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MASTER'S THESIS

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Writer: Terese Habbestad	…… (Writer's signature)
Faculty supervisor: Steinar Sanni External supervisor(s): Odd Ketil Andersen	
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Abstract

Due to regulatory requirements of monitoring the marine environment, and the development of offshore petroleum activities in new and more remote locations, there is at present an emphasis on implementing biological responses in monitoring systems to reflect the environmental conditions over a period of time. Choosing Darwinian fitness parameters that reflect the health status of individuals, such as growth and reproductive potential is highly interesting.

The current study used clearance rate and ingestion rate as parameters to illustrate the feeding behaviour of individual *Chlamys islandica*, which may be used in estimations of energy turnover (availability of energy to growth and reproduction). A laboratory feeding experiment was set up and the parameters were studied over the course of 12 days. The change in the environmental conditions, from water with low particle concentration to water with medium particle concentration, was reflected in the filtration rates of the individuals. Large fluctuations in the feeding behaviour within and between individual *C. islandica* were detected in each treatment. Maximum clearance rates varied from 4.20 l/h to 16.50 l/h within a single individual and average clearance rate varied between 2.31 l/h and 10.33 l/h between individuals. Average clearance rate and standard deviation of groups with different size, converged towards the population mean (n=40) with increasing number of individuals in the group. Based on the standard deviation and coefficient of variation, the results indicate that a group of 15-20 scallops give reasonable estimates that can be representative for larger populations.

All of the individuals changed their feeding behaviour from one treatment to another, and more than 87% increased their clearance rates when exposed to water with low particle concentration. This is a good indication that individual responses can be utilised as biosensor units that compare present responses to responses back in time. An individual scallop with high/low clearance rate (compared to the average) in one treatment did not necessarily show the same pattern of high/low clearance in the other treatment. A natural next step to investigate this further will be to continue the experiment with more time-series measurements for each individual, and include treatments with higher particle concentration.

Acknowledgement

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Terese Habbestad
June 2012

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1. Introduction

1.1 Background

As the petroleum industry is expanding into new and more sensitive areas, there is a need for new and better methods for monitoring the marine environment. Vulnerable regions in the Arctic and Subarctic areas are under close watch of environmental agencies, and a law of zero harmful effect discharges has been implemented as an environmental goal (OLF, 2004). The increasing use of subsea installations and unmanned stations in remote places has resulted in requirement for a new generation of monitoring systems that focus on the biological composition of the ecosystems and possible effects from disturbance.

Biotatools AS is developing 3rd generation biosensors for application in real-time monitoring of marine environments. Their aim is to offer a system that delivers continuous data to local or remote stations of integrated operations (IO-centres). *Chlamys islandica* is widespread in Arctic and Subarctic regions and can serve as an indicator species in the biosensor technology for northern marine areas. Parameters of interest are related to the growth of the organisms, as these can be implemented in environmental risk assessment models. The background for this thesis is thereby to contribute with relevant information and new insight for the development of the biosensor technology.

1.2 Objectives

The objective of the study is to investigate variability within and between individuals of *Chlamys islandica*, and compare these to group responses. Clearance rate and ingestion rate are utilised as response-parameters as they are indirect measurement of energy turnover and growth potential. Furthermore the aim is to see if there is an advantage of

using time-series data, where individual responses are compared back in time, to evaluate the state/health of an individual and the influence from its surroundings.

1.3 Scope of study

The following tasks were included to achieve the objectives of the thesis:

- Carry out an experiment with *Chlamys islandica* to acquire a data set for analysis.
- Extract and organize data.
- Analyse data with.
    i. Infometric principal component analysis.
    ii. Standard statistical methods.

1.4 Report outline

The next chapter is a background study of concepts that are relevant for the thesis. An overview of three main topics is included:

- Environmental monitoring with focus on biological monitoring systems.
- Individual variability.
- Methods of analysing univariate and multivariate data (statistical and infometric).

The methodology chapter is a description of how the experiment was put together and how the data was treated. The results are presented in chapter 4 and discussed in the following chapter. Conclusions with proposed further work is shown in chapter 6.

2. Theoretical Background

2.1 Environmental monitoring

Environmental monitoring can be defined as time-series measurements of physical, chemical, and biological variables, designed to answer questions about environmental changes (Lovett et al., 2007). It is a tool for assessing and evaluating the state of the environment and is performed by implementing repeated or continuous measurements that can be compared with reference data.

Oil companies operating in the Norwegian offshore regions have to follow regulations and guidelines from the authorities and international agreements such as the climate and pollution agency (KLIF) and Oslo and Paris Commissions (OSPAR). Monitoring of the water column and sediments must be conducted regionally, and the surveys are used to see whether the environmental status is changing as a result of oil and gas activities (Iversen et al., 2011; KLIF, 2011). Monitoring of the offshore continental shelf of Norway started in 1968 when Phillips produced an Environmental Impact Assessment for the Ekofisk oil field. Since then, field monitoring of the marine environment has developed and environmental practices and goals have improved from discharging oil-based drill cuttings directly to sea, to the present aim of having zero harmful effect from discharges (Gray et al., 1999). Initially, a goal of zero discharge to the environment was set in policy, however, due to difficulty in interpreting "zero discharges", the statement was changed to put stronger focus on the measurement of effects on biological ecosystem components. Thus, the need to develop biological monitoring systems emerged.

Developing good and labour efficient monitoring systems that provide data in real-time for quick and easy interpretation of the environmental conditions of the biological components, is especially important when the petroleum industry is expanding into new potentially vulnerable and more distant locations, such as Lofoten and the Barents Sea. It is important to get knowledge about ecosystems in new areas so that the necessary

protection can be implemented. The difficulty in using traditional monitoring systems in remote places such as very deep waters or ice-covered locations also encourages the development of new monitoring methods. Arctic and Subarctic areas can be very sensitive, and vulnerable species and protected spawning grounds are under close watch from environmental organisations. Environmental monitoring is a way of building up knowledge about the quality of an ecosystem, which is especially important when new areas are explored.

During the history of environmental monitoring there has been a general shift from using conventional methods based on routine measurements of physical and chemical parameters, to a more bio-ecological view. The traditional methods are based on measurements of abiotic factors like temperature, salinity, dissolved oxygen, nutrients and chemical contaminants like heavy metals. Surveys of the biotic abundance and diversity of the benthic communities are conducted, and the sediments are often tested for organic matter (Lam, 2009). These chemical methods to monitor the environment are well developed, but they do not necessarily give a real picture of the ecological state of the environment. They might reveal the presence of a toxic chemical, but it is not given that the chemical is bioavailable or harmful to the biological system. A chemical may be non-hazardous alone, but highly toxic in a mixture with others (Gruber et al., 1994).

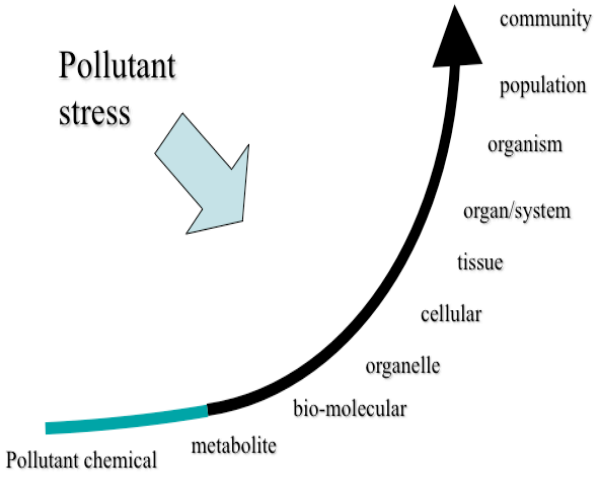

Figure 2.1. Illustration of how pollutant stress evolves in chains of events from lower to higher levels of biological organisation. Modified from van der Oost et al. (2003)

Limitations with the physicochemical methods make it difficult to indicate the real biological status of the ecosystem. Because of this, a new approach for monitoring evolved, that focused mainly on the biological compartments of an ecosystem. Instead of looking at disturbances *a posteriori*, selected biological systems are observed to see if there are on-going changes in the environment (Vasseur & Cossu-Leguille, 2003).

Deviations from normal responses occurring at low levels of biological organisation function as early warning signals for changes at higher levels. Biomarkers are indicators developed for this purpose and can be defined as quantitative measurements in biological systems that respond to exposure of xenobiotic substances (Lam & Gray, 2003). The responses can be measured at different levels within the organism, such as the molecular, biochemical, cellular or physiological (Fig 2.1). Information about the state of the environment is generated through effect measurements within these organisms and thereby useful for protecting and conserving natural ecosystems.

Through the shift from chemical based monitoring to biological effect measurements a multiple set of biomarkers that have been implemented in monitoring of the marine environments has been established. The key function is to provide an early warning signal for disturbances at population and community levels, but their effectiveness as ecologically relevant is discussed between scientists, e.g. Forbes et al. (2006). Although biomarker responses at molecular or submolecular levels are more predictable and repeatable than responses at physiological levels, Lam (2009) highlights some of the issues regarding their usefulness:

- Few biomarkers are specific enough to identify the nature of the stressor.
- The physiological responses are less detectable, but more relevant when looking at impact at the whole ecosystem.
- The ability of organisms to repair damage induced by toxic insults and thereby adjust biological responses may produce false negatives in experiments.
- Factors such as non-target chemicals, food availability, water temperature and reproductive activity may, conceivably, influence the responses and lead to wrong interpretations.

Even though it is generally believed that biomarkers at lower levels of the biological hierarchy respond faster than those at higher levels, the scepticism to the ecological relevance of biomarkers has lead to a focus of implementing living whole-organisms into monitoring systems (Gruber, et al., 1994; Lam, 2009; Wu et al., 2005). Changes in the physiological and behavioural responses of selected organisms appear to be very fast and sensitive, and may thereby function as early warning signals for toxicity. As canary

birds in cages were used as sentinel organisms in the coalmines to detect toxic gases, different responses of these biological sensors are observed and linked to a system to provide continuous real-time information about the surrounding environment (Gerhardt et al., 2006).

Compared to biomarkers, which are exposure or effect measurements within biological components (biochemical, metabolic, enzymes, cells, organs), the term biosensor generally include an analytical device incorporated with a sensitive biological component (van der Schalie et al., 2001). A transducing element transforms and processes the signal from the detection unit at the biological sensor and displays it in an appropriate manner (Kröger & Law, 2005). This element may be electrochemical, optical, piezoelectric, thermometric or magnetic, and the biological component may range from enzymes and antibodies to whole-organism sensors (Gruber, et al., 1994). In other words, the biological component of a biosensor is a biomarker that provides repeated signals with non-destructive effects on the organism. The development of biosensors embraces a wide variety of systems that have been successfully implemented in fields such as medicine, food safety, agriculture and environmental monitoring. It seems to be a certain disagreement amongst scientists in defining the concept, as it is referred to in various ways in the literature. Terms such as “automated biomonitors” (Gruber, et al., 1994), “biological early warning system (BEWS)” (van der Schalie, et al., 2001) and “whole organism biomonitor” (Allen et al., 2001), have been applied by different scientists. For the purpose of this thesis the term biosensor will be applied to cover the same concept; living whole-organisms that are incorporated with an analytical device.

2.2 Individual variability

"There is no such thing as an average organism!
Real individuals are unique combinations of traits whose uniqueness should be recognised and used as an advantage"
A. F. Bennett

Biological science treats questions connected to living organisms. Compared to other fields of science, like physics or geology, it is subjected to a different set of issues, such as variability within and between individuals.

Imagine a bag with millions of Lego bricks. The different pieces represent the building blocks of an organism within a species such as *Homo sapiens* or *Chlamys islandica*. There are certain instructions to follow when building the different parts, but there are still countless manners of putting them all together, resulting in variability between populations, communities and individuals. Because of biological complexity involved in things like circadian rhythms, reproductive stages and health situation, individuals are also subjected to intra-individual variability that may further increase inter-individual variations.

This important feature is often overlooked in biological research. The traditional approach of carrying out experiments often emphasises statistical significance between groups of individuals where classical descriptive statistics, such as mean values and standard deviation, are applied. Bayne (1998) highlights this issue in regard to suspension feeding bivalves. It is here pointed out how the wide variability between individuals is mostly treated as a statistical feature of the data instead of looking at how the variety may contribute to further understanding of the attributes of an organism. Bayne (1998, pg. 13) states "by focusing on inter-individual variability rather than population means, and coupling this to rigorous experimental design, we may gain better functional understanding of growth (and feeding) and be able to construct hypothesis concerning the fitness consequences of specific traits".

The subject of using central tendencies in biological studies is addressed by Bennett (1987, pg. 150) where he states that "the tyranny of the golden mean restricts our vision of the data and narrows our conceptual framework so that we cannot take advantage of all analytical possibilities of biological variability". Individual variation does exist and contradicts the view of organisms as ideal or typological. Bennett (1987) further states his opinion about how data often is misinterpreted:

- Extreme values are atypical or abnormal and do not reflect the response of most individuals.
- Observed variability is due to instrumentation or procedural error, and not because of real biological differences.
- The variation measured is real, but reflects random and unrepeatable responses of individuals (high intra-individual differences).

Bennetts examples may not provide the best examples of key issues for this thesis, but the main point is clear and relevant for its topic; when assessing information about individuals by looking at central tendencies of a group, it is likely that information is lost in the process. An analogy from the field of community medicine may provide a better example of how Bennett's opinions are highly relevant for the present thesis. Before a new medication reaches the market, it has to undergo several tests and trials. A common procedure for validation is to look for significant difference between two groups of experimental objects (patients) where one group have been given the medication and the other group a placebo. If the results from the trial show no significant differences between the two groups, the medication is not verified. The rejection is based on the average response of the experimental objects, but it is still likely that some of the patients had a positive experience with the medication. This information is hidden in the calculated values and thereby overlooked.

An alternative method for studying parameters such as health and growth of an organism can be analysis of time-series data (Fig 2.2). An individual will then serve as a control for itself by comparing present responses with responses of the past. In this way individual variation is not ignored or hidden away, but utilised as an advantage. In the development of efficient biosensor technology, this method of employing individual

responses in continuous time-series measurements is highly interesting. It is beneficial and can be more ethical as the methods are non-destructive (compared to using groups of biomarkers where the organisms are dissected for assessment).

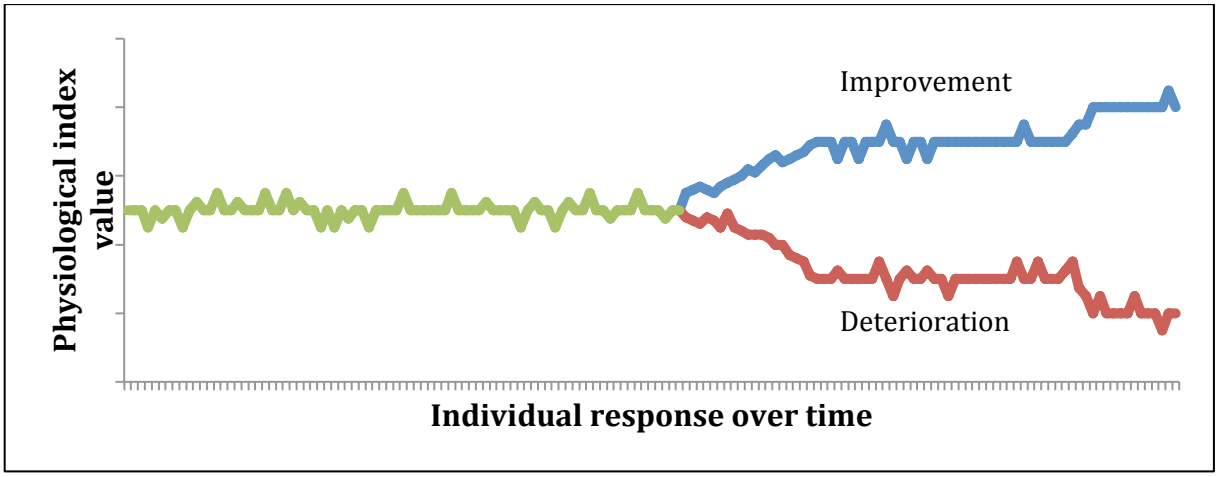

Figure 2.2. Illustration of how a time-series of individual responses can be utilised to see whether the health/growth of an organism show a tendency of improving or deteriorate (Redmond & Andersen, 2012).

2.3 Organisms used for monitoring the environment

Living organisms assimilate the state of the surrounding environment and can be highly sensitive to toxic conditions. Incorporated with an analytical device they can function as an early warning system, and signals may be transmitted to local or remote stations (Gruber, et al., 1994). Various organisms have been utilised as sensors in different biosensor systems. Algae have been applied to measure fluorescence and photosynthesis and bacteria are used to monitor respiration, growth and bioluminescence (Gerhardt, et al., 2006). Measurement of the locomotion, respiration and heart rate in crustaceans (from small daphnids to higher order crawfish) has also been successfully implemented in monitoring systems (Gerhardt, et al., 2006; Gruber, et al., 1994). Fish were among the first organisms that were utilised in a biosensor system, and responses such as rheotaxis, ventilation, swimming behaviour (avoidance), cough rate, heart rate, and electrical discharge (in electric fish) has been monitored (Gerhardt, et al., 2006; Gruber, et al., 1994).

Bivalves have been extensively used for monitoring the marine environments, especially as bioindicator species for effect measurement because of their ability to accumulate toxins to a level that can be easily assessed in the laboratory (Kramer et al., 1989). In the search for suitable organisms for real-time continuous biosensors systems, these organisms are still beneficial. They are sedentary, widely distributed and easy available, as well as occupying a low position in the food chain. Together with their sensitivity to low concentrations of chemicals, they are well suited biosensors (Gruber, et al., 1994). Because of its characteristics, the mussel *Mytilus edulis* has been extensively employed as a sentinel organism to monitor levels of contaminants in many parts of the world (Widdows et al., 1982). Similarly, the Iceland scallop *Chlamys islandica* may serve a similar purpose in Arctic and Subarctic regions where *M. edulis* is less widespread (Hannam et al., 2010). It is the northernmost member of the family Pectinidae and the most abundant scallop in the Arctic regions. The species is found at depth of 10-250 m with the greatest concentration between 20-60 m (Galand & Fevolden, 2000; Pedersen, 1994). It lives on hard or sandy bottoms, attached to stones or other shells by their byssus threads, as the currents may be strong in many of the places. Most population of the species are found in areas with a temperature of 0-8 °C, and a maximum temperature tolerance of 12-15 °C is suggested by Jonasson et al. (2004).

2.4 End point parameters

Different responses have been monitored for the purpose of receiving an early warning signal for stress in the environment. Some of them were mentioned in Section 2.3, others include monitoring of the valve movement or cardiac activity of bivalves and crustaceans. The valve movement of bivalves has been used to study changes in the environment, based on the assumption that the shell will close if the organism is exposed to stress. Bivalves have their shell open for feeding and respiration, and measurements of how they are displaced can be utilised as an indicator for irregularities in the environment (Kramer, et al., 1989). Continuous monitoring of the cardiac activity in selected crustaceans and bivalves has also been applied as a parameter to monitor stress in the environment (Depledge & Andersen, 1990; Fedotov et al., 2000; Fedotov et al., 2006). The system is put together with a transducing element that registers the heart

beat (continual recording of the pulse from a reflected light beam sent out from the sensor and reflected by the heart), and displays the signals as continuous graph on a computer screen (Depledge & Andersen, 1990).

Limitations with monitoring these physiological and behavioural responses are connected to their ecological relevance. Darwinian fitness can be defined as the ability of an organism to successfully grow and reproduce to maintain the size of the population that it is a part of (Bayne et al., 1979; Calow & Forbes, 2008; EC, 2003). Parameters of fitness are important as they may be used to link environmental monitoring to risk assessment models (Calow & Forbes, 2008; EC, 2003). Although being on a physiological level of biological organization, valve movement and cardiac activity are not linked to these parameters, and are thereby not included in the concept. Measurement of growth and energy turnover, on the other hand, may be used as fitness parameters to predict the ecological consequences of pollutant exposures (Depledge & Galloway, 2005). Scope for growth (SFG) is a measure of available energy, as it reflects the balance between energy achieved through feeding and digestion, and energy spent through metabolism and excretion (Widdows et al., 1995). The energy budget (SFG) indicates the potential of an organism to grow and reproduce, and quantitative measurement may serve as an indication of environmental stress in biological monitoring programs. The filtration rate of a bivalve is a parameter of food consumption and hence energy available for growth and reproduction. Mussels and scallops eat by retaining particles from the water that they filtrate through gill-structures in the valve opening. An indication of the filtration efficiency can be obtained by looking at the volume and composition of particles that are ingested through the incurrent siphon, and pumped out through the excurrent siphon (Fig 2.3). Many approaches for monitoring the filtration rates have been made, and one of them is the flow-through chamber method. The method is advantageous for in-situ measurements as it use natural seawater, and allows for exhaustive control of experimental conditions (Filgueira et al.,

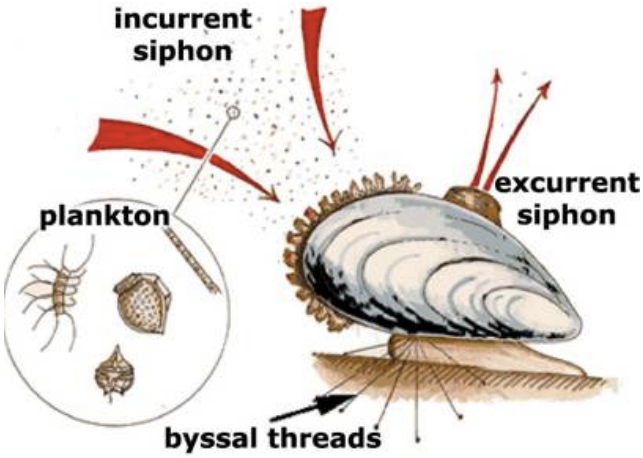

Figure 2.3. Illustration of how a bivalve (here mussel) inhale water with particles through incurrent siphon and pump out water through excurrent siphon.

2006). A bivalve is placed in a chamber with flowing water, and the amount of particles in the inflowing and outflowing water is determined. Retention efficiency is the ratio of particles that the bivalve retains from the inflowing water. Above a certain particle size, the bivalve retain 100% of the incoming particles. Multiplied with the water flow, this gives a number of the clearance rate (CR) which is defined as the volume of water cleared of suspended particles per unit time (MacDonald & Ward, 2009). For *Mytilus edulis*, the limit of 100% retention is suggested at 4 µm (Strohmeier et al., 2012) and for *Chlamys islandica* a limit at 7 µm was proposed by Vahl (1973).
Requirements for the appropriate use of the flow-through chamber method are given by Filgueira et al. (2006):

- The food crossing the chamber should be completely accessible to the mussel; otherwise the available food will be less than the theoretical value.
- The geometry of the chamber should minimize water recirculation to prevent dilution of the incoming food concentration.
- The food has to be completely retained by the gills to reduce underestimation of the measurement.

The clearance rate parameter is important when measuring the energy budget of an organism, as it may account for 60-70% of the SFG (*J. Widdows, pers. comm*). It is debated if the mechanism of retaining particles from filtrating water is a basically autonomous process (Jørgensen, 1996), or whether it is a physiological process where the organism may regulate the capacity of filtrating according to the environmental conditions. Many factors, such as temperature, salinity, water flow, seston availability and composition, have been shown to influence the filtration rate of bivalves. The clearance rate can vary by several orders of magnitude, reflecting that feeding behaviour is not constant and may change as a response to environmental conditions (Strohmeier et al., 2009). Few studies on specifically Iceland scallop have looked at the feeding behaviour in environments with low food availabilities. It is suggested by Strohmeier et al (2009) that the variation in the filtration rates (clearance rate) in these conditions is of great importance as the adjustment will have proportionally more impact on the net energy balance of the organism.

2.5 Analysing biological data; from traditional statistics to infometric principal component analysis.

The science of statistics arose to satisfy a need of scientific research, and the word is derived from its origin when it was developed as a quantitative description of the affairs of a state (Sokal & Rohlf, 1995). Statistical methods are sought as a tool to help decision-making in situations that are unpredictable or causal (Løvås, 2010). From a biometric point of view, statistics can be defined as "the scientific study of data describing natural variation" Sokal and Rohlf (1995, pg. 2).

The aim with statistics is to extrapolate information about larger groups through analysis of smaller and randomly selected sub-sets. A procedure for implementing a statistical analysis is:

- Collect and organize data
- Describe and transform if necessary
- Apply methods of extrapolation
- Look for patterns and significant differences
- Generalize upon the results

The aspect of central tendency is important in statistics. It involves identification of the central value in the distribution, which is found through the mean, mode or median.
How the objects are situated away from the average, indicates the spread in the dataset which is described through the range, variance or standard deviation (Fowler et al., 2003). The mean value and the standard deviation is often used to look for significant differences, and implemented in hypothesis testing. Most statistical techniques are based on an assumption that the random samples are collected from populations that are normally distributed. Non-parametric tests are less sensitive for extreme observations and can be applied if the distribution of the data does not follow a gauss-curve (normal distribution)(Walpole et al., 2007).

The reliability of a statistical analysis depends on the number of objects that are included. Large numbers of objects ($n>30$) give better estimates than a sample that contains fewer objects. In biological statistics it can be difficult to obtain this, as there

may be a lack of available sampling units for a population, or it is ethically wrong to disturb natural compartments. Another issue, that differentiate biometrics from other fields of statistics, is the large variability in the data. “Biological measurements are inherently variable as compared to those made by physicists and chemists. Coefficients of variation of 20 to 30%, values that would cause a physical scientist to blanch, are routine measurements in most physiological measurements” Bennett (1987, pg. 150).

Various statistical methods are used to extract information from a dataset. Estimation (confidence intervals) and statistical testing (testing of null hypothesis) applied with different levels of significance, are common procedures. Other approaches include measurement of correlation, that can reveal whether there is coherence between variables, or regression analysis, which identifies the nature of the correlation by applying a mathematical equation such as the line of best fit or the method of least squares. The methods look for patterns in the dataset or differences between objects or groups of objects, but they do not necessarily indicate the cause of the results (Løvås, 2010). In biological science this subject is important as biological effect should be emphasized rather than statistical significance (Yoccoz, 1991).

When analysing biological data, it is likely that the results are influenced by more than one variable. Multivariate analysis (MVA) is the study of data that includes simultaneous measurements of many variables (Johnson & Wichern, 2002; Wold, 1976). It is an important application as the likelihood of revealing hidden information increase with the number of relevant variables in the dataset (Esbensen, 2000). Examples of fields of study where multivariate analysis is highly beneficial are meteorology (wind, temperature, air pressure, dew point etc.), human health (genes, environment, social position, eating habits, stress) or mollusc-based biosensor technology (climate, seston availability, sex, reproductive phase, seasonal factors, circadian rhythm and external stress factors). Many of the traditional statistical methods were developed for the situation that ruled in the 1930, with many (and often specific) observations (objects) and few variables. Data that are a product of today’s advanced measurement, however, has another set of characteristics that may require an additional set of methods. Many variables, few objects, data noise, collinearity and dependency between the variables are important characteristics that many of the classical methods want to avoid (Nordtvedt

et al., 1996). Fig 2.4 illustrates how a multiple set of variables (exogenous and endogenous) may influence the behaviour/responses of a bivalve and how many of them are linked together through dependency.

When the energy budget (respiration, excretion and growth; Fig 2.4) of a single individual is assessed as an indication of the state of the surrounding environment, it is important to include all of the variables that may influence the measured end parameters. Principal component analysis (PCA) is a tool that can be utilised for this purpose as it constitutes "the most basic "work-horse" of all multivariate data analysis" (Esbensen, 2000, pg. 19). It is used for explorative analysis of a multivariate dataset and is concerned with explaining the variance and covariance of a given dataset (Johnson & Wichern, 2002). Implementation of a PCA prior to classifying raw data may reduce the possibility of losing valuable information (Hand et al., 2001). The method is utilised in traditional statistics, and is also an important tool in the field of infometrics.

The infometric principal component analysis used in the current project, is based on the NIPALS (Non linear Iterative Partial Least Squares) algorithm. It is more robust when it comes to skewed or polymodal distribution, which is often encountered in biological data set (*O.H.J. Christie pers. comm*)

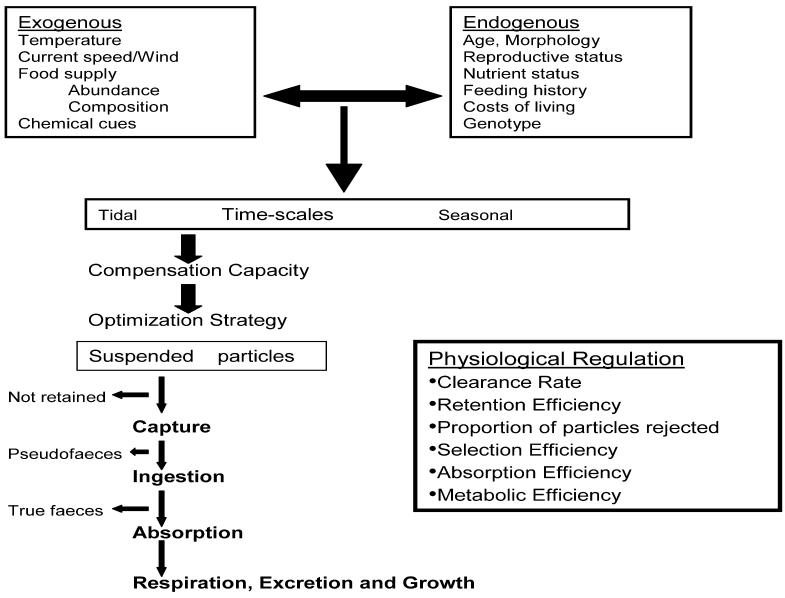

Figure 2.4. A conceptual illustration of how multiple exogenous and endogenous variables can influence the feeding behaviour of bivalves (Brian L. Bayne, 2004).

3. Materials and methods

An experiment with 40 numbered *Chlamys islandica* was carried out at IRIS-Biomiljø (at the Akvamiljø facility), Mekjarvik, Stavanger. The *C. islandica* were exposed to water with two different particle concentrations using the flow-through chamber method. The experiment was carried out under controlled laboratory conditions for the duration of 12 days (from 19.03 – 29.03 and 17.04). Data from the experiment was analysed by using infometric principal component analysis (PCA) and classical descriptive statistics.

3.1 Test organisms

Chlamys islandica (Iceland scallop; Norwegian "Haneskjell") were collected at the inlet to Balsfjorden (N:69.34.56, E:18.55.83) at 30-35 m depth on 06.12.2011. Scallops were held at the University of Tromsø overnight, and transported to Akvamiljø, Stavanger with plane and courier. To minimize stress, they were packed in a polyester box and placed between damp newspapers with crushed ice. At arrival the box was still cold, and the scallops were transferred to a seawater holding tank at 7 °C. Of 50 individuals, one dead scallop was registered the following day, due to damage to shell and mantle. No further mortalities occurred during acclimatization or experiment. The *C. islandica* were kept in a tank with running filtrated water from 78 m depth. Scallops were tagged (number 1-50) with small numbered plastic labels using Pattex superglue (without solvents). Tagging scallops using this method has not been observed to have any damaging effects (*K. Redmond, pers. comm*). Individual scallops were referred to by the label number (#). The height of each shell was measured with a digital calliper, and the gender was determined visually by looking at the colour of the gonad. The image-processing program ImageJ 1.45s was used to estimate the area of exhalant siphon by looking at the pixels-distance of photographs taken.

3.2 Experimental set-up and procedure

A flow-through chamber method (Fig 3.1) was used to obtain data on the feeding behaviour of the *Chlamys islandica* during exposure to water with two different particle concentrations.

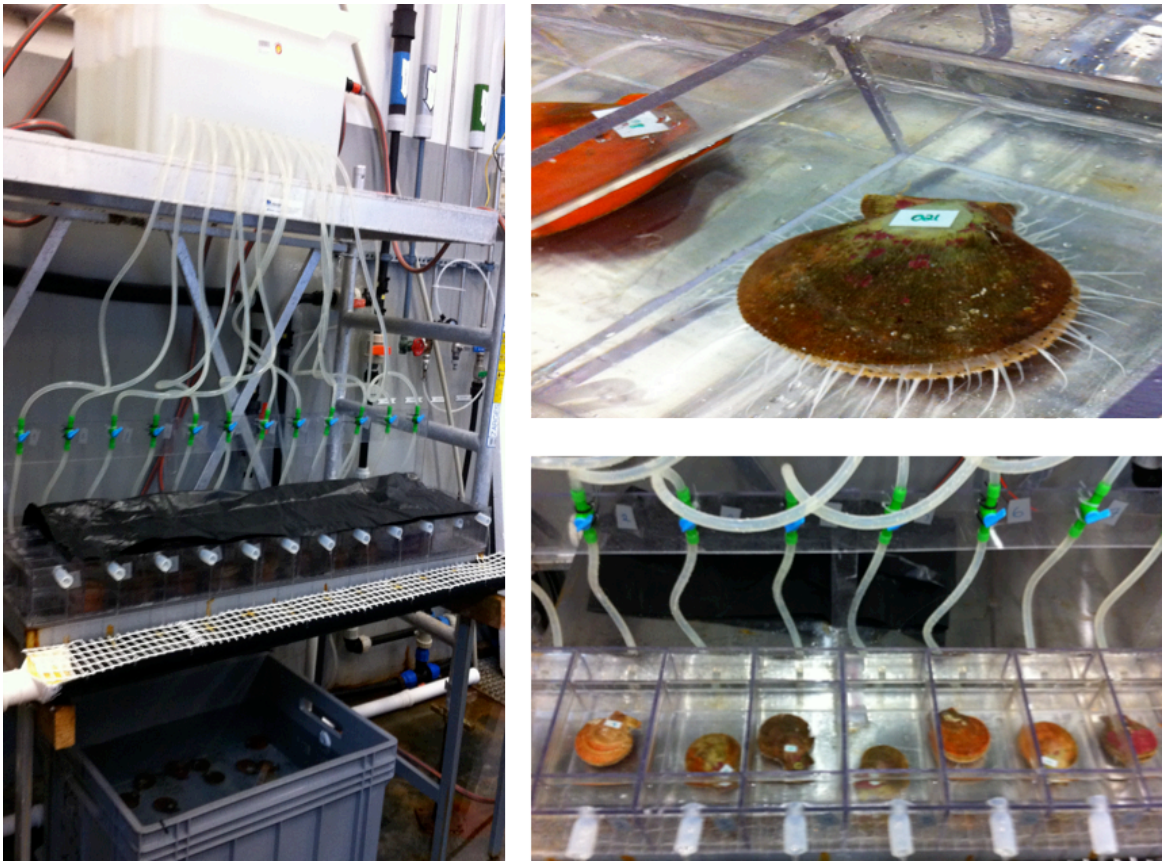

Figure 3.1. Photos of chambers and experimental set-up.

A header tank was linked to 12 flow-through chambers with plastic tubes. Dimension of the chambers were 21.2 cm × 10.3 cm × 10.0 cm (length × width × height). During the first week, the header tank received unfiltered water from 78 m depth, and during the second week it took in filtered water from the same depth. Particle concentration in the two waters (unfiltered versus filtered) was very different, and all of the *C. islandica* were given both of the water treatments to register how they adjusted their feeding behaviour. Two of the chambers were left empty during the experiment for the purpose of measuring the amount of particles in the inflowing water to the Iceland scallops. It

was assumed that water leaving the empty chambers had the same particle concentration as the water entering all of the chambers.

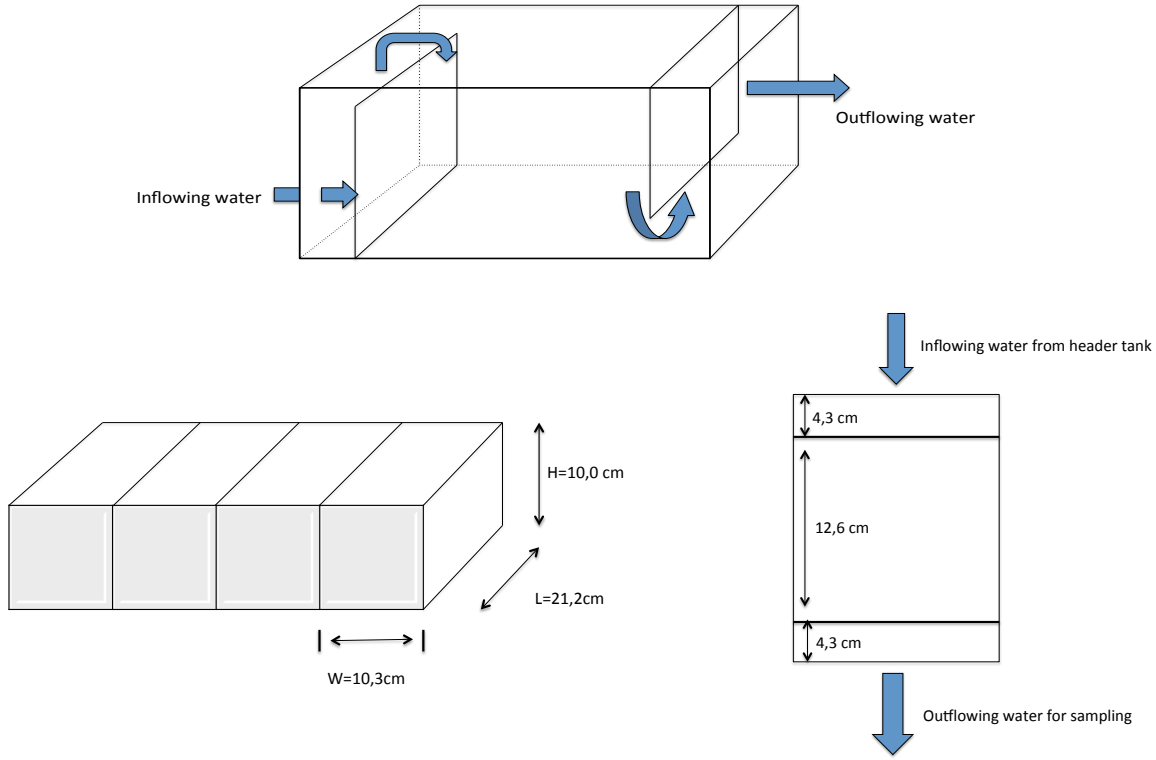

Figure 3.2. Illustration of flow-through chambers with dimensions.

Scallops were randomly allocated to four groups (n=10), and each group was placed in a holding tank with flowing seawater from the treatment water concentration for at least 5 days prior to sampling. This was carried out to avoid sudden change to a new diet affecting the experimental results.

For each treatment one group was used each day, and the 10 scallops were placed in separate chambers. Three to five sampling rounds were taken during the day on the same ten individuals (Table 3.1).

Sampling was originally planned to be carried out five times per day, in order to investigate whether the behaviour changed during the day. However, problems with the Coulter Counter analytical capacity resulted in a reduced number of samples on some of the days. This is marked with a star (*) in the table.

Table 3.1. Schematic overview of sampling procedure in the two treatments. Reduced sampling rounds (*) are explained in the text.

	Day	Date	Individual number (tag)	Group	Number of sampling rounds
First treatment with medium particle concentration	D1	19/03	34, 02, 44, 39, 46, 41, 40, 50, 36, 08	Gr.1	5
	D2	20/03	01, 37, 48, 12, 16, 06, 18, 15, 20, 03	Gr.2	5
	D3	21/03	13, 43, 21, 10, 5, 45, 31, 38, 14, 33	Gr.3	5
	D4	23/03	11, 07, 09, 42, 26, 04, 28, 19, 29, 27	Gr.4	4*
Second treatment with low particle concentration	D1	26/03	34, 02, 44, 39, 46, 41, 40, 50, 36, 08	Gr.1	4*
	D2	17/04	01, 37, 48, 12, 16, 06, 18, 15, 20, 03	Gr.2	3*
	D3	28/03	13, 43, 21, 10, 5, 45, 31, 38, 14, 33	Gr.3	3*
	D4	29/03	11, 07, 09, 42, 26, 04, 28, 19, 29, 27	Gr.4	3*

3.3 Sampling protocol and measurements

The chambers were cleaned before the *Chlamys islandica* were placed with their hinge towards the inflowing water. They were covered with black plastic to minimise disturbance, and left for one hour before the first round of samples were taken. Before each sampling round, the flow from every chamber was measured and the valve opening of the scallops were checked. Samples from the 12 chambers (two control and ten with Iceland scallops) were taken in plastic beakers after they were washed/emptied twice. No pseudofeces was observed in the chambers any of the days.

The particle concentration from each sample was determined by the use of Multisizer 3™ Coulter Counter. The technology is based on suspensions from the water sample to be drawn through the aperture tube of the machine, and metered by electrical

impedance and digital pulse processing. The reported particle size is the diameter of a sphere with the same volume as the particle. Tube size of the aperture was 70 µm, and the program was set to display the amount of particles within each size range (diameter; Ø) in volume (μm^3/ml). Looking at the volume of particles, instead of particle amount, gives a better estimate of the available biomass in the water.

Water temperature and salinity is continuously monitored at the laboratory facility by the use of Continuous Flow System (CFS), measured at 78 m depth (Sanni et al., 1998). The water flowing through the chambers was assumed to have the same temperature and salinity as given by the CFS.

3.4 Calculation of clearance rate

Clearance rate was calculated from the formula: $CR = F\ (1 - \frac{C_2}{C_1})$

where F is the flow rate, and C_1 and C_2 are the concentration of suspended particles in the inflowing and outflowing water respectively (MacDonald & Ward, 2009). The particle concentration in the water was less then assumed in advance of the experiment. There were few particles of relative large size in the water, and the scallops did not reach 100% retention efficiency at a certain particle size, meaning that it was not possible to determine the actual clearance rate. The clearance rate is therefore referred to as clearance rate* (CR*) in the results and discussion.

3.5 Data analysis

Data from the Multisizer 3™ Coulter Counter were extracted and organised in Excel sheets. The machine was set to measure the total volume of particles within 299 size ranges (from 2 µm to 59.3236 µm). Retention efficiency, clearance rate, total volume retained, ingestion rate and flow velocity were calculated with the formulas in Table 3.2.

Table 3.2. Schematic overview of equations and parameters that were implemented in the data analysis.

Variable	Formula	Unit	Parameters
Retention efficiency	$RE= 1-C_2/C_1$	---	C_1= Particles in (μm^3/ml) C_2=Particles out (μm^3/ml) F = Flow PV_1 = Particle volume in PV_2 = Particle volume out A = area of exhalant siphon at Iceland scallop
Clearance rate	CR= RE × F	l/h	
Total volume retained	$TVR= PV_1 - PV_2$	mm^3/l	
Ingestion rate	IR = TVR × F	mm^3/h	
Flow velocity	$F_v=CR/(3,6^{-3}\times A)$	mm/sec	

An infometric principal component analysis was carried out using the software package Sirius version 7.0a. As a first step, temperature, salinity, flow and size of the scallops were implemented as variables together with the parameters in Table 3.2 (except flow velocity). However, some of the variables showed little relevance for the outcome of the analysis, resulting in a smaller set of variables in the final analysis.

The calculated values for clearance rate (CR) and ingestion rate (IR) were analysed by using standard statistical methods. Intra- and inter-individual variability were examined by looking at the response patterns (CR and IR) of scallops that were randomly selected by using a random number generator.

To investigate the effect of group size, the average, standard deviation (SD) and coefficient of variation (CV) of groups with n=5, n=10, n=20 and n=30 scallops were compared to the average, SD and CV of the largest group (n=40). For each group size, eight replicates were generated by randomly selecting measured individual values using a random number generator. Individual values were selected only once within a group, but the same individuals were permitted to be selected in subsequent groups.

The statistical data analysis software R version 2.12.1, was used to look for correlation patterns between the 40 individual Iceland scallops.

4. Results

4.1 Environmental parameters

4.1.1. Temperature and salinity

The temperature varied from 7.83 °C to 8.03 °C with an average of 7.89 ± 0.06 °C (mean ± sd). The salinity of the water did not vary much, with a minimum of 33.28 PSU and a maximum of 33.51 PSU. The average salinity was 33.35 ± 0.07 PSU.

4.1.2. Available particles for the Chlamys islandica

An analysis of the water in the control chambers was carried out to get an overview of the particle volume available for the *C. islandica*. The analysis was also done to verify the results from the particle counter. The blue data series in Fig 4.1 show the available particles (in the control chambers) during the first week of the experiment when the Iceland scallops were exposed to water from 78 m depth. It did not fluctuate much between each day, meaning that all of the individuals (Gr.1 to Gr.4) had similar access to food. The highest volume of particles was found within the size ranges 3.8 – 4.7 µm and the maximum volume was 1795 ± 128 μm^3/ml. The red data series demonstrates the amount of particles in the water after it had passed the chambers with Iceland scallops. It is the average particle volume (μm^3/ml) of the ten scallops in the chambers. The gap between the blue and red data series, illustrates the amount of particles that are retained within each particle size range. The plots are similar, and demonstrate little variability of particles in the water from day to day.

The blue data series in Fig 4.2 shows the available volume of particles during the second week of the experiment when the Iceland scallops were exposed to filtered water from 78 m depth. The red data series illustrates the average particle amount in the water leaving the chambers with Iceland scallops. At the 26.03 (Fig 4.2 A) and 28.03 (Fig 4.2 C) the blue data points are mostly situated over the red data points and the difference between them demonstrates the average retention efficiency of the *C. islandica.* This is not so clear in the plots from the 17.04 (Fig 4.2 B) and 29.03 (Fig 4.2 D). The total volume of particles had decreased more than 15 times, and the peak concentration was

109 ± 25 µm^3/ml. This was found within the particle size-range 3.0 – 4.0 µm, which agree well with the results from the first treatment. It demonstrates that the highest concentration of biomass (particles) in the water was found around the same particle sizes, which validate the results from the particle counter.

Fig 4.3 A demonstrates how the clearance rate* (CR*) of a random *C. islandica,* started to fluctuate above a certain particle size limit (here 6.7 µm). This can also be seen in Fig 4.1 and Fig 4.2. Because of low particle concentration in the water and the relatively large particle size, the retention efficiency went from 0 to 100% from one size range to another in the sample volume analysed. This pattern is repeated in the measurements for all of the Iceland scallops, and particles above 6.0 µm have therefore been excluded when analysing individual CR* and ingestion rate. This is done in Fig 4.3 B.

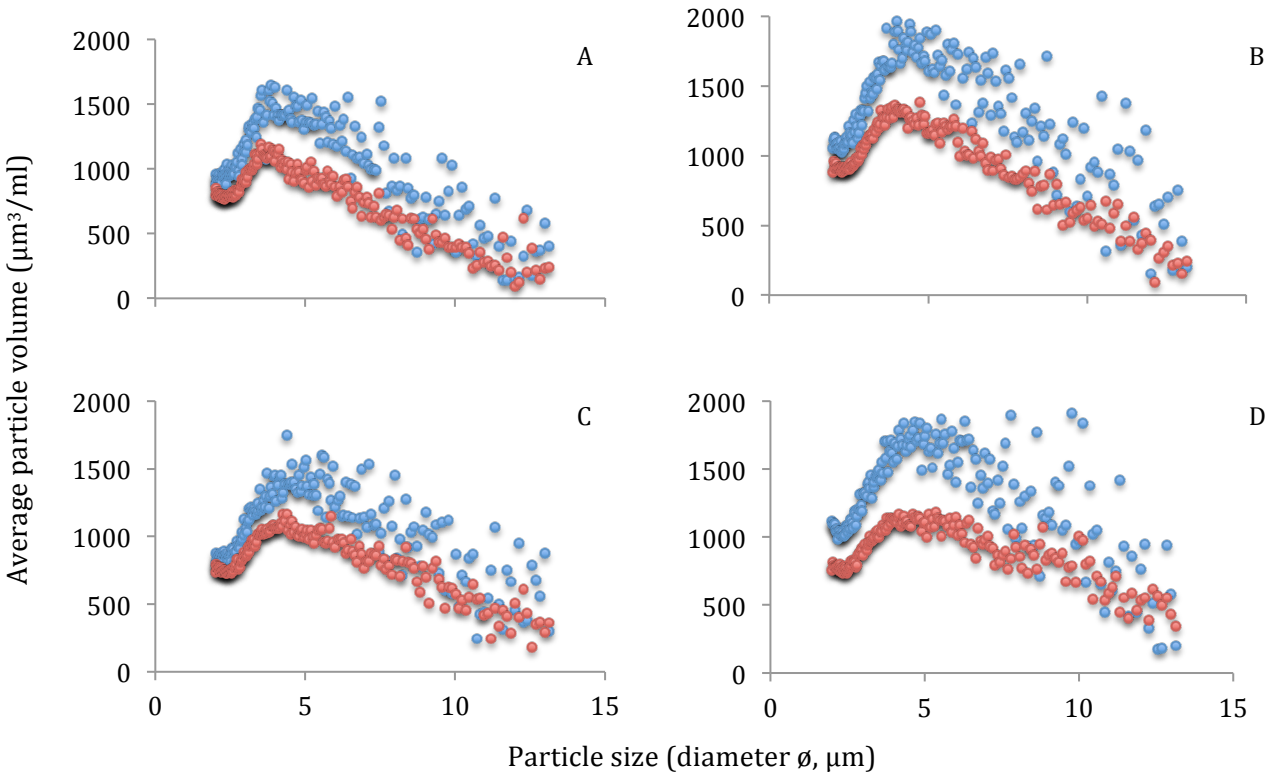

Figure 4.1. Average particle volume during treatment with medium particle concentration, going in (blue) and out (red) of the chambers the four days for A: Group 1(measured at date 19.03; hour 10:13), B: Group 2 (20.03; 09:56), C: Group 3 (21.03; 09:13) and D. Group 4 (23.03; 09:12).

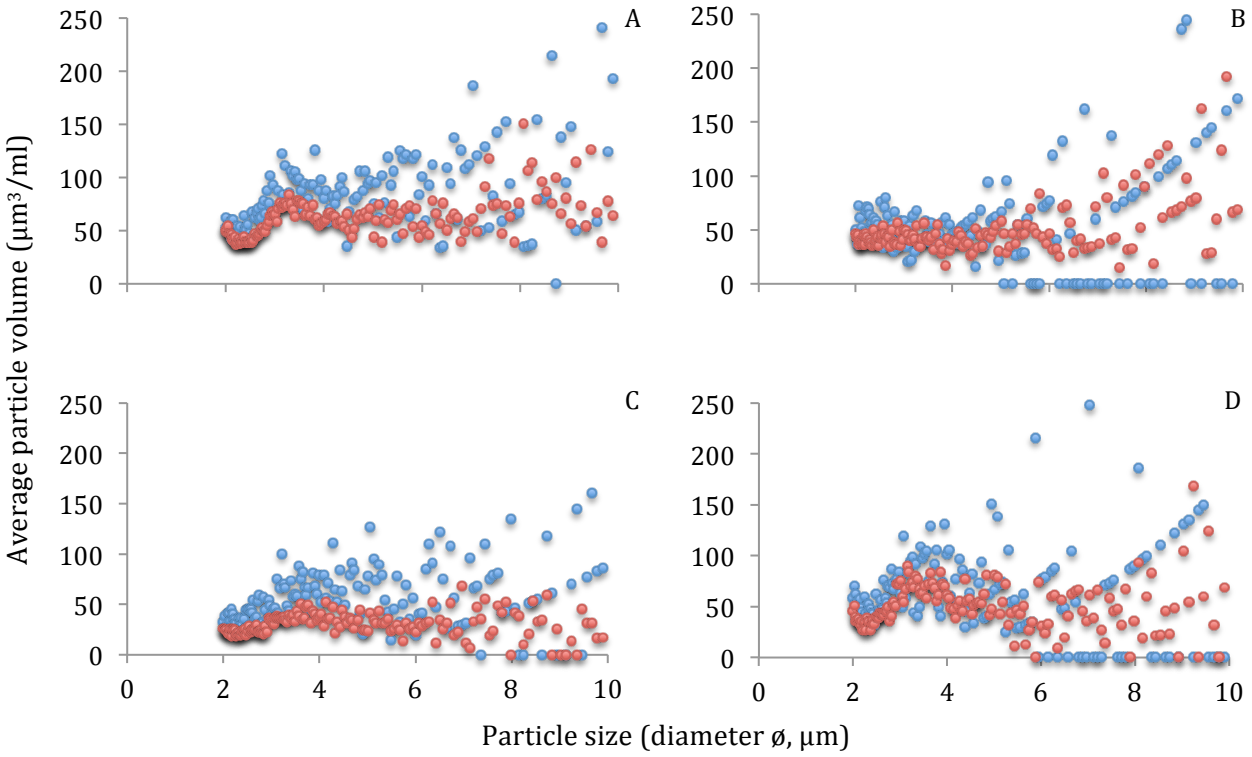

Figure 4.2. Average particle volume during treatment with low particle concentration going in (blue) and out (red) of the chambers the four days of A: Group 1 (measured at date 26.03; hour 10:23), B: Group 2 (17.04; 09:49), C: Group 3 (28.03; 08:45) and D: Group 4 (29.03; 08:58).

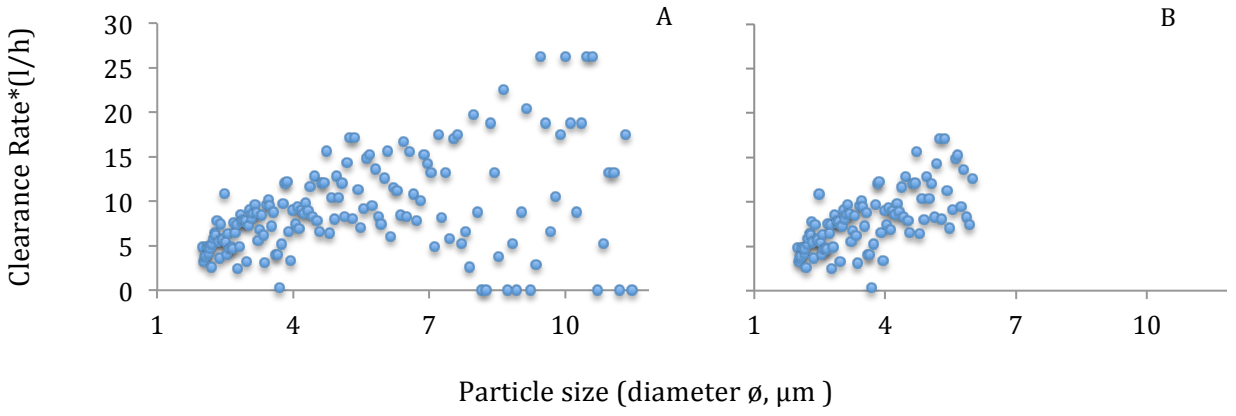

Figure 4.3. Clearance Rate* (l/h) of a random chosen Iceland scallop where plot A includes particle sizes up to 11.5 µm and plot B include particles with sizes up to 6.0 µm.

4.2 Principal Component Analysis

Four principal components (PC) were extracted from the data set, explaining 88.9% of the total variance in the data. The variance explained by each of the components is shown in Table 4.1.

Table 4.1. Principal components and explained variance.

Principal Component	Explained Variance
PC1	65.0%
PC2	11.4%
PC3	7.3%
PC4	5.3%

An outlier detection test was completed using a plot of the Hotelling t-test versus the absolute value of residual standard deviation from the model centre (RSD) for each object. The object MED-46-F came out with a high t-test value (Fig 4.4), indicating that it had an exceptional influence on the model. It was therefore removed from further analysis. Several objects had high RSD (Fig 4.4), but as this is normal in smaller data sets they were included in the analysis (based on acceptable Hotelling t-test values).

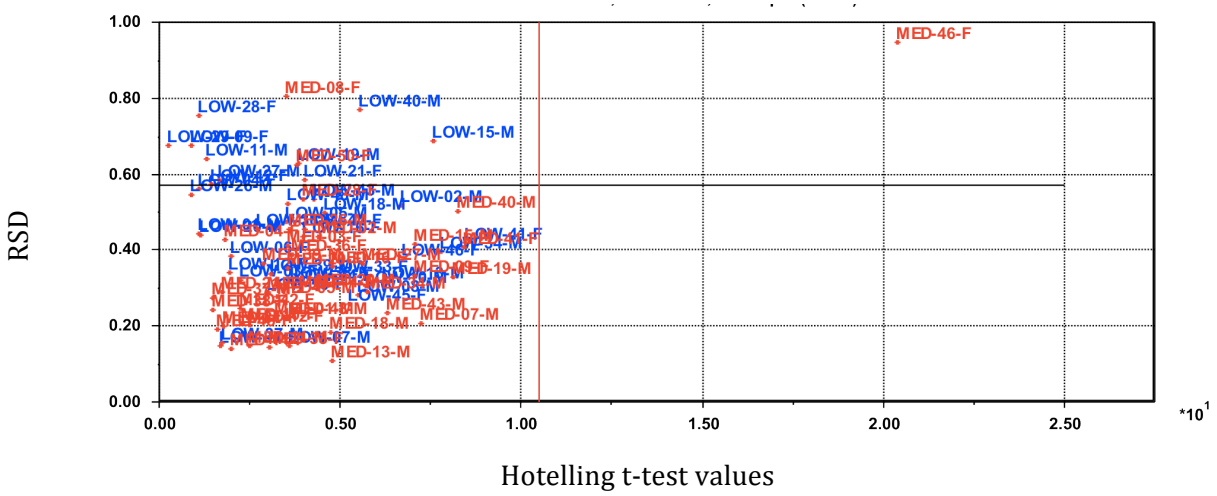

Figure 4.4. Outlier detection test for the 80 objects with individual tag numbers (40 from treatment with medium particle concentration (MED) and 40 from treatment with low particle concentration (LOW). The letters M/F illustrate the gender of the scallop (male/female).

The most interesting information was found in the two first principal components as they explain 76.3% of the total variation (Table 4.1). Principal components that explain less then 10% of the total variance are often categorised as data noise in smaller data sets (*O.H.J. Christie, pers. comm*).

The main partitioning of the objects was found in PC1 (Fig 4.5 and Fig 4.7). The clear separation of the objects can be explained by a correlation pattern between the variables situated on each side of the x-axis in Fig 4.6. The loading values of the variables (Fig 4.8) illustrate a positive correlation between the particle volume in the inflowing water (Mean-in and TV-in for the three measurements 1-3) and particle volume retained by the Iceland scallops (TVR1-TVR3). The negative correlation between these variables and the flow (F1-F3), also explain the partitioning of the objects in PC1 (Fig 4.7). There were no visual grouping of male and females in neither of the principal components.

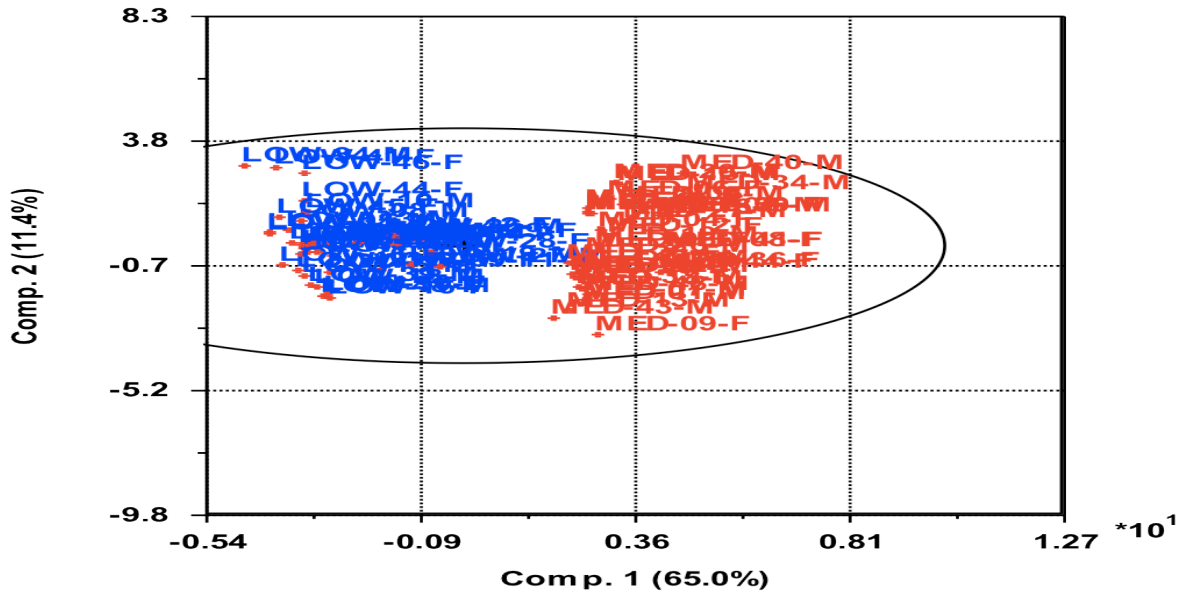

Figure 4.5. Score values of the objects in PC1 and PC2, where the blue data points illustrate the treatment with low particle concentration and the red data series the treatment with medium particle concentration (it is only the object names that overlap and not the corresponding point).

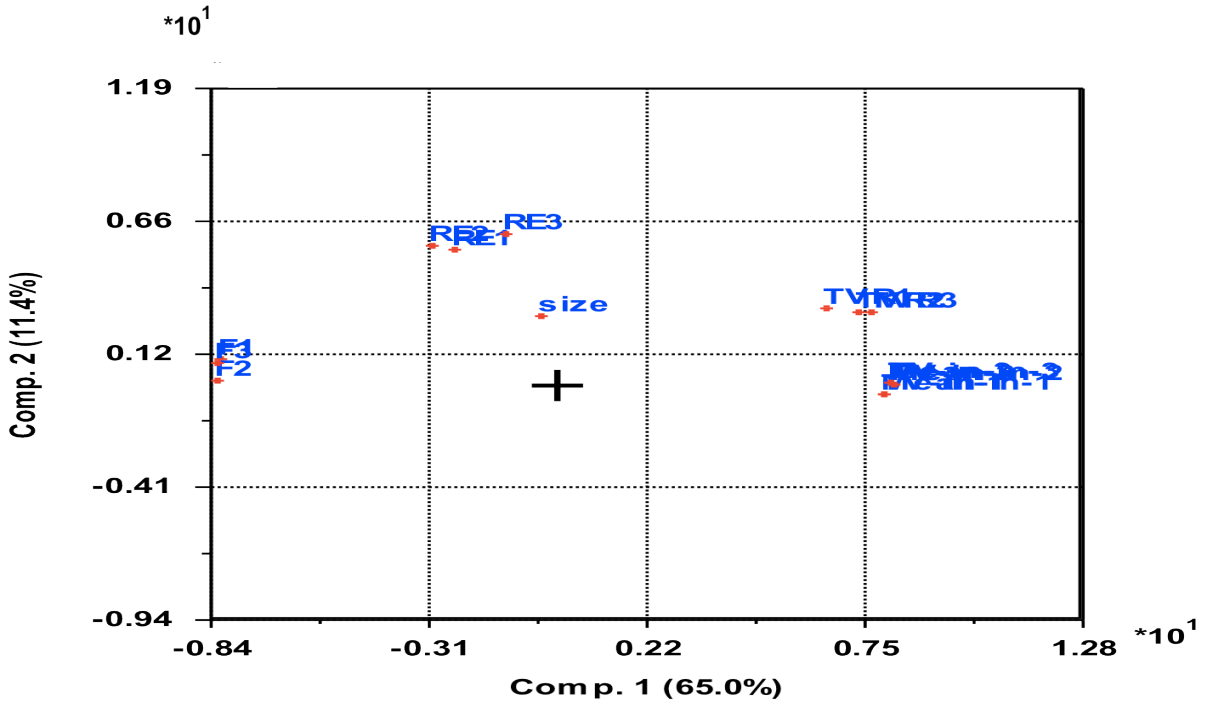

Figure 4.6. Loading values of the variables in PC1 and PC2: Flow (F), retention efficiency (RE), size, total particle volume retained by scallop (TVR), mean particle volume into chamber (Mean-in) and total particle volume into chamber (TV-in). The numbers 1-3 illustrates the sampling rounds (1: first measurement – 3:last measurement).

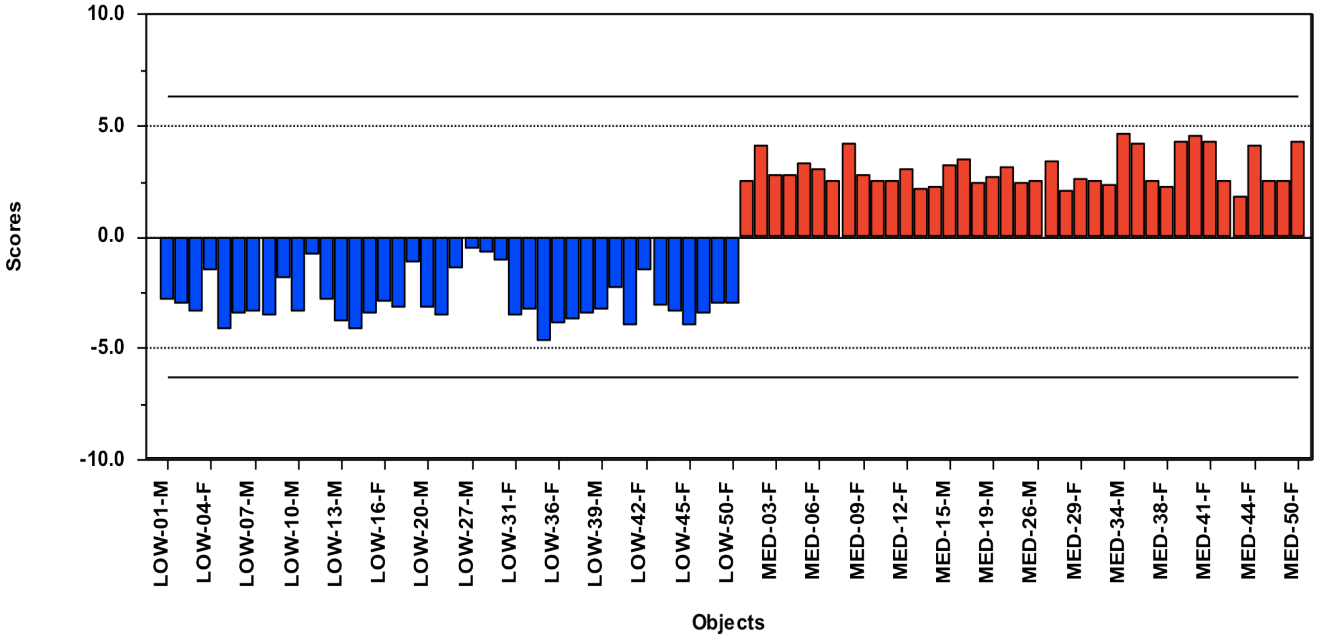

Figure 4.7. Score-values of the 39 objects in PC1, during treatment with low particle concentration (Low; blue) and treatment with medium particle concentration (MED; red). The numbers illustrate the individual tag numbers and the letters M/F demonstrate the sex of the scallop (male/female).

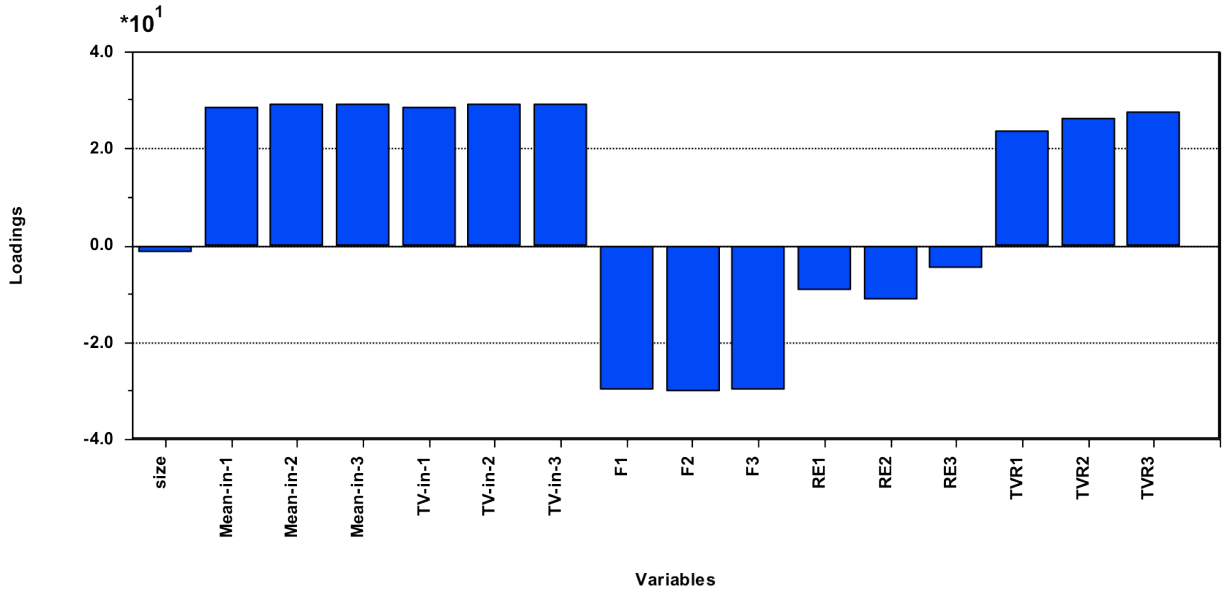

Figure 4.8. Loading values of the variables in PC1: Flow (F), retention efficiency of the scallop (RE), size, total volume retained by scallop (TVR), mean particle volume into chamber (Mean-in) and total particle volume into chamber (TV-in). The numbers 1-3 illustrates the sampling rounds (1: first measurement – 3:last measurement).

The score values of the different objects in PC2 did not show a clear pattern of grouping/partitioning (Fig 4.5 and Fig 4.9). Furthermore, the size-variable had a strong loading value in this principal component (PC2) and was positively correlated with the retention efficiency (RE) of the scallops (Fig 4.10). The different score values of each object indicated that there were individual differences in retaining particles in both of the treatments. The positive correlation between size and RE suggested that large Iceland scallops had a tendency of retaining more particles than smaller ones.

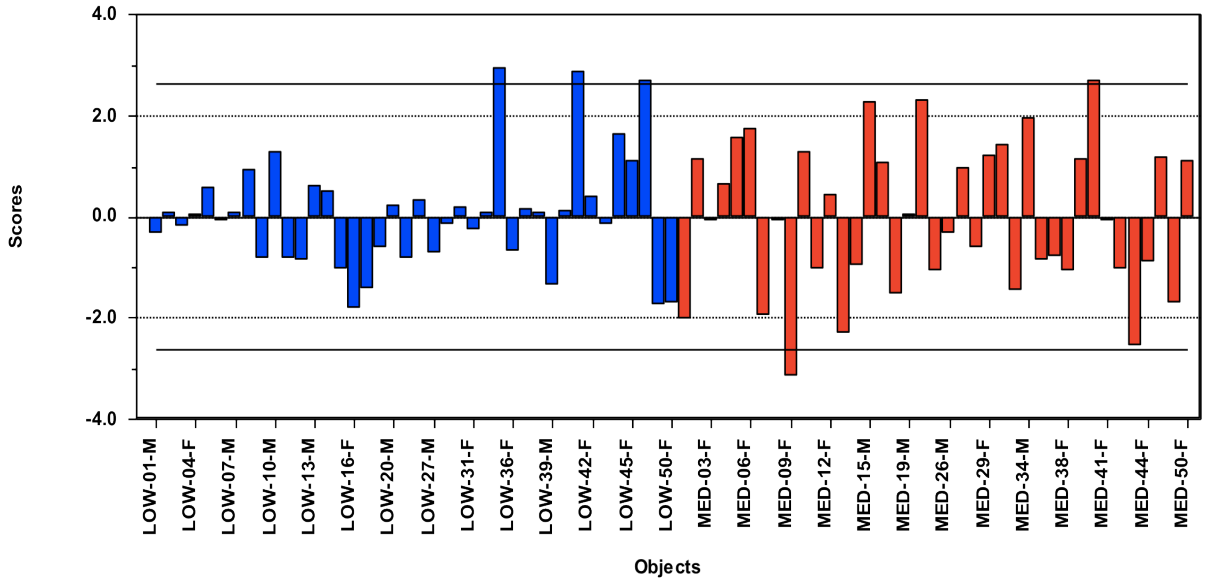

Figure 4.9. Score-values of the 39 objects in PC2, during treatment with low particle concentration (blue) and treatment with medium particle concentration (red). The numbers illustrate the individual tag numbers and the letters M/F demonstrate the sex of the scallop (male/female).

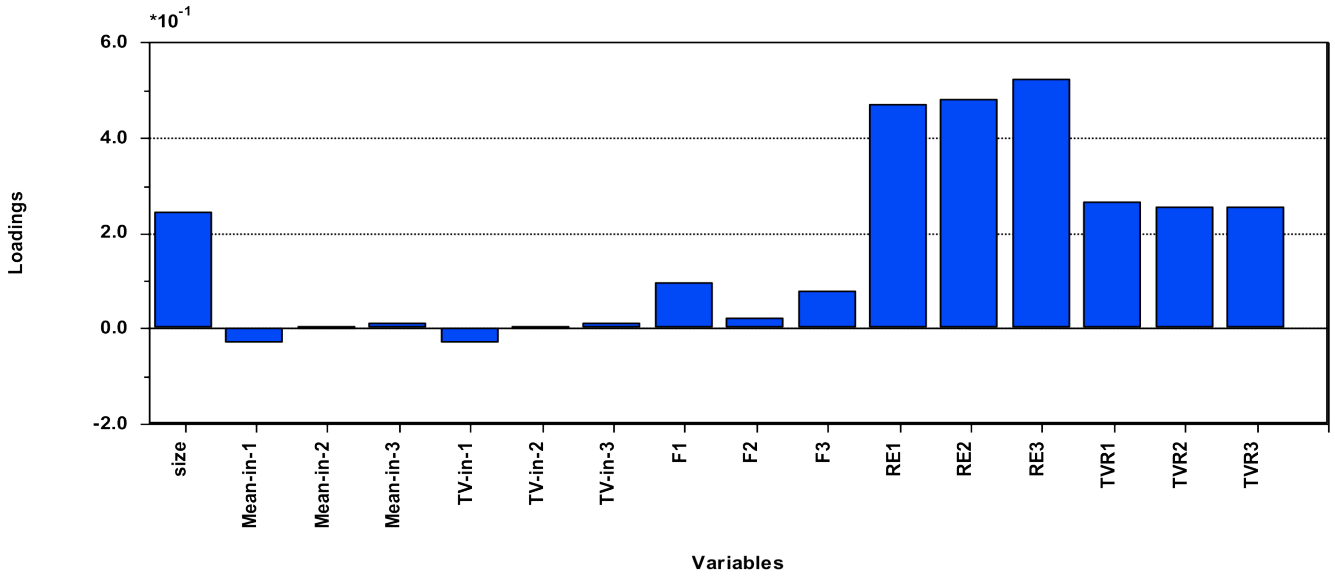

Figure 4.10. Loadings of the variables in PC2: Flow (F), retention efficiency of the scallop (RE), size, total volume retained by scallop (TVR), mean particle volume into chamber (Mean-in) and total particle volume into chamber (TV-in). The numbers 1-3 illustrates the sampling rounds (1: first measurement – 3:last measurement).

Fig 4.11 shows the large spectrum of individual variation as the objects have been sorted and plotted by their PC2 score-values. The red columns (treatment with low particle concentration) for each individual did not follow the green columns (treatment with medium particle concentration), indicating that there was no clear pattern in how a single individual adjusted their feeding behaviour in both of the treatments. A large scallop may have had a high score value (large retention efficiency) in the treatment with low particle concentration and low score value (low retention efficiency) in the treatment with medium particle concentration. See object 15, 16 and 41 (Fig 4.11) as examples.

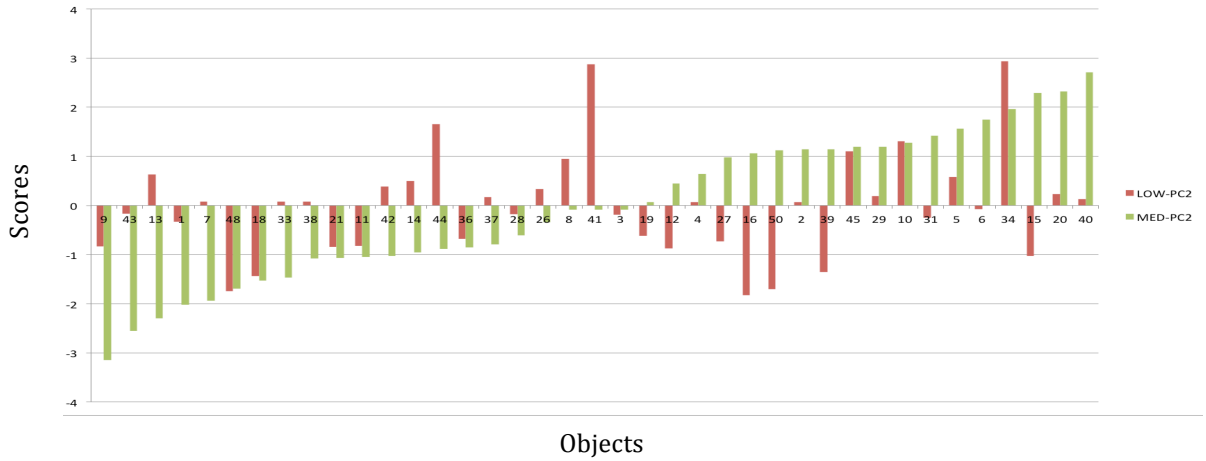

Figure 4.11. Score values of the 39 objects during low particle concentration (red) and medium particle concentration (green).

4.3 Intra-individual variability

The clearance rate* (CR*) of a randomly selected *C. islandica* was not constant at different particle sizes, and also changed between the different measurements. In the course of one day, it fluctuated from 1.21 l/h to 13.09 l/h at 4 µm (Fig 4.12 A, B). Minimum CR* (4.20 l/h at 2.0 µm) and maximum CR* (16.51 l/h at 5.4 µm) showed that there was a tendency of higher CR* with increasing particle size (Fig 4.12 A). The clearance rate was more variable within each particle size during the treatment with low particle concentration (Fig 4.12 E-H).

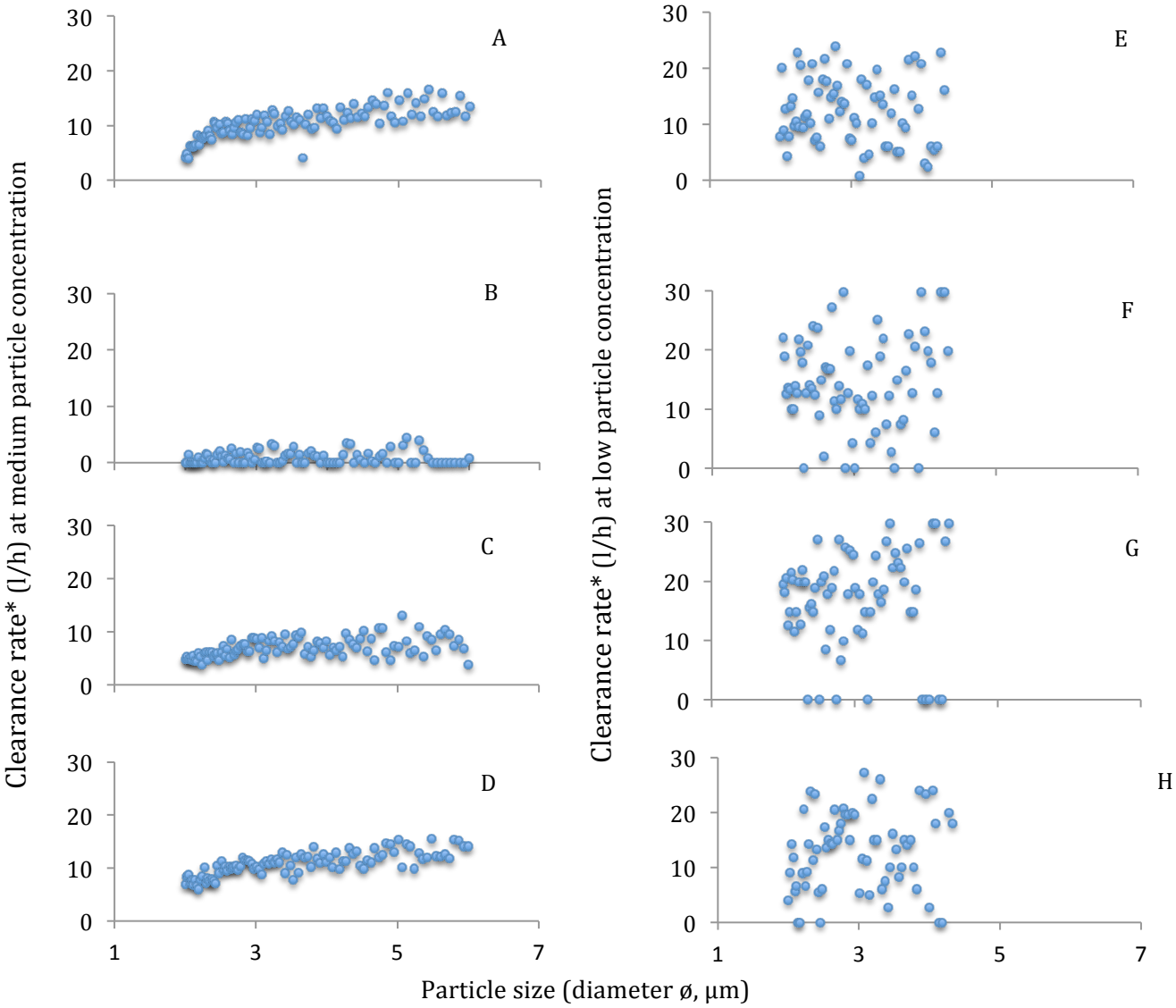

Figure 4.12. Variation in clearance rate* (l/h) of a single Iceland scallop during treatment with medium particle concentration (A-D) and low particle concentration (E-H). Time of sampling was 10:13 (A,E), 12:58 (B,F), 14:04 (C,G) and 15:10 (D,H).

Time-series of maximum clearance rates* and ingestion rates (Fig 4.13) demonstrated how an individual Iceland scallop changed feeding behaviour from one treatment to another. The CR* in the low particle concentration, was considerable higher than the CR* in the medium particle concentration (Fig 4.13 A, blue data vs. red data), while the IR showed the opposite pattern of being substantially lower in the low particle concentration (Fig 4.13 B, blue data vs. red data). It was also clear that the maximum CR* and ingestion rate (IR) fluctuated more when the scallop was exposed to water with medium particle concentration (max CR*: 12.34 ± 4.75 l/h, max IR: 22.41 ± 8.83 mm^3/l), compared to the CR* from water with low particle concentration (max CR*: 28.34 ± 2.61 l/h, max IR: 2.03 ± 0.36 mm^3/l). Similar pattern and change in CR* and IR was registered for most of the individuals.

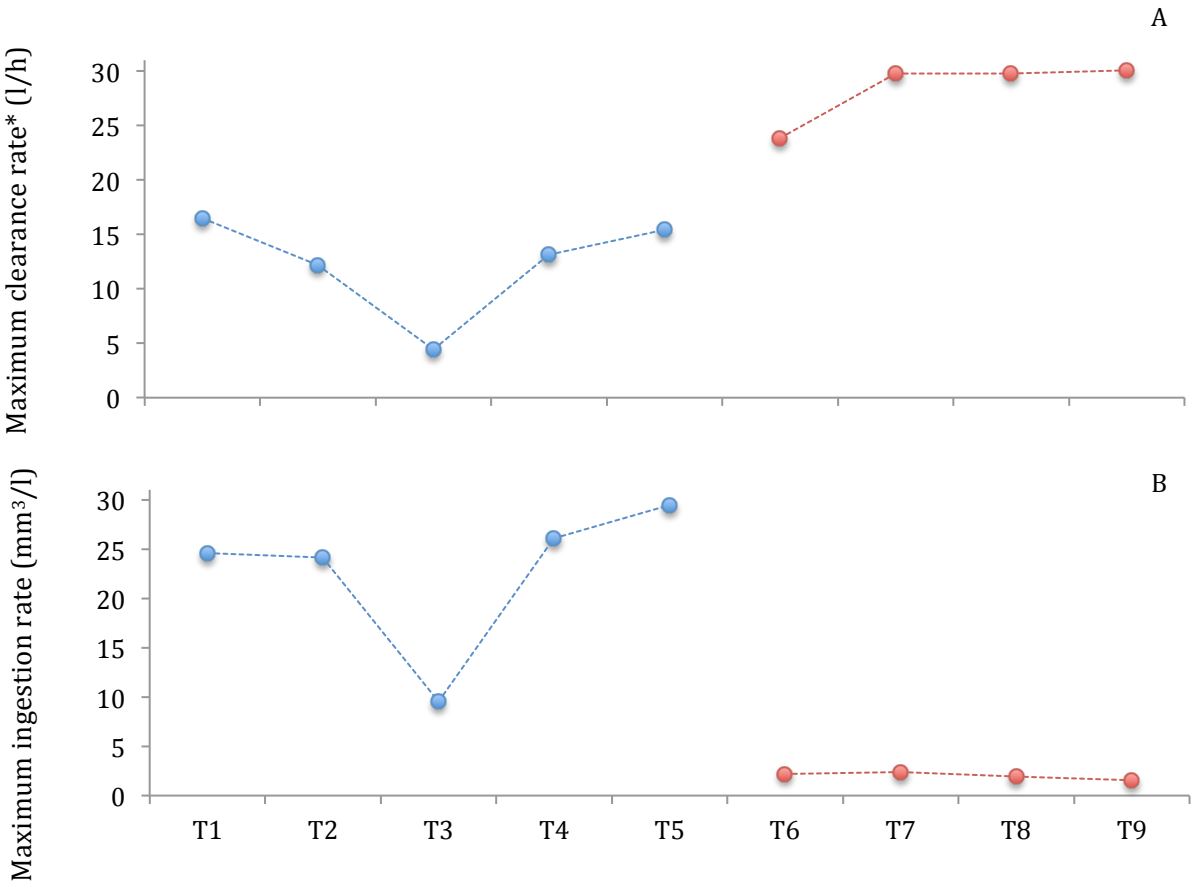

Figure 4.13. Time-series (T1 – T9) of A: maximum clearance rate* (l/h) and B: maximum ingestion rate (mm^3/l) of a single Iceland scallop during treatment with medium particle concentration (blue) and low particle concentration (red).

Even though the CR* of the randomly selected *C. islandica* (Fig 4.12 and 4.13) does not illustrate a typical cycle of feeding behaviour, there was still a correlation pattern in how all of the Iceland scallops adjusted their CR* throughout the day. Fig 4.14 illustrates how the CR* of the 40 individual Iceland scallops became more correlated in the last sampling rounds. There was a weak correlation (r=0.53) between the individuals in the two first sampling rounds (from CR1 to CR2). The two last sampling rounds (from CR4 to CR5), on the other hand, show a strong correlation (r=0.86). This indicated that the 40 individuals adjusted their CR* (intra-individual feeding behaviour) in a similar way after they had been left without disturbance for more than 3 hours.

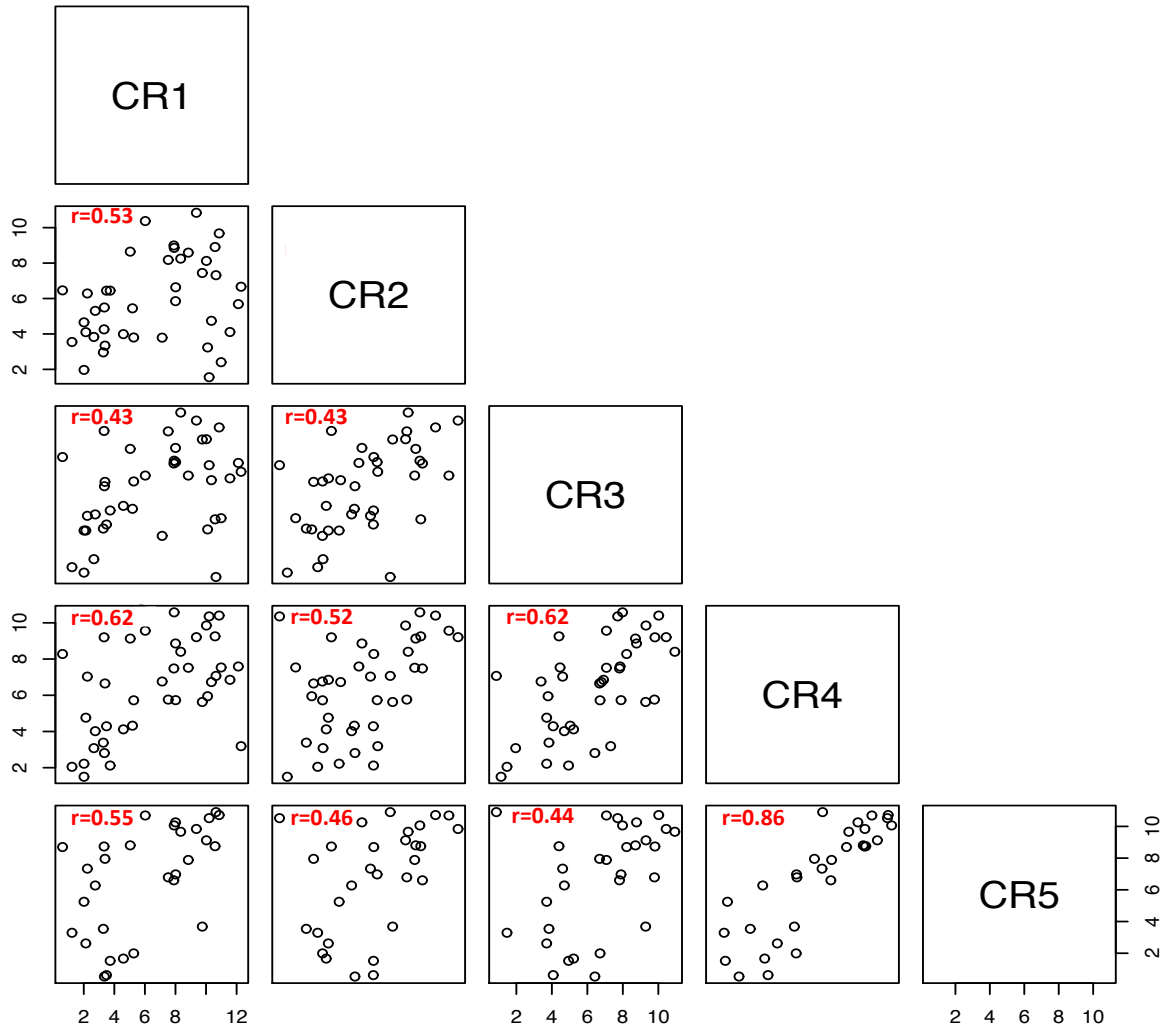

Figure 4.14. Correlation pattern (with correlation coefficient, r) between the clearance rates* (CR1-CR5) of 40 individual Iceland scallops. Numbers 1 to 5 illustrate different sampling rounds (CR1: first sampling round, CR5: last sampling round) and each circle in a square demonstrates one individual Iceland scallop.

4.4 Inter-individual variability

When comparing individual responses from the same sampling-time, the *C. islandica* showed various patterns of feeding behaviour, which was illustrated by their clearance rate* (CR*), and ingestion rate (IR).

4.4.1. Clearance rate

The maximum CR* for particles between 2-6 µm (see Fig 4.3 and explanation in text) ranged between 13.93 l/h at 5.67 µm for individual #12 (Fig 4.15 D) and 18.8 l/h at 5.67 µm for individual #27 (4.15 B). While individual #08 and #34 (Fig 4.15 A, C) had distinctive patterns with higher CR* as particle size increased, individuals #12 and #27 had more constant CR* over the different particle sizes. This was illustrated by the flat scatter of data points (most clear for Iceland scallop #27) (Fig 4.15 B, D). Average CR* values was 10.27 ± 3.53 l/h, 12.11 ± 1.91 l/h, 7.91 ± 3.38 l/h and 7.88 ± 2.53 l/h for individual #08, #27, #34 and #12 respectively (Fig 4.15 A-D).

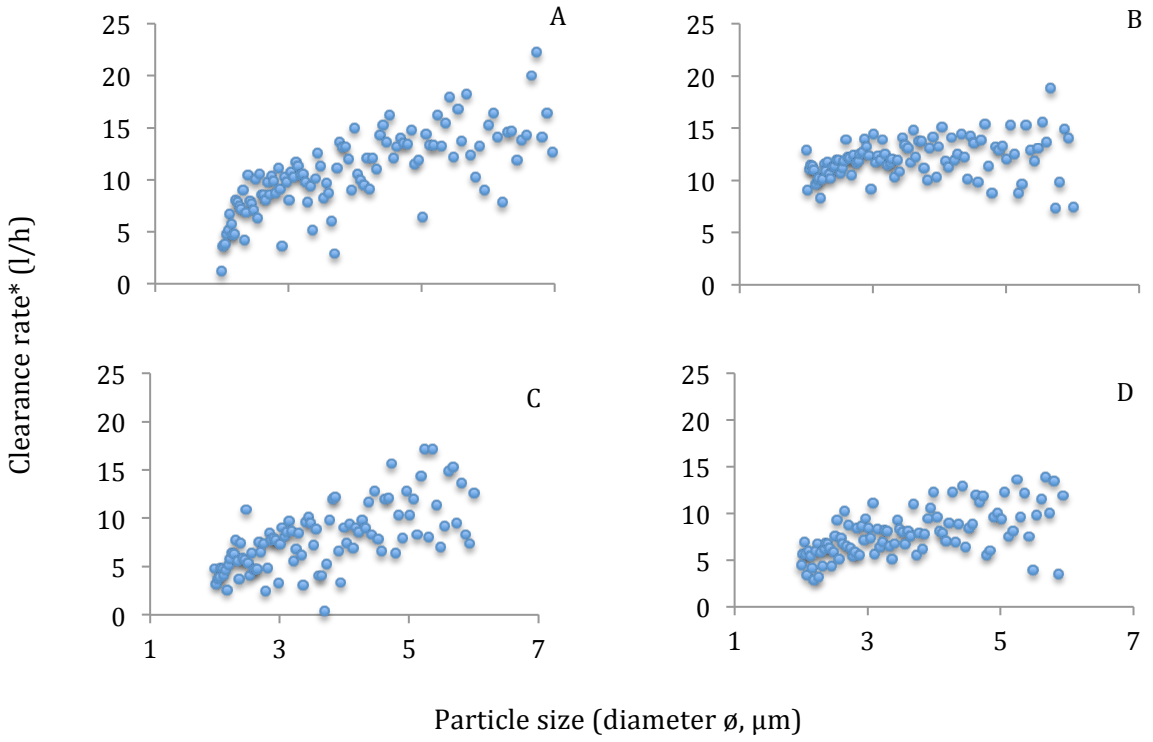

Figure 4.15. Clearance rate* (l/h) at different particle sizes (µm) for the four randomly selected *Chlamys islandica* with tag numbers #08 (A), #27 (B), #34 (C) and #12 (D).

4.4.2. Ingestion rate

The maximum IR for particles between 2-6 µm (see Fig 4.3 and explanation in text) of the four individual *C. islandica* did not vary much and was observed at similar particle sizes (from 5.24 µm to 5.42 µm). The highest IR was registered for individual #27 (31.33 mm^3/l), and the lowest for individual #12 (25.95 mm^3/l) (Fig 4.16 B, D). Average IR values varied more between the individuals; 13.25 ± 6.13 mm^3/l, 17.37 ± 5.42 mm^3/l, 10.33 ± 5.62 mm^3/l and 12.14 ± 5.68 mm^3/l for the four individuals #08, #27, #34 and #12 respectively (Fig 4.16 A-D). The standard deviations were higher here (coefficient of variation: 31-54%) compared to the CR* measurements shown in the former section (coefficient of variation: 15-42%).

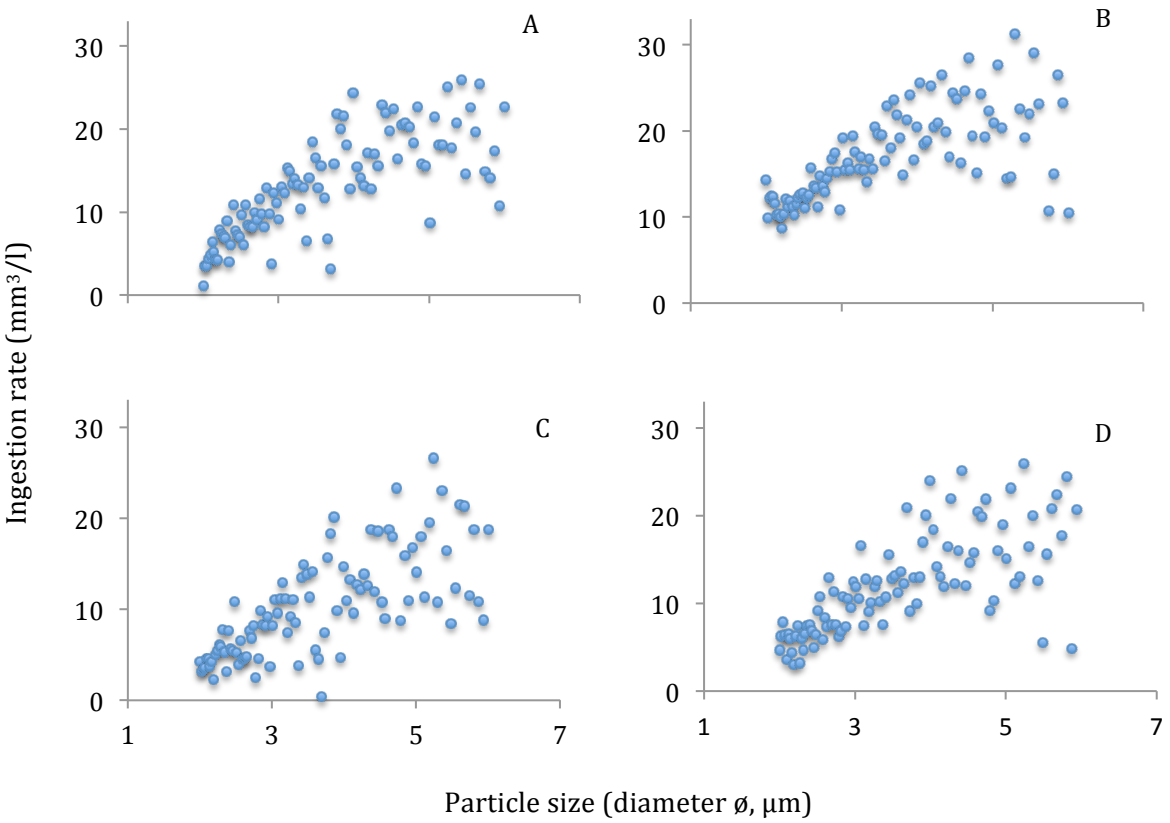

Figure 4.16. Ingestion rate (mm^3/l) at different particle sizes (µm) for the four randomly selected *Chlamys islandica* with tag numbers #08 (A), #27 (B), #34 (C) and #12 (D).

4.4.3. Flow velocities through exhalant siphon

Different size (area) of the exhalant siphon resulted in variations in the flow velocity (F_v) of each individual. With a siphon area of 23.5 mm^2 (Figure 4.17 A), individual #08 had an estimated maximum flow velocity of 211.6 mm/sec. The lowest estimated flow velocity was registered with individual #34 (Fig 4.17 C) at 127.5 mm/sec, while the highest was found with individual #27 at 286.9 mm/sec (Fig 4.17 B).

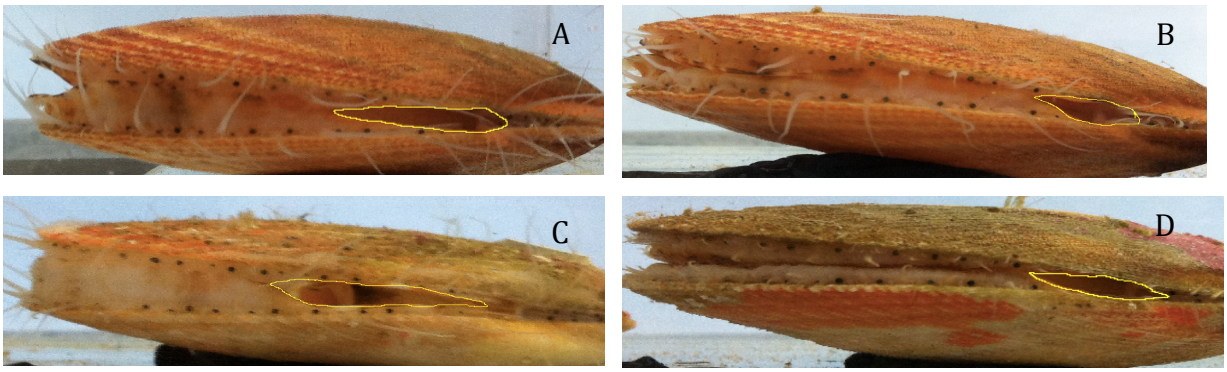

Figure 4.17. Estimates of siphon area of A: individual #08 (Area 23.5 mm^2; Height 69.7 mm), B: individual #27 (18,2 mm^2; 67.6 mm), C: individual #34 (37,4 mm^2; 78.5 mm) and D: individual #12 (17,6 mm^2; 68.5 mm).

4.4.4. Average clearance rate and ingestion rates

The average CR* and IR (Fig 4.18), demonstrated both variability within an individual (intra-individual variability) and between five individuals (inter-individual variability). Almost identical time-series for the average CR* and IR illustrated how closely the two parameters were linked together. Individual #20 had the highest and most stable values of CR* and IR with a daily average of 10.33 ± 0.49 l/h and 12.79 ± 2.20 mm^3/l (Fig 4.18, light blue). Individual #43 had the lowest CR* and IR values (2.31 ± 1.05 l/h and 2.34 ± 1.65 mm^3/l), and neither showed large fluctuations throughout the day (Fig 4.18, dark blue). The three individuals #31, #37 and #42, however, showed large intra-individual variations in feeding behaviour (in addition to inter-individual variations), where CR* values fluctuated between 0.61 l/h and 8.70 l/h (#37) and IR values varied from 8.26 mm^3/l to 1.61 mm^3/l (#42)(Fig 4.18, red and green).

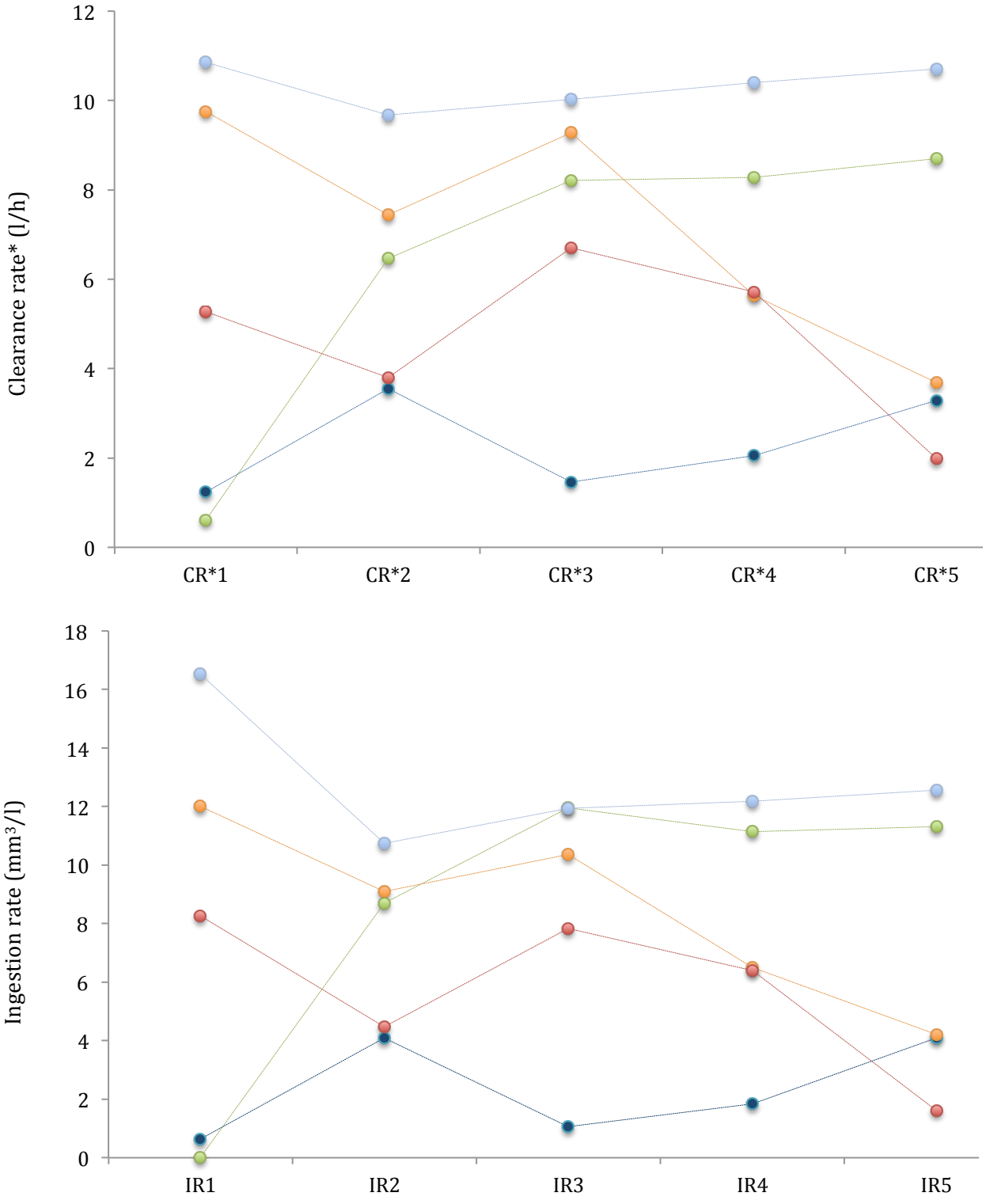

Figure 4.18. Time-series (1-5) of average clearance rates (CR*) and ingestion rates (IR) for the randomly selected individuals #20 (light blue), #42 (green), #31 (orange), #37 (red) and #43 (dark blue).

4.5 Group responses

The average clearance rate* of *C. islandica,* when placed in multiple arbitrary selected groups of 5, 10, 20 and 30 individuals, converged towards 6.33 l/h which was the average value of the largest group (with n=40 individuals)(Fig 4.19 A). Average CR* in the smallest groups (5 individuals) ranged from 4.11 l/h to 8.38 l/h, while the values of groups with n=30 individuals had a much smaller range (from 5.90 l/h to 6.72 l/h). The same pattern of convergence was found when comparing the standard deviations of multiple groups of 5, 10, 20 and 30 individuals (Fig 4.19 B). The coefficient of variation (CV) was 35.7% for the group with n=40 individuals (Fig 4.19 C). When the number of individuals decreased to n=10 the CV varied from 21.7% to 49.0%. For n=20 individuals, it was more stable, and ranged between 28.6% and 40.5%.

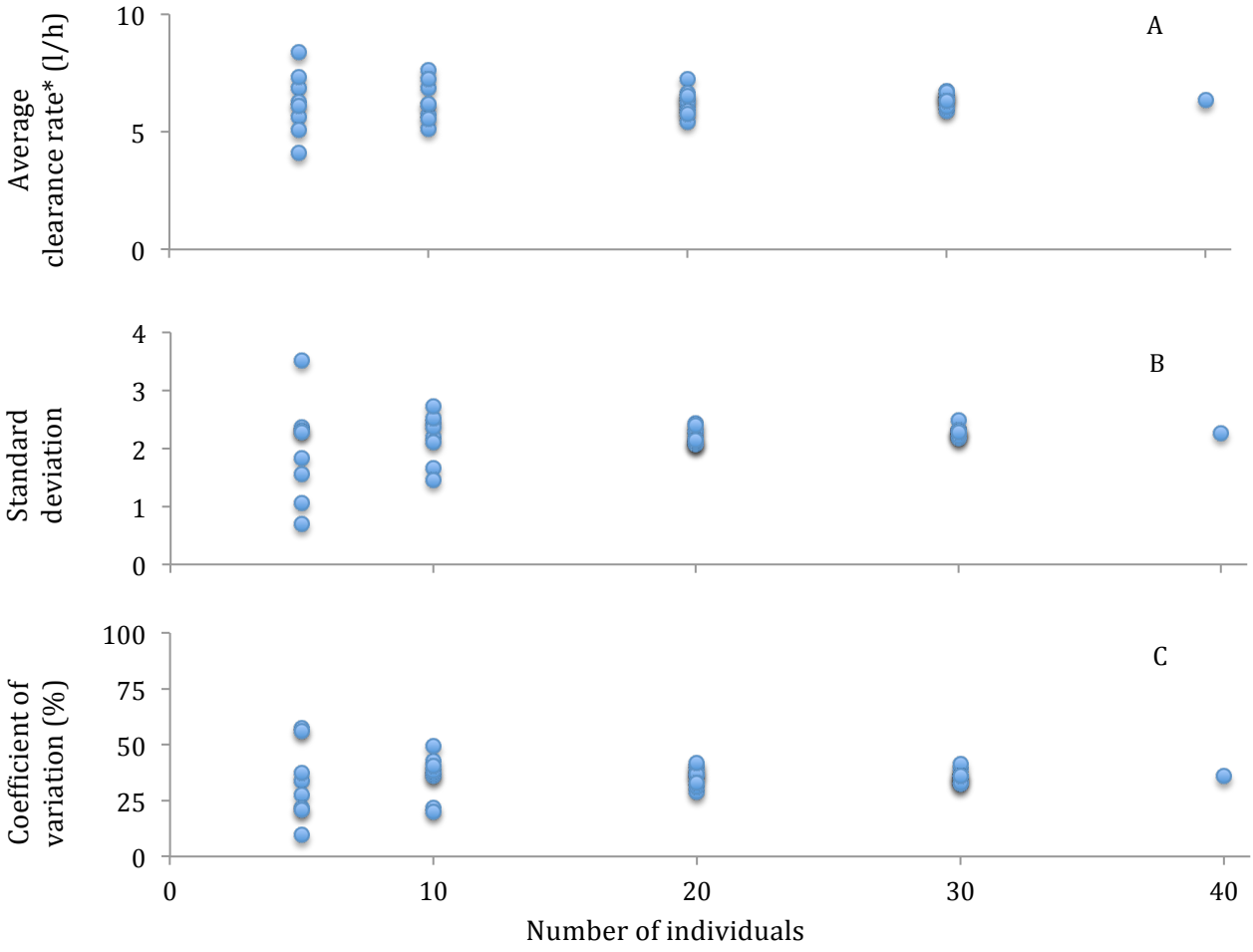

Figure 4.19. Average (A), standard deviation (B) and coefficient of variation(C) in clearance rate* (l/h) of multiple groups of 5, 10, 20, 30 and 40 (only one group) individuals.

The change in environmental conditions from water with medium particle concentration to water with low particle concentration is reflected through the CR* of the 40 individuals (Fig 4.20). There was a clear pattern of higher CR* values during the treatment with low particle concentration as 87.5% of the individuals had higher CR* responses during this treatment compared to treatment with medium particle concentration. Individual differences in CR* (from low particle concentration to medium particle concentration), had a great influence on group responses, as the average ± sd were very different between two groups with same amount of individuals, n=5 (μ = 1.71 ± 1.80 vs. μ = 6.22 ± 2.10) (Fig 4.20).

Most of the individuals had lower CR* during treatment with medium particle concentration, but there was no clear correlation indicating that an individual had similar CR* in both of the treatments compared to the average (higher/lower CR* than the average in both treatments) (Fig 4.20). Looking at the individuals with highest CR* values, only one was present in the upper 90 percentile of both treatments (Fig 4.20, individual #28). The individuals that had the lowest CR* values (10 percentile) during treatment with low particle concentration (#8, #34, #7 and #25), were not the same as the individuals that had the lowest values during medium particle concentration (#1, #36, #6 and #36).

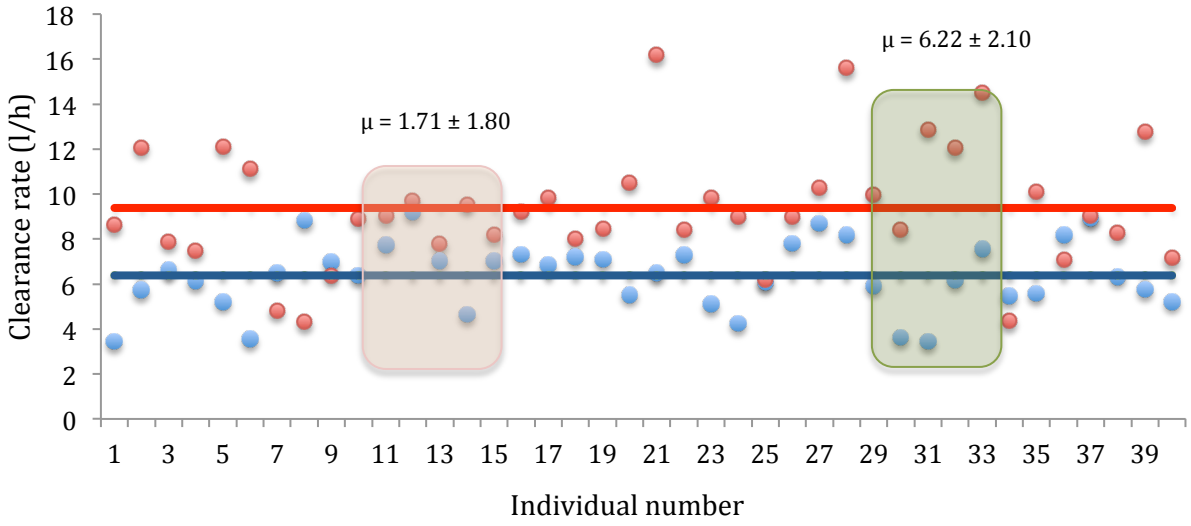

Figure 4.20. Average clearance rate* (l/h) of the 40 individual Iceland scallops during treatment with low particle concentration (red circles) and medium particle concentration (blue circles). Average difference in clearance rate (μ; mean ± sd) from low particle concentration to medium particle concentration, are illustrated for two groups with n=5 individuals (red and green square). Average values for all individuals within each treatment are illustrated with a line (red/blue).

5. Discussion

5.1 Environmental conditions and similar response patterns

Environmental parameters (temperature and salinity) varied little during the experiment. This was expected given the short duration (12 days), and the deep-water intake from 78 m. There is conflicting evidence whether temperature changes may effect the filtration rates of bivalves (Kittner & Riisgård, 2005; Koenhn & Bayne, 1989; Strohmeier, et al., 2009; Widdows, 1978). A study of the temperature tolerance of *Chlamys islandica* showed increased mortality and change in behaviour when temperatures increased towards 12 °C (Jonasson, et al., 2004). In the current study, the temperature was held within the tolerance limit of *C. islandica*, and the low variation was assumed to have no affect on the feeding behaviour.

The results illustrated a stable particle concentration in the water during the two separate treatments. As the volume of particles (μm^3/ml) in the water decreased substantially (total particle volume was more than 15 times lower in the treatment with low particle concentration), two highly different environmental conditions for the Iceland scallops were generated. The large difference in particle volume between the treatments was reflected by the clearance rate* (CR*) and ingestion rate (IR) of the scallops. All of the individuals had lower ingestion rates during the treatment with low particle concentration. This reflects the available amount of particles in the water, as it is difficult to maintain the same ingestion rate when the particle availability declines drastically. Other studies have found similar fluctuations in IR when environmental conditions change (Widdows et al., 1979). Contrary to the change in ingestion rate, the Iceland scallops showed a pattern of higher (and more stable) clearance rates* during treatment with low particle concentration. Clearance rate* increased for 87.5% of the individuals when exposed to water with low particle concentration. This illustrated how the scallops controlled their feeding physiology in different environments, which is consistent with other studies (Clausen & Riisgård, 1996; Strohmeier, et al., 2012; Widdows, 1978; Widdows, et al., 1979). The clear change in CR* responses may reflect

the individual`s necessity to retain as many particles as possible from the water to satisfy nutritional requirements at low particle concentration.

During treatment with medium particle concentration, most of the individuals showed a pattern of higher CR* and IR with increasing particle size (Section 4.4, Fig 4.15 and 4.16, Section 4.3, Fig 4.12). Particles are retained when water is pumped through the organism, and particles with different sizes are retained differently. Other studies have found the same pattern of higher retention efficiency (leading to higher CR*) as particle size increase (Strohmeier, et al., 2012; Vahl, 1973). Small particles are likely to be pumped through the organism without being retained, while particles above a certain size limit are retained effectively. A maximum retention is achieved at a certain particle size. Vahl (1973) suggested that the *Chlamys islandica* effectively retained particles (100% retention) down to 7 µm. In the current study, the scallops did not reach 100% retention as particles above 6 µm were excluded from the analysis (Fig 4.3 and explanation in text).

The first principal component (PC1) in the principal component analysis (PCA), showed a positive correlation between the amount of particles in the water and the scallops ability to retain particles (Section 4.2, Fig 4.8). This is consistent with the results that were discussed above. PC1 also showed a negative correlation between the flow rate and the volume of particles (in the inflowing water and retained by the scallops). A slight change in the flow rate between the treatments with medium and low particle concentration may explain the negative correlation, despite trying to keep the flow rate stable throughout the experiment. The results did not show any visual grouping of males and females, indicating that the gender did not influence the feeding behaviour of scallops.

5.2 Individual and group responses

The results show large variations within and between individuals, which may also influence group responses when average values are utilised. This issue has been recognised in other studies (Brian L. Bayne, 2004; Frechette et al., 2005; Ringwood et al.,

1999), and Kohn & Bayne (1989; pg. 168) stated that "the effect of environmental change and/or stress upon individual variability in physiological performance is an area still in need of investigation".

5.2.1 Intra-individual variability

Intra-individual variability is visualised when responses from an individual organism fluctuate between several measurements. This was illustrated in the results, which showed large variation in individual clearance rate* and ingestion rate over the course of one day. At a certain particle size (4 μm) the clearance rate* for the randomly selected scallop changed with a factor of 10.8 during the measurement period (Section 4.3, Fig 4.12). The low CR* values at 12:58 (Section 4.3, Fig 4.12 B) may indicate that the scallop had closed the valves or stopped filter feeding. Few studies have focused on the topic of intra-individual variability, but it has been acknowledged in the work of other scientists (MacDonald & Ward, 2009; Strohmeier, et al., 2009). *Chlamys islandica,* as other bivalves, are sensitive organisms and may alter feeding behaviour for various reasons such as stress (Kramer, et al., 1989), disturbance (Møhlenberg & Riisgård, 1979; Vahl, 1973) or change in food supply (Pilditch & Grant, 1999), which may result in intra-individual variation on short time scales.

It is interesting to see how the 40 individuals showed similar patterns (r=0.86) of adjusting their clearance rates* between the fourth and fifth measurement (from CR4 to CR5) in the treatment with medium particle concentration (Section 4.3, Fig 4.14). There may be various reasons for this correlation, one being connected to the time they were left undisturbed before sampling. Former studies with bivalves in flow-through chamber, suggest that organisms need at least half an hour without disturbance before measurements are made (Strohmeier, et al., 2012). Riisgård (2004) advocate that bivalves need more than one hour to achieve 100% clearance rate, and in a study of Vahl (1973) the Iceland scallops were left undisturbed for 24 hours. The scallops used in the current study were left for one hour, however, the correlation pattern between all of the 40 individuals (Section 4.3, Fig 4.14) indicated that they could have benefitted from being left a longer period without disturbance (>3 hours). This will be tested in a further study where the scallops will be placed in the individual chambers the day before measurements are made.

In the present study, behaviour of individuals varied over the measurement period (intra-individual variations). This means that a single measurement of clearance rate and ingestion rate was not necessarily representative of the average feeding behaviour over the course of one day. Other studies have suggested that repeated sampling from the same individual is necessary to reduce intra-individual variations (Bennett, 1987; MacDonald & Ward, 2009). Strohmeier (2009, pg. 1789) stated that "a large number of replicate CR measurements (18) adequately constrained inter-individual variations, permitting precise measurements of the mean cohort CR response to experimental conditions". In the present study, at least three sampling rounds were used to investigate fluctuations in the feeding behaviour of a single individual within each treatment. If the aim was to find a critical number of measurements needed to obtain a more accurate average response of an individual, a study with more sampling rounds should be carried out.

The subject of intra-individual variation is of great importance if individual *C. islandica* are used as biosensor units for continuous, real-time monitoring of the environment. If group responses are utilised in the technology, intra-individual variations should be recognised as they may alter inter-individual variations, which again could influence the group response. If time-series of the health status (in this case, feeding physiology) of a single individual will be applied as an indication of environmental status, it is important to determine the range of natural variation in the feeding behaviour of an individual (intra-individual variation). Looking at longer time-series of responses from a single individual than those used in the current study will generate more accurate upper/lower limits for acceptable responses.

5.2.2 Inter-individual variability

The results illustrated large variations in the feeding behaviour of individual *C. islandica.* Maximum values for both CR* and IR showed a tendency of occurring at similar particle size for the randomly selected individuals (Section 4.4, Fig 4.15 and Fig 4.16). This is consistent with other results (Strohmeier, et al., 2012). Average values of CR* and IR, on the other hand, fluctuated between the 40 individuals. From a study of *Mytilus edulis* and *Pecten maximus*, Strohmeier et al. (2009) found large variations between individuals

showing a variety of CR responses over time (stable CR versus erratic changes). As previously mentioned, individuals react differently to variations in environmental conditions, causing inter-individual variation. Factors such as size, health, reproductive phase and circadian rhythm may affect an individual and its filter feeding capacity.

PC2 in the principal component analysis illustrated a positive correlation between the size (shell height) of a scallop and its ability to retain particles from the water (Section 4.2, Fig 4.10). Estimates of the siphon area (Section 4.4, Fig 4.17) also showed that scallops may have a different capacity of pumping or filtrating water (flow velocity) based on the size of the siphon area. The degree of valve opening was uncertain when pictures for estimation were taken, which may have led to underestimates of the siphon size resulting in overestimates of the flow velocities. A positive correlation between the pumping rate and the siphon area has been found in other studies (Jørgensen et al., 1988), although there is conflicting evidence for the effect of individual size on the filter-feeding capacity (Kiørboe & Møhlenberg, 1981; Widdows, 1978). In studies similar to the present study, the size of individuals within an experiment are often standardised to avoid size-related variations. Alternatively, height or weight standardised clearance rate are employed (Møhlenberg & Riisgård, 1979). In the current study the objective was to see how different variables could influence the feeding behaviour of *C. islandica*, and it was therefore not necessary to calculate height or weight standardised responses.

As the principal component (PC2) that illustrated the relationship between size and retention efficiency only accounted for 11.4% of the total variation (Section 4.2, Table 4.1) it is likely that there are individual differences in feeding behaviour of the scallops beyond the correlation pattern, meaning that scallops may have high or low rates of retention efficiency despite their size.

Biosensor technologies for real-time continuous monitoring might employ average responses, where large groups are necessary for minimizing the effect of inter-individual variability. Traditional ways of treating values that demonstrate irregularity, are to remove data with extreme values, increase group size and ignore data that are not repeatable from the same organism (Bennett, 1987). Suggestions of how to treat the subject of individual variation have been made by several scientists. Standardisation of

responses to size or weight, as discussed above, is a widely used procedure to constrain the effect of inter-individual variability in feeding rates (Strohmeier, et al., 2012). Statistical methods, such as ANOVA (one-way, two-way or repeated measures) are often used when the issue of intra- or inter-individual variability is recognised (Frechette, et al., 2005; Strohmeier, et al., 2009). If the aim is to specifically investigate individual responses (such as growth rate or energy turnover), individual-based models could also be utilised, such as the Dynamic Energy Budget model (Martin et al., 2012).

In the development of expensive new biosensor technology, the use of individual responses rather than average responses of large groups is efficient for reducing animal sacrifice and maintaining the resolution of the responses instead of masking them by creating averages.

5.2.3 Group responses

The results illustrated a typical statistical pattern of how group responses converge towards the population mean with increasing group size (Section 4.5, Fig 4.19 A). Standard statistical methods are often concerned with the number (n) of individuals (objects) that should be included in the analysis to make good estimates. According to the law of large numbers, the average value of a population (statistical population) will converge towards the real expected value, only with high values of n ($n \to \infty$). The standard deviation is a better indication of inter-individual variability than the average, because it indicates how responses from single individuals are spread away from the average of the group (hence, an indication of whether there are large variations between the individual responses). When looking for statistically significant differences between groups, it is preferable to have small SD. In the results (Section 4.5, Fig 4.19 B), the lowest value for SD was found in the groups with n=5 and n=10 individuals. This value, however, is subjected to randomness, as the highest SD is also found within these group sizes. In the groups with n=20 individuals, the different SD were more stable (from 2.07 to 2.43), and therefore a better indication of the true value. The coefficient of variation (CV) is a good indication of how many individuals should be included to obtain sufficiently good estimates for a group response. The highest CV for groups with n=20 individuals was 4.8% higher than the CV for the population (n=40), while the CV for a group with n=10 individuals was 13.3% higher. If a maximum increase in CV from the

population (n=40) CV is set to 5%, then a group of 15-20 individuals should give a reasonable estimate that can be representative for larger groups. This is consistent with other studies on clearance rate that suggest a minimum number of 18 individuals in a group (Strohmeier, et al., 2012; Strohmeier, et al., 2009). Other biological responses or environmental conditions may present more or less variation between individuals; therefore, optimal group sizes should be investigated in pilot studies where possible.

It is favourable to implement a small number of individuals when developing new biosensors systems for continuous real-time monitoring of the environment. This is mainly due to economical factors, but also because of time and ethical considerations. Implementing 15-20 individuals as units in the biosensor technology is expensive, indicating that it may be more cost effective to utilise single-individual information that are compared back in time for environmental monitoring.

5.2.4. Time-series analysis

In time-series analysis, responses from an organism are compared back in time to see whether present responses are similar to former response patterns. As organisms are general sensors for toxicity (Gruber, et al., 1994), the behaviour pattern of a single individual can reflect the conditions of the surrounding environment. The results from the current study, where 40 individual *C. islandica* were exposed to two highly different environmental conditions, demonstrated how behaviour of the organisms changed from one treatment to another. As discussed in Section 5.1, all of the individuals showed a clear change in IR, while 87.5% showed an adjustment in CR* between the treatments. This is very interesting with regards to developing biosensor technology that utilise time-series backtracking of individual responses. The purpose of the technology is to look for changes in the environment over time, and the results demonstrate how looking at individual responses can assess this.

Even though an individual had a high/low CR* (compared to the average) in the treatment with medium particle concentration, it did not necessary show a similar pattern (high/low CR* compared to the average) in the treatment with low particle concentration (Section 4.5, Fig 4.20 and Section 4.2, Fig 4.11). More measurements on the same individual (larger time-series) should be completed to investigate this further.

Setting up an additional treatment with high particle concentration as first planned (see (see Section 5.3), will generate more information for analysing the advantages of time-series measurements further.

A possible application of the time-series measurements in the biosensor technology could be to install 4-6 molluscs (as *Chlamys islandica* or *Mytulis edulis*) as individual biosensor units that continuously monitored certain responses of the organism (feeding behaviour, growth, oxygen consumption etc.). These continuous and real-time measurements would be analysed by integrating multivariate analysis (such as infometric principal component analysis) in a computer system. Variables explaining the surrounding environment (temperature, salinity, dissolved oxygen, wind/current etc.) would also be implement in the analysis as they may affect individual behaviour. Variation in intra-individual behaviour would be clarified with time, creating upper/lower limits for acceptable responses. The measured responses and external factors would create an n-dimensional room of variables that would be linked together in correlation patterns and continuously updated with time. Drastic changes in environmental conditions would be indicated by altered behavioural responses of >1 individual, generating an alarm. The strength of the alarm would depend on how many of the individuals that reacted to the environmental change.

5.3 Discussion of methods

The experiment was set up for the purpose of looking at individual variability between individual *C. islandica* held in natural conditions, meaning that no algae was added to the water. Previous studies have shown that using artificial diets, or homogenous diets from cultured algae, can lead to overestimations of actual feeding rates (Cranford et al. 2011 and reference therein). The aim was to measure the feeding behaviour in three different water conditions with low, medium and high particle concentration. Due to problems with the particle counter, there was only time to set up and measure the Iceland scallops responses to the first two water conditions. The Coulter Counter did not work properly, and measuring each water samples took additional time. This resulted in a reduced number of sampling rounds, meaning that the feeding behaviour of some of the

individuals was only measured three or four times during each treatment, instead of five as originally planned. Time-series for the individuals, should originally have been 15 (five from each treatment), but was decreased to 7 or 9 (4 in first treatment and 3 in the second, or 5 in the first and 4 in the second) resulting in less data for investigating time-series measurements.

The particle concentration in the water was much less than expected and because of that, the treatment with medium particle concentration could have been representative for a "low particle concentration", and the treatment with low particle concentration could have described an environment with "very low particle concentration". As there were few particles in the water, there was little consistency in the data above 6 µm (particle size), and they were removed from the analysis. Including bigger particles (> 6 µm) would have lead to higher uncertainty in the data material. A natural next step for completion of the study is to set up the experiment when the seston concentration in the incoming water is higher (closer to summer). Additionally, available seston could be up-concentrated to create a very high concentration of particles. More particles in the water will presumably give clearer patterns of ingestion rates and clearance rates with the possibility of comparing individual pumping rate (clearance rate at 100% particle retention). It will also be important to increase the flow rate in the subsequent studies, as the retention efficiency should not exceed 20-25% (Filgueira, et al., 2006). Such a follow-up study is presently in progress.

As a first approach to see whether underlying variables influenced the feeding behaviour of the Iceland scallops, a principal component analysis (PCA) was carried out. The variables that where measured during the experiment were implemented together with calculated values from the results. Many of the variables were then removed from the analysis because they were almost constant (temperature and salinity), or they were calculated from other values in the analysis (a variable that was linked to another created a correlation pattern that was not interesting). As discussed above, the fourth and fifth measurement from each day was not carried out for all of the individuals (objects). The data from these measurements were removed from the PCA so that all of the individuals had the same basis for the analysis. The final set of variables for the analysis was therefore much smaller than originally planned, resulting in the extraction

of less principal components, or principal components that explained less of the variation. Measuring and implementing a greater number of relevant variables in the principal component analysis (such as suspended particulate matter, chlorophyll a, size (area) of exhalant siphon) would likely have revealed even more information from the dataset.

6. Conclusion

The aim of this study was to investigate inter- and intra-individual variability in the feeding behaviour of *Chlamys islandica* by looking at clearance rates and ingestion rates of 40 individuals, and the possible application of time-series analysis in measuring behavioural changes at the individual level.

The change in the environmental conditions from medium to low particle concentration was reflected through increased clearance rates* and decreased ingestion rates in more than 87% of the individuals studied. Intra-individual variation was higher during treatment with medium particle concentration, compared to treatment with low particle concentration. This is most likely a response to food availability, where the individuals must maintain high (and stable) pumping rates to retain as many particles from the water as possible.

The results demonstrated large variations within and between individual *C. islandica* in the two treatments. The following suggestions are made in how to address this subject of matter:

1. Avoid or reduce variation: Equalise intra- and inter-individual variability by taking the average of multiple samples from one individual (to balance intra-individual variability), or the average of many individuals (to balance inter-individual variability). The results from the current study suggest that a minimum of 15-20 individuals could be used without loosing too much accuracy (reflected through increased CV) as compared to a group of 40 individuals.

2. Recognise variation: Be aware that data material may be subjected to intra- and/or inter-individual variations, and apply proper methods in analysis.

3. Utilise as an advantage: Develop systems where responses from single individuals can be introduced in biosensor technology for continuous real-time monitoring.

Utilising time-series measurements from individual organisms such as *Chlamys islandica* or other bivalves may be beneficial in several ways; improved economical viability, ethical considerations and time efficiency. Applying raw data in an analysing system is also advantageous as the resolution of the response is maintained instead of being masked by averages and standard deviations.

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