Sentinel Sensors:

Revolutionising our understanding and management of the estuarine environment

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Abbreviations

BPM – beats per minute
DO – dissolved oxygen
FRDC – Fisheries Research and Development Corporation
HR – heart rate
MAPE - Mean Absolute Percentage Error
MDC – Woodbridge Marine Discovery Centre
ML – Machine Learning
ORP – oxygen reduction potential

RPi – Raspberry Pi microcomputer
WQ – water quality

Executive Summary

This report documents the 2018/19 field research undertaken by CSIRO in the mollusc biosensor space. The Sentinel Sensors project had two key components, a set of scientific objectives around biosensors as a way of monitoring the environment, and an outreach objective around inspiring young students in the space of marine science.

To demonstrate the utility of using biosensors for environmental monitoring the project deployed sets of biosensors alongside more traditional water quality sensors at two locations for 12 months. It deployed the latest biosensor technology, a version refined specifically for the remote, long-term deployment challenge of this project, to demonstrate that the technology has matured to the point that these devices can be deployed and operated with zero maintenance for up to 12 months, saving the significant cleaning and maintenance costs that traditional sensors require. Data was analysed to show how the physiological and behavioural data from the biosensors can be interpreted in the context of the water quality.

To deliver the outreach component the project worked with the Woodbridge Marine Discovery Centre (MDC) as well as the CSIRO Discovery Centre in Canberra by developing teaching modules and dedicated lab equipment for primary and secondary students and visitors to those centres.

Our current understanding of environmental and anthropogenic impacts on marine life, whether farmed or wild, is limited by a lack of technology to directly measure the perception and responses of the animals themselves. Molluscs have been used as "biological early warning systems" because their swift behavioural response to environmental stressors is predictive of impending effects on other organisms in the ecosystem. Despite this potential, a lack of appropriate technology has prevented measurements of real-time data from animals in parallel with environmental monitoring. This project demonstrated the utility of using sentinel animals with novel biosensors to provide early warning information on the health and status of the marine environment. This project measured the heart rate and gaping behaviour of sentinel pacific oysters, as they respond to multiple and interacting changes in the environment. Such complex monitoring is not possible with infrequent water sample analysis, and the lack of real-time sensors for all potential stressors creates the need for a world-first rapid biological response system.

The "animal-eye" view that biosensors provide adds value to existing ecosystem monitoring and modelling research and provides the crucial missing piece in the puzzle – the biological response to environmental change. This research linked physiological and behavioural responses of bio-filtering molluscs to more traditional water quality measurements, to produce a more complete picture of environmental changes and ecosystem health.

There were four main objectives of this research:

1) The project aimed to demonstrate an operational network of sentinel molluscs providing real-time streaming of physiological and behavioural data providing a biological perspective of the environment. This is a fundamental component of the project. It must demonstrate that it is feasible to build, deploy and maintain a network of sentinel sensors.

2) The project aimed to improve the local economy's capacity to install, operate and maintain realtime, telemetered, environmental sensing systems by demonstrating the commercial operation of telemetered water quality sensor system over an extended 12-month period by a commercial operator. This is also an important component of the research to ensure any ongoing solution can be serviced by the local economy.

3) The project aimed to demonstrate that aspects of the ecosystem can be represented in real-time using sentinel animal data. This data analysis is also a critical component of the Sentinel Sensors concept.

4) Finally, the project aimed to provide an educational outreach program to develop the next generation's interest in the marine environment and marine science. This is an important benefit to all estuarine stakeholders, and to achieve this outcome the project will work with the MDC. The project will deploy sentinel animals and environmental monitoring on-site and develop and deliver learning modules and experiences for students related to the sentinel animal concept as well as more traditional environmental monitoring and caring for the marine environment.

To deliver the field biosensor results, the project first designed and tested remote field deployable sensor systems (both traditional water quality sensors as well as the latest biosensor technology). Two final systems were built and then deployed in the D'Entrecasteaux Channel south of Hobart and operated for 12 months. These systems provide live water quality and biosensor data. Data was collected and analysed to demonstrate relationships between the biosensor data and the underlying water quality the animals were living in.

For the education outreach component, the project developed and delivered teaching modules at the MDC, and subsequently reviewed and refined those modules for future years. The MDC teachers reported that students are engaged with the suite of theoretical and practical biosensor experiments and displays, and that having unique access to world leading technology does inspire the students.

An important result from the field experiments is that CSIRO's novel mollusc biosensors can be deployed and operated for up to 12 months with zero maintenance. Conventional sensor equipment requires costly monthly maintenance and annual calibration, expenses that can potentially be averted, making continual environmental monitoring available to more users.

Data analysis indicated that there are strong links between bio-sensed physiology and behavioural data and water quality, reinforcing that these sensors can be used for environmental monitoring.

These results have implications for all users and managers of marine spaces as they open the possibility for more relevant and lower cost environmental monitoring.

Some further work is recommended to advance the commercialisation of the biosensors and to continue to refine and interpret data coming from the devices.

Keywords

Biosensors, Water Quality, Sensing, Oyster, Blue Mussel

Introduction

Priority R&D focus for many sectors is on improving management and sustainability of marine environments for multiple users and ensuring social licence to operate. This research proposal was developed to validate the novel use of biosensors on sentinel individuals to provide a biological perspective of environmental changes. Data and resulting decision support systems from such monitoring is predicted to add significant value to environment monitoring and modelling, and therefore a more relevant mechanism for managing the environment.

This project was in response to the 2016 FRDC priority R&D call, and consultation with industry and environmental monitoring partners. The project contributed to the Environmental Health priority area, with development of tools for cost-effective environmental monitoring at the ecosystem scale, as well as at local scale. The project evaluated 'smart technologies' to assist environmental monitoring and provide opportunities for improved mitigation responses to biologically relevant changes in environmental conditions.

The research is aligned strongly to the FRDC Environment Program with its focus on improving sustainable use of the marine environment through improved understanding of environmental changes and how these are ‘biologically perceived’. By providing and understanding an animal’s perception of environmental change, rather than an instrument’s measurement of a parameter, this project builds social understanding and confidence in environmental monitoring. The national RD&E Strategy goals include ‘Health of habitats and environments upon which fisheries and aquaculture rely are maintained’. The use of sentinels to better demonstrate biological responses to complex environmental changes clearly fits within this goal.

The sentinel animal monitoring project was undertaken with key partners: Aquenal are specialists in the assessment of coastal environments, playing a major role in monitoring in the selected southeast region, and therefore are potential users of the technology; the MDC is a well-respected educational facility situated in the selected region that provides opportunity for students to learn about the marine environment and how to care for it, therefore providing a critical public education aspect to sentinel biomonitoring; DPIPWE Marine Farming Branch is the key body monitoring the environmental conditions within Tasmania’s marine environments, and a potential key future advocate for incorporation of sentinel sensors in environmental monitoring regulation.

The base biosensor technology had previously been developed by CSIRO with a focus on use of the technology for improving commercial oyster production efficiency. This project focused on trialling the use of biosensor technology for environmental monitoring, utilising a species (oysters and mussels) that could be readily available via aquaculture production for future commercialisation of the sentinel concept.

Our current understanding of anthropogenic impacts on marine life, whether farmed or wild, is limited by a lack of technology to directly measure the responses of the animals themselves. Molluscs have been used as “biological early warning systems” because their swift behavioural response to environmental stressors is predictive of impending effects on other organisms in the ecosystem. Despite this potential, a lack of appropriate technology has prevented measurements of real-time data from animals in parallel with environmental monitoring. This project demonstrates the utility of using sentinel animals with novel biosensors to provide early warning information on the health and status of the marine environment. As human health monitoring relies on ‘vital signs’, this project measures the vital signs of heart rate and behaviour in sentinel animals, as they respond to multiple interacting changes in the environment. Such complex monitoring is not possible with infrequent water sample analysis, and the lack of real-time sensors for all potential stressors creates the need for a world-first rapid biological response system.

Figure 1. Mussel attached to biosensor during testing

The “animal-eye” view provides direct measurements of how animals perceive and respond to their changing environment, thus removing the guesswork in predicting ecosystem health based solely on environmental monitoring data. This project adds value to existing ecosystem monitoring and modelling research and provides the crucial missing piece in the puzzle – the biological response to environmental change. To test the feasibility of using sentinel oysters and mussels as a biological early warning system, the project links physiological and behavioural responses of filter-feeding molluscs to more traditional water quality measurements, producing a more complete picture of environmental changes and ecosystem health.

South-east Tasmania was selected as the study site, as it has a long history of environmental monitoring and physical and biogeochemical modelling, and has multiple key stakeholders in commercial aquaculture, other industries, the community, and the educational sector. A project advisory team comprising key stakeholders was established.

The project also provides data that can be assessed in the context of the trade-off between spatiotemporal data collection by providing high temporal information alongside of the current monthly sampling. This data and collection learnings will feed into any future hybridisation of the monitoring program.

Objectives

There are four project objectives:

1. Demonstrate an operational network of sentinel molluscs providing real-time streaming of physiological and behavioural data providing a biological perspective of the environment.

2. Improve the local economy's capacity to install, operate and maintain real-time, telemetered, environmental sensing systems by demonstrating the commercial operation of telemetered water quality sensor system over an extended 12-month period by a commercial operator.

3. Demonstrate that aspects of the ecosystem can be represented in real-time using sentinel animal data.

4. Provide an educational outreach program to encourage a generation of people interested in the marine environment and marine science.

Methods

Using CSIRO developed biosensors attached to sentinel molluscs, responses in behaviour (i.e. valve gape) and physiology (i.e. heart rate) were collected in parallel with environmental data (e.g. temperature, salinity, dissolved oxygen (DO), depth, chlorophyll). The project deployed real-time telemetered biosensors and water quality sensors (temperature, salinity and DO) for twelve months from autumn 2017 to autumn 2018.

Locations shortlisted for deployment included sites used in the Broad-scale Environmental Monitoring Program (BEMP) managed by Aquenal as a regulatory requirement of the salmon industry, sites within and adjacent to salmon leases, and sites that could be managed by the MDC as part of their educational program. Initial advisory team meetings helped develop the environmental sensing aspect of the project. The team determined the number and location of telemetered WQ sensors that could be installed and maintained with the project resources. Some logging (non- telemetered) environmental sensors were also deployed to provide better depth profiles.

Aquenal was involved in these deployments and the project was able to leverage the environmental data obtained as part of their program, as well as adding value to FRDC 2015-024 (Managing ecosystem interactions across differing environments: building flexibility and risk assurance into environmental management strategies). The project worked with Aquenal to add real-time sensing capability to selected BEMP monitoring locations to acquire high resolution temporal data at some sites where sentinel sensor strings are located.

Data was managed by Data61's Senaps (Sensor Cloud) platform. Data61 is CSIRO's business unit focused on digital disruption in the data and analytics spaces. The Senaps technology has been used internally in CSIRO for several years to ingest, store, process, and access data from a wide variety of sources. It was used for both the real-time and archival sentinel mollusc nodes and environmental sensors to provide tools to visualise the data.

CSIRO has been using in-house developed telemetry equipment in the field for several years. The project assessed that technology as well as the current market options to implement real-time telemetry.

In parallel with field data acquisition, limited lab-based experiments were conducted at CSIRO to tease apart the independent and synergistic effects that different environmental variables had on mollusc behaviour and physiology. This part of the proposal builds on the Sense-T project (June 2015 to Dec

2016) focused on determining the metabolic and stress responses of Pacific Oysters under commercial production models. This FRDC project adds value to the Sense-T project by extending the work to mussels and assessing the potential utility of the sentinel mollusc beyond assisting husbandry in production systems and demonstrating the potential role that biological early warning systems could play in whole-ecosystem management.

Members of Data61's analytics and machine learning group applied their skills to interpret the sentinel animal and environmental data in ways that provided simple outputs to members of the project advisory team. These algorithms were then uploaded into the Senaps infrastructure, executed in real time and linked to simple visualisation tools as a way of demonstrating the potential of this sentinel animal concept to provide ecosystem health awareness.

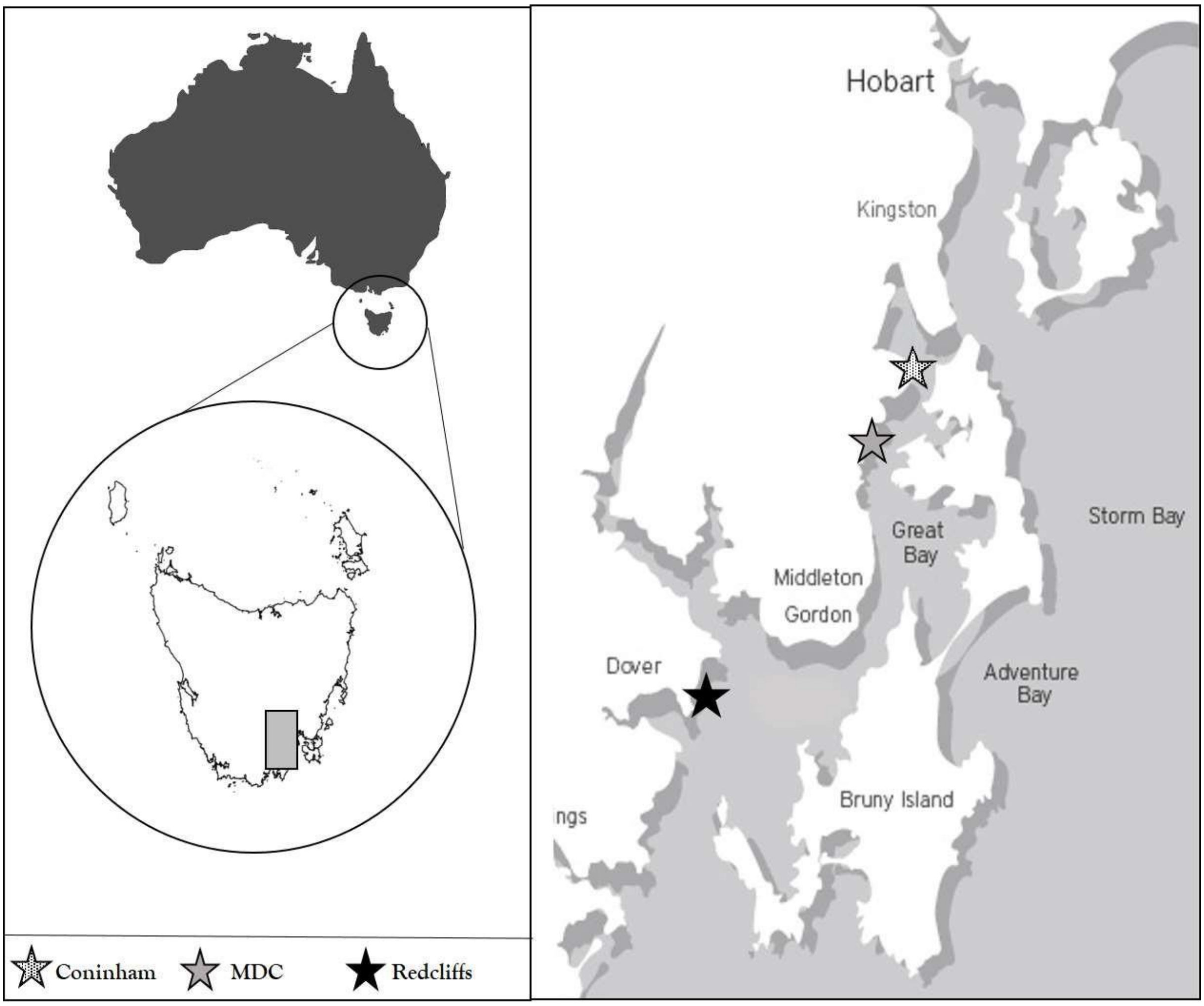


Figure 2. Field Deployment locations at Coningham and Redcliffs

Field Deployments

Planning

A significant component of the project was the development and deployment of the field equipment. Meetings were held with the key internal and external stakeholders to coordinate details of the field deployment. Some of the plans changed through the project as biosecurity issues along with options for suitable deployment sites and available infrastructure were worked through. This was a collaborative approach, initially with two of the key project proponents (the State government and Aquenal). It became apparent that commercial fish-farm leases may be the ideal field locations, and a working relationship was developed with Tassal to facilitate the deployment. Planning, resourcing and developing the equipment across organisational boundaries was a major challenge and led to significant delays in getting equipment ready for deployment. The initial concept was to use independent biosensor and water quality measurement systems mounted on buoys near BEMP sampling locations, but this was changed to combined systems mounted on barges.

In terms of placement, it was desirable to cover locations that gave different perspectives on the channel area, ideally one to the north of the channel and one further south. After considering the options such as water conditions, available/required infrastructure, budget, other sensor coverage, site depth, accessibility for servicing and physical marine conditions, the working group determined that the two locations for the field deployments would be Coningham and Redcliffs. Coningham is in the north of the channel and has different water conditions to Redcliffs in the south (where the water is influenced by meeting of the Huon mouth to north, mid-channel to north east and the sea from the south). At both sites the seabed was accessible, should issues arise with the deployed gear. Figure 2 shows the key locations (Coningham, Redcliffs and the MDC). GPS coordinates of the deployments are: Coningham Lon / Lat: -43.0915, 147.3015 and Redcliffs Lon / Lat: -43.3120, 147.0830. Initial plans were to use a Tinderbox site with a dual mooring buoy instead of the Coningham site, but complexities with the deployment of the moorings led to a decision to switch to the nearby Coningham site to allow a simpler barge mounting. A third field location, on infrastructure maintained by the MDC, had been considered as an extra element to the teaching modules and as a backup biosensor storage area for the main deployments, but as the project developed it was decided that the internal facilities of the MDC were a more convenient testing and storage location for the spare biosensors.

The suite of sensors to be deployed was partially dictated during the initial project planning phase but there was scope for the working group to refine the complete data acquisition configuration. The main WQ sensing components (two Xylem EXO2 multi-parameter sondes with Storm3 3G/4G telemetry systems) were selected by CSIRO as the project was being planned. These components represented a major capital expenditure, which was out of scope of the budget for this project and required significant pre-planning to ensure procurement. One of these sensors was made available for deployment at each deployment site. Similarly, the biosensor component was selected by CSIRO as it is the developer of that technology. Six biosensors were available to be deployed at each site. To complete the sensing, and get better water column profile information, the working group added the requirement of a series of non-telemetered logger units to be added alongside of each of the biosensor units to get better water column profile information.

The objective was to have five sets of loggers at each site alongside each of the biosensors (noting the EXO2 units are alongside the sixth biosensor). The project was able to fund six Hobo DO loggers and six Hobo CT (Conductivity/Temperature) loggers for this purpose. It was proposed that the industry partners could supply the remaining four DO and CT loggers to complete the coverage.

The high-quality telemetered WQ sensor was placed at a depth of 5m. The biosensors were deployed at a range of depths (0.5m, 1.5m, 3.5m, 5m, 7m, 10m) to provide a wider understanding of the environment. Figure 3 shows the final configuration of field deployed sensors.

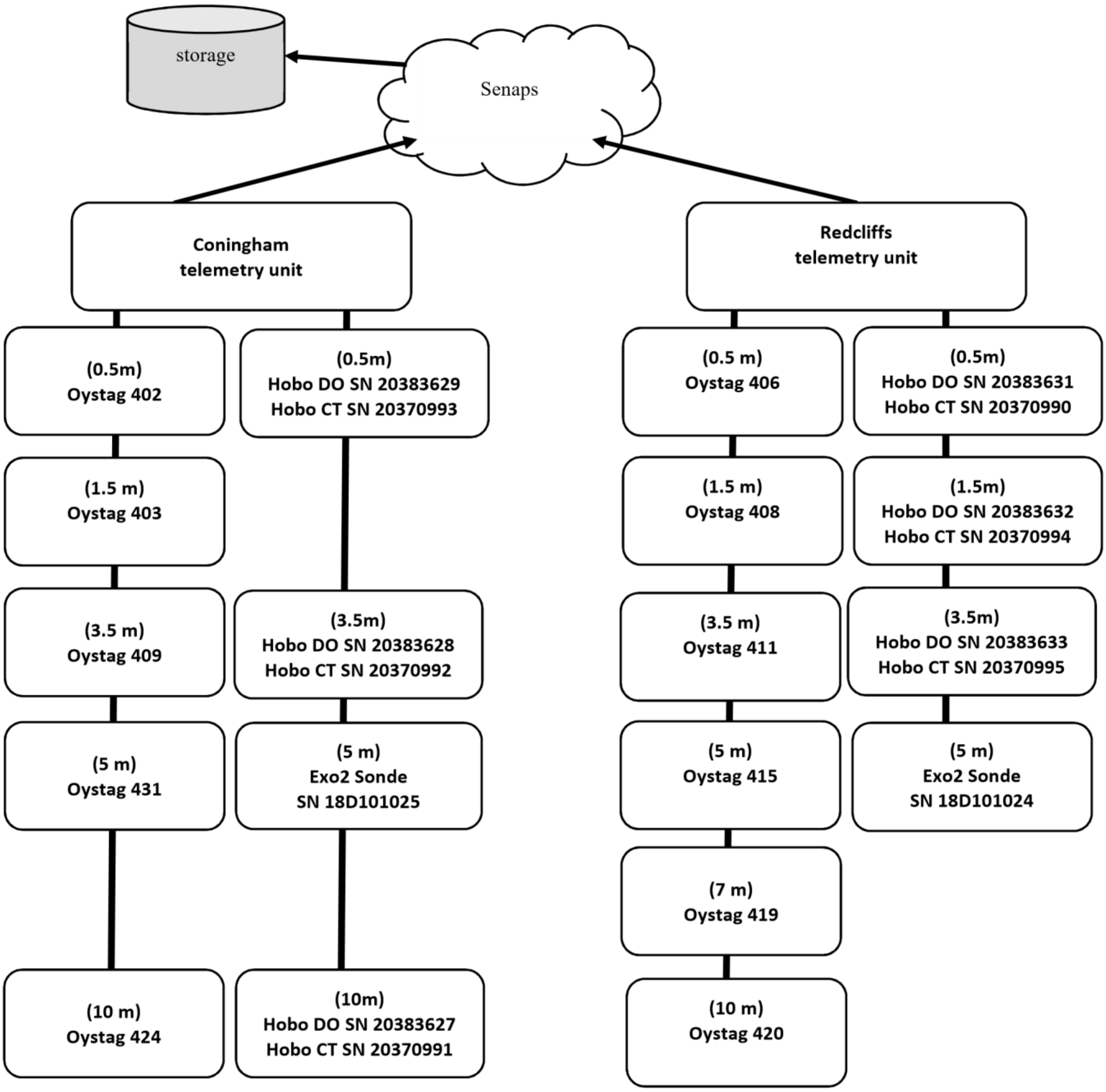


Figure 3. Connectivity of sensor systems deployed in D'Entrecasteaux Channel

In the final configuration we were unable to source the extra Hobo logger units so each of the sites had some depths not covered by water quality sensors. The uncertainty about accessing the extra loggers and time between deployments were factors that resulted in the difference between the two sites in terms of deployed Hobo logger depths. On-the-day deployment issues (human oversight) prevented one of the 7m biosensors from being deployed.

Equipment - Biosensors

The biosensors that were built for the field deployment are an upgrade of designs used in earlier projects. The basic sensing methodologies are the same; they use an infra-red optical reflectance plethysmograph sensor to measure heart motion, and a magnetic field hall-effect sensor to measure valve (shell) gape angle. The design used in this project has an upgrade to the plethysmograph as well as the addition of a new external interface and processing module. The plethysmograph change was to do away with the external leads to the sensor head, as they were prone to damage during lengthy deployments. This change puts the sensor head as a permanent attachment (or part of) the main sensor board, which changes the physical mounting of the oyster on the biosensor, changes which may affect the quality of both the heart and gape signals, so testing was required.

The other change was to assist with remotely retrieving data from multiple devices. Having multiple units with the original RS-232 interface added complexity to surface electronics – especially from a power management, cabling and data transfer perspective. Also, multiple units created more data and the requirement to use a low cost IoT style telemetry system. There was a need to reduce the amount of data transmitted. To address the interface and cabling issues, the new sensors were designed to be daisy-chained over a Controller Area Network (CAN) bus. To address the data communications challenges, an on-board signal processing algorithm was introduced to allow processed heart rate data — a beat-per-minute (BPM) statistic — to be sent, rather than high-rate raw heart signal data. Previously, heart rate calculations were performed in the cloud.

To include the CAN bus and the required heart rate calculation, a second microprocessor board was added to each sensor, rather than redesigning the biosensor board itself. This reduced technical risk but did make for a slightly larger package as both microprocessor boards (the original sensor and the CAN interface) were encapsulated in resin together. This version of the Biosensor board was ~10mm thicker. The daisy-chain configuration also had these Biosensors manufactured with ‘in’ and ‘out’ (male and female) underwater connectors rather than a single fixed-length interface cable. Figure 4 shows the various versions of the biosensor designs.

An algorithm was developed and deployed on the devices to analyse the raw heart signals and provide a rate statistic in BPM. The algorithm also provided three diagnostic signals to assist with validating the result of the calculation: a metric indicating the periodicity of the heart signal (a measure of the extent to which the signal contains a repetitive pattern such as expected from a heart signal), a metric indicating the maximum range or strength of the raw heart signal, and a similar metric indicating the variance in the raw heart signal. Implementing the algorithm on board the small sensor system microprocessor was a significant technical achievement as the microprocessor is limited in memory and power. It required, among other things, an efficient in-place implementation of an autocorrelation function.

At power-up, this new microprocessor activated the second biosensor ‘sensor’ board, recorded 30 seconds of raw data, powered down the biosensor ‘sensor’ board, calculated the HR (in BPM), and then transmitted a single value for all the sensors (with heart rate instead of raw data) on the CAN bus. If the system remained powered, it repeated this process every minute. (Note - this repeating functionality was not utilised in the complete system as the surface node powered down sensors after receiving the first lot of data.) Sampling for thirty seconds was a trade-off between calculation accuracy and system power usage. When only acquiring 30 seconds of 20 samples per second (20Hz) data, the resulting resolution of the BPM calculation suffers from quantisation errors at higher (but feasible) heart rates/signal frequencies. To counter this quantisation error, without increasing power usage, a mathematical technique reliant on the noise in the system was added to subsample the autocorrelation result, giving a much-improved result.

Figure 4. Evolution of biosensors– top left original flying lead version, top right new heart sensor version, bottom new field version with daisy chain Controller Area Network (CAN) interface

Equipment Surface Node/IoT – Connectivity

As part of the project, CSIRO developed a new interface for the biosensors. The original form factor of the biosensors was a device that live streamed raw data out a standard RS-232 serial port. This interface was not suitable for field deployment of larger numbers of devices due to requirement for individual cabling and interface ports as well as the larger volumes of data in the raw signals that needed to be transmitted. CSIRO augmented the individual biosensors with a new CAN bus interface to allow a multi-drop (daisy-chain) topology of sensors so a single cable and single surface interface was required. These smart interfaces also had the heart rate calculation algorithms installed and executed on board to remove the need to transmit the high volumes of raw heart data over the airwaves.

CSIRO also developed the new surface module (Figure 5) to interface to this daisy-chain of biosensors. This interface controlled the sampling and sensor power, received data from the biosensors and transmitted the data over the airwaves. It was configured to power up the biosensor string every 15 minutes, wait for it to sample the animals (in the order of 1 minute), power down the biosensor string, transmit the results and go back to sleep until the next 15-minute trigger occurs. This surface node also had the ability to monitor its own system health and performance (up-time and power availability).

To assist in demonstrating the feasibility of installing multiple sensors in a commercial setting a lowcost telemetry interface was required, so the surface module was developed to use an emerging IoT technology called LoRaWAN. LoRaWAN is suitable as it has a long radio range (10’s of km in ideal conditions) is extremely low power (important for remote devices) and has a low running cost (no SIM cards like 3G/4G). This technology does require base station infrastructure to operate, and whilst LoRaWAN technologies are now being rolled out across the country, none were available in Tasmania for this project, so CSIRO also needed to install off the shelf base stations for the purpose of the project.

Figure 5. Field biosensor interface and LoRaWAN telemetry unit. External connectors visible at right are for solar panel and daisy-chained sensor cable

Initial Field Tests – Biosensor + Telemetry

The early stage of the project involved field trials of the new biosensor and telemetry interface designs in Hobart. Field tests were completed to verify performance of the new animal mounting system (new heart sensor and gaping sensor), the heart rate calculation, the multi-drop CAN interface and the IoT connectivity before larger scale manufacture was undertaken. Testing off the CSIRO wharf was undertaken in two stages to confirm system wide operation.

The first stage involved deploying four newly manufactured units off the CSIRO wharf (Biosensor #416, Biosensor #423, Biosensor #427, & Biosensor #429) at 1m depth intervals, with the first one being just intertidal. Oysters were placed on the sensors a couple of hours before deployment, but two units had animals transferred from other sensors in the lab i.e. there was no drilling/minimal handling for these two animals. Deployment was on the 21st Dec 2017 and competed on 12th Jan 2018. Stage 2 redeployed three of these sensors on January 12th and continued until January 22nd.

Equipment – Field Stations

As indicated, the two field sites each had biosensors and WQ sensors deployed. The biosensor equipment included a solar power system, the surface node described above, and the string of biosensors. The WQ sensors consisted of a commercial WQ sensor and telemetry unit along with several self-contained Hobo loggers (dissolved oxygen and Temperature/Conductivity models). The commercial system contained a YSI Storm3 telemetry system with its own solar-based power system, and a Xylem EXO2 multi-parameter sonde fitted with temperature, conductivity, dissolved oxygen, chlorophyll, pH, oxidation-reduction potential (ORP) and turbidity sensors.

For robustness and simplicity, the initial surface design had the two telemetry and power surface components as separate items. As it was to be mounted on a buoy where orientation could not be controlled, each system had dual back-to-back solar panels. To reduce the risk of the buoys inverting in severe weather, the centre of mass was lowered by combining the two systems into one enclosure. The two battery and charge systems were electrically isolated for reliability. However, the system was finally deployed on tethered barges, and redundant systems were retained. The buoy mount was simply fastened to the edge of the barge decks.

Finally, as planning evolved it became clear that complexities of getting LoRaWAN base stations installed on private property were more significant than switching the biosensor telemetry solution to use 3G/4G connectivity. This required adding a 3G serial-to-TCP/IP modem per unit.

Pre deployment biosensor preparation

As a method of testing of the biosensors and in preparation for system deployment, a third biosensor surface node/telemetry system was set up at the MDC. For biosecurity reasons, permits were obtained to translocate oysters directly from the North East of the state to the MDC and then to our deployment locations. We deployed approximately 20 animals and biosensors at the MDC in the weeks leading up to deployment so we could choose the best performing ones for deployment. Prior to deployment the selected oysters were attached to biosensors and allowed to acclimatise in aquaria.

Field Deployment 1 - Coningham

Oyster biosensors, EXO2 multiparameter sondes and Hobo loggers were deployed at marine farm lease no. 94, west of Sheppards Hill near Coningham (-43.0915 longitude, 147.3015 latitude) on 9 November 2018. Deployment used four personnel and one vessel. Due to a logistical error, five biosensors were deployed at this site instead of the planned six. The telemetry unit was secured to a feed barge deck, and sensor string shackled nearby. The sensors were inspected and cleaned again in December 2018, March 2019, and August 2019 (at which time the Hobo loggers were retrieved). At each inspection, the EXO2 sonde's instrument heads were found to be clear of biofouling and obstruction, due to the inbuilt cleaning brush working well. Oysters and biosensors tended to accumulate biofouling, but this cleaned easily and did not appear to affect oyster growth.

Figure 6. Coningham surface equipment

Field Deployment 2 - Redcliffs

The second set of sensors and telemetry system were deployed at marine farm lease no. 201, Redcliffs (-43.3120 longitude, 147.0830 latitude) on 18 December 2018. As before, four personnel were used. A heavier end weight was selected on this site due to more exposed location and currents. The telemetry system was mounted to a small floating platform alongside a salmon pen. The HOBO loggers were retrieved in May 2019.

Figure 7. Redcliffs surface equipment

Data Analysis Methods

The primary objective of the data analysis was to understand the relationship between oyster's physiological behaviour and WQ. Driven by this objective, the analysis aimed to study: 1) how the environmental conditions modulated physiological response of oysters by utilising the data from EXO2 and biosensors, and 2) what happened to WQ variables during the sudden and abrupt changes in HR.

The analysis of data from multiple sources (e.g. biosensors, EXO2 multiparameter sondes) was performed in several different steps: data collection, data pre-processing, model development, event driven approach, and result analysis. The first four steps are separately described below whereas the result analysis is presented in the next section.

Data Collection

As a part of testing long-term low-maintenance biosensor systems neither the biosensor strings nor biosensor telemetry systems had any maintenance since deployment (although they were 'handled' when the WQ sensors were cleaned).

Table 1 below provides the list of biosensors and their depth in water column at both sites.

Table 1. Oyster biosensor Id vs depth, at Redcliffs and Coningham sites

Depth [m]	Redcliffs biosensor Id	Coningham biosensor Id
0.5	406	402
1.5	408	403
3.5	411	409
5	415	431
7	419	-
10	420	424

Data Pre-processing

The pre-processing of data involved time synchronization and cleaning. The WQ data was uniformly sampled every 15 minutes whereas the sampling interval for physiological data varied from 5 to 15 minutes depending on oysters. To time synchronize the physiological data for all oysters with WQ data at each site, a sliding window technique was applied. A window of 15 minutes was moved along the physiological time series data such that it did not include any overlapping data samples. If there were multiple measurements within any 15 minutes segment of a time series, they were replaced by the measurement which is closest (in time) to the end-boundary of the window.

Model Development

The model development exercise involved developing mapping functions to translate WQ variables to physiology and vice-versa. The goal was to develop data driven machine learning models for this purpose. It is important to understand how well the water quality variables relate to physiology before they can be used for modelling. Hence, the model development approach first evaluated the merit of each WQ variable for predicting HR (and vice-versa) by assessing the pair-wise correlation between them. It then formulated a multivariate regression problem based on machine learning models to predict HR that used WQ variables showing high level correlations with HR as inputs, and to predict WQ variables using physiological data (both HR and gape) as inputs.

Machine Learning Based Mapping Model: Data Set Preparation and Validation

A machine learning model learns a mapping from input to output based on historical data. We developed two data sets. In the first one, the input side consisted of WQ and the target was HR. In the second one, the input side consisted of HR and gape, and the target was WQ.

In the first data set, a separate set of input-output samples was prepared for each oyster where the input side was constructed using the most recent 1-hour measurements (i.e. last 4 measurements) of the WQ variables that had correlation coefficient higher than a threshold (set as 0.5). For example, the input side for oyster with biosensor Id 402 consisted of 4 most recent measured values of Conductivity, ORP, pH, and Temperature (thus 4×4=16 inputs altogether) whereas the input side of oyster with biosensor Id 406 consisted of only 4 most recent measured values of Temp (thus only 4×1=4 inputs). The output side was the HR for the oyster in consideration. The samples for each oyster were then fed into machine learning algorithms to develop prediction models.

In the second data set, the WQ variables whose mean correlation with HR for all oysters was higher than a threshold (0.5) at both sites were considered. Only two (Temperature and Conductivity) out of eleven WQ parameters satisfied this condition. Hence the subsequent description of the prediction approach and analysis of results for WQ prediction only focused on these two variables. For each WQ variable, we first prepared a separate set of input-output samples where the input side consisted of physiological data (i.e. HR and shell gape) recorded in the last 1-hour (i.e. 4 recent measurements for both HR and gape) for an oyster. The output side consisted of the measurement of the WQ variable of interest. For each WQ variable, it constructed N sets (one for each oyster) of input-output pairs, where N was the number of oysters. A machine learning algorithm was then applied to N data sets separately that resulted in N different prediction models. The N outputs from N models were then combined into a single prediction by taking their weighted average where the weights were the correlation between the HR data for an oyster and the considered WQ parameter. A 10-fold cross-validation was then applied to evaluate the predictions.

The machine learning algorithms that were applied for prediction of HR and WQ included Neural Networks (NNs), Support Vector Regression (SVR), Random Forest (RF), and Linear Regression (LR). The rationale behind using different machine learning algorithms was to find the algorithm that best suited the data set for each oyster and develop a customised prediction model for each oyster. All the prediction models were evaluated by following 10-fold cross-validation technique.

NNs are state-of-the-art machine learning models. They can estimate any complex functional relationship (both linear and nonlinear) and can learn this relationship from data. NNs based prediction models were developed based on Multi-Layer Perceptron (MLP) NNs that are considered universal function approximators.

SVR is another popular machine learning algorithm for time series prediction. Like NNs, SVRs also can learn complex input-output relationships and extract patterns from examples. The key idea of SVR is to map the input data into a higher dimensional feature space using a non-linear transformation and then apply linear regression in the new space. The linear regression in the new space corresponds to non-linear regression in the original space.

RF is a representative of the rule based and ensemble learning algorithms. The basic idea of RF is to create a forest by fitting several Decision Tree (DT) based learners that estimate the value of the target variable. They generate a set of rules learned from the input features and then combine the prediction of all the individual decision trees by taking their average. Each tree in the forest was trained using a randomly drawn subset of input-output examples. All the subsets were equal in size and drawn using bootstrapping (i.e. sampling with replacement). The features for splitting the nodes of the tree were always randomly permuted at each split. These increased the diversity in the learning process and led to more robust overall predictions compared to the single DT based prediction algorithm.

LR is a classical algorithm that assumes a linear relationship between predictors and target variable. It uses the least square method to find the regression coefficients.

Event Driven Approach

To investigate the changes in WQ variables during the events in the HR data, a separate analysis was carried out. The analysis manually identified the sudden and abrupt changes in HR data and considered any such change as an event. It then evaluated the relationship between changes in HR/gape during the events and any changes in WQ at same time as well as up to 2-h before the events. This events-based analysis utilised the WQ data measured by EXO2 sensor deployed at 5.0 meters depth at Coningham site and HR/gape data for an oyster (biosensor Id 431) at the same depth and location.

Figure 8. Marine Discovery Centre at Woodbridge, Tasmania

Photo credits: Woodbridge Marine Discovery Centre

Education Outreach

To deliver the goal of inspiring the next generation to a career in marine science, the project developed and delivered teaching modules around environmental monitoring and sentinel animals.

These modules were developed in coordination with educators from the MDC (Figure 8). A series of meetings were held to organise the teaching module. Additionally, visits to the centre were organised to assess facilities.

The teaching module plans were prepared according to students' ages and knowledge levels. The teaching modules covered diverse activities such as lectures, experimental activities, and field activities. The full educational modules included details on the preparation for class delivery, the educational material itself, equipment for experimental activities, and the biosensor technologies with the associated interaction software that the students would interact with.

Appendix B and Appendix C show the educational materials, and Appendix D shows an extended proposed future module outline.

The program covered daily classes of 2 hr per group of students. As part of the activities, students were able to understand basic information of bivalve's biology, physiology, and anatomy (bivalves' dissection). Other relevant topics such as environmental issues, sensor technology, history of oyster farms in Tasmania, etc. were also delivered. Altogether this gave an experiential understanding of why biosensors are important, how they can be used and why technology is necessary.

Figure 9. Facilities at the Marine Discovery Centre at Woodbridge, Tasmania Photo credits: Woodbridge Marine Discovery Centre

MDC demonstrations

To give students a diverse and practical experience during this course work the project planned to deliver biosensor hardware and software that they could use in coursework. The initial plan was to provide an interface for individual sensors to individual PCs to provide live viewing of the heart signal. As discussions with the MDC evolved it emerged that that configuration had limitations around continuous data streaming and online access to the data. A complimentary system that could also stream data and provide data access via a cloud service would also be useful to allow students to access historical data as well as real time data both at and beyond their physical visit to the MDC. For this reason, two interactive biosensor hardware and software systems were built specifically for the MDC. One configuration was for continuous laboratory operation and observation and one for interactive use in an individual class session. This enabled students and visitors to visualize animals, connections and signals at the same time, providing an understanding of the animal physiology and how it can be applied to inform environmental conditions, as well as allowing students to interact with the technology to learn more.

MDC Biosensors

The biosensors used with both systems were like the original Oystag design built for the Sense-T stage 1 project, with one key difference. With the newer design the optical heart sensor was a part of the main device as opposed to being on a flexible flying lead. This change was made because the flying lead had robustness issues as it was mechanically vulnerable to damage. This new design required the animals to be mounted differently so testing was required to ensure the biosensor operated correctly. This testing is described in detail in the Initial Field Tests – Biosensor + Telemetry section as that biosensor had the same configuration.

The biosensors used at the MDC all had a serial communications interface over a fixed 5m cable with a 4-pin IP67 Hirschman plug on the end. The interface on the cable was the original RS-232 protocol which was suitable for the data rates and cable lengths used. For this reason, they required USB to serial converters to interface to modern devices or PCs. The units were configured to stream out the full set of sensor data (heart, gape, internal temperature, water temperature, pressure/depth, light, and several engineering/diagnostics parameters) 20 times every second.

MDC Live Data Webserver

The first of the systems built for MDC was a real-time (running 24/7) biosensor web server/interface that connected to four biosensors and provided a web interface to display live data. It allowed people to connect via Wi-Fi and a web browser to see live streaming and historical data from any of the four animals that were permanently wired up and living in aquaria in the lab. It was designed this way to work with the existing infrastructure at the MDC. The MDC had wall-mounted tablet devices next to the aquaria that the animals were in. These tablets were configured to connect to the Wi-Fi interface and display the live heart and gape signals from any selected animal (Figure 10). Students and visitors could visualize a longer-term view of data from the biosensors alongside the source animals and sensors. This helped them understand animal physiology and how it can be applied to understand change in the environment.

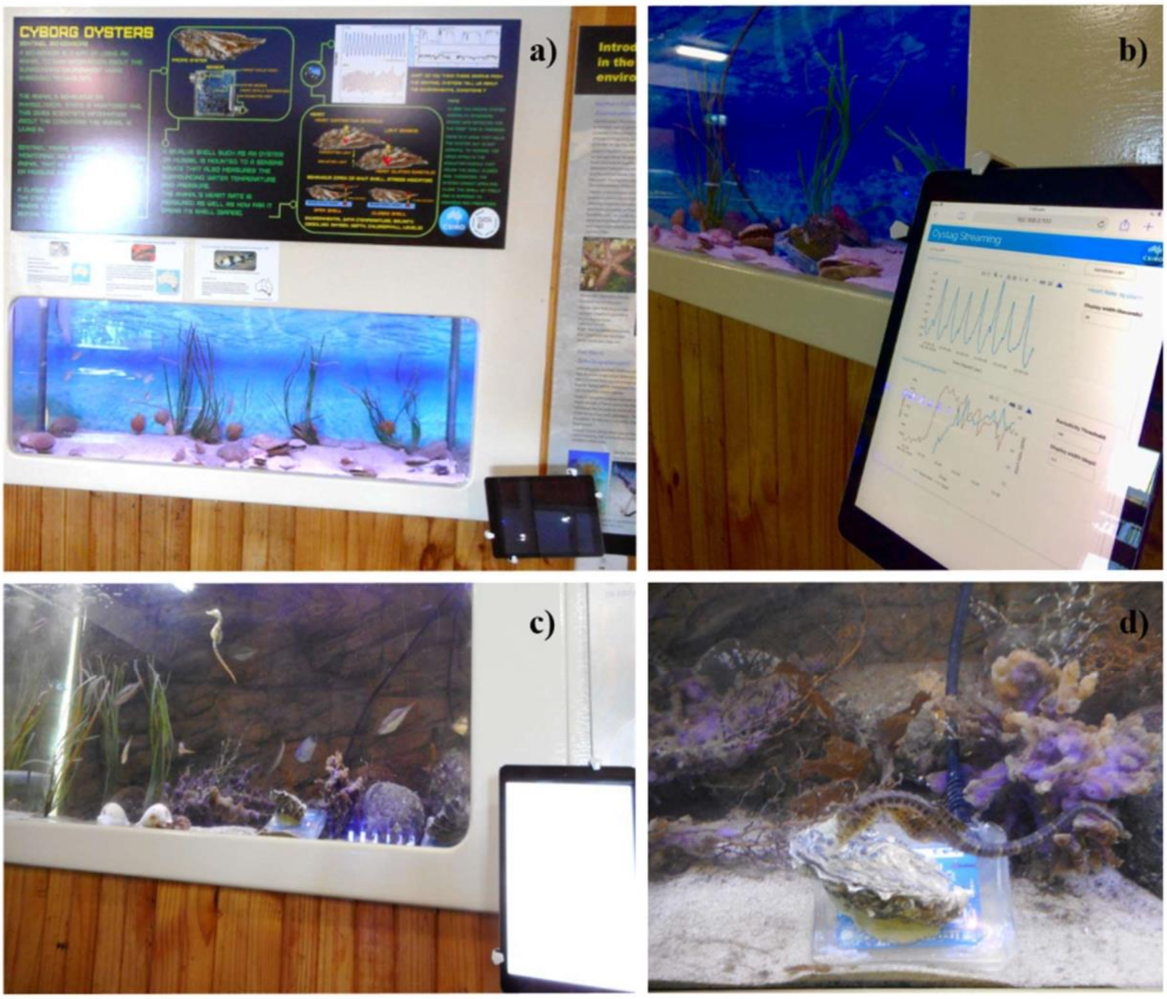


Figure 10. Educational material at the Marine Discovery Centre. a) Display set upper educational aquarium, with sensor and iPad showing real-time data, b) Real-time data from biosensor, c) General view of educational aquarium, sensor and display, d) Biosensor deployed at indoor aquarium

This unit was based on a RPi 3+. The required USB-RS232 adaptors connected the RPi's four USB ports to Hirschman connectors on the case to allow the four biosensors to be plugged in. Internet connectivity was obtained internally via a TP-link router and a Sierra Wireless 3G/4G modem. Alternatively, the system could be directly connected to the MDC's internet via an ethernet port/cable. The longer-term plan was to move to using that configuration, but it required a security audit to ensure the system was not vulnerable to external cyber challenges. The unit required an external 2A 12v supply and included an internal 5v regulator for the RPi. Figure 11 shows the unit with lid off and one biosensor attached.

A common problem with embedded RPi based devices (devices with no keyboard/screen connected) is how to shut the system down safely before unplugging the power. If there is no mechanism to do this then the system is vulnerable to corruption when power outages are required. To ensure the system could be shut down cleanly a reed switch was wired to the RPi, and a script written that monitored the status of that switch. If the reed switch was activated the script did a safe shutdown in software. There was a marked location on the outside of the enclosure to enable activation of the reed switch with an external magnet. To shut the system down you waved a magnet over that location and then waited 30 seconds for the system to shut down before you could safely remove the system power.

There were two main software components running on the RPi. They were both quite sophisticated, but essentially, the first one read the live data from the sensors and stored it locally as well as uploading it to CSIRO's cloud data and analytics service called Senaps. The second hosted and served up a web page that displayed the historical and live data coming from the attached sensors.

Figure 11. Left: Oysters with laboratory biosensors attached, Right: Telemetry system with a biosensor connected

To read the data from the sensors the first script monitored the four USB ports for connected biosensors. It was supervised to ensure that hot plugging sensors in and out worked seamlessly. As the data arrived from the sensors (updates 20 times per second) it was processed. The raw heart data was kept in its entirety and processed using an on-board signal processing algorithm which generates the heart rate in beats per minute (BPM). The gape data was stored once per second, and the remaining, slowly varying, signals (light, temperature etc) were stored once per minute. The script stored this data in a local database as well as optionally sending it to the Senaps cloud platform. The local storage was used by the second software component to serve up the web portal, and the data in the Senaps platform was remotely available for viewing or historical analysis.

The script used a sophisticated caching mechanism around uploading of data into Senaps. It had the ability to automatically forward historical and live data to Senaps even under intermittent internet connectivity. This provided continuous historical data should internet access be interrupted. Upload priority (to Senaps) was given to live data so as soon as the unit came back online after an internet outage, the live data stream was sent preferentially whilst the backlog was sent in the background.

The second software component used the Plotly/Dash webserver software framework to generate the visuals and serve up the live data streams. This system allowed you to select any of the connected biosensors from a drop-down menu and then displayed both a 30-second trace of the raw heart signal and a beats per minute display (updated live) as well as a longer 5-day trace of the gaping behaviour and calculated heart rate (also updated live). Both displays had configurable time windows to allow examination of longer / shorter term behaviours. The RPi was configured to run a wi-fi hotspot to allow third party devices to connect in and access the web page. The Dash software was configured to use server-side data caching to give an acceptable viewing performance (minimising on-air traffic and load on the RPi). Figure 10-b shows the system operating at the MDC with one of their wall-mounted tablets connected to the RPi's wifi and displaying a live display of heart signal (top graph of the screen shot) and gaping and heart rate (bottom graph of the screen shot). Figure 10-a shows a poster display about the system at MDC, and Figure 10-c & d show the biosensors with animals in their tanks.

MDC Laptop Interface

The second system developed for the MDC was a laptop application that showed live streaming of data from a single connected sensor. From a hardware perspective this was much simpler. Adaptors were made that provided 12v power from a plug-pack as well as converted the RS232 data interface to USB so they could be plugged into a standard laptop. LabVIEW software was written that reads and visualises data from any connected biosensors. To make it as easy as possible to use, off the shelf USB converters were used that install automatically under windows, and some smarts were installed into the software to automatically monitor and detect USB devices and biosensors as they are connected/disconnected from a system. Also, to make it simple to configure, an installer was produced for the software to allow it to be easily installed on most PCs. The software interface was similar to the webserver version, but it had the ability to display the full range of signals coming from the biosensors (heart, gape, temperature, pressures, & light + diagnostics). This system allowed students to explore how the sensors work (e.g. they can detect their own pulse by placing a finger on the sensor, or use a magnet to emulate gaping behaviour) as well as connect up individual animals to see signals.

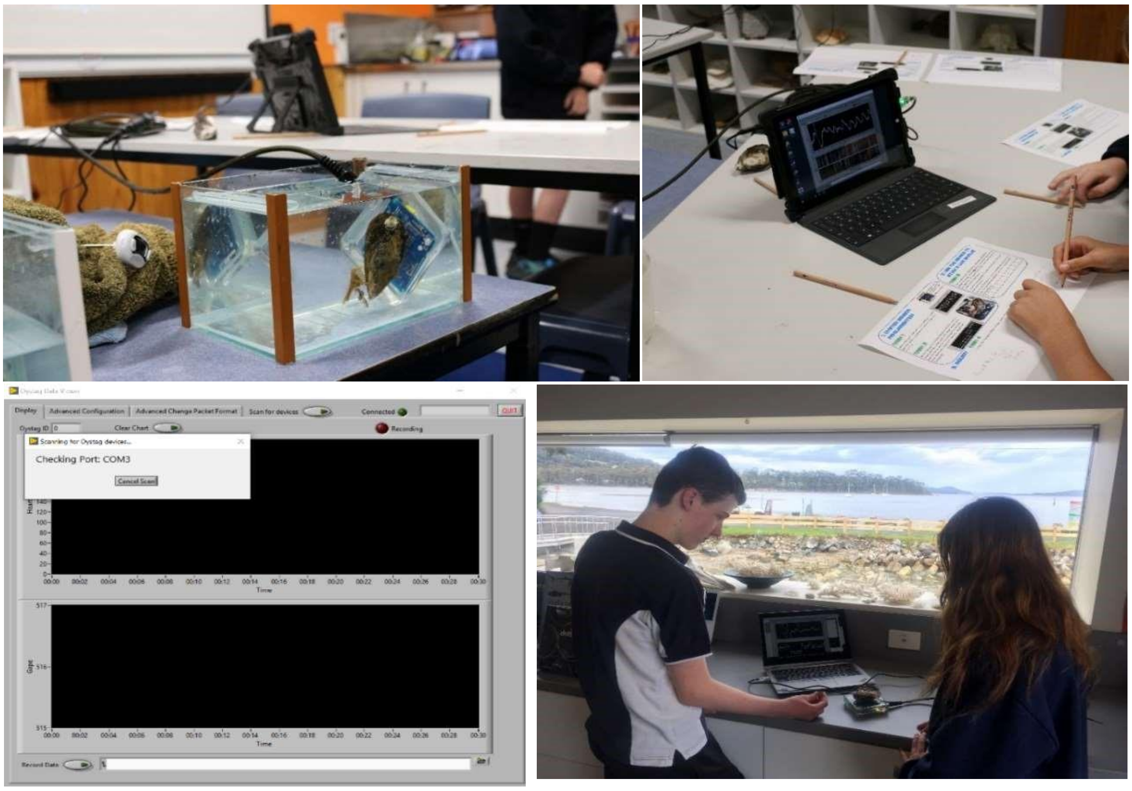


Figure 12. Lab biosensor setup showing students using the system and the default LabVIEW display whilst the automated sensor detection is running

Photo credits: Woodbridge Marine Discovery Centre

Figure 12 shows students working with the laptop interface in action at the MDC (bottom left is a screenshot of the system in the mode of automatically searching for and detecting sensors).

Both systems were developed, tested, delivered and used at the MDC as part of the education outreach component of the project.

Results and Discussion

Initial Field Tests – Biosensor + Telemetry

Analysis of LoRaWAN Field Telemetry System

Figure 13 shows the elapsed time between data points arriving via the LoRaWAN radio system. The three levels were expected due to different wake up and transmit behaviours (the node transmits initial data after waking but before powering up the Biosensor bus to facilitate remote debugging of issues).

The large gap in the timeline on the chart was due to a failure of the backend system over the Christmas break (that was not rectified until staff returned from leave). Other than that, and an occasional manual intervention of the system for testing (the random dots on the plot), there was 100% throughput. It should be noted that the system does not guarantee 100% throughput, however good throughput is still expected based on other LoRaWAN radio testing. The large outage was due to a hard drive that filled up due to logging of debugging information. It was rectified and systems put in place to prevent its reoccurrence. The tests also confirmed solar charging and battery voltage reporting were operating correctly, and that the incorporated GPS was successfully reporting location to the cloud.

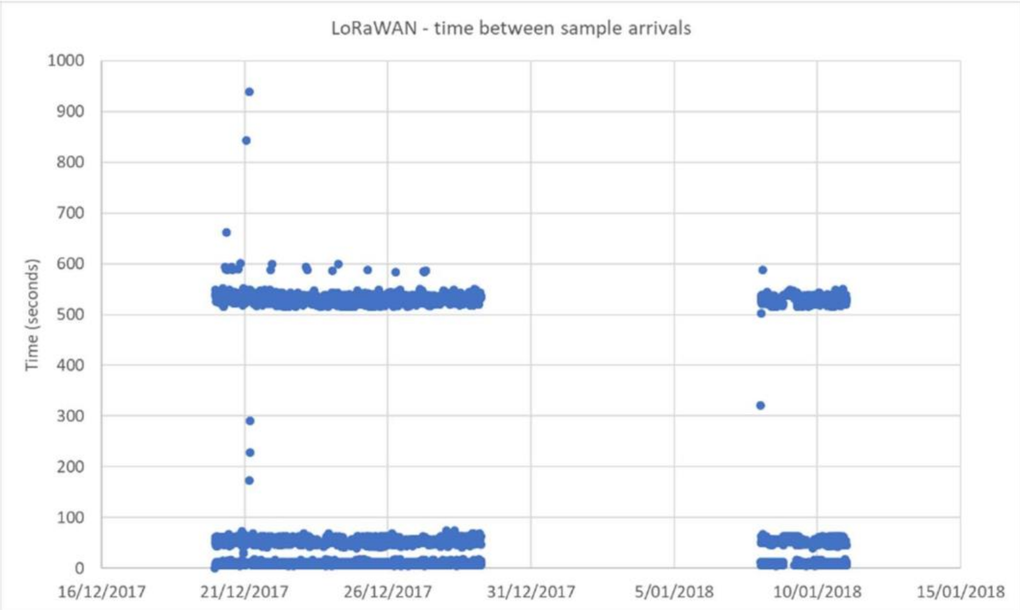


Figure 13. Elapsed time between data samples arriving from the surface node during initial field testing

Analysis of the mounting system

The new mounting system had the potential to affect the quality of both the heart and gape signals. The heart signals collected during this deployment were good (further details and examples are in the section on the analysis of the heart rate algorithm below). Similarly, with a little practice in animal placement the gape signals were also strong. Figure 14 shows a classic gaping pattern from one of the animals during the deployment. Whilst the polarity of these gape signals is determined by the polarity of the attached magnet, the recommended approach is placing the magnet such that shell closed gives lower signals and higher signals occur as the shell opens. The signal values are raw counts from a 10bit analogue to digital converter (ADC), so a value of approximately 512 represents no magnet detected. The step change in behaviour on the 12th January was a result of relocation of this animal to a different depth.

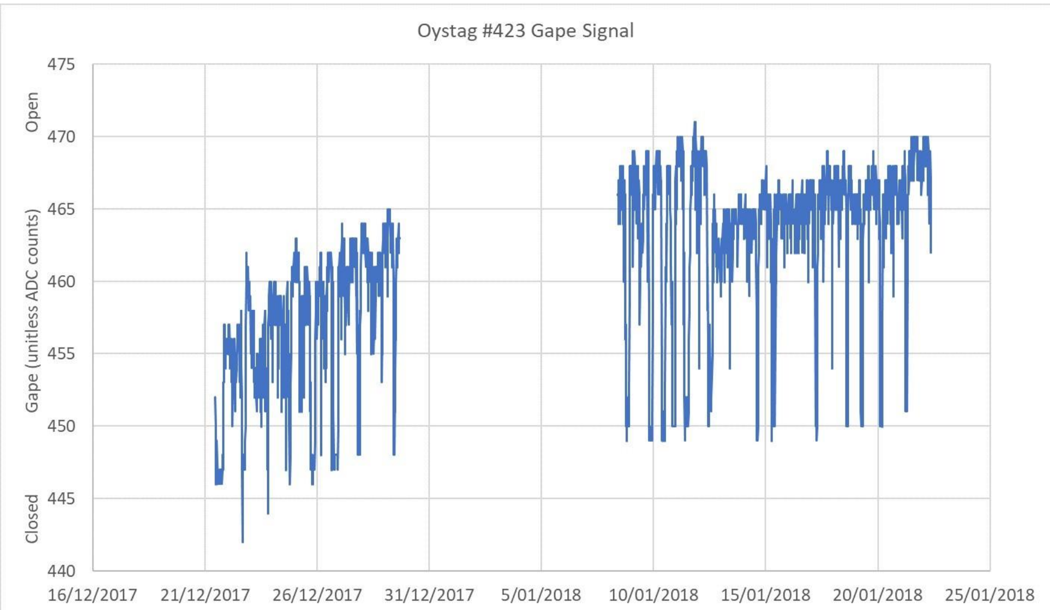


Figure 14. Gape signal (low values closed, high values open)

Analysis of the CAN bus daisy-chain system

The surface telemetry node was able to successfully control and receive data from the four daisychained biosensors for the duration of the trial. Specialised cables had been manufactured to reduce risk of long cables causing issues, and the test verified that signals could be successfully received over the required 10 meters of cable.

Analysis of the heart rate algorithm

Figure 15 shows a filtered version of the heart rates reported by the devices. These show clear trends that have some correlation to water temperature as expected. The plots only include the HR values after a simple threshold was applied to the corresponding periodicity values (to visualise results when clean heart signals were detected). Noisy signals can occur when shells are closed, or sometimes due to interference from direct sunlight on the sensors. This effect can be mitigated by shading but that was not done for these trials. The results show a mixture of strong signals and intermittent (especially after initial sensor attachment) signals, but overall represent a good result for the biosensor systems.

Whilst not telemetered, individual 30 second snapshots of the raw heart signal were manually obtained to verify the algorithm was operating correctly. Figure 16 shows an example from the deployment. The algorithm reported a heart rate of 24.5 BPM for that signal. Verification of this calculated value is achieved by manually inspecting the signal. In Figure 16 you can see the 30 second snapshot (600 samples at 20 samples per second) contains a repetitive signal with 12 and a bit cycles of that signal. When doubled (extrapolated to a sixty-second or one-minute time span) this agrees with the 24.5 beats per minute the algorithm produced. The algorithm produced a periodicity of 0.77 which indicates that it did find a good repetitive signal. Periodicity was transmitted as an 8-bit value representing the range 0 -> 1.

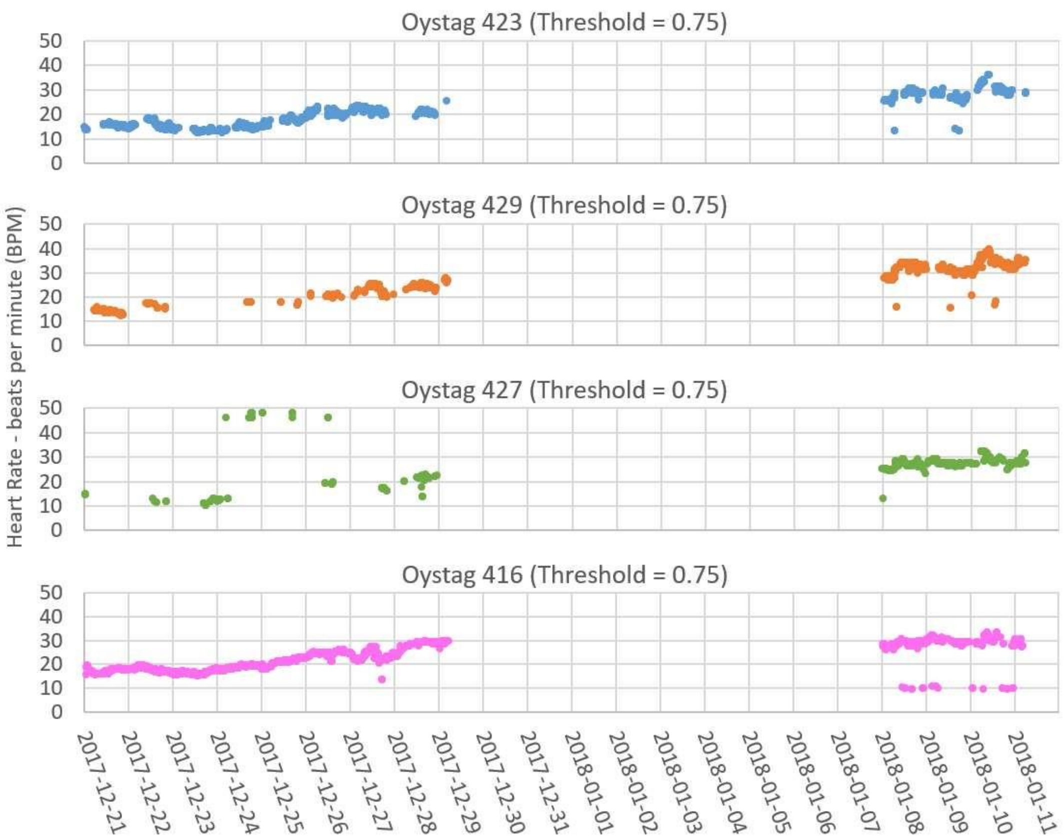


Figure 15. Filtered heart rate (HR) data

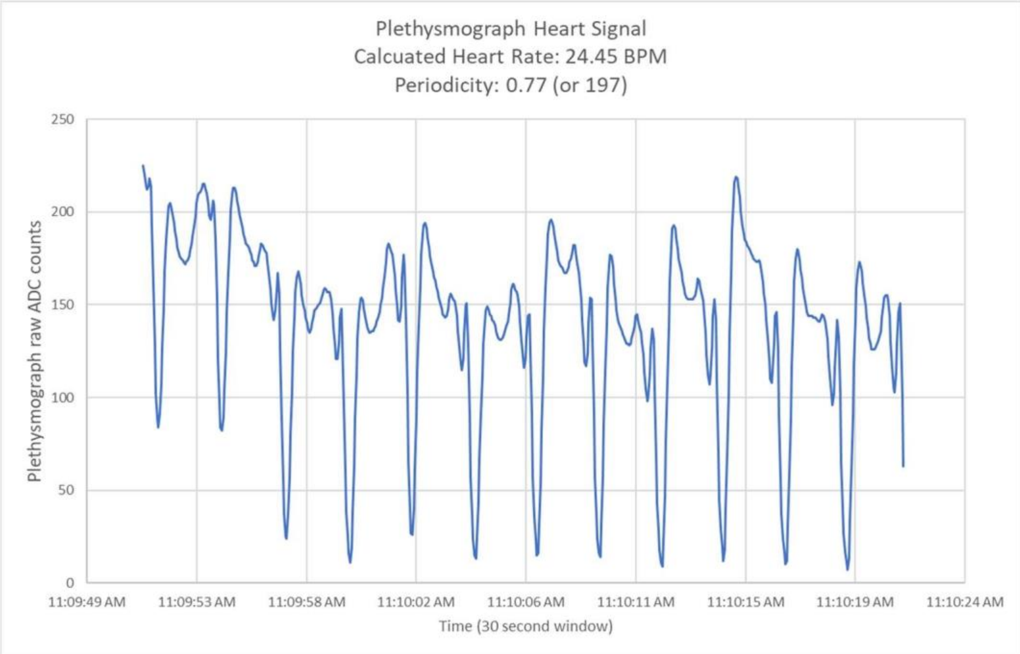


Figure 16. Raw heart rate data showing calculated heart rate

Analysis of the field deployments

Biosensors

Each biosensor telemetry system performed well after deployment. One unit functioned flawlessly - not even restarting once in over 12 months (either pre or post deployment) whilst the other system had a few outages. Through the winter the system voltage dipped due to a lack of power where the solar power system temporarily could not keep continuous power. On all occasions the system restarted correctly when energy returned. These telemetry/control systems also monitored power flowing to the biosensor strings (load current and both loaded and unloaded battery voltages). Both systems showed slightly increased power usage after 10-11 months of deployment, most likely an indication that one or more biosensors were degraded or failing.

Table 2 provides a summary of the performance of the biosensors used in the project. Overall the results were very good, but some issues did occur. Primarily the project demonstrated that it is possible for deployed biosensors to operate without maintenance for 12 months. This was a surprising result as it was expected animal growth would require repositioning of the animal on the sensor to ensure a clean heart rate signal could be detected long before the 12-month time elapsed. Lack of growth over the winter months did help with this. The engineering data from the sensors indicated that 12 months is the likely limit, though. Nine out of the eleven units deployed provided useable physiological data for long periods. One unit stopped working completely after 2 months and one unit failed to provide valid heart data. On multiple units the water pressure/temperature sensor failed before or during the deployment (only two remain functional after 12 months). This was a design/manufacturing issue with water leaking sooner or later near the exposed pressure port.

Table 2. Oyster biosensor performance summary

ID	summary	Gape Sensor	Heart Sensor	Internal Temp Sensor	Environmental Temp/Pressure Sensor	Light Sensor
#402 Coningham 0.5m	Excellent 1-year operation (>75,000 samples)	Excellent	Good, intermittent through winter	Good	Good for 8 months then failed	Good
#403 Coningham 1.5m	Excellent 1-year operation (>75,000 samples)	Excellent	Good, intermittent through winter, still operating	Good	Failed on deployment	Good
#409 Coningham 3.5m	Average 1-year operation (>75,000 samples) Unexplained gap in data for first two weeks of deployment.	Excellent	Good for 3 months, then failed operating correctly but intermittent data for a further 3 months.	Good	Failed on deployment possible reason for initial 2-week data gap.	Good

ID	summary	Gape Sensor	Heart Sensor	Internal Temp Sensor	Environmental Temp/Pressure Sensor	Light Sensor
#431 Coningham 5.0m	Excellent 1-year operation (>75,000 samples)	Excellent	Excellent, bit intermittent through winter, still operating	Good	Failed predeployment	Good
#424 Coningham 10.0m	Average 1-year operation (>75,000 samples) Looks like animal was disturbed on 14th August during servicing.	Good (Odd after 14th August)	Good for 7 months, nothing over winter, intermittent at 12 months.	Good	Failed predeployment	Good

#406 Redcliffs 0.5m	Average 1-year operation (>75,000 samples)	Excellent	Average Not operating correctly (insufficient plethysmograph light being detected) but still provided a valid signal for 6 months, big gap through winter, still operating after 12 months.	Good	Good for 8 months then failed.	Good
#408 Redcliffs 1.5m	Excellent 1-year operation (>75,000 samples)	Excellent, (strange event Nov 2019)	Average. Initially did not function for first 3 months then worked well since. Suspected too much light in plethysmograph.	Good	Failed before deployment	Good
#411 Redcliffs 3.5m	Poor 2-months intermittent operation then no data other than a few samples after 4 months on 17 July 2019 (10,000 samples)	Good (whilst unit operated)	No data once deployed in field (problematic whilst testing in lab prior to deployment.	Good (whilst unit operat ed)	Failed before deployment	Good (whilst unit operat ed)
ID	summary	Gape Sensor	Heart Sensor	Internal Temp Sensor	Environmental Temp/Pressure Sensor	Light Sensor
#415 Redcliffs 5.0m	Excellent 1-year operation (>75,000 samples)	Excellent	Excellent, data all year.	Good	Good	Good
#419 Redcliffs 7.5m	Good 1-year operation (>75,000 samples)	Excellent	Excellent, data all year.	Good	Worked for approximately 1 month before failing.	Good
#420 Redcliffs 10.0m	Poor 1-year operation (>75,000 samples)	Excellent	Bad data	Bad	Excellent	

Figure 17 shows the range (max – min) of the raw plethysmograph heart sensor signal from a typical biosensor over the 12-month deployment. The signal dropped off rapidly as the animal grew and stayed constant during the non-growing winter months. This is due to layers of shell growing over the sensor as well as physical growth of the animal which both contribute to the sensor not seeing the flesh movements from the heart. It is unlikely that the signal strength will be good enough to operate over another growing period. Across the board heart rate signals became intermittent through the winter months. This does happen as animals spend a higher percentage of the time closed during these months and this version of the sensors cannot detect heart activity during closures.

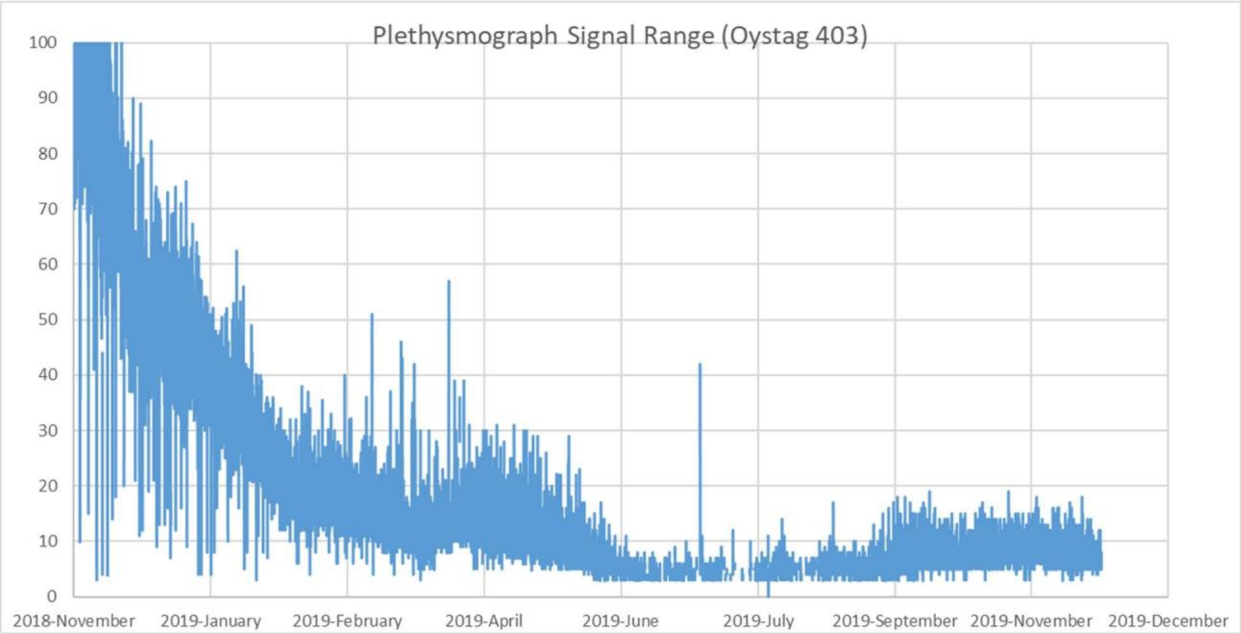


Figure 17. Typical Plethysmograph signal range over time

Figure 18 shows a typical gaping pattern over a 12-month period. It clearly shows the growth up until winter and then a steady state through to summer.

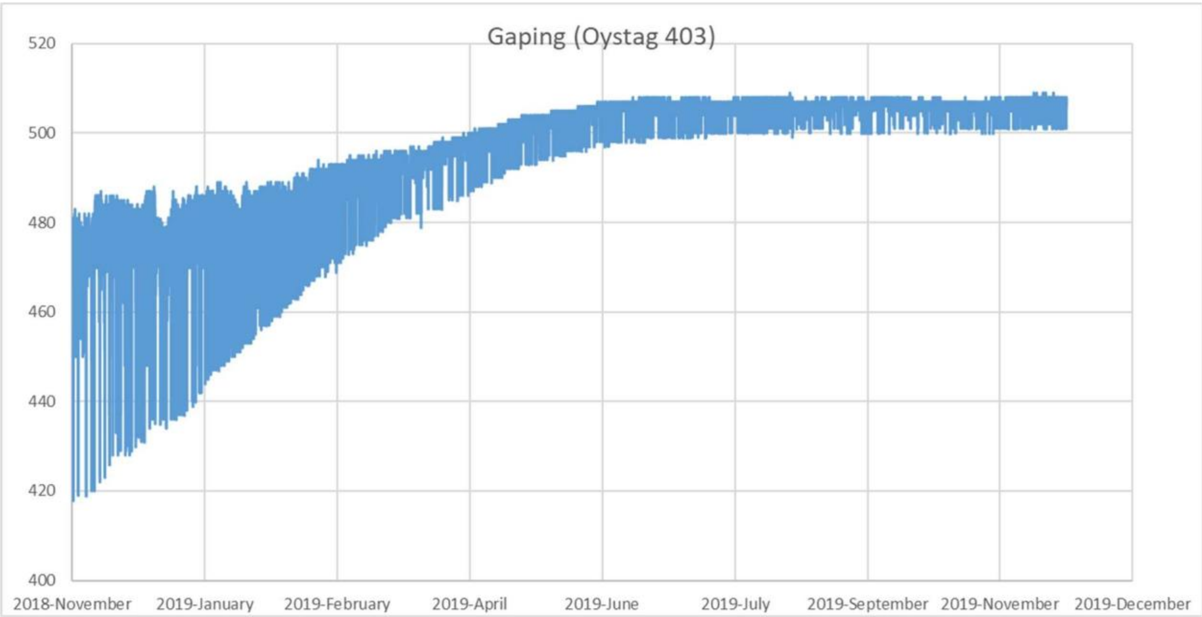


Figure 18. Typical oyster gaping pattern

Figure 19 shows the light sensor signal for biosensor 403 as well. This was deployed at 1.5m deep and contrasting the November 2018 signals (new biosensor deployed) with the November 2019 signals (been in the water for 12 months) clearly shows how biofouling prevents light from penetrating to the unit. The advantage of the biosensors is that this biofouling does not affect the sensor's ability to measure the animal physiology like it does with standard water quality sensors (when they try to measure water quality parameters).

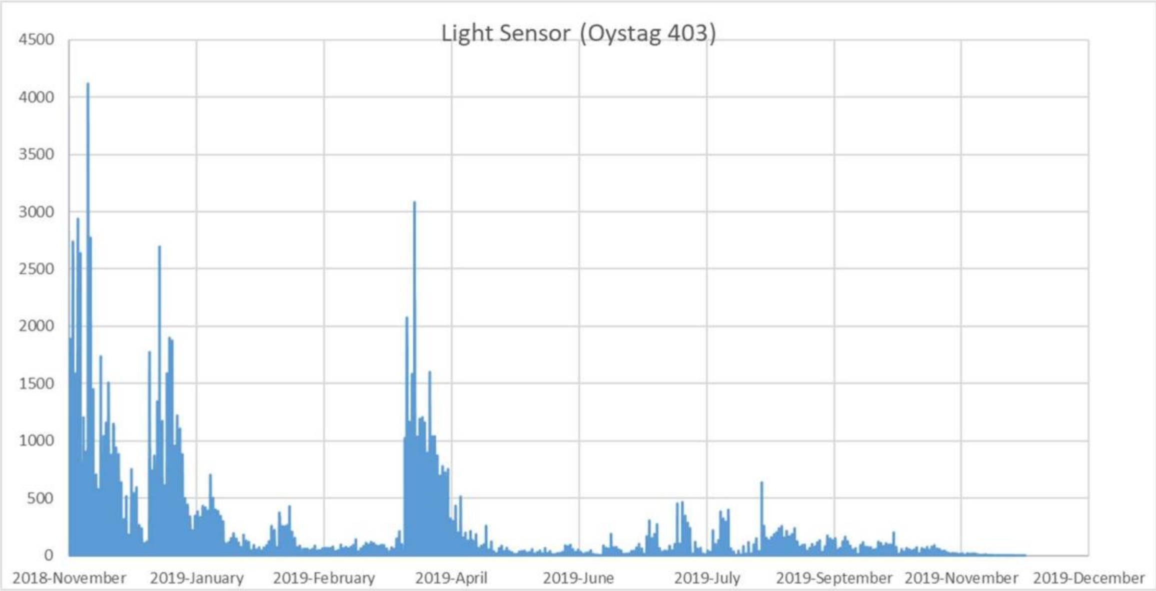


Figure 19. Typical light sensor reading

EXO2 Sensors

The EXO2 sensors measured a broad category of WQ variables that influence well-being of oysters and consequently affect their physiology. The measured WQ variables included Temperature (Temp), Conductivity (Cond), Dissolved Oxygen (DO), salinity, pH, Oxidation-reduction potential (ORP), turbidity, pressure, chlorophyll a concentration, and phycoerythrin concentration. Figure 20 shows example data obtained from the sensors.

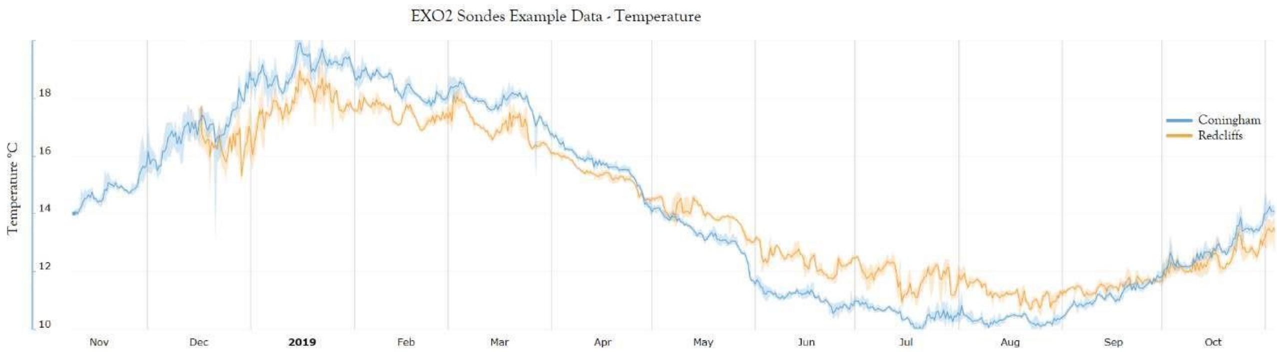


Figure 20. Example data from the deployed EXO2 sensors

Biosensor – Water Quality

The following subsections provide the details of WQ variable selection approach and data driven machine learning methods used in the study.

Data pre-processing

Both the physiological and WQ data had issues with quality and noise. To make the data suitable for further analysis, statistical methods were applied to identify and remove the outliers. Specifically, the physiological data was cleaned based on Hampel identifier technique and WQ time series data was cleaned based on adjusted boxplot method. Figure 21 and Figure 22 respectively show some outliers detected and removed from HR data for oyster with biosensor Id 408 and Chlorophyll concentration data at Redcliffs.

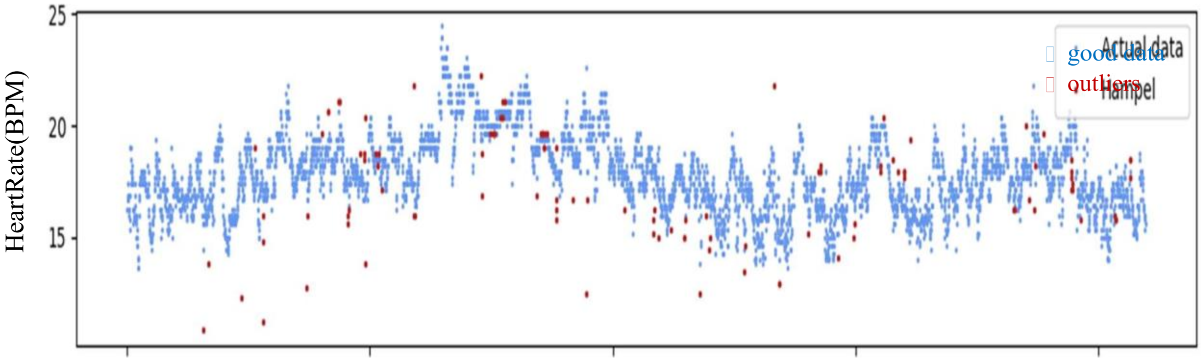


Figure 21. Outliers (red dots) in heart rate (HR) data of oyster with biosensor Id 408

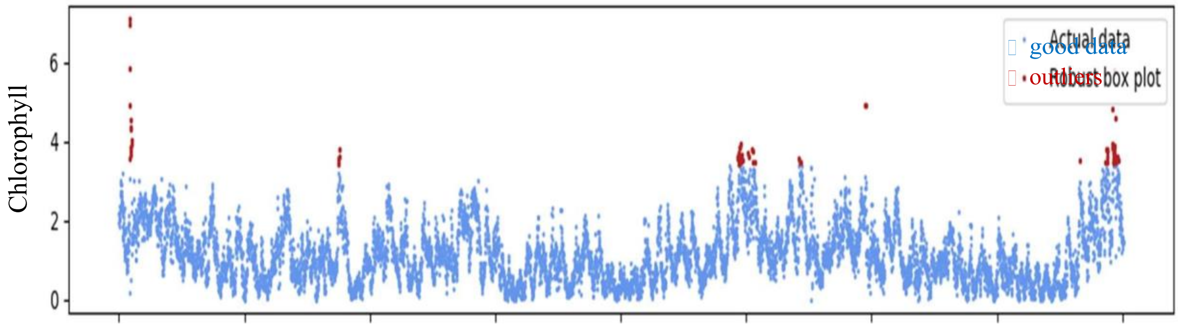


Figure 22. Outliers (red dots) in Chlorophyll concentration data at Redcliffs

To address whether it was possible to accurately predict the HR of oysters as a function of WQ variables, a data driven machine learning approach was developed (as described in previous section (Methods)).

Water Quality Variable Selection

The correlation matrices between HR and WQ variables for the oysters at Coningham and Redcliffs are visually presented in Figure 23 and Figure 24, respectively. The pressure data for Coningham site is not available due to sensor malfunctioning, and hence does not include the correlation between pressure and HR of the oysters at that site. It was found that : 1) Conductivity and Temperature were the two WQ variables that had the highest level of correlation with HR of oysters at both sites; 2) ORP and pH were also highly correlated with HR for three out of five oysters at Coningham site; and 3) pH was highly correlated with HR for only one oyster (biosensor Id 408) at Redcliffs.

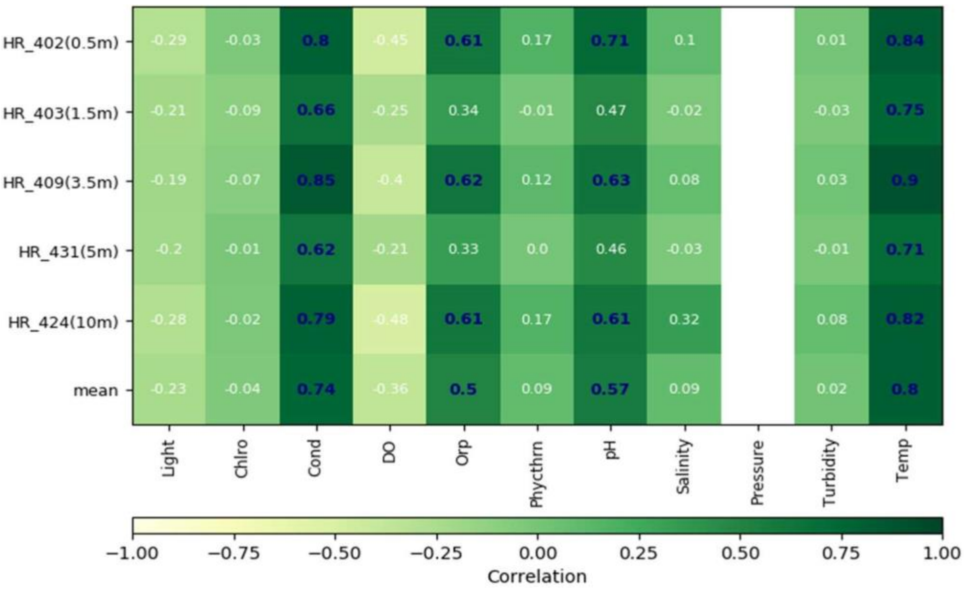


Figure 23. Correlation between water quality (WQ) variables and heart rate (HR) of oysters at Coningham

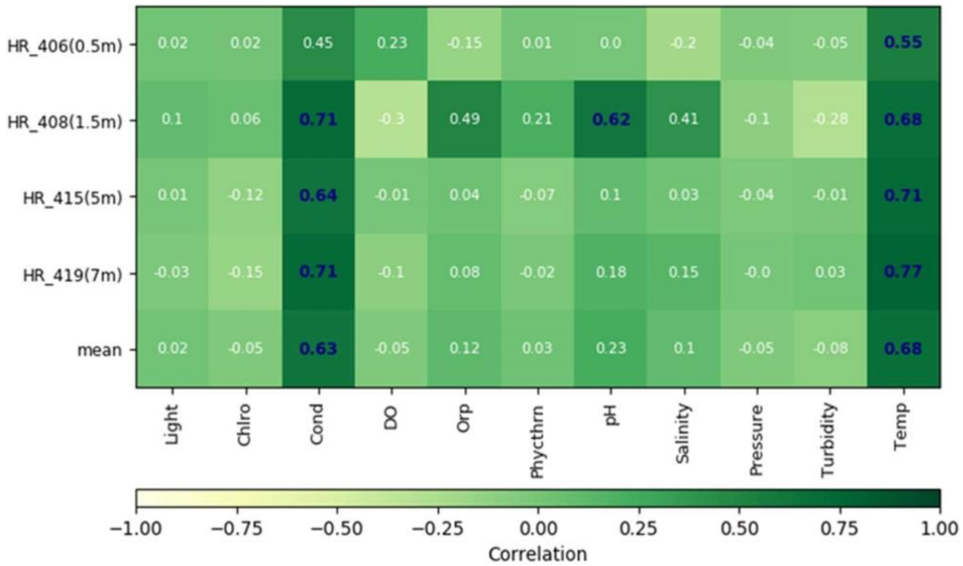


Figure 24. Correlation between water quality (WQ) variables and heart rate (HR) of oysters at Redcliffs

Heart Rate Prediction

Table 3 and Table 4 present the results for first set of experiments that aimed to predict HR of oysters as a function of different WQ parameters. The text highlighted in blue italics indicates the best prediction accuracy in terms of Mean Absolute Percentage Error (MAPE) achieved for each of the oysters at two sites (Coningham and Redcliffs). The subsequent analysis of the results only focuses on the best prediction accuracy achieved for each oyster. Figure 25 and Figure 26 show the actual vs predicted HR data for two oysters – one from each site.

Table 3. Accuracy of heart rate (HR) prediction for oysters at Coningham

Oyster biosensor Id	Target	Inputs	Model	MAPE [%]
402	HR	Cond, ORP, pH, Temp	LR	6.8
			NN	*5.9*

			RF	6.43
			SVR	6.83
403	HR	Cond, Temp	LR	6.9
			NN	5.9
			RF	6.66
			SVR	6.99
409	HR	Cond, ORP, pH, Temp	LR	3.91
			NN	3.17
			RF	4.45
			SVR	3.75
431	HR	Cond, Temp	LR	6.97
			NN	6.98
			RF	7.06
			SVR	7.33
424	HR	Cond, ORP, pH, Temp	LR	5.61
			NN	5.08
			RF	5.81
			SVR	5.63

Table 4. Accuracy of heart rate (HR) prediction for oysters at Redcliffs

Oyster biosensor Id	Target	Inputs	Model	MAPE [%]
406	HR	Temp	LR	6.97
			NN	6.38
			RF	6.83
			SVR	6.98
408	HR	Cond, pH, Temp	LR	10.41
			NN	12.77
			RF	8.21
			SVR	9.72
415	HR	Cond, Temp	LR	9.02
			NN	6.12
			RF	6.51
			SVR	8.62
419	HR	Cond, Temp	LR	7.86
			NN	6.13
			RF	6.84

SVR	7.52

Results show that all machine learning models achieved good performance and could predict HR of the oysters with high level of accuracy. The prediction error (in terms of MAPE) was in the range of 3.17%-6.97% for oysters in Coningham and 6.12%-8.21% for oysters in Redcliffs. The low prediction error implies that there are inherent patterns in WQ data that can explain the HR patterns. This implies that physiological behaviour (HR) of oysters can be explained by the environmental conditions.

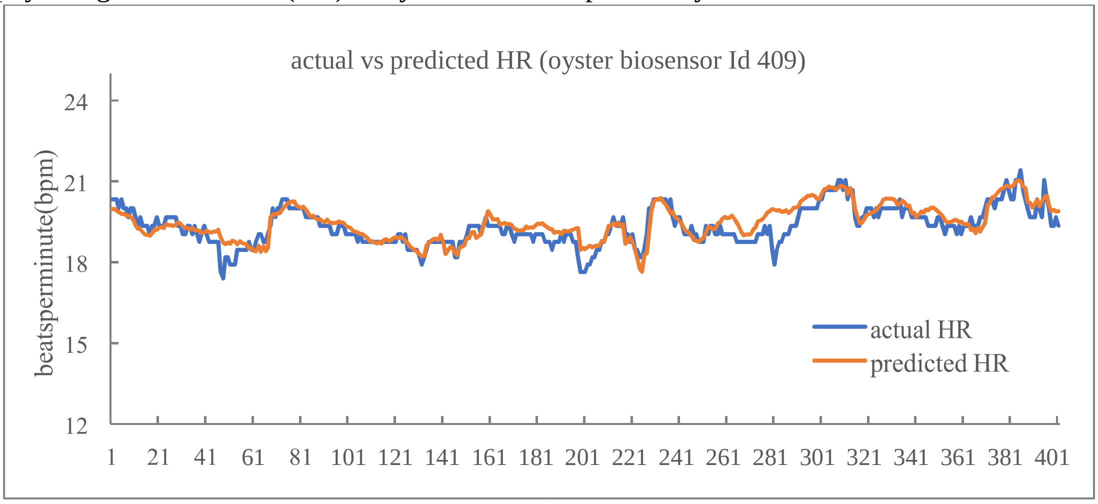


Figure 25. Actual vs predicted heart rate (HR) for an oyster with biosensor Id 409, at Coningham

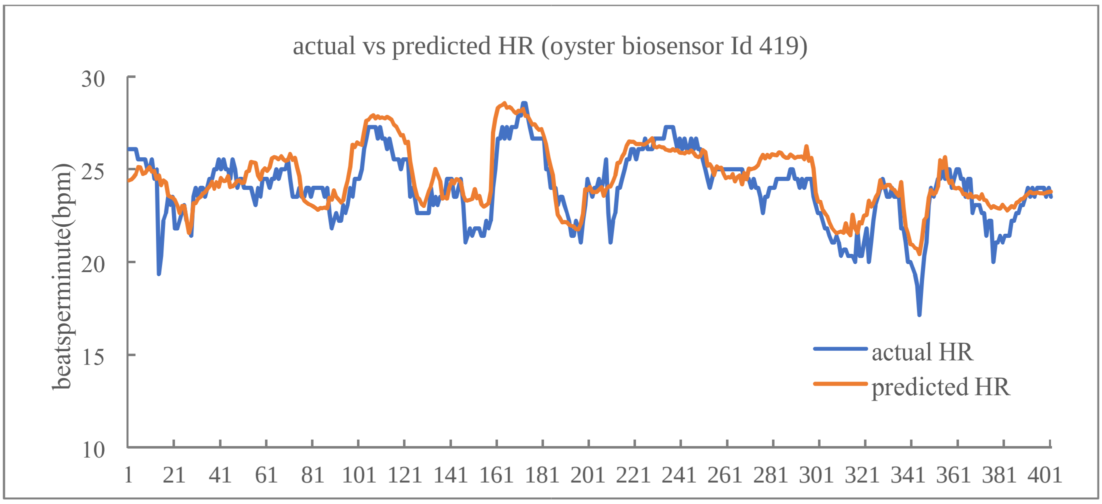


Figure 26. Actual vs predicted heart rate (HR) for an oyster with biosensor Id 419, at Redcliffs

Moreover, it is observed that the inputs to prediction models varied for different oysters (see Table 3 and Table 4). For example, the input WQ variables for the HR prediction model for oyster 402 were Cond, ORP, pH, and Temp (Table 3) whereas Temp was the only input WQ variable for oyster 406 (Table 4). This is because the correlation between WQ variables and HR of individual oysters were different, and the customised prediction model for each oyster only considered the WQ variables that had a correlation higher than a pre-set threshold. It was found that Temperature is one of the main environmental factors that has the most significant relationship with physiological responses of the oysters. Thus, it was selected as input to the prediction models for all oysters. Conductivity was also shown to be highly correlated with HR and was selected as input to prediction models for all but one oyster (biosensor Id 406). This was expected as these two WQ parameters can affect oxygen consumption rate, metabolic rate, food intake, and growth of the oysters.

Water Quality Prediction

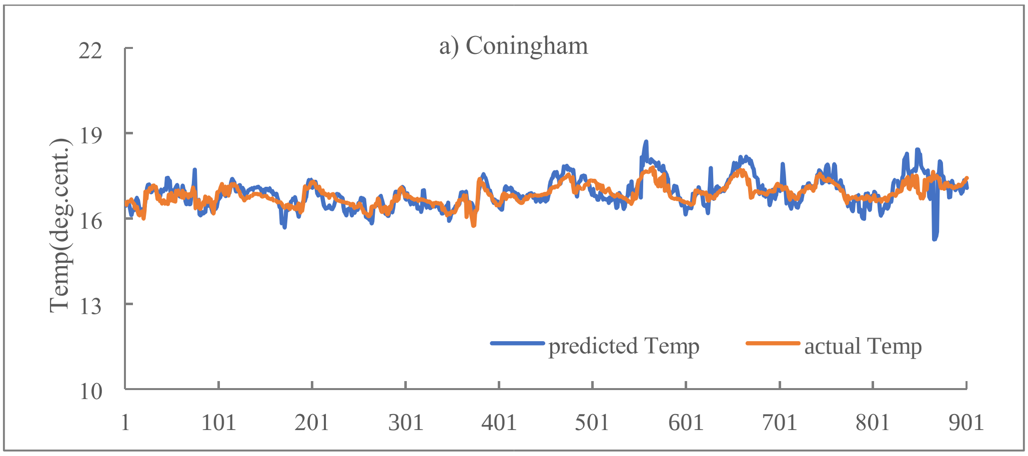
The second set of experiments were conducted to investigate the second research question i.e. whether it is possible predict WQ variables using the physiological data (HR and shell gape) of oysters as inputs. This report only presents the results for Temp and Cond. The subsequent discussion is confined accordingly since only these two WQ variables were found to be highly correlated with HR data and satisfy the filtering criteria imposed as part of model development. Table 5 and Table 6 present the prediction accuracy for these two WQ variables at Coningham and Redcliffs, respectively. Again, the text in blue italics in these two tables indicates the best prediction accuracy achieved. The actual vs predicted data for Temp and Cond are shown in Figure 27 and Figure 28, respectively.

Table 5. Prediction accuracy for water quality (WQ) variables (temperature and conductivity) at Coningham

Site	Target	Inputs	Model	MAPE [%]
Coningham	Temp	HR, gape	LR	3.11
			NN	2.48
			RF	2.60
			SVR	3.12
	Cond	HR, gape	LR	1.39
			NN	1.01
			RF	1.12
			SVR	1.39

Table 6. Prediction accuracy for water quality (WQ) variables (temperature and conductivity) at Redcliffs

Site	Target	Inputs	Model	MAPE [%]
Redcliffs	Temp	HR, gape	LR	2.32
			NN	1.94
			RF	2.01
			SVR	2.32
	Cond	HR, gape	LR	1.00
			NN	0.74
			RF	0.72
			SVR	1.01



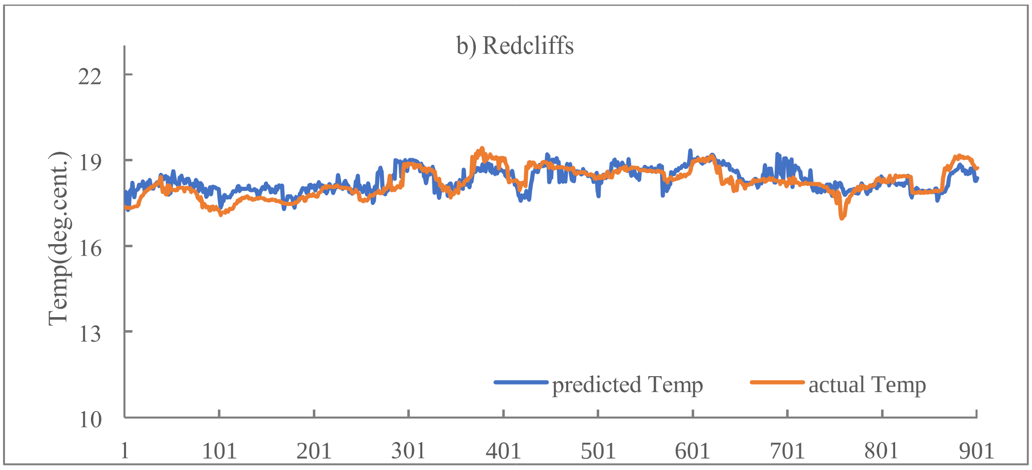
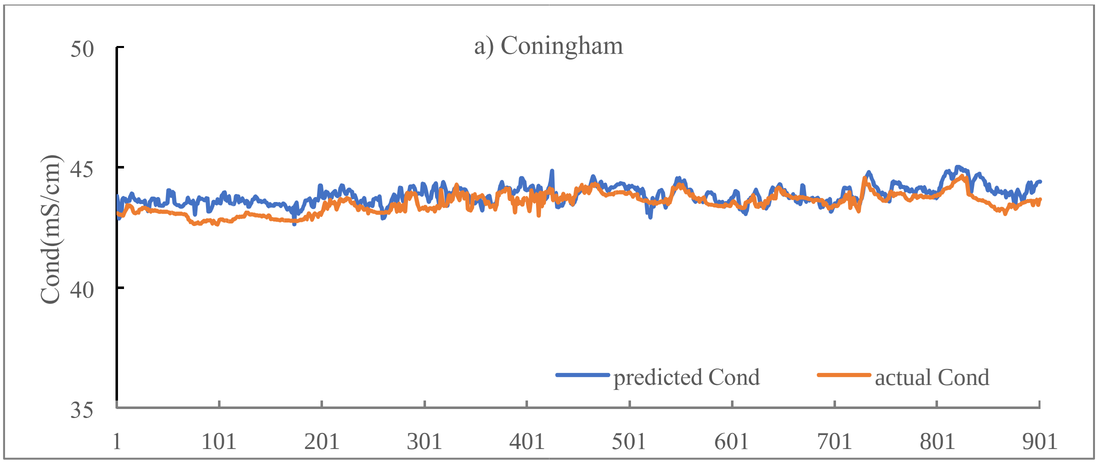


Figure 27. Actual vs predicted Temperature for: a) Coningham and b) Redcliffs

The results indicate that the machine learning models incurred low error and provided reasonably accurate predictions for both Temp and Cond using physiological data as input. The prediction error for Temp and Cond are 2.48% and 1.01% at Coningham; and 1.94% and 0.72% at Redcliffs. The low prediction error indicates that it is possible to explain changes in WQ variables using physiological data of oysters. The predictions for WQ variables obtained using machine learning models possibly could be used to characterise these fundamental environmental WQ parameters and provide a basic status of the environment without regular sampling of water.



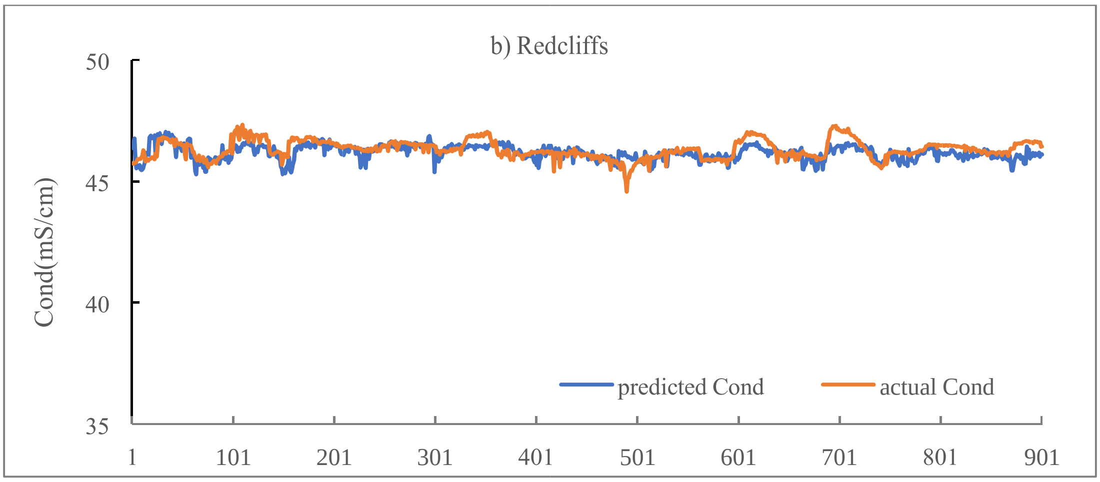


Figure 28. Actual vs predicted Conductivity for: a) Coningham and b) Redcliffs

Event-driven Analysis

The primary aim of the event driven approach was to analyse the changes in WQ variables around the time of sudden and abrupt change in the HR data. To do this, 20 events were manually identified in the HR data for the oyster with biosensor Id 431 at Coningham. HR showed an upward trend for 9 of these events and a downward trend for remaining 11 events.

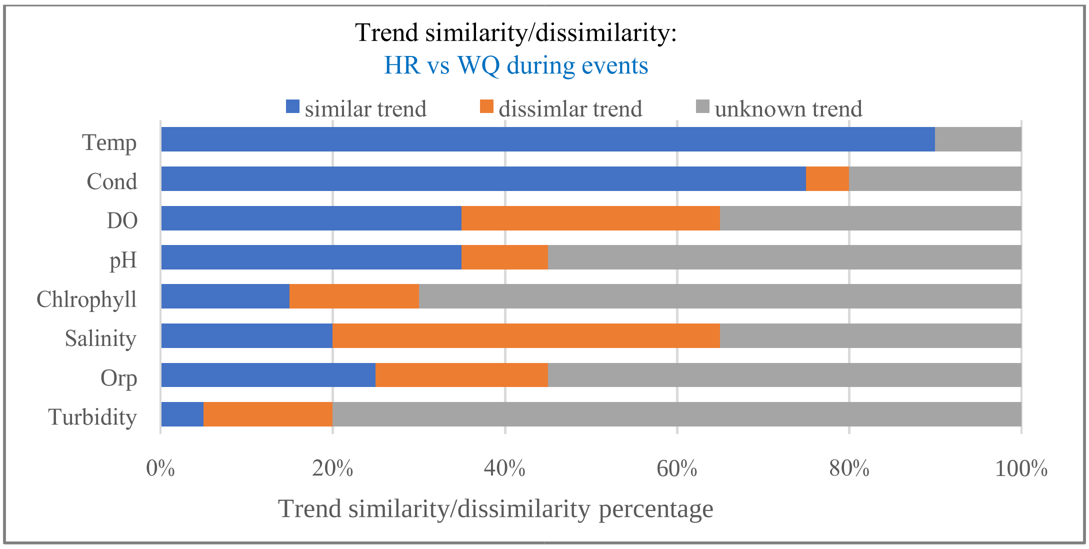


Figure 29. Trend similarity/dissimilarity between heart rate (HR) and water quality (WQ) during events

Figure 29 visually presents the similarity/dissimilarity of trend between HR and WQ during the time of the events. It shows that HR had similar trends (upward/downward) to that of Temp and Cond during most of the events: 90% for HR and Temp; 75% for HR and Cond. DO and pH came next in the order. HR followed similar trends to that of DO and pH in 35% of the events. Similarity of trend between HR and each of the remaining WQ variables was very low (<=25%). Trend dissimilarities with HR were the highest for Salinity, followed by DO and ORP: 45%, 30% and 20% of the events, respectively. Turbidity, Chlorophyll, ORP, and pH did not show any clear trend in most of the cases: 80%, 70%, 55%, and 55% of the events respectively.

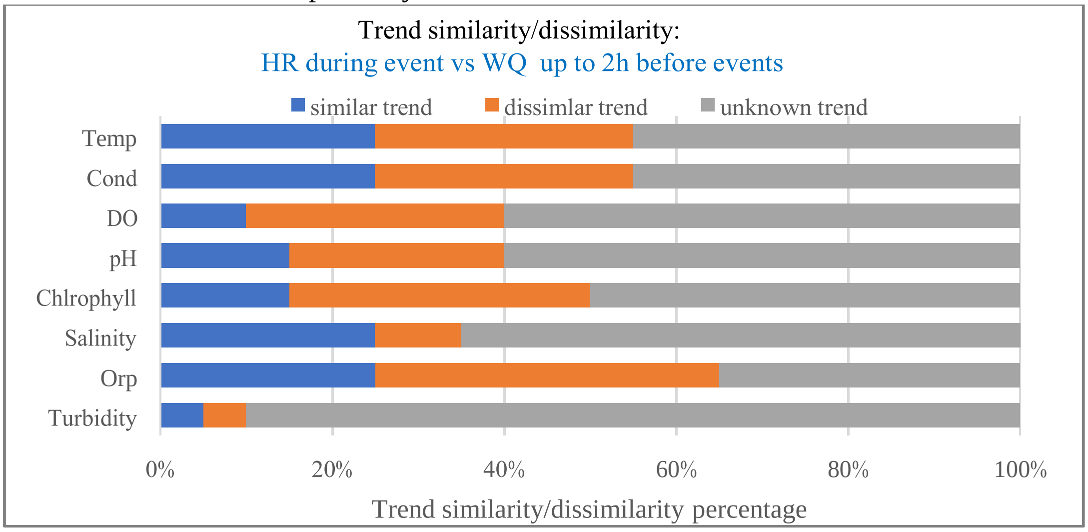


Figure 30. Trend similarity/dissimilarity between heart rate (HR) during events and water quality (WQ) up to 2h before events

Figure 30 presents the similarity/dissimilarity of trend between HR during the events and WQ up to 2h before the events. It is observed that the similarity of trend between 2-h lagged WQ variables with that of HR during events was low. Maximum similarity was 25% for Temp, Cond, Salinity, ORP. Either there was no clear trend in the lagged WQ variables, or they had dissimilarity of trend with that of HR during events.

A similar analysis was also conducted to see the relationship between the Gape Open Rate (GOR) during the events and WQ around the time of the events. Regardless of the trend in WQ during the events, the GOR was found to be very high (>=80%) during 90% of the events. Figure 31 shows the changes in WQ variables happened during the events for the cases (i.e. events) when the gape open rate is ≥ 80%. For Temp and Cond, the cases with high GOR was the maximum when they had downward trend during the events. For DO, the cases with high GOR were the maximum if it had upward trend during the events. For remaining WQ variables, the cases with high GOR had been found unrelated to the trend of these WQ variables.

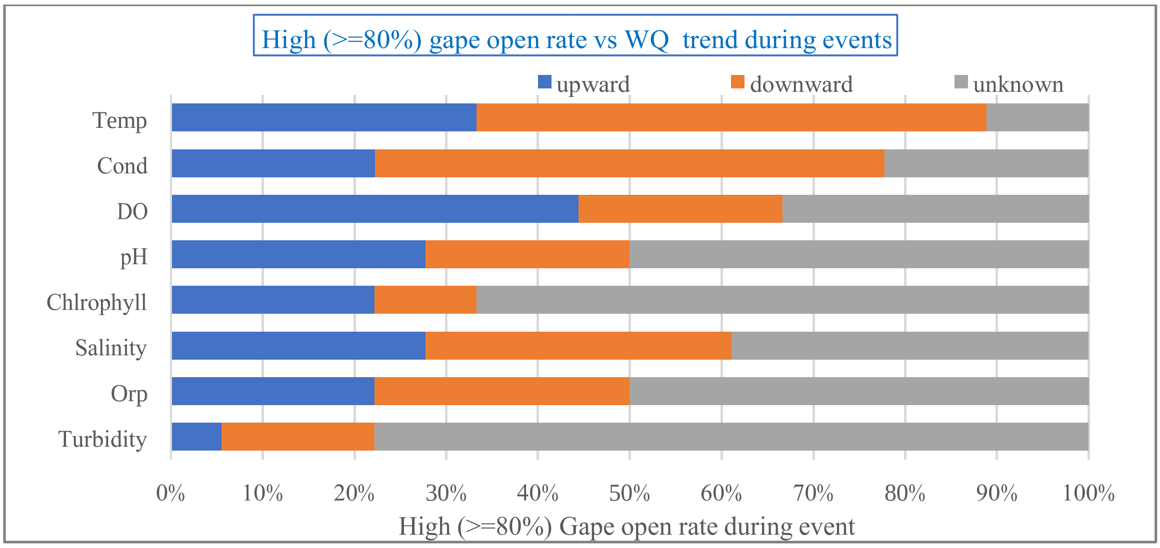


Figure 31. Relationship between high gape open rate (GOR) and water quality (WQ) during the time of the events

Figure 32 shows the changes (in terms of trend) in WQ variables up to 2-h before the events for the cases when the gape open rate was ≥ 80%. For Temp and Cond, it shows that the cases with high GOR increased if they had upward trend and decreased if they had downward trend up to 2-h before the events. On the other hand, it is opposite for DO - the cases with high GOR decreasing if it had upward trend up to 2-h earlier before events. However, for all WQ variables, in maximum cases the high GOR was unrelated to the trend (upward/downward) of lagged WQ variables.

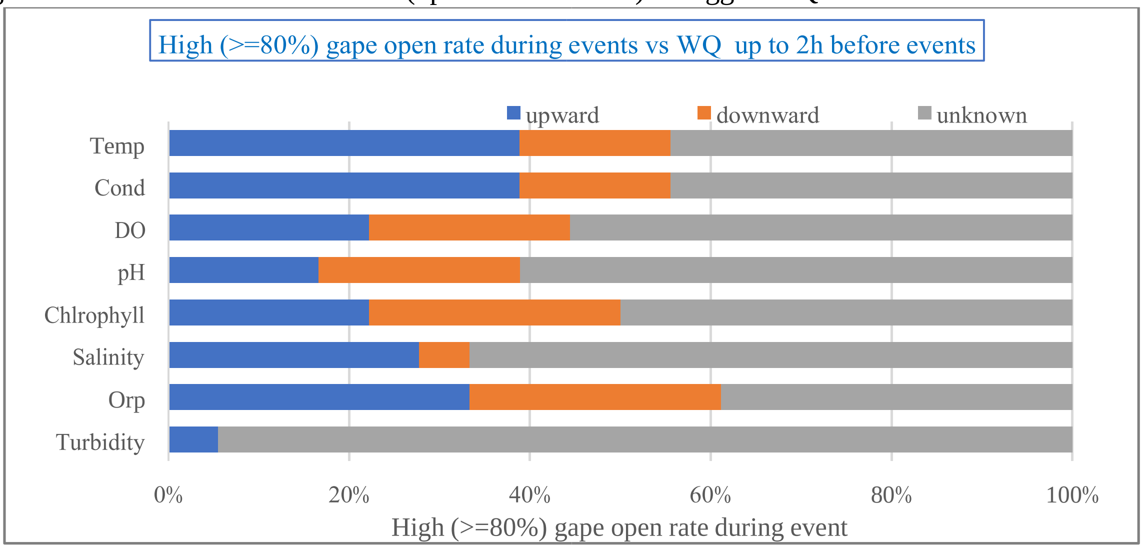


Figure 32. Relationship between high gape open rate (GOR) and water quality (WQ) up to 2-h before the events

We met the objectives of the Field Biosensors part of the project. We successfully demonstrated an operational network of sentinel molluscs in the field. Physiological and behavioural data was gathered from 10 animals at different depths across two field sights for 12 months. This data was streamed live so changes could be observed in real time. We demonstrated it is feasible to build, deploy and maintain a network so sentinel sensors. In fact, we highlighted that it was possible for the sentinel sensors to operate for up to 12 months with <u>no</u> maintenance, something that is very difficult to do with traditional water quality monitoring equipment. During this work we exposed commercial operators (Aquenal and Tassal) to the requirements of assembling, deploying and maintaining both traditional and biosensor field deployments, improving their capacity to do this work. In terms of the data acquired we demonstrated that changes in behaviour and physiology can be measured and interpreted and that the sentinel sensor data can give an indication of changes in the water quality.

Outreach Program

The outreach program at the MDC directly reached over 1,500 students who undertook teaching modules related to this project in its first year. Our static displays at both the MDC (see Figure 33 & Figure 35) and the CSIRO Discovery Centre in Canberra (see Appendix F) had over 30,000 visitors during the year (over 25,000 people visit Canberra each year and over 5,000 visit Woodbridge each year).

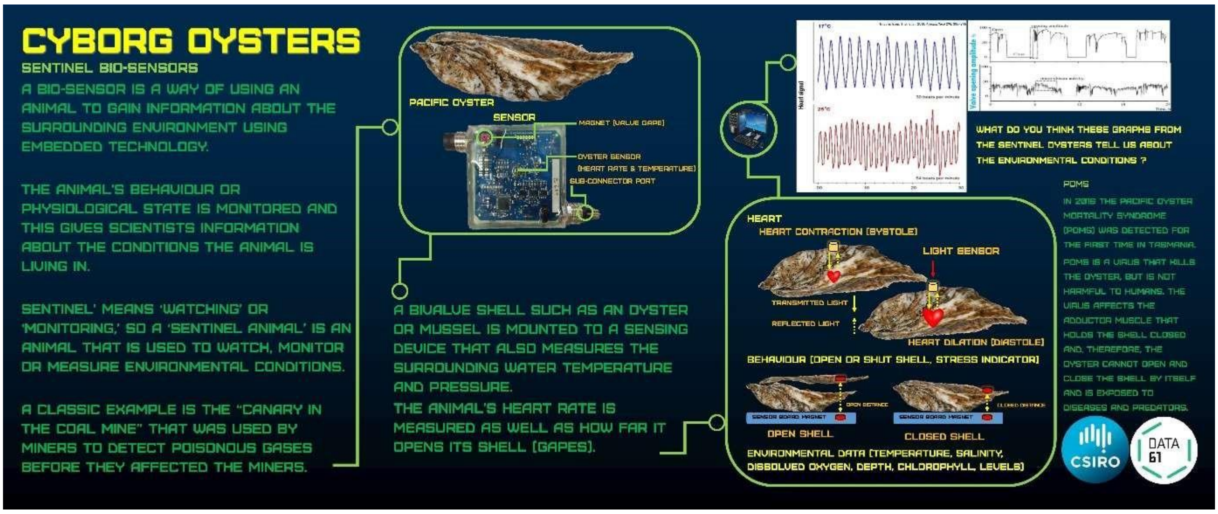


Figure 33. Marine Discovery Centre (MDC) poster describing biosensors

At a primary level the concepts and equipment were only used with Year 6 students as the technical aspects fit with the science curriculum content descriptor, "The growth and survival of living things are affected by physical conditions of their environment." The total number of primary students that worked with the biosensors was relatively small (<150) as the Tasmanian year 6 curriculum tends not to drive traffic to the MDC. Those that did visit worked through the worksheet shown in Figure 34. Mussels were used as they are easier to collect and work with. The Grade 6 Students were interested in the technology as it is pitched as cutting-edge CSIRO developed technology and how it can be used in the field. The software output was not intuitively obvious, so they required some help in initially interpreting the data and to prompt their thinking. Once students started to see changes in the mussel heart rate, they began to think about the kind of tests that they could do and started to experiment. This activity was a part of a longer day at the Marine Discovery Centre, so it formed an introduction, or taster, to this sort of technology that they could go away and think about more.

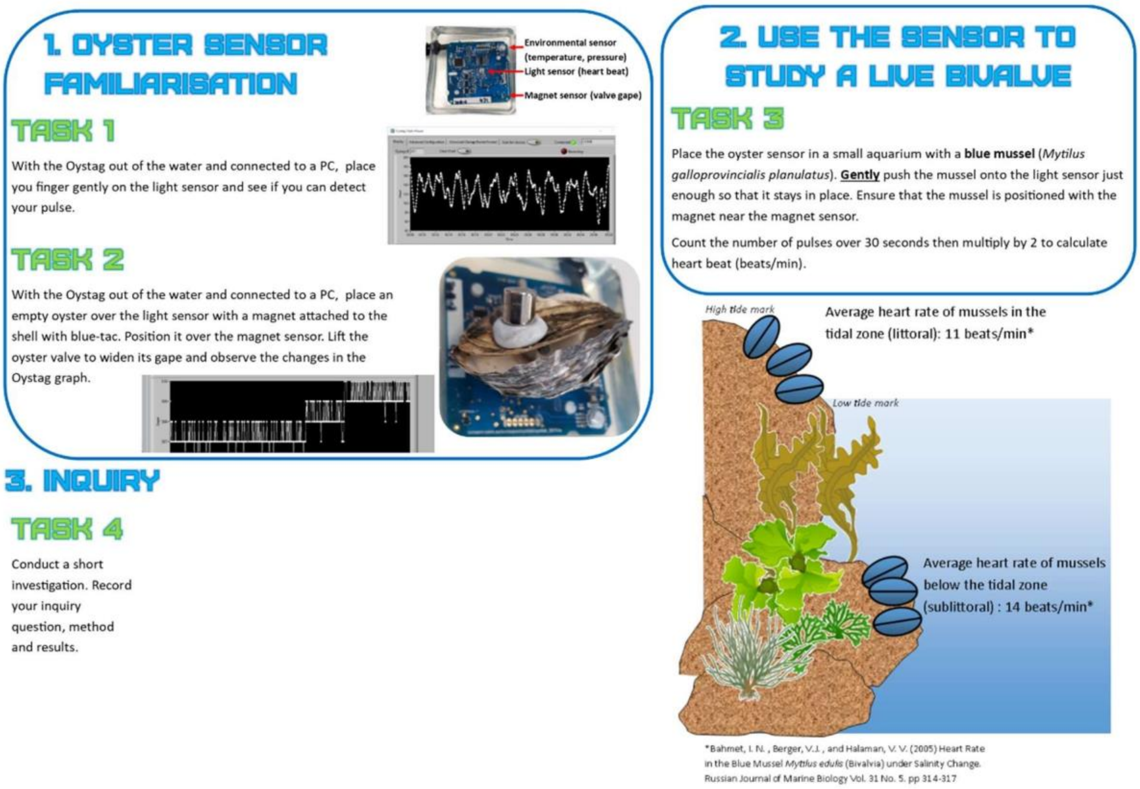


Figure 34. Primary (Grade 6) module plan

Most exposure at the MDC was at secondary level, typically at least two classes per week over 24 teaching weeks, with approximately 1,440 students in total. All year levels looked at abiotic factors in the marine environment and used the oyster's response to temperature as part of investigations on the East Australian Current and climate change. The material was also useful for lesson plans on introduced marine pest species as well as understanding human impacts on marine ecosystems.

This project was an excellent STEM example and very engaging for students. The class groups were able to observe the live oysters and monitor their physiological response to changes in water temperature and gape response to being fed with plankton. They enjoyed using the biosensor test unit and Oystag software for measuring their own pulses and testing out how the magnetic sensors pick up gape of the 'demo oyster'.

There were several learning outcomes from classes: making predictions from data, observing and analysing data in real-time, analysing difference scenarios of environmental changes, and learning about biotechnology applications. Example activities are provided in the Appendix of this report.

Table 7 shows the focus of the various year groups.

Table 7. Biosensor focus of each year group

Year		Focus

Grade 6	Physiological responses	The growth and survival of living things are affected by physical conditions of their environment.
Grade 8	Biology	Anatomy and body systems – show body parts of the mollusc and look at heart rate and physiological response.
Grade 9	Ecosystems	Introduced marine pest species in marine ecosystems – looking at biosensors.
Grate 10	Evolution & genetics	Biotechnology
Year 11-12 Biology		Technology as a tool to measure, analyse and monitor biodiversity.

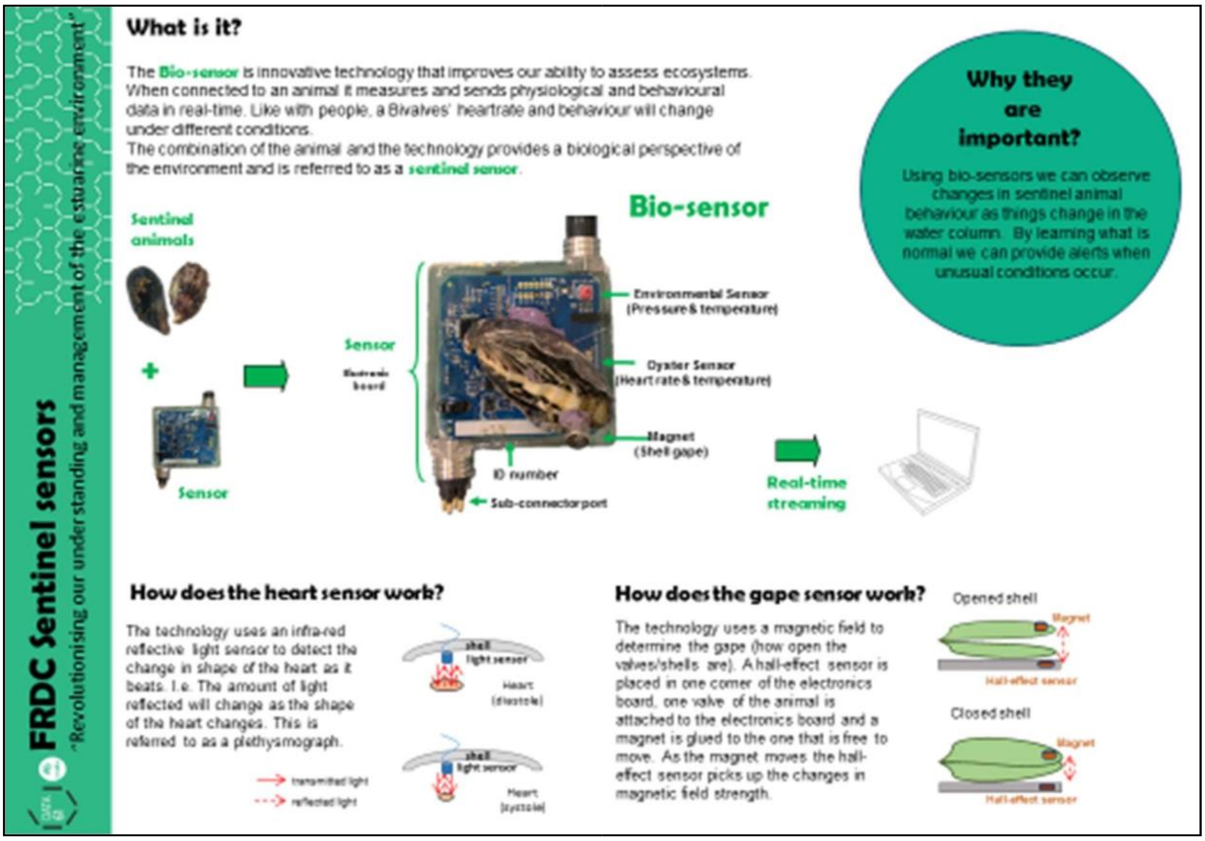


Figure 35. Example of graphic educational material displayed at the Marine Discovery Centre (MDC)

We met the Education Outreach objectives of the project by developing and delivering biosensor teaching modules at the Woodbridge Marine Discovery Centre to over 1,500 students. We extended these initial objectives by also providing educational material to the CSIRO Discovery Centre in Canberra. Sensors and oysters were displayed at the centre with a graphic material that explains to visitors our project (Figure 37).

Conclusion

The main objective of field deployments was to investigate the physiological response (especially HR) of oysters with respect to the changes in surrounding environment (given by different WQ variables) they are exposed to. Two data driven machine learning approaches were developed to predict HR of sentinel oysters using WQ variables as inputs, and WQ variables using HR and shell gape as inputs. The influence of WQ on oyster's physiology was described by demonstrating how accurately they can predict each other. Experimental results obtained using data from two different deployment sites indicated that Temperature and Conductivity were the two WQ variables that showed the highest level of correlation with HR. These two variables are likely driving factors that influence the physiological and behavioural patterns of oysters in these two sites. Results also showed that it is possible to predict HR of oysters with good level of accuracy using the WQ variables as inputs – the prediction error in terms of MAPE is 3.17%-6.97% for the oysters in Coningham and 6.12%-8.21% for the oysters in Redcliffs. This implies that the physiology of oysters can be explained by the environment (WQ) they belong to. The predictions for WQ variables (Temperature and Conductivity) also closely followed the actual data. Hence there were small prediction errors for these two WQ variables – MAPE is in the range of 0.72%-2.48%. This implies that it is possible to infer the surrounding environment (WQ) of the oysters from their physiology. Overall, these results support the feasibility of applying machine learning approaches to explain the interactions between oyster's physiology and WQ.

Moreover, from the results of event driven analysis it was found that HR had similar trend (upward/downward) with that of Temperature and Conductivity during most of the manually identified events. The similarity of trend between WQ variables 2-hours prior to the event with that of HR during event are low, indicating that responses were immediate. Also, the gape open rate was found to be high (>80%) during 90% of the events irrespective of trend in WQ during the time of events.

Importantly, the biosensors have continued to operate and report data. These physiological data are relevant (directly or indirectly) to oysters' health, and hence can provide vital signs of responses of sentinel oysters to multiple and interacting changes in the environment.

This project has proven that field deployed biosensors can operate in harsh marine environments without servicing or maintenance far longer than typical water quality sensors.

Staff at the MDC report that the Biosensors were a highly valuable teaching resource. They met several important science curriculum outcomes such as science as a human endeavour, inquiry skills, and science understanding, plus, importantly, also crossed with the use of digital technology, and was relevant and authentic. The MDC exists to provide students learning opportunities that they would not otherwise be able to have in the everyday classroom and this activity is a classic example. We are reaching our next generation and hopefully inspiring a few to continue and strike up a career in marine science to help Tasmania and Australia continue to have world best practice in marine science.

Implications

Whilst further work would be required to commercialise the technology, we have demonstrated that it is now feasible to deploy biosensor technologies and have them operate unattended for long periods of time. We demonstrated that the use of biosensors can alleviate one of the most significant barriers to continual marine sensing (the need for regular service and cleaning visits), providing a significant cost benefit. As new biosensor technologies become available, it may revolutionise how we monitor marine environments.

Recommendations

To reach the full potential of this research, the biosensor technology needs to be commercialised. Commercially available sensors would also help maintain the momentum we have developed at the Marine discovery centre, as they need a way to maintain and upgrade the equipment they use for teaching and the current arrangement is for CSIRO to continue to provide in-kind to maintain sensors. Extending the teaching material to classrooms prior to visiting will allow students to arrive better prepared and give them time to do much more interesting scientific investigations with the equipment on site.

Further development

The biosensor technology needs support to move from a scientific custom-made sensor to a commercially available device. Engineering solutions around pressure ports need to be developed, as well as finding a manufacturing partner to handle production.

Since the beginning of this project, CSIRO has also developed the next generation biosensor technology. This integrates an enhanced sensing technique that now allows us to monitor physiology with greater precision (it provides insights during low activity periods such as shell closures) which will add further modalities to the acquired data and potentially provide a much richer interpretation of the environment. To refine the insights to be gleaned from these new sensors and the data driven ML approaches, much larger datasets will be required.

Extension and Adoption

The outreach part of the project has developed equipment and teaching modules for the MDC which continue to be used. It also provided a static display to the CSIRO marine discovery centre, collectively these centres receive over 30,000 visitors per year.

The machine learning science will be communicated in peer review publications.

Project materials developed

Project materials developed consist of software, hardware, scientific literature, research data sets and teaching materials.

Teaching materials

Teaching and display materials are included in this report (some are available from the MDC – see the references section):

Display Material

- Educational material at the Marine Discovery Centre. a) Display set upper educational aquarium, with sensor and iPad showing real-time data, b) Real-time data from

biosensor, c) General view of educational aquarium, sensor and display, d) Biosensor deployed at indoor aquarium (see Figure 10 page 23)
- Biosensor display at CSIRO Discovery Centre (see Appendix F page 64)

Student workbooks

- Educational Program unit 1 of Sentinel sensors: “Revolutionising our understanding and management of the estuarine environment” (see Appendix B page 49)
- Educational Program unit 2 of Sentinel sensors: “Revolutionising our understanding and management of the estuarine environment” (see Appendix C page 60)
- Educational Program unit 3 of Sentinel sensors: “Revolutionising our understanding and management of the estuarine environment” (See Appendix D page 62)

Hardware and Software

The project delivered hardware and software systems for the MDC for teaching and display purposes, as well as developed sensor and telemetry packages for the field deployments. LabVIEW based software for interacting with the biosensors is available from CSIRO or MDC.

Teaching Materials

- Educational material at the Marine Discovery Centre. a) Display set upper educational aquarium, with sensor and iPad showing real-time data, b) Real-time data from biosensor, c) General view of educational aquarium, sensor and display, d) Biosensor deployed at indoor aquarium (see Figure 10 page 23)
- Left: Oysters with laboratory biosensors attached, Right: Telemetry system with a biosensor connected (See Figure 11 page 24)

Field Equipment

- Mussel attached to biosensor during testing (See Figure 1 page 9)
- Field Deployment locations at Coningham and Redcliffs (See Figure 2 page 11)
- Field biosensor interface and LoRaWAN telemetry unit. External connectors visible at right are for solar panel and daisy-chained sensor cable (See Figure 5 page 16)
- Coningham surface equipment (See Figure 6 page 17)
- Redcliffs surface equipment (See Figure 7 page 18)
- Lab biosensor setup showing students using the system and the default LabVIEW display whilst the automated sensor detection is running (See Figure 12 page 25)

Scientific Literature

The following paper has been published:

M. Rana, A. Rahman, D. Hugo, J. McCulloch, and A. Hellicar, “Investigating data-driven approaches to understand the interaction between water quality and physiological response of sentinel oysters in natural environment,” Accepted for publication at Elsevier Computers and Electronics in Agriculture https://doi.org/10.1016/j.compag.2020.105545

Research Data

Data acquired from both the environmental and biosensors deployed in the field is available from CSIRO’s publicly available Data Access Portal, link provided in the references section.

Collection description

Physiological data (such as heart rate, shell gape) of sets of oysters deployed at two marine sites in the D'Entrecasteaux Channel, Tasmania, Australia: Coningham (approximately 43.091 S 147.303 E), and Redcliffs (approximately 43.310 S 147.085 E). Water quality data (such as DO, Salinity, pH, Turbidity, Temp, Cond) for both Redcliffs and Coningham (also known as Tinderbox) site. Sensors were deployed on 9th Nov 2018 at Coningham, and 18th Dec at Redcliffs. The dataset contains the measured water quality and oyster's physiological data up to 15 March 2019. At Redcliffs, there were four functional biosensors placed at the depth of 0.5, 1.5, 5, and 7 meters to measure the oyster's physiological data. On the other hand, there were five biosensors deployed at Coningham at the depth of 0.5, 1.5, 3.5, 5, and 10 meters. The water quality at each site was measured using a YSI EXO2 multiparameter sonde at 5 m depth.

https://doi.org/10.25919/5ed45737b2d98

Appendices

APPENDIX A – List of researchers and project staff

Principal Investigator	John McCulloch	Senior Research Engineer	CSIRO Data61 Hobart
Co-Investigator	Daniela Farias Aqueveque	Research Lead	CSIRO Aquaculture Hobart
Co-Investigator	Ashfaqur Rahman	Principal Research Scientist	CSIRO Data61 Hobart
Co-Investigator	Daniel Hugo	Research Engineer	CSIRO Data61 Hobart
Co-Investigator	Md Mashud Rana	Postdoctoral Fellow	CSIRO Data61 Sydney
Collaborator	Chloe Simons	MDC Coordinator/Teacher	Education Department of Tasmania

Collaborator	Andrew Walsh	Teacher	Education Department of Tasmania
Collaborator	Joe Valentine	Environmental Scientist	Aquenal
Collaborator	Jeremy Lane	Operations Manager	Aquenal
Collaborator	Sam Gray	Marine Scientist	Aquenal
Collaborator	Matt Barrenger	Senior Manager - Environment	Tassal
Collaborator	Will Perry	Team Leader - Environmental Compliance	Tassal
Collaborator	Anthony Ashe	Environmental Project Officer	Tassal
Collaborator	Stephen Witkowski	Environmental Officer	Tassal
Advisor	David Horner	Scientific Officer (Water)	Environment Protection Authority DPIPWE
Advisor	Greg Dowson	Water Specialist	Environment Protection Authority, DPIPWE

APPENDIX B – Educational Program unit 1 of Sentinel sensors: “Revolutionising our understanding and management of the estuarine environment”

Sentinel sensors:

“Revolutionising our understanding and management of the estuarine environment”

Editor:

Daniela Farias

Educational program unit 1

The proposed activities will be delivered depending on the student's grade, knowledge and other courses/ activities that are part of the Educational institution to avoid repetition.

Course Outline:

1. Introduction
1.1 Oyster farms in Tasmania
1.2 Oysters diseases
1.3 Environmental monitoring
2. Biosensors
2.1 What is a biosensor?
2.2 Why biosensors are important?

3. Sentinel animals
3.1 What is a sentinel animal?
3.2 Bivalve anatomy / physiology (Animal dissection)
4. Hands to work
4.1 Real time monitoring (Deployments)
4.2 Data stream analysis (Lab experiment, i.e. Feeding oyster)

1. Introduction

1.1 Oyster farms in Tasmania

The Tasmanian oyster industry is an important part of aquaculture in Australia. The cultured oyster is the Pacific oyster (scientific name: Crassostrea gigas), which is an introduced species from Japan. The first Tasmania's hatcheries were in 1980s.

Oyster farms in Tasmania provide:

- Employment for ~300 people
- Produce around 4 million dozen oysters each year (estimated value of $24 million)

Oyster farms are in the north, east and south-east coasts of Tasmania. Most of the oysters grow in the intertidal zone.

1.2 Oyster diseases

In 2016 the Pacific Oyster Mortality Syndrome (POMS) was detected for the first time in Tasmania. POMS is a virus that can kill oysters but is not harmful for humans. The virus effects the adductor muscle; therefore, the oyster cannot open and close the shell by itself and is exposed to diseases and predators.

1.3 Environmental monitoring

Environmental monitoring is the activity that helps to characterise the quality of the environment. Monitoring the environment is often used to evaluate any impacts of human activities or natural events. Monitoring the environment consists of looking at water, soil, forests, and the air.

Derwent Estuary Program

In Tasmania the Derwent Estuary has been monitored for 30 years by the Derwent Estuary Program (DEP). The DEP keeps community and scientist informed about overall health of the water. E.g. if there are high levels of pollution in the river or in the estuary (which can happen after heavy rains for example) a public health warning is issued to prevent people swimming or fishing.

2. Biosensors

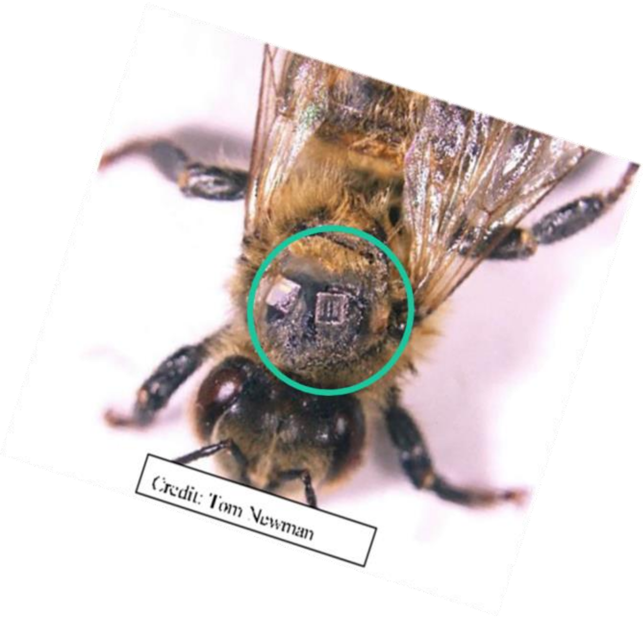
2.1 What is a biosensor?

A biosensor combines biology and technology for monitoring. Sometimes biosensors are attached to a few individuals and the data they provide gives us an understanding of the wider population. These animals are called “sentinel animals”. Sentinel means ‘watching’ or ‘monitoring’. Biosensors can provide information about the animals themselves (behaviour or physiology) or the environment (e.g. temperature) that those animals are in. It is also possible, to use behavioural or physiological information to infer information about the environment without having to directly measure it.

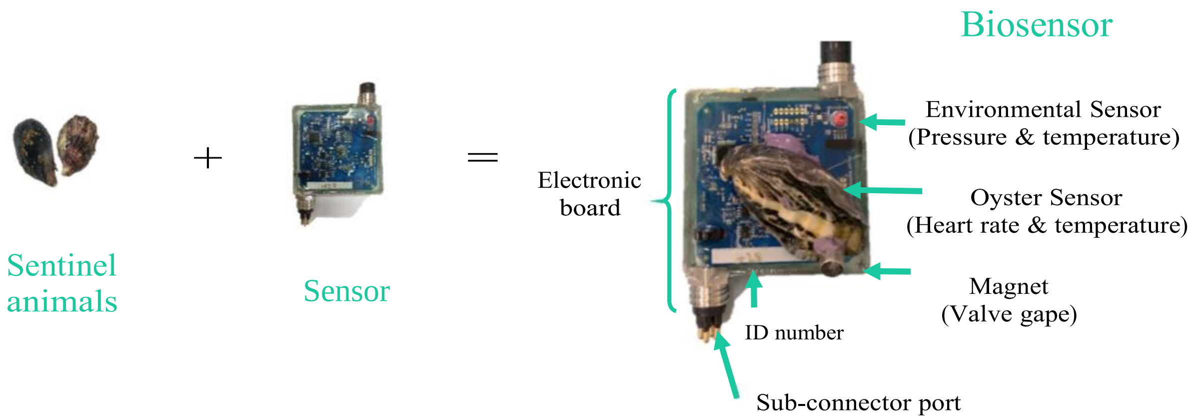
There are various types of life used as biosensors such as bacteria and enzymes. But, the most common are animals.

The humble Honeybee

Honeybees are well-known biosensors of air pollution. Nowadays, they are also used to assess pollination.



BIOSENSOR is a biosensor designed for oysters, which can alert changes in the environment.



2.2 Why biosensors are important?

Sometimes it is difficult to monitor the environment with traditional sensors. Using biosensors, it is possible observe changes in animals' behaviour that can alert us to changes in the environment (for example changes in temperature), or diseases (for example harmful algae blooms).

3. Sentinel animals

3.1 What is a sentinel animal?

A sentinel animal is an organism that is used for providing an early warning system. They could be placed in a given environment to monitor diseases or harmful agents in the environment.

Up until 1986 canaries were used as biosensors to save coal miners' lives. Because they are more sensitive to the poisonous carbon monoxide (CO) gas, which sometimes builds up in coal mines but cannot seen or smelt, the birds would get sick or die alerting the miners to get out whilst they still could.

In the ocean, there are many sentinel animals that have been used for a long time to evaluate environmental changes. The most common marine sentinel animals are bivalves such as oyster and mussels.

Bivalves are animals (Molluscs) with two shells.

Bi = two

Valve = Shell

3.2 Bivalve anatomy/ physiology (Animal dissection)

Objective

Recognise bivalves' anatomy with the aim of identifying where a sensor should be attached and why we are using bivalves as sentinel animals.

This activity involves the dissection of mussels and oysters for identifying internal organs, especially heart location.

Mussel's anatomy

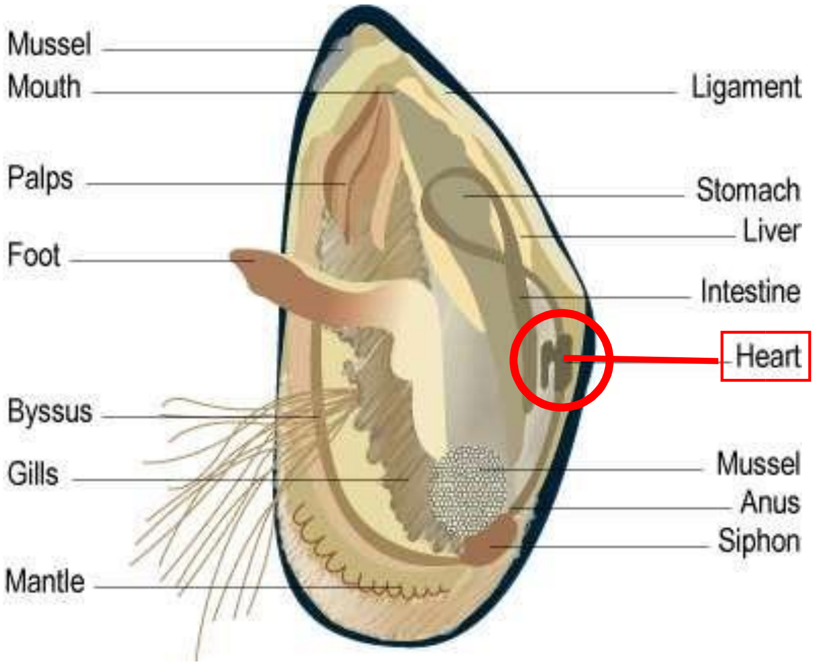
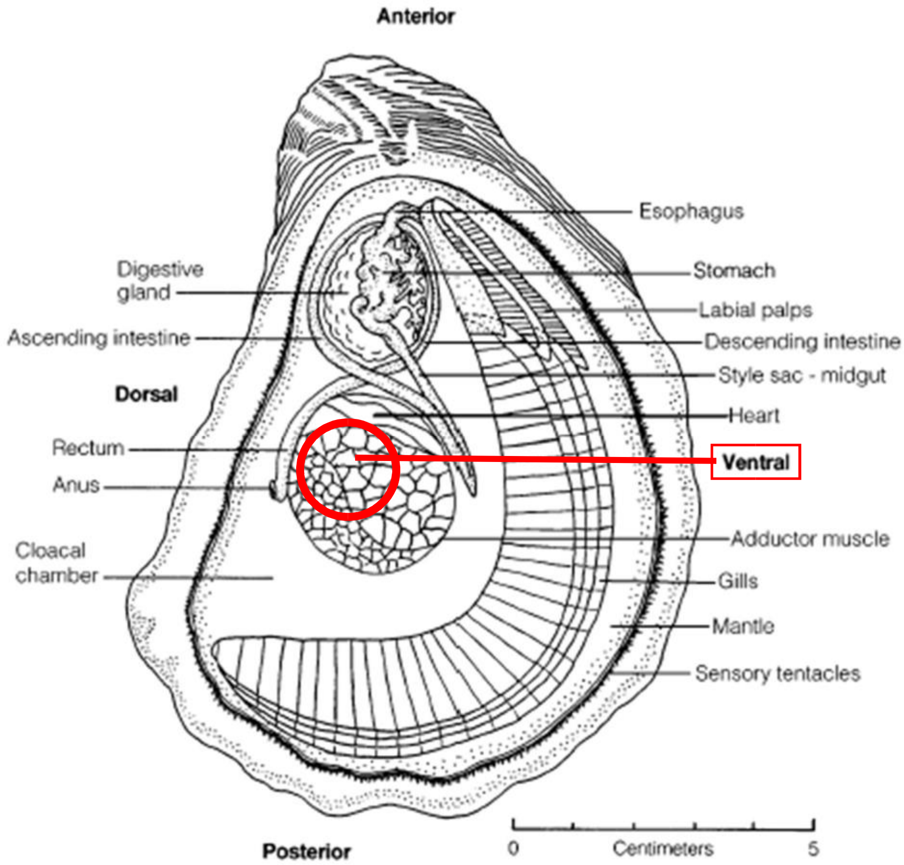


Image from: www.irish-mussels.com

Oyster's anatomy



Images taken from Eble and Scro 1996 .

4. Hands to work

4.1 Real-time monitoring

Objective

Learn how to use real-time stream data.

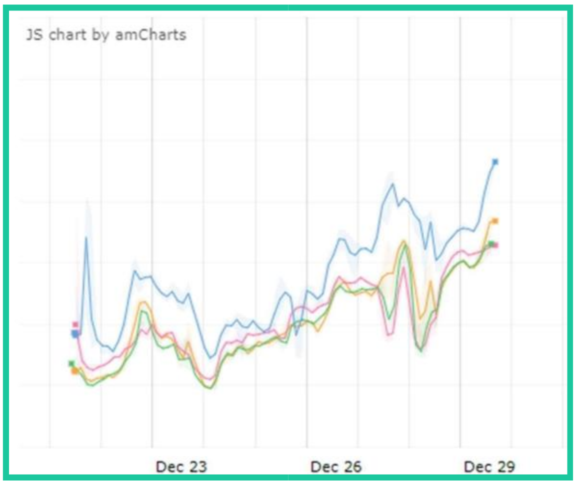
Biosensors can provide different information from the animal and from the environment. BIOSENSOR was developed to provide animal information such as:

- "Physiological data" (heart rate and internal temperature),
- Behavioural data (valve gape)
- Environmental data (light, salinity, temperature and pressure).

Animal internal temperature - Physiological data:

Deployed biosensors allow scientist to observe in real-time the internal temperature of an animal.

Figure 1. Graph of internal temperature of four animals deployed between 0 and 3m underwater.



Did you know?

The normal internal human body temperature is 37°C.

Over 37°C = Sickness sign

Light - Environmental data:

BIOSENSOR can also provide environmental information such as water temperature and light.

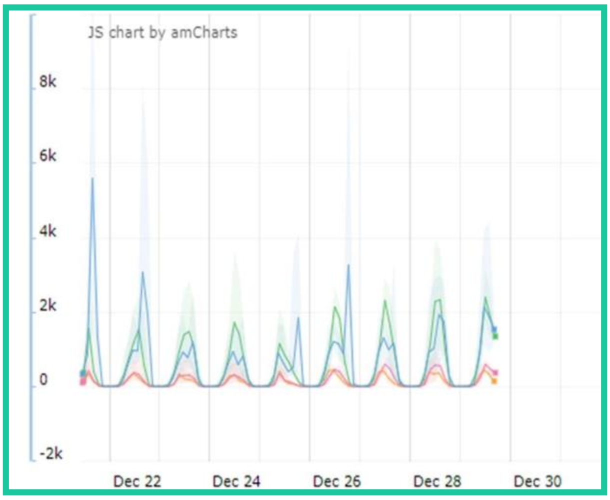


Figure 2. Light received by four animals deployed between 0 and 3m underwater.

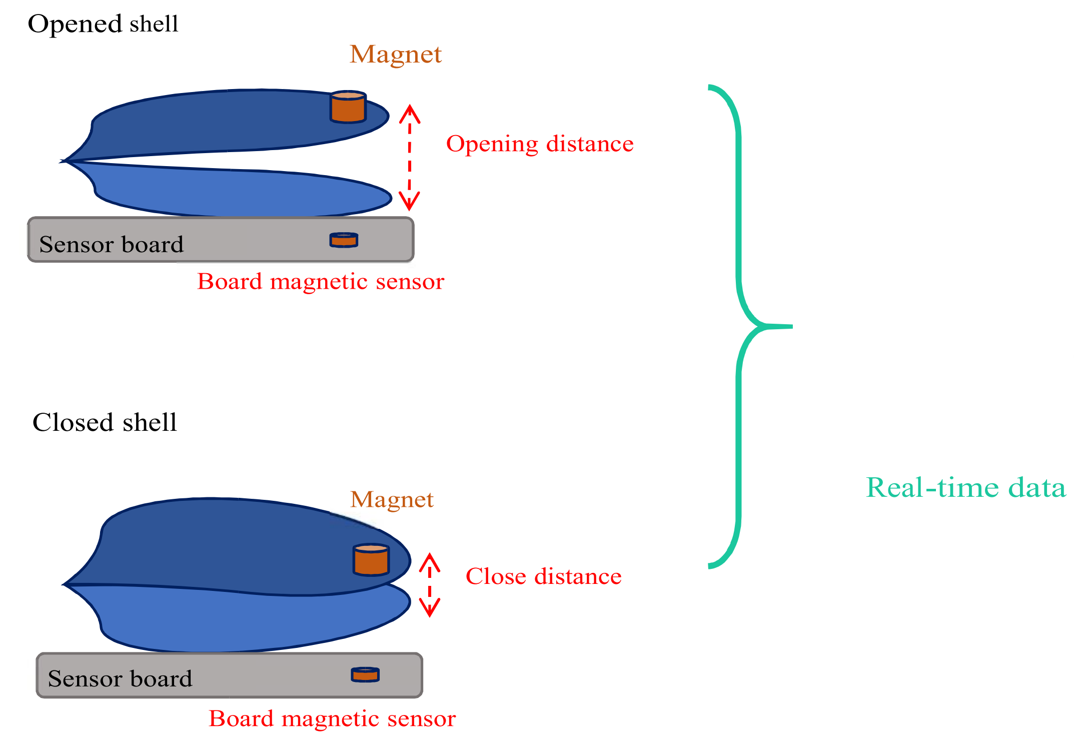
4.2 Data streams

Objective

Observe deployed animals in aquariums and in the field, to evaluate in real-time their behaviour. Part of the activities will be to feed bivalves with microalgae to evaluate heart rate and valve gape. But, how does the sensor BIOSENSOR work?

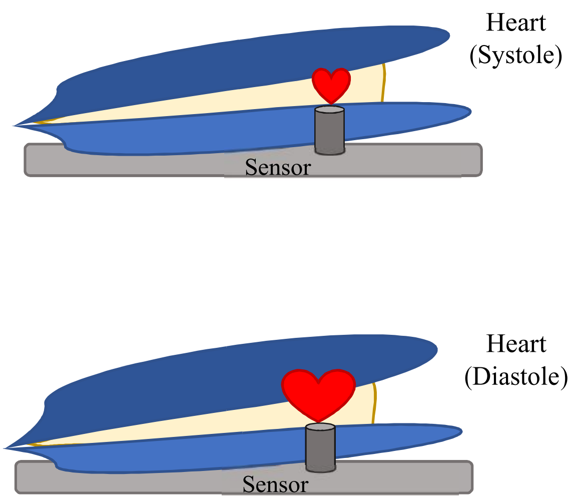
- Valve gape

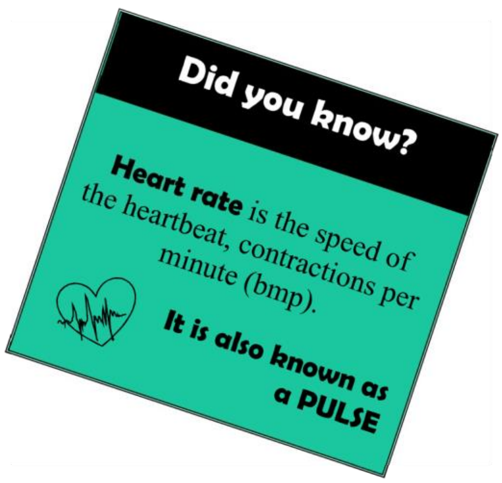
The valve gape is calculated using a magnet that is attached to the shell, which is connected to another magnet in the electronic board. The magnet in the sensor-board sends a signal every time the animal opens and closes the shell. The signal is sent it to a computer which calculates how many times the shell opens and closes, and how wide it was open.



- Heart rate

Like in humans, Bivalves' heartbeat changes under different conditions. When changes in the heartbeat are visible, it is an alert that something has changed, or the animal is under stress. BIOSENSOR was designed to identify changes in the environment that can alert diseases or changes in temperature.





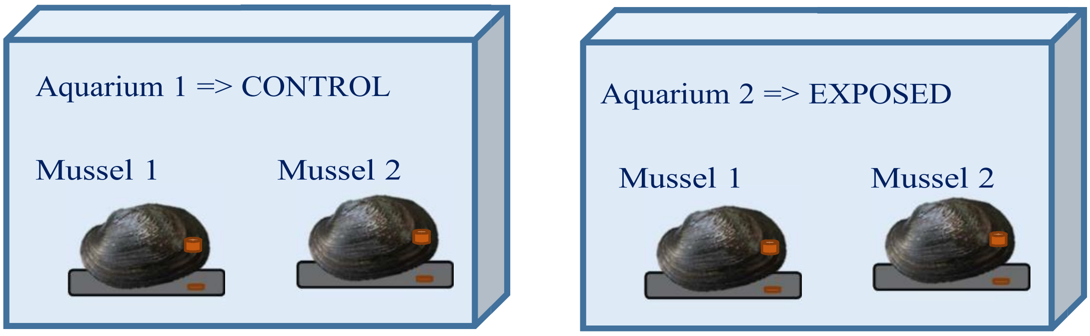
APPENDIX C – Educational Program unit 2 of Sentinel sensors: "Revolutionising our understanding and management of the estuarine environment"

Activity 1:

In this activity, you will find two experiments with real-time data that was taken from freshwater mussel from South Australia exposed to pollutants from mine runoff waters.

Aquarium 1: Two mussels has been set up in a sensor, they were leave in an aquarium with clean freshwater. This aquarium is call ‘CONTROL’.

Aquarium 2: Two mussels set up in sensors were leave in an aquarium with runoff water from a mine industry from South Australia. This aquarium is call ‘EXPOSED’.

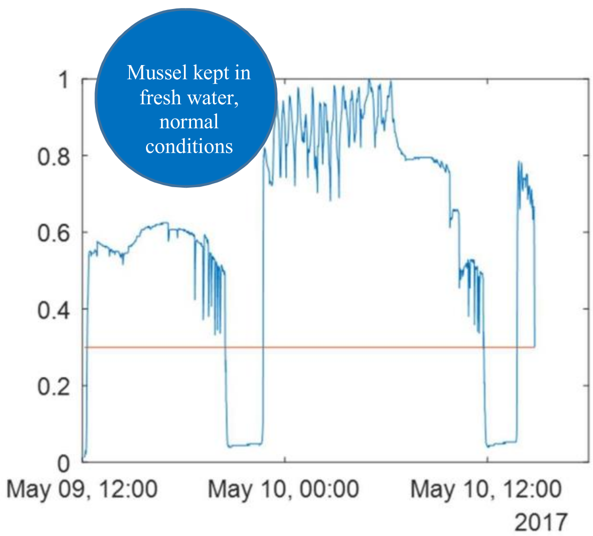


☐ SCENARIO 1

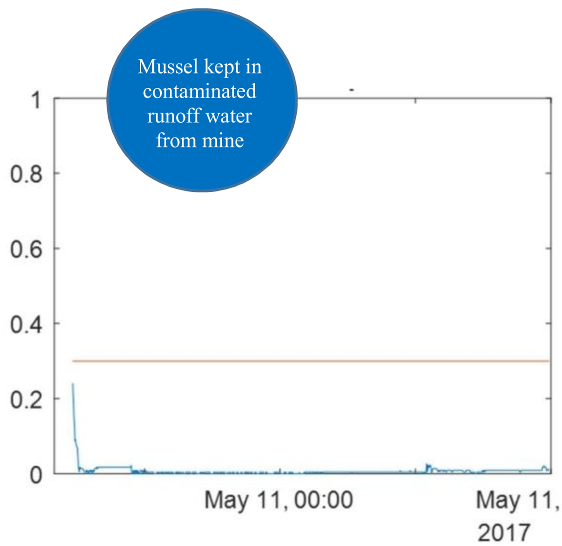
SCENARIO 1:

Real time results obtained from Mussel 1 from aquarium 1 ‘CONTROL’ and from aquarium 2 ‘EXPOSED’. Blue line is the actual gaping (open/closed) and the orange line is a threshold we use to determine if the animal is open or closed.

Mussel 1 - CONTROL



Mussel 1 - EXPOSED

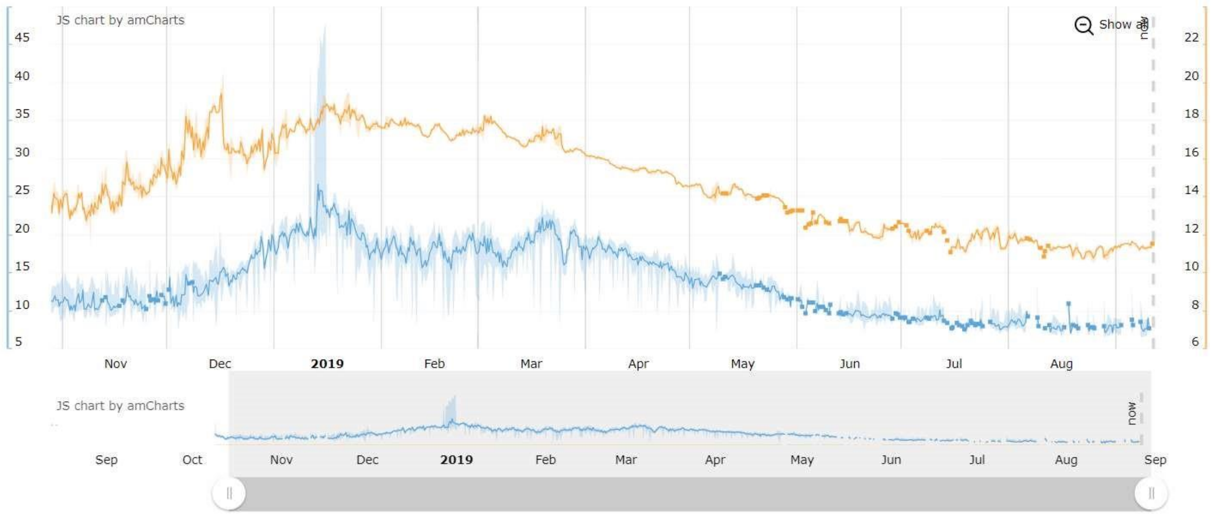


Observed results showed, discuss:

- Mussel 1 from CONTROL aquarium open and close their shell normally
- During EXPOSED time the mussel close their shell as a protection
- During EXPOSED time sensor shows low signal as animal keeps the shell close

Activity 2:

The graph below shows environmental water temperature (Orange line) and oyster heart rate (Blue line) of an oyster deployed at the D’Entrecasteaux Channel for long-term monitoring, period of observation from November 2018 to August 2019. Vertical axis shows Temperature in Celsius degrees (°C) and heart rate in Beats per minute (BPM). (Biosensor 415)



Using the information above analyse the questions of different scenarios below:

- What changes would you expect to see if the temperature increased drastically?
- Would the oyster open or close in case of an unexpected contaminant is present in the seawater?
- Oyster and sensors are deployed in the Derwent estuary close to the Zinc smelter located in the Princes of Wale Bay, an unexpected spill of heavy metals (principally Zinc) has occurred. What do you think oysters will behave? It will keep the shell open or shut?

APPENDIX D – Educational Program unit 3 of Sentinel sensors: “Revolutionising our understanding and management of the estuarine environment”

This is a proposed third (more intense) future course based on earlier modules.

1. Introductory class
a) Oyster farms in Tasmania: a short story about the relevance of oysters in Tasmania.
b) Oyster diseases: a short story of oyster diseases and how diseases affect the oysters, in the context of farms productions and climate change.
c) Environmental monitoring: The importance of environmental monitoring, how they are done and who does it.
2. Biosensor
a) What is a biosensor: An explanation of biosensors technology

b) Why biosensors are important: The importance on biosensors for the environment, with the emphasis on marine species.
3. Sentinel animals
a) What is a sentinel animal: The explanation of sentinel animals with examples of historical and relevant animals used as a biosensor.
b) Bivalve anatomy/ physiology: The identification of bivalves' anatomy with the aim to identify relevant organs that are used for biosensor application.
4. Experimental activities "Hands to work"
a) Animal dissection: Oysters and mussels will be opened with the objective of identify internal organs, localise heart and evaluate sensor location.
b) Real time monitoring: Deployments. Sensors will be deployed in aquariums (indoor) and in mussel raft (Outdoor). Students will be able to see how sensor technology works.
c) Data stream analysis: Evaluation of real-time data. There will be biosensors deployed to allow students evaluate in real-time how the sensors work.
d) Experimentation: oysters/ mussels will be fed with the objective that students will observe in real time changes in behaviour (Valve gape), and heartrate. Additionally, animals will be relocated to an aquarium with different water temperature to evaluate changes. At the end of the experimentation, the students will be able to understand bivalves' responses under different environmental conditions.

APPENDIX E – Example of outreach news

The Culture, issue-31, CSIRO Aquaculture Agriculture and Food (Fig. 11) newsletter

The Culture

INTERNAL NEWSLETTER **ISSUE 31**

As a part of our FRDC project "Sentinels sensors" we have been working with the Woodbridge Marine Discovery Centre (MDC) in Tasmania, to develop an educational outreach program to teach to young generations about the importance of using technology

Photo: John McCulloch, Daniela Farias, and Andrew Walsh from the Woodbridge Marine discovery Centre

and sentinels animals to understand changes in the environment, temperatures, and diseases as POMS. Our program is designed for kids from grade 6 to 12, students will be able to set oysters in sensors, see real-time data, test oyster's heartbeat, and see how oysters open and shut the shells according to environmental changes.

Photo: IPad set it up next to aquarium showing signal of oyster sensors "Oystag" in educational aquariums.

The sensors are installed in aquariums where students can see shells opened and closed, and at the same time, they can see on an IPad a graph with oysters' heartbeat and movements. Andrew, from the MDC, was the most excited about the use of this wonderful innovative technology (You can see his face in the photo) ☺

information and to register click here and type **Virtual Working Smart with Outlook**.

The next **Productive Collaborations** program will be running at St Lucia (Women's College) on 26th and 27th June (two days face to face). This program is designed to build your communication skills and the ability to work more effectively with others. More specifically, the program will enabling you to improve your conflict management skills, develop your emotional intelligence and practice mindfulness techniques to build personal resilience. Cost: $850. For more information and to register click here and type **Productive Collaborations**.

CERC Postdoctoral Fellows Procedure: all staff consultation - You may have seen on the MyCSIRO site that the Student & CERC Postdoctoral Fellow Office are seeking feedback on the CSIRO Early Research Career Postdoc Fellows procedure. The draft procedure is available here, if you have any constructive feedback or suggestions feel free to leave a comment. Formal consultation will end on 11th April.

Career Opportunities - RP6 does not have any open vacancies this month. However, if you're interested to see what vacancies are available in CSIRO click here.

Seminars and Conference Attendance

"Famelab 2019 semi-finals (by Paula Lima)

FameLab is the world's largest live science communication competition that aims to discover charismatic early career scientists who can (in 3 minutes) inspire people to see the world from new perspectives. This month I had the chance to participate as one of the 10 semi-finalists of Famelab 2019 in an event that took place during the World Science Festival Brisbane (WSFB) at the Queensland Museum. It was an amazing experience and I strongly encourage other early career scientists from our program to participate in the following year. It is not only a really fun event, but also a good training opportunity as semi-finalists participate of a one day Science Communication training session with

Figure 36. Extract of 'the Culture' issue-31, April-2019, showing part of the work delivered at the Marine Discovery Centre

APPENDIX F – Biosensor display at CSIRO Discovery Centre

Figure 37. Static Biosensor Display at the CSIRO Discovery Centre in Canberra

APPENDIX G – References

Teaching Materials

Educational resources can be found on the Woodbridge Marine Discovery Centre Teacher Resources page at: https://www.woodbridge.education.tas.edu.au/wp-content/uploads/2020/05/Educationalprogram-complementary-information_25_02_2019.pdf

Additional information about incorporating Biosensors into Australian Curriculum Science or for examples of how this program is used in a secondary science class, please contact:

Chloe Simons – Science Programs Coordinator (Years 7-12) Woodbridge Marine Discovery Centre.

Scientific Literature

M. Rana, A. Rahman, D. Hugo, J. McCulloch, and A. Hellicar, "Investigating data-driven approaches to understand the interaction between water quality and physiological response of sentinel oysters in natural environment," Accepted for publication at Elsevier Computers and Electronics in Agriculture

Research Data

Data from the project is accessible through CSIRO's Data access portal:

Collection description

Physiological data (such as heart rate, shell gape) of sets of oysters deployed at two marine sites in the D'Entrecasteaux Channel, Tasmania, Australia: Coningham (approximately 43.091 S 147.303 E), and Redcliffs (approximately 43.310 S 147.085 E). Water quality data (such as DO, Salinity, pH, Turbidity, Temp, Cond) for both Redcliffs and Coningham (also known as Tinderbox) site. Sensors were deployed on 9th Nov 2018 at Coningham, and 18th Dec at Redcliffs. The dataset contains the measured water quality and oyster's physiological data up to 15 March 2019. At Redcliffs, there were four functional biosensors placed at the depth of 0.5, 1.5, 5, and 7 meters to measure the oyster's physiological data. On the other hand, there were five biosensors deployed at Coningham at the depth of 0.5, 1.5, 3.5, 5, and 10 meters. The water quality at each site was measured using a YSI EXO2 multiparameter sonde at 5 m depth.

https://doi.org/10.25919/5ed45737b2d98