Effects of Seismic on Snow Crab - Final Report

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1 Summary

Objectives of the study

The objectives of the project are chosen to help shed light on whether Snow Crabs *(Chionoecetes opilio)* are susceptible to exposure to sound energy generated by seismic exploration. The research performed in this project is designed to address and resolve many of the questions raised in recent studies on Snow Crabs. The approach taken introduces a system that combines advanced biosensor technology capable of monitoring physiological and behavioural traits in crustaceans and molluscs with conventional oceanographic sensors to continuously monitor the marine environment.

Specifically the objective in this study is to map possible effects of seismic exposures on Snow Crab. The research will offer new insights to the science of the Snow Crab species for use in environmental effects monitoring in Nova Scotia, Canada and help identifying the possible environmental effects in local areas exposed to seismic activity and thus enhance Offshore Energy Research Association's (OERA) ability to assess any potential impacts on the marine environment in a fashion that could enable a responsible development of seismic operations in the waters of offshore Nova Scotia.

Methodology

In total two experiments were performed, where one was a lab experiment and one a field trial. The laboratory experiment tested the biosensors response to seismic noise and also allowed visual inspection of reactions. This test provided important insight and experience with the responses to be expected in the field trial. The test also provided valuable experience with using Snow Crab as a biosensor with regards to both biological and technical aspects. The field trial took place in the *Høgsfjord* in the south western Norway. The activities included re-design and re-configuration of equipment and methods, in addition to manufacturing of new equipment needed for the field trial. Biota Guards subsea Sensor Array were utilized as a base for the lander where also the new Snow Crab biosensors were connected. A seismic exposure was conducted half way in the field trial. The data from all sensors were transferred to Biota Guards expert centre in real time for analysis.

A biosensor is an analytical device, used for the detection of an analyte, that combines a biological component with a physicochemical detector.

Results

The findings from both trials points to a common conclusion that there are no obvious short or longer term changes in the heart rate of Snow Crabs exposed to the test conditions presented to them. The results indicate that shorter physiological changes might be linked to the seismic discharges. These changes persisted for 24 hours, before returning to pre discharge conditions.

Laboratory experiment

The laboratory experiment was successful as the objectives were met with regards to identifying response from novel stimuli in both Snow Crab heart rate pattern and in the blue mussel valve gape activity. An expected, but important result of the experiment is that it provided valuable insight and knowledge about new specie for use as a biosensor. The experience from the laboratory experiment was

highly valuable in the following work with developing a suitable method for adapting the biosensor to the Biota Guard system.

The responses shown both in the Snow Crab and the mussel in the laboratory experiment are strong indicators of what kind of an immediate response to expect in the field trial. While there are no findings which indicate a lasting effect from the seismic exposure in the laboratory experiment, one should note that the experiment was not designed with this objective in mind. An interesting observation was the circadian patterns that were registered in both species. The pattern correlated with light conditions changing between day and night. This rhythm seems to be stronger when directly exposed to the variations in the daylight like they were during the lab experiment.

Field trial

There appears to be no significant difference in heart rate pattern in the period immediately coincident with the discharge of the air gun when compared against heart rate patterns immediately prior and post to the discharge. The results from the field trial support the conclusion that there are no obvious short or longer term changes in the heart rate of Snow Crabs exposed to the test conditions presented to them.

The results from the multivariate analysis of the field trial data supports the conclusions made by Dr. Bamber but indicates that the heart activity for two of the five crabs that were analysed might have been changed when exposed to seismic activity. The possible change observed in the results from the analysis is small but it persists throughout the rest of the trial, however the magnitude of the change is considered to have statistically very low significance and caution is advised in interpreting much from this. This result is interesting and further research is advised in order to be able to uncover whether this is a reaction pattern or not.

Discussion of results

The findings from both trials points to a common conclusion that there are no obvious short or longer term changes in the heart rate of snow crabs exposed to the test conditions presented to them. The results indicate that shorter physiological changes might be linked to the seismic discharges. These changes persisted for 24 hours, before returning to pre discharge conditions.

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The Biota Guard system

Biota Guard has gained considerable experience with the use of Snow Crabs as a biosensor. The process with adapting the specie to our system has provided important knowledge about physical, mechanical and biological limitations and requirements as well as signal interpretation and adaption to multivariate modelling tools. The trials have shown that the Snow Crab proves a reliable biosensor as long as the knowledge gained during this project is applied.

Conclusions and recommendations

The results does not show any evidence that shorter exposure of seismic activity causes prolonged physiological changes or damage to Snow Crab *(Chionoecetes opilio).*

Further research work is advised in order to establish whether a longer trial including the use of an industrial size air gun array will show clearer long term effects. An underwater camera should be used during such a trial in order to enable visual inspection and recording of behaviour and reactions.

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3 Introduction

Historical background

The use of seismic surveys in oil or gas exploration offshore Nova Scotia has been at times controversial, due to competing interests between the fishing and oil and gas industries and the lack of definitive scientific data on whether or not these types of surveys have a negative impact on the environment, and particularly the ecology and sustainability of fisheries of Snow Crab *(Chionoecetes opilio)* in Canadian waters.

The issue of whether or not Snow Crabs are susceptible to exposure to sound energy generated by seismic exploration is a technically challenging task to resolve. Past research has provided a considerable amount of insight into the subject, however there remain many gaps to fill and the work performed in this project will try to fill some of them.

Snow Crab (Chionocetes opilio) (Fisheries and Oceans Canada, 2013)
Snow Crab, also referred to as Queen Crab, are found in the North Atlantic and North Pacific oceans. In the North Atlantic, they are found from Greenland in the northeast Atlantic and from southern Labrador to the Gulf of Maine in the northwest Atlantic. They prefer deep, cold-water conditions. Canada is the world's largest supplier of Snow Crab, accounting for about two-thirds of the global supply. In 2011, almost 73 percent of all Snow Crab exports from Canada were destined for the United States. China and Japan are also major markets.

Landings: In 2010, the total landings in Atlantic Canada were 83,584 tonnes, with total allowable catch set at 87,952 tonnes. In 2011 landings were 84,139 tonnes.
Value: Snow Crab is the second most valuable Canadian fishery export product, with 2011 exports valued at $613.5 million. The 2011 landed value was $459 million.
Abundance Status and Trends: The size of Snow Crab stocks is naturally variable and cyclical. Between 1990 and 2002, landings quadrupled from just over 26,000 tonnes to a peak of almost 107,000 tonnes. Since then, landings have fluctuated but remain high. There appears to be a general upward trend in biomass in some areas, although some fishery areas have seen recent declines.
Fisheries: There are approximately 60 Snow Crab Management Areas in Canada spanning four DFO regions. In 2010, 4,326 Snow Crab fishery licenses were issued.
Conservation Measures: The management of the Snow Crab fishery is based on annual total allowable catch, quotas, effort controls, minimum legal size, minimum mesh size of traps, seasons, areas and soft-shelled (also known as white crab) protocols.

Purpose

The objective in this study is to map possible effects of seismic exposures on Snow Crab. The research will offer new insights to the science of the Snow Crab species for use in environmental effects monitoring in Nova Scotia, Canada and help identifying the possible environmental effects in local areas exposed to seismic activity. The results will broaden the understanding of how and if Snow Crabs may be susceptible to exposure from sound energy, and also enhance OERA's ability to assess any potential impacts on the marine environment in a fashion that could contribute to responsible development of seismic operations in the waters offshore Nova Scotia. The project will aim to simulate conditions of a seismic survey and habitat conditions typically experienced at the seabed by the benthic animals that live there, during seismic survey operations.

Scope of work

The first phase of the project was focused on engineering and fabrication of equipment for the field trial as well as establishing an execution plan. Development of the execution plan included preparing procedures for deployment/demobilization, seismic noise methodology, securing availability of suitable vessel(s), permits and time plan.
Modifications shall be made to the Biota Guard subsea Sensor Array (sSA) to prepare for monitoring of new specie. A lab test shall be performed to study Blue Mussels and Snow Crabs' response to seismic noise. Endpoints to study are behaviour and heart rate. The setup shall include 16 Snow Crabs with cardiac monitoring and 16 bivalves with both cardiac and behaviour monitoring.

The next phase is preparation and setup of one common field trial assumed to happen in Norway. Final assembly of all Biota Guard equipment will be conducted in a workshop in Norway. The Biota Guard system will undergo a final test before being deployed offshore. The system consists of the sSA, umbilical, top side computer, real-time communication to the Biota Guard Expert Centre (BGEC), data collection/data mining and multivariate analysis capabilities in BGEC.

The field trial shall last for 2-4 weeks. After deployment and a period of acclimatization, a seismic vessel shall perform an agreed seismic exposure scenario. This is assumed to include at least 3 survey lines, one being at a distance, one survey line directly above and a final survey line at a distance from the crabs, but still close relative to the first survey line.

Background information

Biota Guard

A description of Biota Guard follows to ensure the reader has a basic conception of our services and technology. The following description of the service is based on a conventional industrial application. In this project, changes were made to the system in order to suit the projects specifications.

History

Biota Guard is a service company offering a novel method for integrated environmental monitoring. Biota Guard was spun-out from IRIS (International Research Institution of Stavanger) in 2005 following more than 10 years of R&D within marine biology and ecotoxiology. The basic premise of introducing instrumented living marine organisms (biosensors). The meaning of a biosensor in this circumstance is a blue mussel or a snow crab with sensors for heart rate and/or valve gape to measure biological functions and features that are related to the ability to absorb the effects of the total water ambient.

Relevant capabilities

Biota Guards core business objective is to offer the marine industries and governmental bodies the opportunity to enhance environmental management capabilities and provide a fact-based response to environmental monitoring needs. Sustainable development and operations in environmentally-sensitive regions require new technologies and methods for the monitoring and control of the marine environment. The ability to monitor responses in live animals coupled with oceanographic data makes the technology suitable for use in this project. Below are some of the systems capabilities listed.

Biota Guard enables a multi-sensor-based, real-time service for monitoring day-to-day operations that delivers:

- A leak detector with high sensitivity. Oil in water leak detection with sensitivity at 0,06 ppm

- Long-term environmental effect monitoring enables documentation of an environmental footprint of a given oil & gas related operation in real time
- Environmental analysis available on multiple platforms, including an operator's Integrated Operations room

Biota Guard adopts a cause-and-effect approach to environmental monitoring, enabling remedial action to be taken before an operational event can develop into a serious environmental problem.

Biota Guard's multi-sensor-approach allows for long term environmental effects to be detected throughout the life-cycle of a field, and the system delivers a factual based response to unmet environmental monitoring questions. A key output generated by the system is the Biota Guard EPI (Environmental Performance Index) – reflecting chemical changes in water over time as a function of biosensor responses and direct health parameters.

Data Collection
The Biota Guard adaptive subsea Sensor Array provides real-time chemical, physical and biological data in addition to accommodating offline biomarkers. Other relevant process data from daily operations such as water sample analysis, event notifications from discharges, topographical and hydrographical measurements can be included to strengthen the analysis.

Data Analysis and Management
The automated analytical framework is managed by the Biota Guard Expert Centre. Both mathematical and statistical tools are coupled with proprietary algorithms and methodologies, supporting an integrated overview of the environmental "footprint" of a given field or operation.

End User
Biota Guard provides a user interface for communicating analysis for fast and reliable decision support. The Biota Guard solution includes a time slider functionality that gives the user the opportunity to play back incidents that have occurred. The Biota Guard solution provides a tool for integration, sharing, collaboration and documentation of environmental information.

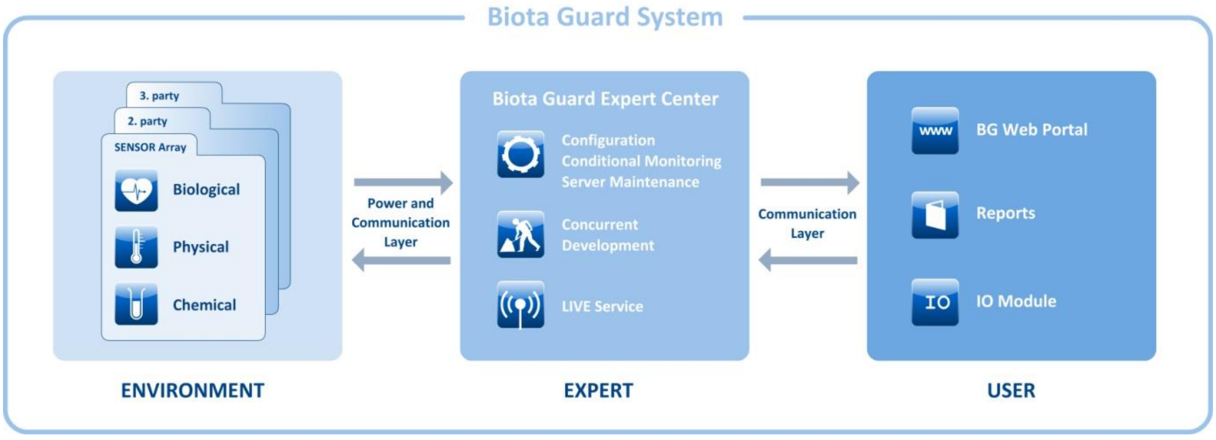

Figure 1 Biota Guard system overview

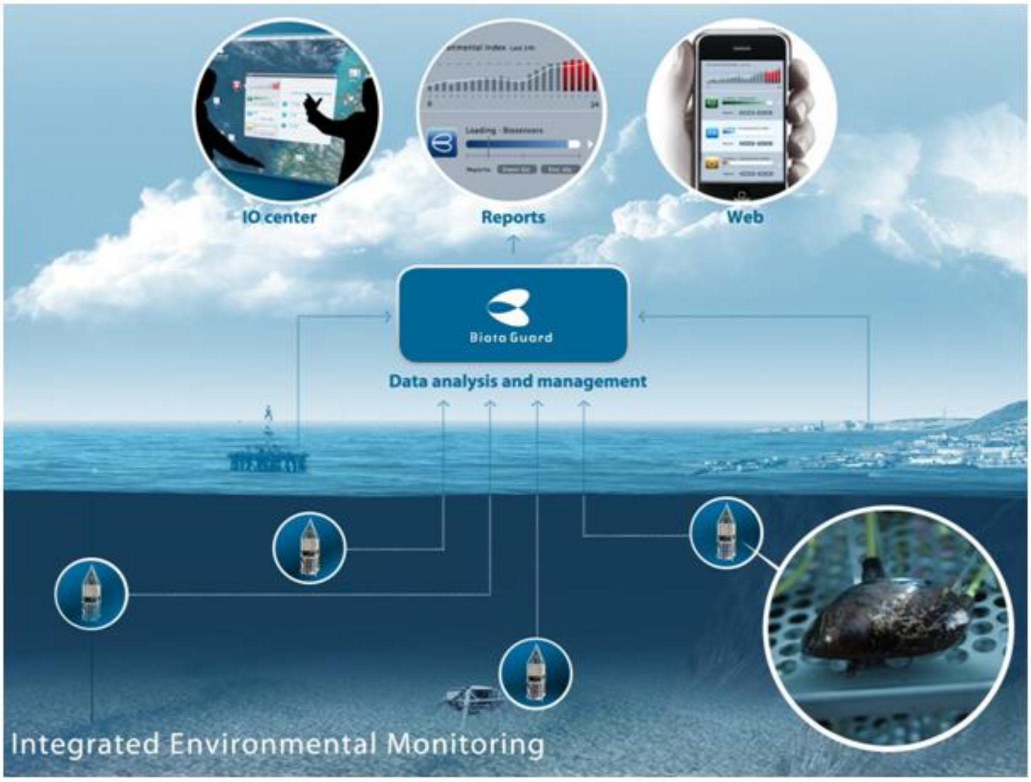

Figure 2 High level overview of the Biota Guard system

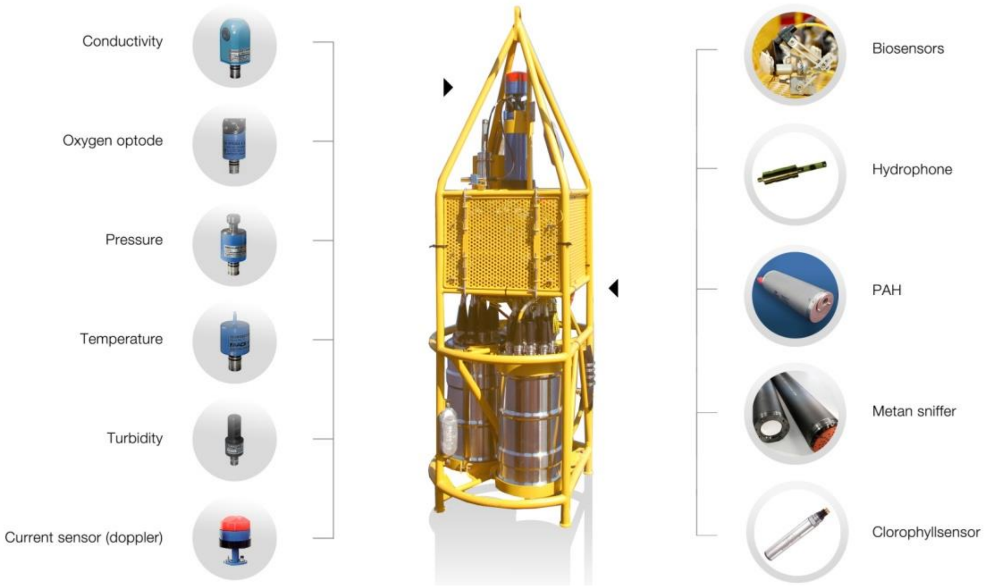

Figure 3 Biota Guard subsea Sensor Array (sSA)

4 Discussion of Objectives, Methodology and Results

The objective in this study is to map possible effects of seismic exposures on Snow Crab *(Chionoecetes opilio)*. The research will offer new insights to the science of the Snow Crab species for use in environmental effects monitoring in Nova Scotia, Canada and help identifying the possible environmental effects in local areas exposed to seismic activity. The methodology involved using Biota Guards expertise with biosensors, their multisensory approach to monitor both biota and environment. Biota Guards capabilities of multivariate statistical analysis has proven an important approach when analysing a multi parameter environment.

The objectives are clearly defined, but methodology was discussed throughout the project in order to find good ways to solve tasks that were new to both Biota Guard and OERA. Cooperation and communication with OERA and resources made available to the project has worked well in spite of the great distance between the parties. Both Dr. Lee and Dr. Moriyasu in Fisheries and Oceans Canada have proven valuable resources.

4.1 Methodology: Phase 1a - Laboratory experiment

Objective
The objective of this phase was to do detailed planning and to perform laboratory testing of biosensor response to seismic noise.

A laboratory experiment was planned to be conducted before the field trial in order to register response patterns to seismic noise. The monitoring of heart activity and behaviour of eight Blue Mussels *(Mytilus edulis)* and eight Snow Crabs should provide the necessary data and experience while exposing the population to seismic noise.

Changes to the initial objective
One change was made to the initial objective. The laboratory seismic noise study was conducted at Norwegian Institute for Nature Research's (NINA) facilities, and not IRIS'. The reason for this change was that NINA's facilities provided a better environment for the laboratory test and good access to a close by field test site.

R&D and engineering
According to the SoW, the air gun specifications for both the lab and field test were not initially established. These details were established through discussion in phone meetings with OERA. A commercially available paintball gun was chosen for the laboratory experiment. The "Planet eclipse - Etha" delivers an even air volume burst which is regulated so that the pressure is also the same. The chamber volume and chamber pressure can be regulated down but this was not done. The lab test conditions are not easily compared to natural surroundings and would be very difficult to imitate. The test itself was more a verification of the setup and to verify any response pattern. The air gun is easily available and reasonably affordable. The air gun was modified to accompany a remote control and a mount.

Signal analysis shows that the sound pressure from the paintball gun measured at 2 metres from the gun is approximately 55dB SPL (Sound Pressure Level).

Sourcing Snow Crabs

A Norwegian fishing boat, the "Arctic Wolf" did a test catch for Snow Crabs just before Christmas. As it turned out, the Barents Sea Snow Crab population was higher than earlier estimated (researched in fall 2012). Biota Guard was able to source Snow Crabs from the boat when it went back out on the banks in the end of March 2013. The Snow Crabs were successfully transported to Stavanger by air freight and put in a holding tank at NINA's facilities. The transportation duration was about 12 hours in total.

Description of the laboratory experiment

We received 47 crabs of which 2 died the first week, and 3 died the following two weeks. This is about a 10% mortality rate which is to be expected (Dr. Moriyasu, Fisheries and Oceans Canada). The total catch on the boat that trip was 18 tonnes, caught in baited pots. The crabs were packed in two Styrofoam boxes with cooling elements in the bottom. The climate in the boxes was cool and damp.

Figure 4 Snow Crabs arriving NINA's facilities

The lab experiment was performed at NINA's facilities at Ims near Stavanger which is adjacent to the Ims bay where the field test also was conducted.

In addition to provide the sea water tanks, NINA provides the project with on-site practical support and facility services when needed. IRIS is Biota Guards research partner and has been preparing and conducting the lab test together with Biota Guard. IRIS was in charge of conducting the laboratory experiment as well as the analysis of the data from both trials.

The crabs were left to acclimatize in a holding tank for over two weeks before they were prepared for the lab experiment in a separate testing tank. Both tanks are 10 metres in diameter and the water depth in both tanks was approximately 70 cm. Sea water is continuously taken in from 40 m depth in the Høgsfjord. The water was further filtered and sterilized with UV light. The crabs had access to food in the holding tank at all times. The appetite was low to begin with, but got better after approximately two weeks. Both shrimp and cod were given.

The biosensor setup was tested after the acclimatizing period. The heart rate sensors were fitted to the carapace directly above the heart using a plastic bracket that was glued on using cyanoacrylate glue (super glue). The sensor was secured in the bracket by tightening a set screw against it. The sensors is based on infrared light reflection and is therefore non invasive. This is a laboratory setup also used in an earlier research project where king crabs were observed. See Figure 5 for an illustration of sensor mount.

Figure 5 Heart rate sensor secured in bracket

The sensor setup was thoroughly tested on two crabs. A crab with a sensor fitted to it was placed in a cage. The test was performed to ensure that the setup was suitable for this specie. The sensor wire was feed through the centre of the cage lid and tied off with enough cable to let the crab reach all corners in the cage. The crabs were observed for several days using an underwater camera to see how it behaved and reacted in the cage with the sensor wire attached to it, see Figure 6. The recordings were observed while fast forwarding through the footage. Movement was observed at first, but the crab settled down after a while. Some entanglement occurred on occasions which were manually sorted out. The setup did not seem to result in any major mechanical stress on the sensor, but the wire slack were somewhat shortened to reduce the risk of entanglement. Wire length after shortening it was about 35cm.

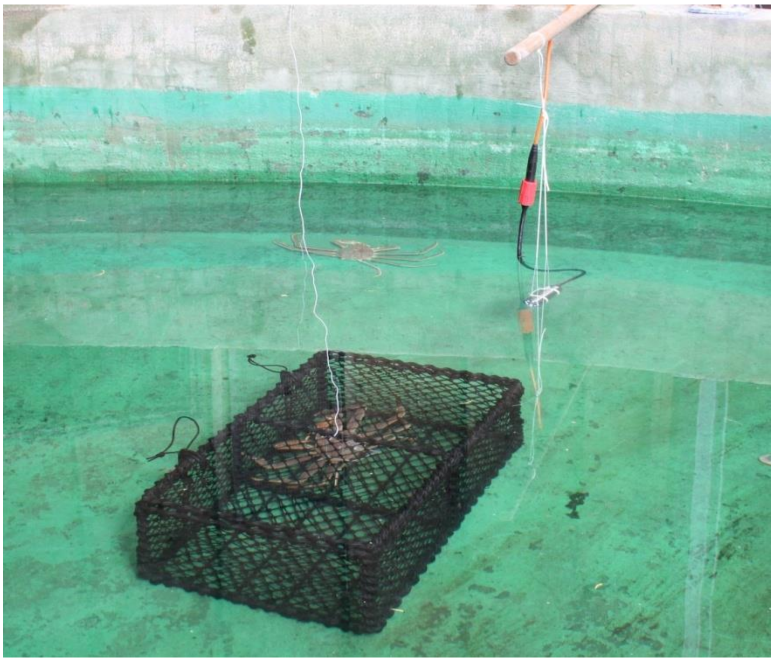

Figure 6 Observation of sensor setup

The laboratory experiment was conducted by IRIS at NINA's facilities. Personnel from IRIS prepared all 16 biosensors and ran the experiment. The equipment which IRIS provided for use in the test setup included sensors, sensor mounts and data gathering equipment. The equipment were of laboratory type and therefore quite different from the equipment used in the field test.

The test was set up in the separate testing tank identical to the holding tank. 8 male Snow Crabs were prepared with heart rate sensors. Eight Blue Mussels *(Mytilus Edulis)* were prepared with both heart rate sensors and valve gape sensors, mounted on painted metal plates providing a stable base for the setup. The plates with instrumented mussels were placed in cages for the ease of handling and to make acoustic conditions the same as for the crabs.

The crabs were placed in individual cages in a circle around the seismic source. The mussels were also placed in cages at the same distance from the source as the crabs. The seismic source was mounted from the testing tanks bridge with the barrel pointing downwards to the centre of the circle on which the biosensors were placed in. The placement of the cages was not put in any particular pattern around the circle as the distance to the seismic source in the middle would have been the same all around. Also the cables to the blue mussel biosensors were not long enough to be stretched from the other side of the circle to the control unit.

The seismic source was remotely discharged by a manual hand control. The source was discharged in several series and the crabs were left to regain normal heart rate between each series. All biosensors (mussels and crabs) were left in the testing tank for three days after the setup to gather background data.

The exposure was performed on April 19, 2013. The exposure sequence was videotaped to record any movement. No visible movement either startled reactions or other limb movement were observed before, during or immediately after exposure. One video camera was mounted under water pointing at two cages from about a metres distance from the cage wall. The other camera was mounted on a tripod on positioned on the floor besides the tank pointing downwards to the four left crabs. The cameras would capture movement of limbs. The underwater camera also captured movement of the mandibles of at least one crab. A DVD with video from this experiment is attached to the hardcopy of this report. (Attachment 3).

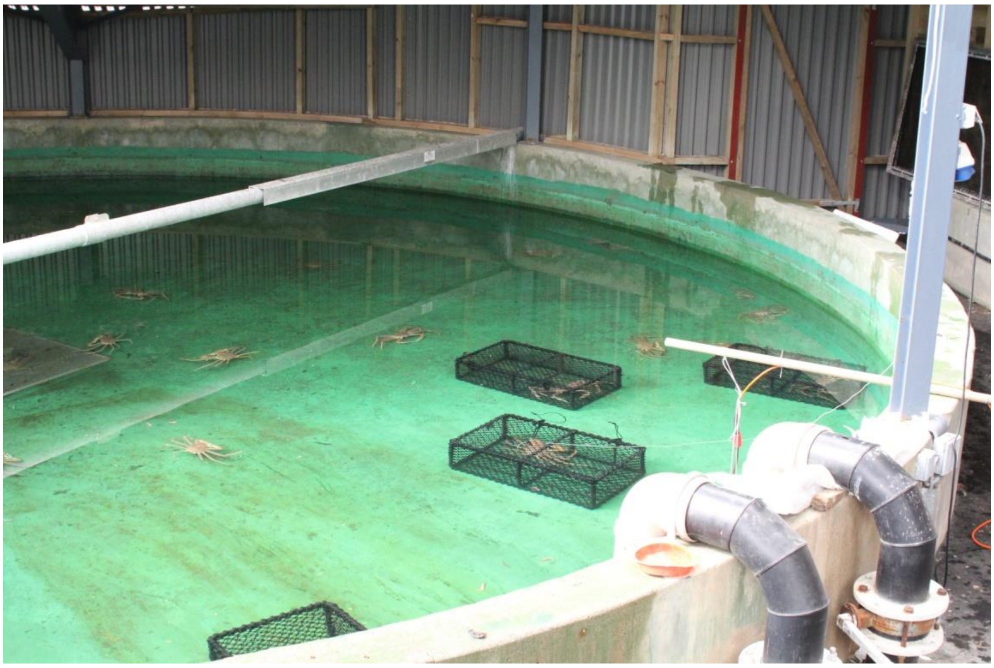

Figure 7 Holding tank

Table 1 Laboratory experiment time phases

Phase description	Time period
Acclimatization in holding tank (From crabs received at facility to lab test preparation)	26.03 - 16.04
Lab test acclimatization in testing tank: (exposure performed 19.04)	16.04* - 19.04
Lab test recovery period:	19.04 - 22.04**

*16.04 at 13:52

**22:04 at 01:51

Table 2 Water temperatures

Information	Average temp.	Max. temp.	Min. Temp.
Temperature in holding tank from 26.03 to 16.04	7.27 deg. Celsius	8.3	5.1
Temperature in holding tank from 26.03 to 07.05	7.39 deg. Celsius	9.14	5.1
Temperature in testing tank from 16.04 to 22.04	6.75 deg. Celsius	8.92	6.0

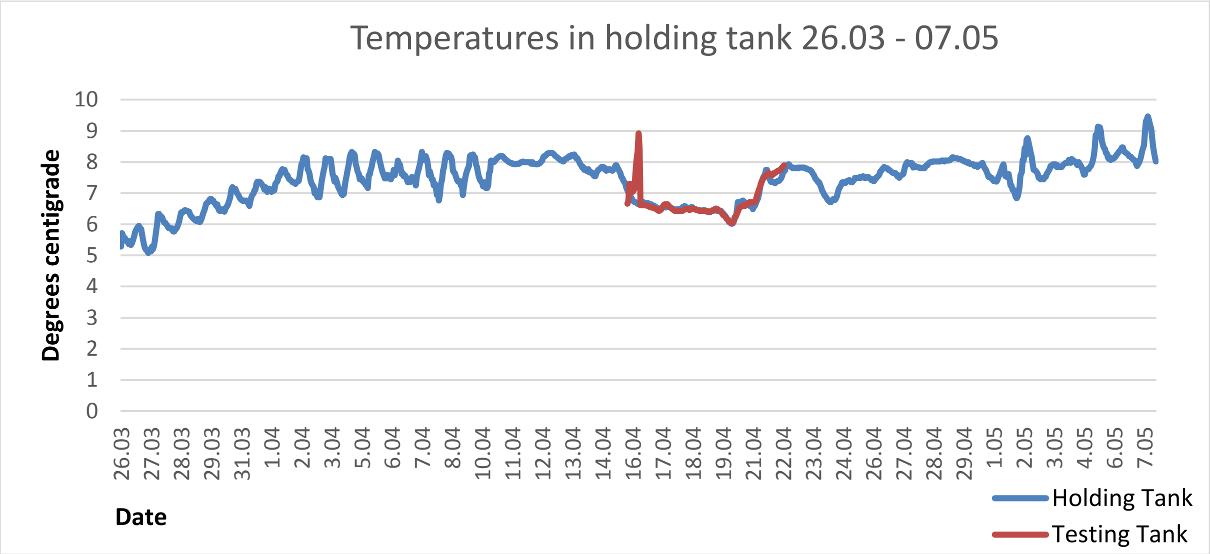

Figure 8 Temperature log, holding tank

Exposure

The seismic exposure sequence was performed on April 19, 2013. The plan was to deliver one exposure series consisting of 5 pulses in 10 second intervals and to let the crabs regain close to normal heartbeat pattern between the exposures. 4 series of 5 pulses were given over a period of two hours, as seen below.

Distance from the seismic source to the cages was 2.2 metres +-0.2 metres.
All exposure series consisted of 5 pulses. 1 pulse was given each 10 seconds. All four series lasted for 40 seconds.

Signal analysis shows that the sound pressure from the paintball gun measured at 2 metres from the gun is approximately 55dB SPL. See Figure 9. In the field test levels around 50dB SPL was registered using a conventional seismic source at around 400 meters. See Figure 38. This indicates that the crabs would experience similar sound pressure somewhere between 400 and 300 meters when deployed at 50 meters depth using the specific air gun used in the field trial.

Table 3 Overview of exposure series

Exposure start time (am) CEST	Exposure end time (am) CEST	Number of pulses given	Time between pulses (s)
10:05.00	10:05:40	5	10
11:05.00	11:05:40	5	10
11:35.00	11:35:40	5	10
12:05.00	12:05:40	5	10

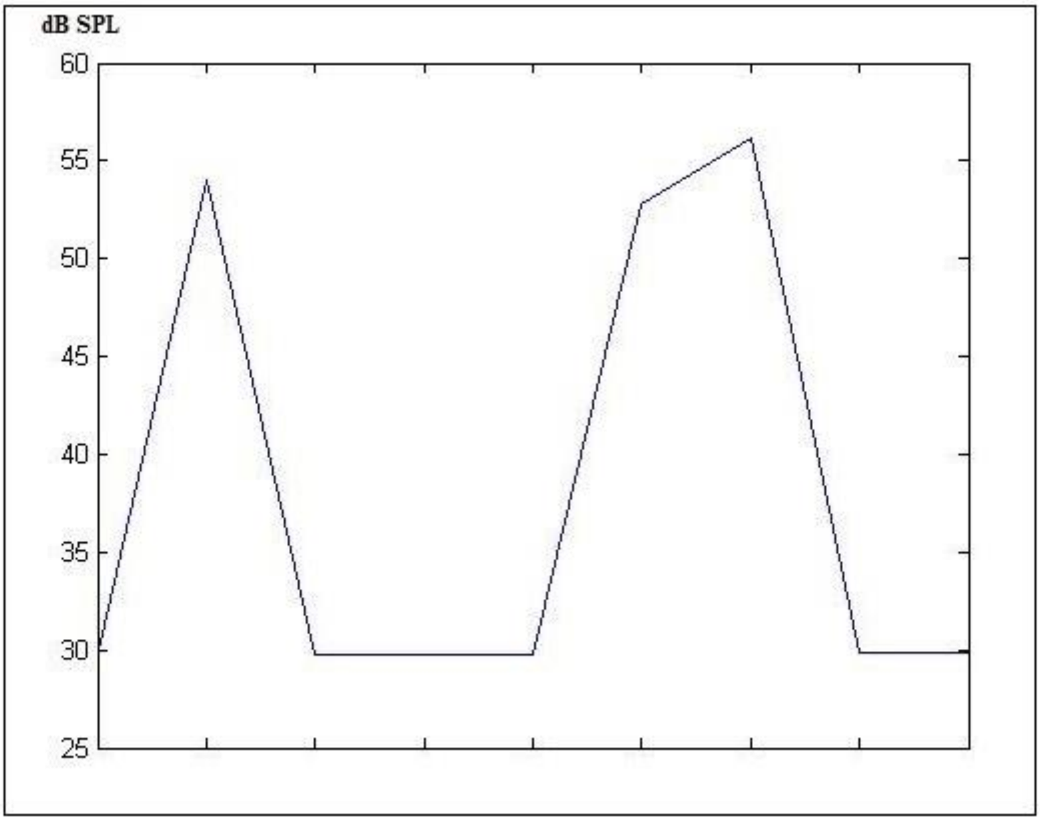

Figure 9 Plot of hydrophone recording of three air gun discharges from the air gun used in the laboratory experiment. The discharges X-axis represents time, but the plotting method does not provide a relevant time stamp

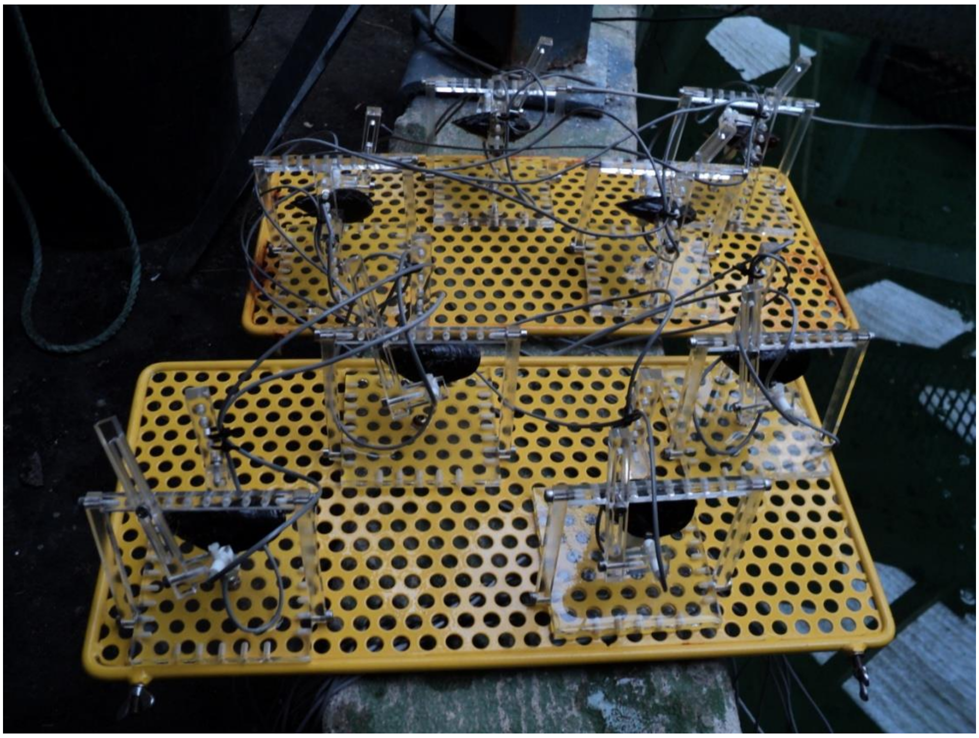

Figure 10 Blue mussel biosensors

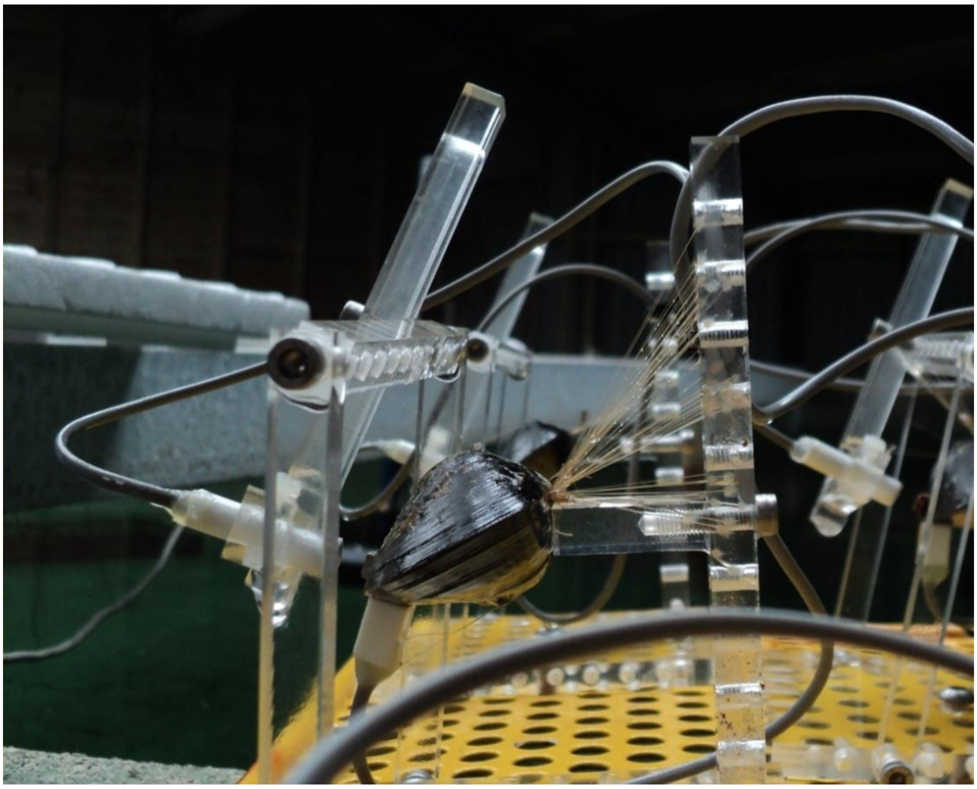

Figure 11 Close up of instrumented blue mussel

Figure 12 Test setup left half circle

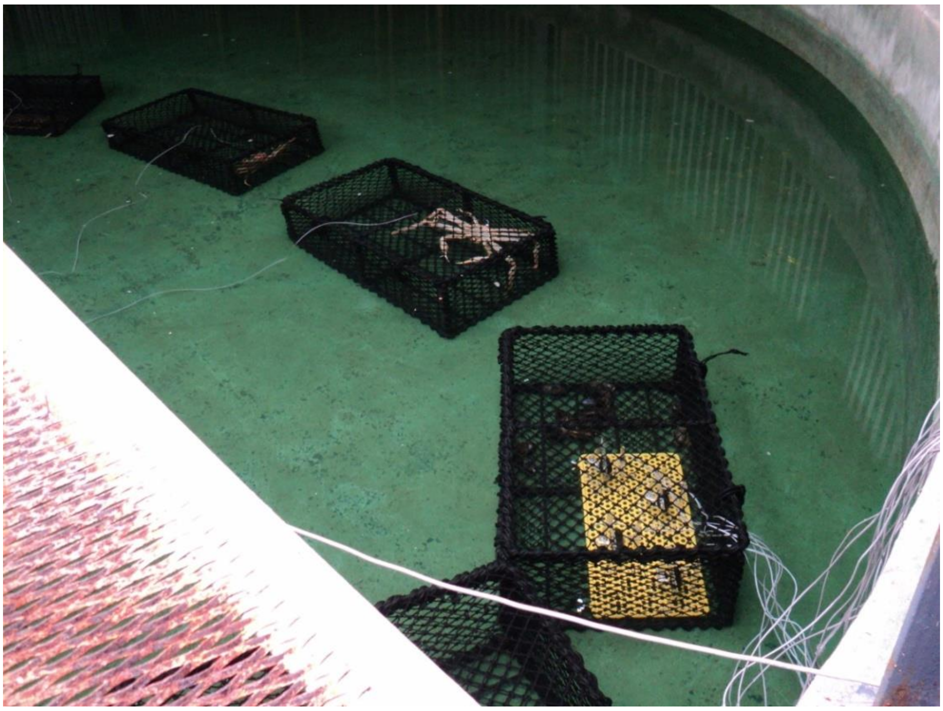

Figure 13 Test setup right half circle

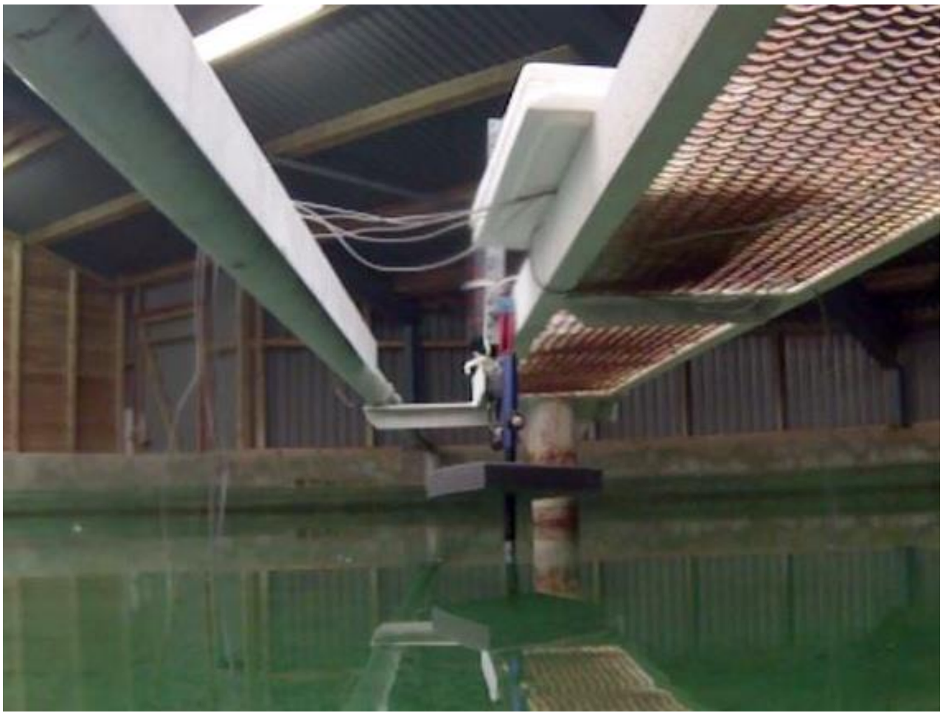

Figure 14 The air gun was placed in the centre of the testing tank containing the crab and mussel biosensors.

Figure 15 Remote controlled air gun

4.2 Results Laboratory experiment

- Dr. Shaw Bamber, IRIS October 30, 2013

Summary of findings from experiments where Snow Crabs were exposed to simulated seismic survey air gun in the laboratory

All eight of the crabs prepared for the experiment initially provided reliable signals from their heart rate sensors, but during the run of the experiment three crabs lost their sensors due to detachment from the carapace. The plots provided below have been compiled to take these numbers into account. Even with the progressive loss of three of the eight sensors the remaining fully functioning sensors have provided a sufficiently robust indication of heart beat behaviour before, during and after the sequence of seismic discharges.

A circadian pattern was observed in the crabs, with mean heart rate reaching its peak during darkness. The roof over the testing tank was fitted with transparent plastic panels so natural daylight levels were present throughout the exposure.

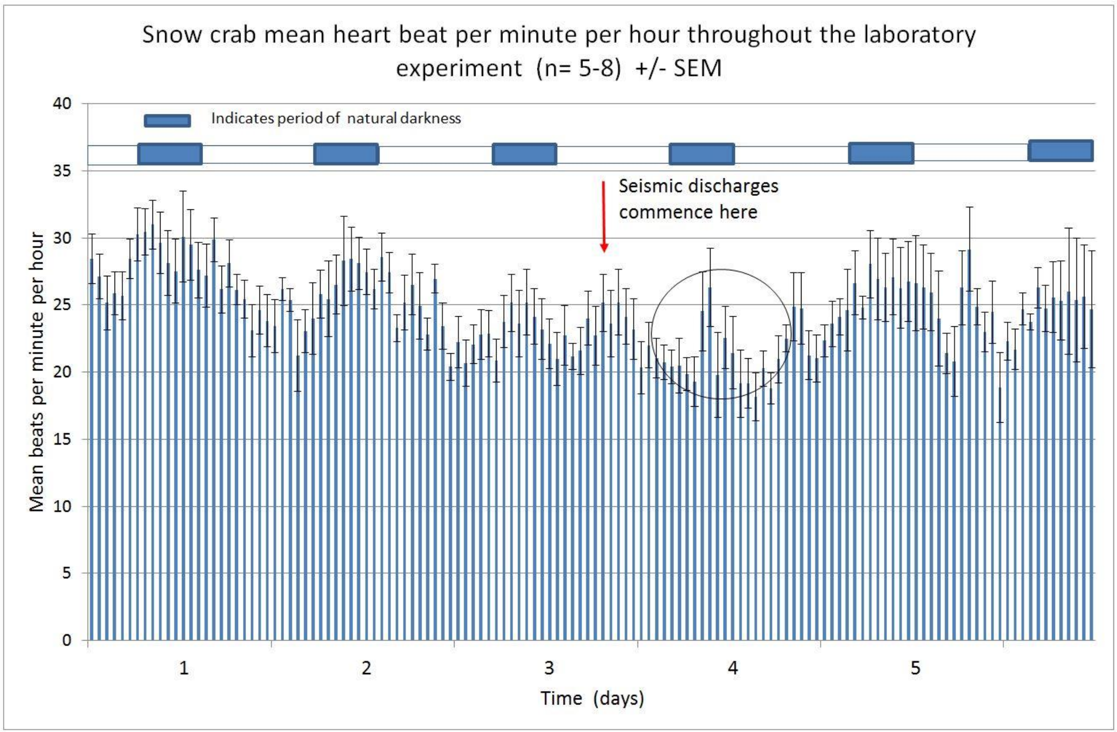

Figure 16 Snow Crab mean heart beat throughout the lab experiment. The data used for this analysis is gathered from April 16. 01:52pm to April 22. 01:51am

Some, but not all of the Snow Crabs were observed to reduce heart rate immediately the air gun was discharged. These initial reductions, when present, lasted just a few seconds to a few minutes before pre-exposure levels returned and in some cases increased beyond the initial rest rate, though these increases were not large. This effect can be seen in Figure 16, where, beneath the arrow indicating the start of the discharge sequence, there is a small rise in mean heart rate during the 'light' period. This is followed by a lower than expected mean heart rate in the following dark period, shown circled in the plot. This change is not sustained and the increased heart rate in dark periods observed before the seismic discharging, returns during the following two cycles.

Sudden changes in heart rate are not unusual in crabs exposed to similarly sudden changes in their local environment. Novel stimuli such as shading of captive animals or gentle tapping on tanks can lead to sudden heart stops which can last up to a minute (personal observation). The triggering of the air gun above the caged crabs in the laboratory would also be experienced as a novel stimulus. A second plot showing heart rate changes at the scale of beats per minute over 24 hours which includes the period of simulated air gun discharge is shown in Figure 17.

There is no clear sustained response in the heart rate of the exposed crabs.

A sustained response is one which continues over a period of time deemed to be significant in the context of the study. A long term effect in the context of this study is a physiological or behavioural change in the test animal that could have a negative impact on its fitness.

An important goal in the laboratory study was to determine whether possible effects resulting from discharging the air gun persisted. It is apparent that no gross changes in heart rate occurred within the scope of the laboratory exposure, as heart rate returned to its pre-exposure circadian pattern within 24 hours.

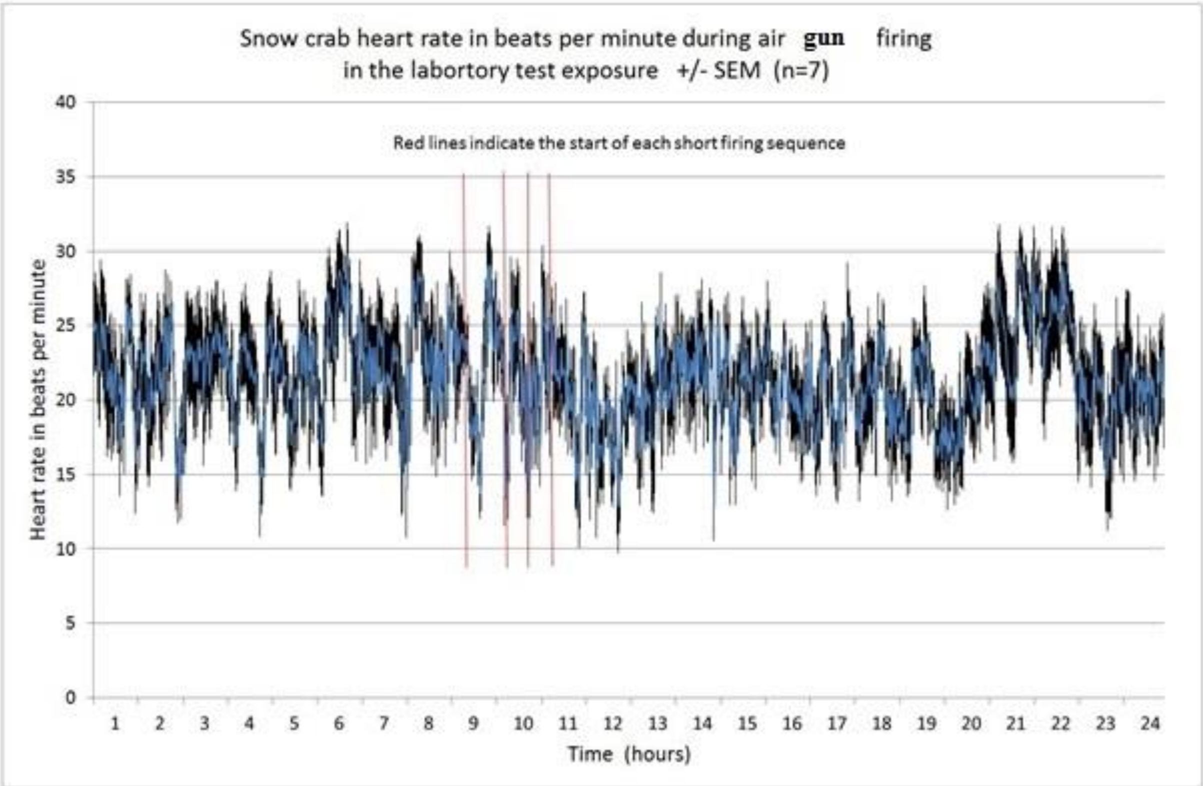

Figure 17 Snow Crab heart beats during air gun discharge

Summary of findings from experiments where blue mussels were exposed to simulated seismic survey air gun in the laboratory

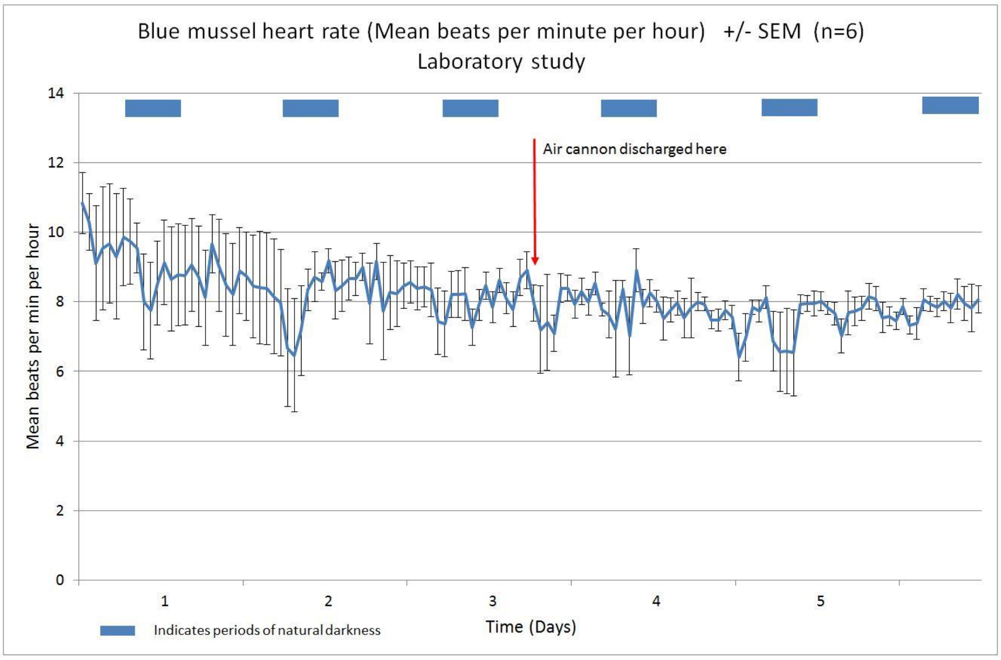

Figure 18 Blue mussel heart rate

In Blue Mussels there was no obvious change in heart rate observed during the exposure sequence and nor was there a clear correlation between heart rate and light level changes. This is shown in Figure 18.

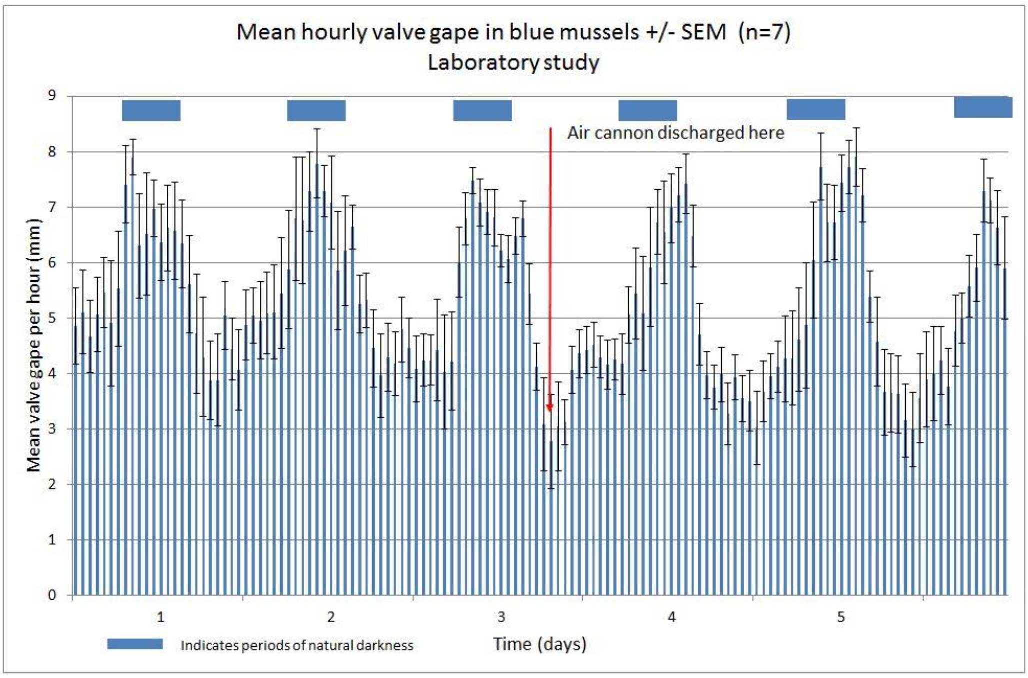

Figure 19 Mean hourly valve gape in blue mussels

Valve gape and activity did however show very clear circadian patterns with maximum gape and activity occurring during the hours of darkness. This is shown in Figure 19.

There is an immediate reduction in valve gape observed at the discharge point but this is soon recovered and mean valve gape returns to its previous condition within 1 to 2 hours, see Figure 19. This type of novel response typically leads to temporary reduction in valve gape in mussels, indeed similar to that observed in heart rate in the Snow Crabs. So shading and physical disturbance will generate a similar response in mussels to that observed immediately following the air gun discharge.

A similar situation occurs with valve gape activity, with a reduction observed around the time of discharge followed by an apparent return to pre-discharge conditions, see Figure 19.

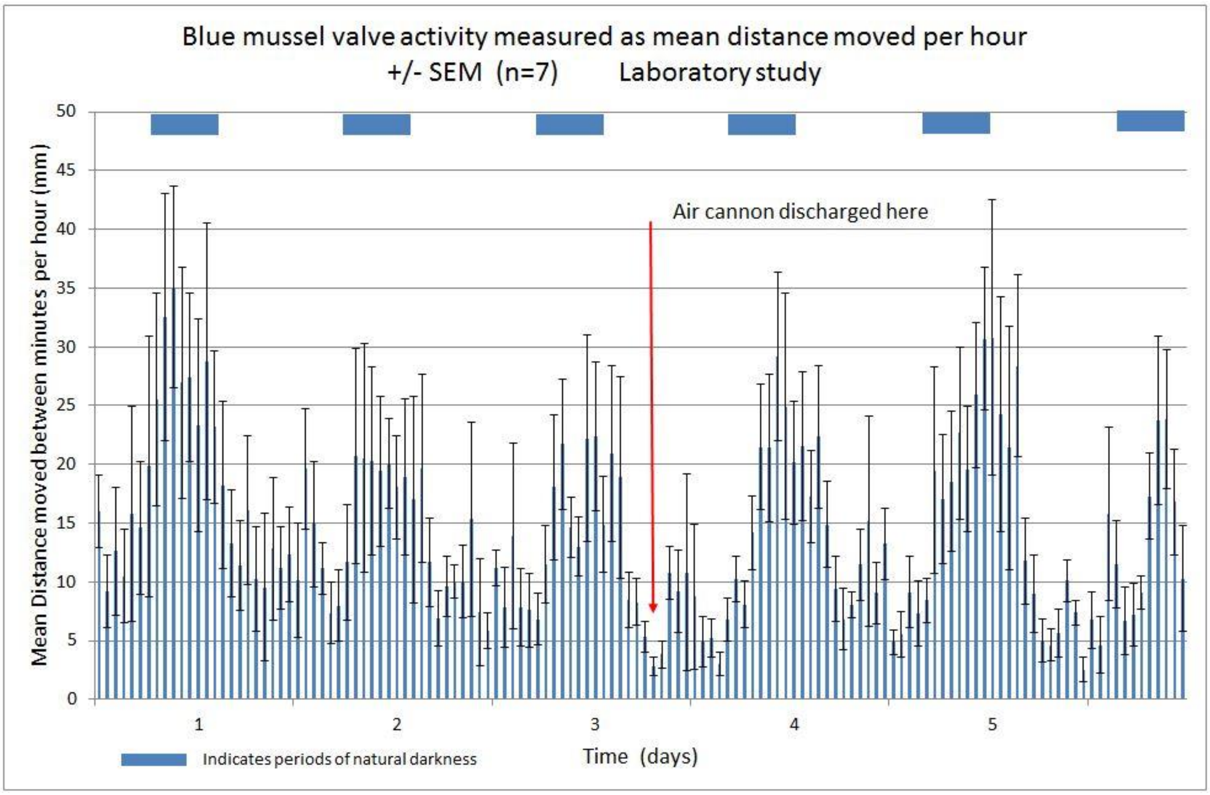

Figure 20 Blue mussel valve activity

The Blue Mussels were included in the study to observe whether a different phylum of marine animal, in addition to the crabs, would be susceptible to simulated seismic discharge in the laboratory. What was observed in both cases was a short lived physiological or behavioural response to a novel stimuli with a return to pre-exposure status within hours of the treatment. Possible further chronic effects of the exposure were beyond the remit of the laboratory study.

Conclusion from mussel laboratory study

Mussel heart rate does not appear to be influenced by the exposure to seismic discharge, though valve gape and valve activity both showed short term responses over a few hours before recovery to pre-exposure patterns.

4.3 Methodology: Phase 1b & 2/3 - Engineering and field trial

Objectives

Phase 1b

Phase 1b focuses on engineering and fabrication of equipment for the field trial as well as establishing an execution plan for the field trial. Development of execution plan includes preparing procedures for deployment/demobilization, seismic noise methodology, securing availability of suitable vessels, permits and time plan.

Equipment engineering includes adaption to the Biota Guard subsea Sensor Array for crab monitoring, crab cages and mooring. The setup is assumed to include 16 crabs with cardiac monitoring and 16 bivalves with both cardiac and behaviour monitoring.

Changes to original objectives in phase 1b:
The time line of the field trial has been changed during the course of the project due to several delays as described under "Changes in the project schedule" in chapter 3 in the first interim report.

Phase 2/3
In phase 2/3 a field deployment will be carried out during a 2-4 week period. After deployment and a period of acclimatization, a seismic vessel shall perform an agreed seismic exposure scenario.

This is assumed to include at least 3 survey lines, one being at a distance, one survey line directly above and a final survey line at a distance from the crabs, but still close relative to the first survey line. See Figure 21 for an overview of the survey plan.

Changes to original objectives in phase 2/3:

A working ROV was not used as a diver was deployed instead to verify that the bottom conditions for the lander was as optimal as possible (no rocks and pits). The diver also inserted sand into each cage to recreate a more natural like habitat. An inspection ROV was used during demobilization in order to inspect the conditions at the lander.

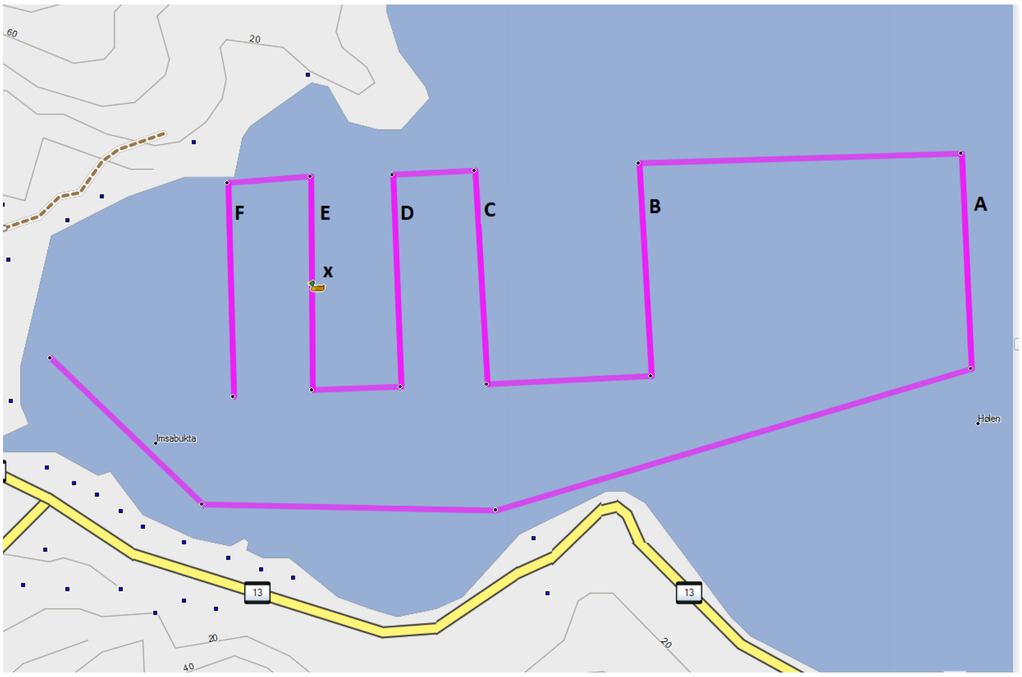

Figure 21 Survey plan

R&D and engineering

Some of the necessary equipment and unique parts have been designed or redesigned, tested and manufactured specially for this project as stated in the first interim report. The following is a description of this work.

A platform structure for the subsea lander was designed and manufactured specifically for this project. The result from the integration of the platform with the sSA can be seen in Figure 22 and Figure 29. The lander was designed by Biota Guard and underwent a thorough design review before it was produced by EB Marine, the same company which was performing offshore operations during deployment and demobilisation. The frame is constructed of metal beams and a metal mesh floor. It is anchored to the Biota Guard subsea sensor array (yellow structure) at the centre point and supported by wires from each corner. The lander is protected from corrosion by anodes and measures (l x d x h) 4.5 x 4.5 x 2.2 metres.

The heart rate sensor and the bracket which holds it had to be redesigned and manufactured so that the mechanical abilities suited field conditions. The version of the sensor configuration for heart rate monitoring of crabs only existed in a laboratory version as shown in Figure 5. It consists of a plastic bracket which holds the sensor in place and works well in the conditions for which it was made, but for the field trial a new sensor bracket with a better mechanical anchor had to be developed to ensure a good connection and ergonomically fit of the sensor to the crab carapace. The bracket was glued to the carapace above the heart. The glue used was a quick setting, non hazardous epoxy glue to make a cast, which was then glued to the carapace using cyanoacrylate (super glue) after the cast had settled. The cylindrical sensor was attached in its correct position by tightening a set screw. See Figure 23 for a close up view. This principle is the same as for the brackets used in the laboratory experiment. The bracket used in the laboratory experiment were made of plastic where the field bracket is heavier due to the bigger size and denser material but still it is not likely to cause added stress.

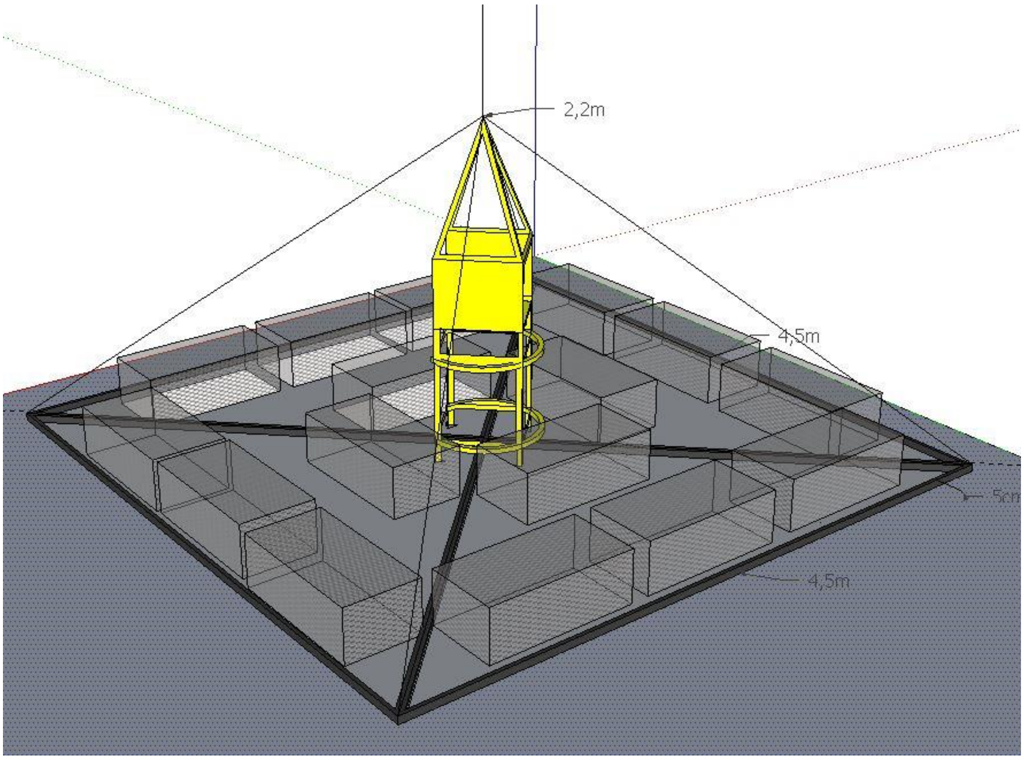

Figure 22 Subsea Lander

Figure 23 Heart rate bracket

Figure 24 Heart rate bracket mounted with sensor on Snow Crab

Figure 25 Dorsal view of sensor bracket during mounting process

Description of the field trial

The trial was performed in the Ims bay in the Høgsfjord, see Figure 26 and Figure 27. The trial site is located about 20 km south-east from Stavanger and 20 km north-east from Sandnes. The deployment site for the lander is situated close to NINA's facilities where the laboratory trial was held.

Figure 26 Map of northern Europe

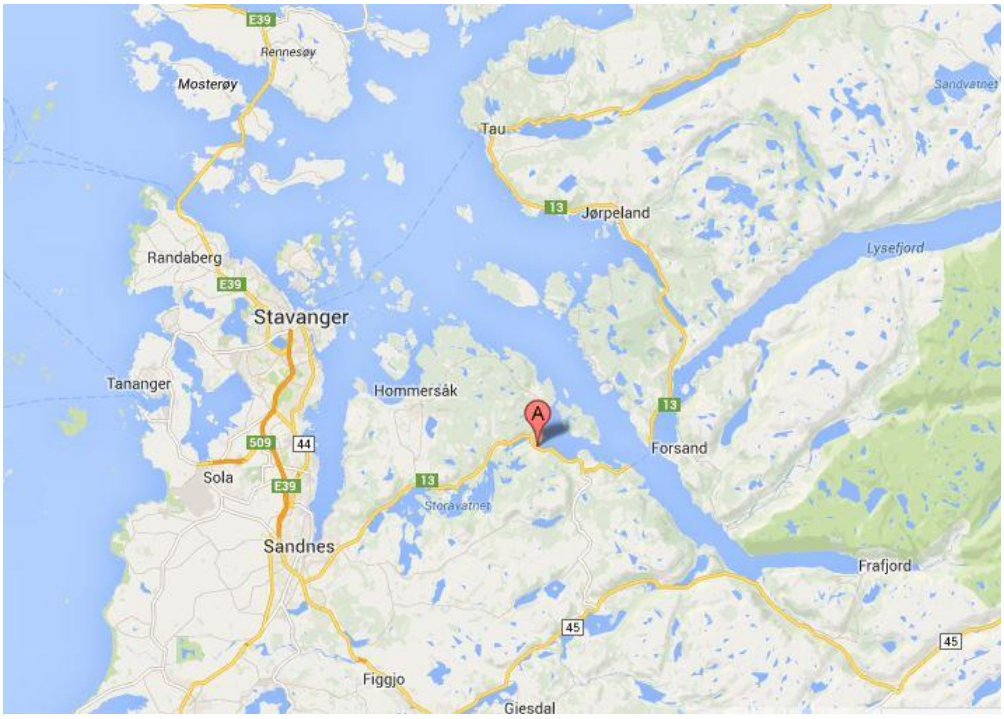

Figure 27 Field trial site - Ims Bay

All biosensors were prepared with sensors at NINA's facilities. The 16 Blue Mussels were fitted with heart rate and valve gape sensors and mounted in a bio module compartment prior to deployment. The 16 Snow Crabs were fitted with heart rate sensors and placed in individual cages the same way as in the laboratory experiment as shown in Figure 6.

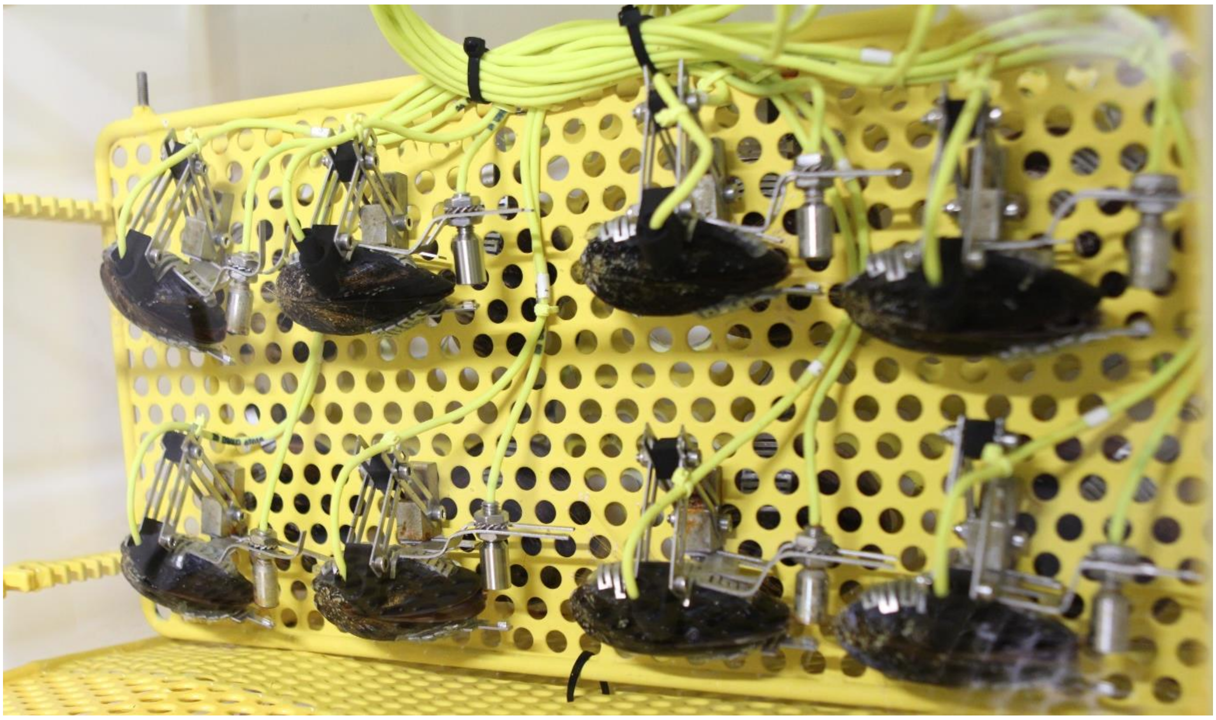

Figure 28 Eight blue mussel biosensors prepared in bio module compartment

Deployment - May 7, 2013

The Biota Guard subsea Sensor Array (sSA) system was integrated with the lander platform and positioned in the middle of the platform. The lander with the sSA and all peripheral equipment were prepared and assembled on NINA's docks the day before. The diving company EB Marine was hired to assist with the deployment. EB Marine has long experience with similar operations, has worked in the Ims bay earlier and is familiar with the topography and bottom conditions. EB Marine arrived early the day of deployment with a main deployment vessel equipped with a lifting crane, a diving station, a crew of five and an assisting boat. A decompression chamber was also installed next to the docks as a safety precaution because of the diving operations to be performed.

A safety meeting was held with EB Marine some days earlier where Biota Guard's safe job analysis was performed as a part of our QHSE procedures. The deployment procedure was also discussed. A safety briefing was also held the day of deployment to ensure that all participants were informed of the procedure and to map possible hazards connected to the operation.

The biosensors were mounted to the lander and sensors were connected to the control canisters located in the lower section of the sSA the day of deployment as seen in Figure 29. The crab cages were placed around the sSA and the bio module holding the mussels were placed in the bio module compartment in the middle section of the sSA. A system test was performed after installation to verify signal throughput on selected sensor data streams as shown in Figure 30.

The lander and the cable were both lifted directly from the dock on board the deployment vessel using the boats crane and transported directly out to the deployment site. The Snow Crab and Blue Mussel biosensors were out of water in about one hour.

Figure 29 Lander assembly

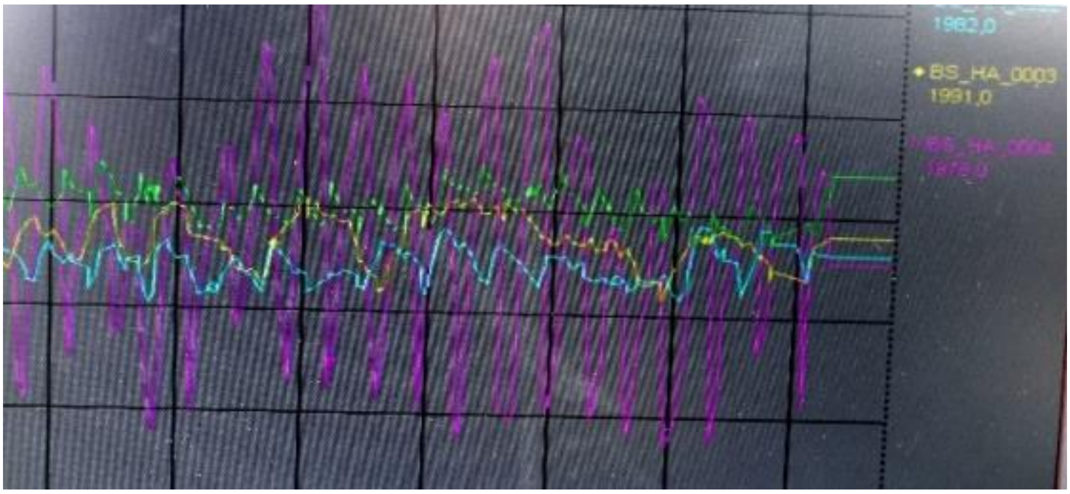

Figure 30 System test. Raw heart rate data of crabs shown

Figure 31 Lifting operations

Figure 32 Lowering of the lander

Figure 33 Diver prepares for inspection of bottom conditions and final lander placement

The lander was deployed about 500 metres from shore at 51 metres depth. The main deployment vessel ensured steady lowering of the lander while a smaller assisting vessel controlled a hold back line to the lander in order to ensure that it did not rotate during lowering.

A diver inspected the bottom conditions before the final lowering of the lander to verify that no stones or other objects were present at the bottom. A short (0,5 m) sideways adjustment was made. The diver put sediments from the nearby bottom in each cage as planned. This was done to make the conditions in the cages more like the crabs natural habitat, and in accordance with advice from Mikio Moriyasu, an expert in this field. Sediment depth varied, but was approximately 1-5mm. There were no way to monitor the sediment depth throughout the trial. The numbers are based on assumptions from the diver.

The subsea umbilical from the sSA to shore was pre-attached to the lander. It was rolled out of the cable drum and deployed along the bottom back to shore as the deployment vessel went back to the docks. The cable was further rolled out along the docks and into the control room at NINA's facilities where the topside communication computer had been placed. The data were continuously transferred wireless from the topside computer to our servers throughout the campaign.

Table 4 Field trial activities

Field trial activities	Time period*
Acclimatization in holding tank (From crabs received at facility to field trial preparation)	March 26 - May 1
Sensors attached and crabs put in individual cages**	May 1 - May 4
Acclimatization in holding tank before field trial	May 1 - May 7
Deployment and acclimatization commences	May 7
Seismic exposure performed	May 15
Recovery period	May 15 - May 27
Demobilization	May 27

*All dates in 2013

**Sensor attachment is a two stage process due to the use of two types of glue. This is the reason for this process to last for several days. The crabs were placed in individual cages from May 1, 2013.

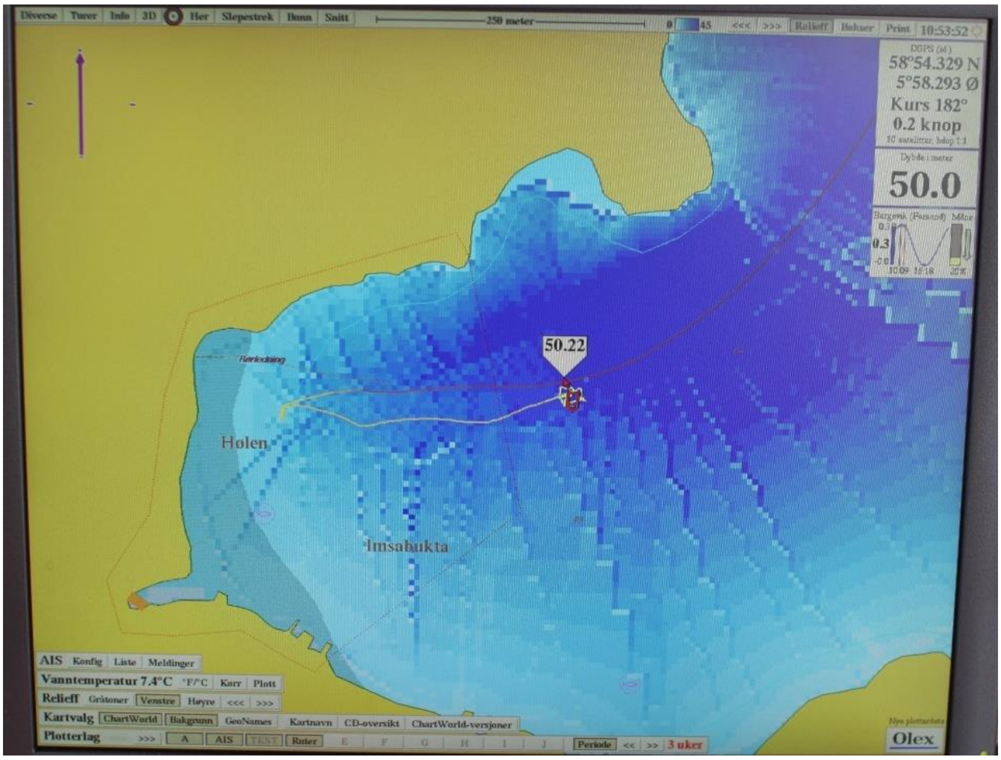

Figure 34 Position and depth of lander

Seismic exposure - May 15, 2013

The seismic exposure were performed on May 15, eight days from the date of deployment. Read Well Services conducted the seismic operation under guidance from Biota Guard. A 40cu. inch sleeve air gun from ION (earlier named Input/Output) was used. Model name “sleeve 40”. Air gun size and configurations for seismic exposure campaigns vary greatly depending on the target of the campaign, depth of water and bottom properties. The air gun used was chosen in cooperation with the air gun supplier, Read Well Services. The target was to set up a trial that would simulate a seismic survey dimensioned for the depth at the test site. The exposure test should include at least three survey lines as described in the "Objectives" section.

Biota Guard and the representative from Read Well Services performed a safety briefing before the work with assembling the equipment started. The equipment was placed and assembled in a 14 ft open motor boat which was also used to tow the air gun.

The air gun was hung two metres below a circular buoy and towed approximately 10 metres behind the boat. The air gun was manually controlled by the operator from Read Well Services onboard the boat. The air gun operated at 2000 psi feed by two 200 litre, 3000 psi air tanks onboard the boat. A third bottle was brought onboard as a backup in case additional air was needed. The third bottle of compressed air was not connected to the system. 2000 psi is standard pressure for several air guns used in seismic surveys. The chamber volume and the number of air guns are varied in order to obtain a certain energy output from an air gun array so that it suits the requirements in a specific survey. An array of several air guns is often used to cover a bigger area when conducting exploration surveys.

The planned survey consisted of 6 survey lines which were planned to be performed along a heading which is perpendicular to the direction from the lander to the bay opening, see an overview of the survey plan in Figure 21 Survey plan. This way a seismic survey would be simulated where a seismic vessel gradually would close in on the lander as it progressed back and forth over an area.

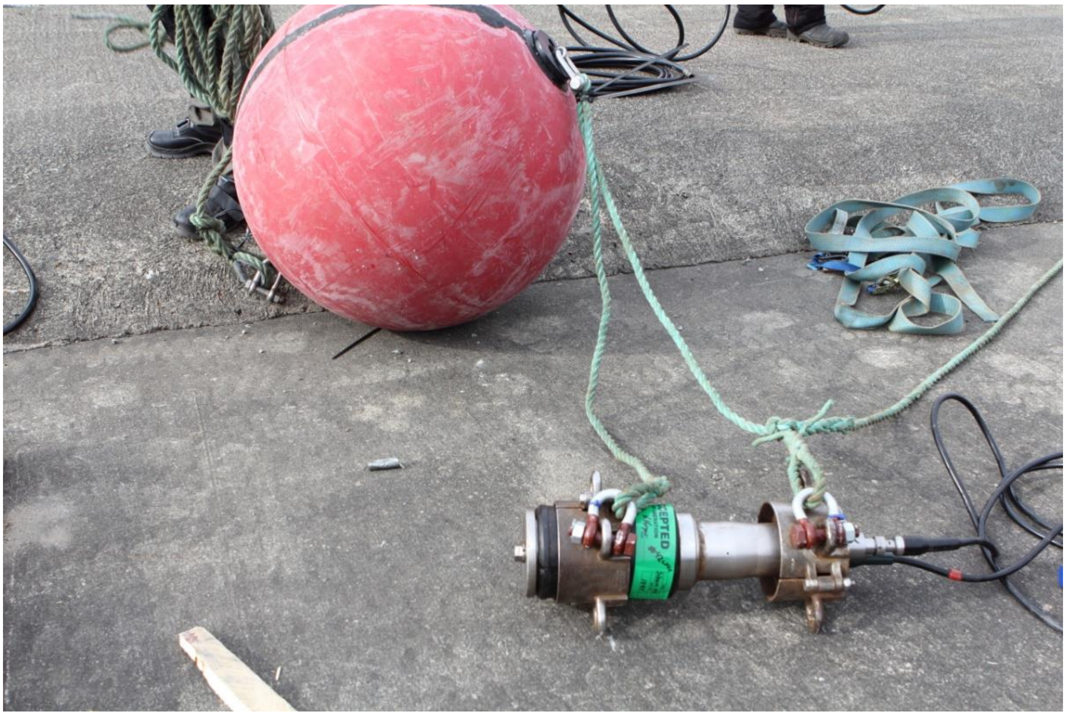

Figure 35 "ION Sleeve 40cu. inch air gun attached to buoy

Figure 36 Buoy and air burst visible from boat right after discharge

Table 5 Seismic exposure, Ims Bay, Sandnes - Norway May 15, 2013

Survey start time H:M.S (am)	Survey line id	Distance* from lander (metres)	Air pressure - air gun (psi)
10:05.30	A	800	2000
10:10.28	B	400	2000
10:14.44	E	0	1900
10:17.26	F	100	1850
10:31.18	E	0	2000
10:33.38**	F	100	2000

*Measured in the horizontal plane. 0 is actually 50 metres above the lander.

**End time of the last survey line was 10:35.27

The plan for the survey lines was set up so that the distance from the seismic source to the subsea lander would decrease during the exposure. The exposure was planned to be carried out as one continuous exposure. Due to a more rapid drop in pressure than what was planned survey line C and D was cancelled. See Figure 37 for a GPS track of the performed survey line. Also e second pass along survey line E and F were made after changing to a backup air tank to ensure that also these survey lines were passed with the air gun at full power. See further details in interim report 2.

Figure 37 GPS track from performed survey. The black dot represents the position of the lander

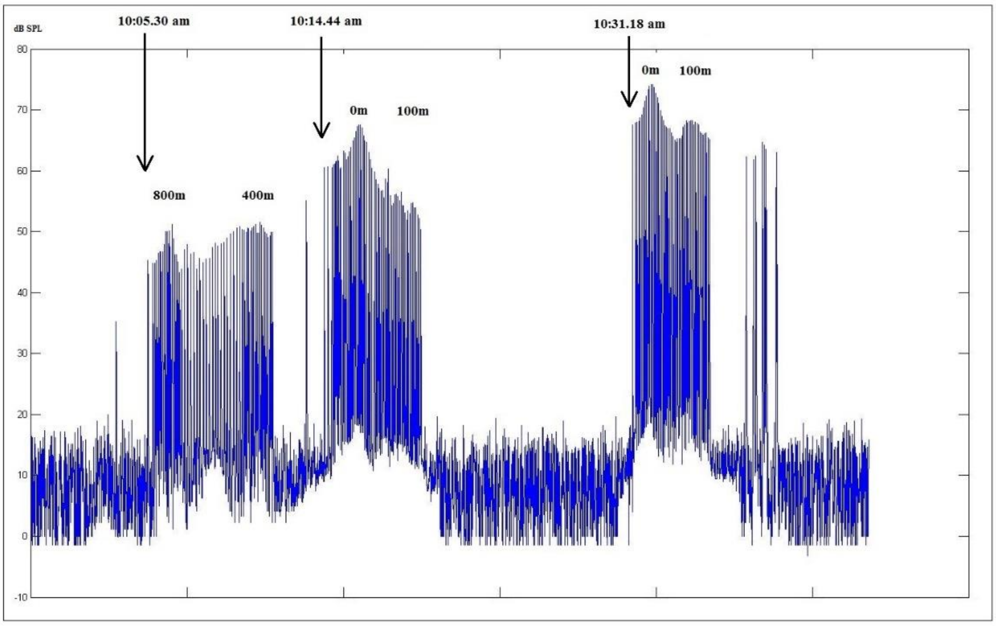

Figure 38 Plot of hydrophone recording of all air gun discharges during air gun exposure. Time stamps above corresponds to time stamps in Table 5. X-axis represents time, but the plotting method does not provide a relevant time stamp

The plot of the hydrophone recording from the time of the exposure period is shown in Figure 38. X-axis is time; Y-axis is acoustic amplitude in decibel. The first of the three thick sections is the exposures from the first and second survey line (A & B). The discharges during transportation can be seen between A and B. During each of the survey lines about 20 shots were fired. The plot makes it clear that the amplitude registered by the hydrophone on the lander increases and decreases gradually as the air gun approaches and passes above. This is particular clear for survey line E and F. The highest sound level reached directly above the lander was appx. 75dB SPL as shown in Figure 38 and Figure 39. The last few peaks are recordings of discharges performed by the dock in order to empty the compressed air in the air gun's camber and air supply system. This had to be done as part of a safety procedure before disassembling of the system could start. This occurred between 10:37.24 and 10:39.04. The exposure was conducted between 10:00 am and 10:30 am CEST.

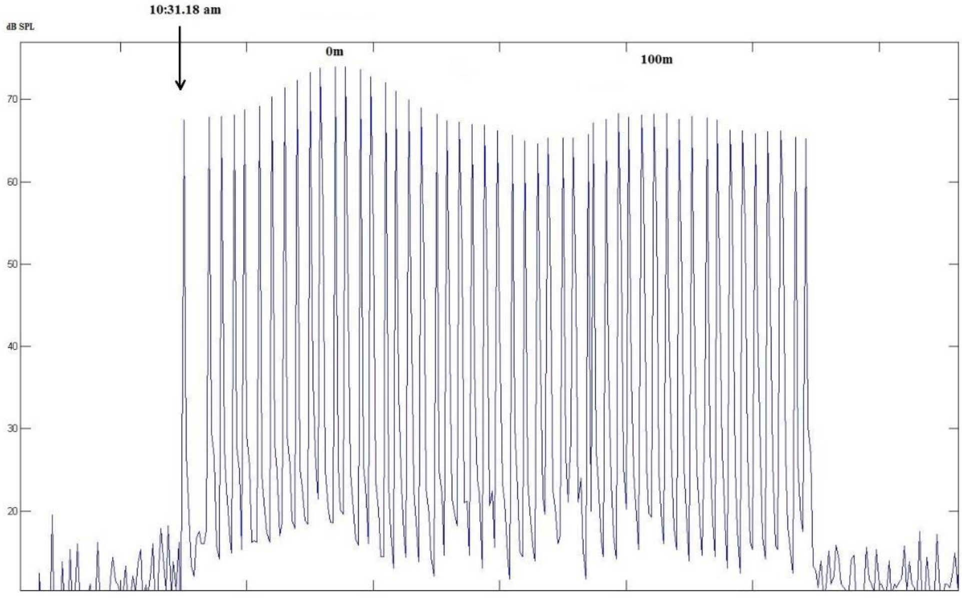

Figure 39 Close up of air gun discharges from the final passes along survey line lines E and F. X-axis represents time, but the plotting method does not provide a relevant time stamp

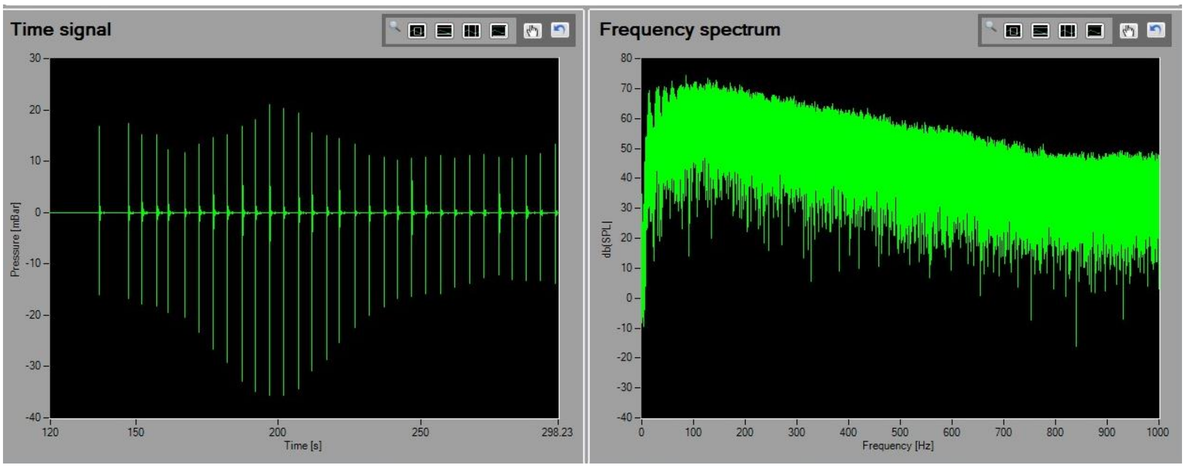

Figure 40 - Frequency and loudness analysis of discharges directly above the lander

Demobilization - May 27, 2013

The demobilization was a reverse operation of the deployment. EB Marine also assisted in this operation. An inspection ROV inspected the lander and the surroundings before the lander was retrieved to document the conditions in the area and to see if there had been any sediment build-up or other major changes since the deployment. The conditions were about the same as after deployment of lander.

The crabs and mussels were quickly taken into the holding tanks after returning to the dock. The time out of water was about one hour.

Four days after demobilization, on May 31, all crabs were euthanized, and tissue samples were taken according to recommended practice (RSPCA, 2012). Both operations were performed by Dr. Bamber (IRIS).

Knowledge gained from using Snow Crab as a biosensor

One of the important lessons learned has been that the sensor should have been attached in the same process as the sensor bracket is glued to the carapace. Since this was done in two stages, some of the excess glue used to fasten the bracket to the carapace had cured inside the lower part of the bracket cylinder and kept the sensor from being positioned all the way through the bracket which in several cases caused it to stop around one millimetre from the carapace's surface when it was inserted the day of deployment. Salt deposits in the aluminium bracket could also have added to this although the brackets were brushed and rinsed with clean water just before the sensors were inserted. This left several of the heart rate signals from the crabs unusable. See plot of raw data for two heart beat sensors in Figure 41 and Figure 42. The difference in amplitude shows how a correctly connected sensor produces a healthy signal with high amplitude (A) and data from a sensor with bad connection where the signal consists of noise with low amplitude (B).

Biota Guard have made design and procedure changes which will ensure that this error will not occur in future work.

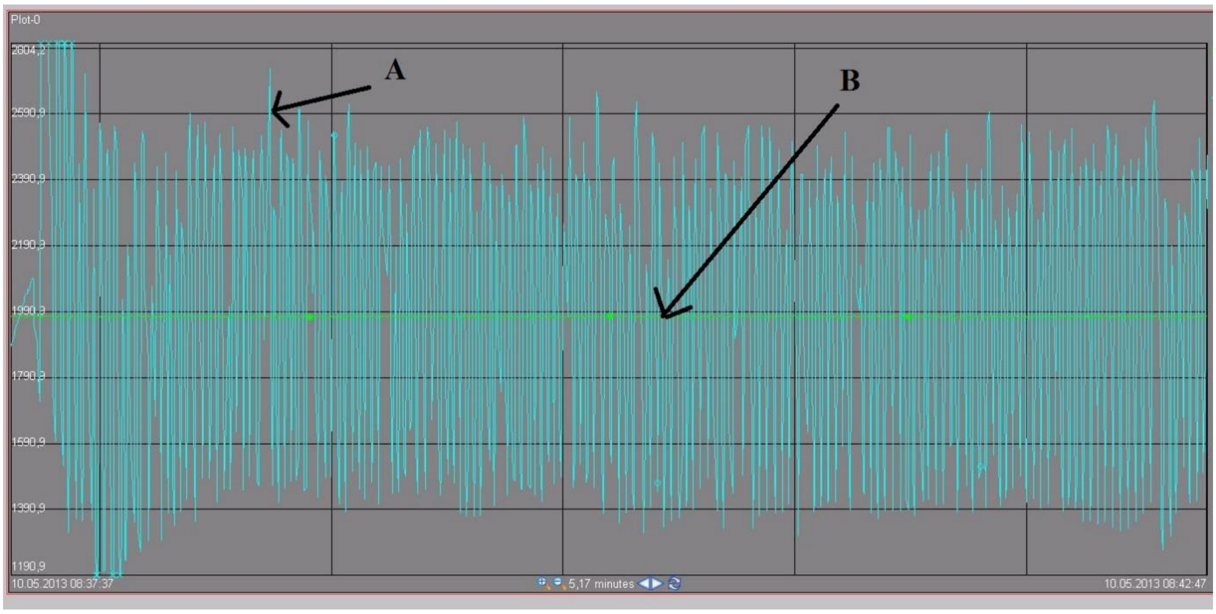

Figure 41 Raw snow crab heart rate data. A represents a healthy signal, B represents an unhealthy signal

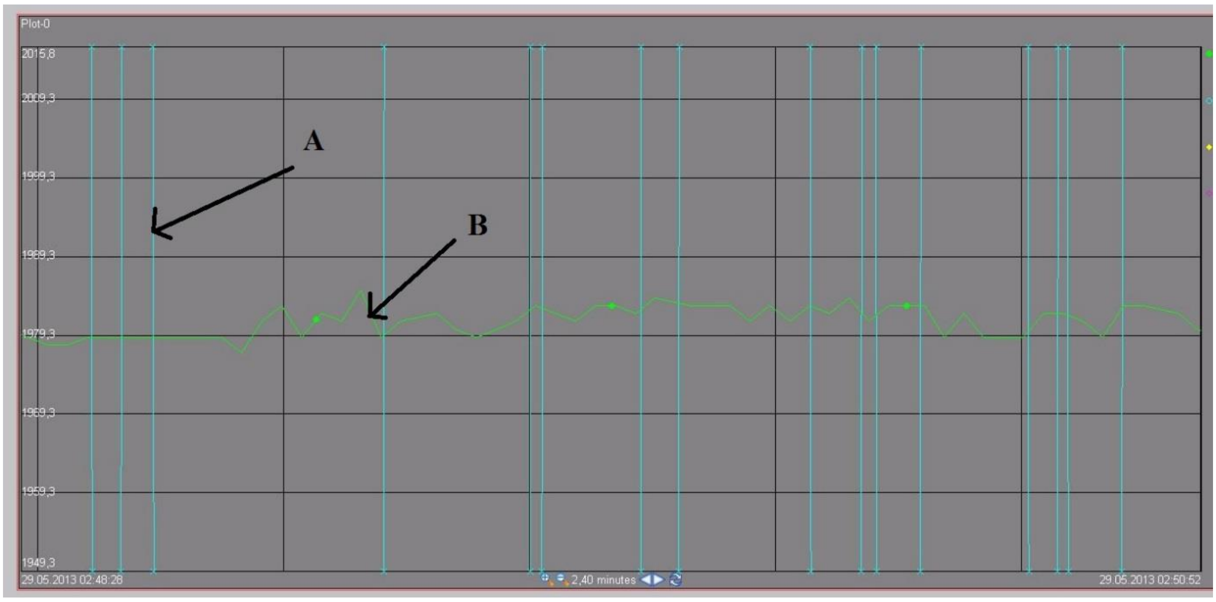

Figure 42 Close up of raw snow crab heart rate data. A represents a healthy signal, B represents an unhealthy signal (noise)

4.4 – Results field trial

- Dr. Shaw Bamber, IRIS October 30, 2013

Summary of findings on the effect of seismic discharge in the field on Snow Crab heart rate

Five of the 16 crabs that had been set up for the field trial generated near continuous data throughout the procedure. The majority of the others contained long periods with no recording of heart rate throughout many days. The reason for this is explained in the last paragraph on the previous page of this report. ”Experience with Snow Crab as biosensor”.

Trying to incorporate these data into the assessment is likely to create more problems than benefit. The five remaining animals appear to have maintained a steady heart beat throughout the course of the trial. A high initial rate, presumably due to handling, settled into a steady rate for all of the crabs. The generally low variability seen in the plot indicates their performance was similar. There are no large changes in the plot that might suggest a sustained stress response. The lack of a sustained response does indicate that the discharge is likely to have little long term effect on the crabs.

There were two periods late in the field trial when all data logging stopped temporarily. The basal heart rate level seen in the field deployed crabs was slightly lower than that observed in the laboratory maintained animals. The housing conditions in the aquarium (shallow depth and higher light fluctuation intensity) are the likely causes for this relatively small change.

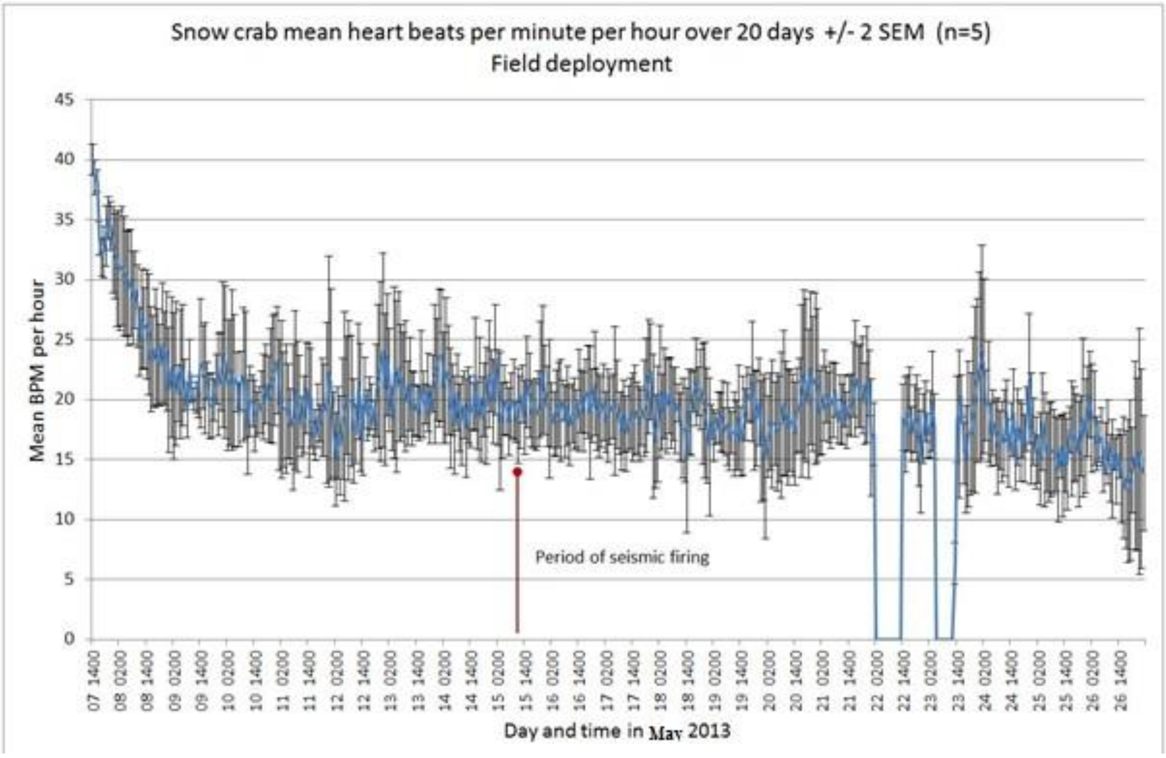

Figure 43 Snow Crab mean heart beat Field Trial. 20 days. SEM (Standard Error of Mean)

Figure 43 shows mean beats per minute per hour to give a general overview of the heart rate over the entire timeline.

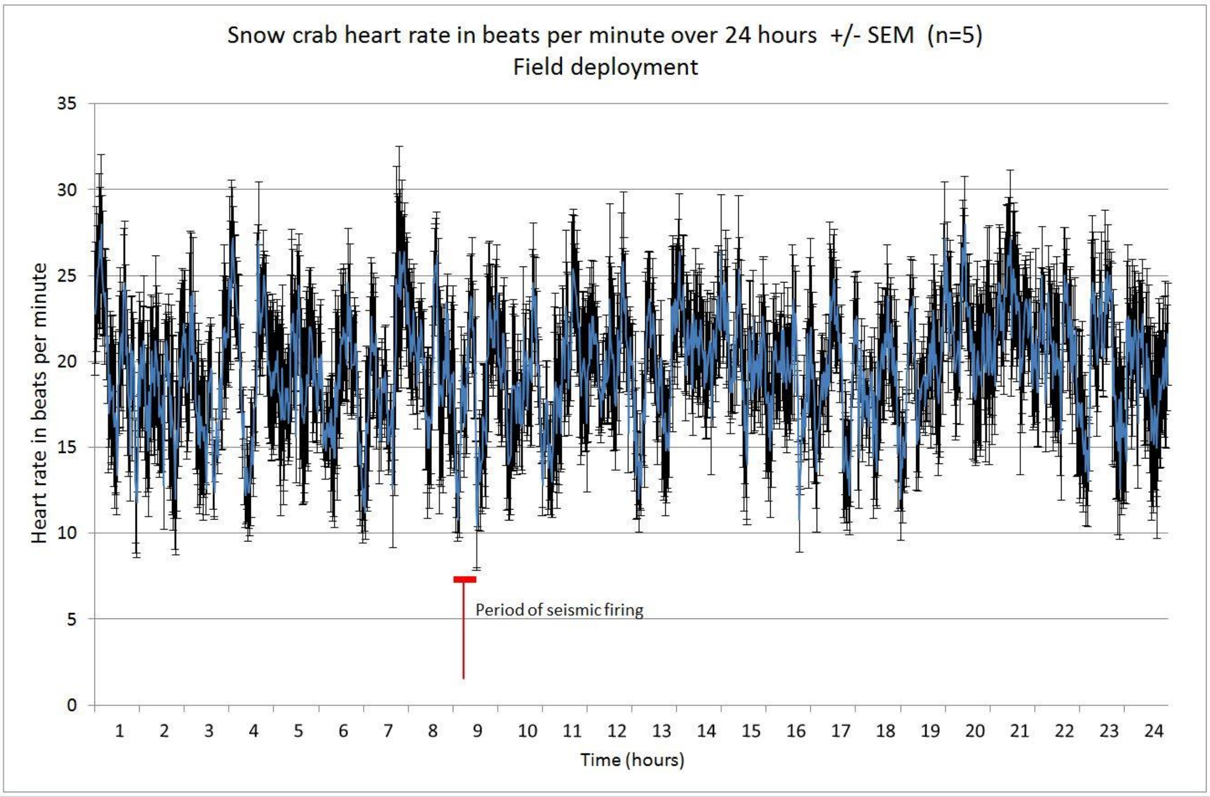

Figure 44 Snow Crab heart rate in beats per minute over 24 hours Field Trial. SEM (Standard Error of Mean)

In order to check whether a rapid response to the seismic discharge occurred in the Snow Crabs that were not evident in the hourly mean plots, a further plot was prepared to show heart rate changes at the scale of beats per minute, within a 24 hour period that included the seismic discharge. These findings are presented in Figure 44. There appears to be no significant difference in heart rate pattern in the period immediately coincident with the discharge of the air gun when compared against heart rate patterns immediately prior and post to the discharge.

Summary of findings on the effect of seismic discharge in the field on Snow Crab

The findings from the field test therefore support the conclusion that there are no obvious short or longer term changes in the heart rate of Snow Crabs exposed to the test conditions presented to them. It should be noted that these findings are based on results taken from just five crabs. The heart rate recordings from all the crabs included within the analysis of the field data were similar in pattern over an extended period of testing, as can be seen from the relatively narrow standard error of the means provided in the plots above. Thus, even though the sample size is small, there is a likelihood that these animals are representative of a larger population. A further trial with a greater sample size would of course be necessary to establish this categorically.

Summary of findings on the effect of seismic discharge in the field on blue mussels.

Heart rate and valve gape parameters were measured in mussels deployed at 50 metres depth exposed to discharges from an air gun, towed behind a small craft above.

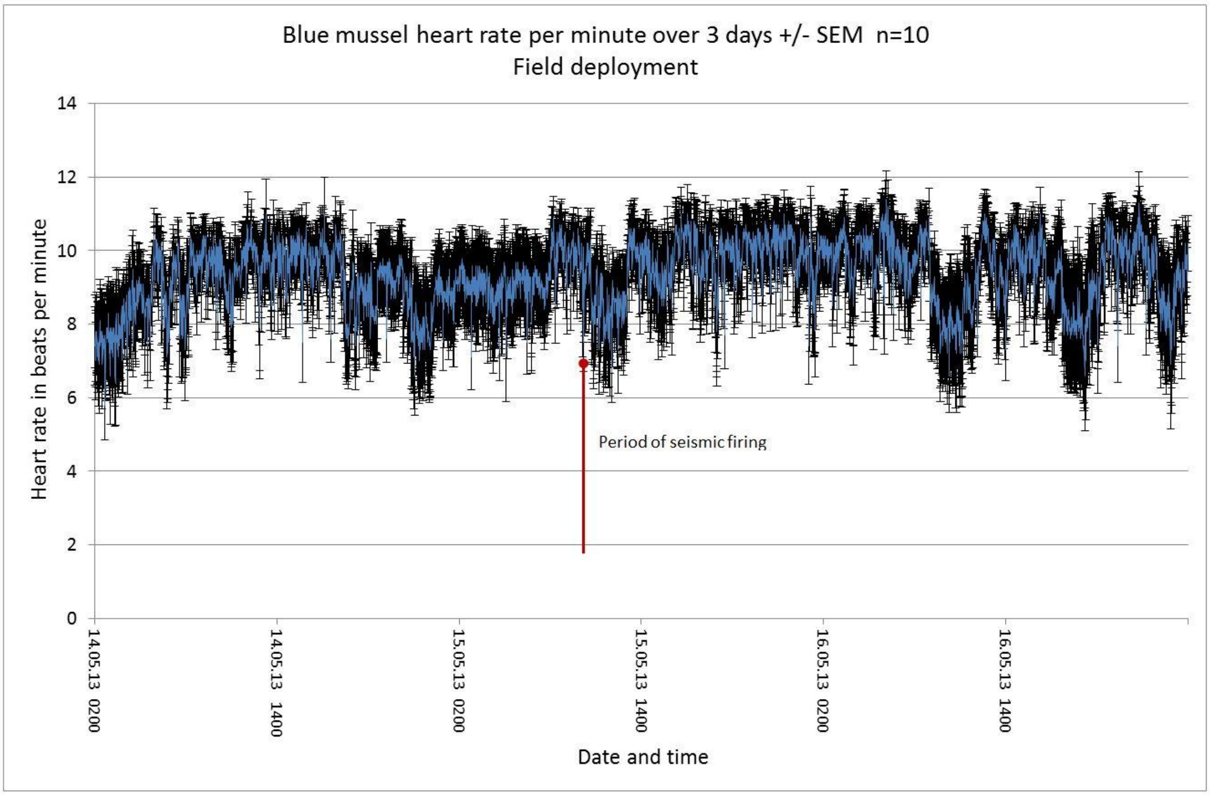

Figure 45 Blue Mussel heart rate, Field trial. 3 days.

Heart rate was successfully recorded from 10 mussels and Figure 45 shows mean heart beat per minute for these animals. The figure covers three days of measurement, which included the air gun discharge period.

No significant changes in heart rate were observed during or following the discharge of the air gun.

Mussel valve gape was seen to change during air gun discharge in the field. Figure 46 shows a time period of 8 hours which includes the repeated air gun discharges. There appears to be a close correlation between discharge and valve closure. The first semi-closure occurred with the air gun 800 metres distance from the mussels and the extent of closure increased as this distance shortened. The extent of valve closure is then seen to reduce when the air gun discharges are repeated, suggesting perhaps a rapid acclimation to the disturbance.

There is also a valve closing response a little later in the sequence where no discharge took place. This may perhaps have been associated with boat traffic directly overhead.

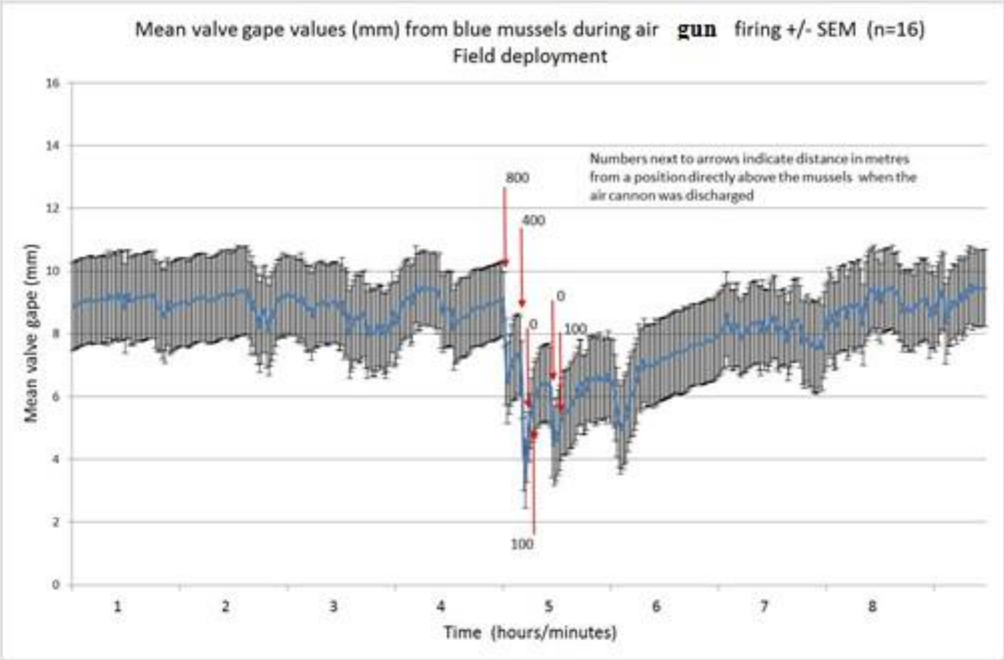

Figure 46 Mean valve gape during air gun discharge. A section showing the time of discharge +- 4 hours is chosen in order to enable showing the changes within the discharge sequence.

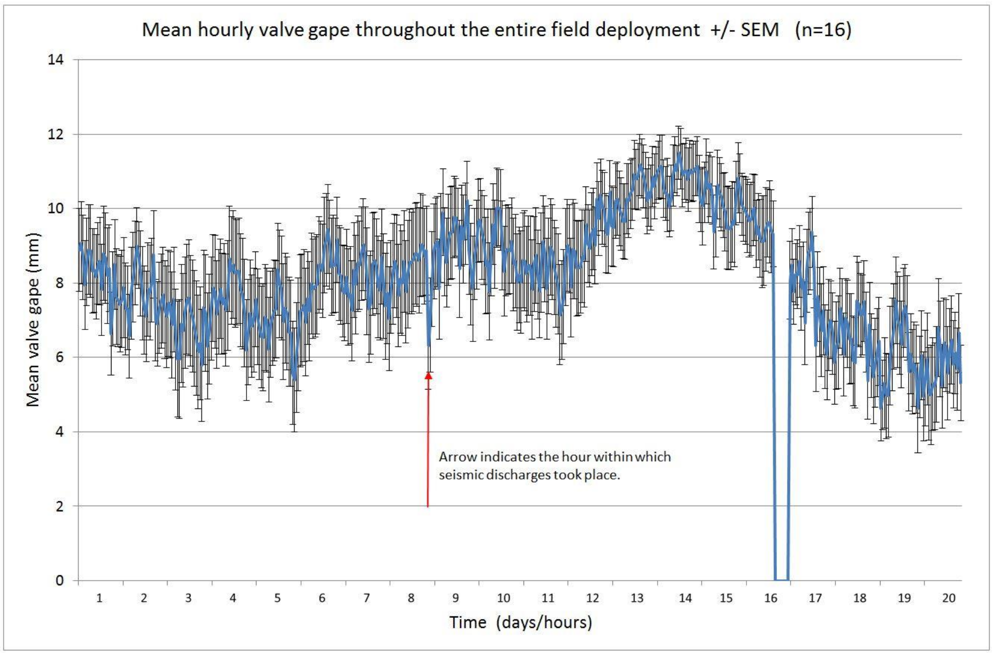

Figure 47 Mean valve gape throughout the entire field trial

The extent of variation in valve gape over the entire period of the field deployment is shown in Figure 47.

There is just under 20 days of near continuous data included in the plot. There are periods of increase and decrease as time progresses, though with no obvious pattern. The very clear circadian rhythm observed in the laboratory test is not clear in the field plot, though these animals were of course at a depth of 50 metres. The period when the air gun was discharged is marked on the plot and there is a clear reduction in valve gape here. There are however, similar events earlier and later in the plot though these may be explained by boat traffic alone.

Conclusions from blue mussel field deployment.

There was no significant change in the heart rate of the mussels monitored during the air gun discharges in the field. This was also the case in the laboratory study and this underlines that this particular endpoint in mussels is not a good indicator of seismic disturbance. The valve gape measures did however indicate quite clearly a response to the air gun discharges in the field and to a lesser extent in the laboratory. The recovery from the disturbance was rapid and there was no indication of any longer term changes in the valve behaviour of these mussels. The mussel valve closure response is used by these animals in response to various signals such as shading or mechanical disturbance and it seems clear that seismic survey activity also triggers this response, though once the discharge was completed they return to their previous state quite rapidly.

4.5 Multivariate analysis

As described in the introduction, Biota Guard uses a multivariate analytical framework to further utilize the information contained in all data sources available in the Biota Guard system from the field trial. All the data is added to a multivariate model where it is treated with multivariate statistical tools to extract all relevant information.

Figure 48 through Figure 53 are also presented in landscape in attachment 4 as figure 1 through figure 6.

In order to evaluate any given effects following the seismic shootings in the field trial, multivariate methods were used. Multivariate analytical methods are powerful tools in revealing trends over time and important relations in complex datasets from multiple diverse sensor sources. Data is transformed into information and categorized in context of the most dominant processes affecting the system in question. The responses from the instrumented Snow Crabs and blue mussels are governed by several stressors. The main stressors addressed in this analysis are stress introduced by seismic shooting and general oceanographic conditions such as oxygen, temperature, salinity and food availability.

In order to evaluate the possible relation between biosensor responses/effects and seismic shooting, two different multivariate techniques were used. A general PCA (Principal Component Analysis) analysis was used to investigate the oceanographic conditions during the experiment. It was important for the analysis that responses connected with oceanographic variations was filtered out. A multivariate technique called OPLS was used in order to highlight possible relations between biosensor responses and seismic activity.

In the following section the results and basic principles of the above mentioned multivariate techniques will be presented, for further details and background regarding the analytical approach and methodologies, an in depth report is required, and hence out of scope.

Oceanographic conditions during the test

Analytical approach: PCA

Table 6 Overview of input data to the oceanographic PCA analysis

Data table: 7 variables 56 Observations	
Conductivity	Oxygen
Pressure	Temperature
Turbidity	Current Speed
Current Direction	

PCA gives an overview of the information in a data table. This summary shows how the observations are related and if there are any deviating observations or groups of observations in the data. Of particular interest in process data analysis is the ability of PCA to uncover both smooth time trends and sudden shifts in the data. In addition, with PCA we also gain an understanding of the relationships among the variables: which variables contribute similar information to the PCA model, and which provide unique information about the observations. PCA describes the correlation structure in the data. Geometrically, PCA finds lines, planes and hyperplanes in the K-dimensional variable space that approximate the data as well as possible in the least squares sense.

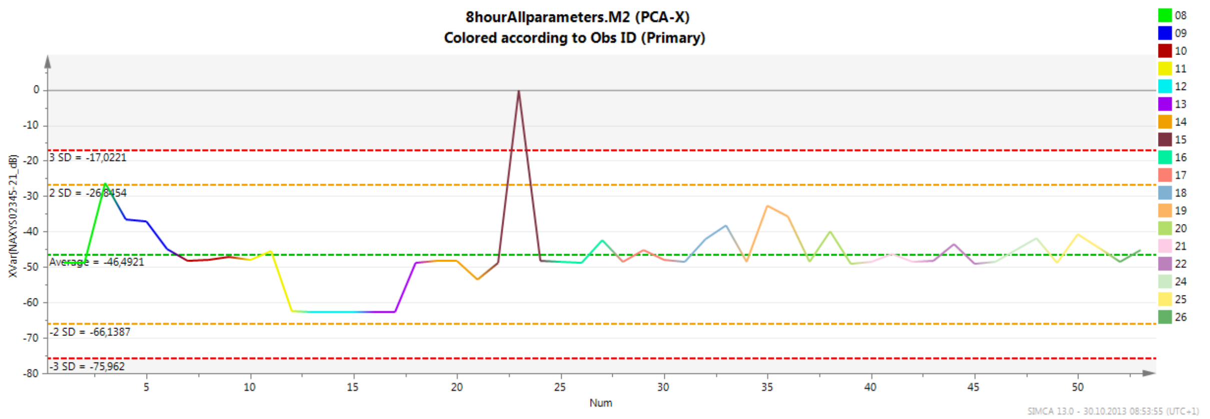

Figure 48 Overview of sound pressure throughout the field trial (May 8 to May 26). Each coloured section represents a 24 hour interval. The exposure day is identified by where the graph passes the upper red line.

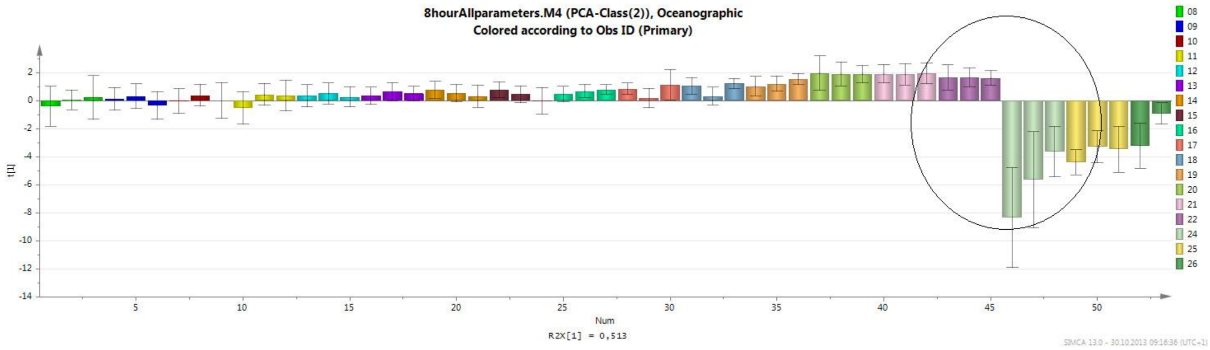

Figure 49 - Score plot of the oceanographic parameters May 8 to May 26. The bars indicate how the oceanographic parameters change in time throughout the trial. High bars represent changes. Low bars represent unchanged state. Each coloured section represents a 24 hour interval

Figure 48 presents the sound pressure from May 8 to May 26, 2013 and Figure 49 supported by the same timeline represents changes in the oceanographic parameters. The oceanographic conditions proved stable throughout the test with exception of change of water masses on May 24, providing colder and more oxygen rich water to the site. The oceanographic changes will in no manner interfere with the final results of the analysis.

Responses/effects connected to seismic shooting

The target of the analysis is to evaluate if there are any significant responses or effects as a result of the seismic shootings conducted on May 15. In order to remove responses or effects not connected to the seismic test and ease the interpretation, the OPLS algorithm (Eriksson, Byrne, Johansson, Trygg, & Wikström, 2013) was used in the analysis of both carbs and mussels.

The OPLS model is an extension of the PLS model. It separates the systematic variation in X (input data) into two parts, one that is linearly related (and therefore predictive) to Y (sound) and one that is orthogonal to Y. The X/Y predictive variation is modeled by the predictive components. The variation in X which is orthogonal to Y is modeled by the orthogonal components. The interpretation of scores and loadings and other OPLS model parameters is analogous to that of a PLS model.

Table 7 gives an overview of the data used in the analysis of crabs' responses/effects to seismic sound. Data that was found erroneous was removed from the dataset. The reason for the erroneous data is explained in chapter 4.3 at the end of the section called "Description of the field trial".

Crabs

Table 7 Overview of data input to crab analysis

Data table: 8 X variables, 1 Y 56 Observations	Features
16 crabs	Beats per minute (X – variable)
	Interpulse duration (X – variable)
Sound pressure	Max dB per 8 hour (Y – variable)

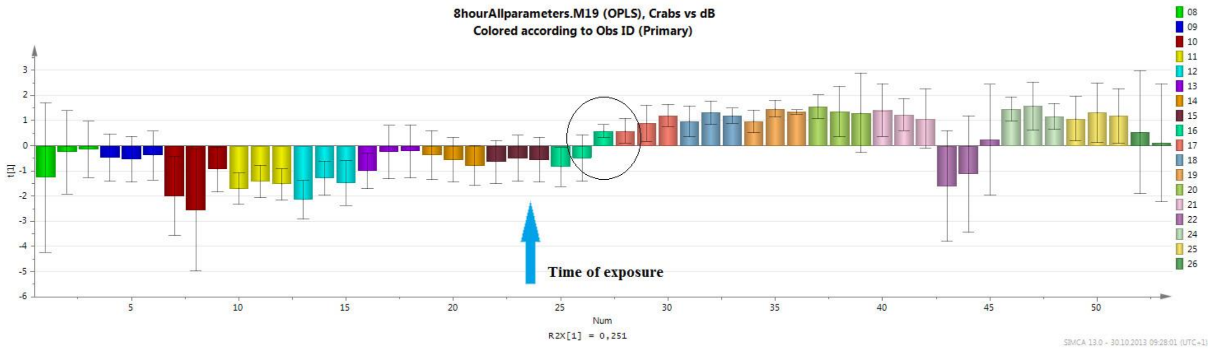

Figure 50 - OPLS Score plot of crabs. The plot represents a multivariate time series analysis of crab responses throughout the field trial. Each coloured section represents a 24 hour interval

Figure 50 represents a multivariate time series analysis of the crabs response to the sound pressure displayed in Figure 48. The multivariate model provided one significant principal component. A time shift is present in the data set, going from negative bars to positive. The turning point occurs at May 16 ref. circle in Figure 50. No acute responses connected to the seismic shooting is present, but the distinct shift at May 16. promotes further analysis in order to investigate possible long term effects.

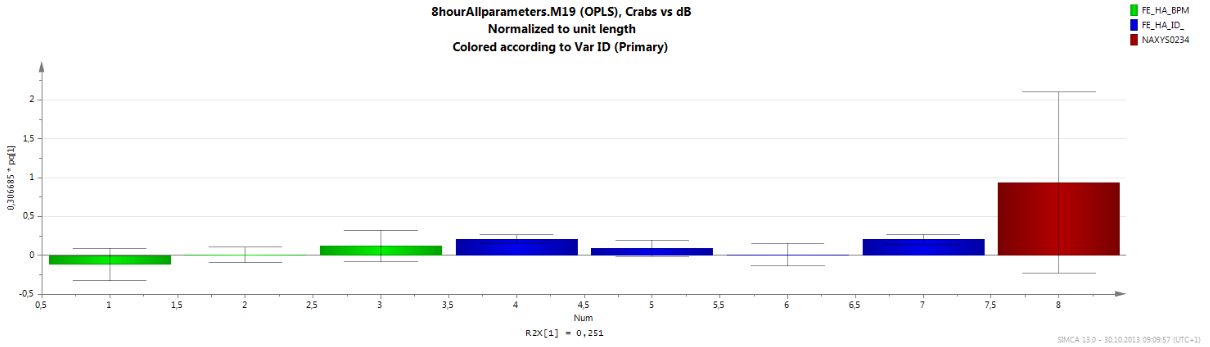

Figure 51 OPLS Contribution plot - Crabs

Figure 51 displays the heart activity endpoints that contribute the most to the pre/post seismic shooting time shift. All though not significant, the analysis indicates that there might be a change in interpulse duration after the seismic shooting.

Blue mussels

Table 8 Overview of input data for the blue mussel analysis

Data table: 46 X variables, 1 Y 56 Observations	Features
16 blue mussels	Beats per minute (X-variable)
Pressure	Interpulse duration (X-variable)
Turbidity	Distance moved (X-variable)
Sound pressure	Max dB per 8 hour (Y-variable)

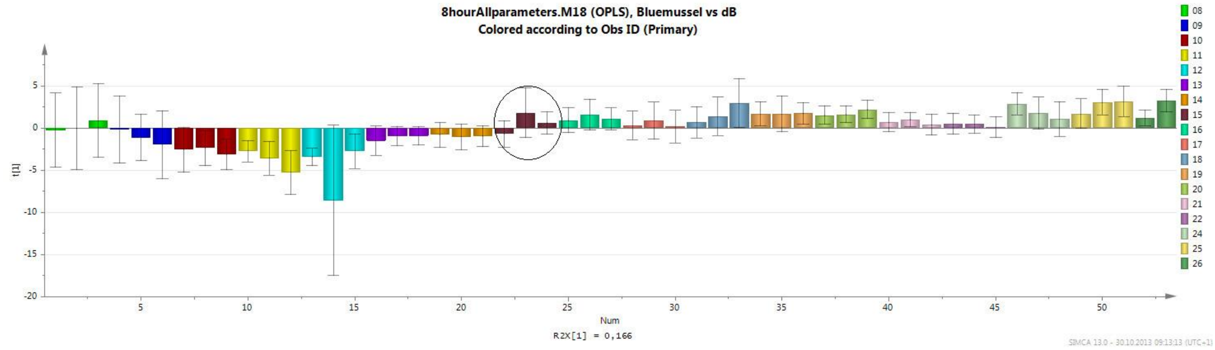

Figure 52 OPLS Score plot of blue mussels. The plot represents a multivariate time series analysis of blue mussel's responses throughout the campaign. Each coloured section represents a 24 hour interval

Figure 52 represents a multivariate time series analysis of the blue mussel's response to the sound pressure displayed in Figure 48. The OPLS multivariate model provided one significant principal component. A time shift is present in the data set, going from negative bars to positive. The turning point occurs at the time of seismic shooting. No acute responses connected to the seismic shooting is present at a population level, but some responses at an individual level and the distinct shift at May 15 promotes further analysis in order to investigate possible long term effects.

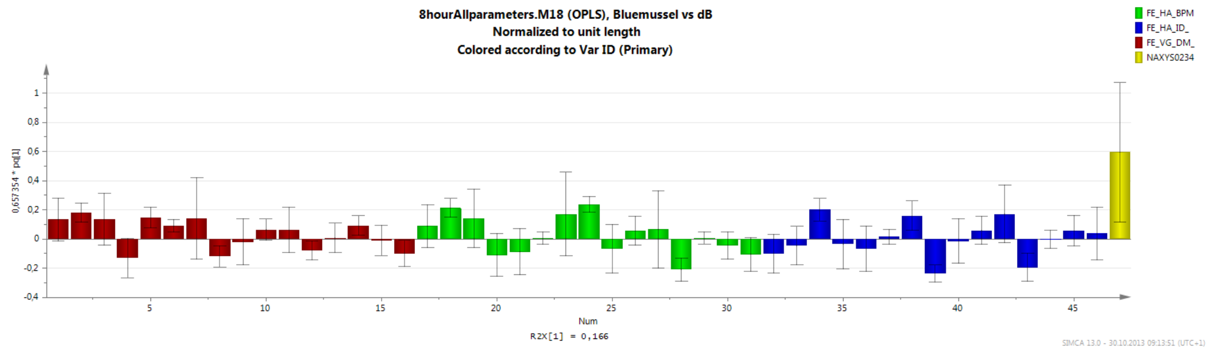

Figure 53 OPLS Contribution plot - blue mussels

Figure 53 displays how the endpoints produced by the blue mussels react to the exposure of increased sound pressure. Although there were no acute significant responses, the analysis indicate that behavioural activity and heart rate change when exposed to seismic activity.

Tissue samples

Samples of the hepatopancreas were taken from all the 16 crabs. Gill samples were taken from random animals where signs of necrosis were observed. The samples were at first immersed for several days in formalin and then in 70% alcohol for 24 hours before they were packed in vinyl bags and sent to the project consultant Dr. Mikio Moriyasu in Fisheries and Oceans Canada for analysis. The samples were prepared and preserved for future analysis. The tissue samples has not yet been analysed, this will be done later this year.

Dr. Moriyasu's preliminary notes:

"Based on my preliminary observation of gills under a light microscope, I do not see any damage to gills. Partially blackened gills are not due to damage by seismic noise but rather a necrosis caused by 'unknown' causes (possibly some bacteria, but needs to be observed under a scanning electron microscope in order to determine the possible causes). We often see the same necrosis on the gills in Canadian Snow Crabs."

Discussion of results

The findings from both trials points to a common conclusion that there are no obvious short or longer term changes in the heart rate of Snow Crabs exposed to the test conditions presented to them. The results indicate that shorter physiological changes might be linked to the seismic discharges. These changes persisted for 24 hours, before returning to pre discharge conditions.

Laboratory experiment

The laboratory experiment was successful as the objectives were met with regards to identifying response from novel stimuli in both Snow Crab heart rate pattern and in the blue mussel valve gape activity. An expected, but important result of the experiment is that it provided valuable insight and knowledge about new specie for use as a biosensor. The experience from the laboratory experiment was highly valuable in the following work with developing a suitable method for adapting the biosensor to the Biota Guard system.

The responses shown both in the Snow Crab and the mussel in the laboratory experiment are strong indicators of what kind of an immediate response to expect in the field trial. While there are no findings which indicate a lasting effect from the seismic exposure in the laboratory experiment, one should note that the experiment was not designed with this objective in mind. An interesting observation was the circadian patterns that were registered in both species. The pattern correlated with light conditions changing between day and night. This rhythm seems to be stronger when directly exposed to the variations in the daylight like they were during the lab experiment.

Field trial

The results from the field trial support the conclusion that there are no obvious short or longer term changes in the heart rate of Snow Crabs exposed to the test conditions presented to them.

The results from the multivariate analysis of the field trial data supports the conclusions made by Dr. Bamber but indicates that the heart activity for two of the five crabs that were analysed might have been changed when exposed to seismic activity. The possible change observed in the results from the analysis is small but it persists throughout the rest of the trial, however the magnitude of the change is considered to have statistically very low significance and caution is advised in interpreting much from this. This result is interesting and further research is advised in order to be able to uncover whether this is a reaction pattern or not.

The Biota Guard system

Biota Guard has gained considerable experience with the use of Snow Crabs as a biosensor. The process with adapting the specie to our system has provided important knowledge about physical, mechanical and biological limitations and requirements as well as signal interpretation and adaption to our multivariate modelling tools. The trials have shown that the Snow Crab proves a reliable biosensor as long as the knowledge gained during this project is applied.

5 Dissemination and Technology Transfer

The project has held a low public profile.
To be discussed in the near future.

6 Conclusions and Recommendations

The results does not show any evidence that shorter exposure of seismic activity causes prolonged physiological changes or damage to Snow Crab *(Chionoecetes opilio).*

Further research work is advised in order to establish whether a longer trial including the use of an industrial size air gun array will show clearer long term effects. An underwater camera should be used during such a trial in order to enable visual inspection and recording of behaviour and reactions.

7 Publications

Publication of results are under planning.

8 Bibliography

Eriksson, L., Byrne, T., Johansson, E., Trygg, J., & Wikström, C. (2013). *Multi- and Megavariate Data Analysis Basic Principles and Applications, Third revised editionMulti- and Megavariate Data Analysis Basic Principles and Applications, Third revised edition.* Umetrics.

Fisheries and Oceans Canada, F. a. (2013, 06 28). *Snow Crab - Species at a Glance*. Retrieved 10 30, 213, from Fisheries and Oceans Canada: http://www.dfo-mpo.gc.ca/fm-gp/sustainable-durable/fisheries-peches/snow-crab-eng.htm

RSPCA, T. (2012). *Humane electrical stun/killing of crustacea.*

9 Appendices

Attachment 1: Deployment procedure

Attachment 2: Safe job analysis – SJA

Attachment 3: DVD with video from the laboratory experiment

Attachment 4: Landscape presentations of multivariate plots. Figure 48 through Figure 53.