TRANSFORMING AUSTRALIAN SHELLFISH PRODUCTION

Gogleys Lagoon Harvest Area, Camden Haven River.

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

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

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Transforming Australian Shellfish Production: Gogleys Lagoon Harvest Area, Camden Haven River. Report on Stage 1, October 2017-March 2021

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Contents

Executive Summary

This report presents results from Camden Haven 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 Gogleys Lagoon harvest area, Camden Haven River, recording high-resolution temperature, salinity and depth data. Oyster farmers collected weekly biological samples (600 environmental DNA samples and 279 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

8

Available data indicated that eight harvest area downgrades could have potentially been avoided between March 2018 and September 2022

100%

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

E. coli was highly variable, while cow and human bacteria were generally low and linked to rainfall

Bird contamination became elevated in summer

x2.5

Cumulative oyster mortality in Camden Haven from August 2018 to February 2020 was 40% which is more than two and a half times greater than background farming mortality (estimated at 10% per annum).

1. Introduction

1.1 Transforming Australian Shellfish Production

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

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

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

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

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

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

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

1.2 Camden Haven River

Camden Haven River (-31.64478° S, 152.8282° E) is an open and trained intermediate wave dominated barrier estuary located in the Mid North Coast region of New South Wales, approximately 380 km north of Sydney (Roy et al. 2001). It has a catchment area of 589 km^2, total estuary area of ~32 km^2 and a flushing rate of ~81 days (Roy et al. 2001, Roper et al. 2011) (Fig. 1). The surrounding catchment is mainly undeveloped (70% covered in native vegetation), with the remaining ~30%, disturbed due to grazing (21%) and urban development (4%). The aquatic system supports many significant areas of seagrass (10 km^2) as well as mangroves (1.4 km^2), saltmarsh (~1 km^2) and wetlands (Roper et al. 2011). Seven major tributaries enter the Camden Haven in the tidal zone (~29km from the mouth) including Queens Lake, Limeburners Creek, Herons Creek, Camden Haven River, Watson Taylor Lake, Washtub Inlet. Stewarts River, Gogleys Lagoon, Mud Bay, Bensons Inlet, Herons Creek.

1.3 Oyster Production in Camden Haven River

Camden Haven River is considered one of the ‘safer’ growing areas in the state, with no incidence of the two major oyster diseases (QX and winter mortality) (Camden Haven River Oyster Farmers Environmental Management System 2011). Camden Haven River production value today is estimated to be ~154K dozens and valued at ~$1.4 Mil (NSW DPI 2023).

FINDINGS

2. Findings

2.1. The data assessment from this report supports implementing a change to the Gogleys Lagoon harvest for management of harvest area downgrades, subject to agreement by the local shellfish industry. Available data indicated that eight harvest area downgrades could have potentially been avoided between March 2018 and September 2022. The existing rainfall closure limits are well placed for Gogleys Lagoon harvest area and these limits would still apply to any request for a management plan change.

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 Camden Haven over the biological sampling period, September 2018 to September 2020.

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

2.4. The maximum predictive capability for each bacterial group were 44% for *E. coli*, 31% for cow, 65% for bird, and 100% for human at the sensor site (this latter model was unstable and should be interpreted with caution).

2.5 Where the models were predictive, they suggested bacteria (*E. coli)* increased with higher salinity and temperature (a possible lack of flushing at the sensor site), or increased with increasing rainfall (cow and human).

2.6. Shell length increased steadily in Camden Haven from August 2018 to March 2019 and November 2019 to January 2020. The greatest growth in terms of oyster whole weight was recorded from August 2018 to August 2019, with oysters doubling in weight over this time (22.6 g to 42.5 g, respectively). Oyster whole weight plateaued between February to June 2020, corresponding with a period of reduced salinity (<25 ppt).

2.7. Cumulative mortality in Camden Haven reached 40% by February 2020. This is above the level of mortality expected for background farming of the Sydney Rock Oyster (approximately 10% per annum). A major proportion of this mortality occurred during December 2018 to March 2019 (27%). Across this period, the average water temperature was high (24°C), with a maximum of 26.7°C, but no reduction in salinity was recorded. Mortality was low (<3%) between all other sampling occasions.

ACKNOWLEDGEMENTS

3. Acknowledgements

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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.

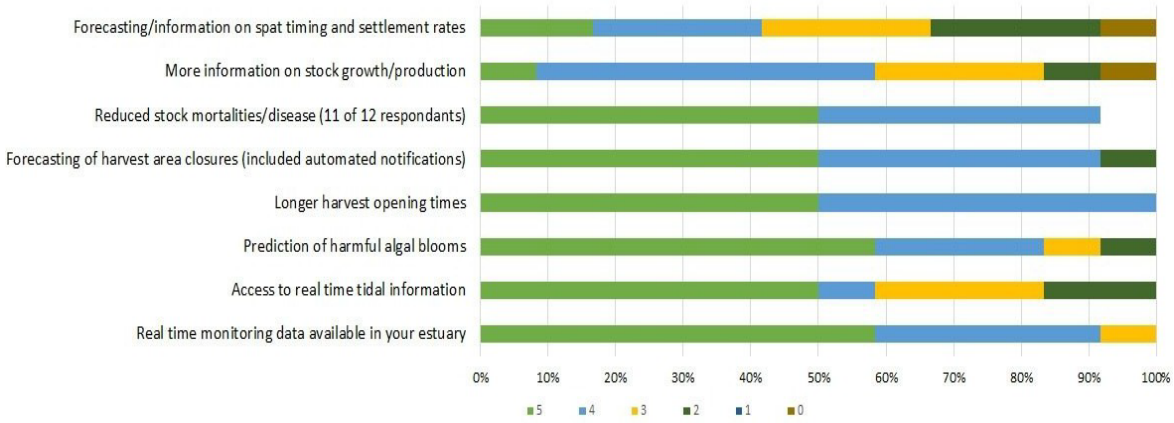


Figure 4.1. The importance of factors as rated by farmers in relation to their business operations. Light green is most important and brown is least important.

RESULTS

5. Results

5.1 High resolution temperature and salinity data

High-resolution real time data summaries for Camden Haven River for the period 13 Mar 2018 to 31 Mar 2021 are shown in Figs. 5.1A-C. Depth recordings ranged from 0 m (21 Nov 2018) to 2.6 m (19 Mar 2021). The lowest and highest daily average salinity recordings were 0.5 ppt (21 Mar 2021) and 37.3 ppt (30 Dec 2019) respectively, while the lowest and highest daily average temperature recordings were 12.8℃ (11 Aug 2019) and 27.97℃ (22 Jan 2020) respectively.

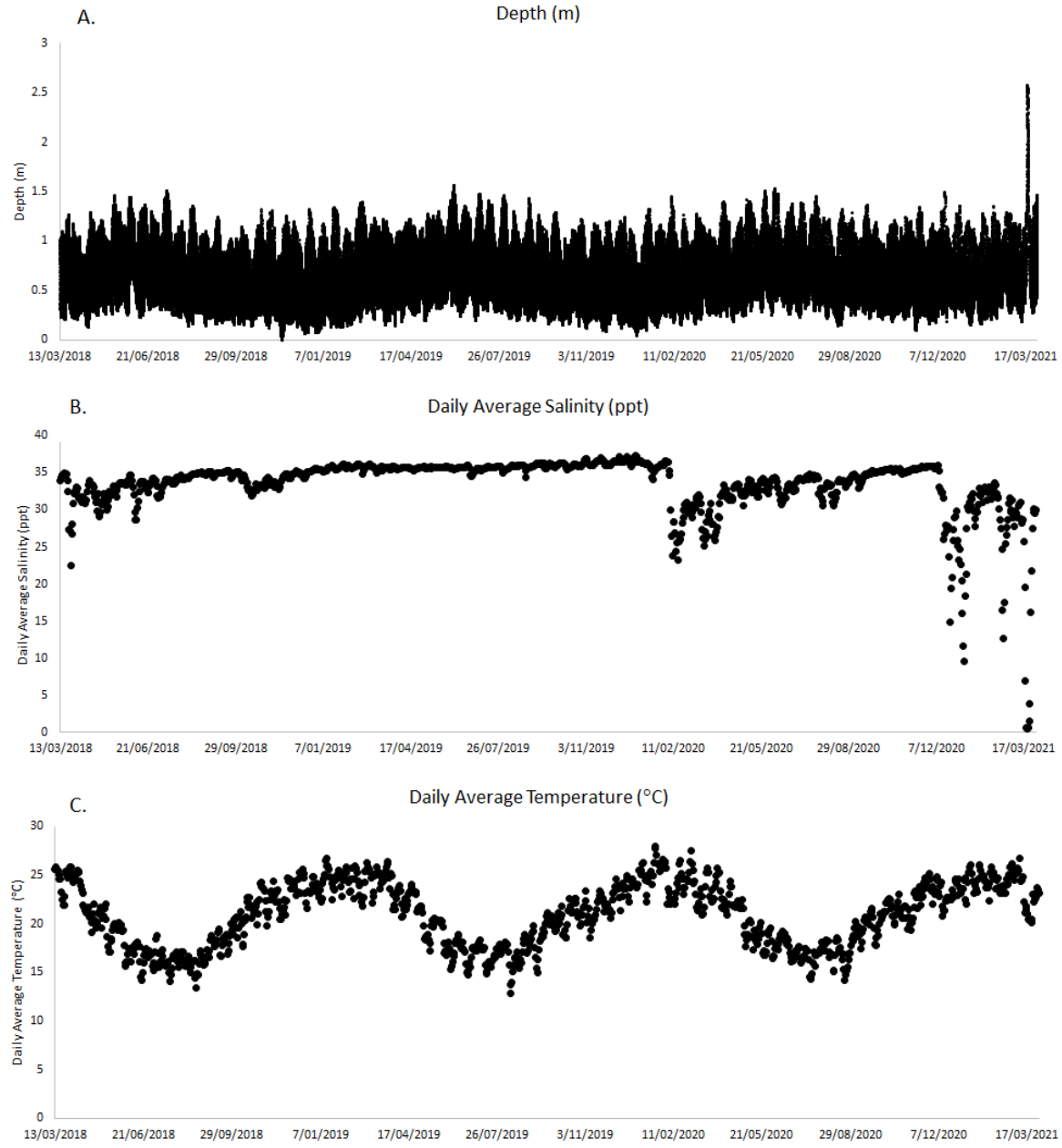


Figure 5.1A-C. Real time sensor data from Camden Haven River sensor 13 Mar 2018 to 31 Mar 2021 A. Depth (m); B. Daily average salinity (ppt); and C. Daily average temperature (°C).

The maximum daily rainfall at the Laurieton PMHC rainfall gauge (560018) occurred on 20 Mar 2021 and was reported as 439.6 mm (Fig. 5.2).

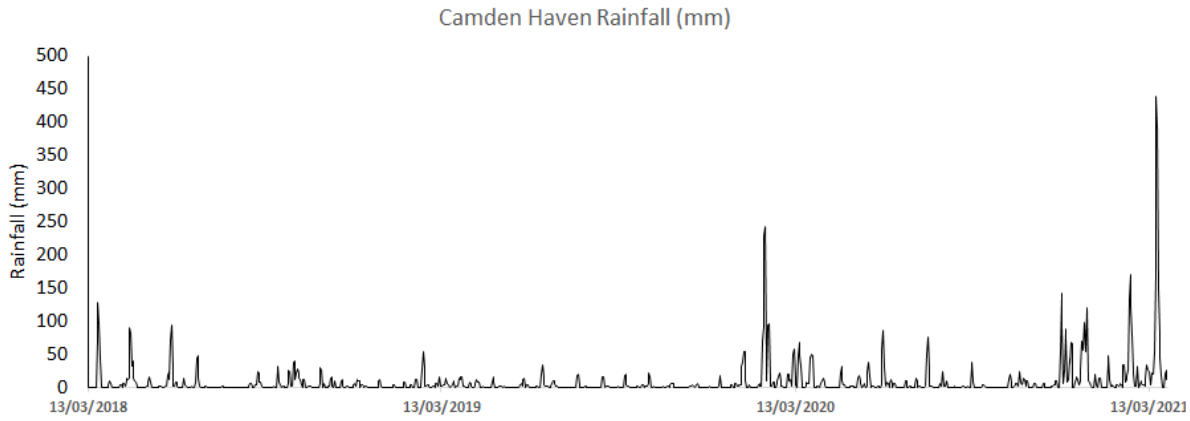


Figure 5.2. Daily rainfall (mm) from the Laurieton PMHC (560018) rainfall gauge (~-31.64° S, 152.79° E) from Mar 2018 to March 2021.

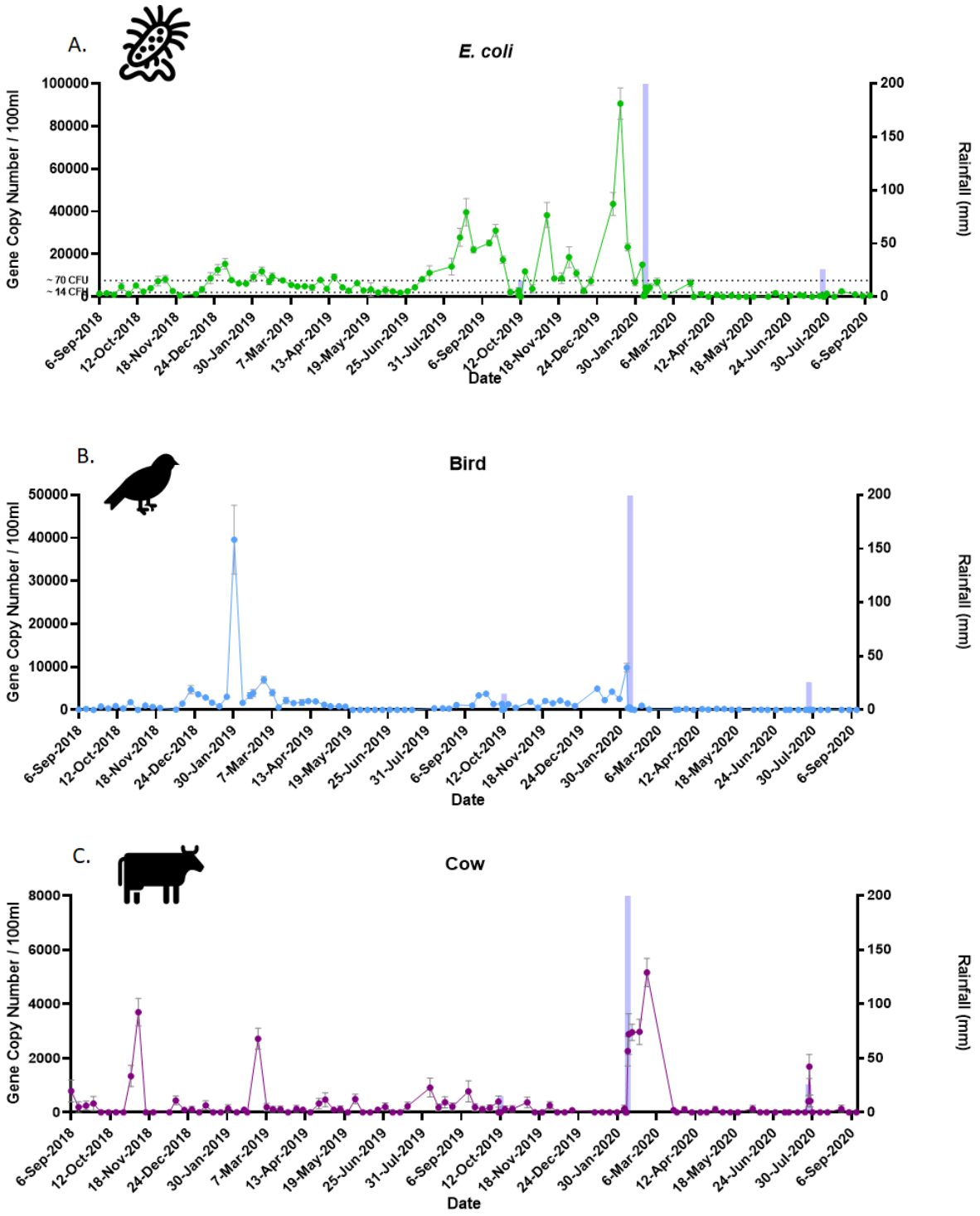
5.2 Management Plan

Data analysed during the 2022 annual review of Gogleys Lagoon harvest area indicated that there could have been fewer harvest area downgrades since the sensor was installed, if downgrades were based on salinity sensor data. Seventeen harvest area rainfall downgrades occurred between 14 March 2018 and 30 September 2022. Based on a management plan downgrade limit of 30 ‰, harvest area downgrades were reviewed based on available salinity sensor data and shellfish program microbiological results since March 2017. Forty-two days of harvest area downgrade occurred over eight rainfall downgrades, although salinity sensor data did not decline below 30 ppt and microbiological sample data collected between zero- and eight-days post downgrade met Approved harvest criteria. Time periods where salinity is slower to recover may require additional sampling to meet management plan requirements. The existing rainfall closure limits are well placed for Gogleys Lagoon harvest area and these limits would still apply to any request for a management plan change.

5.3 Bacterial source tracking

A total of 600 water samples and 279 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 Camden Haven (Fig. A1).

The maximum *E. coli* reached 90,612 gene copies 100 mL^{-1} on 16 Jan 2020, 39,593 copies 100 mL^{-1} for *Helicobacter* (bird) on 31 Jan 2019, 5,164 gene copies 100 mL^{-1} for bovine faecal pollution (cow) on 27 Feb 2020, and finally, 132 copies 100 mL^{-1} for human faecal pollution on 11 Oct 2019 (Fig. 5.3 A-D).


A.
E. coli
Gene Copy Number / 100ml
Rainfall (mm)
~ 70 CFU
~ 14 CFU
Date
B.
Bird
Gene Copy Number / 100ml
Rainfall (mm)
Date
C.
Cow
Gene Copy Number / 100ml
Rainfall (mm)
Date

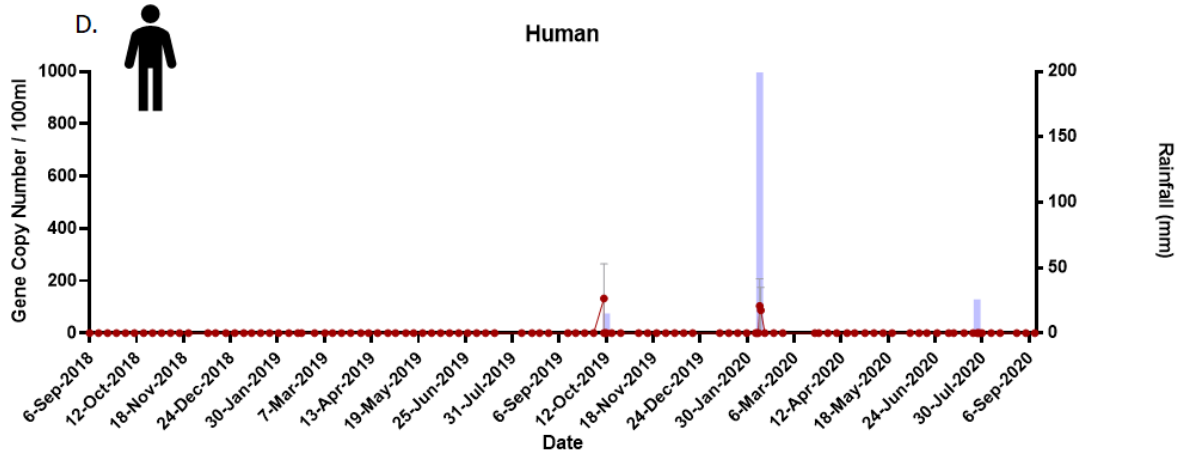


Figure 5.3 A-D. Weekly *E. coli* data from the sensor location, Camden Haven River, using A. *E. coli* assay; B. Bird assay; C. Cow assay; C2. Cow assay with different y-axis scale to show low levels of bovine contamination across sampling period; and D. Human assay. Dotted lines in Fig. A at 14 and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

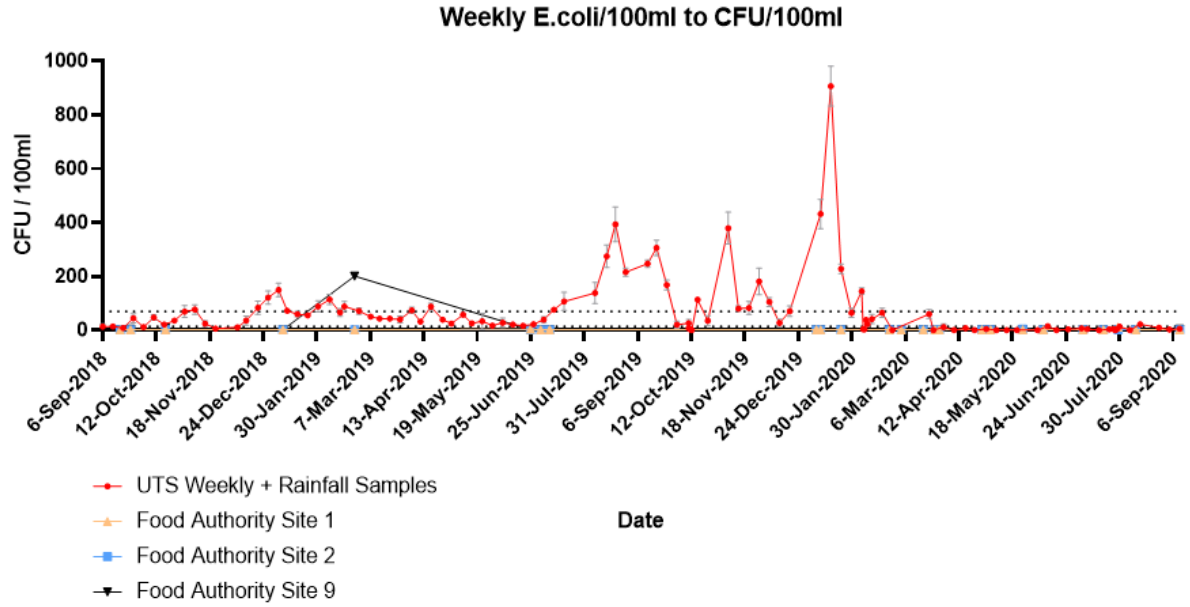
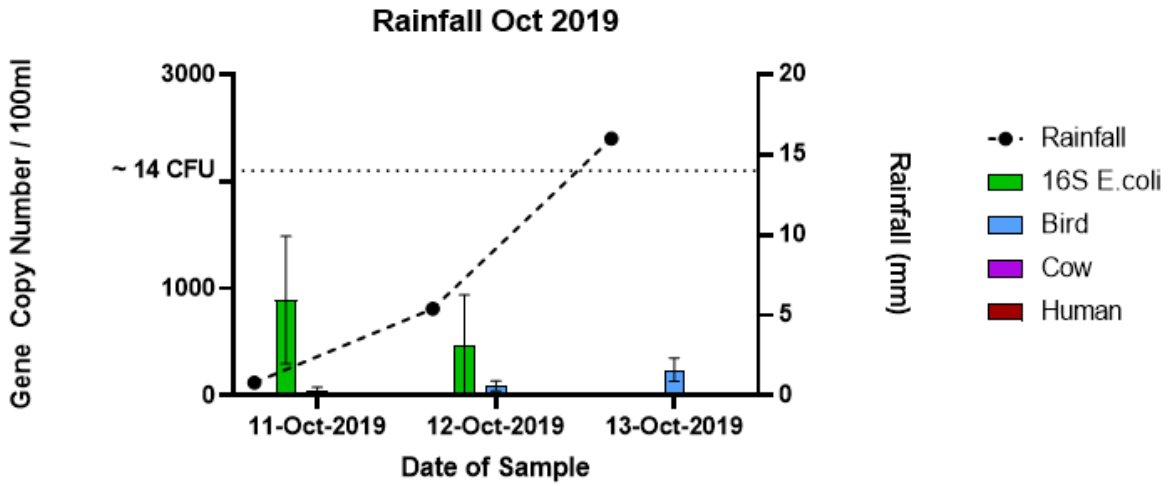


Figure 5.4 Weekly faecal coliform counts (cfu/100 mL) from water samples collected by DPI Food Authority at three sites in Camden Haven River compared to Oyster Transformation Project weekly sampling results. Dotted lines at 14 and 70 cfu/100 mL (Fig. 5.4B) are the operational limits for direct or restricted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

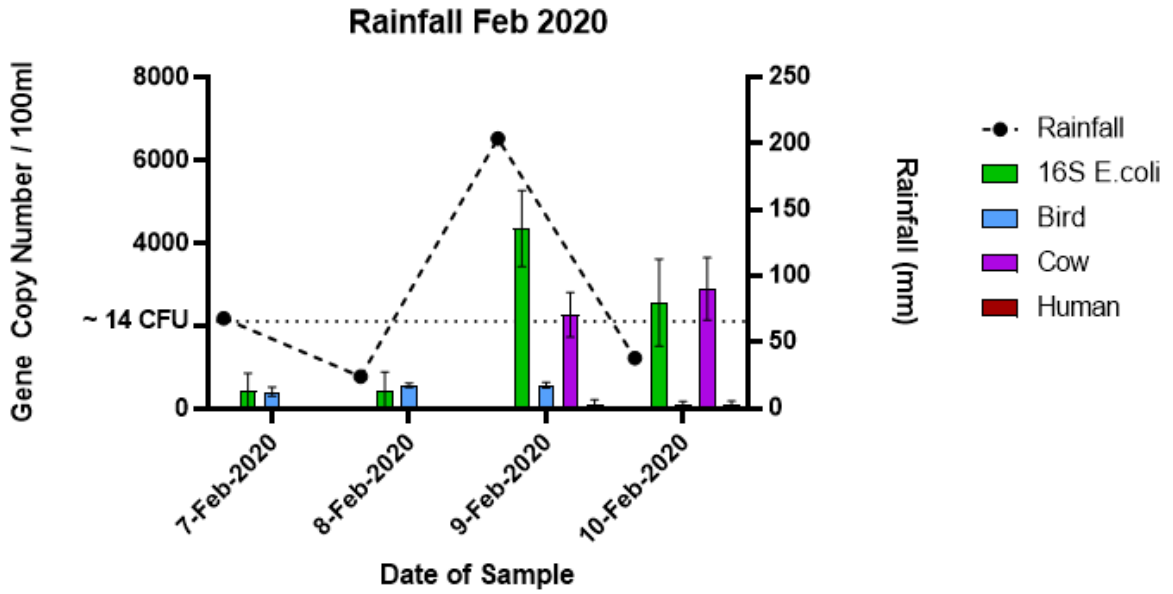
Elevated faecal coliform counts reported from the CRC project were noted across the period Jun 2019 to Jan 2020 (Fig 5.4) but were not clearly associated with spikes in the bird, cow or human assay results. Other possible sources of *E. coli* could be native wildlife (e.g., flying foxes). The CRC project test methods appeared more sensitive than routine testing on some occasions.

Three rainfall events were also sampled across the study period (see purple bars in Fig 5.3 A-D). These included 11-13 Oct 2019 (Fig 5.5A), 7-10 Feb 2020 (Fig. 5.5B) and 27-29 Jul 2020 (Fig. 5.5C).

A.



B.



C.

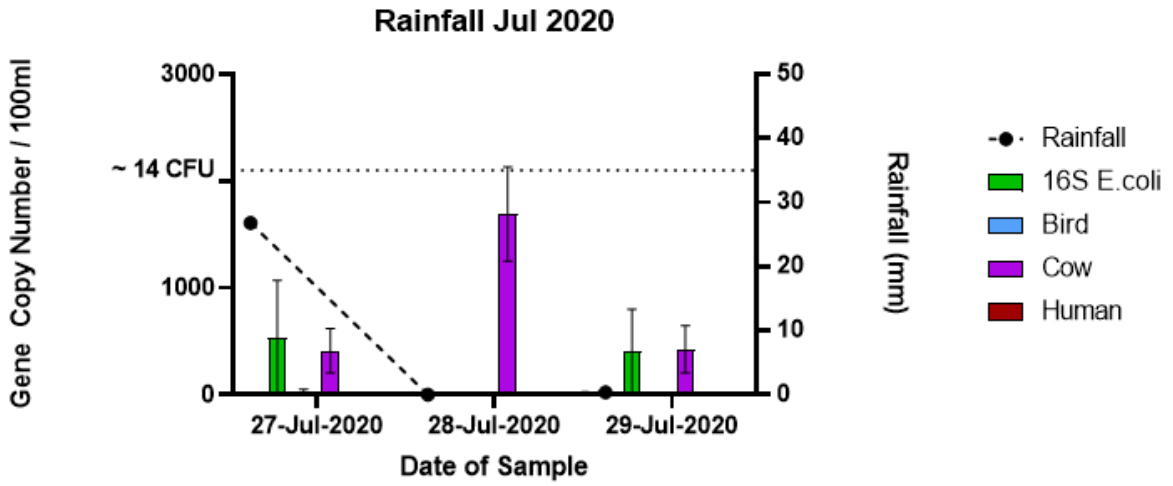


Figure 5.5 (A-C) Rainfall events sampled for *E. coli* assays. Green bar = E. coli; blue bar = bird assay; purple bar = cow assay; grey bar = human assay. Dotted line is rainfall (mm) obtained from the closest rainfall gauge at Laurieton PMHC (560018). All bars are the mean value of nine replicate samples (3 biological x 3 technical) and the error bars are the standard error of all nine replicates.

During these rainfall events, *E. coli* became elevated with increasing rainfall but generally decreased by day 3-4 of sampling (Fig. 5.5 A-C). Cow bacteria generally became elevated after rainfall declined, suggesting a lag effect, while both bird and human bacteria were either absent or very low concentration across the events sampled.

5.4 Phytoplankton enumeration and HAB events

The maximum phytoplankton cell concentration across the sampling period (March 2018 to March 2021) occurred on 6 Jan 2020 (Fig. 5.6). Total cell concentrations reached 7.5E +06 cells L^{-1} and the sample was dominated by the planktonic diatom *Chaetoceros* and with some *Skeletonema* and flagellates (cryptomonads, dinoflagellates, prasinophtyes). Sediment and organic detritus were also observed, which comprised planktonic diatoms (*Cerataulina*, *Leptocylindrus Dactyliosolen* and *Chaetoceros*) and some benthic diatoms (*Cylindrotheca*) and small flagellates (dinoflagellates). This bloom did not coincide with any significant rainfall event.

Only one other potentially harmful bloom occurred cross the sampling period. This was due to the toxic diatom *Pseudo-nitzschia delicatissima* gp., but it only occurred at sites away from the sensor (NSW Food Authority sampling sites 22 and 23). Maximum cell densities of 700,000 cells L^{-1} and 860,000 cells L^{-1} were reported for each of these respectively on 17 Oct 2018. NSW Food Authority trigger levels for flesh testing are 500,000 cells L^{-1} for the *P. delicatissima* group. No positive biotoxin results were reported in routine monitoring samples collected by CHSP during the same period.

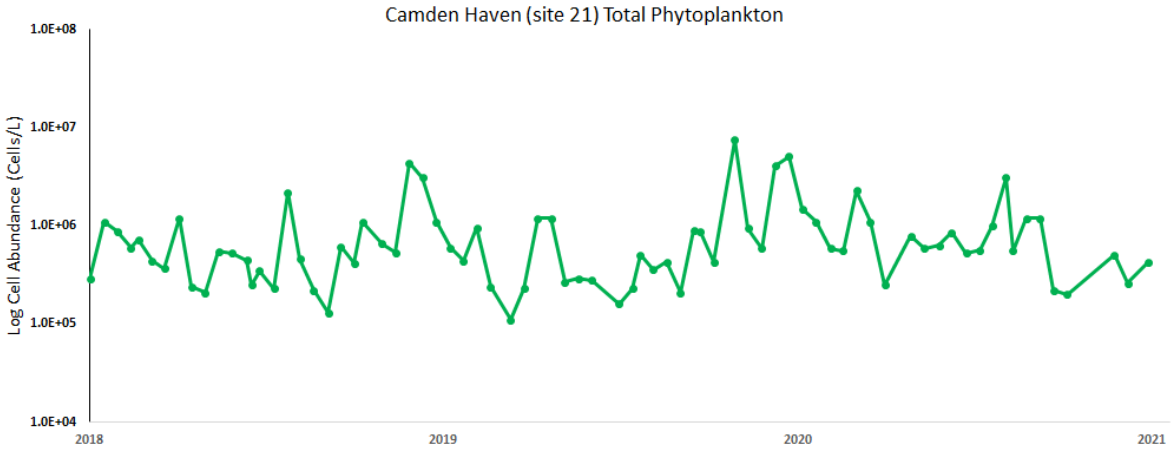


Figure 5.6 Log abundance of total phytoplankton sampled approximately fortnightly from 13 Mar 2018 to 31 Mar 2021.

5.6 Oyster Growth and Mortality

5.6.1 Oyster Growth

Average oyster whole weight increased by 29.2 g from deployment in August 2018 to June 2020 (Fig. 5.7A). Oyster whole weight was 51.8 ± 6.6 g at the end of the experiment (June 2020). The greatest increase in oyster whole weight occurred between August 2018 to August 2019, with oyster whole weight increasing by 19.9 g in 12 mo. Increases in oyster whole weight slowed dramatically from February 2020 to June 2020, increasing only 1.5 g over this time. Oysters deployed in Camden Haven attained a large size grade of greater than 70 mm total length and greater than 50 g whole weight in January to February 2020, when they were 37 mo and 38 mo old, respectively.

Oyster shell length was 53 ± 4 mm at the start of the experiment and increased to 76 ± 2 mm in June 2020 (Fig. 5.7 B). The greatest increase in shell length in Camden Haven was recorded from November 2019 to January 2020, with shell length increasing by 10 mm in 2 mo. Shell lengths were measured more frequently than whole weight and fluctuated throughout the experiment. No increase in shell length occurred between March and November 2019 and a decrease in shell length was recorded between January and February 2020.

5.6.2 Mortality

From August 2018 to February 2020, cumulative oyster mortality was 40% in Camden Haven. A period a high oyster mortality occurred between December 2018 to March 2019, with 27% mortality recorded over this period (Fig 5.7 C-D). This level of mortality was greater than the background Sydney Rock Oyster farming mortality level which is estimated to be approximately 10% per annum. Oyster mortality was minimal (<3%) between all other sampling occasions. Oysters from this site remain frozen for future analyses.

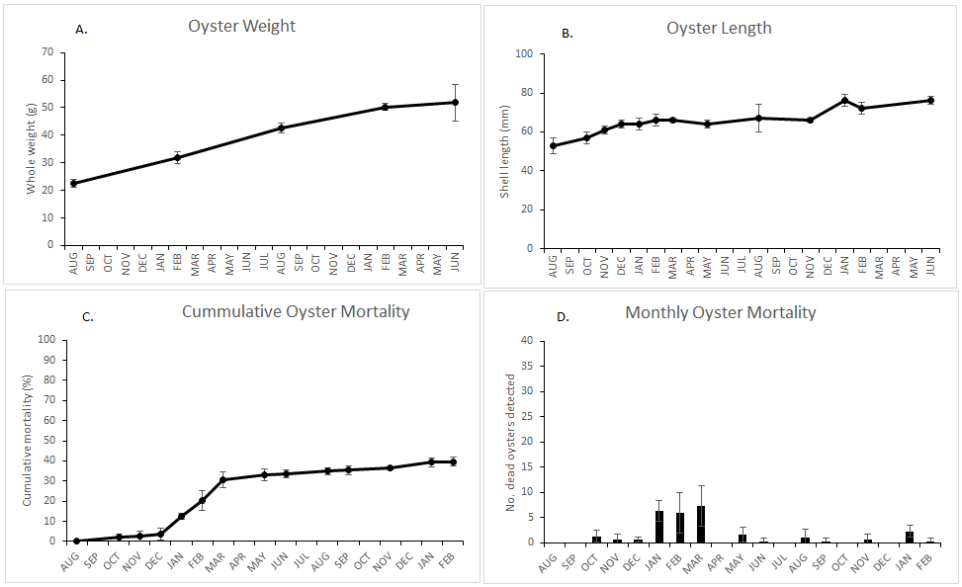


Figure 5.7 A-D. Oysters deployed at the sensor site, Camden Haven River. A. whole weight; B. shell height; C. cumulative mortality, and D. monthly mortality.

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 few strong positive relationships overall ($r > 0.7$). 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: 44% for *E. coli* (sensor + total phytoplankton), 31% for cow (sensor + total phytoplankton), 65% for bird (sensor + total phytoplankton) and 100% for human (sensor + total phytoplankton) although this latter model was unstable (Table 1A).

The abundance of *E. coli* at the sensor site was significantly better explained by the sensor data compared to the rainfall data (44% deviance explained as compared to 5%), and was linked to increasing salinity and temperature over the previous 72 hours (Table 1, Figures 5.8 A-D, 5.9 A-D). Cow bacterial abundance was also better predicted using the sensor data compared to rainfall data (31% compared to 14% with rainfall data), however this time declining salinity over the previous 72 hours and increasing water temperature were important predictor variables (Table 1, Figures 5.8 A-D, 5.9 A-D). Faecal contamination from birds at the sensor site was significantly better explained by the salinity model (65% deviance explained, compared to 4% using rainfall data), with higher salinity and temperature over the previous 72 hours linked to higher abundance (Table 1, Figures 5.8 A-D, 5.9 A-D). Finally, an increase in human bacteria was best explained by the sensor data (100% compared to sensor data 34%), however this model was unstable and therefore should be interpreted with caution (Table 1, Figures 5.8 A-D, 5.9 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 80% 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 Camden Haven River. Only significant variables are shown for each model.

Bacteria	Variables	No. of observations	Significant Variables	Deviance Explained
E. coli	Salinity, Depth, Temp	103	Depth72***, Salinity72***, Temp72***	43.1%
E. coli	Salinity, Depth, Temp, logPhytoplankton	103	logPhytoplankton***, depth***, salinity***, temp***	43.8%
E. coli	Rainfall72	103	Rainfall72***	3.01%
E. coli	Rainfall72, logPhytoplankton	103	Rainfall72***, logPhytoplankton ***	5.1%
Bird	Salinity, Depth, Temp	103	Salinity***, Depth***, Temp***	64.1%
Bird	Salinity, Depth, Temp, logPhytoplankton	103	Salinity***, Depth***, Temp***, logPhytoplankton ***	64.5%
Bird	Rainfall72	103	Rainfall72***	1.63%
Bird	Rainfall72, logPhytoplankton	103	Rainfall72***, logPhytoplankton***	3.87%
Cow	Salinity, Depth, Temp	103	Salinity***, Depth***, Temp***	30%
Cow	Salinity, Depth, Temp, logPhytoplankton	103	Salinity***, Depth***, Temp***, logPhytoplankton***	31%
Cow	Rainfall24	105	Rainfall24***	10.3%
Cow	Rainfall24, logPhytoplankton	105	Rainfall24***, logPhytoplankton**	14.4%
Human	Salinity, Depth, Temp	105	Salinity, Depth, Temp	100%*
Human	Salinity, Depth, Temp, logPhytoplankton	105	Salinity, Depth, Temp, logPythoplankton	100%*
Human	Rainfall48	104	Rainfall48**	33.5%
Human	Rainfall48, logPhytoplankton	104	Rainfall48**, logPythoplankton*	33.9%

***** Unstable fit

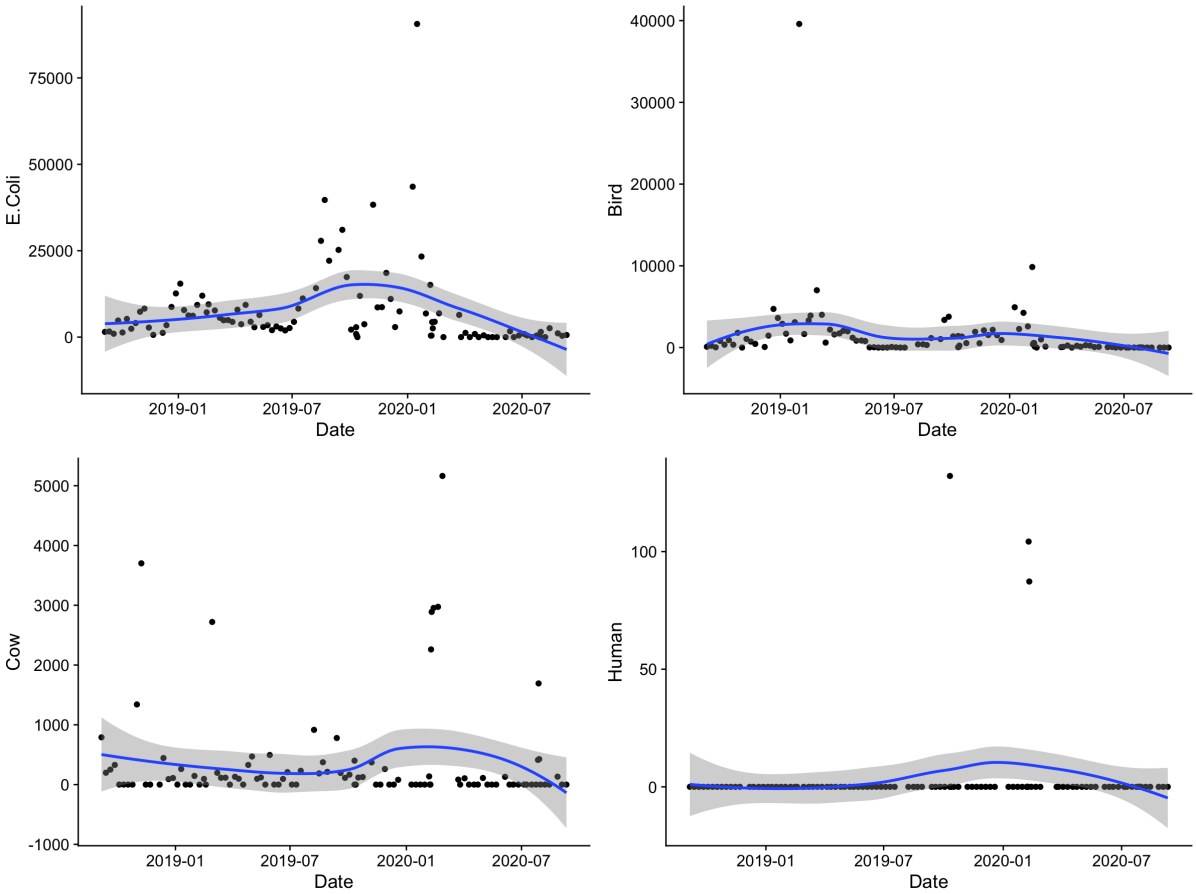


Figure 5.8 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. *E. coli*, B. Bird, C. Cow, and D. Human bacterial load as measured by weekly sampling at the sensor site, Camden Haven River.

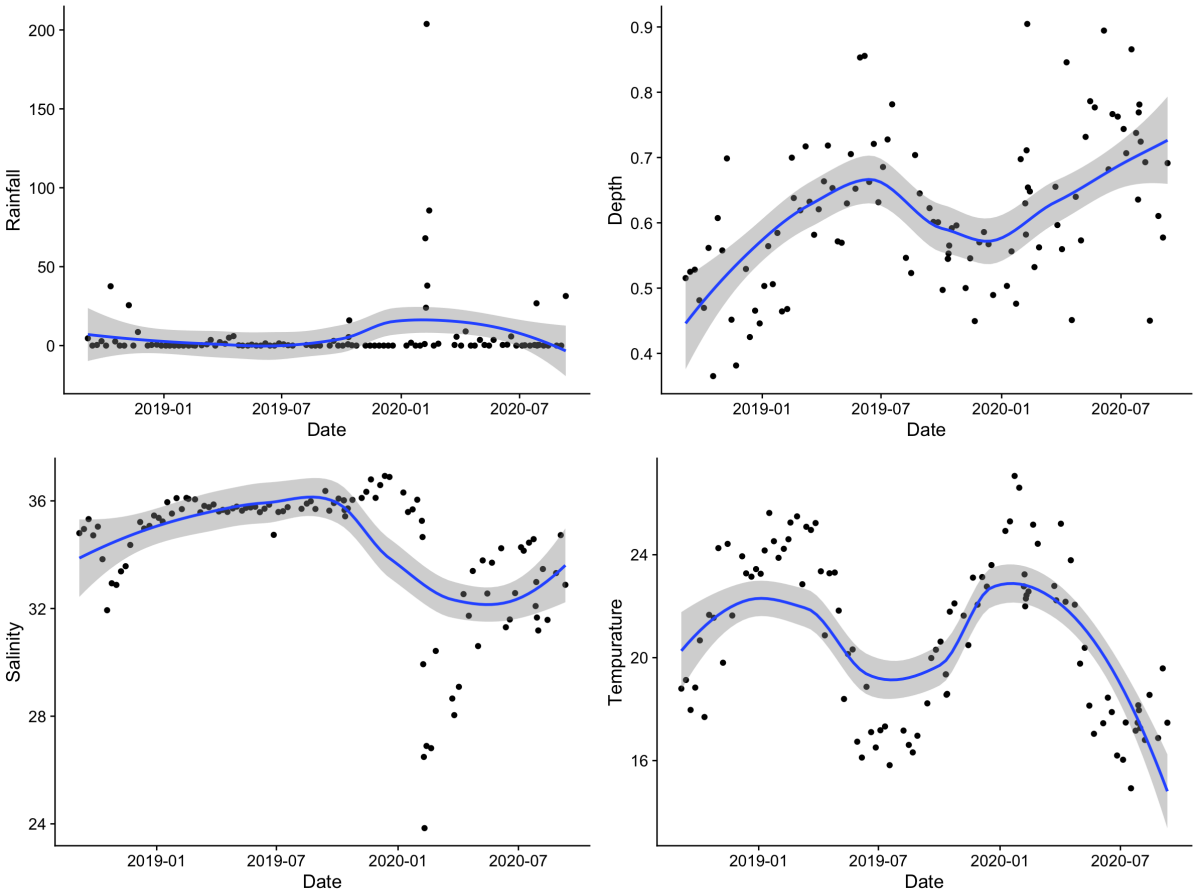


Figure 5.9 A-D. Data points (black dots), average (blue line) and standard error (shaded area) of A. Rainfall, B. Depth, C. Salinity, and D. Temperature values measured in at the sensor site, Camden Haven River.

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 harvest area downgrades for Gogleys Lagoon harvest area. Based on the available data, up to eight harvest area downgrades could have potentially been avoided between 14 March 2018 and 30 September 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. Camden Haven Shellfish Program (CHSP) were consulted about the option of a salinity-only management plan component for harvest area downgrades in Gogleys Lagoon harvest area following the 2022 annual review, but a decision has not yet been reached. If CHSP did not wish to pursue the implementation of a management plan that is based on sensor salinity for harvest area downgrades, or if the salinity sensor data were not accessible, the Gogleys Lagoon harvest area management plan would revert to the current management plan that is based on both rainfall and salinity downgrade and closure limits.

6.2 Phytoplankton and HABs

The most common HAB species that bloomed in Camden Haven River during this study was *Pseudo-nitzschia*. Although this did not occur in significantly high numbers during our sampling period, *Pseudo-nitzschia* is a high-risk HAB group in SE Australia for the shellfish aquaculture industry, and both estuaries and coastal waters in this area remain under threat (Ajani et al., 2013, 2020). Blooms within the Hawkesbury River estuary (330 km south of Wallis River), a high-risk area in SE Australia for HAB events, recently experienced a very dense bloom of *P. delicatissima* gp., with one out of seven strains isolated to produce domoic acid (Ajani, 2020). Fifteen years of modelled data in the Hawkesbury River estuary revealed that *Pseudo-nitzschia* was linked to an increase in soluble reactive phosphorus and a decrease in nitrogen at all six sites sampled (via rainfall/nutrient runoff). There is contrasting evidence, however, of which environmental conditions promote the blooming of the different species complexes (Dermastia et al., 2020). In response to a toxic bloom of *Pseudo-nitzschia delicatissima* gp. (dominated by *P.* cf. *cuspidata)* in Wagonga Inlet in April 2019, we have now successfully developed a rapid, sensitive and efficient quantitative real-time polymerase chain reaction (qPCR) assay to detect *P. pseudodelicatissima* complex Clade I, to which *P.* cf. *cuspidata* belongs (Ajani et al. 2021).

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

Dinophysis is common in Australian waters, with 36 species reported (Ajani et al., 2011; Hallegraeff and Lucas, 1988; McCarthy, 2013). Toxic species include *D. acuminata*, *D. acuta*, *D. caudata*, *D. fortii*, *D. norvegica*, and *D. tripos*. There have been three serious human DSP poisoning events in Australia. The first episode was caused by contamination of Pipis (*Plebidonax deltoides*) in New South Wales in 1997 (NSW) by *D. acuminata* (Quaine et al., 1997). One hundred and two people were affected and 56 cases of gastroenteritis reported. A second episode occurred again in NSW in March 1998, this time with 20 cases of DSP poisoning reported (Madigan et al., 2006). The final event occurred in Queensland in March 2000, when an elderly woman became seriously ill after eating local Pipis (Burgess and Shaw, 2001). While no human fatalities from DSP are known globally, DSTs continue to be a major food safety challenge for the shellfish industry. In response to elevated cell densities of a toxic algal species *Dinophysis* in February 2019 in the Manning River, we have also successfully developed a rapid qPCR assay to detect species belonging to the genus *Dinophysis* in environmental samples (Ajani et al. 2022).

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

Quantitative PCR is an efficient and powerful tool to identify and enumerate HAB species, especially those that are difficult to distinguish using routine methods (Handy et al. 2008, Penna and Galluzzi 2013). For this reason, this method is used routinely in certain monitoring programs around the world (Clarke & Gilmartin 2020). We have now developed qPCR assays for *Alexandrium* (sxtA gene) (Ruvindy et al. 2018), *Dinophysis* spp. (Ajani et al. 2022) and *Pseudo-nitzschia pseudodelicatissima* complex Clade 1 (Ajani et al. 2021). The qPCR assays can be used on-farm, allow for automation, are easy to use without specialist knowledge, and provide an early warning that harmful algae are present in the water column. It is envisaged that high-resolution, real-time environmental data, combined with sensitive, specific and efficient molecular tools such as we have developed in the current study, will enable us to effectively predict and manage these blooms into the future.

6.3 Assay Development and Faecal Pollution in Camden Haven River

Molecular assays for the detection of faecal bacterial contamination in Camden Haven 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 detection in estuarine waters. In this study, several primer pairs were trialled which targeted 3 different genes within *E. coli*, with the final *E. coli* assay selected being the most efficient and specific only to the target organism (Tesoreiro 2020).

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

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

Salinity was a more reliable predictor than rainfall for all four faecal indicators tested in Camden Haven. Elevated *E. coli* was highly variable, and linked to increasing salinity and temperature, suggesting a lack of flushing at the sensor site. When *E. coli* did become elevated with rainfall (event sampling), it generally decreased by day 3-4 of sampling. On the other hand, when cow bacteria became elevated (relatively low compared to other estuaries), it was linked to rainfall, and a possible lag effect was observed.

Avian faecal pollution was observed to peak during the summer months. The first 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 in Camden Haven. 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 Camden Haven were greatest in the first half of the experiment from August 2018 to August 2019. However, growth, in terms of shell length, was greatest from November 2019 to January 2020. The salinity during the period of maximum shell growth was very stable and remained above 33 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 first 12 months of the experiment which was also characterised by stable salinity levels above 30 ppt (Fig. 5.1B). The period of minimum whole weight increase, coupled with a shell height decrease, occurred between January and February 2020, and coincided with a rapid drop in salinity below 25ppt. This was following an intense rain event where approximately 500 mm was recorded by the Laurieton PMHC (560018) rainfall gauge between the 7th to 10th of February 2020.

Low levels of mortality were recorded for most of the experiment, between August to December 2018 and March 2019 to February 2020. Mortality during this period was similar to background mortality (approximately 10% per annum) commonly experienced when farming Sydney Rock Oysters. High levels of mortality were recorded, however, between December 2018 to March 2019, with a cumulative mortality of 27% during this period. This was the highest mortality event to occur in any monitoring site during the experiment. Cumulative mortality at the end of the sampling period (February 2020) was 39.7% and exceeded the cumulative mortality measured in all other monitoring sites on the same date. Oysters held in the Hastings River experienced a similar spike in mortality from January to February 2019 (19.7%), however, the cumulative mortality of oysters in this monitoring site only reached 34% by February 2020. High levels of cumulative mortality were also measured at Camden Haven in a previous study, with 67% mortality over a 26-month period from April 2004 to April 2006 (Dove and O'Connor, 2009).

The cause of high cumulative mortality in the Camden Haven during the experiment is unknown. Unlike estuaries such as the Georges River, the Camden Haven does not experience known outbreaks of QX or winter mortality disease, both of which cause significant mortality in Sydney Rock Oysters (Nell et al. 2000; Dove et al. 2013). Further, salinity measured during December 2018 to March 2019, when peak mortality occurred, was approximately 35 ppt, suggesting that salinity did not impact survival (Nell and Holliday, 1988; Sarwer 2020).

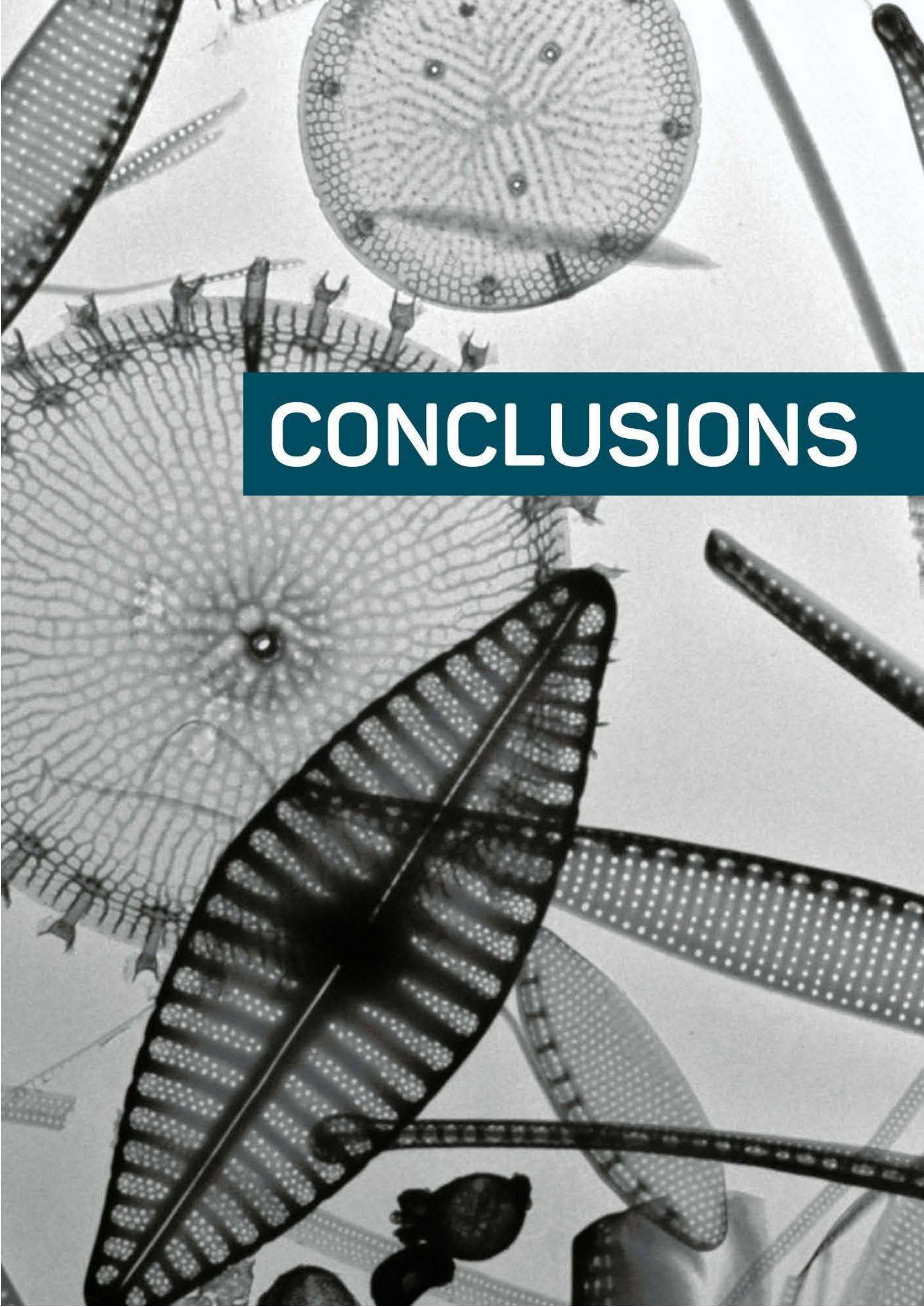
The batch of oysters used for this experiment were a random mix of families taken from the 2016-year class of the Sydney Rock Oyster Breeding program. This particular year class had 86% of the parents selected from wild and QX disease resistant genetic groups. Only 14% of the parents for this year class were sourced from the fast growth genetic group. It took this year class approximately 3 years and 2 months to reach the large oyster size grade (> 70 mm total length or > 50 g whole weight). The only estuaries where this same batch of oysters reached the large oyster size grade benchmark faster were the Georges River, Hawkesbury River, and Manning River. High growth rates of the Sydney Rock Oyster in the Camden Haven were recorded in a previous study, with oyster whole weight and shell length being higher than the same batch of oysters in all other estuaries tested in the study (Shoalhaven River, Wagonga Inlet, Merimbula Lake, Lake Conjola, Kalang River and Wallis Lake; Dove and O'Connor, 2009).

The Camden Haven is ranked 10th in the state for Sydney Rock Oyster production with 153,699 dozen oysters sold annually worth ~$1.4 Mil (NSW DPI 2023). Despite oysters in the Camden Haven being the 4th fastest to reach the large size grade among the twelve estuarine sites in this study, most Sydney Rock Oysters in Camden Haven (55%) are sold at the small size grade, when oysters are less than 55 mm in total length and less than 30 g whole weight (NSW Department of Primary Industries 2022).

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 harvest area downgrades in Gogleys Lagoon harvest area, subject to agreement by the local shellfish industry. No change to the existing harvest area rainfall closure limits is proposed. Available data indicated that eight harvest area downgrades could have potentially been avoided between March 2018 and September 2022. As of August 2023, nineteen salinity-only management plans had been offered for harvest areas in participating NSW estuaries, with seven being taken up and the remaining twelve under consideration.

Compared to the other monitoring sites in NSW, Camden Haven was ranked 4th fastest in terms of oysters reaching the large size grade (> 70 mm total length and > 50 g whole weight). Low levels of mortality were recorded over most of the monitoring period, except for December 2018 to March 2019, where mortality increased by 27%. This was the largest mortality event recorded among the monitoring sites, with cumulative mortality at the end of the experiment (February 2020) being two and a half times 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 and whole-body weight, but not mortality.

The pollution source tracking results were highly variable across the study period, most likely attributable to the extreme variation in environmental conditions experienced (drought, bush fires, floods). Real time sensor data showed a higher predictive capability than rainfall for all four faecal indicator bacteria (although the human model was unstable). Elevated levels of *E. coli* were variable, while cow and human bacterial loads most often corresponded to rainfall events. Furthermore, contamination from bird sources was observed at relatively higher concentrations compared to other estuaries, showing a distinct increase throughout the summer months.

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 Camden Haven River.

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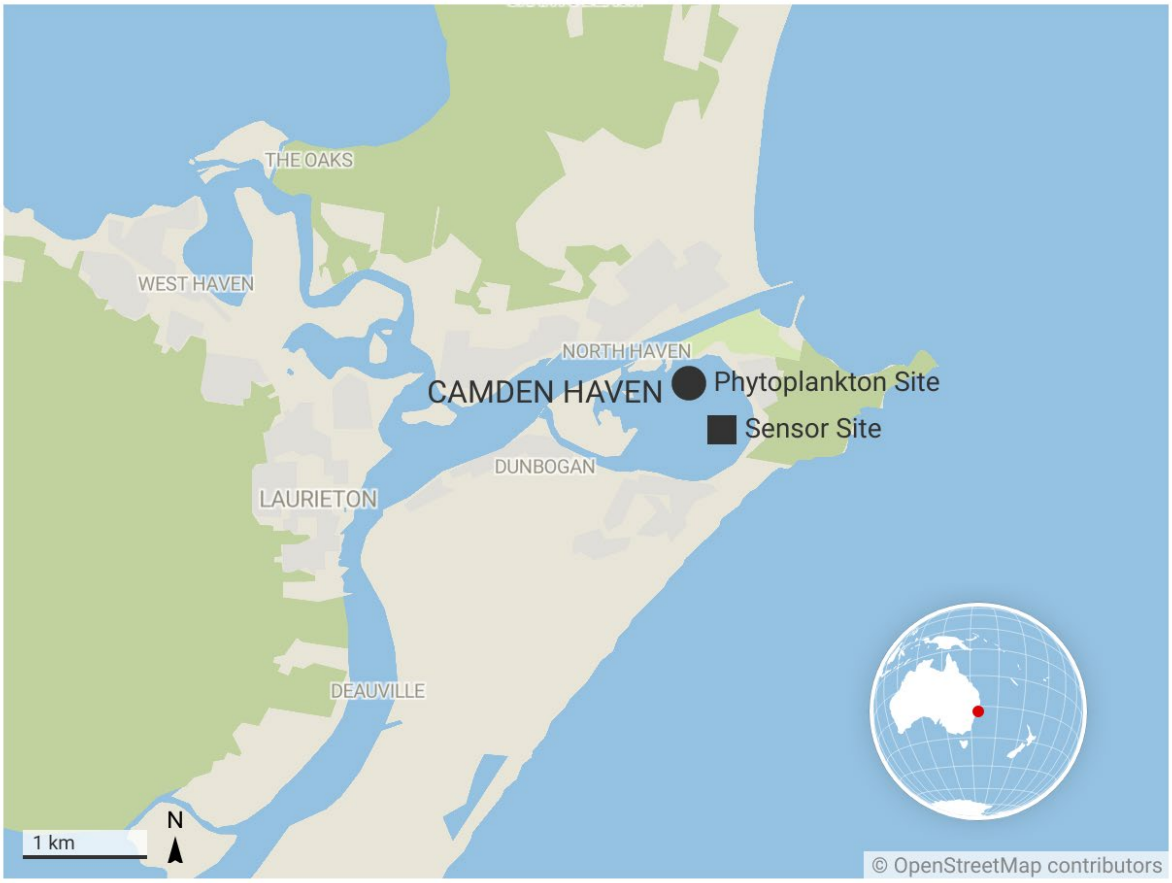
9. Appendices

A1. Methods

A1.1 Sampling locations in Camden Haven River

Data used in this report originates from locations within Camden Haven River over the period 13 Mar 2018 to 31 March 2021. High-resolution temperature, salinity and depth data were obtained from a sensor located in Gogleys Lagoon harvest area, located within Camden Haven 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).



Created with Datawrapper

Figure A1: Map of Camden Haven River highlighting the sensor located in Gogleys Lagoon (black square) and the phytoplankton sampling location (black circle).

A1.2 High-resolution sensor data

High-resolution temperature (°C), salinity and water depth (m) data were collected from the sensor site using Seabird SBE 37-SM/SMP/SMP-ODO MicroCAT high accuracy conductivity, temperature and

depth (CTD) field sensors. This sensor was deployed using a fixed installation, with the inlet 60 cm above the seabed and at least 30 cm below the estimated Lowest Astronomical Tide (LAT) (Fig. A2). This fully autonomous instrument collected and transmitted data every 10 minutes (24 h day^{-1}) to Microsoft Azure cloud storage before downstream quality checking and analysis. Sensor data was then packaged into RO-Crates by the e-Research team at UTS, which are then uploaded to an Arkisto-based website. This website allows for the filtering and downloading of these crates based on both time and location, for use in research and analysis (Fig. A3). Finally, rainfall data were obtained from the closest rainfall gauge at Laurieton PMHC (560018) (~-31.64° S, 152.79° E) from March 2018 to March 2021.

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

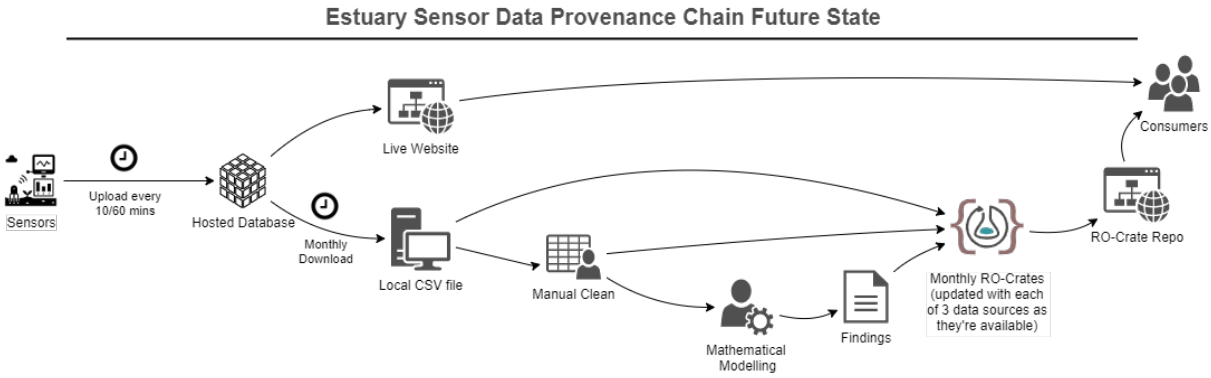


Figure A3. Camden Haven River data provenance chain from source of data (sensor), via quality assurance processes, data analyses, to consumers.

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 Camden Haven annual review is 1 October. As part of the most recent (2022) annual review for Gogleys Lagoon harvest area, all salinity data from the monitoring sensors during the 2018, 2019, 2020, 2021 and 2022 annual review periods were assessed in relation to microbiological

samples collected by the local shellfish program during the same period. There was a gap in data collection between 1 and 28 April 2021, while the sensor was transitioned to a new provider. Salinity data collected between 1 and 18 February 2022 were excluded from the analysis, as data collected during this period was not representative of local conditions. The sensor returned to normal operations after maintenance/cleaning. Other occasional salinity data points removed from the analysis were generally elevated above expected salinity levels, and indicative of debris interfering with the sensor reporting.

A1.4 Biological sampling, eDNA extraction and nutrient analyses

Estuarine water samples were collected weekly by Camden Haven 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 the closest available rainfall gauge at Laurieton PMHC (560018) (~-31.64° S, 152.79° E) from March 2018 to March 2021.

A1.5 qPCR assays for bacterial source tracking

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

A1.6 Phytoplankton enumeration

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

A1.8 Oyster Growth and Mortality

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

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

A1.8.1 Oyster Whole Weight

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

A1.8.2 Shell Length

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

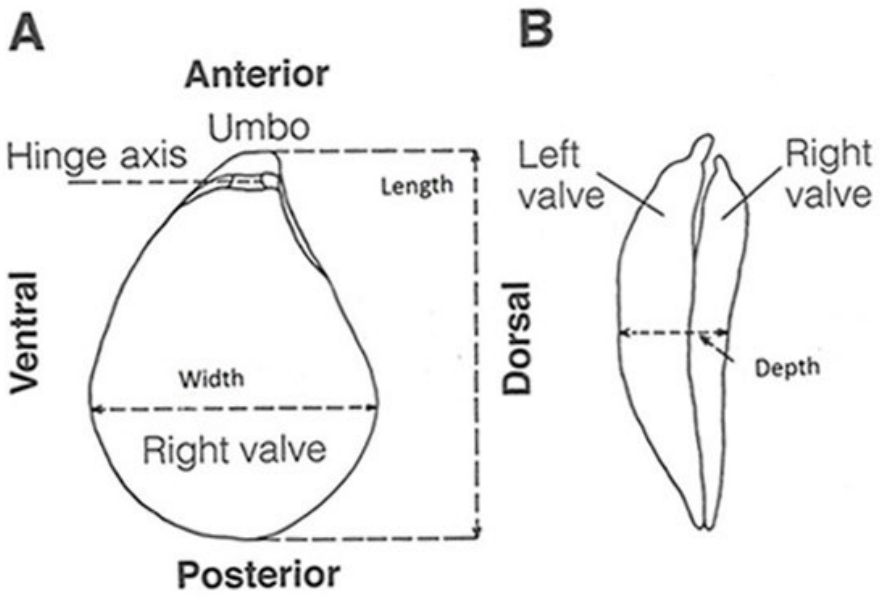


Figure A4. Oyster shell dimensions (Carriker 1996)

A1.8.3 Oyster Mortality

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

A1.9 Modelling

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

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

Rainfall data from the closest rainfall gauge at Laurieton PMHC (560018) (~-31.64^{o} S, 152.79^{o} E) from Mar 2018 to March 2021, which was the official management plan gauge for this harvest area, were averaged over the 24 h, 48h, 72 h and 7 days prior to the water sampling each day, to incorporate a measure of exposure of the bacterial community and deployed oysters. Total phytoplankton (and log transformed total phytoplankton) from microscopic phytoplankton enumeration was also included in the modelling as a potential predictor variable. Finally, week of the year and water depth were included in the models to understand any seasonality or tidal variability that was present in the data.

To model the relationship between bacteria (*E. coli*, bird, cow, human) abundance and/or oyster growth (response variables) and environmental variables (temperature, salinity, week, depth, total phytoplankton and rainfall) at the sensor location within Camden Haven 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, Camden Haven River

Variable	Mean	Standard Error	Median	Standard Deviation	Minimum	Maximum	Count	Missing
average_cfu	69.28	11.56	33.18	118.47	0.00	906.79	105	0
bird	1458.80	398.46	406.64	4082.97	0.00	39592.70	105	0
cow	351.85	82.87	83.10	849.16	0.00	5163.63	105	0
depth24	0.62	0.01	0.60	0.11	0.37	0.90	105	0
depth48	0.62	0.01	0.61	0.10	0.42	0.85	105	1
depth72	0.62	0.01	0.61	0.09	0.45	0.82	105	2
ecoli	7229.78	1160.73	3457.71	11893.91	0.00	90612.17	105	0
human	3.08	1.79	0.00	18.33	0.00	132.16	105	0
logPhytoplankton	13.32	0.09	13.29	0.89	11.61	15.83	105	0
Phytoplankton	962095.24	124274.06	590000.00	1273430.18	110000.00	7500000.00	105	0
rainfall24	6.15	2.26	0.20	23.20	0.00	203.80	105	0
rainfall48	6.04	1.80	0.50	18.40	0.00	120.90	105	1
rainfall72	6.04	1.71	0.87	17.50	0.00	109.13	105	2
salinity24	34.24	0.24	35.37	2.51	23.84	36.93	105	0
salinity48	34.24	0.24	35.36	2.44	25.16	36.91	105	1
salinity72	34.24	0.23	35.39	2.40	25.74	36.80	105	2
temp24	20.83	0.30	20.87	3.07	14.92	27.06	105	0
temp48	20.86	0.29	21.33	2.95	16.04	26.83	105	1
temp72	20.88	0.28	21.41	2.90	16.15	26.32	105	2

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

Author(s)	Title	Bibliographic details	Status (Submitted, Accepted, Published)
Penelope Ajani, Hernan Henriquez-Nunez, Arjun Verma, Satoshi Nagai, Matthew Tesoriero, Hazel Farrell, Anthony Zammit, Steve Brett and Shauna Murray	Mapping the development of *Dinophysis* spp. HABs using a novel molecular qPCR assay	*Harmful Algae* 116 (2022) 102253	Published
DPI Food Authority	Foodwise - Issue 60	https://www.foodauthority.nsw.gov.au Winter 2022	Published
Penelope Ajani, Arjun Verma, Jin Ho Kim, Hazel Farrell, Anthony Zammit, Steve Brett & Shauna Murray	Using qPCR and high-resolution sensor data to model a multi-species *Pseudo-nitzschia* (Bacillariophyceae) bloom in southeastern Australia	*Harmful Algae* 108 (2021) 102095	Published
DPI Food Authority	Foodwise - Issue 56	https://www.foodauthority.nsw.gov.au Autumn 2021	Published
NSW DPI	Net Returns of Real-Time Sensors and Salinity-Based Management Plans in NSW Oyster Production - Report	https://www.foodauthority.nsw.gov.au/about-us/science/science-in-focus/real-time-sensors-shellfish-harvest-area-management	Published
NSW DPI	Net Returns of Real-Time Sensors and Salinity-Based Management Plans in NSW Oyster Production - Factsheet	https://www.foodauthority.nsw.gov.au/about-us/science/science-in-focus/real-time-sensors-shellfish-harvest-area-management	Published
The Team	Oyster Transformation Project	NSW Oyster Newsletter https://www.nswoysters.com.au/nsw-oyster-newsletter.html July 2020	Published
Penelope A. Ajani, Michaela E. Larsson, Stephen Woodcock, Ana Rubio, Hazel Farrell, Steve Brett, &Shauna A. Murray.	Fifteen years of *Pseudo-nitzschia* in an Australian estuary, including the first potentially toxic *P. delicatissima* bloom in the southern hemisphere	*Estuarine, Coastal and Shelf Science*, 236 (2020) 106651.	Published

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

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

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

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