Quantifying physiological and behavioural responses of cultured abalone to stress events

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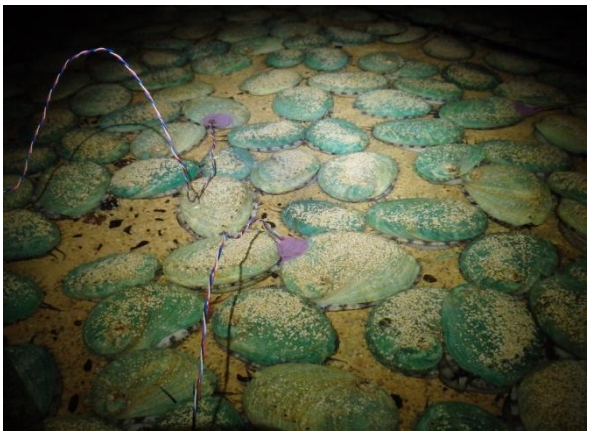

AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

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Non-Technical Summary

Quantifying physiological and behavioural responses of cultured abalone to stress events

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PROJECT OBJECTIVES:

1. To determine the physiological coping ranges and responses of temperate abalone to various environmental and production stressors measured under controlled laboratory conditions.
2. To attempt to monitor in-situ farmed temperate abalone under commercial conditions to identify and understand the key physiological and behavioural responses to a variety of production stressors.
3. To develop preliminary algorithms to enable interpretation of data from biosensors in the context of physiological and behavioural response to identified stressors.
4. To identify any potential applications of existing biosensors to improve current farm management protocols.

NON-TECHNICAL SUMMARY

Knowledge remains limited on the underlying physiological responses to stress by molluscs, and in particular abalone. This is the case for both wild and cultured individuals. A better understanding of, and the ability to monitor in real-time, an individual's response to stress events will assist in improving farming practices that result in minimised stress, and so lead to better health, increased productivity and high quality product.

This research project provided an important preliminary step in the process of quantifying and understanding the responses of cultured abalone to stress events.

The research project undertook the first comparative study between the two main cultured temperate abalone species in Australia, and their commercial inter-species hybrid. This work was at the juvenile stage of growout as the animals move from the more controlled nursery phase to the growout system. Importantly, at this age a critical variable governing biological performance, dissolved oxygen, is not limited, and the individuals are not subject to sub-optimum conditions. The research found that at this early stage of growth and under optimum conditions there was no significant difference in metabolic rate between the three genetically different cohorts. This work will be followed up with further comparative studies with different ages of the same pedigree of the three cohorts and under varying optimal and sub-optimal conditions to better define the coping ranges and responses.

A primary focus for this project was to modify an existing animal physiological biosensor to allow on-farm real-time monitoring of the key physiological parameter, heart rate, during commercial growout and harvesting. This was achieved and demonstrated.

To achieve this result laboratory trials were conducted on both wild and cultured abalone of varying sizes and conditions to ensure that the biosensor was optimally and efficiently placed

on the animals, and that the resulting signal and interpretation of the data was accurate and meaningful under a range of conditions.

Following this developmental stage, the biosensor was tested on-farm and delivered for the first time, real-time heart rate data for abalone under farming conditions. These initial activities monitored individual abalone under commercial growout conditions and then during a harvesting and processing activity for live shipment. Further on-farm trials have been planned with key stakeholders and additional laboratory data are undergoing analyses.

OUTCOMES ACHIEVED

The expected outcome from this project will be further on-farm trials of the modified and tested abalone biosensor, coupled with ongoing physiological data over the full size range, to better understand the impacts of the farm environment on the abalone under culture and so improve productivity and product quality. In addition, its future use with wild individuals could lead to better understanding of the impacts of the environment as well as harvesting and transport.

Further trials on-farm have been planned with key industry stakeholders.

LIST OF OUTPUTS PRODUCED

A research grade abalone specific biosensor, for collecting continuous heart-rate data as well as environmental variables, was modified and tested in the laboratory and on-farm, and was shown to deliver sound and useful data.

Data on individual animal responses to stress during grow-out, and the harvesting and holding for live shipment, have been recorded and analysed.

Comparative metabolic rate measurements of cultured juvenile blacklip, greenlip and their interspecies hybrid reveal no differences between the three at this early age.

ACKNOWLEDGEMENTS

The authors would like to thank Great Southern Waters for allowing access to their farm and their commercial abalone for on-farm trials and experimentation, AbTas and Cold Gold for access to cultured farm animals, and IMAS University of Tasmania for access to wild abalone, and John McCulloch (CSIRO) and Brian Taylor (University of Tasmania) for electronics and data analysis capability.

1. Introduction and Background

The project proponents (CSIRO/University of Tasmania) have developed a research group and facilities to investigate and understand the interaction of physiology and the environment of aquaculture animals, in particular abalone, oysters and fish. Knowledge is very sparse on the fundamental physiological aspects and responses to stress in a commercial aquaculture environment, and this is especially true for molluscs.

The concept of in-situ measuring and monitoring of physiology and behaviour of cultured abalone to improve production efficiency and ensure optimal abalone performance was presented by the project proponents to the Australian Abalone Growers Association (AAGA) annual meeting in 2011. In January 2012 AAGA invited the research proponents to develop the concept plan for a project for funding through the Seafood CRC. This research project was designed to provide preliminary steps to improved knowledge on the interaction between the animal under culture and its environment, leading eventually to increased production efficiency. The project therefore fits with the proposed outcome of the Seafood CRC Program 1 - Production Innovation. The research was directed at starting to understand the physiological responses of abalone to key production and environmental interactions/stressors, therefore allowing the industry to adapt their protocols and processes, where necessary, to improve production efficiency.

Growth of individual animals is limited by their energetic capacity. Those individuals that obtain, process and utilize energy the most efficiently and have an optimum balance between growth and other physiological functions (reproduction, movement, etc.) will have the highest growth rate (Tolkamp et al., 2003; Butler and Green, 2004). Therefore, measuring the energetic state (metabolic rate) and the allocation of energy are key in understanding an animal's physiology. Measuring an individual's metabolic rate directly can be accomplished under controlled conditions in the laboratory, but it requires expensive specialized equipment and a lot of time. Measuring metabolic rate in the field, for example under commercial farming practices, is not very practical or efficient. Instead, researchers have found numerous other techniques to indirectly measure the energetic state (Speakman 1997). One of the most common methods is the heart rate method for estimating metabolic rate; it is faster, more efficient and can be performed on many animals simultaneously (Butler and Green, 2004). This method is based on the Fick convection equation for the cardiovascular system:

$$V_{O_2} = f_h \times V_s(C_aO_2 - C_vO_2)$$

Where f_h is heart rate, V_s is cardiac stroke volume (the amount of blood pumped per heart beat), C_aO_2 is oxygen content of arterial blood and C_vO_2 is the oxygen content of mixed venous blood. This method relies on the premise that a change in f_h is a major component in the response of the cardiovascular system of an individual to an increase in the demand for oxygen. If the oxygen pulse ($V_s(C_aO_2 - C_vO_2)$; the amount of oxygen consumed per heart beat; Henderson & Prince 1914) remains constant or changes in a systemic fashion than there will be a linear relationship between metabolic rate (V_{O_2}) and heart rate (f_h) which can be exploited (Butler 1993). This method has been used since 1915 in a variety of animals (See Butler et al (2004) for review), most of which have been vertebrates, but to date it has not been used in molluscs. This relationship has not been developed in abalone, nor has there been a comprehensive set of metabolic measurements across developmental stages for this mollusc.

Heart rate is a strong indicator of stress and responds quickly to changing environments. Therefore, measuring heart rate in-situ will allow farmers to monitor abalone and determine their metabolic status in real-time allowing them to adjust farm conditions and protocols to maintain optimal metabolic and growth conditions throughout the life of the abalone. This is a huge advantage over the current assessment methods which can only measure growth and survival periodically, and through intervention resulting in handling stress, which gives no indication as to whether they are achieving continual maximal growth rates.

1.1 Need

The challenge in any aquaculture system is 'observing' the physiological and behavioural responses associated with environment, production and other stressors; all factors that impact the animal health and welfare and overall production efficiency. Suboptimal health is often associated with culturing conditions, and this is predicted to become more prevalent and unpredictable with a changing climate. There is therefore an immediate and long-term need to overcome the 'observation' challenge and monitor relevant animal metrics remotely over relatively long time scales. Understanding stock health and welfare is vital for any primary producer to ensure optimal production and return on investment.

How do we know if conditions are optimal, and the performances observed are efficient and sustainable? Generally, for aquaculture species such as molluscs, it is through measurements of growth rate and survival, equating to biomass produced, rather than on metabolic and behaviour observations on the animal which are difficult to observe and poorly understood. Therefore there is limited information available for optimising the commercial environment from the animal's perspective. Sub-optimal conditions lead to stress, and there are multiple (observed and unobserved) stressors or stress events within a commercial growout system, the impact of which on an abalone's physiology is poorly understood. Measurement of an animal's response to stress is usually retrospective of the event and via invasive sample collection (an additional stressor).

Visual monitoring of general health and responses to environmental change is not possible when animals are intensively reared in water. Consequently, traditional aquaculture monitoring has focused on utilising easily-measured, important environmental parameters such as water temperature and quality (pH, oxygen level, turbidity) as a proxy for animal health (Schneider et al. 2012). Recent developments in integrated sensor networks have greatly improved the applications of this technology. Key environmental variables can be measured on farms in real time and monitored remotely via a PC interface (e.g. Zhu et al. 2010). However, although environmental sensors are often relatively cheap, easy to maintain and provide valuable insight into stock living conditions they fail to measure the animals themselves. Understanding how their animals respond to environmental changes is of great interest to commercial farmers, particularly as our climate changes and becomes more unpredictable. The development of small biosensors has enabled long-term, non-invasive monitoring of a range of variables that are relevant to animal health and productivity including heart rate, body temperature, water depth and light level. Real time physiological data that provides insight into animal health has the ability to assist and drive management practices. Environmental sensors are still required to interpret the physiological data and thus, the sentinel animals become another sensor in the network.

This proposal takes advantage of a newly developed research tool ("biosensors") that measures physiological and behavioural parameters in-situ providing an understanding of the response of the individual to a range of commonly experienced and predicted stressors in a commercial system. This research will provide preliminary knowledge for refining farm management protocols, and in the longer-term for developing real-time bio-monitoring of farm management protocols.

1.2 Objectives

1. To determine the physiological coping ranges and responses of temperate abalone to various environmental and production stressors measured under controlled laboratory conditions.
2. To attempt to monitor in-situ farmed temperate abalone under commercial conditions to identify and understand the key physiological and behavioural responses to a variety of production stressors.
3. To develop preliminary algorithms to enable interpretation of data from biosensors in the context of physiological and behavioural response to identified stressors.
4. To identify any potential applications of existing biosensors to improve current farm management protocols.

2. Methods

The research consisted of two components. Laboratory based equipment development and testing experiments conducted with full environmental control, and on-farm experiments and real-time physiological monitoring of abalone. Experiments were planned in consultation with industry partners at the 2013 AAGA meeting to address initial key stressors and production cycle events which included live transport.

Stressors that were to be included in these initial experiments were water temperature, and dissolved oxygen, exposure times and air temperature, and disturbance.

Experimental animals, included the blacklip abalone (*H. rubra*), the greenlip abalone (*H. laevigata*), and their inter-species hybrid. All individuals for which data is reported were from cultured stock, in addition significant preliminary biosensor testing used wild harvest blacklip abalone as well as cultured pure species and hybrids (data not reported here).

The Biosensors

The biosensors are a combination of plethysmography technology to measure heart rate and thermistors to measure body temperature. External environmental sensors were used to measure oxygen content and temperature of the water immediately adjacent to the test animal.

The wired biosensors are connected to the abalone shell using dental impregum (flexible, removable adhesive) around a small hole in the shell exposing the heart (see cover photo). To do this, abalone are removed from the water and a small hole (<5mm) is made using a dremmel. The sensor is inserted flush with the shell and glued in place. After the glue is cured the abalone is placed back in the water. This refined and well practiced process can now be achieved in under 10 minutes per individual animal. This method was refined both in the laboratory and on the farm. The bio- and environmental sensors are connected to a printed circuit board which is interfaced through PowerLab to collect the data on a PC.

Laboratory experiments using cultured and wild abalone and semi-controlled environmental conditions were used to validate the abalone version of the biosensor, its location on the shell, the signal intensity, accuracy and efficiency, and to obtain preliminary base physiological knowledge before embarking on the full scale on-farm demonstration experiments.

Comparative juvenile metabolic rate

Controlled environment based experiments focussed on the juvenile stage of production in this initial project. To undertake this research individual metabolic rate (oxygen consumption) chambers were designed and tested. Within these sealed chambers the water temperature and dissolved oxygen needed to be able to be monitored and manipulated, and the activity of the individual animal monitored. This custom designed system and protocol consisted of six chambers that were embedded in a galvanized aluminium plate which was placed in a temperature controlled environment. Each chamber was individually closed with an acrylic lid and was equipped with two pumps. One pump allowed water flow from a fresh seawater tank through the chamber during incubation. The seawater tank was also placed in the temperature controlled environment and supplied with an air stone to maintain the experimental temperature and 100% oxygen saturation, respectively. The second pump maintained water circulation within the chamber when it was sealed during the experimental period. The chambers were kept in the dark throughout the experiment.

For the experiments, individuals (*H. laevigata, H. rubra* and their hybrid (n=3 to 9 individuals per species; ~10mm shell length) were transferred into chambers after being levered off the algae plates by using a blunt spatula. At the time of the experiments the ambient water

temperature in the tanks was ~20°C. Animals were incubated for 12 h at the experimental temperature (10, 15, 19, 23°C: a typical range of temperatures experienced on-farm by this cohort of juveniles) to allow them to recover from handling and were unfed during this time. Oxygen content of the water was measured with a sensor dish reader (PreSens, Germany) throughout the experimental period at a time interval of 15 sec until 90-100% of the oxygen was consumed from the water in the chamber. Within each temperature treatment oxygen consumption was also measured in chambers without individuals (n=3 to 6 chambers) as well as chambers with shells only (n=3 to 6 chambers with shell) to account for microbial respiration. No differences in oxygen consumption between chambers without individuals and chambers with shells only were detected so results were pooled and used as blanks. At the conclusion of each experiment, tissues were separated from the shells to obtain tissue weight, both parts were frozen in liquid nitrogen and transported to CSIRO for future analyses (outside of this project plan). Wet weights (WW) of frozen tissues and shells were determined to the nearest mg. Subsequently, tissues were freeze dried at -60°C for 48 h and dry weights (DW) were determined to the nearest mg. Shells were air dried at 60°C until DW were stable. Shells were photographed for morphological examination and shell lengths and widths were measured using the software tpsDig 2 (data yet to be fully analysed).

On farm *in situ* proof of concept of biosensor

Following the laboratory testing on a range of sizes of cultured and wild abalone, the first proof-of-concept of the research grade abalone biosensor was conducted on-farm at Great Southern Waters (Victoria) on commercial cultured hybrid abalone (~80.4 mm shell length).

Six abalone were fitted with wired biosensors (biosensor wired direct to computer rather than data logged and stored within the biosensor) for real-time data collection for the duration of the experiments.

After the sensors were attached, the six abalone were allowed to rest and recover in the same grow out tank from which they were obtained at ambient temperature (~ 20.5C) in full darkness. Abalone were fed once daily at approximately 3:00pm and uneaten food pellets were washed away during morning cleaning cycles at 8:00am. To simulate a major stress event, four abalone were then moved into live holding tanks (~14.5C, 100% oxygen, natural 12:12 light:dark cycle) for 48 hours. These same wired abalone were removed from the water and then packaged according to standard live shipping protocols (12kg inside of a plastic bag filled with oxygen, covered by two ice packs and sealed in a styrofoam box) and left in the shipment container for 18 hours to simulate a live shipment. After 18 hours the abalone were removed from the packaging and placed back into the live holding tanks (~14.5C, 100% oxygen, natural 12:12 light:dark cycle) to simulate arrival at their destination.

The other two wired abalone remained in the commercial growout tank (slab tank) for five days and real-time heart rate measured once daily for 1 hour prior to feeding. These were control animals to examine long-term recover y following implanting of the sensor, and to gather data on daily variation.

Heart rate, temperature and oxygen were continuously measured during all stages of the harvest and live shipment experiments including the time in the raceways, live holding tanks, live shipment packaging and return to live holding tanks.

Analysis

Heart rate and corresponding environmental data was analysed using Chart software and a newly designed CSIRO analytical program which can interpret complex heart beat data to yield heart rate and interbeat variability across long periods of continuous data. This program has been used to interpret similar data from oysters and was modified for use in abalone (Hellicar, et al, 2014).

The abalone heart rate signal was sampled 1000 times per second over timeframes of many hours and exhibited a large range of heart rates as a consequence of the abalone's exposure to a range of environmental conditions. The heart rate was estimated from 20 second

sections. Within the 20 seconds a single heart beat was calculated as the time period between two similar signals (ie heart beats). 20 second overlapping sections were selected throughout the entire data set to generate the full heart rate results. Sections over time or during specific events on the farm were then averaged.

Additional detailed analyses of the extensive data collected from the biosensors both on-farm and in the laboratory are undergoing additional analyses beyond this project timeframe.

3. Results

A. Comparative juvenile metabolic rate

We have collected metabolic rate data on known pedigree juvenile greenlip, blacklip and hybrid abalone across a range of water temperatures (10-23°C). No significant differences in metabolic rate were observed between the species or the hybrid at this young age at any of the temperatures examined or at decreased oxygen levels (Fig. 1).

At 100% oxygen saturation weight adjusted metabolic rate was highest at 23°C (~12umol/g/h) and lowest at 10°C (~4.5umol/g/h). Juvenile abalone appear to be oxygen regulators with a critical oxygen value of approximately 40% saturation, after which metabolic rate begins to decline rapidly.

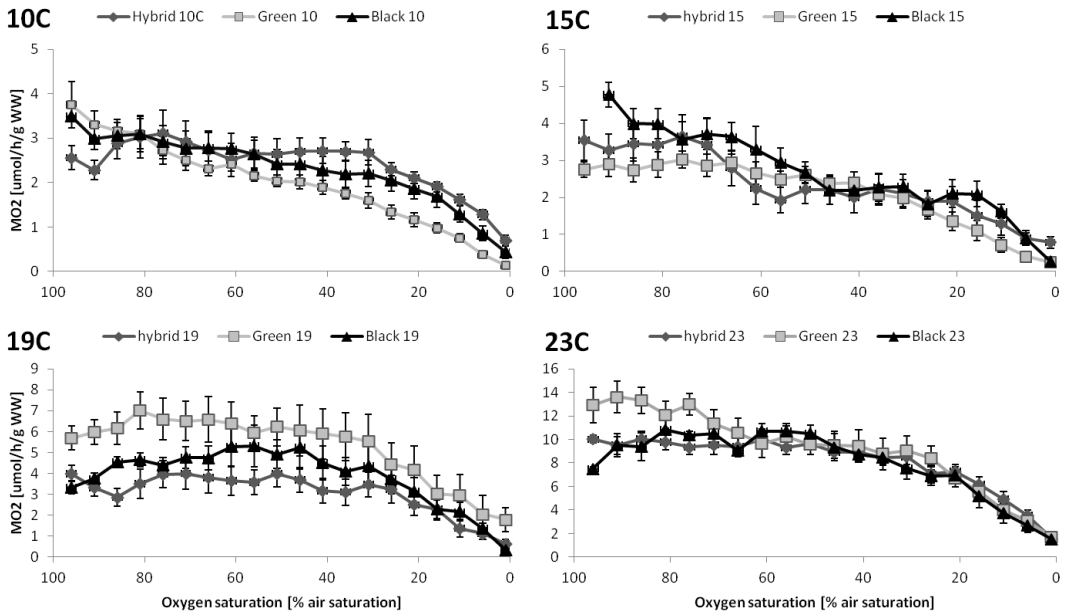

Fig. 1 – Metabolic rate (weight adjusted) of juvenile greenlip, blacklip and hybrid abalone at 10, 15, 19 and 23°C from 100% to 0% oxygen saturation. Data points are mean ± S.E., n = 4)

B. Biosensor real time data

Heart rate data from wired individual hybrid abalone was collected from individuals within commercial growout tanks and during mock harvest and live transport shipments. Overall, we measured continuous heart rate of four individuals from the commercial raceway through to in-air shipment and re-immersion, and found that heart rate was controlled and varied depending on the environment Fig. 2. Each bar represents the mean of four individual abalone as they progressively encountered each new area of the farming/harvesting/shipping process. Heart rate was highest in the higher water temperature in the raceways and was progressively lower when moved into the live holding tanks and packaged for shipping. Each section is elaborated below.

The two abalone left for five days in the raceway after sensor attachment allowed measurement of basal levels and any changes in heart rate due to handling and recovery time. Fig. 3 shows that there was no change in mean heart rate over the five day recovery period.

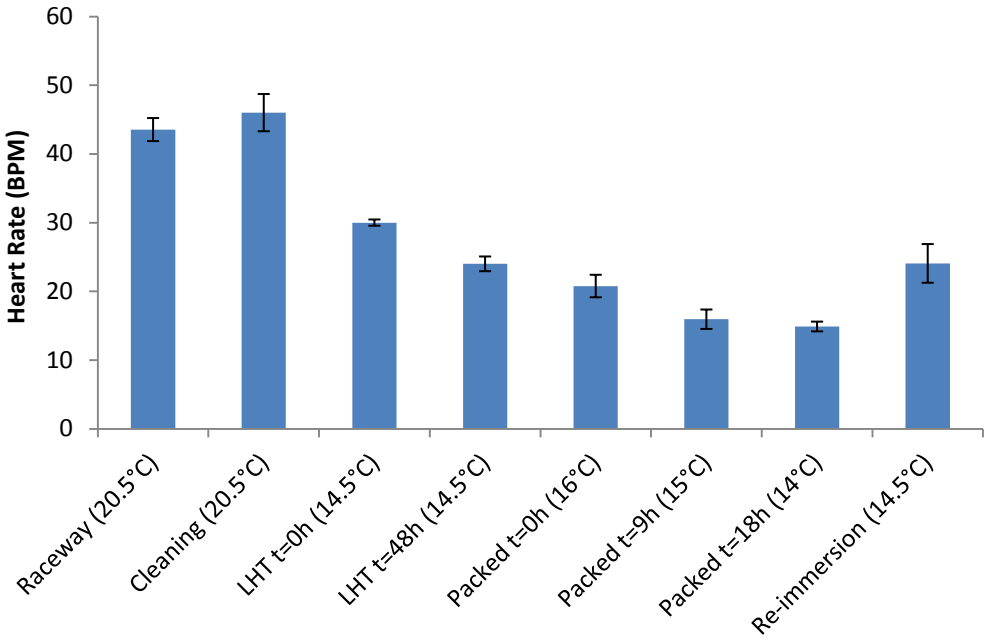

Fig. 2 – Mean heart rate over 1 hour across the range of commercial environments and stress events. (n = 4). Raceway = commercial raceway; Cleaning = mean value during raceway cleaning and flushing LHT = live holding tank, Packed = packaged for live shipment, Re-immersion = abalone removed from in-air and return to 14.5°C water t = time in each condition prior to measurement.

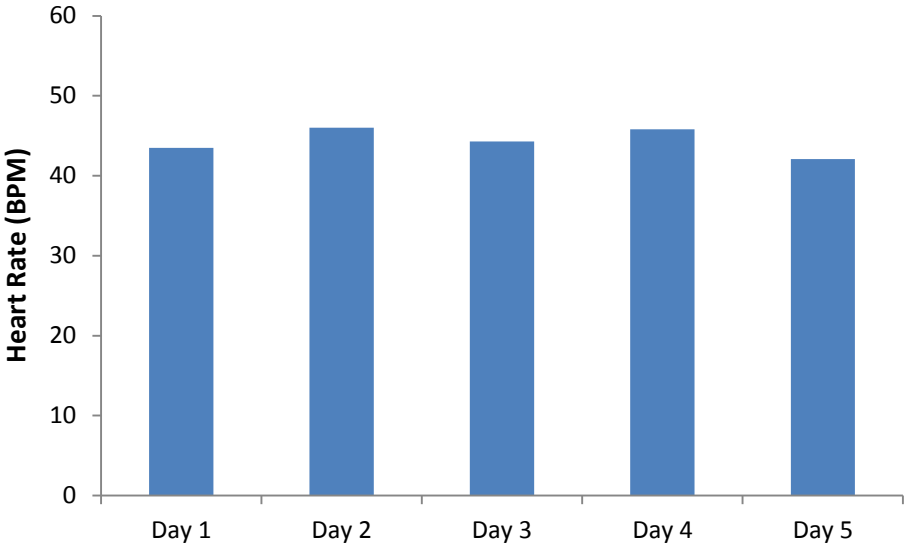

Fig. 3 – Abalone daily mean heart rate during recovery from acute handling, air exposure and sensor attachment. Mean of two individuals. The range over the 5 days was 37-51bpm.

Commercial Raceway

Within the raceway, data was collected continuously including during cleaning cycles in which large volumes of water were tipped into the raceway. Results indicate that on average "resting" abalone at 20.5°C have a heart rate of 43.5 beats per minute (bpm) and remains relatively constant throughout day and night as well as during cleaning events. Despite disturbance by large volumes of flowing water and small changes in oxygen content the average heart rate during cleaning only rose to 46bpm but with increased variability between individuals. Heart rate does not appear to be affected by each individual cleaning cycle (Fig. 4). Fresh water is dumped on one end of the raceway at approximately 3.5 minute intervals every morning but the number of cycles can vary day to day. Oxygen fluctuates between 80-85% during each cycle.

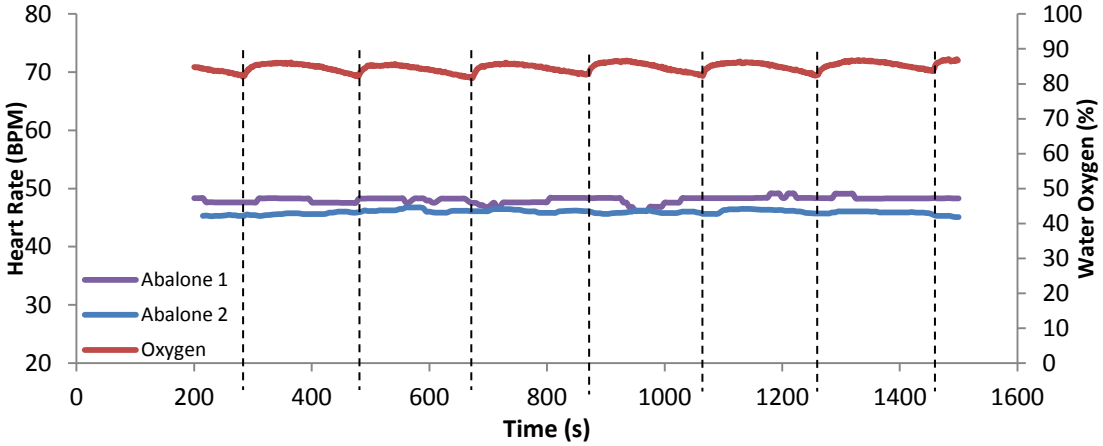

Fig. 4 – Example of heart rates of two abalones during cleaning cycles.

Oxygen content of the water was variable throughout the day and night and ranged from a minimum of 60.1% at night to a maximum of 84.9% during cleaning cycles in the morning. Water temperature did not fluctuate as dramatically as oxygen content and only varied between 19.5°C at night to 21.5°C during the day. Heart rate remained relatively constant as episodes of extremely low oxygen were short lived (60% for several minutes).

Live Holding Tanks

Wired abalone were moved to live holding tanks at 14.5°C for 2 days without feed. Oxygen content (100%) and temperature are very stable in these systems due to the constant aeration of the tanks and chilling units to regulate temperature. Light cycles at this stage of the farm follow the natural seasonal light cycle as the unit is in an open air facility (~12:12 light:dark). Immediately there is a drop in heart rate to 30.7 ± 0.5 bpm associated with a 6°C drop a temperature. Heart rate declined slightly to 27.1 ± 1 bpm after 48 hours in the live holding tanks. Fig. 5 shows an example of the variability in heart rate during an hour in the live holding tanks for one abalone; analysis of this variability required development of new software, and its application and interpretation of the data will be on-going beyond the timeframe of this project.

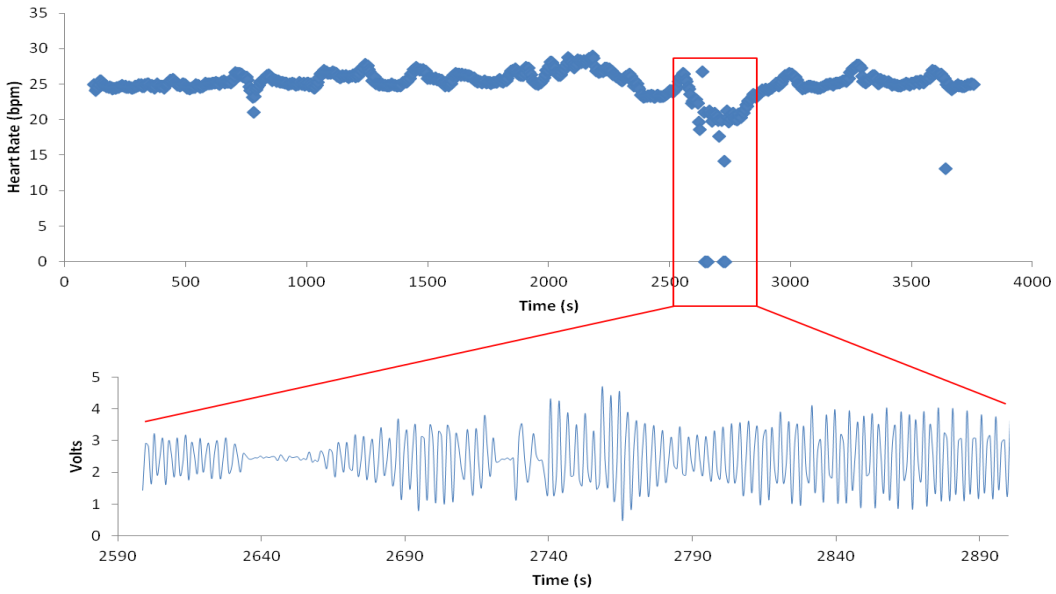

Fig. 5 – Example of variation in heart rate over a one hour period in the live holding tanks at 14.5°C. The graph highlights an example of an area of variability in heart rate trace in which the rate drops to zero.

Live Shipment

Following live holding in cooler water for 2 days, the abalone were packaged according to standard shipping protocols for 18 hours. The air inside of the container was 315% oxygen at the onset of the mock shipment and dropped to 211% just prior to opening. The temperature inside of the box started at 16.8°C and cooled to 13.4°C over the 18 hours (Fig. 6). Initial heart rate upon air exposure was 22.7 bpm. Throughout the trial, heart rate became increasing erratic with longer and variable interbeat times. After 9 hours of air exposure the mean heart rate had dropped to 15 bpm and fluctuated around that mean rate for the remaining 9 hours (Fig. 1). The frequency and duration of variability in the heart rate increased with time in the shipping boxes (Fig. 7). By 15 hours the heart rate was very unstable.

Abalone were immersed back into the live holding tanks at 14.5°C following 18 hours of air exposure. Heart rate returned to 24bpm upon re-entry and increased to 30bpm after 3 hours.

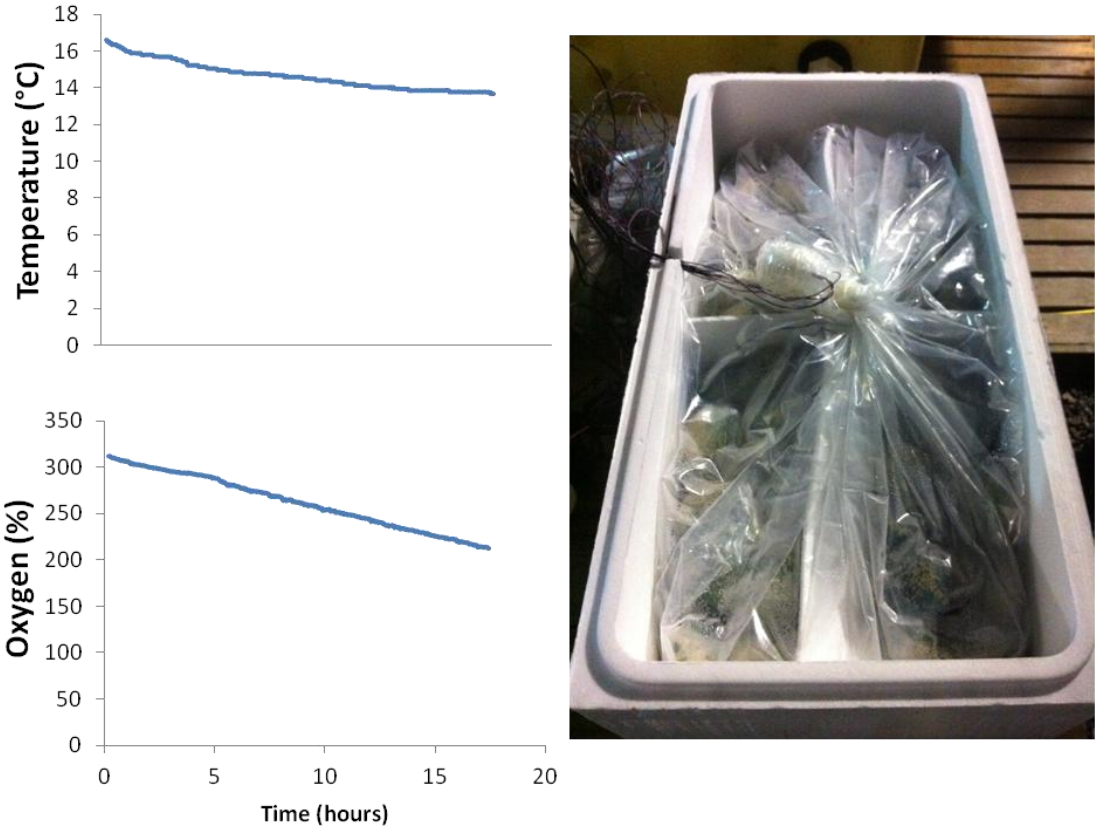

Fig. 6 – Oxygen and temperature profile of the air during an 18 hour mock live shipment of 12kg of hybrid abalone.

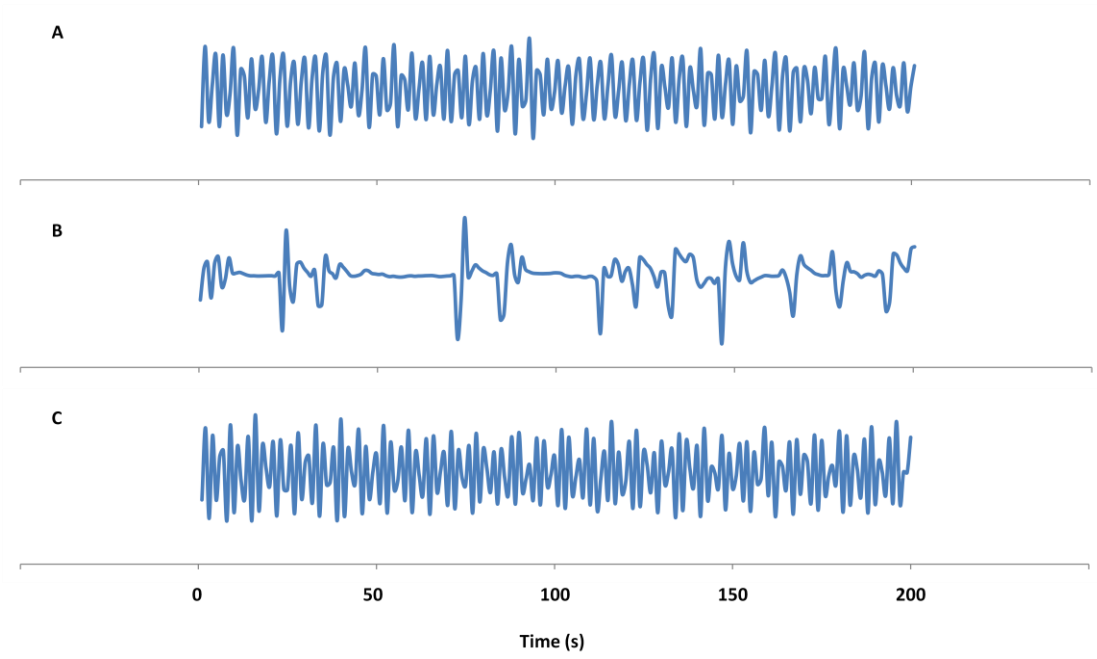

Fig. 7 – Example of an abalone heart rate trace upon initial entry into air (A), after 15 hours in air (B), and after re-immersion into water (C).

4. Discussion

We have successfully used the UTas/CSIRO mollusc biosensors on a commercial abalone farm to monitor heart rate in commercial growout, and throughout harvesting and live transport of hybrid abalone which is a major stress event. The present data provides baseline data for all areas of the farm and has enabled us to pinpoint sensitive areas to address in future experiments. The data collected from the biosensors, both on-farm and in the laboratory testing was extensive and is undergoing more detailed analyses, beyond the scope and timeline of this project. We also investigated the metabolic rate of juveniles of both species of abalone and their hybrid across a range of temperatures and water oxygen as the first component of the laboratory data.

Objective 1: Physiological coping ranges and responses

We began controlled environment laboratory testing of abalone metabolic rate with juvenile abalone (of known pedigree) at the end stages of their time on algal plates. Metabolic rate declined with decreases in both temperature and oxygen as expected but we found no significant differences between species at this age (Fig. 1).

Metabolic rate is often correlated with shell growth rate (e.g. Lewis and Cerrato 1997), and thus we expected to find a higher metabolic rate in the hybrids which are known to have a higher growth rate. However, the correlation may not hold for soft tissue growth (weight) that was the trait of measure at this time with the small individuals, and a species difference in metabolic rate may be apparent when shell length is considered rather than weight (planned work). It is also possible that hybrids are consuming more food and moving less which would contribute to faster growth. A difference in metabolic rate may also not be apparent until later life stages where abalone are growing in the raceways and conditions are less than optimal (fluctuating oxygen and temperature).

This initial measurement of juveniles will be complimented in the future with data collected from larger and older abalone, from the same pedigree, to provide a comprehensive data set for the development of the metabolic algorithms and better determining coping ranges and responses to various environmental stressors. As significant project resources were put into ensuring delivery of Objective 2, and this comparative work is best undertaken on the same pedigree lines as they age (outside time frame of this project), this project has only provided the start to the gathering of this critical physiological knowledge.

Objective 2: Attempt in-situ monitoring

The technique of using heart rate to estimate metabolic rate has not been employed in molluscs previously, and thus we took a significant amount of time in generating a robust and reliable biosensor for abalone of varying size and history.

The sensor is now of an appropriate size and shape for attachment to the abalone and the interface between the sensor and the computer has been refined to provide the clearest visualization of the heart rate. The interface required significant alterations to optimize the signal shape and strength to allow for simple interpretation of the data. The interface and the biosensor were both tested through several trials to determine the best water-proofing method which kept the units dry and functional but still allowed for manipulation of the components. The current research version is now fully functional and was used on the farm for the first time (see below).

The biosensors have been trialled on a small set of abalone on the farm across a range of commercial conditions which has provided baseline heart rate data for future large scale trials. Significant amounts of data were collected during the on-farm and laboratory trials, and analyses of this will be on-going beyond the time frame of this project. The specific details of each condition are outlined below.

Grow-out Raceways

Resting heart rate values in ~80mm hybrid abalone at ~20°C water temperature were roughly 43bpm in six individuals and this did not change over a 5 day period after the sensor was attached (Fig 2). This indicates that the abalone, at least in terms of heart rate was likely not affected by the addition of the sensor. Behaviourally, the abalone with the sensors returned to normal and moved throughout the raceways at night. For several hours in the morning the raceways were flushed with large volumes of water every 3-5 minutes. This flushing had no effect on the heart rate throughout the experiment, despite the higher flow of water and small changes in water oxygen the abalone remained undisturbed (Fig. 4).

The grow-out raceways (slab tanks) where the abalone spend the majority of their time are a critical area of interest for increasing growth and production efficiency. In particular, oxygen concentration fluctuates throughout the day and can drop to as low as 60% in some cases. The frequency or duration of occurrence of these drops in oxygen content are unknown, but should they be frequent (or be isolated within a raceway), they could be severely limiting the performance of the abalone. Oxygen is a limiting factor for growth (Fry, 1971) as it limits the capacity for aerobic metabolism and ultimately growth. Generally, anything below 80% is considered hypoxic and can have an effect on metabolic rate, and thus, growth rate. Over a 24 hour period oxygen in the water on average is roughly 80% which is potentially limiting their growth, particularly during summer where metabolic rate will be elevated due to the higher temperature. Growth rates in greenlip abalone have been found to decline by 50% when oxygen levels fall to between 70-80%, making this a critical issue that needs to be addressed on the farm. Future controlled laboratory test could further address this issue in both species and the hybrid, and at all ages, sizes, maturation stages, nutritional status, and temperatures.

Live Holding Tanks

The live holding tanks are the one area of the farm where oxygen and temperature are regulated, thus providing a stable environment for the abalone prior to shipping. Current practices require abalone to be in the live holding tanks for 5 days at ~14°C. During summer months, this is an immediate minimum of 6°C drop in temperature from the raceway. As expected the heart rate slows upon entry into the colder water by 30%. This heart rate is similar to other species of abalone tested at this temperature (Ragg and Taylor, 2006). Aside from anecdotal evidence, it is presently unclear if this is the best temperature and time duration for the abalone to be acclimated in prior to shipment. Further research in this area for longer periods of time and at various temperatures should help to determine the best conditions for the abalone prior to shipment.

Live Shipment

Oxygen levels inside the shipping containers were maintained significantly above 100% throughout the 18 hour mock shipment in contrast to what was previously thought to occur ie. the oxygen would diffuse away through the plastic or the abalone would consume it over that period of time. Current packaging methods are therefore assumed to be sufficient to prevent hypoxia/anoxia during transport. The current experiment cannot discern whether the decline in oxygen is due to diffusion through the plastic or consumption by the abalone, but could be addressed in future trials.

While abalone seem to be able to tolerate long exposures to air, there are major consequences for heart rate. Initially, heart rate is similar to that in the live holding tanks (24bpm) but by 9 hours drops to ~15bpm and remains at this rate for the duration of the shipment. As time passes there are increasingly more episodes of irregular heartbeats with varying interbeat times (Fig. 7). It is currently unclear as to whether or not abalone are able to sufficiently acquire oxygen from the air, therefore, despite having higher amounts of oxygen in the air it may be unavailable to the abalone which would reduce the heart rate. Alternatively, abalone out of water tend to lose water mass which may cause a shift in the location of the heart relative to the sensor giving “false zero” readings.

Upon immersion back into the live holding tanks abalone resumed a cyclical heart beat pattern with a rate similar to that before they were exposed to air. The conditions that abalone are exposed to upon arrival at their destinations are currently unknown and water variables could range from location to location having an effect on the mortality and physiology of the abalone on arrival. Determining the optimal water conditions for abalone post shipment could reduce mortality and yield higher quality abalone. The current live holding tanks on the farm appear to be suitable in terms of heart rate, but it will be important to assess the abalone quality under varying conditions to discern the optimal combination of parameters for optimal survival.

Objective 3: Preliminary algorithms for data interpretation

We have developed a new software program for the interpretation of the abalone heart rate data which has been tested and can now be used in future in-situ experiments in the laboratory and on-farm. The greater than expected commitment of resources to ensuring delivery on Objective 2, meant that the amount of controlled data collected and fully analysed in laboratory or on farm trials was limited and this objective was not fully achieved.

Objective 4: Potential applications

We have identified the key aspects of the farming process for which the biosensors will be most useful for improving production efficiency and product quality. Namely, the use of the biosensor in the growout tanks will provide information relating to the farm management practices and changing environmental parameters that will impact on optimal growth. Any application of the data obtained from the biosensor in this phase will lead to optimized growth and survival. Secondly, using the biosensor during live shipment will give information on the environment and the physiological condition of the animals which has previously never been determined. This information is critical in determining the optimal shipping conditions as well the optimal conditions for re-immersion on arrival which will help prevent mortalities and keep the abalone in good condition for sale.

Critical research areas to address in the short term:

1) We need further testing to understand the meaning of small gaps in the heart rate traces. Are they a true drop in heart rate? Due to movement and displacement of the sensor? A result of an environmental cue? A behavioural pattern? And what are the potential implications on growth and survival? On-going data analyses are continuing beyond the conclusion of this project.
2) We need to continue the laboratory based trials to cover the suite of environmental and biological conditions experienced on the farm in order to further develop the algorithms for on-going interpretation of the heart rate data collected during this project.
3) Further biosensor trails are proposed with both wired (real-time) and 'logger style' (data stored in on-board unit attached to shell) units; the latter will allow continuous commercial data to be captured during handling/ transitions periods on the farm, ie. from raceway to grading, to live holding tanks and packaging, which cannot be done when wired.

5. Benefits and Adoption

This project has enabled us to further the limited understanding of how animals respond physiologically to environmental and farm stressors. In particular this project has highlighted the issue of fluctuating oxygen conditions in growout tanks, and this needs further investigation on whether there are potential impacts for optimal growth and survival, and this may lead to changes to the design and management of the growout system. In addition the research has provided the first insight into the animal's response to a live shipment protocol, involving harvesting, depuration under colder temperatures, out of water packing and re-

immersion. These finding can be used, along with additional data to improve the processing protocols and ensure higher survival and quality abalone for the market.

The project has demonstrated the potential application of physiological measurements for research on key production issues in abalone farming. The project successfully used the collection of heart rate data in real-time during phases of the production and harvesting cycle. Given the relationship between heart rate and metabolism this research tool provides a unique opportunity for addressing in more detail a range of production issues including the influence of temperature, DO, density, nutrition, feeding pattern and handling.
Ultimately the aim is the adoption of a commercialised form of the technology in sentinel animals across a farm site and linked to environmental sensors, providing a continuous 'picture' of the health and welfare of the farm stock and its environment.

6. Further Development

We aim to progress this research through on-going collaborative funding to:

- Conduct the additional required laboratory trials with an extended range of environmental, biological and physiological variables,
- Conduct further on-farm experiments with the 'wired' biosensor to understand additional stressors, including changing of feed ingredients and feeding regimes, stocking densities, DO levels, etc
- Test and use a refined logger biosensor on the abalone which will enable us to capture data continuously without the issue of power. These loggers can also be shipped on the abalone to local and international buyers and returned, allowing us to fully understand the effects of live shipment on the abalone and how best to mitigate any stress that may affect the quality and survival of the abalone when they arrive
- Test the benefit of sentinel abalone with biosensors within commercial raceways to help understand unknown and unexpected events or behaviours, and potential to predict these with real-time data
- Apply the biosensor technology with the wild abalone sector to increase understanding of impacts of harvesting and live transport.

For long-term development please see "Linkages with CRC Milestone Outcomes" below.

7. Planned Outcomes

Public Benefit Outcomes

There is an increased understanding of abalone physiology and behaviour in response to changing environment and production stressors. The future development of more refined and efficient production protocols reduces the strain on natural resources and the environment surrounding the farm.

Private Benefit Outcomes

Abalone farmers will see production gains e.g. increased growth rate due to better detection of stressful environments for abalone and mitigation of these adverse conditions. Increased growth and survival rates will mean higher yield, reduced growth time and increased profitability for the farmers.

Expected Outcomes

Short term (1-2 years): Some Improved farm management practices (enabling reduced cost of production) based on enhanced understanding of the effect of these practices on key physiological and behavioural indicators in abalone. Potential further R&D on the application of in-situ physiology and behavioural monitoring.

Medium term (3-5 years): Further improvement in farm management practices (further reducing costs of production) resulting from additional knowledge of the impacts of multiple farm practices on economically important production characteristics of cultured abalone using real time data from sentinel animals. Depending on need and demand, a commercially available in-situ biosensor systems.

Longer term (5-10 years): Further reductions in production costs arising from application of full decision support systems in abalone farms based on complex data management systems including data from biosensors. The research is likely to identify some key physiological and behavioural responses that may enable farms to adapt practices to reduce stress in farmed abalone and this knowledge will be communicated to industry and provide them with some confidence in the use physiological data as a monitoring and measuring platform to address production issues. It is possible that the existing biosensor may have applications on farm depending on whether the variables it can measure are shown to be key variables. A subsequent research proposal may be developed to pursue the application of this technology.

Linkages with CRC Milestone Outcomes

CRC Outputs and Milestones
The project has contributed to the following Seafood CRC outputs and milestones

Output	Milestone
1,3 - Removal or reduction of key production constraints in existing aquaculture systems	1.3.3 - Strategic disease management approaches and technologies developed for at least two aquaculture species
	1.3.5 - Production efficiency gains from genetic, health management and nutritional Interventions quantified to inform long-term strategies and estimate commercial benefits
1.5 - Production interventions that add value to Australian seafood milestone	1.5.2 - Management systems for improved and more uniform condition of selected aquaculture species at harvest developed for at least two aquaculture species

8. Conclusion

Successful implementation of abalone biosensors on the farm has allowed us to demonstrate its potential, and gain an initial better understanding of the interaction between the abalone and their environment. The technology will be useful both as a research tool, to address specific issues (e.g. low DO, handling) and add value to other research (e.g. diet research), and as continuous monitor of animal welfare and performance in commercial growout.

Laboratory testing and validation of the individual metabolic rate chambers has allowed us to complete the first full scale comparative study between the two species and their hybrid.

Using data from the farm we have completed the first steps in designing the software programs to manage, analyse and interpret very large data sets from heart rate traces.

9. Publication

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11. Appendices

Appendix 1. Intellectual property.

The intellectual property associated with the biosensor resides with the University of Tasmania and CSIRO.

The intellectual property and valuable information arising from this research lies with the Seafood CRC on behalf of all its participants. At the time of this Report no specific intellectual property is identified as being generated from the results of this research.

Appendix 2. Staff

Staff engaged on the project:

Principal Investigator

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Co-Investigators

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