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Preface

The following thesis is a part of the South Africa – Norway research co-operation on Blue Economy, Climate Change, the Environment and Sustainable Energy (SANOCEAN) funded by the Research Council of Norway and the National Research Foundation of South Africa. The thesis was supervised by Professor Daniela M. Pampanin.

Abstract

In this thesis, farmed Mytilus edulis were caged and deployed at three locations at the coastline of the North Sea to monitor the potential biological effect related to the presence of pharmaceuticals in the marine environment. Station 1 was selected west of Kvitsøy, Rogaland, and was used as a reference location. Another site east of Kvitsøy was also included (station 2) located close to residences and an aquaculture facility. Finally, station 3 was by the wastewater outlet of IVAR.

Prior to the field deployment, a group of mussels were sampled for a time zero (T0) biological determination (pre-deployment data collection). After 1, 2, and 4 weeks of transplantation, mussels were sampled to evaluate time-integrated biological responses. A multi-biomarker approach was used to assess the biological effect on the mussels, and the different field locations were compared. The selected biomarkers were analysed: lysosomal membrane stability (LMS), evaluated by the neutral red retention time assay; catalase (CAT) activity, a measurement of oxidative stress; acetylcholinesterase (AChE), a measurement of neurotoxicity; condition index (CI), and stress on stress (SoS), the last two as physiological parameters of general health.

After 4 weeks of deployment, mussels from stations 2 and 3 had a significantly lower value ofCI, compared to station 1 and T0. For the SoS assay, a significant decrease in the median survival time was observed in mussels caged at stations 2 and 3 after 4 and 2 weeks of deployment, compared to station 1 and T0. AChE activity was significantly increased in mussels caged at station 3 after 1 week and 2 weeks of deployment compared to organisms from station 1 and T0, while station 2 organisms had significantly higher activity compared to T0 samples. After 4 weeks of translocation, organisms from all stations had significantly increased AChE activity compared to T0; however, there was no significant difference between the samples. CAT activity significantly decreased in mussels caged at for all stations after 1 week of deployment compared to T0 samples. After 2 weeks, organisms caged at station 2 had significantly lower CAT activity compared to the ones from stations 1, 3, and T0. Samples after 4 weeks of deployment were not significantly different. However, CAT activity in mussels caged at station 3 was significantly decreased in time (2 to 4 weeks).

A principal component analysis (PCA) was carried out to summarize all the biomarker responses. The PCA clearly distinguished the stations from each other, separating station 3 clearly from station 1 and T0 after 1, 2, and 4 weeks deployment. The biomarker responses at station 2 were in between stations 1 and 3 after 1 week of deployment, most similar to station 1 and T0 after 2 weeks of deployment, and after 4 weeks, more similar to station 3 responses. The overall results showed that organisms close to the wastewater outlet (station 3) were affected by the surrounding environmental conditions, while tend to compensate towards the end of the transplantation time.

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List of Acronyms

AChE Acetylcholinesterase

BAC Background assessment concentration

CAT Catalase

CEC Contaminants of emerging concern

CI Condition Index

DDD Defined daily dose

EAC Environmental assessment criteria

GST Glutathione-S-Transferase

LMS Lysosomal membrane stability

LOD Level of detection

LOQ Level of quantification

NRRT Neutral red retention time

PCA Principal component analysis

PBTs Persistent bio-accumulative, and toxic chemicals

POPs Persistent organic pollutants

SOS Stress on stress

WWAP United nations water assessment programme

1. Introduction

Ecosystems provide essential services, such as food and habitat provisions, purification of water, erosion control, nutrient cycling, and climate regulation, but these services are completely dependent on a rich biodiversity. Pollution, overexploitation of natural resources, and climate change are putting high pressures on the ecosystems and are causing biodiversity to decline worldwide. Marine, coastal and freshwater biodiversity is crucial for the livelihood of over 3 billion people in the world. (OECD, 2018).

One of the ways contaminants are finding their way into the marine environment is through wastewater outlets. Discharged wastewater may contain biotic and abiotic stressors such as nutrients, persistent organic pollutants (POPs) persistent, bioaccumulative, and toxic chemicals (PBTs) and contains low levels of complex mixtures of contaminants of emerging concern (CECs). CECs include chemical compounds such as pharmaceuticals and personal care products, androgens and oestrogens, pesticides, industrial by-products, and their metabolites (Archer et al., 2017; Jasinska et al., 2015; Jjemba, 2018). CECs are often not removed by secondary treatments due to their physio-chemical properties. There are no regulations monitoring the release of those chemicals in the recipient ecosystem. As wastewater is continuously pumped out in the sea, the pharmaceuticals become pseudo-persistent; the rate of biotransformation and removal rates are counterbalanced (Petrovic et al., 2003). Pharmaceuticals are usually present in low concentrations in the environment and sometimes they are below the limit of detection (LOD) for some analytical methods. Even though the pharmaceuticals may not cause an observable acute effect, they can have long-term effects, and harming the organisms by affecting fecundity and reproductive success (Galus et al., 2013; Kidd et al., 2007; Lister et al., 2009; David & Pancharatna, 2009; Mimeault et al., 2005). An effect on the individual organism can cause a chain reaction where it affects the population size, community, and biodiversity of the ecosystem. Another aspect of water contamination is the bioaccumulation and biomagnification potential, where contaminants are accumulating in the tissue of organisms and then move up the food chain.

In 2015, the United Nations water assessment programme (WWAP) published a water report calling for a focus on wastewater discharges, as poor wastewater management has a direct effect on the degradation of ecosystems (UNESCO, 2015). As it is more expensive to

rehabilitate an ecosystem than to preserve it, one has to recognize healthy ecosystems' economic and social value. There has to be an increased understanding of the symbiotic relationship between human uses of water and environmental needs.

1.1 Aim of the Study

The aim of this study is to assess how organisms living in the surrounding area of a marine wastewater discharge are affected by environmental concentrations of pharmaceuticals. Mussels *Mytilus edulis, were* used as a sentinel species and deployed at three different stations. To evaluate the potential effects related to the presence of pharmaceuticals, biomarkers at several biological levels of organization were assessed. Biomolecular, cellular and physiological alterations were monitored over a four-week deployment period for a time-integrated response. These obtained results were used to:

1) evaluate if the biomarkers chosen were capable of providing a response
2) evaluate if the responses obtained are over threshold levels, and consequently of concern
3) evaluate the potential effect on populations and ecosystems
4) evaluate if action is necessary to mitigate the presence of pharmaceuticals
5) suggest which biomarkers that should be included in similar environmental studies in the future
6) suggest analysis to close the remaining knowledge gap

1.2 Pharmaceuticals in wastewater discharges

Common sources of wastewater are domestic wastewater from residential properties, commercial, institutional, and public facilities, industrial wastewater, infiltration/inflow to the collection system (indirectly or directly), and stormwater. The latter is a result of rain or melting snow (Tchobanoglous, et al., 2014).

Wastewater contains nutrients, POPs, PBTs, and CECs. A nutrient-rich effluent, mainly nitrogen and phosphorous, can cause dead zones by eutrophication and water quality being

degraded (Tchobanoglous et al., 2014). Sources for POPs are pesticides, industrial chemicals, and oil production. Due to their persistence, toxicity, global distribution, and potential for bioaccumulation and biomagnification, they are considered hazardous. The production, import, and use of POPs are banned by the Stockholm convention 2004 (Verhaert et al., 2017; EEA, 2010). Despite the ban, POPs still find their way into aquatic environments.

Although still relevant due to their persistence in the environment, scientists' focus of interest and awareness has shifted the last two decades to CECs. These compounds find their way to the aquatic environment through wastewater effluents, sewer overflow, septic tank release, industrial outfall pipes, manufacturing of narcotics, aqua- and agriculture, landfill leachate, ship waste, and aquatic recreation (Prichard & Granek, 2016; Petrovic et al., 2003). Globally, pharmaceuticals are detected frequently in coastal waters, with concentrations varying from nanograms per litre to micrograms per litre. These concentrations are low, however potentially relevant as contaminants as pharmaceuticals are designed to be effective at low dosages. Pharmaceuticals are designed to evoke certain effects on target organisms, potentially causing unwanted effects in nontarget organisms. When medications are used by target organisms such as humans and farmed animals, the bioactive ingredients are only partially metabolized (Daughton & Ternes, 1999). Additionally, when active pharmaceuticals ingredients (APIs) are metabolized in phase I and II in the target organism, the APIs can be transformed to a more toxic metabolite (Gonzalez-Rey & Bebianno, 2012). The APIs and their metabolites are then excreted through faeces and urine. Further decomposition might happen in the wastewater treatment, before the release into the aquatic environment. The release through wastewater is causing marine organisms to bioaccumulate these compounds and their metabolites. Also, they can be biomagnified in the food chain. (Gilroy et al., 2012; Fabbri & Franzeletti, 2016). By exposure to environmentally relevant concentrations of pharmaceuticals, specific animal functions such as development, growth, and reproduction can be affected. This can potentially affect the populations' genetic diversity; by reducing the number of reproducing individuals, the inbreeding in a population will increase (Bickley et al. 2013). Effects from pharmaceuticals can cause secondary effects, such as disturbing the natural balance in the food web, which can decrease or promote the ability for other species to survive.

As an example of behavioural effects, Matus, et al. (2018) found a significantly altered swimming pattern and pigmentation in the fish *Phalloceros harpagos* by exposure to

propranolol. The same study found a preference for the dark compartment in the fish when exposed to paracetamol. These effects can decrease the fish's ability to feed, escape or hide from predators, which affects their chance of survival and reproduction.

Globally the usage of pharmaceuticals has increased the last 20 years (Fabbri & Franzeletti, 2016). Populations are expected to increase, and thereby the impact of CECs on coastal environments is also expected to increase. In Norway, the sale of pharmaceuticals has nearly doubled since 2000. From 2018, to 2019, the increase was at 2.6 % measured in defined daily dose (DDD) (Sommerschild, 2020).

1.3 Wastewater treatment plants

Data from the United Nations World Water assessment programme (WWAP) report from 2015, shows that approximately 70% of wastewater discharges are released untreated into the waters, such as rivers, lakes and sea. By Norwegian law, wastewater treatment facilities are required to remove 70% of biologically degradable organic material from the water (IVAR, n.d.). According to Statistics Norway (SSB, 2018), 62% of the population in Norway is connected to advanced wastewater treatment facilities such as biological or chemical treatment. In Rogaland, the percentage is at 61. However, SSB reported in 2018 that the compliance with the treatment permits is at 58%, increasing by 3% since 2017. In the same time period, the compliance was 22% in Rogaland county.

The wastewater at Nord-Jæren in Rogaland is treated by IVAR at IVAR Sentralrenseanlegg Nord-Jæren (SNJ). This is one of the largest and advanced treatment facilities in Norway (IVAR, n.d.). The facility receives wastewater from the 300 000 inhabitants of Randaberg, Sola, Stavanger, Sandnes and Gjesdal municipalities. IVAR states that they often achieve up to 80% removal of organic material by using their biological treatment.

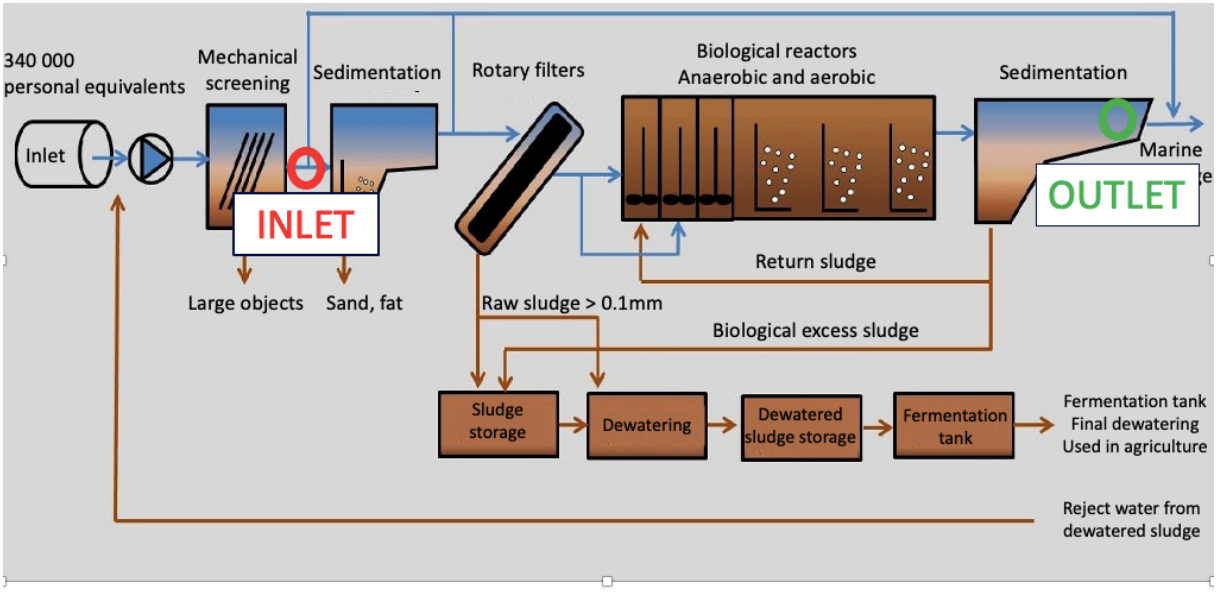

Figure 1: The wastewater treatment process at IVAR (SNJ) adapted from ppt presentation by Leif Ydstebø, the process engineer at IVAR.

The wastewater is first mechanically screened through grates with 6 mm pores, where objects such as paper, plastics, cloths, wet wipes and q-tips are removed and sent for combustion. In the second step of treatment (see figure 1 above), fat and sand from the wastewater are removed in an aerated sedimentation tank, where fat is scraped off from the top and sand sediments to the bottom while aeration keeps the organic particles suspended. Approximately 50% of the particles in the water is then removed in the filter system containing 20 rotary filters with a pore size of 0.1 mm. From the rotary filters, the water goes to bioreactors where the organic material is broken down by bacteria. The bioreactors are divided into two parts, first an anaerobic part where phosphorous accumulating bacteria will grow and remove fatty acids and releasing their own storage of phosphorous. The second, and largest part of the bioreactor is aerated to give the bacteria oxygen to break down the organics and let the phosphorous accumulating bacteria store phosphorous again. The phosphorous will thereby be removed, as the amounts of bacteria is multiplying (IVAR, n.d.). After the bioreactor, the colonies of bacteria are removed from the water through sedimentation. The colonies are either recycled back to the reactor or removed as slam. This type of treatment is defined as secondary treatment. The treated wastewater is discharges in Håsteinfjorden, at 80 m dept, 1.6 km from the coast.

A study done by Angeles et al., (2019) assessing the removal of pharmaceuticals by advanced wastewater treatment, found that less than 50 % were removed by biological treatment.

1.4 Environmental monitoring

To classify the environmental quality of an ecosystem, there are five different approaches. One is the chemical monitoring, where one measures some specific chemicals in the abiotic environment such as water and sediment samples. However, these samples may represent just snapshots in time, and there can be large variations in concentrations over time as they are affected by currents, wind, temperature, pH, UV exposure. The last three abiotic conditions, in turn, affect the degradation and metabolism of the parent compound into metabolites, which can be more harmful than the initial contaminant. (Pampanin & Sydnes, 2013; Prichard & Granek, 2016)

Monitoring the bioaccumulation of contaminants in biota is another form of monitoring. By measuring the levels of contaminants accumulated in biota, the exposure to these can be assessed. (Pampanin & Sydnes, 2013). However, the analysis of CECs is challenged by complex mixtures, the high diversity of chemical properties, and usually as low concentrations as parts per billion (ppb) and parts per trillion (ppt) (Petrovic et al., 2003). Due to these low concentrations, there is a lack of analytical methods for the CECs present in the wastewater effluent. The contaminants' different properties challenge monitoring programs designed to monitor CECs and legacy contaminants' presence in the ecosystem. Causing the chemicals to require different methods for sample preparation, pre-treatment, and/or measurement conditions. Additionally, one parent compound may have several metabolites that may need different methods to detect but are still biologically active (Jjemba, 2019). This means that it is complicated, extremely time consuming, and very costly to analyse for all chemicals present in an environmental sample.

Another form of monitoring is represented by the biological effect monitoring, where the exposure and effect in organisms are assessed. This type of monitoring will be further explained in chapter 1.6 and 1.7.

Examining organisms for the occurrence of irreversible diseases or tissue damage is categorized as health monitoring.

At last, there is ecosystem monitoring, which implies looking at the species composition, density, and diversity and thereby assessing an ecosystem's integrity (Pampanin & Sydnes, 2013).

The fate, distribution, and exposure risk of pharmaceuticals in the aquatic environment is dependent on the physical-chemical properties of the chemicals, coastal mixing patterns, and residence times. Hydrodynamic models predicting the dispersion of chemicals are, therefore, a handy tool in multidisciplinary approaches. The fate of these chemicals is however hard to predict, as the mixtures are complex and vary between populations (Petrovic et al., 2003).

The SANOCEAN programme, is a collaboration between South Africa and Norway that started in 2018 to advance cooperation between the two countries. The University of Stavanger and the University of the Western Cape got granted a project to evaluate the environmental impact of wastewater outfalls. In 2019, as a part of this project, an environmental monitoring was carried out in Stavanger and some pharmaceutical compounds were detected. Acetaminophen, atenolol, atorvastatin, caffeine, carbamazepine, diclofenac, ibuprofen, naproxen, sulfamethoxazole and trimethoprim were found in the wastewater outlet of IVAR. However, most targeted pharmaceuticals were not detected in environmental water and sediment samples (Bøe, 2020, *thesis unpublished*). These findings increased the concern of the fate of the pharmaceuticals, and if there is a potential for biological effects in the surrounding environment.

1.5 Biological monitoring

Due to the high cost of analysis and lack of analytical methodologies for some pharmaceuticals it is impossible to analyse for every single contaminant and their metabolites in an environmental sample. (Hecker & Hollert, 2009; Petrovic et al., 2003). One therefore has to choose a certain amount of indicator chemicals to analyse for. The chemicals detected will then be evaluated against exposure studies in the laboratory for each chemical found and this will not account for the effect a complex mixture will have. Another challenge is that

chemicals with a short half-life biologically can still exert a long-term effect, such as affecting the ability to reproduce (Walker et al., 2012). As content and concentrations of different pharmaceuticals vary depending on populations, wastewater treatment, as well as abiotic conditions, local environmental monitoring is essential to make a risk assessment. Concentrations and bioavailability of compounds can vary in the environment, and therefore biomonitoring using biomarkers will give a more accurate understanding of the impact of exposure. Although the concentrations of contaminants may be found lower than the no observed effect concentrations (NOEC) for each chemical, the combined effect of a complex mixture may cause toxic effects (Beyer et al., 2014). It is important to note however, that using biomarkers should not replace chemical monitoring, but contribute to give a better understanding of the status of the environment.

1.6 Biomarkers

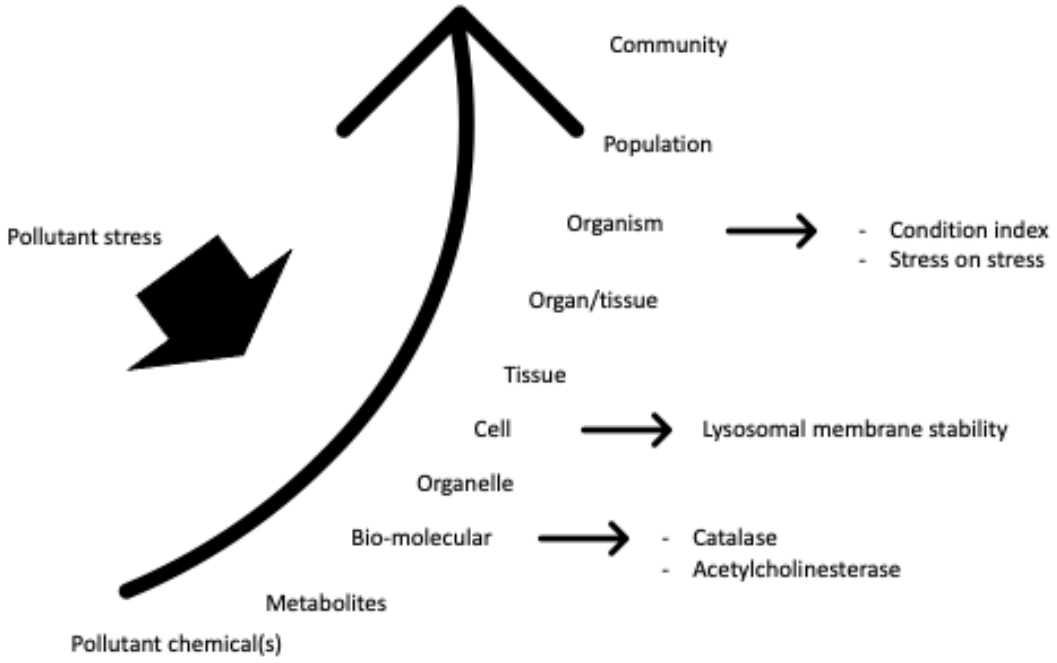

Figure 2: Schematic illustration of the response order after pollution, with the level of organization of the biomarker responses chosen for this thesis. Modified from van der Oost et al. (2003).

A change in a biological response that can be correlated to exposure to contaminants is defined as a biomarker (van der Oost et al., 2003). Biomarkers are early indicators of pollution, giving responses at low concentrations. They can be used to predict long-term effects of pollution, while the effects may still be reversible (Sanni et al., 2017). Biomarkers

are crucial for distinguishing between pollution and contamination of the environment. By also integrating chemical analysis with the use of biomarker responses, an environmental risk evaluation can be made.

To be able to measure sublethal or chronic toxicity of contaminants in the aquatic environment, several standardized, short-term, sensitive and cost-effective biomarkers have been developed (Viarengo et al., 2007; Aguirre- Martinez et al., 2013). A battery of biomarkers should include parameters that assess the different levels of organization shown in figure 2. Firstly, biomarkers at molecular and cellular levels that are sensitive to stress should be included. These biomarker responses act as a first evidence of an effect on the organisms, and thereby provide an early warning, before the effects of toxic chemicals become irreversible or too costly to restore. Secondly, assessment of damage by contaminants on tissue and effects on the tissue level, and thirdly biomarkers assessing an effect on the whole organism. Important effects on the whole organisms are the ability to reproduce offspring, maintain energy balance, and survival capacity. These effects can be extrapolated to population level effects, and if that occur the whole ecosystem can be affected. Predicting if an effect on the population will have an effect on an ecosystems structure and function, is a complicated task as it depends on the biodiversity of the ecosystem (Oskarsson et al., 2014). The consequences of contaminant exposure can vary within the population, in the community and in the whole ecosystem. A negative effect on one species might give a positive or a negative outcome for another species. It depends on the ecosystems buffer capacity, and the interactions between species. Some species are dependent on others to survive, while others are competing for the same resources.

A multiple biomarker approach is essential when dealing with a mixture of compounds, as different compounds have different modes of action (Fabbri & Franzellitti, 2016) The total strain on the health of organisms caused by contamination cannot be assessed by one single biomarker (Regoli et at., 2004).

1.6.1 Condition index

The condition index is a parameter that summarise the general health of mussels or in other words, the degree of nutrition and welfare (Sanni et al., 2017; Pampanin et al., 2005). This biomarker assesses the eco-physiological health of the animal, by measuring the ratio between

the weight of shell and weight of tissue or weight shell and volume of the whole organism. The condition index is a summary of growth, reproduction, and secretion, under the environmental conditions it is exposed to. When the mussel is breaking down contaminants, it is using a fraction of its energy budget, thereby reducing the energy available for growth and reproduction. This parameter is of high ecological relevance as it shows an effect on the organism level and can cause a decrease in the population.

1.6.2 Stress on stress

When organisms are exposed to contamination, the physiological status changes, reducing their ability to tolerate natural environmental fluctuations (De Zwaan et al., 1995; Viarengo et al., 1995). By keeping their valves closed under anoxic conditions, mussels can tolerate anoxia for extended periods (Thain et al., 2019). To keep the valves closed, they need to fuel the adductor muscle using adenosine triphosphate (ATP). However, when mussels experience pollution, elimination and detoxification of contaminants are using metabolic energy, decreasing ATP available for the adductor muscle. The ability to survive without oxygen is vital for mussels in situations where oxygen availability varies. Reduced tolerance for anoxia may, therefore, lead to population decrease.

Consequently, the biomarker stress on stress (SoS) has been used in several studies as an evaluation of the effect of pollutants on the whole organism level (Viarengo et al., 1995, Eertman et al., 1993; Pampanin et al., 2005; Moles & Hale, 2003). It is a sensitive, dose-dependent, and low-cost biomarker that shows an early warning of pollution.

1.6.3 Lysosomal membrane stability

Lysosomal membrane stability (LMS) evaluated *in vitro* by the neutral red retention time (NRRT) is a widely used and sensitive biomarker on invertebrates such as mussels (Bocchetti et al., 2008; Pampanin et al., 2005; Viarengo, et al., 2007). Many contaminants induce destabilization of lysosomal membrane, causing leakage of acid hydrolases to the cytosol (Lowe et al., 1995). This leakage can be visualized under the microscope by adding toxic neutral red dye to a cell medium. The dye is confined in the lysosomes by hydronation, which is essential in toxic defence. In time, the lysosomes will leak the neutral red into the cytosol,

killing the cells. The level of up-take and retainment of the dye is, therefore, correlated to the health of the cell, as already compromised cells (by contamination) will leak the dye faster (Beyer et al., 2017; Martínez-Gómez et al., 2015). The role of the lysosomal system in cell physiology, food digestion, intracellular turnover, immune function and the sequestering and excretion of toxic contaminants is crucial, and therefore this is an important biomarker to include when assessing ecotoxicity (Bochetti et al., 2008).

1.6.4 Acetylcholinesterase

Several natural chemicals and xenobiotics can impact the nervous system (Walker et al., 2012). A biomarker of neurotoxicity is, consequently, essential when looking at the effect of CECs on organisms. AChE has an affinity to hydrolyse the neurotransmitter acetylcholine (ATC), and is, therefore, a crucial enzyme for neurofunction (Ghisi et al., 2016). Organophosphates (OP) pesticides/insecticides, carbamates and copper are well-known toxicants that inhibit AChE, and the degree of inhibition is directly related to the concentrations of these. The toxicants react with the hydroxyl group on the AChE, the functional group, and produces an unreactive phosphorylated enzyme. Acetylcholine then builds up in the synapsis, resulting in an overstimulation of the receptor. (Walker et al., 2012).

Several studies (Mezzelani et al., 2016, Yaqin & Hansen, 2010) have demonstrated that the gill is the most sensitive organ for AChE inhibition, and therefore it is the target organ for neurotoxic stress.

1.6.5 Catalase

Many contaminants' mode of action is to elevate the intracellular generation of reactive oxygen species (ROS), thereby activating antioxidant defences (Regoli et al., 2004). Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione s-transferase (GST) are some important antioxidant enzymes to defend the organism against ROS. The superoxide anion radical (O^{-}_{2}) is converted by SOD to H_2O_2. The enzymes CAT and GPx detoxify H_2O_2 and organic hydroperoxides. CAT, which is mainly a peroxisomal enzyme, is immensely active in reducing H_2O_2 to water. When concentrations of H_2O_2 are low, it is used as a substrate to break down phenols, alcohols, etc., by reducing H_2O_2 to water. When concentrations are high, CAT is a catalyst for the reaction between two H_2O_2

molecules, resulting in two water molecules and oxygen (Regoli & Guiliani, 2014). If CAT does not remove H_2O_2, it can cause the formation of hydroxyl radicals, resulting in lipid peroxidation. CAT thereby prevents ROS formation and is vital in the antioxidant response. The conjugation of several electrophilic products to glutathione (GSH) is catalysed by GST (Barata et al., 2005).

When ROS production exceeds the antioxidant defences, there is potential for oxidative damage to molecules at the cellular level, causing DNA damage, lipid peroxidation, degradation of proteins, and enzyme inhibition. (Carney Almroth et al., 2008). Alterations at several subcellular targets, such as lysosomal membrane stability and DNA, have been correlated to a reduced ability to neutralize ROS (Regoli et al., 2004). Organisms can become adjusted to high production of ROS by up regulating the activities of SOD, CAT, GPx and GST enzymes.

The digestive gland of mussels is the primary site of xenobiotic uptake and oxyradical-generating biotransformation enzymes (Livingstone et al., 1992). It is the model tissue for oxidative stress biomarkers, as this tissue has the highest antioxidant enzyme activities (Livingstone et al., 1990; Faggio et al., 2018)

1.7 Mussels as sentinel species

Through numerous studies, mussels have been proved to be sensitive and suitable sentinel species (Viarengo et al., 2007; Mezzelani et al., 2016; Beyer et al., 2017). There are several advantages with using mussels in monitoring programs. Due to the abundance globally and easy obtainability both in the field and from aquaculture, they are suitable for both in situ studies and laboratory exposure studies (OSPAR convention, UNEP, Beyer et al., 2017). In the North Atlantic region, the native mussel species is *Mytilus edulis*. Distributed along the Norwegian coast one can also find *M. galloprovincialis* (Mediterranean) and *M. trossulus* (Baltic Sea), as well as fertile hybrids of the three congeneric sub-species' (Pampanin et al 2013; Väinölä & Strelkov, 2011). *M. galloprovincialis* has spread globally due to human activities such as global maritime transport, and this sub-species is invasive to many marine ecosystems around the world (Beyer et al., 2017)

Another benefit of using mussels is that they are a sessile species and can easily be caged, and thereby one can easily standardize the results by using farmed mussels, as they then provide information specific to a location. Mussels are primary consumers and feed on phytoplankton by filtering water through their gills. By filter-feeding, they are bioaccumulating pollutants, and in a field experiment, they thereby give a time-integrated indication of contamination in the environment they are deployed (Regoli, 1998). Their role in the ecosystem is important, as they provide food and habitat for several species. Being primary consumers, the levels of pollutants in their bodies are directly correlated with the bioavailability of pollutants in the environment. Xenobiotics move up to higher trophic levels as mussels are food for many species, including humans, and are thereby potentially biomagnified (Beyer et al., 2017)

The size of the mussels is another advantage, as tissue from one individual provide enough material for chemical analysis or for several biological indicators (Beyer et al., 2017). By using mussels, one can optimize the sampling in such a way that the mussels that are sacrificed are used for several purposes. Giving as much information on the environmental status as possible, while reducing the number of individuals sacrificed.

2 Materials and methods

2.1 Sampling sites

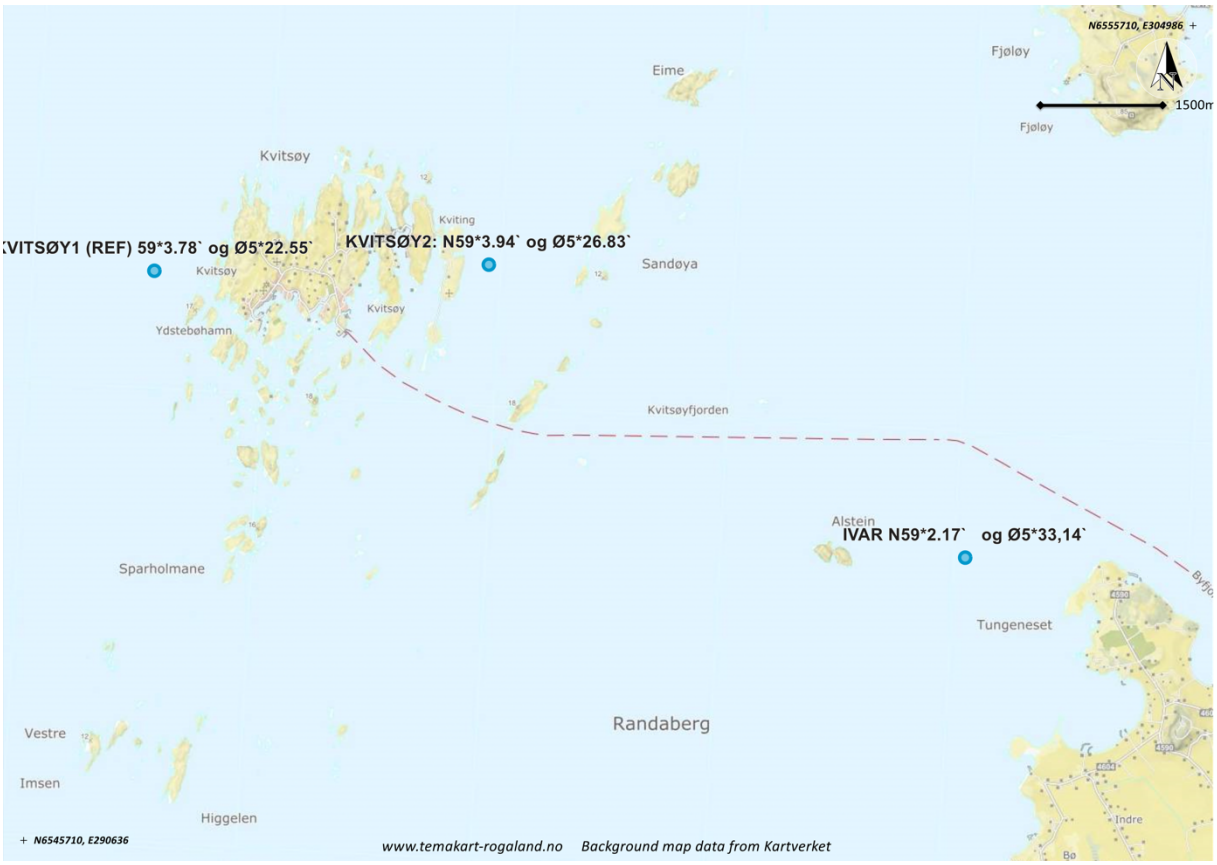

Figure 3: Map over sampling locations, from the left, station 1, 2 and 3

The mussels were caged at three different locations in Håsteinfjorden to assess the effect on organisms by pharmaceuticals in wastewater discharges. Station 1 was located at west of Kvitsøy, (59° 3,78'N, 5° 22,55'E), a quite clean area far from anthropogenic sources of contamination. The second location chosen for this study is located east of Kvitsøy (59°3,94N, 5°26,83′E), relatively clean, but potentially affected by domestic activities and an aquaculture farm.

Figure 4: Up from the left; on the boat "The scallop", that was used to deploy and pick up the mussel cages, station 1 at the day of deployment, station 3 at the day of deployment, and a picture of the mussel cage marking.

The third location, hereby referred to as station 3, is located at 80 m depth by the discharge point of wastewater from IVAR ($59°2{,}17'N, 5°33{,}14$). Models were used to predict where the plume is going to know where to deploy the cages, ensuring that the mussels were exposed to wastewater (Espeland et al., 2020a; 2020b)

2.2 Mussel exposure

Figure 5: From the left: A picture of the metal cage the mussels were distributed in, the mussels inside the cage, and the caged being deployed at station 1.

Mussels (5.99±0.02 cm shell length) were obtained from a farm (near Kvitsøy). At each station three metal cages (figure 3) containing mussels were distributed, and 90 mussels sampled at each station after 1, 2, and 4 weeks. The seawater at station 1, 2 and 3 had a temperature of 13.92±0.03°C, 13.88±0.03°C, and 13.18±0.04°C, salinity 33.82±0.05, 32.62±0.02 and 33.21±0.04, and dissolved oxygen level at 7.96±0.01,7.70±0.01, 7.42±0.01 mg/L. The cages were deployed at 15-20 meters depth.

The mussels were transported to the laboratory in a Styrofoam box with cooling elements. At each sampling, 30 specimens from each site were immediately measured and placed in incubators for the survival in air test. Another 30 specimen were used for the condition index. For chemical analysis 5 mussels were pooled together in triplicate, and the last 15 mussels were utilised for biomarker analyses. Sampling sheets with more detailed information and overview of mussels can be found in the appendix 1.

A group of mussels were sampled at day 0 (T0) and analysed for biological responses to provide information of mussel health pre-deployment.

2.3 Biomarkers/Biological assays

2.3.1 Condition Index

Figure 6: Separated tissues and shells after dissection before drying

Procedure derived from Pampanin et al., 2005

The mussels collected for condition index was rinsed carefully using fresh water and opened. The shells and soft tissue were completely separated (figure 6) and dried at 90°C for 48 h.

To calculate the condition index Lucas & Beninger (1985) formula was used.

CI = dry weight of meat / dry weight of shell

The calculated data from the 30 specimens are reported as mean±standard error. Raw data used in calculation can be found in appendix 2.

2.3.2 Stress on stress

The stress on stress (SoS) test was performed by placing 30 mussels in a humidity chamber at 10°C. The mussels were checked daily, and the death was recorded when the valves gaped, and a response in the mussel was not produced by external stimulus. The experiment ended after 35 days.

2.3.3 Lysosomal membrane stability

This method was originally developed by Lowe, Fossato et al., 1995.

A stock solution of Neutral Red was prepared by dissolving Neutral red dye (20 mg) in dimethyl sulfoxide (1 ml) and stored in a light proof Eppendorf tube. The fresh working solution was then prepared by adding the stock solution ($5\mu l$) to filtered seawater (995 μl), keeping it in a light proof container.

Haemolymph from 15 individual per sample set were drawn from the posterior abductor, using a syringe with filtered sea water (0.1 ml). The samples were then transferred to Eppendorf tubes. The tubes were inverted gently a couple of times to mix the cell suspension before the cell suspensions (30 μl) was transferred to the center of the corresponding microscope slides. The cells adhered to the slides, by keeping the slides in a light proof humidity chamber for 15 minutes at room temperature (20 degrees Celsius). The excess suspension was tapped off gently and working solution of Neutral red (30 μl) was added to the cell layer and covered with a cover slip. The slides were incubated for 15 minutes and then examined individually in a microscope at x40/100 magnification. The samples were examined again after 30, 60, 90, 120, 150 and 180 minutes of incubation time. When the dye loss from the lysosomes to the cytosol were evident in more than 50% of the granular haemocytes, the test was terminated, and the retention time noted. The median retention time for the 15 individuals per sampling was then calculated.

2.3.4 Acetylcholinesterase

This method is a modified procedure derived from Ellman, et al., (1961) and Bocquené & Galgani (1998) (Pampanin et al., 2019). The activity of acetylcholinesterase is measured photometrically by the increase yellow colour caused by the production of 5-thio-2-nitrobenzoic acid (TNB). The following reaction happens:

Acetylthiocholine (ATC) → thiocholine + acetate

Thiocholine + dithiobisnitrobenzoate (DTNB) → 5-thio-2-nitrobenzoic acid (TNB)

Acetylthiocholine is hydrolysed by the enzyme acetylthiocholinesterase (AChE), inhibition of AChE will therefore lead to a decreased production of TNB.

Frozen gills (0.1-0.4 g tissue) from 15 individuals were kept on ice and Tris/HCl buffer (pH 8.0, 100 mM, 0.1% triton X100) was added (1:4 w/v). The samples were homogenized using Omni Tissue Homogenizer (TH), and then centrifuged at 10 000 x g for 20 minutes at 4℃ (. .). The supernatants (S9) from each individual were then obtained and an aliquot from each individual was used for total protein content determination and the remains divided into quadruplicates for analysis of AChE.

Tris/HCl buffer (pH 8.0, 220 $\mu l, spiked\ with$ 0.1 % $Triton\ X$ 100), DTNB (20 μl, 7.89 mM) and S9 (50 μl) was added in a 96-microplate sample well (VWR Tissue culture plates) at room temperature. After 5 minutes incubation ATC (10 μl, 78.9 mM) was added to start the reaction. The enzyme activity was then determined by measuring the increase of absorbance (405 nm) during 10 minutes at room temperature (SpectraMax Paradigm, Molecular Devices).

The following formula (Pampanin et al., 2019) was used to calculate the AChE activity:

AChE activity (umol ATC/min/mg protein) = $\frac{[\Delta A * Vol_T * 1000]}{\varepsilon * light\ path * Vol_S * [Protein]}$

Where,

$\Delta A = change\ in\ absorbance$ (OD) per minute at 405 nm

Vol_T = total assay volume (0.300 ml)

$\varepsilon = extinction\ coefficient\ of\ TNB\ \ (1.36 * 10^4\ M/cm)$

Vol_T = sample volume (0.05 ml)

$[Protein] = concentration\ of protein\ in\ the\ supernatant (mg/ml)$

To determine the protein concentration, the Pierce Modified Lowry Protein Assay kit (ThermoFisher Scientific) was used. Bovine Serum Albumin was used as protein standard to make a standard curve (0-1500 ug BSA/ml). A series of dilutions of one of the samples were

made to decide the dilution of the S9 for protein determination. The S9 had to be diluted to 15 % using Tris/HCl buffer to be within the standard curve.

2.3.5 Catalase

Sample preparation procedure derived from Regoli et al., 2004.

Stock solutions of potassium phosphate buffer (100.29mM, 2.51% NaCl, pH 7.5), bacitracin (100mg/ml), aprotinin (10 mg/ml), Leupeptin (5 mg/ml), Pepstatin (1 mg/ml) were made prior sample preparation and stored at appropriate temperatures. The working solution was prepared right before homogenization to avoid any destabilization.

Digestive gland from mussels from each sampling group (n=7) were homogenized (1:4 (w/v) ratio) in 100mM K-phosphate buffer (pH 7.5), 0.1 mM phenylmethylsulponyl fluoride (PMSF), 0.1 mg/ml bacitracin, 0.008 TIU/ml aprotinin, 1 ug/ml leupeptin, 0.5 ug/ml pepstatin, NaCl 2.5%, using an Omni Tissue Homogenizer (TH). The samples were then centrifuged first at 10 000 x g for 20 min at 4℃. The resulting supernatant (S9) was frozen at -80 ℃. $After\ thawing,$ the S9 samples were centrifuged at 110 000 x g for 1 h at 4℃ to obtain the S100 fraction. After ultracentrifugation, the supernatants (S100) were frozen in aliquots at -80℃.

Catalase (CAT, EC 1.11.1.6) activity was measured by the decrease of H_2O_2, determined spectrophotometrically as a decrease in absorbance at 240 nm. The procedure reported by Hara, 2014 (*Master thesis, unpublished*) was adapted for microplates.

Phosphate buffer (100mM, pH 7.4) 270 μl, 15 μl H_2O_2 buffer (1.674%) and 15 μl sample (S100) was added to each well in a 96-microplate sample well (Greiner, UV-Star® Microplate). Each sample was analysed in quadruplicates. Every plate included a blank consisting of 285 μl phosphate buffer and 150 μl H_2O_2 and a positive control containing 270 μl phosphate buffer, 15 μl H_2O_2 and 15 μl CAT (1 mg/ml). The change in absorbance was measured by measuring the microwell plate every 1 min. The average change of absorbance was corrected against the blank and following formula was used to calculate the CAT activity:

CAT activity (µmol/min/mg proteins) = $\frac{(\Delta A_{240nm}/min)*Vol_T}{\varepsilon*[Protein]*Vol_{s100}}$

Where

$\Delta A_{240nm}/min$ = the change of absorbance at 240 nm per min

Vol_T = total assay volume (0.300 ml)

ε = the extinction coefficient of H_2O_2 (0.040 mM^{-1} cm^{-1})

Vol_{s100} = sample volume (0.015 ml)

$[Protein]$ = protein concentration in mg/ml

The protein concentration of the cytosolic fraction (S100) of digestive glands were determined by Lowry protein assay. A standard curve using BSA (0 - 1500ug/ml) were made to obtain the formula for calculating protein concentration (y = 172,21x3 + 40,649x2 + 939,82x - 17,205). A serial dilution of random digestive glands was made to determine the dilution factor needed. The aliquots of S100 were thawed and diluted 1/10 (w/v) with the potassium phosphate buffer (100 mM, pH = 7.4). Quadruplicates of each sample were measured, and the average was blank corrected before calculation.

2.3.6 Statistical analysis

For analysis of CI, the three different sampling locations after 1, 2 and 4 weeks were compared using one-way analysis of variance (ANOVA) in SPPS software. Levene's test was used to check for homogeneity of variance. Significant differences between stations were checked using Tukey HSD post-hoc test, where $p<0.05$ was set as the level of significance.

For statistical analysis, the survival curves from SOS from the three different locations and sampling times were estimated by Kaplan-Meier in SPSS. The median survival time (LT_{50}), the number of days when 50% of the mussels from the group was dead was estimated. The groups were compared using pairwise comparisons by Breslow (generalized Wilcoxen test).

To analyse the LMS, AChE and CAT results, the different stations after 1, 2 and 4 weeks were compared using Kruskal Wallis non-parametric test in SPSS for significant differences. Non-parametric data is common in ecotoxicological studies. To visualize the distribution of the data collected the results are given in box plots.

3 Results and Discussion

3.1 Condition index

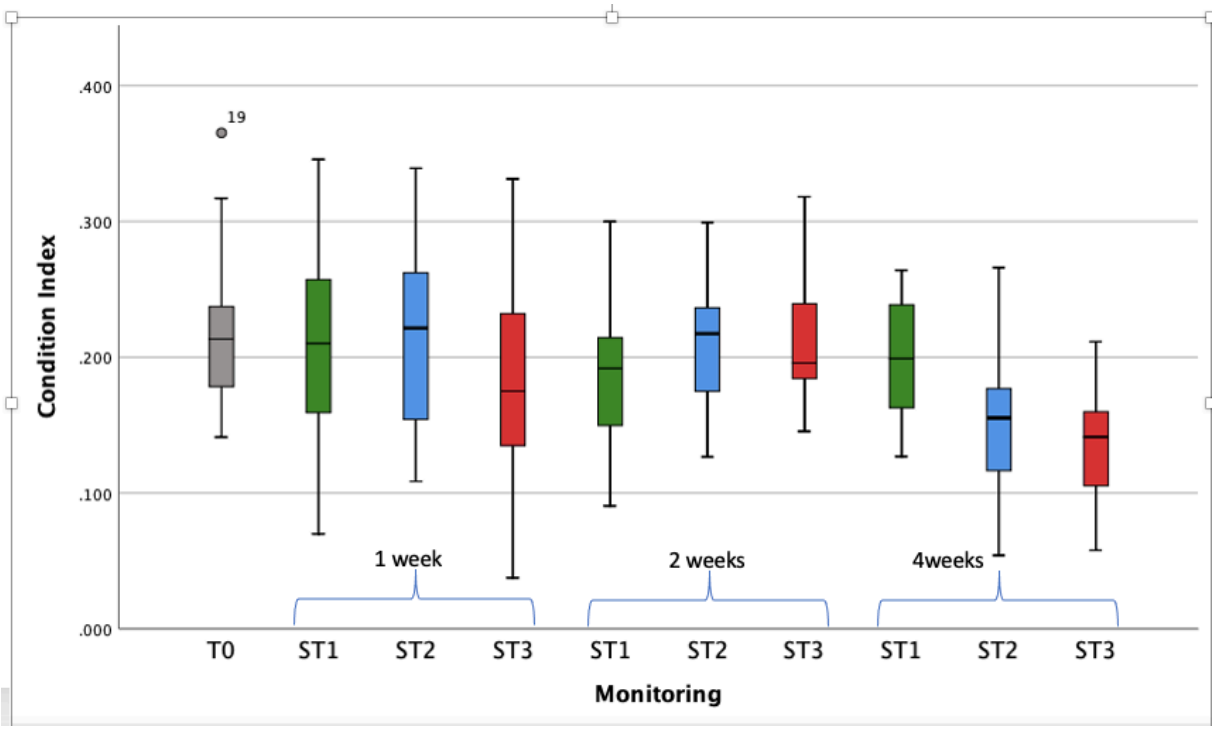

Figure 7: Condition index in percent (w/w), from 1-, 2- and 4-weeks deployment in field, where T0 is the pre-deployment group, ST1 is the from the station west of Kvitsøy, ST2 is the station east of Kvitsøy, and ST3 is the station in the wastewater outlet.

Figure 5 illustrates the CI of the mussels at T0, and for station 1, 2 and 3 after 1, 2, and 4 weeks in field. In the box summarises the distribution of the CI at the different station and times; the median, the interquartile where 50% of the values are found, the whiskers where all values that are 1.5 times lower or higher than the interquartile. The circle above the whiskers at T0, shows an outlier, that are more that 1.5 times the interquartile.

At T_0, the mean CI was calculated to be 0.215±0.009. After 1-week deployment the mean CI at station 1, 2 and 3 were 0.210±0.012, 0.213±0.011, and 0.184±0.012. After 2 weeks of deployment the CIs for station 1, 2 and 3 were 0.184±0.009, 0.212±0.008 and 0.210±0.008 illustrated in the box plot in figure 5.

After 4 weeks after the deployment of the mussel cages, the mean CI for station 1, station 2 and station 3 were 0.200±0.007, 0.150±0.009 and 0.136±0.008. Station 2 and 3 had a significantly lower CI compared to station 1 and T0 and compared to week 1 and 2, showing

that a considerable amount of biological energy has been utilized for the mussels at these stations. The reason for the stress observed is most likely either connected to gametogenesis or contamination (Lucas & Beninger, 1985). Therefore, the gonadal development should be further evaluated through histological analysis of gonads. Several other factors may affect CI, such as salinity (Marsden, 2004) food availability (Hickman et al., 1991; Helson et al., 2007) temperature (Amiard et al., 2004; Lucas & Beninger (1985). The values are within the same range as the CI reported in previous studies (Pampanin et al., 2019) and are considered values of relatively healthy mussels.

3.2 Stress on stress

The median survival time (LT_{50}) for the mussels at time zero, was calculated to be 16 days.

The stress-on-stress test after 1 week of employment shows no significant difference between locations ($p>0.05$). For station 1, 2 and 3 the LT_{50} was 21 days, 14 days and 16 days respectively. The survival curves are illustrated in figure xx.

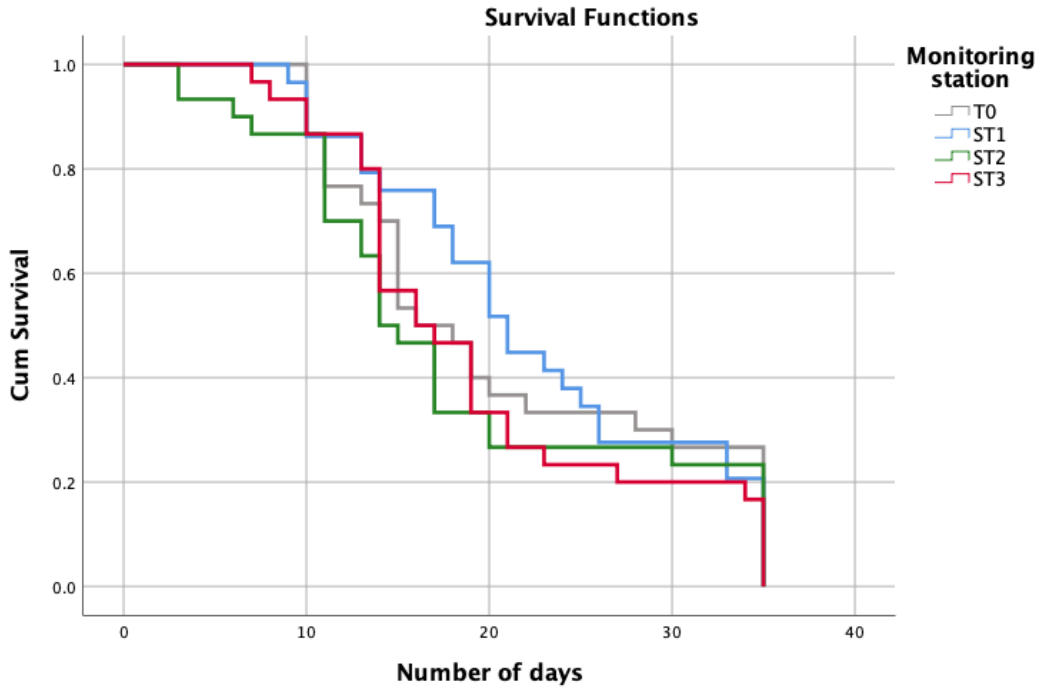

Figure 8: Cumulative survival curve (Cum Survival) in number of days for mussels sampled after 1 week of employment, T0 is pre-deployment group at day zero in grey. Station 1 (ST1) in green, station 2 (ST2) in blue and station 3 (ST3) in red. The experiment lasted for 35 days. There was no significant difference between stations ($p>0.05$).

After 2 weeks of translocation the LT_{50} for station 1, 2 and 3 was 25 days, 20 days and 11 days respectively. The median LT_{50} at station 3 had significantly decreased compared to station 1, 2 and T0 as visualized in figure 7.

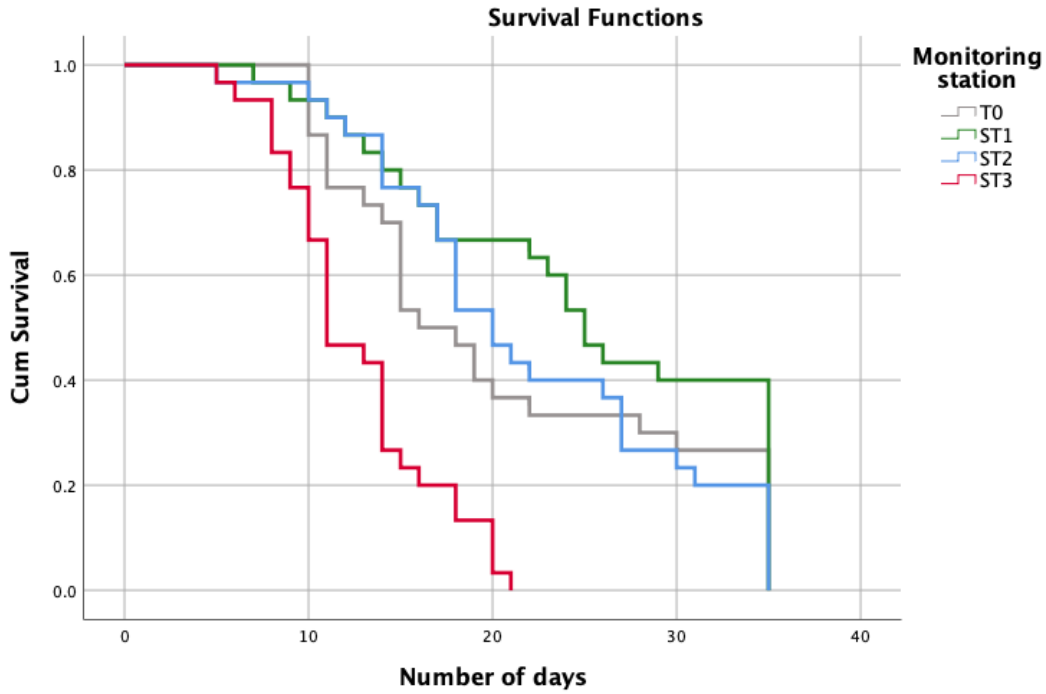

Figure 9: Cumulative survival (Cum Survival) curve in number of days for mussels sampled 2 weeks after employment, T0 is the pre-deployment group at day zero in grey. Station 1 (ST1) is the line in green, station 2 (ST2) is the blue line, and station 3 (ST3) is the red line. Station 3 had significantly lower survival compared to station 1, 2 and T0 ($p<0.05$). The experiment lasted 35 days.

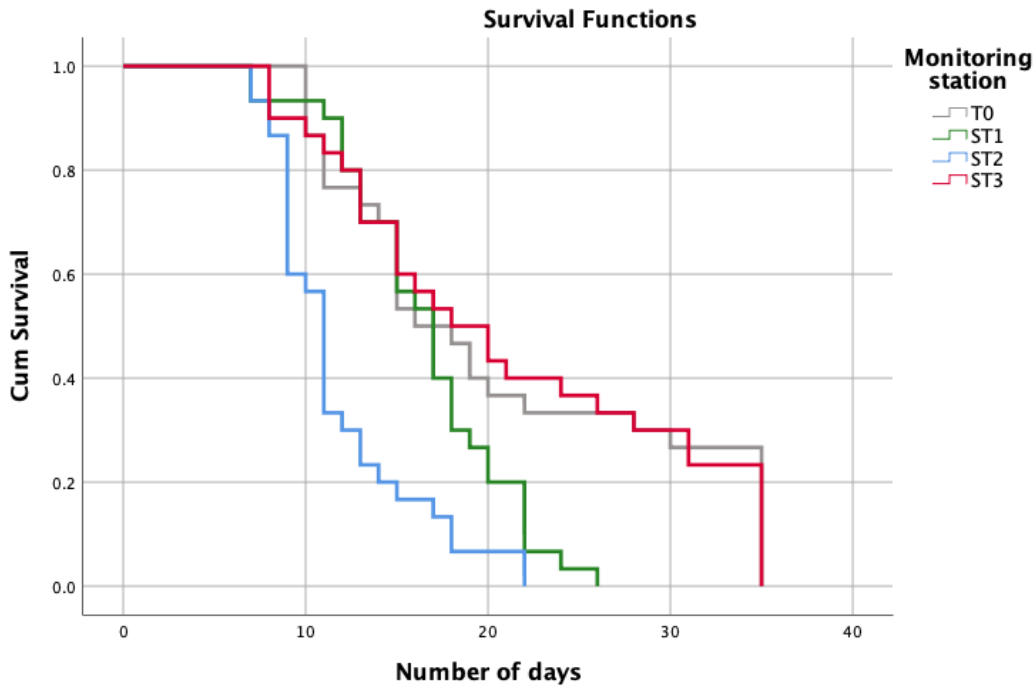

Figure 10: Cumulative survival (cum survival) curve for mussels from T0 (pre-deployment, grey line) and station 1(green line), station 2 (blue line) and station 3 (red line) sampled 4 weeks after translocation. Station 2 had a significantly lower LT_{50} compared to station 1, 3 and T0.

After 4 weeks of deployment, the calculated LT_{50} for station 1, 2 and 3 were 17 days, 11 days and 18 days respectively. The mussels at station 2 had significantly lower LT_{50} compared to station 1, 3 and T0.

To assess biological effects ICES developed background assessment concentration (BAC) and environmental assessment criteria (EAC) for several biological effects (Davies and Vethaak, 2012). For SoS, the BAC is reported to be 10 days and 5 days for EAC for mussels. Mussels are considered healthy if LT_{50} is more than above BAC, stressed but compensating if LT_{50} is between BAC and EAC, and severely stressed if LT_{50} is less than 5 days. Following these criteria, all stations at all sampling times are considered healthy. However, they also report (Davies & Vethaak, 2012) that for *M. edulis* the BAC may be as high as 16 days, indicating that mussels from station 3 after 2 weeks and station 2 after 4 weeks, fall within the category of stressed but compensating.

Moles & Hale (2003) demonstrated a high sensitivity of this physiological biomarker for mussels (*Mytilus trossulus*) exposed to both sewage and secondary wastewater. The median survival time (LT_{50}) was reduced significantly for both stations compared to reference sites, with the sewage decreasing LT_{50} more than the wastewater.

Seasonal variations in the environment can affect the SoS test. Higher LT_{50} has been observed when temperatures are low compared to summer (Davies & Vethaak, 2012). Another confounding factor is the development of gonads. Additionally, Thomas et al., (1999) found that smaller mussels has a significantly higher tolerance to air exposure compared to larger mussels

3.3 Lysosomal membrane stability

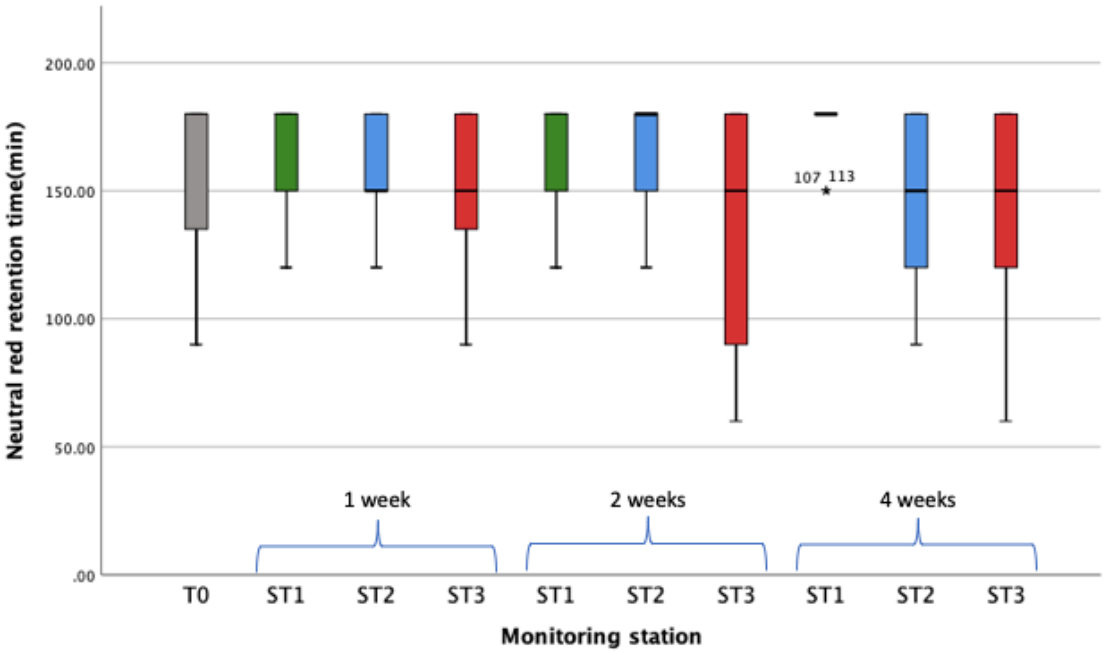

Figure 11:Lysosomal membrane stability expressed as neutral red retention time (NRRT) in minutes in mussel haemocytes, at T0, pre-deployment, and the three stations 1,2 and 3 after one-, two- and four-weeks deployment. No significant difference between stations. The IQ range (box) shows where 50% of the recorded NRRT lays. The whiskers (lines) represent all samples that are less than 1.5xIQ range and ranges from 60 to 180 min. The star at station 1 (ST1) after 4 weeks is an extreme outlier, representing two values that were more than three times the IQ range.

Figure 11 describes the neutral red retention time in minutes for the mussels at pre-deployment (T0, in grey), and the three locations the mussels were deployed at: station 1(in green); station 2 (in blue); and station 3 (in red), after 1 week, 2 week and 4 weeks of deployment. The assay was stopped after 180 minutes. At T0 the median NRRT was 180 min, with whiskers from 90-180 min. After 1-week deployment the median NRRT for station 1, 2 and 3 were 180 min, 150 min and 150 min respectively. After 2 weeks of deployment, the

median NRRT for station 1, 2 and 3 were 180 min, 180 min and 150 min. Finally, after 4 weeks of deployment the mussels from station 1, 2 and 3 had a median of 180 min,150 min and 150 min. As seen in the figure above 11, the variability at station 3 is a bit higher after 2 and 4 weeks (60-180 min). At station 1 after 4 weeks, over 50% of the samples had NRRT of 180 min, with two extreme outliers at 150 min. For station 2, the variability in the samples is similar to other stations (120-180), except a bit higher after 4 weeks (90-180 min). The neutral red retention time of the mussels at the different stations over time were not significantly different. Mussels are considered healthy if NRRT is above 120 min (Davies & Vethaak, 2012; Pampanin et al., 2013).

Factors that may affect lysosomal membrane stability are prolonged hypoxia, malnutrition, extreme overheating and the reproduction cycle (Davies & Vethaak, 2012; Banni et al., 2015).

A significant decrease in LMS in the mussel *M. galloprovincialis* after exposure to environmental concentrations of fluoxetine, propranolol, acetaminophen, diclofenac, ibuprofen, ketoprofen and nimesulide and the antibiotic oxytetracycline has been observed in several studies (Banni et al., 2015; Franzeletti et al., 2015; Mezzelani et al., 2016)

3.4 Acetylcholinesterase

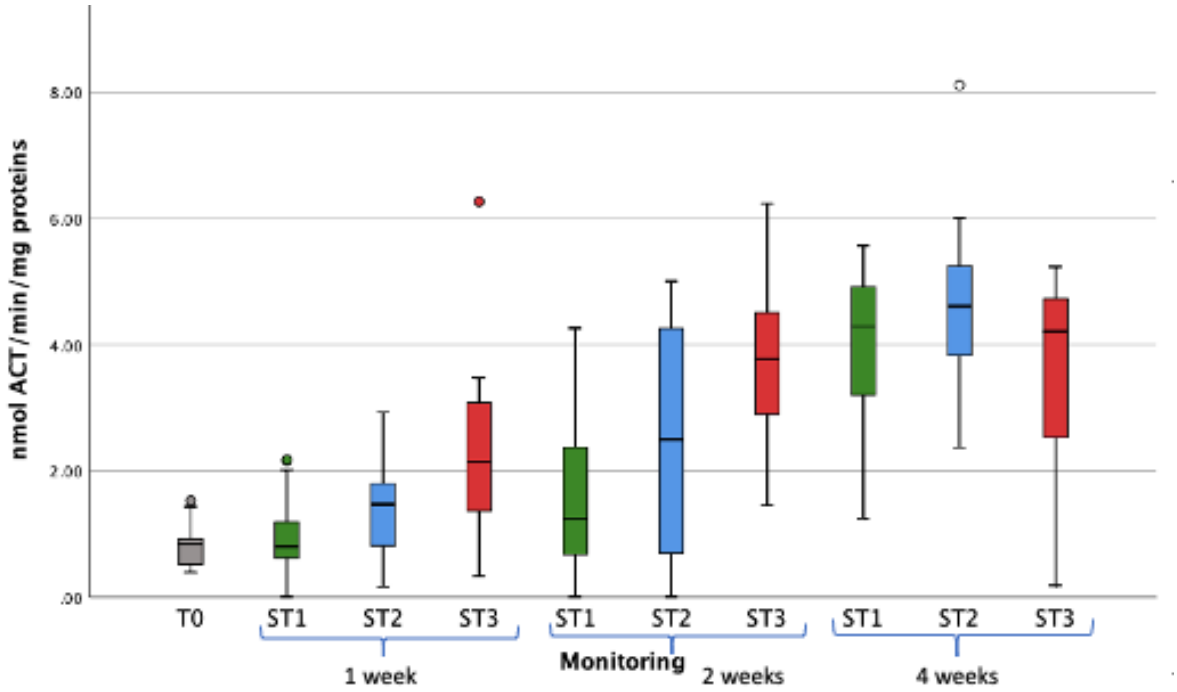

Figure 12: AChE activity expressed as nmol ATC/min/mg protein, for; T0, the pre-deployment group, station 1 (ST1, in green), station 2(ST2 in blue) and station 3 (ST3 in red), after 1, 2 and 4 weeks of deployment. The IQ range (box) indicates where 50% of the values for that station is, and the whiskers (lines) indicates where values that are less than 1.5xIQ are found. The circles above the whiskers shows outliers that are more than 1.5 times IQ.

The activity at pre-deployment (T0) was 0.822 ± 0.087 $nmol\ ATC/min/mg\ protein$. After one-week field deployment, the activity at station 1, 2 and 3 was 0.917±0.214, 1.074±0.500 and 2.342±0.374 nmol ATC/min/mg protein. Station 3 had significantly increased activity compared to T0 and station 1, but not significantly compared to station 2. Station 2 was significantly higher than T0.

After two weeks of deployment the AChE activity at station 1, 2 and 4 was 1.582±0.370, 2.520±0.497 and 3.704±0.339 nmol ATC/min/mg protein. There was a significant increase at station 2 compared to T0, but not to the other stations. Station 3 had a significant increase in AChE activity compared to station 1 and T0.

After four weeks exposure, the AChE activities at station 1, 2 and 3 were 4.008±0.322, 4.576±0.370 and 3.432±0.418 nmol ATC/min/mg protein. None of the stations were significantly different to each other, but all were significantly higher than T0.

The low activity in T0 may indicate that there is some contamination at this site decreasing the AChE activity. A chemical analysis of the tissue bioaccumulation is necessary to evaluate any background contamination. However, these results also indicate that contamination by the wastewater outlet (station 3) is significantly enhancing the increase of AChE activity compared to station 1. Station 2 might be more affected by contamination than anticipated, as the increase at this station was also enhanced compared to station 1.

All of the stations had significantly lower activity than reported BAC and EAC, which is 30 nmoles ATC/min/mg protein and 21 nmol/min/protein (Davies & Vethaak, 2012). However, these values are for *Mytilus edulis* in the Atlantic Ocean by the coast of France and Portugal and not the Norwegian coast. Background levels in along the Norwegian coast needs to be further researched. Pampanin et al., (2019) measured AChE activities at reference stations to be between 11-33 nmol ATC/min/mg protein, and between 4-13 nmol ATC/min/mg protein for the T0. Comparing the results obtained in this thesis, to these values, T0, station 1 and 2 are lower, and not comparable to T0 or reference values. The results obtained for station 3, after 1 week, and station 1, 2 and 3 after 2 and 4 weeks of deployment are comparable to the T0 AChE activity Pampanin et al., (2019) measured, but not comparable to reference stations. Bocquené et al. (2004) measured low AChE activities between 5-20 nmol/min/mg protein in *M. edulis* in late autumn, but it is uncertain if these low values are due to contamination from an oil spill, or natural seasonal fluctuations.

A considerable amount of research (Gill et al 1991; Zatta et al 2002; Flora & Seth 2000; Martinez-Tabche et al., 2001; Thaker & Haritos 1989) shows that some metals (Cd, Al, Hg, Mg, Sn and Ca) can increase AChE activity in some tissues, by increasing the binding efficiency. Thaker & Haritos (1989) saw inhibition of AChE *in vitro* in the shrimp *Callianassa tyrrhena* after exposure to mercury, but a significant increase *in vivo*. A significant inhibition of AChE activity was seen after oligochaetes was exposed to Pb, however a decrease was also observed that likely was caused by Al, Hg, Mg, Sn, Ca or other contaminants in the sediments (Martinez-Tabche et al.,2001). Bainy, et al., (2006) demonstrated an increase of AChE synthesis in the mussel *Perna perna*, after exposure to

lead and cadmium. The metal concentration in the tissues of the mussels at the farm should therefore be determined. Wan et al., 2014 found that exposure of the bivalve *Meretrix meretrix* to municipal wastewater increased the AChE activity in the gills. A significant increase in AChE activity was seen in the haemolymph of *M. galloprovincialis* after exposure to acetaminophen (Mezzelani et al., 2016). The AChE activity in *M. galloprovincialis* also increased after three days exposure to the selective reuptake inhibitor fluoxetine, but after 15 days a significant down regulation was observed (Gonzalez-Rey & Bebianno, 2014). Exposure to the hormone 17β- estradiol (E2), resulted in a significant increase in AChE activity in *Lateolabrax japonicus* and the female rat hippocampus (Pereira et al., 2008; Thilagam et al., 2014). Gagné et al., (2011) examined the effect of municipal wastewater on the freshwater mussel *Elliptio complanata* and found significant neuroendocrinal alterations and oxidative stress after exposure. Al-Ghais (2013) exposed the freshwater fish *Tilapia mossambica* to sewage and measured a decrease in the activity of AChE.

Brown et al., (2003) evaluated the activity of AChE in subcellular fractions of several tissues and found that for gills, the highest activities were measured in the microsomal fraction. Environmental concentrations of pharmaceuticals might be too low to give a strong effect. Centrifugation at higher speed would therefore have given a higher activity.

3.5 Catalase

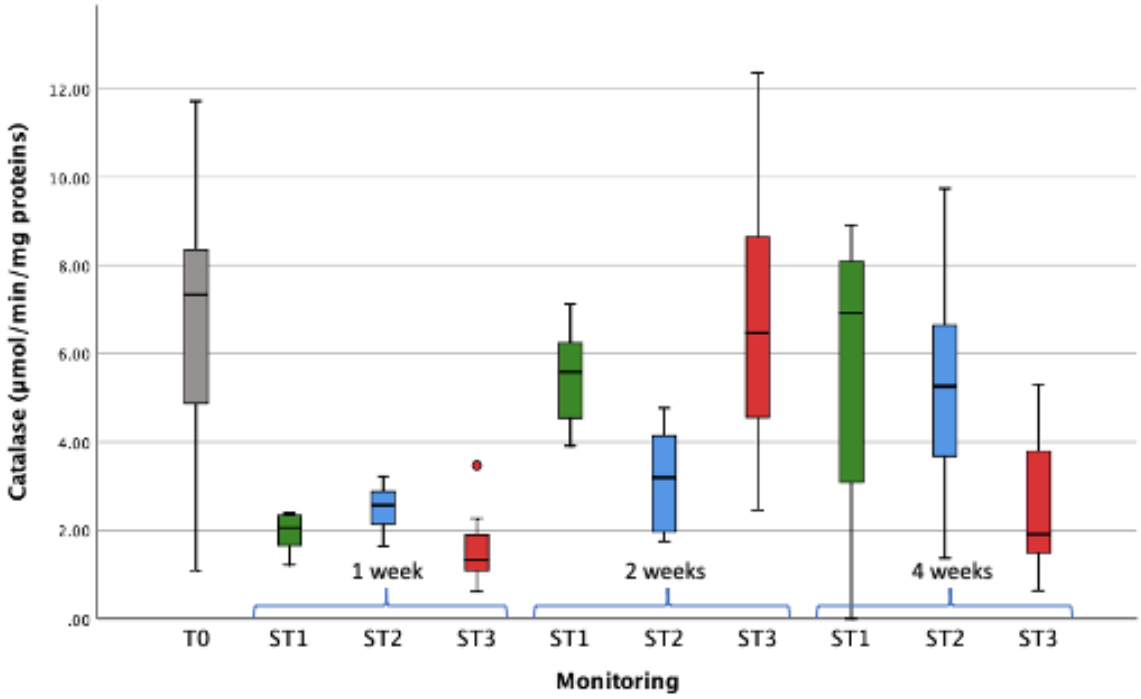

Figure 13: CAT activity ($\mu mol\ H_2O_2/min/mg\ protein$), pre-deployment (T0, in grey), and for station 1 (ST1 in green), station 2, ST2, in blue) and station 3 (ST3 in red) after 1, 2 and 4 weeks of deployment. The median value for the station is shown by the line in the box. The IQ range (boxes) show where 50% of the values are, and the whiskers (lines) show values less than 1.5xIQ range. The circles above the whiskers show outliers (more than 1.5xIQ range).

Pre-deployment (T0), the CAT activity was 6.660±1.326 µmol/min/mg protein. After 1 week of deployment, the CAT activities for station 1, 2 and 3 were 1.953±0.184, 2.500±0.230 and 1.627±0.363 µmol/min/mg protein. Station 1 and 3 was significantly different to T0. None of the stations were significantly different to each other.

After 2 weeks of deployment the CAT activities for mussels at station 1, 2 and 3 were 5.457±0.459, 3.137±0.477 and 6.815±1.300 µmol/min/mg protein. Mussels at station 2 were significantly lower that organisms at T0, station 1 and 3. The higher variability observed for samples that had higher CAT activity could be linked to the bubble formation when O_2 is formed from H_2O_2, influencing the readings in the spectrophotometer (figure 13).

Finally, after 4 weeks of deployment the CAT activities were 5.316±1.463, 5.288±1.058 and 2.631±0.712 µmol/min/mg protein. None of the stations were significantly different. The CAT activities obtained are lower than Livingstone et al., (1992) have reported for microsomal fractions of digestive gland in *M. edulis*. It is also significantly lower than

reported CAT activities in *M. galloprovincialis* (Gonzalez-Rey & Bebianno, 2012; 2014; Mezzelani et al., 2018), although there might be interspecies differences.

Regoli & Principato (1995) suggested that an inhibition of CAT activity is a temporary response to acute pollution. However, a transient decrease CAT activity 3 days after translocation was measured by Regoli et al., (2004) in both reference site and at the polluted site. Therefore, it may be more likely that the initial decrease in CAT activity for organisms at all stations is due to the translocation and change of environment. The results from station 3 follows the same biphasic pattern reported by Regoli et al., (2004) for their pollutes site, with a significant increase in activity after 2 weeks compared to one week, followed by a significant decrease after 4 weeks compared to 2 weeks. Station 1 and 2 shows a steady increase in activity from 1 week to four weeks of deployment. The recovery to similar values as other stations and to T0, indicate adaptive or counteractive mechanisms (Regoli & Guilani, 2014). CAT activity in *M. galloprovincialis* was significantly inhibited by exposure to ketoprofen and nimesulide (Mezzelani et al., 2016).

CAT activity was significantly increased when the mussels *M. galloprovincialis* were exposed to a mixture of fluoxetine and propranolol, but there were no significant alterations compared to controls when exposed to each pharmaceutical alone (Franzeletti et al., 2015).
Canesi et al., (2008) exposed *M. galloprovincialis* to environmental concentrations of endocrine-disrupting compounds (EDCs). They recorded a significant decrease in CAT activity and a dose-dependent response in GST levels. A significant increase in MDA content was also observed in their exposure study. A significant increase in MDA content in *M. galloprovincialis* has been reported by several studies (Banni et al., 2015; Martin-Diaz et al., 2009; Solé et al., 2010;) after exposure to the pharmaceutical's carbamazepine, acetaminophen, and oxytetracycline. Carbamazepine also significantly increased CAT and GST levels, while no effect was seen in activity after oxytetracycline. Propanol (147ug/L) gave no effect on CAT activity, but depleted GST activity. Further research evaluating GST activity and MDA content in in the digestive gland of the mussels sampled for this thesis is therefore planned.

CAT activity measured in *M. galloprovincialis* were lower during autumn and winter (Regoli, 1998; Viarengo et al., 1991). Gametogenesis is reported to start during autumn to winter for mussels in the Ligurian sea, British waters and in Iceland, and therefore the reproduction

cycle might affect the results (Viarengo et al., 1991; Vlahogianni et al., 2007; Thorarinsdóttir et al., 2013). Gametogenesis is naturally stressful and is a major confounding factor in biological monitoring (Davies & Vethaak, 2012).

3.6 Principal component analysis

A principal component analysis (PCA) was done on the different sampling times, summarizing the biomarker analysis for each station.

The scatter plot of the PCA of the mean/median values of the biomarker data for time 0 (T0), station 1, station 2 and station three (Figure 14 below) show how the first component was able to clearly separate station 3 from station 1 and T0. Station 2 is situated more to the middle left side, and thereby showing that after 1 week at this station, the biomarker battery also differentiates this station from station 1 and T0, although not to the same degree as station 3. This component explains 60.5% of the variance in the biomarkers.

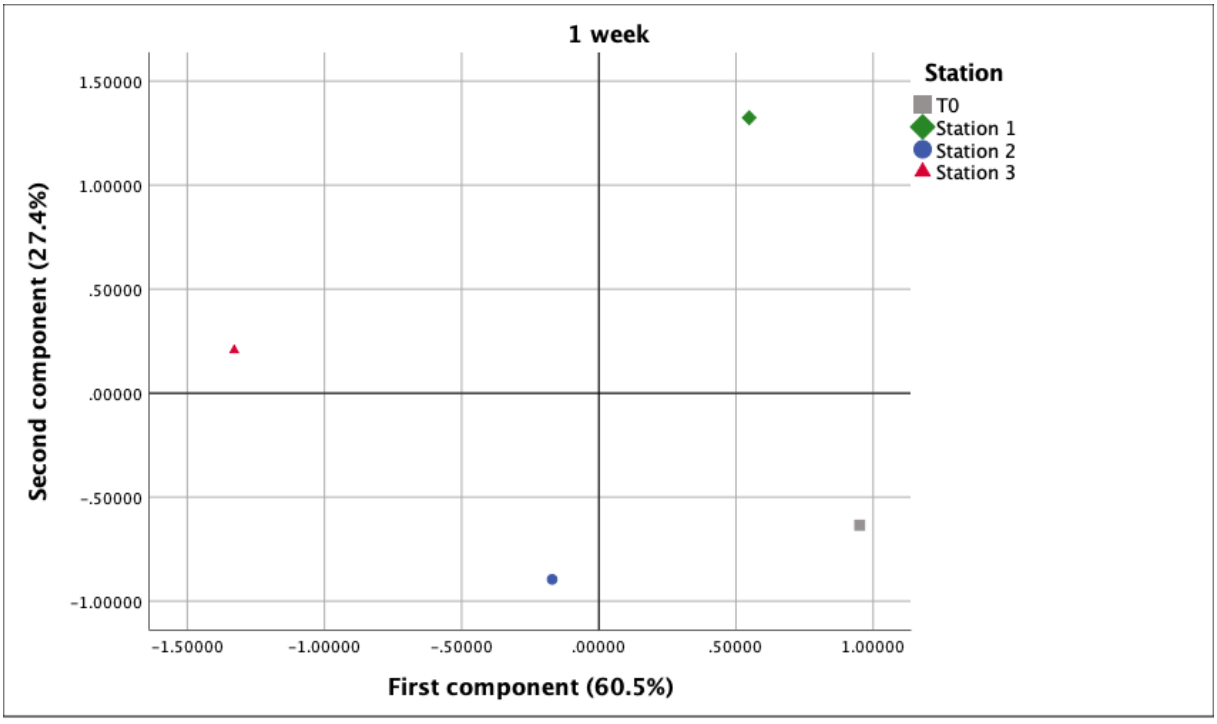

Figure 14: Score plot of the components derived from PCA for the mean/median values of the biomarkers after 1 week of deployment. Where T0 is time 0, pre-deployment in grey to the lower right, station 1 is in green to the higher right, station 2 is in blue to the lower left and

station 3 is in red to the middle left. The first component explains 60.5% of the variance, while the second component explains 27.4 %.

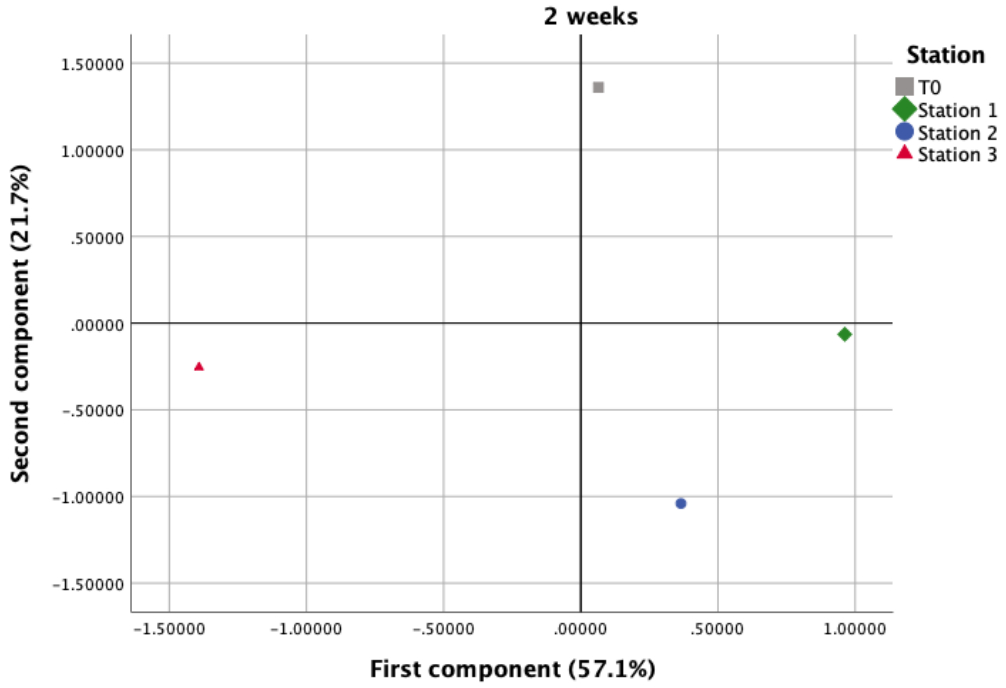

Figure 15: Scatter plot of PCA components from mean/median of biomarkers from T0 (pre-deployment, in grey), station 1 (in green), station 2 (in blue) and station 3 (in red). The first component explains 57.1% of the variance, clearly differentiating station 3 from station 1, 2 and T0. The second component explains 21.7 % of the variance in biomarkers.

The scatter plot from 2 weeks deployment shows that the sum of biomarkers derived from PCA at station 1 (green) are more to the lower right and the responses similar to station 2 (lower right blue). Responses from T0 are found to the upper right in grey. While station 3 can be found in the middle right. Showing that after 2 weeks, the biomarker battery was able to distinguish between the stations.

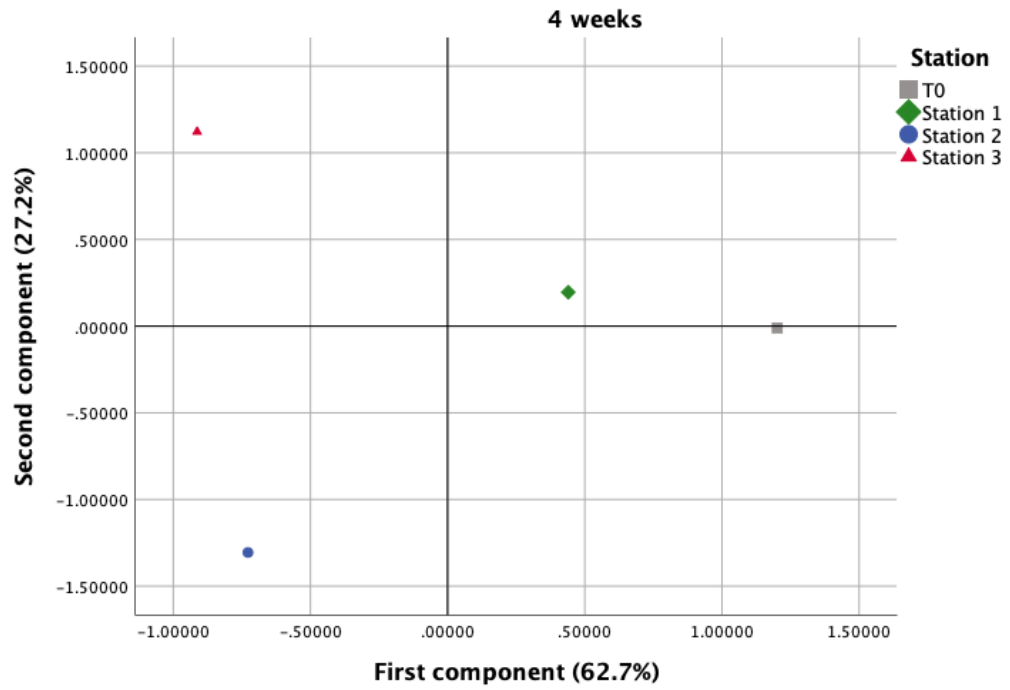

Figure 16: Scatter plot of the PCA components summarizing the mean/median biomarker responses of T0, Station 1, station 2 and station 3. Where T0 is the mussel group pre-deployment (grey to the middle right), station 1 is in green to the middle right, station 2 is in blue to the lower left and station 3 in red to upper left corner. The first component shows 62.7% of the variance between stations and was able to clearly separate station 1 and T0 from station 1 and 3. The second component explains 27.2% of the variance.

After 4 weeks deployment, the biomarker battery shows a clear difference between responses from station 1 and T0, which are quite close, and station 2 and 3 on the opposite side. The components derived explained 89.9% of the variance between stations and are illustrated in figure 16. PCA components derived from all the biomarker data is summarized in scatter plots of each station in the appendix.

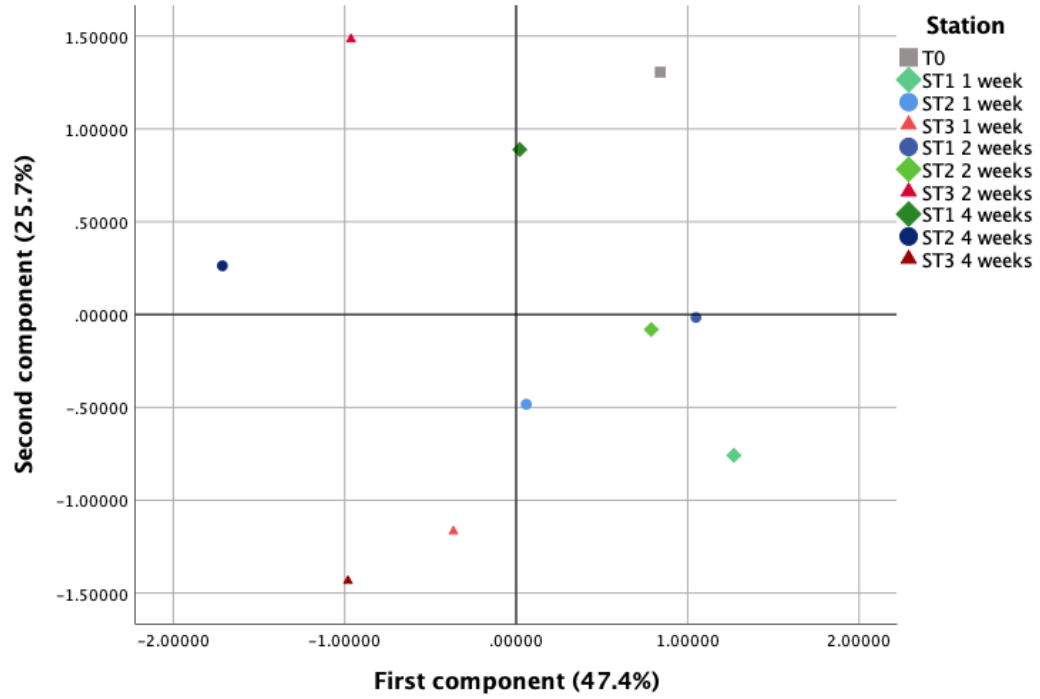

Figure 17: Scatter plot of PCA components summarizing all the biomarker responses, to show how the stations responses changes over time. Pre-deployment (T0) biomarker responses are found to the right in grey. Station 1 in green can at all times be found on the right side, the responses from station 2 keeps on the right side after 1 and 2 weeks but shifts to the left after 4 weeks. Station 3 in red.

In figure 16 illustrates the components derived from PCA of all stations at all times (1, 2 and 4 weeks). T0 and station 1 are at all times to the right (in grey and in green), while station 2 are to the right after 1 and 2 weeks but shifts to the left after 4 weeks. Station 3 is at all times located to the left. The components derived from this analysis explain 73.1% of the total variances between stations in time.

3.7 Perspectives and proposals for the future

Even if this thesis focuses on pharmaceuticals, there several other contaminants that can be found in wastewater effluents, as mentioned in the introduction. CECs are putting a major strain on marine organisms, this makes them more vulnerable to other stressors, such as anoxic conditions, overexploitation, other pollution, invasive species and other intrinsic factors (Franzellitti et al., 2015) However, further studies higher up in food chain and ecological monitoring over time needs to be done to fully understand if the recipient ecosystem is gradually affected. The changes may be small, but over a long period it might

decrease the populations ability to survive and affect the whole compositions of the ecosystem. Studies have shown that the long-term exposure to pollutants have greater effects than short term exposure (Keiter et al 2012; Tassou and Schulz 2013).

A macrocosm study by Lagesson et al. 2016 demonstrated how the bioaccumulation of pharmaceuticals is species dependant and that those pharmaceuticals remain persistent for a long time. The bottom dwellers were the ones that had the highest bioaccumulation. Although this study did not look at the pharmaceuticals' biological effects, it is useful to understand how organisms bioaccumulate CEC's differently from sediments, food, and water.

The importance of microcosm studies was demonstrated by Oskarsson et al., 2014 when they examined the effect of propranolol (100 and 1000 microg/L) on the mussel *Mytilus edulis trossulus*, the microalgae *Ceramium tenuicorne*, and the amphipods *Gammarus spp*. This study showed that the effect on the mussels was comparable to single-species studies. Propranolol increased the mortality, excretion, and respiration in the mussels. However, it seemed like the amphipods and microalgae experienced a neutral or positive effect, the microalgae by increased carbon content and biomass, and the amphipods by biomass and decreased mortality. The increase in carbon content in the microalgae may be due to the increase in nitrogen by mussels. Although this microcosm study showed positive effects on amphipods and microalgae, single-species studies have shown negative effects (Eriksson Wiklund et al., 2011, Oskarsson et al., 2014). This study clearly shows how the food web in an ecosystem can be altered by pharmaceuticals and how needed more microcosm research is to understand the complicated effects of pollution.

Angeles et al., 2019 reports that it is possible to remove 95% pharmaceuticals by activated carbon and ozone. Further investigation into the long-term effect of pharmaceuticals on organisms, and ecosystem composition are needed to evaluate if using this advanced tertiary treatment will improve the health of the organisms. Additionally, there is a need to investigate seasonal differences, as the solubility and concentration of pharmaceuticals vary between seasons.

As most studies on the biological effects of pharmaceuticals on mussels have been using *M. galloprovincialis*, there is a need for more studies on *M. edulis*, to see if there are any interspecies differences.

Chemical analysis of the tissues in the mussels sampled in this thesis are planned and will see if the biomarker responses obtained can be linked to pharmaceuticals in the tissues or if the responses are due to other contamination at these sites. Histological evaluation of the tissues is also planned, and to investigate if any damage on tissue level. There is also a need to look deeper into the effects on reproduction. Bickley et al. (2013) found that inbreeding due to low genetic biodiversity in addition to environmental stress by contamination decreased embryo viability, and inbred males get fewer offspring compared to outbred.

4. Conclusion

In this thesis the biological effects of a discharge from a municipal wastewater were assessed on the mussel, *M. edulis,* using a battery of biomarkers. All obtained responses from the biomarker battery were summarized using the PCA. The 2 first components were able to clearly distinguish station 3 by the wastewater outlet from station 1(reference) and T0(pre-deployment). The overall of biomarker responses also separated individuals from station 2, with responses after 4 weeks shifting and being more similar to organisms from station 3. These findings show that the municipal wastewater discharges have the potential to decrease the general health of mussels, at least temporarily. However, further research is needed to evaluate the long-term effects at populations and ecosystem levels.

The data also showed that CI and SoS were more sensitive than LMS to detect targeted environmental contamination. In details, mussels from station 2 and 3 had significantly lower CI values after 4 weeks. LT_{50} values were significantly lower after 2 weeks for organisms at station 3 and after 4 weeks for organisms at station 2, as calculated in the SoS assay. The AChE activity was significantly enhanced in mussels at station 3 compared to the ones at station 1 and T0, and in organisms from station 2 compared to T0, after 1 and 2 weeks of deployment. The CAT activity was significantly decreased in mussels from station 2 compared to the ones at stations 1, 3 and T0. There was no significant difference between organisms from the stations 1, 2 and 3 for both CAT activity and AChE activity after 4 weeks of deployment. This may indicate that the mussels are compensating for the effects of contamination.

Further studies are planned to look at tissue samples taken from the mussels to evaluate if there are any damage at tissue level. Samples should also be analysed for the bioaccumulation of pharmaceuticals and check for correlations between chemical content of pharmaceuticals and biomarker responses. When these results will be combined, they will provide valuable knowledge that can be used when making environmental risk assessments for wastewater outlets.

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Appendices

Appendix A1: Detailed sampling sheet pre-deployment (T0)

Mussel code	date	station/time	Sex	lenght (cm)	volume (ml)	Analysis
1	2.10.20	T0	F	7	29	Biochemistry (LMS, AChE)
2	2.10.20	T0	M	6,3	20	Biochemistry (LMS, AChE)
3	2.10.20	T0	M	6,18	20	Biochemistry (LMS, AChE)
4	2.10.20	T0	/	6,06	19	Biochemistry (LMS, AChE)
5	2.10.20	T0	F	6,5	22	Biochemistry (LMS, AChE)
6	2.10.20	T0	F	6,9	16	Biochemistry (LMS, AChE)
7	2.10.20	T0	F	5,83	14	Biochemistry (LMS, AChE)
8	2.10.20	T0	M	5,55		Biochemistry (LMS, AChE)
9	2.10.20	T0	M	5,9		Biochemistry (LMS, AChE,CAT)
10	2.10.20	T0	M	6,66		Biochemistry (LMS, AChE,CAT)
11	2.10.20	T0	M	5,77		Biochemistry (LMS, AChE,CAT)
12	2.10.20	T0	M	7		Biochemistry (LMS, AChE,CAT)
13	2.10.20	T0	M	6,9		Biochemistry (LMS, AChE,CAT)
14	2.10.20	T0	M	6,7		Biochemistry (LMS, AChE,CAT)
15	2.10.20	T0	F	7		Biochemistry (LMS, AChE,CAT)
16	2.10.20	T0	M	5,53		Chemistry 1-5
17	2.10.20	T0	M	5,51		Chemistry 1-5
18	2.10.20	T0	M	5,37		Chemistry 1-5
19	2.10.20	T0	M	5,39		Chemistry 1-5
20	2.10.20	T0	M	6,61		Chemistry 1-5
21	2.10.20	T0	M	5,61		Chemistry 6-10
22	2.10.20	T0	M	4,91		Chemistry 6-10
23	2.10.20	T0	M	5,91		Chemistry 6-10
24	2.10.20	T0	M	6,46		Chemistry 6-10
25	2.10.20	T0	M	6,66		Chemistry 6-10
26	2.10.20	T0	M	5,99		Chemistry 11-15
27	2.10.20	T0	M	6,76		Chemistry 11-15
28	2.10.20	T0	M	6,67		Chemistry 11-15
29	2.10.20	T0	F	6,19		Chemistry 11-15
30	2.10.20	T0	M	5,69		Chemistry 11-15
31	2.10.20	T0	**F**	**7,37**		CI
32	2.10.20	T0	M	6,2		CI
33	2.10.20	T0	M	5,56		CI
34	2.10.20	T0	F	5,67		CI
35	2.10.20	T0	F	5,56		CI
36	2.10.20	T0	M	6,35		CI
37	2.10.20	T0	M	5,57		CI
38	2.10.20	T0	M	5,52		CI
39	2.10.20	T0	M	6,85		CI
40	2.10.20	T0	M	5,49		CI
41	2.10.20	T0	M	5,75		CI
42	2.10.20	T0	M	5		CI
43	2.10.20	T0	M	6,1		CI
44	2.10.20	T0	F	5,51		CI
45	2.10.20	T0	M	7,22		CI
46	2.10.20	T0	M	5,23		CI
47	2.10.20	T0	M	5,58		CI
48	2.10.20	T0	M	5,51		CI
49	2.10.20	T0	M	5,29	22	CI
50	2.10.20	T0	M	6,18	28	CI
51	2.10.20	T0	M	5,25	20	CI
52	2.10.20	T0	M	6,96	34	CI
53	2.10.20	T0		5,24	20	CI
54	2.10.20	T0		5,64	24	CI
55	2.10.20	T0		5,61	22	CI
56	2.10.20	T0		6,87	32	CI
57	2.10.20	T0		6,67	28	CI
58	2.10.20	T0		5,71	22	CI
59	2.10.20	T0		5,39	20	CI
60	2.10.20	T0		4,9	18	CI

Appendix A1 Detailed sampling sheet pre-deployment (T0)

61	2.10.20	T0		6,11		Stress on stress
62	2.10.20	T0		7,14		Stress on stress
63	2.10.20	T0		5,67		Stress on stress
64	2.10.20	T0		5,87		Stress on stress
65	2.10.20	T0		5,79		Stress on stress
66	2.10.20	T0		6,8		Stress on stress
67	2.10.20	T0		5,53		Stress on stress
68	2.10.20	T0		6,73		Stress on stress
69	2.10.20	T0		7,05		Stress on stress
70	2.10.20	T0		6,35		Stress on stress
71	2.10.20	T0		6,23		Stress on stress
72	2.10.20	T0		6,06		Stress on stress
73	2.10.20	T0		6,92		Stress on stress
74	2.10.20	T0		5,7		Stress on stress
75	2.10.20	T0		6,74		Stress on stress
76	2.10.20	T0		6,13		Stress on stress
77	2.10.20	T0		5,74		Stress on stress
78	2.10.20	T0		5,3		Stress on stress
79	2.10.20	T0		6,82		Stress on stress
80	2.10.20	T0		6,4		Stress on stress
81	2.10.20	T0		6,45		Stress on stress
82	2.10.20	T0		6,52		Stress on stress
83	2.10.20	T0		5,83		Stress on stress
84	2.10.20	T0		5,07		Stress on stress
85	2.10.20	T0		6,44		Stress on stress
86	2.10.20	T0		6,17		Stress on stress
87	2.10.20	T0		6,47		Stress on stress
88	2.10.20	T0		5,51		Stress on stress
89	2.10.20	T0		5,4		Stress on stress
90	2.10.20	T0		6,77		Stress on stress
Average		Ratio: F= 11/ M		6,077	22,632	

Appendix A2 Detailed sampling sheet station 1, week 1

Mussel code	date	station/time	Sex	lenght (cm)	volume (ml)	comments
91	07.10.20	Station 1 T1	F	6,1		Biochemistry (LMS, AChE,CAT)
92	07.10.20	Station 1 T1	M	5,66		Biochemistry (LMS, AChE,CAT)
93	07.10.20	Station 1 T1	F	7,27		Biochemistry (LMS, AChE,CAT)
94	07.10.20	Station 1 T1	M	5,94		Biochemistry (LMS, AChE,CAT)
95	07.10.20	Station 1 T1	M	7		Biochemistry (LMS, AChE,CAT)
96	07.10.20	Station 1 T1	F	6,1		Biochemistry (LMS, AChE,CAT)
97	07.10.20	Station 1 T1	F	5,69		Biochemistry (LMS, AChE,CAT)
98	07.10.20	Station 1 T1	M	6,15		Biochemistry (LMS, AChE)
99	07.10.20	Station 1 T1	M	6,3		Biochemistry (LMS, AChE)
100	07.10.20	Station 1 T1	F	6,33		Biochemistry (LMS, AChE)
101	07.10.20	Station 1 T1	M	5,9		Biochemistry (LMS, AChE)
102	07.10.20	Station 1 T1	M	6,66		Biochemistry (LMS, AChE)
103	07.10.20	Station 1 T1	M	5,5		Biochemistry (LMS, AChE)
104	07.10.20	Station 1 T1	F	5,68		Biochemistry (LMS, AChE)
105	07.10.20	Station 1 T1	M	5,6		Biochemistry (LMS, AChE)
106	07.10.20	Station 1 T1		6,1		Chemistry 1-5
107	07.10.20	Station 1 T1		6,66		Chemistry 1-5
108	07.10.20	Station 1 T1		6,66		Chemistry 1-5
109	07.10.20	Station 1 T1		6,7		Chemistry 1-5
110	07.10.20	Station 1 T1		6,94		Chemistry 1-5
111	07.10.20	Station 1 T1		8,03		Chemistry 6-10
112	07.10.20	Station 1 T1		8,27		Chemistry 6-10
113	07.10.20	Station 1 T1		6,77		Chemistry 6-10
114	07.10.20	Station 1 T1		7,27		Chemistry 6-10
115	07.10.20	Station 1 T1		7		Chemistry 6-10
116	07.10.20	Station 1 T1		7,94		Chemistry 11-15
117	07.10.20	Station 1 T1		7		Chemistry 11-15
118	07.10.20	Station 1 T1		5,99		Chemistry 11-15
119	07.10.20	Station 1 T1		5,5		Chemistry 11-15
120	07.10.20	Station 1 T1		5,66		Chemistry 11-15
121	07.10.20	Station 1 T1	**F**	**6,19**	**14**	CI
122	07.10.20	Station 1 T1	F	5,91	20	CI
123	07.10.20	Station 1 T1	F	5,62	13	CI
124	07.10.20	Station 1 T1	F	6,69	15	CI
125	07.10.20	Station 1 T1	M	5,42	10	CI
126	07.10.20	Station 1 T1	M	6,56	20	CI
127	07.10.20	Station 1 T1	M	5,95	20	CI
128	07.10.20	Station 1 T1	M	4,85	8	CI
129	07.10.20	Station 1 T1	F	5,81	20	CI
130	07.10.20	Station 1 T1	F	4,66	10	CI
131	07.10.20	Station 1 T1	M	5,37	14	CI
132	07.10.20	Station 1 T1	M	6,97	16	CI
133	07.10.20	Station 1 T1	F	6,13	14	CI
134	07.10.20	Station 1 T1	F	6,36	16	CI
135	07.10.20	Station 1 T1	M	6,43	22	CI
136	07.10.20	Station 1 T1	M	6,52	25	CI
137	07.10.20	Station 1 T1	F	6,9	28	CI
138	07.10.20	Station 1 T1	F	7,9	22	CI
139	07.10.20	Station 1 T1	M	5,7	20	CI
140	07.10.20	Station 1 T1	M	7,29	30	CI
141	07.10.20	Station 1 T1	F	8	40	CI
142	07.10.20	Station 1 T1	M	6,55	20	CI
143	07.10.20	Station 1 T1	F	6,2	22	CI
144	07.10.20	Station 1 T1	M	6,19	20	CI
145	07.10.20	Station 1 T1	F	6,89	22	CI
146	07.10.20	Station 1 T1	F	6,49	20	CI
147	07.10.20	Station 1 T1	M	6,14	16	CI
148	07.10.20	Station 1 T1	M	7,74	35	CI
149	07.10.20	Station 1 T1	M	6,77	20	CI
150	07.10.20	Station 1 T1	F	5,95	16	CI

Appendix A2 Detailed sampling sheet station 1, week 1

151	07.10.20	Station 1 T1		6,06		Stress on stress
152	07.10.20	Station 1 T1		7,06		Stress on stress
153	07.10.20	Station 1 T1		5,61		Stress on stress
154	07.10.20	Station 1 T1		7		Stress on stress
155	07.10.20	Station 1 T1		6,92		Stress on stress
156	07.10.20	Station 1 T1		5,35		Stress on stress
157	07.10.20	Station 1 T1		6,05		Stress on stress
158	07.10.20	Station 1 T1		6,03		Stress on stress
159	07.10.20	Station 1 T1		4,52		Stress on stress
160	07.10.20	Station 1 T1		6,02		Stress on stress
161	07.10.20	Station 1 T1		5,67		Stress on stress
162	07.10.20	Station 1 T1		6,29		Stress on stress
163	07.10.20	Station 1 T1		5,53		Stress on stress
164	07.10.20	Station 1 T1		7,27		Stress on stress
165	07.10.20	Station 1 T1		6,7		Stress on stress
166	07.10.20	Station 1 T1		5,5		Stress on stress
167	07.10.20	Station 1 T1		7,02		Stress on stress
168	07.10.20	Station 1 T1		7,27		Stress on stress
169	07.10.20	Station 1 T1		6,33		Stress on stress
170	07.10.20	Station 1 T1		6,18		Stress on stress
171	07.10.20	Station 1 T1		6,34		Stress on stress
172	07.10.20	Station 1 T1		4,17		Stress on stress
173	07.10.20	Station 1 T1		6,52		Stress on stress
174	07.10.20	Station 1 T1		6,21		Stress on stress
175	07.10.20	Station 1 T1		6,21		Stress on stress
176	07.10.20	Station 1 T1		7		Stress on stress
177	07.10.20	Station 1 T1		7,59		Stress on stress
178	07.10.20	Station 1 T1		6,27		Stress on stress
179	07.10.20	Station 1 T1		6,83		Stress on stress
180	07.10.20	Station 1 T1		5,54		Stress on stress
Average			Ratio F = 21 / M=23	6,351	19,600	

Appendix A 3 Detailed sampling sheet station 2, week 1

Mussel code	date	station/time	Sex	lenght (cm)	volume (ml)	Analysis
181	07.10.20	Station 2 T1	F	6,76		Biochemistry (LMS, AChE,CAT)
182	07.10.20	Station 2 T1	M	6,8		Biochemistry (LMS, AChE,CAT)
183	07.10.20	Station 2 T1	F	6.61		Biochemistry (LMS, AChE,CAT)
184	07.10.20	Station 2 T1	F	5,7		Biochemistry (LMS, AChE,CAT)
185	07.10.20	Station 2 T1	M	5,52		Biochemistry (LMS, AChE,CAT)
186	07.10.20	Station 2 T1	M	6,81		Biochemistry (LMS, AChE,CAT)
187	07.10.20	Station 2 T1	M	6,51		Biochemistry (LMS, AChE,CAT)
188	07.10.20	Station 2 T1	M	5,64		Biochemistry (LMS, AChE)
189	07.10.20	Station 2 T1	F	6,44		Biochemistry (LMS, AChE)
190	07.10.20	Station 2 T1	M	6,25		Biochemistry (LMS, AChE)
191	07.10.20	Station 2 T1	F	5,57		Biochemistry (LMS, AChE)
192	07.10.20	Station 2 T1	M	5		Biochemistry (LMS, AChE)
193	07.10.20	Station 2 T1	M	6,13		Biochemistry (LMS, AChE)
194	07.10.20	Station 2 T1	M	7,11		Biochemistry (LMS, AChE)
195	07.10.20	Station 2 T1	F	6,76		Biochemistry (LMS, AChE)
196	07.10.20	Station 2 T1	M	6,94		Chemistry 1-5
197	07.10.20	Station 2 T1	F	7,18		Chemistry 1-5
198	07.10.20	Station 2 T1	F	4,71		Chemistry 1-5
199	07.10.20	Station 2 T1	M	7,99		Chemistry 1-5
200	07.10.20	Station 2 T1	M	6,77		Chemistry 1-5
201	07.10.20	Station 2 T1	M	4,99		Chemistry 6-10
202	07.10.20	Station 2 T1	M	4,91		Chemistry 6-10
203	07.10.20	Station 2 T1	M	6,05		Chemistry 6-10
204	07.10.20	Station 2 T1	F	6,72		Chemistry 6-10
205	07.10.20	Station 2 T1	M	5,18		Chemistry 6-10
206	07.10.20	Station 2 T1	M	5,22		Chemistry 11-15
207	07.10.20	Station 2 T1	M	7,16		Chemistry 11-15
208	07.10.20	Station 2 T1	M	5,7		Chemistry 11-15
209	07.10.20	Station 2 T1	F	6,17		Chemistry 11-15
210	07.10.20	Station 2 T1	M	5,9		Chemistry 11-15
211	07.10.20	Station 2 T1	**F**	**6,5**	**24**	CI
212	07.10.20	Station 2 T1	M	6,58	20	CI
213	07.10.20	Station 2 T1	M	6,28	20	CI
214	07.10.20	Station 2 T1	F	5,24	14	CI
215	07.10.20	Station 2 T1	M	5,83	16	CI
216	07.10.20	Station 2 T1	M	7,8	OR	CI
217	07.10.20	Station 2 T1	F	5,55	18	CI
218	07.10.20	Station 2 T1	M	5,6	12	CI
219	07.10.20	Station 2 T1	M	6,5	20	CI
220	07.10.20	Station 2 T1	M	6,48	10	CI
221	07.10.20	Station 2 T1	M	5,76	10	CI
222	07.10.20	Station 2 T1	M	6,86	30	CI
223	07.10.20	Station 2 T1	M	5,5	10	CI
224	07.10.20	Station 2 T1	M	6,56	26	CI
225	07.10.20	Station 2 T1	M	6	20	CI
226	07.10.20	Station 2 T1	F	7,67	35	CI
227	07.10.20	Station 2 T1	M	4,77	8	CI
228	07.10.20	Station 2 T1	M	5,02	10	CI
229	07.10.20	Station 2 T1	M	5,54	16	CI
230	07.10.20	Station 2 T1	M	8,22	40	CI
231	07.10.20	Station 2 T1	M	6,49	20	CI
232	07.10.20	Station 2 T1	F	6,53	16	CI
233	07.10.20	Station 2 T1	M	5,07	10	CI
234	07.10.20	Station 2 T1	M	5,54	11	CI
235	07.10.20	Station 2 T1	F	7,15	30	CI
236	07.10.20	Station 2 T1	M	4,87	8	CI
237	07.10.20	Station 2 T1	M	4,99	9	CI
238	07.10.20	Station 2 T1	F	6,91	25	CI
239	07.10.20	Station 2 T1	M	5,42	16	CI
240	07.10.20	Station 2 T1	F	4,48	8	CI

Appendix A 3 Detailed sampling sheet station 2, week 1

241	07.10.20	Station 2 T1		6,31		Stress on stress
242	07.10.20	Station 2 T1		6,66		Stress on stress
243	07.10.20	Station 2 T1		7,05		Stress on stress
244	07.10.20	Station 2 T1		4,73		Stress on stress
245	07.10.20	Station 2 T1		4,91		Stress on stress
246	07.10.20	Station 2 T1		6,67		Stress on stress
247	07.10.20	Station 2 T1		5,9		Stress on stress
248	07.10.20	Station 2 T1		6,21		Stress on stress
249	07.10.20	Station 2 T1		4,91		Stress on stress
250	07.10.20	Station 2 T1		6,11		Stress on stress
251	07.10.20	Station 2 T1		6,1		Stress on stress
252	07.10.20	Station 2 T1		5,81		Stress on stress
253	07.10.20	Station 2 T1		5,23		Stress on stress
254	07.10.20	Station 2 T1		5,83		Stress on stress
255	07.10.20	Station 2 T1		5.96		Stress on stress
256	07.10.20	Station 2 T1		7,9		Stress on stress
257	07.10.20	Station 2 T1		5,62		Stress on stress
258	07.10.20	Station 2 T1		6,71		Stress on stress
259	07.10.20	Station 2 T1		5,39		Stress on stress
260	07.10.20	Station 2 T1		5,39		Stress on stress
261	07.10.20	Station 2 T1		5,68		Stress on stress
262	07.10.20	Station 2 T1		6,79		Stress on stress
263	07.10.20	Station 2 T1		7,59		Stress on stress
264	07.10.20	Station 2 T1		5,82		Stress on stress
265	07.10.20	Station 2 T1		6,04		Stress on stress
266	07.10.20	Station 2 T1		4,76		Stress on stress
267	07.10.20	Station 2 T1		4,54		Stress on stress
268	07.10.20	Station 2 T1		5,86		Stress on stress
269	07.10.20	Station 2 T1		5,91		Stress on stress
270	07.10.20	Station 2 T1		5,21		Stress on stress
Average			Ratio: F=18/M=42	6,045	17,655	

Appendix A 4 Detailed sampling sheet station 3, week 1

Mussel code	date	station/time	Sex	lenght (cm)	volume (ml)	Analysis
271	08.10.20	Station 3 T1	F	7,2		Biochemistry (LMS, AChE,CAT)
272	08.10.20	Station 3 T1	M	7,27		Biochemistry (LMS, AChE,CAT)
273	08.10.20	Station 3 T1	F	6,82		Biochemistry (LMS, AChE,CAT)
274	08.10.20	Station 3 T1	F	5,99		Biochemistry (LMS, AChE,CAT)
275	08.10.20	Station 3 T1	F	5,88		Biochemistry (LMS, AChE,CAT)
276	08.10.20	Station 3 T1	F	7,28		Biochemistry (LMS, AChE,CAT)
277	08.10.20	Station 3 T1	M	6,54		Biochemistry (LMS, AChE,CAT)
278	08.10.20	Station 3 T1	F	6,51		Biochemistry (LMS, AChE)
279	08.10.20	Station 3 T1	M	6,38		Biochemistry (LMS, AChE)
280	08.10.20	Station 3 T1	F	6,77		Biochemistry (LMS, AChE)
281	08.10.20	Station 3 T1	M	7,13		Biochemistry (LMS, AChE)
282	08.10.20	Station 3 T1	F	5,73		Biochemistry (LMS, AChE)
283	08.10.20	Station 3 T1	F	7,28		Biochemistry (LMS, AChE)
284	08.10.20	Station 3 T1	M	5,25		Biochemistry (LMS, AChE)
285	08.10.20	Station 3 T1	M	5,32		Biochemistry (LMS, AChE)
286	08.10.20	Station 3 T1	F	6,35		Chemistry 1-5
287	08.10.20	Station 3 T1	F	6,64		Chemistry 1-5
288	08.10.20	Station 3 T1	M	9,41		Chemistry 1-5
289	08.10.20	Station 3 T1	F	6,61		Chemistry 1-5
290	08.10.20	Station 3 T1	M	5,7		Chemistry 1-5
291	08.10.20	Station 3 T1	M	5,2		Chemistry 6-10
292	08.10.20	Station 3 T1	M	8		Chemistry 6-10
293	08.10.20	Station 3 T1	M	4,69		Chemistry 6-10
294	08.10.20	Station 3 T1	M	4,12		Chemistry 6-10
295	08.10.20	Station 3 T1	M	6,3		Chemistry 6-10
296	08.10.20	Station 3 T1	M	5,06		Chemistry 11-15
297	08.10.20	Station 3 T1	M	4,94		Chemistry 11-15
298	08.10.20	Station 3 T1	F	7,8		Chemistry 11-15
299	08.10.20	Station 3 T1	M	6,31		Chemistry 11-15
300	08.10.20	Station 3 T1	M	5,5		Chemistry 11-15
301	08.10.20	Station 3 T1	F	**5**	**10**	CI
302	08.10.20	Station 3 T1	F	7	20	CI
303	08.10.20	Station 3 T1	M	6,8	20	CI
304	08.10.20	Station 3 T1	F	6	20	CI
305	08.10.20	Station 3 T1	M	6,2	16	CI
306	08.10.20	Station 3 T1	F	6,41	22	CI
307	08.10.20	Station 3 T1	F	6,43	20	CI
308	08.10.20	Station 3 T1	F	6,11	18	CI
309	08.10.20	Station 3 T1	F	6,33	20	CI
310	08.10.20	Station 3 T1	M	5	10	CI
311	08.10.20	Station 3 T1	M	5,98	14	CI
312	08.10.20	Station 3 T1	M	5,81	12	CI
313	08.10.20	Station 3 T1	F	5,72	14	CI
314	08.10.20	Station 3 T1	F	6,22	22	CI
315	08.10.20	Station 3 T1	M	6,4	24	CI
316	08.10.20	Station 3 T1	F	7,86	35	CI
317	08.10.20	Station 3 T1	M	4,83	12	CI
318	08.10.20	Station 3 T1	M	5	10	CI
319	08.10.20	Station 3 T1	M	4,84	8	CI
320	08.10.20	Station 3 T1	M	5,17	13	CI
321	08.10.20	Station 3 T1	M	4,8	8	CI
322	08.10.20	Station 3 T1	M	5,3	10	CI
323	08.10.20	Station 3 T1	M	5,99	14	CI
324	08.10.20	Station 3 T1	M	4,58	8	CI
325	08.10.20	Station 3 T1	M	5,35	10	CI
326	08.10.20	Station 3 T1	M	4,76	8	CI
327	08.10.20	Station 3 T1	F	4,79	9	CI
328	08.10.20	Station 3 T1	M	4,82	9	CI
329	08.10.20	Station 3 T1	M	4,72	7	CI
330	08.10.20	Station 3 T1	M	4,69	8	CI

Appendix A 4 Detailed sampling sheet station 3, week 1

331	08.10.20	Station 3 T1		5,44		Stress on stress
332	08.10.20	Station 3 T1		8.36		Stress on stress
333	08.10.20	Station 3 T1		6,28		Stress on stress
334	08.10.20	Station 3 T1		6,33		Stress on stress
335	08.10.20	Station 3 T1		7,04		Stress on stress
336	08.10.20	Station 3 T1		6,57		Stress on stress
337	08.10.20	Station 3 T1		5,41		Stress on stress
338	08.10.20	Station 3 T1		5,6		Stress on stress
339	08.10.20	Station 3 T1		7,86		Stress on stress
340	08.10.20	Station 3 T1		6,63		Stress on stress
341	08.10.20	Station 3 T1		6,9		Stress on stress
342	08.10.20	Station 3 T1		6,23		Stress on stress
343	08.10.20	Station 3 T1		6,51		Stress on stress
344	08.10.20	Station 3 T1		6,13		Stress on stress
345	08.10.20	Station 3 T1		7,11		Stress on stress
346	08.10.20	Station 3 T1		6,15		Stress on stress
347	08.10.20	Station 3 T1		7,4		Stress on stress
348	08.10.20	Station 3 T1		5,01		Stress on stress
349	08.10.20	Station 3 T1		5,54		Stress on stress
350	08.10.20	Station 3 T1		5,64		Stress on stress
351	08.10.20	Station 3 T1		5,85		Stress on stress
352	08.10.20	Station 3 T1		7,41		Stress on stress
353	08.10.20	Station 3 T1		7,18		Stress on stress
354	08.10.20	Station 3 T1		5,84		Stress on stress
355	08.10.20	Station 3 T1		7,06		Stress on stress
356	08.10.20	Station 3 T1		6,12		Stress on stress
357	08.10.20	Station 3 T1		5,52		Stress on stress
358	08.10.20	Station 3 T1		7,25		Stress on stress
359	08.10.20	Station 3 T1		5,1		Stress on stress
360	08.10.20	Station 3 T1		5,44		Stress on stress
Average			Ratio F = 24 / M= 36	6,084	14,367	

Appendix A 5 Detailed sampling sheet station 1, week 2

Mussel code	date	station/time	Sex	lenght (cm)	volume (ml)	Analysis
451	14.10.20	Station 1, T2	F	6,96		Biochemistry (LMS, AChE,CAT)
452	14.10.20	Station 1, T2	M	4,84		Biochemistry (LMS, AChE,CAT)
453	14.10.20	Station 1, T2	M	5,55		Biochemistry (LMS, AChE,CAT)
454	14.10.20	Station 1, T2	M?	5,88		Biochemistry (LMS, AChE,CAT)
455	14.10.20	Station 1, T2	F	5,97		Biochemistry (LMS, AChE,CAT)
456	14.10.20	Station 1, T2	F	5,61		Biochemistry (LMS, AChE,CAT)
457	14.10.20	Station 1, T2		5,55		Biochemistry (LMS, AChE,CAT)
458	14.10.20	Station 1, T2	M	5,9		Biochemistry (LMS, AChE)
459	14.10.20	Station 1, T2		6,2		Biochemistry (LMS, AChE)
460	14.10.20	Station 1, T2	M	6,43		Biochemistry (LMS, AChE)
461	14.10.20	Station 1, T2	M	5,84		Biochemistry (LMS, AChE)
462	14.10.20	Station 1, T2	M	5,31		Biochemistry (LMS, AChE)
463	14.10.20	Station 1, T2	M	5,75		Biochemistry (LMS, AChE)
464	14.10.20	Station 1, T2	M	5,99		Biochemistry (LMS, AChE)
465	14.10.20	Station 1, T2	M	5,54		Biochemistry (LMS, AChE)
466	14.10.20	Station 1, T2		5,64		Chemistry 1-5
467	14.10.20	Station 1, T2		6,63		Chemistry 1-5
468	14.10.20	Station 1, T2		5,98		Chemistry 1-5
469	14.10.20	Station 1, T2		7,78		Chemistry 1-5
470	14.10.20	Station 1, T2		5,76		Chemistry 1-5
471	14.10.20	Station 1, T2		6,57		Chemistry 6-10
472	14.10.20	Station 1, T2		6,8		Chemistry 6-10
473	14.10.20	Station 1, T2		6,15		Chemistry 6-10
474	14.10.20	Station 1, T2		7,05		Chemistry 6-10
475	14.10.20	Station 1, T2		6,72		Chemistry 6-10
476	14.10.20	Station 1, T2		7,54		Chemistry 11-15
477	14.10.20	Station 1, T2		7.34		Chemistry 11-15
478	14.10.20	Station 1, T2		6,67		Chemistry 11-15
479	14.10.20	Station 1, T2		6,08		Chemistry 11-15
480	14.10.20	Station 1, T2		5,73		Chemistry 11-15
481	14.10.20	Station 1, T2	**F**	**5,48**	**15**	CI
482	14.10.20	Station 1, T2	M	6,2	14	CI
483	14.10.20	Station 1, T2	F	5,61	16	CI
484	14.10.20	Station 1, T2	M	5,88	14	CI
485	14.10.20	Station 1, T2	F	5,33	13	CI
486	14.10.20	Station 1, T2	M	5,79	15	CI
487	14.10.20	Station 1, T2	M	5,34	12	CI
488	14.10.20	Station 1, T2	F	5,5	14	CI
489	14.10.20	Station 1, T2	M	5,37	14	CI
490	14.10.20	Station 1, T2	F	4,9	12	CI
491	14.10.20	Station 1, T2	M	5,14	10	CI
492	14.10.20	Station 1, T2	M	5,04	11	CI
493	14.10.20	Station 1, T2	M	5,19	10	CI
494	14.10.20	Station 1, T2	M	5,09	8	CI
495	14.10.20	Station 1, T2	M	5,47	12	CI
496	14.10.20	Station 1, T2	F	7,36	30	CI
497	14.10.20	Station 1, T2	M	5,88	16	CI
498	14.10.20	Station 1, T2	M	5,49	8	CI
499	14.10.20	Station 1, T2	M	5,4	10	CI
500	14.10.20	Station 1, T2	M	4,82	6	CI
501	14.10.20	Station 1, T2	M	5,51	16	CI
502	14.10.20	Station 1, T2	F	6,45	20	CI
503	14.10.20	Station 1, T2	F	5,54	11	CI
504	14.10.20	Station 1, T2	M	5,43	10	CI
505	14.10.20	Station 1, T2	M	6,67	18	CI
506	14.10.20	Station 1, T2	M	4,96	10	CI
507	14.10.20	Station 1, T2	M	4,54	8	CI
508	14.10.20	Station 1, T2	F	4,4	6	CI
509	14.10.20	Station 1, T2	M	5,61	14	CI
510	14.10.20	Station 1, T2	M	6,11	16	CI

Appendix A 5 Detailed sampling sheet station 1, week 2

511	14.10.20	Station 1, T2		5,59		Stress on stress
512	14.10.20	Station 1, T2		6,22		Stress on stress
513	14.10.20	Station 1, T2		8,05		Stress on stress
514	14.10.20	Station 1, T2		7,02		Stress on stress
515	14.10.20	Station 1, T2		5,94		Stress on stress
516	14.10.20	Station 1, T2		6,51		Stress on stress
517	14.10.20	Station 1, T2		6,01		Stress on stress
518	14.10.20	Station 1, T2		6,04		Stress on stress
519	14.10.20	Station 1, T2		6,97		Stress on stress
520	14.10.20	Station 1, T2		6,45		Stress on stress
521	14.10.20	Station 1, T2		6,45		Stress on stress
522	14.10.20	Station 1, T2		6,36		Stress on stress
523	14.10.20	Station 1, T2		6,52		Stress on stress
524	14.10.20	Station 1, T2		5,02		Stress on stress
525	14.10.20	Station 1, T2		6,87		Stress on stress
526	14.10.20	Station 1, T2		5,82		Stress on stress
527	14.10.20	Station 1, T2		5,47		Stress on stress
528	14.10.20	Station 1, T2		5,86		Stress on stress
529	14.10.20	Station 1, T2		7,72		Stress on stress
530	14.10.20	Station 1, T2		5,73		Stress on stress
531	14.10.20	Station 1, T2		6,43		Stress on stress
532	14.10.20	Station 1, T2		5,94		Stress on stress
533	14.10.20	Station 1, T2		5,66		Stress on stress
534	14.10.20	Station 1, T2		5,79		Stress on stress
535	14.10.20	Station 1, T2		4,94		Stress on stress
536	14.10.20	Station 1, T2		5,59		Stress on stress
537	14.10.20	Station 1, T2		6,07		Stress on stress
538	14.10.20	Station 1, T2		4,83		Stress on stress
539	14.10.20	Station 1, T2		4,72		Stress on stress
540	14.10.20	Station 1, T2		5,47		Stress on stress
Average			Ratio: F=12/M	5,910	12,967	

Appendix A 6 Detailed sampling sheet station 2, week 2

Mussel code	date	station/time	Sex	lenght (cm)	volume (ml)	Analysis
361	13.10.20	Station 2, T2	M	6.22		Biochemistry (LMS, AChE,CAT)
362	13.10.20	Station 2, T2	M	5.29		Biochemistry (LMS, AChE,CAT)
363	13.10.20	Station 2, T2	M	5.32		Biochemistry (LMS, AChE,CAT)
364	13.10.20	Station 2, T2	M	5.4		Biochemistry (LMS, AChE,CAT)
365	13.10.20	Station 2, T2		7.18		Biochemistry (LMS, AChE,CAT)
366	13.10.20	Station 2, T2	F	6.55		Biochemistry (LMS, AChE,CAT)
367	13.10.20	Station 2, T2	M	4.99		Biochemistry (LMS, AChE,CAT)
368	13.10.20	Station 2, T2		4.63		Biochemistry (LMS, AChE)
369	13.10.20	Station 2, T2	F	6.88		Biochemistry (LMS, AChE)
370	13.10.20	Station 2, T2	F	5.85		Biochemistry (LMS, AChE)
371	13.10.20	Station 2, T2	M	5.32		Biochemistry (LMS, AChE)
372	13.10.20	Station 2, T2		5.94		Biochemistry (LMS, AChE)
373	13.10.20	Station 2, T2	M	6.94		Biochemistry (LMS, AChE)
374	13.10.20	Station 2, T2	F	5.33		Biochemistry (LMS, AChE)
375	13.10.20	Station 2, T2	F	6.49		Biochemistry (LMS, AChE)
376	13.10.20	Station 2, T2		7,08		Chemistry 1-5
377	13.10.20	Station 2, T2		7,05		Chemistry 1-5
378	13.10.20	Station 2, T2		5,72		Chemistry 1-5
379	13.10.20	Station 2, T2		6,22		Chemistry 1-5
380	13.10.20	Station 2, T2		5,61		Chemistry 1-5
381	13.10.20	Station 2, T2		6,38		Chemistry 6-10
382	13.10.20	Station 2, T2		5,59		Chemistry 6-10
383	13.10.20	Station 2, T2		5,95		Chemistry 6-10
384	13.10.20	Station 2, T2		6,78		Chemistry 6-10
385	13.10.20	Station 2, T2		6,64		Chemistry 6-10
386	13.10.20	Station 2, T2		5,84		Chemistry 11-15
387	13.10.20	Station 2, T2		7,08		Chemistry 11-15
388	13.10.20	Station 2, T2		5,82		Chemistry 11-15
389	13.10.20	Station 2, T2		6,88		Chemistry 11-15
390	13.10.20	Station 2, T2		6,33		Chemistry 11-15
391	13.10.20	Station 2, T2	**M**	**7,7**	**30**	CI
392	13.10.20	Station 2, T2	F	7,41	34	CI
393	13.10.20	Station 2, T2	F	6,5	22	CI
394	13.10.20	Station 2, T2	M	6,6	24	CI
395	13.10.20	Station 2, T2	F	7,32	32	CI
396	13.10.20	Station 2, T2	M	6,15	16	CI
397	13.10.20	Station 2, T2	F	5,81	20	CI
398	13.10.20	Station 2, T2	M	6,2	20	CI
399	13.10.20	Station 2, T2	F	6,81	22	CI
400	13.10.20	Station 2, T2	M	7	26	CI
401	13.10.20	Station 2, T2	M	6,6	24	CI
402	13.10.20	Station 2, T2	M	6,22	22	CI
403	13.10.20	Station 2, T2	F	6,7	24	CI
404	13.10.20	Station 2, T2	F	5,6	16	CI
405	13.10.20	Station 2, T2	M	6,17	18	CI
406	13.10.20	Station 2, T2	M	5,73	14	CI
407	13.10.20	Station 2, T2	F	6,17	16	CI
408	13.10.20	Station 2, T2	M	5,01	12	CI
409	13.10.20	Station 2, T2	M	6,25	8	CI
410	13.10.20	Station 2, T2	F	5,33	14	CI
411	13.10.20	Station 2, T2	F	7,01	28	CI
412	13.10.20	Station 2, T2	M	4,47	8	CI
413	13.10.20	Station 2, T2	M	5,3	12	CI
414	13.10.20	Station 2, T2	M	5,36	14	CI
415	13.10.20	Station 2, T2	M	5,4	10	CI
416	13.10.20	Station 2, T2	M	5,15	10	CI
417	13.10.20	Station 2, T2	M	5,79	16	CI
418	13.10.20	Station 2, T2	M	5,12	10	CI
419	13.10.20	Station 2, T2	M	4,57	6	CI
420	13.10.20	Station 2, T2	F	4,61	10	CI

Appendix A 6 Detailed sampling sheet station 2, week 2

421	13.10.20	Station 2, T2		7,1		Stress on stress
422	13.10.20	Station 2, T2		5,51		Stress on stress
423	13.10.20	Station 2, T2		4,99		Stress on stress
424	13.10.20	Station 2, T2		5,97		Stress on stress
425	13.10.20	Station 2, T2		6,73		Stress on stress
426	13.10.20	Station 2, T2		6,49		Stress on stress
427	13.10.20	Station 2, T2		5,8		Stress on stress
428	13.10.20	Station 2, T2		5,17		Stress on stress
429	13.10.20	Station 2, T2		6,44		Stress on stress
430	13.10.20	Station 2, T2		6,06		Stress on stress
431	13.10.20	Station 2, T2		7,39		Stress on stress
432	13.10.20	Station 2, T2		6,66		Stress on stress
433	13.10.20	Station 2, T2		8		Stress on stress
434	13.10.20	Station 2, T2		5,8		Stress on stress
435	13.10.20	Station 2, T2		6,05		Stress on stress
436	13.10.20	Station 2, T2		6,59		Stress on stress
437	13.10.20	Station 2, T2		7,43		Stress on stress
438	13.10.20	Station 2, T2		5,5		Stress on stress
439	13.10.20	Station 2, T2		5,95		Stress on stress
440	13.10.20	Station 2, T2		6,45		Stress on stress
441	13.10.20	Station 2, T2		6,43		Stress on stress
442	13.10.20	Station 2, T2		4,79		Stress on stress
443	13.10.20	Station 2, T2		5,49		Stress on stress
444	13.10.20	Station 2, T2		5,7		Stress on stress
445	13.10.20	Station 2, T2		5,54		Stress on stress
446	13.10.20	Station 2, T2		6,28		Stress on stress
447	13.10.20	Station 2, T2		6,06		Stress on stress
448	13.10.20	Station 2, T2		6,14		Stress on stress
449	13.10.20	Station 2, T2		5,27		Stress on stress
450	13.10.20	Station 2, T2		5,81		Stress on stress
Average			Ratio: F= 16/M= 26	6,115	18,276	

Appendix A 7 Detailed sampling sheet station 3, week 2

Mussel code	date	station/time	Sex	lenght (cm)	volume (ml)	Analysis
541	30.10.20	Station 3, T2	F	6,6		Biochemistry (LMS, AChE,CAT)
542	30.10.20	Station 3, T2	M	6,92		Biochemistry (LMS, AChE,CAT)
543	30.10.20	Station 3, T2	F	8		Biochemistry (LMS, AChE,CAT)
544	30.10.20	Station 3, T2	F	6,5		Biochemistry (LMS, AChE,CAT)
545	30.10.20	Station 3, T2	M	5,72		Biochemistry (LMS, AChE,CAT)
546	30.10.20	Station 3, T2	M	6		Biochemistry (LMS, AChE,CAT)
547	30.10.20	Station 3, T2	M	5,99		Biochemistry (LMS, AChE)
548	30.10.20	Station 3, T2	M	6,01		Biochemistry (LMS, AChE)
549	30.10.20	Station 3, T2	M	7,5		Biochemistry (LMS, AChE)
550	30.10.20	Station 3, T2	M	6,21		Biochemistry (LMS, AChE,CAT)
551	30.10.20	Station 3, T2	F	7,61		Biochemistry (LMS, AChE)
552	30.10.20	Station 3, T2	M	8,1		Biochemistry (LMS, AChE)
553	30.10.20	Station 3, T2	M	6,1		Biochemistry (LMS, AChE)
554	30.10.20	Station 3, T2	F	5,48		Biochemistry (LMS, AChE)
555	30.10.20	Station 3, T2	M	5,48		Biochemistry (LMS, AChE)
556	30.10.20	Station 3, T2	M	4,79		Chemistry 1-5
557	30.10.20	Station 3, T2	M	4,89		Chemistry 1-5
558	30.10.20	Station 3, T2	F	7,19		Chemistry 1-5
559	30.10.20	Station 3, T2	M	5,13		Chemistry 1-5
560	30.10.20	Station 3, T2	M	4,96		Chemistry 1-5
560A	30.10.20	Station 3, T2	M	5,55		
561	30.10.20	Station 3, T2	M	5,19		Chemistry 6-10
562	30.10.20	Station 3, T2	M	5,54		Chemistry 6-10
563	30.10.20	Station 3, T2	M	4,86		Chemistry 6-10
564	30.10.20	Station 3, T2	M	4,87		Chemistry 6-10
565	30.10.20	Station 3, T2	M	4,91		Chemistry 6-10
565A	30.10.20	Station 3, T2	M	4,56		
566	30.10.20	Station 3, T2	M	4,76		Chemistry 11-15
567	30.10.20	Station 3, T2	F	4,72		Chemistry 11-15
568	30.10.20	Station 3, T2	M	4,71		Chemistry 11-15
569	30.10.20	Station 3, T2	F	4,75		Chemistry 11-15
570	30.10.20	Station 3, T2	F	4,76		Chemistry 11-15
570 A	30.10.20	Station 3, T2	M	4,59		Chemistry 11-15
571	30.10.20	Station 3, T2	**M**	**6,5**	**26**	CI
572	30.10.20	Station 3, T2	F	6,22	16	CI
573	30.10.20	Station 3, T2	F	5,22	14	CI
574	30.10.20	Station 3, T2	F	6,93	26	CI
575	30.10.20	Station 3, T2	M	5,6	18	CI
576	30.10.20	Station 3, T2	F	7,5		CI
577	30.10.20	Station 3, T2	M	5,9	12	CI
578	30.10.20	Station 3, T2	M	5,9	18	CI
579	30.10.20	Station 3, T2	M	6,6	26	CI
580	30.10.20	Station 3, T2	M	6,1	20	CI
581	30.10.20	Station 3, T2	F	6,4	20	CI
582	30.10.20	Station 3, T2	M	5,98	12	CI
583	30.10.20	Station 3, T2	M	6	14	CI
584	30.10.20	Station 3, T2	M	4,9	12	CI
585	30.10.20	Station 3, T2	F	5,83	18	CI
586	30.10.20	Station 3, T2	M	5,22	12	CI
587	30.10.20	Station 3, T2	M	5,2	10	CI
588	30.10.20	Station 3, T2	M	5,11	12	CI
589	30.10.20	Station 3, T2	M	5,11	10	CI
590	30.10.20	Station 3, T2	M	4,9	11	CI
591	30.10.20	Station 3, T2	M	5,1	10	CI
592	30.10.20	Station 3, T2	M	5,11	12	CI
593	30.10.20	Station 3, T2	M	5,32	13	CI
594	30.10.20	Station 3, T2	M	4,99	9	CI
595	30.10.20	Station 3, T2	M	4,9	6	CI
596	30.10.20	Station 3, T2	M	5,16	10	CI
597	30.10.20	Station 3, T2	M	5	9	CI
598	30.10.20	Station 3, T2	F	5,2	12	CI
599	30.10.20	Station 3, T2	M	4,8	8	CI
600	30.10.20	Station 3, T2	M	4,9	10	CI

Appendix A 7 Detailed sampling sheet station 3, week 2

871	30.10.20	Station 3, T2		6,15		Stress on stress
872	30.10.20	Station 3, T2		6,53		Stress on stress
873	30.10.20	Station 3, T2		4,92		Stress on stress
874	30.10.20	Station 3, T2		5,38		Stress on stress
875	30.10.20	Station 3, T2		5,29		Stress on stress
876	30.10.20	Station 3, T2		5,85		Stress on stress
877	30.10.20	Station 3, T2		6,67		Stress on stress
878	30.10.20	Station 3, T2		5,61		Stress on stress
879	30.10.20	Station 3, T2		6,53		Stress on stress
880	30.10.20	Station 3, T2		5,86		Stress on stress
881	30.10.20	Station 3, T2		5,74		Stress on stress
882	30.10.20	Station 3, T2		5,16		Stress on stress
883	30.10.20	Station 3, T2		5,94		Stress on stress
884	30.10.20	Station 3, T2		5,53		Stress on stress
885	30.10.20	Station 3, T2		5,72		Stress on stress
886	30.10.20	Station 3, T2		5,71		Stress on stress
887	30.10.20	Station 3, T2		5,08		Stress on stress
888	30.10.20	Station 3, T2		5,7		Stress on stress
889	30.10.20	Station 3, T2		6,25		Stress on stress
890	30.10.20	Station 3, T2		4,72		Stress on stress
891	30.10.20	Station 3, T2		5,79		Stress on stress
892	30.10.20	Station 3, T2		5,88		Stress on stress
893	30.10.20	Station 3, T2		6,65		Stress on stress
894	30.10.20	Station 3, T2		5,43		Stress on stress
895	30.10.20	Station 3, T2		5,88		Stress on stress
896	30.10.20	Station 3, T2		6,21		Stress on stress
897	30.10.20	Station 3, T2		6		Stress on stress
898	30.10.20	Station 3, T2		6,1		Stress on stress
899	30.10.20	Station 3, T2		6,25		Stress on stress
900	30.10.20	Station 3, T2		5,58		Stress on stress
Average			Ratio: F= 16 /M= 47	5,706	14,000	

Appendix A 8 Detailed sampling sheet station 1, week 4

Mussel code	date	station/time	Sex	lenght (cm)	volume (ml)	Analysis
691	28.10.20	Station 1, T4	F	8,08		Biochemistry (LMS, AChE,CAT)
692	28.10.20	Station 1, T4	M	5,4		Biochemistry (LMS, AChE)
693	28.10.20	Station 1, T4	M	6,21		Biochemistry (LMS, AChE,CAT)
694	28.10.20	Station 1, T4	F	6,58		Biochemistry (LMS, AChE)
695	28.10.20	Station 1, T4	F	5,92		Biochemistry (LMS, AChE,CAT)
696	28.10.20	Station 1, T4	M	6,57		Biochemistry (LMS, AChE)
697	28.10.20	Station 1, T4	M	5,93		Biochemistry (LMS, AChE,CAT)
698	28.10.20	Station 1, T4	F	6,83		Biochemistry (LMS, AChE)
699	28.10.20	Station 1, T4	F	5,55		Biochemistry (LMS, AChE)
700	28.10.20	Station 1, T4	M	5,16		Biochemistry (LMS, AChE,CAT)
701	28.10.20	Station 1, T4	M	6,7		Biochemistry (LMS, AChE)
702	28.10.20	Station 1, T4	M	6,58		Biochemistry (LMS, AChE,CAT)
703	28.10.20	Station 1, T4	M	7,29		Biochemistry (LMS, AChE,CAT)
704	28.10.20	Station 1, T4	F	5,96		Biochemistry (LMS, AChE)
705	28.10.20	Station 1, T4	M	6,05		Biochemistry (LMS, AChE)
706	28.10.20	Station 1, T4	M	6,22		Chemistry 1-5
707	28.10.20	Station 1, T4	M	5,49		Chemistry 1-5
708	28.10.20	Station 1, T4	M	5,33		Chemistry 1-5
709	28.10.20	Station 1, T4	M	6,01		Chemistry 1-5
710	28.10.20	Station 1, T4	M	6,37		Chemistry 1-5
711	28.10.20	Station 1, T4	M	6,79		Chemistry 6-10
712	28.10.20	Station 1, T4	M	5,69		Chemistry 6-10
713	28.10.20	Station 1, T4	M	5,72		Chemistry 6-10
714	28.10.20	Station 1, T4	M	5,94		Chemistry 6-10
715	28.10.20	Station 1, T4	F	8,2		Chemistry 6-10
716	28.10.20	Station 1, T4	M	7,25		Chemistry 11-15
717	28.10.20	Station 1, T4	M	7,16		Chemistry 11-15
718	28.10.20	Station 1, T4	M	6		Chemistry 11-15
719	28.10.20	Station 1, T4	F	6,74		Chemistry 11-15
720	28.10.20	Station 1, T4	F	7,01		Chemistry 11-15
721	28.10.20	Station 1, T4	**M**	**6**	**18**	CI
722	28.10.20	Station 1, T4	M	5,3	14	CI
723	28.10.20	Station 1, T4	F	6,5	20	CI
724	28.10.20	Station 1, T4	M	5,8	12	CI
725	28.10.20	Station 1, T4	F	6,8	20	CI
726	28.10.20	Station 1, T4	F	5,4	14	CI
727	28.10.20	Station 1, T4	M	6	12	CI
728	28.10.20	Station 1, T4	M	6,5	22	CI
729	28.10.20	Station 1, T4	M	6,61	20	CI
730	28.10.20	Station 1, T4	M	6,62	20	CI
731	28.10.20	Station 1, T4	M	5,91	14	CI
732	28.10.20	Station 1, T4	M	5,5	14	CI
733	28.10.20	Station 1, T4	M	5,63	16	CI
734	28.10.20	Station 1, T4	M	5,87	16	CI
735	28.10.20	Station 1, T4	M	5,43	10	CI
736	28.10.20	Station 1, T4	F	5,47	10	CI
737	28.10.20	Station 1, T4	M	6,3	24	CI
738	28.10.20	Station 1, T4	M	6,59	22	CI
739	28.10.20	Station 1, T4	M	6,24	18	CI
740	28.10.20	Station 1, T4	M	6,44	18	CI
741	28.10.20	Station 1, T4	M	6,49	18	CI
742	28.10.20	Station 1, T4	M	6,34	24	CI
743	28.10.20	Station 1, T4	F	5,84	16	CI
744	28.10.20	Station 1, T4	M	6,09	16	CI
745	28.10.20	Station 1, T4	M	6,74	22	CI
746	28.10.20	Station 1, T4	M	5,74	16	CI
747	28.10.20	Station 1, T4	M	6	14	CI
748	28.10.20	Station 1, T4	F	5,16	10	CI
749	28.10.20	Station 1, T4	F	6,15	12	CI
750	28.10.20	Station 1, T4	M	5,19	12	CI

Appendix A 8 Detailed sampling sheet station 1, week 4

751	28.10.20	Station 1, T4		6,22		Stress on stress
752	28.10.20	Station 1, T4		6,62		Stress on stress
753	28.10.20	Station 1, T4		5,75		Stress on stress
754	28.10.20	Station 1, T4		6,61		Stress on stress
755	28.10.20	Station 1, T4		6,04		Stress on stress
756	28.10.20	Station 1, T4		6,95		Stress on stress
757	28.10.20	Station 1, T4		5,44		Stress on stress
758	28.10.20	Station 1, T4		6,03		Stress on stress
759	28.10.20	Station 1, T4		7,23		Stress on stress
760	28.10.20	Station 1, T4		5,69		Stress on stress
761	28.10.20	Station 1, T4		6,3		Stress on stress
762	28.10.20	Station 1, T4		5,71		Stress on stress
763	28.10.20	Station 1, T4		6,71		Stress on stress
764	28.10.20	Station 1, T4		6,39		Stress on stress
765	28.10.20	Station 1, T4		5,77		Stress on stress
766	28.10.20	Station 1, T4		7,53		Stress on stress
767	28.10.20	Station 1, T4		5,98		Stress on stress
768	28.10.20	Station 1, T4		7,01		Stress on stress
769	28.10.20	Station 1, T4		6,15		Stress on stress
770	28.10.20	Station 1, T4		5,5		Stress on stress
771	28.10.20	Station 1, T4		6,79		Stress on stress
772	28.10.20	Station 1, T4		5,59		Stress on stress
773	28.10.20	Station 1, T4		6		Stress on stress
774	28.10.20	Station 1, T4		5,19		Stress on stress
775	28.10.20	Station 1, T4		6,78		Stress on stress
776	28.10.20	Station 1, T4		6,21		Stress on stress
777	28.10.20	Station 1, T4		6,19		Stress on stress
778	28.10.20	Station 1, T4		5,74		Stress on stress
779	28.10.20	Station 1, T4		5,88		Stress on stress
780	28.10.20	Station 1, T4		5,83		Stress on stress
Average			Ratio: F=16 /M=44	6,191	16,467	

Appendix A 9 Detailed sampling sheet station 2, week 4

Mussel code	date	station/time	Sex	lenght (cm)	volume (ml)	Analysis
601	27-Oct	Station 2, T4	F	6,51		Biochemistry (LMS, AChE,CAT)
602	27-Oct	Station 2, T4	M	6,77		Biochemistry (LMS, AChE)
603	27-Oct	Station 2, T4	M	5,46		Biochemistry (LMS, AChE)
604	27-Oct	Station 2, T4	M	6,95		Biochemistry (LMS, AChE,CAT)
605	27-Oct	Station 2, T4	M	6,18		Biochemistry (LMS, AChE)
606	27-Oct	Station 2, T4	M	6,21		Biochemistry (LMS, AChE,CAT)
607	27-Oct	Station 2, T4	F	7,31		Biochemistry (LMS, AChE,CAT)
608	27-Oct	Station 2, T4	M	5,81		Biochemistry (LMS, AChE)
609	27-Oct	Station 2, T4	F	5,8		Biochemistry (LMS, AChE,CAT)
610	27-Oct	Station 2, T4	M	5,63		Biochemistry (LMS, AChE)
611	27-Oct	Station 2, T4	F	7,05		Biochemistry (LMS, AChE,CAT)
612	27-Oct	Station 2, T4	M	6		Biochemistry (LMS, AChE,CAT)
613	27-Oct	Station 2, T4	M	7,01		Biochemistry (LMS, AChE)
614	27-Oct	Station 2, T4	F	6,5		Biochemistry (LMS, AChE)
615	27-Oct	Station 2, T4	M	5,78		Biochemistry (LMS, AChE)
616	27-Oct	Station 2, T4	M	7,02		Chemistry 1-5
617	27-Oct	Station 2, T4	F	5,7		Chemistry 1-5
618	27-Oct	Station 2, T4	F	6,53		Chemistry 1-5
619	27-Oct	Station 2, T4	M	5,91		Chemistry 1-5
620	27-Oct	Station 2, T4	M	6,04		Chemistry 1-5
621	27-Oct	Station 2, T4	M	4,88		Chemistry 6-10
622	27-Oct	Station 2, T4	M	5,34		Chemistry 6-10
623	27-Oct	Station 2, T4	F	5,95		Chemistry 6-10
624	27-Oct	Station 2, T4	F	5,68		Chemistry 6-10
625	27-Oct	Station 2, T4	F	5,31		Chemistry 6-10
626	27-Oct	Station 2, T4	M	5,95		Chemistry 11-15
627	27-Oct	Station 2, T4	F	6,77		Chemistry 11-15
628	27-Oct	Station 2, T4	M	5,33		Chemistry 11-15
629	27-Oct	Station 2, T4	M	5,4		Chemistry 11-15
630	27-Oct	Station 2, T4	M	5,24		Chemistry 11-15
631	27-Oct	Station 2, T4	**M**	**6,5**	**20**	CI
632	27-Oct	Station 2, T4	F	6,1	20	CI
633	27-Oct	Station 2, T4	F	7	40	CI
634	27-Oct	Station 2, T4	M	6,51	20	CI
635	27-Oct	Station 2, T4	F	6,4	20	CI
636	27-Oct	Station 2, T4	M	6	16	CI
637	27-Oct	Station 2, T4	M	6,51	16	CI
638	27-Oct	Station 2, T4	M	6,32	18	CI
639	27-Oct	Station 2, T4	F	5,7	12	CI
640	27-Oct	Station 2, T4	M	6,4	22	CI
641	27-Oct	Station 2, T4	M	6,3	22	CI
642	27-Oct	Station 2, T4	F	5,6	16	CI
643	27-Oct	Station 2, T4	F	5,56	14	CI
644	27-Oct	Station 2, T4	F	6,41	20	CI
645	27-Oct	Station 2, T4	M	6,2	18	CI
646	27-Oct	Station 2, T4	M	6,02		CI
647	27-Oct	Station 2, T4	M	6,35		CI
648	27-Oct	Station 2, T4	M	7,19		CI
649	27-Oct	Station 2, T4	M	5,94		CI
650	27-Oct	Station 2, T4	M	5,47		CI
651	27-Oct	Station 2, T4	M	5,47		CI
652	27-Oct	Station 2, T4	M	5,05		CI
653	27-Oct	Station 2, T4	M	5,55		CI
654	27-Oct	Station 2, T4	M	5,2		CI
655	27-Oct	Station 2, T4	M	4,76		CI
656	27-Oct	Station 2, T4	M	5,34		CI
657	27-Oct	Station 2, T4	M	5,96		CI
658	27-Oct	Station 2, T4	F	6,57		CI
659	27-Oct	Station 2, T4	F	4,8		CI
660	27-Oct	Station 2, T4	M	4,97		CI

Appendix A 9 Detailed sampling sheet station 2, week 4

661	27-Oct	Station 2, T4		5,45		Stress on stress
662	27-Oct	Station 2, T4		5,38		Stress on stress
663	27-Oct	Station 2, T4		5,06		Stress on stress
664	27-Oct	Station 2, T4		5,7		Stress on stress
665	27-Oct	Station 2, T4		7,91		Stress on stress
666	27-Oct	Station 2, T4		4,81		Stress on stress
667	27-Oct	Station 2, T4		7,22		Stress on stress
668	27-Oct	Station 2, T4		6,21		Stress on stress
669	27-Oct	Station 2, T4		5,23		Stress on stress
670	27-Oct	Station 2, T4		6,14		Stress on stress
671	27-Oct	Station 2, T4		5,63		Stress on stress
672	27-Oct	Station 2, T4		4,68		Stress on stress
673	27-Oct	Station 2, T4		4,85		Stress on stress
674	27-Oct	Station 2, T4		7,8		Stress on stress
675	27-Oct	Station 2, T4		5,9		Stress on stress
676	27-Oct	Station 2, T4		5,35		Stress on stress
677	27-Oct	Station 2, T4		4,88		Stress on stress
678	27-Oct	Station 2, T4		6,11		Stress on stress
679	27-Oct	Station 2, T4		6,13		Stress on stress
680	27-Oct	Station 2, T4		5,01		Stress on stress
681	27-Oct	Station 2, T4		7,12		Stress on stress
682	27-Oct	Station 2, T4		5,54		Stress on stress
683	27-Oct	Station 2, T4		5,46		Stress on stress
684	27-Oct	Station 2, T4		5,54		Stress on stress
685	27-Oct	Station 2, T4		5,55		Stress on stress
686	27-Oct	Station 2, T4		5,6		Stress on stress
687	27-Oct	Station 2, T4		6,28		Stress on stress
688	27-Oct	Station 2, T4		6,24		Stress on stress
689	27-Oct	Station 2, T4		5,67		Stress on stress
690	27-Oct	Station 2, T4		5,49		Stress on stress
Average			Ratio: F= 20/M= 40	5,935	19,600	

Appendix A 10 Detailed sampling sheet station 3, week 4

Mussel code	date	station/time	Sex	lenght (cm)	volume (ml)	Analysis
781	10.11.20	Station 3, T4	M	5,93		Biochemistry (LMS, AChE,CAT)
782	10.11.20	Station 3, T4	F	6,35		Biochemistry (LMS, AChE,CAT)
783	10.11.20	Station 3, T4	M	6		Biochemistry (LMS, AChE,CAT)
784	10.11.20	Station 3, T4	F	7,5		Biochemistry (LMS, AChE,CAT)
785	10.11.20	Station 3, T4	M	5,84		Biochemistry (LMS, AChE)
786	10.11.20	Station 3, T4	M	5,75		Biochemistry (LMS, AChE)
787	10.11.20	Station 3, T4	M	5,85		Biochemistry (LMS, AChE)
788	10.11.20	Station 3, T4	F	6,02		Biochemistry (LMS, AChE,CAT)
789	10.11.20	Station 3, T4	M	6,21		Biochemistry (LMS, AChE,CAT)
790	10.11.20	Station 3, T4	F	5,31		Biochemistry (LMS, AChE)
791	10.11.20	Station 3, T4	M	5,72		Biochemistry (LMS, AChE)
792	10.11.20	Station 3, T4	M	6,17		Biochemistry (LMS, AChE)
793	10.11.20	Station 3, T4	M	5,05		Biochemistry (LMS, AChE)
794	10.11.20	Station 3, T4	M	6,2		Biochemistry (LMS, AChE,CAT)
795	10.11.20	Station 3, T4	M	5,27		Biochemistry (LMS, AChE)
796	10.11.20	Station 3, T4	M	5,07		Chemistry 1-5
797	10.11.20	Station 3, T4	M	5,59		Chemistry 1-5
798	10.11.20	Station 3, T4	M	6,24		Chemistry 1-5
799	10.11.20	Station 3, T4	M	5,14		Chemistry 1-5
800	10.11.20	Station 3, T4	M	5		Chemistry 1-5
801	10.11.20	Station 3, T4	M	4,8		Chemistry 6-10
802	10.11.20	Station 3, T4	F	5,58		Chemistry 6-10
803	10.11.20	Station 3, T4	F	5,38		Chemistry 6-10
804	10.11.20	Station 3, T4	M	5,37		Chemistry 6-10
805	10.11.20	Station 3, T4	M	5,69		Chemistry 6-10
806	10.11.20	Station 3, T4	M	5,8		Chemistry 11-15
807	10.11.20	Station 3, T4	M	5,11		Chemistry 11-15
808	10.11.20	Station 3, T4	M	5,13		Chemistry 11-15
809	10.11.20	Station 3, T4	F	5,09		Chemistry 11-15
810	10.11.20	Station 3, T4	F	5,57		Chemistry 11-15
811	10.11.20	Station 3, T4	**M**	**5,77**	**14**	CI
812	10.11.20	Station 3, T4	F	6,15	20	CI
813	10.11.20	Station 3, T4	M	5	8	CI
814	10.11.20	Station 3, T4	F	4,91	8	CI
815	10.11.20	Station 3, T4	M	5,15	10	CI
816	10.11.20	Station 3, T4	M	5,8	20	CI
817	10.11.20	Station 3, T4	F	6,04	16	CI
818	10.11.20	Station 3, T4	M	5,69	14	CI
819	10.11.20	Station 3, T4	M	5,08	10	CI
820	10.11.20	Station 3, T4	M	5,69	14	CI
821	10.11.20	Station 3, T4	M	5	10	CI
822	10.11.20	Station 3, T4	M	4,82	8	CI
823	10.11.20	Station 3, T4	F	5,42	12	CI
824	10.11.20	Station 3, T4	M	5,97	16	CI
825	10.11.20	Station 3, T4	M	5,05	10	CI
826	10.11.20	Station 3, T4	F	5,09	10	CI
827	10.11.20	Station 3, T4	M	4,96	10	CI
828	10.11.20	Station 3, T4	F	4,97	11	CI
829	10.11.20	Station 3, T4	M	4,89	9	CI
830	10.11.20	Station 3, T4	M	4,9	10	CI
831	10.11.20	Station 3, T4	M	4,89	8	CI
832	10.11.20	Station 3, T4	M	5,89	10	CI
833	10.11.20	Station 3, T4	M	5,2	10	CI
834	10.11.20	Station 3, T4	F	6,55	22	CI
835	10.11.20	Station 3, T4	F	5,47	12	CI
836	10.11.20	Station 3, T4	M	5,02	10	CI
837	10.11.20	Station 3, T4	M	5,73	13	CI
838	10.11.20	Station 3, T4	F	5,05	10	CI
839	10.11.20	Station 3, T4	M	5,78	13	CI
840	10.11.20	Station 3, T4	M	5	9	CI

Appendix A 10 Detailed sampling sheet station 3, week 4

841	10.11.20	Station 3, T4		5,1		Stress on stress
842	10.11.20	Station 3, T4		5,89		Stress on stress
843	10.11.20	Station 3, T4		5,01		Stress on stress
844	10.11.20	Station 3, T4		6,53		Stress on stress
845	10.11.20	Station 3, T4		5,23		Stress on stress
846	10.11.20	Station 3, T4		5,9		Stress on stress
847	10.11.20	Station 3, T4		4,85		Stress on stress
848	10.11.20	Station 3, T4		4,96		Stress on stress
849	10.11.20	Station 3, T4		6,71		Stress on stress
850	10.11.20	Station 3, T4		5,53		Stress on stress
851	10.11.20	Station 3, T4		6,07		Stress on stress
852	10.11.20	Station 3, T4		5,34		Stress on stress
853	10.11.20	Station 3, T4		5,9		Stress on stress
854	10.11.20	Station 3, T4		5,51		Stress on stress
855	10.11.20	Station 3, T4		5,12		Stress on stress
856	10.11.20	Station 3, T4		4,99		Stress on stress
857	10.11.20	Station 3, T4		5,6		Stress on stress
858	10.11.20	Station 3, T4		5,64		Stress on stress
859	10.11.20	Station 3, T4		5,19		Stress on stress
860	10.11.20	Station 3, T4		5,43		Stress on stress
861	10.11.20	Station 3, T4		5,6		Stress on stress
862	10.11.20	Station 3, T4		4,95		Stress on stress
863	10.11.20	Station 3, T4		5,54		Stress on stress
864	10.11.20	Station 3, T4		5,92		Stress on stress
865	10.11.20	Station 3, T4		5,4		Stress on stress
866	10.11.20	Station 3, T4		5		Stress on stress
867	10.11.20	Station 3, T4		5,28		Stress on stress
868	10.11.20	Station 3, T4		6,45		Stress on stress
869	10.11.20	Station 3, T4		5,86		Stress on stress
870	10.11.20	Station 3, T4		5,87		Stress on stress
Average			Ratio: F= 17 /M=43	5,523	11,900	

Appendix B 1 CI raw data, pre-deployment (T0)

Mussel #	Dry Weight Tissue (g)	Dry Weight Shell (g)	Condition Index
31	1,872	10,933	0,171
32	1,039	6,479	0,160
33	0,682	4,288	0,159
34	1,065	4,608	0,231
35	1,130	5,050	0,224
36	1,470	7,421	0,198
37	1,550	6,736	0,230
38	0,951	4,977	0,191
39	1,925	6,073	0,317
40	1,043	5,108	0,204
41	1,024	4,891	0,209
42	0,610	4,249	0,144
43	1,500	6,326	0,237
44	0,711	4,131	0,172
45	1,447	10,248	0,141
46	1,133	5,081	0,223
47	0,865	3,624	0,239
48	1,110	5,836	0,190
49	1,553	4,253	0,365
50	2,092	9,291	0,225
51	0,919	5,154	0,178
52	2,536	9,204	0,276
53	1,051	4,821	0,218
54	1,235	6,256	0,197
55	1,008	5,439	0,185
56	2,148	8,387	0,256
57	0,959	5,993	0,160
58	1,418	4,913	0,289
59	1,012	4,653	0,217
60	0,842	3,471	0,243
Average			0,22
Std. Dev			0,05
Std. Error			0,01

Appendix B 2 CI raw data, station 1, week 1

Mussel #	Dry Weight Tissue (g)	Dry Weight Shell (g)	Condition Index
121	1,607	5,761	0,279
122	1,745	7,583	0,230
123	1,448	5,219	0,278
124	2,067	8,169	0,253
125	1,102	4,747	0,232
126	2,230	8,323	0,268
127	1,254	7,056	0,178
128	0,894	3,121	0,287
129	0,793	6,072	0,131
130	0,204	2,922	0,070
131	0,714	4,484	0,159
132	1,840	5,323	0,346
133	0,420	5,027	0,083
134	1,673	6,268	0,267
135	1,127	7,389	0,153
136	0,983	9,368	0,105
137	1,958	10,831	0,181
138	1,705	8,233	0,207
139	1,152	5,741	0,201
140	3,667	14,808	0,248
141	3,115	15,731	0,198
142	1,785	6,942	0,257
143	1,095	7,392	0,148
144	1,476	6,532	0,226
145	1,899	9,495	0,200
146	1,377	6,589	0,209
147	1,750	5,641	0,310
148	2,384	16,066	0,148
149	2,187	10,347	0,211
150	1,619	6,645	0,244
Average			0,210
Std dev			0,066
std error			0,012

Appendix B 3 CI raw data, station 2, week 1

Mussel #	Dry Weight Tissue (g)	Dry Weight Shell (g)	Condition Index
211	1,707	8,627	0,198
212	2,005	7,447	0,269
213	1,062	6,880	0,154
214	1,189	5,692	0,209
215	0,445	3,512	0,127
216	3,007	11,472	0,262
217	1,097	5,978	0,183
218	0,606	5,024	0,121
219	1,706	6,798	0,251
220	1,787	6,893	0,259
221	0,913	6,165	0,148
222	2,661	11,006	0,242
223	3,069	9,047	0,339
224	1,370	4,980	0,275
225	1,837	9,396	0,196
226	3,270	12,231	0,267
227	0,421	3,195	0,132
228	1,088	3,601	0,302
229	1,762	6,173	0,285
230	3,200	12,476	0,256
231	2,171	8,402	0,258
232	0,868	6,871	0,126
233	0,788	4,125	0,191
234	1,084	4,305	0,252
235	1,880	10,788	0,174
236	0,645	3,682	0,175
237	0,898	3,408	0,263
238	2,077	8,878	0,234
239	0,681	6,275	0,109
240	0,427	3,114	0,137
Average			0,213
Std dev			0,063
Std error			0,011

Appendix B 3 CI raw data, station 3, week 1

Mussel #	Dry Weight Tissue (g)	Dry Weight Shell (g)	Condition Index
301	0,311	3,349	0,093
302	0,254	6,746	0,038
303	1,085	7,342	0,148
304	2,049	7,964	0,257
305	1,561	4,713	0,331
306	1,753	7,568	0,232
307	1,327	6,217	0,213
308	1,398	7,171	0,195
309	1,001	5,176	0,193
310	0,366	2,902	0,126
311	0,728	4,841	0,150
312	1,219	5,256	0,232
313	0,937	5,313	0,176
314	1,064	8,995	0,118
315	0,709	5,536	0,128
316	1,729	14,647	0,118
317	0,415	3,078	0,135
318	0,479	2,757	0,174
319	0,846	2,987	0,283
320	0,666	4,490	0,148
321	0,805	3,390	0,238
322	1,124	3,634	0,309
323	0,724	4,262	0,170
324	0,517	3,057	0,169
325	0,959	3,759	0,255
326	0,611	3,068	0,199
327	0,227	2,766	0,082
328	0,659	3,483	0,189
329	0,515	2,992	0,172
330	0,756	3,099	0,244
Average			0,184
Std dev			0,067
Std error			0,012

Appendix B 4 CI raw data, station 1, week 2

Mussel #	Dry Weight Tissue (g)	Dry Weight Shell (g)	Condition Index
481	1,000	5,657	0,177
482	0,920	4,401	0,209
483	0,548	5,223	0,105
484	0,461	3,957	0,116
485	0,743	3,807	0,195
486	1,213	6,479	0,187
487	0,965	4,854	0,199
488	0,668	5,315	0,126
489	0,666	4,447	0,150
490	0,742	3,965	0,187
491	0,425	4,699	0,091
492	0,921	3,916	0,235
493	0,800	3,411	0,235
494	0,603	2,965	0,203
495	0,636	4,171	0,152
496	2,441	10,146	0,241
497	1,478	6,886	0,215
498	0,727	3,388	0,215
499	0,987	3,289	0,300
500	0,353	2,892	0,122
501	0,826	6,597	0,125
502	1,369	7,637	0,179
503	1,090	5,385	0,202
504	0,912	4,672	0,195
505	1,305	7,865	0,166
506	1,150	4,182	0,275
507	0,642	2,993	0,214
508	0,349	3,412	0,102
509	1,542	7,479	0,206
510	1,231	6,541	0,188
Average			0,184
Std dev			0,051
Std error			0,009

Appendix B 5 CI raw data, station 2, week 2

Mussel #	Dry Weight Tissue (g)	Dry Weight Shell (g)	Condition Index
391	3,410	11,397	0,299
392	1,290	9,760	0,132
393	1,363	8,306	0,164
394	2,131	9,668	0,220
395	2,783	11,269	0,247
396	1,899	6,404	0,297
397	1,511	7,046	0,214
398	1,730	7,685	0,225
399	1,836	10,790	0,170
400	1,752	7,905	0,222
401	2,151	9,103	0,236
402	1,954	7,660	0,255
403	1,830	8,476	0,216
404	0,895	5,514	0,162
405	1,390	7,444	0,187
406	0,897	4,849	0,185
407	1,737	8,299	0,209
408	0,637	4,216	0,151
409	1,565	7,036	0,222
410	0,627	4,953	0,127
411	1,783	10,318	0,173
412	0,523	2,269	0,230
413	0,959	4,656	0,206
414	1,259	4,901	0,257
415	0,555	2,791	0,199
416	0,867	3,243	0,267
417	1,219	5,574	0,219
418	0,877	3,264	0,269
419	0,434	2,481	0,175
420	0,930	4,039	0,230
Average			0,212
Std dev			0,044
std error			0,008

Appendix B 6 CI raw data, station 3, week 2

Mussel #	Dry Weight Tissue (g)	Dry Weight Shell (g)	Condition Index
571	2,130	8,497	0,251
572	1,257	6,129	0,205
573	0,725	4,424	0,164
574	2,281	12,176	0,187
575	1,108	6,300	0,176
576	2,685	13,844	0,194
577	1,260	6,815	0,185
578	0,820	4,951	0,166
579	2,261	8,318	0,272
580	1,692	7,788	0,217
581	1,490	7,599	0,196
582	0,897	4,870	0,184
583	1,447	6,667	0,217
584	0,879	4,805	0,183
585	1,518	5,487	0,277
586	0,969	3,821	0,254
587	1,037	3,534	0,294
588	0,948	5,715	0,166
589	0,735	3,882	0,189
590	0,863	4,042	0,213
591	1,024	4,228	0,242
592	0,764	4,677	0,163
593	1,239	3,894	0,318
594	0,757	3,937	0,192
595	0,877	4,205	0,208
596	0,694	3,550	0,196
597	0,781	4,099	0,191
598	1,057	5,186	0,204
599	0,744	3,106	0,239
600	0,529	3,639	0,145
Average			0,210
Std dev			0,041
Std error			0,007

Appendix B 7 CI raw data, station 1, week 4

Mussel #	Dry Weight Tissue (g)	Dry Weight Shell (g)	Condition Index
721	1,117	6,271	0,178
722	0,742	4,557	0,163
723	1,638	7,353	0,223
724	1,031	4,671	0,221
725	1,660	7,574	0,219
726	0,556	4,176	0,133
727	1,269	4,840	0,262
728	1,342	7,119	0,189
729	1,677	6,984	0,240
730	1,516	7,601	0,199
731	1,787	6,768	0,264
732	1,085	5,028	0,216
733	0,704	4,335	0,162
734	1,011	5,459	0,185
735	0,673	4,234	0,159
736	0,578	3,770	0,153
737	1,750	7,215	0,243
738	1,440	7,557	0,191
739	0,888	4,808	0,185
740	0,681	5,370	0,127
741	1,533	6,412	0,239
742	2,112	8,188	0,258
743	1,285	5,387	0,239
744	1,215	6,114	0,199
745	1,862	8,388	0,222
746	1,197	5,930	0,202
747	1,272	5,264	0,242
748	0,731	4,836	0,151
749	0,849	4,337	0,196
750	0,553	3,957	0,140
Average			0,200
Std dev			0,038
Std error			0,007

Appendix B 8 CI raw data, station 2, week 4

Mussel #	Dry Weight Tissue (g)	Dry Weight Shell (g)	Condition Index
631	1,043	7,947	0,131
632	1,190	6,750	0,176
633	0,807	6,769	0,119
634	1,035	6,767	0,153
635	1,543	7,467	0,207
636	0,297	5,501	0,054
637	1,397	5,731	0,244
638	0,790	6,991	0,113
639	1,254	5,549	0,226
640	1,227	9,598	0,128
641	1,664	8,189	0,203
642	0,773	4,694	0,165
643	0,512	6,000	0,085
644	1,009	8,659	0,116
645	1,098	6,559	0,167
646	0,449	5,937	0,076
647	1,671	9,251	0,181
648	1,395	8,784	0,159
649	1,038	6,555	0,158
650	0,944	5,164	0,183
651	0,747	4,302	0,174
652	0,899	3,380	0,266
653	0,722	5,305	0,136
654	0,381	4,217	0,090
655	0,465	3,215	0,145
656	0,566	7,097	0,080
657	0,866	6,578	0,132
658	1,358	7,681	0,177
659	0,346	3,399	0,102
660	0,397	2,523	0,157
Average			0,150
Std dev			0,051
Sts error			0,027

Appendix B 8 CI raw data, station 2, week 4

Mussel #	Dry Weight Tissue (g)	Dry Weight Shell (g)	Condition Index
811	1,096	5,264	0,208
812	1,184	8,319	0,142
813	0,500	2,797	0,179
814	0,313	4,093	0,076
815	0,390	3,701	0,105
816	0,430	5,880	0,073
817	1,398	6,481	0,211
818	1,007	6,298	0,160
819	0,777	5,370	0,145
820	0,891	5,833	0,153
821	0,677	3,905	0,173
822	0,312	2,813	0,111
823	0,946	5,115	0,185
824	0,987	6,610	0,149
825	0,234	4,042	0,058
826	0,474	3,735	0,127
827	0,710	3,576	0,199
828	0,677	4,925	0,137
829	0,594	4,024	0,148
830	0,357	4,546	0,078
831	0,649	3,200	0,203
832	0,357	5,609	0,064
833	0,420	3,615	0,116
834	1,046	7,461	0,140
835	0,679	4,436	0,153
836	0,501	5,099	0,098
837	0,331	4,997	0,066
838	0,575	4,146	0,139
839	0,803	5,280	0,152
840	0,340	2,748	0,124
Average			0,136
Std dev			0,045
Std error			0,008

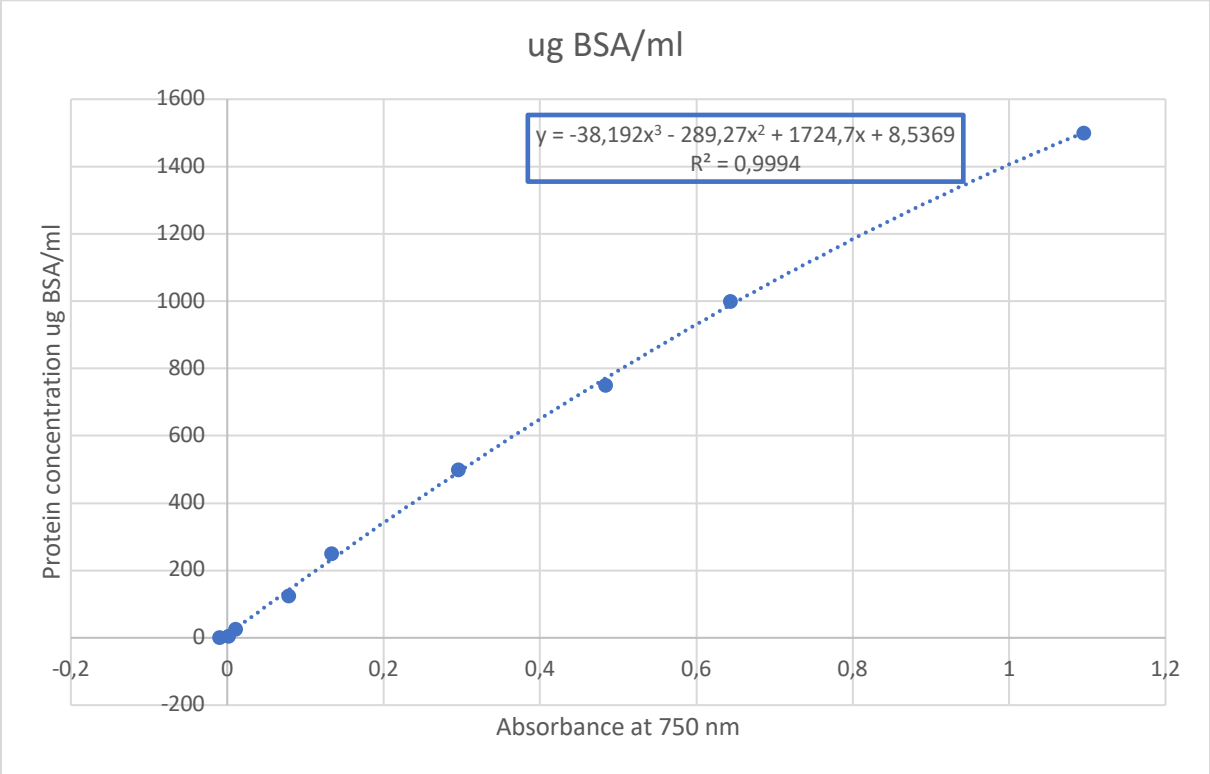
Appendix C SoS raw data

SoS: Survival time for all stations, in days									
T0	Station 1, T1	Station 2, T1	Station 4, T1	Station 1, T2	Station 2, T2	Station 3, T2	Station 1, T4	Station 2, T4	Station 3, T4
10	9	3	7	7	5	5	7	7	8
10	10	3	8	9	10	6	7	7	8
10	10	6	10	11	11	8	11	8	8
10	10	7	10	12	12	8	12	8	10
11	13	11	13	13	14	8	12	9	11
11	13	11	13	14	14	9	12	9	12
11	14	11	14	15	14	9	13	9	13
13	17	11	14	16	16	10	13	9	13
14	17	11	14	17	17	10	13	9	13
15	18	13	14	17	17	10	15	9	15
15	18	13	14	22	18	11	15	9	15
15	20	14	14	23	18	11	15	9	15
15	20	14	14	24	18	11	15	10	16
15	20	14	16	24	18	11	16	11	17
16	21	14	16	25	20	11	17	11	18
18	21	15	17	25	20	11	17	11	20
19	23	17	19	26	21	13	17	11	20
19	24	17	19	29	22	14	17	11	21
20	25	17	19	35	26	14	18	11	24
22	26	17	19	35	27	14	18	11	26
28	26	20	21	35	27	14	18	12	28
30	33	20	21	35	27	14	19	13	31
35	33	30	23	35	30	15	20	13	31
35	35	35	27	35	31	16	20	14	35
35	35	35	34	35	35	18	22	15	35
35	35	35	35	35	35	18	22	17	35
35	35	35	35	35	35	20	22	18	35
35	35	35	35	35	35	20	22	18	35
35	35	35	35	35	35	20	24	22	35
35		35	35	35	35	21	26	22	35

Appendix D LMS raw data

Slide	T0	Station 1, T1	Station 2, T1	Station 3, T1	Station 1, T2	Station 2, T2	Station 3, T2	Station 1, T4	Station 2, T4	Station 3, T4
1	150	180	150	180	180	180	60	180	150	150
2	180	180	150	180	180	180	60	150	180	60
3	180	150	150	150	150	180	90	150	120	180
4	180	180	120	180	180	180	180	180	120	180
5	120	180	180	150	180	180	180	180	150	
6	120	150	180	150	150	150	180	180	180	120
7	180	150	120	120	150	120	150	180	90	150
8	180	120	150	180	120	150	120	150	180	180
9	90	180	180	120	150	180	150	180	180	60
10	120	180	180	90	180	180	60	180	150	150
11	180	180	120	180	180	180	180	180	120	120
12	150	150	180	150	180	150	90	180	180	180
13	180	150	150	120	180	180	90	180	120	120
14	180	180	180	180	180	150	180	180	120	180
15	180	180	150	180	150	180	180	180	180	180
Median value	180	180	150	150	180	180	150	180	150	150

Appendix E Protein concentration determination gill, plot used for AChE calculation



Protein standard curve using Bovine Serum Albumin as protein standard, the formula obtained was used to calculate the protein content of the gills.

Appendix F 1 AChE activity, T0(1-15), ST1 (91-105), ST2(181-195) and ST3 (271-285)

Mussel number	Average Absorbance 750 Blank corrected	protein concentra	15:100 dilution	mg/ml	Average abs 405 Blank corrected	umol ATC/min/	nmol ATC/min/
1	0,58743	914,10936	6094,06239	6,09406	0,01103	0,00080	0,79833
2	0,77658	1155,56576	7703,77170	7,70377	0,01634	0,00094	0,93575
3	0,90808	1307,56915	8717,12767	8,71713	0,00779	0,00039	0,39413
4	0,69158	1050,31923	7002,12821	7,00213	0,02277	0,00143	1,43465
5	0,86608	1260,47410	8403,16065	8,40316	0,02004	0,00105	1,05199
6	0,75258	1126,39675	7509,31166	7,50931	0,01500	0,00088	0,88097
7	1,03358	1439,95866	9599,72441	9,59972	0,01885	0,00087	0,86618
8	0,98883	1394,20177	9294,67848	9,29468	0,01907	0,00091	0,90517
9	0,97108	1375,60465	9170,69768	9,17070	0,01052	0,00051	0,50597
10	0,85933	1252,77696	8351,84643	8,35185	0,01417	0,00075	0,74838
11	0,80808	1193,18830	7954,58870	7,95459	0,00958	0,00053	0,53146
12	0,89733	1295,64651	8637,64341	8,63764	0,01655	0,00085	0,84531
13	0,69483	1054,44217	7029,61444	7,02961	0,00717	0,00045	0,44999
14	0,72308	1089,95088	7266,33921	7,26634	0,02518	0,00153	1,52850
15	0,83258	1221,92675	8146,17831	8,14618	0,00849	0,00046	0,45966
91	0,71208	1076,19480	7174,63203	7,17463	0,02112	0,00130	1,29885
92	0,82508	1213,17815	8087,85435	8,08785	0,03966	0,00216	2,16324
93	0,83883	1229,18419	8194,56125	8,19456	0,01377	0,00074	0,74121
94	0,92208	1322,95998	8819,73321	8,81973	0,04347	0,00217	2,17456
95	0,90258	1301,48053	8676,53690	8,67654	0,01572	0,00080	0,79919
96	0,87558	1271,24711	8474,98071	8,47498	0,01996	0,00104	1,03904
97	0,60033	931,41118	6209,40785	6,20941	0,02834	0,00201	2,01373
98	1,03333	1439,70755	9598,05035	9,59805	0,01579	0,00073	0,72591
99	0,55423	869,05328	5793,68853	5,79369	0,01355	0,00103	1,03142
100	0,54498	856,36113	5709,07417	5,70907	0,01397	0,00108	1,07936
101	0,55148	865,28623	5768,57489	5,76857	0,00759	0,00058	0,58080
102	0,55523	870,42179	5802,81194	5,80281	0,00682	0,00052	0,51857
103	0,56573	884,74851	5898,32342	5,89832	-0,00078	-0,00006	-0,05803
104	0,54298	853,60896	5690,72638	5,69073	-0,01311	-0,00102	-1,01617
105	0,44348	713,17644	4754,50960	4,75451	0,00715	0,00066	0,66299
181	0,46298	741,23601	4941,57338	4,94157	0,02163	0,00193	1,93132
182	0,54648	858,42341	5722,82271	5,72282	0,02154	0,00166	1,66086
183	0,58298	908,11565	6054,10434	6,05410	0,02213	0,00161	1,61230
184	0,51673	817,22678	5448,17851	5,44818	0,00203	0,00016	0,16438
185	0,52848	833,57175	5557,14501	5,55715	-0,06741	-0,00535	-5,35162
186	0,49173	782,13054	5214,20358	5,21420	0,01026	0,00087	0,86768
187	0,45573	730,83392	4872,22610	4,87223	0,01559	0,00141	1,41144
188	0,56898	889,16716	5927,78110	5,92778	0,03426	0,00255	2,54999
189	0,53423	841,53518	5610,23453	5,61023	0,03242	0,00255	2,54944
190	0,56323	881,34446	5875,62974	5,87563	0,02102	0,00158	1,57830
191	0,70198	1063,47887	7089,85915	7,08986	0,02354	0,00146	1,46497
192	0,58448	910,13758	6067,58385	6,06758	0,01044	0,00076	0,75928
193	0,46098	738,37006	4922,46704	4,92247	0,00585	0,00052	0,52431
194	0,70723	1070,09481	7133,96538	7,13397	0,02354	0,00146	1,45591
195	0,57498	897,30499	5982,03330	5,98203	0,03969	0,00293	2,92733
271	0,58448	910,13758	6067,58385	6,06758	0,00453	0,00033	0,32974
272	0,42360	684,31114	4562,07424	4,56207	0,03068	0,00297	2,96692
273	0,46050	737,68899	4917,92660	4,91793	0,01727	0,00155	1,54881
274	0,46073	738,01162	4920,07746	4,92008	0,02060	0,00185	1,84695
275	0,32858	542,64537	3617,63579	3,61764	0,00693	0,00085	0,84512
276	0,46343	741,88047	4945,86980	4,94587	0,02898	0,00259	2,58504
277	0,54265	853,16146	5687,74309	5,68774	0,01529	0,00119	1,18560
278	0,54303	853,67779	5691,18530	5,69119	0,02310	0,00179	1,79050
279	0,45725	733,02493	4886,83284	4,88683	0,03847	0,00347	3,47279
280	0,40315	654,33218	4362,21456	4,36221	0,03399	0,00344	3,43710
281	0,42325	683,80042	4558,66946	4,55867	0,02218	0,00215	2,14652
282	0,59655	926,35563	6175,70423	6,17570	0,01621	0,00116	1,15818
283	0,43935	707,20756	4714,71709	4,71472	0,02524	0,00236	2,36158
284	0,31715	525,21120	3501,40801	3,50141	0,04973	0,00627	6,26565
285	0,57023	890,86465	5939,09768	5,93910	0,04299	0,00319	3,19344

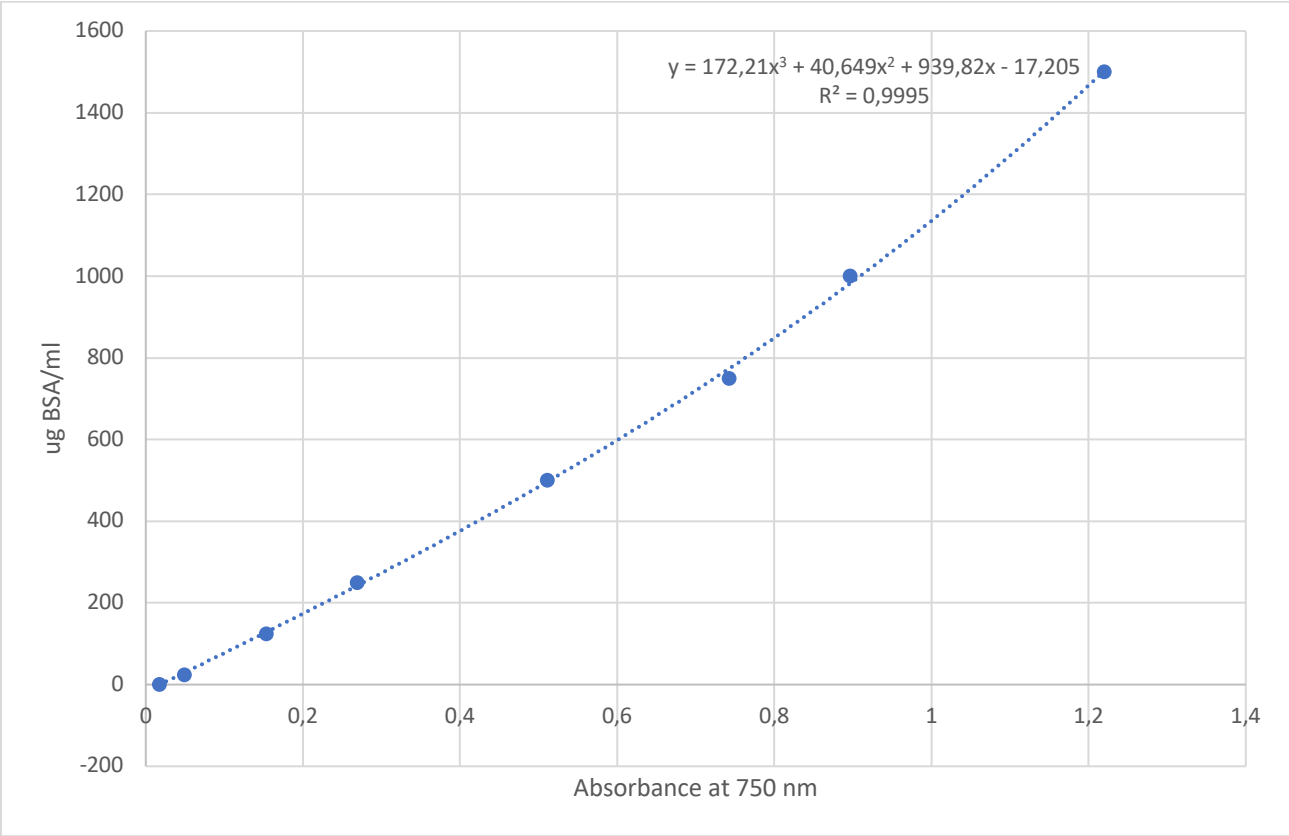
Appendix F 2: AChE activity, ST2 (361-475), ST1 (451-455) and ST3 (541-555)

361	0,43645	703,00434	4686,69561	4,68670	0,04999	0,00471	4,70551
362	0,52795	832,84350	5552,29002	5,55229	0,05277	0,00419	4,19322
363	0,46768	747,96021	4986,40141	4,98640	0,05660	0,00501	5,00729
364	0,59898	929,60012	6197,33410	6,19733	0,03502	0,00249	2,49319
365	0,51255	811,39591	5409,30603	5,40931	0,02441	0,00199	1,99065
366	0,46508	744,24231	4961,61540	4,96162	0,03896	0,00346	3,46402
367	0,49528	787,14060	5247,60402	5,24760	0,05140	0,00432	4,32095
368	0,60438	936,80981	6245,39873	6,24540	0,06882	0,00486	4,86146
369	0,32223	532,96599	3553,10663	3,55311	0,03045	0,00378	3,78056
370	0,46943	750,46004	5003,06691	5,00307	0,00276	0,00024	0,24338
371	0,41143	666,49694	4443,31293	4,44331	0,01503	0,00149	1,49258
372	0,61503	950,96761	6339,78409	6,33978	0,00974	0,00068	0,67797
373	0,46330	741,70147	4944,67644	4,94468	0,00383	0,00034	0,34195
374	0,45085	723,81921	4825,46143	4,82546	0,00774	0,00071	0,70741
375	0,58215	907,00291	6046,68605	6,04669	-0,00658	-0,00048	-0,47991
451	0,55060	864,08651	5760,57674	5,76058	-0,00528	-0,00040	-0,40456
452	0,39145	637,05410	4247,02734	4,24703	-0,00179	-0,00019	-0,18568
453	0,59423	923,24100	6154,93998	6,15494	0,00994	0,00071	0,71248
454	0,40520	657,35010	4382,33397	4,38233	0,02064	0,00208	2,07761
455	0,50715	803,83619	5358,90793	5,35891	0,01101	0,00091	0,90620
456	0,51825	819,35359	5462,35725	5,46236	0,00790	0,00064	0,63765
457	0,50243	797,20482	5314,69878	5,31470	0,02424	0,00201	2,01225
458	0,27888	466,18739	3107,91595	3,10792	0,02139	0,00304	3,03636
459	0,51023	808,14351	5387,62342	5,38762	0,01522	0,00125	1,24632
460	0,54743	859,72870	5731,52465	5,73152	0,03151	0,00243	2,42506
461	0,48643	774,63451	5164,23008	5,16423	0,02715	0,00232	2,31919
462	0,30928	513,14471	3420,96471	3,42096	0,03008	0,00388	3,87952
463	0,37490	612,45761	4083,05072	4,08305	0,03942	0,00426	4,25909
464	0,55275	867,03344	5780,22291	5,78022	-0,00124	-0,00009	-0,09483
465	0,39170	637,42425	4249,49499	4,24949	0,00868	0,00090	0,90063
541	0,50210	796,74812	5311,65415	5,31165	0,03519	0,00292	2,92261
542	0,56785	887,63848	5917,58986	5,91759	0,04077	0,00304	3,03985
543	0,47000	751,28095	5008,53966	5,00854	0,04916	0,00433	4,32981
544	0,66053	1010,53154	6736,87694	6,73688	0,02215	0,00145	1,45070
545	0,56528	884,13611	5894,24074	5,89424	0,05046	0,00378	3,77649
546	0,40668	659,51977	4396,79849	4,39680	0,04637	0,00465	4,65228
547	0,28588	477,05278	3180,35188	3,18035	0,03361	0,00466	4,66271
548	0,52998	835,65140	5571,00933	5,57101	0,01920	0,00152	1,52048
549	0,67778	1032,71960	6884,79734	6,88480	0,05680	0,00364	3,63957
550	0,54915	862,09721	5747,31473	5,74731	0,05432	0,00417	4,16934
551	0,65120	998,44661	6656,31075	6,65631	0,04083	0,00271	2,70635
552	0,43925	707,06272	4713,75146	4,71375	0,03068	0,00287	2,87098
553	0,54820	860,79307	5738,62047	5,73862	0,05667	0,00436	4,35690
554	0,56760	887,29865	5915,32432	5,91532	0,07014	0,00523	5,23099
555	0,46103	738,44174	4922,94492	4,92294	0,06959	0,00624	6,23596

Appendix F 3: AChE activity, ST2 (601-615), ST1 (691-705) and ST3 (781-795)

601	0,42733	689,74149	4598,27658	4,59828	0,05499	0,00528	5,27571
602	0,50953	807,16356	5381,09041	5,38109	0,05861	0,00481	4,80543
603	0,42070	680,07699	4533,84659	4,53385	0,06172	0,00601	6,00532
604	0,46938	750,38864	5002,59095	5,00259	0,06320	0,00557	5,57358
605	0,45313	727,09476	4847,29840	4,84730	0,04947	0,00450	4,50251
606	0,47745	761,89659	5079,31059	5,07931	0,04533	0,00394	3,93704
607	0,46855	749,21039	4994,73591	4,99474	0,05465	0,00483	4,82692
608	0,48255	769,14166	5127,61106	5,12761	0,06067	0,00522	5,22008
609	0,36900	603,64501	4024,30008	4,02430	0,07399	0,00811	8,11166
610	0,50000	793,79540	5291,96933	5,29197	0,02845	0,00237	2,37138
611	0,52720	831,80281	5545,35209	5,54535	0,03510	0,00279	2,79222
612	0,49070	780,68235	5204,54898	5,20455	0,05437	0,00461	4,60853
613	0,55355	868,12913	5787,52757	5,78753	0,05105	0,00389	3,89123
614	0,67145	1024,60927	6830,72848	6,83073	0,04549	0,00294	2,93801
615	0,49495	786,68230	5244,54865	5,24455	0,04486	0,00377	3,77360
691	0,55628	871,85797	5812,38646	5,81239	0,03345	0,00254	2,53895
692	0,47790	762,53658	5083,57719	5,08358	0,04149	0,00360	3,60048
693	0,63300	974,67782	6497,85214	6,49785	0,04128	0,00280	2,80274
694	0,58940	916,76496	6111,76642	6,11177	0,05599	0,00404	4,04163
695	0,54503	856,42989	5709,53262	5,70953	0,05552	0,00429	4,29004
696	0,50048	794,46355	5296,42363	5,29642	0,05113	0,00426	4,25919
697	0,53995	849,44096	5662,93972	5,66294	0,06602	0,00514	5,14354
698	0,59083	918,67930	6124,52869	6,12453	0,06798	0,00490	4,89672
699	0,56185	879,47035	5863,13565	5,86314	0,06293	0,00474	4,73503
700	0,55490	869,97710	5799,84735	5,79985	0,06705	0,00510	5,10029
701	0,38693	630,34721	4202,31477	4,20231	0,05303	0,00557	5,56678
702	0,50803	805,06252	5367,08346	5,36708	0,05618	0,00462	4,61822
703	0,66375	1014,69629	6764,64191	6,76464	0,03598	0,00235	2,34638
704	0,54418	855,26060	5701,73730	5,70174	0,01610	0,00125	1,24556
705	0,51973	821,40912	5476,06082	5,47606	0,06127	0,00494	4,93626
781	0,55115	864,84069	5765,60457	5,76560	0,03549	0,00272	2,71565
782	0,50208	796,71299	5311,41992	5,31142	0,00220	0,00018	0,18294
783	0,45140	724,61143	4830,74286	4,83074	0,02631	0,00240	2,40258
784	0,47945	764,73993	5098,26618	5,09827	0,00554	0,00048	0,47962
785	0,44073	709,19847	4727,98982	4,72799	0,02854	0,00266	2,66335
786	0,44915	721,36925	4809,12834	4,80913	0,02493	0,00229	2,28678
787	0,47343	756,16603	5041,10686	5,04111	0,04926	0,00431	4,31125
788	0,42145	681,17257	4541,15048	4,54115	0,05144	0,00500	4,99695
789	0,38360	625,41024	4169,40161	4,16940	0,04946	0,00523	5,23324
790	0,40973	664,00159	4426,67727	4,42668	0,04527	0,00451	4,51125
791	0,47303	755,59592	5037,30616	5,03731	0,05640	0,00494	4,93940
792	0,49100	781,10628	5207,37522	5,20738	0,05187	0,00439	4,39408
793	0,51870	819,98086	5466,53907	5,46654	0,05219	0,00421	4,21159
794	0,48088	766,76413	5111,76084	5,11176	0,05808	0,00501	5,01223
795	0,47743	761,86103	5079,07353	5,07907	0,03617	0,00314	3,14157

Appendix G: Protein concentration determination digestive gland, plot



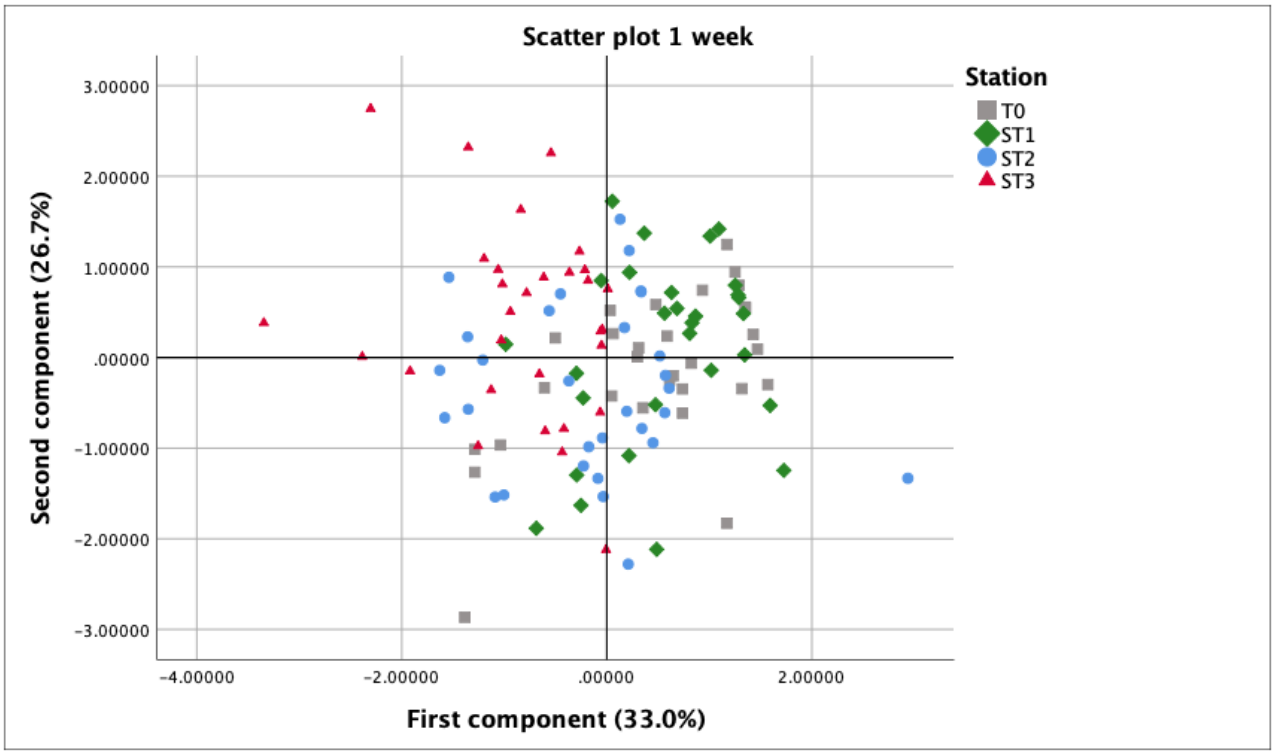
Appendix H 1: Catalase activity, see appendix A for station and time

protein calculation	ΔAbs750	BSA ug/ml	BSA ug/ml	BSA mg/ml	dilution	ΔAbs240	Cat activity
9	1,062	1232,998	12329,976	12,330	0,616	0,181	7,339
10	0,962	1078,359	10783,586	10,784	0,539	0,253	11,721
11	1,105	1302,783	13027,828	13,028	0,651	0,167	6,394
12	0,970	1089,274	10892,742	10,893	0,545	0,183	8,423
13	1,105	1303,073	13030,733	13,031	0,652	0,216	8,294
14	0,945	1053,109	10531,093	10,531	0,527	0,023	1,095
15	0,724	750,394	7503,941	7,504	0,375	0,050	3,354
91	1,196	1460,377	14603,771	14,604	0,730	0,039	1,338
92	0,996	1128,642	11286,420	11,286	0,564	0,053	2,337
93	1,216	1495,679	14956,789	14,957	0,748	0,036	1,219
94	1,229	1519,593	15195,933	15,196	0,760	0,071	2,348
95	1,290	1632,964	16329,643	16,330	0,816	0,067	2,064
96	1,306	1662,562	16625,624	16,626	0,831	0,066	1,985
97	0,838	899,944	8999,435	8,999	0,450	0,043	2,383
181	1,143	1367,143	13671,433	13,671	0,684	0,086	3,151
182	1,265	1584,943	15849,431	15,849	0,792	0,080	2,512
183	0,998	1131,902	11319,017	11,319	0,566	0,040	1,778
186	1,187	1443,558	14435,583	14,436	0,722	0,075	2,614
189	1,224	1510,280	15102,805	15,103	0,755	0,078	2,575
194	1,104	1302,326	13023,263	13,023	0,651	0,043	1,646
195	1,257	1570,035	15700,345	15,700	0,785	0,101	3,227
271	0,951	1061,702	10617,019	10,617	0,531	0,074	3,472
272	0,989	1118,321	11183,209	11,183	0,559	0,034	1,536
273	0,719	743,423	7434,226	7,434	0,372	0,020	1,333
274	0,936	1039,332	10393,323	10,393	0,520	0,047	2,272
275	1,009	1149,591	11495,912	11,496	0,575	0,023	0,993
276	1,189	1447,266	14472,659	14,473	0,724	0,034	1,177
277	1,040	1197,135	11971,347	11,971	0,599	0,015	0,613
361	0,879	956,591	9565,907	9,566	0,478	0,061	3,199
362	0,927	1026,377	10263,765	10,264	0,513	0,045	2,176
363	0,955	1067,872	10678,720	10,679	0,534	0,102	4,771
364	1,192	1451,776	14517,758	14,518	0,726	0,051	1,752
365	0,780	821,979	8219,787	8,220	0,411	0,072	4,404
366	0,788	833,308	8333,075	8,333	0,417	0,030	1,772
367	0,654	663,056	6630,559	6,631	0,332	0,052	3,885
451	0,946	1054,514	10545,143	10,545	0,527	0,083	3,913
452	0,837	898,231	8982,311	8,982	0,449	0,122	6,805
453	0,948	1057,401	10574,011	10,574	0,529	0,091	4,296
454	1,125	1336,231	13362,308	13,362	0,668	0,191	7,128
455	0,917	1011,871	10118,713	10,119	0,506	0,115	5,685
456	1,159	1395,318	13953,184	13,953	0,698	0,134	4,792
457	0,861	932,310	9323,103	9,323	0,466	0,104	5,583
541	0,879	957,119	9571,195	9,571	0,479	0,100	5,229
542	1,105	1303,239	13032,394	13,032	0,652	0,064	2,461
543	0,663	674,135	6741,347	6,741	0,337	0,052	3,887
544	1,192	1452,307	14523,070	14,523	0,726	0,256	8,812
545	0,806	856,532	8565,321	8,565	0,428	0,228	12,361
546	1,054	1220,245	12202,445	12,202	0,610	0,212	8,482
550	1,273	1599,517	15995,174	15,995	0,800	0,207	6,471

Appendix H 1: Catalase activity, see appendix A for station and time

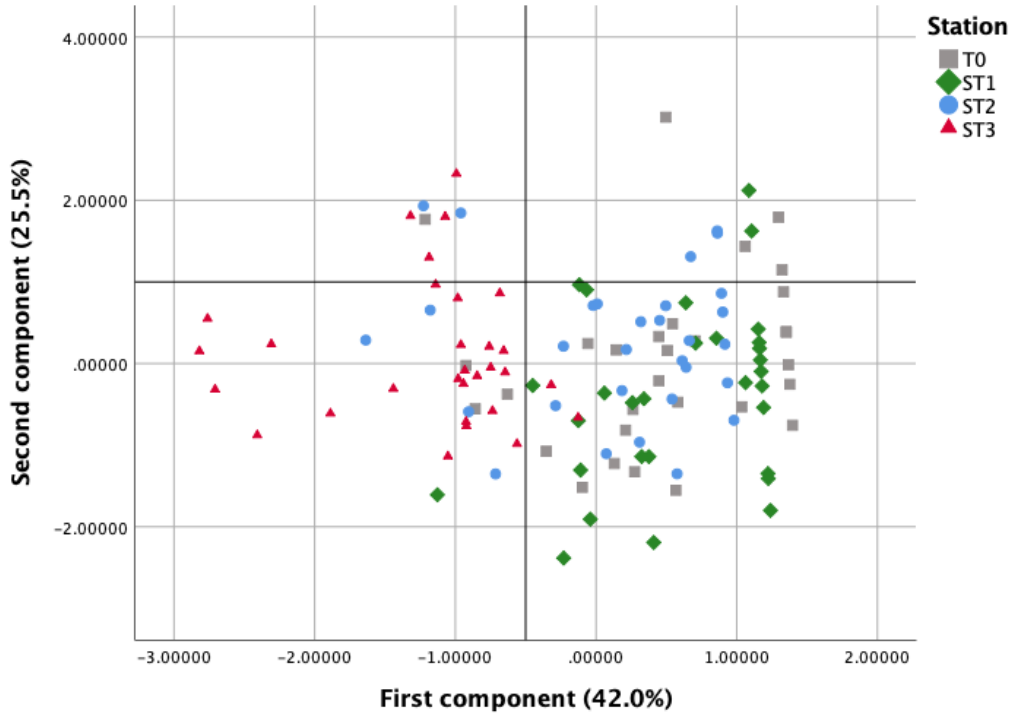
601	0,836	897,889	8978,888	8,979	0,449	0,141	7,852
604	0,962	1077,124	10771,243	10,771	0,539	0,210	9,742
606	1,298	1648,583	16485,831	16,486	0,824	0,045	1,370
607	1,112	1314,475	13144,753	13,145	0,657	0,143	5,436
609	1,113	1316,604	13166,039	13,166	0,658	0,094	3,560
611	1,134	1351,826	13518,259	13,518	0,676	0,142	5,256
612	1,111	1313,766	13137,662	13,138	0,657	0,100	3,801
691	0,595	592,478	5924,782	5,925	0,296	0,061	5,144
693	0,915	1007,999	10079,989	10,080	0,504	-0,020	-1,002
695	1,054	1220,484	12204,844	12,205	0,610	0,169	6,931
697	0,654	663,450	6634,505	6,635	0,332	0,115	8,679
700	1,054	1219,485	12194,849	12,195	0,610	0,025	1,040
702	0,917	1011,473	10114,729	10,115	0,506	0,152	7,507
703	0,726	752,363	7523,633	7,524	0,376	0,134	8,916
781	0,788	832,645	8326,451	8,326	0,416	0,088	5,307
782	0,868	941,315	9413,154	9,413	0,471	0,036	1,914
783	0,895	979,919	9799,194	9,799	0,490	0,012	0,634
784	0,841	904,368	9043,684	9,044	0,452	0,021	1,151
788	0,736	765,596	7655,959	7,656	0,383	0,036	2,372
789	0,737	766,812	7668,121	7,668	0,383	0,028	1,822
794	0,857	927,128	9271,281	9,271	0,464	0,097	5,218

Appendix I 1: Scatter plots of the individuals derived from PCA week 1



Scatter plot from the components obtained from PCA, summarizing all biomarker responses for time zero (T0, grey), station 1 (green), station 2(blue) and station 3(red). Showing that component 1 differentiate between station three (found at left side) and station 1 and T0, which mostly can be found at right side. Station 2 has values mostly on the lower left side.

Appendix I 2: Scatter plots of the individuals derived from PCA week 2



Appendix I 3: Scatter plots of the individuals derived from PCA week 4