TRANSFORMING AUSTRALIAN SHELLFISH PRODUCTION

Quibray Bay Harvest Area - Georges River

Report on Stage 1, October 2017-March 2021

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

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UTS

NSW GOVERNMENT

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2023

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Contents

Executive Summary

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

We installed a real-time sensor in the Quibray Bay harvest area, Georges River, recording high-resolution temperature, salinity and depth data. Oyster farmers collected weekly biological samples (618 environmental DNA samples and 291 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.

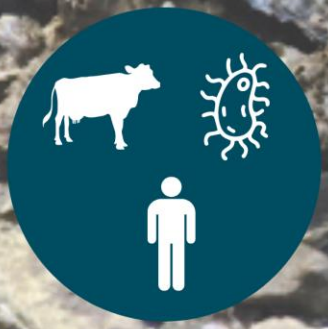
MAJOR FINDINGS

10

Available data indicated that ten harvest area closures could have potentially been avoided between October 2017 and March 2021

25%

Salinity was a more reliable predictor than rainfall of faecal bacteria at 1 out of 4 indicators tested



Elevated E. coli, cow and human bacteria were linked to elevated rainfall and nutrients inputs

0

Oyster mortality during the study did not exceed background farming mortality (estimated at 10% per annum) in Georges River and no activity of winter mortality disease was detected in Quibray Bay

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.*, P. minimum*), which were developed during Phase 1, will also be implemented.

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

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

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

1.2 Georges River

Georges River (-34.0153° S, 151.1847° E) is the main tributary leading into Botany Bay, Sydney. It is a highly modified drowned river valley, with a catchment area of 931 km^2, total estuary area of ~27 km^2 and a flushing rate of ~63 days (Roy et al. 2001, Roper et al. 2011) (Fig. 1). Botany Bay, on the other hand, has a catchment area of 55 km^2, a total estuary area of ~40 km^2, and a flushing rate of ~40 days (Roper et al. 2011). Both systems support seagrass (2, 5.3 km^2 respectively), mangroves (4, 2 km^2), and saltmarsh (~1 km each) areas (Roper et al. 2011). Both Georges River and Botany Bay are surrounded by urban development and are susceptible to stormwater pollution and faecal contamination (DPE 2022). Both the Georges River and Botany Bay also support a range of recreational and commercial uses. Recreational fishing is notably popular in the Georges River, with species caught including bass, bream, whiting, yellowtail, jewfish and flathead.

1.3 Oyster Production in Georges River

The Georges River has a long history of (non-indigenous) oyster farming, with leases established as far back as the 1870s, whereby oysters were grown for their meat and the shells used in lime production for building (Ogburn 2011, Barclay et al. 2017). With adaptation to challenges such as winter mortality and mudworm, the 1970s saw the peak of oyster production in this river, one of the top producing estuaries in NSW at this time. Since then however, an increasing in disease, including QX, winter mortality, and Pacific Oyster Mortality Syndrome, have significantly reduced the productivity of oysters in this river.

Today only one commercial oyster farm exists in the Botany Bay area (Quibray Bay), and detailed production value is not available due to confidentiality reasons (≤ 5 current permit holders in the estuary). Total production value across 11 estuaries with ≤ 5 permit holders is estimated to be ~224,672 dozens and valued at ~$2,208,619 (NSW DPI Aquaculture Production Report 2021/22).

FINDINGS

2. Findings

2.1. The data assessment from this report supports implementing a harvest area management plan based on sensor salinity data for Quibray Bay harvest area, Georges River, subject to agreement by the local shellfish industry. Available data indicated that ten harvest area closures could have potentially been avoided between October 2017 and March 2022.

2.2. We developed rapid, efficient, and sensitive qPCR assays for *E. coli*, cow, bird, and human faecal indicators, and used these rapid genetic tools to track these sources of pollution in 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 one (bird) out of the four faecal indicator bacteria.

2.4. The maximum predictive capability for each bacterial group were 52% for *E. coli*, 94% for cow, 53% for bird, and 92% for human at the sensor site.

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

2.6. The greatest increase in shell length in Georges River was recorded from February to August 2019. Georges River had the heaviest oysters (68.5 g) overall when compared to all other estuary monitoring sites measured for this project. None of the environmental variables measured/modelled were predictive of oyster growth.

2.7. No oyster mortality events that exceeded background farming Sydney Rock Oyster mortality (approximately 10% per annum) occurred in Georges River and no winter mortality disease was detected over the period from August 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 Robert Hill 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 Georges River were funded by the NSW Food Authority and the shellfish industry. We thank Kyle Johnston and Brandt Archer (DPI) for oyster stock preparation and growth/survival data collection, and Dr Nahshon Siboni and Prof Justin Seymour (UTS) for source tracking assistance. Finally, we would like to thank Dr Torri Callan (UTS) for statistical analyses and Chris Komorek (Food Agility CRC) for report layout.

FEEDBACK

4. Feedback

In May 2018, the Oyster Transformation Team held information workshops to allow farmers to have their say in the project. The workshops were held in Pambula (Pambula Fishing Club) and Bateman's Bay (Catalina Country Club).

Farmers were asked to rate the following factors in order of importance and benefit to their business operations (Fig. 4.1). In order of importance (highest to lowest) was the potential to predict algal blooms, longer harvest opening times, reduced stock mortality/disease, forecasting of harvest area closures, and access to real time tidal and monitoring data.

Group discussions followed, whereby additional issues that farmers raised were: the suitability of the sensor location and BOM rainfall gauge; and the breakdown of bacterial data into human and animal sources.

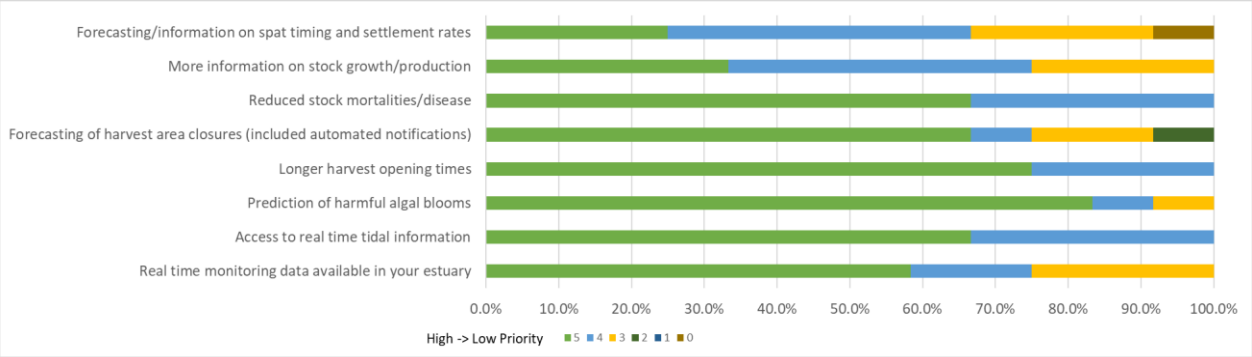


Figure 4.1. The importance of factors as rated by farmers in relation to their business operations. Green is most important and brown is least important.

RESULTS

5. Results

5.1 High resolution temperature and salinity data

High-resolution real time data summaries for the Georges River for the period 12 Oct 2017 to 31 Mar 2021 are shown in Figs. 5.1A-C. Data between 09/08/2019 and 8/10/2019 was removed from 'working data' due to sensor fault/odd salinity data and there was a data gap from 28 June - 23 July 2020 due to technical issues. Depth recordings ranged from 0 m (17 Dec 2020) to 2.5 m (2 Jan 2018). The lowest and highest daily average salinity recordings were 9.6 ppt (11 Feb 2020) and 36.7 ppt (28 Dec 2019) respectively, while the lowest and highest daily average temperature recordings were 11.3℃ (17 Jul 2018) and 29.3℃ (1 Feb 2020) respectively.

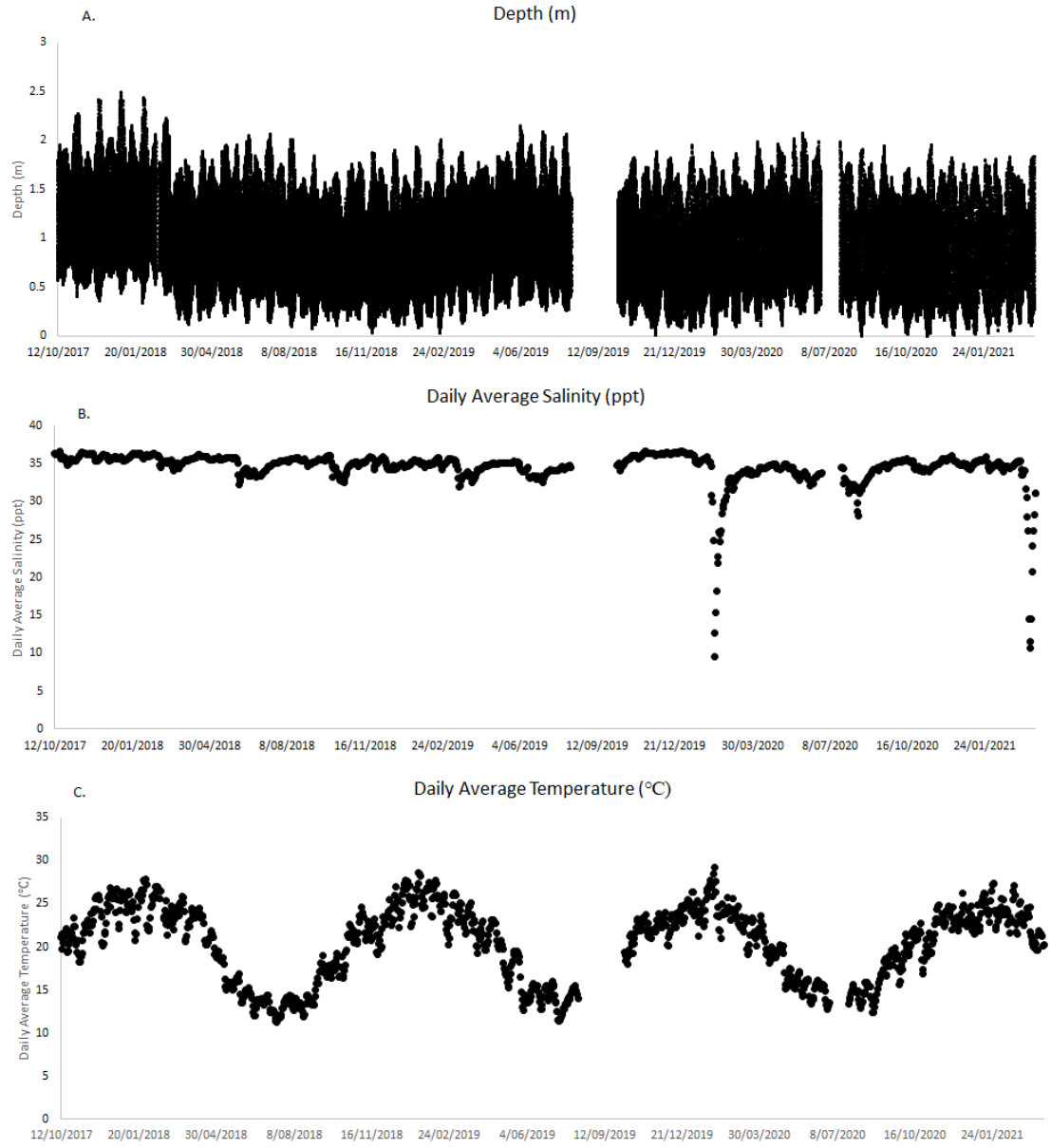


Figure 5.1A-C. Real time sensor data from Georges River sensor 12 Oct 2017 to 31 Mar 2021 A. Depth (m); B. Daily average salinity (ppt); and C. Daily average temperature (°C).

The maximum daily rainfall at the BOM San Souci Public School rainfall gauge occurred on 10 Feb 2020 and was reported as 100 mm (Fig. 5.2).

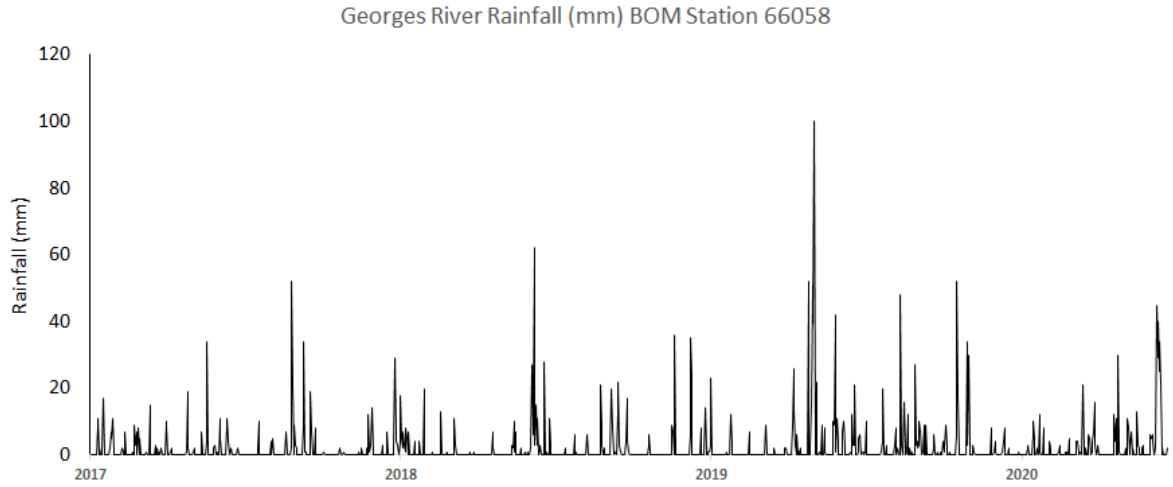


Figure 5.2. Daily rainfall (mm) from Bureau of Meteorology site number 66058 (~-33.99°S, 151.13°E) from Oct 2017 to March 2021.

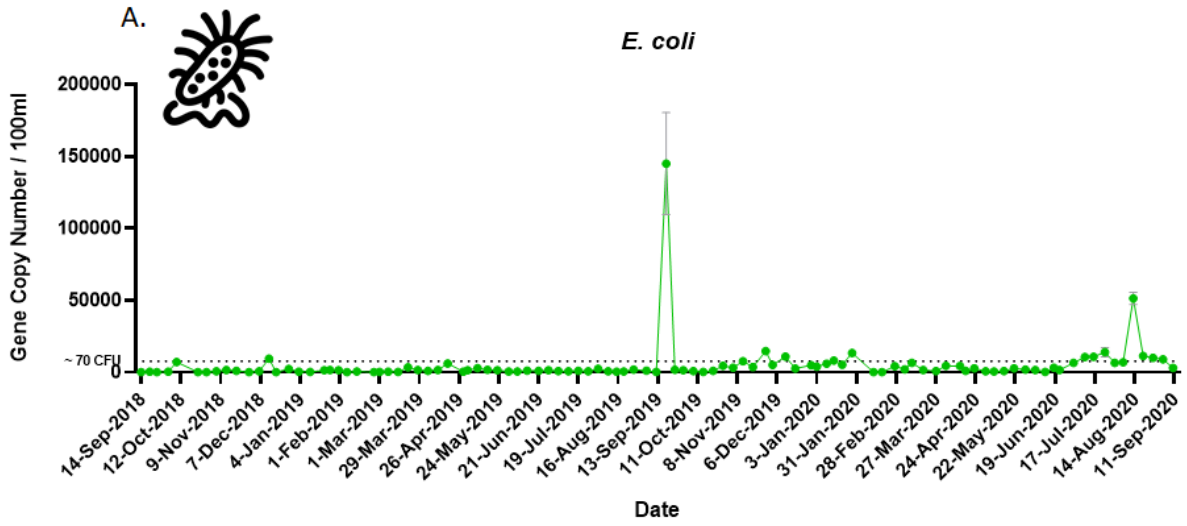
5.2 Management Plan

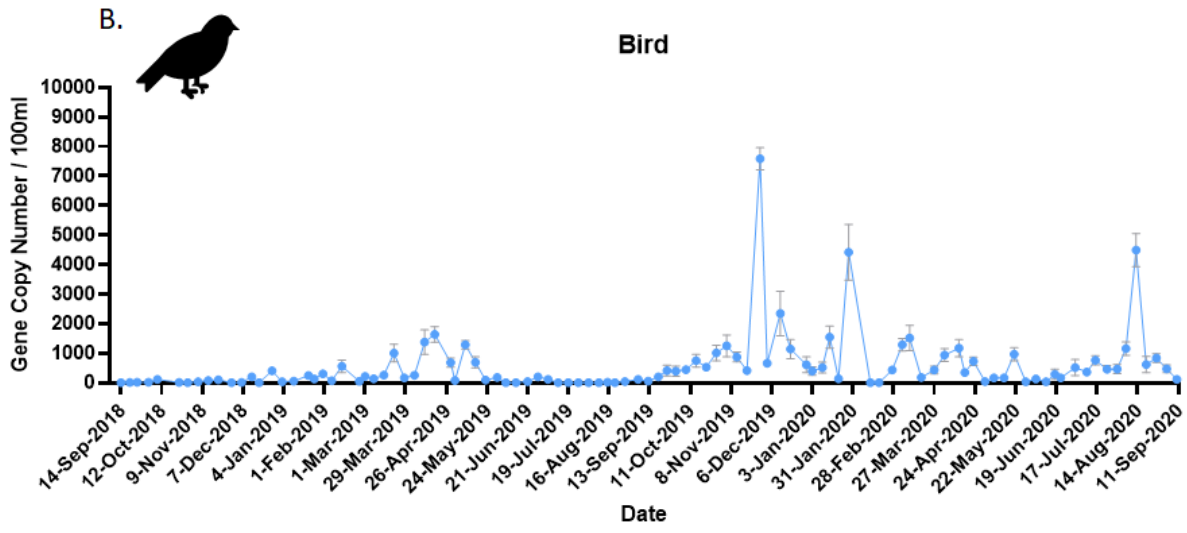
Data analysed during the 2021 and 2022 annual reviews of Quibray Bay harvest area indicated that there could have been fewer harvest area closures since the sensor was installed, if closures were based on salinity sensor data. Sixteen harvest area rainfall closures occurred between October 2017 and March 2022. Based on a management plan closure limit of 30 ‰, harvest area closures were modelled based on available salinity sensor data and shellfish program microbiological results since October 2017. Eighty-seven harvest closure days occurred over ten rainfall closures, although salinity sensor data did not decline below 30 ‰ and microbiological results from samples collected between 2-19 days post closure met Restricted harvest criteria. Time periods where salinity is slower to recover may require additional sampling to meet management plan requirements.

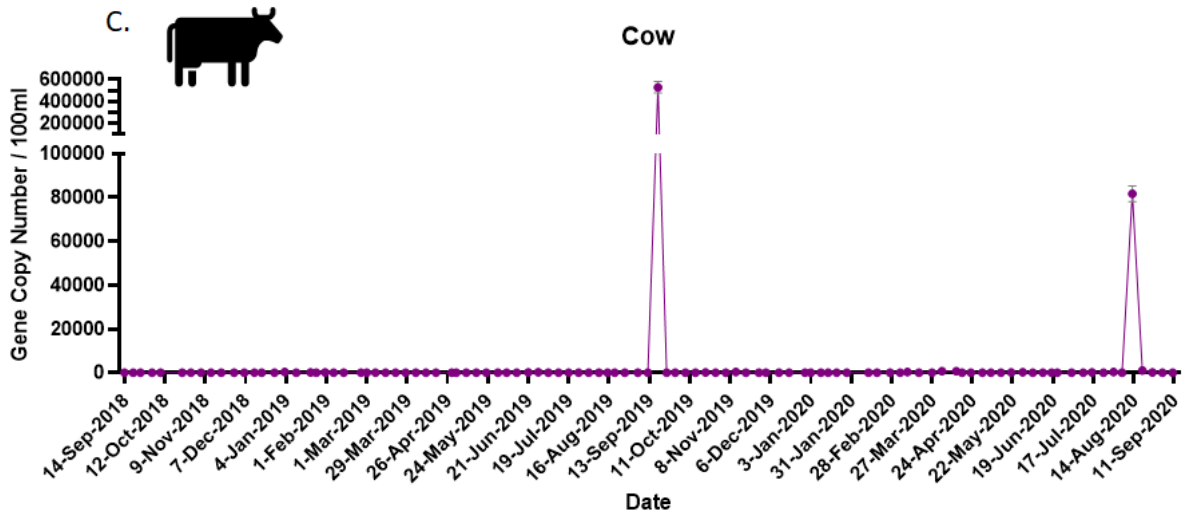
5.3 Bacterial source tracking

A total of 618 water samples and 291 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 Georges River (Fig. A1).

For the Georges River the maximum *E. coli* reached 144,865 gene copies 100 mL^{-1} on 19 Sept 2019, 7,588 copies 100 mL^{-1} for *Helicobacter* (bird) on 28 Nov 2019, 529,176 gene copies 100 mL^{-1} for bovine faecal pollution (cow) on 19 Sept 2019, and finally, 452,515 copies 100 mL^{-1} for human faecal pollution also on 19 Sept 2019 (Fig. 5.3 A-D).


A.
E. coli
Gene Copy Number / 100ml
200000
150000
100000
50000
~ 70 CFU
0
14-Sep-2018
12-Oct-2018
9-Nov-2018
7-Dec-2018
4-Jan-2019
1-Feb-2019
1-Mar-2019
29-Mar-2019
26-Apr-2019
24-May-2019
21-Jun-2019
19-Jul-2019
16-Aug-2019
13-Sep-2019
11-Oct-2019
8-Nov-2019
6-Dec-2019
3-Jan-2020
31-Jan-2020
28-Feb-2020
27-Mar-2020
24-Apr-2020
22-May-2020
19-Jun-2020
17-Jul-2020
14-Aug-2020
11-Sep-2020
Date


B.
Bird
Gene Copy Number / 100ml
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
14-Sep-2018
12-Oct-2018
9-Nov-2018
7-Dec-2018
4-Jan-2019
1-Feb-2019
1-Mar-2019
29-Mar-2019
26-Apr-2019
24-May-2019
21-Jun-2019
19-Jul-2019
16-Aug-2019
13-Sep-2019
11-Oct-2019
8-Nov-2019
6-Dec-2019
3-Jan-2020
31-Jan-2020
28-Feb-2020
27-Mar-2020
24-Apr-2020
22-May-2020
19-Jun-2020
17-Jul-2020
14-Aug-2020
11-Sep-2020
Date


C.
Cow
Gene Copy Number / 100ml
600000
400000
200000
100000
80000
60000
40000
20000
0
14-Sep-2018
12-Oct-2018
9-Nov-2018
7-Dec-2018
4-Jan-2019
1-Feb-2019
1-Mar-2019
29-Mar-2019
26-Apr-2019
24-May-2019
21-Jun-2019
19-Jul-2019
16-Aug-2019
13-Sep-2019
11-Oct-2019
8-Nov-2019
6-Dec-2019
3-Jan-2020
31-Jan-2020
28-Feb-2020
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17-Jul-2020
14-Aug-2020
11-Sep-2020
Date

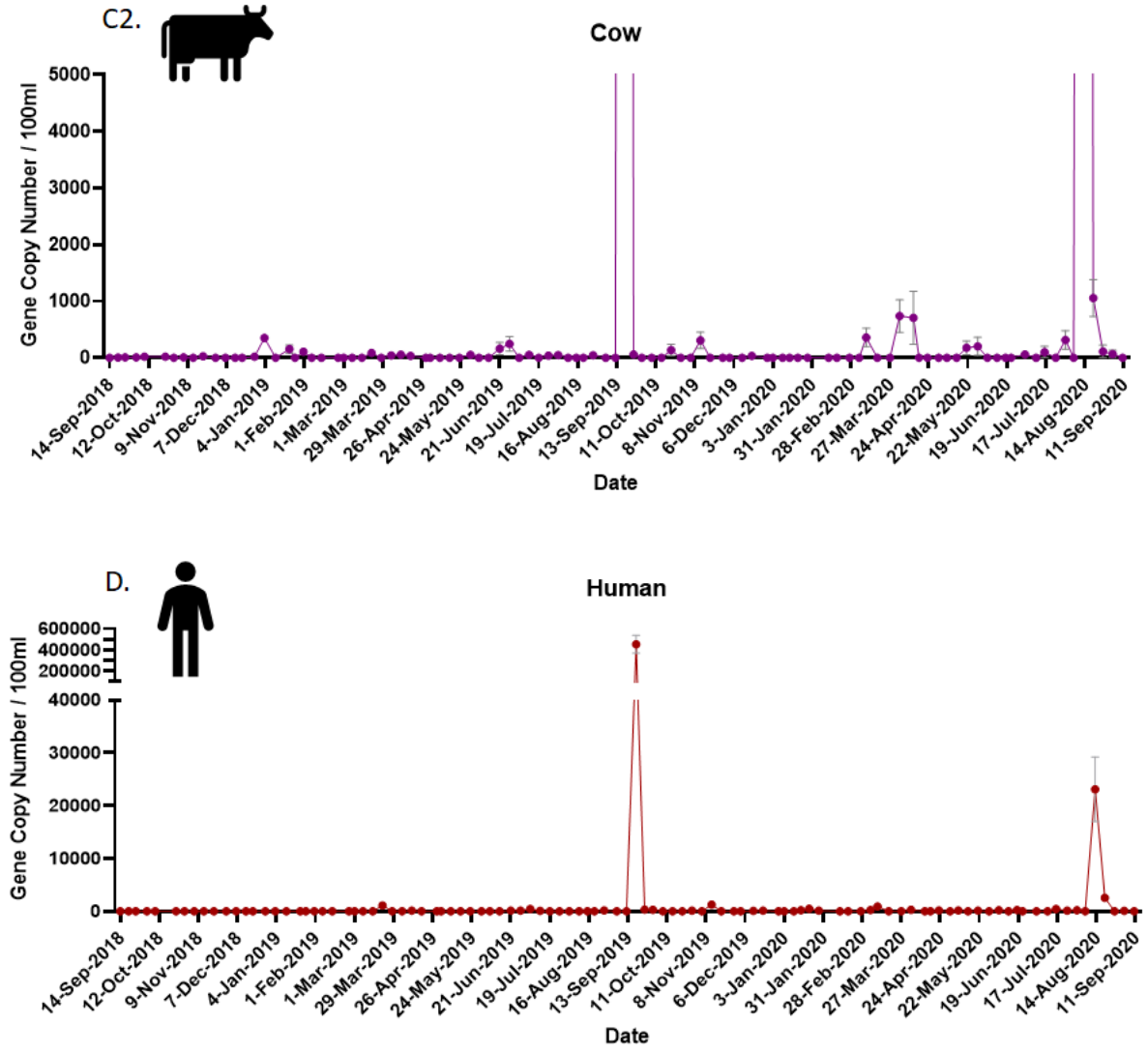


Figure 5.3 A-D. Weekly *E. coli* data from the sensor location, Georges River, using A. *E. coli* assay; B. Bird assay; C. Cow assay; C2. Cow assay with different y-axis scale to show low levels of bovine contamination across sampling period; and D. Human assay. Dotted lines in Fig. A at 14 and 70 cfu/100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

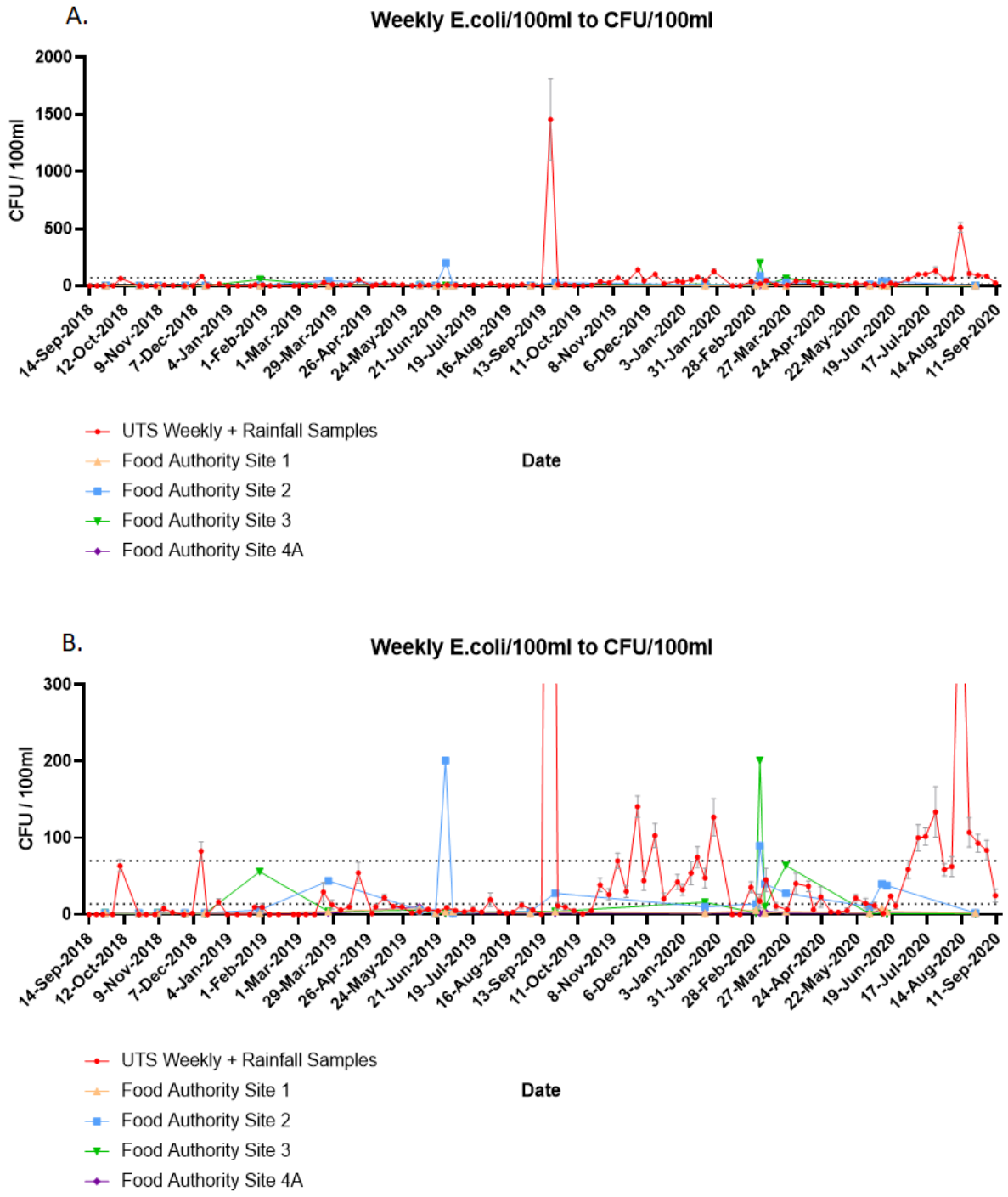


Figure 5.4A-B Weekly faecal coliform counts (cfu/100 mL) from water samples collected by DPI Food Authority at four sites in the Georges River compared to Oyster Transformation Project weekly sampling results. Dotted lines at 14 and 70 cfu/100 mL (Fig. 5.4B) are the operational limits for direct or restricted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

Sampling days with elevated faecal coliform counts reported from CRC project did not correspond to sampling days by the DPI Food Authority. On the other hand, samples collected on the same days by both DPI Food Authority and CRC generally were within the normal range of variability found between sites (Fig. 5.4A-B).

5.4 Phytoplankton enumeration and HAB events

The maximum phytoplankton cell concentration across the sampling period (Oct 2017 to March 2021) occurred on 16 Nov 2020 (Fig. 5.5). Total cell concentrations reached 2.5E +06 cells L^{-1} and the sample was dominated by planktonic diatoms (*Cerataulina*, *Leptocylindrus Dactyliosolen* and *Chaetoceros*) with some benthic diatoms (*Cylindrotheca*) and small flagellates (dinoflagellates). This bloom did not coincide with any significant rainfall event.

Other potentially harmful bloom events across the sampling period exclusively were due to the toxic dinoflagellate *Alexandrium pacificum.* This species reached elevated cell densities (above trigger level – see below) during Oct-Nov 2017 (max cell count of 250 cells L^{-1}), Jan 2018 (350 cells L^{-1}), Nov 2018 (2,600 cells L^{-1}), Jan – Feb 2019 (350 cells L^{-1}), Oct 2019 (300 cells L^{-1}), Jan 2020 (850 cells $L^{-}1$), and Oct – Nov 2020 (2400 cells L^{-1}). *Dinophysis acuminata* cell densities were elevated on 21 Aug 2020 at 500 cells L^{-1}. NSW Food Authority trigger levels for flesh testing are 200 cells L^{-1} for *Alexandrium pacificum* and 500 cells L^{-1} for *Dinophysis acuminata* (NSWFA 2015). A positive detection of paralytic shellfish toxins (PSTs) of 0.64 saxitoxin equivalent (STX eq.) mg/kg total PST was reported in a shellfish sample collected 1 Oct 2019. Shellfish samples collected 13 Jan 2020 and 26 Oct 2020 reported detections of PSTs reported at 0.032 and 0.035 STX eq. mg/kg total PST, respectively.

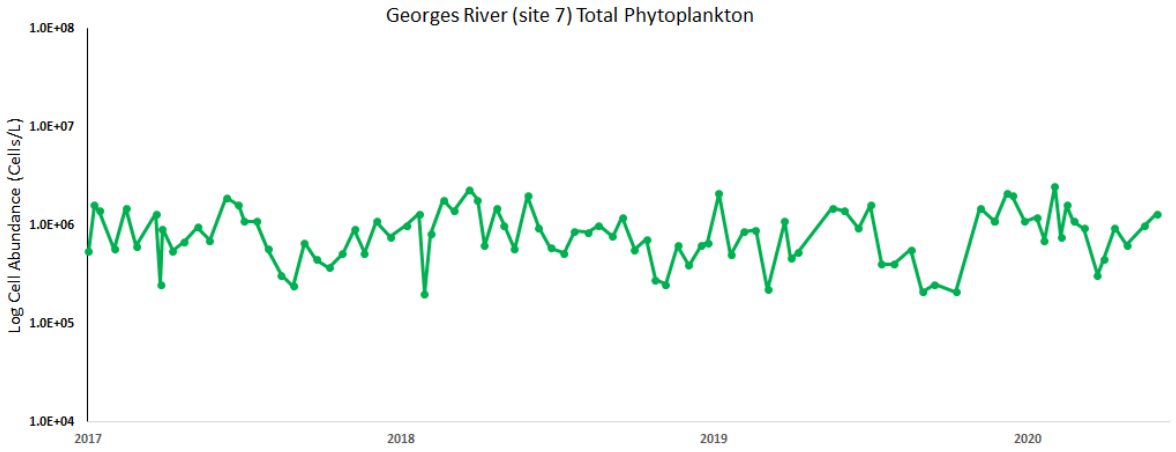


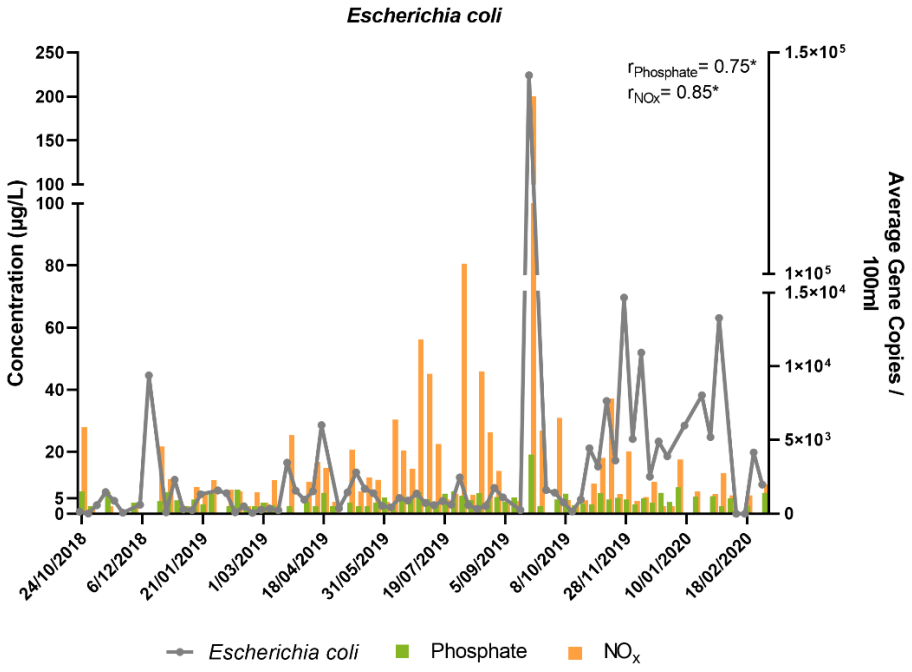
Figure 5.5 Log abundance of total phytoplankton sampled approximately fortnightly from 12 Oct 2017 to 31 Mar 2021.

5.5 Nutrients

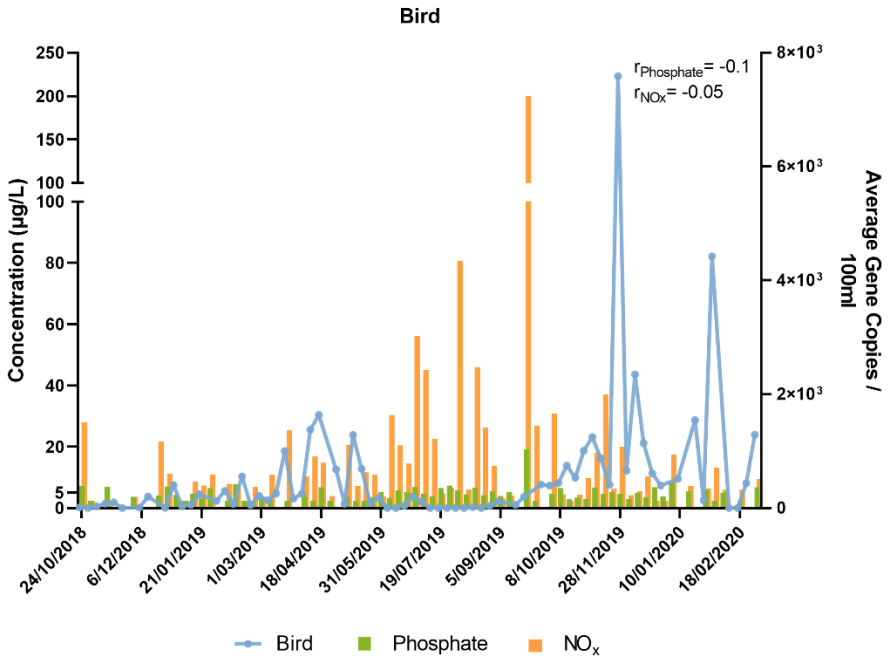
A total of 195 nutrient samples were collected over the sampling period, 24 Oct 2018 to 5 Mar 2020. Mean phosphate concentrations ranged from as low as the detection limit, to a maximum of 19.13 µg L^{-1} on 19 Sept 2019. Similarly, mean NO_x concentrations ranged from the detection limit to a maximum of 200.1 µg L^{-1}, also on 19 Sept 2019. Nitrite concentrations ranged from the detection limit to a maximum of 8.8 µg L^{-1} on 11 April 2019. Significant correlations were observed between *E. coli* and phosphate (r = 0.8) and *E. coli* and NO_x (r =0.8); cow bacteria with phosphate (r = 0.8) and with NO_x (r = 0.9); and human bacteria with phosphate (r = 0.8) and with NO_x (r = 0.9). No bacterial indicators correlated with nitrite concentrations (Fig. 5.6 A-D).

Where phytoplankton and nutrient samples were collected over the same 24-hour period, correlations were calculated. Total phytoplankton and total *Pseudo-nitzschia* spp. both had significant, negative correlations with phosphate (r = -0.5 and -0.6 respectively), while *A. pacificum* had a significant, positive correlation with phosphate (r = 0.5). Total *Dinophysis* spp. had a significant, positive correlation with NO_x (r = 0.5) (Fig. 5.6 E-I).

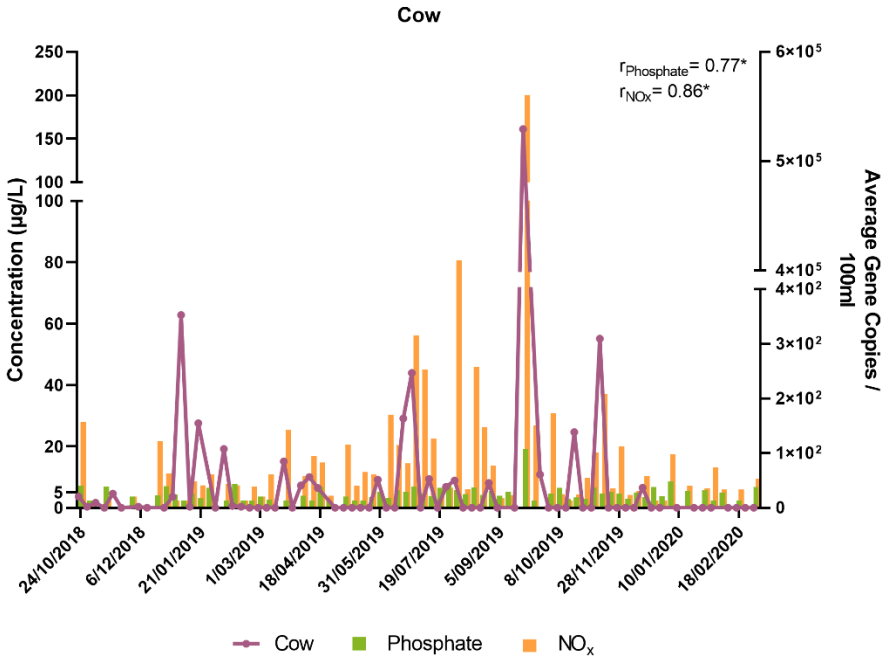
A.



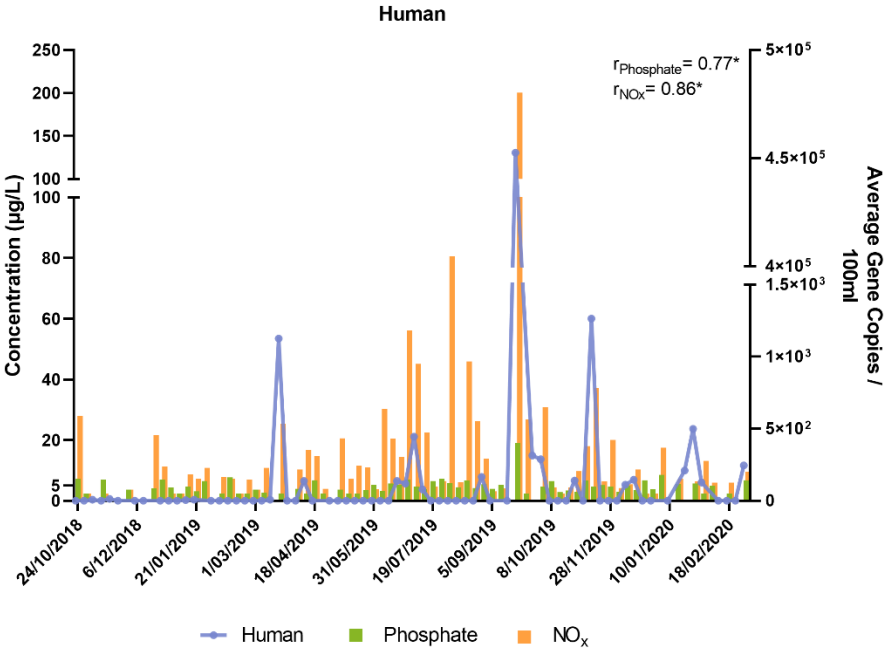
B.



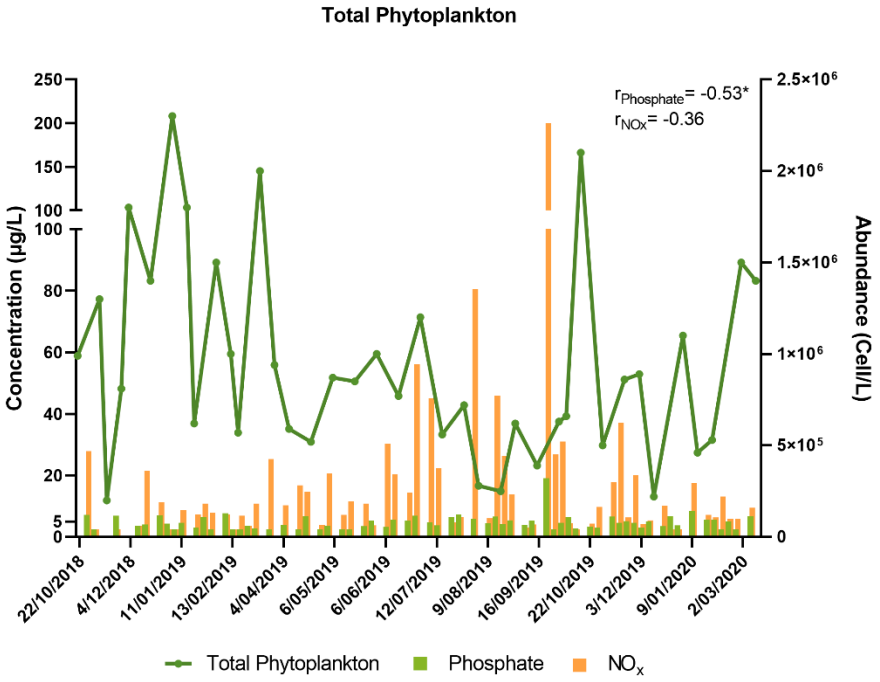
C.


Cow
$r_{Phosphate}$= 0.77*
r_{NOx}= 0.86*
Concentration (µg/L)
Average Gene Copies / 100ml
250
200
150
100
100
80
60
40
20
5
0
6×10^5
5×10^5
4×10^5
4×10^2
3×10^2
2×10^2
1×10^2
0
24/10/2018
6/12/2018
21/01/2019
1/03/2019
18/04/2019
31/05/2019
19/07/2019
5/09/2019
8/10/2019
28/11/2019
10/01/2020
18/02/2020
Cow
Phosphate
NO_x

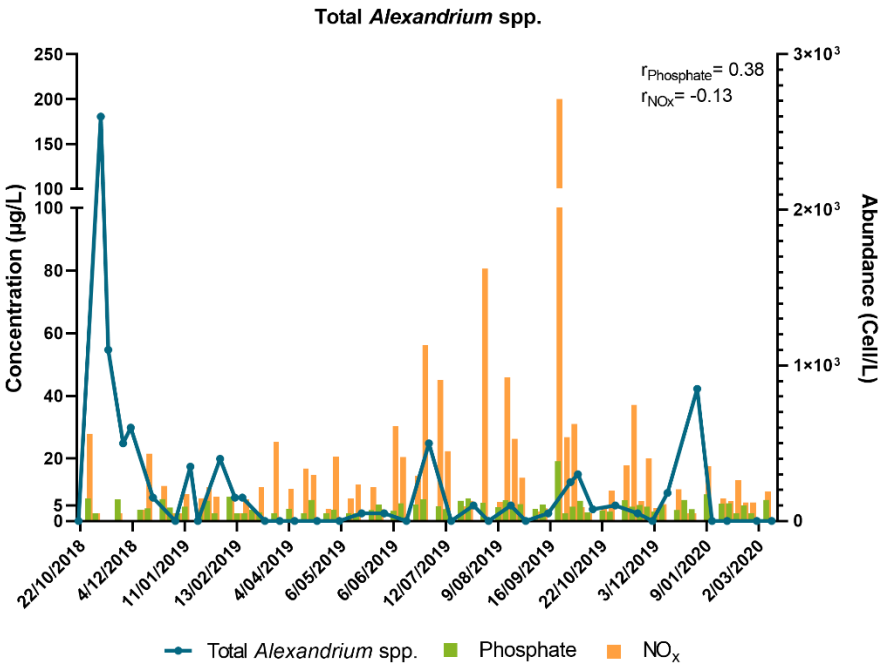
D.


Human
$r_{Phosphate}$= 0.77*
r_{NOx}= 0.86*
Concentration (µg/L)
Average Gene Copies / 100ml
250
200
150
100
100
80
60
40
20
5
0
5×10^5
4.5×10^5
4×10^5
1.5×10^3
1×10^3
5×10^2
0
24/10/2018
6/12/2018
21/01/2019
1/03/2019
18/04/2019
31/05/2019
19/07/2019
5/09/2019
8/10/2019
28/11/2019
10/01/2020
18/02/2020
Human
Phosphate
NO_x

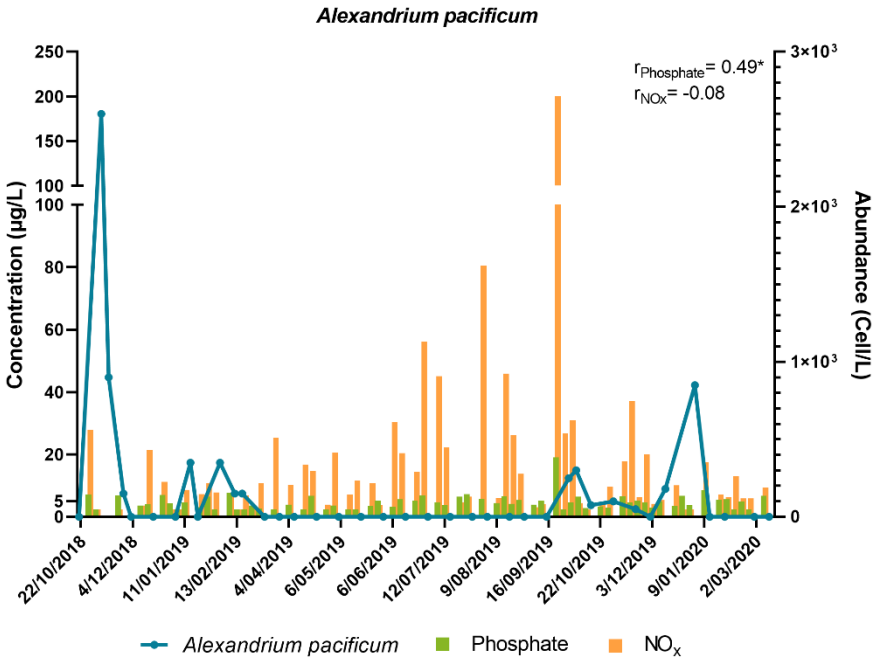
E.


Total Phytoplankton
$r_{Phosphate}$= -0.53*
r_{NOx}= -0.36
Concentration (µg/L)
Abundance (Cell/L)
250
200
150
100
100
80
60
40
20
5
0
2.5×10⁶
2×10⁶
1.5×10⁶
1×10⁶
5×10⁵
0
22/10/2018
4/12/2018
11/01/2019
13/02/2019
4/04/2019
6/05/2019
6/06/2019
12/07/2019
9/08/2019
16/09/2019
22/10/2019
3/12/2019
9/01/2020
2/03/2020
Total Phytoplankton
Phosphate
NO_x

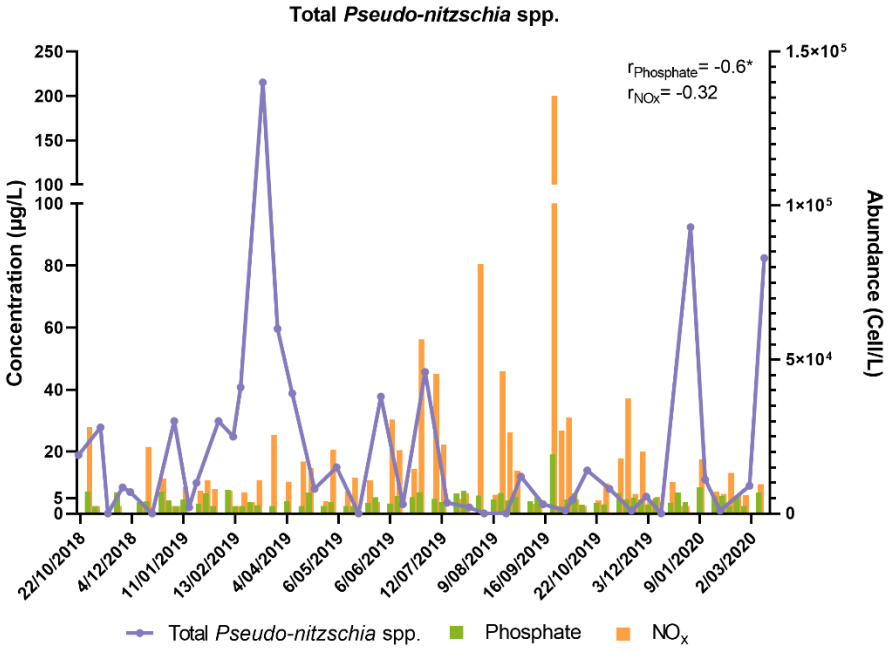
F.


Total *Alexandrium* spp.
$r_{Phosphate}$= 0.38
r_{NOx}= -0.13
Concentration (µg/L)
Abundance (Cell/L)
250
200
150
100
100
80
60
40
20
5
0
3×10³
2×10³
1×10³
0
22/10/2018
4/12/2018
11/01/2019
13/02/2019
4/04/2019
6/05/2019
6/06/2019
12/07/2019
9/08/2019
16/09/2019
22/10/2019
3/12/2019
9/01/2020
2/03/2020
Total *Alexandrium* spp.
Phosphate
NO_x

G.


Alexandrium pacificum
r_Phosphate= 0.49*
r_NOx= -0.08
Concentration (µg/L)
Abundance (Cell/L)
250
200
150
100
100
80
60
40
20
5
0
3×10^3
2×10^3
1×10^3
0
22/10/2018
4/12/2018
11/01/2019
13/02/2019
4/04/2019
6/05/2019
6/06/2019
12/07/2019
9/08/2019
16/09/2019
22/10/2019
3/12/2019
9/01/2020
2/03/2020
Alexandrium pacificum
Phosphate
NOx

H.


Total Pseudo-nitzschia spp.
r_Phosphate= -0.6*
r_NOx= -0.32
Concentration (µg/L)
Abundance (Cell/L)
250
200
150
100
100
80
60
40
20
5
0
1.5×10^5
1×10^5
5×10^4
0
22/10/2018
4/12/2018
11/01/2019
13/02/2019
4/04/2019
6/05/2019
6/06/2019
12/07/2019
9/08/2019
16/09/2019
22/10/2019
3/12/2019
9/01/2020
2/03/2020
Total Pseudo-nitzschia spp.
Phosphate
NOx

I.

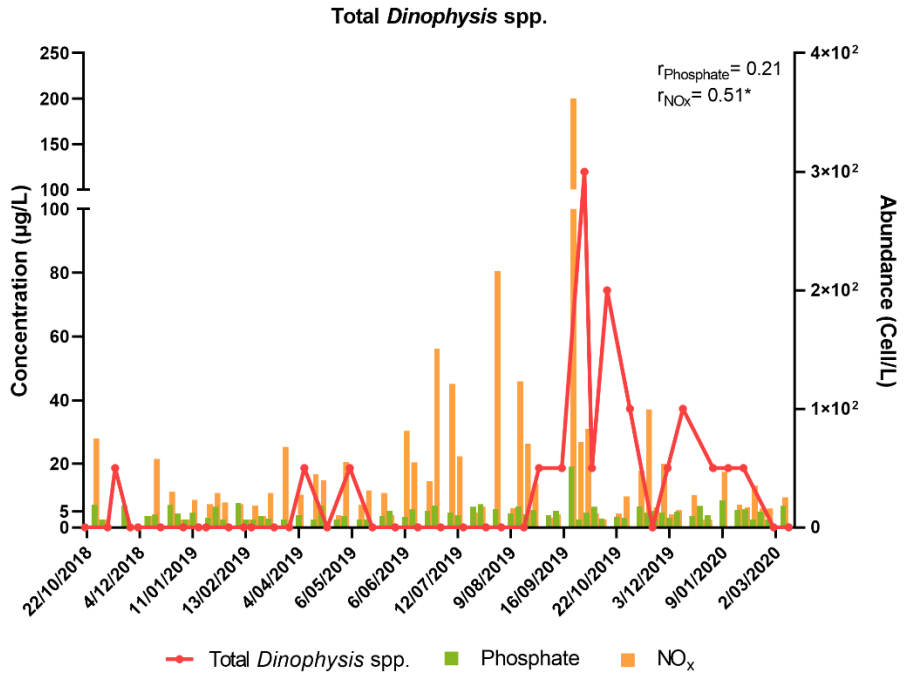


Figure 5.6 A-D Abundance of *E. coli*, bird, cow, and human bacteria; and **Figure 5.6 E-I** Total phytoplankton, total *Alexandrium* spp., *A. pacificum*, total *Pseudo-nitzschia* spp., and total *Dinophysis* spp., each with nutrient concentrations (phosphate and NO_x) over the sampling period (2018-2020) in the Georges River.

5.6 Oyster Growth and Mortality

5.6.1 Oyster Growth

Average oyster whole weight increased by 45.9 g from deployment in August 2018 to June 2020 (Fig. 5.7 A). Oyster whole weight was 68.5 ± 6.3 g at the end of the experiment (June 2020). Oysters deployed in Georges River attained a large size grade where average shell length was > 70 mm in July 2019 and exceeded 50 g whole weight in approximately October 2019. The age of oysters at each of these milestones was 31 mo and 34 mo, respectively.

Oyster shell length was 57 ± 2 mm at the start of the experiment and increased to 77 ± 3 mm in June 2020 (Fig. 5.7 B). The greatest increase in shell length in Georges River was recorded from February to August 2019. The increase in size through this period was 24 mm. Shell lengths were measured more frequently than whole weight and fluctuated throughout the experiment. Periods of shell length decreases were recorded between October and December 2018, January and February 2019 as well as August and November 2019.

5.6.2 Mortality

From August 2018 to February 2020, cumulative oyster mortality was 16% in Georges River. Low levels of mortality were recorded throughout the experiment (Fig 5.7 C-D). The month that had the highest level of mortality recorded was December 2019, however, mortality on this date was less than 5%. Oyster mortality over the study period in Georges River was less

than the background Sydney Rock Oyster farming mortality level which is estimated to be approximately 10% per annum. Oysters from this site remain frozen for future analyses.

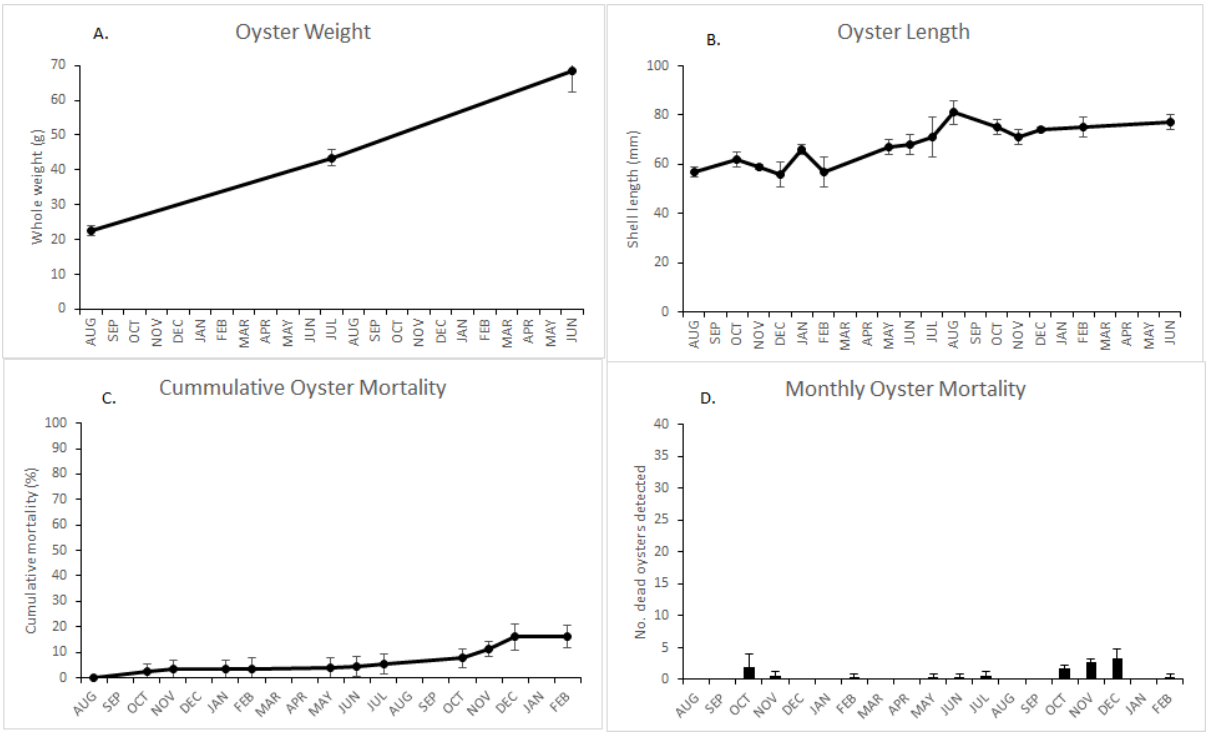


Figure 5.7 A-D. Oysters deployed at the sensor site, Georges River. A. whole weight; B. shell height; C. cumulative mortality, and D. monthly mortality.

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 *E. coli* and bird bacteria (r = 0.85). 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: 52% for *E. coli* (rainfall + total phytoplankton), 94% for cow (rainfall + total phytoplankton), 53% for bird (sensor + total phytoplankton) and 92% for human (rainfall + total phytoplankton) (Table 1A). The models for cow and human bacteria were run again, this time without nutrient data, which allowed for a longer time series to be examined, and to investigate the contribution that nutrients had on the initial model results (Table 1B).

The abundance of *E. coli* at the sensor site was best explained by the rainfall data compared to the sensor data (52% deviance explained as compared to 33%) and was strongly linked to rainfall over the past 72 hours with a concomitant increase in NO_x concentrations (Table 1A, Figures 5.8 A-D, 5.9 A-D).

Cow bacterial abundance was better predicted using rainfall data compared to sensor data (94% compared to 37% with sensor data), with rainfall over the past 24 hours being an important predictor variable along with nutrients (phosphate, NO_x and nitrite) (Table 1A, Figures 5.8 A-D, 5.9 A-D). When the model was run again without nutrients, the rainfall model reduced marginally from 94% deviance explained to 92%, while the sensor model significantly increased from 37% (with nuts) to 76% (without) (Table 1B).

Faecal contamination from birds at the sensor site was best explained by the salinity model (53% deviance explained, compared to 11% using rainfall data), with a peak salinity around 31 ppt, a temperature of ~21℃, and nutrient peaks of phosphate ~4 µg L^{-1} and NO_x ~40 µg L^{-1} (Table 1A, Figures 5.8 A-D, 5.9 A-D).

An increase in human bacteria abundance was best explained by the rainfall data (92% compared to sensor data 59%), and was strongly linked to rainfall over the past 24 hours and nutrient load (Table 1A, Figures 5.8 A-D, 5.9 A-D). When the model was run again without nutrients, the rainfall model reduced from 92% deviance explained to 84%, while the sensor model reduced marginally from 59% to 57% (Table 1B).

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 48% of the deviance, with the daily maximum salinity (maximum growth at <34 ppt and >36 ppt), and a decreasing rainfall over the past week being the best predictive variables of oyster growth.

Table 1A. Modelling results (including nutrient data) for bacterial source tracking at the sensor site in the Georges River. Only significant variables are shown for each model.

Bacteria	Variables	No. of observations	Significant Variables	Deviance Explained
E. coli	Salinity, Depth, Temp, Phosphate, NO_x, Nitrite	47	Depth72**, Salinity72***, Temp72***, Phosphate***, NO_x***	29.6%
E. coli	Salinity, Depth, Temp, Phosphate, NO_x, Nitrite logPhytoplankton	47	logPhytoplankton ***, depth**, salinity***, temp***, Phosphate***, NO_x***, Nitrite***	33%
E. coli	Rainfall72, Phosphate, NO_x, Nitrite	54	Rainfall72***, Phosphate***, NO_x***, Nitrite***	51.7%
E. coli	Rainfall72, Phosphate, NO_x, Nitrite logPhytoplankton	54	Rainfall72***, Phosphate*, NO_x***, Nitrite***	51.9%
Bird	Salinity, Depth, Temp, Phosphate, NO_x, Nitrite	47	Salinity***, Depth***, Temp***, Phosphate***, NO_x***, Nitrite***	51.5%
Bird	Salinity, Depth, Temp, Phosphate, NO_x, Nitrite logPhytoplankton	47	Salinity***, Depth***, Temp***, Phosphate***, NO_x***, Nitrite***, logPhytoplankton ***	52.5%
Bird	Rainfall72, Phosphate, NO_x, Nitrite	54	Rainfall72***, Phosphate***, NO_x***, Nitrite***	10.1%
Bird	Rainfall72, Phosphate, NO_x, Nitrite logPhytoplankton	54	Rainfall72***, Phosphate***, NO_x***, Nitrite***, logPhytoplankton***	11%
Cow	Salinity, Depth, Temp, Phosphate, NO_x, Nitrite	47	Salinity***, Depth***, Temp***, NO_x***, Nitrite***	36.7 %
Cow	Salinity, Depth, Temp, Phosphate, NO_x, Nitrite logPhytoplankton	47	Salinity***, Depth***, Temp***, Phosphate***, NO_x***, Nitrite***, logPhytoplankton***	37.0%
Cow	Rainfall24, Phosphate, NO_x, Nitrite	54	Rainfall24***, Phosphate***, NO_x***, Nitrite***	93.8%
Cow	Rainfall24, Phosphate, NO_x, Nitrite logPhytoplankton	54	Rainfall24***, Phosphate***, NO_x***, Nitrite***, logPhytoplankton***	94.2%
Human	Salinity, Depth, Temp, Phosphate, NO_x, Nitrite	49	Salinity***, Depth***, Temp***, Phosphate***, NO_x***, Nitrite***	58.6%
Human	Salinity, Depth, Temp, Phosphate, NO_x, Nitrite logPhytoplankton	49	Salinity***, Depth***, Temp***, logPhytoplankton***	58.7%

Human	Rainfall24 Phosphate, NO_x, Nitrite	54	Rainfall24***, Phosphate***, NO_x***, Nitrite***	91.5%
Human	Rainfall24, Phosphate, NO_x, Nitrite logPhytoplankton	54	Rainfall24***, Phosphate***, NO_x***, Nitrite**, logPhytoplankton***	91.5%

Table 1B. Modelling results (without nutrient data) for bacterial source tracking at the sensor site in the Georges River. Only significant variables are shown for each model.

Bacteria	**Variables**	**No. of observations**	**Significant Variables**	**Deviance Explained**
Cow	Rainfall24, logPhytoplankton	78	Rainfall24***, logPhytoplankton***	92.4%
Cow	Salinity, Depth, Temp, logPhytoplankton	86	logPhytoplankton ***, depth**, salinity***, temp***	76.4%
Human	Rainfall24, logPhytoplankton	78	Rainfall24***, logPhytoplankton***	84.3%
Human	Salinity, Depth, Temp, logPhytoplankton	92	logPhytoplankton ***, depth**, salinity***, temp***	57.4%

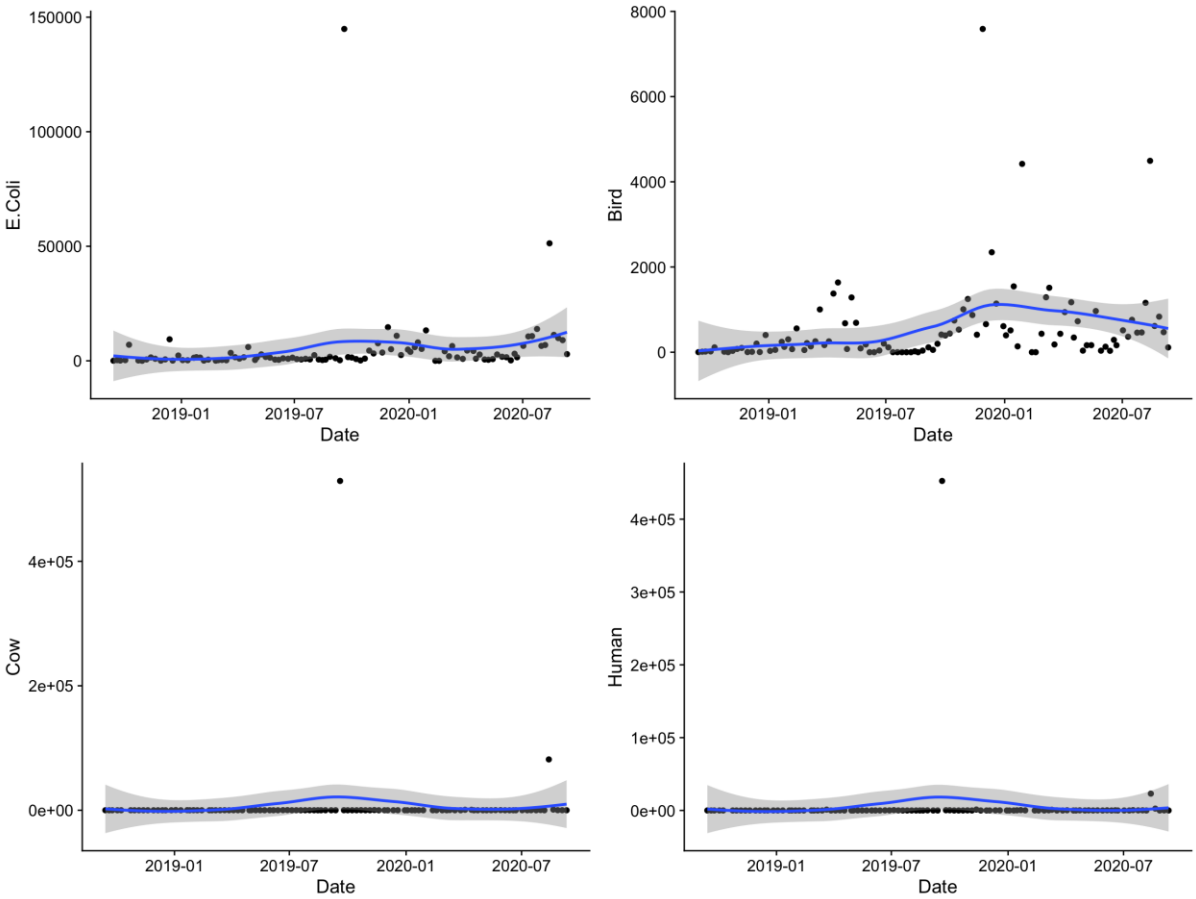


Figure 5.8 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. *E. coli*, B. Bird, C. Cow, and D. Human bacterial load as measured by weekly sampling at the sensor site, Georges River.

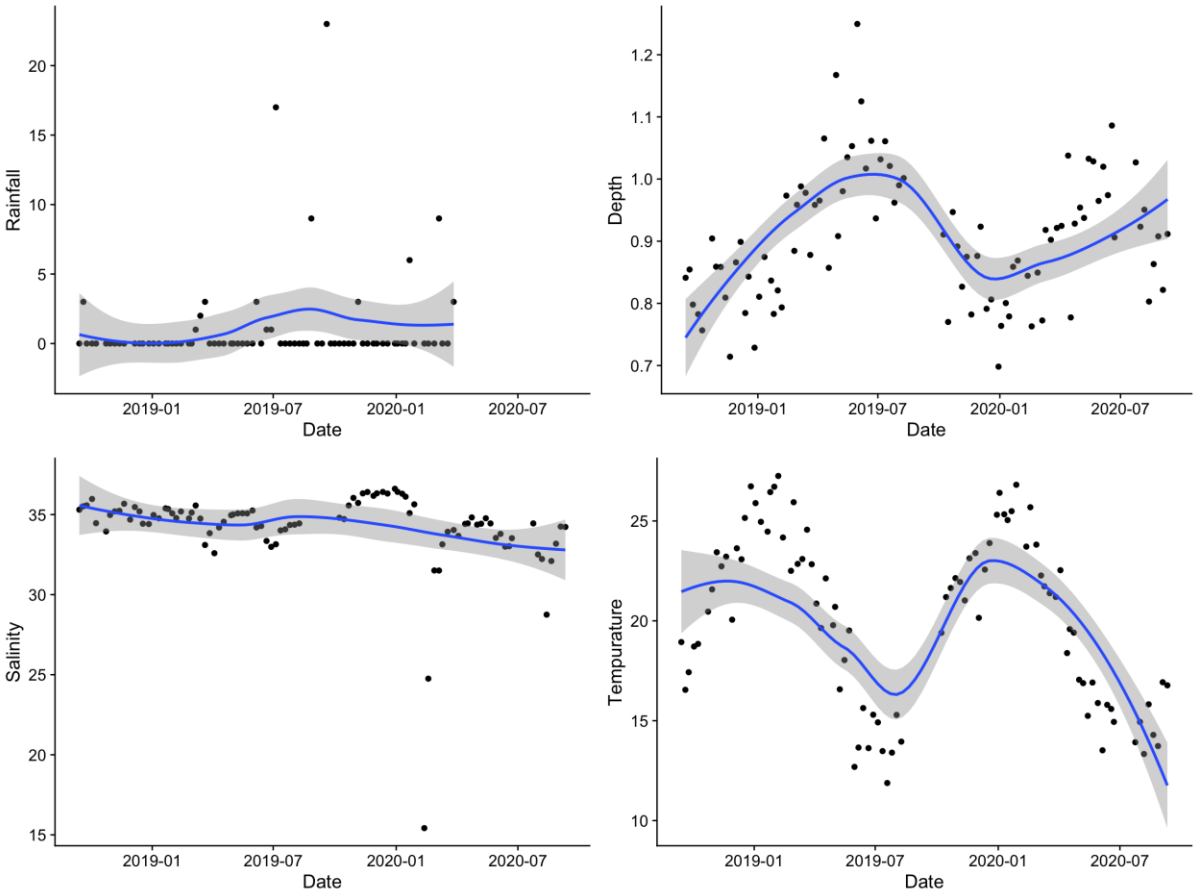


Figure 5.9 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. Rainfall, B. Depth, C. Salinity, and D. Temperature values measured in at the sensor site, Georges River.

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 Quibray Bay harvest area. Based on the available data, up to ten harvest area closures could have potentially been avoided between 12 October 2017 and 31 March 2022. During the initial implementation of such a management plan change, rainfall events would continue to be monitored to increase the database to support the change. Georges River Shellfish Program (GRSP) were consulted about the option of a salinity-only management plan for Quibray Bay harvest area following the 2021 annual review, but a decision has not yet been reached. If GRSP 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 Quibray Bay harvest area management plan would revert to the current management plan that is based on both rainfall and salinity closure limits.

6.2 Phytoplankton and HABs

The most common HAB species that bloomed in the Georges River during this study was the toxic 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 2010. This toxic event led to a worldwide product recall and it was estimated that this toxic event cost the Australian industry AUD ~$23 M in lost revenue (Campbell et al. 2013).

Another HAB group which was observed in the Georges River samples belonged to 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 HAB group to watch is *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).

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 Georges River

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

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

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

While salinity was a more reliable predictor than rainfall in only one out of four of the faecal indicators tested. Elevated *E. coli*, cow and human bacteria concentrations were detected on two occasions (~Aug 19 and July 20). These high concentrations were linked to increasing rainfall and nutrients. The position of the sensor relatively close to Botany Bay, and its oceanic influence, is reflected in the overall high salinity profile and apparent rapid recovery after rainfall events. Under an operational salinity only management plan, adverse sampling continues to provide protection and gather data to better understand the effect of rainfall.

Georges River Council is undertaking large scale landscape and stormwater treatment projects to prevent litter, sediments, nutrients (phosphorus and nitrogen) and oil entering the waterways. This work includes removing concrete stormwater infrastructure, and constructing/reinstating natural waterway features including swales, wetlands, ponds and bioretention systems. Gross pollutant traps, foreshore remediation and naturalisation works as well as redirecting stormwater through swales and detention points before it reaches the waterway, are other activities currently being undertaken to reduce aquatic pollution in this river system (DPE 2022).

Avian faecal pollution in Georges River was linked to rainfall, but was observed to peak during the summer months. This peak coincided with the Australian forest mega-fires of 2019/2020 (Boer et al. 2020), whereby coastal areas may have been a relatively safer refuge during that extreme period. The molecular marker used in this study, however, does not discriminate between avian species (gulls, geese, chickens, ducks etc), so it is uncertain what percentage of the bacterial load is attributable to terrestrial birds and that of aquatic birds. Further discrimination into the breakdown of the faecal load would be required for this elucidation.

The 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 Georges 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 Georges River were greatest in the second half of the experiment from July 2019 to June 2020. However, growth, in terms of shell length, was greatest in the 5-month period leading up to July 2019. The salinity level during the period of

maximum shell growth was very stable and remained above 32 ppt. Higher salinities increase seawater alkalinity providing more calcium carbonate available for oyster shell deposition. The salinity level that promotes the greatest growth rates in Sydney Rock Oyster spat is 30 ppt for small spat (1.3 mg) and 35 ppt for larger spat (0.61 g) (Nell and Holliday, 1988). The period of maximum whole weight increase occurred over the last 11 months of the experiment which was also characterised by stable salinity levels above 32 ppt other than in February 2020 where salinity dropped rapidly to approximately 10 ppt and then quickly recovered to levels above 30 ppt (Fig. 5.1B). This was following an intense rain event where approximately 330 mm was recorded at the Airport Bureau of Meteorology station between the 7th and 10th of February 2020.

Survival of oysters during the experiment was high from deployment until February 2020. Mortality during this period was below the background farming mortality (approximately 10% per annum) commonly experienced when farming Sydney Rock Oysters. Oyster mortality measured on each sampling occasion did not exceed 5%. Cumulative mortality in February 2020 was 16% and comparable to cumulative mortality measured on the same date in Wallis Lake (14%), Manning River (15%), Port Stephens (16%), Pambula River (16%) and Wapengo Lake (15%).

The Georges River sensor site is situated in Quibray Bay. Quibray Bay is a location known to experience recurrent outbreaks of winter mortality disease and has been the primary field exposure site to develop winter mortality resistance in Sydney Rock Oysters since 1997 (Nell et al. 2000; Dove et al. 2013). Winter mortality infections generally commence in autumn and most mortality occurs in spring (Nell, 2006). The Quibray Bay site used in this project experienced increased levels of mortality during October, November and December of 2019 with the highest mortalities occurring in December 2019. However, the oyster mortality level was only 7% during this three-month period. Winter mortality disease can cause over 70% mortality in Sydney Rock Oysters in severe outbreaks (Lauckner, 1983).

Oyster families from the Sydney Rock Oyster breeding program were deployed at this site from April 2018 to December 2018 (2016-year class) and from April 2019 to December 2019 (2017-year class). The average mortality over the duration of the experiment in the 2016- and 2017-year class families was 10% and 8%, respectively. These data indicate that winter mortality disease at the sensor in Quibray Bay was not occurring at all or only occurring at very low levels during the monitoring conducted for this project.

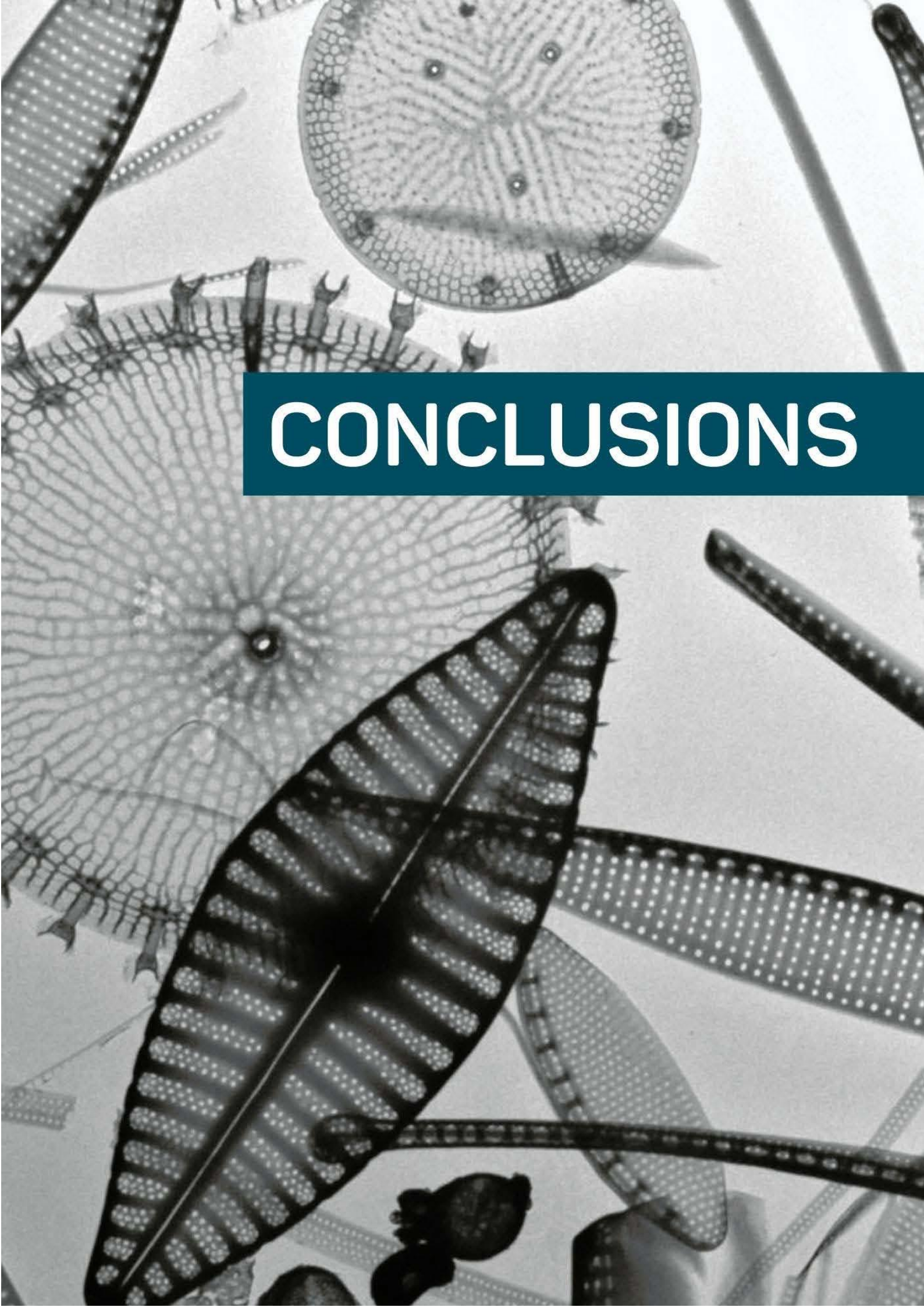
The batch of oysters used for this experiment were a random mix of families taken from the 2016-year class of the Sydney Rock Oyster Breeding program. This particular year class had 86% of the parents selected from wild and QX disease resistant genetic groups. Only 14% of the parents for this year class were sourced from the fast growth genetic group. It took this year class approximately 2 years and 10 months to reach the large oyster size grade (> 70 mm total length or > 50 g whole weight). This site had the heaviest oysters at the end of the experiment in June 2020 (68.5 g average whole weight). Hawkesbury River and Wagonga Inlet were the only other two sites that had comparable growth in terms of whole weight with oysters attaining 63.2 g in the Hawkesbury River and 61.9 g in Wagonga Inlet.

Both Sydney Rock Oysters and Pacific Oysters are cultured in Georges River and Quibray Bay is an important area for oyster culture in Georges River. This location is the only site from which oysters can be harvested and then depurated prior to sale for human consumption. For Sydney Rock Oyster culture, all other oyster growing areas in Georges River are impacted by seasonal QX disease outbreaks and Quibray Bay has had no recorded outbreaks of QX disease. However, winter mortality disease is a known threat to Sydney Rock Oyster in Quibray Bay. The spatial and temporal characteristics of winter mortality disease outbreaks at this location, and in many other estuaries from Port Stephens to Wonboyn Lake, are mysterious and unpredictable. Farming triploid Pacific Oysters in Georges River is one way that growers can mitigate against Sydney Rock Oyster losses in Georges River from QX disease and winter mortality. However, *Ostreid herpesvirus* is a known disease threat for Pacific Oysters cultured in all areas of Georges River. Strategies to manage the oyster disease threats in Georges River are securing disease resistant oysters and using window farming techniques in areas where disease is known to occur.

Winter mortality of Sydney Rock Oysters has low to moderate heritability and would respond to genetic selection. Despite considerable effort, the Sydney Rock Oyster breeding program has not been able to incorporate winter mortality resistance for Sydney Rock Oysters due to low levels of winter mortality at field test sites which dampens response to selection. Given that winter mortality is a significant issue for sections of the Sydney Rock Oyster industry, field trials continue in Quibray Bay each year to gain a better understanding of this trait for inclusion into the breeding goals.

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 Quibray Bay harvest area, subject to agreement by the local shellfish industry. Available data indicated that ten harvest area closures could have potentially been avoided between October 2017 and March 2022. As of August 2022, seventeen salinity-only management plans had been offered for harvest areas in participating NSW estuaries, with six being taken up and the remaining eleven under consideration.

Quibray Bay is an important location for oyster culture in Georges River as it is the only location in this estuary where oysters can be harvested and then depurated prior to sale for human consumption. Compared to the other monitoring sites in NSW, oyster growth in Georges River ranked first overall in terms of whole oyster weight and 3rd overall in terms of shell length. Low levels of mortality were recorded over the period from August 2018 to February 2020 and mortality was below the level accepted as background farming mortality (approximately 10% per annum). Although Quibray Bay is often impacted by outbreaks of winter mortality disease, no significant oyster mortality events occurred to indicate that this disease was active during the study.

The pollution source tracking results were highly variable across the study period, most likely attributable to the extreme variation in environmental conditions experienced (drought, bush fires, floods). Real time sensor data showed a higher predictive capability than rainfall for one (bird) out of the four faecal indicator bacteria. Elevated levels of *E. coli*, cow and human bacterial corresponded to high rainfall and subsequent nutrient inputs. 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 Georges River.

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9. Appendices

A1. Methods

A1.1 Sampling locations in the Georges River

Data used in this report originates from locations within the Georges River over the period 12 Oct 2017 to 31 March 2021. High-resolution temperature, salinity and depth data were obtained from a sensor located in Quibray Bay harvest area, located within the Georges 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).

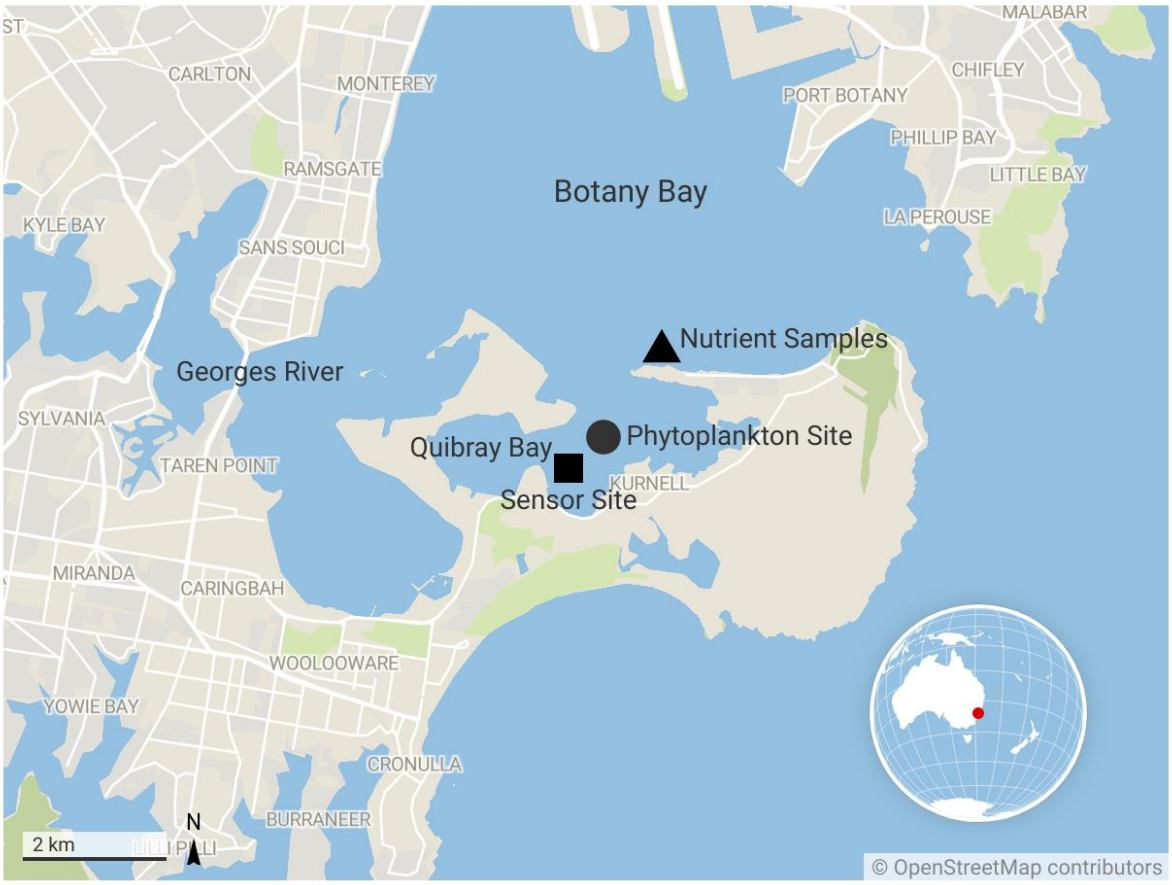

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Figure A1: Map of the Georges River highlighting the sensor located in Quibray Bay (black square), the phytoplankton sampling location (black circle), and location of nutrient sampling (black triangle).

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 BOM rainfall station at San Souci Public School (BOM 66058 ~-33.99°S, 151.13°E) from Oct 2017 to March 2021.

Figure A2 Seabird MicroCAT high accuracy conductivity, temperature and depth (CTD) field sensor deployed in the Georges River.

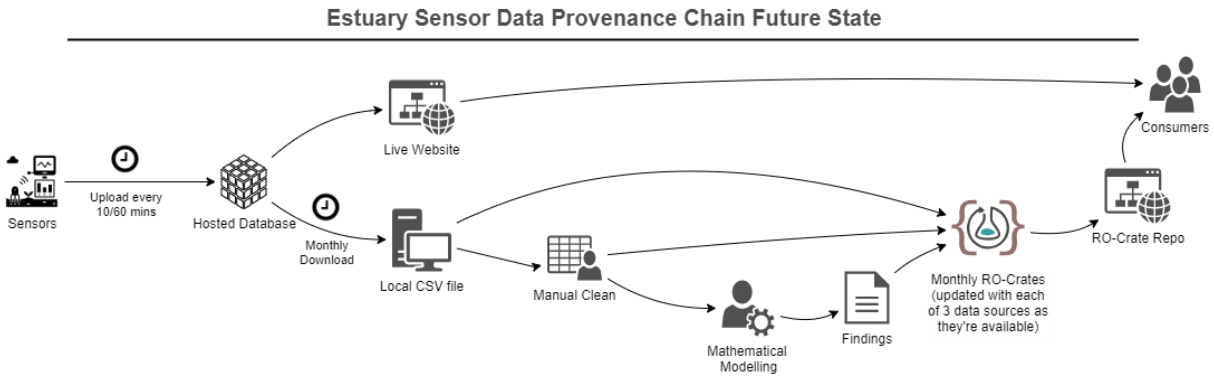

Figure A3. Georges River data provenance chain from source of data (sensor), via quality assurance processes, data analyses, to consumers.

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 Georges River annual review is 1 April. As part of the most recent (2022) annual review for Quibray 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 technical issues with the sensor, there were gaps in data collection between 9 August and 8 October 2019 and 28 June and 23 July 2020. The original sensor ceased reporting 1 April 2021 and data collection resumed with a new sensor 15 April 2021. There were gaps in salinity on 5 October 2021, 8-11 December 2021 and 3 January 2022 due to instrument error, most likely the sensor coming out of the water at lower tides.

A1.4 Biological sampling, eDNA extraction and nutrient analyses

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

In the case of a rainfall event, water samples were collected for bacterial analysis (only) every 24 h over a two-day period commencing on the first day of rainfall and processed as described above. Daily rainfall measurements were taken from the closest available weather station at Bureau of Meteorology site number 66204 (Oyster Bay, Green Point Rd, ~-34.02°S, 151.07°E) from Oct 2017 to March 2021.

Triplicate water samples were also collected for nutrient analyses approximately weekly (n= 65) between September 2018 and March 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.

Orthophosphate [PO_4^{3-}], NO_x (nitrate [NO_3^-] + nitrite [NO_2^-]) and nitrite concentrations were measured colourimetrically using Lachat QickChem 8500 Series 2 flow injection analysis (FIA) and Omnion 4.0 (Lachat Instruments, Loveland CO, USA). Soluble or dissolved reactive phosphorus (SRP) consists mostly of inorganic orthophosphate, which is the form of phosphorus that is directly available for algae (Carlson and Simpson, 1996; Koenig et al., 2014). The concentration of SPR (µg P L^{-1}) was determined using the phosphomolybdenum blue method; reagents were prepared as per QuikChem Method 31-115-01-1-G (Diamond 2003). The reagents used to measure NO_x concentration (µg N/L) were prepared based on QuikChem Method 31-107-05-1-A (Schroeder 2003). The limit of detection (LOD) was 5 µg L^{-1} for all nutrients. Throughout the sample analysis, regular quality control methods were applied, such as calibration standards at the beginning of each analysis furthermore duplicates, field blanks and spike recoveries after every ~20 samples. The efficiency of the cadmium column, installed in the

NO_x line, was checked at least 3 times per analysis. All glassware used during the preparation of reagents was acid-washed using 10 %v/v hydrochloric acid (HCl) solution and rinsed at least 3 times with Milli-Q water to prevent any contamination. Light-sensitive reagents were stored in dark glass containers.

The results were monitored in Omnion 4.0 and peak captures were adjusted when necessary. The data were further processed in Microsoft Excel (2019). In order to mitigate bias towards lower values, concentrations below 4 µg L^{-1} were substituted with 2.5 µg L^{-1}, 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.

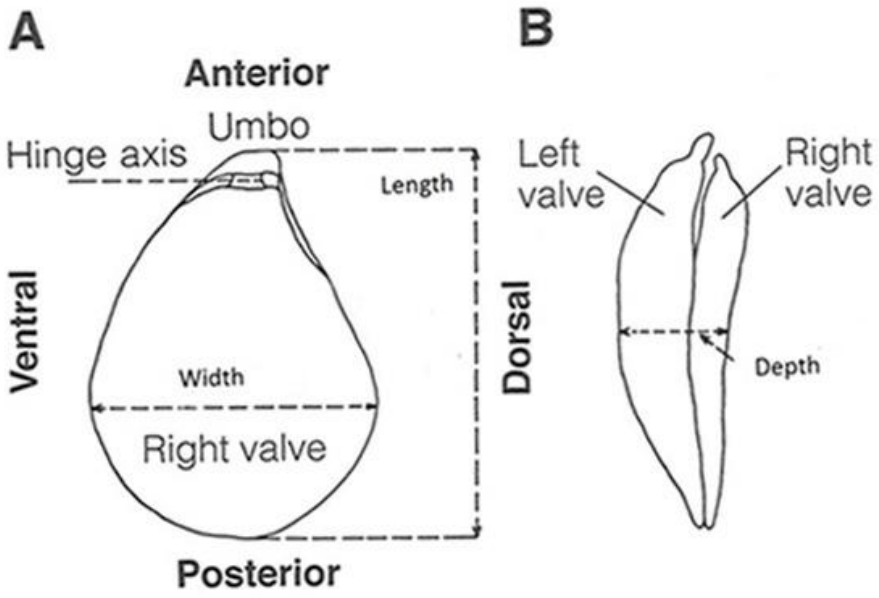

Figure A4. Oyster shell dimensions (Carriker 1996)

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 closest Bureau of Meteorology site (No. 66204, Oyster Bay, Green Point Rd, ~-34.02°S, 151.07°E) from Oct 2017 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, rainfall and nutrients) at the sensor location within Georges 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, total phytoplankton (logged or unlogged) and nutrients. For comparison to current (non-sensor-based) practice, models were also run using only rainfall data. Again, these included depth, week, total phytoplankton and nutrients. 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. Finally, cow and human bacteria models were run again this time without nutrient data, to observed the impact these additional variables had on bacterial abundance.

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. Models were rerun for cow and human bacterial abundance without nutrients - this extended the dataset and revealed the difference with/without nutrient data included.

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

Variable	Mean	Standard Error	Median	Standard Deviation	Minimum	Maximum	Count	Missing
average_cfu	43.17	14.98	9.96	152.07	0.00	1454.73	103	0
average_nitrite	2.81	0.11	2.50	1.14	2.50	8.76	103	49
average_nox	17.75	2.90	9.10	29.48	2.50	200.15	103	49
average_phosphate	4.73	0.25	4.37	2.57	2.50	19.13	103	49
bird	543.28	99.46	203.54	1009.36	0.00	7587.97	103	0
cow	5989.56	5190.16	0.00	52674.41	0.00	529176.18	103	0
depth24	0.90	0.01	0.90	0.11	0.70	1.25	103	11
depth48	0.90	0.01	0.88	0.09	0.73	1.19	103	14
depth72	0.90	0.01	0.88	0.09	0.75	1.14	103	17
ecoli	4743.94	1491.42	1474.62	15136.25	0.00	144865.41	103	0
human	4727.08	4395.87	0.00	44613.23	0.00	452515.12	103	0
logPhytoplankton	13.41	0.07	13.40	0.66	12.21	14.65	103	0
Phytoplankton	821650.49	51370.72	660000.00	521355.88	200000.00	2300000.00	103	0
rainfall24	1.08	0.35	0.00	3.56	0.00	23.00	103	25
rainfall48	1.09	0.24	0.00	2.46	0.00	11.50	103	27
rainfall72	1.09	0.19	0.00	1.96	0.00	7.67	103	29
salinity24	34.23	0.25	34.70	2.57	15.42	36.61	103	11
salinity48	34.22	0.22	34.69	2.26	20.09	36.52	103	14
salinity72	34.22	0.20	34.64	2.06	23.89	36.45	103	17
temp24	20.10	0.42	20.78	4.24	11.88	27.25	103	11
temp48	20.23	0.41	21.29	4.12	12.64	26.98	103	14
temp72	20.36	0.40	21.55	4.06	12.92	26.80	103	17

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

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

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

Farrell, Swami Palanisami & Wayne O'Connor		
Penelope Ajani, Michaela Larsson, Ana Rubio, Stephen Bush, Steve Brett, Stephen Woodcock, Hazel Farrell & Shauna Murray	Australian Shellfish Quality Assurance Advisory Committee Science Day 2018	Modelling harmful algal blooms in the Hawkesbury River, Australia
Hazel Farrell, Grant Webster, Phil Baker, Anthony Zammit, Penelope Ajani, Shauna Murray & Steve Brett	Australian Shellfish Quality Assurance Advisory Committee Science Day 2018	Developing phytoplankton and biotoxin risk assessments for both shellfish aquaculture and wild harvest shellfish in New South Wales.
Wayne O'Connor	SIMS, July 2017	Oyster Research Overview Presentation

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