r = 0.8). A total of 4 models were developed for each of the bacterial sources: sensor + nutrients only; sensor, nutrients and total phytoplankton (logged or unlogged); rainfall and nutrients only; and rainfall, nutrients 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 were: 36% for *E. coli* (salinity + nutrients + total phytoplankton), 91% for cow (salinity + nutrients + total phytoplankton), 58% for bird (salinity + nutrients + total phytoplankton) and 98% for human (salinity + nutrients + total phytoplankton) (Table 1). The model for human bacteria and rainfall could not find an estimate with ammonia included (possibly due to the high correlation to other nutrient variables, so was dropped for the final model).

The abundance of *E. coli* at the sensor site was best explained by the sensor data compared to rainfall data (36% deviance explained as compared to 18%) and was strongly linked to

decreasing salinity (~20 ppt)/increasing rainfall, as well as increasing water temperature and varying nutrient load (Table 1, Figures 5.9 A-D, 5.10 A-D).

Cow bacterial abundance was better predicted using sensor data compared to rainfall data (91% compared to 69% with sensor data), with decreasing salinity and marginally increasing temperature over the past 72 hours and varying nutrient load being significant predictors (Table 1, Figures 5.9 A-D, 5.10 A-D).

Faecal contamination from birds at the sensor site was best explained by the salinity model (58% deviance explained, compared to 41% using rainfall data), with a peak in salinity ~22 ppt and an increasing temperature (over the past 72 hours), as well as a varying nutrient load (Table 1, Figures 5.9 A-D, 5.10 A-D).

An increase in human bacteria abundance was best explained by the sensor data (98% compared to rainfall data 74%), and was strongly linked to a decreasing salinity over the past 24 hours, a water temperature between 20-22℃ and a varying nutrient load (Table 1, Figures 5.9 A-D, 5.10 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 84% 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 Hawkesbury River. Only significant variables are shown for each model.

| Bacteria | Variables | No. of observations | Significant Variables | Deviance Explained |
|---|---|---|---|---|
| *E. coli* | Salinity72, Depth72, Temp72, Phosphate, NOx, Nitrite, Ammonia | 81 | Depth72**, Salinity72***, Temp72***, Phosphate***, NOx***, Ammonia*** | 34.8% |
| *E. coli* | Salinity72, Depth72, Temp72, Phosphate, NOx, Nitrite, Ammonia, logPhytoplankton | 81 | logPhytoplankton ***, depth72**, salinity72***, temp72***, Phosphate***, NOx***, Nitrite***, Ammonia*** | 35.5% |
| *E. coli* | Rainfall72, Phosphate, NOx, Nitrite, Ammonia | 97 | Rainfall72***, Phosphate***, NOx***, Nitrite***, Ammonia*** | 17.3% |
| *E. coli* | Rainfall72, Phosphate, NOx, Nitrite, Ammonia, logPhytoplankton | 97 | Rainfall72***, Phosphate***, NOx***, Nitrite***, Ammonia*** | 17.5% |
| Bird | Salinity72, Depth72, Temp72, Phosphate, NOx, Nitrite, Ammonia | 81 | Salinity72***, Depth72***, Temp72***, Phosphate***, NOx***, Nitrite***, Ammonia*** | 58.1% |
| Bird | Salinity72, Depth72, Temp72, Phosphate, NOx, Nitrite, Ammonia, logPhytoplankton | 81 | Salinity***, Depth***, Temp***, Phosphate***, NOx***, Nitrite***, Ammonia***, logPhytoplankton*** | 58.1% |
| Bird | Rainfall72, Phosphate, NOx, Nitrite, Ammonia | 97 | Rainfall72***, Phosphate***, NOx***, Nitrite***, Ammonia*** | 40.9% |
| Bird | Rainfall72, Phosphate, NOx, Nitrite, Ammonia logPhytoplankton | 97 | Rainfall72***, Phosphate***, NOx***, Nitrite***, Ammonia***, logPhytoplankton*** | 40.9% |
| Cow | Salinity, Depth, Temp, Phosphate, NOx, Nitrite, Ammonia | 81 | Salinity72***, Depth72***, Temp72***, NOx***, Nitrite***, Ammonia*** | 90.8% |
| Cow | Salinity, Depth, Temp, Phosphate, NOx, Nitrite, Ammonia logPhytoplankton | 81 | Salinity***, Depth***, Temp***, Phosphate***, NOx***, Nitrite***, Ammonia***, logPhytoplankton*** | 90.9% |
| Cow | Rainfall24, Phosphate, NOx, Nitrite, Ammonia | 98 | Rainfall24***, Phosphate***, NOx***, Nitrite***, Ammonia*** | 69.2% |
| Cow | Rainfall24, Phosphate, NOx, Nitrite, Ammonia logPhytoplankton | 98 | Rainfall24***, Phosphate***, NOx***, Nitrite***, Ammonia***, logPhytoplankton*** | 69.3% |
| Human | Salinity24, Depth24, Temp, Phosphate, NOx, Nitrite, Ammonia | 86 | Salinity24***, Depth24***, Temp24***, Phosphate***, NOx***, Nitrite***, Ammonia*** | 94.5% |
| Human | Salinity, Depth, Temp, Phosphate, NOx, Nitrite, | 86 | Salinity***, Depth***, Temp***, Phosphate***, | 98.3% |

| | | | | |
|---|---|---|---|---|
| | Ammonia logPhytoplankton | | NOx***, Nitrite***, Ammonia***, logPhytoplankton*** | |
| Human | Rainfall24 Phosphate, NOx, Nitrite, Ammonia | 98 | Rainfall24***, Phosphate***, NOx***, Nitrite***, Ammonia*** | 73.7% |
| Human* | Rainfall24, Phosphate, NOx, Nitrite, logPhytoplankton | 98 | Rainfall24***, Phosphate***, NOx***, Nitrite**, logPhytoplankton*** | 73.7% |

***** Model did not converge with Ammonia included

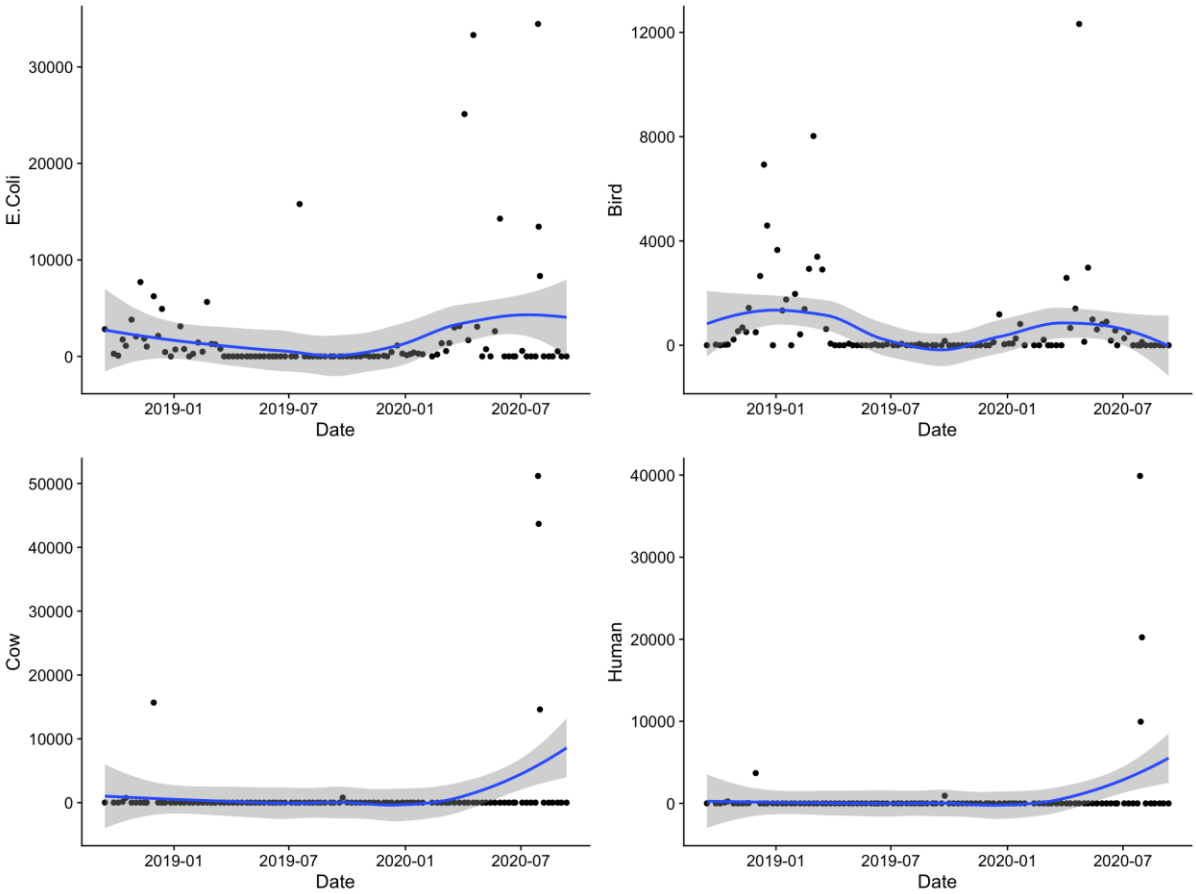



**Figure 5.9 A-D.** Data points (black dots), average (blue line) and standard error (shaded area) of A. *E. coli*, B. Bird, C. Cow, and D. Human bacterial load as measured by weekly sampling at the sensor site, Hawkesbury River.

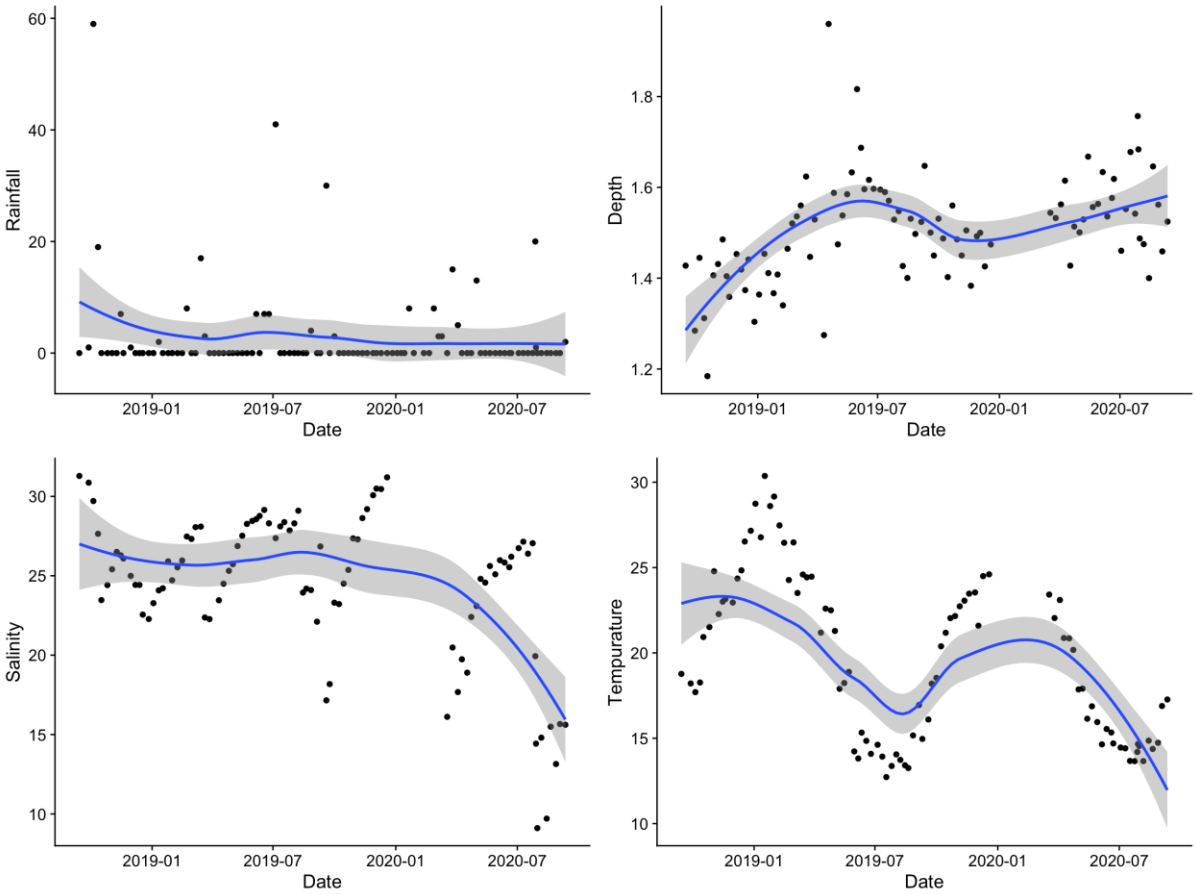



**Figure 5.10 A-D.** Data points (black dots), average (blue line) and standard error (shaded area) of A. Rainfall, B. Depth, C. Salinity, and D. Temperature values measured in at the sensor site, Hawkesbury River.

# DISCUSSION

# 6. Discussion

## 6.1 High Resolution Sensor Data and Management Plan

Analysis of sensor data during the annual review process demonstrated that there was potential to implement a salinity sensor-based management plan for Coba Bay harvest area. During the 2020 annual review assessment, results to date from the sensor supported a change to a salinity only based management plan closure limit for Coba Bay harvest area. Based on the available data at that time, five harvest area closures could have potentially been avoided between 13 October 2017 and 30 June 2020. HRSP were consulted about this option and HRSP requested the management plan change, which was implemented 7 December 2020. Since July 2020, and following the implementation of a salinity-based management plan in December 2020, there were no occasions when the harvest area would have remained open when comparing operations under a rainfall or salinity-based management plan. This reflected wetter conditions during this period. 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 Coba Bay harvest area management plan would revert to the previous management plan that is based on both rainfall and salinity closure limits.

## 6.2 Phytoplankton and HABs

The most common HAB species that bloomed in the Hawkesbury 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).

Another common toxic species 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).

Finally, *Takayama pulchella* (syn. *Gymnodinium pulchellum*) has been implicated in fish kills but the toxins involved in blooms of this species are as yet unknown. This species has been widely reported from southeastern Australia, including the Hawkesbury River, but no toxic effects have been reported in Australia to date. *Takayama pulchella* was first described from Australian (Victorian) sites, as *Gymnodinium pulchellum* (Larsen 1994). It was thought to be linked to fish kills in the region, however, no molecular genetic data, cultures or toxicity information was available from the time to verify either the species identification or the toxins. *Takayama pulchella* has been widely reported along the NSW coastline from the Hastings River to Wonboyn (Ajani 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 Hawkesbury River

Molecular assays for the detection of faecal bacterial contamination in the Hawkesbury 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. 2020).

Salinity was a more reliable predictor than rainfall for all four of the faecal indicators tested in the Hawkesbury River. Elevated *E. coli* was variable, and at times linked to rainfall events. When consecutive days were sampled during a rainfall event, faecal bacteria increased immediately in response to heavy rainfall (with the exception of avian bacteria), yet decreased immediately after the rainfall subsided.

Cow and human bacteria were generally low across the sampling period, yet dramatically increased during one rainfall event on 28 Jul 2020 (see Fig. 5.3), and to a much lesser extent during another event on 30 Nov 2018 (~50 mm over two days). On the other hand, avian faecal pollution was observed to peak during the summer/autumn months only. 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 and failure of onsite sewage management systems present the highest impact/risk for human contamination in the Hawkesbury 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 Hawkesbury River were greatest in the second half of the experiment from August 2019 to June 2020. However, growth, in terms of shell length, was

greatest in the 4-month period leading up to December 2018. The salinity level fluctuated substantially during the experiment with frequent episodes of reduced salinity (15-20 ppt) observed (Fig. 5.1B). These episodes of reduced salinity had no effect on oyster whole weight but appeared to coincide with the multiple periods of shell length decreases recorded in oysters throughout the trial. Lower salinities decrease seawater alkalinity providing less 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).

Low levels of mortality were recorded for most of the experiment, between November 2018 to January 2020. Mortality during this period was less than background mortality (approximately 10% per annum) commonly experienced when farming Sydney Rock Oysters. High levels of mortality were recorded, however, between August to November 2018, with a cumulative mortality of 17% during this period. Cumulative mortality at the end of the sampling period (January 2020) was 22%. This was the third highest cumulative mortality recorded across the monitoring sites, behind Camden Haven and Hastings River, which had a cumulative mortality level of 40% and 34%, respectively. High levels of cumulative mortality were also measured at Hawkesbury River in a previous study, with 80% and 22% mortality measured in wild and QX resistant Sydney Rock Oysters, respectively, over a 19-month period from October 2005 to May 2007 (Dove et al., 2013).

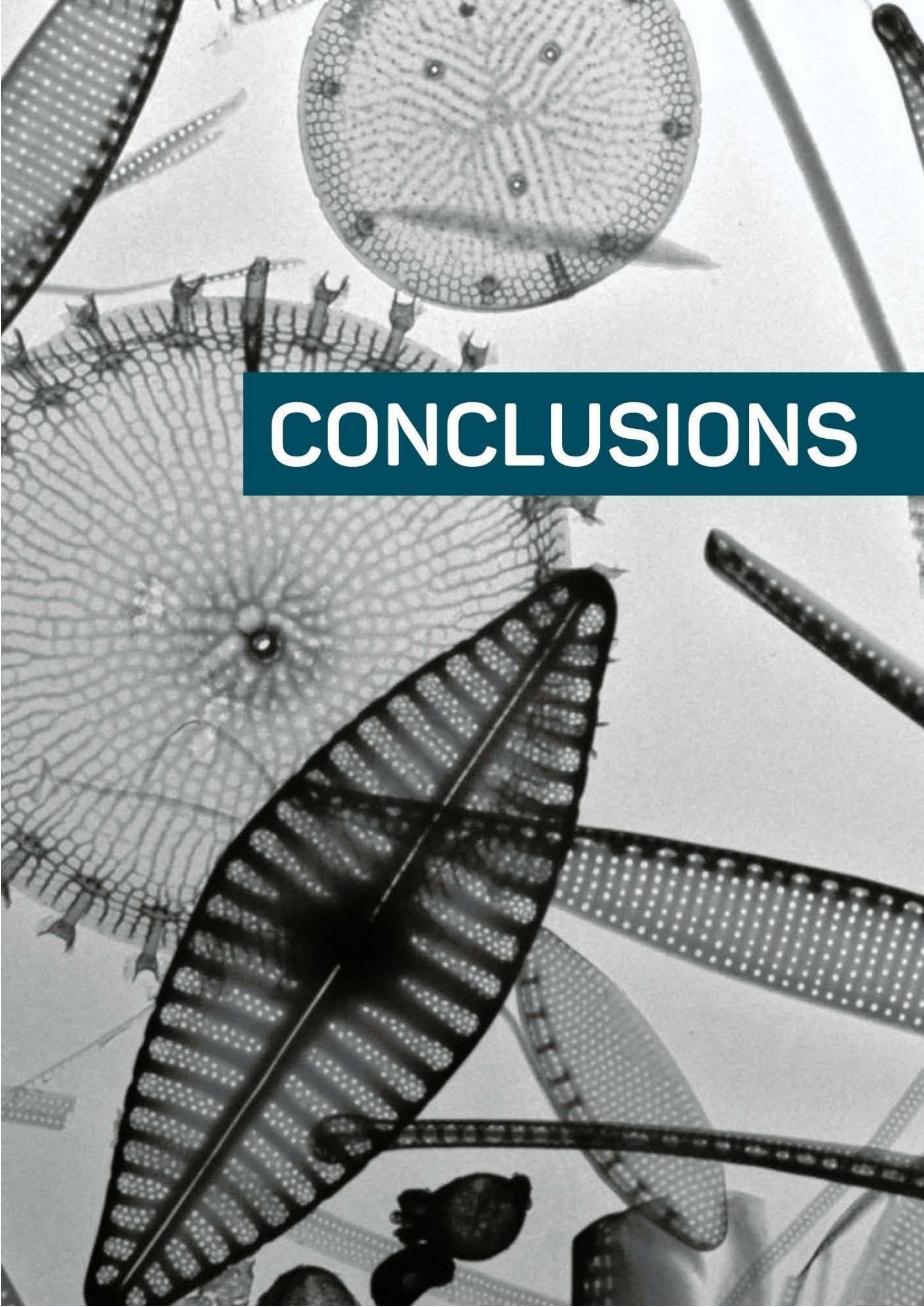
The current Hawkesbury River sensor site is situated in Marramarra harvest area. This location is known to experience recurrent outbreaks of QX disease with mortality rates of up to 100% recorded for wild oysters farmed in this location (Nell et al. 2007). The highest mortality event recorded in this experiment (17%) occurred from August to November 2018. This is outside the typical QX infection window for this location, which has been found previously to peak in mid-February to mid-March (Dove et al. 2013) and suggests that QX disease may not the driver of mortality. However, *M. Sydneyi* infections have been detected in other NSW estuaries outside of the typical QX disease window of infection period (for example, Port Stephens). Frozen archived samples could be examined to investigate possible factors for the mortality that occurred in October 2018. Mortalities were not detected in the oysters used in this trial in the 2019 and 2020 main QX disease risk period (late summer to early autumn) indicating that QX disease may not have occurred, or only occurred at very low levels, during this trial.

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 approximately 2 years and 9 months to reach the large oyster size grade (> 70 mm total length or > 50 g whole weight). Oyster at this site had the fourth largest shell length and were the second heaviest at the end of the experiment in June 2020 (76 mm average shell length and 63.5 g average whole weight). Georges River and Wagonga Inlet were the only other two sites that had comparable growth in terms of whole weight with oysters attaining 68.5 g in the Georges River and 61.9 g in Wagonga Inlet.

The Hawkesbury River is ranked 15th in the state for Sydney Rock Oyster production with 49,052 dozen oysters sold annually worth $659,752 (NSW DPI, 2023). Many oyster growers in this estuary also produce 46,486 dozen triploid Pacific Oysters worth $664,517. Most Sydney Rock Oysters sold from Hawkesbury River are either at the medium or large size grades (NSW Department of Primary Industries 2023). This likely reflects the high growth rates of oysters in this estuary and the need to farm and market oysters around disease windows.

### 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 supports implementing a harvest area management plan based on sensor salinity data for Coba Bay harvest area. This was agreed by the local shellfish industry, and implemented during December 2020. Available data indicated that five harvest area closures could have potentially been avoided between October 2017 and June 2020. Since July 2020, and following the implementation of a salinity-based management plan in December 2020, there were no occasions when the harvest area would have remained open when comparing operations under a rainfall or salinity-based management plan. Further assessment of sensor data demonstrated also supported implementing a salinity-based management plan in the adjacent Marramarra harvest area management plan, and this was adopted by HRSP during December 2021. 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, oyster growth in Hawkesbury River ranked second overall in terms of whole weight and fourth overall in terms of shell length. Low levels of mortality were recorded over most of the monitoring period (< 5% per annum), except from August to November 2018, where cumulative mortality was 17%. The cumulative mortality at the end of the experiment (January 2020) was 22%. This was the third highest cumulative mortality recorded among the monitoring sites but was only slightly higher than 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 weight or mortality. Although Marramarra in Hawkesbury River is often impacted by outbreaks of QX disease, the peak QX infection window for this location (mid- February to mid-March) did not coincide with the timing of the mortality event observed in this study, indicating that QX disease was not likely to be the cause of mortality. Low levels of mortality at all other times of this trial indicates that impacts from QX disease were minor.

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 four faecal indicator bacteria. Elevated levels of *E. coli* were highly variable, at times increasing with rainfall across the sampling period, while bird bacteria fluctuated seasonally. Cow and human bacterial corresponded to one high rainfall event and subsequent nutrient inputs. Furthermore, while contamination from bird sources was observed at levels similar to other estuaries, a distinct presence throughout the summer/autumn 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 Hawkesbury River.

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# 9. Appendices

# A1. Methods

### A1.1 Sampling locations in the Hawkesbury River

Data used in this report originates from locations within the Hawkesbury River over the period 13 Oct 2017 to 31 March 2021. High-resolution temperature, salinity and depth data were obtained from a sensor located in Coba Bay harvest area, located within the Hawkesbury 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).

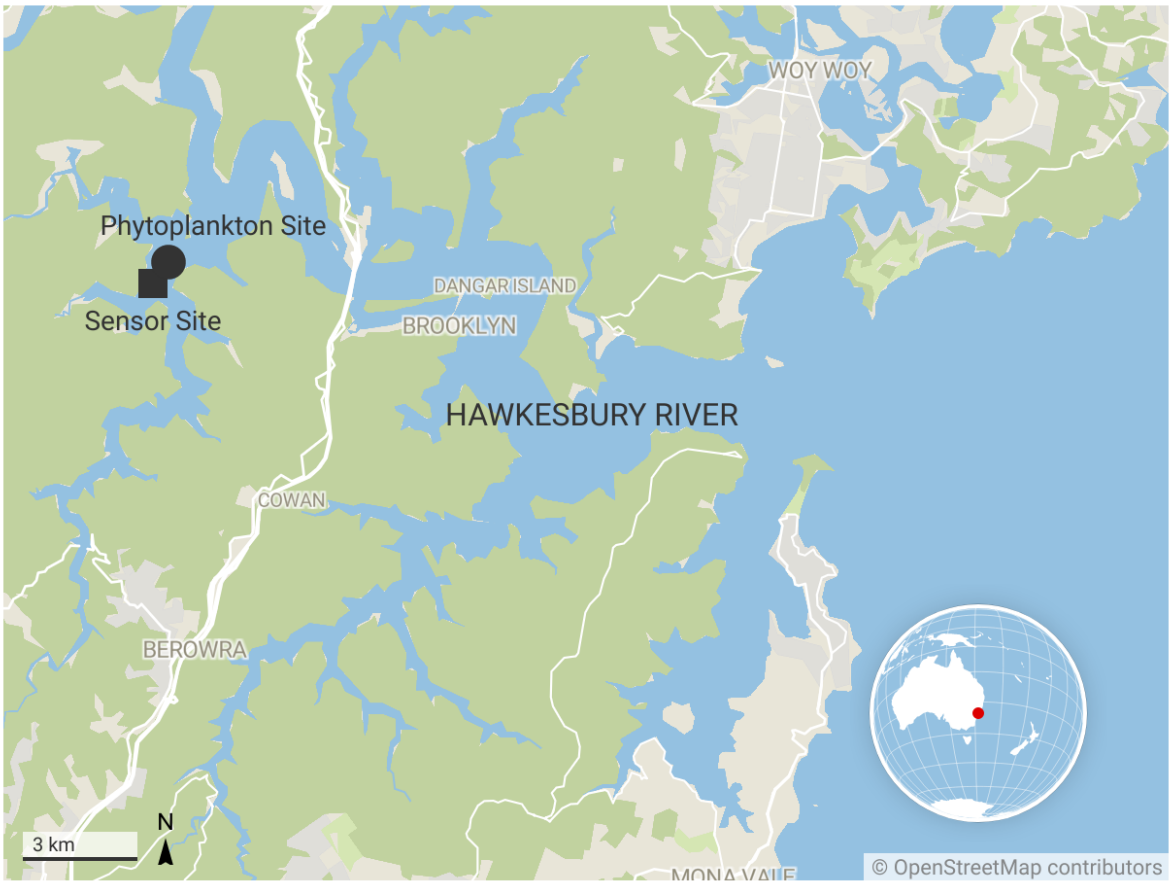


**Figure A1**: Map of the Hawkesbury River highlighting the sensor located in Coba Bay (black square) and the phytoplankton sampling location (black circle).

### 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 HRSP rainfall gauge (Mooney) (~-33.52°S, 151.20°E) from 13 Oct 2017 to 31 March 2021.

**Figure A2** Example of a Seabird MicroCAT high accuracy conductivity, temperature and depth (CTD) field sensor deployed in NSW estuaries.

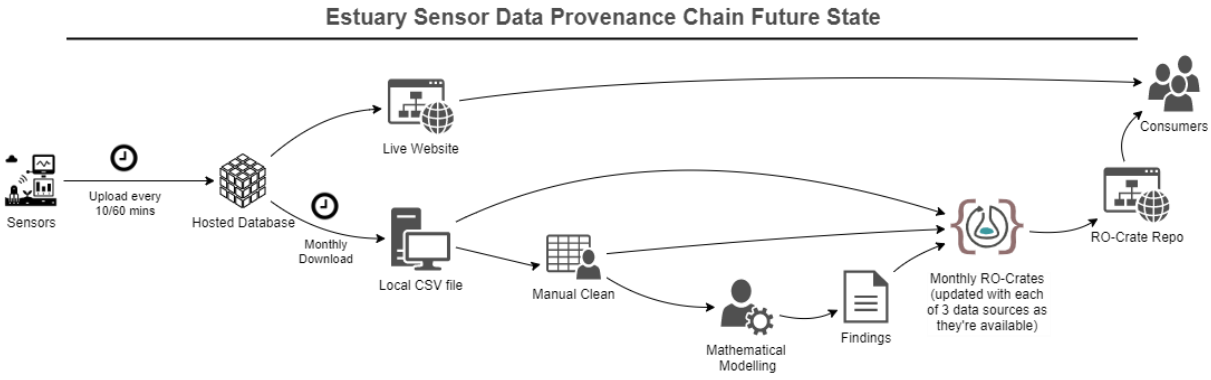


**Figure A3.** Hawkesbury River data provenance chain from source of data (sensor), via quality assurance processes, data analyses, to consumers.

### 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 Hawkesbury River annual review is 1 July. As part of the most recent (2022) annual review for Coba Bay 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. Due to hardware issues with the sensor, a portion of data between 25 December 2019 and 13 March 2020 was unavailable. Data collection from the original project sensor in Coba Bay was scheduled to cease at the end of March 2021, due to a change in sensor provider from the original project. Data collection (temperature and salinity, currently at 30-minute intervals) from a buoy, which was within the nearby Marramarra harvest area, was ongoing since 2010 via Hornsby Shire Council (HSC). NSWSP, in consultation with HSC, compared data from the two sensors. The analysis demonstrated that data from the HSC sensor was suitable for use in the Coba Bay harvest area management plan. This HSC operated sensor in Marramarra harvest area became the official Coba Bay management plan sensor during April 2021. During the 2021 annual review period, data were also assessed to determine the suitability of a salinity only management plan for Marramarra harvest area. The analysis demonstrated that data from the HSC sensor was suitable for use in the Marramarra harvest area management plan, and this was adopted by HRSP during December 2021.

### A1.4 Biological sampling, eDNA extraction and nutrient analyses

Estuarine water samples were collected weekly by Hawkesbury 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 ℃ 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 HRSP rainfall gauge (Mooney) (~-33.52°S, 151.20°E) from 13 Oct 2017 to 31 March 2021.

Triplicate water samples were also collected for nutrient analyses approximately weekly between Sept 2018 and Sept 2020 (Fig. A1). For each sample (~150 m apart), a one 10 L acid washed container of water was collected from ~2 meters depth, ~5 meters offshore. From each of these containers, five litres of sample water was transported to the laboratory at UTS, where it were filtered through a 100 µm mesh. Subsequently, 100 ml of water per sample was filtered through a sterile, 0.22 µm Sterivex-GP pressure filter (Merck) using a MasterFlex L/S Multichannel Peristaltic Pump 7535-08 (Cole-Parmer, Vernon Hills IL, USA). Each triplicate filter was stored in 2 x 50 ml Falcon tubes at -20 °C until further analysis. Sample blanks were then prepared and treated in an identical manner following collection, using ultra-pure water (18.2 MΩ-cm) sourced from an in-lab water purification system.

All glassware used during the preparation of standards and reagents were acid-washed twice using 10 %v/v hydrochloric acid (HCl) solution and rinsed at least three times with ultra-pure water (18.2 MΩ-cm) to prevent any contamination. Light-sensitive reagents were stored in amber glass containers.

The concentrations of free aqueous ammonium ($NH_4^+$), orthophosphate ($PO_4^{3-}$), NOx (as a sum of nitrate and nitrite, i.e. $NO_3^-$ and $NO_2^-$) and nitrite ($NO_2^-$) were quantified colourimetrically using a

Seal Analytical AA 500 Nutrient Analyser and Seal Analytical AS4 Autosampler. Methods for nutrient detection were based on those supplied in the Seal Analytical Nutrient Analysis manual for low level nutrient detection.

Briefly, analysis of ammonium (as µg N/L) required preparation of a phenate reagent (containing 2.8 % w/v sodium hydroxide, 5 % w/v phenol), a sodium nitroprusside complexing reagent (containing 0.05 % w/v sodium nitroprusside, 3 % w/v disodium-EDTA and 12% w/v trisodium citrate dihydrate) and a 1 % w/v dichloro isocyanuric acid solution. Analysis of phosphate (as µg P/L) (Method no. A-005-19 Rev. 2, Murphy and Riley 1962, Drummond and Maher 1995) required preparation of an ammonium molybdate solution (containing 0.3 % w/v ammonium molybdate tetrahydrate, 3.2 % v/v concentrated sulphuric acid and 0.0253 % w/v antimony potassium tartrate in ultra-pure water), an ascorbic acid solution (containing 0.2 % w/v ascorbic acid, 0.18 % w/v SDS and 1 % v/v acetone SDS in ultra-pure water), and surfactant solution (containing 1% w/v SDS in ultra-pure water). Analysis of both $NO_x$ and $NO_2^-$ (as µg N/L) (Method no. A-002-21 Rev. 1; Rice et al. 2017) required preparation of an ammonium chloride solution (containing 4 % w/v ammonium chloride, adjusted to pH 7.5 with 25% ammonia, and 0.25 % w/v Brij-35 surfactant), a colour reagent solution (containing 10 % v/v 85 % phosphoric acid, 1 % w/v sulphanilamide and 0.5 % w/v N-(1-naphthyl)-ethylenediamine dihydrochloride solution (NED)), and surfactant solution (containing 0.25 % w/v Brij-35 surfactant). The absorbances of the resulting colour complexes were monitored at 880 nm and 540 nm respectively. Sampling was conducted at a rate of 45 samples per hour, with a sampling time of 64 seconds and a wash time of 16 seconds.

Limits of detection (LODs) for all monitored analytes were determined experimentally through assessing precision at the low end of the calibration curve. These were 1.73 µg $L^{-1}$ for $NH_4^+$, 1.05 µg $L^{-1}$ for $PO_4^{3-}$, 0.46 µg $L^{-1}$ for $NO_x$, and 0.65 µg $L^{-1}$ for $NO_2^-$. These were determined as equivalent to an error of 3.3 standard deviations in four measurements of a 5-ppb standard. Analysis accuracy and precision were validated through spiked recovery, and regular quality controls were monitored throughout analysis, consisting of a baseline, blank, and low and high calibration points. The calibration curve and efficiency of the cadmium column, installed in the NOx line, were re-assessed every 60 samples. For data analyses purposes, any value under the LOD was assigned ½ of the LOD.

### 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.

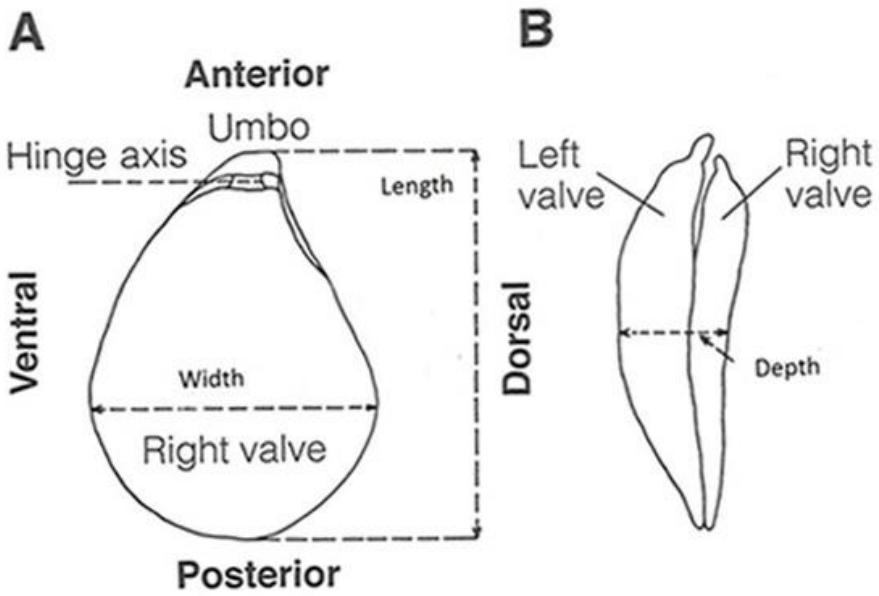


**Figure A4**. Oyster shell dimensions (Carriker 1996)

### *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 HRSP rainfall gauge (Mooney) (~-33.52$^{o}$S, 151.20$^{o}$E) from 13 Oct 2017 to 31 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 Hawkesbury 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, Hawkesbury River

| Variable | Mean | Standard Error | Median | Standard Deviation | Minimum | Maximum | Count | Missing |
|---|---|---|---|---|---|---|---|---|
| **average_ammonia** | 26.0 | 2.5 | 17.2 | 25.4 | 0.9 | 123.0 | 105 | 7 |
| **average_cfu** | 18.9 | 5.5 | 0.0 | 56.5 | 0.0 | 340.4 | 105 | 0 |
| **average_nitrite** | 5.3 | 0.5 | 3.5 | 4.9 | 0.3 | 26.8 | 105 | 7 |
| **average_nox** | 41.1 | 6.1 | 18.2 | 62.4 | 3.6 | 362.2 | 105 | 7 |
| **average_phosphate** | 2.6 | 0.3 | 2.0 | 2.7 | 0.5 | 20.4 | 105 | 7 |
| **bird** | 712.4 | 171.0 | 20.5 | 1752.5 | 0.0 | 12324.5 | 105 | 0 |
| **cow** | 1208.8 | 665.3 | 0.0 | 6817.4 | 0.0 | 51179.9 | 105 | 0 |
| **depth24** | 1.5 | 0.0 | 1.5 | 0.1 | 1.2 | 2.0 | 105 | 12 |
| **depth48** | 1.5 | 0.0 | 1.5 | 0.1 | 1.2 | 1.8 | 105 | 15 |
| **depth72** | 1.5 | 0.0 | 1.5 | 0.1 | 1.3 | 1.7 | 105 | 18 |
| **ecoli** | 2083.7 | 560.5 | 73.3 | 5743.8 | 0.0 | 34456.1 | 105 | 0 |
| **human** | 715.2 | 434.4 | 0.0 | 4451.6 | 0.0 | 39895.9 | 105 | 0 |
| **logPhytoplankton** | 14.6 | 0.1 | 14.6 | 0.8 | 13.1 | 16.9 | 105 | 0 |
| **Phytoplankton** | 3160857 | 340224 | 2200000 | 3486258 | 490000 | 22000000 | 105 | 0 |
| **rainfall24** | 2.8 | 0.8 | 0.0 | 8.3 | 0.0 | 59.0 | 105 | 0 |
| **rainfall48** | 2.8 | 0.6 | 0.0 | 6.2 | 0.0 | 39.0 | 105 | 1 |
| **rainfall72** | 2.8 | 0.5 | 0.3 | 5.1 | 0.0 | 26.3 | 105 | 2 |
| **salinity24** | 24.5 | 0.5 | 25.5 | 4.6 | 9.1 | 31.3 | 105 | 12 |
| **salinity48** | 24.5 | 0.4 | 25.4 | 4.4 | 11.8 | 31.1 | 105 | 15 |
| **salinity72** | 24.6 | 0.4 | 25.4 | 4.2 | 11.2 | 30.7 | 105 | 18 |
| **temp24** | 19.6 | 0.5 | 18.8 | 4.7 | 12.7 | 30.4 | 105 | 12 |
| **temp48** | 19.5 | 0.5 | 18.8 | 4.7 | 13.1 | 29.5 | 105 | 15 |
| **temp72** | 19.4 | 0.5 | 19.0 | 4.7 | 13.3 | 29.4 | 105 | 18 |

## 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<br><br>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<br><br>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<br><br>https://www.nswoysters.com.au/nsw-oyster-newsletter.html<br><br>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 |

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|---|---|---|---|
| DPI Food Authority | Foodwise - Issue 46 | https://www.foodauthority.nsw.gov.au<br><br>Feb 2018 | Published |
| Shauna Murray & Penelope Ajani | Ah shucks, how bushfires can harm and even kill our delicious oysters | The Conversation<br><br>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<br><br>(Supervisors: Arjun Verma and Shauna Murray) | Final Hons Seminar,<br><br>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 |

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|---|---|---|
| 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 |