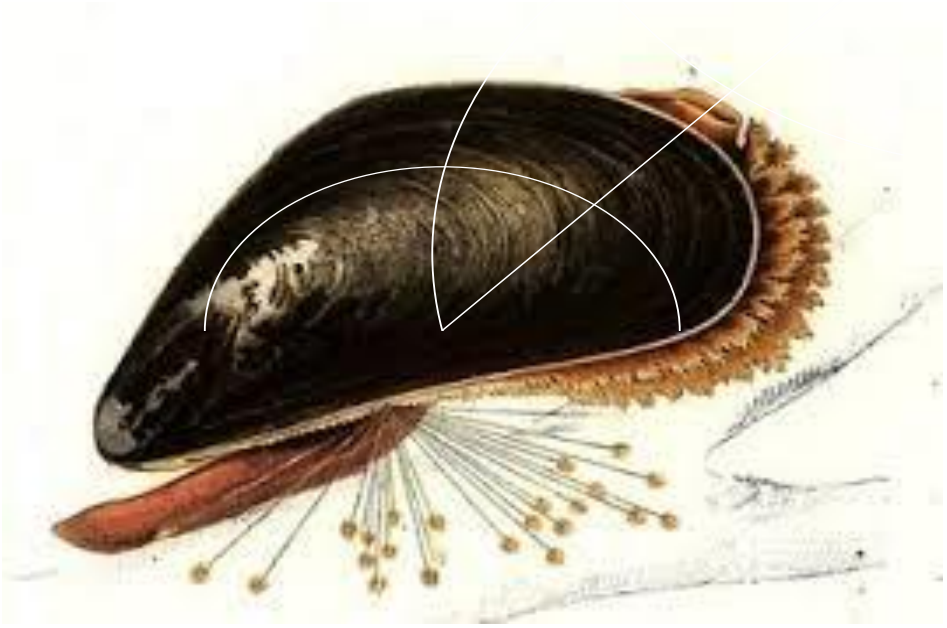
FACULTY OF SCIENCE
UNIVERSITY OF COPENHAGEN

Master's thesis

Flemming Aanæs

Algal stimuli based variability in the clearance rate and pseudo faeces production of the blue mussel (*Mytilus edulis*)



Academic advisor: Bent Vismann

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Abstract

M. edulis was fed with 6 types of algae; different in structure of surface and size, and a description of feeding behavior was developed. It was found, that *M. edulis* is capable of regulating the clearance rate based on such differences in the algae filtrated. The upper threshold, where *M. edulis* is protecting against overloading the gut system, is found to be regulated but lowering the clearance rate in most cases, and in a few cases by pseudo faeces. Production of pseudo faeces is also observed in cases, where the purpose is not to protect against overloading the gut system but when fed with not preferred algae. The upper threshold is also found to be variable depending on the characteristics of the algae filtrating. The lower threshold, were the concentrations of algae is so low, that the *M. edulis* stops filtrating, is found to be depending on how many algae *M. edulis* has in its gut system and of the characteristic of the algae filtrating. It was tested if *M. edulis* has a preference for algae with a high content of Chlorophyll *a* and/or Carbon. This was found not to be the case in these experiments, which covered the first 3 hours of feeding. It was also tested, if *M. edulis* was performing particle selection between 2 algae of different size, which was found not to be the case.

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Introduction

Mytilus edulis Linneaus 1758

The blue mussel *Mytilus edulis* is a bivalve with a distribution given by its requrements to salinity, temperature and depth. *Mytilus edulis* is not found in areas where water temperature exceeds 27 ^{o}C (Wells and Gray, 1960). *Mytilus edulis* can tolerate low temperatures, even beeing frozen down (Gonzales and Yevich, 1976), but the distribution in areas dominated by low temperatures requires sufficient periods with temperatures above 5 ^{o}C allowing the blue mussel to grow and reproduce (Newell, 1989). *Mytilus edulis* can be found from the intertidal zone and down to depths of approximately 30 meters (Kautsky, 1982A). The maximum shell length of *M. edulis* depends on the salinity of the sorrounding water (kautsky, 1982A) where *M. edulis* reaches a maximum shell length of 40 mm at a salinity of 4 - 5 (Remane and Schlieper, 1971) but can be up to 100 mm in the higher salinity of the North Atlantic sea (Køie et al., 2000). *Mytilus edulis* can become more than 15-20 years old depending on environmental parameters such as salinity and temperature and biological

conditions such as predators and food avaiability (Sukhotin et al., 2002). The dry weight of soft parts of a 60 mm long *M. edulis* can due to seasonal conditions vary from 0.3 – 1.1 g (Bayne and Worral, 1980), and the mussel is able to survive a loss in meat biomass of up to 78 % (Kautsky, 1982A). *Mytilus edulis* colonizes nearly all hard substances (kautsky, 1982A) where it settles using the sticky threads (byssus) which is produced by the byssus gland (Køie et al., 2000). After settlement *M. edulis* is able to move by breaking the byssus and then use the foot to move and attach with new byssus (Køie et al., 2000).
The yearly cyclus of *M. edulis* is that in early April (synchronized with the spring bloom and increasing temperature) the gonads of *M. edulis* build up and spawning occours in May to early June, where sperm and egg are released into the sorrounding water (Kautsky 1982B). Usually a second spawning period is observed during summer (Kautsky 1982B). The settlement takes place in June – September (Kautsky 1982B).

Mytilus edulis is a filter feeder where water is pumped in through the inhalant region, past the gill filaments, where particles are retained, and out through the exhalant region of the mantle. In the process of filter feeding, the filtration rate (FR) denotes the volume of water pumped per time unit through the gills. Whereas the clearance rate (CR) denotes the water volume cleared for particles per time unit. The relationship between the two is CR = FR * RE, where RE is retention efficiency (i.e., the efficiency of the gills to retain certain particles). However, it is 'generally accepted' (e.g., Riisgård et al., 2003; Strohmeier et al., 2008) that for *M. edulis* the RE is 100 % for particles larger that 4 µm in diameter and in this case FR and CR becomes synonymous. The force which drives the water flow past the gill filaments is provided by the strokes of the lateral cilia located at the entrance to the interfilamental canals (Clemmesen and Jørgensen, 1987). It has been suggested that both the effective and the recovery stroke contributes to the pressure generated and driving the water flow (Clemmesen and Jørgensen, 1987). The strokes of the lateral cilia is controlled by the central nervous system and it has been shown that the beat frequence of the lateral cilia is stimulated by 5-hydroxytryptamine (HT) (or serotonin) which acts as a nerve transmitter (Aiello et. al, 1988; Clemmesen and Jørgensen, 1987), as well as dopamine (Aiello et. al, 1988; Stefano and Catapane, 2005). The same nerve transmitters are involved in stimulating the cells, which produces the mucus needed to produce pseudo faeces (Aiello et. al, 1988; Clemmesen and Jørgensen, 1987, Stefano and Catapane, 2005). These cells are located among the frontal ciliated cells and in the area between the frontal cilia and the latero-frontal cells. (Aiello et. al, 1988).

Jørgensen (1981) has shown that secretion of muscus is positive correlated with the concentration of algae and that the most efficient feeding takes place without secretion of mucus. When a small amount of mucus was present, particles arriving at the ventral edge of the gill were carried to the tips of the filaments and into the food groove, and futher on into the mouth (Jørgensen, 1981). When a heavy layer of mucus was present, particles remained outside the food groove and were carried along the tips of the filaments to the palps and from there into the rejection tract (Jørgensen, 1981). Finally, the particles are released into the water as pseudo faeces (Jørgensen, 1981).
The pass way from the inhalant region to the mouth is well described and understood, but several open questions and theories exist concerning if or how *M. edulis* is able to control this particle transport system.

Mytilus edulis as a filter feeder and with its high abundance in many coastal areas makes it an important factor in the marine eco-system by transporting organic material from the pelagic system and into the benthic system. *Mytilus edulis* is one of the marine species, which have managed to adapt to the low salinity in the Baltic Sea (Kautsky, 1982B) making it an important faunal factor, since it can make up to more than 80 % of the total animal biomass (Jansson and Kautsky, 1977). In 2014, Denmark was catching and producing 41.363.201 ton of *M. edulis* (Naturerhvervsstyrelsen, 2015), which shows, that *M. edulis* also plays an important role in human consumption and the economy within the fishing industry. In addition, *M. edulis* plays an important environmental role in connection with fish farming, where it is used to avoid algae blooms as a result of the waste products from the many fish hold and feed at a very narrow space. It is therefore natural that *M. edulis* in general has the attention by many marine biologists, and in particular for them to be able to predict the influence of *M. edulis* by environmental changes and to give input to politicians on restrictions concerning *M. edulis* in determining the balance between the economic interest in fishing bivalves and the interest in preserving a high population in order to protect against algae blooms. However, there are several conceptions of the functionality of the filtration capabilities of *M. edulis* according to literature which makes a scientific consensus difficult to achieve.

Some authors find that the CR has a constant relationship with the weight or the shell length of the *M. edulis* (Winter, 1973; Kiørboe and Møhlenberg, 1981; Famme et al., 1986; Strohmeier et. al., 2009) without discussing if other important factors than scaling can make a significant difference on

the CR. Such a constant relationship description implies that the CR of *M. edulis* is regulated by an on/off pump which cannot be adjusted according to external or internal stimuli.

Others (Petersen et. al 2004; Cranford, 1999; Widdows 2001) show that the CR varies depending on many factors such as water temperature, salinity and concentration of suspended material

In a study, of the effect of the concentration of *Rhodomonas baltica* (4 – 5 µm) on the CR of *M. edulis*, Riisgård (1991) defined the CR in relation to 3 different concentration ranges of algae:

High: At algal concentrations > 15,000 cells ml^{-1} *M. edulis* will after a short period of active clearing the water reduce the clearance rate, because of satiation of the digestive system (Riisgård 1991).
Medium: At algal concentrations within an interval of 2000 – 6000 ml^{-1} *M. edulis* will display a high and constant clearance rate. (Riisgård, 1991)
Low: Algal concentrations where *M. edulis* will reach a lower threshold where it stops clearing the water because the energetically expense to pump water exceeds the energy gained from digestion of the captured algae (Riisgård et. al, 2003).

However, it has not been tested (nor discussed) if such a regulation is dependent on algal species (Riisgård, 1991, Riisgård and Randløv, 1981; Riisgård et. al, 2003). The change in filtration rate is explained as a sencondary result of a reduced pump potential caused by the reduction in valve gape, which shortens the gill axes, and results in the interface between opposite bands of lateral cilia becomes negative with decreasing width of the interfilament canals which reduces the pumppotential (Riisgård, 1991). The discussion is therefore whether the filtration rate is physiological regulated (Bayne et al., 1993; Cranford and Hill, 1999) or it is a basic autonomous process (Jørgensen et al., 1986).

A third conception concerns, if *M. edulis* is able to perform particle selection. The above cited studies together with e.g. Foster-Smith (1975) do implicit or explicit not believe so. However, other studies have shown particle selections to take place (Ward & Targett, 1989, Bourgrier et al., 1997). In addition, *M. edulis* has been observed to preferentially capture chlorophyll containing particles relative to particles not containing chlorophyll (Newell et al., 1989). The discrepancy is very well

Feltkode ændret

expressed by Beringer et al. (1995) who state 'Reports of selection in mussels are inconsistent'. If particle selection takes place, then the palps are the only possible site for such an activity (Beringer et al., 1995). Recently, it has been shown that *M. edulis* offered microspheres with different surface chemistry rejects microspheres with surface containing e.g. aluminum oxide, but ingests microspheres with polystyrene (Rosa et al., 2013). However, Rosa et al. (2013) could only conclude that 'non-specific physicochemical interactions can play a role in mediating selection' (Rosa et al., 2013). In all, no agreement in literature has been reached concerning the mechanism by which *M. edulis* can select between different suspended particles. If the retention efficiency is less than 100 % it can be impossible to distinguish particle selection from retention efficiency unless you quantify pseudo faeces. Until recently it has generally been assumed that the retention efficiency is 100 % for particles larger than 4 μm (Møhlenberg and Riisgard, 1978), but this assumption has been questioned by Stromeier et al. (2012) who found the smallest particles to be 100 % retained were close to 7 μm.

Another discussion concerns the role of pseudo faeces. Some authors advocate for pseudo faeces to be the only regulation mechanism against overload of the digesting system and that production of pseudo faeces starts when a threshold in particle concentration is exceeded (Kiørboe et al., 1980; Widdows et al., 1979). However, others authors propose pseudo faeces production to be a result of particle selection because the chemicals contained in the mucus produced by *M. edulis* reacts with the coating of the surface of the filtered particles, and the not ingested particles is rejected with the mucus as pseudo faeces (Espinosa et al., 2010).

The above conceptions have served as the main motivation behind the present Master thesis. The objectives of this Master thesis were to contribute to the clarification of 1) parameters important for the regulation of the clearance rate, 2) the mechanism behind protection against overload of the digestive system and 3) the algal characteristics which are important for determining the lower threshold for clearance. The overall aim was to add information to our present knowledge on the feeding behavior of *M. edulis*. The objectives were studied in a series of experiments designed to test the following hypotheses:

Regulation of Clearance rate

It was tested if *M. edulis* shows different CR when fed different algae species with same size.

It was tested if *M. edulis* shows different CR when fed algae species of different size.

In both cases the hypothesis was that *M. edulis* is capable of regulating the CR based on stimuli produced by the algae and therefore the CR will be different when fed with algae with different surface structure or different in size.

It was tested if RE is the same with algae of different size and/or if there is evidence of particle selection between algae of different size.

Based on results from literature, the hypothesis was that the RE is similar for algae species > 4µm in diameter.

Protection against algal overload of the digestive system

It was tested to what extent *M. edulis* is protecting against overload of the digestive systems by regulation of the CR or by producing pseudo faeces.

The hypothesis was that *M. edulis* primarily adjust the CR in favor of producing pseudo faeces in order to protect against overloading the gut system. This hypothesis is interconnected to the above hypotheses that *M. edulis* is capable of regulating the CR and thereby saves energy compared to maintaining a high CR and rejecting the surplus food by producing mucus.

Lower threshold for clearance rate of *Mytilus edulis*

It was tested at which algal concentration *M. edulis* stops filtrating (lower threshold). It was also tested if this threshold is determined only by the break-even between energy gained from captured algae and the energy expense for pumping water - or the feeding history and the amount of food in the gut system (short time feeding history) is a more causal factor.

The hypothesis was that the short time feeding history is a significant factor in determining the lower threshold. The reason behind selecting this hypothesis was the idea that *M. edulis* is capable of feeling hunger and the more hungry it gets the more effort it will spend on achieving additional food.

Material and methods

Mytilus edulis

The *Mytilus edulis* used in experiments were collected by SCUBA divers the 13th of March 2015 in Øresund off the harbor Nordhavn (55°43'14.67"N; 12°36'57.27"Ø). After collection, the mussels were transported to the Marine Biological Section, Helsingør. Prior and between experiments the mussels were kept in aquariums (10 l) with running and aerated seawater (temp10 ± 0.1 °C, salinity 30 ± 2 and pH 7.9 ± 0.1). From the specimens collected, 6 were used in all experiments. The selected *M. edulis* (shell length 6.0 ± 0.3 cm and a dry weight of soft parts of 1.979 ± 0.230 g) were cleaned and all *Balanus spp*. were removed from the shells. A piece of plastic labeled with a unique number was glued onto the umbo of all experimental mussels using waterproof glue. The decision to use 6 individual *M. edulis* specimens in all experiments was made since this optimized the probability of changes observed in behavior and clearance rate were due to differences in the algal species offered and not due to biological variation between *M. edulis* specimens. To avoid results being biased due to individual specimen participation in earlier experiments, a 48 hours long period separated each experiment. To ensure an empty gut system at the start of each experiment the *M. edulis* were not fed between the experiments. In addition, bias was minimized by randomizing the order by which the different experiments were done.

The algae

The algae used in the present study were selected on basis of their individual characteristic by which they can be grouped into two groups: 1) algal species of similar bio volume but with different surface characteristics and 2) algal species with ten – twenty times larger bio volume than group 1. The first group was used to study the effect of different surface characteristics on the blue mussel clearance rate. The second group was together with the first group used to study the effect of algal size on the blue mussel clearance rate. None of the algal species selected are forming colonies, aggregates or toxins.

Group 1:

This group consisted of 4 algae which are approximately equal in size but have different surface characteristic. (All 4 algae have a medium Equivalent Spherical Diameter (ESD) range of (6.7 ± 0.4µm).

Rhodomonas salina (Wislouch) (Cryptophyta, Cryptophyceae) (cell volume: 151.8 ± 52.72 μm^3) has a single boat-shaped, red-colored plastid with a pyrenoid. *Rhodomonas salina* has several to many large ejectisomes which lines the vertical oriented furrow. An ejectisome is a cytoplasmic structure that can be violently discharged. Each ejectisome consist of two unequal coiled ribbons. When discharged both ribbons unfurl, forming a narrow, kinked barb (Graham et al. 2009). *Rhodomonas salina* (K-1487) was obtained (February 2015) from the Marine Biological Section, Helsingør.

Chroomonas vectensis (N. Carter) (Chryptophyta, Chryptophycaea), (cell volume: 159.4 ± 86.76 μm^3) has two flagella of unequal length covered with hairs. *Chroomonas vectensis* was selected due to the physical characteristic that the plasma membrane is sandwiched between protein layers (periplast). This should give a different surface structure than the other algae in the group and at the same time also a different digestion speed by the *M. edulis*. The periplast plates are rectangular and there are two large ejectisomes in the vicinity of the shallow vestibular depression. It has a single blue-green H-shaped plastid with a pyrenoid on the bridge. Located at the vestibulum are rows of ejectisomes. It has an eyespot composed of lipid droplets which makes it possible to orientate according to light sources (Graham et al. 2009). *Chroomonas vectensis* (K-0432) was obtained (February 2015) from the Scandinavian Culture Collection for Algae and Protozoa (SCCAP), Copenhagen.

Tetraselmis levis (Butcher) (Chlorophyta, Chlorodendraceae) (cell volume: 198.0 ± 17.17 μm^3) has four flagella emerging from the pit in two pairs. *Tetraselmis levis* was selected due to the physical characteristic that the cell is covered with a wall (theca) composed of scale like particles in a crystalline array (Graham et al. 2009). This should also give a new different surface structure and a different impact on the digestion system of *M. edulis*. *Tetraselmis levis* (K-0937) was obtained (February 2015) from SCCAP, Copenhagen.

Dunaliella tertiolecta (Butcher) (Chlorophyta, Clorophyceae) (cell volume: 139.5 ± 94.17 μm^3) is an unicellular, naked green algae with two flagella, (Francavilla et al, 2010). *Dunaliella tertiolecta* has a high content of β-carotene and glycerol. (Francavilla et al. 2010). The surface structure and

chemical composition is therefore very different from *R. salina*, *Tetraselmis levis* and *Croomonas vectensis*. *Dunaliella tertiolecta* (K-0591) was obtained (February 2015) from Scandinavian Culture Collection for Algae and Protozoa (SCCAP), Copenhagen.

Group 2:

This group consisted of two algae which are significant larger (10 – 20 times) than the four similar sized algae in group 1 and they were selected to test if size of algae is a regulating factor for the CR of *M. edulis*.

Tetraselmis contracta (N. Carter) **(**Chlorophyta, Chlorodendraceae**)** (cell volume: 4204 ± 1300 μm^3). *Tetraselmis contracta* was selected because its volume is app. 20 times larger than that of group 1 (see table x). *Tetraselmis contracta* belongs to the same genus as *T. levis*, and comparison between these two algal species excluded as many characteristics as possible other than size. *Tetraselmis contracta* (K-0011) was obtained (May 2015) from SCCAP, Copenhagen.

Heterocapsa triquetra (Ehrenberg) (Dinophycaea, heterocapsaceae) (cell volume: 2440 ± 1037μm^3) was selected because it is covered with three-dimensional scales *(Spector, 1984)* and it has a size in the middle between *T. contracta* and the group 1 algae. *Heterocapsa triquetra* (K-0447) was obtained (May 2015) from SCCAP, Copenhagen.

All algal species were cultured in artificial F/2 medium (Guillard 1975). The medium was prepared by taking 10 liter of autoclaved (121 °C, 40 minutes) seawater (salinity = 31, pH = 7.9), and after cooling adding 5 ml of each of the following 3 mixtures:

1. Salt mixture: the following salts are added to 500 ml of distilled water:

75 g $NaNO_3$
5 g $NaH_2PO_4 \cdot H_2O$

2. Metal mixture: the following metals are added to 500 ml of distilled water

4.36 g Na_2 •EDTA
3.15 g $FeCl_3 \cdot 6\ H_2O$
0.01 g $CuSO_4 \cdot 5\ H_2O$
0.022 g $ZnSO_4 \cdot 7\ H_2O$
0.01 g $CoCl_2 \cdot 6\ H_2O$

0.18 g $MnCl_2 \cdot 4\ H_2O$
0.006 g $Na_2MoO_4 \cdot 4\ H_2O$

3. Vitamins: the following vitamins are added to 500 ml of distilled water:

0.1 g Thiamin • HCl
0.0005 g Biotin
0.0005 g B_{12}

All cultures received an irradiance of 90 µmol photons m^{-2} s^{-1} using cool white fluorescent light. They were kept in a climate room with a temperature of 10 °C and under constant filtered aeration.

Figure 1. The experimental set-up for measuring clearance rate.

Experimental set-up and experiments concerning measuring CR.

The experimental set-up consisted of six aquariums each filled with 3 liter of filtered seawater (10 °C ± 0, salinity 31 ± 2 and pH 7.9 ± 1). The set-up was placed in a climate room (10 °C). Adequate water mixing was ensured simultaneously in all six aquariums by a propelling system (Fig. 1). In a pilot experiment the mixing capacity of the propelling system was tested. Water samples were taken at 5 different locations in the aquariums at 30 minutes intervals. The algal concentration of the samples was measured in triplicates using an electronic particle counter (Beckman Coulter Counter, Multisizer 3). The pilot study showed no difference in algal concentration measured at the 5 locations in the aquariums. Hence, all experimental water samples were decided to be taken in the center of each aquarium.

In the experiments the mussels were exposed to the different algal species at one of the three different concentration ranges (*Sensu* Riisgård et al. 2003): Low: < 1.6 $x10^3$ cells ml^{-1}; medium: 2 x 10^3 – 6 x 10^3 cells ml^{-1} and High: > 15 $x10^3$ cells ml^{-1}. Throughout the rest of the thesis these ranges of algal concentrations will be referred to as low, medium and high.

Prior to each experiment the desired algal concentration of the appropriate algal species was established in each aquarium. The algal concentrations were controlled by measurements in the Coulter Counter. Hereafter one *M. edulis* was randomly placed in each aquarium away from the propeller and in an orientation that allowed possible pseudo faeces production to be visually observed. In each experiment, the decrease in algal concentration over time was measured by taking water samples (10 ml) from each aquarium every 30 min for a total period of maximum 5 hours or until one of the following conditions occurred 1) faces in the water 2) pseudo faeces in the water 3) the algae concentration decreased under the level which was tested or 4) The algal concentration had been constant for a period of 1 hour (testing lower threshold). At the start and end of experiments it was verified that the water was fully saturated with oxygen using an oxygenmeter (WTW pH/Oxi 340i).

The different types of experiments were made using algae from the two groups at the different algal concentration ranges (i.e, low, medium and high) and were terminated as describe below.

Regulation of clearance rate.

CR of *M. edulis* fed algae of same size (group 1)

The objective of these experiments was to describe the CR at medium algal concentrations of the similar sized algal species, but with difference surface characteristics. This was done in order to test, if different algal surface stimuli had an impact on the CR of *M. edulis*. The experiments were terminated, when the concentration of algae no longer were within the medium concentration range.

CR of *M. edulis* fed different sized algae.

The objective of these experiments was to describe the CR and bio volume retained at the medium and high algal concentration ranges of an algal species in the low end of size (*R. salina*) and algal species of significantly larger size (*T. contracta* and *H triquetra*) . This was done in order to test if different sized algae would have an impact on the CR and of bio volume retained of *M. edulis*. The experiments were terminated, when the concentration of algae no longer were within the defined concentration ranges.

CR of *M. edulis* fed with a mixture of algae of different size.

The objective of these experiments was to test if the algae, within the sizes used in this study, were retained by *M. edulis* with the same efficiency (RE). The concentration of two algal species of different size in mixture was measured as a function of time, when *M. edulis* was feeding on the mixture. If the ratio between the concentrations of the two algal species was constant with time, then *M. edulis* is not showing any difference in retention efficiencies of the two algal species. The presence or absence of differences in retention efficiency was studied using two different approaches. In both approaches different sized algae were used in order to make it possible to measure the concentration of the two algal species simultaneously in the Coulter Counter, which besides counting the particles also differentiate them according to their size.

Approach 1: Each aquarium was added a mixture of *Rodomonas salina* and *Heterocapsa triquetra* to a final concentration of app. 6000 cells ml^{-1} and 4000 cells ml^{-1}, respectively. One *M. edulis* was transferred to each aquarium. The concentration of *R. salina* and *H. triquetra* was measured every 30 minutes for a period of three hours using the Coulter Counter as described above.

Approach 2: In one aquarium was added a mixture of *Rhodomonas salina* and *Tetraselmis contracta* to a final concentration of appx. 50,000 and 4000 cells ml^{-1}, respectively. Then *M. edulis* (n=6) were transferred into the aquarium. The concentration of the two algal species was measured as a function of time as described for approach 1.

Protection against algal overload of the digestive system

The objective was to test the hypothesis that *M. edulis* prefers to protect the digestive system against overload by regulation of the CR in favor of rejecting the surplus of food as pseudo faeces.

This was not tested with a specific designed series of experiments. Instead, the presence or absence of pseudo faeces was visually observed every half hour when water samples were taken for algal concentration measurements in all experiments on regulation of CR. In addition, pseudo faeces could be seen in the Coulter Counter measurements as a temporal increase in algal concentration. This was a result of algae contained in the pseudo faeces being separated from the mucus by the propeller and released back into the water giving the temporal increase in the algae concentration (Fig. 2).

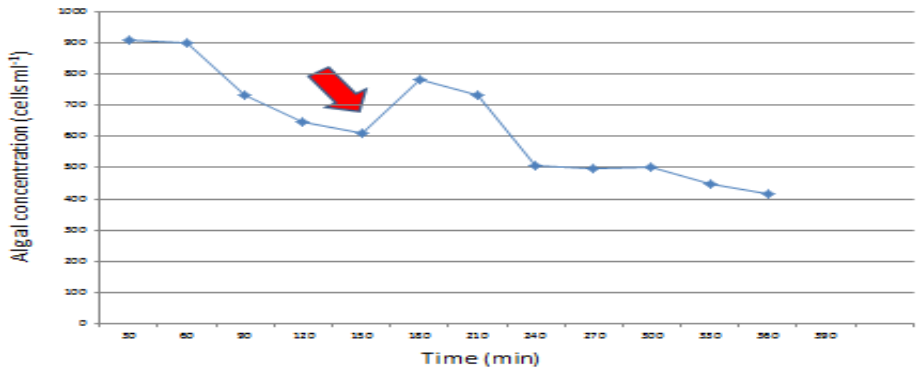

Figure 2. A typical example where *M. Edulis* is regulating using pseudo faeces with a low concentration of the algae *Chroomonas vectensis* (< 1500 number of algae per ml) and are releasing

the content of the pseudo faeces into the water again in the interval 150-180 minutes (red arrow) after the start of the experiment.

Lower threshold for clearance of *Mytilus edulis*.

The objective of this experiment was to test the hypothesis that *M. edulis* stops filtrating when the energy used to filtrate (i.e., pump water past the gills) exceeds the energy obtained from digestion of the captured algae. The *M. edulis* were fed with group 1 algae at start concentrations between 500 and 4000 cells ml^{-1}. The experiments were stopped when the algal concentration had not decreased for an hour.

Additional measurements

Chlorophyll *a.*

The chlorophyll *a* concentration of the different experimental algal species was measured according to Strickland and Parson (1972). The method is a spectrophotometric analysis in which the absorption maximum of Chlorophyll *a* is measured at 665 nm. At this wavelength 1 g of Chlorophyll *a* per liter will give absorption of 83.4 at a light distance of 1 cm. This value is the absorption coefficient of Chlorophyll *a*.

From each algal culture a 300 ml sample was taken and the algal concentrations were measured using the Coulter Counter. After the algal concentrations were measured the algae were filtrated from the samples onto Whatman glass microfiber filter using a vacuum pump (500 mm Hg). In order to reduce the filtration time two filter units were used. The glass microfiber filters with the filtrated algae were put into test tubes filled with 15 ml methanol. The test tubes were closed with a lid and wrapped in aluminum foil to protect against daylight. The test tubes were left for chlorophyll extraction at 5^{o} C for 48 hours. During the extraction period the test tubes were shaken 5 times.

After extraction, the methanol from the test tubes were transferred to new test tubes and methanol were added to a total volume of 15 ml. The test tubes were centrifuged for 5 minutes at 4500 g (5000 rpm) in a Sigma 3 centrifuge. The supernatants were carefully transferred to new test tubes. From the test tubes, 3 ml were transferred into cuvettes and the absorption was measured spectrophotometrically at wavelengths 665 nm and 750 nm. Finally, the degree to which

Chlorophyll *a* was degraded to phaeophytin was measured. This was done by adding 1 drop of HCl (1 N) to the samples followed by a new measurement of the absorbance at wavelengths 665 nm and 750 nm. If the ratio of absorbance before and after acidification was 1.7 (which was the case for all the algal species) then no degradation to phaeophytin had occurred.
According to Strickland and Parson (1972) the chlorophyll *a* concentration (Chl *a*, g $cell^{-1}$) can be calculated by the equation:

$$\text{Chl } a = (Ab_{665} - Ab_{750}) * df / 83.4 * algae$$

where Ab_{665} = the absorbance wavelength 665 nm, Ab_{750} = the absorbance at wavelength 750 nm df = the dilution factor, 83.4 = the absorbance coefficient of Chlorophyll *a*, and algae = the number of algae from which chlorophyll *a* had been extracted.

Algal dry weight, ash free dry weight and carbon content

From each algal culture a 300 ml sample was taken and the algal concentration was measured using the Coulter Counter. The algal samples were filtrated onto Whatman glass microfiber filters as described above. All filters had been heated in an oven at 540 °C for 24 hours before use. The filters with the algae were dried in an oven at 60^{o} C for 24 hours and the dry weight was measured using an electronic scale. Then the filters were returned to the oven and heated in an additional 24 hours at 540^{o} C. After cooling the filters the ash free dry weight was measured using the electronic scale. The temperature 540 °C was chosen because it has been shown that using temperatures higher than 550 °C will cause loss of sodium and potassium (Grove et al., 1961).
The dry weight of the different algal species (W_{dw}, pg $cell^{-1}$) was calculated according to the following equation:

$$W_{dw} = (W_{fa} - W_f)/Alg$$

where W_{fa} = Weight of the filter with the dried algae on (mg), W_f = weight of the filter(mg), and Alg = number of algae in the filtration.

The ash free dry weight of the different algal species (W_{af}, pg cell^{-1}) was calculated according to the equation below:

$$W_{af} = (W_{fa} - W_f)/Alg$$

Where W_{aa} = weight of the filter with the algae ashes on (mg), W_f = weight of the filter (mg), Alg = number of algae in the filtration

Finally, the carbon content (C, pg cell^{-1}) of the different algal species was calculated:

$$C = W_{dw} - W_{af}$$

Calculation of clearance rate.

If a mussel in a closed system clears algae form the water at a constant rate, the decrease in algal concentration with time will be exponentially. The clearance rate (CR, l min^{-1}) was calculated according to Coughlan (1969):

$$CR = V/(n*t) * \ln(algae_{t=n}/algae_{t=n+1})$$

where V = volume (3 l), n = number of *M. edulis* (in the present study n = 1), t = time (min), $algae_{t=n}$ and $algae_{t=n+1}$ = algal concentration (cells ml^{-1}) at time = n and time = n+1, respectively.

Statistics

The statistical tool used for performing students 2-tailed t-test and calculating mean and SD is free software on the internet: http://www.physics.csbsju.edu/stats/t-test.html

Feltkode ændret

For performing the Kruskal-wallis one way analysis of variance when the data is not normal distributed: http://www.mathcracker.com/kruskal-wallis.php.

Feltkode ændret

Whether the slope of the regression line was different from 0 was tested with a two-sided t-test

Results

Regulation of Clearance Rate

CR of *M. edulis* fed algae of same size

The clearance rate of *M. edulis* was at medium concentrations shown to be constant with time for each of the similar sized algae; *T. levis* and *R. salina* (Fig. 3) (slope = -0.05 ± 0.03, n= 4, t stat = -1.5, p=0.28) and (slope= -0.05± 0.1, n=4, t stat -0.46, p= 0.69) respectively. CR also found to be constant when fed with *D. tertiolecta* and *C. vectensis* (not shown) The average clearance rate of *M. edulis* fed the four similar sized algal species with different surface characteristics (i.e., group 1) at medium concentrations for 2.5 hours (Fig. 4) was 75 ± 5, 49 ± 8.5, 28 ± 6 and 24 ± 6 ml min^{-1} for *R. salina*, *D. tertiolecta*, *C. vectensis* and *T. levis*, respectively. Except for the clearance rate found for *C. vectensis* and *T. levis* all clearance rates of the algal species were significantly different (Table 1).

The different contents of carbon were found for the group 1 Algae and the rank was:

1). *T.levis* (104 pg $cell^{-1}$) 2) *C.vectensis* (95 pg $cell^{-1}$) 3) *D. tertiolecta* (76 pg $cell^{-1}$) and 4).. *R. salina* (44 pg $cell^{-1}$) (fig. 5). The content of Chlorophyll *a* was also found with the same ranking as result (82.6; 39.5; 36.3 and 10.2 pg $cell^{-1}$ respectively). The Carbon/ Chlorophyll *a* relation was calculated (fig. 6).The different CRs found for the above similar sized algal species were compared for correlations to the carbon and/or chlorophyll *a* content of the different algal species (fig. 5 and 6). The ranking of preference for the same four algae as expressed in CR (fig. 4) was exactly the reverse as found with the content of Carbon and Chlorophyll a (A formal ranked correlations test was not performed since such a test requires minimum 5 pairs and there were only 4 available)

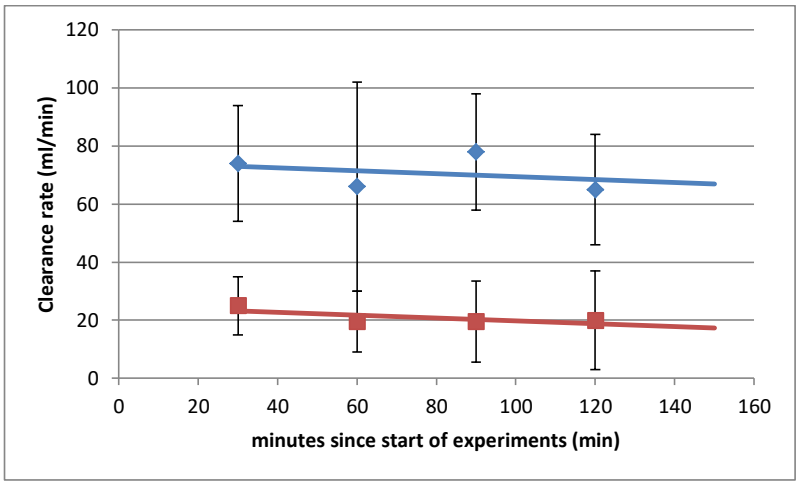

Figure 3. The mean clearance rate of *Mytilus edulis* (n=6) fed medium concentrations (2000 - 6000 cells ml^{-1}) of *Tetraselmis levis* (red bars) and *Rhodomonas salina* (blue bars) (n = 24) during a period of 2 hours. The results are presented with standard deviation bars. Regression lines inserted

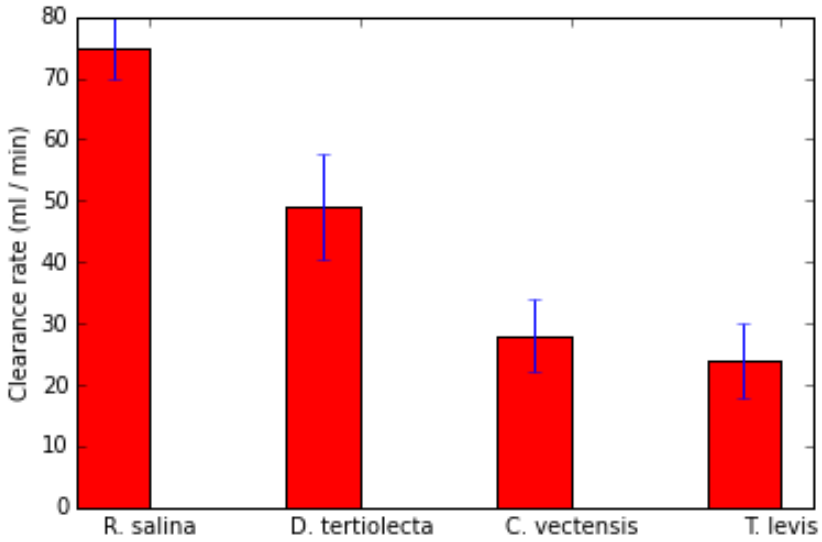

Figure 4. The clerance rate of *M. edulis* (n=6) during 2.5 hours fed four different algal species. (n-total = 44). Startconcentrations between 2000 cells ml^{-1} and 6000 cells ml^{-1}(medium). Bars represent the mean of a 2.5 hour period and are presented with standard deviation bars. P-values shown in table 4.

		p value
R. salina	*D. tertiolecta*	< 0,001
R. salina	*C. vectensis*	< 0,001
R. salina	*T. levis*	< 0,001
D. tertiolecta	*C. vectensis*	0,002
D. tertiolecta	*T. levis*	< 0,001
C. vectensis	*T. levis*	0,233

Table 1. Two-sided t-test of clearance of *M. Edulis* (n=6) fed with 4 different algae within the medium concentration range.

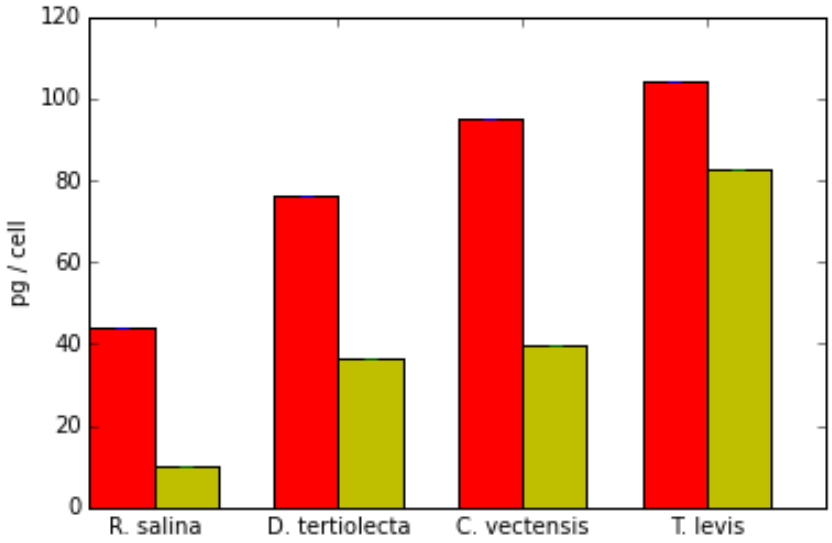

Figure 5 . Carbon content (red) and Chl *a* (green) of the 4 algal species of group 1.

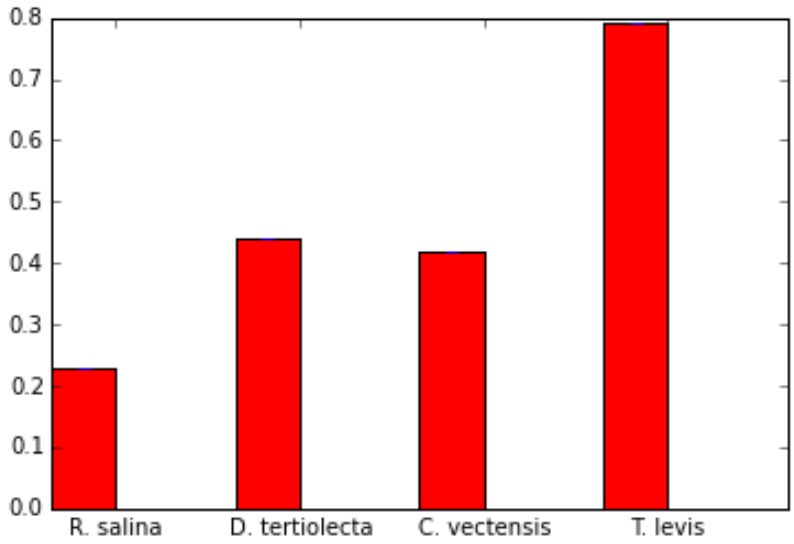

Figure 6 . The Chl *a*: C relation of the 4 algal species of group 1.

CR of *M. edulis* fed different sized algae.

It was found that the clearance rate of *M. edulis* for the different sized algae *H. triquetra* (2440 ± 1037 μm^3) and *R. salina* (151.8 ± 52.72 μm^3) at appx. the same start cell concentration measured in cell density (*H. triquetra*: 2011 ± 950 cells ml^{-1}; *R. salina*: 1901 ± 274 cells ml^{-1}) was 24.5 ± 4.9 and 40 ± 4.5 ml min^{-1} (n = 6), respectively (Fig. 7). The two CRs were not significantly different (p = 0.05), but the biovolume retained ($7.75 * 10^5$ μm^3 ± 4.25 and $1.1 * 10^5$ μm^3 ± 0.6) where significant different (p<0.001) (Fig 8).

The clearance rate of *M. edulis* fed the same two different sized algae algae *H. triquetra* and *R. salina* but at appx. the same start concentration measured in bio volume (*H. triquetra*: $3.6 * 10^6$ ± $0.9 * 10^6$ μm^3 ml^{-1} and *R. salina*: $3.0 * 10^6$ ± $1.0 * 10^6$ μm^3 ml^{-1}) was 14.03 ± 0.74 and 24.37 ± 2.78 ml min^{-1} (n = 6), respectively (Fig. 9). The two CRs were significantly different (p = 0.01), but the bio volume filtered ($9.4 * 10^5$ ±4.25 and $14.1 * 10^5$ ±6.6 μm^3) were not significant difference (P = 0.05). (Fig. 10).

The CR of *M. Edulis* (n=6) fed with the two different sized algae of the same genus, *Tetraselmis levis* (198 ± 17.17 μm^3) and *Tetraselmis contracta* 4204 ± 1300 μm^3) at appx. the same

startconcentrations measured in bio volume (8.4 *10^5 ± 1.7 * 10^5 and 7.3*10^5 ± 1.6*10^5 µm^3) were 37.7 ± 28.1 and 21.5 ± 7.3 ml min^{-1}, respectively (Fig 11A). The total volume of *T. levis* and *T. contracta* eaten was 486*10^3 ± 225*10^3 and 1554*10^3 ± 600*10^3 µm3, respectively (Fig 11B). Both the average CR (p = 0.023) and the total volume of algae eaten (p =0.002) were significantly different.

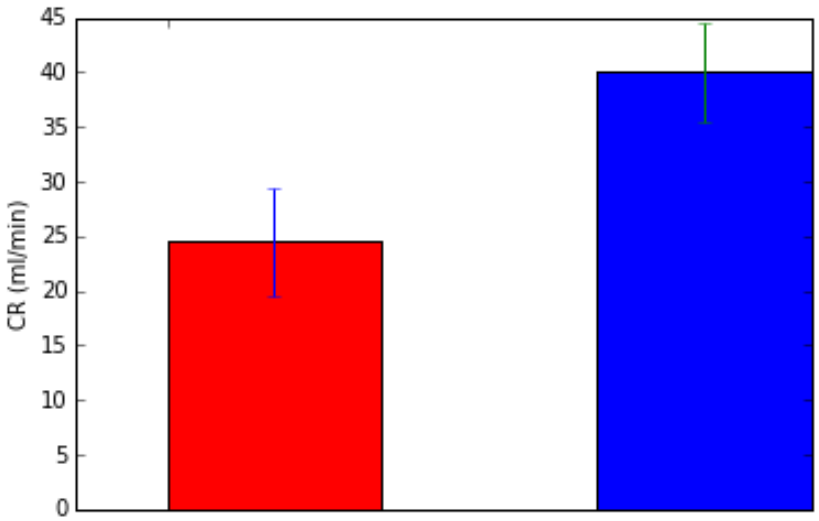

Figure 7 . The Average clearance rate of *M. edulis* (n=6) during first 2 hours of experiments when fed with *H. Triquetra* (red) or *R. Salina* (blue) at a similar start concentration of app 2000 cells ml^{-1}. Bars are presented with standard deviations.

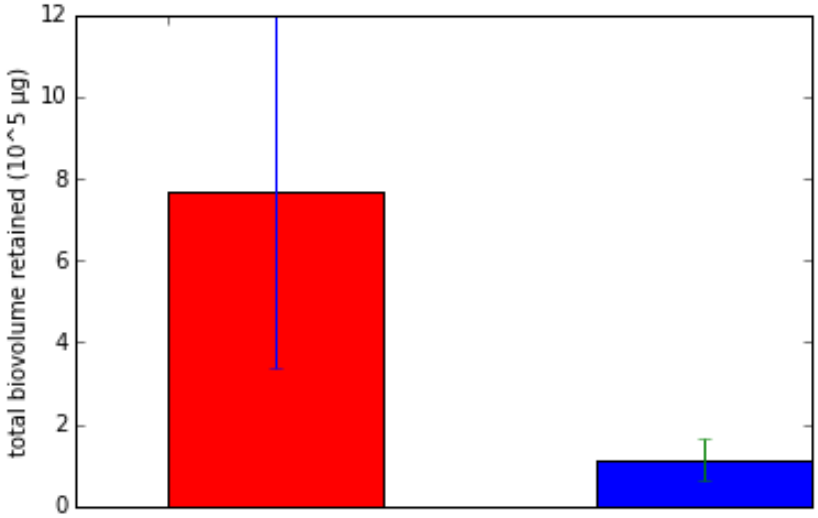

Figure 8 .The total volume of algae retained during first 2 hours by *M. Edulis* (n=6) fed with *H. Triquetra* (red) or *R. Salina* (blue) at a start concentration of app 2000 cells ml^{-1}. Bars are presented with standard deviations.

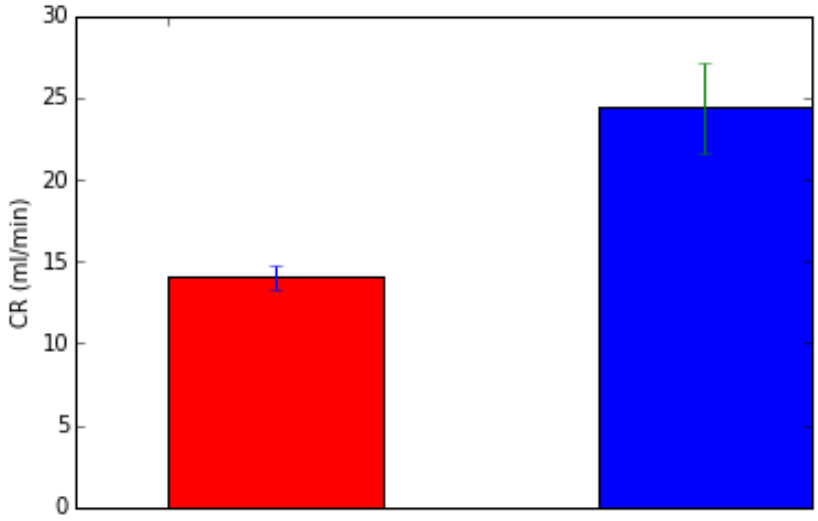

Figure 9 . The Average clearance rate of *M. Edulis* (n=6) during first 2 hours of experiments when fed with *H. Triquetra* (red) or *R. Salina* (blue) at a start algal volume of app 3.3 * 10^6 μm^3 ml^{-1}. Bars are presented with standard deviations. ,

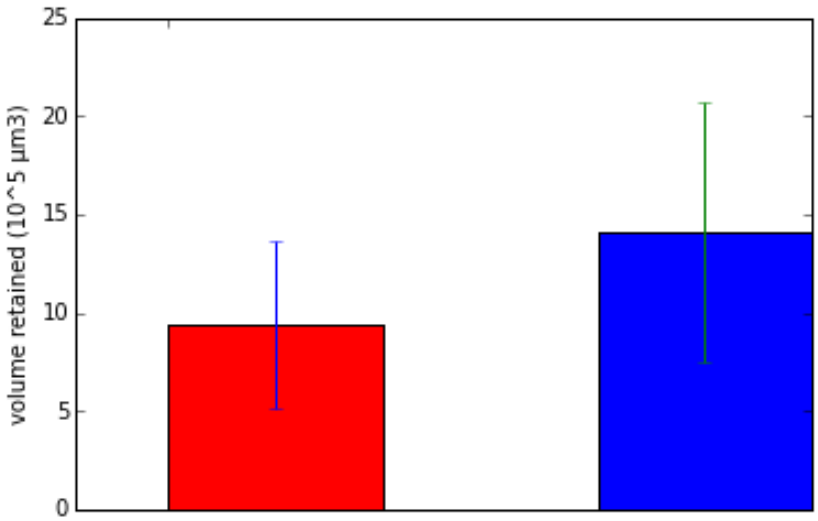

Figure 10 . The total volume of algae retained by *M. edulis* (n = 6) during first 2 hours of experiments when fed with *H. Triquetra* (red) or *R. Salina* (blue) at a start algal volume of app 3.3 * 10^6 μm^3 ml^{-1}. Bars are presented with standard deviations.

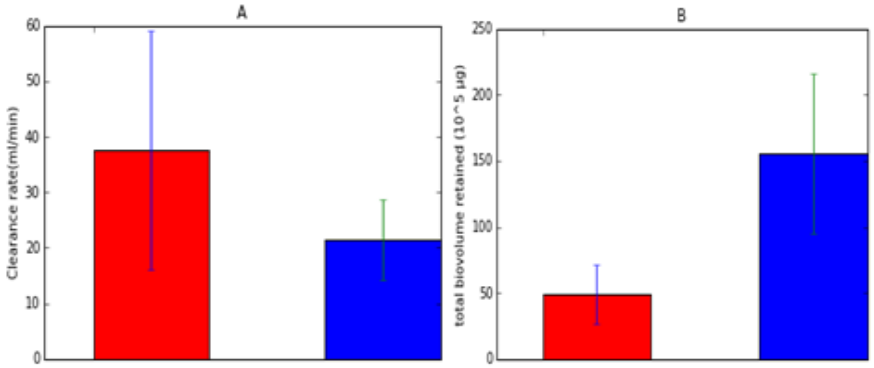

Figure 11 . The average CR of *M. edulis* (n = 6) during first 90 minutes of experiments (A) and the total bio volume retained (B) (n=6) when fed with appx. same start concentration measured in bio volume of the

two different sized algae of the same genus, *T. Levis* (red) ($7.3*10^5 \pm 1.6*10^5$) *T. Contracta* (green) ($8.4*10^5 \pm 1.7*10^5$). Bars are presented with standard deviations.

CR of *M. edulis* fed with a mixture of algae of different size.

The CRs of six individual *M. edulis* when fed 6 different randomly selected mixtures of *R. salina* (151.8 ± 52.72 μm^3) and *H. triquetra* (2440 μm^3 ± 1037 μm^3) (Table 2) were when calculated separately for the two algal species not significant different ($p = 0.70$). Therefore, the RE of the two algal species was indentical.

Another experiment was performed with *M. edulis* (n=6) fed one random selected mixture of *R. salina* (151.8 ± 52.72 μm^3) and *T. contracta* (4204 μm^3 ± 1037 μm^3) the CRs were, when calculated individually for *R. salina* 6.8 ± 2.5 ml min^{-1} and for *T. contracta* 6.3 ± 1.9 ml min^{-1} . were not significantly different ($p = 0.85$) as above.

	R. salina CR (ml min-1)	*H. triquetra* CR (ml min-1)
A	12 ± 5.5	13 ± 7.8
B	11 ± 4.9	13 ± 5.5
C	28 ± 4.5	25 ± 4.6
D	13 ± 6.7	19 ± 5.7
E	50 ± 15.3	46 ± 27.9
F	6 ± 1.5	10 ± 2.1

Table 2. The average CR (ml min^{-1}) during 3 hours in 6 (A-F) experiments, where *M. edulis* (n=1) was fed with mixture of two algae of different size (*R. salina* (151.8 ± 52.72 μm^3) and *H. triquetra* (4204 μm^3 ± 1037 μm^3)) at random start concentrations within the medium range (2000 – 6000 cells ml^{-1}).

Protection against overloading the gut system

It was found that *M. edulis* (n=6) fed with 3 different start concentrations of *R. salina* (29000; 19000 and 17000 cells ml^{-1}) all defined within the high concentration range the CRs became constant or slowly decreasing with time after an adjustment period of 60-40 minutes (Fig. 12) ((slope -0.003 ± 0.03; n = 6; t stat =-0.62; p = 0.56)(slope -0.027 ± 0.04; n = 6; t stat = -0.12; p =

0.90) and (slope -0.06 ± 0.02; n = 6; t stat = -3.01; p = 0.03) respectively). The CRs of the three start concentrations were 29065 ± 1375, 19830 ± 700 and 17219 ± 697, respectively. The CRs were all found to be significant different (P<0.01). In addition, the CR was seen to decrease with increasing algal start concentration. However, after the adjustment period the bio volume filtrated (not shown) during the experiments were not significantly different (P=0.47)

It was found that *M. edulis* (n=6) fed 5 different algal species (group 1 and the larger sized *H. triquetra*) at start concentrations within the defined high range measured in bio volume, the upper threshold concerning how large a volume (µmm^3 min^{-1}) *M. edulis* has retained in 6 out of 10 combinations were significant different (p <0.009) (fig. 13 and table 3).

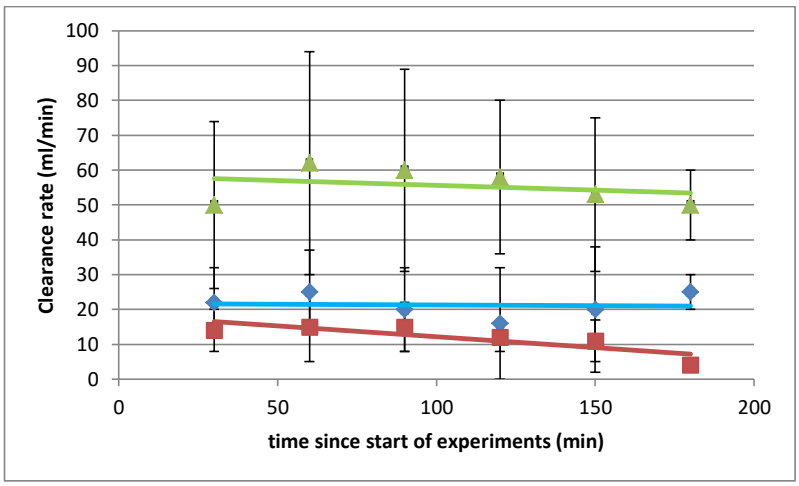

Figure 12 . The mean clearance rate of *M. Edulis* (n=6) fed with *R. salina* at start concentrations of29.000 (red line) , 20.000 (blue line) and 17.000 cells ml^{-1} (green line) . Points are presented with standard deviation bars. Regression lines inserted

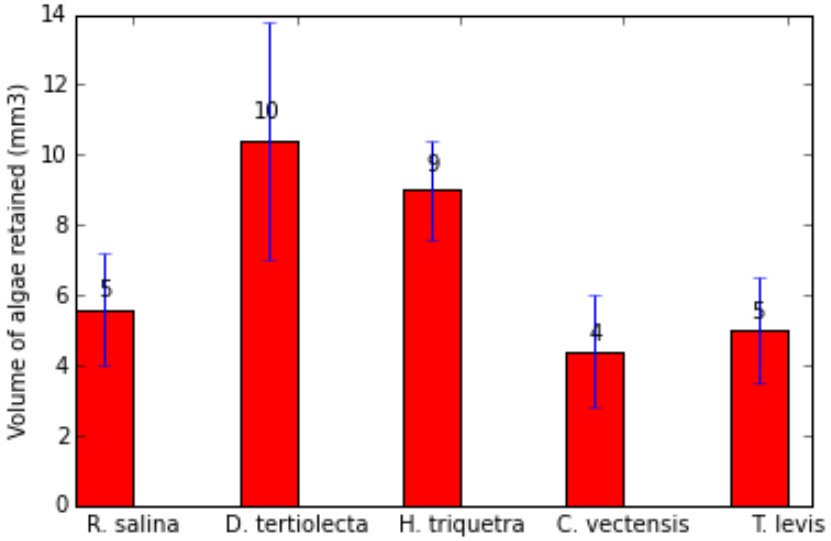

Figure 13. The algal volume retained by *M. Edulis* (n=6) during the first 2,5 hours of experiments when fed with 5 different algal species (n-total = 32). Concentrations of *H. triquetra* = 2,000 ± 300 cells ml^{-1} . Concentrations of the other four algal species were within the range defined as high (> 17.000 cells ml^{-1}).

	D. tertiolecta	*H. triquetra*	*C. vectensis*	*T. levis*
	p – values			
R. salina	0.009	0.008	0.300	0.700
D. tertiolecta		0.320	0.003	0.005
H. triquetra			0.001	0.002
C. vectensis				0.427

Table 3 . Two sided t-test of algal volume filtrated by *M. edulis* (n=6) fed with 5 different algal species at same concentration measured in biovolume.

pseudo faeces production

The observed pseudo faeces production of *M. edulis* (n=6) when fed with the four group 1 algal species at start concentrations (n=42) defined within the high range (>15000 cells ml^{-1}) was highly dependent on the algal species. The highest number of pseudo faeces occurances were 83 % when fed with *C. vectensis* and the lowest number of occurances were 8 % when fed with *D. Tertiolecta* (Fig. 14).

It was found that *M. edulis* fed with *C. vectensis* were producing pseudo faeces in both low, medium and high start concentrations, but when fed with *D. Tertiolecta* it was only in a few cases (8 %) and only when fed high start concentrations that pseudo faeces was observed.

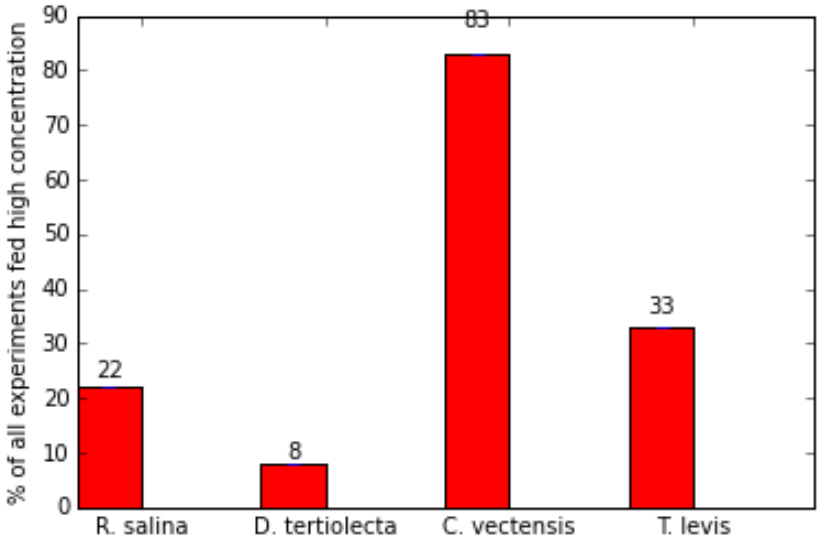

Figure 14 . The procentage of experiments (n=42) where *M. edulis* (n=6) fed high concentrations (> 15000 cells ml^{-1}) of the four group 1 algal species was observed to produce pseudo faeces.

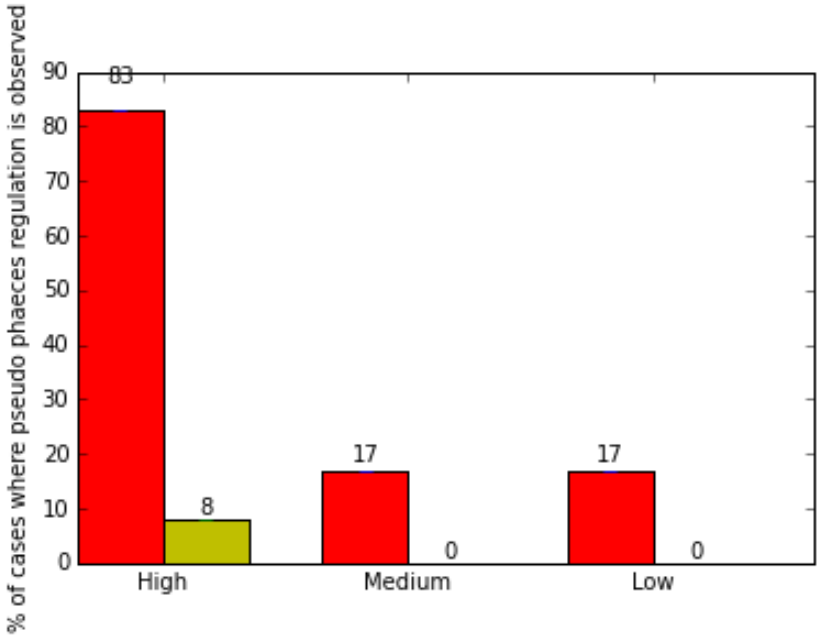

Figure 15. The percentage of experiments (n_{total}=360) where production of pseudo faeces by *M. edulis* (n=6) were observed when fed with *C. vectensis* (red) and *D. tertiolecta* at the high, medium and low algal concentration ranges

Lower threshold for clearance of *Mytilus edulis*.

When *M. edulis* (n=6) was fed with *R. salina* at different start concentrations (n=21) it was shown that the lower threshold for clearance was linearly related (R^2 = 0.64) to the start concentration (Fig. 16).

When fed with the similar sized algal species, *R. salina*, *D. tertiolecta*, *C. vectensis* and *T. levis* at the same low start concentration (app. 1700 cells ml^{-1}) the lower threshold for clearance was found to be 84 ± 42, 48 ± 36, 110 ± 28 and 176 ± 104 cells ml^{-1}. The lower thresholds for clearance found for the four algal species were not significantly different (p >0.05) (Fig 17 and Table 4).

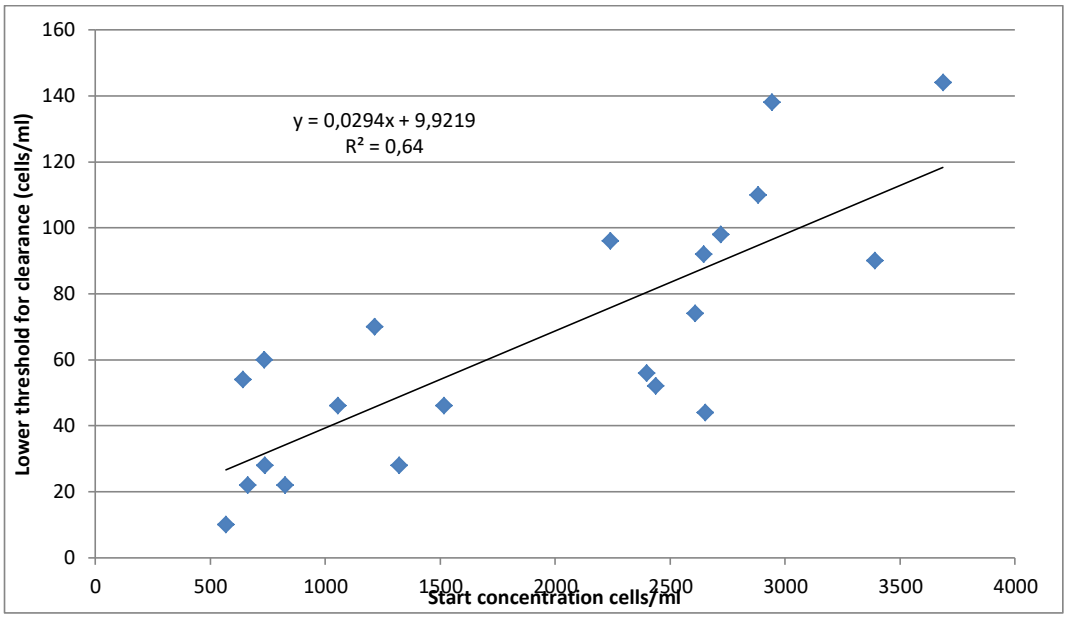

Figure 16. The lower threshold for clearance (i.e. stop algal concentration) for *M. edulis* (n=6) as a function of different start concentrations of *R. salina* (n=21).

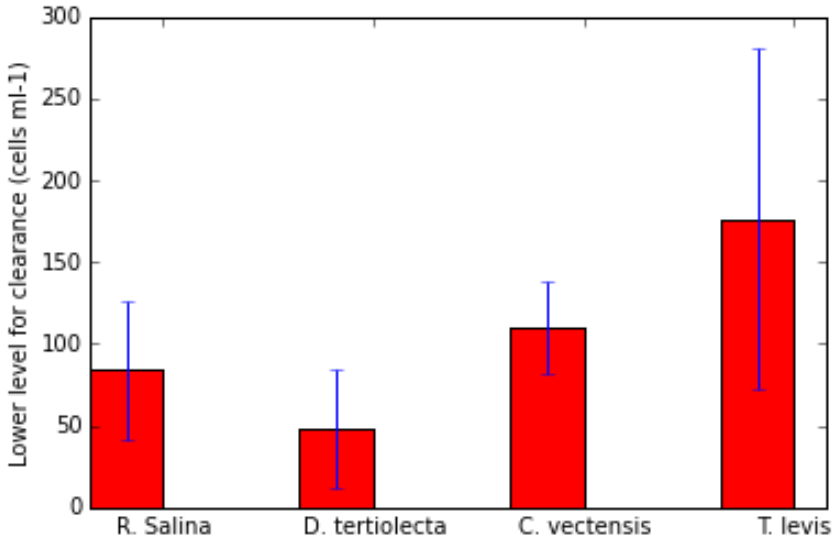

Fig. 17 Lower threshold for clearance of *Mytilus edulis* (n = 6)when fed with the 4 similar sized group 1 algal species at an appx. start concentration of 1700 cells ml^{-1}stopped filtrating (84 SD 42, 48 SD 36, 110 SD 28 and 176 SD 104 respectively). Bars are represented with standard deviation.

		Probability
R. salina	*D. tertiolecta*	0.32
R. salina	*C. vectensis*	0,40
R. salina	*T. contracta*	0,21
D. tertiolecta	*C. vectensis*	0,05
D. tertiolecta	*T. levis*	0,09
C. vectensis	*T. levis*	0,25

Table 4 Two sided t-test of the lower threshold for clearance of *M. edulis* (n=6) fed with 4 similar sized algal species at the same start concentrations.

Discussion

In the present study the CR of *M. edulis* fed medium concentrations of the four similar sized algal species with different surface characteristics became (after a short adjustment period) constant with time (Fig. 3). However, all the CRs were significantly different for the four algal species (except for *C. vectensis* compared to *T. levis*) (Fig. 4). Therefore, the present study showed that *M. edulis* has the capability to sense different stimuli based on the surface characteristics (organic and non-organic) of the different algal species used and to regulate the CR accordingly. The nature of such stimuli could be, that some algae have organic surfaces others have non-organic, some are naked others have armour, all of which can immediately be sensed by *M. edulis* if it has the capability. It should therefore be noticed that two of the 4 similar sized algae with armour (*D. tertiolecta* with a protein layers and *T. levis* with a crystalline theca) are less preferred as compared to the two algae (*D. tertiolecta* and *R. salina*) without armour. However, the amount of data is not enough to go a step deeper and show specifically how the stimuli from such differences in surface characteristics can be sensed and affect the CR. (Espinosa et al., 2010; Rosa et al., 2013).

Besides surface characteristics, the content of the algae may play a role. In literature it has been showed that *M. edulis* has a preference - and thereby can sense – algae with a high content of Chlorophyll *a* (Newell et al., 1989). Another (more obvious) parameter would be carbon content (as a proxy for energy). It has been shown that *Mytilus trossulus* can adjust the CR in a changing silt environment in such a way, that the intake of carbon is constant (Arifin and Bendell-Young, 1997). However, in the present study no significant correlations between the CR of *M. edulis* and the content of carbon (or Chlorophyll *a*) carbon were found.

Feltkode ændret

The above is not the same as saying that the CR does not correlate with the Chlorophyll *a* or the carbon content, because if *M. edulis* is regulating the CR with preferences for Chlorophyll *a* and/or carbon content, the stimuli causing such a regulating most likely come from the metabolism. Therefore, the absence of correlation in the present study could be a result of the experimental time was too short (3 hours) for such effects to be observed.

Algal size is an additional parameter which can influence the CR of *M. edulis*. However, an experimental problem in the study of the effect of different sized algae is how to establish equal experimental conditions since there will be difference between having the same start concentration in cell concentration or in bio volume. The other problem is how to measure the results since there can be differences if the same experiment is measured in CRs or in bio volume retained. In the present study this was addressed by conducting parallel experiments where equal conditions were established by measuring the algal start concentration in cell density (cells ml^{-1}) (Fig. 7 and 8) or in biovolume mg ml^{-1} (Fig. 9 and 10). At the same time the results of the two experiments are measured both in biovolumes (Fig. 8 and 10) and in CRs (Fig. 7 and 9). In the first serie of experiment with algae different size *M.edulis* where fed with *R. Salina* (152 ± 53 μmm^3) or *H. triquetra* (2440 ± 1037 μmm^3)

Only in the case where the start concentration were the same in density (and thereby having a much higher bio volume per ml of water *M.edulis* retained a higher biovolume ($p=0.01$) of *H. Triquetra* than *R.salina* (fig. 8) despite the CR was higher ($p=0.05$) when fed with *R. Salina*.

When equal start condition was established by measuring in bio volume *M. edulis* retained more *R salina* than *H. Triquatra* ($p=0.05$) mesured in bio volume and the CR were significant higher ($p=0.01$) when fed with *R. salina*.

This together shows a clear preference in favour of *R. Salina* against the much larger *H. Triquetra* .

Even though there were found significant differences it can not be concluded if this significant difference is due to difference in size or difference in surface structue, since this study have shown that difference in surface structure can make a significant difference.

Therefore, another serie of experiments were performed with two different sized algal species belonging to the same genus, *T. Levis* and *T. Contracta*, the latter being 20 times as big, measured in bio volume. Also here the CRs were significantly different regardless of the measurements were made in CR or in bio volume (Fig. 11). Although, differences in surface characteristics of the two

algal species can not be completely excluded, the experiment showed that algal size is only likely to have an effect on the CR.

These results generate an issue in relation to the feeding behaviour described by Riisgård (1991) (and used init this study) since Riisgård (1991) used only one algal species *(Rhodomonas baltica)* and the present result with algae of different size shows, that the defined densities (high, medium and low) may not be correct, if transferred to algae of different size by using the bio equavalient technique.

All algal species used in the present study were larger than 4 µm, and should according to Møhlenberg and Riisgård (1978) be retained by *M. edulis* with 100 % efficiency. In full accordance with Møhlenberg and Riisgård (1978) the REs of the different sized algae used in the present study were shown to be identical (Table 2 and Fig. 10). Therefore the differences in CRs found in the present study were a result of regulation of the pumping rate and not due to differences in the RE of the different algal species.

The overall conclusion is therefore, that *M. edulis* are capable of regulating the CR based on external stimuli produced by the algae. Therefore the present study supports the hypothesis by Rosa et al. (2013) that *M. edulis* can sense and react on the physical and/or chemical structure of the surface of the algae, rather than being a basic autonomous process.

How *M. edulis* protects its digestive system against overload when feed high algal concentrations has been discussed in the literature (e.g., Widdows et al. 1979; Kiørboe et al., 1980; Riisgård et al 2011; Riisgård 1991; Widdows et al. 1979). Part of the discussion is related to whether CR represents an autonomous or physiologically regulated process. It has been suggested that production of pseudo faeces is the only mechanism by which *M. edulis* can protect the digestive system against overload when feeding on high algal concentrations above a certain threshold (Kiørboe et al., 1980; Bougrier et al., 1996; Widdows et al., 1979). The threshold has been published to be 12.000 cells ml^{-1} of *R. salina* (Riisgård et al., 2011). The conception is based on the hypothesis that the *M. edulis* pump works as a basic autonomous process (Jørgensen et al., 1986; Riisgård et al., 2003). On the other hand, Widdows et al. (1979) showed that with increasing concentration of organic material the production of pseudo faeces will increase until a maximum and then the production of pseudo faeces will start to decrease. This decrease in pseudo faeces production was shown to be correlated to a decrease in CR (Widdows et al, 1979). The study by

Widdows et al. (1979) was done with seston containing not digestible material, but if fed only with digestible algae it seems counterintuitive to produce pseudo faeces instead of regulating the CR, because if CR is not regulated, *M. edulis* will spend energy on clearing more particle than it can handle and use even more energy on pseudo faeces production to get rid of the surplus. Espinosa et al. (2010) reached the conclusion, that the production of pseudo faeces is a result of unidentified chemical reaction between the mucus and the surface of the algae, and others have suggested that *M. edulis* is capable of performing particle selection (Ward & Targett, 1989; Bourgrier et al., 1997) and production of pseudo faeces is the mechanism by which the mussel gets rid of the not wanted particles.

In the present study, *M. edulis* was shown to be capable of regulating the CR and the question is therefore if this capability is used to protect against overload of the digestive system. The CRs of *M. edulis*, when feed the different experimental algal species in high concentrations (bio volume ml^{-1}), were significantly different and decreased with increasing algal concentrations (Fig. 12). In addition, the volume of how many algae (bio volume $time^{-1}$) *M. edulis* were filtrating was found to depend on the algal species (Fig. 13 and table 3). The latter shows that the threshold for overload was not just determined by the volume of the digestive system but most likely also by the speed by which the food passes through the digestive system.

In the present study *M. edulis* was shown to decrease the CR significantly with increasing high concentrations of *R. salina* and only in 22 % of the experiments production of pseudo faeces was observed (Fig. 14). Similar, when *M. edulis* was fed high concentrations of *D. teriolecta* and *T. levis*, pseudo faces was only observed in 8 % and 33 % of the experiments, respectively (Fig. 14). In contrast to this, *M. edulis* produced pseudo faces in 83 % of the experiments when fed *C. vectensis* (Fig. 14). Further, it was shown that when fed *C. vectensis* pseudo faeces was also produced at algal concentrations where there was no need for protection against overload of the digestive system. The observations on 1) *C. vectensis* combined with the knowledge, that this algal species has a non-digestible surface, and 2) the low percentage of pseudo faeces production in high concentrations of the other algal species used, supports, that pseudo faeces production primarily is a mechanism by which *M. edulis* discards unwanted algae.
The conclusion is therefore, that *M. edulis* most likely protects the digestive system against overload by adjusting the CR according to the algae concentration (i.e., constant ingestion) rather than producing pseudo faeces. In addition, the present study supports the hypothesis (Ward &

Targett, 1989; Bourgrier et al., 1997) that production of pseudo faeces can be a part of performing particle selection.

All organisms have to be energetically efficient in order to be competitive and ultimately to survive. In terms of bivalves this implies that they will stop filtrating (i.e., lower threshold), when the energy used for filtration becomes lower than the energy gained from the ingested food. In other words, this leads to the conclusion, that *M. edulis* is able to react on the energy in the food and adjust the CR accordingly as discussed by e.g. Riisgård (1991) and Strohmeier et al. (2009).
In the present study, the lower threshold for the 4 similar sized algal species was found significantly different (Fig. 17 and Table 4) when *M. edulis* was fed with the algae in the same start concentrations (Fig. 17). This could imply that the different algal species have different content of energy, which *M. edulis* can sense and adjust the CR accordingly. However, taking the actual measured carbon content of the same 4 algae into account (Fig. 5) such a correlation between preference and content of energy was not found, neither was a correlation between Chl *a* and preference found. (Fig. 5 and 6). An explanation could be, that the experiments in this study was only short time experiments (less than 3.5 hours) and if such a preference according to energy content of the algae comes as a metabolic response , it is not likely to be found within the first 3 hours fed with a specific algal.

Another finding was, that if the lower thresholds were represented as a function of the start algal concentration (fig. 16) it was clearly seen, that the lower thresholds were highly dependent on the start concentration.

The present study did not identify a specific energetical lower threshold where *M. edulis* stops filtration due to low algal concentration. On the contrary, it was shown that the lower limit where *M. edulis* stops filtrating depended on the algal species it was feeding on (Fig. 17) and the experimental algal start concentration (Fig. 16). However, as mentioned earlier, the stimulus concerning energy content must come from the metabolism and most likely such a stimulus could not be seen in 3 hours experiments.

The findings, that the lower threshold varies both with the type of algae eaten and the feeding history prior to the threshold is met, could lead to the theory, that both the amount and the quality of the food within the gut system of *M. edulis* are determining factors concerning how much energy *M.edulis* will spend in getting additional food at very low concentrations of algae.

In conclusion, a specific lower threshold related to the energy content of the different algal species used was not identified. However, the experiments clearly showed that in the study of the lower threshold the experimental methodology and the bivalve feeding history are most important parameters.

Based on the present discussion the feeding behavior of *M. edulis* concerning the CR as described by Riisgård (1991, 2003) can be modified as follows:
High : At algal concentrations > 15000 ml^{-1} *M. edulis* will after a short period of active clearing the water reduce the clearance rate, because of satiation of the digestive system (Riisgård 1991)(Fig. 14). In addition, the present study showed that the CR is dependent of the alga species (Fig. 13).

Medium: At algal concentrations in the range of 2000 – 6000 cells ml^{-1}*M. edulis* will display a high and constant clearance rate. (Riisgård, 1991)(Fig. 3). However, the present study showed that such a constant rate is highly dependent on the algal species being filtered (Fig. 4).

Low: According to Riisgård (Riisgård et. al, 2003) when lowering the algal concentrations, there will be a lower threshold, determined by when *M. edulis* will reach a point where it will stop clearing the water because the energetically expense to pump water across the gills exceeds the energy gained from digestion of the captured algae. In addition, the present study showed that such a lower threshold also depends on how much *M. edulis* have eaten prior to reaching the threshold (Fig. 16), and on the algal species being filtered (Fig. 17).

The present study has demonstrated that *M. edulis* has the capability to sense different stimuli based on the characteristics of the different algal species and thereby a preference which is used to regulate CR. However, particle selection requires both the ability to sense the differences and the physical capability to sort away the non-preferred particle. In principle, there only exists 2 ways by which particles can be selected either in filtering or by being rejected as pseudo faeces. The present study showed that the algae were filtrated equally efficient (Table 2). The study also showed, that the production of pseudo faeces were significant different (Fig.4) depending on the algal species and a correlation between high pseudo faeces production and non preferred algae (*C. vectensis*) could be observed. This supports the theory, that pseudo faeces is a mechanism used by *M. edulis* primarily to perform particle selection.

Perspectivation

Mytilus edulis is an important organism, both economical, and as a major component of the marine fauna in many coastal areas. Therefore, there have been performed a high number of studies in order to understand the feeding behavior of *M. edulis.* In spite of the high number of studies many key points still remain unresolved - as addressed in the introduction of the present thesis. It is of course too optimistic to believe that the present study, should find all the missing answers. However, several pieces have been added to the puzzle of understanding the feeding behavior of *M. edulis.* Among these are the variation in CR based on differences in surface characteristics and size of algal species and important observations concerning the functionality of pseudo faeces. In addition, the study can serve as inspiration on where to go next and where not to go in search for the answers. This study is based on a method with a closed system, which limits the observation period due to the change in algal concentration. It is therefore recommended, that the experiments are supplemented with same sort of experiments, but in an open system, where the algae concentration can be kept constant. By using such a method, the long time response to different algal species can be studied. This should give further pieces to the puzzle by adding results on differences caused by responses to stimuli produced by the metabolism.

This study can also give inspiration to additional experiments, which may give new and important answers. An obvious example is to repeat the experiment where CR is measured in a mixture of two algal species. The two algal species in such future studies could be *C. vectensis* and *T. contracta* (or *R. salina*). The reason is, that in the present study it was shown, that *C. vectensis* is a non-preferred algal which courses a high degree of pseudo faeces, and *T. contracta* is a preferred algae, which – even at high concentrations – do not courses generation of pseudo faeces. If *M. edulis* are able to perform particle selection, one should expect in such a combination, that the non-preferred would be transported back into the water column via pseudo faeces, and the preferred would be digested.

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