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Abstract

The oil and gas industry is required to have a monitoring capacity that makes it possible to fulfill the regulatory requirements in the areas they operate today and intend to operate in the future. Because of the relative scarcity of new oil and gas fields in already explored offshore areas, the oil and gas industry is now moving to more challenging areas such as the Barents Sea where potential new resources may exist. Cold Water Corals (CWC) are present in the Barents Sea.

The development of CWC as part of a whole organism biosensor can provide a new tool to be used for early warning of leaks allowing implementation of corrective actions before an operational event develops into a serious environmental problem. Consequences of a late response can include breach of environmental permits, environmental harm and clean-up costs, loss of production and damage to reputation.

The oil and gas industry is obliged to have control of their environmental footprint. Current technology is limited to short and offline environmental effect campaigns using sampled bivalves followed by analysis in lab. CWC can provide a real-time presentation of analyzed behavioral data. This will be in line with anticipated future requirements from the Authorities.

One of the major environmental target compounds that can result from accidental discharges at sea is drill cuttings (DC). The thesis experiment is using DC to expose the CWC as the impurity to be tested against. The development of a real time biosensor capable of capturing images of coral nubbins and measuring the individual polyp activity was accomplished. This was done by utilizing pixel area fraction calculations on thresholded images of *L. pertusa* polyp images. The software was developed and run entirely through Matlab.

Further research on reducing uncertainties and making the sensor more reliable by removal of false signals from the image analysis are advised with suggestions for improvements.

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Acronyms

.avi	- Audio video interleave (file format)
AWACSS	- Automated Water Analyzer Computer Supported System
BEWS	- Biological early warning systems
C1P1	- Coral 1 Polyp 1
C1P2	- Coral 1 Polyp 2, etc.
.csv	- Comma separated value (file format)
CWC	- Cold water corals
CCD	- : Charge-coupled device
DC	- Drill cuttings
DVR	- Digital Video Recorder
ESP	- Environmental Sample Processors
HD	- High Definition
HDD	- Hard disk drive
.ifv	- Indeo Video Format (file format)
IRIS	- International Research Institute of Stavanger
LBP	- Local Binary Pattern
LED	- Light emitting diode
MIPs	- molecular imprinted polymers
OBM	- Oil based mud
OSPAR	- Oslo Paris convention
PAH	-Polycyclic aromatic hydrocarbons
RIANA	- River Analyzer
SWaP	- Size, weight, area, power
TVL	- Television line resolution
WBM	-Water based mud

1 Introduction

Management of the marine environment with focus on anthropogenic influenced hazardous pollution will require an array of different sensors in order to evaluate possible impacts a potential hazardous source may have. Emphasis is put on biosensors for monitoring of marine waters with respect to mainly anthropogenic instigated contaminants.

In the literature the biosensor is in general described as a self-sufficient device consisting of a biological recognition component directly connected to a transduction element which can convert a biological event or activity into a comprehensible output signal (Rodriguez-Mozaz et al., 2005). The oceans cover vast areas and any anthropogenic or natural contaminants will ensure large dilution, thus as the pollution has a biological significance it may occur in very low concentrations. Depending on the possible origin, severity and concentration of a contaminant, different strategies would need to be evaluated. (Mills and Fones, 2012) A biosensor is an *in-situ* sensor giving often a high sampling resolution and repeatable monitoring. Some of the foreseen obstacles for the biosensor are area coverage, size, weight, power consumptions, (SWaP-factor), biofouling and simplicity (Zielinski et al., 2009, Rogers, 2006, Rodriguez-Mozaz et al., 2007).

1.1 Biosensor technology

Biosensors are analytical sensors based on bio recognition elements like antibodies, molecular receptors, enzymes or DNA coupled together with transducers based on acoustic, chemical, electrochemical, spectroscopic, thermal, microbalance, optical piezoelectric or magnetic principal that convert the elements to electrical signals. The sensors do not automatically measure only biological parameters but can also indicate what the actual analyte of interest is (Zielinski et al., 2009, Kröger et al., 2002, Mills and Fones, 2012). Currently there are not many commercially available biosensors compared to what is being researched upon as instruments for environmental applications. However most have been developed for the measuring of contaminants in freshwater and wastewater (Mills and Fones, 2012).

RIANA and AWACSS are two immunosensor technologies with optically linked multichannel sensors for measuring pesticides and organic pollutants in rivers and in wastewaters, respectively. A biosensor based on immobilization of bacteria to measure Nitrate/Nitrite/Nitrous Oxide is commercially available. Developed originally for wastewater measurements, but now is also utilized for marine services up to 30 meters deep. Other developments are the Environmental Sample Processors (ESPs) which automatically collect water samples and ensures DNA probe-based immunoassays and sample filtrations to measure the potential toxicity of harmful algal blooms. Results are then sent remotely to a nearby vessel or to the shore and samples are stored internally for future lab analysis. (Mills and Fones, 2012, Doucette et al., 2009, Unisense.com, 2014)

Whole organism sensors are being developed to include fish monitoring systems which take advantage of avoidance behavior. In a dual-fluvarium set-up where one stream is contaminated and the other is uncontaminated, fish may show a distinct avoidance or

attraction behavior where the swimming and positioning patterns are analyzed as on-line biomonitors. There have been experiments with several elaborative designs, coupled with the measure of behavioral responses, that fish will tend to avoid oil-contaminated water, gas supersaturated waters, heavy metals, pesticides and rotenone. A different setup is the monitoring of fish ventilation frequency and gill movement, or the sensing of gill muscle activity by use of sensitive electrodes near the gill muscles. (Kane et al., 2005, Allan et al., 2006).

Mosselmonitor[®] is a commercially available freshwater sensor for the detection of contaminants in water. One of the bivalve responses to stresses and contaminants in water is valve closure, which is being monitored and continuously observed with the use of electromagnetic sensors. This is being done on freshwater mollusc bivalves or *Unio pictorum*, *Dreissena polymorpha* and marine blue mussel, *Mytilus edulis*. Also, the measurement of pulse with infrared-sensors together with this valve response has been tested for toxicity measurements on the marine blue mussel (Allan et al., 2006, Curtis et al., 2000, AquaDect, 2014, Gnyubkin, 2009).

Biota Guard AS is a technology and service company offering an *in-situ* real time leak detection system and environmental decision support. Part of the company technology is based on using biosensors, capable of providing input to the environmental monitoring system in use during offshore drilling and production operations, but also near-, sensitive coastal areas, rivers and harbors. The sensor station combines traditional oceanographic and chemical sensors with instrumented whole organisms. -The most common biosensor used in Norwegian shallow waters is the blue mussel- or *Mytilus edulis*. The organism's heart rate and valve gaping activities are constantly monitored as indicators for stress that can relate to abnormal constituents in the surrounding waters. This multi-sensor-approach allows for an environmental performance index to be generated from the data that can reflect the chemical changes in the waters over time. In short the company delivers a sensor array with oil in water leak detection up to 0.06 ppm, as well as a means of real time documentation of what an environmental footprint an oil and gas related operation might have (Biotaguard, 2014, Lingjerde, 2014).

Several other monitoring technologies are based on the luminescence or oxygen production of micro-organisms like algae, to detect the effect of harmful constituents like herbicides or other environmental toxicants. Lee et al. (2005) describe a study where the use of micro-organisms reactions to toxins that induce superoxic, DNA or protein damage. The measurement is done by recording bioluminescence with a CCD camera and the images are then processed through computer image software analysis to obtain relevant data.

Recent development has been done in producing materials dubbed Molecular Imprinted Polymers (MIPs), which mimic the biological activity of antibodies. By using MIPs as recognition units in biosensors, so in a way not being a true biosensor, the sensors become even more resistant to thermal, mechanical and thermal stress. The sensor is already developed for PAH's and pesticides. (Rodriguez-Mozaz et al., 2005, Mills and Fones, 2012, Rickerby, 2009)

1.2 Sensor Information Depth

In order to get a broad and rapid view of the ocean in a large scale the use of aircraft and satellites are most common. Satellites will show the surface films from oil-spills and give a good indication of the extent of the contaminated areas in real time, while accommodating for conditioning daylight and clear skies. Remote sensing gives a valuable and inevitable resource for oil-spill combat due to the information it gives. But due to the high attenuation in the sea, optic and radiographic signals used with the remote sensors are only able to perform surface layer sensing, giving a wide but shallow information depth (Zielinski et al., 2009).

In the opposite side of this resolution scale are the biosensors. These sensors are *in-situ* and can often measure pollutants in complex mixed mediums. At the same time that they offer determination of specific chemicals some also offer measurement of the direct response of an organism from contaminants such as toxicity, cytotoxicity, genotoxicity or endocrine disruptors. In many cases these effects are even more valuable than knowing the specific composition of a pollution, as it in the end it is the ecosystem and its inhabitant's response to xenobiotics that are of the greater importance, (Rodriguez-Mozaz et al., 2007). In addition the effects from these parameters are often virtually impossible to characterize using conventional analysis (Kröger et al., 2002). An important drive in the development of new biosensor technology is the multi-analyte determination by the use of arrays of miniaturized transducer elements. This research is done in order to record several parallel measurements of different species in a single sample analysis, increasing the detailed sensor properties of a biosensor unit even further (Rodriguez-Mozaz et al., 2007, Rowe-Taitt et al., 2000a, Rowe-Taitt et al., 2000b).

1.3 Sensor simplicity

Advances in microprocessors, electronics and microfluidics continue to give reductions in the SWaP-factor, and due to the size and composition of the biosensors they are often amendable to mass production. Each sensor is made up to do a specific assay(s), thus the design will be fixed, leading to improved reproducibility and almost complete removal of operator errors. Due to the effect of macro-kinetic transport time reduction, rapid measurements are produced which are then delivered in real time and can be utilized to give a complete visualization of any abnormal activity of unknown pollutants in the biosensors *in-situ* area (Kröger et al., 2002, Rodriguez-Mozaz et al., 2007). Still increase of sensors durability, selectivity, concentration ranges and biofouling resistance, compared to a simple pH electrode, are likely to limit acceptance for the use of them for environmental monitoring (Rogers, 2006).

1.4 Sensor networking

In-water platforms, bottom-tethered deep sea and coastal moorings and offshore power cables to observatories, together with high bandwidth are the strategic technologies providing integrated observing networks which enable longer deployments, and giving rapid dual-way communications with sensors and operators onshore. The utilization of different ocean observing systems like drifters gliders, floats, and autonomous underwater vehicles, offer the

possibility to complement each other in terms of data network distribution and real-time continuous observations of the ocean over a larger area, (Paul et al., 2007). The ability of monitoring physical and environmental conditions in spatial-distributed locations could be one of the main advances for continuous environmental monitoring (Farré et al., 2009).

1.5 Biological early warning systems

Sensory systems set up as biological early warning systems (BEWS) base it's response on a biological organism's toxicological reaction towards a contaminant or a complex matrix of contaminants. By analyzing an organisms physiological or behavioral changes and linking it to acute toxicity effects it is possible to provide a rapid warning in terms of water quality. BEWS systems need to have a quick response time and be online in order to give an alert or warning in case of altered environmental conditions. Therefore an elaborate network for data treatment and response co-ordination to stop or minimize a potential hazardous contamination is necessary. In addition BEWS systems need to be relatively cheap, reliable, and low maintenance with minimal operational requirements so that the systems can be installed in remote unsecured sites. Organisms that have been used for BEWS systems include different species of fish, daphnia, larvae, microorganisms, and bivalve molluscs. (Allan et al., 2006, Gnyubkin, 2009, Curtis et al., 2000)

As mentioned Cho et al. (2004), measurements of luminescence of microorganisms can similarly be used in a BEWS setup for early toxicity testing. Also Lee et al. (2005) CCD camera based bioluminescence can be utilized as an early warning system due to its quick response and portability.

1.6 Drill Cuttings

Drill waste consist of DC which are crushed rock residues from the bore hole mixed together with different chemical constituents residing in the drill mud. The drill fluid is used for lubrication and cooling of the drill crown, stabilization and pressure control of bore hole and transport of the DC to the platform. The drilling mud consists of a liquid part, being either water, oil or other organic material and a weighted material, typically barite, (BaSO_4), (Bakke et al., 2013, NFR, 2012).

Currently only Water based mud (WBM) are allowed to be discharged in UK and Norwegian offshore waters due to the stringency of the country's legislations and OSPAR decisions putting limitations on type of chemicals allowed to be discharged.

1.7 Future and current challenges

The oil and gas industry will face increasingly strict regulations related to their activity in environmentally sensitive areas. According to the Activities Regulations § 57(2), set by the Norwegian Environment Agency, the operator shall monitor the external environment and shall set criteria for acute pollution from the offshore and onshore facility. The Norwegian Environment Agency has indicated that a prudent operator should be able to identify and report disturbance to the environment both subsea, at sea surface and on land within a

timeframe of three hours. The industry is obliged to have control of their environmental footprint. Current technology is limited to short and offline environmental effect campaigns like discrete sampling of bivalves following return to laboratory for proper analysis. Process is very expensive and does not focus towards new paradigm shift in ocean observations where it is sought to have data returning to shore and not samples, i.e. *in-situ* analysis returning real time data. (Baussant and Bamber, 2013, KLIF, 2011, OG21, 2013)

Biosensor technologies focused towards the marine environment are a rapidly expanding field of research, with special attention towards offshore oil and gas production. Operational discharges of produced water and DC are meeting increasing demands for monitoring in some cases also complete removal of harmful discharges to the sea. In the Norwegian governmental White Paper Report number 26, it is clearly stated that a zero discharge policy should be enforced for the petroleum industry. The Paper goes in detail to describe the more stringent zero discharge requirements aimed specifically for the Barents Sea area. Closing in on the arctic regions which are areas becoming of increasing interest for offshore oil explorations (Parlament, 2006-2007).

There is a need to minimize the complexity of the biosensors and their processes, reduce regular calibrations, stabilize biological reagents as well as reduce energy costs. In order to achieve more reliable and improved devices future research will need to focus on continuous real time monitoring with multi-analyte sensors with even more stable transducers. Wireless networks of biosensors measuring physical and biological impact connected to onshore or offshore marine vessels, giving the operator full overview of a larger ocean area, has great potential to be the future of real time monitoring. (Farré et al., 2009).

1.8 Cold water coral biosensor

Cold Water Coral (CWC) reefs are found in most benthic regions of the ocean from 39 to 2000 meters in for example Trondheim's fjord and the Pacific or Atlantic oceans respectively. Generally these are areas where seasonal storm wave bases do not affect the sea bed. Topographically guided bottom currents will funnel the water flow through straight channels and fjords, preventing the deposition of sediments creating a harder substrate for coral colonization and the stimulation of phyto- and zooplankton growth due to the nutrient rich water. In the North Atlantic the stony coral *Lophelia pertusa* is the main reef-building organism. (Hovland, 1999) and (Freiwald et al., 2004). Normal temperature ratings tied to the altering water masses are identified to be around 4–12 °C. Salinity levels are also widespread for the *L. pertusa* which is reported to thrive in salinity levels from 32 ‰ to 38.78 ‰. Salinity and temperature levels are usually found to be stable at a corals location and depth. But they are reportedly less tolerable to changes on the individual level, (Dodds et al., 2007, Roberts et al., 2006, Freiwald et al., 2004, Forsgren et al., 2009). Growth rates are of the *Lophelia*, i.e. the skeletal linear extension is estimated to be between 5 and 25 mm per year. The growth rates are derived from studying corals growing and colonizing on manmade structures like cables, ship wrecks and buoys to analysis of carbon and oxygen stable isotopes (Freiwald et al., 2004)

More and more focus is put on the CWC as it is just recently that the ubiquity, biodiversity and the paleoclimatic value is being understood. For these reasons many nations worldwide are putting restrictions on activity in these habitats (Roberts et al., 2006). Also OSPAR has listed *L. pertusa* (CWC reefs) as a habitat of urgent need of protection, and “coral gardens” as endangered and in decline (gorgonian octocoral dominated) (Nilsen et al., 2010). Special areas of conservation are established for reefs with important CWC that requires protection, by the EU Habitats Directive (EU, 1992). Even further work is being done by the EU Marine Strategy Framework Directive (EU, 2008) by establishment of monitoring programs, environmental targets and similar within 2020.

As corals are seen to be the of an utmost ecological significance it would be of keen interest if it was possible to evaluate the direct response on environmental stresses that for example DC would give. There exist some research on the effects that DC may have on reef building corals, but relation to the ecological effects are never straight forward (Dodge, 1982). The surface attached animals have no way of escaping and their inflexible bodies make them vulnerable to any physical disturbances. Since they are suspension feeders they may be susceptible to increased concentration of inedible particles that may reside in the water, (Nilsen et al., 2010) and (Mortensen, 2007).

1.9 Coral symptoms

Before analyzing images of corals it's necessary to know what information one would be looking for in a stressed coral. The *L. Pertusa* captures phyto- and zooplanktons drifting by with their tentacles and pull them into their mouths. As the corals only have one opening into their system the mouth will also function as the path for excretion. A healthy individual is an opportunistic predator believed to be feeding at a normalized rate, while a stressed individual is believed to have this feeding pattern altered, that be increased or decreased activity for feeding or excretion, (Hovland, 1999, Dodds et al., 2009).

Externally, the polyps of a coral are connected by a tissue resembling a thin layer or membrane with mucus producing cells called the coenosarc. The coenosarc acts as a protective membrane for the coral skeleton, against parasites and symbionts, and other external stressors like sedimentation from drill cutting particles. Production of slime from the membrane is an efficient way of rejecting particles building up on the coral. When severely stressed the coenosarc may reduce to a thin membrane at the polyp calyx edges of the coral skeleton. (Baussant, Baussant, 2012)

Previous experiments have found that the *L. pertusa* is able to survive repeated exposure and slight smothering from both natural sediments and DC. Also the efficiency of rejecting deposited material from the coral surface even after repeated exposure was found to be indifferent to it being natural sediments or DC completely covered or buried coral lead to polyp mortality and tissue smothering, (Larsson and Purser, 2011). Environmental monitoring studies with video observation platforms and lipid class and fatty acid analysis of coral was done at the Morvin field in 2009 and 2010. The study found no significant differences to corals exposed to DC and corals not exposed. Image analysis also revealed no apparent behavioral differences between exposed and non-exposed corals. Immediate damage was not

observed but there was recommended to revisit the field to obtain information on long-term effects. (Buhl-Mortensen et al., 2010, Bakke et al., 2013)

Previous research at IRIS on polyp activity has shown indications of behavioral differences in the coral polyps during exposure to DC but no significant differences in terms of long term effects. During DC exposure polyp activity was seen to be at its lowest, while returning to normal shortly after exposure periods, (Baussant, 2012).

1.10 Objectives

Discussions with the University of Stavanger, Biota Guard and IRIS resulted in the idea of expanding the multi-sensor monitoring station of Biota Guard AS to include a biosensor that utilizes the reef building CWC species *Lophelia pertusa* as part of their sensor array.

The overall objective of this thesis is therefore to produce a real time *in-situ* biosensor. The sensor to be developed, hereby referred to as the biosensor, is an analytical device that will combine nubbins of *L. pertusa*, together with an image capturing device as transducer measuring individual polyp activity. In order to reach this overall objective several sub objectives needs to be fulfilled.

The primary sub-objective is to create a software program that can take a picture of a coral and convert this graphical image to generate a dataset based on well-defined endpoints enabling to inform about the corals physical state. By taking a picture of a coral in a time series and comparing the results over time during periods of exposure to external stressors, it is believed that sufficient data can be generated and related to the coral activity prior or during exposure.

To facilitate the completion of the overall objective, the activity was divided into several sub objectives.

- Evaluate best suitable image analysis method for converting an image to comprehensible data.
- Evaluate a software or programming environment suitable for designing biosensor software.
- Design software able to utilize the image analysis method of converting images to data
- Find and get a hold of an image capturing device suitable for *in-situ* subsea environment to represent the transducer for the biosensor.
- Construct a lab setup that will simulate an *in-situ* environment.
- Develop a secondary system for long term analysis of the effect of sedimentation.

The last sub objective was added in order to further increase the usage of the biosensor as an environmental monitoring. Being able to predict other impacts DC could have on the corals.

2 Methods

A non-intrusive and intuitive way of monitoring or evaluating the wellbeing of a coral would be to capture pictures of the coral and study its behavioral responses to stresses over a given time period without physical interactions. Just looking at a organisms response is easy when considering a highly advanced organism like for instance a fish, which have distinct evasive behavioral responses (Kane et al., 2005). Doing the same with corals, considering its slow response, would be a daunting task even in controlled environment. It has long been known that filming and recording corals over time could be a solution to intensive monitoring (Fosså and Mortensen, 1998). A technique for making the task manageable would be to take a photo in a specific time period and producing a time lapse with the pictures for future analysis by a panel of experts. More so, by applying different image analysis techniques on the pictures taken one could proceed to automate the process. A software analysis could then calculate a scaled number to represent the information that is of interest in the image, which could be used for a calculated evaluation of the organism response.

Following are considerations to different image analysis techniques that could be possible to use in order to obtain functioning and computable information from an image.

2.1 Computer Vision applications

In this chapter a brief review of different image analysis methods are described with examples on utilization in comparable applications. The assessments will not go into detail in the programming or theory of the method but rather compare an example to application on coral stress measurements based on the analysis of an image.

2.1.1 Local Binary Pattern (LBP)

Each pixel of an image is replaced by a binary valued label, and the technique used to get the binary value is by thresholding neighboring pixels around a center pixel and then to represent this string as a binary number. This LBP label will store information on distribution of the gray level or pattern in an image, which can be utilized to characterize the texture of the image. Multi-resolution analysis is carried out by combining the different obtained LBP operators. From the accumulated LBP values a LBP histogram is calculated in order to analyze the texture presented in an image. (Kotu et al., 2012)

In Kotu et al. (2012) the LBP image analysis is used on Cardiac Magnetic Resonance (CMR) images, Figure 2-1, in order to classify patients with high and low risk of getting life threatening arrhythmia. Standard methods for classification of these patients are by evaluating reduced left ventricular ejection fraction and scar size of the hearth. Concluding remarks show that textural differences of scarred myocardium were able to be captured with LBP and contrast measurements.

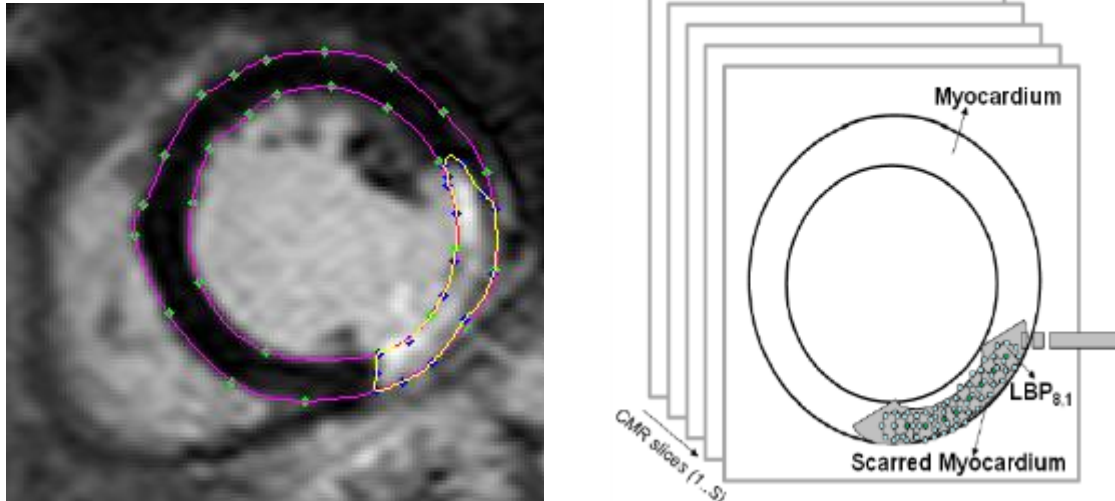


Figure 2-1. Left: Cropped short-axis CMR image showing manual segmentation of myocardium and scar tissues. The green and blue dots in the image are manually marked (by Cardiologist) coordinates to segment myocardium and scar. The magenta and yellow contours generated by cubic spline interpolations of the above coordinates show myocardium and scar tissues respectively. Right: The LBP values are calculated for each pixel in scarred myocardium using LBP operator, LBP_{8,1}. The LBP values are accumulated into a column vector from all the CMR slices (where scar is visible) belonging to a particular patient (Kotu et al., 2012).

2.1.2 Image gradient computation

Before the image gradients are estimated the image needs to be filtered. Simple finite differences in image pixels may result in a strong noise response. Among others the most common is the use of the additive stationary Gaussian noise model. The image gradient can be represented by two different principles, edge computation or the use of gradient orientations, which are usually differentiated by fast changes in brightness or illumination intensity respectively. See Figure 2-2 and Figure 2-3 for the gradient magnitude method and Figure 2-4 for review of the gradient orientations. (Forsynth and Ponce, 2012)



Figure 2-2. The gradient magnitude can be estimated by smoothing an image and then differentiating it. The smoothing will affect the gradient magnitude as seen above. At the center the gradient magnitude is estimated with a Gaussian smoothing scale of $\sigma = 1$ pixel. On the Right the gradient magnitude is estimated with a smoothing scale of $\sigma = 2$ pixel (Forsynth and Ponce, 2012).



Figure 2-3. Left: Gaussian smoothing scale of $\sigma = 1$ pixel with high threshold for the gradient magnitude edge point determination. Center: $\sigma = 4$ pixels with high threshold for the gradient magnitude edge point determination. Right: Low threshold testing and of $\sigma = 4$ pixels for the gradient magnitude edge point detection (Forsynth and Ponce, 2012).

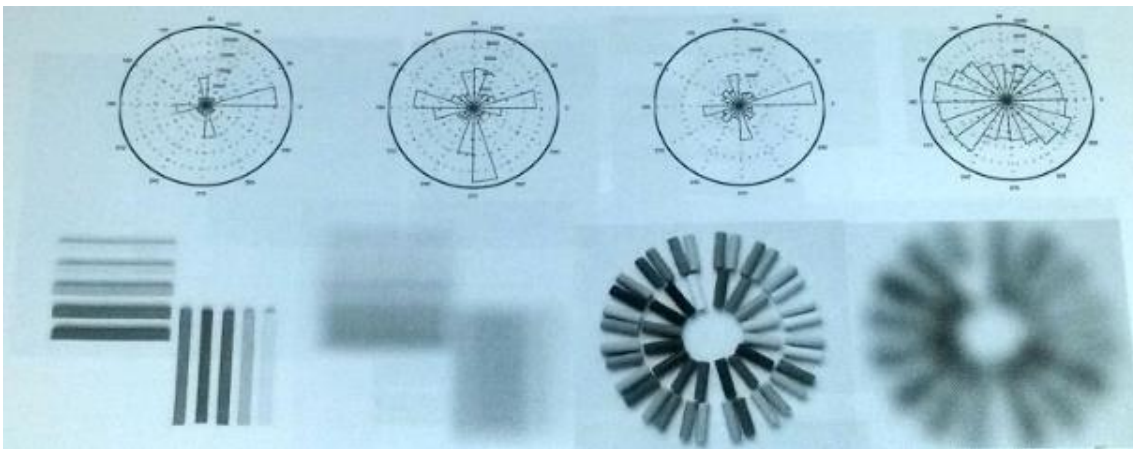


Figure 2-4. The orientation histograms are showing that the seemingly similar patterns are quite different. Left: images and rose plots of pastelles at two different scales. Right: The pastelles arranged in different pattern together with rose plots. Both the different orientation patterns and the changes in scales give are very unlike (Forsynth and Ponce, 2012).

2.1.3 Area Fraction

To calculate the area fraction of an image the sum of pixels within a certain area of the image is divided by the total number of pixels in that image. In order to get a computable histogram from the area fraction calculation it is often necessary to threshold the image into pseudo colors, (a term usually used to distinguish it from actual color, as seen in the microscope), see Figure 2-5 and Figure 2-6 (Friel et al., 2000).

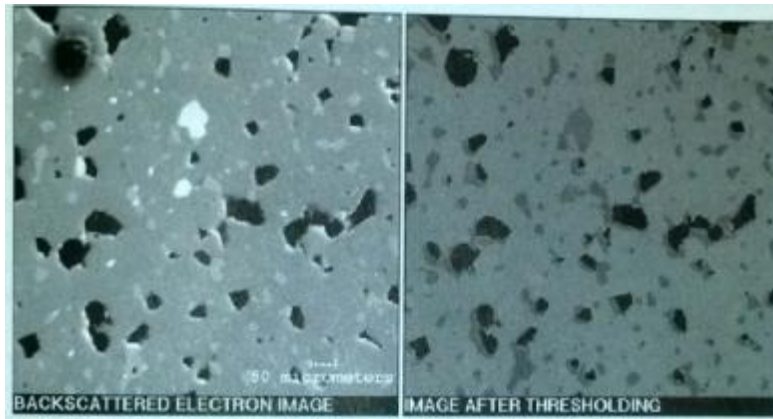


Figure 2-5. Scanning electron microscope image of a multiphase ceramic material. Left: Image in grayscale. Right: Image in pseudo color (Friel et al., 2000).

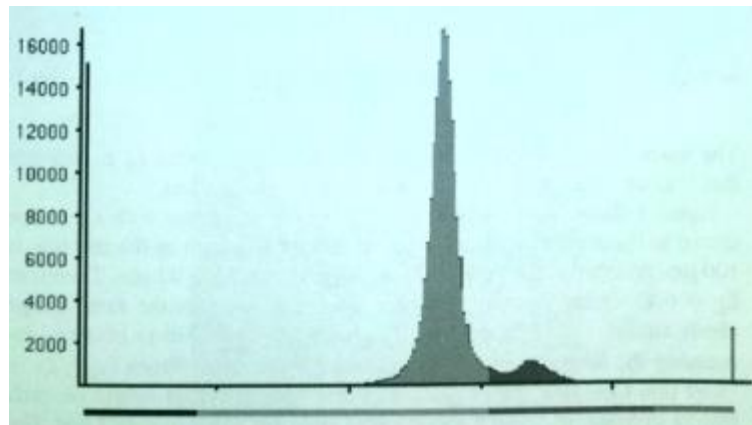


Figure 2-6. The Histogram readout from the threshold image in Figure 2-5, (Friel et al., 2000).

2.1.4 The color of surfaces

Surface colors vary due to many different mechanisms, for instance differential absorption, diffraction, refraction and bulk scattering. The reflection of an object is often divided into two types of specular reflection color regimes. Dielectric surfaces, reflecting light with the tendency of same color as light source and conducting surfaces where the color reflected will depend heavily on the wavelength of the emitted light, see Figure 2-7 for example, on spectral reflectance from some selected natural objects (Forsynth and Ponce, 2012).

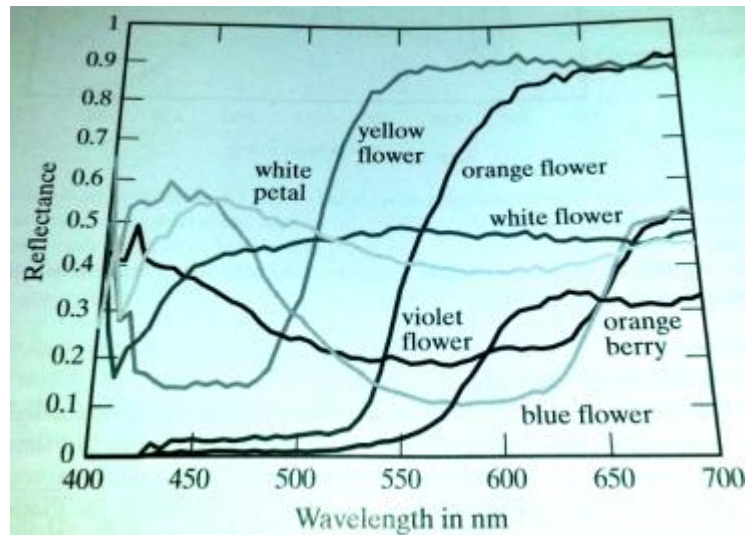


Figure 2-7. Spectral albedos for a variety of natural surfaces measured by Esa Koivisto, Department of Physics, University of Kuopio, Finland, (Forsynth and Ponce, 2012).

2.2 Method Evaluation

The chapter attempts to evaluate the four different methods presented above in terms of utilization for image analysis of *in-situ* and *in-vitro* setups. The Evaluation is based upon discussion with UIS professor Ivar Austvoll, (Austvoll, 2013).

2.2.1 Simplicity of implementation

The first assessment of an image is often to be able to segment the part of the image that is of interest, i.e. segment out the background from the part that is of interest. In an ideal condition this can usually be done in a pretty straight forward manner without much trial and error. However, in real *in-situ* situations, this may come to be one of the main problems as unforeseen situations like obstructions, altered picture quality, resolutions, interference, etc. might prove to be hard to preprogram. In such situations it is often difficult to know the impediments without actual field trials to get images which to test the software on. There exist several different pre-made powerful algorithms for this purpose.

The Following segmentation is area calculations. Depending on the success of the segmentation area calculations will be simple and straightforward. There already exist *in-vitro* images that utilize this method, with success.

Texture analysis is probably the most demanding method to use in this case. There exist several different analysis methods and algorithms, based on frequency calculations, statistical method, and more. The simplicity of implementation is still very hard to evaluate without actually doing any experiments on real images. The same criteria are put on the usage of color analysis.

2.2.2 Computational requirements

Matlab is a powerful editorial tool that should be able to handle this kind of image analysis without difficulty during the testing phase. For easier and faster computational

calculations in a product construction phase the conversion to C language and transferring and compiling into a μ computer should be feasible without any problems.

2.2.3 Editor requirements

Matlab is recommended. The software has its own routines that are highly effective and have high efficiency for matrix and vector processing.

3 Materials and experiment

The experiment is divided into two main parts.

- Programming and testing of software
- Setting up a lab experiment where the actual images and exposure of the CWC are to be performed. The chapter describes the Matlab program functionality with associated equipment and lab setup.

3.1 Pseudo code

A high level general pseudo code was prepared in order to show thoughts and ideas of the system in a feasible way. The overview has been edited and modified numerous times since first drafted, mainly during the programming and testing phase. As several ideas and improvements on the initial functions became clearer and limitations and advances were better known and assessed. The finished program functions in the principal manner described below.

Main program:

- Take photo every minute.
- Store photo in a local hard drive, dedicated folder with incremented order.
- Loop, image analysis:
 - Open image in dedicated folder, oldest image to be analyzed first.
 - Do image analysis
 - Move analyzed image to new folder.

Image analysis:

- Cut out part of the image to be analyzed, i.e. polyp or coral.
- Turn image into grayscale image.
- Threshold the cut out image.
 - Different methods to be evaluated and tested during experiment:
 - Manually adjusting the set level.
 - Altering the contrast level of the image before thresholding.
 - Otsu's Method, method that evaluates the goodness of the threshold by evaluating the measure of separability of classes for each grayscale image, (Otsu, 1979).
- Perform area calculation of the cropped out image.
- Save data for each image descending in txt file.
- Move analyzed image to new folder

3.2 Software module

The software was programmed entirely through Matlab. The image capturing was for practical and financial reasons run through a free and independent image capturing software. Doing this through Matlab would require Matlab Image toolbox bundle, which is not supplied in Matlab student version. Labview with IMAQdx extension was also evaluated but seen to be

too demanding in terms of computer processing requirements. Limitations on use of imaging hardware renders the software a less popular alternative. Several free image acquisition software programs were evaluated. Yawcam (Lundvall, 2013) was assessed to be the simplest and fastest way to implement image capturing from a PC when a standard web-camera was used for the image capturing. Requirements for the image capturing software were:

- Time lapse function
- Increment function
- IP webcam function
- Easy to use and connect

Other software evaluated was VideoVelocity, BooruWebcam, Flix (CandyLabs, 2014, Lumai, 2006, Butler, 2014).

The actual biosensor program was developed entirely through Matlab. It consists of one script, Biologger.m, to start the program and three functions with their own respective tasks, ToDo.m, PolypMonitor.m and AreaCalc.m. Before startup the program needed calibration and adjustment according to the positioning of the corals that was being analyzed. A separate script called iCrop.m was used to decide which corals and polyps to analyze.

Following is a rough description of the function and tasks performed by the different parts of the software module.

- Biologger.m initiates a timer sequence on how often an image analysis is to be performed. Every pre-set time it will call the ToDo.m function and initiate it's given tasks. During this experiment it was set to perform an iteration every 5 minutes.
- ToDo.m creates necessary file paths and files according to current dates. The function then calls PolypMonitor.m and stores the returned data from this function in specified files.
- PolypMonitor.m opens the oldest image in the folder where the time lapse images of the corals are stored. Pre specified pieces of the image are cut out and sent to AreaCalc.m where the actual calculations of the images are performed. Performs a filtering of the images, completely black or too dark images are removed before image analysis. A verification of there being any images in destination folder is performed to discontinue image analysis.
- AreaCalc.m takes a given image, thresholds it and performs an area calculation. The number returned is a scalar of the amount of pixels in the image that is "on".
- iCrop.m was developed in order to set which part of the corals to analyze. Figure 3-1 shows how the program cut out parts of the original image to do several analyses per image. Every rectangle will represent its own dataset to be evaluated and analyzed and resulting in its own independent biosensor. The script is also used to visualize parts of the image that is analyzed as seen in Figure 3-1 below.



Figure 3-1. The image analysis is performed on the part of the image that is inside each rectangle. In short, every rectangle will represent a single individual sensor contributing to data for the whole biosensor arrangement. The large red rectangle represents the entire Coral referred to as Coral 1. The smaller rectangles represent the different polyps on the corals. I.e. CIP1 abbreviates Polyp 1 on Coral 1.

Thresholding the image was best solved by use of Otsu's method (Otsu, 1979). This function will evaluate each and every image before threshold, resulting in consecutively better thresholding result. In addition altering the contrast before thresholding was tested but left out due to increasing variations of area calculations. The thresholding method has a built in function in Matlab and further evaluations of similar methods were not evaluated.

The actual calculation of the pixel was performed by using an image property function in Matlab called `bwarea()`. The function returns a scalar with a value corresponding to the amount of pixels contained in the image. Pixel area is determined by looking at its 2-by-2 neighborhood, giving a different area to pixels to that are "on" with different weighing for different patterns (Pratt, 1991).

For detailed understanding and to further look into the software scripts and programs, see Appendix A where the entire program is attached. The program could be copied into Matlab and run as it is, though folder and file path directories needs to be edited into the correct destinations to allow for the software to find images to analyze. In script, comments are added to ease the overall understanding of the software and functions. Also changes made during two mile stones of experiment 1 and experiment 2 which is described later in the thesis, can be found in the software to be able to repeat the experiment that was performed

3.3 Transducer

The Sensors tested for the biosensor setup were two types of images capturing devices. Two web cameras of same type and one subsea web camera. Effort was put in to acquire an image capturing device which could readily be transferrable to an *in-situ* situation on a subsea floor.

3.3.1 Web Camera

Two identical web cameras of type Logitech HD C525 (Logitech, 2014) were used. The webcam's are simple 720p cheap over the counter plug and play devices. Still images can be

recorded up to 8 megapixels. With the chosen web camera software Yawcam by Lundvall (2013), both cameras were controlled and set to sample an image every minute. Default image size obtained by Yawcam software was a 640 x 480 pixel width x height resolution image.

3.3.2 Subsea Camera

Biota Guard AS assisted in borrowing a subsea video camera from MacArtney A/S, a global supplier of underwater technology. The camera model C400 manufactured by Remote Oceans Systems is a low light monochrome camera for underwater inspections. The camera has a depth rating of up to 3000 meters, is suitable for very low light conditions, has a water corrected lens and delivers a 650 Television line resolution (TVL). TVL is commonly used for describing resolutions on analogue surveillance video and 650 TVL roughly correlates to an 811 x 508 pixel resolution (Macartney, 2014, ROS, 2011).

The camera was connected with a coaxial cable to a Digital Video Recorder (DVR), DVRS-S08H. The software for the DVR made it possible to connect and format a Hard Disk Drive (HDD) of 150GB. The size of the HDD made it possible to record up to six days of monochrome video before a backup had to be made. When surpassing the six day recording limit the software would loop and record over the oldest video archives.

Every six days the captured video had to be copied onto a separate HDD and then transferred to a PC for image analysis. The biosensor software developed was only programmed to utilize still images, therefore the video files would need further processing. Every minute an image from the video was sampled to be used in the image analysis software. The DVR stored the video files in an Indeo Video Format (.ifv). This file type made it complicated to integrate automatic editing in Matlab. A separate software FFmpeg (Zerane ffmpeg, 2013), a command line tool with Windows build that converts multimedia files between formats, was used to convert the video file to an Audio video interleave (.avi) file extension. Whereas Image Grabber (ERGUN, 2006) could then be utilized to produce the time lapse image sampling. Subsea camera video feed was sampled equal to the images supplied from the web cameras, only with a different angle, superior image quality and light sensitivity.

3.4 Experimental setup

Four separate white corals of the *L. pertusa* CWC species was fitted in rubber mounting pods and transferred in submerged containers over to an aquarium tank filled with seawater from the IRIS Mekjarvik sea water supply. The glass aquarium tank was used to maintain the coral branches and expose them to DC particles in laboratory flow-through conditions as adequate as possible for CWC. Flow conditions and feeding with freshly hatched (1 day) brine shrimp *Artemia salina* nauplii were provided during the entire course of the experiment. Seawater was pumped from 75 meters depth in the Byfjord next to the IRIS Mekjarvik facility. The seawater was sand-filtered and temperature regulated to 7.5 ± 0.5 degrees Celsius before reaching the aquarium. The aquarium was located in a temperature-regulated room set at 8°C. The aquarium used was of size 75 cm x 25 cm x 30cm (length × width × height) see Figure 3-2, containing about 53 liters of water.

Figure 3-3 shows the coral tank setup and positioning of camera sensors inside and outside of the aquarium. The two web cameras were connected to a PC via USB cable while the subsea camera is connected with a burton cable 5500 series, to a power supply and coaxial cable to a DVR recorder. See Cooper Interconnect (2012) for a guide on burton cable system.

The side mounted web camera images were used for individual polyp and whole coral analysis, while the top mounted web camera was used for the monitoring of sedimentation of particles onto the corals.

The lighting was supplied by two overhead light sources. One Cotech LED Work Light with 60 powerful LEDs and one Cotech Fluorescent Work Light. Both were connected to 230 Volt power supply with a mechanical timer set to an interval of 15 minutes on and 15 minutes off. The on and off sequence was chosen as it was the highest frequency made possible with inexpensive mechanical switches. The light could not be left on during the whole experiment due to the risk of fouling on corals. The corals are adapted to a life in complete darkness and are believed to not be physically affected or stressed by a light source (Baussant).

Matlab was run from a stationary PC connected to two USB web cameras with a 500GB internal HDD storage space. A connection through Team Viewer and Dropbox was intended to assimilate the *on-line in-situ* biosensor capabilities the system could give, communications to the system and its given parameters live from any suitable location with internet access.

In this setup, we used 4 coral nubbins with a number of polyps between 7 and 12. These were originally collected from 4 larger coral individuals collected by divers in the Tautrafjord (Trondheim) in May 2013. At IRIS, these corals were maintained in flow-through conditions and fed regularly. They were used as reference corals for an experiment performed by the staff of IRIS in 2013 and minimal handling or stress was applied towards these corals. Their polyps were active and extended during their maintenance at IRIS, a sign of well-being. In the tank, these nubbins were aligned diagonally in order to allow full overview of all corals and as many polyps silhouettes as possible with black background from both web camera and subsea camera

3.4.1 Exposure system

The exposure system was designed to assimilate the spreading of DC particles that might be experienced from offshore drilling activity. The corals were exposed to DC collected from a field in the North Sea (kindly supplied by Statoil). DC stock samples were collected 30th of August 2012 and transported to IRIS Mekjarvik 31th of August 2012 where it has been stored in a room at 4 °C. DC was supplied to the main flow through chamber via a 520S Watson Marlow pump set to 4ml/min. A capillary rubber hose was used to lead the cuttings into the chamber with the output just in front of the seawater inlet nozzle for best spreading of the cuttings. An optimal drill cutting mixture was prepared in two stages. For the first experiment a DC portion of 200 grams was added into 1 liter of sea water and mixed overnight on a shaker. The following day the mixture was diluted into a mixture tank with 20 liters of seawater and constantly stirred mechanically to keep the particles in suspension. For the setup an IKA Eurostar power control-visc stirrer was used and set to 365 rpm. Same procedure was done for the second experiment but with different concentrations, see Table 3-1.

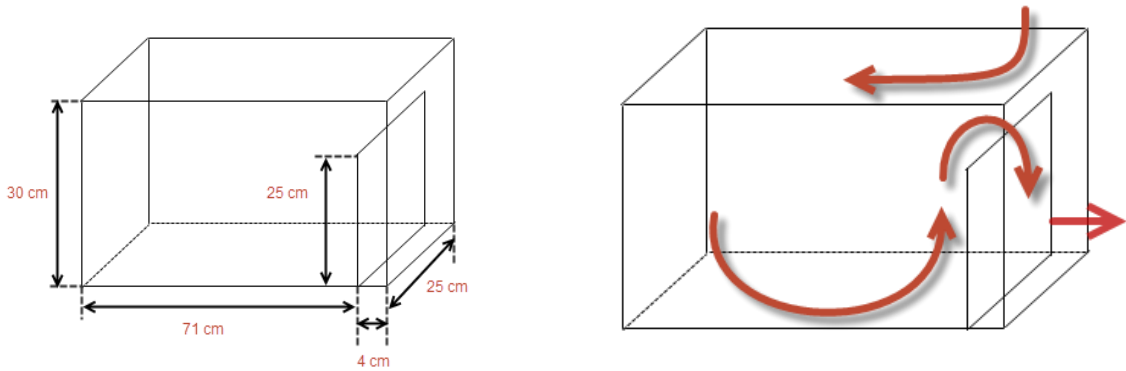


Figure 3-2 The illustration above shows the dimensions and the flow path in the aquarium.

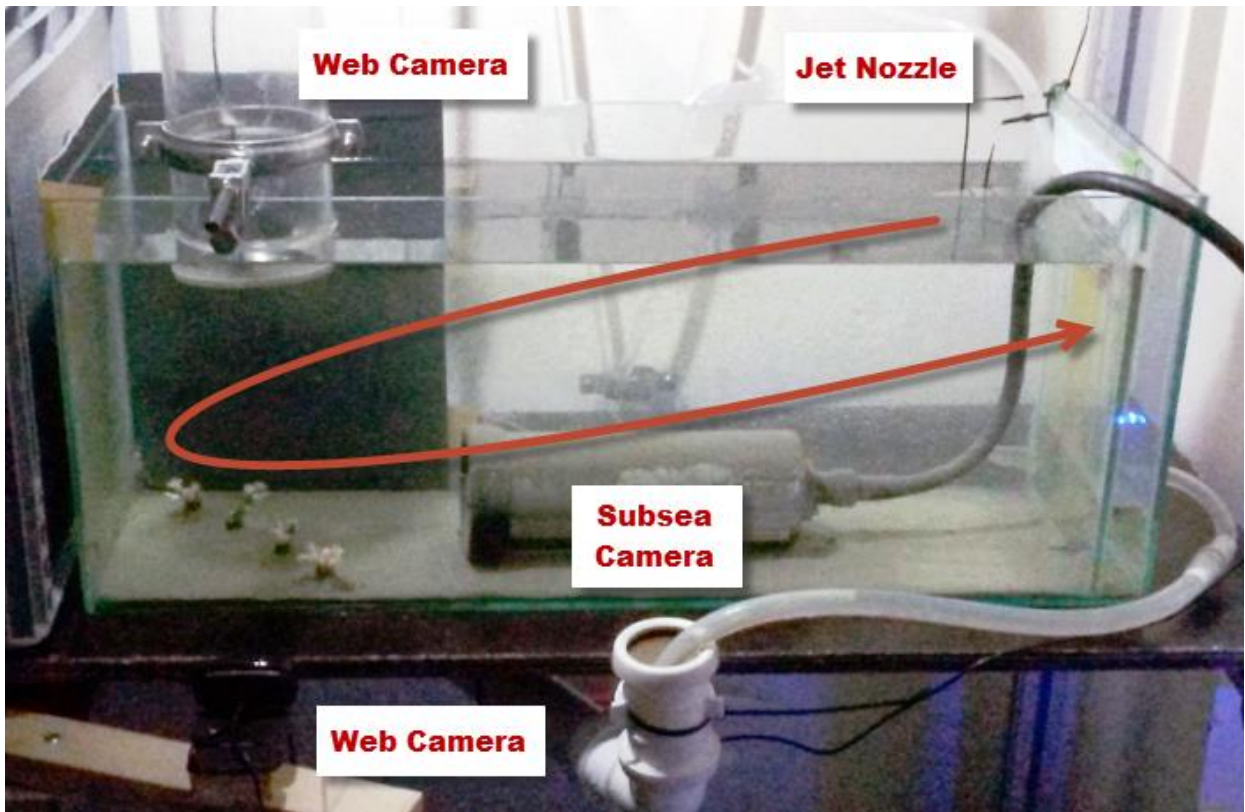


Figure 3-3. A current is induced by jet nozzle in top right. Flowing at approximate 500mL/min. the flow path is indicated by the red arrow. Three different cameras were used to capture images of the corals, two web cameras and a subsea black and white navigation camera from Macartney. The top web camera is looking through a periscope submerged in the water, while the outside mounted camera is looking from the outside of the aquarium.

3.5 Procedure

The procedure was designed in order to both assess the functionality and the degree of response that the biosensor could give. Due to the high degree of experimentation and the factor of unknown end result, exposure periods vs. no exposure periods were chosen as a best qualified time available and from reasonability.

3.5.1 Biosensor assessment and exposure

Two separate experiments were performed. The first experiment was a test done in order to assess that both the setup and image analysis were able to get a feasible response from exposing the corals to DC. The second experiment, if the first was a success, was to expose the corals for different concentrations of the DC. The same tank with all corals was exposed by DC with stepwise increased concentration. The entire experiment consisted of 3 periods with 4 days of exposure and 3 days of no exposure each. The stepwise exposures started with the use of 66.7 grams of dry weight DC, second stage with 133.3 grams of DC and last stage of exposure with 200 grams DC, see Table 3-1. Water flow was maintained constant during the entire experiment, mud flow was kept equal for all exposure periods in both experiments.

During the experiment the biosensor ran continuously. Recording images from web cameras every minute and constant video feed from subsea camera. The biosensor software ran on the PC and would every 5 minutes initiate the analysis of all images that had been captured since last software run. The result was stored locally in .csv files, (comma separated value), where a new file was created for every day of the experiment.

Table 3-1 Overview of exposure periods and amount of cuttings the corals were suspected to. *Target concentrations are not confirmed and only a qualified estimate due to many uncertainties regarding the exposure system. One large factor is DC sedimenting out of suspension before leaving the DC mixture tank.

	Date	Target Concentration ppm	Flow water mL/min	Flow mud L/day	DC mg/L	DC g/day	DC total g in 21 Liters
Experiment 1	27.03-01.04	0	500	0	0	0	0
	01.04-06.04	50	500	5.76	9523.81	54.86	200
	06.04-22.04	0	500	0	0	0	0
Experiment 2	22.04-25.04	15*	500	5.76	3174.76	18.29	66.7
	25.04-29.04	0	500	0	0	0	0
	29.04-02.05	30*	500	5.76	6347.62	36.56	133.3
	02.05-06.05	0	500	0	0	0	0
	06.05-09.05	50*	500	5.76	9523.81	54.86	200.0
	09.05-12.05	0	500	0	0	0	0

4 Results and discussions

The study lasted from 26th of March to 12th of May representing a total of 48 days. For each day 1440 images were recorded, half when the light was off, giving an average total of 720 area calculation datasets per day. A total of 34560 images were analyzed with 15 pixel area calculations performed per image during experiment 1 and 20 per image for experiment 2. Each individual pixel area calculation performed per image is sought to be a biosensor indicator, experiment 2 totaled 4 indicators incorporating a coral nubbin and its polyps and 16 individual polyp indicators.

Every 6 days the DVR HDD had to be copied and the pc would be restarted in order to connect and disconnect the HDD. During these offline periods less images has been recorded.

Following errors resulting in unpredictable data losses were experienced:

- Programming flaws.
- Computer crashes and unscheduled restarts.
- Random overwriting of images, user errors.

Initial startup problems and programming errors were dealt with as the experiment was running but resulted in non-salvageable image losses. Data is presented without further emphasis on days with errors and data loss. Mainly initial and startup days were affected by these errors. Records for the first 3 days are removed from data results due to changes in environment background, completely altering the data. Images are non-comparable and will not be evaluated or presented as part of the experiment.

This section will present and discuss how the thesis project was executed according to the plan and goals. Important observations and personal remarks are mentioning and reviewed in an attempt to provide a critical assessment of the work performed. The main attention is weighted towards the overall aim of an operative biosensor for coral health monitoring

4.1 Methodological issues

A short description on how the flow of the thesis was from start to finish. Chapter is divided into the different sub objective of the project described in the introduction.

At the onset of the thesis work, a number of issues were not clearly solved. For example, the organism chosen for the biological part of the biosensor was the marine blue mussel *Mytilus edulis* and the transducer part was built on radar based technology. As with this thesis both projects were sprung from brainstorming sessions together with Biota Guard AS. Following discussions regarding the use of the reef building stony coral *Lophelia pertusa*, a kick off meeting was held in September 2013 and preliminary milestones for the different sub goals were established.

4.1.1 Software development

Below the differences that were recorded in the corals is presented. Figure 4-1 is showing the polyp activity changes that is captured by the web cameras.

For each image and rectangle that the software cuts out and processes the resulting threshold image may vary as shown in Figure 4-2. This method creates temporary image matrices from an original image in order to produce data. The cut out images before and after threshold are discarded and not saved, but analyzed image is renamed and stored in designated folder.

Subsequent change in image for the different rectangles were recorded in .csv files. Corresponding to the images shown in Figure 4-2 for C2P4, the software counted the amount of pixels that were on (white). For the presented images the value is 90 for the top thresholded image and 223 for the bottom image. Before averaging maximum value during experiment 1 was showing a peak at 376 and a bottom at 17. Smoothed values with an average done for every 15 processed images shows a maximum value of 261.9 and a minimum of 64.8. Introducing a moving average on top of these data gives a lowered maximum to 250.2 and an increased minimum to 78.5 for the same polyp.

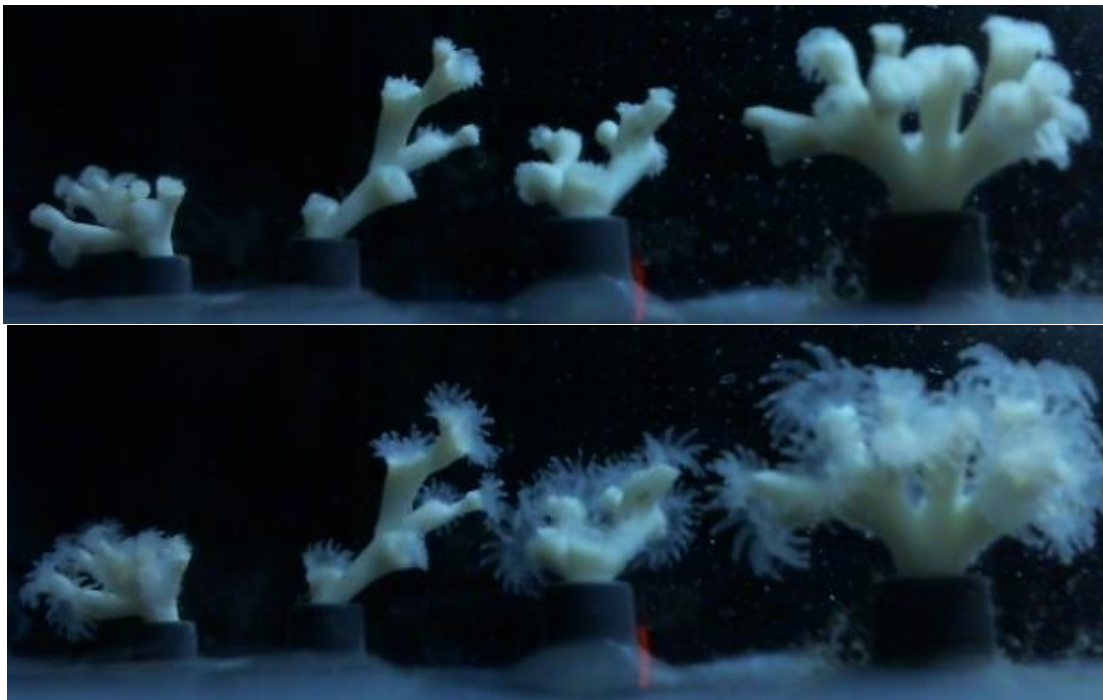


Figure 4-1. The images above are recordings of the corals with 24 hours difference. From almost completely contracted polyps to apparently fully extended. The images are from the 29th of March, just before corals are exposed to DC on day 3.



Figure 4-2. The two top images are showing Coral 2, Polyp 4 as seen in Figure 4-7. The cut out image that's being analyzed has a dimension of 20 x 19 pixels. Top left image is showing the image inside red rectangle before threshold. Top right is the same image after threshold. Bottom left is also showing Coral 2 polyp 4 and its thresholded image. The images are showing the results from the software analysis performed on the exact images seen in Figure 4-1.

Programming of the software through Matlab was completed with satisfactory results. The program was able to perform real time analysis of images taken by the independent image capturing software and generate .csv files for storing the data. For an *in-situ* sensor, a good solution could be to add a compressing feature for the stored data and send packages of information at timed intervals. The ability to easily process the information in a microcontroller where the image is taken would reduce the amount of data to send and reduce possible power consumption issues related to image data processing. One should note that power limitations and low bandwidth capacities are becoming less of an obstacle due to the development of shore-powered, cabled observatories where two way communications are a possibility (Paul et al., 2007).

During experiment several modifications to the software was made, and many unknown programming errors were discovered. Even after experiment, there was still an unknown issue with the software, forcing it to stop randomly after running problem free for days. Alterations of the data series from each image should be done so that date- and timestamps were stored. This would make it easier when evaluating interesting data, to go back and compare results with the actual images from that period while studying data that look wrong.

Another error that was discovered and could alter the end results are the removal of over exposed images. This is due to the light being turned on in the whole coral room due to feeding, routine inspections and other random intrusions. Figure 4-3 shows an example of how images are returned when light was left on in the coral room and Figure 4-4 is showing how the actual image analysis error looked like in some instances. The pixel area count is a relative number and hard to compare up against the different sensors. Looking at the number

for the same sensor, one can see that the value almost doubles and the images are taken with only a minute difference. There could exist an easy way to insert a condition on the program, somewhat similar to the method to remove images taken when the lights were off, i.e. completely black images giving close to zero output values. Looking at the results many of these peaks of errors will anyway be mitigated due to the smoothing of data done and reviewed in this thesis. The biosensor should be able to provide data to provide rapid indication of coral status changes enabling to take rapid counteractions by operators e.g. when DC plumes is drifting to close to an area with corals.

During the complete experiment two separate light sources were used to give local lighting above the corals for the 15 min on/off intervals. These two light sources were controlled by independent mechanical switches where the on/off periods were set manually using a non-digital timer. Due to this system it was hard to get the two light sources to synchronize the periods of on/off lights and transition phases where only one of the lights were on at a time occurred. Images recorded from this phase can not only give different data compared to a normally lighted image, it will also count as extra images in addition to the averaged 720 images per day. Ultimately, this contributed to create an apparent time shift in the resulting graphs.

An issue during the analysis of the results was the way the software was developed regards the creation of a new folder to save all the images and produced data for each new day. Initially this was believed to be a good solution to keep track of where the data were saved. This was partly tied up to the fact that the images were recorded using a separate software. The software did not keep track of the time that passes by checking a world clock or other time function. This means that it is expected approximately 720 images for every day, if there are fewer images one day due to for example system restarts, power shortages or similar, this will create an apparent lag when analyzing the resulting data..



Figure 4-3. Web camera time lapse image showing reflection from aquarium glass wall created due to the light left on in the coral room at IRIS lab.

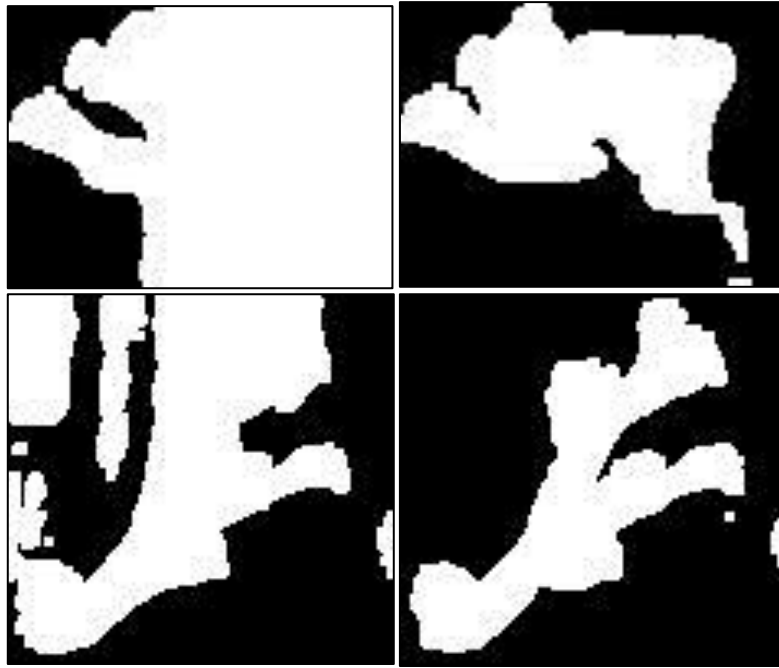


Figure 4-4. Top left image is showing a threshold image of coral 1 where the reflection in aquarium wall clearly has altered the end result. The right image is the threshold image of coral 1 from an original image without reflections. When counting the pixel images the relative pixel area value for this dataset will be a lot higher than for the image on the top right. The bottom two images are showing the same error being processed by the software for coral 2. The actual pixel area values from software was calculated to be 5307, 2833, 6511 and 3912 respectively from top left to bottom right.

4.1.2 Subsea camera

At first a subsea image capturing device from Imenco of the model Silvertip Shark Range was borrowed, (Imenco, 2013). The camera fulfilled all criteria's in terms of being suitable as an *in-situ* device and much effort was put in making it possible to communicate with the camera via Matlab, command line tools and associated software. The camera had both RS-232, network connection possibilities and a manual remote control that would allow access and image control. Eventually even after assistance from several different sources and expertise the solutions had to be abandoned and the transducer unit needed to be completely re-evaluated due to both lack of time and the complexity of using a subsea camera

The main goal was to develop a biosensor as close as possible to as an *in-situ* sensor and a subsea camera from Macartney was borrowed according to the sub objective set. As mentioned the solutions was not entirely successful both in terms of being an "offline" solutions and due to the video sampling not functioning properly. Some interesting observations were however made. The light sensitivity of the subsea camera was great and the lighting conditions did not seem to be of an issue. The lens of the camera was dome shaped and it was observed that DC was stuck to half of the lens from top and down leading to a slight reduced clarity of half of the image, see Figure 4-5. Camera was facing the current which could affect the attachment of cuttings but this is an observation that should be addressed in a potential subsea biosensor array. Research into existing technologies to keep camera window clean, usage of water jets or keeping the camera inclined could be possible solutions.

The Navigator subsea camera managed to record video images for both experiments. Acquisition of software to convert the video into still images that could be utilized in the image analysis program was however not so successful. In the end the procedure was abandoned and data could not be generated as it became too time consuming to find or develop software capable of the conversion.



Figure 4-5. DC sticking to the subsea camera dome shaped protective lens.

A secondary plan developed alongside experimenting with the Silvertip camera was the usage of a standard web camera. The web camera acted as an easy and reliable image capturing device and should have been evaluated as a separate sub objective in order to start the design and testing of the biosensor software at an earlier stage. As hindsight more research upfront and detailed discussions with camera vendors in terms of connectivity or actual examples on communication protocols with the subsea camera should have been performed

4.1.1 Lab setup

The lab was set up to simulate as close as possible the *in-situ* environment of deep-sea corals. This was maybe the most difficult task to complete in a satisfactory way and many simplifications were made. Some of them were the lighting, water flow path, logging of temperature, water flow rate and DC concentration in the aquarium. The corals were also affected by a routine feeding schedule which could have an effect on how the corals could respond. All of the parameters mentioned above could in their way affect the end result and will have to be part of the evaluation when looking critically at the results.

The flow path created by the jet nozzle for the water inlet was not very well controlled e.g. with addition of a dye. Looking at the diagonal setup of the corals as seen in Figure 3-3 it is possible that some of the corals are more affected by the DC than others. Some are positioned in the direct current while others could be positioned in a more sheltered area of the aquarium. Consequently the polyps themselves on the coral may have different levels of stresses caused by the amount of DC affecting them. Some polyps could be facing towards the current and may be more exposed to cuttings during the exposure creating larger stresses for the

individual. While other polyps are facing away from the current and thus could possibly be less affected. The same could be with polyps in areas with less current or pockets with no currents, polyps facing upwards could be more affected by the effects of sedimenting DC than polyps facing sideways or more downward. This variability is however expected in an *in situ* situation. Hence, it is important to evaluate the position and current flow experienced by corals in the field for future *in situ* operation of the biosensor.

The particle concentration could have been measured with turbidity meters or manual sampling/filtration of water. Unfortunately portable meters were not available and stationary turbidity meters at the University were not available. Sampling during exposure periods could have helped to verify the DC concentration in the aquarium during the experiment, confirming the different levels of contamination they were exposed to.

4.1.2 Secondary sensor system

The last sub objective mentioned was the camera system to measure how corals could be affected by DC sedimentation or sediment coverage quantification of the coral. Sediment coverage or burial of a coral or some of the polyps have been observed with smothering of tissues (Larsson and Purser, 2011, Baussant, 2011 - 2014) and an early measurement would be of interest. The building of a submersible chamber was considered which ended up with the construction of a periscope with an additional web camera looking through the Plexiglas window. See Appendix D for a copy of the draft made for designing a model of a shallow water chamber. A simple periscope solution was constructed in the end due to the size of aquarium that was chosen for the lab setup and we abandoned the earlier designs.

During the entire study the second web camera were recording images at the same intervals as the side mounted web camera. Figure 4-6 shows a comparison of the changes that occurred from start to the finish. Software to analyze these images was developed using the same principal as for the polyp activity method. The result was promising at first but as DC started to sediment and cover the area around the coral, issues with the background changing due to sedimentation of DC at the bottom of the aquarium created growing challenges. This resulted in problem to distinguish coral from the background by the image analysis software. There was sedimentation on the coral and there is a great difference in the two presented images (Figure 4-6). Hence getting a calculated value between the two pictures should be possible. The method used for the polyp activity did not give a satisfactory solution.

There is a seemingly great difference in the two images presented and getting a calculated value between the two should be a possibility. It is evaluated that copying the method used for the polyp activity is not the best solution though with better lighting system or a different threshold method this could still be possible. A solution could be to make a new program able to generate a frame from the contour of the coral before addition of DC as visualized in the early image in Figure 4-6. Further, replacing the red rectangle window with the actual coral frame contour, a “shrinkage” of the initial coral area (dark spots) could reveal the sedimentation effect.

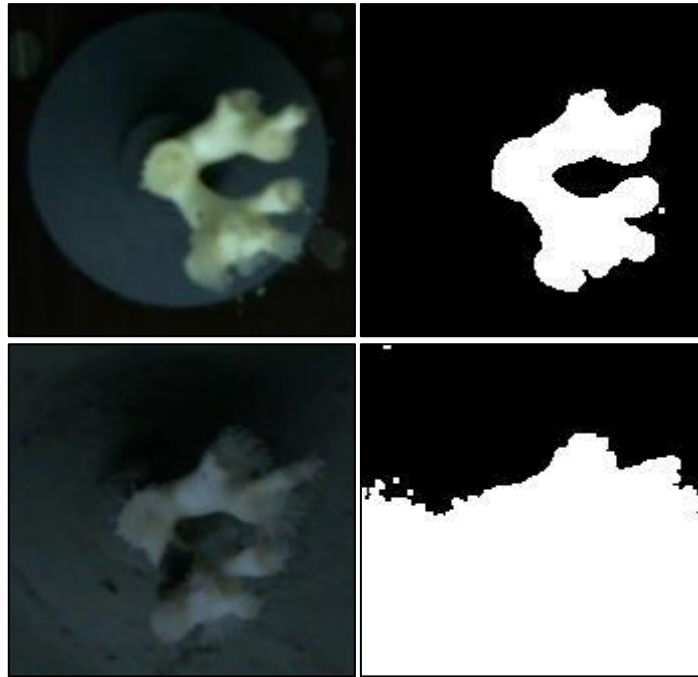


Figure 4-6. Top left image is from the startup of the experiment on the 28th of March. Bottom left image was taken the 12th of May. Thresholded versions of the images are shown to the right of the original images.

4.2 Exposure periods

The different exposure periods for the corals are shown in Table 4-1. The exposure periods are shown in number of days after the initial startup of the experiment on April 1st. During the last exposure period between 6th and 9th of May the following unwanted events occurred. Evening of the 06th of May the seawater pump was turned off resulting in no water flow. Sea water pump was turned on again 7th of May at 22:00. Sea water pump was turned off again the 8th of May for two hours from 16:00 to 18:00.

Table 4-1. Exposure period for both experiments of the study.

	Description	Date	Time	Day
Experiment 1	Exposure start	01.04	10:20	3.2
	Exposure stopped	07.04	08:37	8.3
Experiment 2	1/3 Exposure started	22.04	10:11	23.6
	1/3 Exposure stopped	25.04	14:00	27.2
	2/3 Exposure started	29.04	08:14	31.1
	2/3 Exposure stopped	02.05	09:27	34.1
	3/3 Exposure started	06.05	09:39	38.3
	3/3 Exposure stopped	09.05	16:30	41.6

The first stage experiment was initially planned without thoughts about performing a second experiment. As the initial results were showing indications of reduced activity and increased activity during exposure period, it was decided to try a new experiment to 1) confirm the results observed in the first experiment and 2) perform further tests with different

DC concentrations. The second stage experiment was made with an increasing gradient of DC concentration in an attempt to study the dose-response of the tested biosensor.

Other types of experiments that were evaluated were to increase the resolutions used for the image acquisition device. This could be done fairly easy with Booru Webcam, a different image capturing software that was tested early in the project, (Lumai, 2006). One issue with the choice of doing this experiment would be that the data from the first experiment could be more difficult in comparing. Increasing the resolutions of the images taken would require a recalibration of the software module through the use of iCrop.m script. This in term would lead to a greater resolution on each image that was analyzed completely altering the data output with a greater value span. Still if a baseline of the coral behavior is established, a higher resolution is not a disadvantage and it could still have been used to compare the first results as the pixel area values in the y-axis are removed from the presented graphs in the results. A minor setback that also made this experiment less attractive was the increased demand on storage space, which already was an issue with the existing setup. Then again using a inbuilt image quality reduction function this could greatly reduce the storage requirements on the system without affecting the resolution of the images. Further research into the effects of quality reduction would need to be done to be able to describe this in greater detail. Also independent image programs or solutions through image processing functions in Matlab could give solutions or increased data storage efficiency for the system.

4.1 Experimental data

One of the main improvements that should have been done was the calibration or removal of unwanted data that would pass a given maximum or minimum pixel area limit. This in term could be implemented in the software, resulting in the capturing of a more accurate response. The maximum and minimum pixel area values for C2P4 first trial experiment were 376 and 17, and 262 and 65 after averaging. A way to get a more rapid response would be to find and calculate the polyp's minimum and maximum states and set the program to dismiss any values not fitting these limits. The results presented in the report was able to make a qualified conclusion on if the method will be a feasible way to monitor if the corals are stressed or affected in any way by the DC.

The polyp's indicators C2P1 and C2P4 were chosen as main part of discussion due to the seemingly clear responses seen in the indicator for polyp 1, and the not so evident results shown for polyp 4. Other indicators might as well have been chosen, or a systematical discussion of every single indicator could have been done, but that would have resulted in much tedious and unnecessary work

4.1.1 Experiment 1: Biosensor assessment phase

The complete software together with the experimental setup was to present evidence of a functioning principal. Figure 4-7 shows the polyps and the cut out parts of the images that were analyzed during this experiment.



Figure 4-7. The image shows the cut out of which polyps that was analyzed and presented in the software calibration used during the first experiment. The acronym C2P1 relates to Coral 2 Polyp 1 and so on.

For both graphs Figure 4-8 and Figure 4-9 on the vertical axis, positive value upwards relates to the polyp tentacles extending out from the polyp skeleton, and a negative value downwards movement relates to the retraction of the tentacles into the polyp.

The graphs in Figure 4-8 and Figure 4-9 are averaged from the original data, where the average of each 15 images from the same polyp are calculated and presented. 15 images represent a monitoring during a 30 minute period due to the sensor light being on in 15 minutes and off in 15 minutes. The inbuilt software will filter out the black images. Only selected data are presented. For a full overview of all polyps biosensor readouts as shown in Figure 4-7 see Appendix B.

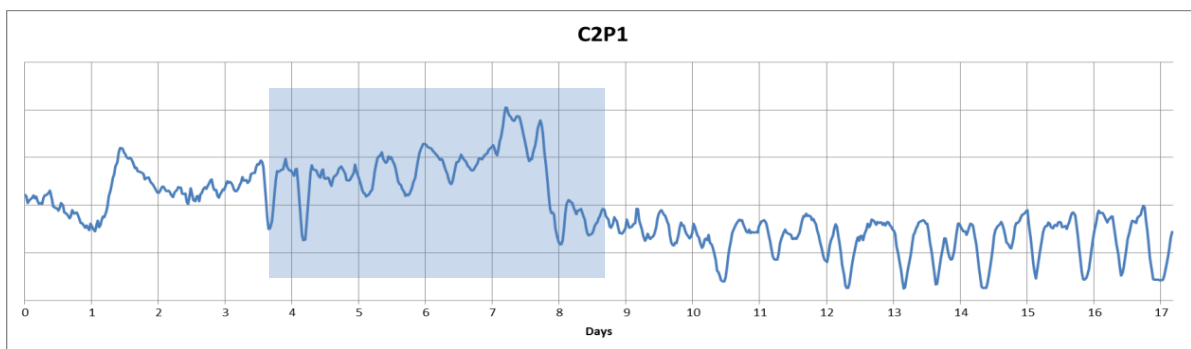


Figure 4-8. Coral 2 Polyp 1 relative change in pixel area. The exposure period for experiment 1 is highlighted in blue and the periods not marked in blue are periods with no exposure at all, only clean sea water. Horizontal axis is showing amount of days from the initial startup of the experiment, day 0 to day 17. The vertical axis is representing the polyp pixel area within the rectangle defining C2P1 as seen in Figure 4-7.

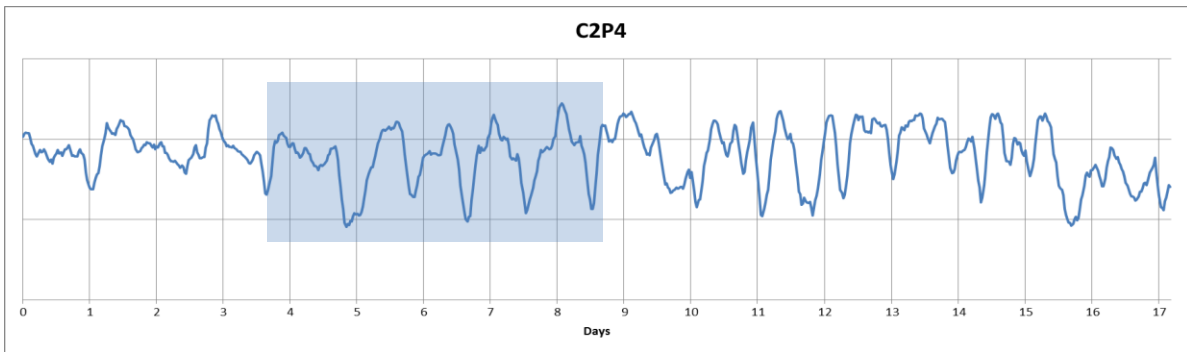


Figure 4-9. Coral 2 polyp 4 relative change in pixel area. The DC exposure period for experiment 1 is highlighted in blue and the periods not marked in blue are periods with no exposure at all, only clean sea water. Axis have same legend as figure 4.4, generated by the rectangle defining C2P4 in Figure 4-7.

The first stage was run in order to assess the biosensor functionality and test the lab and biosensor setup. All the graphs in Appendix B Stage 1 biosensor assessment phase were analyzed for this assessment. For the detailed discussion focus is put on polyp sensor C2P1 and C2P4 shown in Figure 4-8 and Figure 4-9. In the figure the exposure period is marked with a transparent square roughly between day 3 and day 8 see Table 4-1.

Studying this period for C2P1 it looks like there is a trend of increasing value from the start of the exposure period. And looking at the end of the exposure period the value drops rather drastically. The value seems to drop almost half a day before the exposure period is over, this could be easily explained if the exposure was stopped for some reason. The DC were mixed together in a bucket, totaling 21 liters, and the pump was set to distribute the cuttings at 4 ml/min equaling approximately 5.76 liters per day. The bucket was refilled with cuttings on day 4 even though it was supposed to be done on day 3 but as there was still enough cuttings left this was postponed 1 day. In the end of the exposure period there was still plenty of cuttings left. This could indicate that the flow rate was not maintained stable over the exposure period which could be reasonable as the DC is of a sticky substance and could easily be clogging or creating extra frictions internally in the rubber capillary tubes used for the exposure. A likely reason to the apparent too early drop in the coral activity was the loss of images or datasets due to the following documented event. On 02.04 at 03:11 the computer had a system restart stopping all images being recorded. The recording were not turned on again before 03.04 at 08:31 resulting in the loss of approximately 809 images using an average of 720 per day. As mentioned before the two independent light sources were constantly adding to the average of 720 images analyzed per day. Given an addition of 1 extra image per 30 min a total of 48 images per day or 384 additional images within day 8 of the experiment. Thus the seemingly early drop in coral activity could be explained by the lack in a proper time bound analysis of the images that were recorded.

Following day 8 after the rather drastic drop in activity, day 8 to 10 gradually decreased activity, move into a steady state condition throughout the entire first period experiment. A peculiar observation is that this steady state is remarkably lower than what it was before the exposure period. Peculiar is it that this steady state is remarkably lower than what it was before the exposure period. Though looking at the short period before exposure a steady state was not as clearly defined as it is after the exposure. There even seems to be a activity peak half ways out in day 2, a period with no exposure what so ever. Looking at all the polyps'

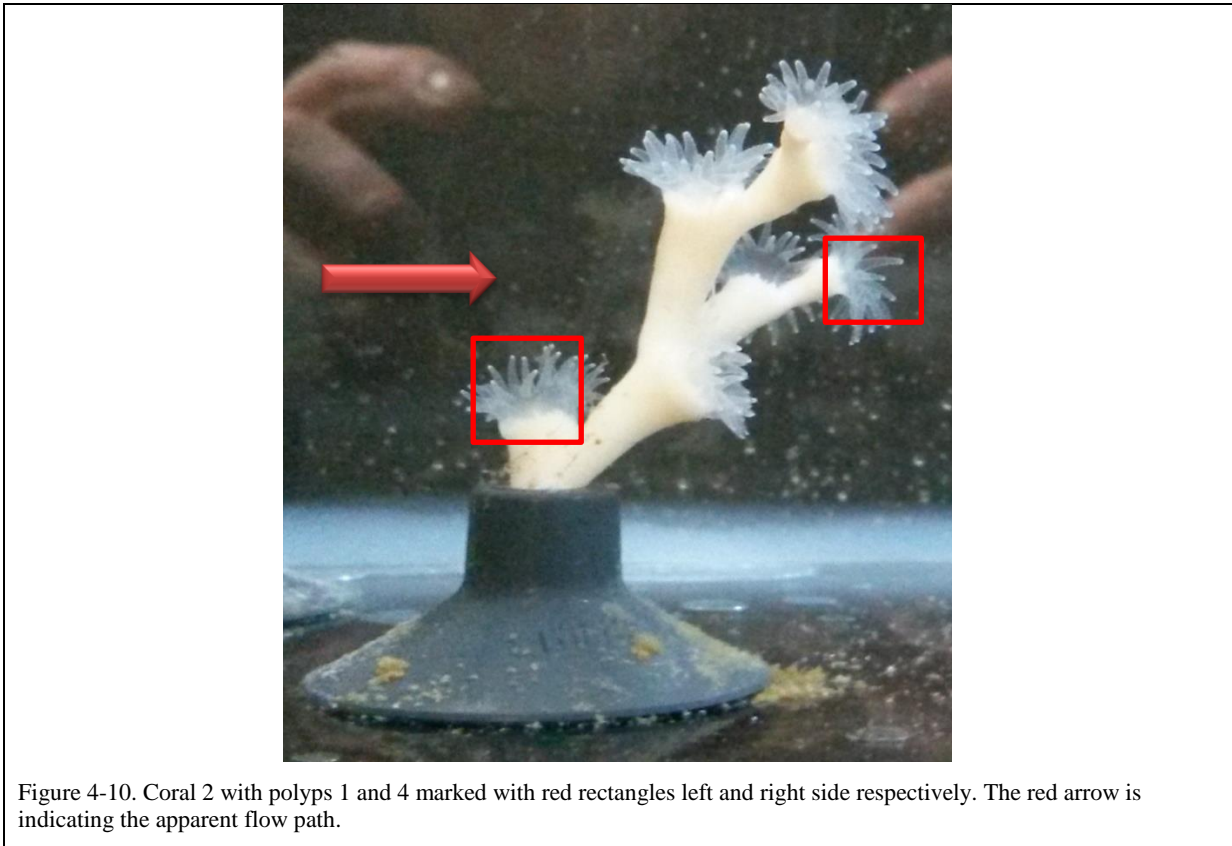
activity for coral 2 they seem to share this peak half ways in day 2. In coral 3 this peak seem to also be evident though in some polyps there is a valley instead, see C3P1, C3P4 and C3P5. A similar trend is found in polyp sensors from coral 4, with peaks or valleys a day and a half into the experiment. There are no obvious explanation to this apparently shared response so early but it is reasonable to say that there is something affecting all of the polyps at more or less the same time in terms of giving an increased or reduced activity. When starting the experiment a much longer period to document and record that the corals were in a steady “normalized” state should have been performed before initiating the exposure of DC. This could have helped determining the corals homeostasis situation i.e. normal diurnal resting and feeding routines before inflicting the external stressors.

Throughout the whole experiment there are both peaks and valleys representing the polyp’s background activity as their tentacles extend or retract. Looking at the different steady states after exposure for the polyps a pattern can be revealed. Reasonable explanations for these could be activity alterations due to external stimuli caused by feeding, or likely normal diurnal rhythm for the corals for feeding and resting. Water flow in was not monitored and daily varying flow could also be a cause to the observable responses on the coral. The same could be said for the temperature of the water or ambient temperature, possibly affecting the water and thus inflicting the activity of the corals, though this is rather unlikely due to temperatures between 4 and 12 degree Celsius are documented to be of normal conditions, (Dodds et al., 2007) and (Roberts et al., 2003).

Figure 4-9 is showing the gathered data C2P4. It is hard to see that there are any variations from the exposure. From day 5 there seems to be a more or less complete stabilization of the polyp’s diurnal activity. This apparent no response is also seen in C2P5 though this polyp can be said to have a slight decrease in activity from the start of the exposure period. Looking at the activity levels from before and after the exposure period for C2P4, it seems to be in the same area on average, though with deeper valleys which started appearing around day 5. A reasonable explanation to the variation of response to the exposure could be the positioning of the polyps according to both current and sedimentation effects as discussed earlier. In Figure 4-10 C2P1 and C2P4 are seen and given the flow path alone C2P4 can be said to be well sheltered as it is facing the completely opposite direction. DC particles being carried by the water flow are this way maybe less likely to get stuck in the polyps tentacles and not be forced into the polyp mouth as it would be if it was facing the opposite direction. C2P1 on the other hand is slightly angled towards the current direction, more so it is facing directly upwards. Sedimenting particles will in this polyp constantly be subsiding not only onto the tentacles, but into the polyp’s mouth. Also here C2P4 seems to be more sheltered than C2P1 as it is facing almost completely sideways, in addition there is a polyp close and straight above that could be shielding for additional particles sedimenting onto its tentacles. Also studying the positioning of the other polyps C2P2, C2P3 and C2P5 a similar relation can be seen for polyp 5, a weaker response but no apparent reduction in overall activity level, though it seems to have a lower peak to bottom variation.

The low amplitude variation could possibly be explained by looking at what is covered inside the red rectangle of C2P5 as seen in Figure 4-7. In the figure the polyp is closed and the fully closing and opening of the coral or the minimum and maximum of the amount of pixels

has a smaller span area than the others. C2P2 and C2P3 can seem to have a lowered averaging cycle following the exposure period than it had before the exposure period, though the first day of data does not support this trend.



Observing the lowered averaged activity that C2P1 is showing after the exposure period, Figure 4-8, and seeing that C2P2 is not showing this lowered average even though this polyp is facing pretty much in the same direction. One difference in the two polyps is the age of them. As polyp 2 is on the bottom makes it the oldest, and the polyps branching out from it are younger clones of it. A possible explanation to the different averaged states of the two polyps could be that the younger polyp manages to dispel DC flowing or sedimenting into its mouth. Though the older polyp cannot get rid of all the mud and is forced to live in a state of lowered activity due to all the cuttings impairing its movement.

4.1.2 Experiment 2: Biosensor exposure evaluation

Results from experiment 1 were evaluated followed by a complete calibration of the software. Figure 4-11 illustrates the new image analysis setup and data to be processed. Polyp and coral labels are the same but rectangles have different shapes and sizes compared to experiment 1. Additional polyps were included while others were randomly omitted. Also the analysis of the whole coral with all the polyps was included as illustrated by the large red rectangles.

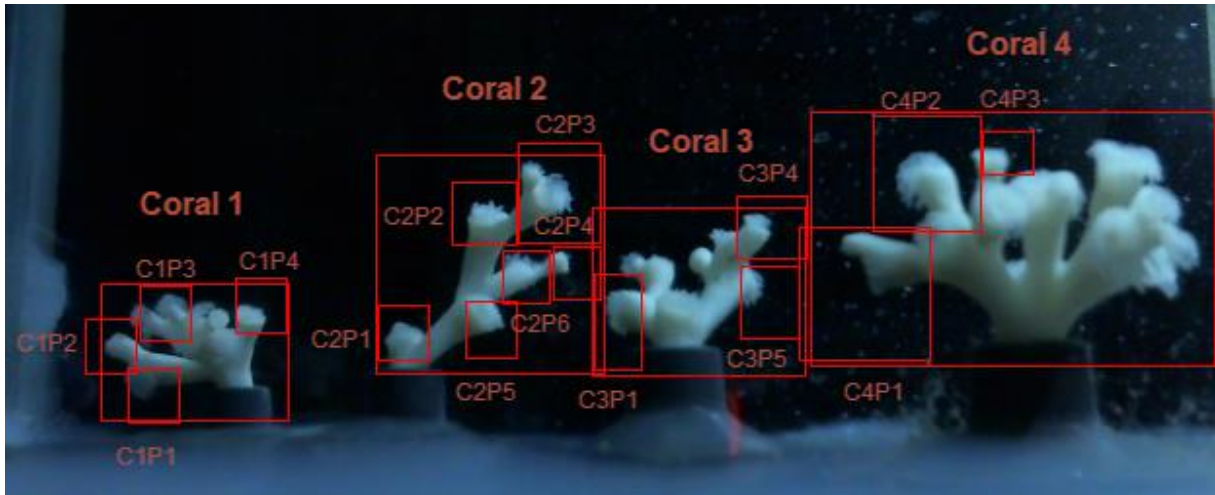


Figure 4-11. Experiment 2, biosensor image analysis setup. Each rectangle corresponds to a set point generating data representing a potential biosensor indicator.

For both graphs Figure 4-12 and Figure 4-13 on the vertical axis, positive value upwards relates to the polyp tentacles extending out from the polyp skeleton, and a negative downwards movement relates to the retraction of the tentacles into the polyp.

Due to recalibrations of the image analysis software for experiment 2 the biosensor software was run manually and all the images from experiment 1 was processed again to generate data for verification purposes. Figure 4-12 and Figure 4-13 shows both experiments from start finish with the new chosen rectangles sets. The remaining coral and polyp graphs are shown in Appendix B, both with experiment 1 only and with all 44 days of the experiment in one graph.

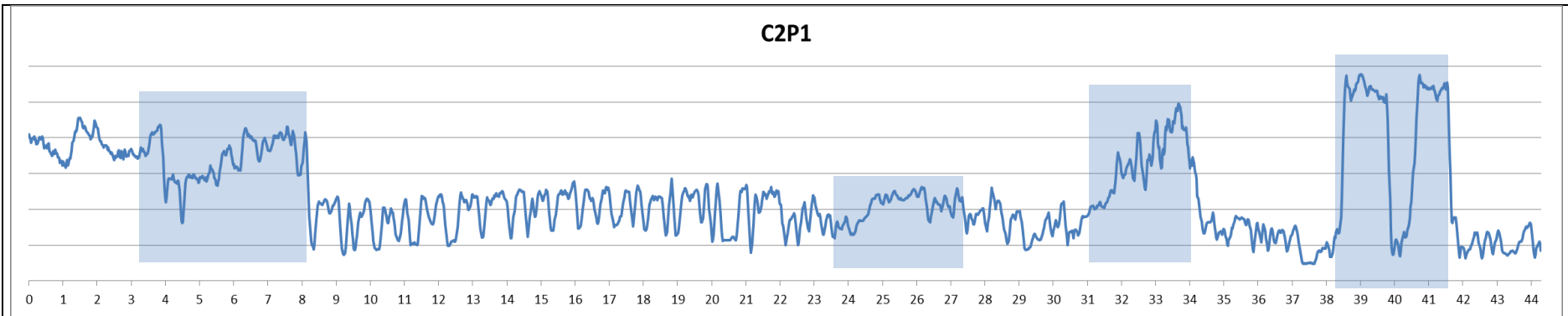


Figure 4-12. Coral 2 polyp 1 pixel area variations. Showing experiment 1 exposure in first blue rectangle from the left, followed by experiment 2 with the 3 periods of increasing concentrations highlighted consecutively. Non highlighted areas are periods without any exposure, only clean sea water. Horizontal axis is showing amount of days representing the whole study, day 0 to 44. The vertical axis is representing the polyp pixel area within the rectangle defining C2P1 as seen in

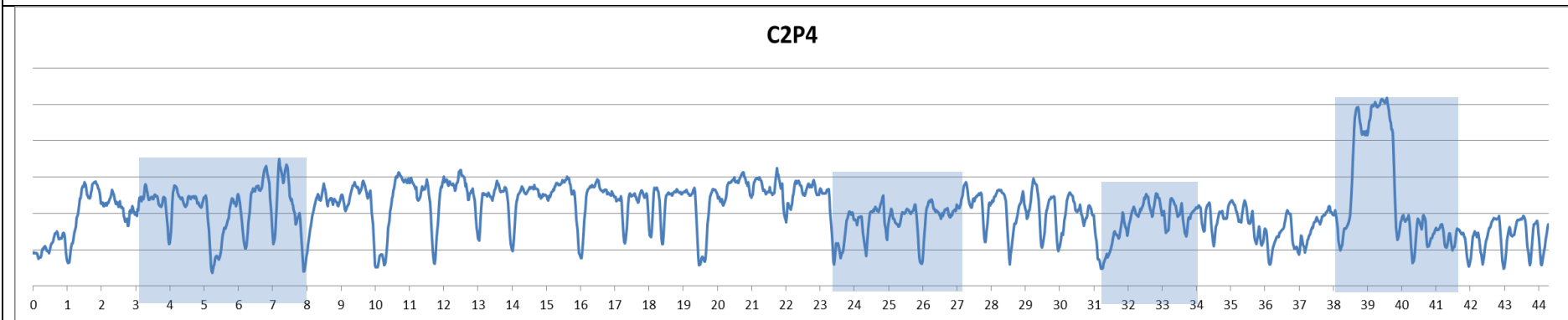


Figure 4-13. Coral 2 polyp 4 pixel area variations. Showing experiment 1 exposure in first blue rectangle from the left, followed by experiment 2 with the 3 periods of increasing concentrations highlighted consecutively. Non highlighted areas are periods without any exposure, only clean sea water. Axis have same legend as Figure 4-12 generated by the rectangle defining C2P4 as seen in Figure 4-11.

Before assessing the results a quick review on time shift compensations are evaluated. As mentioned from experiment 1 biosensor assessment the computer had a restart and there was a period of overlapping with lighting due to the mechanical switches. Giving a total of approximate half a day delay on the graphs compared to exposure days. Continuing into the experiment the extra images gained due to the overlapping lighting would decrease little by little as time went by. Though this error was observed to be reduced more and more as it seemed that the fluorescent lighting source eventually would stop functioning properly. It was not noted when it started to fail, but assumed to be fading gradually over several days. Comparing images from the start of the experiment to the end are showing significant difference in the lighting conditions, see Figure 4-14. It is difficult to say if this has had any effect on the pixel area calculations due to the changing conditions for the corals over this period. It is also difficult to say how many extra images from overlapping light conditions there has been. In addition every five or six days when copying subsea HDD the image sensors were off, from between 30 min to an hour. Looking at the response curves for the different polyps and corals it is not clear that there is much delay present.

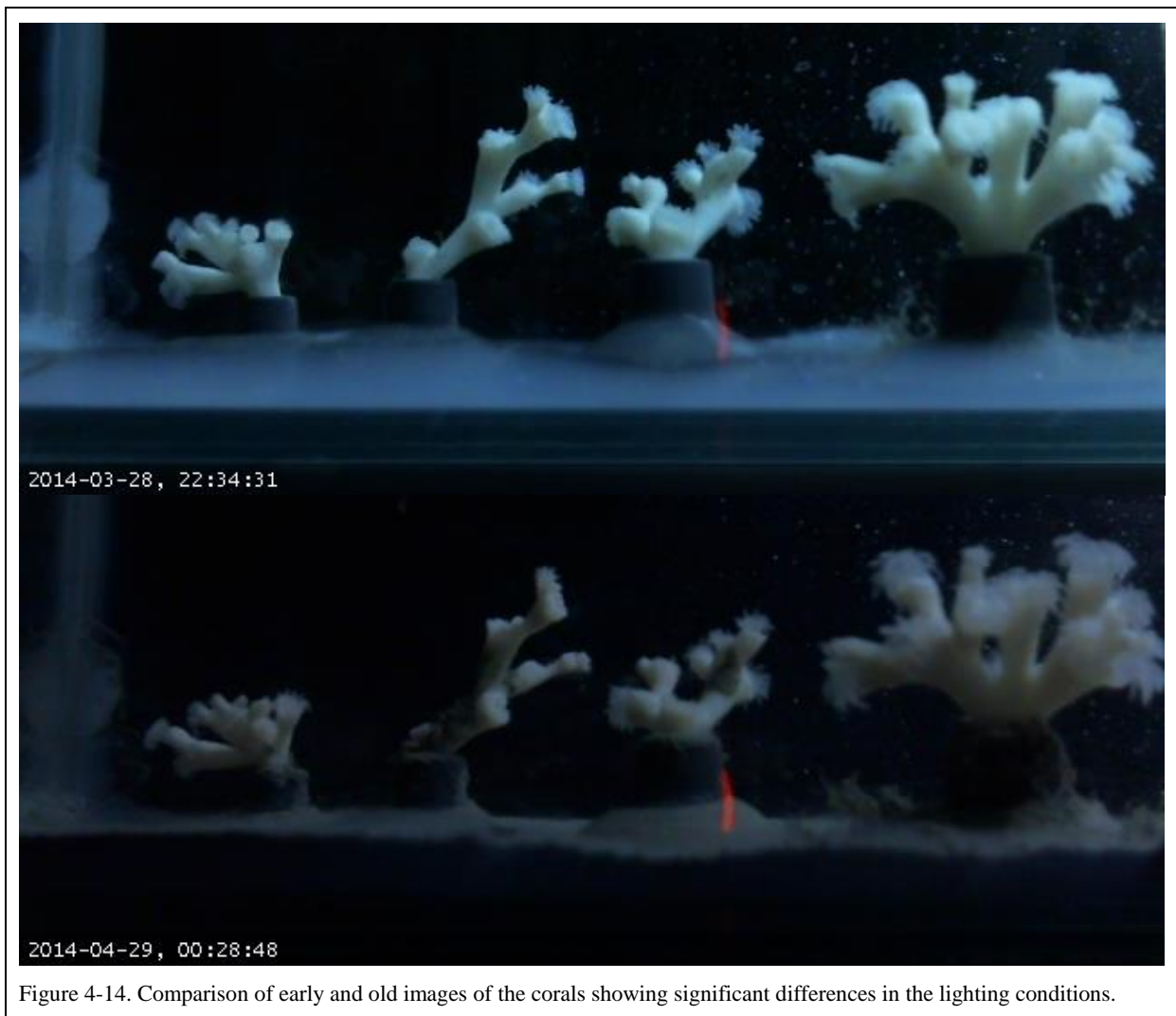


Figure 4-14. Comparison of early and old images of the corals showing significant differences in the lighting conditions.

The lighting conditions that was chosen for the lab was clearly not completely ideal, as its evident that the fluorescent lighting was not able to survive the interval of on and off every 15 minutes. This should maybe have been evaluated before start of the experiment, but a benefit

from doing this showed the advantage of having two independent lighting sources. Even though one of the light sources failed no data was lost, and apparently no significant changes in resulting data was observed. For a new experiment keeping two independent light sources may still be a good idea as long as the overlapping lighting is removed by for example using a mechanical switch with a two socket outlet. Also led lighting has shown to be a robust light source.

A second impediment caused by the failing light intensity over several days might be having an overall contributing effect to less pixels being thresholded to “on” pixels. Studying several of the graphs for the individual polyp and whole coral indicators there is a slight reduction in activity that can be seen during the off periods between the two experiments. This might also be due to other factors mentioned later in the report.

Comparing the new assigned rectangles for experiment 2 with experiment 1 looking at C2P1, Figure 4-12 and Figure 4-8 it is possible to pick up the same pattern and trend. More specifically the peak midways in day two, increase in activity during the exposure period and a drastic drop after exposure period is over which ends in a reduced averaged activity level. Scaling the two, the new rectangle from 2 is seen to give a slightly stronger or clearer impact from the exposure. Figure 4-15 is showing the two rectangles and the thresholded versions of the polyps, though it is not evident by looking at the images its possible for future testing that choosing a rectangle covering more of the area around as much of the polyp as possible is beneficial.



Figure 4-15. Detailed view of C2P1, comparing calibration of new rectangle setup from experiment 1 to experiment 2 image analysis software.



Figure 4-16. Comparing the thresholded images of C2P1 experiment 1 software calibrations on a image where the polyps tentacles are in and out of the polyp mouth two images on the left respectively. And thresholded images from Experiment 2

software calibrations showing tentacles protruding and contracted from the polyp mouth from the right respectively.

It is interesting to note that though there is a similar pattern in both experiment 1 and experiment 2 results the latter experiment seems to have shifted slightly in terms of time. This can easily be seen comparing the drastic activity drop in C2P1 near the exposure stop in day 8, which from experiment 1 occurs before day 8, Figure 4-8, while for experiment 2 this occurs just after, Figure 4-12. The same can be observed comparing Figure 4-9 and Figure 4-13, polyp C2P4 for experiment 1 and experiment 2 respectively. Data from experiment 1 seem to be more delayed than experiment 2, or in other words more images may have been analyzed in the analysis for experiment 2. As stated earlier this data was manually run through the software after calibrations of the biosensor software between the two experiments. It is possible a human error could have altered the amount of images that was treated here and thus causing this apparent change in timescale between the two experiments. A possible solution to this problem has already been partly mentioned and involves adding date- and time-stamps for each dataset, (image analyzed). If this was done for the two experiments it would have been possible to see where and if there was a difference in the two datasets. Also implementing a system of creating blank data or somehow accounting for lost images in periods of maintenance, system failures, human errors or similar could be beneficial when analyzing results as in this thesis.

Comparing the datasets for C2P4 from experiment 1, Figure 4-9 with data obtained in the new software setup, Figure 4-13 day 0 to 17. There is an indication from day 5 a slight increase in overall activity level for the new data shown in Figure 4-13. Studying the difference in the rectangles forming the baseline for the datasets, it is showing a great difference both in terms of size, positioning and coverage, see Figure 4-17 and Figure 4-18. Even though the polyp is facing opposite direction than the prevailing current and sideways which could be protecting it from sedimenting DC particles, experiment 2 software setup seems to be capturing some altered behavior. Even though it is not an immediate reaction from the exposure as other polyps seem to be showing.



Figure 4-17. Detailed view of C2P4, comparing calibration of new rectangle setup from experiment 1 to experiment 2 image analysis software.

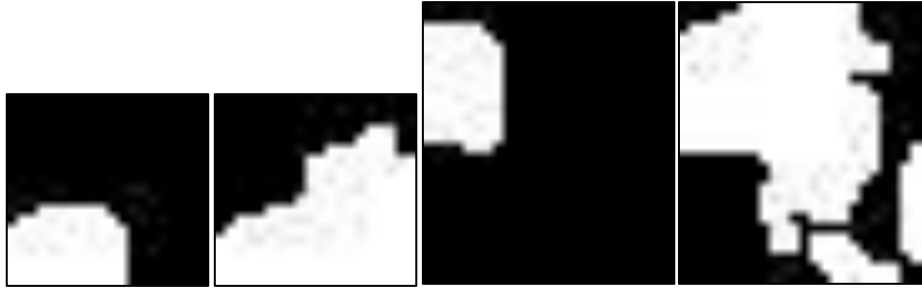


Figure 4-18. Comparing the thresholded images of C2P4 experiment 1 software calibrations on a image where the polyps tentacles are in and out of the polyp mouth from the right respectfully. And thresholded images from Experiment 2 software calibrations showing tentacles protruding and contracted from the polyp mouth from the right respectfully.

Finally, looking at stage 2 exposure periods, a gradient of increasing exposure concentrations was prepared and that should result in an increasing response from the three periods. Unfortunately, the last exposure period was disrupted by the water being turned off leading to uncontrolled levels of DC in the aquarium. Looking at the data for this last exposure period almost all polyps and coral indicators have extreme peaks. This is due to there being close to zero visibility in the water resulting in images almost completely covered in pixels that counted to the area calculation. To support this is the indicator C4P1 which does not have this peak, most likely due to it being positioned so close to the aquarium wall and being closest to the light source making it still possible for the image analysis software to generate a usable threshold.

Both C2P1 and C2P4 in Figure 4-12 and Figure 4-13 respectively are showing indications to responses from the first and second exposure periods in stage 2. C2P1 is showing a clear response with rising activity in both periods and an increased impact on the second exposure period. The C2P4 indicator is not showing an enlarged impact on the second period, but a clearer due to a dramatic drop in activity level before increasing and slightly leveling out before exposure period is over. The increased acidity between the two periods can have several explanations. This can be a natural response of increasing activity where the polyp is trying to push out particles sedimenting or forced into its mouth by the prevailing current. In other cases as seen in experiment 1 for C2P1 the initial response to the exposure seems to be a rapid drop in activity level which could be a way of protecting the polyp (retraction inside the skeleton) to mitigate the impact of DC.

A problem that can be observed in the bottom right threshold image of Figure 4-18 is the inclusion of neighboring polyp's tentacles in the pixel area calculations. It is difficult to say how this could affect the datasets, but it is reasonable to suggest that the data will be affected maybe hiding peaks that should be clearer and valleys showing to be more drastic or complete hiding of both if the opening and closing of the two polyps occur at opposite intervals. A solution to this would be to secure that neighboring corals are positioned away from the others and trying as best possible to have a selected rectangle for the image analysis that only covers the polyp wanted for the indicator. An obvious limiting factor here is the physical shape of the coral, this is an important factor to evaluate when designing an *in-situ* biosensor.

Four of the indicators that was calibrated into the software was the complete coral indicators for coral 1, 2, 3 and 4 and can be seen in Appendix B Experiment 2 Biosensor exposure evaluations. These indicators were chosen to be able to evaluate the difference in

looking at only one polyp at the time compared to the whole coral with all the individual polyps summed up in one data output. The graphs representing the different coral indicators does not stand out in any distinct way and show responses to the exposure periods in a similar manner as the polyp indicators. Though coral 4 indicator seems to be reacting with drastic reduction in activity levels over the whole experiment 1 exposure period, after returning quickly to apparent stable higher activity levels. Experiment 2 exposures show a clear activity drop in the start of the exposure phases with gradual increase of activity. One noticeable factor different from the polyp indicators are the data variations which seem to be more stable, with less variations throughout the entire experiment. This could be due to the actual amount of pixels relative pixels in the image that changes values over time. As the whole skeletal body of the coral is included in the image this will constantly count as part of the area while only the tentacles of the polyps are altering the area which relatively counts for less of the total area thus accounting for the smoother graphs presented. The apparent result looks cleaner but it is possible that the averaging between all the individual polyps can give a less sensitive biosensor than having just one polyp representing the entire dataset. Then again using only one polyp as a biosensor alone makes the sensor more susceptible for an individual's weaknesses like if it was sick or if it died.

4.2 Posterior biosensor assessment

In experiment 2 the software was set to do 20 different image analyses per recorded image. To get a better overview of which polyps or corals that indicated a response to the treatments an ordinal scale is shown in Figure 4-19. The scale is showing the distribution of the different biosensors, i.e. the rectangles shown in Figure 4-11 for software calibrations corresponding to experiment 2. The assessments are made from observing the graphs showing both experiments of the complete study as seen in Appendix B experiment 2 biosensor exposure evaluations. The biosensor indicator is measured on a 3 point ordinal scale with the sensor strength options being: Strong indication, weak indication, no indication. This is an empirical way to describe the data, based mainly on visual observations of the results. With more time, a more quantitative way could be done with a different type of scaling or use of statistical approaches.

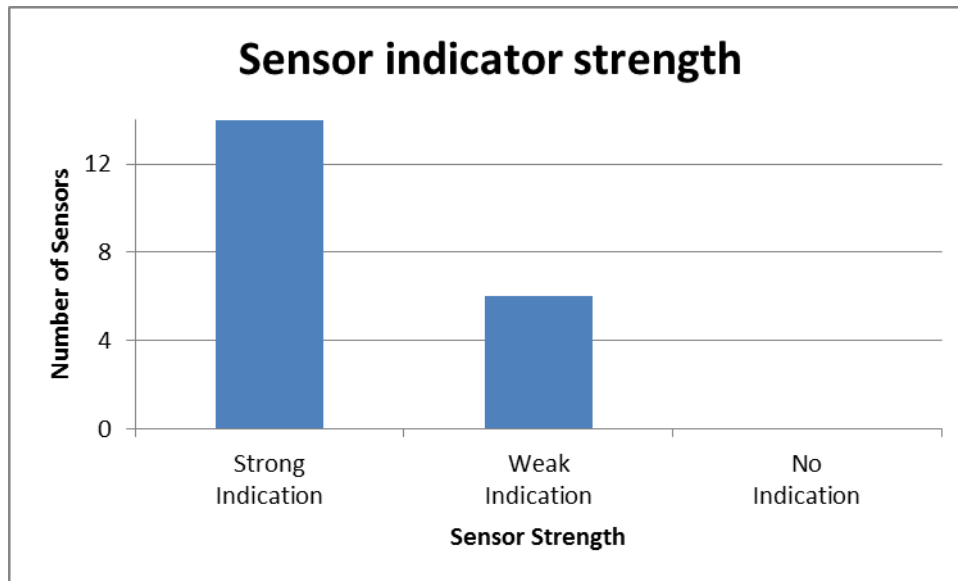


Figure 4-19. The ordinal scale above is showing if the different individual biosensors are showing a strong, weak and no indication of response to stresses, see Appendix C for data on sensor indicator strength choices.

The indicators from experiment 2 was scaled and evaluated in terms of strength. The prevailing judgment of this was that a strong indicator would show a response to the DC in at least two of the 3 exposure phases from experiment 1 and 2, where the last exposure phase was not part of the evaluation. A weak indicator showed a response in at least one of the phases. There exist uncertainties in these judgments but an overall view like shown Figure 4-19 points towards the biosensors being able to pick up on the coral responding to external stressors like DC.

For a clearer verification of the polyp's responses both control samples and replicates could have been included for the entire experiment. Practicalities led to the exclusion of purchasing an additional web camera and setting up separate aquarium systems where some of the four corals used during the experiment could be fitted.

Choosing to have only a few indicators on the first experiment and some more on the second experiment, and not choosing to have an indicator for every single polyp and coral that there were from the start was never a straight forward choice. Uncertainties of the principal actually functioning and the amount of data that would be generated, the fact that several polyps tentacles were overlapping each other or that the polyps did not protrude much outside of the coral. Choosing to ignore these issues could have given the indicator scaling more weight and made it more obvious that some polyp indicators would be preferred in front of others.

4.3 Reflection

Is the use of corals as biosensors a feasible solution? How adaptable is the organism to different water depths, is it at all possible to get a coral of *Lophelia pertusa* to acclimate in a new location with close to similar environmental properties. It is reported low tolerances to changes in water depths giving altered salinity and temperature conditions on the individual levels. Is it possible to implement the biosensor on a coral individual *in-situ* without having to remove it and manually attach it to a biosensor array before re-deploying it? Having to do a

subsea dive two times for implementation of any monitoring device could be a costly affair, possibly ultimately leading to the application of the biosensor never being realized outside of a controlled lab environment.

How much maintenance would be needed for the biosensor once it's deployed on the subsea floor, would it be possible to make it maintenance free for a longer period? Where does there exist a market for constant monitoring of coral reefs? How long would a monitoring period last, is field lifetime monitoring a viable option? The coral is a living organism, growing only a few millimeters per year, but increase in length would be a potential source of error or constant shifting of a data set point. Solutions to long term or apparent neglect able errors should be assessed. Like easy two way communications that would make it possible to calibrate the software real time and do equipment assessment from an onshore location.

5 Conclusions

Development of a real time biosensor capable of capturing images of coral nubbins and measuring the individual polyp activity was accomplished. The overall objective of the thesis was to develop an *in-situ* biosensor, which was not completed. From the start of the thesis the main objective was divided into several smaller objectives to be able to approach the overall aim in a most efficient way as possible

Several different image analysis methods were evaluated. The method of using pixel area calculations together with threshold of images was an easy and relative reliable approach. Other methods could still be used and is advised for future research.

Matlab for the programming environment was chosen on recommendations from Ivar Austvoll, professors at UiS. A large contributing factor was the development help found through Matlab's own expertise readily available as online user guidelines and explanatory forums on the Internet. The success of the thesis is seen to be positively influenced by the choice of this programming environment.

A software or principal approach was programmed and set together in Matlab. The system was able to manipulate images from the transducer and convert them to data representing individual polyp activity.

The main reason not reaching the overall objective of the thesis was due to not completing the sub objective of utilizing an image capturing device suitable for *in-situ* subsea environment to represent the transducer for the biosensor. A subsea camera was operated and run in parallel to the web camera based transducer but failure to connect and utilize images real time with the software was not conducted.

In situ environments are always challenging to mimic or copy in any indoors environment. The laboratory flow-through conditions that were used during the experiment were as adequate as possible to maintain viable conditions for the corals. The only way to get a real *in-situ* condition is to perform a pilot test and put the biosensor in their real environment. This will require extensive research and funding thus far beyond the scope of this thesis.

The last sub-objective for this project was added not to facilitate the overall main objective, but to further increase the usage of the biosensor. Adding a feature of sediment monitoring and being able to tell if the coral could be taking damage in the long run would be a valuable addition to the normal polyp activity monitoring. This sub objective was however not fully completed due to the failure of utilizing the same image analysis method to this principal.

It is strongly advised to continue research on reducing uncertainties and making the sensor more reliable. More real-time testing and mapping of individual polyps behavior is proposed. Issues and problems discussed on the interpretation of the gathered data should be further tested and evaluated. Mapping of different behavioral responses and the degree of effect different stresses can have on the corals should be evaluated and graded.

6 Future Work

More evaluation and research should be put on different image analysis methods. Area calculations seem to hold a great potential for the biosensor task. Testing of a method to analyze variation of the perimeter length around a cut out image of a polyp or the whole coral could be alternatives to test for studying the activity of corals and assessing end-points. Also a method that would utilize the coloring of the images is believed to have a potential in the creating data sets that would show the activity of a coral. A separate study for testing and comparing different methods on a series of time lapse images should be considered. To do this a background in programming and image analysis is strongly advised.

Removal of false signals from the image analysis could be done by mapping the fully extended polyp tentacles to the fully retracted for the different individual poly indicators. Creating a sensor array of several individual polyp indicators with this scale would possibly be an easier way for further utilization of the biosensor to a real in-situ environmental sensor.

Testing with subsea cameras is crucial to get images that will be obtained from a real in-situ biosensor. Ongoing effort is put in producing time lapse images from the subsea video files stored through the DVR recorder and the Navigator subsea camera borrowed from Macartney. Due to the system set up in the experiment being separated from the pc has led to an almost continuous video stream of the experiment. If successful to cut out and create an image for every minute of the video it would be possible to utilize the same image analysis software to produce data from this independent image source. This would make the comparison of the two data sets possible and therefore a good way to verify the image analysis module, enable a better verification of the overall objective of the thesis.

Research could be proposed to develop ways to prepare the individual polyp sensors more easily comparable.. It can be proposed to have the same size on all of the rectangles used in the image analysis software. Another principal could be to compare the polyp size with the diameter or length around or along the polyp calyx edge.

Covering or severely stressing the corals may smother or reduce the tissue covering the corals, i.e. the coenosarc. A method to analyze the physiological changes, reduction of the coenosarc or coverage of the coral by DC, should be evaluated. The secondary sensor system for sediment monitoring was an approach to reveal if the coral was getting covered by particles. A different method using same images as for the polyp indicator would be to utilize a method for analyzing the changes in surface color or texture analysis. When the white corals are covered by gray or dark particles it could be possible to capture either change in color or texture with similar image analysis software.

Further research could include developing a proper User interface, (UI), that would present the polyp state in real time. An UI needs to be both user friendly and give simple and straight forward results. This would in term require a biosensor image analysis software to be thoroughly tested. A start would be to have a way of presenting a live graph of the polyp activity, but in the end given set-points for different stress stages of a polyp would need to be mapped or an approach to creating an environmental performance index similar to Biota Guard's system.

By combining the data from several polyp monitors together by multi-resolutions analysis or other statistical analysis could be proposed as future research. A similar way that is already being used with the blue mussels could be incorporated to use with the data from the coral biosensor.

How would one make the system more robust? If the biosensor should be used as a real time *in-situ* environmental sensor subsea it should be robust and maintenance free. A considerable amount of research should be done in developing a way to protect the coral used as a sensor and the image-capturing device. With current software any alterations or shifts in the positioning of either the coral or the camera would lead to completely altering the data that is produced by the sensor. This could make it difficult to compare new data with historical datasets. At the start of the experiment it was experienced that a slight bump into the side mounted camera lead to a complete recalibrations of the image analysis software due to most of the rectangles used for pixel area calculations were shifted away from their respective corals.

7 References

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8 Appendix A

8.1 Biosensor Software – Matlab

8.1.1 BioLogger.m

```
% Script to initiate and start Biosensor analysis

%To stop timer function use stop(T);

% numberOfExcecutions = 3; % for testing;
limit the timer function to set number of periods. % Limit the timer
% set(T,'TasksToExecute', numberOfExcecutions);
to continue forever
date = '2013-04-25';
timerSeconds = 0.2; % Set timer
period. I.e. the time between every image analysis itteration. % Set =
timerDelay = timerSeconds;
timerSeconds when finished testing. Delay from start of timer first
iteration.
nrImages = 1; % Number of images
to be analyzed per analysis iteration. Keep to 1 during % actual
experiment, bug with blackimage removal function. % Iteration
i = 1;
counter for image file name. % Need to have a
way to check which images has already been analyzed % and to not skip
images not been analyzed yet. % i can not be 0
as sorting algorithm in AnalyseXImages % does not take
this. % Issue with
counter: If matlab stops or have to restarts i will start % counting on 0
again, ultimately leading to overwriting old photos. % Manual fix is to
check .csv file to see iteration it last stopped at and set i= that number.

%Initiation of timer sequence, setting of image analysis function for every
period.
T= timer;
set(T,'executionMode','fixedRate');
set(T,'TimerFcn', '[i,date] = ToDo(nrImages,i,date)'); % Functions to be
run per timer period.
set(T,'StartDelay', timerDelay, 'Period', timerSeconds);
start(T);
```

8.1.2 ToDo.m

```

function [ counter, date ] = ToDo( nrImages, i, setDate)
% ToDo stores the calculated area data to specified txt file. calls
% polypMonitor.m to do activity calculations on each polyp, gets data back
which are written in file.
% Check that filepathways are correct according to specified setup and
% storage of data.

    currentDate = '2013-04-25';           % ##### For modifying and testing
purposes
%currentDate = strcat(datestr(clock,'yyyy-mm-dd'));

if 0 == strcmp(setDate,currentDate) % Link to ref for date capture.
http://programming-tips-and-tricks.blogspot.no/2012/10/matlab-
timestamp.html
                                % If start date is different from
                                % current date, create new folders with
                                % new dates
    date = currentDate;           % #####For modifying and testing
purposes
%    date = strcat(datestr(clock,'yyyy-mm-dd'));
% Set date to new Date. i.e. its a new day and new folder

% is created to store all images and new area data.
    polypImageSource =
strcat('C:\Users\Eric\Desktop\IRIS\',date,'\Polyp\');           % Path
of images to be analysed for polyp activity
    mkdir(polypImageSource);
    polypDestination =
strcat('C:\Users\Eric\Desktop\IRIS\',date,'\PolypTreated\');   %
Destination of polyp images to be moved.
    mkdir(polypDestination);
    sedimentImageSource =
strcat('C:\Users\Eric\Desktop\IRIS\',date,'\Sediment\');       % Path of
images to be analysed for sedimentation effects.
    mkdir(sedimentImageSource);
    sedimentDestination =
strcat('C:\Users\Eric\Desktop\IRIS\',date,'\SedimentTreated\'); %
Destination of images to be analysed for sedimentation effects.
    mkdir(sedimentDestination);
    dataFile = strcat('C:\Users\Eric\Desktop\IRIS\Data\',date, '.csv');
% File to store Area calculation data from images
    %mkdir(dataFile); %make sure directory exists before running program
% Create the detination folder to store analysed images, Gives warning if
folder already exists.

%    % Opens or creates the file to store data in so that append function
can be used later
%    file_id = fopen(dataFile,'a');
##### removed 'w'
%    fprintf(file_id,'\r\n');
##### added line
%    fprintf(file_id,'%s', 'Image #',',', ' ');
%    fprintf(file_id,'%s', 'C1P1',',', ' ');
%    fprintf(file_id,'%s', 'C1P2',',', ' ');
%    fprintf(file_id,'%s', 'C1P3',',', ' ');
%    fprintf(file_id,'%s', 'C1P4',',', ' ');
%    fprintf(file_id,'%s', 'C1P5',',', ' ');

```

```

%     fprintf(file_id,'%s', 'C2P1',',', ');
%     fprintf(file_id,'%s', 'C2P2',',', ');
%     fprintf(file_id,'%s', 'C2P3',',', ');
%     fprintf(file_id,'%s', 'C2P4',',', ');
%     fprintf(file_id,'%s', 'C2P5',',', ');
%     fprintf(file_id,'%s', 'C3P1',',', ');
%     fprintf(file_id,'%s', 'C3P2',',', ');
%     fprintf(file_id,'%s', 'C3P3',',', ');
%     fprintf(file_id,'%s', 'C3P4',',', ');
%     fprintf(file_id,'%s', 'C3P5',',', ');
%     fprintf(file_id,'%s', 'C3P6',',', ');
%     fclose(file_id);

else
    date = setDate;
    polypImageSource =
strcat('C:\Users\Eric\Desktop\IRIS\',date,'\Polyp\');           % Path
of images to be analysed for polyp activity
    polypDestination =
strcat('C:\Users\Eric\Desktop\IRIS\',date,'\PolypTreated\');   %
Destination of polyp images to be moved.
    sedimentImageSource =
strcat('C:\Users\Eric\Desktop\IRIS\',date,'\Sediment\');       % Path of
images to be analysed for sedimentation effects.
    sedimentDestination =
strcat('C:\Users\Eric\Desktop\IRIS\',date,'\SedimentTreated\'); %
Destination of images to be analysed for sedimentation effects.
    dataFile = strcat('C:\Users\Eric\Desktop\IRIS\Data\',date, '.csv');
% File to store Area calculation data from images
end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%% Stage 1 experiment %%%%%%%%%
% %%%%%%%%%
% [noImages,x,C1P1,C1P2,C1P3,C1P4,C1P5, C2P1,C2P2,C2P3,C2P4,C2P5,...
%   C3P1,C3P2,C3P3,C3P4,C3P5,C3P6] = PolypMonitor(nrImages, i,
polypImageSource, polypDestination);
% %%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%% Stage 2 experiment %%%%%%%%%
% %%%%%%%%%
% [noImages,x, C1,C1P1,C1P2,C1P3,C1P4, C2,C2P1,C2P2,C2P3,C2P4,C2P5,...
%   C2P6, C3,C3P1,C3P2,C3P3, C4,C4P1,C4P2,C4P3] = PolypMonitor(nrImages, i,
polypImageSource, polypDestination);
% %%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%% Sediment monitoring %%%%%%%%%
% %%%%%%%%%
[noImages,x, C1] = PolypMonitor(nrImages, i, polypImageSource,
polypDestination);
% %%%%%%%%%

if noImages == 0;           %test to check there is images in
source folder.

    counter=x(end)+1;      % Counts images that has been
processed, the number is printed in display.

```

```

                                % Only counting if noImages = 0, i.e.
there are images in
                                % folder

                                % %creating and writing to file

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Stage 1 experiment %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% %       for a=1:length(x)
% %       m= x(a);
% %       file_id = fopen(dataFile,'a');
% %       fprintf(file_id,'\r\n');
% %       fprintf(file_id,'%u', m);
% %       fprintf(file_id,',');
% %       fprintf(file_id,'%16.f', C1P1(a));
% %       fprintf(file_id,',');
% %       fprintf(file_id,'%16.f', C1P2(a));
% %       fprintf(file_id,',');
% %       fprintf(file_id,'%16.f', C1P3(a));
% %       fprintf(file_id,',');
% %       fprintf(file_id,'%16.f', C1P4(a));
% %       fprintf(file_id,',');
% %       fprintf(file_id,'%16.f', C1P5(a));
% %       fprintf(file_id,',');
% %
% %       fprintf(file_id,'%16.f', C2P1(a));
% %       fprintf(file_id,',');
% %       fprintf(file_id,'%16.f', C2P2(a));
% %       fprintf(file_id,',');
% %       fprintf(file_id,'%16.f', C2P3(a));
% %       fprintf(file_id,',');
% %       fprintf(file_id,'%16.f', C2P4(a));
% %       fprintf(file_id,',');
% %       fprintf(file_id,'%16.f', C2P5(a));
% %       fprintf(file_id,',');
% %
% %       fprintf(file_id,'%16.f', C3P1(a));
% %       fprintf(file_id,',');
% %       fprintf(file_id,'%16.f', C3P2(a));
% %       fprintf(file_id,',');
% %       fprintf(file_id,'%16.f', C3P3(a));
% %       fprintf(file_id,',');
% %       fprintf(file_id,'%16.f', C3P4(a));
% %       fprintf(file_id,',');
% %       fprintf(file_id,'%16.f', C3P5(a));
% %       fprintf(file_id,',');
% %       fprintf(file_id,'%16.f', C3P6(a));
% %       fprintf(file_id,',');
% %
% %       fclose(file_id);
% %
% %     end
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Stage 2 experiment %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%       for a=1:length(x)
%       m= x(a);
%       file_id = fopen(dataFile,'a');

```

```

%      fprintf(file_id, '\r\n');
%      fprintf(file_id, '%u', m);
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C1(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C1P1(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C1P2(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C1P3(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C1P4(a));
%      fprintf(file_id, ',');
%
%      fprintf(file_id, '%16.f', C2(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C2P1(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C2P2(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C2P3(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C2P4(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C2P5(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C2P6(a));
%      fprintf(file_id, ',');
%
%      fprintf(file_id, '%16.f', C3(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C3P1(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C3P2(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C3P3(a));
%      fprintf(file_id, ',');
%
%      fprintf(file_id, '%16.f', C4(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C4P1(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C4P2(a));
%      fprintf(file_id, ',');
%      fprintf(file_id, '%16.f', C4P3(a));
%      fprintf(file_id, ',');
%      fclose(file_id);
%
%      end
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%      Sediment monitoring %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
for a=1:length(x)
    m= x(a);
    file_id = fopen(dataFile,'a');
    fprintf(file_id, '\r\n');
    fprintf(file_id, '%u', m);
    fprintf(file_id, ',');
    fprintf(file_id, '%16.f', C1(a));

```

```
fprintf(file_id, ',');  
    fclose(file_id);  
  
    end  
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%  
  
else disp('No Data Saved')  
    counter = i;  
end  
  
end
```


8.1.3 PolypMonitor.m

```

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Stage 1 experiment %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% function [noImages,x,C1P1,C1P2,C1P3,C1P4,C1P5,
C2P1,C2P2,C2P3,C2P4,C2P5,...
%   C3P1,C3P2,C3P3,C3P4,C3P5,C3P6] = PolypMonitor( nrImages, i,
imageSource, destination)
% %%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Stage 2 experiment %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% function [noImages,x, C1,C1P1,C1P2,C1P3,C1P4,
C2,C2P1,C2P2,C2P3,C2P4,C2P5,...
%   C2P6, C3,C3P1,C3P2,C3P3, C4,C4P1,C4P2,C4P3] = PolypMonitor( nrImages,
i, imageSource, destination)
% %%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Sediment monitoring %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
function [noImages,x, C1] = PolypMonitor( nrImages, i, imageSource,
destination)
% %%%%%%%%%

% nrImages = number of images from image folder in Areacalc.m to analyse.
Set to 1.
% i = counting integer of image in folder to be analysed.
% imageSource = filepath where original image is stored.
% destination = filepath for where processed image is stored.

listCounter = 0;
counter = i;
lightsOnOff = 0;
x = [];

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Stage 1 experiment %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% C1P1 = [];
% C1P2 = [];
% C1P3 = [];
% C1P4 = [];
% C1P5 = [];
%
% C2P1 = [];
% C2P2 = [];
% C2P3 = [];
% C2P4 = [];
% C2P5 = [];
%
% C3P1 = [];
% C3P2 = [];
% C3P3 = [];

```

```

% C3P4 = [];
% C3P5 = [];
% C3P6 = [];
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Stage 2 experiment %%%%%%%%%
% %%%%%%%%%
% C1 = [];
% C1P1 = [];
% C1P2 = [];
% C1P3 = [];
% C1P4 = [];
% C2 = [];
% C2P1 = [];
% C2P2 = [];
% C2P3 = [];
% C2P4 = [];
% C2P5 = [];
% C2P6 = [];
% C3 = [];
% C3P1 = [];
% C3P2 = [];
% C3P3 = [];
% C4 = [];
% C4P1 = [];
% C4P2 = [];
% C4P3 = [];
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Sediment monitoring %%%%%%%%%
% %%%%%%%%%
C1 = [];
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Sort images accoring to date to be sure they are in right order before
analysing
% ref.:
http://www.mathworks.com/matlabcentral/newsreader/view\_thread/291331
dirData = dir(strcat(imageSource,'*.jpg')); % Get the selected file data
Ref. : http://stackoverflow.com/questions/2004211/rename-image-file-name-in-matlab
S = [dirData(:).datenum].';
[S,S] = sort(S);
sortedFileNames = {dirData(S).name} ; % Create a Cell array of names
in order by datenum. Putting all images in a list ensures that no images
are left behind, and that all images are sorted to date so they are
analysed in correct order.

if length(sortedFileNames) >= nrImages % Test to check there is
images in source folder.
    noImages = 0; % i.e. nrImages = 1; If the
folder containing images has less than 1 image set noImages to 1
% when returned to ToDo.m this
% will not add to the image
% incemental, and not force
% program to go in error

```

```

while listCounter < nrImages

    oldPathOldName= strcat(imageSource,sortedFileNames{listCounter+1}); %
(iterator+1) to ensure no 0 in list calling. select original file according
to integercounter to move and rename
    newName = sprintf('image%d.jpg',counter); %
Make the new name, %05D gives 5 numbers i e image00001.jpg
    newPathNewName = strcat(destination,newName);
    movefile(oldPathOldName,newPathNewName); %
Move image to new folder with new name.

    RGBimage = imread(newPathNewName); %
Read image from file

    % Test if image is black, i.e. the light is not on and not to be
analyzed. Image to be moved to
    % new folder but overwritten with next file.
    [blackImage,blackTresh] = AreaCalc(RGBimage, counter);

    if blackImage > 10000 % An area of less than 10000 indicates almost
only black pixels on image. Number found from testing. should maybe me
revised.

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Stage 1 experiment %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%       Coral1Polyp1 = imcrop(RGBimage,[211.5 324.5 24 20]); % Cropping
area is desided manually through use of iCrop.m
%       Coral1Polyp2 = imcrop(RGBimage,[253.5 263.5 24 20]); % I.e.
Camera and coral setup must be fixed during experiment.
%       Coral1Polyp3 = imcrop(RGBimage,[279.5 238.5 24 20]); % Cropped
out polyp
%       Coral1Polyp4 = imcrop(RGBimage,[297.5 288.5 19 18]); % Cropped
out polyp
%       Coral1Polyp5 = imcrop(RGBimage,[255.5 316.5 23 23]); % Cropped
out polyp
%
%       Coral2Polyp1 = imcrop(RGBimage,[323.5 310.5 23 23]);% Cropped out
polyp
%       Coral2Polyp2 = imcrop(RGBimage,[330.5 287.5 21 20]);% Cropped out
polyp
%       Coral2Polyp3 = imcrop(RGBimage,[395.5 264.5 25 24]);% Cropped out
polyp
%       Coral2Polyp4 = imcrop(RGBimage,[363.5 283.5 19 19]);% Cropped out
polyp
%       Coral2Polyp5 = imcrop(RGBimage,[398.5 299.5 23 22]);% Cropped out
polyp
%
%       Coral3Polyp1 = imcrop(RGBimage,[439.5 275.5 23 24]);
%       Coral3Polyp2 = imcrop(RGBimage,[476.5 238.5 34 23]);
%       Coral3Polyp3 = imcrop(RGBimage,[512.5 229.5 29 23]);
%       Coral3Polyp4 = imcrop(RGBimage,[534.5 243.5 42 26]);
%       Coral3Polyp5 = imcrop(RGBimage,[572.5 223.5 48 34]);
%       Coral3Polyp6 = imcrop(RGBimage,[597.5 288.5 33 33]);
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

```

```

%%%%%%%% Stage 2 experiment %%%%%%%%%
%%%%%%%%
%       Coral_1 = imcrop(RGBImage, [69.5 316.5 96 70]);
%       Coral1Polyp1 = imcrop(RGBImage, [83.5 359.5 26 28]); % Cropping
area is desided manually through use of iCrop.m
%       Coral1Polyp2 = imcrop(RGBImage, [61.5 334.5 26 28]); % I.e. Camera
and coral setup must be fixed during experiment.
%       Coral1Polyp3 = imcrop(RGBImage, [89.5 317.5 26 28]); % Cropped out
polyp
%       Coral1Polyp4 = imcrop(RGBImage, [138.5 313.5 26 28]); % Cropped
out polyp
%
%       Coral_2 = imcrop(RGBImage, [210.5 250.5 117 112]);
%       Coral2Polyp1 = imcrop(RGBImage, [211.5 327.5 26 28]); % Cropped
out polyp
%       Coral2Polyp2 = imcrop(RGBImage, [249.5 264.5 33 32]);% Cropped out
polyp
%       Coral2Polyp3 = imcrop(RGBImage, [283.5 244.5 42 51]);% Cropped out
polyp
%       Coral2Polyp4 = imcrop(RGBImage, [301.5 297.5 24 27]);% Cropped out
polyp
%       Coral2Polyp5 = imcrop(RGBImage, [275.5 299.5 24 27]);% Cropped out
polyp
%       Coral2Polyp6 = imcrop(RGBImage, [256.5 325.5 26 29]);% Cropped out
polyp
%
%       Coral_3 = imcrop(RGBImage, [321.5 277.5 109 86]);
%       Coral3Polyp1 = imcrop(RGBImage, [322.5 311.5 24 49]);
%       Coral3Polyp2 = imcrop(RGBImage, [395.5 271.5 36 32]);
%       Coral3Polyp3 = imcrop(RGBImage, [397.5 307.5 30 37]);
%
%       Coral_4 = imcrop(RGBImage, [433.5 228.5 207 130]);
%       Coral4Polyp1 = imcrop(RGBImage, [427.5 287.5 67 68]);
%       Coral4Polyp2 = imcrop(RGBImage, [465.5 230.5 55 59]);
%       Coral4Polyp3 = imcrop(RGBImage, [521.5 238.5 26 22]);
%%%%%%%%

%%%%%%%%
%%%%%%%% Sediment monitoring %%%%%%%%%
%%%%%%%%
Coral = imcrop(RGBImage, [210.5 175.5 171 164]);
%%%%%%%%

        x = [x, counter];                                % Vector
X to store integer.

%%%%%%%%
%%%%%%%% Stage 1 experiment %%%%%%%%%
%%%%%%%%
%       [Coral1Polyp1A, Tresh11] = AreaCalc(Coral1Polyp1, counter);%
Perform Areacalc of each polyp
%       [Coral1Polyp2A, Tresh12] = AreaCalc(Coral1Polyp2, counter);%
Perform Areacalc of each polyp
%       [Coral1Polyp3A, Tresh13] = AreaCalc(Coral1Polyp3, counter);%
Perform Areacalc of each polyp
%       [Coral1Polyp4A, Tresh14] = AreaCalc(Coral1Polyp4, counter);%
Perform Areacalc of each polyp
%       [Coral1Polyp5A, Tresh15] = AreaCalc(Coral1Polyp5, counter);%
Perform Areacalc of each polyp
%

```

```

%      [Coral2Polyp1A,Tresh21] = AreaCalc(Coral2Polyp1, counter);%
Perform Areacalc of each polyp
%      [Coral2Polyp2A,Tresh22] = AreaCalc(Coral2Polyp2, counter);%
Perform Areacalc of each polyp
%      [Coral2Polyp3A,Tresh23] = AreaCalc(Coral2Polyp3, counter);%
Perform Areacalc of each polyp
%      [Coral2Polyp4A,Tresh24] = AreaCalc(Coral2Polyp4, counter);%
Perform Areacalc of each polyp
%      [Coral2Polyp5A,Tresh25] = AreaCalc(Coral2Polyp5, counter);%
Perform Areacalc of each polyp
%
%      [Coral3Polyp1A,Tresh31] = AreaCalc(Coral3Polyp1, counter);%
Perform Areacalc of each polyp
%      [Coral3Polyp2A,Tresh32] = AreaCalc(Coral3Polyp2, counter);%
Perform Areacalc of each polyp
%      [Coral3Polyp3A,Tresh33] = AreaCalc(Coral3Polyp3, counter);%
Perform Areacalc of each polyp
%      [Coral3Polyp4A,Tresh34] = AreaCalc(Coral3Polyp4, counter);%
Perform Areacalc of each polyp
%      [Coral3Polyp5A,Tresh35] = AreaCalc(Coral3Polyp5, counter);%
Perform Areacalc of each polyp
%      [Coral3Polyp6A,Tresh36] = AreaCalc(Coral3Polyp6, counter);%
Perform Areacalc of each polyp
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Stage 2 experiment %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%      [Coral_1A,TreshC1] = AreaCalc(Coral_1, counter);
%      [Coral1Polyp1A,Tresh11] = AreaCalc(Coral1Polyp1, counter);%
Perform Areacalc of each polyp
%      [Coral1Polyp2A,Tresh12] = AreaCalc(Coral1Polyp2, counter);%
Perform Areacalc of each polyp
%      [Coral1Polyp3A,Tresh13] = AreaCalc(Coral1Polyp3, counter);%
Perform Areacalc of each polyp
%      [Coral1Polyp4A,Tresh14] = AreaCalc(Coral1Polyp4, counter);%
Perform Areacalc of each polyp
%
%      [Coral_2A,TreshC2] = AreaCalc(Coral_2, counter);
%      [Coral2Polyp1A,Tresh21] = AreaCalc(Coral2Polyp1, counter);%
Perform Areacalc of each polyp
%      [Coral2Polyp2A,Tresh22] = AreaCalc(Coral2Polyp2, counter);%
Perform Areacalc of each polyp
%      [Coral2Polyp3A,Tresh23] = AreaCalc(Coral2Polyp3, counter);%
Perform Areacalc of each polyp
%      [Coral2Polyp4A,Tresh24] = AreaCalc(Coral2Polyp4, counter);%
Perform Areacalc of each polyp
%      [Coral2Polyp5A,Tresh25] = AreaCalc(Coral2Polyp5, counter);%
Perform Areacalc of each polyp
%      [Coral2Polyp6A,Tresh26] = AreaCalc(Coral2Polyp6, counter);%
Perform Areacalc of each polyp
%
%      [Coral_3A,TreshC3] = AreaCalc(Coral_3, counter);
%      [Coral3Polyp1A,Tresh31] = AreaCalc(Coral3Polyp1, counter);%
Perform Areacalc of each polyp
%      [Coral3Polyp2A,Tresh32] = AreaCalc(Coral3Polyp2, counter);%
Perform Areacalc of each polyp
%      [Coral3Polyp3A,Tresh33] = AreaCalc(Coral3Polyp3, counter);%
Perform Areacalc of each polyp
%
%      [Coral_4A,TreshC4] = AreaCalc(Coral_4, counter);

```

```
% [Coral4Polyp1A,Tresh41] = AreaCalc(Coral4Polyp1, counter);%
Perform Areacalc of each polypp
% [Coral4Polyp2A,Tresh42] = AreaCalc(Coral4Polyp2, counter);%
Perform Areacalc of each polypp
% [Coral4Polyp3A,Tresh43] = AreaCalc(Coral4Polyp3, counter);%
Perform Areacalc of each polypp
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Sediment monitoring %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
[Coral_A,TreshC] = AreaCalc(Coral, counter);
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Stage 1 experiment %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% % C1P1 = [C1P1, Corall1Polyp1A];% Store area coverage of each
polypp in vectors
% % C1P2 = [C1P2, Corall1Polyp2A];% Store area coverage of each
polypp in vectors
% % C1P3 = [C1P3, Corall1Polyp3A];% Store area coverage of each
polypp in vectors
% % C1P4 = [C1P4, Corall1Polyp4A];% Store area coverage of each
polypp in vectors
% % C1P5 = [C1P5, Corall1Polyp5A];% Store area coverage of each
polypp in vectors
% %
% % C2P1 = [C2P1, Coral2Polyp1A];% Store area coverage of each
polypp in vectors
% % C2P2 = [C2P2, Coral2Polyp2A];% Store area coverage of each
polypp in vectors
% % C2P3 = [C2P3, Coral2Polyp3A];% Store area coverage of each
polypp in vectors
% % C2P4 = [C2P4, Coral2Polyp4A];% Store area coverage of each
polypp in vectors
% % C2P5 = [C2P5, Coral2Polyp5A];% Store area coverage of each
polypp in vectors
% %
% % C3P1 = [C3P1, Coral3Polyp1A];% Store area coverage of each
polypp in vectors
% % C3P2 = [C3P2, Coral3Polyp2A];% Store area coverage of each
polypp in vectors
% % C3P3 = [C3P3, Coral3Polyp3A];% Store area coverage of each
polypp in vectors
% % C3P4 = [C3P4, Coral3Polyp4A];% Store area coverage of each
polypp in vectors
% % C3P5 = [C3P5, Coral3Polyp5A];% Store area coverage of each
polypp in vectors
% % C3P6 = [C3P6, Coral3Polyp6A];% Store area coverage of each
polypp in vectors
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Stage 2 experiment %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% C1 = [C1, Coral_1A];
% C1P1 = [C1P1, Corall1Polyp1A];% Store area coverage of each polypp
in vectors
```

```

%          C1P2 = [C1P2, Coral1Polyp2A];% Store area coverage of each polypp
in vectors
%          C1P3 = [C1P3, Coral1Polyp3A];% Store area coverage of each polypp
in vectors
%          C1P4 = [C1P4, Coral1Polyp4A];% Store area coverage of each polypp
in vectors
%
%          C2 = [C2, Coral_2A];
%          C2P1 = [C2P1, Coral2Polyp1A];% Store area coverage of each polypp
in vectors
%          C2P2 = [C2P2, Coral2Polyp2A];% Store area coverage of each polypp
in vectors
%          C2P3 = [C2P3, Coral2Polyp3A];% Store area coverage of each polypp
in vectors
%          C2P4 = [C2P4, Coral2Polyp4A];% Store area coverage of each polypp
in vectors
%          C2P5 = [C2P5, Coral2Polyp5A];% Store area coverage of each polypp
in vectors
%          C2P6 = [C2P6, Coral2Polyp6A];% Store area coverage of each polypp
in vectors
%
%          C3 = [C3, Coral_3A];
%          C3P1 = [C3P1, Coral3Polyp1A];% Store area coverage of each polypp
in vectors
%          C3P2 = [C3P2, Coral3Polyp2A];% Store area coverage of each polypp
in vectors
%          C3P3 = [C3P3, Coral3Polyp3A];% Store area coverage of each polypp
in vectors
%
%          C4 = [C4, Coral_4A];
%          C4P1 = [C4P1, Coral4Polyp1A];% Store area coverage of each polypp
in vectors
%          C4P2 = [C4P2, Coral4Polyp2A];% Store area coverage of each polypp
in vectors
%          C4P3 = [C4P3, Coral4Polyp3A];% Store area coverage of each polypp
in vectors
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Sediment monitoring %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
C1 = [C1, Coral_A];
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% save cropped and tresholded images, and store them in destination
% folder

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Stage 1 experiment %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% %
imwrite(Coral1Polyp1, strcat(destination, sprintf('image%d_Coral_2_Polyp1.jpg
',counter)));
% %
imwrite(Coral1Polyp2, strcat(destination, sprintf('image%d_Coral_2_Polyp2.jpg
',counter)));

```

```

% %
imwrite(Coral1Polyp3, strcat(destination, sprintf('image%d_Coral_2_Polyp3.jpg
', counter)));
% %
imwrite(Coral1Polyp4, strcat(destination, sprintf('image%d_Coral_2_Polyp4.jpg
', counter)));
% %
imwrite(Coral1Polyp5, strcat(destination, sprintf('image%d_Coral_2_Polyp5.jpg
', counter)));
% %
% %
imwrite(Coral2Polyp1, strcat(destination, sprintf('image%d_Coral_3_Polyp1.jpg
', counter)));
% %
imwrite(Coral2Polyp2, strcat(destination, sprintf('image%d_Coral_3_Polyp2.jpg
', counter)));
% %
imwrite(Coral2Polyp3, strcat(destination, sprintf('image%d_Coral_3_Polyp3.jpg
', counter)));
% %
imwrite(Coral2Polyp4, strcat(destination, sprintf('image%d_Coral_3_Polyp4.jpg
', counter)));
% %
imwrite(Coral2Polyp5, strcat(destination, sprintf('image%d_Coral_3_Polyp5.jpg
', counter)));
% %
% %
imwrite(Coral3Polyp1, strcat(destination, sprintf('image%d_Coral_4_Polyp1.jpg
', counter)));
% %
imwrite(Coral3Polyp2, strcat(destination, sprintf('image%d_Coral_4_Polyp2.jpg
', counter)));
% %
imwrite(Coral3Polyp3, strcat(destination, sprintf('image%d_Coral_4_Polyp3.jpg
', counter)));
% %
imwrite(Coral3Polyp4, strcat(destination, sprintf('image%d_Coral_4_Polyp4.jpg
', counter)));
% %
imwrite(Coral3Polyp5, strcat(destination, sprintf('image%d_Coral_4_Polyp5.jpg
', counter)));
% %
imwrite(Coral3Polyp6, strcat(destination, sprintf('image%d_Coral_4_Polyp6.jpg
', counter)));
% %
% %
imwrite(Tresh11, strcat(destination, sprintf('image%d_Coral_2_Polyp1Tresh.jpg
', counter)));
% %
imwrite(Tresh12, strcat(destination, sprintf('image%d_Coral_2_Polyp2Tresh.jpg
', counter)));
% %
imwrite(Tresh13, strcat(destination, sprintf('image%d_Coral_2_Polyp3Tresh.jpg
', counter)));
% %
imwrite(Tresh14, strcat(destination, sprintf('image%d_Coral_2_Polyp4Tresh.jpg
', counter)));
% %
imwrite(Tresh15, strcat(destination, sprintf('image%d_Coral_2_Polyp5Tresh.jpg
', counter)));
% %

```



```

% %
imwrite(Tresh21, strcat(destination, sprintf('image%d_Coral_3_Polyp1Tresh.jpg
', counter)));
% %
imwrite(Tresh22, strcat(destination, sprintf('image%d_Coral_3_Polyp2Tresh.jpg
', counter)));
% %
imwrite(Tresh23, strcat(destination, sprintf('image%d_Coral_3_Polyp3Tresh.jpg
', counter)));
% %
imwrite(Tresh24, strcat(destination, sprintf('image%d_Coral_3_Polyp4Tresh.jpg
', counter)));
% %
imwrite(Tresh25, strcat(destination, sprintf('image%d_Coral_3_Polyp5Tresh.jpg
', counter)));
% %
% %
imwrite(Tresh31, strcat(destination, sprintf('image%d_Coral_4_Polyp1Tresh.jpg
', counter)));
% %
imwrite(Tresh32, strcat(destination, sprintf('image%d_Coral_4_Polyp2Tresh.jpg
', counter)));
% %
imwrite(Tresh33, strcat(destination, sprintf('image%d_Coral_4_Polyp3Tresh.jpg
', counter)));
% %
imwrite(Tresh34, strcat(destination, sprintf('image%d_Coral_4_Polyp4Tresh.jpg
', counter)));
% %
imwrite(Tresh35, strcat(destination, sprintf('image%d_Coral_4_Polyp5Tresh.jpg
', counter)));
% %
imwrite(Tresh36, strcat(destination, sprintf('image%d_Coral_4_Polyp6Tresh.jpg
', counter)));
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%
imwrite(Coral_1, strcat(destination, sprintf('image%d_Coral_1.jpg', counter)))
;
%
imwrite(Coral1Polyp1, strcat(destination, sprintf('image%d_Coral_1_Polyp1.jpg
', counter)));
%
imwrite(Coral1Polyp2, strcat(destination, sprintf('image%d_Coral_1_Polyp2.jpg
', counter)));
%
imwrite(Coral1Polyp3, strcat(destination, sprintf('image%d_Coral_1_Polyp3.jpg
', counter)));
%
imwrite(Coral1Polyp4, strcat(destination, sprintf('image%d_Coral_1_Polyp4.jpg
', counter)));
%
%
imwrite(Coral_2, strcat(destination, sprintf('image%d_Coral_2.jpg', counter)))
;
%
imwrite(Coral2Polyp1, strcat(destination, sprintf('image%d_Coral_2_Polyp1.jpg
', counter)));

```

```

%
imwrite(Coral2Polyp2, strcat(destination, sprintf('image%d_Coral_2_Polyp2.jpg
', counter)));
%
imwrite(Coral2Polyp3, strcat(destination, sprintf('image%d_Coral_2_Polyp3.jpg
', counter)));
%
imwrite(Coral2Polyp4, strcat(destination, sprintf('image%d_Coral_2_Polyp4.jpg
', counter)));
%
imwrite(Coral2Polyp5, strcat(destination, sprintf('image%d_Coral_2_Polyp5.jpg
', counter)));
%
imwrite(Coral2Polyp6, strcat(destination, sprintf('image%d_Coral_2_Polyp6.jpg
', counter)));
%
%
imwrite(Coral_3, strcat(destination, sprintf('image%d_Coral_3.jpg', counter)))
;
%
imwrite(Coral3Polyp1, strcat(destination, sprintf('image%d_Coral_3_Polyp1.jpg
', counter)));
%
imwrite(Coral3Polyp2, strcat(destination, sprintf('image%d_Coral_3_Polyp2.jpg
', counter)));
%
imwrite(Coral3Polyp3, strcat(destination, sprintf('image%d_Coral_3_Polyp3.jpg
', counter)));
%
%
imwrite(Coral_4, strcat(destination, sprintf('image%d_Coral_4.jpg', counter)))
;
%
imwrite(Coral4Polyp1, strcat(destination, sprintf('image%d_Coral_4_Polyp1.jpg
', counter)));
%
imwrite(Coral4Polyp2, strcat(destination, sprintf('image%d_Coral_4_Polyp2.jpg
', counter)));
%
imwrite(Coral4Polyp3, strcat(destination, sprintf('image%d_Coral_4_Polyp3.jpg
', counter)));
%
%
imwrite(TreshC1, strcat(destination, sprintf('image%d_Coral_1_Tresh.jpg', coun
ter)));
%
imwrite(Tresh11, strcat(destination, sprintf('image%d_Coral_2_Polyp2Tresh.jpg
', counter)));
%
imwrite(Tresh12, strcat(destination, sprintf('image%d_Coral_2_Polyp3Tresh.jpg
', counter)));
%
imwrite(Tresh13, strcat(destination, sprintf('image%d_Coral_2_Polyp4Tresh.jpg
', counter)));
%
imwrite(Tresh14, strcat(destination, sprintf('image%d_Coral_2_Polyp5Tresh.jpg
', counter)));
%

```

```
%
imwrite(TreshC2, strcat(destination, sprintf('image%d_Coral_2_Tresh.jpg', counter)));
%
imwrite(Tresh21, strcat(destination, sprintf('image%d_Coral_2_Polyp1Tresh.jpg', counter)));
%
imwrite(Tresh22, strcat(destination, sprintf('image%d_Coral_2_Polyp2Tresh.jpg', counter)));
%
imwrite(Tresh23, strcat(destination, sprintf('image%d_Coral_2_Polyp3Tresh.jpg', counter)));
%
imwrite(Tresh24, strcat(destination, sprintf('image%d_Coral_2_Polyp4Tresh.jpg', counter)));
%
imwrite(Tresh25, strcat(destination, sprintf('image%d_Coral_2_Polyp5Tresh.jpg', counter)));
%
imwrite(Tresh26, strcat(destination, sprintf('image%d_Coral_2_Polyp6Tresh.jpg', counter)));
%
%
imwrite(TreshC3, strcat(destination, sprintf('image%d_Coral_3_Tresh.jpg', counter)));
%
imwrite(Tresh31, strcat(destination, sprintf('image%d_Coral_3_Polyp1Tresh.jpg', counter)));
%
imwrite(Tresh32, strcat(destination, sprintf('image%d_Coral_3_Polyp2Tresh.jpg', counter)));
%
imwrite(Tresh33, strcat(destination, sprintf('image%d_Coral_3_Polyp3Tresh.jpg', counter)));
%
%
imwrite(TreshC4, strcat(destination, sprintf('image%d_Coral_4_Tresh.jpg', counter)));
%
imwrite(Tresh41, strcat(destination, sprintf('image%d_Coral_4_Polyp1Tresh.jpg', counter)));
%
imwrite(Tresh42, strcat(destination, sprintf('image%d_Coral_4_Polyp2Tresh.jpg', counter)));
%
imwrite(Tresh43, strcat(destination, sprintf('image%d_Coral_4_Polyp3Tresh.jpg', counter)));
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Sediment monitoring %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
imwrite(Coral, strcat(destination, sprintf('image%d_Coral.jpg', counter)));
imwrite(TreshC, strcat(destination, sprintf('image%d_Coral_Tresh.jpg', counter)));
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

```

listCounter=listCounter+1;
counter=counter+1;

else
    disp('Image in source folder is black, indicating light source
is turned off, throwing away image and data.')
    noImages = 1;
    listCounter=listCounter+1;
end

end

else disp('Not enough images in source folder to perform image analysis,
analysis skipped til next timer execution')

    noImages = 1;    % Test to check there is images in source folder. when
1 there is no images in folder.
    % Set all data to return 0 i.e. no data.
    x = [x, counter];

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Stage 1 experiment %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% %    C1P1 = [C1P1, 0];% Store area coverage of each polypp in vectors
% %    C1P2 = [C1P2, 0];% Store area coverage of each polypp in vectors
% %    C1P3 = [C1P3, 0];% Store area coverage of each polypp in vectors
% %    C1P4 = [C1P4, 0];% Store area coverage of each polypp in vectors
% %    C1P5 = [C1P5, 0];% Store area coverage of each polypp in vectors
% %
% %    C2P1 = [C2P1, 0];% Store area coverage of each polypp in vectors
% %    C2P2 = [C2P2, 0];% Store area coverage of each polypp in vectors
% %    C2P3 = [C2P3, 0];% Store area coverage of each polypp in vectors
% %    C2P4 = [C2P4, 0];% Store area coverage of each polypp in vectors
% %    C2P5 = [C2P5, 0];% Store area coverage of each polypp in vectors
% %
% %    C3P1 = [C3P1, 0];% Store area coverage of each polypp in vectors
% %    C3P2 = [C3P2, 0];% Store area coverage of each polypp in vectors
% %    C3P3 = [C3P3, 0];% Store area coverage of each polypp in vectors
% %    C3P4 = [C3P4, 0];% Store area coverage of each polypp in vectors
% %    C3P5 = [C3P5, 0];% Store area coverage of each polypp in vectors
% %    C3P6 = [C3P6, 0];% Store area coverage of each polypp in vectors
% %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%% Stage 2 experiment %%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%    C1 = [C1, 0];
%    C1P1 = [C1P1, 0];% Store area coverage of each polypp in vectors
%    C1P2 = [C1P2, 0];% Store area coverage of each polypp in vectors
%    C1P3 = [C1P3, 0];% Store area coverage of each polypp in vectors
%    C1P4 = [C1P4, 0];% Store area coverage of each polypp in vectors
%
%    C2 = [C2, 0];
%    C2P1 = [C2P1, 0];% Store area coverage of each polypp in vectors
%    C2P2 = [C2P2, 0];% Store area coverage of each polypp in vectors
%    C2P3 = [C2P3, 0];% Store area coverage of each polypp in vectors
%    C2P4 = [C2P4, 0];% Store area coverage of each polypp in vectors
%    C2P5 = [C2P5, 0];% Store area coverage of each polypp in vectors
%    C2P6 = [C2P6, 0];% Store area coverage of each polypp in vectors

```

```
%  
% C3 = [C3, 0];  
% C3P1 = [C3P1, 0];% Store area coverage of each polypp in vectors  
% C3P2 = [C3P2, 0];% Store area coverage of each polypp in vectors  
% C3P3 = [C3P3, 0];% Store area coverage of each polypp in vectors  
%  
% C4 = [C4, 0];  
% C4P1 = [C4P1, 0];% Store area coverage of each polypp in vectors  
% C4P2 = [C4P2, 0];% Store area coverage of each polypp in vectors  
% C4P3 = [C4P3, 0];% Store area coverage of each polypp in vectors  
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%  
  
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%  
%%%%%%%% Sediment monitoring %%%%%%%%%  
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%  
C1 = [C1, 0];  
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%5  
  
end  
  
end
```

8.1.4 AreaCalc.m

```

function [ Area, FilledImage5] = AreaCalc( RGB1, integer )
% Calculates relative pixel area density.
%
% Object identification
% ref http://www.mathworks.se/help/images/examples/detecting-a-cell-using-
image-segmentation.html?prodcode=IP&language=en
% #1 Collect image
%
% % data_source = 'C:\Users\Eric\Desktop\Timelapse\' ;
% % filename = 'image%d.jpg';
% % filename = sprintf(filename,integer);      % Add correct ingeger of
image to read.
% % path = strcat(data_source,filename);      % add two strings
horisontally to make full path to image.
% % RGB1 = imread(path);
%
% #2 Threshold with Otsu's method, (see help on im2bw)
I = rgb2gray(RGB1);
iContrast= imadjust(I, [0 0.9], [0 1]); %contrast adjustment 0.5 is 50%
graytresh = graythresh(I);
ThresholdImage2 = im2bw(I,graytresh); %Set threshold sensitivity level. Use
graytresh for Ostu's method.
%contrast treshing
graytreshContrast = graythresh(iContrast);
ThresholdContrast = im2bw(I,graytreshContrast); %Set threshold sensitivity
level. Use graytresh for Ostu's method.
%
% % #3 Remove bordering objects (can maybe be run earlier?)
% % NoBorderImage3 = imclearborder(ThresholdImage2,4);
%
% #4 Dilation, increase line density to reduce noise.
se90 = strel('line', 3, 100);
se0 = strel('line', 3, 0);
DilutionImage4 = imdilate(ThresholdImage2, [se90 se0]);
%
% #5 Fill holes in outline
FilledImage5 = imfill(DilutionImage4, 'holes');
%
% Area calculations
Area = bwarea(FilledImage5);
%
% %Image analysis visualisation, remove comments to view images as they are
% analysed
%
subplot (2,3,1), subimage(RGB1), title('Original image'); %remove the
plotting of image on later stage
subplot (2,3,2), subimage(ThresholdImage2), title('Thresholded
Otsu');%remove the plotting of image on later stage
subplot (2,3,3), subimage(iContrast), title('Manual Contrast
adjust');%remove the plotting of image on later stage
subplot (2,3,4), subimage(ThresholdContrast), title('Thresholded
contrast');%remove the plotting of image on later stage
subplot (2,3,5), subimage(DilutionImage4), title('dilated gradient
mask');
subplot (2,3,6), subimage(FilledImage5), title('filled holes');%remove the
plotting of image on later stage

```

end

8.1.5 iCrop.m

```

imageSource = 'C:\Users\Eric\Desktop\IRIS\2013-04-25\Polyp\';           % Path
of images to be analysed
destination = 'C:\Users\Eric\Desktop\IRIS\2013-04-25\Polypcropped';   %
Destination of images to be moved.
% listCounter = 1;                               % Simple counter
% nrImages = listCounter+2;                       % Simple counter
%
% while listCounter < nrImages

%     oldName = sprintf('image%d.jpg', listCounter);
% oldName = 'image1.jpg';
% oldPathOldName= strcat(imageSource,oldName);
%     % newName = sprintf('Cropped_image%d.jpg',listcounter);       % Make the
new name, %05D gives 5 numbers i e image00001.jpg
%     % newPathNewName= strcat(destination,newName);
%     % movefile(oldPathOldName,newPathNewName);                   % Move image to
new folder with new name.
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%     Experiment 2, new image analysis coordinates.
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%

    RGBimage = imread('image1.jpg');                               % Read image from file
% imshow(RGBimage);
f = figure, imshow(RGBimage, 'Border', 'tight');
% rectangle('Position', [100, 100, 10, 10]);

rectangle('position',[69.5 316.5 96 70],'edgecolor','r','LineWidth',1)
rectangle('position',[83.5 359.5 26 28],'edgecolor','r','LineWidth',1)
rectangle('position',[61.5 334.5 26 28],'edgecolor','r','LineWidth',1)
rectangle('position',[89.5 317.5 26 28],'edgecolor','r','LineWidth',1)
rectangle('position',[138.5 313.5 26 28],'edgecolor','r','LineWidth',1)

rectangle('position',[210.5 250.5 117
112],'edgecolor','r','LineWidth',1)
rectangle('position',[211.5 327.5 26 28],'edgecolor','r','LineWidth',1)
rectangle('position',[249.5 264.5 33 32],'edgecolor','r','LineWidth',1)
rectangle('position',[283.5 244.5 42 51],'edgecolor','r','LineWidth',1)
rectangle('position',[301.5 297.5 24 27],'edgecolor','r','LineWidth',1)
rectangle('position',[275.5 299.5 24 27],'edgecolor','r','LineWidth',1)
rectangle('position',[256.5 325.5 26 29],'edgecolor','r','LineWidth',1)

rectangle('position',[321.5 277.5 109 86],'edgecolor','r','LineWidth',1)
rectangle('position',[322.5 311.5 24 49],'edgecolor','r','LineWidth',1)
rectangle('position',[395.5 271.5 36 32],'edgecolor','r','LineWidth',1)
rectangle('position',[397.5 307.5 30 37],'edgecolor','r','LineWidth',1)

rectangle('position',[433.5 228.5 207
130],'edgecolor','r','LineWidth',1)
rectangle('position',[427.5 287.5 67 68],'edgecolor','r','LineWidth',1)
rectangle('position',[465.5 230.5 55 59],'edgecolor','r','LineWidth',1)
rectangle('position',[521.5 238.5 26 22],'edgecolor','r','LineWidth',1)

    print(f, '-r80', '-dtiff', 'image2.tif');
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%

```



```

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% Experiment 1, image analysis coordinates.
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%

%   RGBimage = imread('image1.jpg');           % Read image from
file                                         %
%   imshow(RGBimage);
%   f = figure, imshow(RGBimage, 'Border', 'tight');
%   rectangle('Position', [100, 100, 10, 10]);
%   rectangle('position', [211.5 324.5 24
20], 'edgecolor', 'r', 'LineWidth', 1)
%   rectangle('position', [253.5 263.5 24
20], 'edgecolor', 'r', 'LineWidth', 1)
%   rectangle('position', [279.5 238.5 24
20], 'edgecolor', 'r', 'LineWidth', 1)
%   rectangle('position', [297.5 288.5 19
18], 'edgecolor', 'r', 'LineWidth', 1)
%   rectangle('position', [255.5 316.5 23
23], 'edgecolor', 'r', 'LineWidth', 1)
%
%   rectangle('position', [323.5 310.5 23
23], 'edgecolor', 'r', 'LineWidth', 1)
%   rectangle('position', [330.5 287.5 21
20], 'edgecolor', 'r', 'LineWidth', 1)
%   rectangle('position', [395.5 264.5 25
24], 'edgecolor', 'r', 'LineWidth', 1)
%   rectangle('position', [363.5 283.5 19
19], 'edgecolor', 'r', 'LineWidth', 1)
%   rectangle('position', [398.5 299.5 23
22], 'edgecolor', 'r', 'LineWidth', 1)
%
%   rectangle('position', [439.5 275.5 23
24], 'edgecolor', 'r', 'LineWidth', 1)
%   rectangle('position', [476.5 238.5 34
23], 'edgecolor', 'r', 'LineWidth', 1)
%   rectangle('position', [512.5 229.5 29
23], 'edgecolor', 'r', 'LineWidth', 1)
%   rectangle('position', [534.5 243.5 42
26], 'edgecolor', 'r', 'LineWidth', 1)
%   rectangle('position', [572.5 223.5 48
34], 'edgecolor', 'r', 'LineWidth', 1)
%   rectangle('position', [597.5 288.5 33
33], 'edgecolor', 'r', 'LineWidth', 1)
%   print(f, '-r80', '-dtiff', 'image2.tif');

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%
%   Cropped_RGB1 = imcrop(RGBimage);           %   Get
coordinates from image with this function.

%   Coral_1 = [69.5 316.5 96 70]
%   C1P1= [83.5 359.5 26 28]
%   C1P2= [61.5 334.5 26 28]
%   C1P3= [89.5 317.5 26 28]
%   C1P4= [138.5 313.5 26 28]

%   Coral_2 = [210.5 250.5 117 112]
%   C2P1= [211.5 327.5 26 28]
%   C2P2= [249.5 264.5 33 32]
%   C2P3= [283.5 244.5 42 51]

```

```

%      C2P4= [301.5 297.5 24 27]
%      C2P5= [275.5 299.5 24 27]
%      C2P6= [256.5 325.5 26 29]

%      Coral_3 = [321.5 277.5 109 86]
%      C1P1= [322.5 311.5 24 49]
%      C1P1= [395.5 271.5 36 32]
%      C1P1= [397.5 307.5 30 37]

%      Coral_4 = [433.5 228.5 207 130]
%      C1P1= [427.5 287.5 67 68]
%      C1P1= [465.5 230.5 55 59]
%      C1P1= [521.5 238.5 26 22]

%      Cropped_RGB2 = imcrop(RGBimage, [1185.5 2204.5 372 521]);
%      Cropped_RGB3 = imcrop(RGBimage, [1563.5 2042.5 444 659]);
%      Cropped_RGB4 = imcrop(RGBimage, [2493.5 2168.5 360 551]);
%      Cropped_RGB5 = imcrop(RGBimage, [3033.5 2210.5 582 503]);
%      Cropped_RGB6 = imcrop(RGBimage, [3624.5 2074.5 458 669]);

%      subplot (2,3,1), subimage(Cropped_RGB1);
%      subplot (2,3,1), subimage(Cropped_RGB2);
%      subplot (2,3,3), subimage(Cropped_RGB3);
%      subplot (2,3,4), subimage(Cropped_RGB4);
%      subplot (2,3,5), subimage(Cropped_RGB5);
%      subplot (2,3,6), subimage(Cropped_RGB6);

%      listCounter=listCounter+1;
% end

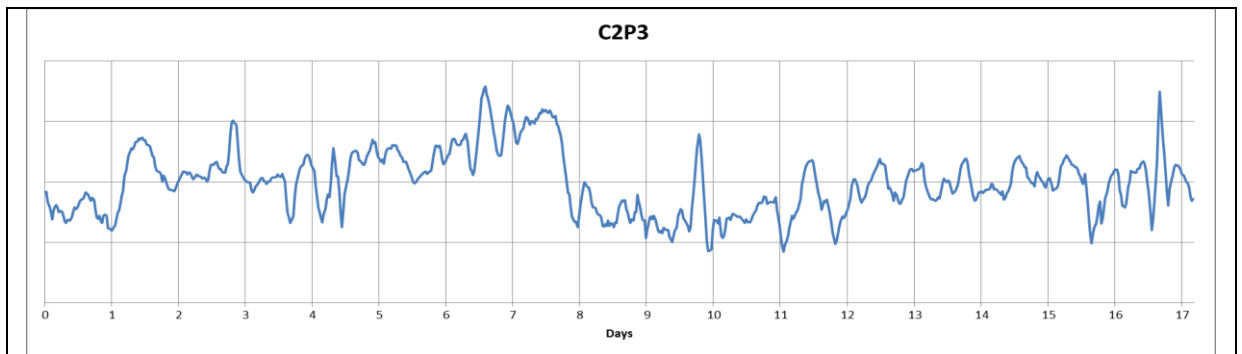
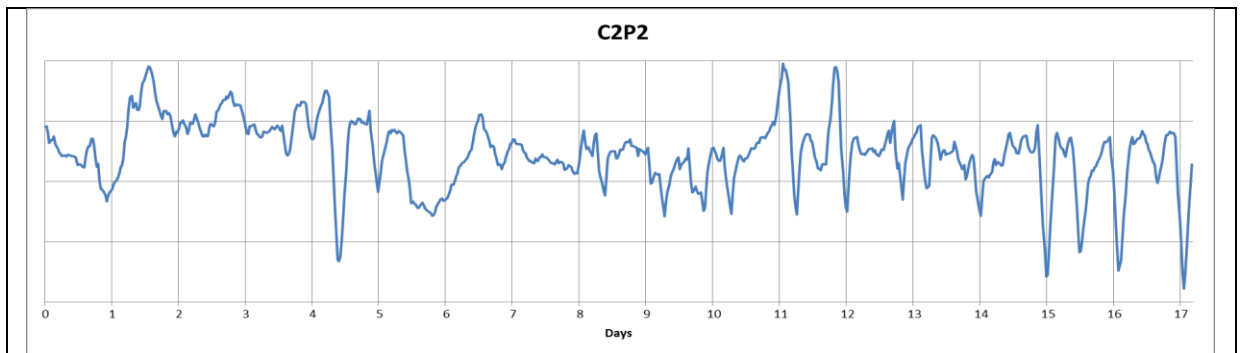
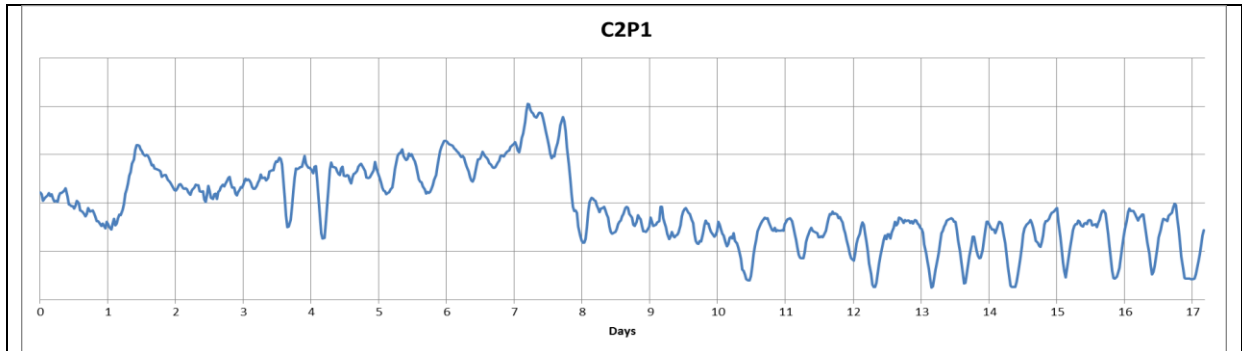
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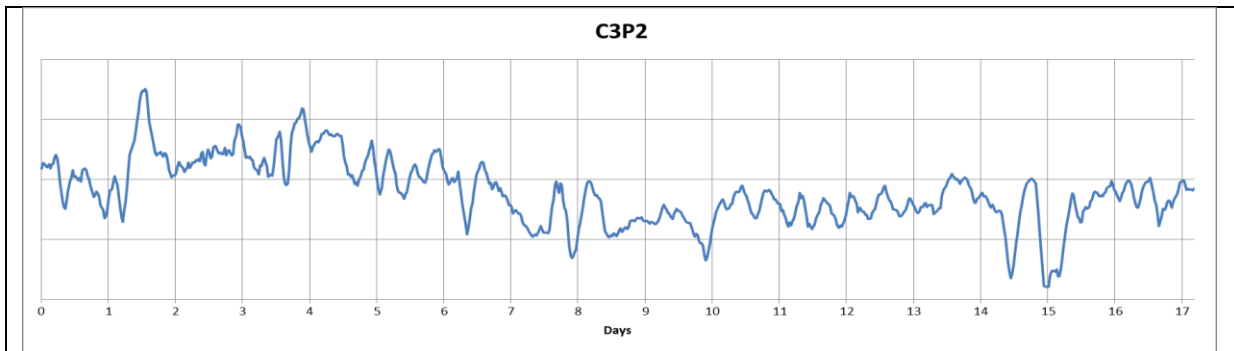
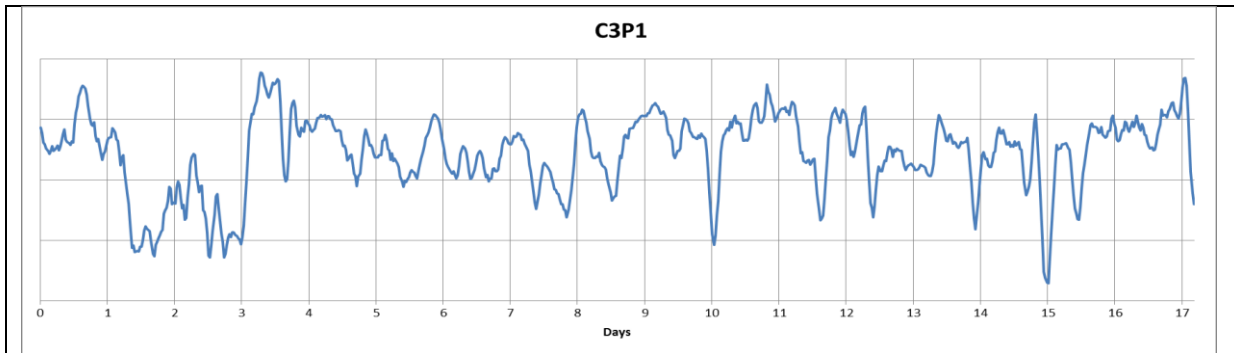
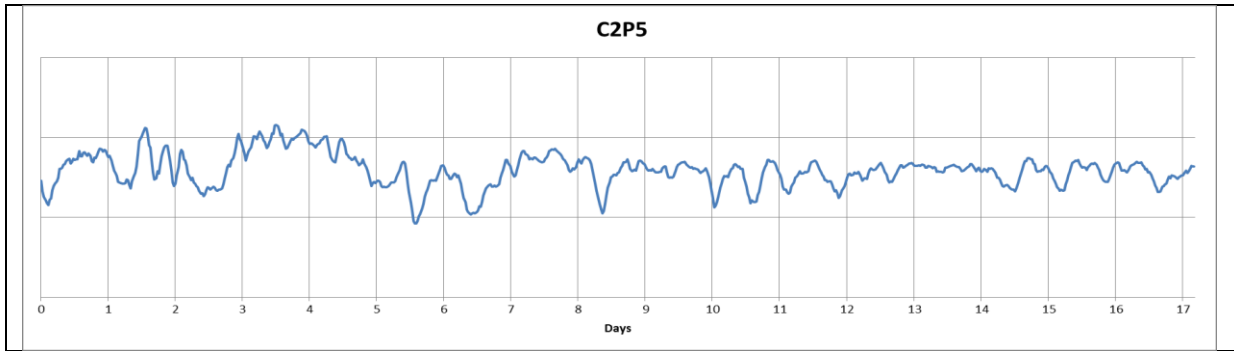
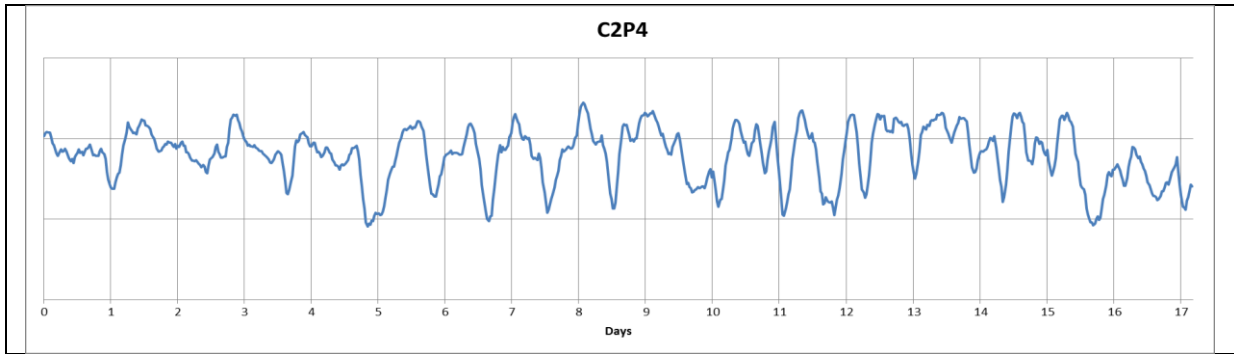
9 Appendix B

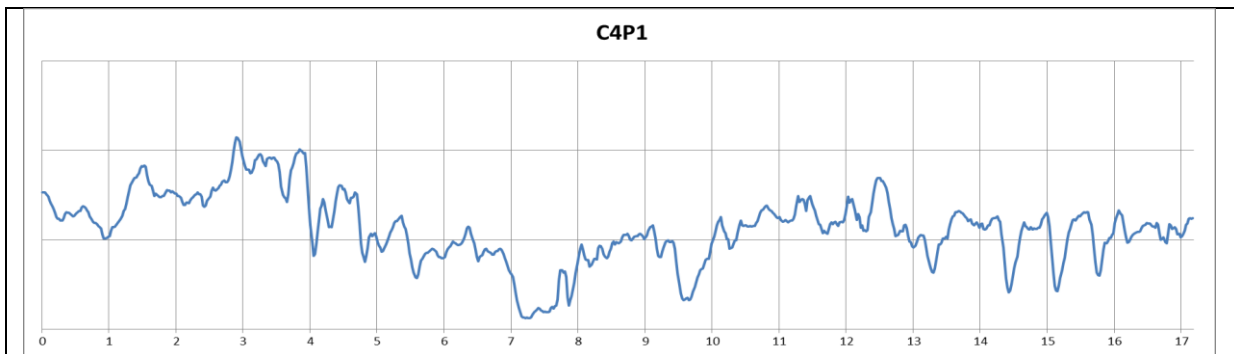
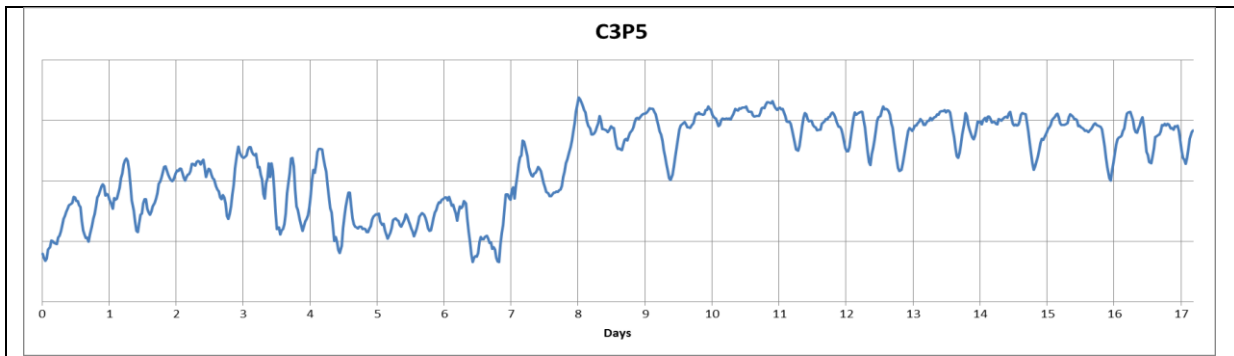
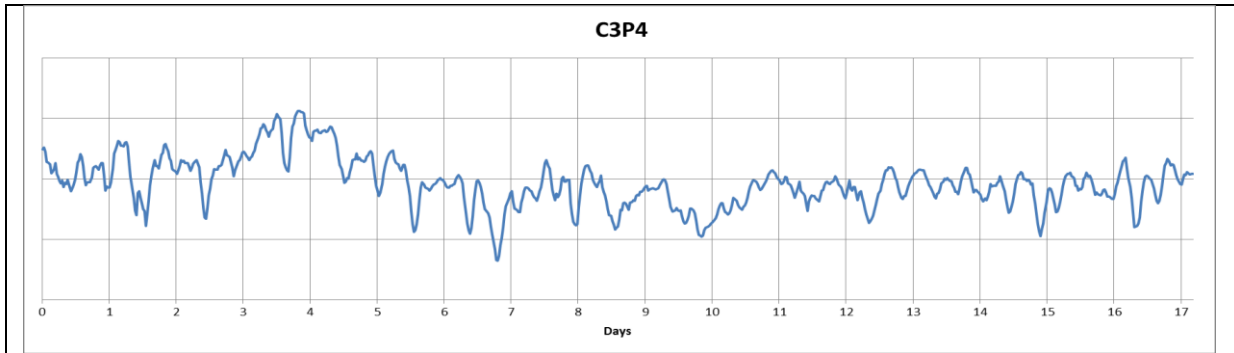
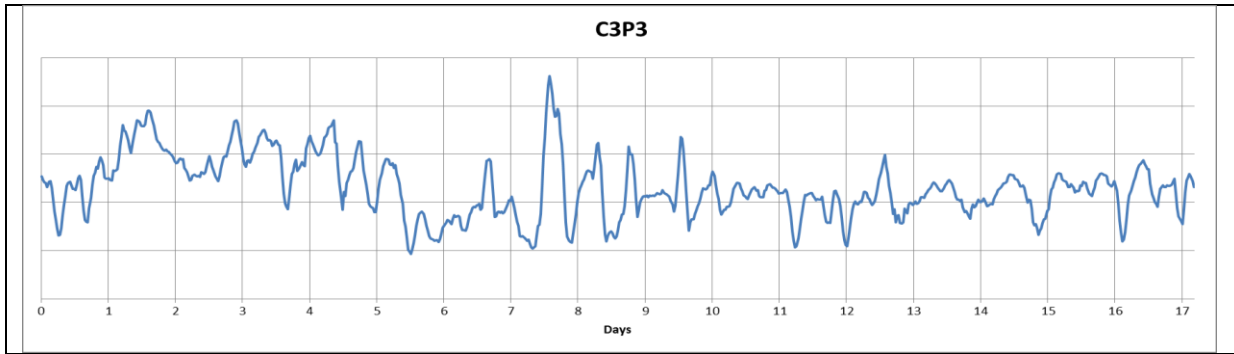
9.1 Experiment 1 Biosensor assessment phase

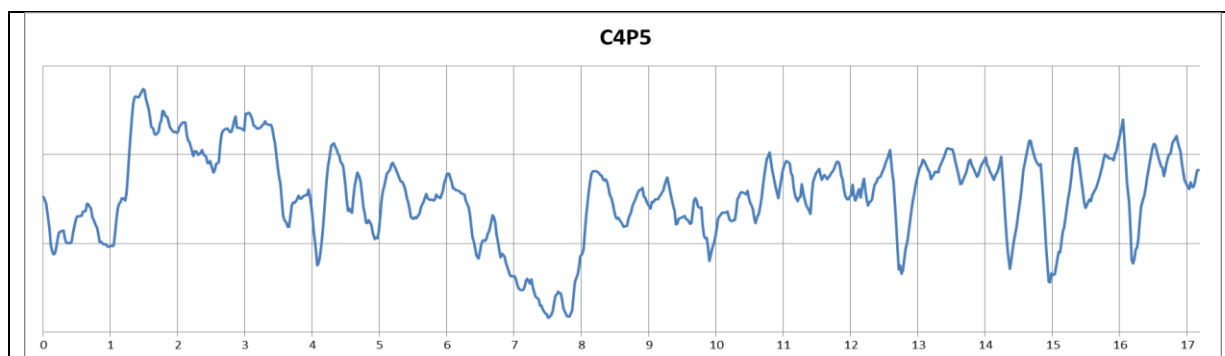
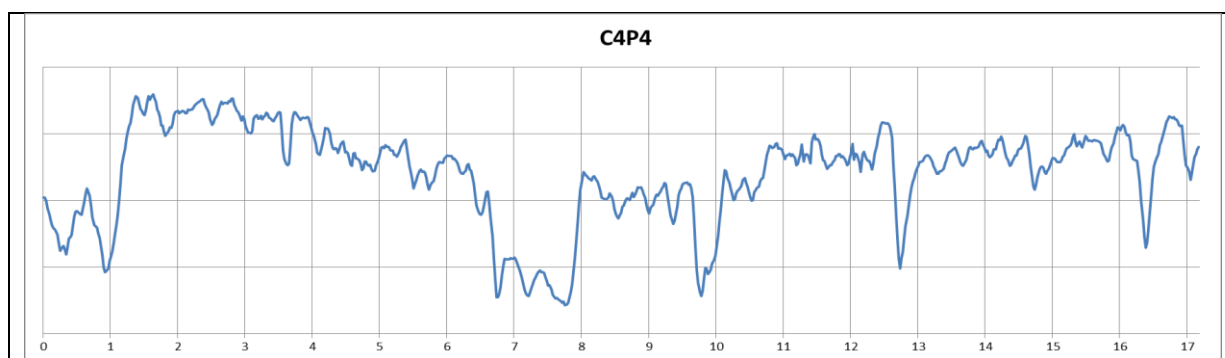
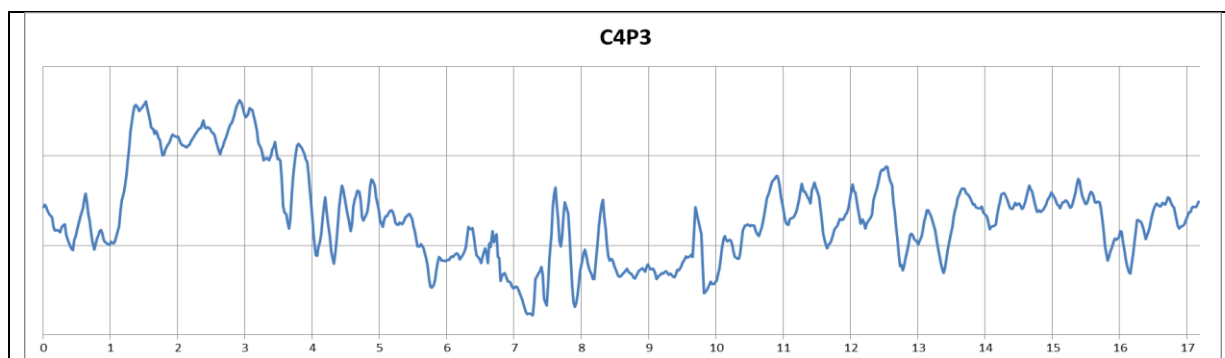
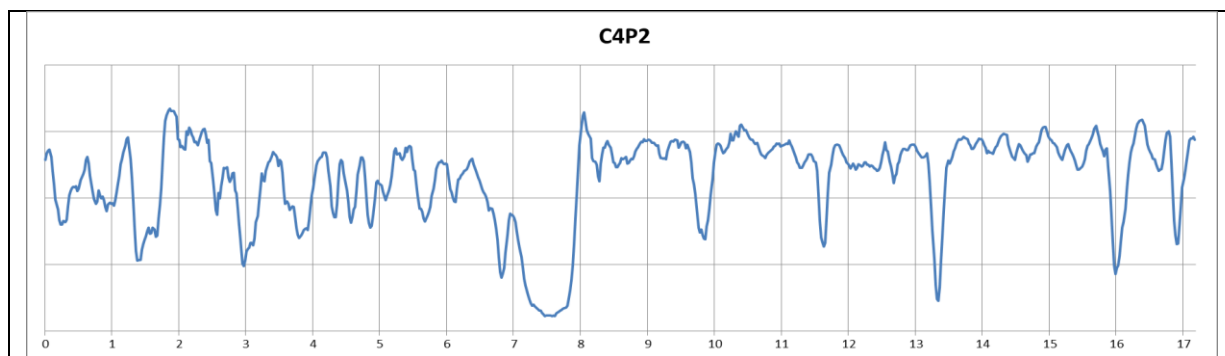
The following graphs are showing all analyzed polyps and corals from the first experiment

On all Following graphs vertical axis, positive value upwards relates to the polyp tentacles extending out from the polyp skeleton, and a negative downwards movement relates to the retraction of the tentacles into the polyp.





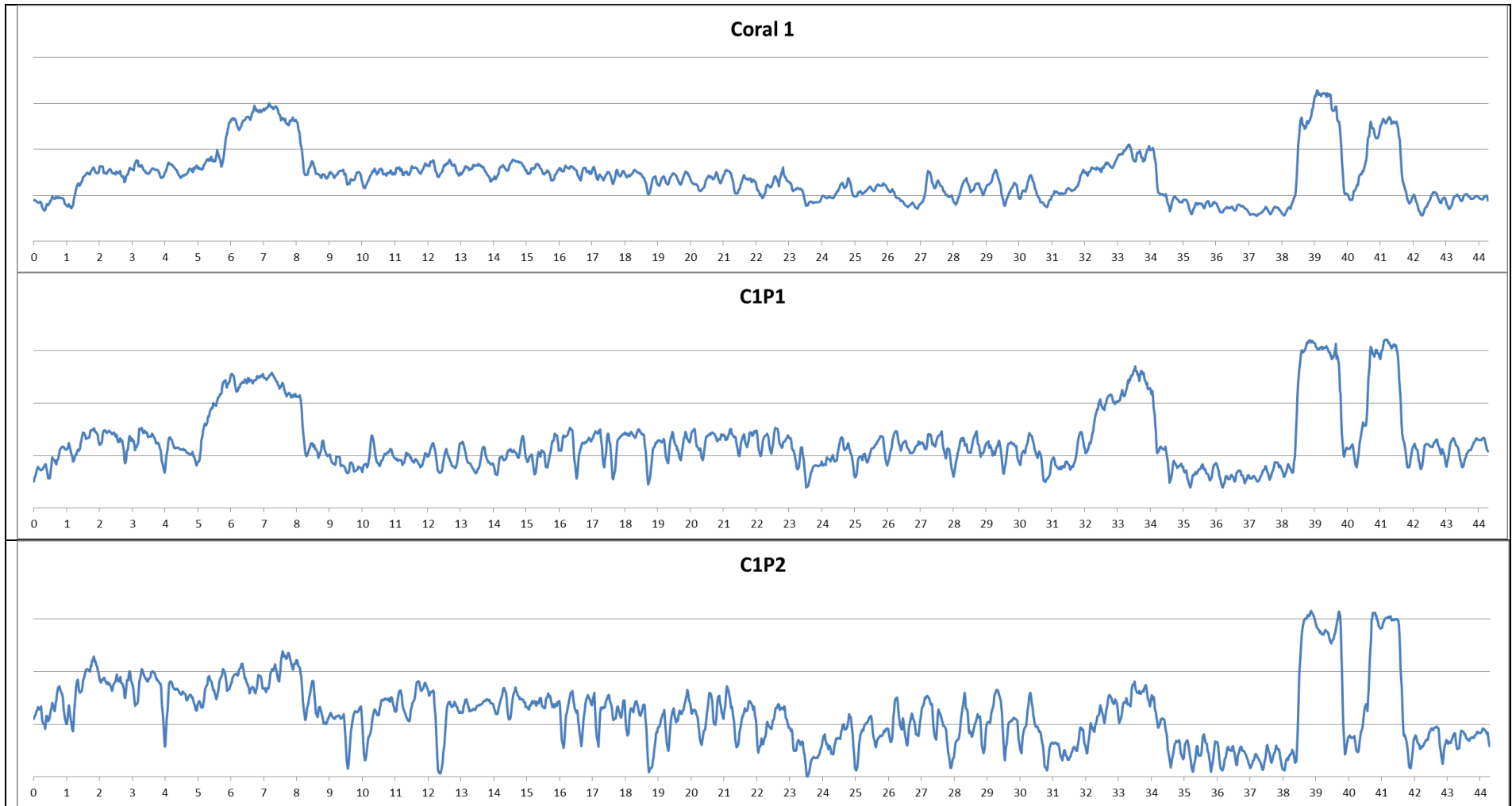


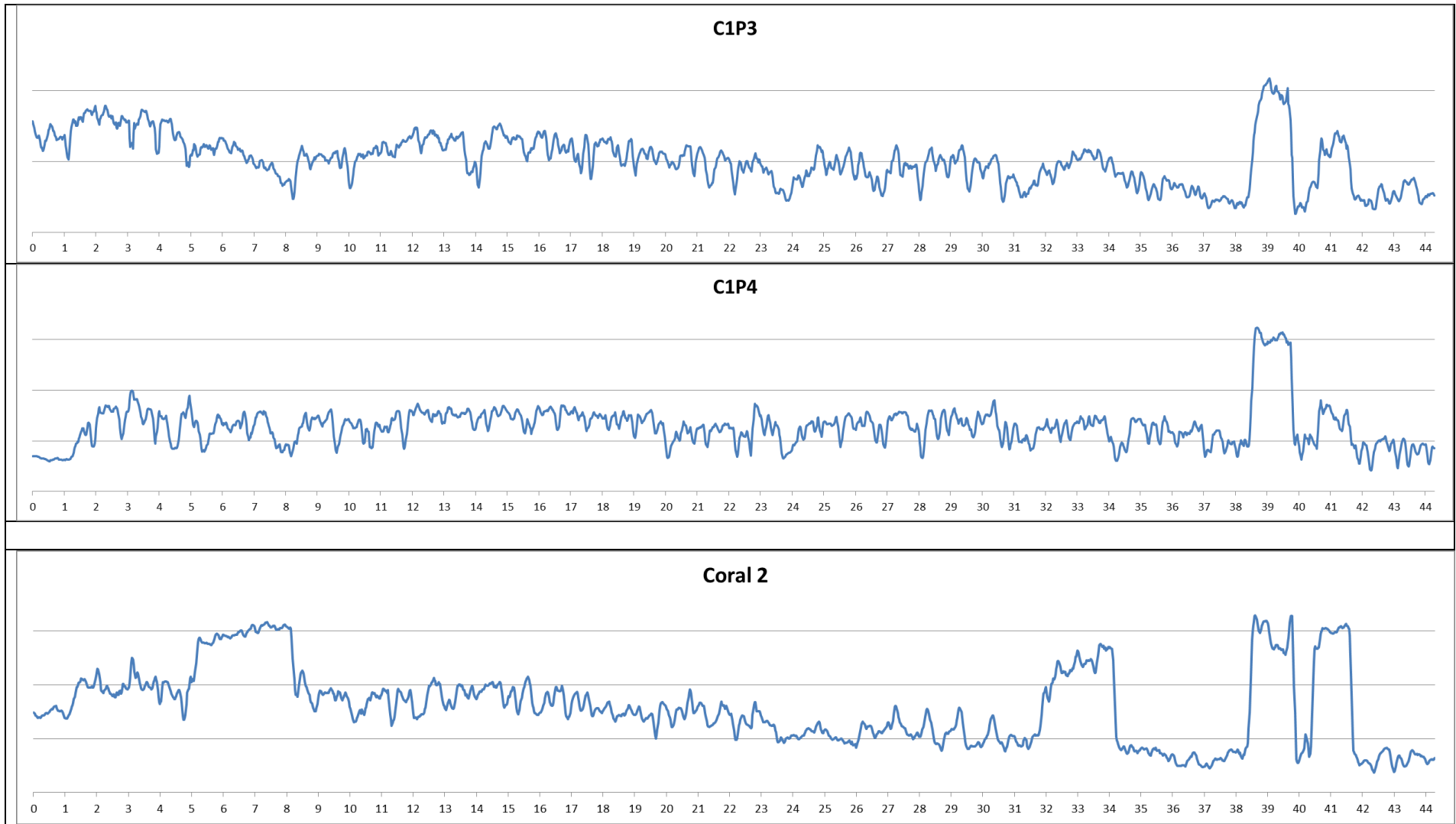


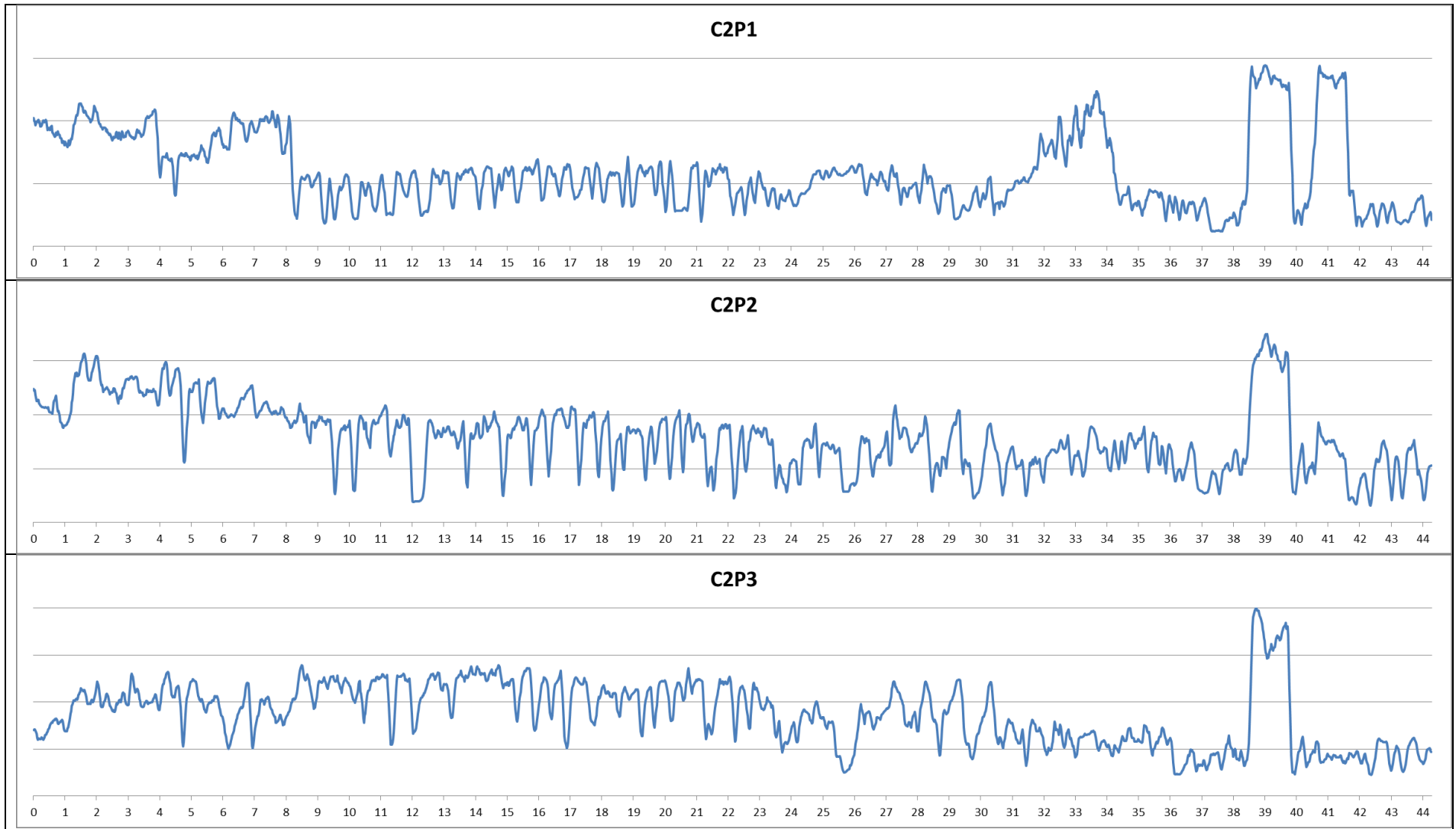
9.2 Experiment 2 Biosensor exposure evaluation

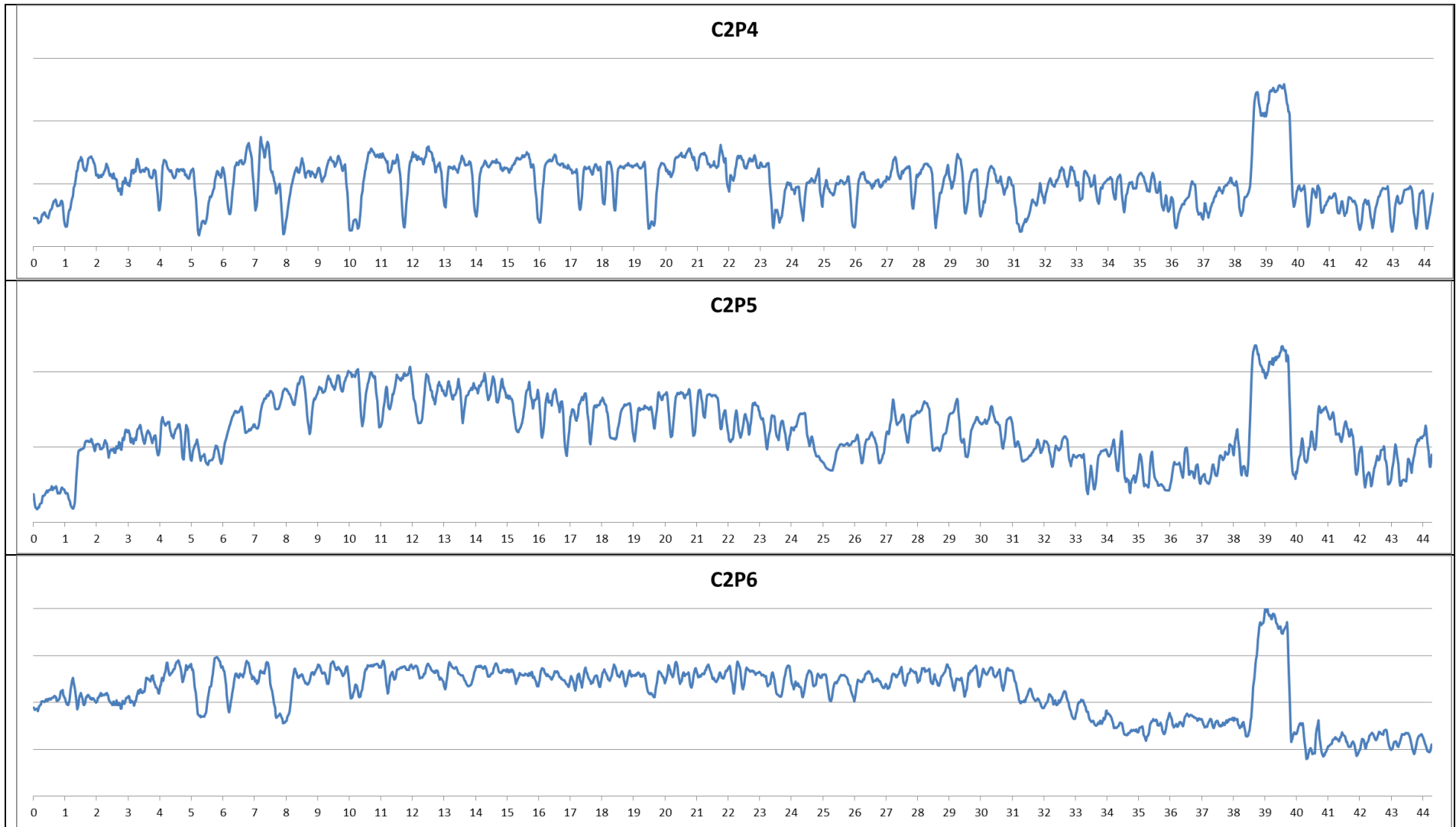
The graphs are showing the complete study from day 1 to 45 with the software configurations from experiment 2.

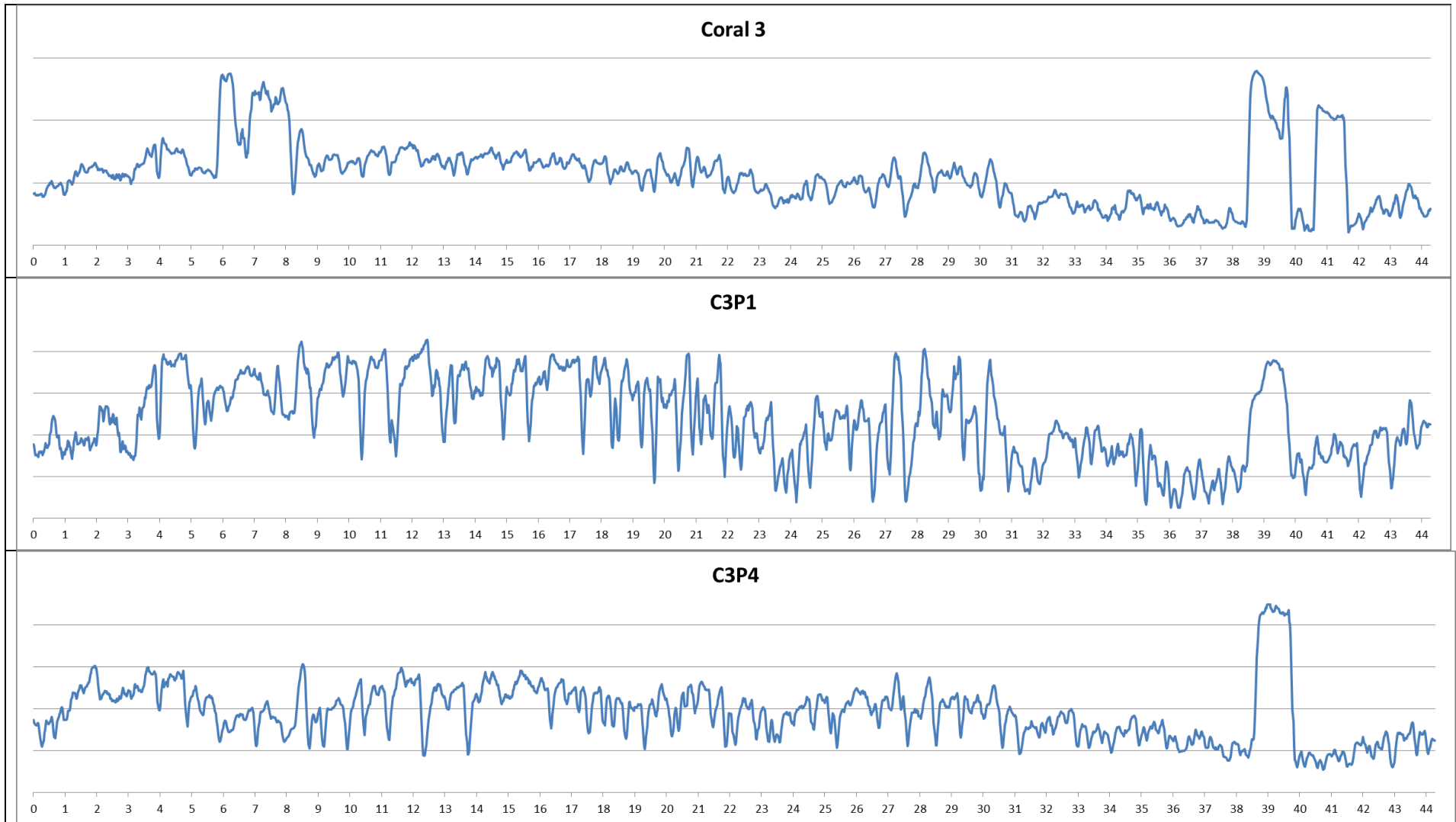
On all Following graphs vertical axis, positive value upwards relates to the polyp tentacles extending out from the polyp skeleton, and a negative downwards movement relates to the retraction of the tentacles into the polyp.



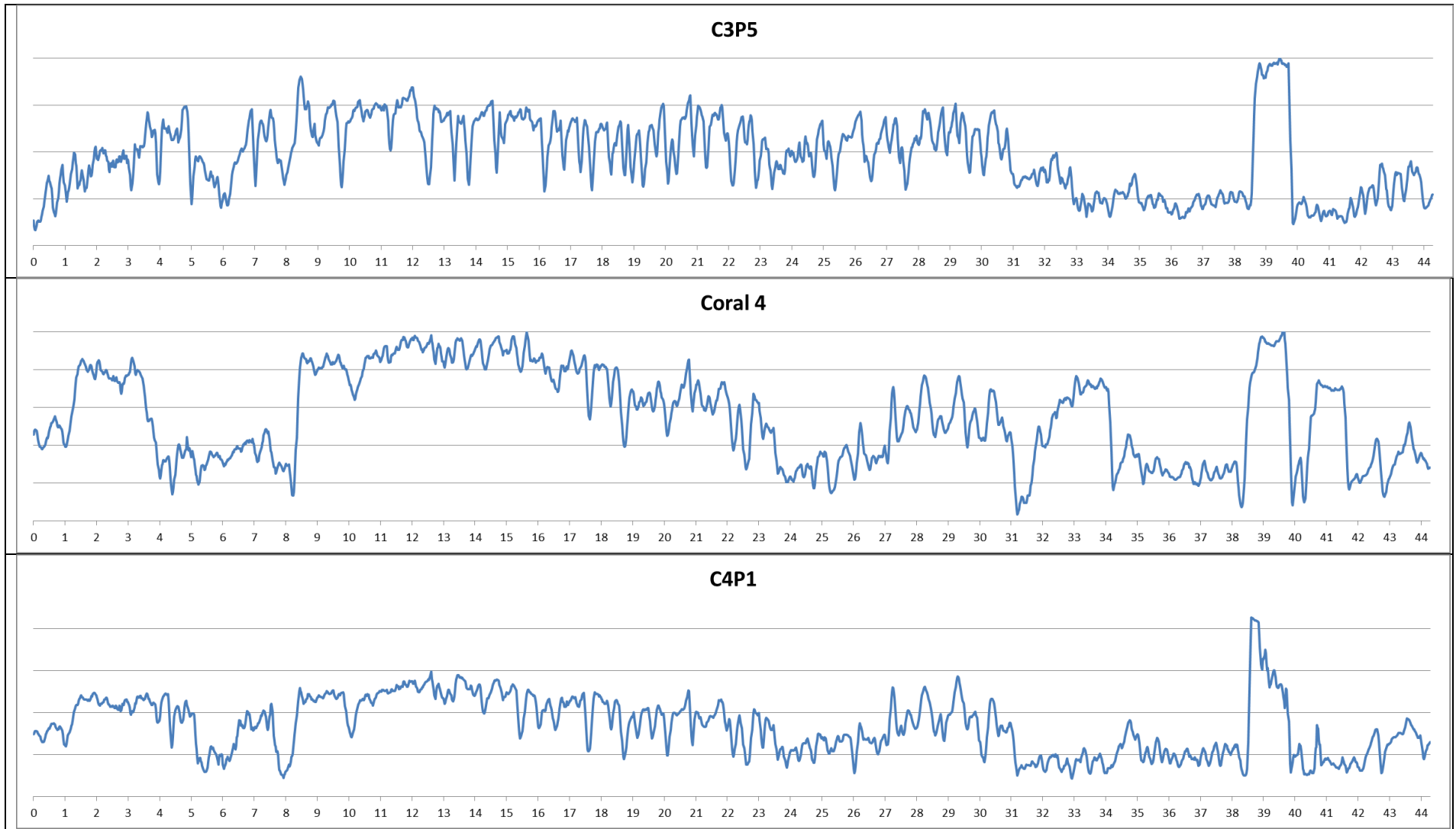




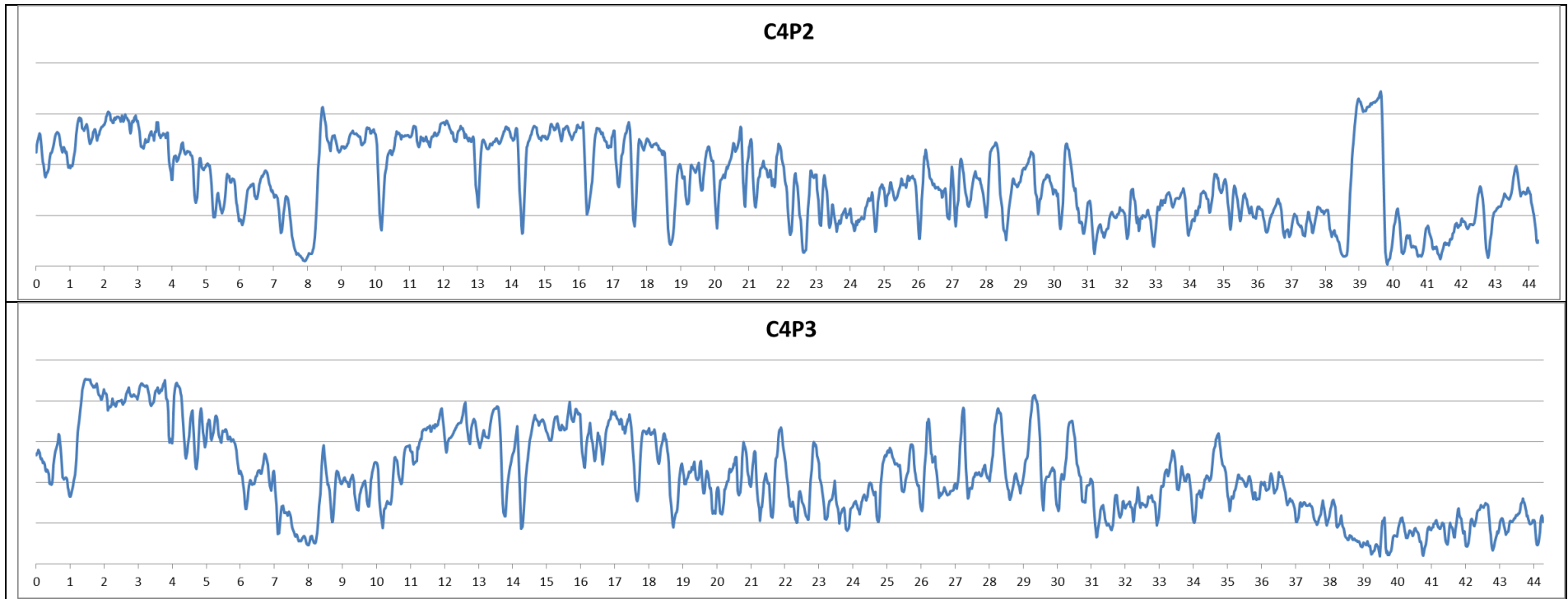




Cold Water Coral *Lophelia Pertusa* Biosensor



Cold Water Coral *Lophelia Pertusa* Biosensor



10 Appendix C

10.1 Ordinal scale evaluations

Table below is showing which biosensor indicators scored which strength on the ordinal scale evaluations.

Indicators	Strong Indication	Weak Indication	No Indication
Coral 1	1		
C1P1	1		
C1P2	1		
C1P3	1		
C1P4		1	
Coral 2	1		
C2P1	1		
C2P2	1		
C2P3		1	
C2P4		1	
C2P6		1	
C2P5	1		
Coral 3	1		
C3P1	1		
C3P4		1	
C3P5		1	
Coral 4	1		
C4P1	1		
C4P2	1		
C4P3	1		
sum indicators	14	6	0

11 Appendix D

11.1 Submersible web camera

Below is a copy of the preliminary drawings made in order to construct a shallow water chamber for the web camera.

